Supporting Information

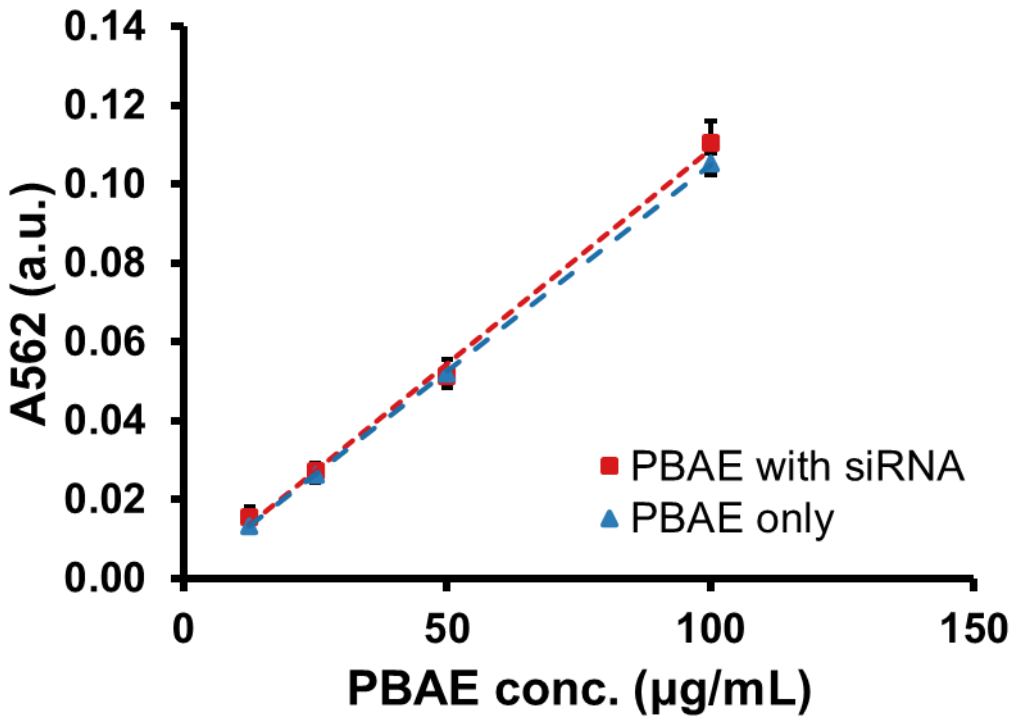


Figure S1. Micro BCA Assay for PBAE Quantitation. Serial two-fold dilutions of PBAE were made with siRNA (red squares) and without siRNA (blue triangles). siRNA was added at 12.9 µg/mL. The Micro BCA assay was performed following manufacturer's instructions. The linear regressions (represented by dashed lines) show that the presence of siRNA does not affect Micro BCA assay readings. We found that the Micro BCA assay can be appropriately repurposed to quantify PBAE loading in the LbL film.

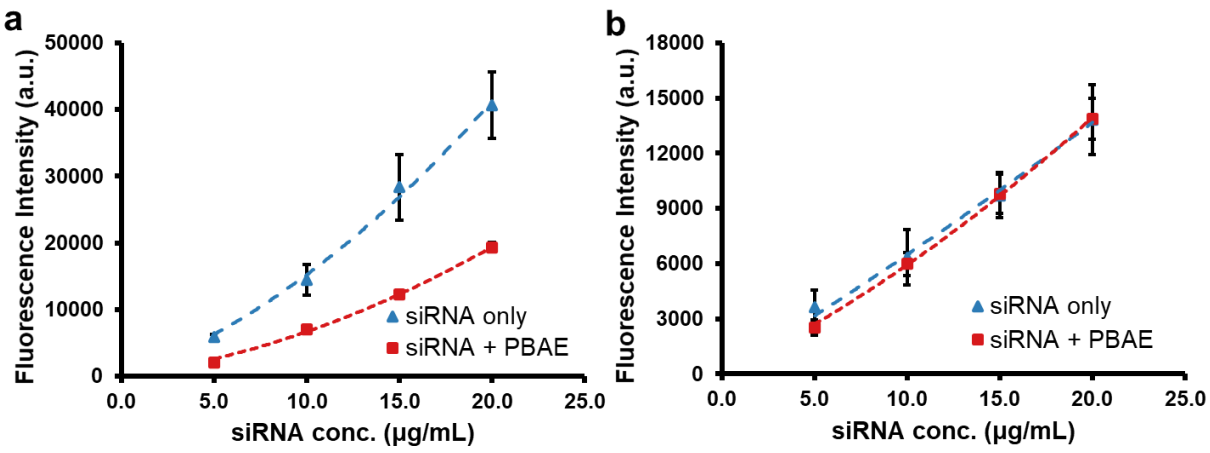


Figure S2. Ribogreen Assay for siRNA Quantitation. Standard curves with known siRNA amounts were made with PBAE (red squares) and without PBAE (blue triangles). PBAE concentration was at 100 µg/mL and siRNA was at varying concentrations. The Ribogreen assay was performed following manufacturer's instructions. Without modification (a), the presence of PBAE quenches the Ribogreen fluorescent signal considerably. To address the quenching, the PBAE was hydrolyzed using base treatment followed by acid neutralization. After hydrolysis of the PBAE (b), interference from the PBAE is shown to be minimized.

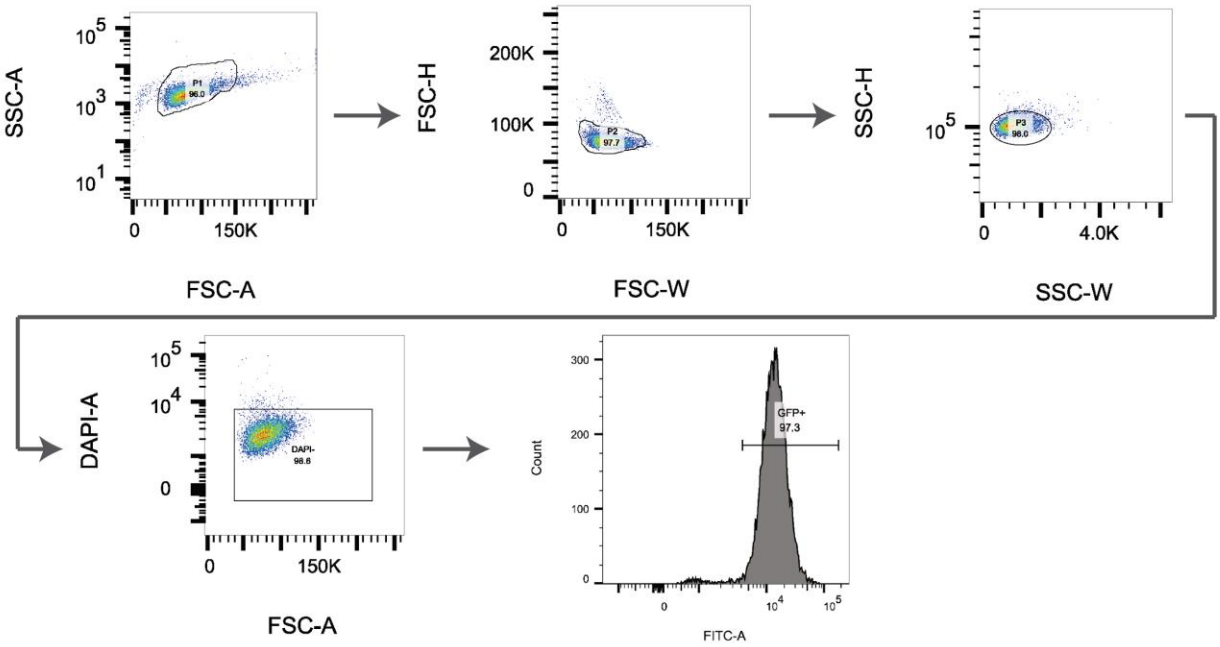
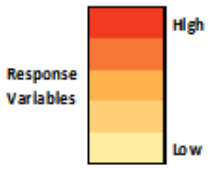


Figure S3. Gating Strategy for Flow Cytometry

Table S1. Fractional factorial design parameters and response variable data. The parameters tested include pH, ionic strength, PBAE concentration, and siRNA concentration. Ranges were determined based on parameters previously published by our lab. Response variables of PBAE loading, siRNA loading, w/w ratio, knockdown, and viability are reported for each replicate. Cells are colored to depict differences in magnitude. JMP was used to determine Runs 1a - 8c for the fractional factorial design. Runs 9a – 12c were added as midpoints for pH and polymer concentration to test for non-linearity.

Run	pH	Ionic Strength (mM)	PBAE Conc. (mg/mL)	siRNA Conc. (µg/mL)	PBAE Loading (µg/cm)	siRNA Loading (µg/cm)	w/w ratio	Knockdown (%)	Viability (%)
1A	4.5	150	2.0	20	1.02	0.64	1.58	25.52	90.0
1B	4.5	150	2.0	20	1.18	0.57	2.06	9.65	95.8
1C	4.5	150	2.0	20	1.35	0.77	1.76	18.46	96.9
2A	4.5	150	2.0	30	0.88	1.37	0.64	8.61	87.4
2B	4.5	150	2.0	30	0.88	1.02	0.86	3.73	94.9
2C	4.5	150	2.0	30	0.94	1.52	0.62	12.14	86.1
3A	4.5	250	0.5	20	0.64	1.07	0.60	2.80	95.3
3B	4.5	250	0.5	20	0.52	1.02	0.51	4.46	92.7
3C	4.5	250	0.5	20	0.55	1.17	0.47	25.21	97.1
4A	4.5	250	0.5	30	0.77	1.97	0.39	13.17	93.0
4B	4.5	250	0.5	30	0.83	2.02	0.41	8.20	95.9
4C	4.5	250	0.5	30	0.81	1.86	0.44	6.33	87.4
5A	6.0	150	0.5	20	1.44	1.60	0.90	3.32	88.8
5B	6.0	150	0.5	20	1.54	1.55	0.99	12.55	88.8
5C	6.0	150	0.5	20	1.67	1.10	1.51	6.74	83.6
6A	6.0	150	0.5	30	1.43	2.24	0.64	56.02	87.4
6B	6.0	150	0.5	30	1.29	2.06	0.63	12.86	81.2
6C	6.0	150	0.5	30	1.18	2.20	0.53	42.01	79.1
7A	6.0	250	2.0	20	0.69	0.05	12.76	0.00	92.0
7B	6.0	250	2.0	20	0.78	0.09	8.24	0.00	95.7
7C	6.0	250	2.0	20	0.53	0.11	4.86	0.00	95.4
8A	6.0	250	2.0	30	1.01	0.30	3.36	0.00	95.6
8B	6.0	250	2.0	30	0.91	0.28	3.22	0.50	93.1
8C	6.0	250	2.0	30	0.76	0.29	2.65	2.62	89.4
9A	5.2	150	1.0	20	1.08	0.99	1.09	4.42	97.9
9B	5.2	150	1.0	20	1.05	0.89	1.18	0.60	91.5
9C	5.2	150	1.0	20	1.08	0.77	1.40	1.77	94.4
10A	5.2	150	1.0	30	1.20	1.31	0.92	0.00	95.7
10B	5.2	150	1.0	30	1.18	1.63	0.73	3.04	93.7
10C	5.2	150	1.0	30	1.17	1.29	0.91	3.25	90.1
11A	5.2	250	1.0	20	1.31	0.77	1.69	0.00	89.5
11B	5.2	250	1.0	20	1.42	0.83	1.72	1.03	93.3
11C	5.2	250	1.0	20	1.51	0.57	2.65	0.00	97.2
12A	5.2	250	1.0	30	1.14	1.23	0.92	4.74	87.5
12B	5.2	250	1.0	30	1.26	1.43	0.88	5.91	89.6
12C	5.2	250	1.0	30	1.08	1.36	0.80	0.18	58.8



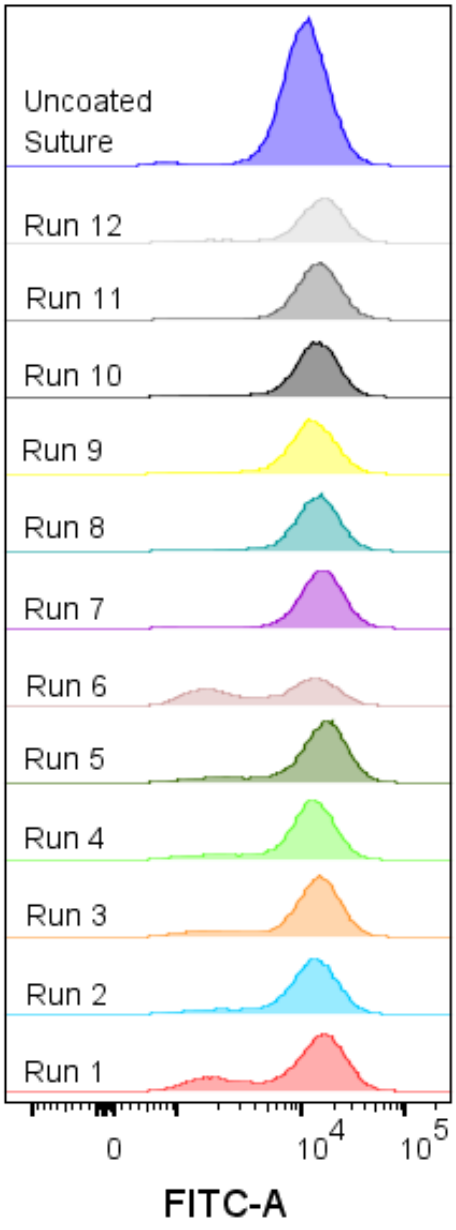


Figure S4. Averaged histogram of GFP fluorescence by run. Flow cytometry was used to measure GFP fluorescence upon treatment of cells with sutures created with different formulation conditions (different runs). A histogram of GFP (FITC channel) fluorescence averaged across the three replicates of each set of formulation conditions is shown and compared to that from an uncoated suture.

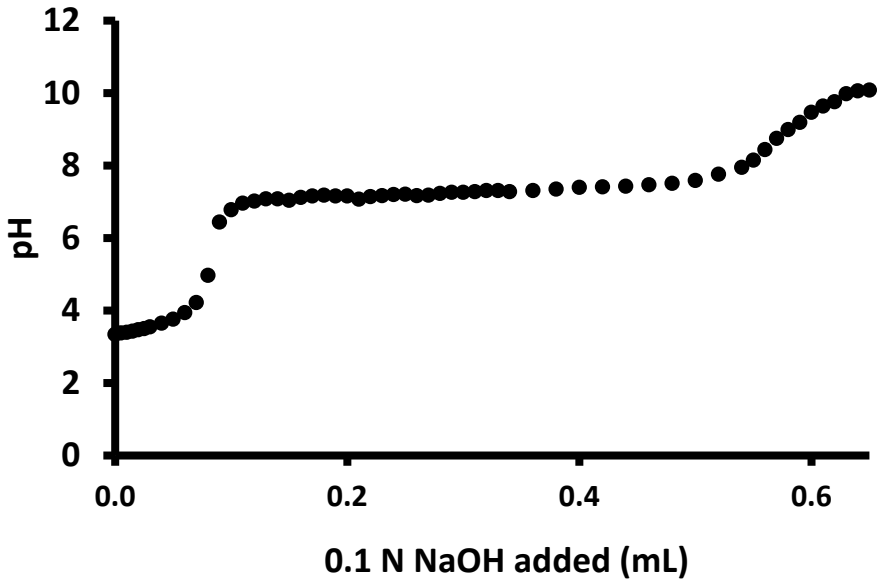


Figure S5. pH Titration of PBAE. 10.1 mg PBAE was dissolved at 1 mg/mL in water with 75 µL of 1 M HCl. 0.1 N NaOH was used for titration. The pKa relevant to our pH-responsive system was found to be 7.4. It is believed that this pKa represents the tertiary amine in the backbone of the PBAE.

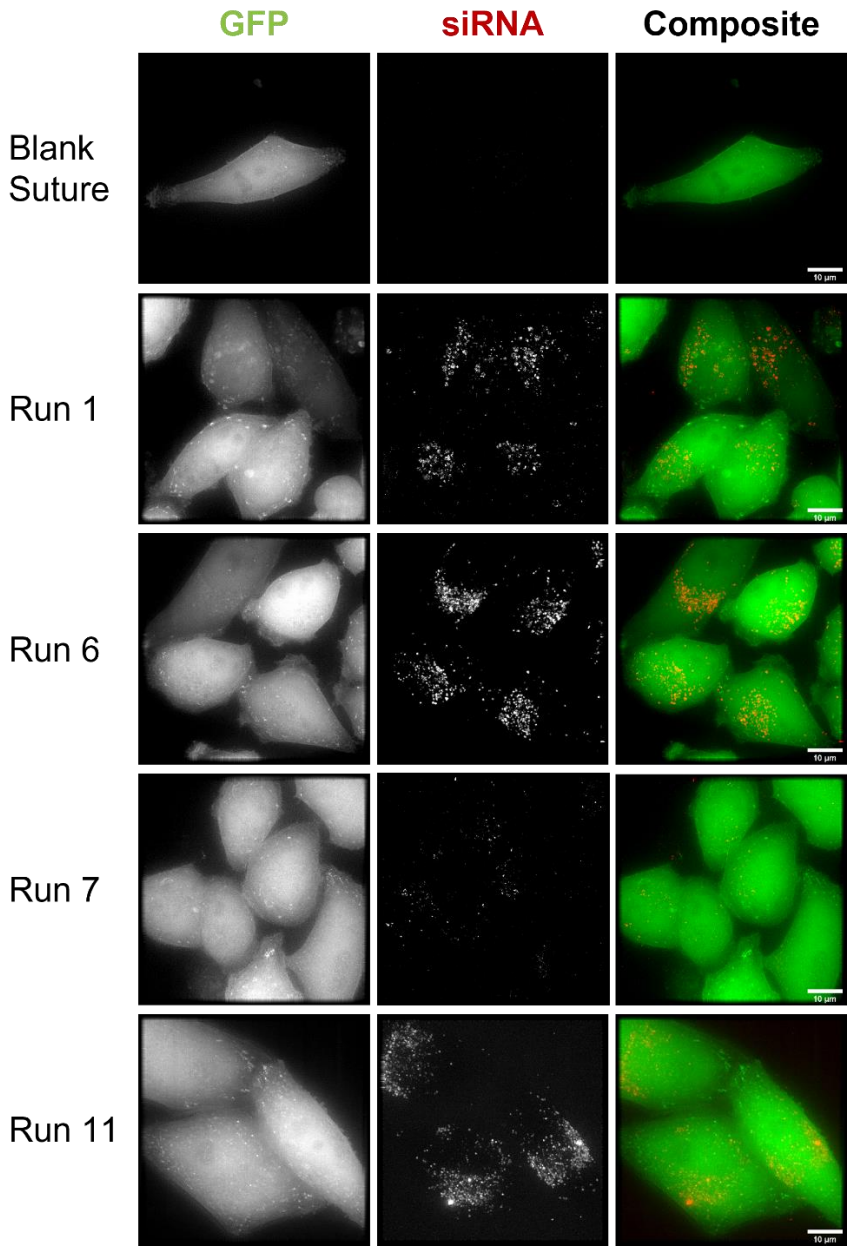


Figure S6. Fluorescence microscopy of LbL sutures with HeLa cells. siRNA-coated LbL sutures were incubated in glass chamber slides with HeLa d2eGFP cells for 24 hours. In addition to an uncoated suture, four different runs were imaged: 1 (pH 4.5, 150 mM ionic strength, 2.0 mg/mL PBAE, 20 μg/mL siRNA), 6 (pH 6.0, 150 mM ionic strength, 0.5 mg/mL PBAE, 30 μg/mL siRNA), 7 (pH 6.0, 250 mM ionic strength, 2.0 mg/mL PBAE, 20 μg/mL siRNA), and 11 (pH 5.2, 250 mM ionic strength, 1.0 mg/mL PBAE, 20 μg/mL siRNA). Scale bar indicates 10

μm.

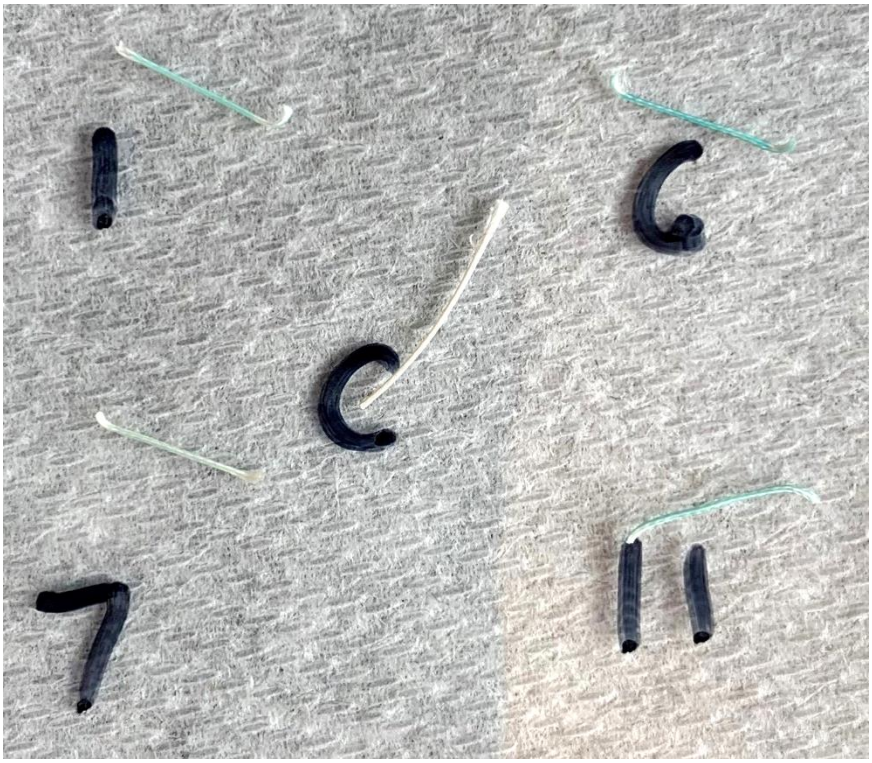
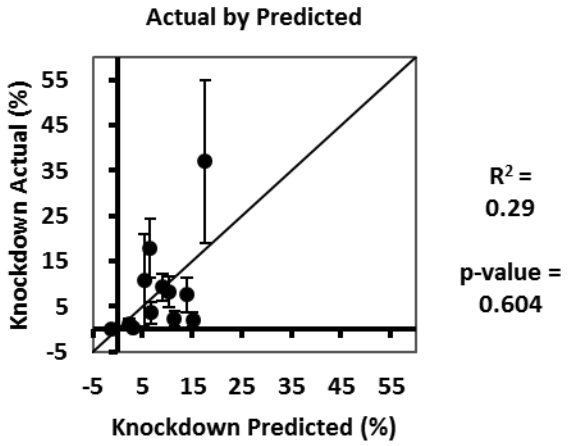


Figure S7. Digital imaging of fluorescently labeled siRNA sutures. siRNA-coated LbL sutures using an AlexaFluor647 labeled control siRNA were imaged and compared to an uncoated suture (C). Representative pieces of suture from 4 sets of solution conditions (1, 6, 7, and 11) are demonstrated. Blue color is indicative of the amount of fluorescent siRNA loaded onto the suture.

a	Knockdown	
Term	Norm. Coeff.	p-value
pH	+0.33 ↑	0.967
Ionic Strength	-8.31 ↓	0.233
PBAE Conc.	-7.03 ↓	0.389
siRNA Conc.	+3.71 ↑	0.579



b	Knockdown	
Term	Norm. Coeff.	p-value
PBAE Loading	+1.83 ↑	0.862
siRNA Loading	+25.24 ↑	0.118
w/w Ratio	+7.16 ↑	0.662

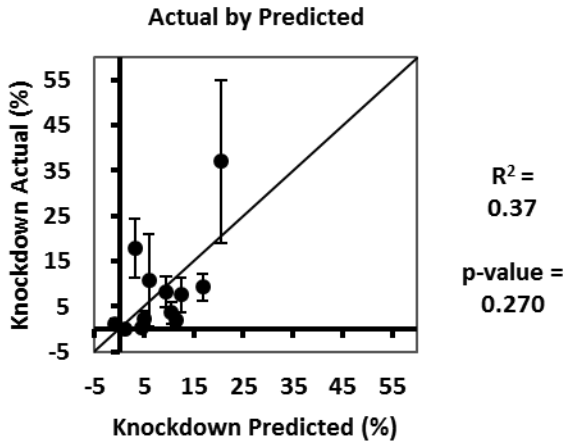


Figure S8. Standard Least Squares Fit on Knockdown. A standard least squares fit was performed for in vitro knockdown with (a) assembly parameters as explanatory variables and (b) PBAE loading, siRNA loading, and w/w ratio as explanatory variables. "Actual by Predicted" plots are shown with R^2 and p-values reported. The coefficients for each parameter were normalized by the ranges of the explanatory variables and p-values are reported. No significant parameter terms were identified for either regression (correlation p-value < 0.05). Error bars represent standard deviation.

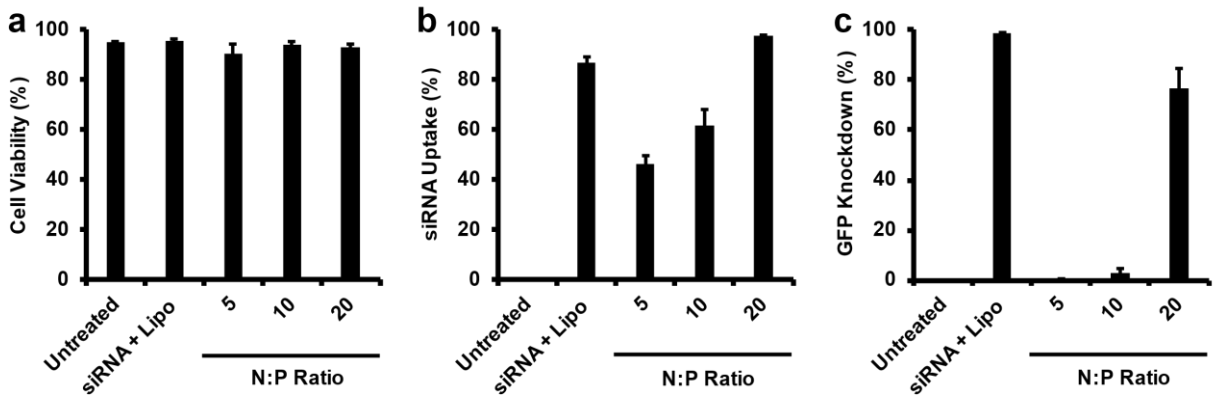


Figure S9. Polyplex transfection studies. Polyplexes were assembled by mixing PBAE and siRNA at predetermined N:P ratios of 5, 10, and 20. (A 1:1 weight ratio of PBAE to siRNA roughly equates to an N:P ratio of 2). HeLa cells expressing destabilized GFP were treated with these polyplexes, and cell viability, siRNA uptake, and GFP silencing were measured through flow cytometry. Flow cytometry was performed three days after treatment. (a) NucBlue assay was used to measure viability. (b) Alexa Fluor 647-labeled siRNA was used to track siRNA uptake. (c) GFP-targeting siRNA was used in studies of siRNA knockdown efficacy. Untreated cells served as negative controls. For positive controls, cells were treated with siRNA + Lipofectamine RNAiMAX. Error bars represent standard deviation.

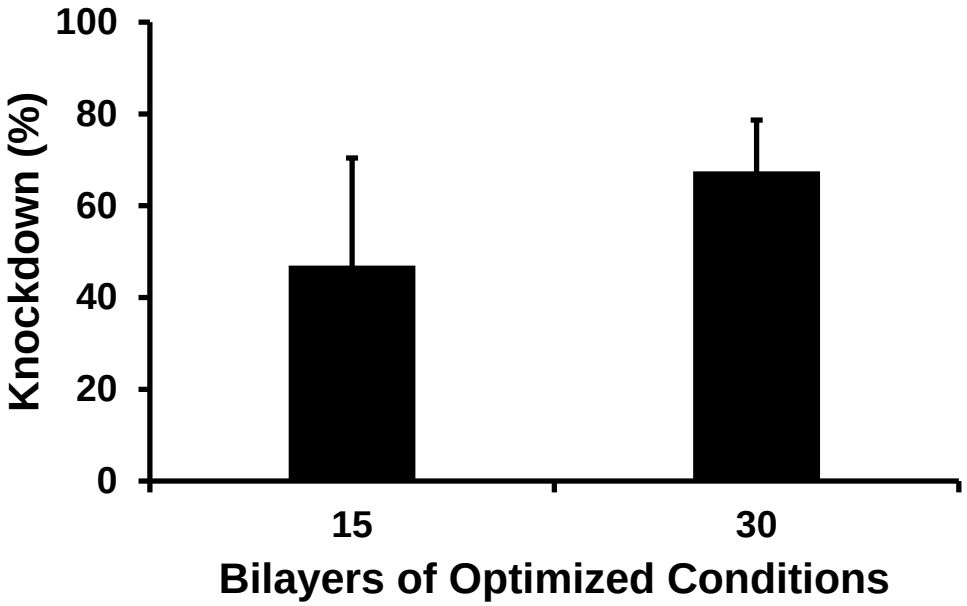


Figure S10. Knockdown comparison of optimized formulation by number of bilayers. As the number of bilayers is increased to 30, nearly 70% of siRNA knockdown is achieved. Error bars represent standard deviation.