

Supporting Information

“Biophysical and Structural Characterization of Polyethylenimine-Mediated siRNA Delivery in Vitro”

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Supporting Table: Summary of transfection conditions used for various commercial reagents.

Reagent	Reagent (μ L)	siRNA (μ L)	Cell Stock (μ L)	OPTI-MEM [®] I (μ L)	Cell Culture Medium (μ L)	Total Transfectio n Volume (μ L)
siPORT [™] <i>Amine</i> ^a	1, 1.5, 2	0.25	80	18.75, 18.25, 17.75	0	100
siPORT [™] <i>Lipid</i> ^a	0.3, 0.45, 0.6	0.25	80	19.45, 19.3, 19.15	0	100
RNAiFect [™] ^b	0.1995, 0.399, 0.599	0.25	40	24.5505, 24.351, 24.151	35	100
GeneSilencer ^{®b,c}	0.3325, 1.33, 2.66, 4.655	0.25	40	39.4175, 38.42, 37.09, 35.095	20	100
X-tremeGENE ^{a,c}	0.2394, 0.5985, 1.197	0.45	80	29.3106, 28.9515, 28.353	70	180
<i>TransIT</i> [®] -siQUEST ^{™b}	0.09, 0.2, 0.4, 0.6	0.15	40	9.76, 9.65, 9.45, 9.25	10	60

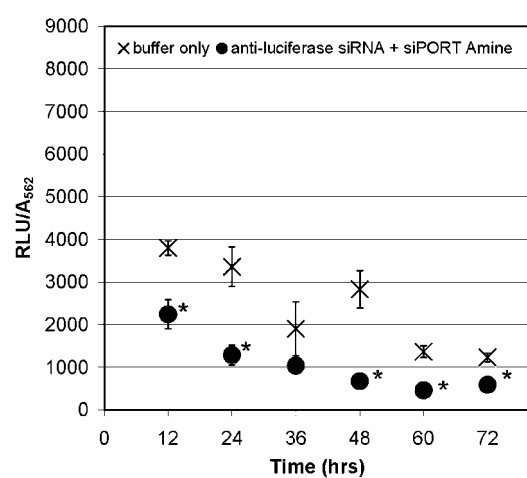
^a 50,000 cells/mL stock

^b 100,000 cells/mL stock

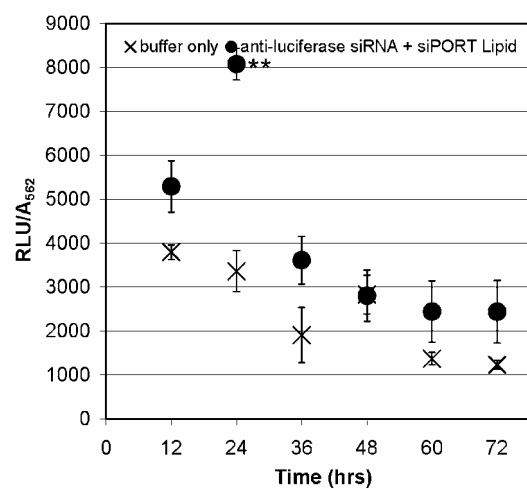
^c dilute reagent and siRNA in OPTI-MEM[®] I separately, then combine

SUPPORTING FIGURE 1: Luciferase expression knockdown kinetics for NIH/3T3 cells. Luciferase expression (RLU) normalized by protein content (A_{562}) over time for NIH/3T3 cells transfected with a selected volume of each reagent. On each panel, symbols indicate cells transfected with pCMV-luc and *TransIT*[®]-LT1 (positive controls, ×), and transfected with pCMV-luc and *TransIT*[®]-LT1 followed by anti-luciferase siRNA and a selected volume of each reagent (●). Statistical significance between sample groups indicated by * ($p < 0.05$) or ** ($p < 0.01$). Error bars are the standard error of the mean, $n = 3$. a) 2 μ L/well siPORT[™] *Amine*, b) 0.6 μ L/well siPORT[™] *Lipid*, c) 0.399 μ L/well RNAiFect[™], d) 4.655 μ L/well GeneSilencer[®], e) 0.5985 μ L/well X-tremeGENE, f) 0.2 μ L/well *TransIT*[®]-siQUEST[™].

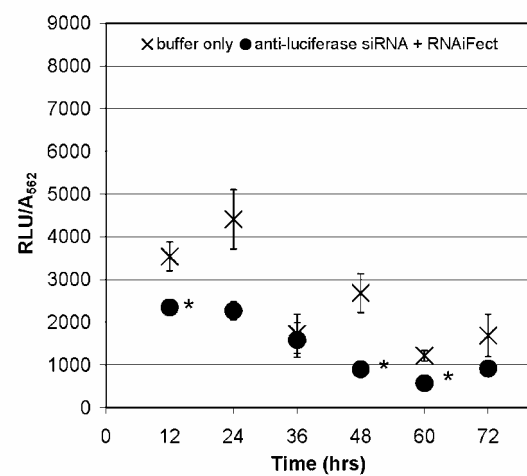
SUPPORTING FIGURE 2: Cytotoxicity of siRNA delivery reagents. Cell viability, as measured by normalized absorbance at 490 nm (A_{490}), as a function of reagent concentration after 48 h incubation. On each panel, symbols indicate results for HR5-CL11 (■), HeLa (□) and NIH/3T3 (×) cells. Quadratic fits were performed to data points spanning 0–2.5 μ L/well for *TransIT*[®]-siQUEST[™] and 0–10 μ L/well for all other reagents. IC_{50} values were calculated using the Solver function in Excel as the concentration of reagent (% v/v) at which the calculated A_{490} was one-half the measured A_{490} for cells that received 0 μ L of reagent. Error bars are one standard deviation, $n = 3$. a) siPORT[™] *Amine*, b) siPORT[™] *Lipid*, c) RNAiFect[™], d) GeneSilencer[®], e) X-tremeGENE, f) *TransIT*[®]-siQUEST[™].



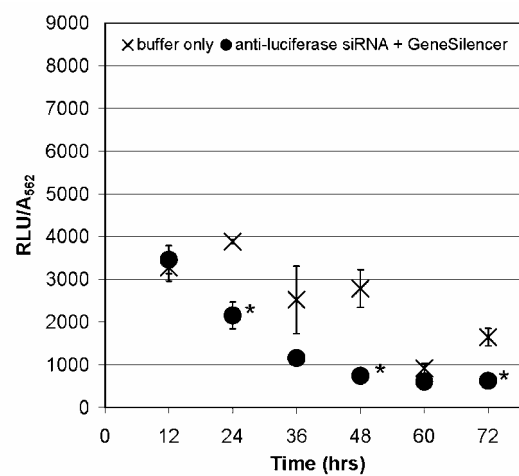
a)



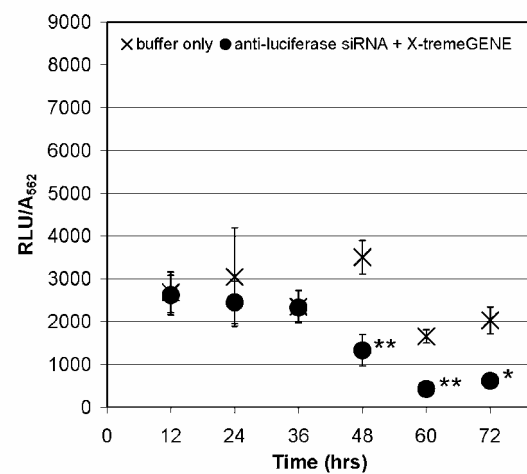
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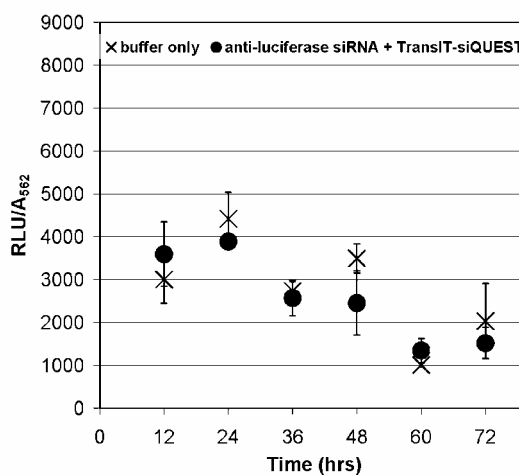
c)



d)

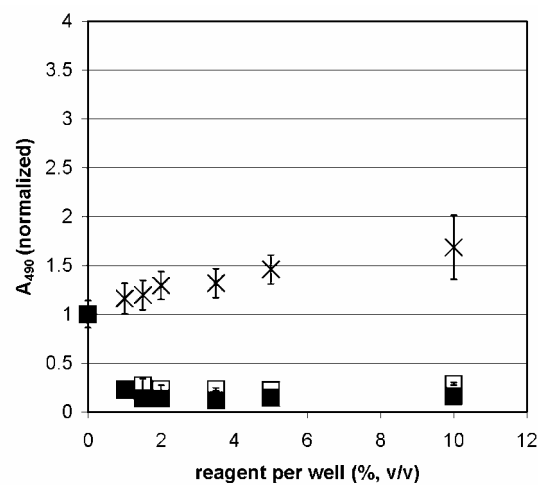


e)

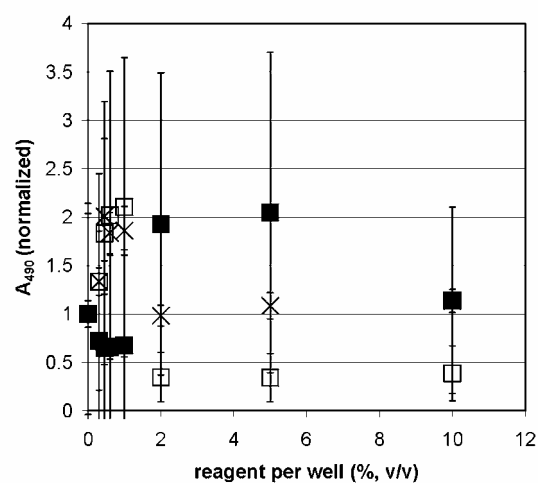


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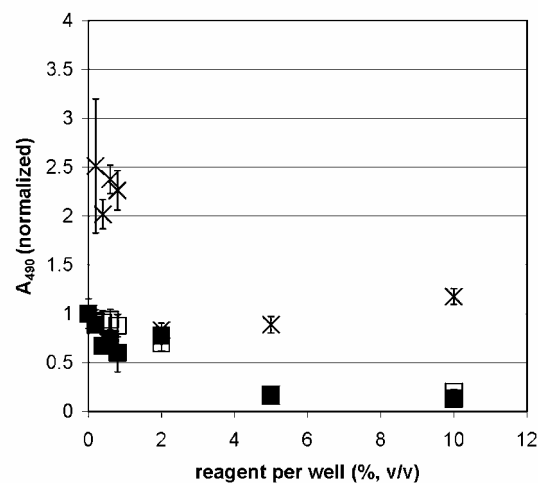
SUPPORTING FIGURE 1



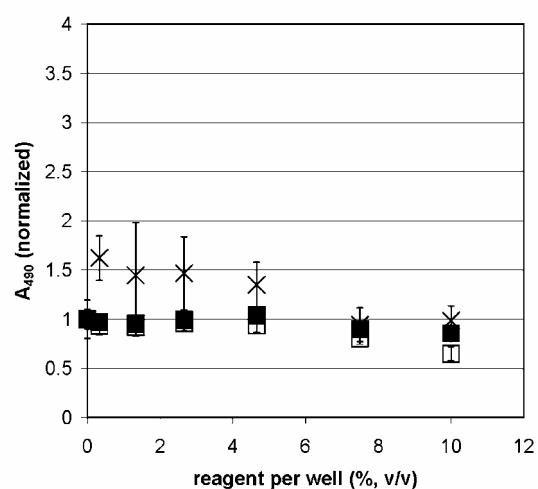
a)



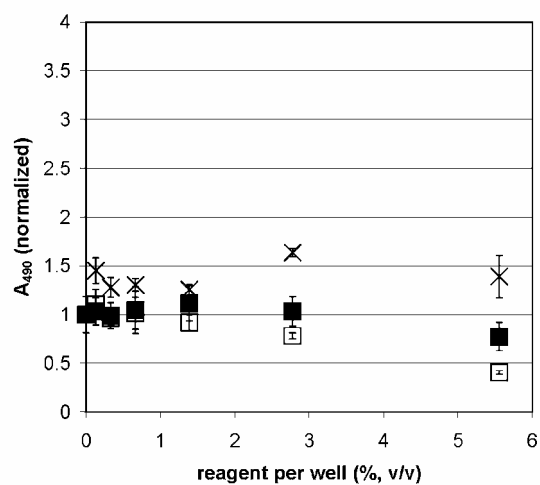
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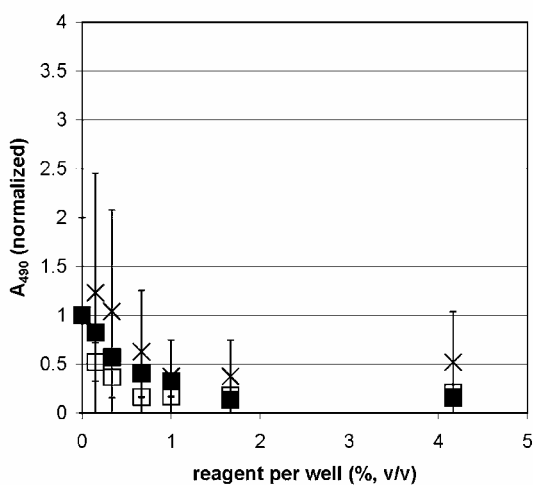
c)



d)



e)



f)

SUPPORTING FIGURE 2