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High Density Orthogonal Surface Immobilization via Photoactivated Copper-Free Click Chemistry

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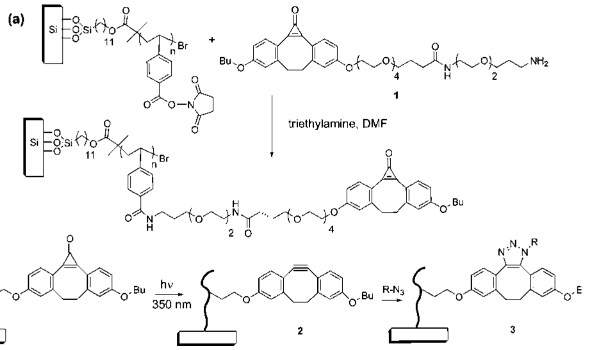
Abstract: Surfaces containing reactive ester polymer brushes were functionalized with cyclopropenone-masked dibenzocyclooctynes for the light activated immobilization of azides using catalyst-free click chemistry. The photodecarbonylation reaction in the amorphous brush layer is first order for the first 45 s with a rate constant of 0.022 s^{-1}. The catalyst-free cycloaddition of surface bound dibeznocyclooctynes proceeds rapidly in the presence of azides under ambient conditions. Photolithography using a shadow mask was used to demonstrate patterning with multiple azide containing molecules. This surface immobilization strategy provides a general and facile platform for the generation of multicomponent surfaces with spatially resolved chemical functionality.

The advancement of engineered particles and surfaces with spatially resolved chemical functionality is of interest to many areas of science and technology including the fabrication of biochips, microfluidic devices, targeted drug delivery, and microelectronic devices. Among several immobilization strategies developed,[1,2] the alkyne−azide Huisgen 1,3-dipolar cycloaddition is emerging as an ideal coupling approach.[2−4] These “click” reactions are especially appealing for biological attachment due to their quantitative reactivity, small size, and the ability to incorporate azides in biomolecules through native cell machinery.[5] While conventional copper(I)-catalyzed click chemistry is ideal for many applications,[6−8] the cytotoxicity of the Cu catalyst can limit bioorthogonal conjugation. Recently, catalyst-free click reactions have emerged that utilize a reactive cycloalkyne to promote the [3+2] cycloaddition with comparable reaction rates.[9,10]

Herein, we report the functionalization of activated ester polymer brushes with a cyclopropenone masked dibenzocyclooctyne compound that allows selective immobilization of azido-containing substrates only upon activation with light. A poly(*n*-hydroxysuccidimide 4-vinyl benzoate) (poly(NHS4VB)) brush coating was chosen as a versatile surface platform because it is densely packed and provides a facile template for postfunctionalization.[11] The electrophilic *n*-hydroxysuccinimide (NHS) ester pendant group allows coupling of a wide variety of functional groups, and the controlled nature of surface initiated polymerization allows for homopolymer, copolymer, and block copolymer coatings with precise control of functionality and microenvironment. These surface bound polymer coatings also allow one to decouple sensitive chemistry or multistep monomer synthesis from the polymer brush geometry.

Scheme 1[a]



[a] (a) Attachment of cyclopropenone (**1**) to poly(NHS4VB) brushes. (b) Subsequent photoactivation (**2**) and functionalization (**3**) of the polymer brush pendant groups with azide-derived fluorescent dyes azido-FL and azido-RB (see Figure SI.1 for structures).

Scheme 1 outlines the surface immobilization strategy. First poly(NHS4VB) coatings (125 nm) were prepared using surface-initiated ATRP.[11] Cyclopropenone **1** was then immobilized to the brush matrix under aminolysis conditions with quantitative conversion (Scheme 1a). When irradiated with UV light (350 nm, 3.5 mW/cm^2), **1** undergoes rapid decarbonylation to yield the reactive dibenzocyclooctyne **2**. Cyclooctyne **2** can then undergo catalyst-free cycloadditions with azides to yield the triazole-linked conjugate in quantitative yield under ambient conditions (Scheme 1b). Unexposed cyclopropenone **1** does not react with azides and is thermally stable at 60 °C for 12 h without decomposition.[12,13] Unreacted **1** can be subsequently decarbonylated by further irradiation with UV light.

The consecutive functionalization steps of the brush coatings were characterized using ellipsometry, contact angle, and grazing-incidence attenuated total reflectance Fourier transform infrared spectroscopy (GATR-FTIR). Upon functionalization, polymer brush thickness increases with the additional molecular weight of the pendant group and static contact angle measurements confirm anticipated surface wettability changes (Table SI.1). Figure 1 shows the progression from the poly(NHS4VB) brush to the covalent attachment of a fluorescein−azide conjugate (azido-FL) via photoactivated click chemistry. Upon functionalization with **1**, the disappearance of the NHS C═O stretch at 1738, 1769, and 1801 cm^{-1} is observed along with the appearance of the cyclopropenone C═O stretch at 1846 cm^{-1} and conjugation of the C═C−C═O stretch at 1608 cm^{-1} (Figure 1a and b). Upon irradiation, the cyclopropenone C═O stretch disappears yielding cyclooctyne **2** (Figure 1c). After the click reaction, the appearance of carboxylic acid stretches at 1757 and 1447 cm^{-1} indicate the attachment of the azido-FL (see Table SI.2 for complete peak assignments).

The photodecarbonylation of cyclopropenones to alkynes in solution proceeds quantitatively on the order of picoseconds with high quantum efficiency ($\Phi = 0.2-1.0$).[13] In the solid crystalline

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state, it has also been observed that diarylcyclopropenones can undergo decarbonylation with a remarkable quantum efficiency ($\Phi > 1$).[14] In order to examine the kinetics of photodecarbonylation in the amorphous brush layers, time dependent UV−vis spectroscopy on quartz substrates was performed. Absorption spectra were recorded at light exposure (350 nm, 3.5 mW/cm^2) intervals of 1 s up to 150 s and are shown in Figure 2. The bands at 335 and 353 nm of cyclopropenone **1** rapidly decrease upon initial exposure to 350 nm light along with the simultaneous emergence of bands at 308 and 326 nm for dibenzocyclooctyne **2**. The spectral observations in the brush layer are consistent with the photodecarbonylation of **1** in solution.[12]

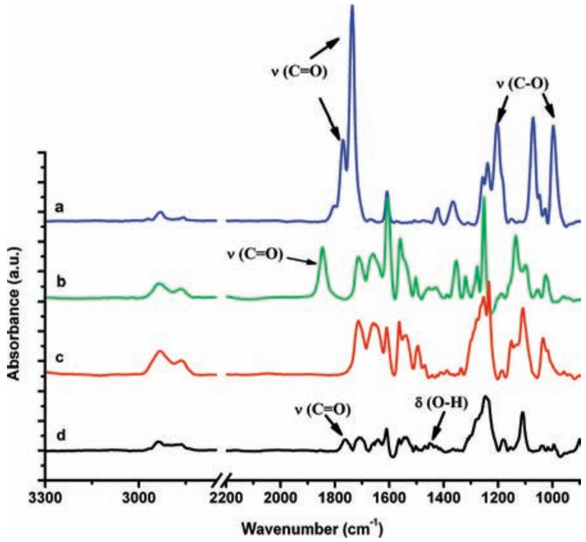

Figure 1. Grazing incidence attenuated total reflection Fourier transform infrared spectroscopy (GATR-FTIR) of the original poly(NHS4VB) brush functionalized (a), postfunctionalization with cyclopropenone **1** (b), conversion of cyclopropenone to dibenzocyclooctyne **2** (c), and functionalization with azido-FL **3** (d).

The decay of the cyclopropenone absorbance at 353 nm was found to be first order for the first 45 s of exposure with a rate constant of 0.022 s^{-1} (Figure SI.3). Decarbonylation after 45 s deviates from first-order behavior even though the photoconversion is unimolecular. The alteration of decarbonylation kinetics is likely due to nonequivalent sites in the polymer matrix that influence the absorption coefficient and/or quantum yield such as differing states of aggregation or a heterogeneous free volume distribution in the amorphous film.[15,16] Overall, 95% of the brush decarbonylation is complete within 90 s of irradiation with a hand-held UV lamp and quantitative conversion occurs within 150 s. No photodegradation of the polymer substrate or cyclooctyne moieties was observed in the UV−vis spectra after several minutes of exposure to 350 nm light.

To further demonstrate the versatility of the photoclick substrates, cyclopropenone functionalized substrates were irradiated through a shadow mask to form multicomponent surfaces with spatially resolved chemical functionality. A square patterned transmission electron microscope grid (12 μm pitch) was used to mask **1** during irradiation. Substrates were irradiated through the shadow mask and then immersed in a solution of Lissamine Rhodamine B-azide conjugate (azido-RB). The cycloaddition is complete within 20 min and occurred only in the exposed areas, where cyclopropenone groups underwent decarbonylation to generate dibenzocyclooctyne. A subsequent flood of irradiation of the substrate liberates the remaining cyclooctyne groups and allows for the functionalization of the protected regions for further click reactions. Azido-FL was then immobilized to generate a multifunctional substrate. Figure 3 shows a fluorescence microscopy image of the photopatterned substrates. There is negligible cross-contamination between the two dyes, with excellent segregation between the selectively activated regions.

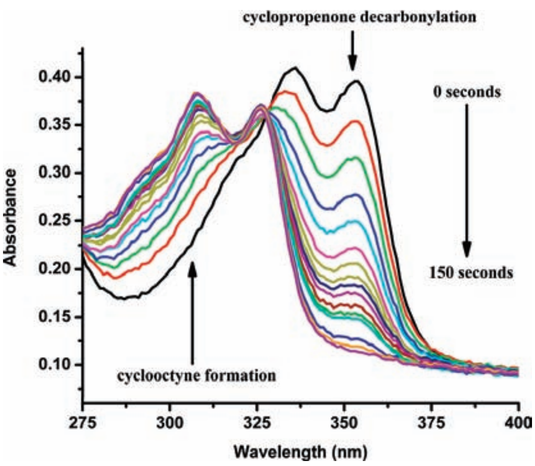

Figure 2. UV−vis absorption spectroscopy of the simultaneous decarbonlyation of cyclopropenone and formation of cyclooctyne on the polymer brush irradiated with 350 nm UV light. Spectra shown are every 5 s until complete disappearance of cyclopropenone after 150 s.

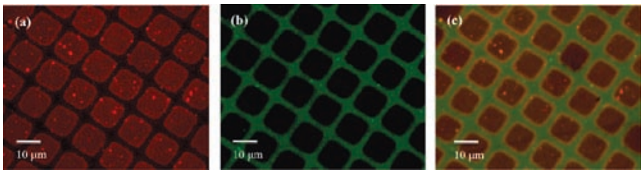

Figure 3. Fluorescence microscope images of a photopatterned surface fabricated by sequential photoactivation of dibenzocycloocytnes. (a) Click functionalized azido-RB excited at 550 nm, (b) azido-FL excited at 477 nm, and (c) both dyes imaged under wide UV excitation (350 nm).

In conclusion, we have demonstrated the surface immobilization of cyclopropenones that undergo photoinduced decarbonylation to yield dibenzocyclooctynes for catalyst-free cycloaddition with azides. The decarbonylation reaction occurs quickly and quantitatively with low power UV irradiation. This photoactivated surface platform allows the creation of multifunctional surfaces with spatially resolved chemical functionality and can be extended to biological, nanoparticle, and materials science applications.

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Supporting Information Available: Detailed experimental procedures, ellipsometry and contact angle measurements, decarbonylation kinetics, and FTIR peak assignments. This material is available free of charge via the Internet at http://pubs.acs.org.

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