Supporting Information

Effect of Polyplex Morphology on Cellular Uptake, Intracellular Trafficking, and Transgene Expression

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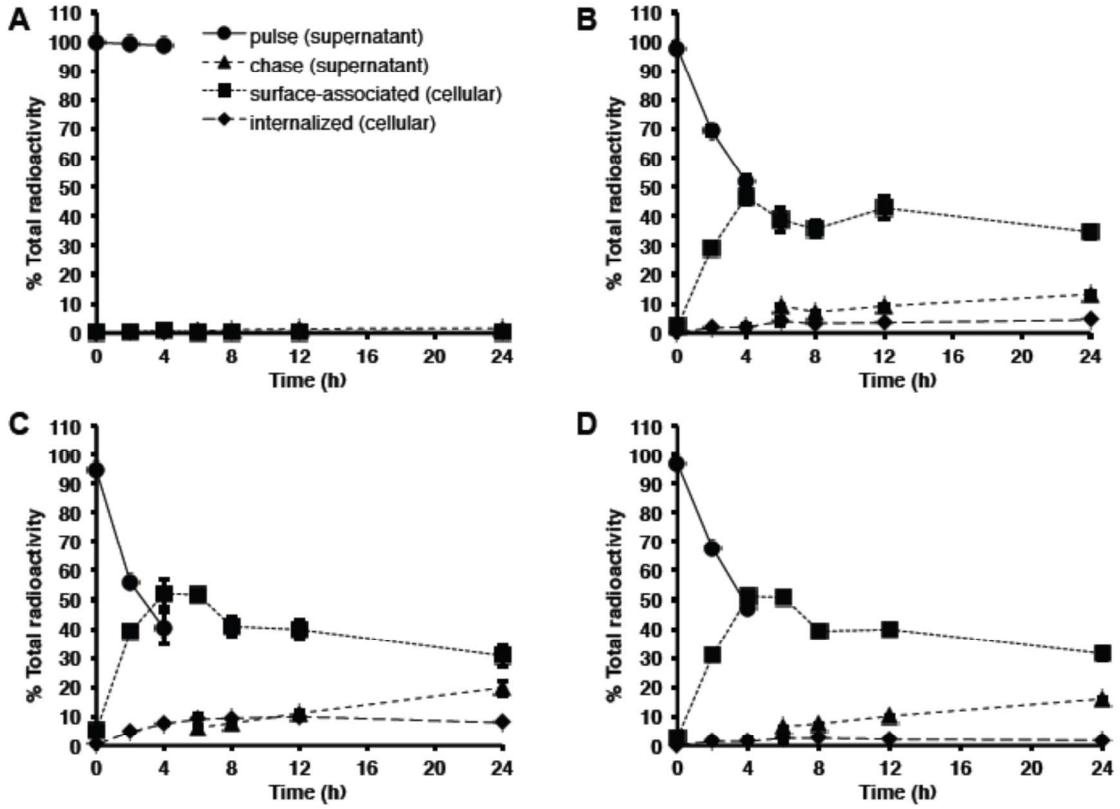
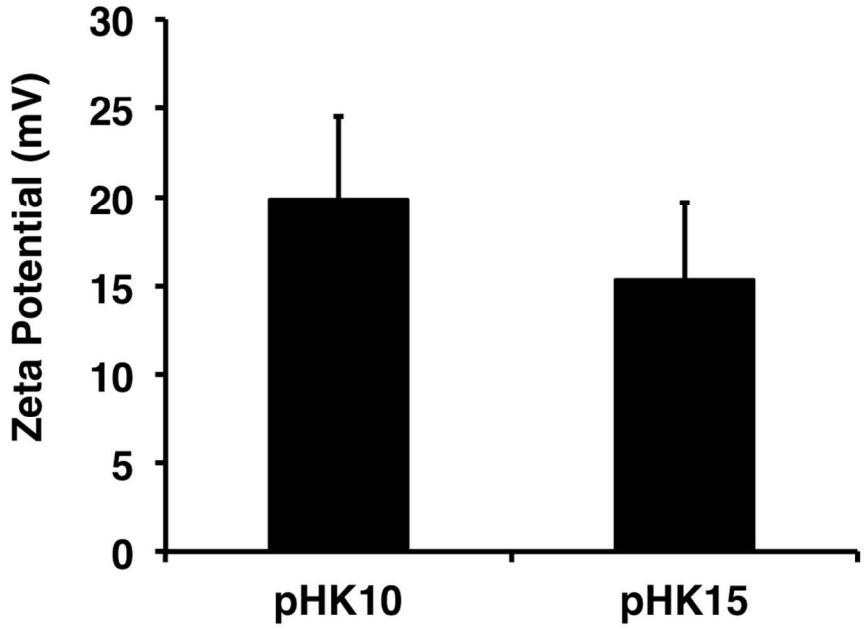
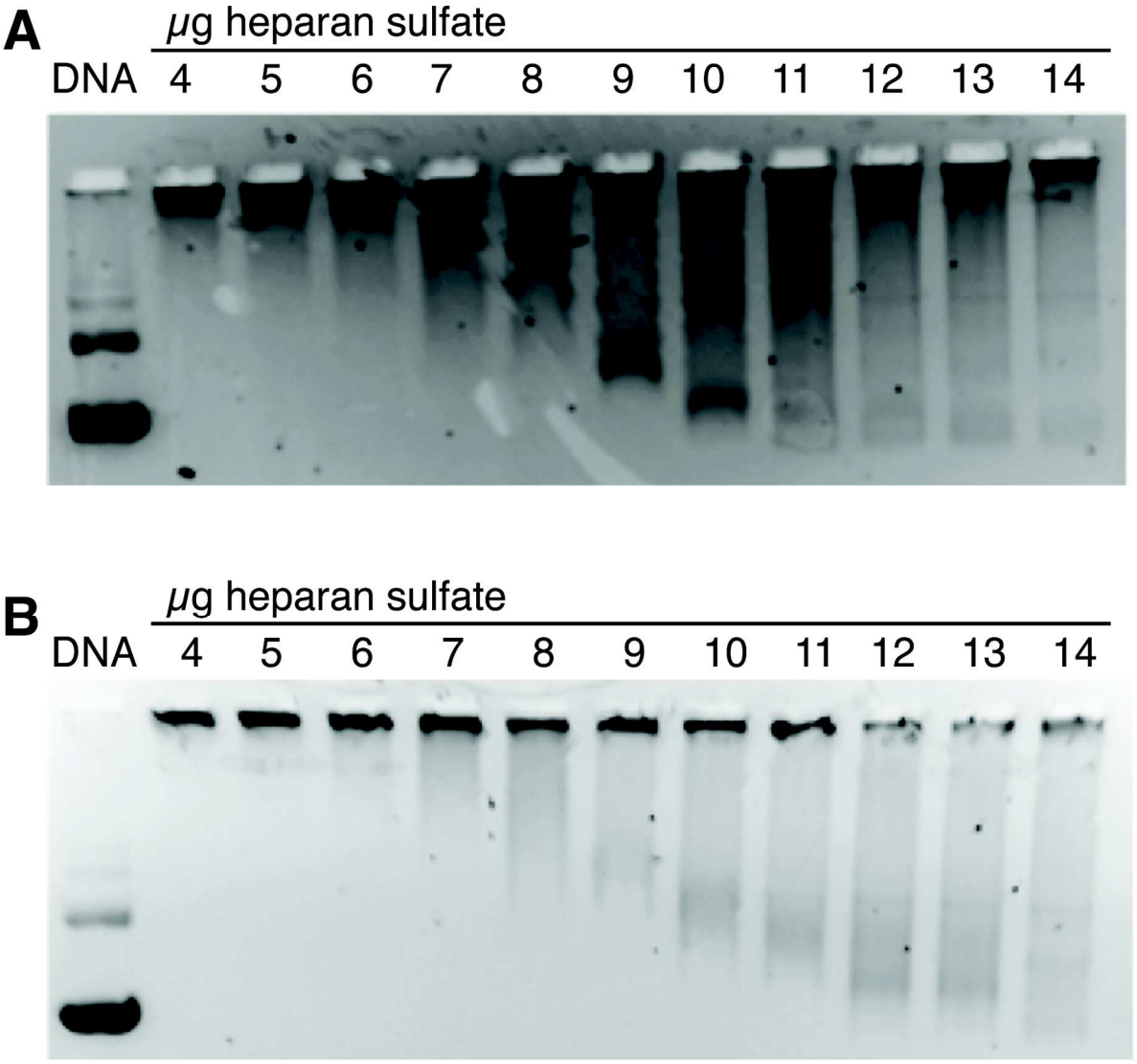


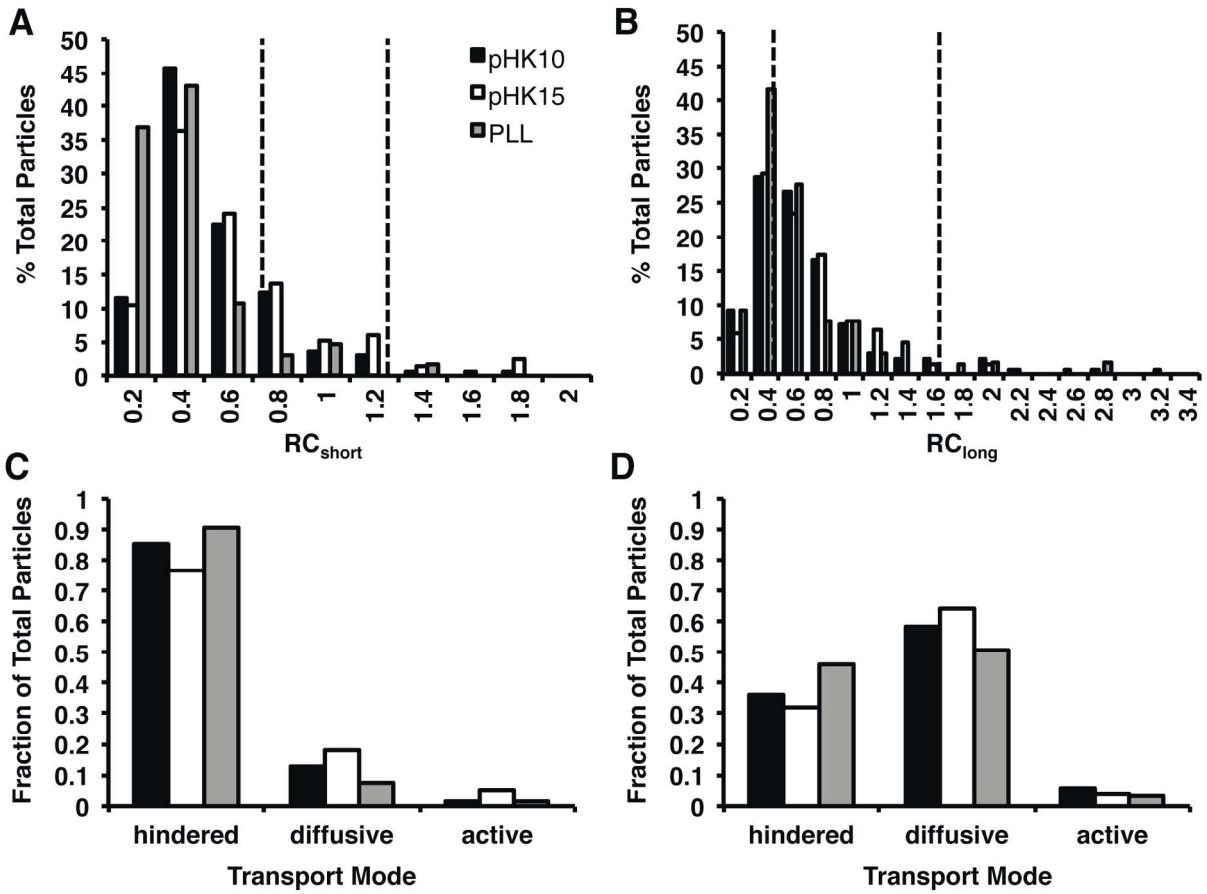
Supplemental Figure 1. [^{3}H]DNA/polymer complexes in pulse, chase, surface-associated, and internalized fractions over time. HeLa cells were treated with [^{3}H]DNA/polymer complexes for 4 h in serum-free media, rinsed, and then allowed to incubate for an additional 20 h. In addition to trypsinized cells ("internalized"), the supernatant in both pulse and chase, as well as washes with CellScrub to remove extracellularly-bound polyplexes ("surface-associated), was collected at various times for (A) DNA, (B) PLL, (C) pHK10, and (D) pHK15. Data are presented as mean ± S.D., $n = 3$.



Supplemental Figure 2. Zeta potential of pHK10 and pHK15 polyplexes. Polyplexes were formulated at N/P 5 with 1 μg of DNA and diluted in 10 mM NaCl. Data are presented as mean ± S.D., $n = 3$.



Supplemental Figure 3. Heparan sulfate decomplexation assay. Polyplexes with (A) pHK10 and (B) pHK15 (containing 0.5 µg DNA) were treated with 4-14 µg heparan sulfate prior to gel electrophoresis. Free DNA is represented for comparison.



Supplemental Figure 4. Transport mode of pHK10, pHK15, PLL particles using multiple particle tracking (MPT). HeLa cells were incubated with Alexa Fluor 568-labeled polymer/DNA complexes for 1 h in serum-free media, rinsed, and then allowed to incubate in complete media for an additional 2 h. Afterwards, 20 s videos of particle trajectories were captured. The relative change (RC) value for polymers calculated at (A) short time scales (τ = 0.2 s) and (B) long time scales (τ = 5 s), where dotted lines mark the bounds of random diffusive motion. The particle tracks were further characterized as hindered, diffusive, or active transport for (C) short and (D) long time scales.