Supporting Information for the article:

Enhanced Endosomal Escape and Efficient siRNA Delivery by Optimization of Cationic Shell Crosslinked Knedel-like Nanoparticles with Tunable Buffering Capacities

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Synthesis of poly(tert-butyl acrylate)$_{160}$ (PtBA$_{160}$) ***(S1)***

Bulk polymerization of *tert*-butyl acrylate by atom transfer radical polymerization: To a flame-dried 100 mL Schlenk flask, *tert*-butyl acrylate (56.6 g, 44.2 mmol) was added along with copper (I) bromide (0.41 g, 2.9 mmol). The reaction mixture was sealed and three freeze pump thaw cycles were performed with N_2 backfilling. N,N,N',N',N''-pentamethyldiethylenetriamine (0.49 g, 2.9 mmol) and 2-ethylbromo propionate (0.40 g, 2.2 mmol) were added to the reaction mixture *via* a syringe and the reaction mixture was allowed to undergo two more freeze pump cycles. The reaction mixture was brought to room temperature and stirred to ensure homogeneity. Following this, the Schlenk flask was immersed into a preheated oil bath at 50 °C. Aliquots were taken at predetermined time interval and conversion was monitored *via* NMR and GPC. After the expected conversion was reached, the polymerization was quenched by immersing the reaction flask into liquid nitrogen. The reaction mixture was passed through a short alumina plug to remove copper and three consecutive precipitations were performed in methanol and ice (80:20). After the final precipitation, removal of solvent and subsequent drying *in vacuo*, a white solid polymer was obtained (18.0 g, 50% yield). $(M_n)_{\mathrm{NMR}}$ = 21.2 kDa. $(M_n)_{\mathrm{GPC}}$ = 14.9 kDa. $(M_w)_{\mathrm{GPC}}$ = 17.7 kDa. PDI= 1.18. IR: 3000-2750, 1710, 1500, 1370, 1220, 1150, 880, 810 cm^{-1}. ^{1}H-NMR (CD_2Cl_2): δ 1.5 (s, $(C\boldsymbol{H}_3)_3COC$-), 1.8, (br, -C$\boldsymbol{H}$$CH_2$-), 2.2 ((br, -CHC$\boldsymbol{H}_2$-), 4.1 ($CH_3C\boldsymbol{H}_2OCO$-) ppm. ^{13}C NMR ($CD_2Cl_2$): δ: 26.7, 31.6–38.0, 80.2, 174.6 ppm. DSC: (T_g) = 58 °C. TGA: T_{onset} = 118 °C; $T_{\mathrm{decomposition}}$: [(118-248 °C) 31 % mass loss; (248-457 °C) 43% mass loss; 26% mass remaining].

Synthesis of poly(tert-butyl acrylate)$_{160}$-b-poly(styrene)$_{30}$ (PtBA$_{160}$-b-PS$_{30}$) ***(S2)***

The block copolymer poly(*tert*-butyl acrylate)$_{160}$-*b*-poly(styrene)$_{30}$ (P*t*BA$_{160}$-*b*-PS$_{30}$) was prepared using atom transfer radical polymerization. To a flame-dried 25 mL Schlenk flask, P*t*BA$_{160}$ (8.00 g, 0.37 mmol) was added along with copper (I) bromide (108 mg, 0.75 mmol), styrene (1.96 g, 18.9 mmol) and anisole (8.00 g). The reaction mixture was sealed and three freeze pump thaw cycles were performed with N_2 backfilling. N,N,N',N',N''-pentamethyldiethylenetriamine (130 mg, 0.75 mmol) was added to the reaction mixture *via* a syringe and the reaction mixture was treated by two more freeze pump thaw cycles. The reaction mixture was brought to room temperature and stirred to ensure homogeneity. Following this, the Schlenk flask was immersed into a preheated oil bath at 82 °C. Aliquots were taken at predetermined time intervals and conversions were monitored *via* ^{1}H-NMR spectroscopy and GPC. After the expected conversion was reached, the polymerization was quenched by immersing the reaction flask into liquid nitrogen. The reaction mixture was passed through a short alumina plug to remove copper and three consecutive precipitations were performed in methanol and ice (80:20). After the final precipitation, removal of solvent and subsequent drying *in vacuo*, a white powder was obtained (6.08 g, 97% yield). $(M_n)_{\text{NMR}}$ = 24.4 kDa. $(M_n)_{\text{GPC}}$ = 23.4 kDa. $(M_w)_{\text{GPC}}$ = 25.9 kDa. PDI= 1.11. IR: 3200-3050, 3000-2750, 1730, 1460, 1380, 1280, 1150, 870, 770 cm^{-1}. ^{1}H-NMR (CD_2Cl_2): δ 1.5 (s, $(C\boldsymbol{H}_3)_3COC$-), 1.8, (br, -$CHC\boldsymbol{H}_2$-), 2.2 ((br, -$C\boldsymbol{H}CH_2$-), 4.1 ($CH_3C\boldsymbol{H}_2OCO$-), 6.2-7.3 (br, Ar***H***) ppm. ^{13}C NMR (CD_2Cl_2): δ: 26.7, 31.6–38.0, 80.2, 126.4–128.1, 174.6 ppm. DSC: $(T_g)_{\text{P}t\text{BA}}$ = 51 °C; $(T_g)_{\text{PS}}$ = not observed. TGA: T_{onset} = 136 °C; $T_{\text{decomposition}}$: [(136-245 °C) 36 % mass loss; (245-464 °C) 43% mass loss; 21% mass remaining].

Synthesis of poly(acrylic acid)$_{160}$-b-poly(styrene)$_{30}$ (PAA$_{160}$-b-PS$_{30}$) ***(S3)***

Block copolymer **S2** (2.1 g, 0.09 mmol) was dissolved in trifluoroacetic acid (70 g, 0.6 mol) and stirred at room temperature for 16 h. The next day, TFA was evaporated under a N2 stream, and the polymer was dissolved in THF and transferred to presoaked dialysis tubing (MWCO 6-8000 Da). The mixture was extensively dialyzed for 4 days against nanopure water followed by lyophilization to obtain white fluffy polymer of PAA$_{164}$-*b*-PS$_{31}$ (1.0 g, yield = 78%). $(M_n)_{\mathrm{NMR}}$ = 15.2 kDa. IR: 3700-2400, 1720, 1488, 1280, 1205, 860 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.0-2.0 (br, polymer backbone), 2.2 (br, -CHC$\boldsymbol{H}_2$-), 4.1 (CH$_3$C$\boldsymbol{H}_2$OCO-), 6.2 - 7.3 (br, Ar***H***), 12.2 (br, COO***H***) ppm. ^{13}C NMR (DMSO-d_6) δ: 31.6–38.0, 126.4–128.1, 175.6 ppm. DSC: $(T_g)_{\mathrm{PAA}}$ = 127 °C; $(T_g)_{\mathrm{PS}}$ = not observed. TGA: T_{onset} = 154 °C; $T_{\mathrm{decomposition}}$: [(154-289 °C) 6 % mass loss; (289-482 °C) 40% mass loss; 40% mass remaining].

Characterization of micelles of PAEA$_{160}$-b-PS$_{30}$ ***(S4)***

$(D_h)_{\mathrm{num}}$ (DLS) = 28 ± 5 nm; $(D_h)_{\mathrm{vol}}$ (DLS) = 36 ± 23 nm; $(D_h)_{\mathrm{int}}$ (DLS) = 170 ± 150 nm. Zeta potential = 47 ± 5 mV (in nanopure water pH 5.5).

Characterization of micelles of (PAEA$_{0.85}$-g-PAEI$_{0.15}$)$_{160}$-b-PS$_{30}$ ***(S5)***

$(D_h)_{\mathrm{num}}$ (DLS) = 14 ± 4 nm; $(D_h)_{\mathrm{vol}}$ (DLS) = 21 ± 4 nm; $(D_h)_{\mathrm{int}}$ (DLS) = 150 ± 160 nm. Zeta potential = 57 ± 2 mV (in nanopure water pH 5.5).

Characterization of micelles of $(PAEA_{0.50}\text{-}g\text{-}PAEI_{0.50})_{160}\text{-}b\text{-}PS_{30}$ ***(S6)***

$(D_h)_{num}$ (DLS) = 11 ± 3 nm; $(D_h)_{vol}$ (DLS) = 16 ± 12 nm; $(D_h)_{int}$ (DLS) = 160 ± 170 nm. Zeta potential = 38 ± 1 mV (in nanopure water pH 5.5).

Characterization of micelles of $PAEI_{160}\text{-}b\text{-}PS_{30}$ ***(S7)***

$(D_h)_{num}$ (DLS) = 11 ± 3 nm; $(D_h)_{vol}$ (DLS) = 17 ± 11 nm; $(D_h)_{int}$ (DLS) = 100 ± 100 nm. Zeta potential = -34 ± 2 mV (in nanopure water pH 5.5).

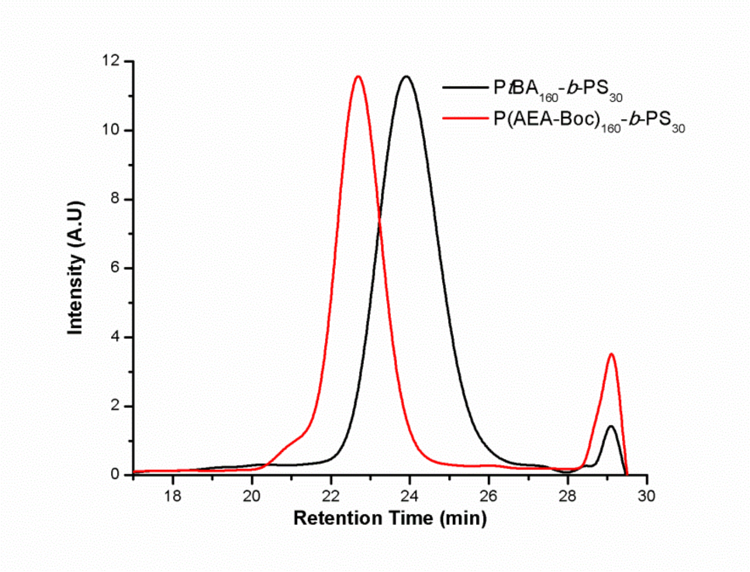

Figure S1. GPC of P*t*BA$_{160}$-*b*-PS$_{30}$ (**S2**) and P(AEA-Boc)$_{160}$-*b*-PS$_{30}$ (**1**).

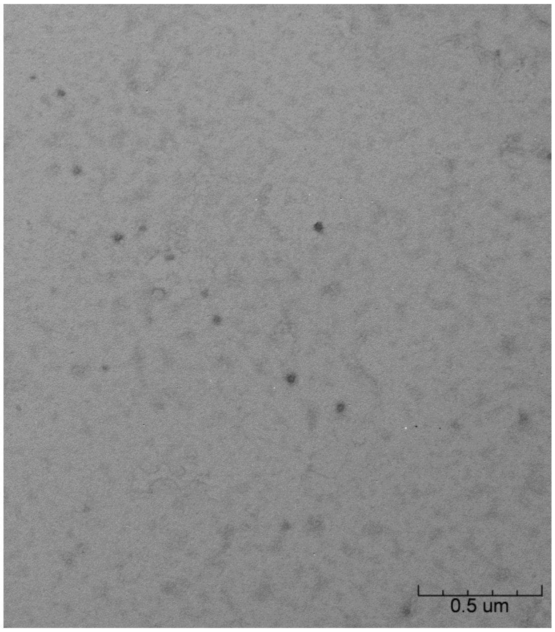

Figure S2. TEM micrograph of cSCKs (**12**) composed of PAEA$_{160}$-*b*-PS$_{30}$.