Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Star polymers with acid-labile diacetal-based cores synthesized by aqueous RAFT polymerization for intracellular DNA delivery

Thomas J. Gibson, Peter Smyth, Mona Semsarilar, Aidan P. McCann, William J. McDaid, Michael C. Johnston, Christopher J. Scott and Efrosyni Themistou*

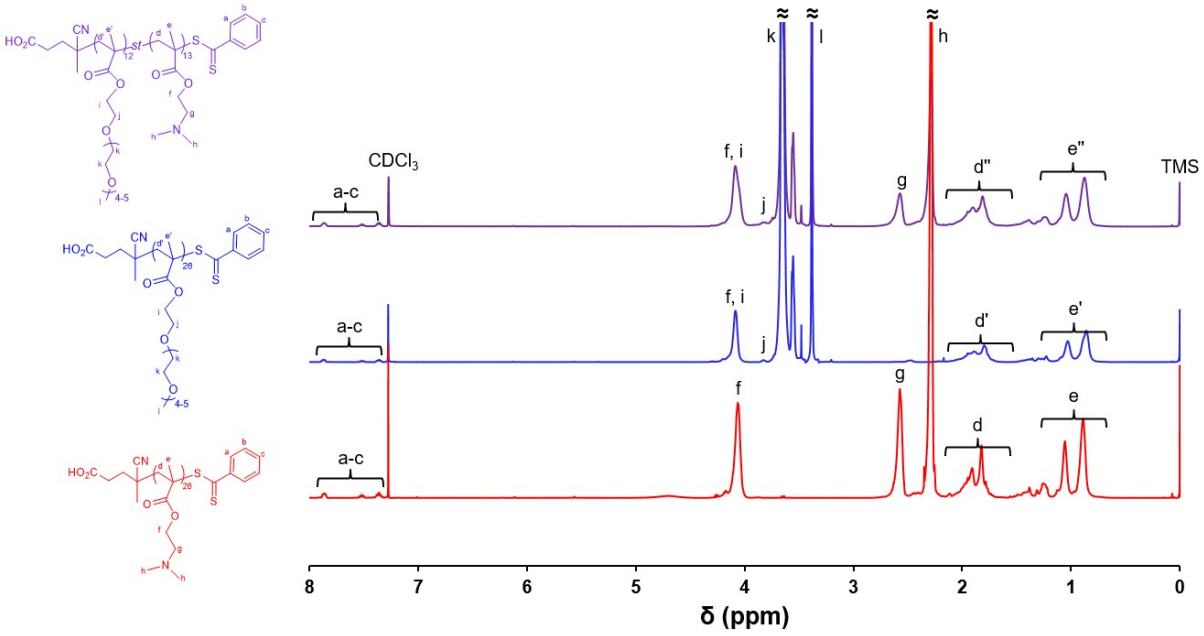

Figure S1. ^{1}H NMR ($CDCl_3$) spectra of P($OEGMA_{12}$-*st*-$DMAEMA_{13}$), $POEGMA_{26}$ and $PDMAEMA_{26}$ macro-CTAs.

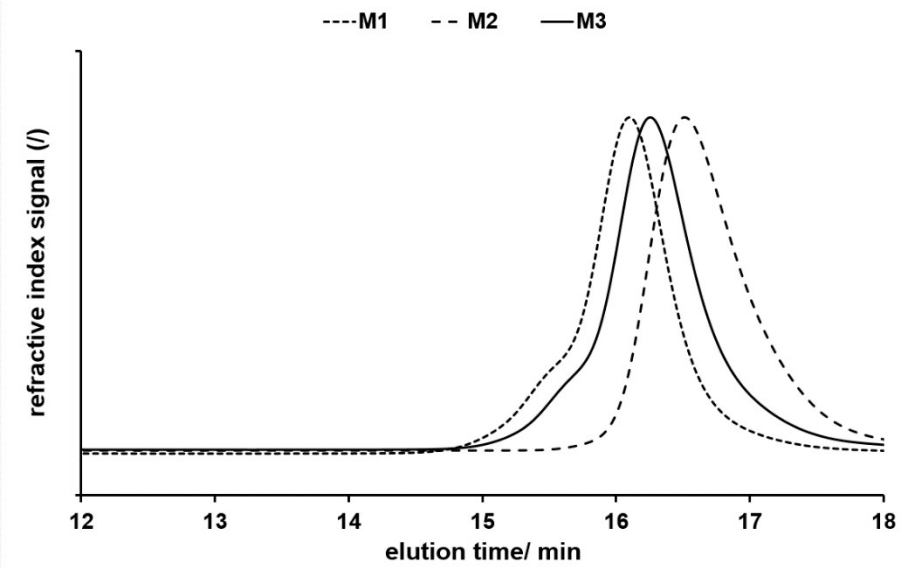

Figure S2. SEC (THF) chromatograms of $POEGMA_{26}$ (M1), $PDMAEMA_{26}$ (M2) and $P(OEGMA_{12}$-*st*-$DMAEMA_{13})$ (M3) macro-CTAs.

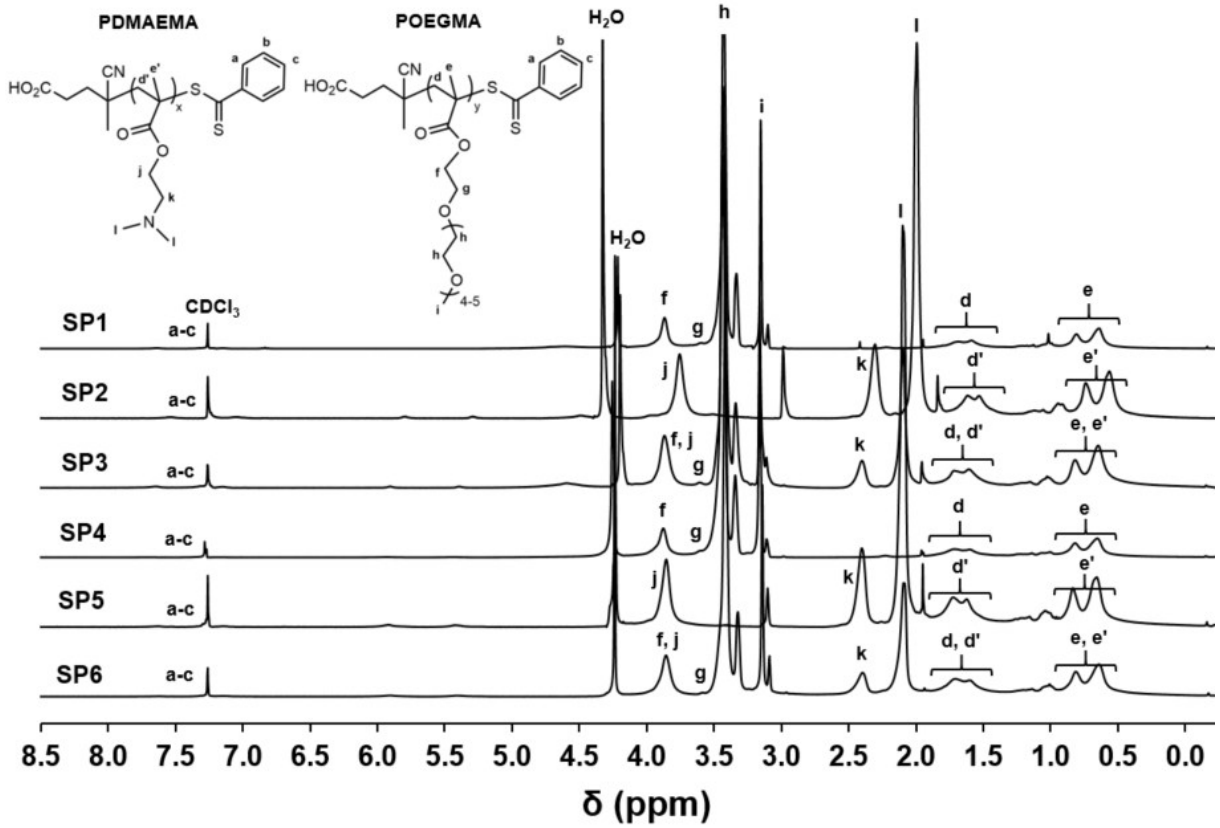

Figure S3. ^1H NMR ($CDCl_3$/MeOD mixture 3/1 v/v) spectra of the purified $POEGMA_{26}$-$MOEME_4$-*star* (SP1), $PDMAEMA_{26}$-$MOEME_4$-*star* (SP2), $P(OEGMA_{12}$-*st*-$DMAEMA_{13})$-$MOEME_4$-*star* (SP3), $POEGMA_{26}$-$EGDMA_6$-*star* (SP4), $PDMAEMA_{26}$-$EGDMA_6$-*star* (SP5) and $P(OEGMA_{12}$-st-$DMAEMA_{13})$-$EGDMA_6$-*star* (SP6) star polymers.

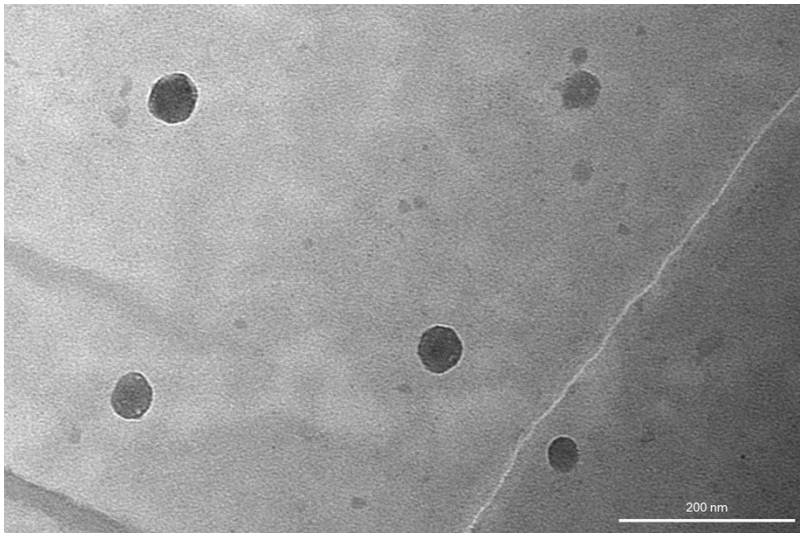

Figure S4. TEM image of PDMAEMA$_{26}$-MOEME$_4$-*star* in water. Ammonium molybdate was used as the positive staining agent.

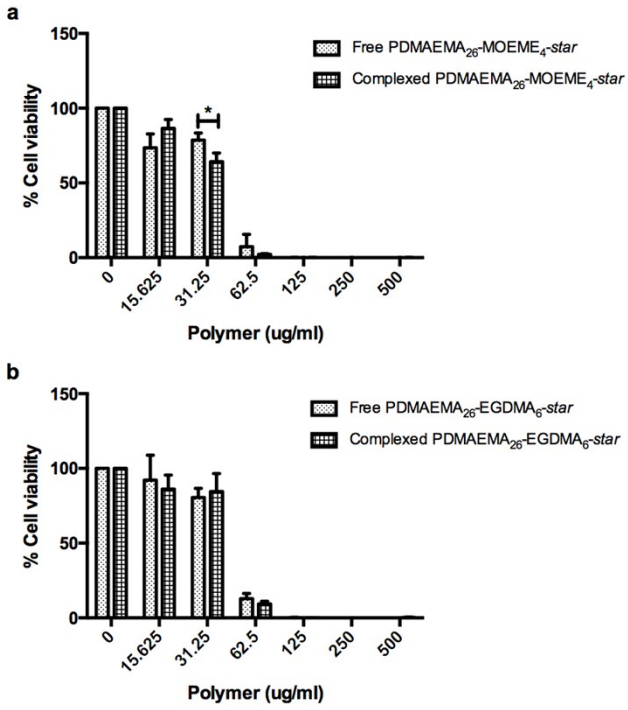

Figure S5. Cell viability data for complexed (DNA:star polymer ratio 1:40) and free, (a) purely cationic acid-labile PDMAEMA$_{26}$-MOEME$_4$-*star,* and (b) purely cationic non-labile PDMAEMA$_{26}$-EGDMA$_6$-*star* polymers. RAW 264.7 cells were treated with varying concentrations of star polymer (0-500 μg mL^{-1}) and cell viability assessed *via* CTG assay 48 h post treatment. Assay performed in triplicate. Representative of two independent experiments. Statistical significance was established by two-way ANOVA (*$p \leq 0.05$).

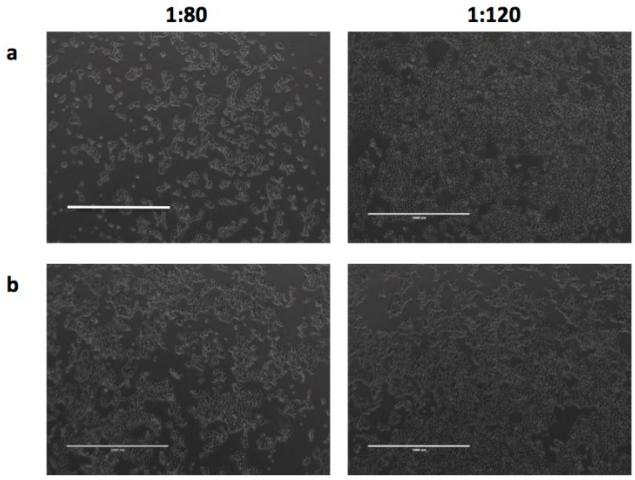

Figure S6. Fluorescent microscopy images of 293T cells transfected for 48 h with eGFP plasmid DNA-star polymer complexes of (a) partially cationic acid-labile P(OEGMA$_{12}$-*st*-DMAEMA$_{13}$)-MOEME$_4$-*star* and (b) partially cationic non-labile P(OEGMA$_{12}$-*st*-DMAEMA$_{13}$)-EGDMA$_6$-*star* star polymers at DNA:star polymer ratios of 1:80 and 1:120. Scale bars = 1000 μm.

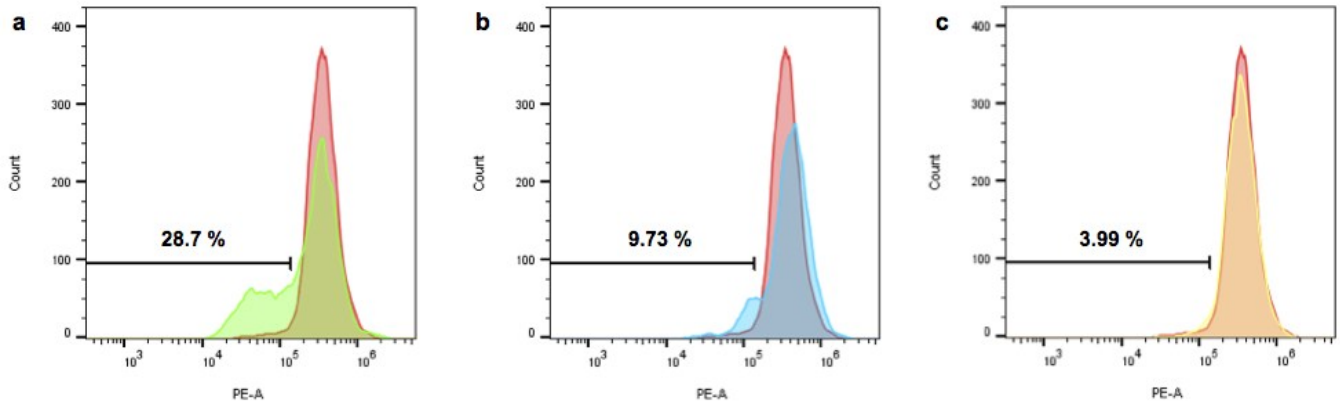

Figure S7. Endo/lysosome destabilization as assessed using acridine orange**.** RAW 264.7 cells were left untreated and then incubated with acridine orange (1 μg mL^{-1}) alone (red histogram) or treated with (a) PDMAEMA$_{26}$-MOEME$_4$-*star* (0.125 mg mL^{-1}) (green histogram) (b) PDMAEMA$_{26}$-MOEME$_4$-*star* (0.0625 mg mL^{-1}) (blue histogram) or (c) PDMAEMA$_{26}$-MOEME$_4$-*star* (0.03125 mg mL^{-1}) (orange histogram) for 24 h and then incubated with acridine orange (1 μg mL^{-1}). Histograms of acridine orange fluorescence intensity as determined by flow cytometry are displayed with gated regions indicating percentage of cell population exhibiting low acridine orange signal intensity. Assay performed in duplicate. Representative of three independent experiments.