DOI: 10.1002/ange.201302040

Photopatterning

Spatially Controlled Surface Immobilization of Nonmodified Peptides**

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Controlled surface modification through the precise positioning of chemical functionalities or relevant biomolecules holds enormous promise for advances in ever-growing research areas such as biointerface science, point-of-care applications, biosensors, and nanotechnology.[1] Among the myriad of modification techniques that have been developed, photochemical approaches play a key role. They contribute not only in a significant way to the existing repertoire of carbon–carbon bond-forming reactions,[2] but also inherently confer spatiotemporal control to chemical reactions and are as such highly appealing for the production of patterns of carbohydrates, proteins, DNA fragments, or multiple cell lines.[3] The use of light has a tremendous influence on the way biomaterials scientists can perform experiments, e.g., to control the photodegradation of hydrogels[4] or the activity of cells.[5] Today chemists have a number of methods available for spatially controlled surface grafting, which mostly rely on the release of appropriate functional groups upon a light stimulus, such as azide-reactive cyclooctynes, ene-reactive nitrile imines, or aminooxy-reactive aldehydes.[6] A novel approach that photogenerates highly reactive intermediates with short lifetimes, such as *o*-naphtoquinone methides, was also reported.[7] Regeneration of the initial photoactive moiety occurs through hydration resulting often in improved selectivity and orthogonality as well as a better handling in an environment that is not protected from light. Popik, Locklin, and co-workers have followed such a path when they used 3-(hydroxymethyl)-naphthalene-2-ol derivatives with vinyl ether moieties for covalent surface derivatization.[8] In the same context, we have recently employed the photoisomerization of *o*-methylbenzaldehyde to *o*-quinodimethanes and demonstrated its ease and efficiency in the light-induced modular ligation of polymers prepared by reversible addition-fragmentation chain transfer polymerization (RAFT) and in spatially controlled surface grafting.[9] Altogether, these different photoreactions can be applied to photopatterning in different contexts with high efficiency. A limiting factor of their applicability in novel technologies is, however, that all of the aforementioned transformations involve complex multistep synthesis of surface anchors functionalized with photochemically active moieties and, often even more challenging, individual modifications of (biologically) relevant substrates with appropriate functionalities for coupling. These compounds have to be precisely tailored to the concrete needs, thus limiting their versatility. The idea of the current study is to use potent dienes, namely phencyclone derivatives, as a novel platform for light-triggered modifications in solution and on surfaces. The outcomes of these chemical transformations were assessed separately; specifically, the approach initially investigated in solution proceeds in three steps: A) a versatile and highly efficient Diels–Alder-based formation of a photoactive precursor, B) a fast and mild photoactivation to induce spatiotemporal control, and C) the nucleophilic attachment of amines (Figure 1 a). The construction of patterns of nonmodified biomolecules such as peptides and proteins by means of their inherent amine functionality would be highly desirable,[10] especially if the procedure does not involve extremely reactive intermediates such as radicals, which would clearly result in limitations of selectivity and orthogonality. Upon performing a screening study of poten-

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[**] C.B.-K. acknowledges funding from the Karlsruhe Institute of Technology (KIT) in the context of the BioInterfaces program of the Helmholtz association.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201302040.

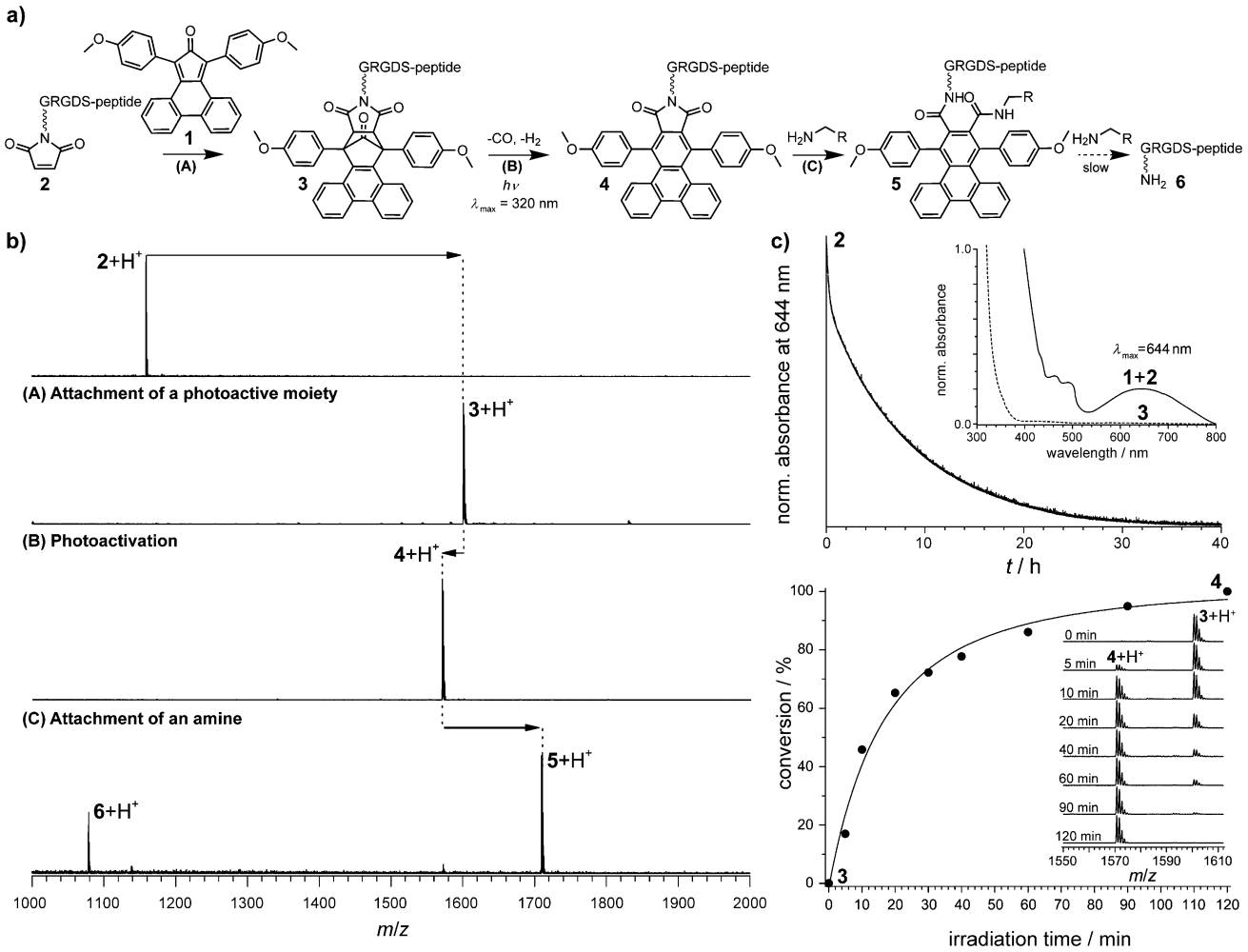


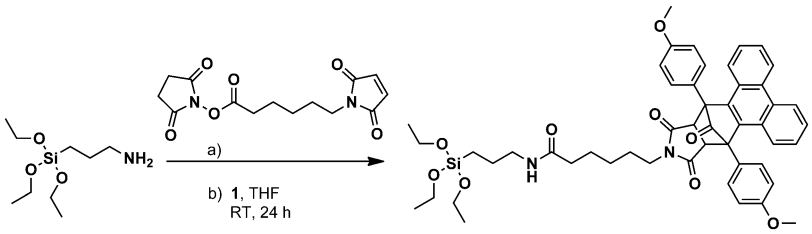
Figure 1. a) Overall concept for light-triggered modifications of the GRGDS peptide **2**. b) ESI-MS spectra of a maleimide end-capped GRGDS peptide **2** and after reaction with phencyclone derivative **1** (1.05 equiv). Subsequent decarbonylation and dehydrogenation to **4** was performed in water by an in situ photoreaction (2 h, 320 nm) in the presence of 1,4-benzoquinone (3 equiv). Amine attachment to **5** was achieved by ring opening employing an excess of an amine derivative at 45 °C. c) Kinetic investigations of the Diels–Alder reaction of **1** with **2** by UV/Vis spectroscopy (top) and of the in situ activation of **3** by irradiation in the presence of 1,4-benzoquinone (3 equiv) to give **4** (bottom). The structural formulas of all compounds are included in Scheme S1 in the Supporting Information.

tial phencyclone candidates, we have identified the easily synthesizable[11] 1,3-bis(4-methoxyphenyl)-2*H*-cyclopenta-[l]phenanthren-2-one (MCPO) **1** compound to be the most efficient precursor (see Figure S13 in the Supporting Information).

The initial light-triggered modifications were performed in solution utilizing the maleimide-functionalized GRGDS peptide **2**, which aided us to evaluate the possibility of performing our proposed reaction sequence in the presence of diverse amino acid residues in polar solvents. The synthetic procedure for the attachment of the photoactive moiety is straightforward and was achieved by stirring **2** with **1** (1.05 equiv of **1**, MeCN/H_2O 7:1 v/v). UV/Vis spectroscopy is a useful technique for the online measurement of the concentration of **1**, which is colored by virtue of its n→π* transition. The progress of the cycloaddition reaction could thus be monitored by measuring the loss of the long-wavelength absorbance of the phencyclone chromophore in the visible spectrum (644 nm, see Figure 1 c) while the initially completely dark green solution turned colorless within 40 h reaction time, thereby indicating the efficient consumption of **1**. As a further test for a successful attachment of the photoactive precursor, an electrospray mass spectrometry (ESI-MS) analysis was performed. Figure 1 b depicts the mass spectra of the starting GRGDS peptide **2** alongside the quantitatively formed Diels–Alder cycloadduct **3**. Upon confirming the successful attachment of the photoactive species, we turned our attention to systematically investigating the photochemical behavior of the latter by employing a low-cost 36 W compact fluorescent lamp ($\lambda_{max} = 320$ nm; Figure S9 in the Supporting Information) as the UV source. In general, bridged carbonyl compounds such as norbornen-7-one derivatives can eliminate carbon monoxide upon heating or mild irradiation.[12] In the case of **3** we observed light-triggered decarbonylation, accompanied by a low amount of dehydrogenation product **4** (see Figure S14 in the Supporting Information). To produce the fully conjugated triphenylene imide **4** in a quantitative manner, one can perform a one-pot

in situ decarbonylation/dehydrogenation photoreaction. In detail, **3** was irradiated at ambient temperature in water for 2 h in the presence of 1,4-benzoquinone (1:3 mol/mol). Inspection of the ESI-MS spectra in Figure 1 b reveals a full peak shift of 30 amu towards lower mass-to-charge ratios, which corresponds to the loss of CO and H_2. Complete disappearance of the quasimolecular ion $[\mathbf{3}+H]^+$ leaving $[\mathbf{4}+H]^+$ demonstrates that full conversion was achieved after less than 120 min irradiation time at ambient temperature (Figure 1 c). A more in-depth analysis of the photoreaction in various solvents, through a two-step procedure (adding the dehydrogenation agent after the light-triggered decarbonylation) and the utilization of different dehydrogenation agents (e.g., alkylmaleimides) may be found in the Supporting Information. We observed in all of these cases an efficient transformation from the photoactivatable compound **3** to photoactivated **4** in a quantitative and solvent-independent manner. Secondary photolysis, which can be a major issue in the widely employed *o*-nitrobenzyl photoprotecting moiety,[13] is avoided even after prolonged irradiation by means of the overall high photolytic and thermal stability of all products, which are namely CO, H_2, and the triphenylene imide **4**. The latter moiety is however capable of undergoing ring-opening reactions with amines in analogy to the well-known phtalimide system.[14] Inspection of Figure 1 b (for the attachment of other amines refer to Figure S19 in the Supporting Information) reveals the successful formation of the monoadduct of 2-(4-fluorophenyl)ethanamine **5** as the main product, whereas double attachment resulted in cleavage and a minor fraction of amine end-capped peptide **6**. The solution-based experiments convincingly demonstrate the highly efficient attachment of a photoactive precursor (which does not require complex synthesis), its rapid and quantitative photoactivation, and its catalyst-free coupling with amines. A limiting factor of the approach in solution is, however, the necessity to employ an excess of amine to efficiently ring-open the triphenylene imide **4**. The current strategy is thus not the system of choice in cases where product separation is difficult, e.g., when polymer–polymer conjugations are targeted. However, the full strength of the given method for light-directed functionalization is evidenced when the reaction sequence is performed on a surface. Excess of amine reagent is easily removed by simple washing, and double nucleophilic ring-opening by amines is less pronounced owing to steric hindrance. The clear dichotomy between the photoactivation and the chemical attachment in the current strategy would also allow for the construction of patterns of light-sensitive (bio)molecules that cannot be obtained by more conventional strategies. Importantly, and being the basis for any spatially resolved grafting of molecules onto surfaces, the nonirradiated photosensitive precursor **3** was found to be fully inert in the presence of excess 2-(4-fluorophenyl)ethanamine at 45 °C (see Figure S20 in the Supporting Information).

These findings and the potential ability to pattern nonmodified biomolecules by means of their inherent amine functionality prompted us to translate it to the spatially constrained immobilization of amines onto surfaces to produce molecular patterns. Note that, in contrast to the initial solution experiments where the peptide was bearing the photoactive moiety, this time we employed a nonmodified peptide able to react with photoactivated substrates through its lysine residue. The synthetic route to functionalized silicon surfaces is straightforward. A maleimide end-capped silane was prepared from commercially available substances (Scheme 1) and was converted to the MCPO end-capped silane in a Diels–Alder cycloaddition with **1**. Finally the silane was dissolved in anhydrous toluene and employed to treat activated silicon wafers. Upon successful silanization, the photopatterning was achieved by irradiation of the silicon wafers for 3 h under normal atmospheric conditions (no inert gas, ambient temperature) and without solvent. Two shadow masks were utilized for the locally constrained surface activation: one featuring squares with 50 μm pitches in *x*/*y* coordinates and one with a macroscopic pattern. After irradiation, patterning was achieved by immersing the wafers in a 1,4-benzoquinone solution (dehydrogenation) and finally in a methanolic solution of the respective amine for 18 h at 45 °C. Analysis of the photopatterning was achieved by imaging time-of-flight secondary-ion mass spectrometry (ToF-SIMS), which is a highly surface-sensitive and label-free technique for the spatially resolved analysis of solid substrates.[15] A ToF-SIMS composition analysis of the surface reproduced the shadow mask structures with an excellent spatial resolution (edge steepness: 4.5 μm) between irradiated and nonirradiated areas (see Figure 2 b, top). Indeed, only the nonirradiated zone showed the presence of the maleimide–phencyclone Diels–Alder cycloadduct ($C_{35}H_{24}NO_5^-$), while only the irradiated squares exhibited a fragment at 30 amu lower mass-to-charge ratio, corresponding to triphenylene imide ($C_{34}H_{22}NO_4^-$), which is formed by loss of H_2 and CO. Bromine compounds with their inherent isotopic pattern can be unambiguously detected by ToF-SIMS, therefore, the photopatterned wafer was immersed in a solution of 2-(4-bromophenyl)ethanamine as a molecular marker to spatially map the amine-reactive areas. Clearly, only the irradiated part exhibited bromine functionalization after immersion of the wafer in the solution of 2-(4-bromophenyl)ethanamine (Figure 2 b, bottom). To demonstrate the feasibility of a covalent and spatially resolved attachment of nonmodified peptides by this phototriggered approach, we repeated the irradiation/dehydrogenation procedure (irradiation: no inert gas, ambient temperature, no solvent; dehydrogenation in a 1,4-



Scheme 1. Synthesis of the MCPO-functionalized silane.

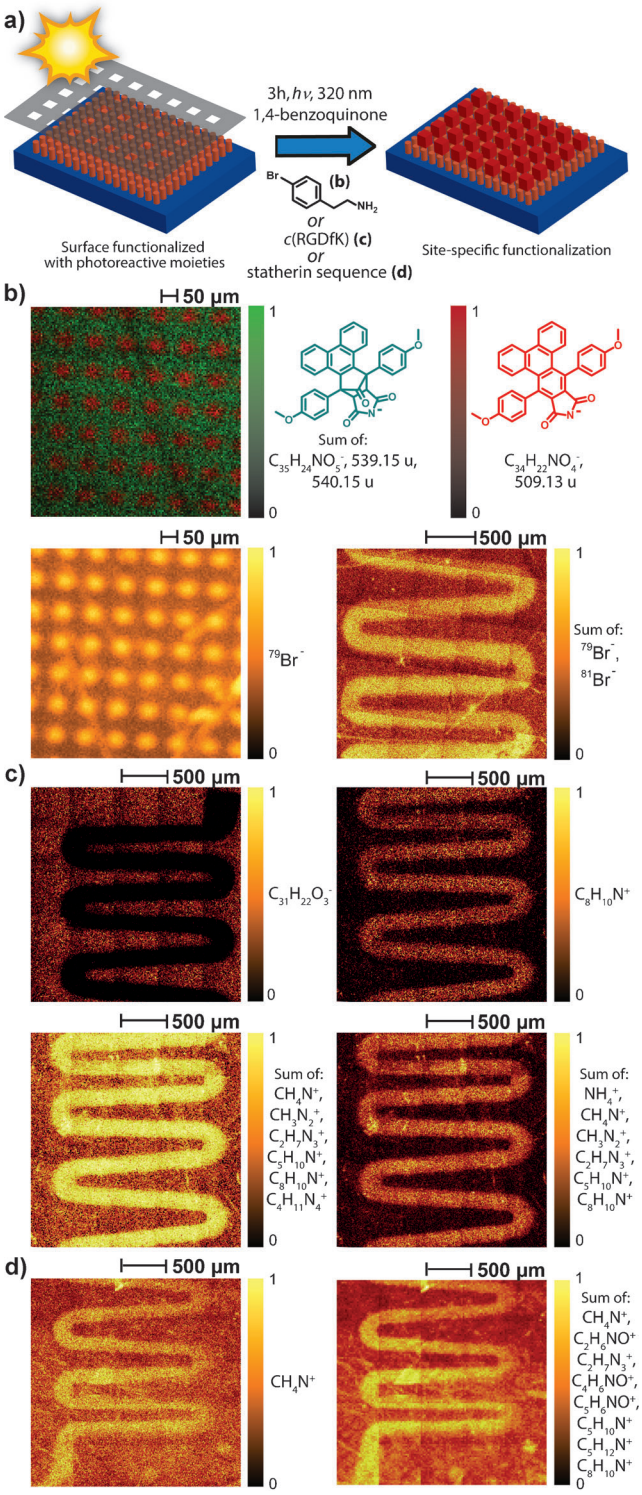


Figure 2. a) Representation of the phototriggered surface grafting. b) ToF-SIMS images of silicon wafers patterned utilizing two shadow masks. Photopatterning was achieved as described in (a) by immersing the wafers in a 1,4-benzoquinone solution (top). The patterned surfaces were then immersed in 2-(4-bromophenyl)ethanamine to visualize the amine-reactive areas (bottom). c) ToF-SIMS images of silicon wafers patterned with c(RGDfK) and d) with a statherin sequence utilizing a shadow mask.

benzoquinone solution) for a freshly prepared MCPO-functionalized wafer. Finally, the surface was treated with a solution of *N*,*N*-diisopropylethylamine (DIPEA) and the extracellular matrix protein-mimicking c(RGDfK) peptide in DMF. In a ToF-SIMS analysis we could confirm the successful photodeprotection by using a negative contrast for the Diels–Alder cycloadduct ($C_{35}H_{24}NO_5^-$, see Figure S24 in the Supporting Information) as well as by its retro-Diels–Alder follow-up product **1** ($C_{31}H_{22}O_3^-$, see Figure 2c, top left) formed during analysis. ToF-SIMS also provided evidence that the peptide was immobilized in a pattern corresponding to the mask features. In that case, composition analysis was based on the presence of $CH_3N_2^+$ and $C_8H_{10}N^+$ (Figure 2c, top right), characteristic secondary ions for arginine- or phenylalanine-containing peptides, respectively.[16] Sums of all positive ion fragments that can be assigned to the peptide are depicted in Figure 2c. The same surface modification sequence can also be applied for the locally constrained immobilization of a highly charged peptide comprising the active amino acid sequence of statherin, which inhibits secondary calcium phosphate precipitation. In this case again, the substrate was efficiently immobilized in a patterned way (see Figure 2d, for a full overview of all observed fragments for both peptides in ToF-SIMS negative and positive modes, refer to Figures S22, S25, and S26 in the Supporting Information).

Herein we introduced a novel technique allowing the photopatterning of peptides strictly composed of naturally occurring residues, representing the first important feature of our method, since it avoids the expensive preparation or purchase of nonnatural amino acids or the modification of peptide C or N termini. In addition, the synthetic route towards the relevant photoreactive compounds is technically simple and achieved at a rather low cost. Finally, the photopatterning sequence itself involves catalyst-free and rather mild conditions. The photoreaction is fully compatible to all investigated solvents including water, avoids the formation of harmful photolysis by-products, and can be performed under ambient atmosphere and temperature. The combination of these features renders the system extremely attractive for industrially relevant peptide micro- or nanoarray fabrication.

Received: March 11, 2013
Revised: May 31, 2013
Published online: July 24, 2013

Keywords: patterning · peptides · phencyclone · photochemistry

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