Electronic Supporting Information (ESI)

Near-Infrared Fluorescent Ribonuclease-A-Encapsulated Gold Nanoclusters: Preparation, Characterization, Cancer Targeting and Imaging

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Experimental Section

***Chemical Reagents*:** Bovine pancreatic ribonuclease A (RNase A), Gold (III) chloride ($AuCl_3$), sodium hydroxide (NaOH) were purchased from Sigma-Aldrich. All chemicals were used as received without further purification. Ultrapure water with a resistivity of ~18.2 $m\Omega \cdot cm^{-1}$ at 25 ºC was used as the solvent throughout the experiments.

Synthesis of RNase-A-AuNC and VB_{12}-R-AuNC: In a typical synthesis, the precursor solution (RNase-A-Au^{3+}) was prepared by mixing 10 mL $AuCl_3$ aqueous solution (10 mM) with 10 mL RNase-A solution (50 mg/mL) under vigorous stirring. Next, 1 mL aqueous NaOH solution (1 M) was injected into RNase-A-Au^{3+} solution in 15 min to adjust the pH to ~11. Thereafter, the mixture was incubated for 12 h at psychological temperature (37 °C) to yield bright emissive Au NCs. Vitamin B_{12} (VB_{12}; hydroxocobalamin) obtained commercially was bound to the RNase-A-AuNCs using EDC/NHS-mediated covalent coupling, according to a modified method described elsewhere.[1] The RNase-A-AuNCs and VB_{12}-R-AuNCs were purified *via* ultracentrifugation with a 10 kDa MWCO membrane to remove both residual Au^{3+} ions and un-reacted VB_{12}.

Characterizations of the Au NC: Fluorescence spectra were measured on a Hitachi F-4600 fluoro-spectrometer, and UV-visible spectra were obtained on a Shimadzu UV-2450 spectrophotometer. Fluorescence lifetime was determined on a FLS 920 fluorescence lifetime and steady state spectroscopy. MALDI-TOF MS spectra were obtained with an AXIMA CFR MALDI-TOF mass spectrometer. Electrophoresis analysis of the RNase-A-AuNCs was carried out in 10% native polyacrylamide gel at a constant voltage of 110 V for 30 min. BF TEM imaging was conducted on a JEOL JEM-2010F field emission TEM with an accelerating voltage of 200 kV. Scanning transmission electron microscopy (STEM) was performed in a FEI Tecnai F20 field emission gun TEM/STEM microscope operated at 200 kV and fitted with a Fischione HAADF detector and an 80 mm^2 Oxford Instruments X-Max Silicon Drift Detector (SDD) and a Gatan Orius SC600A CCD camera. Nanocluster aqueous dispersions were drop-cast onto TEM grids coated with ultrathin carbon support (Agar Scientific); samples were dried under an ultraviolet lamp. Dynamic light scattering (DLS) was performed at a fixed scattering angle of 90° on a Brookhaven light scattering system (BI-200SM Laser Light Scattering Goniometer) with a BI-APD detector using a He-Ne laser at 633 nm. Optical and fluorescence photographs of the RNase-A-AuNCs and VB_{12}-R-AuNCs were captured with a digital color camera (Olympus U7010). X-ray photoelectron spectra (XPS) were measured on a Thermo ESCALab 250 system with Al Kα radiation (1486.6 eV). Binding energy was calibrated by setting the carbon 1s peak to 285 eV. Detailed spectra had a Shirley or Linear background fitted to them and the data was analysed using mixed Gaussian-Lorentzian fits (using CASA XPS). Raman spectra were recorded with Renishaw inVia Raman Microscope containing a Leica DM2500 microscope.

Cell Proliferation and Viability Measurement: *In vitro* cytotoxicity of RNase-A-AuNCs was evaluated on A549 human lung carcinoma cells. The cells were maintained in RPMI-1640 medium (Invitrogen) supplemented with 10% calf serum, penicillin (100 U/mL) and streptomycin (100 μg/mL) in a humidified atmosphere containing 5% CO_2 at 37°C. A549 cells were seeded at 1.5×10^5

cells/well in 96-well plates and allowed to attach overnight. The RNase-A-AuNCs were purified *via* cryodesiccation from water solution to form lyophilized powders that were then redispersed in phosphate buffered saline (PBS) buffer solution (0.3 M NaCl, 10 mM phosphate, pH=7.4) and stored at 4 °C. For the toxicity assessment, the culture medium was diverted away from the wells individually and replaced with 200 μL Dulbecco's modified Eagle's medium (DMEM, Thermo Scientific) containing serial dilutions of RNase-A-AuNCs (e.g., 1, 0.25, 0.05 mg/mL). Finally, the cancer cells were allowed to grow for another 24 h. 20 μL MTT solution as a 5 mg/mL stock in PBS was added to each well. After incubating for 24 h, the medium containing unreacted MTT was carefully withdrawn. The obtained blue formazan crystals were dissolved in dimethyl sulfoxide (DMSO, 200 μL/well), the absorbance of which was subsequently measured in a Thermo Labsystems Multiskan Mk3 plate reader.

In Vitro Cancer Cells Targeted Imaging: The human colonic adenocarcinoma Caco-2 cells were obtained from the cell resource center of Shanghai Institutes for Biological Sciences affiliated to the Chinese Academy of Sciences (CAS). The cells were cultured in 75 cm^2 culture flasks (Corning Incorporated Life Sciences) using DMEM supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acids, 1% L-glutamine, penicillin (100 IU/mL), and streptomycin (100 μg/mL), and maintained in a Thermo Scientific Forma Direct-Heat CO_2 incubator at 37 °C with 5% CO_2 in air (v/v) and 95% relative humidity. The growth medium was changed daily until the day of the test. During the course of imaging for Caco-2 cells, the old medium was replaced by freshly prepared ones that contained an appropriate amount of RNase-A-AuNCs or VB_{12}-R-AuNCs. After ~2 h of incubation, the two groups of Caco-2 cells were washed three times with PBS buffer to free the unbound Au NCs in advanced of direct diagnostic observation under the epi-fluorescence microscopy with 488 nm illumination with a mercury vapor lamp.

References

[1] (*a*) D. J. Zhou, L. M. Ying, X. Hong, E. A. Hall, C. Abell, D. Klenerman, *Langmuir*, 2008, **24**, 1659; (*b*) K. B. Chalasani, G. J. Russell-Jones, A. K. Jain, P. V. Diwan, S. K. Jain, *J. Control. Release*, 2007, **122**, 141.

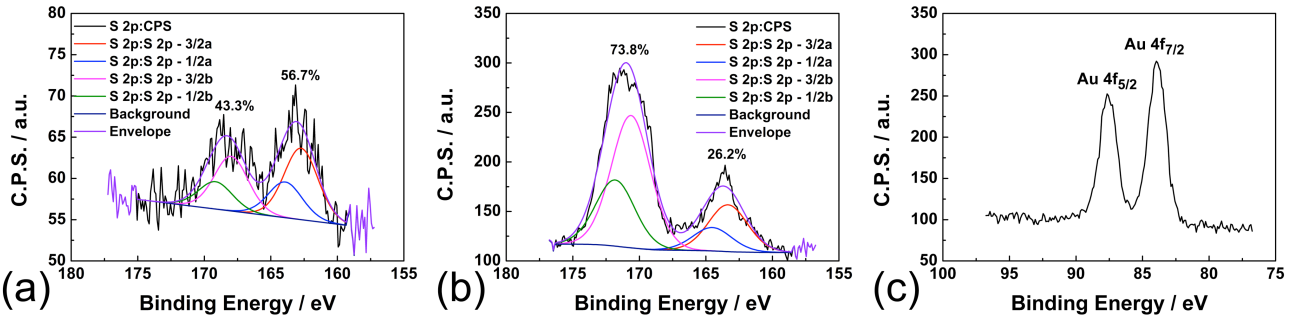

Fig. S1 Representative X-ray photoelectron spectroscopy (XPS) spectra of S 2p region for (**a**) the natural withered and (**b**) lyophilized RNase-A-AuNCs. The corresponding fits with relevant abundance of S 2p 1/2 and 3/2 species in both samples are shown. (**c**) XPS spectrum of Au 4f region for the lyophilized RNase-A-AuNC.

The fits can provide an estimation of the relevant abundances of the sulfur species in the reduced (BE ~ 163 eV, typical for sulfur bind to Au, *e.g.* S-Au) and oxidized state (BE 168-171 eV, sulfur not bind to Au). Based on the above fits, the reduced and oxidized sulfur contents are estimated as 57% and 43% in (**a**); and 26% and 74% in (**b**), respectively.

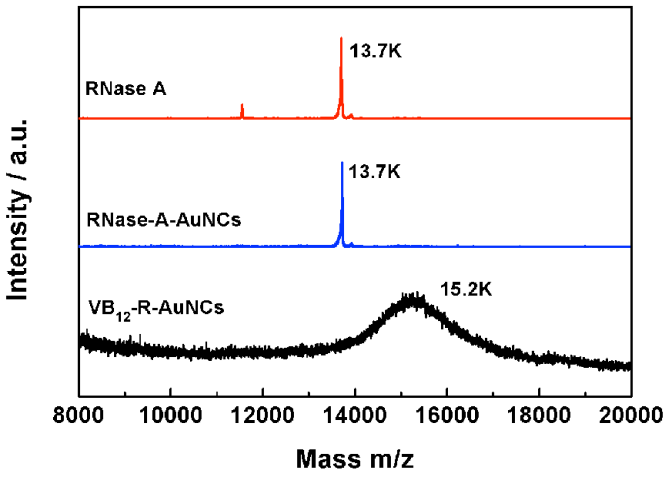

Fig. S2 Typical MALDI-TOF mass spectra of the RNase A (**blue line**), the RNase-A-AuNC (**red line**) and the VB_{12}-R-AuNC (**black line**).