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. Several approaches work by photochemically modifying surfaces to either promote or deter nonspecific protein or cell adsorption. 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 nitrines and carbenes. Benzophenone groups have been employed to create reactive benzhydrol radicals. Various forms of deprotection chemistry using nitrobenzene as a caging moiety have also been used to pattern active surfaces. Two particularly popular strategies employ caged biotin and photobiotin, 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. While this effect is usually an undesirable byproduct in most applications, photobleaching could also be exploited to create photogenerated radicals 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. 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
was shining 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 species from the same solution. The red cross corresponds to the Alexa 488 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.

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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.

References

(35) A deposition process using whole proteins can lead to multilayer formation because nascently photobleached molecules can attach to proteins already patterned on the surface. This will usually not be the case when small molecules are patterned. In fact, we have demonstrated that patterning bait-4-fluorescein leads to saturation behavior for streptavidin (see Supporting Information).
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