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Light Activated Patterning of Dye-Labeled Molecules on Surfaces

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Since the late 1980s, a number of photoimmobilization strategies have been developed for producing patterns of proteins on a variety of surfaces.[1,2] Several approaches work by photochemically modifying surfaces to either promote or deter nonspecific protein or cell adsorption.[1,3−9] Other methods employ a variety of photoactive moieties, which can link specific ligands or proteins of interest to the surface. For example, aryl azides and aryldiazirine moieties have been used to capture proteins in patterns via light activation to highly reactive nitrenes[10−12] and carbenes.[13−18] Benzophenone groups have been employed to create reactive benzhydrol radicals.[19−22] Various forms of deprotection chemistry using nitrobenzene as a caging moiety have also been used to pattern active surfaces.[23−27] Two particularly popular strategies employ caged biotin[28,29] and photobiotin,[30−33] which exploit the ubiquitous biotin−avidin/streptavidin interaction.

Although these diverse methods have proven very useful, they nevertheless possess certain drawbacks. For example, all of them require the use of UV radiation (typically 350 nm) to perform the immobilization. Also, many of the small molecule cross-linkers are not readily available in highly water-soluble form and are often spun on and dried before irradiation. It would be exceedingly desirable to develop methods by which ligands and/or proteins of interest could be patterned in aqueous environments with longer and less damaging wavelengths. In fact, if linking chemistries were available at a variety of longer wavelengths, it should be possible to pattern several different species onto the substrate from a single solution simply by exposing different wavelengths of light to different portions of the surface. Below, we lay out a general strategy for light-induced surface patterning by employing commercially available dye molecules that react over a broad range of wavelengths. This method also enjoys the benefit of being compatible with aqueous solution chemistries.

Fluorescent dyes represent an incredible diversity of compounds excitable over a wide range of wavelengths. Prolonged exposure of these molecules to light generally leads to photobleaching and loss of fluorescence.[34] While this effect is usually an undesirable byproduct in most applications, photobleaching could also be exploited to create photogenerated radicals[35] for attaching organic linker molecules to substrates. Indeed, it should be possible to covalently pattern dye-conjugated ligands to a substrate under aqueous conditions by following the simple strategy outlined in Figure 1.

As a demonstration of this principle, surface patterning was performed by bleaching two fluorophore-labeled species sequentially onto a BSA coated substrate from a single phosphate buffer solution (Figure 2). This process is quite general; therefore, it is not only possible to link small molecule ligands to the surface via photobleaching, but also to directly attach whole fluorescently labeled proteins.[35] We demonstrated this process by using a PBS buffer solution containing 0.025 mg/mL of biotin-4-fluorescein and 0.25 mg/mL of Alexa 594 labeled anti-dinitrophenyl IgG. Fluorescein can be photobleached with blue light, while the Alexa 594 can facilely bleach in the yellow/green region. Alexa 594 labeled IgG was patterned first by passing 560 nm light through a cross shaped photomask for 2 h. Because each IgG contained 3−4 fluorescent labels, the protein pattern could clearly be visualized on the surface by its characteristic red fluorescence, because not all fluorophores were consumed during the attachment process. The photomask was then rotated 45°, and the biotin-4-fluorescein was patterned for 30 min using 470 nm light. Control experiments performed by washing out the bulk solution showed no evidence for green fluorescence from the bleached species after surface patterning of the fluorescein dye. At this point, the aqueous solution was rinsed out, and a solution containing Alexa 488 labeled streptavidin was introduced for 2 min. The sample was then rinsed with pure buffer, and the surface was imaged. As can be clearly seen, the streptavidin was patterned only where the 470 nm light

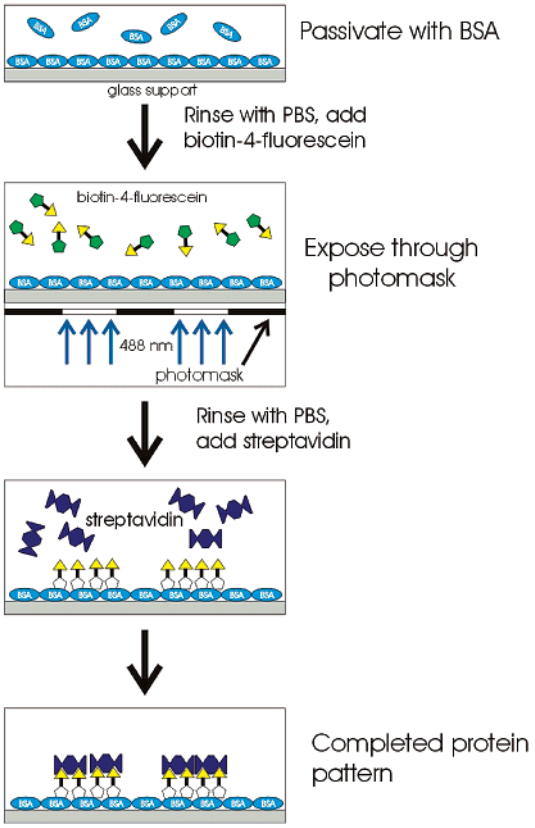

Figure 1. First, a passivation layer of bovine serum albumin (BSA) is deposited onto a glass substrate from a buffer solution. The excess BSA is washed out followed by the introduction of an organic fluorophore containing a covalently attached ligand. Fluorophores are excited on resonance causing photobleaching and attachment to the passivated substrate via a mechanism involving singlet oxygen.[35] Although the fluorophore is destroyed in this process, the ligand molecule is bound to the interface and available for attachment by a protein.

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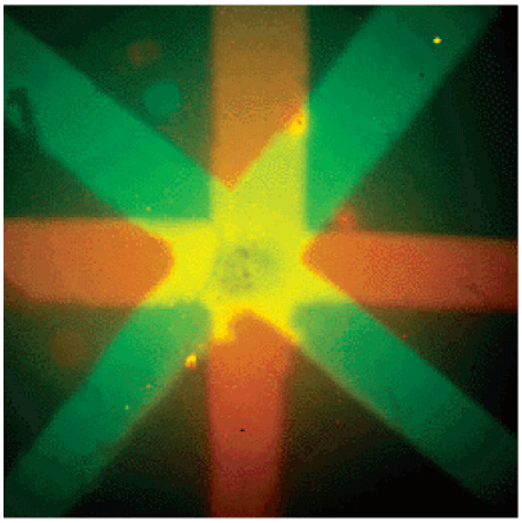

Figure 2. Sequential photopatterning of two different fluorophore linked species from the same solution. The red cross corresponds to the Alexa 594 labeled IgG, and the green cross corresponds to the Alexa 488 labeled streptavidin. Each patterned line is 250 μm wide, although line widths of 7 μm could be easily resolved with this technique (see Supporting Information). The dark region in the center of the intersecting lines is due to a reference dot (through which light could not pass during surface patterning) used to align the mask.

was shined onto the surface, while the IgG was patterned where 560 nm light was irradiated.

To help elucidate the reaction mechanism for surface patterning, experiments were performed in buffer solutions with varying oxygen concentrations (see Supporting Information). The results indicated that solutions saturated with oxygen led to much more rapid photopatterning than buffer solutions that were oxygen depleted. This result is consistent with the idea that photobleaching of fluorescein (or Alexa dye) occurs through the formation of a triplet state, the binding of triplet oxygen, and the subsequent formation of singlet oxygen.[36] The highly reactive singlet oxygen probably attacks the fluorophore to generate a free radical species that can facilely bind with the BSA coated surface. This mechanism is also consistent with the observation that fluorophore attachment to bare glass was much slower than to a protein layer as was found by additional control experiments. This is almost certainly because glass contains relatively few electron-rich sites for attack by free radicals.

The technique described above has many potential applications. For example, biomolecules could be patterned inside microfluidic devices in an online fashion without the need to expose the materials to anything other than aqueous solution conditions. Furthermore, the technique could be expanded to the immobilization of DNA, peptide, or other material arrays. Finally, although the proteins patterned here were deposited sequentially from solution, it should be possible to deposit materials in parallel from a single solution provided light sources at multiple frequencies can be trained onto the surface simultaneously.

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Supporting Information Available: Experimental procedures for photopatterning as well as oxygen and time dependence of the photobleaching/photopatterning process (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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