Capturing Protein Dynamics with Time-Resolved Luminescence 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

2013

(Defended May 1, 2013)

Abstract

The presented doctoral research utilizes time-resolved spectroscopy to characterize protein dynamics and folding mechanisms. We resolve millisecond-timescale folding by coupling time-resolved fluorescence energy transfer (trFRET) to a continuous flow microfluidic mixer to obtain intramolecular distance distributions throughout the folding process. We have elucidated the folding mechanisms of two cytochromes—one that exhibits two-state folding (cytochrome cb_{562}) and one that has both a kinetic refolding intermediate ensemble and a distinct equilibrium unfolding intermediate (cytochrome c_{552}). Our data reveal that the distinct structural features of cytochrome c_{552} contribute to its thermostability.

We have also investigated intrachain contact dynamics in unfolded cytochrome cb_{562} by monitoring electron transfer, which occurs as the heme collides with a ruthenium photosensitizer, covalently bound to residues along the polypeptide. Intrachain diffusion for chemically denatured proteins proceeds on the microsecond timescale with an upper limit of 0.1 μs. The power-law dependence (slope = -1.5) of the rate constants on the number of peptide bonds between the heme and Ru complex indicate that cytochrome cb_{562} is minimally frustrated.

In addition, we have explored the pathway dependence of electron tunneling rates between metal sites in proteins. Our research group has converted cytochrome b_{562} to a *c*-type cytochrome with the porphyrin covalently bound to cysteine sidechains. We have investigated the effects of the changes to the protein structure (i.e., increased rigidity and potential new equatorial tunneling pathways) on the electron transfer rates, measured by transient absorption, in a series of ruthenium photosensitizer-modified proteins.

Acknowledgements

When I think back on my time in graduate school, I remember how my friends, coworkers, and family have been there to support me when times were tough. So I wanted to say "thanks" to anyone and everyone who has ever been a part of my support network.

First and foremost, I could not have asked for a more supportive advisor than Harry Gray. My graduate school experience has been defined by being a member of the Gray Nation. Harry keeps an open door and a smile on his face. He cares about the people in his group, not just results. Thanks also, Harry, for giving me the opportunity to go to conferences and to Japan. I appreciate your mentoring during group meetings, while writing papers, and in professional matters.

Jay Winkler has been an excellent advisor in all things technical, from lasers to paper-writing. Discussions with Jay about my results have influenced how I think about biophysical problems. Thanks, Jay, for welcoming me into the group when I became interested in the protein folding projects.

I have been fortunate to have all of my committee members at my candidacy exam, fourth-year meeting, proposal exam, and thesis defense. Jackie Barton, my committee chair, has given me focus and helpful advice. Tom Miller has given

my research and proposals considerable thought and always has an insightful, complex physical question to ask. Douglas Rees has been helpful in discussions of protein dynamics.

My work with Sony has really broadened my graduate school experience. Seiji Yamada is a good friend of mine with whom I enjoyed collaborating. Thank you, Seiji, for inviting me to experience Japan with you and your family. I would like to thank Tokita-san for supporting me while I worked in his group at Sony. Discussions with Goto-san about science and culture contributed to my experience at Sony. I also appreciate the rest of the biomaterial research group who welcomed me into their group. Arigatou gozaimasu. I would also like to acknowledge my collaborator on the electron tunneling project, Tatiana Prytkova.

I have been fortunate to work with the wonderful members of the Gray Group, and especially closely with the bio subgroup: Bert, Tetsu, Melanie, Heather, Keiko, Crystal, Gretchen, Charlotte, Kyle, Maraia, Lionel, Matt, Jeff, Astrid, Peter, Oliver, and Kana. We had great times, in and out of lab. Bert recruited me into the group and taught me how to get around lab, express proteins, and think about life after graduate school. Tetsu taught me how to use the microfluidic mixer. Melanie has been a good friend, exercise buddy, and fellow biotech enthusiast. Heather was a great roommate and is my favorite person to talk to about cats, then science, and then cats. Gretchen was my partner in learning the intricacies of the picosecond laser system, and Kana has been a fun laser buddy. I appreciate the feedback while writing my proposals from my labmates: Peter, Maraia, Kana, Melanie, Heather,

Bert, and Gretchen. I would also like to acknowledge the hard work of the two undergraduate researchers whom I have mentored—Dong Woo Shin and Yuehan Huang.

My friends outside of lab have been a great support group for me. Andrea, Alma, Eldar, and Artur were great fun and helped me to relax at the end of the week. I love catching up with Lisa, Caitlin, Chethana, Amanda, Leslie, and Linhda (my link to Los Angeles). The Miller group has always welcomed me into their fun troupe: Artur, Nick, Jason, Josh, and Connie. My fellow classmates, the "first years," helped in the transition to Caltech—to Long, you are missed. Involvement with the Caltech Y has been a source of great enjoyment and a productive break from work; I would like to thank the Y employees (Greg, Athena, Liz), my fellow Y Outdoor Committee members, especially Deva and Avni, and the students who planned cultural and service events.

Many people in my past have believed in me and helped me make it to Caltech. My mom has always been supportive of me and shows her love through the little things, like letters, homemade pie, and chocolate. My dad has shown me how important it is to keep learning throughout life and was influential in my decision to go to college at Trinity, where there were many opportunities for me. My sisters, Regina and Daphne, were always excited about me doing cool science. They have grown up from "PIGs" to be smart, beautiful ladies. My brother-in-law David has been a wonderful addition to the family. My friends Elizabeth and Rebecca "dreamed big" with me during the difficult years of high school. My college

roommates Lauren, Christie, and Sarah are amazing people and my closest friends. Trinity University chemistry professors (Drs. Urbach, Bachrach, Pursell, and Mills) are great teachers and scientists who inspired me to go to graduate school.

And most importantly, I do not know where I would be without Will, my love and partner who believes in me. He helped me get through candidacy and has shared with me his knowledge of kinetics, data fitting, and biology. He has emotionally supported me throughout. Life is more enjoyable with Will, and Caltech would not have been what it is for me without him.

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