CHAPTER SIX

Future Directions

6.1 SYSTEMS TO STUDY

There are many electron tunneling pathways on which to study multistep tunneling on *Pseudomonas aeruginosa* azurin. While many mutants were prepared for study, not all planned studies were accomplished. **Table 6.1** outlines mutants that were given only cursory study and their stages of preparation; it is hoped that these materials will be useful for future studies done on azurin.

Protein	Stage of Preparation	Remark
Ru107/$YNO_2$109/Cu^{2+}	Pure protein, ready for study	Cys112 arm
Re107/$YNO_2$109/Cu^{2+}	Studied once by laser spectroscopy	Cys112 arm
H107/W109/Cu^{2+}	Expressed	Cys112 arm
H107/Y109/W110/Cu^{2+}	Expressed	Double Hop
Ru107/W109/Y110/Cu^{2+}	Expressed	Double Hop
H126/W122/Zn^{2+}	Expressed	Met121 arm
H126/F122/Zn^{2+}	Expressed	Met121 arm
H83/Y48/Zn^{2+}	Expressed	
H83/F48/Zn^{2+}	Expressed	

Table 6.1. Azurin mutants which were expressed but not discussed in this dissertation

Multistep tunneling through the Cys112 arm of azurin was not at all addressed in this dissertation, which is a shame; the mutants were prepared, but there was insufficient data to draw real conclusions. However, the tentative studies on the site indicated that electron transfer occurred between Ru107 and $YNO_2$109. The 109 site was simply too

far away from the copper center to facilitate the subsequent electron transfer. It was postulated that the system would be ideally suited to study a double hop. Proteins to investigate this possibility were expressed, but never nitrated or labeled. Given the success of the Re124/W122 system, a Re107/W109 system also ought to be studied.

Along the Met121 arm, more studies need to be done to confirm the second hopping system. A Re126/W122/Zn^{2+} mutant should be prepared; it is hoped that the tryptophan radical cation's spectrum, which was masked by Re^{0} in the Re124/W122/Cu^{2+} system, can be measured in this mutant. Double hopping systems should also be investigated in this system, using 124/122 residues as hopping sites, and 126 for labeling.

6.2 THESIS CONCLUSIONS

The goal of the research done in this dissertation was to demonstrate multistep electron tunneling in a model system installed on the protein *Pseudomonas aeruginosa* azurin. This system has been successfully made and characterized. The electron transfer kinetics of system Re124/W122/Az(Cu^{+}) have been studied by different methods of spectroscopy, and all data support the same multistep tunneling model. Furthermore, the electron transfer time observed in the studies is consistent with the time calculated from theory.

This system was varied to test its robustness; the potential of both label and amino acid were perturbed. It was through these investigations that it was demonstrated that 3-nitrotyrosine could participate in electron transfer reactions, substantiating the possibility of tyrosine and its analogs being utilized as hopping intermediates in nature. It remains

unclear how the residue participates, and it is hoped that the promising initial observations will inspire future investigations of hopping through that amino acid.

When the label was installed another 5 Å away at the 126 site, electron transfer between excited state rhenium and tryptophan shut down; the centers were simply too far apart. However, once an exogenous quencher was utilized to oxidize the rhenium into its higher potential Re^{2+} state, the Re126/W122/Az(Cu^{+}) system exhibited hopping kinetics, giving the multistep tunneling program its second working system.

I hope it is clear to the reader how invigorating studies in this system are; each insight yields substantial information, which makes each experiment quite exciting to execute! There are still many unexplored possibilities in studying multistep electron tunneling on azurin and much more information on multistep tunneling to be gained. It is hoped that this report will give the reader the inspiration and methods to study them.