Communication

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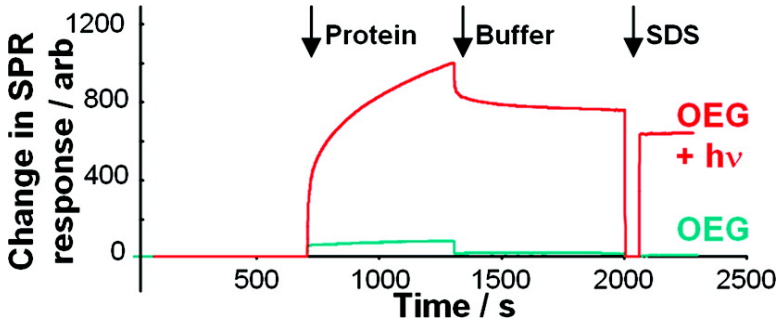

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One-Step Photochemical Introduction of Nanopatterned Protein-Binding Functionalities to Oligo(ethylene glycol)-Terminated Self-Assembled Monolayers

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The problem of nonspecific adsorption, in which biomolecules adsorb via physical forces of attraction to solid surfaces, is a ubiquitous one in the design of biochips and other devices that require precision placement of biological entities such as proteins, oligonucleotides, and cells. Proteins, in particular, adsorb irreversibly to most surfaces. Oligo(ethylene glycol) (OEG)-terminated self-assembled monolayers (SAMs) of alkylthiols on gold surfaces[1,2] have become the "gold standard" for protein resistance. Extensive studies by Whitesides and co-workers have established that protein adsorption on OEG-terminated SAMs is effectively undetectable within the sensitivity limits of surface plasmon resonance (SPR).[3] Consequently, there has been a great deal of interest in the use of OEG-terminated SAMs for applications in which nonspecific adsorption must be eliminated.

There is widespread interest in the formation of nanostructured biological materials, for new applications in sensing and in fundamental studies of interfacial phenomena,[4−10] prompting a search for versatile methods for patterning the adhesiveness of materials toward protein adsorption. The most successful strategies to date have been based upon OEG-terminated SAMs,[11,12] but typically, covalent attachment of proteins using such approaches has involved the execution of several sequential steps (including patterning of the OEG-terminated adsorbates and the subsequent introduction of contrasting species to which biological species may be attached).[12,13] In this communication, we demonstrate that photochemical modification of OEG-terminated SAMs facilitates the patterning of surface adhesiveness and the introduction of groups capable of covalent anchoring of biomolecules in a single step. The covalent attachment of streptavidin is demonstrated. The simplicity of the process makes it an attractive one for many potential applications.

On exposure to UV light with a wavelength of ca. 250 nm, alkylthiolate adsorbates are converted photochemically to alkylsulfonates.[14] Recently, however, it was suggested that an alternative, competitive process occurs when OEG-terminated SAMs are exposed to UV light, leading to the degradation of the OEG chain.[13] Cai and co-workers similarly noted that OEG degradation occurred during exposure of OEG-terminated monolayers on Si to an AFM tip biased relative to the substrate.[15] We have found that the OEG degradation process is in fact substantially quicker than the photoconversion of the adsorbate head group to a sulfonate and, at very short exposures, leads to the introduction of significant numbers of aldehyde groups to the surface. These are able to covalently bind proteins.

(1-Mercaptoundec-11-yl)tri(ethylene glycol) ($C_{11}(OEG)_3$) was synthesized using the method of Pale-Grosdemange et al.[1] Figure 1 shows X-ray photoelectron spectroscopy (XPS) data of fresh and exposed monolayers.[16] To demonstrate the capacity of the surface aldehyde groups for covalent attachment, samples were incubated with a 10 mM solution of 2-amino-1,1,1-trifluoroethane. Spectrum

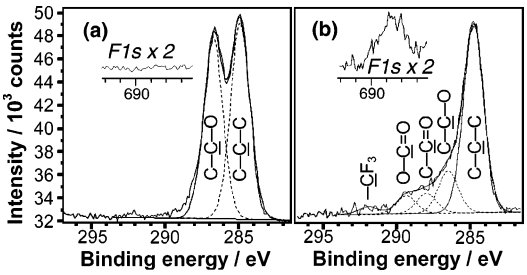

Figure 1. C1s and (inset) F1s XPS spectra of (a) and OEG-terminated SAM; (b) an OEG-terminated SAM exposed to illumination at 254 nm following exposure to a solution of a fluorinated primary amine.

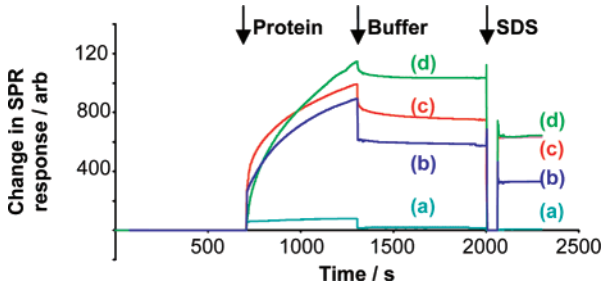

Figure 2. SPR traces for (a) an OEG-terminated SAM; (b) the *N*-hydroxysuccinimidyl ester of mercaptoundecanoic acid; (c) an OEG-terminated SAM following exposure to UV light at 0.4 J cm^{-2}; (d) an OEG-terminated SAM following UV exposure and activation of EDC/NHS.

(a) is the C1s spectrum of the as-received monolayer and exhibits large components due to the alkyl chain and ether carbon atoms at 285.0 and 286.6 eV, respectively. After a UV exposure of 4 min at 96 mW, the spectrum is significantly different (Figure 2b). The ether component is substantially reduced in size, and new components are evident at 287.8 and 289.4 eV, corresponding to C−C═O and O−C═O species, respectively. A small peak at 292.3 eV corresponds to the CF_3 carbon atom, indicating covalent attachment of the amine to the surface, presumably via imine bond formation. The ratio of this peak to the main peak at 285 eV is 1:25, suggesting that ca. 1 in 3 adsorbates has been derivatized and confirming that OEG-terminated SAMs covalently bind amines following UV exposure.

Protein binding was studied using SPR (Figure 2). For the virgin OEG-terminated monolayer (trace (a)), injection of a solution of streptavidin (100 μg mL^{-1} in phosphate-buffered saline solution (PBS)) led to a small rise in the SPR signal, which was reduced to zero on injection of further buffer, reflecting the weak interaction between the protein and the OEG-terminated surface even at this high concentration. Trace (b) was acquired for a monolayer of mercaptoundecanoic acid that was activated, following procedures similar to those used by other workers, by immersion in a solution of *N*-hydroxysuccinimide (NHS, 20 mM) and 1-ethyl-3,3-dimethyl

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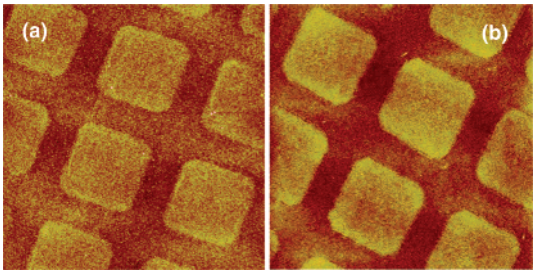

Figure 3. Tapping mode ($40 \times 40\ \mu m^2$) AFM images of OEG-terminated monolayers after exposure to UV light through a mask and immersion in solutions of streptavidin (a) and IgG (b); *z*-range: 0−10 nm.

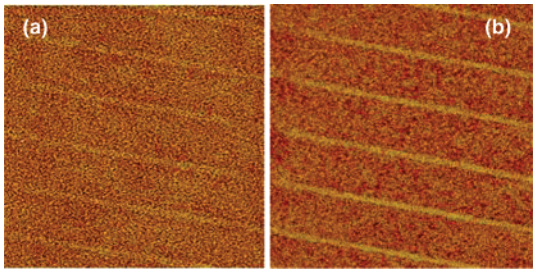

Figure 4. Tapping mode height (a) and phase (b) images of nanolines formed by exposure of OEG-terminated SAMs using SNP and immersion of the samples in a solution of streptavidin. Image sizes: $10 \times 10\ \mu m^2$; *z*-range: 0−10 nm (a) and 0−120° (b).

carbodiimide (EDC, 20 mM),[13] to yield protein-binding active ester groups. The initial rise in the SPR signal is much larger for this sample than for the OEG-terminated SAM. A small fall is observed in the SPR signal following injection of buffer, indicating elution of some weakly bound protein. To ensure complete removal of noncovalently bound proteins, the sample was further exposed to a 3% solution of sodium dodecyl sulfate (SDS). A slightly larger reduction is observed in the SPR signal after injection of the detergent, but a substantial fraction of the streptavidin clearly remains at the surface, indicative of covalent attachment.

Trace (c) shows the behavior observed for an OEG-terminated SAM exposed to a UV dose of 0.4 J cm^{-2} and rinsed in PBS. The initial rise in the SPR signal is similar to that measured for the active ester surface. A small reduction in the signal occurs after injection of buffer, and a further small fall occurs following introduction of SDS. However, the SPR signal at the end of the procedure is larger than that measured for the active ester surface, indicating a high degree of efficiency of attachment despite the much greater simplicity of the process. The failure of the SDS to displace the streptavidin from the surface indicates a strong bond between the protein and the modified SAM, strongly suggesting that extensive covalent attachment has occurred. Trace (d) suggests that activation of the carboxylate groups formed following UV exposure leads to no further increase in binding.

Micrometer-scale patterns were readily formed by exposure of OEG-terminated SAMs to UV light through an electron microscope grid for 90 s, at a power of 100 mW (Figure 3). This exposure is significantly less than that required for extensive photo-oxidation of the adsorbate head group (ca. 8 min). The sample was then rinsed in PBS buffer and immersed in a solution of streptavidin in HEPES-buffered saline solution containing 0.1% bovine serum albumin for 10 min. The sample was removed and rinsed before being imaged by atomic force microscopy (AFM) in tapping mode. The height contrast is clear. The mean heights of the features (1.2−2 nm) were consistent with the attachment of no more than a monolayer of streptavidin. To test for retention of biological activity by the bound streptavidin, binding of biotinylated IgG was measured (see Supporting Information).

Nanometer-scale patterns were fabricated using scanning near-field photolithography (SNP), in which the sample is exposed to the evanescent field associated with the aperture of a scanning near-field optical microscope (SNOM). Because the exposure occurs in the optical near-field, the diffraction limit associated with conventional optical processes is lifted and high resolution may be achieved (as good as $\lambda/30$). SNP of OEG-terminated monolayers to UV light presents an attractive single-step route to protein-binding nanostructures. Figure 4 shows tapping mode height and phase images of structures formed by writing a series of lines in an OEG-terminated SAM and immersing the sample in a solution of streptavidin. In this case, the lines have a full width at half-maximum height of 150 nm. The contrast is superior in the phase image, likely because the height contrast in a feature so small in a $10 \times 10\ \mu m^2$ image is small given the intrinsic roughness of the polycrystalline substrate. The phase image displays clear contrast because of the differences in the rates of energy dissipation during contact between the tip and the protein-covered and monolayer regions of the sample.

In summary, these data indicate that UV modification of OEG-terminated SAMs presents a simple, one-step route to the introduction of functional groups capable of covalently anchoring proteins that should have widespread utility. The method facilitates the formation of nanometer-scale patterns. Given that the process does not depend upon the modification of the Au−S bond, it seems likely that the methodology may be extended to monolayers of OEG-terminated adsorbates on other substrates.

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Supporting Information Available: AFM measurements of the binding of biotinylated IgG to immobilized streptavidin. This material is available free of charge via the Internet at http://pubs.acs.org.

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