Supporting Information

Fabrication of PεCL-AuNP-BSA Core-Shell-Corona Nanoparticles for Flexible Spatiotemporal Drug Delivery and SERS Detection

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CONTENTS

1. Materials.

$HAuCl_4 \cdot 4H_2O$ (Heowns, 99%), trisodium citrate dehydrate (Tianjin Chemical Reagent Company, AR), $NaBH_4$ (Tianjin Chemical Reagent Company, AR), 2-naphthalenethiol (NPT, Sigma-Aldrich, 99%), folic acid (FA, Heowns, 97%), bovine serum albumin (BSA, Dingguo Biotechnology Co. Ltd., 96%), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (EDC·HCl, Macklin, 98.5%), *N*-hydroxysuccinimide (NHS, Heowns, 98%), Nile red (NR, Sigma-Aldrich, 98%), fluorescein isothiocyanate (FITC, Alfa Aesar, 95%), tris(2-carboxyethyl phosphine hydrochloride (TCEP·HCl, Sigma-Aldrich, 98%)，curcumin (CUR, MERYER, 98%), lipase from *pseudomonas cepacia* (PS-lip, Sigma-Aldrich, 40 U/mg), trypsin from porcine pancreas (TRY, MACKLIN, 250 U/mg), and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI, Solarbio, 90%) were used as received. Ultrapure water (18.2 MΩ.cm) was obtained via a UPH water purifying system (ULUPURE). $P\varepsilon CL_{376}$-s-s-py_{18} with pendant pyridyl disulfide groups was synthesized according to our previous paper.[1] AuNPs and functionalized BSA (BSA-SH, BSA-FITC, BSA-FA and BSA-FA-FITC) were synthesized according to previous research paper.[2-3]

2. Characterizations.

^{1}H NMR measurements. ^{1}H NMR spectra of the polymers synthesized in this research were collected on a Varian UNITY-plus 400 M nuclear magnetic resonance spectrometer, using deuterated $CDCl_3$ as the solvents.

UV−vis measurements. UV−vis analysis was performed on a Shimadzu UV-2450 spectrometer using quartz cuvettes with 10 mm path length.

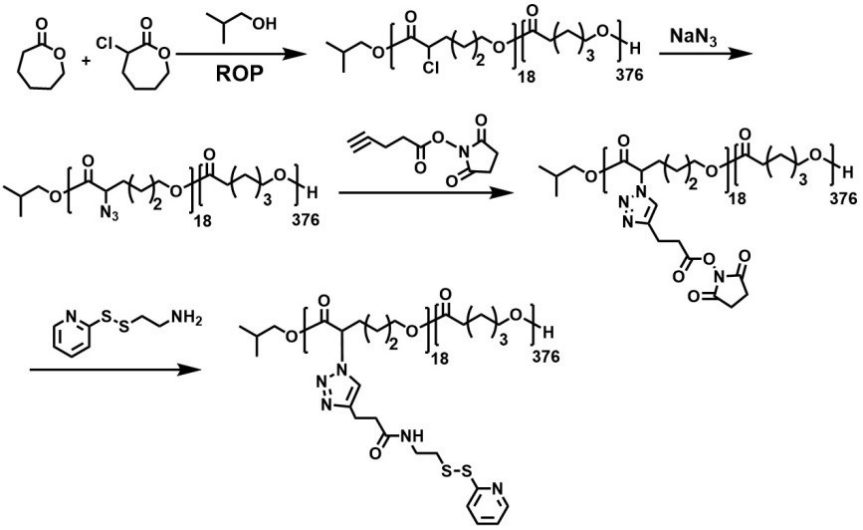
Size Exclusion Chromatography (SEC). The apparent molecular weights and dispersities of the polymers synthesized in this research were collected on a SEC, which was equipped with a Hitachi L-2130 HPLC pump, a Hitachi L-2350 column oven operated at 50 ℃, a Hitachi L-2490 refractive index detector and three Shodex columns (5000-5K, 400-0.5K, and 5-0.15K molecular ranges). DMF was used as eluent and the flow rate was set at 1.0 mL/min. Molecular weights were calibrated by using linear poly (methyl methacrylate) (PMMA) as standards.

Transmission Electron Microscopy (TEM). The morphologies of the hybrid nanoparticles were characterized on a Tecnai G2 20 S-TWIN electron microscope operating at 200 kV. The TEM specimens were prepared by depositing aqueous solutions of hybrid assemblies on Formvar Carbon-coated copper grids, and the solvent was evaporated in air at room temperature. The TEM specimens of the hybrid nanoparticles were stained with OsO_4.

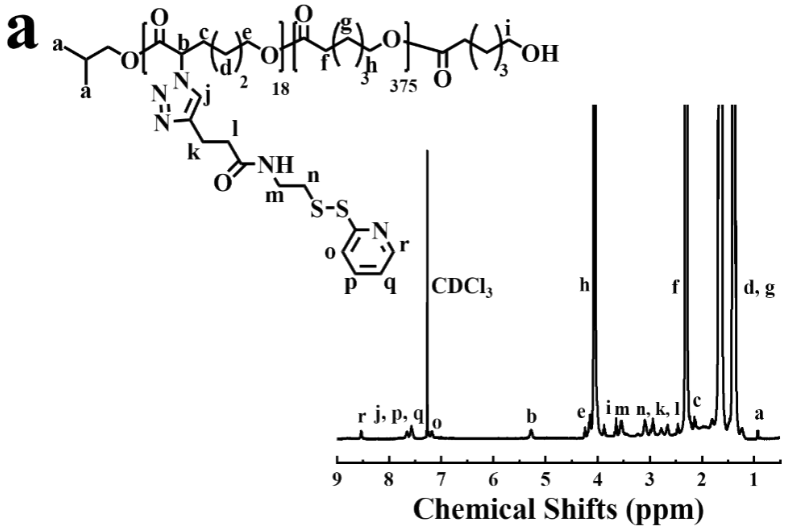
Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF). MALDI-TOF analyses were carried on a Bruker Autoflex III LRF200-CID spectrometer, which is equipped with a 337 nm nitrogen laser.

3. Synthesis of PεCL with pendant pyridyl disulfide groups ($PεCL_{376}$-s-s-py_{18}).

$PεCL_{376}$-s-s-py_{18} random copolymer was prepared by ring opening copolymerization of εCL and αCl-εCL, Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction (click reaction) and thiol-disulfide exchange reaction. A scheme for the synthesis of the copolymer is shown in **Scheme S1**. The synthetic details can be found in our previous publication.[1] ^{1}H NMR and SEC curve of the copolymer are shown in **Figure S1a and Figure S1 b**, respectively. The average repeating unit numbers of εCL and αPy-εCL calculated based on ^{1}H NMR result are 376 and 18, respectively. The number-average molecular weight (Mn) and PDI of the copolymer detected on SEC is around 38.3 k and 1.28.



Scheme S1. Synthetic route for the synthesis of $PεCL_{376}$-s-s-py_{18}.



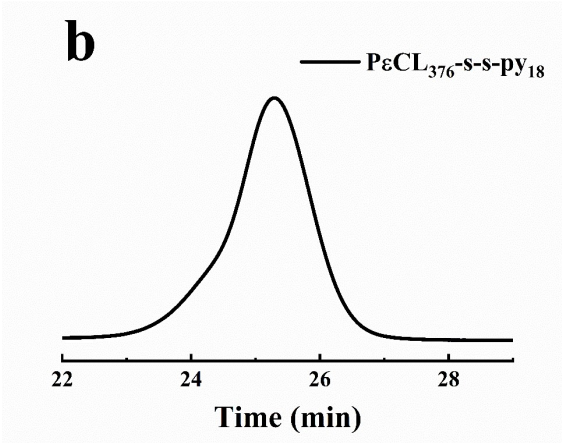


Figure S1. (a) ^{1}H NMR and (b) SEC curve of $P\varepsilon CL_{376}$-s-s-py_{18}.

4. Synthesis of gold nanoparticles (AuNPs).

The citrate-capped AuNPs were prepared by reduction of $HAuCl_4$ with borohydride in aqueous solution.[2] $HAuCl_4 \cdot 4H_2O$ (30 mg, 0.075 mmol) and trisodium citrate dehydrate (22 mg, 0.075 mmol) were dissolved in 100 mL of deionized water. After vigorous stirring for 1 h, ice cold $NaBH_4$ (1.5 mL, 0.45 mmol) was added to the solution and the solution turned burgundy immediately. The solution was vigorously stirred for 4 h at room temperature. On the basis of the TEM result, the average size of AuNPs is about 5 nm (**Figure S2**). The SERS-encoded AuNPs were obtained by adding NPT (1 mg/mL，10 μL, dissolved in DMF) into the AuNPs dispersion and NPT-decorated AuNPs (AuNPs-NPT) were obtained. The concentration of AuNPs in the solution was calculated by using the following equations.

$$V_{AuNP} = \frac{4}{3}\pi R^3 \quad (1)$$

$$m_{AuNP} = \rho_{AuNP} \times V_{AuNP} \quad (2)$$

$$m_{AuNPs} = m_{HAuCl4 \cdot 4H2O} \times Au\% \quad (3)$$

$$N_{AuNPs} = \frac{m_{AuNPs}}{m_{AuNP}} \quad (4)$$

$$n_{AuNPs} = \frac{N_{AuNPs}}{N_A} \quad (5)$$

$$C_{AuNPs} = \frac{n_{AuNPs}}{V} \quad (6)$$

where V_{AuNP} is the volume of one AuNP, m_{AuNP} is the mass of one AuNP, m_{AuNPs} is the total mass of all AuNPs in the aqueous solution, N_{AuNPs} is the number of AuNPs in the aqueous solution, n_{AuNPs} is the mole of the AuNPs in the solution, and C_{AuNPs} is the molar concentration of the AuNPs in the solution.

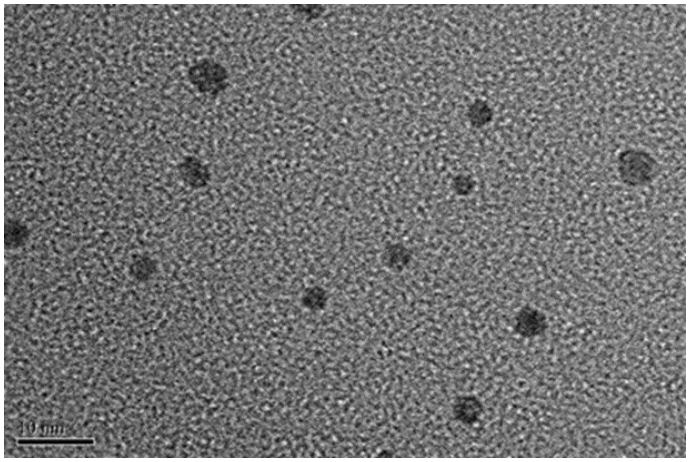


Figure S2. TEM image of AuNPs. Scale bar: 10 nm.

5. Synthesis of reduced BSA.

BSA was dissolved in degassed PBS (pH 8.0, 10 mM) at 5 mg/mL. TCEP (1 mg/mL) in degassed PBS (pH 8.0, 10 mM) was added to the protein solution under an argon atmosphere. The mixture was stirred at 4 ˚C for 6 h, and was purified by dialyzing (MWCO 7000) against degassed deionized water for 3 days. Reduced BSA was used in the following experiments.

6. Conjugation of FA to BSA.

The carboxylic acid groups of FA were reacted with NHS to form active ester intermediates (FA-NHS), which was subsequently reacted with the amine groups on BSA.[3] FA (33.1 mg, 0.075 mmol) was dissolved in 20 mL of PBS (pH 7.4, 10 mM), and EDC (71.9 mg, 0.376 mmol) and NHS (43.2 mg, 0.376 mmol) were added to the solution under stirring at room temperature. The solution was stirred in the dark for 1 h, and subsequently the solution was added slowly to 30 mL of PBS (pH 7.4, 10 mM) solution of BSA (500 mg, 0.0075 mmol). The solution was stirred at 4 ˚C for 6 h in the dark. The mixture was dialyzed against deionized water (MWCO 7000) for 3 days to remove organic solvents and small molecular compounds. As calculated by MALDI-TOF (**Figure S3**), the average number of FA grafted to BSA is about 2.0.

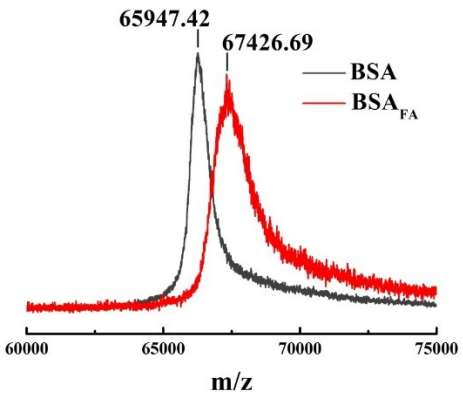


Figure S3. MALDI-TOF mass spectra of BSA and BSA_{FA}.

7. Synthesis of FITC-Labeled BSA and FITC-Labeled BSA_{FA}.

FITC in DMSO (50 μL, 1 mg/mL) was added to buffer solution (pH 9.0, 100 mM) of BSA or BSA_{FA} (5 mg/mL) at 4 ˚C. The solutions were stirred in the dark for 2 h, and subsequently, the solution was dialyzed against deionized water (MWCO 7000) for 2 days at 4 ℃. The MALDI-TOF spectra of FITC-labeled BSA and BSA_{FA} are shown in **Figure S4**.

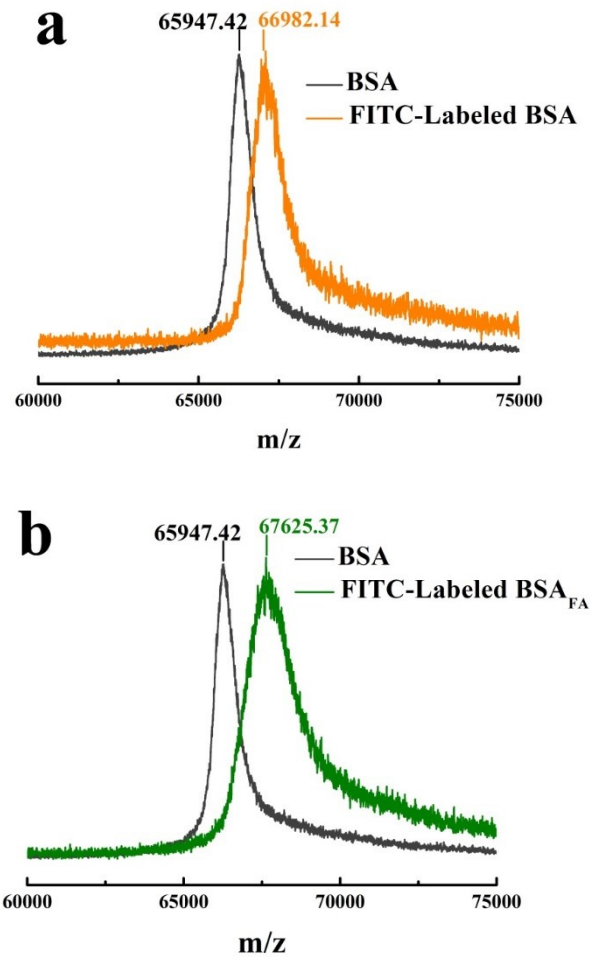


Figure S4. MALDI-TOF spectra of (a) BSA and FITC-labeled BSA, and (b) BSA and FITC-labeled BSA_{FA}.

8. Fabrication of PεCL-AuNPs-BSA CSCNPs.

A typical procedure for the fabrication of PεCL-AuNPs-BSA core-shell-corona nanoparticles (CSCNPs) was described as follows. Briefly, 2.5 mg of $PεCL_{376}$-s-s-py_{18} dissolved in 1 mL of DMF was added by drop into the aqueous dispersion of AuNPs (189 nM, 10 mL), and the mixture was kept stirring at room temperature for 4 h. After centrifugation, the obtained PεCL-AuNPs core-shell nanoparticles (CSNPs) were dispersed in water, and the particle solution was slowly dropped into aqueous solution of BSA (2 mg/mL, 10 mL). The mixture was stirred at 4 ˚C in the dark overnight. After centrifugation, PεCL-AuNPs-BSA CSCNPs were obtained.

9. Preparation of Curcumin-loaded CSCNPs.

A typical procedure for the synthesis of curcumin (CUR) loaded CSCNPs was described as follows. PεCL$_{376}$-s-s-py$_{18}$ (2.5 mg) and CUR (0.5 mg) were dissolved in 1 mL of DMF. The polymer solution was added into aqueous dispersion of AuNPs (189 nM, 10 mL), and the solution was stirred at room temperature for 6 h. After centrifugation, CUR-loaded PεCL-AuNPs CSNPs were obtained. The collected NPs were dispersed into 10 mL of BSA solution (2 mg/mL) at 4 ˚C, and the solution was stirred at room temperature overnight. To determine the CUR encapsulation efficiency (EE) and loading content (LC) of the obtained CUR-loaded CSCNPs, the CSCNPs were dissolved in DMF, and the UV-Vis absorbance of CUR at 420 nm was measured. The EE and LC of CUR were calculated according to the following equations:

$$EE\ \% = \frac{CUR\ loading}{Cur\ fed} \times 100\% \quad (7)$$

$$LC\ \% = \frac{CUR\ loading}{Mass\ of\ nanoparticles\ recovered} \times 100\% \quad (8)$$

10. Enzyme-triggered CUR Release from CSCNPs.

CUR-loaded CSCNPs (4.3 mg) were dispersed in 1 mL PBS of lipase (2 mg/mL, pH=7.4), or trypsin (1 mg/mL, pH=7.4). The solutions were transferred to dialysis bags with a molecular cutoff of 3500 Da, and the dialysis bags were immersed in 15 mL of PBS (pH=7.4) containing 0.1% w/v Tween-80 at 37 ˚C. At designated time intervals, the release medium (1.0 mL) was withdrawn and replaced by equal volume of fresh medium. CUR concentration in the medium was measured by UV-vis at 420 nm. All the release experiments were carried out in triplicate.

11. Folate receptor-mediated cellular uptake.

NIH 3T3 and 4T1 cells were seeded in a 35 mm glass-bottom culture dish at a density of 2×10^5 cells per dish and cultured in DMEM supplemented with 10 % FBS and 1% antibiotics (37 ˚C, 5% CO_2) for 24 h. The medium was then replaced by 1 mL of NR- and FITC-labeled CSCNPs at a concentration of 100 μg/mL. After 4 h of incubation, the dishes were washed with PBS for three times and the cells were fixed with 4% formaldehyde for 10 min and washed by PBS. Cell nuclei were counterstained with DAPI (10 μg/mL, 1 mL) for 10 min. Then the cells were washed with PBS for 3 times and observed on a confocal laser scanning microscope (Leica TCS SP8).

To quantify cellular uptake of CSCNPs, cells were treated with NR- and FITC-labeled PεCL-AuNP-BSA$_{FA}$ and PεCL-AuNP-BSA CSCNPs for 4 h, respectively. Subsequently, the cells were washed with PBS for 3 times and collected following trypsin-EDTA digestion. The obtained cells were re-suspended in 500 μL of PBS containing 10% FBS, and analyzed by flow cytometry (BD Accuri C6, BD biosciences, San Jose, CA). All the experiments were carried out in four replicates.

12. Cytotoxicity assays and tumor cells growth suppression *in vitro*.

Tumor 4T1 cells were seeded in a 96-well plate at a density of 5000 cells per well. The cells were cultured in DMEM supplemented with 10% FBS at 37 ˚C in 5% CO_2 atmosphere for 24 h. Afterwards, the culture medium was replaced with fresh medium containing CUR-free or CUR-loaded CSCNPs at different concentrations. Cells were maintained in culture for 24 h, after which media were removed and 10 μL of CCK-8 reagents (Dojindo Laboratories) and 90 μL of fresh medium were added. After 30 min, the plates were gently shaken for 2 min to dissolve the formazan crystals. The absorbance of each well was measured at 450 nm using a multifunctional ELISA plate reader (Thermo Varioskan Flash). The cell viability (%) was calculated using the following formula:

$$Cell\ viability\ \% = \frac{A_{Sample} - A_{Blank}}{A_{Control} - A_{Blank}} \times 100\ \% \qquad (9)$$

Where A_{Blank} is the absorbance of CCK-8 reagents without cells, A_{Sample} is the absorbance of cells treated with CSCNPs, and $A_{Control}$ is the absorbance of the untreated cells. All experiments were carried out in four replicates.

13. SERS Detection and Dark-Field Imaging.

For SERS detection of folate receptor-targeting tumor cells, PεCL-Au_{NPT}-BSA_{FA} and PεCL-Au_{NPT}-BSA CSCNPs were prepared. 4T1 cells were seeded onto 14 mm tissue culture glass slides at a density of 2 × 10^5 cells per slide and maintained in 24-well plates under the same cell culture condition for 24 h. Then, the medium was replaced with serum-free medium containing CSCNPs at a concentration of 100 μg/mL. After 4 h of incubation, the glass slides were washed with PBS for three times and SERS detections were made. SERS spectra were recorded on a Raman spectrophotometer (Horiba HR evolution, 600 grooves/mm grating) with a laser excitation wavelength of 532 nm (He-Ne laser). A 10× (NA 0.5) microscope objective, 6.7 mW laser beam and 10 s acquisition time were applied to measure the solution samples. A 50× (NA 0.5) microscope objective, 5.4 mW laser beam and 0.5 s acquisition time were applied to measure the cell samples.

The enhancement factor (EF) of the individual nanoparticles was determined by computing the ratio of SERS to normal Raman scattering of NPT by using the following equation:

$$EF = \frac{I_{SERS} \times C_{Normal}}{I_{Normal} \times C_{SERS}} \qquad (10)$$

Where, I_{SERS} and I_{Normal} are the Raman intensities of 2-naphthalenethiol (NPT) on hybrid nanoparticles and in NPT solution at 1420 cm^{-1}. C_{SERS} and C_{Normal} are the concentrations of NPT on nanoparticles and in solution. C_{SERS} was calculated by using the equation $C_{SERS} = N \times C_{AuNPs}$, where N is the average number of NPT on a AuNP.

14. Statistical Analysis.

All data points were represented as mean ± standard deviation (SD). Statistical significance was evaluated by Student's *t*-test. The confidence interval was 95%. The difference was considered to be significant when p value was smaller than 0.05 (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

15. References

1) Y. Ju; M. Zhang and H. Zhao, *Polym. Chem.*, 2017, **8**, 5415-5426.
2) J. Tian, F. Zheng, Q. Duan and H. Zhao, *J. Mater. Chem.*, 2011, **21**, 16928-16934.
3) N. Ma, J. Liu, W. He, Z. Li, Y. Luan, Y. Song and S. Garg, *J. Colloid and Interface Sci.*, 2017, **490**, 598-607.