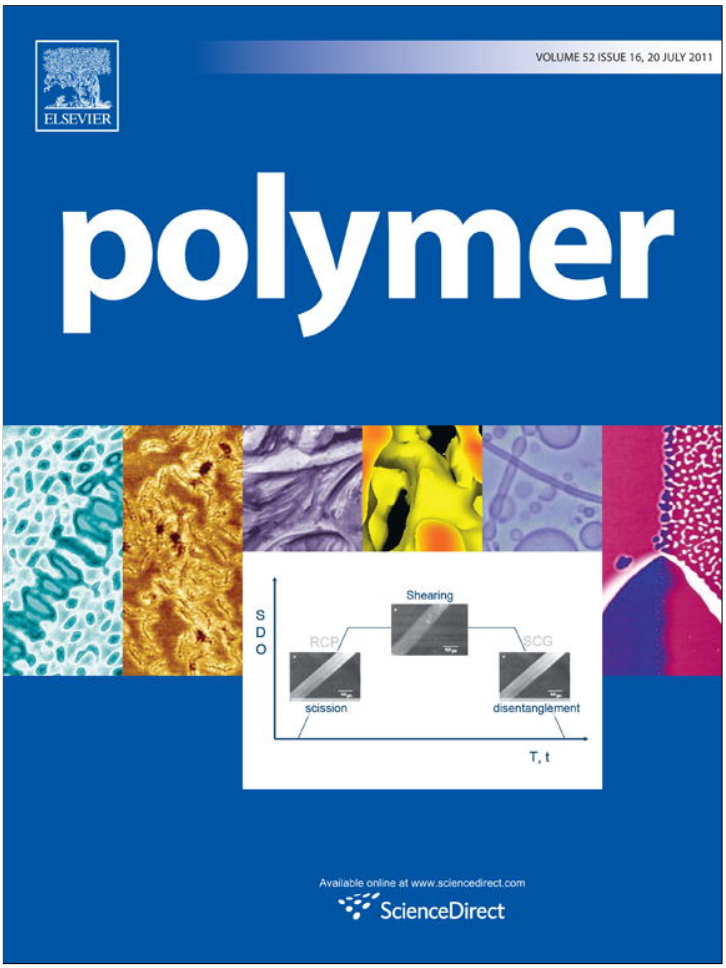

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Does PNIPAM block really *retard* the micelle-to-vesicle transition of its copolymer?

Kongchang Wei, Lu Su, Guosong Chen**, Ming Jiang*

The Key Laboratory of Molecular Engineering of Polymers, Ministry of Education and Department of Macromolecular Science, Fudan University, Shanghai 200433, PR China

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ABSTRACT

The well-known coil-to-globule transition of poly(*N*-isopropyl acrylamide) (PNIPAM) at its LCST lasts as short as hundred of seconds with fully reversibility. However, for the PNIPAM-containing block copolymers, thermal transformation from micelles to vesicles caused by the conformation transition of PNIPAM took as long as several weeks, even at the temperatures much higher than the LCST, and without satisfactory reversibility. In the literature, this slow process has been attributed to the strong interchain hydrogen bonding in PNIPAM, which retards the transition. In this work, asymmetrically modified PNIPAM (Mw 10K), *i.e.* C_{12}-PNIPAM-CA with a hydrophobic hydrocarbon chain $-C_{12}H_{25}$ (C_{12}) at one end and a hydrophilic carboxyl group –COOH (*CA*) at the other, was prepared and found to form micelles with a core of the lightly associated hydrocarbon chains. When temperature is increased to the LCST of PNIPAM, the transformation from micelles to vesicles can be realized within 30 min, while the reverse process only takes a few minutes. Based on full monitoring of the transition process, it is proposed that the micelles serve as building blocks in constructing the vesicles via processes of combination, fusion, and *etc*., in which only local conformation adjustment of PNIPAM is involved. Therefore, reducing the restriction to the conformation change of PNIPAM chains, which is imposed by the micellar core, is one of the key factors in realizing the fast transition.

1. Introduction

Vesicles deserve great research efforts as it provides plentiful information for deepening our understanding of the nature as well as a broad range of potential applications. In the researches for vesicles made of amphiphilic block copolymers, so called polymersomes [1–3], the morphology transition of the vesicles with stimuli-responsiveness has been of particular interest [4–8]. Recently, several groups addressed their attempts to thermally induced morphology transition from micelles to vesicles [9,10]. However, the whole transformation process is not rapid and well-controlled as desired and one lacks of deep understanding about the transition mechanism so far. In these researches, amphiphilic block copolymers with thermo-responsive poly(*N*-isopropyl acrylamide) (PNIPAM) as the central block, which connects a hydrophobic side block and a hydrophilic side block or functional group have been intensively studied. Here the well-known thermally induced coil–globule transition of PNIPAM around the lower critical solution temperature (LCST, 32 °C) has been utilized to adjust the amphiphilic balance of the block copolymers and the packing parameter *p*, leading to the morphology transition. Grubbs and his coworkers [9] reported first that triblock copolymer PEO-*b*-PNIPAM-*b*-PI (poly(ethylene oxide)-*b*-PNIPAM-*b*-poly(isoprene)) in water formed small spherical micelles at low temperatures, which turned to large vesicles after heating at 65 °C, for as long as 3 weeks. After cooling to 20 °C for about 48 h, the vesicles returned to assemblies with much smaller sizes. Then O'Reilly et al. [10] demonstrated that diblock copolymer P*t*BA-*b*-PNIPAM (poly(*tert*-butylacrylate)-*b*-PNIPAM), with a quaternary amine end to the PNIPAM block, realized a micelle-to-vesicle transition at 65 °C, which was faster but one week was needed. Both of the results were successful in realizing the transition, but still suffered from the long duration for the transition and unsatisfactory reversibility. Further efforts from the two groups targeted the problem from different directions. Replacing the hydrophobic P*t*BA block by PMA (poly(methyl acrylate)) with a lower glass transition temperature (T_g), O'Reilly's group achieved even faster morphology transition in about one day [11]. Very recently, Grubbs and his coworkers claimed that it was the strong interchain hydrogen bonding between the amide groups of PNIPAM during the dehydration

* Corresponding author. Tel./fax: +86 21 65640293.
** Corresponding author.
E-mail addresses: guosong@fudan.edu.cn (G. Chen), mjiang@fudan.edu.cn (M. Jiang).

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slowed down further rearrangement, resulting in a week-long transition [12]. So they used a random copolymer of ethylene oxide and butyl oxide as the central block with a much lower T_g to replace the PNIPAM block ($T_g = 145$ °C). It resulted in a fast 'initial morphology change' in 2 h at 70 °C but the subsequent evolution of the vesicle size lasted a few weeks. PNIPAM is a well-known temperature-sensitive polymer showing a sharp conformation change from coil to globule at its LCST around 32 °C, which takes place in hundreds of seconds only [13]. However, the reported thermally induced micelle–vesicle transition of the PNIPAM-containing block copolymers was performed in much longer time, days or even weeks, at temperatures much higher than the LCST. In fact, such a slow and incompletely reversible transition process has seriously hindered deep investigation on the transition mechanism and made the applications almost impractical. In this work, we tried to solve the problem from a point of view differing from the previously reported efforts, *i.e.* in our opinion, in order to realize the fast and reversible micelle–vesicle transition, it seems necessary to greatly reduce the restriction to the mobility of the PNIPAM chains imposed by the solid micellar core. Thus, an asymmetrically end-modified PNIPAM with a short hydrocarbon chain (C_{12}) at one end and carboxyl group (*CA*) at the other, was designed and employed (Scheme 1). As a result, a fast, in about 30 min, and fully reversible micelle–vesicle transition was realized for the first time, which enabled continuous monitor of the transition process by light scattering and, therefore induced a new mechanism for the transition. Furthermore, C_{12}-PNIPAM-BA with a phenylboronic acid (*BA*) replacing the carboxyl group displayed similar fast and reversible transition and the resultant vesicles coated by BA can be easily modified by oligosaccharides through boron–oxygen ether bond, showing promising functionalities and applications.

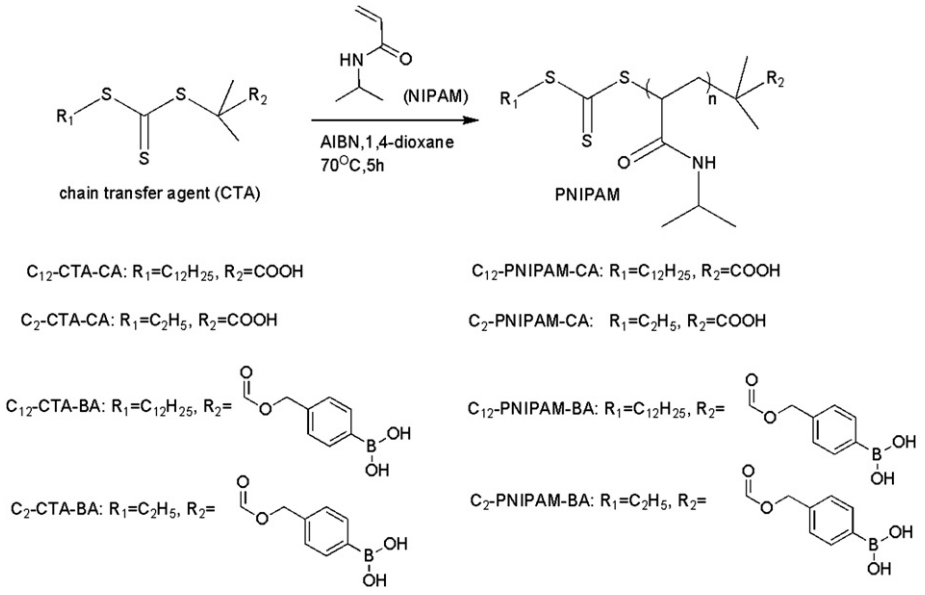
2. Results and discussion

2.1. Design and synthesis of asymmetrically modified PNIPAM

It has been known for a long time that modification of PNIPAM with a hydrocarbon chain (usually 12–18 carbons) would lead to a downward shift of LCST [14] and micelle-formation due to the hydrophobic association of the hydrocarbon chains in water. In the present work, a short hydrocarbon chain ($-C_{12}H_{25}$) was used to replace the solid core-forming blocks such as PMA [11], PI [12] and P*t*BA [10] mentioned above, in constructing our PNIPAM-based polymer. The hydrocarbon chains are expected to provide enough hydrophobic interaction for micelle-formation as well as the least interaction to affect the PNIPAM mobility responding to temperature change. A carboxyl group was selected as a simple hydrophilic end of the polymer. As shown in Scheme 1, a RAFT chain transfer agent C_{12}-CTA-CA was synthesized and used in polymerizing NIPAM monomer leading to our target polymer C_{12}-PNIPAM-CA. Meanwhile, C_2-PNIPAM-CA with a short hydrocarbon group ($-C_2H_5$) was prepared for studying the effect of hydrocarbon chain. Both polymers have the same molecular weight of 10 K. In addition, phenylboronic acid was introduced as Lewis acid to replace the carboxyl group. C_{12}-PNIPAM-BA and C_2-PNIPAM-BA were also synthesized by the same method. Details of the synthesis and characterization data are in supporting information (Figs. S1–S11).

2.2. Rapid and reversible transition from micelles to vesicles

Dynamic light scattering (DLS) and static light scattering (SLS) were employed to study the thermally induced aggregation of C_{12}-PNIPAM-CA in dilute aqueous solution (0.1 mg/mL). Temperature was controlled to increase stepwise and kept for 30 min at each given temperature before measurements. Hydrodynamic radius R_h, polydispersity index (PDI) and relative scattering intensity (I_s/I_0) of the solution as functions of temperature are shown in Fig. 1a. At low temperatures below 33.5 °C, the R_h was around 10 nm with a relatively large PDI (ca 0.25, autocorrelation function in Fig. S12). Considering that the molecular weight of the polymer is 10 K only, this obtained R_h value indicates the micellar structure due to the hydrophobic interaction of the $-C_{12}H_{25}$ chains. It is remarkable to find that when the solution was heated to and kept at 34 °C for 30 min, R_h of the assembly increased dramatically from 10 nm to around 80 nm, accompanied by an obvious increase of the scattering intensity and a decrease of PDI from 0.25 to 0.1 (also shown in Fig. S13). Meanwhile, radius of gyration R_g at 34 °C was determined to be 83 nm by SLS (Fig. S14), thus R_g/R_h was 1.04, which generally indicates vesicle morphology of the aggregates. Thus it is clear that in such a small temperature interval between 33.5 °C and



Scheme 1. Preparation of asymmetrically end-modified PNIPAM.

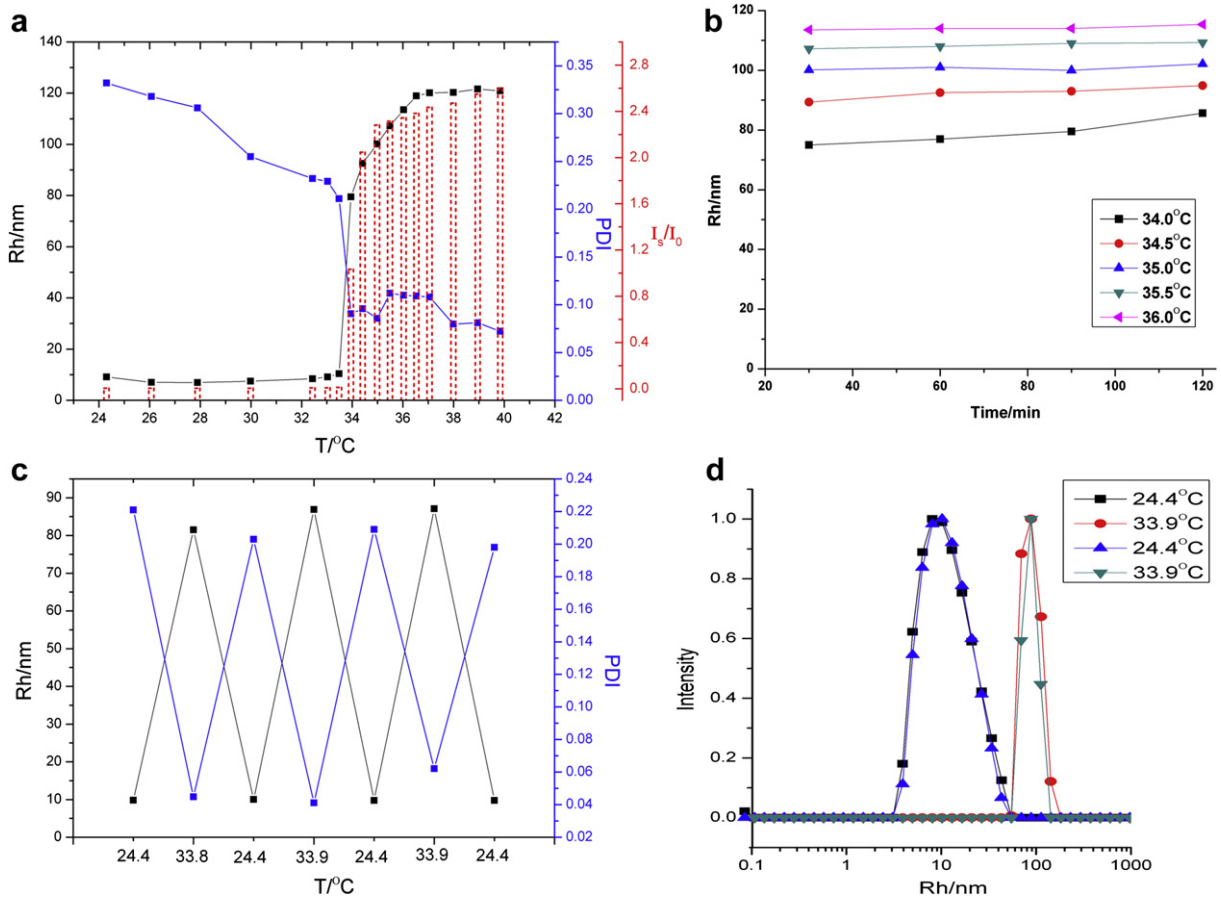


Fig. 1. Light scattering study of C_{12}-PNIPAM-CA (0.1 mg/mL) in water. (a) R_h, PDI and relative scattering light intensity recorded after 30 min heating at a given temperature, (b) R_h evolution at different temperatures within 2 h, (c) reversible changes in R_h and PDI with temperature cycles between 24.5 °C and 34 °C, (d) size distributions by weight recorded in one heating–cooling–heating cycle.

34 °C, a sharp morphology transition from polydisperse micelle-like structures to uniform vesicles takes place in about 30 min.

We also found that the R_h value of the vesicles was very sensitive to temperature and could keep constant at a given temperature. As shown in Fig. 1b, at 34 °C, the initial R_h was 80 nm and changed a little to 82 nm after 2 h. However, R_h was measured to be 93 nm, 30 min after the temperature reached 34.5 °C, and kept stable in the following 1.5 h (Fig. 1b). This precise control over the size of vesicles in solution was achieved by changing temperatures with only 0.5 °C interval until 37 °C. This result means that the vesicles obtained in this temperature program are very close to their equilibrium state. To the best of our knowledge, polymeric vesicles with such temperature sensitivity and delicate size-controllability have not been reported so far. It is worth noting that in our LS measurements, it took about 3 min to make temperature increase 0.5 °C and we found that the corresponding size increment reached almost simultaneously. So the size change of the vesicles with temperature is a very fast process (see Fig. S15).

Several successive morphology transition cycles were also monitored by DLS and SLS showing robust reversibility (Fig. 1c). The temperature program for each cycle was as follows: the temperature was increased from 24.5 °C within 15 min to and then kept at 34.0 °C for 30 min before the measurement. Then the temperature was decreased from 34.0 °C to 24.5 °C, which needed 50 min in our LS equipment, and the measurement was performed (temperature variation history in Figs. S16 and S17). For the first, second and third cycles, at 34 °C, R_h was 81.5 nm, 86.9 nm and 87.1 nm, respectively. And their corresponding R_g was 78.7 nm (R_g/R_h 0.96), 85.2 nm (R_g/R_h 0.98) and 93.8 nm (R_g/R_h 1.07), respectively (details are in Figs. S18–S23). These results show that the vesicular structure can easily be reproduced in heating–cooling cycles. Fig. 1c also shows that R_h measured at 24.5 °C gave the same value around 9.9 ± 0.1 nm in each cycle, which was also forcible stable. It is worth to mention that, the PDI is also reversible and reproducible, *i.e.* with low PDI less than 0.1 after heating and higher PDI around 0.2 after cooling. The corresponding size distributions shown in Fig. 1d demonstrate the complete reversibility of the transition. It is important to mention that when the vial of the vesicle solution at 34 °C was immersed into a water bath at 24 °C, the light opalescence immediately disappeared and the solution became transparent. It means that the transition from vesicles to micelles was very fast. In short, our DLS and SLS measurements demonstrated the great advantage of our designed C_{12}-PNIPAM-CA that, the transitions between micelles and vesicles are sharp, rapid and fully reversible. These merits enable us to monitor the whole process of the transition from micelles to vesicles by various techniques, which was almost impractical in the previously reported slow transition cases.

The morphology of the vesicles was observed by AFM. The sample was prepared from C_{12}-PNIPAM-CA solution in water, which was kept at 35 °C for 30 min. A drop of the solution was deposited onto a fresh mica surface and dried in air at the same temperature. As shown in Fig. 2, the radius of the vesicles is around 92 nm, which is in accordance with the DLS results, while the

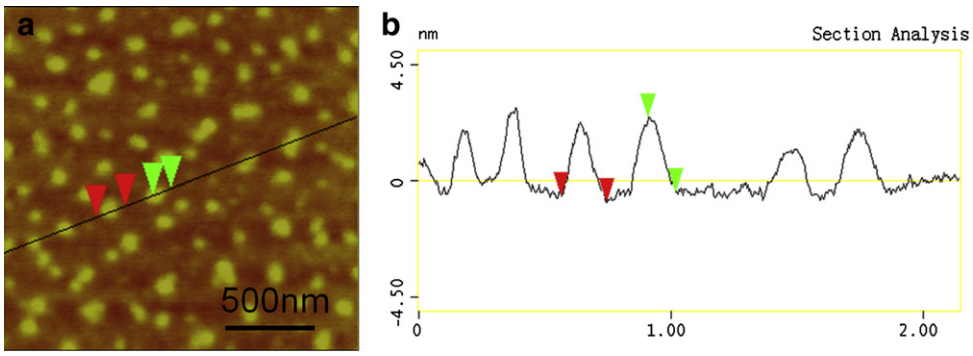


Fig. 2. (a) AFM image of vesicles formed while C_{12}-PNIPAM-CA in deionized water (0.1 mg/mL) was kept at 35 °C for 30 min before dripping onto mica; (b) section analysis of the image with horizontal distance of 184 nm (red arrows) and vertical distance of 3 nm (green arrows). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

height is only 3 nm indicating that the soft vesicles have collapsed. As reported before [15], in tapping mode AFM for soft materials, the measured height does not exactly correspond to the true height and is usually less, due to the deformation of the soft surface by the AFM tip. This phenomenon is more pronounced for vesicles made of PNIPAM, as observed by O'Reilly et al. [10,11]. It has been proved that the highly collapsed PNIPAM globule still contains about 80% water in its hydrodynamic volume [13] above LCST. Then the vesicles formed by C_{12}-PNIPAM-CA are reasonably very soft, due to the high water content of the PNIPAM wall.

2.3. Hydration of PNIPAM in vesicles by VT-^{1}H NMR

It was impressive that the size of the vesicles of C_{12}-PNIPAM-CA formed at different temperature from 34 °C to 37 °C can be precisely controlled. VT-^{1}H NMR studies were employed to explore the difference in molecular states of the PNIPAM chains among the vesicles formed at different temperatures. I_{NIPAM}%, the ratio of the peak intensity of the C***H***$(CH_3)_2$ protons (δ 3.7 ppm) of PNIPAM to the solvent (1 mg/mL in D_2O) with increasing temperature using the similar program as that used in LS studies, was recorded in Fig. 3. At low temperatures, I_{PNIPAM}% is around 95%, indicating full hydration of the PNIPAM units in the micelles. Then a sharp decrease of I_{PNIPAM}% was observed at 34 °C, where the micelles turned to vesicles as demonstrated by our LS studies. It is interesting to see that at 34 °C, although vesicles formed, 80% of NIPAM units remained hydrated. When temperature increased further, the degree of hydration decreased rapidly, which certainly altered the amphiphilic balance of the polymer and as a result, the vesicle diameter would increase. That was what observed by LS shown in Fig. 1b. In the temperature range from 38 °C to 42 °C, a small I_{NIPAM}% decrease was detected but the vesicle size no longer changed. That is probably because in this stage far above LCST, PNIPAM chains were almost fully de-hydrated and then lost their ability to adjust their conformation to make the vesicle expansion.

2.4. The role of alkyl chains

C_2-PNIPAM-CA was selected as the counterpart of C_{12}-PNIPAM-CA to explore the role of the alkyl chains in the vesiculation process. Fig. 4 compares the two traces of the transmittance variations of C_{12}-PNIPAM-CA and C_2-PNIPAM-CA solutions at the same concentration (0.2 mg/mL) as a function of temperature. The temperature variation program for the measurements was similar to that for light scattering studies shown in Fig. 1. As shown in Fig. 4, at low temperatures, both polymer solutions, C_{12}-PNIPAM-CA and C_2-PNIPAM-CA, showed almost 100% transmittance although the former existed as micelles with R_h of 10 nm and the latter as single chains with R_h of 2.7 nm (Figs. S24 and S25). It is clear that the transmittance of C_{12}-PNIPAM-CA began to decrease at a temperature of 2 °C lower than that of its counterpart, which can be

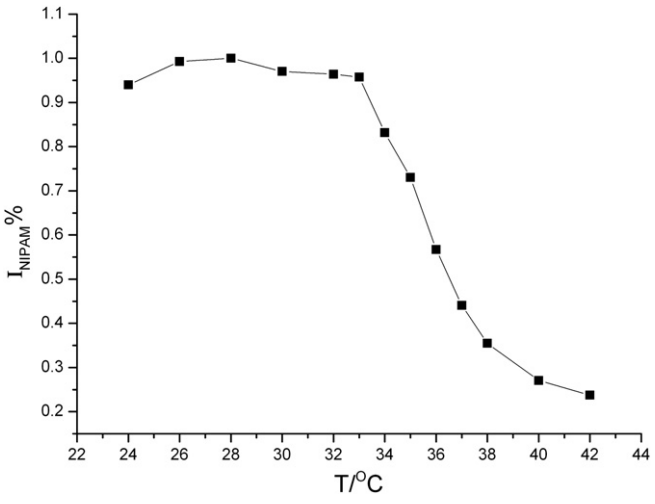


Fig. 3. VT-^{1}H NMR of C_{12}-PNIPAM-CA in D_2O (1 mg/mL).

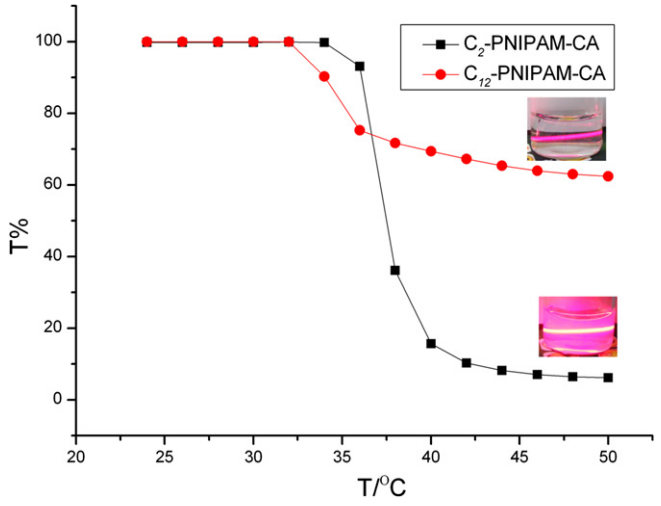


Fig. 4. Transmittance at different temperatures and photos taken at 45 °C of C_{12}-PNIPAM-CA and C_2-PNIPAM-CA in deionized water at the concentration of 0.2 mg/mL.

attributed to the higher hydrophobicity of $-C_{12}H_{25}$ than that of $-C_2H_5$ [14]. However, a remarkable difference appeared at temperatures above LCST: macroscopic precipitate formed by C_2-PNIPAM-CA leading to about 5% transmittance while the solution of C_{12}-PNIPAM-CA kept transparent with 60% transmittance and a strong Tyndall effect indicated the vesicle formation (photos in Fig. 4). Such a difference between the two solutions was observed for the solutions at higher concentrations (0.5 mg/mL in Fig. S26 and 1 mg/mL in Fig. S27) as well.

It is clear that when the temperature was increased to above LCST, the discrete chains of C_2-PNIPAM-CA aggregated rapidly as a result of the polar carboxyl end groups not being able to prevent them from precipitation. However, due to the association of the long alkyl chains, C_{12}-PNIPAM-CA existed as micelles at low temperature. As temperature was increased to above LCST, the PNIPAM chains collapsed but the carboxyl groups enriched on the micelle surface prevent them from further precipitation. Therefore, it was obvious that the micellar structure existing below LCST plays a key role in the further morphology change when temperature increases, *i.e.* the micelles might serve as building blocks in the further process of forming vesicles.

2.5. Morphology transition kinetics

The rapid morphology transition of C_{12}-PNIPAM-CA at temperature above the LCST makes tracing the transition process by light scattering possible. As the increase of R_h with time around 34 °C was too quick to follow experimentally, the time dependence of the relative scattering intensity I_s/I_0 was monitored instead, which was indicative of the size change of the aggregates. Here I_s and I_0 refer to the intensities of scattering light and incident light respectively. I_s/I_0 was recorded immediately after the C_{12}-PNIPAM-CA solution was heated from 33.5 °C to 34 °C. As shown in Fig. 5, in the first 500 s, I_s/I_0 gradually and slowly increased and was then followed by a rapid, dramatic increase during 500–1200 s. Such an aggregate size increase process resembles the well-known polycondensation kinetics. This initial slow increase may be ascribed to the formation of small aggregates such as dimer, trimer, *etc.*, from micelles as building units. And the following dramatic increase could be attributed to the formation of large aggregates. The slight size fluctuation in the last stage may be attributed to the final formation of the vesicles.

It is also noteworthy that, in our measurements of DLS, either with continuous temperature increase or jumping to a fixed temperature above the LCST, all the parameters we measured, *i.e.* light intensity, particle size and its polydispersity *etc.*, changed continuously. Neither a sudden drop in light intensity and particle size nor broadening of the size distributions was observed. It implies that in the micelle–vesicle transition, the dissociation of the pre-existed micelles and reorganization of the individual chains, as possible steps during the long morphology transition, could be excluded.

2.6. Possible mechanism of the rapid and reversible micelle–vesicle transition

Combining all the results addressed above, we would propose a possible mechanism for the micelle–vesicle transition, which is schematically presented in Fig. 6. First, at temperatures below the LCST, C_{12}-PNIPAM-CA forms micellar structure with a lightly associated core of the hydrocarbon chains. When the temperature has been increased to above the LCST of C_{12}-PNIPAM-CA, *i.e.* 34 °C, the PNIPAM chains rapidly collapse but are still highly hydrated and the carboxyl groups on micelle surface prevent them from macroscopic precipitation. Driven by minimizing the surface energy between the collapsed PNIPAM chains and water, the micelles as building blocks tend to combine to each other and then fusion between the connected micelles takes place. Fusion reduces the surface areas of the original micelles, which makes the density of hydrophilic moieties increase nearby. The densified hydrophilic moieties prevent further fusion close to this area and, as a result, direct the next step of the micelle combination into two dimensional patches. In fact, that soft species such as lipids [16], reactive cucurbit[6]uril [17] and polymeric micelles [18] connect to each other laterally rather than randomly has been widely observed and theoretically proved. Finally the assembled patch starts to bend forming vesicles driven by reducing the total energy. Further minor temperature increase makes R_h increase due to the PNIPAM chains becoming less hydrated and so increasing the packing parameter. We would emphasize that in this mechanism, dissociation of the micelles and reorganization of the dissociated species, may take long time, do not exist. And in each step of the whole process, *i.e.* combination, fusion, bending, *etc.*, local conformation adjustment of the PNIPAM chains is certainly involved. In the present case, the restriction to the conformation change exerted by the connected hydrocarbon chains is tiny, compared to that from the solid micellar cores of

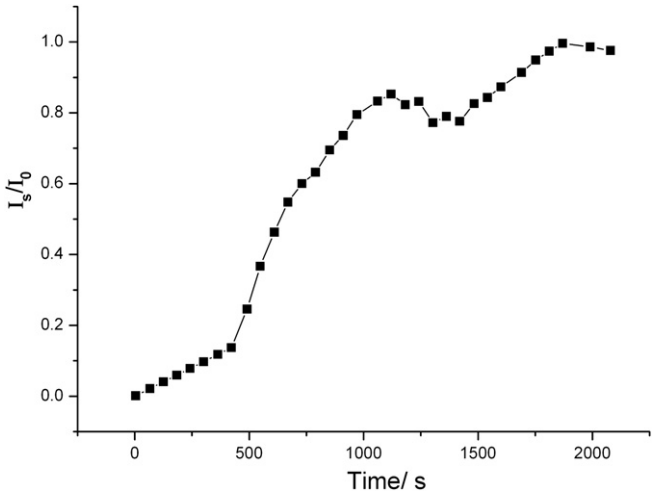


Fig. 5. Evolution of scattering intensity of C_{12}-PNIPAM-CA in water (0.1 mg/mL) heated at 34 °C.

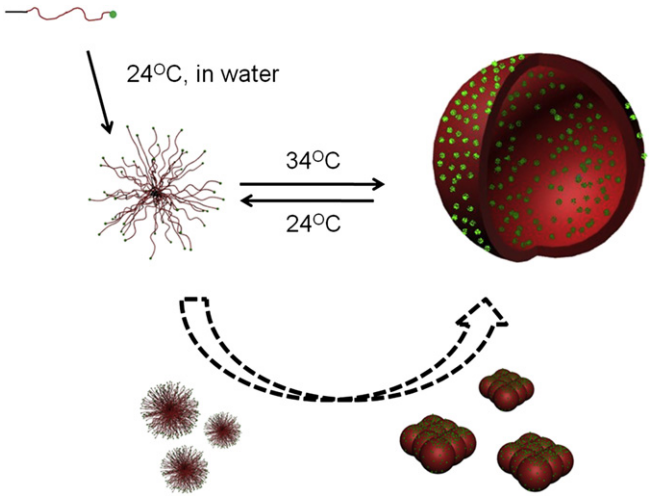


Fig. 6. Schematic illustration of the thermally induced morphology transition.

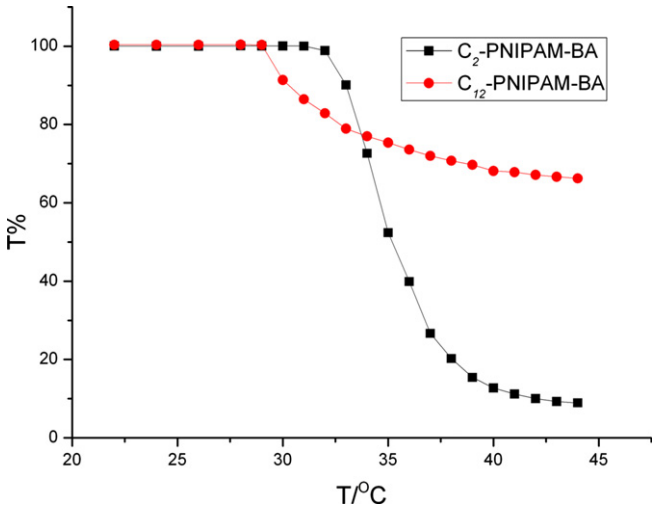


Fig. 7. Transmittance of C_{12}-PNIPAM-BA (0.2 mg/mL) and C_2-PNIPAM-BA (0.2 mg/mL) at different temperatures.

PMA, PI and P*t*BA, which were used in the previous reports. This could be one of the major reasons for us to realize the really rapid and fully reversible thermo-induced transitions from micelles to vesicles for the PNIPAM-based copolymer. The reverse process, *i.e.* transition from vesicles to micelles, when temperature is reduced to lower than the LCST, proceeds extremely quick, which demonstrates that in the process of vesicle formation, the micelles serve as building units and keep its basic structure. So the reverse transition is just a dissociation of the vesicles into micelles.

2.7. Asymmetric modification of PNIPAM with phenylboronic acid

As discussed above, the vesicles formed from C_{12}-PNIPAM-CA have carboxyl groups decorated on the surface. In order to confirm the proposed mechanism with different polymers as well as to explore the possibility of surface functionalization of the vesicles, we studied another pair of asymmetric end-modified PNIPAM polymers by replacing the carboxyl group with phenylboronic acid as a Lewis acid. Phenylboronic acid is well known as a carbohydrate acceptor through the reversible boron–oxygen cyclic ether bond. Considering the hydrophilicity of phenylboronic acid in basic aqueous solutions, solutions of C_2-PNIPAM-BA and C_{12}-PNIPAM-BA at pH 9 were studied by turbidimetry, DLS and SLS. As shown in Fig. 7, transmittance curves of C_2-PNIPAM-BA and C_{12}-PNIPAM-BA present very similar thermo-responsive behavior as those of C_2-PNIPAM-CA and C_{12}-PNIPAM-CA, respectively. Compared to C_2-PNIPAM-BA, C_{12}-PNIPAM-BA has a lower LCST but forms stable aggregation instead of precipitation above the LCST.

We also traced this thermally induced aggregation of C_{12}-PNIPAM-BA (0.1 mg/mL in aqueous sodium hydroxide, pH 9) by DLS in detail. It was found that, similarly, micelle-like structures with R_h around 10 nm (PDI 0.4–0.5) were formed by hydrophobic end-group association below LCST (Fig. 8a). While heating at 28 °C for 20 min, R_h increased dramatically to around 110 nm with PDI decreasing from 0.45 to 0.13. R_g was determined by SLS to be 116 nm thus R_g/R_h was 1.05, which indicates the vesicle structure. As shown in Fig. 8b, several successive morphology transition cycles were also monitored by DLS showing satisfactory reversibility. This morphology transition shows the similar rapid and reversible merits as that of C_{12}-PNIPAM-CA.

Furthermore, the obtained vesicles with phenylboronic acid on surface can be modified with either sugars or a potential fluorescent species, based on the dynamic covalent bond of boron–oxygen cyclic ether. This modification can be proved by Alizarin Red S (ARS) competitive binding assay [19]. ARS is a derivative of anthraquinone, the catechol of which also owns the binding equilibrium with phenylboronic acid. When this binding happens, the non-fluorescent ARS will emit strong fluorescence at the wavelength of 565 nm. This method has been employed to prove the surface modification of our vesicles. When ARS was added into the solution of C_{12}-PNIPAM-BA vesicles at 28 °C, a strong fluorescence emission appeared (Fig. 9). Then, at the same temperature, after addition of a model sugar (Galactose) to this solution, the fluorescence emission was almost quenched because of the competitive binding ability of galactose with phenylboronic acid. It shows that, the vesicle surface can be either modified by an indicator to induce its fluorescence, or bind to "sweet" oligosaccharides, which compete with the indicator and quench the fluorescence.

3. Experimental

3.1. Materials

N-Isopropylacrylamide (NIPAM) purchased from Tokyo Kasei Kagyo Co. was recrystallized three times from benzene/hexane (65:35 v/v) prior to use. Azobisisobutyronitrile (AIBN, CP) supplied by Sinopharm Chemical Reagent Co. was recrystallized from ethanol before use. Unless specially mentioned, all other chemicals were used as received.

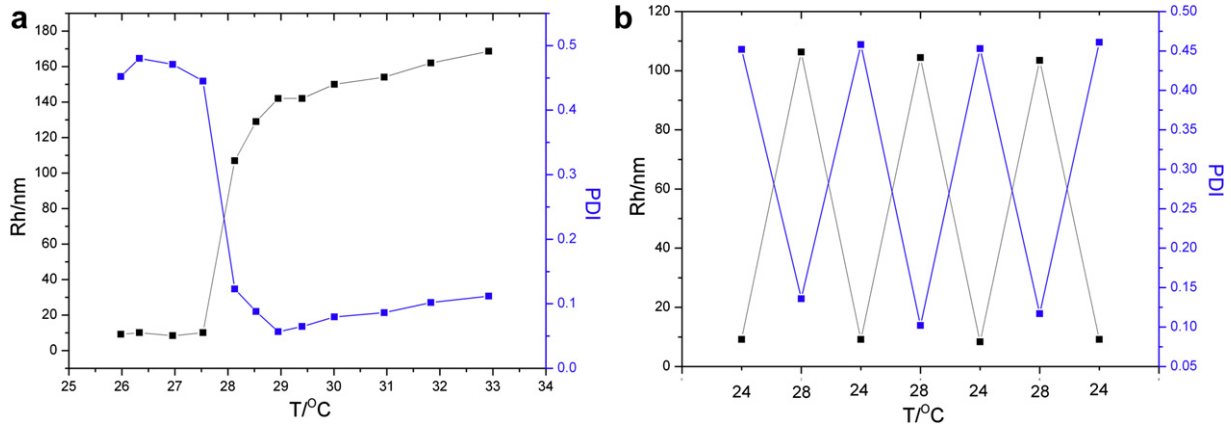


Fig. 8. Light scattering study of 0.1 mg/mL C_{12}-PNIPAM-BA in water: (a) R_h and PDI recorded after 30 min heating at a given temperature, (b) morphology transition cycles between 24 °C and 28 °C.

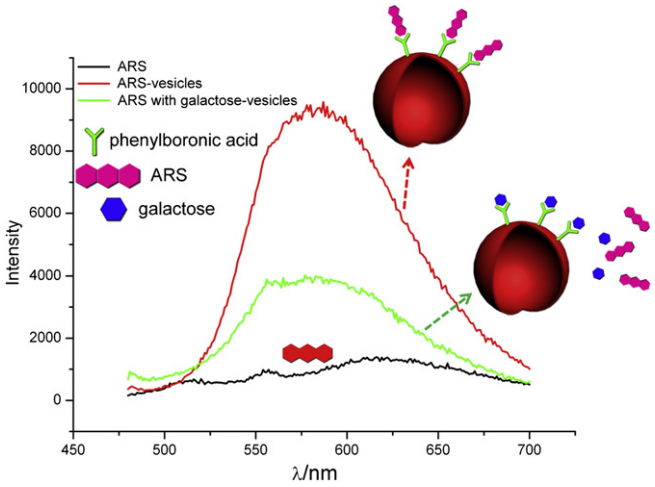


Fig. 9. ARS competitive binding assay of vesicles with phenylboronic acid on surface.

3.2. Characterization methods

^{1}H NMR spectra were recorded with a JEOL ECA-400 spectrometer. Gel permeation chromatography (GPC) analysis was carried out with a Waters Breeze 1525 GPC analysis system with two PL mix-D column, using DMF with 0.5 M LiBr as eluents at the flow rate of 1 mL/min at 80 °C and PEO calibration kit (purchased from TOSOH) as the calibration standard. Turbidimetry was recorded in a conventional quartz cell (light path 10 mm) on a Perkin–Elmer Lambda 35 spectrophotometer. Dynamic and Static light scattering studies were conducted using ALV/5000E laser light scattering (LLS) spectrometers at scattering angle of 90°, CONTIN analysis was used for the extraction of R_h data. In this paper, we use the R_h result collected at 90°, because only slight scattering angle dependence of R_h has been observed (a typical example in Table S1). The four PNIPAM samples were dissolved in deionized water (pH = 6) at different concentrations for studies of their thermo-responsive property utilizing turbidimetry, dynamic light scattering (DLS) and static light scattering (SLS). The AFM images were acquired in tapping mode by using a Nanoscope IV from Digital Instruments equipped with a silicon cantilever with 125 imand E-type vertical engage piezoelectric scanner.

3.3. Synthesis of chain transfer agents

C_2-CTA-CA [20] and C_{12}-CTA-CA [21] were synthesized according to procedures described in the literature. ^{1}H NMR characterizations are shown in supporting information. 4-bromomethylphenyboronic acid was synthesized via two steps according to the literature [22], then reacted with C_{12}-CTA-CA to obtain a new CTA, C_{12}-CTA-BA [23]. Another CTA, C_2-CTA-BA was synthesized following the same procedure started from C_2-CTA-CA. Their synthesis procedure and ^{1}H NMR spectra are in supporting information.

^{1}H NMR of C_{12}-CTA-BA: (400 MHz, $CDCl_3$, ppm): δ8.21 (d, 2H, C2***H***-Ar and C6***H***-Ar); 7.46 (d, 2H, C3***H***-Ar and C5***H***-Ar); 5.22 (s, 2H, Ar-C***H***$_2$OCO), 3.25–3.28 (t, 2H, $-CH_2-CH_2-S-C(=S)$), 1.74 (s, 6H, $-S-C(CH_3)_2-CO$), 1.64–1.61 (t, 2H, $-CH_2-CH_2-S-C(=S)$), 1.22 (s, 18H, $CH_3-C_9H_{18}-CH_2-CH_2S-C(=S)$), 0.91–0.87 (t, 3H, $CH_3-C_9H_{18}-CH_2-CH_2S-C(=S)$).

^{1}H NMR of C_2-CTA-BA: (400 MHz, $CDCl_3$, ppm): δ 8.18 (d, 2H, C2***H***-Ar and C6***H***-Ar); 7.46 (d, 2H, C3***H***-Ar and C5***H***-Ar); 5.19 (s, 2H, Ar-C***H***$_2$OCO), 3.30 (q, 2H, $CH_3-CH_2-S-C(=S)$), 1.74 (s, 6H, $-S-C(CH_3)_2-CO$),1.34 (t, 3H, $CH_3-CH_2-S-C(=S)$).

3.4. RAFT polymerization method for asymmetrical modified PNIPAM

Briefly, polymerizations were conducted under argon at 70 °C in 1,4-dioxane employing various CTAs as RAFT agents and AIBN as initiator. A representative polymerization procedure is as follows. NIPAM (1 g, 8.85 mmol), CTA (0.089 mmol), AIBN (0.045 mmol) and 3 mL 1,4-dioxane were sealed in a flask equipped with a magnetic stir bar, followed by three freeze–thaw cycles. The reaction flask filled with argon was placed in a preheated oil bath at 70 °C. The polymerization was quenched after 5 h by removing the reaction flask from heat followed by cooling in liquid nitrogen immediately. The polymer was precipitated into cold ethyl ether, filtrated and then dissolved in THF and precipitated again. The procedure was repeated for three times and the polymer was obtained as white powder after drying under vacuum at room temperature for 12 h. GPC (Figs. S10 and S11) exhibited satisfactory PDI < 1.2. The polymers were also characterized by ^{1}H NMR (Figs. S6–S9).

4. Conclusion

A real rapid and fully reversible thermally induced morphology transition can be realized for asymmetrically end-modified PNIPAM. The micelle-like structure turns to vesicle while it is heated above the LCST of PNIPAM within 30 min, which is much faster than the reported results ever. Based on this suitable time scale for morphology transition, after careful monitor of the transition process by light scattering and NMR above the LCST of PNIPAM, a new mechanism is proposed: when temperature is increased to above the LCST, the pre-existing micelles as building blocks, could combine and fuse to each other to form patches and then vesicles. This new mechanism can also explain the previously reported slow transitions and opens a new window for us to understand self-assembly and related morphology transitions. The vesicles can also be expanded by other functionalities modified on the surface, including fluorescence indicators and oligosaccharides.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.polymer.2011.06.005.

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