Electric Supporting Information (ESI)

Facile Synthesis of β-Lactoglobulin Capped Ag_2S Quantum Dots for *In Vivo* Imaging in the Second Near-Infrared Biological Window

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1. Methods

1.1 The Quantum yield of QDs

Absorption spectra were recorded on a LAMBDA 950 UV/Vis/NIR spectrophotometer equipped with an indium gallium arsenide (InGaAs) 150 mm integrating sphere (PerkinElmer); NIR-II fluorescence spectra were measured on a NS1 NanoSpectralyzer fluorimetric analyzer (Applied NanoFluorescence).

The quantum yield (QY) of the prepared Ag_2S QDs is calculated *via* the following formula:

$$QY_S = QY_{ref} \times (I_s/I_{ref}) \times (A_{ref}/A_s) \times (n_s/n_{ref})^2$$

Where the subscripts 's' and 'ref' denote sample and reference, *I* is the integrated intensity, *A* is the absorbance at the excitation wavelength, and *n* is the refractive index of the solvent. Using a recently reported QY_{ref} of 0.5% for the IR-26 dye[1] , the QY_s of the prepared Ag2S QDs is determined.

1.2 Cell Culture

Human embryonic kidney (293T) and mouse calvaria-derived cell lines (MC3T3-E1) were purchased from the Cell Bank of the Chinese Academy of Sciences Type Culture Collection. Human gastric epithelial cells (GES-1) were preserved in our institute and maintained as recommended. All of the cell lines were cultured in DMEM supplemented with 10% FBS (Sigma Chemical, St. Louis, MO), 100 units mL^{-1} penicillin and 0.1 mg mL^{-1} streptomycin. The cells were stored at 37°C in a humidified chamber with 5% CO_2 and then culture expanded with moderate changes every three days.

1.3 Genotoxicity Assay (Single Cell Gel Electrophoresis)

The comet assay was developed following the approach of Guidi *et al* with slight modifications [2]. The culture medium was remove by centrifugation at 3,000 rpm for 2 min, and cells were re-suspended in 100 µL of 0.5 % low melting agarose. Two drops were placed on a slide coated with 1% normal melting agarose, a coverslip was placed on top of each slide, and the slides were chilled to allow for cover-slip removal. After storage at 4°C for a few minutes, the slides without coverslips were placed in a chilled lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-base, 8 g NaOH and 1 L ddH_2O; 1% Triton X-100 and 9% DMSO were added immediately prior to use) for 1 h, and then placed in an electrophoresis buffer (300 mM NaOH and 1 mM EDTA) for 20 min at 0°C. Electrophoresis was performed for 20 min (25 V and 300 mA; 1.0 V/cm). Then, the slides were neutralized with a Tris-HCl buffer (0.4 M Tris; pH 7.5), and stained

with 10% ethidiumbromide for 10 min. Each slide was analyzed using an inverted Nikon fluorescence microscope. DNA damage (percentage of DNA in the tail) was calculated by analyzing 100 sildes.

2. Results and Discussion

Table S1. Comparison of quantum yields (QDs) of those reported protein capped NIR-II Ag_2S QDs.

Sample	QY	Refence
BSA capped NIR-II Ag_2S QDs	2.3 %	Yang[3]
GSH capped NIR-II Ag_2S QDs	1.8 %	Gui[4]
β-LG capped NIR-II Ag_2S QDs	5.68%	This work

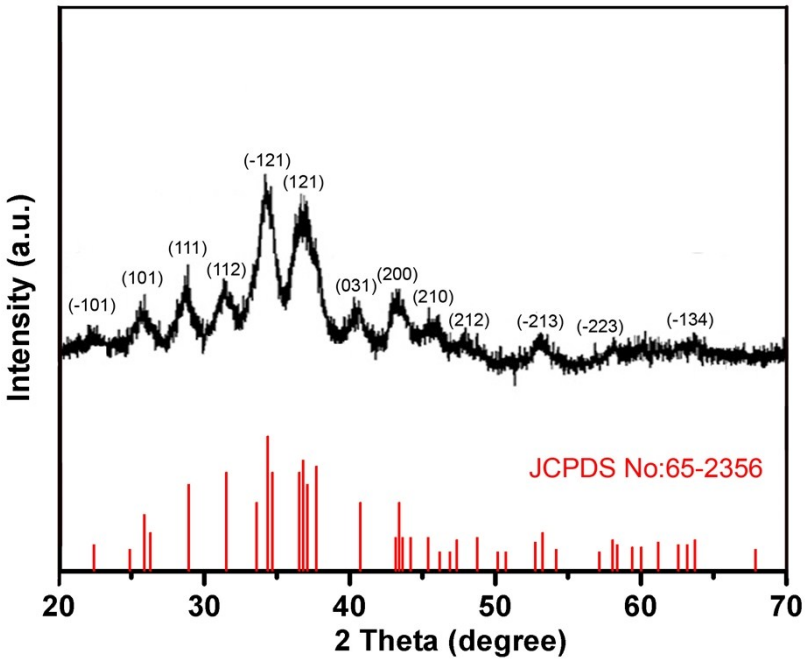

Fig. S1. Powder X-ray diffraction (XRD) pattern of freshly prepared β-lactoglobulin (β-LG)-Ag_2S QDs. The corresponding diffraction peaks expected for monoclinic Ag_2S structure is also shown for comparison.

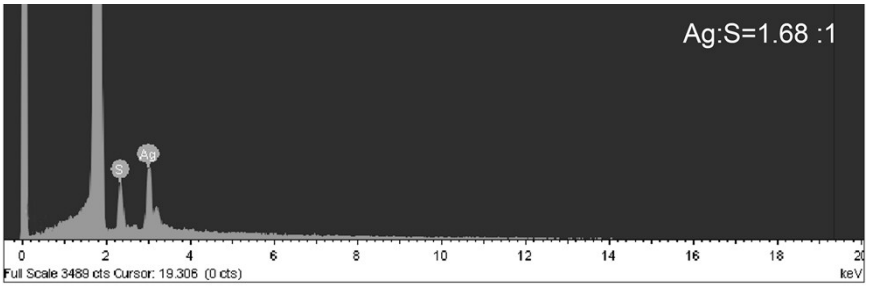

Fig.S2 Energy dispersive spectroscope (EDS) spectra of freshly prepared β-LG-Ag_2S QDs.

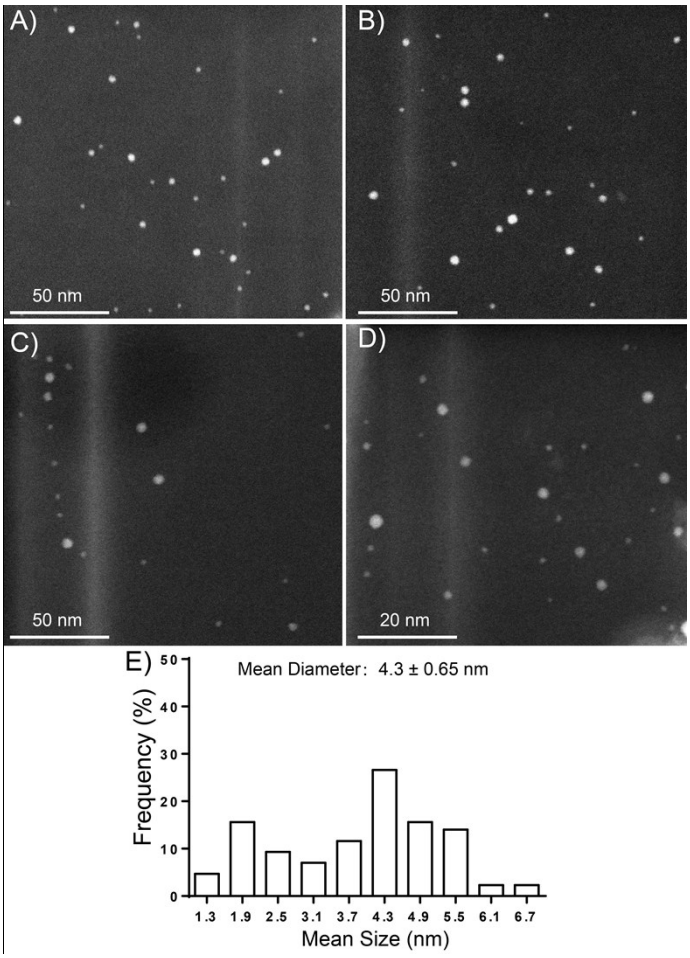

Fig.S3 A-D) High-angle annular dark-field scanning transmission electron microscopy (HAAD-TEM) image of as-prepared β-LG-Ag_2S QDs. E) Corresponding size distribution analysis of the Ag_2S cores.

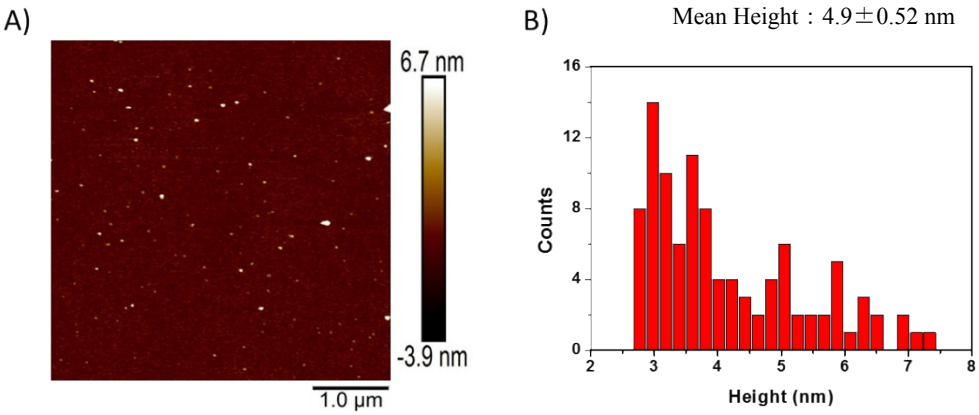

Fig. S4 (A) Typical AFM topographic image of β-LG-Ag_2S QDs measured by contact mode in water. (B) Height profile histogram of the β-LG-Ag_2S QDs obtained from the AFM image.

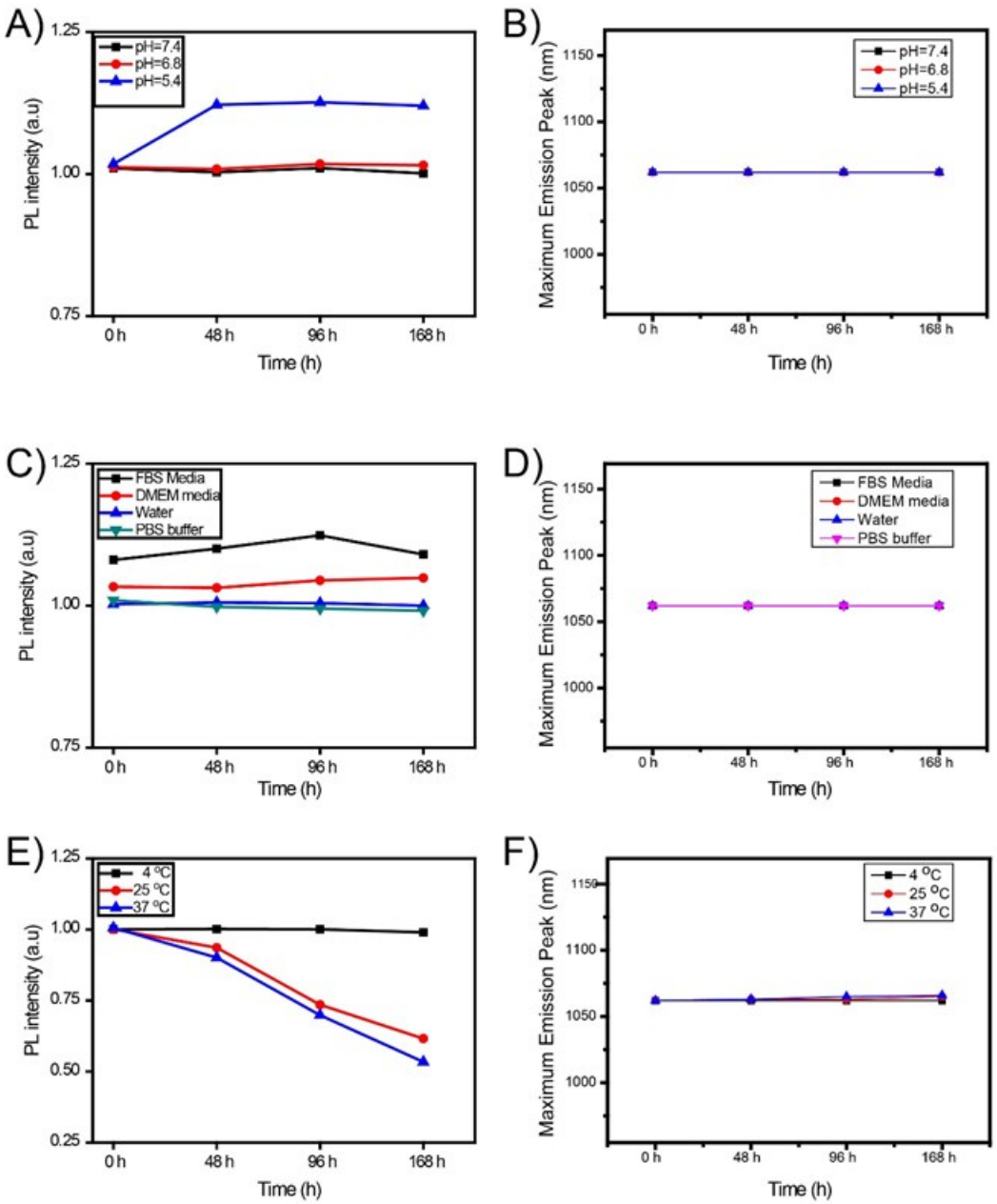

Fig. S5. PL stability of LG-Ag2S QDs under test conditions of different pH (A), buffer (C), and temperature (E) over 7 days. The corresponding maximum emission peaks of Ag2S QDs are shown in (B), (D) and (F).

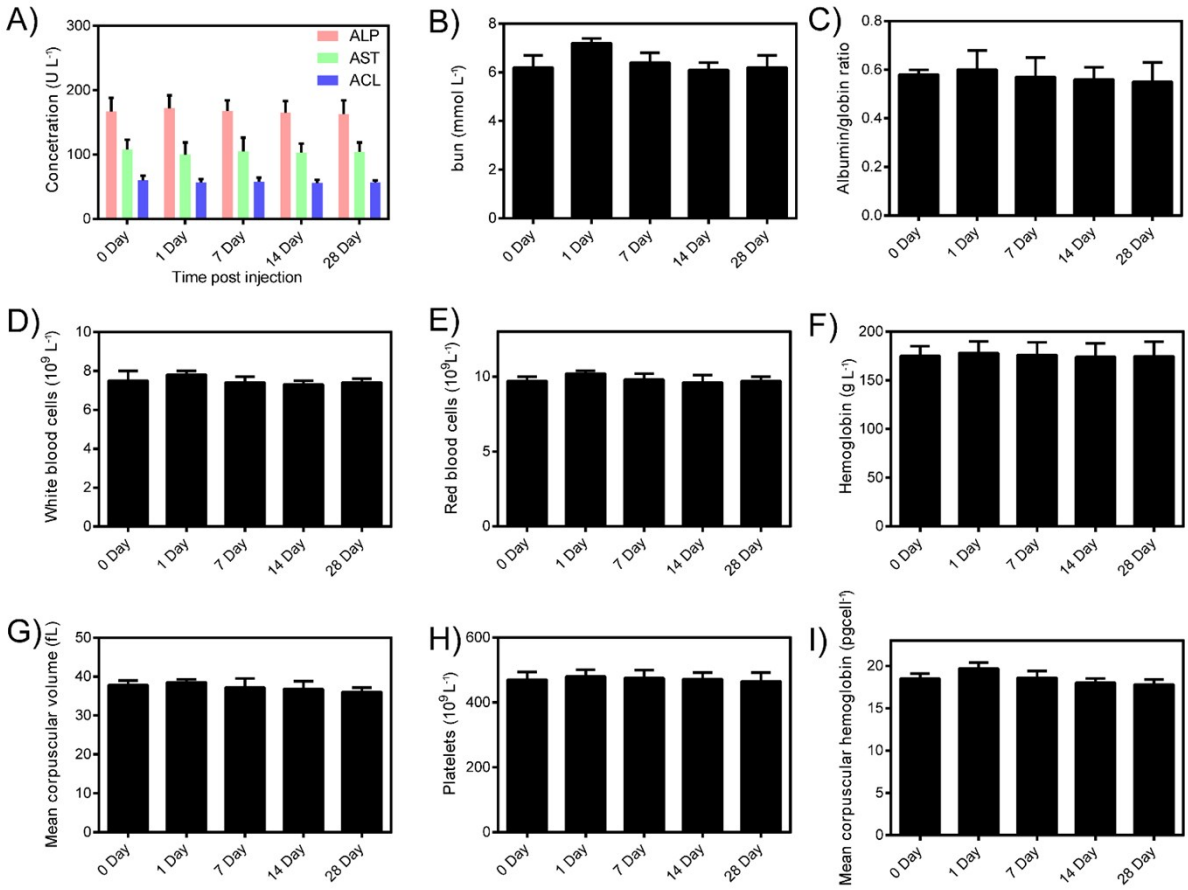

Fig. S6. Blood biochemical and hematological analysis of Balb/c mice treated with β-LG-Ag_2S QDs over a 28-day period. This result shows the main blood biomarkers, *i.e*. alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), kidney function marker, urea nitrogen (BUN) and albumin/globulin ratios for the QD-treated mice over 28 days, which are all within the normal range. The routine blood examination results were also normal for white blood cells, red blood cells, hemoglobin, mean corpuscular volumes, mean corpuscular hemoglobin and platelet counts.

Table S2. Comparison of infrared emitting rare earth doped nanoparticle and NIR-II Ag2S QDs

Sample	Laser λ_{EX}	Laser power density	resolution	dose concentration	Reference
shortwave infrared emitting rare earth doped nanoparticles	980 nm	0.14 W cm^{-2}	?	?	Naczynski[5]
rare earth doped nanoparticles	800 nm	0.2 W cm^{-2}	?	5 nm	Wang[6]
β-LG-Ag_2S QDs	808 nm	0.015 W cm^{-2}	~ 1 mm	0.2 μg/ml	This work

Reference:

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