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Clickable Surfaces

Adding Spatial Control to Click Chemistry: Phototriggered Diels–Alder Surface (Bio)functionalization at Ambient Temperature**

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The recent years have witnessed a substantial increase of attention in the modification of materials by the highly efficient chemical methods often referred to as click chemistry.[1] The set of reactions belonging to this class exhibit specific features such as fast reaction kinetics, high yields, orthogonal reactivity, and tolerance to a wide range of solvents. However, in some applications featuring surface patterns or three-dimensional scaffolds, these characteristics are not sufficient, because spatial control is also required. Furthermore, temporal control of chemical reactions could offer the significant advantage of triggering reactions at any time, which would be particularly interesting when a specific sequence of events is required. The use of light as a temporal and spatial trigger has very recently been applied to click methods.[2] For instance, Bowman and co-workers reported patterns of hydrogels fabricated by azide–alkyne cycloaddition cross-linking initiated by the phototriggered reduction of Cu^{II} into Cu^{I}, the latter being the catalyst for this reaction.[3] However, their method presents the disadvantage of involving many species, such as the different copper species and the radical photoinitiator required to generate Cu^{I}. Although no such effect was reported, these species are able to diffuse in the medium and could hinder the spatially resolved character. The same applies to the widely used photoinitiated radical thiol–ene and thiol–yne reactions.[4]

The key to achieve full spatial control is to immobilize one of the two components and to directly activate it. Popik, Locklin, and co-workers followed such a path when they used immobilized cyclopropenone-masked cyclooctynes.[5] Under UV light, decarbonylation occurs, releasing strained alkynes reacting rapidly with azides in solution and producing fluorophore patterns. Following the inspiring work of Lin and co-workers,[6] who resurrected the seminal work of Huisgen and Sustmann,[7] we recently explored the photogeneration of nitrile imines from immobilized diaryl tetrazoles to pattern different polymers on cellulose by 1,3-dipolar cycloaddition with maleimide-functionalized macromolecules.[8] Importantly, we have additionally introduced a novel procedure for click conjugations based on Diels–Alder addition of hydroxy-*o*-quinodimethanes (photoenols) generated by photoisomerization of *o*-methylphenyl ketones or aldehydes and demonstrated its ease and efficiency in light-induced conjugations of polymeric building blocks.[9] The latter strategy fulfills the harsh set of click conditions required for polymer–polymer ligation.[1b] Upon performing a screening study of potential photoenol candidates, we have now identified the 2-formyl-3-methylphenoxy (FMP) moiety to be an even more efficient precursor compared to our previously reported 2-methylbenzophenone derivative. Promotion of the Diels–Alder *endo* addition occurs based on hydrogen bond formation in the photoenol intermediate by increasing both its lifetime and the amount of formed *Z* isomer (Scheme 1), which is, in contrast to the *E* isomer,[10] highly reactive towards dienophiles (for example, a maleimide derivative).

Initial model reactions were performed in solution, as mass spectrometry of samples in solution is an efficient method for identifying potential side-product formation with much higher sensitivity and specificity than for example ^{1}H NMR spectroscopy.[11] A 36 W compact fluorescent lamp at the absorbance maximum of FMP ($\lambda_{max} = 320$ nm; Supporting Information, Figure S1) was employed as UV source. The outcome of such an investigation is shown in Figure 1a, which depicts the mass spectra of starting FMP-capped poly(ethylene glycol) methyl ether before irradiation **2** alongside the photostable Diels–Alder cycloadduct **3**. Full conversion with maleimides is typically achieved in less than 15 minutes at ambient temperature (for a kinetic investiga-

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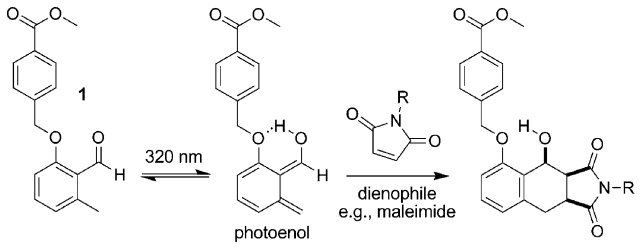


Scheme 1. Photoinduced isomerization of a 2-formyl-3-methylphenoxy (FMP) derivative **1** and subsequent Diels–Alder [4+2] cycloaddition with a dienophile.

tion of the photoreaction in acetonitrile and dichloromethane, see the Supporting Information, Figures S9 and S10). Importantly, the photoinduced reactions were also compatible with polar solvents, such as water (Supporting Information, Figure S11) and DMF (for example, in the phototriggered reaction with *N*-(5-fluoresceinyl)maleimide; Supporting Information, Figure S12). It should however be noted that longer irradiation times are typically required with these solvents for quantitative product formation. As water-borne catalyst-free conjugation reactions are highly interesting for biological applications, we also placed our attention on the attachment of biomolecules, for example, peptides. Figure 1b depicts the mass spectra of a maleimido-GRGSGR peptide **4** before and after **5** conjugation with a low-molecular-weight FMP derivative **1**. In detail, we irradiated **4** in the presence of **1** (1:1.2 mol/mol) in acetonitrile/phosphate-buffered saline (PBS; 3:1 v/v) and could again observe quantitative formation of the expected Diels–Alder adduct (for a detailed investigation, see the Supporting Information, Figure S13).

The model reactions in solution provided a proof-of-principle that phototriggered (bio)conjugation can be achieved without catalyst yet with high efficiency. The light-based nature of the conjugation technique prompted us to translate it to the spatially constrained grafting of molecules onto surfaces to obtain molecular patterns. During the writing of this manuscript, Popik et al. presented surface grafting by photoinduced hetero-Diels–Alder reaction onto vinyl ether-coated slides[12] involving a type of chemistry similar to our proposed system. However, the photoreactive moiety used was present as a soluble species, which is detrimental with respect to the spatial resolution. The method also requires multistep incorporation of the photoactive 3-hydroxymethyl-2-naphthol moiety for each different coupling. In contrast, our key idea is the immobilization of the photoactive FMP moiety directly onto the surface to perform spatially resolved in situ grafting of maleimide derivatives, for example sensitive biomolecules. With the existence of a broad spectrum of readily available maleimido-functionalized (bio)molecules one also avoids the complicated incorporation of the photoactive species mentioned above.

The synthetic route to functionalize silicon surfaces is rather straightforward. The FMP-functionalized silane **6** was prepared (Scheme 2), dissolved in anhydrous toluene, and reacted with activated silicon wafers. Upon confirmation of effective surface silanization by X-ray photoelectron spectroscopy (XPS; Supporting Information, Figure S14), we performed light-directed Diels–Alder cycloaddition on the surface using different maleimide derivatives. Imaging time-of-flight secondary-ion mass spectrometry (ToF-SIMS) is a commonly utilized and sensitive technique that allows the highly spatially resolved analysis of molecular patterns on

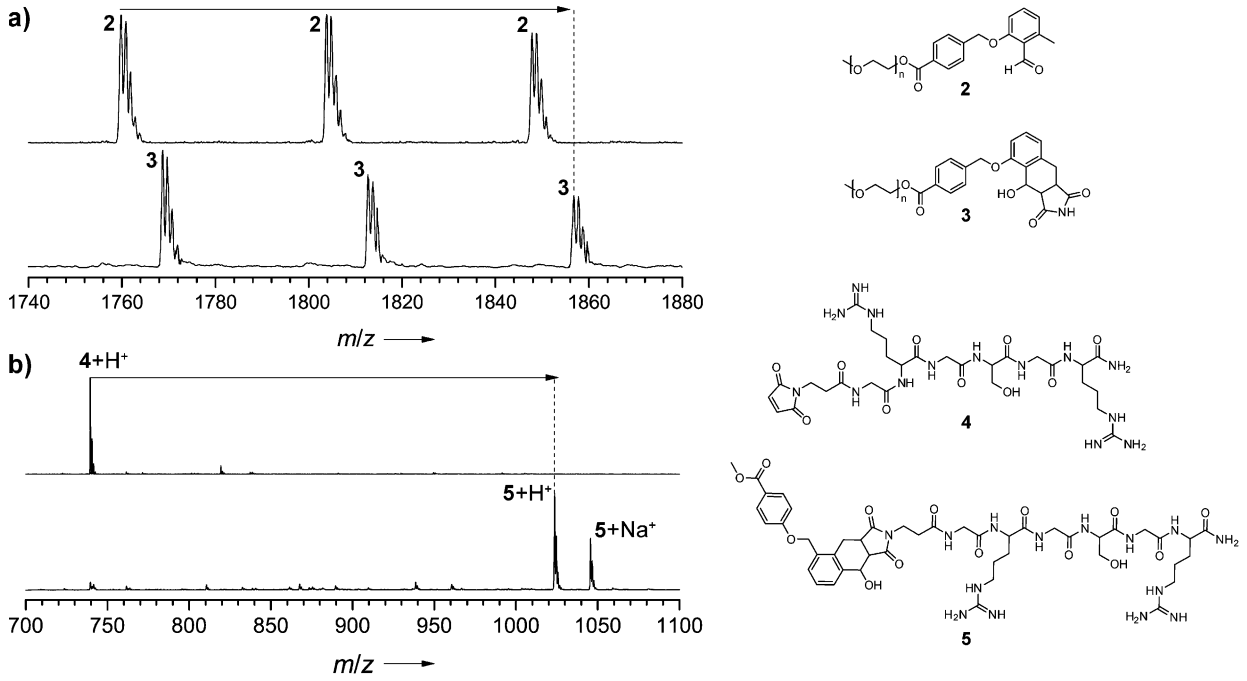
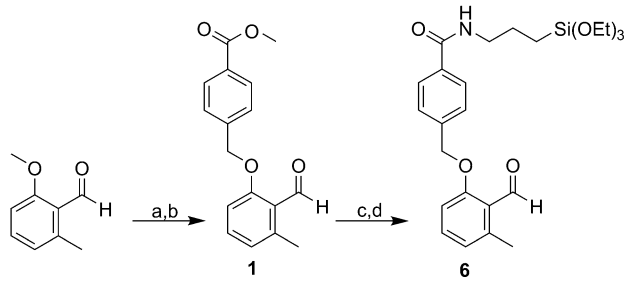


Figure 1. ESI-MS spectra of a) the photoconjugation of 2-formyl-3-methylphenoxy PEG (with repeating unit *n*) before (above, **2**) and after (below, **3**) 15 min photoconjugation with maleimide, and b) maleimido-GRGSGR peptide before (above, **4**) and after (below, **5**) 2 h photoconjugation with FMP derivative **1**.



Scheme 2. Synthesis of the FMP-functionalized silane **6**. Reagents and conditions: a) $AlCl_3$, CH_2Cl_2; b) 4-bromomethylbenzoate, K_2CO_3, acetone; c) NaOH, CH_2Cl_2/methanol (9:1 v/v); d) ethyl chloroformate, 3-triethoxysilylpropan-1-amine, THF.

solid substrates.[13] In contrast to traditional fluorescence imaging, ToF-SIMS data provides detailed information on the chemical composition essential for analysis of non-fluorescent (bio)molecules. For instance, bromine compounds with their inherent isotopic pattern can be unambiguously detected by ToF-SIMS; therefore, the bromine-containing dienophile **7** (Figure 2) was utilized in the current study as a molecular marker to spatially map the locally constrained surface grafting. The photopatterning was achieved by irradiation of the functionalized silicon wafers immersed in a solution of **7** by utilizing two shadow masks: first a macroscopic structure with a square cut into a metal plate and then one with a micropattern (Figure 2).

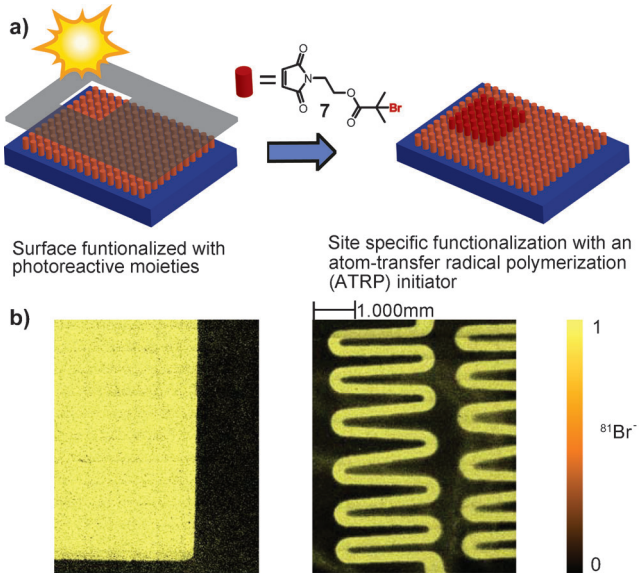


Figure 2. a) Representation of the phototriggered Diels–Alder surface grafting of bromine-containing maleimide derivative **7**. ATRP = atom-transfer radical polymerization. b) ToF-SIMS images of silicon wafers patterned with **7** utilizing two shadow masks.

After the phototriggered reaction, it is not necessary to deactivate or cap the remaining FMP groups on the surface, as the formation of reactive diene species formed during irradiation is fully reversible. The FMP surface is also photochemically inert (in the absence of a dienophile during irradiation, no chemical changes of the FMP layer were detectable by ToF-SIMS) and only reacts in presence of a suitable dienophile, thus simplifying preparation, handling, and storage. Subsequent ToF-SIMS composition analysis of the bromine content on the surface readily reproduced the shadow mask structures (Figure 2b) of irradiated and non-irradiated areas with good spatial resolution. This type of pattern could potentially be used to grow polymers by surface-initiated metal-catalyzed radical polymerization in a "grafting-from" approach.[14] In fact, the enormous applicability of polymer surface patterns at different length scales is well-known in research fields including tissue engineering, cell biology, and medicinal science.[15]

Apart from the aforementioned "grafting-from" approach, our phototriggered strategy can also be utilized to directly functionalize the FMP surface with polymers, for example maleimido-capped poly(ethylene glycol) methyl ether (PEG-Mal) **8** by the "grafting-to" approach (Figure 3a). By utilizing this methodology, we could find characteristic mass fragments of PEG only in the irradiated square (Figure 3b, left), thus confirming the site-specific immobilization. PEG is commonly used to locally reduce non-specific binding[16] and to spatially control adhesive and non-adhesive areas on surfaces.

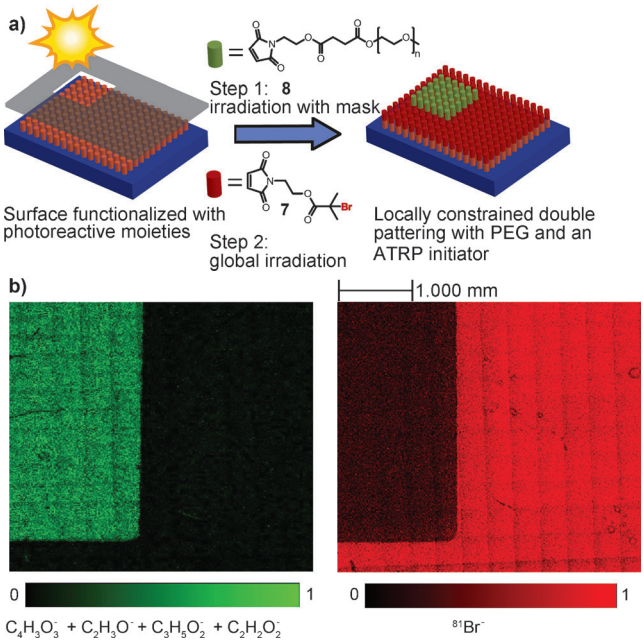


Figure 3. a) Representation of the phototriggered double patterning of ω-maleimido poly(ethylene glycol) **8** and ATRP initiator **7**. b) ToF-SIMS images demonstrating successful photopatterning of **8** (left). The initial photoreactivity is retained on non-irradiated parts and can be reutilized for further Diels–Alder functionalization by global irradiation with **7** (right).

Importantly, the initial photoreactivity of non-irradiated FMP-functionalized areas is retained and can be utilized for further Diels–Alder functionalization with a second dienophile. In this context, a preformed PEG-patterned surface

received a subsequent flood exposure in presence of **7**. We could indeed verify successful sequential photopatterning by bromine and PEG composition analysis (Figure 3b).

To also demonstrate the feasibility of covalent and site-specific attachment of peptides by this phototriggered approach (which also avoids protection chemistry), we irradiated a freshly prepared FMP-functionalized surface with maleimido-GRGSGR **4** in acetonitrile/PBS (3:1 v/v). ToF-SIMS again provided evidence that the peptide was immobilized in a patterned way that corresponded to the mask features. In that case, composition analysis was based on the presence of $C_4H_8N^+$, a secondary-ion characteristic for peptides (Figure 4).[17] The successful biopatterning was also confirmed by characteristic mass fragments for the arginine groups of **4** for example ($C_4H_{11}N_3^+ + C_5H_8N_3^+ + C_5H_{11}N_4^+$; Supporting Information, Figure S15).

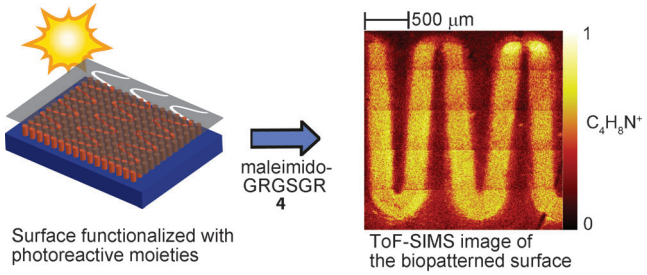


Figure 4. Locally constrained biofunctionalization on a surface. ToF-SIMS image (on the right) for bioconjugation with maleimido-GRGSGR **4**.

The photoenol-mediated conjugation strategy possesses many interesting features, making it a first-class click reaction. It can proceed rapidly at ambient temperature in a wide range of (polar) solvents. No catalyst is required and no by-product is formed, and thus no purification steps are required. Most importantly, control over time and space is inherent owing to its light-induced nature. Herein, we have demonstrated the high efficiency and speed of the phototriggered Diels–Alder (bio)conjugation in solution as well as on a surface by means of the covalent attachment of three dienophiles: a small-molecule ATRP initiator, a polymer, and a peptide. We envisage many applications for this procedure ranging from the photolithographic grafting of conductive polymers for LED development to the generation of a variety of complex architectures, including polymer–protein conjugates. Our efforts are currently directed towards the production of functional patterned substrates to control cell behavior by the introduced technique.

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