Cellular and Nuclear Uptake Rate Evaluation of Exogenous DNA via Flow Cytometry

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Supplementary Data

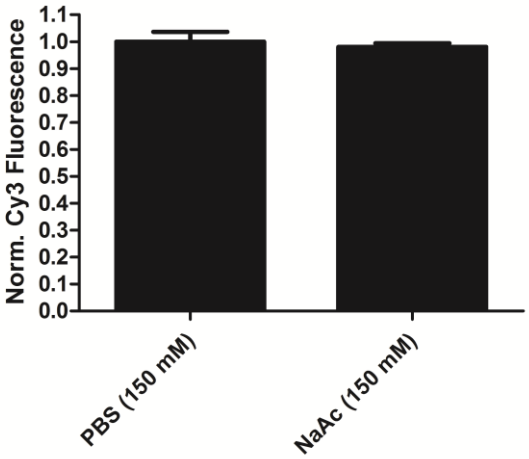

Figure S1. Normalized fluorescence of Cy3, showing no statistical difference at pH 7.4 (150 mM PBS) and 5.2 (150 mM NaAc).

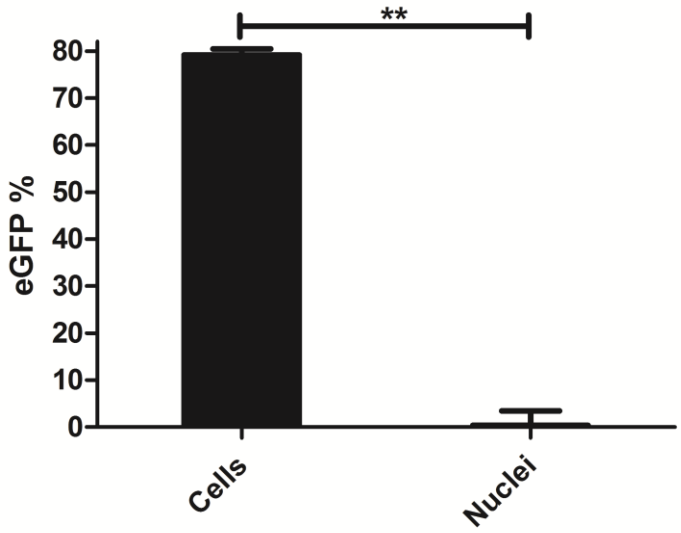

Figure S2. Purity of nuclei. Flow cytometry was used to assess the eGFP presence of eGFP-stably expressing cells and their isolated nuclei to ascertain the purity of the nuclei; the eGFP protein in adhered cytoplasm would have caused nuclei to fluoresce.

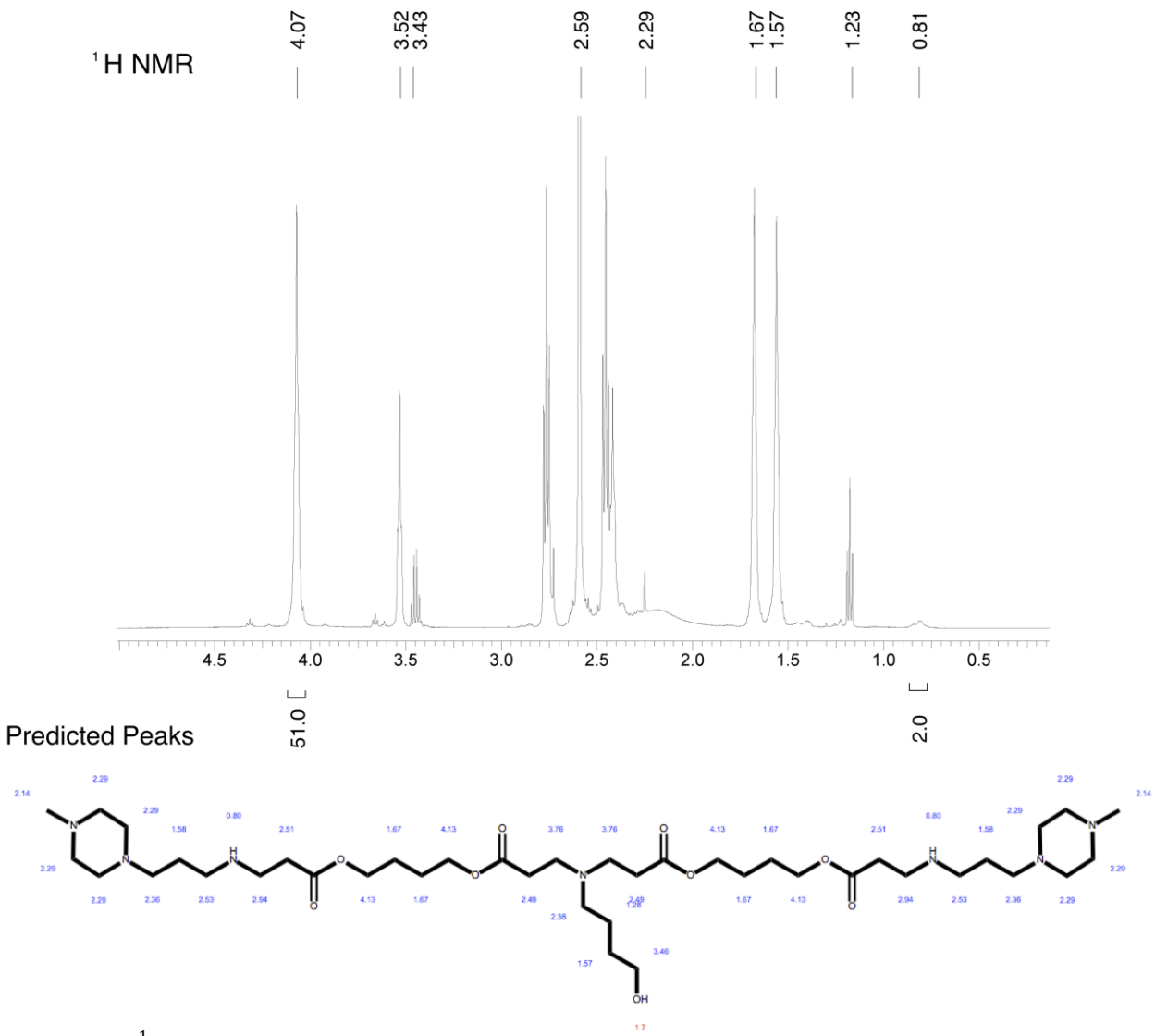

Figure S3. ^{1}H NMR in $CDCl_3$ was used to measure 447 polymer molecular weight as 7.6 kDa. The mean repeat unit (MW = 287.36 g/mol) was determined to be 25.5 using the ratio of area between peaks for the endcap secondary amine hydrogen at 0.81 ppm and the hydrogens of the α-carbons of the B repeat units at 4.07 ppm. The peak and satellites at 2.59 ppm were due to DMSO contamination following isolation of the polymer from a DMSO stock.

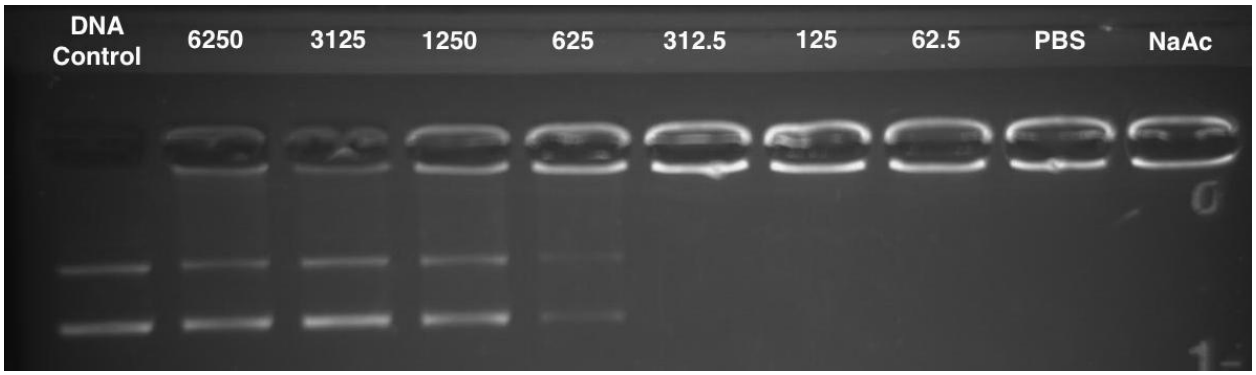

Figure S4. Particles of 447 and DNA were formed at 30 w/w in 25 mM NaAc at a DNA concentration of 16 µg/mL. Heparin sulfate was diluted in PBS in a serial dilution then 20 µL were mixed in a 1:1 ratio with the nanoparticles to assess its ability to disrupt 447 interaction with DNA. Thirty-percent glycerol (8 µL) was added to the 40 µL of nanoparticles and heparin as a loading buffer and 15 µL of each sample were loaded in a 1% agarose gel. The gel contained 100 ng DNA and 3000 ng of 447 per well. Notations on the figure denote the amount of heparin sulfate per well (ng). At close to a 1:1 mass ratio of heparin:PBAE (3125 ng heparin), the nanoparticles were sufficiently disrupted to prevent retention in the gel

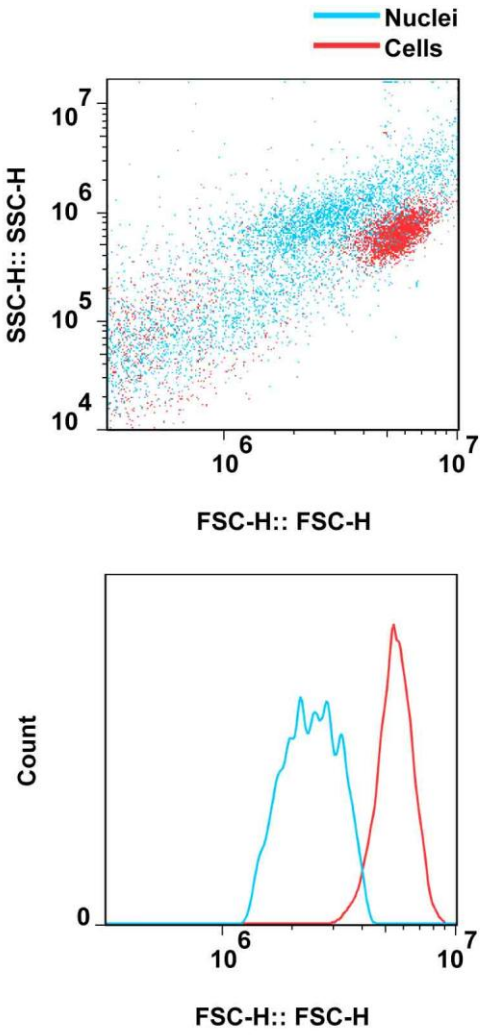

Figure S5. Top: Ungated cells and nuclei (FSC-H vs SSC-H). The nuclei had a lower FSC-H value as expected due to their smaller size. Bottom: FSC-H Histogram for gated cells and nuclei.

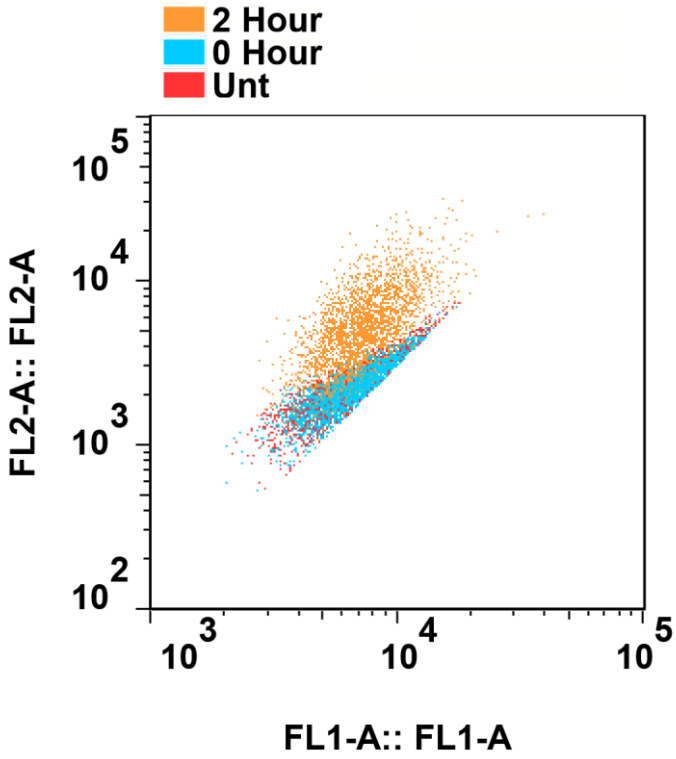

Figure S6. Cell flow cytometry data for the untreated, and the 0 and 2 hour time points. There is a great deal of population overlap for the untreated and the 0 hour time point, as would be expected.

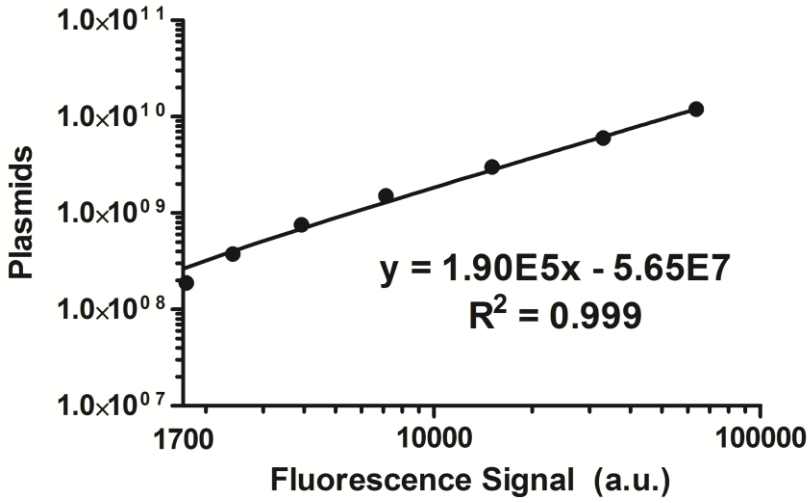

Figure S7. Calibration curve for converting bulk fluorescence on a plate reader to a plasmid number.

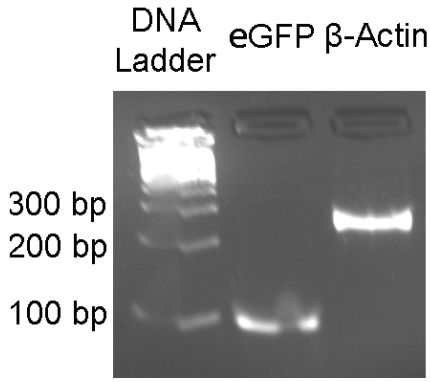

Figure S8. Gel electrophoresis of qPCR products (left, middle and rights lanes: DNA ladder, and qPCR products of eGFP and β-actin, respectively).

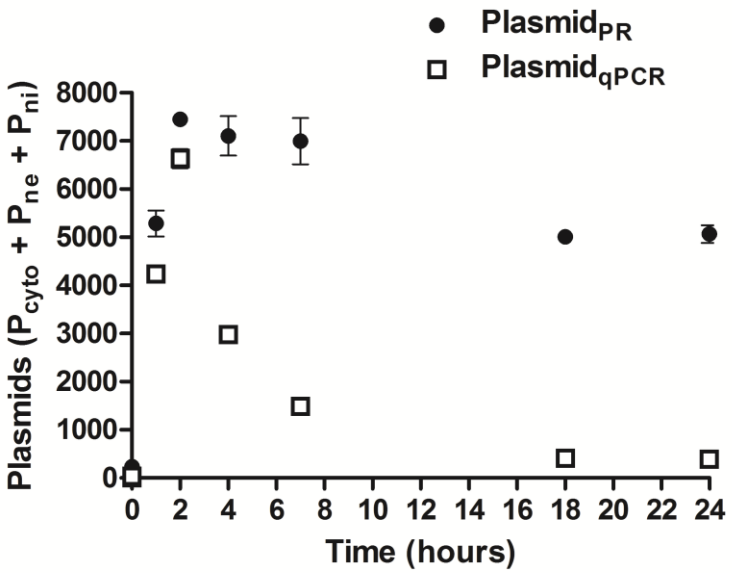

Figure S9. Plasmids in time calculated using the plate reader ($Plasmid_{PR}$) method compared to qPCR ($Plasmid_{qPCR}$).

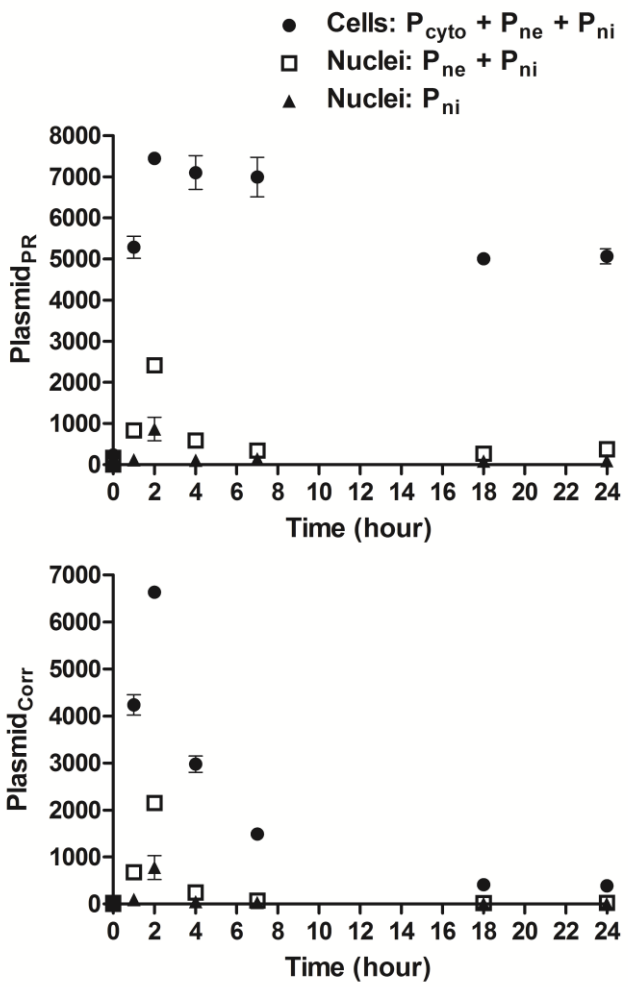

Figure S10. $Plasmid_{PR}$ (top) and $Plasmid_{Corr}$ (bottom) in time for cells and nuclei.

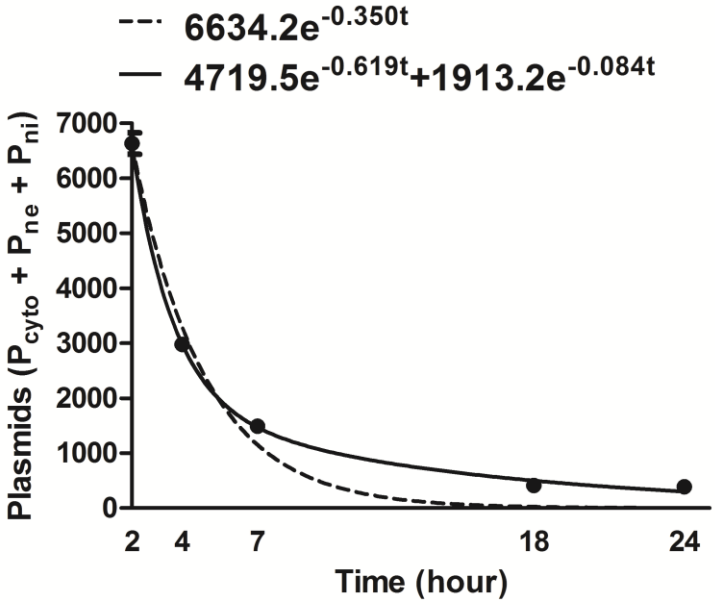

Figure S11. Plasmid$_{qPCR}$ values from 2 to 24 hours for fitting the degradation constant, k_{deg}, showing mono- (dotted line) and bi-exponential degradation fittings.

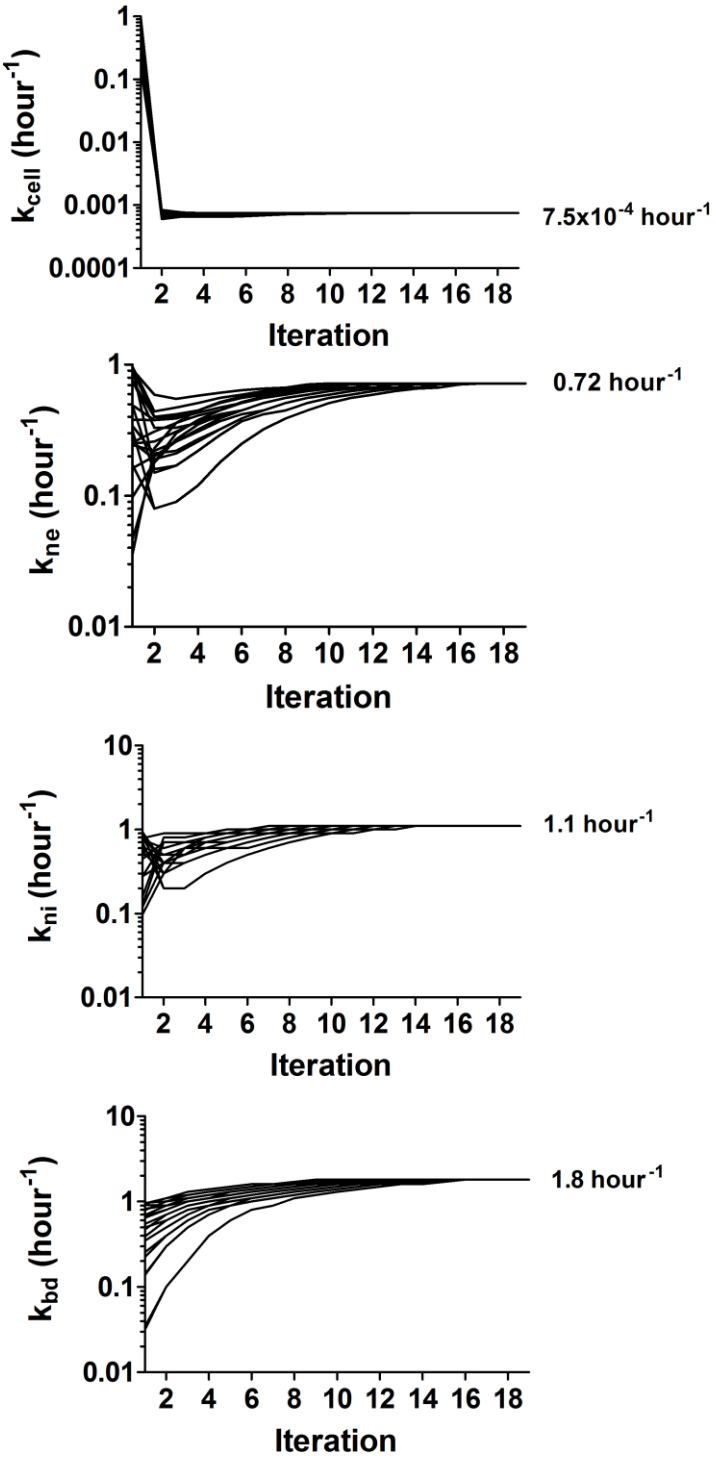

Figure S12. Convergences of k_{cell}, k_{ne}, k_{ni}, and k_{bd} through optimization iterations.

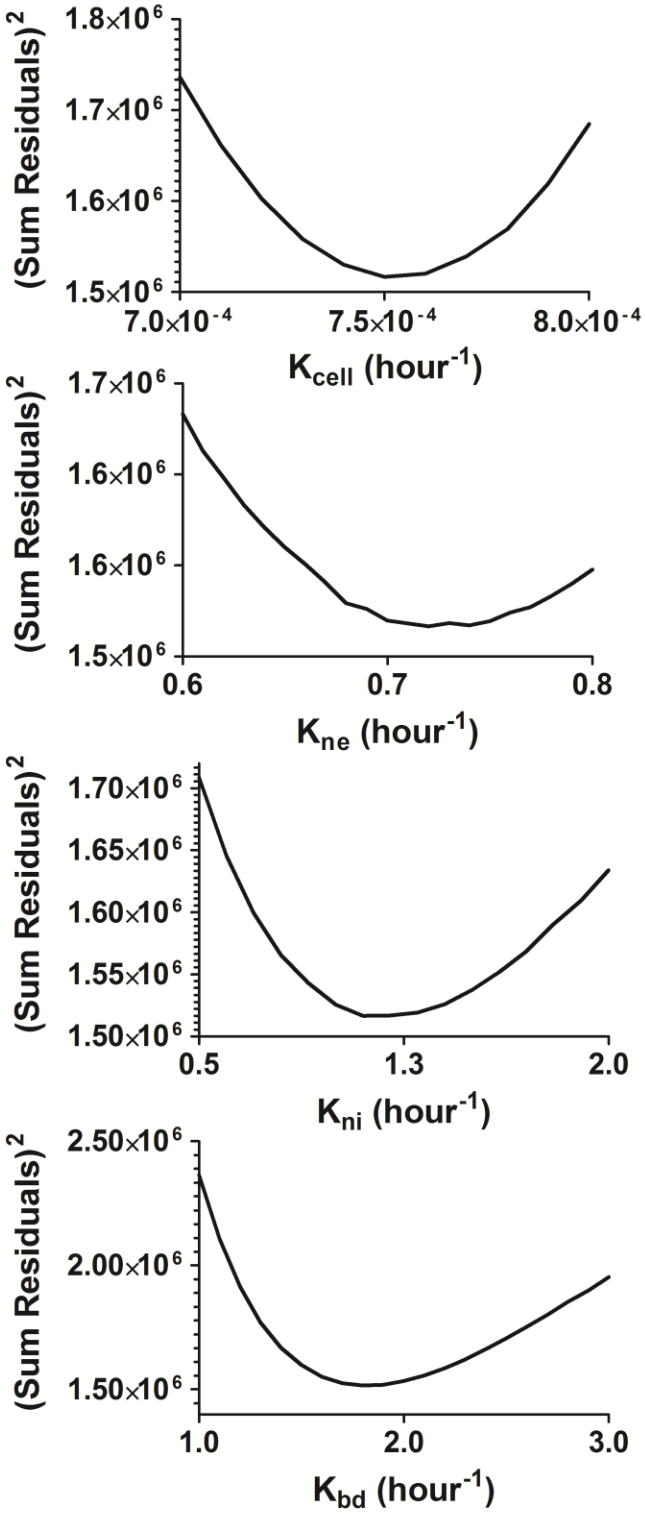

Figure S13. Error minimizations for the 20th or last iteration of optimization for k_{cell}, k_{ne}, k_{ni}, and k_{bd}.

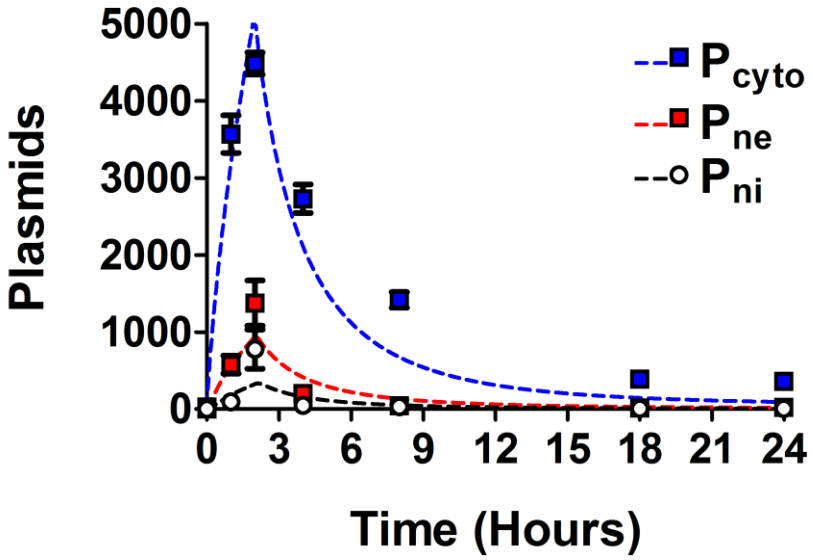

Figure S14. First order mass-action model (dotted lines; mono-exponential degradation) for the P_{cyto}, P_{ne}, and P_{ni} experimental data points.

Supplemental derivation 1

Derivation of degradation term in the differential equations:

$$P = Ae^{k_{deg1}t} + Be^{k_{deg2}t}$$

$$\ln P = \ln(Ae^{k_{deg1}t} + Be^{k_{deg2}t})$$

$$d(\ln P) = d\left(\ln\left(Ae^{k_{deg1}t} + Be^{k_{deg2}t}\right)\right)$$

$$\frac{dP}{dt}d(\ln P) = \frac{dP}{dt}d\left(\ln\left(Ae^{k_{deg1}t} + Be^{k_{deg2}t}\right)\right)$$

$$\frac{dP}{dt}\frac{d(\ln P)}{dP} = \frac{d\left(\ln\left(Ae^{k_{deg1}t} + Be^{k_{deg2}t}\right)\right)}{dt}$$

$$\frac{dP}{dt}\frac{1}{P} = \frac{d\left(\ln\left(Ae^{k_{deg1}t} + Be^{k_{deg2}t}\right)\right)}{dt}$$

$$\frac{dP}{dt} = \frac{d\left(\ln\left(Ae^{k_{deg1}t} + Be^{k_{deg2}t}\right)\right)}{dt}P$$

$$\frac{dP}{dt} = \frac{d(\ln u)}{dt}P; u = Ae^{k_{deg1}t} + Be^{k_{deg2}t}$$

$$\frac{du}{dt} = Ak_{deg1}e^{k_{deg1}t} + Bk_{deg2}e^{k_{deg2}t}$$

$$\frac{du}{Ak_{deg1}e^{k_{deg1}t} + Bk_{deg2}e^{k_{deg2}t}} = dt$$

$$\frac{dP}{dt} = \frac{d(\ln u)}{\frac{du}{Ak_{deg1}e^{k_{deg1}t} + Bk_{deg2}e^{k_{deg2}t}}}P$$

$$\frac{dP}{dt} = \frac{d(\ln u)\left(Ak_{deg1}e^{k_{deg1}t} + Bk_{deg2}e^{k_{deg2}t}\right)}{du}P$$

$$\frac{dP}{dt} = \frac{\left(Ak_{deg1}e^{k_{deg1}t} + Bk_{deg2}e^{k_{deg2}t}\right)}{u}P$$

$$\frac{dP}{dt} = \frac{\left(Ak_{deg1}e^{k_{deg1}t} + Bk_{deg2}e^{k_{deg2}t}\right)}{Ae^{k_{deg1}t} + Be^{k_{deg2}t}}P$$