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Supplementary Information

Protein-modified conjugated polymer nanoparticles with strong near-infrared absorption: a novel nanoplatform to design multifunctional nanoprobes for dual-modal photoacoustic and fluorescence imaging

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Experimental Section

Materials: Bovine serum albumin (BSA) was purchased from Aladdin. Polyethylene glycol (PEG, MW 3350), Sephacryl S-300 HR, HEPES buffer (1 M), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were all obtained from Sigma-Aldrich. Tetrahydrofuran (THF) was received from Tokyo Chemical Industry Co. Ltd. Chloroauric acid ($HAuCl_4$) was purchased from Sinopharm Chemical Reagent Co. Ltd. Poly((E)-3-(5-([8,8′-biindeno[2,1-b]thiophenylidene]-2-yl)thiophen-2-yl)-alt-2,5-bis(2-octyldodecyl)-6-(thiophen-2-yl)-pyrrolo[3,4-c]pyrrole-1,4(2H,5H)dione) (PBTP-DPP) was prepared according by the literature reported by us previously.[1] All chemicals were used as received in this study were of analytical reagent grade and used without further purification. Ultrapure water (18.25 MΩ cm, 25 °C) was used in all experiments.

Synthesis and purification of BSA-modified PBTP-DPP conjugated polymer (CP) nanoplatform: The BSA-modified CP nanoplatform was synthesized under ultrasonic in the water bath. Different from traditional mini-emulsion and nanoprecipitation, BSA (30 mg/mL) and PBTP-DPP polymer (10 μg/mL) were dissolved in pure water and THF, respectively. And then the PBTP-DPP polymer (2 mL) was injected swiftly into the BSA solution (20 mL) under sonication for 3 min. Nitrogen (N_2) was then introduced to remove the THF for 30 min. After that, BSA-modified CP nanoplatform was prepared. Rotary evaporation was used to obtain high concentration nanoplatform which was further purified by gel filtration using Sephacryl HR-300 gel media according to our previous report. The purified nanoplatform was stored at 4 °C for further application.

Synthesis and purification of Au clusters-functionalized BSA-modified CPNs: The as-prepared BSA-modified PBTP-DPP nanoplatform and $HAuCl_4$ (10 mM) were mixed under 37 °C for about 2 min. Then NaOH (1M) was used to adjust the pH value to 10. Au clusters can be formed on the nanoplatform at 37 °C for 12 h under vigorous stirring. After that, the pH was adjusted to 7.4 by PBS using ultrafiltration (MWCO = 10 kDa, Millipore). Finally, gel filtration was introduced to

remove the possible free Au clusters. The Au clusters-functionalized BSA-modified CPNs was synthesized and stored at 4 °C for further use.

Characterization of the nanomaterials: Ultraviolet–visible (UV-vis) absorbance spectra of the nanoparticles including BSA-modified PBTP-DPP nanoplatform, Au clusters and Au clusters-functionalized BSA-modified CPNs were recorded using a Shimadzu UV-1800 UV-Vis absorption spectrophotometer. Photoluminescence (PL) spectra were recorded with a Horiba FluoroLog spectrometer. The hydrodynamic diameters were measured using Malvern Nano-ZS Particle Sizer. Transmission electron microscopy (TEM) image was obtained on a FEI Tecnai G20 transmission microscope at 200 kV. 10 μL of the sample was dropped on a carbon-coated grid and then dried for the TEM observation.

Cell culture: The human embryonic kidney 293T cell line and the 4T1 mammary carcinoma cell line were incubated in Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin, and 1% (v/v) streptomycin. The cells were cultured in a humidified incubator at 37 °C with 5% CO_2.

In vitro cytotoxicity: The human embryonic kidney 293T cell line and the 4T1 mammary carcinoma cell line were involved to evaluate the cytotoxicity of the as-prepared BSA-modified PBTP-DPP nanoplatform. First, 293T cells and 4T1 cells (5×10^3 cells/per well, 100 μL) were respectively cultured on 96-well plates in the medium mentioned above overnight. Then, the medium was replaced with mediums containing different concentrations of the BSA-modified PBTP-DPP nanoplatform (0, 2.5, 5, 10, 20, 40 μg/mL). After 12 and 24 h incubation, the cell viabilities were assessed by the MTT assay. The cells incubated with DMEM without any treatment as the blank control.

In vitro PA imaging: PA signals of the as-prepared BSA-modified conjugated polymer nanoplatform were obtained by measuring an agarose gel phantom containing different does of the nanoplatform ranging from 0.9375 μg/mL to 30 μg/mL. The PA stability of the nanoplatform was also measured by recording the PA intensities under the irradiation of the laser. All the PA data were from our home-made PA system reported previously.[2]

Animals and tumor model: Animals involved in the experiments were all treated strictly in compliance with the Suggestions for the Care and Use of Laboratory Animals. The protocols were

approved by Institutional Animal Care and Use Committee of the University of Macau and Shenzhen Institutes of Advanced Technology (SIAT), Chinese Academic of Sciences (CAS). 6-8 weeks nude mice were introduced to establish the subcutaneous animal tumor model for in vivo PA and FL imaging. 100 μL four million 4T1 tumor cells dispersed in PBS were injected subcutaneously in the hind leg of the mouse. Tumors were grown until the size was about 100 mm^3 before applied for in vivo imaging experiments. It should be noted that the tumor size was calculated according to the traditional formula $V = [(length) \times (width)^2]/2$.

In vivo PA imaging: 4T1 breast tumor-bearing mouse model was established to evaluate the in vivo PA imaging ability of the BSA-modified conjugated polymer nanoplatform. 100 μL of the nanoplatform was intravenously (i.v.) injected into the mouse with a concentration of 30 μg/mL. In vivo PA imaging was first conducted before the injection which denoted as control (0 h) at the wavelength of 790 nm. And then the images were obtained at different time points including 2h, 4h, 6h, 12h, and 24h under the same condition.

In vivo FL imaging: The procedure of in vivo FL imaging was carried out in accordance with PA imaging mentioned above. Briefly, the Au clusters-functionalized BSA-modified CPNs was intravenously (i.v.) injected into the mouse. In vivo FL images were also obtained by a Maestro GNIR Flex imaging system (CRi) at different time points.

Biodistribution Analysis: The tumor-bearing mice with the injection of the dual-modal Au clusters-functionalized BSA-modified CPNs were sacrificed after in vivo FL imaging. The tumor and major organs including heart, live, spleen, lung, and kidney were collected. The PA signals and FL images were recorded later using the PA imaging system and optical imaging system, respectively.

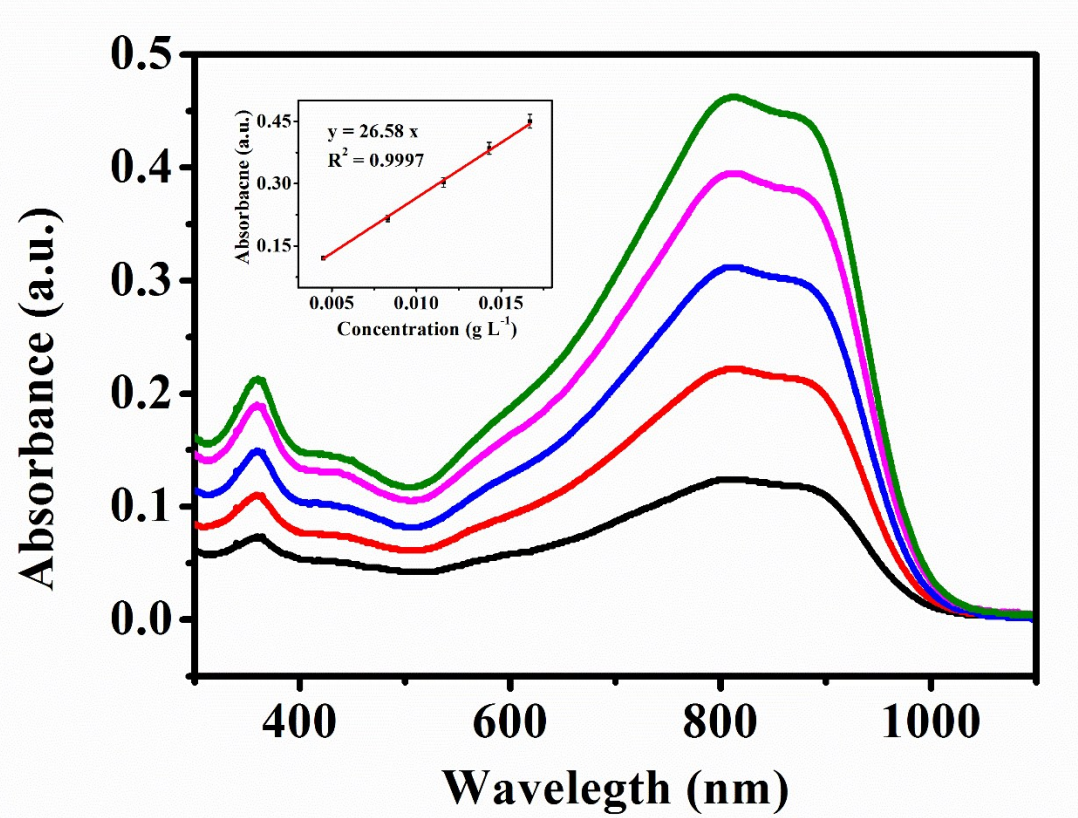

Figure S1. Uv-vis-NIR absorption spectra and linear relationship between the absorption at 790 nm and the concentrations (insert) of the PBTP-DPP dissolved in THF.

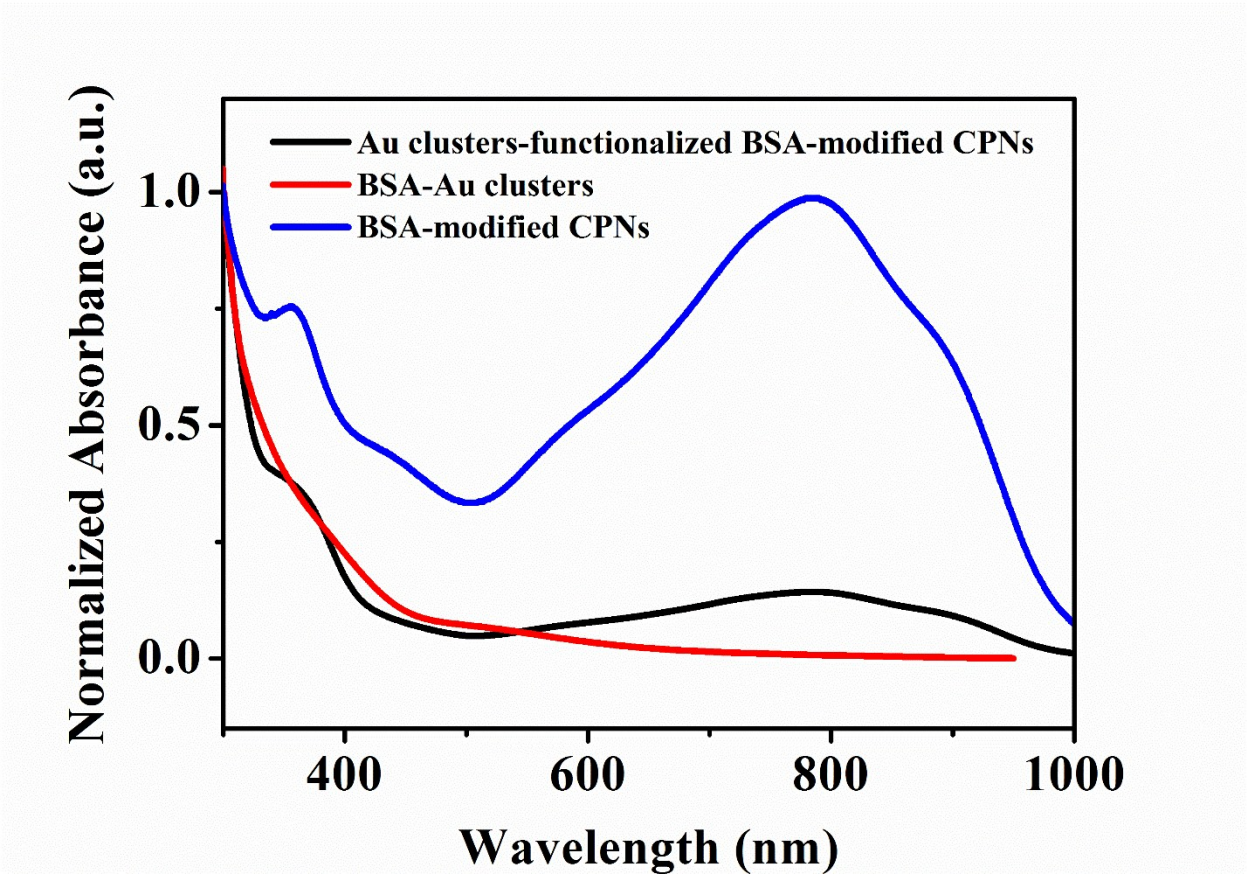

Figure S2. Absorption spectra of the BSA-Au clusters, BSA-modified CPNs, and Au clusters-functionalized BSA-modified CPNs.

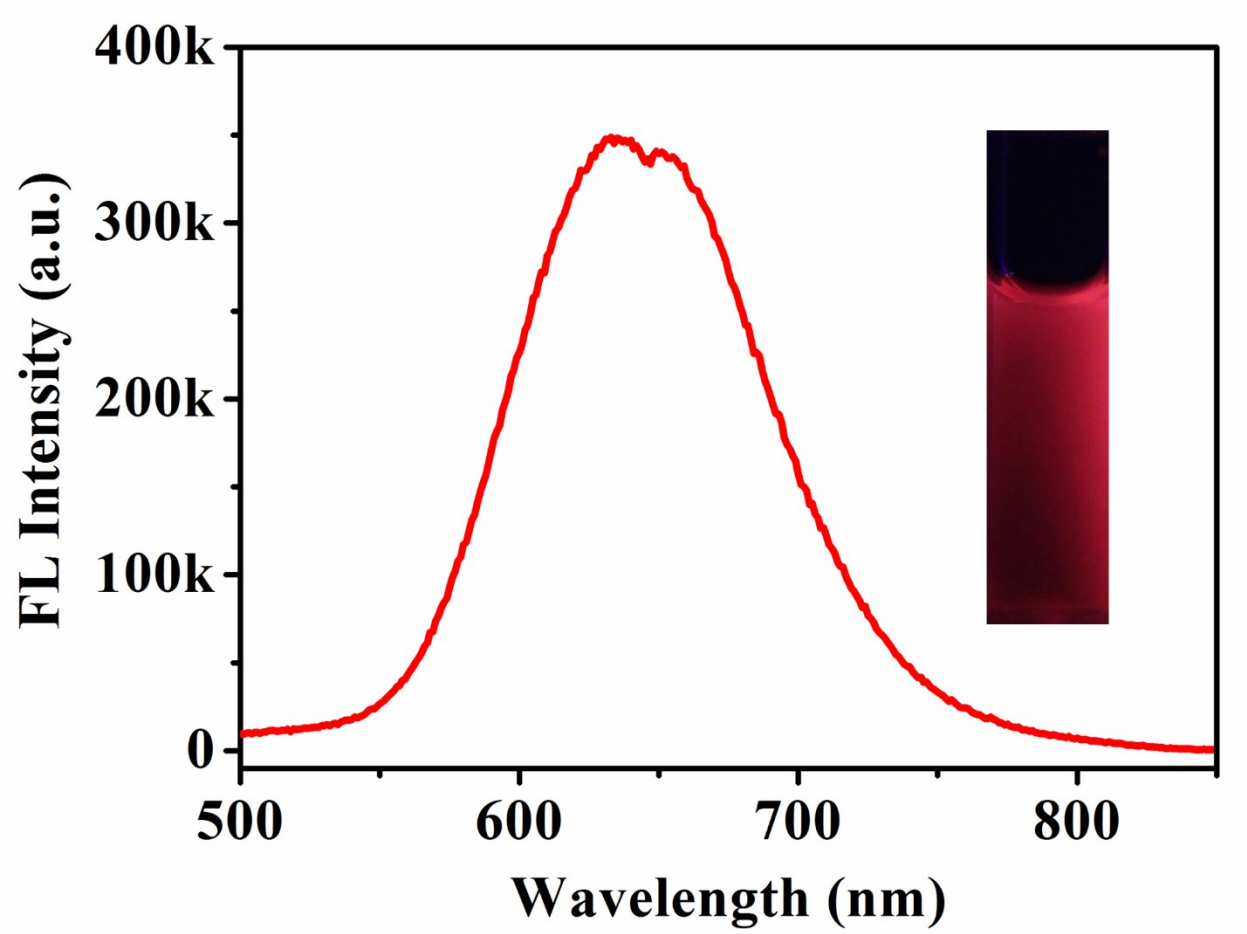

Figure S3. FL spectrum and photograph under uv-light of the Au clusters-functionalized BSA-modified CPNs.

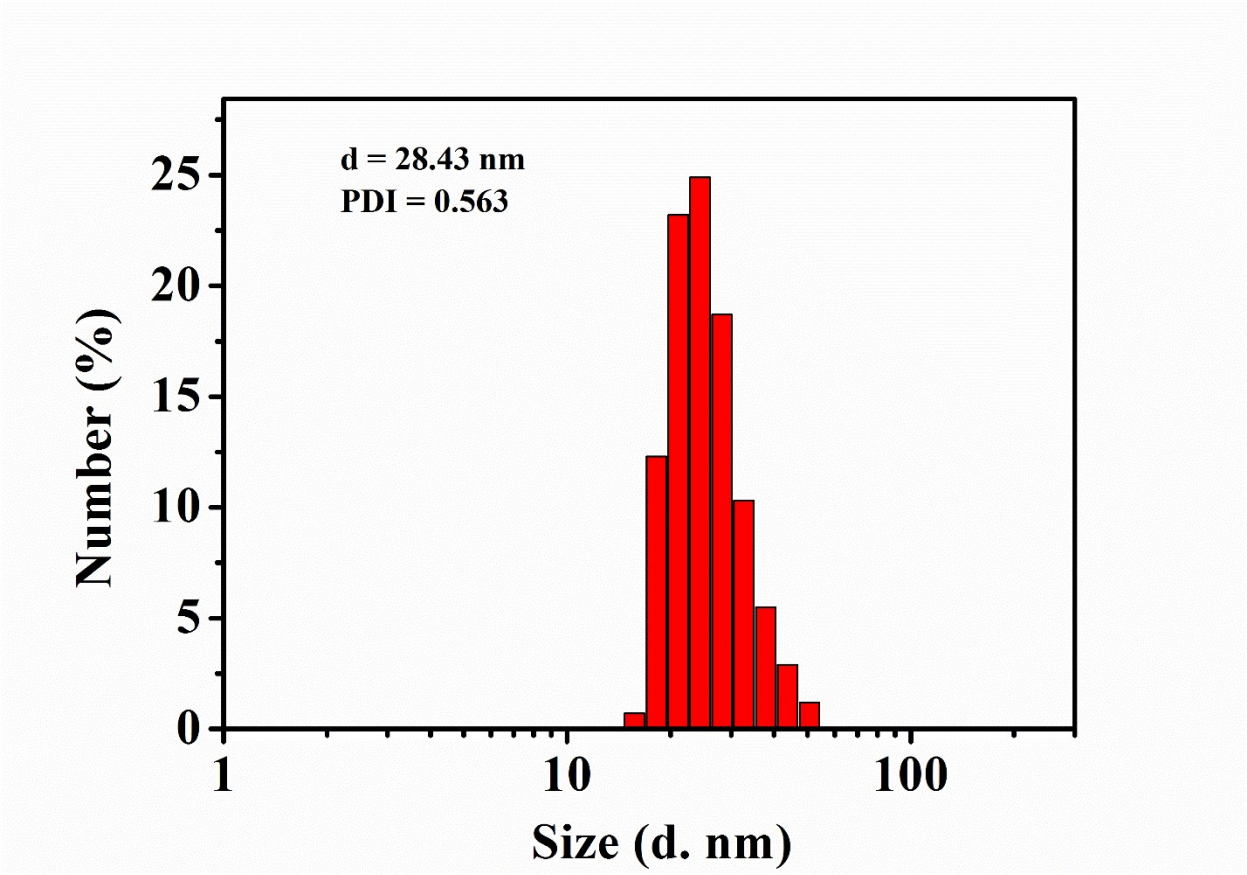

Figure S4. Hydrodynamic diameter of the Au clusters-functionalized BSA-modified CPNs measured by DLS

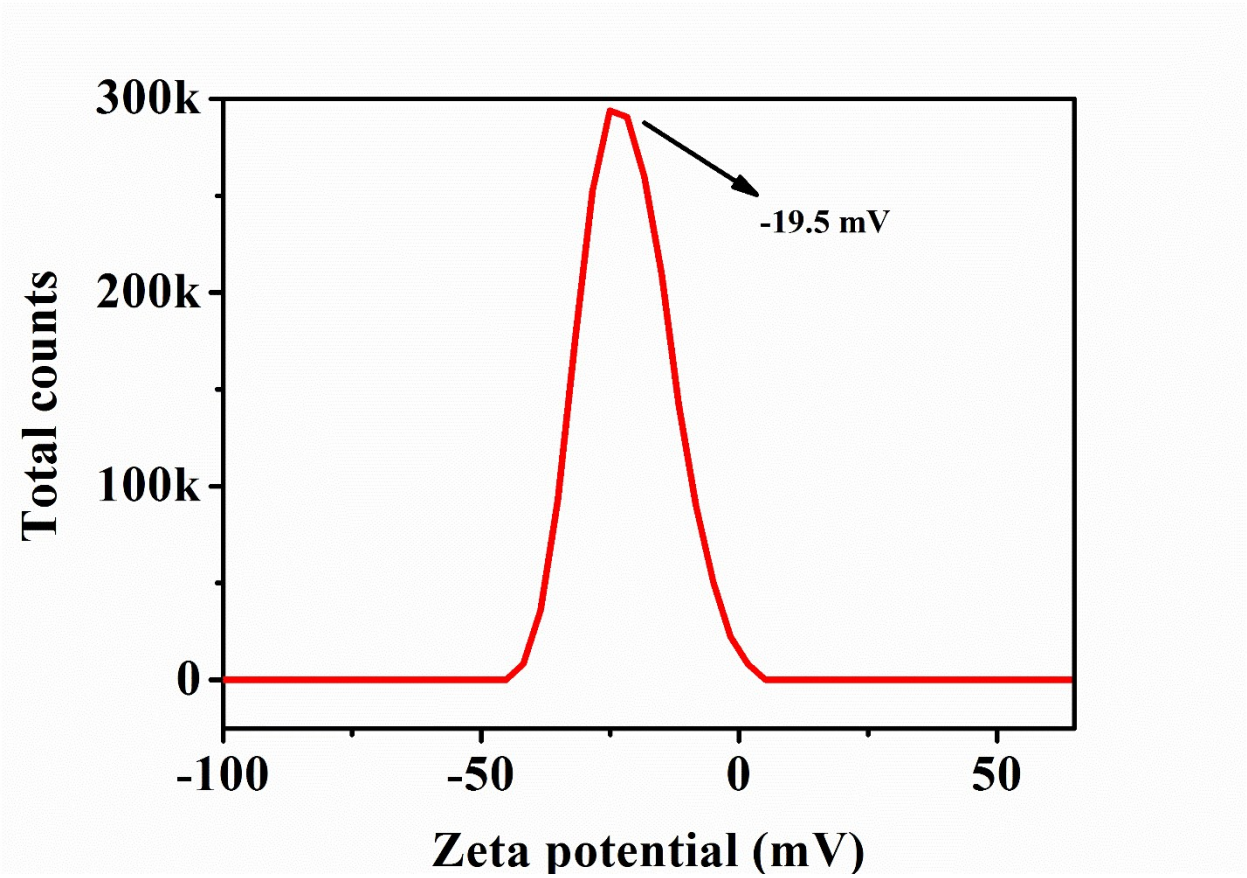

Figure S5. Zeta potential of the Au clusters-functionalized BSA-modified CPNs measured by DLS

References

1. C. Li, Z. Mao, H. Chen, L. Zheng, J. Huang, B. Zhao, S. Tan and G. Yu, *Macromolecules,* 2015, **48**, 2444-2453.

2. Y. Liu, H. Jiang and Z. Yuan, *Med. Phys.*, 2016, **43**, 3987-3997.