Investigating DNA-Mediated Charge Transport by Time-Resolved Spectroscopy

Thesis by

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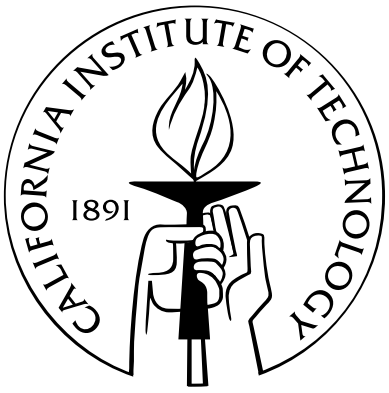
In Partial Fulfillment of the Requirements

for the Degree of

Doctor of Philosophy



California Institute of Technology

Pasadena, California

2012

(Defended Sept 30, 2011)

Acknowledgements

As is true for any graduate student, most of this work could not have been completed without assistance from a large number of people. First and foremost, I want to thank my advisor, Jackie Barton, not only for her scientific guidance and her unwavering enthusiasm, but also for the subtle instruction she provides in the many duties of academia. Not every graduate student is given the opportunity to communicate their research, participate in grant writing, or critically review manuscripts, but proficiency in these areas is necessary to succeed in the academic world. Jackie has also been a strong role model. In my future endeavors, I will seek to emulate the tenacity with which she pursues answers to scientific questions, the boldness with which she follows her intuition, and the graciousness she shows even at the highest levels of professional success.

I also want to express my sincere gratitude to my thesis committee and to my collaborators. Although I didn't have as much interaction with them as I would have liked, I appreciate the input and encouragement of my committee: Harry Gray, Doug Rees, Tom Miller, and formerly Pat Collier. My collaborator Tony Vlček introduced the Barton Group to a new experimental method for the time-resolved observation of DNA-mediated CT. Besides providing access to a sensitive TRIR instrument in the UK and facilitating the measurement of our samples, Tony was a fantastic resource when the time came to work up and interpret the data. He was unfailingly cheerful and he always treated me as a scientific equal. Without his interest, generosity, and guidance, much of the work that is presented here would simply not have been possible. Similarly, I am indebted to Mike Hill for his assistance in conducting electrochemical and spectroelectrochemical measurements at Occidental College. Mike was always convivial, enthusiastic, and generous with his time. He made me feel at home each time I visited his laboratory, introducing me to his colleagues

and showing me what life is like as a chemistry professor at a liberal arts college.

Closer to home, I received a great deal of support and guidance from Eric Stemp and Jay Winkler. Eric, a perennial visitor and collaborator with the Barton Group, was always happy to discuss photophysics, group lore, and baseball. In addition, my work owes much to fundamental aspects of DNA-mediated CT that he established years ago. I am grateful to Jay partly for sharing his expertise in instrumentation and data analysis, but mostly for providing a strong example of scientific integrity. As difficult as it is to be held to the high standards that Jay demands, such attention to detail and logic is necessary to rigorously test complex scientific theories. I will continue to pursue that ideal.

I must thank the tireless administrators and adjuncts of Caltech who keep the Institute running. Thanks to Steve, Joe, and Ron for making sure that the materials I order find their way to me. Thanks to Agnes and her predecessors Laura and Dian for always knowing where I should go and what I should do next. Thanks to Mona, Dave, Scott, and Angelo for keeping the instruments running. Thanks to Tom, Rick, and Mike for fixing everything I broke. Thanks to Ernie for the food. Finally, thank you Mo, Administrative Assistant to Prof. Barton, and Mo, Administrative Assistant to the Chair, for being the glue that holds the whole place together.

While at Caltech, I have had the distinction of experiencing almost every facet of Chem 1, from grader to recitation TA to head TA. I have also observed the increase in clarity and organization that took place when Mike Vicic began his tenure as course coordinator. I want to thank Mike for showing me how to properly design and run a course. I also want to thank Sarah Reisman for taking me on as head TA during her first term teaching Chem 1b. These experiences have largely directed my ambition to establish a career in academia.

Music has served as a counterpoint to my scientific life at Caltech. My gratitude goes to Bill Bing, who encouraged me to join the concert band and later the jazz band. It has been a weekly pleasure to play percussion with Scott, Jeff, Dan, Joe, Wesley, Gregory, Heather, Chris, Mike, and Kris. I also want to thank Rob Usiskin, Brett Babin, and James McKone, who have given me the opportunity to explore jazz in a less structured setting.

Life is comprised of a series of interactions with other people, and the enjoyment

of life depends mainly on the quality of those interactions. I am fortunate to have been part of a supportive, creative, and at times eccentric lab group throughout my time in grad school. Of course I appreciate the help and advice they have given me in the lab, but I am more thankful for the personal memories. I will never forget Eddie's enthusiasm during summer softball and how he convinced every single person in the lab to play that first year; Brian's ability to make Pumpkinpalooza and Lablympics come to life and his even more impressive ability to convince us all that we wanted to participate; Paul's cat suit, paper cutouts, clay sculptures, skull stickers, suicide bunnies, and secret messages; physics discussions with Joey; 4th of July at the Rose Bowl; spending ages in the laser lab with Ben; the vacation cottage at ACS; Yosemite and King's Canyon, talent shows, long car rides, Whiskey Rock, fire breathing, and Christine's camp cooking; the cake-baking committee; the lunch train; late night trips to Pinkberry and Papa George's; karaoke and balls; Curtis's brews; crossword puzzles; flag football; beer and cheese pairings; and so much more. Thanks everyone.

I also want to thank my friends outside of lab for giving me some much needed perspective. Thanks to Arthur, Puneet, Andrew, Kathleen, Erik, Mary, Diana, Yvonne, Havala, and Claire for trips to the beach, hikes, and cookouts. Thanks Morgan, Andy, Rob, John, Val, and Sam for an amazing time in Korea. Thanks to the rest of the 2005 entering class, especially Gretchen, Alex, Jillian, and Matt for inviting me to your parties and poker games. Thanks Chithra and Keith for operas, plays, dinners out, and dinners in.

Thanks to my family. I am impressed by my mom, who has continued earning professional degrees while working full-time and raising three boys. I respect my dad for his thoughtfulness and broad knowledge of the world. I am proud of Matt for following his dreams to L.A. and for maintaining his ambition and integrity. I appreciate Rob's constant friendship and appropriate balance of support and criticism. I love you all.

Finally, I want to thank my fianceé, complement, and best friend Chethana Kulkarni for her constant understanding and love. Throughout the time I have been with her, she has reminded me to focus on what is important in life, and she has shown me so much of the world and of life that I would not have experienced on my own. Thank you.

Abstract

In all organisms, oxidation threatens the integrity of the genome. Numerous studies have suggested that DNA-mediated charge transport (CT) may play an important role in the sequestration, detection, and repair of oxidative damage. To fully understand the mechanism of DNA-mediated CT, it is necessary to characterize transient intermediates that arise during the reaction and to determine the lifetimes of these intermediates. Time-resolved spectroscopy is the most appropriate experimental method for such observations. Each intermediate has a characteristic spectrum. By observing time-dependent changes in the absorption spectrum of the sample, it is therefore possible to determine what species are present at a particular time and how long it exists in solution. Experiments presented here involve the use of time-resolved spectroscopy to better understand the process of DNA-mediated CT.

The study of DNA-mediated CT requires a robust and consistent method for triggering the CT reaction. The metal complexes that have traditionally been used for this purpose provide several advantages over organic phototriggers: they are synthetically versatile, they are stable in solution, they exhibit rich photophysics, and many are strong photooxidants. However, the spectroscopic features used to follow the photochemical processes triggered by these probes are generally broad optical bands. These can be difficult to resolve in samples that contain several absorbing species. For this reason, we have developed a Re photooxidant bearing a set of vibrationally active carbonyl ligands that can be covalently tethered to DNA. Unlike many absorption bands in the visible range, the vibrational absorption bands of these ligands are narrow, well-resolved, and specific. Such probes can be used to follow the complex photophysical pathways observed in biochemical systems with good precision, making them useful for the study of DNA-mediated CT.

Specifically, the complex $[Re(CO)_3(dppz)(py\text{-}OR)]^+$ (dppz = dipyrido[3,2-*a*:2′,3′-*c*]-phenazine; py′-OR = 4-functionalized pyridine) offers IR sensitivity and can oxidize DNA directly from the excited state. The behavior of several covalent and noncovalent Re-DNA constructs was monitored by time-resolved IR (TRIR) and UV/visible spectroscopies, as well as biochemical methods, confirming the ability of the complex to trigger long-range oxidation of DNA. Optical excitation of the complex leads to population of metal-to-ligand charge transfer excited states and at least two distinct intraligand charge transfer excited states. Several experimental observations are consistent with charge injection by excited Re*. These include similarity between TRIR spectra and the spectrum of reduced Re observed by spectroelectrochemistry, the appearance of a guanine radical signal in TRIR spectra, and the eventual formation of permanent guanine oxidation products. The majority of reactivity occurs on the ultrafast time scale, although processes dependent on slower conformational motions of DNA, such as the accumulation of oxidative damage at guanine, are also observed.

The photooxidation activity of this Re complex was compared directly to that of other metallointercalators that have been used previously in our laboratory to oxidize DNA. The complexes $[Rh(phi)_2(bpy')]^{3+}$ (phi = 9,10-phenanthrenequinone diimine; bpy′ = 4-methyl-4′-(butyric acid)-2,2′-bipyridine), $[Ir(ppy)_2(dppz')]^+$ (ppy = 2-phenylpyridine; dppz′ = 6-(dipyrido[3,2-*a*:2′,3′-*c*]phenazin-11-yl)hex-5-ynoic acid), and $[Re(CO)_3(dppz)(py'\text{-}OH)]^+$ (py′-OH = 3-(pyridin-4-yl)-propanoic acid) were each covalently tethered to DNA. Biochemical studies show that upon irradiation, the three complexes oxidize guanine by long-range DNA-mediated CT with the efficiency: Rh > Re > Ir. Comparison of spectra obtained by spectroelectrochemistry after bulk reduction of the free metal complexes with those obtained by transient absorption (TA) spectroscopy of the conjugates suggests that excitation of the conjugates at 355 nm results in the formation of the reduced metal states. Electrochemical experiments and kinetic analysis of the TA decays verify that the primary factors responsible for the trend observed in the guanine oxidation yield of the three complexes are the thermodynamic driving force for CT, variations in the efficiency of back electron transfer, and coupling to DNA.

The ability of redox-active DNA-binding proteins to act as hole sinks in DNA-mediated CT systems was also studied by time-resolved spectroscopy. Such experiments are designed to provide support for the utilization of DNA-mediated CT in biological systems. In studies involving the cell cycle regulator p53, photoexcitation results in the formation of a weak transient band at 405 nm. This band, which is not observed in samples lacking the protein, resembles the primary spectral feature of the tyrosine cation radical. Although the signal is weak and reproducibility is inconsistent, these results suggest that photolysis of the sample leads to DNA-mediated oxidation of tyrosine in p53. Similar experiments were conducted on the transcriptional activator SoxR. Here, the presence of dithionite, required in solution to keep the protein reduced, complicates the photochemistry of the system considerably. Regardless, a weak absorbance at 418 nm that develops following photolysis at 355 nm provides evidence for the DNA-mediated oxidation of the protein. The behavior of the base excision repair protein endonuclease III was also observed in the presence of DNA and metal complex oxidants. In flash-quench studies, addition of the protein results in the formation of a strong negative signal at 410 nm in TA traces. In studies involving direct photooxidation by Rh, Ir, and Re complexes, no new transients are detected upon the addition of protein, but changes in the intensities of the resultant TA spectra and in the steady-state absorbance spectra following photolysis indicate that DNA-mediated oxidation of the protein may be taking place.

The experiments described here comprise several new developments in the story of DNA-mediated CT. First, proof of concept has been given for a valuable new vibrationally-active Re probe. Further modifications on the characteristics of this complex and further study by time-resolved vibrational spectroscopy will allow us to observe DNA-mediated CT with high spectral resolution. Second, comparison between this Re probe and established photooxidants shows that the Re complex is a strong photooxidant in its own right and that this complex can be added to our growing toolbox of CT phototriggers. Third, time-resolved studies involving redox-active proteins have provided preliminary direct evidence for the ability of these proteins to serve as CT probes themselves. Further refinement of the experimental methods used in these experiments will allow us to observe such processes

with greater sensitivity, increasing our knowledge of the mechanism and applications of DNA-mediated CT.

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