Electrochemical Sensors Based on DNA-Mediated Charge Transport Chemistry

THESIS BY
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Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Chemistry

California Institute of Technology, Pasadena, CA 91125
August 2002
(defended August 7, 2002)

ACKNOWLEDGMENTS

I first want to thank my research advisor, Jackie Barton. Thank you for your enthusiasm and energy, which are contagious, as well as the tremendous encouragement and guidance you have given me. Thanks especially for allowing me to be independent in my research while still insisting on certain things (done yesterday).

I have been exceptionally privileged to work with a number of small school faculty members during my stay at Caltech. In particular I want to thank Mike Hill at Occidental College. Nearly this whole thesis has been completed in close collaboration with him; he has been not only a teacher and collaborator, but also a good friend. Reef Hardy and Eileen Spain are two other members of the faculty at Occidental that have been instrumental in various parts of this thesis work. Working with Eric Stemp, in the cold and dark sub-basement of the BI, I have learned a whole lot about science, but I have also learned, from observation and conversation, about leading a balanced and sane life and viewing one's career as service, wisdom I hope I have taken to heart.

I have terrific committee members in Harry Gray, Fred Anson, and David Tirrell. I want to thank them for taking so much time to really read my props and thesis and also for offering great advice on my current and future work (and also for passing me). Furthermore, I want to thank Mo Renta and Dian Buchness for taking care of so many administrative tasks for me, making my life leagues easier.

To take nothing away from the science done in the Barton Labs, one of my favorite things about working for Jackie is that she attracts a wonderful group of people and I have truly enjoyed my experience in lab for that reason. I am sure I could list everyone that I have worked with over the last five years along with something for which I should thank that person, but for the sake of brevity, here is the short list. Shana Kelley and Scott Rajski helped me to settle in to lab and learn the skills I needed to get started. Donato Ceres and Greg Drummond have been excellent co-DNA electrochemists and friends. Julia Salas is a talented undergraduate student who worked with me for two years. Pratip Bhattacharya, Duncan Odom, Chris Treadway, Melanie O'Neill, Matthias Pascaly, Dave Vicic, Eva Rueba, and Jon Hart have all contributed in various ways to increase both my knowledge of science and research as well as my enjoyment of graduate school. I particularly want to thank Kim Copeland, Sarah Delaney, Megan Nunez and Tashica Williams for their close friendship. Last, but certainly not least, I would like to thank each and every member of The Peasant Cuisine for making the summer of 2002 enjoyable even as I slaved away on my thesis and props.

I have made many great friends at Caltech. I would like to thank Niki Zacharias, Jason Belitsky, Doan and Peter Hackley, Catherine Baker, Soojin Kwon, Mike Farwell, Carlos Bosques, Gabriel Brandt and Jill Sakata for many wonderful lunches, camping, etc. trips, coffee breaks, and conversations. Jae Yoo, Rachel Feldmann, and especially Sarah Delaney and Jill Sakata have been my faithful running buddies over the years, helping me to maintain healthy stress and body fat levels, for which I am very appreciative.

Finally, I want to thank my parents for unconditional love and support and everything else that really matters. Thanks to Middleton and Read for lots of love, making me laugh, and keeping my ego in check. Deciding to come to Caltech was one of the best decisions I have ever made, because it is here that I found my husband. Isaac, thank you for being my best friend and supporter and most stubborn critic; thanks for making me go to lab when I didn't want to and for making me take a break when I needed it. Thanks for promising to love me even if I had failed my thesis defense and run away from Caltech never to be seen in Pasadena again. Mostly, thank you for marrying me and promising love and stick with me forever.

ABSTRACT

The base pair stack within double helical DNA provides an effective medium for charge transport. The π-stacked DNA base pairs mediate charge transport chemistry over long molecular distances in a reaction that is exquisitely sensitive to DNA sequence dependent conformation and dynamics. This sensitivity to minor perturbations in DNA structure and base stacking makes DNA-mediated charge transport chemistry an ideal platform for DNA sensing. Electrochemical methods through DNA-modified electrode surfaces that exploit this sensitivity for efficient biosensing are described. Gold electrodes are modified with DNA double helices and used to monitor the electrochemistry of bound redox active intercalators. The efficiency of electrochemical reduction of the intercalated redox probe, in a DNA-mediated reaction, provides an indicator of base stacking within the surface-bound duplexes. Perfectly stacked DNA is capable of mediating the electrochemical reduction, while duplexes containing π-stacking perturbations, such as single base mismatches, do not support current flow to the intercalator.

This sensitive assay of DNA stacking is improved through electrocatalysis. Electrochemically reduced methylene blue, a redox active intercalator, bound to a DNA film, is capable of reducing solution-borne $Fe(CN)_6^{3-}$. Upon reoxidation, the methylene blue is available for electrochemical reduction and ensuing electrocatalysis. Because the electrochemical reduction of methylene blue takes place by DNA-mediated

charge transport, the π-stack is repeatedly sampled during electrocatalysis, making this assay extremely sensitive to even very minor perturbations in DNA structure and stacking. All single base mismatches, including thermodynamically stable GT and GA mismatches, as well as many common base damage products can be detected within DNA and DNA/RNA hybrid duplexes using this assay. Moreover, mismatches can be detected as a small percentage of a perfectly matched film, making it possible to detect mutations associated with genetic disorders in only a small fraction of cells. This assay is also compatible with DNA based chip technology.

Electrochemical DNA-mediated charge transport on surfaces also provides a tool for directly characterizing small perturbations in DNA stacking and structure. The preferred base stacking orientation of a conformationally constrained nucleotide within A- and B-form DNA duplexes is assayed using electrocatalysis methodology; the conformation of the sugar is seen to sensitively determine the local stacking of the duplex. Furthermore, electrochemistry at DNA films is found to provide a novel and sensitive method for probing protein dependent changes in DNA structure and enzymatic reactions. DNA charge transport chemistry allows the rapid determination of structural perturbations in a DNA site associated with binding of a given protein. Charge transport chemistry also facilitates the real time monitoring of enzymatic reactions on DNA. As DNA-modified electrodes are amenable to array formats, this provides a practical tool for the selection and assay of proteins based upon their sequence specific interactions with DNA as well as a sensitive route to test for inhibitors of such protein-DNA interactions. Hence DNA charge transport not only provides a novel

strategy for the structural analysis of how individual proteins bind DNA but also a remarkably sensitive tool in real time for DNA based proteomics.

Fundamental aspects of this technology are also explored. The alkanethiol tether used to assemble the DNA duplexes on gold electrode surfaces is varied to establish the importance of the length, orientation and flexibility of the linker in forming densely packed DNA films. Results presented here demonstrate that redox probes that bind to DNA by intercalation (themselves becoming a part of the DNA base pair stack) are critical for efficient detection of base stacking perturbations using DNA-mediated charge transport chemistry. An analysis of the kinetics and mechanism of the electrocatalytic assay is also presented. Electrocatalysis requires an intercalator that binds reversibly to the DNA monolayer and in the MB^+/ferricyanide electrocatalysis system, the rate of catalysis depends on the total concentration of MB^+, the rate of MB^+ intercalation and the rate of reduced MB^+ diffusion away from the monolayer.

The efficient transport of charge through self-assembled monolayers of thiol-terminated duplexes on gold therefore offers an extremely sensitive probe for the integrity of DNA sequences. Completely new approaches to single base mismatch detection as well as assaying protein-DNA interactions and reactions on surfaces are now available. This technology is generally applicable as a tool for directly measuring base pair stacking in nucleic acid duplexes.

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