Selective Interfacial Olefin Cross Metathesis for the Preparation of Hollow Nanocapsules

Kerstin Malzahn,[†,‡] Filippo Marsico,[†,‡] Kaloian Koynov,[†] Katharina Landfester,[†] Clemens K. Weiss,*[,†,§] and Frederik R. Wurm*[,†]

[†]Max-Planck-Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany
[‡]Graduate School, Materials Science in Mainz, Staudinger Weg 9, 55128 Mainz, Germany
[§]University of Applied Sciences Bingen, Berlinstrasse 109, 55411 Bingen am Rhein, Germany

Ⓢ Supporting Information

ABSTRACT: The first synthesis of hollow nanocapsules with an aqueous core via olefin cross metathesis is presented. The reaction was tailored such that it proceeds selectively at the oil–water interface of aqueous nanodroplets in an inverse miniemulsion. The cross metathesis takes place between an acrylated polysaccharide and unsaturated organophosphates under mild conditions. This general protocol allows the synthesis of biocompatible and polyfunctional nanocapsules via the bioorthogonal olefin metathesis, thus generating a highly versatile methodology for the design of future materials for biomedical applications but also for materials science. Functionalization of the nanocapsules was demonstrated with fluorescent labels, which can be attached to the pendant phosphoester either within the cross-linker, exploiting the versatility of the phosphorus chemistry, or via coupling to the capsules' surface.

Degradable and renewable polymers are a growing field in modern materials science because conventional/fossil carbon sources are limited and commodity plastics typically have long half-life times in nature.[1] Moreover, in the biomedical field, e.g., for drug delivery and release, tissue engineering, or to ensure renal clearance of (macro)molecules, degradable polymers are of high interest.[1,2] Within the field of degradable polymers with biomedical application, polyesters and polysaccharides,[3] such as poly(lactide) or dextran, are the most prominent materials.[2,4] Another, yet rather unexplored, class of biodegradable polymers are poly(phosphoester)s (PPEs)[5] that are chemically much more versatile than conventional polyesters because phosphorus can form triesters allowing the inherent functionalization of every ester moiety along a polymer backbone to precisely control their hydrophilicity or functionality.[6]

Heterogeneous polymerization techniques allow the defined generation of (nano)particles and capsules,[7] which can be loaded in situ with drugs, biomolecules, etc. A well-known example is the formation of polyurethane/polyurea nanocapsules via a polyaddition reaction of isocyanates and alcohols or amines at the interface of (mini)emulsion droplets.[8,9] This strategy, however, has only limited use for the encapsulation of pharmaceutically active biomolecules, as nucleophiles of peptides and proteins, especially amines, thiols, or alcohols, will participate in the reaction,[10] and might decrease their biomedical function. Thus, bio-orthogonal reactions are demanded, leaving the biomolecules' functionalities unaltered. Prominent examples are "click" reactions, between thiols and alkenes or alkynes and azides which were also successfully used for the formation of nanocapsules.[11]

Another example for a bio-orthogonal reaction is olefin metathesis. As metathesis can be performed under mild conditions, tolerating water, oxygen, and a variety of functional groups,[12] it can be easily conducted in aqueous emulsions under ambient conditions. Furthermore, the introduction of olefins to the monomers is a straightforward process and superior to typical azidation reactions as it proceeds in one step avoiding large amounts of activators. Several reports include ring-closing metathesis, acyclic diene metathesis, and ring-opening metathesis polymerization that were conducted in emulsion to generate low molecular weight materials but also polymers and lattices.[12a,13] Breitenkamp et al. reported the formation of microcapsules by ring-opening polymerization, whereas the reaction takes place in a single phase and the molecules are driven to the interface due to their amphiphilic character.[13b]

Instead of relying on the above-mentioned assembly process, we go one step further by designing the cross olefin metathesis in such a way that it can only proceed at the oil–water interface. The hydrophilic/hydrophobic character of the respective monomers limits the reaction to the droplets interface of stable miniemulsions. Defined nanocapsules are generated, whereas the potential payload, like enzymes or peptides, is left unaffected during the reaction. The fast and convenient protocol was used to prepare potentially biodegradable and biocompatible nanocapsules consisting of a hydrophilic core and a shell based on

polysaccharides (dextran) and PPEs. Additionally, we use a dye-functionalized organophosphate to underline the convenient introduction of functional groups via the pendant phosphoester. The possibility to create nanocapsules by cross metathesis reaction gives rise to a new quality of nanocapsules in biomedical applications as the hydrophobic catalyst remains in the continuous phase and is easily removed after the reaction. The shell is generated by cross metathesis of acrylated dextran (DexA, **1**) and an unsaturated organophosphate **2** (for experimental details see the Supporting Information).

Using the procedure reported in the Supporting Information for the preparation of DexA (M_w = 40 000 g·mol^{-1}) a degree of substitution (DS) between 0.13 and 0.55 was realized and quantified by ^{1}H NMR (Figure S1a, Supporting Information) and IR spectroscopy. In the IR spectrum the characteristic carbonyl stretching of the acrylate can be detected at 1723 cm^{-1}. This confirms the successful modification of dextran and gives a qualitative measure for the DS (the spectrum shows two batches with variable DS).

For the fabrication of the nanocapsules, an inverse (water-in-oil) miniemulsion was prepared. Therefore, an aqueous solution of DexA is dispersed in cyclohexane as an organic solvent generating stable aqueous droplets. Subsequently, a hydrophobic unsaturated organophosphate (**2**) is added to the emulsion followed by the addition of the oil-soluble catalyst (Scheme 1 and Figure 1). A Grubbs−Hoveyda second-generation catalyst was chosen as it has a high tendency for selective cross metathesis of acrylates (type 2 olefins) with type 1 olefins.[14]

Scheme 1. Schematic Representation of the Interfacial Reaction between Acrylated Dextran (1) and Phenyl-di(undec-10-en-1-yl)-phosphate (2) Leading to a Cross-Linked Polymer Network (3)^a
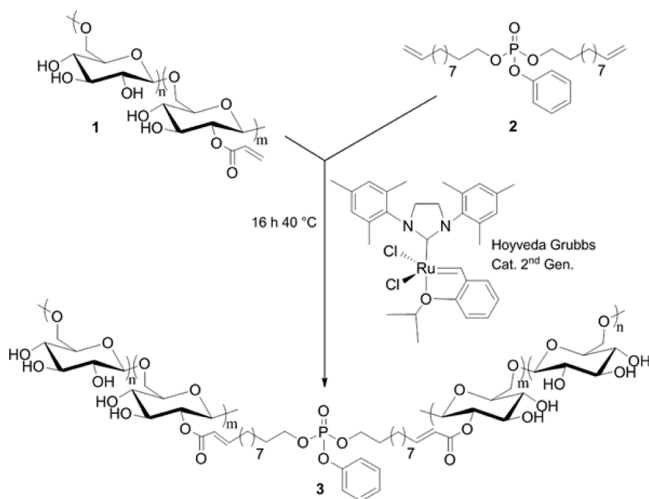
^{a}Note: dextran is modified statistically with acrylate groups.

Mediated by the catalyst, the cross metathesis takes place at the interface only, cross-links DexA with biodegradable phosphoesters, and forms a shell surrounding the water droplet. The reaction was allowed to proceed at 40 °C for 16 h. Within 30 min after addition of the catalyst a color change (from green to purple) suggested that the reaction was in progress. Subsequently, the nanocapsules were purified by repetitive centrifugation and washing to remove residual catalyst and monomer from the organic phase.
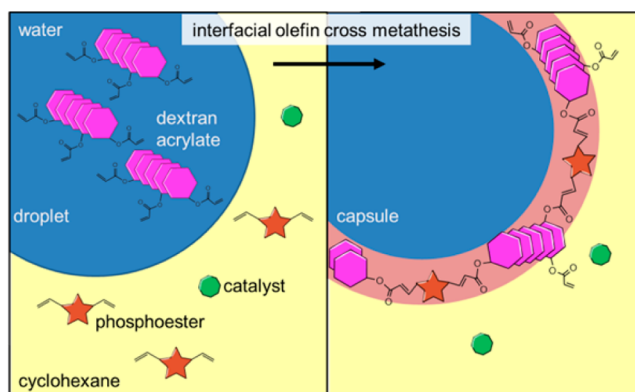
Figure 1. Schematic representation of the interfacial olefin cross metathesis at the water−oil interface of a nanodroplet in an inverse miniemulsion process for the formation of stable nanocapsules.

The resulting colorless dispersion was stable for several weeks, and the nanocapsules could easily be transferred to an aqueous environment by redispersion in 0.3 wt % SDS solution. This aqueous dispersion was also stable for several weeks. Excess surfactant can be removed by exhaustive dialysis. Dynamic light scattering (DLS) was used to determine the average size of the nanocapsules in cyclohexane (270 nm ± 50 nm) which is representative for the droplet size obtained during the miniemulsion process. After redispersion in water the diameter was unchanged (280 nm ± 50 nm); however, a slight increase in size could be attributed to the SDS hydration layer surrounding the nanocapsules after transferring into water, as reported earlier.[15] As the size of the nanocapsules does not change significantly after transfer to an aqueous solution a high degree of cross-linking is assumed, hindering the swelling which is common for dextrans.[16]

To support the DLS results, scanning electron microscopy (SEM) was used to image the nanocapsules. The image in Figure 2a shows the characteristic appearance of a nanocapsule with a
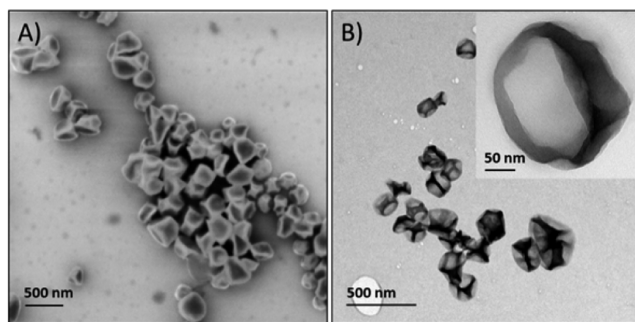
Figure 2. (a) Scanning electron microscopy image and (b) transmission electron microscopy image of the nanocapsules prepared in a minimulsion process by olefin cross metathesis.

thin wall, which deflates partially in vacuo during the sample preparation process. The size of the displayed nanocapsules is ca. 300 nm, which resembles the results obtained by DLS. Additionally, transmission electron microscopy (TEM) analysis (Figure 2b) confirms the hollow capsule morphology (see Figure S5, Supporting Information, for further results).

The polymer obtained after the capsule formation was insoluble in all common solvents, which indicated that DexA was cross-linked during the reaction, as expected. Accordingly, direct proof that metathesis reaction occurred can only be

demonstrated via ^{13}C and ^{31}P solid-state NMR. Prior to NMR analysis, a Soxhlet extraction with methanol and dichloromethane (good solvents for monomer and homopolymer, respectively) was performed to purify the capsules from unbound material and residual monomer. No polymeric material was recovered in the organic solvent which is in good agreement with the literature and proves that homopolymerization of **2** is not occurring under these condititons.[14b] The ^{31}P NMR spectrum unambiguously confirms the incorporation of the phosphate into the cross-linked polymer (Figure 3a) showing a resonance for the phosphoester at −7.7 ppm.
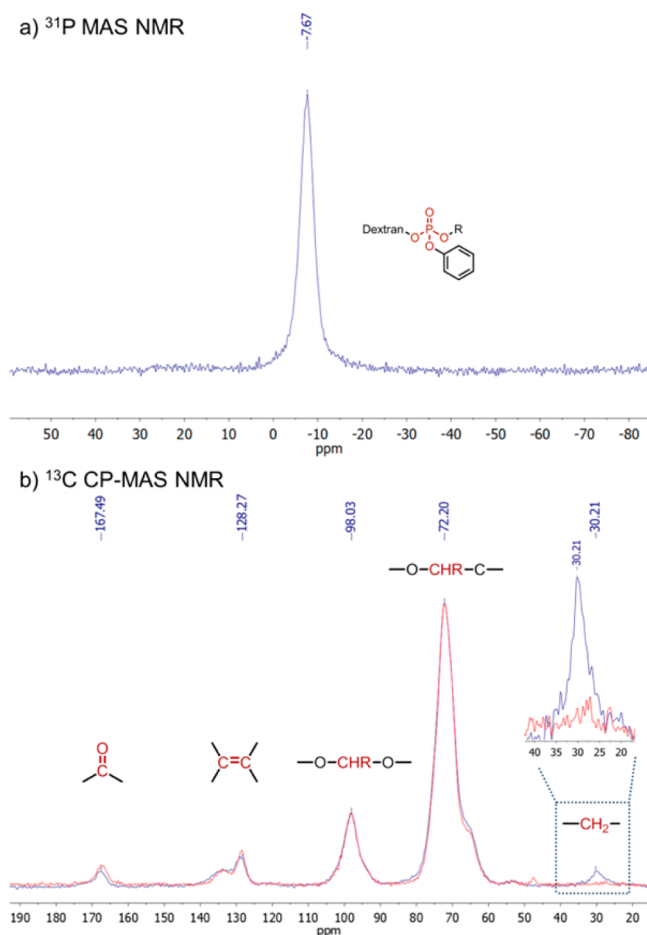

Figure 3. ^{31}P (a) and ^{13}C (b) solid-state NMR spectra of the nanocapsules (note: (b) shows the superimposed ^{13}C NMR spectra of DexA (red) with the nanocapsules (blue), magnified area highlighting the methylene resonances stemming from the metathesis reaction with **2**).

The appearance of methylene resonances in the ^{13}C NMR spectrum further proves the incorporation of **2**, while no aliphatic methylene resonances can be detected in the reference sample (DexA, Figure 3b). The limited resolution of SS-^{13}C NMR does not allow quantification of the saccharide to phosphate ratio in the polymer network. Further, the signals originating from the internal postmetathesis double bond and the benzyl group overlap with the signal of the terminal olefin around 128 ppm.

Furthermore, the elementary composition of the capsules was investigated with microanalysis (EDX). A peak at 2 keV gave evidence for the presence of the phosphate in the capsule structure. Simultaneously, no ruthenium (Ru) was found in the

material by EDX, which indicates that the catalyst was efficiently removed after the reaction within the organic phase. Using highly sensitive inductively coupled optical emission spectroscopy (ICP-OES) only trace amounts of Ru were detected in a low parts per million regime with respect to the dry weight of the nanocapsules. For further removal of catalyst traces the use of chelating agents and subsequent dialysis is proposed. The optimization of the purification process was considered beyond the scope of this paper.

To exclude reactions other than the desired cross metathesis for the capsule formation, the miniemulsion process was conducted without the addition of **2**, or without catalyst. To ensure that the DexA did not polymerize initiated by temperature, light exposure, or the catalyst alone, NMR of the droplets was recorded. In contrast to the cross-linked nanocapsules, all freeze-dried samples of the control reactions were soluble in D$_2$O, and the characteristic peaks for the acrylates (6.5, 6.25, and 6.1 ppm) were unchanged after the miniemulsion process (Figure S6, Supporting Information). This is a direct proof that the nanocapsules were generated by olefin cross metathesis that can take place only at the interface.

To demonstrate the potential of the metathesis protocol for modification with functional groups or targeting moieties, a fluorescent marker was chosen as a model. Two possibilities for labeling were investigated: First, in addition to **2**, an unsaturated BODIPY derivative (λ_{max} = 540 nm, Figure S7, Supporting Information) was added, which should act as a chain termination agent during the polycondensation and serve as a model for the modification from the "outside". Second, the versatility of the phosphate was exploited, and the fluorescent label was attached via the third (pendant) ester into the polymer to act as a model for modification within the shell.[17]

The labeled nanocapsules were studied by various spectroscopic techniques including UV/vis, fluorescence, and fluorescence correlation spectroscopy (FCS). Using UV/vis and fluorescence spectroscopy, well-defined absorption (λ_{max} = 520 nm) and emission peaks (λ_{max} = 540 nm) were obtained at the expected wavelengths (Figure S7, Supporting Information). This gives a first implication that the dye is bound to the capsules.

Fluorescence correlation spectroscopy (FCS)[18] measurements were used to prove that the dyes are covalently bound to the nanocapsules. Additionally, the pH-induced degradation of the capsules was studied by acidic hydrolysis. Figure 4 shows the fluorescence intensity autocorrelation curves (ACCs) recorded before and after hydrolysis of the nanocapsule dispersion. The initial ACC represents fluorescent species with hydrodynamic diameter of ∼230 nm; this value is very close to the hydrodynamic diameters of the capsules from DLS and proves incorporation of the dye into the capsule shell.

Upon lowering of the pH, the corresponding autocorrelation curve (Figure 4) shifts to shorter lag times indicating the appearance of smaller fluorescent species with hydrodynamic diameter of around 5 nm. This decrease of the hydrodynamic diameter could be caused by the hydrolysis of the dextran[19] or the phosphoesters, thus proving the degradability of the nanocapsules. Further studies proved the degradation of the nanocapsules under various conditions, e.g., at pH = 4 and pH = 2, respectively (Figure S8, Supporting Information).

In conclusion, the first nanocapsules with an aqueous core prepared by olefin metathesis are presented. This convenient protocol allows creating nanocapsules with a mild bioorthogonal cross-linking of degradable and biocompatible polymers at the oil−water interface. It combines two materials
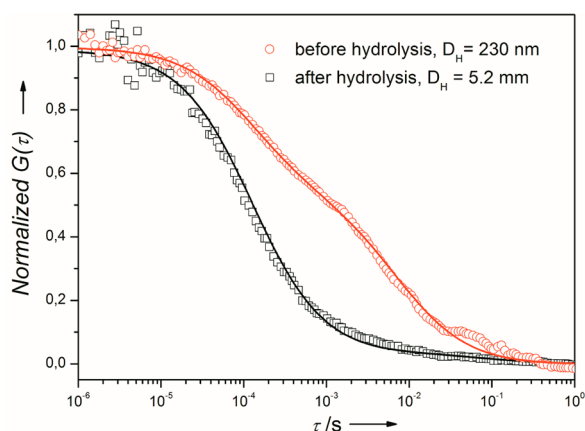
Figure 4. Normalized FCS autocorrelation curves recorded for aqueous dispersions of the fluorescently functionalized nanocapsules before (red circles) and after (black squares) acid hydrolysis with HCl. The solid lines represent the corresponding fits with eq S1 (Supporting Information).

with high potential for future applications: poly(phosphoester)s are a currently developing and highly versatile class of biocompatible and degradable polymers,[6,20] which were combined via olefin metathesis with polysaccharides, here dextran, which are known for their biocompatibility.

The nanocapsules, created from cross-linking acrylated dextran with unsaturated organophosphates in the presence of a metathesis catalyst, feature defined shell morphology with a hollow core, and the capsule-forming reaction was confirmed by several techniques, such as solid-state NMR. Further, their potential for modification with dyes or other functional groups was demonstrated. Given by their potential biodegradability and the bio-orthogonal cross metathesis, we are currently deeply investigating this general protocol, which is not limited to polysaccharides, to generate biomolecule-loaded nanocapsules for targeted drug delivery and release.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental protocols for characterization methods, dextran modification, nanocapsule preparation, and characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors
*E-mail: c.weiss@fh-bingen.de (C.K.W.).
*E-mail: wurm@mpip-mainz.mpg.de (F.R.W.)

Notes
The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

■ REFERENCES

(1) Shah, A. A.; Hasan, F.; Hameed, A.; Ahmed, S. *Biotechnol. Adv.* **2008**, *26* (3), 246−265.

(2) Nair, L. S.; Laurencin, C. T. *Prog. Polym. Sci.* **2007**, *32* (8−9), 762−798.

(3) (a) Williams, C. K. *Chem. Soc. Rev.* **2007**, *36* (10), 1573−1580. (b) Mizrahy, S.; Peer, D. *Chem. Soc. Rev.* **2012**, *41* (7), 2623−2640.

(4) Mehta, R.; Kumar, V.; Bhunia, H.; Upadhyay, S. N. *J. Macromol. Sci., Polym. Rev.* **2005**, *45* (4), 325−349.

(5) Wang, Y.-C.; Yuan, Y.-Y.; Du, J.-Z.; Yang, X.-Z.; Wang, J. *Macromol. Biosci.* **2009**, *9* (12), 1154−1164.

(6) (a) Steinbach, T.; Alexandrino, E. M.; Wurm, F. R. *Polym. Chem.* **2013**, *4* (13), 3800−3806. (b) Marsico, F.; Turshatov, A.; Weber, K.; Wurm, F. R. *Org. Lett.* **2013**, *15*, 3844−3847.

(7) Yang, S.; Liu, H.; Zhang, Z. *J. Polym. Sci., Part A: Polym. Chem.* **2008**, *46* (12), 3900−3910.

(8) Landfester, K.; Weiss, C. Encapsulation by Miniemulsion Polymerization. In *Modern Techniques for Nano- and Microreactors/reactions*; Caruso, F., Ed.; Springer: Berlin Heidelberg, 2010; Vol. 229, pp 1−49.

(9) Crespy, D.; Stark, M.; Hoffmann-Richter, C.; Ziener, U.; Landfester, K. *Macromolecules* **2007**, *40* (9), 3122−3135.

(10) Andrieu, J.; Kotman, N.; Maier, M.; Mailänder, V.; Strauss, W. S. L.; Weiss, C. K.; Landfester, K. *Macromol. Rapid Commun.* **2012**, *33* (3), 248−253.

(11) (a) Chen, C.-K.; Law, W.-C.; Aalinkeel, R.; Yu, Y.; Nair, B.; Wu, J.; Mahajan, S.; Reynolds, J. L.; Li, Y.; Lai, C. K.; Tzanakakis, E. S.; Schwartz, S. A.; Prasad, P. N.; Cheng, C. *Nanoscale* **2013**. (b) Siebert, J. M.; Baier, G.; Musyanovych, A.; Landfester, K. *Chem. Commun.* **2012**, *48* (44), 5470−5472. (c) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36* (8), 1249−1262. (d) DeForest, C. A.; Polizzotti, B. D.; Anseth, K. S. *Nat. Mater.* **2009**, *8* (8), 659−664. (e) Zou, J.; Hew, C. C.; Themistou, E.; Li, Y.; Chen, C.-K.; Alexandridis, P.; Cheng, C. *Adv. Mater.* **2011**, *23* (37), 4274−4277.

(12) (a) Burtscher, D.; Grela, K. *Angew. Chem., Int. Ed.* **2009**, *48* (3), 442−454. (b) Schuster, M.; Blechert, S. *Angew. Chem., Int. Ed. Engl.* **1997**, *36* (19), 2036−2056.

(13) (a) Chemtob, A.; Héroguez, V.; Gnanou, Y. *J. Polym. Sci., Part A: Polym. Chem.* **2004**, *42* (5), 1154−1163. (b) Breitenkamp, K.; Emrick, T. *J. Am. Chem. Soc.* **2003**, *125* (40), 12070−12071. (c) Gulajski, L.; Sledz, P.; Lupa, A.; Grela, K. *Green Chem.* **2008**, *10* (3), 271−274.

(14) (a) Hoveyda, A. H.; Gillingham, D. G.; Van Veldhuizen, J. J.; Kataoka, O.; Garber, S. B.; Kingsbury, J. S.; Harrity, J. P. A. *Org. Biomol. Chem.* **2004**, *2* (1), 8−23. (b) Demel, S.; Slugovc, C.; Stelzer, F.; Fodor-Csorba, K.; Galli, G. *Macromol. Rapid Commun.* **2003**, *24* (10), 636−641.

(15) Baier, G.; Cavallaro, A.; Vasilev, K.; Mailänder, V.; Musyanovych, A.; Landfester, K. *Biomacromolecules* **2013**, *14* (4), 1103−1112.

(16) Klinger, D.; Aschenbrenner, E. M.; Weiss, C. K.; Landfester, K. *Polym. Chem.* **2012**, *3* (1), 204−216.

(17) Marsico, F.; Turshatov, A.; Weber, K.; Wurm, F. R. *Org. Lett.* **2013**, *15* (15), 3844−3847.

(18) Koynov, K.; Butt, H.-J. *Curr. Opin. Colloid Interface Sci.* **2012**, *17* (6), 377−387.

(19) Basedow, A. M.; Ebert, K. H.; Ederer, H. J. *Macromolecules* **1978**, *11* (4), 774−781.

(20) (a) Steinbach, T.; Schroeder, R.; Ritz, S.; Wurm, F. R. *Polym. Chem.* **2013**, *4*, 4469−4479. (b) Marsico, F.; Wagner, M.; Landfester, K.; Wurm, F. R. *Macromolecules* **2012**, *45*, 8511−8518.