Supporting information for:

Supramolecular Assemblies for Transporting Proteins Across an Immiscible Solvent Interface

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General methods: All reagents were commercially available and used as received unless stated otherwise. ^{1}H-NMR spectra were recorded on a 400 MHz or a 500 MHz NMR spectrometer using residual proton resonance of the solvents as internal standard. Chemical shifts are reported in parts per million (ppm). Mass spectra were obtained by a Bruker AmaZon quadrupole ion trap mass spectrometer coupled with electrospray ionization source. Gel permeation chromatography (GPC) was used to estimate the molecular weight of polymers using THF/DMF as eluent and 1 μL of toluene was added as the internal reference. Polystyrene standards were used for calibration and data analysis.

Dynamic Light Scattering (DLS)

For the DLS measurements, the polymers were dissolved in toluene, and one equivalent of water per hydrophilic unit was added to form the water pool inside the reverse micelles. The samples were sonicated until clear solutions were formed. DLS measurements were carried out in a quartz cuvette at room temperature. The sizes of each solution were recorded overtime by a Malvern Nanozetasizer ZS90 with a 637-nm laser source with non-invasive backscattering technology detected at 173^o using quartz cuvette. Standard operating procedures (SOP) are set up including following parameters: the sample was equilibrated for 120 s at 25 ^{o}C before each measurement; the sizes were reported as the hydrodynamic diameter (D_H) and each measurement average 16 runs were repeated three times.

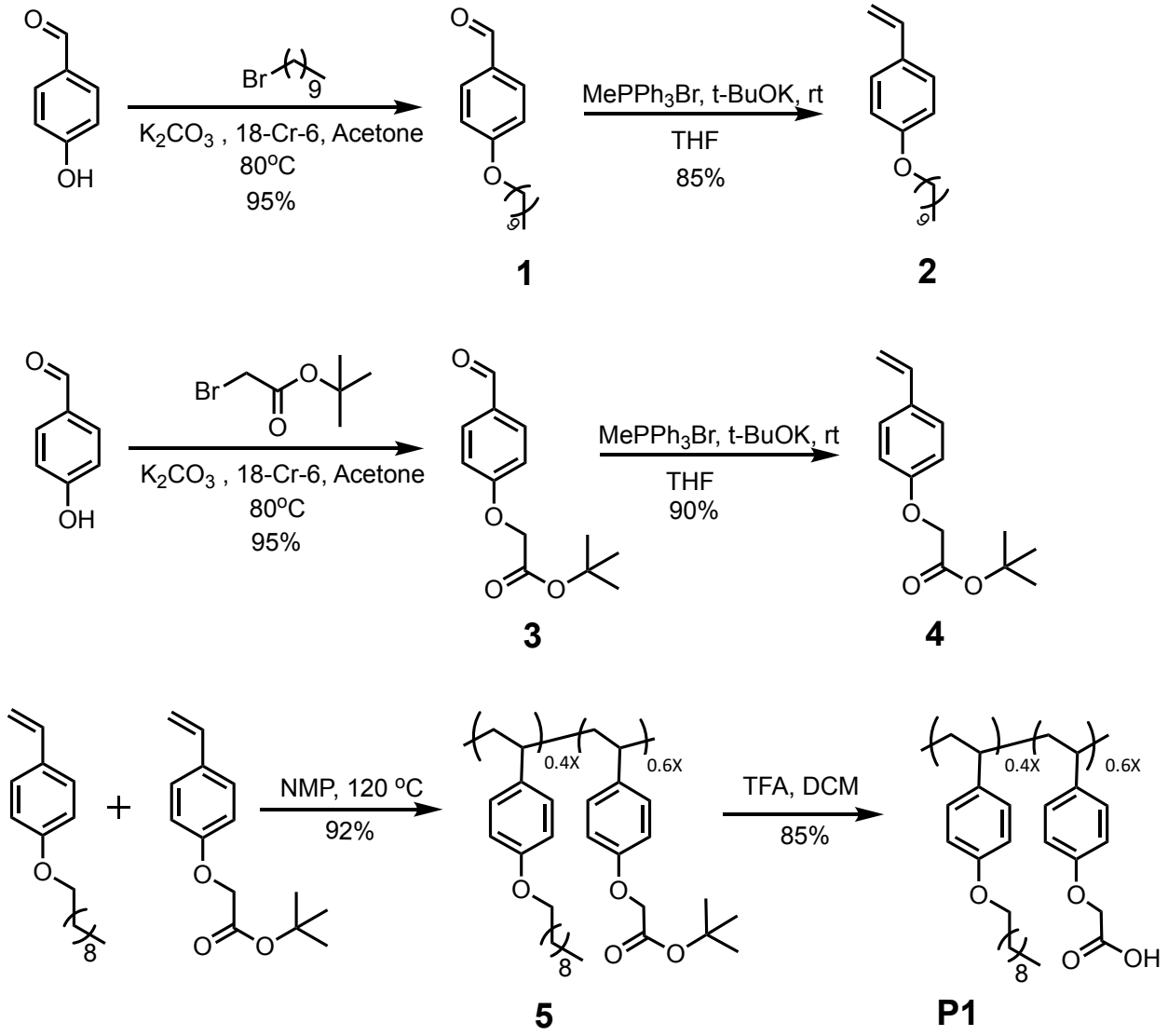
Transmission Electron Microscope (TEM)

The same sample for DLS measurement was dropped onto carbon-coated copper grid. The grid was dried by slow evaporation in air, and then dry separately in a vacuum overnight. Images were recorded on a JEOL-2000FX electron microscopy operated at 200 kV and at a nominal magnification of 5000X. At least 10 locations on the TEM grid were examined. The assembly diameter was calculated using ImageJ software.

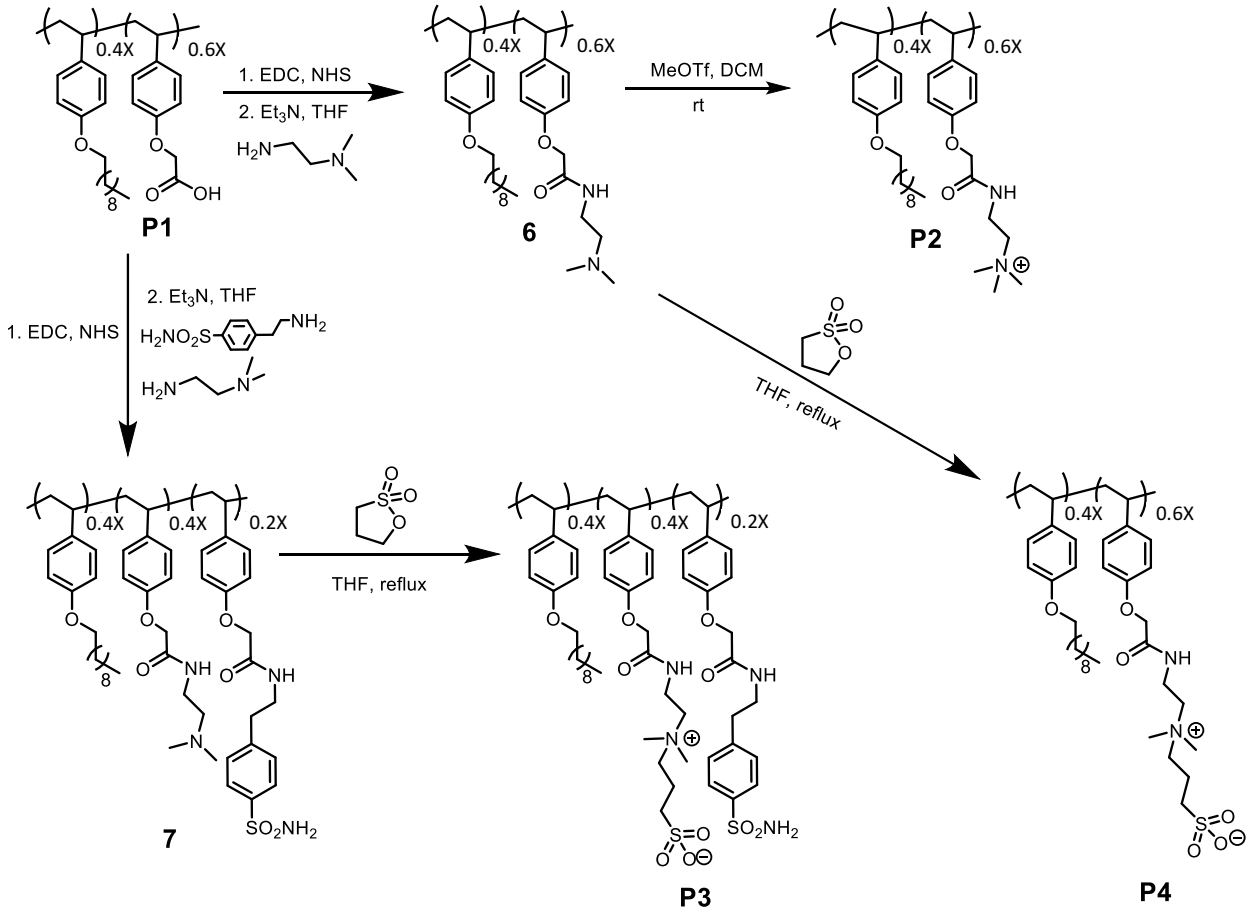
Matrix Assisted Laser Desorption/Ionization Mass Spectrometry (MALDI-MS)

MALDI-MS analysis of samples before and after transport were performed with Bruker Autoflex III time-of-flight mass spectrometer. The matrix was prepared with a solvent mixture of acetonitrile, water and trifluoroacetic acid (with a ratio 50:47.5:2.5) containing 10 mg/mL sinapic acid. The matrix and samples from aqueous or organic phase were mixed at 1:1 ratio and spotted on the MALDI target for analysis.

Polymer synthesis



Scheme 1. Synthetic route for polymer **P1**



Scheme 2. Synthetic route for polymer **P2**, **P3** and **P4**

Synthesis procedure

Synthesis of compound 1: According to previous procedure[S1], to a solution of acetone mixed with K_2CO_3 (11.84 g, 85.65 mmol) and 18-crown-6 (1.13 g, 4.28 mmol), 4-hydroxybenzaldehyde (5.23 g, 42.83 mmol) was added and stirred for 5 min. To this mixture, 1-bromodecane (14.21 g, 64.24 mmol) was added and stirred while refluxing for 20 h. The reaction mixture was then cooled to room temperature and filtered to afford the crude product in acetone solution. The solvent was evaporated to dryness and purified by silica gel column chromatography (8-10% ethyl acetate in hexanes) to obtain 8.8 g (79% yield) of **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (s, 1H), δ 7.83-7.81 (d, J = 8.0 Hz, 2H), δ 7.00-6.98 (d, J = 8.0 Hz, 2H), δ 4.02-4.05 (t, J = 6.6 Hz, 2H), δ 1.76-1.83 (quint, 2H), δ 1.47-1.26 (m, 14H), δ 0.87-0.91 (t, J = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.7, 164.2, 131.9, 129.7, 114.7, 68.4, 31.9, 29.5, 29.63, 29.32, 29.31, 29.1, 25.9, 22.7, 14.1. ESI-MS (expected: $[m+H]^+$= 263.19, obtained: $[m+Na]^+$= 285.2)

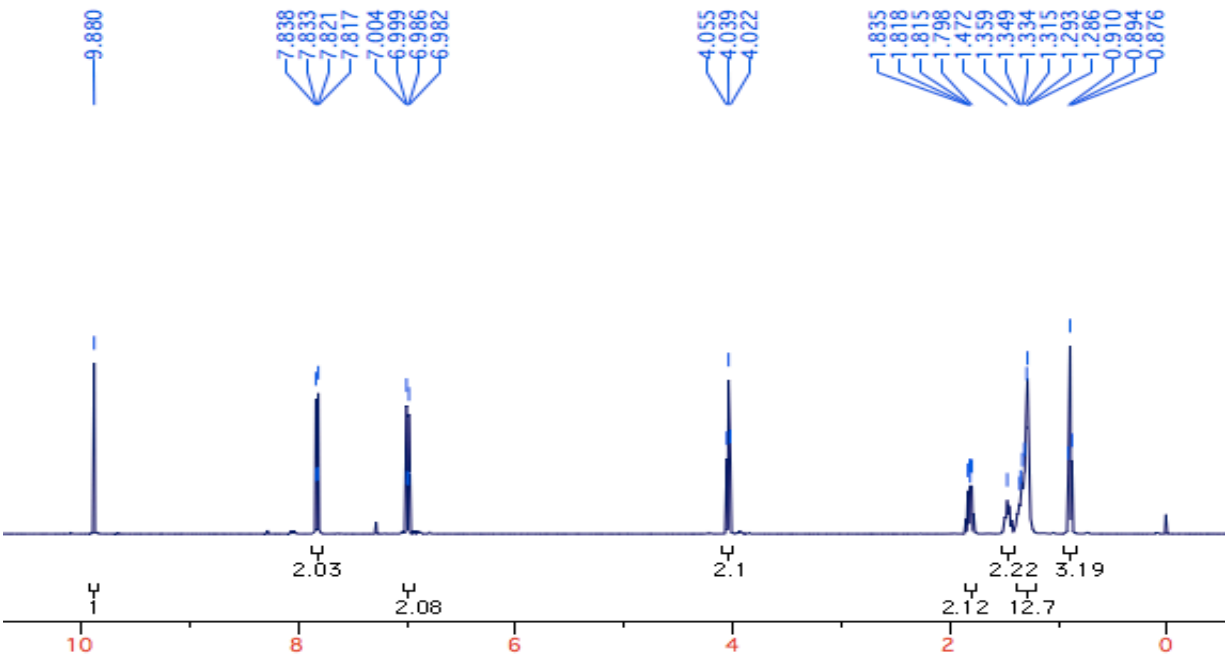
Synthesis of compound 2: Methyltriphenylphosphonium bromide (6.58 g, 25.11 mmol) and potassium tert-butoxide (3.94 g, 35.15 mmol) were mixed in a round bottom flask, and dry THF (20 mL) was added to the mixture. The mixture was stirred under argon atmosphere in an ice bath for 15 min to yield a bright yellow solution. **1** (6.58 g, 25.11 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, saline and ethyl acetate were added for extraction. The combined organic layer was separated and washed with saline (3

times). The organic layer was evaporated to dryness and purified by silica gel column chromatography (3-5% ethyl acetate in hexanes) to afford 5.7 g (88% yield) of **2**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31-7.33 (d, J = 8.0 Hz, 2H), δ 6.83-6.85 (d, J = 3.4 Hz, 2H), δ 6.61-6.68 (q, 1H), δ 5.57-5.61 (d, J = 7.2 Hz, 1H), δ 5.09-5.12 (d, J = 4.4 Hz, 1H), δ 3.93-3.96 (t, J = 5.4 Hz, 3H), δ 1.73-1.80 (quint, 2H), δ 1.27-1.46 (m, 14H), δ 0.86-0.89 (t, J = 6.8 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.1, 136.4, 130.3, 127.4, 114.6, 111.5, 68.1, 32.1, 29.7, 29.7, 29.5, 29.4, 29.4, 26.1, 22.8, 14.2. ESI-MS (expected: $[m+H]^+$= 261.21, obtained: $[m+Na]^+$= 283.2).

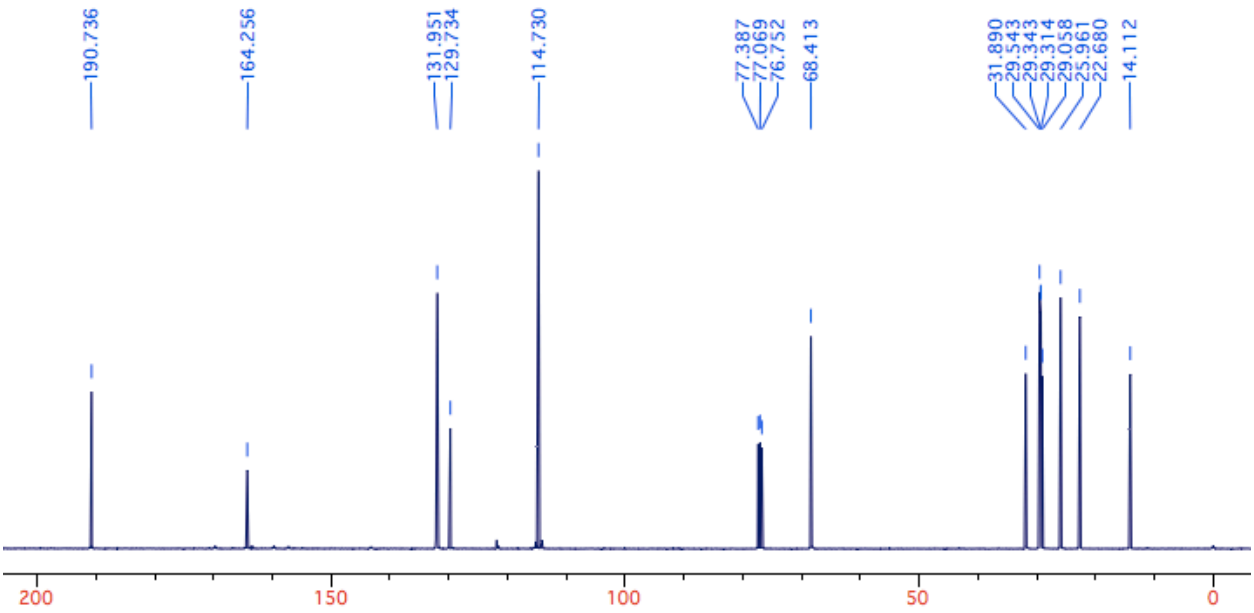
Synthesis of compound 3: To a solution of acetone mixed with K_2CO_3 (6.79 g, 49.13 mmol), NaI (7.36 g, 49.13 mmol) and 18-crown-6 (0.65 g, 2.46 mmol), 4-hydroxybenzaldehyde (3.00 g, 24.57 mmol) was added and stirred for 5 min. To this mixture, tert-butyl bromoacetate (9.58 g, 49.13 mmol) was added and stirred while refluxing for 20 h. The reaction mixture was then cooled to room temperature and filtered to afford the crude product in acetone solution. The solvent was evaporated to dryness and purified by silica gel column chromatography (10-13% ethyl acetate in hexanes) to obtain 5.3 g (91% yield) of **3**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.88 (s, 1H), δ 7.82-7.84 (d, J = 4.2 Hz, 2H), δ 6.97-6.99 (d, J = 4.4 Hz, 2H), δ 4.59 (s, 2H), δ 1.47 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.8, 167.2, 162.8, 132.0, 130.7, 114.9, 83.0, 65.6, 28.1. ESI-MS (expected: $[m+H]^+$= 237.1, obtained: $[m+Na]^+$= 259.1)

Synthesis of compound 4: Methyltriphenylphosphonium bromide (7.94 g, 22.24 mmol) and potassium tert-butoxide (2.50 g, 22.24 mmol) were mixed in a round bottom flask, and dry THF (15 mL) was added to the mixture. The mixture was stirred under argon atmosphere in an ice bath for 15 min to yield the bright yellow solution. **3** (3.5 g, 14.83 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, saline and ethyl acetate were added for extraction. The combined organic layer was separated and washed with saline (3 times). The organic layer was evaporated to dryness and purified by silica gel column chromatography (3-5% ethyl acetate in hexanes) to afford 3.3 g (95% yield) of **4**. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.33-7.35 (d, J = 1.0 Hz, 2H), δ 6.84-6.87 (d, J = 1.0 Hz, 2H), δ 6.63-6.68 (dd, J = 17.6, 10.9 Hz, 1H), δ 5.60-5.64 (dd, J = 17.6, 0.9 Hz, 1H), δ 5.13-5.15 (dd, J = 10.9, 0.8 Hz,), δ 4.51 (s, 2H), δ 1.49 (s, 9H). ^{13}C NMR (100MHz, $CDCl_3$) δ 168.0, δ 157.7, δ 136.2, δ 131.3, δ 127.48, δ 114.7, δ 112.1, δ 82.4, δ 65.8, δ 28.1. ESI-MS (expected: $[m+H]^+$= 235.1, obtained: $[m+Na]^+$= 257.1)

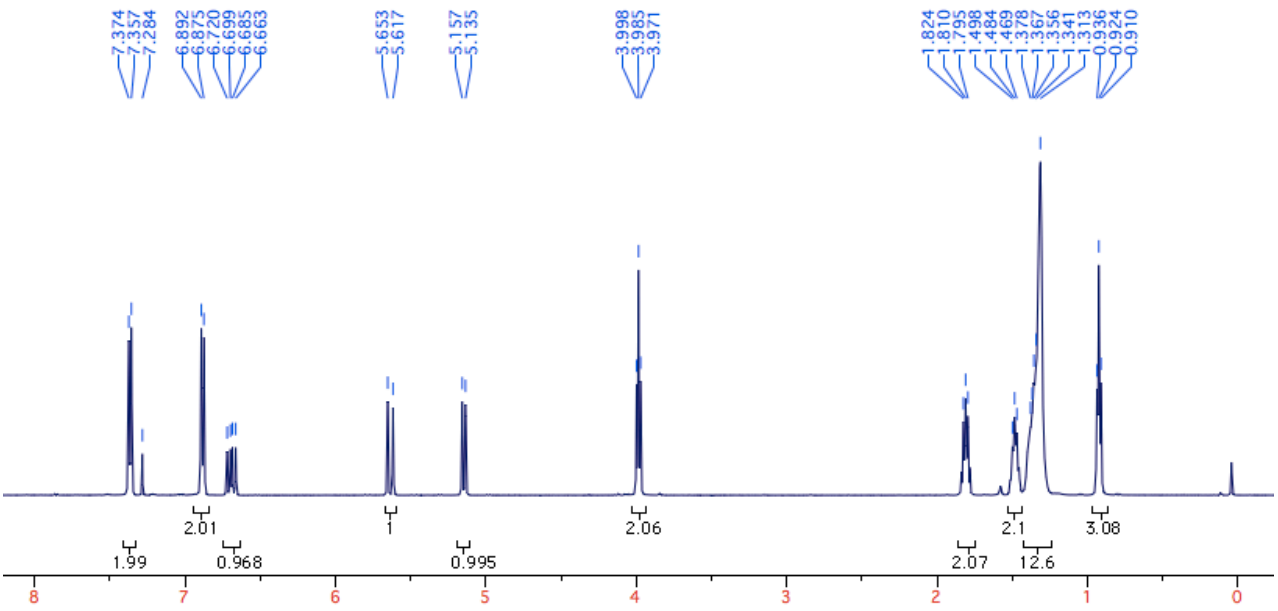
^{1}H NMR of compound **1**



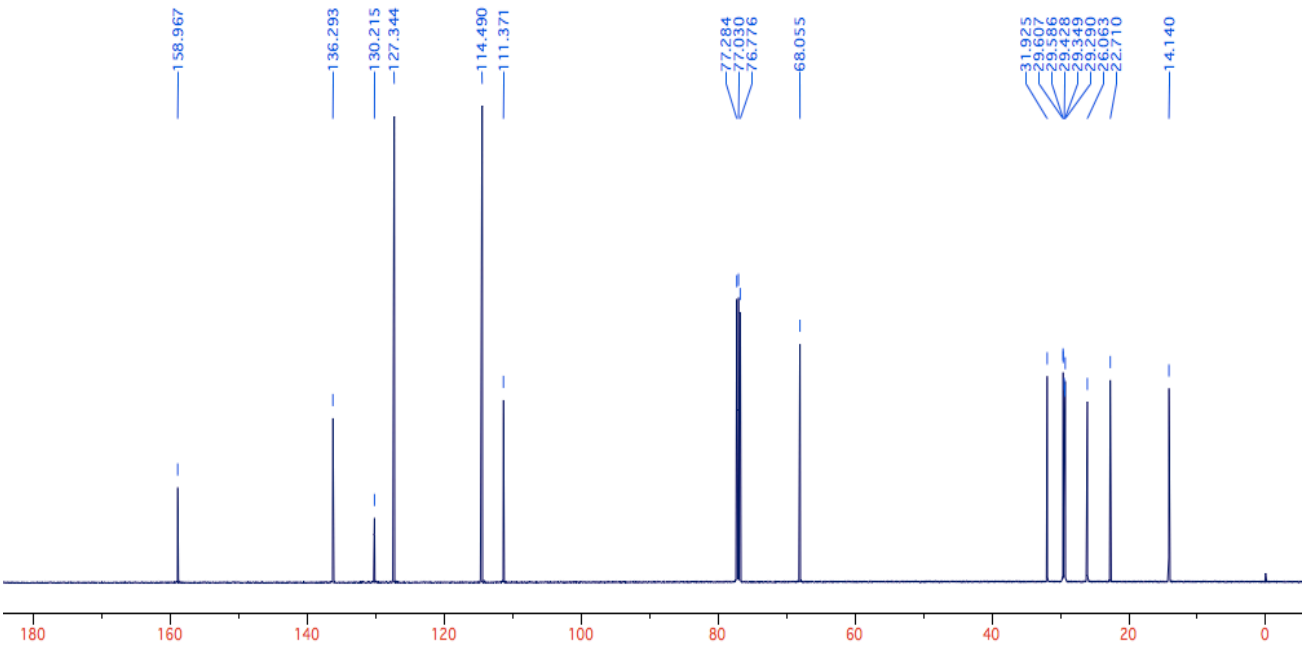
^{13}C NMR of compound **1**



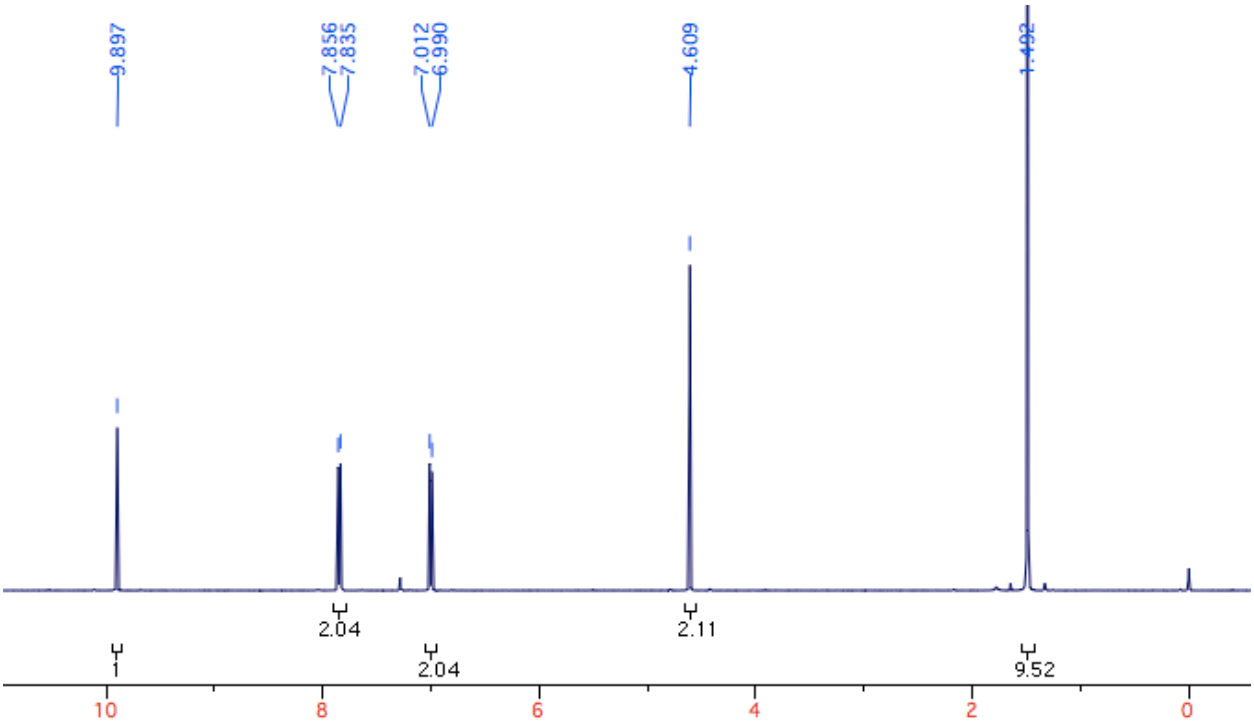
^{1}H NMR of compound **2**



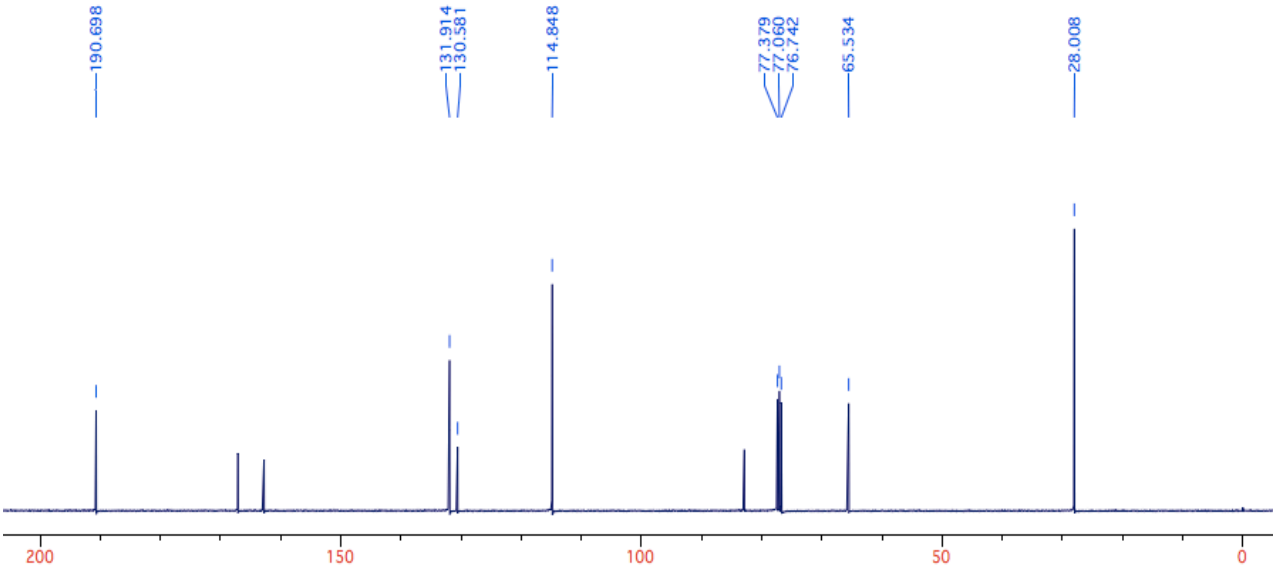
^{13}C NMR of compound **2**



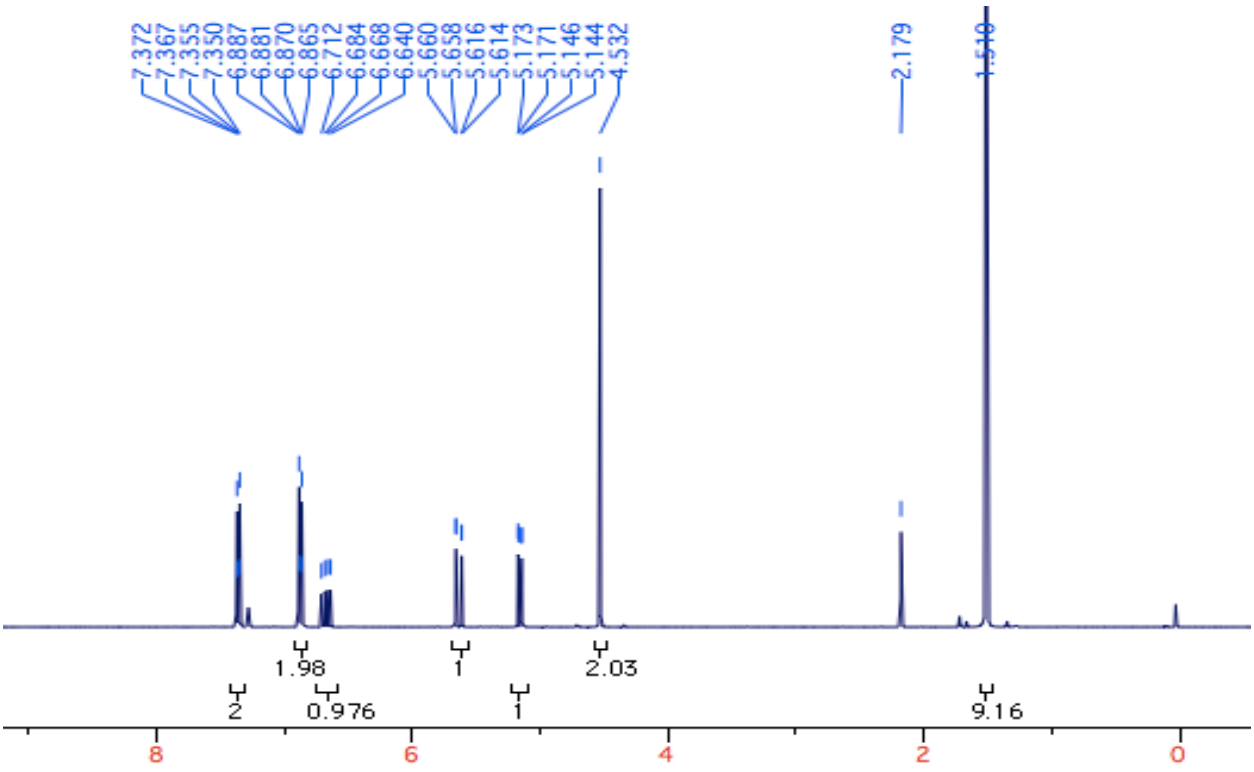
^{1}H NMR of compound **3**



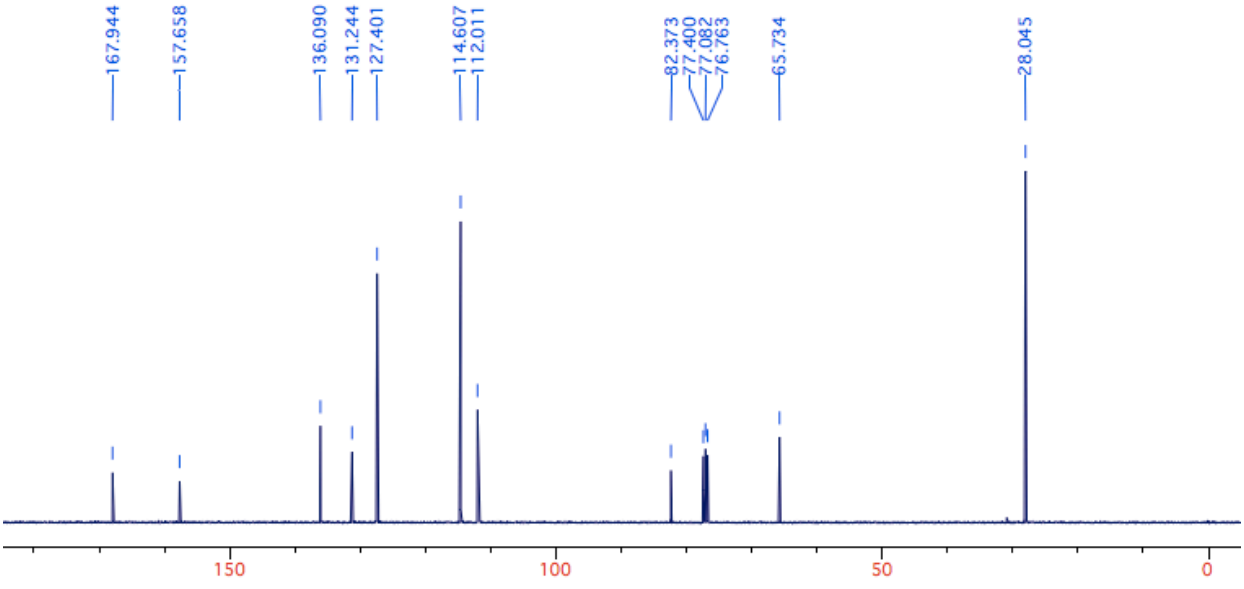
^{13}C NMR of compound **3**



^{1}H NMR of compound **4**

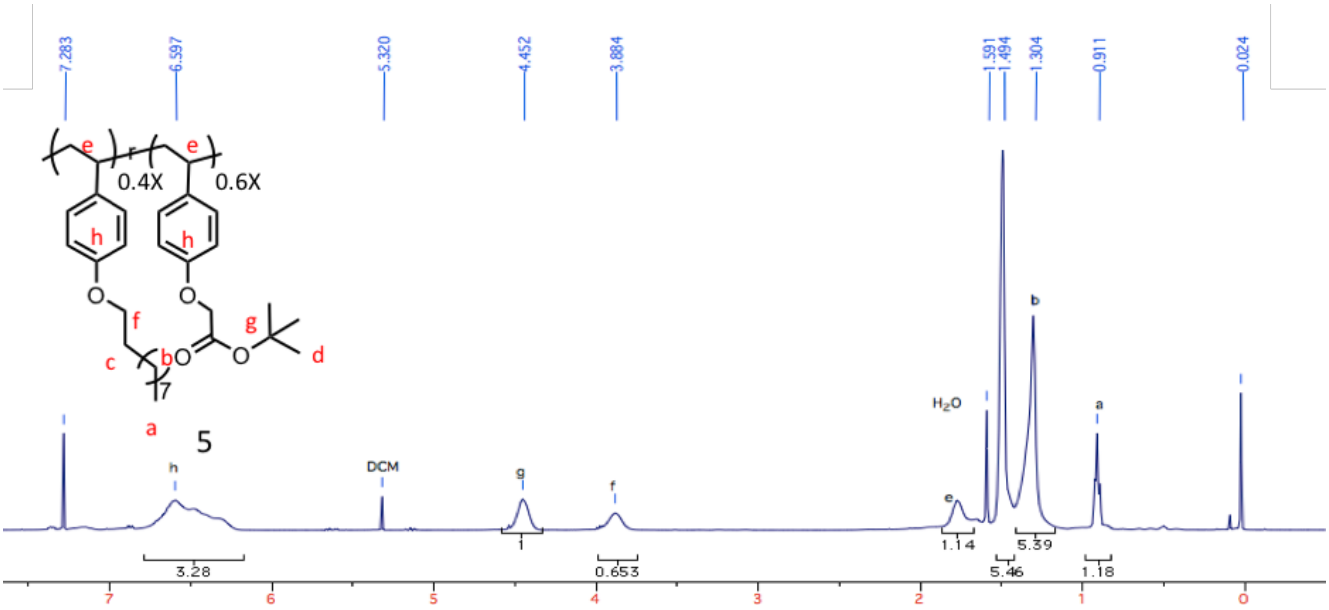


^{13}C NMR of compound **4**

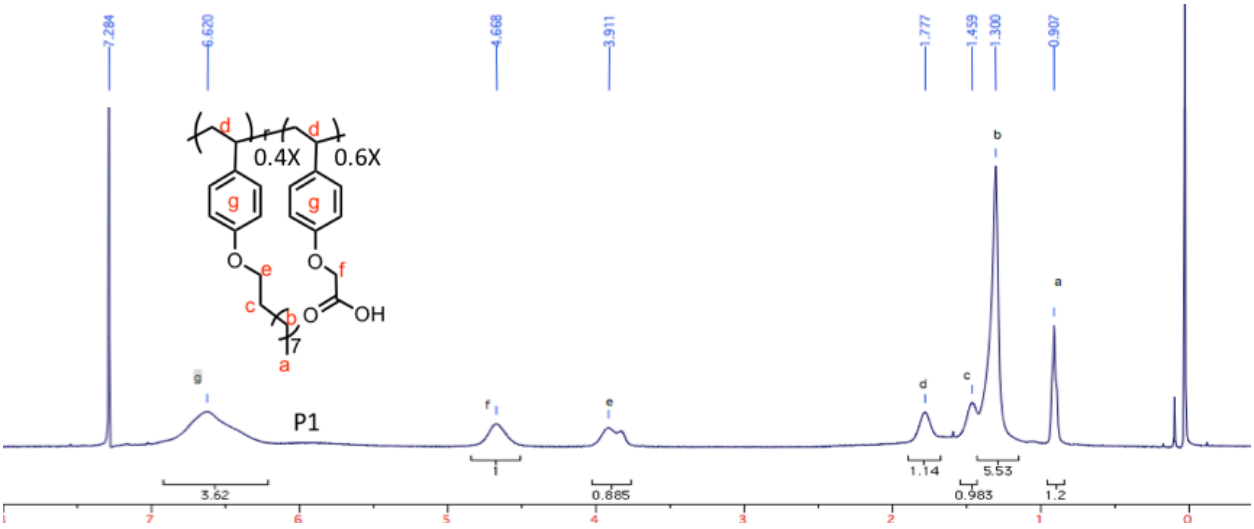


Synthesis of random copolymer 5: A mixture of the compound **1** (500 mg, 1.92 mmol), **2** (675 mg, 2.88 mmol) and *N*-*tert*-butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl) hydroxylamine

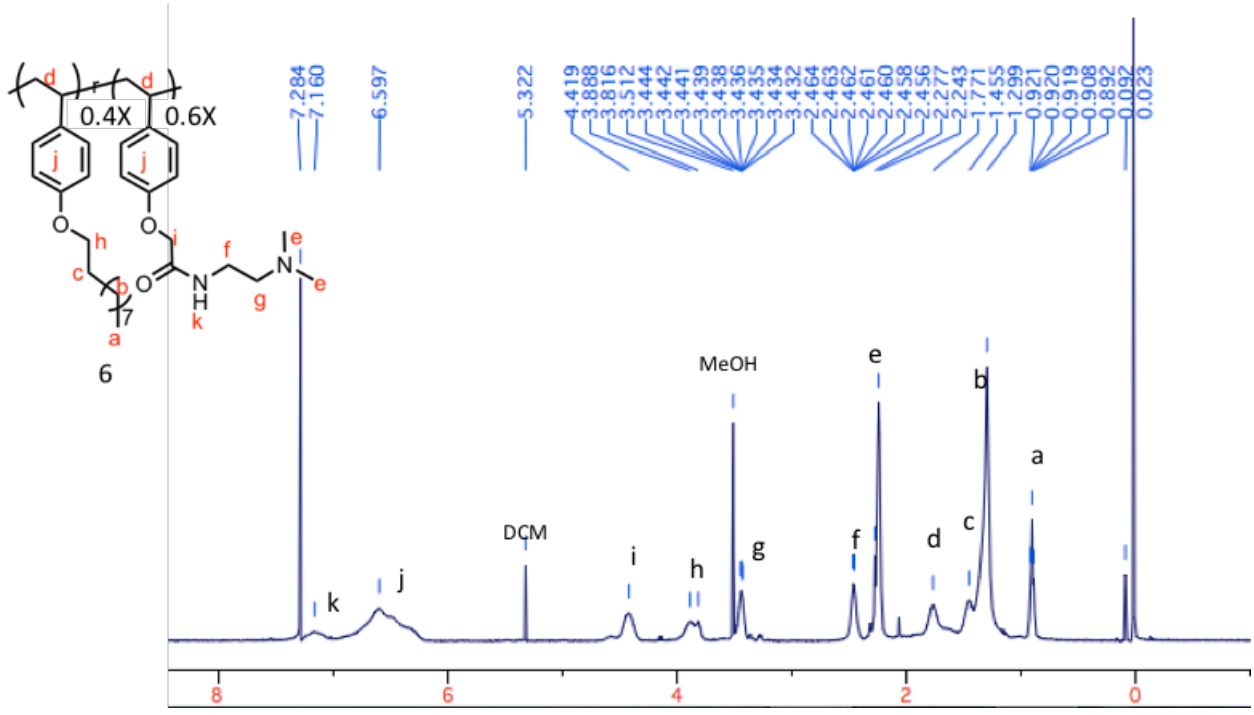
(NMP initiator, 25 mg, 0.077 mmol) were degassed by three freeze/thaw cycles, sealed under argon, and heated at 120 °C under argon for 12 h. After the reaction cooled down to room temperature, the reaction mixture was dissolved in minimal amount of DCM, and precipitated 3 times in MeOH. The precipitate was collected and dried under vacuum to yield 988 mg (84% yield) of **5**. GPC (THF): M_n= 11 K Da, Đ= 1.09. ^{1}H NMR (400MHz, $CDCl_3$) δ 6.59-6.25, 4.42, 3.85, 1.75, 1.48-1.21, 0.88. From ^{1}H NMR, integration of methylene proton next to the phenol in both alkyl unit (f) and carboxylate unit (g) provided the molar ratio of monomers to be 4:6 (decyl/carboxylate).



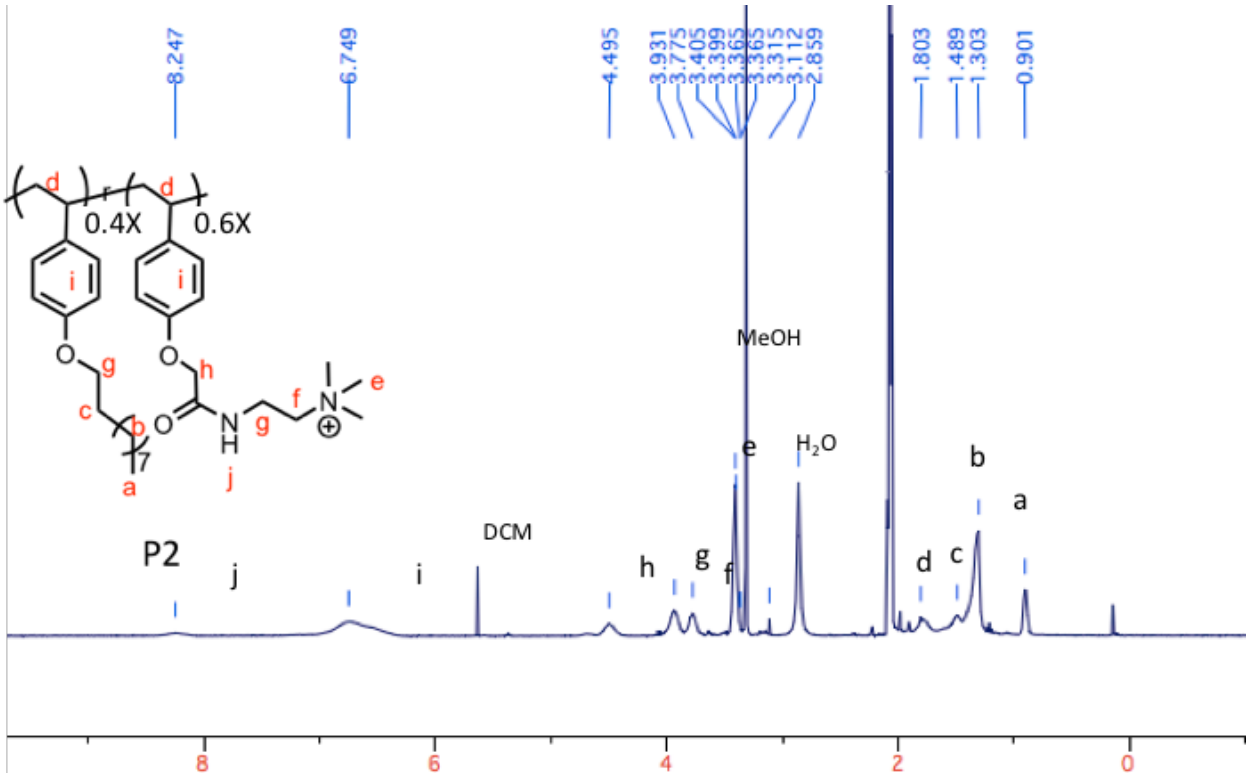
Synthesis of random copolymer P1: Dichloromethane (2 mL) was added to dissolve the dried random copolymer **P1** (200 mg). Trifluoroacetic acid (0.5 mL) was added to the mixture and stirred for 12 h at room temperature. The reaction mixture was evaporated and dried under vacuum to obtain **P1** (95% yield). ^{1}H NMR (400MHz, $CDCl_3$) δ 6.59, 4.65, 3.90, 1.75, 1.48-1.21, 0.88. ^{13}C NMR (100MHz, $CDCl_3$) δ 174.2, 156.6, 154.8, 128.7, 114.2, 68.1, 65.1, 39.9, 31.9, 29.6, 29.3, 26.1, 22.7, 14.1. GPC (DMF): M_n= 11 K Da, Đ= 1.12. From ^{1}H NMR, a sharp decrease in integration at δ 1.48 suggested the successful deprotection of tert-butyl group. From ^{1}H NMR, integration of proton a and f again confirmed the molar ratio of monomers to be 4:6 (decyl/carboxylate)



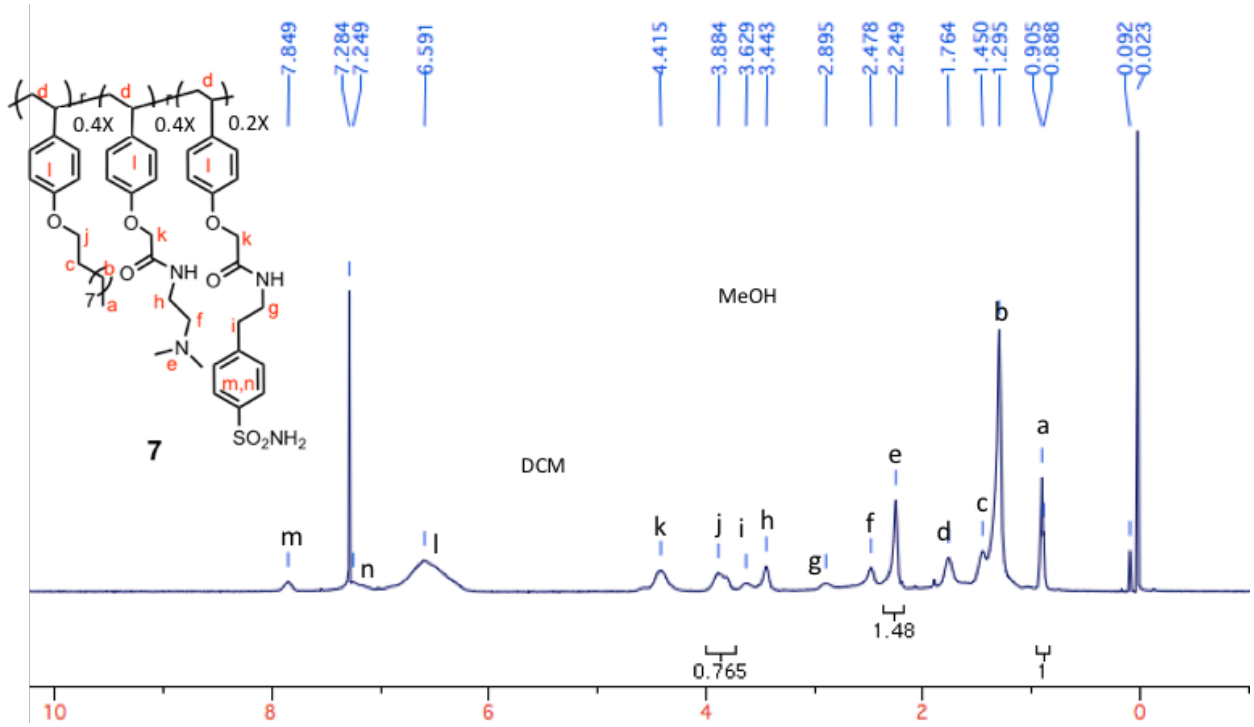
Synthesis of random copolymer 6: Carboxylate polymer **P1** (100 mg, 0.22 mmol carboxylic acid repeat unit) and N-Hydroxysuccinimide (38 mg, 0.33 mmol) was weighed in a 20 mL glass vial and dissolved in 5 mL dry THF and stirred at 0 °C. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC) (63 mg, 0.33 mmol) was added to the mixture and stirred for 4 hours. Afterwards, triethylamine (92 uL, 0.66 mmol) and N, N-dimethylethylenediamine (29 mg, 0.33 mmol) were added dropwise to the reaction mixture and the solution was stirred for 24 h at room temperature. After that, the modified polymer was purified by dialyzing against dichloromethane/methanol using a membrane of MWCO: 3.5 kDa. After dialysis, the solvent was evaporated and the polymer was dried under vacuum for 24 h. Yield: 90%, GPC (THF) Mn: 12 K. Đ: 1.15. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15, 6.57-6.2, 4.39, 3.84, 3.42, 2.44, 2.22, 1.74, 1.43-1.26, 0.88. From ^{1}H NMR, integration of proton a and e again confirmed the molar ratio of these two monomers to be 4:6.



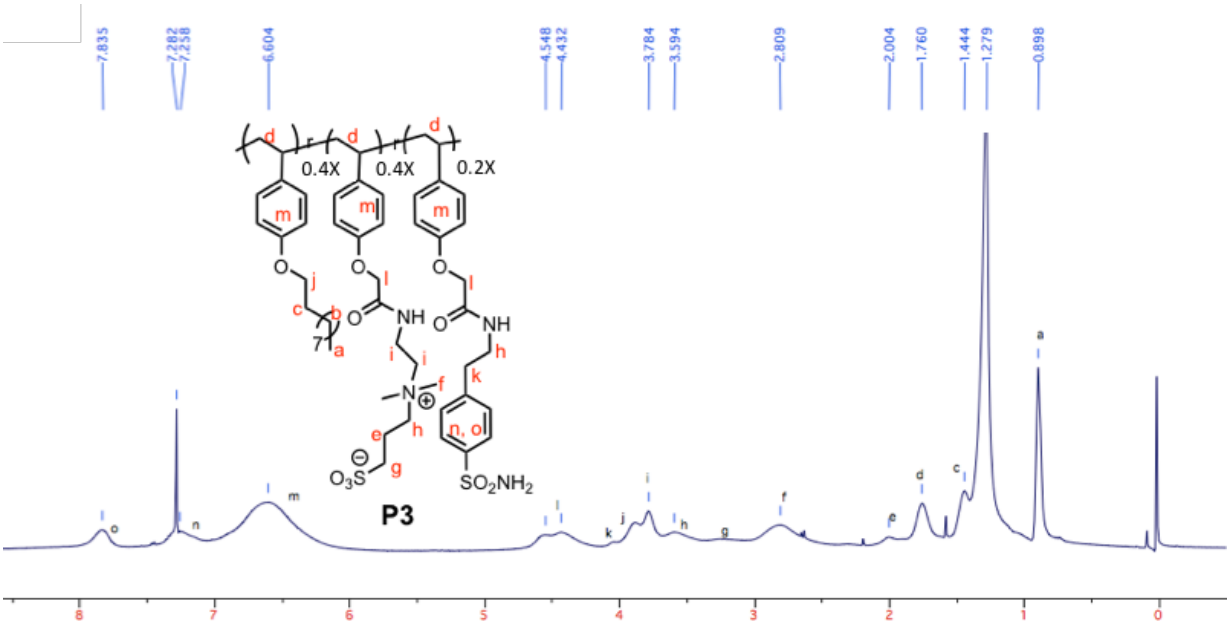
Synthesis of random copolymer P2: Random copolymer **6** (80 mg, 0.16 mmol tertiary amine repeat unit) was weighed in a 20 mL glass vial and dissolved in 5 mL dry THF and stirred at 0 °C under argon protection. Methyl trifluoromethanesulfonate (56 mg, 0.33 mmol) was added to the solution dropwise and stirred for 2 hours. Afterwards, the solvent was evaporated and the polymer was dried under vacuum for 24 h. Yield: 90%, GPC (THF) Mn: 12K. Đ: 1.15. ^{1}H NMR (400 MHz, acetone-d6): δ 8.24, 6.7-6.3, 4.49, 3.92, 3.77, 3.40, 1.78, 1.48-1.26, 0.88. ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.5, 156.6, 122.7, 53.5, 49.8, 31.9, 29.6, 29.35, 29.33, 26.1, 22.7, 14.1. From ^{1}H NMR, proton peak of e shifting downfield suggested the successful conversion of tertiary amine to quaternary ammonium. Ratios of two monomers were calculated based on integration of a and h.



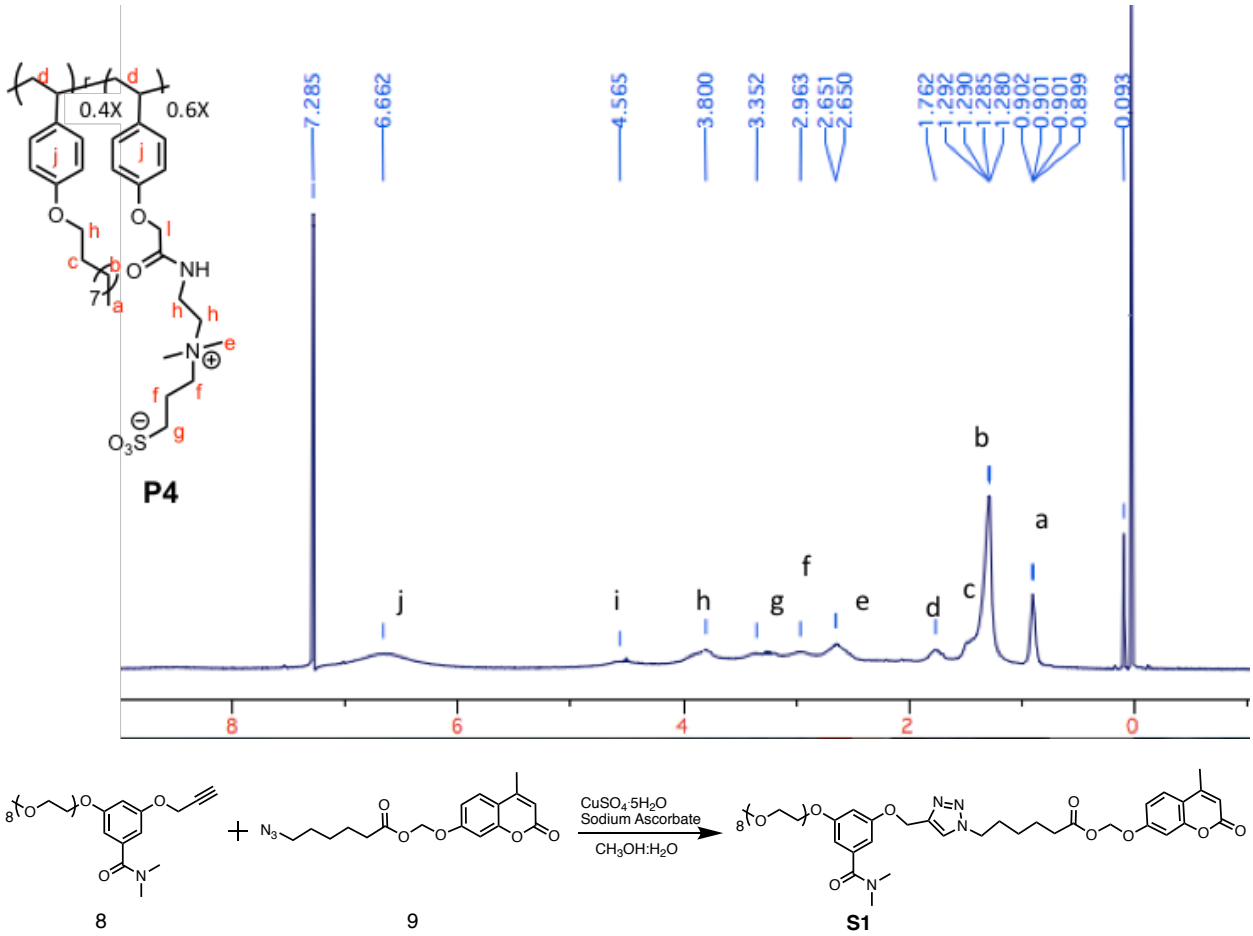
Synthesis of random copolymer 7: P1 (100 mg, 0.22 mmol carboxylic acid repeat unit) and N-hydroxysuccinimide (38 mg, 0.33 mmol) was dissolved in 5 mL dry THF and stirred at 0 °C. EDC (63 mg, 0.33 mmol) was added and stirred for 4 hours. Afterwards, triethylamine (92 uL, 0.66 mmol) was added and stirred for 30 minutes. Then a mixture of 4-(2-aminoethyl) benzenesulfonamide (22 mg, 0.11 mmol) and N, N-dimethylethylenediamine (19.3 mg, 0.22 mmol) in 1 mL DMF were added dropwise and the solution was stirred for 24 h at room temperature. After that, the modified polymer was purified by dialyzing against DCM/methanol using a membrane of MWCO: 3.5 kDa. After dialysis, the solvent was evaporated and the polymer was dried under vacuum for 24 h. Yield: 93%, GPC (THF) Mn: 12 K. Đ: 1.2. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82, 7.15, 6.57-6.2, 4.56-4.2, 3.87-3.78, 3.42, 2.44, 2.22, 1.74, 1.43-1.26, 0.88. Ratios of three components were calculated based on integration of a, e and m.



Synthesis of random copolymer P3: Random copolymer **7** (80 mg, 0.09 mmol tertiary amine repeat unit) was dissolved in 5 mL dry THF, 1,3-propane sultone (39 uL, 0.45 mmol) was added to the solution and the mixture was refluxed at 80 °C overinight. Then the modified polymer was purified by dialyzing against aacetone using a membrane of MWCO: 3.5 kDa. After dialysis, the solvent was evaporated and the polymer was dried under vacuum for 24 h. Yield: 95%, GPC (DMF) Mn: 12 K. Đ: 1.2. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.8, 7.15, 6.57-6.2, 4.52-4.2, 4.05-3.42, 2.78, 1.98, 1.74, 1.43-1.26, 0.88. ^{13}C NMR (100 MHz, $CDCl_3$) δ 181.6, 172.8, 170.4, 156.3, 128.8, 114.2, 100.5, 67.9 43.4, 39.6, 31.9, 29.6, 29.3, 26.15, 26.13, 22.7, 14.1. Ratios of three components were calculated based on copolymer **7**.



Synthesis of random copolymer P4: Random copolymer **6** (80 mg, 0.16 mmol tertiary amine repeat unit) was dissolved in 5 mL dry THF, 1,3-propane sultone (71 uL, 0.80 mmol) was added to the solution and the mixture was refluxed at 80 °C overinight. Then the modified polymer was purified by dialyzing against aacetone using a membrane of MWCO: 3.5 kDa. After dialysis, the solvent was evaporated and the polymer was dried under vacuum for 24 h. Yield: 95%, GPC (DMF) Mn: 11 K. Đ: 1.1. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30, 6.2-6.61, 4.64–4.29, 3.97-2.65, 1.76, 1.54-1.1, 0.87. ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.7, 157.8, 129.0, 114.8, 107.9, 68.2, 50.9, 42.8, 31.9, 29.6, 29.3, 26.2, 22.7, 14.1. Ratios of two components were calculated based on polymer **P1**.

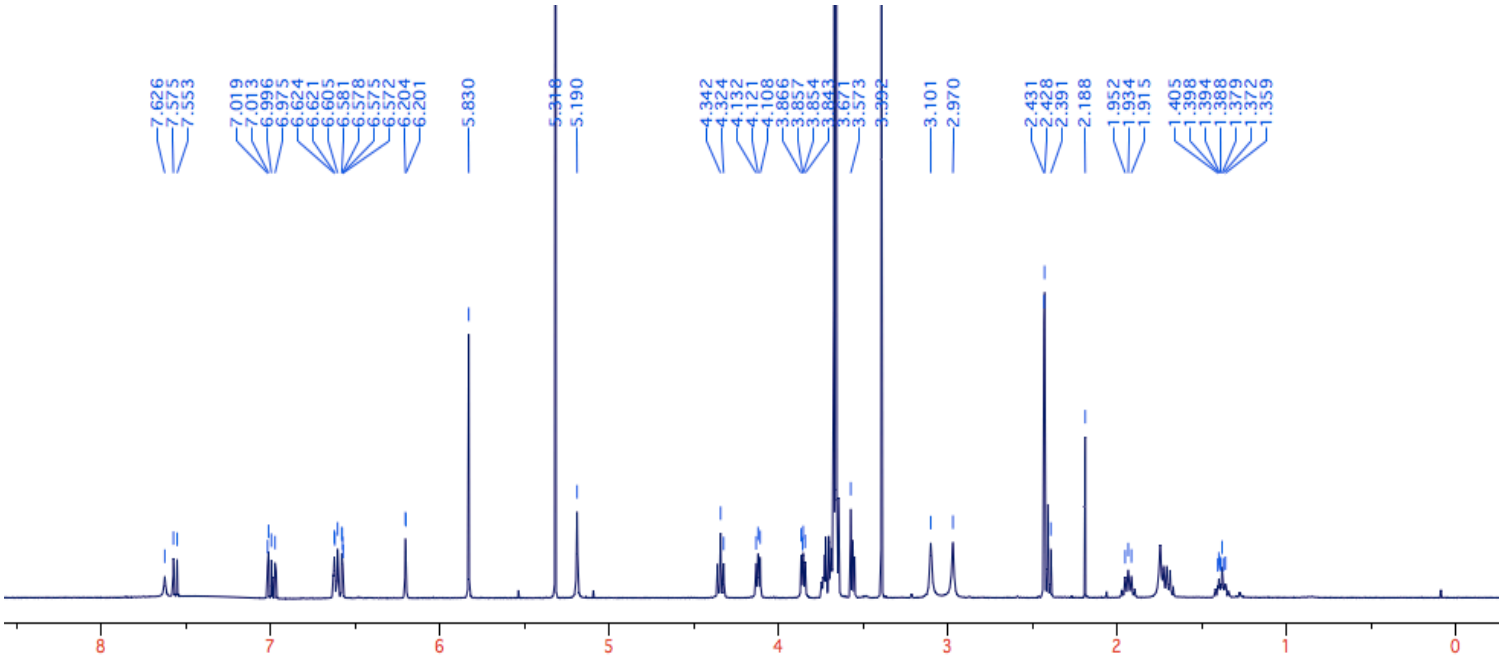


Scheme 3. Synthetic route for substrate S1

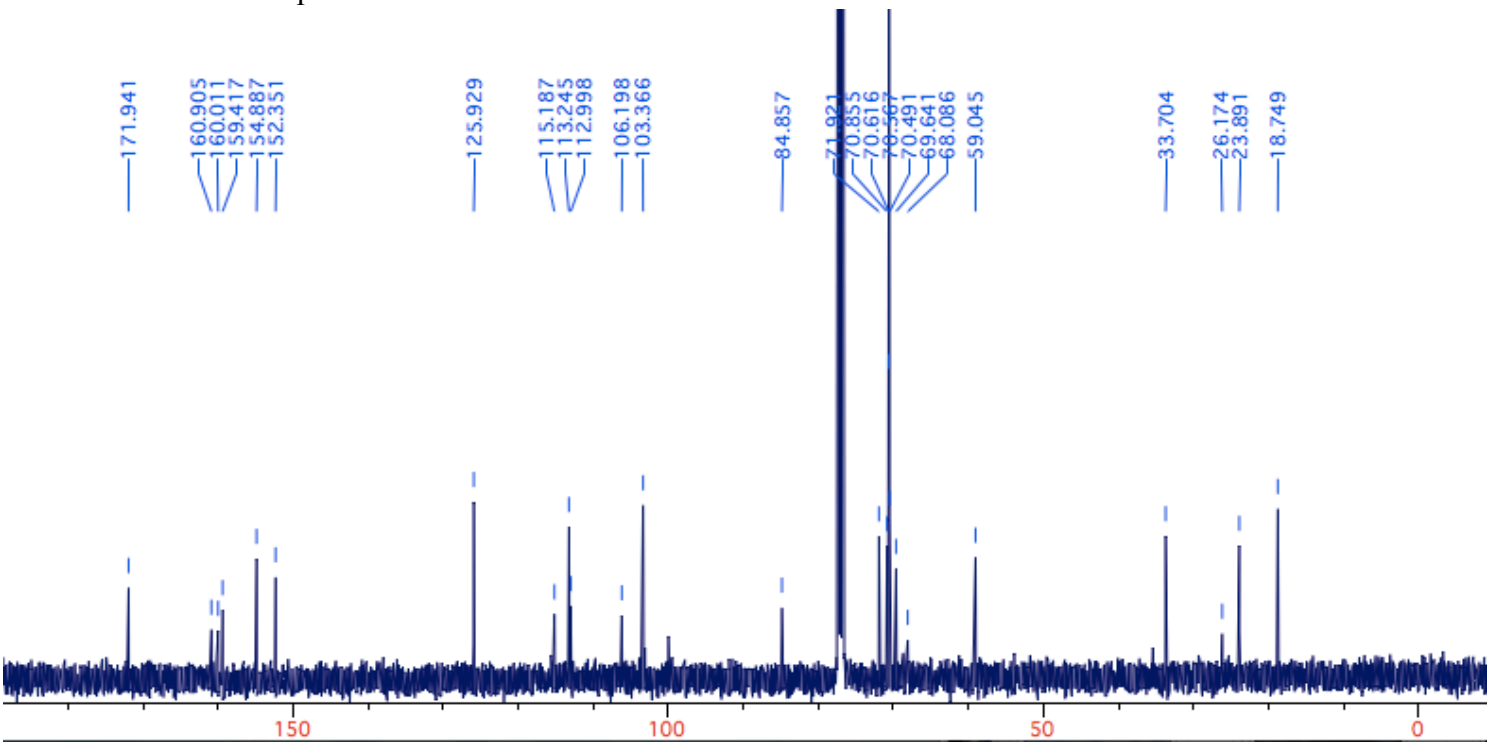
Synthesis of substrate S1: Compound **8** and **9** were synthesized according to previous reported procedures.[S2] The mixture of compound **8** (1.0 eq), compound **9** (2 eq), $CuSO_4{\cdot}5H_2O$ (0.5 equiv.) and sodium ascorbate (0.5 eq.) in MeOH/H_2O (1:1) solvent mixture was heated at 50 °C for 24 h. The reaction progress was monitored by TLC. After completion of the reaction, the reaction mixture was partitioned between ethyl acetate and saturated aqueous NH_4Cl solution. The aqueous layer was extracted twice with ethyl acetate and the combined organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by silica gel column chromatography. Yield: 93%, ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (s, 1H), δ 7.53 (d, J = 4.4 Hz, 1H), δ 6.95 (m, 2H), δ 6.61-6.52 (m, 2H), δ 6.18 (s, 1H), δ 5.80 (s, 2H), δ 5.15 (s, 2H), 4.32 (t, J = 3.6 Hz, 2H), δ

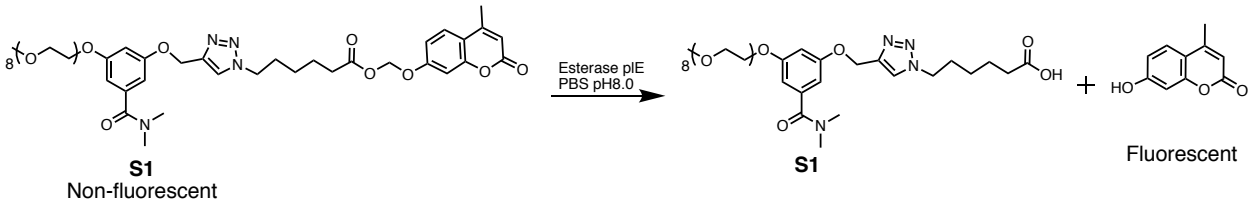
4.12 (t, *J* = 4.8 Hz, 2H), δ 3.85 (t, J = 4.6 Hz, 2H), δ 3.75-3.62 (m, 26H), δ 3.55 (m, 2H), δ 3.36 (s, 3H), δ 3.10 (s, 3H), δ 2.95 (s, 3H), δ 2.4 (m, 4H), δ 1.92 (m, 2H), δ 1.65 (m, 4H), δ 1.35 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 160.9, 160.0, 159.4, 159.2, 154.9, 152.3, 138.4, 125.9, 115.2, 113.2, 113.0, 106.2, 106.0, 103.4, 84.8, 71.9, 70.8, 70.6, 70.58, 70.50, 69.6, 59.1, 40.1, 35.4, 33.7, 29.3, 26.2, 23.9, 18.7. HR-ESI-MS (calculated: $[m+H]^+$= 931.45, obtained: $[m+Na]^+$= 953.3553).

^{1}H NMR of compound **S1**



^{13}C NMR of compound **S1**





Scheme 4. Substrate cleavage in present of esterase

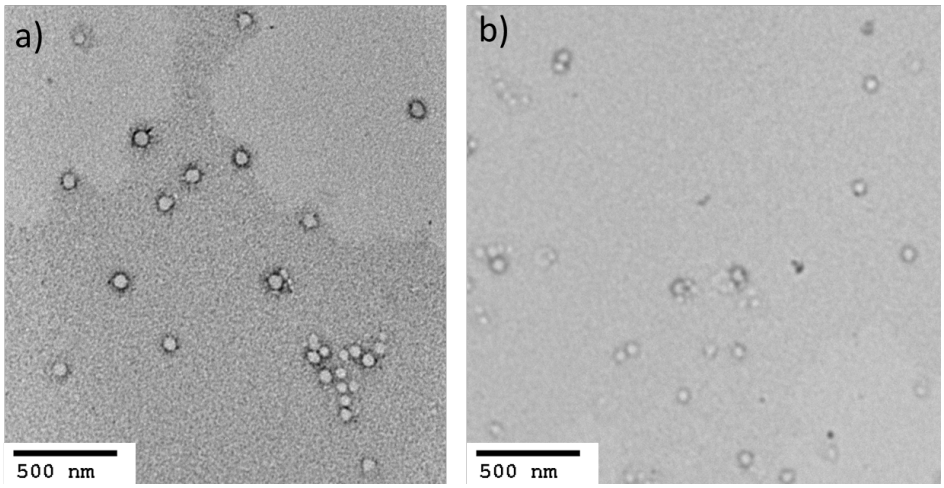


Figure S1: TEM images of **P1** (a) and **P2** (b).

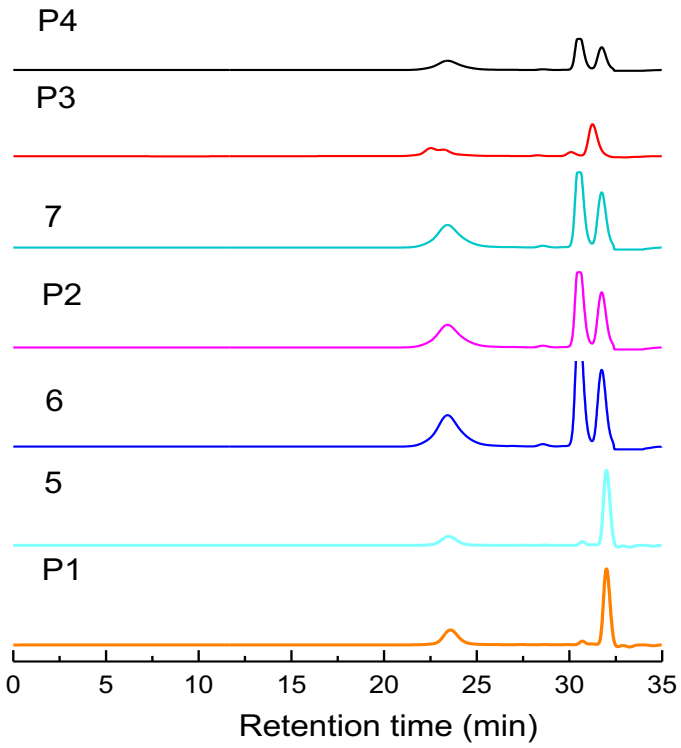


Figure S2: GPC traces of polymers synthesized.

a)

	PDI	Number average (nm)
P1	0.182	50
P2	0.193	37
P3	0.336	40
P4	0.248	43

b)

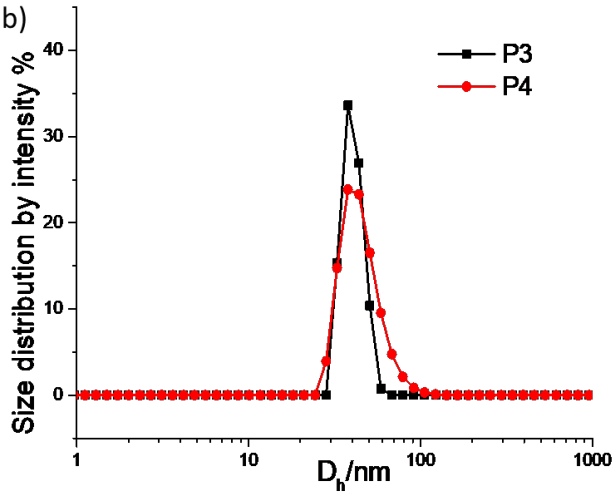


Figure S3. (a) PDI and number average of DLS measurement for P1, P2, P3 and P4. (b) DLS profile of P3 and P4 in volume distribution, number average found for P3 is 40nm, P4 is 43nm

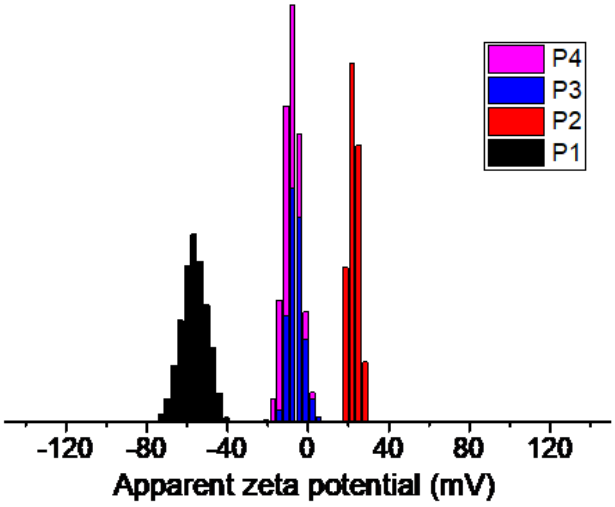


Figure S4. Apparent zeta potential for P1, P2, P3 and P4 in water (1 mg/mL).

Protein transport and release experiments

For protein transport with reverse micelles, 500 uL of a toluene solution of polymers (1 mg/mL) with 1 mL of protein in 10 mM PBS buffer at pH 7.4. The mixture is vortexed for 30 min and centrifuged at 10,000 rpm for 1 h to separate the organic and aqueous layers. The organic phase and aqueous phase is then analyzed by MALDI-MS or fluorimeter.
For the release of proteins back into the aqueous phase, the 500 uL organic phase contains proteins was treated with 100 uL THF and then equilibrated with 400 uL 1 M HCl for 30 minutes. After centrifugation for 30 minutes, the phase was separated. The pH of aqueous phase was adjusted to 7.4 for further analysis.

Quantification of porcine liver esterase in reverse micelles

1) Through the BCA method: The standard curve was made using Pierce BCA assay kit[S3] as following the protocols. Pipette 0.1 mL of each standard sample (0.005, 0.025, 0.05, 0.125, 0.25, mg/mL, three replicates for each sample) and the unknown plE sample into test tube and then add 2.0 mL of the working reagent to each tube and mix well. Cover and incubate tubes at 60 ^{o}C for 30 minutes and then cooled to room temperature. Then took the readings from UV-Vis at 562 nm. The standard curve was prepared plotting the average 562 nm measurement for each standard sample vs. its concentration. Then the plE concentration of aqueous phase after transport was determined using the standard curve. The difference of aqueous phase before and after the equilibration provide the loading capacity of reverse micelles.

2) Through SDS-PAGE method: Standard curves were generated from the known concentrations of protein samples loaded into the gel lanes. Then the samples of aqueous phase before and after the equilibration were loaded to the gel lane. For the organic phase, the samples for gel lanes were dried with air and dissolved in THF/H_2O. The intensities for the band will be used to calculate the

protein concentration of each phase. The concentration of protein for the organic phase after equilibration can provide how much proteins have been transported into the organic phase.

SDS-PAGE Analysis

For the transport and release of plE studies: 20 μL of different samples containing plE were mixed with 20 μL of loading buffer (3% DTT), then incubated 95 ºC for 10 minutes before subjecting 10 μL of each sample to acrylamide gel electrophoresis. Standard curves were generated from the known concentrations of pure protein samples loaded into the gel lanes. The gel image analysis and quantification were performed with Bio-Rad Image LabTM software.

Evaluation of PlE activity in reverse micelles

First, the amount of plE that got transported into the organic phase was calculated based on the SDS-PAGE or BCA assay. The organic phase containing plE was then equilibrated with an aqueous phase of substrate S1 (100 μM) for 30 minutes. After centrifugation for 30 minutes, the fluorescence of aqueous phase was measured over time. The control experiments with the same amount of plE were performed in aqueous phase.

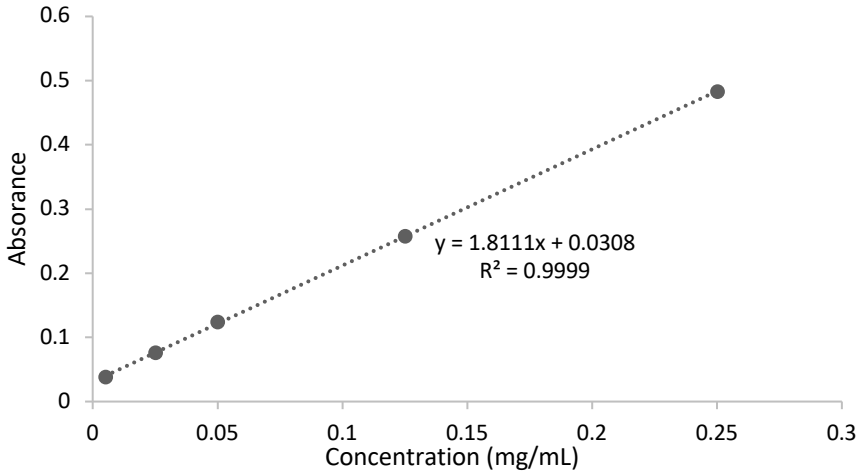


Figure S5. Standard curve of plE using BCA assay.

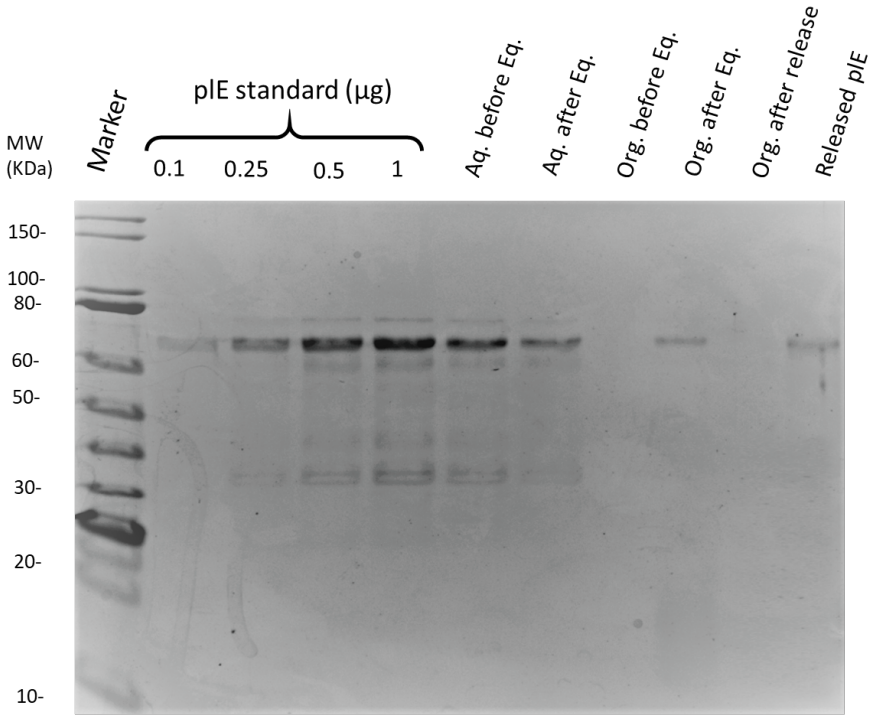


Figure S6. SDS-PAGE for transport and release of plE from reverse micelles.

a)

Lanes	Intensity
plE standard (0.1)	3451
plE standard (0.25)	8390
plE standard (0.5)	13912
plE standard (1)	19848
Aq. before Eq.	13389
Aq. after Eq.	8294
Org. before Eq.	0
Org. after Eq.	7326
Org. after release	0
Released plE	7262

b)

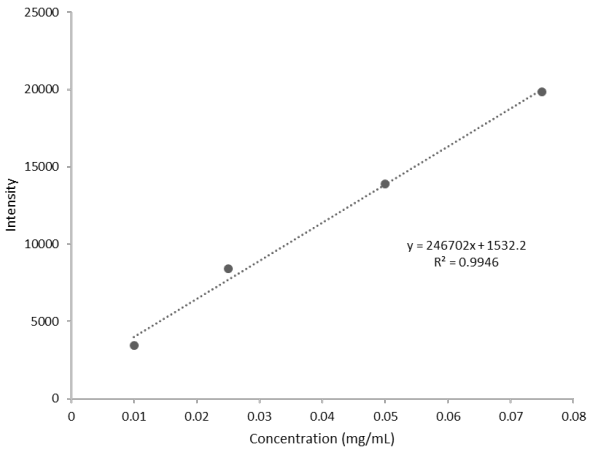


Figure S7. a) Intensity value for each band of SDS-PAGE; b) Standard curve of plE based on SDS-PAGE.

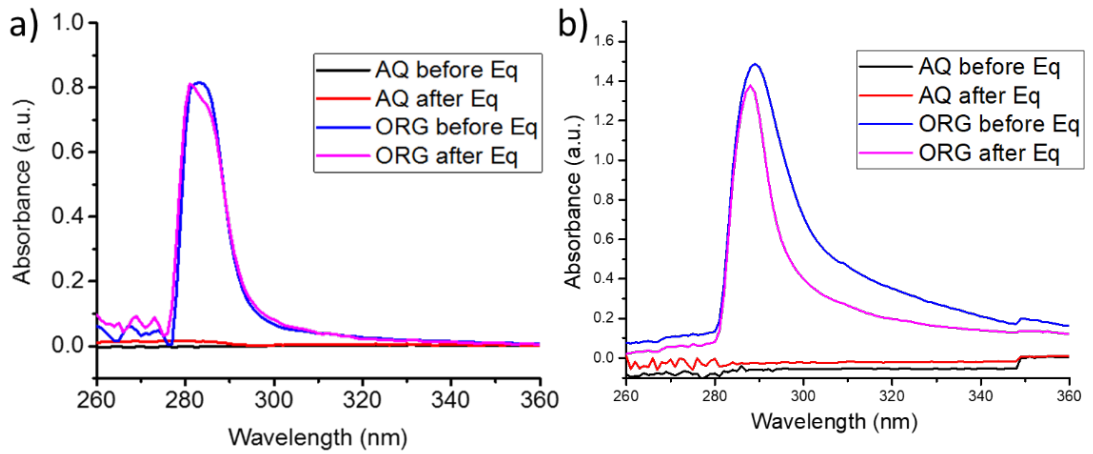


Figure S8: UV-Vis measurements with reverse micelles of a) polymer P1 (1×10^{-4} M), b) polymer P2 (1×10^{-4} M starting in toluene (ORG), before and after equilibration with aqueous phase (AQ).

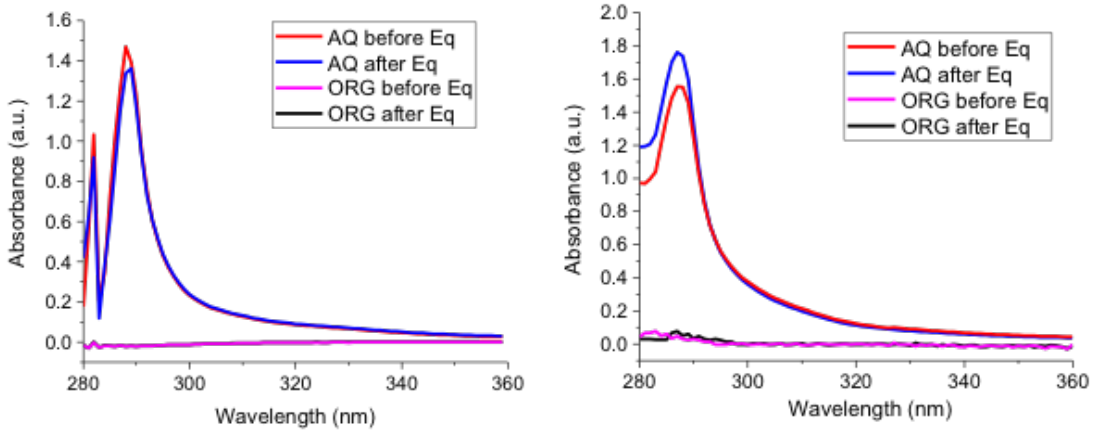


Figure S9: UV-Vis measurements with micelles of a) polymer P1 (1×10^{-4} M), b) polymer P2 (1×10^{-4} M starting in water (AQ), before and after equilibration with apolar phase toluene (ORG).

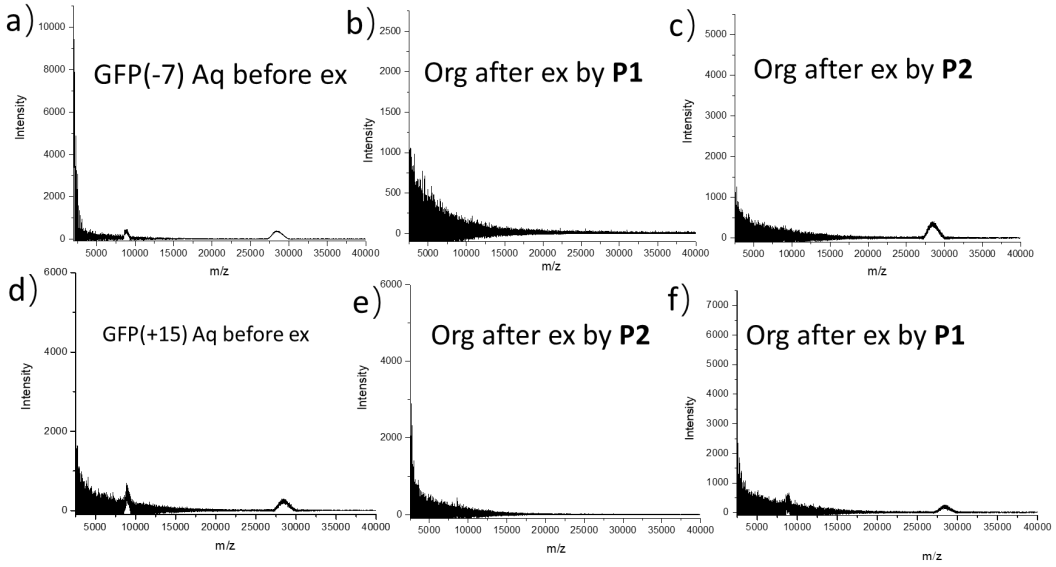


Figure S10. MALDI-MS analysis of GFP before and after transportation a) GFP (-7) before transportation, b) organic phase after transportation GFP (-7) using P1, c) organic phase after transportation GFP (-7) using P2, d) GFP (+15) before transportation, b) organic phase after transportation GFP (+15) using P2, c) organic phase after transportation GFP (+15) using P1.

Protein denaturation

bCA was dissolved in 10 mM PBS (pH 7.4) buffer at a concentration of 1mg/mL, 10% by volume of acetonitrile was added to the solution and stirred at room temperature for 10 minutes. After that, the mixture was heated at 100ºC for 2 minutes and then a buffer exchange was performed using 3 k Da Amicon Ultra Centrifugal Filters to remove acetonitrile. Then the sample was diluted to 0.1 mg/mL in 10 mM PBS (pH 7.4) for CD measurement.

Circular dichroism (CD) spectra

CD spectra bCA and denatured bCA were recorded on JASCO J-1500 spectrophotometer. For recording the spectra, 200 μL 0.1 mg/mL protein solution was injected into a quartz cuvette of 1-mm path length, equilibrated at 25 ºC for 10 min and scanned from 190 to 250 nm (scan rate: 20 nm/min, interval: 0.2 nm, average of three spectra).

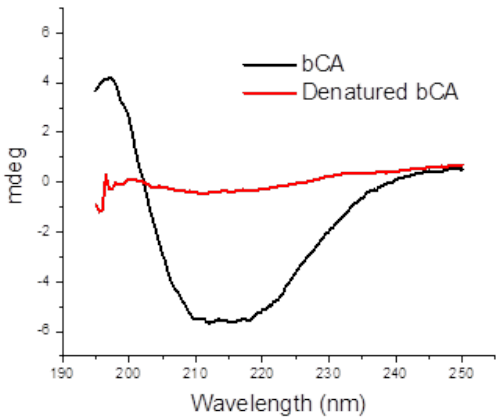


Figure S11. CD Spectra of bCA before and after denaturation.

Fluorescent labeling proteins

Labeling of proteins (lysozyme(Lyz), bovine serum albumin (BSA) and bovine carbonic anhydrase (bCA)) with Tetramethylrhodamine-5-Isothiocyanate (TRITC) or Fluorescein Isothiocyanate (FITC). In a typical labelling procedure, proteins (4 mg) were dissolved separately in 2 mL of 0.1 M $NaHCO_3$ buffer (pH 8.5) and stirred for 15 min at 4 ºC. TRITC/FITC (5 eq. of each protein, 10 mg/mL in DMSO) was added dropwise to each protein solution and stirred at 4 ºC for 2 h protected from light. The labelled-proteins were purified by extensive dialysis with 50 mM PBS pH 7.4 and 50 mM NaCl mixture to remove excess dye and concentrated using 3 kDa Amicon Ultra Centrifugal Filters. Protein concentrations in each labelled conjugate were calculated using UV-Vis spectroscopy.

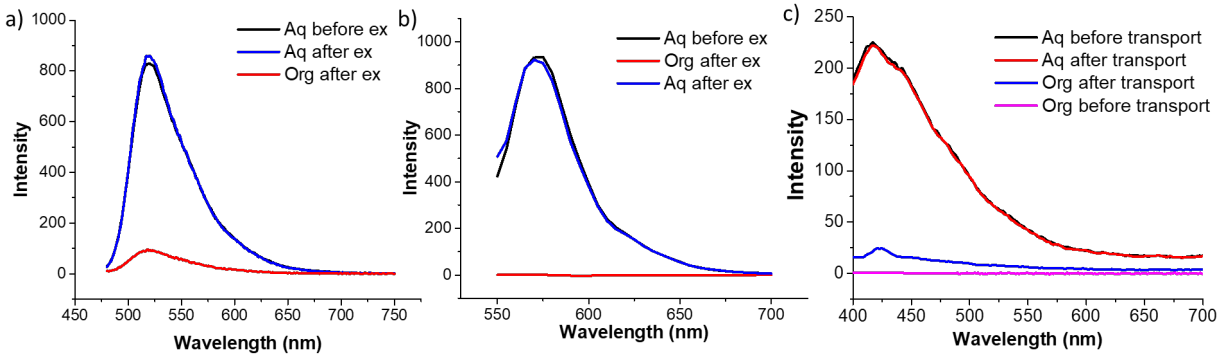


Figure S12. Emission spectra of a) FITC-BSA (excitation wavelength 470 nm), b) RITC-lysozyme (excitation wavelength 535 nm) and myoglobin (excitation wavelength 380 nm) before and after transportation by polymer P3.

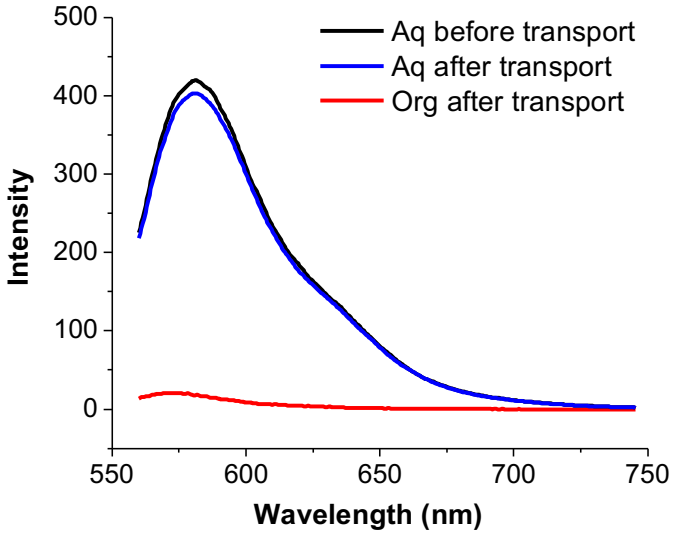


Figure S13. Emission spectra of plE-TRITC (excitation wavelength 535 nm) before and after transportation by polymer **P1**.

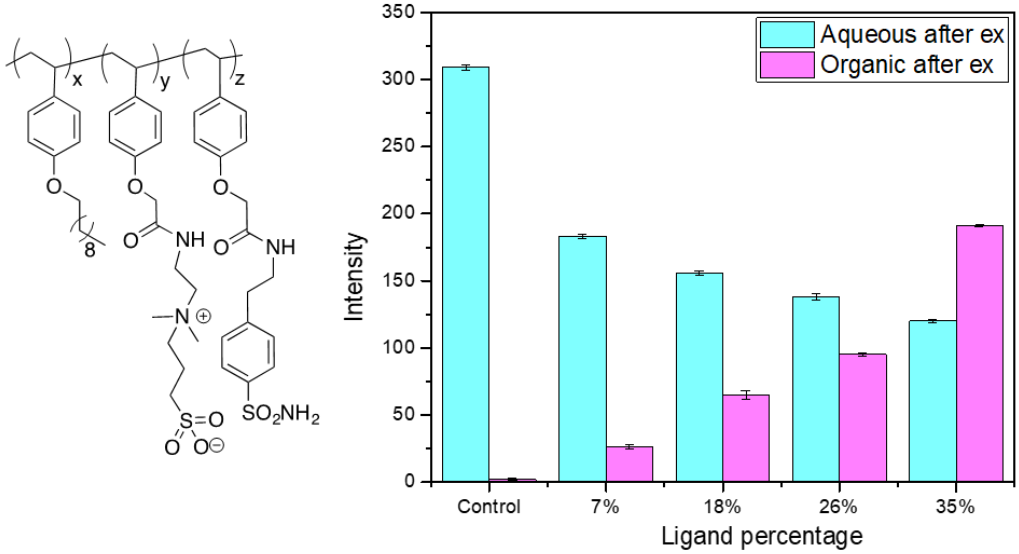


Figure S14. Increase in the ligand intensity (z value) can transport more bCA from aqueous phase to organic phase(x=0.4, y=0.6-z).

Reference

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3. a) https://www.thermofisher.com/order/catalog/product/23225. b) Smith, P. K., Krohn, R. I., Hermanson, G. T., Klenk, D. C et al. *Anal. Biochem.* **1985**, 150 (1), 76-85.