Electronic Supplementary Information

Effects of chain lengths and backbone chirality on the bone-targeting ability of poly(glutamic acid)s

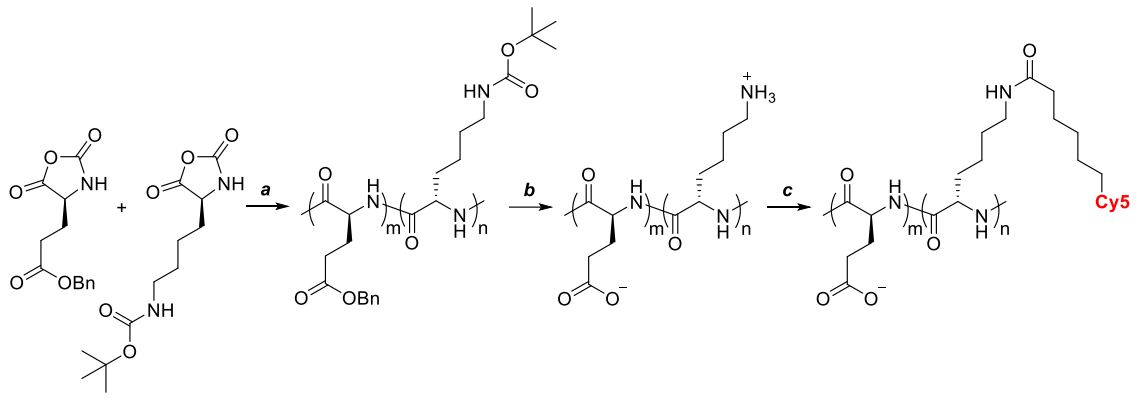
Jianglong Xia,[‡a] Wanying Wang,[‡b] Xiaoxiong Jin,[b] Jing Zhao,[b] Jiaoyu Chen,[b] Ning Li,[b] Shanshan Xiao,[b] Dongjun Lin,*[a] and Ziyuan Song*[b]

[a] Department of Haematology, The Seventh Affiliated Hospital of Sun Yat-sen University, Shenzhen 518107, China.

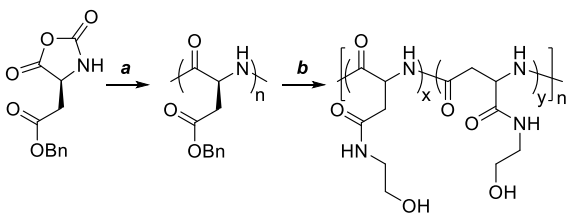
[b] Institute of Functional Nano & Soft Materials (FUNSOM), Jiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Soochow University, Suzhou 215123, China.

*Corresponding email: zysong@suda.edu.cn; lindongjun@163.com

[‡] These authors contributed equally.



Scheme S1. Synthetic routes to Cy5-labelled poly(glutamic acid)s. ***a***. *n*-hexylamine or α-helical PBLG macroinitiators, DCM, rt. ***b***. HBr/HOAc, TFA, 0 ºC, 2 h. ***c***. Cy5 NHS ester, $NaHCO_3$ (aq), rt, overnight.



Scheme S2. Synthetic routes to PHEA. ***a***. *n*-hexylamine, DCM/DMF (1:1, v/v), rt. ***b***. ethanolamine (3 equiv.), DMF, 35 ºC, 12 h.

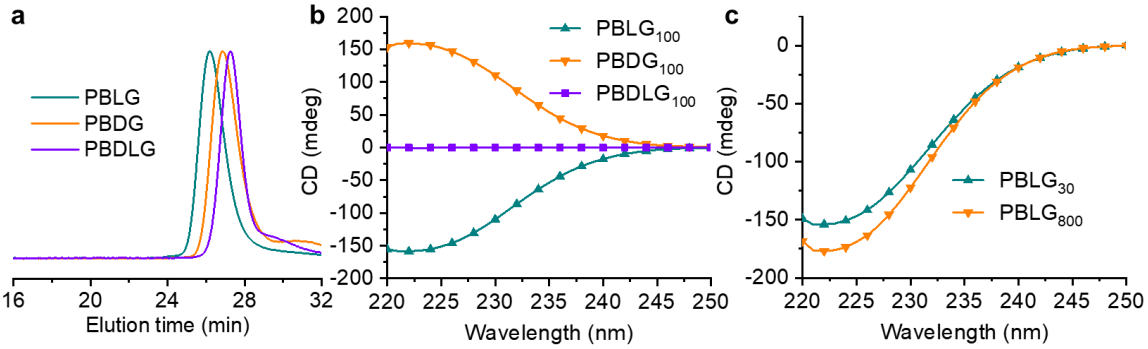


Figure S1. Characterization of poly(γ-benzyl glutamate)s precursors before side-chain deprotection. (a) Normalized GPC-LS traces of poly(γ-benzyl glutamate)s with different backbone chirality. (b, c) CD spectra of poly(γ-benzyl glutamate)s in DCM with different backbone chirality (b) and chain lengths (c).

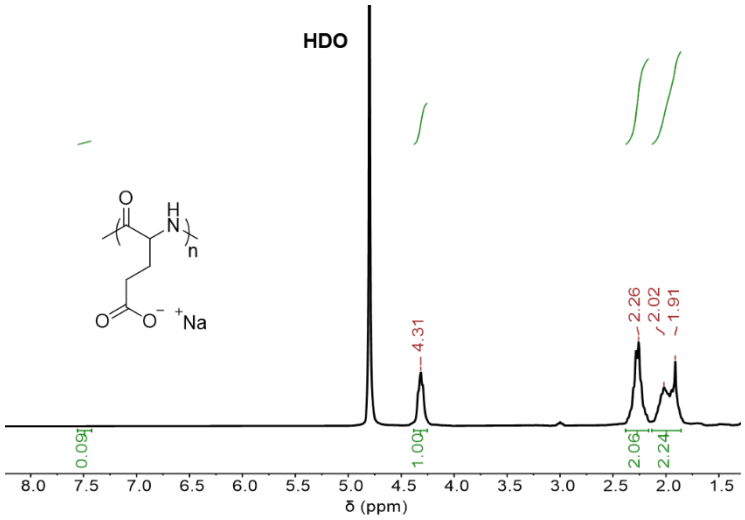


Figure S2. ^{1}H NMR spectrum (300 MHz) of representative poly(glutamic acid) (PL100) after side-chain deprotection in D_2O.

The integral of the residue peak at 7.5 ppm was used for the calculation of side-chain deprotection efficiency.

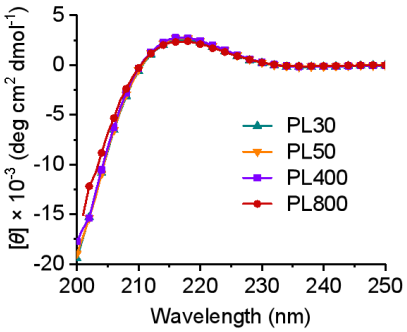


Figure S3. CD spectra of poly(L-glutamic acid)s in water at pH = 7.4 with different chain lengths.

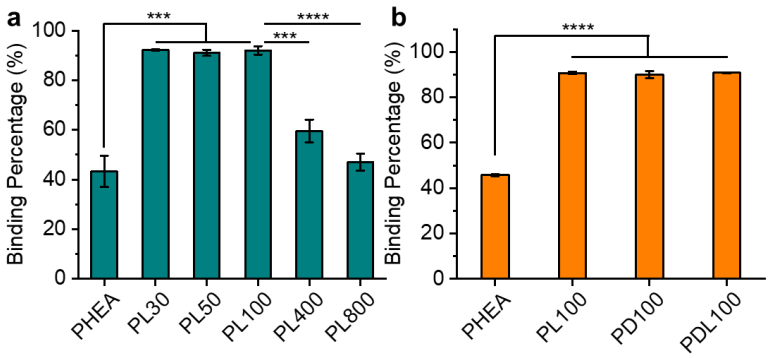


Figure S4. HA binding affinity of poly(glutamic acid)s with different chain lengths (a) and backbone chirality (b) after 30-min incubation (n = 3). PHEA with neutral side-chain hydroxyl groups was used as a control. ***$p < 0.001$, ****$p < 0.0001$.

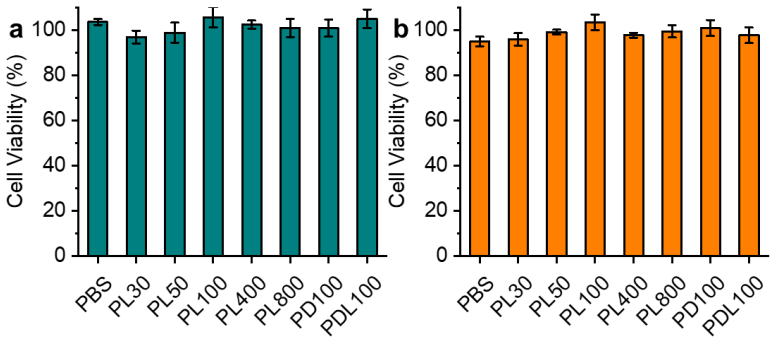


Figure S5. Cell viability of RAW 264.7 cells after 48-h incubation with various poly(glutamic acid)s at 0.01 (a) or 0.1 mg/mL (b) (n = 3). Cells treated with PBS were used as control groups.

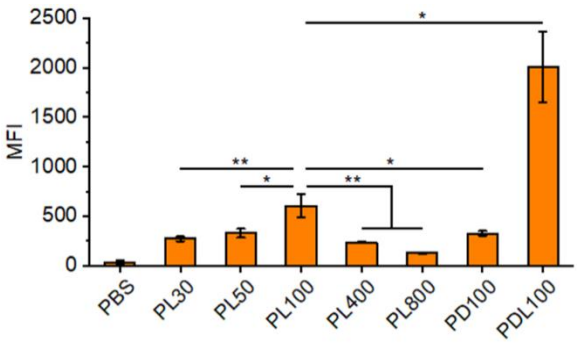


Figure S6. Mean fluorescent intensity of RAW 264.7 cells after 16-h incubation with various poly(glutamic acid)s. Cells treated with PBS were used as control groups (n = 3). *p < 0.05, **p < 0.01.

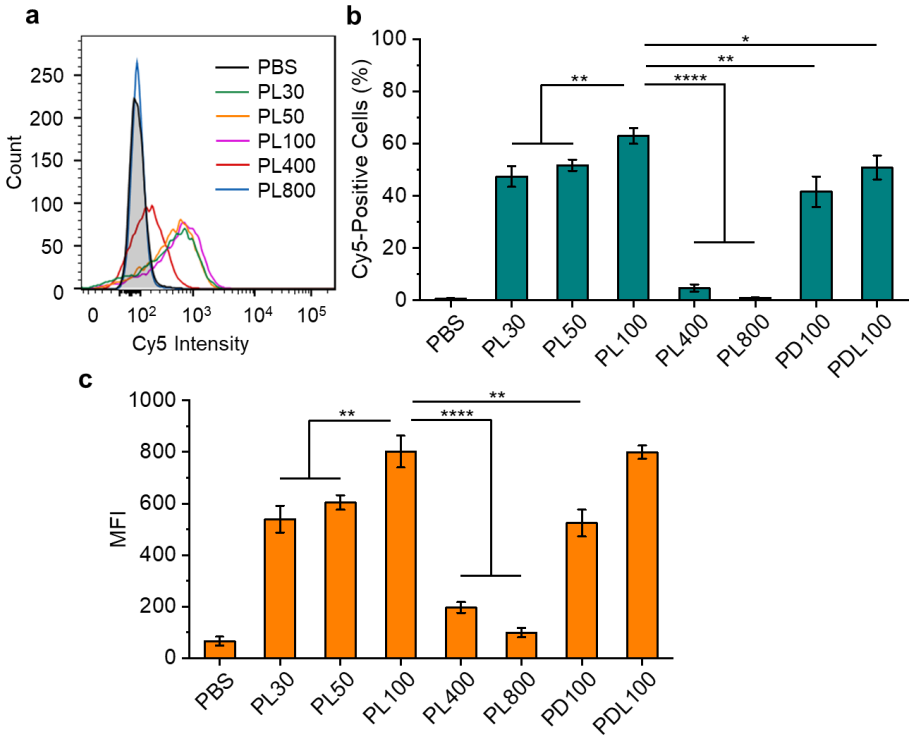


Figure S7. Cellular uptake of poly(glutamic acid)s by MV-4-11 cells. (a) Flow cytometric analysis of MV-4-11 cells after 16-h incubation with various poly(glutamic acid)s. (b) Cellular uptake efficiency of poly(glutamic acid)s by MV-4-11 cells (n = 3). (c) Mean fluorescent intensity of MV-4-11 cells after 16-h incubation with various poly(glutamic acid)s (n = 3). Cells treated with PBS were used as control groups. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

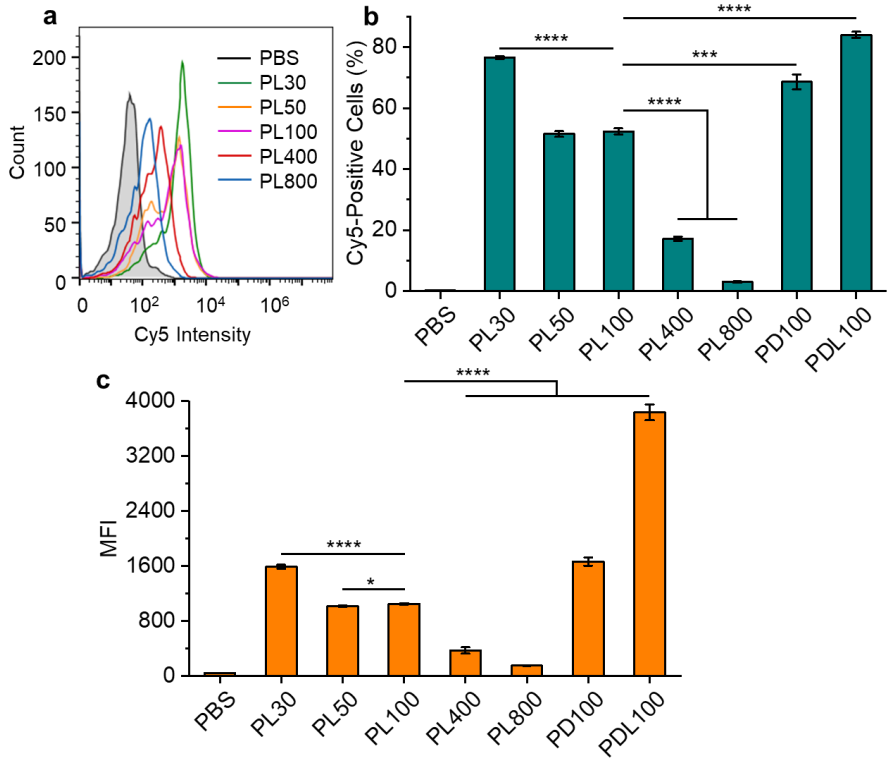


Figure S8. Cellular uptake of poly(glutamic acid)s by NIH/3T3 cells. (a) Flow cytometric analysis of NIH/3T3 cells after 16-h incubation with various poly(glutamic acid)s. (b) Cellular uptake efficiency of poly(glutamic acid)s by NIH/3T3 cells (n = 3). (c) Mean fluorescent intensity of NIH/3T3 cells after 16-h incubation with various poly(glutamic acid)s (n = 3). Cells treated with PBS were used as control groups. *$p < 0.05$, ***$p < 0.001$, ****$p < 0.0001$.

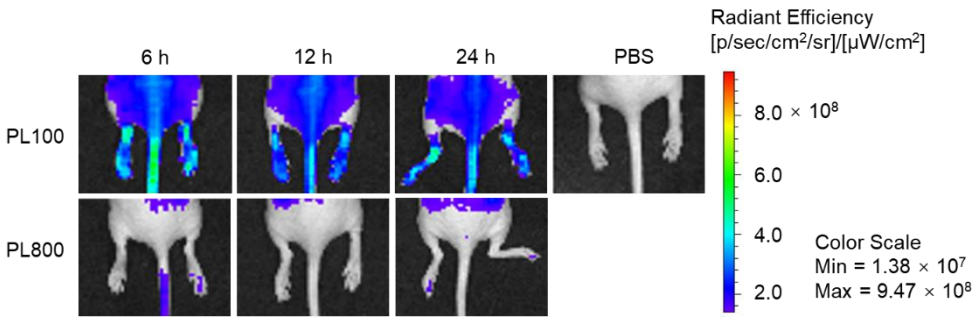


Figure S9. *In vivo* fluorescent imaging of mice at 6, 12, and 24 h post injection of Cy5-labelled PL100 and PL800. Mice treated with PBS were used as control groups.

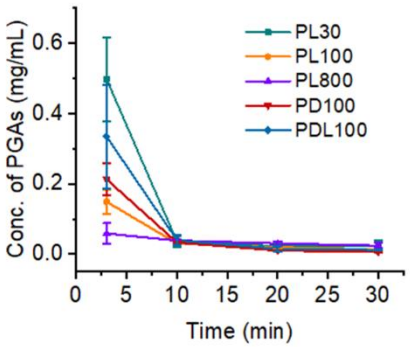


Figure S10. The change in plasma concentration of Cy5-labelled PGAs in mice over time (n = 3).

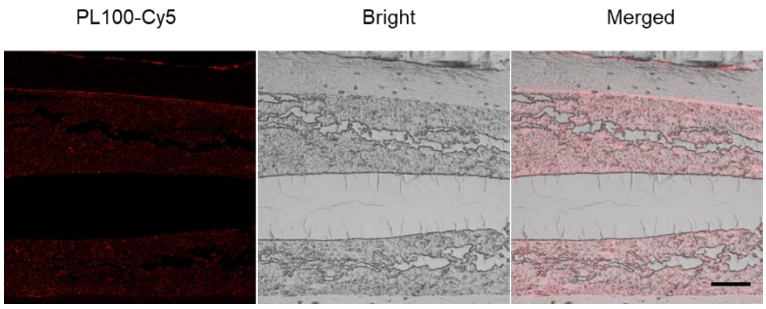


Figure S11. CLSM images of femur bone slices at 24 h post injection of Cy5-labelled PL100. Scale bar = 100 μm.

Supporting Tables

Table S1. The estimation of PGA size.

Entry	PGAs	DP[a]	$\langle r^2 \rangle_0^{1/2}$ (nm)[b]
1	PL30	30	5.4
2	PL50	63	7.9
3	PL100	108	10.4
4	PL400	515	22.7
5	PL800	766	27.7
6	PD100	83	9.1
7	PDL100	70	8.4

[a]Degree of polymerization of PGAs calculated from molecular weights by GPC analysis.

[b]Estimated size of PGAs calculated through equation: $\langle r^2 \rangle_0^{1/2} \sim (DP)^{1/2}$.