Photochemistry

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UV-Induced Tetrazole-Thiol Reaction for Polymer Conjugation and Surface Functionalization**

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Abstract: *A UV-induced 1,3-dipolar nucleophilic addition of tetrazoles to thiols is described. Under UV irradiation the reaction proceeds rapidly at room temperature, with high yields, without a catalyst, and in both polar protic and aprotic solvents, including water. This UV-induced tetrazole-thiol reaction was successfully applied for the synthesis of small molecules, protein modification, and rapid and facile polymer–polymer conjugation. The reaction has also been demonstrated for the formation of micropatterns by site-selective surface functionalization. Superhydrophobic–hydrophilic micropatterns were successfully created by sequential modifications of a tetrazole-modified porous polymer surface with hydrophobic and hydrophilic thiols. A biotin-functionalized surface could be fabricated in aqueous solutions under long-wavelength UV irradiation.*

Ever since the first reported photoreaction of an organic compound, santonin, in 1834 by Trommsdorf,[1] the spatially and temporally controllable photochemistry has found diverse and widespread applications,[2] including surface functionalization to create patterned or gradient immobilization of various substrates.[3] Photo-induced click reactions have been actively investigated during the last decade in attempts to combine the benefits of click reactions with the excellent spatial and temporal controllability of photochemical processes.[4] UV-induced thiol-ene and thiol-yne reactions are the most known radical photo-click reactions.[5] Non-radical photoreactions have also attracted a lot of attention in recent years.[6] For instance, Lin et al.,[7] introduced a photo-click 1,3-dipolar tetrazole-ene reaction based on Huisgen's studies.[8] The tetrazole-ene reaction presents several advantages: simplicity of implementation, fast reaction kinetics, and high yields; it is catalyst-free, yields inoffensive by-products (N_2), and is therefore biocompatible. This and other photoreactions have been implemented in many different applications such as dendrimers synthesis,[9] bioconjugation,[6b,d,e,10] in situ biolabeling,[6c,11] hydrogels formation,[12] and surface functionalization.[13] Nevertheless, the implementation of photoreactions in bioapplications is still limited, partly because unnatural functional groups have to be first introduced to a biomolecule. In addition, only a few photoreactions could be applied for polymer–polymer coupling and site-selective conjugation of biomolecules on surfaces.[13b,14] Thus, despite the progress in the field of photoinduced reactions, there is a clear need for novel efficient photoreactions that are selective toward different types of functionalities, compatible with polymer–polymer conjugation, and bioapplications.

About 50 years ago, Huisgen et al. reported that thiophenol could be added to the intermediate nitrilimine generated by decomposition of 2,5-diphenyltetrazole in boiling thiophenol.[8a,b] Photolytic decomposition of tetrazoles with release of nitrogen and nitrilimines was also described.[8c] In this work, we report a UV-induced tetrazole-thiol reaction (Figure 1 A) that allows for rapid catalyst-free polymer–polymer conjugation, efficient surface functionalization, and patterning as well as opens the way to direct functionalization of biomolecules bearing periphery thiol groups.

To initially examine the kinetics of the UV-induced tetrazole-thiol reaction, a solution of methyl 4-(2-phenyl-2*H*-tetrazol-5-yl)benzoate **1** in ethyl acetate and 5 equiv of 2-mercaptoethanol **2** were subjected to irradiation at 260 nm UV light (Figure 1 B). The results showed the UV-triggered rupture of the tetrazole ring evidenced by the gradual decrease in the tetrazole absorption at 278 nm. At the same time a new absorption band around 369 nm was observed only in the presence of a thiol, confirming the formation of a tetrazole-thiol adduct (Figures 1 C,D and S1). Both steps were rapid, achieving complete conversion within 10 s based on UV/Vis spectroscopy. The resulting tetrazole-thiol adduct, meanwhile, showed a strong fluorescent emission band at 480 nm (Figure S1). The thiohydrazonate structure of the product **3** was confirmed by ESI-MS and NMR (Figures S2–S4). The kinetics of the UV-induced reaction was also investigated by ^{1}H NMR spectroscopy (Figure S5).

Table S1 shows yields of isolated product after UV irradiation of tetrazole **1** in the presence of thiol **2** in both polar protic (ethanol) and aprotic (ethyl acetate) solvents

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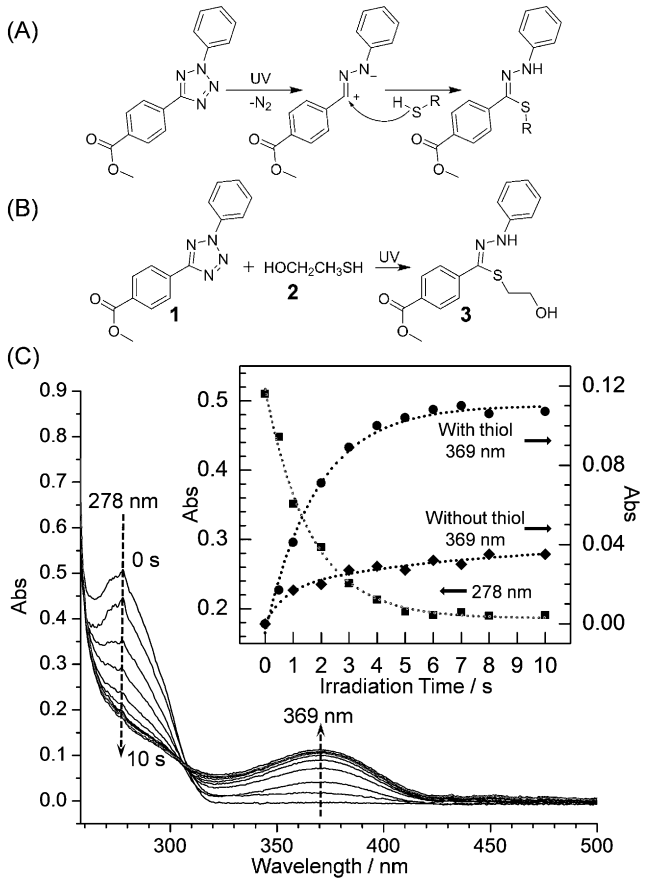

Figure 1. Representation of A) UV-induced formation of the nitrilimine intermediate from tetrazole **1** and subsequent nucleophilic thiol addition; B) the UV-induced tetrazole-thiol reaction between tetrazole **1** and thiol **2**. C) UV/Vis absorbance of the tetrazole-thiol reaction mixture as a function of UV irradiation time. The evolution of the absorbance peaks at 278 nm (■) as well as the 369 nm (●) with irradiation time (inset). The control experiments (369 nm peak is shown as ◆) were performed under the same conditions but without **2**.

under 260, 312, and 365 nm UV light. Since the photoactivity of diaryltetrazoles depends on their UV absorption properties[15] and **1** absorbs strongly at shorter wavelengths (λ_{max} = 278 nm), 260 nm and 312 nm irradiation afforded the highest yields in the range of 75–95%. The reaction between **1** and an equimolar amount of **2** in ethanol leads to 87% of the isolated product. The thiols could be used in this reaction even in the presence of amine,[16] another nucleophile, in polar protic solvents. The tetrazole-thiol adduct **3** was isolated with a yield of 70% from a mixture of **1** with **2** (2.5 equiv) and ethanolamine (2.5 equiv) after UV irradiation (Figure S6).

Most surface immobilizations or chemical modifications of biomolecules require aqueous conditions to avoid possible protein denaturation and loss of activity. To demonstrate that UV-induced tetrazole-thiol reaction can proceed in water, a tetrazole-bearing poly(ethylene glycol)methyl ether (MW 5000 g mol^{-1}; PEG-tetrazole **4**; Figure S7) was reacted with **2** (5 equiv) in water under 312 nm UV light for 3 h. The conversion of **4** to the corresponding thiohydrazonate was as high as 95% based on NMR spectroscopy (Figure S8). In addition, bovine serum albumin (BSA), a protein containing one free peripheral thiol cysteine group,[17] was reacted with **4** in aqueous PBS buffer under 312 nm UV light. The presence of a fluorescent higher-molecular-weight band in the gel electrophoresis of the product confirmed BSA–PEG conjugation in aqueous medium (Figure S9).

We further investigated the efficiency of the UV-induced tetrazole-thiol reaction for macromolecular conjugation. Figure 2A shows a model polymer conjugation experiment that

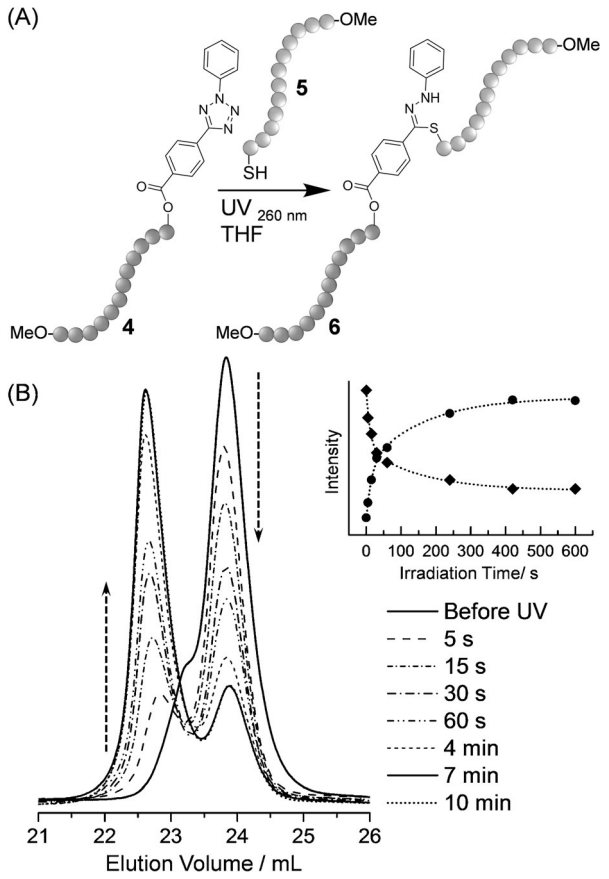

Figure 2. A) UV-induced conjugation of two polymers using the tetrazole-thiol reaction to form PEG-*block*-PEG copolymer **6**. B) GPC monitoring of the block copolymer formation. Evolution of the intensity of elution volumes at 23.8 mL (◆) and 22.6 mL (●) with irradiation time (inset).

was conducted. *O*-(2-mercaptoethyl)-*O*′-methyl poly(ethylene glycol) **5** (MW 5000 g mol^{-1}) was utilized as the thiol-terminated polymer. Equimolar amounts of **4** and **5** were dissolved in THF (3 mg mL^{-1}) and subsequently irradiated with $UV_{\lambda=260\,nm}$ light. The reaction mixture was analyzed by gel permeation chromatography (GPC) at different irradiation times (Figure 2B). A distinct shift of the GPC traces to lower elution volumes indicates successful formation of a polymer–polymer conjugate **6** already after 7 min of UV irradiation. The remaining small GPC peak corresponding to the starting material could be due to the incomplete tetrazole functionalization of **4** as well as the nonequimolar ratio of reactant.

Next, the performance of the UV-induced tetrazole-thiol reaction for surface functionalization was also examined. A

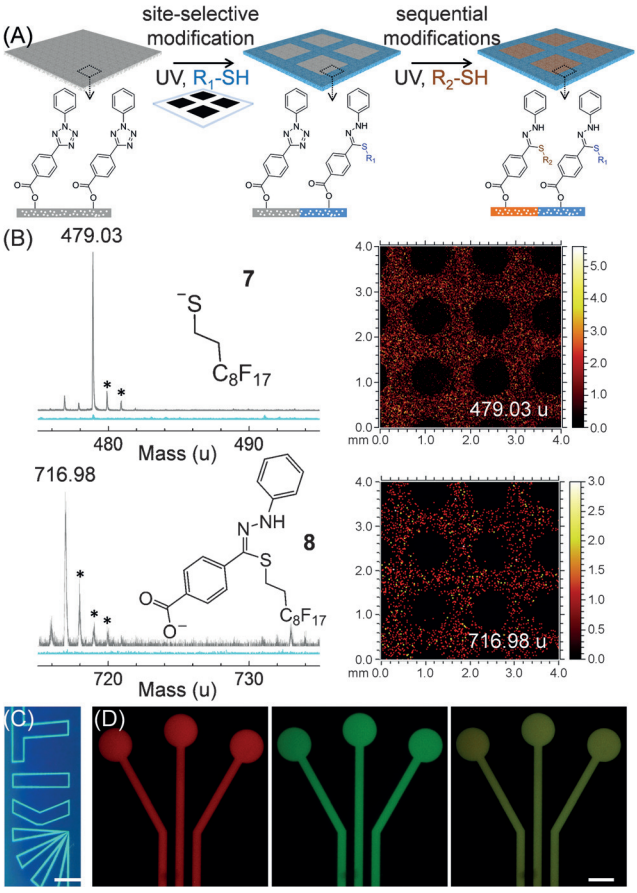

Figure 3. A) Surface micropatterning through the UV-induced tetrazole-thiol reaction. B) ToF-SIMS (negative polarity) spectra of the tetrazole surface before (blue line) and after (dark line) functionalization with thiol ***7***. Isotopic peaks are marked with asterisks. The ToF-SIMS images (fragments 479.03 u and 716.98 u, corresponding to the thiol **7** ion and the conjugation product **8**, respectively) of the polymer layer are inserted. Scale bars: 1 mm. C) Photograph of a thiol **7** patterned tetrazole surface under 365 nm UV light. Scale bar: 3 mm. D) Red (left), green (middle), and red/green overlay (right) fluorescence microscope images of the tetrazole surface patterned by Rhodamine-SH showing both red and green fluorescence. Scale bar: 300 μm.

porous polymer layer functionalized with tetrazole (tetrazole surface, Figure S10) was applied as the substrate. With the aid of a photomask, the surface was site-selectively modified by 1*H*,1*H*,2*H*,2*H*-perfluorodecanethiol **7** under $UV_{\lambda=260\,nm}$ irradiation (Figure 3A). The successful immobilization and patterning of thiol was confirmed by time-of-flight secondary ion mass spectrometry (ToF-SIMS) (Figures 3B and S11). X-ray photoelectron spectroscopy revealed the presence of F 1s, F KLL as well as S 2p peaks after thiol modification. Integration of N 1s and S 2p peak areas showed the evolution of conversion with UV irradiation time reaching 88% after 20 min of UV irradiation (Figure S12). After the tetrazole-thiol reaction, the appearance of fluorescence provides a visualization method to assess the success of surface modification (Figure 3C). Tetrazole surface modification with a thiol-containing fluorophore, Rhodamine-SH, was also shown in Figure 3D. The produced pattern shows a perfect superimposition of both red and green fluorescence. Surface micropatterns with feature sizes down to 10 μm could be obtained using an appropriate photomask (Figure S13).

The surface modification could be performed using different thiols in various common solvents (Figure S14). Moreover, when a fluorinated thiol **7** was employed, the hydrophobic tetrazole surface was transformed into a superhydrophobic surface exhibiting water contact angles θ_{st}, θ_{adv}, and θ_{rec} as high as 167°, 170°, and 161°, respectively. The SEM analysis in Figure 4A did not show any changes of the surface

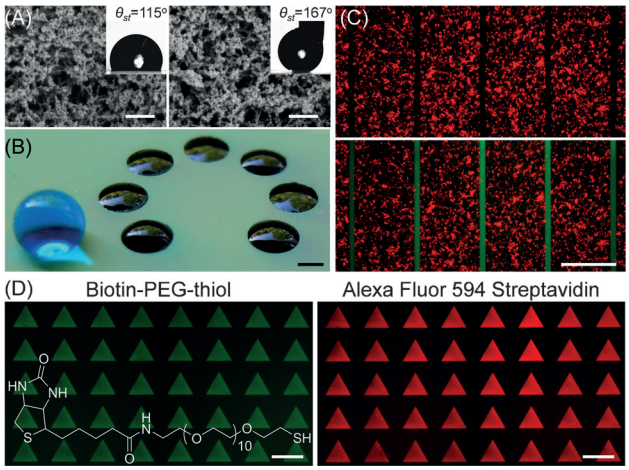

Figure 4. A) SEM images of the tetrazole surface before (left) and after (right) modification with thiol **7**. The images of a water droplet on the corresponding surfaces are inserted. B) Optical images of superhydrophobic-hydrophilic micropatterns. C) Red (top) and red/green overlay (bottom) fluorescence microscope images of the mCherry-expressing rat mammary carcinoma cells after growing for 30 h on a thiol **7** patterned tetrazole surface. The areas modified with **7** emit green fluorescence and show cell-repellent properties. D) Fluorescence microscopy image showing the immobilization of biotin-PEG-thiol in water under 365 nm UV light and Alexa Fluor 594-labeled streptavidin binding. Scale bars: 500 nm (A) and 1 mm (B–D).

morphology after the thiol modification. Hence, the UV-induced tetrazole-thiol reaction could be used to create well-defined superhydrophobic–hydrophilic micropatterns of different geometries after sequential modifications (Figures 4B and S15). The produced superhydrophobic barriers show good cell-repellent properties and mCherry cells only adhered well to the hydrophilic areas (Figure 4C), which is important for a variety of different biotechnological applications ranging from sensors to cell screening microarrays.[18]

To demonstrate that the tetrazole-thiol reaction could be used for the in situ immobilization of biomolecules in aqueous solutions under long-wavelength UV irradiation, we patterned biotin-PEG-thiol onto the tetrazole surface in water under 365 nm UV light (Figure 4D). The surface was then incubated with Alexa Fluor 594-labeled streptavidin solution. Fluorescence microscopy revealed a two-color green-red fluorescence pattern (Figure 4D), in which the green fluorescence originated from the thiohydrazonate product of the tetrazole-thiol reaction, whereas the red fluorescence originated from the Alexa Fluor 594-labeled streptavidin bound to the biotinylated pattern.

In conclusion, we have presented a new versatile UV-induced tetrazole-thiol reaction for conjugation of polymers as well as surface functionalization. The reaction proceeds very rapidly at ambient temperature with high efficiency and in the absence of any catalyst. Furthermore, this photo-based approach can be performed in aqueous conditions, making it a promising tool for diverse biological and biotechnological applications such as protein modification and surface bio-functionalization. The formation of a fluorescent product omits the necessity of using fluorescent labels and can be convenient for tracking the reaction or for multicolor labeling. Because of the above-mentioned advantages of this method, we believe that the UV-induced tetrazole-thiol reaction will become a valuable tool for different applications.

Keywords: nucleophilic addition · photochemistry · polymer conjugation · surface modification · tetrazole-thiol reaction

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[1] a) H. Trommsdorff, *Ann. Chem. Pharm.* **1834**, *11*, 190–208.
[2] a) H. Okabe, **1978**; b) N. J. Turro, *Modern molecular photochemistry*, University Science Books, **1991**; c) S. L. Murov, I. Carmichael, G. L. Hug, *Handbook of photochemistry*, CRC, Boca Raton, **1993**; d) J. P. Knowles, L. D. Elliott, K. I. Booker-Milburn, *Beilstein J. Org. Chem.* **2012**, *8*, 2025–2052; e) K. A. Mosiewicz, L. Kolb, A. J. van der Vlies, M. M. Martino, P. S. Lienemann, J. A. Hubbell, M. Ehrbar, M. P. Lutolf, *Nat. Mater.* **2013**, *12*, 1072–1078; f) C. Li, A. Glidle, X. Yuan, Z. Hu, E. Pulleine, J. Cooper, W. Yang, H. Yin, *Biomacromolecules* **2013**, *14*, 1278–1286; g) S.-H. Lee, J. J. Moon, J. L. West, *Biomaterials* **2008**, *29*, 2962–2968; h) T. A. Martin, S. R. Caliari, P. D. Williford, B. A. Harley, R. C. Bailey, *Biomaterials* **2011**, *32*, 3949–3957; i) Y. Li, B. H. San, J. L. Kessler, J. H. Kim, Q. Xu, J. Hanes, S. M. Yu, *Macromol. Biosci.* **2015**, *15*, 52–62.
[3] a) C.-M. Chan, T.-M. Ko, H. Hiraoka, *Surf. Sci. Rep.* **1996**, *24*, 1–54; b) L. Spanhel, M. Haase, H. Weller, A. Henglein, *J. Am. Chem. Soc.* **1987**, *109*, 5649–5655; c) Y. Ikada, *Biomaterials* **1994**, *15*, 725–736; d) J. A. Rogers, K. E. Paul, R. J. Jackman, G. M. Whitesides, *Appl. Phys. Lett.* **1997**, *70*, 2658–2660; e) F. L. Geyer, E. Ueda, U. Liebel, N. Grau, P. A. Levkin, *Angew. Chem. Int. Ed.* **2011**, *50*, 8424–8427; *Angew. Chem.* **2011**, *123*, 8575–8578; f) L. Li, J. Li, X. Du, A. Welle, M. Grunze, O. Trapp, P. A. Levkin, *Angew. Chem. Int. Ed.* **2014**, *53*, 3835–3839; *Angew. Chem.* **2014**, *126*, 3914–3918.
[4] a) M. A. Tasdelen, Y. Yagci, *Angew. Chem. Int. Ed.* **2013**, *52*, 5930–5938; *Angew. Chem.* **2013**, *125*, 6044–6053; b) S. Arumugam, V. V. Popik, *J. Am. Chem. Soc.* **2012**, *134*, 8408–8411; c) S. Arumugam, V. V. Popik, *J. Am. Chem. Soc.* **2011**, *133*, 5573–5579; d) S. Arumugam, V. V. Popik, *J. Am. Chem. Soc.* **2011**, *133*, 15730–15736; e) T. Pauloehrl, G. Delaittre, M. Bruns, M. Meißler, H. G. Börner, M. Bastmeyer, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2012**, *51*, 9181–9184; *Angew. Chem.* **2012**, *124*, 9316–9319; f) T. Pauloehrl, G. Delaittre, V. Winkler, A. Welle, M. Bruns, H. G. Börner, A. M. Greiner, M. Bastmeyer, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2012**, *51*, 1071–1074; *Angew. Chem.* **2012**, *124*, 1096–1099; g) T. Pauloehrl, A. Welle, K. K. Oehlenschlaeger, C. Barner-Kowollik, *Chem. Sci.* **2013**, *4*, 3503–3507.
[5] a) R. Hoogenboom, *Angew. Chem. Int. Ed.* **2010**, *49*, 3415–3417; *Angew. Chem.* **2010**, *122*, 3489–3491; b) A. B. Lowe, C. E. Hoyle, C. N. Bowman, *J. Mater. Chem.* **2010**, *20*, 4745–4750; c) C. E. Hoyle, C. N. Bowman, *Angew. Chem. Int. Ed.* **2010**, *49*, 1540–1573; *Angew. Chem.* **2010**, *122*, 1584–1617; d) A. Dondoni, *Angew. Chem. Int. Ed.* **2008**, *47*, 8995–8997; *Angew. Chem.* **2008**, *120*, 9133–9135.
[6] a) B. J. Adzima, Y. Tao, C. J. Kloxin, C. A. DeForest, K. S. Anseth, C. N. Bowman, *Nat. Chem.* **2011**, *3*, 256–259; b) W. Song, Y. Wang, J. Qu, Q. Lin, *J. Am. Chem. Soc.* **2008**, *130*, 9654–9655; c) Z. P. Yu, L. Y. Ho, Q. Lin, *J. Am. Chem. Soc.* **2011**, *133*, 11912–11915; d) Y. Wang, W. Song, W. J. Hu, Q. Lin, *Angew. Chem. Int. Ed.* **2009**, *48*, 5330–5333; *Angew. Chem.* **2009**, *121*, 5434–5437; e) W. Song, Y. Wang, J. Qu, M. M. Madden, Q. Lin, *Angew. Chem. Int. Ed.* **2008**, *47*, 2832–2835; *Angew. Chem.* **2008**, *120*, 2874–2877.
[7] R. K. V. Lim, Q. Lin, *Acc. Chem. Res.* **2011**, *44*, 828–839.
[8] a) R. Huisgen, R. Grashey, M. Seidel, H. Knupfer, R. Schmidt, *Justus Liebigs Ann. Chem.* **1962**, *658*, 169–180; b) R. Huisgen, J. Sauer, M. Seidel, *Chem. Ber.* **1961**, *94*, 2503–2509; c) J. S. Clovis, A. Eckell, R. Huisgen, R. Sustmann, *Chem. Ber.* **1967**, *100*, 60–70.
[9] K. L. Killops, L. M. Campos, C. J. Hawker, *J. Am. Chem. Soc.* **2008**, *130*, 5062–5064.
[10] J. Wang, W. Zhang, W. Song, Y. Wang, Z. Yu, J. Li, M. Wu, L. Wang, J. Zang, Q. Lin, *J. Am. Chem. Soc.* **2010**, *132*, 14812–14818.
[11] a) F. Li, H. Zhang, Y. Sun, Y. Pan, J. Zhou, J. Wang, *Angew. Chem. Int. Ed.* **2013**, *52*, 9700–9704; *Angew. Chem.* **2013**, *125*, 9882–9886.
[12] a) Y. Fan, C. Deng, R. Cheng, F. Meng, Z. Zhong, *Biomacromolecules* **2013**, *14*, 2814–2821; b) C.-C. Lin, A. Raza, H. Shih, *Biomaterials* **2011**, *32*, 9685–9695.
[13] a) E. Blasco, M. Piñol, L. Oriol, B. V. K. J. Schmidt, A. Welle, V. Trouillet, M. Bruns, C. Barner-Kowollik, *Adv. Funct. Mater.* **2013**, *23*, 4011–4019; b) M. Dietrich, G. Delaittre, J. P. Blinco, A. J. Inglis, M. Bruns, C. Barner-Kowollik, *Adv. Funct. Mater.* **2012**, *22*, 304–312; c) T. Tischer, C. Rodriguez-Emmenegger, V. Trouillet, A. Welle, V. Schueler, J. O. Mueller, A. S. Goldmann, E. Brynda, C. Barner-Kowollik, *Adv. Mater.* **2014**, *26*, 4087–4092; d) W. Feng, L. Li, E. Ueda, J. Li, S. Heißler, A. Welle, O. Trapp, P. A. Levkin, *Adv. Mater. Inter.* **2014**, DOI: 10.1002/admi.201400269.
[14] a) M. Glassner, K. K. Oehlenschlaeger, T. Gruendling, C. Barner-Kowollik, *Macromolecules* **2011**, *44*, 4681–4689; b) K. K. Oehlenschlaeger, J. O. Mueller, N. B. Heine, M. Glassner, N. K. Guimard, G. Delaittre, F. G. Schmidt, C. Barner-Kowollik, *Angew. Chem. Int. Ed.* **2013**, *52*, 762–766; *Angew. Chem.* **2013**, *125*, 791–796.
[15] a) Y. Wang, W. J. Hu, W. Song, R. K. V. Lim, Q. Lin, *Org. Lett.* **2008**, *10*, 3725–3728; b) Z. Yu, T. Y. Ohulchanskyy, P. An, P. N. Prasad, Q. Lin, *J. Am. Chem. Soc.* **2013**, *135*, 16766–16769.
[16] Y. X. Zhang, W. J. Liu, Z. B. K. Zhao, *Molecules* **2014**, *19*, 306–315.
[17] a) A. Svenson, J. Carlsson, *Biochim. Biophys. Acta Protein Struct.* **1975**, *400*, 433–438; b) M. Oblak, A. Prezelj, S. Pecar, T. Solmajer, *Z. Naturforsch. C* **2004**, *59*, 880–886.
[18] E. Ueda, P. A. Levkin, *Adv. Mater.* **2013**, *25*, 1234–1247.

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