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Functional Choline Phosphate Polymers

Gaojie Hu and Todd Emrick*

Polymer Science and Engineering Department, University of Massachusetts Amherst, 120 Governors Drive, Amherst, Massachusetts 01003, United States

Supporting Information

ABSTRACT: Zwitterionic polymers have been widely implemented in surface modification and as biomaterials that exhibit exceptional hydrophilicity, biocompatibility, and antifouling properties. However, a wider breadth of applications for polymer zwitterions is hindered by their inherent lack of functionality, requiring the integration of chain-end functionality or incorporation of functional comonomers along the polymer chain. Here we demonstrate the facile placement of unsaturated groups directly into zwitterionic methacrylate monomers, specifically choline phosphate structures, and show the utility of these monomers in controlled free radical polymerization. These versatile functional zwitterions are converted easily to polymer prodrugs, hydrogels, and other derivatives that establish the versatility of this novel materials platform.

Polymers having zwitterionic moieties fixed pendent to the backbone grow in importance owing to their numerous useful features including nonfouling properties and biocompatibility. For example, poly(2-methacryloyloxyethyl phosphorylcholine) (polyMPC, **1**, Figure 1), a synthetic polymer inspired by the phosphorylcholine (PC) headgroup of the bilayer cell membrane, has proven successful as a component of implant materials such as coronary stents and artificial hips.[1] Ishihara's facile preparation of the MPC monomer[2] accelerated the entry of polyMPC into applications and moreover opened opportunities for advancing polyMPC chemistry into living polymerization[3,4] and bioconjugation strategies.[5−14] Successful use of MPC in controlled free radical polymerization led to polyMPC-based prodrugs,[5−7,9] while functional polyMPC-containing copolymers afford access to hydrogels and cell-adhering zwitterionic polymers.[15,16]

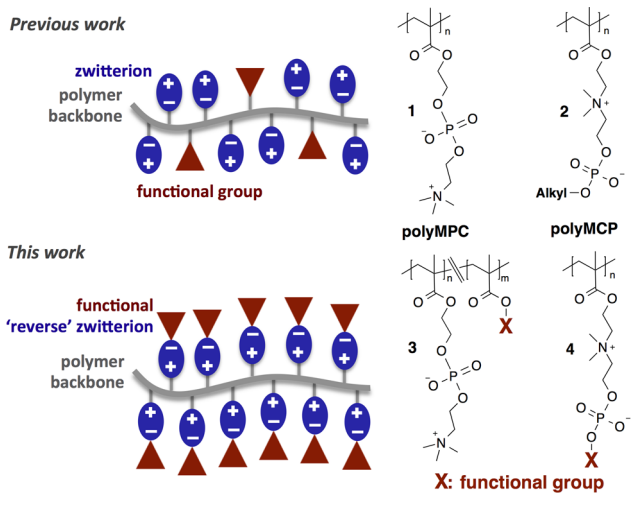

Figure 1. Integration of functionality (red triangles) into polymer zwitterions. Top: conventional copolymers. Bottom: embedded functionality.

There is a long-standing interest in choline phosphate (CP) molecules as "reverse PC"-zwitterions, with synthetic advances reported by Ishihara, Nakaya, and others.[17−22] For CP-polymers, recent reports of polymer **2** (Figure 1), where alkyl = CH_3, attribute red blood cell aggregation to dipole pairing between the CP groups of the polymer and the PC groups of the cell membrane.[20] We found that CP polymer **2** with alkyl = *n*-Bu (polyMBP) did not cause RBC aggregation,[22] suggesting CP polymer solution behavior to hinge on the selection of the phosphate alkyl group. The more facile synthesis of the *n*-Bu vs methyl-substituted polyMCP is crucial for future scalability. However, neither the methyl nor *n*-Bu substituent of **2** constitutes useful functionality to facilitate further chemical functionalization. Noting that there are few examples of functional zwitterions in general, the CP-based polymers we report provide an opportunity for convenient entry into a novel polymer zwitterion platform. Prior to this work, functionality was introduced into PC or CP polymer zwitterions by copolymerization, whereas the route we describe embeds functionality into the zwitterionic moiety directly. As illustrated in Figure 1, we connect functional groups directly to the zwitterion, selecting alkenes and alkynes for their potential use in subsequent reactions such as thiol−ene and azide−alkyne cycloaddition.

As shown in Scheme 1, accessing CP methacrylate monomers depends largely on selection of the "X" group of structure **5** that originates from the alcohol (X−OH) used to displace the chloride of 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP). Phospholanes of type **5** undergo ring-opening with tertiary amines, and when *N*,*N*-dimethylaminoethyl methacrylate (DMAEMA, **6**) is employed the "endocyclic" product **7** represents the desired CP monomer. Unfortunately, ring-opening of COP with amines is complicated by a competing "exocyclic" reaction that yields ammonium phosphate salt **8** that is difficult to separate from the ring-opened product. The use of *n*-alkyl "X"-groups, in place of methyl, promotes the desired ring-opening over salt formation. However, long alkyl chains preclude the favorable hydrophilicity of polymer zwitterions. In the *n*-butyl substituted MBP synthesis, only traces of salt formed, and the resultant polymers were water-soluble.[22]

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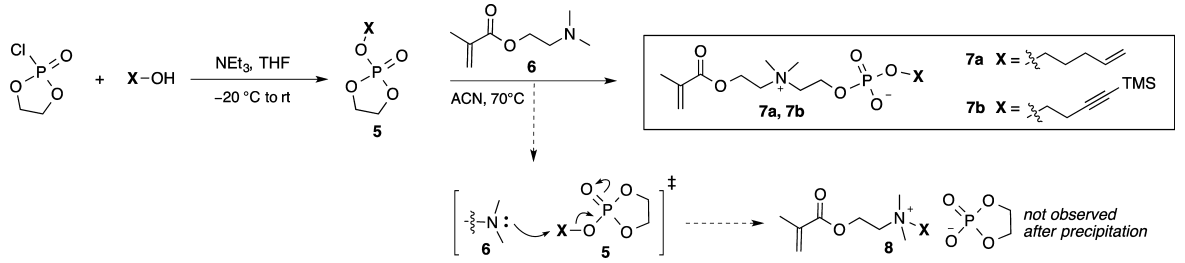
Scheme 1. Synthesis of Functional Choline Phosphate Methacrylates 7a and 7b, and Structure of the Ammonium Phosphate Side Product 8



We reasoned that success with polyMBP would translate to functional CP monomers prepared using unsaturated alcohols in the synthesis of **5**, leading us to the preparation of alkene-substituted **7a** and alkyne-substituted **7b**. Typical reaction conditions involved stirring the substituted phospholane **5** with DMAEMA **6** in anhydrous acetonitrile in a sealed flask under nitrogen atmosphere at 70 °C. Adding anhydrous diethyl ether to the reaction mixture caused precipitation of the monomers as pure solids, which were isolated in >50% yield over the two steps. *In each case there was a notable absence of the competing side product salt* ***8*** *or other contaminants.* In the ^{31}P NMR spectra of **7a** and **7b** (Figure 2), clean signals at 0 ppm represent the phosphorus

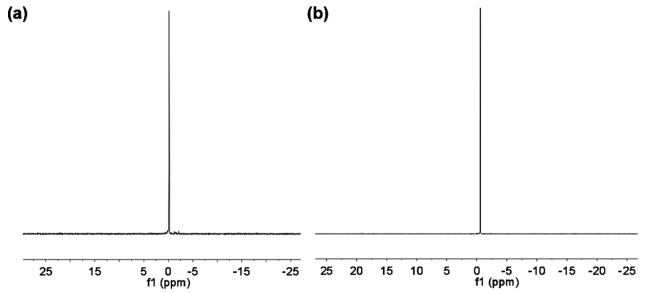
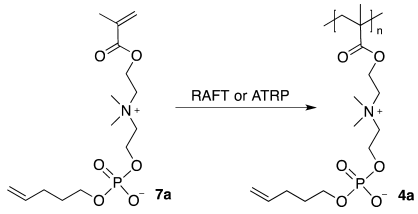


Figure 2. ^{31}P NMR spectra of (a) **7a** and (b) **7b**.

atoms of the desired CP-monomers, whereas phosphorus resonances of the salt, compound **8**, would appear near 18 ppm.[22] These reactions proved scalable to 10 g batches in our laboratories.

Homopolymerization of methacrylates **7a** and **7b** was examined by controlled free radical methods, with results given in Tables 1 and 2 and Figure S1. Reversible addition−fragmentation chain transfer (RAFT) polymerization was performed in methanol at 70 °C, with 4,4′-azobis(4-cyanovaleric acid) (ACVA) as initiator and 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid as chain transfer agent (CTA). Atom transfer radical polymerization (ATRP) was conducted in methanol at room temperature, using CuBr as catalyst, 2,2′-bipyridine (bpy) as ligand, and ethyl α-bromoisobutyrate (EBiB) as initiator. Monomer conversion was monitored by ^{1}H NMR spectroscopy, integrating the methacrylate olefin (5.72 ppm) against the methyl group resonances of the polymer (0.91−1.47 ppm). Polymer molecular weight was estimated by gel permeation chromatography (GPC) with refractive index detection using 1,1,1-trifluoroethanol (TFE) as the mobile phase. Polymerization of the alkene-functionalized CP methacrylate **7a** by RAFT or ATRP (Table 1, Figure S1) afforded CP polymer **4a** as a water-soluble polymer of considerable molecular weight (>10 kDa). Interestingly, polydispersity index (PDI) increased markedly at high

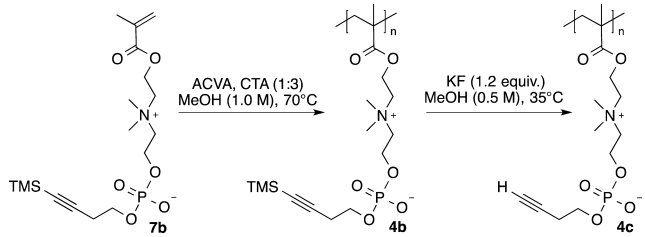
Table 1. Data for Controlled Free Radical Polymerization of CP Methacrylate 7a (RAFT and ATRP)



				TFE GPC[d]	
method	target M_n (kDa)	% conv[c]	theor M_n (kDa)	M_n (kDa)	PDI
RAFT[a]	10.0	56	5.6	4.9	1.23
	20.0	55	11.0	11.1	1.27
	30.0	63	18.9	13.9	1.31
ATRP[b]	20.0	37[e]	7.4	7.5	1.07
	20.0	56[f]	11.2	8.5	1.24
	20.0	72[g]	14.4	13.5	1.45

[a]ACVA, CTA (1:3), MeOH (1.0 M), 70 °C, 14 h. [b]CuBr, bpy, EBiB (1:2:1), MeOH (0.8 M), rt. [c]By ^{1}H NMR. [d]PMMA standards. [e]Reaction time: 2 h. [f]Reaction time: 8 h. [g]Reaction time: 16 h.

Table 2. Synthesis of CP Polymer 4c



			TFE GPC[b]		
entry	target M_n (kDa)	% conv[a]	M_n (kDa)	PDI	% yield
1	8.2	> 95	6.4	1.23	64
2	16.4	> 95	14.4	1.17	75
3	24.6	95	16.3	1.18	72
4	41.0	> 95	22.9	1.16	62

[a]% Conversion of polymerization by ^{1}H NMR. [b]PMMA standards.

conversion, in some cases leading to gel formation due to participation of the pendent alkenes in propagation and likely cross-linking (despite the lower reactivity of this double bond relative to the methacrylate). Fortunately, when monomer conversion was kept lower, PDI values evolved in a fashion typical of finely controlled free radical polymerization (1.1−1.3) and the alkene-substituted CP polymers formed homogeneous solutions in water without observed gelation.

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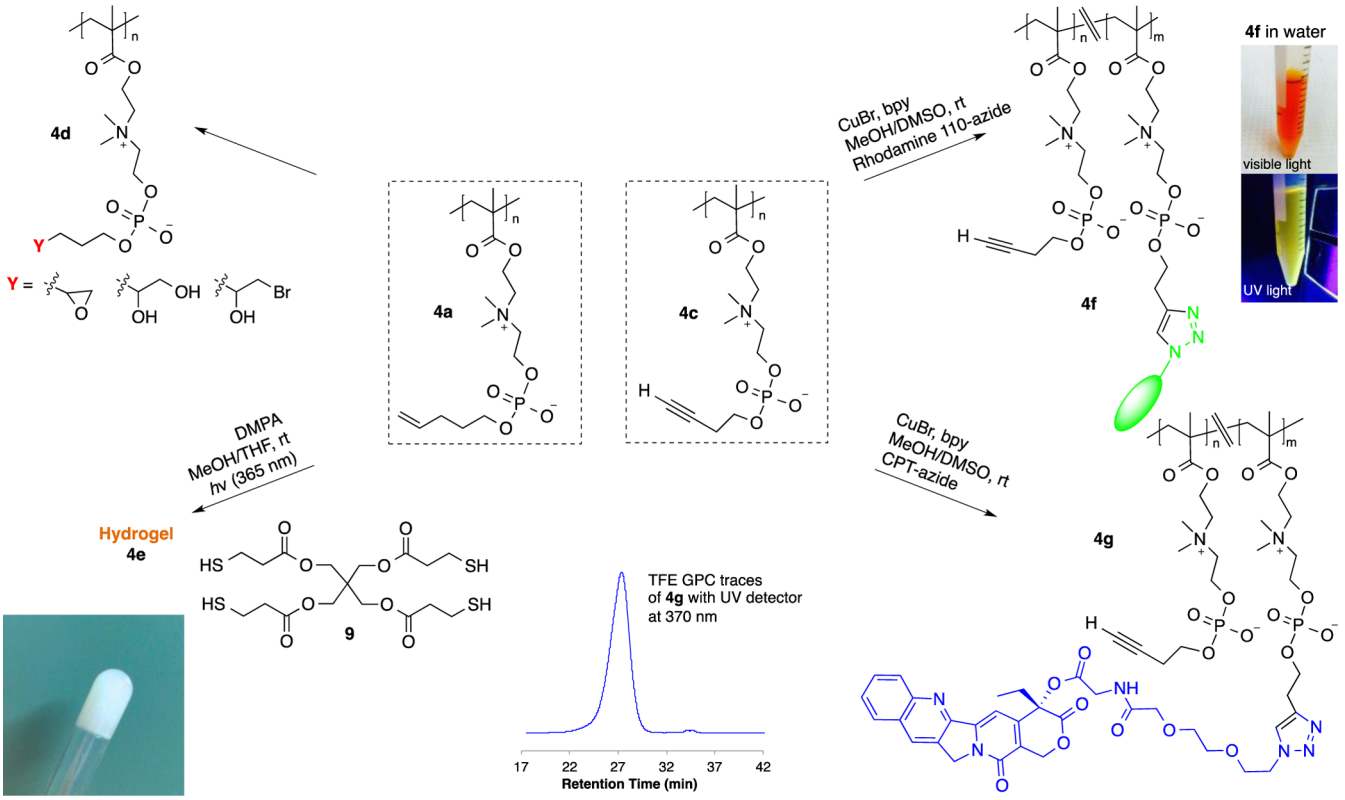
Scheme 2. CP Polymers Offer Access to Functional Zwitterions (4d), Hydrogels (4e), Fluorophores (4f), and Prodrugs (4g)



Homopolymerization of CP-alkyne **7b**, using either ATRP or RAFT, yielded well-controlled polymers even up to 95% monomer conversion (Table 2, Table S1), with no evidence of the PDI broadening as seen in the case of **7a**. Removal of the TMS groups, achieved by stirring the polymer in a 0.5 M methanol solution of KF at 35 °C, afforded deprotected CP polymers that retained low PDI values. The alkyne-substituted CP homopolymers were soluble in methanol and pure water, confirming that the alkyne substituent does not interrupt their desired aqueous solubility. The ability to integrate useful reactive substituents into these polymer zwitterions, while maintaining high levels of water solubility, opens access to further chemistry that we chose to examine initially by azide−alkyne cycloaddition.

Functional CP polymers are amenable to chemical transformations that confirm the robust nature of the zwitterionic moiety and that insert useful functionality for cross-linking and surface functionalization (Scheme 2). For example, the pendent olefins of CP polymer **4a** were converted easily to the corresponding 1,2-diol and 1,2-bromoalcohol, and were additionally useful in thiol−ene "click" reactions by cross-linking polymer **4a** with tetrathiol **9** in methanol/THF (20:1) mixtures. Irradiation of this mixture for 5 min at 365 nm (with 2,2-dimethoxy-2-phenylacetophenone (DMPA) initiator) gave a white hydrogel that absorbed considerable water (>75 wt % for a gel prepared from a 1.5:1 ratio of thiol-to-alkene). Gels synthesized using thiol-to-alkene ratios of 1.5:1 and greater afforded increasingly robust materials able to withstand physical manipulation.

These functional CP polymers are anticipated as versatile precursors to numerous hydrophilic polymers through use of the alkyne groups of CP polymer **4c** in azide−alkyne cycloaddition. For example, fluorescent CP polymer **4f** was generated from the alkyne precursor **4c**, containing ∼1 mol % Rhodamine 110-azide (commercially available from Sigma-Aldrich) as pendent groups. Polymer **4f** had excellent water solubility and exhibited fluorescence emission at 525 nm. Click cycloaddition also afforded CP-polymer prodrug **4g**, containing camptothecin (CPT) pendent groups. This approach to polymer chemotherapeutics simplifies that of polymer prodrugs that require copolymerization and subsequent chemical transformations on the nonzwitterionic subunits.[9] A glycine-linked CPT azide[9] (Figure S2) was selected for reaction with CP polymer **4g**, with successful conjugation confirmed by overlapping of the refractive index and UV-detector (370 nm) signals in GPC characterization. These CPT-containing polymers proved water-soluble even at a CPT loading of 11.5 wt %. ^{1}H NMR spectroscopy of polymer **4g** in 1:1 MeOD-d_4/DMSO-d_6 mixtures ensured solvation of both the polymer backbone and pendent CPT components. Dynamic light scattering (DLS) showed the solution size of **4g** to vary with CPT incorporation. Interestingly, the solution diameter of the CP polymer−CPT prodrugs increased considerably with CPT content, extending to 17 nm diameter for the highest CPT incorporation of 11.5 wt % (Figure 3). This is distinctly different from our prior studies on polyMPC prodrugs[9] in which incorporation of 5−14 wt % CPT caused little-to-no change in hydrodynamic size, with 5−6 nm diameter structures maintained across these polymer compositions.[23] This suggests a more facile access to nanoscale aggregates for CP−polymer prodrugs, which may prove beneficial for prolonging circulation time in injectable applications. Using size exclusion HPLC equipped with UV and fluorescence detectors, CPT release was observed from prodrug **4g** in ethanol/PBS solution (pH 7.4), and an approximately six times faster CPT release in acidified ethanol/PBS solution (pH 2−3, SI, part III). While this communication centers primarily on the synthetic chemistry of CP zwitterions, and specifically new access to functional

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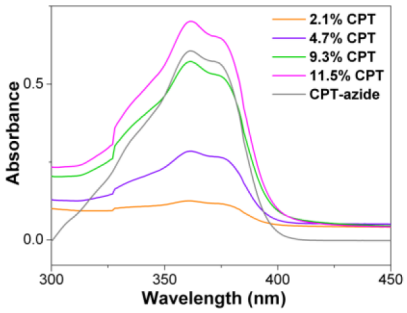

CP-CPT Prodrug (4g)

%CPT	diameter (nm)
0	10.1
2.1	10.1
4.7	13.8
9.3	14.1
11.5	16.8

Figure 3. Left: UV−vis absorbance of CP−CPT prodrug **4g** having 2.1, 4.7, 9.3, and 11.5 wt % CPT at 0.1 mg/mL and CPT-azide at 0.01 mg/mL in 1:1 MeOH/DMSO solution. Right: sizes of CP−CPT prodrug **4g** in DI water determined by dynamic light scattering.

zwitterionic structures accessed through the CP subunit, future work will examine the efficacy of these polymers as prodrugs.

In summary, we have presented a synthesis of novel, functional polymeric choline phosphates featuring alkene and alkyne pendent groups. This "reverse zwitterionic" platform overcomes prior limitations in the area of functional polymer zwitterions and provides clean, high yield access to useful and functional choline phosphate monomers and polymers. The polymers obtained proved amenable to diverse chemical modification, hydrogel preparation, fluorophore labeling, and prodrug formation. Overall, these polymers open new opportunities in bioconjugate chemistry, functional materials preparation, and surface modification and will allow researchers facile entry into such structures without the need for non-zwitterionic components.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b13156.

Experimental procedures, spectroscopic data, and other experimental details (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*tsemrick@mail.pse.umass.edu

Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Iwasaki, Y.; Ishihara, K. *Sci. Technol. Adv. Mater.* **2012**, *13*, 064101.
(2) Ishihara, K.; Ueda, T.; Nakabayashi, N. *Polym. J.* **1990**, *22*, 355−360.
(3) Ma, Y.; Tang, Y.; Billingham, N. C.; Armes, S. P.; Lewis, A. L.; Lloyd, A. W.; Salvage, J. P. *Macromolecules* **2003**, *36*, 3475−3484.
(4) Bhuchar, N.; Deng, Z.; Ishihara, K.; Narain, R. *Polym. Chem.* **2011**, *2*, 632.
(5) McRae Page, S.; Martorella, M.; Parelkar, S.; Kosif, I.; Emrick, T. *Mol. Pharmaceutics* **2013**, *10*, 2684−2692.
(6) Wang, H.; Xu, F.; Li, D.; Liu, X.; Jin, Q.; Ji, J. *Polym. Chem.* **2013**, *4*, 2004.
(7) Chen, X.; Parelkar, S. S.; Henchey, E.; Schneider, S.; Emrick, T. *Bioconjugate Chem.* **2012**, *23*, 1753−1763.
(8) McRae, S.; Chen, X.; Kratz, K.; Samanta, D.; Henchey, E.; Schneider, S.; Emrick, T. *Biomacromolecules* **2012**, *13*, 2099−2109.
(9) Chen, X.; McRae, S.; Parelkar, S.; Emrick, T. *Bioconjugate Chem.* **2009**, *20*, 2331−2341.
(10) Samanta, D.; McRae, S.; Cooper, B.; Hu, Y.; Emrick, T.; Pratt, J.; Charles, S. a. *Biomacromolecules* **2008**, *9*, 2891−2897.
(11) Lewis, A.; Tang, Y.; Brocchini, S.; Choi, J.-W.; Godwin, A. *Bioconjugate Chem.* **2008**, *19*, 2144−2155.
(12) Miyamoto, D.; Watanabe, J.; Ishihara, K. *Biomaterials* **2004**, *25*, 71−76.
(13) Sakai-Kato, K.; Kato, M.; Ishihara, K.; Toyo'oka, T. *Lab Chip* **2004**, *4*, 4−6.
(14) Sakaki, S.; Nakabayashi, N.; Ishihara, K. *J. Biomed. Mater. Res.* **1999**, *47*, 523−528.
(15) Xu, Y.; Jang, K.; Konno, T.; Ishihara, K.; Mawatari, K.; Kitamori, T. *Biomaterials* **2010**, *31*, 8839−8846.
(16) McRae Page, S.; Parelkar, S.; Gerasimenko, A.; Shin, D. Y.; Peyton, S. R.; Emrick, T. *J. Mater. Chem. B* **2014**, *2*, 620−624.
(17) Kusumi, A.; Singh, M.; Tirrell, D. A.; Oehme, G.; Singh, A.; Samuel, N. K. P.; Hyde, J. S.; Regen, S. L. *J. Am. Chem. Soc.* **1983**, *105*, 2975−2980.
(18) Yasuzawa, M.; Nakaya, T.; Imoto, M. *Makromol. Chem., Rapid Commun.* **1985**, *6*, 721−726.
(19) Kiritoshi, Y.; Ishihara, K. *Polymer* **2004**, *45*, 7499−7504.
(20) Yu, X.; Yang, X.; Horte, S.; Kizhakkedathu, J. N.; Brooks, D. E. *Chem. Commun.* **2013**, *49*, 6831−6833.
(21) Chen, X.; Chen, T.; Lin, Z.; Li, X.; Wu, W.; Li, J. *Chem. Commun.* **2015**, *51*, 487−490.
(22) Hu, G.; Parelkar, S. S.; Emrick, T. *Polym. Chem.* **2015**, *6*, 525−530.
(23) For DLS studies on polyMPC and polyMBP aqueous solutions, see: Morozova, S.; Hu, G.; Emrick, T.; Muthukumar, M. *ACS Macro Lett.* **2016**, *5*, 118−122.

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