Electronic Supplementary Information

of

Dual Stimuli-responsive Multi-drug Delivery System for Individual Controlled Release of Anti-cancer Drugs

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1. Materials

Poly(acrylic acid) (PAA) was purchased from Sigma-Aldrich. The average molecular weight of PAA was reported to be 1.0×10^6 by the supplier. 3-Amino-3-deoxy-α-cyclodextrin (3-NH_2-α-CD) was purchased from Tokyo Chemical Industries (TCI). N-hydroxysuccinimide (NHS), N,N'-dicyclohexylcarbodiimide (DCC), ethylcarbodiimide (EDC), 1,10-Phenanthroline, p-aminoazobenzene, poly(allylamine hydrochloride) (PAH), propargylamine (PA) were obtained from Sigma-Aldrich and used as received. Succinic anhydride (SA) and rhodamine B (RhB) were obtained from Shanghai Aladin Co., Ltd. (China) and used directly. N,N'-dimethylformamide (DMF), ethylenediamine (EDA), tetrahydrofuran (THF) were obtained from Shanghai Chemical Reagent Company and used after distillation. N-Fluorenyl-9-methoxycarbonyl (Fmoc) protected L-amino acids, 2-chlorotrityl chloride resin (100–200 mesh, loading: 0.4 mmol g-1, 1% DVB), o-benzotriazole-N,N,N',N'-tetramethyluroniumhexafluorophosphate (HBTU), N,N-diisopropylethylamine (DIEA), N-hydroxybenzotrizole (HOBt) and piperidine were purchased from GL Biochem Ltd. (Shanghai, China) and used as received. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin streptomycin, trypsin, and Dulbecco's phosphate buffered saline (PBS) were purchased from Invitrogen Corp. Ethylenediamine modified β-cyclodextrin (EDA-β-CD), polyasparticacid (PASP) and $dextran_{5000}$-fluorescein isothiocyanate (Dex_{5000}-FITC) were synthesized in our lab previously according to literature. [S1]

2. Synthesis of N_3-PLGVR-AD

The peptide was synthesized manually in 0.6 mmol scale on the 2-chlorotrityl chloride resin, employing a standard Fmoc chemistry by solid phase peptide synthesis

(SPPS) method. The coupling of the first residue used 4 equiv of Fmoc-protected amino acid (Fmoc-Lys(N_3)-OH) relative to resin substitution degree with 6 equiv of DIEA in a DMF solution. Other amino acid couplings and 1-Adamantoic acid were carried out with 4 equiv of Fmoc-protecting amino acid or 1-Adamantoic acid, 4 equiv of HBTU, and 6 equiv of DIEA for 4 h. Through the synthesis, the Fmoc protecting groups were deprotected with 20% (v/v) piperidine/DMF twice. Cleavage of the peptide was performed in a mixture of TFA, deionized water, and TIS in the ratio of 95:2.5:2.5. After 2 h stirring at room temperature, the cleavage mixture was collected. Excess TFA was removed by rotary evaporation, the remaining viscous peptide solution was precipitated with cold ether, the resulting white product was collected and vacuum dried, then dissolved in distilled water, and freeze-dried. The molecular weight of N_3-PLGVR-AD ($[M+H]^+$) measured by ESI-MS is 914.5 (theoretical value is 914.7).

3. Synthesis of Azo-SA-EDA

Azo-SA-EDA was synthesized according to the literature.S1 Briefly, 2.18 g SA, 3.57 g p-aminoazobenzene and 1.43 g pyridine were dissolved in 30 mL distilled acetone at 60 ^oC. The solution was stirred for 6 hours and thereafter the Azo-SA was obtained as red precipitate by centrifugation.

0.3 g Azo-SA,0.48 g HBTU, 0.17 g HOBt and 0.3 mL DIEA were dissolved in 8 mL DMF/THF (v:v=1:1). The solution was added in 4 mL EDA slowly and then stirred for 24 hours. The product solution was concentrated and then dropped into $CHCl_3$. In dissolvable precipitate was filtered out and the solution was concentrated by rotatory vaporization again. The concentrated solution was precipitated with

diethyl ether to obtain Azo-SA-EDA.

4. Synthesis of PAA-g-Azo-g-PLGVR-AD

300 mg PAA was dissolved in 10 mL distilled DMF, 525 mg DIC, 525 mg HOBT, 23 mg PA and 71 mg Azo-SA-EDA were added. The reaction continued for 24 hours. The resulting mixture was first dialyzed against DMF for 2 days and then against de-ionized water for 4 days. PAA-g-Azo-g-PA was obtained after lyophilised.

100 mg PAA-g-Azo-g-PA was dissolved in 5 mL distilled DMF, 5 mg CuBr and 48 mg N_3-PLGVR-AD was added. The mixture was stirred at room temperature for 24 hours with nitrogen protection. The resulting mixture was first dialyzed against DMF for 2 days and then against de-ionized water for 4 days. PAA-g-Azo-g-PLGVR-AD was obtained after lyophilised. The substitution degree of Azo and PLGVR-AD were calculated as 4.7% and 9.8% respectively from the ^{1}H NMR spectrum (Fig. S2).

5. Synthesis of PASP-EDA-β-CD

PASP-EDA-β-CD was synthesized according to the literature.[S1] Briefly talking, 400 mg EDA-β-CD and 65 mg PASP were dissolved in 10 mL water, after the solution's pH value was adjusted to 5, 125 mg EDC was added into this solution and shrilled for 24 h. This solution was purified by dialysis against water in a dialysis tube (MWCO: 3500) for 48 h. PASP-EDA-β-CD was obtained after lyophilized. The substitution degree was calculated as 45% from the ^{1}H NMR spectrum (Fig. S3).

6. Synthesis of α-CD-rhodamine B (α-CD-RhB)

To synthesize α-CD-RhB, 60 mg 3-NH_2-α-CD and 148 mg RhB were dissolved in 5 mL deionized water, after the solution's pH value was adjusted to 5, 80 mg EDC

was added into this solution and stirred for 24 h at room temperature. The reaction mixture was poured into a large excess of acetone to recover the product. The residue was washed with acetone four times and dried for 2 days under vacuum drying.

7. Prepration of ~5 μm $CaCO_3$ Particles

5 mL 0.33 M K_2CO_3 solution was rapidly poured into 5 ml 0.33 M solution of $CaCl_2$ containing 10 mg PAH and 5 mg Dex_{5000}-FITC at room temperature. After intense agitation for 30 s, the reaction mixture was left still for about 2 min. Then the precipitate was filtered off, thoroughly washed with DI water and acetone, and dried in air. The whole process was protected from light wherever possible.

8. Fabrication of $(PASP\text{-}g\text{-}\beta\text{-}CD)_5/(PAA\text{-}g\text{-}Azo\text{-}g\text{-}PLGVR\text{-}AD\&\alpha\text{-}CD\text{-}RhB)_5$ microcapsules

PAH and Dex_{5000}-FITC captured $CaCO_3$ particles were used as colloid template for the fabrication of microcapsules. Briefly, a total of 150 mg of $CaCO_3$ particles were symmetrically dispersed in 1 mL of PAA-g-Azo-g-PLGVR-AD&α-CD-RhB solution (1 mg/mL). And the suspension was shaken constantly for 15 min to establish a PAA-g-Azo-g-PLGVR-AD&α-CD-RhB layer. After adsorption, the particles were isolated by centrifugation (4,000 rpm for 1 min), followed by washing with 1 mL of DI water thrice. For adsorption of the next layer, 1 mL of PASP-g-β-CD solution (1 mg/mL) was added, followed by the same washing protocol. The LbL process was repeated to get the microcapsules with a designed numbers of layers. The whole process was protected from light wherever possible.

Hollow microcapsules were formed by dissolving the $CaCO_3$ core using 0.4 M EDTA solution with pH = 7.4. Three centrifugation (10,000 rpm for 3 min) and water washing steps were applied to remove the EDTA and isolate the microcapsules for analysis. Structure of these microcapsules was observed by confocal laser scanning microscopy (CLSM) (Fig. S4).

9. In vitro drug release under different conditions

80 mg of $CaCO_3$ particles coated with 5 bilayers of (PASP-g-β-CD)/(PAA-g-Azo-g-PLGVR-AD&α-CD-RhB) films were dispersed in 1 mL of 0.4 M pH = 7.4 EDTA solution to remove the core. Then the hollow microcapsules were isolated with centrifugation (10,000 rpm for 3 min) and were dispersed in 1 mL of DI water. The $(PASP\text{-}g\text{-}\beta\text{-}CD)_5$/ $(PAA\text{-}g\text{-}Azo\text{-}g\text{-}PLGVR\text{-}AD\&\alpha\text{-}CD\text{-}RhB)_5$ capsules solution were departed into three parts evenly and were respectively put into three dialysis tubes (MWCO: 8000-14,000 Da) quickly. These dialysis tubes were immersed into 10 mL 0.1 M pH = 7.4 buffer solution (in dark or irradiated by 365 nm UV light for 7 min per hour) or MMP solution (1 ug/mL, without UV irradiation) respectively, and then stirred for 12h. The liquid in the bottles was collected and renewed after 1 hour periodically. The fluorescent intensities of α-CD-RhB and Dex_{5000}-FITC were measured by SHIMADZU RF-530/PC spectrofluorophotometer (Fig. S5).

10. Cellular uptake study

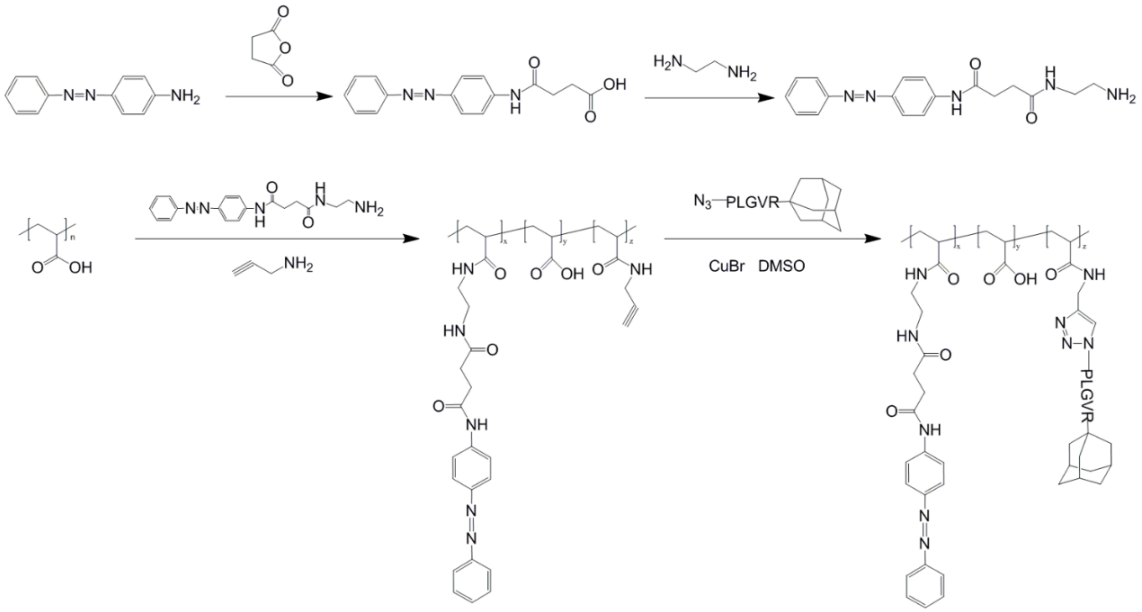
The SCC-7cells were allowed to grow to ~70% confluence in 24-wellplate. Then the culture medium was replaced, 1 mL DMEM (with or without 20 ug/mL 1,10-Phenanthroline) containing $(PASP\text{-}g\text{-}\beta\text{-}CD)_5$/ $(PAA\text{-}g\text{-}Azo\text{-}g\text{-}PLGVR\text{-}AD\&\alpha\text{-}CD\text{-}RhB)_5$ capsules was added into each well. After the capsules were incubated in serum-

containing DMEM for 5 h (some wells were incubated in dark, the other wells were irradiated by 365 nm UV light for 7 min per 1h), The DMEM was replaced and the cells were washed with PBS solution for four times. Then 1 mL PBS solution was added in each well. The fluorescent images of SCC-7 cells were observed by confocal laser scanning microscopy (Nikon C1-si, BD Laser at 543 nm).

Supplementary References

[S1] H. Lin, W. Xiao, S. Y. Qin, S. X. Cheng and X. Z. Zhang, *Polym. Chem.,* 2014, **5**, 4437.

Supplementary Fig.s and Schemes



Scheme S1. Synthesis route of PAA-g-Azo-g-PLGVR-AD.

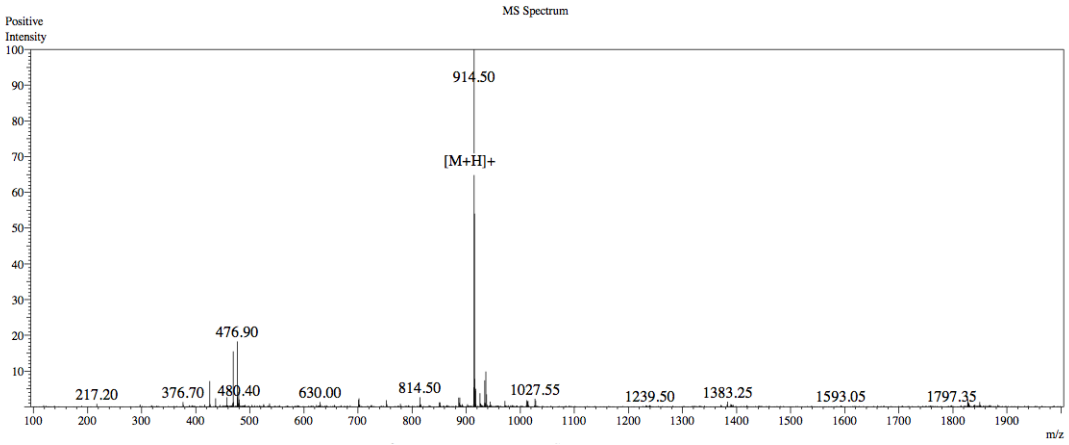


Fig. S1 ESI-MS spectrum of N_3-PLGVR-AD.

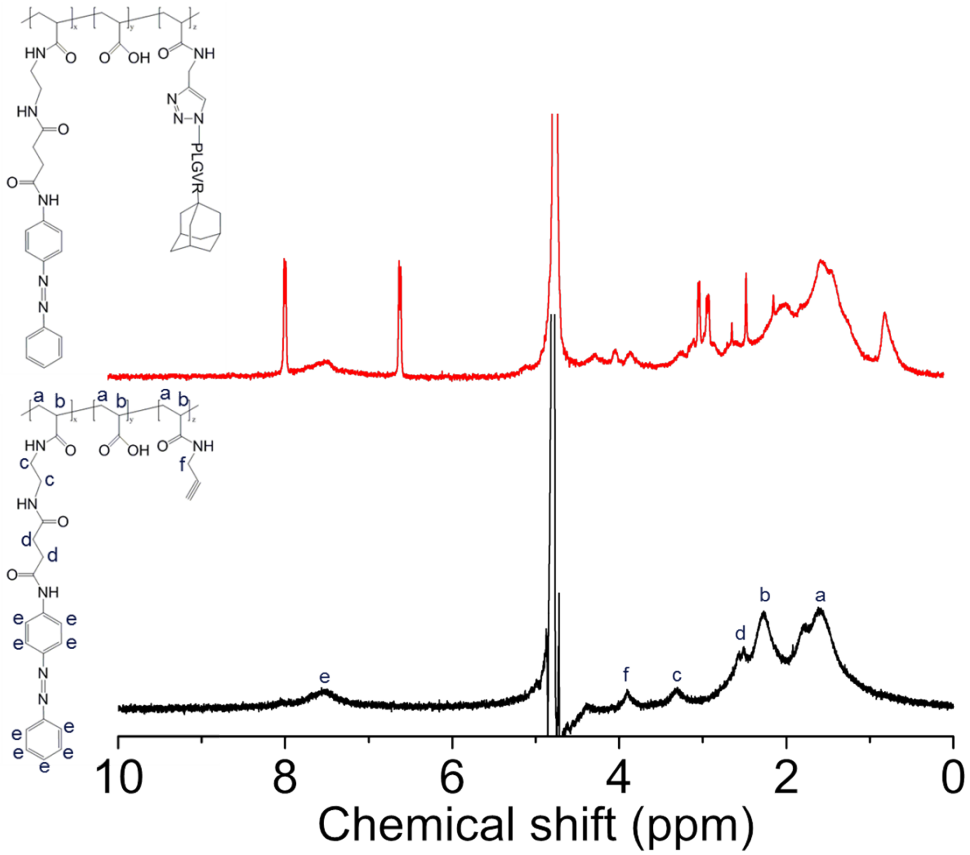


Fig. S2 The [1]HNMR spectra of PAA-g-Azo-g-PA and PAA-g-Azo-g-PLGVR-AD.

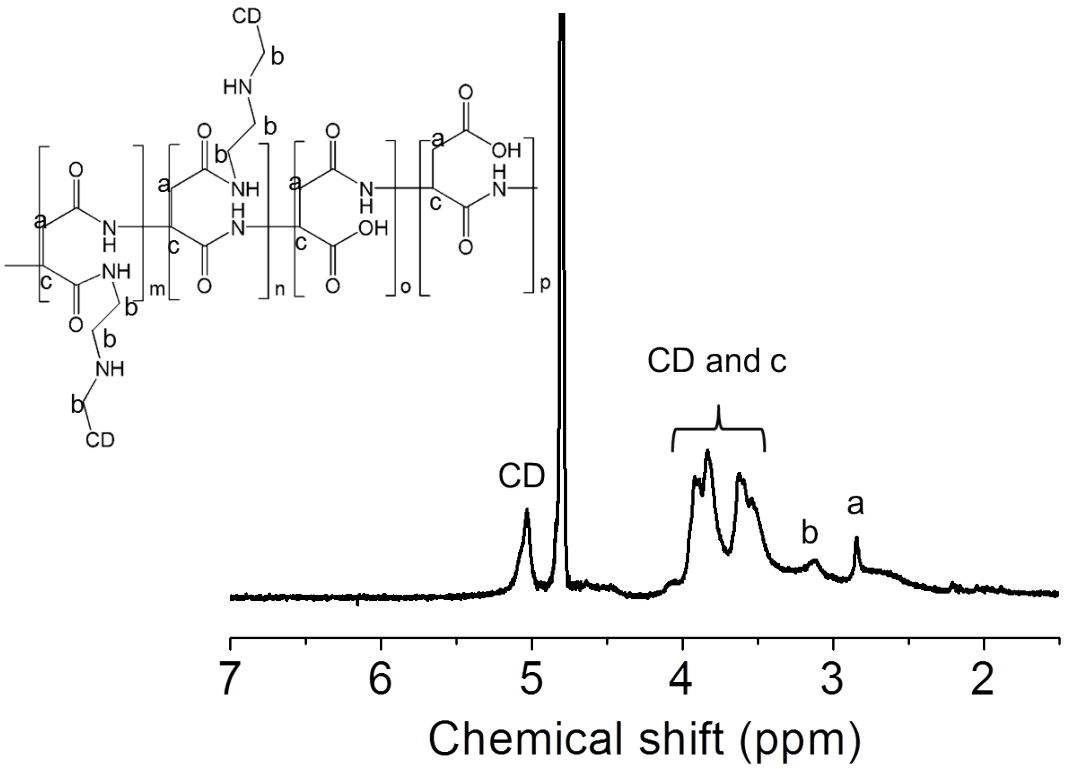


Fig. S3 The [1]HNMR spectra of PASP-EDA-β-CD.

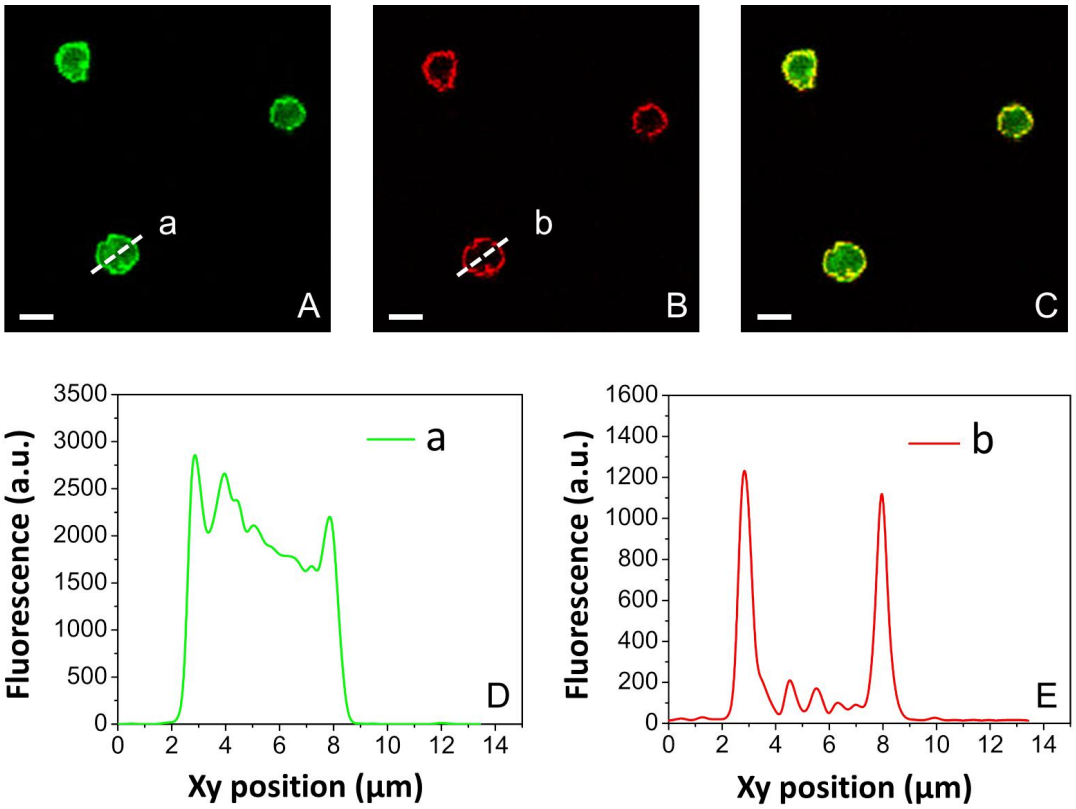


Fig. S4 The green fluorescent (A), red fluorescent (B) and merged field (C) CLSM images of $(PASP\text{-}g\text{-}\beta\text{-}CD)_5/(PAA\text{-}g\text{-}Azo\text{-}g\text{-}PLGVR\text{-}AD\&\alpha\text{-}CD\text{-}RhB)_5$ microcapsules. The profiles in D and E correspond to line a and b, respectively. (The scale bar is 5 μm).

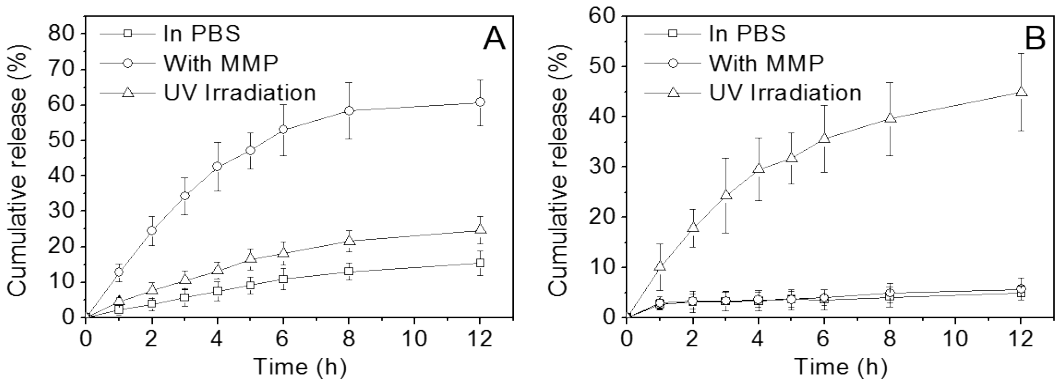


Fig. S5 Drug release profiles of Dex_{5000}-FITC (A) and α-CD-RhB (B) under different stimuli.