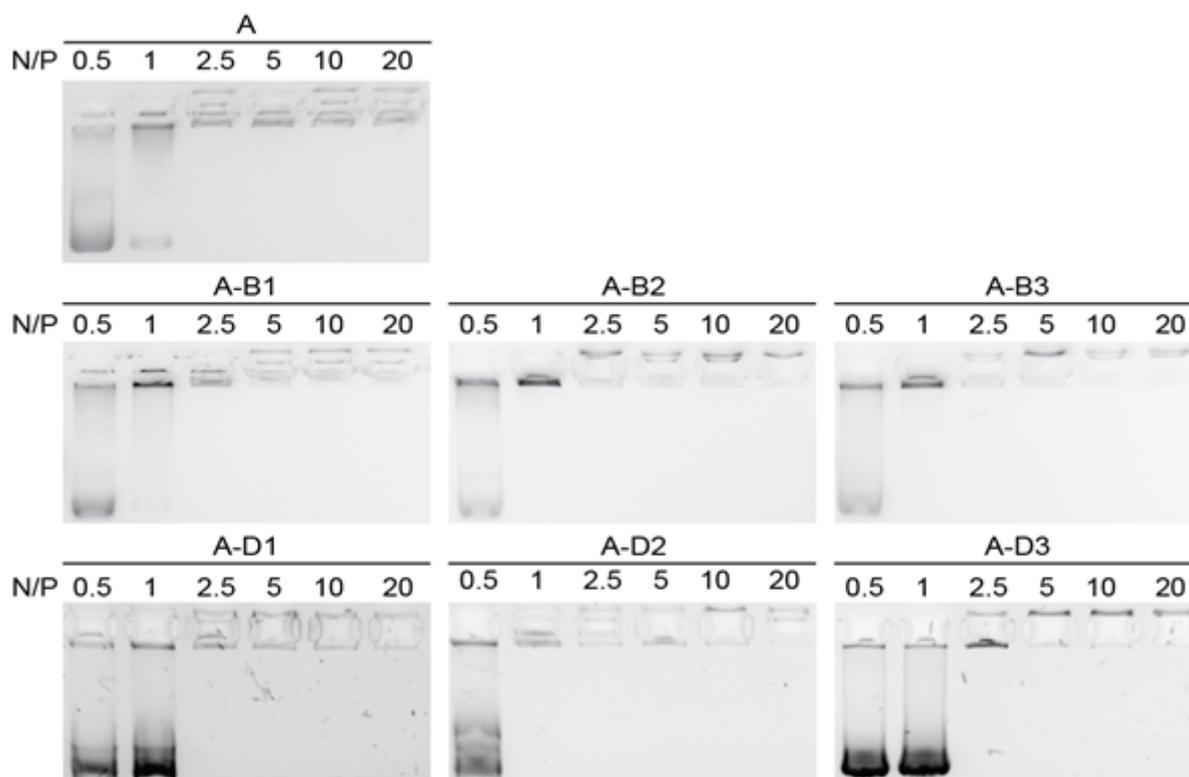


**Electronic Supplementary Information to**

**Timed-Release Polymers as Novel Transfection Reagents**

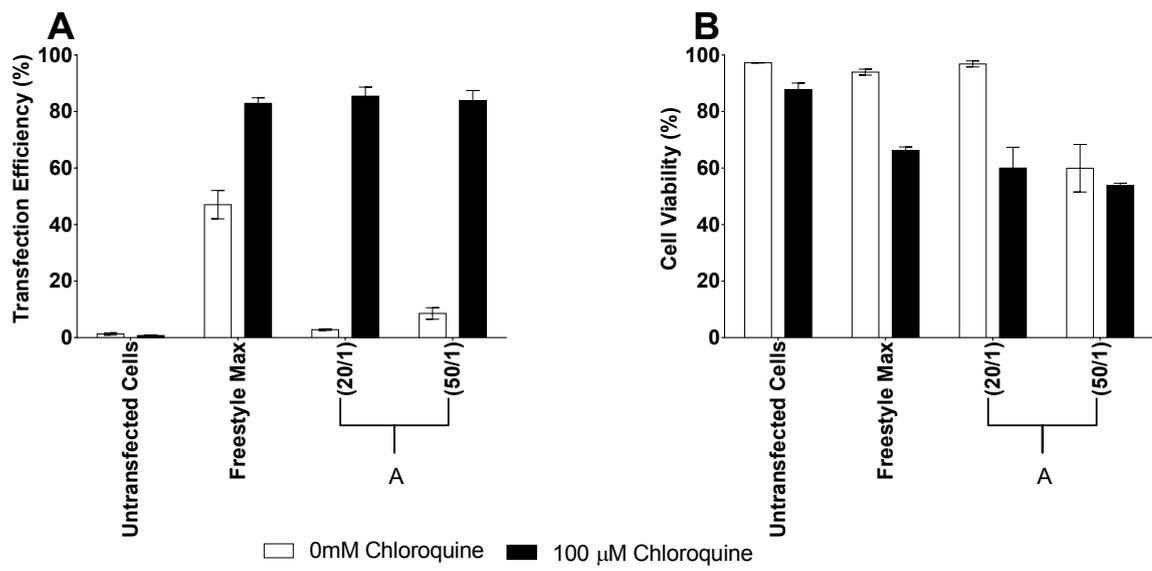
Marianne Gillard,<sup>a</sup> Zhongfan Jia,<sup>a</sup> Peter P. Gray,<sup>a</sup> Trent P. Munro<sup>ab</sup> and Michael J. Monteiro<sup>\*a</sup>



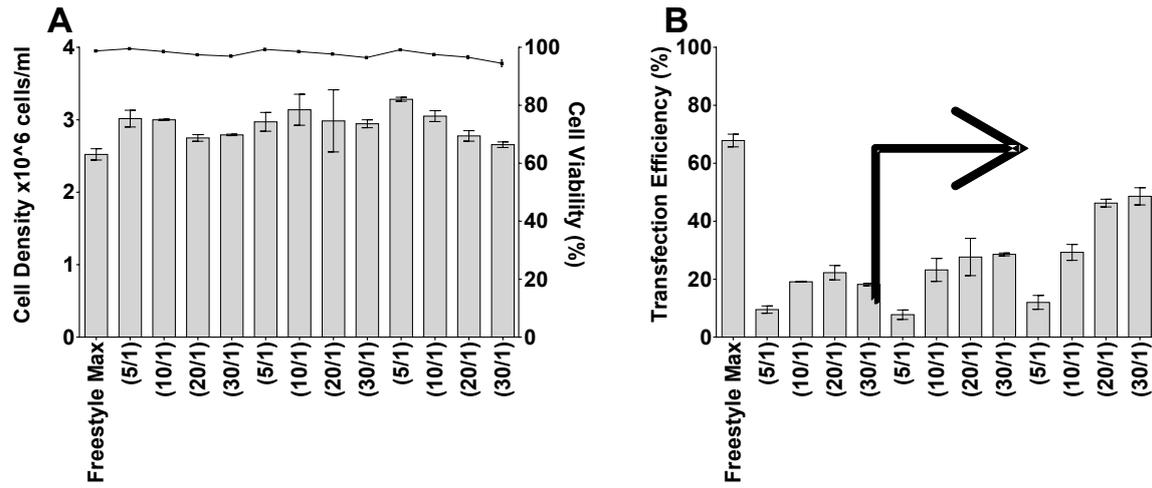
**Supplementary Figure S1.** Agarose gel retardation assay of P(DMAEA) (A), P(DMAEA-b-ImPAA) (A-B1, A-B2 and A-B3), and P(DMAEA-b-(DMA-co-BA)) (A-D1, A-D2 and A-D3) /pDNA complexes. Complexes formed using 1  $\mu$ g of pDNA at different N/P ratios (0.5-20). Complexes incubated at room temperature for 30 minutes before running on a 1% agarose gel at 90 V for 30 minutes.

**Supplementary Table S1.** Zeta potential of P(DMAEA) (A), P(DMAEA-b-ImPAA) (A-B1, A-B2 and A-B3), P(DMAEA-b-(ImPAA-co-BA)) (A-C1, A-C2 and A-C3), and P(DMAEA-b-(DMA-co-BA)) (A-D1, A-D2 and A-D3) complexes with pDNA (16 $\mu$ g) in water at different N/P ratios (5, 25 and 50). PEI/pDNA complex 4:1 (w:w) 35.2 mV. Zeta potential is an average of three measurements.

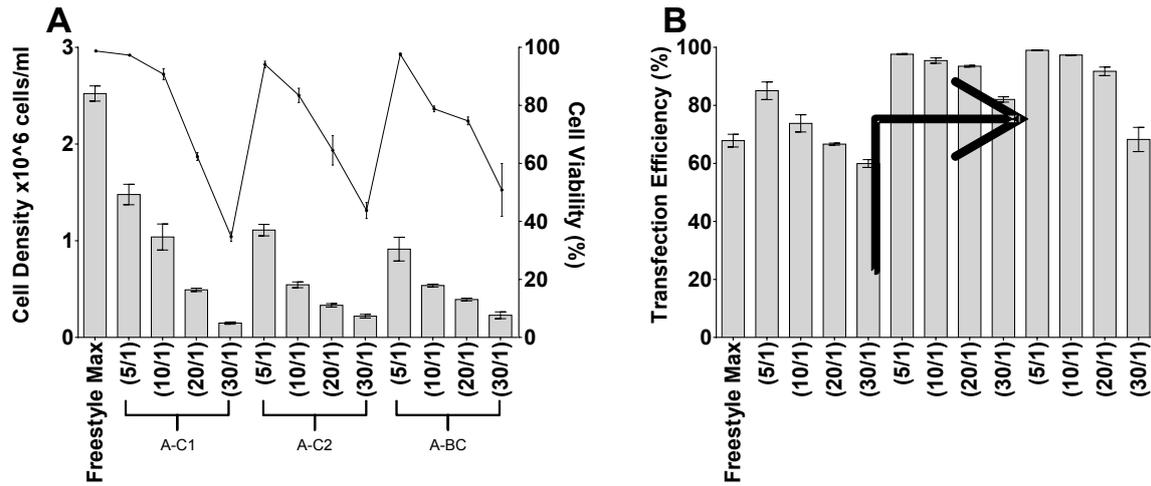
N/P Ratio	Zeta Potential mV									
	A	A-B1	A-B2	A-B3	A-C1	A-C2	A-C3	A-D1	A-D2	A-D3
5	29.5	21.87	24.17	24.30	26.60	31.57	32.67	29.63	38.13	39.80
25	23.9	22.13	25.70	23.77	36.00	34.27	37.83	33.70	35.43	33.30
50	7	15.60	18.00	15.37	30.40	28.70	33.83	34.93	30.63	31.20



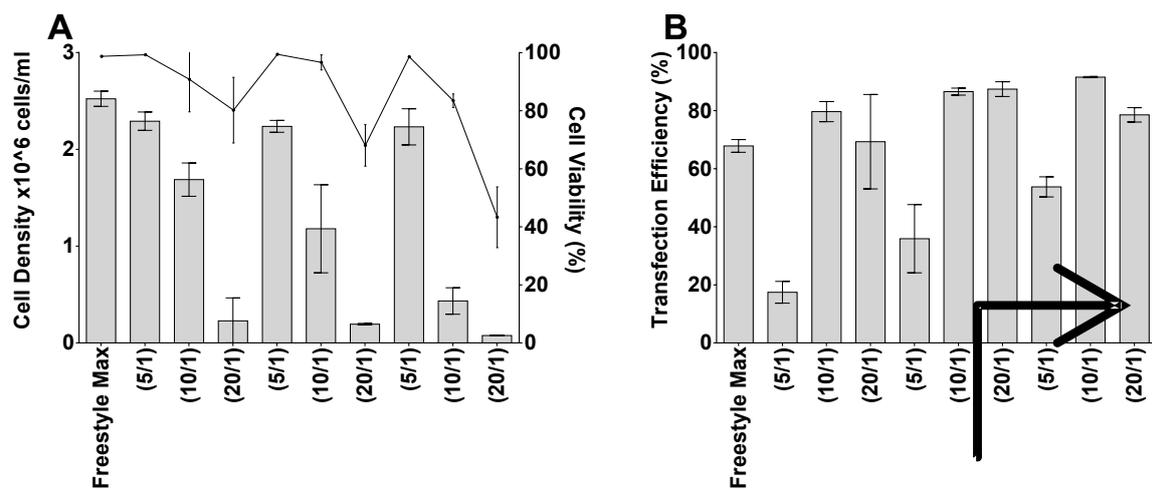
**Supplementary Figure S2.** Endosomal escape assays for P(DMAEA) (A) at various N/P ratios in HEK293 cells pre-treated with and without 100  $\mu$ M chloroquine 48 h post-transfection. (A) Transfection efficiency and (B) cell viability. Data reported as the mean  $\pm$  standard error of the mean of two replicates. N/P ratios are in parenthesis.



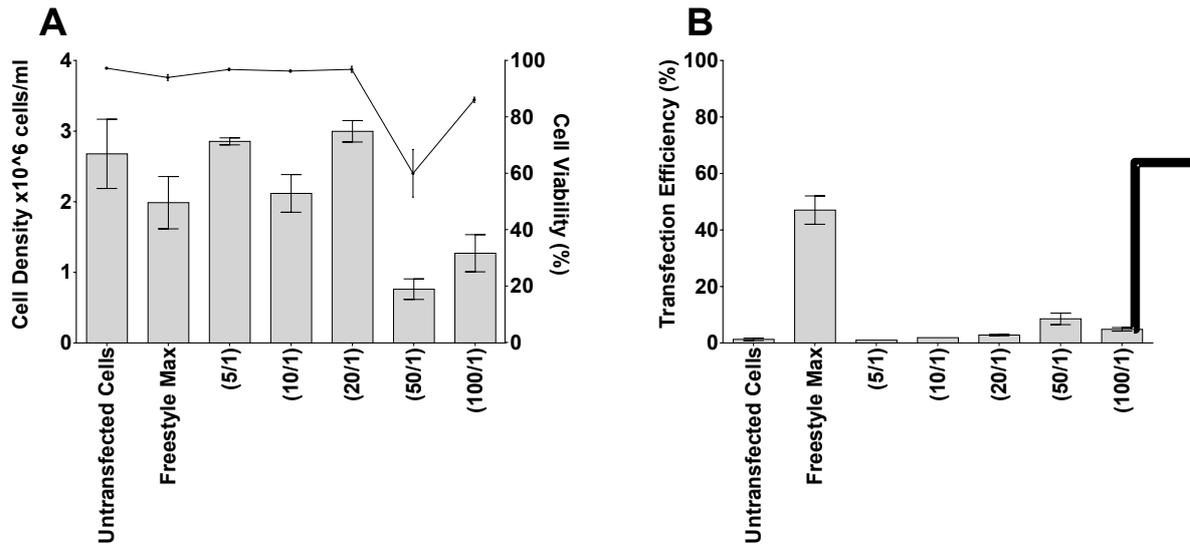
**Supplementary Figure S3.** Transfection efficiency and cellular viability of P(DMAEA-b-ImPAA) (A-B1, A-B2 and A-B3) at various N/P ratios in HEK293 cells 48 h post-transfection. (A) Cell density and viability and (B) transfection efficiency. Data reported as the mean  $\pm$  standard error of the mean of two replicates. N/P ratios are in parenthesis.



**Supplementary Figure S4.** Transfection efficiency and cellular viability of P(DMAEA-b-(ImPAA-co-BA)) (A-C1, A-C2 and A-C3) at various N/P ratios in HEK293 cells 48 h post-transfection. (A) Cell density and viability and (B) transfection efficiency. Data reported as the mean  $\pm$  standard error of the mean of two replicates. N/P ratios are in parenthesis.



**Supplementary Figure S5.** Transfection efficiency and cellular viability of P(DMAEA-b-(DMA-co-BA)) (A-D1, A-D2 and A-D3) at various N/P ratios in HEK293 cells 48 h post-transfection. (A) Cell density and viability and (B) transfection efficiency. Data reported as the mean  $\pm$  standard error of the mean of two replicates. N/P ratios are in parenthesis.



**Supplementary Figure S6.** Transfection efficiency and cellular viability of P(DMAEA) (A) at various N/P ratios in HEK293 cells 48 h post-transfection. (A) Cell density and viability and (B) transfection efficiency. Data reported as the mean  $\pm$  standard error of the mean of two replicates. N/P ratios are in parenthesis.