Supporting information

Self-Assisted Membrane-Penetrating Helical Polypeptides Mediate Anti-Inflammatory RNAi against Myocardial Ischemic Reperfusion (IR) Injury

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Instrumentation

^1H 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 dn/dc 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 0.05 mg/mL. The solution was placed in a quartz cell with a light path of 1 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]

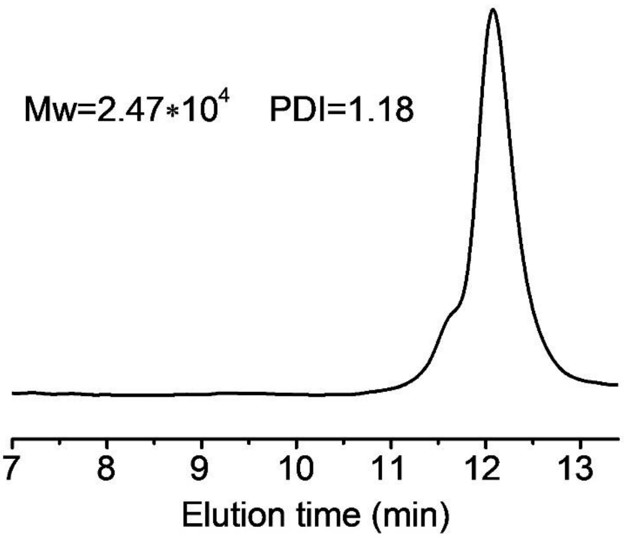

Fig. S1. GPC trace of the PPLG polymer.

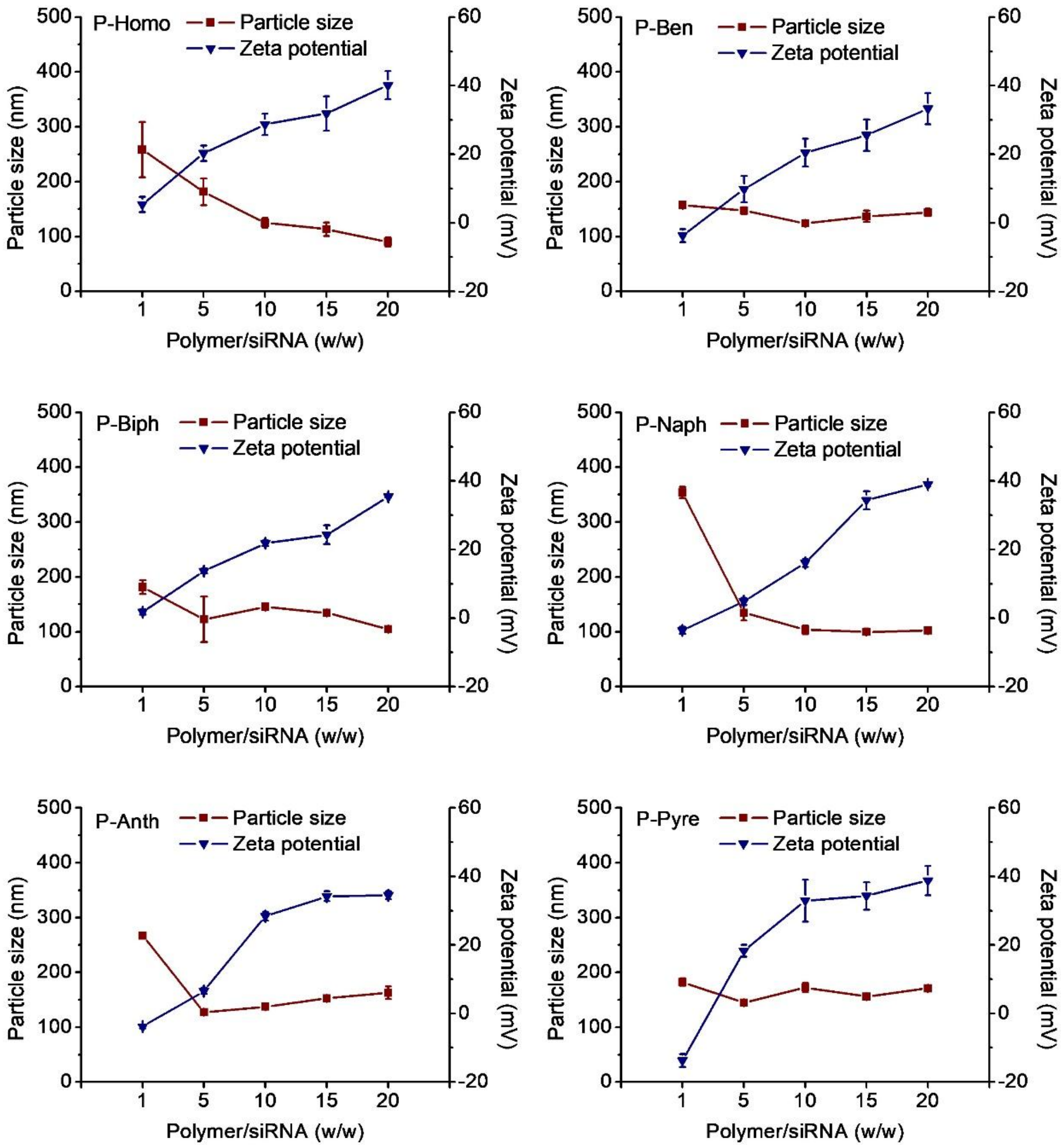

Fig. S2. Particle size and zeta potential of polypeptide/siRNA polyplexes in DEPC at various polymer/siRNA ratios as determined by DLS measurement.

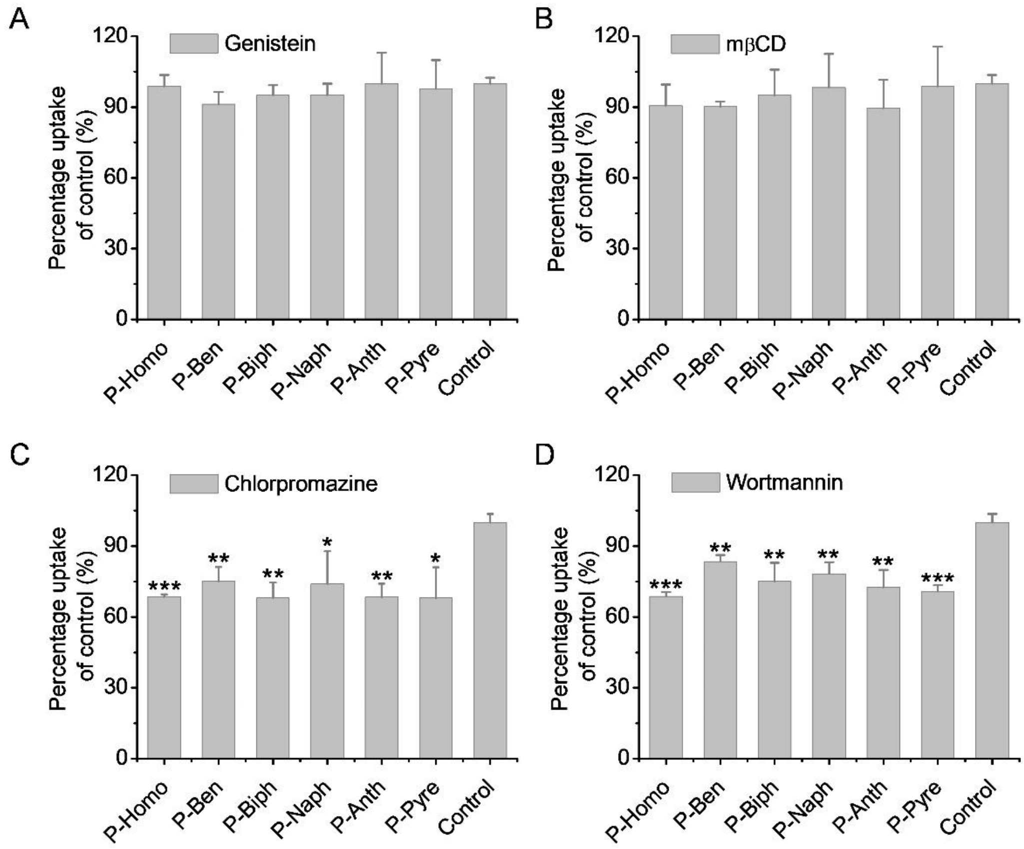

Fig. S3. Relative uptake levels of different polypeptide/FAM-siRNA polyplexes (w/w = 15) in H9C2 cells in the presence of various endocytic inhibitors (n = 3).

Table S1. Sequences of siRAGE and siScr.

		Sequence
siRAGE	Sense	5’-CACUCUACGAUCCCAAUUCAAdTdT-3’
	Anti-sense	5’-UUGAAUUGGGAUCGUAGAGUGdTdT-3’
siScr	Sense	5’-UUCUCCGAACGUGUCACGUTT-3’
	Anti-sense	5’-ACGUGACACGUUCGGAGAATT-3’

Table S2. Primer sequences of RAGE and GAPDH.

		Sequence
RAGE	Forward	5’-GAATCCTCCCCAATGGTTCA-3’
	Reverse	5’-GCCCGACACCGGAAAGT-3’
GAPDH	Forward	5’-CATGCCGCCTGGAAACCTGCCA-3’
	Reverse	5’-TGGGCTGGGTGGTCCAGGGGTTTC-3’

Table S3. ^{1}H NMR spectral data of chemicals synthesized.

Chemicals	^{1}H NMR spectral data
N_3-HG	(400 MHz, $CDCl_3$, δ, ppm): 3.34 (t, 2H, $-CH_2NHC(NH)NH_2$), 3.21 (t, 2H, $-CH_2N_3$), 1.76 (m, 2H, $-CH_2CH_2NHC(NH)NH_2$), 1.65 (m, 2H, $-CH_2CH_2N_3$), 1.32 (m, 4H, $-(CH_2)_2(CH_2)_2N_3$).
Ben-N_3	(400 MHz, $CDCl_3$, δ, ppm) : 7.78 (m, 2H, ArH), 7.31 (m, 3H, ArH), 4.62 (s, 2H, $-CH_2Ar$)
Naph-N_3	(400 MHz, $CDCl_3$, δ, ppm) : 8.30 (1H, d, ArH), 7.85 (2H, m, ArH), 7.54 (2H, m, ArH), 7.43 (2H, m, ArH), 5.44 (s, 2H, $-CH_2Ar$)
Biph-N_3	(400 MHz, $CDCl_3$, δ, ppm) : 7.60 (m, 4H, ArH), 7.46 (t, 2H, ArH), 7.37 (m, 3H, ArH), 4.38 (s, 2H, $-CH_2Ar$)
Anth-N_3	(400 MHz, $CDCl_3$, δ, ppm) : 8.56 (s, 1H, ArH), 8.33 (d, 2H, ArH), 8.10 (d, 2H, ArH), 7.60 (t, 2H, ArH), 7.53 (t, 2H, ArH), 5.39 (s, 2H, $-CH_2Ar$)
Pyre-N_3	(400 MHz, $CDCl_3$, δ, ppm) : 8.31 (m, 4H, ArH), 8.16 (m, 5H, ArH), 4.94 (s, 2H, $-CH_2Ar$)
PPLG	(400 MHz, $CDCl_3$, δ, ppm) : 4.57 (s, 1H, −CH2C≡CH), 3.98 (s, 1H, α-H), 2.60−2.47 (br, 3H, $-CH_2CH_2COO-$ and −C≡CH), 2.28−2.10 (br, 2H, $-CH_2CH_2COO-$).
P-Homo	[400 MHz, TFA-d/D2O (9:1, v/v), δ, ppm]: 8.47 (s, 1H, triazole-H), 5.5 (s, 2H, $-COOCH_2-$), 4.5−4.8 (t, 3H, α-H and $-CH_2(CH_2)_4CH_2$-$NHC(NH)NH_2$), 3.3 (t, 2H, $-CH_2(CH_2)_4CH_2NHC$-$(NH)NH_2$), 2.76 (t, 2H, $-CH_2CH_2COO-$), 2.34−2.15 (m, 4H, $-CH_2CH_2COO-$ and $-CH_2CH_2(CH_2)_3CH_2NHC(NH)NH_2$), 1.76 (m, 2H, $-CH_2(CH_2)_3$-$CH_2CH_2NHC(NH)NH_2$), 1.43−1.58 (m, 4H, $-CH_2CH_2(CH_2)_2CH_2$-$NHC(NH)NH_2$).
P-Ben	[400 MHz, TFA-d/D2O (9:1, v/v), δ, ppm]: 8.1 (s, 1H, triazole-H), 7.2−7.0 (m, 1H, ArH), 5.1 (s, 2H, $-COOCH_2-$), 4.48−4.2 (t , 2.8H, α-H, and $-CH_2(CH_2)_4CH_2NHC(NH)NH_2$), 3.4 (m, 0.2H, $-CH_2Ar$), 2.9 (t, 1.8H, $-CH_2CH_2(CH_2)_3CH_2NHC(NH)NH_2$), 1.51−1.32 (m, 2H, 1.8H, $-CH_2(CH_2)_3CH_2CH_2NHC(NH)NH_2$), 1.1 (m, 3.6H, $-CH_2CH_2(CH_2)_2CH_2CH_2NHC(NH)NH_2$).
P-Biph	[400 MHz, TFA-d/D2O (9:1, v/v), δ, ppm]: 8.1 (s, 1H, triazole-H), 7.52−6.91 (m, 0.9H, ArH), 5.1 (s, 2H, $-COOCH_2-$), 4.47−4.21 (t, 2.8H, α-H, and $-CH_2(CH_2)_4CH_2NHC(NH)NH_2$), 3.51 (m, 0.2H, $-CH_2Ar$), 2.92 (t, 1.8H, $-CH_2CH_2(CH_2)_3CH_2NHC(NH)NH_2$), 1.52−1.3 (m, 1.8H, $-CH_2(CH_2)_3CH_2CH_2NHC(NH)NH_2$), 1.12 (m, 3.6H, $-CH_2$ $CH_2(CH_2)_2CH_2CH_2NHC(NH)NH_2$).
P-Naph	[400 MHz, TFA-d/D2O (9:1, v/v), δ, ppm]: 8.1 (s, 1H, triazole-H), 7.91−7.12 (m, 0.7H, ArH), 5.1 (s, 2H, $-COOCH_2-$), 4.45−4.19 (t, 2.8H, α-H and $-CH_2(CH_2)_4CH_2NHC(NH)NH_2$), 3.41 (m, 0.2H, $-CH_2Ar$), 2.9 (t, 1.8H, $-CH_2CH_2(CH_2)_3CH_2NHC(NH)NH_2$), 1.51−1.32 (m, 1.8H,

	$-CH_2(CH_2)_3CH_2CH_2NHC(NH)NH_2$), 1.12 (m, 3.6H, $-CH_2CH_2(CH_2)_2$ $-CH_2CH_2NHC(NH)NH_2$).
P-Anth	[400 MHz, TFA-d/D2O (9:1, v/v), δ, ppm]: 8.1 (s, 1H, triazole-H), 7.91−6.02 (m, 0.9H, ArH), 5.1 (s, 2H, $-COOCH_2-$), 4.46−4.22 (t, 2.8H, α-H and $-CH_2(CH_2)_4CH_2NHC(NH)NH_2$), 3.52 (m, 0.2H, $-CH_2Ar$), 2.9 (t, 1.8H, $-CH_2CH_2(CH_2)_3CH_2NHC(NH)NH_2$), 1.50−1.31 (m, 1.8H, $-CH_2(CH_2)_3CH_2CH_2NHC(NH)NH_2$), 1.13 (m, 3.6H, $-CH_2CH_2(CH_2)_2CH_2CH_2NHC(NH)NH_2$)
P-Pyre	[400 MHz, TFA-d/D2O (9:1, v/v), δ, ppm]: 8.1 (s, 1H, triazole-H), 8.02−6.90 (m, 0.9H, ArH), 5.12 (s, 2H, $-COOCH_2-$), 4.45−4.20 (t, 2.8H, α-H and $-CH_2(CH_2)_4CH_2NHC(NH)NH_2$), 3.51 (m, 0.2H, $-CH_2Ar$), 2.93 (t, 1.8H, $-CH_2CH_2(CH_2)_3CH_2NHC(NH)NH_2$), 1.51−1.31 (m, 1.8H, $-CH_2(CH_2)_3CH_2CH_2NHC(NH)NH_2$), 1.1 (m, 3.6H, $-CH_2CH_2(CH_2)_2CH_2CH_2NHC(NH)NH_2$).

References:

1. R. Zhang, N. Zheng, Z. Song, L. Yin and J. Cheng, *Biomaterials*, 2014, **35**, 3443-3454.