APPENDIX A

Excited States of Zn-Cytochrome c’

Acknowledgment

Dr. Judy Kim collaborated on various experiments in this chapter.

A.1 INTRODUCTION

A.1.2 Isotope Effect of Zn-Cyt c'

An analysis of the amino acid content of cytochrome *c*' reveals that it is much more hydrophobic than horse heart cytochrome *c*. The higher hydrophobicity might contribute to the presence of compact denatured structures even under fully unfolded conditions. Studies of the folding of Fe(II)-cyt *c*' show that there are populations

Population	**k_1/k_2 (s^{-1})**	**Overall Percentage**
Fast	5700/7000	9/8
Intermediate	15-900	24
Slow	0.59/0.48	16/43

Figure A1. Structure of Cyt *c*' and Rate constants for the Folding of Fe(II)-cyt *c*'.

folding at different rates due to misligation pathways which can be broken into three main groups: fast-folders, intermediate folders, and slow-folders. The slow folding population is the dominant pathway, taking several second to form the fully folded structure. The main barrier to folding in Fe(II)-cyt *c*' is a misligated methionine residue (possibly Met-15 or Met-25). This problem is not present in the monoexponential folding of Fe(III)-cyt *c*', which folds on the order of one second[1].

A.2 RESULTS AND DISCUSSION

The combination of slow folding and compact unfolded populations makes cyt *c*' an interesting protein to study in H_2O and D_2O. However, the substitution of zinc in cyt *c*' causes an increase in the triplet state decay rate (~500 s^{-1}) compared to Zn-cyt *c* (105 s^{-1}). Initially, this fast rate constant was believed to be due to quenching of the triplet state by extraneous oxygen, however the addition of oxygen scavengers (glucose oxidase

and catalase) reduces the rate constant of half the population to 370 s^{-1}, while the triplet state decay of the other half of the population remains approximately the same. The longer lifetime, assumed to be the true lifetime of Zn-cyt *c*' in the absence of oxygen, is still shorter than Zn-cyt *c*. In addition, no isotope effect is apparent in Zn-cyt *c*' under the same experimental conditions used for Zn-cyt *c* and ZnAcMP8. From these initial experiments it is not clear why no isotope effect is observed, but perhaps it is due to total protection of the Zn-porphyrin from the solvent.

	Cyt c'	**Cyt c' (no O_2)**
k_1 (s^{-1})	118.291 (5 %)	314.677 (52 %)
k_2 (s^{-1})	541.525 (95 %)	463.585 (45 %)

Figure A2. Rate Constants for Zn-cyt c' in phosphate and under oxygen free conditions.

A similar method was used for zinc incorporation of R. palustris cyt c'(Q1A-modified, Jennifer Lee), which was monitored by a shift in absorbance from 402 nm to 417 nm.

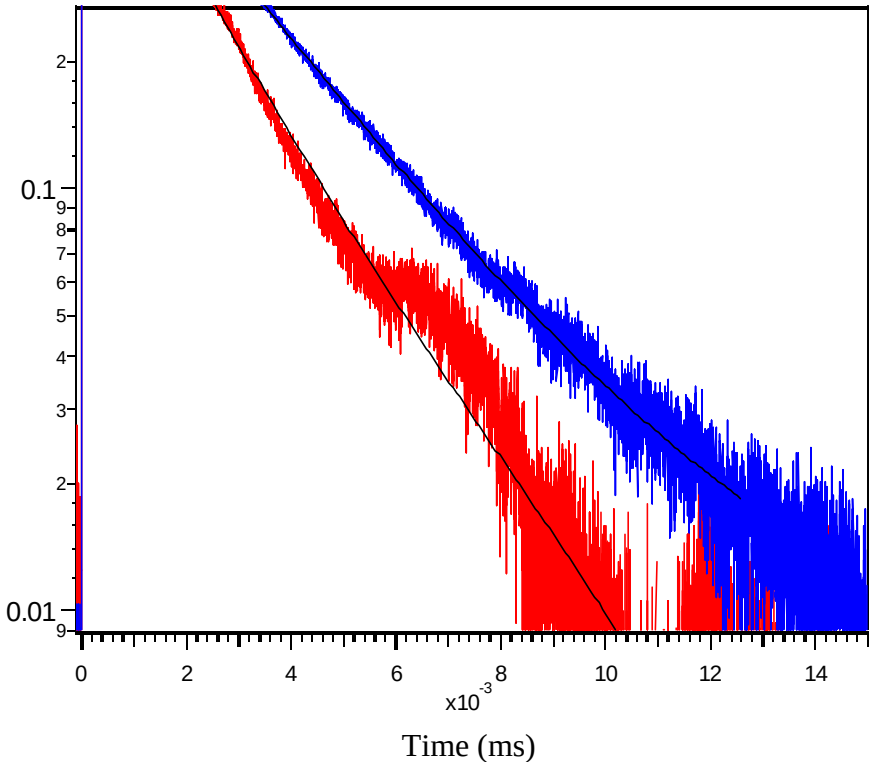

Figure A3. Decay kinetics of triplet Zn-cyt c' in 20 mM phosphate (red) and with oxygen scavenging enzymes (blue).

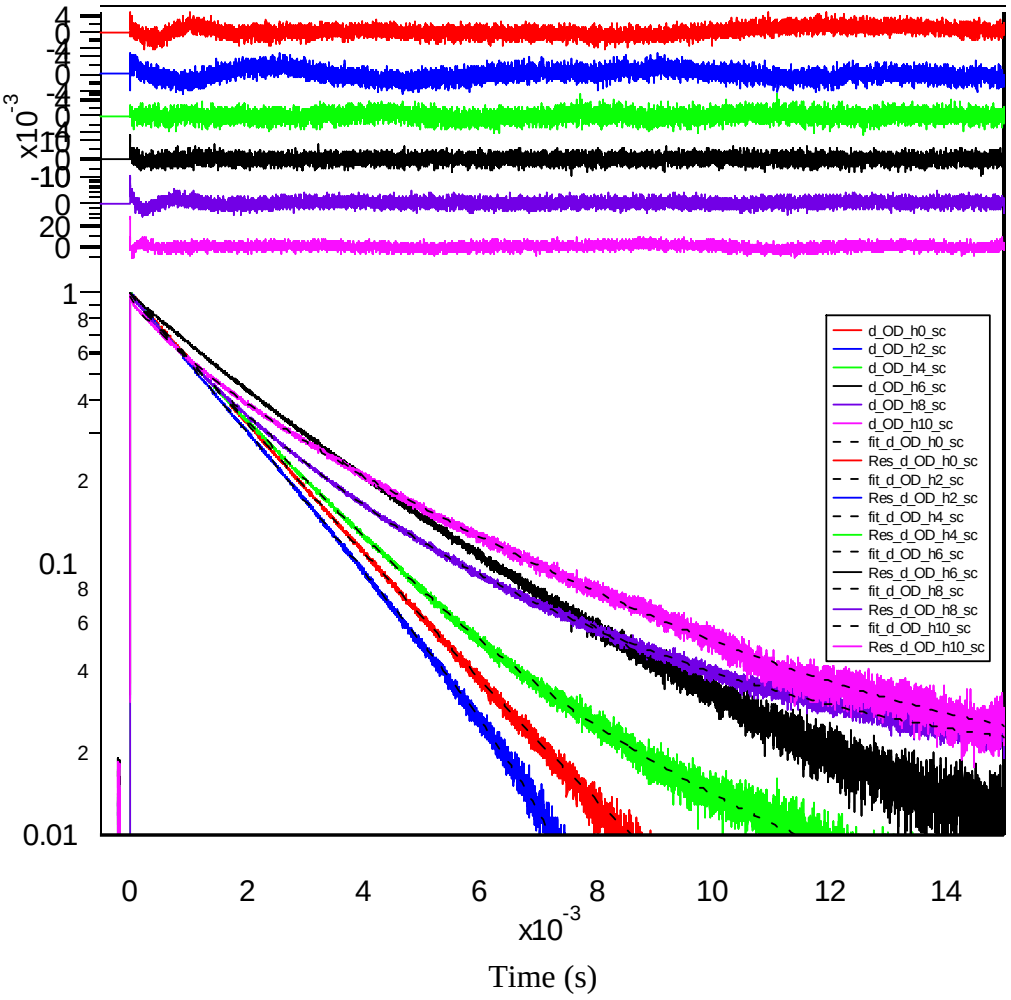

Figure A4. Decay kinetics of triplet Zn-cyt c' in 20 mM phosphate (H_2O).

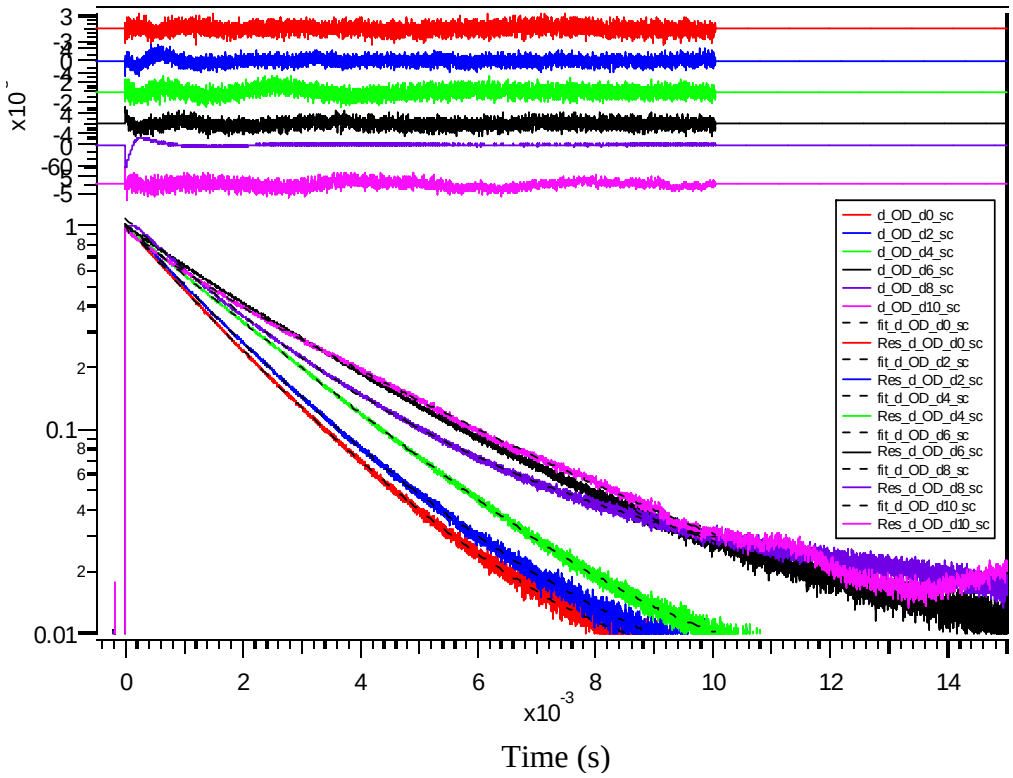

Figure A5. Decay kinetics of triplet Zn-cyt c' in 20 mM phosphate (D_2O).

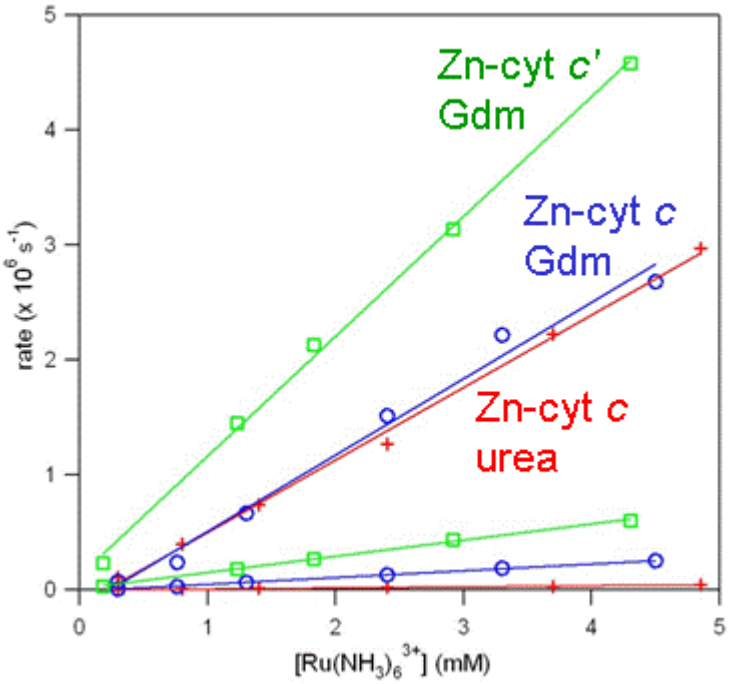

Figure A6. Bimolecular quenching rates of cytochrome *c* and *c*' in urea and GdmCl.

Gdm	Gdm	urea
Fast: 1.0×10^9 s^{-1} M^{-1}	Fast: 6.6×10^8 s^{-1} M^{-1}	Fast: 6.3×10^8 s^{-1} M^{-1}
Slow: 1.4×10^8 s^{-1} M^{-1}	Slow: 5.9×10^7 s^{-1} M^{-1}	Slow: 6.6×10^8 s^{-1} M^{-1}
$k_u = 761$ s^{-1}	$k_u = 810$ s^{-1}	$k_u = 720$ s^{-1}