Nanopatterning of Bioelectronic Components via Multiphoton-Induced Photocrosslinking

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Efficient electron transfer is often compromised in bioelectronic systems due to protein/enzyme denaturing upon immobilization at the electrode surface (1). To address this challenge, biomaterials may be either co-immobilized with moieties such as redox polymers or directly "wired" to the electrode surface in such a manner as to prevent denaturing. For example, effective wiring has been achieved by reconstitution of apo-enzymes onto cofactor-functionalized electrodes (2). Nevertheless, current immobilization and wiring methods are nontrivial in that they require extensive modification of either the electrode or biomolecule. The ability to immobilize functional biomaterials directly onto electrodes without intensive processing of either electrode or biomolecule should facilitate more effective electrochemical communication, as well as provide a more efficient preparation route for assembly of bioelectronic components.

Currently, we are exploring the feasibility of using multiphoton excitation (MPE) (3) as a means for direct assembly of functional bioelectronic components on unmodified electrode supports. Various proteins deposited onto electrodes in this manner have been shown to retain their chemical functionalities despite the use of high intensity lasers (3b,c). For bioelectrode fabrications, described herein a femtosecond pulsed laser is directed into an inverted microscope containing a high NA objective, and photocrosslinked structures are directly "written" by using a mechanical xy stage to scan the laser beam across the surface of an ITO-coated coverslip immersed in a solution containing the protein and a photoinitiator.

In this presentation, we describe the electrochemical characterization of novel, redox-active and conductive photocrosslinked protein structures. Additionally, the novel structures' morphologies have been elucidated using SEM and in situ AFM. In particular, we have extensively investigated direct electron transfer between photocrosslinked cytochrome *c* and an ITO substrate. The electrochemical data obtained from our structures is in close agreement with previously published findings (4). We also present biometallic conduits comprised of metal nanowires selectively bound to photocrosslinked protein structures, a schematic of which is shown in Fig. 1. Specifically, we have used avidin-biotin interactions to link protein-conjugated Au nanoparticles to photocrosslinked bovine serum albumin (BSA) structures functionalized with the complementary protein. Silver or Au electroless deposition solutions are then used to selectively overcoat nanoparticle-modified BSA structures to form continuous metallized nanowires (Fig. 2). We are currently investigating the compatibility of these materials in living cell environments. One goal is to use these functional bioelectronic assemblies as microenvironmental sensors for localized monitoring of cellular processes.

References:

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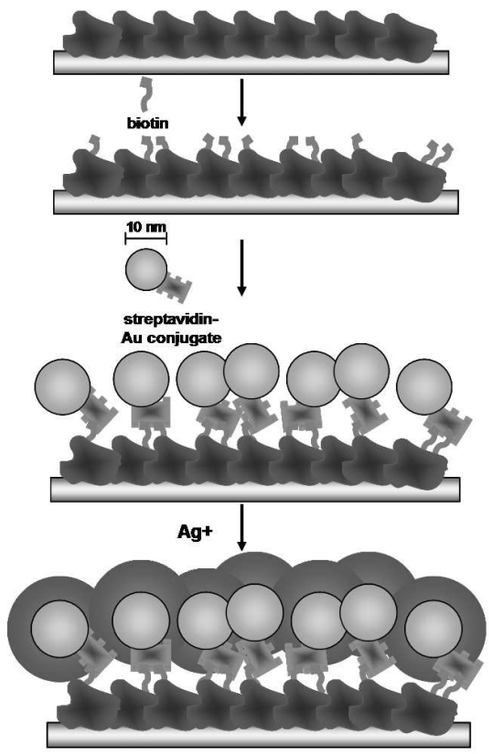

Figure 1. Schematic of metal deposition onto MPE-photocrosslinked protein structures.

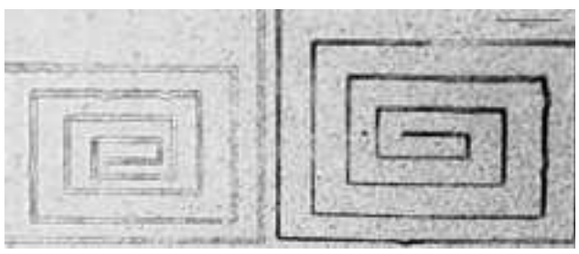

Figure 2. Photocrosslinked BSA structures with (right) and without (left) biotin-bound streptavidin-Au conjugates, immersed in Ag deposition solution for ~12 min.