Supporting Information

Biodegradable DNA Nanoparticles that Provide Widespread Gene Delivery in the Brain

Panagiotis Mastorakos, Eric Song, Clark Zhang, Sneha Berry, Hee Won Park, Young Eun Kim, Jong Sung Park, Seulki Lee, Jung Soo Suk, Justin Hanes**

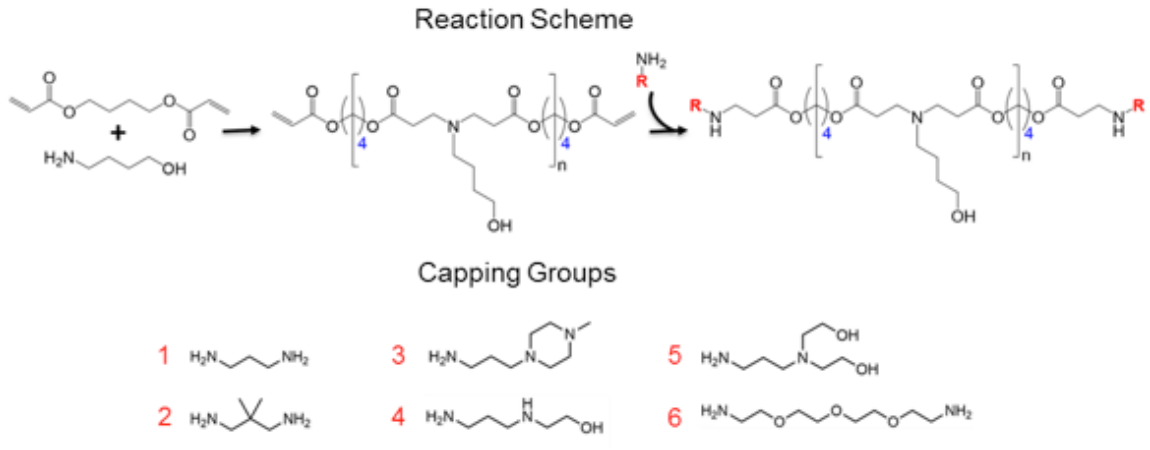

Figure S1. PBAE polymer synthesis. Uncapped PBAE polymers were synthesized by a Michael addition reaction of 1,4-butanediol diacrylate and 4-amino-1-butanol at a ratio of 1.1 or 1.2 to yield PBAE of 6 kDa (n=19-20) and 4kDa (n=13-14), respectively. Subsequently, 4 kDa PBAE was capped with group 1 and and 6 kDa PBAE was capped with one of the selected capping groups 1-6. Of note, PBAE-BPN formulated with PBAE polymer with the capping group 4 were further explored *in vitro*, *ex vivo* and *in vivo* in this study.

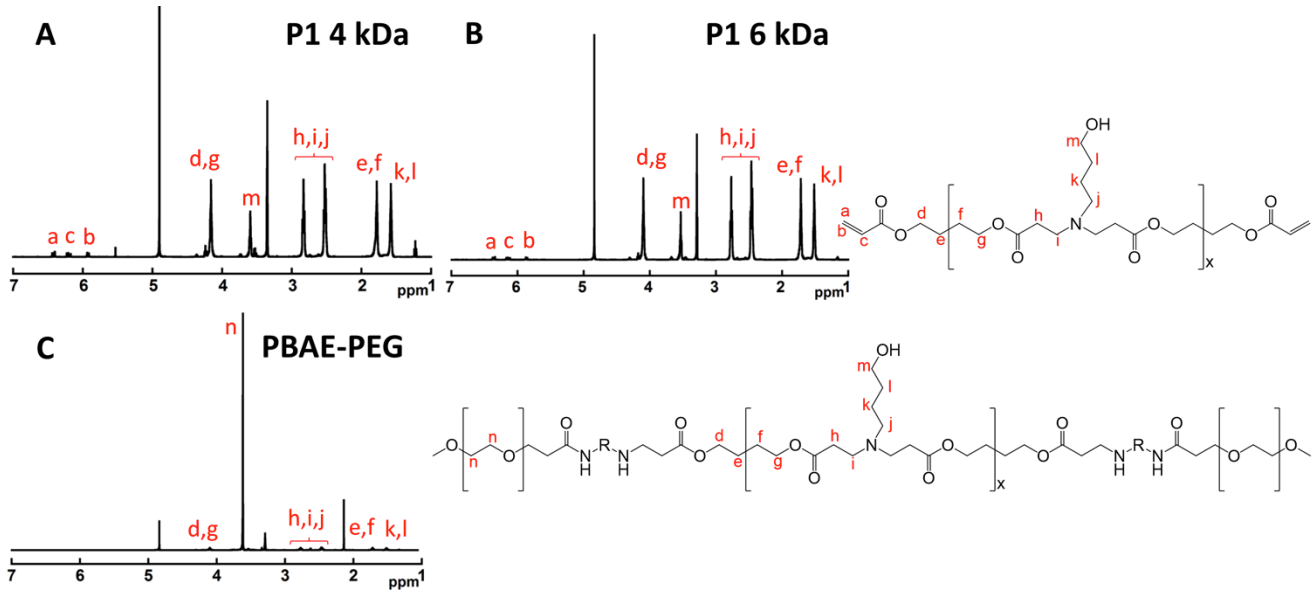

Figure S2. Structures and ^{1}H NMR spectra of respective PBAE polymers and intermediates. NMR spectra of **(A)** 4 kDa uncapped PBAE polymer synthesized by a Michael addition reaction of 1,4-butanediol diacrylate and 4-amino-1-butanol (PBAE; x = 19-20), **(B)** 6 kDa PBAE (x = 13-14), **(C)** PEGylated PBAE at a 2:1 PEG to PBAE molar ratio.

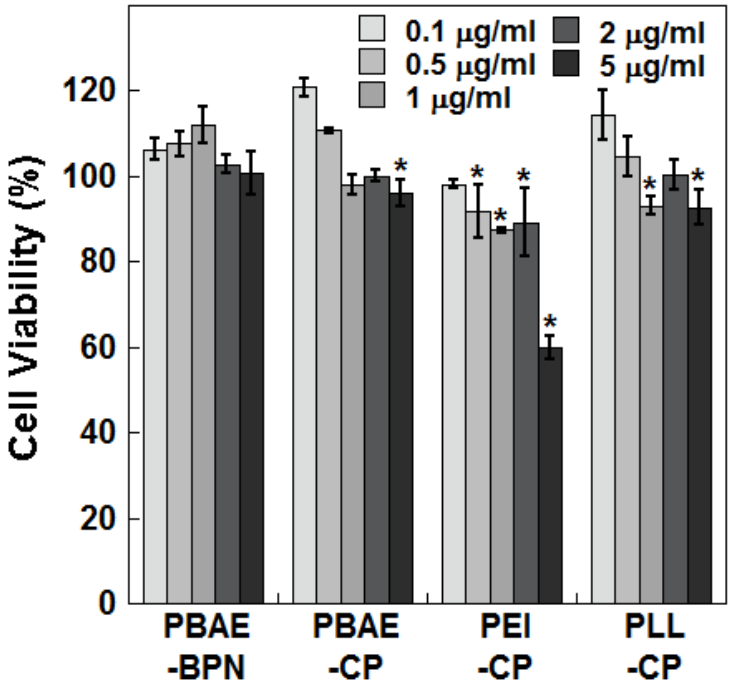

Figure S3. DNA-NP safety profile. HT22 hippocampal neuronal cells were treated with varying concentrations of PBAE-BPN and conventional DNA-NP (PBAE-CP, PEI-CP and PLL-CP). Cell viability was measured after 24 h of incubation and compared to untreated controls. Data are represented as mean ± SEM. *Denotes statistically significant difference from 100% viability ($p < 0.05$).

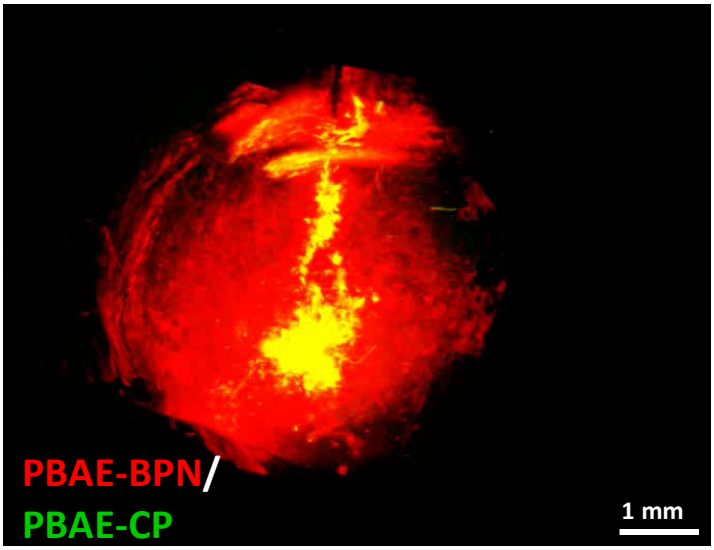

Figure S4: 3D re-constitution of representative Cy3-labeled PBAE-CP (green) and Cy5-labeled PBAE-BPN (red) distribution in the rat striatum following co-administration using CED. Co-localization is depicted in yellow. Scale bar = 1 mm.

Table S1. Effect of PBAE weight % on DNA-NP formulation.

PBAE weight % of PEG-PBAE:PBAE	Hydrodynamic Diameter ± SEM (nm)	ζ-potential ± SEM (mV)	PDI[a]
100:0	107 ± 10	-0.5 ± 0.2	0.36
60:40	56 ± 2.0	0.9 ± 0.3	0.16

Table S2. Physicochemical properties and stability in aCSF of PBAE-BPN formulations based on PBAE polymers with varying capping groups (Figure S1).

Capping group	Hydrodynamic Diameter ± SEM (nm)	PDI	ζ-potential ± SEM (mV)	Hydrodynamic Diameter ± SEM (nm) in aCSF (1 h)
1	49 ± 2	0.20	2.1 ± 0.1	55 ± 18
2	52 ± 3	0.14	0.9 ± 0.5	57 ± 3
3	50 ± 1	0.15	2.2 ± 0.2	49 ± 3
4	55 ± 1	0.15	1.7 ± 0.4	48 ± 5
5	56 ± 1	0.12	1.3 ± 0.6	53 ± 1
6	50 ± 2	0.14	0.9 ± 5	47 ± 3