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JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
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Attach, Remove, or Replace: Reversible Surface Functionalization Using Thiol–Quinone Methide Photoclick Chemistry

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Supporting Information

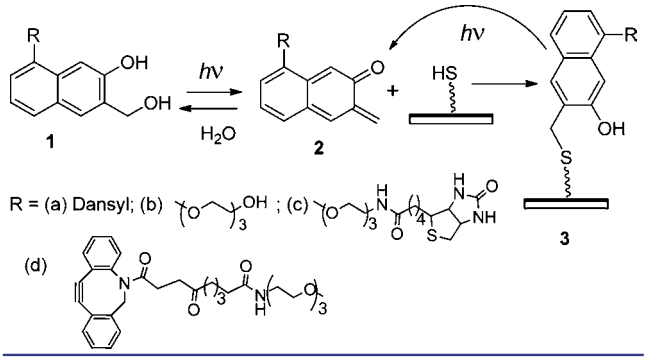
ABSTRACT: A very facile reaction between photochemically generated *o*-naphthoquinone methides (*o*NQMs) and thiols is employed for reversible light-directed surface derivatization and patterning. A thiol-functionalized glass slide is covered with an aqueous solution of a substrate conjugated to 3-(hydroxymethyl)-2-naphthol (NQMP). Subsequent irradiation via shadow mask results in the efficient conversion of NQMP into reactive *o*NQM species in the exposed areas. The latter react with thiol groups on the surface, producing thioether links between the substrate and the surface. Unreacted *o*NQM groups are rapidly hydrated to regenerate NQMP. The short lifetime of *o*NQM in aqueous solution prevents its migration from the site of irradiation, thus allowing for the spatial control of the surface derivatization. A two-step procedure was employed for protein patterning: photobiotinylation of the surface with an NQMP–biotin conjugate followed by staining with FITC–avidin. The orthogonality of *o*NQM–thiol and azide click chemistry allowed for the development of a sequential click strategy, which might be useful for the immobilization of light-sensitive compounds. The thioether linkage produced by the reaction of *o*NQM and a thiol is stable under ambient conditions but can be cleaved by UV irradiation, regenerating the free thiol. This feature allows for the removal or replacement of immobilized substrates.

Photochemical surface derivatization allows for patterned or gradient immobilization of various substrates with high spatial resolution. Light-directed immobilization of carbohydrates,[1] proteins,[2,3] DNA fragments,[3,4] antibodies,[2,5] cells, and other substrates[6,7] is employed in the development of novel biotech and analytical tools.[8] Several recently developed "photoclick" ligation techniques combine the efficiency of click chemistry with the high spatial resolution of photolithography. Some of these methods rely on the photochemical generation of appropriate functional groups on the surface, such as azide-reactive cyclooctynes,[9] alkene-reactive nitrile imines,[10] hydroquinone dienophiles,[8] and reactive heterodienes.[11] Light-induced generation of short-lived surface-reactive species provides an alternative approach to patterning.[12,13] Thus, popular UV-initiated thiol–ene[14] and thiol–yne[15] strategies depend on photochemical homolysis of S–H bonds. Reactions proceeding via reactive species such as radicals, carbenes, nitrenes, etc., often suffer from inadequate selectivity. Photoreduction of Cu(II) to Cu(I) enables spatial control of the copper-catalyzed azide click reaction.[16] All of these photoimmobilization techniques are usually irreversible, as they produce a covalent bond between the surface and the substrate. In some cases, the immobilized substrate can be cleaved from the surface,[13,17] but the surface cannot be reused for subsequent immobilizations.

On the other hand, light is often used for the reagent-free and spatially controlled release of the substrates. In this approach, substances are immobilized using conventional ("dark") chemistry via a photolabile linker. Irradiation cleaves the bond between the surface and the substrate.[18] As in the previous cases, the surface cannot be reused.

Here we report a new surface photoderivatization strategy that allows for not only the patterned immobilization of various substrates on the surface but also the light-directed release or replacement of the immobilized substances. This method is based on the very facile reaction between 2-napthoquinone-3-methides (*o*NQMs) and thiols ($k_{RSH} \approx 2.2 \times 10^5$ M^{-1} s^{-1}).[19] A thiol-derivatized glass slide[20] is immersed in an aqueous solution of a substrate conjugated to 3-(hydroxymethyl)-2-naphthol [the *n*aphtho*q*uinone *m*ethide *p*recursor (NQMP) **1**] and irradiated for 2 min via a shadow mask using a 350 nm fluorescent lamp.[20] The NQMP moiety undergoes efficient photochemical dehydration ($\Phi = 0.20$)[19] in the exposed areas to produce *o*NQM **2** (Scheme 1). In the presence of a surface thiol, the *o*NQM undergoes Michael addition to yield thioether **3**. Unreacted *o*NQM is rapidly hydrated ($k_{H_2O} \approx 145$ s^{-1})[19] to regenerate NQMP **1**. The very short lifetime of *o*NQM species in aqueous solution prevents their migration from the site of

Scheme 1. Reversible Surface Derivatization Using Thiol–*o*NQM Photoclick Chemistry



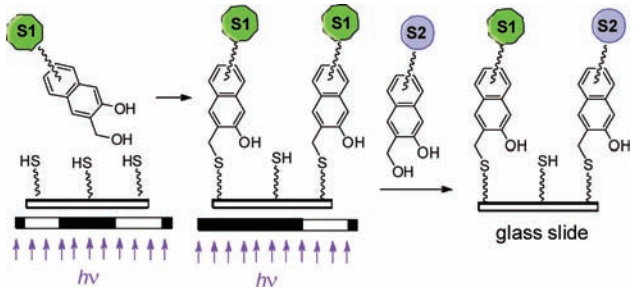
Received: March 27, 2012
Published: May 8, 2012

   dx.doi.org/10.1021/ja302970x | *J. Am. Chem. Soc.* 2012, 134, 8408–8411

irradiation, thus allowing for the high spatial resolution of the derivatization (see below). Because of the much higher nucleophilicity of thiols, their quantitative conversion in the exposed areas is achieved despite the large excess of the nucleophilic solvent.

The thioether **3** produced in the reaction of a thiol with *o*NQM **2** is hydrolytically stable but can be quantitatively cleaved under 300 or 350 nm irradiation back to **2** with a quantum yield of 10%.[21] Thus, subsequent irradiation of the photoderivatized surface **3** in an aqueous solution containing no NQMP results in the release of the NQMP-derivatized substrate (Scheme 1). This process also regenerates free thiols on the surface. In essence, the formation of **3** is a photochemically driven equilibrium between a thiol and NQMP **1** on one side and thioether **3** and water on the other. Since *o*NQM **2** reacts ~5 orders of magnitude faster with thiols ($k_{RSH} \approx 2.2 \times 10^5$ M^{-1} s^{-1})[19]than with water ($k_{H_2O} \approx 2.6$ M^{-1} s^{-1})[19] and thioether **3** is 50% less prone to photoelimination than NQMP **1**,[21] the equilibrium is shifted toward the formation of **3**. However, when no NQMP is present in the solution, irradiation of **3** results in complete photohydrolysis. If a different NQMP-tagged substrate is present in solution, a quantitative substitution (with substrate S1 replaced with substrate S2) takes place in the exposed areas (Scheme 2).

Scheme 2. Immobilization and Replacement of NQMP-Derivatized Substrates on a Thiol-Functionalized Surface



To demonstrate the efficiency of this photoclick strategy, we patterned NQMPs **1a–d** on commercially available thiol-derivatized microscopic glass slides.[20] A transmission electron microscopy (TEM) grid (12 μM pitch) was employed as a shadow mask for patterned irradiation of the slides. Conjugation of 3-(hydroxymethyl)-2-naphthol to the substrates of interest to give **1a–d** was achieved in a few simple steps,[20] and the resulting NQMP-derivatized compounds were stable under ambient conditions and required no special handling.

We began with photopatterning of the dansyl fluorophore. Two procedures were employed for dansyl derivatization of thiol-coated glass slides. In method A, slides were immersed in a 0.2 mM solution of (5-dansyloxy-3-hydroxynaphthalen-2-yl)methanol (DNS-NQMP, **1a**) and irradiated through the TEM grid mask using a hand-held fluorescent UV lamp (350 nm, 4W) for 4 min (Figure 1A). Alternatively (method B), slides were covered with a thin layer of the same DNS-NQMP solution and irradiated from below through the TEM grid mask (Figure 1B). The patterned slides were rinsed with water and methanol and blow-dried under a stream of nitrogen. Images were obtained using fluorescence microscopy.

The two methods produced similar, if not identical, brightness and resolution of the dansyl fluorescent dye pattern. This experiment demonstrates that patterning of the dye is

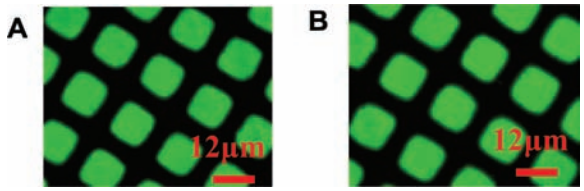


Figure 1. Fluorescence microscope images of thiol-derivatized glass slides irradiated in the presence of DNS-NQMP (**1a**) through a 12 μm pitch TEM grid mask (A) from above and (B) from below.

achieved by selective light exposure and not by squeezing out the reagent when the mask is placed on the slide. The second procedure is more convenient because the mask is not immersed in the reagent solution. We employed method B for all subsequent experiments. Thiol-derivatized glass slides irradiated for 2 min in 0.2 mM and 0.4 mM solutions of **1a** produced identical fluorescence intensities within the experimental uncertainty.[20] This experiment shows that an NQMP concentration of 0.2 mM and 4 min of irradiation are enough for functionalization of all the available thiol groups. Slides incubated in 0.4 mM **1a** solution overnight in the dark did not show detectable DNS fluorescence.

The quinone methide–thiol click reaction is orthogonal to the majority of other derivatization techniques, including the well-developed alkyne–azide click chemistry. Concurrent or sequential applications of photoclick and alkyne–azide click ligations permits one-pot derivatization of substrates with multiple moieties or for the light-directed patterning of photosensitive substances. To demonstrate the efficiency of such sequential click immobilization, we employed the heterobifunctional click reagent NQMP-ADIBO (**1d**), which contains both a photoreactive NQMP group and a strained alkyne (azadibenzocyclooctyne).[22] The latter moiety permits efficient conjugation with azide-tagged substances via the strain-promoted azide–alkyne click reaction (SPAAC). The NQMP–cyclooctyne conjugate was photopatterned onto the thiol-coated surface, washed, and immersed for 1 h in a 0.1 mM solution of rhodamine B azide in DMF. The fluorescence microscopy image of the resulting slide is presented in Scheme 3 (top left). This image shows that a sequential click strategy permits the clean and selective immobilization of azide-tagged substrates.

The *o*NQM–thiol click chemistry was also found to be suitable for protein immobilization. Thus, FITC–avidin was photopatterned on a thiol-coated glass slide using a two-step procedure. First, NQMP-biotin (**1c**) was micropatterned on a slide using the thiol photoclick reaction (Scheme 3, right). The resulting biotinylated slide was treated with FITC–avidin and washed.[20] The fluorescence microscopy image (Scheme 3, top right) demonstrates that FITC–avidin was immobilized only in the exposed areas.

Protein patterning procedures often require extensive washing to remove substrate that is nonspecifically absorbed on the surface. In our experiments, washing for at least 16 h was required to bring the signal-to-noise ratio into the 70:1 range.[13] From a practical point of view, a shorter washing procedure could enhance the efficiency of the photoclick protein patterning. To reduce nonspecific protein binding, we tested two PEGylation procedures. A control FITC–avidin-patterned slide (Figure 2A), prepared as described above, as well as two PEGylated slides (see below), were rinsed with water and incubated in a fresh phosphate-buffered saline solution for 1 h

dx.doi.org/10.1021/ja302970x | J. Am. Chem. Soc. 2012, 134, 8408–8411

Scheme 3. Sequential Click Derivatizations: Thiol–*o*NQM Click Reaction Followed by (left) SPAAC or (right) Biotin–Avidin Ligation

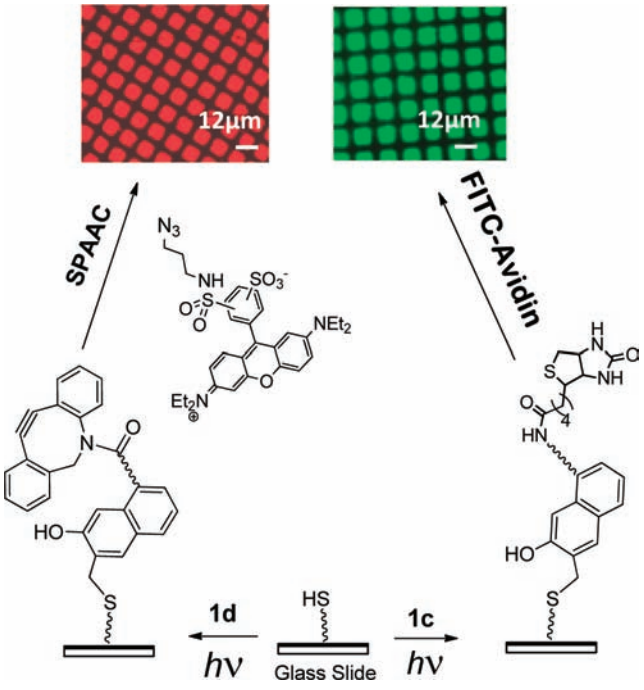


Figure 2. Specific vs nonspecific binding of FITC–avidin to biotin-photopatterned slides: (A) with no PEGylation; (B) with post-photolysis treatment using maleimide–PEG2000; (C) with NQMP-TEG groups selectively replaced with NQMP-biotin.

after FITC–avidin treatment. This washing time was clearly insufficient to obtain a clear FITC–avidin pattern on the control slide, as significant amounts of nonspecifically bound protein produced a fuzzy image under the fluorescence microscope (Figure 2A). The image quality gradually improved with increased washing time. In the first PEGylation method, thiol-derivatized glass slides were patterned with **1c**, rinsed, and treated with maleimide–PEG2000 conjugate to block all of the remaining thiol groups on the surface. The fluorescent image obtained after FITC–avidin staining showed high contrast between the biotinylated and biotin-free areas (Figure 2B).

In the second PEGylation method, we employed the unique reversibility of the *o*NQM–thiol click chemistry. First, a thiol-derivatized slide was flood-irradiated in a 0.2 mM aqueous solution of NQMP-TEG (**1b**). This procedure covered the surface with highly hydrophilic NQMP-TEG moieties and significantly reduced the protein binding.[13] The resulting TEGylated slide was then immersed in a 0.2 mM solution of NQMP-biotin **1c** and irradiated through the TEM grid mask. The fluorescence microscopy image of the slide after FITC–avidin development (Figure 2C) demonstrated that NQMP-TEG **1b** was replaced with NQMP-biotin **1c** in the exposed areas. The postphotolysis PEGylation and photochemical NQMP-TEG replacement procedures both produced fluorescent protein patterns with high contrast and resolution after a simple 1 h wash.[20]

The reversibility of the thiol–*o*NQM chemistry permits straightforward generation of a positive or negative pattern. Thus, a thiol-coated glass slide was exhaustively photo-TEGylated by flood irradiation in **1b** solution. The biotin pattern was introduced by irradiation of the slide in **1c** solution through a shadow mask. FITC–avidin treatment produced a high-resolution protein pattern (Figure 3A). The high contrast

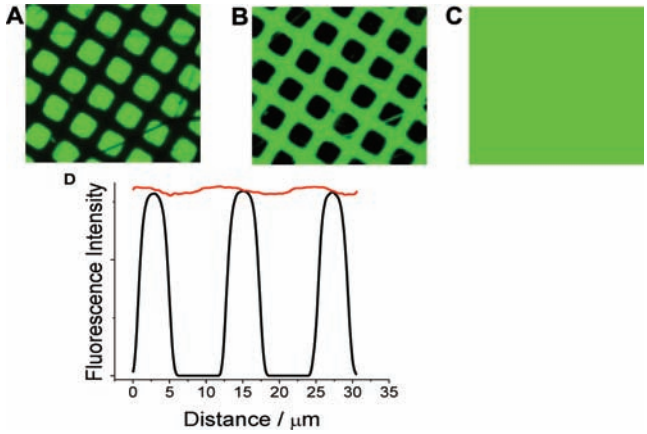


Figure 3. (A–C) Fluorescence images of FITC–avidin-stained photobiotinylated slides: (A) flood irradiation with **1b** and masked irradiation with **1c**; (B) flood irradiation with **1c** and masked irradiation with **1b**; (C) flood irradiation with **1c**, masked irradiation with **1b**, and subsequent flood irradiation with **1c**. (D) Fluorescence intensity profiles along the line perpendicular to the patterns in images B (black) and C (red).

of the image indicates the efficient replacement of **1b** with **1c** in the exposed areas (Figure 3D). The reverse process, involving flood irradiation of the slide in **1c** solution followed by NQMP-TEG photopatterning in **1b** solution, produced a negative image (Figure 3B). To demonstrate further the reversibility of this patterning strategy, the slide shown in Figure 3B was flood-irradiated in **1c** solution and stained with FITC–avidin. The uniform fluorescence of the resulting slide (Figure 3C) demonstrates the complete replacement of NQMP-TEG fragments with NQMP-biotin moieties.

The fluorescence intensity profile of the image in Figure 3B, given by the black curve in Figure 3D, shows that the *o*NQM–thiol photoclick patterning technique readily reproduces features as small as 5 μm. This is a remarkable result, since the photoreactive compound is not immobilized on the surface but rather dissolved in a low-viscosity solvent. The short lifetime of *o*NQM in aqueous solution ($\tau \approx 7$ ms) prevents migration of reactive species from the site of irradiation and makes high-resolution photopatterning feasible. The red curve in Figure 3D, which is the intensity profile for Figure 3C, reveals that there was no appreciable difference between the fluorescence intensities of the surface treated with **1c** and that treated first with **1b** and then with **1c**. This observation underscores the efficiency of the reversible immobilization and indicates that the surface density of thiol groups is not significantly affected by multiple formations and photohydrolyses of the thioether linkages.

In summary, we have developed an efficient photoclick immobilization strategy based on the very fast reaction of photochemically generated *o*-naphthoquinone methides

dx.doi.org/10.1021/ja302970x | *J. Am. Chem. Soc.* 2012, 134, 8408–8411

(*o*NQMs) with thiol-functionalized surfaces. Since the latter are readily available and a wide variety of substrates can be derivatized with naphthoquinone methide precursor (NQMP) group 3-(hydroxymethyl)-2-naphthol, this method offers a new platform for light-directed surface functionalization. The *o*NQM−thiol click photopatterning approach is orthogonal to other derivatization techniques and can be used in conjunction with the well-developed azide−alkyne click chemistry. A solution of NQMP-conjugated substrate can be reused numerous times without loss of efficiency because a very minute amount of the reagent is consumed for the derivatization of the thiol-coated surface, and all of the unreacted *o*NQMs are quenched with water to regenerate NQMP. The short lifetime of the photogenerated reactive species (*o*NQMs) limits their migration from the site of irradiation and permits high spatial resolution of the patterning process. A unique feature of the *o*NQM−thiol photoclick chemistry is the reversibility of the process, which allows for the release of immobilized substrates from a surface or for the replacement of one substrate with another. This feature can be used in the development of light-healable surface coatings, time-resolved photorelease of bioactive molecules, and renewable and repairable microarray technologies. The high stability and robustness of the NQMP group and the compatibility of *o*NQM−thiol chemistry with aqueous solutions makes photoclick immobilization suitable for biological applications.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and preparation and NMR spectra of newly synthesized compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank NSF (CHE-0842590) for support of this project.

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