Spectroelectrochemistry in Low-Dimensional NanoOptoFluidic Devices for Chemical and Biochemical Sensing

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We are interested in fabricating nanostructured architectures so that molecular transport (analyte/reagent delivery), chemical sensing (optical or electrochemical) and subsequent chemical conversion can all be coupled in the same physical construct. The ultimate goal of these experiments is to produce chemical reagents *in situ* and consume them directly at a proximal reaction site, so that chemical transformations may be realized with optimal efficiency. To realize this goal, we are developing sensing strategies that efficiently match electron transfer and spectroscopic probing to low-dimensional, *i.e.* zero- and one-dimensional nanostructures.

In one version of the experiment, vertically-oriented nanopores provide fluidic communication in nanocapillary array membranes supporting embedded annular nanoband electrodes (EANEs) fabricated on the interior of the nanopores. In these structures, electroosmotic flow (EOF) is used to enhance the delivery of electroactive species to the EANE, and the same potential used to drive EOF also provides for electron transfer. Because transport and electron transfer are intimately coupled, high efficiency electrochemical conversions can be achieved. Conversion efficiency is improved by approximately 10-fold compared to a comparable microfluidic structure.

In a different experiment, we exploit the localization of optical fields to reduce spectroscopic background signals and enable studies of single electroactive fluorophores. Zero-mode waveguides (ZMWs) strongly confine optical fields to zeptoliter volumes and can be coupled with fluorescence microscopy to study the dynamics of single enzyme molecules, due to their excellent optical confinement, precise positioning, and massive parallelism. The redox enzyme, monomeric sarcosine oxidase (MSOX) contains a covalently bound flavin adenine dinucleotide (FAD) cofactor which is highly fluorescent in the oxidized state and dark in the reduced state, thus producing a characteristic on-off fluorescence signal synchronous with transitions between oxidation states. For MSOX reactions involving both the nominal substrate (sarcosine) and an analogous substrate (proline), statistical analysis of single-molecule temporal trajectories reveal the static heterogeneity of single enzyme reaction rates, but no dynamic disorder. In addition, the single molecule data confirm the independence of reductive and oxidative reactions. These structures open the way for systematic studies of the effect of molecular crowding on enzyme dynamics.

Furthermore, the electron transfer can occur between solution-phase substrate and immobilized enzyme, or, if redox species are immobilized on the metallic sidewalls of the ZMW, it is possible to observe the single molecule fluorescence signatures resulting from direct heterogeneous electron transfer from metallic electrodes to single redox-active molecules. Starting with a relatively simple construct based on the MSOX-FAD-$FADH_2$ system, we have isolated the FAD cofactor on the surface of the Au sidewalls of the ZMWs. A Au surface is derivatized with a self-assembled monolayer (SAM) presenting a terminal amine for derivatization with (1) pyrroloquinoline quinone, PQQ, and (2) *m*-aminophenylboronic acid (APBA), which then binds the sugar ring of FAD. We have prepared ZMW arrays with a significant number of single FAD chromophores bound to the Au sidewalls (see Figure). Experiments to illustrate potential control over electron transfer to single FAD molecules in ZMWs suggest that the transition rates between luminescent (oxidized, FAD^+) and dark (reduced, $FADH_2$) states can be modulated with electrochemical potential.