SUPPLEMENTAL INFORMATION for

Polycation-induced Cell Membrane Permeability Does Not Enhance Cellular Uptake or Transfection Efficiency

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Figure S1. Flow Cytometry data for Cos-7 cells transfected with CFP-encoding pDNA and various vectors at +/- charge ratio = 10 in the absence of dye molecules. Controls are included.

Figure S2. Flow Cytometry data for M4A4 cells transfected with CFP-encoding pDNA and various vectors at +/- charge ratio = 10 in the absence of dye molecules. Controls are included.

Figure S3. Flow Cytometry data for M4A4 cells treated with propidium iodide and transfected with CFP-encoding pDNA and various vectors at +/- charge ratio = 10. Controls are included.

Figure S4. Flow Cytometry data for M4A4 cells treated with 2MDa tetramethylrhodamine dextran and transfected with CFP-encoding pDNA and various vectors at +/- charge ratio = 10. Controls are included.

Table S1. Statistical Analysis of Anti-correlation of Membrane Permeability to Dye Molecule and CFP Expression in Cos-7 Cells

		Pearson's r	t-value
PI	jetPEI	- 0.01	1.65
	Lipofectamine 2000	- 0.01	0.43
	G5-NH2	- 0.63	18.93
	G7-NH2	- 0.74	22.38
2 MDa Dextran	jetPEI	- 0.08	4.13
	Lipofectamine 2000	- 0.22	8.33
	G5-NH2	- 0.54	14.11
	G7-NH2	- 0.67	13.24

Table S1. Statistical Analysis of Anti-correlation of Membrane Permeability to Dye Molecule and CFP Expression in M4A4 Cells

		Pearson's r	t-value
PI	jetPEI	- 0.68	14.46
	Lipofectamine 2000	- 0.52	10.76
	G5-NH2	- 0.20	7.68
	G7-NH2	- 0.23	10.25
2 MDa Dextran	jetPEI	- 0.62	24.14
	Lipofectamine 2000	- 0.58	11.67
	G5-NH2	- 0.49	11.04
	G7-NH2	- 0.31	8.32

Table S1. Statistical Analysis of Anti-correlation of Membrane Permeability to Dye Molecule and pDNA Internalization

		Pearson's r	t-value
PI	jetPEI	- 0.67	20.51
	Lipofectamine 2000	- 0.68	13.00
	G5-NH2	- 0.54	28.68
	G7-NH2	- 0.48	20.99
2 MDa Dextran	jetPEI	- 0.33	23.35
	Lipofectamine 2000	- 0.31	19.40
	G5-NH2	- 0.20	19.16
	G7-NH2	- 0.65	40.60

Figure S5. Flow cytometry plots and gating used to perform cell sorting based on retention (R2) or leakage (R3) of fluorescein no treatment of Cos-7 cells (a), treatment with DNA only (b) or treatment with polyplexes at +/- = 10 formed using jetPEI (c), Lipofectamine 2000 (d), G5-NH₂ (e) or G7-NH₂ (f).

Figure S6. Flow Cytometry data for Cos-7 cells transfected with CFP-encoding pDNA labeled with FITC and various vectors at +/- charge ratio = 10 in the absence of PI or dextran. Controls are included.

Figure S7. Flow Cytometry control data comparing propidium iodide uptake in the absence and presence of the plasmid DNA label in Cos-7 cells after 3 hours of transfection with CFP-encoding pDNA and various vectors at +/- charge ratio = 10. The cells only control is also included.

Figure S7. Continued.

Figure S8. Flow Cytometry control data showing the release of uptaken propidium iodide over time in Cos-7 cells after transfection with CFP-encoding pDNA and G5-NH₂ at +/- charge ratio = 10.

Figure S9. Flow Cytometry control data showing the release of uptaken 2 MDa tetramethylrhodamine dextran over time in Cos-7 cells after transfection with CFP-encoding pDNA and G5-NH₂ at +/- charge ratio = 10.