

# Spatially Controlled Surface Immobilization of Nonmodified Peptides\*\*

Thomas Pauloeuhl, Alexander Welle, Michael Bruns, Katharina Linkert, Hans G. Börner, Martin Bastmeyer, Guillaume Delaittre, and Christopher Barner-Kowollik\*

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.<sup>[1]</sup> 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,<sup>[2]</sup> 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.<sup>[3]</sup> The use of light has a tremendous influence on the way biomaterials scientists can perform experiments, e.g., to control the photodegradation of hydrogels<sup>[4]</sup> or the activity of cells.<sup>[5]</sup> 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.<sup>[6]</sup> A novel approach that photogenerates highly reactive intermediates with short lifetimes, such as *o*-naphthoquinone methides, was also reported.<sup>[7]</sup> 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.<sup>[8]</sup> In the same context, we have recently employed the photoisomeri-

zation 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.<sup>[9]</sup> 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 multi-step 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,<sup>[10]</sup> 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-

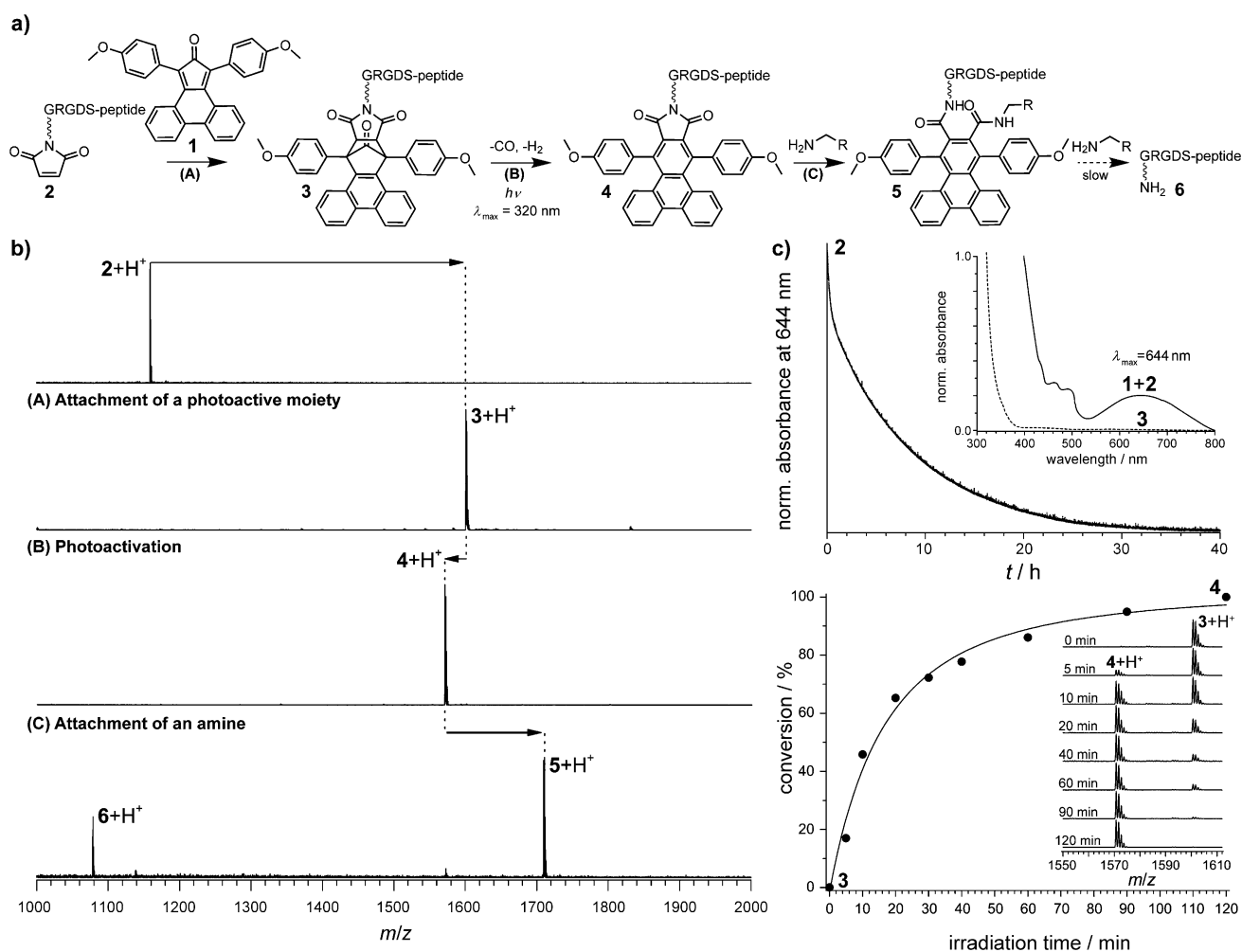
[\*] T. Pauloeuhl, Dr. G. Delaittre, Prof. Dr. C. Barner-Kowollik  
Preparative Macromolecular Chemistry  
Institut für Technische Chemie und Polymerchemie  
Karlsruhe Institute of Technology (KIT)  
Engesserstraße 18, 76128 Karlsruhe (Germany)  
E-mail: christopher.barner-kowollik@kit.edu  
Homepage: <http://www.macroarc.de>  
Dr. A. Welle, Prof. Dr. C. Barner-Kowollik  
Institut für Biologische Grenzflächen (IBG), KIT  
Hermann-von-Helmholtzplatz 1  
76344 Eggenstein-Leopoldshafen (Germany)  
Dr. M. Bruns  
Institut für Angewandte Materialien (IAM) and  
Karlsruhe Nano Micro Facility (KNMF), KIT  
Eggenstein-Leopoldshafen (Germany)  
K. Linkert, Prof. Dr. H. G. Börner  
Laboratory for Organic Synthesis of Functional Systems  
Department of Chemistry  
Humboldt-Universität zu Berlin (Germany)

Prof. Dr. M. Bastmeyer, Dr. G. Delaittre  
Zoologisches Institut, Zell- und Neurobiologie, KIT  
Karlsruhe (Germany)  
Prof. Dr. M. Bastmeyer  
Institut für Funktionelle Grenzflächen, KIT  
Eggenstein-Leopoldshafen (Germany)  
Dr. G. Delaittre  
Institute of Toxicology and Genetics (ITG), KIT  
Eggenstein-Leopoldshafen (Germany)

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**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<sup>[11]</sup> 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).

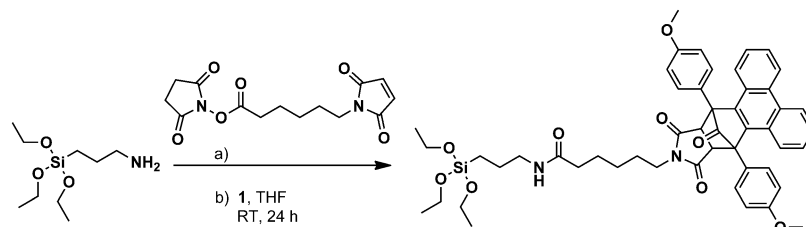
The initial light-triggered modifications were performed in solution utilizing the maleimide-functionalized GRGDS peptide **2**, which aided us to evaluate the possibility of performing our proposed reaction sequence in the presence of diverse amino acid residues in polar solvents. The synthetic procedure for the attachment of the photoactive moiety is straightforward and was achieved by stirring **2** with **1** (1.05 equiv of **1**, MeCN/H<sub>2</sub>O 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  $n \rightarrow \pi^*$  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 1c) 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 1b 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 ( $\lambda_{\text{max}} = 320$  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.<sup>[12]</sup> 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 1b reveals a full peak shift of 30 amu towards lower mass-to-charge ratios, which corresponds to the loss of CO and H<sub>2</sub>. Complete disappearance of the quasimolecular ion [3+H]<sup>+</sup> leaving [4+H]<sup>+</sup> demonstrates that full conversion was achieved after less than 120 min irradiation time at ambient temperature (Figure 1c). 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,<sup>[13]</sup> is avoided even after prolonged irradiation by means of the overall high photolytic and thermal stability of all products, which are namely CO, H<sub>2</sub>, and the triphenylene imide **4**. The latter moiety is however capable of undergoing ring-opening reactions with amines in analogy to the well-known phthalimide system.<sup>[14]</sup> Inspection of Figure 1b (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).

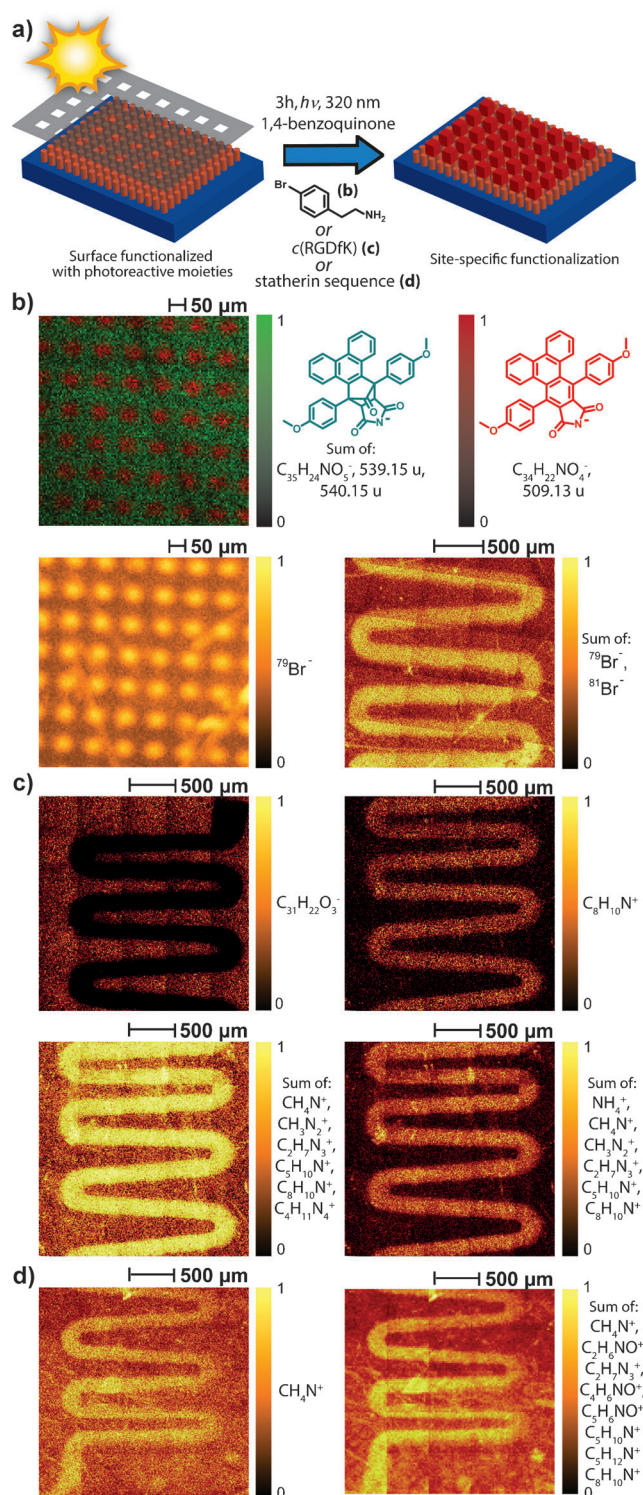
These findings and the potential ability to pattern non-modified biomolecules by means of their inherent amine

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



Scheme 1. Synthesis of the MCPO-functionalized silane.

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.<sup>[15]</sup> 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 2b, top). Indeed, only the nonirradiated zone showed the presence of the maleimide–phencyclone Diels–Alder cycloadduct (C<sub>35</sub>H<sub>24</sub>NO<sub>5</sub><sup>−</sup>), while only the irradiated squares exhibited a fragment at 30 amu lower mass-to-charge ratio, corresponding to triphenylene imide (C<sub>34</sub>H<sub>22</sub>NO<sub>4</sub><sup>−</sup>), which is formed by loss of H<sub>2</sub> 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 2b, 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-



- Esashi, *Sens. Actuators B* **2002**, 83, 67; c) S. Yamaguchi, S. Yamahira, K. Kikuchi, K. Sumaru, T. Kanamori, T. Nagamune, *Angew. Chem.* **2012**, 124, 132; *Angew. Chem. Int. Ed.* **2012**, 51, 128.
- [4] a) A. M. Kloxin, A. M. Kasko, C. N. Salinas, K. S. Anseth, *Science* **2009**, 324, 59; b) C. A. DeForest, K. S. Anseth, *Angew. Chem.* **2012**, 124, 1852; *Angew. Chem. Int. Ed.* **2012**, 51, 1816.
- [5] a) M. P. Lutolf, *Nature* **2012**, 482, 477; b) A. Levskaya, O. D. Weiner, W. A. Lim, C. A. Voigt, *Nature* **2009**, 461, 997.
- [6] a) A. A. Poloukhine, N. E. Mbua, M. A. Wolfert, G.-J. Boons, V. V. Popik, *J. Am. Chem. Soc.* **2009**, 131, 15769; b) M. Dietrich, G. Delaittre, J. P. Blinco, A. J. Inglis, M. Bruns, C. Barner-Kowollik, *Adv. Funct. Mater.* **2012**, 22, 304; c) T. Pauloeherl, G. Delaittre, M. Bruns, M. Meißler, H. G. Börner, M. Bastmeyer, C. Barner-Kowollik, *Angew. Chem.* **2012**, 124, 9316; *Angew. Chem. Int. Ed.* **2012**, 51, 9181.
- [7] S. Arumugam, S. V. Orski, J. Locklin, V. V. Popik, *J. Am. Chem. Soc.* **2012**, 134, 179.
- [8] a) S. Arumugam, V. V. Popik, *J. Am. Chem. Soc.* **2011**, 133, 15730; b) S. Arumugam, V. V. Popik, *J. Am. Chem. Soc.* **2011**, 133, 5573.
- [9] a) K. K. Oehlenschlaeger, J. O. Mueller, N. B. Heine, M. Glassner, N. K. Guimard, G. Delaittre, F. G. Schmidt, C. Barner-Kowollik, *Angew. Chem.* **2013**, 125, 791; *Angew. Chem. Int. Ed.* **2013**, 52, 762; b) T. Pauloeherl, G. Delaittre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, *Angew. Chem.* **2012**, 124, 1096; *Angew. Chem. Int. Ed.* **2012**, 51, 1071.
- [10] a) R. M. Broyer, G. N. Grover, H. D. Maynard, *Chem. Commun.* **2011**, 47, 2212; b) K. L. Christman, V. D. Enriquez-Rios, H. D. Maynard, *Soft Matter* **2006**, 2, 928.
- [11] H. Wettach, S. S. Jester, A. Colsmann, U. Lemmer, N. Rehmann, K. Meerholz, S. Höger, *Synth. Met.* **2010**, 160, 691.
- [12] a) D. Chaudhuri, H. Wettach, K. J. van Schooten, S. Liu, E. Sigmund, S. Höger, J. M. Lupton, *Angew. Chem.* **2010**, 122, 7880; *Angew. Chem. Int. Ed.* **2010**, 49, 7714; b) B. Fuchs, *J. Chem. Soc. C* **1968**, 68.
- [13] Y. V. Il'ichev, M. A. Schwörer, J. Wirz, *J. Am. Chem. Soc.* **2004**, 126, 4581.
- [14] M. K. Hargreaves, J. G. Pritchard, H. R. Dave, *Chem. Rev.* **1970**, 70, 439.
- [15] C.-Y. Lee, G. M. Harbers, D. W. Grainger, L. J. Gamble, D. G. Castner, *J. Am. Chem. Soc.* **2007**, 129, 9429.
- [16] a) M. S. Wagner, D. G. Castner, *Langmuir* **2001**, 17, 4649; b) H. E. Canavan, D. J. Graham, X. Cheng, B. D. Ratner, D. G. Castner, *Langmuir* **2007**, 23, 50; c) V. L. Sedman, L. Adler-Abramovich, S. Allen, E. Gazit, S. J. B. Tendler, *J. Am. Chem. Soc.* **2006**, 128, 6903.