Self-assembled structures using CABC multi-block copolymers

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Self-Assembled Structures Using CABC Multi-Block Copolymers

Jie ZHENG

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

2020

Self-Assembled Structures Using CABC Multi-Block Copolymers

Jie ZHENG

SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

A thesis submitted to the Nanyang Technological
University in partial fulfilment of the requirement for the
degree of Doctor of Philosophy

2020

Statement of Originality

I hereby certify that the work embodied in this thesis is the result of original research done by me except where otherwise stated in this thesis. The thesis work has not been submitted for a degree or professional qualification to any other university or institution. I declare that this thesis is written by myself and is free of plagiarism and of sufficient grammatical clarity to be examined. I confirm that the investigations were conducted in accord with the ethics policies and integrity standards of Nanyang Technological University and that the research data are presented honestly and without prejudice.

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[Assoc. Prof. Atsushi Goto]

Authorship Attribution Statement

This thesis contains material from 1 paper(s) published in the following peer-reviewed journal(s) / from papers accepted at conferences in which I am listed as an author.

Chapter 2 is published as Zheng, J.; Wang, C. G.; Yamaguchi, Y.; Miyamoto, M.; Goto, A. Temperature-Selective Dual Radical Generation from Alkyl Diiodide: Applications to Synthesis of Asymmetric CABC Multi-Block Copolymers and Their Unique Assembly Structures. *Angew. Chem. Int. Ed.* **2018**, 57, 1552 -1556.

The contributions of the co-authors are as follows:

- Assoc. Prof. A. Goto provided the initial project.
- I performed all the experimental work and analysis at the Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Singapore.
- Assoc. Prof. A. Goto and Dr. C. G. Wang provided guidance in the interpretation of the experimental data.
- I prepared the manuscript drafts. Assoc. Prof. A. Goto and Dr. C. G. Wang edited and finalized the manuscript.
- Mr Y. Yamaguchi and Mr M. Miyamoto provided the initiator.

[07-11-2019]

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Date

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[Zheng Jie]

Abstract

This thesis describes the self-assembly of block copolymers and their applications. Controlled radical polymerizations, in particular, the reversible complexation mediated polymerization (RCMP), were introduced as well. By applying a new temperature-selective dual initiator, the CABC multi-block copolymer was prepared using RCMP. Taking advantage of the asymmetric structure of the CABC multi-block copolymer, novel structural nanoparticles, interesting micellar morphological transformation, and the toroidal nanostructure were achieved in the thesis.

In Chapter 1, I described the block copolymer self-assembly properties and their applications, especially in the drug delivery and stimuli-responsive systems. The assembly morphology influence factors were discussed as well. Controlled radical polymerizations, especially the reversible complexation mediated polymerization (RCMP), have been introduced. I also introduced the motivations and purposes of Chapters 2, 3 and 4.

In Chapter 2, temperature-selective radical generation from a newly designed alkyl diiodide ($I-R_2-R_1-I$) was studied. R_1-I and $I-R_2$ had different reactivities for generating alkyl radicals in the presence of a tetraoctylammonium iodide (ONI) catalyst. Taking advantage of the temperature selectivity, I used the alkyl diiodide as a dual initiator in ONI-catalyzed living radical polymerization to uniquely synthesize CABC non-symmetric multi-block copolymers. Because of their non-symmetric structure, CABC multi-block copolymers form unique assemblies, i.e., Janus-type particles with hetero-segment coronas and flower-like particles with hetero-segment petals.

In Chapter 3, I developed a temperature-directed micellar morphological transformation using CABC multi-block copolymers with a hydrophobic block A, a hydrophilic block B, and a thermally responsive block C with a lower critical solution temperature (LCST). The micellar structure was switched from a star (below LCST) to a flower

(above LCST). The transition-temperature was tuneable in a wide range (11-90 °C) by varying the C monomer composition. The large difference in the loading capacity between the star and flower enabled efficient encapsulation and controlled release of external molecules. Unlike conventional systems, the present star-to-flower transformation keeps micellar structures and hence does not liberate polymers but only external molecules selectively. Another application is a hidden functional segment. A functional segment is hidden (shielded) below LCST and exposed to interact with external molecules or surfaces above LCST, which may serve as a new temperature-directed interface for, e.g., biological and sensing applications.

Chapter 4 described the assembled morphologies evolution using CABC block copolymers with different length of each segment. The CABC block copolymer composing of hydrophobic block A, hydrophilic block B, and thermo-responsive block C with LCST. Varying the lengths of A and C segments (varying the hydrophobicity), the assembly morphological change from spherical micelles to discs, toroids, oval toroids, and cage-like structures. I also crosslinked toroids and studied their size change below and above LCST with keeping the toroid structures.

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Chapter 1. General Introduction

1.1. Introduction.

Polymeric products are widespread in our lives. With the development of synthetic technology, not only homopolymers and random copolymers but also gradient copolymers, block copolymers and graft copolymers (Figure 1.1) can be prepared. Block copolymers have drawn substantial attention for their self-assembly ability in selective solvents.¹ Depending on the kind and length of each block, polymer concentration, solvent, temperature, and ionic strength, various morphologies such as micelles, rods, vesicles, toroids, discs, Janus-type micelles, and other complex morphologies can be obtained (Figure 1.2).²⁻⁹ Those polymeric nanostructures are widely used as nanocarriers, nanoreactors and sensors in biological and biomedical fields, for example.¹⁰⁻¹³

Figure 1.1. Several polymer structures.

Figure 1.2. Morphologies formed by block copolymers.

1.2. Self-Assemblies of Block Copolymers.

The self-assembly of block copolymers in bulk state or in a solvent is widely used to prepare well-defined architectures in nano- or micrometre scale. Due to the mechanical and physical properties of block copolymers, their self-assemblies exhibit higher stability and durability, providing promising applications in varieties fields, such as biomedicine, biotechnology, and biomaterials fields.¹⁴ Thus, studying the self-assembly of block copolymers not only because of the academic interest, but also due to their widely promising potential applications.^{1,10-14}

Block copolymers self-assembly in the solid state has been widely studied since the 1960s.¹⁵ In the bulk state, block copolymers undergo microphase separate because of the immiscibility of each segment. The phase separation leads to the same chain segment aggregating and formation of different morphologies. By changing the composition of the

block copolymers, diverse morphologies, including spheres, cylinders, and lamellae, can be prepared in the solid state.¹⁴⁻¹⁷

The self-assembly of block copolymers not only occurs in the bulk state, but also exists in a solvent. The study of block copolymers self-assembly in a selective solvent is started in 1995.¹⁸ Compared to the block copolymer self-assembly in the solid-state, the self-assembly in solution exhibit a higher level of complexity, as the interaction between the polymer segment and the solvent as well as each other.^{1,14}

In aqueous media, amphiphilic block copolymers self-assemble above the critical micelle concentration (CMC), yielding core-shell structures.¹ The insoluble hydrophobic segment aggregates into the core and the hydrophilic segment dissolves in water to form the shell. The assembly morphology is determined by several factors. One of the most important factors is the assembly hydrophilic/hydrophobic interfacial curvature. This interfacial curvature can correlate with the polymer chain packing parameter (P) defined by equation (1):

$$P = v / (a_0 \times l_c) \quad (1)$$

where v is the hydrophobic core volume, a_0 is the hydrophilic headgroup area, and l_c is the fully extended length of the hydrophobic chain. The assembly morphology can be predicted using P . For example, spherical micelles form at $P \leq 1/3$, cylindrical micelles (rods) form at $1/3 \leq P \leq 1/2$, and vesicles form at $1/2 \leq P \leq 1$.^{1,2} It is notable that the packing parameter is only useful to predict the assembly morphology at the equilibrium state, where polymer chains are able to rapidly exchange among the assembled structures. Experimentally, the self-assembling behaviour of the block copolymer is often much more complicated because the high molecular weight of the polymer reduces the exchange of the polymer chain among the assembled

structures (non-equilibrium state). The assembly morphology also strongly depends on the solvent, polymer concentration, and ionic strength.^{1,2}

A variety of morphologies, such as spherical micelles, rods-like micelles, and vesicles, have been obtained via amphiphilic block copolymers self-assembly in aqueous solution. Generally, the spherical micelle contains a spherical core which surrounded by a hydrophilic shell is the first aggregates formed by the block copolymer. By increasing the hydrophobicity of the block copolymer, the spherical micelles connect and fuse to form rods (cylindrical or wormlike micelles), which consist of a cylindrical core and a surrounding shell. Rods contain a similar diameter of the spherical micelle but with a widely variable length. Vesicles, which are hollow spheres with a bilayer wall sandwiched by internal and external shells, represent the next morphology in the increase of the hydrophobic segment of block copolymer. For example, with the increase of the length of the relative hydrophobic segment (PHPMA, red segment) of the poly(glycerol monomethacrylate)-*block*-poly(2-hydroxypropyl methacrylate) (PGMA-*b*-PHPMA) di-block copolymer from 90 units to 140 units to 220 units, the spherical micelles changed to wormlike micelles to vesicles in an aqueous environment (Figure 1.3).¹⁹ Besides, other complex nanostructures, including large compound micelles,²⁰ large compound vesicles²¹ and hexagonally packed hollow hoop structures,²² can be prepared with well-designed block copolymers as well.

The nanostructures formed by block copolymers are relatively stable compare with their low molar mass counterparts (low molar mass surfactants and lipids), facilitating their potential applications in various fields. In particular, in the drug delivery systems, block copolymer nano-carriers are more stable, avoiding undesirable release (leak) of the drugs.^{10,11,23,24} In addition, the morphology, size, and property of the polymeric self-assembly can be precisely tuned via the composition, length, and functional group in each segment, enabling smart applications for, e.g., molecular templates, nano-devices, and stimuli-responsive systems.²⁵⁻²⁹

Figure 1.3. Schematic illustration of the self-assemblies formed di-block copolymers.

Because both the hydrophobic and hydrophilic segments of block copolymers can be biocompatible, the assemblies may be used for drug delivery nano-carriers. Generally, the core of the spherical micelle works as a container of hydrophobic drugs, and the surrounding shell acts as a protector to avoid unsolicited interactions between loading drugs and surrounding tissues as well as recognize specific organs for target delivery.²³ Moreover, in practical use, two or more drugs are required to load at the same time. However, the two drugs may react with each other if they are encapsulated (coexist) in the same core. To avoid such unfavourable contact of the two drugs, multi-compartment micelles were designed, which offered the segregated storage of different drugs and the simultaneous or sequential release of the drugs upon the need. For example, the micelle generated with mikto-arm star copolymers (μ -polyethylethylene-polyethylene oxide-polyperfluoropropylene oxide, μ -EOF) (Figure 1.4),

enable segregated encapsulation of two different molecules (i.e. pyrene and naphthyl perfluoroheptanyl ketone) in the same micelle.³⁰

Figure 1.4. Schematic illustration of selective storage of two molecules in the same assembly.

The assemblies can respond to external stimuli such as temperature, pH, and light using stimuli-responsive polymers (Figure 1.5). For example, poly(*N*-isopropylacrylamide) (PNIPAM), poly(*N*-(1-hydroxymethyl)propylmethacrylamide) (PHMPMA), poly(dimethylaminoethyl methacrylate) (PDMAEMA), poly(*N,N*-diethylacrylamide) (PDEAM), and poly(ethylene glycol) (PEG) are responsive to temperature and have lower critical solution temperatures (LCST), and poly(*N*-acryloylasparaginamide) (PNAAAM) has an upper critical solution temperature (UCST).³¹⁻³⁴ Block copolymers composed of these thermo-responsive polymer chains can switch their solubility (from hydrophobic to hydrophilic or vice versa) at the critical solution temperatures, altering the morphologies, sizes and properties of the assemblies. Block copolymers with protonated groups are able to respond to pH. For example, the micelle generated by a poly(2-vinylpyridine)-block-poly((dimethylamino)ethyl methacrylate) (P2VP-*b*-PDMAEMA) block copolymers responded to pH because the P2VP and PDMAEMA segments are protonated at different pH, which resulted in the change in solubility.³⁵ Light-sensitive block copolymers such as poly(ethylene oxide)-*b*-PSPA (PEO-*b*-PSPA) exhibited photo-switched morphological transition, where SPA is a spiropyran-based monomer. PEO-*b*-PSPA underwent a photo-triggered isomerization between the hydrophobic spiropyran and the zwitterionic merocyanine by irradiating at

different wavelengths of light.³⁶ Multi-responsive assemblies were also reported. They responded to a range of different external stimuli. For example, the polybutadiene-*b*-poly(*tert*-butyl methacrylate)-*b*-poly(2-(dimethylamino)ethyl methacrylate) (PB-*b*-PtBMA-*b*-PDMAEMA) triblock copolymer responded to pH, temperature, and salinity.³⁷

Figure 1.5. Schematic illustration of the assembly stimuli responsive.

1.3. Controlled Radical Polymerization (CRP).

Block copolymers such as AB diblock, ABA triblock, and ABC triblock copolymers are accessible via controlled radical polymerization (CRP) (also termed as reversible-deactivation radical polymerization (RDRP)).³⁸⁻⁴⁷ The CRP systems include nitroxide-mediated radical polymerization (NMP),⁴⁸⁻⁴⁹ atom transfer radical polymerization (ATRP),⁵⁰⁻⁵² and reversible addition–fragmentation chain transfer polymerization (RAFT).⁵³⁻⁵⁵

The basic principle underlying in CRP is the reversible activation of the dormant species (Polymer-X with a capping agent X) to the propagating radical (Polymer•) (Scheme 1.1). Polymer• propagates until it deactivated with X to form Polymer-X. By repeating the activation and deactivation processes, the polymer chain can grow in a step-by-step manner, yielding a polymer with low dispersity ($D = M_w/M_n$), where M_n and M_w are the number- and weight-average molecular weights, respectively. Because the deactivation process is much faster than the activation process, the concentration of Polymer• is much lower than that of

Polymer-X. Therefore, the amount of dead chains generated via radical-radical termination is usually much smaller than that of Polymer-X.

Scheme 1.1. General scheme of the reversible activation.

NMP was pioneered by Solomon, Rizzardo and Cacioli in the mid-1980s and has attracted great attention since the first report of Georges et al., who synthesized low dispersity polystyrene in 1993 (Scheme 1.2). NMP uses a nitroxide ($\text{R}_1\text{R}_2\text{N-O}^{\bullet}$) as an X. Figure 1.6 shows several nitroxides developed for NMP.

Scheme 1.2. Reversible activation in NMP.

Figure 1.6. Chemical structures of some nitroxides.

ATRP was first reported by Matyjaszewski and Sawamoto in 1995. ATRP uses a halide (usually bromides or chlorides) as an X (Scheme 1.3). The transition metal halide complex ($\text{Mt}^{\text{m}}\text{X}_n/\text{L}_n$) is used as a catalyst. ATRP has widely been used, because it is amenable to a wide range of monomers such as styrene, acrylates, methacrylates, and acrylamides.

Scheme 1.3. Reversible activation in ATRP.

RAFT polymerization was first reported by Moad, Rizzard and Thang in 1998. The RAFT polymerization uses the SC(=S)Z groups such as dithioesters, trithiocarbonates, xanthates, and dithiocarbamates as X. In the RAFT polymerization (Scheme 1.4), the propagating radical ($\text{P}_n^\bullet$) adds to the dormant species ($\text{P}_m\text{-SC(=S)Z}$) to form an intermediate radical. The intermediate radical subsequently undergoes fragmentation to generate a new radical ($\text{P}_m^\bullet$).

Scheme 1.4. RAFT.

Our research group developed an organocatalyzed control radical polymerization using an alkyl iodide (R-I) as an initiator (X = iodide) and an organic molecule as a catalyst.⁵⁶⁻⁶⁵ The catalysts include organic salts such as tetrabutylammonium iodide ($\text{Bu}_4\text{N}^+\text{I}^-$). Mechanistically, the dormant species (polymer-iodide (polymer-I)) and the catalyst are supposed to form a complex (polymer-I $\cdots$ catalyst) via halogen bonding. The complex subsequently reversibly generates Polymer $^\bullet$ (Scheme 1.5). This polymerization is termed reversible complexation mediated polymerization (RCMP). RCMP is attractive because no special capping agents or metals are required while it is amenable to a wide range of monomers and polymer structures.

Scheme 1.5. Reversible activation in RCMP.

1.4 Aim in Chapter 2.

In chapter 2, I aimed to synthesize CABC multi-block copolymers. To this end, I designed a temperature-selective alkyl diiodide initiator (I-R²-R¹-I, methyl 2-iodo-2-(4'(2''-iodopropionyloxy)phenyl)acetate (I-MEPE-I)) (Scheme 1.6) as a conceptually new dual initiator.⁶⁶ The initiator bears two initiation sites (I-R² and R¹-I sites) with largely different reactivities. The R¹-I site can initiate at a given temperature and the I-R² site can initiate at an elevated temperature selectively. This temperature selectivity enabled the synthesis of CABC asymmetric multi-block copolymers. At a mild temperature (60 °C), two monomers A and B are successively polymerized from the R¹-I site to yield an AB diblock copolymer. Importantly, the I-R² site remains unreacted at this mild temperature. Subsequently, monomer C is polymerized at an elevated temperature (110 °C), where the propagation occurs at both chain ends. Therefore, CABC block copolymers are prepared by simply altering the temperature.

Scheme 1.6. Temperature-Selective Polymerization for Synthesis of CABC Multi-Block Copolymer.

Figure 1.7. Structures of the Janus-type particle and flower-like particle.

Taking advantage of the non-symmetric structure of the CABC block copolymer, I synthesized a Janus-type particle with hetero-segment coronas and a flower-like particle with heterosegment petals (Figure 1.7) as unique applications. The B segment was crosslinked for the Janus-type particle, which has two different coronas (CA hetero-segment and C homo-segment). The C segment was crosslinked for the flower-like particle, which has AB hetero-segment petals. Chapter 2 describes the synthesis of the CABC multi-block copolymers and the preparation of the Janus-type particle and the Flower-like particle.

1.5 Aim in Chapter 3.

Chapter 3 aimed to use the CABC block copolymer for creating stimuli-responsive micellar morphological transformation systems. The assembled structures of CABC block copolymers were reversibly switched between Janus-type star micelles and flower-like micelles by changing the temperature (Scheme 1.7). Unlike Chapter 2, I did not crosslink, enabling the morphological transformation. The CABC block copolymer was designed to possess three distinct segments. Block A is hydrophobic (coloured in blue in Scheme 1.7), block B is hydrophilic (red), and block C is thermosensitive (green) having an LCST in water. Below LCST, block C is soluble in water, and thus only block A is insoluble in water and forms a core, yielding a Janus-star micelle with the BC hetero-segment and C homo-segment in the

shell (Scheme 1.7a). Above LCST, block C is insoluble in water, and thus both block A and block C form a core, yielding a flower micelle with the B segment in the shell (petal) (Scheme 1.7a). This type of star-flower transformation is achievable only with (1) a hydrophobic block A to retain the core, (2) a hydrophilic block B to retain the shell, and (3) a thermosensitive block C to switch the morphology. Therefore, this is unique to CAB block copolymers. ABC tri-block copolymers might be an alternative but can not provide an asymmetric star.

Interestingly, the physicochemical properties of the assemblies can be changed by the morphological transformation. The large difference in the core volumes of the star and flower leads to the difference in the loading capacity of external molecules, enabling the reversible encapsulation and release of external molecules (Scheme 1.7b and Figure 1.8a). Another interesting application is the hidden functional segment. In the star, block B (the hidden segment coloured in red) is shielded (hidden) by block C (green) (Scheme 1.7c and Figure 1.8b) and is prevented from the contact with external substrates. Upon heating, due to the transformation from the star to the flower, block B turned to be exposed, enabling block B to contact with external substrates. Block B can possess functional groups. The functional block B segment can selectively be shielded and exposed by the temperature change, offering a novel functional interface on the assembly (Figure 1.8b). Chapter 3 describes the temperature-directed morphological transformation and the applications to the encapsulation-release and hidden-segment systems.

Scheme 1.7. (a) Reversible morphological transformation between star micelle (left) and flower micelle (right) self-assembled by CABC multi-block copolymer. (b) Reversible encapsulation and release of external hydrophobic molecules. (c) Hidden and exposed functional segment.

Figure 1.8. Schematic illustration of the reversible encapsulation-release system (a) and the hidden segment interfacial system (b).

1.6 Aim in Chapter 4.

Chapter 4 aimed to extensively study the assembled morphologies using CABC block copolymers, particularly to explore discs, toroids, oval toroids, and cage-like structures. As in Chapter 3, the polymer contains hydrophobic block A (coloured in blue in Figure 1.9), hydrophilic block B (red), and thermo-responsive block C (green) with LCST. I varied the chain lengths of blocks A and C (varied the hydrophobicity) and observed the morphological change from spherical micelles to discs, toroids, oval toroids, and cage-like structures (Figure 1.9). I also crosslinked toroids and studied their size change below and above LCST with keeping the toroid structures. Chapter 4 describes the morphology evolution with varying the lengths of blocks A and C and the preparation of crosslinked toroids.

Figure 1.9. Assembly morphologies formed by CABC block copolymers.

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Chapter 2. Temperature-Selective Dual Radical Generation from Alkyl Diiodide: Applications to Synthesis of CABC Multi-Block Copolymers and Their Unique Assembly Structures

Abstract.

Temperature-selective radical generation from a newly designed alkyl diiodide ($I-R_2-R_1-I$) was studied. R_1-I and $I-R_2$ had different reactivities for generating alkyl radicals in the presence of a tetraoctylammonium iodide (ONI) catalyst. Taking advantage of the temperature selectivity, I used the alkyl diiodide as a dual initiator in ONI-catalyzed living radical polymerization to uniquely synthesize CABC non-symmetric multi-block copolymers. Because of their non-symmetric structure, CABC multi-block copolymers form unique assemblies, i.e., Janus-type particles with hetero-segment coronas and flower-like particles with hetero-segment petals.

2.1. Introduction.

The generation of a carbon-centered radical ($R^\bullet$) from an alkyl halide ($R-X$) is a fundamental reaction in organic synthesis, and its reactivity significantly depends on the alkyl group (R). An alkyl di-halide ($X-R_2-R_1-X$) with largely different structures at R_1 and R_2 can selectively generate an alkyl radical from one site (R_1-X) at a given temperature and then another radical from the other site ($X-R_2$) at an elevated temperature. This temperature dependence enables each site to be independently transformed to a different functional group by simply altering the temperature.

Living radical polymerization (LRP) is a useful method for preparing block copolymers.¹⁻¹¹ LRP is based on the reversible activation of a dormant species (Polymer- X) to a propagating radical (Polymer $^\bullet$) (Scheme 2.1a). I found that the iodide anion (I^-) works as a catalyst for the reversible generation of $R^\bullet$ from an alkyl iodide ($R-I$).⁹ I used this reaction for the reversible activation of Polymer- I to Polymer $^\bullet$ and developed a new class of LRP, i.e., organocatalyzed LRP (Scheme 2.1b).^{9,10}

(a) Reversible activation (general scheme)

(b) Organocatalyzed LRP

Scheme 2.1. Reversible activation: (a) General scheme and (b) Organocatalyzed LRP.

Scheme 2.2. Temperature-Selective Polymerization for Synthesis of CABC Multi-Block Copolymer.

In the present work, I propose a temperature-selective dual initiator as a conceptually new initiator in LRP. I designed an alkyl di-iodide (I-R₂-R₁-I) (Scheme 2.2) with considerably different reactivities of R₁-I and I-R₂; thus, it can initiate at a given temperature (from R₁-I) and an elevated temperature (from I-R₂) selectively. Interestingly, this initiator enables the synthesis of CABC non-symmetric multi-block copolymers (Scheme 2.2). At a mild temperature, two monomers A and B are successively polymerized from R₁-I, whereas I-R₂

remains unreacted. Another monomer C is subsequently polymerized at an elevated temperature, whereupon propagation occurs from both chain ends. CABC block copolymers are thus obtainable by simply altering the temperature.

Diblock, triblock, and multi-block copolymers have garnered increasing attention for their assembly structures. However, only several examples have been reported for the synthesis of CABC multi-block copolymers and experimental studies on their phase separation and vesicle and micelle formation.¹²⁻¹⁵ The present thesis reports the first synthesis of a Janus-type particle with hetero-segment coronas and a flower-like particle with hetero-segment petals (Scheme 2.3) by utilizing the non-symmetric structure of CABC block copolymer. For the Janus-type particle, the B segment is crosslinked to form a particle with two distinguishable hetero-CA-segment and homo-C-segment coronas. For the flower-like particle, the C segment is crosslinked to form a particle with hetero-AB-segment petals. The temperature-selective synthesis of CABC block copolymers and the preparation of these two unprecedented particles are reported in the present thesis.

Scheme 2.3. Janus particles and flower-like particles synthesized in this work (top) and possible particles obtainable by AB diblock and ABC triblock copolymers (bottom).

2.2. Results and discussion.

I previously experimentally determined the activation rate constant k_a (Scheme 2.1b) of several alkyl iodides catalyzed by tributylmethylphosphonium iodide (BMPI) at 70 °C.¹⁶ The k_a value ($M^{-1} s^{-1}$) (Scheme 2.2) was more than 30 times different for R = propionate (4×10^{-4}) and phenylacetate (130×10^{-4}). Exploiting this large difference, I combined these two R groups and designed an alkyl diiodide dual initiator, methyl 2-iodo-2-(4'-(2''-iodopropionyloxy)phenylacetate) (I-MEPE-I) (Scheme 2.2). The phenylacetate site (PE-I) can initiate at a mild temperature 60 °C, whereas the propionate site (I-ME) can initiate at only an elevated temperature 110 °C, thereby providing temperature selectivity. Importantly, the k_a value was similar for R = poly(methyl methacrylate) (PMMA) (70×10^{-4}) and phenylacetate (130×10^{-4}). This means that PMMA-I generated from PE-I has a similar initiation ability to PE-I, ensuring that I-ME-PMMA-I (accessible after the first block polymerization) can also work as a temperature-selective dual macroinitiator. In the present work, the more soluble tetraoctylammonium iodide (ONI) was used as a catalyst instead of BMPI. I may reasonably assume a similar magnitude of the k_a values for BMPI and ONI, because I^- (not the counter cations) serves as the catalyst in both cases.

I synthesized a CABC block copolymer using methyl methacrylate (MMA), butyl methacrylate (BMA), and butyl acrylate (BA) as the A, B, and C monomers, respectively, as a proof of principle (Table 2.1 (entry 1) and Figures 2.1 and 2.2). I heated a mixture of MMA (8 M), I-MEPE-I (160 mM) (initiator), and ONI (80 mM) (catalyst) at 60 °C. To retain the high chain-end fidelity of iodide, the polymerization was intentionally stopped at a reasonably short time 3 h (monomer conversion = 68%). I obtained an I-ME-PMMA-I polymer with $M_n = 4100$ and PDI = 1.13 before purification and $M_n = 4100$ (36 monomer units of MMA) and PDI = 1.13 after purification by reprecipitation, where M_n is the number-average molecular weight and PDI is the polydispersity index. Figures 2.2a and 2.2b show that 100% of PE-I initiated

and that 96% of I-ME remained unreacted, demonstrating the high initiation-selectivity of the two sites.

Using the purified I-ME-PMMA-I macroinitiator, I polymerized BMA at 60 °C for 6 h and obtained an I-ME-PMMA-PBMA-I diblock copolymer with $M_n = 7400$ and PDI = 1.15 before purification and $M_n = 7800$ (23 monomer units of BMA) and PDI = 1.15 after purification, where PBMA is poly(butyl methacrylate). GPC traces (Figure 2.1) show that a large fraction of the I-ME-PMMA-I macroinitiator was extended to the I-ME-PMMA-PBMA-I block copolymer, confirming the high block-efficiency of the PMMA-I site. Moreover, 92% of I-ME remained unreacted after the two polymerizations (Figure 2.2c).

Table 2.1. Synthesis of CABC multi-block copolymers.

Entry	Monomer	I-R-I	$[M]_0/[I-R-I]_0/[ONI]_0$ (mM) ^[a]	T (°C)	<i>t</i> (h)	conv (%)	M_n (GPC) ($M_{n,theo}$) ^[b]	DP (¹ H NMR) ^[c]	PDI (GPC)
1	MMA	I-MEPE-I	8000/160/80	60	3	68	4100 (3900)	–	1.13
						After purification	4100	36	1.13
	BMA	I-PMMA-I	8000/160/80	60	6	47	7400 (7400)	–	1.15
						After purification	7800	+ 23	1.15
	BA	I-PMMA-PBMA-I	8000/160/320	110	24	59	11800 (11600)	–	1.33
						After purification	12800	+ 22	1.27
2	MMA	I-MEPE-I	8000/53/80	60	3	63	7900 (9900)	–	1.12
						After purification	8300	82	1.10
	AMA	I-PMMA-I	8000/27/13	60	1.3	12	13000 (13000)	–	1.19
						After purification	13000	+ 36	1.19
	BA	I-PMMA-PAMA-I	8000/8/32	110	5	7	22000 (20000)	–	1.40
						After purification	23000	+ 50	1.34
						After reaction with <i>n</i> -hexylamine followed by purification	22000	–	1.36
3	MMA	I-MEPE-I	8000/20/20	60	3	30	12000 (12000)	–	1.14
						After purification	12000	105	1.12
	BMA	I-PMMA-I	8000/16/24	60	4	16	22000 (23000)	–	1.16
						After purification	22000	+ 90	1.16
	DGDA	I-PMMA-PBMA-I	8000/16/320	110	4	10	29000	+ 50	1.24
					16	14	59000	+ 70	2.33

[a] M = monomer. [b] Theoretical M_n calculated with $M_n = ([M]_0/[I-R-I]_0) \times \text{conversion} \times (\text{molecular weight of monomer}) + (\text{molecular weight of I-R-I})$. [c] DP obtained from the peak areas of the initiating PE unit (phenyl group) and the monomer units (with $\pm 10\%$ experimental error).

In the third step, I used an elevated temperature 110 °C. Using the purified I-ME-PMMA-PBMA-I macroinitiator, I polymerized BA at 110 °C, yielding an I-PBA-PBMA-PMMA-PBA-I tetrablock copolymer with $M_n = 11800$ and PDI = 1.33 before purification and $M_n = 12800$ (11 monomer units in each BA segment) and PDI = 1.27 after purification, where PBA is poly(butyl acrylate). At this elevated temperature, 100% of I-ME initiated (Figure 2.2d), meaning that the polymer bears PBA segments on both sides of the polymer. Thus, a CABC block copolymer was successfully synthesized by simply changing the temperature.

Figure 2.1. GPC curves for the synthesis of PBA-PMMA-PBMA-PBA (Table 2.1 (entry 1)).

Figure 2.2. ^1H NMR spectra for the synthesis of PBA-PMMA-PBMA-PBA (Table 2.1 (entry 1)).

A Janus-type particle was synthesized by using a post-crosslinkable monomer, allyl methacrylate (AMA), in the B segment (36 units). The A segment was MMA (82 units), and the C segment was BA (25 units of each C segment). After the polymerization, the iodides at the chain ends were removed via a reaction with *n*-hexylamine.¹⁷ The M_n and PDI of the obtained CABC block copolymer were 23000 and 1.34, respectively (Table 2.1 (entry 2) and Figure 2.3a).

The obtained CABC block copolymer (30 wt%) was heated with benzoyl peroxide (BPO) as a radical source (3 wt%) in ethylbenzene (67 wt%) at 80 °C for 1 h. The B segment was crosslinked via the radical addition of the allyl groups (Figure 2.3a). A majority of the allyl groups remained unreacted during the polymerizations, because the methacrylic (B monomer) and acrylic (C monomer) C=C bonds are much more reactive than the allylic C=C bond (B monomer). I used a relatively large amount of BPO (0.2 equiv to the AMA units) and exploited the high reactivity of the generated oxygen-centered radical from BPO in the post-crosslinking to facilitate the radical addition of the allyl groups.

After crosslinking, the obtained polymer was fractionated with preparative GPC (Figure 2.3b). The lower-molecular-weight fraction (fraction 1) contains unreacted and intramolecularly crosslinked chains. The higher-molecular-weight fraction (fraction 2) contains intermolecularly crosslinked particles. The weight ratio was 35:65 (fraction 1: fraction 2).

Dynamic light scattering (DLS) analysis (Figure 2.3c) showed that the hydrodynamic size (DLS peak top) in THF increased from 9 nm (before crosslinking) to 19 nm (after crosslinking). The contour length of the CABC block copolymer (168 units) is 42 nm. The particle size (19 nm) was reasonably smaller than the contour length, indicating that the aggregation among the particles was negligible.

Figure 2.3. (a) GPC curves for the synthesis of PBA-PMMA-PAMA-PBA (Table 1 (entry 2)), (b) GPC curves after fractionation, (c) DLS curves after fractionation, (d) TEM image of generated particles, (e) possible future application as a co-delivery container.

Figure 2.3d shows a TEM image of the particles (fraction 2). The particles were stained as follows. The particles (after crosslinking) still contained unreacted allyl groups (68% of the AMA units, as estimated with ^1H NMR). The remaining allyl groups were reacted with $\text{HSCH}_2\text{CH}_2\text{NH}_2$ via the thiol-ene Michael addition to functionalize the B segment with the NH_2 groups. The particles were subsequently immersed in an I_2 solution (solvent = THF), and the B segment was stained through the I_2/NH_2 halogen bonding. The TEM image (Figure 2.3d) shows the stained B segment as the darkest dot in each particle. The A segment was observed as a gray domain neighboring the B domain. The C domain was observed as the lightest gray domain surrounding the A and B domains, because the C segment (butyl acrylate units) tended to spread on the grid due to the low glass transition temperature of the butyl acrylate segment.

Janus-type particles have gained widespread attention for their anisotropic properties and have been used as, e.g., solid surfactants, nano-probes, bio-sensors, electronic paper, micro-motors, and drug delivery vehicles. ABC triblock copolymers have been used in many cases.¹⁸⁻²⁰ As shown in Scheme 2.3, while AB diblock copolymers can form symmetric particles, ABC triblock copolymers can form Janus particles with one core (B) domain and two distinguishable shell (A and C) domains. Unlike ABC triblock copolymers, CABC tetrablock copolymers can provide Janus-type particles with two core (A and B) domains and one shell (C) domain. With this special structure, I may uniquely encapsulate two different types of molecules such as hydrophilic and hydrophobic molecules in the two core (A and B) domains and can release these molecules by a function of the shell (C) domain (Figure 2.3e). Such nano-containers can simultaneously carry two different molecules and may find unprecedented applications as co-delivery systems in, e.g., cancer therapy and agrochemical release.

A flower-like particle with hetero-petals was synthesized by using a crosslinkable monomer, diethylene glycol diacrylate (DGDA) in the C segment. The A segment was MMA (105 units), and the B segment was BMA (90 units). After the second polymerization, the M_n and PDI of the AB diblock copolymer were 22000 and 1.16, respectively (Table 2.1 (entry 3) and Figure 2.4a). The polymer chain was crosslinked during the third polymerization with DGDA (Figure 2.4a), and the resulting polymer was fractionated with preparative GPC (Figure 2.4b). The lowest-molecular-weight fraction contained non-crosslinked chains. The higher-molecular-weight fractions (fractions a-d) contained flower-like particles with different sizes. The weight ratio was 37:63 (fraction 1: fractions a-d).

Figure 2.4. (a) GPC curves for the synthesis of PDMA-PMMA-PAMA-PDMA (Table 1 (entry 3)), (b) GPC curves after fractionation, (c) DLS curves after fractionation, and (d) TEM image of generated particles.

Figure 2.4c shows the DLS curves with the peak tops at 8 nm (fraction 1), 16 nm (fraction a), 21 nm (fraction b), 29 nm (fraction c), and 50 nm (fraction d). Figure 2.4d shows the TEM images of the particles (fractions a-d). The particles in fractions a-c with different particle sizes were not aggregated. The particles in fraction d were interconnected. The contour length of the AB segment (totally 195 units) is 49 nm. Because the AB segment should be looped, the length of the petal is at most 24.5 nm. The sizes of particles a-c (16-29 nm) were reasonably smaller than the maximum particle size (two petals (49 nm) + core), confirming that the aggregation among the particles was negligible for these particles. The length of the petal segment was able to be made longer (242 A units and 137 B units) and shorter (53 A units and 30 B units) (Figure 2.5, 2.6 and Table 2.2 (entries 1 and 2) in the Experimental section). The core of the particle was made hydrophilic by using a hydrophilic monomer instead of DGDA (Figure 2.7 and Table 2.2 (entry 3)). These results demonstrate accessibility to a range of particle design.

Flower-like particles have no chain ends in the petals and can exhibit properties between those of star-like particles and single-molecular macrocycles.²¹⁻²³ The lack of chain ends results in no entanglement with other polymers and particles and can exhibit lubrication. The diffusivity and biological recognition through the cell membranes can be tuned by the topological effects, and hence flower-like particles may serve as new vectors for drug delivery.²¹ Our flower-like particle bears hetero-petals that can contain different functionalities in the A and B segments, thereby offering a new material design of flower-like particles.

Figure 2.5. (a) GPC curves for the synthesis of the flower-like particle with a longer segment on the corona (I-PMMA-I, I-PMMA-PBMA-I, and I-PDGDGDA-PMMA-PBMA-PDGDGDA-I) (Table 2.2 (entry 1)), and (b) GPC curves and (c) DLS curves after the fractionation with a preparative GPC.

Figure 2.6. (a) GPC curves for the synthesis of the flower-like particle with a shorter segment on the corona (I-PMMA-I, I-PMMA-PBMA-I, and I-PDGDGDA-PMMA-PBMA-PDGDA-I) (Table 2.2 (entry 2)), and (b) GPC curves and (c) DLS curves after the fractionation with a preparative GPC.

Figure 2.7. (a) GPC curves for the synthesis of the flower-like particle with hydrophilic core (I-PMMA-I, I-PMMA-PBMA-I, and I-PPDGDA-PMMA-PBMA-PPDGDA-I) (Table 2.2 (entry 3)), and (b) GPC curves and (c) DLS curves after the fractionation with a preparative GPC. The solvent for the GPC and DLS measurement was DMF.

2.3. Conclusion.

In summary, the temperature-selective radical generation from a designed alkyl diiodide (I–MEPE–I) was utilized to synthesize CABC non-symmetric block copolymers. Exploiting the unique non-symmetric structure, I synthesized two new types of particles, i.e., a Janus-type particle with hetero-segment coronas and a flower-like particle with hetero-segment petals. Such particles may find promising applications as new co-delivery containers and dual-functional vectors.

2.4. Experimental Section.

Materials

Methyl methacrylate (MMA) (>99.8%, Tokyo Chemical Industry (TCI), Japan), butyl methacrylate (BMA) (>99.0%, TCI), butyl acrylate (BA) (>99.0%, TCI), allyl methacrylate (AMA) (98%, Aldrich, USA), di(ethylene glycol) diacrylate (DGDA) (75%, Aldrich), poly(ethylene glycol) diacrylate (PEGDA) (average $M_n = 700$) were purified through an alumina column before use. Benzoyl peroxide (BPO) (>75.0%, TCI) was purified through recrystallization in chloroform/methanol mixture before use. Tetra-*n*-octylammonium iodide (ONI) (>98.0%, TCI), *n*-hexylamine (>99%, TCI), 1-butanol (BuOH) (>99.0%, TCI), diethylene glycol dimethyl ether (diglyme) (>99%, TCI), tetrahydrofuran (THF) (>99.5%, Kanto, Japan), hexane (>99%, International Scientific, Singapore), methanol (>99%, International Scientific), acetonitrile (HPLC grade, Anhui Fulltime Specialized Solvent & Reagent, China), and ethylbenzene (>99%, TCI) were used as received.

Measurement

The GPC analysis was performed on a Shodex GPC-101 liquid chromatograph (Tokyo, Japan) equipped with two Shodex KF-804L mixed gel columns (300 × 8.0 mm; bead size = 7 μm ; pore size = 20–200 Å). The eluent was tetrahydrofuran (THF) or dimethyl formamide

(DMF) at a flow rate of 1.0 mL/min (THF) or 0.8 mL/min (DMF) (40 °C). The DMF eluent included LiBr (10 mM). Sample detection and quantification were conducted using a Shodex differential refractometer RI-101 calibrated with known concentrations of polymer in solvent. The column system was calibrated with standard poly(methyl methacrylate)s (PMMA)s. The crosslinked polymers were fractionated with a preparative GPC (LC-9204, Japan Analytical Industry, Tokyo) equipped GPC KF-2004 column (column size = 20 mmID × 300 mm). Chloroform was used as the eluent at a flow rate of 3 mL/min (room temperature). The NMR spectra were recorded on a Bruker (Germany) AV500 spectrometer (500 MHz) or Bruker BBFO400 spectrometer (400 MHz) at ambient temperature; Bruker AV500, ¹H: spectral width 5000.00 Hz, acquisition time 6.554 sec, and pulse delay 1.000 sec; Bruker BBFO400, ¹H: spectral width 4000.00 Hz, acquisition time 8.192 sec, and pulse delay 1.000 sec. CDCl₃ and acetone-*d*₆ were used as the solvents for the NMR analysis, and the chemical shift was calibrated using residual undeuterated solvents or tetramethylsilane (TMS) as the internal standard. The monomer conversions were determined from ¹H NMR spectra. The DLS measurement was carried out with a Malvern Zetasizer Nano ZSP (Worcestershire, UK). A temperature-controlled quartz cell was employed. The test angle for the DLS analysis was 173° (backscattering detection). THF was used as the solvent. In the case of the flower-like particle with a hydrophilic core (Table S1 (entry 3)), DMF was used as the solvent for the DLS measurement. The TEM images were obtained on a JEM-1400 transmission electron microscope (JEOL, Japan) operated at 100 kV. The TEM grid was carbon-coated on 200 mesh (copper) (Ted Pella, USA).

Synthetic Procedures

Synthesis of methyl 2-iodo-2-(4'-(2''-iodopropionyloxy)phenylacetate (I-MEPE-I)

To the solution of methyl 2-(4'-hydroxyphenyl)acetate (25 g, 150 mmol) in diethyl ether (100 mL), 2-bromopropionyl bromide (39 g, 180 mmol) in diethyl ether (50 mL) was

dropwisely added over 20 min at 0 °C and stirred at 25 °C for 30 min. The reaction mixture was washed with aqueous HBr (5%), saturated aqueous Na₂CO₃ solution, saturated aqueous NaHSO₃ solution, and water, and dried over Na₂SO₄. Removal of the solvent under reduced pressure afforded methyl 2-(4'-(2''-bromopropionyloxy)phenylacetate (95% yield), which was used in the subsequent reaction without further purification. ¹H NMR (CDCl₃): δ = 1.91 (d, 3H, CH₃CHBrCOO), 3.61 (s, 2H, CH₂COOCH₃), 3.67 (s, 3H, CH₂COOCH₃), 4.55 (q, 1H, CH₃CHBrCOO), 7.06 (d, 2H, PhH), 7.29(d, 2H, PhH). ¹³C NMR (CDCl₃): δ = 21.4 (CH₃CHBrCOO), 39.6 (CH₃CHBrCOO), 40.5 (CH₂COOCH₃), 52.1 (CH₂COOCH₃), 121.1 (Ph), 130.4 (Ph), 132.0 (Ph), 149.5(Ph), 168.7 (CH₃CHBrCOO), 171.6 (CH₂COOCH₃). *N*-bromosuccinimide (7.0 g, 39.6 mmol) was added to the solution of methyl 2-(4'-(2''-bromopropionyloxy)phenylacetate (9.9 g, 33 mmol) in dichloroethane (66 mL) and the mixture was reacted at 80 °C for 2 h under irradiation with an LED lamp. The reaction mixture was washed with saturated aqueous Na₂SO₃ solution and water, and dried over Na₂SO₄. Removal of the solvent under reduced pressure afforded methyl 2-bromo-2-(4'-(2''-bromopropionyloxy)phenylacetate (93% yield), which was used in the subsequent reaction without further purification. ¹H NMR (CDCl₃): δ = 1.92 (d, 3H, CH₃CHBrCOO), 3.77 (s, 3H, CHBrCOOCH₃), 4.55 (q, 1H, CH₃CHBrCOO), 5.33 (s, 1H, CHBrCOOCH₃), 7.12(d, 2H, PhH), 7.57 (d, 2H, PhH). ¹³C NMR (CDCl₃): δ = 21.4 (CH₃CHBrCOO), 39.4 (CH₃CHBrCOO), 45.4 (CHBrCOOCH₃), 53.5 (CHBrCOOCH₃), 121.5 (Ph), 130.1 (Ph), 133.7 (Ph), 151.0(Ph), 168.5 (CH₃CHBrCOO), 168.6 (CHBrCOOCH₃). The mixture of methyl 2-bromo-2-(4'-(2''-bromopropionyloxy)phenylacetate (9.0 g: 23.7 mmol) and NaI (28.4 g: 190 mmol) were stirred in acetonitrile (50 mL) at 0 °C for 1 h. The reaction mixture was diluted with dichloromethane, washed with saturated aqueous Na₂SO₃ solution, water and brine, and dried over Na₂SO₄. After removal of solvent under reduced pressure, the purification by flash chromatography (silica gel; ethyl acetate/hexane) afforded I-MEPE-I (12% yield). ¹H NMR (CDCl₃): δ = 2.05 (d, 3H,

CH_3CHICOO), 3.76 (s, 3H, CHICOOCH_3), 4.67 (q, 1H, CH_3CHICOO), 5.52 (s, 1H, CHICOOCH_3), 7.07 (d, 2H, PhH), 7.63 (d, 2H, PhH). ^{13}C NMR (CDCl_3): δ = 12.2 (CH_3CHICOO), 18.7 (CH_3CHICOO), 23.0 (CHICOOCH_3), 53.4 (CHICOOCH_3), 121.2 (Ph), 130.2 (Ph), 135.1 (Ph), 150.6 (Ph), 170.1 (CH_3CHICOO), 170.2 (CHICOOCH_3).

Synthesis of I-PBA-PMMA-PBMA-PBA-I (Table 2.1 (entry 1) and Figures 2.1 and 2.2)

Preparation of I-PMMA-I. A mixture of MMA (2.48 g, 8000 mM), I-MEPE-I (0.235 g, 160 mM) and ONI (0.147 g, 80 mM) in a Schlenk tube was heated at 60 °C for 3 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-I polymer with $M_n = 4100$ and $M_w/M_n = 1.13$ (monomer conversion = 68%). The reaction mixture was diluted with THF (15 mL). Then, the polymer was reprecipitated in hexane (150 mL) twice, giving a purified I-ME-PMMA-I with $M_n = 4100$ and $M_w/M_n = 1.13$.

Preparation of I-PMMA-PBMA-I. A mixture of BMA (1.76 g, 8000 mM), I-ME-PMMA-I (0.992 g, 160 mM) and ONI (0.073 g, 80 mM) in a Schlenk tube was heated at 60 °C for 6 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-PBMA-I block copolymer with $M_n = 7400$ and $M_w/M_n = 1.15$ (monomer conversion = 47%). The reaction mixture was diluted with THF (10 mL). Then, the polymer was reprecipitated in hexane (100 mL) twice, giving a purified I-ME-PMMA-PBMA-I with $M_n = 7800$ and $M_w/M_n = 1.15$.

Preparation of I-PBA-PMMA-PBMA-PBA-I. A mixture of BA (0.696 g, 8000 mM), I-ME-PMMA-PBMA-I (0.792 g, 160 mM) and ONI (0.129 g, 320 mM) in a Schlenk tube was heated at 110 °C for 24 h under argon atmosphere with magnetic stirring, yielding an I-PBA-PMMA-PBMA-PBA-I block copolymer with $M_n = 11800$ and $M_w/M_n = 1.33$ (monomer conversion = 59%). The reaction mixture was diluted with THF (5.0 mL). Then, the polymer was reprecipitated in hexane (50 mL) twice, giving a purified I-PBA-PMMA-PAMA-PBA-I with $M_n = 12800$ and $M_w/M_n = 1.27$.

Preparation of Janus-type particle (Table 2.1 (entry 2) and Figures 2.3 and 2.8)

Preparation of I-PMMA-I. A mixture of MMA (2.00 g, 8000 mM), I-MEPE-I (0.0631 g, 53 mM) and ONI (0.119 g, 80 mM) in a Schlenk tube was heated at 60 °C for 3 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-I polymer with $M_n = 7900$ and $M_w/M_n = 1.12$ (monomer conversion = 63%). The reaction mixture was diluted with THF (10 mL). Then, the polymer was reprecipitated in hexane (100 mL) twice, giving a purified I-ME-PMMA-I with $M_n = 8300$ and $M_w/M_n = 1.10$.

Preparation of I-PMMA-PAMA-I. A mixture of AMA (4.61 g, 8000 mM), I-ME-PMMA-I (1.01 g, 27 mM) and ONI (0.036 g, 13 mM) in a 100 mL flask was heated at 60 °C for 1.3 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-PAMA-I block copolymer with $M_n = 13000$ and $M_w/M_n = 1.19$ (monomer conversion = 12%). The reaction mixture was diluted with THF (15 mL). Then, the polymer was reprecipitated in hexane (150 mL) twice, giving a purified I-ME-PMMA-PAMA-I with $M_n = 13000$ and $M_w/M_n = 1.19$.

Preparation of I-PBA-PMMA-PAMA-PBA-I. A mixture of BA (10.6 g, 8000 mM), I-ME-PMMA-PAMA-I (1.08 g, 8 mM) and ONI (0.197 g, 32 mM) in a 100 mL flask was heated at 110 °C for 5 h under argon atmosphere with magnetic stirring, yielding an I-PBA-PMMA-PAMA-PBA-I block copolymer with $M_n = 22000$ and $M_w/M_n = 1.40$ (monomer conversion = 7%). The reaction mixture was diluted with THF (10 mL). Then, the polymer was reprecipitated in methanol/H₂O (= 2/1 (v/v), 100 mL) twice, giving a purified I-PBA-PMMA-PAMA-PBA-I with $M_n = 23000$ and $M_w/M_n = 1.34$.

Remove iodide of I-PBA-R-PMMA-PAMA-PBA-I with *n*-hexylamine. A mixture of I-PBA-PMMA-PAMA-PBA-I (1.00 g, 8000 mM) (10wt%), *n*-hexylamine (0.132 g, 24000 mM) and diglyme/1-butanol (45wt%/45wt%) in a 100 mL flask was heated at 100 °C for 12 h under argon atmosphere with magnetic stirring, yielding a CH₃(CH₂)₅NH-PBA-PMMA-

PAMA-PBA-NH(CH₂)₅CH₃ copolymer with $M_n = 22000$ and $M_w/M_n = 1.36$. The reaction mixture was diluted with THF (10 mL). Then, the polymer was reprecipitated in methanol/H₂O (= 2/1 (v/v), 100 mL), giving a purified CH₃(CH₂)₅NH-PBA-PMMA-PAMA-PBA-NH(CH₂)₅CH₃ with $M_n = 22000$ and $M_w/M_n = 1.36$.

Cross-linking reaction of CH₃(CH₂)₅NH-PBA-PMMA-PAMA-PBA-NH(CH₂)₅CH₃. A mixture of CH₃(CH₂)₅NH-PBA-PMMA-PAMA-PBA-NH(CH₂)₅CH₃ (0.778 g, 8 M, 30wt%), BPO (0.086 g, 80 M, 3wt%) and ethylbenzene (1.73 g, 67wt%) in a Schlenk tube was heated at 80 °C for 1 h under argon atmosphere with magnetic stirring, yielding a crosslinked polymer. The reaction mixture was diluted with THF (3.0 mL). Then, the polymer was reprecipitated in methanol/H₂O (= 2/1 (v/v), 100 mL), giving a purified crosslinked polymer (particle). Then, the polymer (particle) was fractionated with a preparative GPC.

Staining of the Janus-type particle with I₂. A mixture of the fractionated particle (0.150 g, 8 M, 10wt%), HSCH₂CH₂NH₂ (0.025 g, 1600 M), BPO (0.0391 g, 80 M) and acetonitrile (1.29 g) in a Schlenk tube was heated at 70 °C for 12 h under argon atmosphere with magnetic stirring. The reaction mixture was diluted with THF (1.0 mL). Then, the particle was reprecipitated in methanol/H₂O (= 2/1 (v/v), 20 mL), giving a purified particle containing the NH₂ functionality in the B segment. Then, the particle was immersed in an I₂/THF solution (10wt%) for 3 days to stain the B segment through the I₂/NH₂ halogen bonding. The particle solution was dropped on a TEM grid and dried, and the residual I₂ was removed under vacuum. The particle on the TEM grid was subjected to the TEM measurement.

Preparation of flower-like particle (Table 2.1 (entry 3) and Figures 2.4 and 2.9)

Preparation of I-PMMA-I. A mixture of MMA (3.00 g, 8000 mM), I-MEPE-I (0.036 g, 20 mM) and ONI (0.045 g, 20 mM) in a Schlenk tube was heated at 60 °C for 3 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-I polymer with $M_n = 12000$

and $M_w/M_n = 1.14$ (monomer conversion = 30%). The reaction mixture was diluted with THF (15 mL). Then, the polymer was reprecipitated in hexane (150 mL) twice, giving a purified I-ME-PMMA-I with $M_n = 12000$ and $M_w/M_n = 1.12$.

Preparation of I-PMMA-PBMA-I. A mixture of BMA (1.82 g, 8000 mM), I-ME-PMMA-I (0.311 g, 16 mM) and ONI (0.023 g, 24 mM) in a Schlenk tube was heated at 60 °C for 4 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-PBMA-I block copolymer with $M_n = 22000$ and $M_w/M_n = 1.16$ (monomer conversion = 16%). The reaction mixture was diluted with THF (7 mL). Then, the polymer was reprecipitated in hexane (70 mL) twice, giving a purified I-ME-PMMA-PBMA-I with $M_n = 22000$ and $M_w/M_n = 1.16$.

Preparation of I-PDGDA-PMMA-PBMA-PDGDA-I. A mixture of DGDA (0.393 g, 8000 mM), I-ME-PMMA-PBMA-I (0.081 g, 16 mM), ONI (0.044 g, 320 mM) and ethylbenzene (1.10 g) in a Schlenk tube was heated at 110 °C for 16 h under argon atmosphere with magnetic stirring, yielding an I-PDGDA-PMMA-PBMA-PDGDA-I block copolymer (particle) with $M_n = 59000$ and $M_w/M_n = 2.33$ (monomer conversion = 14%). The reaction mixture was diluted with THF (3.0 mL). Then, the polymer was reprecipitated in hexane (30 mL) twice, giving a purified I-PDGDA-PMMA-PBMA-PDGDA-I (particle), which was then fractionated with a preparative GPC.

Preparation of flower-like particle with longer sgement on the corona (242 A units and 137 B units) (Table 2.2 (entry 1) and Figures 2.5 and 2.10)

Preparation of I-PMMA-I. A mixture of MMA (3.00 g, 8000 mM), I-MEPE-I (0.0947 g, 5 mM) and ONI (0.0119 g, 5 mM) in a Schlenk tube was heated at 60 °C for 4 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-I polymer with $M_n = 25000$ and $M_w/M_n = 1.24$ (monomer conversion = 18%). The reaction mixture was diluted with THF (10 mL). Then, the polymer was reprecipitated in hexane (100 mL) twice, giving a purified I-ME-PMMA-I with $M_n = 25000$ and $M_w/M_n = 1.22$.

Preparation of I-PMMA-PBMA-I. A mixture of BMA (1.56 g, 8000 mM), I-ME-PMMA-I (0.186 g, 5 mM) and ONI (0.0652 g, 8 mM) in a Schlenk tube was heated at 60 °C for 4 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-PBMA-I block copolymer with $M_n = 44000$ and $M_w/M_n = 1.20$ (monomer conversion = 7%). The reaction mixture was diluted with THF (5 mL). Then, the polymer was reprecipitated in hexane (50 mL) twice, giving a purified I-ME-PMMA-PBMA-I with $M_n = 44000$ and $M_w/M_n = 1.20$.

Preparation of I-PDGDA-PMMA-PBMA-PDGDA-I. A mixture of DGDA (0.324 g, 8000 mM), I-ME-PMMA-PBMA-I (0.133 g, 16 mM), ONI (0.0359 g, 320 mM) and ethylbenzene (1.17 g) in a Schlenk tube was heated at 110 °C for 17 h under argon atmosphere with magnetic stirring, yielding an I-PDGDA-PMMA-PBMA-PDGDA-I block copolymer (particle) with $M_n = 92000$ and $M_w/M_n = 2.48$ (monomer conversion = 13%). The reaction mixture was diluted with THF (3.0 mL). Then, the polymer was reprecipitated in hexane (30 mL) twice, giving a purified I-PDGDA-PMMA-PBMA-PDGDA-I (particle), which was then fractionated with a preparative GPC.

Preparation of flower-like particle with shorter sengerment on the corona (53 A units and 30 B units) (Table 2.2 (entry 2) and Figures 2.6 and 2.11)

Preparation of I-PMMA-I. A mixture of MMA (2.00 g, 8000 mM), I-MEPE-I (0.0947 g, 80 mM) and ONI (0.0593 g, 40 mM) in a Schlenk tube was heated at 60 °C for 3 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-I polymer with $M_n = 5900$ and $M_w/M_n = 1.17$ (monomer conversion = 54%). The reaction mixture was diluted with THF (10 mL). Then, the polymer was reprecipitated in hexane (100 mL) twice, giving a purified I-ME-PMMA-I with $M_n = 6400$ and $M_w/M_n = 1.12$.

Preparation of I-PMMA-PBMA-I. A mixture of BMA (2.70 g, 8000 mM), I-ME-PMMA-I (0.809 g, 53 mM) and ONI (0.0375 g, 27 mM) in a Schlenk tube was heated at 60 °C for 3 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-PBMA-I

block copolymer with $M_n = 10000$ and $M_w/M_n = 1.17$ (monomer conversion = 21%). The reaction mixture was diluted with THF (15 mL). Then, the polymer was reprecipitated in hexane (150 mL) twice, giving a purified I-ME-PMMA-PBMA-I with $M_n = 11000$ and $M_w/M_n = 1.15$.

Preparation of I-PDGDA-PMMA-PBMA-PDGDA-I. A mixture of DGDA (0.509 g, 8000 mM), I-ME-PMMA-PBMA-I (0.0523 g, 16 mM), ONI (0.0565 g, 320 mM) and ethylbenzene (2.00 g) in a Schlenk tube was heated at 110 °C for 24 h under argon atmosphere with magnetic stirring, yielding an I-PDGDA-PMMA-PBMA-PDGDA-I block copolymer (particle) with $M_n = 43000$ and $M_w/M_n = 5.67$ (monomer conversion = 14%). The reaction mixture was diluted with THF (5.0 mL). Then, the polymer was reprecipitated in hexane (50 mL) twice, giving a purified I-PDGDA-PMMA-PBMA-PDGDA-I (particle), which was then fractionated with a preparative GPC.

Preparation of flower-like particle with a hydrophilic core (Table 2.2 (entry 3) and Figures 2.7 and 2.12)

Preparation of I-PPEGDA-PMMA-PBMA-PPEGDA-I. I-ME-PMMA-I ($M_n = 12000$ and $M_w/M_n = 1.12$) and I-ME-PMMA-PBMA-I ($M_n = 22000$ and $M_w/M_n = 1.16$) were synthesized as shown in Table 2.1 (entry 3). A mixture of PEGDA (1.47 g, 8000 mM), I-ME-PMMA-PBMA-I (0.0923 g, 16 mM), ONI (0.0498 g, 320 mM) and ethylbenzene (0.234 g) in a Schlenk tube was heated at 110 °C for 24 h under argon atmosphere with magnetic stirring, yielding an I-PPEGDA-PMMA-PBMA-PPEGDA-I block copolymer (particle) with $M_n = 70000$ and $M_w/M_n = 6.29$ (monomer conversion = 9%). The reaction mixture was diluted with CHCl_3 (6 mL). Then, the polymer was purified and fractionated with a preparative GPC.

Table 2.2. Synthesis of the flower-like particles with different chain lengths on the corona and with a hydrophilic core.

Entry	Monomer	I-R-I	$[M]_0/[I-R-I]_0/[ONI]_0$ (mM) ^[a]	T (°C)	t (h)	conv (%)	M_n (GPC) ($M_{n,theo}$) ^[b]	DP (¹ H NMR) ^[c]	PDI (GPC)
1	MMA	I-MEPE-I	8000/5/5	60	4	18	25000 (28000)	–	1.24
							After purification	25000	1.22
	BMA	I-PMMA-I	8000/5/8	60	4	7	44000 (43000)	–	1.20
							After purification	44000	1.20
	DGDA	I-PMMA-PBMA-I	8000/16/320	110	17	13	92000	+ 137	2.48
								+ 66	
2	MMA	I-MEPE-I	8000/80/40	60	3	54	5900 (5900)	–	1.17
							After purification	6400	1.12
	BMA	I-PMMA-I	8000/53/27	60	3	21	10000 (11000)	–	1.17
							After purification	11000	1.15
	DGDA	I-PMMA-PAMA-I	8000/16/320	110	24	14	43000	+ 70	5.67
3	MMA	I-MEPE-I	8000/20/20	60	3	30	12000 (12000)	–	1.14
							After purification	12000	1.12
	BMA	I-PMMA-I	8000/16/24	60	4	16	22000 (23000)	–	1.16
							After purification	22000	1.16
	PEGDA	I-PMMA-PBMA-I	8000/16/320	110	24	9	70000 ^[d]	+ 46	6.29 ^[d]

[a] M = monomer. [b] Theoretical M_n calculated with $M_n = ([M]_0/[I-R-I]_0) \times \text{conversion} \times (\text{molecular weight of monomer}) + (\text{molecular weight of I-R-I})$. [c] DP obtained from the peak areas of the initiating PE unit (phenyl group) and the monomer units (with $\pm 10\%$ experimental error). [d] The M_n and PDI of I-PPEGDA-PMMA-PBMA-PPEGDA-I were obtained with PMMA-calibrated DMF-GPC. PMMA-calibrated THF-GPC was used for the other polymers.

¹H NMR Spectral Data

(a)

(b)

(c)

Figure 2.8. ¹H NMR spectra (acetone-*d*₆) for the synthesis of the Janus-type particle: (a) I-PMMA-I, (b) I-PMMA-PAMA-I, and (c) I-PBA-PMMA-PAMA-PBA-I (Table 2.1 (entry 2)).

Figure 2.9. ^1H NMR spectra (acetone- d_6) for the synthesis of the flower-like particle: (a) I-PMMA-I, (b) I-PMMA-PBMA-I, and (c) I-PDGDA-PMMA-PBMA-PDGDA-I (Table 2.1 (entry 3)).

Figure 2.10. ^1H NMR spectra (CDCl_3) for the synthesis of the flower-like particle with a longer segment on the corona: (a) I-PMMA-I, (b) I-PMMA-PBMA-I, and (c) I-PDGDA-PMMA-PBMA-PDGDA-I (Table 2.2 (entry 1)).

Figure 2.11. ^1H NMR spectra (CDCl_3) for the synthesis of the flower-like particle with a shorter segment on the corona: (a) I-PMMA-I, (b) I-PMMA-PBMA-I, and (c) I-PDGDA-PMMA-PBMA-PDGDA-I (Table 2.2 (entry 2)).

Figure 2.12. ^1H NMR spectra (acetone- d_6) of the flower-like particle with a hydrophilic core: I-PPEGDA-PMMA-PBMA-PPEGDA-I (Table 2.2 (entry 3)).

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Chapter 3. Temperature-Directed Micellar Morphological Transformation Using CABC-Block Copolymers and its Applications in Encapsulation and Hidden Segment

Abstract: I developed a temperature-directed micellar morphological transformation using CABC multi-block copolymers with a hydrophobic block A, a hydrophilic block B, and a thermally responsive block C with a lower critical solution temperature (LCST). The micellar structure was switched from a star (below LCST) to a flower (above LCST). The transition-temperature was tuneable in a wide range (11-90 °C) by varying the C monomer composition. The large difference in the loading capacity between the star and flower enabled efficient encapsulation and controlled release of external molecules. Unlike conventional systems, the present star-to-flower transformation keeps micellar structures and hence does not liberate polymers but only external molecules selectively. Another application is a hidden functional segment. A functional segment is hidden (shielded) below LCST and exposed to interact with external molecules or surfaces above LCST, which may serve as a new temperature-directed interface for, e.g., biological and sensing applications.

3.1. Introduction.

Morphological transformation of polymer nanoparticles, where the particle structures reversibly change in response to external stimuli such as temperature, light, and pH, has attracted great attention for diversifying smart responsive materials in, e.g., bio-sensor, fluorescence thermometer, and drug delivery applications.¹⁻⁷ Such morphological transformation enables the control of physicochemical properties of the nanoparticles such as rheological, optical, encapsulation, and bio-interaction properties.⁸⁻¹¹

Block copolymers are of growing interest for preparing self-assembled nanostructures such as micelles, rods, and vesicles.¹² Their morphological transformation such as micellar inversion,¹³ micelle-to-vesicle transformation,^{14,15} and assembly-dissolution conversion¹⁶⁻²² are widely studied using di- and tri-block copolymers.^{12,23,24} However, there are scarce examples studying morphological transformation of nanostructures prepared with more complex multi-block copolymers such as CABC tetra-block copolymers.²⁵⁻²⁷

Living (or reversible-deactivation) radical polymerization²⁸⁻³⁶ is a useful method for synthesizing block copolymers. Our research group developed an organocatalyzed living radical polymerization using an alkyl iodide ($R-I$) as an initiator and an organic molecule as a catalyst.³⁷⁻⁴¹ In this system, polymer-iodide dormant species ($Polymer-I$) reversibly generates the propagating radical ($Polymer^{\bullet}$) by the work of the organic catalyst (Scheme 2.1b). The catalysts include organic salts such as tetrabutylammonium iodide ($Bu_4N^+I^-$).³⁸ Previously, I studied a temperature-selective radical generation from a newly designed initiator, i.e., alkyl diiodide ($I-R^2-R^1-I$) ($I-MEPE-I$ in Scheme 2.2).⁴² Because of the largely different reactivities of $I-R^2$ and R^1-I , this initiator can selectively initiate from the R^1-I site at a mild temperature (60 °C) and from the $I-R^2$ site at an elevated temperature (110 °C), opening up the synthesis of unique CABC asymmetric multi-block copolymers (Scheme 2.2).

Scheme 3.2. (a) Reversible morphological transformation between star micelle (left) and flower micelle (right) self-assembled by CABC multi-block copolymer. (b) Reversible encapsulation and release of external hydrophobic molecules. (c) Hidden and exposed functional segment.

The present work aims to use CABC block copolymers to create novel micellar morphological transformation systems, which is a new topic. The nanostructures are reversibly switched from Janus-type star micelles to symmetric flower-like micelles directed by temperature (Scheme 3.2a). Block A is a hydrophobic segment (coloured in blue in Scheme 3.2a), block B is a hydrophilic segment (red), and block C is a thermosensitive segment (green) having a lower critical solution temperature (LCST) in water. Below LCST, block C is soluble

in water. Hence, only block A is insoluble to form a core, which is expected to give a Janus-star micelle with blocks BC and C in the shell (Scheme 3.2a). Above LCST, block C is insoluble in water. Blocks A and C are insoluble to form a core, which is expected to give a flower micelle with block B in the shell (petal) (Scheme 3.2a). To form the flower as expected, blocks A and C should show good compatibility. This type of star-flower transformation is achievable by combining a hydrophobic block A to retain the core, a hydrophilic block B to retain the shell, and a thermosensitive block C to switch the morphology, and hence is unique to CABC block copolymers. ABC tri-block copolymers might be an alternative but can not give an asymmetric star.

The star-flower transformation can tune physicochemical properties of the nanostructures. The large difference in the core size between the star and flower (Scheme 3.2a) can bring a large difference in the loading capacity in the core (Scheme 3.2b) and is expected to give the reversible encapsulation and release of external molecules. This system is unique in that, in the release process, the system is expected to liberate only external molecules but not to liberate the polymer in the solvent, because the polymer can remain forming the nanostructure. This system is not a mere aggregation-dissolution system, where the polymer is liberated (dissolved in the solvent) as a contaminant in the release process, but a novel system that retains the nanostructure, where only external molecules are liberated.

Another interesting application is a hidden functional segment. In the star, block B (red segment) is expected to be shielded (hidden) by block C (green segment) (Scheme 3.2c). Upon heating, because of the transformation to the flower, block B is expected to be exposed (Scheme 3.2c). Recently, a few elegant works were reported on hidden segments on nanostructures by mixing two different diblock copolymers.^{43,44} In the present work, only a single polymer (CABC block copolymer) is used. The hidden segment can interact with external molecules or surfaces, providing a novel interface on the nanostructure. The present thesis describes the

encapsulation- release and the hidden segment as unique applications of CABC block copolymers.

Figure 3.1. (a) The deprotection of PEEA-PMMA-PSMA-PEEA to afford PEEA-PMMA-PGLMMA-PEEA. (b) GPC (DMF eluent) curves for the synthesis of PEEA-PMMA-PGLMMA-PEEA (Table 3.1 (entries 1, 2, and 3-1)). For I-PMMA-I, the chromatogram of polymer overlapped with those of small molecules or LiBr-cluster in the DMF eluent in the low-molecular-weight region. To avoid the overlap, I-PMMA-I was also analyzed with THF-GPC, giving accurate M_n (= 3000) and D (= 1.10) values, and these values are given in Figure 3.1b and Table 3.1 (entry 1). For the GPC curve comparison (Figure 3.1b), the DMF-GPC curves are given for I-PMMA-I and the three other polymers.

3.2. Results and Discussion.

Synthesis of CABC Block Copolymer. Figure 3.1a shows the synthesis of a CABC block copolymer. The precursor CAB'C block copolymer was prepared by using methyl methacrylate (MMA) (block A), solketal methacrylate (SMA) (block B'), and 2-(2-ethoxyethoxy)ethyl acrylate (EEA) (block C) (Table 3.1 (entries 1, 2, and 3-1) and Figure 3.1b). A mixture of MMA (50 equiv), I-MEPE-I (1 equiv) (alkyl di-iodide initiator), and

tetraoctylammonium iodide (ONI) (0.5 equiv) (catalyst) was heated at 60 °C, yielding an I-ME-PMMA-I polymer with $M_n = 3000$ (25 monomer units of MMA) and dispersity $\mathcal{D}$ ($= M_w/M_n$) = 1.10 after the purification by reprecipitation, where PMMA is poly(methyl methacrylate) and M_n and M_w are the number- and weight-average molecular weights, respectively. The M_n and M_w values were obtained with PMMA-calibrated GPC, and the number of the monomer units was obtained by ^1H NMR (from the signal areas of the monomer units and the initiator (phenyl group)) (Experimental and Calculation Section) throughout the thesis. ^1H NMR showed that 100% of the PE-I site initiated and 97% of the I-ME site remained unreacted, demonstrating the high selectivity of the initiation from the two sites (Experimental and Calculation Section, Figure 3.10b). The purified I-ME-PMMA-I was used as a macroinitiator in the polymerization of SMA at 60 °C to obtain a diblock copolymer I-ME-PMMA-PSMA-I with $M_n = 9300$ (27 monomer units of SMA) and $\mathcal{D} = 1.24$ after the purification, where PSMA is poly(solketal methacrylate). The GPC traces (Figure 3.1b) show that a large fraction of the macroinitiator was extended to the diblock copolymer, confirming the high block-efficiency of the PMMA-I site of the macroinitiator. 95% of the I-ME site remained unreacted after the two polymerizations (Experimental and Calculation Section, Figure 3.10c). In the third step, the purified I-ME-PMMA-PSMA-I was used as a macroinitiator in the polymerization of EEA at an elevated temperature 110 °C, yielding an I-PEEA-PMMA-PSMA-PEEA-I tetrablock copolymer with $M_n = 48000$ (82 monomer units in each EEA segment) and $\mathcal{D} = 1.52$ after purification, where PEEA is poly(2-(2-ethoxyethoxy)ethyl acrylate) (Experimental and Calculation Section, Figure 3.10d). Subsequently, the obtained precursor CAB'C copolymer was hydrolyzed to remove the acetal group in the B' segment to produce the desired CABC block copolymer (PEEA-PMMA-PGLMMA-PEEA) with $M_n = 26000$ and $\mathcal{D} = 1.48$ after purification, where PGLMMA is poly(glycerol monomethacrylate) (Experimental and Calculation Section, Figure 3.10e).

PMMA (block A) is hydrophobic, and PGLMMA (block B) is hydrophilic. PEEA (block C) is temperature-responsive.⁴⁵

Table 3.1. Synthesis of CABC multi-block copolymers.

Entry	Monomer	I-R-I	[M] ₀ /[I-R-I] ₀ /[ONI] ₀ (mM) ^[a]	T (°C)	t (h)	conv (%)	M _n (GPC) ^[b] (M _{n,theo}) ^[c]	DP (¹ H NMR) ^[d]	D (GPC) ^[b]
1	MMA	I-MEPE-I	8000/160/80	60	2	57	2900 (3000)		1.11
					After purification		3000	25	1.10
2	SMA	I-PMMA-I	8000/80/80	60	4	30	8800 (8400)		1.28
					After purification		9300	+ 27	1.24
3-1	EEA	I-PMMA-PSMA-I	8000/13/52	110	19	29	48000 (39000)		1.53
					After purification		48000	+ 164	1.52
					After deprotection		26000		1.48
3-2	EEA/PEGA	I-PMMA-PSMA-I	(7600/400)/11/44	110	22	23	46000 (40000)		1.61
					After purification		49000	+ 146/8	1.57
					After deprotection		28000		1.48
3-3	EEA/PEGA	I-PMMA-PSMA-I	(7200/800)/9/36	110	22	17	46000 (42000)		1.56
					After purification		47000	+139/15	1.52
					After deprotection		29000		1.54
3-4	EEA/PEGA	I-PMMA-PSMA-I	(6400/1600)/9/36	110	24	15	45000 (46000)		1.61
					After purification		50000	+122/30	1.56
					After deprotection		31000		1.48
3-5	EEA/PEGA	I-PMMA-PSMA-I	(5600/2400)/8/32	110	24	15	49000 (43000)		1.44
					After purification		50000	+88/37	1.53
					After deprotection		28000		1.43
3-6	EEA/PEGA	I-PMMA-PSMA-I	(4800/3200)/7/28	110	24	13	50000 (44000)		1.51
					After purification		49000	+70/47	1.54
					After deprotection		29000		1.50
3-7	EEA/PEGA	I-PMMA-PSMA-I	(4000/4000)/7/28	110	24	12	37000 (47000)		1.60
					After purification		40000	+58/58	1.59
					After deprotection		28000		1.52
3-8	PEGA	I-PMMA-PSMA-I	8000/7/28	110	36	8	33000 (52000)		1.53
					After purification		35000	+90	1.51
					After deprotection		28000		1.53

^[a]M = monomer, ^[b]PMMA-calibrated value by THF-GPC for entry 1 and DMF-GPC for all other entries, ^[c]Theoretical M_n calculated with M_n = ([M]₀/[I-R-I]₀) × conversion × (molecular weight of monomer) + (molecular weight of I-R-I), ^[d]DP obtained from the peak areas of the initiating phenyl group and the monomer units (with ±10% experimental error).

Figure 3.2. (a and b) DLS analysis of PEEA-PMMA-PGLMMA-PEEA in an aqueous solution at different temperatures. TEM image of the particles generated at (c) 4 °C and (d) 21 °C (1 wt% polymer in water/DMF = 10/1 (v/v)). For (c), PMMA and PEEA domains are not able to be clearly distinguished with TEM and the blue-coloured domain given in the figure is just for the guide for the eyes.

Temperature-Directed Micellar Morphological Trans-formation. The self-assemblies of the obtained block copolymer were studied. I used *N,N*-dimethyl formamide (DMF) to dissolve all of the three segments and water to aggregate the hydrophobic segments to generate the assemblies. The polymer was first homogeneously dissolved in DMF, to which water was added to generate the assemblies (1 wt% polymer in water/DMF = 10/1 (v/v)). If PEEA (block C) is hydrophilic, the star is expected to form (Scheme 3.2a and Figure 3.2a). The dynamic light scattering (DLS) analysis (Figure 3.2a) showed that the hydrodynamic size (diameter) of the assembly was 21 nm (peak top) at a low temperature 4 °C. PEEA is expected to be hydrophilic at 4 °C, because the LCST of PEEA in the studied solution is 11 °C as shown below. The contour length of the CABC copolymer (216 units) is 54 nm (= 0.25 nm x 216 units). The particle size (21 nm) was reasonably smaller than the contour length, indicating that the assembly was a micelle (sphere) (not vesicle) and that the aggregation among the micelles was negligible. Figure 3.2c shows the TEM image of the micelle. PGLMMA (block B) was stained with FeCl₂ through a strong affinity between 1,2-diol groups in PGLMMA and Fe²⁺ (Experimental Section below). The dark domain in the TEM image (PGLMMA segment) was surrounded by the light grey domain (PMMA and PEEA segments), which is consistent with the expected star structure (Figure 3.2a).

Above LCST, PEEA is hydrophobic, and the nanostructure is expected to be the flower (Figure 3.2a). Arising the temperature to 24 °C (above LCST), the hydrodynamic size of the assembly changed (from 21 nm) to 48 nm, indicating the change in the morphology. The TEM image (Figure 3.2d) of the assembly prepared at 21 °C (above LCST) shows that the dark domain (PGLMMA segment) covers the whole surface in each sphere, which is consistent with the expected flower structure (Figure 3.2a).

Figure 3.2b shows the change in the hydrodynamic size with temperature. A sharp change in the size was observed between 9 °C (24 nm) and 12 °C (51 nm), showing the

morphological change by the LCST transition. I determined the LCST to be 11°C at a half change in the size (38 nm = (24+51)/2 nm), which is consistent with the LCST (11 °C) of the PEEA homopolymer (not CABC block copolymer) as determined below. Importantly, the change in the micellar size was reversible upon the heating (square in Figure 3.2b) and cooling (triangle) processes, demonstrating the reversible morphological transformation. The sizes of the flower (48-51 nm) and the star (21-24 nm) were considerably different, suggesting that the morphological transition may accompany with an inter-particle re-organization. The flower size (48-51 nm) was still within the contour length of the polymer (54 nm), indicating negligible vesicle formation. According to the diameter of the star and flower, one single flower could transform to around 12 stars.

The formation of the star and flower was further rationalized in two ways using ¹H NMR and fluorescence. Figure 3.3a shows the variable-temperature (VT) ¹H NMR spectra of the CABC block copolymer in D₂O/dimethyl sulfoxide (DMSO)-*d*₆ (v/v = 10/1). At 4 °C (below LCST), I clearly observed the characteristic proton peaks of PEEA (a methine proton **a** at 2.25-2.05 ppm and methylene protons **b** at 4.04-3.82 ppm), meaning that PEEA was dissolved in D₂O and located in a hydrated shell. This result matches the expected star structure (Scheme 3.2a). The two characteristic peaks became smaller with an increase in temperature from 4 °C (below LCST) to 24 °C (above LCST), meaning that PEEA was undissolved in D₂O and formed the core. This result also agrees with the expected flower structure (Scheme 3.2a).

The other way is to use a special fluorophore, i.e., 4-(*N,N*-dimethylaminosulfonyl)-7-(2-aminoethylamino)-2,1,3-benzoxadiazole (DBDED), whose fluorescence is quenched in an aqueous environment (quenched by hydrogen bonding).⁴⁶ The iodides at the CABC polymer chain ends were converted to DBDED (Figure 3.3b and Experimental and Calculation Section).^[47] At 24 °C (above LCST), the micellar solution exhibited a strong fluorescence (Figure 3.3b (square)), since the DBDED chain ends and hence PEEA (block C) segments were

in the hydrophobic core of the flower. At 4 °C (below LCST), the fluorescence intensity significantly dropped, suggesting that the DBDED chain ends and hence PEEA segments extended to the aqueous environment and formed the hydrated shell of the star. This change in the fluorescence intensity clearly demonstrates the change in the location of the PEEA segments, supporting the expected flower and star structures (Scheme 3.2a). A sharp change in the fluorescence intensity was observed between 10 °C and 15 °C (Figure 3.3b (square) (left vertical axis)), which is also consistent with the micellar size transition observed with DLS between 11 °C and 14 °C (Figure 3.3b (triangle) (right vertical axis)). The assembly size at 24 °C (above LCST) increased from 48 nm without DBDED (Figure 3.2b) to 68 nm with DBDED (Figure 3.3b) in the solution. I suppose that the assembly with DBDED was still the flower but that the core tended to be swollen because DBDED is hydrophilic. The TEM image in the dry state (Figure 3.4) showed similar assembly sizes with and without DBDED, suggesting that the attachment of DBDED did not significantly impact the actual assembly size (and hence the morphology) at 24 °C. The assembly size at 4 °C (below LCST) was unchanged with and without DBDED (21 nm (Figures 3.2b and 3.3b)), suggesting no impact of the attachment of DBDED on the morphology (star) at 4 °C. In the star, DBDED is located in the already fully hydrated shell and would not further swell the shell. The observed increase in the flower size and no change in the star size in the solution would also be consistent with the locations of DBDED. The mentioned DLS, TEM, ^1H NMR, and fluorescence results proved the successful star-flower transformation directed by temperature.

Figure 3.3. (a) Variable-temperature (VT) ^1H NMR spectra (1 wt% polymer in $\text{D}_2\text{O}/\text{DMSO-}d_6 = 10/1$ (v/v)) of PEEA-PMMA-PGLMMA-PEEA. (b) Fluorescence intensity and particle size of DBDED-

PEEA-PMMA-PGLMMA-PEEA-DBDED in an aqueous solution at different temperatures ($\lambda_{\text{ex}} = 450$ nm for fluorescence) (1 wt% polymer in water/DMF = 10/1 (v/v)).

Figure 3.4. TEM images of particles generated with (a) PEEA-PMMA-PGLMMA-PEEA and (b) DBDED-PEEA-PMMA-PGLMMA-PEEA-DBDED (1 wt% polymer in water/DMF = 10/1 (v/v)) at 21 °C. The particles were not stained with Fe^{2+} .

The mentioned assemblies were studied in the water/DMF mixture (10/1 (v/v)). By removing DMF through dialysis, I also studied the assemblies in pure water. I observed a sharp change in the size between 11 °C and 14 °C with DLS (Figure 3.5). The assembly sizes in pure water (14-18 nm (star) and 28-36 nm (flower) (Figure 3.5)) were smaller than those in the water/DMF mixture (21-24 nm (star) and 48-51 nm (flower) (Figure 3.2b)), because the hydrophobic cores would shrink more in the absence of DMF (pure water). The assemblies described below were studied in the water/DMF mixture.

Figure 3.5. DLS results of PEEA-PMMA-PGLMMA-PEEA at different temperatures in pure water (1 wt% polymer).

Tailoring of Transition Temperature. The transition temperature was able to tailor by changing the monomer composition of block C. I used poly(ethylene glycol) methyl ether acrylate (PEGA) as a co-monomer of EEA to provide EEA/PEGA random copolymers in block C. The EEA/PEGA composition was varied from 100/0 to 50/50. With an increase in the hydrophilic PEGA composition, LCST increased from 11 °C (EEA/PEGA = 100/0) to 81 °C (EEA/PEGA = 50/50) (Figure 3.6 and Figure 3.7b (circle)) for the EEA/PEGA random copolymers (not CABC multi-block but only block C polymers). The LCST of the CABC multi-block copolymer was slightly higher than that of the only block C polymer due to the presence of the other segments (A and B blocks). With an increase in the hydrophilic PEGA composition, the LCST of the CABC multi-block copolymer increased from 11 °C (EEA/PEGA = 100/0) to 90 °C (EEA/PEGA = 60/40), and no transition was observed at EEA/PEGA = 50/50 (Figures 3.7a and 3.7b (square) and Table 3.1 (entries 3-1 to 3-7)).

Therefore, I were able to tune the morphological transition in a wide range of temperature (between 11 °C and 90 °C).

Figure 3.6. DLS analysis of PEEA_m/PPEGA_n random copolymers (m/n = 100/0, 95/5, 90/10, 80/20, 70/30, 60/40, and 50/50) at different temperatures (1 wt% polymer in water/DMF = 10/1 (v/v)).

(a) CABC block copolymers

Figure 3.7. (a) DLS analysis of PEEA_m/PPEGA_n-PMMA-PGLMMA-PEEA_m/PPEGA_n (m/n = 100/0, 95/5, 90/10, 80/20, 70/30, 60/40, and 50/50) at different temperatures. (b) LCST of PEEA_m/PPEGA_n-PMMA-PGLMMA-PEEA_m/PPEGA_n (CABC) block copolymers and PEEA_m/PPEGA_n (only block C) random copolymers (1 wt% polymer in water/DMF = 10/1 (v/v)).

Figure 3.8. (a) Reversible encapsulation and release of hydrophobic Nile Red through the morphological transformation. (b) Fluorescence intensity of Nile Red at different temperatures, (c) fluorescence spectra of Nile Red at 24 °C and 4 °C, and (d) fluorescence intensity of Nile Red at 24 °C and 4 °C altered in a recycled manner, using PEEA-PMMA-PGLMMA-PEEA (1 wt% polymer in water/DMF = 10/1 (v/v), $\lambda_{\text{ex}} = 550 \text{ nm}$).

Reversible Encapsulation and Release of External Molecule. An interesting application of this morphological transformation is the reversible encapsulation and release of external molecules. As mentioned above, in the release process, unlike mere micellization-dissolution systems, this system does not liberate the polymer but liberates only external molecules, which is a unique and useful aspect in applications. I studied the encapsulation and release of a hydrophobic fluorescence dye Nile Red (Figure 3.8a). To a DMF solution of Nile Red and CABC block copolymer (PEEA-PMMA-PGLMMA-PEEA with LCST of 11 °C), I added water (1 wt% polymer in water/DMF = 10/1 (v/v)), vigorously stirred for 1 h, and removed a precipitated excess amount of Nile Red using a filter (pore size = 450 nm) at room temperature (21 °C) (above LCST). At room temperature (above LCST), both PMMA (block A) (25 units) and PEEA (block C) (164 units) segments formed the core and encapsulated the hydrophobic Nile Red (Figure 3.8a), and hence a strong fluorescence was observed (Figure 3.8b). By cooling down (below LCST), PEEA (block C) became hydrophilic and released the hydrophobic Nile Red (Figure 3.8a). Because the fluorescence of the released Nile Red was partially quenched in an aqueous environment, the fluorescence intensity was reduced (Figure 3.8b). This result demonstrates the release of Nile Red. Additionally, the maximum fluorescence peak shifted from 627 nm (more hydrophobic environment) at 24 °C to 639 nm (more aqueous environment) at 4 °C (Figure 3.8c).

Importantly, the morphological change was reversible. Upon heating from 4 °C to 24 °C, the flower (large loading capacity) was regenerated, recovering the high fluorescence intensity (Figure 3.8d). After 7 repeating cycles, approximately 73% of Nile Red was still re-encapsulated successfully (629 arbitrary intensity (eighth run) / 857 arbitrary intensity (first run) = 73%) (Figure 3.8d). Thus, I successfully demonstrated the reversible encapsulation and release of external molecules in a controlled manner using this thermal responsive morphological change.

I also prepared a Nile-Red-containing purified star (i.e., I removed extra Nile Red at 0-4 °C (below LCST)). The fluorescence intensity for the Nile-Red-containing purified star (Figure 3.8b (triangle) at 4 °C) was 27% of that for the Nile-Red-containing flower (Figure 3.8b (circle) at 24 °C) ($230 \text{ arbitrary intensity} / 857 \text{ arbitrary intensity} = 27\%$), suggesting that approximately 27% of Nile Red was retained in the PMMA core in the release process in the first run.

Hidden Segment and Interaction with External Substrate. Another interesting application is a hidden functional segment. The PGLMMA segment (block B) with a diol functionality was used as the hidden segment (colored in red in Scheme 3.2c). Below LCST, PGLMMA is shielded (hidden) by hydrophilic block C (colored in green in Scheme 3.2c), because block C forms the outermost surface of the particle. PGLMMA is difficult to contact external substrates because of the steric hindrance of block C. In contrast, above LCST, block C becomes hydrophobic and forms the core. PGLMMA is exposed to the outermost surface (Scheme 3.2c) and is able to interact with external substrates.

I studied CABC block copolymers with four different EEA/PEGA compositions in block C (100/0 (polymer **1** (LCST = 11 °C)), 95/5 (polymer **2** (LCST = 20 °C)), 80/20 (polymer **3** (LCST = 64 °C)), and 0/100 (polymer **4** (no LCST)) (Table 3.1 (entries 3-1, 3-2, 3-4, and 3-8)). The self-assembly was prepared in an aqueous buffer solution at pH ~10 (1 wt% polymer in buffer water/DMF (10/1)). At a low temperature 8 °C, all of the four polymers formed the star with shielded PGLMMA. At a higher temperature 24 °C, polymers **1** and **2** turned to form the flower with exposed PGLMMA, while polymers **3** and **4** kept forming the star with shielded PGLMMA.

Figure 3.9. (a) No attachment of the star to the QCM sensor due to the hidden block B (left), attachment of the flower to the QCM sensor due to the exposed block B (middle), and release of the flower from the QCM sensor in an acidic condition (right). (b and c) QCM frequency change for PEEA_m/PPEGA_n-PMMA-PGLMMA-PEEA_m/PPEGA_n block copolymers with $m/n = 100/0, 95/5, 80/20$, and $0/100$ at (b) 8 °C and (c) 24 °C (1 wt% polymer in buffer water/DMF = 10/1 (v/v)) at pH ~10. The pH was decreased to ~5 after 2000 s for $m/n = 100/0$ in (c).

The interaction of the micelle with an external substrate was studied with quartz crystal microbalance (QCM). I studied the interaction between the micelle and the QCM sensor surface. A gold-coated QCM sensor was fabricated with a boronic acid (Figure 3.9a) by immersing the sensor in an ethanol solution of 4-mercaptophenylboronic acid (Experimental and Calculation Section). Upon the exposure, PGLMMA segment can chemically attach on the sensor surface through a condensation of the diol in PGLMMA and the boronic acid on the sensor surface (Figure 3.9a). Figures 3.9b and 3.9c show the QCM frequency change at 8 °C and 24 °C, respectively, where time zero is a time when the micelle solution was injected to the QCM cell. At 8 °C, PGLMMA was shielded for all of the four polymers, giving no change in the frequency (no attachment of the micelle on the QCM sensor) (Figure 3.9b). No obvious

change was observed even after 2000 s, meaning that PGLMMA was shielded in a stable manner. By heating to 24 °C, the frequency gradually changed with time for polymers **1** and **2** (Figure 3.9c (red and orange lines)), meaning that PGLMMA was exposed and reacted with the boronic acid to attach on the sensor surface. After 2000 s, the frequency change (ΔF) was 15.1 and 10.1 Hz for polymers **1** and **2**, respectively, which correspond to 89 and 59 ng/cm² mass changes according to the Sauerbrey equation⁴⁸ (Experimental and Calculation Section). The theoretically possible maximum mass change is calculated to be 1500-2900 and 1400-2700 ng/cm², respectively, assuming that the micelle is a swollen sphere (density = 0.5-1 g/cm³) and is attached on the sensor surface in a close packing manner (Experimental and Calculation Section). The experimental surface occupancy was thus 3-6% (89/2900 to 89/1500) and 2-4% (59/2700 to 59/1400), respectively. The value of 2-6% would be in a reasonable range for actual micellar attachment. For polymers **3** and **4**, PGLMMA was shielded even at 24 °C, giving no change in the frequency (Figure 3.9c (blue and green lines)). This result confirms that the micellar attachment of polymers **1** and **2** at 24 °C is ascribed to the morphological change but not the temperature change in the same star morphology. Thus, I successfully demonstrated the concept of the hidden functional segment, its controlled exposure by temperature, and its interaction with the external substrate upon the exposure. Additionally, the attached micelle (polymer **1** at 24 °C) was able to release from the sensor surface by decreasing the pH from 10 to 5 (Figure 3.9c (red line after 2000 s)) through an acid-catalyzed hydrolysis of the boronic ester to the original diol and boronic acid.

3.3. Conclusion.

As highlighted in this thesis, I developed a reversible temperature-directed micellar morphological transformation using CABC block copolymers with a hydrophobic block A, a hydrophilic block B, and a thermally responsive block C. The assembly structure was reversibly switchable between the star (below LCST) and the flower (above LCST) (Scheme

3.2a). The LCST of the CABC block copolymer was tuneable in a wide range from 11 °C to 90 °C by varying the EEA/PEGA composition in block C.

The morphological transformation enabled us to tune the physical property (loading capacity) and the chemical property (surface reactivity) of the micelle. The different loading capacity in the star and flower allowed the encapsulation and release of external molecules in a recycled manner. Unlike mere aggregation-dissolution systems, the present star-flower transformation keeps micellar structures and liberates only external molecules but not the polymer. This advantage charms for drug delivery and separation applications. Another interesting application was a hidden functional segment. Block B (PGLMMA) was hidden below LCST and exposed to interact with external substrates above LCST, serving as a new temperature-directed interface. The functional block B can possess zwitterionic, electron donor/acceptor, and ligand groups for biological, electronic, and sensing applications. Also interestingly, block B forms a loop structure (petal) in the flower, and such topology can provide ultra-low friction and high surface permeability and may find applications in lubrication and biological fields.

3.4. Experimental and Calculation Section.

Materials and Measurements

Materials. Methyl methacrylate (MMA) (>99.8%, Tokyo Chemical Industry (TCI), Japan), 2-(2-ethoxyethoxy)ethyl acrylate, (EEA) (>99.8%, TCI), and poly(ethylene glycol) acrylate (PEGA) (average molecular weight = 480) (98%, Aldrich, USA) were purified through an alumina column before use. Solketal methacrylate (SMA) (50 wt% in dichloromethane, Aldrich) was purified with rotary evaporator to remove dichloromethane before use. Nile Red (TCI), tetra-*n*-octylammonium iodide (ONI) (>98.0%, TCI), ethyl α -iodophenylacetate (EPh-I) (>98%, TCI), 4-mercaptophenylboronic acid (MPBC) (90%, Aldrich), 4-(*N,N*-

dimethylaminosulfonyl)-7-(2-aminoethylamino)-2,1,3-benzoxadiazole (DBDED) (TCI), ferrous chloride (FeCl_2) (98%, Aldrich), sodium acetate (>98.5%, TCI), acetic acid ($\geq 99.7\%$, Aldrich), sodium hydrogen carbonate (>99.0%, TCI), sodium carbonate (>99.0%, TCI), ammonia solution (28% in water, TCI), hydrogen peroxide (35 % in water, TCI), hydrochloric acid (HCl) (1 mol/L, TCI), sodium hydroxide (NaOH) (2 mol/L in water, TCI), 1-butanol (>99.0%, TCI), diethylene glycol dimethyl ether (diglyme) (>99%, TCI), ethanol ($\geq 99.5\%$, absolute, Fisher Scientific), tetrahydrofuran (THF) (>99.5%, Kanto, Japan), *N,N*-dimethylformamide (DMF) (>99.5%, Kanto), hexane (>99%, International Scientific, Singapore), acetonitrile (HPLC grade, Anhui Fulltime Specialized Solvent & Reagent, China) and dialysis tubing (benzoylated, pore size: 2000 MWCO, Aldrich) were used as received. Methyl 2-iodo-2-(4'-(2''-iodopropionyloxy)phenyl)acetate (I-MEPE-I) (>92.0%) was provided through the courtesy of Godo Shigen, Japan. A gold coated quartz crystal microbalance (QCM) sensor (QSX 301 Gold, surface roughness < 1 nm RMS) was purchased from Biolin Scientific (Gothenburg, Sweden).

^1H NMR. The ^1H NMR spectra were recorded on a Bruker (Billerica, US) AV500 spectrometer (500 MHz) (ambient temperature) or JEOL (Tokyo, Japan) ECA-400 (variable-temperature); Bruker AV500, spectral width 5000.00 Hz, acquisition time 6.554 sec, and pulse delay 1.000 sec; JEOL ECA-400, spectral width 4000.00 Hz, acquisition time 8.192 sec, and pulse delay 1.000 sec. Acetone- d_6 , DMSO- d_6 and D_2O were used as solvents for the NMR analysis, and the chemical shift was calibrated using residual undeuterated solvents as the internal standards. The monomer conversions were determined with ^1H NMR.

GPC. The GPC analysis was performed on a Shimadzu (Kyoto, Japan) LC-2030C Plus liquid chromatograph equipped with two Shodex (Tokyo, Japan) LF-804 mixed gel columns (300×8.0 mm; bead size = 6 μm ; pore size = 3000 Å). The eluent was *N,N*-dimethyl formamide (DMF) (containing LiBr (10 mM)) at a flow rate of 0.5 mL/min (40 °C). Sample detection was

conducted using a Shimadzu differential refractometer RID-20A. The column system was calibrated with standard PMMAs. I-PMMA-I (Table 1 (entry 1)) was also analyzed using tetrahydrofuran (THF) eluent at a flow rate of 0.7 mL/min (40 °C), for which the columns were a Shodex LF-804 mixed gel column (above) and a Shodex KF-804L mixed gel column (300 × 8.0 mm; bead size = 7 μm; pore size = 1500 Å).

DLS Analysis. The DLS measurement was carried out using a Malvern Zetasizer Nano ZSP (Worcestershire, UK). A temperature-controlled quartz cell was employed. The test angle for the DLS analysis was 173° (backscattering detection). The sample was equilibrated at each temperature for 5 min.

Fluorescence. The fluorescence spectra were measured using a Varian Cary Eclipse optical spectrometer (Agilent, Santa Clara, US) with a temperature controller. The sample was equilibrated at each temperature for 5 min. The excitation wavelengths were 450 nm and 550 nm for DBDED and Nile Red, respectively.

TEM. The TEM images were obtained on a JEOL (Tokyo, Japan) JEM-1400 transmission electron microscope operated at 100 kV. The TEM grid was carbon-coated on 200 mesh (copper) (Ted Pella, Redding, US). The TEM sample at 4 °C (Figure 3.2c) was prepared by freeze-drying. A micelle solution, a TEM grid in a vial, and a micropipette tip were placed in a fridge at 4 °C. A 100 mL flask was placed in a dry ice/acetone mixture bath. Then, the micelle solution was quickly dropped on the TEM grid in the vial using the micropipette, the TEM grid in the vial was quickly placed in the flask in the dry ice/acetone mixture bath, and the droplet was frozen. The sample in the flask was vacuumed with a pump overnight and lyophilized.

Preparation of Micelles. To a DMF solution (0.1 mL) of a CABC block copolymer (0.0110 g), water (1 mL) was added slowly under stirring at room temperature (21 ± 1 °C)

(water/DMF = 10/1 (v/v)). The obtained micellar solutions were studied with DLS, fluorescence, and QCM at prescribed temperatures using temperature controllers.

Staining of Micelle with FeCl₂. To a micellar solution of PEEA-PMMA-PGLMMA-PEEA (1 wt% polymer in water/DMF = 10/1, 1 mL), an FeCl₂ solution (0.5 wt% FeCl₂ in water, 0.02 mL) was added under stirring for 6 h at room temperature (21 ± 1 °C). Then, insoluble impurities were removed with a filter (pore size = 450 nm). A half portion of the solution was kept at 21 ± 1 °C and the other half was kept at 4 °C. The solutions were dropped on the TEM grid.

Preparation of Nile Red Loaded Micelles. To a DMF solution (0.4 mL) of Nile Red (0.0020 g) and PEEA-PMMA-PGLMMA-PEEA (Figure 3.8 and Figure 3.25 (circle) in the Experimental section) (0.0440 g) or PPEGA-PMMA-PGLMMA-PPEGA (Figure 3.25 (square)) (0.0440 g), water (4 mL) was added slowly under stirring for 1 h at room temperature (21 ± 1 °C) (water/ DMF = 10/1 (v/v)). Then, the precipitated excess amount of Nile Red was removed with a filter (pore size = 450 nm). The obtained micellar solution was studied with the fluorescence spectrometer at prescribed temperatures using a temperature controller.

Preparation of Nile-Red-Containing Purified Star. A DMF solution (0.4 mL) of Nile Red (0.0020 g) and PEEA-PMMA-PGLMMA-PEEA (0.0440 g) was stirred in an ice bath (0 °C) for 0.5 h. To this solution, ice water (4 mL) was added slowly under stirring for 1 h (water/ DMF = 10/1 (v/v)) in an ice bath (0 °C). The precipitated excess amount of Nile Red was quickly removed with a filter (pore size = 450 nm), where the filter was pre-cooled in a refrigerator at 4 °C before use. The sample was studied with the fluorescence spectrometer at 4 °C (Figure 3.8b (triangle)).

Preparation of 4-Mercaptophenylboronic Acid (MPBC)-Immobilized QCM Sensor. A gold-coated QCM sensor was treated with an ozone cleaner (PSD Pro Series, Novascan Technologies, Ames, US) for 10 min. Then, the QCM sensor was placed in a

preheated (75 °C) mixture of deionized water, In the present study, the third overtone ($n = 3$) was used. The QCM channel was washed with deionized water, and subsequently the micelle solution was injected (at pH ~ 10). Then, a buffer solution (pH ~ 5) was injected at a flow rate of 50 $\mu\text{L min}^{-1}$ to detach the micelles from the QCM sensor.

Calculation of Theoretical Maximum Micellar Binding Amount. Assuming that the micelle is a swollen sphere (density (d) = 0.5-1 g/cm^3) is attached on the QCM sensor surface in a close packing manner (the two dimensional close-packing factor is 0.906). The maximum binding amount (m) of the micelle per area is

$$m = \frac{0.906}{A_0} \times m_0 = \frac{0.906}{\pi \times r^2} \times \frac{4}{3} \times \pi r^3 \times d$$

where A_0 and m_0 are the cross-sectional area and mass of a single spherical micelle, respectively, and r is the radius of the micelle. The hydrodynamic diameters ($2r$) experimentally measured by DLS in the aqueous solution were 48 nm and 44 nm for polymer **1** (PEEA-PMMA-PGLMMA-PEEA) and polymer **2** (PEEA_m/PPEGAn-PMMA-PGLMMA-PEEA_m/PPEGAn with $m/n = 95/5$), respectively. The calculated m values were 1500-2900 ng/cm^2 and 1400-2700 ng/cm^2 for polymers **1** and **2**, respectively.

Synthetic Procedures

Preparation of I-PMMA-I (Table 3.1 (entry 1)). A mixture of MMA (1.00 g, 8000 mM), I-MEPE-I (0.0947 g, 160 mM), and ONI (0.0593 g, 80 mM) in a Schlenk tube was heated at 60 °C for 2 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-I polymer with $M_n = 2900$ and $D = 1.11$ (monomer conversion = 57%). The reaction mixture was diluted with THF (2 mL). Then, the polymer was reprecipitated in hexane (20 mL) twice, giving a purified I-ME-PMMA-I with $M_n = 3000$ and $D = 1.10$.

Preparation of I-PMMA-PSMA-I (Table 3.1 (entry 2)). A mixture of SMA (1.00 g, 8000 mM), I-ME-PMMA-I (0.195 g, 80 mM), and ONI (0.0297 g, 80 mM) in a Schlenk tube

was heated at 60 °C for 4 h under argon atmosphere with magnetic stirring, yielding an I-ME-PMMA-PSMA-I block copolymer with $M_n = 8800$ and $D = 1.28$ (monomer conversion = 30%). The reaction mixture was diluted with THF (2 mL). Then, the polymer was reprecipitated in hexane (20 mL) twice, giving a purified I-ME-PMMA-PSMA-I with $M_n = 9300$ and $D = 1.24$.

Preparation of I-PEEA-PMMA-PSMA-PEEA-I (Table 3.1 (entry 3-1)). A mixture of EEA (1.00 g, 8000 mM), I-ME-PMMA-PSMA-I (0.0824 g, 13 mM), and ONI (0.0210 g, 52 mM) in a Schlenk tube was heated at 110 °C for 19 h under argon atmosphere with magnetic stirring, yielding an I-PEEA-PMMA-PSMA-PEEA-I block copolymer with $M_n = 48000$ and $D = 1.53$ (monomer conversion = 29%). The reaction mixture was diluted with THF (3 mL). Then, the polymer was reprecipitated in hexane (30 mL) twice, giving a purified I-PEEA-PMMA-PSMA-PEEA-I with $M_n = 48000$ and $D = 1.52$.

Conversion of iodide of I-PEEA-PMMA-PSMA-PEEA-I with DBDED. A mixture of I-PEEA-PMMA-PSMA-PEEA-I (0.300 g, 1 equiv) (10 wt%), DBDED (0.0535 g, 30 equiv), diglyme (45 wt%), and 1-butanol (45 wt%) in a Schlenk tube was heated at 100 °C for 4 h under argon atmosphere with magnetic stirring, yielding a DBDED-PEEA-PMMA-PSMA-PEEA-DBDED block copolymer. Then, the polymer was reprecipitated in hexane (20 mL) twice, giving a purified DBDED-PEEA-PMMA-PSMA-PEEA-DBDED with $M_n = 48000$ and $D = 1.52$.

Preparation of I-PEEA-PMMA-PGLMMA-PEEA-I and DBDED-PEEA-PMMA-PGLMMA-PEEA-DBDED (Table 3.1 (entry 3-1)). A mixture of I-PEEA-PMMA-PSMA-PEEA-I (0.300 g) or DBDED-PEEA-PMMA-PSMA-PEEA-DBDED (0.300 g) and acetonitrile (4 mL) and an aqueous HCl solution (1 mol/L, 2 mL) in a 25 mL flask was stirred at room temperature for 12 h, yielding an I-PEEA-PMMA-PGLMMA-PEEA-I or DBDED-PEEA-PMMA-PGLMMA-PEEA-EBDED block copolymer. Then, the polymer solution was neutralized with an aqueous NaOH solution (2 mol/L) and transferred to a dialysis tube (pore

size: 2000 MWCO) to dialyze in water for 24 h, giving a purified I-PEEA-PMMA-PGLMMA-PEEA-I with $M_n = 26000$ and $\bar{D} = 1.48$ or a purified DBDED-PEEA-PMMA-PGLMMA-PEEA-DBDED with $M_n = 25000$ and $\bar{D} = 1.50$.

Preparation of I-PEEA_m/PPEGAn-PMMA-PGLMMA-PEEA_m/PPEGAn-I (Table 3.1 (entries 3-2 to 3-8)). The polymers were prepared in similar manners, where EEA and PEGA with prescribed ratios were used for the synthesis of block C.

Preparation of PEEA_m/PPEGAn-I (only C segment) random copolymers (Table 3.2). A mixture of EEA and PEGA with a prescribed ratio (totally 600 equiv), EPh-I (1 equiv), and ONI (4 equiv) in a Schlenk tube was heated at 110 °C for a prescribed time under argon atmosphere with magnetic stirring, yielding a PEEA/PPEGAn-I random copolymer. The reaction mixture was diluted with THF (3 mL). Then, the polymer was reprecipitated in hexane (30 mL) twice, giving a purified PEEA/PPEGAn-I random copolymer.

¹H NMR Analysis of I-MEPE-I, I-ME-PMMA-I, I-ME-PMMA-PSMA-I, I-PEEA-PMMA-PSMA-PEEA-I and I-PEEA-PMMA-PGLMMA-PEEA-I. Figure 3.10a shows the ¹H NMR spectrum of I-MEPE-I (initiator). Figure 3.10b shows the spectrum of I-ME-PMMA-I polymer. The PE-I site proton (I-CH, proton **d**, 5.89 ppm) completely disappeared, meaning 100% initiation from the PE-I site. 97% of the I-ME site remained unreacted, as calculated from the signal areas of the I-ME site (I-CH, proton **b**, 4.95 ppm) and the phenyl group in the initiator (-C₆H₄-, proton **c**, 7.42-7.13 ppm). The number of MMA units (25 units) was calculated from the signal areas of the MMA units (-OCH₃, protons **e** and **h**, 3.79-3.59 ppm) and the phenyl group in the initiator (-C₆H₄-, proton **c**, 7.42-7.13 ppm). Figure 3.10c shows the spectrum of I-ME-PMMA-PSMA-I (a trace amount of the monomer SMA remained). 95% of the I-ME site remained unreacted, as calculated in a similar manner. The number of the SMA units (27 units) was calculated from the signal areas of the SMA units (-CH, proton **i**, 4.45-4.34 ppm) and the phenyl group in the initiator (-C₆H₄-, proton **c**, 7.42-7.13 ppm). Figure 3.10d

shows the spectrum of I-PEEA-PMMA-PSMA-PEEA-I. The I-ME site proton (I-CH, proton **b**, 4.95 ppm) completely disappeared, meaning 100% initiation from the I-ME site. The number of the EEA units (164 units) was calculated from the signal areas of the EEA units (-CH in the backbone, proton **o**, 2.56-2.34 ppm) and the SMA units (-CH, proton **I**, 4.45-4.34 ppm) (27 units). Figure 3.10e shows the spectrum of I-PEEA-PMMA-PGLMMA-PEEA-I. The SMA units (-CH, proton **I**, 4.45-4.34 ppm) completely disappeared, meaning a full conversion of the SMA units to the GLMMA units.

¹H NMR Analysis of I-PEEA_m/PPEGAn-PMMA-PSMA-PEEA_m/PPEGAn-I (m/n = 95/5, 90/10, 80/20, 70/30, 60/40, 50/50, and 0/100) and I-PEEA_m/PPEGAn-PMMA-PGLMMA-PEEA_m/PPEGAn-I. Figures 3.11a, 3.12a, 3.13a, 3.14a, 3.15a, 3.16a, and 3.17a show the ¹H NMR spectra of I-PEEA_m/PPEGAn-PMMA-PSMA-PEEA_m/PPEGAn-I (m/n = 95/5, 90/10, 80/20, 70/30, 60/40, 50/50, and 0/100) tetrablock copolymers, respectively. The total number of the EEA and PEGA units was calculated from the signal areas of the EEA/PEGA units (-CH in the backbone, protons **o** and **w**, 2.56-2.34 ppm) and the SMA units (-CH, proton **I**, 4.45-4.34 ppm) (27 units). Because the signals of EEA and PEGA can not be distinguished in ¹H NMR, I assumed that the EEA/PEGA ratio in the polymer was the same as the feed monomer ratio. Figures 3.11b, 3.12b, 3.13b, 3.14b, 3.15b, 3.16b, and 3.17b show the spectra of I-PEEA_m/PPEGAn-PMMA-PGLMMA-PEEA_m/PPEGAn-I (m/n = 95/5, 90/10, 80/20, 70/30, 60/40, 50/50, and 0/100) tetrablock copolymers, respectively.

¹H NMR Analysis of PEEA-I (only C segment). Figure 3.18a shows the ¹H NMR spectrum of EPh-I (initiator). Figure 3.18b shows the spectrum of PEEA-I. The number of EEA units (129 units) was calculated from the signal areas of the EEA units (-CH in the backbone, proton **f**, 2.57-2.34 ppm) and the phenyl group in the initiator (-C₆H₅, proton **c**, 7.39-7.28 ppm).

¹H NMR Analysis of PEEA_m/PPEGAn-I (only C segment) (m/n = 95/5, 90/10, 80/20, 70/30, 60/40, 50/50). Figures 3.19, 3.20, 3.21, 3.22, 3.23, and 3.24 show the ¹H NMR spectra

of PEEA_m/PPEGA_n-I (m/n = 95/5, 90/10, 80/20, 70/30, 60/40, and 50/50), respectively. The total numbers of the EEA and PPEGA units were calculated from the signal areas of the EEA/PEGA units (-CH in the backbone, protons f and m, 2.57-2.34 ppm) and the phenyl group in the initiator (-C₆H₅, proton c, 7.39-7.28 ppm). Because the signals of EEA and PEGA can not be distinguished in ¹H NMR, I assumed that the EEA/PEGA ratio in the random copolymer was the same as the feed monomer ratio.

Table 3.2. Synthesis of PEEA/PPEGA (only C segment) polymers.

Entry	Monomer	[M] ₀ /[EPh-I] ₀ /[ONI] ₀ (mM) ^[a]	T (°C)	t (h)	conv (%)	M _n (GPC) (M _{n,theo}) ^[b]	DP (¹ H NMR) ^[c]	Đ (GPC)
1	EEA	8000/13/52	110	17	19	21000 (25000)		1.33
				After purification		22000	129	1.31
2	EEA/PEGA	7600/400/13/52	110	18	21	27000 (27000)		1.30
				After purification		26000	126/7	1.25
3	EEA/PEGA	7200/800/13/52	110	18	17	25000 (25000)		1.29
				After purification		25000	101/11	1.23
4	EEA/PEGA	6400/1600/13/52	110	20	21	32000 (33000)		1.49
				After purification		32000	106/26	1.48
5	EEA/PEGA	5600/2400/13/52	110	20	19	32000 (34000)		1.48
				After purification		32000	85/37	1.42
6	EEA/PEGA	4800/3200/13/52	110	22	22	38000 (43000)		1.65
				After purification		38000	84/56	1.54
7	EEA/PEGA	4000/4000/13/52	110	24	17	35000 (36000)		1.63
				After purification		39000	53/53	1.44

[a] M = monomer. [b] Theoretical M_n calculated with M_n = ([M]₀/[EPh-I]₀) × conversion × (molecular weight of monomer) + (molecular weight of EPh-I). [c] DP obtained from the peak areas of the initiating phenyl group and the monomer units (with ±10% experimental error).

¹H NMR Spectral Data

Figure 3.10. ¹H NMR spectra (acetone-*d*₆) of (a) I-MEPE-I, (b) I-ME-PMMA-I, (c) I-ME-PMMA-PSMA-I, (d) I-PEEA-PMMA-PSMA-PEEA-I, and (e) I-PEEA-PMMA-PGLMMA-PEEA-I (Table 3.1 (entries 1, 2, and 3-1)).

Figure 3.11. ^1H NMR spectra (acetone- d_6) of (a) I-PEEA $_m$ /PPEGAn-PMMA-PSMA-PEEA $_m$ /PPEGAn-I and (b) I-PEEA $_m$ /PPEGAn-PMMA-PGLMMA-PEEA $_m$ /PPEGAn-I (m/n = 95/5) (Table 3.1 (entry 3-2)).

Figure 3.12. ^1H NMR spectra (acetone- d_6) of (a) I-PEEA $_m$ /PPEGAn-PMMA-PSMA-PEEA $_m$ /PPEGAn-I and (b) I-PEEA $_m$ /PPEGAn-PMMA-PGLMMA-PEEA $_m$ /PPEGAn-I (m/n = 90/10) (Table 3.1 (entry 3-3)).

Figure 3.13. ^1H NMR spectra (acetone- d_6) of (a) I-PEEA_m/PPEGA_n-PMMA-PSMA-PEEA_m/PPEGA_n-I and (b) I-PEEA_m/PPEGA_n-PMMA-PGLMMA-PEEA_m/PPEGA_n-I ($m/n = 80/20$) (Table 3.1 (entry 3-4)).

Figure 3.14. ^1H NMR spectra (acetone- d_6) of (a) I-PEEA_m/PPEGA_n-PMMA-PSMA-PEEA_m/PPEGA_n-I and (b) I-PEEA_m/PPEGA_n-PMMA-PGLMMA-PEEA_m/PPEGA_n-I ($m/n = 70/30$) (Table 3.1 (entry 3-5)).

Figure 3.15. ^1H NMR spectra (acetone- d_6) of (a) I-PEEA $_m$ /PPEGAn-PMMA-PSMA-PEEA $_m$ /PPEGAn-I and (b) I-PEEA $_m$ /PPEGAn-PMMA-PGLMMA-PEEA $_m$ /PPEGAn-I ($m/n = 60/40$) (Table 3.1 (entry 3-6)).

Figure 3.16. ^1H NMR spectra (acetone- d_6) of (a) I-PEEA $_m$ /PPEGAn-PMMA-PSMA-PEEA $_m$ /PPEGAn-I and (b) I-PEEA $_m$ /PPEGAn-PMMA-PGLMMA-PEEA $_m$ /PPEGAn-I ($m/n = 50/50$) (Table 3.1 (entry 3-7)).

Figure 3.17. ^1H NMR spectra (acetone- d_6) of (a) I-PPEGA-PMMA-PSMA-PPEGA-I and (b) I-PPEGA-PMMA-PGLMMA-PPEGA-I (Table 3.1 (entry 3-8)).

Figure 3.18. ^1H NMR spectra (acetone- d_6) of (a) Eph-I (initiator), and (b) PEEA-I (Table 3.2 (entry 1)).

Figure 3.19. ^1H NMR spectrum (acetone- d_6) of PEEA_m/PPEGA_n-I (m/n = 95/5) (Table 3.2 (entry 2)).

Figure 3.20. ^1H NMR spectrum (acetone- d_6) of PEEA_m/PPEGA_n-I (m/n = 90/10) (Table 3.2 (entry 3)).

Figure 3.21. ^1H NMR spectrum (acetone- d_6) of PEEA_m/PPEGA_n-I (m/n = 80/20) (Table 3.2 (entry 4)).

Figure 3.22. ^1H NMR spectrum (acetone- d_6) of PEEA $_m$ /PPEGA $_n$ -I (m/n = 70/30) (Table 3.2 (entry 5)).

Figure 3.23. ^1H NMR spectrum (acetone- d_6) of PEEA $_m$ /PPEGA $_n$ -I (m/n = 60/40) (Table 3.2 (entry 6)).

Figure 3.24. ^1H NMR spectrum (acetone- d_6) of PEEA $_m$ /PPEGA $_n$ -I (m/n = 50/50) (Table 3.2 (entry 7)).

Fluorescence Intensity of Nile Red in Aqueous Solution of PPEGA-PMMA-PGLMMA-PPEGA (with no LCST) Solution at Different Temperatures.

To a DMF solution of Nile Red and PPEGA-PMMA-PGLMMA-PPEGA (with no LCST), water was added slowly under stirring for 1 h at room temperature (21 ± 1 °C) (1 wt% polymer in water/DMF = 10/1 (v/v)). The precipitated excess amount of Nile Red was removed with a filter (pore size = 450 nm) at room temperature. In this case, only block A (PMMA) formed the hydrophobic core retaining Nile Red at all temperatures. No obvious change in the fluorescence intensity was observed at 1-40 °C (Figure 3.25 (square)), meaning an insignificant effect of temperature to the fluorescence intensity. Thus, the observed reduction in the fluorescence intensity by lowering the temperature in the PEEA-PMMA-PGLMMA-PEEA (with LCST of 11 °C) system (Figure 3.25 (circle)) is ascribed to the morphological change but not to the temperature change.

Figure 3.25. The fluorescence intensity of Nile Red in an aqueous solution of PEEA-PMMA-PGLMMA-PEEA (with LCST of 11 °C) (circle) and PPEGA-PMMA-PGLMMA-PPEGA (with no LCST) (square) at different temperatures (1 wt% polymer in water/DMF = 10/1 (v/v), $\lambda_{\text{ex}} = 550$ nm).

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Chapter 4. Effect of Segment Lengths on Self-Assembled Structures of CABC Block Copolymers

4.1. Introduction

Amphiphilic block copolymers have drawn substantial attention for preparing self-assembled nanostructures upon dispersing in selective solvent.¹⁻³ With varying the length and composition of each segment, polymer concentration, solvent, temperature, and ionic strength, various morphologies such as micelles, cylinders, vesicles, discs, Janus micelles, toroids, tubes, and other complex structures can be prepared.⁴⁻⁷ Among them, the toroidal (donut or ring-shaped) nanostructures are of growing interest as their unique geometry and potential applications in biological and nanotechnology materials.⁸⁻¹⁰ For example, toroids are found in DNA packing structures in bacteria and used for artificial gene delivery.¹¹ Additionally, because of the hollow structure, toroids are used for fabricating pores in the nanomaterials.¹²

The formation of polymeric toroids depends on several factors such as the length and composition of each block segment, the preparation procedure, and the solvent property.¹³⁻¹⁵ The type of block copolymer also significantly affects the morphology. Di- and tri-block copolymers were used to create toroids. For example, an AB diblock copolymer (i.e. polyisoprene-*block*-poly(2-vinylpyridine) (PI-P2VP)) self-assembled in THF/ethanol mixture to yield toroids with highly uniform shape and size.¹⁶ Toroids were also obtained using an ABA tri-block copolymer (i.e. polystyrene-*block*-poly(4-vinylpyridine)-*block*-polystyrene (PS-P4VP-PS)).¹⁷ Via the interaction with a counterion additive, an ABC tri-block copolymer (i.e. poly(acrylic acid-*b*-methyl acrylate-*b*-styrene) (PAA-PMA-PS)) generated toroids with high control and uniformity.⁸ Nevertheless, to date, toroids formed by more complex block copolymers, such as CABC multi-block copolymers, has not been reported.

Control radical polymerization (CRP) has gained increasing attention as an effective tool for preparing a wide range of well-defined block copolymers.¹⁸⁻²⁵ CRP is based on the reversible generation of the dormant species (Polymer-X) and the propagating radical (Polymer•). Our research group was the first to utilize organic catalysts in CRP and developed an organocatalyzed control radical polymerization, termed as reversible complexation mediated polymerization (RCMP).²⁶⁻³² In RCMP, an alkyl iodide (R-I) is used as an initiator (X = iodide) and an organic molecule (such as tetrabutylammonium iodide (Bu₄N⁺I⁻)) served as a catalyst. Mechanistically, in RCMP, dormant species (Polymer-I) reversibly activated by the organic catalyst to generate the propagating radical (Polymer•) (Scheme 2.1b). In Chapter 2 and 3, I synthesized CABC multi-block copolymers using a newly designed alkyl diiodide initiator (I-R²-R¹-I, methyl 2-iodo-2-(4'(2''-iodopropionyloxy)phenyl)acetate (I-MEPE-I))³³ (Scheme 2.2) and studied micellar morphological transformation.

Scheme 4.2. Assembly morphologies formed by CABC block copolymers.

In Chapter 4, I comprehensively studied the self-assembly morphology evolution using CABC asymmetric tetra-block copolymers with different lengths of A and C segments. I studied amphiphilic CABC block copolymers composing of hydrophobic block A (coloured in blue in Scheme 2.2), hydrophilic block B (red), and thermosensitive block C (green) with a lower critical solution temperature (LCST). By varying the lengths of A and C segments (varying the hydrophobicity), the morphology changed from spherical micelles to discs, toroids,

oval toroids, and cage-like structures (Scheme 4.2a) upon heating because of the LCST of block C.

By introducing the post-crosslinkable monomer in the A segment, I synthesized a cross-linkable CABC block copolymer and prepared a crosslinked toroid. I generated the toroid above LCST, crosslinked the A segment, and cooled down. Below LCST, the crosslinked toroid can increase its size because the C segment is soluble in water and swollen but retain the ring shape because of the crosslinking. Therefore, I were able to reversibly transform the morphology (such as toroid-to-star transformation) without the crosslinking and fix the toroid with crosslinking. The crosslinked toroid may be used as a red blood cell-like container in the drug delivery and a donut structure molecular template in material design.

4.2. Results and discussion.

Synthesis of CABC Block Copolymers. According to the synthetic procedure shown in Chapter 3, I synthesized CABC (PEEA-PMMA-PGLMMA-PEEA) block copolymers with different chain lengths of the PMMA (block A) and PEEA (block C) segments, where PMMA is poly(methyl methacrylate), PGLMMA is poly(glycerol monomethacrylate) and PEEA is poly(2-(2-ethoxyethoxy)ethyl acrylate). PMMA (block A) is hydrophobic, PGLMMA (block B) is hydrophilic, and PEEA (block C) is temperature-responsive. I synthesized a series of $C_x-A_n-B_m-C_x$ block copolymers with different degrees of polymerization (DPs), where n, m, and x denote for the degree of polymerization (DPs) of block A, B, and C, respectively. Table 4.2 (Experimental section) shows the $C_x-A_n-B_m-C_x$ ($PEEA_x-PMMA_n-PGLMMA_m-PEEA_x$) polymers with varying x (block C) and fixing n and m. Table 4.3 (Experimental section) shows the polymers with varying n (block A) and fixing m and x.

Phase Diagram. The obtained $C_x-A_n-B_m-C_x$ ($PEEA_x-PMMA_n-PGLMMA_m-PEEA_x$) block copolymer formed self-assemblies in an aqueous solution (Figure 4.1). Because the

LCST of PEEA is 11 °C (as shown in Chapter 3), PEEA (block C) is hydrophobic at 60 °C. In the present work, *N,N*-dimethyl formamide (DMF) was used to dissolve all of the three (PMMA, PGLMMA, and PEEA) segments, and water was used to aggregate the hydrophobic segments to form the assemblies. I first dissolved the polymer in DMF to obtain a homogeneous solution, to which water was added to generate the assemblies at 60 °C (0.5 wt% polymer in water/DMF = 10/1(v/v)). At this temperature, the PMMA (block A) and PEEA (block C) segments are hydrophobic and can form the core, and the PGLMMA (block B) segment is hydrophilic and can form the shell, and hence, the flower micelle (Scheme 4.2) can be formed. I first studied PEEA₆₆-PMMA₂₅-PGLMMA₁₀-PEEA₆₆. The dynamic light scattering (DLS) analysis shows that the hydrodynamic size (diameter) of the assembly was 50 nm (peak top) (Table 4.1, entry 1). The contour length of this block copolymer (167 units) is 42 nm (= 0.25 nm x 167 units). The particle size (50 nm) was reasonably smaller than the twice of contour length (84 nm), indicating that the assembly was a micelle (sphere) (not vesicle or other structures). Transmission electron microscope (TEM) image also shows spheres (Figure 4.2A).

I fixed the 25 DP of PMMA (block A) and the 10 DP of PGLMMA (block B) and varied the DP of PEEA (block C) from 132 (66 for each block) to 340 (170) (Figure 4.1, Table 4.1, entries 1-9, and Table 4.2). The sphere size increased from 50 nm to 91 nm with an increase in the DP of PEEA from 122 (66 for each block) to 184 (92) (Figures 4.1, 4.2A and 4.2B and Table 4.1, entries 1-3).

Table 4.1. DLS analysis of self-assemblies using CABC multi-block copolymers.

Entry	Code of C _x -A _n -B _m -C _x block copolymer ^[a]	<i>f</i> _{PMMA}	<i>f</i> _{PGLMMA}	<i>f</i> _{PEEA}	Hydrodynamic diameter (nm) ^[b]	DLS size Distribution index	Assembly structure	Code in Figures 4.1 and 4.2
1	PEEA ₆₆ -PMMA ₂₅ -PGLMMA ₁₀ -PEEA ₆₆	0.09	0.06	0.85	50	0.263	Micelle	A
2	PEEA ₈₁ -PMMA ₂₅ -PGLMMA ₁₀ -PEEA ₈₁	0.07	0.05	0.88	79	0.153	Micelle	B
3	PEEA ₉₂ -PMMA ₂₅ -PGLMMA ₁₀ -PEEA ₉₂	0.06	0.04	0.90	91	0.160	Micelle	
4	PEEA ₁₀₃ -PMMA ₂₅ -PGLMMA ₁₀ -PEEA ₁₀₃	0.06	0.04	0.90	106	0.182	Disc	C
5	PEEA ₁₁₂ -PMMA ₂₅ -PGLMMA ₁₀ -PEEA ₁₁₂	0.05	0.03	0.92	122	0.265	Disc	
6	PEEA ₁₂₈ -PMMA ₂₅ -PGLMMA ₁₀ -PEEA ₁₂₈	0.05	0.03	0.92	122	0.275	Disc	D
7	PEEA ₁₄₀ -PMMA ₂₅ -PGLMMA ₁₀ -PEEA ₁₄₀	0.04	0.03	0.93	200	0.277	Toroid	E
8	PEEA ₁₅₀ -PMMA ₂₅ -PGLMMA ₁₀ -PEEA ₁₅₀	0.04	0.03	0.93	154	0.164	Toroid	
9	PEEA ₁₇₀ -PMMA ₂₅ -PGLMMA ₁₀ -PEEA ₁₇₀	0.04	0.02	0.94	190	0.316	Toroid	F
10	PEEA ₁₅₀ -PMMA ₃₃ -PGLMMA ₁₀ -PEEA ₁₅₀	0.05	0.03	0.92	164	0.254	Toroid	
11	PEEA ₁₅₀ -PMMA ₅₀ -PGLMMA ₁₀ -PEEA ₁₅₀	0.08	0.03	0.89	130	0.188	Oval Toroid	G
12	PEEA ₁₆₁ -PMMA ₆₀ -PGLMMA ₁₀ -PEEA ₁₆₁	0.09	0.02	0.89	164	0.178	Oval Toroid	
13	PEEA ₁₅₆ -PMMA ₉₈ -PGLMMA ₁₀ -PEEA ₁₅₆	0.14	0.02	0.84	149	0.049	Cage	H

[a] The CABC block copolymer was dissolved in DMF at 60 °C, to which hot water (60 °C) was added to generate the assemblies at 60 °C (0.5 wt% polymer in water/DMF = 10/1 (v/v)). [b] The DLS peak top value, and all the samples were determined at 60 °C.

Figure 4.1. Phase diagram of the self-assemblies generated by CABC block copolymers, (keep PGLMMA (block B) ~ 10 units).

A further increase in the DP of PEEA to 206 (103 for each block), 224 (112) and 256 (128) led to a different morphology (Figures 4.1C, 4.1D, 4.2C and 4.2D and Table 4.1, entries 4-6). DLS showed that the assembly size was 106, 122 and 122 nm, respectively (Table 4.1, entries 4-6). These sizes were around the twice of the contour length (60 nm, 65 nm and 73 nm, respectively), suggesting that the assembly was not a spherical micelle. The TEM images (Figures 4.2C and 4.2D) suggest the formation of disc with a flat bilayer structure.

Interestingly, a further increase in the DP of PEEA to 280 (140 for each block), 300 (150), and 340 (170) provided a toroid (ring-shaped structure) (Figures 4.1E, 4.1F, 4.2E and 4.2F and Table 4.1, entries 7-9). The size of the assembly (154-200 nm) determined with DLS

was much larger than the contour length of the polymer (79-94 nm). The TEM image clearly showed the ring-shaped structure with a hole in the center (Figures 4.2E and 4.2F). The atomic force microscopy (AFM) height profiles (Figures 4.2I and 4.2J) for PEEA₁₇₀-PMMA₂₅-PGLMMA₁₀-PEEA₁₇₀ (Figure 4.2F) show that the center of the assembly was lower than the edges, confirming the presence of the hole in the center.

Two mechanisms have been proposed for the formation of toroids.³⁴ Most of the toroids reported in the literature are believed to be generated via end-to-end coalescence of cylindrical micelles. A long flexible thread-like micelle is connected at the chain ends, giving a flexible toroid, for example. The other lesser-reported mechanism is a sphere-disc-toroid transition proposed by He and Schmid.³⁴ A spherical micelle grows to a disc-like micelle, which further develops a hole in the center to form a donut-like rigid toroid. The present toroids seemed to be generated via the sphere-disc-toroid transition system, because no cylindrical precursors were observed. Also, the inner diameters of the toroids (Figures 4.2E and 4.2F) were smaller than the cross sectional diameters of the circular rings. It would be unlikely that such toroids were generated by end-to-end coalescence of very short cylinders bending at a very high curvature. Therefore, the present toroids would most likely be generated by the sphere-disc-toroid transition. It was empirically suggested that the sphere-disc-toroid mechanism is operational when the core-forming segment has a low glass transition temperature (T_g).^{9,16} The core-forming PEEA (block C) segment in the present system has a low T_g (-70 to -60 °C),³⁵ which would be consistent with the empirical implication.

The mass (weight) fraction of PMMA (block A), PGLMMA (block B) and PEEA (block C) segments in the CABC block copolymer are defined as f_{PMMA} , f_{PGLMMA} , and f_{PEEA} , respectively. As a rough estimate, the block copolymer formed spheres with $f_{\text{PEEA}} < 90$ %, discs with $f_{\text{PEEA}} = 90$ -92 %, and toroids with $f_{\text{PEEA}} \geq 93$ % in this studied system, where the hydrophilic fraction (f_{PGLMMA}) was approximately 3 % (Table 4.1).

Figure 4.2. TEM images of the self-assemblies generated by CABC block copolymers (Tables 4.1, 4.2, 4.3 and Figure 4.1), where A = PEEA₆₆-PMMA₂₅-PGLMMA₁₀-PEEA₆₆, B = PEEA₈₁-PMMA₂₅-PGLMMA₁₀-PEEA₈₁, C = PEEA₁₀₃-PMMA₂₅-PGLMMA₁₀-PEEA₁₀₃, D = PEEA₁₂₈-PMMA₂₅-PGLMMA₁₀-PEEA₁₂₈, E = PEEA₁₄₀-PMMA₂₅-PGLMMA₁₀-PEEA₁₄₀, F = PEEA₁₇₀-PMMA₂₅-PGLMMA₁₀-PEEA₁₇₀, G = PEEA₁₅₀-PMMA₅₀-PGLMMA₁₀-PEEA₁₅₀, H = PEEA₁₅₆-PMMA₉₈-PGLMMA₁₀-PEEA₁₅₆, I = AFM height image of F, J = cross-sectional height profile along the red line in I.

As mentioned, a toroid was generated with PEEA₁₇₀-PMMA₂₅-PGLMMA₁₀-PEEA₁₇₀ (Figure 4.2E). Based on this block copolymer, I kept the DPs of PGLMMA (block B) (DP = approximately 10) and PEEA (block C) (DP = approximately 170 for each block), varied the DP of the PMMA (block A) segment from 25 to 98, and studied their morphologies (Figures 4.2E, 4.2G and 4.2H and Table 4.1, entries 7 and 10-13). While circular ring toroids were obtained with 25 and 33 DPs of PMMA (Figure 4.2E), an increase in the DP of PMMA to 50 and 60 gave oval ring toroids (Figure 4.2G). A further increase in the DP of PMMA to 98 led to cage-like nanostructure (Figure 4.2H). Via the sphere-disc-toroid transition mechanism, the disc is favoured to develop one or more holes, when the shell-forming segment is more segregated from the core-forming segment to allow a larger surface area. The punctured disc subsequently grows into a toroid. I suppose that with an increase in the DP of PMMA (block A), the shell-forming segment (PGLMMA (block B)) is more segregated from the core-forming segments (PMMA (block A) and PEEA (block C)), forcing the circular ring toroid to deform to the oval ring toroid, and the cage-shaped structure with more holes. Therefore, circular ring toroids, oval ring toroids, and a cage-shaped structure obtained by varying the DP of PMMA (block A).

Morphological Transformation of Non-Crosslinked Structure. As mentioned, PEEA (block C) has a LCST at 11 °C. Above the LCST (as studied above), PEEA is insoluble in water and hence both PMMA (block A) and PEEA (block C) form the core, generating the flower micelles, toroids, and cage with PGLMMA (block B) in the corona (shell). Below the LCST, PEEA (block C) turns to be soluble in water and only PMMA (block A) is expected to form the core for generating a Janus-star micelle with blocks BC and C in the corona. I tested the morphological transformation using PEEA₁₄₀-PMMA₂₅-PGLMMA₁₀-PEEA₁₄₀ at 60 °C (above LCST) and 4 °C (below LCST) (Figure 4.3). As mentioned, at 60 °C, the size of the assembly determined with DLS was 200 nm and the morphology determined with TEM was a

circular ring toroid (Figure 4.3a), where Figures 4.2E and 4.3a are identical. At 4 °C, the size sharply decreased to 20 nm and the morphology turned to a sphere (Figure 4.3b), demonstrating the morphological transformation driven by the LCST transition of PEEA. Upon heating the solution to 60 °C again, the toroid was regenerated, as confirmed with the DLS and TEM analyses (Figure 4.3c). Thus, I successfully attain the reversible toroid-star transformation directed by temperature.

Figure 4.3. (a, b, c) TEM images of the self-assemblies generated by CABC block copolymers (PEEA₁₄₀-PMMA₂₅-PGLMMA₁₀-PEEA₁₄₀) at 60 °C, 4 °C, and 60 °C, respectively.

Morphological Transformation of Crosslinked Structure. The polymers studied above contained a non-crosslinkable monomer MMA in the A segment, enabling the reversible morphological transformation. Here, to fix the assembly structure, I used a post-crosslinkable monomer, i.e., allyl methacrylate (AMA) in block A and obtained a cross-linkable CABC block copolymer (PEEA₁₅₀-PAMA₃₀-PGLMMA₉-PEEA₁₅₀), where PAMA is poly(allyl

methacrylate) (Figure 4.4a, and Table 4.3, entries 5-1 to 5-3). The obtained polymer formed a toroid with the size of 187 nm (DLS value) at 60 °C. To this solution, I added a radical source, i.e., 4,4'-azobis(4-cyanovaleric acid) (V501) and crosslinked the AMA (block A) segment at 60 °C. The size of the assembly was 177 nm (DLS value) (Figure 4.4b), which is slightly smaller than that before crosslinking (187 nm) because of the reduced core volume via the crosslinking. The crosslinking did not change the morphology, as a toroid was observed with TEM (Figure 4.4c, 60 °C). By cooling the temperature to 4 °C (below LCST), the size significantly increased to 340 nm (Figure 4.4b), because the PEEA segment turned to be soluble in water and swelled the assembly. I froze and dried the 4 °C sample and analysed the sample with TEM. I observed the expanded toroid containing a smaller (occupied) hole (Figure 4.4c (4 °C)). This temperature-directed size change was reversible (Figure 4.4b), Thus, the circular ring toroid was successfully fixed by the crosslinking.

Figure 4.4. (a) Schematic illustration of toroids morphology transformation after crosslinking; (b) and (c) DLS curves and TEM images of the self-assemblies generated by PEEA₁₅₀-PAMA₃₀-PGLMMA₉-PEEA₁₅₀ after crosslinking at 60 °C and 4 °C, respectively.

4.3. Conclusion.

The CABC (PEEA-PMMA-PGLMMA-PEEA) block copolymers self-organized into micelles, discs, toroids, and a cage-shaped structure. Above LCST, with an increasing the chain length of PEEA (hydrophobic block C), the spherical micelle was transformed to discs and circular ring toroids. With an increasing the chain length of PMMA (hydrophobic block A), the toroids deformed to oval ring toroids and then generated cage-shaped structures. The toroid was also crosslinked. The crosslinked toroid may be used as a red blood cell-like container of external molecules and a ring-shaped molecular template for producing inorganic nanoparticles, for example.

4.4. Experimental Section.

Materials.

Methyl methacrylate (MMA) (>99.8%, Tokyo Chemical Industry (TCI), Japan), 2-(2-ethoxyethoxy)ethyl acrylate, (EEA) (>99.8%, TCI), and allyl methacrylate (AMA) (98%, Aldrich, USA) were purified through an alumina column before use. Solketal methacrylate (SMA) (50 wt% in dichloromethane, Aldrich) was purified with rotary evaporator to remove dichloromethane before use. Tetra-*n*-octylammonium iodide (ONI) (>98.0%, TCI), hydrochloric acid (HCl) (1 mol/L, TCI), sodium hydroxide (NaOH) (2 mol/L in water, TCI), tetrahydrofuran (THF) (>99.5%, Kanto, Japan), *N,N*-dimethylformamide (DMF) (>99.5%, Kanto), hexane (>99%, International Scientific, Singapore), acetonitrile (HPLC grade, Anhui Fulltime Specialized Solvent & Reagent, China) and dialysis tubing (benzoylated, pore size: 2000 MWCO, Aldrich) were used as received. Methyl 2-iodo-2-(4'-(2''-iodopropionyloxy)phenyl)acetate (I-MEPE-I) (>92.0%) was provided through the courtesy of Godo Shigen, Japan.

Measurements.

¹H NMR. The ¹H NMR spectra were recorded on a Bruker (Billerica, US) AV500 spectrometer (500 MHz) (ambient temperature); Bruker AV500, spectral width 5000.00 Hz, acquisition time 6.554 sec, and pulse delay 1.000 sec. Acetone-*d*₆ was used as solvents for the NMR analysis, and the chemical shift was calibrated using residual undeuterated solvents as the internal standards. The monomer conversions were determined with ¹H NMR.

GPC. The GPC analysis was performed on a Shimadzu (Kyoto, Japan) LC-2030C Plus liquid chromatograph equipped with two Shodex (Tokyo, Japan) LF-804 mixed gel columns (300 × 8.0 mm; bead size = 6 μm; pore size = 3000 Å). The eluent was *N,N*-dimethyl formamide (DMF) (containing LiBr (10 mM)) at a flow rate of 0.5 mL/min (40 °C). Sample detection was conducted using a Shimadzu differential refractometer RID-20A. The column system was calibrated with standard PMMAs. I-PMMA-I (Table 4.2 (entry 1) and Table 4.3 (entry 1-1)) were also analyzed using tetrahydrofuran (THF) eluent at a flow rate of 0.7 mL/min (40 °C), for which the columns were a Shodex LF-804 mixed gel column (above) and a Shodex KF-804L mixed gel column (300 × 8.0 mm; bead size = 7 μm; pore size = 1500 Å).

DLS Analysis. The DLS measurement was carried out using a Malvern Zetasizer Nano ZSP (Worcestershire, UK). A temperature-controlled quartz cell was employed. The test angle for the DLS analysis was 173° (backscattering detection). The sample was equilibrated at each temperature for 5 min.

TEM. The TEM images were obtained on a JEOL (Tokyo, Japan) JEM-1400 transmission electron microscope operated at 100 kV. The TEM grid was carbon-coated on 200 mesh (copper) (Ted Pella, Redding, US). The TEM sample at 4 °C (Figure 4.4c) was prepared by freeze-drying. A micelle solution, a TEM grid in a vial, and a micropipette tip were placed in a fridge at 4 °C. A 100 mL flask was placed in a dry ice/acetone mixture bath. Then, the micelle solution was quickly dropped on the TEM grid in the vial using the micropipette,

the TEM grid in the vial was quickly placed in the flask in the dry ice/acetone mixture bath, and the droplet was frozen. The sample in the flask was vacuumed with a pump overnight and lyophilized.

AFM. AFM analyse was performed using a Bruker Multimode 8 atomic force microscope (Billerica, US) equipped with a SuperSharpSilicon SSS-NCH probe (tip radius, 2 nm) (Neuchâtel, Sweden). A micelle solution (sample F) and a mica in a vial were placed in an oil bath at 60 °C. Then, one drop (ca. 10 μ l) of the solution was dropped on the mica in the vial, and dried in the oil bath (60 °C). The AFM image was conducted in the air at ambient temperature.

Synthetic Procedures.

The synthesis of CABC block copolymers followed the steps described Chapter 3.

Table 4.2. Synthesis of CABC multi-block copolymers.

Entry	Monomer	I-R-I	$[M]_0/[I-R-I]_0/[ONI]_0$ (mM) ^[a]	T (°C)	t (h)	conv (%)	M_n (GPC) ^[b] ($M_{n,theo}$) ^[c]	DP (¹ H NMR) ^[d]	$\bar{D}$ (GPC) ^[b]
1	MMA	I-MEPE-I	8000/160/80	60	2	57	2900 (3000)		1.11
					After purification		3000	25	1.10
2	SMA	I-PMMA-I	8000/80/80	60	1.5	22	5200 (5000)		1.17
					After purification		5100	10	1.16
3-1	EEA	I-PMMA-PSMA-I	8000/13/52	110	13	20	26000 (30000)		1.35
					After purification		27000	131	1.35
					After deprotection		25000		1.24
3-2	EEA	I-PMMA-PSMA-I	8000/13/52	110	15	30	36000 (35000)		1.49
					After purification		37000	162	1.43
					After deprotection		33000		1.37
3-3	EEA	I-PMMA-PSMA-I	8000/10/40	110	18	30	43000 (40000)		1.57
					After purification		45000	184	1.45
					After deprotection		38000		1.63
3-4	EEA	I-PMMA-PSMA-I	8000/13/52	110	18	34	45000 (44000)		1.64
					After purification		46000	206	1.60
					After deprotection		38000		1.60
3-5	EEA	I-PMMA-PSMA-I	8000/13/52	110	19	35	47000 (47000)		1.88
					After purification		50000	224	1.81
					After deprotection		46000		1.76
3-6	EEA	I-PMMA-PSMA-I	8000/10/40	110	20	34	49000 (53000)		1.52
					After purification		50000	256	1.50
					After deprotection		47000		1.60
3-7	EEA	I-PMMA-PSMA-I	8000/10/40	110	21	37	51000 (58000)		1.61
					After purification		53000	280	1.49
					After deprotection		47000		1.69
3-8	EEA	I-PMMA-PSMA-I	8000/10/40	110	22	40	56000 (61000)		1.89
					After purification		57000	300	1.86
					After deprotection		51000		2.04
3-9	EEA	I-PMMA-PSMA-I	8000/8/32	110	24	40	64000 (69000)		2.01
					After purification		63000	340	1.95
					After deprotection		57000		1.88

[a] M = monomer. [b] PMMA-calibrated value by THF-GPC for entry 1 and DMF-GPC for all other entries. [c] Theoretical M_n calculated with $M_n = ([M]_0/[I-R-I]_0) \times \text{conversion} \times (\text{molecular weight of monomer}) + (\text{molecular weight of I-R-I})$. [d] DP obtained from the peak areas of the initiating phenyl group and the monomer units (with $\pm 10\%$ experimental error).

Table 4.3. Synthesis of CABC multi-block copolymers.

Entry	Monomer	I-R-I	$[M]_0/[I-R-I]_0/[ONI]_0$ (mM) ^[a]	T (°C)	t (h)	conv (%)	M_n (GPC) ^[b] ($M_{n,theo}$) ^[c]	DP (¹ H NMR) ^[d]	$\bar{D}$ (GPC) ^[b]
1-1	MMA	I-MEPE-I	8000/160/80	60	2.5	68	3600 (3800)		1.11
							After purification 37000	33	1.10
1-2	SMA	I-PMMA-I	8000/160/160	60	1.3	20	5400 (5800)		1.20
							After purification 5400	10	1.19
1-3	EEA	I-PMMA-PSMA-I	8000/8/32	110	20	27	60000 (62000)		1.93
							After purification 60000	300	1.92
							After deprotection 55000		2.17
2-1	MMA	I-MEPE-I	8000/160/80	60	3	60	5700 (5500)		1.18
							After purification 5800	50	1.15
2-2	SMA	I-PMMA-I	8000/100/100	60	1.6	11	7900 (7500)		1.31
							After purification 8100	10	1.28
2-3	EEA	I-PMMA-PSMA-I	8000/8/32	110	21	30	62000 (64000)		2.11
							After purification 63000	300	2.00
							After deprotection 58000		2.00
3-1	MMA	I-MEPE-I	8000/80/40	60	3.5	67	6800 (6500)		1.19
							After purification 6700	60	1.16
3-2	SMA	I-PMMA-I	8000/80/80	60	1.6	10	8300 (8500)		1.21
							After purification 8500	10	1.20
3-3	EEA	I-PMMA-PSMA-I	8000/5/20	110	26	25	58000 (69000)		2.24
							After purification 58000	322	2.05
							After deprotection 55000		2.02
4-1	MMA	I-MEPE-I	8000/53/53	60	3.5	70	10000 (10000)		1.17
							After purification 11000	98	1.17
4-2	SMA	I-PMMA-I	8000/53/53	60	1.6	6	13000 (12000)		1.38
							After purification 14000	10	1.38
4-3	EEA	I-PMMA-PSMA-I	8000/5/20	110	24	19	56000 (71000)		2.11
							After purification 58000	311	1.83
							After deprotection 51000		2.00
5-1	AMA	I-MEPE-I	8000/160/80	60	2.5	45	6100 (4300)		1.22
							After purification 6100	30	1.17
5-2	SMA	I-PAMA-I	8000/182/182	60	1.5	15	8300 (6100)		1.26
							After purification 8700	9	1.25
5-3	EEA	I-PAMA-PSMA-I	8000/4/16	110	20	17	67000 (63000)		2.21
							After purification 66000	300	2.38
							After deprotection 60000		2.02

[a] M = monomer. [b] PMMA-calibrated value by THF-GPC for entry 1 and DMF-GPC for all other entries. [c] Theoretical M_n calculated with $M_n = ([M]_0/[I-R-I]_0) \times \text{conversion} \times (\text{molecular weight of monomer}) + (\text{molecular weight of I-R-I})$. [d] DP obtained from the peak areas of the initiating phenyl group and the monomer units (with $\pm 10\%$ experimental error).

Preparation of Micelles. To a DMF solution (0.2 mL) of a CABC block copolymer (0.0110 g) was prewarmed in an oil bath (60 °C), hot water (2 mL, 60 °C) was added slowly under stirring at 60 °C (water/DMF = 10/1 (v/v)). The obtained micellar solutions were studied with DLS at prescribed temperatures using temperature controllers.

Preparation of crosslinked micelles. The micellar solution was transferred to a reaction tube contained the 4,4'-azobis(4-cyanovaleric acid) (V501) (1 equiv). The reaction was conducted under argon atmosphere with magnetic stirring for 2 h at 60 °C.

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Chapter 5. Conclusions

An unprecedented temperature-selective radical generation was studied and its use for synthesizing non-symmetric CABC multi-block copolymers and creating unique assembly structures. The CABC asymmetric multi-block copolymers was used to attain temperature-directed micellar morphological transformation. This transformation was unique and opened up conceptually new controlled encapsulation-release system and hidden-segment interfacial system. A comprehensive study was conducted on the self-assembly morphology evolution using CABC asymmetric tetra-block copolymers with different lengths of A and C segments.

In Chapter 2, the temperature-selective radical generation from a newly designed alkyl diiodide (I-MEPE-I) was utilized to synthesize CABC non-symmetric block copolymers. The I-ME and PE-I sites had different reactivities for generating alkyl radicals in the presence of an organic catalyst (ONI). Taking advantage of the temperature selectivity, I used the alkyl diiodide as a dual initiator in RCMP to uniquely synthesize CABC multi-block copolymers. Exploiting the unique non-symmetric structure, I synthesized two new types of particles, i.e., a Janus-type particle with hetero-segment coronas and a flower-like particle with hetero-segment petals. Such particles may find promising applications as new co-delivery containers and dual-functional vectors.

In Chapter 3, a reversible temperature-directed micellar morphological transformation was attained successfully using CABC block copolymers with a hydrophobic block A, a hydrophilic block B, and a thermally responsive block C with a LCST transition. The assembly structure was reversibly switchable between the star (below LCST) and the flower (above LCST). The LCST of the CABC block copolymer was tuneable in a wide range from 11 °C to 90 °C by varying the EEA/PEGA composition in block C. The morphological transformation enables to tune the physical property (loading capacity) and the chemical property (surface reactivity) of the micelle. The different loading capacity in the star and flower allowed the

encapsulation and release of external molecules in a recycled manner. Unlike mere aggregation-dissolution systems, the present star-flower transformation keeps micellar structures and liberates only external molecules but not the polymer. This advantage charms for drug delivery and separation applications. Another interesting application was a hidden functional segment. Block B (PGLMMA) was hidden below LCST and exposed to interact with external substrates above LCST, serving as a new temperature-directed interface. The functional block B can possess zwitterionic, electron donor/acceptor, and ligand groups for biological, electronic, and sensing applications.

In Chapter 4, I comprehensively studied the self-assembly morphology evolution using CABC asymmetric tetra-block copolymers with different lengths of A and C segments. The amphiphilic CABC block copolymers composing of hydrophobic block A, hydrophilic block B, and thermosensitive block C with a LCST transition. Above LCST, with an increasing the chain length of PEEA (hydrophobic block C), the spherical micelle was transformed to discs and circular ring toroids. With an increasing the chain length of PMMA (hydrophobic block A), the toroids deformed to oval ring toroids and then generated cage-shaped structures. I also crosslinked toroids and studied their size change below and above LCST with keeping the toroid structures. The crosslinked toroid may be used as a red blood cell-like container of external molecules as well as a ring-shaped molecular template for producing inorganic nanoparticles.

RCMP serves as an effective method to prepare well-defined block copolymers with great prospective. By designing new initiators, various polymers can be prepared, such as linear multi-block copolymers, mikto-arm star copolymers, and graft copolymers. The self-assembly property of the copolymer exhibits great promise in the fabrication of precise nanostructures in delivery and biotechnology application. In the future, novel stimuli-responsive nanoparticles can be prepared using the polymers prepared by RCMP.