# **Supporting Information**

# Direct Optical Observations of Vesicular Self-Assembly in Large-Scale Polymeric Structures During Photocontrolled Biphasic Polymerization

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### Materials

Poly(ethylene glycol) methyl ether ( $M_w$ = 1900, Alfa Aesar) was dried at 60 °C overnight. Hydroxypropyl methacrylate (Alfa Aesar), elemental sulfur (Sigma), benzyl chloride (Sigma), sodium methoxide (30% solution in methanol, Sigma), anhydrous methanol (Sigma), potassium hexacyanoferrate (Sigma), ethyl acetate (Alfa Aesar), 4,4'-azobis(4-cyano)pentanoic acid (Alfa Aesar) and tris(2,2'-bipyridyl)ruthenium(II) chloride hexahydrate (Sigma) (Ru(Bpy)) were used as received. The stock solution of Ru(Bpy) was prepared in deionized water and the final concentration of the complex was 8.5 mmol/L.

### Instrumentation

The microscope observations were conducted on a Zeiss microscope (Axio Observer A1) equipped with a fluorescence light source (X-Cite 120Q) and filter setup (BD Carv II) as well as a CCD camera optimized for capturing the emitted light. The used filters provided for excitation wavelengths of 450 nm (blue light) and 508 nm (green light). The proton NMR spectra were acquired in dimethyl sulfoxide- $d_6$  or methanol- $d_4$  at 25 °C on a 500 MHz Varian Unity/Inova spectrometer courtesy of the Laukien-Purcell Instrumentation Center at the Department of Chemistry and Chemical Biology, Harvard University.

#### **Experimental Section**

The following three-step synthesis of the RAFT agent has been adapted from literature<sup>1,2</sup>.

**Synthesis of bis(thiobenzoyl) disulfide**. To a three-necked, 50 mL flask equipped with a reflux condenser, a drying tube and a dropping funnel we added 1.6 g (50 mmol) of finely powdered elemental sulfur, 9 mL of methanolic sodium methoxide solution and 15 mL of anhydrous methanol. Benzyl chloride (3.15 g, 25 mmol) was then added dropwise over one hour. Afterwards the flask was refluxed at 65 °C for 10 hours. After cooling, any precipitate was filtered off from the dark-purple liquid and methanol was removed under reduced pressure. The residue was dissolved in 25 mL of water and washed with diethyl ether (3 x 10 mL). The aqueous layer was subjected to two phase-transfer procedures: first, 10 mL of ether and 25 ml of 1.0 mol/L hydrochloric acid were added, the organic layer was extracted and kept. Then, 15 mL of water and 30 mL of 1.0 mol/L sodium hydroxide were added and the product transferred back to the aqueous phase to finally give an aqueous solution of sodium dithiobenzoate. Separately, potassium hexacyanoferrate(III) (4.234 g) was dissolved in 65 mL of water

and this solution was added to the one obtained above, dropwise over 1 hour under vigorous stirring. The purple precipitate was filtered, dried in a vacuum line and stored in a refrigerator (yield: 26%).

**Synthesis of 4-cyano-4-(phenylcarbonoylthio)pentanoic acid.** 17 mL of ethyl acetate was placed in a 50 mL three-necked flask. To the flask was added 1.24 g (4.45 mmol) of 4,4'-azobis(4-cyano)pentanoic acid and 0.9 g bis(thiobenzoyl) disulfide (2.965 mmol). The mixture was refluxed overnight for 18 hours in total. The solvent was removed under reduced pressure to give a deep red residue. This was subjected to column chromatography using silica gel (60 Å, mesh 230-400) as the stationary phase and ethyl acetate/hexanes (2:3) as eluent. The product was loaded on the column using ethyl acetate/hexanes (1:9). The red fraction was dried over anhydrous calcium chloride overnight, followed by solvent removal under reduced pressure. The product was a red, thick oil. It was stored in a refrigerator (yield: 31%).

**Synthesis of the PEG-based RAFT chain transfer agent.** A three-necked, 25 mL flask equipped with a drying tube and a dropping funnel was charged with 10 mL of anhydrous dichloromethane, N,N'- dimethylaminopyridine (0.017 g, 0.137 mmol), poly(ethylene glycol) methyl ether ( $M_w$ = 1900, 0.74 mmol, 1.41 g) and 4-cyano-4-(phenylcarbonoylthio)pentanoic acid (1.48 mmol, 0.413 g). The contents were cooled to 0 °C using an ice bath and stirred at this temperature for 10 minutes. N,N'- dicyclohexylcarbodiimide (1.48 mmol, 0.305 g) in 5 mL of dichloromethane was added through the dropping funnel. The mixture was then stirred at room temperature for 20 hours. The precipitated side product was filtered off and the liquid was poured into a large excess of diethyl ether. The light-pink precipitate was filtered, dried and stored in a refrigerator (yield = 85%).

**Light-controlled polymerization.** In a typical procedure (20 ppm Ru(Bpy) relative to monomer, 10% monomer by weight, target degree of polymerization 200), 0.2 g of hydroxypropyl methacrylate and 0.0139 g PEG-CTA were dissolved in a glass vial, in 1.9968 mL of water, followed by addition of 3.26  $\mu$ L aqueous (8.5 mM) Ru(bpy) solution. The polymerization was conducted under magnetic stirring (250 r.p.m.), and the vial was placed in a secondary container equipped with a blue LED strip (cumulative LED power: 5 W, Figure S7). After a predetermined amount of time, a 9  $\mu$ L aliquot of the mixture was transferred to a microscope slide without dilution and observed under a cover slip. For NMR analysis of the phase-separated fraction, the upper layer was removed and the remainder dissolved in deuterated methanol.

**Table S1.** Efficiency of photocontrolled RAFT polymerization of hydroxypropyl methacrylate (HPMA), initiated by  $PEG_{43}$ -dithiobenzoate chain transfer agent, for different loadings of tris(2,2'-bipyridine) ruthenium(II) chloride<sup>a</sup>

Ru(II)	<i>Conversion<sup>b</sup></i>	M <sub>n,theo</sub> c	$M_{n,NMR}^{b}$
concentration		[g/mol]	[g/mol]
vs. HPMA			
24 ppm	44 %	14,860	11,410
32 ppm	84 %	26,380	21,730
48 ppm	11 %	5,360	4,050

<sup>*a*</sup>Experimental conditions: target degree of polymerization 200, room temperature, dimethyl sulfoxide as 4solvent, 22 hours of reaction time.

<sup>b</sup>As determined by <sup>1</sup>H NMR measurements.

<sup>c</sup>Calculated from the formula  $Mw = M_{w,CTA} + \alpha M_{w,M}([M]/[CTA])$ , where  $M_{w,CTA}$  and  $M_{w,M}$  denote the molecular weight of the RAFT chain transfer agent and the monomer, respectively,  $\alpha$  stands for monomer conversion, [M]/[CTA] for the molar ratio of the monomer and the RAFT chain transfer agent.





**Figure S1.** Typical structures revealed by phase-contrast optical microscopy at *t* = 18 hours in the polymerization medium (20 ppm Ru(Bpy) relative to monomer, 10% monomer by weight, target degree of polymerization 200).





**Figure S2.** Fluorescence microscopy images of an aggregate observed during RAFT polymerization of 2hydroxypropyl methacrylate (HPMA) in water photoinitiated by  $Ru(bpy)_3^{2+}$  and controlled by the  $PEG_{43}^{-}$ functionalized dithiobenzoate chain transfer agent (20 ppm Ru(Bpy) relative to monomer, 10% monomer by weight, target degree of polymerization 200). The observed sample was removed from the bulk at 18 hours reaction time.



**Figure S3.** Phase separation occurring on a microscope slide during RAFT polymerization of 2hydroxypropyl methacrylate (HPMA) in water photoinitiated by  $Ru(bpy)_3^{2+}$  and controlled by the  $PEG_{43}$ functionalized dithiobenzoate chain transfer agent (20 ppm Ru(Bpy) relative to monomer, 10% monomer by weight, target degree of polymerization 200), as revealed by phase contrast microscopy. The observed sample was removed from the bulk at 2 hours reaction time.



**Figure S4.** Influence of illumination wavelength on the rate of dynamic changes in the RAFT polymerization of 2-hydroxypropyl methacrylate (HPMA) in water photoinitiated by  $Ru(bpy)_{3}^{2+}$  and controlled by the  $PEG_{43}$ -functionalized dithiobenzoate chain transfer agent (20 ppm Ru(Bpy) relative to monomer, 10% monomer by weight, target degree of polymerization 200), as revealed by phase contrast microscopy. Top row: blue light illumination after 15, 30 and 45 minutes. Bottom row: green light illumination after 15, 30 and 45 minutes. The observed sample was removed from the bulk at 7 hours reaction time.



**Figure S5.** <sup>1</sup>H NMR spectrum of the bottom, polymer-rich layer of the reaction medium (20 ppm Ru(Bpy) relative to monomer, 10% monomer by weight, target degree of polymerization 200) at 18 hours since starting the reaction. The signals of the vinyl protons are clearly visible at  $\delta$  = 5.65 ppm and  $\delta$  = 6.15 ppm. The peak at  $\delta$  = 3.64 ppm corresponds to the protons from the poly(ethylene glycol) chain, whereas water swelling is attested by the area around  $\delta$  = 4.80 ppm.



**Figure S6.** The proposed mechanism of the light-induced processes taking place during biphasic polymerization of 2-hydroxypropyl methacrylate, adapted from Xu *et al., Macromolecules*, **2014**, *47*, 4217-4229 (ref. 14 in the main text).



**Figure S7.** The illumination setup used for polymerization before the contents are transferred to a microscope slide.



**Figure S8.** Fluorescence microscopy image of the RAFT polymerization mixture: 2-hydroxypropyl methacrylate (HPMA) in water,  $Ru(bpy)_3^{2+}$  and  $PEG_{43}$ -functionalized dithiobenzoate chain transfer agent (20 ppm Ru(Bpy) relative to monomer, 10% monomer by weight, target degree of polymerization 200). The sample was observed immediately after mixing the reagents.

#### **Movie Clips**

**Video SM1.** Morphology changes during RAFT polymerization of 2-hydroxypropyl methacrylate (HPMA) in water photoinitiated by  $Ru(bpy)_3^{2+}$  and controlled by the PEG43-functionalized dithiobenzoate chain transfer agent. The video, taken by a CCD camera at the photoinitiator emission wavelength (620 nm) corresponds to the vicinity of the middle panel in Figure 1 and presents accelerated changes occurring over 43 minutes.

**Video SM2.** Continued morphology changes during RAFT polymerization of 2-hydroxypropyl methacrylate (HPMA) in water photoinitiated by  $Ru(bpy)_3^{2+}$  and controlled by the PEG43-functionalized dithiobenzoate chain transfer agent. The video, taken by a CCD camera at the photoinitiator emission wavelength (620 nm), corresponds to the vicinity of the bottom panel in Figure 1 and presents accelerated changes occurring over 43 minutes.

**Video SM3**. Comparison of the behavior of a polymer aggregate (after 18 hours of polymerization) between a situation in which it is illuminated with diffuse transmitted light (first 8 seconds of the video) and with blue light corresponding to the absorption maximum of the ruthenium(II)-based photoinitiator. The video presents accelerated changes occurring over 15 minutes.

#### References

- 1. Y. Mitsukami, M. S. Donovan, A. B. Lowe, and C. L. McCormick, *Macromolecules*, 2001, **34**, 2248-2256.
- J. Rieger, F. Stoffelbach, C. Bui, D. Alaimo, C. Jérôme, and B. Charleux, *Macromolecules*, 2008, 41, 4065-4068.