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Sequential Nucleophilic "Click" Reactions for Functional Amphiphilic Homopolymers

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KEYWORDS: Activated alkyne, thiol-yne click reaction, sequential nucleophilic addition, multi-functionality.

Abstract

Amphiphilic homopolymers with high densities of functional groups are synthetically challenging. Thiol-yne nucleophilic click reactions have been investigated to introduce multiple functional groups in polymers with high density. An electron deficient alkyne group bearing methacrylate monomer was polymerized using reversible addition–fragmentation chain-transfer (RAFT) polymerization. Subsequently, the electron deficient alkyne group on polymer side chain was readily reacted with a thiol reagent using triethylamine (TEA) as the organocatalyst. This reaction was found to be very efficient under mild conditions. The resultant homopolymer bearing thiol vinyl ether functional groups could perform a second thiol addition with a stronger base, such as triazabicyclodecene (TBD), to prepare multifunctional homopolymers. This stepwise addition process was monitored by ^{1}H NMR as well as gel permeation chromatography. The fidelity of this method was

demonstrated by attaching four different functionalities, including both hydrophobic and hydrophilic moieties. Furthermore, these dual functionalized polymers bearing dithio-acetal groups are sensitive to reactive oxygen species (ROS), which compromises the host-guest properties of the assembly in response to this stimulus. The ROS responsive polymers reported here may have potential use in therapeutic delivery.

Introduction

Classical homopolymers have been quite featureless by definition, because of their rather monotonous repeat unit patterns. There has been an increased interest in introducing greater functional group diversity in homopolymers. Prominent among this class of polymers are amphiphilic homopolymers that contain both hydrophilic and hydrophobic groups in the same repeating unit[1,2]. These amphiphilic polymers can form micellar or vesicular aggregates in polar solvent[3–7] and reverse micelle like structures in nonpolar solvent[4,8,9]. Due to their high density of functional groups and stable self-assemblies, amphiphilic homopolymers have been used in materials science for applications in areas such as separations[10,11] and catalysis[12,13] and in biology in fields such as protein sensing[14] and DNA detection[1,15].

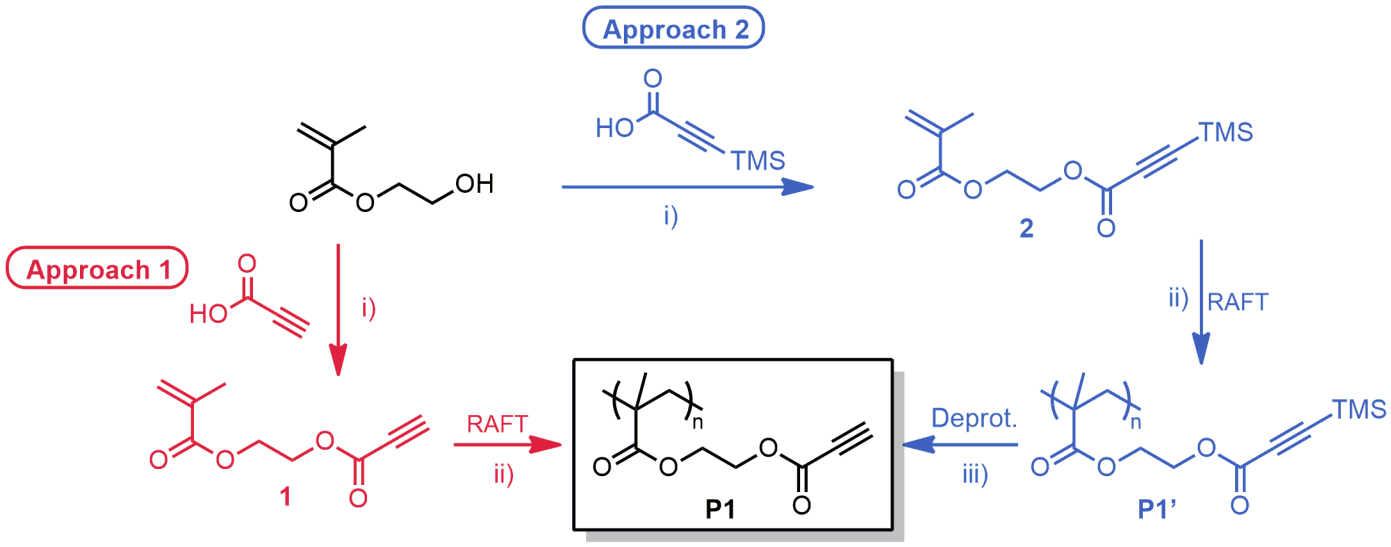
Moreover, amphiphilic homopolymers with stimuli responsive features can undergo changes in morphological and physicochemical properties[16] upon external or internal trigger application, which makes them useful for designing stimuli sensitive drug delivery systems[17–21]. Because of these novel properties, significant effort has been devoted to the syntheses of amphiphilic homopolymers. The commonly used method for amphiphilic homopolymer preparation involves either polymerization of a pre-functionalized amphiphilic monomer[22–24] or post modification of a precursor polymer with functionalities of interest[5,25,26]. In the former case, 100% amphiphilicity in each repeating unit can be potentially achieved. However, the drawbacks of this approach include low

polymerization efficiency and low polymeric molecular weights, owing to the steric hindrance effect from the functional groups. Also, significant synthetic challenges involved in making multifunctional monomers may limit their broad applications. On the other hand, post modification can lead to incomplete functionalization due to inefficient reactions. Therefore, developing an efficient method for the synthesis of homopolymers using an environmentally friendly approach is needed. We envisaged that a synthetic route that can overcome these obstacles would pave the way for expanding the existing polymerization toolbox.

Our solution is to use efficient click chemistry to post-modify the polymer substrate with different functionalities of interest. Click chemistry is increasingly useful in polymer and material science due to its speed, atom economy, easy operation and high reaction yields. Different click chemistry strategies have been developed for this purpose, e.g. Cu-catalyzed[27,28] or Cu free[29] Huisgen 1,3-dipolar cycloaddition, Staudinger ligation[30] and thiol-ene[31] click chemistry and thiol-yne photo click chemistry[32]. We were inspired by a recent report on a nucleophilic thiol-yne click reaction, involving α,β-unsaturated esters.[33] In this reaction, the electron deficient alkyne group of an α,β-unsaturated ester can readily react with a thiol reagent under mild conditions to form a mixture of *cis* and *trans* thiol vinyl esters. This functional group is then poised to undergo another thiol-ene addition reaction but only in the presence of a much stronger base. This latter requirement offers the opportunity to attach two different thiol groups on the same carbon to afford a hetero-dithiol adduct. This

organocatalytic thiol-yne and a subsequent thiol-ene reaction has been reported for small molecule synthesis, but its use in polymer synthesis has been very limited to the best of our knowledge.[34,35] In this work, we utilize this concept, where the pre-installed electron deficient alkyne groups on polymer side chains are used as a handle for post modification with different thiol species to introduce the functionalities of interest. Furthermore, these customized polymers bearing dithioacetal groups are sensitive to ROS stimulus as the oxidation of the sulfide or degradation in response to it. [36-39] In this work, we have shown how thiol-alkyne click chemistry is used for efficient post-polymerization modification with different functional groups, both hydrophobic and hydrophilic. We demonstrate the ease of this approach and its potential for the design and syntheses of stimuli responsive materials.

Results and Discussion

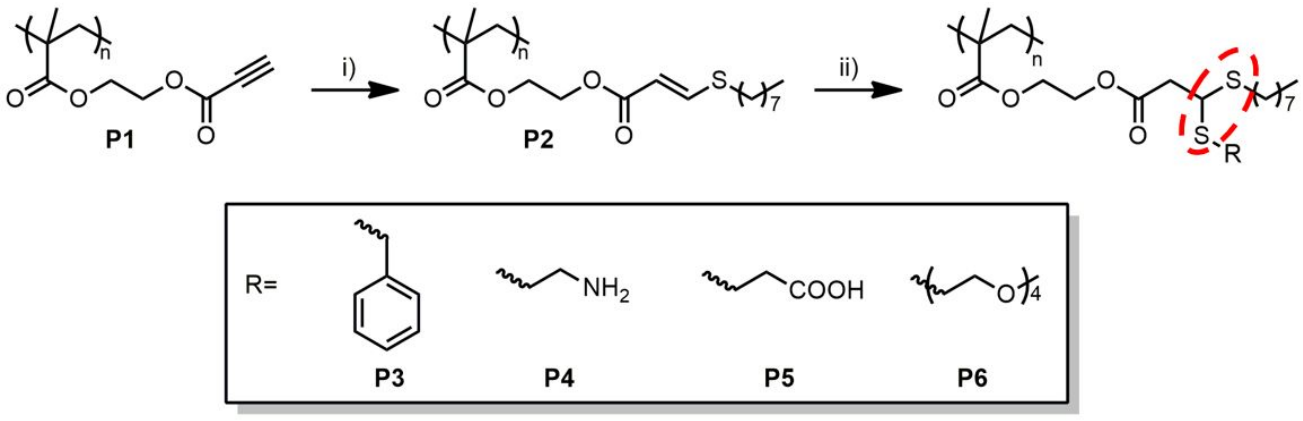


Scheme 1. Synthetic approaches for the targeted α,β-alkynoate ester-based polymer **P1**. Approach 1: i) H_2SO_4 (cat.), toluene, reflux, 42%; ii) CTR, AIBN, THF, 30%. Approach 2: i) DCC, DMAP, DCM, 52%; ii) CTR, AIBN, THF, 80%; iii) AgF, MeCN/THF, then 1M HCl, 99%.

The precursor polymer for the targeted functionalization with two different functionalities is shown as polymer **P1** in Scheme 1. This structure provides us with the opportunity for installing functional moieties of interest through a simple addition of thiol species. Two approaches were explored to synthesize the target polymer (**P1**). Approach 1 involved direct polymerization of the unprotected monomer **1**, which was prepared through the esterification reaction of hydroxyethyl methacrylate (HEMA) and propiolate acid (Scheme 1, approach 1). **P1** was obtained through RAFT polymerization of monomer **1** with 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid as chain transfer agent. Different solvents (DMF or THF), monomer concentrations (500 mg/mL or 100 mg/mL) and reaction times (12 h or 24 h) were investigated to avoid crosslinking during the polymerization reaction. However, even under the optimized conditions, mildly crosslinked products (10 % crosslinking degree) were still produced. The crosslinking can be identified by the alkene proton peaks (6.12 ppm, 5.63 ppm) from NMR spectrum. Moreover, due to the low monomer concentration and short reaction times used here, the final polymerization yield was relatively low (<30%).

The second approach that we investigated was to use a protection group for the active alkyne group to prevent crosslinking during polymerization. In this process, TMS-protected propiolate acid reacted with hydroxyethylmethacrylate (HEMA) to prepare monomer **2**. By protecting the alkyne group, monomer **2** can be conveniently polymerized with no crosslinking observed from NMR. Note that the esterification reaction in refluxing toluene can produce ethylene glycol dimethacrylate through

trans-esterification of HEMA. This byproduct could then act as a crosslinker later in the polymerization reaction. Indeed, this byproduct was observed under the reaction conditions and was also found to exhibit a similar R_f value (R_f = 0.6 in 10% ethyl acetate/hexane) as that of the desire product in chromatography, which made the purification of monomer **2** in this reaction difficult. To address this challenge, a base catalyzed esterification reaction was utilized with DCC and DMAP as the coupling reagents. After polymer **P1'** was acquired through the polymerization of monomer **2**, several deprotection methods were attempted. The reagent tetra-*n*-butylammonium fluoride (TBAF) yielded insoluble products, presumably due to the strong base-catalyzed crosslinking. The reaction yield under acid conditions (*p*TsOH, MeOH/DCM) was also very poor. We were gratified to find that a metal fluoride (AgF) in MeCN/THF can be used to achieve 100% deprotection. By choosing the protection and deprotection approach, we could successfully obtain the target polymer **P1** with no crosslinking and high overall yield (>70%).



Scheme 2. Schematic structure of multifunctional polymers: i) 1st thiol addition reaction with octyl thiol and triethylamine in $CHCl_3$. ii) 2nd thiol addition reaction with different thiol reagents (HS-R) and 1,5,7-Triazabicyclo [4.4.0] dec-5-ene (TBD) in $CHCl_3$.

To test the possibility of sequential nucleophilic addition to **P1**, we treated the polymer with octane-1-thiol (1.0 equiv. respect to alkyne group) as the nucleophile in the presence of triethylamine (0.1 equiv.) as the organocatalyst. The reaction progress was monitored by ^{1}H NMR (Figure 1A). The evolution of the alkyne proton signal at 3.0 ppm and the new alkene proton signals at 7.73 ppm and 5.75 ppm were monitored. The former peak decreased in signal intensity with time, while the latter ones concurrently increased in intensity. Complete disappearance of the alkyne peak and saturation of the alkene peaks within 80 minutes indicated a quantitative conversion within this timeframe (Figure 2A). To check whether the thio-vinyl product is available for a second nucleophilic addition under the same reaction conditions, the polymer was reacted with excess octane-1-thiol (2.0 equiv. to alkene group) for a much longer reaction time (up to 24 h) with TEA as the catalyst. NMR spectra of these reactions showed that the alkene proton peaks were intact, which suggests that the addition reaction stopped only at the first step and did not go further for a second addition under the mild base conditions (Figure 2B). Also, the GPC profiles of polymers from the tested reactions revealed the similar shape and same retention time (see Supporting Information (SI) Figure S1). Together, these results suggested that the degree of the first thiol addition reaction is base-mediated and is independent of the amount of thiol reagent added to the system. This feature is considered critical, as this provides us with the opportunity to attach a different thiol reagent onto the same repeat unit by simply altering the reaction conditions for the second addition.

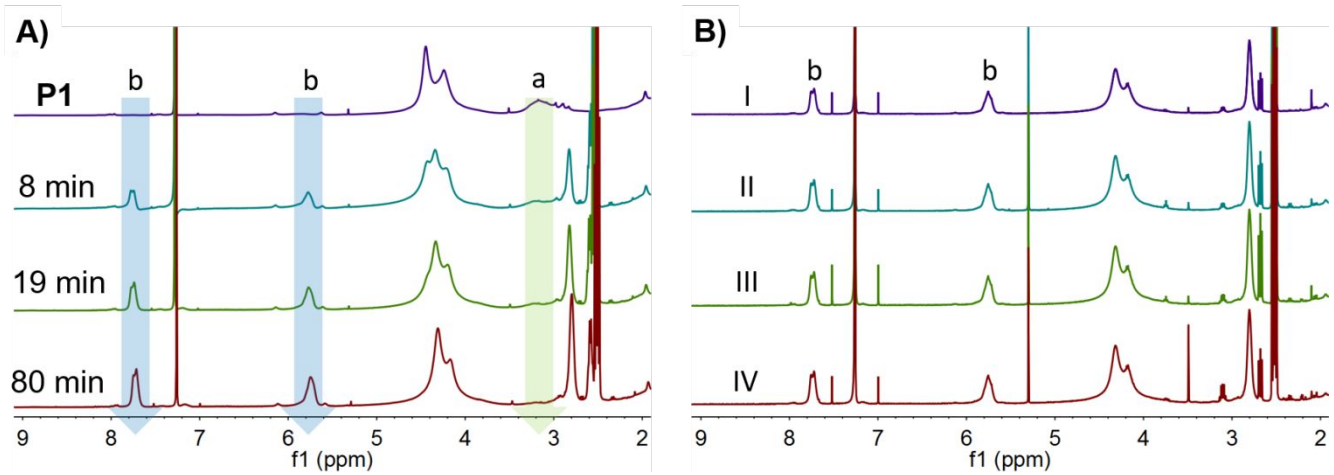


Figure 1. A) ^{1}H NMR spectrum for 1st thiol addition reaction with octyl thiol over time. B) ^{1}HNMR spectrum for 1st thiol addition with different amount of octyl thiol: (I) 1:1_24 h; (II) 1:2_4 h; (III) 1:2_9 h; (IV) 1:2_24 h. (e.g. 1:1 is respect to the ratio of alkyne to thiol reagent, solvent: $CDCl_3$, base: TEA (0.1 eq respect to alkyne))

Next, the possibility of a second addition of a different thiol onto the vinyl side chains of polymer **P2** was tested. The weak electrophilicity of the double bonds in **P2** is attributed to its relatively electron-rich character, owing to the vinyl-thioether functionality. We envisaged that the use of a stronger base would overcome this reactivity hurdle. Thus, polymer **P2** was treated with benzyl-thiol in the presence of triazabicyclodecene (TBD) as the organocatalytic base. TBD was chosen as the base, as it is considered a strong base with the pK_a of ~23.5 in DMSO. In the presence of this base, the progress of the second addition to **P2** was monitored by ^{1}H NMR. Total disappearance of the alkene proton signals from NMR spectrum indicated the complete addition of a second thiol species (Figure 2A). Since the double bonds in **P2** are based on an α,β-unsaturated ester, the second thiol is expected to add to the same carbon through a Michael-type addition. Indeed, the proton signals at 4.15 ppm confirm this

expectation, where the product is a dithioacetal. The decrease in alkene proton signal was monitored over time in an NMR tube. The complete disappearance of alkene proton signals was considered to be 100% conversion, and the reaction process over time was plotted in Figure 2B for benzyl mercaptan. The alkene proton signals disappeared within 160 min, showing that the second thiol addition reaction was complete to afford polymer **P3** within 3 h under these reaction conditions. A similar trend was observed when a different thiol nucleophile, cysteamine, was used where the reaction completed in about 120 min (Figure 2C).

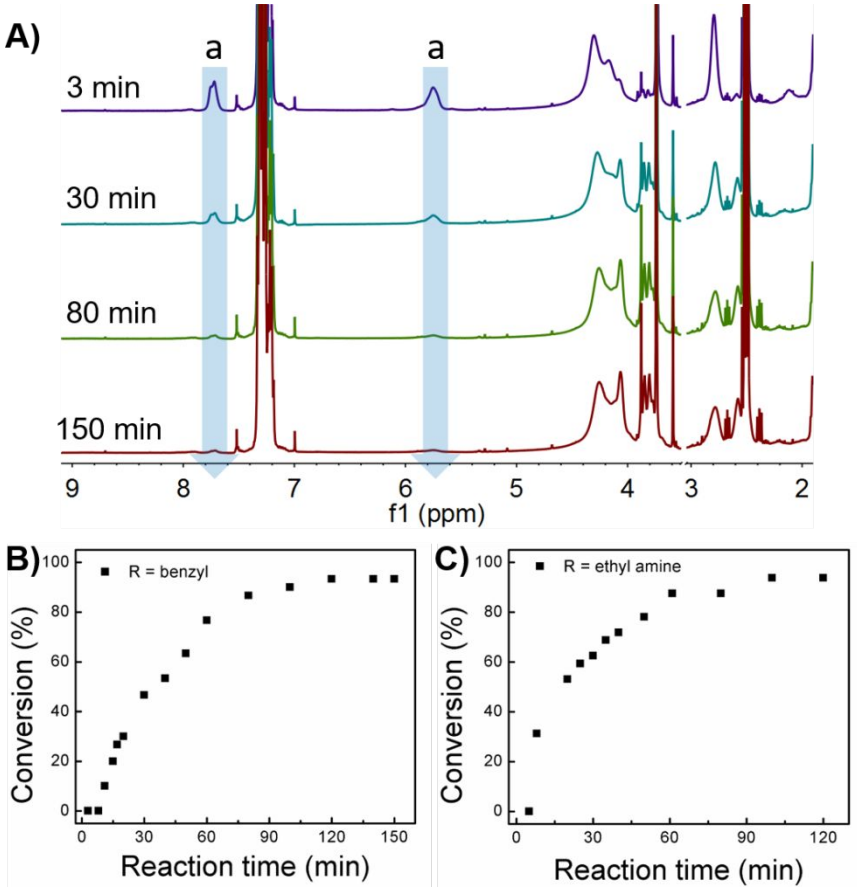


Figure 2. A) ^{1}H NMR evidence for 2nd thiol addition using benzyl mercaptan. Kinetic study of 2nd addition with benzyl mercaptan (B) and cysteamine (C).

We next sought to examine whether this methodology would translate to useful applications, such as peptide separation and detection. Peptide detection in biological fluids, especially of disease-relevant biomarkers, can be challenging due to their low concentration and inherent sample complexity.[40,41] There have been several approaches that have used supramolecular chemistry to predictably simplify complex mixtures through selective sequestration of peptides in a liquid-liquid extraction process for detection by matrix assisted laser desorption ionization mass spectrometry (MALDI-MS)[10,11,42,43]. To demonstrate the potential utility of the bifunctional homopolymers synthesized here, we synthesized a negatively charged amphiphilic homopolymer **P5**. To synthesize this polymer, *tert*-butyl-3-sulfanylpropanoate was used as the second thiol addition reagent. Deprotection of *tert*-butyl group from the resultant polymer using trifluoroacetic acid produced the targeted polymer **P5**, as evidenced by the complete disappearance of *tert*-butyl proton signal at 1.41 ppm (Figure 3A).

The negatively charged, amphiphilic homopolymer **P5** is expected to form micelle-like aggregates in water and reverse-micelle-like aggregates in toluene. The size of micellar aggregate was about 190 nm with negative charged zeta potential (-76.8 ± 4.05 mV), while the size of the reverse micelle state in toluene was found to be ~260 nm (Figure S3). Reverse micelles of such polymers can be used to selectively enrich peptides according to charge from an aqueous phase into an organic phase. To identify whether these reverse micelle assemblies would be capable of selectively sequestering molecules, we first investigated the extraction capability and selectivity of **P5** toward water-soluble dyes as the model

analyte. A positively charged dye molecule, rhodamine 6G (R6G), and a negatively charged molecule, calcein, were chosen as the candidates for this study due to their distinct absorption spectrum. A 200 µL toluene solution with a 2.3 mM concentration of **P5** was used as the apolar phase, while the aqueous phase contained 1 mL of 4 µM calcein or 2.5 µM R6G in PBS buffer (pH = 7.4, 150 mM NaCl). After a two-phase liquid-liquid extraction procedure for 10 minutes, the apolar organic and the aqueous phases were separated. The UV-visible absorption spectra of the aqueous solutions before and after the liquid-liquid extraction procedure were compared, and the overall efficiency of dye extraction was estimated based on the amount of dye molecule left in the aqueous phase. The binding efficiency and the amount of dye molecule remaining in the aqueous phase is expected to be inversely correlated. As discerned from the data in Figures 3B and 3C, the **P5** reverse micelle could efficiently extract the complementarily charged analyte R6G from the aqueous phase to organic phase. In contrast, a negligible amount of calcein is extracted by the same polymer **P5**. These data indicate that the amphiphilic homopolymer **P5** could also be used to selectively separate analyte molecules of interest.

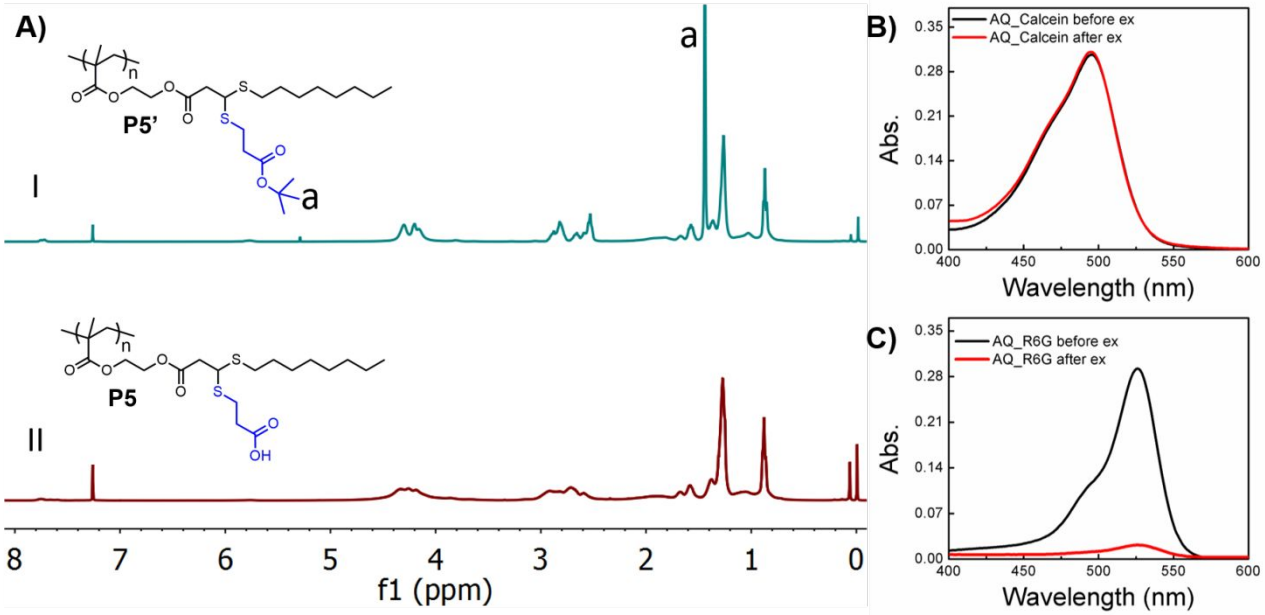


Figure 3. A) ^{1}H NMR spectrum of polymer **P5** before (I) and after (II) deprotection. UV-Vis spectrum of Calcein (B) and Rhodamine 6G (C) in aqueous solution before (black line) and after (red line) extraction with reverse micelles made from **P5**.

After testing the selectivity with dye molecules, we evaluated the potential peptide enrichment selectivity of polymer **P5**. The peptides preproenkephalin, β-amyloid (1-11), kinetensin and bradykinin were chosen as candidate peptides for their different pI values. These peptides were dissolved in MOPS buffer at pH 7. A toluene solution containing the reverse micelles of **P5** was used as the organic phase. As shown in Figure 4, after the liquid-liquid extraction process, only peptides with pI values higher than 7.0, *viz.* kinetensin (pI 10.84) and bradykinin (pI 12.0) (Figure 4C), were selectively extracted into the organic phase and detected by MALDI-MS. The more negatively charged peptides with pI values lower than 7, *viz.* preproenkephalin (pI 3.71) and β-amyloid (1-11) (pI 4.31) (Figure 4B), remained in the aqueous

phase. This experiment shows that polymer **P5** could be potentially useful in peptide separation and detection.

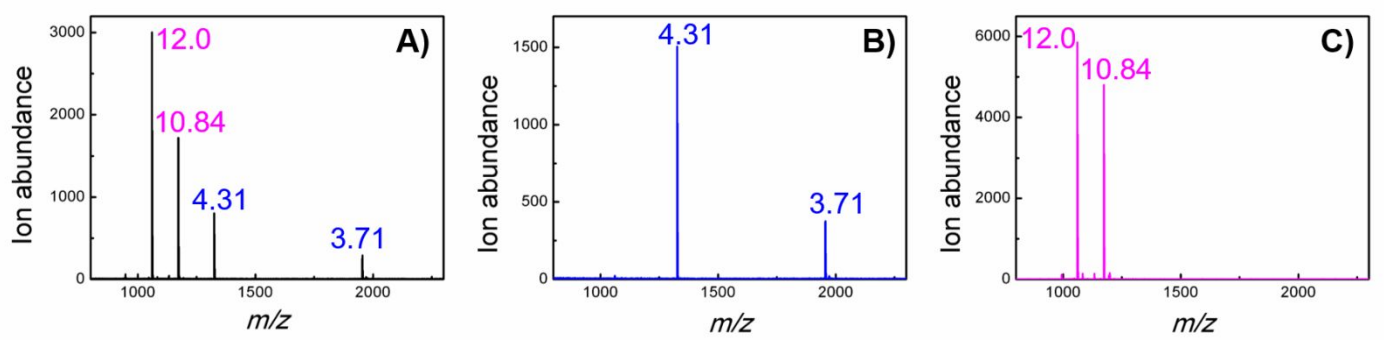


Figure 4. MALDI mass spectra of selective extraction of peptides in a mixture with reverse micelles made from **P5**. A) A mixture of four peptides labeled with their pIs in a buffer of pH 7 (Bradykinin *m/z* 1060.5, Kinetensin *m/z* 1172.7, β-amyloid (1-11) *m/z* 1326.3 and Preproenkephalin *m/z* 1954.7). B) Peptides left in aqueous solution after extraction using reverse micelles made by polymer **P5**. C) Peptides extracted by **P5**. * pI numbers are calculated by ExPASy.

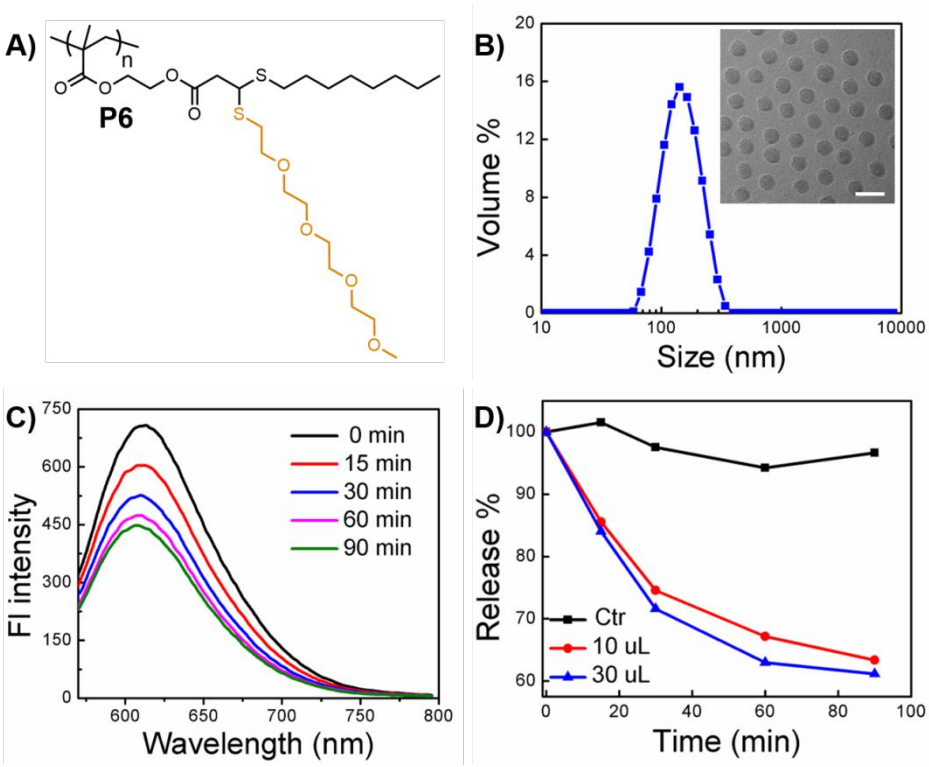


Figure 5. A) Chemical structure of polymer **P6**. B) DLS size distribution curve and TEM image (insert) of **P6** micelle in aqueous solution. (scale bar =100 nm). C) Fluorescence

emission spectra of Nile Red (NR) at different intervals in the presence of H_2O_2 of aqueous solution of **P6**. D) NR release profile at different time intervals in the presence of different amount of H_2O_2 in **P6**.

Finally, a charge neutral amphiphilic homopolymer **P6** was prepared using tetra-ethyleneglycol monothiol (TEG-SH) as a second thiol agent (Figure 5A and S2, see SI for details). The amphiphilic polymer **P6** bears TEG as the hydrophilic moiety and octyl chain as hydrophobic moiety in each repeating unit. This polymer, too, forms micelle-like aggregates in aqueous solution. These aggregates can encapsulate water-insoluble guest molecules, such as small molecule drugs, in their hydrophobic pockets. We were interested in utilizing this feature to test whether the ROS-sensitive nature of the dithioacetal moiety can be utilized to cause molecular release from these aggregates. Accordingly, Nile red (NR) was utilized as the model guest molecule due to its hydrophobic and fluorescent nature. Also, NR is more fluorescent when present inside the hydrophobic core of the micellar aggregate, whereas very little fluorescence would be observed if NR is in a more polar environment, such as in water[44]. This property could be used for monitoring NR release in response to ROS stimulus. First of all, the morphological characterization on **P6** micelle showed that they form spherical aggregates with size ~130 nm (Figure 5B, enlarged TEM image shown in Figure S4). The assembly was found to be stable in solution for over a month. When 10 µL of H_2O_2 was added as the ROS trigger to a solution of 250 µM concentration of **P6** in water, the fluorescence of the encapsulated NR was found to decrease with time (Figure 5C). The control sample, on the other hand, showed very little change in fluorescence

properties if any over the same timeframe (Figure 5D). When the amount of H_2O_2 was increased to 30 µL, the kinetics of NR release was only moderately higher. Overall, the highest molecular release obtained was ~40%. Moreover, the size of the aggregate was found to slightly increase, while the morphology remained spherical shape, with time upon exposure to H_2O_2 (Figure S5). The thioacetal containing polymer can be oxidized to forms sulfoxides and sulfones, while dithioketals can be oxidized and further degraded under H_2O_2 conditions.[36,37] In this paper, as the sequential substitution of the electron deficient alkyne with thiols will result in the formation of the dithioacetal, we tested if this release was due to the oxidation or the degradation of the dithioacetal. We designed two small molecules (dithioacetal and dithioketal) to study the oxidation process with time dependent NMR. The results are shown in Figure S6 and S7. We found that the dithioketal can be degraded to produce the ketone precursor. However, dithioacetal can just be oxidized and cannot be further degraded to produce the aldehyde precursor. This oxidation induces change in the hydrophilicity of the polymer. These results correlated well with the DLS and TEM data, which showed that increased hydrophilicity of the nanoassembly will induce swelling of the particles. Furthermore, the increased hydrophilicity of the nanoaggregate reduces the ability of the polymeric aggregate as a host for hydrophobic guest molecules, which results in release of the guest molecules albeit with a limited amount (~40%). Overall, these observations suggest that the oxidation of the dithioacetal by H_2O_2 will result in the increase of the hydrophilicity of the nanoassembly. The

molecular release shows that the host-guest capacity of these swollen assemblies is much worse than the parent assembly.

Conclusion

In summary, a novel method for the convenient preparation of bifunctional polymers using sequential thiol-yne nucleophilic addition reactions is used in a post-polymerization modification process. We have developed a procedure for introducing an activated terminal alkyne as the polymer side chain functionality. This moiety can then be sequentially reacted with two different thiols under mild reaction conditions. Since the base strength requirement for the first step and the second step are considerably different and since the first addition is quantitative under the optimized conditions, this sequential addition paved the way for introducing two different side chain functional groups in every repeat unit of the polymer. The utility of this methodology has been demonstrated by generating amphiphilic homopolymers, where hydrophilic and hydrophobic functional groups have been introduced as the two different side chains. In addition to the functional utility of these amphiphilic homopolymers, we have also provided a preliminary demonstration of the possible ROS-induced release of guest molecules from the polymeric aggregates, as the synthetic methodology naturally lends itself to the introduction of dithioacetal moieties in each of the repeat units in the polymer. Overall, syntheses of highly functional polymeric materials using the environmentally-friendly nucleophilic thiol-yne click approach could pave the way for expanding the functional polymer toolbox.

Note:

Acknowledgments

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References

(1) Kale, T. S.; Klaikherd, A.; Popere, B.; Thayumanavan, S. *Langmuir* 2009, **25**, 9660–9670.

(2) Zhang, J.; Liu, K.; Müllen, K.; Yin, M. *Chem. Commun.* 2015, **51**, 11541–11555.

(3) Cha, J. N.; Birkedal, H.; Euliss, L. E.; Bartl, M. H.; Wong, M. S.; Deming, T. J.; Stucky, G. D. *J. Am. Chem. Soc.* 2003, **125**, 8285–8289.

(4) Savariar, E. N.; Aathimanikandan, S. V.; Thayumanavan, S. *J. Am. Chem. Soc.* 2006, **128**, 16224–16230.

(5) Laurent, B. A.; Grayson, S. M. *Polym. Chem.* 2012, **3**, 1846–1855.

(6) Zhou, Y.; Liu, B.; Wang, X. *Polymer* 2016, **97**, 1–10.

(7) Manojkumar, K.; Mecerreyes, D.; Taton, D.; Gnanou, Y.; Vijayakrishna, K. *Polym. Chem.* 2017, **8**, 3497–3503.

(8) Kohut, A.; Hevus, I.; Voronov, S.; Voronov, A. Amphiphilic Invertible Polymers and Their Applications. In *Industrial Applications for Intelligent Polymers and Coatings*; Springer International Publishing: Cham, 2016; pp 399–415.

(9) Qiu, L.; Zhang, J.; Yan, M.; Jin, Y.; Zhu, K. *Nanotechnology* 2007, **18**, 475602.

(10) Rodthongkum, N.; Chen, Y.; Thayumanavan, S.; Vachet, R. W. *Anal. Chem.* 2010, **82**, 8686–8691.

(11) Rodthongkum, N.; Ramireddy, R.; Thayumanavan, S.; Richard, W. V. *Analyst* 2012, **137**, 1024–1030.

(12) Arumugam, S.; Vutukuri, D. R.; Thayumanavan, S.; Ramamurthy, V. *J. Am. Chem. Soc.* 2005, **127**, 13200–13206.

(13) Miao, W. K.; Yan, Y.-K.; Wang, X. L.; Xiao, Y.; Ren, L. J.; Zheng, P.; Wang, C. H.; Ren, L. X.; Wang, W. *ACS Macro Lett.* 2014, **3**, 211–215.

(14) Sandanaraj, B. S.; Demont, R.; Thayumanavan, S. *J. Am. Chem. Soc.* 2007,

129, 3506–3507.

(15) Yu, Y.; Yin, M.; Müllen, K.; Knoll, W. *J. Mater. Chem.* 2012, **22**, 7880–7886.

(16) Li, N.; Ye, G.; He, Y.; Wang, X. *Chem. Commun.* 2011, **47**, 4757–4759.

(17) Changez, M.; Kang, N. G.; Lee, C. H.; Lee, J. S. *Small* 2010, **6**, 63–68.

(18) Liu, J.; Huang, W.; Pang, Y.; Huang, P.; Zhu, X.; Zhou, Y.; Yan, D. *Angew. Chem., Int. Ed.* 2011, **50**, 9162–9166 DOI: 10.1002/anie.201102280.

(19) Mane, S. R.; Rao, V. N.; Shunmugam, R. *ACS Macro Lett.* 2012, **1**, 482–488.

(20) Mane, S. R.; Rao N, V.; Chaterjee, K.; Dinda, H.; Nag, S.; Kishore, A.; Das Sarma, J.; Shunmugam, R. *Macromolecules* 2012, **45**, 8037–8042.

(21) Zhuang, J.; Garzoni, M.; Torres, D. A.; Poe, A.; Pavan, G. M.; Thayumanavan, S. *Angew. Chem., Int. Ed.* 2017, **56**, 4145–4149.

(22) Basu, S.; Vutukuri, D. R.; Shyamroy, S.; Sandanaraj, B. S.; Thayumanavan, S. *J. Am. Chem. Soc.* 2004, **126**, 9890–9891.

(23) Luo, C.; Liu, Y.; Li, Z. *Macromolecules* 2010, **43**, 8101–8108.

(24) Wang, Y.; Alb, A. M.; He, J.; Grayson, S. M. *Polym. Chem.* 2014, **5**, 622–629.

(25) Ramireddy, R. R.; Prasad, P.; Finne, A.; Thayumanavan, S. *Polym. Chem.* 2015, **6**, 6083–6087.

(26) Kubo, T.; Bentz, K. C.; Powell, K. C.; Figg, C. A.; Swartz, J. L.; Tansky, M.; Chauhan, A.; Savin, D. A.; Sumerlin, B. S. *Polym. Chem.* 2017, **8**, 6028–6032.

(27) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* 2007, **36**, 1249–1262.

(28) Meldal, M.; Tornøe, C. W. *Chem. Rev.* 2008, **108**, 2952–3015.

(29) Jewett, J. C.; Bertozzi, C. R. *Chem. Soc. Rev.* 2010, **39**, 1272–1279.

(30) Schilling, C. I.; Jung, N.; Biskup, M.; Schepers, U.; Bräse, S. *Chem. Soc. Rev.* 2011, **40**, 4840.

(31) Hoyle, C. E.; Bowman, C. N. *Angew. Chem., Int. Ed.* 2010, **49**, 1540–1573.

(32) Lowe, A. B. *Polymer,* 2014, **55**, 5517–5549.

(33) Truong, V. X.; Dove, A. P. *Angew. Chem., Int. Ed.* 2013, **52**, 4132–4136.

(34) Kuroda, H.; Tomita, I.; Endo, T. A *Macromolecules* 1995, **28**, 6020–6025.

(35) Yao, B.; Sun, J.; Qin, A.; Tang, B. Z. *Chinese Sci. Bull.* 2013, **58**, 2711–2718.

(36) Napoli, A.; Valentini, M.; Tirelli, N.; Müller, M.; Hubbell, J. A. *Nat. Mater.* 2004, **3**, 183-189.

(37) Wilson, D. S.; Dalmasso, G.; Wang, L.; Sitaraman, S. V; Merlin, D.; Murthy, N. *Nat. Mater.* 2010, **9**, 923–928.

(38) Shim, M. S.; Xia, Y. A *Angew. Chem., Int. Ed.* 2013, **52**, 6926–6929.

(39) Martin, J. R.; Gupta, M. K.; Page, J. M.; Yu, F.; Davidson, J. M.; Guelcher, S. A.; Duvall, C. L. *Biomaterials* 2014, **35**, 3766–3776.

(40) Hanash, S. M.; Pitteri, S. J.; Faca, V. M. *Nature*. 2008, **452**, 571–579.

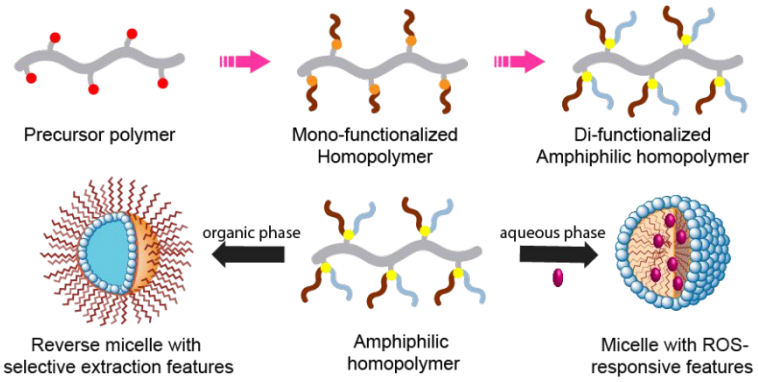
(41) Liotta, L. A.; Petricoin, E. *Cancer Cell*. 2011, **20**, 279–280.

(42) Serrano, M. A. C.; He, H.; Zhao, B.; Ramireddy, R. R.; Vachet, R. W.; Thayumanavan, S. *Analyst* 2016, **142** 118-122.

(43) Combariza, M. Y.; Savariar, E. N.; Vutukuri, D. R.; Thayumanavan, S.; Vachet, R. W. *Anal. Chem.* 2007, **79**, 7124–7130.

(44) Greenspan, P.; Fowler, S. D. *J. Lipid Res.* 1985, **26**, 781–789.

TOC graphic


Precursor polymer
Mono-functionalized Homopolymer
Di-functionalized Amphiphilic homopolymer
organic phase
aqueous phase
Reverse micelle with selective extraction features
Amphiphilic homopolymer
Micelle with ROS-responsive features