

Scanning Electrochemical Microscopy of DNA  
Monolayers Modified with Nile Blue

Krzysztof Slowinski<sup>1</sup>, Alon A. Gorodetsky<sup>2</sup>, William J. Hammond<sup>1</sup>, Michael G. Hill<sup>3</sup>, and Jacqueline K. Barton<sup>2</sup>

<sup>1</sup>Department of Chemistry & Biochemistry,  
California State University, Long Beach, CA 90840

<sup>2</sup>Division of Chemistry and Chemical Engineering,  
California Institute of Technology  
Pasadena, CA 91125

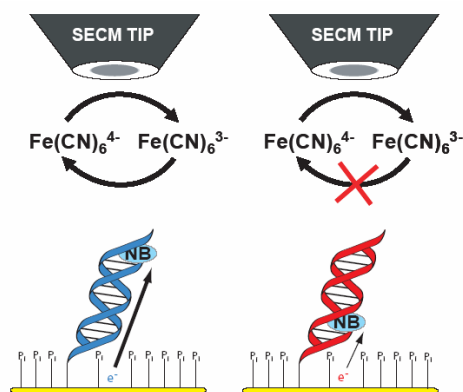
<sup>3</sup>Department of Chemistry, Occidental College  
Los Angeles, CA 90041

The use of scanning electrochemical microscopy (SECM) to probe long-range charge transport across a ds-DNA monolayer containing the redox-active intercalator Nile Blue (NB) covalently attached to discrete sites within the individual DNA helices is reported. By coupling Nile Blue with ferricyanide in an electrocatalytic cycle (Figure 1), the NB-DNA monolayers can be interrogated with SECM.

The cyclic voltammetry of DNA films modified with NB at the top or bottom of the DNA monolayer show a reversible redox couple at -240 mV vs. Ag/AgCl representing the reduction/oxidation process of NB. The SECM feedback mode allows charge transfer across the NB modified DNA monolayers to be probed as a function of the both NB geometric position within the film and its redox state. Here, the  $\text{Fe}(\text{CN})_6^{4-}$  dissolved in a solution is oxidized at a Pt SECM tip yielding steady-state diffusionaly controlled current. The SECM tip is then brought to the DNA covered surface at a constant speed and the current-distance feedback curve is recorded. For DNA films containing the NB intercalator attached in the "bottom" position within the DNA helix, negative feedback is found regardless of the substrate bias consistent with the pure negative feedback response over an insulator. This behavior, reported previously for unmodified DNA films, is consistent with restricted access of  $\text{Fe}(\text{CN})_6^{3-}$  due to the electrostatic repulsion between the negatively charged phosphates and the SECM tip generated probe.

A markedly different behavior is observed for DNA films modified with top-NB. For those films polarized to potentials allowing reduction of the NB intercalator, positive feedback is obtained indicating significant charge transfer between the SECM tip generated  $\text{Fe}(\text{CN})_6^{3-}$  and the DNA covered gold surface. This behavior is consistent with the regeneration of the mediator via a bimolecular reaction with the surface-bound NB: the reduced form of NB reacts with the tip generated  $\text{Fe}(\text{CN})_6^{3-}$  leading to regeneration of  $\text{Fe}(\text{CN})_6^{4-}$  and resulting in a positive feedback observed in the approach experiment. Significantly, only Nile Blue attached at the solution-exposed periphery of the film is capable of supporting catalytic regeneration of the redox mediator present in the solution, confirming that Nile Blue-DNA monolayers adopt an upright orientation and that charge transfer proceeds via a DNA mediated pathway.

To gain further insight into the morphology of NB-DNA monolayers, the imaging mode of the SECM was utilized to examine top-NB and bottom-NB DNA films. The analysis of a large collection of images indicates that



**Figure 1.** Schematic illustration of SECM imaging of DNA monolayers modified with Nile Blue (NB) at the top or the bottom; the negatively charged backfilling underlayer is also shown. The sequence was 5'-NGC GTG CTT TAT ATC TC-3' (top NB) and 5'-TGC GTG CTT TAT ATC NC-3' (bottom NB) where the bold N indicates the location of the NB moiety.

some electrodes are completely and uniformly covered by a DNA film. On the other hand, some images indicate that parts of the electrode surface are either DNA-free or contain thinner and less organized DNA assemblies. In fact, such partially covered or imperfect DNA monolayers were found to correlate well with incomplete passivation of ferricyanide at the substrate, allowing for a detailed analysis of these surfaces. The analysis of images for the bottom-NB DNA film indicates that the modulation of the substrate bias had a small effect on the steady state current at the tip with nearly identical images at 0 mV and -400 mV. For the top-NB DNA film, altering the substrate bias from 0 mV to -400 mV caused the entire area under the scan to "switch" with a significant enhancement of the steady state current. These observations are in close agreement with those obtained via the feedback mode SECM. Notably, the most efficient "switching" occurs within these regions of the DNA film that exhibit the lowest currents at substrate potentials of 0V. Clearly, the SECM allows for investigation of *imperfect* DNA films since it affords the ability to *differentiate* between catalytically active DNA spots and defects within the film. This is a significant advantage of SECM which is not shared by conventional voltammetric techniques.

In summary, at substrate potentials negative of the formal potential of covalently attached NB, the electrocatalytic reduction of  $\text{Fe}(\text{CN})_6^{3-}$  generated at the SECM tip is observed only when NB is located at the DNA/solution interface; for DNA films containing NB in close proximity to the DNA/electrode interface, the electrocatalytic effect is absent. This behavior is consistent with both rapid DNA-mediated CT between the NB intercalator and the gold electrode and a strongly distance-dependent electron transfer between NB and the solution phase  $\text{Fe}(\text{CN})_6^{3-}$ . The NB/ferricyanide catalytic cycle furthermore allows for the local investigation of the surface characteristics of DNA monolayers. These experiments highlight the utility of DNA-modified electrodes as flexible platforms for SECM detection schemes that take advantage of charge transfer mediated by the DNA base pair stack.