Enzyme-assisted Photoinitiated Polymerization-induced Self-assembly in Continuous Flow Reactors with Oxygen Tolerance

Wei-Bin Cai, Dong-Dong Liu, Ying Chen, Li Zhang, and Jian-Bo Tan

Department of Polymeric Materials and Engineering, School of Materials and Energy, Guangdong University of Technology, Guangzhou 510006, China

Abstract

Polymerization-induced self-assembly (PISA) is an emerging method for the preparation of block copolymer nano-objects at high concentrations. However, most PISA formulations have oxygen inhibition problems and inert atmospheres (e.g., argon, nitrogen) are usually required. Moreover, the large-scale preparation of block copolymer nano-objects at room temperature is challenging. Herein, we report an enzyme-assisted photoinitiated polymerization-induced self-assembly (photo-PISA) in continuous flow reactors with oxygen tolerance. The addition of glucose oxidase (GOx) and glucose into the reaction mixture can consume oxygen efficiently and constantly, allowing the flow photo-PISA to be performed under open-air conditions. Polymerization kinetics indicated that only a small amount of GOx (0.5 μmol/L) was needed to achieve the oxygen tolerance. Block copolymer nano-objects with different morphologies can be prepared by varying reaction conditions including the degree of polymerization (DP) of core-forming block, monomer concentration, reaction temperature, and solvent composition. We expect this study will provide a facile platform for the large-scale production of block copolymer nano-objects with different morphologies at room temperature.

Keywords

Polymerization-induced self-assembly; Reversible addition-fragmentation chain transfer (RAFT) polymerization; Photoinitation; Oxygen tolerance

INTRODUCTION

Block copolymer nano-objects with various morphologies are of widespread interest for applications in the field of drug delivery, catalysis, optoelectronic device, biomineralization, functional coating, etc. Cosolvent self-assembly is one of the most commonly used methods for the preparation of block copolymer nano-objects, which involves the synthesis of well-defined amphiphilic block copolymers and the subsequent self-assembly in a selective solvent. The morphology of block copolymer nano-objects can be controlled by changing packing parameter of the block copolymer. However, a diluted solution (solids content <1%) is required in the cosolvent self-assembly method, making the large-scale production of block copolymer nano-objects challenging. Moreover, organic solvents are usually needed, which is not beneficial to the preparation of biofunctional block copolymer nano-objects.

Over the past decade, the development of reversible addition-fragmentation chain transfer (RAFT)-mediated polymerization-induced self-assembly (PISA) by Pan, Armes, Charleux and others has enabled the preparation of block copolymer nano-objects at much higher polymer concentrations (10 wt%–50 wt%). In RAFT-mediated PISA, a soluble homopolymer prepared by RAFT polymerization is chain-extended with a second block. The second block becomes insoluble in a selective solvent when the molecular weight increases to a critical value, leading to the occurrence of in situ self-assembly and therefore the formation of block copolymer nano-objects. The morphology of block copolymer nano-objects can be easily controlled by varying reaction parameters (e.g., molecular weight of the core-forming block, monomer concentration, temperature etc.). Most RAFT-mediated PISA formulations are performed via thermal initiation at relatively high temperatures (e.g., 70 °C). The high temperature feature of thermally initiated RAFT-mediated PISA limits the preparation of various functional block copolymer nano-objects e.g. biofunctional block copolymer nano-objects. In 2015, our group and others have developed a room-temperature PISA method based on type I photoinitiation, termed photoinitiated polymerization-induced self-assembly (photo-PISA). The photo-PISA method has greatly expanded the scope of RAFT-mediated PISA for the preparation of functional block copolymer nano-objects. Several novel PISA systems such as sequence-controlled PISA, temperature-programmed PISA, and Z-RAFT-mediated PISA have also been developed based on photo-PISA.

Despite the fact that many achievements have been made...
in photo-PISA, external light irradiation is inevitable to perform the polymerization. Therefore, light penetration is a critical issue that should be considered in photo-PISA, which may limit the large-scale production of block copolymer nano-objects. To solve the light penetration problem, conducting photo-PISA in continuous flow reactors is an attractive strategy to achieve uniform light irradiation of the reaction mixture.[54–56] Currently, several flow photo-PISA formulations have been developed by Boyer, Junker, and others.[57–58] However, prior deoxygenation with nitrogen and sealed environment are required in these flow photo-PISA systems. The oxygen-sensitive characteristic of RAFT-mediated PISA greatly reduces the applicability of flow photo-PISA for the preparation of block copolymer nano-objects. In this study, we report an enzyme-assisted photo-PISA method using continuous flow reactors in the presence of glucose oxidase (GOx) and glucose. Oxygen and glucose can be converted into hydrogen peroxide and gluconolactone via the catalysis of GOx, respectively.[59] Therefore, the flow photo-PISA reported in this study exhibited excellent oxygen-tolerant property. The effect of GOx on the polymerization kinetics was investigated in detail, demonstrating the importance of GOx on the oxygen-tolerant property. By changing the length of core-forming block, monomer concentration, reaction temperature, and solvent composition, block copolymer nano-objects with various morphologies can be readily prepared and two morphological phase diagrams were constructed.

**EXPERIMENTAL**

**Materials**

2-Hydroxypropyl methacrylate (HPMA, Aladdin), monomethoxy poly(ethylene glycol) (mPEG, 2000 and 5000 g/mol, Sigma-Aldrich), GOx (Aladdin), glucose (Aladdin), anhydrous dichloromethane (DCM, Tianjin Zhuyuan), hydroquinone (Aladdin), N,N-dimethylformamide (DMF, Aladdin), dicyclohexylcarbodiimide (DCC, Aladdin), 4-dimethylaminopyridine (DMAP, Aladdin), 1,3,5-trioxacyclohexane (Aladdin), and 4,4'-azobis(4-cyanovaleric acid) (ACVA, Aladdin) were used as received. 4-Cyano-4-(ethyliothiocarbonothioylthio) pentanoic acid (CEPA) was synthesized according to a literature procedure.[33] Sodium phenyl-2,4,6-trimethylbenzoylphosphinate (SPTP) was synthesized according to a literature procedure.[34] Silicone tubes were purchased from Maijialong (Shanghai). LED lamps (405 nm) were purchased from Jincheng LED Lighting (Shenzhen, Guangdong). LED strips (405 nm) were purchased from Maijialong (Shanghai). LED lamps (405 nm) were purchased from Guangdong). LED strips (405 nm) were purchased from Jincheng LED Lighting (Shenzhen, Guangdong).

**Characterization**

The obtained samples were diluted with water. A drop of the dispersion was placed on a copper grid for 3 min and then blotted with filter paper to remove excess solution. A drop of uranyl acetate solution (0.5 wt%) was soaked on the same copper grid for another 3 min, and then blotted with filter paper to remove excess strain. Transmission electron microscopy (TEM) observations were carried out on an HT7700 instrument operated at 100 kV. 

$^1$H-NMR spectra were recorded in CDCl$_3$, D$_2$O or DMSO-d$_6$ using a Bruker Avance III 400 MHz NMR spectrometer at 25 °C. The molecular weight and polydispersity of the block co-polymers were measured by gel permeation chromatography (GPC) using a Waters 1515 GPC instrument with DMF as the mobile phase and Waters styragel HR1 and HR4 columns. The eluent was DMF containing 10 mmol/L LiBr and was filtered prior to use. The flow rate of DMF was 1.0 mL/min. Linear poly(methyl methacrylate) polymers with narrow molecular weight distributions were used as standards to calibrate the apparatus.

UV-Visible absorption spectra were recorded with a 1.0 cm quartz cuvette using a UV2450 spectrometer.

Intensity-average hydrodynamic diameters of the copolymer dispersions were determined by dynamic light scattering (DLS) using a Brookhaven nanoparticle size-zeta potential and molecular weight analyzer. Dilute aqueous dispersions were analyzed using disposable cuvettes and all data were averaged over three consecutive runs.

**Synthesis of mPEG$_{113}$-CEPA**

A solution of CEPA (2.37 g, 9 mmol) in anhydrous DCM (60 mL) was added in a dry flask under nitrogen atmosphere containing mPEG$_{113}$ (30.0 g, 6 mmol). Then a solution of DCC (1.86 g, 9 mmol) and DMAP (0.11 g, 0.9 mmol) in anhydrous DCM (20 mL) was added dropwise to the reaction mixture at 0 °C. The esterification reaction proceeded with stirring at room temperature for 48 h. The polymer was collected by precipitation of the reaction mixture in cold hexane. The product was then further purified by silica column chromatography (DCM:CH$_3$OH=15:1, V/V), and finally dried at 45 °C under vacuum to obtain a yellow powder.

**Enzyme-assisted Photo-PISA in a Flow Reactor**

In a typical experiment for the synthesis of mPEG$_{113}$-HPMA$_{200}$ (10 wt%) at room temperature, HPMA (0.4 g, 2.77 mmol), mPEG$_{113}$-CEPA (0.073 g, 0.014 mmol), glucose (0.072 g, 0.1 mol/L), and GOx solution (64 μL, 5 mg/mL) were added into a 25 mL round bottom flask. Then a certain amount of water (3.249 g in this case) was added to the flask to dissolve all reagents to form a homogeneous solution. The reaction mixture was incubated for 15 min to remove dissolved oxygen. Then a SPTP solution (287 μL, 5 mg/mL) was added to the reaction mixture. The reaction mixture was then pumped into the flow reactor via a peristaltic pump while the LED strips ($\lambda$=405 nm) were on. By regulating the speed of the peristaltic pump, the reaction mixture was exposed to purple light ($\lambda$=405 nm) at room temperature for 30 min. The polymerization was quenched by exposure to air and the addition of a small amount of hydroquinone.

**Kinetic Study of Enzyme-assisted Photo-PISA in a Flow Reactor**

In a typical experiment for the synthesis of mPEG$_{113}$-HPMA$_{200}$ (10 wt%, [GOx]$_0$=0.5 mmol/L), HPMA (20.0 g, 13.9 mmol), mPEG$_{113}$-CEPA (0.243 g, 0.046 mmol), SPTP solution (956 μL, 5 mg/mL), glucose (0.36 g, 0.1 mol/L), and water (16.40 g in this case) were added into a 25 mL round bottom flask to form a homogeneous solution. The reaction mixture was then separated into ten centrifugate tubes. For each centrifugate tube, a certain amount of GOx solution glucose (2.5 mg/mL) to ensure the final concentration of GOx is 0.5 mmol/L was added and incubated for 15 min to remove dissolved oxygen. The reaction
mixture was pumped into the flow reactor via a peristaltic pump at room temperature while the LED strips ($\lambda=405$ nm) were on. The irradiation time in the flow reactor was controlled by changing the flow rate. The reaction was quenched by exposure to air and the addition of a small amount of hydroquinone. The samples were then analyzed by $^1$H-NMR spectroscopy and GPC.

**Enzyme-assisted Photo-PISA in Batch**

In a typical experiment for the synthesis of mPEG$_{113}$-PHPMA$_{200}$ (20 wt%), HPMa (1.2 g, 8.3 mmol), mPEG$_{113}$-CEPA (0.218 g, 0.042 mmol), glucose (0.108 g, 0.1 mol/L) and GOx solution (96 $\mu$L, 5 mg/mL) to ensure the final concentration of GOx is 0.5 $\mu$mol/L were weighed into a 10 mL round bottom flask. Then a certain amount of water (3.844 g in this case) was added to the flask. The reaction mixture was incubated for 15 min to remove dissolved oxygen. SPTP solution (860 $\mu$L, 5 mg/mL) was then added to the reaction mixture. The reaction mixture was exposed to a LED lamp ($\lambda=405$ nm, 0.9 mW/cm$^2$) at room temperature for 30 min. The polymerization was quenched by exposure to air and the addition of a small amount of hydroquinone.

**RESULTS AND DISCUSSION**

**Synthesis and Characterization of Macro-RAFT Agent**

The macro-RAFT agent was prepared by coupling mPEG$_{113}$ with CEPA in dry DCM for 48 h using a DCC/DMAP chemistry, denoted as mPEG$_{113}$-CEPA. Fig. 1 shows $^1$H-NMR spectra of CEPA, mPEG$_{113}$, and mPEG$_{113}$-CEPA in CDCl$_3$. A new $^1$H-NMR signal (signal f in Fig. 1C) at 4.25 ppm was observed in the $^1$H-NMR spectrum of mPEG$_{113}$-CEPA, indicating the successful esterification between mPEG$_{113}$ and CEPA. The esterification efficiency can be determined by comparing integral areas of signal a (or f) and signal i in Fig. 1(C), and a value of ~98% was obtained. UV-Vis spectroscopy was also utilized to measure the esterification efficiency (Fig. 2). Fig. 2(a) shows a Lambert-Beer linear calibration plot obtained for CEPA at a wavelength of 310 nm. By comparing the calibration plot of CEPA with the absorbance of mPEG$_{113}$-CEPA (Fig. 2c), an esterification efficiency of ~98% was also obtained.

**Enzyme-assisted Photoinitiated Polymerization-induced Self-assembly in Continuous Flow Reactors**

The obtained mPEG$_{113}$-CEPA was then used to mediate enzyme-assisted flow photo-PISA of HPMA in water using SPTP as a water-soluble photoinitiator (Scheme 1). To ensure good RAFT control during the polymerization, the [mPEG$_{113}$-CEPA]/[SPTP] ratio was maintained at 3/1 in this study. Because of the amphiphilic feature, mPEG$_{113}$-PHPMA, diblock copolymer nano-objects would be formed after the chain extension of PHPMA in

![Figure 2](https://doi.org/10.1007/s10118-021-2533-z)
water. Purple light ($\lambda=405$ nm) was used to perform the aqueous flow photo-PISA, since radicals can be generated rapidly via the decomposition of SPTP under purple light irradiation.[60] Fig. 3 shows a custom-made flow reactor, which consists of silicone tubing wrapped inside a crystallizing dish. A LED strip (purple light, $\lambda=405$ nm) wrapped inside a transparent beaker was placed in the middle of the crystallizing dish and faced toward the silicone tubing. Reaction solutions were pumped into the reactor via a peristaltic pump under open-air conditions and the irradiation time was controlled by changing the flow rate. In our experiments, we found that the change of flow rate had little effect on the morphology of block copolymer nano-objects. The light intensity in the flow reactor was maintained at 0.9 mW/cm$^2$.

In this study, GOx was utilized to consume oxygen in the presence of glucose. Mild reaction conditions of aqueous flow photo-PISA such as aqueous medium, room temperature (30 °C in this study), and visible light are critical to maintaining the bioactivity of GOx and ensuring good oxygen tolerance throughout the polymerization. In the GOx deoxygenation process (Scheme 1), it is clear that a small amount of H$_2$O$_2$ would be generated. In our previous study,[60] we have demonstrated that H$_2$O$_2$ generated from the GOx deoxygenation process had no effect on the RAFT controllability. To evaluate the effect of GOx on the flow photo-PISA, polymerization kinetics of aqueous flow photo-PISA of HPMA (10 wt%, target DP of 300) mediated by mPEG$_{113}$-CEPA were studied by using different GOx concentrations. It should be noteworthy that the glucose concentration was maintained at 0.1 mol/L to ensure constant removal of oxygen during the polymerization. Samples collected at different irradiation time points were dissolved in DMSO-$d_6$ immediately and characterized by $^1$H-NMR spectroscopy. Monomer conversions can be determined by monitoring the decrease in vinyl signals relative to an internal standard. Fig. 4(a) shows $^1$H-NMR spectra of reaction mixtures collected at different time points during the kinetic study of aqueous photo-PISA with 0.5 μmol/L GOx. A small amount of N,N-dimethylformamide (DMF) was added as the internal standard. Vinyl signals of HPMA at 5.65 and 6.05 ppm decreased significantly as the polymerization proceeded, indicating the rapid consumption of HPMA during enzyme-assisted flow photo-PISA. Fig. 4(b) shows plots of monomer conversion versus irradiation time for enzyme-assisted flow photo-PISA with different GOx concentrations. In the absence of GOx, only 38.2% monomer conversion was achieved within 30 min of purple light irradiation. In our previous research on batch photo-PISA with nitrogen deoxygenation, we have demonstrated that full monomer conversions could be achieved within 15 min of purple light irradiation.[28] The low polymerization rate and incomplete monomer conversion in the flow photo-PISA can be ascribed to the oxygen inhibition toward radicals. When 0.1 μmol/L GOx was added, a monomer conversion of 59.4% was achieved within 30 min of purple light irradiation and a significantly higher polymerization rate was observed compared to that of 0 μmol/L GOx. Upon increasing the GOx concentration to 0.5 μmol/L, near quantitative monomer conversion was achieved within 30 min of purple light irradiation, exhibiting a fast polymerization behavior. Further increasing the GOx concentration to

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**Scheme 1** Schematic illustration for the enzyme-assisted photoinitiated polymerization-induced self-assembly of HPMA mediated by mPEG$_{113}$-CEPA in continuous flow reactors.

**Fig. 3** (a) A digital image of the flow reactor used in enzyme-assisted flow photo-PISA. (b) Schematic illustration of the flow reactor including all dimensions.
2.0 μmol/L has little influence on the polymerization kinetics. These results suggest that flow photo-PISA with excellent oxygen tolerance can be achieved by adding a small amount of GOx (0.5 μmol/L or higher in this case) in the reaction. Fig. 4(c) shows semilogarithmic plots of enzyme-assisted flow photo-PISA with 0.5 and 2.0 μmol/L GOx. Two distinct regimes were observed in both cases, which correspond to homogenous polymerization stage and heterogenous polymerization stage. The rate enhancement observed during the heterogenous polymerization stage can be attributed to the high local monomer concentration in the micelles. These results indicate that self-assembly can still occur even conducting photo-PISA in a flow reactor with the addition of GOx under open-air conditions.

Samples collected during the kinetic study of enzyme-assisted flow photo-PISA (with 0.5 μmol/L GOx) were freeze-dried directly and characterized by DMF GPC. As shown in Fig. 5(a), each GPC trace shifted gradually to a higher molecular weight as the irradiation time increased, indicating the successful chain extension under enzyme-assisted flow photo-PISA conditions. It was found that mPEG₁₁₃-CEPA consumed gradually and two GPC peaks were observed during the early stage of the polymerization. A monomodal and symmetric GPC curve was observed for the final sample, indicating the achievement of a relatively high blocking efficiency. It should be noteworthy that the amount of GOx used in this study was too small to be detected by GPC equipment. A linear increase in number-average molecular weight ($M_n$) with monomer conversion and narrow molecular weight distributions ($M_w/M_n < 1.30$) confirmed that good RAFT control was maintained under the enzyme-assisted flow photo-PISA conditions (Fig. 5b).

One significant advantage of RAFT-mediated PISA is its ability to produce a variety of block copolymer nano-objects at high concentrations. A series of mPEG₁₁₃-PHPMA₃ diblocks...
lock copolymer nano-objects were then prepared by systematically changing the target DP of PHPMA block and the HPMA concentration. Mean DPs were determined by measuring monomer conversions via $^1$H-NMR spectroscopy and the morphology was checked by transmission electron microscopy (TEM). A morphological phase diagram was then constructed, as shown in Fig. 6. Similar to other PISA phase diagrams, the phase diagram reported here is strongly concentration-dependent. Spheres can only be prepared at a HPMA concentration of 10 wt%. In contrast, pure vesicles can only be obtained at a HPMA concentration of 15 wt% or higher with a high DP of PHPMA (500 in this case). This can be explained by the fact that higher monomer concentrations in RAFT-mediated PISA favor the plasticization of micelles with monomer as well as the sphere-sphere fusion, promoting the formation of higher-order morphologies. Moreover, a large region of mixed morphology was also observed in this phase diagram. Comparing this phase diagram with our previous phase diagram obtained in batch photo-PISA, it can be concluded that conducting RAFT-mediated PISA in bath or in flow has a significant influence on the morphology of block copolymer nano-objects. To make a direct comparison between these two techniques, enzyme-assisted photo-PISA of HPMA mediated by mPEG$_{113}$-CEPA was also conducted in batch (a 10 mL round bottom flask). Fig. 7 shows DMF GPC traces of mPEG$_{113}$-PHPMA$_n$ ($n=200, 300, 400, 500$) diblock copolymers (20 wt% HPMA) prepared by enzyme-assisted flow photo-PISA (top) and enzyme-assisted batch photo-PISA (bottom). It can be seen that the GPC data of mPEG$_{113}$-PHPMA$_n$ diblock copolymers prepared in the flow reactor were very close to those prepared in the batch reactor. These results suggest that conducting enzyme-assisted photo-PISA in flow
or in batch had little effect on the composition of the diblock copolymers. These samples were also characterized by TEM, as shown in Fig. 8. However, it was found that performing enzyme-assisted photo-PISA in batch was more likely to obtain higher-order morphologies. For example, a mixed morphology consisting of spheres and short worms was obtained at a DP of 300 when the enzyme-assisted photo-PISA was performed in flow (Fig. 8b). In contrast, pure vesicles were obtained at a DP of 300 when the enzyme-assisted photo-PISA was performed in batch (Fig. 8f). Moreover, for the enzyme-assisted flow photo-PISA, DP of PHPMA up to 500 was required to obtain pure vesicles (Fig. 8d). The morphological difference between flow photo-PISA and batch photo-PISA can be attributed to two reasons: (i) Continuous magnetic stirring in batch photo-PISA can enhance the sphere-sphere fusion and therefore the formation of higher-order morphologies; (ii) Fluidic dynamic is different in both cases, for a lamellar flow exists in the flow photo-PISA and a turbulent flow exists in the batch photo-PISA.

Temperature is another important factor that can further control the morphology of block copolymer nano-objects prepared by RAFT-mediated PISA. In our previous research, we have demonstrated that higher-order morphologies are likely to form at a higher reaction temperature in RAFT-mediated PISA. Herein, the effect of reaction temperature on enzyme-assisted flow photo-PISA of HPMA (20 wt%) was also investigated by varying the reaction temperature from 30 °C to 50 °C. Near quantitative monomer conversions were achieved in all cases when the reaction temperature was 50 °C or lower. Low monomer conversions were observed when the
reaction temperature was higher than 50 °C (e.g. 55 °C). This can be ascribed to the loss of bioactivity of GOx at such high temperatures, and thereby leading to the occurrence of oxygen inhibition during aqueous flow photo-PISA. Fig. 9(a) shows that GPC traces of mPEG<sub>113</sub>-PHPMA<sub>500</sub> diblock copolymers prepared at different temperatures are very close, suggesting the change of reaction temperature had little effect on the composition of the formed diblock copolymers. Figs. 8(d) and 9(b)–9(e) show TEM images of mPEG<sub>113</sub>-PHPMA<sub>500</sub> diblock copolymer nano-objects prepared by enzyme-assisted flow photo-PISA of HPMA (20 wt%) at different temperatures. Pure vesicles were obtained when the reaction temperature was 35 °C or lower (Figs. 8d and 9b). When the reaction temperature was increased to 40 or 45 °C (Figs. 9c and 9d), mixed morphologies containing vesicles and large compound vesicles (LCVs) were obtained. Further increasing the reaction temperature to 50 °C led to the formation of LCVs (Fig. 9e). A morphological phase diagram was also constructed by varying the reaction temperature and the DP of PHPMA (Fig. 9f). It is clear that increasing the reaction temperature facilitates the formation of higher-order morphologies. This phenomenon can be explained by the fact that increasing the reaction temperature can decrease the solubility of mPEG<sub>113</sub> and HPMA in the reaction medium.<sup>[50]</sup> Therefore, the packing parameter of mPEG<sub>113</sub>-PHPMA will increase as the reaction temperature increases, favoring the formation of higher-order morphologies.

To further tune the morphology of block copolymer nano-objects prepared by enzyme-assisted flow photo-PISA, water mixed with a certain amount of non-functionalized mPEG (2000 g/mol) was then used as the solvent. The Zhang group<sup>[63]</sup> reported that the addition of solvophilic homopolymer in RAFT-mediated PISA can promote the formation of block copolymer nano-objects with higher-order morphologies. Similar results were observed in our enzyme-assisted flow photo-PISA of HPMA (20 wt%, target composition of mPEG<sub>113</sub>-PHPMA<sub>500</sub>). Quantitative monomer conversions were observed in all cases, suggesting that the addition of mPEG in water did not lead to the loss of GOx activity. Fig. 8(d) shows that pure vesicles were obtained in the absence of mPEG. When the mPEG content was 5%, similar vesicular morphology was obtained (Fig. 10a). When the

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**Fig. 9** (a) DMF GPC traces of mPEG<sub>113</sub>-PHPMA<sub>500</sub> diblock copolymers prepared by enzyme-assisted flow photo-PISA of HPMA (20 wt%) at different temperatures. (b–e) TEM images of mPEG<sub>113</sub>-PHPMA<sub>500</sub> diblock copolymer nano-objects prepared by enzyme-assisted flow photo-PISA of HPMA (20 wt%) at different temperatures. (f) Morphological phase diagram constructed for enzyme-assisted flow photo-PISA of HPMA (20 wt%) by varying the DP of PHPMA and reaction temperature. Phase regions consist of worms (W), mixed morphologies (Mixed), vesicles (V), and large compound vesicles (LCV).

**Fig. 10** TEM images of mPEG<sub>113</sub>-PHPMA<sub>500</sub> diblock copolymer nano-objects prepared by enzyme-assisted flow photo-PISA of HPMA (20 wt%) at room temperature using water mixed with different amounts of mPEG (2000 g/mol) as solvents: (a) 5% mPEG, (b) 10% mPEG, (c) 15% mPEG, (d) 20% mPEG.
REFERENCES

1. Zhu, Y.; Yang, B.; Chen, S.; Du, J. Polymer vesicles: mechanism, preparation, application, and responsive behavior. Prog. Polym. Sci. 2017, 64, 1–22.

2. Zhang, W. J.; Hong, C. Y.; Pan, C. Y. Polymerization-induced self-assembly of functionalized block copolymer nanoparticles and their application in drug delivery. Macromol. Rapid Commun. 2019, 40, 1800279.

3. He, J.; Cao, J.; Chen, Y.; Zhang, L.; Tan, J. Thermoresponsive block copolymer vesicles by visible light-initiated seeded polymerization-induced self-assembly for temperature-regulated enzymatic nanoreactors. ACS Macro Lett. 2020, 9, 533–539.

4. Jin, X. H.; Price, M. B.; Finnegan, J. R.; Boott, C. E.; Richter, J. M.; Rao, A.; Menke, S. M.; Friend, R. H.; Whitwell, G. R.; Manners, I. Long-range exciton transport in conjugated polymer nanofibers prepared by seeded growth. Science 2018, 360, 897–900.

5. Ning, Y.; Fielding, L. A.; Ratcliffe, L. P.; Wang, Y. W.; Meldrum, F. C.; Armes, S. P. Oxidation of sulfate-based diblock copolymer nanoparticles within calcite: effect of varying the surface density of anionic stabilizer chains. J. Am. Chem. Soc. 2016, 138, 11734–11742.

6. Nguyen, D.; Such, C.; Hawkett, B. Polymer-TiO2 composite nanorattles via RAFT-mediated emulsion polymerization. J. Polym. Sci., Part A: Polym. Chem. 2012, 50, 346–352.

7. He, J.; Xu, Q.; Tan, J.; Zhang, L. Ketone-functionalized polymer nano-objects prepared via photoinitiated polymerization-induced self-assembly (photo-PISA) using a poly(diacetone acrylamide)-based macro-RAFT agent. Macromol. Rapid Commun. 2019, 40, 1800296.

8. Tian, H.; Qin, J.; Hou, D.; Li, Q.; Li, C.; Wu, Z. S.; Mai, Y. General interfacial self-assembly engineering for patterning two-dimensional polymers with cylindrical mesopores on graphene. Angew. Chem. Int. Ed. 2019, 58, 10173–10178.

9. Mai, Y.; Eisenberg, A. Self-assembly of block copolymers. Chem. Soc. Rev. 2012, 41, 5969–5985.

10. Wang, W. M.; Hong, C. Y.; Pan, C. Y. One-pot synthesis of nanomaterials via RAFT polymerization induced self-assembly and morphology transition. Chem. Commun. 2009, 5883–5885.

11. Wang, W. M.; Pan, C. Y. One-pot synthesis of polymeric nanoparticles via RAFT dispersion polymerization induced self-assembly and re-organization. Polym. Chem. 2010, 1, 1475–1484.

12. He, W. D.; Sun, X. L.; Wan, W. M.; Pan, C. Y. Multiple morphologies of PAA-b-PSt assemblies throughout RAFT dispersion polymerization of styrene with PAA macro-CTA. Macromolecules 2011, 44, 3358–3365.

13. Boissé, S.; Rieger, J.; Belal, K.; Di-Cicco, A.; Beaunier, P.; Li, M. H.; Charleux, B. Amphiphilic block copolymer nano-fibers via RAFT-mediated polymerization in aqueous dispersed system. Chem. Commun. 2010, 46, 1950–1952.

14. Charleux, B.; Delaître, G.; Rieger, J.; D’Agosto, F. Polymerization-induced self-assembly: from soluble macromolecules to block copolymer nano-objects in one step. Macromolecules 2012, 45, 6753–6765.

15. Li, Y.; Armes, S. P. RAFT synthesis of sterically stabilized methacrylic nanolatexes and vesicles by aqueous dispersion polymerization. Angew. Chem. Int. Ed. 2010, 49, 4042–4046.

16. Blanazs, A.; Madsen, J.; Battaglia, G.; Ryan, A. J.; Armes, S. P. Mechanistic insights for block copolymer morphologies: how do worms form vesicles? J. Am. Chem. Soc. 2011, 133, 16581–16587.

17. Ding, Z.; Ding, M.; Gao, C.; Boyer, C.; Zhang, W. In situ synthesis of coil-coil diblock copolymer nanotubes and tubular Ag/polymer nanocomposites by RAFT dispersion polymerization in poly(ethylene glycol). Macromolecules 2017, 50, 7593–7602.

18. Huang, J.; Guo, Y.; Gu, S.; Han, G.; Duan, W.; Gao, C.; Zhang, W. Multicompartent block copolymer nanoparticles: recent advances and future perspectives. Polym. Chem. 2019, 10, 3426–3435.

19. Li, S.; Nie, H.; Gu, S.; Han, Z.; Han, G.; Zhang, W. Synthesis of multicompartent nanoparticles of ABC miktoarm star polymers by seeded RAFT dispersion polymerization. ACS Macro Lett. 2019, 8, 783–788.

20. Li, S.; Han, G.; Zhang, W. Cross-linking approaches for block copolymer nano-assemblies via RAFT-mediated polymerization-induced self-assembly. Polym. Chem. 2020, 11, 4681–4692.

21. Wang, X.; Shen, L.; An, Z. Dispersion polymerization in environmentally benign solvents via reversible deactivation radical polymerization. Prog. Polym. Sci. 2018, 83, 1–27.

22. Wang, X.; Man, S.; Zheng, J.; An, Z. Alkyl-o-hydroxymethyl acrylate monomers for aqueous dispersion polymerization-induced self-
assembly. *ACS Macro Lett.* 2018, 7, 1461–1467.

23 Lv, F.; An, Z.; Wu, P. What determines the formation of block copolymer nanotubes? *Macromolecules* 2020, 53, 367–373.

24 Chen, X.; Liu, L.; Huo, M.; Zeng, M.; Peng, L.; Feng, A.; Wang, X.; Yuan, J. Direct synthesis of polymer nanotubes by aqueous dispersion polymerization of a cyclodextrin/styrene complex. *Angew. Chem. Int. Ed.* 2017, 56, 16541–16545.

25 Huo, M.; Zeng, M.; Wu, D.; Wei, Y.; Yuan, J. Topological engineering of amphiphilic copolymers via RAFT dispersion copolymerization of benzyl methacrylate and 2-(perfluoroctyl)ethyl methacrylate for polymeric assemblies with tunable nanostructures. *Polym. Chem.* 2018, 9, 912–919.

26 Huo, M.; Ye, Q.; Che, H.; Wang, X.; Wei, Y.; Yuan, J. Polymer assemblies with nanostructure-correlated aggregation-induced emission. *Macromolecules* 2017, 50, 1126–1133.

27 Zeng, R.; Chen, Y.; Zhang, L.; Tan, J. R-RAFT or Z-RAFT? Well-defined star block copolymer nano-objects prepared by RAFT-mediated polymerization-induced self-assembly. *Macromolecules* 2020, 53, 1557–1566.

28 Tan, J.; Sun, H.; Yu, M.; Sumerlin, B. S.; Zhang, L. Photo-PISA-shielding light on polymerization-induced self-assembly. *ACS Macro Lett.* 2015, 4, 1249–1253.

29 Dai, X.; Zeng, J.; Yu, L.; Li, X.; Zhang, L.; Tan, J. Seeded photoinitiated polymerization-induced self-assembly: cylindrical micelles with patchy structures prepared via the chain extension of a third block. *ACS Macro Lett.* 2019, 8, 955–961.

30 Liu, D.; He, J.; Zhang, L.; Tan, J. 100th Anniversary of macromolecular science viewpoint: heterogenous reversible deactivation radical polymerization at room temperature. Recent advances and future opportunities. *ACS Macro Lett.* 2019, 8, 1660–1669.

31 Zhang, Y.; Yu, L.; Dai, X.; Zhang, L.; Tan, J. Structural difference in macro-RAFT agents redirects polymerization-induced self-assembly of polymeric micelles. *Chem. Commun.* 2020, 56, 11920–11923.

32 Yu, L.; Dai, X.; Zhang, Y.; Zeng, Z.; Zhang, L.; Tan, J. Better RAFT control is better? Insights into the preparation of monodisperse surface-functional polymeric microparticles by photoinitiated RAFT dispersion polymerization. *Macromolecules* 2019, 52, 7267–7277.

33 Yu, L.; Zhang, Y.; Dai, X.; Xu, Q.; Zhang, L.; Tan, J. Open-air preparation of cross-linked CO2-responsive polymer vesicles by enzyme-assisted photoinitiated polymerization-induced self-assembly. *Chem. Commun.* 2019, 55, 11920–11923.

34 Liu, D.; Cai, W.; Zhang, L.; Boyer, C.; Tan, J. Efficient photoinitiated polymerization-induced self-assembly with oxygen tolerance through dual-wavelength type I photoinitiation and photoinduced deoxygenation. *Macromolecules* 2020, 53, 1212–1223.

35 Zeng, R.; Chen, Y.; Zhang, L.; Tan, J. Uncontrolled polymerization that occurred during photoinitiated RAFT dispersion polymerization of acrylic monomers promotes the formation of uniform raspberry-like polymer particles. *Polym. Chem.* 2020, 11, 4591–4603.

36 Jiang, Y.; Xu, N.; Han, J.; Yu, Q.; Guo, L.; Gao, P.; Lu, X.; Cai, Y. The direct synthesis of interface-decorated reactive block copolymer nanoparticles via polymerisation-induced self-assembly. *Polym. Chem.* 2015, 6, 4955–4965.

37 Yu, Q.; Ding, Y.; Cao, H.; Lu, X.; Cai, Y. Use of polyon complexation for polymerization-induced self-assembly in water under visible light irradiation at 25 °C. *ACS Macro Lett.* 2015, 4, 1293–1296.

38 Huang, L.; Ding, Y.; Ma, Y.; Wang, L.; Liu, Q.; Lu, X.; Cai, Y. Colloidal stable PIC vesicles and lamellae enabled by wavelength-orthogonal disulfide exchange and polymerization-induced electrostatic self-assembly. *Macromolecules* 2019, 52, 4703–4712.

39 Ding, Y.; Cai, M.; Cui, Z.; Huang, L.; Wang, L.; Lu, X.; Cai, Y. Synthesis of low-dimensional polymer complex nanomaterials via polymerization-induced electrostatic self-assembly. *Angew. Chem. Int. Ed.* 2018, 57, 1053–1056.

40 Gao, P.; Cao, H.; Ding, Y.; Cai, M.; Cui, Z.; Lu, X.; Cai, Y. Synthesis of hydrogen-bonded pore-switchable cylindrical vesicles via visible-light-mediated RAFT room-temperature aqueous dispersion polymerization. *ACS Macro Lett.* 2016, 5, 1327–1331.

41 Varlas, S.; Foster, J. C.; Georgiou, P. G.; Keogh, R.; Husband, J. T.; Williams, D. S.; O'Reilly, R. K. Tuning the membrane permeability of polymersome nanoreactors developed by aqueous emulsion polymerization-induced self-assembly. *Nanoscale* 2019, 11, 12653–12654.

42 Blackman, L. D.; Doncom, K. E. B.; Gibson, M. I.; O'Reilly, R. K. Comparison of photo- and thermally initiated polymerization-induced self-assembly: a lack of end group fidelity drives the formation of higher order morphologies. *Polym. Chem.* 2017, 8, 2860–2871.

43 Xu, S.; Ng, G.; Xu, J.; Kuchel, R. P.; Yeow, J.; Boyer, C. 2-(Methylthio)ethyl methacrylate: a versatile monomer for stimuli responsiveness and polymerization-induced self-assembly in the presence of air. *ACS Macro Lett.* 2017, 6, 1237–1244.

44 Yeow, J.; Boyer, C. Photoinitiated polymerization-induced self-assembly (photo-PISA): new insights and opportunities. *Adv. Sci.* 2017, 4, 1700137.

45 Yeow, J.; Shanmugam, S.; Corrigan, N.; Kuchel, R. P.; Xu, J.; Boyer, C. A polymerization-induced self-assembly approach to nanoparticles loaded with singlet oxygen generators. *Macromolecules* 2016, 49, 7277–7285.

46 Tan, J.; Liu, D.; Huang, C.; Li, X.; He, J.; Xu, Q.; Zhang, L. Photoinitiated polymerization-induced self-assembly of glycidyl methacrylate for the synthesis of epoxy-functionalized block copolymer nano-objects. *Macromol. Rapid Commun.* 2017, 38, 1700195.

47 Xu, Q.; Zhang, Y.; Li, X.; He, J.; Tan, J.; Zhang, L. Enzyme catalysis-induced RAFT polymerization in water for the preparation of epoxy-functionalized triblock copolymer vesicles. *Polym. Chem.* 2018, 9, 4908–4916.

48 Tan, J.; Bai, Y.; Zhang, X.; Huang, C.; Liu, D.; Zhang, L. Low-temperature synthesis of thermoresponsive diblock copolymer nano-objects via aqueous photoinitiated polymerization-induced self-assembly (photo-PISA) using thermoresponsive Macro-RAFT agents. *Macromol. Rapid Commun.* 2016, 37, 1434–1440.

49 Wang, L.; Ding, Y.; Liu, Q.; Zhao, Q.; Dai, X.; Lu, X.; Cai, Y. Sequence-controlled polymerization-induced self-assembly. *ACS Macro Lett.* 2019, 8, 623–628.

50 Zhang, Y.; He, J.; Dai, X.; Yu, L.; Tan, J.; Zhang, L. Combining the power of heat and light: temperature-programmed photoinitiated RAFT dispersion polymerization to tune polymerization-induced self-assembly. *Polym. Chem.* 2019, 10, 3902–3911.

51 Ma, Y.; Gao, P.; Ding, Y.; Huang, L.; Wang, L.; Lu, X.; Cai, Y. Visible light initiated thermoresponsive aqueous dispersion polymerization-induced self-assembly. *Macromolecules* 2019, 52, 1033–1041.

52 Zhao, Q.; Liu, Q.; Li, C.; Cao, L.; Ma, L.; Wang, X.; Cai, Y. Noncovalent structural locking of thermoresponsive polyion complex micelles, nanowires, and vesicles via polymerization-induced electrostatic self-assembly using an arginine-like monomer. *Chem. Commun.* 2020, 56, 4954–4957.

53 Tan, J.; Li, X.; Zeng, R.; Liu, D.; Xu, Q.; He, J.; Zhang, Y.; Dai, X.; Yu, L.; Zeng, Z.; Zhang, L. Expanding the scope of polymerization-induced self-assembly: Z-RAFT-mediated photoinitiated
dispersion polymerization. *ACS Macro Lett.* 2018, 7, 255–262.

54 Zhou, Y.; Gu, Y.; Jiang, K.; Chen, M. Droplet-flow photopolymerization aided by computer: overcoming the challenges of viscosity and facilitating the generation of copolymer libraries. *Macromolecules* 2019, 52, 5611–5617.

55 Reis, M. H.; Leibfarth, F. A.; Pittet, L. M. Polymerizations in continuous flow: recent advances in the synthesis of diverse polymeric materials. *ACS Macro Lett.* 2020, 9, 123–133.

56 Zaquen, N.; Rubens, M.; Corrigan, N.; Xu, J.; Zetterlund, P. B.; Boyer, C.; Junkers, T. Polymer synthesis in continuous flow reactors. *Prog. Polym. Sci.* 2020, 107, 101256.

57 Zaquen, N.; Azizi, W. A. A. W.; Yeow, J.; Kuchel, R. P.; Junkers, T.; Zetterlund, P. B.; Boyer, C. Alcohol-based PISA in batch and flow: exploring the role of photoinitiators. *Polym. Chem.* 2019, 10, 2406–2414.

58 Zaquen, N.; Yeow, J.; Junkers, T.; Boyer, C.; Zetterlund, P. B. Visible light-mediated polymerization-induced self-assembly using continuous flow reactors. *Macromolecules* 2018, 51, 5165–5172.

59 Tan, J.; Liu, D.; Bai, Y.; Huang, C.; Li, X.; He, J.; Xu, Q.; Zhang, L. Enzyme-assisted photoinitiated polymerization-induced self-assembly: an oxygen-tolerant method for preparing block copolymer nano-objects in open vessels and multiwell plates. *Macromolecules* 2017, 50, 5798–5806.

60 Tan, J.; Liu, D.; Bai, Y.; Huang, C.; Li, X.; He, J.; Xu, Q.; Zhang, X.; Zhang, L. An insight into aqueous photoinitiated polymerization-induced self-assembly (photo-PISA) for the preparation of diblock copolymer nano-objects. *Polym. Chem.* 2017, 8, 1315–1327.

61 Blanazs, A.; Ryan, A. J.; Armes, S. P. Predictive phase diagrams for RAFT aqueous dispersion polymerization: effect of block copolymer composition, molecular weight, and copolymer concentration. *Macromolecules* 2012, 45, 5099–5107.

62 Zhang, Y.; Han, G.; Cao, M.; Guo, T.; Zhang, W. Influence of solvophilic homopolymers on RAFT polymerization-induced self-assembly. *Macromolecules* 2018, 51, 4397–4406.

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