Electronic Supplementary Material (ESI) for Chemical Communications
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# Electronic Supplementary Information

## Fabrication of Vascular Endothelial Growth Factor Antibody Bioconjugated Ultrasmall Near-Infrared Fluorescent $Ag_2S$ Quantum Dots for Targeted Cancer Imaging in Vivo

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## EXPERIMENTAL SECTION

**Materials and Reagents.** All reagents used were of at least analytical grade. Ultrapure water (Hangzhou Wahaha Group Co. Ltd., Hangzhou, China) was used throughout. $Na_2HPO_4 \cdot 12H_2O$, $NaH_2PO_4 \cdot 2H_2O$, NaCl, NaOH, $AgNO_3$ and $Na_2S \cdot 9H_2O$ were purchased from Guangfu Fine Chemical Research Institute (Tianjin, China). Bovine serum albumin (BSA, fraction V) was from Sangon Biotech Co., Ltd. (Shanghai, China). N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC) and N-Hydroxysulfosuccinimide sodium salt (Sulfo-NHS) were purchased from Aladdin Industrial Inc. (Shanghai, China). Vascular endothelial growth factor (VEGF) and its antibody were obtained from R&D systems (Minneapolis, MN, USA). 10 mM phosphate buffer solution (PBS) (pH 7.4) was purchased from Shanghai Hongbei Reagent Co. (Shanghai, China).

**Instrumentation and Characterization.** The morphology and microstructure of the QDs were characterized by a JEM-100CXII (Jeol, Tokyo, Japan) field emission high-resolution transmission electron spectroscopy (HRTEM) with an accelerating voltage of 100 kV. X-ray photoelectron spectroscopy (XPS) measurements were carried out on a Axis Ultra DLD spectrometer fitted with a monochromated Al $K_\alpha$ X-ray source (hν = 1486.6 eV), hybrid (magnetic/electrostatic) optics, and a multichannel plate and delay line detector (Kratos Analytical, Manchester, UK). Fourier transform infrared (FTIR) spectra (4000–400 $cm^{-1}$) in KBr were recorded on a Magna-560 spectrometer (Nicolet, Madison, WI, USA). The concentration of $Ag_2S$ QDs was determined on an X series inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Elemental, UK). Absorption spectra were recorded on a UV-3600

UV–vis–NIR spectrophotometer (Shimadzu, Kyoto, Japan). The photoluminescence measurements were performed on an F-4500 spectrofluorometer (Hitachi, Tokyo, Japan) equipped with a plotter unit and a quartz cell (1 cm×1 cm). Photoluminescent measurements were taken at an excitation wavelength of 570 nm. The slit width was 10 and 20 nm for excitation and emission, respectively. The photomultiplier tube (PMT) voltage was set at 700 V. Fluorescence decay curves were measured on a FELIX32 system (Photon Technology International, NJ, USA). The fluorescence quantum yield of $Ag_2S$ QDs was determined on an FLS920 spectrometer with an integration sphere attachment under excitation of 380 nm (Edinburgh, UK). The fluorescence images of the mice were obtained with a Bethold NightOWL LB 983 in vivo Imaging System (Bad Wildbad, Germany). The excitation filter was set as 630 nm, the emission filter was set as 820 nm. Fluorescence images were recorded by the CCD camera with constant exposure time.

**Synthesis of Near-Infrared Fluorescent $Ag_2S$ QDs.** All glassware was washed with Aqua Regia (HCl: $HNO_3$ volume ratio = 3:1), and rinsed with ethanol and ultrapure water. *(Caution: Aqua Regia is a very corrosive oxidizing agent, which should be handled with great care.)* In a typical experiment, aqueous $AgNO_3$ solution (5 mL, 10 mM, 37 °C) was added to BSA solution (5 mL, 50 mg $mL^{-1}$, 37 °C) under vigorous stirring, NaOH solution (0.5 mL, 1 M) was introduced 2 min later. Next, 0.5 mL of 0.1 M $Na_2S$ was quickly injected into the solution, and the reaction was allowed to proceed under vigorous stirring at 37 °C for 12 h. The color of the solution changed from light yellow to colorless, and then to reddish brown. The resulting NIR

$Ag_2S$ QDs were purified via centrifugal filtration at 6000 g for 30 min with Amicon Ultra-15 Centrifugal Filter Unit with Ultracel-50 membrane (Millipore, Billerica, MA, USA).

**Bioconjugation of NIR $Ag_2S$ QDs.** Typically, 7.5 mg EDC and 8.5 mg Sulfo-NHS were dissolved in 250 μl PBS and added to 1.2 μg of antiVEGF mixed well and incubated for 15 min at room temperature. After 15 min incubation, the solution was added to 2.5 ml $Ag_2S$ QDs (from 25 mg $ml^{-1}$ stock) and incubated at 4 °C for 12 h. The final conjugates were purified by a centrifugal filter device (Amicon Ultra-15; 50 KD), lyophilized and resuspended in 2 ml PBS. The conjugates are hereafter referred to as antiVEGF-$Ag_2S$ QD.

**Biodistribution and Toxicity Analysis.** (Gao, J.; Chen, K.; Luong, R.; Bouley, D. M.; Mao, H.; Qiao, T.; Gambhir, S. S.; Cheng, Z. *Nano Lett.* **2012**, *12*, 281.) Athymic nude mice (female, age 6-8 weeks) were obtained from Beijing HFK Bioscience Co., Ltd. (Beijing, China) and were intravenously injected with 500 μL of PBS buffer or $Ag_2S$ QDs (0.1 μmol as Ag). At each time point after injection (1 day, 1 week, 2 weeks, 3 weeks and 4 weeks, respectively), mice were euthanized humanely via carbon dioxide asphyxiation. Blood was collected immediately once mice died. Necropsies were performed for collecting major organs and tissues (heart, lung, liver, kidney, spleen, stomach, intestine and femoral bone). The organs were weighed and dissolved in Aqua Regia for two weeks. The organs were then diluted 2000× in water and submitted for ICP-MS. The %ID/gram values of $Ag_2S$ QDs in all analyzed organs were determined by comparing the Ag concentration to the injected solution as

reported by ICP-MS, giving a quantitative measure of QD biodistribution.

**In Vivo Imaging with $Ag_2S$ QDs.** Nude mice harboring U-87 MG tumors (8 mm) were obtained from the Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union Medical College (Licence Number: SCXK-2004-001, Tianjin, China). All animal studies were conducted according to protocols approved by the Animal Ethics Committee of the Institute of Hematology & Hospital of Blood Diseases, Chinese Academy of Medical Sciences & Peking Union Medical College. For in vivo imaging, BSA-stabilized $Ag_2S$ QDs (0.02 μmol, 500 μL) and antiVEGF-$Ag_2S$ QDs (0.02 μmol, 500 μL) were intravenously injected into U-87 MG tumor-bearing mice. As a negative control, antiVEGF-$Ag_2S$ QDs (0.02 μmol, 500 μL) were intravenously injected into the normal nude mice. The mice were anesthetized with intraperitoneal administration of 4% chloral hydrate at a dosage of 400 mg $kg^{-1}$. At the special time points after injection, the fluorescence profiles in normal and U-87 MG tumor-bearing mice were imaged. The resulting images were processed by subtracting the background tissue autofluorescence from the fluorescence from $Ag_2S$ QDs with the software of the imaging system.

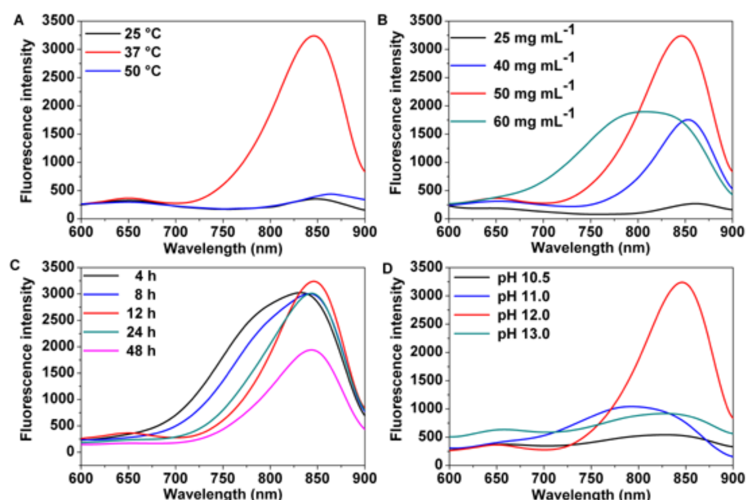


**Fig. S1** Evolution of the fluorescence spectra of BSA-stabilized $Ag_2S$ QDs under different synthesis conditions: (A) temperature; (B) the concentration of BSA; (C) time; (D) pH. The synthesis was initiated by incubating BSA (5 mL, 50 mg $mL^{-1}$) with $AgNO_3$ (5 mL, 10 mM) and $Na_2S$ (0.5 mL, 0.1 M) at pH=12, and the mixture was incubated at 37 °C for 12 h.

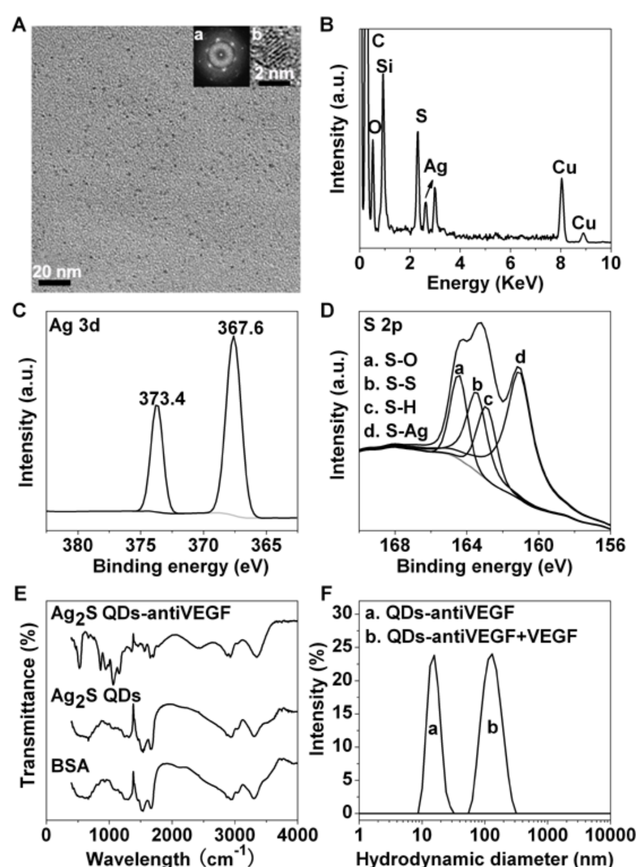


**Fig. S2** (A) HRTEM image of the as-prepared BSA-stabilized $Ag_2S$ QDs. Inset:

(a) SAD of the QDs; (b) HRTEM image of single QDs. (B) EDX spectra of BSA-stabilized $Ag_2S$ QDs. (C) XPS spectra of Ag 3d for BSA-stabilized $Ag_2S$ QDs. (D) XPS spectra of S 2p for BSA-stabilized $Ag_2S$ QDs. (E) FTIR spectra of BSA, BSA-stabilized $Ag_2S$ QDs, antiVEGF-$Ag_2S$ QDs. (F) Dynamic light scattering spectra of antiVEGF-$Ag_2S$ QDs (a) and antiVEGF-$Ag_2S$ QDs in the presence o f VEGF (10 μg $mL^{-1}$) (b). The characteristic stretching vibrations of antiVEGF such as –C–C–C– (1159 $cm^{-1}$), –C–O–C– (1062 $cm^{-1}$ and 862 $cm^{-1}$), and –S–S– (490 $cm^{-1}$) observed in the antiVEGF-$Ag_2S$ QDs (Fig. S2E) indicate that the presence of antiVEGF in the prepared antiVEGF-$Ag_2S$ QDs. The characteristic bands for primary amines at 1545 $cm^{-1}$, 1664 $cm^{-1}$ and 3406 $cm^{-1}$, –CH vibration band at 2959 $cm^{-1}$, and the broad –$NH_2$ and –NH wagging band at 702 $cm^{-1}$ in the FTIR spectra of BSA-stabilized $Ag_2S$ QDs indicate that the presence of BSA in the prepared BSA-stabilized $Ag_2S$ QDs (Fig. S2E).

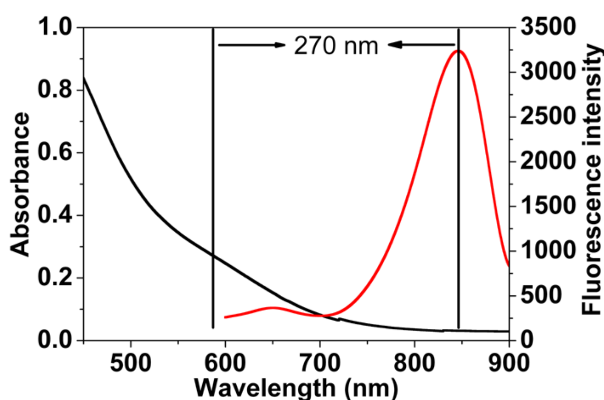


**Fig. S3** Absorption and fluorescence spectra of the as-prepared BSA-stabilized $Ag_2S$ QDs with emission at 840 nm. The large value of the Stokes shift (~270 nm) between the emission and absorption peaks indicates the dominant band edge luminescence in the obtained $Ag_2S$ QDs.

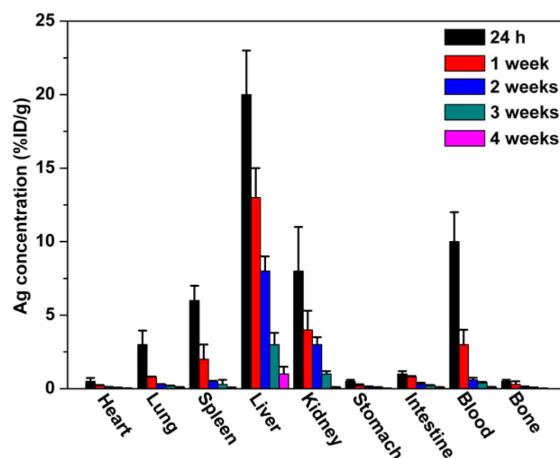


**Fig. S4** In vivo biodistribution and toxicity analysis of BSA-stabilized $Ag_2S$ QDs over a period of 4 weeks in athymic nude mouse. The Ag concentration in the organs was determined at different time points after tail-vein injection of the $Ag_2S$ QDs (0.1 μmol) using ICP-MS (n = 6). Here %ID/g indicates the Ag element concentration in terms of the percentage of the injected dose (ID) per gram of tissue.

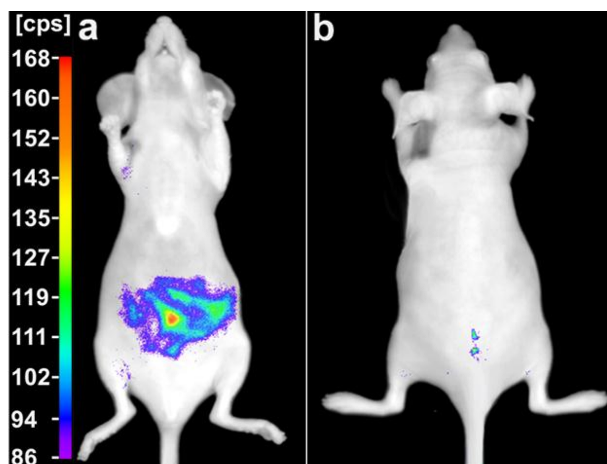


**Fig. S5** Fluorescence imaging from the abdominal cavity (a) and dorsal side (b) of the nude mouse with $Ag_2S$ QDs with emission at 840 nm injected into the abdominal cavity. The results show that the NIR fluorescent $Ag_2S$ QDs are promising for targeted cancer imaging in vivo.

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**Table S1 Decay Parameters of $Ag_2S$ QDs with Emission at 840 nm Obtained by Three-Exponential Fitting[a]**

| | $\tau_1$(ns) | $A_1$ (%) | $\tau_2$(ns) | $A_2$(%) | $\chi^2$ | $\tau_{av}$(ns) |
|---|---|---|---|---|---|---|
| $Ag_2S$ QDs | 144.7 ± 44.9 | 72.7 ± 37.6 | 961.7 ± 79.2 | 27.3 ± 2.7 | 1.028 | 728.1 ± 30.8 |

[a] $F(t) = A_1 e^{-t/\tau 1} + A_2 e^{-t/\tau 2}$