# Supporting Information

## Exogenous vitamin C triggered structural changes of redox-activated dual core-crosslinked biodegradable nanogels for boosting the antitumor Efficiency

Yutong Zhu,[a, b, #] Yanmei He,[a, #] Ting Su,[a] Congrui Li,[b] Shensheng Cai,[a] Zhengzhong Wu, [c] Dennis Huang,[d] Xuequan Zhang,[a] Jun Cao [a,*], Bin He [a]

[a] National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, PR China

[b] College of Chemistry, Sichuan University, Chengdu 610064, PR China

[c] College of polymer science and Engineering, Sichuan University, Chengdu 610065, PR China

[d] Department of Biomedical Engineering, the University of Texas at Austin, Austin 78712, USA

[#] These authors contributed equally.

[*] Corresponding author: caojun@scu.edu.cn

## 1. EXPERITMENTAL SECTION

**1.1 Synthesis of Biodegradable star-shaped 4s(PEG-PLLA)-OH and 4s(PEG-PDLA)-OH Copolymers.** The biodegradable star-shaped poly (ethylene glycol-L-lactide)-poly (ethylene glycol-D-lactide) copolymer (4sP(EG-LLA)-OH or 4s(PEG-DLA)-OH) was synthesized via ring opening polymerization as shown in Scheme S1. Prescribed amount of 4sPEG-OH (0.5 g) and L-lactide (0.2 g) with stannous octoate (0.1 wt%) as catalyst, were added into the polymerization tube. After the tube was degassed three times to remove water and oxygen by vacuum oil pump, the tube was sealed immediately under vacuum and placed into oil bath at 120 ℃ for 48 h. Subsequently, the copolymer was dissolved into dichloromethane and precipitated into diethyl ether. The white precipitates (4sP(EG-LLA)-OH) were dried under vacuum to remove solvents for 12 h. The similar method described above was applied to the synthesis of star-shaped poly(ethylene glycol-D-lactide) (4s(PEG-DLA)-OH).

**1.2 Modification of Biodegradable star-shaped 4s(PEG-PLLA)-OH and 4s(PEG-PDLA)-OH Copolymers.** 4sP(EG-PLLA)-OH and TsCl (g) (4sP(EG-PLLA)-OH: TsCl =1:12) were added into round-bottom flask with $CH_2Cl_2$ as the organic solvent, then triethylamine dissolved in $CH_2Cl_2$ (triethylamine : TsCl = 1:1) was dropwise added into round-bottom flask while the mixture was stirred moderately. The mixture was kept stirred for 24 h under room temperature, followed by the filtration of resulting solution to remove the solid impurities. Then the filtrate was washed by saturated sodium chloride solution, followed by $MgSO_4$ powder removement by dispersion within solution. After filtration, most of $CH_2Cl_2$ solution was removed by rotary evaporation. Then the 4sP(EG-LLA)-OTs product was precipitated into diethyl ether, and then kept under vacuum to remove organic solvents. Subsequently, sodium azide and 4sP(EG-LLA)-OTs ($NaN_3$: 4sP(EG-LLA)-OTs =12:1) were put into round-bottom flask with DMF as organic

solvent, and the mixture was stirred for 24 h under room temperature, followed the addition of distilled water into the mixture while stirring. Mixture was extracted by $CH_2Cl_2$ three times and after that, $MgSO_4$ was added into the organic solution to remove water. The solution was concentrated by rotary evaporation after being filtrated to remove solid impurities, and then the concentrated solution was added into diethyl ether dropwise to remove impurities. After filtration, product was kept under vacuum to remove organic solvents. Then target product (4sP(EG-PLLA)-$N_3$) was obtained. The synthesis of 4sPEG-PDLA-$N_3$ adopted the similar method as described above.

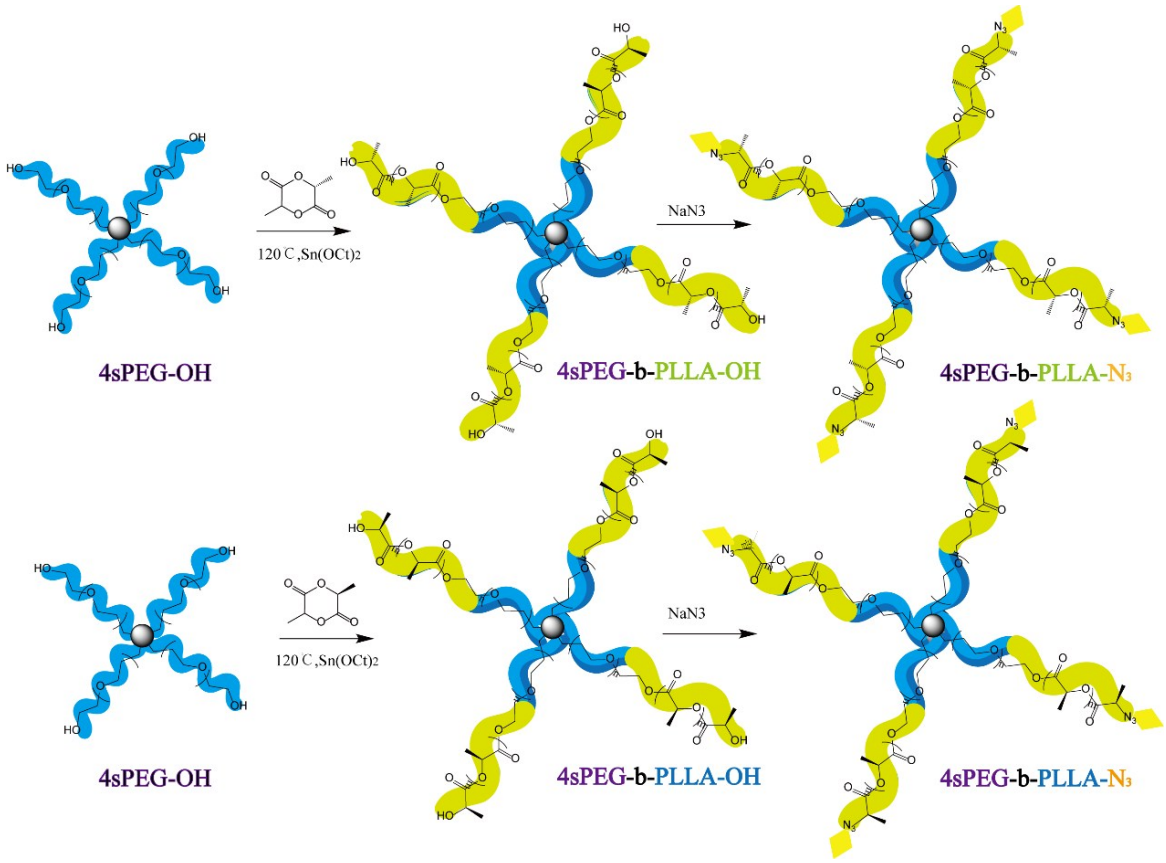
**1.3 Characterizations.** The $^1$HNMR spectra were performed on Bruker Avance 400 MHz NMR spectrometer with $CDCl_3$ as solvents and 0.5% tetramethylsilane was used as the internal standard. FTIR spectra were carried out on a Fourier transform infrared (FTIR) spectrometer (Thermo Fisher Scientific Nicolet 8700) between 500~4000 $cm^{-1}$.

The size and size distribution of nanogels were recorded by dynamic light scattering (DLS) spectrometer (Malvern ZetasizerNano ZS) at 25 ℃ while each sample was measured three times to attain the average value. Scanning electronic microscopy (SEM, Nova NanoSEM450) was employed to observe the morphology of nanogels.

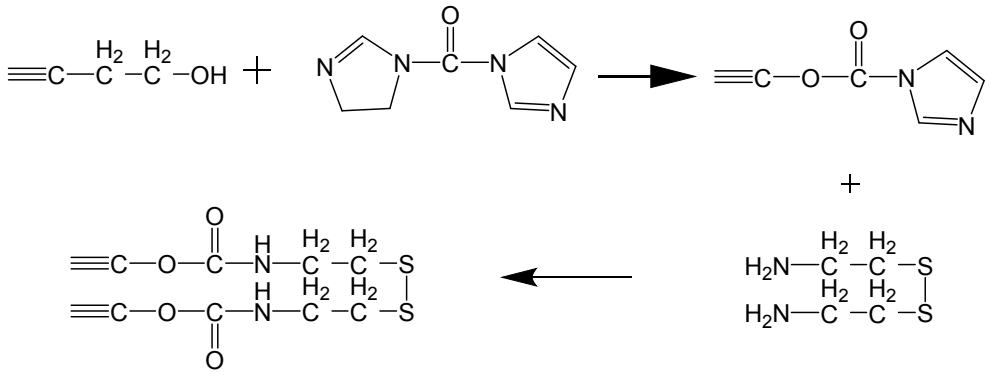
**1.4 Preparation of non-crosslinked, single cross-linked Blank and DOX-loaded Nanogels.** Firstly, the non-crosslinked polymeric micelles (D) and single crosslinked nanogels were prepared by dialysis method. 10 mg of 4sP(EG-DLA)-OH was thoroughly dissolved in 1 mL DMSO, and then slowly added into distilled water (10 mL) after being processed ultrasonic for 30 min. After stirred several hours, then the mixture was transferred into a dialysis tubing (MWCO = 2000) and dialyzed against distilled water to remove DMSO, the outer phase was

replaced by fresh distilled water every 2 h and the resulting solution was lyophilized to obtain the solid powder of blank micelles.

The preparation of single cross-linked nanogels (DL) was prepared as follows procedure: 10 mg of amphiphilic copolymers (5 mg of 4sP(EG-LLA)-$N_3$ and 5 mg of 4s P(EG-DLA)-$N_3$) were dissolved in 1 mL of DMSO and the mixed solution was processed ultrasonic for 30 min before being added dropwise into 10 mL of distilled water. After stirred several hours, the mixture was transferred into a dialysis tubing (MWCO = 2000) and dialyzed against distilled water to remove the organic solvent while the outer phase was replaced by fresh distilled water every 4 h. Then the resulting solution was lyophilized to obtain the solid powder of single cross-linked blank nanogels. The loading of DOX into uncross-linked polymeric micelles (DX) and single cross-linked nanogels (DLX) was similar as that of blank micelles and cross-linked nanogels. The different between DOX loaded and unloaded nanogels was that DOX (3 mg) was added into the mixture. The whole procedure was performed in a dark environment.




**Scheme S1.** the synthetic routes of 4sP(EG-b-LLA)-$N_3$ and 4sP(EG-b-DLA)-$N_3$ copolymers.



**Scheme S2.** the synthetic routes of redox-sensitive cross-linker molecules (d-ss-Bu).

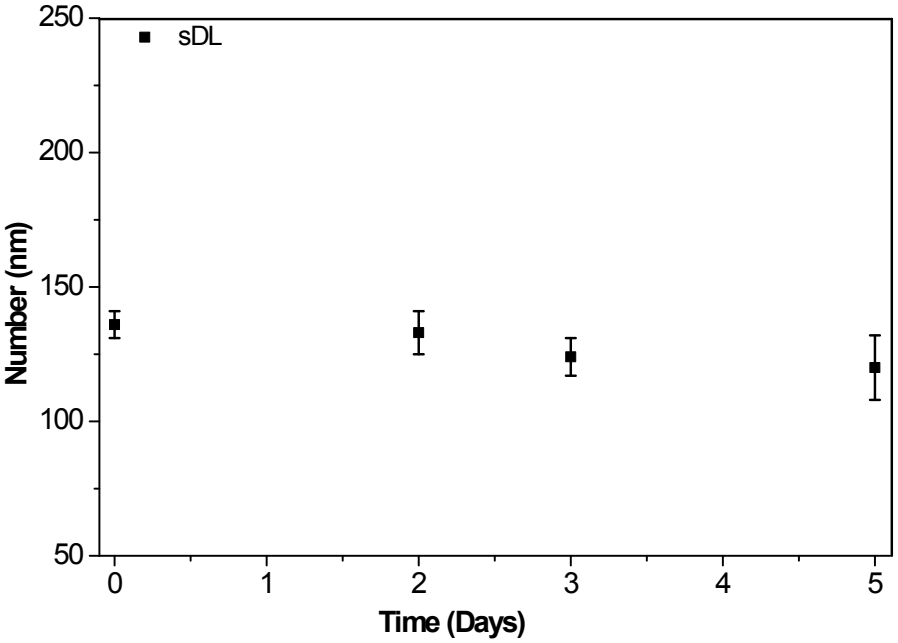



**Figure S1.** the size of sDL at different incubation times.

**Table S1** The properties of DX, DLX and sDLX nanogels**.**

| Nanocarriers | Size (nm) | PDI | DLC% |
|---|---|---|---|
| DX | 240 | 0.31 | 5.76±1.45 |
| DLX | 260 | 0.25 | 11.93±2.10 |
| sDLX | 190 | 0.27 | 13.96±2.01 |