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J. Am. Chem. Soc., **2007**, 129 (30), 9252-9253• DOI: 10.1021/ja072250m • Publication Date (Web): 10 July 2007

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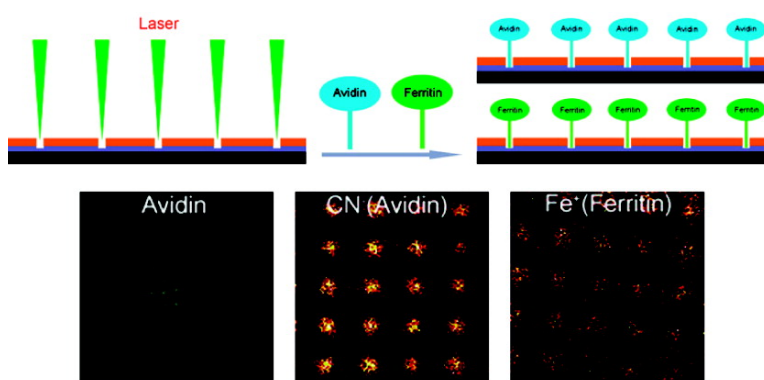

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Published on Web 07/10/2007

Direct Adsorption and Detection of Proteins, Including Ferritin, onto Microlens Array Patterned Bioarrays

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A variety of methods have been described for preparing protein arrays,[1−4] including photolithography,[5−7] microcontact printing,[8] microspotting,[9] pin spotting,[10] and microfluidics.[11] These methods allow different substrate properties and attachment chemistries.[12,13] However, many of the arrays that have been described cannot be made in an industrially viable manner. For real world use, a protein array would be simple and inexpensive to manufacture, its fabrication amenable to automation, the size and shape of the features could be controlled, and the resulting arrays could be used for high throughput and rapid analyses.

Here we describe a technologically viable platform for producing protein arrays that appears to possess all of these virtues. This method consists of coating a silicon oxide surface with a polyethylene glycol (PEG) terminated silane monolayer, known to resist protein adsorption,[14] and then modifying it by microlens array (MA) patterning.[15] In MA patterning, an MA is positioned a short distance over a monolayer-coated substrate. A nanosecond pulse of laser light is then directed through this optic. Each lens in the MA focuses the light it receives onto the substrate, which burns away the protective monolayer near the focus of the light. In this manner 10000 spots/cm^2 (for 100 μm spacing between microlenses) can be made on a surface in ca. 4 ns. Arrays of microbeads have also been employed for surface micromachining.[16,17] The exposed spots in the monolayer show excellent affinity for the direct adsorption of proteins, while the background PEG layer maintains excellent resistance to protein adsorption. Protein adsorption and/or surface modification are confirmed by time-of-flight secondary ion mass spectrometry (TOF-SIMS), which is a powerful tool for analysis of immobilized proteins,[18] as well as X-ray photoelectron spectroscopy (XPS), spectroscopic ellipsometry (SE), fluorescence microscopy, and wetting. It is shown that avidin retains its activity after immobilization. All of the immobilized proteins show good stability to soaking in buffer. Protein localization using a microfluidic spotter is also shown. Finally, we demonstrate that ferritin adsorbs onto PEG coated substrates after MA patterning and that TOF-SIMS reveals that the metal atoms are located inside the protein shell and not at the surface of the protein.

We begin by noting that the PEG terminated monolayers used for MA patterning exhibit the expected resistance to protein adsorption. To within experimental error, spectroscopic ellipsometry showed no change in PEG monolayer thickness after immersion in dilute protein solutions. The protein resistance of these films was further confirmed by XPS, which showed no N 1s signal from PEG monolayers that were immersed in solutions of proteins, but strong N 1s signals from bare, clean silicon oxide control surfaces.

PEG monolayer coated Si/SiO$_2$ slides were then patterned with an MA by placing it over the substrate and firing a 4 ns pulse of 532 nm laser light through the optic. TOF-SIMS ion images of H$^-$, CH$^-$, CH$_2^-$, OH$^-$, C$_2$H$^-$, and the total ion image showed good contrast between the spots and the backgrounds, that is, the spots and backgrounds were chemically distinct. Almost no contrast and little signal was found for the CN$^-$ (see Figure 1a) and CNO$^-$ ions on this surface, which are characteristic of proteins.[19] MA patterned PEG monolayers were then immersed in solutions of various proteins chosen to have a wide range of pI values and molecular weights. All of the proteins studied adsorbed to the spots with strong preference over the background, as shown by the CN$^-$ (see Figure 1) and CNO$^-$ ions in TOF-SIMS imaging of these surfaces. This adsorption appears to be general and nonspecific and based on van der Waals and electrostatic interactions with the exposed substrate. As suggested in the figure, the size of the spots could be controlled by changing the laser power and the focus of the MA.[15] The S$^-$ ion image also showed good contrast in a number of the protein array images. Among the proteins studied were some with useful function in bioconjugate chemistry. For example, avidin and streptavidin have a well-known, and high affinity for biotin. Protein A binds IgG antibodies, and BSA is employed as a blocking agent in enzyme-linked immunosorbent assays (ELISA).

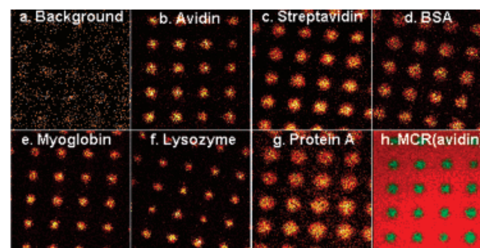

Figure 1. TOF-SIMS negative ion, CN$^-$, images (500 μm × 500 μm) of (a) a PEG silane monolayer patterned with a microlens array, and (b−g) a PEG silane monolayer patterned with a microlens array after immersion in a solution of the protein indicated in each panel. Panel h shows an AXSIA multivariate curve resolution MCR analysis of the negative ion spectra from the avidin image.

Multivariate curve resolution (MCR) of the TOF-SIMS images further confirmed protein adsorption in the spots and not in the backgrounds of the arrays. MCR, which has been shown to be a valuable tool for TOF-SIMS image analysis,[20] was possible because an entire mass spectrum was saved at each pixel in the raw data file. MCR was performed on all of the spectral images of all of the adsorbed proteins in MA patterned protein arrays. A representative example of these results is shown in Figures 1h and 2 and demonstrates that the surfaces are primary composed of two surface species: a spectrum corresponding to the PEG background, and a spectrum corresponding to the adsorbed protein. These assignments were confirmed for avidin arrays (see Figure 2) by comparing these two MCR components to the TOF-SIMS spectra of planar Si/SiO$_2$ that

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10.1021/ja072250m CCC: $37.00 © 2007 American Chemical Society

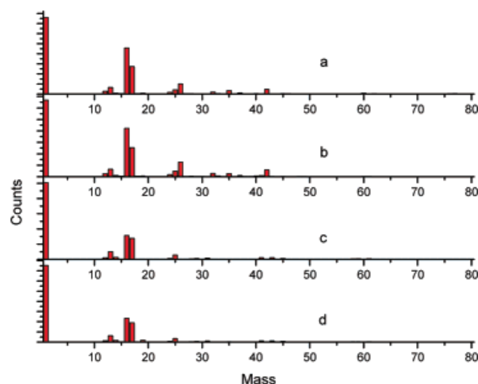

Figure 2. ToF-SIMS spectra of (a) the MCR component corresponding to the avidin spot in a protein array, (b) an avidin-coated, planar, native oxide terminated silicon surface, (c) the MCR component corresponding to the background area in a protein array, and (d) a PEG silane coated surface. Only the low mass region of the spectra are shown.

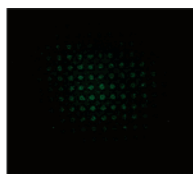

Figure 3. Fluorescence microscopy image of an avidin array made by MA patterning after exposure to a fluorescein-biotin conjugate.

was coated with the PEG silane monolayer and avidin adsorbed onto planar Si/SiO_2.

We next determined whether the proteins were stably bonded to the spots in the arrays and whether they retained their activity after binding. TOF-SIMS imaging of bioarrays containing lysozyme and myoglobin, both deposited at two different pH values (5.6 and 8.0) showed that little change occurred in the protein spots after immersion in PBS buffer for 1 or 3 days. To assay the activity of an avidin array, a control experiment was performed. The surface was incubated in a dilute solution of fluorescein and rinsed. As expected, no array was detected by fluorescence microscopy. However, when the array was incubated with a fluorescein-biotin conjugate and rinsed, the array was seen by fluorescence microscopy (see Figure 3).

Localized protein deposition was demonstrated using a microfluidic spotter at multiple points on the surface.[11] This polydimethylsiloxane device was pressed against an MA patterned surface, which allowed a dilute solution of Cy3 tagged protein A to be circulated over selective regions (about 500 μm in diameter) on the surface. Selective adsorption of protein A at the MA patterned spots was confirmed by fluorescence microscopy.

Ferritin also adsorbs directly onto spots on PEG monolayer substrates after MA patterning. Ferritin is an iron storage protein found in most biological systems.[21] Ferritin molecules are hollow protein shells that can store 2500−4000 iron atoms. Apoferritin is the protein shell, which is ca. 2 nm thick. The cavity inside apoferritin is ca. 8 nm in diameter.

After ferritin binds to the patterned substrates, TOF-SIMS, using a Ga^+ primary ion beam, shows the presence of the protein in the spots, but no iron can be detected (see Figure 4, #1 Fe^+). This is consistent with the extremely shallow information depth of TOF-SIMS (ca. 2 nm).[22] These results also suggest that, like avidin, ferritin does not denature upon adsorption. After heating to 500 °C in an inert atmosphere to remove ferritin's protein shell, TOF-SIMS reveals iron at the surface (Figure 4, #2 Fe^+). Recently, there has been interest in replacing the iron in ferritin with other metals.[23−25] The results from analytical methods used to determine

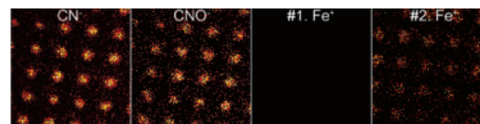

Figure 4. TOF-SIMS negative ion CN^- and CNO^- images and positive ion Fe^+ image after ferritin deposition. The right most panel shows the Fe^+ image after the array was heated to 500 °C under Ar for 1 h. Images are 500 μm × 500 μm.

whether the new metal is deposited within the ferritin shell, or whether it remains outside, are sometimes ambiguous. This new approach should shed light on this problem.

We have demonstrated the rapid formation of protein arrays using MA patterning of PEG monolayer substrates. All of the proteins studied under all conditions adsorb spontaneously and with good stability into these arrays. Protein configuration appears to be retained. Selective protein deposition is shown. A new method for detecting metals in ferritin is suggested. This method appears to be quite general.

Acknowledgment. The authors thank LaserArray Technologies for their support of this work. The protein spotter was provided by Wasatch Microfluidics.

Supporting Information Available: Experimental details and additional results. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA072250M