## **Supporting Information**

of

## **Optimization of Brush-like Cationic Copolymers for Non-viral Gene Delivery**

Hua Wei, Joshuel A. Pahang, Suzie H. Pun\*

1. Table S1 & S2

2. Figure S1-S5

Condition	No.	Time (min)	Conv. (%)	Real structure determined by NMR
[M]:[Macro- CTA]:[I] = 300:1:0.33 [M] = 1.0 M, T = 70 °C	1	40	5.8	$P(GMA)_{50}$ - $b$ - $P(HPMA)_{18}$
	2	90	9.9	$P(GMA)_{50}$ - $b$ - $P(HPMA)_{33}$
	3	280	16.8	$P(GMA)_{50}$ - $b$ - $P(HPMA)_{49}$
	4	480	27.2	$P(GMA)_{50}$ - $b$ - $P(HPMA)_{83}$

**Table S1**. Summary of  $P(GMA)_{50}$ -*b*-P(HPMA) prepared at different polymerization time using  $P(GMA)_{50}$  as a macro-CTA.

**Table S2**. Summary of  $P(GMA)_{50}$ -*b*-P(OEGMA) prepared at different polymerization time using  $P(GMA)_{50}$  as a macro-CTA.

Condition	No.	Time (min)	Conv. (%)	Real structure determined by NMR
[M]:[Macro- CTA]:[I] = 200:1:0.33, [M] = 1.0 M, T = 70 °C	1	10	3.0	$P(GMA)_{50}$ - $b$ - $P(OEGMA300)_6$
	2	20	5.5	$P(GMA)_{50}$ - $b$ - $P(OEGMA300)_{11}$
	3	30	7.5	$P(GMA)_{50}-b-P(OEGMA300)_{15}$
	4	40	11.5	$P(GMA)_{50}$ -b- $P(OEGMA300)_{23}$



Figure S1.<sup>1</sup>H NMR spectra of (a1) P(GMA)<sub>50</sub> in DMSO; (a2) P(HPMA)<sub>33</sub>-*b*-P(GMA)<sub>50</sub> in DMSO; (a3) P(OEGMA)<sub>15</sub>-*b*-P(GMA)<sub>50</sub> in CDCl<sub>3</sub>; (b1) P(GMA-TEPA)<sub>50</sub> in D<sub>2</sub>O; (b2)
P(HPMA)<sub>33</sub>-*b*-P(GMA-TEPA)<sub>50</sub> in D<sub>2</sub>O; (b3) P(OEGMA)<sub>15</sub>-*b*-P(GMA-TEPA)<sub>50</sub> in D<sub>2</sub>O.



**Figure S2**. Buffering capacity of various P(GMA-oligoamine)<sub>50/100</sub> obtained by titrating polymer aqueous solution (0.2 mg/mL) in 0.15 M aqueous NaCl (pH 10, adjusted with NaOH) with 0.1 M HCl. As a reference, the titration curve of 25 kDa bPEI is also presented.



N/P 0 0.5 1 1.5 2 2.5 3 3.5 4 N/P 0 0.5 1 1.5 2 2.5 3 3.5 4 N/P 0 0.67 1.33 2 2.67 3.33 4 4.67 5.33

**Figure S3**. Agarose gel electrophoresis of various P(GMA-oligoamine) polyplexes formed by complexation with plasmid DNA at N/P ratios ranging from 0/1 to 5.5/1.



**Figure S4**. Transfection efficiency (A) and relative cell viability (B) of polyplexes formed by  $P(HPMA)_{33}$ -*b*-P(GMA-TEPA/PEHA)\_{50}. Data are shown as mean  $\pm$  SD (n = 3).



Figure S5. Average size distributions (PDIs) of various polyplexes formed at different N/P ratios in 150 mM PBS.