Supporting information for:

Self-assembly of random co-polymers for selective binding and detection of peptides

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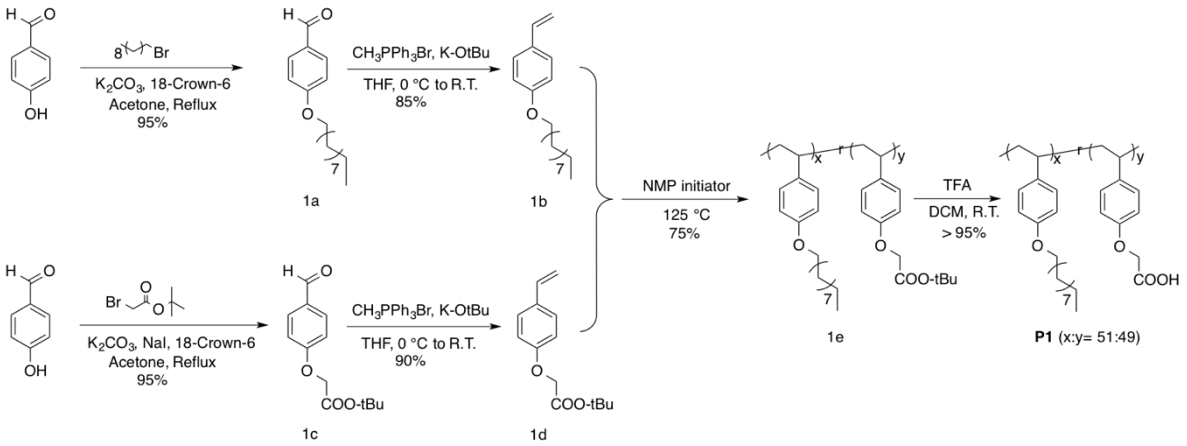
Synthesis of poly(dialkoxystyrene) (PDAS) amphiphilic homopolymer

The PDAS carboxylate homopolymer **P7** was synthesized as previously described elsewhere[1].

Synthesis of random co-polymer P1



Synthesis of compound **1a**:

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 with reflux 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 10.5 g (95% yield) of **1a**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.86 (s, 1H), δ 7.80-7.82 (d, 2H), δ 6.96-6.99 (d, 2H), δ 4.00-4.04 (t, 2H), δ 1.76-1.83 (quint, 2H), δ 1.47-1.26 (m, 14H), δ 0.85-0.89 (t, 3H).

Synthesis of compound **1b**:

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 the bright yellow solution. **1a** (6.58 g, 25.11 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl 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 5.5 g (85% yield) of **1b**. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.31-7.33 (d, 2H), δ 6.83-6.85 (d, 2H), δ 6.61-6.68 (q, 1H), δ 5.57-5.61 (d, 1H), δ 5.09-5.12 (d, 1H), δ 3.93-3.96 (t, 3H), δ 1.73-1.80 (quint, 2H), δ 1.27-1.46 (m, 14H), δ 0.86-0.89 (t, 3H).

Synthesis of compound **1c**:

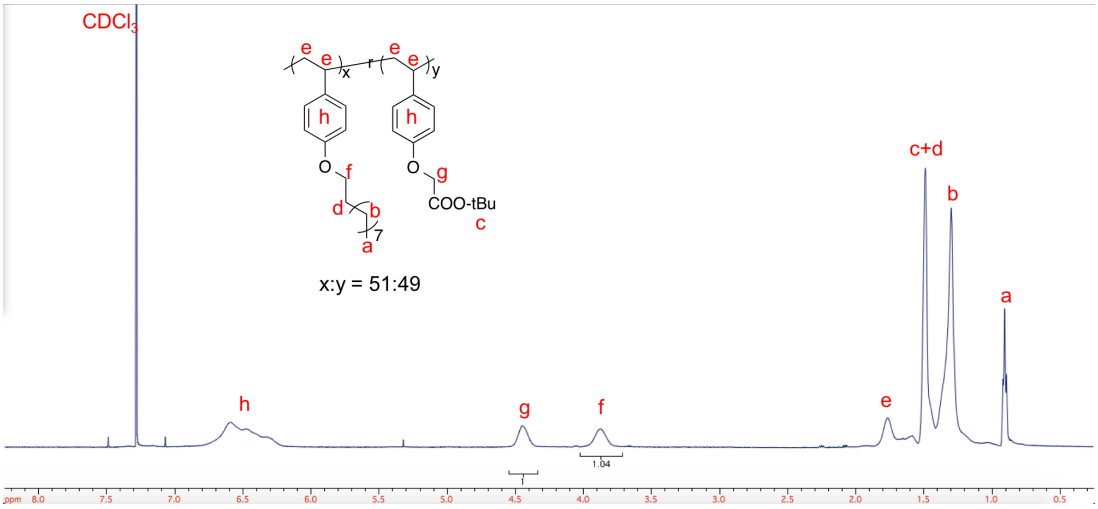
To a solution of acetone mixed with K_2CO_3 (6.79 g, 49.13 mmol), NaI (7.36 g, 49.13 mmol) and 18-crown-6 (0.65 g, 2.46 mmol), 4-Hydroxybenzaldehyde (3.00 g, 24.57 mmol) was added and stirred for 5 min. To this mixture, tert-Butyl bromoacetate (9.58 g, 49.13 mmol) was added and stirred with reflux for 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 5.5 g (95% yield) of **1c**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.88 (s, 1H), δ 7.82-7.84 (d, 2H), δ 6.97-6.99 (d, 2H), δ 4.59 (s, 2H), δ 1.47 (s, 9H); ESI-MS (expected: $[m+H]^+$= 237.1, obtained: $[m+Na]^+$= 259.1)

Synthesis of compound **1d**:

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. **1c** (3.5 g, 14.83 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl 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 3.1 g (90% yield) of **1d**. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.33-7.35 (d, 2H), δ 6.84-6.87 (d, 2H), δ 6.63-6.68 (q, 1H), δ 5.60-5.64 (q, 1H), δ 5.13-5.15 (q, 1H), δ 4.51 (s, 2H), δ 1.49 (s, 9H); ESI-MS (expected: $[m+H]^+$= 235.1, obtained: $[m+Na]^+$= 257.1)

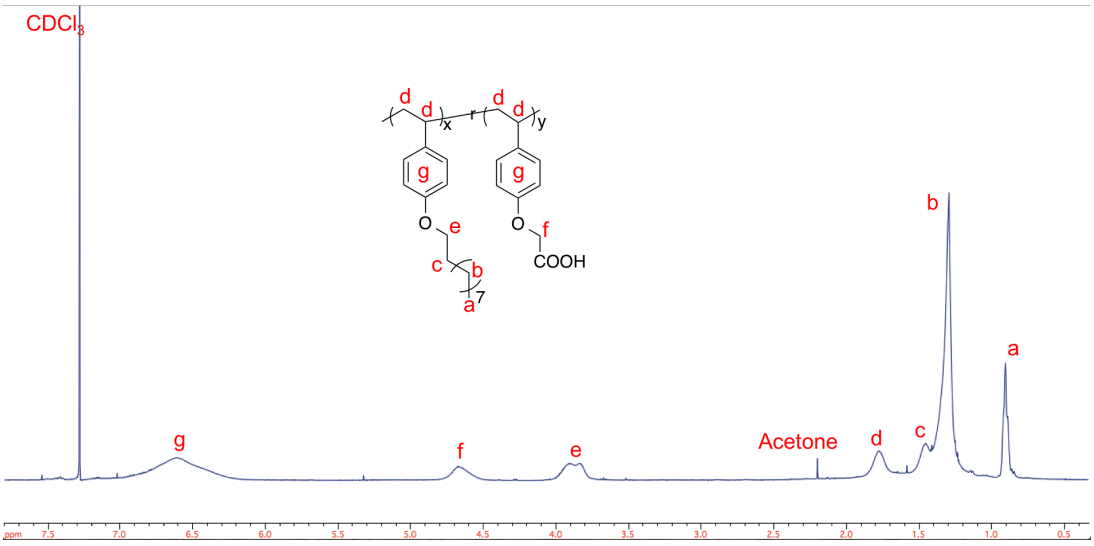
Synthesis of random co-polymer **1e**:

A mixture of the compound **1b** (300 mg, 1.15 mmol), **1d** (269 mg, 1.15mmol) and *N*-*tert*-Butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl)hydroxylamine (NMP initiator, 15 mg, 0.046 mmol) were degassed by three freeze/thaw cycles, sealed under argon, and heated at 125 ^{o}C under argon for 12 h. After the reaction cool down to room temperature, the reaction mixture was dissolved in minimal amount of DCM, and precipitated 3 times in the MeOH. The precipitate was collected and dried under vacuum to yield 430 mg (75% yield) of **1e**. GPC (PMMA/THF): M_n= 10K Da, Đ=1.1;

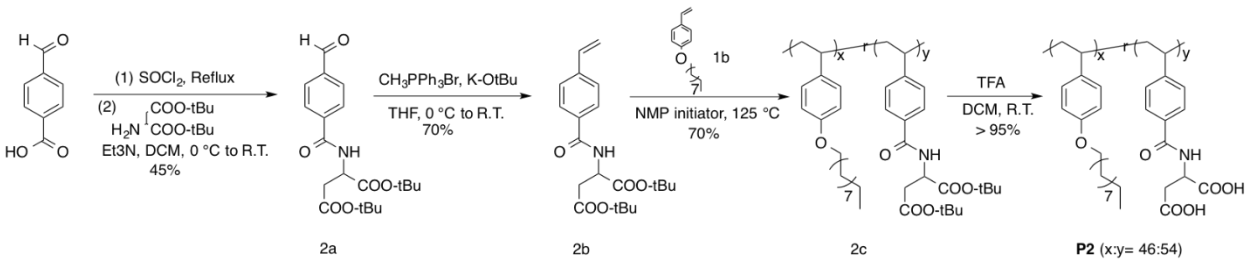


Synthesis of random co-polymer **P1**:

DCM (2 mL) was added to dissolve the dried random co-polymer **1e** (420 mg). Trifluoroacetic acid (0.5 mL) was added to the reaction, and stirred for 12 h. The reaction mixture was evaporated and dried under vacuum to obtain the final product **P1**.



Synthesis of random co-polymer P2



Synthesis of compound **2a**:

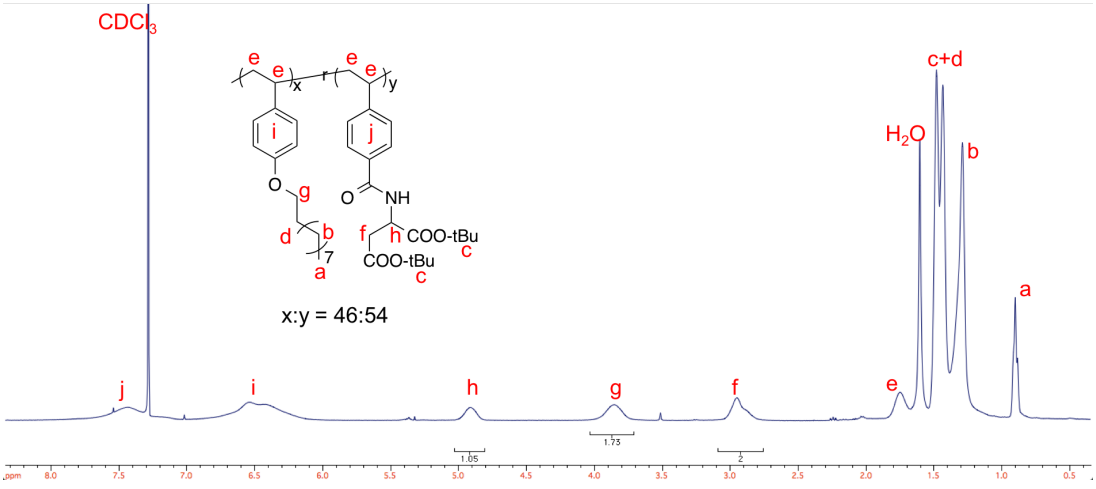
To a solution of Thionyl chloride (5.0 g, 3 mL, 42.03 mmol), 4-Formylbenzoic acid (1.0 g, 6.66 mmol) was added to afford a cloudy solution. This mixture was stirred with reflux for 18 h. The reaction mixture was then cooled to room temperature and filtered to afford the brown clear solution. The reaction mixture was evaporated and dried under vacuum for 3 h. Then the crude product was dissolved in DCM, and mixed with Triethylamine (2.02 g, 19.98 mmol) for 30 min in an ice bath. To the reaction mixture, commercial available compound L-Aspartic acid di-tert-butyl ester hydrochloride (2.06 g, 7.33 mmol) was added and stirred for 12 h. The mixture was evaporated and purified by silica gel column chromatography (10-15% ethyl acetate in hexanes) to obtain 1.1 g (overall 45% yield) of **2a**. ^{1}H NMR (400MHz, $CDCl_3$) δ 10.07 (s, 1H), δ 7.95 (s, 4H), δ 7.30-7.32 (d, 1H), δ 4.84-4.88 (quint, 1H), δ 2.83-3.02 (m, 2H), δ 1.44-1.48 (d, 18H); ESI-MS (expected: $[m+H]^+$= 378.2, obtained: $[m+Na]^+$= 400.2)

Synthesis of compound **2b**:

Methyltriphenylphosphonium bromide (1.56 g, 4.37 mmol) and Potassium tert-butoxide (0.49 g, 4.37 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. **2a** (1.1 g, 2.92 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl 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 (5-8% ethyl acetate in hexanes) to afford 0.77 g (70% yield) of **2b**. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.61-7.78 (q, 2H), δ 7.45-7.47 (d, 2H), δ 7.19-7.20 (d, 1H), δ 6.70-6.77 (q, 1H), δ 5.81-5.85 (d, 1H), δ 5.33-5.36 (d, 1H), δ 4.85-4.89 (quint, 1H), δ 2.82-3.00 (m, 2H), δ 1.44-1.48 (d, 18H); ESI-MS (expected: $[m+H]^+$= 376.2, : obtained: $[m+Na]^+$= 398.2)

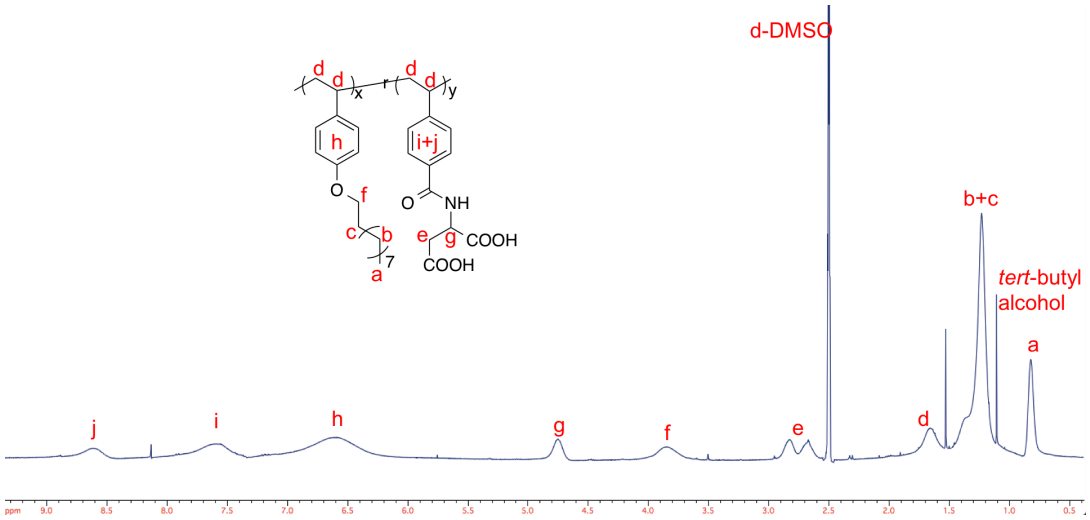
Synthesis of random co-polymer **2c**:

A mixture of the compound **1b** (150 mg, 0.58 mmol), **2b** (216 mg, 0.58mmol) and *N*-*tert*-Butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl)hydroxylamine (NMP initiator, 7.5 mg, 0.023 mmol) were degassed by three freeze/thaw cycles, sealed under argon, and heated at 125 °C under argon for 12 h. After the reaction cool down to room temperature, the reaction mixture was dissolved in DCM, and dialyzed against DCM/MeOH (v/v= 6/1) for 2 days. The solution was collected and dried under vacuum to yield 260 mg (70% yield) of **2c**. GPC (PMMA/THF): M_n= 12K Da, Đ=1.2;

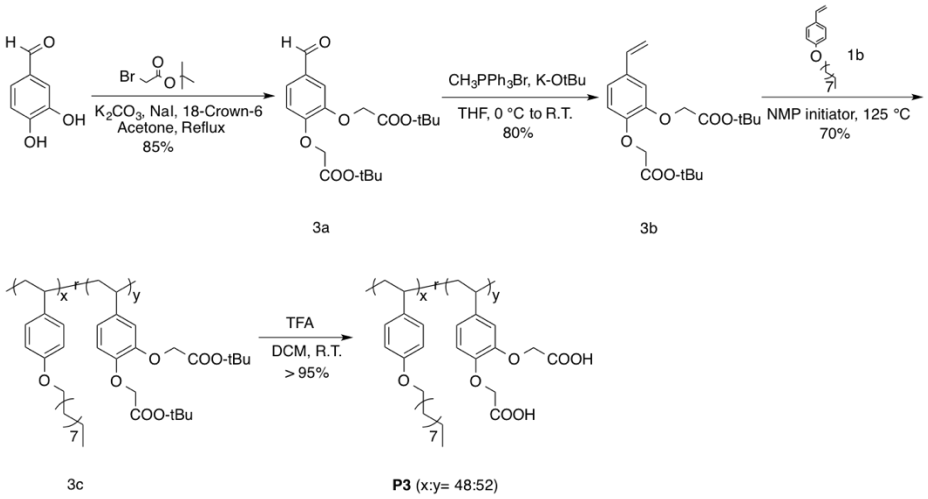


Synthesis of random co-polymer **P2**:

DCM (2 mL) was added to dissolve the dried random co-polymer **2c** (250 mg). Trifluoroacetic acid (0.5 mL) was added to the reaction, and stirred for 12 h. The reaction mixture was evaporated and dried under vacuum to obtain the final product **P2**.



Synthesis of random co-polymer P3



Synthesis of compound **3a**:

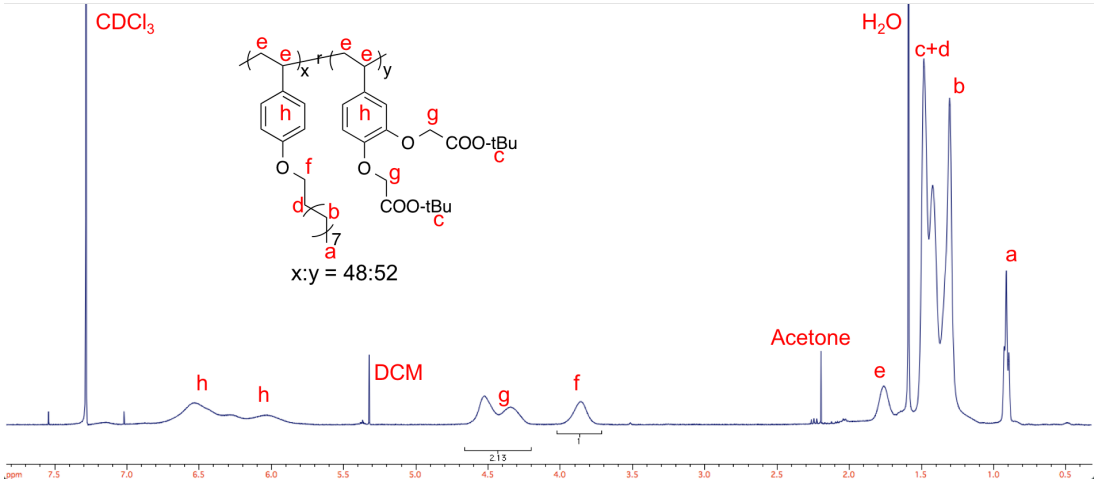
To a solution of acetone mixed with K_2CO_3 (6.00 g, 43.44 mmol), NaI (3.91 g, 26.06 mmol) and 18-crown-6 (0.57 g, 2.17 mmol), 3,4-Dihydroxybenzaldehyde (1.50 g, 10.86 mmol) was added and stirred for 5 min. To this mixture, tert-Butyl bromoacetate (5.08 g, 26.06 mmol) was added and stirred with reflux 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 3.4 g (85% yield) of **3a**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.83 (s, 1H), δ 7.45-7.47 (q, 1H), δ 7.35-7.36 (d, 1H), δ 6.88-6.91 (d, 1H), δ 4.66-4.69 (d, 4H), δ 1.47-1.48 (d, 18H); ESI-MS (expected: $[m+H]^+$= 367.2, obtained: $[m+Na]^+$= 389.2)

Synthesis of compound **3b**:

Methyltriphenylphosphonium bromide (4.24 g, 11.88 mmol) and Potassium tert-butoxide (1.33 g, 11.88 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 the bright yellow solution. **3a** (2.9 g, 7.92 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl 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 2.3 g (80% yield) of **3b**. ^{1}H NMR (400MHz, $CDCl_3$) δ 6.94-6.96 (t, 2H), δ 6.78-6.80 (d, 1H), δ 6.57-6.64 (q, 1H), δ 5.55-5.60 (q, 1H), δ 5.13-5.16 (q, 1H), δ 4.59-4.61 (d, 4H), δ 1.47-1.48 (d, 18H); ESI-MS (expected: $[m+H]^+$= 365.2, obtained: $[m+Na]^+$= 387.2)

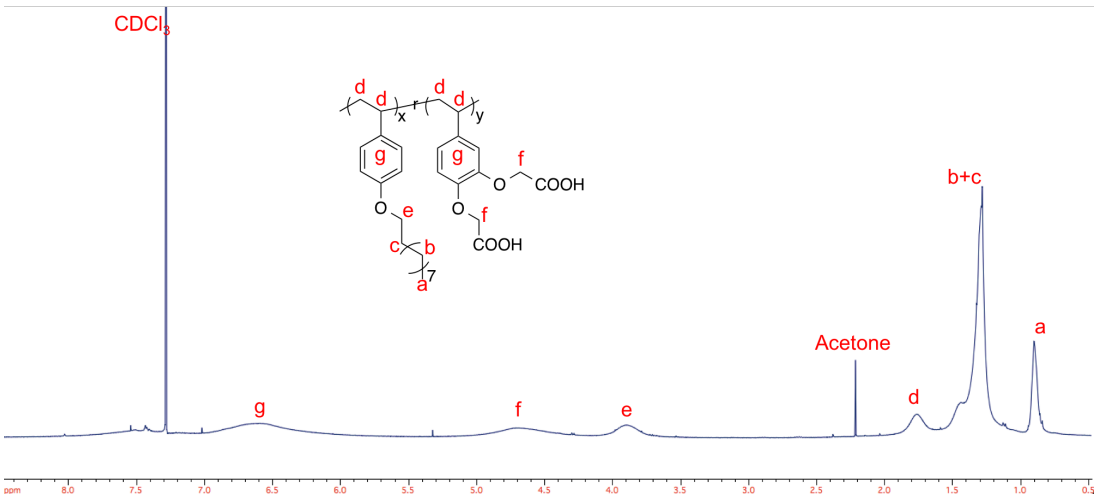
Synthesis of random co-polymer **3c**:

A mixture of the compound **1b** (200 mg, 0.77 mmol), **3b** (280 mg, 0.77mmol) and *N*-*tert*-Butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl)hydroxylamine (NMP initiator, 10 mg, 0.031 mmol) were degassed by three freeze/thaw cycles, sealed under argon, and heated at 125 ^{o}C under argon for 12 h. After the reaction cool down to room temperature, the reaction mixture was dissolved in minimal amount of DCM, and precipitated 3 times in the MeOH. The precipitate was collected and dried under vacuum to yield 340 mg (70% yield) of **3c**. GPC (PMMA/THF): M_n= 14K Da, Đ=1.1;

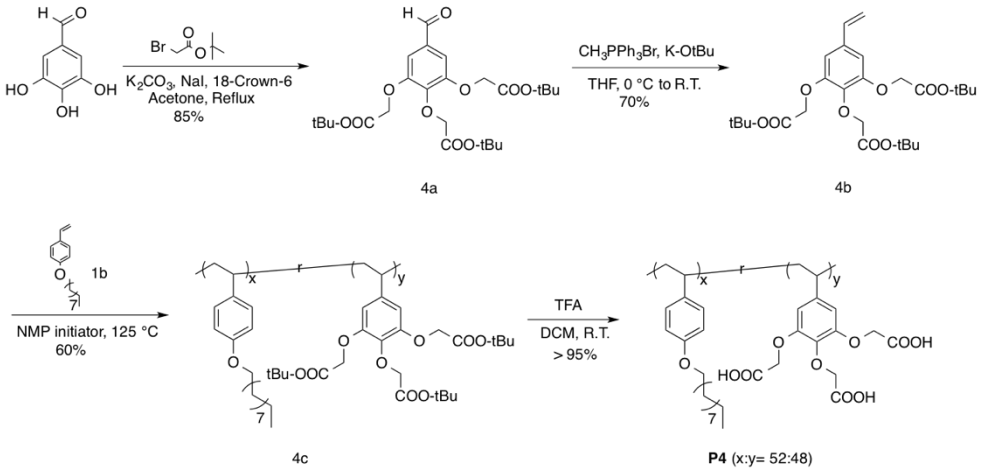


Synthesis of random co-polymer **P3**:

DCM (2 mL) was added to dissolve the dried random co-polymer **3c** (330 mg). Trifluoroacetic acid (0.5 mL) was added to the reaction, and stirred for 12 h. The reaction mixture was evaporated and dried under vacuum to obtain the final product **P3**.



Synthesis of random co-polymer P4



Synthesis of compound **4a**:

To a solution of acetone mixed with K_2CO_3 (3.85 g, 27.88 mmol), NaI (2.50 g, 16.73 mmol) and 18-crown-6 (0.37 g, 1.39 mmol), 3,4,5-Trihydroxybenzaldehyde (0.8 g, 4.65 mmol) was added and stirred for 5 min. To this mixture, tert-Butyl bromoacetate (3.26 g, 16.73 mmol) was added and stirred with reflux 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 1.9 g (85% yield) of **4a**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.78 (s, 1H), δ 7.06 (s, 2H), δ 4.82 (s, 2H), δ 4.64 (s, 4H), δ 2.17 (s, 9H), δ 1.47-1.48 (d, 27H); ESI-MS (expected: $[m+H]^+$= 497.2, obtained: $[m+Na]^+$= 519.2)

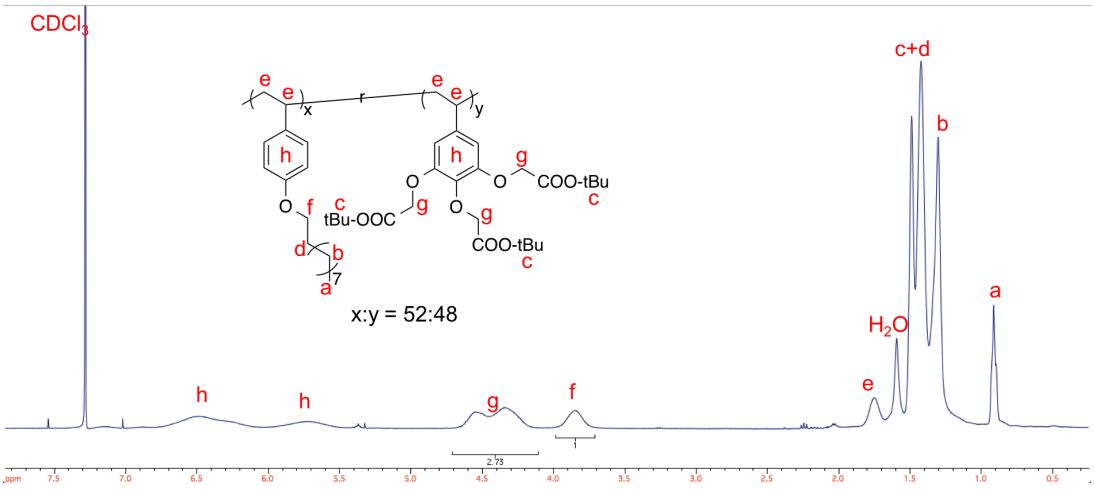
Synthesis of compound **4b**:

Methyltriphenylphosphonium bromide (2.05 g, 5.74 mmol) and Potassium tert-butoxide (0.64 g, 5.74 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 the bright yellow solution. **4a** (1.9 g, 3.93 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl 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 1.3 g (70% yield) of **4b**. ^{1}H NMR (400MHz, $CDCl_3$) δ 6.50-6.58 (quint, 3H), δ 5.55-5.59 (q, 1H), δ 5.16-5.19 (d, 1H), δ 4.59-4.63 (d, 6H), δ 1.46-1.48 (d, 27H); ESI-MS (expected: $[m+H]^+$= 495.3, obtained: $[m+Na]^+$= 517.3)

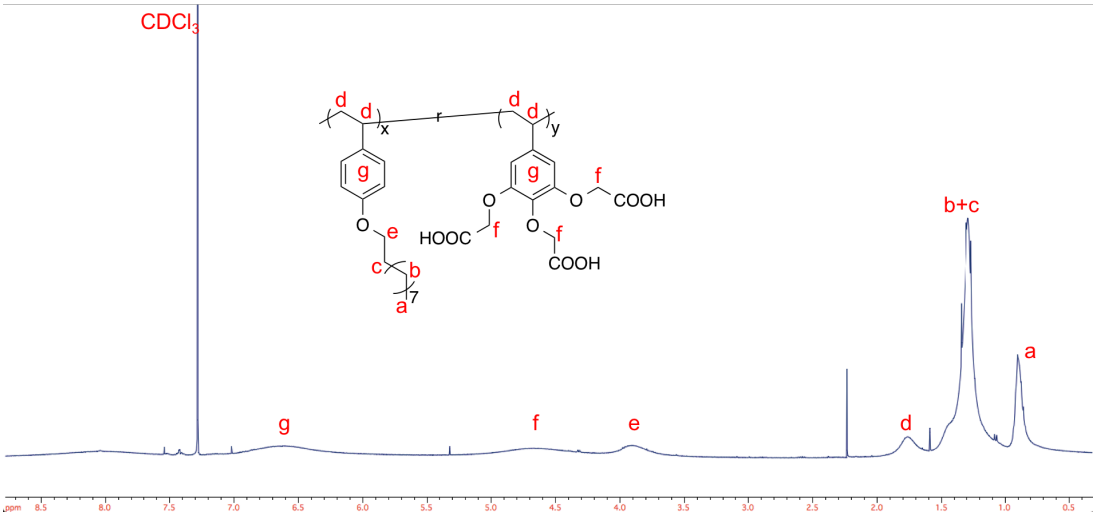
Synthesis of random co-polymer **4c**:

A mixture of the compound **1b** (100 mg, 0.38 mmol), **4b** (190 mg, 0.38 mmol) and *N*-*tert*-Butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl)hydroxylamine (NMP initiator, 5 mg, 0.015 mmol) were degassed by three freeze/thaw cycles, sealed under argon, and heated at 125 °C under argon for 12 h. After the reaction cool down to room temperature, the reaction mixture was dissolved in minimal amount of DCM, and precipitated 3 times in the MeOH. The precipitate was collected and dried under vacuum to yield 170 mg (60% yield) of **4c**. GPC (PMMA/THF): M_n= 15K Da, Đ=1.2;

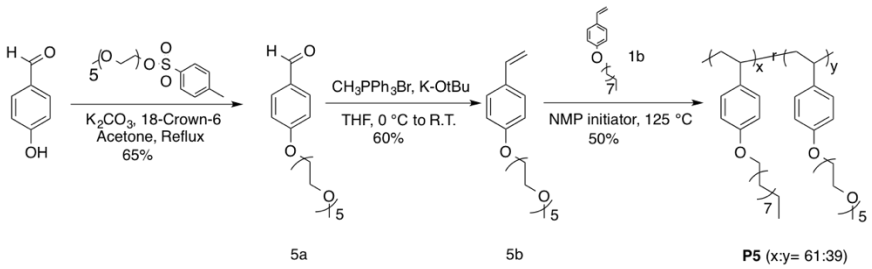


Synthesis of random co-polymer **P4**:

DCM (2 mL) was added to dissolve the dried random co-polymer **4c** (160 mg). Trifluoroacetic acid (0.5 mL) was added to the reaction, and stirred for 12 h. The reaction mixture was evaporated and dried under vacuum to obtain the final product **P4**.



Synthesis of random co-polymer P5



Synthesis of compound **5a**:

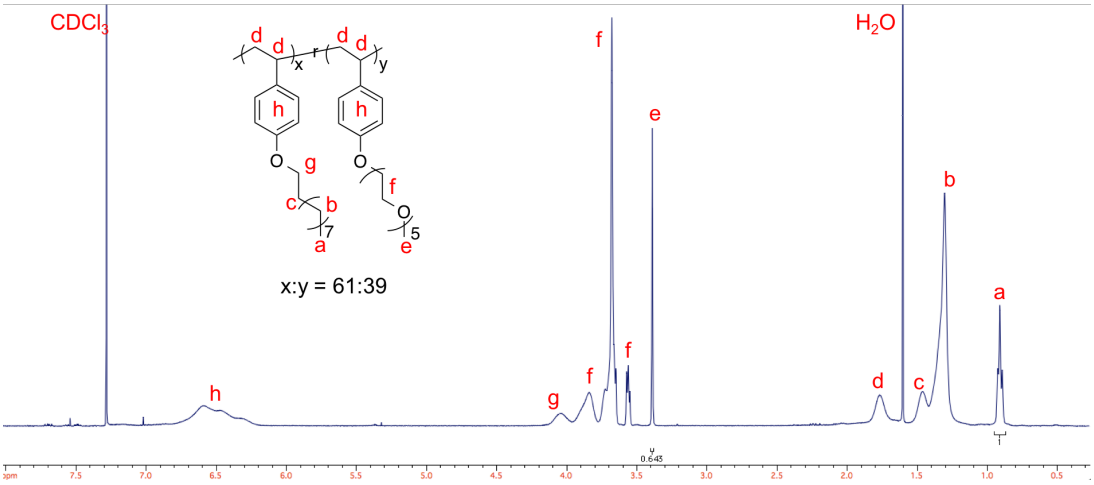
To a solution of acetone mixed with K_2CO_3 (1.17 g, 8.45 mmol), and 18-crown-6 (0.56 g, 2.11 mmol), 4-Hydroxybenzaldehyde (0.52 g, 4.23 mmol) was added and stirred for 5 min. To this mixture, tosylate of pentaethylene glycol monomethyl ether (2.06 g, 5.07 mmol) was added and stirred with reflux for 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 (3-5% MeOH in DCM) to obtain 1.0 g (65% yield) of **5a**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.88 (s, 1H), δ 7.82-7.84 (t, 2H), δ 7.01-7.03 (d, 2H), δ 4.20-4.22 (t, 2H), δ 3.88-3.90 (t, 2H), δ 3.53-3.73 (m, 16H), δ 3.37 (s, 3H); ESI-MS (expected: $[m+H]^+$= 357.2, obtained: $[m+Na]^+$= 379.2)

Synthesis of compound **5b**:

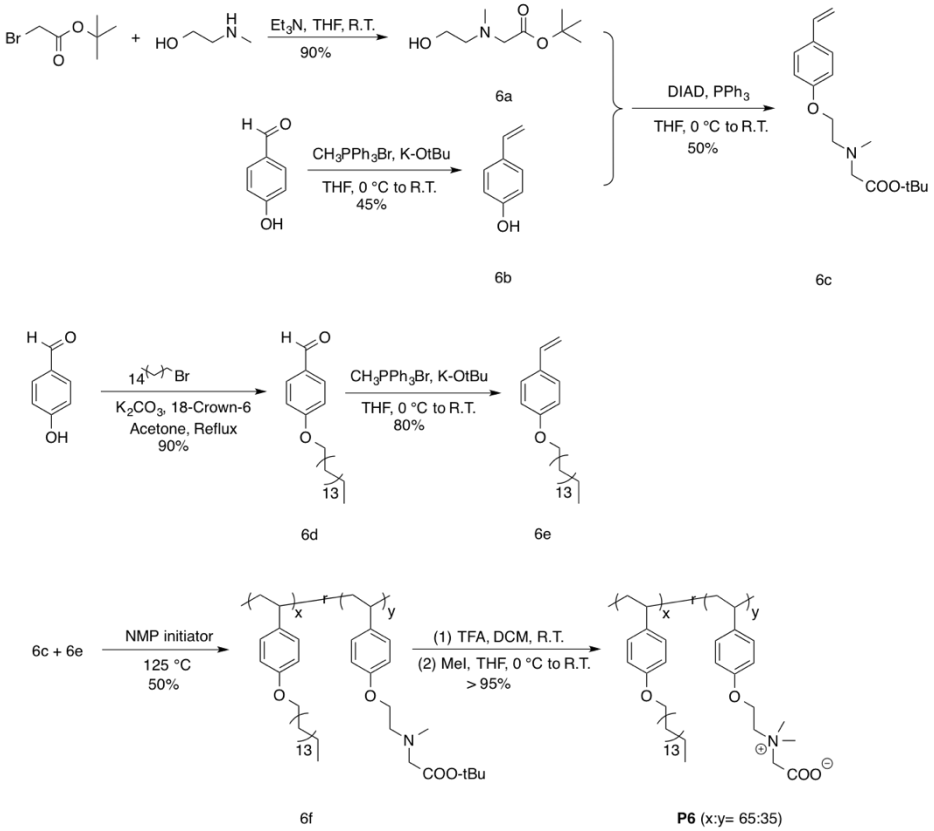
Methyltriphenylphosphonium bromide (1.35 g, 3.79 mmol) and Potassium tert-butoxide (0.42 g, 3.74 mmol) were mixed in a round bottom flask, and dry THF (15 mL) was added to the mixture. The mixture was stirred under argon atmosphere in an ice bath for 15 min to yield the bright yellow solution. **5a** (0.9 g, 2.53 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl 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 (30-40% ethyl acetate in hexanes) to afford 0.54 g (60% yield) of **5b**. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.31-7.32 (d, 2H), δ 6.85-6.86 (d, 2H), δ 6.61-6.67 (q, 1H), δ 5.57-5.61 (q, 1H), δ 5.09-5.12 (q, 1H), δ 4.10-4.12 (t, 2H), δ 3.83-3.85 (t, 2H), δ 3.53-3.72 (m, 16H), δ 3.37 (s, 3H); ESI-MS (expected: $[m+H]^+$= 355.2, obtained: $[m+Na]^+$= 377.2)

Synthesis of random co-polymer **P5**:

A mixture of the compound **1b** (200 mg, 0.77 mmol), **5b** (272 mg, 0.77 mmol) and *N*-*tert*-Butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl)hydroxylamine (NMP initiator, 10 mg, 0.030 mmol) were degassed by three freeze/thaw cycles, sealed under argon, and heated at 125 ^{o}C under argon for 12 h. After the reaction cool down to room temperature, the reaction mixture was dissolved in minimal amount of DCM, and precipitated 3 times in the MeOH. The precipitate was collected and dried under vacuum to yield 240 mg (50% yield) of **P5**. GPC (PMMA/THF): M_n= 10K Da, Đ=1.3;



Synthesis of random co-polymer P6



Synthesis of compound **6a**:

To a solution of 2-(Methylamino)ethanol (5.37 g, 71.45 mmol) in dry THF, Triethylamine (10.89 g, 107.6 mmol) was added and stirred for 15 min at room temperature. To the reaction mixture, tert-Butyl bromoacetate (15.40 g, 78.97 mmol) was added and stirred for 12 h. After the reaction, NaCl saline and ethyl acetate were added for extraction. The combined organic layer was separated and washed with saline 3 times. The combined organic phase was evaporated to dryness to obtain 11.9 g (90% yield) of **6a**.

^{1}H NMR (400MHz, $CDCl_3$) δ 3.55-3.57 (t, 2H), δ 3.19 (s, 2H), δ 2.66-2.68 (t, 2H), δ 2.40 (s, 3H), δ 1.45 (s, 9H); ESI-MS (expected: $[m+H]^+$= 190.1, obtained: $[m+Na]^+$=212.1)

Synthesis of compound **6b**:

Methyltriphenylphosphonium bromide (17.55 g, 49.13 mmol) and Potassium tert-butoxide (5.54 g, 49.37 mmol) were mixed in a round bottom flask, and dry THF (40 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. 4-Hydroxybenzaldehyde (3.0 g, 24.57 mmol) solution (in dry THF) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl 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 (8-9% ethyl acetate in hexanes) to afford 1.3 g (45% yield) of **6b**. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.29-7.32 (q, 2H), δ 6.78-6.80 (q, 2H), δ 6.61-6.69 (q, 1H), δ 5.58-5.62 (d, 1H), δ 5.11-5.14 (d, 1H), δ 4.71 (s, 1H); ESI-MS (expected: $[m+H]^+$= 121.1, obtained: $[m+Na]^+$= 143.1)

Synthesis of compound **6c**:

6a (0.62 g, 3.30 mmol) and **6b** (0.33 g, 2.75 mmol) and Triphenylphosphine (0.86 g, 3.29 mmol) were mixed in a round bottom flask, and dry THF (10 mL) was added to the mixture. The mixture was stirred under argon atmosphere in an ice bath for 10 min. Diisopropyl azodicarboxylate (0.70 g, 3.47 mmol) was added dropwise to the mixture. The reaction mixture was further stirred for 12 h. Then the solvent was evaporated. 3 mL of Ethyl acetate was added to dissolve the mixture, and 30 mL of Hexanes was added, while white solids precipitated from the solution. The mixture was filtered, evaporated to dryness and purified by silica gel column chromatography (14-17% ethyl acetate in hexanes) to afford 0.4 g (50% yield) of **6c**. ^{1}H NMR (400MHz, $CDCl_3$) δ 7.31-7.33 (q, 2H), δ 6.84-6.87 (q, 2H), δ 6.61-6.68 (q, 1H), δ 5.57-5.61 (q, 1H), δ 5.09-5.12 (q, 1H), δ 4.07-4.10 (t, 2H), δ 3.30 (s, 2H), δ 2.97-3.00 (t, 2H), δ 2.49 (s, 3H), δ 1.46 (s, 9H); ESI-MS (expected: $[m+H]^+$= 292.2, obtained: $[m+Na]^+$= 314.2)

Synthesis of compound **6d**:

To a solution of acetone mixed with K_2CO_3 (4.97 g, 35.96 mmol) and 18-crown-6 (0.79 g, 2.99 mmol), 4-Hydroxybenzaldehyde (3.66 g, 29.97 mmol) was added and stirred for 5 min. To this mixture, 1-Bromohexadecane (10.98 g, 35.96 mmol) was added and stirred with reflux 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 9.0 g (90% yield) of **6d**. ^{1}H NMR (400MHz, $CDCl_3$) δ 9.88 (s, 1H), δ 7.81-7.84 (q, 2H), δ 6.98-7.00 (q, 2H), δ 4.02-4.05 (t, 2H), δ 1.79-1.83 (quint, 2H), δ 1.26-1.55 (m, 26H), δ 0.88-0.90 (t, 3H).

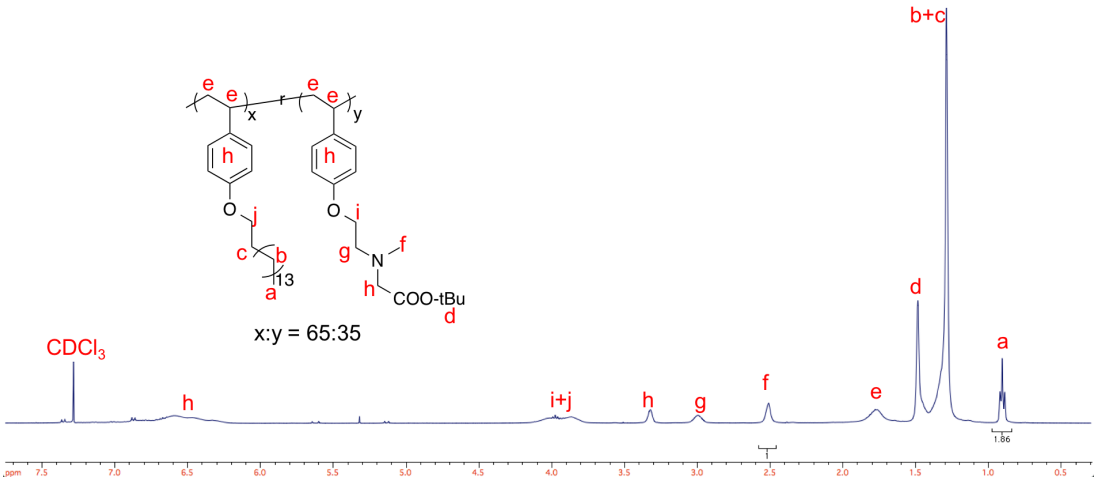
Synthesis of compound **6e**:

Methyltriphenylphosphonium bromide (7.69 g, 21.53 mmol) and Potassium tert-butoxide (2.48 g, 22.10 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 the bright yellow solution. **6d** (4.78 g, 14.37 mmol) was slowly added to the mixture. The reaction mixture was further stirred for 5 h. After the reaction, NaCl 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 (1-3% ethyl acetate in hexanes) to afford 3.8 g (80% yield) of **6e**. ^{1}H

NMR (400MHz, $CDCl_3$) δ 7.32-7.34 (q, 2H), δ 6.84-6.86 (q, 2H), δ 6.62-6.69 (q, 1H), δ 5.58-5.62 (q, 1H), δ 5.10-5.13 (q, 1H), δ 3.94-3.97 (t, 2H), δ 1.76-1.79 (quint, 2H), δ 1.26-1.54 (m, 26H), δ 0.87-0.90 (t, 3H).

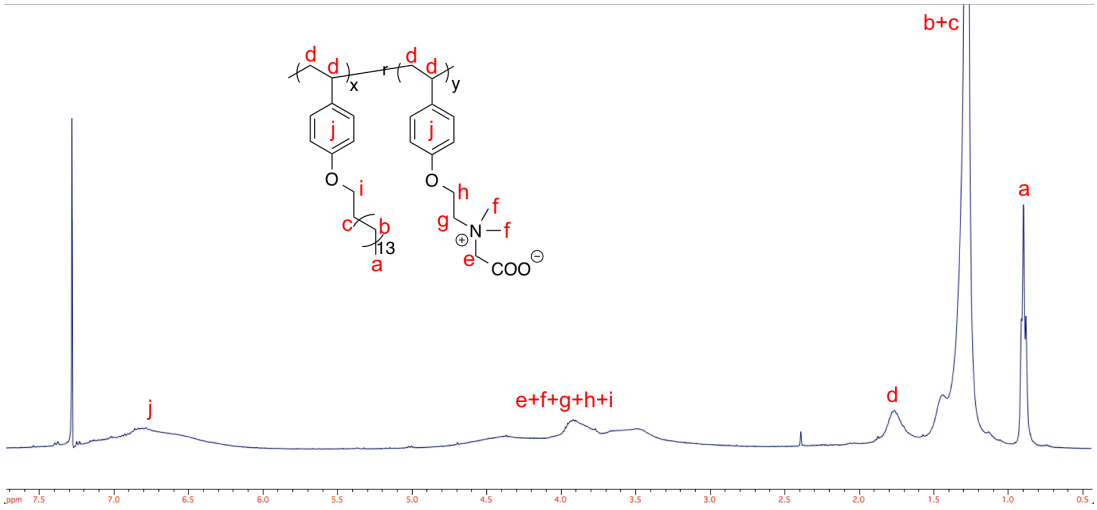
Synthesis of random co-polymer **6f**:

A mixture of the compound **6e** (150 mg, 0.45 mmol), **6c** (132 mg, 0.45 mmol) and *N*-*tert*-Butyl-*N*-(2-methyl-1-phenylpropyl)-*O*-(1-phenylethyl)hydroxylamine (NMP initiator, 5.9 mg, 0.018 mmol) were degassed by three freeze/thaw cycles, sealed under argon, and heated at 125 °C under argon for 24 h. After the reaction cool down to room temperature, the reaction mixture was dissolved in minimal amount of DCM, and precipitated 3 times in the MeOH. The precipitate was collected and dried under vacuum to yield 140 mg (50% yield) of **6f**. GPC (PMMA/THF): M_n= 20K Da, Đ=1.4;



Synthesis of random co-polymer **P6**:

DCM (2 mL) was added to dissolve the dried random co-polymer **6f** (130 mg). Trifluoroacetic acid (0.5 mL) was added to the reaction, and stirred for 12 h. The reaction mixture was evaporated and dried under vacuum. THF (2 mL) was then added to dissolve the solid polymers. Methyl iodide (0.2 mL) was added in the ice bath, and the reaction mixture was further stirred for 12 h. The solvent was evaporated, and the brown solids were dried under vacuum to afford the random co-polymer **P6**.



Dynamic light scattering (DLS)

The sizes of different polymer-based reverse micelles were measured by DLS. The polymers were dissolved in toluene, and one equivalents of aqueous NaOH (or H_2O) per carboxylate (or charge neutral functional group) unit were added to form the water pool inside the reverse micelles. The samples were sonicated until the reverse micelles were homogeneously dispersed. DLS measurements were carried out in a quartz cuvette. The particle sizes obtained for all reverse micelles are shown below based on an average of 3 correlations of 10 measurements each.

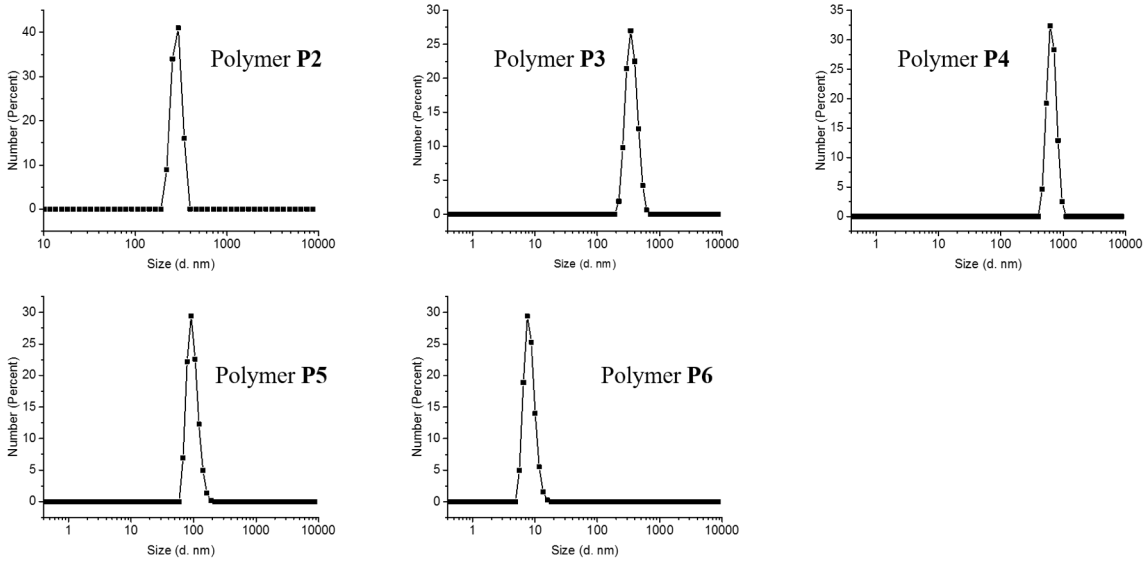


Figure S1. DLS of reverse micelles of polymers **P2-P6**.

Stability of reverse micelles

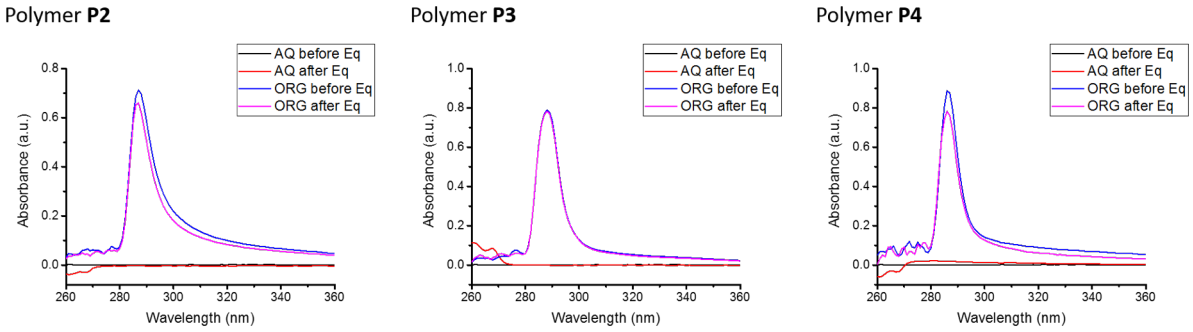


Figure S2. UV-Vis measurements with reverse micelles of **P2-P4** starting in toluene (ORG), before and after equilibration (Eq) with aqueous phase (AQ).

Stability of reverse micelles using DCM as organic solvent

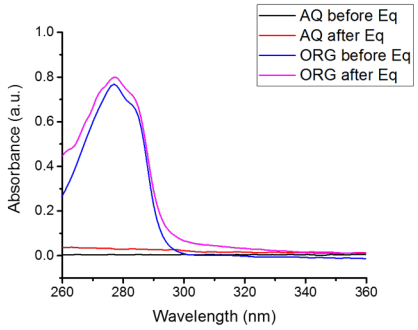


Figure S3. UV-Vis measurements with reverse micelles of **P1** starting in DCM (ORG), before and after equilibration (Eq) with aqueous phase (AQ).

Extraction selectivity of peptides

A mixture of peptides was extracted by reverse micelles of polymer **P2** at pH 7.0 to determine the extraction selectivity. After extraction, only the peptides with positive net charge were extracted to the organic phase. Peptides with negative net charge remained in the aqueous phase. Some of the positively charged peptide peaks seen in the aqueous phase is due to the low capacity of the reverse micelles. Identical results were seen for polymers **P3** and **P4**.

Table S1. Peptides used for determining the selectivity of extraction. ([a] calculated using the program available at http://pepcalc.com.)

Peak in Figures	Peptide	Sequence	Concentration (μM)	M_w	pI[a]	Net charge at pH 7.0
1	Bradykinin	RPPGFSPFR	0.1	1060.57	12.5	+
2	Kinetensin	IARRHPYFL	0.1	1172.67	11.1	+
3	Angiotensin I	DRVYIHPFHL	0.1	1269.68	7.7	+
4	Malantide	RTKRSGSVYEPLKI	0.1	1633.94	10.7	+
5	β-Amyloid 1-11	DAEFRHDSGYE	0.15	1325.54	4.1	-
6	Preproenkephalin	SSEVAGEGDGDSMGHEDLY	0.15	1954.76	3.6	-

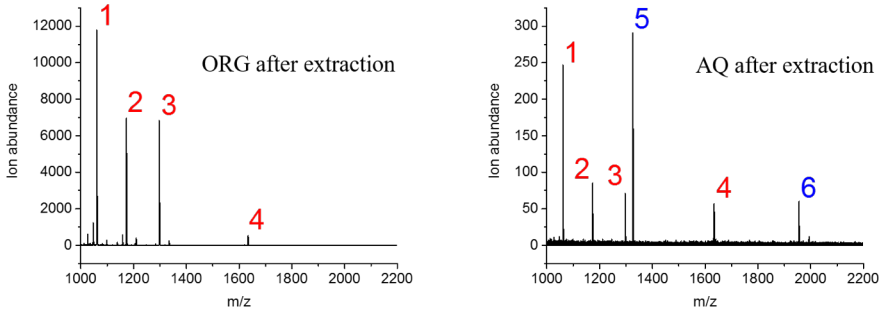


Figure S4. Example of MALDI mass spectra of organic phase (ORG) and aqueous phase (AQ) after extraction at pH 7.0 using reverse micelles of **P2**.

3D Structure of Bradykinin

Although we recognize that the peptides studied here are too short to have an observable secondary structure, we were interested in evaluating the possibility of studying secondary structures of peptides upon binding to polymers inside the reverse micelles. For this propose, we probed the peptides studied, bradykinin (9 amino acids). We noticed from circular dichroism (CD) and from computation peptide structure predictions (based on PEP-FOLD server, http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/) that bradykinin is quite unstructured.

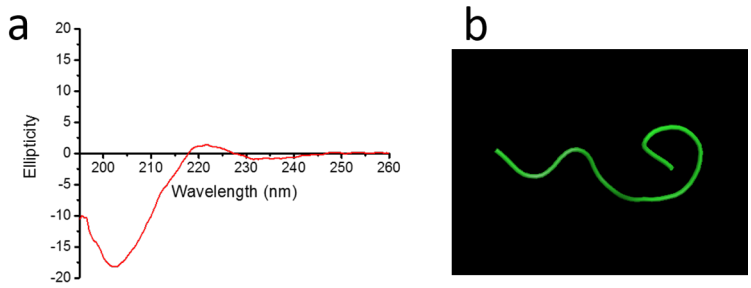


Figure S5. (a) CD spectrum of bradykinin in aqueous phase before extraction. (b) 3D structure of bradykinin predicted by PEP-FOLD Peptide Structure Prediction Server (http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD/).

Extraction capacity determination

Increasing concentrations of the peptide bradykinin was extracted using 100 µL of each polymer and the leftover peptide in the aqueous phase was mixed on a 1:1 volume ratio with ACN CHCA matrix solution, then analyzed by MALDI-MS. The extraction capacity is taken as the concentration of peptide at which a significant increase in peptide signal is seen remaining in the aqueous phase, suggesting that the reverse micelles are saturated and can no longer accommodate more peptides in the organic phase.

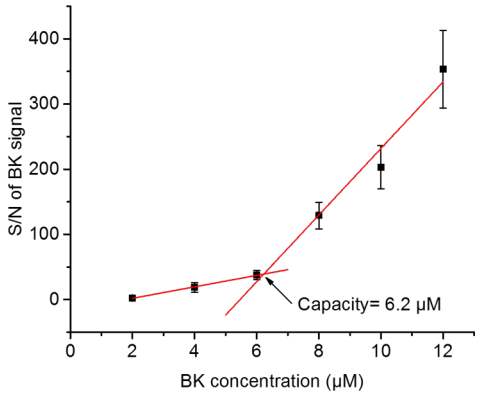


Figure S6. Example of peptide extraction capacity measurement using reverse micelles of homopolymer **P7** at pH 7.0.

Fluorescence microscopy

50 nM of TAMRA-labeled Bradykinin (TMR-BK) were prepared in 1 mL 50 mM Tris buffer. After extraction, all the TMR-BK were extracted in organic phase. The organic phase was dried by blowing N_2 gas. The dried residue of the mixture of polymer and the extracted peptides was re-dissolved in 10 µL of THF, giving a final TMR-BK concentration of 5 µM. For the control experiment, 5 µM unextracted TMR-BK was compared to all the extracted results.

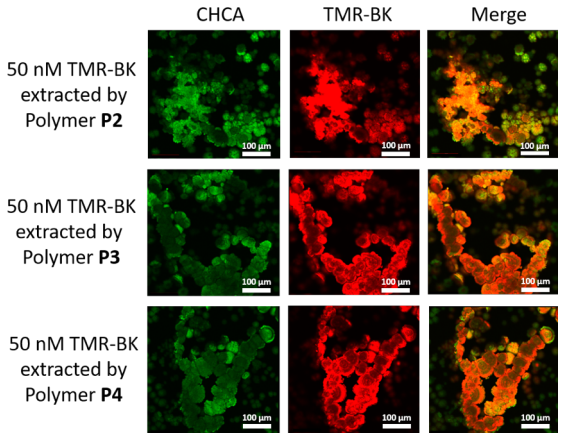


Figure S7. Fluorescence microscopy images showing the degree of clustering and co-localization of TMR-BK and CHCA matrix in extracted samples (50 nM) using **P2-P4**. Scale bar = 100 µm.

Structure of TMR-Bradykinin

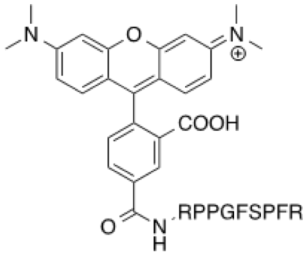


Chart S1. Structure of the TMR-Bradykinin.

Limit of detection analysis

To better understand the sensitivity of this method, we performed extractions for a series of bradykinin peptide concentration (from 1 fM to 1μM) using 100μL of 1.75×10^{-4} M of copolymer **P1**. Signal to noise (S/N) value was measured for peptide signal in the organic phase. We have found that we were able to detect as low as 10 pM of peptide using this experimental condition (Figure S8). Note that this detection limit is highly dependent on (i) the inherent sensitivity of MALDI instrument; (ii) the "hot spot" formation in the final sample.

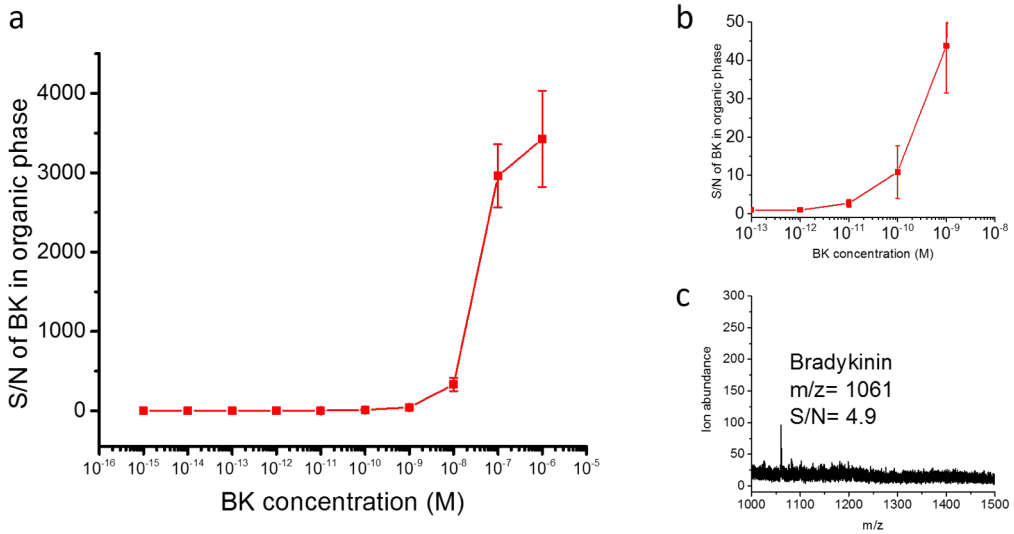


Figure S8. (a) Signal to noise ratio (S/N) of bradykinin signal in the organic phase after extraction using 1.75×10^{-4} M of copolymer P1. (b) Zoom-in region of (a) shows the detection limit is as low as 10 pM. (c) Exemplified spectrum of 10 pM of bradykinin detected in the organic phase after extraction.

References

[1] S. Basu, D. R. Vutukuri and S. Thayumanavan, *J. Am. Chem. Soc.,* 2005, **127**, 16794–16795.