Company, are also appreciated.

Registry No. I, 140925-97-9; II, 140925-99-1.

Supplementary Material Available: Crystallographic details including positional parameters, thermal parameters, intra- and intermolecular bond lengths and bond angles, and unit cell drawings, experimental section for I and II, and listings of bond distances of the third form of CHD and CHD derivatives (26 pages); listing of observed and calculated structure factors for I and II (22 pages). *Ordering information is given on any current* masthead page.

New Approach To Producing Patterned Biomolecular Assemblies

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Received February 3, 1992

A key requirement to the integration of biomolecules into complex structures and materials is the ability to specify the site of immobilization, preferably on a submicron scale.[1] A major difficulty in immobilizing proteins in specific patterns on a surface is not the chemistry required for immobilization but the methodology for preventing proteins from absorbing to unwanted regions. Previous methods for forming protein patterns required lift-off procedures or exposure to denaturants to remove proteins adsorbed in the wrong areas.[2-6] We have created photolithographically patterned surfaces with clearly defined regions of high and low protein adsorptivity by exposing silica surfaces treated with (3-mercaptopropyl)trimethoxysilane (MTS) to UV light through a mask. Upon exposure of an MTS-coated surface to UV light in the presence of oxygen, X-ray photoelectron spectroscopy (XPS) indicates that the thiol terminal is oxidized. The resulting surface is resistant to protein adsorption. Antibodies immobilized in patterns using this method retain their antigen binding capability.

One approach to the creation of a self-assembling three-dimensional structure using biomolecules as building blocks begins with a two-dimensional template which has the ability to direct the construction of subsequent layers. For making patterns of biomolecules, such a template must include areas which resist nonspecific adsorption. To this end, we investigated the extent of protein adsorption on MTS, (3-aminopropyl)trimethoxysilane, [3-[*N*-(2-aminoethyl)amino]propyl]trimethoxysilane, [3-(glycidyloxy)propyl]trimethoxysilane, and (tridecafluoro-1,1,2,2-tetrahydrooctyl)dimethylchlorosilane films before and after deep UV exposure. Glass surfaces coated with silane films were irradiated with a low-pressure Hg(Ar) lamp for 10 min and incubated with ^{125}I-labeled goat immunoglobulin G (IgG). The amounts of radiolabeled protein adsorbed onto the unirradiated and irradiated surfaces were calculated in each case using radioactive counts from the scintillation counter.[7]

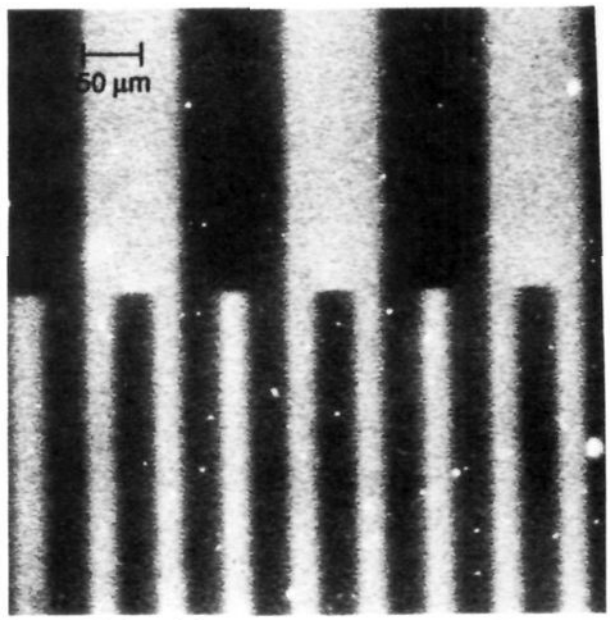

Figure 1. High-resolution pattern of fluorescent protein. Phycoerythrin was covalently immobilized[9] on a patterned thiol silane film and examined using confocal fluorescence microscopy.

With amine, epoxy, and perfluoro silane films, little or no change in the protein adsorption occurred as a function of irradiation. However, the amount of radiolabeled protein adsorbed on MTS dropped from 1.2 ± 0.2 ng/mm^2 to 0.17 ± 0.02 ng/mm^2 following irradiation, corresponding to an 86% reduction compared to the unirradiated surface. In similar experiments, irradiation of an MTS-coated slide reduced adsorption of BSA, a protein well-known for its propensity to adhere to surfaces,[8] by 75% and that of glucose oxidase by 90%. Subsequent treatment of irradiated MTS films with the heterobifunctional cross-linker *N*-[(γ-maleimidobutyryl)oxy]succinimide (GMBS) did not increase the amount of radiolabeled IgG bound to the irradiated surface (0.15 ± 0.02 ng/mm^2). Thus, the thiol groups with which GMBS specifically reacts[9,10] were no longer available after irradiation of the coated surface.

Patterns of covalently attached proteins were produced by placing a high-resolution mask in mechanical contact with an MTS-coated substrate and irradiating the surface with UV light. The irradiated films were then incubated separately with and without GMBS and a fluorescent protein, phycoerythrin.[11] Subsequent visualization using a confocal fluorescence microscope indicated selective attachment of the protein to the unirradiated areas (Figure 1).

Irradiation of an MTS-coated substrate through a low-resolution mask and incubation with GMBS[9] and radiolabeled IgG resulted in selective attachment of the antibody to unirradiated areas visualized by autoradiography. Retention of function following covalent immobilization was also demonstrated by attaching the unlabeled antibody, rabbit anti goat IgG, to a patterned silane film using GMBS. Radiolabeled antigen, goat IgG, was then added. Autoradiographs indicated that antibody immobilized in the regions protected from UV light was still capable of binding

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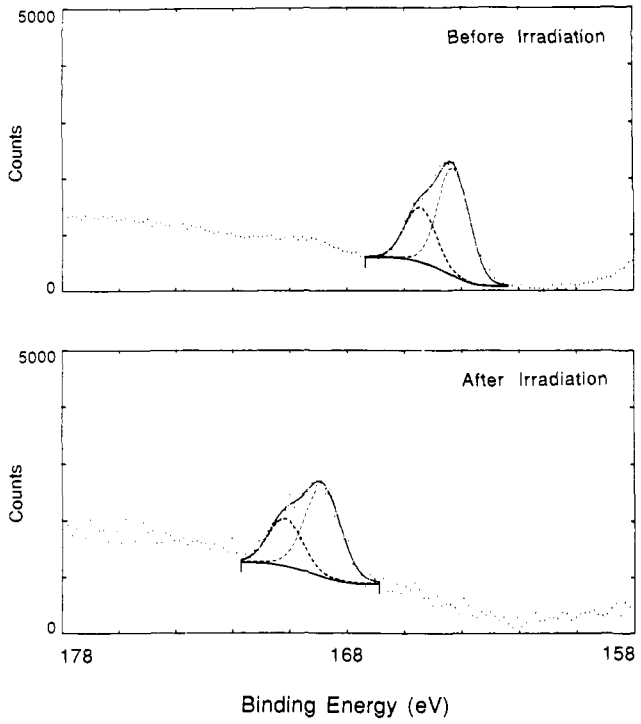

Figure 2. Oxidation of thiol to sulfonate. The XPS spectra of MTS monolayers are shown before (top) and after (bottom) irradiation with a Hg(Ar) UV light source. X-ray damage was minimized by limiting acquisition time. The take-off angle was 35°, and the operating pressure was less than 10^{-8} Torr. All spectra were referenced to the Si 2p(3/2) peak of the quartz sample, and charging problems were neutralized with a 2.7-eV electron beam.

antigen, while very little antigen was present in the areas exposed to UV light.

To characterize the film surfaces before and after irradiation, contact angle measurements and X-ray photoelectron spectroscopy (XPS) were used. Using the sessile drop method,[12] the water contact angle on an unirradiated thiol surface was 58 ± 3°. This was reduced to 30 ± 6° after irradiation. Previous work involving the irradiation of aromatic, amine, and other silane monolayers with UV light has shown that a photocleavage reaction results in contact angles of 10° or less.[13-17] XPS was used to investigate the chemical nature of the irradiated silane surface because the contact angle measurements suggested an alternate mechanism than photocleavage. Figure 2 shows the S 2p region before (top) and after (bottom) irradiation. As can be seen, the reduced thiol at 164 eV is not removed by irradiation but quantitatively converted to an oxidized form of sulfur at 169 eV. This is consistent with the conversion of the thiol to a sulfonate group.[18]

The technique outlined here for the preparation of protein patterns on a surface circumvents many of the problems encountered in earlier attempts at protein patterning,[2-4] because the irradiated thiol silane inhibits nonspecific protein adsorption. This sulfonated surface may have further applications in the protection of implants or prostheses against biofouling[19] and in the protection of critical sensor components during short-term clinical or environmental use. We have also developed a means of patterning a silane film prior to exposure of the surface to proteins which prevents any possible protein denaturation due to exposure to UV light. Such patterns may be useful for building multifunctional biosensors on a single substrate. Furthermore, because photolithography using ultrathin films has been proven to produce submicrometer patterns,[13,15] this technique should be usable to achieve patterns at much smaller dimensions.

Acknowledgment. We thank J. M. Calvert, C. S. Dulcey, P. E. Schoen, J. H. Georger, L. C. Shriver-Lake, and M. A. Anderson for their contribution to ideas and technical support. Financial support was provided by the Office of Naval Research, through the Naval Research Laboratory. The views, opinions, and/or findings contained in this report are those of the authors and should not be construed as an official Department of the Navy position, policy, or decision.

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Far-Infrared Magnetic Resonance of Matrix-Isolated Nickelocene

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Received February 18, 1992

The large value of the zero-field splitting (zfs) parameter, D, of nickelocene (bis(cyclopentadienyl)nickel) prevents this triplet ground-state molecule from being observed by conventional electron-spin-resonance (ESR) spectroscopy. Here D has been directly determined in argon and krypton matrices at 4 K by far-infrared absorption in applied magnetic fields up to 4 T. D is customarily obtained from magnetic susceptibility (χ)[1] or inelastic neutron scattering (INS) data[2] on the solid powder or single crystal over a range of temperatures. In the case of nickelocene, a series of investigations have culminated in the thorough studies of Baltzer et al.,[3] where χ was measured on a diamagnetic host crystal (ruthenocene or ferrocene) doped with varying concentrations (up to ~6% by weight) of nickelocene. From these measurements, a value of the zfs parameter $D_0 = +33.6 \pm 0.3$ cm^{-1} for isolated nickelocene was deduced by extrapolation to infinite dilution. Also, a value of 31.6 ± 1.0 cm^{-1} was obtained from INS measurements, but it was not considered as comparable since it was not for an isolated molecule and could also show some influence of deuterated ligands.[3]

Some time ago, Brackett, Richards, and Caughey[4] measured the far-infrared transmission spectra of polycrystalline compounds and directly obtained D (and E) for magnetic ions in molecular sites with large ligand fields. Here we have extended their procedure to study matrix-isolated molecules so as to obtain essentially unperturbed molecular parameters.

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