Heterofunctional polymers and core–shell nanoparticles *via* cascade aminolysis/Michael addition and alkyne–azide click reaction of RAFT polymers†

Zesheng An,*[a] Wei Tang,[b] Minghong Wu,[a] Zheng Jiao[a] and Galen D. Stucky*[b]

Received (in Cambridge, UK) 22nd September 2008, Accepted 21st October 2008
First published as an Advance Article on the web 11th November 2008
DOI: 10.1039/b816578e

A convenient methodology involving cascade aminolysis/Michael addition and alkyne–azide click reaction was developed for polymers and polymeric core–shell nanoparticles, synthesized *via* RAFT-mediated homogeneous and heterogeneous polymerisation processes, respectively, to provide well-defined heterofunctional polymeric materials.

Reversible addition–fragmentation chain transfer (RAFT) polymerisation, amenable to a wide range of monomers and easily performed under various conditions, has proven to be a highly versatile technique for the preparation of well-defined polymers.[1] It also provides easy access to virtually unlimited types of telechelic polymers due to the high degree of end functionalisation of the RAFT process.[2] Telechelic polymers can be obtained by using pre-functionalised chain transfer agents or through post modification processes of the obtained RAFT polymers.[2] In the latter case, simple and efficient chemical approaches are highly desirable to provide polymers with a high degree of functionality, eliminating unnecessary laborious purification procedures.

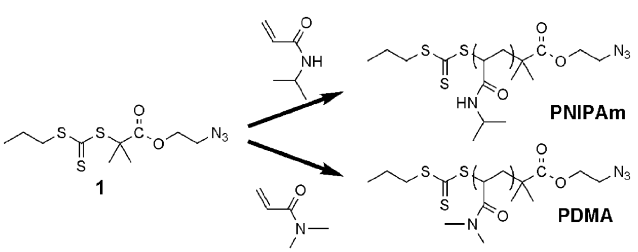
While well-defined functional polymers, enabled by various living polymerisation processes,[3] have attracted attention in a number of important technological fields,[4] nearly monodisperse multivalent polymeric nanoparticles are particularly interesting in nanobiotechnologies ranging from bioimaging to targeted cancer therapy.[5] In this regard, great effort has been directed towards the development of novel strategies for the production of narrowly disperse nanoparticles with distinct functionalities that can be subsequently employed to interact with biological entities.[6]

Among various chemical approaches for the modification of polymers, the highly modular alkyne–azide click reaction is perhaps the most frequently employed process to efficiently functionalise a variety of polymers,[7] which otherwise is unfortunately difficult to realise. For the RAFT polymers in particular, the dithioester or trithiocarbonate group at the polymer end can be readily modified through aminolysis[8] and the generated thiol can be used for further modification by Michael addition process.[9]

We report the development of a novel methodology for the preparation of heterofunctional polymers *via* cascade aminolysis/Michael addition and alkyne–azide click reaction for RAFT polymers. Further, we apply this methodology to nearly monodisperse core–shell nanoparticles, synthesized *via* facile RAFT-mediated precipitation polymerisation process,[10] to afford multifunctional nanoparticles with selectively defined heterofunctional groups in the core and on the surface.

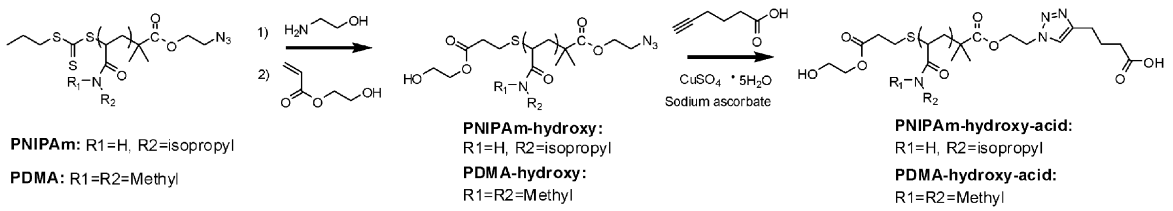
In order to demonstrate our strategy for the preparation of heterofunctional polymers, we used azido-functionalised chain transfer agent, **1**, to prepare well-defined polymers with different molecular weights, poly(*N*-isopropylacrylamide) (PNIPAm) and poly(*N*,*N*-dimethylacrylamide) (PDMA) (Scheme 1). These telechelic polymers, bearing a trithiocarbonate group at one end and an azido group at the other, have low polydispersities ($M_w/M_n < 1.15$) with molecular weights ranging from 6900 to 13 000. Chain extension of these homopolymers as macromolecular chain transfer agents to prepare block copolymers was successfully performed as indicated by the narrow polydispersity (<1.15) of the resultant block copolymers (see ESI†), suggesting a high degree of end functionalisation of the RAFT polymers.

Heterofunctional polymers were effected by sequential modification of the trithiocarbonate and the azido group at the ends of the RAFT polymers (Scheme 2). In the one-pot aminolysis/Michael addition process, PNIPAm or PDMA polymers were dissolved in THF in the presence of the antioxidant agent, trioctylphosphine, followed by treatment with 5 equivalents of 2-hydroxyethylamine and 10 equivalents of 2-hydroxyethylacrylate at room temperature. The characteristic yellow color of the trithiocarbonate group quickly faded away and UV-Vis measurements for PNIPAm-hydroxy (PDMA-hydroxy) showed that 96% (88%) of the trithiocarbonate groups at the end of polymers were aminolysed. PNIPAm-hydroxy (PDMA-hydroxy) was then subject to click reaction with 5 equivalents of 5-hexynoic acid using $CuSO_4$/sodium ascorbate catalyst system in water to furnish



Scheme 1 Synthesis of azido-functionalised RAFT polymers.

[a] *Institute of Nanochemistry and Nanobiology, School of Environmental and Chemical Engineering, Shanghai University, Shanghai, 200444, China. E-mail: an.zesheng@shu.edu.cn*
[b] *Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA 93106, USA. E-mail: stucky@chem.ucsb.edu*
† Electronic supplementary information (ESI) available: Preparation and characterisation of polymers and nanoparticles. See DOI: 10.1039/b816578e



Scheme 2 Preparation of heterofunctional polymers *via* cascade aminolysis/Michael addition and alkyne–azide click reaction.

heterofunctional polymer PNIPAm-hydroxy-acid (PDMA-hydroxy-acid). The molecular weights of PNIPAm (PDMA), PNIPAm-hydroxy (PDMA-hydroxy) and PNIPAm-hydroxy-acid (PDMA-hydroxy-acid) experienced a slight increase in that order due to further purification after each step, but the polydispersity of the polymers remained low (<1.2), indicating that no coupling between polymers occurred during the transformation processes and thus demonstrating the orthogonal nature of these processes. ^{1}H NMR (Fig. 1) showed that after one-pot aminolysis/Michael addition process, the methylene (3.28 ppm) and methine (4.55 ppm) protons next to the trithiocarbonate group of PNIPAm disappeared in PNIPAm-hydroxy, accompanied by the obvious appearance of the methylene groups at 3.82 ppm (–CH_2–OH), 2.79 ppm (–CH_2–S–), and 2.64 ppm (–OC(O)–CH_2–) in PNIPAm-hydroxy. After click reaction, the methylene group at 3.47 ppm (–CH_2–N_3) in PNIPAm-hydroxy underwent a notable shift to 4.62 ppm for the methylene protons next to the heterocyclic aromatic ring, formed during the click reaction, in PNIPAm-hydroxy-acid. Click reaction between chain transfer agent **1** and 5-hexynoic acid was also performed, and the ^{1}H NMR spectrum of the product (Fig. S1, ESI†) was used for comparison to ensure correct assignment of ^{1}H NMR for the products of the polymer click reactions. Similarly, the end group transformation processes for the PDMA series also led to distinct changes in ^{1}H NMR spectra with somewhat lower efficiencies (see ESI†).

Having demonstrated the success of the methodology for the production of heterofunctional polymers *via* aminolysis/Michael addition and click reaction on RAFT polymers, we next showed that the same methodology can be applied to prepare multifunctional core–shell nanoparticles (Scheme 3). First, nearly monodisperse core–shell nanoparticles (see ESI†) were synthesized using our previously established approach,[10] namely RAFT-mediated precipitation polymerisation in which PDMA RAFT polymers were used as both chain transfer agents and stabilising agents during the precipitation polymerisation of *N*-isopropylacrylamide. The PNIPAm generated during the precipitation polymerisation process constituted the core in which the trithiocarbonate groups were located while the PDMA blocks formed the shell with the azido groups on the surface of the shell. Core–shell nanoparticles of different sizes, 65 nm (polydispersity 6.5%) and 95 nm (polydispersity 7.1%), were obtained using PDMA macromolecular chain transfer agents with molecular weights of 12 980 and 6900, respectively. First, the core of the nanoparticles was functionalised with fluorescein through one-pot aminolysis, with 10 equivalents of 2-hydroxyethylamine, and Michael addition, with 10 equivalents of fluorescein *o*-acrylate (**2**), in the presence of sodium bisulfite antioxidant to obtain NP-fluorescein. The purified NP-fluorescein was then subject to further modification with 10 equivalents of dansyl probe (**3**) *via* click chemistry using $CuSO_4$/sodium ascorbate catalyst system to obtain NP-fluorescein-dansyl. The purified NP-fluorescein at pH = 7.0 showed the characteristic absorption spectrum of fluorescein with a functionalisation efficiency of 36%. The NP-fluorescein-dansyl showed absorption of both fluorescein and the dansyl probe and the conversion efficiency of the click reaction step was essentially quantitative. Therefore, the prepared heterofunctional core–shell nanoparticles were decorated with fluorescein in the core and dansyl probe on the surface. Both NP-fluorescein and

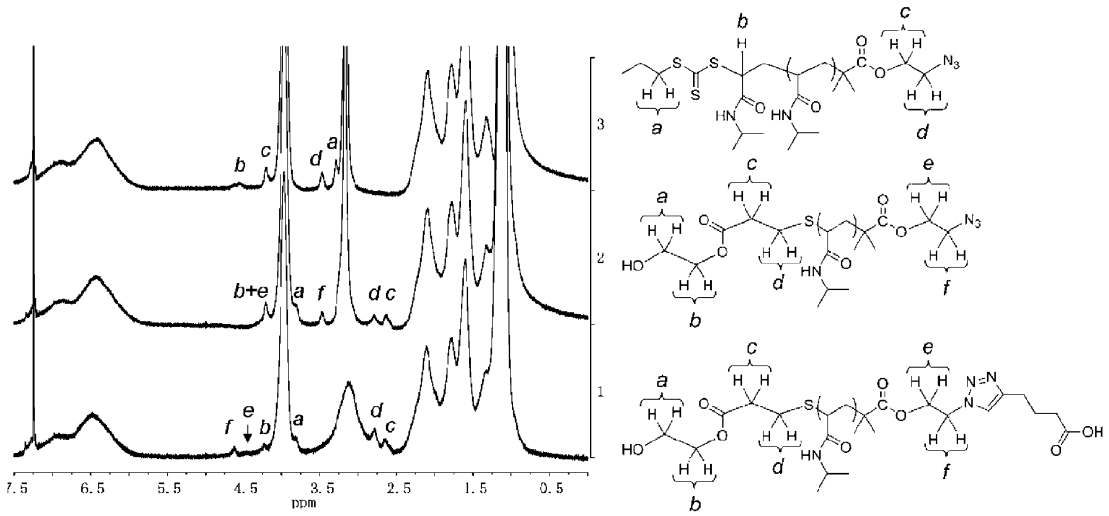
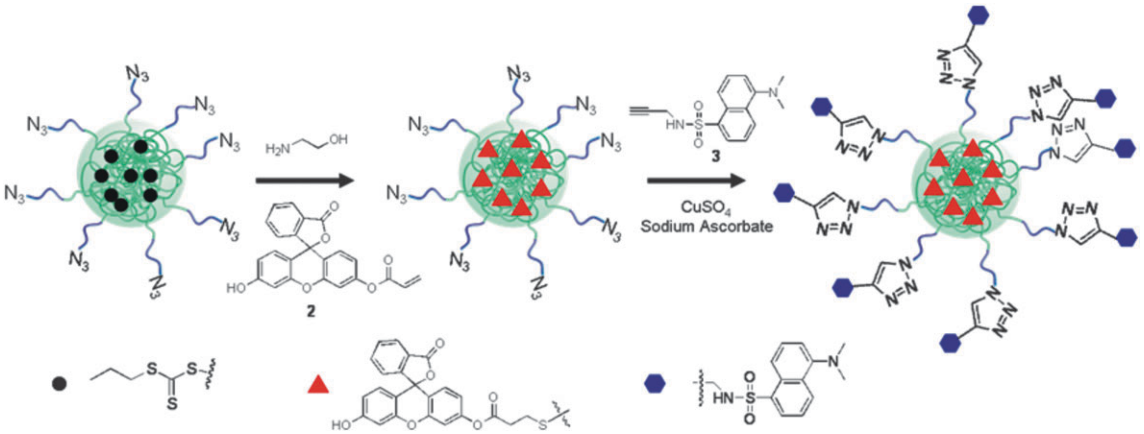


Fig. 1 ^{1}H NMR spectra of PNIPAm (top), PNIPAm-hydroxy (middle) and PNIPAm-hydroxy-acid (bottom).



Scheme 3 Preparation of heterofunctional core–shell nanoparticles *via* cascade aminolysis/Michael addition and click reaction.

NP-fluorescein-dansyl were similar in size to the original nanoparticles and could be well dispersed in water.

In conclusion, we have demonstrated a facile methodology for the preparation of well-defined, heterofunctional polymers and core–shell nanoparticles, using the easily performed reactions of one-pot aminolysis/Michael addition coupled with the efficient click reaction. It is anticipated that this methodology should be readily adopted to functionalise polymers and polymer nanoparticles with a wide variety of functional groups, thus greatly expanding the applications of these materials.

We acknowledge financial support by the Ministry of Science and Technology of China 973 program (2009CB930200) and by the Institute for Collaborative Biotechnologies through grant DAAD19-03-D-0004 from the U.S. Army Research Office.

Notes and references

1 J. Chiefari, Y. K. Chong, F. Ercole, J. Krstina, J. Jeffery, T. P. T. Le, R. T. A. Mayadunne, G. F. Meijs, C. L. Moad, G. Moad, E. Rizzardo and S. H. Thang, *Macromolecules*, 1998, **31**, 5559; S. Perrier and P. Takolpuckdee, *J. Polym. Sci. Part A: Polym. Chem.*, 2005, **43**, 5347; A. Favier and M.-T. Charreyre, *Macromol. Rapid Commun.*, 2006, **27**, 653; A. B. Lowe and C. L. McCormick, *Prog. Polym. Sci.*, 2007, **32**, 283; G. Moad, E. Rizzardo and S. H. Thang, *Polymer*, 2008, **49**, 1079.

2 J. Liu, C. Y. Hong and C. Y. Pan, *Polymer*, 2004, **45**, 4413; R. Wang, C. L. McCormick and A. B. Lowe, *Macromolecules*, 2005, **38**, 9518; Y.-Z. You, D. S. Manickam, Q.-H. Zhou and D. Oupicky, *Biomacromolecules*, 2007, **8**, 2038; T. M. Legge, A. T. Slark and S. Perrier, *Macromolecules*, 2007, **40**, 2318; C. Boyer, J. Liu, V. Bulmus, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Macromolecules*, 2008, **41**, 5641; K. L. Heredia, T. H. Nguyen, C. W. Chang, V. Bulmus, T. P. Davis and H. D. Maynard, *Chem. Commun.*, 2008, 3245.

3 C. J. Hawker, A. W. Bosman and E. Harth, *Chem. Rev.*, 2001, **101**, 3661; N. V. Tsarevsky and K. Matyjaszewski, *Chem. Rev.*, 2007, **107**, 2270; C. W. Bielawski and R. H. Grubbs, *Prog. Polym. Sci.*, 2007, **32**, 1; R. G. Lopez, F. D'Agosto and C. Boisson, *Prog. Polym. Sci.*, 2007, **32**, 419.

4 C. J. Hawker and K. L. Wooley, *Science*, 2005, **309**, 1200; D. Y. Ryu, K. Shin, E. Drockenmuller, C. J. Hawker and T. P. Russell, *Science*, 2005, **308**, 236; J. B. McLeary and B. Klumperman, *Soft Matter*, 2006, **2**, 45; Y. Z. You, C. Y. Hong and C. Y. Pan, *Adv. Funct. Mater.*, 2007, **17**, 2470; J. Liu, V. Bulmus, D. L. Herlambang, C. Barner-Kowollik, M. H. Stenzel and T. P. Davis, *Angew. Chem., Int. Ed.*, 2007, **46**, 3099; T. Hasell, K. J. Thurecht, R. D. W. Jones, P. D. Brown and S. M. Howdle, *Chem. Commun.*, 2007, 3933; P. De, M. Li, S. R. Gondi and B. S. Sumerlin, *J. Am. Chem. Soc.*, 2008, **130**, 11288.

5 L. Brannon-Peppas and J. O. Blanchette, *Adv. Drug Delivery Rev.*, 2004, **56**, 1649; M. Ferrari, *Nat. Rev. Cancer*, 2005, **5**, 161; G. Gaucher, M.-H. Dufresne, V. P. Sant, N. Kang, D. Maysinger and J.-C. Leroux, *J. Controlled Release*, 2005, **109**, 169; G. Sun, J. Xu, A. Hagooly, R. Rossin, Z. Li, D. A. Moore, C. J. Hawker, M. J. Welch and K. L. Wooley, *Adv. Mater.*, 2007, **19**, 3157.

6 R. K. O'Reilly, M.J. Joralemon, K. L. Wooley and C. J. Hawker, *Chem. Mater.*, 2005, **17**, 5976; E. S. Read and S. P. Armes, *Chem. Commun.*, 2007, 3021; J. K. Oh, R. Drumright, D. J. Siegwart and K. Watyjaszewski, *Prog. Polym. Sci.*, 2008, **33**, 448; A. W. York, S. E. Kirkland and C. L. McCormick, *Adv. Drug Delivery Rev.*, 2008, **60**, 1018; M. H. Stenzel, *Chem. Commun.*, 2008, 3486.

7 J.-F. Lutz, H. G. Börner and K. Weichenhan, *Macromol. Rapid Commun.*, 2005, **26**, 514; J. A. Opsteen and J. C. M. van Hest, *Chem. Commun.*, 2005, 57; G. Mantovani, V. Ladmiral, L. Tao and D. M. Haddleton, *Chem. Commun.*, 2005, 2089; D. Quémener, T. P. Davis, C. Barner-Kowollik and M. H. Stenzel, *Chem. Commun.*, 2006, 5051; M. R. Whittaker, C. N. Urbani and M. J. Monteiro, *J. Am. Chem. Soc.*, 2006, **128**, 11360; S. R. Gondi, A. P. Vogt and B. S. Sumerlin, *Macromolecules*, 2007, **40**, 474; B. Li, A. L. Martin and E. R. Gillies, *Chem. Commun.*, 2007, 5217; J. A. Opsteen, R. P. Brinkhuis, R. L. M. Teeuwen, D. W. P. M. Löwik and J. C. M. van Hest, *Chem. Commun.*, 2007, 3136; W. H. Binder and R. Sachsenhofer, *Macromol. Rapid Commun.*, 2008, **29**, 952; J. A. Johnson, M. G. Finn, J. T. Koberstein and N. J. Turro, *Macromol. Rapid Commun.*, 2008, **29**, 1052.

8 V. Lima, X. L. Jiang, J. Brokken-Zijp, P. J. Schoenmakers, B. Klumperman and R. V. D. Linde, *J. Polym. Sci. Part A: Polym. Chem.*, 2005, **43**, 959; D. L. Patton, M. Mullings, T. Fulghum and R. C. Advincula, *Macromolecules*, 2005, **38**, 8597; M. R. Whittaker, Y.-K. Goh, H. Gemici, T. M. Legge, S. Perrier and M. J. Monteiro, *Macromolecules*, 2006, **39**, 9028; J. Xu, J. He, D. Fan, X. Wang and Y. Yang, *Macromolecules*, 2006, **39**, 8616; Y.-Z. You, Q.-H. Zhou, D. S. Manickam, L. Wan, G.-Z. Mao and D. Oupicky, *Macromolecules*, 2007, **40**, 8617.

9 M. Li, P. De, S. R. Gondi and B. S. Sumerlin, *J. Polym. Sci. Part A: Polym. Chem.*, 2005, **43**, 5093; X.-P. Qiu and F. M. Winnik, *Macromol. Rapid Commun.*, 2006, **27**, 1648.

10 Z. S. An, Q. H. Shi, W. Tang, C.-K. Tsung, C. J. Hawker and G. D. Stucky, *J. Am. Chem. Soc.*, 2007, **129**, 14493.