# Rational Design of Drug Delivery Systems for Potential Programmable Drug Release and Improved Therapeutic Effect

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## Methods

### Instrument

Dynamic light scattering (DLS) was performed on a laser light scattering spectrometer (BI-200SM) and the zeta potential analysis was performed on Brookhaven ZetaPALS (Brookhaven Instrument, USA). Transmission Electron Microscopy (TEM) measurement was performed on a commercial Talos F200C electron microscope (120 kV). $^{1}$H NMR analysis was performed on a nuclear magnetic resonance spectrometer (AVANCE III 400MHz). Flow cytometry analysis was performed on flow cytometry (LSR Fortessa, BD, USA). CLSM images were captured on Confocal Laser Scanning Microscopes (TCS SP8, Leica Camera). Ex vivo and in vivo imaging was conducted by the Kodak IS in vivo FX imaging system (IVIS Lumina II, Xenogen, USA).

### Synthesis of Block Copolymers.

**PAE-*b*-PEG.** PEG monoacrylate (PEG-A) was synthesized as shown in Fig. S1. Briefly，poly (ethylene glycol) methyl ether ($PEG_{5k}$-OH, 5 g) was dissolved in $CH_2Cl_2$ (25 mL) in a round bottom flask. Subsequently, TEA (2.5 eq.) and acryloyl chloride (2.0 eq.) were added in succession. The mixture was allowed to react for 12 h at room

temperature. Then, the solvent was removed under reduced pressure and the residue was quickly filtered to remove the ammonium salts. The filtrate was precipitated in cold diethyl ether and the precipitate was dried under vacuum.[1] Then, PAE-*b*-PEG was synthesized by a Michael-type addition polymerization of PEG-A, HDD and TDP. In brief，PEG-A (1g, 0.2 mmol), HDD (1.13 g, 5 mmol) and TDP (1.12 g, 5.2 mmol) were dissolved in 10 ml of $CHCl_3$. After stirring at 55 °C for 3 days, the solution was precipitated in excess diethyl ether and the precipitate was dried under vacuum.[2] The products in each step were presented by $^1$H NMR spectra (Fig S4).

**PAE-*b*-Plys.** PAE-*b*-Plys was synthesized by ring-opening polymerization (ROP) and Michael-type addition polymerization referring to previous work of our group[3] as shown in Fig. S2. The products in each step were presented by $^1$H NMR spectra (Fig S5 and S6).

**PCL-*b*-Plys.** PCL-*b*-Plys was also synthesized referring to previous work as shown in Fig. S3. First, PCL-NHBoc was synthesized by ring opening polymerization (ROP) of *ε*-CL monomer with *t*-Bocaminoethyl alcohol as an initiator and $Sn(Oct)_2$ as a catalyst in refluxed toluene. The PCL-$NH_2$ was acquired from the deprotection of PCL-NHBoc in trifluoroacetic acid.[4] PCL-*b*-Plys(Z) was synthesized by ring opening polymerization (ROP) of Lys(Z)-NCA. Briefly, PCL-$NH_2$ (1 g, 0.2 mmol) and Lys(Z)-NCA (1.25 g, 5 mmol) were dissolved in DMF (10 mL) and allowed to stir for 72 h at 35 °C. The resulting product was precipitated in excess cold diethyl ether, the precipitate was dried under vacuum. Subsequently, the product was dissolved in 5mL of trifluoroacetic acid (TFA). 1 mL hydrogen bromide (HBr) (45% in acetic acid) was added and the mixture

was allowed to stir for further 2 h at room temperature. Then, the mixture was transferred into a dialysis bag (MWCO 3500) and dialyzed against water for 2 days. Finally, the aqueous solution of purified product was lyophilized to obtain PCL-*b*-Plys. The products in each step were presented by $^{1}$H NMR spectra (Fig S7). [5]

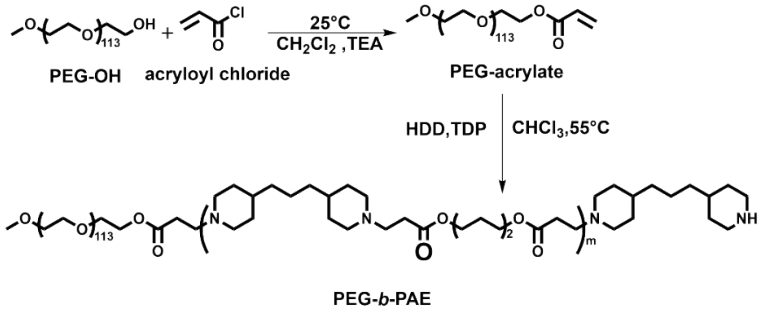


**Fig. S1** Synthesis of PAE-*b*-PEG.

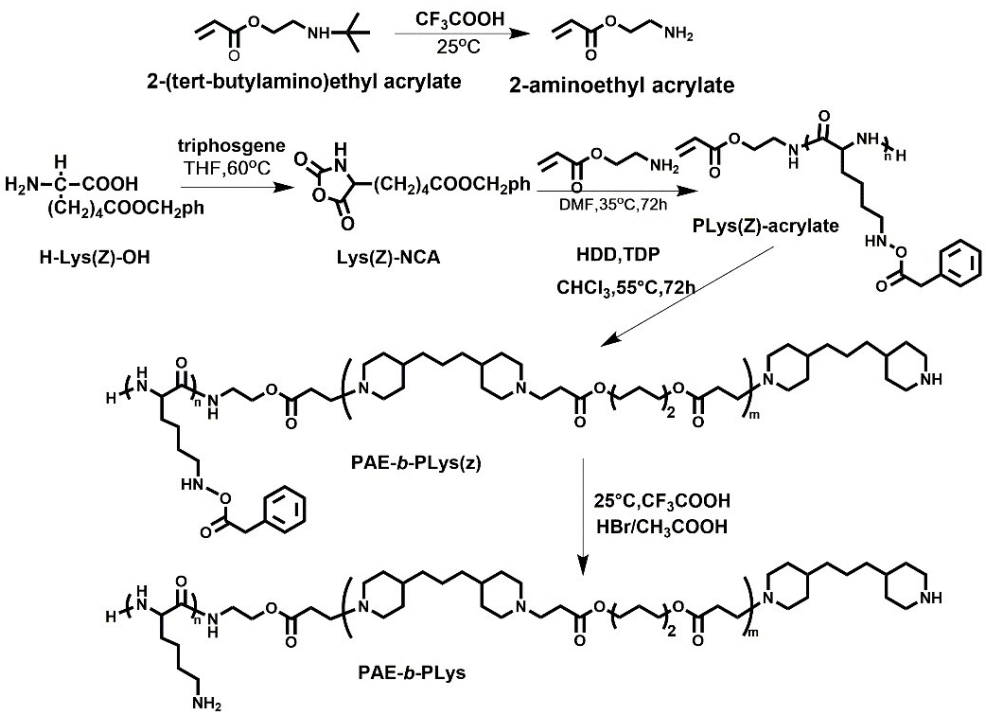


**Fig. S2** Synthesis of PAE-*b*-Plys.

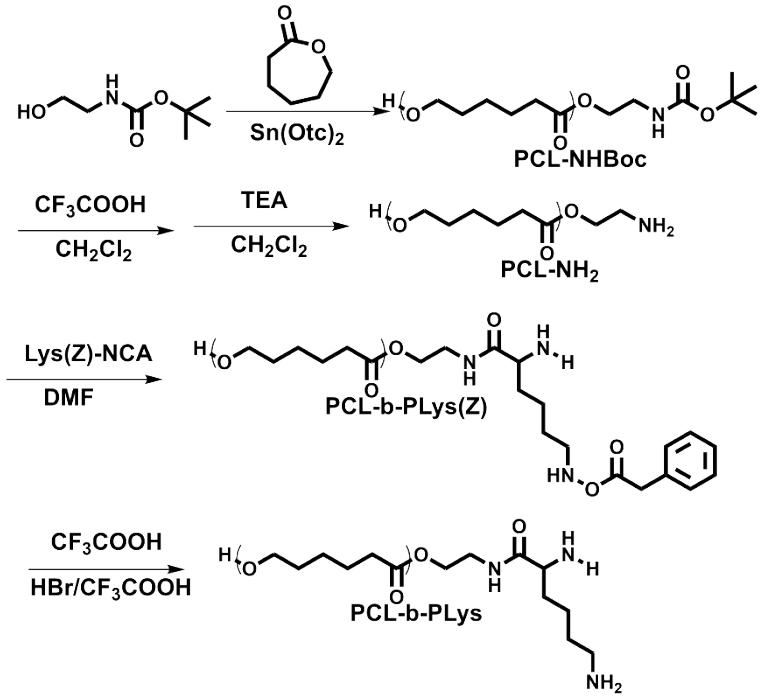


**Fig. S3** Synthesis of PCL-*b*-Plys.

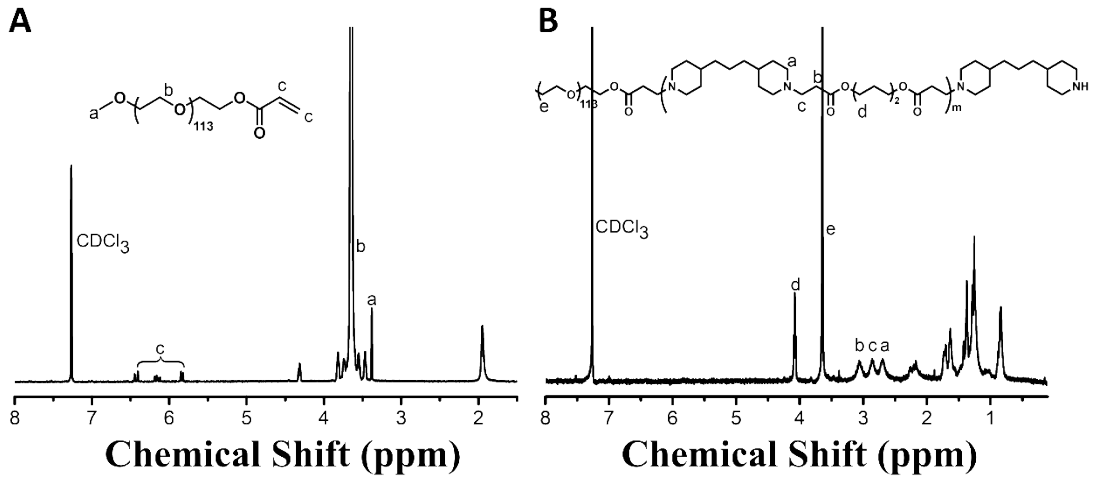


**Fig. S4** The $^{1}$H NMR spectra of polymers. (A). PEG monoacrylate in $CDCl_3$. (B). PAE-*b*-PAE in $CDCl_3$

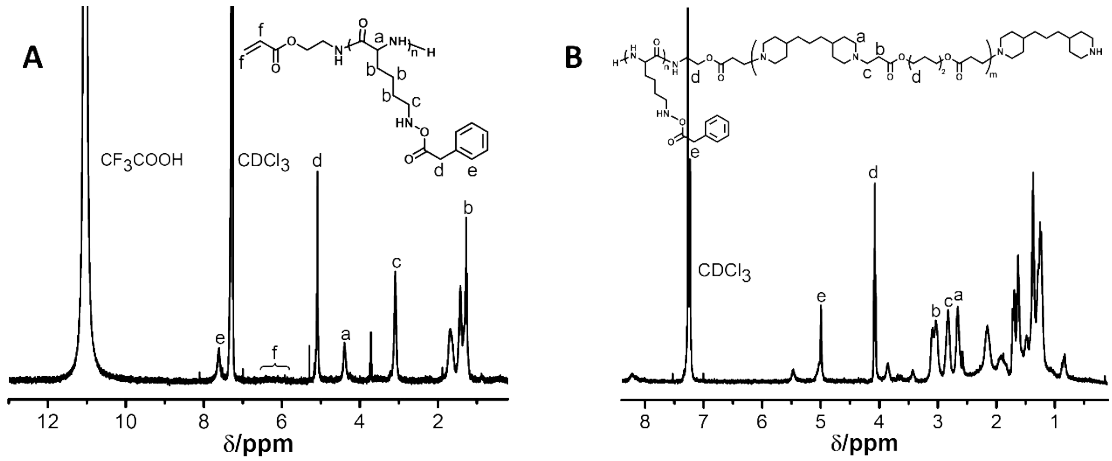


**Fig. S5** The $^1$H NMR spectra of polymers. (A). PLys(z) monoacrylate in $CDCl_3$. (B). PAE-*b*-PLys(Z) in $CDCl_3$

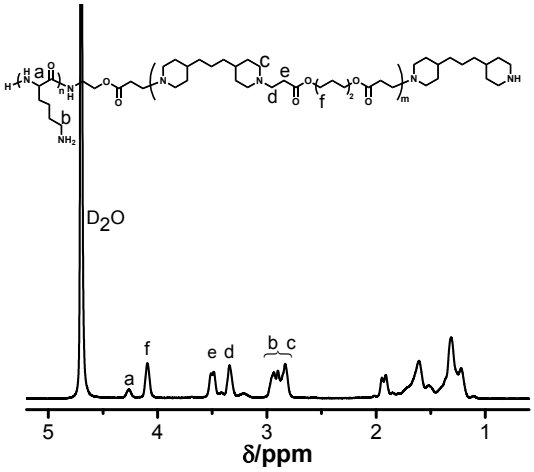


**Fig. S6** The $^1$H NMR spectra of PAE-*b*-PLys in $D_2O$

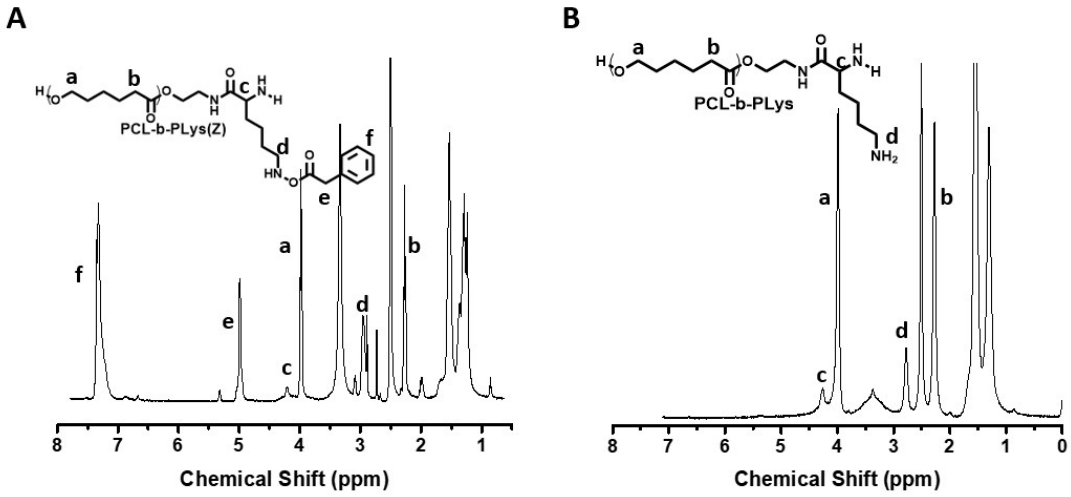


**Fig. S7** The $^1$H NMR spectra of PCL-*b*-PLys(Z) (**A**) and PCL-*b*-PLys (**B**) in DMSO-$d_6$.

**Table S1.** Formulations of fluorescently-labeled nanocarriers.

| | PAE-*b*-PEG | PAE-b-Plys + SA | CDDP | Cy3 NHS | Cy5.5 |
|---|---|---|---|---|---|
| DRNs | 2.5 mg | 2.5 mg | 1 mg | 0 mg | 0 mg |
| DRNs/Cy5.5 | 2.5 mg | 0 mg | 1 mg | 0 mg | 0.1 mg |
| Cy3-DRNs/Cy5.5 | 2.5 mg | 2.5 mg | 0 mg | 0.1 mg | 0.1 mg |

**Table S2** Formulations of Cy5.5-labeled nanocarriers.

| | PAE-*b*-PEG | PAE-b-Plys + SA | PCL-b-Plys + SA | CDDP | CA4 | Cy5.5 NHS |
|---|---|---|---|---|---|---|
| Cy5.5-DRNs | 2.5 mg | 2.5 mg | 0 mg | 1 mg | 0 mg | 0.1 mg |
| Cy5.5-DRNs/CA4 | 2.5 mg | 2.5 mg | 0 mg | 1 mg | 1 mg | 0.1 mg |
| Cy5.5-R-RNs | 2.5 mg | 0 mg | 2.5 mg | 1 mg | 0 mg | 0.1 mg |

**Table S3** Formulations of CA4-loaded nanocarriers.

| | PAE-*b*-PEG | PAE-b-Plys + SA | CDDP | CA4 |
|---|---|---|---|---|
| DRNs/CA4 | 2.5 mg | 2.5 mg | 1 mg | 1 mg |
| pH-RNs/CA4 | 2.5 mg | 2.5 mg | 0 mg | 1 mg |

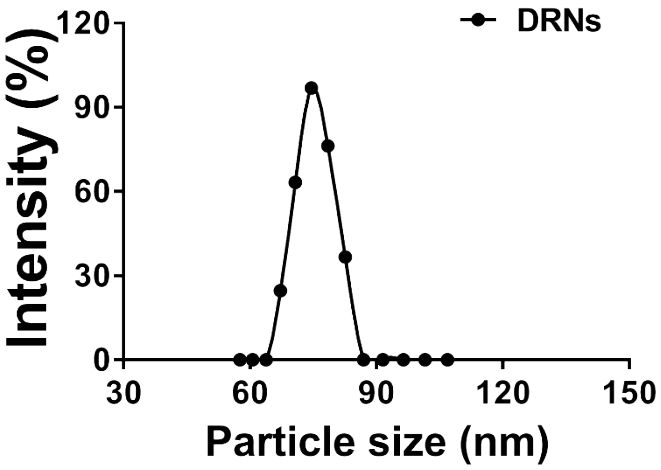


**Fig. S8** The particle size of DRNs in pH 7.4.

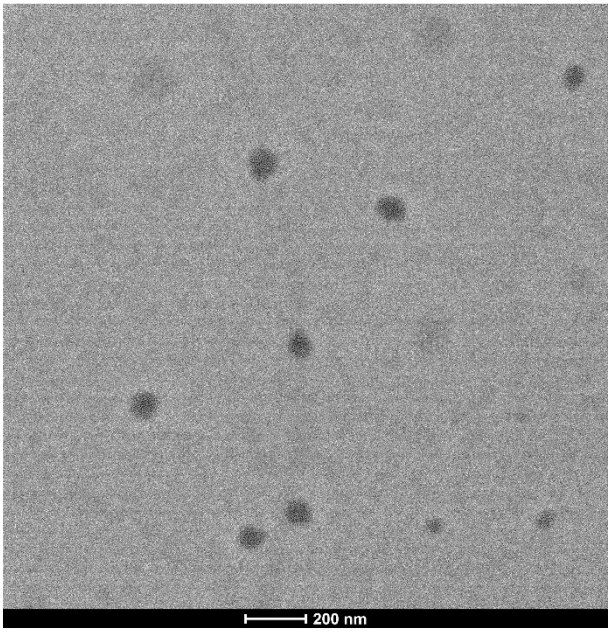


**Fig. S9** The TEM images of DRNs in pH 7.4.

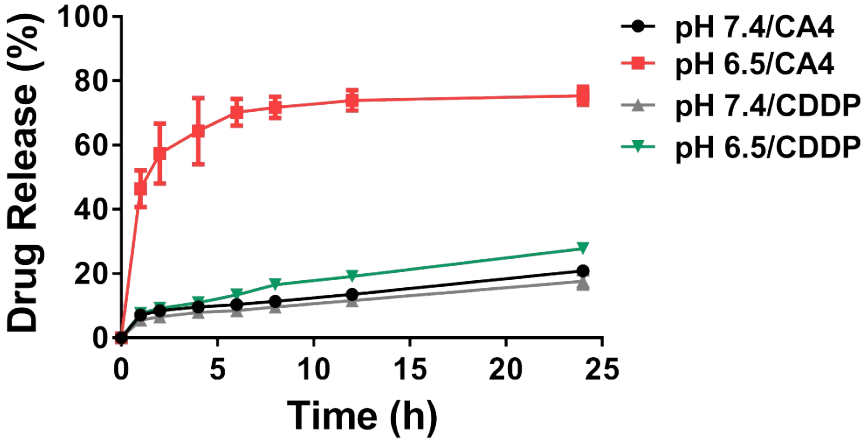


**Fig. S10** The release of CA4 and CDDP at pH 7.4 and 6.5.

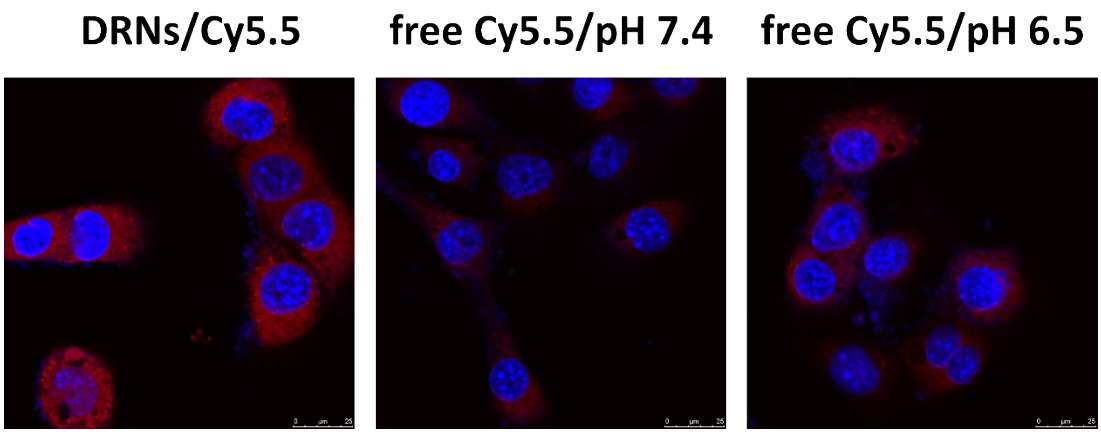


**Fig. S11** Confocal microscopy images of 4T1 cells cultured with DRNs/Cy5.5 at pH 7.4 and free Cy5.5 at pH 7.4 and 6.5.

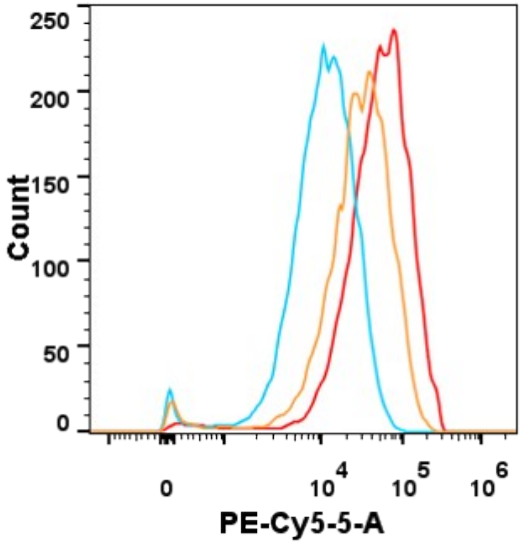


**Fig. S12** Flow cytometry of 4T1 cells cultured with DRNs/Cy5.5 (red) at pH 7.4 and free Cy5.5 at pH 7.4 (blue) and 6.5 (yellow).

## Notes and references

1. M. Winkler, L. M. de Espinosa, C. Barner-Kowollik and M. A. R. Meier, *Chem Sci*, 2012, **3**, 2607-2615.
2. T. Cheng, Y. Zhang, J. Liu, Y. Ding, H. Ou, F. Huang, Y. An, Y. Liu, J. Liu and L. Shi, *ACS Appl Mater Interfaces*, 2018, **10**, 5296-5304.
3. T. J. Cheng, R. J. Ma, Y. M. Zhang, Y. X. Ding, J. J. Liu, H. L. Ou, Y. L. An, J. F. Liu and L. Q. Shi, *Chem. Commun.*, 2015, **51**, 14985-14988.
4. F. Huang, Y. Gao, Y. Zhang, T. Cheng, H. Ou, L. Yang, J. Liu, L. Shi and J. Liu, *ACS Appl Mater Interfaces*, 2017, **9**, 16880-16889.
5. H. Gao, J. Xiong, T. Cheng, J. Liu, L. Chu, J. Liu, R. Ma and L. Shi, *Biomacromolecules*, 2013, **14**, 460-467.