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PAPER

Fabrication of vesicle-like dual-responsive click capsules by direct covalent layer-by-layer assembly†

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We report a click chemistry approach for the consecutive layer-by-layer assembly of thermo- and pH-sensitive clickable copolymers on silica particles and the subsequent formation of vesicle-like dual-responsive click capsules. These click capsules exhibit both thermo- and pH-responsive behaviors by elevating the solution temperature and incubating in acidic or basic solutions respectively. We also demonstrate the *in situ* preparation of silver nanoparticles within the multilayer of the click capsules. These stimuli-responsive behaviors were examined by using confocal laser scanning microscopy (CLSM), transmission electron microscopy (TEM), and atomic force microscopy (AFM). This approach provides potential applications in preparing well-defined vesicle-like capsules with covalent stabilization and flexibility in introducing a range of new materials including different functional polymers.

Introduction

In the early 1990s, Decher and co-workers developed a technique for constructing ultrathin organic films, creating multilayer assemblies by consecutive, layer-by-layer (LbL) adsorption of anionic and cationic polyelectrolytes.[1] So far, the field of LbL assembly has expanded to include films prepared using other non-electrostatic interactions-such as hydrogen bonding,[2] host–guest interactions,[3] and hybridization of DNA base pairs.[4] For over a decade, the LbL assembly has also been a versatile method for the fabrication of hollow capsules with well-defined structures, composition and tailorable physicochemical properties.[5] To prepare stable polymer capsules under various conditions (*e.g.*, temperature, ionic strength), the use of covalent bonds to enhance the capsules' binding strength has been proved to be an efficient method.[6] These strategies include post-modifications through oxidization,[7] UV-irradiation,[8] carbodiimide formation,[9] and glutaraldehyde modification.[10] Furthermore, the direct covalent LbL assemble performed through chemical reaction between two kinds of functional groups on polymer materials allows new properties to be engineered into the capsules.[11]

These strategies have opened the door to the fabrication of directly covalently bonded multilayer films. Recently, Caruso and co-workers demonstrated a promising approach to introduce covalent bonds in capsules *via* click chemistry,[12] the philosophy of being selective and high yielding under mild conditions,[13] based on the promising Huisgen 1,3-dipolar cycloaddition of azides and alkynes and thiol-ene click reactions. This general applicable and modular approach opened the door to the fabrication of directly covalently bonded multilayer films.

We recently reported the feasibility of direct covalent LbL assembly using click chemistry to fabricate ultrathin thermoresponsive capsules.[14] It was demonstrated that single component poly(*N*-isopropylacrylamide) (PNIPAm) capsules stabilized by aromatic 1,2,3-triazole linkages exhibited unique thermo-sensitive assembly behavior and thermo-reversible swelling/deswelling transition upon changing the temperature of the medium. We sought to exploit this LbL approach to fabricate dual-component polymer capsules that have the same basic architecture as polymer vesicles. In most cases, the polymer vesicles,[15] also known as "polymersomes",[16] are generally built through self-assembly from amphiphilic block copolymers, which phase separate in selective solvents as a result of the solubility difference between the blocks. Although polymer vesicles self-assembled through phase separation in dilute solution can be tailored for response to external stimuli including pH,[17] light,[18] and temperature,[19] some of these vesicles can be susceptible to disassembly upon a variation of such conditions, which may limit their use in biomedical applications. There are a limited number of literature reports on the cross-linking of polymersome walls and the stability of their construction, these reports include the use of radical polymerization,[20] photoinduced [2 + 2] cycloaddition,[21] base-catalyzed self-condensation of siloxanes,[22] and ring opening of epoxides.[23] Our studies are motivated in part by the potential for the controllable cross-linking of bilayers as drug-delivery vehicles.

To broaden the realm of multilayer architectures, herein we report the use of the LbL technique *via* click chemistry to alternately assemble thermo and pH responsive clickable copolymers

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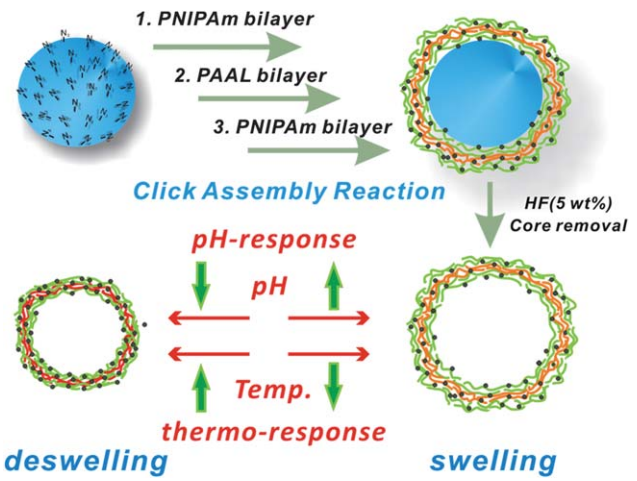
on a silica template, leading to a vesicle-like ultrathin multilayer with dual stimuli-responsive behaviors, as illustrated in Scheme 1. Such LbL assembled polymer capsules not only possess a robust covalent-stabilized nanostructure but also exhibit reversible swelling/deswelling behaviors upon changing the condition of the medium.

In this paper, we also extend our work in preparing inorganic nanoparticles within the multilayer. Nanometre-sized inorganic particles have unique properties stemming from quantum confinement effects and their large surface areas relative to their volumes.[24] Our objective is to demonstrate that the ion exchange and reduction of silver cations to zerovalent Ag nanoparticles can be carried out within the inner pH responsive layer of the capsules by using its carboxylic acid groups as reaction sites, and which provides a confined space in which organic or inorganic materials with unique properties can be produced.

Experimental section

Materials

All reagents and solvents were purchased from commercial suppliers and used as received unless otherwise noted, including L-ascorbic acid sodium salt (TCI, >98%), acryloly chloride (Alfa Aesar, 96%), cupric sulfate pentahydrate (SHOWA, 99.5%), sodium azide (SHOWA, 98%), silver acetate (Sigma-Aldrich, 99%), sodium borohydride (Sigma-Aldrich, 99%), and 2-propanol (Tedia, 99.5%). The monomer *N*-isopropylacrylamide (NIPAm, 99%, TCI) was recrystallized in hexane/toluene and dried under vacuum before use. *N*,*N*-dimethylformamide (DMF) (Tedia, 99.8%) and toluene (Tedia, 99.5%) were dried over CaH_2 (Acros, 93%) and distilled under reduced pressure. Tetrahydrofuran (Tedia, 99.8%) was distilled over Na/benzophenone. Deionized (DI) water was purified to a resistance of 18 MΩ (Milli-Q Reagent Water System, Millipore Corporation) and was used in all the reactions, solution preparations, and polymer isolations. Copper(I) bromide (Acros, 98%) was washed with glacial acetic acid for the removal of any soluble oxidized species, filtered, washed with 2-propanol, and dried. *N*-Acryloylalanine (AAL),[25] dansyl-labeled ATRP initiator,[26] 2-(1-carboxy-1-methylethylsulfanylthiocarboylsulfanyl)-2-methylpropionic acid (CMP),[27] hexamethylated tris(2-(dimethylamino)ethyl)amine (Me_6TREN),[28] (trimethylsilyl)propargyl acrylamide,[29] and 5 μm azido-modified silica particles[30] were synthesized according to previously described procedures.



Scheme 1 Schematic representation of the preparation of covalently stabilized dual responsive polymer capsules through LbL assembly using click chemistry.

Procedure for layer-by-layer multilayer coating of azide modified silica particles

An aqueous solution (8 mL) containing the azido-modified silica particles (20 mg) and the alkyne-functionalized PNIPAm copolymers (5 mg) was combined with copper(II) sulfate (4 mg). The suspension was sonicated for 5 min, sodium ascorbate solution (5 mg mL^{-1}, 2 mL) was added, and then the mixture was incubated for 8 h. After the click reaction was complete, the dispersion of particles was centrifuged and the supernatant was removed and replaced with DI water. This rinsing process, aided by ultrasonication, was repeated three times to ensure removal of the excess copolymer; at this point, the resulting particles were redispersed by combining with an azido-functionalized PNIPAm copolymer solution, and the dispersion was allowed to proceed the second layer assembly by adding copper(II) sulfate and sodium ascorbate solution. The inner core pH-responsive PAAL bilayer was assembled at pH 3 to ensure that the carboxylic acid groups were in the protonated state. After the desired number of bilayers had been formed, the capsules were obtained by immersing the coated silica particles in 2 M HF for 5 min at ambient temperature to etch out the silica cores completely. The resulting capsules were purified through extensive dialysis against DI water.

Synthesis of silver nanoparticles within the PAAL bilayer

1.5 mL of a silver acetate solution (0.05 g mL^{-1}) was added to the three bilayers PNIPAm/PAAL/PNIPAm coated silica particle aqueous suspension. The dispersion was vigorously agitated on a shaking apparatus for 12 h to allow the silver ions to adsorb on to the carboxylic acid groups of the PAAL. The dispersion was centrifuged at 6000 *g* for 3 min, the supernatant was removed, and 1.5 mL of water was added. This rinsing step was repeated three times. The Ag(I)-containing silica particles were reduced in 1 mM $NaBH_4$ solution for 30 min and a rinsing step was performed.

Characterizations

Analytical TLC was performed on commercial Merck Plates coated with silica gel GF254 (0.24 mm thick). Silica gel for flash chromatography was Merck Kieselgel 60 (230–400 mesh, ASTM). ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) measurements were carried out on a Varian Unity Inova spectrometer using $CDCl_3$ or D_2O as solvents. The gel permeation chromatography (GPC) instrument comprised a HITACHI L-7100 pump and a RI 2000 refractive index detector (Schambeck SFD GmbH). For PNIPAm random copolymers, the applied columns were a Polymer Laboratories PLgel guard column (5 μm particles, 50 × 7.5 mm), followed by two PLgel 5 μm Mixed-D columns (300 × 7.5 mm, particle size 5 μm) in series, and the DMF elution rate was 1.0 mL min^{-1} at 80 °C. For PAAL random

copolymers, the applied columns were a Polymer Laboratories PLgel guard column (5 μm particles, 50 × 7.5 mm), followed by a Shodex OHpak SB-803 HQ column (300 × 8.0 mm) in series using an aqueous eluent of 20% acetonitrile/80% 0.1 M Na_2SO_4 at 25 °C. The molecular weight calibration curve was obtained using poly(ethylene oxide) standards of defined molecular weights (1010–163,000 g mol^{-1}) (Polymer Laboratories Inc., MA). Transmission electron microscopy (TEM) images were obtained by using a JEOL TEM-1200EX II electron microscope instrument operated at 120 KV. The height measurements for microcapsules were determined by tapping-mode atomic force microscopy (AFM) under ambient conditions in air. The AFM instrumentation consisted of a Digital Instrument Dimension 5000 scanning probe microscope (Veeco-Digital Instruments, Santa Barbara, CA) using silicon cantilevers (Pointprobe® Silicon AFM Probe) at room temperature, in air. The swelling samples of PNIPAm microcapsules were prepared by drying a droplet of capsule suspension on the surface of a clean silicon wafer or copper grid and allowing it to dry freely in air. In order to maintain its shrunk morphology, the de-swelling samples were prepared by heating the capsules suspension solution up to 50 °C for 20 min and allowing a droplet of heated capsule suspension to be dried quickly in this oven at 50 °C on a silicon wafer or copper grid. Confocal laser scanning microscopy (CLSM) images were taken with a Leica TCS SP5 confocal microscope imaging system equipped with a diode laser (excitation wavelength = 403 nm). The thermal analyses of copolymer solutions were performed on a Q-20 (TA instruments, Delaware, USA) calorimeter. Heating scans were recorded in the range of 0–50 °C at a scan rate of 2 °C min^{-1}.

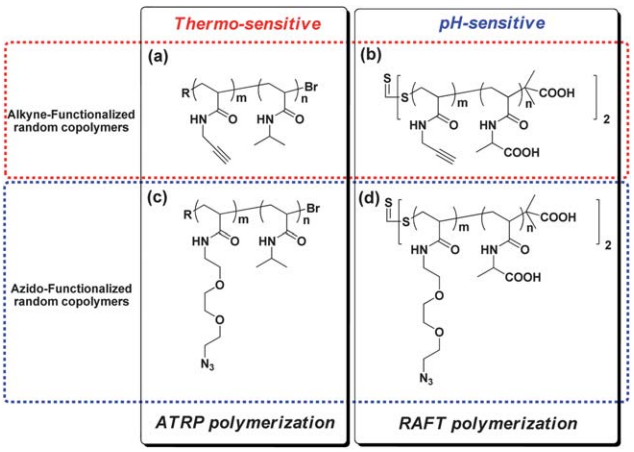


Fig. 1 Chemical structures of the thermo and pH sensitive (a, b) alkyne-functionalized and (c, d) azido-functionalized random copolymers prepared by ATRP and RAFT polymerization.

Results and discussion

In order to obtain capsules with desired properties resulting in their "smart" response, one of the prerequisites is to design their built-up polymer materials. To implement this idea, we prepared alkyne- and azido-functionalized stimuli-responsive random copolymers *via* controlled radical polymerization, as shown in Fig. 1.

We first synthesized a water-soluble azide-containing monomer, *N*-(2-(2-(2-azidoethoxy)ethoxy)ethyl)acrylamide, containing an ethylene oxide repeat unit to increase the hydrophilicity (see ESI†). These thermo-sensitive poly(*N*-isopropylacrylamide) (PNIPAm) and pH-sensitive poly(*N*-acryloylalanine) (PAAL) clickable random copolymers were successfully prepared *via* atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) polymerization. Controlled radical copolymerization *via* ATRP and RAFT was chosen because of its monomer compatibility, good control of molecular weight distribution, and chain composition.[31] This allowed us to design and synthesize the molar ratio of either the azide or the alkyne while retaining its stimuli-responsive behavior. These copolymer materials were designed to contain 20 mol % of either azide or the alkyne functional monomer. By nuclear magnetic resonance (NMR) spectroscopy, we found that the compositions of azide and alkyne moieties were close to their monomer contents of 20 mol %. Table 1 provides a detailed summary of the results and the polymer characterization. These novel clickable functionalized PNIPAm and PAAL random copolymers provided the capsules with covalent linkages and also retained their responsive characteristics, thereby allowing them to exhibit swelling/deswelling behaviors.

In this study, consecutive layer-by-layer assembly was performed with a sequence of PNIPAm/PAAL/PNIPAm to fabricate vesicle-like click capsules with thermo-sensitive outer shell bilayers and a pH-responsive inner core bilayer (Scheme 1). The first layer of alkyne-functionalized PNIPAm was assembled through 1,3-dipolar cycloaddition on the azido-modified silica particles close to the lower critical solution temperature (LCST) of PNIPAm-*r*-PPAm at 32 °C. In our previous study, we have demonstrated a promising method for low surface roughness and thick multilayer films of PNIPAm single component capsules.[14] Therefore, the PNIPAm LbL process was facilitated by adjusting the solution temperature closely to LCST as a result of coil-to-globule transition characteristic of PNIPAm leading to the assembly of a tighter packed thin film structure. The remaining

Table 1 Summary of alkyne- and azido-functionalized random copolymers characterization

Click-functionalized copolymer samples	$M_{n,\ NMR}$ (kg mol^{-1})	F_{azide}[a]	F_{alkyne}[b]	Maximum of endothermic peak (°C)
PNIPAm79-r-PPAm15	10,500	—	16	32.7
PNIPAm86-r-PEOAm21	14,500	20	—	33.9
PAAL45-r-PPAAm9	7,700	—	17	—
PAAL50-r-PEOAm11	9,700	18	—	—

[a] F_{azide} = mole percentage of azide functionality. [b] $F_{acetylene}$ = mole fraction of alkyne functionality measured by ^{1}H NMR in D_2O at 20 °C.

clickable groups in the multilayers on the surface of the particles are available for covalent attachment of subsequent layers because of the steric hindrance of adsorbed layers. Accordingly, the second layer of PNIPAm-*r*-PEOAm was assembled at 33 °C by using the remaining free alkyne groups to establish the first PNIPAm bilayer. The pH-responsive PAAL was introduced at pH 3 to ensure that the carboxylic acid groups were in the protonated state. As a result, the deposition of PAAL on PNIPAm surface was facilitated by the hydrogen bonding between the amide hydrogen of the PNIPAm and the acid carbonyl of the PAAL and the acid hydrogen of the PAAL and amide carbonyl of the PNIPAm.[32] Finally, the outer PNIPAm bilayer was assembled to build up the three bilayers core-shell particles.

After removing the silica template by hydrogen fluoride etching and incubation at pH 7, the vesicle-capsules were obtained. A uniform coverage with fluorescently labeled polymer, a regular spherical shape, and a uniform size of the capsules were observed as shown in Fig. 2. Electrostatic interactions[33] and hydrogen bonding[12b] induced by changing the pH are known to be a driving force for the swelling and shrinkage of capsules. The pH-sensitivity of the click capsules was first examined by immersion of the capsules into low and high pH solutions, where the carboxylic acid side chains of PAAL copolymers protonate or deprotonate. As shown in Fig. 2a, the capsule diameter at pH 3 of approximately 5.5 μm (Fig. 2a, inset) is larger than the 5.0 μm particle template.

The covalently bonded linkage between the multilayer and the silica sphere surface was removed after removing the silica template. Below LCST, the PNIPAm outer bilayers become hydrophilic and are able to swell as a result of globule-to-coil thermo reversible transition. In contrast to the swelling of PNIPAm outer shells, PAAL intramolecular association of the protonated carboxylic acid units and hydrogen bonding complex of PAAL with PNIPAm under acidic conditions at pH 3 leads to shrinkage of the inner core bilayer of the click capsules. Therefore, we can ascribe the slight swelling rather than shrinkage of the click capsules at pH 3 to the equilibrium between the swelling of PNIPAm outer shells after core removing and the shrinkage of PAAL inner core. In comparison with acidic conditions, the carboxylic acid groups are deprotonated under basic conditions leading to electrostatic repulsion (negative charge) in the PAAL bilayer. As shown in Fig. 2b, the click capsules swelled to 6.5 μm at pH 11 after treating with sodium hydroxide solution. Similar swellability was also observed for the single-component poly (acrylic acid) (PAA) click capsules[12b] and two-component pH-responsive capsules which reversibly respond to changes in environmental pH by variation in diameter and permeability as a function of pH.[34] In comparison with the highly swelling/deswelling behaviors of the PAA click capsules, we may attribute the reduced swellability in this study to the higher degree of cross-linking density of the PAAL bilayer and the outer encompassing PNIPAm shells. These results demonstrated that the PAAL bilayer was successfully incorporated within the click multilayer and exhibited pH response.

The morphologies of the air-dried capsules in acidic, basic, and thermo conditions were also examined by using TEM and AFM. AFM analyses of the air-dried click capsules shown in Fig. 3a–c reveal spherical structures with features of folds and creases that are typical of polymer capsules prepared by LbL assembly on particle templates.[35] TEM analyses shown in Fig. 3d–f also reveal folds and creases in the click capsules, and the diameters are similar to those obtained by AFM. These dried click capsules display smooth surfaces as a result of the outer tighter packing PNIPAm shells as we discussed earlier. AFM and TEM measurements provided stimuli-responsive behaviors as is evident from variation of the size and thickness of these dried click capsules. The dimension of the dried click capsules was increased considerably upon changing the pH value from 3 to 11. By increasing the temperature to 50 °C, the outer PNIPAm shells were also shrunk and the dimension of the click capsules at pH 3 was further decreased. Fig. 4 shows the AFM section analyses of stimuli-responsive samples indicating that the increase in wall thickness correspond with the degree of the shrinkage of these click capsules. The higher degree of shrinkage, the thicker the multilayers observed.

In addition to fully deswelled state of the click capsules at pH 3 and temperature above LCST, we also investigated the thermo-responsive behavior at pH 11 where the PAAL inner core bilayer was in swelling state. After elevating the solution temperature of the swelled PAAL core click capsules to 50 °C, only a slight increase in wall thickness was observed(Fig. 4d) compared with fully deswelling capsules at pH 3 and 50 °C (Fig. 4c) this is probably due to the tendency of the hydrophilic deprotonated PAAL inner core bilayer to raise the LCST of the PNIPAm outer

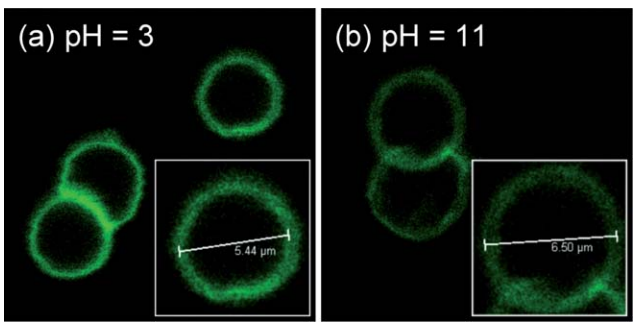


Fig. 2 Confocal laser scanning microscopy (CLSM) images of vesicle-like PNIPAm/PLAA/PNIPAm click capsules obtained from 5 μm diameter silica particles at (a) pH 3 and (b) pH 11.

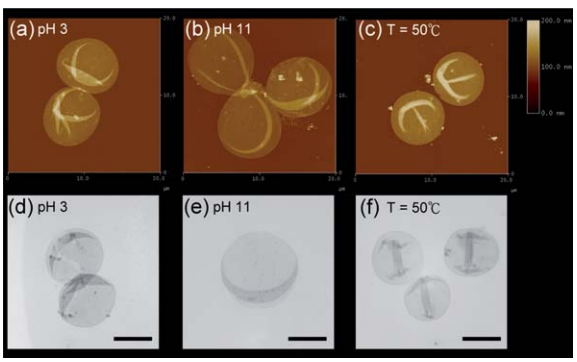


Fig. 3 (a–c) AFM and (d–f) TEM images of the three-bilayer PNIPAm/PAAL/PNIPAm click capsules. Samples prepared at 25 °C (a, b, d, e) and at 50 °C (pH 3) (c, f). The AFM images for scanned areas of 20 × 20 μm^2. The scale bar in TEM images: 2 μm.

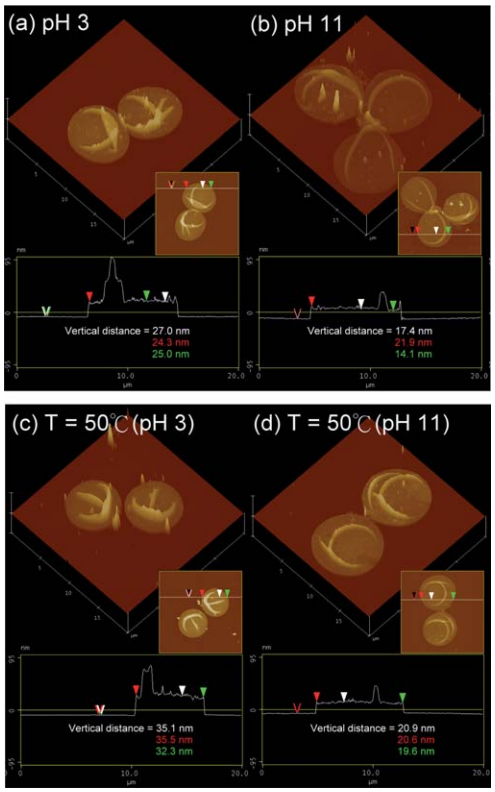


Fig. 4 (a–d) AFM images and section analyses of the three-bilayer PNIPAm/PAAL/PNIPAm click capsules at (a) pH 3, (b) pH 11, (c) 50 °C (at pH 3), and (d) 50 °C (at pH 11).

shells. It's well-known that the LCST of PNIPAm copolymer is strongly influenced by the overall hydrophilicity of the copolymer.[36] In general, hydrophobic compounds lower the LCST, whereas hydrophilic compounds raise it.[37] Consequently, we suggest that the LCST of PNIPAm outer shells in this covalently bonded multilayer is substantially affected by the hydrophilicity of the PAAL inner core bilayer.

In this study, we chose an SiO_2 template with a diameter of up to 5 μm because the pH- and thermo-response swelling/deswelling behaviors could be clearly distinguished and characterized by CLSM and AFM under micro-scale dimension. However, we found that the ultra-thin multilayer shells of microcapsules with thickness of less than 20 nm were easily deformed after removing the silica template etching with dilute HF. On the other hand, the ultra-thin multilayer shells of the microcapsules emitted weak fluorescence because only one fluorescent dansyl group was attached at the chain end of each responsive copolymer. Therefore, the fluorescence was quenched rapidly as a result of the high power of the excitation laser at 403 nm of CLSM. As a result, we failed to observe, *in situ*, the reversible stimuli-responsive behaviors in aqueous solution and to gather statistical data on pH and temperature induced swelling/deswelling behaviors by CLSM.

In order to further confirm the three bilayer structure multilayer of these dual pH- and thermo-responsive capsules, we also demonstrated the *in situ* preparation of inorganic silver nanoparticles within the PAAL bilayer. Details of the *in situ* nanoparticles synthesis methodology can be found in ref. 38. Silver

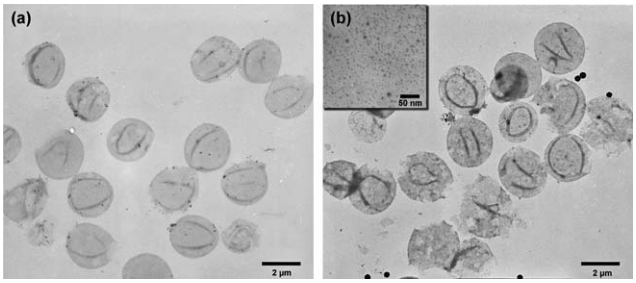


Fig. 5 TEM images of 1.5 μm three-bilayer PNIPAm/PAAL/PNIPAm click capsules at (a) pH 3, (b) containing silver nanoparticles.

acetate was previously found to be a good source for silver binding to PAAL carboxylic acid groups in polyelectrolyte multilayers.[38] The PNIPAm/PAAL/PNIPAm coated 1.5 μm silica particles were immersed in $Ag(ac)_{aq}$ where the acid protons of PAAL bilayer were exchanged for silver cations. The Ag(I)-containing particles were reduced in $NaBH_4$ solution forming Ag (0) nanoparticles and regenerating the carboxylic acid protons. The TEM image in Fig. 5 shows that abundance of stable silver nanoparticles produced by $NaBH_4$ reduction are attached within the multilayer and dispersed throughout the thin film. The inset shown in Fig. 5b also reveals that the surrounding PAAL polymer limits particle aggregation and thus yields a small particle size. Consequently, the PAAL interior layer of the click capsules can be used as reaction sites providing a confined space in which organic or inorganic materials with unique properties can be produced.

Conclusions

We have developed a new and convenient method based on click chemistry for the direct covalent layer-by-layer assembly of vesicle-like capsules with dual stimuli-responsive characteristics. Consecutive layer-by-layer of these synthesized responsive clickable random copolymers was performed with a sequence of PNIPAm/PAAL/PNIPAm to fabricate vesicle-like click capsules consisting of thermo-sensitive outer shell bilayers and a pH-responsive inner core bilayer. The vesicle-like click capsules exhibited thermo and pH-responsive behaviors by elevating the solution temperature and incubating in acidic or basic solutions respectively. This combination of two widely employed techniques, layer-by-layer and copper-catalyzed 1,3-dipolar cycloaddition, is promising for introducing a broad range of new materials including different functional polymers with covalent stabilization. Furthermore, excess azide/alkyne groups that have not been utilized in the assembly can be used to postfunctionalize the outer multilayer films of the click capsules. We also report the *in situ* preparation of silver nanoparticles within the multilayer of the click capsules. The versatility and generality of this approach is expected to enable us to further design advanced and stimuli-responsive capsules.

Acknowledgements

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Notes and references

1 (*a*) X. Y. Shi, M. W. Shen and H. Mohwald, *Prog. Polym. Sci.*, 2004, **29**, 987; (*b*) G. Decher, J. D. Hong and J. Schmitt, *Thin Solid Films*, 1992, **210–211**, 831.

2 (*a*) Y. J. Zhang, Y. Guan, S. G. Yang, J. Xu and C. C. Han, *Adv. Mater.*, 2003, **15**, 832; (*b*) V. Kozlovskaya, S. Ok, A. Sousa, M. Libera and S. A. Sukhishvili, *Macromolecules*, 2003, **36**, 8590; (*c*) S. Y. Yang, D. Lee, R. E. Cohen and M. F. Rubner, *Langmuir*, 2004, **20**, 5978.

3 Z. Wang, Z. Feng and C. Gao, *Chem. Mater.*, 2008, **20**, 4194.

4 (*a*) A. P. R. Johnston, E. S. Read and F. Caruso, *Nano Lett.*, 2005, **5**, 953; (*b*) A. P. R. Johnston, H. Mitomo, E. S. Read and F. Caruso, *Langmuir*, 2006, **22**, 3251.

5 (*a*) C. S. Peyratout and L. Dahne, *Angew. Chem., Int. Ed.*, 2004, **43**, 3762–3783; (*b*) A. P. R. Johnston, C. Cortez, A. S. Angelatos and F. Caruso, *Curr. Opin. Colloid Interface Sci.*, 2006, **11**, 203; (*c*) B. G. De Geest, S. De Koker, G. B. Sukhorukov, O. Kreft, W. J. Parak, A. G. Skirtach, J. Demeester, S. C. De Smedt and W. E. Hennink, *Soft Matter*, 2009, **5**, 282.

6 J. F. Quinn, A. P. R. Johnston, G. K. Such, A. N. Zelikin and F. Caruso, *Chem. Soc. Rev.*, 2007, **36**, 707.

7 S. Moya, L. Dähne, A. Voigt, S. Leporatti, E. Donath and H. Möhwald, *Colloids Surf., A*, 2001, **183–185**, 27.

8 (*a*) C. Nardin, T. Hirt, J. Leukel and W. Meier, *Langmuir*, 2000, **16**, 1035; (*b*) I. Pastoriza-Santos, B. Schöler and F. Caruso, *Adv. Funct. Mater.*, 2001, **11**, 122; (*c*) H. G. Zhu and M. J. McShane, *Langmuir*, 2005, **21**, 424.

9 (*a*) W. J. Tong, C. Y. Gao and H. Möhwald, *Chem. Mater.*, 2005, **17**, 4610; (*b*) Y. J. Zhang, Y. Guan and S. Zhou, *Biomacromolecules*, 2005, **6**, 2365.

10 (*a*) Z. Feng, Z. Wang, C. Gao and J. Shen, *Adv. Mater.*, 2007, **19**, 3687; (*b*) W. Tong, C. Gao and H. Möhwald, *Macromol. Rapid Commun.*, 2006, **27**, 2078; (*c*) L. Duan, Q. He, X. H. Yan, Y. Cui, K. W. Wang and J. B. Li, *Biochem. Biophys. Res. Commun.*, 2007, **354**, 357.

11 (*a*) P. Schuetz and F. Caruso, *Adv. Funct. Mater.*, 2003, **13**, 929; (*b*) V. Kozlovskaya, S. Ok, A. Sousa, M. Libera and S. A. Sukhishvili, *Macromolecules*, 2003, **36**, 8590; (*c*) D. Lee, M. F. Rubner and R. E. Cohen, *Chem. Mater.*, 2005, **17**, 1099; (*d*) S. Y. Yang, D. Lee and R. E. Cohen, *Langmuir*, 2004, **20**, 5978.

12 (*a*) G. K. Such, J. F. Quinn, A. Quinn, E. Tjipto and F. Caruso, *J. Am. Chem. Soc.*, 2006, **128**, 9318; (*b*) G. K. Such, E. Tjipto, A. Postma, A. P. R. Johnston and F. Caruso, *Nano Lett.*, 2007, **7**, 1706; (*c*) C. J. Ochs, G. K. Such, B. Staedler and F. Caruso, *Biomacromolecules*, 2008, **9**, 3389; (*d*) L. A. Connal, C. R. Kinnane, A. N. Zelikin and F. Caruso, *Chem. Mater.*, 2009, **21**, 576; (*e*) C. R. Kinnane, G. K. Such, G. Antequera-Garcia, Y. Yan, S. J. Dodds, L. M. Liz-Marzan and F. Caruso, *Biomacromolecules*, 2009, **10**, 2839; (*f*) C. R. Kinnane, K. Wark, G. K. Such, A. P. R. Johnston and F. Caruso, *Small*, 2009, **5**, 444.

13 (*a*) H. C. Kolb, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2001, **40**, 2004; (*b*) H. C. Kolb and K. B. Sharpless, *Drug Discovery Today*, 2003, **8**, 1128; (*c*) W. G. Lewis, L. G. Green, F. Grynszpan, Z. Radic, P. R. Carlier, P. Taylor, M. G. Finn and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 1053.

14 C. J. Huang and F. C. Chang, *Macromolecules*, 2009, **42**, 5155.

15 D. E. Discher and A. Eisenberg, *Science*, 2002, **297**, 967.

16 (*a*) B. M. Discher, Y. Y. Won, D. S. Ege, J. C. Lee, F. S. Bates, D. E. Discher and D. A. Hammer, *Science*, 1999, **284**, 1143.

17 (*a*) L. Zhang, K. Yu and A. Einsenberg, *Science*, 1996, **272**, 1777; (*b*) H. Lomas, I. Canton, S. MacNeil, J. Du, S. P. Armes, A. J. Ryan, A. L. Lewis and G. Battaglia, *Adv. Mater.*, 2007, **19**, 4238; (*c*) J. Du, Y. Tang, A. L. Lewis and A. P. Armes, *J. Am. Chem. Soc.*, 2005, **127**, 17982; (*d*) J. Du and S. P. Armes, *J. Am. Chem. Soc.*, 2005, **127**, 12800.

18 (*a*) X. Liu and M. Jiang, *Angew. Chem., Int. Ed.*, 2006, **45**, 3846; (*b*) T. Ikeda, J. I. Mamiya and Y. Yu, *Angew. Chem., Int. Ed.*, 2007, **46**, 506; (*c*) G. Wang, X. Tong and Y. Zhao, *Macromolecules*, 2004, **37**, 8911; (*d*) C. Konak, R. C. Rathi, P. Kopeckova and J. Kopecek, *Macromolecules*, 1997, **30**, 5553.

19 (*a*) Y. Li, B. Lotiz and C. L. McCormick, *Angew. Chem., Int. Ed.*, 2006, **45**, 5792; (*b*) Y. Li, A. E. Smith, B. S. Lokitz and C. L. McCormick, *Macromolecules*, 2007, **40**, 8524.

20 (*a*) B. M. Discher, H. Bermudez, D. A. Hammer, D. E. Discher, Y. Y. Won and F. S. Bates, *J. Phys. Chem. B*, 2002, **106**, 2848; (*b*) A. Kros, J. G. Linhardt, H. K. Bowman and D. A. Tirrell, *Adv. Mater.*, 2004, **16**, 723.

21 (*a*) R. J. Thibault, O. Uzun, R. Hong and V. M. Rotello, *Adv. Mater.*, 2006, **18**, 2179; (*b*) J. Ding and G. Liu, *J. Phys. Chem. B*, 1998, **102**, 6107; (*c*) R. Zheng and G. Liu, *Macromolecules*, 2007, **40**, 5116; (*d*) A. Walther, A. S. Goldmann, R. S. Yelamanchili, M. Drechsler, H. Schmalz, A. Eisenberg and A. H. E. Müller, *Macromolecules*, 2008, **41**, 3254.

22 (*a*) J. Du and S. P. Armes, *J. Am. Chem. Soc.*, 2005, **127**, 12800; (*b*) J. Du, Y. Chen, Y. Zhang, C. C. Han, K. Fischer and M. Schmidt, *J. Am. Chem. Soc.*, 2003, **125**, 14710.

23 H. Zhu, Q. Liu and Y. Chen, *Langmuir*, 2007, **23**, 790.

24 (*a*) A. Henglein, *Chem. Rev.*, 1989, **89**, 1861; (*b*) L. Brus, *Appl. Phys. A: Solids Surf.*, 1991, **53**, 465; (*c*) A. P. Alivisatos, *Science*, 1996, **271**, 933.

25 L. S. Brad, A. J. Convertine, R. G. Ezell, A. Heidenreich, Y. Li and C. L. McCormick, *Macromolecules*, 2006, **39**, 2113.

26 C. J. Huang and F. C. Chang, *Macromolecules*, 2008, **41**, 7041.

27 J. T. Lai, D. Filla and R. Shea, *Macromolecules*, 2002, **35**, 6754.

28 M. Ciampolini and N. Nardi, *Inorg. Chem.*, 1966, **5**, 41.

29 M. Malkoch, R. J. Thibault, E. Drockenmuller, M. Messerschmidt, B. Voit, T. P. Russell and C. J. Hawker, *J. Am. Chem. Soc.*, 2005, **127**, 14942.

30 R. Ranjan and W. J. Brittain, *Macromolecules*, 2007, **40**, 6217.

31 (*a*) N. V. Tsarevsky and K. Matyjaszewski, *Chem. Rev.*, 2007, **107**, 2270; (*b*) W. A. Braunecker and K. Matyjaszewski, *Prog. Polym. Sci.*, 2007, **32**, 93.

32 J. F. Quinn and F. Caruso, *Langmuir*, 2004, **20**, 20.

33 V. Kozlovskaya, E. Kharlampieva, M. L. Mansfield and S. A. Sukhishvili, *Chem. Mater.*, 2006, **18**, 328.

34 (*a*) V. Kozlovskaya and S. A. Sukhishvili, *Macromolecules*, 2006, **39**, 5569; (*b*) V. Kozlovskaya, A. Shamaev and S. A. Sukhishvili, *Soft Matter*, 2008, **4**, 1499.

35 E. Donath, G. B. Sukhorukov, F. Caruso, S. A. Davis and H. Mohwald, *Angew. Chem., Int. Ed.*, 1998, **37**, 2201.

36 H. Feil, Y. H. Bae, J. Feijen and S. W. Kim, *Macromolecules*, 1993, **26**, 2496.

37 (*a*) G. Chen and A. S. Hoffman, *Macromol. Chem. Phys.*, 1995, **196**, 1251; (*b*) D. Kuckling, H. J. Adler, K. F. Arndt, L. Ling and W. D. Habicher, *Macromol. Symp.*, 1999, **145**, 65; (*c*) S. Kunugi, Y. Yamazaki, K. Takano and N. Tanaka, *Langmuir*, 1999, **15**, 4056; (*d*) Y. Deng and R. Pelton, *Macromolecules*, 1995, **28**, 4617.

38 S. Joly, R. Kane, L. Radzilowski, T. Wang, A. Wu, R. E. Cohen, E. L. Thomas and M. F. Rubner, *Langmuir*, 2000, **16**, 1354.