

# Electrochemical Sensors Based on DNA-Mediated Charge Transport Chemistry

THESIS BY  
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## ABSTRACT

The base pair stack within double helical DNA provides an effective medium for charge transport. The  $\pi$ -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  $\pi$ -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  $\text{Fe}(\text{CN})_6^{3-}$ . 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  $\pi$ -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  $\text{MB}^+$  / ferricyanide electrocatalysis system, the rate of catalysis depends on the total concentration of  $\text{MB}^+$ , the rate of  $\text{MB}^+$  intercalation and the rate of reduced  $\text{MB}^+$  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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