Controllable Vesicular Size and Shape in Polymerization-Induced Self-assembly Aided by Aromatic Interactions

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Abstract

The size and shape of polymeric vesicles have great impact on their physicochemical and biological properties. Polymerization-induced self-assembly (PISA) is an efficient method to fabricate vesicles. In most PISA-cases, the formation of vesicles is driven by the solvophobic interactions which are lack of versatility on finely structural regulation. Herein, controlling vesicular size and shape is realized in PISA aided by aromatic interactions. Aromatic interactions between the membrane-forming blocks contribute to the augments of membrane tension which lead to the formation of smaller vesicles (as small as 70 nm), but overly enhanced aromatic interactions result in vesicle fusion rather than size decreasing. When the membrane tension is dominated by aromatic interactions and meanwhile high enough to overcome the energetic barriers of fusion, the aromatic interactions drive vesicle fusion in a directional manner to form tubular structures. The precise regulation of vesicular size and shape in PISA would pave the way to fabricate vesicles for a series of size/shape-dependent applications.

1. Introduction

Polymeric vesicles have attracted great attention in drug delivery, diagnostic imaging, and nanoreactors, etc. due to their unique hollow structures.¹⁻⁶ The size and shape of polymeric vesicles have significant impact on their physicochemical and biological properties, such as photoconductivity, in vivo distribution, the pathways of cellular uptake, and depth of tumor penetration.⁷⁻¹¹ Many cells or organelles adopt complex shape and varying size to aid the regulation of cell function.¹²⁻¹⁴ However, artificial vesicles from amphiphilic block copolymer are usually prepared in spherical structures and uncontrollable size, which greatly limited their size/shape-dependent applications. Recently, some inspiring strategies, such as using cross-linkers,¹⁵ adjusting the osmotic pressure,¹⁶⁻¹⁸ and membrane-incorporation of liquid crystalline or aromatic moieties,¹⁹⁻²¹ are reported to induce shape transformation of spherical vesicles to anisotropic ones. Notwithstanding significant advances, the preparation of anisotropic vesicles usually encounters problems of low concentrations (<1%) and multi-step processes (typically including precise sequential syntheses, tedious purification and sophisticated post-polymerization assembly), making it formidable for scalability.

In the last two decades, polymerization-induced self-assembly (PISA), which combines polymerization and self-assembly in one-pot in a concentrated solution (up to 50 wt % solid content), has been established as a powerful strategy for fabrication of polymeric vesicles.²²⁻⁴¹ Although the size and shape of vesicles are significantly important, regulation of vesicular size/shape in PISA system has been rarely reported.⁴²⁻⁴⁵ Particularly, formation of sub-100nm vesicles or vesicles with anisotropic membrane structures via PISA is quite an unusual phenomenon.^{25,44,45} Generally, morphological transition in most PISA-cases is driven by variation of the volume fraction ratio of the solvophobic and solvophilic blocks. Gradual growth of the solvophobic blocks results in the alteration of packing parameter and thus morphological transition from spheres to worms, vesicles, and some inverse structures. The lack of directionality of solvophobic interactions results in the formation of vesicles with spherical shape in most

cases. The weakly intermolecular interactions between the solvophobic blocks lead to enough chain mobility for morphological transition, but sacrifice the membrane tension of the resultant vesicles, which makes forming and maintaining the high surface curvature of small vesicles very difficult. In addition to solvophobic effects, some other non-covalent interactions can also drive/influence self-assembly of block copolymers.⁴⁶⁻⁴⁸ PISA aided by hydrogen bonding,⁴⁹ static electricity,⁵⁰ host-guest complexation,⁵¹ crystallization,⁵² and liquid crystal ordering,⁵³ obviously improve the accessibility of anisotropic nano-objects. Applying cooperativity of multiple non-covalent interactions generates abundant structures and even functional optimization of the cells and organelles in nature.¹²⁻¹⁴ Introducing multiple driving forces in PISA syntheses would hopefully facilitate the possibility in complex size/shape regulation of artificial assemblies.

Herein, synergetic effects of solvophobic and aromatic interactions (or called π - π interactions) on the vesicular size and shape were investigated in PISA system. Vesicles of about 77 ± 26 nm (number-average diameter by

Figure 1. Scheme of PISA to fabricate sub-100 nm vesicles with strong aromatic interactions in the membrane-forming blocks, and TEM images of PEO-*b*-PCMA nanoparticles obtained via RAFT dispersion polymerization of CMA using PEO₄₅-CPADB as the macro RAFT agent (feed molar ratio of CMA/PEO₄₅-CPADB = 80) at different polymerization time, (A) 1.25 h (Conversion_{CMA} = 18%), (B) 1.5 h (Conversion_{CMA} = 29%), (C) 3.0 h (Conversion_{CMA} = 68%) , (D) 4.0 h (Conversion_{CMA} = 85%), (E) 6.0 h (Conversion_{CMA} = 94%).

DLS) were fabricated by RAFT dispersion polymerization of the 7-(2-methacryloyloxyethoxy)-4-methylcoumarin (CMA) using poly(ethylene oxide) (PEO-CPADB) as macro RAFT agent at DP_{PCMA} = 80. CMA was selected as the monomer to fabricate the solvophobic block due to the aromaticity of the coumarin units. Strong aromatic stacking and solvophobicity of the membrane-forming poly(7-(2-methacryloyloxyethoxy)-4-methylcoumarin) (PCMA) blocks in the vesicles facilitate the formation of sub-100 nm vesicles. The aromatic interactions of the membrane-forming blocks were adjusted by copolymerization of CMA with nonaromatic monomer 2-(diisopropylamino)ethyl methacrylate (DIPEMA), or weakly aromatic monomer benzyl methacrylate (BzMA), or strongly aromatic monomer 2-(methacryloyloxy) ethyl anthracene-9-carboxylate (ACMAE). Variations in aromatic interactions in the vesicular membrane contributed to the discrepancy in membrane-tension which led to the formation of vesicles with controllable size. Generally, strong aromatic interactions result in the formation of smaller vesicles, but overly enhanced aromatic interactions lead to vesicle fusion rather than size decreasing of

the vesicles. The aromatic interactions drive vesicle fusion in a directional manner to form tubular structures. Our results indicate that sufficient frequency of inelastic collision, enough membrane tension to overcome the energetic barriers of vesicle fusion, and aromatic interactions rather than the solvophobic interactions dominated the membrane tension to provide directionality of vesicle fusion, play significant roles in the formation of tubular structures.

2. Results And Discussion

2.1 PEO₄₅-CPADB mediated RAFT dispersion polymerization of CMA.

Firstly, PEO₄₅-CPADB with $M_n = 2900$ and $M_w/M_n = 1.05$ (Figure S1) was synthesized via esterification reaction of mPEO and 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CPADB). Figure S2 shows the ¹H NMR spectrum of resultant PEO₄₅-CPADB. High end-functionality ($F_{CPADB} = 97\%$) was obtained according to Equation S1 based on the integral values of signals of ester methylene protons at $\delta = 4.3$ ppm (c), aromatic protons at $\delta = 7.3 - 8.0$ ppm (e, f, g), and methoxy protons of PEO₄₅ at $\delta = 3.4$ ppm (a). The monomer CMA was successfully synthesized using the reagents 7-hydroxy-4-methylcoumarin, 2-bromoethanol, and methacryloyl chloride, the structure of CMA and the intermediate were confirmed by ¹H and ¹³C NMR spectra (Figure S3, S4 and S5). Afterwards, PEO₄₅-CPADB was used as macro-RAFT agent to mediate RAFT dispersion polymerization of CMA in the ethanol/water mixture (7/3, w/w). To investigate the polymerization kinetics, the polymerization conducted under $[CMA]_0 : [PEO_{45}\text{-CPADB}]_0 : [AIBN]_0 = 80 : 1 : 0.2$ was monitored by ¹H NMR spectroscopy (Figure S6), and the conversions of CMA at different polymerization time were calculated based on Equation S2. The $\ln([M]_0/[M])$ vs. time plot demonstrates two stages during the whole polymerization process (Figure S7). Homogeneous polymerization conducted in the first stage of polymerization within 1 h, the monomer was consumed slowly and the formed copolymer might be molecularly dissolved in the ethanol/water mixture (7/3, w/w). Spherical micelles were formed at 1.25 h of polymerization with monomer conversion getting 18 % at this time (Figure 1A, S6 and S7), then heterogeneous polymerization was conducted. The apparent enhancement of polymerization rate after phase separation might be induced by the relatively high local monomer concentration in the nanoparticles.^{54,55} The GPC traces of PEO₄₅-*b*-PCMA obtained at different polymerization time are summarized in Figure S8, which shows narrow molecular weight distribution ($M_w/M_n \leq 1.10$) for all samples.

The morphologies of PEO₄₅-*b*-PCMA assemblies obtained at different polymerization time were traced by TEM (Figure 1), which go through an evolution from spherical micelles, to nanoworms, to lamellae and semi-closed

Figure 2. (A) ^1H NMR spectra of the monomer CMA (red line) and the polymer $\text{PEO}_{45}\text{-}b\text{-PCMA}_{80}$ (black line) in CDCl_3 . The concentrations of monomer CMA or equivalent CMA units in $\text{PEO}_{45}\text{-}b\text{-PCMA}_{80}$ block copolymer are both 5 mg/mL. (B) Normalized fluorescence emission spectra of CMA monomer in CH_2Cl_2 (red line), $\text{PEO}_{45}\text{-}b\text{-PCMA}_{80}$ block copolymer in CH_2Cl_2 (black line) and $\text{PEO}_{45}\text{-}b\text{-PCMA}_{80}$ vesicles in ethanol/water (blue line). The concentrations of monomer CMA, equivalent CMA units in $\text{PEO}_{45}\text{-}b\text{-PCMA}_{80}$ block copolymer or vesicles, are all 0.5 mg/mL.

vesicles, and finally to vesicles with the growth of PCMA blocks. What's surprising, sub-100 nm vesicles with a number-average diameter of 76.8 ± 26.3 nm based on DLS are obtained herein (volume-average diameter of 97.7 ± 17.2 nm, intensity-average diameter of 132.7 ± 12.5 nm). Strong aromatic interactions and solvophobicity of the membrane-forming PCMA blocks herein may generate sufficient membrane tension to form and maintain the high surface curvature of such small vesicles.

2.2 Characterization of the aromatic interactions

It has been well-established that aromatic interactions between aromatic rings result in shielding of the protons due to the anisotropy of the ring current effect, which reduce the chemical shifts of aromatic protons.^{56,57} Herein, ^1H NMR spectra of CMA monomer at concentration ranging from 5 mg/mL to 200 mg/mL (Figure S9 and S10) show that the chemical shift of coumarin protons gradually shifted to a lower δ value with increasing of the CMA concentration, indicating an enhancement in aromatic interactions. Compared to the coumarin units in CMA monomer, the coumarin units in the $\text{PEO}_{45}\text{-}b\text{-PCMA}_{80}$ chains are greatly restrained, resulting in enhancement of aromatic interactions. The coumarin protons in diblock polymer $\text{PEO}_{45}\text{-}b\text{-PCMA}_{80}$ are more shielded and obviously lower chemical shift of coumarin protons in $\text{PEO}_{45}\text{-}b\text{-PCMA}_{80}$ than that of CMA monomer is observed in ^1H NMR spectra (Figure 2A and S6). Besides, fluorescence emission spectrum can also be used to examine the aromatic interactions because the emission maximum is also influenced by the strength of the aromatic interactions.¹⁹ When the concentration of CMA increases, the fluorescence emission spectra show gradual red shift as a result of the formation of a low-energy excimer state due to stronger $\pi\text{-}\pi$ electronic overlap between coumarin units (Figure S11). Compared to the fluorescence emission spectrum of CMA monomer in CH_2Cl_2 (Figure 2B), the fluorescence emission spectra of diblock copolymer $\text{PEO}_{45}\text{-}b\text{-PCMA}_{80}$ in CH_2Cl_2 also show a red shift as a result of the stronger aromatic interactions between coumarin units (Figure 2B), which is consistent with the results of ^1H NMR spectra (Figure 2A and S6). Greater red shift in the fluorescence emission spectrum of $\text{PEO}_{45}\text{-}b\text{-PCMA}_{80}$ vesicles was observed (Figure 2B), indicating a more pronounced aromatic interactions in the assemblies than that in CH_2Cl_2 . This phenomenon is reasonable, because inter-chain interactions between coumarin units are greatly enhanced in vesicles, while that between coumarin units in CH_2Cl_2 is greatly reduced due to the molecularly dissolving state of

the PEO₄₅-*b*-PCMA₈₀ block copolymer. The above results demonstrate that there are strong aromatic interactions in the membrane forming PCMA blocks of the PEO₄₅-*b*-PCMA₈₀ vesicles, and the strength of the aromatic interactions can be characterized by ¹H NMR spectrum and fluorescence emission spectrum. In general, stronger aromatic interactions result in lower chemical shifts of the aromatic coumarin protons as well as red shifts of the fluorescent emission maximum of coumarin.

2.3 Controlling vesicular size

Combining the fact that membrane tension has great influence on the vesicular size and that aromatic interactions in vesicular membrane significantly impact the membrane tension, we proposed that adjustment of aromatic interactions may achieve the controllability of vesicular size. In order to adjust the aromatic interactions in vesicular membrane, RAFT dispersion copolymerization of CMA and nonaromatic monomer DIPEMA was performed (Figure 3A, Table S1 and S2). Insertion of DIPEMA units into the solvophobic PCMA block is expected to reduce the aromatic interactions between CMA units to some extent, and the overall aromatic interactions between the solvophobic blocks decrease accordingly. Fixing DP of solvophobic block P(CMA-*co*-DIPEMA) at 80, a series of vesicles were prepared with varying feed molar ratio of DIPEMA/(CMA+DIPEMA) from 0 to 0.40 (Figure 3B₁-B₅).

Figure 3. (A) Scheme of RAFT dispersion copolymerization of CMA and DIPEMA with various molar ratio of DIPEMA/(CMA+DIPEMA) (*x* values) to adjust the aromatic interaction of the membrane-forming blocks P(CMA_{1-*x*}-*co*-DIPEMA_{*x*})₈₀. TEM images of the PEO₄₅-*b*-P(CMA_{1-*x*}-*co*-DIPEMA_{*x*})₈₀ vesicles with different *x* values (B₁) *x* = 0.05, (B₂) *x* = 0.10, (B₃) *x* = 0.15, (B₄) *x* = 0.25, and (B₅) *x* = 0.40. (C) Chemical shift of H_b and H_d (protons in coumarin units of P(CMA_{1-*x*}-*co*-DIPEMA_{*x*})₈₀ as shown in Figure 3A) in ¹H NMR spectra (CDCl₃ as the solvent). (D) Normalized fluorescence emission spectra of PEO₄₅-*b*-P(CMA_{1-*x*}-*co*-DIPEMA_{*x*})₈₀ vesicles in ethanol/water (7/3, w/w). (E) US-DSC curves of the PEO₄₅-*b*-P(CMA_{1-*x*}-*co*-DIPEMA_{*x*})₈₀ vesicles in ethanol/water (7/3, w/w) at 50 mg/mL. (F) DLS diameter of the PEO₄₅-*b*-P(CMA_{1-*x*}-*co*-DIPEMA_{*x*})₈₀ vesicles with different *x* values.

The conversions of CMA and comonomer DIPEMA by ¹H NMR spectra of all the samples were above 82% as shown in Table S2, and the molar contents of comonomer DIPEMA (*x*) were roughly identical to the targeted ones. The corresponding diblock terpolymers were denoted as PEO₄₅-*b*-P(CMA_{1-*x*}-*co*-DIPEMA_{*x*})₈₀, wherein *x* represents molar content of DIPEMA in the solvophobic block, and *x* = 0.05, 0.10, 0.15, 0.25,

and 0.40, respectively. ^1H NMR spectra were used to study the variations of aromatic interaction strength for a series of $\text{PEO}_{45}\text{-}b\text{-P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$ diblock terpolymers by examining the changes in chemical shift of coumarin protons (Figure 3C and S12). Insertion of DIPEMA units in solvophobic PCMA block resulted in deshielding of the protons on the coumarin ring to some extent. The chemical shift of coumarin protons H_b and H_d gradually move to higher δ values with increasing molar content of DIPEMA units (Figure 3C and S12), suggesting the aromatic interactions are gradually weakened. Fluorescence spectra of the vesicles in ethanol/water (7/3, w/w) were used to study the effect of DIPEMA content on the variation of aromatic interactions of the solvophobic blocks $\text{P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$ in the vesicles (Figure 3D). Gradual blue shifts of the emission maximum from 401 nm ($x = 0$) to 390 nm ($x = 0.40$) for $\text{PEO}_{45}\text{-}b\text{-P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$ vesicles reveal that the aromatic interactions are more disturbed at higher content of DIPEMA. Ultra-sensitive differential scanning calorimetry (US-DSC) analysis was used to study the variations of the solvated T_g (defined as T_{sg} herein) of $\text{P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$ in the polymerization medium ethanol/water (7/3, w/w). The dispersions of $\text{PEO}_{45}\text{-}b\text{-P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$ vesicles (50 mg/g) in ethanol/water (7/3, w/w) were directly used for US-DSC analysis. PEO is soluble in ethanol/water and shows no signal, so US-DSC curves only exhibit the T_{sg} of the membrane-forming block $\text{P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$. The same DP (80) of $\text{P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$ in these samples eliminates the impact of the molecular weight on the T_{sg} value. Figure 3E shows that the T_{sg} values of $\text{P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$ at $x = 0$, $x = 0.10$, and $x = 0.25$ were determined to be 62.3, 58.1 and 49.0 °C, respectively. The decreased T_{sg} with increasing of the x values indicates that the membrane-forming blocks $\text{P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$ are much easier to be plasticized by the solvent, which illustrates that the flexibility of the membrane-forming blocks increase in these conditions. As the insertion of DIPEMA units in PCMA blocks greatly weakened the aromatic interactions in vesicular membrane, the stacking of polymer chains is supposed to be less compact in this situation which accordingly increases the mobility of membrane-forming blocks. In addition to the decrease in aromatic interactions strength, reduction in solvophobicity of $\text{P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$ blocks may partly account for the rising flexibility. The weakened interactions of the membrane-forming blocks $\text{P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$ with increasing of the x values resulted in reduction of the membrane tension of the resultant vesicles, which favored the formation of larger vesicles (Figure 3B₁-B₅). DLS results (Figure 3F and S13) exhibit that the number-average diameter of the $\text{PEO}_{45}\text{-}b\text{-P}(\text{CMA}_{1-x}\text{-}co\text{-DIPEMA}_x)_{80}$ vesicles increases from 76.8 ± 26.3 nm at $x = 0$ to 333.5 ± 158.9 nm at $x = 0.40$, volume-average diameter increases from $97.7.8 \pm 17.2$ nm at $x = 0$ to 722.8 ± 120.2 nm at $x = 0.40$, and intensity-average diameter increase from 132.7 ± 12.5 nm at $x = 0$ to 1061.2 ± 632.0 nm at $x = 0.40$.

Figure 4. (A) Scheme of RAFT dispersion copolymerization of CMA and BzMA with various molar ratio of BzMA/(CMA+BzMA) (x values) to adjust the aromatic interactions of the membrane-forming polymers $P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$. TEM images of the $PEO_{45}\text{-}b\text{-}P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$ vesicles with different x values (B_1) $x = 0.05$, (B_2) $x = 0.10$, (B_3) $x = 0.15$, (B_4) $x = 0.25$, and (B_5) $x = 0.40$. (C) Chemical shift of H_b and H_d (protons in coumarin units of $P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$ as shown in Figure 4A) in 1H NMR spectra ($CDCl_3$ as the solvent). (D) Normalized fluorescence emission spectra of $PEO_{45}\text{-}b\text{-}P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$ vesicles in ethanol/water (7/3, w/w). (E) US-DSC curves of the $PEO_{45}\text{-}b\text{-}P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$ vesicles in ethanol/water (7/3, w/w) at 50 mg/mL. (F) DLS diameter of the $PEO_{45}\text{-}b\text{-}P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$ vesicles with different x values.

RAFT dispersion copolymerization of CMA and the weakly aromatic monomer BzMA was also carried out to fabricate a series of $PEO_{45}\text{-}b\text{-}P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$ vesicles (Figure 4A, 4B₁-B₅, Table S3 and S4). The conversions of CMA and comonomer BzMA of all the samples were above 82% (Table S4), and the molar contents of BzMA units (x) in the membrane-forming blocks were roughly identical to the targeted ones. 1H NMR results (Figure 4C and S14) of the $PEO_{45}\text{-}b\text{-}P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$ diblock terpolymers show that the chemical shift of coumarin protons H_b almost unchanged at varying x values. The possible reason is that anisotropy of the ring current effect provided by phenyl of BzMA was also able to shield coumarin protons, which can offset part of weakened π - π interactions between coumarins units. The phenyl of BzMA is overlapped less with coumarin protons H_d than that of H_b , so the chemical shift of coumarin protons H_d slightly increases with the increasing of molar content of BzMA units (x values). For the fluorescence spectra of $PEO_{45}\text{-}b\text{-}P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$ vesicles, only slight shift of the emission maximum was observed (401 nm at $x = 0$ to 398 nm at $x = 0.40$), which further demonstrated that the phenyl ring of BzMA can offset the broken π - π interactions between coumarins units. The results of 1H NMR spectra (Figure 4C and S14) and fluorescence spectra (Figure 4D) demonstrate that there is no significant influence of the BzMA units on the aromatic interactions between the membrane-forming blocks. However, US-DSC results show that the T_{sg} values of $P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$ still decreased with increasing of the x values, which are 62.3 °C at $x = 0$, 59.9 °C at $x = 0.10$ and 54.1 °C at $x = 0.25$. The increased flexibility of the membrane-forming block $P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$ is possibly due to the relatively weak solvophobicity of the membrane-forming block. Similarly, the higher mobility of membrane-forming block results in lower membrane tension, and thus size increasing (Figure 4B₁-4B₅, 4F, S15 and Table S4). By contrasting vesicles prepared from copolymerization of CMA with BzMA and DIPEMA, it can be identified that $PEO_{45}\text{-}b\text{-}P(CMA_{1-x}\text{-}co\text{-}DIPEMA_x)_{80}$ vesicles generally have larger size than that of the $PEO_{45}\text{-}b\text{-}P(CMA_{1-x}\text{-}co\text{-}BzMA_x)_{80}$ vesicles at the same x value (Table S2 and S4). Concurrent reduction of the aromatic interactions and solvophobicity was induced by copolymerization of CMA with DIPEMA, while only the solvophobicity was reduced in copolymerization of CMA with BzMA, the former generated lower membrane tension to facilitate the formation of larger vesicles.

The above results indicate that controlling vesicular size can be realized by RAFT dispersion copolymerization of CMA and DIPEMA or BzMA to adjust the interactions between the membrane-

forming blocks, and the diameter of the resultant vesicles increased due to the reducing of membrane tension. In order to demonstrate whether controlling vesicular size with a decreasing trend can be realized by increasing the membrane tension, RAFT dispersion copolymerization of CMA with a strongly aromatic monomer ACMAE (Figure S16 and 17) were carried out to fabricate a series of $\text{PEO}_{45}\text{-}b\text{-P}(\text{CMA}_{1-x}\text{-}co\text{-ACMAE}_x)_{80}$ vesicles (Figure 5A, 5B₁-B₅, Table S5 and S6). The conversions of CMA and comonomer ACMAE of all the samples were above 93 % as shown in Table S6 and the molar contents of ACMAE units (x) were roughly identical to the targeted ones. Although the interplanar distance between the coumarin units was enlarged as a result of the insertion of ACMAE in solvophobic $\text{P}(\text{CMA}_{1-x}\text{-}co\text{-ACMAE}_x)_{80}$ blocks, anthracene rings of ACMAE, which took the

Figure 5. (A) Scheme of RAFT dispersion copolymerization of CMA and ACMAE with various molar ratio of ACMAE/(CMA+ACMAE) (x values) to adjust the aromatic interactions of the membrane-forming polymers $\text{P}(\text{CMA}_{1-x}\text{-}co\text{-ACMAE}_x)_{80}$. TEM images of the $\text{PEO}_{45}\text{-}b\text{-P}(\text{CMA}_{1-x}\text{-}co\text{-ACMAE}_x)_{80}$ vesicles with different x values (B₁) x = 0.05, (B₂) x = 0.10, (B₃) x = 0.15, (B₄) x = 0.25, and (B₅) x = 0.40. (C) Chemical shifts of H_b and H_d (protons in coumarin units of $\text{P}(\text{CMA}_{1-x}\text{-}co\text{-ACMAE}_x)_{80}$ as shown in Figure 4A) in ¹H NMR spectra (CDCl₃ as the solvent). (D) Normalized fluorescence emission spectra of $\text{PEO}_{45}\text{-}b\text{-P}(\text{CMA}_{1-x}\text{-}co\text{-ACMAE}_x)_{80}$ vesicles in ethanol/water (7/3, w/w). (E) US-DSC curves of the $\text{PEO}_{45}\text{-}b\text{-P}(\text{CMA}_{1-x}\text{-}co\text{-ACMAE}_x)_{80}$ vesicles in ethanol/water (7/3, w/w) at 50 mg/mL. (F) DLS diameter of the $\text{PEO}_{45}\text{-}b\text{-P}(\text{CMA}_{1-x}\text{-}co\text{-ACMAE}_x)_{80}$ vesicles with different x values.

place of coumarin units, have stronger aromatic interactions with the remaining coumarin, leading to greater shielding of the coumarin protons. ¹H NMR spectra show that the chemical shifts of coumarin protons H_b and H_d gradually moved to lower δ values with increasing molar content of the ACMAE units (Figure 5C and S18), which indicate that the aromatic interactions were enhanced upon insertion of ACMAE in the solvophobic block. Red shifts of the emission maximum in fluorescence spectra of the $\text{PEO}_{45}\text{-}b\text{-P}(\text{CMA}_{1-x}\text{-}co\text{-ACMAE}_x)_{80}$ vesicles (Figure 5D) are observed as the molar content of ACMAE increase. The great red shift of the emission maximum in fluorescence spectra from 401 nm at x = 0 to 450 nm at x = 0.05 is mainly due to the fluorescence resonance energy transfer (FRET) effect,⁵⁸ resulting from the significant spectral overlap between the emission spectrum of CMA and the absorption spectrum of ACMAE (Figure S19) as well as the close distance between the two fluorophores in vesicular membrane. However, the red shift of the emission maximum in fluorescence spectra from 450 nm at x =

0.05 to 464 nm at $x = 0.40$ (Figure 5D) further indicated that the aromatic interactions in the solvophobic blocks were gradually enhanced as the molar content of ACMAE increase. Moreover, US-DSC results (Figure 5E) show that the T_{sg} values of $P(CMA_{1-x}-co-ACMAE_x)_{80}$ at $x = 0$, $x = 0.10$ and $x = 0.25$ were determined to be 62.3, 64.1 and 66.4 °C, respectively. The higher T_{sg} of $P(CMA_{1-x}-co-ACMAE_x)_{80}$ with bigger x values indicates that the membrane-forming blocks are harder to be plasticized by the solvent with increasing of the x values. The enhancement of the interactions between the $P(CMA_{1-x}-co-ACMAE_x)_{80}$ blocks results in greater membrane tension, which is favorable to form smaller vesicles. TEM (Figure 5B₁-B₆) and DLS results (Figure 5F, S20 and Table S6) show that the diameter of the resultant vesicles becomes smaller and smaller as increasing of x values (from 0 to 0.25) in the solvophobic blocks $P(CMA_{1-x}-co-ACMAE_x)_{80}$, and the number-average diameter of the $P(CMA_{1-x}-co-ACMAE_x)_{80}$ vesicles decreases from 76.8 ± 26.3 nm at $x = 0$ to 64.8 ± 16.8 nm at $x = 0.25$, volume-average diameter decreases from 97.7 ± 17.2 nm at $x = 0$ to 79.3 ± 6.3 nm at $x = 0.25$, and intensity-average diameter decrease from 132.7 ± 12.5 nm at $x = 0$ to 95.4 ± 5.0 nm at $x = 0.25$. Although the aromatic interactions of the membrane-forming blocks continue to increase from $x = 0.25$ to 0.40 (Figure 4C, 4D and S18), the size of the $PEO_{45}-b-P(CMA_{1-x}-co-ACMAE_x)_{80}$ vesicles increases with number-/volume-/intensity-average diameter getting to 87.6 ± 33.5 nm/ 258.5 ± 32.8 nm/ 250.1 ± 136.5 nm (Figure 5F, S20, and Table S6), respectively. The possible reason is that the membrane tension at this condition is too high to maintain the thermodynamic stabilization of the vesicles, which may result in part of vesicle fusion rather than size decreasing of the vesicles. Continuous increasing the molar content of strongly aromatic ACMAE units in the membrane-forming blocks $P(CMA_{1-x}-co-ACMAE_x)_{80}$ to $x = 0.6$ results in further increase of the aromatic interactions as demonstrated by 1H NMR spectra (Figure S18) and fluorescence spectra (Figure S21), and DLS results reveal further increases in number-/volume-/intensity-average diameter at this condition (Figure 5F, S20, and Table S6). Obvious vesicle fusion to form necklace-like structures can be observed from the TEM image (Figure S22), which indicates that the aromatic interactions drive vesicle fusion in a directional manner. However, the fusion of the vesicles is incomplete and tubular structures were not formed at this condition (Figure S22), which may be due to the insufficient enhancement of membrane tension by only introducing strongly aromatic units (ACMAE).

According to the above results, both the membrane tension and aromatic interactions can be weakened by insertion of DIPEMA units into PCMA blocks, weakened membrane tension but without significant influence on the aromatic interactions is induced by insertion of BzMA units into PCMA blocks, both the membrane tension and aromatic interactions can be enhanced by insertion of ACMAE units into PCMA blocks. The weakened membrane tension can not maintain the high curvature of the small vesicles and then lead to the formation of bigger vesicles. The enhanced membrane tension results in size decreasing of the resultant vesicles, but excessive enhancement of membrane tension leads to vesicle fusion rather than size decreasing. Moreover, the aromatic interactions seem to drive the vesicle fusion in a directional manner (Figure S22).

2.4 Controlling the directionality of vesicle fusion.

Alternatively, increasing the DP of the solvophobic blocks is a more direct strategy to enhance the membrane tension of the vesicles.^{24,43} When the membrane tension is high enough to overcome the energetic barriers of vesicle fusion, vesicle fusion occurs to reduce the total surface area of the vesicles and simultaneously releases part of the membrane tension to minimize the total free energy in the system. Besides sufficient membrane tension, enough frequency of inelastic collision is also very important to induce vesicle fusion.⁴³ RAFT dispersion polymerizations of CMA with varying DP_{PCMA} of 100, 120, 150 and 200 were carried out at 5% and 10 % solid

Figure 6. TEM images of the PEO₄₅-*b*-PCMA_x vesicles obtained at 5% solid content with different DP_{PCMA}, (A₁) DP_{PCMA} = 100, (A₂) DP_{PCMA} = 120, (A₃) DP_{PCMA} = 150, (A₄) DP_{PCMA} = 200. TEM images of the PEO₄₅-*b*-PCMA_x vesicles obtained at 10% solid content with different DP_{PCMA}, (B₁) DP_{PCMA} = 100, (B₂) DP_{PCMA} = 120, (B₃) DP_{PCMA} = 150, (B₄) DP_{PCMA} = 200. (C) The concentration of polymer chains for the samples of PEO₄₅-*b*-PCMA_x fabricated at different conditions. TEM images of (D₁) PEO₄₅-*b*-PCMA₁₅₀ vesicles and (D₂) PEO₄₅-*b*-PCMA₂₀₀ vesicles fabricated at a fixed concentration of polymer chains at 3.01×10^{-3} mmol/g (the concentration of the polymer chains is same to that of the sample B₂, PEO₄₅-*b*-PCMA₁₂₀).

content (Figure 6, S23 and Table S7). The formation of anisotropic structures by vesicle fusion was not observed at 5 % solid content from DP_{PCMA} = 100 to DP_{PCMA} = 200 (Figure 6A₁-A₄). However, at 10 % solid content, nanotubes mixed with vesicles were formed at DP_{PCMA} = 100 (Figure 6B₁), and most of the vesicles transformed to nanotubes at DP_{PCMA} = 120 (Figure 6B₂). What is surprising, the proportion of the nanotubes in the mixture of vesicles and nanotubes obviously decreased at DP_{PCMA} of 150 and 200 (Figure 6B₃ and 6B₄). The width of the nanotubes gradually decreased from DP_{PCMA} 120 to 200 (Figure S24-S26), which illustrated the membrane tension was still obviously enhanced during the growth of DP_{PCMA} from 120 to 200. But why the enhancement of membrane tension did not induce the continuous

fusion of the vesicles? Although the solid content kept at 10 % for the samples of $DP_{PCMA} = 100, 120, 150$ and 200, the concentration of the polymer chains obviously decreased in turn for these samples (Figure 6C and Table S7). In this regard, fixing the chain concentration during the adjustment of DP_{PCMA} might be a better strategy to provide high frequency of inelastic collision than fixing the solid content. Then the samples of $DP_{PCMA} = 150$ and 200 with a fixed chain concentration at 3.01×10^{-3} mmol/g (the chain concentration is equal to the samples of $DP_{PCMA} = 120$ in Figure 6B₂) were prepared (Figure 6D₁, 6D₂, S27, S28, and Table S7) to demonstrate the above speculation, and nanotubes with negligible residual of vesicles were formed at these conditions. The above results indicate that sufficient membrane tension and high frequency of inelastic collision are the prerequisites for vesicles fusion.

In order to demonstrate the importance of aromatic interactions in providing directionality of vesicle fusion to form the tubular structures, RAFT dispersion

Figure 7. TEM images of (A₁) PEO₄₅-*b*-P(CMA_{0.75}-*co*-DIPeMA_{0.25})₈₀, (A₂) PEO₄₅-*b*-P(CMA_{0.75}-*co*-DIPeMA_{0.25})₁₀₀, (A₃) PEO₄₅-*b*-P(CMA_{0.75}-*co*-DIPeMA_{0.25})₁₂₀, (A₄) PEO₄₅-*b*-P(CMA_{0.75}-*co*-DIPeMA_{0.25})₁₅₀, (A₅) PEO₄₅-*b*-P(CMA_{0.75}-*co*-DIPeMA_{0.25})₂₀₀, (B₁) PEO₄₅-*b*-P(CMA_{0.75}-*co*-BzMA_{0.25})₈₀, (B₂) PEO₄₅-*b*-P(CMA_{0.75}-*co*-BzMA_{0.25})₁₀₀, (B₃) PEO₄₅-*b*-P(CMA_{0.75}-*co*-BzMA_{0.25})₁₂₀, (B₄) PEO₄₅-*b*-P(CMA_{0.75}-*co*-BzMA_{0.25})₁₅₀, (B₅) PEO₄₅-*b*-P(CMA_{0.75}-*co*-BzMA_{0.25})₂₀₀, (C₁) PEO₄₅-*b*-P(CMA_{0.75}-*co*-ACMAE_{0.25})₈₀, (C₂) PEO₄₅-*b*-P(CMA_{0.75}-*co*-ACMAE_{0.25})₁₀₀, (C₃) PEO₄₅-*b*-P(CMA_{0.75}-*co*-ACMAE_{0.25})₁₂₀, (C₄) PEO₄₅-*b*-P(CMA_{0.75}-*co*-ACMAE_{0.25})₁₅₀, and (C₅) PEO₄₅-*b*-P(CMA_{0.75}-*co*-ACMAE_{0.25})₂₀₀. The subscript number is the DP of the corresponding block.

copolymerizations of CMA and DIPeMA/BzMA/ACMAE with a fixed chain concentration at 3.01×10^{-3} mmol/g and varying DP of the solvophobic block at 80, 100, 120, 150, 200 were carried out (Figure 7, S29, and Table S8). For all the above samples, the molar content of CMA was kept at 75% and the molar content of the comonomer (DIPeMA or BzMA or ACMAE) was kept at 25 %, in the solvophobic blocks. For the samples of PEO₄₅-*b*-P(CMA_{0.75}-*co*-DIPeMA_{0.25}), vesicles are formed at $DP_{P(CMA-co-DIPeMA)} = 80$ and 100 (Figure 7A₁ and 7A₂), compound vesicles are formed at $DP_{P(CMA-co-DIPeMA)} = 120$ and 150 (Figure 7A₃

and 7A₄), and inverse structures are formed at $DP_{P(CMA-co-DIPEMA)} = 200$ (Figure 7A₅). Although the membrane tension is weakened by DIPEMA units, the formation of compound vesicles and inverse structures illustrates that it is still high enough to overcome the energetic barriers of vesicles fusion. However, the weakened aromatic interaction leads to the loss of directionality of vesicle fusion. The above results demonstrate that aromatic interactions play a crucial role in driving the directional fusion of vesicles. For the samples of $PEO_{45}-b-P(CMA_{0.75}-co-BzMA_{0.25})$, the morphology gradually changed from vesicles at $DP_{P(CMA-co-BzMA)} = 80$ (Figure 7B₁), to the mixture of nanotubes and vesicles at $DP_{P(CMA-co-BzMA)} = 100$ and 120 (Figure 7B₂ and 7B₃), and then to the nanotubes at $DP_{P(CMA-co-BzMA)} = 150$ and 200 (Figure 7B₄ and 7B₅). Comparing the samples of $PEO_{45}-b-P(CMA_{0.75}-co-BzMA_{0.25})$ and $PEO_{45}-b-PCMA$, the insignificant influence of BzMA units on the aromatic interactions of the membrane-forming blocks remains the directionality of vesicles fusion, but the weakened membrane tension results in obviously higher width and lower aspect ratio of the $PEO_{45}-b-P(CMA-co-BzMA)_{150\sim 200}$ nanotubes (Figure 7B₄, 7B₅, S30, S31) than those of the $PEO_{45}-b-PCMA_{150\sim 200}$ nanotubes (Figure 6D₁, 6D₂, S27, S28). For the $PEO_{45}-b-P(CMA_{0.75}-co-ACMAE_{0.25})$ samples, anisotropic vesicles are formed even at $DP_{P(CMA-co-ACMAE)} = 80$ (Figure 7C₁), considerable amount of nanotubes with minority of vesicles are formed at $DP_{P(CMA-co-ACMAE)} = 100$ (Figure 7C₂), and nanotubes are formed at $DP_{P(CMA-co-ACMAE)} = 120, 150$ and 200 (Figure 7C₃-7C₅ and S32-S34). Moreover, the $PEO_{45}-b-P(CMA-co-ACMAE)_{150\sim 200}$ tubes (Figure 7C₄, 7C₅, S33 and S34) have obviously higher aspect ratio than that of the $PEO_{45}-b-P(CMA-co-BzMA)_{150\sim 200}$ (Figure 7B₄, 7B₅, S30 and S31) and $PEO_{45}-b-PCMA_{150\sim 200}$ nanotubes (Figure 6D₁ and 6D₂, S27 and S28).

The above results indicate that enough membrane tension to overcome the energetic barriers of vesicles fusion, aromatic interactions rather than the solvophobic interactions dominated the membrane tension, and sufficient frequency of inelastic collision, are three prerequisites to drive directional fusion of vesicles to form tubular structures. In order to further demonstrate the above conclusions, RAFT dispersion copolymerizations of CMA and DIPEMA/BzMA/ACMAE with the molar content of CMA remaining at 60 % and the molar content of the comonomer (DIPEMA or BzMA or ACMAE) keeping at 40 % in the solvophobic blocks, were also carried out (Figure S35-S41, and Table S9). Increasing the DP of the solvophobic blocks to induce vesicle fusion, compound vesicles of $PEO_{45}-b-P(CMA_{0.6}-co-DIPEMA_{0.4})$ are formed due to the loss of aromatic interactions, nanotubes with low aspect ratio of $PEO_{45}-b-P(CMA_{0.6}-co-BzMA_{0.4})$ are formed due to the relatively weakened membrane tension and still remained aromatic interactions, and nanotubes with obviously higher aspect ratio of $PEO_{45}-b-P(CMA_{0.6}-co-ACMAE_{0.4})$ are formed due to the concurrent enhancement of membrane tension and aromatic interactions (Figure S35-S41).

Conclusions

In summary, controlling vesicular size to the minimum of about 70 nm and directionality of vesicle fusion to form nanotubes is realized in PISA aided by aromatic interactions. The strong membrane tension generated by the great aromatic and solvophobic interactions of the membrane-forming block is evidenced to be a major factor for the formation of such small vesicles, but overly enhanced membrane tension results in vesicle fusion rather than size decreasing. The aromatic interactions play a crucial role in driving the vesicle fusion in a directional manner to form tubular structures. There are three prerequisites for the formation of tubular vesicles: (1) sufficient frequency of inelastic collision to induce vesicles fusion, (2) enough membrane tension to overcome the energetic barriers of vesicles fusion, (3) aromatic interactions rather than solvophobic interactions dominated the membrane tension to drive vesicle fusion in a directional manner. In consideration of the aromaticity of many drug molecules and fluorophores, the reported approach herein with the distinct mechanism in precise regulation of vesicular size and shape, and the intrinsic scalability of PISA, paves the way to fabricate vesicles for a series of size/shape-dependent applications including drug delivery and biological in vivo imaging.

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Figures

Figure 1

Scheme of PISA to fabricate sub-100 nm vesicles with strong aromatic interactions in the membrane-forming blocks, and TEM images of PEO-b-PCMA nanoparticles obtained via RAFT dispersion polymerization of CMA using PEO45-CPADB as the macro RAFT agent (feed molar ratio of CMA/PEO45-CPADB = 80) at different polymerization time, (A) 1.25 h (Conversion_{CMA} = 18%), (B) 1.5 h (Conversion_{CMA} = 29%), (C) 3.0 h (Conversion_{CMA} = 68%) , (D) 4.0 h (Conversion_{CMA} = 85%), (E) 6.0 h (Conversion_{CMA} = 94%).

Figure 2

(A) ¹H NMR spectra of the monomer CMA (red line) and the polymer PEO₄₅-b-PCMA₈₀ (black line) in CDCl₃. The concentrations of monomer CMA or equivalent CMA units in PEO₄₅-b-PCMA₈₀ block copolymer are both 5 mg/mL. (B) Normalized fluorescence emission spectra of CMA monomer in CH₂Cl₂ (red line), PEO₄₅-b-PCMA₈₀ block copolymer in CH₂Cl₂ (black line) and PEO₄₅-b-PCMA₈₀ vesicles in ethanol/water (blue line). The concentrations of monomer CMA, equivalent CMA units in PEO₄₅-b-PCMA₈₀ block copolymer or vesicles, are all 0.5 mg/mL.

Figure 3

(A) Scheme of RAFT dispersion copolymerization of CMA and DIPEMA with various molar ratio of DIPEMA/(CMA+DIPEMA) (x values) to adjust the aromatic interaction of the membrane-forming blocks $\text{P}(\text{CMA}_{1-x}\text{-co-DIPEMA}_x)_{80}$. TEM images of the $\text{PEO}_{45}\text{-b-P}(\text{CMA}_{1-x}\text{-co-DIPEMA}_x)_{80}$ vesicles with different x values (B1) $x = 0.05$, (B2) $x = 0.10$, (B3) $x = 0.15$, (B4) $x = 0.25$, and (B5) $x = 0.40$. (C) Chemical shift of H_b and H_d (protons in coumarin units of $\text{P}(\text{CMA}_{1-x}\text{-co-DIPEMA}_x)_{80}$ as shown in Figure 3A) in ^1H NMR spectra (CDCl_3 as the solvent). (D) Normalized fluorescence emission spectra of $\text{PEO}_{45}\text{-b-P}(\text{CMA}_{1-x}\text{-co-DIPEMA}_x)_{80}$ vesicles in ethanol/water (7/3, w/w). (E) US-DSC curves of the $\text{PEO}_{45}\text{-b-P}(\text{CMA}_{1-x}\text{-co-DIPEMA}_x)_{80}$ vesicles in ethanol/water (7/3, w/w) at 50 mg/mL. (F) DLS diameter of the $\text{PEO}_{45}\text{-b-P}(\text{CMA}_{1-x}\text{-co-DIPEMA}_x)_{80}$ vesicles with different x values.

Figure 4

(A) Scheme of RAFT dispersion copolymerization of CMA and BzMA with various molar ratio of BzMA/(CMA+BzMA) (x values) to adjust the aromatic interactions of the membrane-forming polymers P(CMA $_{1-x}$ -co-BzMA $_x$)₈₀. TEM images of the PEO₄₅-b-P(CMA $_{1-x}$ -co-BzMA $_x$)₈₀ vesicles with different x values (B₁) $x = 0.05$, (B₂) $x = 0.10$, (B₃) $x = 0.15$, (B₄) $x = 0.25$, and (B₅) $x = 0.40$. (C) Chemical shift of H_b and H_d (protons in coumarin units of P(CMA $_{1-x}$ -co-BzMA $_x$)₈₀ as shown in Figure 4A) in ¹H NMR spectra (CDCl₃ as the solvent). (D) Normalized fluorescence emission spectra of PEO₄₅-b-P(CMA $_{1-x}$ -co-BzMA $_x$)₈₀ vesicles in ethanol/water (7/3, w/w). (E) US-DSC curves of the PEO₄₅-b-P(CMA $_{1-x}$ -co-BzMA $_x$)₈₀ vesicles in ethanol/water (7/3, w/w) at 50 mg/mL. (F) DLS diameter of the PEO₄₅-b-P(CMA $_{1-x}$ -co-BzMA $_x$)₈₀ vesicles with different x values.

Figure 5

(A) Scheme of RAFT dispersion copolymerization of CMA and ACMAE with various molar ratio of ACMAE/(CMA+ACMAE) (x values) to adjust the aromatic interactions of the membrane-forming polymers P(CMA_{1-x}-co-ACMAE_x)₈₀. TEM images of the PEO₄₅-b-P(CMA_{1-x}-co-ACMAE_x)₈₀ vesicles with different x values (B₁) $x = 0.05$, (B₂) $x = 0.10$, (B₃) $x = 0.15$, (B₄) $x = 0.25$, and (B₅) $x = 0.40$. (C) Chemical shifts of H_b and H_d (protons in coumarin units of P(CMA_{1-x}-co-ACMAE_x)₈₀ as shown in Figure 4A) in ¹H NMR spectra (CDCl₃ as the solvent). (D) Normalized fluorescence emission spectra of PEO₄₅-b-P(CMA_{1-x}-co-ACMAE_x)₈₀ vesicles in ethanol/water (7/3, w/w). (E) US-DSC curves of the PEO₄₅-b-P(CMA_{1-x}-co-ACMAE_x)₈₀ vesicles in ethanol/water (7/3, w/w) at 50 mg/mL. (F) DLS diameter of the PEO₄₅-b-P(CMA_{1-x}-co-ACMAE_x)₈₀ vesicles with different x values.

Figure 6

TEM images of the PEO45-b-PCMA_x vesicles obtained at 5% solid content with different DPPCMA, (A1) DPPCMA = 100, (A2) DPPCMA = 120, (A3) DPPCMA = 150, (A4) DPPCMA = 200. TEM images of the PEO45-b-PCMA_x vesicles obtained at 10% solid content with different DPPCMA, (B1) DPPCMA = 100, (B2) DPPCMA = 120, (B3) DPPCMA = 150, (B4) DPPCMA = 200. (C) The concentration of polymer chains for the samples of PEO45-b-PCMA_x fabricated at different conditions. TEM images of (D1) PEO45-b-PCMA150 vesicles and (D2) PEO45-b-PCMA200 vesicles fabricated at a fixed concentration of polymer chains at 3.01×10^{-3} mmol/g (the concentration of the polymer chains is same to that of the sample B2, PEO45-b-PCMA120).

Figure 7

TEM images of (A1) PEO45-b-P(CMA0.75-co-DIPEMA0.25)80, (A2) PEO45-b-P(CMA0.75-co-DIPEMA0.25)100, (A3) PEO45-b-P(CMA0.75-co-DIPEMA0.25)120, (A4) PEO45-b-P(CMA0.75-co-DIPEMA0.25)150, (A5) PEO45-b-P(CMA0.75-co-DIPEMA0.25)200, (B1) PEO45-b-P(CMA0.75-co-BzMA0.25)80, (B2) PEO45-b-P(CMA0.75-co-BzMA0.25)100, (B3) PEO45-b-P(CMA0.75-co-BzMA0.25)120, (B4) PEO45-b-P(CMA0.75-co-BzMA0.25)150, (B5) PEO45-b-P(CMA0.75-co-BzMA0.25)200, (C1) PEO45-b-P(CMA0.75-co-ACMAE0.25)80, (C2) PEO45-b-P(CMA0.75-co-ACMAE0.25)100, (C3) PEO45-b-P(CMA0.75-co-ACMAE0.25)120, (C4) PEO45-b-P(CMA0.75-co-ACMAE0.25)150, and (C5) PEO45-b-P(CMA0.75-co-ACMAE0.25)200. The subscript number is the DP of the corresponding block.

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