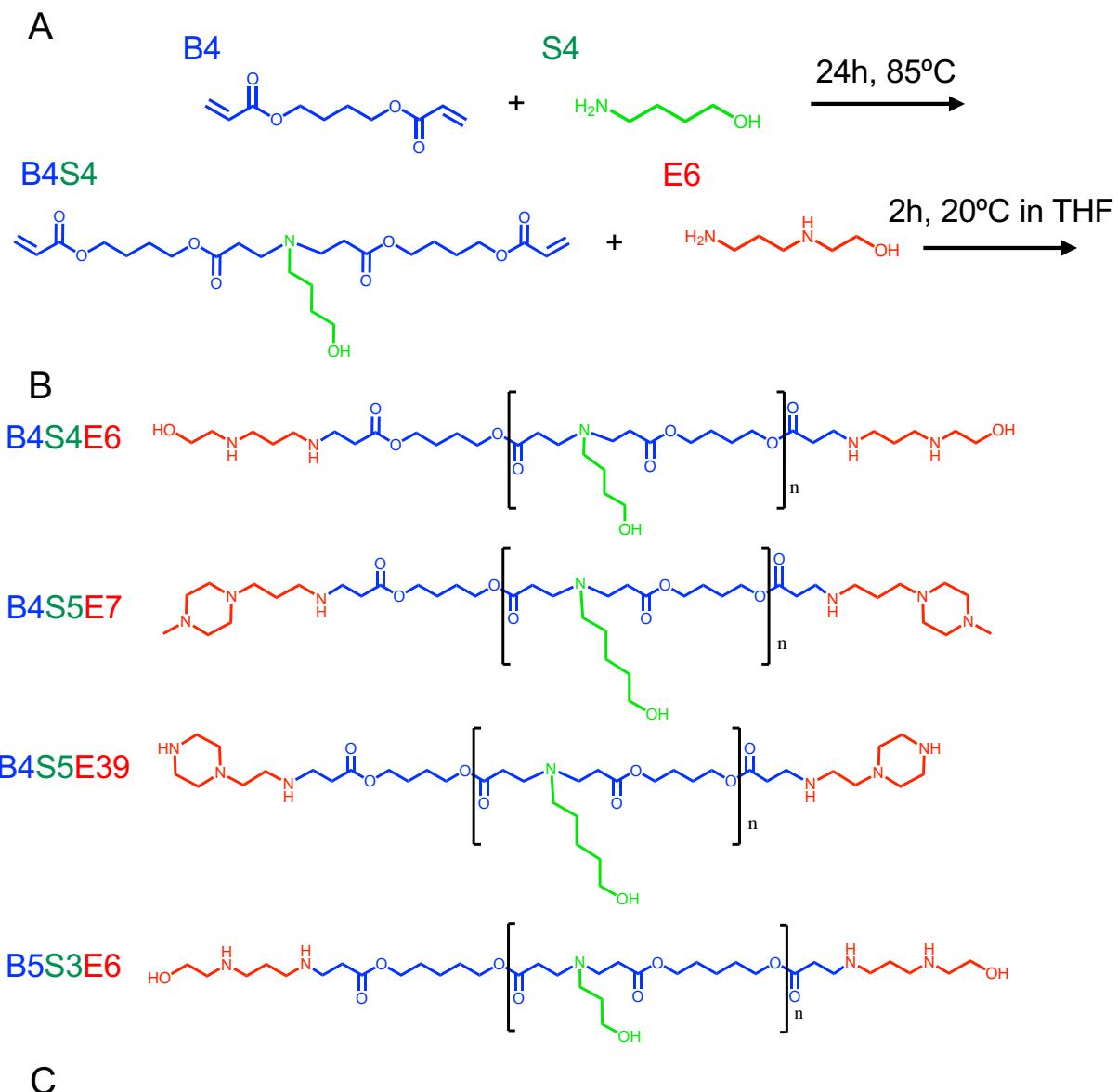


## Figure S1



C

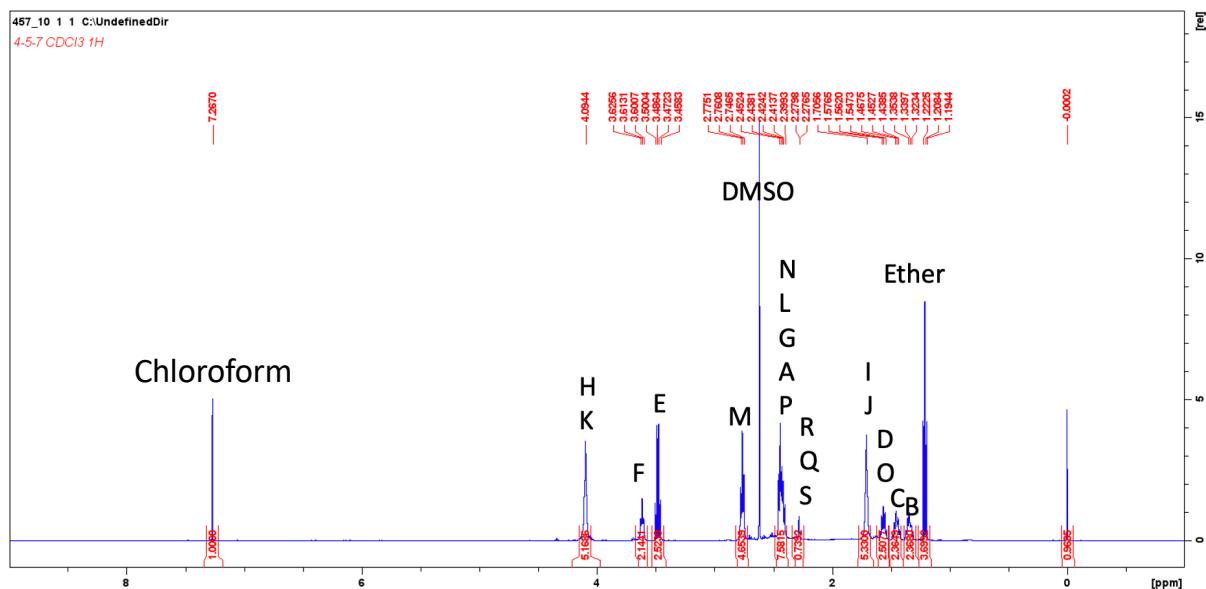
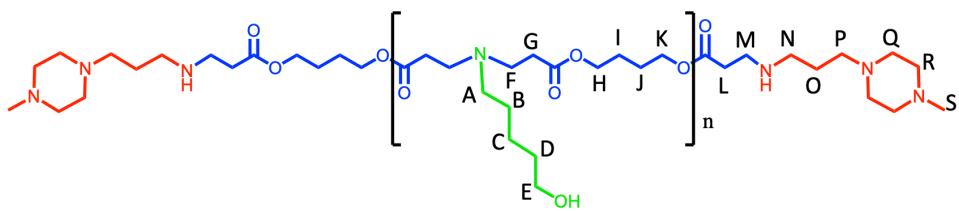
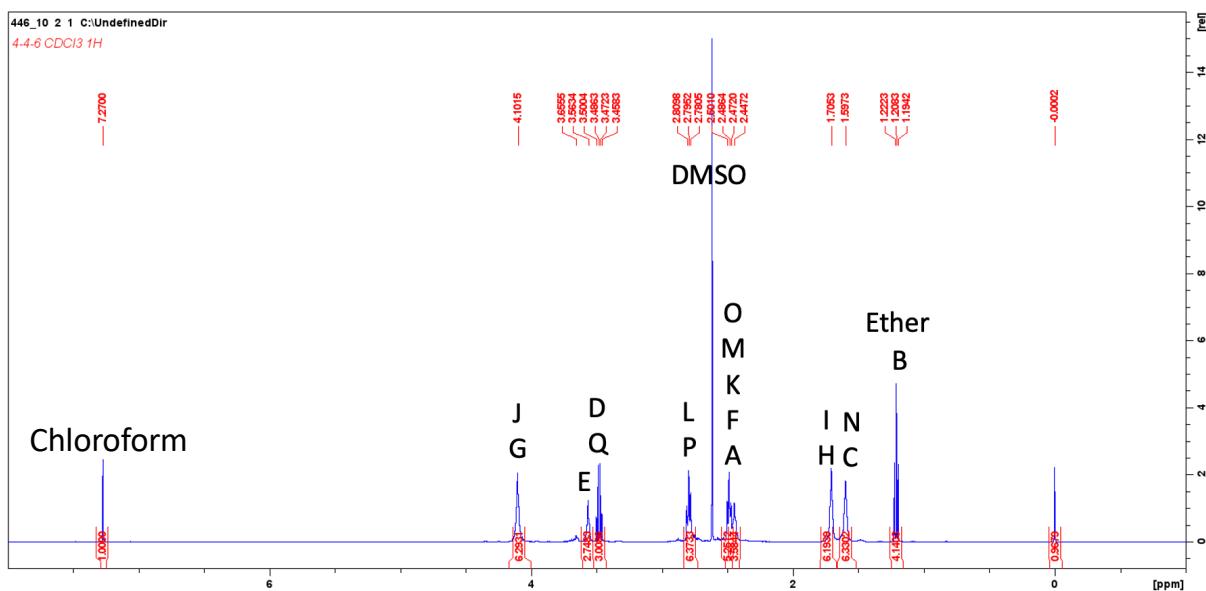
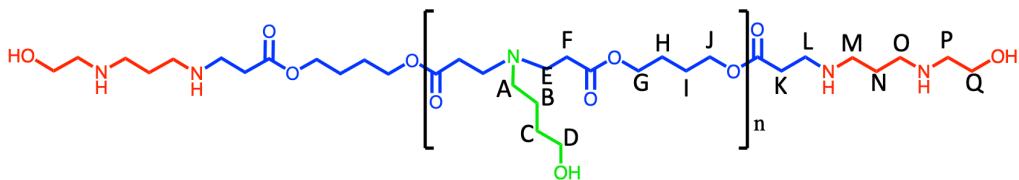
	Z Average Mean Size (nm)		PDI		Zeta Potential (mV)		
	HEK Medium	CHO Medium	HEK Medium	CHO Medium	PBS	HEK Medium	CHO Medium
4-4-6	256	335	0.09	0.12	25.5	18.4	17.3
4-5-7	291	293	0.15	0.14	25.5	8.66	18.3
4-5-39	184	248	0.07	0.13	25.8	16.7	19.5
5-3-6	214	268	0.14	0.15	26.2	17.2	17.3
PEI	1095	1274	0.33	0.71	15.0	7.49	4.62

Figure S1. PBAE synthesis and resulting polymer structures. (A) Synthesis of 4-4-6 PBAE. Acrylate and amine monomers react for 24h at 85°C, followed by an end capping reaction, resulting in a linear capped polymer. (B) Resulting structures of 4-4-6, 4-5-7, 4-5-39, and 5-3-6 polymers. (C) Dynamic light scattering measurements and electrophoretic mobility for PBAE and PEI nanoparticles in HEK or CHO medium for size measurements, or PBS, HEK, or CHO medium for zeta potential. Measurements represent mean values from  $n=3$  individually prepared replicates.

## Figure S2

A

	<b>M<sub>n</sub> (g/mol)</b>	<b>M<sub>w</sub> (g/mol)</b>	<b>PDI</b>
4-4-6	4290	15100	3.52
4-5-7	8610	43500	5.05
4-5-39	4640	14500	3.13
5-3-6	4370	10500	2.40



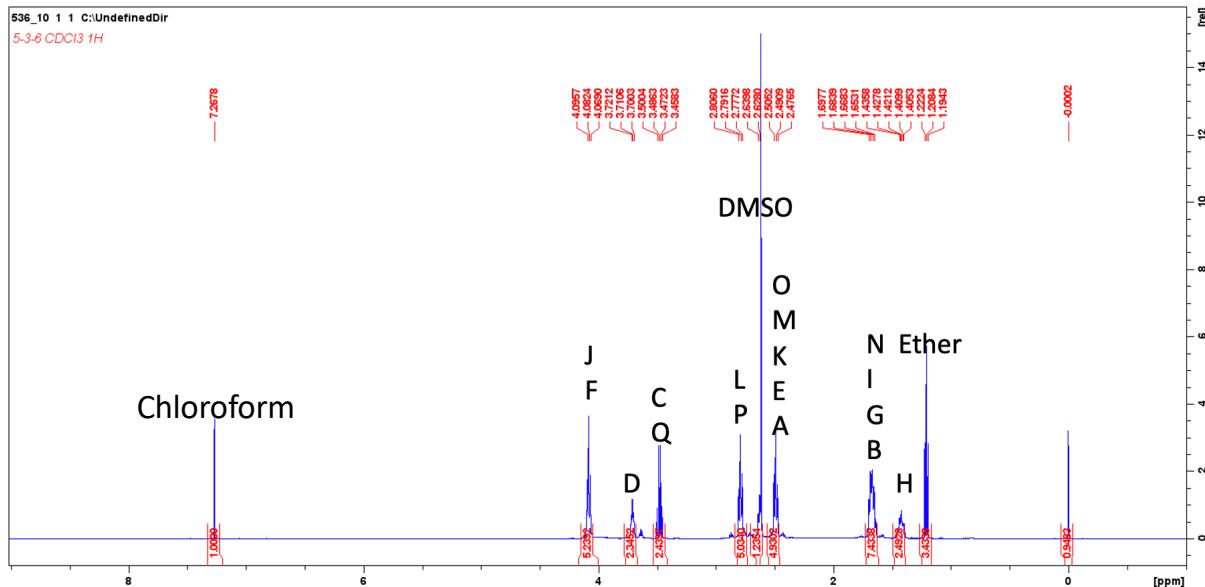
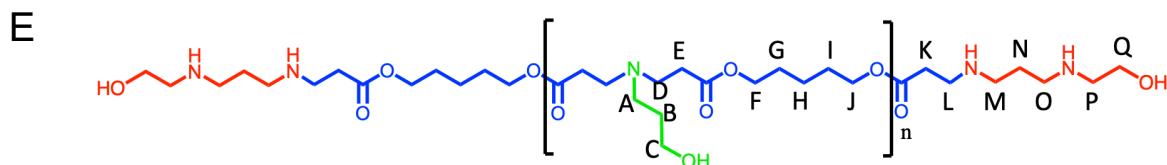
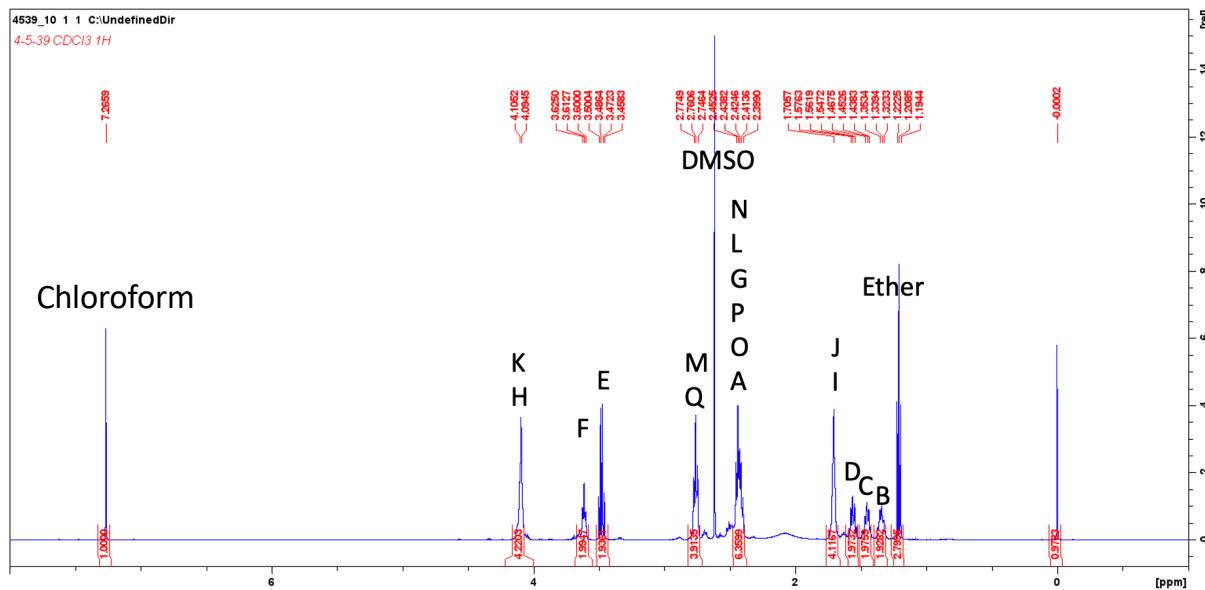
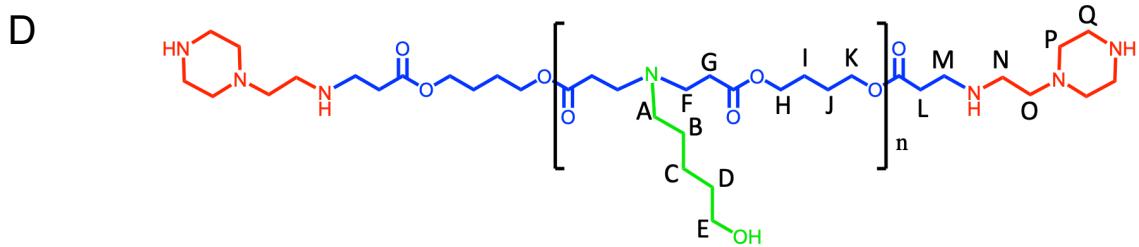


Figure S2. PBAE characterization. (A) GPC analysis of PBAE polymers. Number averaged molecular weight ( $M_n$ ), weight averaged molecular weight ( $M_w$ ), and polydispersity (PDI;  $M_w/M_n$ ). (B) NMR spectrum and peak assignments for 4-4-6. (C) NMR spectrum and peak assignments for 4-5-7. (D) NMR spectrum and peak assignments for 4-5-39. (E) NMR spectrum and peak assignments for 5-3-6.

**Figure S3**

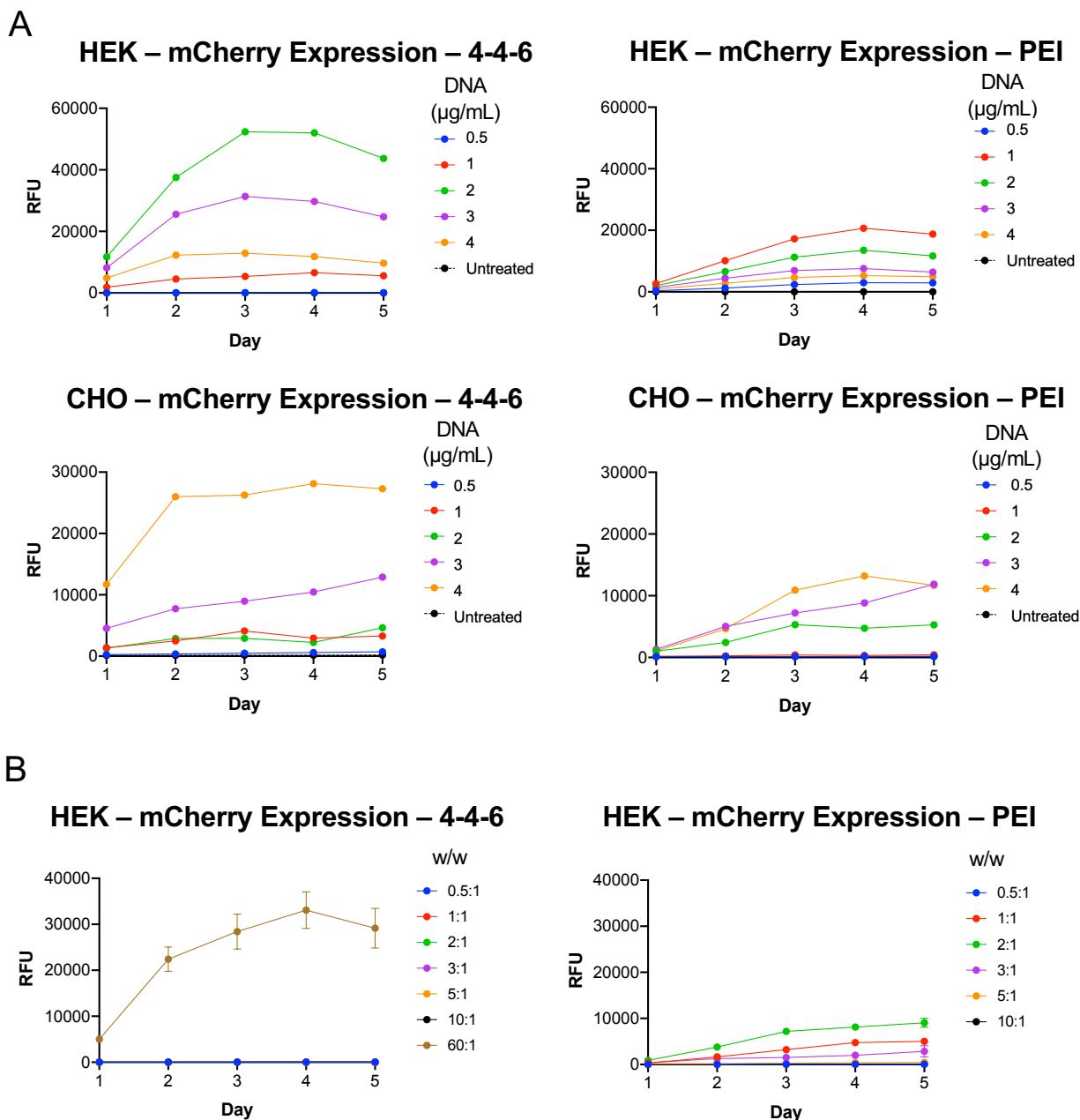


Figure S3. 4-4-6 and PEI DNA dose optimization in HEK and CHO cells. (A) DNA dose optimization in HEK and CHO cells over 5-day time courses. Cells were transfected with varying amounts of mCherry DNA and fluorescence was assessed via plate reader on each day ( $n=1$ ). In HEK cells, PEI was used at a 2:1 polymer:DNA w/w ratio, whereas in CHO cells, PEI was used at a 3:1 polymer:DNA w/w ratio. All PBAEs were used at a 60:1 polymer:DNA w/w ratio in both cell lines. (B) Polymer:DNA w/w ratio was optimized for 4-4-6 and PEI. For all conditions, 2 $\mu$ g/mL DNA was dosed with varying ratios of 4-4-6 or PEI. With 4-4-6, all conditions other than 60:1 w/w are overlapping.

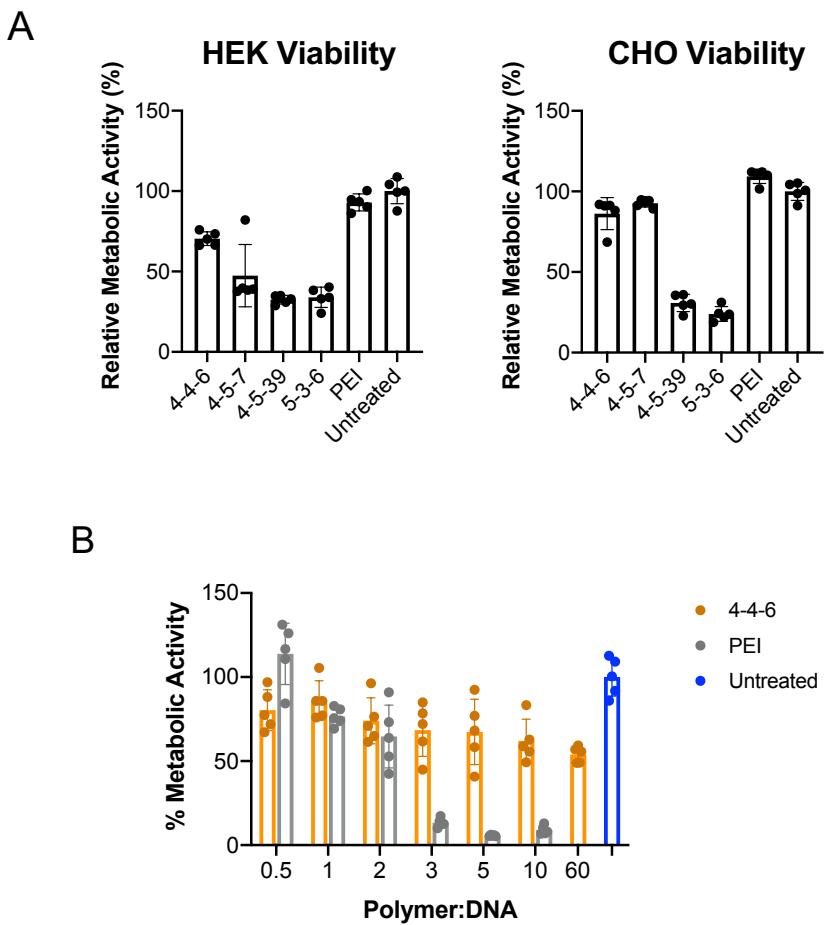
**Figure S4**

Figure S4. Viability assessment following administration of PBAE or PEI nanoparticles (A) Cell viability was assessed via MTS assay 24 h following transfection with 2  $\mu$ g/mL mCherry DNA for HEK cells or 4  $\mu$ g/mL mCherry DNA for CHO cells using 4-4-6 or PEI nanoparticles ( $n=5$ ). (B) Long term cell viability was assessed via MTS assay 5 d following transfection with 2 $\mu$ g/mL mCherry DNA in HEK cells and either 4-4-6 or PEI at various polymer:DNA w/w ratios ( $n=5$ ). PEI was not tested at 60 w/w. Error bars represent SD.

## Figure S5

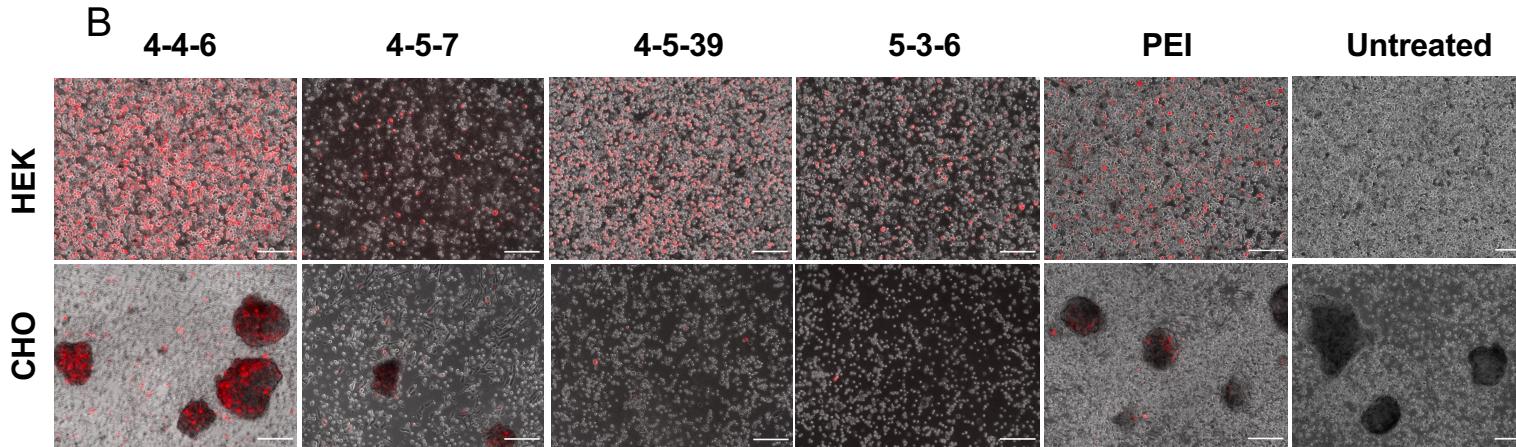
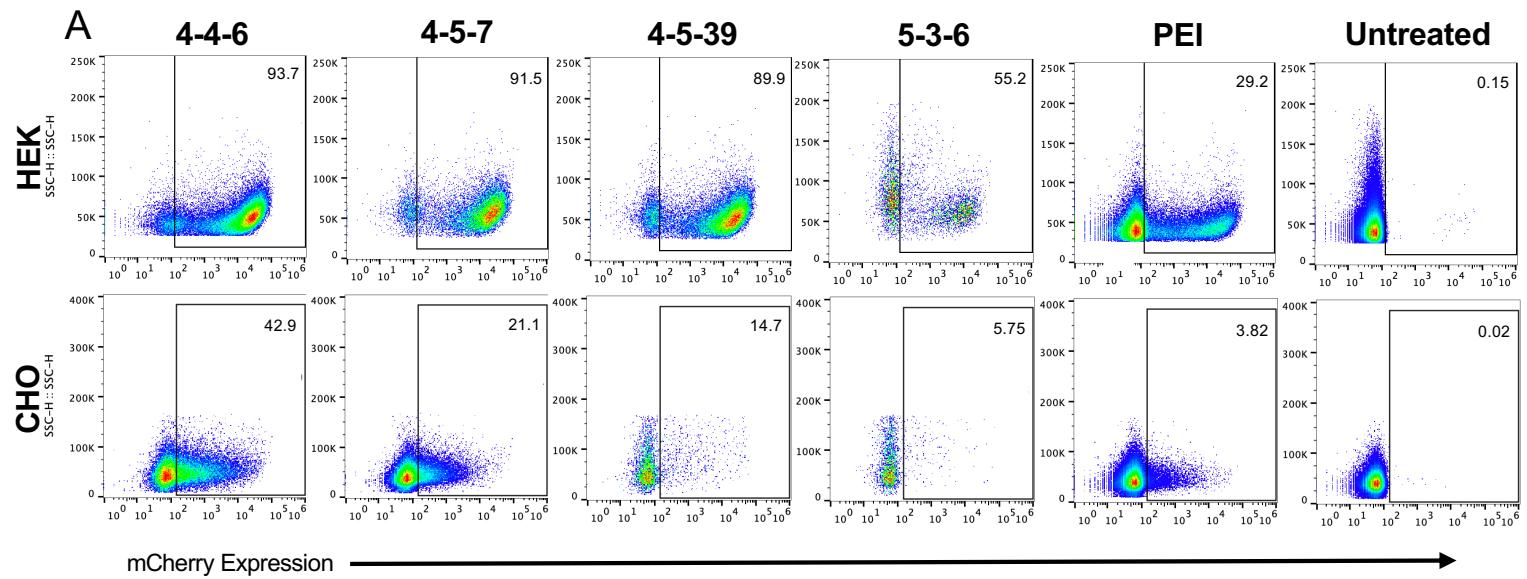
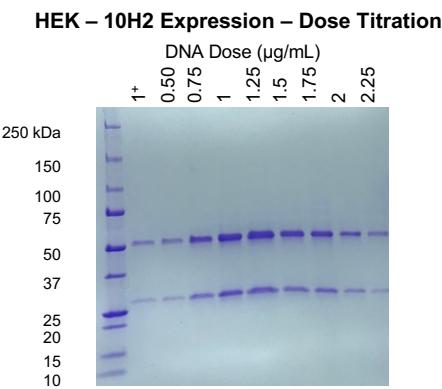


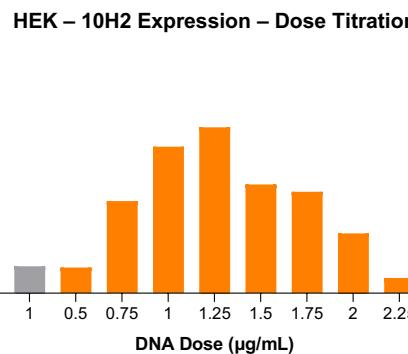
Figure S5. (A) mCherry transfection efficiency determined via flow cytometry 5 days following transfection with 2  $\mu$ g/mL mCherry DNA for HEK cells or 4  $\mu$ g/mL mCherry DNA for CHO cells using PBAE or PEI nanoparticles. mCherry+ cells were gated on live cells. Representative plots are presented (n=5). (B) Representative fluorescence microscopy images (n=5) of HEK and CHO cells 5 days following transfection with 2  $\mu$ g/mL mCherry DNA for HEK cells or 4  $\mu$ g/mL mCherry DNA for CHO cells using PBAE or PEI nanoparticles. Scale bars are 200  $\mu$ m.

## Figure S6

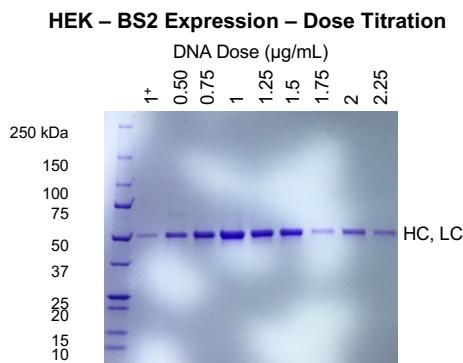
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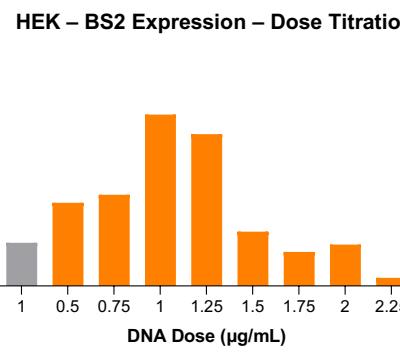
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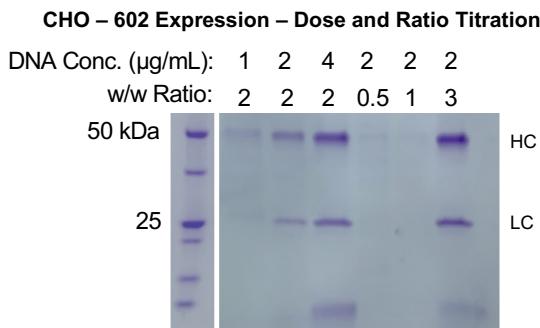
C



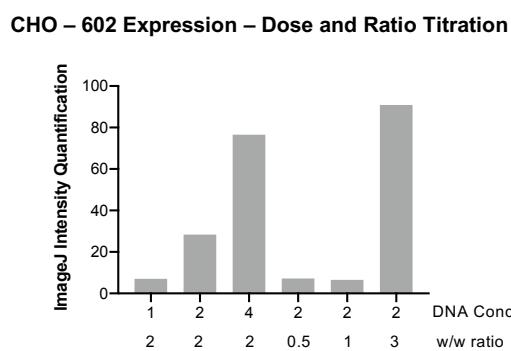
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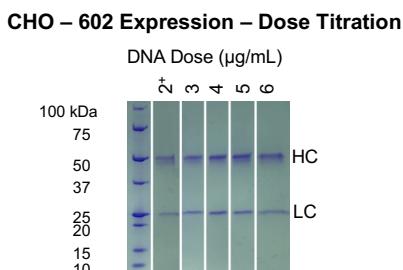
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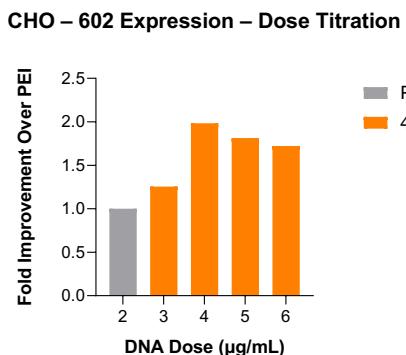
F



G



H



I

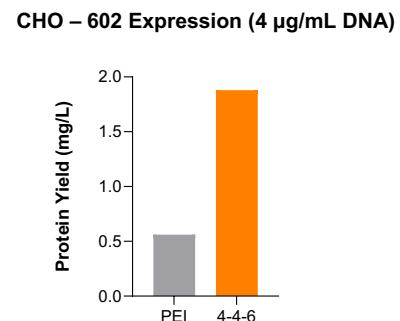


Figure S6. DNA dose optimization for transient transfection of secreted recombinant antibodies. In HEK cells, PEI was used at a 2:1 polymer:DNA w/w ratio, whereas in CHO cells, PEI was used at a 3:1 polymer:DNA w/w ratio. 4-4-6 was used at a 60:1 polymer:DNA w/w ratio in both cell lines. (A) Reducing SDS-PAGE analysis showing expression of the 10H2 monoclonal antibody following transient transfection of HEK cells with the indicated doses of DNA encapsulated in PEI (+) or 4-4-6 nanoparticles. (B) Quantification of 10H2 expression from (A), presented as fold improvement over PEI. (C) Reducing SDS-PAGE analysis showing expression of the BS2 bispecific antibody following transient transfection of HEK cells with the indicated doses of DNA encapsulated in PEI (+) or 4-4-6 nanoparticles. (D) Quantification of BS2 expression from (C), presented as fold improvement over PEI. (E) Reducing SDS-PAGE analysis showing expression of the 602 monoclonal antibody following transient transfection of CHO cells with the indicated doses of DNA encapsulated in PEI (+) or 4-4-6 nanoparticles at various w/w ratios. (F) Quantification of 602 expression from (E). (G) Reducing SDS-PAGE analysis showing expression of the 602 monoclonal antibody following transient transfection of CHO cells with the indicated doses of DNA encapsulated in PEI (+) or 4-4-6 nanoparticles. (H) Quantification of 602 expression from (G), presented as fold improvement over PEI. (I) Comparative yield (pre-FPLC) from transient transfection of CHO cells with the 602 monoclonal antibody utilizing 4-4-6 or PEI DNA-containing nanoparticles at 50 mL scale (4 µg/mL DNA dose). HC, heavy chain; LC, light chain.

## Supplemental Table S1

Table S1. Masses of monomers and volumes of solvent used to synthesize 1g batches of 4-4-6, 4-5-7, 4-5-39, and 5-3-6.

	<b>4-4-6</b>	<b>4-5-7</b>	<b>4-5-39</b>	<b>5-3-6</b>
<b>B4 (mg)</b>	706.02	674.80	674.80	
<b>B5 (mg)</b>				753.20
<b>S3 (mg)</b>				246.80
<b>S4 (mg)</b>	293.98			
<b>S5 (mg)</b>		325.20	325.20	
<b>E6 (mg)</b>	236.36			236.36
<b>E7 (mg)</b>		314.50		
<b>E39 (mg)</b>			258.39	
<b>THF (mL)</b>	6.00	6.00	6.00	6.00

## Supplemental Table S2

Table S2. Quantities of DNA, 4-4-6, and MgAc<sub>2</sub> used for expression of secreted proteins in various transfection volumes.

DNA (μg)	4-4-6 (mg)	Total volume NPs in MgAc <sub>2</sub> (μL)	Cell volume to be transfected (mL)
1000	60	40000	1000
200	12	8000	200
100	6.0	4000	100
20	1.2	800	20

### **Supplemental Table S3**

Table S3. Quantities of DNA, PEI, and OptiPro used for expression of secreted proteins in various transfection volumes.

DNA ( $\mu\text{g}$ )	PEI ( $\mu\text{g}$ )	Total volume NPs in OptiPRO ( $\mu\text{L}$ )	Cell volume to be transfected (mL)
1000	2000	40000	1000
200	400	8000	200
100	200	4000	100
20	40	800	20