

**Metallointercalator-DNA Conjugates: Synthesis and Application in
Investigations of DNA-Mediated Electron and Energy Transfer**

Thesis by

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DEDICATION

To my grandparents.

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ABSTRACT

The DNA base stack offers a unique medium for long-range charge transfer (CT). Dipyridophenazine (dppz) complexes of Os(II) were characterized as a new class of luminescent DNA probes and applied in mechanistic studies of DNA-mediated electron transfer (ET). The product of ET between $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ (phi = phenanthrenequinone diimine) was spectroscopically characterized to confirm the mechanism of ET in quenching reactions on DNA. The rate of photoinduced ET is $> 3 \times 10^{10} \text{ s}^{-1}$ and the rate of recombination is $\sim 10^{10} \text{ s}^{-1}$ for several donor-acceptor pairs. The rate and efficiency of ET depend sensitively on the DNA binding properties of the reactants and DNA sequence, which underscores the importance of π -stacking interactions in ET through the DNA base stack.

To investigate long-range DNA-mediated CT in well defined molecular assemblies, a methodology was developed to tether metallointercalators to the 5', 3', or both termini of oligonucleotides on a solid support. This method permits the construction of metallointercalator-DNA conjugates possessing metal complexes that vary in photochemical and photophysical properties, as well as structure. These diverse properties were exploited to characterize the intercalation of the tethered complexes and the chemical composition of the materials.

Several CT reactions in modified duplexes were investigated. Rh-modified oligonucleotides were employed to introduce a photoexcited hole into the DNA π -stack at a precise location and initiate remote oxidative repair of thymine dimer lesions in duplex DNA. The yield of repair was sensitive to the stacking of the photooxidant and the intervening bases, but not distance. Here, the DNA bases mediate CT over long-range (26 Å) and serve as a reactant. The yield of photoinduced ET between $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ ($\text{M} = \text{Os}, \text{Ru}$) and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ derivatives tethered to opposite ends of oligoduplexes (14-18 bp) was modest ($\leq 15\%$) and sensitive to sequence and

orientational isomer. This lower efficiency of ET compared with studies of metallointercalators bound noncovalently to DNA may reflect the inhibition of stacking introduced by the tether.

The DNA base stack has been shown also to mediate triplet energy transfer (TET), which consists of simultaneous hole transfer and ET. The yield of subnanosecond DNA-mediated TET between Ru(II) and Os(II) metallointercalators tethered to DNA is found to be remarkably sensitive to the stacking of the metal complexes in the duplex, and the stacking of the intervening bases. TET is observed over a distances ranging from 27-40 Å and requires intercalated reactants. The distance dependence for subnanosecond TET in duplexes 14-18 bp in length is extremely shallow, especially for TET, which required two CT events. These studies illustrate the facility of CT through the DNA base stack and the sensitivity of several DNA-mediated CT reactions to π -stacking of the reactants and the medium.

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Chapter 1: Double Helical DNA: Remarkable Medium For Electron Transfer and Conduit for Chemistry at a Distance[#]

1.1. INTRODUCTION

As the molecular library of the cell the DNA double helix is thought of primarily as a substrate for molecular recognition. Genetic information must be accessed and expressed through an array of noncovalent interactions between proteins and nucleic acid.¹ Rational drug design targeted to DNA depends upon determining the general principles that govern the selective recognition of a nucleic acid site.² In order to understand in detail the remarkable variety of reactions involving the double helix within the cell, such as those to repair DNA damage, to coordinate the transcription of different genes, or to screen the fidelity of the DNA library, it becomes important to explore and consider the rich chemistry of DNA.³ The work described in this thesis is aimed at gaining an understanding of the ability of the π -stacked DNA bases to facilitate charge transfer reactions over large molecular distances.

In our laboratory we have focused on the DNA double helix as a medium for charge transfer chemistry. From this perspective, the DNA double helix may be viewed as an extended array of stacked, electronically coupled, aromatic heterocycles within a polyanionic sugar-phosphate helical backbone (Figure 1.1). Extended π -systems in the solid state facilitate charge transport and are a major focus of study in materials research.^{4,5} The DNA double helix is a molecular π -stacked array, and represents a unique, structurally well-defined system to explore charge transfer mediated by such an array in solution. We sought to ask whether the DNA double helix facilitates charge

[#]Adapted from: Holmlin, R. E.; Dandliker, P. J.; Barton, J. K. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 2714-2730.

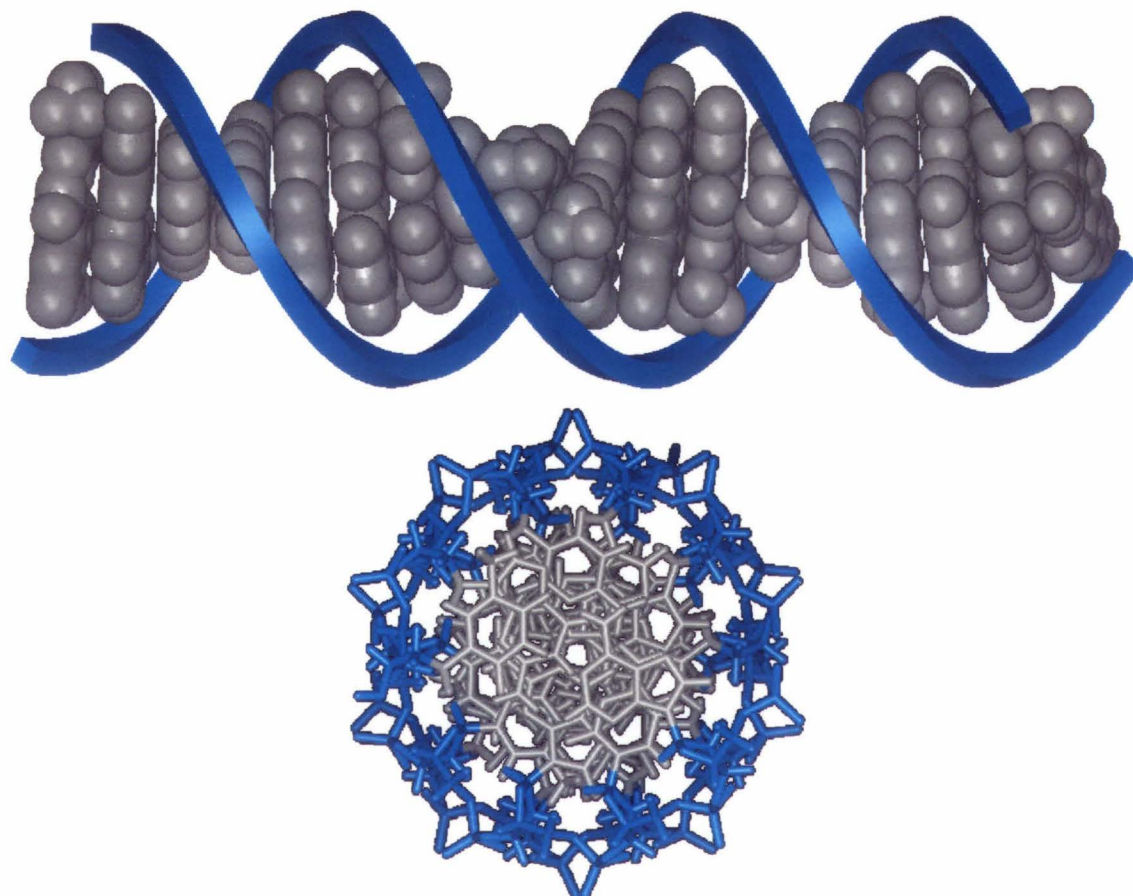


Figure 1.1. Schematic representation of double helical DNA emphasizing the linear array of π -stacked aromatic heterocycles (gray) and the sugar-phosphate backbone (blue). Views are perpendicular to the helical axis (top) and down the helical axis (bottom).

transfer over long distances, and indeed, whether the base stack can act as a conduit for chemistry at a distance.

Over the forty years since Watson and Crick first proposed the double helical structure for DNA, scientists have considered the possibility of charge transfer through the base stack. Early theoretical models predicted that electron hopping from base to base might occur on a timescale as fast as a few picoseconds.⁶ Conductivity measurements on DNA fibers bracketed a "DNA band gap" at ~ 2 eV.⁷ The question of whether radicals, generated by high energy irradiation of DNA, migrate over distances of 2 or 200 base pairs has been the subject of intense debate amongst radiation biologists.⁸ In the last decade, experimental studies of DNA as a medium for charge transfer by chemists have centered on photoinduced electron transfer reactions between donors and acceptors bound to DNA in solution.^{9,10}

In our laboratory we have designed small, coordinatively saturated transition-metal complexes to interrogate nucleic acid structure and function.¹¹ To characterize DNA-mediated charge transfer reactions, we exploit the well-characterized chemical and photophysical properties of polypyridyl metal complexes.^{9,12} Studies by Barton and coworkers revealed that the presence of the DNA enhanced the efficiency of photoinduced electron transfer between complexes bound weakly to the polymer;¹³ the rate of luminescence quenching of photoexcited $[\text{Ru}(\text{phen})_3]^{2+}$ by tris(phenanthroline) complexes of Co(III) and Rh(III) is enhanced by two orders of magnitude in the presence of DNA. Moreover, this electron transfer reaction can be enantioselective: for Δ - $[\text{Ru}(\text{phen})_3]^{2+}$, which binds to DNA more tightly than the Λ enantiomer, electron transfer is more efficient. These first studies demonstrated the utility of chiral, octahedral metal complexes in probing electron transfer through the DNA π -stack. Since DNA binding by tris(phenanthroline) metal complexes is not restricted to intercalation, several factors could contribute to the enhanced reactivity in the presence of DNA: (a) the increased local concentration of reactants near the helix; (b) the possibility of facilitated diffusion

of species along the polymer; and, perhaps most intriguingly, (c) the possibility that electron transfer may occur over long range through the π -stack.

Since these early studies, we have focused on metallointercalators—complexes which bind to DNA by classical intercalation—to explore charge transfer chemistry through the π -stack.⁹ The tight binding and rigid association of metallointercalators with the helix restricts diffusion along the DNA polymer. Moreover, intercalative stacking of the donors and acceptors in the DNA helix provides a direct probe of the DNA π -stack. We find, in each system below and in the work described in the following chapters, that the charge transfer chemistry mediated by the DNA is most affected by how the donors and acceptors are coupled into the DNA π -stack. DNA-mediated electron transfer depends very sensitively upon π -stacking.

The goal of this chapter is to introduce the approach we developed to investigate DNA-mediated charge transfer. We summarize the results in our laboratory that guided the work presented in this thesis, as well as some results that were made possible or inspired by work in this thesis. We also summarize work from other groups that has contributed to our understanding of the problem of charge transfer mediated by the DNA base stack.

1.2. Metallointercalators as Probes of DNA-mediated Electron Transfer

In probing DNA-mediated electron transfer chemistry, we focused on octahedral metal complexes which bind tightly to DNA by intercalation. NMR studies have been useful in characterizing structurally the noncovalent association of these metal complexes with the DNA duplex, while photophysical and photochemical studies have been exploited in defining the sites intercalation and the factors affecting association of the complexes along the helical polymer. These companion experiments have proven to be powerful tools in delineating the charge transfer chemistry. Figures 1.2 and 1.3 illustrate schematically some of the complexes we may employ as donors and acceptors for

photoinduced electron transfer.

1.2.1. Dipyridophenazine Complexes of Ruthenium and Osmium

The family of mixed-ligand complexes $[M(L)_2(dppz)]^{2+}$ ($M = Ru, Os$; $L = phen, bpy$; $dppz = dipyrido[3,2-a:2',3'-c]phenazine$) containing the $dppz$ ligand represents a straightforward extension from the tris(phenanthroline) metal complexes, but one in which the aromatic surface area of the $dppz$ ligand provides an expansive platform for intercalation (Figure 1.2). $Dppz$ complexes of ruthenium, osmium, and more recently cobalt, nickel, and rhenium, have been shown to bind to DNA by preferential intercalation of the $dppz$ ligand within the helical stack.¹⁴⁻¹⁷ Binding affinities for duplex DNA are found to be $\geq 10^7 M^{-1}$, approximately three orders of magnitude higher than the simple tris(phenanthroline) metal complexes. NMR studies of selectively deuterated $[Ru(phen)_2(dppz)]^{2+}$ isomers bound to a DNA hexamer have indicated intercalation from the major groove side of the DNA helix with a population of intercalative orientations.¹⁸ Although some sequence preferences are evident in binding of the complexes to DNA by NMR, high sequence selectivity is not apparent, consistent with biophysical studies which indicate classical intercalation essentially randomly along a helical polymer. Because of the low sequence-selectivity, however, a detailed structural determination of how these complexes stack in a helix is precluded.

The utility of the $dppz$ complexes for our studies rests not only in the tight intercalative binding by the complexes but also in their unique photophysical properties. Irradiation of complexes in this class with visible light leads to efficient population of a metal-to-ligand charge-transfer (MLCT) excited state, localized on the $dppz$ ligand.^{14,19,20} Although luminescence can be readily detected upon excitation in organic solvents, in aqueous solution the luminescence is quenched by proton transfer from solvent to the phenazine nitrogens.^{19,20} The DNA π -stack provides protection from this quenching. Therefore, when the $dppz$ ligand intercalates into the base pair stack, the phenazine

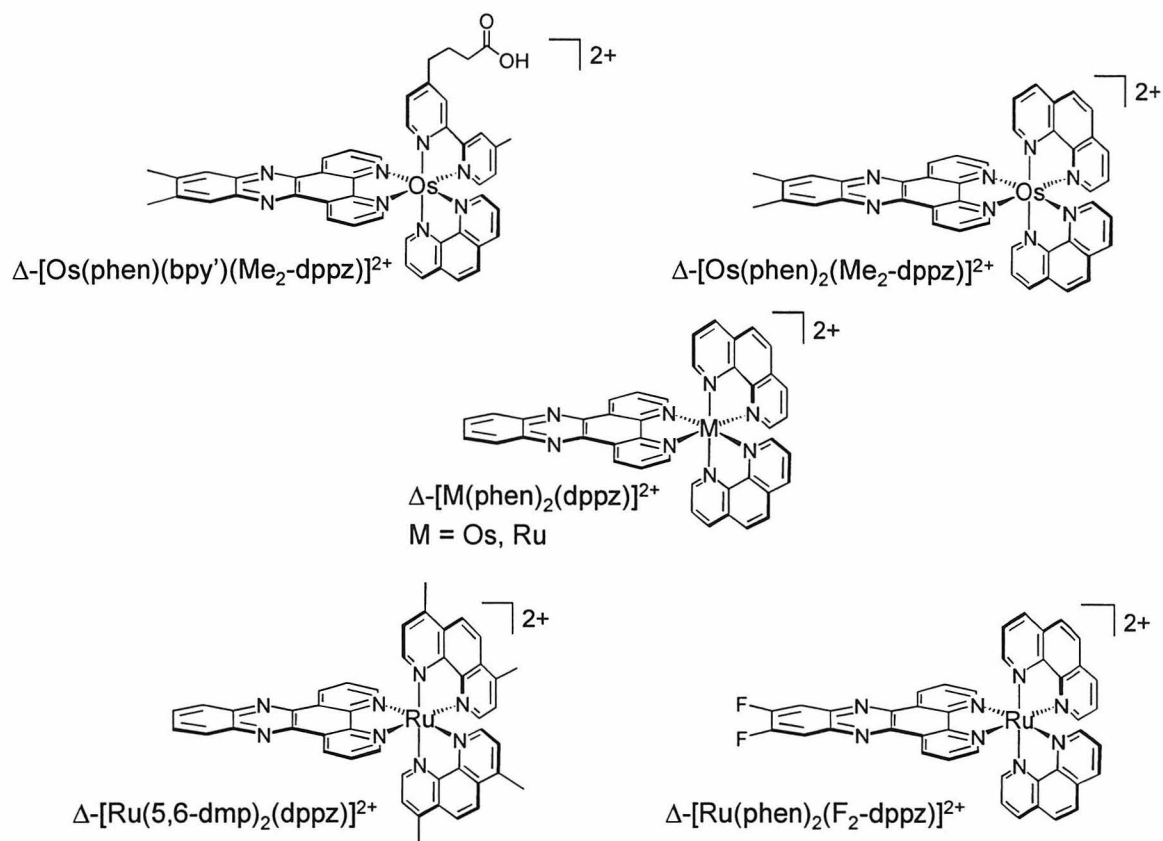


Figure 1.2. Derivatives of $\Delta\text{-}[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ that bind DNA by preferential stacking of the dppz ligand between adjacent DNA base pairs. In the metal-to-ligand charge-transfer excited state, these complexes direct an electron into the DNA π -stack.

nitrogens are protected from water, and the complex is again emissive. This effect has proven to be a sensitive diagnostic for nucleic acids, and the term "molecular light switch" has been adopted to describe this interesting luminescence property. Moreover, these molecules have become generally useful in characterizing hydrophobic matrices, for example SDS micelles²¹ and Nafion[®] films.²²

Owing to the light switch character of $[\text{Ru}(\text{L})_2(\text{dppz})]^{2+}$ complexes, the steady-state and time-resolved emission profiles are particularly sensitive to orientation of the metal complex within the DNA π -stack.¹⁴ For *rac*- $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to mixed sequence B-form DNA (nucleotide:Ru = 100:1), the emission follows a biexponential decay with $\tau_1 = 130$ ns (80%) and $\tau_2 = 730$ ns (20%). For the pure isomers Δ - and Λ - $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA, biexponential decays are also observed,²³ which likely reflect the population of binding orientations observed by NMR.¹⁸ For Δ - $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA, the emission decay is described by $\tau_1 = 150$ ns (80%) and $\tau_2 = 800$ ns (20%), while for Λ - $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to mixed sequence DNA, the luminescence decays with $\tau_1 = 30$ ns (80%) and $\tau_2 = 160$ ns (20%). This difference in emission decay profiles, manifested as an increase in the steady-state emission yield for the Δ enantiomer bound to DNA, reflects the greater protection of the intercalating ligand from solvent. Such a propensity for intimate association between Δ - $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and double helical DNA follows from the matching of symmetry between the right-handed DNA duplex and the right-handed metal complex.²⁴ It is also noteworthy that the excited state lifetimes and relative distributions vary with the concentration of bound species, which may reflect the sensitivity of these decay parameters to the rigidity of the helix, which increases with intercalator loading.

Substitution of the third-row transition element osmium for ruthenium in $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ complexes does not alter the light switch characteristic or the DNA binding properties of the complex but provides an isostructural analogue for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ with distinct photophysical and electrochemical properties. Like the

ruthenium analogue, no photoluminescence is detected upon irradiation of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in aqueous solution in the absence of DNA.^{15,25} In the presence of DNA, however, excitation of the complex affords significant, long-wavelength ($\lambda_{\text{max}} = 738 \text{ nm}$) emission. A biexponential decay in emission is observed for Δ - $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA with excited state lifetimes below 10 ns. The osmium center stabilizes the 3+ oxidation state of the complex by $\sim 500 \text{ mV}$ relative to ruthenium, causing the oxidation of the osmium excited state ($E_{1/2}^* \text{Os(II)/Os(III)} = 0.78 \text{ V vs. NHE}$) to be $\sim 200 \text{ mV}$ more favorable compared to ruthenium ($E_{1/2}^* \text{Ru(II)/Ru(III)} = 0.60 \text{ V vs. NHE}$). We are also able to utilize phen or bpy analogues bearing electron donating or withdrawing substituents to tune further the electrochemical properties of these complexes over a range of potentials. Substitution of methyl groups onto the 7 and 8 positions of the dppz ligand increases the excited state lifetimes for both osmium and ruthenium derivatives bound to DNA [$M = \text{Ru}$: $\tau_1 = 370 \text{ ns}$ (65%), $\tau_2 = 1170 \text{ ns}$ (35%); $M = \text{Os}$: $\tau_1 = 2.18 \text{ ns}$ (50%), $\tau_2 = 10.1 \text{ ns}$ (50%)]. This preferential charge transfer onto the dppz is an important characteristic which we exploit in our studies of DNA-mediated electron transfer, in that, for the intercalated complex, the charge transfer upon excitation is directed into the π -stack. These complexes are therefore particularly well suited to probe DNA-mediated charge transfer chemistry.

In Chapter 2, we present a comprehensive investigation of the synthesis, characterization, and photophysical properties of a family of dppz complexes of Os(II). These metallointercalators provide a new class of luminescent DNA probes. Their full characterization has illuminated the fundamental properties of the excited state of dppz complexes of Os(II) and laid a foundation to understand their behavior in electron transfer reactions bound to DNA.

1.2.2. Phenanthrenequinone diimine Complexes of Rh(III)

The rhodium intercalators we employ all contain the phenanthrenequinone-diimine (ϕ) ligand. Several complexes of Rh(III) we constructed are schematically illustrated in Figure 1.3. For these complexes it is the ϕ ligand that provides an extended, aromatic surface for intercalation. All of the ϕ complexes of rhodium we prepared thus far bind tightly to DNA by preferential intercalation of the ϕ ligand. Affinities have varied from 10^6 M^{-1} to 10^8 M^{-1} and the sites recognized may be tuned selectively by varying substituents on the ancillary, non-intercalating ligands.^{26,27} Some of these complexes display binding affinities for DNA and site specificities that rival those of DNA-binding proteins.²⁷

NMR studies have been particularly valuable in delineating structurally how these complexes are associated with DNA.^{28,29} The complexes all appear to bind DNA from the major groove. With the complex anchored by intercalation, functional groups incorporated on the ancillary ligands can interact with major groove sites so as to determine binding site selectivity.^{26,27} In the case of $\Delta\text{-}\alpha\text{-}[\text{Rh}[(R,R)\text{-Me}_2\text{trien}]\phi]^{3+}$, we constructed the complex so as to specify an ensemble of non-covalent contacts in the major groove for predictive targeting of the sequence 5'-TGCA-3'. Recently, a high resolution structural model of this complex bound site-specifically to a decamer was determined using NMR.²⁹ In this structure, the metal complex is well stacked and bound symmetrically to the double helix, with the different non-covalent contacts between the ancillary ligand and major groove site 5'-TGCA-3' apparent. Importantly, in the context of applying ϕ complexes in studies of DNA-mediated charge transfer, the DNA helix is unwound to accommodate the intercalator, but little perturbation to the DNA base-stack is evident beyond the intercalated base step.²⁹

In studying DNA-mediated electron transfer between metallointercalators, we frequently employ $[\text{Rh}(\phi)_2(\text{L})]^{3+}$ ($\text{L} = \text{phen}, \text{bpy}$) complexes as ground-state electron-acceptors. $[\text{Rh}(\phi)_2(\text{L})]^{3+}$ complexes are primarily sequence neutral in association with

DNA and therefore bind randomly to the duplex.³⁰ Characterization of $[\text{Rh}(\text{phi})_2(\text{L})]^{3+}$ electrochemical properties by cyclic voltammetry reveals that in DMF the formally

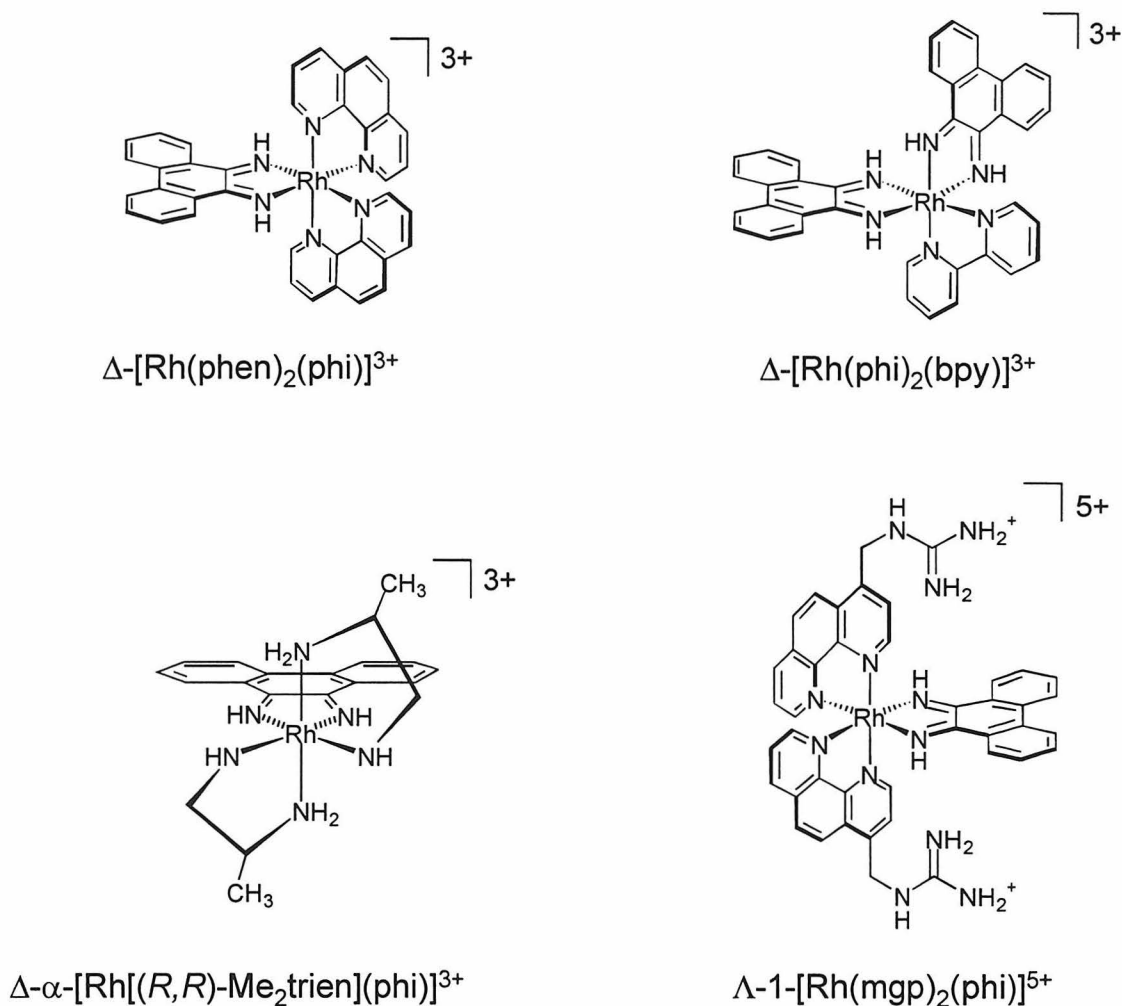


Figure 1.3. Illustrations of phi complexes of Rh(III) that bind to DNA by intercalation. The common feature across this family of metallointercalators is the phi ligand, which serves to anchor the complex in the major groove of DNA. Systematic variations in the ancillary ligands permit the tuning of binding affinity and site selectivity.

Rh(III)/Rh(II) reduction occurs at ~ 0 V vs. NHE.³¹ Hence, electron transfer from photoexcited $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ to $[\text{Rh}(\text{phi})_2(\text{L})]^{3+}$ bound to DNA is thermodynamically favorable ($\text{M} = \text{Ru}$: $\Delta G^\circ = 0.56$ V; $\text{M} = \text{Os}$: $\Delta G^\circ = 0.73$ V).

The photochemistry of these phi complexes of rhodium has proven to be quite valuable first in delineating where along the helical polymer the metal complex is bound in a given electron transfer reaction and secondly in providing potent photooxidants which are intercalated in DNA. Given the rich photochemistry associated with these complexes, we are able to utilize light at different wavelengths to induce selectively different chemical reactions.^{30,32}

Irradiation of the complexes bound to DNA with ultraviolet (UV) light ($\lambda = 313$ nm) leads to direct scission of the DNA sugar-phosphate backbone in a reaction consistent with abstraction of the C3'-H atom from the deoxyribose moiety by the photoactivated phi at the intercalation site.³⁰ This strand scission event marks the site of intercalation. This photochemistry has been used profitably in establishing the site-selectivities of a range of complexes.

If the $[\text{Rh}(\text{phi})_2(\text{L})]^{3+}$ complexes are excited with visible light ($\lambda \geq 365$ nm), a powerfully oxidizing intraligand excited state is populated.³² Based on quenching studies in solution in the absence of DNA, the excited state reduction potential of $[\text{Rh}(\text{phi})_2(\text{phen})]^{3+}$ ($E_{1/2}^* \text{Rh(III)/Rh(II)}$) is estimated to be ~ 2 V vs. NHE. We can therefore exploit these rhodium intercalators as not only ground state but also excited state electron acceptors.

In Chapter 6, we describe the application of $[\text{Rh}(\text{phi})_2(\text{L})]^{3+}$ complexes to the investigation of thymine dimer repair in duplexes to which the metallointercalator has been covalently attached. These studies are illustrative of reactions in which the DNA base stack not only mediates the charge transfer but serves as a reactant as well.

1.3. DNA as a Bridge for Electron Transfer

Scheme 1 illustrates the cycle of electron transfer reactions between metallointercalators bound to DNA. Irradiation of the M(II) complex with visible light leads to the formation of the photoexcited donor, which, in the absence of acceptor may return to ground state by emission of a photon. In the presence of an acceptor with the appropriate reduction potential, the photoexcited donor may undergo electron transfer to the ground state acceptor with rate constant k_{et} , which results in quenching of donor emission. The products of this forward electron transfer then undergo thermal recombination (k_{rec}) to regenerate the ground state pair. This photoinduced electron transfer chemistry is particularly useful. We are able to use a photon of light to initiate a cascade of electron transfer reactions with the DNA π -stack as the intervening bridge which may be monitored by a variety of spectroscopic methods on several different time scales.

1.3.1. Intercalative Coupling into the Π -Stack

Murphy *et al.* compared the quenching of photoexcited $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA by two acceptors: $[\text{Rh}(\text{phi})_2(\text{phen})]^{3+}$, which binds to DNA by intercalation; and $[\text{Ru}(\text{NH}_3)_6]^{3+}$, which binds to the surface of the double helix (Figure 1.4).³¹ Since the $3+/2+$ reduction potential for $[\text{Rh}(\text{phi})_2(\text{phen})]^{3+}$ ($E_{1/2} \text{ Rh(III)/Rh(II)} = -0.03 \text{ V vs. NHE}$) is nearly identical to that for $[\text{Ru}(\text{NH}_3)_6]^{3+}$ ($E_{1/2} \text{ Ru(III)/Ru(II)} = 0.01 \text{ V vs. NHE}$), the driving force for the reaction does not differ significantly between the two donor/acceptor pairs. When the intercalator $[\text{Rh}(\text{phi})_2(\text{phen})]^{3+}$ or $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ is titrated into solutions of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA, fast, efficient quenching of the ruthenium emission on the subnanosecond time scale is observed.

Over a similar concentration range, $[\text{Ru}(\text{NH}_3)_6]^{3+}$ also quenches the luminescence of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA. However, quenching by $[\text{Ru}(\text{NH}_3)_6]^{3+}$ is less

Scheme 1.1. Cycle of photoinduced electron transfer reactions between metallointercalators bound to DNA.

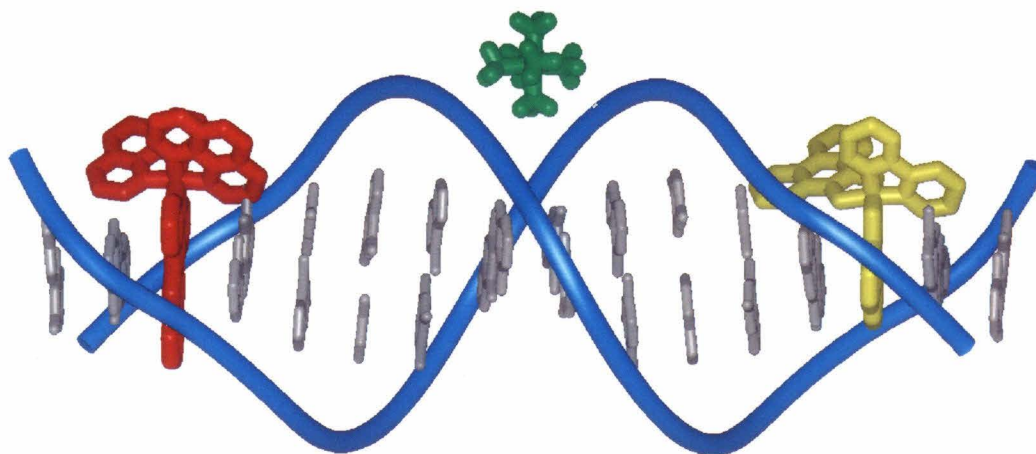
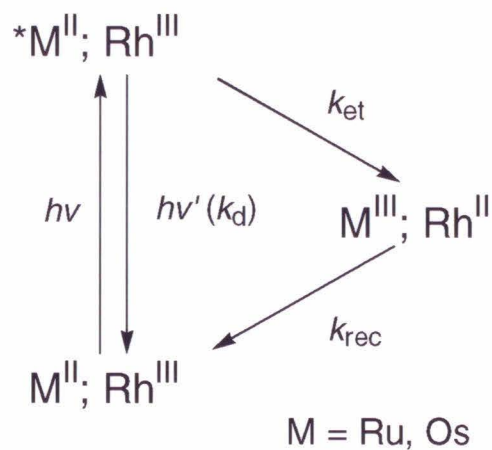


Figure 1.4. Schematic representation of transition metal complexes bound to DNA.

Shown are Δ -[Ru(phen)₂(dppz)]²⁺ (red), Δ -[Rh(phi)₂(bpy)]³⁺ (yellow) and [Ru(NH₃)₆]³⁺ (green). Δ -[Ru(phen)₂(dppz)]²⁺ and Δ -[Rh(phi)₂(bpy)]³⁺ are metallointercalators and probe the DNA base stack directly. [Ru(NH₃)₆]³⁺, which does not have the ligand framework to support intercalation, binds only by surface association, and thus cannot access the π -stack.

efficient, and proceeds on the nanosecond timescale. In contrast to the intercalating acceptor, then, $[\text{Ru}(\text{NH}_3)_6]^{3+}$ exhibits a quenching profile consistent with a reaction which requires diffusion. Here, electron transfer does not proceed through the DNA, rather the DNA merely facilitates diffusion of the acceptor.

The distinction between electron transfer reactions involving intercalated donors and acceptors and those involving groove-bound reactants is evident in data emerging from other laboratories as well.¹⁰ For example, quenching of intercalated ethidium by *N,N'*-dimethyl-2,7-diazapyrenium dichloride (DAP), also an intercalator, is enhanced compared to reaction in the absence of DNA.¹⁰ On the other hand, quenching of ethidium by groove-bound methyl viologen or palladium porphyrins does not exhibit the remarkable efficiency and rate observed with the metallointercalator systems.¹⁰ When methyl viologen is used to quench photoexcited $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA, the quenching is also slow relative to reactions between molecules which access the π -stack directly by intercalation.¹⁰

In fact, coupling into the π -stack is so important that even for molecules which bind to DNA by intercalation, the nature of the stacking interaction seems to affect the reactivity by electron transfer. For example, when $[\text{Rh}(\text{phen})_2(\text{phi})]^{3+}$ is titrated into solutions of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA, we observe no quenching of the ruthenium photoluminescence, despite sufficient driving force for the reaction.³³ Based upon photocleavage studies, $[\text{Rh}(\text{phen})_2(\text{phi})]^{3+}$ prefers to intercalate shape-selectively at sites with an open major groove and hence exhibits a greater sequence selectivity in binding to DNA compared to $[\text{Rh}(\text{phi})_2(\text{L})]^{3+}$ complexes.³³ Even though the complex binds by intercalation, the depth to which the complex intercalates is limited by steric clash between the ancillary phenanthroline ligands and the sugar phosphate backbone of the DNA. Thus, $[\text{Rh}(\text{phen})_2(\text{phi})]^{3+}$ appears unable to couple effectively into the DNA π -stack and fails to quench the ruthenium emission.

The effect of stacking on quenching efficiency is also important for the electron

donor. Owing to symmetry matching between the right-handed octahedral metal complexes and the right-handed DNA duplex,²⁴ the Δ enantiomer binds more deeply within the DNA base stack. It is not surprising then that Δ -[Ru(phen)₂(dppz)]²⁺ is quenched more efficiently by [Rh(phi)₂(bpy)]³⁺ than is Λ -[Ru(phen)₂(dppz)]²⁺.^{33,34} We have also observed significant differences in quenching efficiency in photoinduced reactions with [Ru(phen)₂(dppz)]²⁺ versus ethidium as donor under conditions where all donors and acceptors were fully bound. Here too we estimate no difference in driving force for the two redox couples, yet the intercalators appear to differ in how they stack in the DNA duplex and this affects the electron transfer reaction. It is clear therefore that effective stacking is requisite for coupling into the DNA duplex. A challenge to us is to develop a good understanding of what characterizes this effective stacking and how we may develop quantitative parameters to assess this stacking and its effect on coupling into the helix.

Experiments with [Os(phen)₂(dppz)]²⁺ as the photoexcited donor have been valuable in characterizing the fast, static quenching, and these experiments have ruled out energy transfer and metal-complex diffusion as mechanisms for the efficient quenching between metallointercalators.²⁵ Just as with ruthenium, we observe efficient quenching of the osmium emission in reactions with [Rh(phi)₂(bpy)]³⁺. In fact, over a range of acceptor concentrations, the osmium and ruthenium quenching profiles are remarkably similar. This result is inconsistent with diffusional quenching. If molecular diffusion were important, one would predict a reduction in quenching efficiency for the osmium donor, given its significantly shortened excited state lifetime compared to ruthenium. Moreover, the red-shifted emission spectrum of the osmium complex does not overlap the acceptor absorption, which precludes energy transfer as the quenching mechanism. The correlation between the ruthenium and osmium reaction profiles also rules out the participation of energy transfer in the quenching of either osmium or ruthenium by [Rh(phi)₂(bpy)]³⁺.

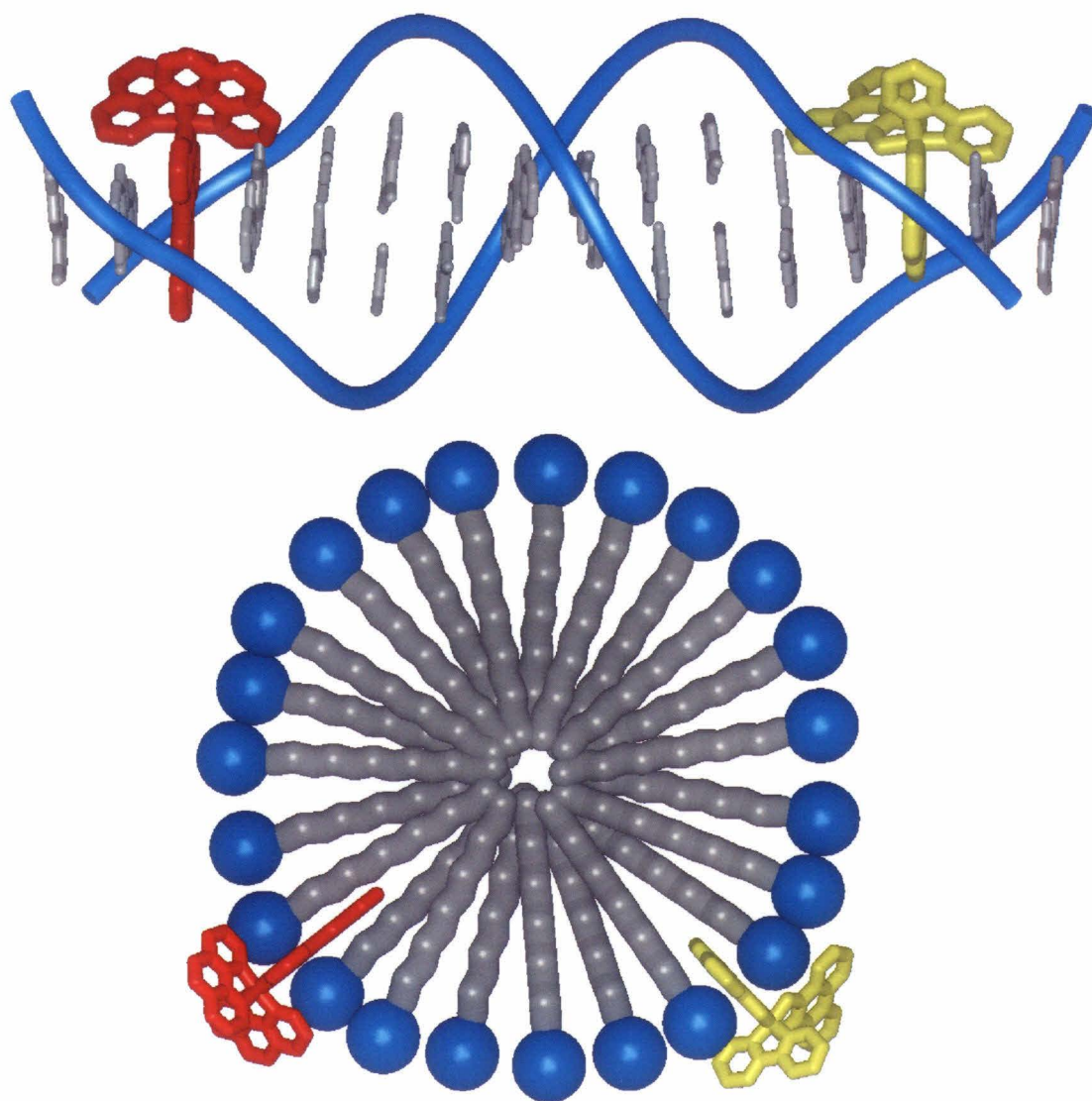


Figure 1.5. Schematic illustration of a Δ -[Ru(phen)₂(dppz)]²⁺ electron donor (red) and a Δ -[Rh(phi)₂(bpy)]³⁺ electron acceptor (yellow) bound to double helical DNA (top) and a sodium dodecyl sulfate micelle (bottom).

A comparison of the quenching of ruthenium donors by rhodium acceptors bound to DNA and SDS micelles further illustrates the importance of the DNA π -stack in mediating fast and efficient electron transfer (Figure 1.5).³³ The DNA double helix and the micelle both provide anionic surfaces to which our cationic donors and acceptors bind and a hydrophobic core region which accommodates their large, intercalating ligands. The primary difference between these two microheterogeneous environments is that the SDS micelle does not contain an organized pathway for electron transfer like the DNA. Accordingly, in the micellar system, rhodium complexes do quench the ruthenium luminescence, but the electron transfer is much less efficient than the DNA-mediated reaction, and proceeds on the nanosecond time scale. In fact, the quenching reaction in the micelle is diffusion-dependent and requires a collision between donor and acceptor. This observation contrasts DNA-mediated electron transfer reactions between metallointercalators where diffusion is clearly not required for quenching.

1.3.2. Rates of DNA-mediated Electron Transfer

To deepen our understanding of electron transfer between metallointercalators, we sought to utilize ultrafast laser spectroscopy to monitor the reaction on the picosecond time scale, and in so doing, hopefully determine the rates for these fast photoinduced quenching processes.³⁴ To examine the rate of forward electron transfer (k_{et}), we employed time-correlated single-photon counting (TCSPC) with ~ 50 ps resolution through which the excited-state decay for the photoexcited $[M(\text{phen})_2(\text{dppz})]^{2+}$ donors could be monitored. Remarkably, when quenching of photoexcited $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ by $[\text{Rh}(\text{phen})_2(\text{bpy})]^{3+}$ bound to DNA is followed by ultrafast TCSPC, no change in excited state decay kinetics was detected as a function of quencher despite significant intensity quenching. Instead, the quenching was manifested primarily as a loss of luminescence intensity at zero time, and this intensity loss increased with increasing $[\text{Rh}(\text{phen})_2(\text{bpy})]^{3+}$ concentration. The same observation is made for the isostructural

analog $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ (see Chapter 3). Such quenching behavior implies that the electron transfer quenching proceeds with a rate faster than the time resolution of this instrument. Hence, we have assigned a lower limit of $3 \times 10^{10} \text{ s}^{-1}$ to the rate constant (k_{et}) describing this ultrafast reaction.

To monitor the back electron transfer reaction, we have utilized ultrafast transient absorption spectroscopy. Following excitation of $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ complexes at 390 nm, we monitored the change in absorbance of the sample at 420 nm as a function of time. In the absence of acceptor, the kinetics of ground state recovery match the luminescence lifetimes of the donors bound to DNA. As $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ is titrated into solutions of donor bound to DNA, a new component is observed, which increases in intensity with increasing rhodium equivalents. Analysis of the transient absorption kinetics reveals that this fast phase is well described by a rate constant $k_{\text{rec}} = 9.0 \times 10^9 \text{ s}^{-1}$ for $\text{M} = \text{Ru}$ and $1.1 \times 10^{10} \text{ s}^{-1}$ for $\text{M} = \text{Os}$. Since such a rate constant could not be detected in the kinetics of luminescence decay, we are confident in our assignment of this rate constant to the recombination reaction.

Transient absorption data for seven donor-acceptor pairs taken on the picosecond time scale appear in Table 1.³⁴ Across this series, the reactants vary with respect to intercalating ligand, ancillary ligands, relative hydrophobicity, chirality, and metal center. Despite such a range of chemical properties, the rate observed by transient absorption is centered around 10^{10} s^{-1} . Even for the osmium complex, in which the 3+ oxidation state is stabilized by $\sim 500 \text{ mV}$ relative to the ruthenium complex, the recombination rate is not significantly perturbed. A significant difference in rate is observed, however, when the chirality of the donor is varied. For the right-handed Δ - $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$, $k_{\text{rec}} = 9.0 \times 10^{10} \text{ s}^{-1}$, while for the left-handed enantiomer k_{rec} is diminished by a factor of 2.5. The faster electron transfer observed for Δ - $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ is consistent with the deeper stacking of this enantiomer into the double helix.

Table 1.1. Rate constant for ground state recombination following electron transfer between photoexcited donors bound to DNA and Δ -[Rh(phi)₂(bpy)]³⁺ as the electron acceptor.^{a,b}

Donor	DNA	k_{rec}^c (10 ⁹ s ⁻¹)	$-\Delta G^{od,e}$ (V)
Δ -[Ru(phen) ₂ (dppz)] ²⁺	calf thymus	9.2	1.66
<i>rac</i> -[Ru(bpy) ₂ (dppz)] ²⁺		7.1	1.69
Δ -[Ru(4,7-dmp) ₂ (dppz)] ²⁺		11	1.59
Δ -[Ru(phen) ₂ (F ₂ -dppz)] ²⁺		7.7	1.68
<i>rac</i> -[Ru(phen) ₂ (Me ₂ -dppz)] ²⁺		9.2	1.67
Δ -[Os(phen) ₂ (dppz)] ²⁺		11	1.21
Λ -[Ru(phen) ₂ (dppz)] ²⁺		4.5	
Δ -[Ru(phen) ₂ (dppz)] ²⁺	poly d(AT)	7.4	
Δ -[Ru(phen) ₂ (dppz)] ²⁺	poly d(GC)	0.21	

- a. Based on ref. 34. Data obtained by transient absorption spectroscopy.
- b. Experimental conditions are as follows: [Ru, Os] = 20 μ M, [DNA] = 2 mM nucleotides (M:Nuc = 1:100), 5 mM Tris, 50 mM NaCl, pH 8.5.
- c. Error in k_{rec} is estimated to be ± 10 %.
- d. $-\Delta G^\circ = [E_{1/2} (M(III)/M(II)) - E_{1/2} (Rh(III)/Rh(II))]$
- e. Error in ΔG° is estimated to be ± 50 mV.

Of particular interest is that these recombination reactions are relatively insensitive to the loading of acceptor on the helix. As the concentration of acceptor is increased throughout a titration, given random occupation of the helix, one expects that the distribution of donor-acceptor separations should decrease. However, we observe in these experiments that, although the amount of recombination reaction on the picosecond timescale increases with increasing acceptor equivalents, we find that the rate constant does not change as a function of acceptor concentration. Such an observation could indicate a rate of reaction that exhibits a shallow dependence on distance.

These fast rates of reaction and insensitivity of fast recombination to loading could also be interpreted in a model where the donor and acceptor bind cooperatively on the DNA helix.³⁴⁻³⁶ With such a clustering model, the distances between metal complexes would be short, and hence, the rates of reaction would be high and would appear to be independent of loading. For the titration of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA with the rhodium quencher, such a clustering model can be fit but would require a cooperativity in binding of more than 1 kcal.³⁵

A perturbation in the circular dichroism (CD) spectrum of $\Delta\text{-}[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to poly d(AT) at high loadings has been interpreted as evidence for preferential binding of the rhodium complex near $\Delta\text{-}[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$.³⁶ It is difficult, however, to reconcile this clustering with several observations: (a) the absence of fast quenching in the micelles where clustering should be encouraged, (b) the presence of this loading-independent kinetics for the full range of donors examined, (c) the random binding to DNA without conformational distortions of the donors and acceptors individually. In addition, there is no apparent structural basis for the cooperative binding between ruthenium and rhodium to satisfy this model.

It is noteworthy that we observed the largest change in electron transfer depending upon the sequence of the DNA bridge.^{34,37} Evident in Table 1 are the rates of recombination from reduced acceptor to oxidized donor (k_{rec}) for poly d(AT) and poly

d(GC). The rate of recombination is 30 times higher with poly d(AT) than with poly d(GC). In addition, the yield of electron transfer from photoexcited $[M(\text{phen})_2(\text{dppz})]^{2+}$ to $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to DNA depends sensitively on the nucleic acid sequence. For donor bound to poly d(AT) the fraction of excited states ($M = \text{Os}$ or Ru) which undergo electron transfer resembles closely the electron transfer yield for donor bound to mixed sequence (calf thymus) DNA. For the same donors bound to poly d(GC), however, the fraction quenched is significantly reduced. Nonetheless, while the yield of photoinduced electron transfer decreases for $[M(\text{phen})_2(\text{dppz})]^{2+}$ bound to poly d(GC) relative to mixed-sequence calf-thymus DNA and poly d(AT), the rate of the forward (k_{et}) reaction remains greater than $3 \times 10^{10} \text{ s}^{-1}$.³⁴ These findings are also hard to reconcile with a clustering model, because both the rate of recombination and yield of electron transfer appear to depend upon the DNA sequence intervening between donor and acceptor.

Given these discrepancies, Franklin *et al.* reinvestigated the CD of Δ - $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and Δ - $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to different DNA polymers as a function of concentration to explore more fully the parameters affecting any possible clustering.³⁸ We find no systematic change in the CD as a function of loading on the helix nor as a function of polymer sequence. The induced CD observed with each intercalator upon the addition of DNA arises whether they are bound together or separately. This induced CD is a function of binding to the chiral interbase pair site and is observed even with achiral intercalators.

These authors tested the clustering model more directly in an NMR experiment. Here, they examined DNA site selection through monitoring the spectral perturbations to the exchangeable imino protons within each DNA base pair of a DNA decamer upon binding the ruthenium and rhodium intercalator separately or together. If anything, the NMR data indicate that the complexes bind anticooperatively to the decamer, binding primarily at sites separated on the helix by four base pairs.

For all of these electron transfer studies, a fundamental understanding of structure, of how the donors and acceptors are associated with the helix, is essential. Moreover, in order to examine in detail the distance dependence of electron transfer, it is important to carry out such studies with donors and acceptors bound at a fixed separation and with a fixed DNA sequence intervening. With molecules bound noncovalently to DNA, a distribution of distances and sites occur, and deconvoluting these different parameters become problematic.

1.3.3. Characterizing the Products of Electron Transfer

Can we provide more evidence that this fast quenching reaction is indeed electron transfer? The fraction of recombination reaction that occurs on the picosecond time scale accounts for the majority of excited states which undergo electron transfer. However, a small fraction of reduced acceptor and oxidized donor appear to recombine on a longer time scale. In order to identify and characterize any long-lived electron transfer intermediates for the reaction between metallointercalators, we monitored the photoinduced electron transfer reaction by transient absorption spectroscopy on the microsecond time scale. For $[\text{Ru}(\text{DMP})_2\text{dppz}]^{3+}$ and $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ donors, we actually observed rhodium-induced transients which persisted well into the microsecond regime.^{25,39} Chapter 3 describes the application of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ as an isostructural analog of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ in mechanistic investigations of DNA-mediated electron transfer between metallointercalators. These studies were essential in establishing unequivocally that electron transfer was the operative mechanism in the quenching of photoexcited donors by $[\text{Rh}(\text{phi})_2(\text{L})]^{3+}$ bound to DNA.

1.3.4. Electron Transfer over a Fixed Donor Acceptor Separation

Experiments in which the donors and acceptors are bound noncovalently to the DNA duplex were all carried out at low reactant loadings in order to maximize donor-

acceptor separation. In a typical experiment, a 1:1 donor/acceptor ratio corresponds to an average separation of 25 base pairs. Remarkably, 50% of the Δ -[M(phen)₂(dppz)]²⁺ excited states undergo electron transfer, indicating that the reaction is very efficient even at low loading. However, under these conditions a distribution of donor-acceptor separations is always present. Moreover, as discussed above, we cannot eliminate the possibility of clustering with metal complexes noncovalently bound to the helix. To establish definitively that photoinduced electron transfer may proceed over some distance, we needed to tether the donor and acceptor intercalators to opposite ends of a DNA duplex, providing an assembly in which the separation between donor and acceptor might be fixed.

Murphy *et al.* constructed a DNA duplex in which derivatives of [Ru(phen)₂(dppz)]²⁺ and [Rh(phi)₂(phen)]³⁺ were tethered by flexible linkers to opposite ends of a 15 base pair oligonucleotide (Figure 1.6).⁴⁰ The complexes were free to intercalate but separated by ~ 40 Å. Companion experiments with duplexes bearing either a tethered ruthenium complex or rhodium complex (but not both) allowed for the integrity of the metal-modified DNA duplex and the intercalation sites to be established. Owing to the sensitivity of the ruthenium luminescence to local environment, the authors could characterize intercalation by the tethered ruthenium within the helix. In the conjugate assembly containing only a rhodium complex, a photocleavage experiment could be used to mark the site of intercalation.

In the 15 base pair assembly shown, intercalation of the metal complexes can occur equally either one or two base pairs in from the end of the duplex, leading to a most probable separation of 41 Å between donor and acceptor.⁴⁰ Remarkably, upon excitation, no ruthenium luminescence was detected in the mixed-metal assembly. Hence, as suggested by experiments with noncovalently bound donors and acceptors, photoinduced electron transfer through the double helix can proceed efficiently between metallointercalators over a long molecular distance. A lower limit of $3 \times 10^9 \text{ s}^{-1}$ was set

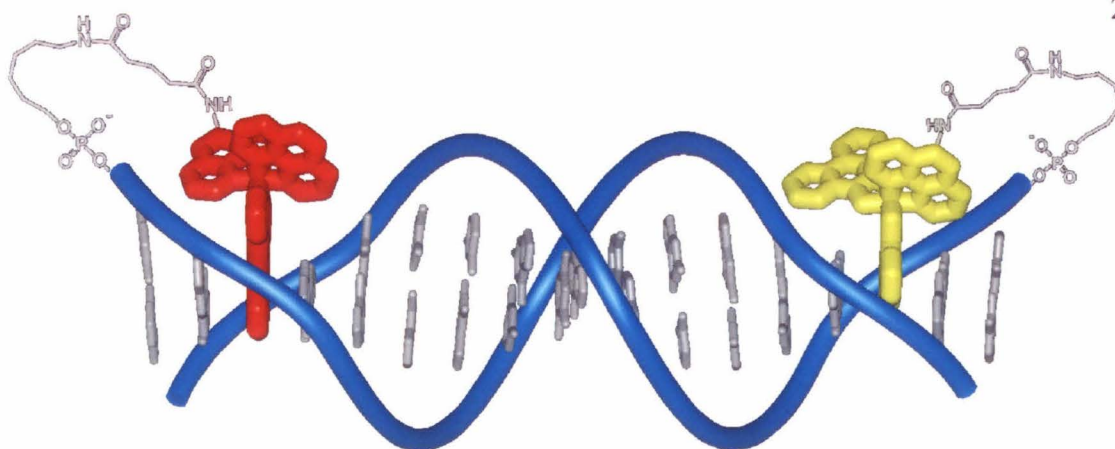


Figure 1.6. Schematic representation of a 15 base pair DNA duplex with an electron donor, Δ -[Ru(phen)₂(dppz)]²⁺ (red), and an electron acceptor, Δ -[Rh(phi)₂(phen)]³⁺ (yellow) tethered to opposite ends of the assembly so the complexes are intercalated but separated by a fixed distance. Shown is the most probable separation of 11 base pairs (41 Å).

Table 1.2. Fluorescence quenching in Et-DNA-Rh mismatch duplexes. ^a

Assembly	Fq ^b
Et'-5'-C-T-A-T-C- T -A-T-C-G-T G-A-T-A-G- A -T-A-G-C-A-5'-Λ-Rh	0.21
Et'-5'-C-T-A-T-C- C -A-T-C-G-T G-A-T-A-G- A -T-A-G-C-A-5'-Λ-Rh	0.04
Et'-5'-C-T-A-T-C- G -A-T-C-G-T G-A-T-A-G- A -T-A-G-C-A-5'-Λ-Rh	0.26

a. Adapted from reference 45.

b. Fq = fraction quenched = fluorescence of Et-Rh doubly modified duplexes compared that for duplexes containing only ethidium.

for the rate constant describing the photoinduced reaction. The DNA double helix therefore, does indeed provide a bridge for long range electron transfer.

Viewed in the context of classical studies of electron transfer through σ -bonded networks,⁴¹ such long range electron transfer through DNA was surprising. Chemists are now beginning to devote attention to developing a theoretical foundation for the consideration of charge transfer mediated by the DNA base stack.⁴² Our studies point to stacking as a critical element in determining the efficiency of the reaction. An efficient reaction mediated by the DNA π -stack requires full intercalation of donors and acceptors. Indeed significant differences in electron transfer are evident not only between groove-bound versus intercalated redox pairs but also among intercalators which stack in the helix to differing extents. Our results highlight the importance of addressing this stacking parameter explicitly when constructing theoretical models of DNA-mediated charge transfer.

Other laboratories have since constructed different DNA assemblies with covalently bound donors and acceptors. At first, the results with these different assemblies appear quite disparate. Meade and Kayyem developed a system in which a nonintercalating ruthenium complex is coordinated directly to amino-modified sugars at the terminal position of oligonucleotides.⁴³ They assert that electron transfer in this system is protein-like.

Lewis and coworkers⁴⁴ measured rates of photo-oxidation of a guanine base in a DNA hairpin by an associated stilbene bound at the top of the hairpin. By systematically varying the position of the guanine base within the hairpin and measuring the rate of electron transfer, a value for β , the electronic coupling parameter, could be made. Here, β was found to be $0.64 \text{ \AA}^{-1}$, something intermediate between that seen in proteins, with σ -bonded arrays, and that expected for a highly coupled π -bonded array.

In another synthetic DNA assembly Fukui^{44b} measured electron transfer rates from guanine to a photoexcited intercalator tethered to the duplex and occupying an

abasic site. These authors also found that electron transfer was slow relative to reactions studied in our laboratory. The value of β determined in these experiments is $1.4 \text{ \AA}^{-1}$.

Using the methodologies described in Chapter 5 and related strategies, Kelley *et al.* synthesized oligonucleotides bearing redox active intercalators tethered to the 5' terminus.⁴⁵ With ethidium tethered and intercalated at one end of DNA oligonucleotides and a $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ derivative covalently bound and intercalated at the opposite end, we also observe fast ($< 10^{10} \text{ s}^{-1}$) photoinduced electron transfer. It is interesting that with excited ethidium as donor, either noncovalently or covalently bound, the efficiency of the reaction is reduced compared to ruthenium, despite similar driving forces. This difference in yield may reflect differences in intercalative stacking between the two donors.

Importantly, a systematic variation of the separation between donor and acceptor over distances ranging from 17-36 $\text{\AA}$ yields fluorescence quenching irrespective of distance, and the dependence of the quenching yield on distance is shallow. Although little sensitivity to distance is apparent, experiments carried out as a function of temperature and with intervening mismatches have underscored the remarkable sensitivity of these reactions to how the base pairs are stacked within the DNA duplex.

As can be seen in Table 2, photoinduced quenching is apparent in an 11 base pair duplex containing tethered ethidium and rhodium. If a single, intervening CA mismatch is introduced, the long range charge transfer is abolished. It is interesting that a GA mismatch, which is known to be well-stacked within B-DNA, actually enhances electron transfer quenching. These results certainly suggest the application of long range electron transfer chemistry in developing new biosensors which are sensitive to the stacking along the DNA bridge.

Kelley and Barton have also used ethidium tethered to DNA in photoinduced ET reactions with 7-deazaguanine (Z). Owing to the substitution of CH for N7 in guanine, Z is more easily oxidized than G by $\sim 150 \text{ mV}$, and therefore the reduction of photoexcited

ethidium by Z is thermodynamically favored. In systematic studies of this reaction as a function of distance and flanking sequence, Kelley and Barton demonstrated again that ET between reactants stacked within the DNA base stack is fast ($> 10^{10} \text{ s}^{-1}$). Furthermore, the reaction exhibits a shallow dependence on distance, however, the distance dependence of the reaction does depend upon the flanking sequence around Z.

We can also begin to reconcile studies in our laboratory and elsewhere on DNA-mediated electron transfer in covalently bound assemblies by focusing on this issue of stacking. We find that electron transfer is most sensitive to the intervening base pair stack. How the donor and acceptor are coupled into this stack varies in each system. In proteins, where the energetic differences in coupling depend upon σ -bonded interactions, small energetic differences between systems do not cause large differences in electronic coupling. In the DNA double helix however, where electronic coupling is mediated by π -stacking, small energetic differences could lead to large differences in coupling efficiency. Hence, with donors and acceptors coordinated to sugar moieties, charge transfer first through a σ -bonded network could be rate-limiting. In the case of the stilbene-modified DNA hairpin, the stilbene is closely associated but unstacked with the DNA base pairs, and hence intermediate coupling might be expected. With well-intercalated donors and acceptors, coupling into the base pair array should be optimum.

1.4. DNA-mediated Electron Transfer involving DNA Bases

In our studies of DNA as a bridge for electron transfer, we have focused on the chemistry of DNA and put aside all considerations of the DNA helix as a biologically important entity. With metallointercalators as donor and acceptor, we have found that the DNA π -stack can promote photoinduced electron transfer at long range. But can the DNA helix serve not only as a conduit for electron transfer but also as a reactant? Are there reactions within the cell in which radicals migrate through the DNA π -stack to effect damage to DNA? The chemistry of the DNA π -stack could indeed be an issue in

carcinogenesis and mutagenesis. Moreover, might this long range chemistry be exploited by nature in sensing or repairing DNA damage or even more generally in transmitting information over long molecular distances with modulation by the DNA π -stack? These intriguing questions were ones suggested by our studies on DNA as a bridge for electron transfer and some appeared ready to explore using chemistry developed in our laboratory. Most importantly, it appeared that the sensitivity of electron transfer to the DNA π -stack provided a route to explore and exploit DNA chemistry at a distance.

1.4.1. Oxidative DNA Damage through Long Range Charge Transfer

The major target of oxidative nucleic acid damage within the cell is guanine (G).⁴⁶ Experimental studies and calculations reveal guanine to be the most easily oxidized of the nucleic acid bases.⁴⁷ A variety of DNA binding agents including riboflavin, anthraquinone derivatives and naphthalimides are known, with photoactivation, to induce oxidative damage specifically at the 5'-G of 5'-GG-3' doublets.⁴⁸ In our laboratories, we recognized that $[\text{Rh}(\text{phi})_2(\text{L})]^{3+}$ metallointercalators, which serve as potent photooxidants ($E_{1/2}^* \text{Rh(III)/Rh(II)} > 2.0 \text{ eV vs. NHE (32)}$) upon irradiation with low energy light ($\lambda \geq 365 \text{ nm}$),³² might be useful in the study of long-range, DNA-mediated oxidative damage to DNA.

We implemented the strategy outlined in Scheme 2 to investigate damage to DNA by long-range oxidation.⁴⁹ For these experiments we prepared DNA duplexes containing 5'-GG-3' doublet sites and a rhodium intercalator, the oxidant, tethered to one end of the duplex. In this DNA assembly, the rhodium is separated spatially, in a well-defined manner, from potential sites of oxidation. Direct strand cleavage of the DNA promoted by 313 nm irradiation of the rhodium-modified duplex is used to mark the site of intercalation and permits the determination of the distance separating the rhodium complex from potential sites of damage. Irradiation of the assembly with low energy (365 nm) light, instead, introduces a photoexcited hole site-specifically into the DNA

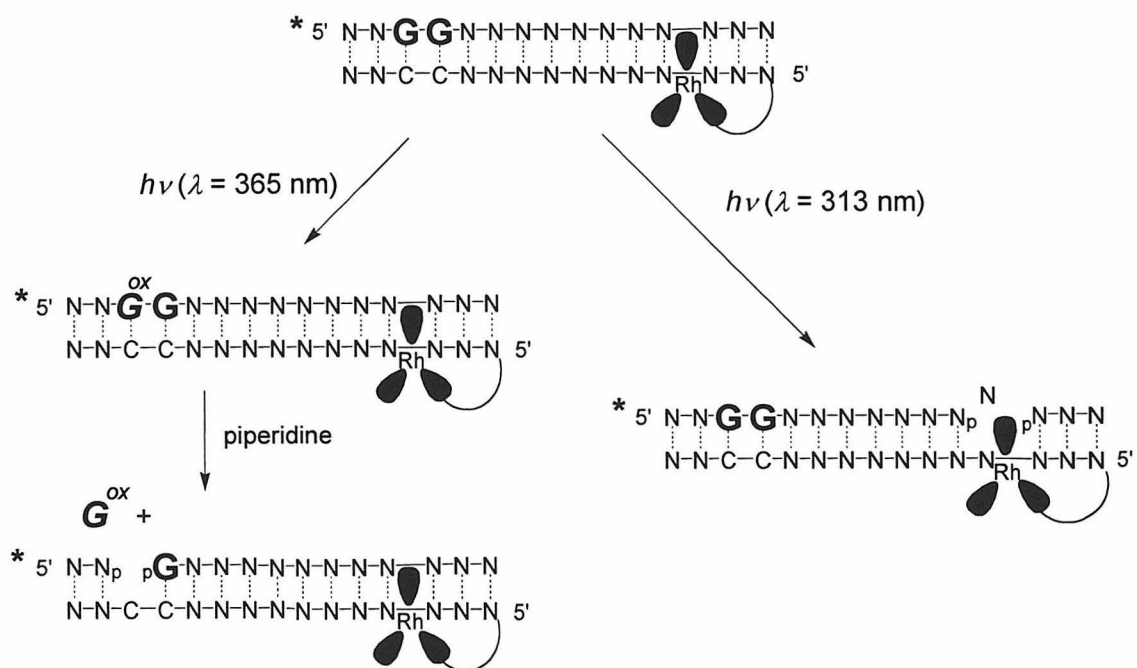




Figure 1.7. Rhodium-modified DNA duplex assembly constructed for the investigation of long-range oxidative DNA damage. The sequence shown contains two 5'-GG-3' doublets (in bold), which are potential oxidation sites, separated from the intercalated rhodium complex by 17 Å and 34 Å.⁴⁹ For clarity the rhodium complex is not shown intercalated.

base stack and effects the oxidation to yield a modified base site. The position and yield of damage is revealed subsequently by treatment of the modified DNA with piperidine, which promotes strand cleavage at the damaged site.

An example of a rhodium-modified assembly containing 5'-GG-3' doublets is illustrated in Figure 1.7.⁴⁹ In this modified duplex, we observe significant oxidative DNA damage specifically at the 5'-G of the 5'-GG-3' sites, upon irradiation at 365 nm. Remarkably, *this oxidative damage must occur from a distance*. With the rhodium intercalator tethered to the 5'-end, photocleavage at 313 nm indicates intercalation primarily 3 base pairs from the modified terminus. In fact, for the duplex shown in Figure 1.7, damage is observed at both 5'-GG-3' sites located 17 Å and 34 Å from the rhodium intercalator. Moreover, the intensity of oxidative damage at the site distal to the rhodium complex is comparable (or even higher) than that of the proximal 5'-GG-3' doublet, suggesting that the yield of damage does not depend sensitively on distance. If we examine an assembly containing only the distal 5'-GG-3' doublet, we still see comparable damage, demonstrating that long range oxidation of the 5'-GG-3' doublet

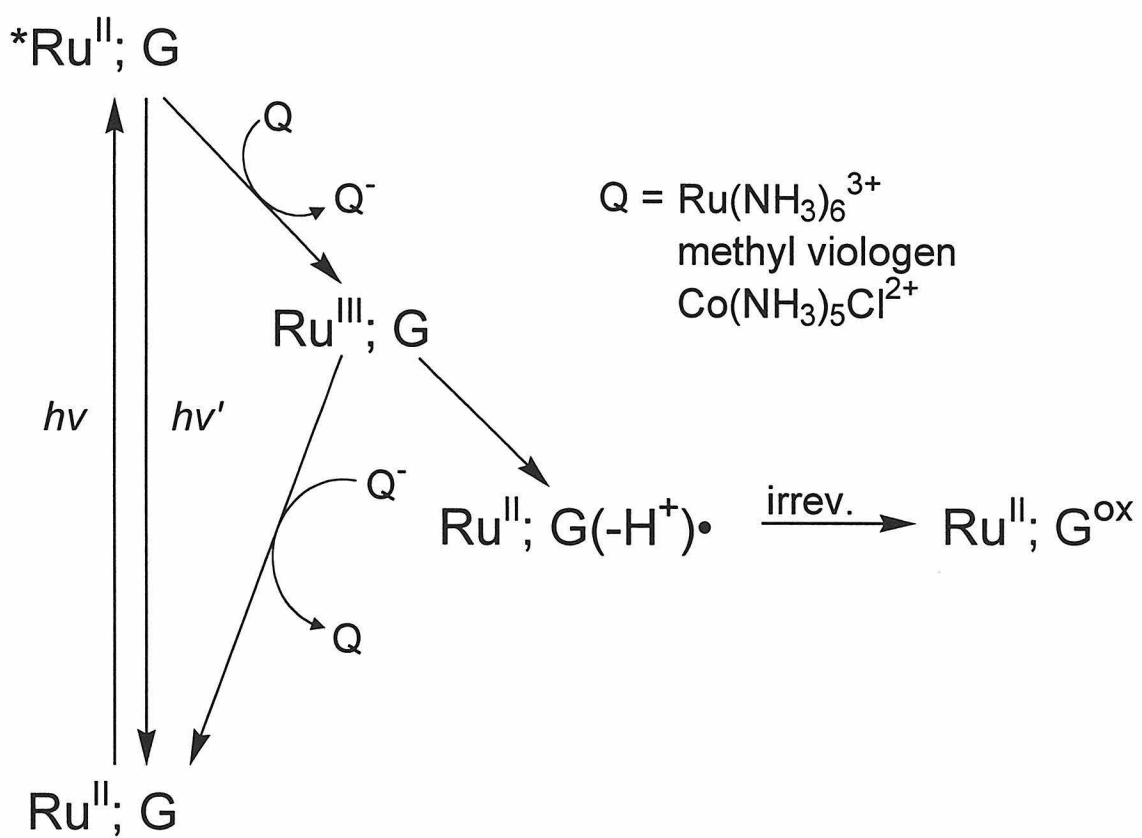
does not require the intervening oxidation site.

Although the yield of oxidative damage does not appear to vary significantly with distance, it does depend sensitively upon sequence, which is known to modulate base oxidation potential.⁴⁸ A rhodium-modified duplex containing a 5'-GGG-3' triplet was also constructed.⁴⁹ Molecular orbital calculations of the ionization potentials of the DNA bases indicated that the 5'-G of the 5'-GGG-3' triplet is even easier to oxidize than the 5'-G of the 5'-GG-3' doublet.⁴⁸ In this assembly, oxidation was observed at the 5'- and central guanines of the triplet and, consistent with the calculated ionization potentials, was greatest at the 5'-G.

Following enzymatic digestion of the damaged duplex, we isolate 8-oxo-G as the oxidative product. 8-oxo-G is the primary lesion in cellular oxidative DNA damage. We find that formation of this product requires O₂. Thus, while the oxidative reaction in our synthetic system employs rhodium photochemistry, one might consider alternative, perhaps more physiologically relevant means to introduce a hole into the π -stack. Our results appear to indicate that, irrespective of the oxidant, once such a hole is introduced into the DNA π -stack, it may migrate, leading effectively to oxidative damage to DNA from a distance. This characteristic of double helical DNA, the extended base paired π -stack which facilitates long range charge transport, is therefore not simply one of chemical novelty. It is instead a characteristic with important implications with respect to DNA mutagenesis and carcinogenesis within the cell.

In fact, many laboratories have shown that a range of oxidants can be used to promote guanine damage in DNA; whether they can also promote damage at long range needs to be established.⁴⁸ We have found most recently that ruthenium intercalators can also be employed to effect long range oxidative DNA damage.⁵⁰ Scheme 3 illustrates the flash quench experiment used to oxidize guanines in DNA with ground state Ru(III) generated *in situ* as oxidant. An analogous flash-quench method was employed in classic studies of protein-mediated electron transfer chemistry.⁵¹

Scheme 1.3. Flash-quench cycle for the generation of Ru(III) and subsequent oxidation of guanine in Duplex DNA.



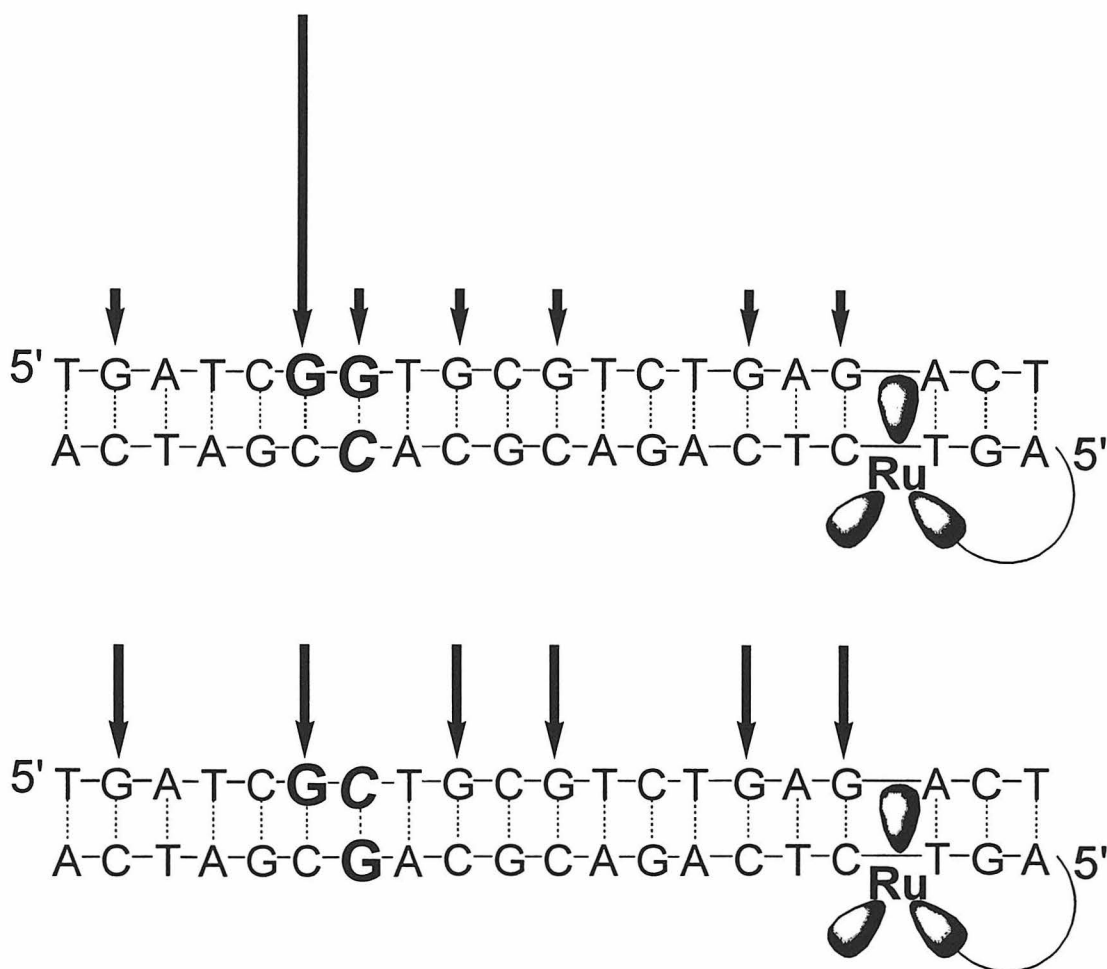


Figure 1.8. Long-range oxidative damage at guanine bases by tethered Ru(III) in modified DNA duplexes with (top) and without (bottom) a 5'-GG-3' guanine doublet. With the 5'-GG-3' site, oxidative damage is observed primarily at the 5'-G of the doublet. Without the 5'-GG-3' site, oxidative damage is observed with equivalent yields (indicated by arrows) at each guanine across the duplex.

This flash-quench chemistry was exciting to us in offering another route to probe oxidative reactions on DNA from a distance. Moreover, the rhodium photochemistry is exceedingly inefficient and involves an excited state oxidant. With ruthenium(III) generated *in situ* more than a thousand-fold increase in quantum yield occurs, and the ruthenium complex represents a ground state oxidant. Moreover, this methodology is particularly well-suited to spectroscopic studies. With ruthenium tethered to a DNA duplex, we also find oxidative damage to DNA from a distance.⁵⁰ Figure 1.8 illustrates a particularly interesting observation made using this chemistry. As with the rhodium photooxidant, reaction occurs preferentially at a 5'-GG-3' doublet. But what if the 5'-GG-3' doublet is removed by mutation? In that case we observed essentially equal oxidative damage distributed over all single G sites. This finding illustrates quite graphically that migration and equilibration of the oxidizing hole across the duplex must occur on a time scale which is fast compared to the trapping reaction. Our finding of damage at a distance with this system also highlights the likely generality of long range oxidation through DNA. We are particularly excited about this system in that this chemistry may allow us to link together the kinetics of these reactions, via spectroscopy and the yield of products of the reaction, using biochemical methods in a detailed mechanistic investigation.

1.4.2. Sensitivity of Long Range Oxidative DNA Damage to the Intervening Base-Paired Stack

Like electron transfer between metallointercalators, we find long-range oxidative damage to be remarkably sensitive to stacking. For rhodium-modified oligonucleotides with tethered Δ -[Rh(phi)₂(bpy)]³⁺, for example, the yield of oxidized product is consistently higher than for conjugates with the rhodium center in the Λ -configuration.⁴⁹ Hence, the deeper stacking of the right-handed metal complex in the DNA appears to enhance the damage efficiency.

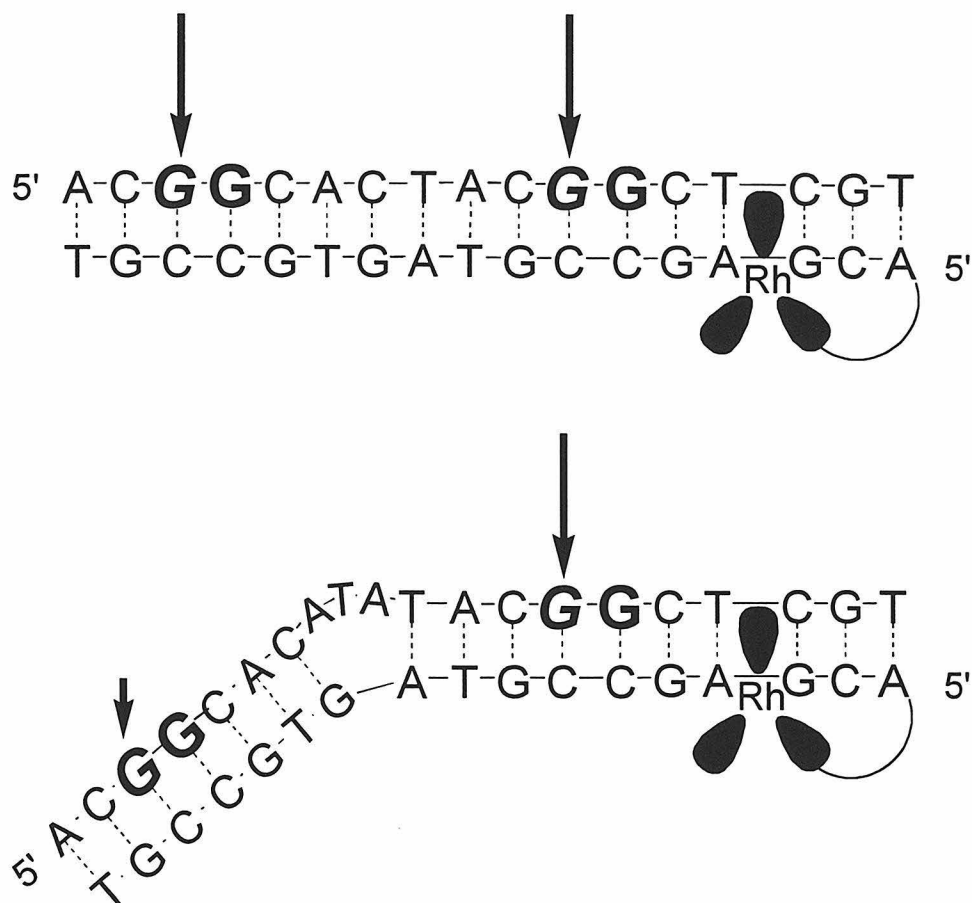


Figure 1.9. Long-range oxidative damage in rhodium-modified duplexes with a fully base-paired and stacked region (top) or a 5'-ATA-3' bulged region (bottom) separating two 5'-GG-3' potential oxidation sites. For the fully stacked duplex, damage yield (indicated by arrows) is nearly identical at both oxidation sites. With the 5'-ATA-3' bulge, which disrupts the stacking between the distal 5'-GG-3' site and the rhodium intercalator but not the proximal, substantial diminution in damage yield at the distal site is observed.⁵²

We wanted to probe the sensitivity of the DNA-mediated reaction to stacking more generally by exploring whether perturbations in the *intervening* π -stack, between the donor and acceptor, would also affect the oxidation.⁵² The assemblies shown in Figure 1.9 are representative of a family of assemblies prepared, each containing a rhodium oxidant and two 5'-GG-3' doublets separated by a bulged base region. By comparing damage yields at the distal and proximal 5'-GG-3' doublets in the presence and absence of the intervening bulged region, we could examine the effect on long range electron transfer of the intervening disruption in base stacking.

Figure 1.9 shows the assembly containing the -ATA- bulge, one of the few bulges which has been characterized using high resolution NMR.⁵³ From those structural studies it was apparent that the bulge leads to a substantial but not complete disruption of the base stack. Consistent with this disruption in stacking, we find ~75% diminution in oxidation of the distal 5'-GG-3' doublet compared to the proximal 5'-GG-3' doublet in the long range oxidation experiment. Hence, perturbations in the intervening π -stack do indeed affect this DNA-mediated electron transfer chemistry. In fact these results indicate directly that the long range hole transfer proceeds through the π -stack.

These results also highlight a novel application of this chemistry. Long range oxidation reactions provide a means to probe the integrity of the base stack in solution. Base pair stacking is not easily assayed by other techniques. What effects on long range oxidation would be expected in the presence of covalent lesions to DNA, or if an intervening base were flipped out of the stack, or simply if a DNA-binding protein such as the TATA-box binding protein, which substantially distorts the duplex, were associated? These experiments are currently underway.

Lastly, these results not only illustrate oxidative reactions on DNA from a remote site, but also suggest strategies for the protection of DNA sequences within the cell from long range radical damage. Oxidative damage to DNA from remote sites may be modulated by the intervening base pair stack. For example, disruptions to the π -stack,

either through transient openings encoded by sequence or through disruptions introduced on protein binding, could actually provide a route to protect DNA.

1.4.3. Repair of the Thymine Dimer mediated by the DNA Base Stack

Electron transfer chemistry may be exploited not only in effecting DNA damage but also in initiating the repair of a common light induced DNA lesion, the thymine dimer.⁵⁴ Bacteria such as *E. coli* utilize photolyase enzymes to repair the thymine dimer photochemically.⁵⁵ In the key step of this process, visible light (350 - 450 nm) induces an electron transfer from an enzyme-bound flavin to the dimer, initiating repair. In model studies using photoexcited reductants, thymine dimer repair in duplex DNA has been observed with high energy irradiation ($\lambda \leq 300$ nm).⁵⁶ Excited state oxidants which remove an electron from the dimer have been shown to repair pyrimidine dimer model compounds when ultraviolet light (< 300 nm) is used to initiate the process.⁵⁷ Based upon these results, we expected that excited state rhodium metallointercalators, as powerful oxidants, might trigger thymine dimer repair in duplex DNA by injecting a photoexcited hole directly into the DNA base stack. Moreover, the thymine dimer and its repair, initiated simply by electron transfer, offered an attractive trapping reaction to characterize parameters of DNA-mediated chemistry.

We found that $[\text{Rh}(\text{phi})_2(\text{dmb})]^{3+}$ (dmb = 4,4'-dimethyl-2,2'-bipyridine), excited with 400 nm light, initiated the repair of a thymine dimer incorporated site-specifically in the center of a synthetic 16-mer oligonucleotide duplex ([duplex]:[$[\text{Rh}(\text{phi})_2(\text{dmb})]^{3+}$] = 1:1) (Figure 1.10).⁵⁴ The repair mechanism is thought to proceed via oxidation of the dimer ($E_{1/2} (^+T \diamond T/T \diamond T) \sim 2.0$ V vs. NHE)⁵⁵ by the intraligand excited state of the rhodium complex, in which an electron deficiency (hole) is localized on the intercalated phi ligand.³² Quantitative repair was also observed with concentrations of rhodium complex as low as 500 nM and a 16-fold excess of DNA duplex (6.25 mole percent $[\text{Rh}(\text{phi})_2(\text{dmb})]^{3+}$), indicating that the process is catalytic in rhodium. Treatment of

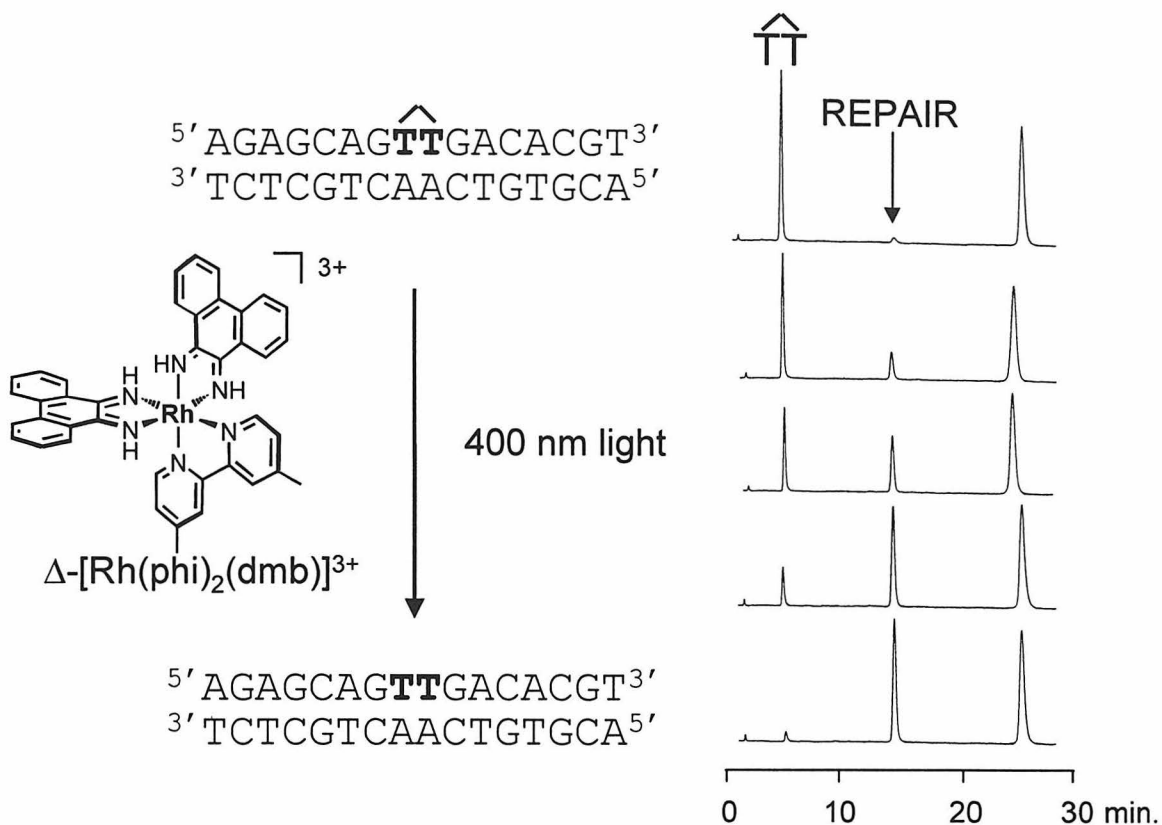


Figure 1.10. Photochemical repair of the thymine dimer in DNA by noncovalently bound *rac*-[Rh(phi)₂(dmb)]³⁺. Shown schematically (left) is the reaction and (right) the HPLC chromatograms at 0, 2, 5, 10, 20 min. irradiation (top to bottom). The thymine dimer-containing strand with retention time (T_R) 6 min. decreased with increasing irradiation time, while the corresponding repaired strand, T_R = 15 min., appeared. The signal at T_R = 26 min. corresponds to the complementary strand. The quantum yield for thymine dimer repair measured for a 20 min. irradiation was 0.0001 moles per Einstein (E) with stoichiometric rhodium.

damaged DNA with the rhodium catalyst and bright sunshine also removed the lesion completely. In these experiments, the noncovalently bound rhodium intercalator is free to sample all intercalation sites along the length of the duplex, and the distance between the complex and the thymine dimer is unknown.

In Chapter 6, we describe an investigation of thymine dimer repair over long-range in the DNA duplex. We synthesized Rh-modified duplexes and characterized the ability of the base stack to facilitate the oxidative repair of the lesion as a function of distance, stacking of the photooxidant, and stacking of the intervening bases.

1.5. Applications of DNA-Mediated Electron Transfer to Sensing Technology

Investigations of charge transfer through the DNA base stack are important to enlarge upon our understanding of the fundamental biological properties of genetic material. From a practical standpoint, DNA-mediated electron transfer can be exploited to develop biosensors that detect disruptions in the base stacking of nucleic acids. Kelley, Hill, and Barton and coworkers have described the adsorption of thiol-modified duplexes on gold surfaces to prepare monolayers of DNA.⁴⁵ The incorporation of intercalators into the monolayers, covalently or noncovalently, creates an electron transfer assembly in which electrons are shuttled from the gold electrode to the intercalator. These authors have shown that despite efficient electron transfer in fully base paired duplexes, when mismatches are incorporated into the assemblies, the electron transfer reaction is shut down. This work has laid the foundation for the first applications of DNA-mediated electron transfer.

DNA-modified gold surfaces were employed in another series of fundamental investigations of the distance dependence of electron transfer through DNA. Here, Kelley *et al.* showed that despite the distance separating the redox active intercalator from the surface, the rate of electron transfer was the same. In fact, this rate was consistent with electron transfer through the alkyl chain that connects the duplex to the surface

acting as a rate limiting step.

1.6. Debate on DNA-mediated Charge Transfer

Although the concept of long-range charge transfer through DNA has been recognized by many researchers, DNA is not seen as a remarkable electron transfer medium by all. The fact that studies of photoinduced electron transfer on DNA appear so different from analogous studies with proteins as a medium is surprising to some. On the other hand, given the chemical differences between the π -stacked DNA helix and largely σ -bonded network of a protein, such differences might be expected. Our understanding of DNA-mediated electron transfer is certainly less well-developed at this point compared to that for proteins as a medium.

As in protein studies, experiments on DNA began using non-covalently bound donors and acceptors,⁵⁸ and here the results obtained have varied enormously depending upon the donors and acceptors employed. While in our laboratory with tightly bound metallointercalators, we have often seen fast, efficient luminescence quenching,^{9,25,31,33,34,38,40} with some organic DNA-binding agents, notably with ethidium as photoinduced donor and DAP as acceptor, fast quenching is not observed.¹⁰ We have attributed such variations in rates and efficiency to the efficacy of donor and acceptor coupling into the π -stack. The comparison in photoinduced quenching of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ luminescence by $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, an avid intercalator, versus quenching by ruthenium hexammine, a groove-binding agent, is a clear cut example.³¹ With $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, photoinduced quenching is faster than expected based upon a collisional mechanism, while the dynamic quenching by ruthenium hexammine is consistent with a collisional mechanism. We also find differences in rates of electron transfer between enantiomeric metal complexes bound to the helix,^{9,34} and these differences reveal a sensitivity in reaction to subtle variations in stacking. However, not all observations may be so neatly categorized. For example, we see no substantive

quenching with $[\text{Rh}(\text{phen})_2(\text{phi})]^{3+}$, and we have ascribed the absence of fast quenching to poor overlap of the shape-selective DNA-binding agent with the π -stack.³³ Brun and Harriman also showed using a metalloporphyrin as donor that the kinetics of electron transfer mediated by DNA need not be fast.^{10h} Here too one may ascribe the lack of a fast reaction to poor overlap of the metal complex with the DNA base pair stack, given the subsequent crystallographic analysis of a metalloporphyrin bound to DNA which shows base displacement rather than stacking.⁵⁹ Certainly, based upon the range of results obtained thus far, a clear *quantitative* parameter that one might use to gauge whether fast DNA-mediated electron transfer should arise has not yet emerged.

Some laboratories have indicated that the fast quenching we have observed might be explained more simply in terms of clustering.^{35,36} A straightforward explanation for static quenching on a timescale which is fast compared to diffusion is that no diffusion is necessary; if the metal complexes bind cooperatively on the helix, they would be placed in a manner to facilitate rapid electron transfer. Barbara and coworkers³⁵ have offered an extensive analysis of the ultrafast quenching data for Δ - $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and Δ - $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to calf thymus DNA and show that these data may be explained based upon cooperative clustering. However, no analysis is provided for Λ - $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ which shows a different, albeit fast quenching profile, nor are data provided for any of the other metal complexes, which differ with respect to hydrophobicity in the ancillary ligands and hence might be expected also to differ with respect to cooperativity.

There are many other questions raised by such a model, as denoted earlier. Why should there be differences in rates of recombination found with the same donor and acceptor but on different DNA sequences? Why is cooperative clustering supposed to occur between ruthenium and rhodium but not for each separately? What is the basis for clustering between ethidium, which lacks ancillary bulk, and rhodium, since such clustering must be invoked here as well to account for the fast quenching observed?

What is the structural basis for this supposed cooperative association? In fact, the only experimental observation provided to support a clustering model is an ill defined perturbation in the circular dichroism of Δ -[Ru(phen)₂(dppz)]²⁺ and Δ -[Rh(phi)₂(bpy)]³⁺ bound to poly d(AT) at high loadings.³⁶ Our more recent investigation of CD shows this earlier study to be incomplete. Both a more extensive CD study and an examination of DNA binding by NMR show that the metallointercalators bind non-cooperatively, indeed perhaps even anticcooperatively, to the DNA duplex. Nonetheless, this whole issue underscores the importance of structural characterizations of DNA-bound donors and acceptors. Our results establish, at the very least, the striking sensitivity of DNA-mediated electron transfer to how donors and acceptors are bound to the helix.

Clearly, a clustering model can never be definitively ruled out for different species bound noncovalently to DNA, but *clustering models cannot be applied in reconciling data in systems where electron donors and acceptors are tethered to DNA duplexes at known, fixed positions*. With tethered intercalators, we have thus far examined electron transfer between metallointercalators tethered to a 15-mer DNA duplex,⁴⁰ and between ethidium and [Rh(phi)₂(bpy)]³⁺ tethered to a series of DNA duplexes.⁴³ These results clearly support long range electron transfer quenching and are fully consistent with observations made with non-covalently bound complexes. In these studies, however, we are able to measure only quenching, the absence of luminescence, and therefore the interpretation of a striking long range electron transfer reaction could be described as resting primarily upon a series of negative results. It will be important to carry out ultrafast transient absorption experiments on these tethered DNA assemblies to measure *rates* of electron transfer. These studies will be challenging ones, however, since fast time resolution and low concentrations (to insure intramolecularity) are needed. In this context, the experiments with non-covalently bound intercalators provide a valuable foundation.

In fact, it was because of the possible ambiguity in interpreting quenching studies

that we first turned to studies with DNA as a reactant.^{48-50,52,54} If we can measure a product of electron transfer at a distance of separation from the source of charge transport, then irrespective of the timescale, one can document definitively a long range effect. Hence, from this perspective, as well as in a biological context, our studies of oxidative damage to DNA from long range using tethered oxidants have been important ones. Here, critics question the poor efficiency of the reaction. The quantum yield for oxidative damage is 10^{-7} for the rhodium oxidants. But in fact, all photochemistry with these rhodium complexes is inefficient (photoaquation = 10^{-4}), owing in part to other overlapping transitions. With ruthenium(III) oxidation, using the flash quench methodology,⁵⁰ we have found a quantum yield for damage over a distance of >30 Å to be 0.002 with methyl viologen as quencher; this yield seems reasonably high considering the alternate pathways available to reaction by ruthenium. Given this increased yield, and the completely parallel observations made with the ruthenium and rhodium photochemistry, it becomes difficult to characterize these studies as merely the result of statistical fluctuations or “leakage”. As with spectroscopic studies, these long range reactions are seen to depend sensitively upon stacking of the donor, acceptor, and structural perturbations in the intervening DNA helix. Moreover, in the case of the long range oxidative repair of thymine dimers in DNA,⁵⁴ the conversion to repaired product is *quantitative* and triggered simply by electron transfer (requiring no additional, diffusing species). While it is still important to emphasize that these experiments do not provide a measurement of the *rate* of long range electron transfer, these experiments unambiguously establish chemistry at a distance.

Few theoretical treatments of DNA as a medium for electron transfer have been described, and here too there is much to debate. Beretan^{42c-d} and coworkers describe calculations which indicate that DNA is protein-like and serves as an insulator. These calculations show agreement with some kinetic data reported by Meade³⁹ and Harriman¹⁰ and differ by several orders of magnitude from our measurements of electron transfer

quenching with covalently bound metallointercalators. These calculations, however, do not take into account any coupling that arises due to stacking, either of the bases or intercalators, nor have variations been made in sequence or base structure to establish a sensitivity in parameters to such changes, as has been seen experimentally. Using calculations based upon Redfield theory, Friesner^{42a} and coworkers have suggested how base stacking could give rise to a delocalized “band” which might facilitate long-range charge transfer without a significant distance dependence. In fact this work provides a prediction for experimentalists to pursue, namely that low temperature should eliminate thermal access to this band and shut down the electron transfer. Actually calculations made more than twenty years ago⁶ also revealed substantial debate regarding both the energetics and mechanisms for long range electron transfer. It is our view that more experimental data are needed to provide a firm foundation upon which theorists may build. At this stage hopping models, charge delocalization, band theory all offer interesting perspectives through which our experiments may be viewed, but experiments to distinguish between these models have not yet been established.

1.7. Summary and Outlook

Here we have described a range of charge transfer reactions mediated by the DNA base pair stack. We have found reactions to proceed fast and efficiently between donors and acceptors which are well-coupled into the π -stack. These reactions do not appear to depend sensitively upon the molecular distances between reactants but are exquisitely sensitive to stacking, the stacking of donor and acceptor but also the stacking of the bases which intervene.

These results provoke a host of questions and new experiments. Over what molecular distances can charge effectively be transported through DNA? We need to develop assays to examine reactions over 50-100 base pairs, not 10-20 base pairs as we have explored thus far using oligonucleotide synthesis. What are the rates for these

reactions? So far, our instrumentation has allowed us only to place limits on rates for photoinduced quenching, and no systematic determination of how rates vary with distance and sequence has been made. Such data are essential in developing a theoretical framework to describe this chemistry. We need also to understand how these reactions vary with temperature and with changes in nucleic acid conformation. Importantly, we need to develop quantitative parameters to describe stacking within the double helix and how such stacking affects electronic coupling in DNA.

Perhaps most intriguing to consider are some of the biological consequences of this chemistry. Does radical damage to DNA arise from a distance within the cell? Are there cellular mechanisms which insulate sequences from such damage or through which the positioning of damage is controlled? Might such long range chemistry be exploited in new chemotherapeutic design or in the development of novel nucleic acid-based sensors? Is long range charge transport through DNA exploited within the cell for long range transmission of information across the DNA library?

These questions are certainly provocative ones. They are nonetheless ones which represent important challenges for the future and ones which, based upon future work, should become implicit to our understanding of the chemistry of DNA.

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Chapter 2: Dipyridophenazine Complexes of Osmium(II) as Red-Emitting DNA Probes: Synthesis, Characterization, and Photophysical Properties.

2.1. INTRODUCTION

Transition metal complexes that bind to DNA and RNA by intercalation (metallointercalators) represent an important class of molecular probes.

Metallointercalators based on ruthenium(II) and rhodium(III) have been useful in studies of nucleic acid (NA) structure and function.^{1,2} As part of an ongoing effort to develop new and effective diagnostics for NAs in solution, we initiated an investigation of dipyridophenazine complexes of osmium(II) as a new class of red-emitting DNA probes. This chapter describes the synthesis, characterization, and photophysical properties of a family of metallointercalators based on osmium(II).^{3,4}

Dipyridophenazine complexes of Ru(II),^{5,6} and Re(I)^{7,8} provide sensitive luminescent probes for double helical DNA in solution. Despite bright luminescence in organic solvents, emission from photoexcited $[\text{Ru}(\text{L})_2(\text{dppz})]^{2+}$ ($\text{L} = \text{bpy}, \text{phen}, \text{dppz} = \text{dipyrido}[3,2\text{-a};2',3'\text{-c}]\text{phenazine}$; see Table 2.1 for abbreviations) is quenched in aqueous solution by the interaction of protons of the solvent with the phenazine nitrogens of the dppz ligand.⁹ For the Re(I) complex, intersystem crossing (ISC) to a $\pi\text{-}\pi^*$ transition on the dppz ligand deactivates the emission. Upon binding to DNA by preferential intercalation of the dppz ligand between adjacent DNA base pairs, these processes are inhibited, and the complexes emit. As a result of quenching by water, the emission from these complexes is sensitive to local environment in terms of solvent accessibility. In fact, dppz complexes of ruthenium have been used to probe hydrophobic matrices in aqueous solution such as Nafion¹⁰ films and SDS micelles.¹¹

There has been a limited amount of detailed investigation of the mechanism that

accounts for the remarkably efficient quenching of photoexcited dppz complexes of Ru(II) by water.^{12,13} Turro *et al.* examined quenching by proton donors and concluded that a discrete proton transfer to the phenazine nitrogen from a sacrificial donor was responsible for deactivating the emission. Olson *et al.* described the first observation of steady state emission from $^*[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ in water. In their analysis, they concluded that the quenching was the result of intramolecular charge transfer (IMCT) involving the dppz ligand. This IMCT is facilitated by hydrogen bonding to the phenazine nitrogens. In each study, it is clear that interactions between the phenazine nitrogens and protons from the solvent will lead to quenching of emission from the lumiphore.

Structural studies of $\Delta\text{-}[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA by $^1\text{H-NMR}$ indicate that intercalation is from the major groove of DNA, with a family of intercalation geometries.^{14,15} This diversity in the orientation of the dppz ligand bound to DNA results in differential exposure of the phenazine nitrogens to solvent and accounts for the biexponential decay in emission observed for the racemic complex and the pure enantiomers. Hartshorn and Barton found that modifications in the structure of the intercalating ligand caused changes in the emission bound to DNA, which also reflected differences in the orientation of the intercalated complex.¹⁶ No systematic investigation of the effect of structural changes in the ancillary phen or bpy ligands on the luminescence profiles of dppz complexes bound to DNA has been reported.

The luminescence signature of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ is sensitive to DNA sequence and conformation.^{6,17-19} Based on comparative luminescence studies of $\Delta\text{-}[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to poly d(AT), poly d(GC), and calf thymus (CT) DNA, we found that this complex binds preferentially to AT sites.¹⁸ Because the complex binds primarily to AT sites, the emission profile of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to T4 DNA,¹⁹ which is glucosylated in the major groove at GC sites, is not changed relative to native

CT at low ratios of metal to DNA. Moreover, groove binding agents that occupy the major groove of DNA displace $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ from the helix, while agents in the minor groove do not induce displacement. These photophysical studies are consistent with the original assignment by NMR of intercalation from the major groove.^{14,15}

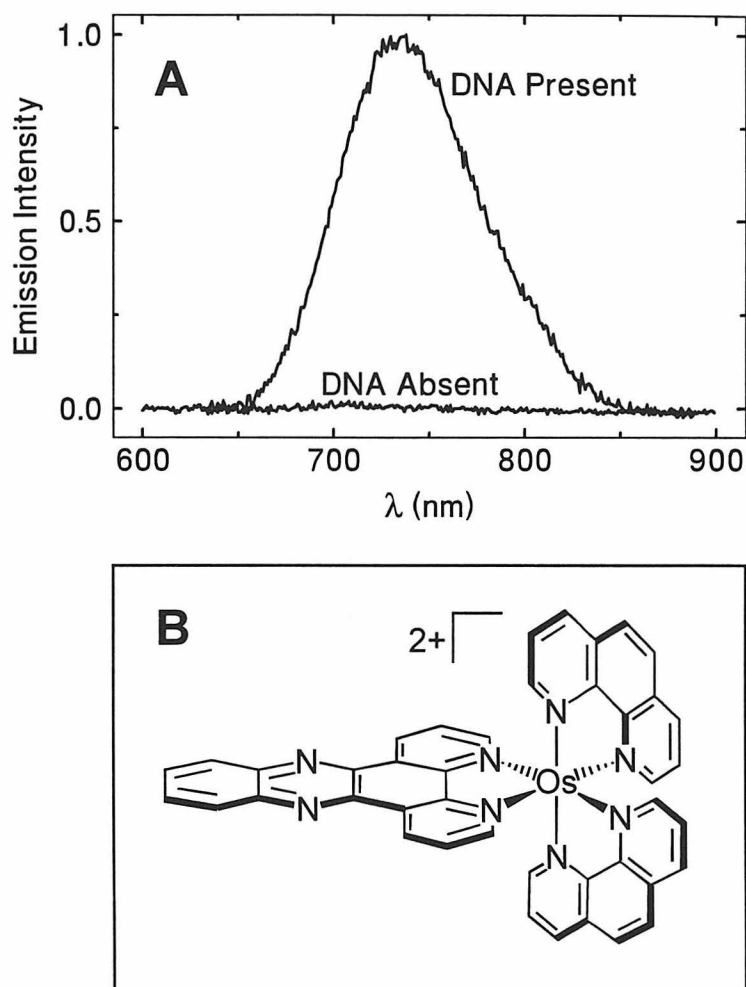


Figure 2.1. (A) Steady state emission spectrum of $\text{rac-}[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in the presence and absence of duplex DNA. (B) Schematic illustration of Δ - $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$. The Δ isomer is shown to illustrate the chirality of the octahedral complex. Conditions: 50 μM Os, 1 mM CT DNA, 5 mM Tris, 50 mM NaCl, pH 8.5.

We prepared $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ as an isostructural analog for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$.^{3,4} $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ exhibits identical DNA binding properties but significantly different photophysical and electrochemical properties compared to $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$. Like its ruthenium analog $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ is also a “molecular light switch” for DNA (Figure 2.1), and emits in aqueous solution only when the phenazine nitrogens of the dppz ligand are protected, as in intercalation. For the osmium complex, the emission is at long-wavelengths ($\lambda_{\text{max}} \sim 740 \text{ nm}$) and the excited state lifetimes are on the order of 10 ns in the presence of DNA. The Os(III) oxidation state is stabilized by $\sim 500 \text{ mV}$ relative to Ru(III) in related complexes and because of the large ligand field splitting for the third row transition element, the Os(II) complexes are photochemically much more stable than their ruthenium counterparts.

The red emission of the osmium complexes makes them attractive for applications in biosensing owing to the large difference in the energy of luminescence from the probe and that of any endogenous fluorescence from the biological matrix of the sample. Therefore, DNA binding agents based on the osmium center constitute a unique class of red-emitting DNA probes.

In this chapter we describe the synthesis and photophysical properties of a family of dppz complexes of Os(II) (Figure 2.2 B). We incorporated simple modifications in the ligand architecture of the DNA binding agents and used absorption and emission spectroscopy to determine the relationship between the structure of the ligands that comprise a complex and its emission profile bound in the presence and absence of DNA. This study provides the first comprehensive characterization of the photophysical and DNA binding properties of dppz complexes of Os(II).

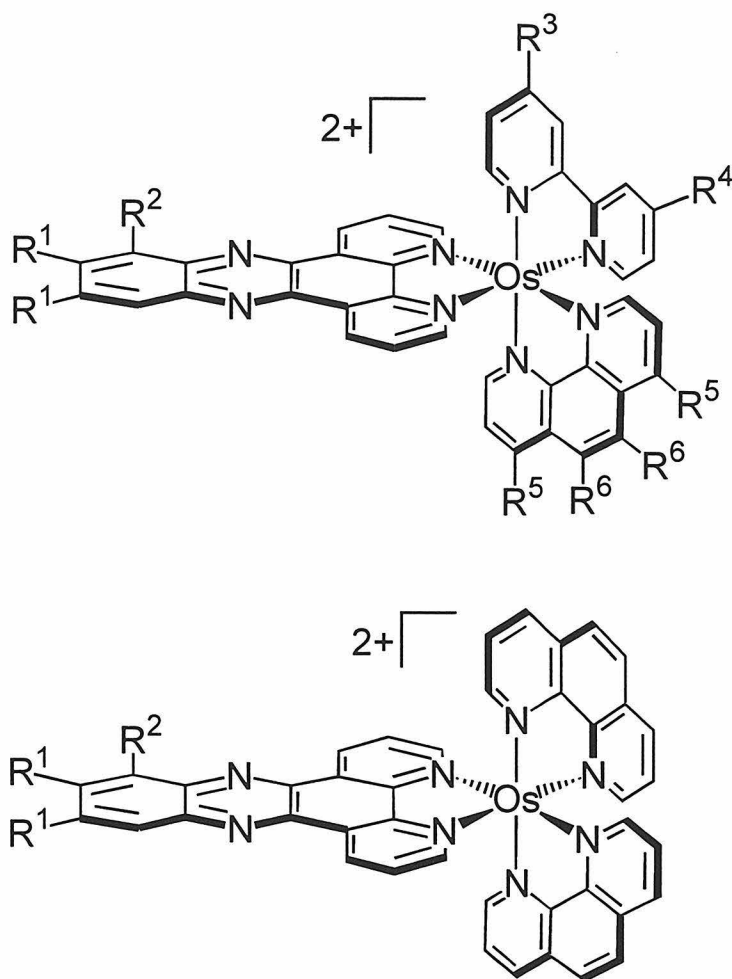


Figure 2.2. Schematic illustrations of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ derivatives. We developed a family of metalointercalators based on the coordination of three different bidentate, polypyridyl ligands to the metal center (top; $[\text{Os}(\text{L}^1)(\text{L}^2)(\text{L}^3)]^{2+}$), or one intercalating ligand and two identical ancillary ligands (bottom; $[\text{Os}(\text{L}^1)_2(\text{R}_n\text{-dppz})]^{2+}$). To illustrate the chirality of the metal complexes, the Δ enantiomers are shown.

2.2. RESULTS

2.2.1. Synthesis

Preparation of Ligands. The preparation of polypyridyl ligands for coordination to metal centers has been described extensively in the literature.^{20,21} In this study we took advantage of commercially available bpy and phen derivatives, and made simple modifications to these parent compounds where necessary using methods described previously.

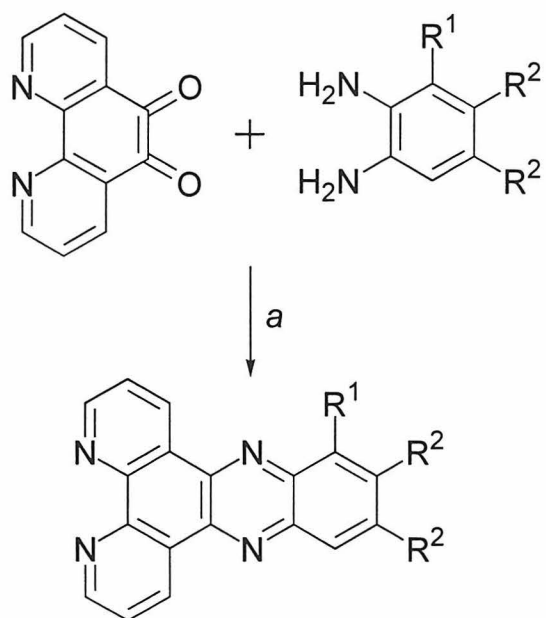
The synthesis of the dppz derivatives for application as intercalating ligands followed the procedure described by Dickeson and coworkers,²² with slight modification. In this approach, the dppz derivative is obtained by condensation of the appropriately substituted ortho diaminobenzene with 5,6-phenanthrolinediquinone in boiling ethanol (Scheme 2.1). These dppz derivatives are isolated by precipitation from the reaction mixture and usually do not require purification, although sublimation can be used if necessary. The yields for these reactions are typically 75% and greater. We found the condition of the commercially available diaminotoluene used to prepare Me-dppz to vary considerably, which affected the yield of this ligand considerably.

Preparation of Metal Complexes. In general, the compounds prepared for this study can be divided into two classes: $[\text{Os}(\text{L}^1)_2(\text{L}^1)]^{2+}$ and $[\text{Os}(\text{L}^1)(\text{L}^2)(\text{L}^3)]^{2+}$. For the $[\text{Os}(\text{L}^1)_2(\text{L}^1)]^{2+}$ class, where L^1 is a phen or bpy derivative and L^2 is a dppz derivative, the complexes are prepared by coordination of the dppz derivative to an $[\text{Os}(\text{L}^1)_2\text{Cl}_2]$ precursor, using well established methods for the synthesis of mixed ligand complexes of Os(II).²³⁻²⁶

To our knowledge, only one method to obtain complexes of the $[\text{Os}(\text{L}^1)(\text{L}^2)(\text{L}^3)]^{2+}$ type, so called tris heteroleptic complexes of Os(II), has been described.²⁷ This approach, which appeared after we began our work, is fairly laborious and requires several steps. Scheme 2.2 outlines the procedure we use to synthesize

polypyridyl complexes of Os(II) that contain three different bidentate polypyridyl

Scheme 2.1. Synthesis of dipyridophenazine derivatives.^a



Reagents and Conditions. (a) EtOH, reflux, 5 min.

ligands. The key intermediate in this approach is $[\text{Os}(\text{phen})\text{Cl}_4]$, which is obtained in high yield by solid phase pyrolysis as described by Meyer and coworkers.²⁶ This method works equally well with several polypyridyl derivatives, although the main requirement is that the ligand dissolve in acid (HCl) to form the phenanthroline or bipyridinium cation. The one condition that must be satisfied in this reaction is that the $(\text{phen-H})(\text{NH}_4)[\text{OsCl}_6]$ must be free of water. We achieved the necessary degree of dryness by pumping the sample down at room temperature on an ultra high vacuum line for 24-48 hours.

With the $[\text{Os}(\text{phen})\text{Cl}_4]$ material in hand, a second polypyridyl ligand (L^2) may be introduced by refluxing the tetrachloro complex and 1 equiv of the desired ligand in DMF for ~4 hours. The complex is isolated from the purple DMF solution by precipitation

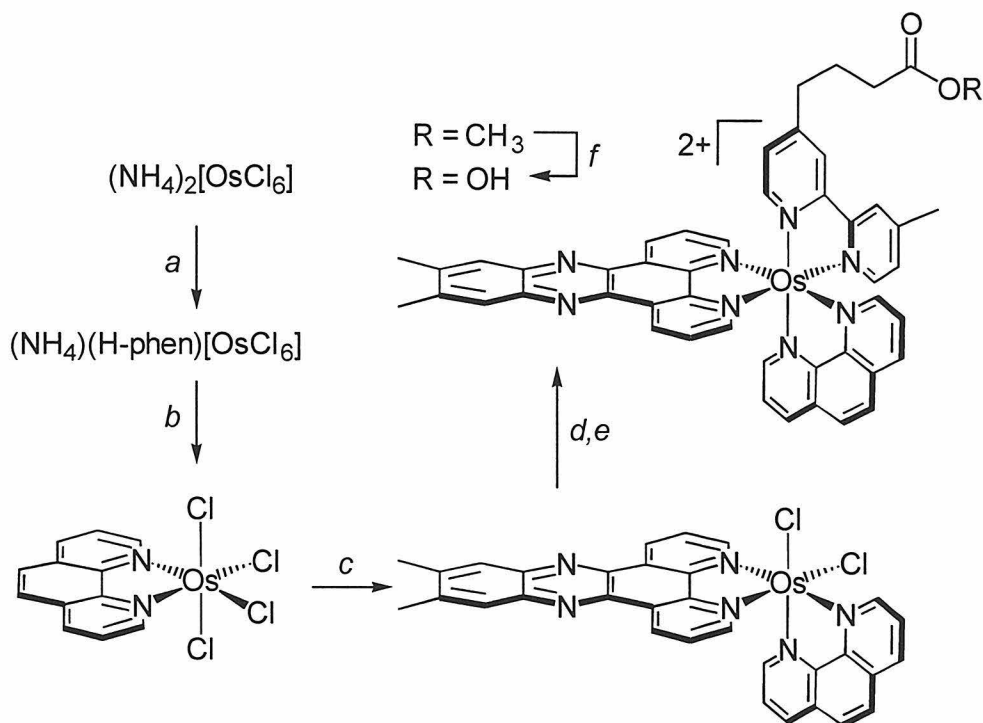
upon addition of excess diethyl ether to the cooled reaction mixture. The solid contains a mixture of Os(II) and Os(III) oxidation states (as evident in ^1H NMR spectra). In order to convert all of the Os(III) to Os(II) the solid is redissolved in fresh DMF, and the Os(III) reduced by adding a saturated aqueous solution of sodium dithionite. The product, $[\text{Os}(\text{phen})(\text{L}^2)\text{Cl}_2]$, precipitates as a dark purple powder, which may be reacted further without purification upon isolation by filtration.

Introduction of the third bidentate ligand follows from protocols established for the preparation of $[\text{Os}(\text{L}^1)_2(\text{L}^1)]^{2+}$ complexes.^{23,26} In our procedure the $[\text{Os}(\text{phen})(\text{L}^2)\text{Cl}_2]$ complex and the appropriate polypyridyl ligand (L^3) are heated at 160 °C in ethylene glycol for three hours. Over the course of the reaction the L^3 dissolves and the solution turns to a characteristic olive green color, consistent with the presence of a tris polypyridyl Os(II) complex. Alternative solvents such as 1:1 water:ethanol (refluxing) may be used if sensitive functionalities are present on L^3 , however, these conditions require as much as 48 hours of reflux.

The product, $[\text{Os}(\text{phen})(\text{L}^2)(\text{L}^3)]^{2+}$, is isolated by precipitation as the hexafluoro phosphate salt upon addition of aqueous NH_4PF_6 to the cooled reaction mixture. In general, we find that the complex usually requires purification before further characterization and use. Two approaches to purification by column chromatography on alumina have been employed. First, if L^3 is a dppz derivative, any excess ligand as well as any $[\text{Os}(\text{L}^1)(\text{L}^2)\text{Cl}_2]$ will be immobile on neutral alumina with neat acetonitrile as the mobile phase. The desired $[\text{Os}(\text{L}^1)(\text{L}^2)(\text{L}^3)]^{2+}$ will be eluted with the solvent front. If L^3 is a bpy derivative, L^3 will also migrate with $R_f = 1$ if acetonitrile is used as the eluent. Therefore, we load the reaction mixture (dissolved in dichloromethane) onto neutral alumina equilibrated in dichloromethane (DCM). Several column volumes of DCM will move the bpy derivative off the column orthogonally to the contents remaining in the reaction mixture. Once the bpy derivative has been eluted, the solvent is changed to

acetonitrile and the desired complex will come off the column.

Scheme 2.2. Synthesis of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$.^{a,b}



Reagents and Conditions: (a) phen (1 equiv), 3 N HCl (aq);²⁶ (b) solid state pyrolysis, 290 °C, molten $\text{KNO}_3/\text{NaNO}_3$ (1:9), 8 h;²⁶ (c) $\text{Me}_2\text{-dppz}$ (0.9 equiv), DMF, reflux, 4 h; (d) b'-OMe, ethylene glycol, 160 °C, 3 h; (e) 10% NH_4PF_6 (aq), metathesis to dichloride salt by anion exchange; (f) LiOH (aq; 10 equiv), 25 °C, 12 h.

Resolution of $\text{rac-}[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$. In order to investigate the importance of the absolute configuration about the octahedral metal center in determining the emission properties of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA, we resolved the racemic

mixture into the pure Δ and Λ enantiomers. We employed a procedure described previously for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$,¹⁵ to resolve the osmium complex by column chromatography on cation exchange resin using an aqueous solution of potassium bis tartratodiantimonate as a chiral eluent (see experimental section for picture of column). Complete resolution was indicated by the presence of baseline separation of the two bands of equal size and color on the column support. Figure 2.3 shows the circular dichroism (CD) spectra of Δ - $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and Λ - $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in water. Owing to the mirror image relationship between these two spectra and the complete baseline resolution of the two bands during chromatography, we conclude that each solution contains a pure enantiomer.

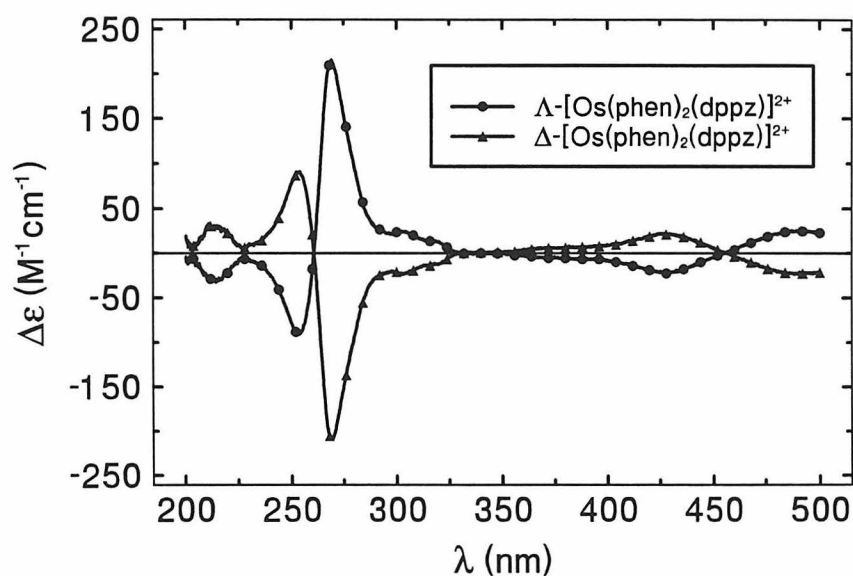


Figure 2.3. CD spectra for the pure Δ (▲) and Λ (●) enantiomers of $[\text{Os}(\text{phen})_2(\text{Me-dppz})]^{2+}$ in water. The complete baseline resolution of the two bands from which these samples were isolated and the mirror image relationship between the CD spectra indicate that these samples are the pure enantiomers. For Δ - $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$, $\Delta\epsilon$ (270 nm) = $-209 \text{ M}^{-1}\text{cm}^{-1}$, and for Λ - $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ $\Delta\epsilon$ (270 nm) = $+214 \text{ M}^{-1}\text{cm}^{-1}$.

2.2.2. Characterization.

Structural Assessment by ^1H -NMR. We employed several analytical methods to characterize the materials synthesized in these studies, including ^1H NMR, mass spectrometry, ultraviolet-visible (UV-vis) spectroscopy, and cyclic voltammetry. The two simplest and most useful structural tools are ^1H NMR and mass spectrometry.

Figure 2.4 shows a ^1H NMR spectrum of $[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})\text{Cl}_2]$ in CD_2Cl_2 taken at 300 MHz after treatment with $\text{Na}_2\text{S}_2\text{O}_4$. Owing to the paramagnetic, d^5 ground state electronic configuration of the Os(III) complex, even trace amounts of this oxidation state will lead to significant perturbations in the NMR spectrum for the complex. Reduction of the Os(III) to Os(II) by dithionite gives the sharp spectrum in Figure 2.4, which is consistent with the diamagnetic d^6 ground state.

In the aromatic region of the spectrum for the fully Os(II) sample, 16 resonances, which integrate to 16 ^1H , are present. Two resonances, which integrate to 3 ^1H each, are present in the aliphatic region. For $[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})\text{Cl}_2]$, *cis* coordination by the two chloro ligands breaks the C_2 symmetry of the coordinated phen and $\text{Me}_2\text{-dppz}$ ligands and makes each proton unique. The observed spectrum is consistent with the inequivalence of all the protons on the two ligands and therefore NMR supports the assignment of the *cis*-dichloro coordination.

The ^1H NMR of $[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})(\text{bpy}')]^{2+}$ in CD_3OD is shown in Figure 2.5. Owing to the significant number of unique protons in this complex, the aromatic region of the ^1H NMR spectrum contains several overlapping resonances. Fortunately, there are well-defined resonances to be used to confirm the identity of the complex in this spectrum. For example, in the aromatic region, the two sets of signals at 10.5 ppm and 10.9 ppm correspond to protons at the 4 and 7 positions of the phenanthroline moiety of the dppz derivative. These signals are shifted downfield by hydrogen bonding with the phenazine nitrogens. It is noteworthy that for this spectrum, the splitting pattern

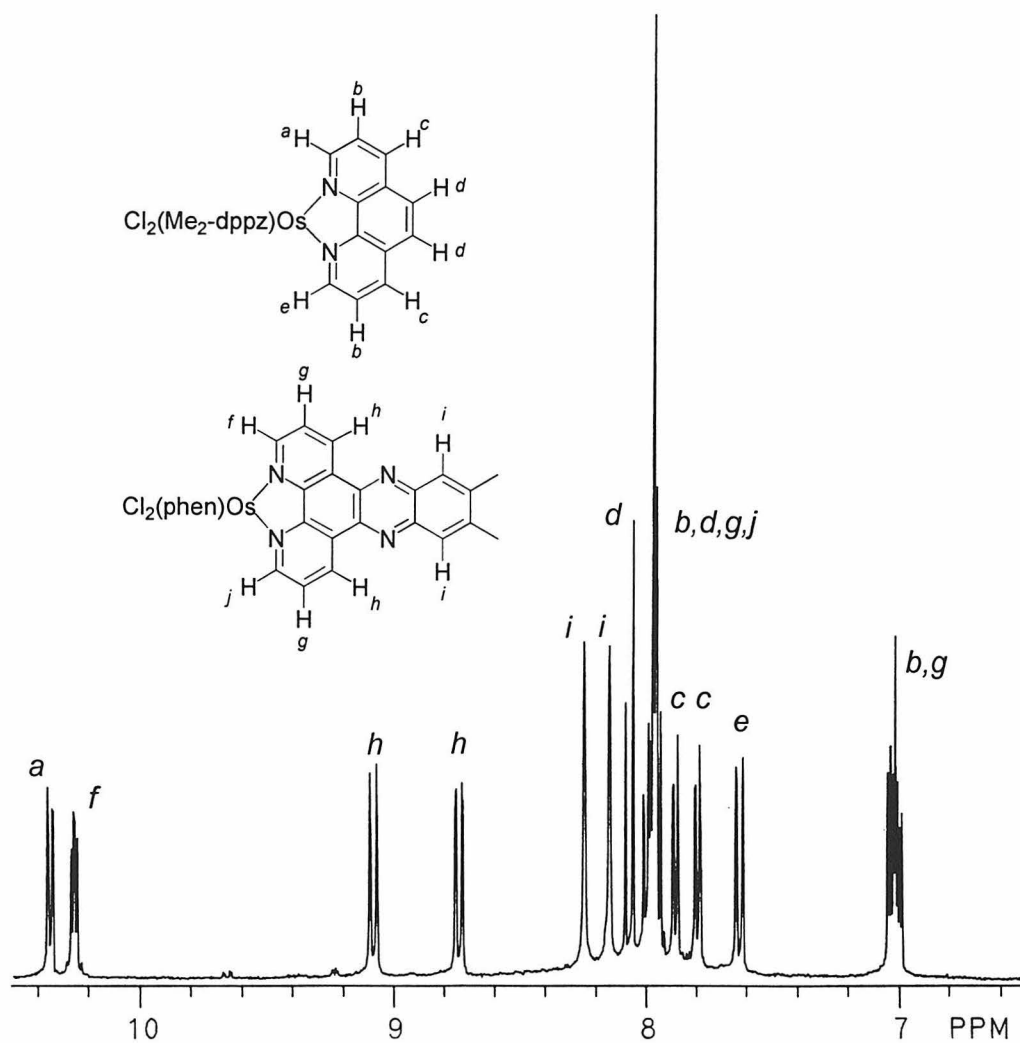


Figure 2.4. ^1H NMR of $[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})\text{Cl}_2]$ in CD_2Cl_2 at 300 MHz. Also shown is a schematic representation of each polypyridyl ligand attached to the metal center with appropriate notations for spectral assignment.

of each signal is different; the resonance appears as a doublet, while the other is a doublet of doublets. For this complex, the spectrum at 300 MHz shows a broad singlet in the aliphatic region, which accounts for all three of the methyl groups (two on Me₂-dppz, one on bpy'). Owing to the asymmetry of this ligand, the complex is present as two diastereomers; one where the butyric acid oriented in an axial direction relative to the Me₂-dppz plane and one where the group is equatorial. The presence of these two isomers is apparent only in the aliphatic region of the NMR spectrum of this compound, which makes sense, since the differences in the isomers are significant only for a substituent that extends away from the metal center.

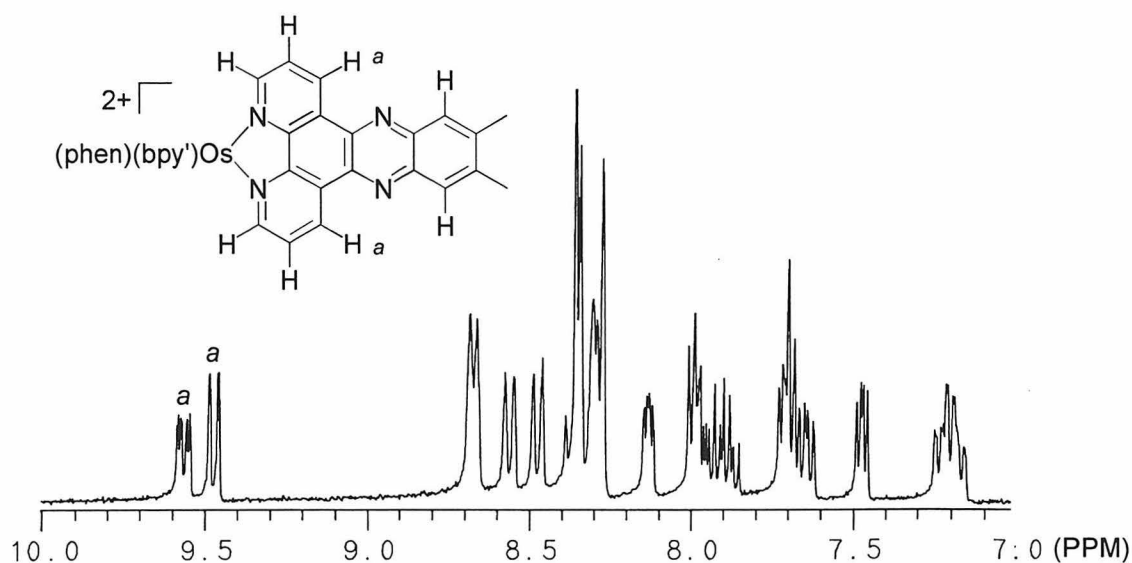


Figure 2.5. ¹H NMR of [Os(phen)(bpy')(Me₂-dppz)]²⁺ in CD₃OD at 300 MHz. The spectrum is complex owing to the inequivalence of all the aromatic protons, however, characteristic signals for the 4,7-protons of the Me₂-dppz ligand are well resolved at ~10.5 ppm.

Characterization by Mass Spectrometry. ^1H NMR data alone are not sufficient to establish the structure of a complex with three different polypyridyl ligands. One can imagine that over the course of the synthesis of an $[\text{Os}(\text{L}^1)(\text{L}^2)(\text{L}^3)]^{2+}$ complex, one obtains only a statistical mixture of complexes with varying degrees in ligand diversity about the metal center. Such a mixture would give rise to an NMR spectrum that resembles the one in Figures 2.4 or 2.5. Therefore, in the absence of a crystal structure, mass spectrometry is an excellent analytical tool to confirm the composition of the complex synthesized.

A high resolution (HR) FABMS of $[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})\text{Cl}_2]$ is shown in Figure 2.6, and a comprehensive summary of the analysis by MS of the family of metallointercalators based on Os(II) is listed in Table 2.1. For the data in Figure 2.6, we find no signals at m/z values that correspond to $[\text{Os}(\text{phen})_2\text{Cl}_2]$ or $[\text{Os}(\text{Me}_2\text{-dppz})_2\text{Cl}_2]$. Thus, we do not believe that any scrambling of the polypyridyl ligands occurs during the synthesis. The HR data in Figures 2.6 A (observed) and 2.5 B (calculated) provide an unambiguous confirmation of the identity of the material and validate our method of introducing one $\text{Me}_2\text{-dppz}$ ligand onto the metal center.

Also shown in Table 2.1 are the MS data for several $[\text{Os}(\text{L}^1)_2(\text{L}^2)]^{2+}$ and $[\text{Os}(\text{L}^1)(\text{L}^2)(\text{L}^3)]^{2+}$ complexes. These data establish the efficacy of our methods to introduce a third polypyridyl ligand onto $[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})\text{Cl}_2]$ to give complexes with three different bidentate, polypyridyl ligands without generating statistical mixtures of homoleptic complexes.

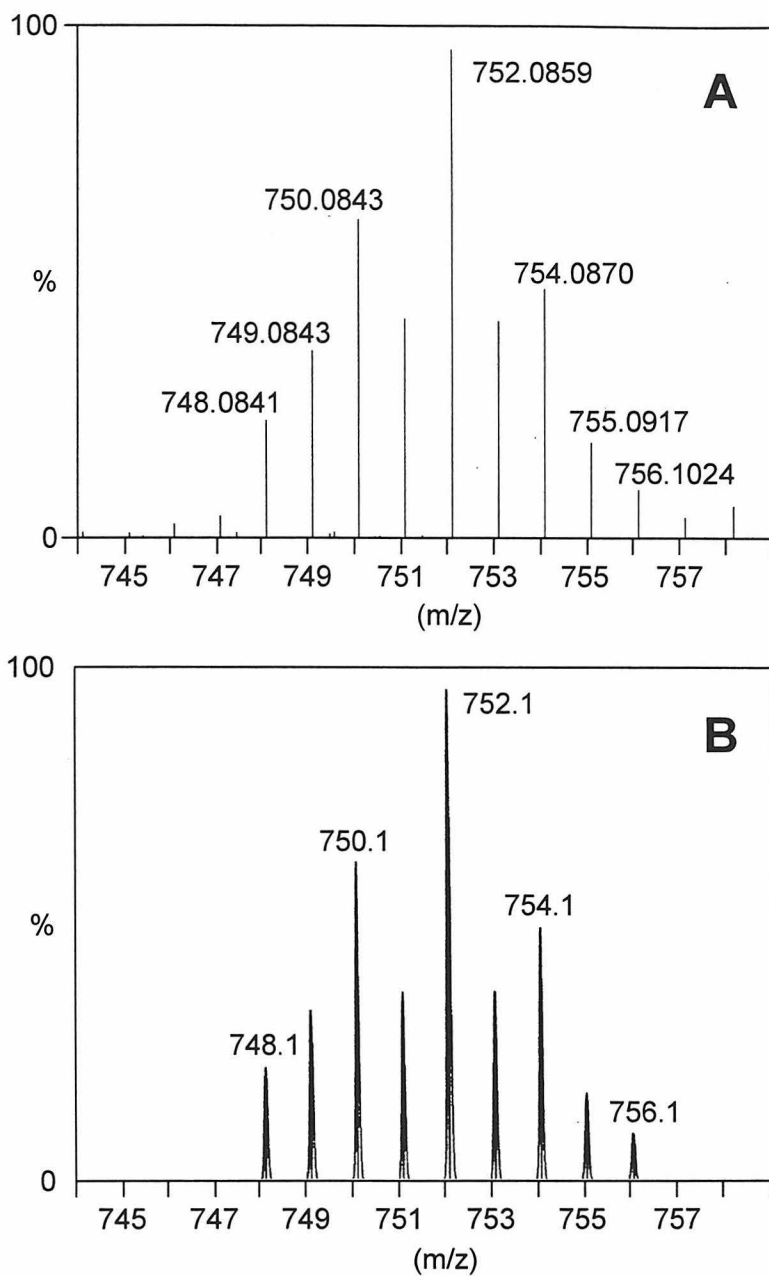


Figure 2.6. (A) Observed spectrum for the analysis of $[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})\text{Cl}_2]$ by high resolution FAB mass spectrometry. (B) Calculated isotopic distribution for the molecular ion $[\text{OsC}_{22}\text{H}_{22}\text{N}_2\text{Cl}_2]^+$. The agreement of the observed and calculated masses and isotopic patterns confirm the composition of the complex.

Table 2.1. Mass Spectrometry Results for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ Derivatives.

Complex ^a	Ion ^b	Formula	Calc'd Mass	Obs'd (m/z) ^c	Method ^d
$[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})\text{Cl}_2]$	$[\text{M}]^+$	$\text{OsC}_{32}\text{H}_{22}\text{N}_6\text{Cl}_2$	752.0905	752.0859	HR FAB
$[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$	$[\text{M}]^+$	$\text{OsC}_{42}\text{H}_{26}\text{N}_8$	834.1902	834	FAB
$[\text{Os}(\text{phen})_2(\text{Me-dppz})]^{2+}$	$[\text{M}]^+$	$\text{OsC}_{43}\text{H}_{28}\text{N}_8$	848.2052	848.2054	HR FAB
$[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$	$[\text{M}]^+$	$\text{OsC}_{44}\text{H}_{30}\text{N}_8$	862.2215	862.2208	HR FAB
$[\text{Os}(4,7\text{-dmp})_2(\text{Me}_2\text{-dppz})]^{2+}$	$[\text{M}]^{2+}$	$\text{OsC}_{48}\text{H}_{38}\text{N}_8$	918.2841	459.2	ESI
$[\text{Os}(5,6\text{-dmp})_2(\text{Me}_2\text{-dppz})]^{2+}$	$[\text{M}]^{2+}$	$\text{OsC}_{48}\text{H}_{38}\text{N}_8$	918.2841	459.1	ESI
$[\text{Os}(\text{dmob})_2(\text{Me}_2\text{-dppz})]^{2+}$	$[\text{M}]^{2+}$	$\text{OsC}_{44}\text{H}_{38}\text{N}_8\text{O}_4$	934.2638	467.1	ESI
$[\text{Os}(\text{phen})(\text{dmb})(\text{Me}_2\text{-dppz})]^{2+}$	$[\text{M}]^+$	$\text{OsC}_{44}\text{H}_{34}\text{N}_8$	866.2521	866.2519	HR FAB
$[\text{Os}(\text{phen})(\text{bpy})(\text{Me}_2\text{-dppz})]^{2+}$	$[\text{M}]^+$	$\text{OsC}_{47}\text{H}_{38}\text{N}_8\text{O}_2$	938.2746	938.2746	HR FAB
$[\text{Os}(\text{phen})(\text{b'-OMe})(\text{Me}_2\text{-dppz})]^{2+}$	$[\text{M}]^+$	$\text{OsC}_{48}\text{H}_{40}\text{N}_8\text{O}_2$	952.2896	952.363	ESI
$[\text{Os}(\text{phen})(\text{dab})(\text{Me}_2\text{-dppz})]^{2+}$	$[\text{M-H}]^+$	$\text{OsC}_{42}\text{H}_{31}\text{N}_{10}$	867.2355	867.2	FAB

a. $\text{Me}_2\text{-dppz}$ = 7,8-dimethyldipyridophenazine; Me-dppz = 6-methyldipyridophenazine; dmob = 4,4'-dimethoxy-2,2'-bipyridyl; 4,7-dmp = 4,7-dimethylphen; 5,6-dmp = 5,6-dimethylphen, bpy = 4-butyric acid-4-methyl-2,2'-bipyridyl; b' - Ome = 4-butyric acid-4-methyl-2,2'-bipyridyl methyl ester, dab = 4,4'-diamino-2,2'-bipyridyl.

b. For $[\text{M}]^+$ ions, the 2+ complex is reduced by the matrix to give a singly charged ion.

c. The techniques employed measure the ratio of mass to charge (m/z) for the ions in the gas phase. The mass of the complex is the product of the charge of the ion and the mass to charge ratio. For $[\text{M}]^{2+}$ ions, $m = 2(m/z)$.

Visible Absorption Properties. Polypyridyl complexes of Os(II) are characterized by intense absorption into metal-to-ligand charge-transfer (MLCT) excited states in the visible region of the electromagnetic spectrum.^{28,29} Figures 2.7 A, B and C show the absorption spectra of Me₂-dppz, [Os(phen)₃]²⁺, and [Os(phen)₂(Me₂-dppz)]²⁺, respectively. The near-visible absorption of Me₂-dppz consists of a π - π^* transition centered on the phenazine portion of the polypyridyl ligand. The absorption profile of [Os(phen)₃]²⁺ is dominated by transitions into the ¹MLCT (400-480 nm) states as well as formally spin forbidden transitions into the ³MLCT states (580-725 nm). Based on the comparison of Figures 2.7 A and 2.7 B, the spectrum of [Os(phen)₂(Me₂-dppz)]²⁺ consists of the sum of two spectral components: the absorption of Me₂-dppz together with that of [Os(phen)₃]²⁺.

Figure 2.8 compares the absorption spectra for [Os(phen)₂(dppz)]²⁺ and [Ru(phen)₂(dppz)]²⁺. Each complex displays visible absorption, however, the absorption by the osmium complex extends out beyond 700 nm, while the absorption by ruthenium is not significant at wavelengths longer than ~550-600 nm. The low-energy absorption by the osmium complex is the result of direct excitation into ³MLCT states.^{28,29} This transition is spin-forbidden for both complexes, however, and therefore is not observed with ruthenium. With osmium, spin orbit coupling results in mixing of the ¹MLCT and ³MLCT states. This mixing gives the triplet state some singlet character and allows for the transition to occur. As a result of absorption across the entire visible region of the spectrum, dppz complexes of Os(II) and polypyridyl complexes of Os(II) in general are darkly colored and appear almost black in concentrated solution.

The transition at 374 nm is centered on the dppz ligand.⁹ The energy of this absorption does not change upon substitution of the metal center. This observation suggests that there is very little electronic coupling between the metal center and the phenazine portion of the molecule.

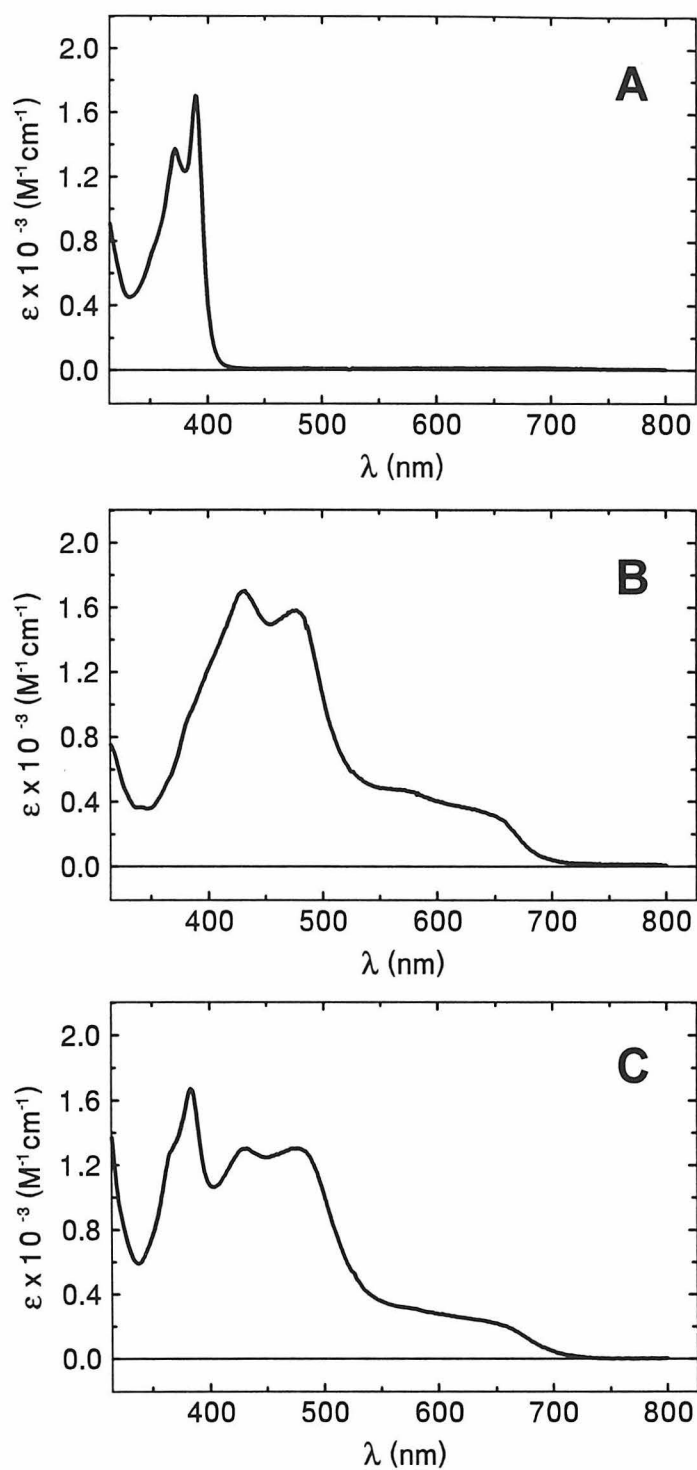


Figure 2.7. Visible absorption spectra of: (A) $\text{Me}_2\text{-dppz}$ in 10:1 water/acetonitrile; (B) $[\text{Os}(\text{phen})_3]^{2+}$ in water; and (C) $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ in water.

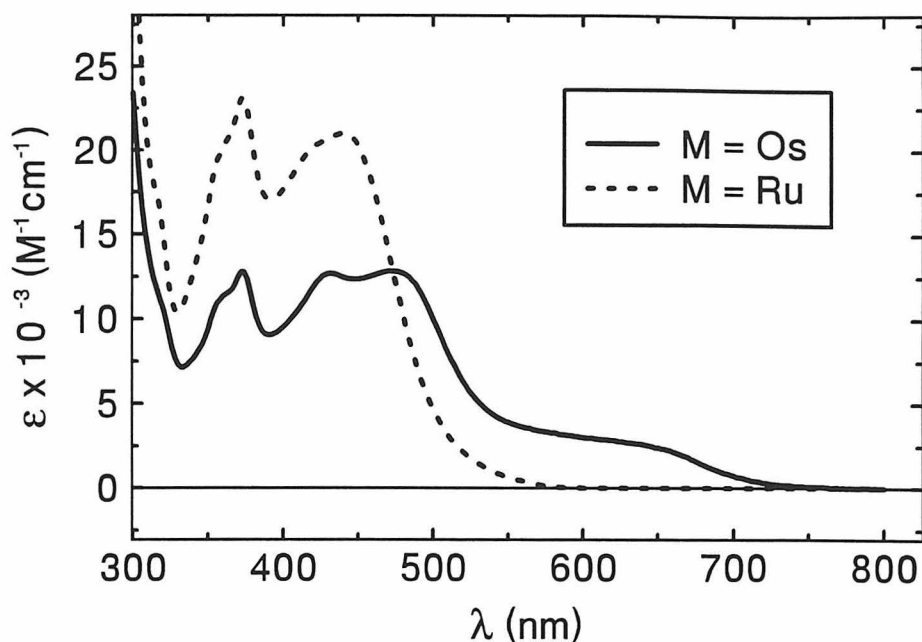


Figure 2.8. Comparison of the visible absorption spectra for $[M(\text{phen})_2(\text{dppz})]^{2+}$ complexes in water.

It is also noteworthy that the lowest energy $^1\text{MLCT}$ transition occurs at lower energy for osmium than for ruthenium. This observation is consistent with the $5d^6$ ground state configuration of Os(II) , which commands a lower third ionization potential compared with Ru(II) .^{26,28}

Table 2.2 lists the wavelength of maximum absorption (λ_{max}) for the $\pi\text{-}\pi^*$ transition centered on the dppz derivative, and the λ_{max} for lowest energy $^1\text{MLCT}$ transition for the series of compounds employed in this study. Across this series, the incorporation of substituents on the phenazine portion of the dppz derivative perturbs the $\pi\text{-}\pi^*$ transition on this ligand, but does not affect the MLCT transitions. Conversely, substituents on the ancillary phen or bpy derivatives affect only the MLCT transitions, but not the absorption centered on the dppz derivative.

Table 2.2. Visible absorption parameters for [Os(phen)₂(dppz)]²⁺ Derivatives in Aqueous Solution.

<u>Complex^a</u>	<u>π-π^* on R_n-dppz^b</u>		<u>¹MLCT^c</u>	
	<u>λ_{max} ($\epsilon \times 10^{-4}$)</u>		<u>λ_{max} ($\epsilon \times 10^{-4}$)</u>	
[Os(phen) ₂ (dppz)] ²⁺	374	(1.27)	473	(1.30)
[Os(phen) ₂ (Me-dppz)] ²⁺	374	(1.15)	475	(1.32)
[Os(phen) ₂ (Me ₂ -dppz)] ²⁺	384	(1.68)	475	(1.30)
[Os(4,7-dmp) ₂ (Me ₂ -dppz)] ²⁺	384	(1.52)	477	(1.23)
[Os(5,6-dmp) ₂ (Me ₂ -dppz)] ²⁺	384	(1.69)	475	(1.34)
[Os(dmob) ₂ (Me ₂ -dppz)] ²⁺	384	(1.84)	485	(1.20)
[Os(phen)(bpy')(Me ₂ -dppz)] ²⁺	384	(1.87)	479	(1.39)
[Os(phen) ₃] ²⁺	-----	-----	477	(1.58)
Me ₂ -dppz ^d	389	(1.7)	-----	-----

a. See Table 2.1 for explanation of ligand abbreviations.

b. For Me₂-dppz, n = 2; R = CH₃; for Me-dppz, n = 1; R = CH₃; for dppz R = H.

c. The ¹MLCT transition has two discernable maxima. The data listed are for the lowest energy transition.

d. For solubility considerations, data were recorded in a mixture of acetonitrile and water.

Investigation of Redox Properties by Cyclic Voltammetry. We employed cyclic voltammetry to measure the relevant reduction and oxidation properties for the osmium complexes prepared in this study. Figures 2.9 A and B show an oxidation wave and a reduction wave, respectively, for $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ in DMF. Scheme 2.3 A and B illustrate the oxidation and reduction processes that account for the flow of current detected in each experiment. Consistent with previous studies of polypyridyl complexes of Os(II),²⁶ oxidation is metal centered and corresponds to the Os(III)/Os(II) couple. The observed reductions are ligand centered and correspond to one electron reductions first on the phenazine portion of the $\text{Me}_2\text{-dppz}$ ligand and then each of the two phenanthrolines.

Table 2.3 shows the reduction potentials for the metal centered and ligand centered processes for the entire family of complexes. As expected, the incorporation of electron donating methyl groups on the 6 or 7 and 8 positions of the dppz derivatives shifts the phenazine centered reduction toward the negative, corresponding to a higher energy for reduction. Each methyl group shifts the potential ~ -50 mV. These substituents do not affect the potential of the metal centered couple.

The incorporation of electron donating substituents on the ancillary phen or bpy ligands shifts the Os(III)/Os(II) reduction to the negative as well. These data are consistent with the electron donors stabilizing the Os(III) state via raising the absolute energy of the highest occupied molecular orbital (HOMO).³⁰

In summary, the redox chemistry of dppz complexes of Os(II) is a superposition of two redox active components. The metal centered ($3+/2+$) couple can be manipulated by incorporating electron donating or withdrawing substituents on the ancillary ligands, without affecting the ligand centered reduction. Substituents on the phenazine portion of the dppz ligand affect the phenazine reduction potential but not the metal centered potential.

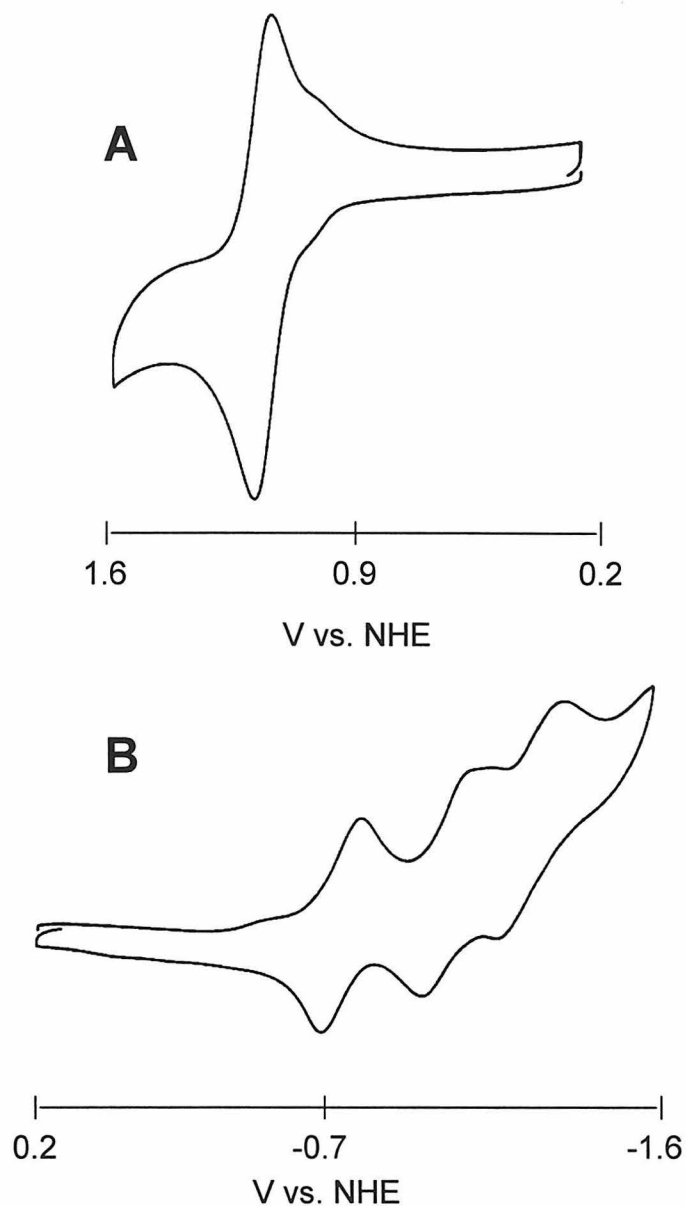


Figure 2.9. Cyclic voltammograms for the oxidation (A) and reduction (B) of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})](\text{PF}_6)_2$ in DMF. The relevant redox properties are outlined in Scheme 2.3. See Table 2.3 for conditions.

Scheme 2.3. Summary of the metal centered (**A**) and ligand centered (**B**) redox processes for $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ measured by cyclic voltammetry (Figure 2.9). See Table 2.3 for conditions.

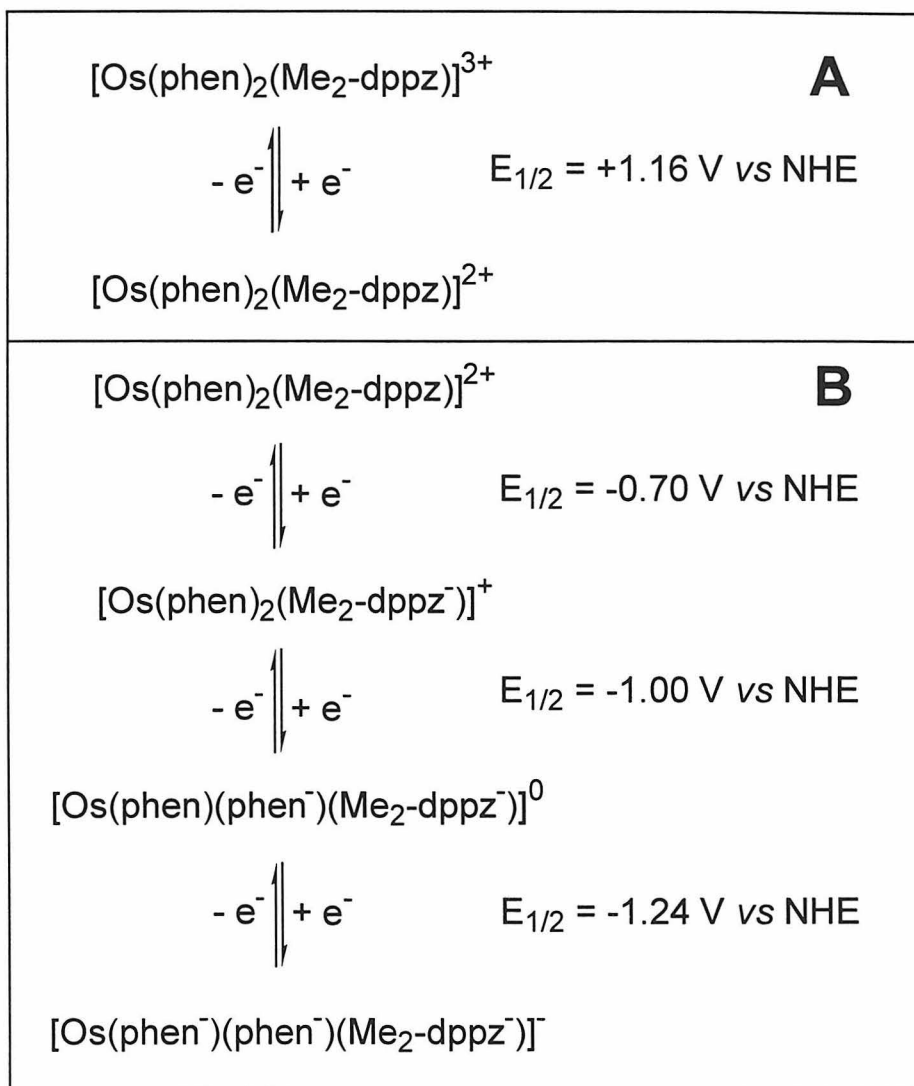


Table 2.3. Ground state reduction and excited state oxidation potentials for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ derivatives.

Complex ^a	Metal Centered ^{b,c}		Ligand Centered ^{b,c}		
	$(^*2+/3+)$	$(3+/2+)$	dppz/dppz-	L2/L2-	L3/L3-
$[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$	0.76	1.16	-0.61	-0.98	-1.21
$[\text{Os}(\text{phen})_2(\text{Me-dppz})]^{2+}$	0.73	1.19	-0.66	-1.00	-1.23
$[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$	0.76	1.16	-0.70	-1.00	-1.24
$[\text{Os}(4,7\text{-dmp})_2(\text{Me}_2\text{-dppz})]^{2+}$	0.89	1.03	-0.74	-1.17	-1.38
$[\text{Os}(5,6\text{-dmp})_2(\text{Me}_2\text{-dppz})]^{2+}$	0.81	1.11	-0.72	-1.06	-1.30
$[\text{Os}(\text{dmob})_2(\text{Me}_2\text{-dppz})]^{2+}$	0.87	0.90	-0.75	-1.16	-1.36
$[\text{Os}(\text{phen})(\text{dmb})(\text{Me}_2\text{-dppz})]^{2+}$	0.81	1.11	-0.70	-1.04	-1.32
$[\text{Os}(\text{phen})(\text{dab})(\text{Me}_2\text{-dppz})]^{2+}$	---	0.86	-0.71	-1.08	-1.39
$[\text{Os}(\text{Me}_2\text{-dppz})_3]^{2+}$	---	1.01	-0.74	-1.00	-----

a. See Table 2.1 for explanation of ligand abbreviations.

b. Potentials are reported in volts vs NHE.

c. Measured against Ag/AgCl (3 M KCl) at a glassy carbon working electrode in dry DMF at a scan rate of $0.1 \text{ V}\cdot\text{s}^{-1}$. The supporting electrolyte was 0.1 M tetrabutylammonium hexafluorophosphate and the auxiliary electrode was Pt wire.

2.2.3. Probing DNA Binding with Visible Absorption Spectroscopy.

Monitoring DNA Intercalation by Hypochromism in the Dipyridophenazine Absorption. We followed the association of the dppz complexes of Os(II) with double helical DNA by absorption spectroscopy. Owing to the well resolved π - π^* transition on the phenazine portion of the dppz derivative in these complexes, one can delineate processes that are localized on this ligand.

The binding of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ to DNA occurs by preferential intercalation of the dppz ligand into the hydrophobic interior of the DNA base stack, and is accompanied by significant hypochromism in the π - π^* transition on the intercalating ligand.^{3,31} Figure 2.10 shows absorption spectra of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ in the presence of increasing concentrations of mixed sequence, CT DNA (A), poly d(AT) (B), and poly d(GC). Like its parent complex, the association of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ with DNA results in a significant perturbation in the π - π^* transition on $\text{Me}_2\text{-dppz}$, consistent with preferential intercalation of this ligand into the base stack of the DNA helix.

A plot of % hypochromism versus the ratio of nucleotides to osmium ($R_{\text{nuc:Os}}$) is shown in Figure 2.11. With increasing DNA concentration, the % hypochromism increases linearly until reaching saturation at $R_{\text{nuc:Os}} = \sim 5$. For $R_{\text{nuc:Os}}$ values of $\sim 100:1$, the % hypochromism actually decreases slightly. Simple fits of these plots as described previously by Thorp and coworkers, indicate binding constants (K_B) with a lower limit of $\sim 10^6 \text{ M}^{-1}$.

Table 2.4 lists the % hypochromism in the π - π^* transition on the dppz derivative for the entire series of Os(II) intercalators in the presence of three different DNA polymers at saturation. For each intercalator, significant hypochromism is observed upon association with DNA. Based on titrations like those in Figure 2.10, the K_B values do not differ significantly from complex to complex. Across this series however, there are noticeable trends with respect to the amount of hypochromism and the ligand architecture

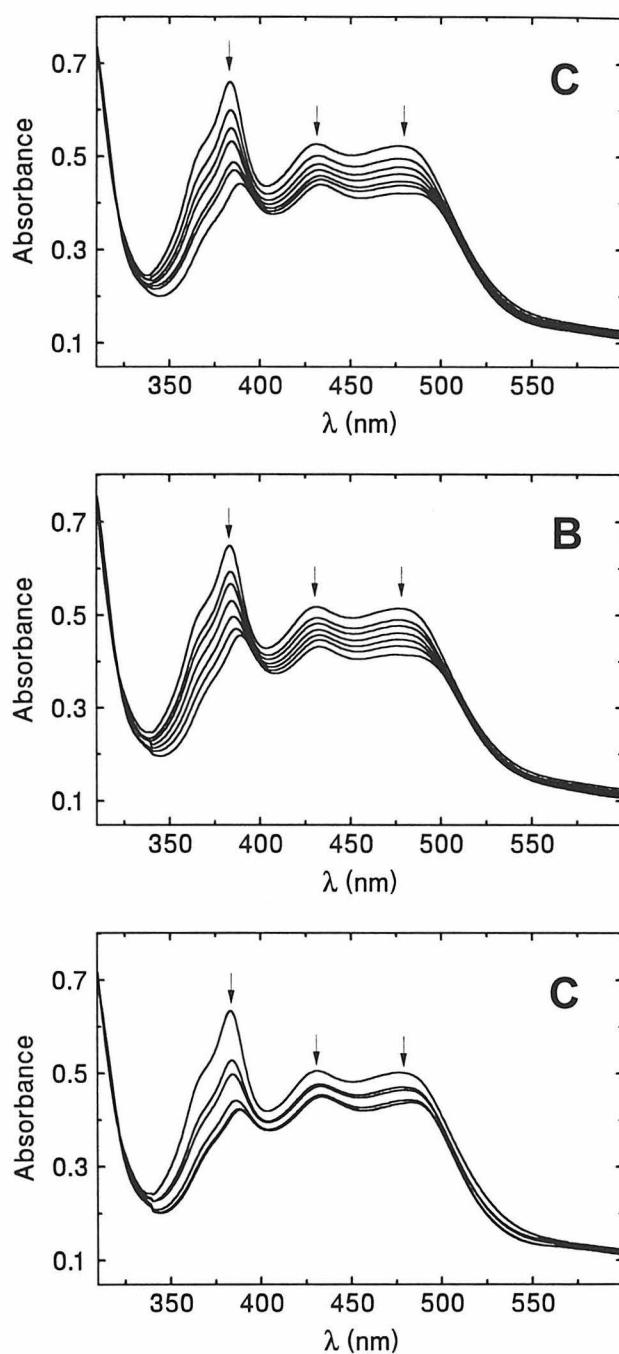


Figure 2.10. Visible absorption spectra of aqueous $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ as a function of increasing concentrations of (A) mixed sequence (CT) DNA; (B) poly d(AT); and (C) poly d(GC). Conditions were as follows: $[\text{Os}] = 30 \mu\text{M}$; $[\text{DNA}] = 0\text{-}0.6$ mM nucleotides in 5 mM Tris, 50 mM NaCl, pH 8.5.

of the metallointercalator. For example, all of the complexes with substituents on the 4 position of the phen or bpy ancillary ligand exhibit less hypochromism than complexes with hydrogen in this position. Based on the similarity in titrations like those in Figure 2.10 for each complex, K_B does not differ significantly across the family of intercalators. Therefore, less hypochromism must reflect less penetration by the dppz ligand into the DNA base stack.

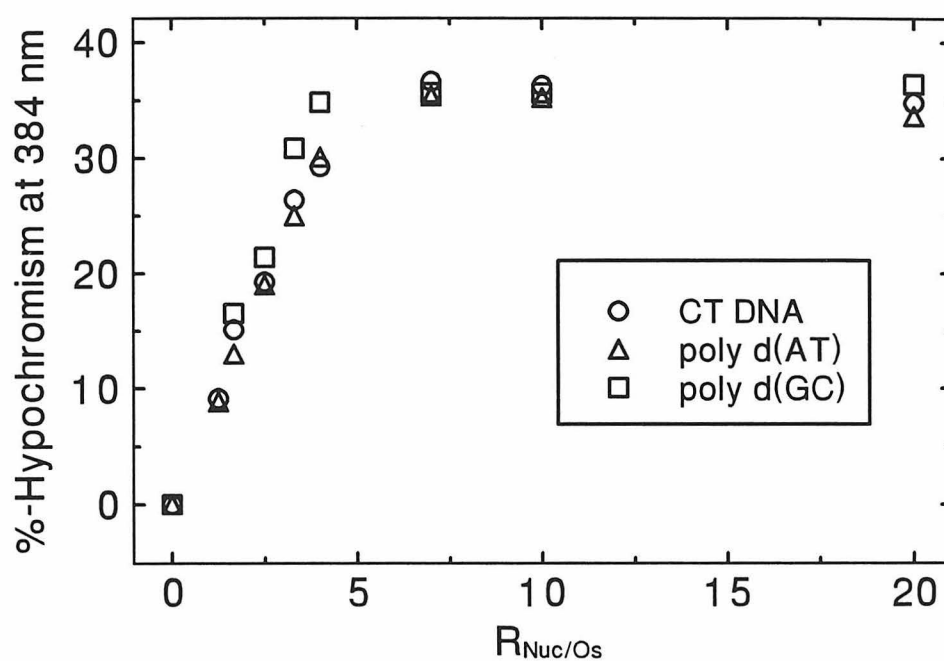


Figure 2.11. Percent hypochromism at 384 nm for $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ in the presence of increasing amounts of duplex DNA (see Figure legend). Conditions are described in Figure 2.10.

Table 2.4. Hypochromism Observed for Association of Dipyrrophenazine Complexes of Os(II) with DNA by Intercalation.

Complex ^b	% Hypochromism ^a		
	CT ^c	poly d(AT) ^c	poly d(GC) ^c
[Os(phen) ₂ (dppz)] ²⁺	31.3	31.5	34.9
[Os(phen) ₂ (Me-dppz)] ²⁺	26.6	31.1	32.8
[Os(phen) ₂ (Me ₂ -dppz)] ²⁺	31.0	33.0	34.8
[Os(4,7-dmp) ₂ (Me ₂ -dppz)] ²⁺	26.7	33.9	35.4
[Os(5,6-dmp) ₂ (Me ₂ -dppz)] ²⁺	30.8	32.4	37.1
[Os(dmob) ₂ (Me ₂ -dppz)] ²⁺	32.8	33.9	33.6
[Os(phen)(b'-Ome)(Me ₂ -dppz)] ²⁺	25.8	27.5	28.7
[Os(phen)(bpy')(Me ₂ -dppz)] ²⁺	27.5	30.9	30.7

a. % Hypochromism = $100 \times \frac{[A(\text{DNA} = 0)] - A(\text{DNA} = 1 \text{ mM nuc})}{A(\text{DNA} = 0)}$. Values are for M:DNA = 1:50 bp.

b. See Table 2.1 for explanation of ligand abbreviations.

c. Conditions: [Os] = 10 μM , [DNA] = 1 mM nuc, in buffer (5 mM Tris, 50 mM NaCl, pH 8.5).

2.2.4. Luminescence Studies in the Presence and Absence of DNA.

We incorporated simple modifications into the ligand sphere of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and related complexes to determine how these perturbations in structure would affect the emission properties of the complexes in the presence and absence of DNA. The luminescence behavior of the resulting series of osmium intercalators was studied as a function of DNA sequence and in nonaqueous solvent by steady-state and time-resolved emission spectroscopy.

Luminescence in Dichloromethane. Luminescence from dppz complexes of transition metals such as Ru(II), Os(II), and Re(I) is remarkably sensitive to water, owing to quenching by proton transfer to the phenazine nitrogens of the photoexcited complex.^{3,5-8,13} In order to measure the inherent quantum yields for emission (Φ_{em}) for each of the osmium complexes without interference by water induced quenching, we sought to study their luminescence profiles in a nonaqueous solvent. Therefore, we chose DCM as a suitable nonaqueous, aprotic solvent that could be rigorously dried.

We measured the excited state lifetime by two time-resolved emission techniques: single photon counting (SPC)⁴ and multifrequency phase-modulation fluorometry (MPMF).³² Results obtained from data collected by both techniques were identical. We also measured the excited state lifetimes of well studied complexes like $[\text{Os}(\text{bpy})_3]^{2+}$ and found that either technique gave results that matched values reported previously.²⁸ The MPMF technique is remarkably simple and allows one to collect and analyze several data sets in the time required for a single data collection run by SPC.

Figure 2.12 shows a data set and computer generated fit for the determination of the excited state lifetime of $[\text{Os}(\text{phen})(\text{dmb})(\text{Me}_2\text{-dppz})]^{2+}$ in DCM collected by MPMF. For this data set we measured the phase and modulation at 25 frequencies. As expected for a pure complex in solution, the data are well described by a single exponential function ($\text{CHI}^2 = 4$).

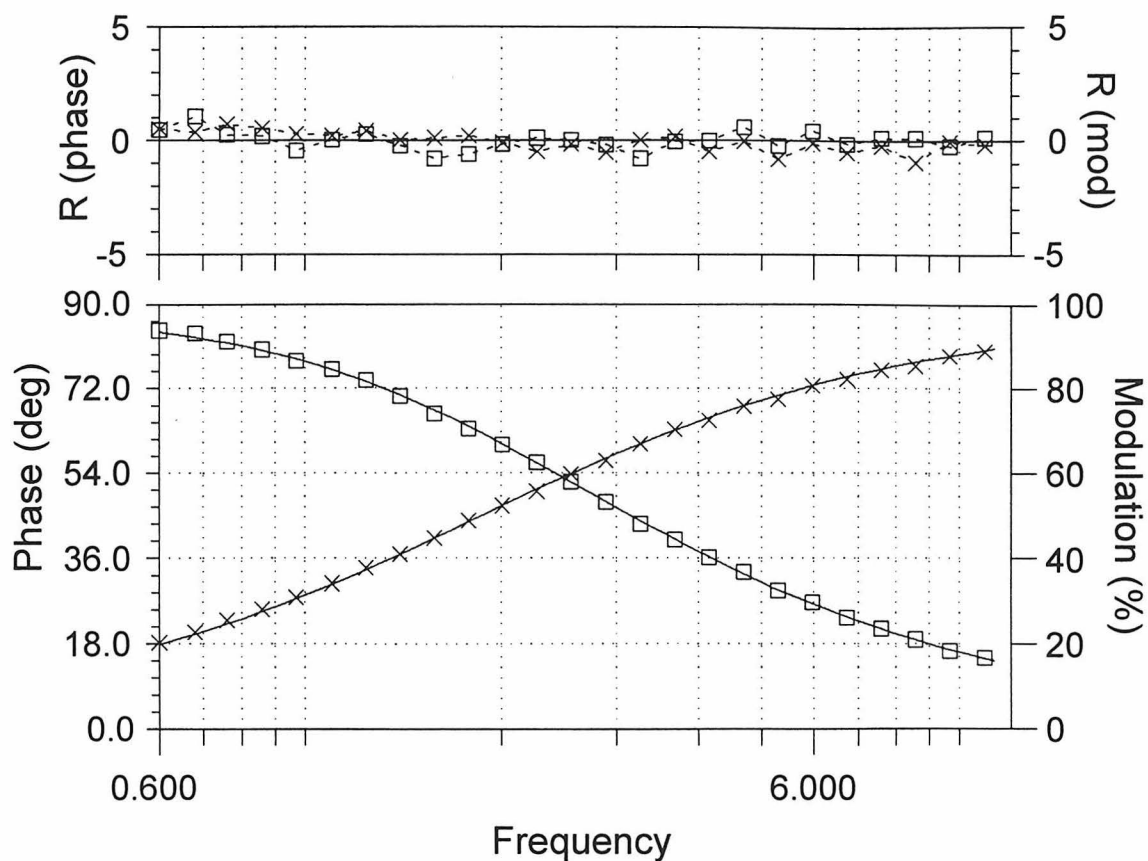


Figure 2.12. Plot of phase (×) and modulation (□) as a function of frequency (MHz) for $[\text{Os}(\text{phen})(\text{dmb})(\text{Me}_2\text{-dppz})]^{2+}$ in dichloromethane. The solid lines correspond to nonlinear least squares analysis of the data according to the expressions below for phase and modulation as a function of frequency. The upper panel shows difference plots for the computer generated fits and the raw data for both phase and modulation. These data are well described by a single exponential decay ($\tau = 84.0$ ns).

The excited state lifetimes for several polypyridyl complexes of Os(II) in DCM solution are listed in Table 2.5. Across this series of complexes, all of the time-resolved emission decays are strictly monoexponential. The observation of single exponential decays for the complexes in a nonaqueous solvent is important since in the presence of DNA, the emission profiles are considerably more complex. Thus, we may conclude that the complexity of the luminescence decays with DNA is not the result of impure samples.

The Φ_{em} values for each complex in Table 2.5 are also listed. Φ_{em} was determined by comparing the steady-state emission yield and the excited state lifetime of each complex to that of $[\text{Os}(\text{bpy})_3]^{2+}$ in acetonitrile and DCM.

Several general observations with respect to Φ_{em} can be made from Table 2.5. First, the emission yield for each complex is within a factor of 1.5 of that for $[\text{Os}(\text{bpy})_3]^{2+}$ in DCM. This observation parallels the fact that the emission yield of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ resembles that of $[\text{Ru}(\text{bpy})_3]^{2+}$. Secondly, it is apparent that the incorporation of methyl groups on the phenazine portion of the dppz derivative leads to an increase in Φ_{em} going from dppz to Me-dppz to Me₂-dppz.

For the series of complexes containing one Me₂-dppz ligand and either two phen derivatives or one phen and a bpy derivative, the emission yields decrease when electron donating substituents are located para relative to the coordinated nitrogen in the ancillary phen (bpy). We attribute this observation to the electron donating substituent pushing the photoexcited electron out onto the phenazine portion of the complex, which results in nonradiative decay.^{9,13} This explanation is consistent with previous studies of dppz complexes of Ru(II) in which the incorporation of multiple dppz ligands through the series of complexes $[\text{Ru}(\text{phen})_{3-n}(\text{dppz})_n]^{2+}$ caused the Ru(II) emission yield to decrease for $n = 0-3$.³³ The addition of a dppz ligand introduced additional nonradiative decay pathways, presumably by forming the phenazine anion. The complex that is outside this trend is $[\text{Os}(\text{phen})(\text{dmb})(\text{Me}_2\text{-dppz})]^{2+}$, which has the highest Φ_{em} overall.

Table 2.5. Monoexponential Luminescence Lifetimes and Quantum Yields for Emission for Osmium Complexes in Dichloromethane.

Complex ^a	τ (ns)	$\Phi_{em}^b \times 10^3$
[Os(phen) ₂ (dppz)] ²⁺	50.4	4.2
[Os(phen) ₂ (Me-dppz)] ²⁺	62.0	5.2
[Os(phen) ₂ (Me ₂ -dppz)] ²⁺	73.8	6.2
[Os(4,7-dmp) ₂ (Me ₂ -dppz)] ²⁺	43.0	3.6
[Os(5,6-dmp) ₂ (Me ₂ -dppz)] ²⁺	63.2	5.3
[Os(dmob) ₂ (Me ₂ -dppz)] ²⁺	13.4	1.1
[Os(phen)(dmb)(Me ₂ -dppz)] ²⁺	85.2	7.1
[Os(bpy) ₃] ²⁺	69.0	5.8
[Os(phen) ₃] ²⁺	143.4	13.3

a. See Table 2.1 for explanation of ligand abbreviations.

b. Quantum yields for emission (Φ_{em}) determined by comparison to Os(bpy)₃²⁺ in acetonitrile.³⁰

Steady-State Emission Yield in the Presence of DNA. Table 2.6 shows the steady state emission yield for the family of osmium based intercalators bound to DNA polymers of various sequence. We also normalized the absolute emission yields against the appropriate Φ_{em} measured in DCM to account for the inherent emission yield of the complex. By comparing directly the values of this corrected emission yield ($\Phi^{CORR(DCM)}$) each complex, the effect of the DNA binding environment on the emission yield can be determined. Therefore, the striking differences in emission yield across this series of compounds must result from differences in their interaction with DNA.

For all of the osmium complexes prepared in this study, no luminescence was

detected with irradiation of the complex free in aqueous solution. Upon addition of double helical DNA to a buffered aqueous solution of osmium complex, significant emission was detected over the wavelength range of ~640 nm – 840 nm. Occasionally, a preparation of metal complex would display luminescence in buffer free of DNA, however, after rigorously excluding any impurities by HPLC, this background emission was no longer present. The low energy emission profile, combined with the remarkable sensitivity to the presence of DNA exhibited by the $[\text{Os}(\text{phen})_2\text{dppz}]^{2+}$ derivatives in aqueous solution makes this class of compounds a unique family of red-emitting DNA probes.

Structural Modification of the Intercalating Ligand. We first examined the effect of incorporating methyl groups at the 6 (Me-dppz) or the 7,8 positions ($\text{Me}_2\text{-dppz}$) of the intercalating, dppz ligand. Figure 2.13 A shows the emission spectra as a function of intercalating ligand for the osmium complexes bound to CT DNA. For this series of complexes the greatest emission yield is observed for $[\text{Os}(\text{phen})_2(\text{Me-dppz})]^{2+}$, followed by $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ and then $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$.

The sensitivity of the emission yield in these complexes to environment arises from the fact that proton transfer or hydrogen bond donation from the solvent to the phenazine nitrogens quenches the emission. If one considers the methyl substituent as an electron donor that might drive an excited state electron off of the phenazine portion of the complex and back into the emissive, tris-phen manifold, one would predict that the complex containing $\text{Me}_2\text{-dppz}$ would have the highest emission yield. However, the Me-dppz ligand produces the brightest emission.³⁴ Thus we can attribute a higher emission yield for an osmium intercalator bound to DNA to an increase in protection of these phenazine nitrogens from quenching by solvent that arises from differential interactions with the DNA duplex.

Effects of Incorporating Substituents on the Ancillary Ligands. Table 2.6 also lists the Φ_{em} and corrected emission yields for several complexes (in the presence of

DNA) that display structural modifications in their ancillary, nonintercalating ligands. We made simple modifications to the ancillary ligands of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ to determine whether these substituents might influence the intercalation of the complexes and affect their emission yield bound to DNA. The $\text{Me}_2\text{-dppz}$ ligand was chosen as the intercalating ligand owing to its higher emission yield compared to the parent dppz ligand, and the fact that its C_2 symmetry would reduce isomer considerations that could be present with Me-dppz .

The emission spectra for the series $[\text{M}(\text{L}^1)_2(\text{Me}_2\text{-dppz})]^{2+}$ complexes bound to CT DNA are shown in Figure 2.13 B. Within this series of complexes, we examined (i) the effects of methyl groups at two positions around the perimeter of the phen backbone; (ii) the importance strongly electron donating substituents such as methoxy and amino groups; and (iii) the role of a carboxylate or methyl ester in the ligand sphere. For $[\text{Os}(\text{L}^1)_2(\text{Me}_2\text{-dppz})]^{2+}$ complexes in which $\text{L}^1 = 5,6\text{-dmp}$ or $4,7\text{-dmp}$, the emission yield is reduced compared to the parent $\text{L}^1 = \text{phen}$ complex. This observation has been made previously for $[\text{Ru}(4,7\text{-dmp})_2(\text{dppz})]^{2+}$,³⁵ which has a reduced quantum yield for emission in the presence of DNA compared to that of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$.

The difference in corrected emission yield for $\text{L}^1 = 5,6\text{-dmp}$ and $\text{L}^1 = \text{phen}$ in the osmium series is significant but small. When $\text{L}^1 = 4,7\text{-dmp}$, the emission yield is reduced dramatically. Such an observation is likely the result of two forces working in tandem. First, the methyl groups at the 4-position prevent intercalation as deeply compared to the phen and 5,6-dmp complexes. Second, the 4,7-complex is more sensitive to quenching by water owing to an increase in the pK_a (the rate of quenching by water in acetonitrile is greater for 4,7-dmp complexes than 5,6-dmp complexes) at the phenazine nitrogens brought on by the electron donating substituents on the ancillary ligand.

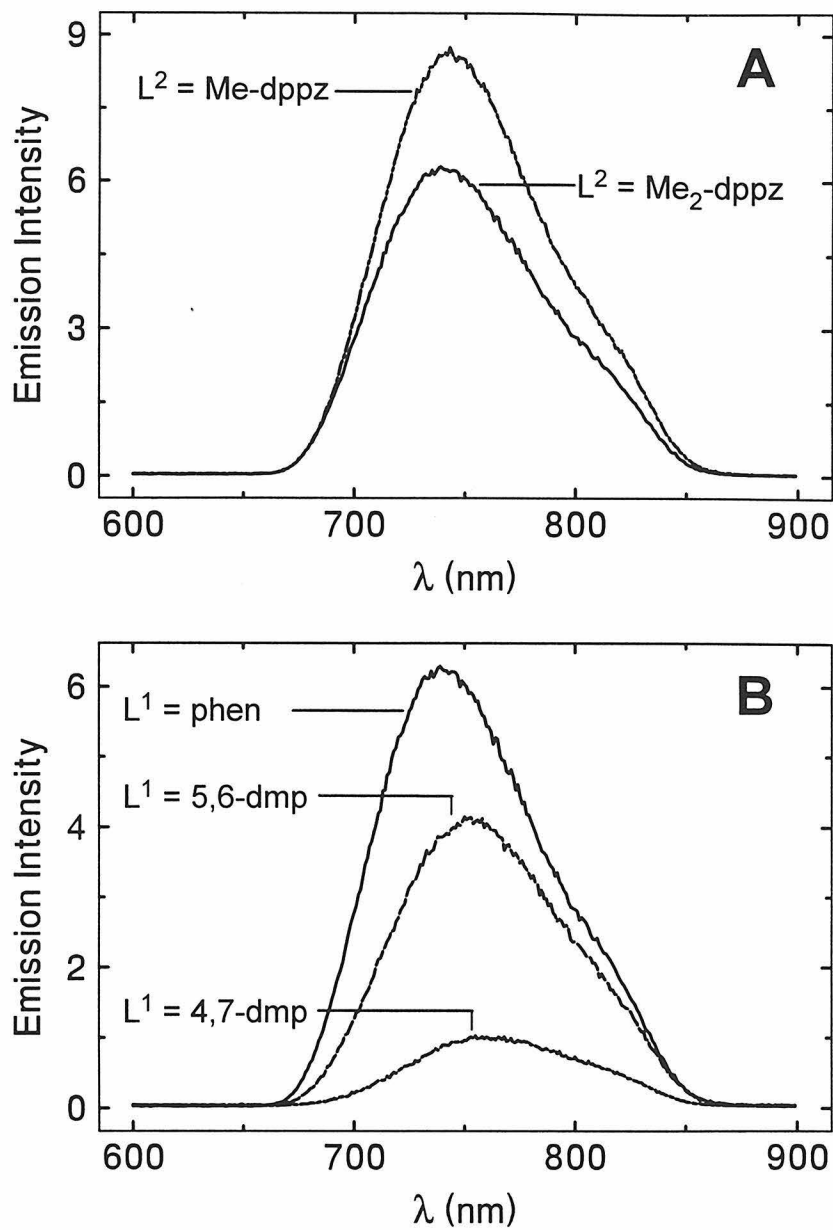


Figure 2.13. Steady state emission spectra for $[\text{Os}(\text{phen})_2(\text{L}^2)]^{2+}$ (**A**) and $[\text{Os}(\text{L}^1)_2(\text{Me}_2\text{-dppz})]^{2+}$ (**B**) complexes bound to CT DNA. Experimental conditions: 10 μM Os, 1 mM nuc, 5 mM Tris, 50 mM NaCl, pH 8.5; $\lambda_{\text{exc}} = 530$ nm.

Table 2.6. Emission intensities of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ Derivatives Bound to DNA Polymers of Varying Sequence.

Complex ^a	DNA Polymer					
	CT (mixed sequence) ^b		poly d(AT) ^b		poly d(GC) ^b	
	$\Phi_{\text{em}} (\times 10^4)^c$	$\Phi^{\text{CORR(DCM)}}d$	$\Phi_{\text{em}} (\times 10^4)^c$	$\Phi^{\text{CORR(DCM)}}d$	$\Phi_{\text{em}} (\times 10^4)^c$	$\Phi^{\text{CORR(DCM)}}d$
$[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$	1.00	1.00	0.34	0.34	1.32	1.32
$[\text{Os}(\text{phen})_2(\text{Me-dppz})]^{2+}$	8.75	7.06	5.79	4.76	13.38	10.80
$[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$	6.29	4.26	2.86	1.94	6.54	4.43
$[\text{Os}(5,6\text{-dmp})_2(\text{Me}_2\text{-dppz})]^{2+}$	4.15	3.29	3.25	2.58	2.82	2.23
$[\text{Os}(4,7\text{-dmp})_2(\text{Me}_2\text{-dppz})]^{2+}$	1.03	1.20	0.84	0.98	1.39	1.62
$[\text{Os}(\text{dmob})_2(\text{Me}_2\text{-dppz})]^{2+}$	0.15	-----	0.12	-----	0.16	-----
$[\text{Os}(\text{phen})(\text{b}'\text{-OMe})(\text{Me}_2\text{-dppz})]^{2+}$	2.39	1.41	1.34	0.80	2.19	1.29
$[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{1+}$	2.55	1.51	1.55	0.92	3.10	1.84

a. See Table 2.1 for explanation of ligand abbreviations.

b. Experimental conditions: $[\text{Os}] = 10 \mu\text{M}$, $[\text{DNA}] = 1 \text{ mM}$ nucleotides ($\text{Os}/\text{nuc} = 1/1000$) in buffer (5 mM Tris, 50 mM NaCl, pH = 8.5).

c. Quantum yield for emission (Φ_{em}) bound to DNA determined by comparison to $[\text{Ru}(\text{bpy})_3]^{2+}$ as described previously.³⁰

d. Corrected emission yield ($\Phi^{\text{CORR(DCM)}}$) = Φ_{em} bound to DNA divided by Φ_{em} in dichloromethane for each complex. The $\Phi^{\text{CORR(DCM)}}$ value allows the emission yields of two complexes to be compared without consideration of the inherent emission yield of the complex.

For ease of comparison, these data were normalized to the $\Phi^{\text{CORR(DCM)}}$ value for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$.

concert: one electronic and the other structural. The presence of the methyl groups in the 4,7 positions leads to a stabilization in the Os(III) oxidation state (*vide supra*), which translates into a more favorable reduction of the phenazine portion of the complex in the excited state. From the structural perspective, substituents at the 4,7 positions may interact disfavorably with the major groove and introduce steric clashes that prevent effective intercalation. Each of these considerations would increase the sensitivity of photoexcited $[\text{Os}(4,7\text{-dmp})_2(\text{Me}_2\text{-dppz})]^{2+}$ to quenching by water and cause a diminution in emission intensity.

In order to examine further the importance of electron donating substituents on the ancillary ligands, we prepared $[\text{Os}(\text{dmob})_2(\text{Me}_2\text{-dppz})]^{2+}$ and $[\text{Os}(\text{phen})(\text{dab})(\text{Me}_2\text{-dppz})]^{2+}$.

We detected no emission for $[\text{Os}(\text{phen})(\text{dab})(\text{Me}_2\text{-dppz})]^{2+}$ bound to DNA (Table 2.6). For $[\text{Os}(\text{dmob})_2(\text{Me}_2\text{-dppz})]^{2+}$, the emission yield is significantly reduced. It is well known that bpy derivatives in the ligand sphere lead to a reduction in emission yield owing to an increase in vibrational deactivation brought about by the flexibility of bpy.²⁸ For this complex, however, the dramatic loss in emission intensity is almost certainly the result of the electron donating substituents influencing the sensitivity of the complex to quenching by water. Of course the methoxy substituents are also in a position to clash sterically with the DNA groove as in the case of 4,7-dmp, however, the loss in intensity for $L^1 = \text{dmob}$ is far more dramatic than that with 4,7-dmp. These results illustrate the powerful sensitivity of luminescent DNA probes based on intercalation of a dppz ligand.

The final issue that we addressed in the investigation of substituents on the ancillary ligands of osmium intercalators was to characterize the emission of $[\text{Os}(\text{phen})(\text{b}'\text{-OMe})(\text{Me}_2\text{-dppz})]^{2+}$ and $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{1+}$. The difference between these complexes lies in the carboxyl substituent on the bpy derivative. For the $\text{b}'\text{-OMe}$, the carboxyl is esterified, while a carboxylate is present with bpy' . The carboxylate is deprotonated at pH 8.5 where we conduct our experiments, which results

in a net reduction in the overall charge of the metal complex. The importance of this study lies in using the difference in charge between the two intercalators to evaluate the relative importance of charge and π -stacking in determining how well these complexes intercalate into DNA.

As shown in Table 2.6 the emission yield for the bpy' and the b'-OMe complexes are not significantly different regardless of DNA sequence. In fact, the emission yield for the bpy' complex is actually somewhat higher. These data suggest that complexes in this class of intercalators derive their binding affinity for DNA primarily from interactions directly with the DNA base stack through intercalation.

Sequence Dependence of Emission Yield. We measured the emission intensity of the complexes listed in Table 2.6 in the presence of three different DNA polymers: calf thymus (CT) DNA; poly d(AT); and poly d(GC). CT DNA is a mixed sequence, natural polymer available commercially with an average strand length of ~2000 base pairs (bp). Poly d(AT) and poly d(GC) are synthetic DNA polymers that consist of alternating AT or GC base steps. The synthetic polymers are commercially available with an average strand length of ~1000 bp. With the exception of $[\text{Os}(5,6\text{-dmp})_2(\text{Me}_2\text{-dppz})]^{2+}$, the sequence dependence of emission intensity for all of the complexes we studied followed the trend: $\Phi_{\text{em}} \text{ poly d(GC)} > \Phi_{\text{em}} \text{ CT} > \Phi_{\text{em}} \text{ poly d(AT)}$. In the case of $[\text{Os}(5,6\text{-dmp})_2(\text{Me}_2\text{-dppz})]^{2+}$, we found the emission yield with poly d(GC) to be similar to that with poly d(AT) while the emission intensity with CT DNA was the highest.

In previous studies of the interaction of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ with polynucleotides, we showed that this complex binds preferentially to AT sites in CT DNA (see Chapter 2 Appendix).¹⁸ In Table 2.6, the similarity between the emission intensities for the series of osmium complexes bound to CT and poly d(AT) suggests that the osmium intercalators are preferentially bound to AT sites within CT. This observation illustrates a general feature in the interaction of dppz complexes of transition metals with DNA polymers.

Picosecond Transient Absorption of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in the Presence and Absence of DNA. No emission has been detected for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in aqueous solution. We used transient absorption spectroscopy on the picosecond time scale to characterize the excited state lifetime of this complex in buffer without DNA. Transient absorption measures the differential absorption for a species before and after a laser pulse. In the case of polypyridyl complexes of Os(II) the excited state is formed within the laser pulse, even for a femtosecond flash.

Owing to the MLCT character of the excited state of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$, we expected to see a negative signal that approaches zero over the course of ground state recovery. Figure 2.14 illustrates the transient signals observed for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in the presence and absence of DNA on the 100 ps (**A**) and 1 ns (**B**) time scales. As expected the laser pulse produces a bleach, which recovers to baseline over time. In the absence of DNA, the disappearance of the bleach signal is fast and can be described by two exponential components with lifetimes of 8 ps (50%) and 28 ps (50%). The presence of two components likely reflects the population of more than one MLCT level. In the presence of DNA however, the lifetime of the complex is significantly enhanced. This observation is consistent with the “molecular light switch” behavior of the steady state luminescence. Given that in the presence of DNA (*vide infra*), the lifetime of the photoexcited complex can be in the range of 10 ns, the presence of DNA produces an enhancement of more 10^3 in excited state lifetime.

Time-Resolved Luminescence of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ Derivatives Bound to DNA. In order to gain insight into the origins of the variations in emission yield observed for the family of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ derivatives, we measured the excited state lifetimes for the complexes bound to DNA. We employed both SPC and MPMF to characterize the time resolved emission decay and found these techniques to give identical results.

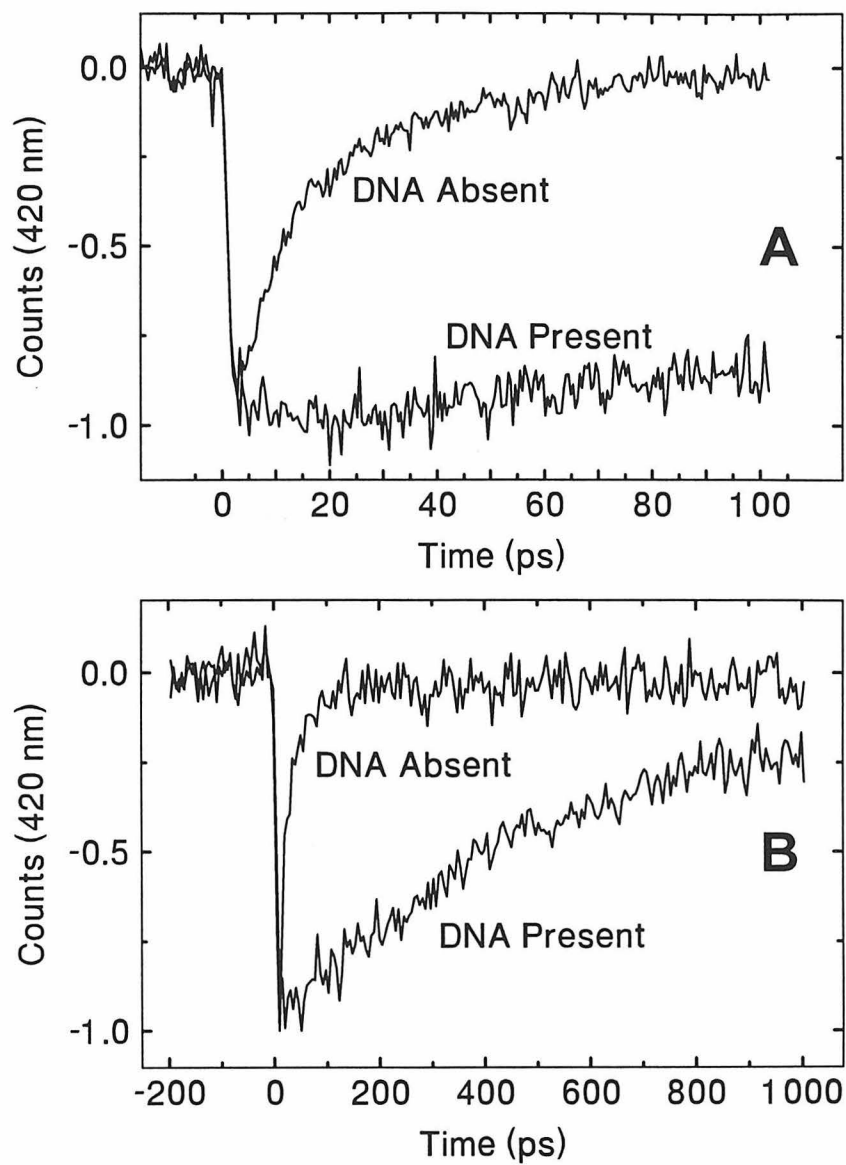


Figure 2.14. Transient absorption signals on the 100 ps (**A**) and 1 ns (**B**) time scales for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in the presence and absence of DNA in aqueous buffer ($\lambda_{\text{exc}} = 390$ nm, $\lambda_{\text{obs}} = 420$ nm). Data are normalized.

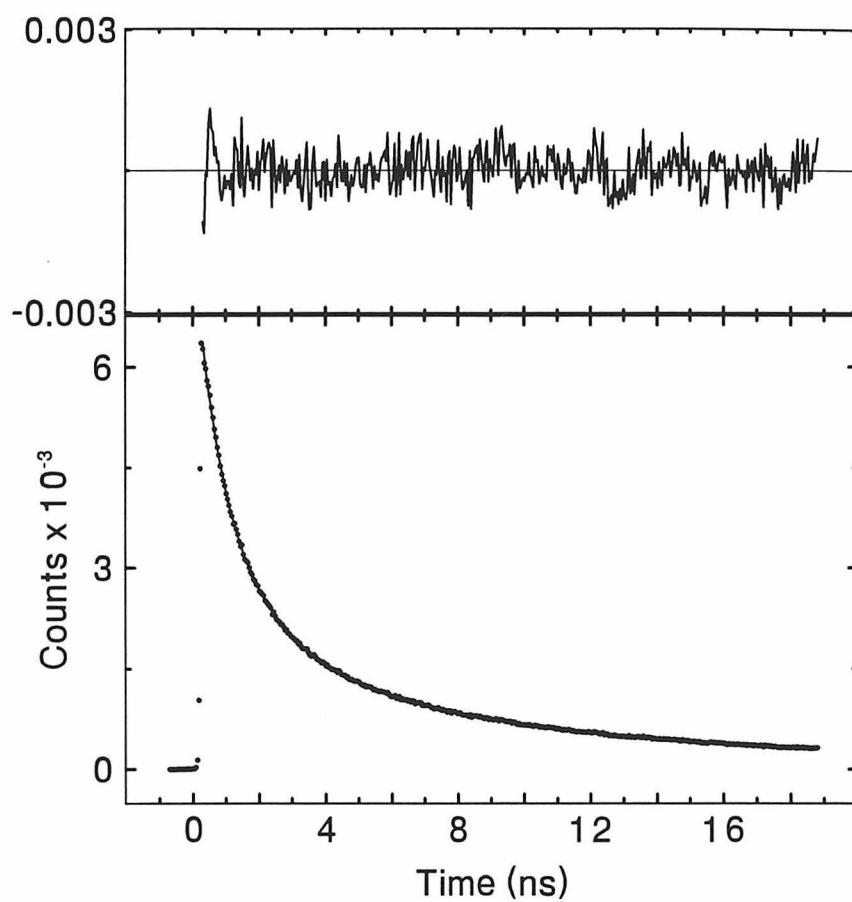


Figure 2.15. Time-resolved emission decay for $rac\text{-[Os(phen)}_2\text{(dppz)]}^{2+}$ bound to CT DNA. A computer generated fit of the triexponential decay overlays the data, and the weighted residuals for the fit appear in the upper panel of the figure.

The time resolved emission decay for *rac*-[Os(phen)₂(dppz)]²⁺ bound to CT DNA measured by SPC is shown in Figure 2.15.

Table 2.7 lists the excited state lifetimes for the series of osmium intercalators bound to double helical DNA as a function of nucleic acid sequence. In sharp contrast to the luminescence in DCM, all of the emission decay profiles for the complexes bound to DNA are multiexponential. As described previously for [Ru(phen)₂(dppz)]²⁺,^{5,6,16} the multiple components in the luminescence decay of the osmium intercalators bound to DNA are manifestations of more than one geometry of intercalation with respect to the base-pair axis of the binding site.

In general the average excited state lifetime is markedly reduced for each complex in the microheterogeneous environment of DNA in aqueous solution. For some complexes the average excited state lifetimes track with the lifetimes in DCM. For example, [Os(4,7-dmp)₂(Me₂-dppz)]²⁺ has the shortest lifetimes in the presence of DNA, and the profiles of [Os(5,6-dmp)₂(Me₂-dppz)]²⁺ [Os(phen)₂(Me₂-dppz)]²⁺ are very similar in the presence of CT and poly d(AT).

Several noteworthy deviations from the trends established in DCM are present as well. First, in DCM the lifetime of photoexcited (*) [Os(phen)₂(Me₂-dppz)]²⁺ is longest for the series of [Os(phen)₂(L₂)]²⁺ complexes. Bound to DNA, however, the lifetime of *[Os(phen)₂(Me-dppz)]²⁺ is significantly longer than that of any other complex. Another remarkable contrast is apparent for [Os(phen)(dmb)(Me₂-dppz)]²⁺ in DCM and [Os(phen)(b'-OMe)(Me₂-dppz)]²⁺ or [Os(phen)(bpy')(Me₂-dppz)]²⁺, where bound to

Table 2.7. Effects of Varying the Intercalating Ligand of Os(phen)₂L²⁺ Complexes on the Luminescence Lifetimes Bound to Natural and Synthetic DNA Polymers.

Complex ^a	DNA Polymer					
	CT (mixed sequence) ^b			poly d(AT) ^b		
	τ1 (%)	τ2 (%)	τ3 (%)	τ1 (%)	τ2 (%)	τ3 (%)
[Os(phen) ₂ (dppz)] ²⁺	14.9 (10.0)	2.9 (28.9)	0.8 (61.2)	5.0 (54.1)	1.1 (45.9)	1.1 (45.9)
[Os(phen) ₂ (Me-dppz)] ²⁺	49.8 (12.9)	19.9 (46.6)	4.1 (40.5)	28.7 (34.7)	9.9 (29.0)	3.8 (36.3)
[Os(phen) ₂ (Me ₂ -dppz)] ²⁺	34.8 (10.4)	11.1 (32.3)	2.9 (57.3)	23.6 (10.0)	4.6 (57.4)	0.8 (32.7)
[Os(4,7-dmp) ₂ (Me ₂ -dppz)] ²⁺	6.4 (31.4)	2.0 (68.6)	-----	3.9 (59.3)	1.6 (40.7)	-----
[Os(5,6-dmp) ₂ (Me ₂ -dppz)] ²⁺	30.1 (7.3)	10.0 (36.9)	2.3 (55.7)	25.2 (7.3)	10.7 (31.4)	2.9 (61.3)
[Os(phen)(b'-OMe)(Me ₂ -dppz)] ²⁺	23.4 (6.5)	6.9 (37.0)	1.7 (56.5)	8.3 (23.9)	2.2 (76.1)	-----
[Os(phen)(bpy')(Me ₂ -dppz)] ²⁺	15.1 (13.7)	4.3 (51.2)	1.4 (35.1)	6.2 (39.3)	2.1 (60.7)	-----
				11.1 (31.0)	2.3 (69.0)	

a. See Table 2.1 for explanation of ligand abbreviations.

b. Experimental conditions: [Os] = 30 μM; [DNA] = 3 mM nucleotides (Os/nuc = 1/1000) in buffer (5 mM Tris, 50 mM NaCl, pH = 8.5). For data collected by multifrequency phase-modulation fluorometry (MPMF), λ_{exc} = 480 nm, observation > 650 nm; data collected by single photon counting (SPC) λ_{exc} = 670 nm, λ_{obs} = 740 nm. Data fitting achieved by nonlinear least squares method using commercial software (ISS (MPMF); AXUM (SPC)).

DNA the complexes containing a bpy' derivative have significantly shorter lifetimes than the parent dmb complex in DCM. This observation is consistent with the alkyl chain on the bpy' ligand causing a steric clash with the DNA groove, and preventing deep intercalation of Me₂-dppz.

Time-resolved emission spectroscopy is useful to identify subtle differences in the way luminescent compounds associate with DNA. An example of this application lies in the comparison of the emission of [Os(phen)(b'-OMe)(Me₂-dppz)]²⁺ and [Os(phen)(bpy')(Me₂-dppz)]²⁺ bound to CT DNA. For these two complexes the steady state emission yields are almost identical, however, the time-resolved profiles reveal important differences. Bound to CT DNA the lifetimes of the bpy' containing complex are significantly shorter than those of the b'-OMe. Such an observation is likely derived from the negative charge on the bpy' preventing the complex from intercalating as deeply as the complex with the methyl ester. The fact that the steady-state yields are similar results from a change in the distribution of lifetimes in the multiexponential decay to favor of the long lifetime.

Effect of DNA Sequence on Luminescence Lifetime. As indicated in Table 2.7, the luminescence lifetimes for the osmium intercalators bound to DNA depend significantly on the sequence of the polymer. In general, the distribution and magnitude of the lifetimes for the complexes bound to CT DNA resemble very closely those in the presence of poly d(AT). The longest lifetimes are observed with CT DNA. Bound to poly d(GC), the excited state decays can all be described as biexponential, with the majority of the luminescence coming from the population with the shortest lifetime. When the complexes are bound to poly d(GC), the average excited state lifetimes are shortest when compared with data for the other two sequences, despite greater yields for emission. This disparity arises from a greater average excited state lifetime.

Previous studies of [Ru(phen)₂(dppz)]²⁺ luminescence as a function of DNA sequence revealed that this complex binds preferentially to AT sites.¹⁸ Therefore, the

similarities in the data with CT and poly d(AT) for the osmium intercalators indicates that these complexes prefer AT sites as well.

Luminescence Lifetimes of Δ and Λ -[Os(phen)₂(dppz)]²⁺ Bound to CT DNA.

We measured the luminescence lifetimes of the pure enantiomers of [Os(phen)₂(dppz)]²⁺ bound to DNA in order to investigate the role of absolute configuration about the metal center in determining the luminescence lifetimes of the complexes bound to DNA. Owing to the right-handed chirality of the DNA duplex, we expected the interaction of the right-handed Δ -isomer to be favored.³⁶ Indeed, a large body of evidence illustrates that the of the interaction of metalointercalators with DNA is favored for the Δ -isomer on the basis of symmetry matching and shape selection.^{1,2}

For both enantiomers of this complex the luminescence decay can be described by a biexponential function. For Δ -[Os(phen)₂(dppz)]²⁺ the decay contains 50 % of a 13 ns component and 50 % of a 2.0 ns component. For the Λ isomer, the lifetimes are significantly shorter. The decay profile for this complex consists of a 70 % contribution by a 750 ps component and a 30 % contribution by a 1 ns lifetime. The excited state lifetimes and hence the quantum yield for emission are much greater for the Δ isomer of this complex compared with those for the Λ isomer. Importantly, when taken as a linear combination the time-resolved emission profiles for the pure enantiomers of [Os(phen)₂(dppz)]²⁺ are consistent with the data recorded for the racemic mixture.

Low Temperature Luminescence Studies. In order to determine whether [M(phen)₂(dppz)]²⁺ complexes might be useful in studying reactions with DNA at low temperature, we measured the luminescence lifetimes of [Os(phen)₂(dppz)]²⁺ in the presence and absence of DNA at 77 K (Figure 2.16). The solvent we used for these studies was a mixture of glycerol and buffer.

At ambient temperature the osmium emission

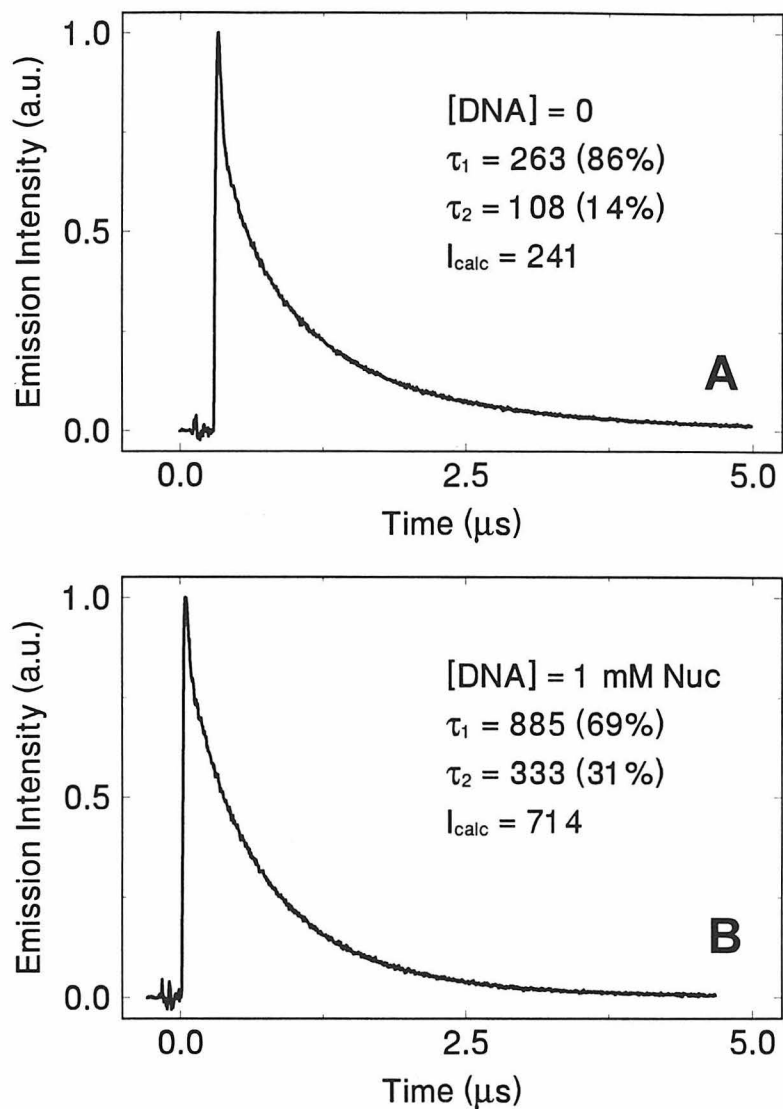


Figure 2.16. Time resolved luminescence decay of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ in the absence (A) and presence (B) of DNA at 77 K. Experimental conditions: 15 μM Os, 1.5 mM nuc, 5 mM Tris, 50 mM NaCl, pH 8.5 in 60 % glycerol, 40 % water; $\lambda_{\text{exc}} = 480 \text{ nm}$, $\lambda_{\text{obs}} = 740 \text{ nm}$. Samples were contained in NMR tubes and frozen slowly in liquid N_2 . The samples did not always form uniform glasses, instead the solid was a heterogeneous mixture of frozen sections.

is quenched in the cryosolvent. At 77 K, however, the complex emits strongly even in the absence of DNA (Figure 2.16). This observation suggests that quenching of the excited state by the interactions of protons with the phenazine nitrogens is inhibited at low temperature. In fact, this observation is consistent with the quenching of the emission depending upon discrete electron transfer onto the dppz ligand, since such an electron transfer would be inhibited significantly at 77 K, whereas hydrogen bonding might not. It is noteworthy that in the absence of DNA, the excited state decays as a biexponential. This observation resembles the data at ambient temperature in aqueous solution.

In the presence of DNA at 77 K, the emission of the osmium decays also as a biexponential, however, the lifetimes are enhanced relative to those in the absence of DNA. The observation of enhanced lifetime and in the presence of DNA suggests that the complex remains bound to DNA even at 77 K in the cryosolvent.

2.3. DISCUSSION

2.3.1. Synthetic Methods

The preparation of mixed ligand complexes of Os(II) and Ru(II) has been the subject of considerable interest for many years, dating back to the work of Dwyer and coworkers.^{23-25,37} We employed these strategies to prepare complexes of the $[\text{Os}(\text{L}^1)_2(\text{L}^2)]^{2+}$ type. Until recently, no methods for the preparation of polypyridyl complexes of osmium bearing three different bidentate ligands had been described. We developed the simple strategy outlined in Scheme 2.2 as a general approach to obtain complexes of the $[\text{Os}(\text{L}^1)(\text{L}^2)(\text{L}^3)]^{2+}$ type. With this method, we are able to introduce one bidentate, polypyridyl ligand onto the osmium center, selectively without over reaction.

We found that the key to this approach is the incorporation of the second ligand onto $[\text{Os}(\text{phen})\text{Cl}_4]$ in DMF. In solvents such as ethylene glycol (EG), heating the

mixture results in the incorporation of two ligands onto the metal center. We suspect that the selectivity in this reaction arises from the role that the solvent plays in reducing the osmium center, which affects the lability of the chloride ligands. The lower oxidation state of the metal center is more susceptible to loss of chloride, and therefore more reactive. Both DMF and EG (at reflux) will reduce Os(IV) to Os(III) and Os(III) to Os(II), however, DMF must be less reactive than EG in this regard and prevent the accumulation of Os(II) while there is an excess of bidentate ligand in solution. This methodology is convenient and general. We have employed it to prepare a variety of complexes with many functionalities present on the coordinating phen, bpy, or dppz derivatives.

2.3.2. Electronic Structure of Ground-State $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ Derivatives

The assembly of several dppz complexes of Os(II) and the characterization of their electrochemical properties has allowed us to develop a picture of the electronic structure of these complexes. We find that substituents on the phen or bpy ligands of the complex affect the reduction potential of the metal center, but not the phenazine portion of the dppz ligand. By contrast, substituents on the 6 or 7,8 positions of the dppz ligand perturb the reduction potential of phenazine portion of the ligand but not the metal center. This orbital arrangement represents is unique since one has independent, modular control in tuning the energetics of two different states simultaneously

2.3.3. Excited State Properties in the Absence of DNA

Role of Ligand Architecture in Determining Φ_{em} in Dichloromethane. Our goal in this study is to use the emission properties dppz complexes of Os(II) in the presence of DNA to determine how substituents in the ligand sphere affect DNA binding properties. We need to understand the inherent photophysical characteristics of these complexes before we can interpret their use as luminescent probes for DNA.

In dppz complexes of Ru(II) and Os(II) the MLCT excited state is quenched in aqueous solution. The presence of the dppz ligand introduces a significant perturbation in the excited state properties of complexes with this ligand.^{9,12,13} Owing to the low reduction potential of the phenazine portion of dppz relative to phen or bpy, it is possible for an electron in the MLCT excited state to be localized on this portion of the ligand, which quenches the emission yield. Increasing the number of dppz ligands in $[\text{Ru}(\text{phen})_n(\text{dppz})_{3-n}]^{2+}$ complexes resulted in a decrease in Φ_{em} in acetonitrile.³³ For complexes of Re(I), the similarity in the absolute energy of the MLCT transition and the π - π^* transition centered on the dppz ligand results in quenching of the MLCT state by intersystem crossing to the π - π^* state.^{7,8,38}

In a model proposed by Olson *et al.*, the quenching by water (and other proton donors) of emission from photoexcited $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ is the result of IMCT to an excited state on the dppz ligand that is deactivated on the picosecond time scale.¹³ This process is inhibited in nonaqueous solvents and DNA, since the energy of the deactivating state is higher than the energy of the emissive state under these conditions, which prevents IMCT.

The dependence of the emission yield on ligand architecture for the $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ derivatives in DCM is clearly illustrated in Table 2.5. The presence of electron donating substituents on the ancillary phen or bpy ligand results in a decrease in the emission yield, while electron donating substituents on the dppz derivative causes Φ_{em} to increase. This trend is consistent with a model that involves IMCT to a deactivating state. When a substituent drives electron density into the metal center, crossover to the phenazine portion of the dppz ligand, which results in nonradiative deactivation of the excited state, is favored. Alternatively, when the reduction potential of the phenazine portion of the molecule is disfavored by an electron donating substituent, this pathway of nonradiative decay is inhibited.

Therefore, the *bis*-phen and *bis*-5,6-dmp complexes display similar Φ_{em} values,

while the *bis*-4,7-dmp complex has a shorter excited state lifetime in DCM. The most electron donating ligand is the dmob ligand, and complexes with this ligand have the lowest emission yield overall.

For the series of dppz derivatives with two phen ligands, the lowest Φ_{em} in DCM is observed for dppz, followed by Me-dppz, and in DCM complexes with the Me₂-dppz ligand have the highest Φ_{em} . This trend is consistent with the hypothesis that the transfer of electron density from the excited state to the dppz ligand quenches emission, since the electron donating methyl groups reduce the electron accepting character of the phenazine moiety.

Lastly, the differences in Φ_{em} observed for the series of complexes are inconsistent with the energy gap law,³⁰ since the most significant perturbations arise from substitution of the dppz ligand. As mentioned above, these substituents do not affect the reduction potential of the metal center, and therefore the energy gap of the MLCT does not change. Hence, orbitals on the dppz ligand must be involved in the excited state structure of these complexes.

2.3.4. DNA Binding Properties of [Os(phen)₂(dppz)]²⁺ Derivatives.

Correlations between Absorption and Emission Data and the Effect of Substituents on DNA Binding. The absorption and emission data for the series of dppz complexes of Os(II) are useful to understand how DNA binding is affected by incorporating substituents into the ligand sphere of these complexes.

Structural Modification of the Intercalating Ligand. We considered first the importance of substituents on the intercalating, dppz derivative. Owing to its flat, expansive aromatic surface, the dppz ligand is well suited for intercalation in between adjacent DNA base pairs. Studies of DNA binding by UV-vis spectroscopy indicate that the association of dppz complexes of Os(II) proceeds by preferential intercalation of the dppz derivative, which protects the phenazine nitrogens from solvent. Therefore the

complexes emit in the presence of DNA.

According to the inherent excited state properties in DCM (Table 2.5), one would expect $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ to have the brightest emission bound to DNA, however, the complex with Me-dppz has the greatest Φ_{em} overall. This observation indicates that the Me-dppz complex is intercalated with the phenazine nitrogens deeper in the DNA base stack, and more protected from quenching by water. If we look at the time resolved luminescence data (Table 2.7), we see that not only are the individual lifetimes in the triexponential decay longer, but the distribution of the lifetimes shifts from favoring the shorter lifetime in the dppz complex to favoring a significantly longer intermediate (τ_2) lifetime in the Me-dppz complex.

One consideration could be that the excited state pKa of the phenazine nitrogens is responsible for the differences in emission yield. Owing to the electron donating nature of the methyl groups on the phenazine portion of the dppz ligand, the phenazine nitrogens should increase in basicity going from dppz to Me-dppz to $\text{Me}_2\text{-dppz}$. If this hypothesis were correct, dppz would give the brightest emission followed by Me-dppz and $\text{Me}_2\text{-dppz}$, which does not fit the data.

Impact of Functional Groups on the Ancillary Ligands. We suggest that the primary trend with respect to the role of ligand architecture in determining DNA binding lies in the regiochemistry of substitution. Our data show quite convincingly that substituents on the 4 and 7-positions of a phen ligand (4 and 4' positions on bpy) cause a reduction in emission yield, and a diminution of the hypochromicity associated with intercalation. As depicted schematically in Figure 2.17, substituents on the 4 position of an ancillary phen ligand can cause steric clashes with the wall of the major groove into which the complexes are intercalating. Such steric clashes would not be present with substituents on the 5 and 6-positions. Therefore the diminished hypochromicity results from relatively shallow intercalation. The lower emission yield results from less protection of the phenazine nitrogens from solvent incurred by shallow intercalation.

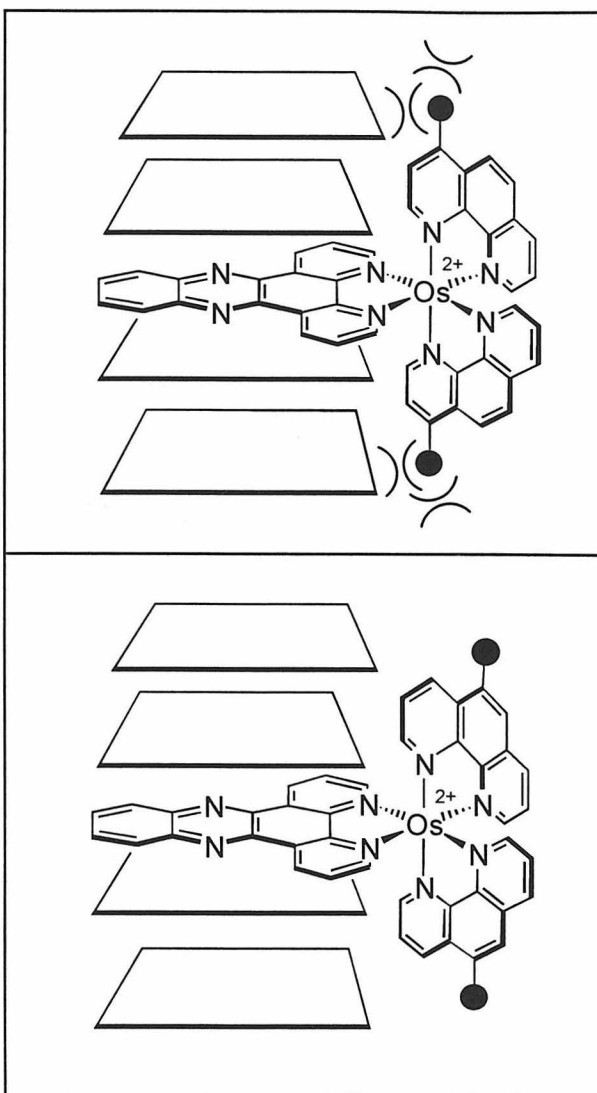


Figure 2.17. Schematic illustration of two metallointercalators bound to DNA. In the upper panel, the substituents on the 4-positions of the ancillary phen ligands clash with the wall of the groove and prevent deep penetration of the dppz ligand into the DNA base stack. In the lower panel, the substituents on the 5-positions are much more innocuous and allow the dppz ligand to intercalate further into the hydrophobic base stack.

Reconciling Emission Data with Models of Intercalation. Previous models of intercalation by dppz complexes assert that each lifetime in the multiexponential decay corresponds to a geometry of intercalation which provides differential exposure of the phenazine nitrogens to water (Figure 2.18).^{5,6,16-18} The two extremes for the different orientations in this model are head-on and side on intercalation. For the head on mode, the phenazine nitrogens are placed deeper in the helix and thus shielded from the solvent. For side-on, the dppz ligand is more exposed to water. In the context of this model, the differences in the luminescence decays for the series of complexes bearing different substituents on the intercalating ligand would indicate that the presence of methyl groups on the 6 or 7,8 positions of dppz forces the complexes to adopt different geometries of intercalation. One could imagine that the fact that the Me-dppz complex displays the greatest Φ_{em} indicates that the methyl substituent at the 6-position forces the complex to adopt an intercalation geometry which buries the phenazine nitrogens in the hydrophobic interior of the base stack.

Given that intercalative binding is driven by the stacking interaction with the bases, the predominant mode of binding in a head-on vs side-on competition would be side on. As illustrated in Figure 2.18, a side-on orientation provides a greater surface area for intercalative stacking; protection of the phenazine nitrogens from solvent does not provide any binding energy. In fact the time-resolved emission data do support the idea of side-on intercalation being preferred. The shorter lifetimes dominate the decay profiles of all the complexes.

Another model proposed to account for the multiexponential decay invokes a distribution effect,³¹ where the presence of more than one excited state lifetime for the intercalated complex arises from the fact that some complexes may bind in the vicinity of others and shield the complexes from solvent. Our data for the entire series of complexes is not consistent with this model since one would not expect to see such dramatic changes in the magnitude and distribution of the lifetimes in the multiexponential decays with

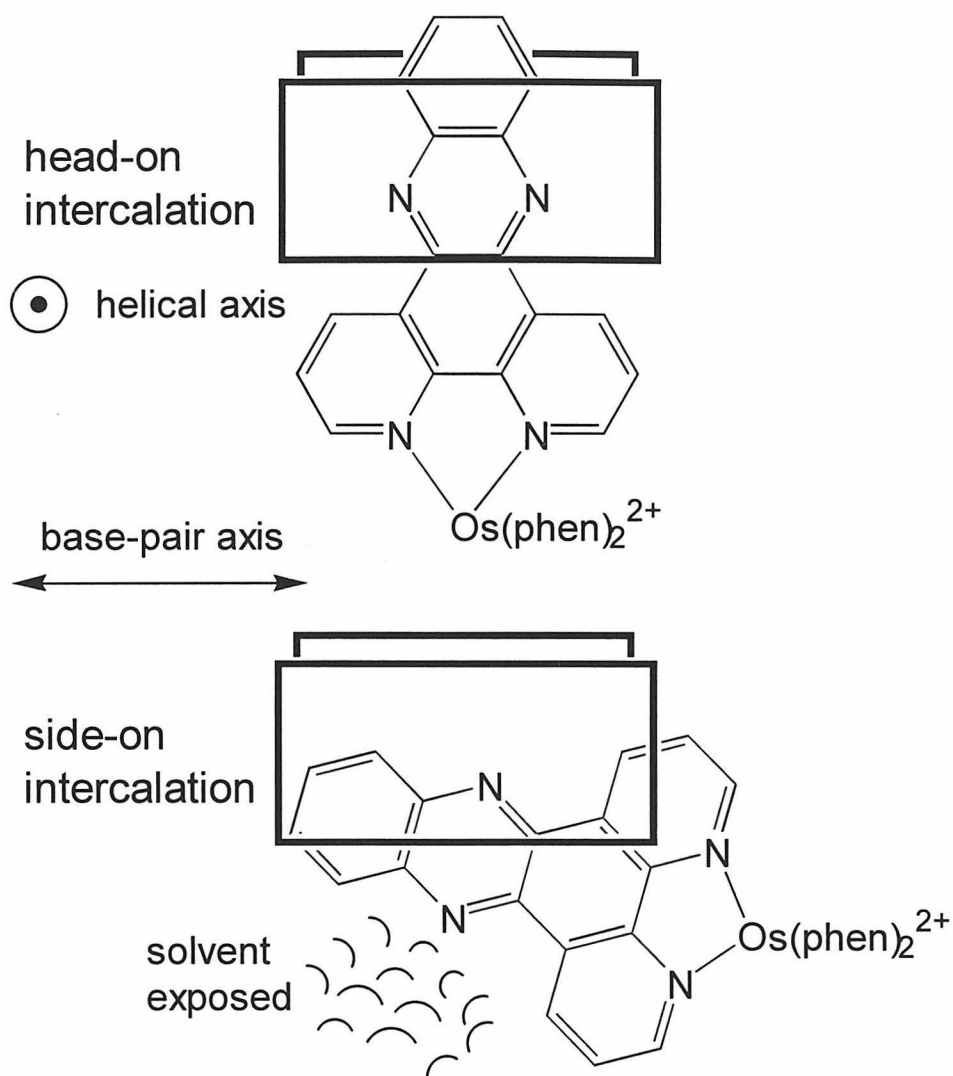


Figure 2.18. Schematic illustration of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ bound to a base pair in the head-on (top) and side-on (bottom) modes of intercalation. Intercalation by the head-on mode results in the phenazine nitrogens being deeper in the base stack, and shielded from solvent. The side-on mode causes the phenazine nitrogens to be more solvent exposed.

such simple changes in ligand architecture. This point is particularly true for the $[\text{Os}(\text{phen})_2(\text{Me}_n\text{-dppz})]^{2+}$ ($n = 0\text{-}3$) series where only the intercalating ligand changes. Therefore, the multiexponential decays observed for the intercalators based on Os(II) and Ru(II) reflects the population of more than one intercalated geometry, which results in differential exposure of the phenazine nitrogens to solvent.

Sequence Dependent Solvent Accessibility of Intercalated Os(II) Complexes.

Since the emission profile of a dppz complex of Os(II) depends so sensitively upon solvent accessibility, we can ask how solvent accessibility varies as a function of DNA sequence. Establishing trends in how sequence predisposes a compound to be solvent exposed upon intercalation may lend insight into the reactivity of molecules with DNA. The solvent accessibility of an agent bound to DNA is an important property of the DNA-drug complex. Species that are tightly bound to DNA and show very little accessibility to solvent are less prone to reactions with exogenous species in solution. Conversely, when the DNA-drug complex is open to solvent, the reactivity of the binding agent with diffusible reactants in solution may be exploited to initiate chemistry on the DNA helix.

The best measure of solvent accessibility for dppz complexes of Os(II) is the excited state lifetime of the metal complex bound to DNA. If we focus on a complex like $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$, we see that the longest lifetimes are observed with CT DNA followed by poly d(AT), while the longest lifetime in the presence of poly d(GC) is significantly reduced compared with the other polymers. So even with a binding mode that favors shielding from solvent, this shielding is sequence dependent.

If we focus on the lifetimes of photoexcited $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ that contribute the most to the overall decay profile, we see that the lifetimes are shortened bound to poly d(AT), followed by CT DNA, and then poly d(GC). In fact for the GC polymer, the decay is biexponential, while with CT and poly d(AT), the excited states decay as triexponentials. This distinction suggests that the sequence affects the orientation of intercalation so significantly that the mode of binding that gives the most

exposure to solvent is not present for poly d(GC). Therefore, despite the fact that poly d(AT) and CT DNA provide orientations for intercalation that maximize protection from solvent, the average complex bound to poly d(GC) is the most protected from water.

2.3.5. Summary and Conclusion

We prepared a variety of polypyridyl metal complexes that contain one dppz derivative and two ancillary ligands derived from phen or bpy. We developed a strategy for the facile synthesis of *tris*-heteroleptic complexes of Os(II) of the type $[\text{Os}(\text{L}^1)(\text{L}^2)(\text{L}^3)]^{2+}$ in high yields where L^1 , L^2 , and L^3 are different bidentate, polypyridyl ligands.

By incorporating simple modifications in the ligand sphere of several complexes, the fundamental excited state properties of these complexes in nonaqueous solvents were characterized. The presence of electron donating substituents that lower the third ionization potential of the osmium metal center, leads to a reduction in the Φ_{em} in DCM by forcing electron density onto the phenazine portion of dppz derivative. Conversely, electron donating substituents on the 6 or 7,8-positions of the dppz derivative raise Φ_{em} by pushing electron density off this ligand.

In the presence of DNA, the emission properties of the complexes depend very significantly on their ligand architecture. The differences in emission yields and lifetimes for the complexes bound to DNA reflect differences in binding orientation and solvent accessibility in the metallointercalator-DNA complex. The emission properties, and hence binding properties depend on the DNA sequence as well. Overall, dppz complexes of Os(II) provide a class of red-emitting intercalators that are sensitive to the ligand sphere around the metal as well as the composition of the matrix to which are associated. Such a family of metallointercalators provides a foundation to develop sensitive diagnostics for nucleic acids in solution.

2.4. EXPERIMENTAL

2.4.1. Materials

Nucleic acid polymers (sonicated, phenol-extracted CT DNA, poly d(AT), and poly d(GC)) were purchased from Pharmacia as lyophilized cakes, dissolved and exchanged into standard buffer (5 mM tris, 50 mM NaCl, pH 8.5) by ultrafiltration (Amicon). Phen, 5,6-dmp, 4,7-dmp, potassium *bis*-tartratodiantimonate were purchased from Aldrich, $K_2[OsCl_6]$ and $(NH_4)_2[OsCl_6]$ were purchased from Johnson Matthey, and all used as received. 4-Methyl-4'-butyric acid-2,2'-bipyridyl (bpy'), 4,4'-dimethoxy-2,2'-bipyridyl, 4,4'-diamino-2,2'-bipyridyl, 1,10-phenanthroline-5,6-dione, and dppz were prepared as described previously.^{20,22} $[Os(phen)_2(dppz)](PF_6)_2$, and $[Os(L)_2Cl_2]$ (L = phen, 4,7-dmp, 5,6-dmp, dmob) complexes were also synthesized by established methods. $[Os(phen)Cl_4]$ was prepared by solid phase pyrolysis according to the method of Kober *et al.*²⁶ Solvents for synthesis were obtained from Aldrich. Solvents for electrochemistry and emission spectroscopy were from Fluka (over molecular sieves). Distilled and deionized water (Millipore: 18 Ω) was used for all experiments in aqueous solution.

Ion exchange resins (Sephadex QAE-A25 (anion), SP-C25 (cation)) were obtained from Sigma or Aldrich and preequilibrated in 0.2 M KCl prior to use. Neutral alumina was obtained from Fluka.

2.4.2. Preparation and Isolation of Compounds.

6-Methyldipyrido[3,2-*a*:2',3'-*c*]phenazine (Me-dppz). 1,10-Phenanthroline-5,6-dione (150 mg; phendione) was dissolved in ethanol (8.6 mL) in a 25 mL round bottom (RB) flask at 90 °C to give an orange solution. 3-Methyl-1,2-phenylenediamine (260 mg) dissolved in ethanol (3.7 mL) was added slowly to the phendione solution with stirring at 90 °C. After refluxing for 30 min., the dark brown solution was cooled to room temperature and the product precipitated overnight. The ligand was isolated on a

scintered glass frit and washed with cold ethanol, yield 75%. ^1H NMR (CDCl_3): δ (ppm) 9.70 (m, 2H), 9.40 (d, 2H), 8.20 (d, 1H), 7.85 (m, 4H), 3.05 (s, 3H). FABMS: Calcd for $[\text{M}+\text{H}]^+$ 297.106, found 297.1.

7,8-Dimethyldipyrido[3,2-*a*:2',3'-*c*]phenazine ($\text{Me}_2\text{-dppz}$). Phendione (660 mg) in a 100 mL RB flask was dissolved in ethanol (35 mL) at 90 °C to give an orange solution, and 4,5-dimethyl-1,2-phenylenediamine (807 mg) dissolved in ethanol (14 mL) was added slowly to the solution. Upon addition of the dimethylphenylenediamine, a burst of dark brown was observed and the $\text{Me}_2\text{-dppz}$ ligand precipitated as faint yellow needles. The solution was stirred at 90 °C for 5 min. and then cooled to room temperature. The precipitate was isolated on a scintered glass frit and washed with excess ethanol, yield 80%. ^1H NMR (300 MHz; CDCl_3): δ (ppm) 9.63 (dd, $J_a = 8$ Hz, $J_b = 2$ Hz, 2H), 9.30 (dd, $J_a = 4$ Hz, $J_b = 2$ Hz, 2H), 8.08 (s, 2H), 7.83 (dd, $J_a = 8$ Hz, $J_b = 4$, 2H), 2.63 (s, 6H). FABMS: m/z Calcd for $[\text{M}+\text{H}]^+$ 311; found 311.

4-Methyl-4'-butyric acid methyl ester-2,2'-bipyridyl ($\text{bpy}'\text{-OMe}$). Bpy' (250 mg) was dissolved in methanol (15 mL) in a 250 mL RB flask and concentrated HCl (15 drops) was added with stirring. The clear solution was heated at reflux for ~30 min. until the starting acid was consumed (TLC (silica): 10:0.95:0.05 DCM/ CH_3OH /triethyl amine; $R_f(\text{bpy}') = 0.1$; $R_f(\text{bpy}'\text{-OMe}) = 0.3$). The methanol was removed *in vacuo* and the residue ($[\text{b}'\text{-OMe}\cdot 2\text{H}]^{2+}$) was dissolved in water (100 mL) and the pH was raised to 10 with saturated NaHCO_3 (aq). The cloudy aqueous solution was extracted with DCM (3 x 100 mL) and the organic layer was reserved and dried over anhydrous Na_2SO_4 . The DCM was removed *in vacuo* to give the product in quantitative yield.

$[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})](\text{PF}_6)_2$ and $[\text{Os}(\text{L})_2(\text{Me}_n\text{-dppz})](\text{PF}_6)_2$ Derivatives. $[\text{Os}(\text{phen})_2\text{Cl}_2]$ (120 mg) and $\text{Me}_2\text{-dppz}$ (65 mg; 1.1 equiv) were suspended in ethylene glycol (16 mL; EG) in a 100 mL RB flask equipped with a magnetic stirrbar. The mixture was heated to 160 °C under argon and stirred for 4 h, during which time the starting materials dissolved to give a dark olive green solution. Following the reaction

the solution was cooled to room temperature and filtered. An equivolume portion of NH_4PF_6 (aq; 10% w/v) was added to the EG solution to precipitate the complex as the hexafluorophosphate salt. The dark brown precipitate was isolated on a medium porosity sintered glass frit and washed extensively with water to remove any soluble impurities and residual EG, and diethyl ether to dry the complex. Purification was achieved on a neutral alumina column using acetonitrile as the eluent. The complex elutes immediately, while unreacted ligand stays on the column. Yield, ~70%. ^1H NMR (300MHz, CD_3CN): δ (ppm) 9.32 (d, $J = 8$, 2H), 8.45 (dd, $J_a = 7\text{Hz}$, $J_b = 5\text{Hz}$, 4H), 8.30 (s, 4H), 8.21 (d, $J = 6\text{Hz}$, 2H), 8.17 (s, 2H), 8.04 (d, $J = 6\text{Hz}$, 2H), 7.98 (dd, $J_a = 5\text{Hz}$, $J_b = 1\text{Hz}$, 2H), 7.60 (m, 6H), 2.66 (s, 6H). High resolution (HR) FABMS: Calcd for $\text{OsC}_{44}\text{H}_{30}\text{N}_8$ ($[\text{M}-2\text{PF}_6]$) 862.2215; found 862.2215.

$[\text{Os}(\text{phen})_2(\text{Me-dppz})](\text{PF}_6)_2$: ^1H NMR (300MHz, CD_3CN): δ (ppm) 9.63 (d, $J = 6\text{Hz}$, 1H), 9.57 (d, $J = 6\text{Hz}$, 1H), 8.55 (m, 4H), 8.40 (s, 4H), 8.25 (m, 2H), 8.19 (m, 2H), 8.10 (m, 4H), 7.85 (m, 2H), 7.75 (m, 4H), 3.10 (s, 3H). HR FABMS: Calcd for $\text{OsC}_{43}\text{H}_{30}\text{N}_8$ ($[\text{M}-2\text{PF}_6]^+$) 848.2059; found 848.2054.

$[\text{Os}(5,6\text{-dmp})_2(\text{Me}_2\text{-dppz})](\text{PF}_6)_2$: ^1H NMR (300MHz, CD_2Cl_2): δ (ppm) 9.47 (d, $J = 8\text{Hz}$, 2H), 8.49 (d, $J = 9\text{Hz}$, 4H), 8.24 (s, 2H), 8.09 (d, $J = 5\text{Hz}$, 2H), 8.00 (d, $J = 5\text{Hz}$, 2H), 7.90 (d, $J = 5\text{Hz}$, 2H), 7.71 (dd, $J_a = 8\text{Hz}$, $J_b = 6\text{Hz}$, 2H), 7.69 (dd, $J_a = 8\text{Hz}$, $J_b = 5\text{Hz}$, 2H), 7.67 (dd, $J_a = 5\text{Hz}$, $J_b = 2\text{Hz}$, 2H), 2.90 (s, 12H), 2.69 (s, 6H). ESI MS: Calcd m/z for $\text{OsC}_{48}\text{H}_{38}\text{N}_8$ ($[\text{M}-2\text{PF}_6]^{2+}$) 459.14; found 459.1 (2+ ion observed; $m = 2(m/z)$).

$[\text{Os}(4,7\text{-dmp})_2(\text{Me}_2\text{-dppz})](\text{PF}_6)_2$: ^1H NMR (300MHz, CD_2Cl_2): δ (ppm) 9.43 (d, $J = 8\text{Hz}$, 2H), 8.38 (s, 4H), 8.24 (s, 2H), 8.07 (d, $J = 5\text{Hz}$, 2H), 7.97 (d, $J = 5\text{Hz}$, 2H), 7.83 (d, $J = 5\text{Hz}$, 2H), 7.74 (dd, $J_a = 8\text{Hz}$, $J_b = 6\text{Hz}$, 2H), 7.51 (t, $J = 5\text{Hz}$, 4H), 3.08 (s, 6H), 3.05 (s, 6H), 2.70 (s, 6H). FABMS: Calcd for $[\text{M}-\text{PF}_6 + \text{H}]^+$ 1063.1; found 1063.5; Calcd for $[\text{M}-2\text{PF}_6]^+$ 918.3; found 918.2. ESI MS: Calcd m/z for $\text{OsC}_{48}\text{H}_{38}\text{N}_8$ ($[\text{M}-2\text{PF}_6]^{2+}$) 459.14; found 459.1 (2+ ion observed; $m = 2(m/z)$).

$[\text{Os}(\text{dmob})_2(\text{Me}_2\text{-dppz})](\text{PF}_6)_2$: ^1H NMR (300MHz, CD_2Cl_2): δ (ppm) 9.36 (d,

$J = 8\text{Hz}$, 2H), 8.22 (s, 2H), 8.19 (d, $J = 5\text{Hz}$, 2H), 7.87 (d, $J = 3\text{Hz}$, 2H), 7.81 (d, $J = 3\text{Hz}$, 2H), 7.78 (dd, $J_a = 8\text{Hz}$, $J_b = 3\text{Hz}$, 2H), 7.60 (d, $J = 6\text{Hz}$, 2H), 7.32 (d, $J = 6\text{Hz}$, 2H), 7.09 (dd, $J_a = 6\text{Hz}$, $J_b = 3\text{Hz}$, 2H), 6.77 (dd, $J_a = 6\text{Hz}$, $J_b = 3\text{Hz}$, 2H), 4.11 (s, 6H), 4.00 (s, 6H), 2.69 (s, 6H). ESI MS: Calcd m/z for $\text{OsC}_{44}\text{H}_{38}\text{N}_8\text{O}_4$ $[(M-2\text{PF}_6)]^{2+}$ 467.13; found 467.1 (2+ ion observed; $m = 2(m/z)$).

***cis*-[Os(phen)(Me₂-dppz)Cl₂].** [Os(phen)Cl₄] (500 mg) and Me₂-dppz (300 mg; 0.95 equiv) were suspended in dry DMF (35 mL) in a 100 mL RB flask equipped with a magnetic stirrer. The mixture was heated to reflux under argon and stirred vigorously for ~4 h (reaction times depend on scale since the reaction involves a suspension). For the first hour, the solution was a dark orange color, consistent with the presence of Os(III). The purple color of the Os(II) was apparent after 2-3 h. Following the reflux the solution was cooled and filtered on a scintered glass frit. Excess diethyl ether was added to precipitate the product. This material can be used for further reactions, however, it contains trace amounts of Os(III). In order to characterize the material by ¹H NMR, the solid was redissolved in DMF and an equivolume portion of Na₂S₂O₄ (aq) was added dropwise to fully reduce the Os(III) to Os(II) and reprecipitate the product. The dark purple solid was isolated on a medium porosity scintered glass frit and characterized without further purification. Yield, ~60%. ¹H NMR (300MHz, CD₂Cl₂): δ (ppm) 10.36 (d, $J = 6\text{Hz}$, 1H), 10.25 (t, $J = 4\text{Hz}$, 1H), 9.08 (d, $J = 4\text{Hz}$, 1H), 8.74 (d, $J = 8\text{Hz}$, 1H), 8.24 (s, 1H), 8.14 (s, 1H), 8.06 (d, $J = 8\text{Hz}$, 1H), 7.98 (dd, $J_a = 8\text{Hz}$, $J_b = 6\text{Hz}$, 1H), 7.95 (dd, $J_a = 4\text{Hz}$, $J_b = 6\text{Hz}$, 1H), 7.96 (s, 2H), 7.88 (d, $J = 5\text{Hz}$, 1H), 7.79 (d, $J = 5\text{Hz}$, 1H), 7.62 (d, $J = 8\text{Hz}$, 1H), 7.02 (dd, $J_a = 6\text{Hz}$, $J_b = 3\text{Hz}$, 1H), 7.00 (dd, $J_a = 6\text{Hz}$, $J_b = 3\text{Hz}$, 1H), 2.70 (s, 3H), 2.67 (s, 3H). HR FABMS: Calcd for $[M]^+$ 752.0905; found 752.0859

[Os(phen)(bpy'-OMe)(Me₂-dppz)](PF₆)₂ and derivatives. [Os(phen)(Me₂-dppz)Cl₂] (200 mg) and bpy'-OMe (75 mg; 1.1 equiv) were suspended in EG (20 mL) in a 100 mL RB flask equipped with a magnetic stirrer. The mixture was heated to 160 °C under argon with stirring for ~4 h. After 1 h of heating, the dark olive green color of the

product can be observed, and continues to intensify over the course of the reaction. Following the heating period, the solution was cooled to room temperature and filtered on a scintered glass frit. The complex was isolated as the hexafluoro phosphate salt as described above. Purification was achieved on neutral alumina, but in contrast to dppz derivatives, bpy'-OMe is eluted from the column with acetonitrile or DCM. Fortunately, the metal complex does not elute off the column with DCM. Therefore a neutral alumina column was assembled with DCM as the mobile phase, and the product, dissolved in DCM, was loaded onto the support. The dark band was washed with copious amounts of DCM to remove unreacted bpy'-OMe, and the desired complex was eluted with acetonitrile. A small amount of purple material ($[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})\text{Cl}_2]$) remained at the top of the column. The acetonitrile solution was evaporated *in vacuo* to give the desired complex in ~70% yield. We found that a convenient way to isolate the complex was to dissolve the material after rotary evaporation in a minimal amount of DCM or acetonitrile and add excess diethyl ether to precipitate the complex, which was isolated on a scintered glass frit. ^1H NMR (300MHz, CD_2Cl_2): δ (ppm) 9.53 (d, $J = 9$ Hz, 1H), 9.42 (d, $J = 8$ Hz, 1H), 8.42 (d, $J = 8$ Hz, 1H), 8.32 (d, $J = 8$ Hz, 3H), 8.22 (m, 6H), 8.01 (d, $J = 5$ Hz, 1H), 7.92 (dd, $J_a = 8$, $J_b = 2$ Hz, 1H), 7.88 (m, 2H), 7.64 (m, 3H), 7.44 (m, 1H), 7.16 (d, $J = 7$ Hz, 2H), 3.66 (s, 1.5H), 3.64 (s, 1.5H), 2.96 (t, 2H), 2.69 (s, 9H), 2.46 (t, 2H), 2.04 (q, 2H). HR FAB MS: m/z Calcd for $\text{OsC}_{48}\text{H}_{40}\text{N}_8\text{O}_2$ ($[\text{M}-2\text{PF}_6]^+$) 952.2896; found 952.363.

$[\text{Os}(\text{phen})(\text{dmb})(\text{Me}_2\text{-dppz})](\text{PF}_6)_2$. ^1H NMR (300MHz, CD_2Cl_2): δ (ppm) 9.51 (dd, $J_a = 10$ Hz, $J_b = 1$ Hz, 1H), 9.41 (dd, $J_a = 8$, $J_b = 1$, 1H), 8.41 (dd, $J_a = 8$ Hz, $J_b = 1$ Hz, 1H), 8.2 (m, 9H), 8.01 (dd, $J_a = 5$ Hz, $J_b = 1$ Hz, 1H), 7.93 (dd, $J_a = 8$, $J_b = 5$ Hz, 1H), 7.88 (dd, $J_a = 10$ Hz, $J_b = 4$ Hz, 1H), 7.87 (d, $J = 4$ Hz, 1H), 7.67 (dd, $J_a = 8$ Hz, $J_b = 6$ Hz, 1H), 7.63 (dd, $J_a = 7$ Hz, $J_b = 4$ Hz, 1H), 7.58 (d, $J = 6$ Hz, 1H), 7.40 (d, $J = 6$ Hz, 1H), 7.14 (t, $J = 7$ Hz, 2H), 2.68 (s, 12H). ESI MS: m/z Calcd for $\text{OsC}_{44}\text{H}_{34}\text{N}_8\text{PF}_6$ ($[\text{M}-\text{PF}_6]^+$) 1011.2170; found 1011.2; m/z Calcd for $\text{OsC}_{44}\text{H}_{34}\text{N}_8$ ($[\text{M}-$

$2PF_6]^{2+}$) 433.1264; found 433.1. HR FAB MS: m/z Calcd for $OsC_{44}H_{34}N_8 ([M-2PF_6]^+)$ 866.2521; found 866.2519.

[Os(phen)(dab)(Me₂-dppz)](acetate)₂. The complex was prepared from [Os(phen)(Me₂-dppz)Cl₂] and dab as described above. The hexafluoro phosphate salt was exchanged to the chloride salt by anion exchange and purified by HPLC to give the acetate salt (*vide infra*). ¹H NMR (300MHz, CD₃OD): δ (ppm) 9.44 (d, $J = 8$ Hz, 1H), 9.36 (d, $J = 8$ Hz, 1H), 8.50 (d, $J = 5$ Hz, 1H), 8.43 (m, 2H), 8.32 (m, 5H), 8.09 (d, $J = 5$ Hz, 1H), 7.95 (m, 3H), 7.57 (m, 4H), 7.07 (d, $J = 7$ Hz, 1H), 6.83 (d, $J = 7$ Hz, 1H), 6.47 (dd, $J_a = 6$ Hz, $J_b = 2$ Hz, 1H), 6.42 (dd, $J_a = 6$ Hz, $J_b = 2$ Hz, 1H), 2.73 (s, 3H), 2.71 (s, 3H) (amino protons are exchanged for deuterium in CD₃OD). FABMS Calcd for $OsC_{42}H_{31}N_{10} [M-2acetate]^+$ 867.0; found 867.2.

[Os(phen)(bpy')(Me₂-dppz)](acetate)₂. [Os(phen)(Me₂-dppz)(bpy'-OMe)](PF₆)₂ (125 mg) was exchanged to the chloride salt by anion exchange chromatography (*vide infra*) and dissolved into 10 mL H₂O in a 25 mL RB flask equipped with a magnetic stirrbar. Aqueous LiCl (1 mL; 1 M) was added to the dark brown/olive solution, and the flask was immersed in an ice bath, with stirring. Stirring was continued overnight as the ice bath warmed to room temperature. The Os(II) solution was diluted to 125 mL with H₂O and neutralized (pH 7) with 1 M HCl. The solution was loaded onto a cation exchange column (Sephadex SP-C25) that was preequilibrated with KCl. The complex adsorbed to the resin and the column was washed with several (>2) column volumes of water to remove any uncharged or negatively charged species. The product was eluted from the column in 1:1 H₂O/acetonitrile (100 mM KCl) and the solvent was removed *in vacuo* and the dry complex was extracted from the salt into DCM. The DCM was removed *in vacuo* and the extraction procedure was repeated to give the dry, salt free product in quantitative yield. For luminescence studies, the complex was further purified by HPLC (*vide infra*). ¹H NMR (300MHz, CD₃OD): δ (ppm) 9.58 (dd, $J_a = 4$ Hz, $J_b = 1$ Hz, 1H), 9.47 (d, $J =$

8Hz, 1H), 8.67 (d, $J = 6\text{Hz}$, 2H), 8.56 (d, $J = 8\text{Hz}$, 1H), 8.47 (d, $J = 8\text{Hz}$, 1H), 8.30 (m, 6H), 8.13 (dd, $J_a = 5\text{Hz}$, $J_b = 3\text{Hz}$, 1H), 7.9 (m, 3H), 7.70 (m, 2H), 7.64 (dd, $J_a = 8\text{Hz}$, $J_b = 5\text{Hz}$, 1H), 7.47 (dd, $J_a = 6\text{Hz}$, $J_b = 4\text{Hz}$, 1H), 2.94 (t, $J = 2\text{Hz}$), 7.20 (m, 2H), 2.73 (s, H), 2.72 (s, 6H), 2.69 (s, 3H), 2.24 (t, $J = 5\text{Hz}$, 2H), 2.00 (q, $J = 8\text{Hz}$, 2H), 1.98 (s, 6H; acetate). ESI MS: m/z Calcd for $\text{OsC}_{47}\text{H}_{37}\text{N}_8\text{O}_2$ ($[\text{M}-2\text{acetate}-\text{H}]^+$) 937.2661; found 937.2; m/z Calcd for $\text{OsC}_{47}\text{H}_{38}\text{N}_8\text{O}_2$ ($[\text{M}-2\text{acetate}]^{2+}$) 469.1370; found 469.1. HR FAB MS: m/z Calcd for $\text{OsC}_{47}\text{H}_{38}\text{N}_8\text{O}_2$ ($[\text{M}-2\text{PF}_6]^+$) 938.2746; found 938.2746.

For applications in luminescence studies metal complexes were exchanged to the chloride salt on Sephadex QAE-A25 anion exchange resin and purified by HPLC (stationary phase: C18 reversed phase; mobile phase: 50 mM ammonium acetate pH 5.5; acetonitrile) to rigorously exclude trace impurities. We found that this procedure always removed luminescent impurities such as *tris*-phen complexes. The test of purity for the complexes was the absence of emission in aqueous solution.

Enantiomer Resolution. The racemic mixture of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ was resolved into its pure Δ and Λ enantiomers by cation exchange chromatography on Sephadex SP-C25 using potassium *bis*-tartratodiantimonate (0.15 M) as a chiral eluent. The pure acetate salt of the complex was adsorbed onto 5 mL of SP-C25 resin that had been equilibrated for 24 h in 0.2 M KCl (aq) and then washed extensively with H_2O to remove excess salt. The Os(II) coated resin was carefully added to a 1 m x 2.5 cm column of resin to form a symmetric disc approximately 2 cm in height. Approximately 10 cm of free resin was loaded on top of the complex to protect the band during solvent additions. The column was washed with ~ 2 L of H_2O and then the chiral eluent was introduced at a gravity driven flow rate and the column thoroughly washed with the antimonate solution (~ 3 column volumes). The antimonate solution was then recirculated through the column using a peristaltic pump. After 10 days of recirculating the chiral eluent through the column at a gravity driven flow rate, and the complex passing over the

column twice, the dark brown band spread into two bands equal in size (5 cm) and color. Resolution was discontinued when the two bands were separated by ~ 3 cm of free column support (baseline resolution; Figure 2.19). The chiral eluent was then washed from the column with several volumes of water, and the stationary phase was pushed out of the column by air pressure while the complex remained adsorbed to the cation exchange resin. The portions of the resin that contained each enantiomer were excised and each isomer was eluted from the resin with KCl (50 mM) in acetonitrile/water (1:1). Upon removing the solvent *in vacuo* the complex was extracted from the salt into DCM. The DCM was removed *in vacuo* to give the dry, salt free enantiomer. Circular dichroism (CD) spectra were recorded on a Jasco J-500A and data for the complexes are as follows: for Δ -[Os(phen)₂(dppz)]²⁺, $\Delta\epsilon$ (270 nm) = -209 M⁻¹cm⁻¹, and for Λ -[Os(phen)₂(dppz)]²⁺ $\Delta\epsilon$ (270 nm) = +214 M⁻¹cm⁻¹. Raw CD data taken on the Jasco were converted to millidegrees according to (1), and millidegrees were converted to $\Delta\epsilon$ (2).

$$\Theta(m \text{ deg}) = \frac{\text{rawdata} \bullet \text{sensitivity}}{500 \bullet \# \text{ scans}} (1) \quad \frac{\Delta\epsilon}{M \bullet \text{cm}} = \frac{\Theta(m \text{ deg})}{\ell(\text{cm}) \bullet c(\text{mM}) \bullet 32.98} (2)$$



Figure 2.19. Sephadex SP-C25 cation exchange column used to resolve *rac*- $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ with *bis*-tartrato diantimonate (aq; 0.15 M) as the chiral eluent. The resolution was complete when the two bands were separated by column support that was essentially free of metal complex. The first band that elutes is the Δ enantiomer, which is followed by the Λ complex. The assignment of absolute configuration was made by comparative CD studies between the bands isolated here and the CD spectra for the pure enantiomers of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$.

2.4.3. Methods

UV-vis. Individual scans were measured on a Beckman DU-7400 diode array spectrophotometer. Titrations were carried out on a Cary 2200, double beam spectrophotometer. To begin, the sample cuvette contained the Os(II) complex in standard buffer (*vide supra*) and the reference cuvette contained only buffer. A concentrated (~15-20 mM nucleotides) aliquot (1-10 μ L) of buffered DNA solution was added to each cuvette and the solutions were mixed by bubbling air into the cuvette with a micropipette equipped with a plastic tip for ~30 s. The absorbance of the sample solution was recorded against the DNA containing reference. The % hypochromism was calculated as: $\% \text{ hyp} = [(A_{\lambda}^{[\text{DNA}] = 0} - A_{\lambda}^{[\text{DNA}] = N}) / A_{\lambda}^{[\text{DNA}] = 0}] \times 100$.

Luminescence Measurements. All samples were air equilibrated. Steady-state emission data were recorded on an SLM-8000 spectrofluorimeter. Emission intensities were determined by integration of the luminescence spectrum, and standardized against $[\text{Ru}(\text{bpy})_3]^{2+}$ as a calibration for the instrument. Spectra were not corrected for instrument sensitivity owing to their near infrared emission. Therefore emission yields represent a minimum absolute value, but relative values are accurate and valid. Quantum yields for emission were determined as described previously by comparison to known standards such as $[\text{Ru}(\text{bpy})_3]^{2+}$ and $[\text{Os}(\text{bpy})_3]^{2+}$.

Luminescence lifetimes were measured by time-correlated single photon counting (TCSPC) and multifrequency phase-modulation fluorometry (MPMF). TCSPC data were collected and analyzed as described previously.⁴ MPMF data were collected on an ISS K2 lifetime fluorometer according to ISS protocol over frequency ranges dictated by the lifetime of the sample.³² Typical frequency ranges spanned 0.1-150 MHz, and data were collected at a minimum of 10 frequencies (5-7 measurements per frequency). Data analysis was achieved on commercial software (ISS) and the quality of the model was judged by the value of chi squared for the least squares minimization. Simulations with chi squared < 10 were accepted as good fits.

Electrochemical Measurements. Reduction potentials were measured by cyclic voltammetry (CV) using instrumentation described previously. Complexes were dissolved in dry DMF (Fluka; stored over molecular sieves) containing 0.1 M tetrabutylammonium hexafluorophosphate as supporting electrolyte. Electrochemical windows ranging from 0.2 to 1.6 V vs NHE for oxidation, 0.2 to -1.55 V vs NHE for reduction were scanned at a rate of 0.1 V/s. $E_{1/2}$ values were taken as the average of the voltage of maximum current for the forward and reverse electrochemical processes.

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Appendix A.2: Ru(phen)₂dppz²⁺ Luminescence: Dependence on DNA Sequences and Groove Binding Agents[#]

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ABSTRACT

Emission of Δ -Ru(phen)₂dppz²⁺ bound to nucleic acid polymers of different sequence has been investigated by time-resolved luminescence spectroscopy, and the effect of major and minor groove DNA binding agents on the luminescence profile of the complex evaluated. In the presence of a 1:1 mixture of poly d(AT) and poly d(GC), the excited state decay of Δ -Ru(phen)₂dppz²⁺ can be described by a linear combination of the decay profiles in the presence of poly d(AT) and poly d(GC) independently. This analysis indicates that ~85% of the complexes are bound to poly d(AT) and indicates that the metallointercalator preferentially occupies AT sites in mixed sequence polymers such as calf thymus or T4 DNA. When *rac*-Ru(phen)₂dppz²⁺ bound to [d(5'-GAGTGCCTC-3')₂] is titrated with the major groove intercalator Δ - α -[Rh[(*R,R*)-Me₂trien]phi]³⁺, the ruthenium emission yield decreases while the absorbance of the π - π^* transition centered on the dppz ligand increases, until saturation behavior is observed at a 1:1 Rh/duplex ratio. These titrations indicate that Ru(phen)₂dppz²⁺ is displaced from the major groove by the rhodium complex. In contrast, for *rac*-Ru(phen)₂dppz²⁺ bound to poly d(AT), addition of the minor groove binding agent distamycin produces an increase in ruthenium emission which saturates at ~1 distamycin/~5 bp, consistent with the double helix being able to accommodate major and minor groove binders simultaneously. Distamycin has no effect on the emission of Ru(phen)₂dppz²⁺ emission bound to poly d(GC). These photophysical studies establish a sequence preference in binding to DNA by Ru(phen)₂dppz²⁺ as well as providing support for the original assignment by NMR of ruthenium intercalation from the major groove side of the DNA helix.

INTRODUCTION

Octahedral transition metal complexes which intercalate into nucleic acids have become useful probes of DNA and RNA.¹ Phenanthrenequinone diimine (phi) complexes of rhodium(III) bind to DNA by intercalation of the phi ligand from the major groove and have been applied in site-specific DNA recognition and oxidation chemistry.^{1,2} Metal complexes containing dppz (dppz = dipyridophenazine) as a ligand also bind to DNA with high affinity,³⁻¹¹ and many have served as luminescent probes.³⁻⁸ This luminescence signature is remarkably sensitive to local environment and depends upon nucleic-acid sequence and structure.³⁻⁵ The wider applicability of dppz complexes as nucleic acid probes requires a firm understanding of the structural basis for their photophysical characteristics.

The lack of apparent DNA sequence-specificity for $\text{Ru(phen)}_2\text{dppz}^{2+}$ makes a detailed structural determination of its interactions with duplex DNA using NMR spectroscopy more difficult. Early ^1H NMR spectroscopic experiments¹¹ of $\text{Ru(phen)}_2\text{dppz}^{2+}$ with DNA showed only broad resonances, due not only to the lack of site-specificity but also to the intermediate exchange kinetics at ambient temperature and to the abundance of overlapping resonances in the aromatic region. Selective deuteration of the coordinating ligands of $\text{Ru(phen)}_2\text{dppz}^{2+}$, variations in temperature to alter the exchange kinetics, and restricting the examination to a DNA hexamer were then applied in carrying out two-dimensional (2D) ^1H -NMR experiments on $\Delta\text{-Ru(phen)}_2\text{dppz}^{2+}$ bound to d(GTCGAC)_2 .^{12,13} This approach yielded NOESY data which were consistent with intercalation of $\Delta\text{-Ru(phen)}_2\text{dppz}^{2+}$ into the 6 base pair (bp) oligonucleotide from the major groove side of the B-form DNA duplex with a family of stacking orientations.

Recently, photophysical studies of Λ - and $\Delta\text{-Ru(phen)}_2\text{dppz}^{2+}$ bound to calf thymus (CT) DNA, T4 DNA, and several synthetic DNA polymers were carried out. Based upon differences in emission characteristics among these DNAs, it was concluded

that $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ isomers bound by intercalation from the minor groove of the DNA helix.¹⁴

Here, we reinvestigate the binding of $\Delta\text{-Ru}(\text{phen})_2\text{dppz}^{2+}$ to poly d(AT), poly d(GC) and calf thymus (CT) DNA by time-resolved luminescence spectroscopy, and describe experiments in which well-characterized major and minor groove DNA binding agents compete with $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ for DNA binding sites. These experiments illuminate the sequence dependence of the $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ emission and support the original assignment¹² of ruthenium intercalation from the major groove side of duplex DNA.

EXPERIMENTAL

Materials. $[\text{Ru}(\text{phen})_2\text{dppz}]\text{Cl}_2$ and $\Delta\text{-}\alpha\text{-}[\text{Rh}[(R,R)\text{-Me}_2\text{trien}]\text{phi}]\text{Cl}_3$ ($[\text{Rh}(\text{MT})\text{phi}]\text{Cl}_3$; Me_2trien = 2,9-diamino-4,7-diazadecane) were prepared as described previously.^{13,15} Resolution of *rac*- $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ was achieved by ion exchange chromatography using antimonyl tartrate (Sigma) as the chiral eluent.¹³ Distamycin was obtained from Sigma, dissolved in minimal ethanol then diluted into buffer (5 mM Tris, 50 mM NaCl, pH 7.8) and quantitated by ultraviolet-visible (UV-vis) absorbance ($\epsilon_{302\text{ nm}} = 3.7 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$; Sigma). To avoid degradation of distamycin, fresh stock solutions were prepared by dissolving solid prior to each experiment. Oligonucleotides were prepared on an ABI 394 automated DNA synthesizer, purified by reversed phase HPLC (triethyl ammonium acetate (100 mM)/acetonitrile mobile phase; C_{18} stationary phase) and converted to the sodium salt as described.¹³ CT DNA, poly d(AT) and poly d(GC) were obtained from Pharmacia and exchanged into buffer (5 mM Tris, 50 mM NaCl, pH 8.5).

Instrumentation. Steady-state emission data were obtained on an SLM 8000 spectrofluorimeter, and absorption data were obtained on a Cary 2200 spectrophotometer.

Time-resolved luminescence data were obtained as described previously⁷ with excitation at 480 nm and observation at 617 nm.

Methods. Stock solutions of metal complex and DNA were quantitated by UV-vis ($\epsilon_{440 \text{ nm}}$ Ru(phen)₂dppz²⁺ = $2.1 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$; $\epsilon_{373 \text{ nm}}$ Rh(MT)phi³⁺ = $1.35 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ (pH 7.0); $\epsilon_{260 \text{ nm}}$ poly d(AT) = $6.6 \times 10^3 \text{ M}^{-1}(\text{nucleotide; nuc})\text{cm}^{-1}$; $\epsilon_{260 \text{ nm}}$ CT = $6.6 \times 10^3 \text{ M}^{-1}(\text{nuc})\text{cm}^{-1}$; $\epsilon_{254 \text{ nm}}$ pd(GC) = $8.4 \times 10^3 \text{ M}^{-1}(\text{nuc})\text{cm}^{-1}$). Oligonucleotide duplex solutions were prepared by annealing the self-complementary strand by heating a solution of strand to 90 °C and cooling to room temperature over ~2 hours. Where appropriate, luminescence samples were prepared by diluting concentrated (~15 mM nuc) DNA solutions into dilute (~10 μM) metal complex solutions. Distamycin titrations were typically carried out by adding concentrated (~0.5 mM) distamycin to solutions of ruthenium bound to DNA, although separate samples prepared in reverse order gave identical results.

To obtain luminescence lifetimes (τ 's), time-resolved emission data were fit to

$$y(t) = 100[C1\exp(-t/\tau1) + (1-C1)\exp(-t/\tau2)]$$

by a nonlinear least-squares method with convolution of the instrument response function using in house (Beckman Institute Laser Resource Center) software as described previously.⁷ For competition experiments involving DNA sequence, luminescence data were fit according to the multiexponential function

$$y(t) = (100/(C1+C2+C3+C4))[C1\exp(-t/\tau1) + C2\exp(-t/\tau2)] + C3\exp(-t/\tau3) + C4\exp(-t/\tau4)]$$

consisting of a linear combination of the decay profiles with poly d(AT) and poly d(GC), separately. The preexponential factors (C1-4) were floating parameters and allowed to converge, and the lifetimes ($\tau1 = 123 \text{ ns}$; $\tau2 = 709 \text{ ns}$; $\tau3 = 44 \text{ ns}$; $\tau4 = 288 \text{ ns}$) were held constant. In the results obtained, the percent contribution of the 123 ns and 709 ns lifetimes reflects the fraction of complex bound to poly d(AT) and the percent contribution of the 44 ns and 288 ns lifetimes reflects the fraction bound to poly d(GC) (Table 1). Error in lifetimes and percent contributions are estimated to be $\pm 10\%$.

For titrations followed by UV-vis, a double beam spectrophotometer was employed. The sample solution contained the 10 bp duplex (10 μ M) and $\text{Ru(phen)}_2\text{dppz}^{2+}$ (10 μ M) and the reference solution contained only the 10 bp duplex. Over the course of the titration, small aliquots of Rh(MT)phi^{3+} (1.5 mM) were added to both reference and sample solutions. Under these conditions, the absorbance of the DNA and the rhodium are subtracted.

RESULTS AND DISCUSSION

Luminescence of $\text{Ru(phen)}_2\text{dppz}^{2+}$ Bound to AT and GC Sites. The luminescence lifetimes for photoexcited $\Delta\text{-Ru(phen)}_2\text{dppz}^{2+}$ bound to a series of nucleic acid polymers are shown in Table 1. For each of the DNA sequences investigated, the emission follows a biexponential decay.^{3,7} As has been noted,^{5,7,14} variations, primarily in the distribution of lifetimes, are observed as a function of loading. Despite the high sensitivity of ruthenium emission to its local environment, in the presence of CT DNA and poly d(AT), the luminescence decays are found to be remarkably similar in both the magnitude and relative distribution of lifetimes. Bound to poly d(GC) however, the excited state lifetimes are shorter and the distribution of lifetimes is strongly shifted in favor of the longer lived species, to give a significantly different profile. These data are fully consistent with those reported earlier,^{3c,14} and over the range of 0.8-4 mJ/pulse, the data are independent of excitation power. Such a lack of a power dependence to the excited state decay argues against the distribution of metal complex across the polymer giving rise to the biexponential decay as proposed¹⁴ since at high laser powers one would expect to observe self quenching even at low loadings.

In order to test the sequence preference of $\Delta\text{-Ru(phen)}_2\text{dppz}^{2+}$ directly in a photophysical experiment, we measured the luminescence decay of the complex in the presence of a 1:1 mixture of poly d(AT) and poly d(GC). These measurements were carried out at low loadings of Ru/DNA to maximize the effect of sequence and to

minimize variations in emission that are associated with high loadings. Here, in the 1:1 mixture of poly d(AT) and poly d(GC), the luminescence decay can be described by a linear combination of the decay profiles in the presence of poly d(AT) and poly d(GC) independently. This analysis indicates that, despite a high affinity for both DNA polymers, ~85% of the complexes are bound preferentially to poly d(AT).

The recent assignment of $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ intercalation from the minor groove side of duplex DNA was based primarily on comparative luminescence studies¹⁴ for the complex bound to CT (42% GC)¹⁶ and T4 DNA (34% GC), a mixed sequence DNA like CT, but one in which 5-hydroxymethylcytosine residues are 100% glucosylated in the major groove.¹⁷ Luminescence titrations and excited state decay kinetics for $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ were essentially the same for both DNAs. These results were interpreted as evidence for intercalation of the complex from the minor groove side of the duplex, since the steric obstruction provided by the sugar units should have prevented binding and caused a significant reduction in the emission yield of the complex bound to T4 DNA compared to CT. Given the finding here of preferential binding of $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ to AT sites, the small differences observed between CT and T4 DNA are not surprising.

We can apply these data in estimating site occupation within both CT and T4 DNA. For $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ bound to CT, the luminescence lifetimes are slightly longer than with poly d(AT). The decay profile may reflect the heterogeneity of AT containing sites within the mixed sequence CT DNA. The ratio of equilibrium constants for $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ binding to poly d(AT) and poly d(GC) ($K_{\text{AT/GC}}$) in the presence of both polymers can be expressed as $K_{\text{AT/GC}} = ([\text{Ru}\bullet\text{AT}])([\text{GC}]) / ([\text{Ru}\bullet\text{GC}])([\text{AT}])$. Assuming a 3 bp binding site for ruthenium, data from the 1:1 poly d(AT):poly d(GC) competition experiment yields $K_{\text{AT/GC}} = 5.9$. Using this constant, the ratio of ruthenium binding to AT vs. GC sites ($[\text{Ru}\bullet\text{AT}] / [\text{Ru}\bullet\text{GC}]$) in CT (42% GC) and T4 (34% GC) DNA are calculated to be 8.2 and 11.5, respectively, or 89% AT occupation in CT and

92% AT occupation in T4. Hence, at low loadings of metal to DNA, it is fully understandable that the emission profiles for these sequences are so similar.

A similarity in emission for $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ bound to CT and T4 DNA would therefore be expected whether the complex were in the major groove or the minor groove. On both DNAs, the luminescence appears to emanate substantially from intercalation within AT sites. Differences in luminescence yield for $\Delta\text{-Ru}(\text{phen})_2\text{dppz}^{2+}$ bound to the two DNAs are observed only at high metal to DNA ratios, where the emission is actually somewhat higher with T4.¹⁴ If the complex were associating from the minor groove, these emission yields should not differ at any loading. At high loadings of ruthenium on T4, the higher emission intensity might result from an increase in protection of the complex from solvent quenching by glucosylated sites in the major groove or follow from an increase in the rigidity^{1,7} of the helix.

The emission profile for ruthenium bound to poly d(IC) (I = inosine) has also been cited in support of intercalation of the complex from the minor groove since the IC base pair lacks the amino group in the minor groove.¹⁴ Bound to poly d(IC), the biexponential decay reported¹⁴ resembles more closely that for ruthenium bound to poly d(AT) than to poly d(GC); as with poly d(AT), emission arises primarily from the shorter excited state decay component. Many factors determine the relative distribution of binding orientations which give rise to the biexponential decay; certainly the dipole characteristics of the different base pairs must affect the relative stability of different stacking orientations, irrespective of whether stacking originates from the major or minor groove side. Based upon steric considerations, one would expect that eliminating the amino group from the minor groove should actually facilitate complete intercalation and an increase in excited state lifetime, yet with poly d(IC), a side-on mode with a shorter excited state lifetime is favored. Hence little support for the minor groove assignment can be gleaned from the observation made with poly d(IC).

Competitive Binding in the Major Groove. We decided to explore the groove assignment for intercalation of $\text{Ru(phen)}_2\text{dppz}^{2+}$ in more detail through competition experiments with DNA-binding molecules where the groove orientation has been well-established. In the case of the interactions of small molecules with DNA, there are in fact only a few examples of species which bind DNA from the major groove side.^{1,18,19}

Phi complexes of rhodium have been shown using a variety of techniques to intercalate in double helical DNA from the major groove side. In particular, 2D NMR studies have established that Rh(MT)phi^{3+} (Figure 1) binds site-specifically ($K_B \sim 10^8 \text{ M}^{-1}$) in the center of a 10 bp duplex $[\text{d}(5'\text{-GAGTGC}^{\text{ACTC}}\text{-3}')_2]$ from the major groove side, with only one equivalent of the rhodium complex bound to the duplex even in the presence of excess Rh(MT)phi^{3+} .²⁰ The sequence selectivity of this intercalation depends upon both hydrogen bonding interactions between the axial amines of the complex and guanines above and below the intercalation site, as well as methyl-methyl interactions between the methyl groups in Rh(MT)phi^{3+} and thymines at the base position once removed from the intercalated base step.

Figure 2 shows the effect of increasing Rh(MT)phi^{3+} concentration on the emission yield (squares) of $\text{Ru(phen)}_2\text{dppz}^{2+}$ bound to $[\text{d}(5'\text{-GAGTGC}^{\text{ACTC}}\text{-3}')_2]$. It should be noted that, based on luminescence titrations, *rac*- $\text{Ru(phen)}_2\text{dppz}^{2+}$ binds well to $[\text{d}(5'\text{-GAGTGC}^{\text{ACTC}}\text{-3}')_2]$ with saturation in luminescence at ~ 3 complexes/duplex, consistent with a 3-4 bp binding site size for the racemic complex. With increasing concentrations of rhodium, the emission yield decreases, which is consistent with displacement of the lumiphore from the DNA since the ruthenium complex emits only when intercalated and protected from water. Such a loss in intensity cannot be a simple charge effect since titrations with the ruthenium(II) complex indicate that at least 2 additional complexes can be accommodated on the duplex. The observed loss of emission intensity also does not arise as a result of quenching by electron transfer. Unlike $\text{Rh(phi)}_2\text{bpy}^{3+}$, which promotes $\sim 70\%$ quenching of ruthenium luminescence on CT

DNA at low loading (1.5 equivalents), $\text{Rh}(\text{MT})\text{phi}^{3+}$, at the same loading shows no detectable quenching of ruthenium emission. In general, phi complexes of rhodium(III) containing saturated amine ligands do not quench the ruthenium excited state in DNA or micelles.^{21,22}

We also followed this titration by UV-vis spectroscopy. Intercalative binding of dppz complexes to DNA is accompanied by significant hypochromism and a red shift in the π - π^* transition centered on the phenazine portion of the ligand.³⁻¹⁰ As expected for a displacement effect, the absorbance at 375 nm increases with increasing rhodium concentration and begins to saturate at 1:1 rhodium/duplex (Figure 2). Given that the increase in absorbance mirrors the decrease in ruthenium emission, both absorption and emission spectroscopies indicate that $\text{Rh}(\text{MT})\text{phi}^{3+}$ displaces $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ from the duplex.

This displacement of ruthenium by rhodium contrasts the cooperative clustering of ruthenium with $\text{Rh}(\text{phi})_2\text{L}^{3+}$ ($\text{L} = \text{bpy}, \text{phen}$) complexes which has been suggested.²³ In fact, owing to the smaller, aliphatic nature of the ancillary ligands of $\text{Rh}(\text{MT})\text{phi}^{3+}$ compared to phen or phi, cooperative clustering of $\text{Rh}(\text{MT})\text{phi}^{3+}$ with $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ might be expected to be far less likely here. Clearly, the decrease in luminescence combined with the loss of hypochromicity is opposite to what would accompany binding cooperativity on the helix. Instead the loss of emission must reflect an anticooperative interaction.

Addition of $\text{Rh}(\text{MT})\text{phi}^{3+}$ does not completely eliminate the ruthenium emission, however. The decrease in emission begins to level off after reaching a 1:1 rhodium:duplex ratio, with slightly more than 50% of the ruthenium emission depleted. Compared to the decay profile in the absence of the rhodium complex, the lifetimes of the biexponential decay in the presence of rhodium are reduced slightly and the distribution is shifted in favor of the shorter lifetime (Table 1). Calculation of a steady-state emission yield from the luminescence decay parameters indicates a decrease in emission of only

35%, and therefore the reduction cannot be accounted for solely on the basis of excited state lifetime. Since the only binding sites present with rhodium bound are near the ends of the duplex, the shorter lifetimes observed could indicate redistribution of ruthenium from the center of the decamer outward in addition to some displacement into solution as observed by UV-vis.

These data are consistent with binding of $\text{Ru(phen)}_2\text{dppz}^{2+}$ from the major groove side of the double helix and its competitive displacement by Rh(MT)phi^{3+} . Based on this result alone, however, this conclusion is not definitive, since intercalation in general can provide a probe of both DNA grooves. Nonetheless, if the ruthenium complex were bound in the minor groove, and displaced because of rhodium intercalation from the other side of the helix, one might have expected only the base step where rhodium intercalated to be somewhat blocked. Instead we observe a larger region of the duplex to be affected.

Competitive Binding in the Minor Groove. We also probed minor groove association of $\text{Ru(phen)}_2\text{dppz}^{2+}$ in titrations with distamycin (Figure 1). Distamycin binds well to poly d(AT) ($K_B \sim 10^7 \text{ M}^{-1}$) but not to poly d(GC) ($K_B \sim 10^4 \text{ M}^{-1}$).^{24,25} As expected, the emission yield of ruthenium bound to poly d(GC) is not significantly affected by the presence of distamycin (Figure 3). Bound to poly d(AT), however, the $\text{Ru(phen)}_2\text{dppz}^{2+}$ luminescence yield is actually increased upon addition of distamycin (Figure 3). This increase in the ruthenium luminescence saturates at ~ 1 distamycin/5 bp, consistent with the ligand binding to the duplex as a monomer and saturating the minor groove.²⁶

That the ruthenium luminescence increases rather than being unaffected by distamycin association likely reflects a stiffening of the duplex on binding distamycin.^{24,25} As indicated in Table 1, the increase in quantum yield with addition of distamycin to $\text{Ru(phen)}_2\text{dppz}^{2+}$ bound to poly d(AT) is derived from a redistribution of the lifetimes in the biexponential decay in favor of the longer lifetime (Table 1). Such an effect on the relative proportion of lifetimes is also observed at high metal complex to

DNA ratios,^{1,5,7,14} which has also been ascribed to duplex stiffening.^{1,7} One might consider that addition of distamycin only concentrates the metal complexes in sections of the minor groove which are uncovered by distamycin. Such a consideration is ruled out, however, since titrations at two ruthenium:bp ratios which differ by a factor of 4 are superimposable until reaching saturation.²⁷ It is noteworthy also that the luminescence data for ruthenium bound to poly d(AT) in the presence of distamycin resemble data for ruthenium in the presence of poly dA•poly dT, which is a more rigid polymer.²⁵

It is also possible to load ruthenium onto the helix when the minor groove is saturated with distamycin. When 20 μM $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ is added to poly d(AT) (250 μM bp) containing saturating concentrations of distamycin (50 μM), bright ruthenium luminescence is observed.

These competitive binding titrations in the minor groove therefore also support intercalation of $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ from the major groove side. If ruthenium were bound in the minor groove, one would have expected a decrease in luminescence as the metal was displaced with increasing distamycin concentrations. Thus these results are certainly consistent with the helix being able to accommodate major and minor groove binding agents simultaneously.

Groove Preference for $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ and Other Metallointercalators. A variety of results have suggested that metallointercalators may in general associate with double helical DNA from the major groove.^{12,13,20,28-30} These observations include the high resolution NMR structure²⁰ of $\text{Rh}(\text{MT})\text{phi}^{3+}$ bound to a DNA decamer and chemical cleavage experiments²⁸ with $\text{Rh}(\text{MGP})_2\text{phi}^{3+}$ (MGP = 4-(guanidylmethyl)-1,10-phenanthroline) and $\text{Rh}(\text{en})_2\text{phi}^{3+}$ (en = ethylenediamine) on synthetic oligonucleotides in the absence and presence of covalent modifications in the major groove. Of particular note is the crystal structure of (terpyridyl)(2-hydroxy-ethanethiolate)platinum(II) intercalated in $\text{d}(\text{CpG})_2$;²⁹ in this case the metallointercalator is primarily planar, and the complex is oriented within the base step with the metal center

on the major groove side. This structure, albeit only of an intercalated dinucleotide, suggests that factors other than steric bulk are important in determining groove preference for intercalation. Indeed, as with organic intercalators,³¹ dipole and induced dipole orientations of the aromatic heterocyclic moiety which intercalates are likely to govern the stacking geometry. Certainly, those factors which dominate groove selectivity for metallointercalators need still to be established.

It is noteworthy that NMR experiments have recently indicated that the metallointercalator, $\text{Ru}(\text{phen})_2\text{dpq}^{2+}$ (dpq = dipyrido[2,2-*d*:2',3'-*f*]quinoxaline), may bind to a 6 bp duplex by intercalation from the minor groove.³² Interestingly, the chemical shift perturbations and nuclear Overhauser effects observed with intercalation of this compound differ significantly from those described for $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$.^{12,13} While it is clear that one binding mode for $\text{Ru}(\text{phen})_2\text{dpq}^{2+}$ involves association from the minor groove, it is not certain from the data presented that this mode is intercalative. If this minor groove association is indeed intercalative, the comparison to $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ must be a consequence of the change in electronic structure between the dppz and dpq ligands. NMR studies of $\Delta\text{-Ru}(\text{phen})_2\text{dppz}^{2+}$ bound to the hexamer duplex $\text{d}(\text{GTCGAC})_2$ clearly show NOE crosspeaks bound adenine proton resonances in the major groove and the upfield-shifted 4',7' protons of intercalated dppz .¹²

The data we present here are fully consistent with intercalation of $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ from the major groove of DNA, as originally described by NMR.^{12,13} These data are also helpful in reconciling a photophysical study¹⁴ which suggested minor groove association. The primary observation put forth in support of minor groove association was the absence of a substantive change in luminescence for T4 DNA compared to CT DNA. Our results here regarding sequence preferences for $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ make such observations understandable. Another suggestion for minor groove association was made based upon the different excited state lifetimes found for ruthenium bound to inosine-containing polymers compared to poly $\text{d}(\text{GC})$, but in general

the excited state lifetimes for $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ is seen to be sensitive to an array of factors, many of which are not yet quantitatively understood. Lastly Tuite et al.¹⁴ have argued that the high luminescence of $\text{Ru}(\text{phen})_2\text{dppz}^{2+}$ bound to triple helical DNA indicates minor groove association since they consider that the third strand must block major groove intercalation. But here too, little difference in emission should be expected between the double and triple helix with binding of ruthenium from the minor groove. Instead we propose that the third DNA strand provides a more substantial platform for intercalative stacking from the major groove (and third strand) side, yielding an increase in protection from quenching by water and thus an increased excited state lifetime.³³

Since photophysical studies provide only an indirect description of structure, their conclusions regarding structural models must be viewed with some caution. High resolution structural information must depend upon NMR and crystallographic studies. Importantly, the photophysical experiments described here and previously are now reconciled with NMR data.

ACKNOWLEDGMENTS

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FIGURE CAPTIONS

Figure 1. Schematic illustrations of DNA binding agents Δ -Ru(phen)₂dppz²⁺, Δ - α -[Rh[(*R,R*)-Me₂trien]phi]³⁺ (Rh(MT)phi³⁺) and distamycin.

Figure 2. Steady-state emission intensity (●) and relative absorbance change at 375 nm (▲) for Ru(phen)₂dppz²⁺ (10 μ M) bound to [d(5'-GAGTGCACTC-3')]₂ (10 μ M duplex; 10 mM phosphate, 20 mM NaCl, pH 7) in the presence of increasing concentrations of Δ - α -[Rh[(*R,R*)-Me₂trien]phi]³⁺ (Rh(MT)phi³⁺). The reduction in emission intensity and the increase in absorbance of the π - π^* transition for the intercalating dppz ligand are consistent with displacement of Ru(phen)₂dppz²⁺ by Rh(MT)phi³⁺, which binds by intercalation in the center of the decamer from the major groove side.²² Error bars represent standard deviations for at least two trials. Emission intensity and absorbance at 375 nm are normalized to the values at [Rh(MT)phi³⁺] = 0 μ M, and absorbance changes are corrected for DNA and Rh(MT)phi³⁺ interference.

Figure 3. Relative emission intensity for Ru(phen)₂dppz²⁺ bound to poly d(AT) (○, ▲) and poly d(GC) (□) in the presence of increasing concentrations of distamycin. On poly d(AT), the changes in ruthenium emission saturate at ~1distamycin/~5 bp, consistent with the helix accomodating major and minor groove binders simultaneously. At concentrations of distamycin > 0.2/bp on poly d(AT), the ruthenium luminescence yield changes with time, and the intensity vs. [distamycin] curves diverge. Error in emission intensities are estimated to be $\pm$ 10%. Conditions are as follows: (○) 10 μ M Ru, 1 mM nucleotides (nuc) poly d(AT); (▲) 20 μ M Ru, 0.5 mM nuc poly d(AT); (□) 10 μ M Ru, 1 mM nuc poly d(GC); in 5 mM tris, 50 mM NaCl, pH 7.8.

Table 1. Luminescence decay parameters for Ru(phen)₂dppz²⁺ bound to DNA polymers.^a

Nucleic Acid	luminescence lifetimes ^b			
	τ_1 (ns)	%	τ_2 (ns)	%
calf thymus ^c	150	(80)	800	(20)
poly d(AT) ^c	123	(82)	709	(18)
poly d(GC) ^c	44	(22)	288	(78)
poly d(GC) + poly d(AT) ^d	126	(79)	599	(21)
poly d(GC) + poly d(AT) ^{d,e}	44	(3)	288	(12)
	[τ_3] 123	(70)	[τ_4] 709	(15)
[d(5'-GAGTGCACTC-3') ₂] ^f	128	(66)	545	(34)
" + Rh(MT)phi ³⁺ g	85	(75)	446	(25)
poly d(AT) + distamycin ^h	120	(65)	671	(35)

a. Data obtained as described previously⁷ with excitation at 480 nm and observation at 617 nm.

b. Data were fit to $y(t) = 100[C1\exp(-t/\tau_1) + (1-C1)\exp(-t/\tau_2)]$ by a nonlinear least-squares method with convolution of the instrument response function using in house software (BILRC) as described previously (Arkin, M. R.; Stemp, E. D. A.; Turro, C.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 2267-2274). Error in lifetimes and percent contributions are estimated to be $\pm 10\%$.

c. Δ -Ru(phen)₂dppz²⁺ (10 μ M) bound to DNA (1 mM nucleotides (nuc); Ru:bp = 1:50) in buffer (5 mM tris, 50 mM NaCl, pH 8.5). Lifetimes are consistent with those reported previously, see reference 1c.

d. Δ -Ru(phen)₂dppz²⁺ (10 μ M) in the presence of 1:1 poly d(AT) and poly d(GC) (1 mM nuc; Ru:bp = 1:50) in buffer. Identical results are obtained at 2 mM nuc (Ru:bp = 1/100).

e. Data fit to multiexponential function consisting of a linear combination of decay profiles for Δ -Ru(phen)₂dppz²⁺ bound to poly d(AT) and poly d(GC), separately. The preexponential factors were allowed to float in the minimization routine, while the lifetimes were fixed (see Experimental).

f. *rac.*-Ru(phen)₂dppz²⁺ (10 μM) in the presence decamer (10 μM duplex) in buffer (10 mM phosphate, 20 mM NaCl, pH 7.0).

g. Rh(MT)phi³⁺ = Δ-α-[Rh[(*R,R*)-Me₂trien]phi]³⁺ (10 μM).

h. *rac.*-Ru(phen)₂dppz²⁺ (10 μM) bound to poly d(AT) (1 mM nucleotides; Ru:bp = 1:50) in buffer (5 mM tris, 50 mM NaCl, pH 7.8) in the presence of distamycin (100 μM).

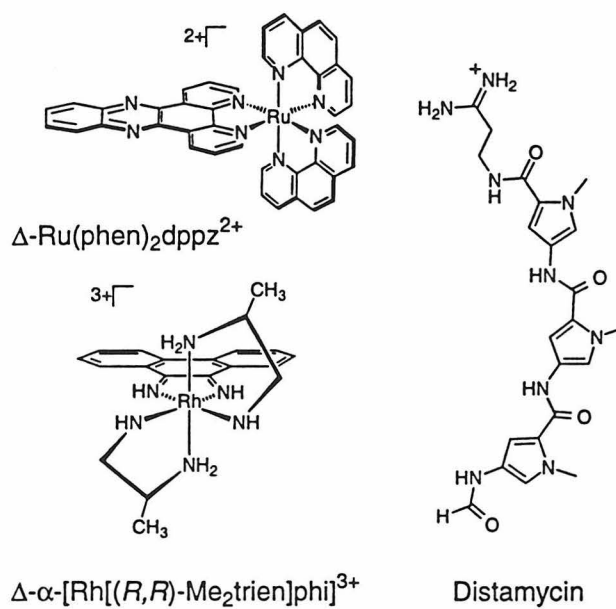


Figure 1

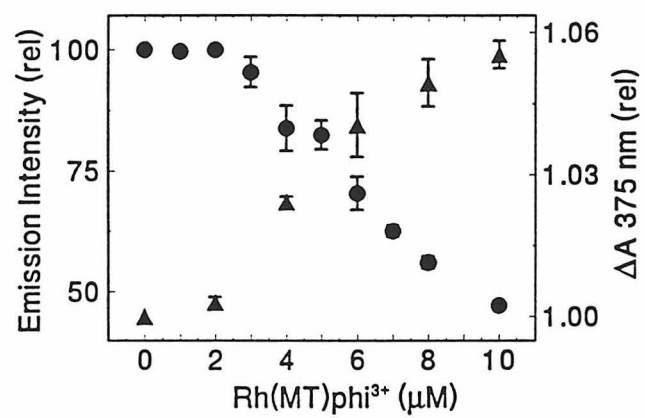


Figure 2

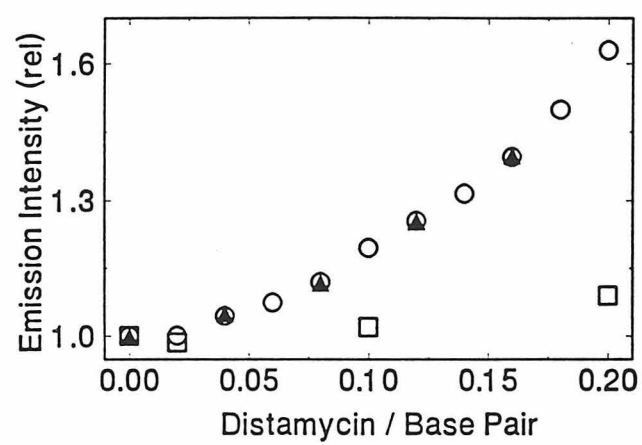


Figure 3

Chapter 3: Applications of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ as an Isostructural Analog of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ in Investigations of DNA-Mediated Electron Transfer between Metallointercalators.[#]

3.1. INTRODUCTION

This chapter describes the application of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in mechanistic investigations of DNA-mediated electron transfer between transition metal complexes bound noncovalently to DNA by intercalation. Theoretical¹⁻³ and experimental⁴⁻¹⁰ studies have been directed toward elucidating the role of the DNA double helix in mediating electron transfer between donors and acceptors bound to the polymer. Experiments in our laboratories focus on electron transfer reactions between transition-metal complexes which bind tightly to DNA by intercalation (metallointercalators).¹⁰ Through their intercalative stacking, these metal complexes provide a direct means to probe the DNA π -stack. Previously, we reported the photoinduced quenching of intercalated $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ (dppz = dipyrdo[3,2-a;2',3'-c]phenazine) by $[\text{Rh}(\text{phi})_2(\text{phen})]^{3+}$ (phi = 9,10-phenanthrene quinonediimine) in DNA.^{10a} Both $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{phen})]^{3+}$ bind tightly to DNA ($K_D < 10^{-6}$ M) by intercalation from the major groove.^{11,12} Additionally, $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ serves as a molecular light switch for DNA.¹¹ Although the complex luminesces in organic solvents, emission from the metal-to-ligand charge-transfer (MLCT) excited state is quenched in aqueous solution owing to interaction between the solvent hydrogens and the phenazine nitrogens of the dppz ligand.¹³ Upon binding to DNA by preferential

[#] Adapted from: Holmlin, R. E., Stemp, E. D. A., Barton, J. K. *J. Am. Chem. Soc.* **1996**, *118*, 5236; Arkin, M. R.; Stemp, E. D. A., Holmlin, R. E.; Hormann, A., Olson, E. J. C.; Barbara, P. F. *Science* **1996**, *273*, 5274.

intercalation of the dppz ligand, these phenazine nitrogens are protected from water, and luminescence returns. Analogous light switch behavior has recently been described for a rhenium(I) complex containing the dppz ligand.¹⁴

DNA-mediated electron transfer quenching of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ by rhodium intercalators appears to be fast^{10a,15} and to occur over some distance.^{10b} At low loadings of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{phen})]^{3+}$ on the helix, where average donor-acceptor separation is large, the photoinduced electron transfer quenching and subsequent recombination¹⁵ were found to proceed on a subnanosecond time scale. In contrast, the groove bound acceptor $[\text{Ru}(\text{NH}_3)_6]^{3+}$ quenches the DNA-bound $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ more slowly through a collisional mechanism.^{10a} Additionally, in an assembly with the ruthenium donor and the rhodium acceptor tethered by a flexible linker to opposite ends of a 15-mer duplex, where the reactants are intercalated but separated by $\sim 40 \text{ \AA}$, the ruthenium steady state emission is fully quenched.^{10b} A lower limit for the quenching rate constant was set at $3 \times 10^9 \text{ s}^{-1}$ over this large molecular distance. Based on energetic considerations, electron transfer was considered the most likely mechanism responsible for this remarkable long-range quenching. Using the donor and acceptor analogues, $[\text{Ru}(\text{dmp})_2(\text{dppz})]^{2+}$ ($\text{dmp} = 4,7\text{-dimethyl phenanthroline}$) and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, respectively, a long-lived intermediate corresponding to the oxidized donor was recently observed by transient absorption spectroscopy, providing the first direct evidence for DNA-mediated electron transfer between metallointercalators.¹⁶

Many issues concerning the nature of the observed DNA-mediated quenching require further consideration. For example, owing to a small but nonzero overlap between donor (Ru(II)) emission and acceptor (Rh(III)) absorption, an energy transfer mechanism may also contribute to the quenching. Additionally, we would like to address the effect of the donor energetics and intrinsic excited-state lifetime on the efficiency of this quenching with DNA as the bridge.

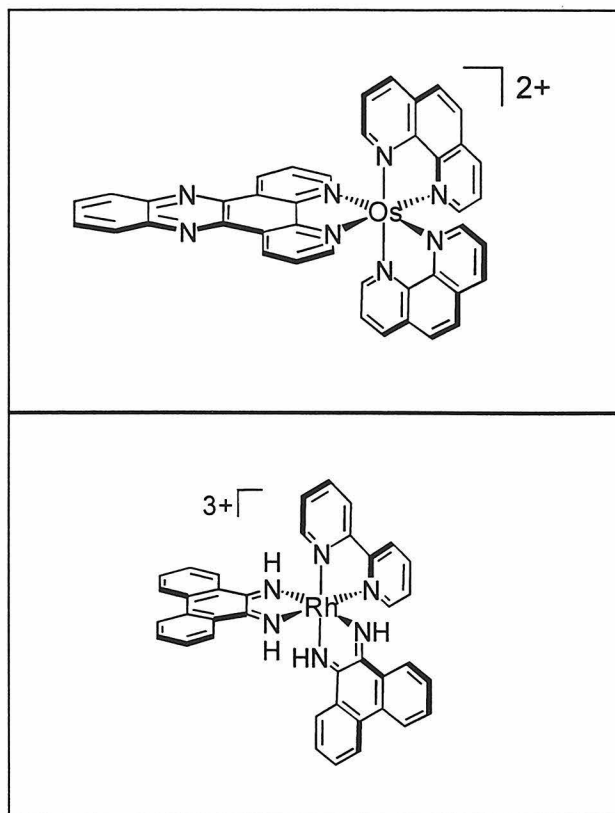


Figure 3.1. Schematic illustration of Δ -[Os(phen)₂(dppz)]²⁺ (top) and Δ -[Rh(phi)₂(bpy)]³⁺ (bottom).

Hence, in order to expand the scope of DNA-mediated electron transfer reactions between metallointercalators and to begin to address these issues, we have now applied dppz complexes of osmium as electron donors. Indeed, [Os(phen)₂(dppz)]²⁺ (Figure 3.1) also serves as a molecular light switch for DNA, providing a red-emitting DNA probe with excited state lifetimes faster than 10 ns.¹⁷ Substitution of osmium should not alter the DNA binding properties of the metal complex, but, rather, provide an isostructural analog for [Ru(phen)₂(dppz)]²⁺ with distinct photophysical and electrochemical properties.¹⁸ Importantly, as shown in Figure 3.2, the emission spectrum of intercalated [Os(phen)₂(dppz)]²⁺ is red-shifted by ~ 130 nm compared to its ruthenium counterpart.

This long-wavelength emission does not overlap the rhodium absorption. Thus, energy transfer¹⁹ is not a consideration when studying the quenching of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ by rhodium intercalators, and the comparison between ruthenium and osmium quenching profiles allows for the identification of any energy transfer quenching in the ruthenium example. Additionally, the fast intrinsic excited-state lifetimes of DNA-bound osmium compared to $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ allow one to evaluate the contribution of dynamic collisional processes to the quenching. In this report, we therefore describe the DNA binding and electrochemical properties of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ as well as the DNA-mediated electron transfer chemistry between intercalated $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ (Figure 3.1) bound to DNA.

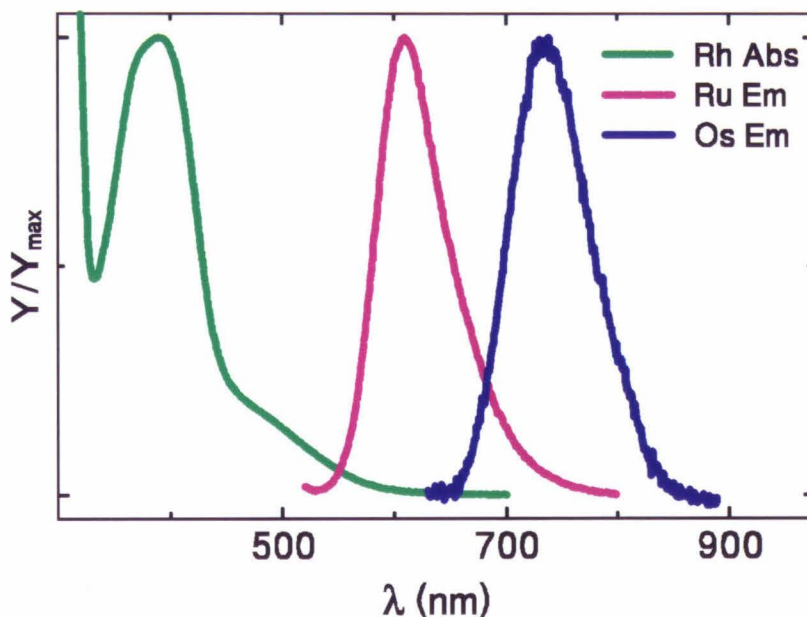


Figure 3.2. Visible absorption spectrum of $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to DNA compared to the emission spectra of intercalated $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ ($\lambda_{\text{max}} = 617 \text{ nm}$) and $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ ($\lambda_{\text{max}} = 738 \text{ nm}$). The red emission from the osmium complex bound to DNA does not overlap with the rhodium absorption.

3.2. RESULTS

3.2.1. DNA Binding Properties of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ as an Isostructural Analog of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$

Titration by Visible Absorption and Time-Resolved Luminescence

Spectroscopy. Visible absorption spectroscopy as well as time-resolved luminescence measurements have been used to characterize the intercalative binding of the $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ to double helical DNA. Figure 3.3 shows the effect of increasing DNA concentration on $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ visible absorption spectrum, consisting of an intraligand (IL) π - π^* transition centered on the phenazine portion of the dppz ligand [$\lambda_{\text{max}} = 372 \text{ nm}$; $\epsilon = 1.3 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$], and $^1\text{MLCT}$ states ($\lambda_{\text{max}} = 430 \text{ nm}, 472 \text{ nm}$). As the DNA concentration is increased, pronounced hypochromism is observed in the IL band as well as in the MLCT transitions, with an isosbestic point at 505 nm. The percent hypochromism at 372 nm, plotted against the nucleotide:osmium ratio ($R_{\text{nuc:Os}}$) in Figure 3.3, increases linearly until reaching saturation with a 38 % decrease in absorbance at $R_{\text{nuc:Os}} \simeq 5$. The IL band is also red shifted $\sim 7 \text{ nm}$ over the course of the titration. Such a hypochromic effect is consistent with the preferential intercalative stacking of the dppz ligand and has also been observed for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ over the same concentration range.^{11,24}

The luminescence intensity for Δ - $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ is plotted as a function of the $R_{\text{nuc:Os}}$ in Figure 3.4. Owing to the molecular light switch characteristic of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+17}$, no emission is detected for the complex in aqueous solution in the absence of DNA. As DNA is titrated into the solution, luminescence is observed. The emission yield increases smoothly with increasing DNA approaching $R_{\text{nuc:Os}} \simeq 5$, consistent with the absorption titration. However, increasing the DNA concentration beyond $R_{\text{nuc:Os}} = 5$ produces a significant increase in the emission yield which maximizes at $R_{\text{nuc:Os}} = 6.25$. Interestingly, with further addition of DNA, the emission yield actually decreases slightly.

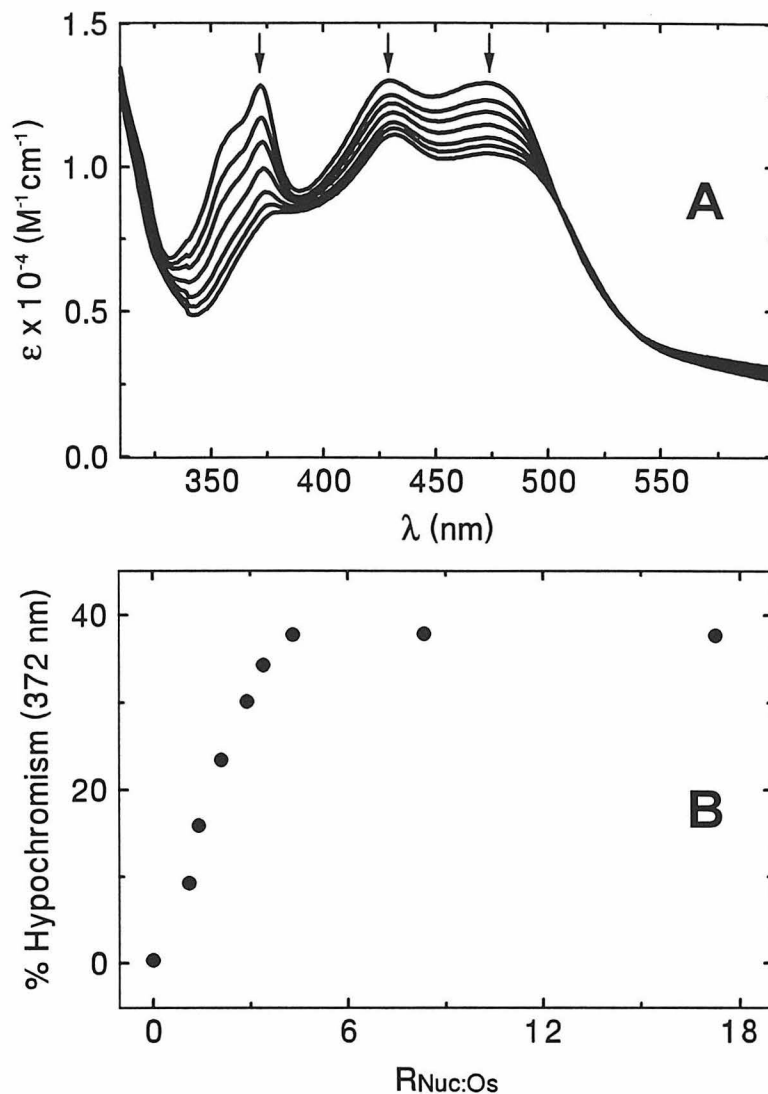


Figure 3.3. Visible absorption spectra of racemic $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in the presence of increasing DNA concentrations. DNA-nucleotide to osmium ratios ($R_{\text{nuc:Os}}$) shown are as follows: 0, 1.1, 1.4, 2.1, 2.9, 3.4, 4.3. The metal concentration was held constant and any absorbance contribution by the DNA subtracted. Conditions: 35 μM Os, 5 mM Tris, 50 mM NaCl, pH 8.5. Inset: Plot of percent hypochromism $[(A_{R=0} - A_{R=x})/A_{R=0}] \cdot 100$ at 372 nm versus $R_{\text{nuc:Os}}$. Shown are $R_{\text{nuc:Os}} = 0, 1.1, 1.4, 2.1, 2.9, 3.4, 4.3, 8.3, 17.2$.

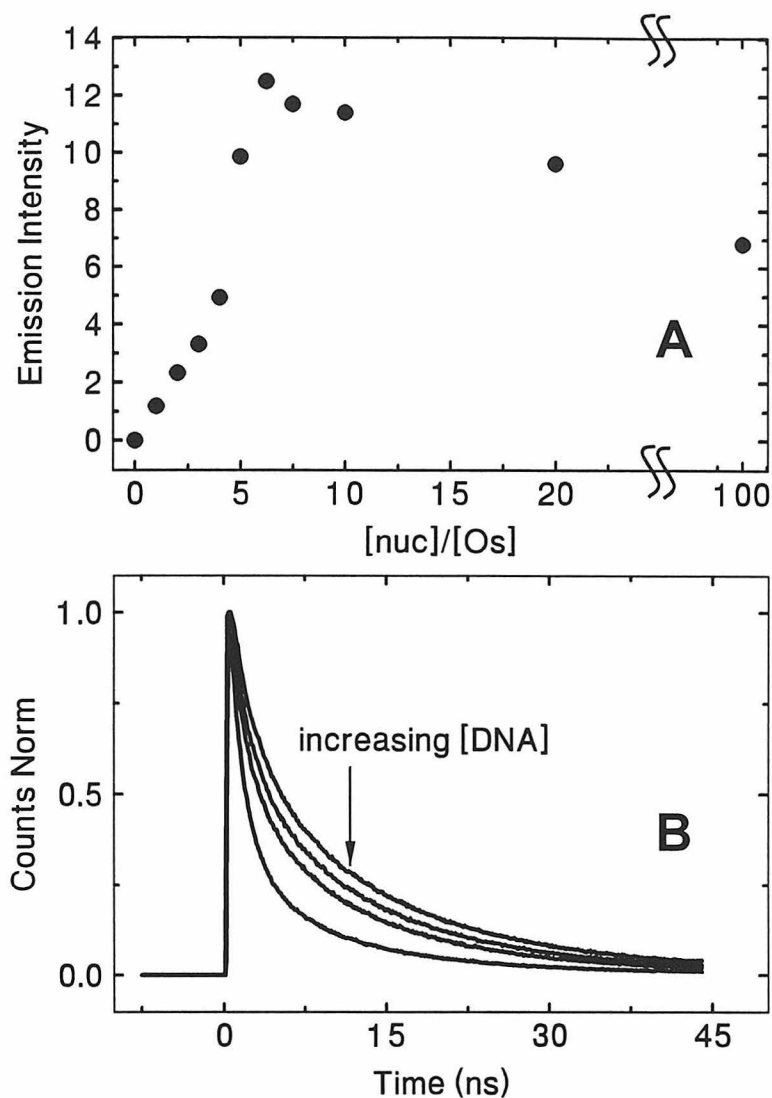


Figure 3.4. Plot of Δ -[Os(phen)₂(dppz)]²⁺ emission intensity as a function of the DNA-nucleotide to osmium ratio ($R_{\text{nuc:Os}}$). Shown are $R_{\text{nuc:Os}} = 0, 1, 2, 3, 4, 5, 6.25, 7.5, 10, 20, 100$. Conditions: 20 μM Os, 5 mM Tris, 50 mM NaCl, pH 8.5. Inset: Plot of emission counts versus time for Δ -[Os(phen)₂(dppz)]²⁺ bound to DNA at $R_{\text{nuc:Os}} = 5, 10, 20, 100$ as measured by TCSPC. Conditions: 50 μM Os, 5 mM Tris, 50 mM NaCl, pH 8.5.

To examine more closely the DNA concentration dependence of the emission yield at the higher DNA concentrations, we measured the time resolved luminescence decay of intercalated Δ -[Os(phen)₂(dppz)]²⁺ by TCSPC, again, as a function of DNA loading. Luminescence decay traces corresponding to R_{nuc:Os} = 5, 10, 20, 100 appear as an inset in Figure 3.4. Emission from Δ -[Os(phen)₂(dppz)]²⁺, like the ruthenium analog, is best described with a biexponential fit, and the results of nonlinear least squares analysis of the decay traces shown are listed in Table 3.1. These time-resolved data are in agreement with similar data for Δ -[Ru(phen)₂(dppz)]²⁺ and indicate identical binding properties for the osmium complex. As is the case for Λ -[Ru(phen)₂(dppz)]²⁺,²⁴ the quantum yield for emission from Λ -[Os(phen)₂(dppz)]²⁺ is significantly lower than that of the Δ -isomer and therefore we have focused our luminescence and quenching studies on Δ -[Os(phen)₂(dppz)]²⁺.

Table 3.1. Excited state lifetimes for Δ -[Os(phen)₂(dppz)]²⁺ Bound to CT DNA as a Function of the Ratio of Nucleotides to Osmium (R_{nuc:Os}).^a

R _{nuc:Os}	τ_1 ns (%)	τ_2 ns (%)	τ avg
5	13.0 (56)	2.3 (43)	8.3
10	12.0 (51)	2.0 (48)	7.1
20	11.0 (46)	1.8 (55)	6.0
100	9.2 (24)	1.0 (76)	3.0

- a. Experimental Conditions: Aerated buffer (50 μ M Os, 5 Tris, 50 mM NaCl, pH 8.5) at ambient temperature; $\lambda_{\text{exc}} = 590$ nm (SPC); $\lambda_{\text{obs}} = 780$ nm. Error in kinetics data is estimated to be $\pm 10\%$.

3.2.2. Emission Intensity Quenching of Δ -[Os(phen)₂(dppz)]²⁺ by Δ -[Rh(phi)₂(bpy)]³⁺ Bound to DNA.

Steady-State Emission Quenching. Emission from [Os(phen)₂(dppz)]²⁺ bound to DNA is quenched by [Rh(phi)₂(bpy)]³⁺. In order to examine this quenching in detail, we have titrated the quencher, Δ -[Rh(phi)₂(bpy)]³⁺, into solutions of donor, Δ -[Os(phen)₂(dppz)]²⁺, bound to sonicated calf-thymus DNA. Importantly, [Ru(phen)₂(dppz)]²⁺ complexes show appreciable luminescence with synthetic polymers of different sequence, and NMR and binding studies of the ruthenium analogue indicate that these dppz complexes bind to the DNA duplex in an essentially random, sequence-neutral fashion.¹¹ Similarly, DNA photocleavage studies of [Rh(phi)₂(bpy)]³⁺ in the absence¹² and presence¹⁵ of [Ru(phen)₂(dppz)]²⁺ or [Ru(bpy)₂(dppz)]²⁺ indicate that the rhodium complex binds DNA with sequence neutrality at these concentrations and there is no perturbation in this binding profile by the ruthenium complexes; hence we find no evidence for binding cooperativity.²⁵ Thus, in an effort to maintain large donor-acceptor separations given the random occupation of both donor and acceptor on the helix, the R_{nuc}:O_s ratio was held constant at 100:1.

The results of such a titration, plotted as the fraction of emission intensity quenched (fraction quenched) as a function of acceptor concentration, are shown in Figure 3.5. With just 1 equivalent of quencher, where the average donor-acceptor separation is 25 base pairs (88 Å), the emission is quenched by almost 50%. The fraction quenched reaches saturation at 0.9 with 6 equivalents of rhodium. Also plotted in Figure 3.5 are the results of the same experiment with Δ -[Ru(phen)₂(dppz)]²⁺ as donor.¹⁵ Over the concentration ranges examined, the osmium and ruthenium quenching profiles are strikingly similar. These results therefore indicate a similar efficiency for the quenching of each donor.

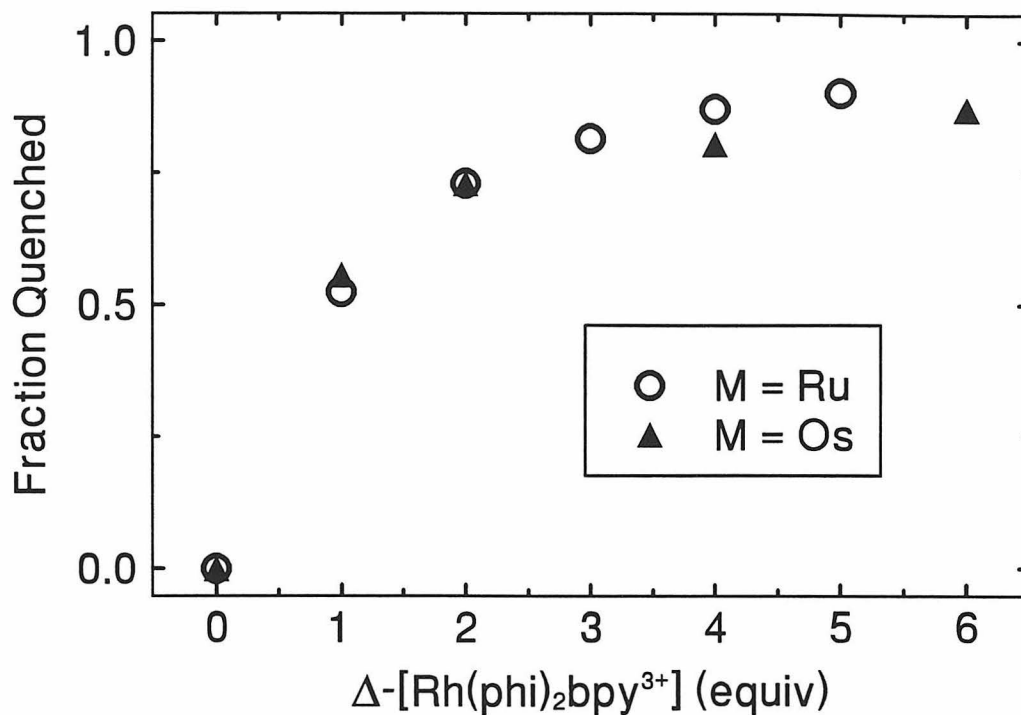


Figure 3.5. Plot of the fraction of emission quenched ($1-I/I_0$) for Δ -[Os(phen)₂(dppz)]²⁺ (s) and Δ -[Ru(phen)₂(dppz)]²⁺ (m) bound to DNA as a function of increasing equivalents of Δ -[Rh(phi)₂(bpy)]³⁺. For the osmium titration ($\lambda_{\text{exc}} = 480 \text{ nm}$; $\lambda_{\text{obs}} = 740 \text{ nm}$), quencher was added to 20 μM Os(II), 2 mM nucleotides in 5 mM tris, 50 mM NaCl, pH 8.5 and in the ruthenium titration ($\lambda_{\text{exc}} = 480 \text{ nm}$; $\lambda_{\text{obs}} = 610 \text{ nm}$), quencher was added to 10 μM Ru(II), 1 mM nucleotides under the same buffer conditions. Equivalent data are observed with 20 μM Ru(II)/2 mM nucleotides.

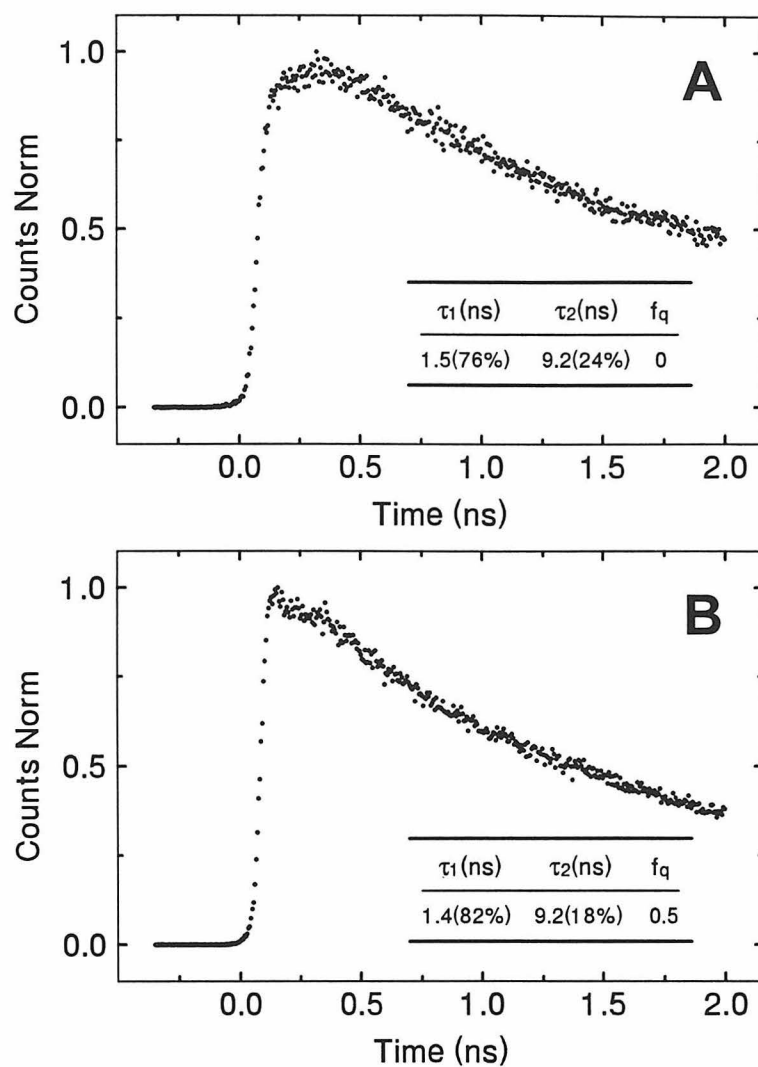


Figure 3.6. Time-resolved luminescence decay of Δ -[Os(phen)₂(dppz)]²⁺ bound to DNA in the absence (**A**) and presence (**B**) of 1 equivalent of Δ -[Rh(phi)₂(bpy)]³⁺. Shown are normalized counts. The lifetimes of the decays and the fraction of emission quenched (f_q) are indicated below each trace. The observed quenching is primarily manifested as a decrease in the intensity at zero time, since the minor changes in decay kinetics for the quenched sample do not account for the 50 % quenching. Conditions: 50 μ M Os, 5 mM nucleotides in 5 mM tris, 50 mM NaCl, pH 8.5; λ_{exc} = 590 nm, λ_{obs} = 775 nm.

Monitoring Luminescence Quenching by Single Photon Counting. We used TCSPC with ~ 150 ps resolution to examine the rate of this $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ quenching. We find that the majority of the quenching is static relative to the resolution of this instrument and thus primarily appears as a decrease in the intensity at time zero. Importantly, the amount of quenching due to this decrease in the intensity at time zero increases with increasing quencher concentration, and no substantial change in decay profile occurs as a function of quencher. Figure 3.6 compares the luminescence decay profile of $\Delta\text{-}[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in the presence and absence of 1 equivalent of $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$. A small amount of response-limited signal is observed for quenched $\Delta\text{-}[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ but the overall kinetic profile is nearly unchanged. This response-limited signal may actually be derived from rhodium-based emission. Therefore, these measurements provide a lower limit of $7 \times 10^9 \text{ s}^{-1}$ for the rate constant describing the ultrafast quenching reaction.

3.2.3. Investigations of Electron Transfer between Metallointercalators by Transient Absorption

Observation and Characterization of Electron Transfer Intermediates on the Microsecond Time Scale. Scheme 1 illustrates the cycle for electron transfer reactions between $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to DNA. In order to attribute the emission intensity quenching described above to photoinduced electron transfer (k_{et}), we have used transient absorption spectroscopy to identify and characterize the intermediate consistent with such a DNA-mediated electron transfer reaction. Figure 3.7 shows the transient signals observed upon 440 nm excitation of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ at 480 nm in the presence of 0, 2 and 4 equivalents $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$. As expected for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in the absence of acceptor, the MLCT excited state is seen as a bleach at 440 nm which returns to baseline within the instrument response, consistent with the short excited-state lifetime of the DNA-bound complex. On the other hand, in

Scheme 3.1. Cycle of photoinduced ET reactions between metallointercalator bound to DNA

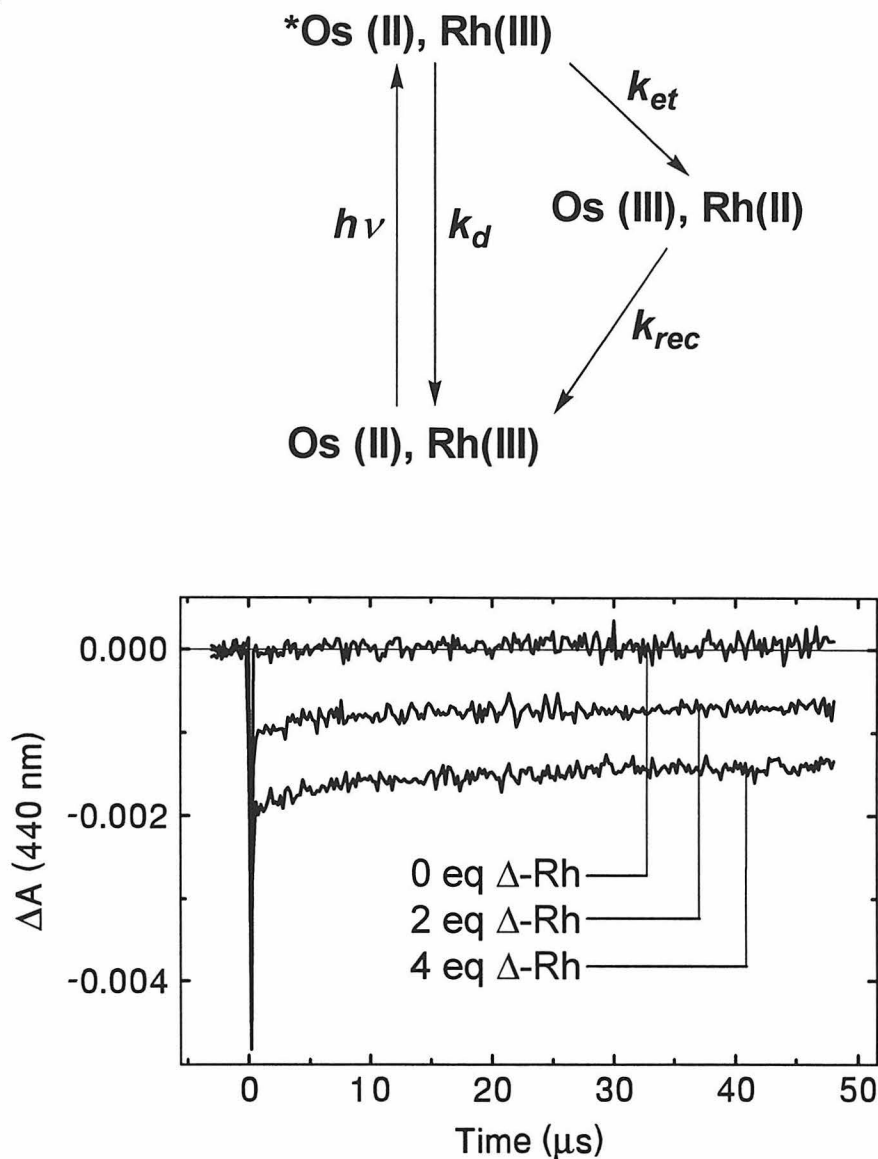


Figure 3.7. Transient signals observed at 440 nm upon excitation (480 nm) of $[\text{Os(phen)}_2(\text{dppz})]^{2+}$ bound to DNA in the presence of 0, 2 and 4 equivalents $\Delta\text{-Rh}$ $[\text{Rh(phen)}_2(\text{bpy})]^{3+}$. As the rhodium concentration is increased, a long-lived signal is observed. The kinetics of these transients are multiphasic with lifetimes on the order of 5 μs and longer. Conditions: 20 μM Os, 2 mM nucleotides in 5 mM tris, 50 mM NaCl, pH 8.5.

the presence of Δ -[Rh(phi)₂(bpy)]³⁺, a long lived signal which persists well into the microsecond regime grows in with increasing acceptor concentration. Owing to the correlation between transient signal intensity and acceptor concentration, such a signal likely reflects an electron transfer intermediate. By analogy to photoinduced electron transfer studies with other polypyridyl complexes,^{18,26} we expected this intermediate to correspond to the oxidized donor.

In an effort to assign unambiguously the observed long-lived transient to the Os(III) oxidized donor, the signal height as a function of wavelength was measured and compared to known Os(III)-Os(II) difference spectra. A static Os(III)-Os(II) difference spectrum was obtained by chemical oxidation of [Os(phen)₂(dppz)]²⁺ to [Os(phen)₂(dppz)]³⁺ with Ce(IV).¹⁸ Additionally, transient absorption spectroscopy was used to observe Os(III) generated by oxidizing photoexcited [Os(phen)₂(dppz)]²⁺ bound to DNA with the well-characterized electron acceptor [Ru(NH₃)₆]³⁺; the wavelength dependence of the signal obtained using this flash-quench methodology provides an Os(III)-Os(II) kinetic difference spectrum.

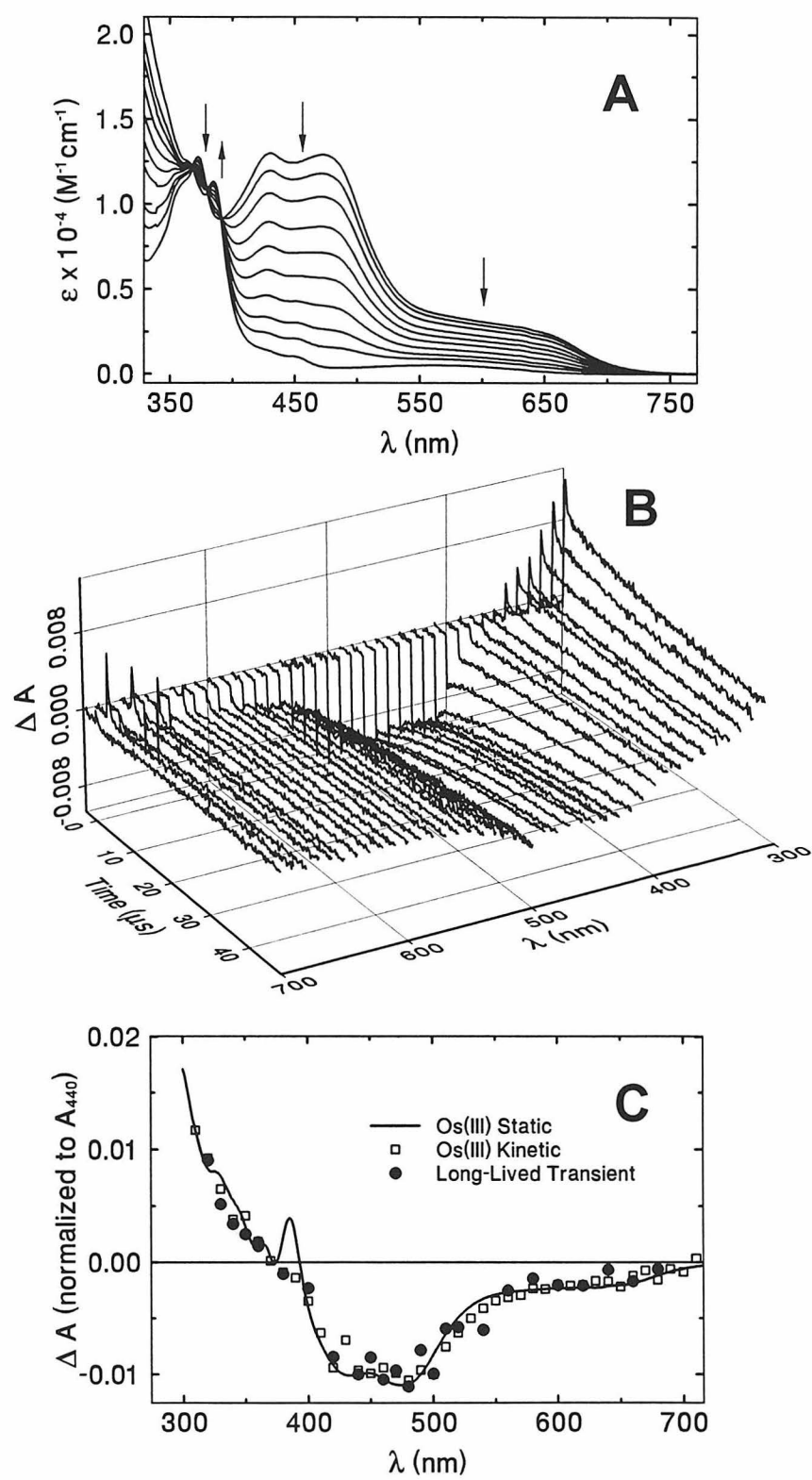
Figure 3.8A shows the spectral changes that accompany the oxidation of [Os(phen)₂(dppz)]²⁺ to [Os(phen)₂(dppz)]³⁺ by Ce(IV) in water. For the Os(II) species, the visible absorption is dominated by the formally singlet MLCT transitions (λ_{max} = 430 nm and 472 nm) and the triplet MLCT transitions which tail out beyond 720 nm. The π - π^* IL transition centered on the phenazine portion of the dppz ligand occurs with λ_{max} = 372 nm. In the oxidized spectrum, the intense charge-transfer transitions are no longer present and a weak transition ($\epsilon < 1000 \text{ M}^{-1}\text{cm}^{-1}$), probably ligand-to-metal charge-transfer (LMCT) in character, occurs around 600 nm.²⁷

Transient signals obtained by oxidation of photoexcited [Os(phen)₂(dppz)]²⁺ bound to DNA by [Ru(NH₃)₆]³⁺ are plotted in Figure 3.8B. In order to show the timecourse of the signal as well as its wavelength dependence, the changes in absorbance are plotted in a three-dimensional representation. To obtain a typical Os(III)-Os(II)

kinetic difference spectrum, we simply extract a time slice ($t > \tau_{\text{MLCT}}$) from this plot.

In Figure 3.8C we show (as a solid line) the static Os(III)-Os(II) difference spectrum constructed by subtracting the $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ absorption spectrum from that of the oxidized complex. Also in Figure 3.8C, plotted as an overlay above the static difference spectrum, is the wavelength dependence in the absorbance changes observed for the oxidation of photoexcited $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA. As expected, both

Figure 3.8. Spectral characterization of the chemical and photochemical oxidation of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$. (A) Spectral changes which accompany the oxidation of aqueous $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ (~ 0.1 mM) to $[\text{Os}(\text{phen})_2(\text{dppz})]^{3+}$ with the addition of dilute $\text{Ce}(\text{NO}_3)_6^{2-}$. (B) Transient absorption signals obtained by oxidation of photoexcited $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ (15 μM) bound to DNA (1.5 mM nucleotides in 5 mM tris, 50 mM NaCl, pH 8.5) by $[\text{Ru}(\text{NH}_3)_6]^{3+}$ (300 μM). The full time course of the transient signal intensity is also plotted as a function of wavelength to show the time dependence of the resulting spectrum. (C) Static Os(III)-Os(II) difference spectrum (solid line) generated by subtracting the Os(II) absorption spectrum from that of the fully oxidized complex. Also shown are the Os(III)-Os(II) kinetic difference spectrum ($\square$) taken from the signal height of the long-lived transient in Figure 3.7B, and the wavelength dependence of the rhodium-induced transient ($\bullet$) observed with quenching of intercalated $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ (Figure 3.6). For ease of presentation, the spectra plotted here are normalized at 440 nm owing to substantial hypochromism from 300 - 500 nm for the complex bound to DNA.



the static and kinetic Os(III)-Os(II) difference spectra are in good agreement. Noteworthy, however, is the deviation at ~ 375 nm which is attributed to the hypochromism of the dppz IL band in the kinetic spectrum where the complex is bound to DNA.

We also present the spectrum of the transient observed for the quenching of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ by $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ in Figure 3.8C. Indeed, throughout the wavelength range investigated, this signal is in excellent agreement with the known Os(III)-Os(II) difference spectra. Thus, as anticipated, we attribute the observed transient to an Os(III) electron transfer intermediate. Like the kinetic difference spectrum obtained by oxidizing photoexcited $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA, the difference spectrum for this Os(III) intermediate deviates slightly from the static Os(III)-Os(II) difference spectrum. This deviation is again derived from the hypochromism in the dppz IL band and, importantly, shows that the complex remains bound throughout the reaction. This spectral feature indicates that neither the transient observed nor the quenching observed arise from a trivial displacement of the complex from the helix, consistent with the $k_{\text{off}} < 100 \text{ s}^{-1}$ determined by NMR¹¹ for the isostructural ruthenium analog. The observation of such an electron transfer intermediate establishes photoinduced electron transfer as the operative mechanism in the fast quenching of emission from intercalated $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ by $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$.

Investigation of Back Electron Transfer by Ultrafast Transient Absorption Spectroscopy on the Picosecond Time Scale. The long-lived electron transfer intermediate formed by the oxidative quenching of photoexcited $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ by $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ in the presence of DNA described above accounts for only $\sim 10\%$ of the total population of donor that undergoes electron transfer. We reasoned that the majority of the back electron transfer reaction was taking place on a time scale faster than the resolution of the instrumentation employed on the microsecond time scale.

Therefore, we used transient absorption spectroscopy with femtosecond resolution to follow the ground state recovery of photoexcited Δ -[M(phen)₂(dppz)]²⁺ bound to DNA in the presence and absence of Δ -[Rh(phi)₂(bpy)]³⁺. Figure 3.9 shows the time-resolved transient absorption data at 420 nm following irradiation of Δ -[Os(phen)₂(dppz)]²⁺ bound to CT DNA in the presence of 0, 1, 2, and 3 equiv of Δ -[Rh(phi)₂(bpy)]³⁺. As expected for either the MLCT excited state of Os(II), or an Os(III) intermediate formed as the product of electron transfer, the ΔA at 420 nm is negative. In the absence of the rhodium complex, the return of this signal to baseline over time exhibits the same kinetics as the luminescence decay of Δ -[Os(phen)₂(dppz)]²⁺ bound to DNA.

As Δ -[Rh(phi)₂(bpy)]³⁺ is titrated into the solution, a fast phase appears in the ground state recovery of the bleach signal. Owing to the fact that this fast phase is not detected in the luminescence of the donor under the same conditions, we assign this component of the ground state recovery to the recombination reaction (k_{rec}). As the concentration of rhodium increases, the contribution of this fast phase to the overall decay increases.

The time constant, or lifetime ($1/k_{\text{rec}}$), that corresponds to this recombination reaction in the presence of 1 equiv of Δ -[Rh(phi)₂(bpy)]³⁺ is 100 ps. Such a short lifetime for the back electron transfer reaction is remarkable under the conditions of this experiment owing to the low loadings of donor and acceptor on the helix. The ratio of metal to DNA is 1:25 with a 1:1 ratio of rhodium to osmium. Even more remarkable, however, is the fact that as the concentration of rhodium increases, the magnitude of k_{rec} remains essentially constant. In other words as the average donor and acceptor separation--based on a random distribution of metallointercalators on the helix--decreases from 25 bp (1 equiv Δ -Rh) to 12.5 bp (3 equiv Δ -Rh), the rate of electron transfer does not change. This loading independent lifetime of ground state recovery is consistent with the DNA base stack serving as a medium for electron transfer reactions with a shallow dependence upon distance.

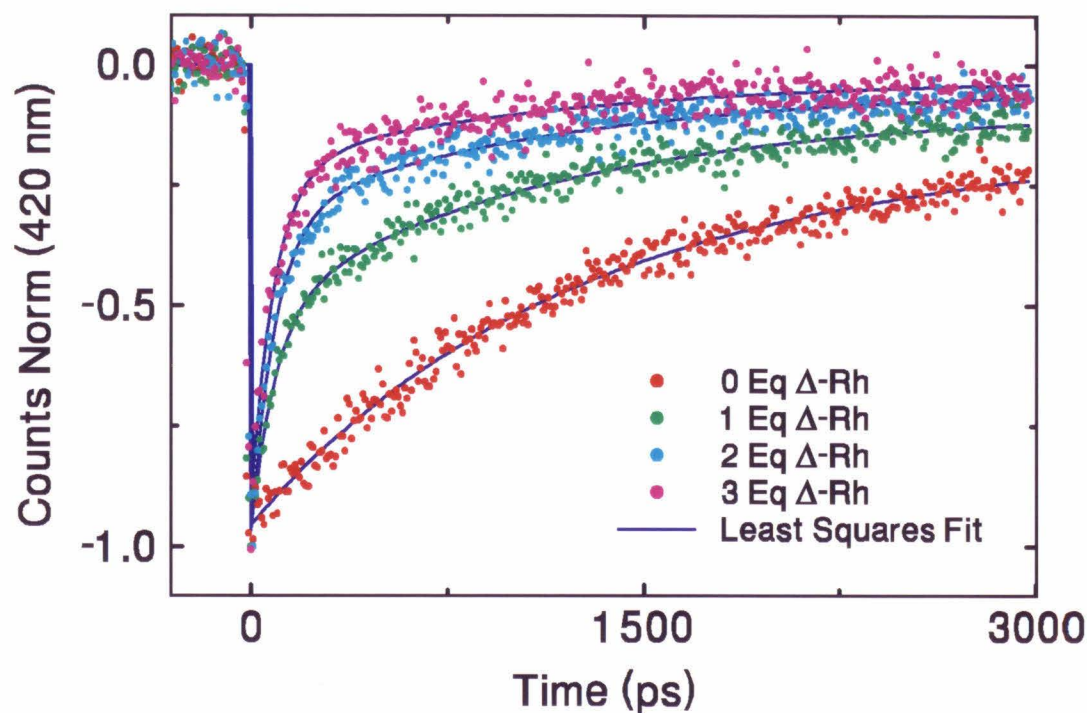


Figure 3.9. Transient absorption at 420 nm as a function of time following the irradiation (390 nm) of Δ -[Os(phen)₂(dppz)]²⁺ bound to CT DNA in the presence of 0, 1, 2, and 3 equiv Δ -[Rh(phi)₂(bpy)]³⁺. A computer generated fit overlays each data set. The Figure Legend indicates the assignments of plot attributes. For clarity the data were normalized to the zero time point.

3.2.4. Redox Properties of $[M(\text{phen})_2(\text{dppz})]^{2+}$ and Related Complexes.

Cyclic Voltammetry. CV was used to characterize the oxidation and reduction potentials of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$. These results along with similar data for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ are listed in Table 3.2.^{10a,18,28} A reversible oxidation wave corresponding to the 3+/2+ couple is observed at 1.1 V (vs NHE). By analogy to the reduction of $[\text{Ru}(\text{bpy})_2(\text{dppz})]^{2+}$ and $[\text{Os}(\text{phen})_3]^{2+}$ measured by CV, the reduction at -0.62 V is assigned to a one-electron reduction centered on the dppz ligand, while the remaining two correspond to one-electron reductions on the phenanthrolines. As expected,¹⁸ oxidation of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ is ~ 500 mV more favorable compared to the ruthenium complex. Additionally, based on an E_{00} value of 1.9 eV for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$,²⁹ the excited state of the osmium complex is ~ 200 mV more reducing compared to that of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$. The reduction potentials of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$, however, are not significantly different from those of the ruthenium analog. These observations are consistent with CV data for other polypyridyl complexes of ruthenium and osmium¹⁸ and indicate a similar absolute energy for the HOMO in the MLCT excited-state of $[M(\text{phen})_2(\text{dppz})]^{2+}$ (M = Os(II), Ru(II)) complexes.

Table 3.2. Electrochemical potentials^a for Osmium and Ruthenium Complexes.

Complex	Metal Centered ^{b,c}		Ligand Centered ^c		
	(*2+/3+)	(3+/2+)	L1/L1--	L2/L2-	L3/L3-
[Os(phen) ₂ (dppz)] ²⁺	0.76	1.16	-0.61	-0.98	-1.21
[Os(phen) ₃] ²⁺		1.11	-0.92	-1.06	
[Ru(phen) ₂ (dppz)] ²⁺ ^d	0.60	1.60			
[Ru(bpy) ₂ (dppz)] ²⁺ ^e		1.48	-0.62	-1.06	-1.27

a. In dry DMF with 0.1 M tetrabutylammonium hexafluorophosphate; scan rate = 0.1 V/s..

b. Excited-state oxidation potential reported in eV

c. Potentials are reported in volts vs NHE.

d. Reference 10a.

e. Reference 28, converted to NHE.

3.3. DISCUSSION

3.3.1. DNA Binding Properties of Isostructural $[M(\text{phen})_2(\text{dppz})]^{2+}$.

A common way to expand the scope of redox reactions between polypyridyl metal complexes is to modify the ligand properties of the reactants. However, in DNA-mediated electron transfer reactions, we find that the DNA binding properties of the reactants play a significant role in determining the efficiency of the reaction, and these binding profiles are sensitive to small perturbations in the ligand structures which make up the complex.^{11,15,25} Thus, in order to change the properties of our electron donor without altering its DNA binding properties, we have substituted osmium for ruthenium in complexes of the type $[M(\text{phen})_2(\text{dppz})]^{2+}$. Based on the lanthanide contraction, there is very little difference in the ruthenium and osmium ionic radii.³⁰ Even if there were a small difference in the size of the metal center, such a difference would be insignificant compared to the full dimensions of the coordination complex. Hence, substitution of osmium for ruthenium should not perturb the binding profile of the metal complex given the same ligand sphere.

Absorption and Emission of $[M(\text{phen})_2(\text{dppz})]^{2+}$ Complexes Bound to DNA.

We have shown $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ to bind to DNA by intercalation of the dppz ligand. Evidence for intercalation is provided by the strong hypochromism in the IL π - π^* transition centered on the dppz ligand observed by visible spectroscopy. This intercalation of the dppz ligand gives rise to the molecular light switch properties of the complex¹⁷ since emission in aqueous solutions is observed only when the dppz nitrogens are protected from solvent, as provided by intercalation. Therefore, since only complexes bound to the polymer emit, we have been able to examine the binding profile of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ by studying the luminescence as a function of DNA concentration by both steady-state and time-resolved techniques.

At intermediate and high $R_{\text{nuc:Os}}$ values, we find that, in the presence of DNA, Δ - $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ emission decays as a biexponential, with lifetimes in the range of

1.5 -2.0 ns and 9.2 - 13 ns (Table 3.1). Such a biphasic emission profile, observed previously for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$, has been attributed to two intercalative binding geometries which provide differential protection of the phenazine nitrogens (see Chapter 2).¹¹ Within this model, which has been supported by two-dimensional ^1H NMR studies,¹¹ the shorter lived species corresponds to an intercalative binding geometry with the dppz ligand oriented along the base-pair axis in a side-on fashion, and the longer lifetime reflects an intercalative geometry where the ligand is intercalated perpendicular to the base-pair axis in a head-on mode. The percent contribution of each lifetime to the overall luminescence decay reflects the relative distribution of side-on and head-on binding populations. As indicated in Table 3.1, at intermediate loadings, the two geometries are present in almost a 1:1 ratio. As the concentration of DNA is increased, the shorter lived species begins to dominate the decay.

As shown in Figure 3.4, the steady-state emission intensity for Δ - $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ increases with increasing DNA concentration for $R_{\text{nuc:Os}}$ values ranging from 0 - ~5, with a sharp increase from 5 - 6.25, where the emission yield is maximized. At $R_{\text{nuc:Os}}$ values greater than 6.25, the emission yield is seen to decrease with increasing DNA concentration over a broad range of $R_{\text{nuc:Os}}$ values. The increase in emission yield with increasing DNA concentration reflects the binding of more complexes to the polymer, since only those complexes bound to DNA show detectable emission. The large enhancement in emission yield going from $R_{\text{nuc:Os}} = 5$ to 6.25 likely reflects self quenching of the emission in the early region of the titration where the complexes are highly loaded onto the DNA.³¹ This titration differs from others²⁴ in that DNA is titrated into lumiphore solution, which results in high loadings of metal on the DNA in the early portions of the titration; high loadings promote self quenching. Self quenching of emission has also been observed for $[\text{Ru}(\text{TMP})_3]^{2+}$ (TMP = 4, 5, 6, 7-tetramethyl-1,10-phenanthroline) complexes bound cooperatively to DNA without intercalation. The quenching experiments performed here with rhodium acceptors are

accomplished at substantially higher ratios of DNA/metal.

The observed decrease in emission yield for $R_{\text{nuc:Os}} > 6.25$ is manifested by a change in luminescence decay profile (Table 3.1). For large $R_{\text{nuc:Os}}$ values, the side-on binding mode, which exhibits a shorter excited-state lifetime, contributes most to the overall decay, leading to a decrease in steady-state emission intensity. The DNA concentration dependence in the relative distribution of side-on and head-on binding populations may reflect spatial constraints on binding geometry when the helix is heavily loaded with complexes. In addition to the changes in binding orientation, the excited-state lifetimes for each binding geometry also decrease slightly with increasing DNA concentration. Such a decrease may also reflect a concomitant decrease in helical rigidity as the metal complexes become more dilute along the polymer, giving rise to a greater propensity for vibrational deactivation of the excited state.

The kinetic analysis of luminescence data which describes the $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ binding characteristics is essentially identical to that used for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ previously.^{11,24} Thus, $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ provides an isostructural analog for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ while at the same time exhibiting a broader absorption profile, red-shifted emission with excited state decay kinetics on a shorter time scale ($\tau < 10$ ns), and access to a 3+ oxidation state which is stabilized by ~ 500 mV compared to the ruthenium species.

3.3.2. DNA-Mediated Photoinduced Electron Transfer

Donor Properties and Quenching Efficiency. As evident in the quenching titrations, emission from intercalated $\Delta\text{-}[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ is significantly quenched by $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, which also binds to DNA by intercalation. The decay profiles for $\Delta\text{-}[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ in the presence and absence of $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ show little difference, as the quenching is revealed as a loss in intensity at zero time. TCSPC with ~ 150 ps resolution allows us to set a lower limit of $7 \times 10^9 \text{ s}^{-1}$ for the quenching constant.

The quenching of Δ -[Os(phen)₂(dppz)]²⁺ as a function of acceptor concentration furthermore matches that of Δ -[Ru(phen)₂(dppz)]²⁺ quite closely. Since the spectral characteristics for the two complexes differ significantly, the quenching cannot depend strongly on spectral overlap of donor emission with acceptor absorption. In fact, spectral overlap of osmium emission and rhodium absorption is vanishingly small. Hence, *this correlation rules out any significant contribution by energy transfer to the quenching of either [Os(phen)₂(dppz)]²⁺ or [Ru(phen)₂(dppz)]²⁺ by [Rh(phi)₂(L)]³⁺ (L = bpy, phen).*

Effect of Donor Excited State Lifetime. Given the two orders of magnitude difference in excited state lifetimes for Δ -[Os(phen)₂(dppz)]²⁺ bound to DNA versus Δ -[Ru(phen)₂(dppz)]²⁺ bound to DNA, the similarity in [M(phen)₂(dppz)]²⁺ quenching profiles demonstrates a lack of sensitivity in quenching efficiency to the excited-state lifetimes of the osmium and ruthenium donors. The absence of such a dependence suggests that diffusion plays only a minor role, if any, in the quenching, a result consistent with the notion that the electron transfer is a first order process, proceeding through the DNA double helix. This observation is in contrast to experiments with tris(phenanthroline) metal complexes as reactants which bind DNA both by intercalation and groove binding.⁹ Interestingly, we observe that the static quenching of Δ -[Ru(phen)₂(dppz)]²⁺ by Δ -[Rh(phi)₂(bpy)]³⁺ is accompanied by a minor (< 10%) component of slower quenching occurring on a time scale of ≥ 100 ns. Since the excited state lifetime of Δ -[Os(phen)₂(dppz)]²⁺ bound to DNA is less than 10 ns, similarly slow processes could not contribute to quenching of the osmium complex.

Considerations of Donor Energetics. Substitution of osmium for ruthenium in [M(phen)₂(dppz)]²⁺ complexes allows evaluation of the role that energetic parameters such as the 3+/2+ redox couple and the excited-state oxidation potential of the donor play in determining electron transfer quenching efficiency. As illustrated in Table 3.2, [Os(phen)₂(dppz)]³⁺ is stabilized by ~500 mV over [Ru(phen)₂(dppz)]³⁺, and the excited state of the osmium complex is ~200 mV more reducing compared to the ruthenium

analog. As described above, the greater potential for reduction by the osmium excited state may give rise to a slight increase in quenching efficiency, but to first order, the efficiency of quenching photoexcited $[M(\text{phen})_2(\text{dppz})]^{2+}$ complexes bound to DNA by intercalated $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ is largely insensitive to both the $3+/2+$ redox couple and the excited-state oxidation potential. Importantly, since we have not resolved the individual rates of forward electron transfer for the osmium and ruthenium donors, the comparison holds here only for the efficiency of the quenching reaction.¹⁵

As an explanation for such an observation, we consider the absolute energies of the $[M(\text{phen})_2(\text{dppz})]^{2+}$ excited state HOMOs. Reduction of the phenazine portion of the dppz ligand occurs at essentially the same potential in $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$. Furthermore, the difference between $E_{00}(\text{Ru})$ and $E_{00}(\text{Os})$ is represented by the difference in the $3+/2+$ reduction potentials for the two complexes. Thus, based on these data and the MLCT character of the $[M(\text{phen})_2(\text{dppz})]^{2+}$ excited states, the excited state HOMOs are judged to be equivalent in absolute energy.³³ Such an orbital arrangement is consistent with the large differences between $E_{\text{MLCT}}(\text{Os})$ and $E_{\text{MLCT}}(\text{Ru})$ being manifested by the difference in energy between the $5d^6$ and $4d^6$ ground state configurations for osmium(II) and ruthenium(II), respectively. Therefore, if the efficiency of photoinduced electron transfer from $[M(\text{phen})_2(\text{dppz})]^{2+}$ were limited by electronic access to a high energy DNA bridge originating from the donor excited state,^{1,2} the efficiency of quenching would be expected to be similar for osmium and ruthenium.

An alternative explanation for the observed leveling effect with respect to free energy for these photoinduced reactions lies in the possibility that electron transfer proceeds from the donor excited state into an acceptor excited state.³⁴ When the formation of reduced acceptor in the excited state via photoinduced electron transfer is thermodynamically favorable, such a reaction may be faster than electron transfer to ground state acceptor if the latter reaction is in the inverted region. Indeed, the inverted

effect, in which rates decrease with increasing driving force (Marcus, R. A. *J. Chem. Phys.* **1965**, *43*, 2654.), has only been observed in recombination reactions. In our case, the $^*\text{Rh(II)}$ excited state energy is likely to be 1.8-2.0 V, thus electron transfer could still be favorable from both $^*[\text{Ru(phen)}_2(\text{dppz})]^{2+}$ and $^*[\text{Os(phen)}_2(\text{dppz})]^{2+}$. For photoinduced electron transfer to ground state rhodium acceptor to fall in the inverted region, the reorganization energy must be low ($\lambda < 0.6$ V), a reasonable possibility for a reaction in which DNA bases act as solvent.

Loading Dependence of Quenching Efficiency. While quenching reactions between donors and acceptors bound noncovalently and randomly to DNA are not intended to provide a quantitative evaluation of the distance dependence of DNA-mediated electron transfer, we may, nonetheless, point to unique aspects of these quenching titrations as a function of loading on the helix. At one equivalent of acceptor, we observe ~50% quenching of the osmium lumiphore, but only 2% of donor-acceptor separations are expected at closest contact with random binding of the metallointercalators on the helix. Importantly, while quenching must be possible at longer distances, it can also not occur over infinite distance, since this latter case would require complete quenching of donor luminescence at 1 equivalent of acceptor. Hence our observation of 50% quenching under these conditions demonstrates that the quenching reaction does indeed exhibit a loading dependence and these quenching titrations are qualitatively consistent with the occurrence of electron transfer events at separations of up to 20 base pairs.³² The application of a sphere of action model to earlier quenching titrations^{10a} of $\text{rac-}[\text{Ru(phen)}_2(\text{dppz})]^{2+}$ bound to DNA with $\text{rac-}[\text{Rh(phi)}_2(\text{phen})]^{3+}$ yielded ~10 base pairs as the critical distance required for reaction. For the quenching titrations described here using enantiomers bound to DNA, deviations from such a model are found, because quenching is more efficient at lower loadings.

3.3.3. Electron Transfer Intermediates and Back Electron Transfer.

Electron Transfer Intermediates on the Microsecond Time Scale. Just as the lack of spectral overlap between the osmium emission and rhodium absorption points to photoinduced electron transfer rather than energy transfer, the observation of an intermediate firmly establishes electron transfer as the quenching mechanism. Transient absorption spectroscopy allowed us to detect a long-lived signal which grows in with increasing quencher concentration. Using Ce(IV) to oxidize $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ free in solution and $[\text{Ru}(\text{NH}_3)_6]^{3+}$ to oxidize $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA photochemically, we have generated Os(III)-Os(II) difference spectra. Comparison of the difference spectrum of the long-lived transient, observed for the quenching of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ by $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to DNA to the known Os(III)-Os(II) difference spectra verifies that the signal observed corresponds to the oxidized donor, $\text{Os}(\text{phen})_2\text{dppz}^{3+}$. Similar spectral evidence for an electron transfer intermediate produced in the DNA-mediated electron transfer quenching of $[\text{Ru}(\text{dmp})_2\text{dppz}]^{2+}$ by $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ has been reported;¹⁶ however, such a full spectral characterization could not be achieved.

While providing a description of the oxidized donor, the difference spectrum of the rhodium-induced intermediate yields little information concerning the reduced acceptor ($[\text{Rh}^{\text{II}}(\text{phi})_2(\text{bpy})]$ or $[\text{Rh}^{\text{III}}(\text{phi})(\text{phi})^-(\text{bpy})]$). Indeed, according to Scheme 1, the spectrum of the electron transfer intermediate should contain some contribution from the reduced acceptor. However, the closeness of the intermediate spectrum to the known Os(III)-Os(II) difference spectrum indicates that the change in extinction coefficient upon reduction of the acceptor bound to DNA must be small relative to that for oxidation of the donor.³⁵ Importantly, transient absorption experiments indicate a value of $\Delta\epsilon \leq 3000 \text{ M}^{-1}\text{cm}^{-1}$ for the reduction of $[\text{Rh}(\text{phi})_2(\text{L})]^{3+}$ ($\text{L} = \text{phen}, \text{bpy}$) free in solution. Based on our lack of observation of the reduced acceptor in these DNA-mediated electron transfer reactions, this change in extinction coefficient must be attenuated for the complex bound to DNA. In addition, some decomposition is possible, since the phi ligand or its radical

anion is expected to be unstable if dissociated from the metal center

In addition to formation of Os(III) via photoinduced electron transfer from the Os(II) excited state to ground state Rh(III), the same intermediate would also be formed by hole transfer if a Rh(III) excited-state were to oxidize ground state Os(II). While such a mechanism has been observed recently in a Ru(II)/Rh(III) dyad,²⁶ we have been able to rule out hole transfer here by shifting the excitation to wavelengths where the Rh(III) complex does not absorb. Indeed, the fraction of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ emission quenched by $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ is not changed whether excitation occurs at 480 nm, 532 nm, or 580 nm, indicating that the Rh(III) excited state does not quench the Os(II) emission. Furthermore, we observe the same long-lived electron transfer intermediate with each of the above excitation wavelengths, and therefore, production of Os(III) must occur by electron transfer rather than hole transfer. Moreover, we can eliminate any questions of multiphoton chemistry in the production of Os(III) since observation of this intermediate is not systematically affected by variations in power or excitation wavelength.

Long-lived species corresponding to the oxidized electron donors have now been observed for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Ru}(\text{dmp})_2(\text{dppz})]^{2+}$, but in the case of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$, no such intermediate can be detected. The distinction between $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ in terms of long-lived intermediates is noteworthy given the similarity in their quenching profiles. To account for these differences, we again consider the relative energetics, or stabilities, of the osmium and ruthenium species. Based on the larger (~ 500 mV) reduction potential for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{3+}$ compared to that for $[\text{Os}(\text{phen})_2(\text{dppz})]^{3+}$ (Table 3.2), the $[\text{Ru}(\text{phen})_2(\text{dppz})]^{3+}$ intermediate is more reactive.

One possible fate of a long-lived Ru(III) species may be reaction with the DNA by oxidation of a guanine base (see Chapter 4).^{8,31} The oxidation potential of guanine has been estimated to be between ~ 1.1 and ~ 1.5 V vs. NHE. Such a reaction would certainly be less favorable, likely prohibitively so, in the case of osmium, as has recently

been observed for intercalated $[\text{Os}(\text{bpy})_2(\text{dppz})]^{2+}$ generated by cyclic voltammetry.³⁷ When photoexcited $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ ($\text{M} = \text{Os}, \text{Ru}$) bound to poly d(GC) is oxidized by $[\text{Ru}(\text{NH}_3)_6]^{3+}$ a long-lived M(III) intermediate is observed only for $\text{M} = \text{Os}$, an observation which suggests that electron transfer from guanine is favorable only for the Ru(III) metallointercalator.

It is also instructive to compare the long-lived intermediates observed for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Ru}(\text{dmp})_2(\text{dppz})]^{2+}$. For the Os(III) intermediate, the long-lived signal observed grows in qualitatively but not quantitatively with quencher concentration. For the ruthenium species, the intermediate signal height parallels the fraction quenched. In contrast, the signal height for the Os(III) intermediate is seen to double going from 2 to 4 equivalents of acceptor while the amount of quenching increases modestly from 73% to 80%. Additionally, the yield ($< 10\%$) of the long-lived Os(III) species is less than the yield (10 - 30%) of long-lived $[\text{Ru}(\text{dmp})_2(\text{dppz})]^{3+}$. We can account for this difference in yields since for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$, more back electron transfer is occurring with k_{rec} in the 10^{10} s^{-1} regime than for $[\text{Ru}(\text{dmp})_2(\text{dppz})]^{2+}$.¹⁵ We do not yet understand the basis for this wide range of back electron-transfer rates. The fast recombination may result in part from electron transfer from the rhodium excited state manifold (*vide supra*) with slower reaction occurring from the rhodium ground state. Additionally, such a distribution may be the consequence of a decoupling of donor and acceptor by defects in base stacking, which arise from the structural polymorphism of DNA and have an increasing probability of occurring with an increase in donor-acceptor separation.

Back Electron Transfer on the Picosecond Time Scale. The back electron transfer reaction that follows photoinduced electron transfer between metallointercalators bound to DNA (Scheme 1) occurs on over two time domains for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ as the donor. In addition to the $\sim 10\%$ ground state recovery on the microsecond time scale, we observed the remaining fraction of the recombination reaction on a time scale of ~ 100

ps. Remarkably, the rate of this ultrafast electron transfer reaction was independent of the loading of acceptor on the helix over the course of a titration.

Such a loading independent electron transfer rate indicates that electron transfer reactions mediated by the DNA double helix exhibit a shallow distance dependence. An alternative explanation for the observation of loading independent kinetics of the recombination reaction is that the donors and acceptors bind cooperatively in adjacent sites so the reaction is actually occurring over only one distance. In fact computer-modeling studies have supported such a proposal, despite a lack of experimental evidence for cooperative binding of metallointercalators.

It is instructive to summarize the physical implications of a cooperative association model in the context of the conditions of the experiments on the picosecond time scale and the observations we recorded. We titrated the acceptor, Δ -[Rh(phi)₂(bpy)]³⁺ into solutions of Δ -[Os(phen)₂(dppz)]²⁺ bound to DNA at a ratio of osmium to bp of 1:50. We demonstrated experimentally that the rhodium complex binds to DNA in a sequence neutral fashion and as described above for the osmium complex, evidence indicates that this complex does not bind in the vicinity of other complexes. When 1 equiv of Δ -[Rh(phi)₂(bpy)]³⁺ was been added to the donor bound to DNA, >50% of the donors are quenched. Moreover, the fraction of the donor population quenched remains ~50% even when the concentration of metal is decreased by a factor of 10, or when the concentrations of metal and DNA are increased by a factor of 5. So, the physical meaning of a cooperative binding model designed to account for the remarkable observations described here is as follows. Even in the presence of a tremendous excess of DNA, for which the metallointercalators have a binding affinity of >10⁷, a cooperative interaction drives half of the population of rhodium to bind near half of the population of osmium. At this point the metal to DNA ratio is 1:25, hardly different from the 1:50 that the first equiv of rhodium encounters, and yet, the second equiv of Δ -[Rh(phi)₂(bpy)]³⁺ only quenches ~50% of the remaining donors (total fraction quenched $\simeq$ 75-80%).

The fact that the modeling studies suggest that cooperative binding accounts for the observations described, only shows that the reactivity between metallointercalators bound noncovalently to DNA does not behave as one would expect for an electron transfer reaction mediated by σ bonds. Our experiments accomplish the same task. If one wants to really consider the distance dependence of charge transfer through DNA, one needs to construct assemblies where the donor-acceptor separations are well-defined and fixed.

Summary on the Assignment of Electron Transfer as the Mechanism by which Metallointercalators are Quenched by Δ -[Rh(phi)₂(bpy)]³⁺. The detection of the long-lived intermediate, in conjunction with ultrafast emission and transient absorption measurements, allows us to assign electron transfer as the mechanism of quenching. Since the long-lived intermediate corresponds to only a small fraction of the electron transfer products, the observation of the transient on the microsecond timescale does not in itself prove an electron transfer mechanism for *all* of the luminescence quenching. Using TCSPC with resolution greater than that employed for this study, we have shown that the quenching constant is $> 3 \times 10^{10} \text{ s}^{-1}$ for both $^*[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and $^*[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$, while transient absorption measurements show that for quenched donors, most of the ground state recovery occurs with a slower rate of $\sim 1 \times 10^{10} \text{ s}^{-1}$. Because no 10^{10} s^{-1} component is present in the emission dynamics, the kinetics measured by picosecond transient absorption must represent the recombination reaction. The fact that not all of the back electron transfer occurs on the picosecond timescale is reflected in the detection of a long-lived intermediate. Taken together, these results clearly show that electron transfer is the quenching mechanism on all timescales.

3.3.4. Implications for Long-Range Electron Transfer. Metallointercalators allow us to probe systematically how the DNA π -stack might mediate electron transfer. Here, we have described the electron transfer reactions between $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$, an

isostructural analog for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$, and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to DNA. For these donors we have observed substantial quenching despite low reactant loadings on the helix. Moreover, we demonstrate here for osmium that the quenching is remarkably fast ($k_{\text{et}} > 7 \times 10^9 \text{ s}^{-1}$).

Given these unique quenching reactions, the results point either to fast quenching processes occurring over large separations of donor from acceptor on the helix or to the cooperative clustering of donor with acceptor on the helix. Spectroscopic and photocleavage studies have been consistent with random binding of donors and acceptors and show no evidence for a cooperative interaction between the intercalators.^{11, 12, 15} Moreover quenching studies in anionic micelles,²⁵ where clustering might be expected to be encouraged, also have shown no evidence for cooperativity and instead reveal dynamic quenching on the nanosecond timescale. Furthermore, the titrations described here for osmium and elsewhere for ruthenium^{10a} are consistent with results obtained on the mixed-metal oligomeric assembly in which ruthenium and rhodium intercalators were covalently attached and stacked at either ends of a 15-mer duplex where quenching was observed on a timescale $> 10^9 \text{ s}^{-1}$.^{10b}

Here osmium analogues have been valuable in elucidating and eliminating mechanistic explanations for this quenching. Importantly, we have established that electron transfer is the mechanism for this fast quenching, and, given the similarity in quenching profiles for osmium and ruthenium, energy transfer in the case of both ruthenium and osmium must be ruled out. Lastly, these studies with osmium illustrate the utility of applying coordination chemistry to the study of DNA-mediated electron transfer. Since the electron transfer reactions depend so sensitively upon coupling of the reactants into the DNA bridge, an isostructural metallointercalator which exhibits distinctive spectroscopic and redox characteristics becomes a valuable tool in dissecting the parameters which determine electron transfer through the DNA helix.

3.4. EXPERIMENTAL

3.4.1. Materials

$[\text{Os}(\text{phen})_2\text{dppz}](\text{PF}_6)_2$ was prepared as described in Chapter 2 and previously¹⁷ and exchanged to the dichloride salt by anion exchange chromatography (1:1 acetonitrile/water eluent) on Sephadex QAE-25 (Aldrich). $[\text{Rh}(\text{phi})_2\text{bpy}]\text{Cl}_3$ was also prepared as reported.²⁰ Trace impurities were rigorously excluded by high-performance liquid chromatography (HPLC). Resolution of the racemic mixtures was achieved using standard methodology.^{11d,e,12b,21} Resolution of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ proceeded with baseline separation of the two enantiomers on the anion-exchange column (see Chapter 2), and absolute configuration was assigned by comparison to Δ - $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$. For Δ - $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$, $\Delta\epsilon(270 \text{ nm}) = -270 \text{ M}^{-1}\text{cm}^{-1}$. Sonicated and phenol extracted calf thymus DNA (Sigma) was exchanged into standard buffer (5 mM tris, 50 mM NaCl, pH 8.5) by ultrafiltration (Amicon). $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$ was purchased from Aldrich and used as received.

3.4.2. Instrumentation

Time-correlated single photon counting (TCSPC) and time-resolved measurements on the nanosecond timescale were carried out using the facilities in the Beckman Institute Laser Resource Center (BILRC) described previously.^{17,22} TCSPC data sets were typically counted until 5,000 - 10,000 counts accumulated in the highest channel. Excitation for transient absorption and nanosecond timescale emission experiments was provided by an excimer-pumped dye laser (1-5 mJ/10 ns pulse) containing either Coumarin 480 (480 nm; Exciton) or Rhodamine 6G (580 nm; Exciton), or by a frequency-doubled Nd:YAG laser which provided 15-25 mJ/8 ns pulse at 532 nm. A pulsed 75 W Xe-arc lamp (Photon Technology International) served as the probe source for transient absorption experiments. Individual data sets were typically the

average of 400 - 2500 shots. Data fitting was accomplished by least-squares method of Marquardt using both in-house (BILRC) and commercial (Axum, Trimetrix) software. Emission spectra were recorded on an SLM 8000 spectrofluorimeter and standardized to $[\text{Ru}(\text{bpy})_3]^{2+}$ (emission maximum = 607 nm).²³ Ultraviolet-visible (UV-vis) spectra were recorded on a Cary 2200 spectrophotometer.

3.4.3. Methods

Photophysical studies were carried out at room temperature using aerated solutions. Emission quenching was quantitated by integration of the response-limited excited-state decays on the nanosecond timescale or by steady-state measurements; both methods gave equivalent results. Plots of the fraction of intensity quenched (fraction quenched = $1 - I/I_0$) versus quencher concentration are reported here, given the substantial quenching evident and the low signal to noise observed at high quencher concentrations which are overemphasized in plots of I_0/I . Importantly, plots of I_0/I for these data curve upward with increasing quencher, indicating that the Stern-Volmer analysis is not appropriate. Quenching titrations were typically carried out with the addition of aliquots of concentrated acceptor solution to samples containing DNA and donor. Identical results were obtained if separate samples were prepared for each quencher concentration.

Absorbance difference spectra of long-lived intermediates were generated as follows. Individual data traces at a given wavelength were fit to an exponential function at times $> 1 \mu\text{s}$ (20 x bandwidth), and the absorbance change obtained by extrapolation of the fit back to time zero, thus eliminating interference from the short-lived $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ excited state. The kinetics of the long-lived transients were independent of wavelength, indicating that a single reaction was being monitored.

Oxidation of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ to $[\text{Os}(\text{phen})_2(\text{dppz})]^{3+}$ was achieved by addition of 10 μL aliquots of a freshly prepared aqueous solution of $(\text{NH}_4)_2[\text{Ce}(\text{NO}_3)_6]$

(10 mg/mL), and monitored by UV-vis spectrophotometry.

3.4.4. Electrochemical Measurements

Reduction potentials were determined by cyclic voltametry (CV) using instrumentation described previously.^{10a} Complexes were dissolved in dry DMF (Fluka; stored over molecular sieves) containing 0.1 M tetrabutylammonium hexafluorophosphate as supporting electrolyte. Electrochemical windows ranging from 0.2 - 1.6 V vs. NHE for oxidation, and from 0.2 - (- 1.55) V vs. NHE for reduction, were scanned at a rate of 0.1 V/s.

3.5. REFERENCES

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Chapter 4: Sequence Dependent Quenching and Reactivity of Metallointercalators Bound to DNA.

4.1. INTRODUCTION

Electron transfer (ET) reactions are remarkably sensitive to the pathway that intervenes the donor and acceptor. In model systems based on small molecules, the chemical composition of the linker separating the two reactants determines the rate of ET over large separations.¹⁻⁶ Owing to the large number of different bonding interactions that comprise the secondary and tertiary structure of a folded protein, this environment has provided a useful matrix to characterize the dependence of ET on medium.^{1,2} In protein systems, the rate of ET depends significantly upon whether the pathway contains specific secondary structures such as β -sheets and α -helices, or combinations of these elements. We sought to investigate the importance of DNA sequence in determining the rate, efficiency, and distance dependence of ET mediated by the stacked bases, to illuminate the ability among all the DNA base stack to facilitate charge transfer reactions.

Nucleic acid polymers are composed of five naturally occurring bases: adenine (A), guanine (G), cytosine (C), thymine (T), and uracil (U). DNA contains A, G, C, and T in the form two base pairs doublets: AT and GC, and RNA contains A, G, C, and U as a substitute for T.⁷ In serving as a substrate for molecular recognition, the DNA helix can be thought of as an array of functional groups. The chemical nature (hydrophobic, hydrogen-bond donor/acceptor) and the relative disposition of these functionalities within a recognition element is determined by the DNA sequence.⁷ DNA sequence is responsible for determining other factors such as persistence length and bending, width of the major and minor grooves, and the flexibility of the helix.⁷ When a base is paired with

a partner other than the natural AT (AU) or GC pairing, the resulting “mismatch” can play an important role in the biological function of the parent nucleic acid.

As a biological matrix for ET, the sequence of the DNA that mediates the charge transfer is certain to play an important role in determining the fate of the reaction. There are large differences in the energy of the lowest unoccupied molecular orbital (LUMO) for each individual base.⁸ Thus the amount of energy required to reduce one base, or sequence will necessarily differ from that of another.

Moreover, it is well known that G is the most easily oxidized of the DNA bases.⁹ A variety of experimental and theoretical studies have shown that the 5'-G in 5'-GG-3' sequences is particularly susceptible to oxidation.¹⁰⁻²⁰ In theoretical studies of ET mediated by the stacked bases of DNA, Friesner and coworkers predicted that a more flexible sequence of DNA would be more effective at facilitating long-range ET.²¹ In addition, the reductive quenching of photoexcited ethidium by amsacrine is more efficient when the intercalators are bound to poly d(AT) compared to poly d(GC).^{22,23} Therefore a DNA base stack of composed of one sequence may have remarkably different ET properties from a base stack of another sequence.

As described in Chapter 3, photoinduced ET from Δ -[Os(phen)₂(dppz)]²⁺ to Δ -[Rh(phi)₂(bpy)]³⁺ in the presence of CT DNA proceeds on the subnanosecond time scale. The majority of the recombination reaction occurs with a lifetime of ~100 ps, while about 10% of the oxidized donors return to the ground state on the μ s time scale.

[Os(phen)₂(dppz)]²⁺ serves as an isostructural analog for [Ru(phen)₂(dppz)]²⁺ with significantly different photophysical and electrochemical properties.^{24,25} The application of these two complexes in comparative studies of ET is a powerful approach to investigate ET between metallointercalators bound to DNA, since the DNA binding

properties of the two probes are identical, but their physical properties differ significantly.

We showed that the efficiency of the photoinduced reaction is identical for the ruthenium and osmium donors (Chapter 3).²⁵ Owing to the red-shifted emission spectrum of the osmium complex, energy transfer to the Rh(III) acceptor is energetically disfavored. Based on the similarity in the quenching profiles for the Ru(II) and Os(II) complexes, energy transfer is not a consideration for either donor. The large difference in excited state lifetime for the two donors rules out diffusion as a mechanistic consideration in the quenching of $[M(\text{phen})_2(\text{dppz})]^{2+}$ by $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, which indicates that ET proceeds through the DNA bases.

This chapter describes our investigations of the effect of DNA sequence on ET reactions between metallointercalators bound to mixed sequence DNA and synthetic DNA polymers of defined sequence. The electron donors and acceptors employed in these studies are shown in Figure 4.1. The dppz complexes of Os(II) and Ru(II) are the parent molecules of a class of metallointercalators that serve as remarkably sensitive luminescent probes for DNA.²⁴⁻²⁷ Although these complexes luminesce upon irradiation in nonaqueous solvents, this emission is quenched in water by proton transfer to the phenazine nitrogens of the photoexcited complex. Upon binding to DNA by preferential intercalation of the dppz ligand in between adjacent base pairs, the phenazine nitrogens are protected and the complex luminesces.

As described in Chapter 2 for a family of dppz complexes of Os(II), the luminescence from these complexes in the presence of DNA is sensitive to a number of factors, especially the solvent accessibility of the phenazine nitrogens. Therefore, the excited state lifetime and luminescence profiles of the $[M(\text{phen})_2(\text{dppz})]^{2+}$ complexes

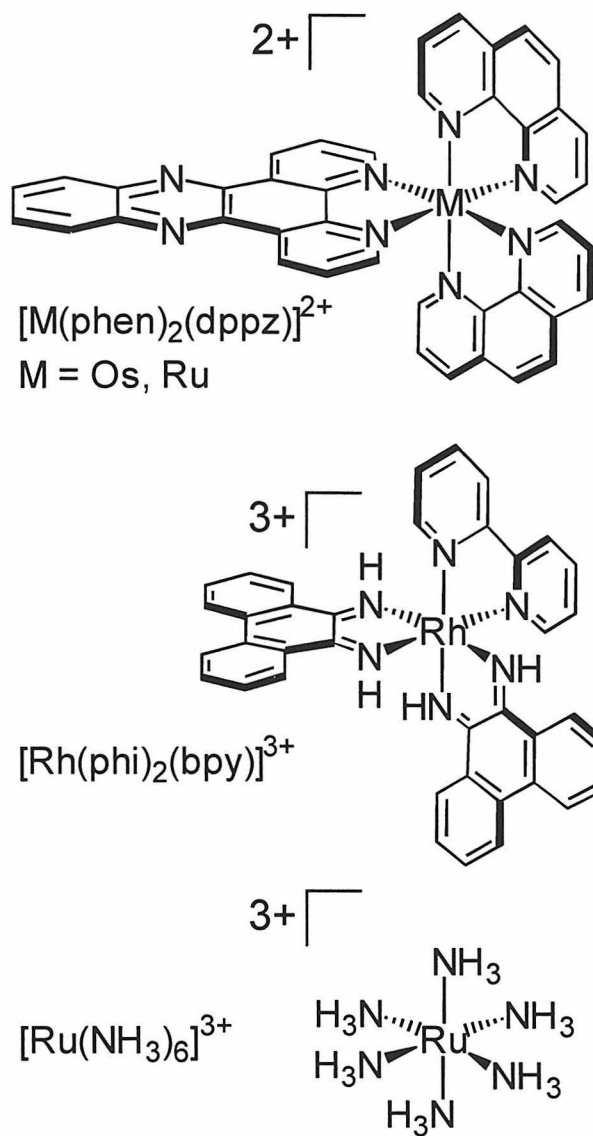


Figure 4.1. Transition metal complexes for DNA-mediated ET reactions. Shown schematically are Δ - $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ (top), Δ - $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ (middle), and $[\text{Ru}(\text{NH}_3)_6]^{3+}$ (bottom).

bound to DNA are indicative of how well the metallointercalator is stacked within the DNA helix and coupled into the π -stack.

We used two ground-state electron acceptors to quench the photoexcited donors bound to DNA (Figure 4.1). The metallointercalator, Δ -[Rh(phi)₂(bpy)]³⁺, binds tightly to DNA in a sequence neutral manner by intercalation of a phi ligand.^{28,29} We also employed [Ru(NH₃)₆]³⁺, which binds in the groove of DNA by a combination of surface binding, electrostatic interactions, and hydrogen bonding as an electron acceptor.³⁰ Reactions in which Δ -[Rh(phi)₂(bpy)]³⁺ is used to quench the intercalated donors are mediated by the DNA base stack, while the reactions with the groove bound acceptor require diffusion.

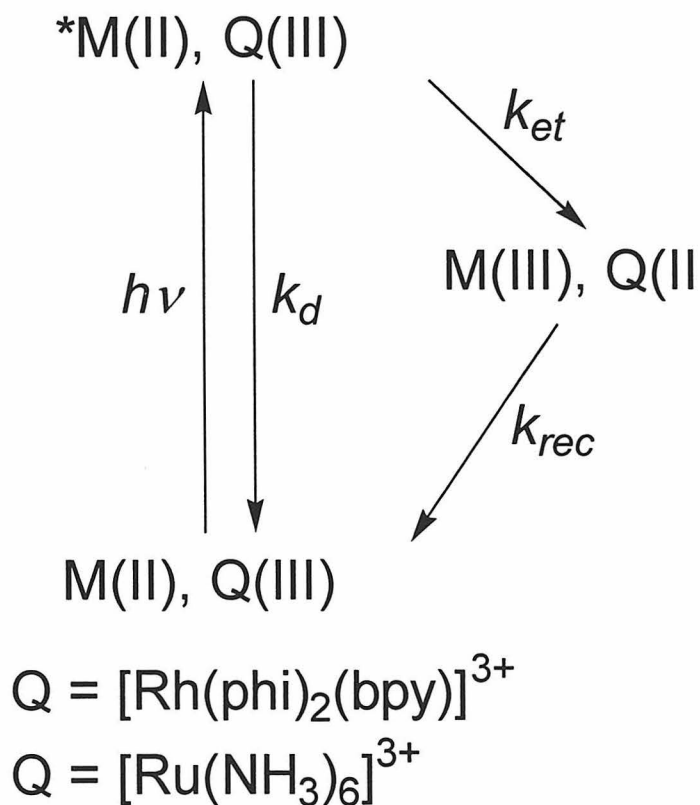
In addition to characterizing the effect of DNA sequence on photoinduced ET from intercalated donors, we also followed the fate of the products of ET. By comparing yields and rates of ground-state recovery of M(III) species formed in photoinduced ET reactions with intercalated or groove bound acceptors, we studied the sequence dependence in the rate and reactivity of these intermediates. Therefore, the comparison of ET reactions with an externally bound acceptor and those with an intercalated acceptor all as a function of DNA sequence provides a comprehensive investigation of how ET reactions between metallointercalators depend upon factors associated with the base sequence that defines the medium.

4.2. RESULTS

4.2.1. Sequence Dependent Quenching of Photoexcited [M(phen)₂(dppz)]²⁺ by Δ -[Rh(phi)₂(bpy)]³⁺ and [Ru(NH₃)₆]³⁺

Scheme 4.1 shows the cycle of photoinduced ET reactions between metal complexes bound to DNA. Upon excitation in the absence of an acceptor, the donor can

Scheme 4.1. Cycle of photoinduced ET reactions between $[M(\text{phen})_2(\text{dppz})]^{2+}$ donors and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, a metallointercalator, or $[\text{Ru}(\text{NH}_3)_6]^{3+}$, a groove bound complex. The forward reaction (k_{et}) quenches the emission of the photoexcited donor $[^*M(\text{II})]$ to give the intermediates $M(\text{III})$ and $Q(\text{II})$. These species return to the ground state by recombination (k_{rec}) or back ET.



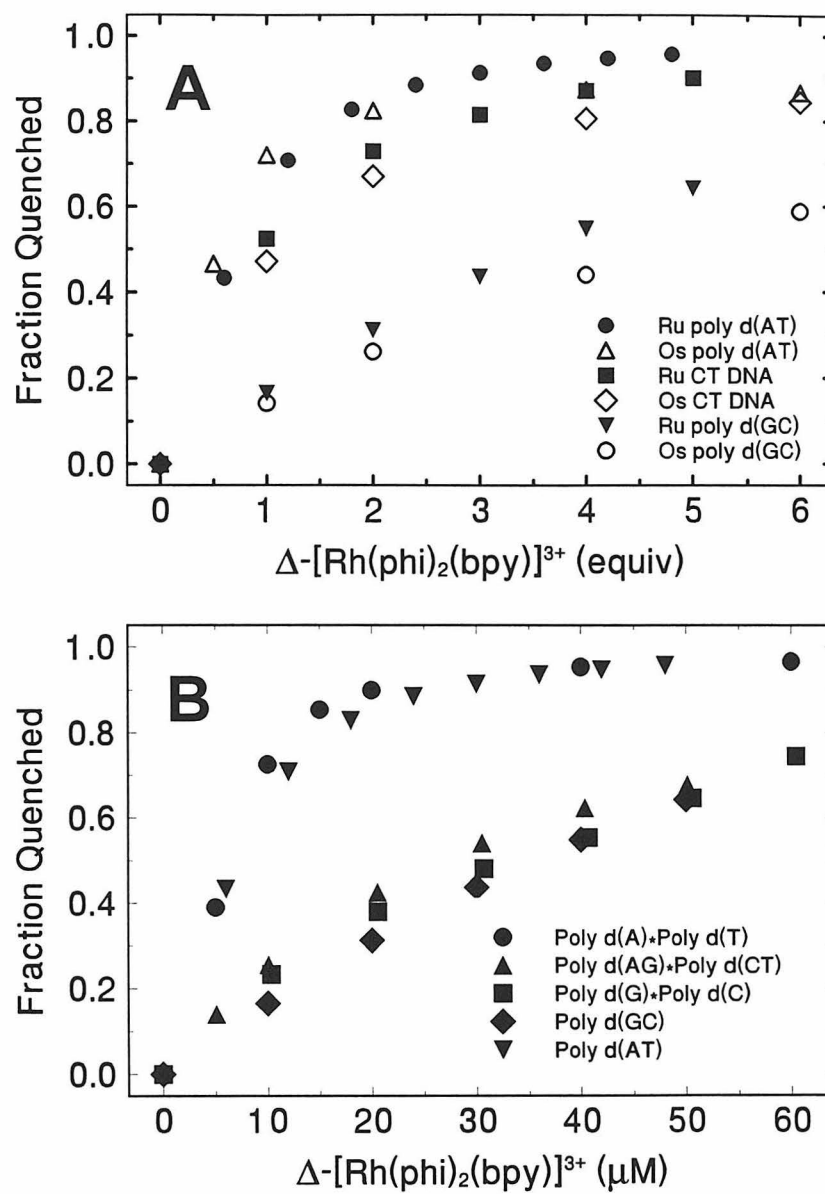
return to the ground state by emitting a photon. In the presence of an electron acceptor with an appropriate reduction potential, the photoexcited donor can reduce the acceptor, which results in quenching of the luminescence from the donor and the formation of two products of ET. These ET intermediates, which are higher in energy relative to their resting state, return to the ground state by thermal, back ET or recombination reaction. We employed fluorescence spectroscopy to monitor the yield and the rate of the forward reaction and transient absorption spectroscopy to identify and characterize the products of photoinduced ET and the rate of the recombination reaction.

Luminescence quenching by Δ -[Rh(phi)₂(bpy)]³⁺. Figure 4.2 shows the yield of photoinduced ET from intercalated Δ -[M(phen)₂(dppz)]²⁺ to Δ -[Rh(phi)₂(bpy)]³⁺ as a function of DNA sequence. In the presence of CT (mixed sequence) DNA and poly d(AT), the quenching profiles are remarkably similar. These titrations are characterized by very efficient quenching (~ 50%) with just 1 equiv of the rhodium complex, and saturation with 90% of the donors quenched with 5 equiv of rhodium.

The yield of ET for the complexes bound to poly d(GC) with increasing concentrations of Δ -[Rh(phi)₂(bpy)]³⁺ is significantly less efficient compared to the yields with CT and poly d(AT). In these titrations 1 equiv of Δ -[Rh(phi)₂(bpy)]³⁺ produces <20% quenching and the fraction of donors quenched increases almost linearly through the titration but never reaches saturation.

Despite the pronounced sensitivity of the photoinduced reaction to the composition of the DNA, the quenching profiles are essentially identical for osmium and ruthenium. As discussed in Chapter 3, this observation points to the yield of ET between metallointercalators having a weak dependence on the driving force for the reaction.

Figure 4.2. (A) Plot of fraction quenched ($F_q = 1 - I/I_0$) as a function of $[\text{Rh}(\text{phen})_2(\text{bpy})]^{3+}$ equivalents for $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ ($\text{M} = \text{Ru}, \text{Os}$) bound to CT DNA, poly d(AT), and poly d(GC). (B) Plot of fraction quenched for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to synthetic polymers as a function of $[\text{Rh}(\text{phen})_2(\text{bpy})]^{3+}$ equivalents. For each plot the relevant DNA sequences and metal centers are indicated in the legend. Samples were aerated and 20 μM Os, 2 mM nucleotides (nuc) in 5 mM Tris, 50 mM NaCl, pH 8.5, or 10 μM Ru, 1 mM nuc, at ambient temperature.



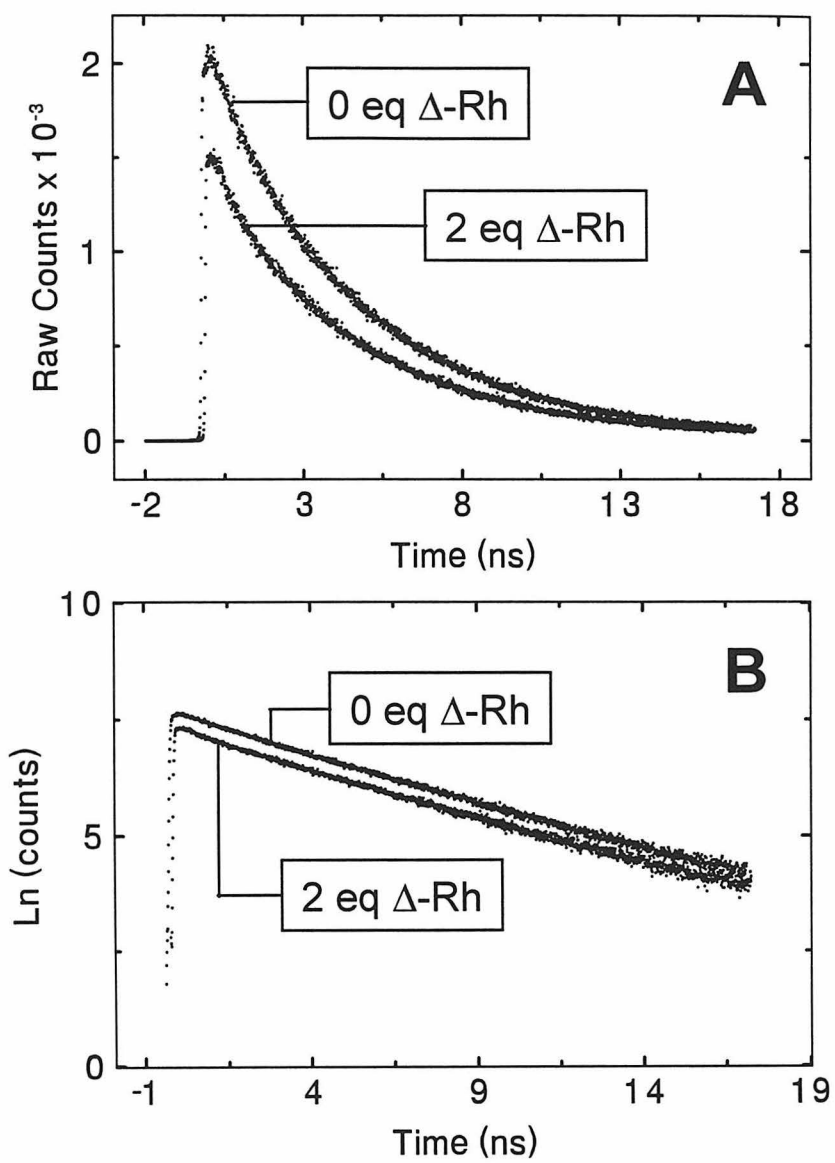
Studies of the luminescence from $[M(\text{phen})_2(\text{dppz})]^{2+}$ complexes and their derivatives bound to DNA reveal that metallointercalators in this class bind preferentially to AT sites.²⁶ At the low ratios of metal complex to base pairs employed in these titrations, the donors occupy primarily AT sites in CT DNA. Therefore the similarity of quenching data with poly d(AT) and CT DNA is a manifestation of the DNA binding properties of $[M(\text{phen})_2(\text{dppz})]^{2+}$ complexes. The fact that there is such a large difference in the titration profiles with AT and GC polymers strongly indicates that the ET step involves ET through the DNA bases.

Figure 4.2 also shows the yield of luminescence quenching for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ donors and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ acceptors in the presence of synthetic polymers of sequences other than the alternating AT or GC polymers. It is noteworthy that all of the quenching profiles are the same with the exception of poly d(AT), which facilitates the reaction most effectively.

Time scale for the quenching reaction between metallointercalators bound to DNA. The results described above illustrate the sensitivity in the yield of photoinduced ET between metallointercalators to DNA sequence. We also sought to determine the rate of ET between the photoexcited donor and the intercalated acceptor. As presented in Chapter 3 for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ bound to CT DNA, a lower limit for the rate of forward ET to $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ was set at 10^{10} s^{-1} .

Figure 4.3 shows the luminescence decay of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ bound to poly d(GC) in the presence and absence of the intercalator $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$. As documented for related systems,^{25,31} the difference in the excited state decay profiles, other than a loss of absolute emission intensity, was detected in these experiments. The observation is that upon addition of the intercalating acceptor the emission yield goes down according to the

Figure 4.3. Plot of emission intensity as a function of time for Δ -[Os(phen)₂(dppz)]²⁺ (20 μ M) bound to poly d(GC) (2 mM nuc, 5 mM Tris, 50 mM NaCl, pH 8.5) in the presence and absence of Δ -[Rh(phi)₂(bpy)]³⁺. For [Rh] = 0 the osmium excited state decays as a biexponential with τ_1 = 4.98 ns (87%) and τ_2 = 1.93 ns (13%). With [Rh] = 40 μ M, τ_1 = 4.89 ns (88%) and τ_2 = 1.24 ns (12%). In (A) the quenching is manifested as a loss of intensity at $t = 0$, which reflects a rate of ET > 10¹⁰ s⁻¹ (instrument response limited). The semi log plot in (B) illustrates graphically the absence of change in lifetime.



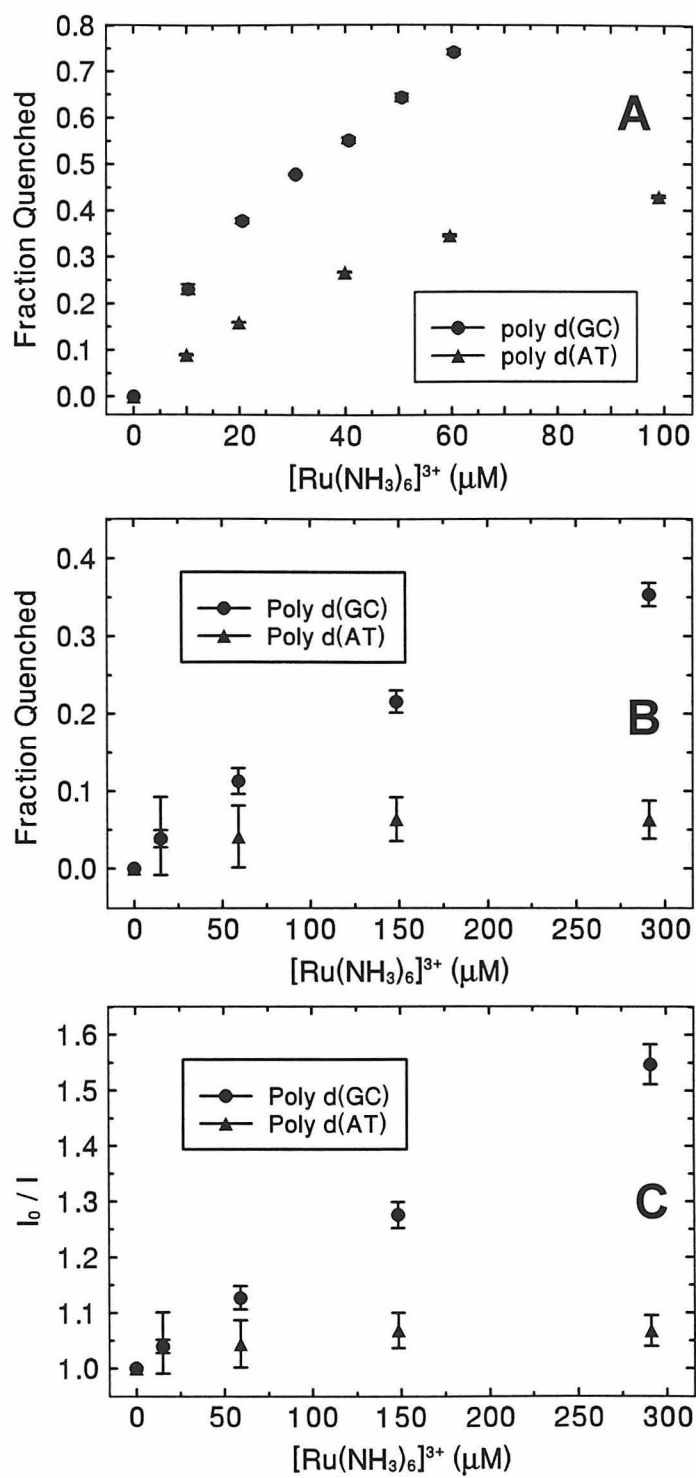
fraction of donors getting quenched, but no change in the kinetics of the emission occurs. Therefore the limiting value for the ET rate, dictated by the instrument response time, is 10^{10}s^{-1} . This fact does not permit us to say that there is no difference in quenching rate despite a significant difference in the yield of ET, however, we can say that the rate remains too fast to measure even when the yield of quenching decreases.

Luminescence quenching by $[\text{Ru}(\text{NH}_3)_6]^{3+}$. We also characterized the yield of photoinduced ET from intercalated donors ($[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$) to the groove bound acceptor, $[\text{Ru}(\text{NH}_3)_6]^{3+}$. Unlike $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, $[\text{Ru}(\text{NH}_3)_6]^{3+}$ does not have the ligand framework to support intercalation. Therefore, in order for ET to occur, the donor and acceptor must collide. Such a reaction relies on diffusion, which may be facilitated by a DNA duplex, but it does not proceed through the DNA bases.³⁰

The fraction of $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ donors quenched by $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in the presence of poly d(AT) and poly d(GC) is shown in Figure 4.4. In contrast to the reaction with $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, for both Ru(II) and Os(II), the quenching yield is significantly greater in poly d(GC) than poly d(AT). Bound to poly d(GC) the quenching of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ (15 μM) increases with increasing concentrations of the acceptor until saturation behavior begins with 40% quenching by 20 equiv of $[\text{Ru}(\text{NH}_3)_6]^{3+}$. Only ~5% of the photoexcited Os(II) population reacts with $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in the presence of poly d(AT), even with 20 equiv of the quencher in solution.

For the ruthenium intercalator, the quenching reaction is more efficient. Lower concentrations of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ produce greater quenching yields. This result contrasts the observation of essentially identical reaction profiles for $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to CT DNA, poly d(AT), and poly d(GC). Bound to poly d(GC) the fraction of photoexcited $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ that react with $[\text{Ru}(\text{NH}_3)_6]^{3+}$ saturates

Figure 4.4. Yield of photoinduced ET between $[\text{Ru}(\text{NH}_3)_6]^{3+}$ and $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ (**A**) and $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ (**B**) in the presence of poly d(AT) and poly d(GC) as a function of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ concentration. The data from (**B**) are plotted in (**C**) as I_0/I versus $[\text{Ru}(\text{NH}_3)_6]^{3+}$ concentration to illustrate the fact that this reaction is dynamic quenching, which is reflected in the linearity of the plots. Conditions: 10 μM Ru, 15 μM Os, $[\text{nuc}]/[\text{M}] = 100$, in 5 mM Tris, 50 mM NaCl, pH 8.5.



with almost 70% quenched at 10 equiv of the acceptor. Like the osmium complex, the reaction is less efficient on poly d(AT), however, 40% of the ruthenium complexes are quenched with 10 equiv of $[\text{Ru}(\text{NH}_3)_6]^{3+}$ on this polymer.

4.2.2. Back Electron Transfer: Sequence Dependent Ground State Recovery of M(III) on the Picosecond and Microsecond Time Scales.

As described above for Scheme 4.1, the cycle of ET reactions between metal complexes bound to DNA requires two ET steps after irradiation to regenerate the ground state reactants. The products of photoinduced ET (so called ET intermediates) return to the resting state in a thermal, recombination reaction (back ET). We concentrated a lot of effort on the identification and characterization of ET intermediates produced in reactions between metallointercalators bound to CT DNA, as well as the rate of the recombination reaction (Chapter 3). In this section, we describe the study of the sequence dependence of the recombination reaction, which takes place on the picosecond time scale for quenching between metallointercalators, and the microsecond time scale for quenching by $[\text{Ru}(\text{NH}_3)_6]^{3+}$. In addition, we investigated the effect of the sequence on the yield of a small fraction of long-lived ET intermediate produced in the quenching of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ by $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$.

Effect of DNA Sequence on the Rate of Ground State Recovery for Ru(III) Produced by ET between Metallointercalators. We measured the rate of recombination for the products of photoinduced ET between $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to poly d(AT) and poly d(GC). As shown in Figure 4.5, the rate of recombination is sequence dependent. Back ET between metallointercalators bound to poly d(AT) occurs with a rate of $\sim 10^{10} \text{ s}^{-1}$, while the rate in the presence of poly d(GC) is

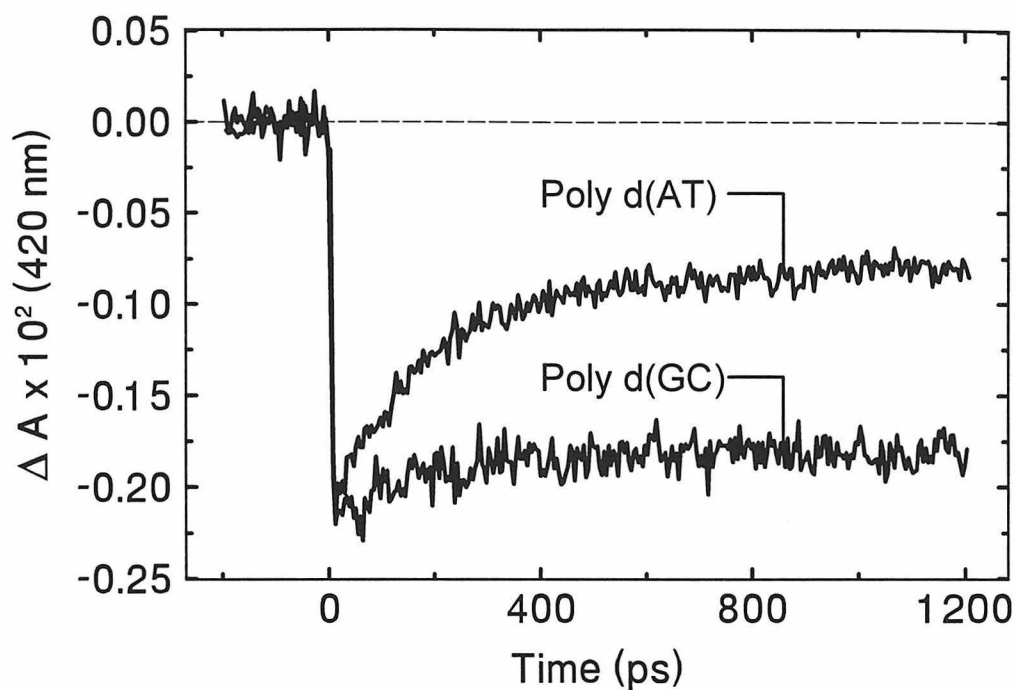


Figure 4.5. Transient absorption at 420 nm as a function of time following the irradiation (390 nm) of $rac\text{-}[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to poly d(AT) or poly d(GC) in the presence of 4 equiv $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$. Bound to poly d(AT) the rate of recombination $k_{\text{rec}} = 7 \times 10^9 \text{ s}^{-1}$, which is faster than with poly d(GC) where $k_{\text{rec}} = 2 \times 10^8 \text{ s}^{-1}$. Conditions: 20 μM Ru, 80 μM Rh, 2 mM nuc, 5 mM Tris, 50 mM NaCl, pH 8.5.

40 times slower. The slower rate of reaction for the complexes bound to the GC polymer is noteworthy given that the excited state lifetimes of the ruthenium complex bound to poly d(GC) are shorter compared to the data for poly d(AT). Longer excited state lifetimes indicate deeper penetration of the dppz ligand of the donor into the DNA base stack.

Sequence dependent yield of long-lived Os(III) intermediates generated in quenching reactions with $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$. Photoinduced ET between $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to DNA leads to the formation of two populations of Os(III) intermediates.^{25,31} We detected the majority (~90%) of the back ET on the picosecond time scale, and assigned a rate constant of $1.1 \times 10^{10} \text{ s}^{-1}$ for the reaction. The remaining fraction of Os(III) lives into the microsecond regime and can be detected by transient absorption spectroscopy on this time scale. As described in Chapter 3 for CT DNA, this long-lived intermediate was unambiguously assigned to Os(III) on the basis of extensive spectroscopic characterization.

As shown in Figure 4.6, we observed the same long-lived transient for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ quenched by $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to poly d(AT) and poly d(GC). The rate of recombination is essentially sequence independent based on the data shown here. Remarkably, the amount of long-lived Os(III) is sequence dependent. The concentration of long-lived intermediate is greater with poly d(GC) than poly d(AT).

Based on the fact that most of the recombination takes place on the picosecond time scale, this observation indicates that the interactions of the complex with the AT polymer facilitate the conversion of Os(III) to Os(II) more efficiently.

Fate of Os(III) and Ru(III) species formed in quenching reactions with $[\text{Ru}(\text{NH}_3)_6]^{3+}$. In addition to monitoring the recombination reaction for M(III)

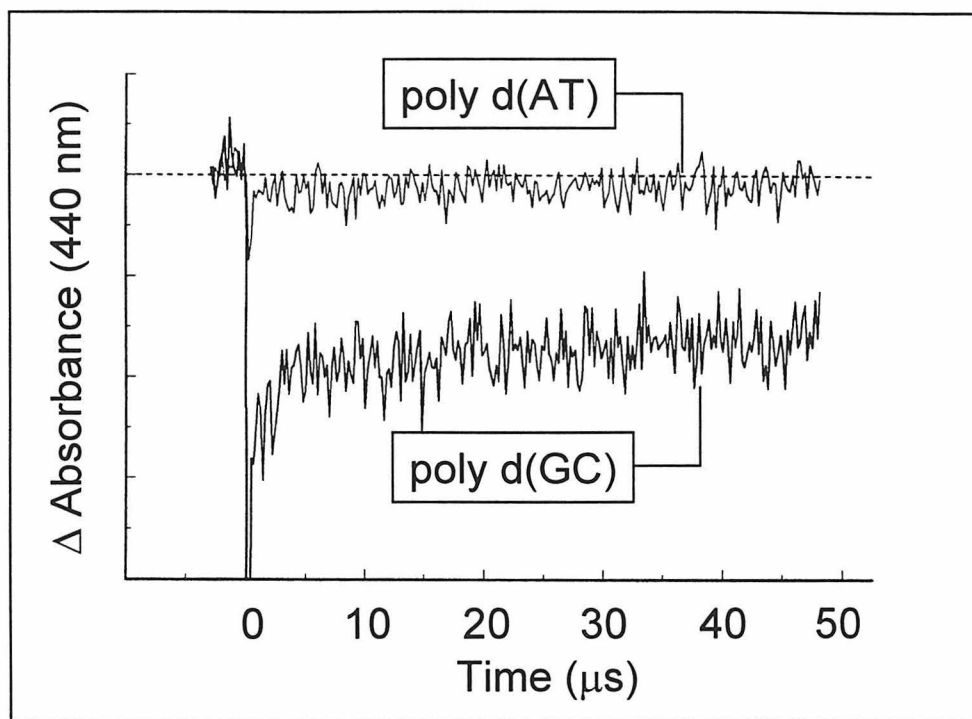
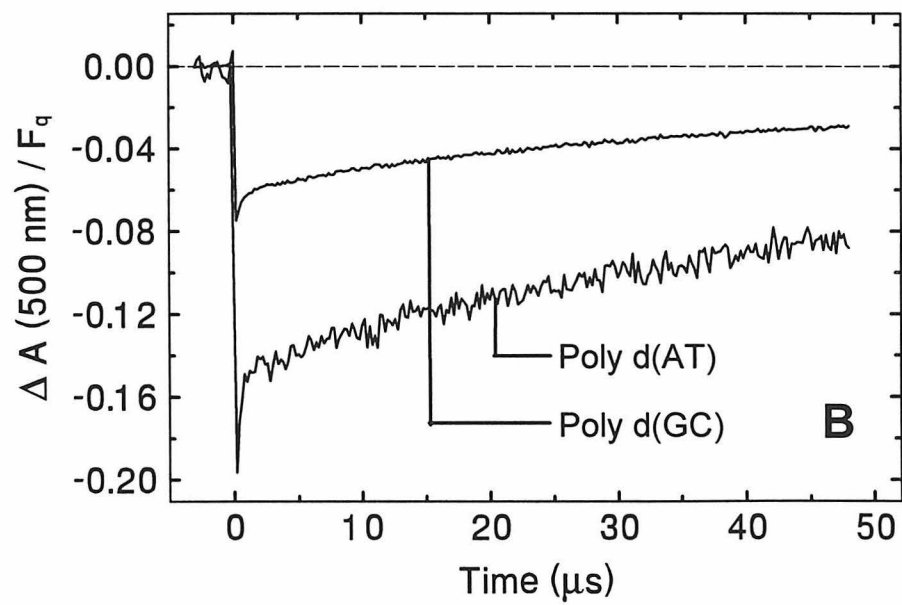
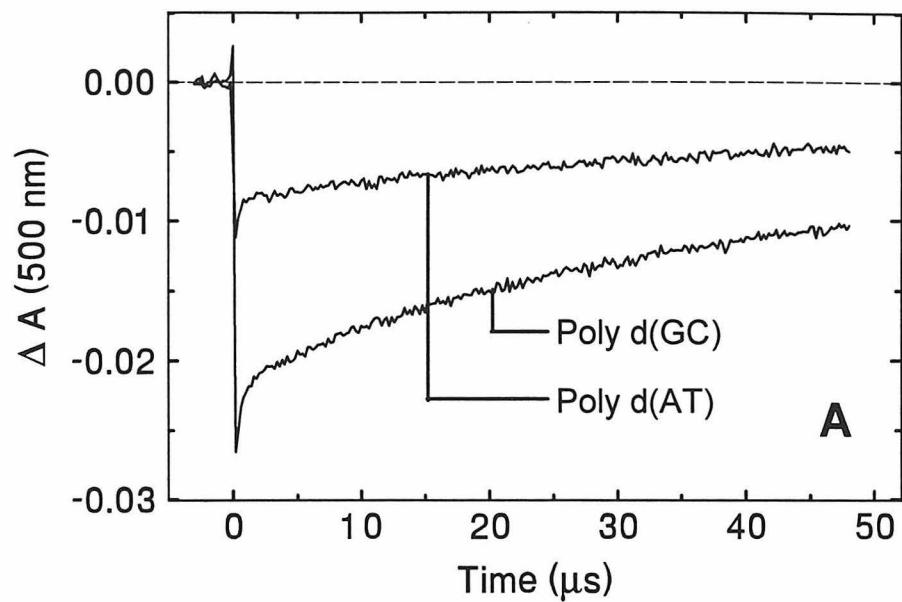


Figure 4.6. Transient absorption signal at 440 nm for long-lived Os(III) intermediates formed by ET between $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ in the presence of poly d(AT) or poly d(GC). Excitation was at 480 nm. Conditions: 15 μM Os, 90 μM Rh, 1.5 mM nuc, 5 mM Tris, 50 mM NaCl, pH 8.5.

metallointercalators formed in reactions with $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, we followed the fate of M(III) species generated in quenching reactions with $[\text{Ru}(\text{NH}_3)_6]^{3+}$. Figure 4.7 shows the transient signals for the ground state recovery of the products of photoinduced ET between $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in the presence of poly d(AT) and poly d(GC). Owing to the differences in excited state lifetimes for the metallointercalators bound to each polymer, the amounts of ET under the same experimental conditions are sequence dependent. In order to account for the effects of excited state lifetime, the data are corrected for the amount of quenching produced in each reaction. Thus, the relative signal intensities in Figure 4.7 can be directly compared and reflect the sequence dependence of M(III) species that lives into the microsecond regime.

For $\text{M} = \text{Ru}$ and Os , the amount of M(III) intermediate is greatest on the AT polymer. In the presence of poly d(GC), however, the concentration of Os(III) is greater than the concentration of Ru(III) . This result is consistent with the Ru(III) species reacting with guanine to form Ru(II) and the G-cation radical. The reduction potential of Os(III) (1.16 V vs. NHE) is not sufficiently oxidizing to react with any of the DNA bases. Therefore, we can observe this species even in the presence of poly d(GC).

Figure 4.7. Time-resolved transient absorption signals observed on the microsecond time scale for the quenching of photoexcited $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ by $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in the presence of poly d(AT) or poly d(GC). **(A)** Absolute signal intensity observed at 500 nm. The amount of Os(III) species formed is greater with poly d(GC) than poly d(AT). **(B)** Signal intensity at 500 nm corrected for the amount of quenching. Since there is less quenching with poly d(AT), this correction makes the relative yield of Os(III) greater with the AT polymer compared to that with poly d(GC).



4.3. DISCUSSION

Owing to the spectroscopic properties of polypyridyl complexes of Os(II) and Ru(II),³²⁻³⁶ we have been able to follow the reactions of these compounds in the presence of DNA by luminescence spectroscopy as well as absorption spectroscopy. We characterized the photoinduced ET quenching of $[M(\text{phen})_2(\text{dppz})]^{2+}$ complexes by metallointercalators and groove bound acceptors. We followed that fate of the ET intermediates produced in reactions of the photoexcited donors as a function of sequence and reaction and found that these systems differ significantly. In this section we compare the reactions of $[M(\text{phen})_2(\text{dppz})]^{2+}$ complexes bound to DNA as a function of metal center, reaction and DNA sequence.

4.3.1. Quenching Efficiencies for Dipyridophenazine Complexes of Os(II) and Ru(II) in Reactions with $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ and $[\text{Ru}(\text{NH}_3)_6]^{3+}$.

Quenching Yields for $[M(\text{phen})_2(\text{dppz})]^{2+}$ as a Function of Acceptor. The yield of photoinduced ET between dppz complexes of Os(II) or Ru(II) and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ is remarkably sequence dependent, however, the reaction does not depend upon whether the donor is the osmium or the ruthenium complex. On the other hand, when the quencher is $[\text{Ru}(\text{NH}_3)_6]^{3+}$, the yield of quenching depends strongly upon sequence and which metal center is at the core of the donor. These observations are important given the significantly different photophysical and electrochemical properties for $[M(\text{phen})_2(\text{dppz})]^{2+}$ derivatives.^{24,25}

Owing to the large differences (three order of magnitude) in the excited state lifetimes for $[M(\text{phen})_2(\text{dppz})]^{2+}$ donors, the absence of metal center dependent yields of quenching in reactions with $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ indicates that the rate of reaction is

significantly faster than the lifetimes of the reactants. Furthermore, this result indicates that the metallointercalators do not diffuse along the helix in order to react with each other. If diffusion was important, the yield of ET for osmium would be less compared with ruthenium.

We illustrated previously for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ that the yield of photoinduced ET was less for $[\text{Ru}(\text{NH}_3)_6]^{3+}$ compared to $[\text{Rh}(\text{phi})_2(\text{phen})]^{3+}$.³⁰ Here, we showed that the same observation is made for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$, however, we also showed that the yield of ET differs significantly for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ versus $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ in reactions with $[\text{Ru}(\text{NH}_3)_6]^{3+}$ but not $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$. This observation further illustrates the importance of having both reactants in DNA-mediated ET accessing the DNA base stack by intercalation.

For $[\text{Ru}(\text{NH}_3)_6]^{3+}$, which does not intercalate, a collision with the donor is required for quenching, whereas with $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, ET can proceed through the DNA base stack. Therefore the yield of ET is less for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ owing to its nanosecond excited state lifetimes.

Sequence Dependent Quenching with $[\text{Ru}(\text{NH}_3)_6]^{3+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$.

Despite remarkably similar quenching profiles for Os(II) and Ru(II) in reactions with $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to a given DNA sequence, the yields of ET do depend upon sequence. We found that the quenching reaction in the presence of poly d(AT) is significantly more efficient compared to poly d(GC). In fact, across a series of DNA polymers, poly d(AT) and CT DNA are very effective in mediating ET, while poly d(GC) and other synthetic polymers have modest quenching profiles.

We looked at the sequence dependence of quenching by $[\text{Ru}(\text{NH}_3)_6]^{3+}$ with $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ complexes to further characterize the reaction between

metallointercalators. In contrast to our observations for $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ as the electron acceptor, the quenching efficiency with $[\text{Ru}(\text{NH}_3)_6]^{3+}$ is significantly more efficient on poly d(GC) than with poly d(AT). The origin of this difference lies in the fact that the average excited state lifetime for the ruthenium and osmium donors is significantly longer in the presence of poly d(GC) (Chapter 2).²⁶ Furthermore, the large difference in excited state lifetime for ruthenium compared to osmium results in the ruthenium complex undergoing much of reaction at significantly lower concentrations of $[\text{Ru}(\text{NH}_3)_6]^{3+}$.

These observations underscore the contrast between photoinduced ET reactions between metallointercalators and those involving groove bound reactants. For the metallointercalators, ET proceeds through the DNA base stack, whereas with the $[\text{Ru}(\text{NH}_3)_6]^{3+}$ bound in the groove, quenching requires diffusion. The remarkable efficiency of the π -stack mediated reaction is clearly evident from these comparative studies.

4.3.2. Back Electron Transfer as a Function of Sequence and Reaction

Quencher Dependent Rate of Back Electron Transfer. We found that the recombination, or back ET reaction, was significantly faster and thus more efficient with $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ as opposed to $[\text{Ru}(\text{NH}_3)_6]^{3+}$. As illustrated clearly for the relative efficiencies of forward ET (quenching), the back ET reaction is also much more efficient when allowed to use the DNA base stack as a conduit for ET. The rate of recombination is $\sim 10^{10} \text{ s}^{-1}$ between $[\text{M}(\text{phen})_2(\text{dppz})]^{3+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{2+}$ bound to CT DNA, compared to reaction well into the microsecond regime with $[\text{Ru}(\text{NH}_3)_6]^{2+}$.

Sequence Dependent Rate of Recombination. Apart from the fact that the efficiency of ET is sequence dependent, we also found that the reate of recombination differs by sequence. The rate of recombination is $\sim 10^{10} \text{ s}^{-1}$ between $[\text{M}(\text{phen})_2(\text{dppz})]^{3+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{2+}$ bound to CT DNA or poly d(AT) while in the presence of poly d(GC) the rate is 40 times slower. It is noteworthy that despite a decrease in the efficiency of forward ET with poly d(GC), the rate remains too fast to measure with our instrumentation ($> 3 \times 10^{10} \text{ s}^{-1}$) for each of the polymers characterized in these studies.

Sequence Dependent Yield of Long-Lived Os(III) for Electron Transfer between $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$. For ET between $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to CT DNA, we observe a small amount of Os(III) that persists into the microsecond time scale. It is noteworthy that we do not detect any long-lived Ru(III) intermediate for the reaction with $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$. We found a sequence dependence to the amount of this long-lived intermediate generated in ET between metallointercalators. We detected almost no long-lived Os(III) with poly d(AT) and a small concentration with poly d(GC) consistent with studies in CT DNA. In fact, this observation suggests that the long-lived species observed with CT DNA reflects complexes bound at GC sites in the mixed sequence polymer. These observations are consistent with the notion that coupling into the π -stack for metallointercalators bound to poly d(GC) is less effective compared with poly d(AT).³¹

Sequence Dependent Yield of M(III) formed in Reactions with $[\text{Ru}(\text{NH}_3)_6]^{3+}$. The ground state recovery of M(III) intermediates formed in ET reactions between $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ donors and $[\text{Ru}(\text{NH}_3)_6]^{3+}$ as an acceptor occurs entirely on the microsecond time scale. We found a significant sequence dependence to the yield of M(III) generated by $[\text{Ru}(\text{NH}_3)_6]^{3+}$ for $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ versus $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$.

For the osmium donor, we detected a greater concentration of Os(III) in the presence of poly d(AT) compared to poly d(GC), after correcting the signal for the amount of quenching. With $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$, we detected the Ru(III) species on the microsecond time scale when the complex is bound to poly d(GC).

On the basis of the relative reduction potentials for osmium ($E_0^{3+/2+} = 1.16 \text{ V}$ vs. NHE) and ruthenium ($E_0^{3+/2+} = 1.6 \text{ V}$ vs. NHE), we attribute the lack of long-lived Ru(III) with poly d(GC) to the oxidation of G by the ruthenium complex. Such a reaction is thermodynamically disfavored for either complex bound to poly d(AT), and the osmium complex is not sufficiently oxidizing to react with G. In subsequent studies, Stemp *et al.* demonstrated the oxidation of G by Ru(III) intercalators by spectroscopically characterizing the G radical produced in the reaction (Chapter 1).

For the reaction between $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Ru}(\text{NH}_3)_6]^{3+}$, it is noteworthy that the yield of long-lived Os(III) is greater for poly d(GC) compared to poly d(AT). This observation is in contrast to the reaction between $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, the two metallointercalators. We suggest that this observation reflects the deeper intercalation of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ into poly d(AT), which means that the complex bound to poly d(GC) is more solvent exposed and thus reacts more easily with the groove bound acceptor.

4.3.3. Stacking Model to Explain the Sequence Dependent Yield of ET between Metallointercalators

We have illustrated the many differences in ET reactions that depend upon whether the reactants are metallointercalators or groove binding agents. Our work has

shown that photoinduced ET and thermal recombination is significantly more efficient for ET *through* the DNA base stack.³⁰

ET between metallointercalators is strongly sequence dependent. It is noteworthy that the yield and rate of ET does not change when osmium is substituted for ruthenium in $[M(\text{phen})_2(\text{dppz})]^{2+}$ donors. Previously we showed that the rate and yield of ET do depend on the absolute configuration of the metal complex (Δ or Λ), which illustrates the importance of stacking into the DNA base stack.^{31,37} Therefore, we propose a model to explain the sequence dependent yield of ET between $[M(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ bound to DNA in terms of differences in the association of the complexes with the polymers.

Sequence Dependent Intercalation Geometries for $[M(\text{phen})_2(\text{dppz})]^{2+}$. Table 4.1 lists the excited state lifetimes of Δ - $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to CT DNA, poly d(GC), and poly d(AT). Lifetimes of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and several derivatives are shown in Chapter 2. It is apparent that the excited state decay profiles for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ in the presence of CT DNA and poly d(AT) are very similar. These decays are biexponential with ~75-85% of a lifetime of ~150 ns, and the remainder of the decay has a lifetime of ~800 ns. In the presence of poly d(GC) as well as the synthetic polymers other than poly d(AT) described in Figure 4.2 B, the decay is dominated by the longer lifetime which is ~250 ns.^{26,27}

As described previously and in Chapter 2, the two lifetimes represent two families of intercalation geometries: one in which the dppz ligand is intercalated ~parallel to the base pair axis (side-on) and another where the complex is perpendicular to the bp axis (head-on). The protection of the phenazine nitrogens from solvent is greater in the head-on mode and therefore this binding orientation is reflected by the longer lifetime. Owing

Table 4.1. Luminescence Decay Parameters for Δ -[Ru(phen)₂(dppz)]²⁺ Bound to DNA polymers.^a

DNA Sequence	luminescence lifetimes ^b			
	τ_1 (ns)	(%)	τ_2 (ns)	(%)
CT DNA ^c	150	(80)	800	(20)
poly d(AT) ^c	123	(82)	709	(18)
poly d(GC) ^c	44	(22)	288	(78)

a. Data obtained as described previously³⁷ with excitation at 480 nm and observation at 617 nm.

b. Data were fit to $y(t) = 100[C_1 \exp(-t/\tau_1) + (1-C_1) \exp(-t/\tau_2)]$ by a nonlinear least-squares method with convolution of the instrument response function using in house software (BILRC) as described previously.³⁷ Error in lifetimes and percent contributions are estimated to be $\pm 10\%$.

c. Δ -[Ru(phen)₂(dppz)]²⁺ (10 μ M) bound to DNA (1 mM nucleotides (nuc); Ru:bp = 1:50) in buffer (5 mM Tris, 50 mM NaCl, pH 8.5). Lifetimes are consistent with those reported previously, see reference 1c.

to the orientation of the dppz ligand in the side-on mode (parallel to the bp axis), the amount of overlap with the bases is larger for this binding orientation.^{38,39}

For $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to poly d(AT) and CT DNA, intercalation occurs primarily in the side-on mode, while with poly d(GC) intercalation is in the head-on mode.^{26,27} Furthermore, the longest lifetimes are observed for the head-on binding in CT DNA and poly d(AT).²⁶ These sequence dependent binding profiles indicate that the stacking of $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ complexes with the base stack of DNA is sequence dependent.

Stacking Model to Explain Sequence Dependent Quenching Yields. A

proposal to explain the greater efficiency of quenching in the presence of poly d(AT) and CT DNA is that the donor is stacked more deeply into the base stack with this polymer. Such a suggestion is supported not only by the luminescence lifetimes but also by the greater concentration of Os(III) generated by $[\text{Ru}(\text{NH}_3)_6]^{3+}$ in the presence of poly d(AT), which indicates that the complex is less accessible to solvent under these conditions. Moreover, not only are the longest luminescence lifetimes observed with poly d(AT), but also the fact that the dominant mode of intercalation is the side-on mode, the amount of overlap between dppz and the bases is greater with poly d(AT) and CT DNA. Thus, sequence dependent stacking is responsible for the sequence dependent quenching yields observed.

Summary. We have employed two ET reactions between transition metal complexes bound to DNA to further investigate the factors that contribute to the remarkable efficiency and sequence dependence of ET between metallointercalators. By substituting Os(II) for Ru(II) in $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ donors, we found that the yield of ET between metallointercalators was not sensitive to the photophysical or redox

properties of the donor. ET reactions involving $[\text{Ru}(\text{NH}_3)_6]^{3+}$ were strongly dependent on the properties of the donor. The contrast between the two system lies in the fact that $[\text{Ru}(\text{NH}_3)_6]^{3+}$ binds only in the groove of the DNA helix and cannot access the DNA base stack.

Photoinduced ET is more efficient for CT DNA and poly d(AT) compared to poly d(GC). We described a model that cites sequence dependent stacking of metallointercalators to account for the differences in efficiency. This similarities between CT DNA and poly d(AT) reflect the fact that $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ complexes are bound (85%) primarily to AT sites in CT DNA.

For back ET between metallointercalators, the reaction is primarily on the picosecond time scale, with a small concentration of Os(III) persisting into the microsecond regime in the presence of poly d(GC). We suspect that the absence of long-lived Os(III) with poly d(AT) reflects more efficient coupling into the π -stack of the AT polymer. When the electron acceptor is $[\text{Ru}(\text{NH}_3)_6]^{3+}$, the back ET reaction proceeds on the microsecond time scale. We observed Ru(III) under these conditions only for poly d(AT), because the trivalent metal complex reacts with G in poly d(GC). Based on the fact that for ET between metallointercalators, long lived Os(III) is observed with poly d(GC) not poly d(AT) and the fact that Ru(III) oxidizes guanine, we now understand why we do not detect long-lived Ru(III) in reactions between metallointercalators bound to CT DNA. The fact that the concentration of Os(III) is greater on poly d(AT) than poly d(GC) in reactions with $[\text{Ru}(\text{NH}_3)_6]^{3+}$, illustrates that the complex is more solvent exposed on GC, and therefore less charge-separated state is observed.

In conclusion, we found that DNA-mediated ET reactions that proceed through the DNA π -stack are remarkably efficient, whereas when one reactant cannot intercalate,

the efficiency is reduced dramatically. Sequence dependent rates and yields of ET between metallointercalators originate in how well the sequence of DNA permits effective stacking of the reactants with the DNA base stack. Furthermore, the reactivity of metallointercalators with DNA bases is sequence dependent owing to the different redox potentials of the transition metal complexes.

4.4. EXPERIMENTAL

4.4.1. Materials

Metallointercalators ($[\text{Os}(\text{phen})_2(\text{dppz})]\text{Cl}_2$, $[\text{Ru}(\text{phen})_2(\text{dppz})]\text{Cl}_2$, $[\text{Rh}(\text{phen})_2(\text{bpy})]\text{Cl}_3$) were prepared as described previously and in Chapter 2.^{28,29,40} Trace impurities were rigorously excluded by high-performance liquid chromatography (HPLC). Resolution of the racemic mixtures was achieved using standard methodology.³⁹ Resolution of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ proceeded with baseline separation of the two enantiomers on the anion-exchange column (see Chapter 2); $[\text{Rh}(\text{phen})_2(\text{bpy})]^{3+}$ was resolved by ion exchange as described previously. Sonicated and phenol extracted CT DNA (Sigma) and poly d(AT), poly d(GC) and other synthetic DNA polymers (Pharmacia) were exchanged into standard buffer (5 mM tris, 50 mM NaCl, pH 8.5) by ultrafiltration (Amicon). $[\text{Ru}(\text{NH}_3)_6]\text{Cl}_3$ was purchased from Aldrich and used as received.

4.4.2. Instrumentation

Time-correlated single photon counting (TCSPC) and time-resolved measurements on the nanosecond timescale were carried out using the facilities in the Beckman Institute Laser Resource Center (BILRC) described previously.³⁷ TCSPC data sets were typically counted until 5,000 - 10,000 counts accumulated in the highest

channel. Excitation for transient absorption and nanosecond timescale emission experiments was provided by an excimer-pumped dye laser (1-5 mJ/10 ns pulse) containing either Coumarin 480 (480 nm; Exciton) or Rhodamine 6G (580 nm; Exciton), or by a frequency-doubled Nd:YAG laser which provided 15-25 mJ/8 ns pulse at 532 nm. A pulsed 75 W Xe-arc lamp (Photon Technology International) served as the probe source for transient absorption experiments. Individual data sets were typically the average of 400 - 2500 shots. Data fitting was accomplished by least-squares method of Marquardt using both in-house (BILRC) and commercial (Axum, Trimetrix) software. Emission spectra were recorded on an SLM 8000 spectrofluorimeter and standardized to $[\text{Ru}(\text{bpy})_3]^{2+}$ (emission maximum = 607 nm).⁴¹ Ultraviolet-visible (UV-vis) spectra were recorded on a Cary 2200 spectrophotometer.

4.4.3. Methods

Titration experiments were carried out by adding concentrated solutions of DNA to dilute solutions of metal where appropriate. For quenching studies, the acceptor ($[\text{Rh}(\text{phen})_2(\text{bpy})]^{3+}$ or $[\text{Ru}(\text{NH}_3)_6]^{3+}$) was added to the solution of donor bound to DNA as small aliquots of concentrated (~ 1 mM) solutions. Identical results were obtained if separate samples were made for each point.

Photophysical studies were carried out at room temperature using aerated solutions. Emission quenching was quantitated by integration of the response-limited excited-state decays on the nanosecond timescale or by steady-state measurements; both methods gave equivalent results. Plots of the fraction of intensity quenched (fraction quenched = $1 - I/I_0$) versus quencher concentration are reported here, given the substantial quenching evident and the low signal to noise observed at high quencher concentrations which are overemphasized in plots of I_0/I . Importantly, plots of I_0/I for these data curve upward with increasing quencher, indicating that the Stern-Volmer analysis is not appropriate.

4.5. REFERENCES

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Chapter 5: Multistep Heterogeneous Coupling of Metal Complexes to DNA on a Solid Support: Synthesis and Characterization of Metallointercalator-DNA Conjugates.

5.1. INTRODUCTION

This chapter describes methods we developed for the preparation and characterization of metallointercalator-DNA conjugates. Electron transfer (ET) reactions between metallointercalators bound to DNA are remarkably fast and efficient.¹⁻⁶ Photoexcited Δ -[M(phen)₂(dppz)]²⁺ (dppz = dipyrido[3,2-a;2',3'-c]phenazine) complexes bound to DNA are quenched by Δ -[Rh(phi)₂(bpy)]³⁺ with a time constant of < 30 ps, even at low ratios of metal to DNA (*e.g.* 1:50).³ Transient absorption on the picosecond time scale provided a means to measure the rate of the recombination reaction between the products of photoinduced ET, as they regenerate the resting state. Across a series of seven pairs of metallointercalators as electron donors and acceptors, we found the rate of recombination (k_{rec}) to be ~100 ps. In titrations of Δ -[Rh(phi)₂(bpy)]³⁺ into solutions of Δ -[M(phen)₂(dppz)]²⁺ (M = Os, Ru) bound to DNA, we found k_{rec} to remain constant over the course of the titration. Even though the average separation between the Rh(III) complexes and the M(II) complexes decreases as the acceptor concentration increases, k_{rec} was centered at 100 ps.³

Two explanations for the loading independent rate of electron transfer between intercalators bound to DNA have been suggested.¹⁻⁷ First, the remarkably fast electron transfer reaction is actually facilitated by the DNA base stack. In this hypothesis, the DNA base stack forms a remarkable medium for ET, which makes long-range electronic coupling between redox active species more efficient compared with other ET media.¹⁻⁶ Alternatively, one might suggest that the loading independent rate is observed because

the donor and acceptor bind cooperatively and thus assume only one donor-acceptor separation over the course of the entire titration.⁷

A well-defined experiment that would permit one to determine whether either of these hypotheses is valid consists of tethering the donor and acceptor at opposite ends of a DNA duplex. In fact, Murphy *et al.* prepared one duplex in which a Ru(II) donor and Rh(III) were covalently attached to a 15 base pair (bp) oligoduplex.⁶ Upon excitation of a duplex to which only Ru(II) had been tethered, bright luminescence was observed. In the duplex to which the donor and acceptor were attached, no emission from the Ru(II) complex could be detected.^{6,8} This observation suggested that 100% of the photoexcited ruthenium complexes, which emitted strongly without rhodium, were quenched when the Rh(III) complex was present. Unfortunately, these experiments were never exhaustively repeated, nor were the samples extensively characterized. This lack of in-depth analysis of the first long-range ET reaction mediated by DNA resulted primarily from the fact that the synthetic methods to prepare the materials for experiments were ineffective and irreproducible.

We sought to develop a strategy that would permit the facile synthesis and isolation of oligonucleotides bearing metallointercalators covalently attached to the terminal hydroxyl group.⁹ Our goal was not only to be able to routinely isolate these materials, but to prepare quantities sufficient for chemical and biochemical analyses such as hybridization studies by polyacrylamide-gel electrophoresis (PAGE), mass spectrometry (MS), enzymatic digests, and photocleavage of modified duplexes by tethered phi complexes of Rh(III).

The methods that resulted from our work are methods that involve derivatization of the single stranded DNA while it is still attached to the control-pore glass (CPG) after automated DNA synthesis. In this chapter, the synthesis and characterization of a variety of metallointercalator-DNA conjugates are described.

5.2. RESULTS

5.2.1. Synthesis of Rhodium-DNA Conjugates on a Solid Support.

Coupling $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ to the 5' Terminus of Oligonucleotides. We developed a multistep heterogeneous coupling method to prepare oligonucleotides on which a metallointercalator, $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ (Figure 5.1), is attached to the 5' or 3' terminus by a flexible linker. Scheme 5.1 outlines our approach to the synthesis of 5'-rhodium-modified oligonucleotides on a solid support. In this strategy, we modify the DNA while it is still attached to the control-pore glass (CPG) beads used during automated DNA synthesis by the phosphoramidite approach.¹⁰

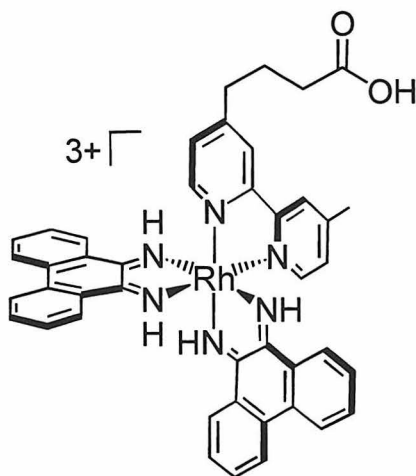
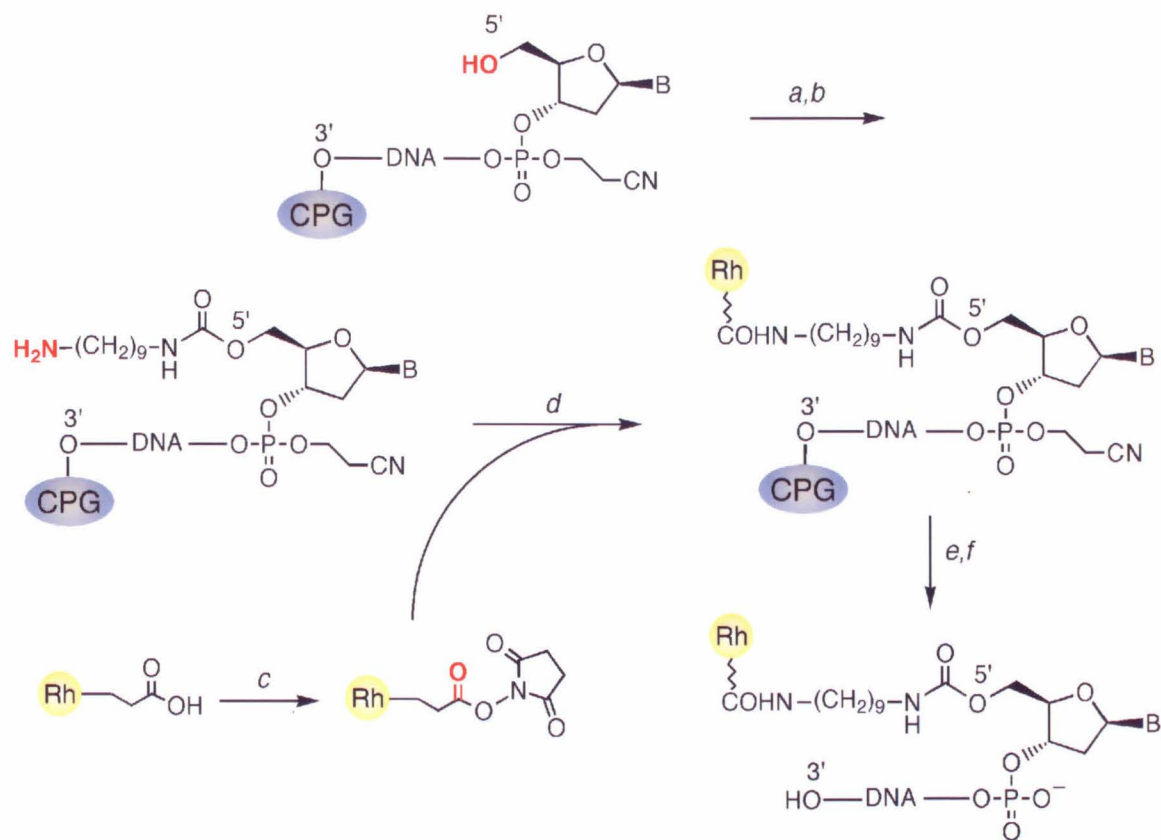


Figure 5.1. Schematic illustration of $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$.

There are several keys to this approach. First, the DNA is attached to the solid support, so each reaction may be driven to completion by employing an excess of reagents since any unreacted material is simply washed away. Second, the phosphate moieties of the DNA backbone are all capped as the cyanoethyl esters so the oligonucleotide is uncharged and therefore well solvated by organic solvents. Thirdly, all

Scheme 5.1. Synthesis of Rhodium-Modified Oligonucleotides on a Solid Support.^{a,b}

Reagents. (a) CDI, dioxane, 30 min. rt.; (b). $\text{H}_2\text{N}(\text{CH}_2)_9\text{NH}_2$, dioxane/ H_2O (9:1), 25 min. rt.; (c) TSTU, DIEA, CH_3OH , CH_3CN , CH_2Cl_2 (1:1:1), rt., 1 h; (d) heterogeneous coupling, DIEA, rt., 12 h; (e) NH_3 (aq), 60 °C, 6 h. **Abbreviations.** CPG = controlled pore glass; Rh = $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$.

of the exocyclic amines are masked with acyl protecting groups so the only reactive functionality is the 5'-OH, which is where we want to carry out reactions.

The first step in the synthesis of 5'-modified oligonucleotides is the introduction of a flexible linker to the 5' terminus of the strand. Using the method of Wachter *et al.*,¹¹ we functionalized the 5'-OH with carbonyl diimidazole (CDI), and attacked the carbamate carbon with a diamino alkane such as diaminononane. This reaction proceeds in >85 % yield and can be followed by reversed phase HPLC (Figure 5.2). At this stage the oligonucleotide is poised for reaction with an electrophile.

In order to introduce the metal complex onto the functionalized oligonucleotide, we exploited the versatile amide bond formation. The metallointercalator, $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$, is functionalized with a carboxylate on the nonintercalating ligand (bpy') and was chosen as a suitable complex for conjugation. In order to activate the carboxyl for the coupling reaction, $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ was treated with N-hydroxy succinimidyl tetramethyluronium tetrafluoroborate (TSTU) to form an N-hydroxy succinimidyl (NHS) ester.^{12,13}

TSTU was used to synthesize an activated ester with a derivative of $[\text{Ru}(\text{phen})_3]^{2+}$ for conjugation to amino-modified DNA in solution.¹²⁻¹⁶ In our work we favored TSTU because we could follow the activation of the carboxylate by thin layer chromatography and isolate the NHS ester for characterization. A matrix-assisted laser-desorption (MALDI) mass spectrum of the NHS ester of $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ is shown in Figure 5.3.

To effect the coupling of the metal complex to the amino-terminated oligonucleotide via formation of a carbamate linkage, the solution containing the NHS ester (without isolation) is combined with the CPG beads coated in amino-modified DNA to form a heterogeneous slurry. In this step the metallointercalator is tethered to the DNA on the CPG bead, which results in the coloration of the beads. The final step is to liberate the conjugate from the bead and simultaneously deprotect the bases by treatment with

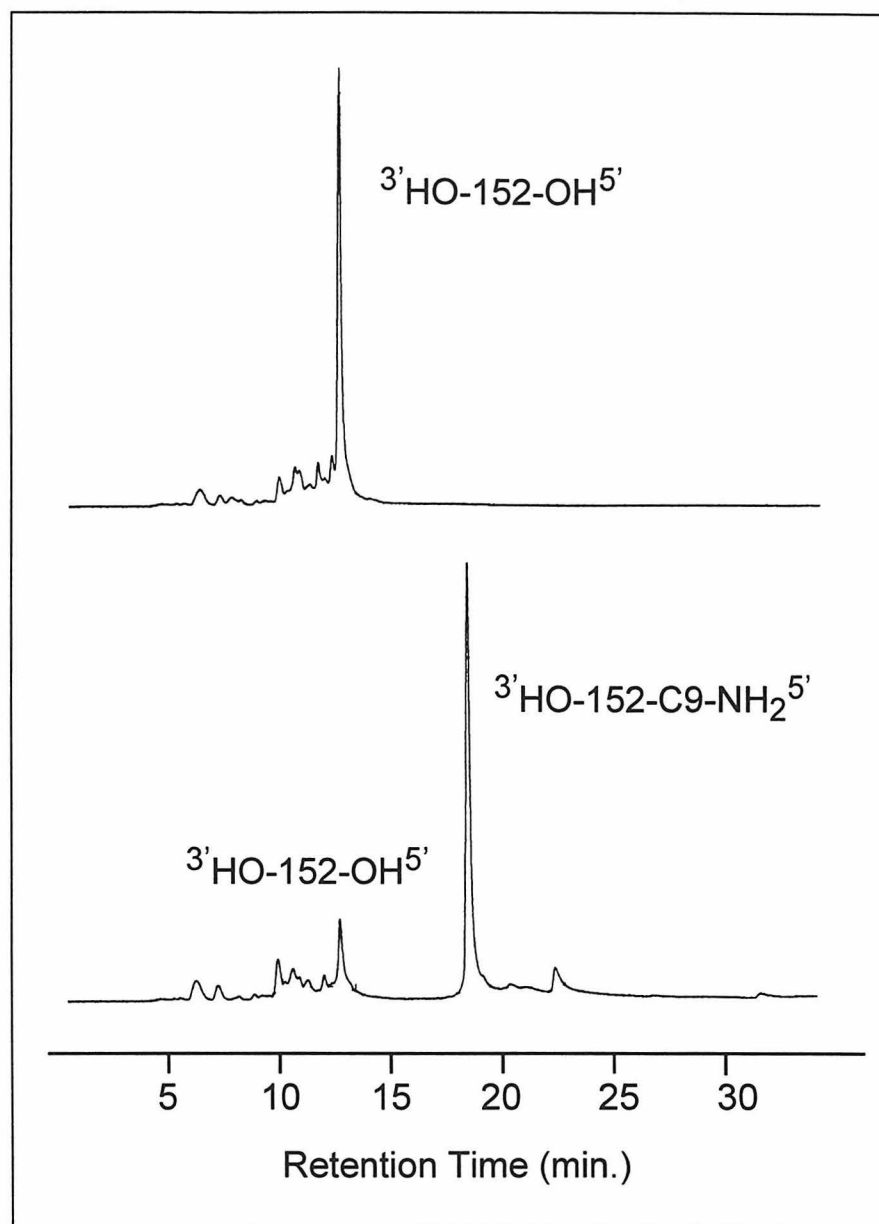


Figure 5.2. HPLC chromatograms of an unmodified (upper trace) and an aminoalkylated (lower trace) 15-mer monitored at 260 nm. HPLC Conditions: C18 stationary phase; 50 mM ammonium acetate, pH 6.5/acetonitrile mobile phase; 0-15 % acetonitrile in 30 min. The aminoalkylated DNA shows a later retention time, consistent with increased hydrophobicity introduced by the linker.

concentrated NH_3 (aq). The conjugates are then purified by reverse phase HPLC on C_{18} or C_4 stationary phases.

Figure 5.4 shows a typical chromatogram of an unpurified reaction mixture for the synthesis of 5'-Rh-modified DNA. We monitored the mobile phase eluted from the column by absorption at 290 nm where everything in the mixture should absorb and at 390 nm where the rhodium complex absorbs. As evident in the figure, the reaction is very clean and produces only two products that contain rhodium.

The absorption spectra (Figure 5.4 B) of the two products are consistent with Rh-modified DNA. The intense absorption centered at 260 nm reflects the contribution of the DNA, while the less intense absorption centered at 390 nm corresponds to absorption of the metal complex. These absorption spectra are essentially identical and appear to be composites of the spectra of DNA and $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ alone, however upon close inspection, it is apparent that there is hypochromism in the transition centered at 390 nm. This hypochromism indicates that the metal complex is associated with the bases of the single-stranded DNA.^{17,18}

In order to answer the question of why there are two products with identical absorption spectra, we measured the circular dichroism (CD) spectra of each species (Figure 5.5). Upon comparing the CD spectra in Figure 5.5 to those for the enantiomers of $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$, and based on their equal and opposite relationship, we concluded that into one product we had incorporated Δ - $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$, while Λ - $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ was introduced into the other. Thus, we had prepared two diastereomeric Rh-DNA conjugates. Remarkably, the achiral HPLC was the only purification method required to separate these two products.

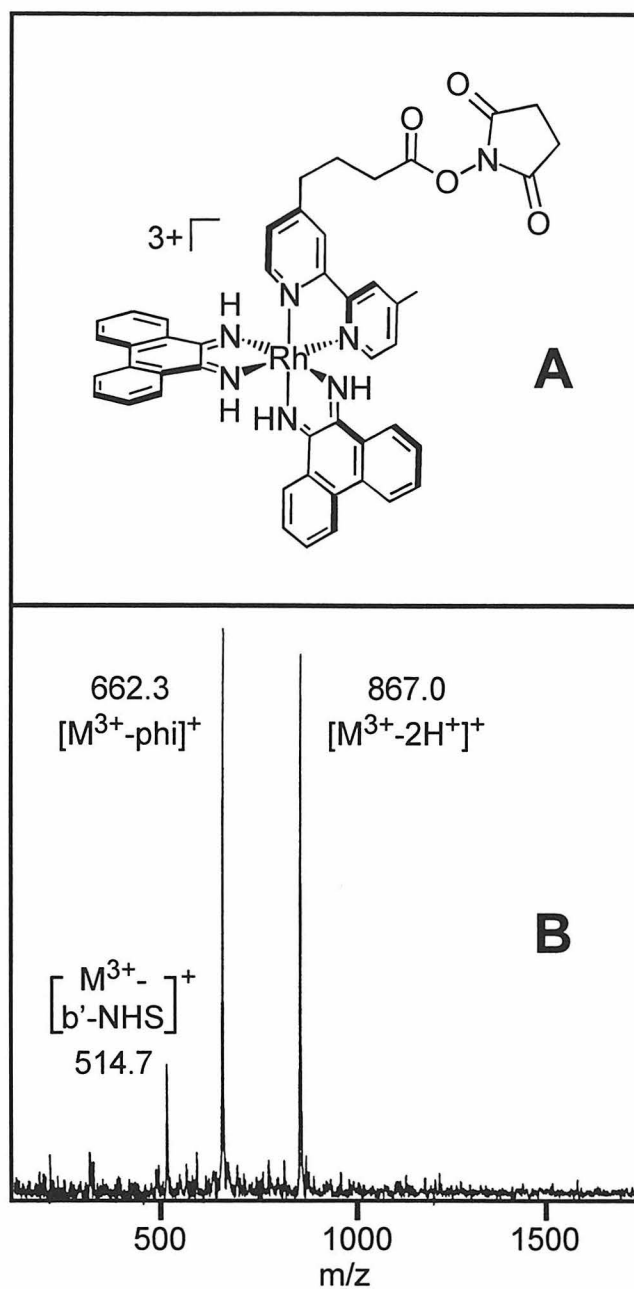


Figure 5.3. (A) Schematic illustration of the N-hydroxysuccinimidyl (NHS) ester of $[\text{Rh}(\text{phi})_2(\text{bpy}')^3]^+$. (B) Matrix assisted laser desorption ionization (MALDI) time of flight (TOF) mass spectrum of $[\text{Rh}(\text{phi})_2(\text{bpy}'\text{-NHS})]^3+$.

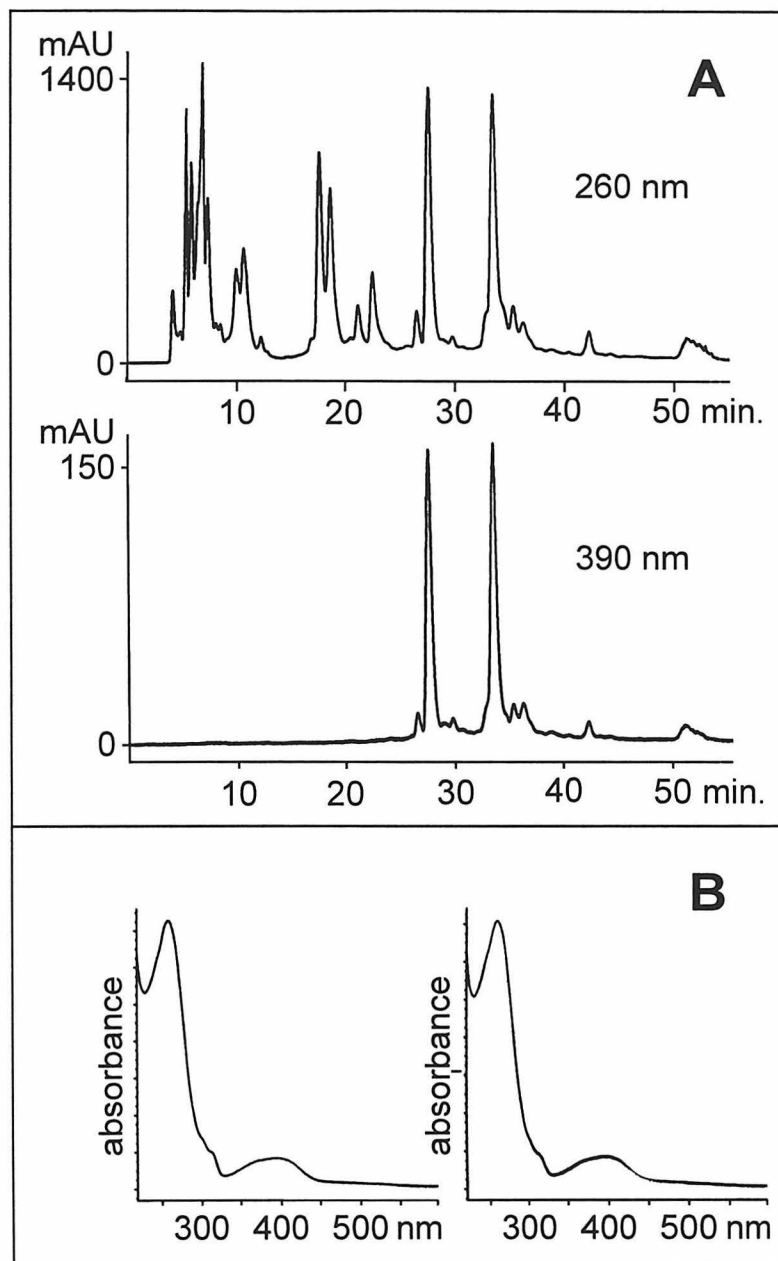


Figure 5.4. HPLC chromatogram of an Rh-DNA conjugate reaction mixture before purification (upper panel). UV-vis absorption spectra of the two rhodium-containing fractions at elution times of 28 min. (left) and 34 min. (right) (lower panel).

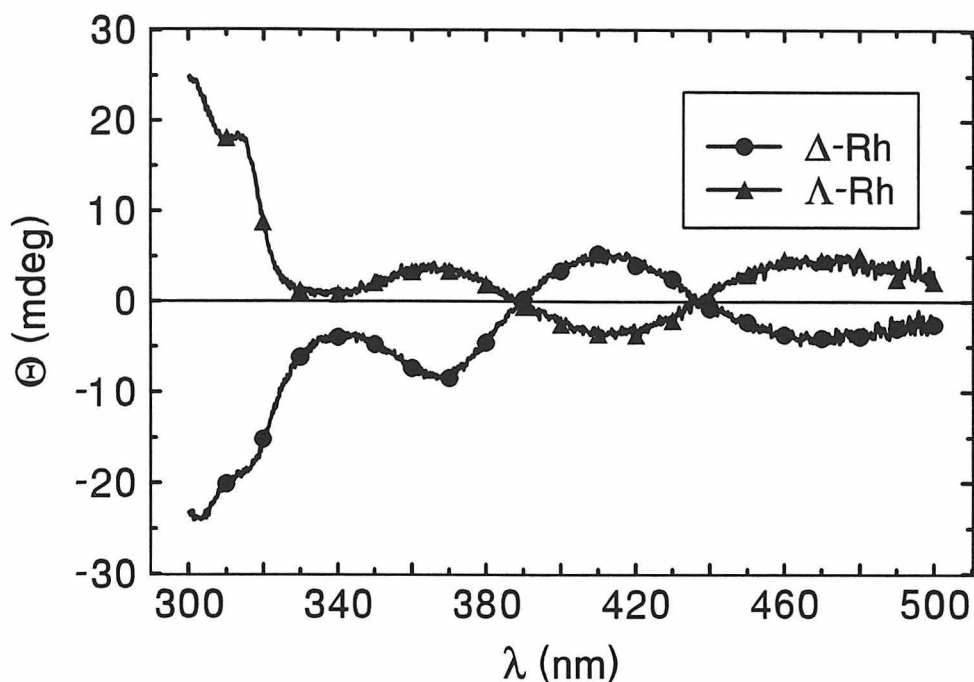


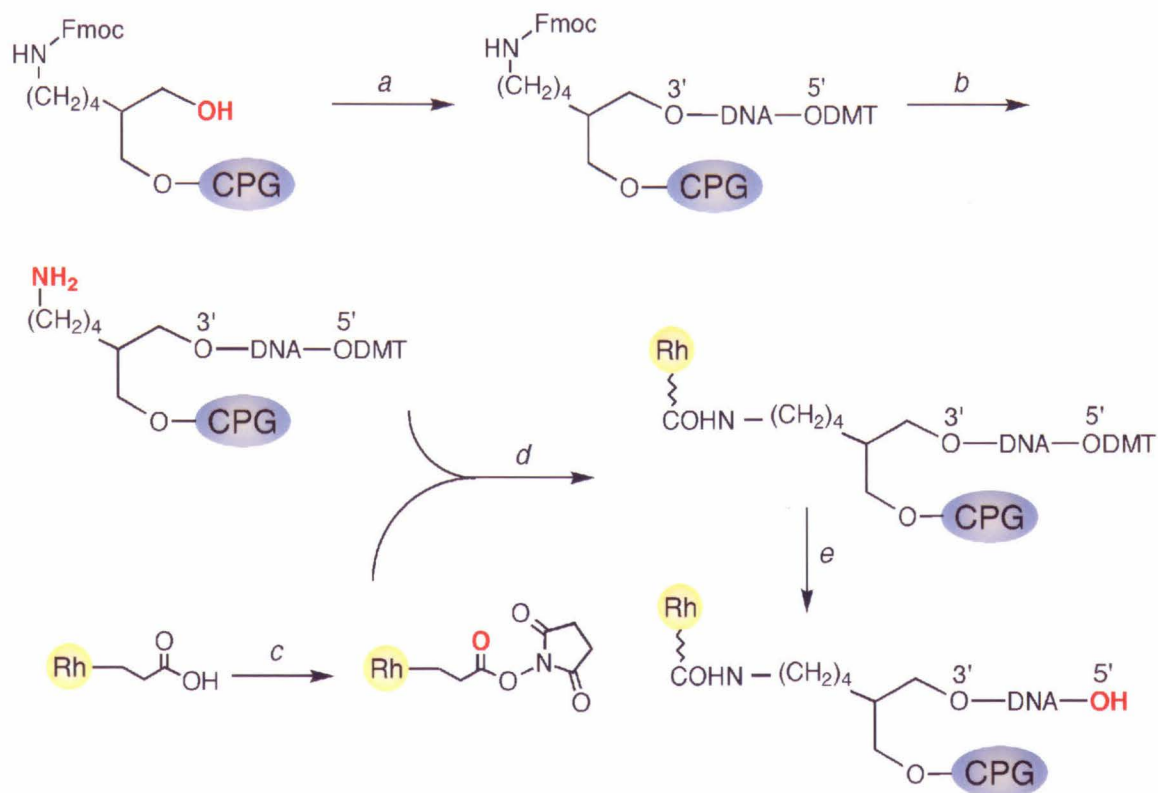
Figure 5.5. Circular dichroism of the two rhodium-containing products (Δ -Rh-151, Λ -Rh-151) isolated from the reaction mixture shown in Figure 5.4.

Synthesis of Rh-Modified Oligonucleotides with the Metallointercalator

Attached to the 3' Terminus. To expand the generality of the heterogeneous coupling method developed for coupling metallointercalators to the 5' terminus of oligonucleotides, we sought to develop a similar approach for modification of the 3' terminus. The strategy we devised is shown in Scheme 5.2. As with conjugation to the 5'-hydroxyl, this approach relies on derivatization of the strand on the CPG resin.

The synthesis of 3'-metallated DNA begins with a commercially available CPG bead that is functionalized with a hydroxy aminoalkane masked with Fmoc (NH_2) and dimethoxy trityl (DMT) protecting groups. The Fmoc group is labile in base but not acid and will therefore withstand the conditions of automated DNA synthesis under the phosphoramidite conditions. The first step in this approach is to remove the DMT group

Scheme 5.2. Synthesis of Rhodium-Modified DNA with the Metallointercalator on the 3' Terminus.^{a,b}



^aReagents. (a) Automated DNA synthesis (ABI 391 DNA synthesizer); (b) piperidine (20% v/v), DMF, rt., 30 min.; (c) TSTU, DIEA, CH₃OH, CH₃CN, CH₂Cl₂ (1:1:1), rt., 1 h; (d) heterogeneous coupling, DIEA, rt., 12 h; (e) TCAA, CH₃CN, 18 s, rt.

^bAbbreviations. CPG = controlled pore glass; DMT = dimethoxy trityl; Rh = [Rh(phi)₂(bpy⁺)]³⁺.

to reveal the hydroxyl group to which the first 3'-terminal base is attached. Next, the DNA strand is synthesized by standard methods with the final 5'-DMT protecting group left intact.

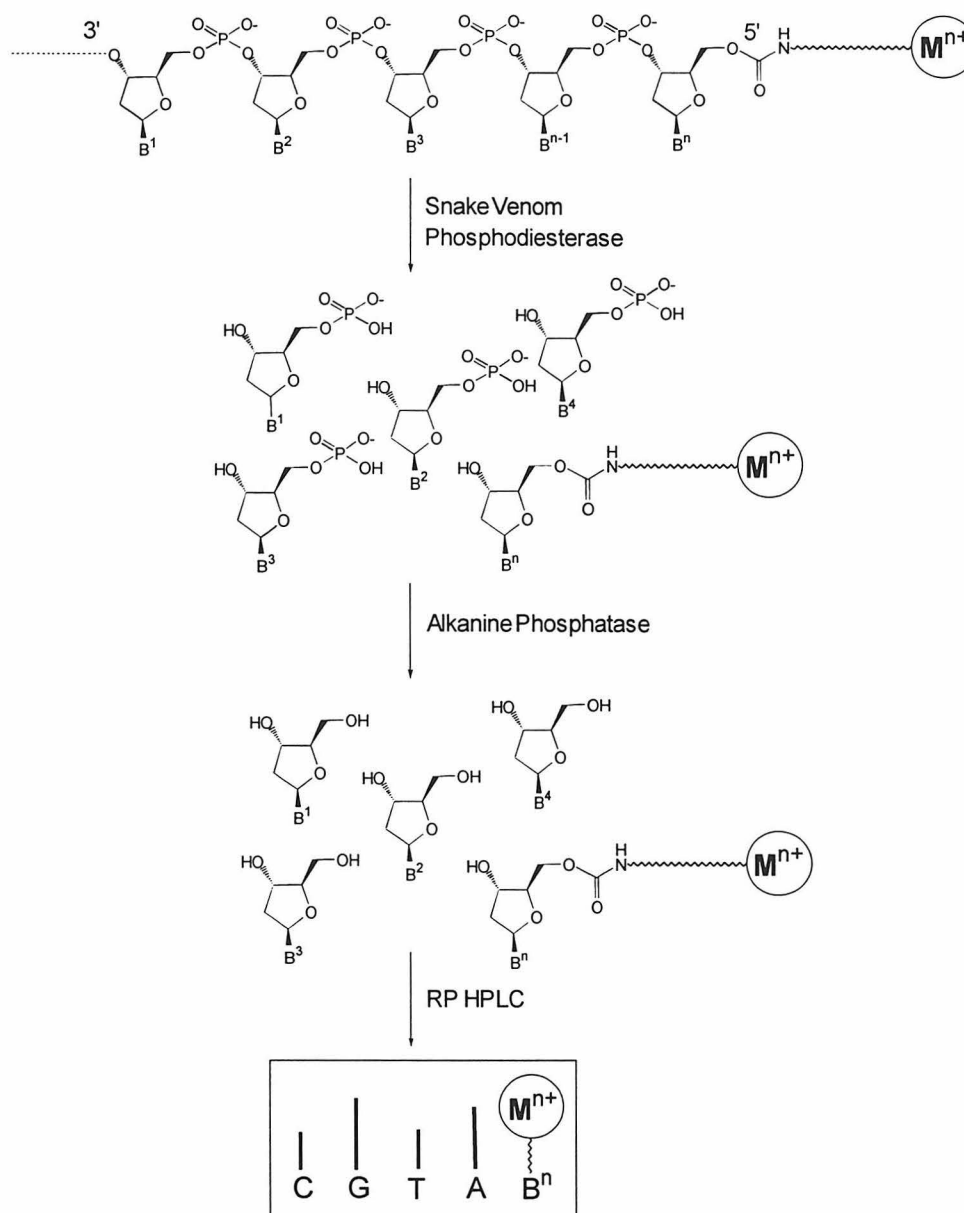
The next step is to selectively unmask the Fmoc-protected amine by treatment with piperidine in DMF. This procedure works well since the acyl protecting groups on the DNA bases will not react with the piperidine and the DMT is not labile in base. Following this deprotection step the oligonucleotide is coupled to the NHS ester of $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ as described for the 5'-modification scheme. Before cleaving the product from the bead, the DMT protecting group is removed from the 5'-hydroxyl by treatment with trichloroacetic acid in acetonitrile. Liberation from the bead and purification also proceed as described for the 5'-modified conjugates. As observed for the conjugates with the complex attached to the 5'-terminus, two products are isolated by HPLC in the purification of 3'-Rh-modified DNA.

5.2.2. Characterization of Rh-Modified Oligonucleotides

Perhaps the most important goal we set for ourselves in developing strategies to prepare metallointercalator-DNA conjugates is that we wanted to be able to subject the products to rigorous chemical and biochemical characterization. We employed well established and newly developed methods from outside our expertise, as well as techniques developed in our laboratories to establish the properties and composition of Rh-modified oligonucleotides.

Enzymatic Digestion of Rh-Modified DNA to Establish Base Content.

Scheme 5.3 outlines an established enzymatic digestion procedure we employed to measure the base content of Rh-modified DNA compared to unmodified DNA. The premise of the assay is to detect the loss of the 5'-terminal base. In this approach the oligonucleotide is treated with a cocktail of snake venom phosphodiesterase (SVP) and alkaline phosphatase (AP).¹⁹ SVP cleaves the phosphodiester bonds of the



Scheme 5.3. Schematic illustration of the enzymatic digestion of oligonucleotides by snake venom phosphodiesterase to give nucleotide monophosphates (NMPs), and hydrolysis of NMPs by alkaline phosphatase to give nucleotides. The 5'-terminal base, remains attached to the metallointercalator because the enzymes do not hydrolyze the carbamate linkage. The products are separated by HPLC and the numbers of each base are counted.

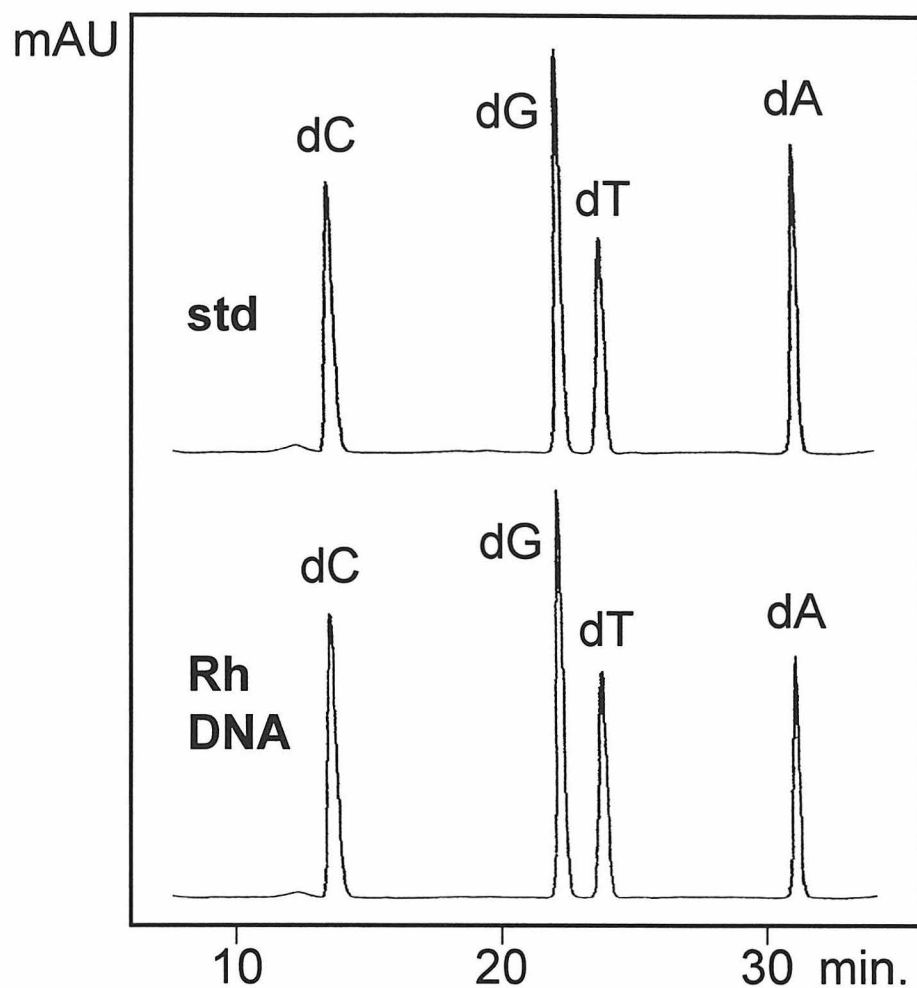


Figure 5.6. HPLC chromatograms monitored at 260 nm of the enzymatic digestion of an unmodified 20-mer (top) and a Rh-modified 20-mer (bottom). The area under the peak for dA is clearly reduced in the data for the conjugate. This loss of dA is consistent with the coupling of the metallointercalator to the 5' terminus of this oligonucleotide.

oligonucleotide to release the nucleotide monophosphates (NMPs). AP then hydrolyzes the NMPs to give the neutral nucleotides, which are significantly more amenable to chromatographic separation. The reaction mixture is filtered, which removes the enzyme, and purified by reversed phase HPLC. Since the enzymes do not recognize the carbamate linkage that joins the nucleotide and the metal complex through an aliphatic linker, the modified DNA will appear to have lost one base in the HPLC analysis.

Figure 5.6 shows the results of the digestion of two oligonucleotides: an unmodified 14-mer and a Rh-modified 14-mer. Given the sequence of the DNA, the rhodium complex is attached to a deoxyadenosine (dA). It is apparent in the figure that the area under the peak corresponding to dA is less for the conjugate compared with that for the unmodified strand. In addition the numbers of each base determined by integration are consistent with the known sequence. These results establish that the conjugation reaction is at the 5' terminus and that no other bases are modified.

Electrospray Ionization Mass Spectrometry of Rh-DNA Conjugates. One of the most powerful analytical methods in modern biochemistry is mass spectrometry (MS).²⁰⁻²³ We used electrospray ionization (ESI) MS to measure the mass of Rh-modified duplexes. The soft ionization of ESI MS is useful for oligonucleotides and the resolution of the technique is easily 1 amu. Therefore ESI MS can be used to confirm not only that the conjugate is intact, but that all of the protecting groups have been eliminated.

Figure 5.7 shows the ESI MS of a 20-mer oligonucleotide attached to $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ by a diaminononane linker and carbamate linkage at the 5'-hydroxyl of the DNA. The data shown is a reconstructed spectrum, convolved from mass:charge ratios for several charge states. The calculated mass for the conjugate is 6964.8, and the observed mass is 6965.0.

Following the Hybridization of Rh-modified Oligonucleotides to their Complements by PAGE. We followed the hybridization of ^{32}P -end labeled DNA to Rh-

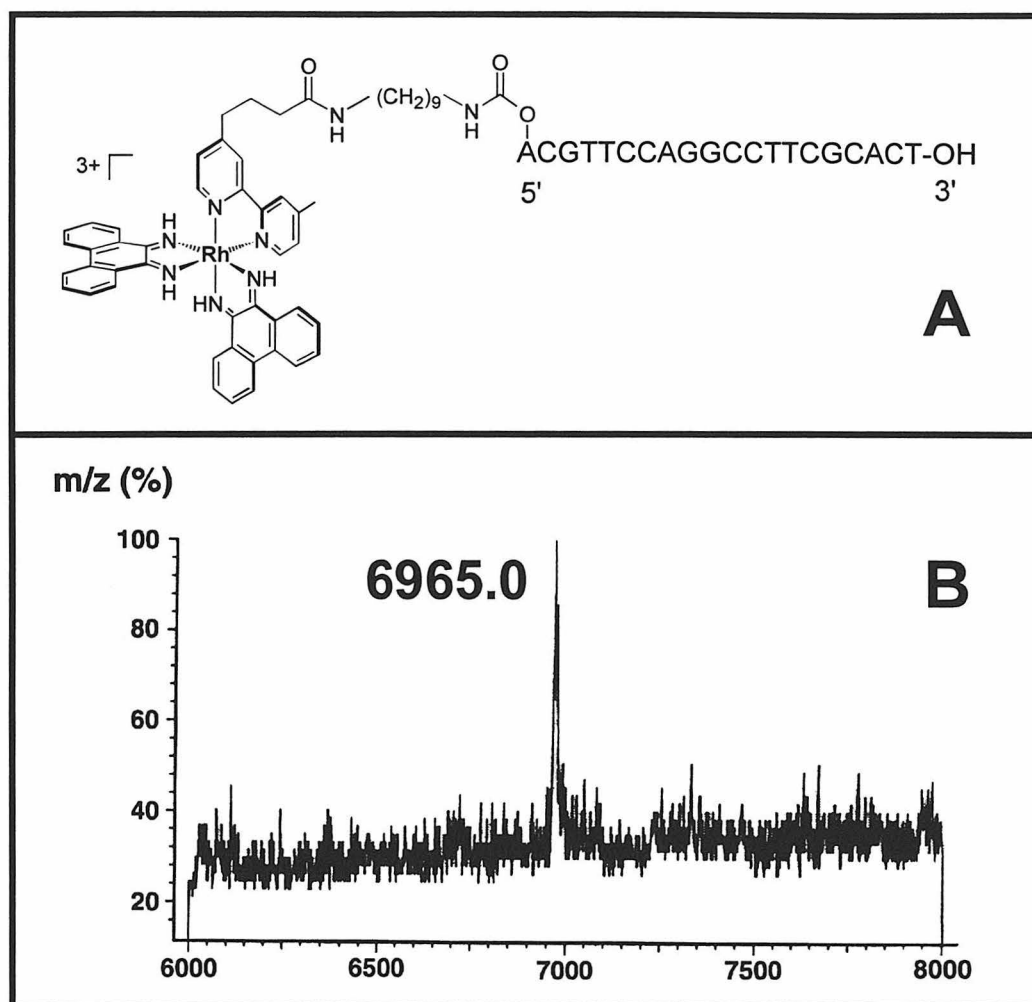


Figure 5.7. Electrospray ionization (ESI) mass spectrum of $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ tethered to a 20-mer oligonucleotide. The calculated mass for this conjugate is 6964.8 amu and the observed mass is 6965.0.

modified DNA on nondenaturing polyacrylamide gels (free of urea). Owing to the stiffness of duplex DNA relative to single strands, double stranded is retarded relative to single stranded DNAs under electrophoresis on polyacrylamide gels.

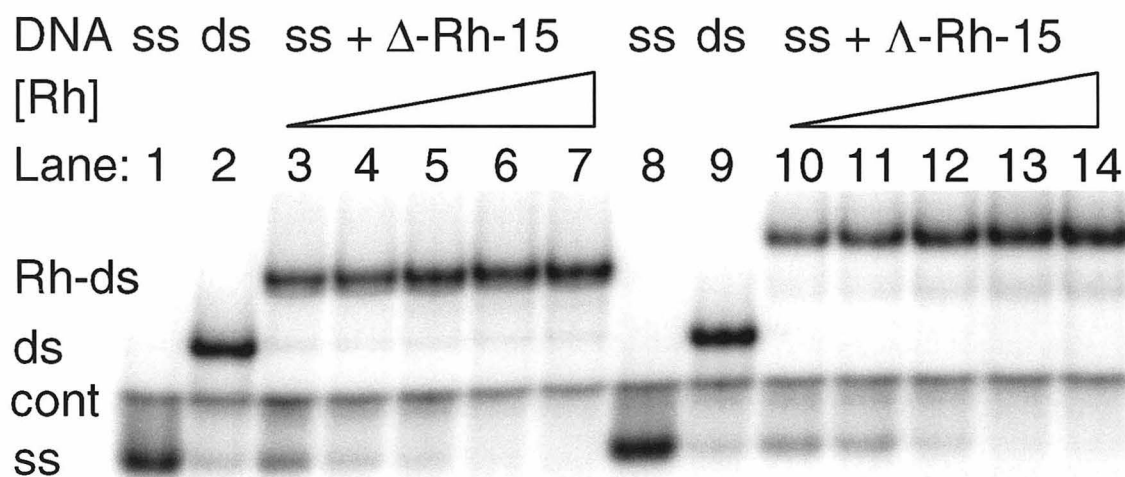


Figure 5.8. Nondenaturing polyacrylamide (20%) gel for following the hybridization of ^{32}P -end labeled complement to Rh-modified DNA. Contents of lanes: (1) labeled complement (single stranded); (2) unmodified duplex (complement plus rhodium strand; 1:1); (3-7) labeled complement (8 μM) plus increasing concentrations of Δ -Rh-DNA (6,7,8,9,10 μM). Lanes 8-14 are the same as 1-7, with Λ -Rh substituted for the Δ isomer.

Figure 5.8 illustrates a typical hybridization experiment for Λ -Rh-154 and Δ -Rh-154. The first lane of each titration contains only the ^{32}P -end labeled complement of the Rh-modified strand (^{32}P -154.C). The second lane contains ^{32}P -154.C hybridized to one equiv of the unmodified rhodium strand. As expected for the formation of a 1:1 complex, a new band appears in the gel retarded relative to the single strand and there is no ^{32}P -154.C remaining. The next five lanes contain ^{32}P -154.C (8 μM) and increasing concentrations of Rh-154 (6, 7, 8, 9, and 10 μM). As in the case of the unmodified

duplex, a new band appears near the top of the gel. The intensity of this band increases with Rh-154 until it reaches saturation at 8 μ M. Similarly, the band for ^{32}P -154.C decreases in intensity until disappearing from the gel when the sample is fully duplexed. There is an exogenous source of radioactivity in the sample that is present in all lanes and migrates just above the single strand.

It is noteworthy that the Rh-modified duplexes migrate above the unmodified duplex. Such an observation is consistent with the positively charged metal complex reducing the negative charge on the duplex and diminishing its attraction to the positive pole of the gel.

Upon comparison of migration profiles of the Δ and Λ -Rh-modified duplexes, the diastereomer containing Λ -Rh moves slower on the gel than does the Δ -Rh containing diastereomer. Owing to the symmetry matching of the right-handed DNA duplex with the right-handed Δ -Rh, we expect the complex to be intercalated more deeply into the duplex than the Λ -isomer. The migration behavior observed in the gels is consistent with this notion, since a more compact structure should be less hindered by the gel, as is the duplex bearing Δ -[Rh(phi)₂(bpy')]³⁺. These experiments demonstrate that the presence of the rhodium complex does not preclude the stoichiometric formation of duplexes, and that the association of the Λ enantiomer of the metallointercalator with the duplex is different from the interactions of the Δ enantiomer.

Photocleavage of Rh-modified DNA Duplexes. Phi complexes of Rh(III) are versatile probes for DNA structure and function.²⁴ Upon photoactivation with UV light in the presence of DNA, the photoexcited complexes can abstract a hydrogen atom from the sugar residue at the base step of intercalation.¹⁷ Such damage to of the sugar-phosphate backbone leads to direct strand scission at the site of intercalation by the rhodium complex and marks the binding site. The ^{32}P -labeled products of the photochemical reaction are separated by PAGE, and visualized by phosphorimager.^{24,25} This approach provides single-base resolution of the site of cleavage.

Figure 5.9. Phosphorimagery results depicting the products of the direct photocleavage of Rh-modified duplexes^a upon irradiation at 313 nm for 8 min. The experiment measures the site of intercalation as a function of configuration about the metal center, linker length, and base sequence for a series of rhodium-DNA conjugates. All samples were 8 μ M in duplex in 5 mM Na₃PO₄, 50 mM NaCl, pH 7.0. The contents of each lane are as follows: **1-3**, 15 bp duplex, 16/15-mer hybrid duplex, 16 bp, duplex, respectively, no irradiation (dark control); **4,5**; Δ -Rh-15 bp duplex, and Δ -Rh-15 bp duplex with C9 linker; **6,7**; Δ -Rh-15 bp duplex, and Δ -Rh-15 bp duplex with C6 linker; **8,9**; Δ -Rh-16/15 hybrid duplex, and Δ -Rh-16/15 hybrid duplex with C9 linker; **10,11**; Δ -Rh-16/15 hybrid duplex, and Δ -Rh-16/15 hybrid duplex with C6 linker; **12,13**, Maxam-Gilbert sequencing lanes for 15-mer (A+G: 12; C+T: 13; **14,15**, Δ -[Rh(phi)₂(dmb)]³⁺ bound noncovalently to 15 bp duplex (14) and 16/15 hybrid (15); **16,17**, Δ -Rh-16 bp duplex, and Δ -Rh-16 bp duplex with C9 linker; **18,19**, Δ -Rh-16 bp duplex, and Δ -Rh-16 bp duplex with C6 linker; **20**, Δ -[Rh(phi)₂(dmb)]³⁺ bound noncovalently to 16 bp duplex; Maxam-Gilbert sequencing lanes for 16-mer (A+G: 21; C+T: 22).

^aSequences of assemblies:

Rh-15 bp duplex:	Rh-16/15 hybrid	R-16 bp duplex
5' -Rh-ACGATATACTAACGT-3'	5' -Rh- T ACGATATACTAACGT-3'	5' -Rh- T ACGATATACTAACGT-3'
3' -TGCTATATGATTGCA-5' *	3' -TGCTATATGATTGCA-5' *	3' -ATGCTATATGATTGCA-5' *

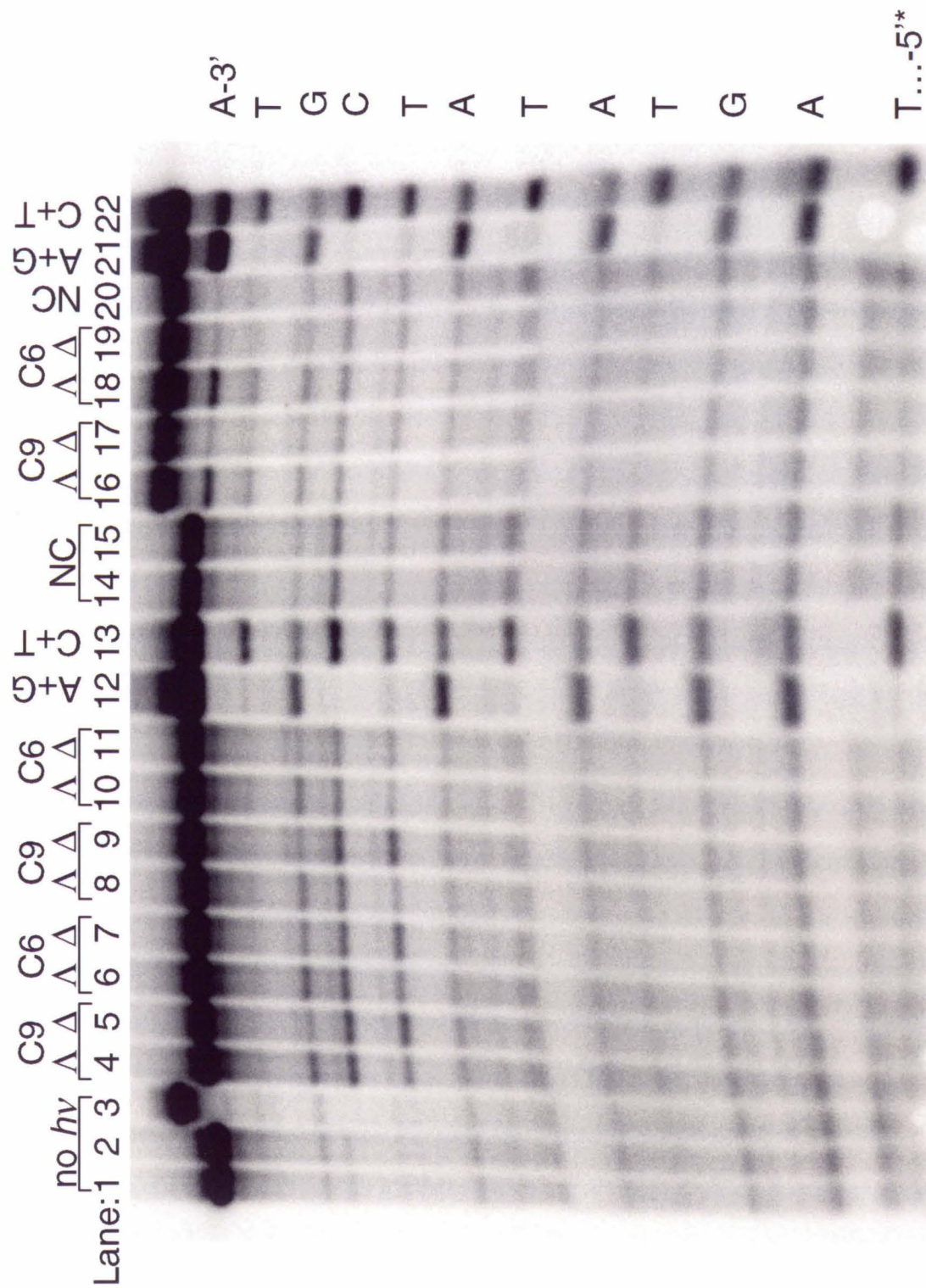


Figure 5.9 shows the polyacrylamide gel used to reveal the site of intercalation in rhodium-modified DNA duplexes as a function of the length of the linker and the binding site. To clarify these data, the results are summarized schematically in Figures 5.10 and 5.11.

We first examined photocleavage by the Δ and Λ enantiomers of $[\text{Rh}(\phi)_2(\text{bpy}')]^{3+}$ tethered by diaminononane (C9) or diaminohexane (C6) linkers to a 15 base pair (bp) duplex (lanes 4-7; Figure 5.9). These reactions were also compared to photocleavage by the parent complex $[\text{Rh}(\phi)_2(\text{dmb})]^{3+}$ bound noncovalently to the unmodified duplexes (lanes 14-15). For the samples where the complex was bound noncovalently, the cleavage is distributed fairly evenly throughout the duplex, with some specificity for binding between the 5'-G(3) and 5'-A(4), as designed. In fact, the 5'-ACGA sequence was chosen to accommodate the tethered complex since this is a preferred binding site of $[\text{Rh}(\phi)_2(\text{dmb})]^{3+}$. Cleavage by the tethered complex is only observed near the end of the duplex to which the metallointercalator is confined by the linker.

For modified duplexes bearing the C9 or C6 linkers, the Λ binds primarily two bases in from the 5'-terminus and the Δ isomer binds three bases from the terminus. This observation has been made repeatedly for rhodium complexes tethered to the sequence 5'-ACGA(T). In quantitative comparisons of cleavage intensities, it is apparent that there is a diminution in the amount of cleavage with the C6 linker, suggesting that this linker might not be long enough to permit efficient access of the preferred binding site.

We next investigated hybrid duplexes composed of a Rh-modified 16-mer hybridized to a 15-mer to give a single base overhang at the end of the duplex to which the metallointercalator was attached (Figure 5.9). The cleavage patterns and hence binding sites for the 16/15 bp hybrids resembled closely those observed with the fully base-paired duplexes. The cleavage patterns exhibited by the complexes attached by C9 linkers did not indicate any significant changes in the site of intercalation by the rhodium

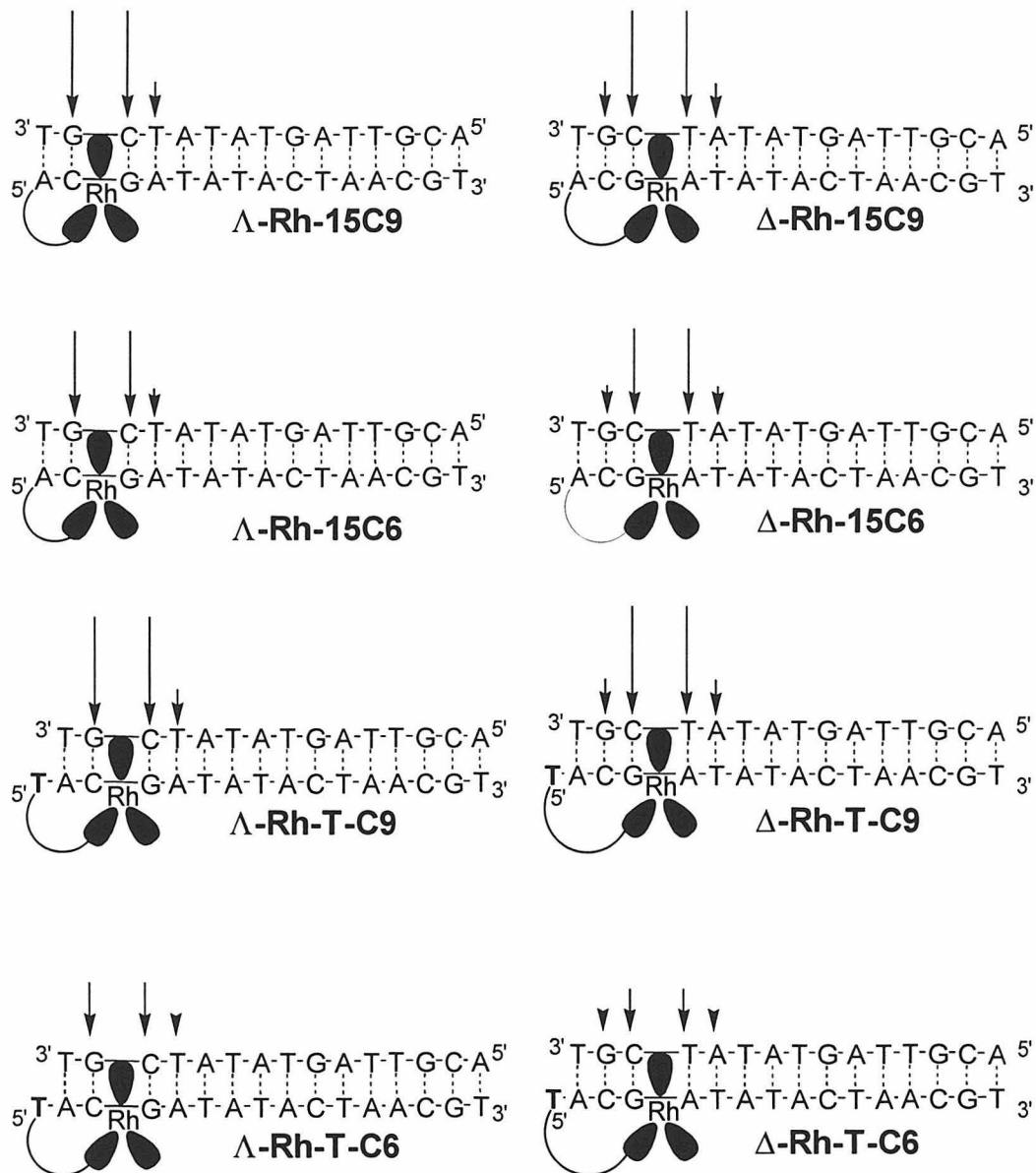


Figure 5.10. Summary of photocleavage of a 15 bp duplex by $[\text{Rh}(\phi)_2(\text{bpy}')]^{3+}$ tethered to the 5'-ACGA terminus as indicated above. The columns correspond to lane numbers in Figure 5.9 as follows: **Left Column** (top to bottom): lane 4, lane 6, lane 8, lane 10; **Right Column** (top to bottom): lane 5, lane 7, lane 9, lane 11. Arrows represent sites of cleavage and their height represents relative cleavage intensity.

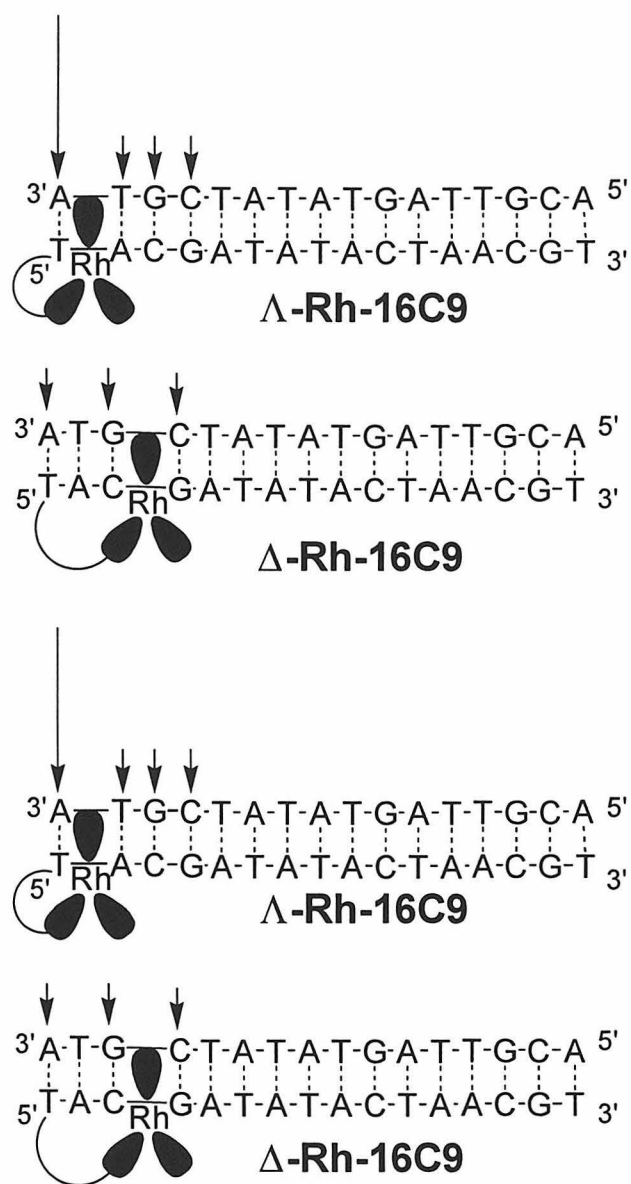


Figure 5.11. Summary of photocleavage results for $[\text{Rh}(\phi)_2(\text{bpy}')]^{3+}$ tethered to 5'-TACG. The column corresponds to lane numbers in Figure 5.9 as follows: (top to bottom): lane 18, lane 19, lane 16, lane 17. Arrows represent sites of cleavage and their height represents relative cleavage intensity.

complex. With the C6 linker, however, the cleavage intensity drops off dramatically, especially for the Δ isomer. This observation parallels the reduction in cleavage intensity described above for the C6 linker in the fully base-paired duplexes, and demonstrates very clearly that the complex is restricted from binding to the duplex. Importantly, we do not see a cleavage pattern in these lanes that indicates intermolecular intercalation either. Thus, the C6 linker combined with a single-base overhang disrupts intercalation significantly.

Finally, we investigated the photocleavage by rhodium tethered to 16 bp duplexes derived from the Rh-modified 16-mer used in the 16/15 bp hybrids. The 5'-terminal sequence for these duplexes is 5'-TACG. Of course, the 5'-ACGA binding site is simply shifted in by one base pair from the Rh-terminus. The photocleavage patterns observed for these samples were strikingly different from those described above. For the Λ isomer, intercalation is almost exclusively at the first base step, between 5'-T(1) and 5'-A(2). The Δ isomer appears not to bind to this sequence very well at all. This pattern is seen for both the C6 and C9 linkers. These results illustrate the importance of the sequence of DNA bases, as well as the length of the linker in determining the site of intercalation by a rhodium complex tethered to the terminus of the duplex by aliphatic linkers.

Quenching the Emission from Photoexcited $[M(\text{phen})_2(\text{dppz})]^{2+}$ Bound to DNA by $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ in Rh-modified Duplexes to Characterize Rhodium Intercalation. We sought to develop an assay that would permit us to correlate the intercalative properties of $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ tethered to DNA duplexes with the ability of the rhodium complex to serve as an electron acceptor in DNA-mediated ET experiments. The efficiency with which the emission from photoexcited electron donors such as $[M(\text{phen})_2(\text{dppz})]^{2+}$ ($M = \text{Ru}, \text{Os}$) is quenched depends sensitively on how well the donors and acceptors intercalate into the DNA base stack.²⁻⁴ Therefore, we compared the quenching of ruthenium and osmium intercalators

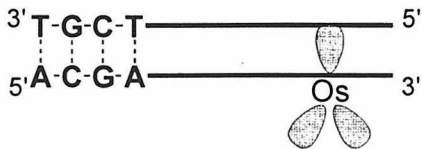
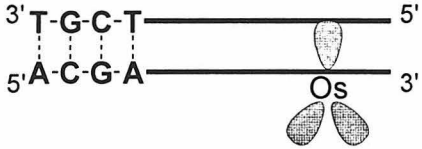
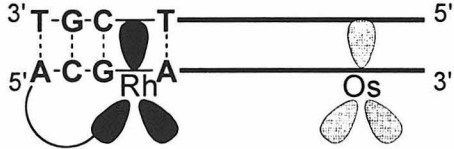
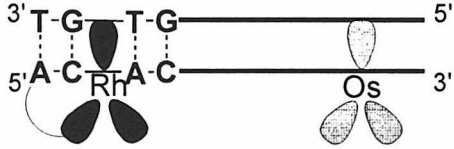
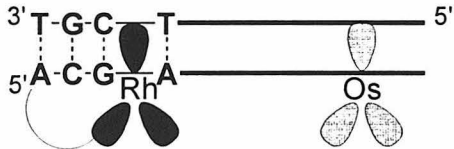
Assembly	Metal	I (rel)	Fq
	<i>rac</i> -Os	1.00	-----
	<i>rac</i> -Os	1.00	-----
	Δ -Rh <i>rac</i> -Os	0.24	0.76
	Λ -Rh <i>rac</i> -Os	0.68	0.32
	<i>rac</i> -Rh <i>rac</i> -Os	0.80	0.20

Figure 5.12. Fraction of photoexcited $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ bound to 15 bp duplexes quenched by $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ tethered to 15 bp duplexes of different sequence. The Os(II) complex is bound noncovalently to the duplex, and thus the actual binding site is not known. Therefore the only sequence shown is that near the terminus to which the rhodium complex is bound (based on photocleavage).

bound to Rh-modified duplexes was compared as a means to extrapolate the coupling of the rhodium complex into the DNA base stack.

The quenching by $[\text{Rh}(\phi)_2(\text{bpy}')]^{3+}$ tethered to DNA was first characterized as a function of the base sequence at the Rh-terminus. Figure 5.12 lists the assemblies characterized and the fraction of photoexcited donors ($[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$) bound to Rh-modified duplexes that are quenched by the metallointercalator. The parent complex $[\text{Rh}(\phi)_2(\text{dmb})]^{3+}$ binds preferentially to the site 5'-ACGA, so this sequence was incorporated into one duplex, and 5'-ACAC into another. In these assemblies, the donor is bound noncovalently, hence the schematic drawing of the duplex in the figure.

When the Δ isomer of the rhodium complex is intercalated into 5'-ACGA, the quenching of the emission from the osmium complex is far more efficient compared with the quenching by the Λ isomer in the same sequence. The Rh-modified oligonucleotide containing 5'-ACAC was isolated as a mixture of diastereomers, so we examined the properties of the mixture. The fraction quenched by $[\text{Rh}(\phi)_2(\text{bpy}')]^{3+}$ intercalated into 5'ACAC is only 20%, which is considerably less efficient compared with 5'-ACGA. These observations indicate that binding of the Δ isomer to 5'-ACGA proceeds with more effective coupling into the base stack compared with the Λ isomer, and with 5'-ACAC.

Figure 5.13 lists the results of this quenching assay applied to the duplexes examined as a function of linker and binding site by the photocleavage assay. These experiments employed $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ as the noncovalently bound luminescent probe. Across this series of duplexes, the efficiency of quenching in the duplexes containing Δ - $[\text{Rh}(\phi)_2(\text{bpy}')]^{3+}$ is always greater than that of the assemblies bearing Λ - $[\text{Rh}(\phi)_2(\text{bpy}')]^{3+}$. Furthermore, as observed in the photocleavage assay with these assemblies, the linker length does not appear to affect significantly the fraction quenched in the 15 bp duplexes or the 16/15 hybrid duplexes. The amount of quenching is reduced in the 16/15 hybrid duplex that contains the C6 linker, which parallels the photocleavage intensity by the rhodium complex. In the 16 bp duplexes with the 5'-TACG binding site

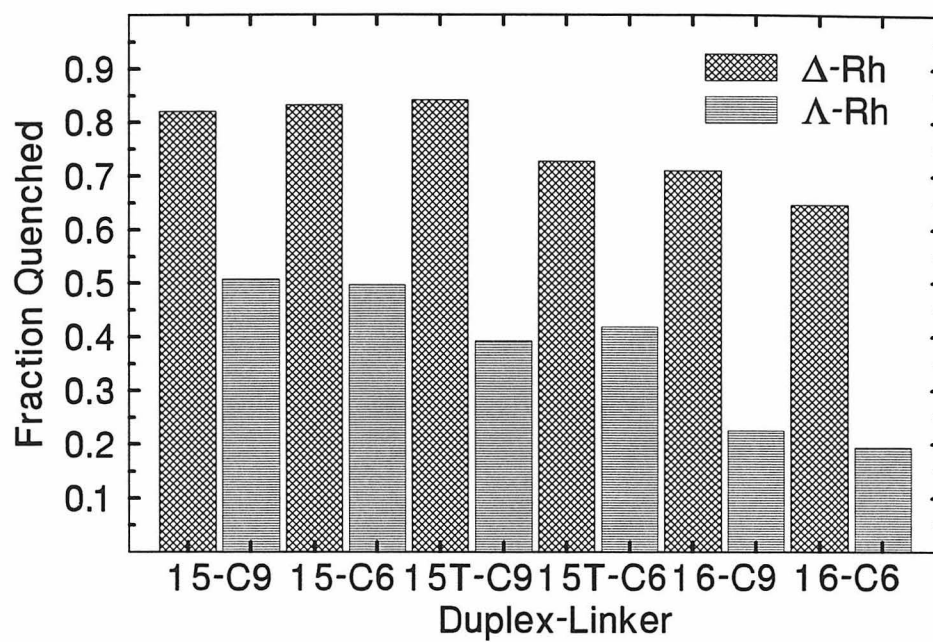


Figure 5.13. Plot of fraction quenched ($F_q = 1-I/I_0$) for $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to Rh-modified oligonucleotides as a function linker length and binding site.

for $[\text{Rh}(\text{phen})_2(\text{bpy}')]^{3+}$, the fraction of ruthenium donors quenched by the tethered complex is reduced slightly for the Δ -Rh and significantly for Λ -Rh. These observations are somewhat surprising given that Λ -Rh appears to be very specific for the 5'-TACG, however, this base step is at the end of the duplex and may not be as effective in supporting reaction with the photoexcited donor.

5.2.3. Synthesis of Os-Modified Oligonucleotides.

Until now our efforts have concentrated on Rh-modified oligonucleotides, which consist of single stranded DNA tethered to a photochemically active metallointercalator that serves as an electron acceptor in DNA-mediated ET reactions. In order to expand the scope of the synthetic methodology, we sought to prepare metallointercalator-DNA conjugates in which the metal complex was an electron donor such as $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ ($\text{M} = \text{Os}, \text{Ru}$), or a suitable analog.

Design and Synthesis of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$. The metal complex pictured schematically in Figure 5.14 was designed to substitute for $[\text{Rh}(\text{phen})_2(\text{bpy}')]^{3+}$ in the modification of oligonucleotides at the 5' terminus. With this complex, each ligand serves a purpose: (i) The $\text{Me}_2\text{-dppz}$ ligand serves as a platform for intercalation; (ii) The phen ligand provides rigidity in the ligand sphere to enhance the excited state lifetime and derivatives of phen can be used to manipulate the redox properties of the metal center; (iii) The bpy' ligand serves as coupling handle for attachment to aminoalkylated oligonucleotides.²⁶⁻²⁸ We synthesized $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ according to methods described in Chapter 2 for the preparation of complexes of the $[\text{Os}(\text{L}^1)(\text{L}^2)(\text{L}^3)]^{2+}$ type where L^1 , L^2 , and L^3 are different bidentate, polypyridyl ligands. As illustrated in Figure 5.14, $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ is isolated as four diastereomers: Δ and Λ enantiomers where the linker on bpy' extends from the metal center in an axial fashion with respect to $\text{Me}_2\text{-dppz}$; and Δ and Λ enantiomers of a complex where the linker is in an equatorial position. Such

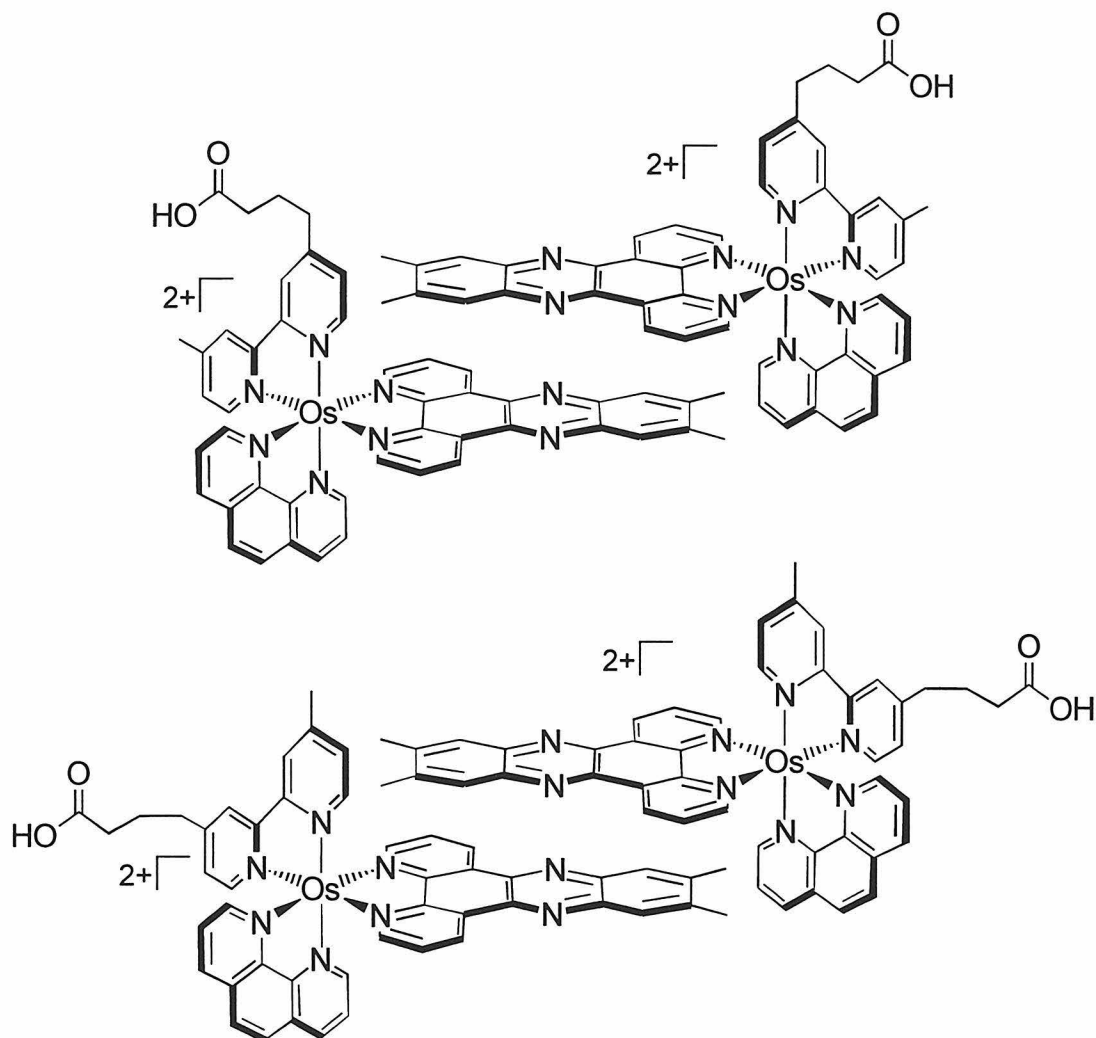


Figure 5.14. Schematic illustrations of the four diastereomers of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$. When the racemic mixture is used to couple the complex to DNA, four products are observed. We designate the sample by Δ or Λ for the orientation about the metal center, and then a number to indicate the order in which that particular conjugate eluted from the HPLC. For example Δ -1-Os-181 corresponds to a Δ isomer of the osmium complex in the first product to elute from the HPLC attached to an 18-mer oligonucleotide. This code is used to designate samples throughout this chapter.

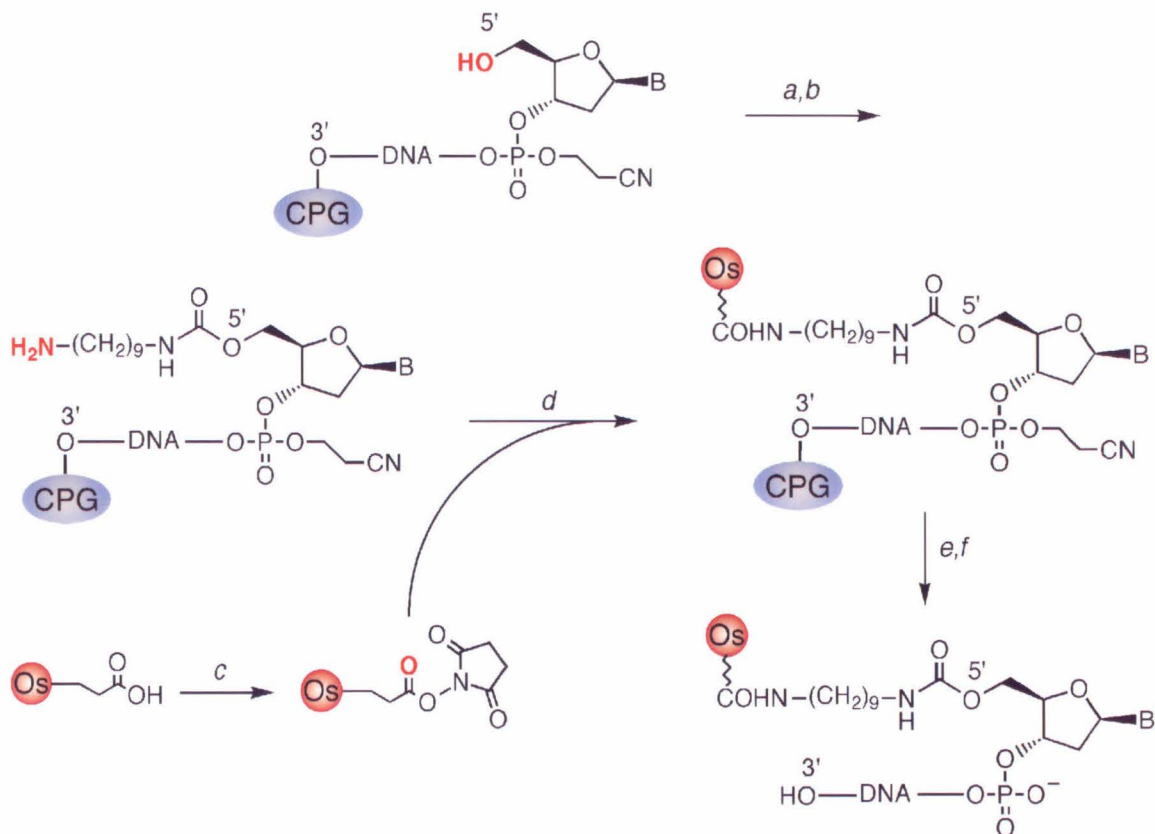
a diversity in the metal complex will give rise to four different conjugates of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ with DNA.

Conjugation of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ to DNA by Heterogeneous Coupling. Scheme 5.4 outlines the method employed to attach $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ to the 5' terminus of oligonucleotides on a solid support. Our goal was to deviate as little as possible from the method developed for $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$, since this approach was so successful. In fact, as shown in Scheme 5.4, we merely substituted the osmium complex for the rhodium intercalator and adjusted the level of DIEA required in order to account for the absence of phi ligands in $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$.

Once again we relied on the formation of an NHS ester to activate the metallointercalator for the amide bond reaction.¹²⁻¹⁶ Figure 5.15 shows a MALDI TOF mass spectrum measured for the NHS ester of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ prepared during the course of synthesizing an Os-modified oligonucleotide.

Having established the ability to activate the bpy' ligand on the osmium complex, coupling to the oligonucleotide was effected without isolation of the NHS-ester in a slurry of DNA coated CPG resin and solution of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ -NHS. Just as with $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$, after the coupling step, and washing the solution of metal complex away from the beads, the resin is left intensely colored. Importantly the colored beads, now coated with conjugate, display very strong solvatochromism. In DCM, the beads containing Os(II) were almost black, whereas if they the same beads are then suspended in methanol, the beads lighten and appear to be colored olive green. This observation is consistent with a molecule on the resin that is well solvated, in contrast to trivial adsorption of metal complex to the bead, which should not be solvatochromic.

We removed the Os-modified DNA from the CPG by treatment with NH_3 (aq) and purified the mixture by HPLC. A typical chromatogram of a reaction mixture obtained in the synthesis of Os-modified DNA is shown in Figure 5.16. We monitored

Scheme 5.4. Synthesis of Osmium-Modified Oligonucleotides on a Solid Support.^{a,b}

Reagents. (a) CDI, dioxane, 30 min. rt.; (b) $\text{H}_2\text{N}(\text{CH}_2)_9\text{NH}_2$, dioxane/ H_2O (9:1), 25 min. rt.; (c) TSTU, DIEA, CH_3OH , CH_3CN , CH_2Cl_2 (1:1:1), rt., 1 h; (d) heterogeneous coupling, DIEA, rt., 12 h; (e) NH_3 (aq), 60 °C, 6 h. **Abbreviations.** CPG = controlled pore glass; Os = $[\text{Os}(\text{phen})(\text{bpy}^*)(\text{Me}_2\text{-dppz})]^{2+}$.

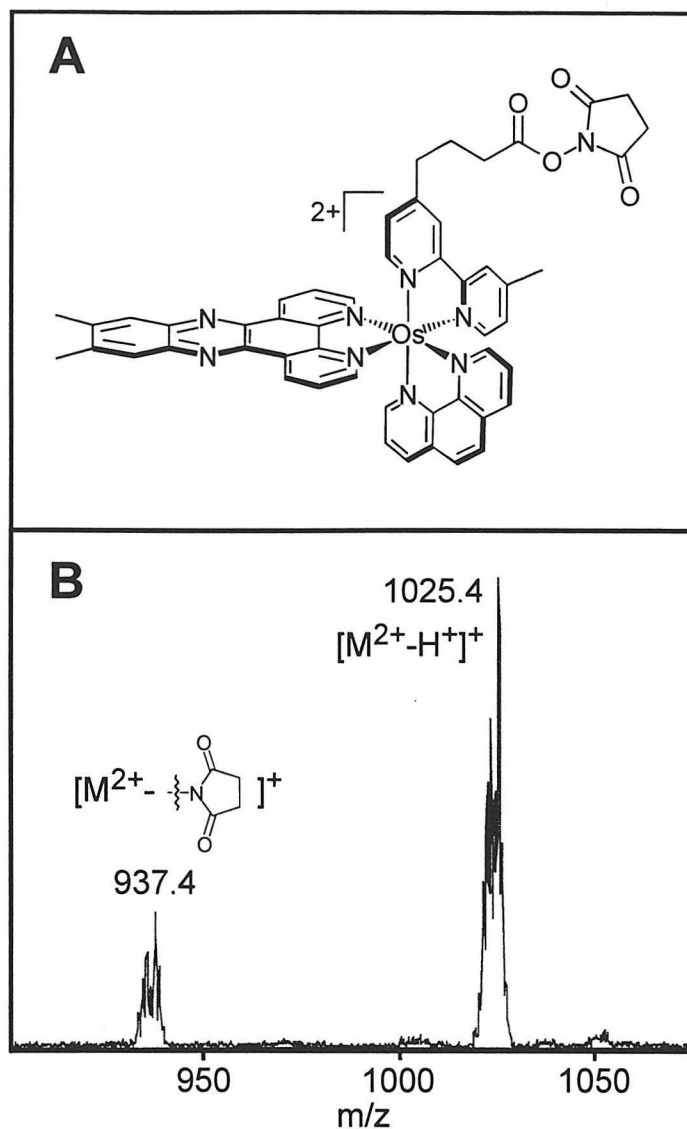


Figure 5.15. (A) Schematic illustration of $[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})(\text{bpy}'\text{-NHS})]^{2+}$. Shown is the Δ -axial isomer of this complex, which is only one of four isomers produced in the reaction of $[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})(\text{bpy}'\text{-NHS})]^{2+}$ with TSTU. (B) ESI mass spectrum for $[\text{Os}(\text{phen})(\text{Me}_2\text{-dppz})(\text{bpy}'\text{-NHS})]^{2+}$. The parent ion and one fragment corresponding to the loss of a succinamidyl group are observed.

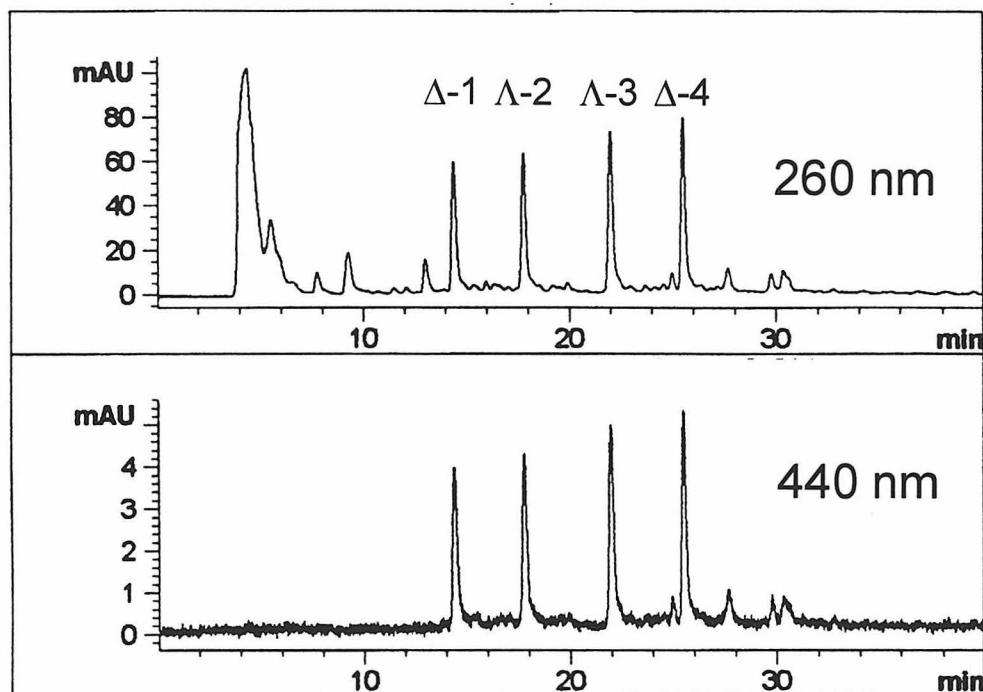


Figure 5.16. HPLC chromatogram of an unpurified Os-modified oligonucleotide reaction mixture. Detection was at 260 nm and 440 nm as indicated. The four Os-containing peaks (identifiable by their 440 nm absorption) contain four different isomers of the complex $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$. Conditions: C18 stationary phase; 50 mM ammonium acetate/acetonitrile mobile phase; 10-27.5% acetonitrile over 30 min.

this mixture at 260 nm where everything in the sample absorbs, and at 480 nm where the Os(II) complex absorbs. As expected based on the structure of the metallointercalator, four products that exhibit absorption at 480 nm were formed in equal amounts in this synthesis.

Shown in Figure 5.17 are absorption spectra for each of the Os-containing products. As expected for structural isomers, the absorption spectra are essentially identical, and any subtle inequivalence arises out of differences in the interaction of the metal complex with the DNA.

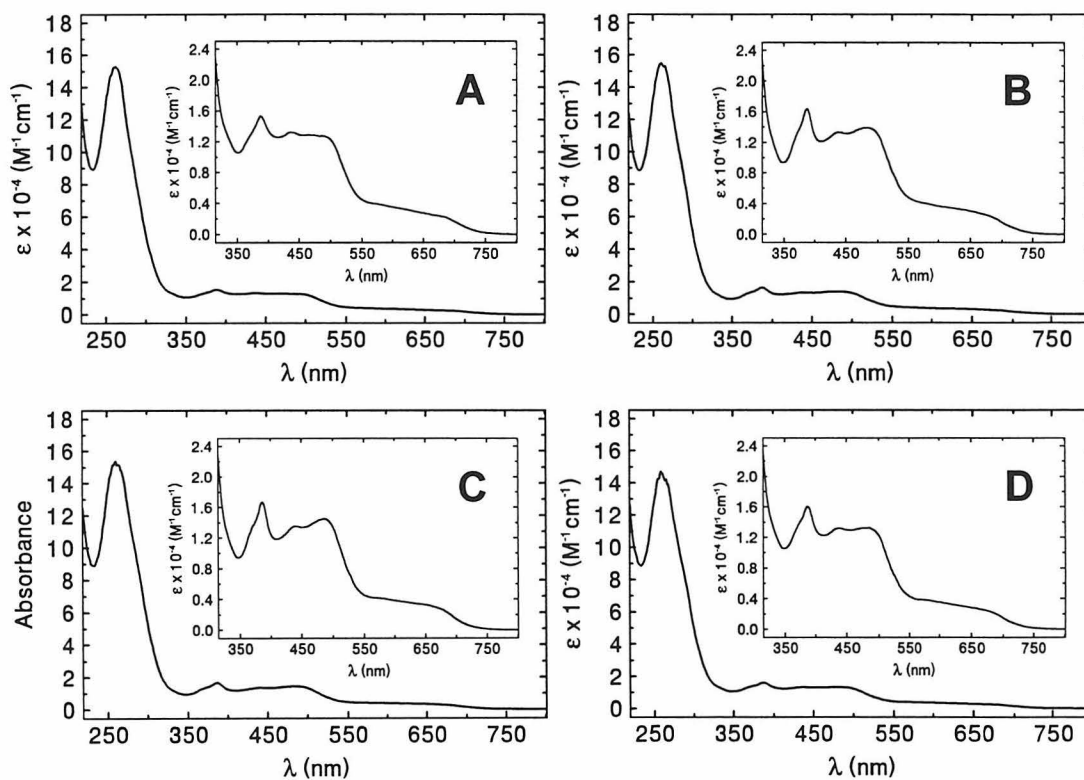


Figure 5.17. UV-vis absorption spectra of Os-modified 16-mers. The four sets of data in A-D correspond to peaks 1-4 in Figure 5.16, respectively. The insets show expansions of the visible absorption spectra.

ESI Mass Spectrometry of Os-Modified DNA. ESI MS was used to characterize the Os-modified oligonucleotides as well. Figure 5.18 shows a schematic illustration of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to a 15-mer, and the ESI mass spectrum for this compound. For this sample, detection in negative ion mode revealed four ions corresponding to the charge states of 7-, 6-, 5-, and 4-. Based on these data, the observed mass is 5684.0, and the expected mass is 5684.2 (for a neutral species *i.e.* $[\text{M}-3\text{H}^+]$).

With the evolution of ESI MS at the UC Riverside facility, the sensitivity of the ESI mass spectrometer in the negative ion mode has improved dramatically from our first studies on Rh-modified DNA. We achieved sufficient resolution and sensitivity to measure the isotopic envelope for the Os-modified oligonucleotide examined above. Figure 5.19 shows a comparison of the observed and calculated isotope distributions for $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to a 15-mer oligonucleotide, at a resolution of 1 amu. Remarkably, the observed mass and calculated mass for the uncharged parent conjugate agree to within 0.2 amu. Furthermore, the isotopic distributions are so similar one might be unable to distinguish the observed data from the calculated data if not for the sodium ion adduct present at $\text{M}+23$. These results confirm unambiguously the composition of the Os-modified DNA and the ability to remove completely the protecting groups from the DNA bases and backbone.

CD Spectroscopy of the Isomers of Os-Modified DNA. In order to confirm our expectation that the four products isolated in the preparation of Os-modified oligonucleotides were two pairs of diastereomers, we measured the CD spectra of each compound. Figure 5.20 shows the CD spectra of peak 1 with that of peak 2, peak 3 with that of peak 4, and a comparison of the spectra for all four products.

Based on the mirror image relationship between the spectra for peaks 1 and 2, and peaks 3 and 4, we concluded that the compounds represent diastereomers that differ (i) in the orientation of the linker relative to the metal center; and (ii) absolute configuration of

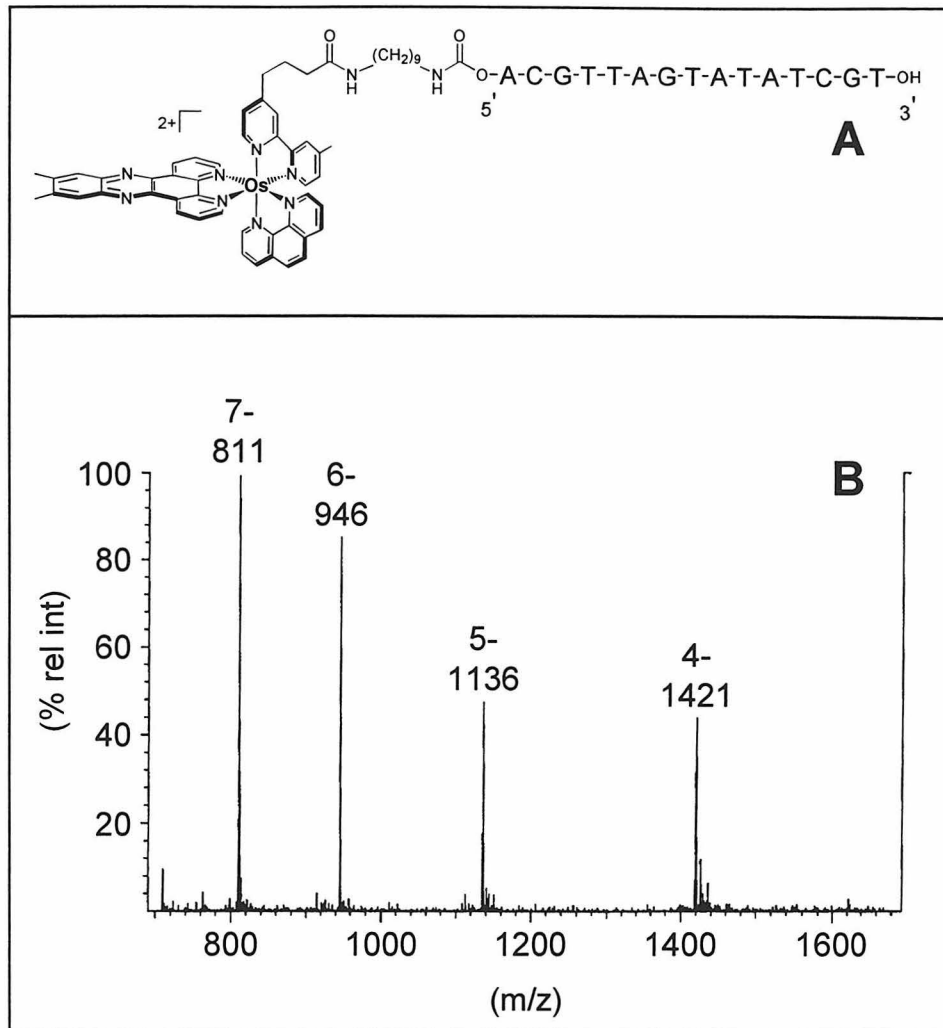


Figure 5.18. (A) Schematic representation of the metallointercalator-DNA conjugate Os.154.C. (B) ESI MS of Δ -1-Os.154.C. The calculated mass for the molecular formula $\text{OsC}_{205}\text{H}_{242}\text{N}_{63}\text{O}_{92}\text{P}_{14} = 5684.2$ (for uncharged parent). The observed mass based on the (m/z) values and charges assigned for the peaks in (B) above is 5684.6 (for the uncharged parent).

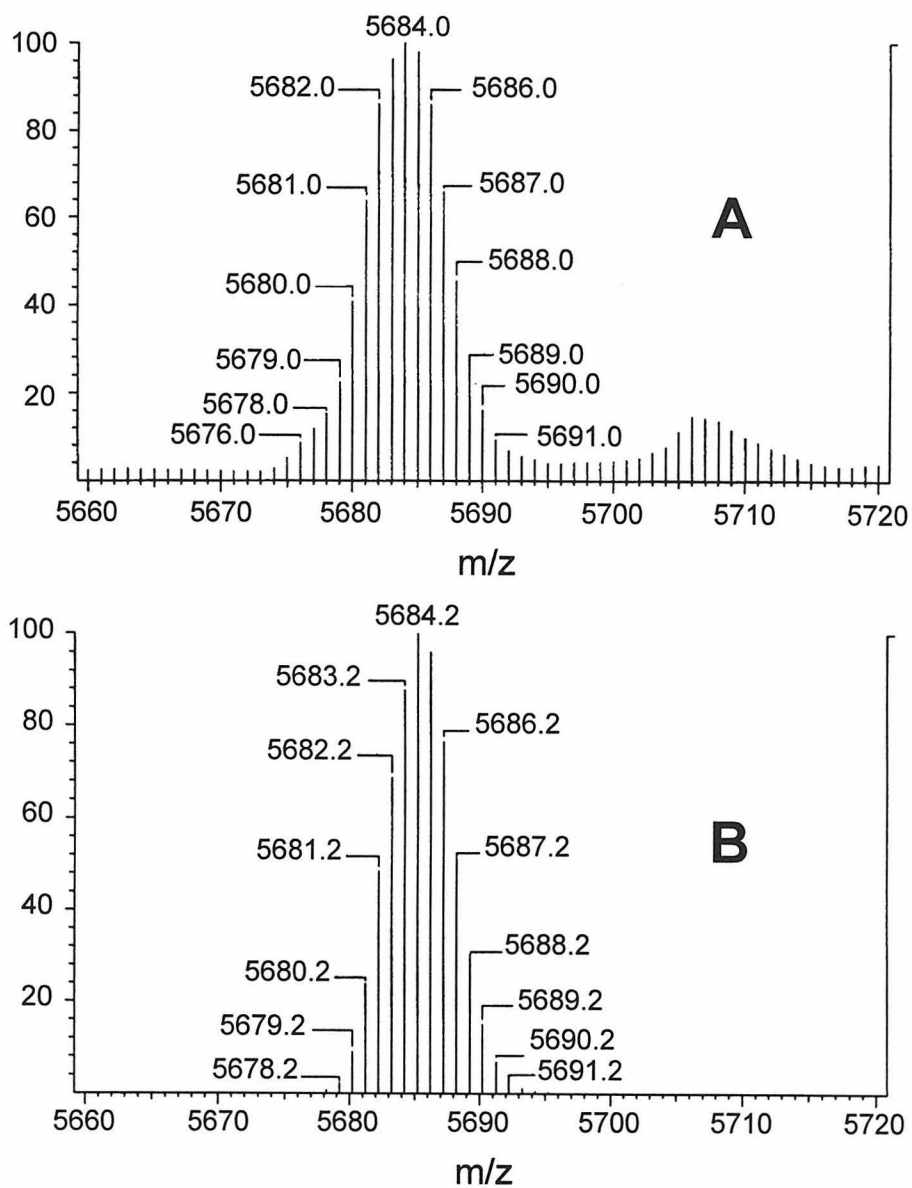


Figure 5.19. (A) Observed isotope distribution in the ESI mass spectrum of Os.154.C; (B) Isotope distribution calculated for $\text{OsC}_{205}\text{H}_{242}\text{N}_{63}\text{O}_{92}\text{P}_{14}$. Units for the y-axes are percent relative abundance; units for the x-axes are mass (m; amu) per charge (z).

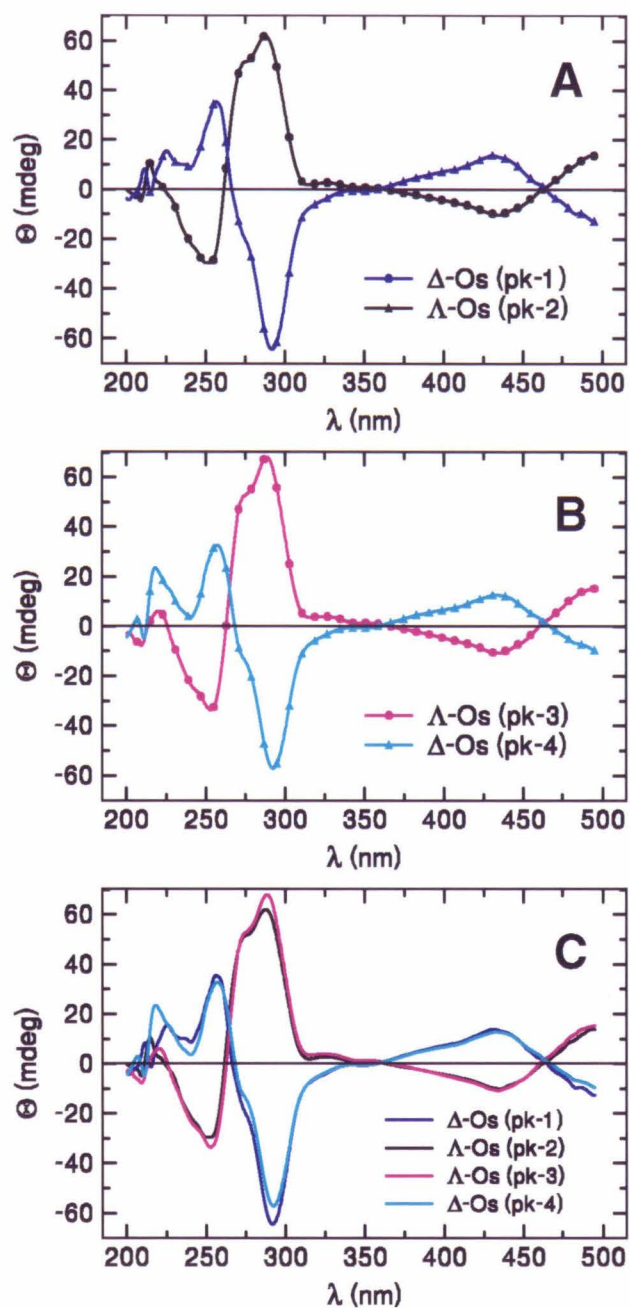


Figure 5.20. Circular dichroism spectra for the isomers of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to a 20-mer oligonucleotide. The order of elution and absolute configuration for each sample are indicated in the figure legends.

the ligands about the metal center. It is not possible from these data alone to assign the axial and equatorial isomers, however, the chirality about the metal center was inferred by comparison of the CD data with data for the pure enantiomers of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$.

Measuring the Base Composition of Os-Modified Oligonucleotides by

Enzymatic Digestion. We employed the assay outlined above in Scheme 5.3 to characterize the base composition of Os-modified oligonucleotides prepared on the solid support.¹⁹ A typical chromatogram of a digestion mixture after filtering is shown in Figure 5.21. Table 5.1 lists the DNA sequence and results of the HPLC analysis of three unmodified DNA strands, and six Os-modified oligonucleotides. As expected, we can account for all of the bases with the exception of the base at the 5' terminus to which the metallointercalator is attached.

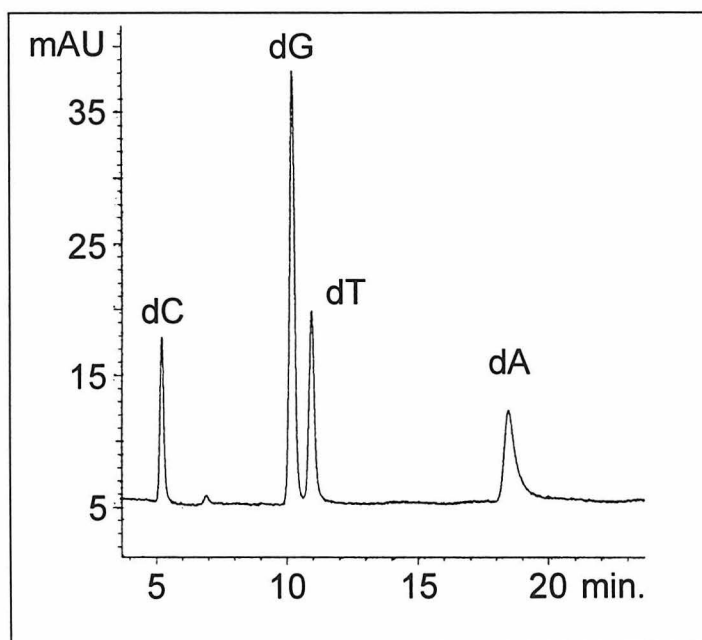


Figure 5.21. HPLC chromatogram for the digestion of Δ -1-Os-181 according to the procedure outlined in Scheme 5.3.

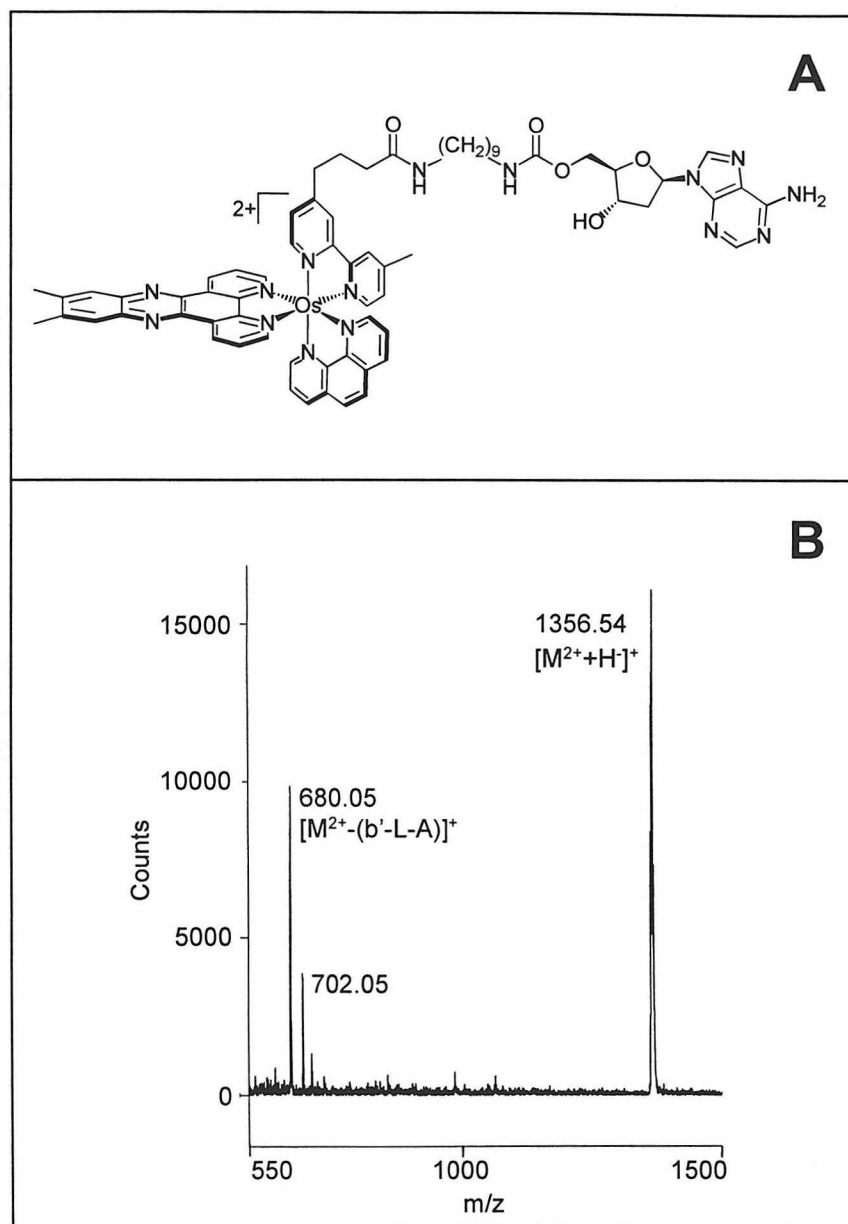


Figure 5.22. (A) Schematic illustration of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to 2'-deoxyadenosine via diaminononane. (B) MALDI TOF mass spectrum of the conjugate represented in (A). For $[\text{M}+\text{H}]^+$ ($\text{OsC}_{67}\text{H}_{70}\text{N}_{15}\text{O}_5$); calculate 1356.5. The spectrum shows evidence for only one species in the sample.

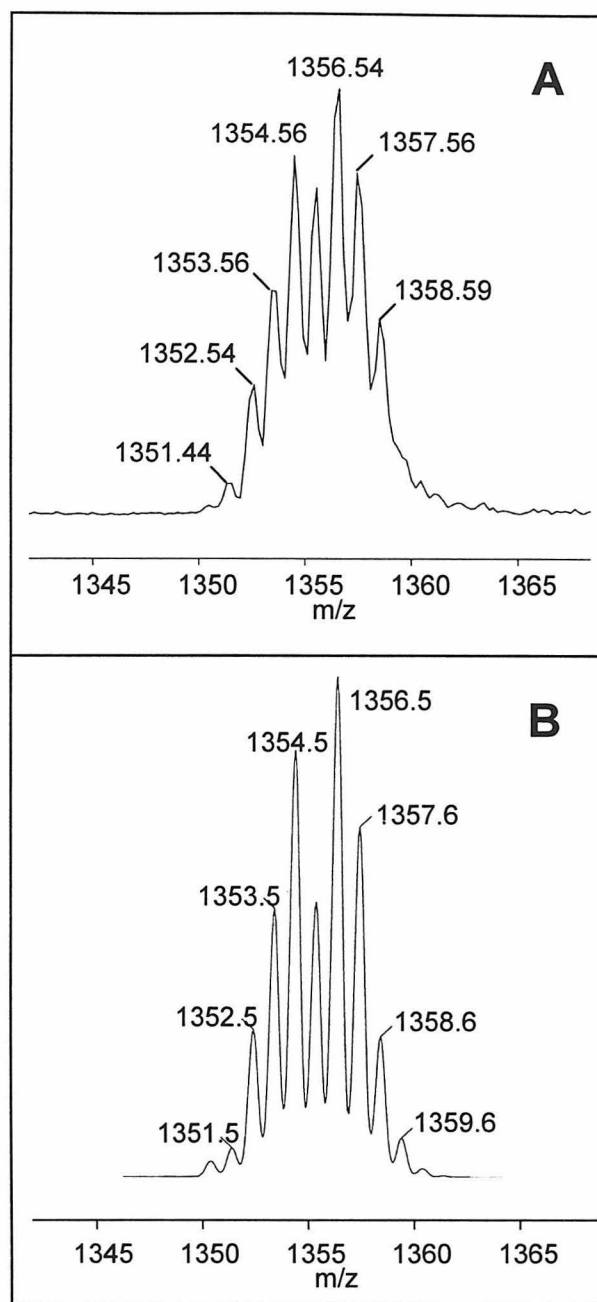


Figure 5.23. Observed (A) and calculated (B) isotope distribution for the conjugate pictured in Figure 5.22 (A): $[M+H]^+$ ($\text{OsC}_{67}\text{H}_{70}\text{N}_{15}\text{O}_5$).

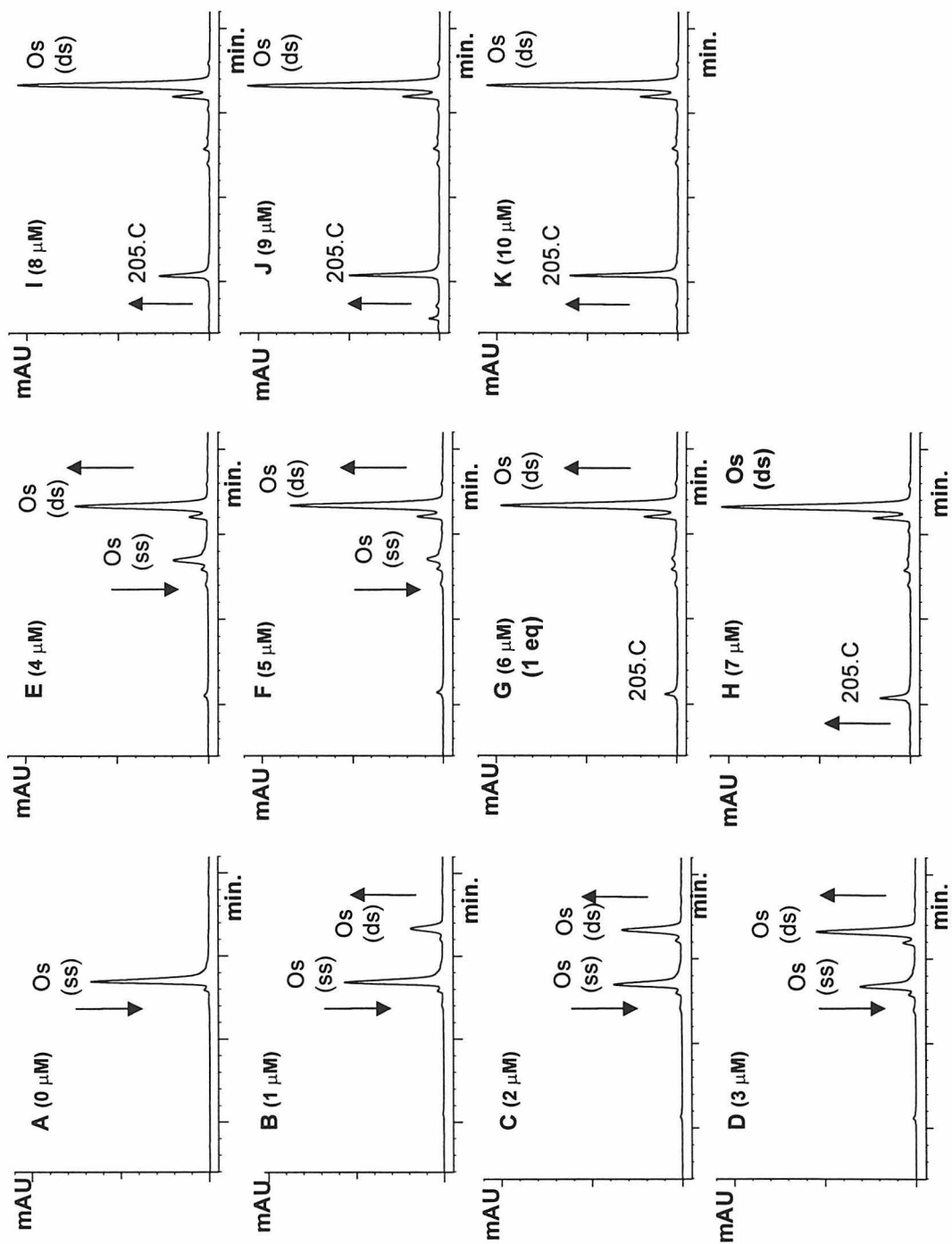
In fact, in analyzing these digestions we expected to see a peak corresponding to the osmium complex coupled to the 5'-terminal nucleoside via the diaminononane linker. Upon close inspection of the nylon filters used to prepare the samples for HPLC analysis, we found that the filters were olive colored, consistent with the presence of an Os(II) complex. Upon treatment with an aliquot of methanol, the color was extracted into the solvent and the filter appeared white.

MALDI MS²⁰ was used to determine the contents of the methanol solution. The structure of the chimera and the results of this analysis are pictured in Figure 5.22, and Figure 5.23 shows a comparison of the observed isotopic distribution with the calculated distribution for the metallointercalator tethered to the nucleoside. Once again we have used MS to unambiguously identify and characterize an important component in the preparation of metallointercalator-DNA conjugates.

HPLC Analysis of the Formation of Os-Modified DNA Duplexes. We sought to develop a versatile and simple method to analyze the hybridization of metallated DNA to complementary strands. It was desirable to avoid using radioactivity, but more importantly, we wanted to be able to follow the fate of both the unmodified strand and the conjugate, in the same experiment.

The method we established is based on simple reversed phase (RP) HPLC with detection by absorption. Fortunately, we found that DNA duplexes remain intact under the conditions of RP HPLC. Figure 5.24 shows an example of a titration of an Os-modified 20-mer (6 μ M) with its complementary strand (0-10 μ M). In the absence of any complement, only one peak is observed in chromatogram. As the concentration of the complement increases, a second peak appears. A comparison of the ratio of absorbance at 260 nm to that at 480 nm for the Os-modified strand and the new species indicates that the absorbance at 260 nm is increasing relative to the absorbance 480 for the new peak. Therefore, the second peak corresponds to the metallated duplex.

Figure 5.24. HPLC analysis of the hybridization of and Os-modified oligonucleotide with its complement. Starting with panel A, which is the chromatogram for a single stranded Os-modified 20-mer, the concentration of complementary strand increases in 1 μM increments from 0-10 μM . As the complement is added, a new species appears in the chromatogram and the signal for the Os-modified 20-mer decreases. The spectral properties of the new peak are consistent with those of an Os-modified duplex. The signal from the duplex increases with increasing complement until reaching 1 equiv (6 μM). At concentrations of complement above 1 equiv, the signal for the complement appears in the chromatogram. This analysis demonstrates the quantitative formation of Os-modified duplex upon incubation with complementary strand. There is no population of Os-single strand in the presence of > 1 equiv of complement.



The area under the peak for the duplex increases as the concentration of the complement increases and the area under the Os-modified strand decreases. The Os-modified strand is fully consumed when 1 equiv (6 μM) of the complementary strand has been added. At concentrations above 1 equiv, a third species, which corresponds to the free complement, begins to appear. The area of each peak as a function of the concentration of complementary strand are plotted in Figure 5.25. These results illustrate clearly that the formation of Os-modified duplexes goes to completion and yields stoichiometric amounts of duplex without leaving multiple populations of single strand and duplex in solution.

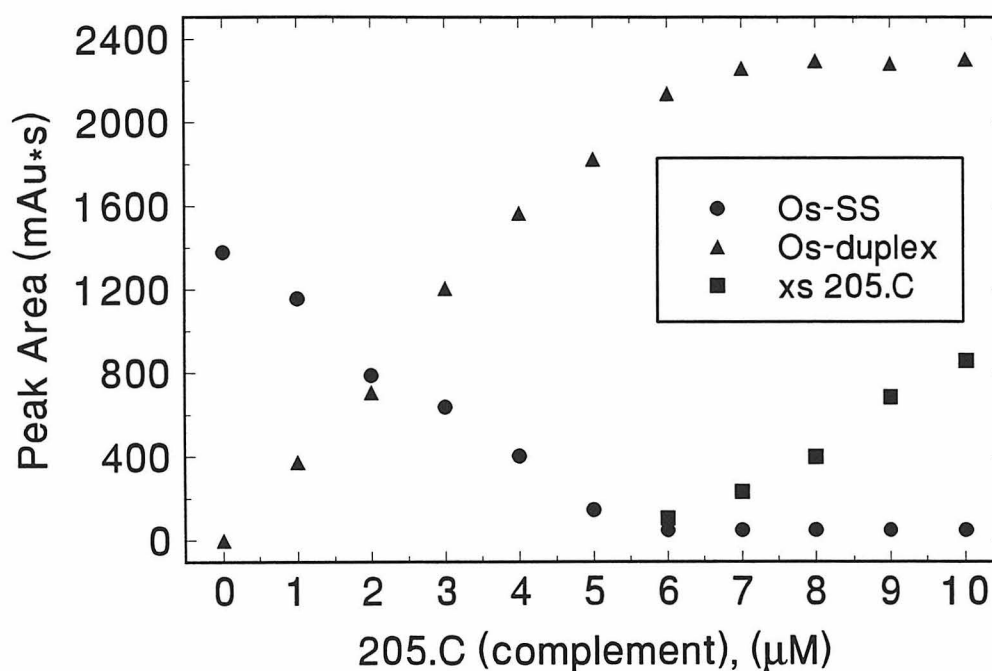


Figure 5.25. Plot of peak area from Figure 5.24 as a function of the concentration of complement added to solutions of Os-modified 20-mer.

5.2.4. Luminescence Properties of Os-Modified DNA Duplexes.

Steady-State Emission as a Function of Binding Site and Duplex Length.

Using the methodology described for the preparation of 5'-Os-modified oligonucleotides, we prepared several duplexes that contained $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to one terminus. We characterized the emission yield and excited state lifetimes as a function of the absolute configuration of the metal center, the base sequence of the binding site, and the length of the duplex. As described in Chapter 2, the emission yield of dppz complexes of Os(II) is a good reporter of the solvent accessibility of the phenazine nitrogens on the intercalated ligand.

Table 5.2 lists the steady-state emission yields for several Os-modified duplexes. In general the Δ isomers show more luminescence than the Λ isomers.²⁹ Furthermore, the isomers that elute latest (peaks 3 and 4) during purification by HPLC tend to exhibit higher emission when compared to the two isomers at earlier times of elution. As expected for intercalative binding that is confined to one site by the tether, the emission yield does not change significantly when the length of the duplex changes but does with the sequence at the terminus to which the metallointercalator is attached.

The two different sequences we examined, 5'-ACGTG and 5'-AGTCT, give rise to similar intensities of emission from the intercalated complex. It is noteworthy that the isomers of the metal complexes attached to the different DNA sequences do not elute from the HPLC column in the same order. This observation underscores the subtlety of the interactions that are responsible for the remarkable separation of the isomers. The main difference is the emission of the second Δ isomer. Bound to 5'-AGTCT the relative emission yield is 1.48 whereas bound to 5'-ACGTG the yield is 2.01. This difference of almost 35 % reflects the sensitivity of intercalation to sequence, and indicates that for this isomer, the 5'-ACGTG sequence is best. The other three isomers are not as sensitive to the sequence.

Table 5.2. Luminescence Intensities for Os-Modified Duplexes as Function of Duplex Length and the Sequence of the Binding Site.

Duplex ^a	I _{rel} ^b	5'-N ¹ -N ⁵
Δ-1-Os_141_ds	1.13	ACGTA
Δ-4-Os_141_ds	1.66	ACGTA
Δ-1-Os_162_ds	1.11	ACGTG
Δ-2-Os_162_ds	1.07	ACGTG
Δ-3-Os_162_ds	1.36	ACGTG
Δ-4-Os_162_ds	2.01	ACGTG
Δ-1-Os_181_ds	1.11	ACGTG
Δ-4-Os_181_ds	2.08	ACGTG
Δ-1-Os_156_ds	0.82	AGTCT
Δ-2-Os_156_ds	1.06	AGTCT
Δ-3-Os_156_ds	1.48	AGTCT
Δ-4-Os_156_ds	1.37	AGTCT

a. Oligonucleotide sequences are given below along with their identification codes.

141: 5' - (Os) -**ACG TAG** TTG AAC GT-3'

156: 5' - (Os) -**AGT CTC** GTA CAT CGT-3'

162: 5' - (Os) -**ACG TGA** GTT GAG ACG T-3'

181: 5' - (Os) -**ACG TGA** TGT TGT AGA CGT-3'

b. I_{rel} refers to the emission intensity under the experimental conditions (8 μM duplex, 5 mM Na₃PO₄, 50 mM Na₃PO₄, pH 7.0; PMT HV = 1000, INT = 0.2, slits = 1.6 mm, polarizers at 9) relative to that of 10 μM [Ru(bpy)₃]²⁺ in water under settings “dppzb”.

Table 5.3. Luminescence Intensities of Os-Modified Duplexes as a Function of Linker Length.

Duplex ^a	I _{rel} ^b
C2: <i>rac</i>-1-Os_181_ds	0.70
C2: Δ-3-Os_181_ds	0.61
C3: Δ-2-Os_181_ds	1.08
C3: Δ-4-Os_181_ds	1.05
C9: Δ-1-Os_181_ds	1.11
C9: Δ-4-Os_181_ds	2.08

^a181: 5' – (Os) – ACGTGATGTTGTAGACGT – 3'

^bSee Table 5.2 legend.

We also examined the effect of linker length on the emission yield of the metallointercalator-DNA conjugates. Table 5.3 lists the emission yields for selected isomers of [Os(phen)(bpy')(Me₂-dppz)]²⁺ tethered to an 18 bp duplex (sequence code: 181) as a function of linker. The three linkers employed, ethylene diamine (C2), diaminopropane (C3), and diaminononane (C9) were chosen to represent two regimes, one in which the linker was too short to accommodate optimal intercalation, and one where the linker was sufficiently long to permit the sampling of a population of binding sites. For each linker we examined only the Δ isomers, except for C2, where the first two isomers eluted from the HPLC together to give a racemic mixture.

As expected for an interaction that requires the linker to be flexible, the emission from the samples with the C2 linker is lowest, followed by C3 and finally C9. Interestingly, the first isomers to elute (*rac*-1 for C2; Δ -2 for C3; and Δ -1 for C9) have similar emission yields. The C2 isomer is significantly lower than C3 and C9, however

this is a racemic mixture. The emission for duplexes constructed from the later eluting isomers (Δ -3 for C2; and Δ -4 for C3 and C9), all Δ -containing diastereomers, increases from C2 to C9. Clearly, the intercalation of the Δ -4 isomer provides the best protection of the phenazine nitrogens from solvent, and requires the greatest amount of linker length.

Time-Resolved Emission Decay of Photoexcited [Os(phen)(bpy')(Me₂-dppz)]²⁺ Tethered to Duplex DNA. We used SPC^{2,30,31} and MPMF to measure the excited state lifetime of [Os(phen)(bpy')(Me₂-dppz)]²⁺ tethered by the C9 linker to a 15 bp duplex (156) and a 16 bp (162) duplex, which differed in the sequence of the intercalation site. For each sequence, we compared the emission of the 4 isomers generated in the synthesis.

The results of nonlinear least squares analysis of the time-resolved emission data are listed in Table 5.4. For all of the duplexes examined, the data were best described by biexponential functions. With 5'-ACGTG, the biexponential decays are described generally as having one lifetime of less than 5 ns and a second lifetime near 20 ± 5 ns. The contribution of the shorter lifetime to the decay is considerably greater than that of the longer lifetime. For 5'-AGTCT, the magnitude of the shorter lifetimes stay the same, but the longer lifetime is significantly shorter, however the distribution of lifetimes is shifted toward the longer lifetimes compared to the data with 5'-ACGTG. In general, the photoexcited complexes bound to the duplex containing 5'-ACGTG display the longest individual and average lifetimes, which suggests that this sequence may better accommodate the tethered metallointercalator.

Table 5.4. Excited state lifetimes for [Os(phen)(bpy')(Me₂-dppz)]-DNA conjugates in modified duplexes.

Duplex ^{a,b}	τ_1 ns (%)	τ_2 ns (%)	τ avg
Δ -1-Os.162.ds ^{c,d}	17.0 (28.6)	3.9 (71.4)	8.2
Λ -2-Os.162.ds	25.2 (8.2)	2.8 (91.8)	6.3
Λ -3-Os.162.ds	21.9 (13.3)	3.2 (86.7)	8.0
Δ -4-Os.162.ds	25.0 (14.8)	4.8 (85.2)	10.2
Λ -1-Os.156.ds ^e	8.0 (14.0)	2.1 (86.0)	4.2
Δ -2-Os.156.ds	6.6 (66.0)	3.0 (34.0)	5.4
Δ -3-Os.156.ds	16.1 (31.0)	3.6 (69.0)	7.5
Λ -4-Os.156.ds	10.9 (50.0)	3.1 (50.0)	7.0

a. Duplex sequences are as follows:

162.ds: 5' -**Os-ACGTG**AGTTGAGACGT-3'
 3' -**TGCAC**TCAACTCTGCA-5'

156.ds: 5' -**Os-AGTCT**CGTACATCGT-3'
 3' -**TCAGA**GCATGTAGCA-5'

b. Experimental Conditions: 8 μ M Os-duplex, 5 mM Na₃PO₄, 50 mM NaCl, pH 7.0.

λ_{exc} = 480 nm (MPMF); 635 nm (SPC); λ_{obs} = 645-900 nm (MPMF); 740 nm (SPC).

c. The key to the sample code is as follows: Δ or Λ refers to absolute configuration of metal center as determined by CD; 1, 2, 3, or 4 refers to the order of elution from the HPLC column for each sample (1 first, etc.); Os indicates osmium complex; 162 or 156 is the sequence code, of which the first two digits indicate the length of the modified strand; ds (vs ss) indicates that the conjugate was hybridized to its complement to give a double stranded assembly.

d. Data collected by MPMF.

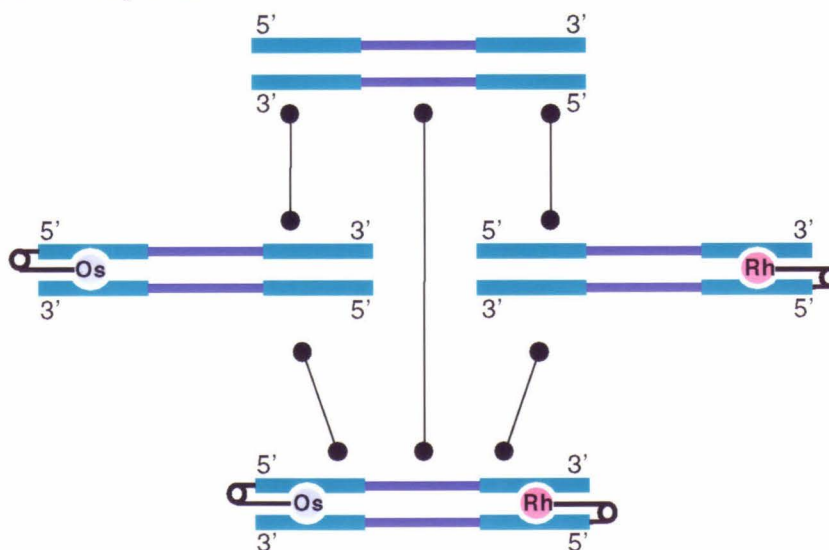
e. Data collected by SPC.

5.2.5. Studies of the Thermal Stability of Metallated DNA Duplexes.

Hybridization of complementary DNA strands, to form ordered structures such as duplexes and triple helices is accompanied by hypochromism in the π - π^* transition on the DNA bases.^{32,33} The origin of this hypochromism associated with base stacking lies in the interactions of the dipole moments on the heterocyclic DNA bases, which affects exciton coupling and in turn perturbs the oscillator strength of the transition.

The study of absorbance (or hypochromism) by DNA in solution as a function of temperature is a classical method to obtain thermodynamic parameters for duplex formation.^{32,33} The melting temperature of a duplex (T_m) is defined as the temperature at which 50 % of duplexes are dissociated into single strands. T_m values across a series of duplexes reveals information about the relative stabilities of the duplexes and the strength of the stacking interactions which dominate the energetics of duplex formation. In addition, agents which bind to DNA and stabilize the duplex, cause the T_m of a duplex to increase.^{9,32,33}

Scheme 5.5. Scheme for the comprehensive evaluation of the thermal stability of metallated DNA duplexes.



We sought to use studies of the T_m of modified duplexes to characterize the integrity of the interaction of tethered metallointercalators with their DNA counterparts. In this section we present an investigation of the relative thermal stability of native and metallointercalator-modified DNA duplexes. Scheme 5.5 illustrates the series of experiments we carried out to evaluate the effect of metallation on a given duplex. The T_m of each duplex pictured in Scheme 5.5 is essential to a comprehensive evaluation of the thermal stability the conjugates, and to the development of a picture of the behavior of these assemblies in solution.

Melting Temperature Studies of Singly and Doubly Modified Duplexes. We measured the T_m values for unmodified (native) duplexes, duplexes modified with the two isomers of Δ -[Os(phen)(bpy')(Me₂-dppz)]²⁺ (Δ -(x)-Os; x = 1 or 4) duplexes tethered to Δ -[Rh(phi)₂(bpy')]³⁺ (Δ -Rh), and duplexes that contained a Δ -Os and a Δ -Rh tethered to opposite 5'-termini. Figure 5.26 shows the absorbance as a function of temperature for one series of 18 bp duplexes. The figure shows two experiments for each duplex: temperature increasing to melt the duplex, and temperature decreasing to follow the rehybridization of the assembly. The similarity in the profiles for the forward and reverse experiments illustrates the reversible nature of duplex formation for the unmodified, as well as the modified helices. The T_m values for 14 bp (141), 16 bp (162), and 18 bp (181) are listed in Table 5.5.

The T_m values for the unmodified duplexes increased with increasing duplex length from 49.6 °C for 141 to 59.7 °C for 181. It is noteworthy that the T_m for 162 (57.6 °C) is very similar to that of 181, which suggests that the sequence of the 18-mer is not as stable than one would predict based on length alone.

The incorporation of Δ -[Rh(phi)₂(bpy')]³⁺ onto the 5' terminus of the duplex affords very little T_m stabilization at this concentration (4 μ M). For 141 and 162, the T_m increased by 1.0 °C, and for 181 the T_m increases by 3.9 °C. These ΔT_m values are modest considering the evidence for intercalation of the rhodium complexes presented in

Figure 5.26. Plots of absorbance as a function of temperature for an unmodified 18 bp duplex (**A**), an Os-modified 18 bp duplex (**B**), and a duplex bearing an osmium intercalator and a rhodium intercalator at opposite 5'-termini (**C**). Observation was at 270 nm and duplexes were at 4 μ M in 5 mM Na_3PO_4 , 50 mM Na_3PO_4 , pH 7.0.

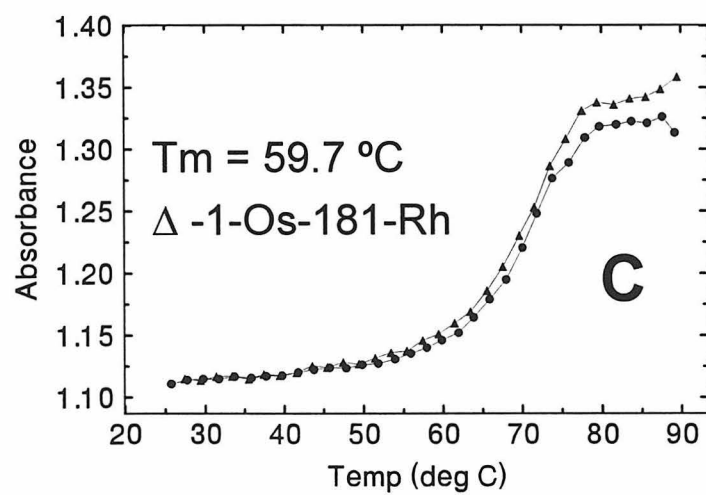
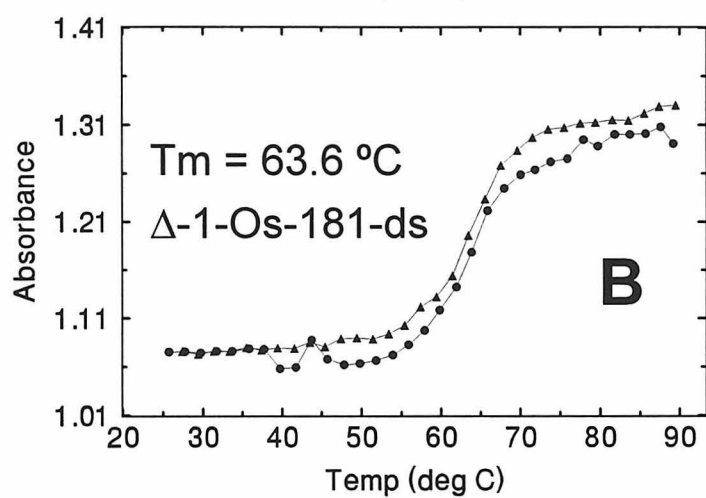
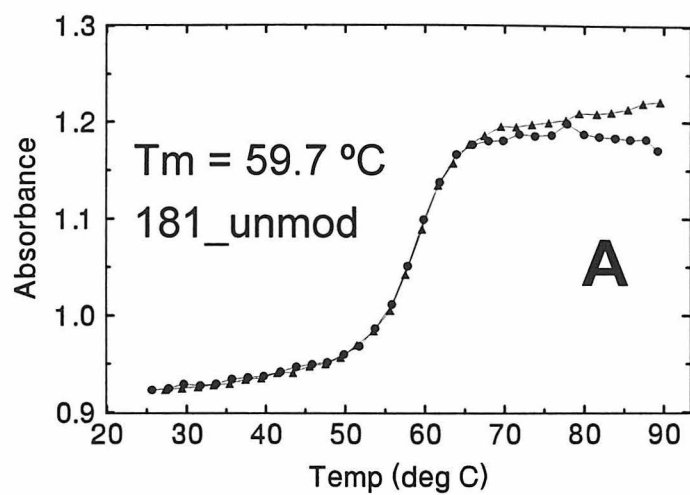


Table 5.5. T_m Values for a Series of Singly and Doubly Metallated Duplexes.

Duplex	T _m (°C)	ΔT _m (°C)
141_unmod	49.6	-----
141_Δ-Rh	50.6	+1.0
Δ-1-Os_141_ds	49.6	+0.0
Δ-1-Os_141_Rh	54.6	+5.0
Δ-4-Os_141_ds	48.6	-1.0
Δ-4-Os_141_Rh	51.6	+2.0
162_unmod	57.6	-----
162_Δ-Rh	58.6	+1.0
Δ-1-Os_162_ds	61.6	+3.0
Δ-1-Os_162_Rh	62.7	+5.0
Δ-4-Os_162_ds	57.6	0.0
Δ-4-Os_162_Rh	60.6	+3.0
181_unmod	59.7	-----
181_Δ-Rh	63.6	+3.9
Δ-1-Os_181_ds	63.6	+3.9
Δ-1-Os_181_Rh	71.8	+12.1
Δ-4-Os_181_ds	59.6	-0.1
Δ-4-Os_181_Rh	67.7	+8.0

141: 5'-(Os)-ACG TAG TTG AAC GT-3'

162: 5'-(Os)-ACG TGA GTT GAG ACG T-3'

181: 5'-(Os)-ACG TGA TGT TGT AGA CGT-3'

See Figure 5.26 for experimental conditions.

this chapter. Other intercalators conjugated to DNA duplexes have afforded more stabilization.

The incorporation of Δ -1 or Δ -4-Os affords some stabilization in the case of the Δ -1-Os-181 and Δ -Os-1-162 duplexes, however Δ -1-Os-141 and Δ -4-Os-162 show no stabilization and Δ -4-Os-141 and Δ -4-Os-181 are slightly destabilized. At least for the 16-mer and 18-mer it seems clear that the Δ -1-isomer of the osmium complex affords some stabilization while the Δ -4 offers very little.

Upon examining the T_m values for the doubly modified duplexes, we find significant stabilization for all of these assemblies. As described above for the Os-modified duplexes, the duplexes that contain Δ -1-Os exhibit more stabilization than those with Δ -2-Os. Remarkably, the amount of T_m stabilization for duplexes bearing two metallointercalators tethered to opposite 5'-termini is greater than the sum of ΔT_m values determined for the singly modified duplexes. This observation suggests that the mechanism by which the unmodified duplexes and singly modified duplexes melt, differs from that of the doubly metallated assemblies.

Following Thermal Denaturation of Os-modified DNA Duplexes by Monitoring Absorption of the Intercalating Ligand. The π - π^* transition centered on the dppz ligand of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ derivatives exhibits significant hypochromism upon preferential intercalation of this ligand into double helical DNA.^{2,30} We monitored the absorbance of this transition (384 nm) as a function of temperature for an Os-modified duplex and compared the T_m determined in this experiment to the value measured at 270 nm.

Figure 5.27 shows a comparison of the melting profiles measured by absorbance for the 16 bp duplex, Δ -1-Os-161. The shape of the two curves in this plot is very similar, although the inflection point (T_m) in the data at 384 nm occurs 4.0 °C higher than in the data at 270 nm. Such an increase in the T_m measured by absorbance centered on the intercalated ligand indicates that the intercalation site melts at a higher temperature

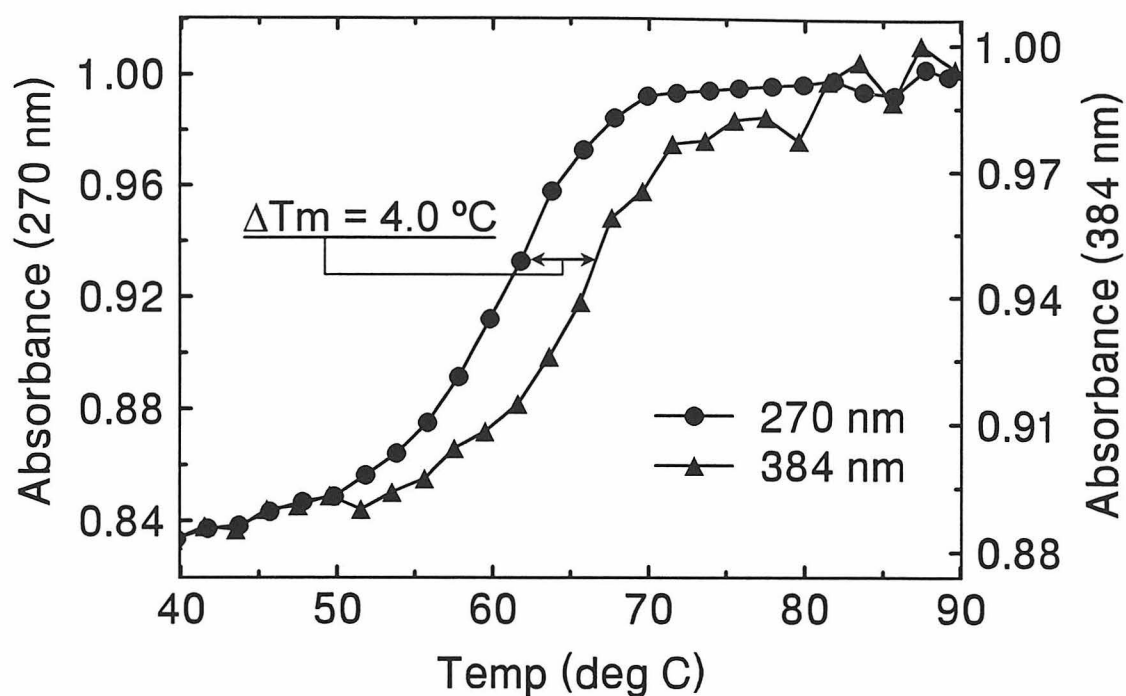


Figure 5.27. Absorbance as a function of temperature for an Os-modified 16 bp duplex monitoring at 270 nm and 384 nm. The curve measured at 384 nm reflects the dissociation of the metallointercalator from the intercalation site. These data indicate the intercalation site melts last.

than does the rest of the duplex. This observation explains the absence of an additive relationship between the T_m values for singly versus doubly modified duplexes. If the intercalation sites melt at higher temperatures, melting must start in the middle of the helix for a doubly metallated assembly, whereas the singly modified duplex can melt from the unmodified terminus first. Therefore, it is not surprising that the duplexes with only one metallointercalator attached do not display a significant amount of stabilization in T_m compared with the native duplexes.

Characterizing the Effect of Linker Length on Thermal Stabilization of DNA Duplexes Tethered to Metallointercalators. We measured the T_m values of several singly and doubly metallated DNA duplexes that contained an osmium intercalator tethered to one 5' terminus by an alkyl linker of variable chain-length. For the doubly modified duplexes, the Os-modified oligonucleotide was hybridized to a Δ -Rh-modified strand that contained the C9 linker.

The T_m values and ΔT_m --the amount of stabilization afforded the modified duplex relative to the native assembly--for the series of assemblies are listed in Table 5.6. The length of the duplexes was 18 bp in each case. For the unmodified duplex, the T_m is 59.7 °C, and the T_m values for the conjugates range from 58.6 °C to 71.8 °C. As described for the T_m as a function of duplex length (*vide supra*), the isomer that elutes first in the purification gives a measurable stabilization to the T_m of the duplex, whereas the later eluting isomer does not, regardless of linker length. The magnitude of ΔT_m for the early moving isomer is 4.0 ± 0.1 °C across the series.

As described above, the doubly modified duplexes exhibit an even greater stabilization of the assembly than one would predict based on ΔT_m values for the singly modified duplexes. The ΔT_m values for the C2 and C3 linkers in doubly modified duplexes (the Δ -Rh is always tethered by C9) are the same for both isomers of the Os complex, however the ΔT_m increases significantly with the C9 linker. These results indicate that the longer linker allows the osmium intercalator to reach a relatively optimal binding configuration, which results in significant increases in the thermal stability of duplexes to which the metallointercalator is tethered.

Table 5.6. Effect of Linker Length on the Thermal Stability of Metallointercalator-modified DNA Duplexes.

Duplex	T _m (°C)	ΔT _m (°C)	Linker
Unmod._181_ds	59.7	-----	C9: H ₂ N(CH ₂) ₉ NH ₂
181_Δ-Rh	63.6	+3.9	
<i>rac</i> -1- C2 -Os_ds	63.8	+4.1	C2: H ₂ N(CH ₂) ₂ NH ₂
<i>rac</i> -1- C2 -Os_Rh	68.8	+9.1	
Δ-3- C2 -Os_ds	58.6	-1.1	
Δ-3- C2 -Os_Rh	65.8	+6.1	
Δ-2- C3 -Os_ds	63.8	+4.1	C3: H ₂ N(CH ₂) ₃ NH ₂
Δ-2- C3 -Os_Rh	68.8	+9.1	
Δ-4- C3 -Os_ds	59.6	-0.1	
Δ-4- C3 -Os_Rh	66.8	+6.1	
Δ-1- C9 -Os_ds	63.6	+3.9	C9: H ₂ N(CH ₂) ₉ NH ₂
Δ-1- C9 -Os_Rh	71.8	+12.1	
Δ-4- C9 -Os_ds	59.6	-0.1	
Δ-4- C9 -Os_Rh	67.7	+8.0	

See Table 5.5 for key to sequence code (181). See Figure 5.26 for experimental conditions.

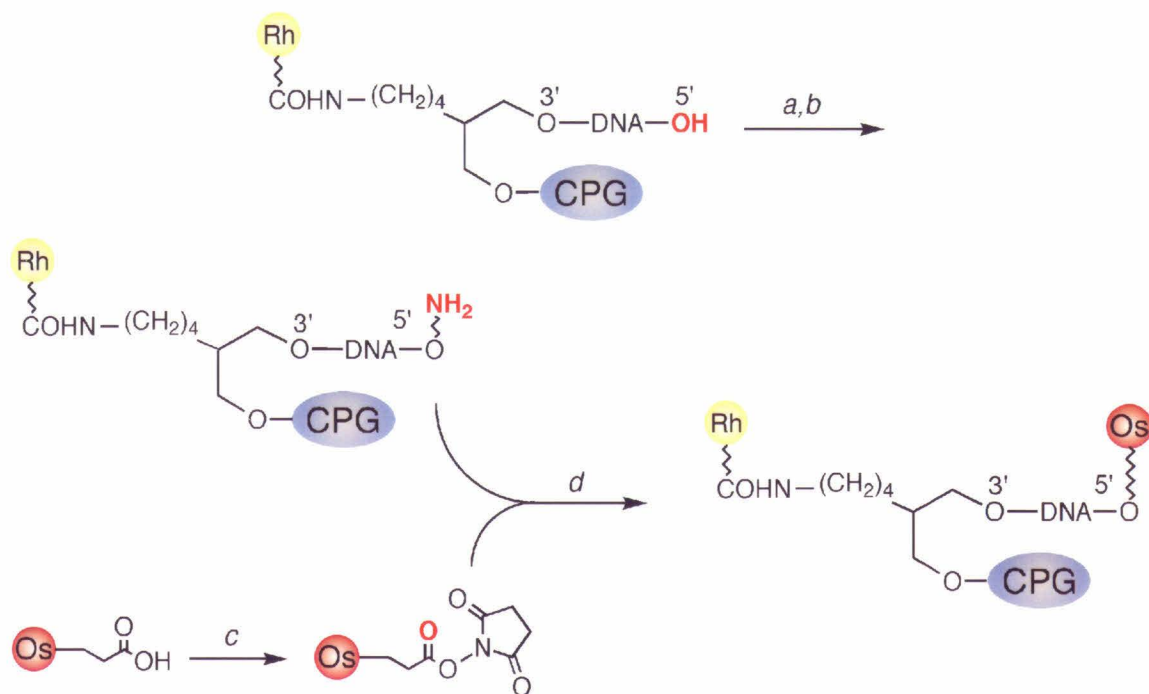
5.2.6. Synthesis of 3',5'-*bis*-Heterometallated Oligonucleotides on A Solid Support

Scheme 5.2 outlines the preparation of oligonucleotides with a metallointercalator attached to the 3'-hydroxyl group. Over the course of these reactions, the 5'-hydroxyl is protected as the DMT ether.¹⁰ The last steps in that procedure involve deprotection of the 5'-hydroxyl group and liberation of the conjugate from the resin. We recognized that if we unmask the 5'-hydroxyl of the 3'-modified oligonucleotide, and leave it on the resin, we are free to functionalize the 5'-hydroxyl as well.⁹ We could synthesize oligonucleotides that contain an electron donor for example at one terminus and an electron acceptor at the other.

We developed the strategy in Scheme 5.6, which permits the synthesis of so called 3',5'-*bis*-heterometallated oligonucleotides. The first phase of the synthesis consists of incorporating a metallointercalator onto the hydroxy aminoalkyl linker tethered to the 3' terminus of the oligonucleotide and removing the DMT group from the 5'-hydroxyl. At this stage the 5'-hydroxyl is functionalized first by treatment with CDI, and then derivatized by adding a diaminononane linker. The second metallointercalator is coupled to the nucleophilic amine by amide bond formation at the carboxylate functionality on the metal complex. Finally, the whole assembly is liberated from the conjugate and deprotected by treatment with NH_3 (aq), and isolated by HPLC.

We carried this procedure out to validate our method and isolated one major product. It is important to note that using racemic mixtures of each metallointercalator gives rise to eight possible isomers of the 3',5'-*bis*-heterometallated oligonucleotide. The ESI MS of the product isolated in this reaction is shown in Figure 5.28. This methodology will be important in the application of DNA-mediated electron transfer in metallointercalator-DNA conjugates in sensing technology.

Scheme 5.6. Synthesis 3',5'-*bis*-heterometallated oligonucleotides on a solid support.^{a,b}



^aReagents. (a) CDI, dioxane, 30 min. rt.; (b).H₂N(CH₂)₉NH₂, dioxane/H₂O (9:1), 25 min. rt.; (c) TSTU, DIEA, CH₃OH,CH₃CN, CH₂Cl₂ (1:1:1), rt., 1 h; (d) heterogeneous coupling, DIEA, rt., 12 h; (e) NH₃ (aq), 60 °C, 6 h. **^bAbbreviations.** CPG = controlled pore glass; Os = [Os(phen)(bpy')(Me₂-dppz)]²⁺.



for M^{5+} calculate mass = 6648.4

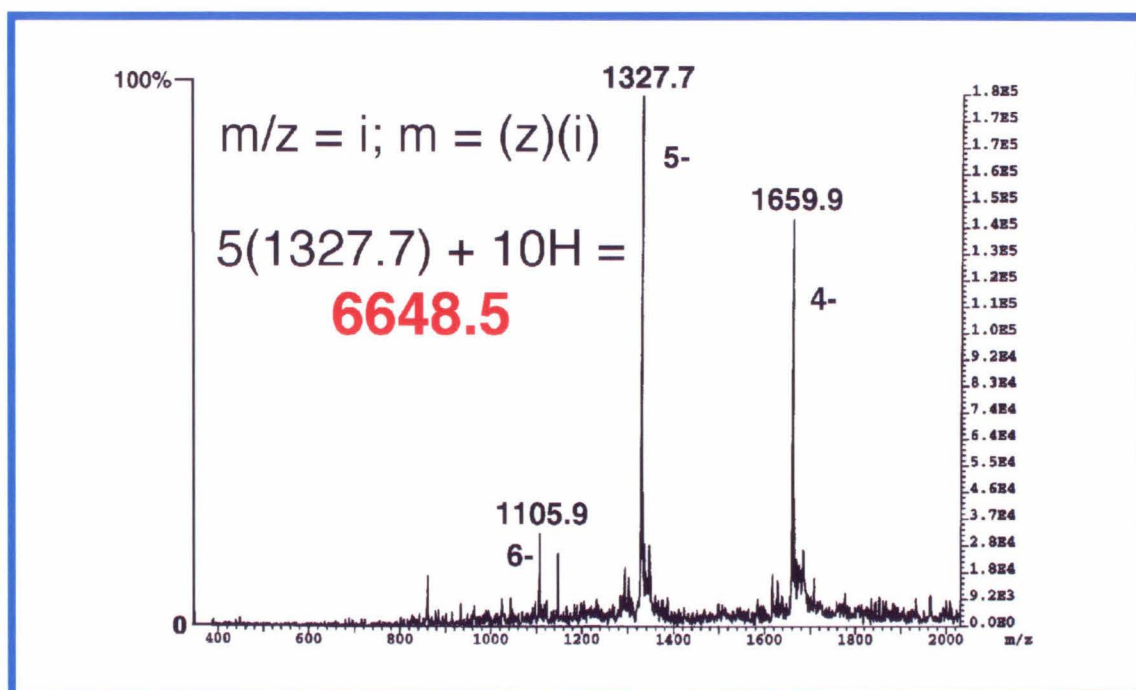


Figure 5.28. ESI mass spectrum of a 3',5'-bis-heterometallated oligonucleotide. The calculated mass for the fully protonated species is $M_{\text{calc}} = 6648.4$. According to the distributions of ions present in the mass spectrum, the mass of the ion plus the appropriate number of hydrogens to achieve the targeted charge, leads to a mass of $M_{\text{obs}} = 6648.5$. This remarkable accuracy confirms that the material is the desired compound, and that all protecting groups have been removed.

5.3. DISCUSSION

5.3.1. Synthesis and Characterization of Metallointercalator-DNA Conjugates.

In this chapter we described a general strategy for the introduction of metallointercalators onto the 5', 3', or both 5' and 3' termini of oligonucleotides. The methods consist of carrying out modifications to the oligonucleotide on a solid support.^{10,34}

The keys to this solid-phase approach are several-fold. First, the oligonucleotide is functionalized and derivatized while it remains attached to the controlled-pore glass beads used during automated DNA synthesis, thus an excess of any reagent may be employed as necessary to drive a reaction to completion since unreacted material is easily washed away from the resin. Second, the phosphate groups of the sugar-phosphate backbone are all capped as the cyanoethyl esters so the oligonucleotide is uncharged and therefore well solvated by organic solvents. Finally, the functional groups on the DNA bases, such as exocyclic amines, are all masked by acyl protecting groups. Thus, the only reactive functional group in the case of 5' and 3',5' modification is the 5'-hydroxyl group. In the 3'-modification strategy, selective deprotection of an amine present during automated DNA synthesis by treatment with piperidine, which does not remove the acyl protecting groups, unmasks to nucleophilic target in the coupling step.

The syntheses we developed are reliable and reproducible. In our laboratory the preparation of metallointercalator-DNA conjugates has become a worker-independent enterprise. The preparations usually generate ~200 nmol of conjugate per 2 μ mol loading on the resin (assuming 100% yield in the DNA synthesis). The overall yield, is only 1%, however this amount of material is not significantly less than the amount of pure, unmodified oligonucleotide one obtains after a typical preparation.

We followed the actual coupling yield by using the unmodified oligonucleotide present after installing the linker as an internal standard. This analysis indicates that the coupling step proceeds in >75% yield. Therefore, the loss of material must be the result

of (i) inhibition of cleavage from resin (the resin usually remains colored), or (ii) loss of material in work up and purification. Nevertheless, the fact that the syntheses work as reproducibly as they do outweighs the yield issue.

Owing to the asymmetry of the bpy' ligand and the octahedral structure of the metal complex, $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ exists as four diastereomers (Figure 5.14). Two isomers are Δ and Λ enantiomers with the linker oriented in an axial fashion with respect to the metal center, and the other two are enantiomers with the linker oriented in an equatorial fashion. Owing to these four diastereomers, we isolate four products (two pairs of two compounds) in the synthesis of oligonucleotides tethered to $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$. We can distinguish between the Δ and Λ enantiomers of each axial or equatorial pair, however we have yet to assign the equatorial and axial isomers.

We purify the products of the heterogeneous coupling procedure by HPLC. In most cases HPLC will separate the four isomers. We have not established an understanding of the factors that control the order in which the isomers elute from the HPLC; different sequences display different orders of elution. The HPLC profile of a given conjugate mixture does not change, given the same purification conditions. We do not know how to predict how a conjugate mixture will be separated in purification.

The ability to isolate the pure diastereomers is an example of capitalizing on the diversity generated in reactions of isomeric mixtures. We have learned a lot from studying the families of isomers, which might not have been learned if more than one reaction had to be carried out to obtain the different samples.

Analysis of the Composition of Metallointercalator-DNA Conjugates. The primary and most useful mode of establishing the composition of metallated DNA is mass spectrometry.²⁰⁻²³ We used ESI MS to characterize Rh-modified and Os-modified oligonucleotides. The importance of the data obtained by MS lies not only in the confirmation that we have the right material, but in the confirmation that we do not have

any contamination by partially reacted or degraded material. We wanted first to confirm that all of the protecting groups had been successfully removed from the DNA bases and backbone, the ESI MS data are of sufficient resolution in m/z to easily detect the acyl protecting groups which weigh ~ 100 amu. Furthermore, we were concerned that the phenazine portion of the dppz ligand might hydrolyze to give phendione, which would not intercalate. We never detected any ions of mass consistent with a phendione derivative in ESI or MALDI experiments.

The application of the enzymatic digestion of the oligonucleotides was useful in demonstrating the localization of the modification chemistry to the 5'-terminus in the case of 5'-modified oligonucleotides.¹⁹ We found that the base to which the metallointercalator complex was attached was always missing in the analysis of the bases, consistent with its modification. The results we obtained by this assay showed excellent agreement between unmodified strands and conjugates, which signifies that the quantitation of the materials is accurate.

Furthermore, the MS analysis of the fraction of the base digest that contained the metallointercalator indicated that we had isolated the adduct of the metal complex, via the diammonononane linker, to an adenosine as the terminal base of that sequence. The high resolution of the instrument employed allowed us to measure the isotopic distribution of the ion, which agreed very closely with the predicted distribution. The MS data were important not only for what they revealed, but for what they did not. We found the metal complex and the base to be fully intact, and detected no degradation products.

Following the Formation of Duplex by PAGE and HPLC. We employed assays based on PAGE and HPLC to follow the formation of Rh-modified duplexes and Os-modified duplexes, respectively. PAGE indicated that Rh-modified oligonucleotides hybridize with their complements in 1:1 stoichiometry as expected. The nondenaturing PAGE experiment revealed differences in the mobility of Δ -rhodium containing diastereomers versus those containing Λ -rhodium. The differences in migration were

consistent with Λ -[Rh(phi)₂(bpy')]³⁺ interacting more with the gel matrix, and hence less with the DNA, than Δ -[Rh(phi)₂(bpy')]³⁺.

Comparisons of the Intercalation of Δ -[Rh(phi)₂(bpy')]³⁺ and Λ -[Rh(phi)₂(bpy')]³⁺ in Rh-modified Duplexes. We studied the intercalation of [Rh(phi)₂(bpy')]³⁺ tethered to DNA duplexes by a photocleavage assay, T_m studies, and an assay that employs DNA-mediated electron transfer as a diagnostic probe of intercalative stacking. Each of these methods indicates that the rhodium complexes intercalate into the helix to which they have been covalently attached. On the basis of the quenching assay and consistent with many studies of the association of octahedral metal complexes with DNA,^{8,24,29,35} we found that the Δ isomer of [Rh(phi)₂(bpy')]³⁺ intercalated more deeply into the base stack than did the Λ isomer.

Photocleavage assays indicated that the binding of rhodium complex is restricted to the end of the duplex at which the complex was tethered. For Δ -[Rh(phi)₂(bpy')]³⁺, intercalation into 5'-ACGA occurs primarily three bases from the tethered end. The Λ isomers intercalates one bp closer to the end at two bases from the tethered end.

When the binding site was changed slightly to 5'-TACG, intercalation by Δ -[Rh(phi)₂(bpy')]³⁺ was disrupted and the Λ isomer shifts its intercalation to just one bp in from the Rh-terminus. These changes in binding, which were first observed in photocleavage, were also detected as reductions in the quenching efficiency of the complex tethered to 5'-TACG.

Luminescence of Osmium-Modified Duplexes. The luminescence of [Os(phen)₂(dppz)]²⁺ and derivatives bound to DNA is sensitive to solvent accessibility owing to quenching by hydrogen bonding to the phenazine nitrogens of the dppz ligand.³⁶⁻⁴⁰ We described an extensive study of the luminescence properties of a family of dppz complexes of Os(II) in Chapter 2. We found that with a particular complex, changes in emission intensity and lifetime bound to DNA reflected how well the

phenazine nitrogens were protected from water by intercalation into the hydrophobic base stack.

The emission yields for $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to oligonucleotides depend upon which diastereomer of the complex is present, the sequence of the intercalation site, and the length of the linker used to join the duplex and the metallointercalator. As discussed above the different isomers consist of two pairs of enantiomers that differ in the orientation of the linker with respect to the metal center. As illustrated in Tables 5.2-5.4, emission intensity and lifetime are greatest for the Δ isomers of each pair of diastereomers and these properties favor the later moving isomers in the HPLC analysis. The fact that emission from Δ complexes tethered to duplexes is greater than that for Λ complexes is consistent with volumes of studies on related metallointercalators and reflects the matching of symmetry for the right-handed complex with the right-handed DNA.^{24,29,35,41-43} It was surprising to us that this property was maintained in the conjugates, since we felt the linker might abrogate such discrimination.

The emission intensity of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to duplexes increases with increasing linker length. Such an observation is to be expected if the emission intensity reflects binding to DNA and protection of the phenazine nitrogens from solvent. Therefore, the difference in the emission of the axial and equatorial isomers is really a manifestation of different effective linker lengths. For complexes tethered to DNA, the effective linker length for the axial isomer is longer than that for the equatorial isomer. Therefore, before experimental verification is obtained, we predict that the earlier moving isomers are the equatorial isomers and the later moving isomers are the axial isomers.

Thermal Stabilization of Duplexes by Tethered Metallointercalators. Several studies have described an increase in the stability of DNA duplexes to which intercalators are attached.⁹ Such stabilization arises from the stacking interaction between the bases

and the binding agent. The stability of duplex DNA is determined mainly from stacking interactions and the introduction of an intercalator increases stacking.

We studied the thermal stabilization of DNA duplexes to which $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ were tethered. We found that the amount of stabilization depended upon which isomer of the complex was tethered and whether there was one or two metallointercalators attached to the duplex. For the singly modified duplexes, Δ - $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ afforded an increase in T_m of only 2 °C, while the Δ -1 isomer (sequences 141, 162, and 181, *vide supra*) of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ increased the T_m by ~4 °C and the Δ -4 isomer of the same sequence offered very little stabilization. The value of ΔT_m increases with increasing linker length.

When the duplex bears two metallointercalators the value for ΔT_m is always greater than the sum of ΔT_m for the singly modified duplexes. Such an observation is not surprising, and reveals that despite the modest stabilization for the single modification, the complexes are intercalated. Thus, the significant ΔT_m values obtained for the thermal denaturation of doubly modified duplexes suggest that the mechanism of melting for these assemblies differs from that with only one complex attached. We showed that the region of the duplex near the metal is the last to melt, thus with one metallointercalator in the assembly, the cooperative dissociation can be freely initiated at the unmodified terminus. With two metallointercalators at opposite ends of the duplex, melting must begin at the center of the assembly.

These values for ΔT_m are also concentration dependent. When we lower the concentration of the duplex, ΔT_m increases. Such an effect is to be expected for an interaction between the duplex and metallointercalator that is independent of concentration over the concentration range investigated.

Correlations Between T_m and Emission Intensity. We can say for certain that when the emission yield for a dppz complex bound to DNA increases, the protection of the phenazine nitrogens from solvent has increased.³⁶⁻⁴⁰ It is more difficult to talk in

terms of the amount of stacking as it relates to emission. Our results clearly show that the earlier moving isomers (in terms of HPLC retention) of $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to DNA exhibit *less* emission than the later moving isomers. We also find repeatedly that the earlier moving isomers provide *more* thermal stabilization of the duplex than do the later moving isomers.

The absolute value of ΔT_m is not as easily assigned to more or less stacking as emission yield is to protection of the phenazine nitrogens. In general more stacking means a greater ΔT_m , however the value of ΔT_m reflects the relative stability of assembly with the metallointercalator attached to that of the unmodified duplex. Thus if a site is inherently destabilized, the presence of the intercalator will appear to enhance the stability more than a site that is not prone to destabilization.

Others described previously,^{36-39,41,42} and we discussed in Chapter 2 the side-on and head-on families of intercalation modes by dppz complexes of Os(II) and Ru(II), which provide differential amounts protection from solvent and different levels of stacking within the duplex. In this model, side-on intercalation results in greater solvent exposure, and thus a lower emission yield, but greater stacking with the base pair. Head-on intercalation buries the phenazine nitrogens more deeply into the base stack, but possibly affords less stacking.

We do not expect such a simplified model of intercalative binding by dppz complexes of Os(II) to fit here, however, it is interesting to speculate that the lower emission yield for the Δ -1 and Λ -2 isomers is a manifestation of more side-on intercalation which provides more stacking interaction. The increased stacking would be manifested as an increase in T_m . This analysis fits with our prediction that the earlier moving isomers are the equatorial isomers and the later moving are the axial, since the equatorial should be inhibited from adopting a head-on mode of intercalation.

5.4. EXPERIMENTAL

5.4.1. Materials

Solvents used in coupling chemistry were anhydrous (Fluka over molecular sieves). DNA synthesis reagents were supplied by Glen Research and chemicals for heterogeneous coupling reactions were obtained from Fluka, Aldrich, and Sigma, and used as received.

Unmodified Oligonucleotides. Native oligonucleotides were prepared by the phosphoramidite approach on an ABI 394 DNA synthesizer. Purification was carried out on the 5'-DMT oligonucleotide by RP HPLC (C18; ammonium acetate/acetonitrile). The DMT group was removed by treatment with 400 μ L 80% (v/v) glacial acetic acid in water for 20 min., followed by precipitation by anhydrous ethanol. The alcoholic acid was removed under reduced pressure and the 5'-OH strand purified a second time by RP HPLC.

Metal Complexes. $[\text{Rh}(\text{phen})_2(\text{bpy}')]\text{Cl}_3$, $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]\text{Cl}_2$, $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]\text{Cl}_2$, $[\text{Ru}(\text{phen})_2(\text{dppz})]\text{Cl}_2$, were all synthesized as described in Chapter 2 for the osmium complexes and previously for the rhodium and ruthenium complexes.^{17,18,44}

Preparation of Conjugates: Aminoalkylation of the 5' Terminus. This procedure followed closely the method of Wachter, with slight modification.¹¹ The desired oligonucleotide was synthesized by automated DNA synthesis using the phosphoramidite approach on CPG resin with 2000 Å pores (Glen Research). The synthesizer was programmed to leave the oligonucleotide on the resin with the DMT group off of the 5' terminus. The DNA coated beads (2 x 1 μ mol loading) were removed from the synthesis columns and added to a 2.5 cm x 10 cm glass cylinder fitted with a coarse glass frit and stopcock at the bottom.

The oligonucleotide on the resin was washed three times with 3 mL dry dioxane (Fluka; over molecular sieves), and a solution of CDI (Sigma) in dry dioxane (50 mg/1 mL) was added to the beads in the cylindrical reaction vessel. The suspension was

agitated by bubbling dry Ar(g) through the glass frit. After 30 minutes of agitation the CDI solution was removed and the resin was washed five times with 3 mL dry dioxane.

A solution of diaminononane in 9:1 dioxane/water (32 mg/1 mL) was added to the functionalized DNA on the resin and the suspension was agitated as above for 25 min. If this reaction proceeds for too long, the amino linker will hydrolyze the DNA from the resin. At the conclusion of the reaction time, the aminononane solution was washed from the resin and the beads were washed with excess dioxane and methanol. The methanol will dissolve unreacted diaminononane. At this point the linker has been installed and the resin may be stored or reacted further.

Coupling $[\text{Rh}(\phi)_2(\text{bpy}')]^{3+}$ to 5'-Aminoalkylated DNA on CPG.

$[\text{Rh}(\phi)_2(\text{bpy}')]\text{Cl}_3$ (10 mg) and TSTU (8 mg; Aldrich) were dissolved in 0.5 mL 1:1:1 $\text{CH}_3\text{OH}/\text{CH}_2\text{Cl}_2/\text{CH}_3\text{CN}$ along with DIEA (6 μL) and stirred at ambient temperature for 1 h. The complete activation of the carboxylate was confirmed by TLC on alumina (CH_3OH), where the R_f for the ester is $\gg$ than the R_f for the acid. This solution of $[\text{Rh}(\phi)_2(\text{bpy}')]^{3+}$ -NHS ester was added to the DNA (aminoalkylated) coated beads in a 5 mL round bottom (rb) flask (after wetting them with 1:1:1 and draining the excess solvent). DIEA (20 μL) were added and the resulting slurry was stirred for 12 h to effect coupling.

Liberating the Conjugate from the CPG Beads. Following the coupling reaction the solution of metal was washed away from the beads on a glass frit, and the beads were washed with excess CH_3OH , CH_2Cl_2 , and CH_3CN . At this stage the beads are colored orange and the intensity of the color is strongly solvatochromic. The color appears darkest in CH_2Cl_2 .

The beads were transferred into a 1.7 mL Eppendorf tube and combined with $\sim 500 \mu\text{L}$ of concentrated NH_3 (aq). The mixture was agitated to wet the beads and then incubated at 60 $^\circ\text{C}$ for 6 h. After 1 h of incubation, the solution is colored red and the intensity of color on the beads is reduced.

After 6 h of incubation, the mixture was cooled to room temperature (very important) and filtered on a centrifuge filter (nylon; Rainin). The ammonia solution was dried down *in vacuo* on a speed vac evaporator.

After the sample was dried, it was redissolved in water (330 μL) and filtered to remove insoluble materials. The mixture was purified by RP HPLC as described in the results section. The extinction coefficient for Rh-modified oligonucleotides is taken as $1.9 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ at 390 nm.

Coupling [Os(phen)(bpy')(Me₂-dppz)]²⁺ to Aminoalkylated DNA on CPG Beads. [Os(phen)(bpy')(Me₂-dppz)]Cl₃ (14 mg) and TSTU (11.4 mg; Aldrich) were dissolved in 0.4 mL 0.5:1:1 *iPr*OH/CH₂Cl₂/CH₃CN along with DIEA (2 μL) and stirred at ambient temperature for 30 min. The complete activation of the carboxylate was confirmed by TLC on alumina (10:1 CH₂Cl₂/CH₃OH), where the R_f for the ester is >> than the R_f for the acid. This solution of [Os(phen)(bpy')(Me₂-dppz)]²⁺-NHS ester was added to the DNA (aminoalkylated) coated beads in a 5 mL round bottom (rb) flask (after wetting them with 0.5:1:1 and draining the excess solvent). DIEA (15 μL) were added and the resulting slurry was stirred for 12 h to effect coupling. Liberation from the beads was achieved as described for [Rh(phi)₂(bpy')]³⁺, and the same solvatochromism was observed. Purification by HPLC followed the description above and in the results section. The extinction coefficient for Os-modified oligonucleotides is taken as $1.3 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ at 430 nm.

Synthesis of 3'-Rh-Modified Conjugates. CPG resin (Glen Research) functionalized with a hydroxyaminoalkyl linker protected at the amine with an Fmoc and at the hydroxyl group with DMT was used for DNA synthesis. A dummy base was incorporated at the 3' terminus of the sequence so the DNA synthesizer would remove the DMT from the linker and build the DNA strand from that branching point. The DNA synthesizer was programmed to leave the oligonucleotide on the resin with the 5' DMT group intact.

After the DNA synthesis was complete, the Fmoc was selectively removed by treatment of the beads with a solution of piperidine (20% v/v) in DMF³⁴ under Ar(g) for 30 min. The DMF solution was washed away from the beads and the resin was transferred to a 5 mL rb flask. A solution of $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ -NHS ester was added to the beads along with DIEA (20 μL) and the mixture was stirred for 12 h to effect coupling. For this procedure we increased the concentration of the solution of NHS ester by reducing the volume to 250 μL and using the same amount of reagents. This modification of the procedure is necessary to accommodate the linker buried in the CPG and the fact that we are using 500 Å beads, which are smaller.

After the coupling reaction we removed the DMT group before cleaving the conjugate from the resin by treatment with trichloroacetic acid in dichloromethane for 18 s on the DNA synthesizer. The conjugate was then liberated from the beads and purified as described above. In general, these conjugates are more hydrophilic than the 5'-modified analogs owing to an extra phosphate in the linker.

Synthesis of 3',5'-bis-Heterometallated Oligonucleotides. A metal complex was introduced onto the 3' terminus of an oligonucleotide using the procedure described above. After removing the DMT protecting group from the 5'-terminal hydroxyl, the oligonucleotide was 5'-aminoalkylated according to the standard procedure, and a second metallointercalator was coupled onto the 5' terminus as described for singly modified strands. Liberation and purification procedures were the same as for the singly metallated DNA, however, higher concentrations of acetonitrile in the mobile phase was required to elute the product.

5.4.2. Methods

ESI and MALDI TOF mass spectrometry was provided by several service facilities. ESI data for oligonucleotides was provided by UC Riverside, MALDI TOF was provided by Colorado State University. ESI of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$

attached to dA was provided by Gary Hathaway at Caltech. CD spectroscopy was carried out on 20 μM samples. PAGE and photocleavage assays were carried out using the methods described previously.^{17,18,24,25}

Enzymatic Digestion of Oligonucleotides. An aqueous solution of oligonucleotide containing ~ 1 nmol of strand was added to 10 μL 1.0 M MgCl_2 (aq), 10 μL AP buffer (10X supplied with enzyme), 3 μL SVP (Sigma), 3 μL AP (Boehringer Mannheim), and water in a 1.7 mL Eppendorf tube to give a volume of 100 μL . The cocktail was incubated at 37 $^\circ\text{C}$ for ~ 8 h.

Following incubation the mixture was filtered on nylon (0.4 μm) centrifuge filters and analyzed by RP HPLC on a 100 $\text{\AA}$, analytical (C18) column. The mobile phase consisted of 50 mM ammonium acetate pH 5.5, and acetonitrile. The gradient started at 0% acetonitrile and went to 5% acetonitrile over 25 min. Some analyses required that the column be heated to 40 $^\circ\text{C}$. The nucleotides elute from the column in the following order: 1-C, 2-G, 3-T, 4-A, as confirmed by coinjection of standard solutions.

The area under each peak (260 nm observation) was normalized to the extinction coefficient of the base ($dC = 7.3 \text{ mM}^{-1}\text{cm}^{-1}$, $dG = 11.7 \text{ mM}^{-1}\text{cm}^{-1}$, $dT = \text{mM}^{-1}\text{cm}^{-1}$, $dA = \text{mM}^{-1}\text{cm}^{-1}$). The fraction of normalized are for a given base was then multiplied by the total number of bases to indicate the number of that component present in the mixture. In general we find the dG and dT numbers to be slightly high ($\sim 10\%$; independent of the conjugation chemistry) and the dA numbers to be slightly low, which probably reflects some error in the extinction coefficients used in the calculations.

Measuring T_m Values by Absorption. The temperature at which 50% of DNA duplexes in solution are dissociated into single strands is defined as the T_m . We measured T_m values by following the absorption of the duplex 270 nm or 384 nm as function of temperature on a Beckman 1400 DU spectrophotometer equipped with temperature control and multisample accessories. All T_m samples were 4 μM duplex in

5 mM Na_3PO_4 , 50 mM NaCl, pH 7.0, unless stated otherwise. The T_m was extracted from a plot of the first derivative of the absorbance vs. temperature curve.

Hybridization of Complementary Oligonucleotides to Form Duplexes.

Complementary DNA strands were dissolved in 5 mM Na_3PO_4 , 50 mM NaCl, pH 7.0 in a 1:1 ratio at the desired concentration. The sample was heated to 90 °C for 5 min. and cooled to ambient temperature (20 °C) by a linear gradient over 180 min.

Luminescence Spectroscopy. The emission properties of Os-modified oligonucleotides were characterized by steady-state and time-resolved emission spectroscopy. Steady-state data were collected on an SLM 8000 spectrofluorimeter and time-resolved data were collected by SPC and MPMF. For steady-state, excitation was at 480 nm or 530 nm. On the SLM we set the high voltage at 1000 V, the polarizers to 9 and the slits to 1.6 mm, and the integration time was 0.2 s. SPC was carried out instrumentation described previously with excitation at 635 nm and observation at 740 nm. SPC data analysis was carried out as described previously. For MPMF, the instrumentation and settings were as described in Chapter 2. All emission data were collected with 8 μM duplex unless stated otherwise.

HPLC Analysis of Hybridization of Metallated DNA with Complements. A sample was dissolved in buffer to a concentration of 1 nmol (duplex) in 300 μL . The concentration of complement was increased and the concentration of conjugate was held constant. The samples were injected onto a 300 Å analytical C18 column while ammonium acetate (50 mM)/acetonitrile flowed through the column at a rate of 1.0 mL/min. For a typical sample the concentration of acetonitrile in the eluent increased from 10% to 30% over 30 min. When the duplex was shorter than 20 bp, we switched to a C4 resin and increased to ammonium acetate concentration to 250 mM to keep the duplex intact on the column. The identities of the samples were confirmed by their absorption spectra.

5.5. REFERENCES

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Chapter 6: DNA-Mediated Charge Transfer Reactions over Fixed Distances in Metallointercalator-DNA Conjugates.

6.1. INTRODUCTION

This chapter describes the investigation of charge transfer reactions in which the reactants are tethered to DNA to give fixed donor-acceptor separations in well-defined assemblies. Photoinduced electron transfer (ET) reactions between metallointercalators bound noncovalently to DNA are remarkably fast and efficient even at low loadings of reactants on the helix.^{1-6 7-9} For example, 50% of photoexcited $[M(\text{phen})_2(\text{dppz})]^{2+}$ donors bound to polynucleotides at a ratio of metal-to-DNA of 1:25 base pairs (bp) (*i.e.* average donor-acceptor separation) are oxidatively quenched by $\Delta\text{-}[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ (1 equiv) with a rate $> 3 \times 10^{10} \text{ s}^{-1}$.^{2,3} The majority of the recombination reaction between the products of photoinduced ET occurs with a rate of $\sim 10^{10} \text{ s}^{-1}$ for several donor acceptor pairs at the same metal-to-DNA ratio.³ In fact, transient absorption studies of the recombination rate as a function of increasing concentrations of acceptor reveal that, despite a decrease in the average donor-acceptor separation, the rate of the recombination reaction (10^{10} s^{-1}) remains constant over the course of the titration. If the donors and acceptors bind randomly to the DNA duplex under the conditions of these titration experiments, our results suggest that the distance dependence of ET mediated by the DNA base stack is shallow relative to ET pathways consisting primarily of σ -bonds.^{10,11}

Another explanation for the efficiency of ET between metallointercalators and the loading independence of the recombination is that the donor and acceptor bind preferentially to adjacent sites along the DNA duplex.¹² In such a model, the cooperativity in intercalation results in one donor-acceptor separation, even over the

course of a titration, and therefore accounts for the fact that the rate of ET does not change.

Using photochemical assays to establish the site of intercalation by the acceptor Δ -[Rh(phi)₂(bpy)]³⁺, in the presence and absence of the donor Δ -[Ru(phen)₂(dppz)]²⁺, we were unable to find any evidence for such a model.³ The pattern of DNA photocleavage produced by irradiation of Δ -[Rh(phi)₂(bpy)]³⁺ bound to DNA did not change if Δ -[Ru(phen)₂(dppz)]²⁺ was added to the system.

Furthermore, studies of ET as a function of DNA sequence and absolute configuration about the metal center revealed that the rate of recombination is sensitive to these factors.³ One would not have expected these subtle changes in the experiment to perturb the proposed preferential binding so significantly.

More recently, conflicting results of spectroscopic studies of metallointercalators bound to DNA have been described by us and others.^{13,14} Franklin *et al.* used NMR spectroscopy in conjunction with circular dichroism to show that Δ -[Rh(phi)₂(bpy)]³⁺ and Δ -[Ru(phen)₂(dppz)]²⁺ do not bind cooperatively to double helical DNA, and in fact, show signs of anitcooperativity.¹⁴

Nevertheless, the argument that photoinduced ET between metallointercalators bound noncovalently to DNA involves preferential intercalation at adjacent sites can only be ruled out definitively by investigating reactions in which the donor and acceptor are separated by known and fixed. In a covalently modified DNA duplex where the reactants were separated by ~40 Å, Murphy *et al.* demonstrated remarkably efficient (100%) quenching of photoexcited [Ru(phen')₂(dppz)]²⁺ by [Rh(phi)₂(phen')]³⁺ on a subnanosecond time scale.⁶ This system could never be fully characterized owing to the lack of reproducible and effective synthetic methods for the preparation of the metallointercalator-DNA conjugates.

Having developed a strategy for tethering metallointercalators to oligonucleotides on a solid support (Chapter 5), we can now prepare synthetic duplexes for investigations of DNA-mediated charge transfer in which the length, sequence, concentration in solution, and reactants may be systematically and easily manipulated. We are now in a position to revisit the question of long-range ET through DNA with well-defined molecular assemblies.

Kelley *et al.* employed the synthetic strategies described in Chapter 5 and an analogous approach to prepare oligonucleotides bearing ethidium or $[\text{Rh}(\text{phi})_2(\text{bpy})]^{3+}$ tethered to the 5' termini.¹⁵ These authors found that the DNA bases did facilitate long-range ET and the distance dependence of this reaction was remarkably shallow. Moreover, the rate of ET was too fast to measure by single photon counting (SPC) with resolution of 150 ps, regardless of the donor acceptor-separation.

In fact, instead of being strongly dependent upon distance, this reaction was far more sensitive to base stacking. A single-base mismatch (TA to CA) blocked the long-range reaction completely.¹⁵ This work constitutes the first systematic study of photoinduced ET through DNA as a function of distance.¹⁵

Hall *et al.* used rhodium-modified oligonucleotides to demonstrate the long-range oxidative damage to guanine (G) in 5'-GG-3' sites initiated by ET from G.^{7,8} In these studies, the yield of oxidative damage was sensitive to base stacking but not distance. In assemblies containing two potential sites of oxidation the yield of damage at the site distal (34 Å) to the photooxidant ($[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$) was equal to or greater than that at the proximal (17 Å) site. When the base stack intervening the rhodium complex and the potential site of oxidation was disrupted by the introduction of bulges, the yield of damage decreased as the size of the disruption increased.

In this chapter, we present the characterization of three systems that involve charge transfer reactions mediated by the DNA base stack. We look first at oxidative

repair of thymine-dimer lesions,⁹ a reaction in which the DNA bases serve not only to mediate the reaction but play the role of reactant as well. We then focus on two groups of assemblies in which the reaction is photoinduced ET between metallointercalators.

6.2. RESULTS AND DISCUSSION

6.2.1. Oxidative Repair of Thymine-Dimers by DNA-Mediated Electron Transfer: Chemistry at a Distance

We sought to unambiguously demonstrate charge transfer over long molecular distances through the DNA base stack. In addition to photoinduced quenching reactions between metallointercalators, we considered also whether we might be able to initiate a chemical reaction (bond breaking and bond formation) at a remote site by ET.

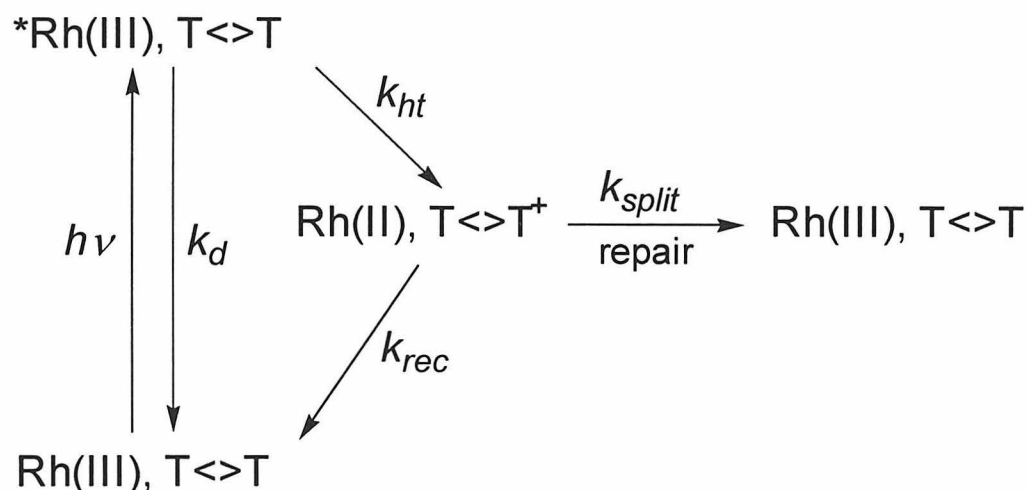
As described above, the irradiation of rhodium intercalators tethered to DNA duplexes with low energy, near visible light (365 nm) resulted in oxidative damage to the 5'-G of 5'-GG-3' sequences.^{7,8} Such oxidation occurred at sites separated by as much as 36 Å from the site of intercalation by the photooxidant ($[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$).

Another target for DNA-mediated charge transfer reactivity is the thymine dimer ($\text{T} \diamond \text{T}$). The $\text{T} \diamond \text{T}$ lesion is the most prevalent photochemical lesion in DNA, consisting of the product of a [2+2] photocycloaddition reaction between adjacent thymines on a polynucleotide strand.¹⁶ In bacteria, photolyase enzymes repair the lesion photochemically in a reaction initiated by the reduction of the dimer.¹⁷⁻²² Model systems have shown that repair of the lesion may also be initiated oxidatively.^{23,24}

We found that irradiation of $[\text{Rh}(\text{phi})_2(\text{dmb})]^{3+}$ bound noncovalently to short DNA duplexes containing a thymine dimer ($\text{T} \diamond \text{T}$) resulted in the catalytic repair of the lesion upon irradiation with low energy (400 nm) light (Chapter 1). Such repair of $\text{T} \diamond \text{T}$ by the photooxidant is consistent with ET from the lesion to the photoexcited rhodium, which is thermodynamically favored based on the relevant redox potentials for the

reactants ($E_{1/2} (^+T \rightleftharpoons T/T \rightleftharpoons T) \sim 2.0$ V vs. NHE;^{17,21,25} ($E_{1/2} ^*Rh^{III}/Rh^{II} > 2.0$ eV vs. NHE (32))²⁶ (Scheme 6.1). In this section we describe the long-range oxidative repair of $T \rightleftharpoons T$ in duplex DNA as a probe of the factors that are important in charge transfer reactions mediated by the DNA base stack.

Scheme 6.1. Cycle of electron transfer reactions in the repair of thymine dimer ($T \rightleftharpoons T$) lesions in DNA.



Thymine Dimer Repair in Rhodium-Modified DNA Duplexes. To explore the remote repair of thymine dimer lesions mediated by DNA, we incorporated a $T \rightleftharpoons T$ site specifically into 16 bp duplexes possessing a Rh(III) intercalator covalently tethered to one end (15) and irradiated these assemblies with 400 nm light. Shown schematically in Figure 6.1 (left) are two DNA assemblies containing tethered Δ -rhodium at either the 5' or the 3' terminus of an oligonucleotide and a thymine dimer in the complementary strand. The HPLC chromatograms (denaturing conditions) at 0, 1, 2, 4, and 6 hours irradiation (top to bottom) for the reaction with the duplex modified with rhodium at the

3'-end are also shown in Figure 6.1. This analysis showed the steady disappearance of the dimer-containing strand ($T_R = 17$ min.) and the corresponding appearance of the repaired strand ($T_R = 26$ min.) as a function of the duration of irradiation at 400 nm (16). The metallated oligonucleotide eluted at $T_R = 45$ min. and is not shown here, however, the visible absorption spectrum of the material showed that the metal complex did not degrade during the course of the reaction. The quantum yield for thymine dimer repair calculated for a 6 hour irradiation is 2×10^{-6} mol E^{-1} , which is two orders of magnitude greater than the quantum yield for oxidative damage by rhodium intercalators.

Establishing the Site of Intercalation by Photocleavage at 313 nm.

Photocleavage of DNA by phi complexes of rhodium with irradiation at higher energies (313 nm) has been useful in marking sites of intercalation,^{27,28} and permits the determination of distances^{6,7} over which DNA-mediated ET reactions can occur. We used this assay to determine the site of intercalation by $[Rh(phi)_2(bpy')]^{3+}$ tethered to the assemblies pictured in Figure 6.1. The arrows indicate relative intensities for the direct photocleavage (14) with 313 nm light.^{7,8} The distribution of intensities indicates the most probable site for intercalation of rhodium is the 5'-CG-3' step for the duplex containing the 5'-Rh modified oligomer and equally in the 5'-CG-3' step and 5'-GT-3' step for the duplex with the 3'-Rh-modified strand. With these intercalation sites, the distance between the catalyst and the cyclobutane ring of the dimer is 19 to 22 Å. These photocleavage results establish that tethering the rhodium complex to the assembly precludes direct contact of the metal center and the thymine dimer within a duplex.

Establishing that the Repair of Thymine Dimers in Rhodium-Modified

Duplexes is Intraduplex. In order to establish that the repair of T<>T lesions in Rh-modified DNA duplexes proceeded in an interduplex reaction, we examined dimer repair for a Rh-modified duplex over a concentration range of 2 - 32 μ M. The repair efficiency by rhodium bound non-covalently to the duplex appears to be 30 times greater than in

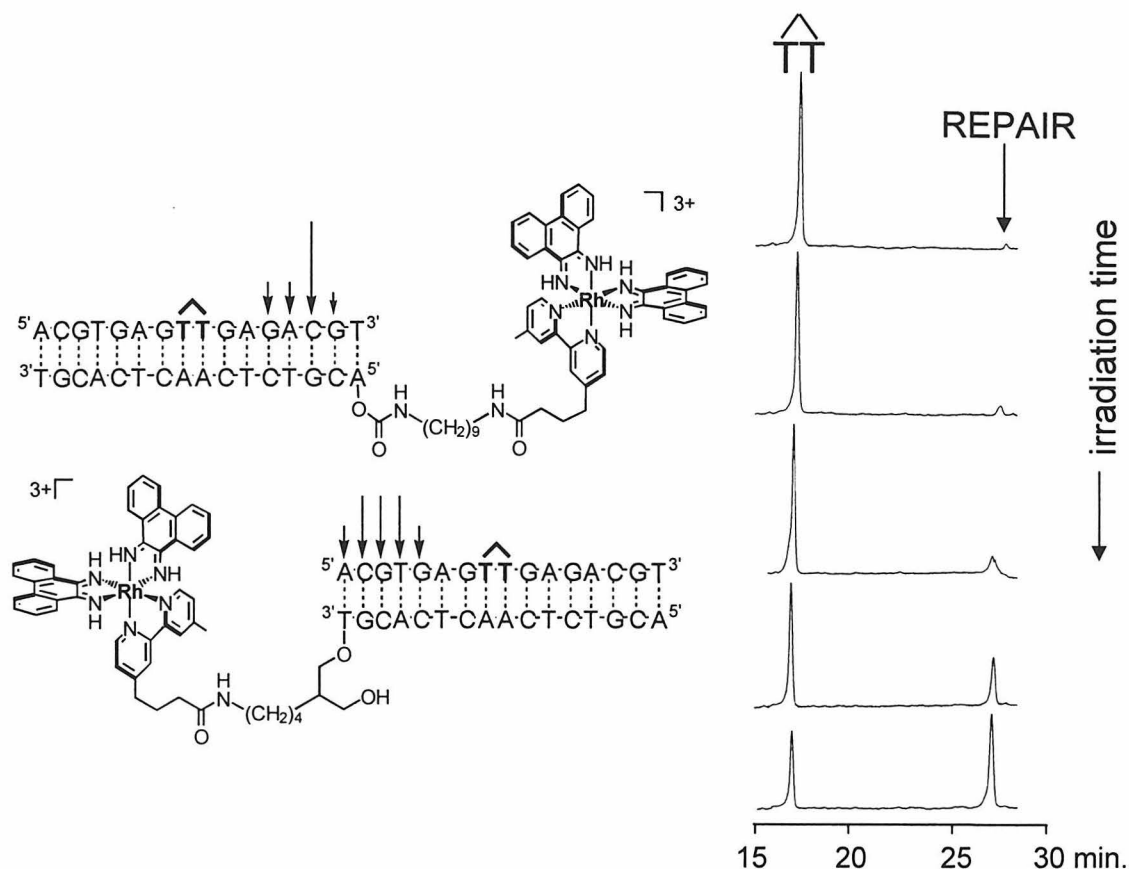


Figure 6.1. Long-range repair of the thymine dimer by rhodium covalently tethered to the DNA duplex. Shown schematically (left) are two DNA assemblies containing tethered Δ -rhodium at either end and a thymine dimer in the complementary strand. The HPLC chromatograms (right) at 0, 1, 2, 4, and 6 hours irradiation (top to bottom) for the reaction with the duplex modified with rhodium at the 3'-end (bottom left) are also shown. The arrows indicate relative intensities for the direct photocleavage (14) with 313 nm light.

between 16 and 32 μM . If the repair were primarily intermolecular, we would have expected an increase in repair efficiency of at least 500% over this concentration range. These data therefore indicate that at 8 μM , where we carry out most repair experiments, duplex dimerization is not significant (17).

We also tested the intermolecularity of the reaction directly in an experiment in which a 16 bp duplex containing a thymine dimer was irradiated in the presence of a separate, undamaged duplex bearing a pendant rhodium complex (Figure 6.2).⁷ After irradiation of this mixture for 6 hours we observed no significant repair above background. A small amount (5%) of repair of the thymine dimer was found in control samples lacking rhodium (light control) and represents the base level of natural repair by light in our experiments.

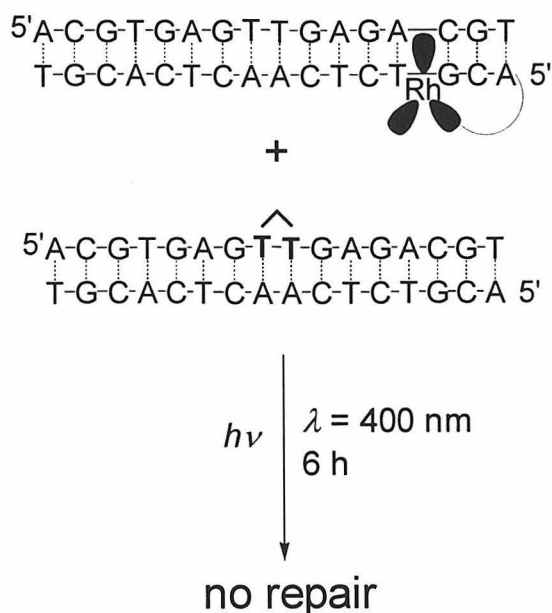


Figure 6.2. Direct test for intramolecular repair of thymine dimer lesions by a rhodium intercalator tethered to another duplex.

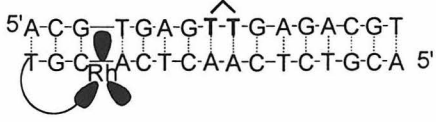
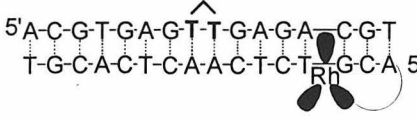
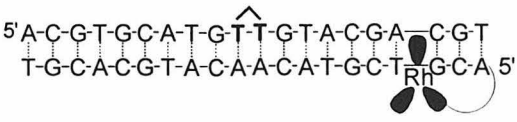
Interduplex intercalation is also inconsistent with DNA photocleavage experiments measured previously as a function of concentration with Rh-modified duplexes.⁷ Hence, oxidative repair of T $\diamond$ T lesions in the Rh-modified duplexes occurs in an intraduplex reaction and is initiated from a remote position 16 -26 Å away through the DNA base stack (19). These results provide an unambiguous demonstration that the DNA helix facilitates charge transfer and mediates the long range repair of the thymine dimer.

Sensitivity of Long-Range Thymine Dimer Repair to Distance and Stacking.

We prepared a series of duplexes, each containing a thymine dimer and a covalently bound rhodium intercalator to test the sensitivity of the reaction to both distance and stacking (Tables 6.1 and 6.2). In all the assemblies, the 4 bp (5'-ACGT-3') intercalation sites at either end of the duplex and the -GAG- sequences flanking the T $\diamond$ T lesion were identical. We systematically varied the duplex length, position of modification by rhodium (3' or 5' terminus) and the configuration about the Rh(III) center (Δ or Λ). We also hybridized the 5'- Δ -Rh-modified 16 nucleotide strand and 18 strand to the 20-mer thymine dimer strand, creating two assemblies with internal bulges between the rhodium intercalator and thymine dimer.

Importance of the Stacking of the Photooxidant and the Dimer. The repair reaction is sensitive to the stacking of the rhodium intercalator into the helix. This is evident in the consistently greater repair efficiency with Δ isomers compared to Λ isomers. As found previously in studies of DNA-mediated ET,²⁻⁴ this result reflects the deeper stacking of the right-handed metal complex into the right-handed DNA helix.²⁹ We see comparable yields of repair in reactions with rhodium tethered to the 3' or 5' side of the dimer. From this observation, we infer that stacking on the 3' and 5' sides of the thymine dimer within the helix is not substantially different.^{16,22}

Table 6.1. Long-Range Repair of Thymine Dimers in Rh-Modified DNA.^a

Assembly	Distance (Å)	T _m (°C)		Repair (%)	
		+TT	-TT	Δ-Rh	Λ-Rh
	19			67	59
	19	59	65	79	47
	22	61	65	91	87
	26	63	68	100	62

a. Repair is expressed as a percentage of thymine dimer repaired after 6 hours of irradiation. Repair efficiencies are given for assemblies containing either Δ - or Λ -Rh. Combined random error in sample preparation, irradiation, and HPLC analysis $\leq 5\%$. Separation distances between catalyst and thymine dimer are based upon intercalation at the sites shown schematically. Melting temperatures (T_m) were determined with 2 μM solutions of modified duplex. Reaction conditions for repair were identical to those of Figure 6.1.

We do find a small difference in the repair efficiency depending on which side of the dimer the photooxidant is tethered. We considered whether these differences might result from the differences in the stacking of the rhodium complex. To evaluate the stacking interaction of tethered rhodium into the DNA duplex for 5' versus 3'-modified

oligonucleotides, we used the luminescence quenching assay described in Chapter 5. The steady-state luminescence quenching of a stoichiometric amount of *rac*-Ru(phen)₂dppz²⁺ bound noncovalently to the duplex by intercalation of the dppz ligand was measured for the individual assemblies. The amount of quenching provides a measure of the efficiency of ET from electronically excited ruthenium to ground state rhodium through the DNA helix and can be assumed, within an individual DNA sequence, to reflect the ability of rhodium to intercalate into the duplex (see ref. 12). For the 3'-Rh tethered 16 bp duplex (Table 6.1, top), luminescence quenching was 58% and 52% for Δ -Rh and Λ -Rh, respectively, versus luminescence of *rac*-Ru(phen)₂dppz²⁺ intercalated into the unmodified duplex (without thymine dimer). For the corresponding 5'-tethered rhodium duplex, luminescence quenching was 49% and 39% for Δ -Rh and Λ -Rh, respectively. Therefore, the higher repair yields correlate with higher quenching of ruthenium luminescence, and reflect the importance of the stacking of the photooxidant in determining the repair efficiency.

Efficiency of Thymine Dimer Repair as a Function of Distance in Rhodium Modified DNA. Table 6.1 also lists the efficiency of T $\diamond$ T repair as a function of distance in Rh-modified duplexes. We find that repair efficiencies do not diminish with increased separation between the thymine dimer lesion and tethered rhodium. In fact, the repair efficiency increased for the Δ -diastereomer over this range of distances, which likely reflects the increased helical stabilization with increased duplex length.^{30,31} These data speak against a mechanism in which fraying of the ends of the DNA duplex might allow the tethered rhodium complex to contact the thymine dimer and initiate repair at close range.

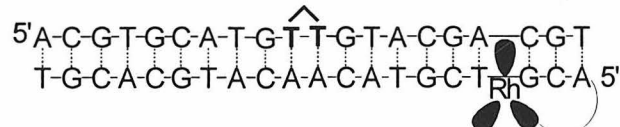
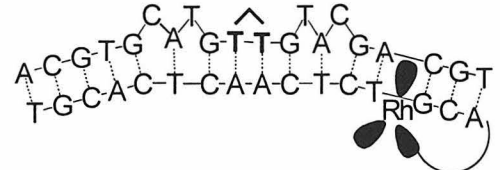
Effect of Bulges Intervening the Dimer and the Photooxidant. While the efficiency of these DNA-mediated repair reactions does not appear to diminish with increasing distance between dimer and oxidative catalyst, the reactions are sensitive to

stacking of the intervening bases. As illustrated schematically in Table 6.2, we compared the efficiency of T $\diamond$ T repair for a dimer containing 20-mer hybridized to a Rh-modified 20-mer (no bulge), a Rh-modified 18-mer (2 single base bulges), and a Rh-modified 16-mer (4 single base bulges).

As the amount of disruption to the base stack increases with more bulges in the assembly, the repair efficiency decreases. The destabilization of the base stack is reflected in the lower melting temperatures (T_m) for the duplex containing bulges. Such a diminution in repair efficiency caused by bulges was described previously for DNA-mediated oxidative damage to DNA.⁸ It is noteworthy that the T_m values for the two assemblies containing bulges are not different, and yet the repair efficiencies differ significantly. Such an observation indicates that it is not the relative concentrations of duplex versus single strands that determines the repair efficiency, but rather, the presence of an optimally stacked pathway for charge transfer that is a determining factor.

One question is why does the remote repair reaction still occur with the disruptions in the base stack. It is important to note that the illustrations in Table 6.2 are two dimensional representations of a complex structure. In fact, the driving force for π -stacking of the bases is so great that we expect some stacking to still occur, even with the bulges present.³⁰ Although single and double base bulges do not completely destack a DNA duplex, they do introduce structural perturbations.³² Such defects in the intervening π -stack interfere with the long-range repair process. These results with disruptions in the base stack provide confirmation that the DNA helix mediates this long range oxidative repair reaction.

Table 6.2. Long-range thymine dimer repair in duplexes with covalently tethered rhodium without and with intervening bulges.^a

Assembly	T _m (°C)	Repair(%)
	+TT	Δ -Rh
	63	100
	45	80
	45	47

a. Repair is expressed as a percentage of thymine dimer repaired after 6 hours of irradiation. Combined random error in sample preparation, irradiation, and HPLC analysis $\leq 5\%$. Reaction conditions for repair were identical to those in Figure 6.1.

Comparisons of Repair Efficiencies for Covalently and Noncovalently

Bound Rhodium. The quantum yields for repair of the T $\diamond$ T lesions by rhodium intercalators bound noncovalently to the DNA duplex is ~ 50 times greater than for the complex tethered to the assembly. Since the yield of repair does not depend on distance, the difference in yields for tethered and noncovalently bound rhodium cannot result from the noncovalently bound oxidant intercalating in sites adjacent to the lesion. It is possible that $[\text{Rh}(\text{phi})_2(\text{dmb})]^{3+}$ bound noncovalently to the duplex can stack directly against the thymine dimer, or, the complex could stabilize the base stack immediately adjacent to the dimer by intercalation. These issues would not be present in the case of the tethered complex, however, photocleavage experiments with $[\text{Rh}(\text{phi})_2(\text{dmb})]^{3+}$ do not indicate intercalation adjacent to the dimer.

We suggest that the difference in repair efficiencies reflects features of the stacking between the metal complex and the base stack. Our concern is that the linker, which anchors $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ to the Rh-modified duplexes, prevents optimal intercalation into the base stack. Since the linker is not present to restrict the intercalation of $[\text{Rh}(\text{phi})_2(\text{dmb})]^{3+}$ bound noncovalently, the repair efficiency is greater. Such a hypothesis is supported in this work by the lower quantum yield for repair by Δ -Rh containing assemblies compared with Λ -Rh. We have also shown in Chapter 2 that osmium intercalators that possess the bpy' ligand do not intercalate as deeply into the π -stack as complexes with less bulky ancillary ligands.

6.2.2. Photoinduced Electron Transfer between Os(II) and Rh(III)

Metallointercalators Tethered to a 15 bp Duplex

Description of the Assemblies. Using the strategies outlined in Chapter 5, we synthesized an Os-modified oligonucleotide and its Rh-modified complement, each 15 nucleotides in length. Instead of isolating four isomers for the Os-modified strand, we

isolated only three since the first two compounds coeluted as a racemic mixture. The rhodium-modified oligonucleotide was isolated as the Δ -Rh and Λ -Rh containing diastereomers. The sequence of the duplex is pictured in Figure 6.3.

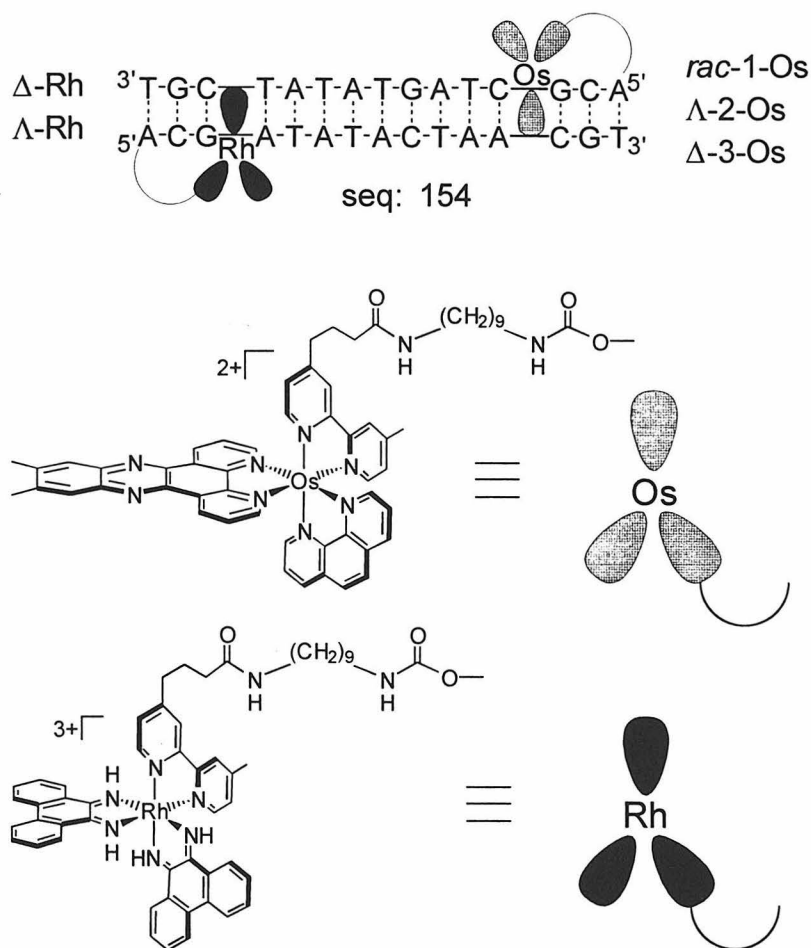


Figure 6.3. Schematic illustration of covalently modified 15 bp duplexes and tethered metallointercalators. For the osmium complex, only one out of four possible isomers is shown (Δ -enantiomer), and for the rhodium complex the Δ -enantiomer is shown.

Luminescence Quenching in the Doubly Modified Duplexes. To determine the efficiency of ET through the DNA base stack in these 15 bp assemblies, we compared the luminescence yield of the duplexes containing only the osmium complex to the intensity of emission from duplexes possessing a rhodium and an osmium complex. The stoichiometry of the complementary strands was confirmed by HPLC analysis of the duplex solutions as described in Chapter 5.

Table 6.3 lists the emission yields and yields of luminescence quenching for the 15 bp assemblies as a function of the isomers of each conjugate used to construct the assemblies. The data for one set of samples taken at 5 °C are also listed. The luminescence yields of the duplexes bearing only osmium depend significantly on the isomer of the complex in the conjugate and are similar to the emission intensities for the duplexes described in Chapter 5.

The yield of luminescence quenching across the series of doubly modified duplexes is incredibly modest. For the samples containing the racemic mixture of Os-modified strands, we find 14% and 12% quenching for Δ -Rh and Λ -Rh, respectively. When the Λ -2-Os isomer is incorporated into the duplexes, there is no detectable quenching outside the error in the luminescence intensity, and the Δ -Os isomer of this pair is quenched by 9% for either Δ or Λ -Rh.

We also cooled the samples that gave 14% and 12% quenching to 5 °C and measured the quenching. Under these conditions the yield of quenching for Δ and Λ -Rh (13% and 10%) did not change significantly.

When compared to the first example of photoinduced ET between donors and acceptors bound covalently to DNA by Murphy,⁶ these duplexes have produced dramatically different results. In fact, the results described here resemble those for several duplexes modified with ruthenium and rhodium described by Arkin in her doctoral thesis.³³

Table 6.3. Luminescence Intensities and Quenching Yields for Duplexes Bearing Osmium and Rhodium Intercalators.^a

Duplex	I_{rel}	F_Q	T (°C)
<i>rac</i> -1-Os_ 154 _ds	1.09	-----	20
<i>rac</i> -1-Os_ 154 - Δ -Rh	0.94	0.14	20
<i>rac</i> -1-Os_ 154 - Λ -Rh	0.96	0.12	20
Λ -2-Os_ 154 _ds	0.74	-----	20
Λ -2-Os_ 154 - Δ -Rh	0.74	0.00	20
Λ -2-Os_ 154 - Λ -Rh	0.71	0.04	20
Δ -3-Os_ 154 _ds	1.55	-----	20
Δ -3-Os_ 154 - Δ -Rh	1.42	0.09	20
Δ -3-Os_ 154 - Λ -Rh	1.46	0.09	20
<i>rac</i> -1-Os_ 154 _ds	1.01	-----	5
<i>rac</i> -1-Os_ 154 - Δ -Rh	0.88	0.13	5
<i>rac</i> -1-Os_ 154 - Λ -Rh	0.91	0.10	5

a. I_{rel} = emission intensity for aerated samples relative to $[\text{Ru}(\text{bpy})_3]^{2+}$ (10 μM) in water.

Duplex solutions were 8 μM in duplex in 5 mM Na_3PO_4 , 50 mM NaCl, pH 7.0. The symbol “ds” indicates the sample is duplex, and contains only the osmium intercalator.

The Δ -Rh or Λ -Rh refers to a doubly modified duplex with osmium and rhodium.

The important question given the data presented here becomes is the quenching we are measuring real? To answer this question we consider some alternative explanations for a loss in emission intensity. Perhaps the small loss in intensity is really the result of an inner filter effect caused by the addition of the Rh-modified strand. In other words, the presence of an additional chromophore reduces the excitation intensity available to the lumiphore, and so less complexes emit because less are excited. We can dismiss this concern for two reasons. The first is that we made these measurements at two excitation energies, where the relative extinction coefficients for each metallointercalator are very different, and saw the same amount of quenching. Second, each of the Os-modified strands has the same extinction coefficient and the same is true for the two Rh-modified oligonucleotides, however, we see isomer dependent yields of quenching.

Another consideration we had was whether the metallointercalator tethered directly to the oligonucleotide might inhibit the formation of duplexes in solution. The consequence of such inhibition would be that the emission we detect is really from a poorly-defined assembly that contains only an Os-modified strand, and the addition of unmodified or Rh-modified strand does not really produce any duplex. The absence of quenching would be the result of incomplete hybridization between complementary strands.

We tested this hypothesis by following the hybridization of Rh-modified oligonucleotides to their Os-modified complements by HPLC (data not shown) as described in Chapter 5 for a singly modified duplex. This analysis was particularly useful and demonstrated quantitative formation of doubly modified duplexes with a 1:1 stoichiometry of modified strand. Thus, the data and discussion above illustrate that quenching in the 15 bp, modified duplexes pictured in Figure 6.3 is real, but much less

than anticipated based on the yield of photoinduced ET previously reported for reactants bound covalently to a 15 bp duplex, or bound noncovalently.

6.2.3. Photophysical Properties of Modified Duplexes with 5'-ACGT Sequences at the Intercalation Sites

Description of the Assemblies. We were surprised to find only 14% quenching in the 15 bp duplexes containing osmium and rhodium intercalators described above. On the basis of our results illustrating the sensitivity of DNA-mediated ET to the binding properties of metallointercalators,^{3-5,7,9} we reasoned that the limited amount of quenching in these assemblies could be the result of poor association of the intercalators with the duplex. If the complexes are restricted in their intercalation, they cannot access the DNA base stack, which facilitates the reaction. Such restriction may be caused by the DNA sequence at the binding site, or the linker may somehow restrict binding.



Figure 6.4. Schematic illustrations of modified 14, 16, and 18 bp duplexes.

Table 6.4. Luminescence Intensities and Quenching Yields for Duplexes Bearing Osmium and Rhodium Intercalators.^a

Duplex	I_{rel}	F_{Q}	F_{Q} (DMB)	$\Delta-T_{\text{m}}$ (°C)
Δ -1-Os_ 141 _ds	1.13	-----	0.48	0.0
Δ -1-Os_ 141 - Δ -Rh	1.08	0.044	0.57	+5.0
Δ -4-Os_ 141 _ds	1.66	-----	0.63	-1.0
Δ -4-Os_ 141 - Δ -Rh	1.54	0.072	0.62	+2.0
Δ -1-Os_ 162 _ds	1.05	-----	0.17	+3.0
Δ -1-Os_ 162 - Δ -Rh	1.01	0.038	0.24	+5.0
Δ -4-Os_ 162 _ds	2.01	-----	0.36	0.0
Δ -4-Os_ 162 - Δ -Rh	1.94	0.030	0.48	+3.0
Δ -1-Os_ 181 _ds	1.11	-----	0.36	+3.9
Δ -1-Os_ 181 - Δ -Rh	1.13	-0.018	0.45	+12.1
Δ -4-Os_ 181 _ds	2.08	-----	0.47	-0.1
Δ -4-Os_ 181 - Δ -Rh	1.99	0.043	0.59	8.0

a. I_{rel} = emission intensity for aerated samples relative to $[\text{Ru}(\text{bpy})_3]^{2+}$ (10 μM) in water.

Duplex solutions were 8 μM in duplex in 5 mM Na_3PO_4 , 50 mM NaCl , pH 7.0. The symbol “ds” indicates the sample is duplex, and contains only the osmium intercalator.

The Δ -Rh or Λ -Rh refers to a doubly modified duplex with osmium and rhodium.

We constructed a family of duplexes that would permit us to examine a different four-base binding site at the 5' termini of the duplexes. We also measured luminescence quenching as a function of the length of the linker joining the donor, $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$, and the duplex. Importantly, to insure the 1:1 stoichiometry of Os-modified strand and complementary or Rh-modified oligonucleotide we purified the intact duplexes by HPLC using the method described in Chapter 5. These assemblies prepared varied in length from 14 to 18 bp.

Luminescence Quenching in Duplexes Terminate in 5'-ACGT-3' Sequences.

Table 6.4 lists the data for a comprehensive examination of the luminescence, luminescence quenching, and thermal stability of a family of DNA duplexes. For each of the assemblies characterized, we focused only on the Δ isomers of the modified conjugates. Owing the asymmetry of the Os-modified strand, two Δ isomers, which differ in the relative orientation of the linker with respect to the metal center, are generated (Δ -1-Os and Δ -4-Os). For the Rh-modified oligonucleotide, there is only one Δ isomer.

Luminescence of Singly Modified Duplexes. As described in Chapter 5 for this series of duplexes, the luminescence yield of the Δ -4-Os isomer is greater than that of the Δ -1-Os isomer. Across the series of duplexes with identical binding sites, the luminescence yield is constant. The Δ -4-Os isomer attached to the 14 bp assembly is somewhat lower. This observation could suggest that the complex is intercalating at the fourth base step (adjacent to the fifth base) from the tethered terminus, which has a sequence different from the 16 and 18 bp duplexes. Studies of DNA damage initiated by photoexcited ruthenium tethered to short duplexes indicate that the complex is intercalated 3 bp from the terminus to which the complex is tethered, however, the resolution of this experiment cannot easily distinguish intercalation 3 bp in from 4 bp in.³⁴

Quenching by $[Rh(phi)_2(bpy')]^{3+}$ Tethered to Osmium Modified DNA.

Table 6.4 also lists the fraction of emission quenched (F_Q) in doubly modified duplexes bearing an osmium and a rhodium intercalator. For six heterometallated duplexes ranging in size from 14 to 18 bp, we found no quenching. These results illustrate that the quenching observed in the 15 bp assemblies described above cannot be the result of inner filtering by the rhodium complex, however, they also illustrate that changing the sequence of the duplex, and trying different lengths, did not lead to efficient long-range ET.

Quenching by $[Rh(phi)_2(dmb)]^{3+}$ Bound Noncovalently to Osmium-Modified DNA. In Chapter 5 we described an experiment where we measured the ability of $[Rh(phi)_2(bpy')]^{3+}$ tethered to DNA duplexes to quench the emission from $[M(phen)_2(dppz)]^{2+}$ derivatives bound noncovalently to the modified duplexes. We used this experiment to infer whether the tethered rhodium complex could intercalate into the duplex and be effective in ET reactions.²⁻⁶

In order to examine whether the lack of ET in the metallated duplexes pictured in Figure 6.4 was the result of the donor not being intercalated, we added $[Rh(phi)_2(dmb)]^{3+}$ to solutions of the osmium-modified duplexes and measured the amount of luminescence quenching by the noncovalently bound electron acceptor. These results are also listed in Table 6.4 in the column labeled F_Q (DMB). Remarkably, we found efficient quenching of the osmium emission across the series of duplexes. The fraction quenched is slightly greater for the doubly modified duplexes than for the duplexes with only the osmium intercalator covalently attached and intercalated.

We suggest that for duplexes with two intercalators tethered and intercalated, the distance between the donor and noncovalently bound acceptor is shorter, therefore the yield of ET is greater. When only the donor is present on the helix, the average separation between donor and acceptor can be greater. Such an explanation would refute

any argument that the quenching observed for $[\text{Rh}(\phi)_2(\text{dmb})]^{3+}$ bound to Os-modified duplexes might involve clustering two reactants near the end of the duplex to which the intercalator is tethered.

It is also noteworthy that the amount of luminescence quenching by $[\text{Rh}(\phi)_2(\text{dmb})]^{3+}$ noncovalently bound to Os-modified duplexes depends upon which Δ isomer of $[\text{M}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ (linker equatorial or axial; Chapter 5) is tethered to the duplex. This observation is independent of whether or not the $[\text{Rh}(\phi)_2(\text{bpy}')]^{3+}$ is tethered to the opposite end of the duplex.

The isomer dependent yield of quenching between $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to duplexes and $[\text{Rh}(\phi)_2(\text{dmb})]^{3+}$ bound noncovalently parallels the isomer dependent luminescence yield. The isomers of the metallointercalator that give the brightest emission in the absence of acceptor are also quenched the most by the noncovalently bound acceptor. The interpretation of these observations is that the brighter emission reflects deeper intercalation within the DNA base stack and thus greater coupling with the pathway for ET. Therefore, emission quenching is favored for reactants that access more effectively the DNA base stack.

In summary, despite the absence of long-range ET between metallointercalators tethered to the DNA duplexes synthesized for this study, efficient quenching of the emission from $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ in Os-modified assemblies by noncovalently bound $[\text{Rh}(\phi)_2(\text{dmb})]^{3+}$ is observed. These results provide evidence that strongly suggests that the osmium complexes are intercalated into the DNA duplexes and capable of undergoing ET. Moreover, the quenching reaction with the noncovalently bound acceptor is sensitive to the structure of the donor that is tethered to the assembly.

Examining the Effect of Linker Length in Long-Range Quenching Reactions.

In order to determine whether changing the length of the tether that attaches $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ to singly and doubly modified duplexes would increase

the yield of ET to $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$, we changed substituted ethylene diamine (C2) and diaminopropane (C3) for diaminononane (C9) in metallointercalator-DNA conjugates. For the series of doubly modified duplexes, the rhodium complex is always attached by the C9 linker.

Table 6.5 lists the emission yields for single-stranded conjugates (18-mers), 18 bp duplexes possessing only $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$, and 18 bp duplexes with osmium and rhodium covalently attached as a function of linker length. We also provide the fraction of osmium luminescence quenched by $[\text{Rh}(\text{phi})_2(\text{dmb})]^{3+}$ bound noncovalently to the duplex assemblies. As described above for the C9 linkers and duplexes ranging from 14-18 bp in length, we find no significant long-range reaction despite efficient quenching by the noncovalently bound acceptor.

It is noteworthy the single stranded conjugates with $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ are all luminescent under the conditions of these experiments. In fact, the double stranded assemblies show less emission than the single strands, which contrasts the properties of single stranded metallointercalator-DNA conjugates described previously by Jenkins³⁵ and Murphy.⁶ This observation may suggest that the tethering of the reactant prevents the optimal association of the donor with the duplex.

Table 6.5. Luminescence Intensities and Quenching Yields as a Function of Linker Length in Duplexes Bearing Osmium and Rhodium Intercalators.^a

Duplex	I_{rel}	F_Q	F_Q (DMB)	$\Delta-T_m$ (°C)
C9: Δ -1-Os_181_ds	1.11	-----	0.36	+3.9
C9: Δ -4-Os_181_ds	2.08	-----	0.47	-0.1
C2: <i>rac</i> -1-Os_181_ss	1.24	-----	-----	-----
C2: <i>rac</i> -1-Os_181_ds	0.70	-----	0.37	+4.1
C2: <i>rac</i> -1-Os_181- Δ -Rh	0.84	-0.20	-----	+9.1
C2: Δ -3-Os_181_ss	1.83	-----	-----	-----
C2: Δ -3-Os_181_ds	0.61	-----	0.43	-1.1
C2: Δ -3-Os_181- Δ -Rh	0.74	-0.22	-----	+6.1
C3: Δ -2-Os_181_ss	1.36	-----	-----	-----
C3: Δ -2-Os_181_ds	1.08	-----	0.36	+4.1
C3: Δ -2-Os_181- Δ -Rh	1.25	-0.16	-----	+9.1
C3: Δ -4-Os_181_ss	1.84	-----	-----	-----
C3: Δ -4-Os_181_ds	1.05	-----	0.34	-0.1
C3: Δ -4-Os_181- Δ -Rh	1.24	-0.18	0.44	+6.1

a. I_{rel} = emission intensity for aerated samples relative to $[\text{Ru}(\text{bpy})_3]^{2+}$ (10 μM) in water. Duplex solutions were 8 μM in duplex in 5 mM Na_3PO_4 , 50 mM NaCl , pH 7.0. The symbol “ss” indicates a single stranded sample, “ds” indicates the sample is duplex, and contains only the osmium intercalator. The Δ -Rh refers to a doubly modified duplex with osmium and rhodium.

6.2.4. Discussion of Long-Range DNA-Mediated Electron Transfer Reactions

In this chapter, we described three charge transfer reactions that are mediated by the DNA base stack. For the first system, thymine dimer repair in the DNA duplex,⁹ we observe repair of the cyclobutane dimer by photoexcited rhodium over substantial distances in Rh-modified duplexes. This reaction is formally hole transfer from the photoexcited rhodium intercalator to the thymine dimer,⁹ which results in the one electron oxidation of the dimer. We have yet to establish this mechanism spectroscopically, however, the hole transfer cycle is the only thermodynamically favored pathway. We illustrated the remarkable sensitivity of the efficiency of repair to the stacking of the photooxidant and the stacking of the intervening pathway. The yield of the repair reaction was not sensitive to the distance separating the rhodium complex and the thymine dimer. Our results underscore a concept that we have been finding in many systems under investigation in our laboratory, namely, that long-range charge transfer through DNA does not bear a large penalty for distance, but does depend significantly on π -stacking interactions.

We also examined photoinduced ET between osmium intercalators and rhodium intercalators tethered to short DNA duplexes. These studies were aimed at further understanding the results described by Murphy *et al.* in 1993.⁶ Murphy found that 100% of ruthenium donors were quenched by rhodium acceptors when the complexes were tethered to a 15 bp DNA duplex and separated by ~ 40 Å. Not only was this reaction remarkably efficient, but, the rate was on the subnanosecond time scale. Our results do not reproduce this single experiment.

Moreover, we found repeatedly in titrations of metallointercalators bound noncovalently to DNA that very efficient quenching (50% by 1 equiv of acceptor) of the donors was observed with a rate $> 3 \times 10^{10} \text{ s}^{-1}$.^{2-4,6} Our data do not follow these

observations either. The question becomes what are the factors that prevent efficient long-range quenching in the assemblies we constructed here.

In order to address this important issue, we consider some concepts that might account for the poor quenching in the assemblies we investigated. We concentrate mainly on the comparison of the long-range quenching results in this work to the titrations on DNA described in Chapters 3 and 4 and previously.^{2-4,6} We found that 50% of photoexcited Δ -[M(phen)₂(dppz)]²⁺ (M = Os, Ru) donors bound to calf thymus (CT) DNA or poly d(AT) were quenched by 1 equiv of Δ -[Rh(phi)₂(bpy)]³⁺, when the average donor acceptor separation was 25 bp. When the analogs of this donor and acceptor, [Rh(phi)₂(bpy')]³⁺ and [M(phen)(bpy')(Me₂-dppz)]²⁺, were tethered to DNA duplexes ranging in size from 14 to 18 bp, the most quenching we detected was ~15%.

One explanation for the lack of quenching is the donor or acceptor or both are not intercalated into the helix at all. We ruled this consideration out by adding a redox partner to bind noncovalently to the duplex and react with the metal complex that is tethered. When [Os(phen)₂(Me₂-dppz)]²⁺ is added to Rh-modified duplexes, we observe relatively efficient (50% or more depending on the sequence) quenching of the photoexcited complex. When [Rh(phi)₂(dmb)]³⁺ is added to Os-modified duplexes, we observe quenching of the tethered donor. These experiments show that each complex tethered to DNA is capable of undergoing ET.

Another concern is by tethering the complexes to the duplex, we have restricted their ability to bind to DNA in a fashion that is optimal for ET. This concept is more subtle than the hypothesis that the complexes are simply not intercalated, but it is supported by results obtained in studies of thymine dimer repair.⁹ In this work we found that the efficiency of dimer repair by the rhodium intercalator bound noncovalently to the duplex was greater (by a factor of ~50) than that observed for the photooxidant tethered to the duplex. We tested several hypothesis concerning the origin of these differences

(*vide supra*), however, we concluded that the diminution in efficiency of repair for the tethered complex was the result of the tether restricting the intercalation of the complex.

If we cite this explanation for the reduction in quenching between metallointercalators tethered to duplexes, can we estimate the quenching yield in these assemblies by taking the results with the reactants bound noncovalently as a starting point? The comparison of the two systems requires that we first reduce the quenching yield taken from the titrations by a factor of 50 for tethering the donor, and again for tethering the acceptor. If the quenching yield at 1 equiv of acceptor in titration experiments is 50%, tethering one reactant reduces the yield to just 1%, which is a negligible amount. Tethering the second reactant would reduce the quenching yield to nothing. This quantitative reasoning is inconsistent with the observation that one redox partner bound noncovalently gives significant quenching, however, it illustrates how tethering the reactant could abolish quenching.

What about comparisons to the work of Murphy *et al.*,⁶ which demonstrated that 100% of photoexcited ruthenium donors were quenched by rhodium acceptors over a distance of 40 Å. Our studies obviously do not reproduce this result. The major drawback with the work of Murphy is that there was really only one experiment. We discussed two systems in this Chapter, but we have examined probably over 50 assemblies and not yet reproduced the remarkable quenching efficiency described by Murphy.

We should cite the other differences between the covalently modified oligonucleotides described in this work and those of Jenkins³⁵ and Murphy.⁶ The most significant difference is the luminescence of single stranded $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ -DNA conjugates. Jenkins and Murphy consistently found that single stranded conjugates of $[\text{Ru}(\text{phen}')_2(\text{dppz})]^{2+}$ and DNA did not emit. Their observation is

remarkable given that the complex emits in the presence of single stranded oligonucleotides without being tethered. Furthermore, Jenkins was never able to observe thermal denaturation of DNA duplexes bearing $[\text{Ru}(\text{phen}')_2(\text{dppz})]^{2+}$ tethered to one terminus.³⁶ We find canonical melting profiles for all of the duplexes under investigation in these studies.

In summary, we demonstrated long-range ET through DNA in a comprehensive investigation of the oxidative repair of thymine-dimer lesions in Rh-modified DNA duplexes. This work provided one of the first studies of charge transfer through DNA as a function of distance and stacking.

What about comparisons to the work of Kelley *et al.*,¹⁵ which showed long-range ET between ethidium and rhodium tethered to short DNA duplexes? This work focused on shorter DNA duplexes, and the most quenching observed was ~30% in an 11-mer. Their results were entirely consistent with the experiments examining quenching between metallointercalators bound noncovalently to DNA. This work suggests that we may need to produce shorter duplexes in order to get quenching in the duplexes bearing $[\text{M}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ and $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$.

We demonstrated long-range quenching of photoexcited $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ by $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ tethered to the opposite ends of a 15 bp duplex. The yield of quenching (~15%) is significantly less than we expected based on the work of Murphy *et al.*⁶ In yet another family of duplexes bearing osmium and rhodium, we found no significant quenching between the tethered complexes, despite efficient quenching of each complex separately by a reactant bound noncovalently to the duplex. Taken together, these results illustrate that long-range charge transfer does occur in DNA, but for some systems we have yet to establish all of the parameters necessary to make the reaction work.

6.3. EXPERIMENTAL

Materials.

Metal complexes ($[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]\text{Cl}_2$, $[\text{Ru}(\text{phen})_2(\text{dppz})]\text{Cl}_2$, $[\text{Rh}(\text{phi})_2(\text{dmb})]\text{Cl}_2$, $[\text{Rh}(\text{phi})_2(\text{bpy}')]\text{Cl}_2$) were all prepared as described previously or in Chapter 2. Oligonucleotides were synthesized by automated DNA synthesis using the phosphoramidite approach on an ABI 384 synthesizer.

Thymine dimer-containing oligonucleotides are prepared by photodimerization of single-stranded DNA at 330 nm with acetophenone as a sensitizer. The chromatogram of the photoproducts and the product ratios are consistent with that reported earlier and support assignment of the major product as the *cis*, *syn* isomer.³⁷ The products were characterized by enzymatic digestion from the 3'-end by T4-DNA polymerase, demonstrating that cleavage did not proceed past the site of dimer incorporation, and by direct photocycloreversion of the dimer-containing strands with 254 nm light to regenerate the undamaged strand.

Rhodium-modified oligonucleotides were prepared by coupling the complex, $\text{Rh}(\text{phi})_2\text{bpy}^{3+}$ activated as the *N*-succinimidyl ester, to alkylamino functionalized oligonucleotides attached to controlled pore glass (CPG), as described in Chapter 5. Osmium-modified oligonucleotides were prepared by the analogous approach using $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ (Chapter 5). The 3'-alkylamino DNA was prepared by standard phosphoramidite synthesis starting from C7-amino-link 500 Å CPG (Glen Research) and selective deprotection of the R-N(H)-Fmoc by treatment with 20% piperidine in dimethylformamide DMF (v/v) at ambient temperature for 30 min. The 5'-alkylamino DNA was prepared from dT 2,000 Å CPG (Glen Research), followed by standard phosphoramidite synthesis and functionalization with a nonamethylene amino-alkyl linker.³⁸ Activation of racemic metal complex, coupling to DNA, and HPLC

purification of the diastereomeric Δ -Rh and Λ -Rh conjugates was identical for both 3'- and 5'- functionalized oligonucleotides.

Methods.

Determination of Metallointercalator-Thymine Dimer Separation. Assuming a 3.4 Å centroid-to-centroid distance between stacked base pairs, the separation between the intercalated phi ligand of the rhodium complex and the center of the thymine dimer is 19 Å for both the 5'-Rh and 3'-Rh tethered assemblies when the complex is intercalated between the third and fourth base pair from the end of the duplex. Based upon the photocleavage pattern (Figure 6.1, arrows), the closest distance between intercalator and thymine dimer along the helical axis appears to be 16 Å for both assemblies with intercalation in the fourth base step. We use the same approach to estimate distances between metallointercalators, assuming the complex intercalate in the third base step from the end of the duplex.

Irradiations for Thymine Dimer Repair. The light source was a Hanovia Hg/Xe arc lamp equipped with monochromator. The samples (40 µL) containing 8 µM DNA duplex, 8 µM *rac*-Rh(phi)₂DMB³⁺, 50 mM NaCl, 5 mM TRIS, pH 8.50 were irradiated at ambient temperature. Product analysis was accomplished by high-temperature reversed phase HPLC on a Microsorb™ MV C₁₈ analytical column (Rainin) maintained at 80 °C; (flow rate = 0.8 mL min⁻¹; we used a linear gradient of 20 mM ammonium acetate containing 2% to 3% CH₃CN over 10 min., to 4.5% CH₃CN over 30 min, and to 20% over 10 min., conditions under which each oligonucleotide elutes with a characteristic retention time. The Rh-modified strand eluted at T_R = 45 min. The identity of the individual compounds was confirmed by coinjection with authentic samples. Electrospray ionization mass spectrometric analysis of the repaired strand (isolated after reaction with noncovalently bound [Rh(phi)₂(dmb)]³⁺) also showed the expected mass.

Hybridization of Complementary Oligonucleotides to Form Duplexes.

Complementary DNA strands were dissolved in 5 mM Na₃PO₄, 50 mM NaCl, pH 7.0 in a 1:1 ratio at the desired concentration. The sample was heated to 90 °C for 5 min. and cooled to ambient temperature (20 °C) by a linear gradient over 180 min.

Luminescence Spectroscopy. The emission properties of Os-modified oligonucleotides were characterized by steady-state spectroscopy on an SLM 8000 spectrofluorimeter with excitation was at 480 nm or 530 nm. On the SLM we set the high voltage at 1000 V, the polarizers to 9 and the slits to 1.6 mm, and the integration time was 0.2 s. All emission data were collected with 8 µM duplex unless stated otherwise.

Measuring T_m Values by Absorption. The temperature at which 50% of DNA duplexes in solution are dissociated into single strands is defined as the T_m. We measured T_m values by following the absorption of the duplex 270 nm as function of temperature on a Beckman 7400 DU spectrophotometer equipped with temperature control and multisample accessories. All T_m samples were 4 µM duplex in 5 mM Na₃PO₄, 50 mM NaCl, pH 7.0, unless stated otherwise. The T_m was extracted from a plot of the first derivative of the absorbance vs. temperature curve.

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Chapter 7: Long-Range Triplet Energy Transfer Mediated by the DNA Base Stack

7.1. INTRODUCTION

This chapter describes the first investigation of DNA-mediated triplet energy transfer (TET) between metallointercalators. The DNA double helix serves as a remarkable medium for electron transfer (ET) reactions.^{1-6 7-9} In Chapters 3, 4 and 6, and in previous reports, we described photoinduced ET between donors and acceptors bound to DNA by intercalation.^{3-6,10} In these reactions, a photoexcited donor is quenched by a ground state acceptor by ET through the DNA base stack.

We also investigated ET reactions in which the DNA base stack not only mediates ET but serves as a reactant as well.^{7-9,11} Examples of these reactions include oxidative repair of thymine-dimer lesions in DNA (Chapter 6), and selective oxidative damage at 5'-GG-3' sites by long-range electron transfer. The reactions involving the DNA bases can be thought of as hole transfer (HT) reactions in which a photoexcited hole on the metallointercalator is migrates to the ground-state electron donor.

We recognized that if the DNA base stack facilitates both HT and ET, it should serve as a similarly effective medium for TET over long-range. TET has been carefully examined in a variety of model organic and inorganic systems.¹²⁻¹⁸ Zewail and coworkers found that TET could occur over modest separations in naphthalene crystals, but even the substitution of deuterium for hydrogen in the aromatic molecules was sufficient to eliminate the long-range interactions.¹⁹ In classic studies^{12,13,20} of TET as a function of distance in steroid derivatives, Closs and Miller demonstrated that the distance dependence of the rate of TET between donors and acceptors separated by fixed distances through molecular spacers behaved as a composite of two distance dependent charge transfers: namely, hole transfer and electron transfer. In fact, the mechanism of TET, described thoroughly by Dexter, is considered to be a double ET reaction, and has

been called electron-exchange energy transfer.²¹ TET is distinguished from singlet energy transfer, which involves the interactions of the dipole moments of the photoexcited donor and the ground-state acceptor. Singlet energy transfer does not involve any transfer of electrons from reactant to reactant.

We sought to develop a system that would permit us to investigate the effect of the DNA base stack on such a “double” ET reaction. Several studies of TET between organic donors and acceptors have been described, however, these systems are not amenable to an investigation of the DNA medium. The lowest excited state of polypyridyl complexes of divalent ruthenium and osmium are nominally triplet metal-to-ligand charge transfer (MLCT) states. In addition, TET between polypyridyl complexes of Ru(II) (donors) and Os(II) (acceptors) has also been described for a number of model systems. Therefore, we initiated a study of DNA-mediated TET between dppz complexes of Ru(II) and Os(II). We used the heterogeneous coupling chemistry described in Chapter 5 to prepare Os- and Ru-modified oligonucleotides for studies of TET over fixed distances through the DNA base stack.

As required for a spontaneous chemical reaction, the net free energy change, ΔG , for TET must be negative in order for a reaction to occur. In order to satisfy this requirement, there must be significant overlap between the emission of the donor and the absorbance of the acceptor.¹⁷ As illustrated in Figure 7.1, the overlap of the emission spectrum of photoexcited $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA with the absorption into the $^3\text{MLCT}$ excited state of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$, makes these $[\text{M}(\text{phen})_2(\text{dppz})]^{2+}$ (M = Ru, Os) derivatives well suited for TET reactions.

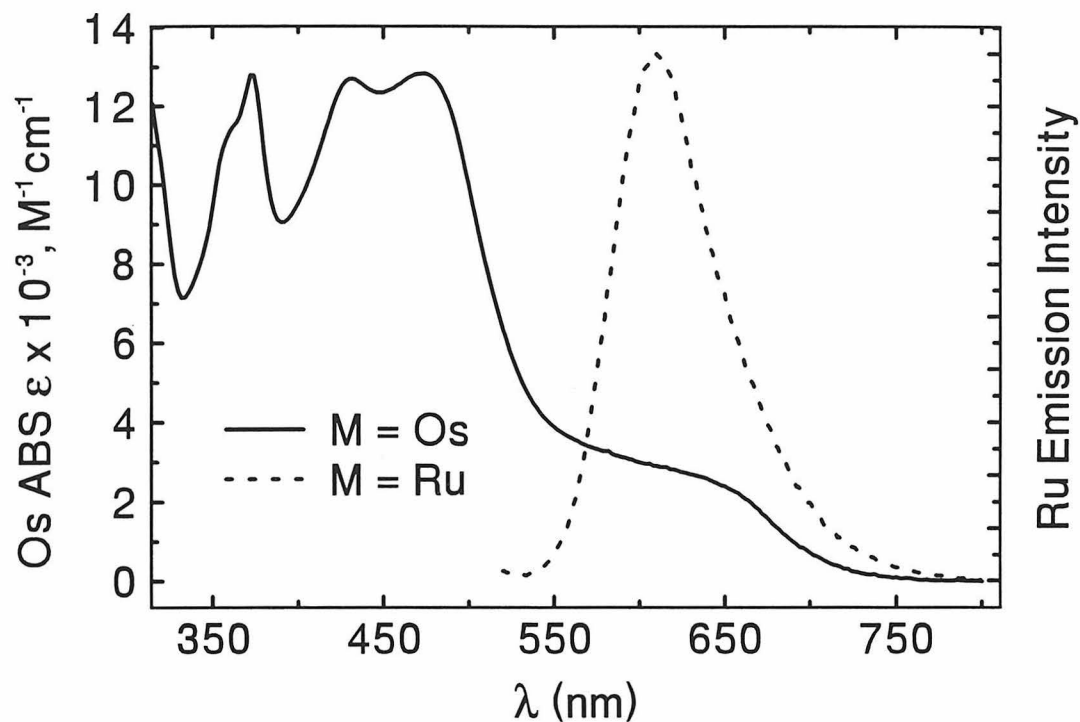


Figure 7.1. Visible absorption spectrum of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ (free in aqueous solution) compared with the emission spectrum of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA. The significant amount of spectral overlap in the wavelength range of 540-725 nm indicates that TET between this pair of donor and acceptor is thermodynamically favored. The ΔG for TET from photoexcited Ru(II) to Os(II) is ~ 0.4 eV.

7.2. RESULTS

7.2.1. Emission Intensity Quenching for $[M(\text{phen})_2(\text{dppz})]^{2+}$ Derivatives Bound Noncovalently to DNA and SDS Micelles

Quenching Titrations on DNA Polymers. Studies of DNA-mediated electron transfer were initiated by characterizing the reaction of photoexcited electron donors with ground state acceptors in titrations on long DNA polymers of varying sequence.^{2,3,5} These titrations are useful to establish the scope and limitations of a reaction on DNA without investing in the preparation of complex molecular assemblies.

We titrated $[\text{Os}(\text{L})_2(\text{Me}_2\text{-dppz})]^{2+}$ ($\text{L} = \text{phen}, 5,6\text{-dmp}, \text{or } 4,7\text{-dmp}$) into solutions of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to CT DNA, poly d(AT), or poly d(GC), as well as oligoduplexes 18 bp in length. Dipyridophenazine complexes bind avidly to these polymers, even at micromolar concentrations of metal (Chapter 2).^{22,23} In order to maximize the average donor-acceptor separation on the duplexes, the titrations with these commercially available polymers were initiated at low metal-to-DNA ratios (1 Ru/50 bp). In experiments with oligonucleotides, the titrations were against a 1:1 ratio of ruthenium to duplex.

Figure 7.2 shows the results of these experiments. In Figure 7.2 A, the emission of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ is significantly quenched as $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ is titrated into the system. The fraction of ruthenium emission that is quenched (F_q) reflects the fraction of excited states that react with the osmium complex. The F_q saturates at ~80% quenched with poly d(AT) and CT DNA, while the saturation point with poly d(GC) is ~60%. The plots of F_q as a function of osmium concentration for poly d(AT) and CT DNA are remarkably similar, whereas the profile with poly d(GC) indicates that the reaction is less efficient on this polymer. The fact that the amount of quenching is sequence dependent establishes immediately that this loss in emission intensity is not a trivial inner-filter effect.

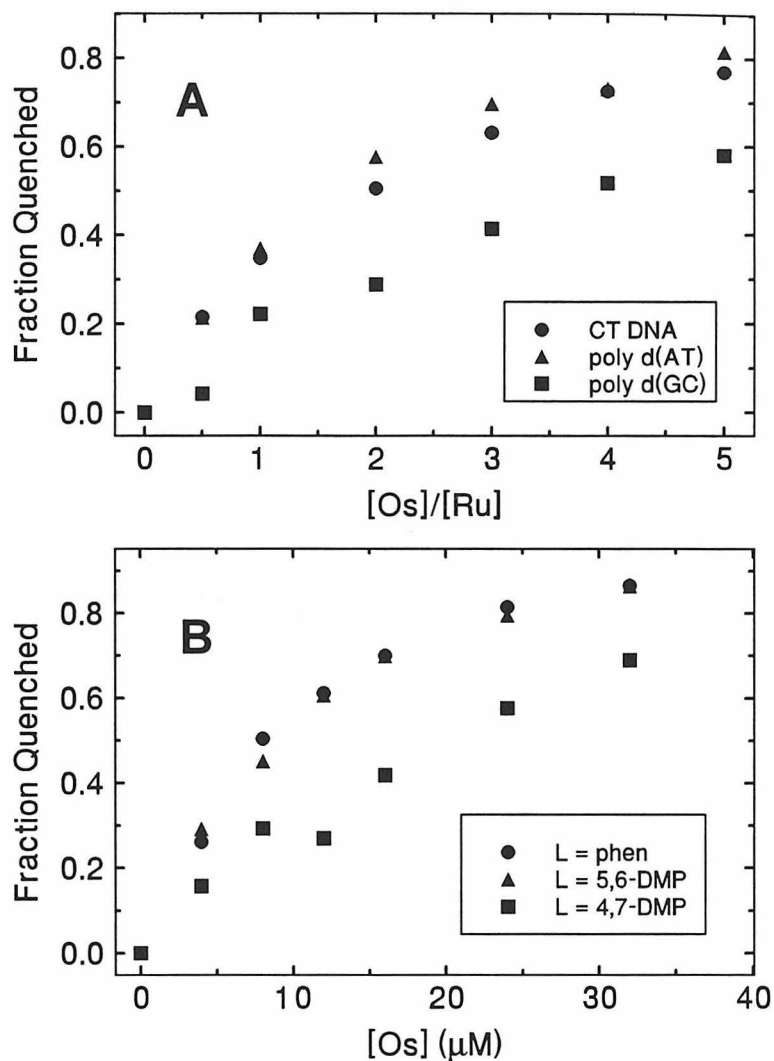


Figure 7.2. (A) Plot of the fraction of emission from $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ quenched by increasing concentrations of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ in the presence of CT DNA, poly d(AT), and poly d(GC). (B) Plot of the fraction of emission from $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ quenched by increasing concentrations of $[\text{Os}(\text{L})_2(\text{Me}_2\text{-dppz})]^{2+}$ (L = phen, 5,6-dmp, 4,7-dmp). The structure of the ancillary ligand affects the quenching efficiency. Conditions: (A) 10 μM Ru, 1 mM nucleotides DNA, 5 mM Tris, 50 mM NaCl, pH 8.5; (B) 10 μM Ru, 10 μM 18 bp duplex, 5 mM Na_3PO_4 , 50 mM NaCl, pH 7.0. Seq: (5'-ACGTGATGTTGTAGACGT-3')•(5'-ACGTCTACAACATCACGT-3').

These titrations as a function of sequence resemble the results described in Chapter 4 for photoinduced ET between metallointercalators bound to DNA.³ As described in Chapter 2, and previously,²³ $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ binds preferentially to AT sites in mixed sequence DNA, which is the reason that the reaction profiles on CT and poly d(AT) look so similar.

We also characterized the quenching reaction on oligoduplexes. Figure 7.2 B shows F_q as a function of osmium concentration in the presence of an 18 bp duplex. Three different complexes were evaluated as quenchers. In this study we examined the effect of different ancillary ligands on the quencher in an effort to determine the how changing the binding properties of one reactant would perturb the amount of reaction.

The reaction profiles of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ and $[\text{Os}(5,6\text{-dmp})_2(\text{Me}_2\text{-dppz})]^{2+}$ are essentially identical, whereas the quenching efficiency of $[\text{Os}(4,7\text{-dmp})_2(\text{Me}_2\text{-dppz})]^{2+}$ is lower on the is oligoduplex. The order of reactivity for this series of metallointercalators tracks with how well the complexes are stacked into the DNA duplex (Chapter 2). As described for a family of $[\text{Os}(\text{phen})_2(\text{dppz})]^{2+}$ derivatives, substituents on the 4 positions of ancillary phen ligands prevent effective intercalation, and hence the ability of the reactant to access the DNA base stack. This is an important result in that it illustrates how coupling into the π -stack favors quenching. If the reaction proceeded by diffusion along the duplex, complexes that were not intercalated as well would be more reactive.

Time Scale of Quenching on DNA. We sought to determine the rate of the quenching reaction between dppz complexes of Os(II) and Ru(II) bound to DNA by time-resolved luminescence spectroscopy on the nanosecond time scale. Figure 7.3 shows the results of a quenching titration monitored at 617 nm over 1.5 μs . As the concentration of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ increases, the initial intensity of the emission immediately following the laser flash decreases. This loss in initial intensity accounts for the major portion of the emission quenching indicated above. Owing to the fact that the reaction

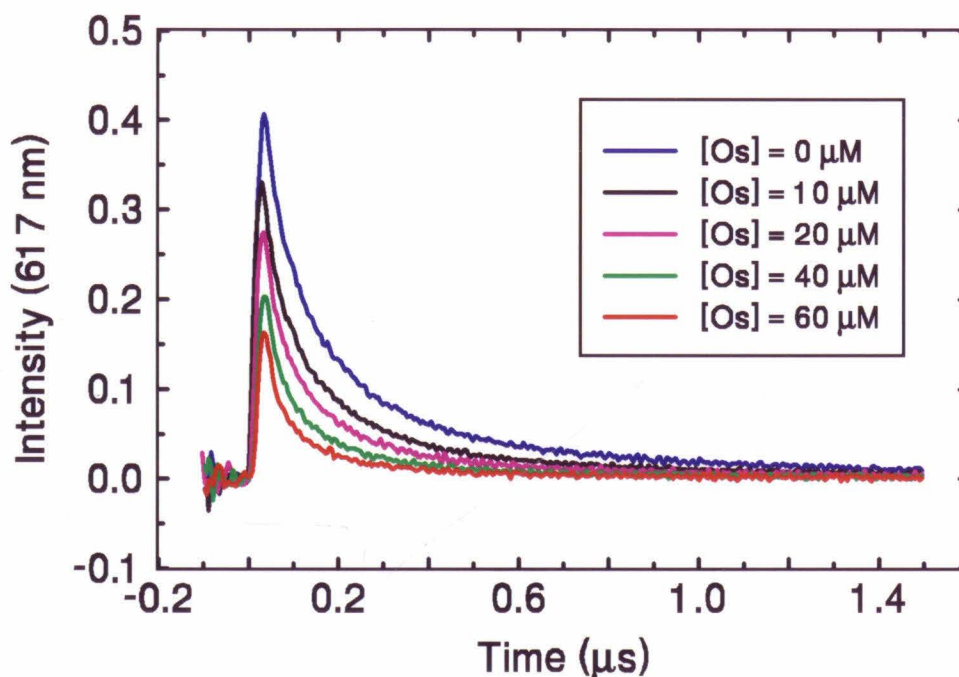


Figure 7.3. Time-resolved luminescence decay of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ (10 μM) bound to CT DNA (1m M nucleotides; 5 mM Tris, 50 mM NaCl, pH 8.5) in the presence of increasing concentrations of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$. The loss of initial emission intensity indicates that the rate of the quenching reaction between photoexcited ruthenium and the osmium complex is faster than the resolution of this instrument (~ 20 ns). Therefore this reaction proceeds on the subnanosecond time scale.

is manifested as a loss of intensity within the response time of the instrument, we can say that this reaction takes place on the subnanosecond time scale.^{2,3}

Comparative Quenching Studies on SDS Micelles and DNA. In order to establish the importance of the stacked DNA bases in facilitating the quenching of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ by $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$, we compared the reactivity on DNA and sodium dodecyl sulfate (SDS) micelles. In its most general form, the DNA duplex might be described as a hydrophobic matrix surrounded by a hydrophilic and negatively

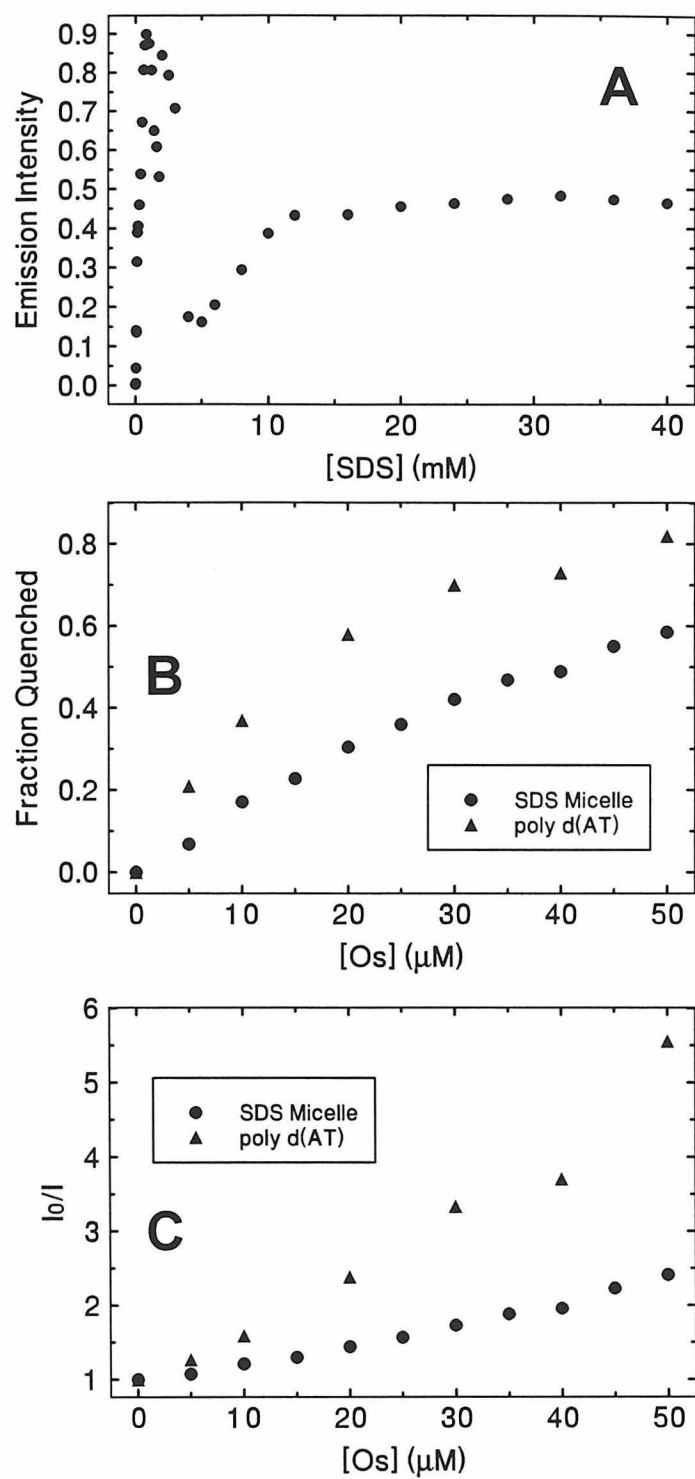
charged shell. The SDS micelle provides an analogous microheterogeneous environment to which metallointercalators bind avidly.^{4,24} The important difference between SDS micelles and DNA in this context is that the micelles do not carry the organized pathway for charge transfer that is present in the DNA base stack.⁴

SDS is a classical surfactant, a hydrophobic chain terminated in a hydrophilic head group (sulfate). In aqueous solution at concentrations below ~8 mM, SDS monomers are in equilibrium with a population at the air/water interface oriented with their hydrophobic tails out of solution, and solvated monomers. As the concentration of monomer increases, more polymers dissolve in solution and partition themselves at the surface. At a certain concentration of monomer, the formation of aggregates (micelles) becomes energetically favored for entropic considerations. This concentration is referred to as the critical micelle concentration (cmc). SDS forms micelles of an aggregation number of ~64.²⁴⁻²⁶ These aggregates are spherical and the SDS monomers are oriented with their hydrophobic chains directed into the interior of the sphere, and separated from water. The hydrophilic headgroups form the shell of the sphere, and are well-solvated by the aqueous medium.

Sauvage,²⁷ then Arkin *et al.*⁴ demonstrated that $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and derivatives bind to SDS micelles in aqueous solution by partial insertion of the dppz ligand into the interior of the aggregate. The association with the micelles affords protection of the phenazine nitrogens from interaction with water and restores the steady-state emission of these complexes.

The association of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ with SDS micelles is illustrated by the increase in emission with increasing concentrations of SDS in solution which levels off at the cmc (Figure 7.4 A). Since the concentration of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ is constant, the emission intensity does not increase beyond the point at which all the complexes are bound to the micelles.

Figure 7.4. (A) Emission intensity of photoexcited $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ (40 μM) as a function of increasing concentration of SDS. (B) Plot of the fraction of photoexcited $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ (10 μM) quenched in the presence of SDS micelles (78 μM) (●) or poly d(AT) (1 mM nucleotides) (▲) by increasing concentrations of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$. (C) Plot of the ratio of luminescence intensity (Ru) in the absence of quencher (I_0) to that in the presence of quencher (I) as a function of increasing concentrations of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$.



We titrated $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ into solutions of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to SDS micelles and followed the quenching of the ruthenium emission. Figures 7.4 B and C compare two representations of the data for the reaction of photoexcited $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ with $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ on poly d(AT) and SDS micelles. In 7.4 B, F_q is plotted as a function of increasing concentration of osmium and in 7.4 C, we plotted I_0/I .

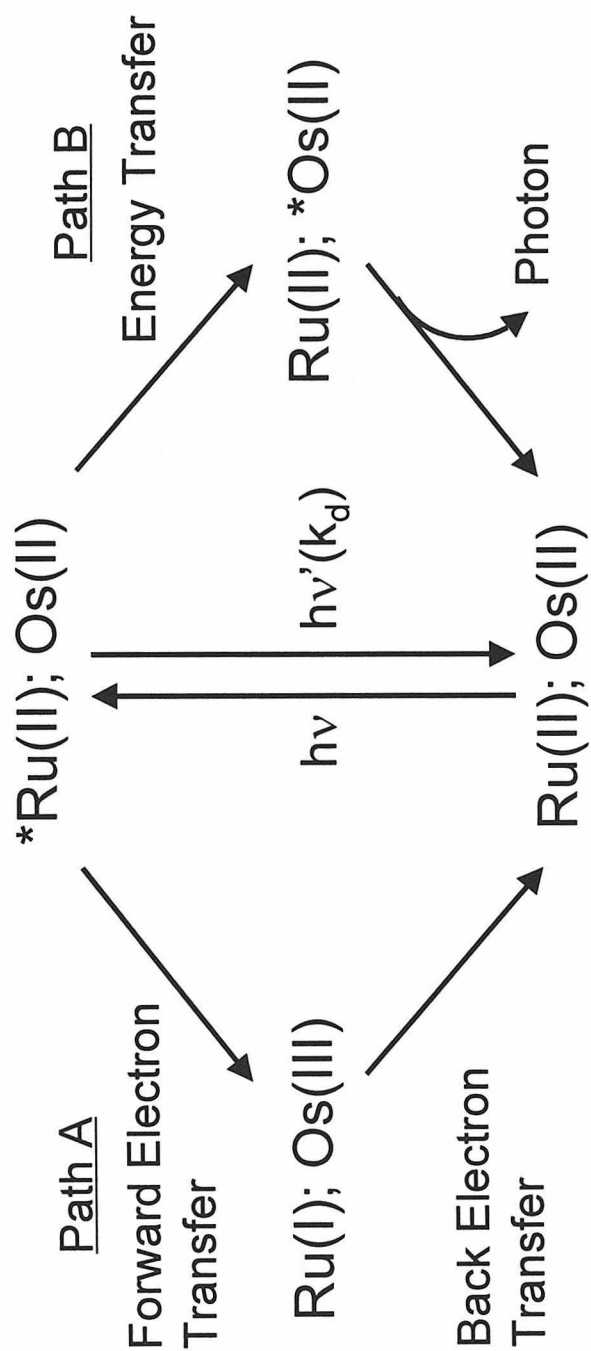
The efficiency of the reaction between the two complexes on DNA is significantly greater than that on the micelles. Furthermore the I_0/I plot of the quenching efficiency with poly d(AT) is upward curving, which is a classical sign of a reaction that is static, or occurs with a fast rate.²⁸ I_0/I for the data on the micelle is linear. Using the Stern-Volmer analysis to treat these data the second order rate constant for the quenching by $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ is on the order of 10^{10} , which is fast, but to be expected for facilitated diffusion on the surface of the micelles.⁴

7.2.2. Characterizing the Mechanism of the Quenching of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ by $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ and Derivatives Bound to DNA.

TET between polypyridyl complexes of Os(II) and Ru(II) is well known.^{14-16,18,29} Several model systems have been developed to characterize the energy transfer reaction over fixed, and large molecular distances.^{12-16,29} These studies have revealed two major pathways by which the reaction of photoexcited Ru(II) complexes react with ground state Os(II).

Scheme 7.1 outlines two thermodynamically favorable reaction cycles for the quenching of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ by $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ and derivatives. In path A, the excited state of the ruthenium complex is reduced by the ground state osmium, which eliminates the emission. In path B, TET is the operative mechanism of quenching. As illustrated in the scheme, the way to differentiate between processes lies in the characterization of their intermediates.

Scheme 7.1. Thermodynamically favored reactions between photoexcited Ru(II) and ground state Os(II) in $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ metallointercalators.



Path A, reductive quenching, gives products that constitute a charge-separated pair. Path B, TET, forms ground state Ru(II) and the excited state of the Os(II) complex. In path A, the intermediates decay to the ground state in a thermal electron transfer, while the product of TET returns to the ground state by emitting a photon. Thus, if path A is relevant, we should be able to observe the charge separated intermediates; if path B is relevant, we should be able to observe emission from the osmium excited state.

We used transient absorption on the microsecond time scale to search for Os(III) or Ru(I) that might be formed as the result of reductive quenching. In these studies we did not find any signal that we could attribute to a product of electron transfer. It is important to note that this negative result is not definitive, however, for all ET reactions between metallointercalators where Os(III) is a product, the trivalent intermediate has been detected. Therefore, although we cannot rule reductive quenching out without further studies on shorter time scales, this mechanism is not favored.

In order to obtain evidence for TET, we monitored the emission from $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ bound to DNA in the presence and absence of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$. Similarities in the Os(II) and Ru(II) absorption profiles precluded the selective excitation of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ over $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$. Therefore, to account for the background emission from the osmium complex, we compared the emission data with and without the donor.

Owing to the large difference in the inherent quantum yield for emission between osmium and ruthenium complexes, we were forced to look at the emission of the osmium complex with only low concentrations of ruthenium present. In this experiment the concentration of ruthenium was 2 μM , and ~25% of the emission was quenched. The concentration of osmium was 8 μM . Therefore, if 100% of the quenching goes by TET, 25% of 2 μM photoexcited Ru(II) should be converted to photoexcited Os(II). In other words, the emission from the osmium should increase by 0.5 μM , or 5%.

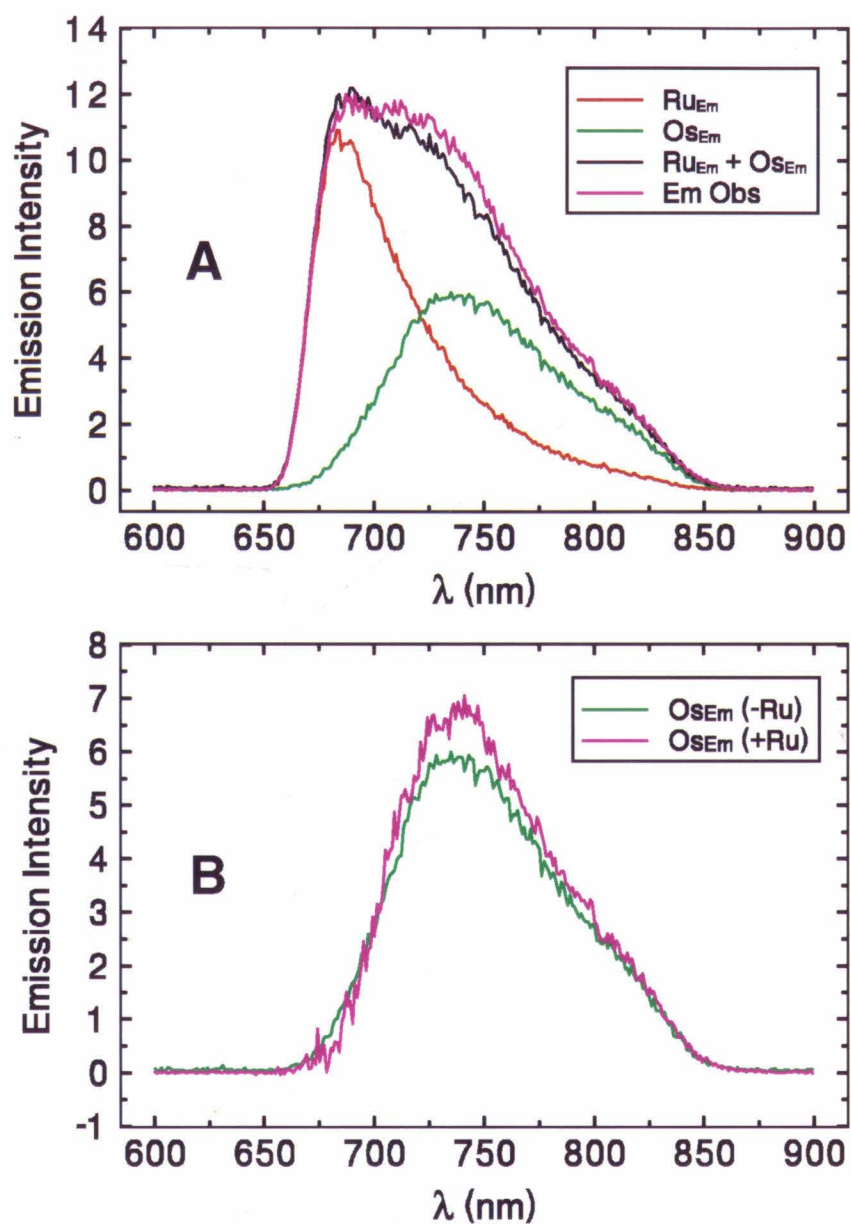


Figure 7.5. Spectroscopic evidence for TET. **(A)** Overlay of the emission intensity of (i) Δ -4-Os.181 (green); (ii) $[Ru(phen)_2(dppz)]^{2+}$ bound to unmodified 181 (red); (iii) sum of (i) and (ii) (black); (iv) observed emission which shows clearly the enhancement as a result of TET (magenta). **(B)** Comparison of the background emission of the osmium complex (green) and the enhanced emission derived from **(A)** (magenta).

Figure 7.5 shows the raw and corrected data that establish the mechanism of the quenching reaction under investigation. If we integrate the derived osmium emission spectrum for the quenching reaction, we find that the increase in emission intensity is $\sim 5\%$ as expected. Therefore, we have positive evidence for TET as one operative mechanism of the quenching reaction between $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ and $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ bound to DNA.

7.2.3. Preparation and Characterization of Os and Ru-Modified Oligonucleotides.

Synthesis. The quenching of emission from photoexcited $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to DNA by $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ at low loadings of metal to DNA is remarkably efficient. We demonstrated that reaction takes place on the subnanosecond time scale. These results strongly suggest that TET proceeds over long range through the DNA base stack. However, since the titrations described above allow only for the average donor-acceptor separation to be known, a system that permits the distance between the reactants to be fixed and well-defined.

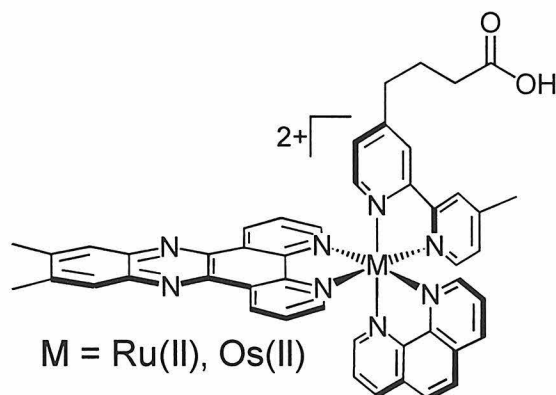


Figure 7.6. Schematic illustration of $\Delta\text{-}[\text{M}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ used in the synthesis of metallointercalator-DNA conjugates for studies of long-range TET. Only one of the four isomers of this complex is depicted.

We used the heterogeneous coupling method presented in Chapter 5 to install dppz complexes of Os(II) and Ru(II) onto the 5' terminus of complementary oligonucleotides (16-mers). The two isostructural complexes $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ were employed as donor ($M = \text{Ru}$) and acceptor ($M = \text{Os}$; Figure 7.6).

Figure 7.7 shows a typical chromatogram for the purification and isolation of Ru-modified DNA. As expected for a metal complex composed of these ligands, four products were formed in equal amounts. As described in Chapter 5, the incorporation of the osmium analog of this complex gives a similar distribution of isomers. Given the diversity afforded by the different isomers, we are able to examine sixteen different hybrid duplexes containing a ruthenium complex one end and an osmium complex on the other.

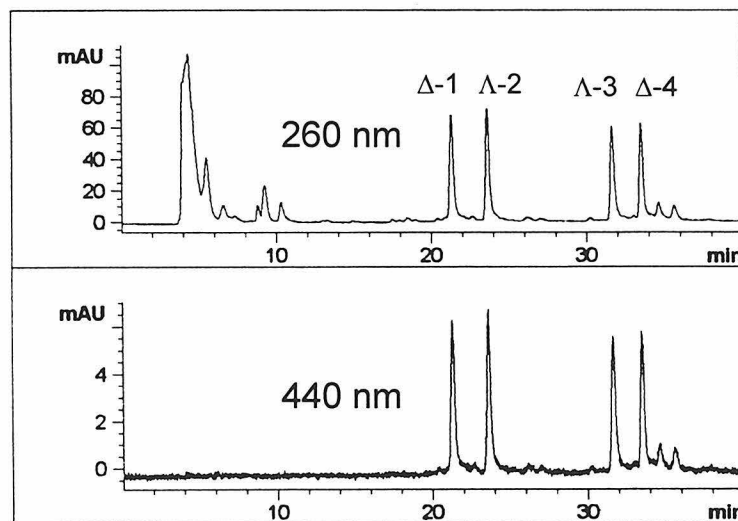


Figure 7.7. HPLC chromatogram of an unpurified Ru-modified oligonucleotide reaction mixture. Detection was at 260 nm and 440 nm as indicated. Each Ru-DNA peak (identifiable by their 440 nm absorption) contains one of four different isomers of the complex $[\text{Ru}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$. Conditions: C18 stationary phase; 50 mM ammonium acetate/acetonitrile mobile phase; 10-27.5% acetonitrile over 30 min.

Probing the Thermal Stability of Singly and Doubly Modified Duplexes

Containing $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$. We carried out a comprehensive investigation of the thermal stability of singly and doubly modified 16 bp duplexes containing $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to the 5' termini. We followed the dissociation of the duplexes by monitoring the absorption at 270 nm as a function of temperature.

The melting temperature (T_m) for 25 duplexes and the change in T_m relative to the native duplex (ΔT_m) are all documented in Table 7.1. The data in the table are presented in the form of a matrix. The Ru-modified strands are along one axis and the Os-modified strands are along the other. The entries in each slot represent the pairing of an oligonucleotide from the row in which the slot occurs with the oligonucleotide from the column in which the strand resides. This system of notation simplifies the reporting of the data.

The presence of the metallointercalator stabilizes the duplex toward melting. As described in Chapter 5 for Os-modified duplexes, the value of ΔT_m depends upon the isomer of the metal complex attached to the duplex. Furthermore, as with oligoduplexes that contain $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ tethered to one terminus and $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to the other, the value of ΔT_m is greatest. For this 16 bp duplex ΔT_m ranges from -1.4 to 16.6 °C. For the four isomers of Ru (or Os) modified DNA, the first isomer to elute from the HPLC (Δ -1-Ru(Os)) affords the greatest T_m stabilization, followed by the fourth isomer (Δ -4-Ru(Os)). For the Λ isomers, the trend is the same, namely, Λ -2-Ru(Os) stabilizes the duplex more than Λ -3-Ru(Os).

Furthermore, this same effect is observed with doubly modified assemblies as well. For example, the highest ΔT_m is seen for the duplex that contains two Δ -1 isomers (Δ -1-Ru-162-Os-1- Δ).

Table 7.1. T_m and ΔT_m^a values for Singly and Doubly-modified Duplexes.^b

Os \ M ₂	T _m value for M ₂ =					
	Δ -1-Ru	Λ -2-Ru	Λ -3-Ru	Δ -4-Ru	Δ -Rh	162.C
Δ -1-Os	74.8	67.8	65.8	68.8	61.6	61.8
Λ -2-Os	70.8	66.8	64.8	63.8	ND	60.8
Λ -3-Os	67.8	61.8	61.8	62.8	ND	56.8
Δ -4-Os	71.8	65.8	63.8	63.8	60.6	58.8
162	67.8	63.7	61.8	62.8	58.6	58.2
Os \ M ₂	ΔT_m value for M ₂ =					
	Δ -1-Ru	Λ -2-Ru	Λ -3-Ru	Δ -4-Ru	Δ -Rh	162.C
Δ -1-Os	16.6	9.6	7.6	10.6	3.4	2.8
Λ -2-Os	12.6	8.6	6.6	5.6	----	2.6
Λ -3-Os	9.6	3.6	3.6	4.6	----	-1.4
Δ -4-Os	13.6	7.6	5.6	5.6	2.4	0.6
162	9.6	5.5	3.6	4.6	0.4	0.0

Shaded areas highlight T_m and ΔT_m values for singly modified duplexes



- a. $\Delta T_m = (T_m \text{ conjugate} - T_m \text{ unmodified duplex})$.
- b. The data are displayed in this matrix for ease of presentation. The contents of an entry are as follows: the metal complex in the column on the left represents one isomer of the 16-mer conjugate which is hybridized to the its complementary strand indicated by the metal complex in the top row. The sequence codes 162 and 162.C refer to the unmodified 16-mer and its complement, respectively. Therefore, a conjugate paired with 162 or 162.C contains only one metallointercalator (singly modified), and 162 paired with 162.C is the native duplex. T_m values above represent averages of at least two trials. The standard deviations are $\sim 1^\circ\text{C}$.

It is noteworthy that the Os-modified duplexes have different T_m values when compared to Ru-modified duplexes of the same isomer. This difference is surprising given the symmetry of the duplexes and binding sites. The same observation is made if the metal complexes are switched. Therefore, there must be an asymmetry present in the 16 bp duplex that cannot be accounted for on the basis of primary sequence alone.

Using Absorbance as a Function of Temperature to Assess Intercalation by Tethered $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$. The significant amount of hypochromism in the $\pi\text{-}\pi^*$ transition centered on the intercalating ligand, $\text{Me}_2\text{-dppz}$, of $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ provides an excellent spectroscopic handle to monitor the dissociation of the metallointercalator from the intercalation site as the duplex is melted.

Figure 7.8 shows the absorbance at 384 nm as a function of temperature for a duplex modified with ruthenium and osmium (A), modified with only ruthenium (B) and with only osmium (C). By comparing the magnitude of the change in absorbance (ΔA) at 384 nm for each assembly, we can see that the total ΔA for the doubly modified duplex is the sum of ΔA for each singly modified conjugate. This observation indicates that each metallointercalator acts independently from the other.

We also compared the T_m determined by monitoring the absorbance at 384 nm and that for the experiment at 270 nm. For the samples in Figure 7.8, the T_m values determined at 270 nm are 74.8 ($\Delta\text{-1-Os-162-}\Delta\text{-1-Ru}$), 61.8 ($\Delta\text{-1-Os-162}$), and 67.8 ($162\text{-}\Delta\text{-1-Ru}$). The values taken from the experiment at 384 nm are 75.7, 65.7, and 69.8 for the same set of samples, respectively.

As described in Chapter 5 for Os-modified duplexes the apparent increase in T_m taken from the data at 384 nm is consistent with this portion of the duplex melting last. For the samples above there is a significant difference in the T_m values at 384 nm from those at 270 nm for only the singly modified duplexes. The doubly modified duplex gives a T_m value which is not too different for either observation wavelength.

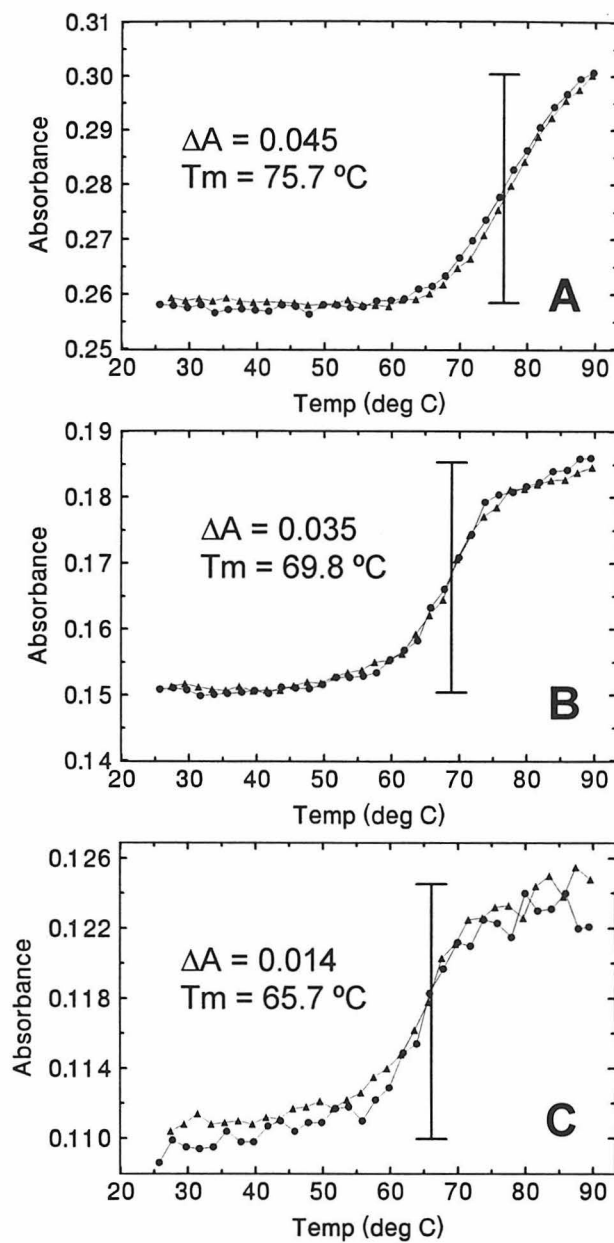


Figure 7.8. Absorbance at 384 nm as a function of temperature for (A) Δ -1-Os-161- Δ -1-Ru, (B) 162- Δ -1-Ru, (C) Δ -1Os-162. Conditions: 8 μM duplex in 5 mM Na_3PO_4 , 50 mM NaCl, pH 7.0. DNA sequence:

$\text{Os-5' - ACGTGAGTTGAGACGT - 3'}$
 $3' - \text{TGCACTCAACTCTGCA - 5' - Ru}$

Two reasons could account for this observation. One is that the doubly modified duplex in this experiment is so significantly stabilized that the small difference in the two experiments is not detectable. Alternatively, this observation might lie in the fact that the singly metallated duplexes melt at the end opposite from the metallointercalator, whereas doubly modified duplexes melt from the center of the duplex, which equalizes the ends of the duplex. One point that is certain is that the melting curves for the doubly modified duplexes do not appear to be biphasic.

7.2.4. Long-Range Triplet Energy Transfer in Metallointercalator-DNA Conjugates

Luminescence Quenching in Heterometallated Duplexes Containing Os(II) and Ru(II). In order to establish TET over long-range mediated by the DNA base stack, we hybridized Os-modified oligonucleotides to their Ru-modified complements. We compared the luminescence intensity of the *bis*-heterometallated duplexes to the luminescence intensity of the duplex containing only ruthenium to determine the yield of energy transfer. Owing to the diversity in the samples available as a result of the different isomers of the metallointercalator-DNA conjugates, we characterized quenching as a function of the absolute configuration about the metal center, and the orientation of the linker with respect to the metal center.

Table 7.2 lists the luminescence intensity and the fraction quenched ($F_q = 1 - I_{Ru}/I_{Ru+Os}$). Again, we present the data in the form of a matrix that compares the data for 25 different samples. The luminescence intensities of the singly modified duplexes are shaded in this table. As described for the Os-modified oligonucleotides in Chapter 5, the emission intensities of the Ru-modified duplexes depend very strongly on whether the metal complex is a Δ or Λ enantiomer and which isomer (equatorial or axial) is tethered to the duplex. We proposed that the earlier moving fractions in the HPLC purification of the Os-modified oligonucleotides were the axial isomers, however, until such a proposal

Table 7.2. Ruthenium and Osmium Luminescence Intensity^a and Fraction Quenched^b for the family of [M(phen)(bpy')(Me₂-dppz)]²⁺-Modified 16 bp Duplexes (sequence below).

Os \ Ru	Luminescence Intensity				
	Δ -1-Ru	Δ -2-Ru	Δ -3-Ru	Δ -4-Ru	162.C
Δ -1-Os	0.61	0.48	0.54	0.89	0.79
Δ -2-Os	0.61	0.48	0.59	0.91	0.76
Δ -3-Os	0.59	0.46	0.57	0.88	0.97
Δ -4-Os	0.57	0.45	0.57	0.83	1.43
162	0.76	0.54	0.67	1.21	-----
Os \ Ru	Fraction Quenched by Os(II)				
	Δ -1-Ru	Δ -2-Ru	Δ -3-Ru	Δ -4-Ru	162.C
Δ -1-Os	0.19	0.11	0.19	0.26	-----
Δ -2-Os	0.19	0.11	0.11	0.24	-----
Δ -3-Os	0.21	0.16	0.15	0.28	-----
Δ -4-Os	0.25	0.17	0.15	0.31	-----
162	-----	-----	-----	-----	-----



- a. The luminescence intensities are relative to [Ru(NH₃)₆]³⁺ (10 μ M) in water. The osmium data were collected at different instrument settings than the data for ruthenium, thus direct comparison of the values does not reveal relative quantum yields for ruthenium compared to osmium. The samples were 8 μ M duplex in 5 mM Na₃PO₄, 50 mM NaCl, pH 7.0.
- b. Fraction quenched (Fq) = $(1 - I_{\text{Ru}}/I_{\text{Ru+Os}})$.

is verified, we refer to each isomer by the order in which it eluted from the HPLC.

The Δ -1 and Λ -2 isomers of Ru-162.C (162.C indicates that the sequence is the complementary sequence to 162) give lower emission intensity, with the Δ isomer being the brighter of the two. The Λ -3 and Δ -4 isomers of this conjugate luminesce more intensely than the first two, and again, the Δ isomer is brighter. The same relationship between isomer and emission intensity is true for the Os-modified strand as well.

The fraction of ruthenium emission for a given isomer that is quenched by a given isomer of the osmium complex when the two complementary conjugates are hybridized together is also presented in Table 7.2. Remarkably, we find significant quenching of the ruthenium emission by TET energy the osmium acceptor over a separation of at least 27 Å.

The distance over which the reaction occurs depends upon where the complexes intercalate. Arkin *et al.* used damage to DNA by singlet oxygen sensitized by photoexcited $[\text{Ru}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to DNA duplexes to identify the site of intercalation by the tethered complex.¹¹ These experiments established that the ruthenium complex binds 3 bp in from the terminus of the duplex to which it is attached. Therefore, on the basis of symmetry in the duplex and the fact that the metal complexes are isostructural, the osmium complex must also bind 3 bp in from the end of the duplex. Hence, in a 16 bp duplex, if the complexes are each intercalated 3 bp in from each end, the separation is 11 bp ($11 \text{ bp} \times 3.4 \text{ Å} = 37.4 \text{ Å}$). The 27 Å value comes from placing the complexes each 4 bp in from the terminus as a conservative estimate.

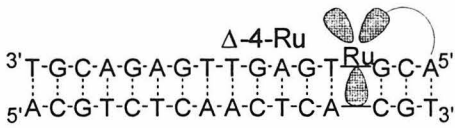
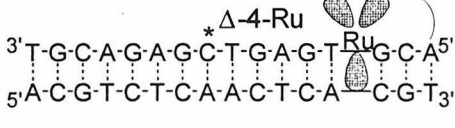
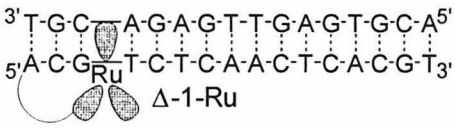
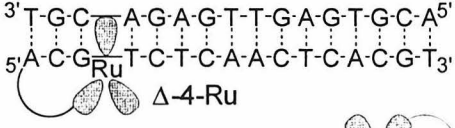
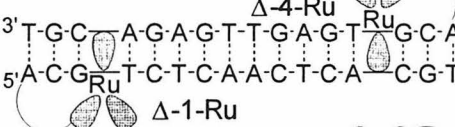
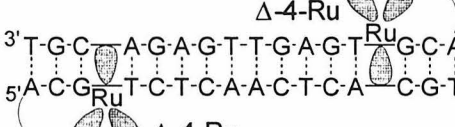
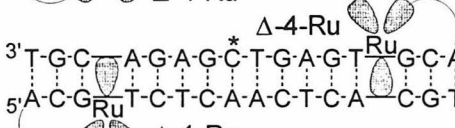
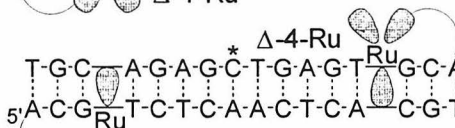
As expected for a reaction that depends upon access to the DNA base stack, the yield of TET depends very strongly on which of the 16 possible combinations of isomers is reacting. Across the entire population of samples, the Δ isomers always produce the greatest quenching compared to the Λ isomers. Furthermore, the Δ -4 isomers, which have the greatest emission yield also exhibit the most quenching. It is noteworthy that quenching efficiency tracks with emission intensity and not ΔT_m .

The quenching yields for the assemblies that contain the Δ -4-Ru isomer are greatest overall. Even the Λ -2 and Λ -3 isomers of the osmium complex paired with the Δ -4-Ru isomer produce 24% and 28% quenching, respectively. When the Λ -2-Ru and Λ -3-Ru isomers are paired with the Δ -4-Os strand, the quenching is not as great. It is difficult to determine whether the reduction in efficiency is on the osmium side or the ruthenium side.

Luminescence Properties in Homometallated Duplexes Containing Two Ru(II) Complexes. One very trivial explanation for the observed quenching is that the association of one intercalator on the DNA duplex is sensed by the other, which results in displacement of one complex. Since these metallointercalators do not luminescence when they are free in aqueous solution, such a displacement would look like quenching. We felt this argument was not important owing to the fact that luminescence titrations dppz complexes of M(II) reveal that DNA can accommodate almost one complex every two base pairs.^{22,23,30,31} Nevertheless, we recognized that such a consideration could be tested experimentally as well.

We synthesized complementary oligonucleotide conjugates bearing $[\text{Ru}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$. We prepared duplexes that contained only one ruthenium and duplexes that contained two rutheniums. If the metallointercalators associate with the DNA duplex independently of one another, the emission yield of a doubly modified duplexes bearing two ruthenium complexes should be the sum of the two singly modified duplexes (*i.e.* $\sim$ twice that of the singly modified duplexes). If a displacement mechanism like the one described above were causing the quenching, we would have expected the emission of the doubly modified duplex containing two ruthenium complexes to be less than the sum of the intensities of the two singly modified assemblies.

Table 7.3. Emission Intensity of Singly and Doubly Modified 16-bp duplexes
Containing only [Ru(phen)(bpy')(Me₂-dppz)]²⁺.

	Sequence Code	I(obs)	I(calc)
	162-Δ-4-Ru	1.19	----
	162(CA)-Δ-4-Ru	1.18	----
	Δ-1-Ru-162.C	0.76	----
	Δ-4-Ru-162.C	1.21	----
	Δ-1-Ru-162-Δ-4-Ru	1.83	1.95
	Δ-4-Ru-162-Δ-4-Ru	2.23	2.40
	Δ-1-Ru-162(CA)-Δ-4-Ru	1.87	1.94
	Δ-4-Ru-162(CA)-Δ-4-Ru	2.24	2.39

The results of these experiments are listed in Table 7.3. As illustrated for two sequences, one of which contains a single base mismatch (CA), the metallointercalators are independent of each other on the assembly.

Time-Resolved Luminescence Spectroscopy of Metallointercalator-Modified DNA. We measured the luminescence lifetimes of selected samples from the matrices in Table 7.2 to establish the time scale of the long-range TET, and to gain further insight into the origin of the sensitivity of quenching to isomer. The results of these studies are listed in Table 7.4.

Table 7.4. Excited state lifetimes of $[\text{Ru}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to DNA duplexes in the absence and presence of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ tethered to the opposite end.

Duplex ^a	τ_1 ns (%)	τ_2 ns (%)	τ_3 ns (%)
ds_162_ Δ -1-Ru	650 (17)	251 (73)	20.0 (10)
ds_162_ Δ -2-Ru	609 (11)	190 (89)	-----
ds_162_ Δ -3-Ru	533 (44)	153 (48)	25.7 (08)
ds_162_ Δ -4-Ru	736 (58)	196 (35)	14.7 (07)
Δ -1-Os_162_ Δ -4-Ru	691 (16)	215 (75)	14.3 (09)
Δ -4-Os_162_ Δ -4-Ru	710 (43)	207 (45)	17.7 (12)

^a162-ds: 5' - (Os) - ACGTGAGTTGAGACGT - 3'
3' - (Os) - TGCACTCAACTCTGCA - (Ru) - 5'

For each sample the luminescence decay is fit best by a triexponential function, however, the third component, with a lifetime of only $\sim 20 \pm 5$ ns, accounts for less than 10% of the duplexes in most assemblies. This short lifetime likely reflects a small percentage of metallointercalators that are not intercalated but associated with the tether or duplex in such a way that a very minimal amount of isolation from solvent is provided.

The other two lifetimes are similar for each duplex, but the distributions are different. The later moving isomers (Λ -3 and Δ -4) contain a much larger fraction of the longer lifetime. Owing to the sensitivity of photoexcited dppz complexes of Ru(II) to quenching by water, the longer lifetime indicates that the phenazine nitrogens of the dppz ligand are buried more deeply in the base stack. When the fraction of longer lifetime increases in the emission decay profile, we conclude that the binding mode that is most deeply intercalated is favored.

The luminescence lifetimes of the ruthenium complex in samples without an osmium complex are not different from those with an osmium complex, despite significant quenching of the emission intensity. As described above for the reaction between complexes bound noncovalently to DNA, the absence of any change in excited state lifetime points to a reaction rate that is remarkably fast. These experiments are sufficient to say that the reaction proceeds at least on a subnanosecond time scale.

7.2.5. Sensitivity of DNA-Mediated Triplet Energy Transfer to Stacking

Effect of Melting the DNA Duplex on the Yield of TET. In order to establish and probe the role of the DNA base stack in mediating TET between metallointercalators, we sought to disrupt the pathway and measure the effect of disruption on the yield of TET. One way to disrupt the DNA base stack is to dissociate the duplex thermally.³²

Figure 7.9 shows the overlay of two plots. The circles represent the emission intensity of the ruthenium complex in the singly modified duplex as a function of temperature, and the triangles represent the fraction quenched, also as a function of temperature. Over the temperature range of 10 °C to 50 °C, the emission intensity remains relatively constant. As the temperature increases above 50 °C, the emission yield begins to decrease. This diminution in intensity is consistent with the duplex beginning to melt, which causes ruthenium to be exposed to solvent.

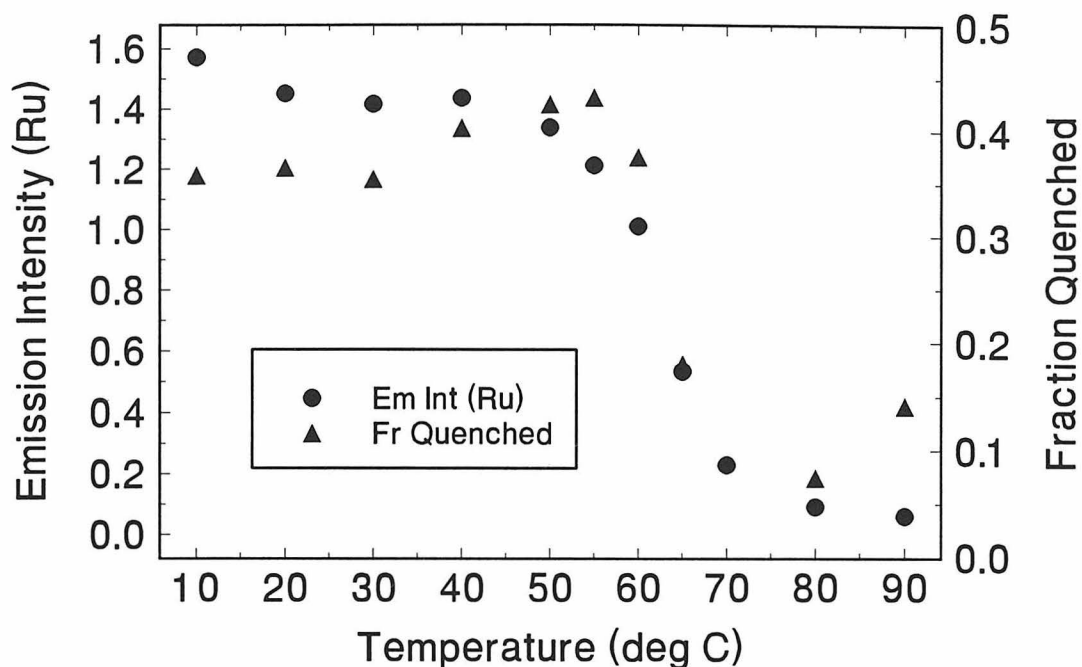


Figure 7.9. Plot of emission intensity (●) and fraction of ruthenium emission quenched (▲) as a function of temperature for 162-Δ-4-Ru (singly modified duplex) and Δ-4-162-Δ-4-Ru (doubly modified duplex), respectively.

The plot of fraction quenched exhibits the same temperature profile as the ruthenium emission. The amount of quenching changes very little until the temperature exceeds ~50 °C, when the fraction quenched drops precipitously. The amount of quenching actually increases slightly before it drops at 50 °C. Such a decrease in the yield of TET with temperature is consistent with a reaction that depends upon having an intact base stack to facilitate the reaction.

It is noteworthy that the temperature at which 50% of the quenching is lost, is less than the melting temperature measured by absorbance at either 270 nm or 384 nm. As we indicated above, the doubly modified duplexes most likely melt in the center first.

Therefore the quenching would diminish at the first loss in stacking. Hence, the “melting temperature” of the quenching reaction is lower than that for complete dissociation of the duplexes.

Perturbations in the Stacking of the Intervening Medium. We employed two methods to introduce perturbations in the stacking of the intervening base stack that are not as drastic as melting duplex, but represent more biologically relevant disruptions. We first examined the effect of single base mismatches in the center of the base stack. We looked at the CA and GA mispairs. In studies of electron transfer between photoexcited ethidium and $[\text{Rh}(\text{phi})_2(\text{bpy}')]^{3+}$ tethered to opposite ends of DNA duplexes, the presence of a CA mismatch blocked the electron transfer so the donor was not quenched at all, whereas the GA did not disrupt quenching.³²

Table 7.5 lists the fraction quenched for the duplexes bearing the mismatches and that for the fully base paired assembly. We carried out the experiment on the CA mismatch twice and got two very different results. We report them both here because we are not certain whether they reflect the true scatter in the data, which would mean that the CA mismatch has a significant effect on the reaction, or that one is wrong. Obviously, a third trial is called for. The amount of reaction with the GA mismatch is essentially identical to that for the fully base paired assembly.

Regardless of which interpretation of the data for the CA mismatch is correct, neither mispair disrupts quenching as seen for ethidium quenched by rhodium.³² It is possible that the sequence context of the mismatch precludes a significant perturbation to stacking. Alternatively, the TET reaction may not be as sensitive to a single base mismatch as photoinduced ET.

We also measured the effect of incorporating a bulged region in the intervening base stack. If a mismatch is not sufficiently disruptive to perturb TET, perhaps a bulge in the base stack, which should interrupt stacking more than a mispair,

Table 7.5. Effect of Mismatches on the Yield Long-Range TET in Metallointercalator-DNA Conjugates.

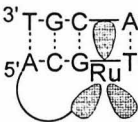
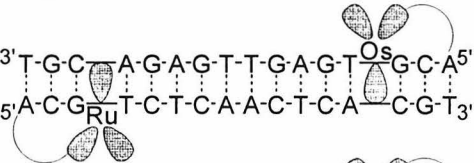
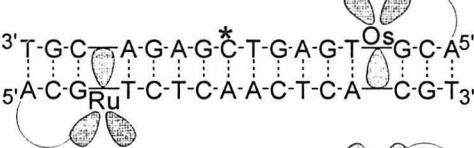
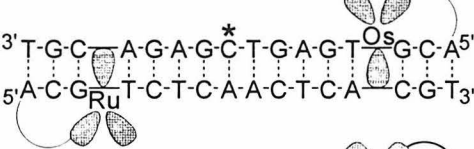
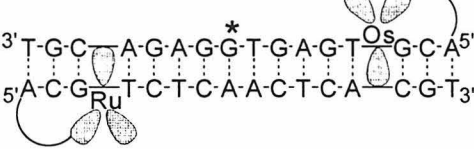
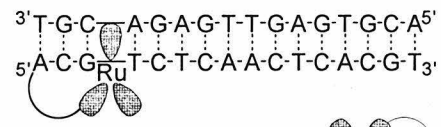
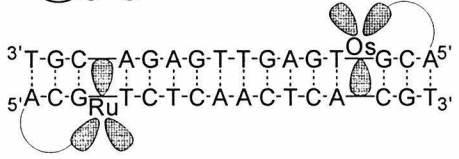
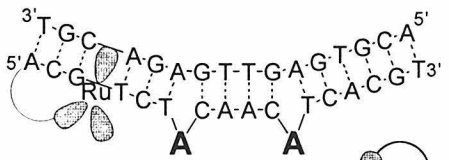
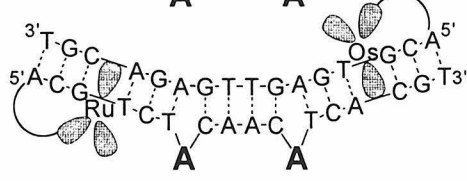
Assembly	Sequence Code	I(obs)	Fq
	Δ-4-Ru-162-ds	1.21	-----
	Δ-4-Ru-162-Δ-4-Os	0.83	0.31
	Δ-4-Ru-162(CA)-Δ-4-Os Trial I	0.50	0.62
	Δ-4-Ru-162(CA)-Δ-4-Os Trial II	0.85	0.29
	Δ-4-Ru-162(GA)-Δ-4-Os	0.86	0.28

Table 7.6. Effect of Bulges on the Yield Long-Range TET in Metallointercalator-DNA Conjugates.

Assembly	Sequence Code	I(obs)	Fq
	Δ-1-Ru-162-ds	0.82	----
	Δ-1-Ru-162-Δ-4-Os	0.64	0.23
	Δ-1-Ru-18-16	0.83	----
	Δ-1-Ru-18-16-Δ-4-Os	0.69	0.17

will affect the yield of TET.³³ Table 7.6 lists the emission yields and amount of TET for samples with and without bulged regions.

As expected, the presence of a bulge reduces the yield of TET. This result combined with the effect of thermal denaturation of the assemblies on the yield of TET support the assignment of the base stack as the medium that facilitates this long-range reaction.

Perturbations in the Stacking of the Reactants. The data in Table 7.2 illustrate how sensitive TET mediated by the DNA base stack is to the stacking of the reactants. For all of those samples, the reactants are intercalators. We sought to establish the requirement for intercalation of donor and acceptor to achieve DNA-mediated TET. Therefore, we used the heterogeneous coupling chemistry outlined in Chapter 5 to synthesize an oligonucleotide tethered to $[\text{Os}(\text{bpy})_2(\text{bpy}')]^{2+}$. This analog of $[\text{Os}(\text{bpy})_3]^{2+}$ does not contain a ligand to promote intercalation into the base stack, however, the photophysical properties of $[\text{Os}(\text{bpy})_2(\text{bpy}')]^{2+}$ are identical to those of $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$. Hence, the effective difference between a Ru-modified duplex tethered to $[\text{Os}(\text{bpy})_2(\text{bpy}')]^{2+}$ compared to a duplex attached to $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ is that $[\text{Os}(\text{bpy})_2(\text{bpy}')]^{2+}$ does not intercalate.

Figure 7.10 illustrates schematically the $[\text{Os}(\text{bpy})_2(\text{bpy}')]^{2+}$ -DNA conjugate used in this study, and the results of the experiment are shown in Table 7.7. As expected, the $[\text{Os}(\text{bpy})_3]^{2+}$ analog does not quench the emission of the ruthenium complex to any significant amount. This result establishes very clearly that in addition to an intact pathway through DNA, TET requires the reactants to intercalate into the base stack.

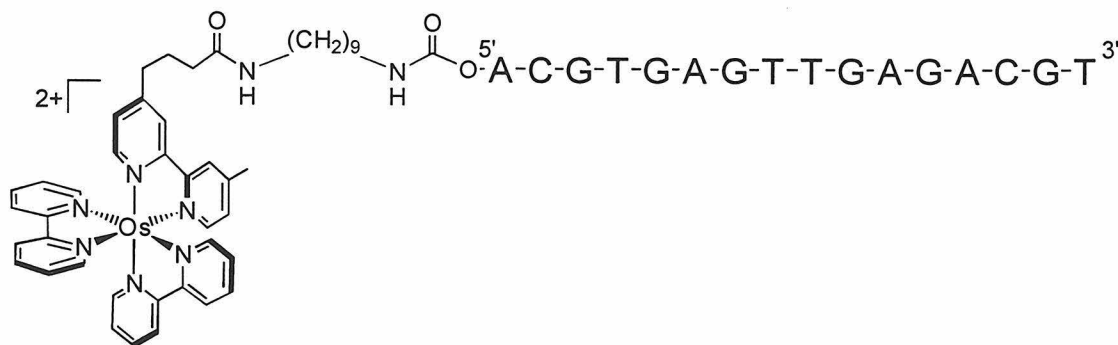


Figure 7.10. $[\text{Os}(\text{bpy})_3(\text{bpy}')]^{2+}$ - DNA conjugate used as an analog to oligonucleotides bearing $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$, which can intercalate. $[\text{Os}(\text{bpy})_3(\text{bpy}')]^{2+}$ does not have the ligand framework to support intercalation.

Table 7.7. The Effect on the Yield of TET of Eliminating Intercalation for the Os(II) Complex.

Assembly	I (rel)	F_q
	1.18	-----
	0.80	0.32
	1.07	0.09

7.2.6. Distance Dependence of DNA-Mediated Triplet Energy Transfer through the DNA Base Stack.

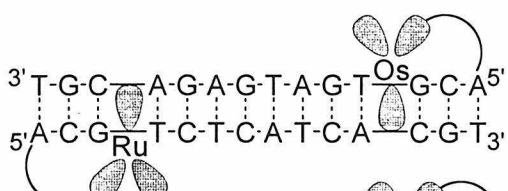
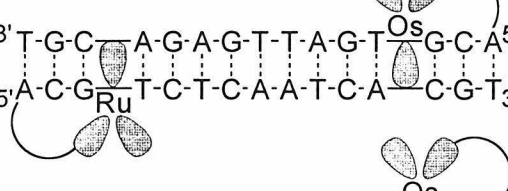
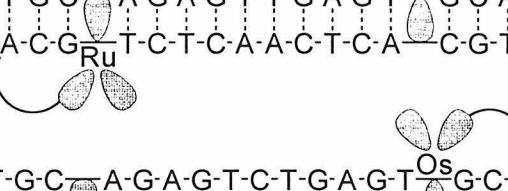
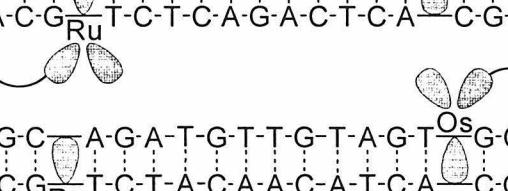
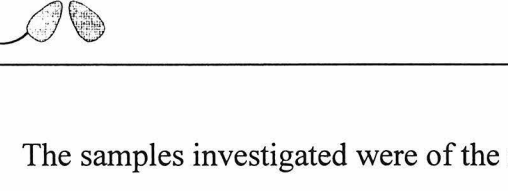
The final experiment in a comprehensive investigation of DNA-mediated TET is the determination of the distance dependence of the reaction. We synthesized a series of Ru and Os-modified oligonucleotides ranging from 14 to 18 bases in length. The yield of TET was characterized as a function of distance in the *bis*-heterometallated duplexes. Unfortunately, not every sequence permitted the purification of four isomers by HPLC. Therefore, we measured the distance dependence of quenching for the racemic mixture of later moving isomers (Λ -3 + Δ -4) for both ruthenium and osmium.

Table 7.8 lists the estimated donor-acceptor separations, and TET yields for each duplex. As the distance separating the reactants in each assembly increases, the yield of TET decreases. The quenching yield is 36% for the 14 bp assembly and 10% for the 18 bp assembly.

A plot of the natural log of $1 - I_{\text{Ru}}/I_{\text{Ru+Os}}$ vs a function of donor-acceptor separation is shown in Figure 7.11. This quantity is derived from a model used to analyze long-range ET in DNA, which relates the probability of all the DNA bases being stacked in a duplex as a function of distance.³² Such a model predicts a linear relationship between the log of the probability of stacking and the yield of quenching. The data in Figure 7.11 are fit reasonably well with a line. The point for the 17 bp duplex lies outside the line, however, the emission yields of the singly modified duplexes were also low, which suggested that this sample might be anomalous.

The slope of the line (slope = $0.1 \text{ \AA}^{-1}$) is a crude measure of the decay in electronic coupling with distance. Since we are not plotting rates, the slope does not equal the parameter for decay in electronic coupling with distance called for in the theory of Marcus.³⁴ Nevertheless, this closely analogous value indicates that the dependence of DNA-mediated TET is remarkably shallow, and may proceed over surprisingly large molecular distances.

Table 7.8. Yield of TET (F_q) in Metallointercalator-DNA Conjugates as a Function of Donor-Acceptor Separation.^a

	Os-Ru dist (Å)	I (rel)	F _q
	24-31	0.607	0.36
	27-34	0.667	0.30
	31-37	0.740	0.24
	34-41	0.889	0.10
	37-44	0.841	0.12

a. The samples investigated were of the racemic mixtures (Os and Ru) for the later eluting isomers in the HPLC purification of each single strand (*i.e.* Λ -3 and Δ -4). The racemic mixtures were used because the Λ -3 and Δ -4 isomers coeluted for some of the sequences.

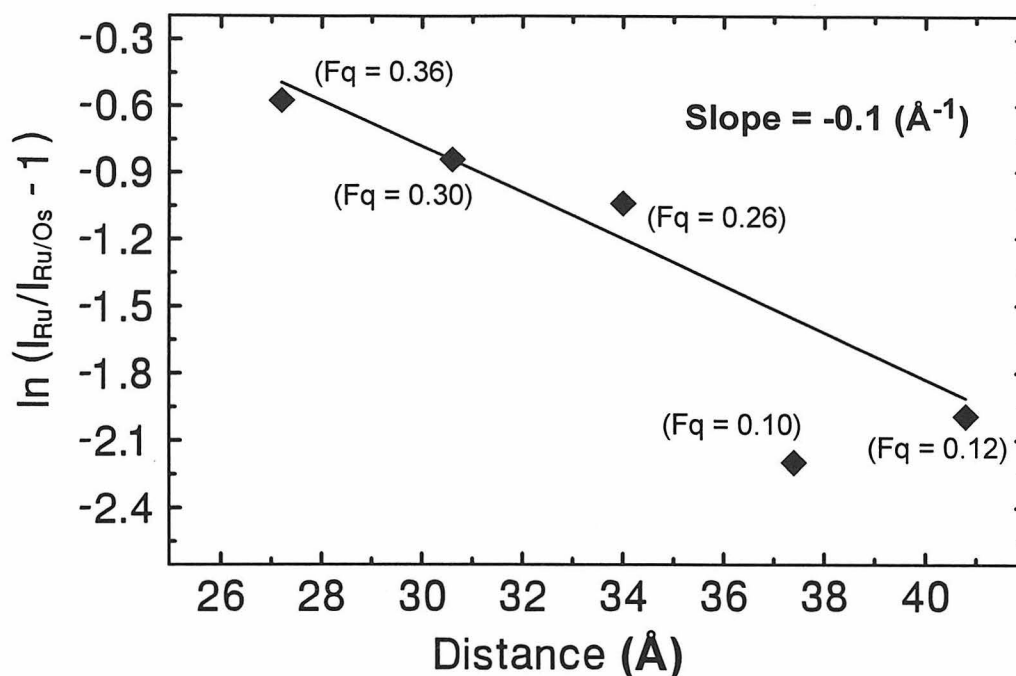


Figure 7.11. Plot of the $\ln(I_{Ru}/I_{Ru+Os} - 1)$ as a function of the distance separating $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ reactants tethered to DNA duplexes ranging in length from 14–18 bp. The fraction quenched (Fq) is shown next to each point. The slope of the best fit line is $-0.1 \text{ (}\text{\AA}^{-1}\text{)}$.

7.3. DISCUSSION

7.3.1. Triplet Energy Transfer between Metallointercalators Bound Noncovalently to DNA

We presented a series of titrations of $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ derivatives as quenchers into solutions of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ bound to polynucleotides of varying sequence. The efficiency of the TET reaction was sensitive to the sequence of the polymer as well as the ligand architecture of the metal complexes.

We understand the sensitivity to the ligands on the metal center as reflecting the importance of stacking of the metallointercalator with the DNA base stack. The complex with ancillary 4,7-dmp ligands is prohibited from stacking deeply within the bases by

steric clash between the methyl groups in the 4-positions and the wall of the major groove.

The sensitivity to sequence follows the same trends established for photoinduced ET between metallointercalators where the yield of ET follows the trend $CT \simeq \text{poly } d(AT) > \text{poly } d(GC)$.

Mechanism of Quenching. We demonstrated experimentally, by observing emission from the product of energy transfer, that the observed quenching is the result of TET from photoexcited Ru(II) to Os(II). The experiment used to demonstrate the formation of excited state Os(II) in the reaction involved adding $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ to a DNA duplex containing an Os(II) attached to one terminus. We employed this approach because the emission yield for the osmium complex in this assembly is greater than that of the complex bound noncovalently to the duplex. The ruthenium complex was not added in a stoichiometric amount, thus statistics favor the presence of 1:1 complexes of $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$ with the Os-modified duplexes, however, even with 25% quenching the amount of excited state Os(II) produced was difficult to measure. Nevertheless, we found that the emission of the tethered $[\text{Os}(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ complex was enhanced over the background emission by an amount that is consistent with expectation based on the amount of quenching.

It is interesting to note that the path A, reductive quenching of the Ru(II) excited state by ET, was not detected. It is possible that this reaction is occurring, but on a time scale faster than we could observe, however this pathway must at least be in competition with TET. We do not understand the factors that partition the two thermodynamically favored reaction manifolds.

7.3.2. Long-Range Triplet Energy Transfer in Metallointercalator-DNA Conjugates

We described significant quenching of ruthenium intercalators by osmium intercalators tethered to opposite ends of a DNA duplex. The reaction takes place over as much as 40 Å and is remarkably sensitive to the structure of the isomers of the metal complexes involved in the reaction. We found that in general, Δ isomers of the metallointercalators favored the TET reaction, whereas the Λ consistently showed less reaction. The yield of TET also depended on whether the linker protruded from the metal center in an axial or equatorial fashion. We believe that the later moving fractions by HPLC are the axial isomers. These isomers display the most luminescence and the Δ isomer leads to the most quenching.

Alternative Explanations for TET in Duplexes Bearing Os(II) and Ru(II) Metallointercalators. Three explanations for the observation of quenching in modified duplexes other than long-range TET mediated by the DNA base stack are considered. First, maybe the quenching observed is a trivial light stealing effect. The fact that the yield of TET is isomer dependent rules out such an explanation. Since the extinction coefficient for each of the Os-modified strands is the same, each sample has the same absorbance at the excitation wavelength and should have produced the same amount of quenching.

The second concern is that the quenching is the result of metallointercalator attached to one duplex intercalating into another, but close to the other reactant. In order to test for such an intermolecular effect we combined the Δ -4-Os-modified duplex (4 μ M) in the same solution with the Δ -4-Ru-modified duplex (4 μ M). The concentration of duplex was held constant (8 μ M), and in this experiment the emission intensity was 45 % of the Δ -4-Ru-modified duplex (8 μ M) (predicted 50%). The small reduction in emission yield from the expected value is probably an inner filter effect.

The third explanation, other than long-range TET, involves the metal complexes confined to one duplex actually managing to collide with each other to produce quenching, despite their restricted mobility. In fact, one could argue that such a mechanism could explain why the isomers with higher emission yields and hence longer excited state lifetimes exhibit greater quenching yields. Since the excited state of the donor is longer, the acceptor has more time to reach it and quench it before it returns to the ground state. It is, however, precisely the relative luminescence yields that rule this consideration out. For example, why should the isomer of the acceptor also play a role in determining the quenching yield if the excited state lifetime of the donor is the primary factor? The case of Λ -2-Ru with Δ -4-Os compared to Λ -3-Ru with Λ -2-Os, where the emission by the Λ -2 isomer is less than that of the Λ -3 isomer, also argues against this point because the quenching in the former case is more than that with the later. Therefore, these plausible explanations for the observed quenching that do not invoke long-range TET are ruled out on the basis of the arguments and experimental evidence provided.

7.3.3. Factors Affecting the Efficiency of Long-Range DNA-Mediated Triplet Energy Transfer

In the section above we addressed some of the best arguments against the idea that the observed quenching is not the result of long-range TET through DNA. So, having established on the grounds of experiment and reason that the quenching of photoexcited Ru(II) by Os(II) tethered to opposite ends of duplex DNA is mediated by the base stack, we can begin to consider the factors that are responsible for the differences in the efficiency of DNA-mediated TET.

Isomer Dependent Yields of Long-Range Triplet Energy Transfer in Metallointercalator-DNA Conjugates. One of the keys to understanding the factors that determine the efficiency of TET mediated by the DNA base stack is the diversity in

the sample population that is generated in the synthesis of metallointercalator-DNA conjugates based on $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ complexes. Owing to the ability of HPLC to separate each of the four isomers for each metal center in the 16-mer assemblies studied here, we looked at quenching in 16 different samples. As described above, the yield of TET depends upon the orientation of the linker with respect to the metal center as well as the absolute configuration of the metal center.

Having eliminated most of the trivial arguments for the observed reactions, the question becomes where do the differences in quenching yield come from? Is it truly stacking of the metallointercalators? Why don't the isomers that provide the most T_m stabilization exhibit the most quenching?

One reasonable answer to the first question above is that TET is distant dependent (albeit shallow as we demonstrated) and the different isomers differ only in their relative separations, and thus, stacking with the bases is not really important. The answer to this question lies in the yield of TET for the family of 16 bp duplexes in Table 7.2. If a simple explanation like different distances for different isomers were applicable, we would expect the same results for Λ -Os quenching Δ -Ru as for Δ -Os quenching Λ -Ru, but we don't.

One possible answer to the second question may lie in the excited state lifetimes for the Ru-modified assemblies (Table 7.4). These data illustrate clearly that the increase in luminescence yield for the later eluting Λ -3 and Δ -4 isomers lies primarily in an increase in the intercalation geometry with the longer excited state lifetime.²³ Such an increase in lifetime is the result of deeper stacking of the phenazine nitrogens into the interior of the base stack. Thus, if the metal complex penetrates into the base stack, TET is more efficient. Hence, by this reasoning and data, the yield of TET is particularly sensitive to the binding properties and therefore stacking of the reactants with the DNA.

Disruptions in Base Stacking Diminish the Yield of Triplet Energy Transfer.

In order to establish that the reaction between photoexcited ruthenium and osmium is

mediated by the base stack, we disrupted the base stack and followed the change in the yield of TET. We used three techniques to perturb base stacking which offer increasing amounts of destabilization to the base stack.

Single Base Mismatch. We first examined the effect of a single base mismatch intervening the donor and acceptor. Studies of DNA-mediated ET revealed that a CA mismatch blocks ET through the base stack, but GA does not.³² Our studies revealed irreproducible amounts of quenching in 16 bp duplexes bearing a CA mismatch, and the duplex with a GA mismatch was not different from the fully base paired assembly. In the case of the CA mismatch, one experiment revealed significantly more quenching than the assembly with TA, and the other experiment revealed only a small reduction in quenching efficiency over the fully base paired duplex. We have not yet fully resolved the question of how mismatches affect TET, but we do not observe complete blocking of TET. Such a difference between TET and ET could be actually a difference in the sequence context of each experiment, or the data may indicate that TET is less sensitive to small changes in stacking.

Bulges in the DNA Base Stack. As illustrated schematically in Table 6, the hybridization of oligonucleotides of one length to shorter strands produces regions in the assembly where bases are pushed out of the stack to give bulges. These bulges cause the stacking through the helix to be disrupted. Studies of DNA-mediated charge transfer involving the DNA bases revealed that bulges, even a single base, perturbed significantly the amount reaction at the DNA bases detected as a result of ET.^{8,9} These observations indicated that the bulges were disrupting the stacking and therefore disrupting charge transfer through DNA.

We found that incorporating a bulge between the Ru(II) donor and Os(II) acceptor caused the efficiency of TET to decrease. The diminution in the yield of TET was ~23%. In fact, this degree of perturbation is analogous to the reduction in the yield of DNA-mediated oxidative thymine dimer repair caused by bulges intervening the reactants.⁹

Thus, these observations support the conclusion that TET between metallointercalators tethered to DNA is mediated by the stacked bases, and, that disruptions in stacking prevent TET.

Thermal Denaturation of the Base Stack. Perhaps the most significant perturbation to the base stack intervening the donor and acceptor in DNA-mediated charge transfer reactions is complete thermal denaturation of the duplex. We measured the yield of TET in a 16 bp assembly as a function of temperature and demonstrated that when the assembly dissociates into single strands, the yield of energy transfer drops off eventually to where no quenching can be detected.

As mentioned above, the temperature at which the yield of TET dropped to 50% of its maximum value was 10-15 °C lower than the temperature at which 50% of the duplexes melted (by UV-vis). One could argue that melting the duplex should eliminate quenching even if the base stack were not mediating the reaction, simply because the donor and acceptor would no longer be close in space. Such an argument is completely ruled out by the fact that the yield of TET drops off with increasing temperature before the duplexes are melted.

The origin of the subtle difference in the yield of TET as a function of temperature and concentration of duplexes as a function of temperature lies in the dependence of TET on base stacking and the mechanism of how duplexes bearing two metallointercalators melt. As described in Chapter 5, the thermal denaturation of metallated duplexes monitored by absorption at 270 nm differs from the profile at 384 nm (absorption centered on the intercalated ligand of the metallointercalator) because the region of the duplex at the intercalation site melts last. Thus, when a duplex is doubly modified with metallointercalators, melting must be initiated in the center of the duplex (Figure 7.12). Hence, the mere initiation of melting blocks TET owing to the disruption of the bases intervening the reactants.

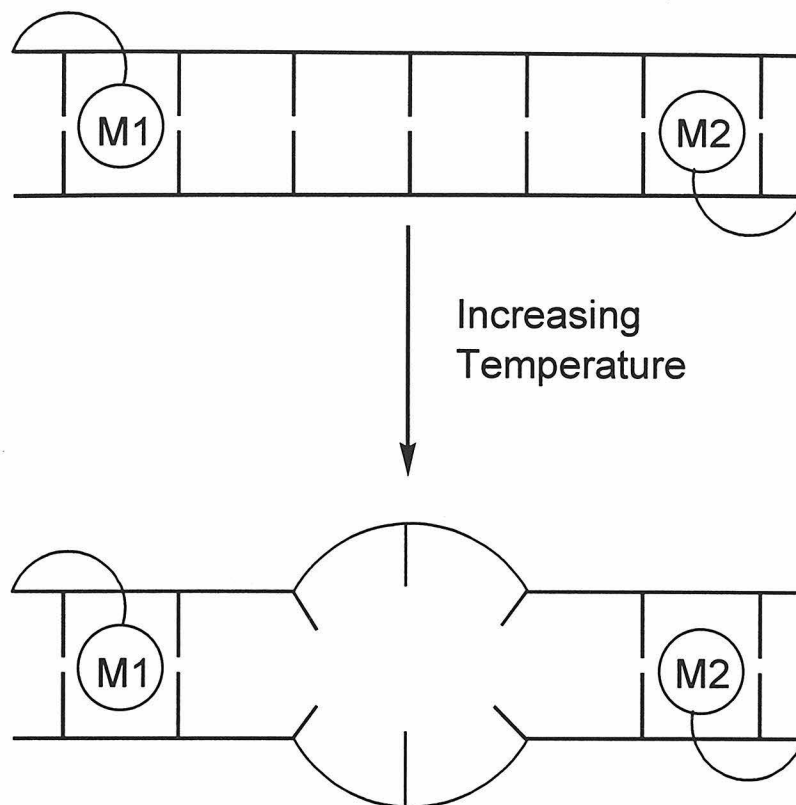


Figure 7.12. Schematic illustration of the melting of the center of doubly modified duplexes with increasing temperature. Such a disruption prevents long-range TET before the two single strands dissociate.

7.3.4. Comparison of DNA-Mediated Triplet Energy Transfer to Triplet Energy Transfer between Os(II) and Ru(II) through an Alkyl Spacer. As described above, several model systems have been employed to study TET between Os(II) and Ru(II). Rather than exhaustively consider each system, we compare the rate of long-range TET for one assembly in the literature to TET mediated by the DNA base stack.

DeCola *et al.* synthesized a bimetallic assembly bearing $[M(bpy)_3]^{2+}$ analogs separated by 17 Å with an adamantyl spacer.¹⁶ The authors observed 100% quenching of the ruthenium emission by the osmium acceptor over this distance. This efficiency of

reaction (at this relatively modest donor-acceptor separation) is significantly greater than the yield of TET in 14 bp duplexes. For the 14 bp duplex, we expect the reactants to be separated by $> 20 \text{ \AA}$. Importantly, the rate of TET across the adamantyl spacer is $\sim 10^6 \text{ s}^{-1}$, which is considerably slower than the DNA-mediated reaction.

The contrast in the efficiency and rate of TET for DNA compared to alkanes is illustrative of the mechanism of charge transport in DNA. Even though the efficiency seems lower for DNA-mediated TET, the rate is on the subnanosecond time scale. This point illustrates the fact that charge transfer through DNA requires that the medium be fully stacked. When base stacking is optimized for a given assembly, the charge transfer occurs, and at a fast rate. DNA-mediated charge transfer serves as ‘snapshot’ of the system at the time of the reaction. For the σ -bonded system, the rate and efficiency are directly connected. If the rate slows down, the efficiency decreases.

7.3.5. Prospects for the Future of DNA-Mediated Triplet Energy Transfer. An excellent system to employ in studies parallel to the investigations of DNA-mediated TET between metallointercalators is the one described recently by Tor and coworkers. These authors synthesized derivatives of nucleotides that are attached via an analog of thymine to an $[\text{M}(\text{bpy})_3]^{2+}$ ($\text{M} = \text{Ru}, \text{Os}$) complex.³⁵ These metallonucleotides are used as building blocks in the synthesis of oligonucleotides with the metal complex located site specifically along the duplex. When such a modified oligonucleotide is hybridized to its complement, the metal complex is fixed in the major groove of the duplex. Importantly, the metal cannot intercalate in these compounds and thus cannot access directly the DNA base stack. We envision a comparison of long-range TET in these assemblies with those containing metallointercalators. Furthermore, hybrid duplexes containing one metallointercalator and one groove bound reactant will be instructive as well.

7.4. EXPERIMENTAL

Materials. Metallated oligonucleotides were synthesized and purified as described in Chapter 5. $[M(\text{phen})(\text{bpy}')(\text{Me}_2\text{-dppz})]^{2+}$ ($M = \text{Ru}, \text{Os}$), $[\text{Ru}(\text{phen})_2(\text{dppz})]^{2+}$, and $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ were all prepared as described in Chapter 2 or previously.³⁶ $[\text{Os}(\text{bpy})_2(\text{bpy}')]^{2+}$ was prepared by coordination of b'-OMe to $[\text{Os}(\text{bpy})_2\text{Cl}_2]$ in ethylene glycol as described for $[\text{Os}(\text{phen})_2(\text{Me}_2\text{-dppz})]^{2+}$ in Chapter 2. The methyl ester was hydrolyzed with LiOH in water as described for $[M(\text{phen})(\text{b}'\text{-OMe})(\text{Me}_2\text{-dppz})]^{2+}$, also in Chapter 2. This complex and the resulting conjugate were characterized by UV-vis spectroscopy ($\epsilon_{430 \text{ nm}} = 1.1 \times 10^4 \text{ cm}^{-1}\text{M}^{-1}$ for the conjugate and the metal complex). SDS was the purest available from Fluka, and the polynucleotides were from Pharmacia and exchanged into working buffer by ultrafiltration (Amicon).

Methods. All quenching experiments with oligonucleotide conjugates were carried out on aerated samples at concentrations of 8 μM duplex in 5 mM Na_3PO_4 , 50 mM NaCl, pH 7.0 unless stated otherwise. Thermal denaturation experiments with observation at 384 nm were also carried out at 8 μM duplex in 5 mM Na_3PO_4 , 50 mM NaCl, pH 7.0; all experiments with observation at 270 nm were carried out at 4 μM duplex in 5 mM Na_3PO_4 , 50 mM NaCl, pH 7.0. Instrumentation for T_m studies was described in Chapter 5.

Luminescence lifetimes were collected by MPMF as described in Chapter 2 and previously. Steady-state emission was collected on an SLM 8000 and analyzed with software provided by the manufacturer. Time-resolved luminescence and transient absorption data were collected in the Beckman Institute on instrumentation described previously.

7.5. REFERENCES

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