Direct Write Photocrosslinked Assembly of Functional Bioelectronic Materials

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Improved, direct electrochemical wiring between biomaterials and electrodes is fundamental to the progression of bioelectronics technology. Because the redox-active centers of proteins are often buried deep within the protein structure, most proteins do not exhibit facile electron transfer upon adsorption to a conductive substrate. Consequently, proteins and enzymes are often "wired" to electrode supports to enable more efficient electron transfer. Some methods currently used for such wiring include tethering biomaterials to the electrode via electron relay units and reconstitution of apo-enzymes onto cofactor-functionalized electrodes (1). However, both of these methods require extensive modification of either the electrode or biomolecule prior to electrochemical analysis in the form of elaborate chemical synthesis, leaving a more direct wiring route to be desired. The ability to prepare functional, conductive bio-architectures directly onto electrodes, without extensive modification schemes, would provide a more efficient alternative to current methods for biomolecule immobilization.

In this presentation, we describe the use of multiphoton excitation (MPE) techniques (2) for direct patterning of bioelectronic components. This approach relies on the high intensity dependence of MPE to confine photochemical reactions to an ultrasmall focal volume (~1 fL) (Fig. 1). Briefly, a femtosecond pulsed laser is directed into an inverted microscope containing a high NA objective, and structures are "written" by photo-induced crosslinking between protein and photoinitiator. Translation of the reaction volume allows for construction of novel bioelectronic structures. We have recently been successful in preparing immobilized cytochrome *c* (cyt *c*) assemblies on conductive, indium tin oxide (ITO) substrates (Fig. 2). Cyt *c* is often studied electrochemically due to its natural conductivity (3); thus our chosen cyt *c* model system can be compared to previously published reports for adsorbed and solution-phase cyt *c* (4). We demonstrate that immobilized cyt *c* remains redox-active after photo-induced crosslinking. As shown in Figure 3, a quasireversible voltammetric response is observed, consistent with literature reports (4) of similar systems. Remarkably, the redox activity remains stable over hours of extended potential cycling. Comparison of the surface coverage calculated from voltammetric measurements to in situ AFM geometrical estimates suggests that nearly all of the cyt *c* molecules remain electroactive. Additionally, AFM measurements indicate that the structures are ~500 nm thick, corresponding to hundreds of cyt *c* monolayers. As expected for a structure of such thickness, the apparent standard ET rate constant, k^o, is slower than that observed for adsorbed monolayers of cyt *c* (0.6 s^{-1} vs. 18 s^{-1}, respectively (4a)).

References:

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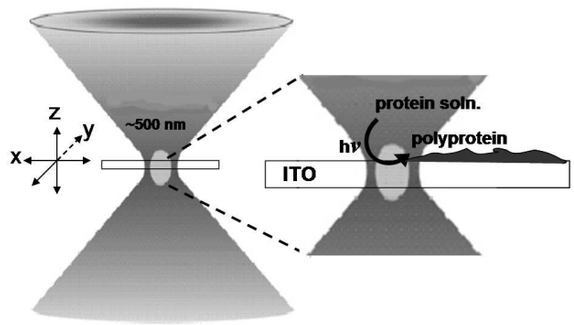

Figure 1. Multiphoton excitation (MPE) fabrication of photcrosslinked protein structures. MPE is contained within the volume depicted by the light grey oval (~1 fL).

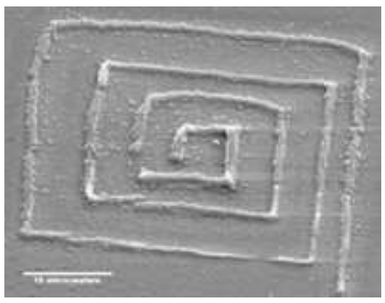

Figure 2. SEM image of MPE-photocrosslinked cyt *c* structure prepared on an ITO electrode. Scale bar is 10 μm.

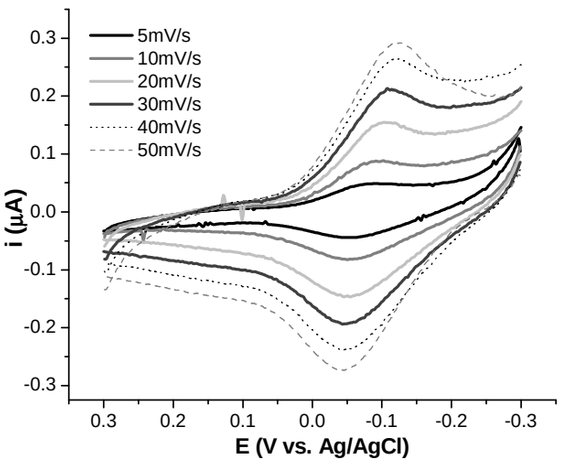

Figure 3. Cyclic voltammograms of photocrosslinked cyt *c* on an ITO electrode.