Supporting information

# Engineering the Aromaticity of Cationic Helical Polypeptides toward "Self-Activated" DNA/siRNA Delivery

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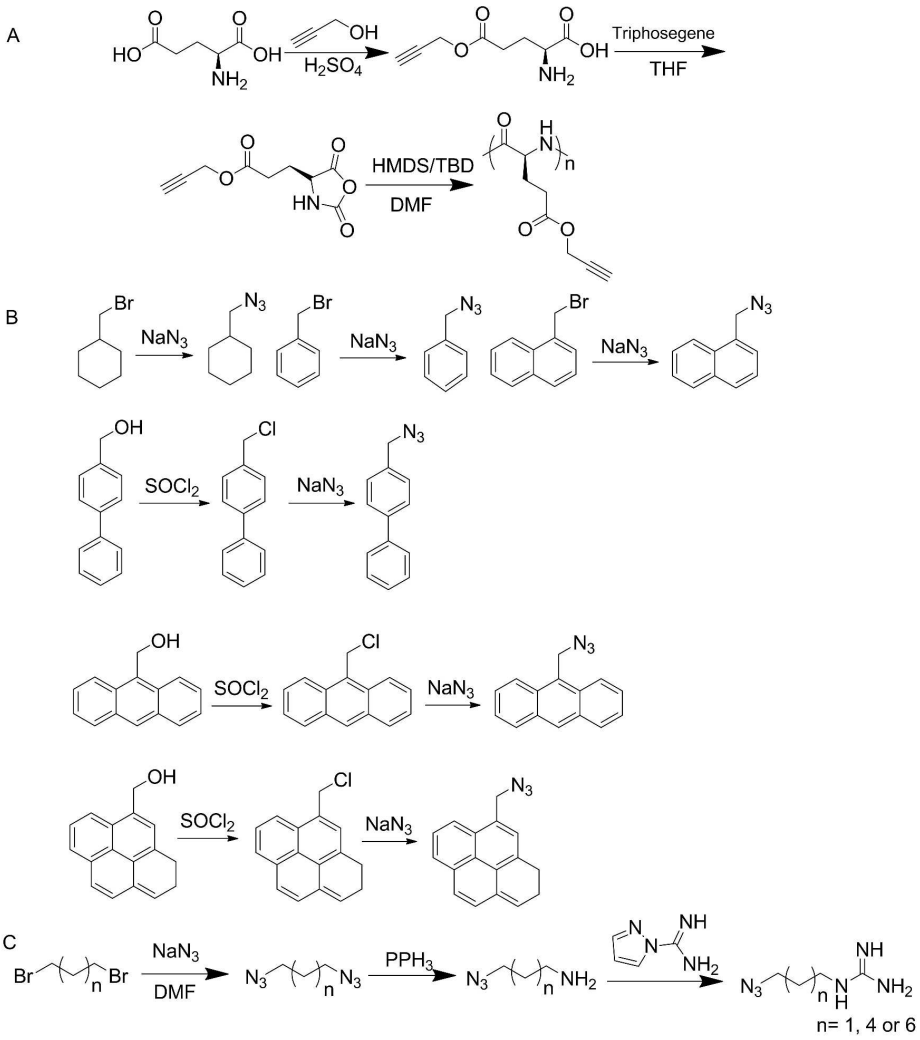
**1. Instrumentation.**

$^1$H NMR spectra were recorded on a Varian U400 MHz spectrometer. Gel permeation chromatography (GPC) experiments were conducted on a system equipped with an isocratic pump (Model 1260, Agilent Technology), a multi-angle laser light scattering (MALLS) detector (Agilent Technology), and a refractive index detector (Agilent Technology). Separations were performed using serially connected size exclusion columns (5 μm, Agilent Technology) using DMF containing 0.05 M LiBr as the mobile phase. The MWs were determined based on the d*n*/d*c* value of polymers calculated offline by using the internal calibration system processed by the same software. Circular dichroism (CD) experiments were performed on a JASCO J-815 CD spectrometer. Polypeptides were dissolved in deionized (DI) water at the concentrations of 0.02 mg/mL. The solution was placed in a quartz cell with a light path of 2 mm. The mean residue molar ellipticity of each polypeptide was calculated based on the measured apparent ellipticity by the following equation: Ellipticity ([θ] in deg·cm$^2$·dmol$^{-1}$) = (millidegrees × mean residue weight)/(pathlength in millimeters concentration of polypeptide in mg mL$^{-1}$). The helicity of the polypeptides was calculated by the following formula: helicity = (-[$\theta_{222}$] + 3000)/39000.[1]




**Scheme S1.** Synthetic routes of alkyne-containing polypeptides (A), small molecular aliphatic and aromatic azides (B), and azide-guanidine small molecules.

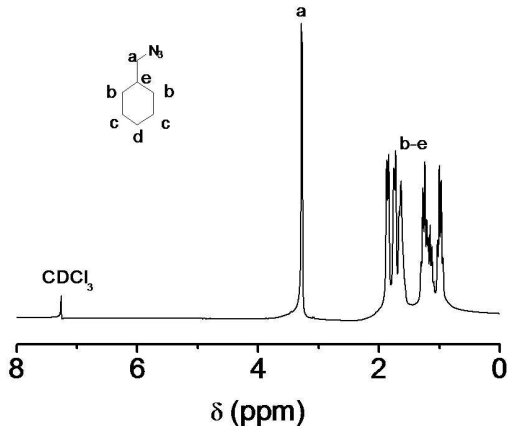



**Figure S1.** $^{1}H$ NMR spectrum of Cy-$N_3$ ($CDCl_3$, 400 MHz).

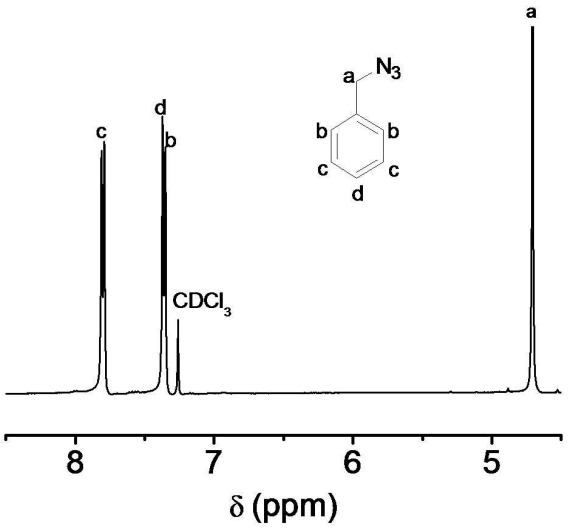



**Figure S2.** $^{1}$H NMR spectrum of Bn-$N_3$ ($CDCl_3$, 400 MHz).

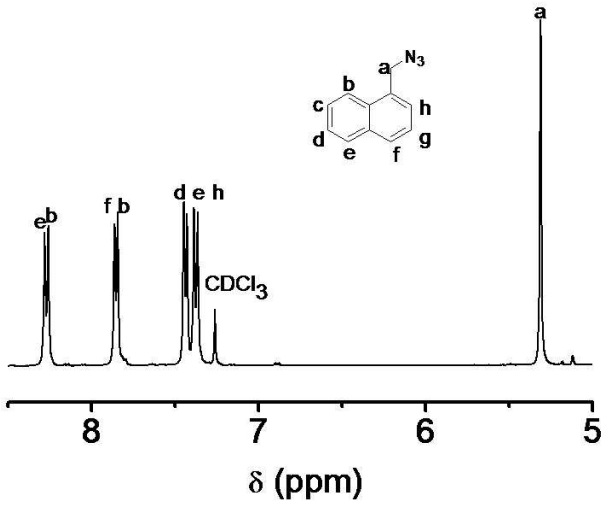



**Figure S3.** $^{1}$H NMR spectrum of Naph-$N_3$ ($CDCl_3$, 400 MHz).

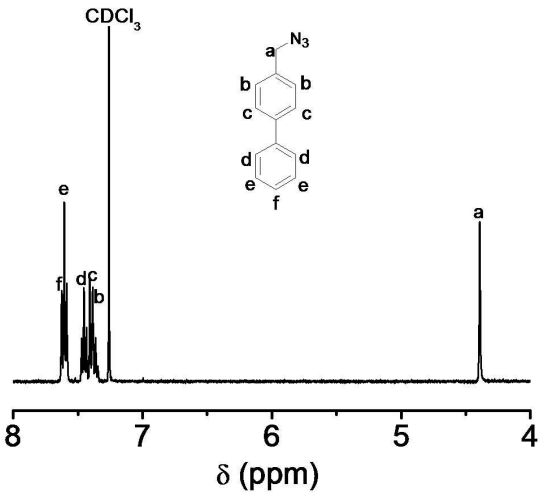



**Figure S4.** $^{1}$H NMR spectrum of Biph-$N_3$ ($CDCl_3$, 400 MHz).

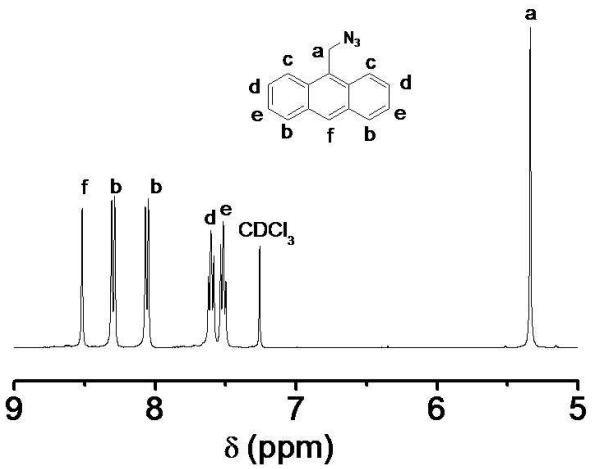



**Figure S5.** $^{1}$H NMR spectrum of Anth-$N_3$ ($CDCl_3$, 400 MHz).

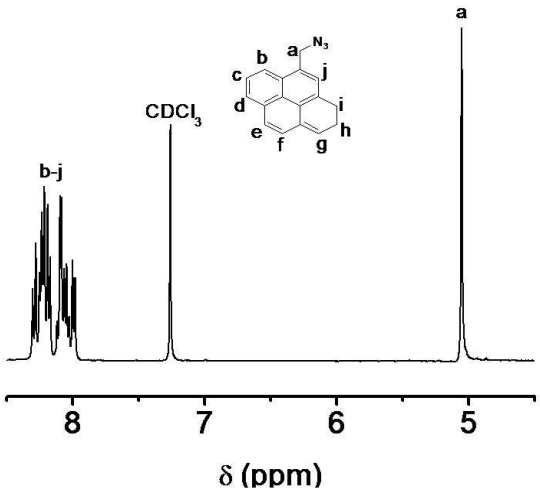



**Figure S6.** $^{1}$H NMR spectrum of Py-$N_3$ ($CDCl_3$, 400 MHz).

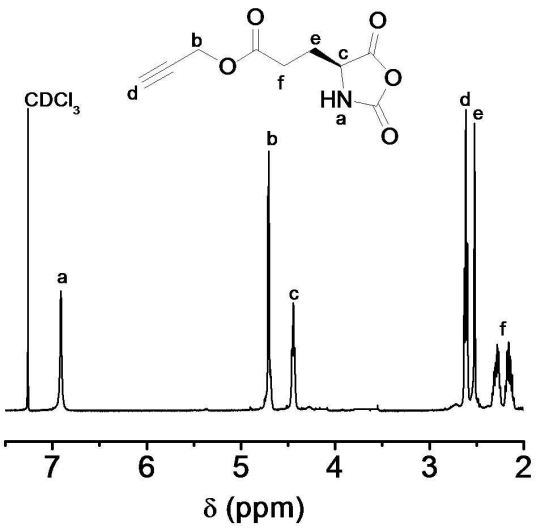



**Figure S7.** $^{1}$H NMR spectrum of PLG-NCA ($CDCl_3$, 400 MHz).

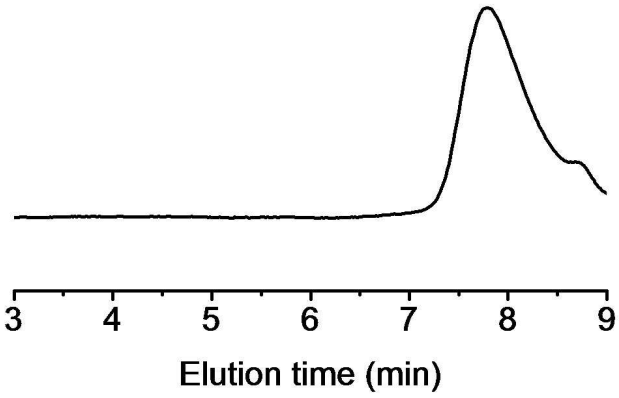



**Figure S8.** The GPC spectrum of polymer PPLG.

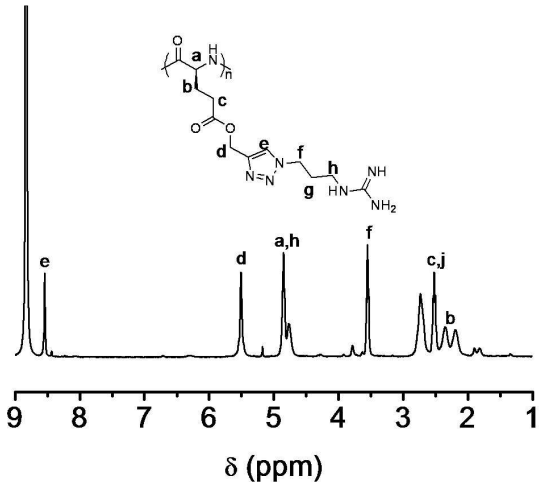



**Figure S9.** $^{1}$H NMR spectrum of P1 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

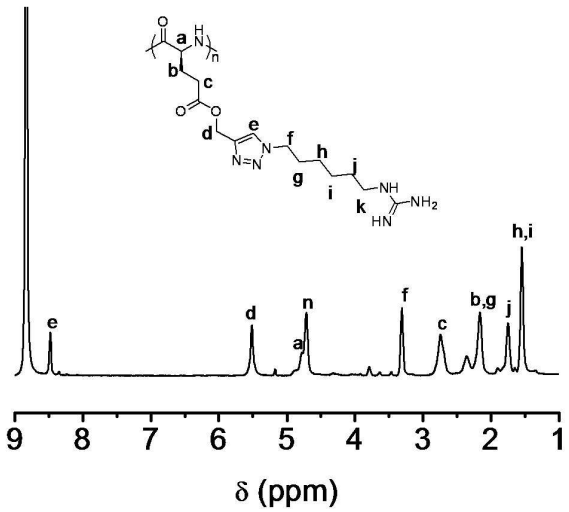



**Figure S10.** $^{1}$H NMR spectrum of P2 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

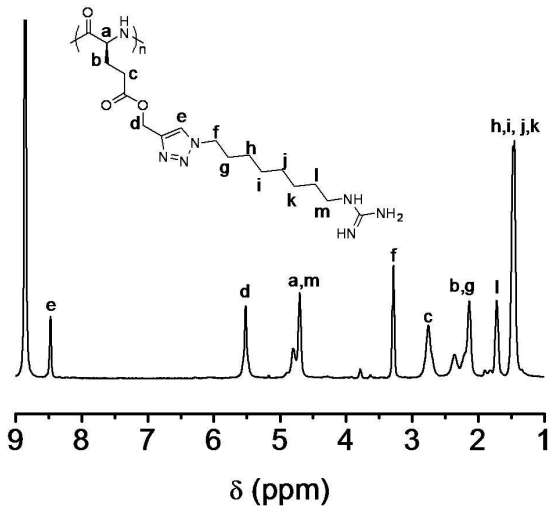



**Figure S11.** $^{1}$H NMR spectrum of P3 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

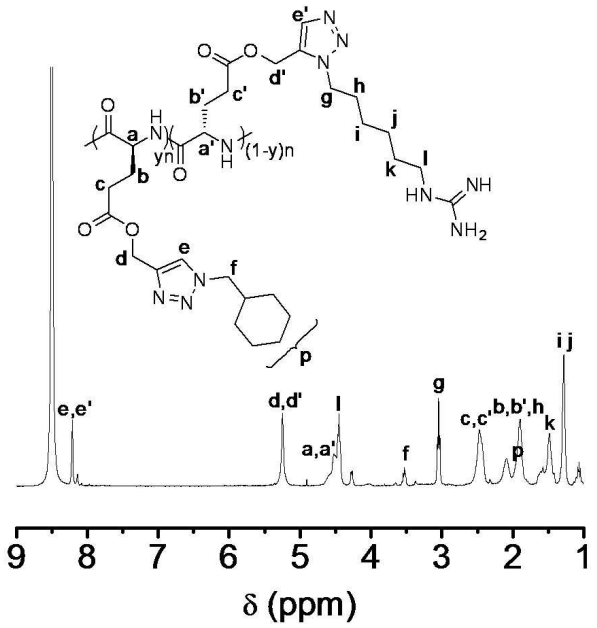



**Figure S12.** $^{1}$H NMR spectrum of Cy10 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

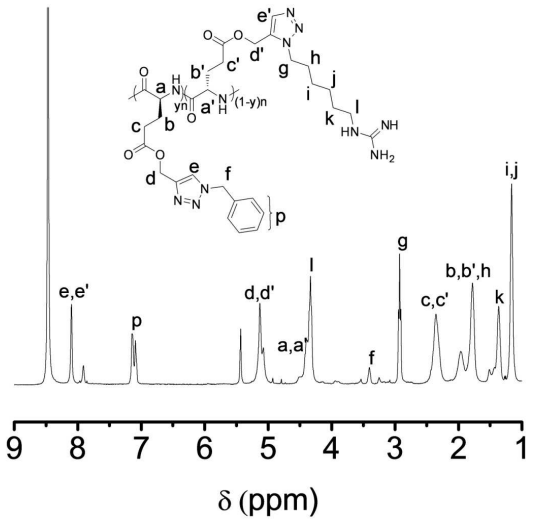



**Figure S13.** $^{1}$H NMR spectrum of Bn10 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

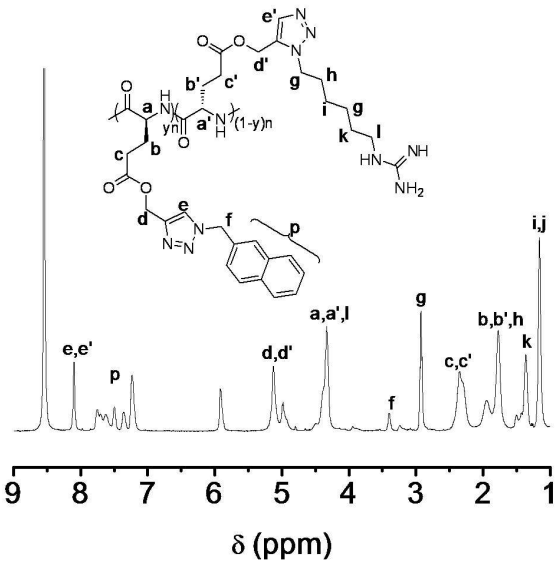



**Figure S14.** $^{1}$H NMR spectrum of Naph10 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

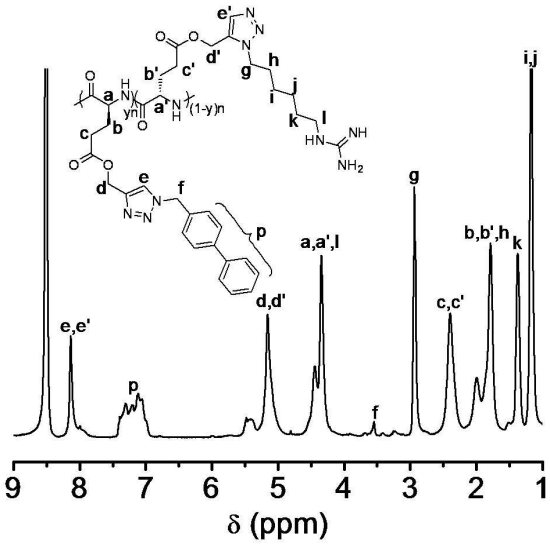



**Figure S15.** $^{1}$H NMR spectrum of Biph10 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

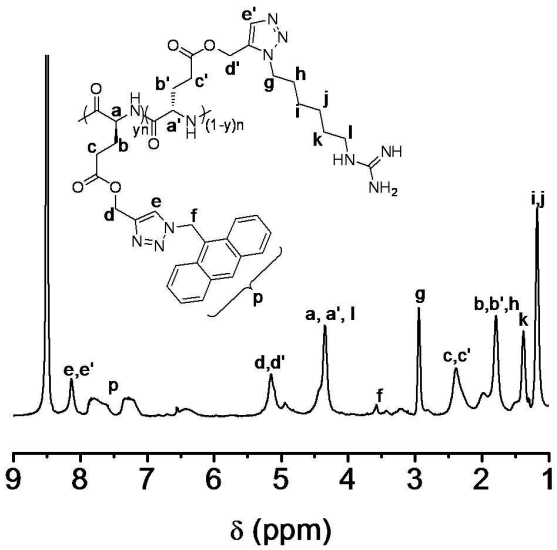



**Figure S16.** $^{1}$H NMR spectrum of Anth10 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

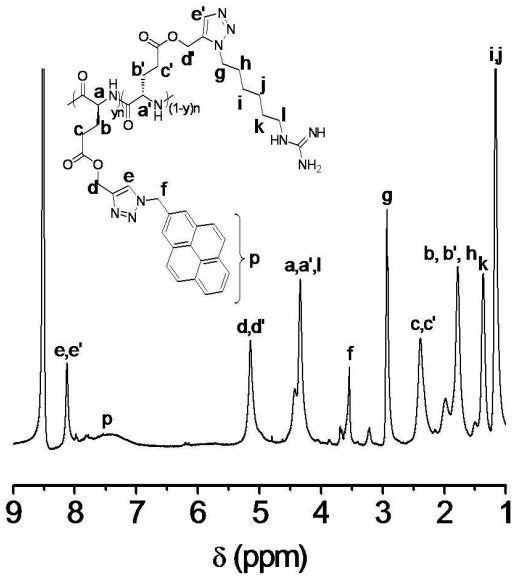



**Figure S17.** $^{1}$H NMR spectrum of Py10 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

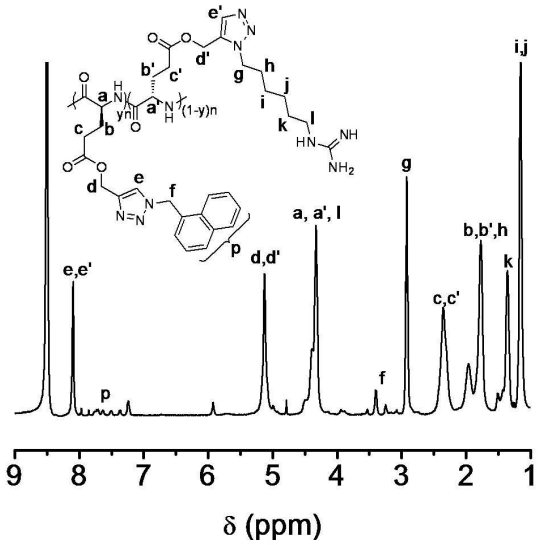



**Figure S18.** $^{1}$H NMR spectrum of Naph2 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

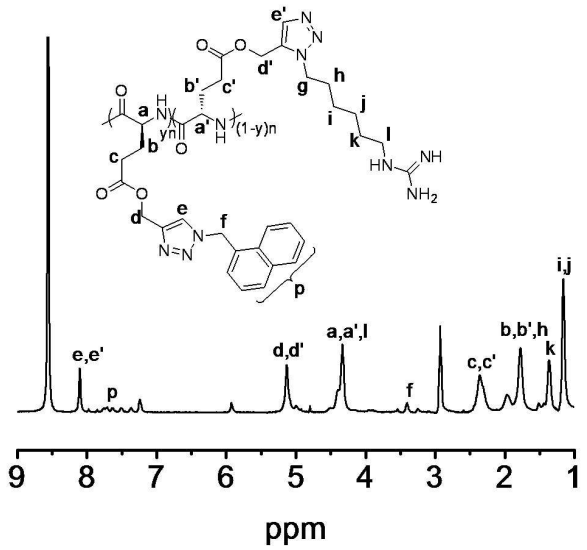



**Figure S19.** $^{1}$H NMR spectrum of Naph5 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

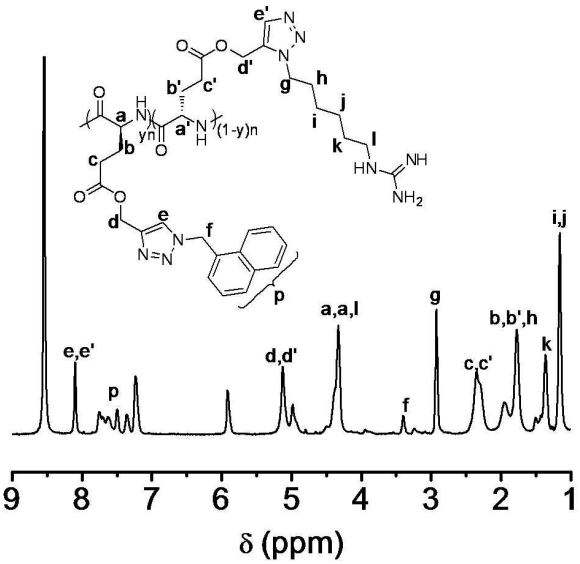



**Figure S20.** $^{1}$H NMR spectrum of Naph15 (400 MHz, TFA-d/$D_2O$ (v/v = 9/1)).

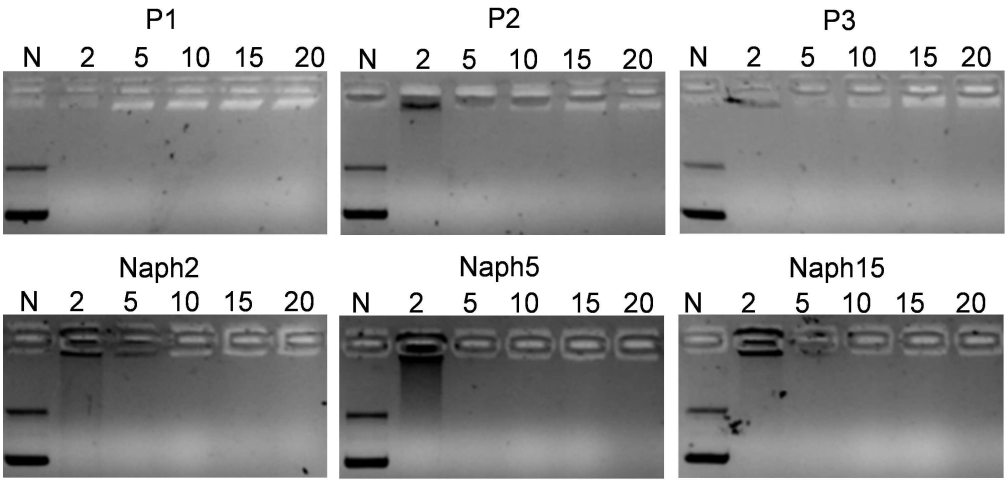



**Figure S21.** DNA condensation by polypeptides at various polymer/DNA weight ratios. N represents naked DNA.

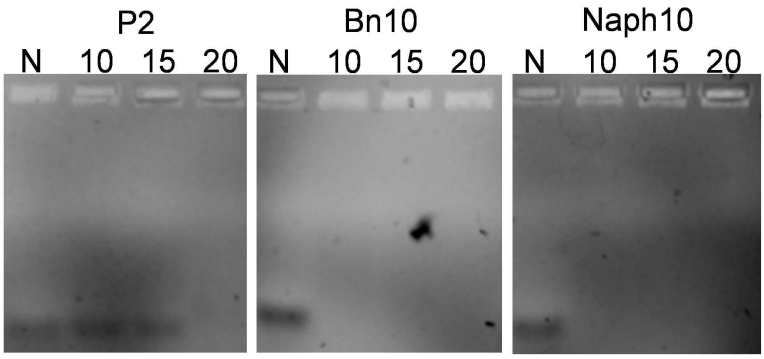



**Figure S22.** siRNA condensation by polypeptides at various polymer/siRNA weight ratios. N represents naked siRNA.

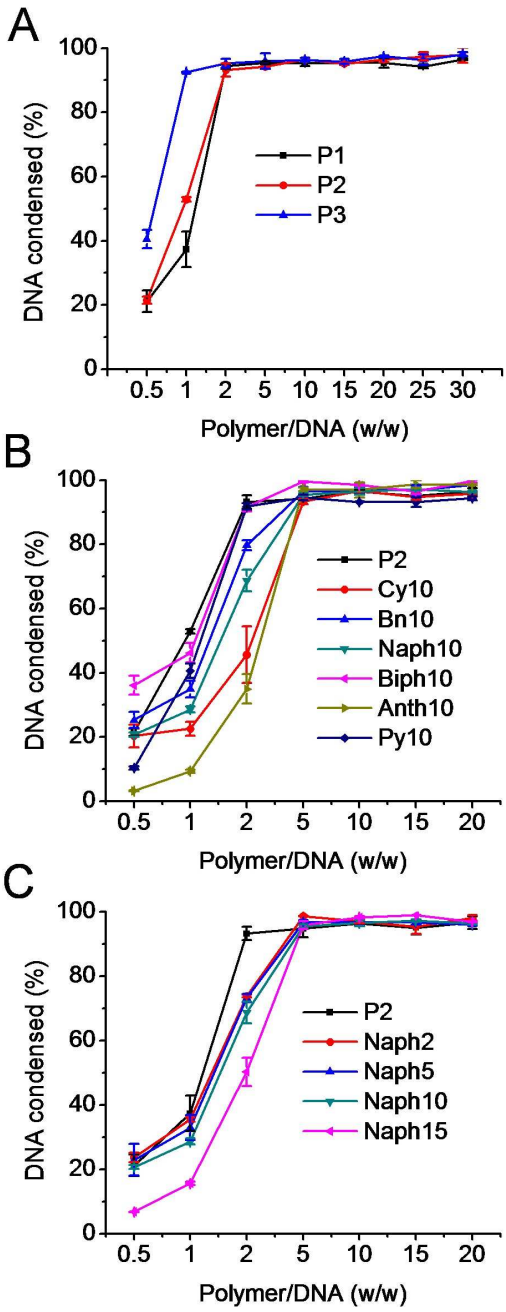



**Figure S23.** DNA condensation levels of polypeptides at various polymer/DNA weight ratios. (A) Homopolypeptides with various triazole-to-guanidine spacer lengths (P1-P3). (B) P2 and co-polypeptides with various aliphatic (Cy10) or aromatic (Bn10, Naph10, Biph10, Anth10, and Py10) side chains at the fixed molar content of 10%. (C) Polypeptides with different molar contents of naphthyl groups on side chains (P2, Naph2, Naph5, Naph10, and Naph15) (n = 3).

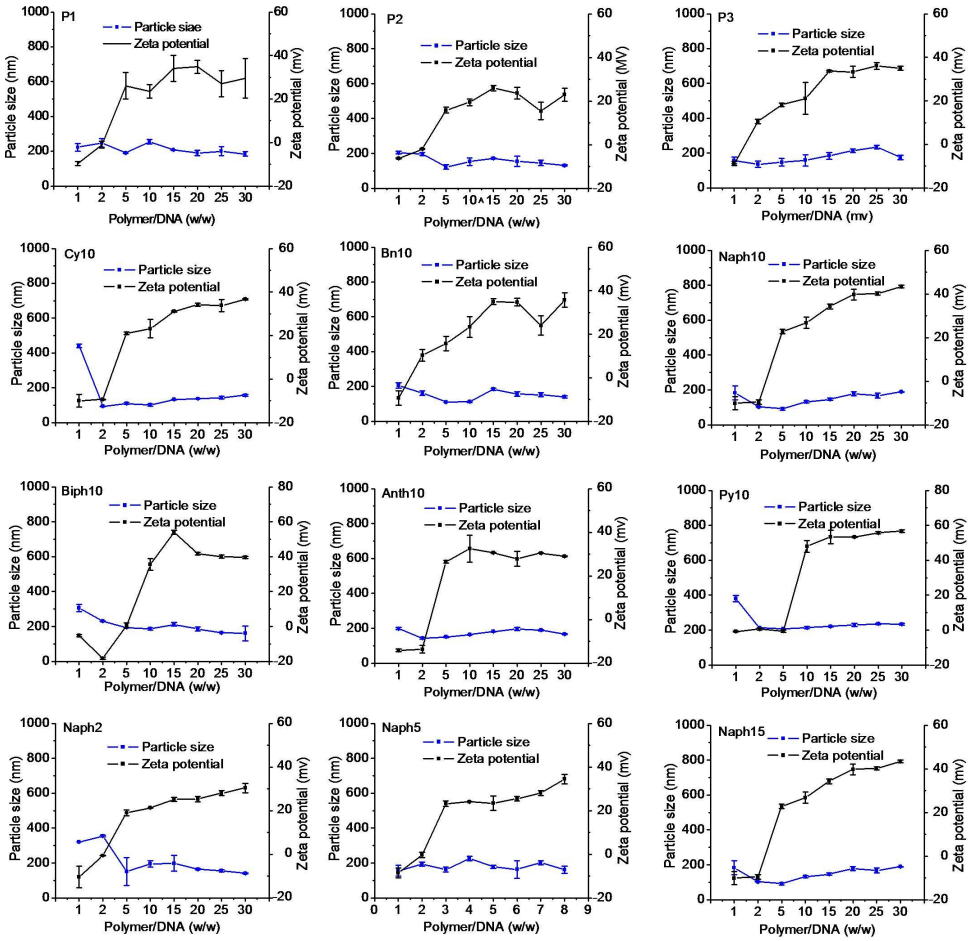



**Figure S24.** Size and zeta potential of polypeptide/DNA polyplexes at various weight ratios.

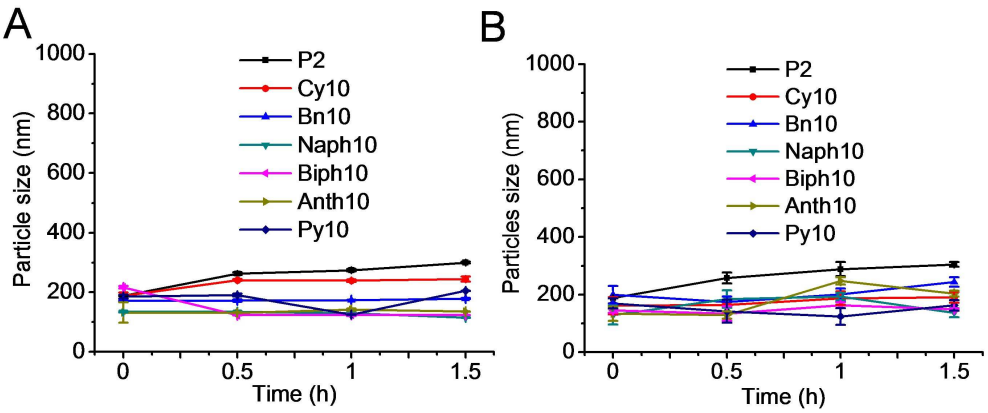



**Figure S25.** Alteration of particle size of polypeptides/DNA polyplexes (w/w = 20, prepared in DEPC) upon dilution with PBS (A) and 10% FBS (B) (10 fold).

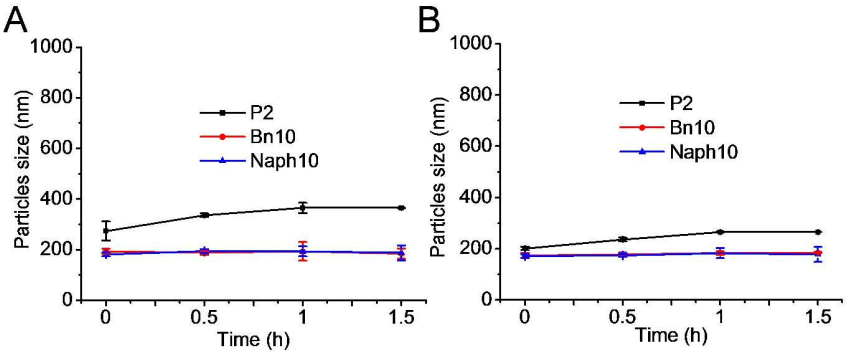



**Figure S26.** Alteration of particle size of polypeptides/siRNA polyplexes (w/w = 20, prepared in DEPC) upon dilution with PBS (A) and 10% FBS (B) (10 fold).

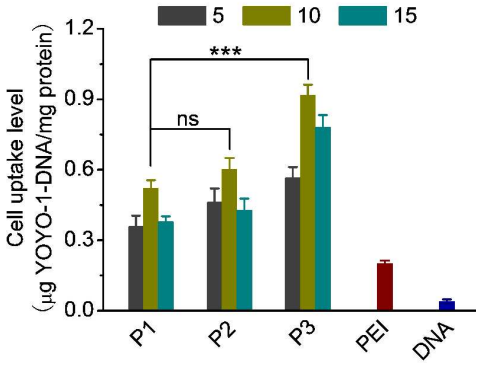



**Figure S27.** Uptake level of polypeptide/YOYO-1-DNA polyplexes at different polymer/DNA weight ratios (0.1 μg DNA/well) following incubation for 4 h at 37 °C in HeLa cells (n = 3).

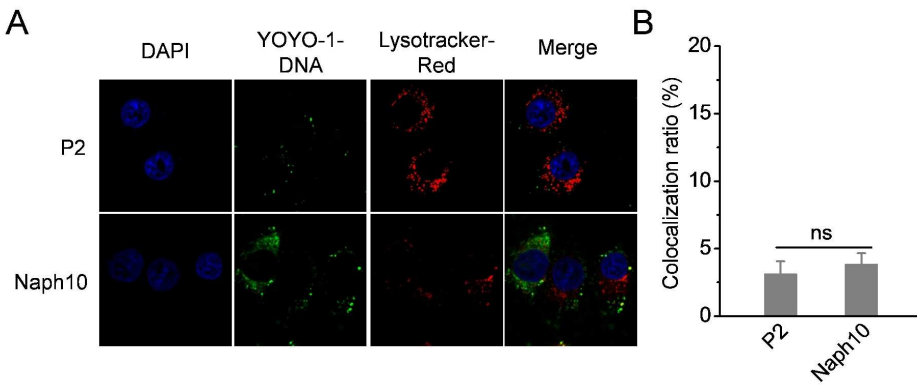



**Figure S28.** Intracellular distribution of polypeptide/DNA polyplexes in HeLa cells. (A) CLSM images of cells treated with Naph10/YOYO-1-DNA and P2/YOYO-1-DNA complexes (w/w = 10) for 4 h at 4 $^{o}$C. Cell nuclei were stained with DAPI, and endosomes/lysosomes were stained with LysotrackerRed (bar = 20 μm). (B) Colocalization ratios between YOYO-1-DNA and Lysotracker Red-stained endosomes as calculated from CLSM images (n = 50).

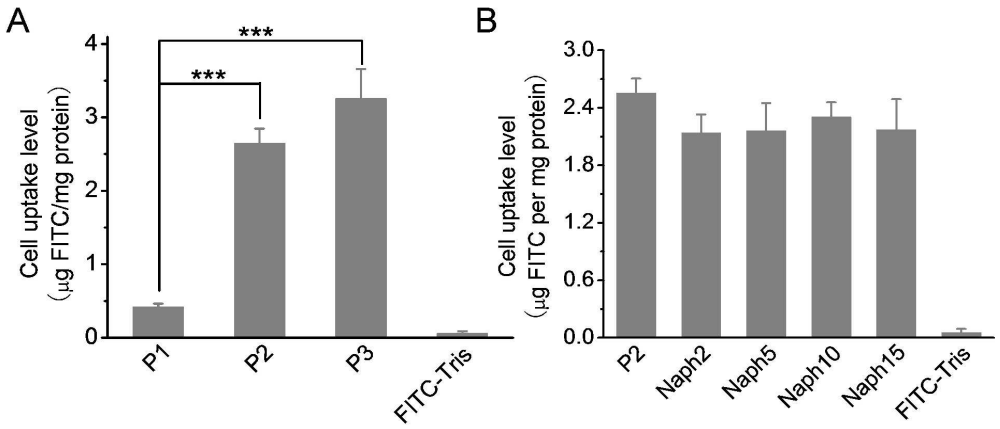



**Figure S29.** Uptake level of FITC-Tris in HeLa cells following co-incubation with polypeptides for 2 h at 37 °C. (A) Homopolypeptides with various triazole-to-guanidine spacer lengths (P1-P3). (B) Polypeptides with different molar contents of naphthyl groups on side chains (P2, Naph2, Naph5, Naph10, and Naph15) (n = 3).

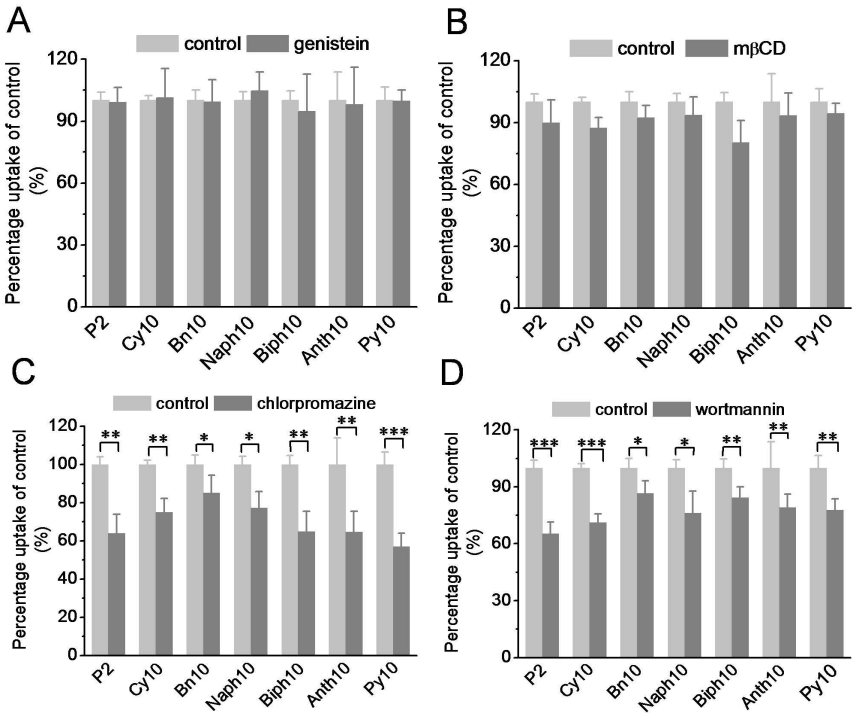



**Figure S30.** Uptake level of polypeptide/DNA polyplexes in HeLa cells in the presence of various endocytic inhibitors including genistein (A), mβCD (B), chlorpromazine (C), and wortmannin (D) (n = 3).

**Table S1.** Sequences of TNF-α and Scramble siRNA (Scr)

| | Sequence |
|---|---|
| TNF-α sense | 5’-GUCUCAGCCUCUUCUCAUUCCUGCT-3’ |
| TNF-α antisense | 5’-AGCAGGAAmUGmAAmGAmGGmGGmCUmGAmGACmAmU-3’ |
| Scr sense | 5’-UUC UCC GAA CGU GUC ACG UTT-3’ |
| Scr antisense | 5’-ACG UGA CAC GUU CGG AGA ATT-3’ |

**Table S2.** Primer sequences of TNF-α and 36B4

| | Sequence |
|---|---|
| TNF-α F | CCCTCACACTCAGATCATCTTCT |
| TNF-α R | GCTACGACGTGGGCTACAG |
| 36B4 F | TCCAGGCTTTGGGCATCAC |
| 36B4 R | CTTTATCAGCTGCACATCACTCAGA |

**REFERENCE**

1. Zhang, R.; Zheng, N.; Song, Z.; Yin, L.; Cheng, J. The Effect of Side-Chain Functionality and Hydrophobicity on the Gene Delivery Capabilities of Cationic Helical Polypeptides. *Biomaterials* **2014**, *35*, 3443-3454.