

Supplementary Information

For

Construction of a versatile and functional nanoparticle platform derived from a helical diblock copolypeptide-based biomimetic polymer

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Experimental section:

Materials

Ethyl acetate, *n*-hexane, isopropyl alcohol, diethyl ether, acetone, sodium hydroxide (NaOH, $\geq 97\%$), sodium chloride ($\geq 99\%$), sodium bicarbonate ($\geq 99.7\%$), trifluoroacetic acid ($\geq 99\%$), magnesium sulfate (MgSO₄, anhydrous, $\geq 99.5\%$) *N,N*-dimethylformamide (DMF, anhydrous, $\geq 99.8\%$), γ -benzyl-L-glutamate ($\geq 99\%$), L-glutamic acid ($\geq 99\%$), propargyl alcohol ($\geq 99\%$), chlorotrimethylsilane ($\geq 99\%$), bis(trichloromethyl) carbonate ($\geq 98\%$), *n*-hexylamine ($\geq 99\%$), 2,2-dimethoxy-2-phenylacetophenone (DMPA, $\geq 99\%$), cysteamine hydrochloride ($\geq 98\%$), 3-mercaptopropionic acid ($\geq 99\%$), pyrene ($\geq 99\%$) were purchased from Sigma-Aldrich company (USA). All chemicals were used without further purification, unless otherwise noted. Nanopure water (18.2 M Ω -cm) was acquired by means of a Milli-Q water filtration system, Millipore Corp. (St. Charles, MO). Dialysis membrane tubing with a molecular weight cut off (MWCO) of 6-8 kDa was purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, CA) and soaked for 5 min in nanopure water at room temperature before usage.

Instrumentation

¹H and ¹³C NMR spectra were recorded on a Varian Inova 300 spectrometer interfaced to a UNIX computer using VnmrJ software. Chemical shifts were referenced to the solvent resonance signals. Attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR) spectra were recorded on an IR Prestige 21 system (Shimadzu Corp., Kyoto, Japan) and analyzed using IRsolution v. 1.40 software. Circular dichroism (CD) spectra were recorded on a Chirascan CD spectrometer from Applied Photophysics, Ltd. (Leatherhead, UK) equipped with a 150 watt xenon arc lamp. The polymer solution samples for CD measurements were prepared at a concentration of 0.1 mg/mL in nanopure water. The samples were placed in a quartz cell with a path length of 1.0 cm and analyzed between 180 and 280 nm with a wavelength step of 1.0 nm. Measurements were analyzed using Pro-Data Version 5 software. Critical micelle concentration determinations were made by pyrene fluorescence measurements that were conducted on a RF-5301PC spectrofluorophotometer system (Shimadzu Corp., Kyoto, Japan) and analyzed using Panorama Fluorescence v. 2.1 software. Fluorescence emission spectra ranging from 360 to 450 nm of the sample solutions were recorded using an excitation wavelength of 334 nm at room temperature. All the measurements were repeated three times.

N,N-Dimethylformamide-based gel permeation chromatography (DMF GPC) was conducted on a Waters Chromatography, Inc. (Milford, MA) system equipped with an isocratic pump model 1515, a differential refractometer model 2414 and a four-column set of 5 μ m Guard (50 \times 7.8 mm), Styragel HR 4 5 μ m DMF (300 \times 7.8 mm), Styragel HR 4E 5 μ m DMF (300 \times 7.8 mm), and Styragel HR 2 5 μ m DMF (300 \times 7.8 mm). The

system was equilibrated at 70 °C in pre-filtered DMF (containing 0.05 mol/L LiBr), which served as the polymer solvent and eluent (flow rate set to 1.00 mL/min). Polymer solutions were prepared at concentrations of ca. 3.0 mg/mL and an injection volume of 0.2 mL was used. Data collection and analysis was performed with Empower Pro software. The system was calibrated with polystyrene standards (Polymer Laboratories, Amherst, MA) ranging from 1480 to 1233000 Da.

Thermogravimetric analysis (TGA) was performed under argon atmosphere using a Mettler-Toledo model TGA/SDTA851^e (Mettler-Toledo, Inc., Columbus, OH), with a heating rate of 10 °C/min. Measurements were analyzed using Mettler-Toledo STAR^e v. 7.01 software. Glass transition temperatures (T_g) were measured by differential scanning calorimetry (DSC) on a Mettler-Toledo DSC822^e (Mettler-Toledo, Inc., Columbus, OH), with a heating rate of 5 °C/min. Measurements were analyzed using Mettler-Toledo STAR^e v. 7.01 software. The T_g was taken as the midpoint of the inflection tangent, upon the second heating scan.

Transmission electron microscopy (TEM) images were collected on a JEOL 1200 EX (Tokyo, Japan) operating at 100 kV and micrographs were recorded at calibrated magnifications using a SLA-15C CCD camera. Samples for TEM measurements were prepared as follows: 10 μ L of the dilute polymer solution was deposited onto a carbon-coated copper grid, and after 1 min, the excess of the solution was quickly wicked away by a piece of filter paper. The samples were then negatively stained with 1 wt % phosphotungstic acid (PTA) aqueous solution. After 30 s, the excess staining solution was quickly wicked away by a piece of filter paper and the samples were left to dry in vacuum overnight.

Dynamic light scattering (DLS) measurements were conducted using a Delsa Nano C from Beckman Coulter, Inc. (Fullerton, CA) equipped with a laser diode operating at 658 nm. Scattered light was detected at 165° angle and analyzed using a log correlator over 70 accumulations for a 0.5 mL of sample in a glass size cell (0.9 mL capacity). The photomultiplier aperture and the attenuator were automatically adjusted to obtain a photon counting rate of ca. 10 kcps. The calculation of the particle size distribution and distribution averages was performed using CONTIN particle size distribution analysis routines using Delsa Nano 2.31 software. The peak averages of histograms from intensity, volume and number distributions out of 70 accumulations were reported as the average diameter of the particles. All determinations were repeated 10 times. The zeta potential values of the nanoparticles were determined by Delsa Nano C particle analyzer (Beckman Coulter, Fullerton, CA) equipped with a 30 mW dual laser diode (658 nm). The zeta potential of the particles in suspension was obtained by measuring the electrophoretic movement of charged particles under an applied electric field. Scattered light was detected at a 30° angle at 25 °C. The zeta potential was measured at five regions in the flow cell and a weighted mean was calculated. These five measurements were used to correct for electroosmotic flow that was induced in the cell due to the surface charge of the cell wall. All determinations were repeated 9 times.

Synthesis of γ -benzyl-L-glutamate *N*-carboxyanhydride (BLG NCA) monomer 1

The BLG NCA monomer 1 was prepared following the literature method.¹ In a 500 mL three-necked round bottom flask equipped with a magnetic stirrer, a septum and a pipet for nitrogen inlet and a condenser with a tubing connector that allows outlet flow through a base solution (NaOH aqueous solution), BLG NCA monomer 1 was synthesized from γ -benzyl-L-glutamate (10.0 g, 42.1 mmol, 3 equiv) and bis(trichloromethyl) carbonate (4.2 g, 14 mmol, 1 equiv) in 300 mL ethyl acetate at 65 °C for 4 h. After cooling to 5 °C, the crude product was extracted with 100 mL 5 °C nanopure water and 100 mL 5 °C 0.5 wt % sodium bicarbonate aqueous solution. The organic layer was then dried over MgSO₄, filtrated and concentrated. The resulting solid was purified by recrystallization three times with ethyl acetate/*n*-hexane 1:1 (v/v), and dried in vacuum to obtain a white crystal (2.6 g, yield: 71%). The product was stored in -20 °C freezer under nitrogen atmosphere. ¹H NMR (300 MHz, CDCl₃, ppm): δ 2.21 (m, 2H, CH₂CH₂COOCH₂), 2.60 (t, *J* = 6.9 Hz, 2H, CH₂CH₂COOCH₂), 4.38 (dt, *J* = 1.0 Hz, *J* = 5.4 Hz, 1H, COCHNH), 5.14 (s, 2H, COOCH₂), 6.42-6.64 (br, 1H, COCHNH), 7.36 (m, 5H, ArH). ¹³C NMR (75 MHz, CDCl₃, ppm): δ 27.1, 30.1, 57.1, 67.3, 128.6, 128.8, 128.9, 135.3, 151.9, 169.5, 172.6. FTIR (cm⁻¹): 3449-2725, 3331, 3251, 2931, 1859, 1773, 1703, 1250, 1185, 1112, 930. HRMS: calculated [M-H]⁻ for C₁₃H₁₃NO₅: 262.0715, found: 262.0710.

Synthesis of γ -propargyl-L-glutamate hydrochloride

In a 1 L round bottom flask, L-glutamic acid (12.0 g, 81.6 mmol) was suspended in propargyl alcohol (400 mL) under nitrogen for 1 h at room temperature.² Chlorotrimethylsilane (28.5 mL, 225 mmol) was added into the suspension dropwise over 1 h under nitrogen atmosphere at room temperature. The resulting solution was stirred at room temperature over 36 h and then precipitated into diethyl ether (1.5 L) giving a white solid. The obtained solid was purified by dissolving into isopropyl alcohol (200 mL), heating to reflux, and precipitating into diethyl ether (1.5 L). The product was filtrated, washed with diethyl ether (200 mL \times 3) and dried in vacuum (12.4 g, yield: 68 %). ¹H NMR (300 MHz, CD₃OD, ppm): δ 2.21 (m, 2H, CHCH₂CH₂), 2.65 (m, 2H, CH₂CH₂CO), 2.95 (t, *J* = 2.5 Hz, 1H, C \equiv CH), 4.06 (t, *J* = 6.7 Hz, 1H, NHCHCO), 4.74 (d, *J* = 2.5 Hz, 2H, COOCH₂). ¹³C NMR (75 MHz, CD₃OD, ppm): δ 26.7, 30.5, 53.2, 53.3, 76.6, 78.8, 171.5, 173.0. FTIR (cm⁻¹): 3337-2154, 3292, 3142, 2906, 2124, 1963, 1737, 1720, 1502, 1489, 1220, 1172, 995, 837. HRMS: calculated [M-Cl]⁺ for C₈H₁₂NO₄Cl: 186.0761, found: 186.0765.

Synthesis of γ -propargyl-L-glutamate *N*-carboxyanhydride (PLG NCA) monomer 2

In a 250 mL three-necked round bottom flask equipped with a magnetic stirrer, a septum and a pipet for nitrogen inlet and a condenser with a tubing connector that allows outlet flow through a base solution (NaOH aqueous solution), PLG NCA monomer **2** was synthesized from γ -propargyl-L-glutamate hydrochloride (4.0 g, 18 mmol, 3 equiv) and bis(trichloromethyl) carbonate (1.8 g, 6.0 mmol, 1 equiv) in 100 mL ethyl acetate at 65 °C for 6 h.³ After cooling to 5 °C, the crude product was extracted with 100 mL 5 °C nanopure water, 100 mL 5 °C saturated sodium bicarbonate aqueous solution, and 100 mL 5 °C saturated sodium chloride aqueous solution. The organic layer was dried over MgSO₄, filtrated and concentrated. The resulting yellow viscous oil was purified by three times precipitation with ethyl acetate/*n*-hexane 1:5 (v/v), and dried in vacuum to obtain viscous oil (0.9 g, yield: 71%). The product was stored in -20 °C freezer under nitrogen atmosphere. ¹H NMR (300 MHz, CDCl₃, ppm): δ 2.21 (m, 2H, CH₂CH₂COOCH₂), 2.52 (t, *J* = 2.5 Hz, 1H, C \equiv CH), 2.60 (t, *J* = 7.1 Hz, 2H, CH₂CH₂COOCH₂), 4.45 (dt, *J* = 5.8 Hz, *J* = 1.0 Hz, 1H, COCHNH), 4.70 (d, *J* = 2.5 Hz, 2H, COOCH₂), 7.00-7.06 (br, 1H, COCHNH). ¹³C NMR (75 MHz, CDCl₃, ppm): δ 26.7, 29.4, 52.6, 56.8, 75.5, 77.2, 152.3, 169.5, 171.8. FTIR (cm⁻¹): 3487-3030, 3284, 2945, 2131, 1854, 1776, 1732, 1163, 1103, 918, 756. HRMS: calculated [M-H] for C₉H₉NO₅: 210.0402, found: 210.0406.

Synthesis of diblock copolypeptide, poly(γ -benzyl-L-glutamate)-*block*-poly(γ -propargyl-L-glutamate) (PBLG-*b*-PPLG) **3, initiated by *n*-hexylamine**

Diblock copolypeptide PBLG-*b*-PPLG **3** was obtained by sequential addition of BLG NCA **1** and PLG NCA **2** monomers into the reaction solution with the method of nitrogen flow accelerated ring-opening polymerization (ROP).⁴ Into a 25 mL flame-dried Schlenk flask equipped with a magnetic stirrer, *n*-hexylamine (10.2 mg, 0.101 mmol, 1 equiv) was added into a solution of BLG NCA **1** (797.6 mg, 3.030 mmol, 30 equiv) in 14.0 mL anhydrous DMF solution. The reaction mixture was stirred at a stir rate of 400 rpm under continuous nitrogen flow (100 mL/min) at room temperature. The Schlenk flask was capped with a rubber stopper with a needle outlet connected with a drying tube. FTIR was used to monitor the polymerization and more than 99% of BLG NCA **1** monomer was consumed in 3.5 h. PLG NCA **2** monomer (639.8 mg, 3.030 mmol, 30 equiv) in 4.0 mL anhydrous DMF was transferred into the reaction solution by syringe and was consumed greater than 99% over another 12 h. The resulting reaction solution was precipitated into diethyl ether under vigorous stirring and a white powder was obtained after centrifuging and drying in vacuum at room temperature (1003.8 mg, yield: 85%). ¹H NMR (300 MHz, TFA-D, ppm): δ 0.71-0.82 (br, 3H, CH₃), 1.75-2.31 (br, 120H, CH₂CH₂COOCH₂), 2.32-2.68 (br, 30H, C \equiv CH, br, 120H, CH₂CH₂COOCH₂), 4.50-4.77 (br, 60H, COCHNH; br, 60H, COOCH₂), 4.93-5.15 (br, 60H, COOCH₂), 7.06-7.30 (br, 150H, ArH). ¹³C NMR (75 MHz, TFA-D, ppm): δ 26.5, 29.6, 29.7, 53.2, 53.3, 68.3, 75.0, 75.2, 127.8, 128.2, 128.4, 134.0, 173.3, 175.1, 175.7. FTIR (cm⁻¹): 3418-3127, 3065, 3034,

2941, 2126, 1730, 1649, 1545, 1159, 1121, 991, 696. GPC: $M_n = 38.5$ kDa, PDI = 1.08.^a DSC: $T_g = 22$ °C, $T_g = 79$ °C. TGA in N₂: 25-240 °C, 3% mass loss; 240-447 °C, 61% mass loss; 447-500 °C, 2% mass loss; 34% mass remaining above 500 °C.

Synthesis of positively-charged diblock copolypeptide 4 via thiol-yne click chemistry between PBLG₃₀-*b*-PPLG₃₀ with cysteamine hydrochloride

Diblock copolypeptide PBLG₃₀-*b*-PPLG₃₀ **3** (200.0 mg, 0.01709 mmol, 1 equiv) and cysteamine hydrochloride (1165.0 mg, 10.254 mmol, 600 equiv) were added into 6.0 mL anhydrous DMF in a 20 mL vial. DMPA (264.0 mg, 1.025 mmol, 60 equiv) was added into the reaction solution after both the solids were dissolved. The reaction solution was then bubbled by nitrogen flow for half an hour, and placed under UV light with a wavelength of 365 nm for 2 h. The crude product was precipitated into acetone and centrifuged to get a white solid. The white solid was then dissolved into 5.0 mL trifluoroacetic acid and the resulting solution was added into 20 mL nanopure water. The mixture was transferred into a presoaked dialysis membrane tubing (MWCO 6-8 kDa), dialyzed against nanopure water for another 3 days, and later lyophilized to get the final product (256.4 mg, yield: 81%). ¹H NMR (300 MHz, TFA-D, ppm): δ 0.74-0.85 (br, 3H, CH₃), 1.80-2.35 (br, 120H, CH₂CH₂COOCH₂), 2.35-2.73 (br, 120H, CH₂CH₂COOCH₂), 2.73-2.86 (br, 30H, SCHCH₂S), 2.86-3.20 (br, 60H, SCHCH₂S; br, 120H, SCH₂CH₂NH₃), 3.30-3.54 (br, 120H, SCH₂CH₂NH₃), 4.10-4.53 (br, 30H, COCHNH; br, 30H, COOCH₂CH), 4.55-4.78 (br, 30H, COCHNH; br, 30H, COOCH₂CH), 4.95-5.25 (br, 60H, COOCH₂Ar), 6.86-7.11 (br, 180H, CH₂CH₂NH₃), 7.11-7.34 (br, 150H, ArH). ¹³C NMR (75 MHz, TFA-D, ppm): δ 26.6, 28.2, 29.7, 29.8, 33.8, 39.4, 39.8, 44.9, 53.2, 66.6, 68.4, 127.9, 128.3, 128.5, 134.1, 173.3, 175.5, 175.8. FTIR (cm⁻¹): 3720-2181, 3288, 3034, 2936, 2129, 1730, 1672, 1651, 1547, 1171, 1126, 835, 799, 721. DSC: $T_g = 29$ °C, $T_g = 70$ °C. TGA in N₂: 25-155 °C, 2% mass loss; 155-242 °C, 20% mass loss; 242-424 °C, 52% mass loss; 424-500 °C, 2% mass loss; 24% mass remaining above 500 °C.

Synthesis of negatively-charged diblock copolypeptide 5 via thiol-yne click chemistry between PBLG₃₀-*b*-PPLG₃₀ with 3-mercaptopropionic acid

Diblock copolypeptide PBLG₃₀-*b*-PPLG₃₀ **3** (200.0 mg, 0.01709 mmol, 1 equiv) and 3-mercaptopropionic acid (1088.3 mg, 10.254 mmol, 600 equiv) into 6.0 ml anhydrous DMF in a 20 ml vial. DMPA (264.0 mg, 1.025 mmol, 60 equiv) was added into the reaction solution after both the solids were dissolved. The reaction solution was then bubbled by nitrogen flow for half an hour, and placed under UV light (365 nm) for 2 h. The crude product was precipitated into diethyl ether three times, centrifuged and dried

^a The molecular weight (M_n) calculated by GPC was higher than that by ¹H NMR, which is attributed to the differences in chemical structures and properties between polystyrene standards and the polypeptides. Therefore, the M_n values by NMR were considered to be more accurate and are reported in the manuscript text. GPC was used primarily for determination of PDI.

in vacuum to get the final product (219.3 mg, yield: 71%). ^1H NMR (300 MHz, TFA-D, ppm): δ 0.72-0.82 (br, 3H, CH_3), 1.65-2.32 (br, 120H, $\text{CH}_2\text{CH}_2\text{COOCH}_2$), 2.33-2.66 (br, 120H, $\text{CH}_2\text{CH}_2\text{COOCH}_2$), 2.67-2.80 (br, 120H, $\text{SCH}_2\text{CH}_2\text{COOH}$), 2.80-2.99 (br, 60H, SCHCH_2S ; br, 120H, $\text{SCH}_2\text{CH}_2\text{COOH}$), 3.08-3.30 (br, 30H, SCHCH_2S), 4.16-4.52 (br, 30H, COCHNH ; br, 30H, COOCH_2CH), 4.55-4.85 (br, 30H, COCHNH ; br, 30H, COOCH_2CH), 4.92-5.22 (br, 60H, COOCH_2Ar), 7.03-7.35 (br, 150H, ArH). ^{13}C NMR (75 MHz, TFA-D, ppm): δ 25.6, 26.5, 26.9, 29.1, 29.7, 31.3, 33.0, 44.5, 53.1, 65.9, 68.3, 127.8, 128.3, 128.4, 134.0, 173.3, 175.7, 179.4. FTIR (cm^{-1}): 3696-2155, 3285, 3034, 2928, 1728, 1649, 1547, 1242, 1163, 1123, 968, 799, 696. DSC: $T_g = 7\text{ }^\circ\text{C}$, $T_g = 63\text{ }^\circ\text{C}$. TGA in N_2 : 25-207 $^\circ\text{C}$, 1% mass loss; 207-340 $^\circ\text{C}$, 69% mass loss; 340-500 $^\circ\text{C}$, 6% mass loss; 24% mass remaining above 500 $^\circ\text{C}$.

Preparation of cationic nanoparticle 6 from self-assembly of positively-charged diblock copolypeptide 4 by direct re-suspension into nanopure water

The positively-charged diblock copolypeptide **4** (1.0 mg) was suspended into nanopure water (1.0 mL), followed by sonication for 10 min at room temperature to obtain a clear aqueous solution of **4** at a concentration of 1.0 mg/mL.

Preparation of anionic nanoparticle 7 from self-assembly of negatively-charged diblock copolypeptide 5 by nanoprecipitation method

The negatively-charged diblock copolypeptide **5** was dissolved into anhydrous DMF to obtain a polymer solution with a concentration of 3.0 mg/mL. The polymer solution (1.0 mL) was then added into nanopure water (4.0 mL) *via* a syringe pump at an addition rate of 0.2 mL/min. The mixture was then transferred into a presoaked dialysis membrane tubing (MWCO 6-8 kDa), and dialyzed against nanopure water for 24 h to remove DMF at room temperature. The final concentration of **5** was adjusted to 0.5 mg/mL by addition of nanopure water.

Determination of critical micelle concentrations (CMCs) for positively-charged 4 and negatively-charged 5 diblock copolypeptides in nanopure water

The CMCs for positively-charged **4** and negatively-charged **5** diblock copolypeptides in nanopure water were determined by using pyrene as the fluorescent probe following the protocol in literature.⁵ The sample solution was prepared by mixing 1.0 mL of a polypeptide aqueous solution with 1.0 mL of a pyrene aqueous stock solution (6.0×10^{-7} mol/L). Samples with polymer concentrations ranging from 0.01 – 1.0 g/L for **4** and 0.01 – 0.25 g/L for **5** were prepared. All the sample solutions were then stored at room temperature overnight to equilibrate the pyrene and the micelles. The fluorescence measurement was conducted at room temperature, in which the pyrene was excited at 334 nm and its emission spectrum was recorded at 373 and 384 nm, corresponding to

the first and third vibrational peaks, respectively. All the measurements were repeated three times and the ratios of intensities of the first (I_I) and third (I_{III}) peaks were plotted against the concentrations of polypeptides in the sample solutions. The CMC was taken as the intersection of the tangent to the curve at the inflection with tangent through the points at high polymer concentration.

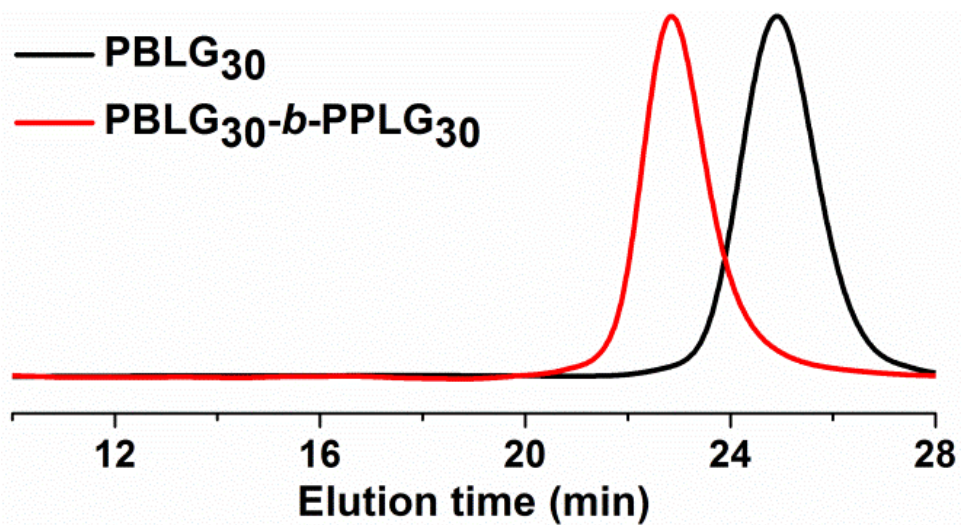


Fig. S1 GPC curves of homopolypeptide PBLG₃₀ at $M_n = 6.7$ kDa (from ^1H NMR) and PDI = 1.10 (from GPC, black line) and diblock copolypeptide PBLG₃₀-*b*-PPLG₃₀ **3** at $M_n = 11.7$ kDa (from ^1H NMR) and PDI = 1.08 (from GPC, red line) *via* one-pot sequential ROPs with the nitrogen flow method.

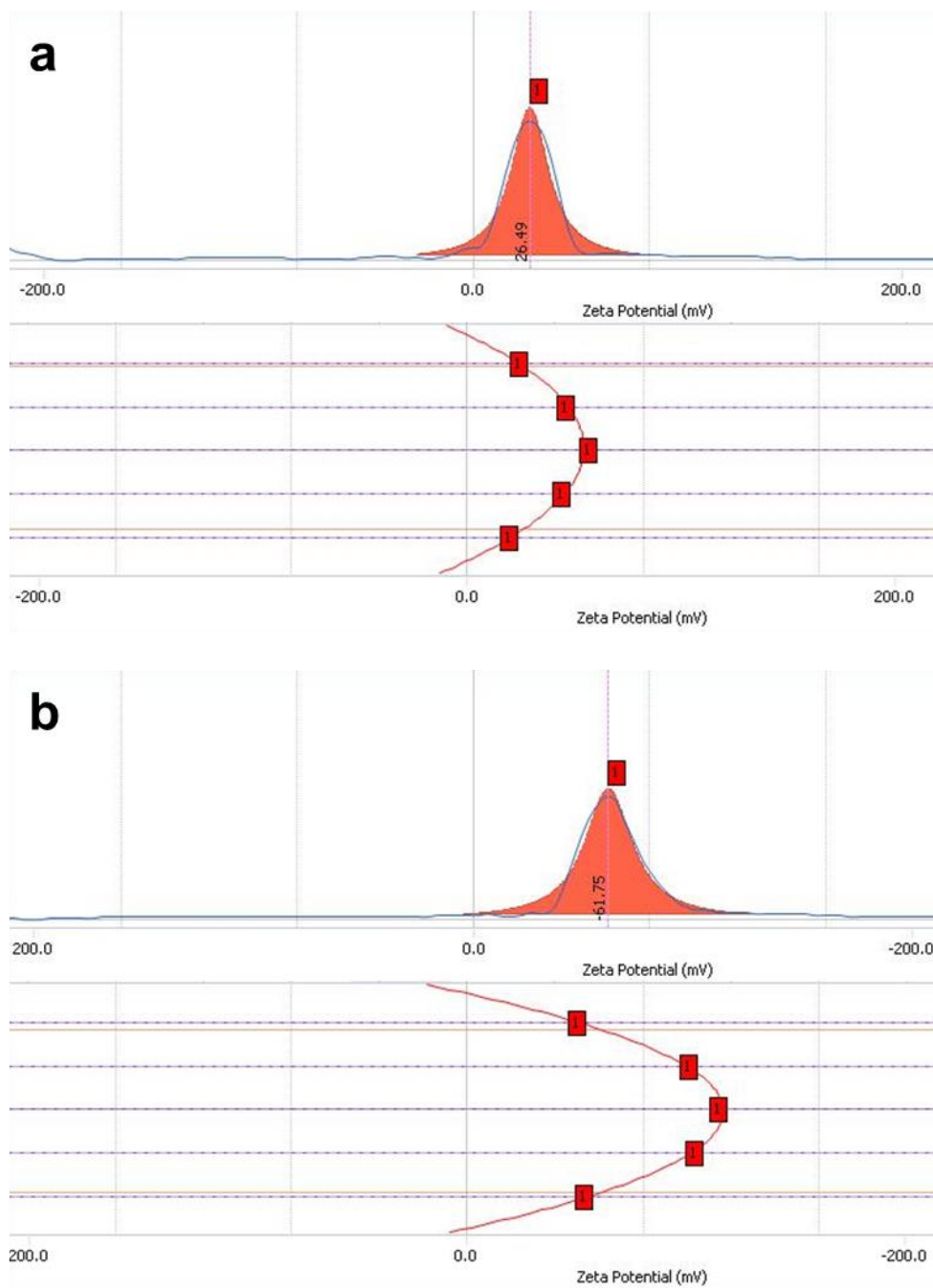


Fig. S2 Zeta potential Lorentzian peak and top view of zeta potential distribution across the cell during electrophoretic light scattering measurement of nanoparticles 6 (a) and 7 (b).

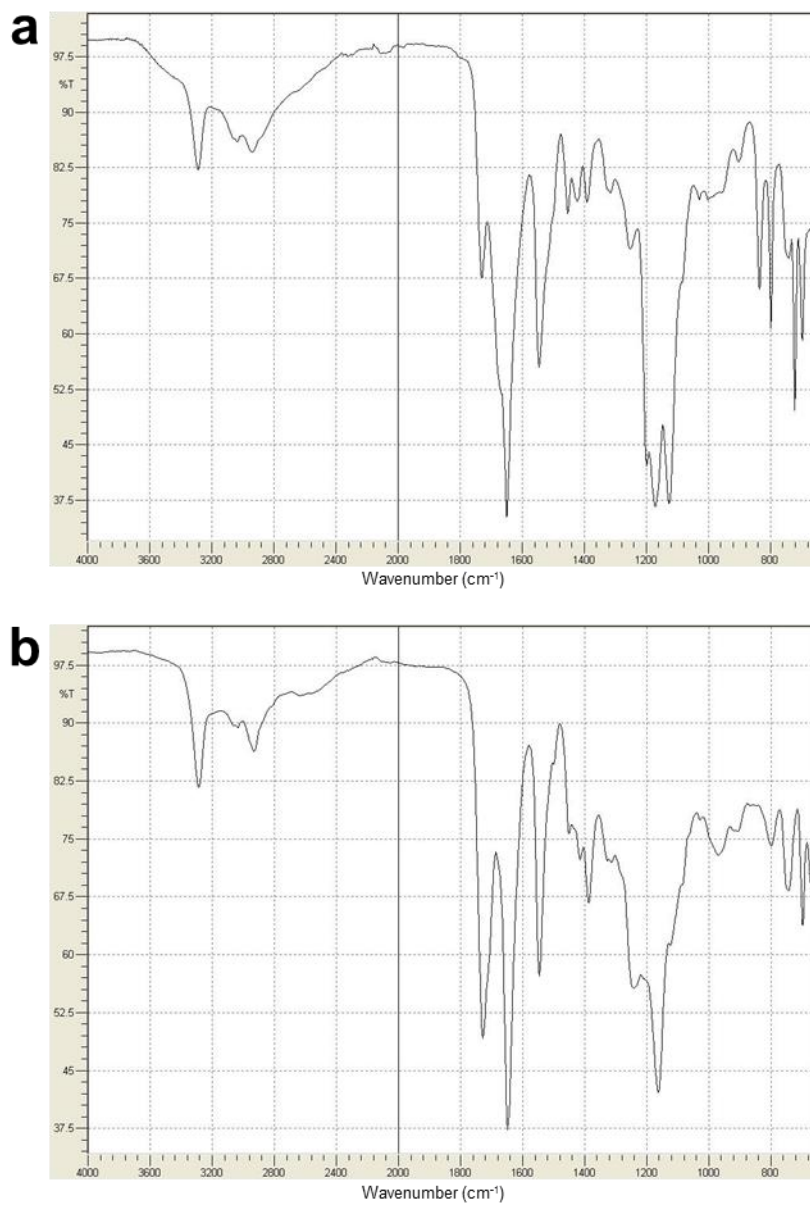


Fig. S3 FTIR spectra of charged diblock copolypeptides **4** (a) and **5** (b) in the solid state.

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NMR spectra:

