Electronic Supplementary Information

Innovative well-defined primary amine-based polyacrylates for plasmid DNA complexation

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1.0 General

1.1 Reagents

Acryloyl chloride (97%, Aldrich), triethylamine (TEA, 99%, Aldrich), *N*-boc-ethanolamine (98%, Aldrich), dichloromethane (DCM, > 99%, Aldrich), trifluoroacetic acid (TFA, 99%, Acros), ethyl acetate (99.8%, Aldrich), *n*-hexane (97%, Aldrich), *N*,*N*-dimethylformamide (DMF, 99.8%, Aldrich), acetone (99.8%, Aldrich), diethyl ether (technical grade, Aldrich), methanol (Prolabo, 99.8%), 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid (CTA1, > 97%, Aldrich), 4-cyano-4-(dodecylthiocarbonothioylthio)pentanoic acid (CTA2, > 97%, Aldrich) and silica gel for column chromatography (Kieselgel 60, 230-400 mesh Merck) were used as received. 2,2'-Azobis(2-methylpropionitrile) (AIBN, 98%, Aldrich) was recrystallized in methanol prior to use. 2-(Dodecylthiocarbonothioylthio)-2-methylpropionic acid (CTA3) was synthesized following a reported procedure.[1] Pure water was obtained from a Millipore Direct Q system and had a conductivity of 18.2 MΩ.cm at 25°C. Ethidium bromide (Sigma, Molecular Biology), plasmid DNA (pDNA, pBR322, 0.5µg.µL^{-1}, EuroMEDEX), Tris acetate EDTA Buffer (TAE 50x, EuroMEDEX), Agarose (EuroMEDEX) were used as received.

1.2 Analyses

Nuclear Magnetic Resonance spectroscopy (NMR). NMR spectra were recorded on a Bruker AC-400 spectrometer for ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz). Chemical shifts are reported in ppm relative to deuterated solvent resonances.

Size Exclusion Chromatography (SEC). Polymers were characterized on a SEC system operating in DMF eluent at 60°C fitted with a guard column (PL Gel 5µm, 1×50×7.5 mm) and two Polymer Laboratories PL Mixed D columns (2×300×7.5 mm), a Waters 410 differential refractometer and a Waters 481 UV detector operating at 309 nm. The instrument

[1] Lai, J. T.; Filla, D.; Shea, R. *Macromolecules,* **2002**, *35*, 6754

operated at a flow rate of 1.0 mL.min^{-1} and was calibrated with narrow linear polystyrene (PS) standards ranging in molecular weights from 580 g.mol^{-1} to 460 000 g.mol^{-1}. Molecular weights and polydispersities ($Đ_M$) were calculated using Waters EMPOWER software.

Fourier Transform Infra-Red (FT-IR) spectroscopy. FT-IR spectra of copolymers were recorded using a ThermoElectron Corp. spectrometer operating with an attenuated total reflection (ATR solid) gate. Spectra were analyzed with OMNIC software.

High Resolution Mass (HR-MS) Spectrometry. HR-MS analysis was measured by a Water Micromass GCT Premier.

Zeta potential (ξ) measurements. ξ were performed using a dynamic light scattering instrument (Zetasizer Nano ZS, Malvern Instrument) equipped with a He-Ne laser beam at 658 nm. The average value was calculated from four replicate measurements.

2.0 Synthesis of N-(*tert*-butoxycarbonyl)aminoethyl acrylate (*t*-BocAEA)

The Boc-AEA has been synthesized using a reported procedure.[2] *N*-Boc-ethanolamine (8.06 g, 5.00x10^{-2} mol), triethylamine (TEA, 8.40 mL, 6.04x10^{-2} mol) and dichloromethane (DCM, 20.00 mL) were added to a round-bottom flask. The resulting solution was then stirred and cooled 0°C under argon using an ice bath in 15 min. Acryloyl chloride (4.10 mL, 5.16x10^{-2} mol) was subsequently added dropwise under stirring while keeping the temperature at 0^{o}C. After completing the addition of acryloyl chloride, the reaction mixture was stirred at 0^{o}C for 2 h and then at room temperature for 24 h (Scheme 1). The solution was then filtered and the white solid was washed with DCM. Solvent of the filtrate was removed under vacuum. The resulting solid was purified by column chromatography on silica gel using ethyl acetate (100%) as the eluent. The solvent was then removed under vacuum. The resulting solid obtained was recrystallized in *n*-hexane and then dried under vacuum to give a white solid

[2] Rodrigues, D. C.; Bader, R. A.; Hasenwinkel, J. M. *Polymer*, **2011**, *52*, 2505

(6.72 g). Yield: 62%. HR-MS: ($[M+H^+]_{cal}$= 216.1230 g.mol^{-1}); $[M+H^+]_{exp}$= 216.1236 g.mol^{-1}. FT-IR (ν, cm^{-1}): ν_{NH}= 3378, $\nu_{(C=O,\ ester)}$ = 1720, $\nu_{(C=C)}$= 1637, $\nu_{(C=O,\ carbamate)}$= 1510. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.40 (s, -C(C**H**$_3$)$_3$, 9H), 3.39 (q,-COOCH$_2$-C**H**$_2$-, 2H), 4.16 (t, -COOC**H**$_2$-CH$_2$-, 2H), 4.88 (s, -N**H**-, 1H), 5.79 (dd, C**H**$_2$=CH-, *J* = 10.4, 1.7 Hz, 1H), 6.04 (dd, CH$_2$=C**H**-, *J* = 17.2, 10.4 Hz, 1H**),** 6.35 (dd, C**H**$_2$=CH-, *J* = 17.2, 1.7 Hz, 1H). ^{13}C NMR (100.62 MHz, $CDCl_3$), δ (ppm): 28.34 (-C(**C**H$_3$)$_3$), 39.65 (-COOCH$_2$-**C**H$_2$-), 63.77 (-COO**C**H$_2$-CH$_2$-), 79.53 (-**C**(CH$_3$)$_3$), 128.08 (CH$_2$=**C**H-), 131.20 (**C**H$_2$=CH-,), 155.78 (-NH-**C**OO-), 166.03 (-**C**OOCH$_2$-).

3.0 RAFT polymerization of t-BocAEA

3.1 General procedure

In a typical RAFT polymerization procedure, in a round-bottom flask along equipped with a magnetic stir bar were charged *t*-BocAEA (1.17 g, 5.44x10^{-3} mol), 4-cyano-4-(dodecylthiocarbonothioylthio)pentanoic acid (CTA2, 0.044 g, 1.09x10^{-4} mol), AIBN (3.6 mg, 2.20x10^{-5} mol) and *N*,*N*-dimethylformamide (DMF, 3.00 mL). The mixture was deoxygenated by bubbling argon for 30 min. The solution was then immersed in an oil bath thermostated at 90^{o}C to allow the polymerization to occur. Samples were removed periodically using a syringe to perform size exclusion chromatography (SEC) analysis and to monitor monomer conversion by ^{1}H NMR spectroscopy. The polymerization was quenched after 120 min by rapid cooling and exposure of the polymerization solution to air. The DMF was removed using a rotary evaporator. The resulting product was then dissolved in acetone and precipitated in cold *n*-hexane, filtered and dried under vacuum. The final product was obtained as a yellow powder with $M_{n,NMR}$ = 8400 g.mol^{-1} ($DP_{n,NMR}$ = 37), $M_{n,SEC}$ = 12500 g.mol^{-1}; $Đ_M$ =1.15. FT-IR (ν, cm^{-1}): ν_{NH} = 3390, $\nu_{(C=O,\ ester)}$ = 1720, $\nu_{(C=O\ ,\ carbamate)}$ = 1510. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 0.86 (C**H**$_3$(CH$_2$)$_{10}$CH$_2$-S-), 1.25

($CH_3(C\mathbf{H}_2)_{10}CH_2$-S-), 1.42 (-COO-C($C\mathbf{H}_3)_3$), 1.51-2.65 (-($C\mathbf{H}_2$-$C\mathbf{H}$)$_n$-), 3.39 (-$COOCH_2$-$C\mathbf{H}_2$-NH- and $CH_3(CH_2)_{10}C\mathbf{H}_2$-S-), 4.14 (-$COOC\mathbf{H}_2$-$CH_2$-NH-), 5.60 (-N**H**-). ^{13}C NMR (100.62 MHz, $CDCl_3$), δ (ppm): 28.34 (-C($\mathbf{C}H_3)_3$), 35.46 (-$\mathbf{C}H_2$-CH-)$_n$, 39.74 (-$COOCH_2$-$\mathbf{C}H_2$-), 41.46 (-CH_2-$\mathbf{C}$H-)$_n$, 64.27 (-$COO\mathbf{C}H_2$-CH_2-), 79.47 (-$\mathbf{C}(CH_3)_3$, 156.18 (-NH-**C**OO-), 175.49 (-**C**$OOCH_2$-).

3.2 Kinetic plots

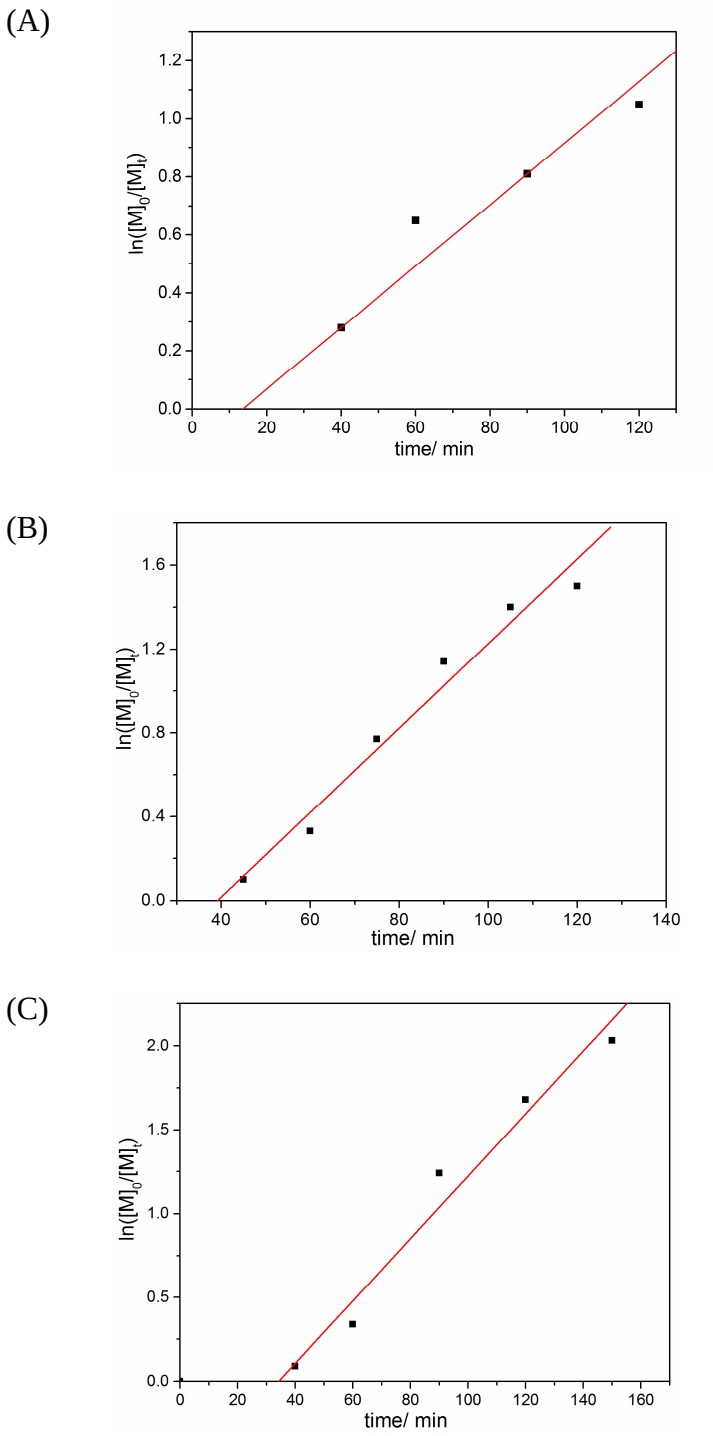

Figure S1. Kinetic plots for RAFT polymerization of *t*-BocAEA using AIBN as initiator and (A) CTA1, (B) CTA2 and (C) CTA3 as chain transfer agents in DMF at 90°C with [*t*-BocAEA]$_0$/[CTA]$_0$/[AIBN]$_0$ = 50/1/0.2.

3.3 Evolutions of $M_{n,SEC}$ and polydispersities with monomer conversion

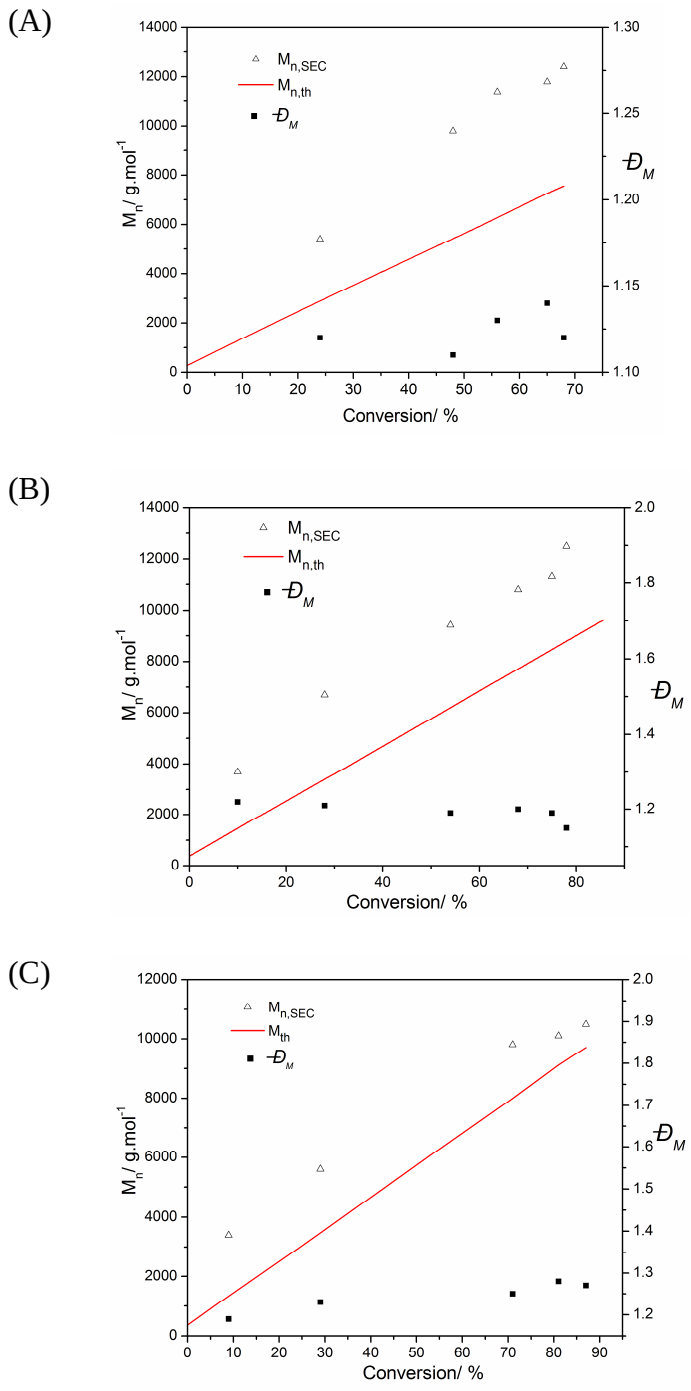

Figure S2. Evolutions of $M_{n,SEC}$ and $Đ_M$ with monomer conversion for RAFT polymerization of *t*-BocAEA using AIBN as initiator and (A) CTA1, (B) CTA2 and (C) CTA3 as chain transfer agents in DMF at 90°C with $[t\text{-BocAEA}]_0/[CTA]_0/[AIBN]_0 = 50/1/0.2$.

4.0 Deprotection of poly(N-(tert-butoxycarbonyl)aminoethyl acrylate) P(t-BocAEA)

4.1 General procedure

In a typical procedure, the P(*t*-BocAEA) of $M_{n,NMR}$ = 13 518 g.mol^{-1} and $DP_{n,NMR}$ = 61 (0.051 g, 3.77x10^{-6} mol) was dissolved in 1.00 mL of DCM. The resulting solution was cooled at 0°C. Trifluoroacetic acid (1.00 mL, 0.013 mol) was added dropwise under stirring while keeping the temperature at 0°C. After complete addition of TFA, the reaction mixture was stirred at room temperature for 5h. The DCM was then removed under vacuum and the resulting polymer was dissolved in methanol, precipitated in diethyl ether, filtered and dried under vacuum. The final product was obtained as a yellow powder. FT-IR (ν, cm^{-1}): ν_{NH} = 3370, $\nu_{(C=O)}$ = 1720, 1680. ^{1}H NMR (400 MHz, $CDOD_3$), δ (ppm): 0.86 ($C\mathbf{H}_3(CH_2)_{10}CH_2$-S-), 1.30 ($CH_3(C\mathbf{H}_2)_{10}CH_2$-S-), 1.42 (-COO-C($C\mathbf{H}_3$)$_3$), 1.41-2.65(-($C\mathbf{H}_2$-C**H**)$_n$-), 3.33 (-$COOCH_2$-$C\mathbf{H}_2$-NH- and $CH_3(CH_2)_{10}C\mathbf{H}_2$-S-), 4.34 (-$COOC\mathbf{H}_2$-$CH_2$-NH-). ^{13}C NMR (100.62 MHz, CD_3OD), δ (ppm): 35.46 (-**C**H_2-CH-)$_n$, 40.63 (-$COOCH_2$-**C**H_2-), 42.02 (-**C**H_2-CH-)$_n$, 63.55 (-COO**C**H_2-CH_2-), 177.45 (-**C**$OOCH_2$-), 163.09 (CF_3**C**OO^-), 116.79 (**C**F_3COO^-).

4.2 FTIR characterization

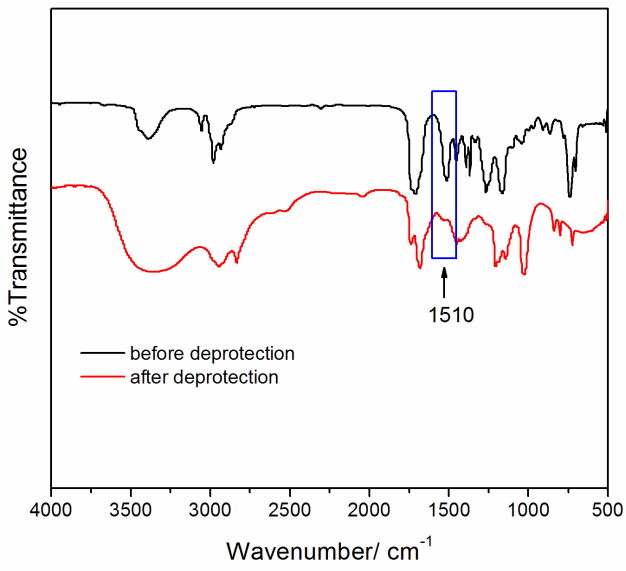

Figure S3. Overlaid FT-IR spectra of P(*t*-BocAEA)$_{61}$ before and after Boc group deprotection.

5.0 Hydrolysis study of quaternized poly(2-aminoethyl acrylate) (PAEA)

The quaternized PAEA of $M_{n,NMR}$ = 7928 g.mol^{-1} and $DP_{n,NMR}$ = 35 (0.010 g, 1.26x10^{-6} mol) was dissolved in D_2O (0.5 mL) and placed in a NMR tube at 37^{o}C. The sample was periodically analyzed by ^{1}H NMR spectroscopy at 0, 1 week, 4 weeks and 8 weeks. The hydrolysis percentage of PAEA polymer was calculated using ^{1}H NMR spectroscopy by comparing the integration areas of –OC**H**$_2$ of AEA units at 4.26 ppm and of, –C**H**$_2$OH of quaternized ethanolamine at 3.72 ppm.

6.0 Preparation of quaternized PAEA/pDNA complex

The quaternized $PAEA_{61}$/pDNA complexes were prepared by mixing 2.0 μg of pDNA (pBR322, 0.5 μg/μL in deionized water) with the desired quantity of $PAEA_{61}$ aliquots (solution in water) to give the desired N/P ratios. The volume of mixture was adjusted to 100 μL with deionized water. The solution was stirred for 10 seconds using a Vortex-2 Genie Scientific Industries. The complex formation was carried out at room temperature for 30 min. After, the complex solution (25 μL) was mixed with DNA loading dye 6X (5 μL) to characterize with agarose gel (1% of agarose in TAE 1X buffer) containing ethidium bromide (0.5 μg.ml^{-1}). The gel electrophoresis was carried out at 80 V with running tris acetate EDTA buffer (1xTAE).