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Photoreactive Polymer Brushes for High-Density Patterned Surface Derivatization Using a Diels–Alder Photoclick Reaction

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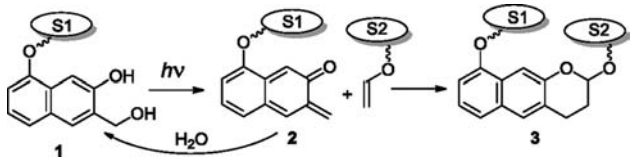
Supporting Information

ABSTRACT: Reactive polymer brushes grown on silicon oxide surfaces were derivatized with photoreactive 3-(hydroxymethyl)naphthalene-2-ol (NQMP) moieties. Upon 300 or 350 nm irradiation, NQMP efficiently produces *o*-naphthoquinone methide (*o*NQM), which in turn undergoes very rapid Diels–Alder addition to vinyl ether groups attached to a substrate, resulting in the covalent immobilization of the latter. Any unreacted *o*NQM groups rapidly add water to regenerate NQMP. High-resolution surface patterning is achieved by irradiating NQMP-derivatized surfaces using photolithographic methods. The Diels–Alder photoclick reaction is orthogonal to azide–alkyne click chemistry, enabling sequential photoclick/azide-click derivatizations to generate complex surface functionalities.

Photochemical immobilization of carbohydrates,[1] proteins,[2−4] DNA fragments,[3,5] antibodies,[4,6] and other substrates[7] allows for the formation of patterned or gradient arrays on various surfaces. These techniques are widely used in the development of novel high-throughput analytical methods.[8] Several photoclick strategies suitable for surface patterning are currently under development. Most of these methods rely on the photochemical generation of reactive functional groups on the surface, such as azide-reactive cyclooctynes,[9] alkene-reactive nitrile imines,[10] or hydroquinone dienophiles.[7] Photoreduction of Cu(II) to catalytically active Cu(I) species also permits spatial control over the alkyne–azide click reaction.[11] While photochemical activation in these techniques is virtually instant, the actual "click" step takes from several seconds to hours, significantly slowing down the overall process. Photoinitiated thiol–ene[12] and thiol–yne[13] reactions proceed via the generation of reactive radicals, allowing for very fast click ligation. The high reactivity of the radical intermediates, on the other hand, is reflected in low selectivity or poor "orthogonality" of the method. In addition, technologies involving photoreactive surfaces often require handling and functionalization of substrates in a light-protected environment.

We have recently developed a photoligation strategy based on very facile ($k \approx 4 \times 10^4$ M^{-1} s^{-1}) hetero-Diels–Alder addition of 2-napthoquinone-3-methides (*o*NQMs, **2**) to vinyl ethers (Scheme 1).[14] In aqueous solutions, *o*NQMs are very selective and undergo cycloaddition only to vinyl ethers to produce a photochemically stable naphthopyran linker, **3**. Unreacted *o*NQMs rapidly hydrate to regenerate the photochemical precursor to *o*NQM, 3-(hydroxymethyl)naphthalene-2-ol [*n*aphtho*q*uinone *m*ethide *p*recursor (NQMP), **1**]. NQMP-derivatized substrates require no special handling because of the excellent stability of NQMP under ambient conditions. Upon irradiation with 300 or 350 nm light, photoconversion results in the efficient generation of *o*NQM moieties (Scheme 1).

Scheme 1. Ligation of Two Substrates (S1 and S2) Using the Diels–Alder Photoclick Reaction between *o*NQM (2) and Vinyl Ether



Here we report the functionalization of activated ester polymer brushes with NQMP groups to allow selective immobilization of vinyl ether-tagged substrates only upon activation with light. A poly(*N*-hydroxysuccinimidyl 4-vinylbenzoate) [poly(NHS4VB)] brush coating was chosen as a versatile surface platform because it is densely packed and provides a facile template for postfunctionalization.[15] The polymer brush platform insures high mechanical and chemical stability of the functionalized surfaces and allows for a much higher surface density of the reactive groups than self-assembled monolayers (SAMs).

Poly(NHS4VB) coatings (50 nm) were prepared using surface-initiated atom-transfer radical polymerization.[15] Overnight incubation of the derivatized silicon wafers in an *N*,*N*-dimethylformamide (DMF) solution of NQMP-TEG-amine derivative **4** (TEG = triethylene glycol) resulted in immobilization of the latter on the brush matrix (Scheme 2). The conversion was quantitative, as evidenced by the complete disappearance of the imide bands in the grazing-angle attenuated total reflectance (GATR)-FTIR spectra (Figure 1a).[16] Removal of acetal protection from immobilized **4** was achieved by treating the wafers with 0.1 HCl in DMF.

The deprotection of surface-immobilized NQMP was confirmed by conversion of the resulting diol group into diacetate by overnight incubation in a dichloromethane solution of acetic anhydride/pyridine. The change in polymer layer thickness and hydrophobicity as well as the appearance of

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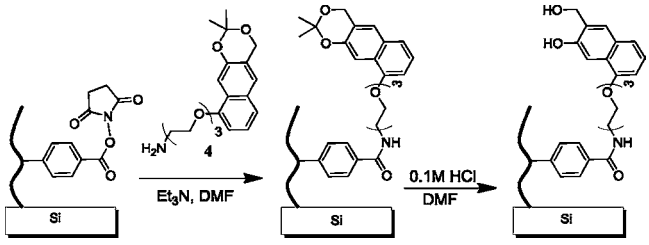
Scheme 2. NQMP Functionalization of a Brush Polymer



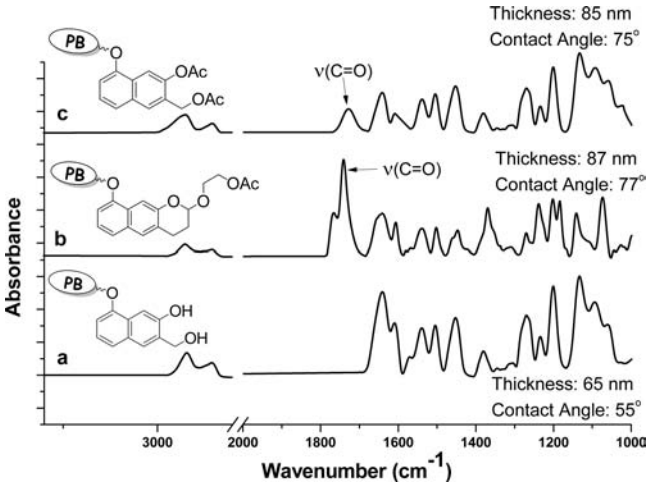
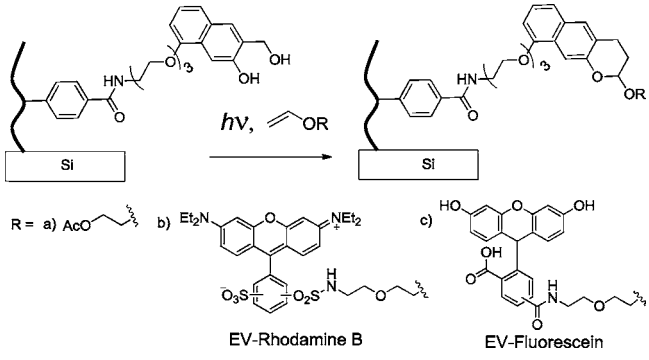


Figure 1. GATR-FTIR spectra and properties of the functionalized polymer brush containing (a) NQMP, (b) NQMP diacetate, and (c) NQMP–acetyl vinyl ether adduct.

ester bands in the IR spectrum (Figure 1b) confirmed the formation of the diacetate.[16]

To test the efficiency of the new photoimmobilization platform, the wafer coated with the NQMP-functionalized polymer brush [poly(NQMP-4VB)] was immersed in an aqueous solution of 2-(vinyloxy)ethyl acetate (0.1 mM) and irradiated at 300 nm using a hand-held lamp (3.5 mW cm^{-2}) for 2 min. Increases in the thickness and hydrophobicity of the polymer layer as well as the appearance of an ester band at 1738 cm^{-1} in the IR spectrum (Figure 1c) clearly indicated formation of naphthopyran **3** on the polymer brushes. Overnight incubation of the irradiated wafer in acetic anhydride/pyridine solution did not change the surface characteristics, confirming complete conversion of the NQMP moieties to **3** (Scheme 3).

Scheme 3. Photo-Diels–Alder Functionalization of a Poly(NQMP-4VB)-Coated Silicon Wafer



To demonstrate the utility of the Diels–Alder photoclick reaction for surface patterning, poly(NQMP-4VB)-coated wafers were irradiated through a shadow mask to form multicomponent surfaces with spatially resolved chemical functionalities. Substrates were immersed in a 0.1 mM aqueous solution of vinyl ether-derivatized fluorescein (EV–fluorescein)[16] and irradiated at 300 nm for 1 min via square-patterned transmission electron microscope (TEM) grids (250 and 12.5 μm pitch) using a hand-held lamp (Scheme 3). The immobilization occurred only in the exposed areas, where reactive *o*NQM moieties were formed upon irradiation (Figure 2a,b). Photoimmobilization of EV–fluorescein was accompanied by a sharp increase of the polymer layer thickness and hydrophobicity.[16]

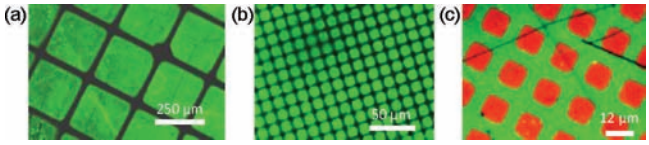


Figure 2. Fluorescence microscopy images of poly(NQMP-4VB)-coated wafers (a, b) photopatterned in 0.1 mM EV–fluorescein solution using (a) 250 and (b) 12 μm pitch TEM grids and (c) upon sequential immobilization of rhodamine B and fluorescein.

The photochemical stability of the naphthopyran linker permitted multiple exposures of photoderivatized areas, simplifying the patterning of multiple substrates. To illustrate this capability of the photo-Diels–Alder immobilization technique, a poly(NQMP-4VB)-coated wafer was irradiated through a TEM grid mask in the presence of a vinyl ether–rhodamine B conjugate (EV–rhodamine B). After the mask was removed, the wafer was thoroughly washed with DMF and flood-irradiated in a solution of EV–fluorescein. Figure 2c shows a fluorescence image of the resulting two-color pattern with negligible amounts of cross contamination between the two dyes.

Diels–Alder photoclick chemistry 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 permit one-pot derivatization of substrates with multiple moieties or for light-directed patterning of photosensitive groups. In such sequential click immobilizations, an azide or alkyne-containing vinyl ether was photopatterned onto a poly(NQMP-4VB)-coated surface. The substrate of interest was then immobilized using Cu(I)-catalyzed (CuAAC) or strain-promoted azide–alkyne cycloaddition (SPAAC) chemistry (Scheme 4).

To test the feasibility of the sequential click surface functionalization, poly(NQMP-4VB)-coated silicon wafers were irradiated in aqueous solutions of the propargyl vinyl ether of diethylene glycol or (2-azidoethoxy)ethyl vinyl ether using a 12 μM pitch TEM grid (Scheme 4). The azide-derivatized wafer was immersed in a 0.1 mM DMF solution of ADIBO–rhodamine B conjugate[17] for 4 h. A fluorescence microscopy image of the resulting slide is shown in Scheme 4a. The substrate containing the propargyl groups was subjected to CuAAC coupling with azidofluorescein[16] to give a clean pattern (Scheme 4b). These images show that a sequential click strategy permits clean and selective immobilization of azide- or alkyne-tagged substrates.

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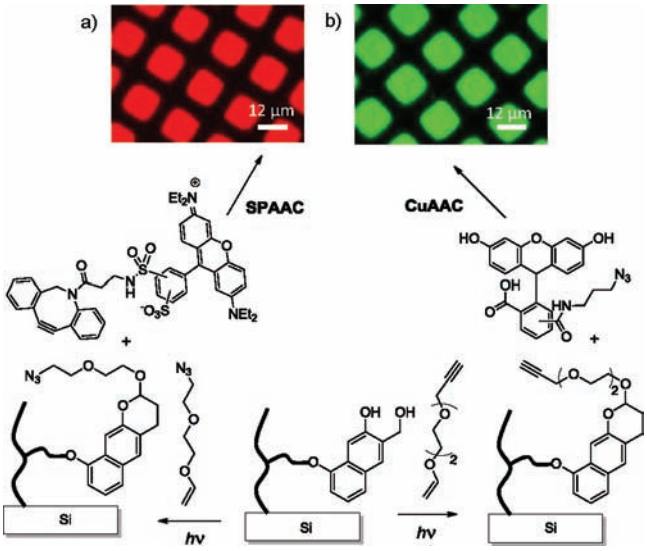
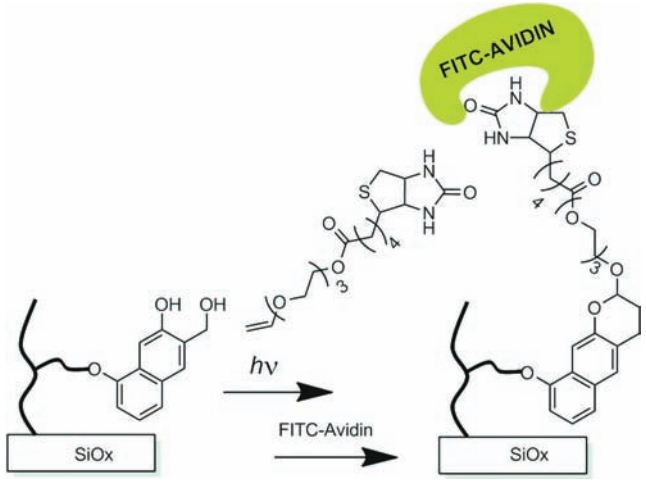
Scheme 4. Sequential Click Functionalization: Photo-Diels–Alder Surface Patterning Followed by Azide–Alkyne Click Immobilization of Fluorescent Dyes via (a) SPAAC of ADIBO–Rhodamine B on an Azide-Derivatized Surface and (b) CuAAC of Azidofluorescein



The relatively low contact angle of the poly(NQMP-4VB) substrate (55°) makes it a suitable platform for protein immobilization. Thus, fluorescein isothiocyanate (FITC)–avidin was photopatterned on the surface using a two-step procedure. First, vinyl ether–biotin conjugate (EV–biotin) was micropatterned on polymer brushes using the Diels–Alder photoclick reaction (Scheme 5). The resulting patterned

Scheme 5. Diels–Alder Photoclick Patterning of FITC–Avidin on a Poly(NQMP-4VB) Surface



biotinylated slide was developed with FITC–avidin. Fluorescence microscopy images demonstrated that avidin was immobilized only in the exposed areas.

One of the most important advantages of the polymer brush platform over SAM-based systems in sensor development is the ability to achieve significantly higher functionalization and therefore a stronger readout signal. We quantitatively compared the binding capacity of the 65 nm poly(NQMP-4VB) layer with a NQMP-TEG monolayer produced by quantitative derivatization[14b] of an epoxide SAM on a glass slide.[16] Both slides were immersed in a 0.1 mM aqueous solution of EV–fluorescein and flood-irradiated using 350 nm light until full conversion was achieved. The average surface emission of the fluorescein-functionalized poly(NQMP-4VB) layer was 61 ± 5 times more intense than that of the NQMP-derivatized SAM (Figure 3B vs

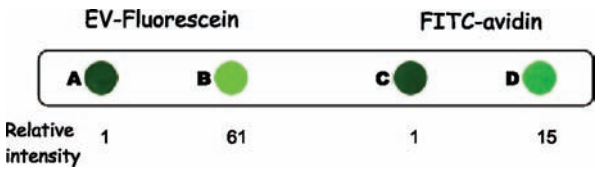


Figure 3. Fluorescent emission of (A, B) EV–fluorescein immobilized on (A) an NQMP-SAM on glass and (B) a poly(NQMP-4VB) layer on a silicon wafer and (C, D) FITC–avidin immobilized on (C) an NQMP-SAM and (D) poly(NQMP-4VB).

A, respectively). This experiment demonstrates the increased surface density of solvent-accessible functional groups in the polymer brush layers in comparison with SAMs.

NQMP-SAM and NQMP-polymer brush platforms were also tested for the immobilization of much larger avidin molecules. Both types of slides were photobiotinylated by exposure to 350 nm light in an EV–biotin solution and then stained with FITC–avidin. The fluorescence intensity of the poly(NQMP-4VB) slide was 15 ± 2 times greater than that of the NQMP-SAM analogue. The biotinylated polymer brush showed a significant and uniform increase in hydrophilicity upon FITC–avidin staining.[16] This observation and GATR-FTIR data indicate uniform protein immobilization on the poly(NQMP-4VB)-coated surface. The appearance and position of amide I and II bands in the FTIR spectra (Table S2 in the Supporting Information) closely matched the absorbance of native avidin, indicating that the protein was not denatured upon immobilization.[18] The changes in layer thickness, on the other hand, were rather small,[16] suggesting a relatively low loading of the protein in the polymer matrix. This conclusion agrees well with the muted fluorescence intensity enhancement for the FITC–avidin-functionalized polymer brush platform relative to the SAM (Figure 3D vs C, respectively).

We presume that the reduction in the loading level of avidin compared to fluorescein is most likely due to the fact that the entropic cost of having polymer brush chains extended from the surface makes it difficult for large molecules to diffuse into the brush.[19] It is also possible that the bound avidin can cross-link to the biotinylated polymer brushes, thereby preventing further diffusion of protein into the matrix. However, it is important to note that the loading level of protein on the surface of the polymer brushes is still more than an order of magnitude greater than that of SAMs.

In summary, we have demonstrated the utility of the light-directed Diels–Alder click reaction for the patterned derivatization of polymer-brush-coated surfaces. The very fast *o*-naphthoquinone methide–vinyl ether cycloaddition allows for temporal control of the immobilization process and can enhance the efficiency of many biotechnological tools, such as microprinting, microarray manufacturing, protein assays, etc. Furthermore, the ability to attach reliably a larger amount of protein than in monolayer methods enhances sensor readout and potentially reduces the cost of biotechnology platforms. This instantaneous photoclick reaction is orthogonal to the majority of modern ligation techniques and can be used in

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parallel or sequential fashion with other click reactions (e.g. alkyne−azide). In addition to its stability and robustness, the NQMP group is readily and inexpensively prepared and can be attached to various substrates. These features make NQMP-based photoclick chemistry a promising tool for ligation and immobilization procedures.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures 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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