Biomimetic controlled radical photopolymerization in the two-dimensional organized environment under visible light.

Sergey A. Dergunov,*

Department of Chemistry, University of Connecticut, 55 North Eagleville Rd, Storrs, CT 06269-3060, USA.

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Methods

Materials

All reagents and solvents were purchased from Sigma-Aldrich and used as received unless otherwise specified. Sodium dodecylbenzenesulfonate (SDBS; an anionic surfactant), cetyltrimethylammonium bromide (CTAB; a cationic surfactant), ethyl α-bromoisobutyrate (EBiB) and tris[2-phenylpyridinato – C^2 N] iridium (III) ($Ir(ppy)_3$) were used as received (Sigma-Aldrich). Methyl methacrylate (MMA), tert-Butyl methacrylate (t-BMA), butyl methacrylate (BMA), hexyl methacrylate, lauryl methacrylate, were purchased from Sigma-Aldrich and were passed through an alumina column to remove the inhibitor shortly before sample preparation. Dichloromethane (DCM) was prepared by drying over $CaSO_4$ for 24 h, and purging with nitrogen gas for 15 minutes. Dry hexanes and ethyl acetate were prepared by passing nitrogen purged solvents through activated alumina columns, dried with $CaSO_4$. All dry solvents were stored over 4Å sieves. All kinetic experiments were performed with the same batch of monomers. $CDCl_3$ (99.8% D), D_2O (99,9% D), were purchased from Cambridge Isotope, USA for NMR study.

Experimental Procedures

The general method for polymerization inside a bilayer

Visible light LED photochemical reactor setup:

The LED reactor (Exitance (E_p): 11 mW/cm^2) is composed of a 10-foot-long adhesive 12V LED strip that emits blue light (SuperBrightLED, λmax = 460 nm) with an intensity of 64 lumens/ft adhered to the inner wall of a 170 x 90 mm crystallization dish (Figure S1). To avoid overheating of the light source a desktop fan is used to provide cooling. The sample is irradiated in a 15 x 100 mm disposable culture tube containing a 10 x 75 mm culture tube. This setup is then capped with a 14/20 rubber septum. Since the inner diameter of the larger test tube is 14 mm, there is a uniform 2 mm path length along the circumference of the setup.

Example procedure of PET-ATRP polymerization within the bilayer

A monomer stock solution containing EBiB (89 µmol), methacrylate (8.9 mmol), was prepared. Afterward, a 2.0 mg/mL solution of $Ir(ppy)_3$ in dichloromethane is prepared separately. Deionized Milli-Q water before mixing was bubbled with N_2 for 15-20 min. 18 mg of CTAB was solubilized in water at 40 °C. In a separate vial, 72 mg of SDBS was mixed with monomer (0.49 mmol), EBiB (4.9 umol), $Ir(ppy)_3$ (0.0245 umol), and 6 mL of deionized water under cover of aluminum foil. Afterward, the SDBS solution gently vortexed until surfactant is dissolved and the solution is homogenous (the solution was briefly sonicated when necessary). Subsequently, both solutions are mixed under slight vortex mixing, then the solution was allowed to mature for 30 mins and then extruded 5 times through a track-etched polyester membrane with 0.2 µm pores using a Lipex stainless steel barrel extruder. The sample is then centered inside the LED reactor and irradiated. In addition, the thickness of the aqueous layer of the reaction mixture was adjusted to 1-3 mm so that light could penetrate deeper and more evenly into the solution. Since the medium for polymerization is not a true solution, but vesicles, with a vesicle size of about 200 nm, more than 95% of the incident radiation is estimated to be absorbed within the first 1-2 mm in the reaction system. This is especially significant if using monomers that are not fully pre-organized in the

bilayer. If the reaction is carried out in large flasks, only a small part of the vesicles located close to the irradiated surface of the reactor will actually be irradiated, and a very strong inhomogeneity should be expected between the irradiated and non-irradiated volume.

DLS analysis was conducted on crude polymerization mixtures without filtration. Time point aliquots during polymerization were taken from the reaction mixture and analyzed by GPC to give the number average molecular weight (Mn), weight average molecular weight (Mw), and molecular weight distribution (Mw/Mn) of the polymer. Conversion of monomers was calculated using GCMS. Following the polymerization, a solution of NaCl (0.02 mL of 3 N) in methanol (10 mL) and acetone (3 ml) was added to the reaction mixture to braking of vesicles and polymer was precipitated by the addition of hexane (20 ml) and vortexing (sometimes with mild sonication). Methacrylates were re-precipitated four times from acetone/hexane and dried under vacuum at room temperature for 6 h. The purified polymers were isolated as white /yellowish-white powder.

Example procedure for EPR studies

18 mg of CTAB was solubilized in water (bubbled with N2 for 15 min) at 40 °C. In a separate vial, 72 mg of SDBS was mixed with monomer (0.49 mmol), EBiB (4.9 umol), PATEMPO (2.82 umol), $Ir(ppy)_3$ (0.0245 umol), and 6 mL of deionized water (bubbled with N2 for 15 min) under cover of aluminum foil. Afterward, the SDBS solution gently vortexed until surfactant is dissolved and the solution is homogenous (the solution was briefly sonicated when necessary). Subsequently, both solutions are mixed under slight vortex mixing, then solution was allowed to mature for 30 mins and then extruded 5 times through a track-etched polyester membrane with 0.2 µm pores using a Lipex stainless steel barrel extruder.

In active free radical probe TEMPO (and derivatives) the hyperfine coupling constant (α_N) of EPR signal is closely related to the nature of charge transfer of free radicals, and, depending on the polarity of the solvent or the inhomogeneity of the medium, a significant change in the shape of EPR spectral lines was observed. Due to this spectra of PATEMPO were recorded in different organic solvents.

Instrumentation

X-band EPR spectroscopy. EPR spectra were recorded at 9.6 GHz with a Bruker EMX EPR spectrometer equipped with a variable temperature controller BVT-3000. The parameters used were: power 0.03162 mW, center field 342 mT, sweep scan 20 mT, 2048 points, scan time 30 s, modulation amplitude 0.1 mT, 16 scans accumulation. Immediately preparation of vesicles was filled in quartz capillary with a diameter of 2 mm and inserted in a quartz tube. The sample was maintained at 25 °C. For initiation of polymerization, a blue LED light strip (λ_{max} = 460 nm, 7.8 mW/cm^2) or Kessil H150 LED Light – Blue 34W (for EPR of pure BMA) were used. The spectra were recorded every 10 min during the first hour.

GPC analysis. Molecular weights and molecular weight distributions were determined by size-exclusion chromatography (SEC) in *N, N*-dimethylacetamide (DMAc), or tetrahydrofuran (THF) at 50 °C using a flow rate of 1 mL min $^{-1}$ (Waters 515 HPLC pump & Waters 717 Autoinjector). Detection was performed using a Waters 2414 Refractive Index Detector operating at λ= 660 nm and Varian 380-LC Evaporative Light Scattering Detector (ELSD) (operating at 3 mW, λ= 670 nm with detection angles of 7^o and 90^o) and a four-capillary viscometer. The system was calibrated using PMMA standards with narrow molecular weight distribution (20 standards used for analyses from 602 g/mol to 120,000 g/mol). Size-exclusion

chromatography (SEC) was performed on a system equipped with size exclusion columns (Two mixed bed Jordi Gel DVB Medium MB columns, 300 X 7.8 mm, and separation over the molecular weight range from 200 to 3,000,000 g /mol) which were maintained at a temperature of 50°C. Samples were filtered through a 0.45 µm PTFE filter before analysis. 100 µl sample loops were used for the majority of SEC analysis. Mw and Mn of polymers were determined using software (Empower 3 Software).

Dynamic Light Scattering (DLS). Hydrodynamic diameter and polydispersity index (PDI) measurements were performed on a Malvern Nano-ZS zetasizer (Malvern Instruments Ltd., Worcestershire, U.K.). The Helium-Neon laser, 4mW, operated at 633 nm, with the scatter angle fixed at 173°, and the temperature at 25 °C. 80 µL samples were placed into disposable cuvettes without dilution (70 µL, 8.5 mm center height Brand UV-Cuvette micro). Each data point was an average of 10 scans. Data were processed using non-negative least squares (NNLS) analysis.

GC–MS analysis. GC–MS was used for both qualitative and quantitative analyses. It consists of a Shimadzu GC-2010 Plus system and a GCMS–QP2010 SE ion trap MS system (Shimadzu, Japan) with an electron impact ionization mode. Chromatographic separations were performed on a Shimadzu SH-Rxi-5SiL MS column (30 m×0.25 mm, 0.25mm film thickness; Non-polar phase: Crossbond™ 100% dimethyl polysiloxane as stationary phase; maximum temperature, 350 °C). Injector and GC–MS transfer line temperatures were 170 and 280◦C, respectively. Ultrahigh purity helium (99.999%) was used as carrier gas at a constant flow of 1.0 ml/min. The electron impact ionization mass spectrometer was operated as follows: ionization voltage, 70 eV; ion source temperature, 200◦C; scan mode, 30.0–500.0 (mass range); scan rate, 5000 amu/s, and 3.68 scans/s; start time 2 min. Electron multiplier (EM) voltage was obtained from autotune. For neutral fraction, the split injection with a ratio of 20:1 was used. The sample volume injected with an AOC-20i Auto-Injector was 1µl. The oven temperature program was 60◦C (2 min)–50◦C/min–300◦C (3 min).

A typical sample is prepared for polymerization and 250 µL aliquots are taken at specified intervals. After the trial is completed 100 µL of each aliquot is combined with 1.9 mL of hexane and stirred for 30 minutes to extract monomers. From this solution, 500 µL of the organic phase was transferred into GC autosampler vials of which 1.0 µL is injected into a Shimadzu QP2010-Se Gas chromatograph mass spectrometer using a split-mode injection (split ratio 20:1). Standards and samples were measured 3 times for each measurement and then the data were averaged. Standard samples of known concentration for methacrylates were prepared via serial dilution using hexane. With these samples, calibration curves are created using the least-squares method by fitting the GC-MS data to a linear curve which was used to interpolate between the points.

Nuclear magnetic resonance spectra (NMR) were recorded on a Bruker AVANCE III 400 MHz NMR spectrometer. Chemical shifts relate to residual protons in deuterated NMR solvents. SpinWorks 4.2.8. was used to analyze all spectra.

UV/vis-absorption spectra were taken with an Agilent Cary 60 UV-Vis spectrophotometer, an aqueous solution of vesicles being placed in a quartz cell of 1; 2; 5; 10 mm optical path.

TEM analysis was performed on carbon grids (300 Mesh, Cu, Electron Microscopy Sciences) coated with a thin layer of carbon. To deposit vesicles, a small drop (ca. 2-10 µL) of the sample was pipetted onto a grid and left to air dry. All samples were examined using a transmission electron microscope (JEOL TEM-1200), operating at 100 kV without the addition of a staining agent.

Figures

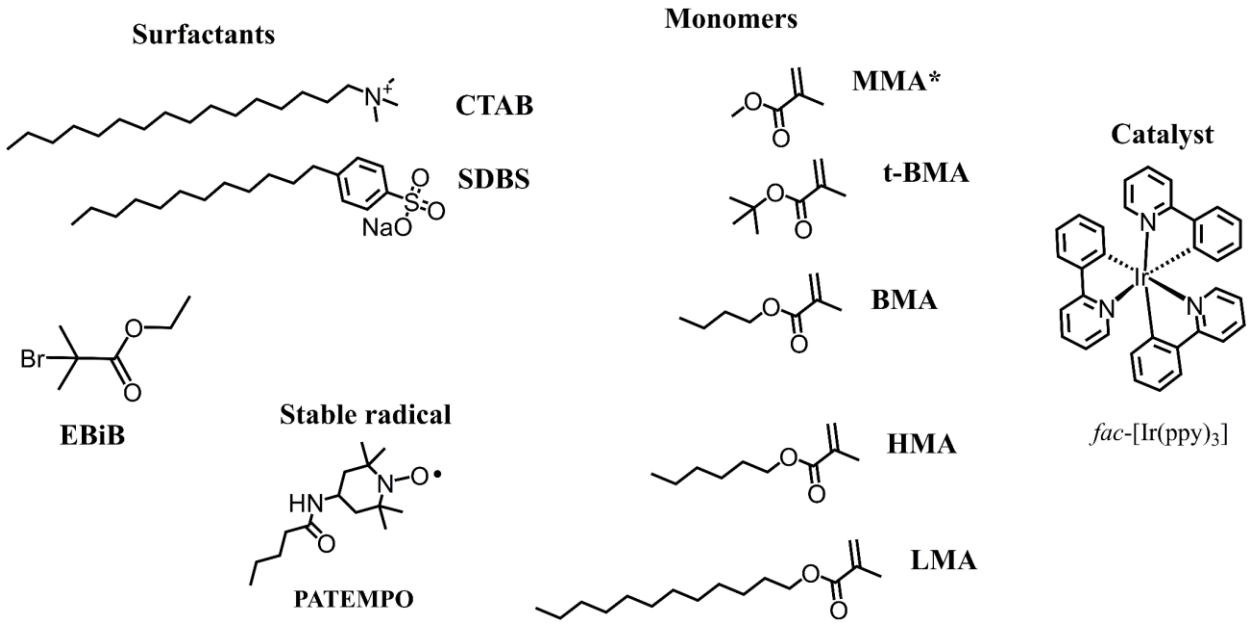

Figure S1. The structures of surfactants, monomers, catalyst, and stable radical.

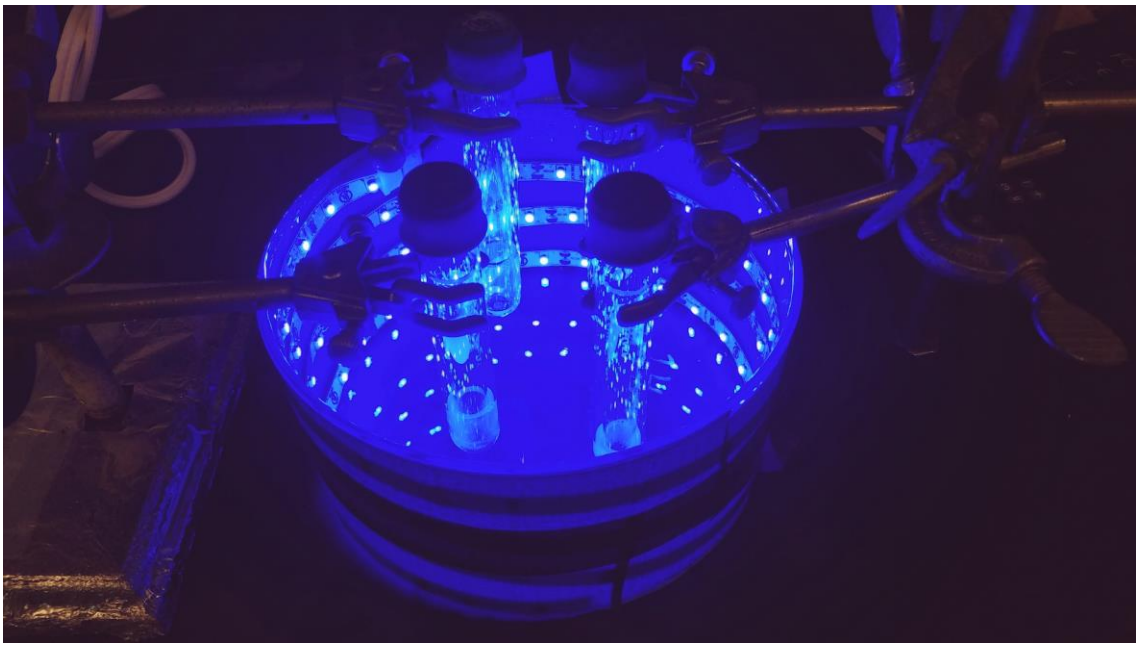

Figure S2. Reaction setup

Figure S3 Influence of the thickness of the water layer on the penetration of the blue LED light

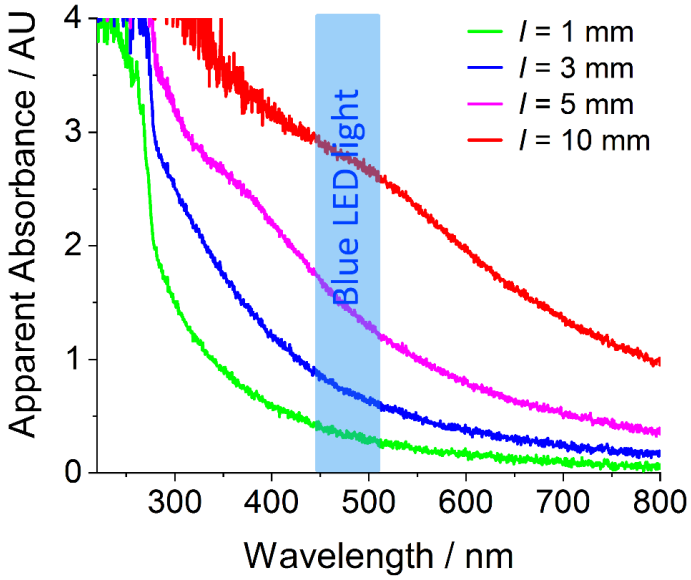

Figure S3a. Absorption spectra of an aqueous solution of vesicles based on 2% surfactants with confined BMA monomers within the bilayer with a vesicle diameter of 220 nm, measured at different path lengths.

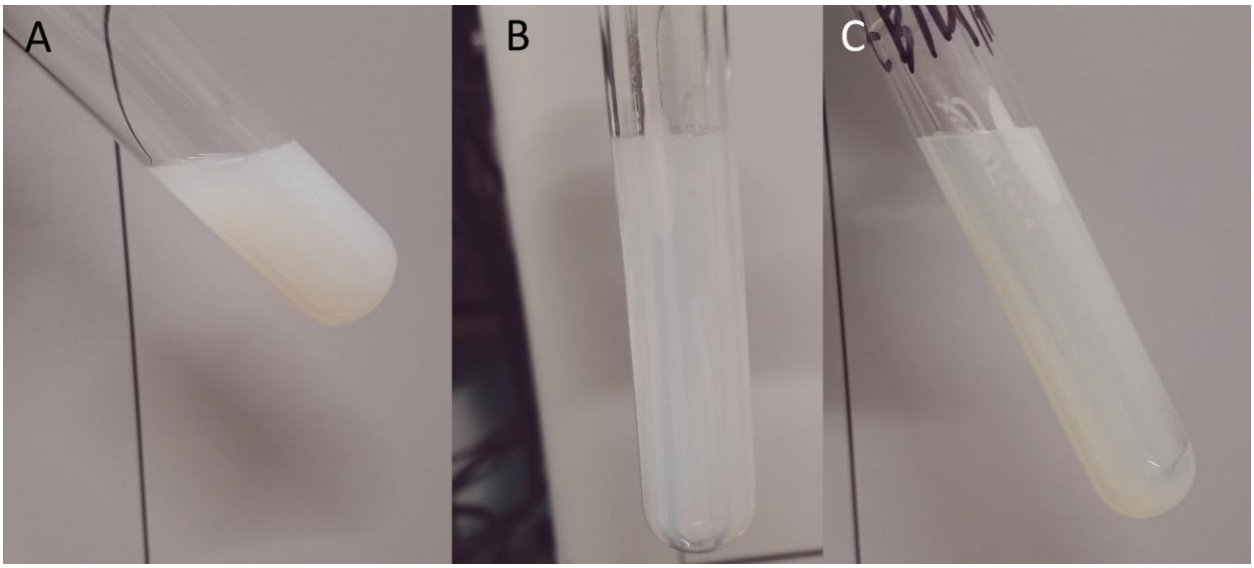

Figure S3b. An example of a photoRDRP in vesicles with a reaction vessel thickness of 1-3 mm is based on the experiment in Figure S3a. Photographs of vesicle solution after preparation (a) and before (b) and after (c) LED polymerization.

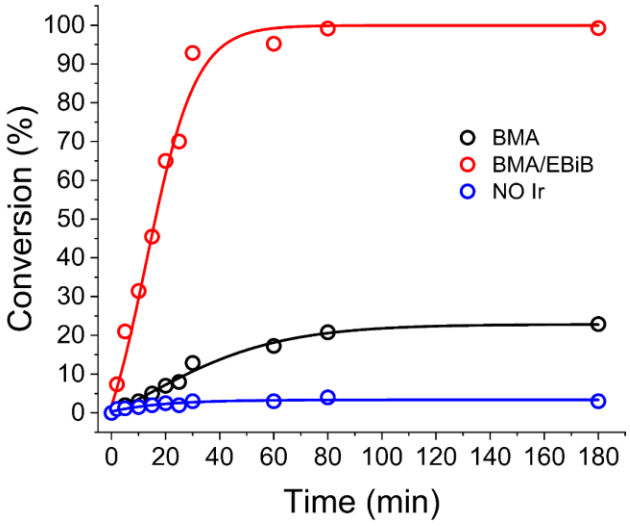

Figure S4. Photopolymerization of confined BMA monomers within the bilayer of vesicles in aqueous solution at 25°C using a *fac-[Ir(ppy)$_3$]* photoredox catalyst (λ_{max}=460 nm, Exitance (E_p): 11 mW/cm^2). [BMA] : [EBiB] : [Ir] = 100 : 1 : 0.005. D_{ave}=220±20nm, CTAB:SDBS=2:8, 2% surfactant solution. The lines are guides to the eye only.

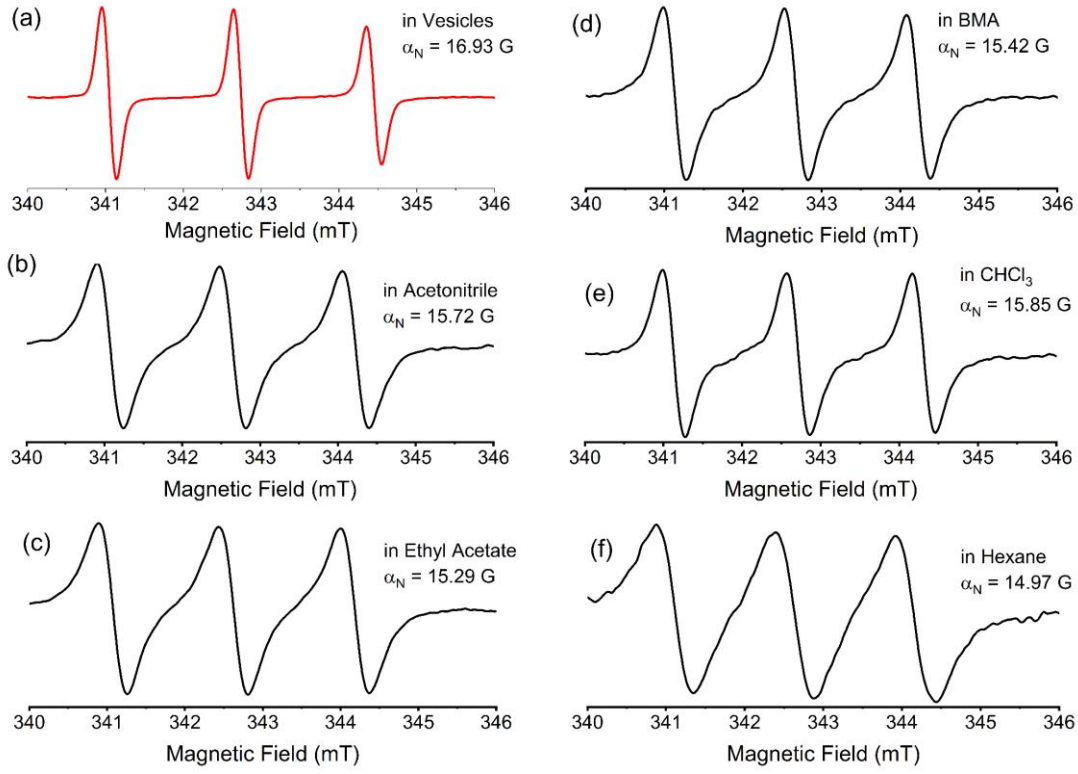

Figure S5. Experimental EPR spectra of 4-pentanamido-TEMPO (PATEMPO) stable radicals in vesicles and different solvents, measured at 25°C. EPR spectrometer settings: ν = 9.651 GHz, power 0.03162 mW, center field 342 mT, sweep scan 20 mT, 2048 points, scan time 30 s, modulation amplitude 0.1 mT, 16 scans accumulation.

Figure S6. EPR spectra of 4-pentanamido-TEMPO (PATEMPO) in the presence of BMA

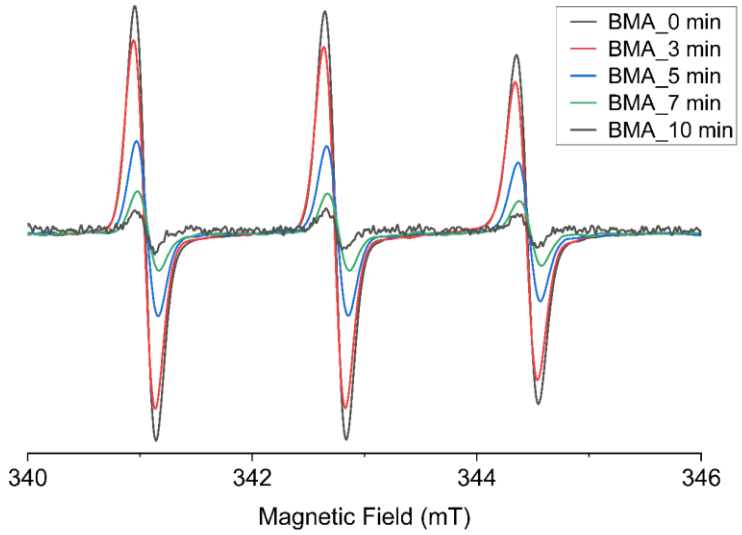

Figure S6a. EPR spectra (ν = 9.651 GHz) of 4-pentanamido-TEMPO (PATEMPO) obtained after exposure of the samples to blue LED light (λ_{max} = 460 nm, 7.8 mW/cm^2) in the presence of *fac*-[Ir(ppy)$_3$]/BMA/EBiB within a bilayer of spontaneously assembled vesicles in an aqueous solution at 25 °C. EPR spectrometer settings: power 0.03162 mW, center field 342 mT, sweep scan 20 mT, 2048 points, scan time 30 s, modulation amplitude 0.1 mT, 16 scans accumulation.

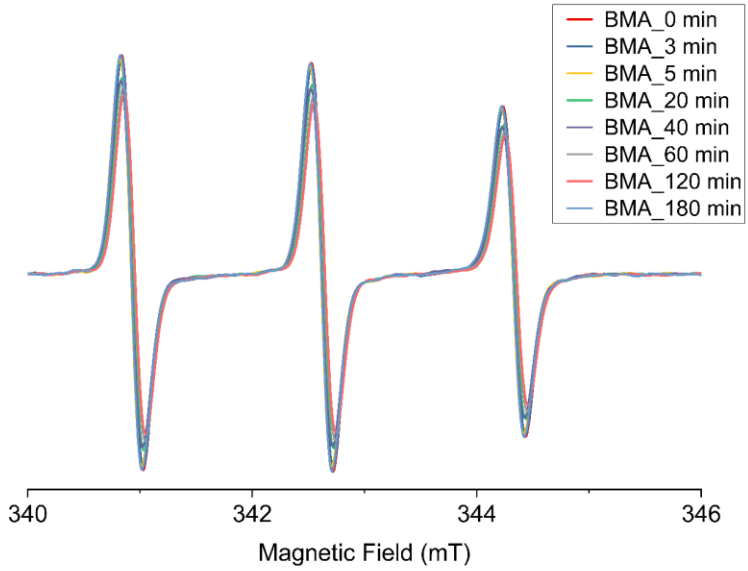

Figure S6b. EPR spectra (ν = 9.651 GHz) of 4-pentanamido-TEMPO (PATEMPO) obtained after exposure of the samples to blue LED light (λ_{max} = 460 nm, 7.8 mW/cm^2) in the presence of *fac*-[Ir(ppy)$_3$]/BMA within a bilayer of spontaneously assembled vesicles in an aqueous solution at 25 °C. NO transfer agent. EPR spectrometer settings: power 0.03162 mW, center field 342 mT, sweep scan 20 mT, 2048 points, scan time 30 s, modulation amplitude 0.1 mT, 16 scans accumulation.

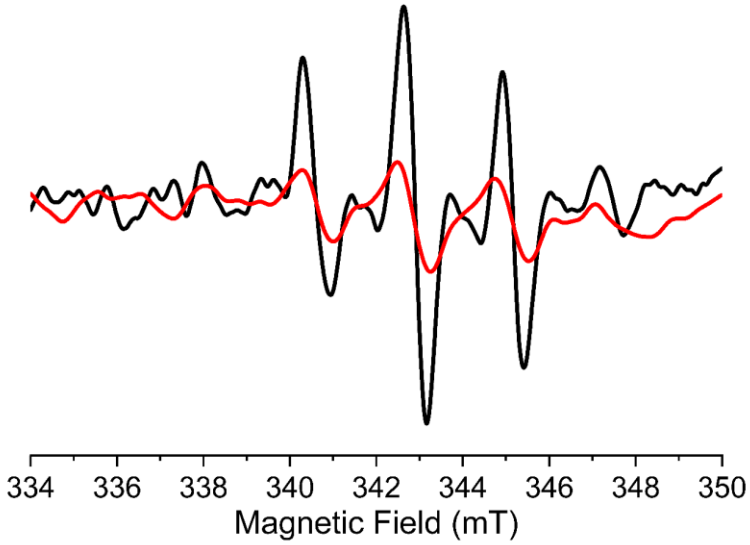

Figure S7. Experimental EPR spectra of BMA confined within the bilayer of vesicles in an aqueous solution, observed at 25 °C after exposure of the samples to blue LED light (5min, λ_{max} = 456 nm, 34 W, 352 mW/cm^2) with a *fac*-[Ir(ppy)$_3$] photoredox catalyst in the presence (solid line) and absence (red line) of EBiB.

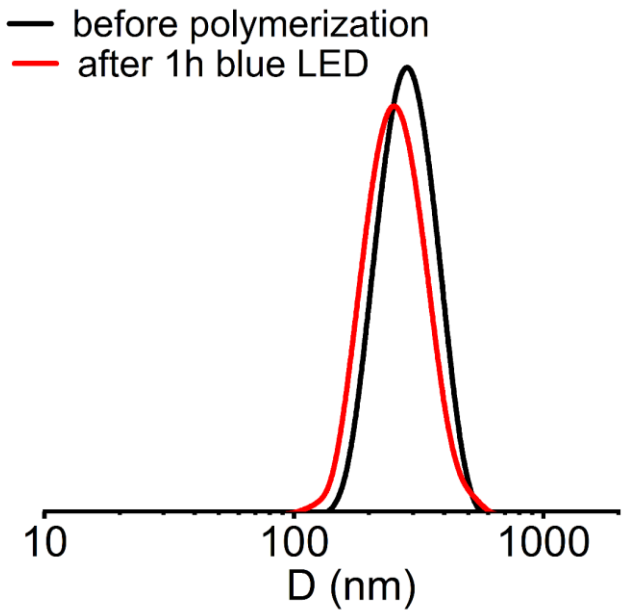

Figure S8. Size distribution of vesicles before (black line) and after (red line) polymerization.

Figure S9. ^{1}H NMR spectra and structures of monomers in $CDCl_3$ and in vesicles in D_2O

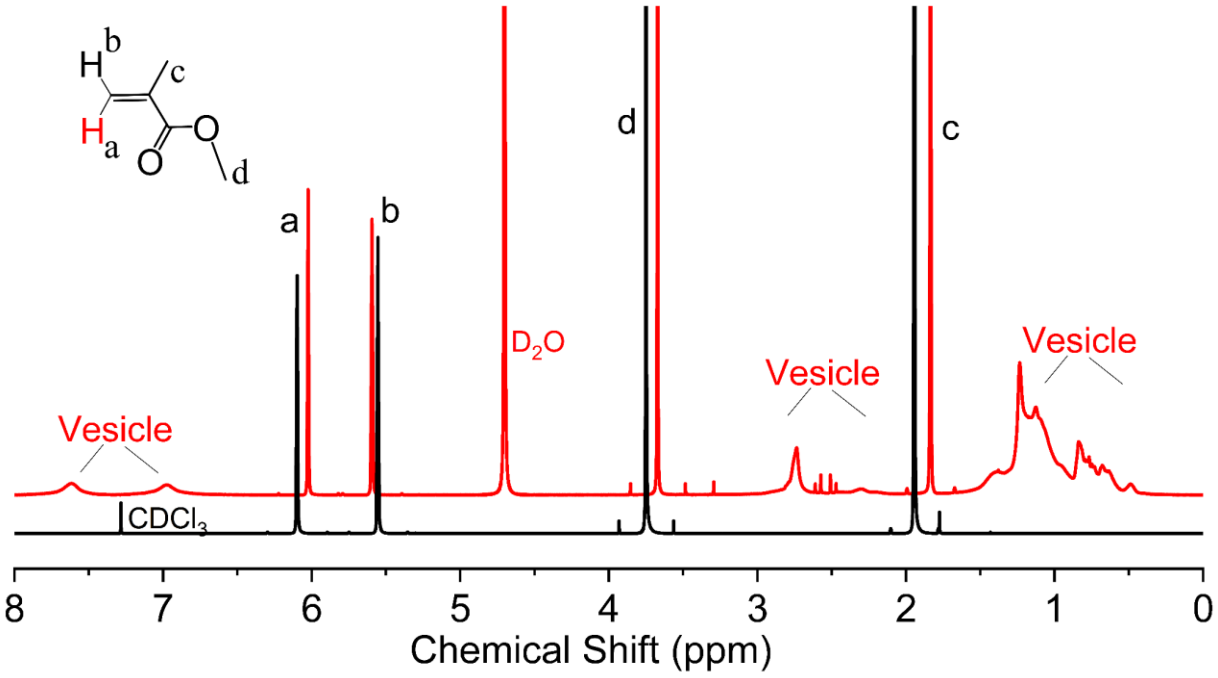

Figure S9a. ^{1}H NMR spectra of MMA in $CDCl_3$ (black line) and synergistically assembled vesicles with MMA in D_2O (red line)

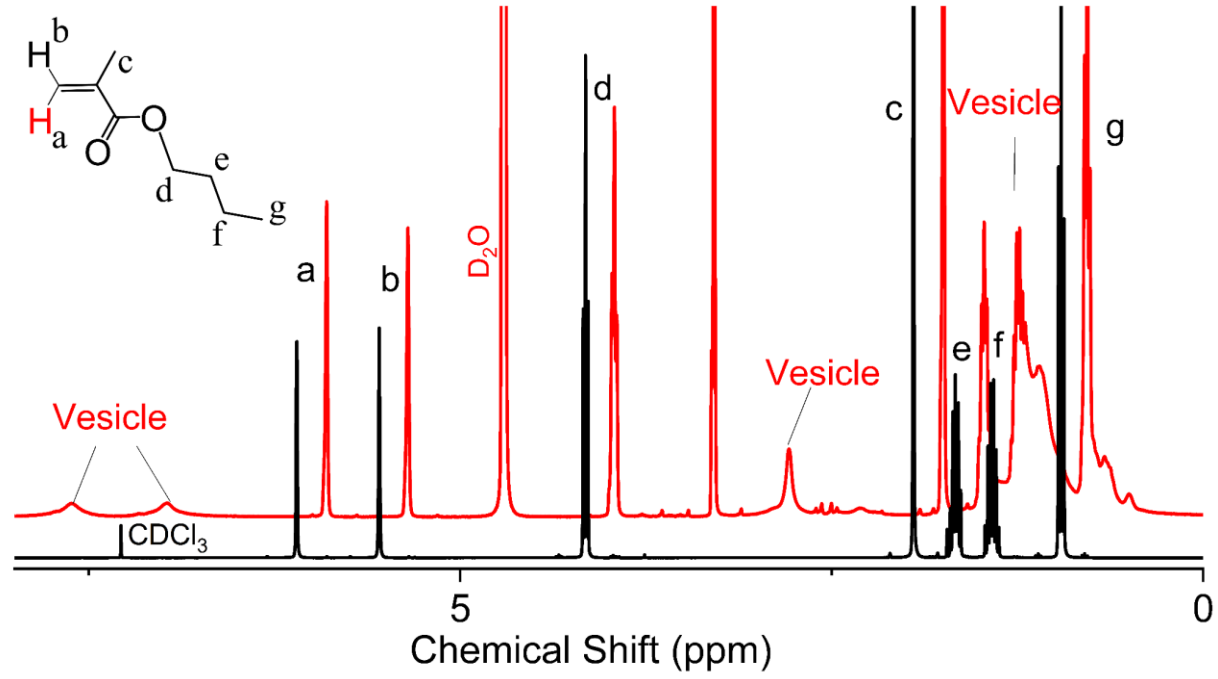

Figure S9b. ^{1}H NMR spectra of BMA in $CDCl_3$ (black line) and synergistically assembled vesicles with MMA in D_2O (red line)

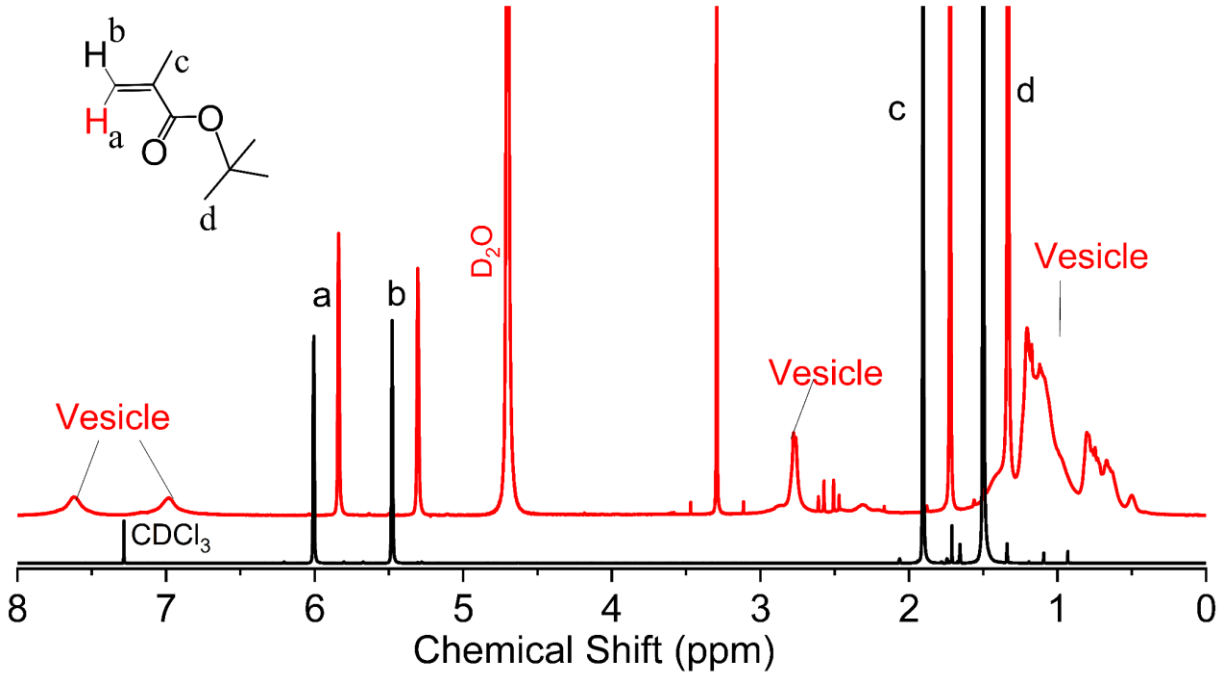

Figure S9c. ^{1}H NMR spectra of t-BMA in $CDCl_3$ (black line) and synergistically assembled vesicles with MMA in D_2O (red line)

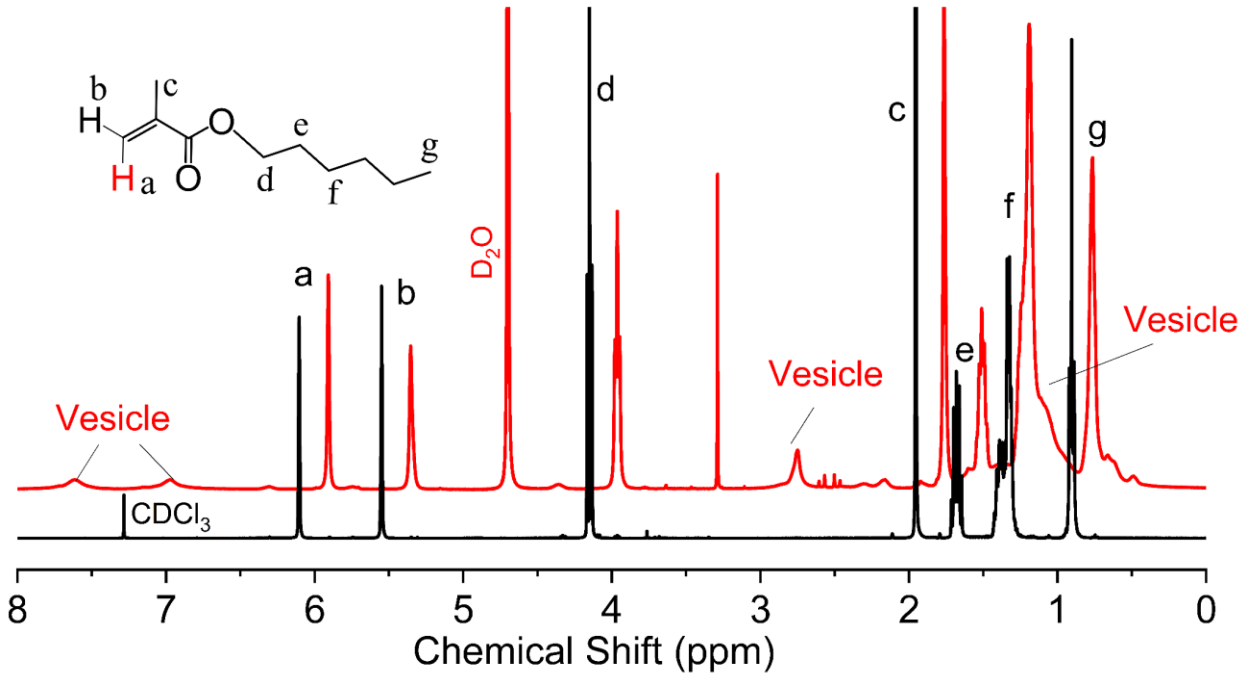

Figure S9d. ^{1}H NMR spectra of HMA in $CDCl_3$ (black line) and synergistically assembled vesicles with MMA in D_2O (red line)

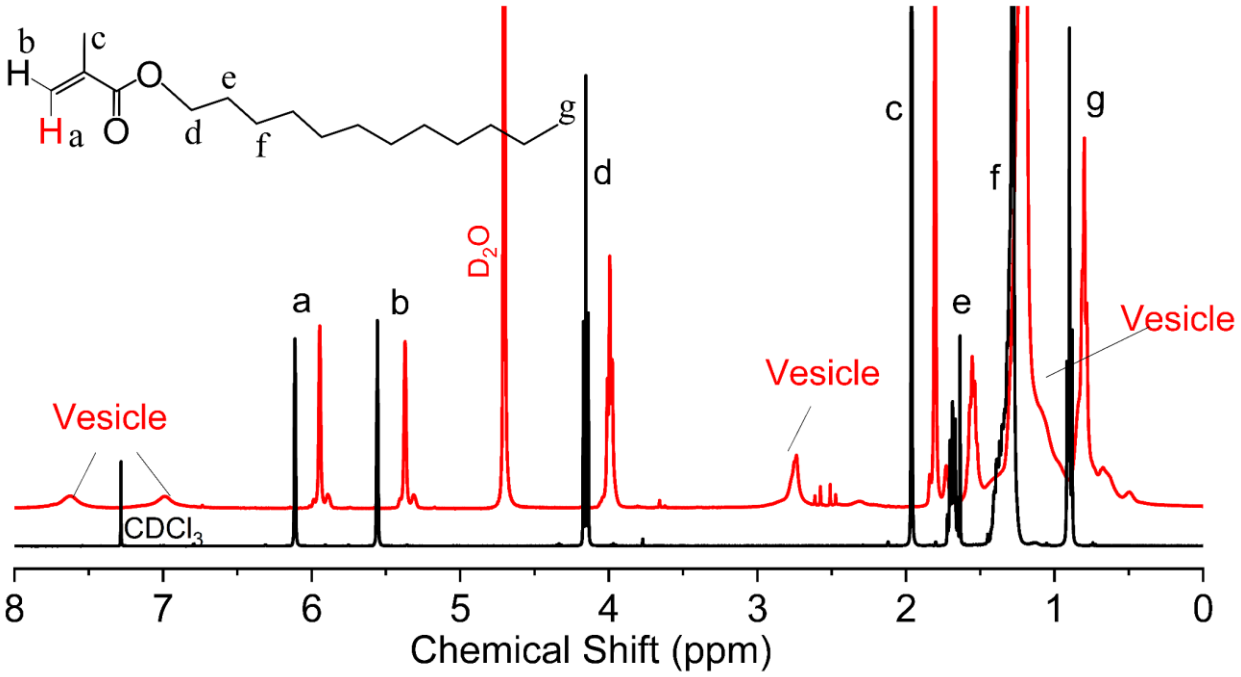

Figure S9e. ^{1}H NMR spectra of LMA in $CDCl_3$ (black line) and synergistically assembled vesicles with MMA in D_2O (red line)

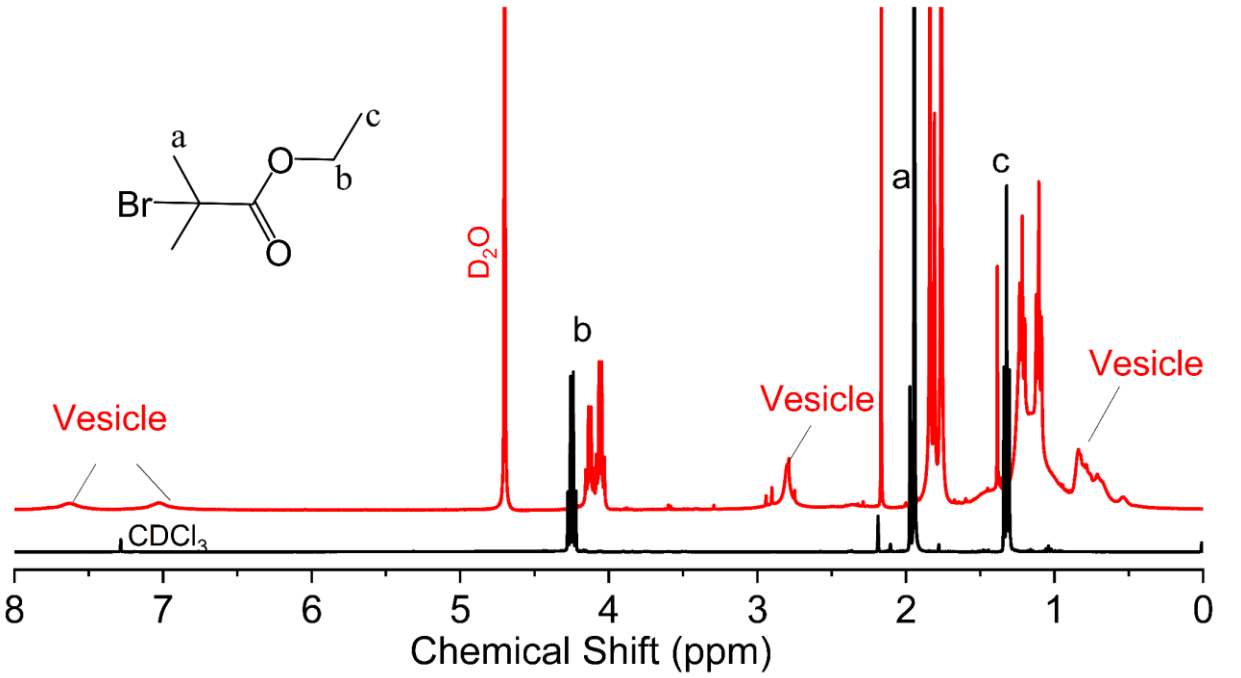

Figure S9f. ^{1}H NMR spectra of EBiB in $CDCl_3$ (black line) and synergistically assembled vesicles with MMA in D_2O (red line)

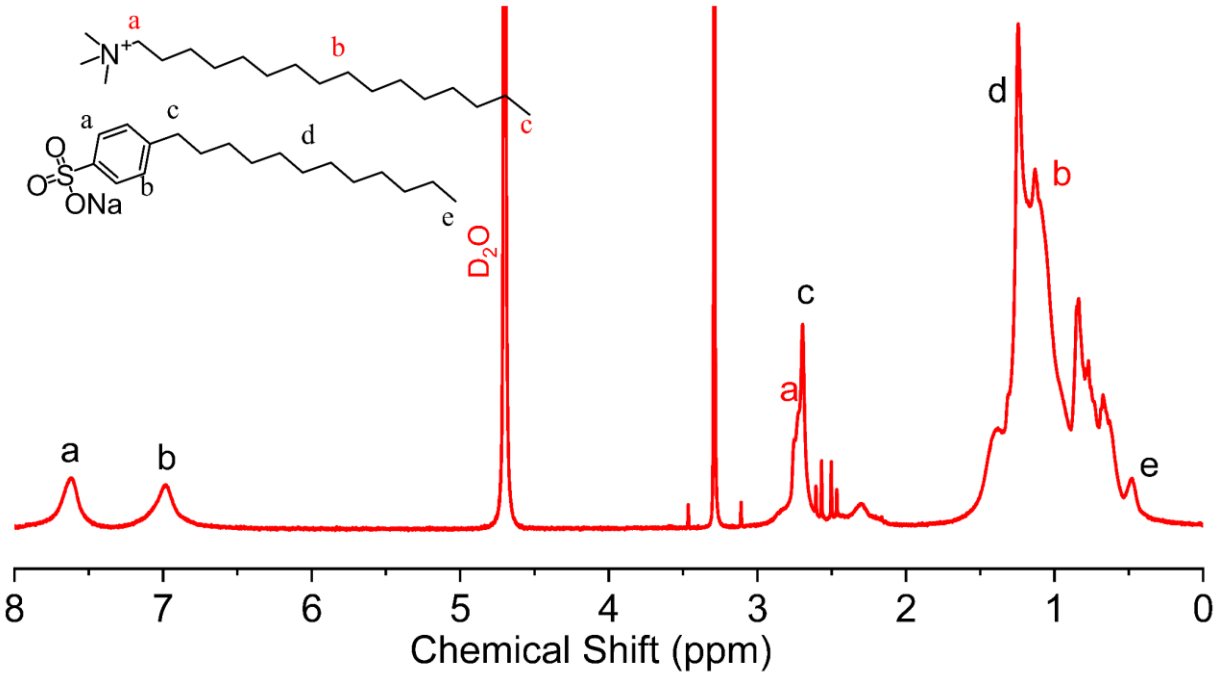

Figure S9g. ^{1}H NMR spectrum of vesicles in D_2O

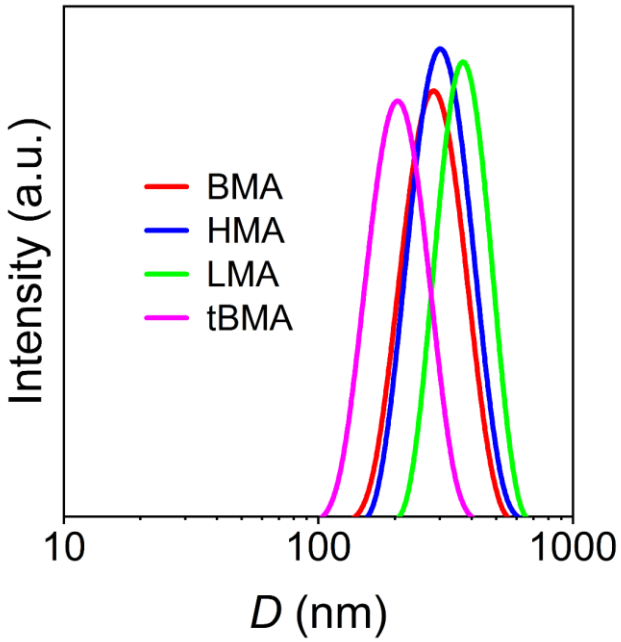

Figure S10. Size distribution of vesicles depending on the alkyl length in methacrylates confined within the bilayer determined by DLS in aqueous solution at 25°C

Figure S11. TEM images of vesicles with confined methacrylates

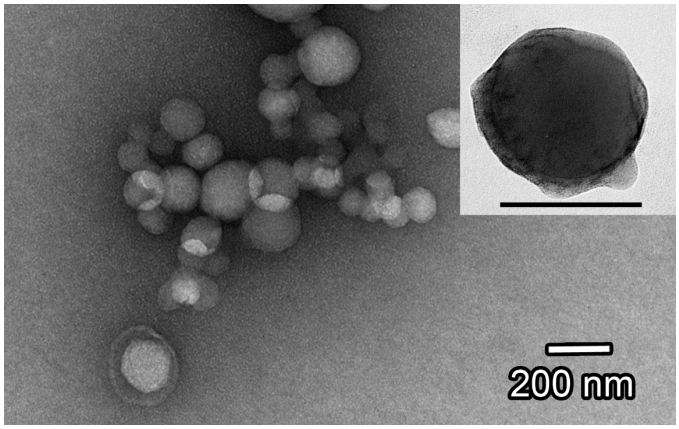

Figure S11a. TEM image of vesicles with confined Ir/BMA/EBiB in the bilayer, Monomers:$Ir(ppy)_3$=4000:1, 2% surfactant solution. CTAB:SDBS=2:8, S:M=1:1, D_{ave}=220±20nm (based on DLS). Inset: TEM image of a single vesicle at higher magnification showing hollow interior.

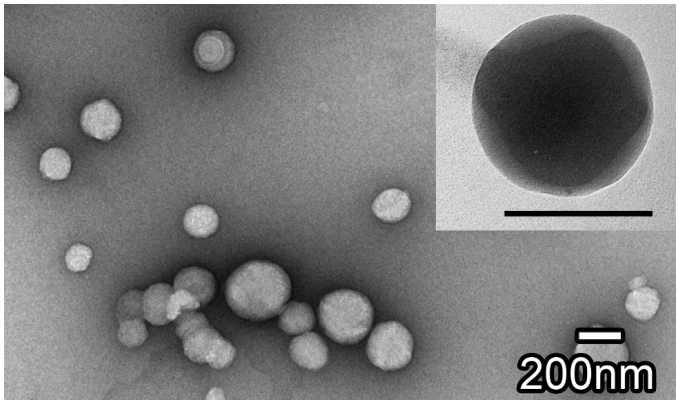

Figure S11b. TEM image of vesicles with confined Ir/HMA/EBiB in the bilayer, Monomers:Ir(ppy)$_3$=4000:1, 2% surfactant solution. CTAB:SDBS=2:8, S:M=1:1, D_{ave}=235±20nm (based on DLS). Inset: TEM image of a single vesicle at higher magnification showing hollow interior.

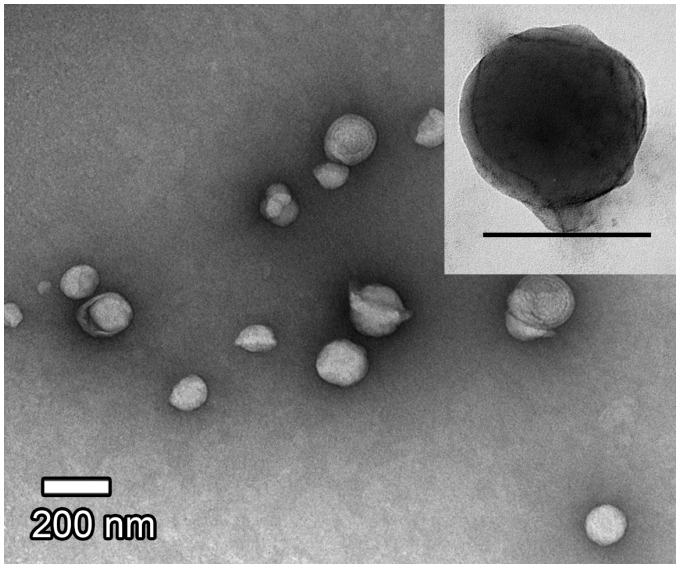

Figure S11c. TEM image of vesicles with confined Ir/LMA/EBiB in the bilayer, Monomers:Ir(ppy)$_3$=4000:1, 2% surfactant solution. CTAB:SDBS=2:8, S:M=1:1, D_{ave}=220±20nm (based on DLS). Inset: TEM image of a single vesicle at higher magnification showing hollow interior.

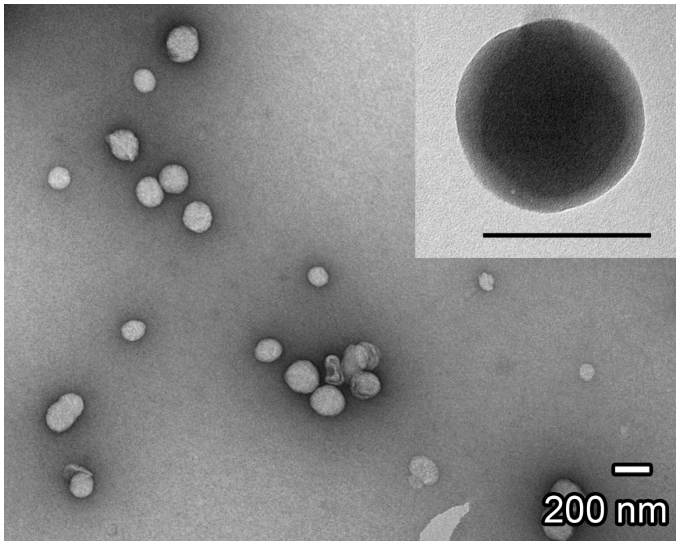

Figure S11a. TEM image of vesicles with confined Ir/tBMA/EBiB in the bilayer, Monomers:Ir(ppy)$_3$=4000:1, 2% surfactant solution. CTAB:SDBS=2:8, S:M=1:1, D_{ave}=190±20nm (based on DLS). Inset: TEM image of a single vesicle at higher magnification showing hollow interior.

Figure S12. SAXS data of vesicles with confined methacrylates in the vesicle bilayer.

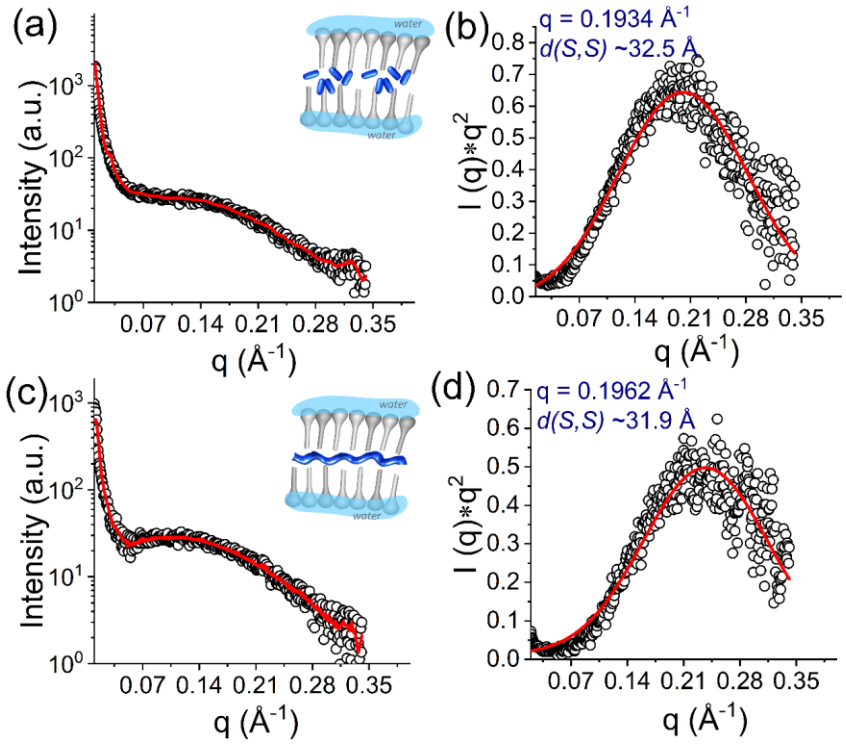

Figure S12a. (a) SAXS data of vesicles with confined Ir/BMA/EBiB in the vesicle bilayer before (a) and after (c) photopolymerization. (b, d) Kratky plots from SAXS data, supporting the formation of unilamellar LUVs, and suggesting vesicles bilayer did not change during polymerization.

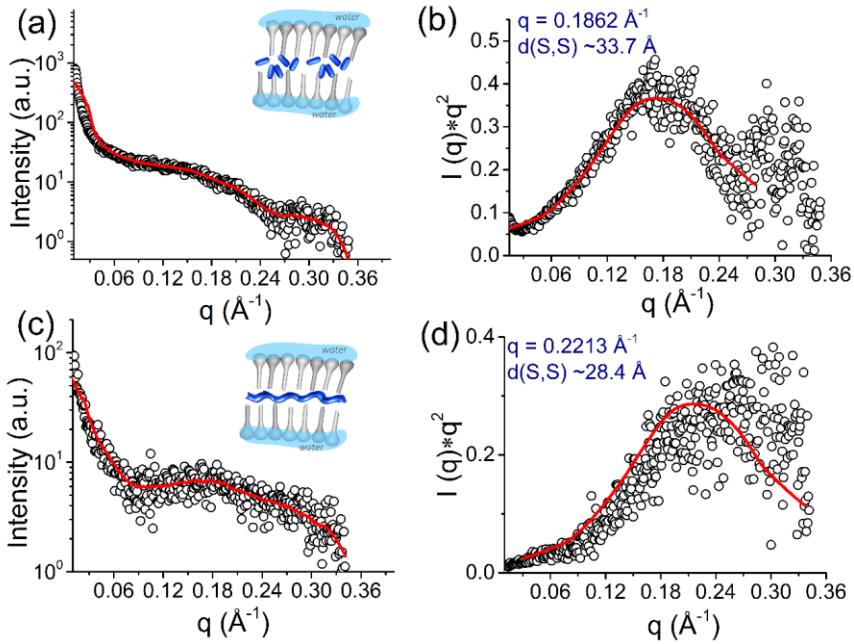

Figure S12b. (a) SAXS data of vesicles with confined Ir/HMA/EBiB in the vesicle bilayer before (a) and after

(c) photopolymerization. (b, d) Kratky plots from SAXS data, supporting the formation of unilamellar LUVs, and suggesting vesicles bilayer did not change during polymerization.

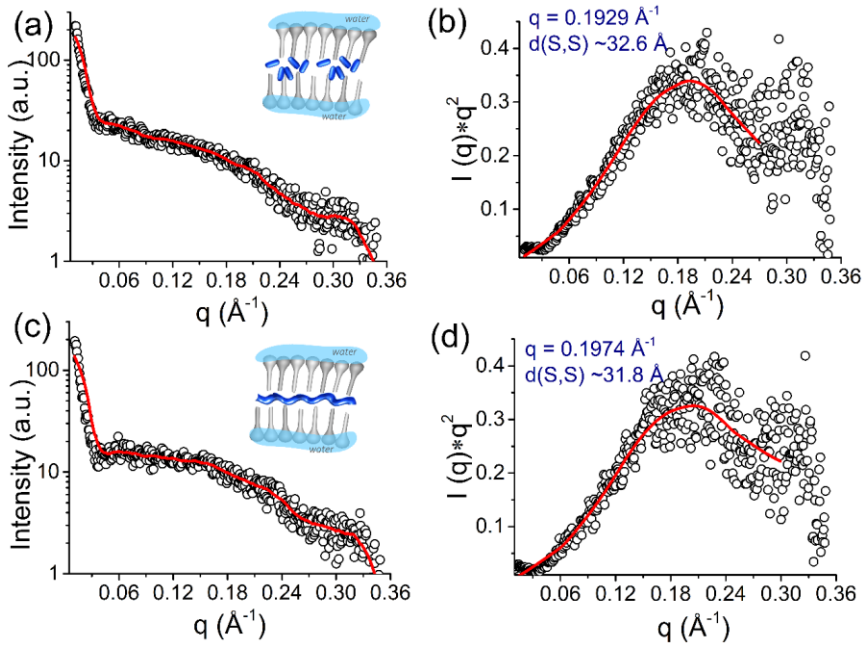

Figure S12C. (a) SAXS data of vesicles with confined Ir/LMA/EBiB in the vesicle bilayer before (a) and after (c) photopolymerization. (b, d) Kratky plots from SAXS data, supporting the formation of unilamellar LUVs, and suggesting vesicles bilayer did not change during polymerization.

Figure S13. Kinetics of photopolymerization of confined methacrylates monomers within the bilayer

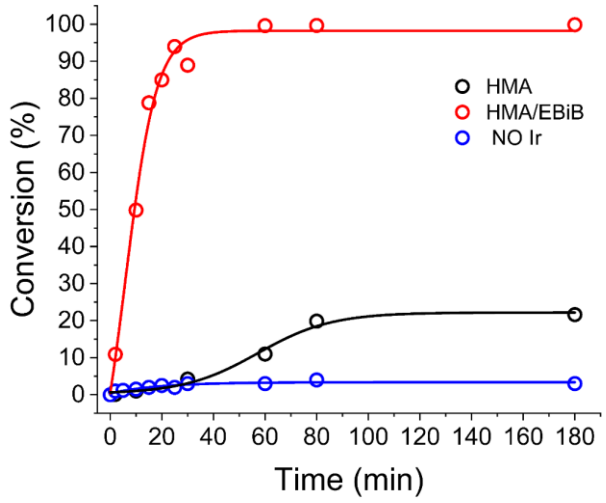

Figure S13a. Photopolymerization of confined HMA monomers within the bilayer of spontaneously assembled vesicles in aqueous solution at 25°C using a *fac-[Ir(ppy)$_3$]* photoredox catalyst (λ_{max}=460 nm, Exitance (E_p): 11 mW/cm^2). [HMA] : [EBiB] : [Ir] = 100 : 1 : 0.005. D_{ave}=220±20nm, CTAB:SDBS=2:8, 2% surfactant solution. The lines are guides to the eye only.

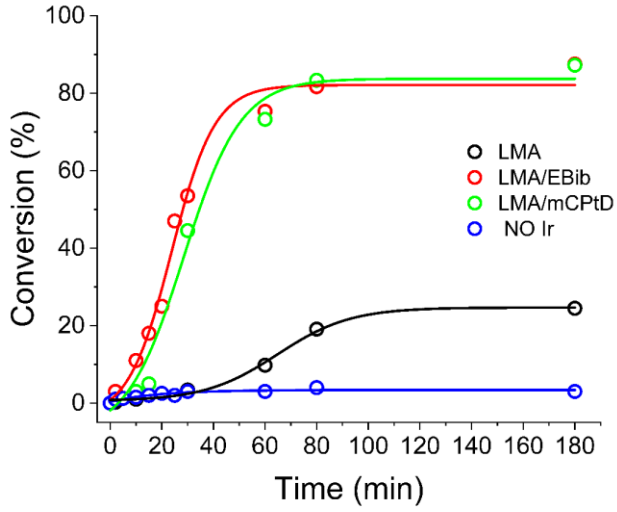

Figure S13b. Photopolymerization of confined LMA monomers within the bilayer of spontaneously assembled vesicles in aqueous solution at 25°C using a *fac-[Ir(ppy)$_3$]* photoredox catalyst (λ_{max}=460 nm, Exitance (E_p): 11 mW/cm^2). [LMA] : [EBiB] : [Ir] = 100 : 1 : 0.005. D_{ave}=220±20nm, CTAB:SDBS=2:8, 2% surfactant solution. The lines are guides to the eye only.

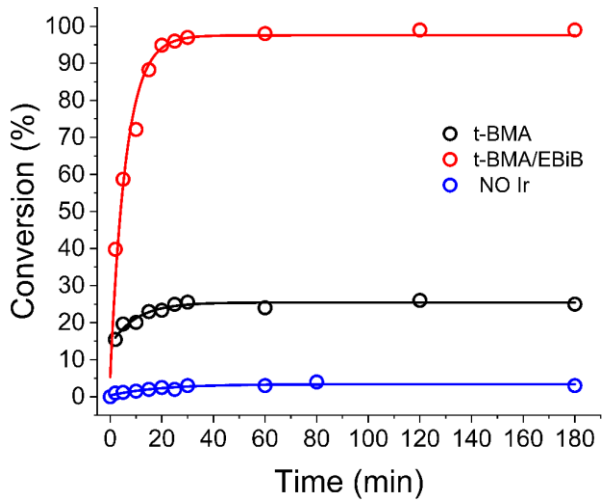

Figure S13c. Photopolymerization of confined LMA monomers within the bilayer of spontaneously assembled vesicles in aqueous solution at 25°C using a *fac-[Ir(ppy)$_3$]* photoredox catalyst (λ_{max}=460 nm, Exitance (E_p): 11 mW/cm^2). [tBMA] : [EBiB] : [Ir] = 100 : 1 : 0.005. D_{ave}=220±20nm, CTAB:SDBS=2:8, 2% surfactant solution. The lines are guides to the eye only.

Table S1. Experimental Results for the PET-ATRP polymerization in two-dimensional organized bilayers of vesicles under blue light (λmax = 460 nm).

#	[M]:[I]:[Ir][a]	M	I	Time (min)	α [b] (%)	$M_{n,th}$ (g mol^{-1})	$M_{n,GPC.}$[c] (g mol^{-1})	M_w/M_n	$D_{h,\ pre}$[d] (nm)	PDI_{pre}	$D_{h,\ post}$ (nm)	PDI_{post}
1	100:1:0.005	BMA	EBiB	30	99	14270	21350	1.25	220	0.2	215	0.23
2	100:0:0.005	BMA	-	180	23	-	61520	1.8	215	0.19	222	0.22
3	100:1:0.005	HMA	EBiB	60	98	16850	18530	1.3	231	0.24	227	0.27
4	100:0:0.005	HMA	-	180	20	-	48700	1.9	237	0.21	233	0.24
5	100:1:0.005	LMA	EBiB	60	95	24360	16280	1.6	251	0.25	255	0.21
6	100:0:0.005	LMA	-	180	24	-	38050	2.7	259	0.27	231	0.23
7	100:1:0.005	t-BMA	EBiB	20	97	14270	23530	1.19	203	0.17	190	0.23
8	100:0:0.005	t-BMA	-	180	28		58103	1.9	224	0.21	215	0.17

[a] The polymerizations were performed at room temperature in water using LED reactor (Exitance (E_p): 11 mW/cm^2) is composed of a 10-foot-long adhesive 12V LED strip that emits blue light (λmax = 460 nm). [b] Monomer conversion was determined by using GCMS. [c] Molecular weight and polydispersity were determined by GPC analysis (THF as eluent) based on polystyrene standards. [d] Diameters and PDI of vesicles before and after polymerization were determined by DLS

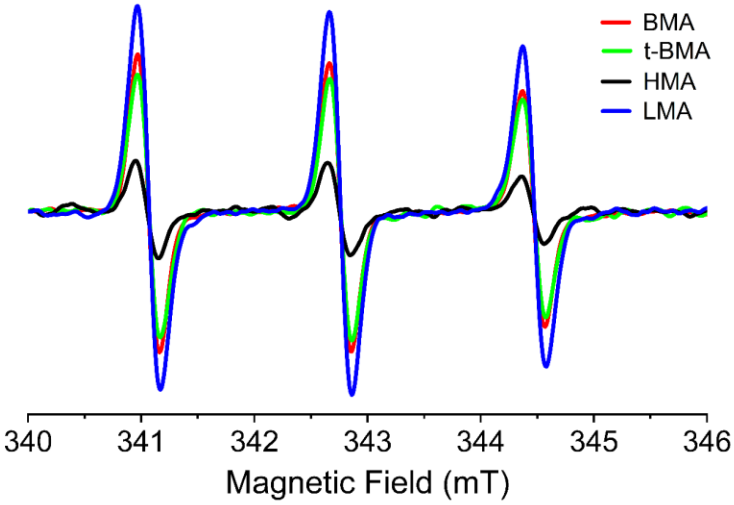

Figure S14. EPR spectra (ν = 9.651 GHz) of PATEMPO obtained after 5 min exposure of the samples to blue LED light (λ_{max} = 460 nm, 7.8 mW/cm^2) in the presence of Ir/M/EBiB within a bilayer of spontaneously assembled vesicles in an aqueous solution at 25 °C. CTAB:SDBS=2:8, 1% surfactant solution

Figure S15. The molecular weight distribution of methacrylates in the range of the target photoRDRP

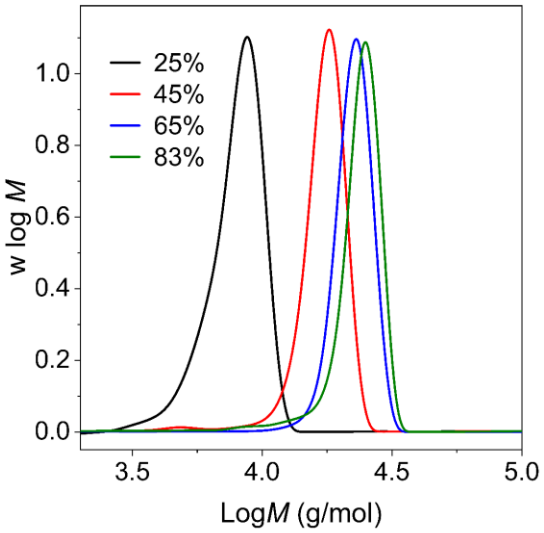

Figure S15a. MWDs at different hexyl methacrylate conversions upon photopolymerization of monomer confined within the bilayer of vesicles in the presence of *fac*-[Ir(ppy)$_3$] as the photoredox catalyst using a molar ratio of [HMA] : [EBiB] : [Ir] = 100 : 1 : 0.005 in aqueous solution at 25°C under blue LED light irradiation (λ_{max}=460 nm, 11 mW/cm2). D_{ave}=235±20nm, CTAB:SDBS=2:8, 2% surfactant solution.

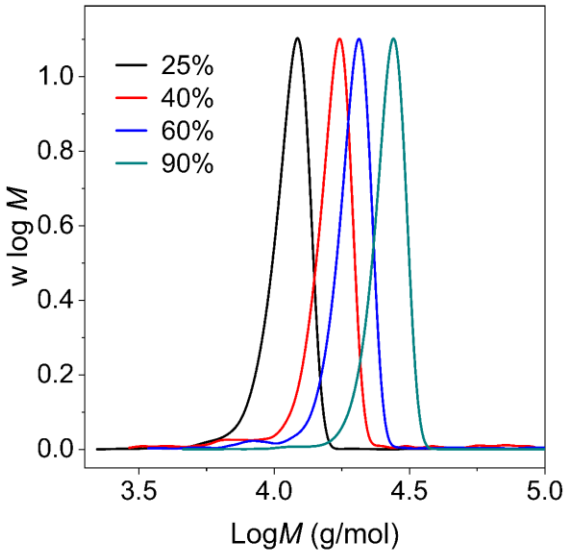

Figure S15b. MWDs at different t-butylmethacrylate conversions upon photopolymerization of monomer confined within the bilayer of vesicles in the presence of *fac*-[Ir(ppy)$_3$] as the photoredox catalyst using a molar ratio of [tBMA] : [EBiB] : [Ir] = 100 : 1 : 0.005 in aqueous solution at 25°C under blue LED light irradiation (λ_{max}=460 nm, 11 mW/cm2). D_{ave}=200±20nm, CTAB:SDBS=2:8, 2% surfactant solution.

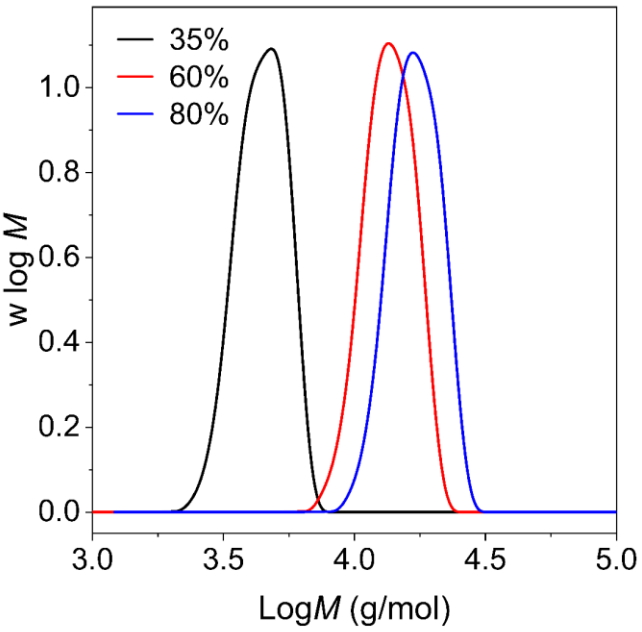

Figure S15c. MWDs at different lauryl methacrylate conversions upon photopolymerization of monomer confined within the bilayer of vesicles in the presence of *fac*-[Ir(ppy)$_3$] as the photoredox catalyst using a molar ratio of [LMA] : [EBiB] : [Ir] = 100 : 1 : 0.005 in aqueous solution at 25°C under blue LED light irradiation (λ_{max}=460 nm, 11 mW/cm2). D_{ave}=250±20nm, CTAB:SDBS=2:8, 2% surfactant solution.