Electronic Supplementary Information

Luminescent $AgGaSe_2$/ZnSe nanocrystals: rapid synthesis, color tunability, aqueous phase transfer, and bio-labeling application

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Experimental section

Materials

Ethylenediamine (EDA, 99%), 3-mercaptopropionic acid (MPA, 99%), tetramethylammonium hydroxide pentahydrate (TMAH, 98%), 11-mercaptoundecanoic (MUA, 95%), N-hydroxysulfosuccinimide (NHS, 98%) and N-(3-dimethylaminopropyl)-N`-ethylcarbodiimide hydrochloride purum (EDC, 98%) were purchased from Energy Chemical. Fetal bovine serum (FBS), Tween-20, Dulbecco`s modified Eagle`s medium (DMEM), cell counting Kit-8/CCK8 were purchased from Dalian Chenyu Biotechnology. Prostate-specific antigen (PSA) was purchased from Sigma-Aldrich. The capture (Ab_1) and signal PSA antibodies (Ab_2) were purchased from Beijing Biosynthesis Biotechnology Co. Ltd. (China). All chemical were used as received without further treatment.

Aqueous phase transfer of oleophilic $AgGaSe_2$/ZnSe NCs via EDA-assisted ligand exchange

A modified method on the basis of Dai`s report[1] was used to achieve the aqueous phase transfer of the oleophilic NCs by replacing the initial hydrophobic ligand (OAm) with hydrophilic Zn-MPA under the assistance of EDA. Typically, 1.5 mL of Zn-MPA aqueous solution (prepared by mixing different ratios of ZnI_2 and MPA into distilled water) was added to the NCs solution (500 μg/mL, 1 mL, ≈ 1.5 μmol) under vigorous stirring. After 2 minutes, EDA (1.5 mmol, ≈ 100 μL) was added to the mixture, and the reaction proceeded until the reddish-brown NCs complete transfer from organic (chloroform) to aqueous phase (distilled water). Finally, the products were precipitated by adding excess anhydrous ethanol and collected by centrifugation at 9000 rpm for 5 minutes. The purified NCs were re-dispersed in distilled water.

Aqueous phase transfer of oleophilic NCs via MUA ligand exchange

Another strategy for the aqueous phase transfer was achieved by replacing the initial hydrophobic surfactant with MUA according to Zhang` reported method.[2] Typically, 0.5 mL of MUA aqueous (dissolving 0.1 g of MUA into 1 mL of methanol and then adjust the pH to 6.8 by TMAH) was added to the NCs solution (500 μg/mL, 5 mL, ≈ 7.5 μmol) under vigorous stirring at 70 °C for 30 minutes. The reddish-brown precipitates appeared in the bottom of the solution. Then, 5.0 mL of distilled water was added to the mixture and the reaction proceeded until forming uniform reddish-brown solution in the aqueous phase. Finally, the products were precipitated by adding excess acetone and collected by centrifugation at 9000 rpm for 5 minutes. The purified NCs

were re-dispersed in distilled water for further use.

Cell viability assay

CCK8 assay was used to assess the cytotoxicity of the water-stable $AgGaSe_2$/ZnSe NCs against Hela cells. Briefly, cells were seeded at 2.5×10^3 cells/well in 96-well plates and incubated at 37 °C for 12 h. Then, a fresh cultured DMEM medium (100 μL) containing NCs samples with concentrations from 20 to 120 μg/mL was added to plate and co-incubated with the cells for 12 h. After incubation, 100 μL fresh DMEM solutions containing 10 μL CCK8 reagent was added to each well and incubated 1h. Finally, a microplate reader (Bio-Rad, Model, 550, USA) was used to measure the absorbance at 450 nm. The free-NCs groups marked as the control were incubated at the same conditions. The relative cell viability can be calculated with the following equation:

$$\text{cell viability (\%)} = \frac{A_s - A_b}{A_c - A_b} \times 100\%$$

where A_s, A_c and A_b are the absorbance of the test, control and blank sample.

Cell luminescence imaging

First, Hela cells were seeded at 1.0×10^4 cells/well in 24-well plates and incubated at 37 °C for 12 h to obtain a suitable cell density. Second, the water-stable $AgGaSe_2$/ZnSe NCs (100 μg/mL) in serum-free DMEM medium were added to each well and incubated 1h. Finally, the stained cells were carefully rinsed with PBS several times and recorded by fluorescent inverted microscope.

Bioconjugation of $AgGaSe_2$/ZnSe NCs with antibodies

The conjugation method of $AgGaSe_2$/ZnSe NCs-Ab_2 was similar with the literature method.[3-4] First, EDC (2 mg/mL, 100 μL) and NHS (1 mg/mL, 100 μL) was added to the water-stable $AgGaSe_2$/ZnSe NCs solution (100 μg/mL, 50 μL, the NCs was dissolved into 10 mM PBS, pH 7.4), and vortexed for 45 min. Next, Ab_2 (2 μg/mL, 1 mL) was added to the mixture. The solution was incubated at 4 °C and shaken overnight. Finally, the $AgGaSe_2$/ZnSe-Ab_2 was separated by centrifugation at 15000 rpm for 10 min and washed several times by PBST (0.05% Tween-20, pH 7.4) to remove unreacted NCs and byproducts. The final product was diluted to 1 mL PBS (10 mM, pH 7.4) and stored at 4 °C for further use.

Preparation of the fluoroimmunoassay (FIA)

First, Ab_1 solution (0.01 mg/mL, 50 μL) was added to 96-well plates and incubated overnight at 4 °C. The physically absorbed Ab_1 was removed by rinsing with PBST. Then, 1% BSA (in PBS) was added to the well and incubated at 37 °C for 2 h to block any remaining activated groups. After washing again, 50 μL of PSA solution with different concentrations was added to the well and incubated at 37 °C for 1 h. After removing the nonspecific absorption, 50 μL of $AgGaSe_2$/ZnSe NCs-Ab_2 was added to the well and incubated at 37 °C for 50 min. Finally, the fluorescence images were recorded by inverted microscope.

Characterization

Powder X-ray diffraction (XRD) analysis was performed on a Rigaku D/max-2500 diffractometer with a graphite monochromator by using Cu-Kα radiation operating at 200 mA and 40 kV. Transmission electron microscopy (TEM), high-resolution transmission electron microscopy (HRTEM) images were recorded on a FEI Tecnai G2 S-Twin with a field emission gun operating at 200 kV. Images were acquired digitally on a Gantan multiple CCD camera. The surface chemical electronic state and composition of the as-synthesized samples were characterized by X-ray photoelectron spectroscopy (XPS) on an ESCALAB 250 X-ray photoelectron spectroscope, using Mg Kα X-ray as the excitation source. Infrared spectra (IR) were measured on a Bruker AXS TENSOR-27 FT-IR spectrometer with pressed KBr pellets in the range of 400-4000 cm^{-1} at room temperature. UV-vis absorption spectra were measured with a Shimadzu UV-3100 spectrophotometer. The photoluminescence (PL) emission spectra were recorded on F-4700 Fluorescence Spectrophotometer. Fluorescence images were taken with an Olympus TH4-200 fluorescent inverted microscope. The images were processed using the ImageJ software to obtain the fluorescence intensity,[5-6] and the error bars were calculated from three independent experiments to represent the standard deviations of the measurements.

The calculation of Bohr exciton radius of tetragonal $AgGaSe_2$

Castro-Rodrigueset al. have calculated the Bohr exciton radius by the following formula:[7]

$$r_B = \frac{\varepsilon}{\mu} \cdot \alpha_B$$

where

$$\mu = \frac{1}{m_e^{*-1} + m_h^{*-1}}$$

α_B is the hydrogen Bohr radius, ε is the dielectric constant of the bulk material, and m_e* and m_h* are the reduced masses of the electron and hole.

For tetragonal $AgGaSe_2$, the m_e* and m_h* are equal to 0.17 and 0.73, respectively,[8]

and the dielectric constant is about 9.8.[9] As a result, its Bohr exciton radius can be estimated to ~3.8 nm.

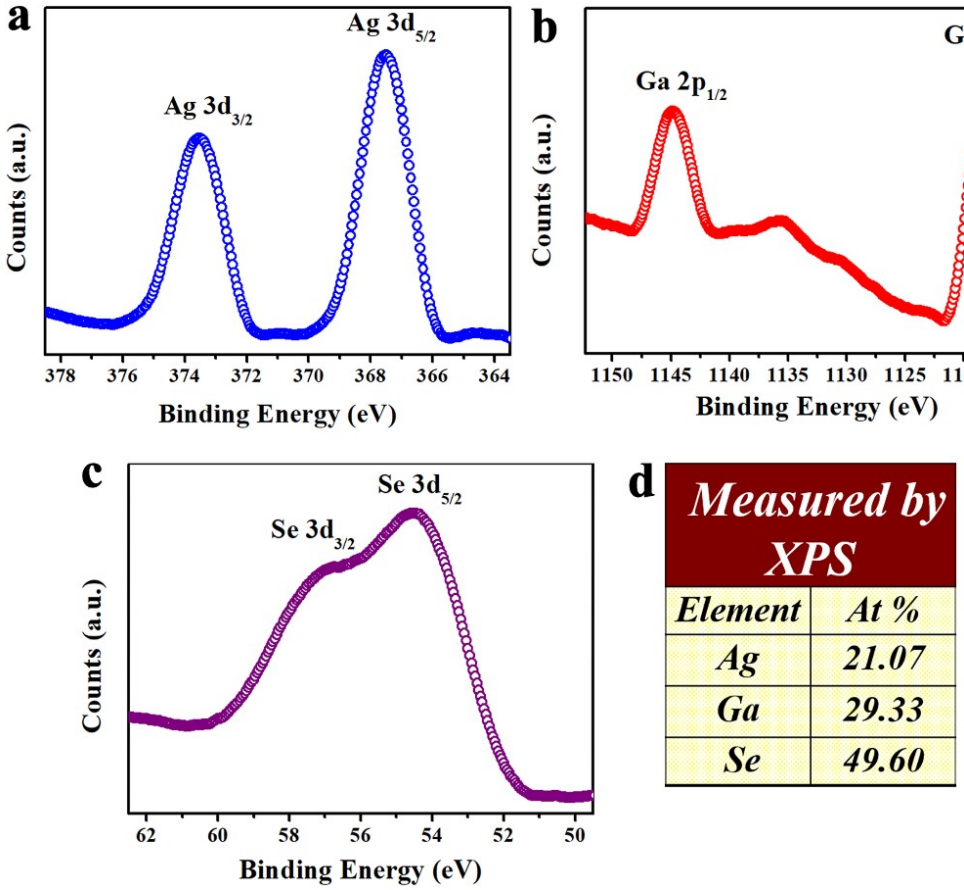

Fig. S1 XPS spectrum of the as-synthesized $AgGaSe_2$ NCs. (a) Ag 3d, (b) Ga 2p, (c) Se 3d core levels, and (d) resulting the average Ag/Ga/Se composition in particles.

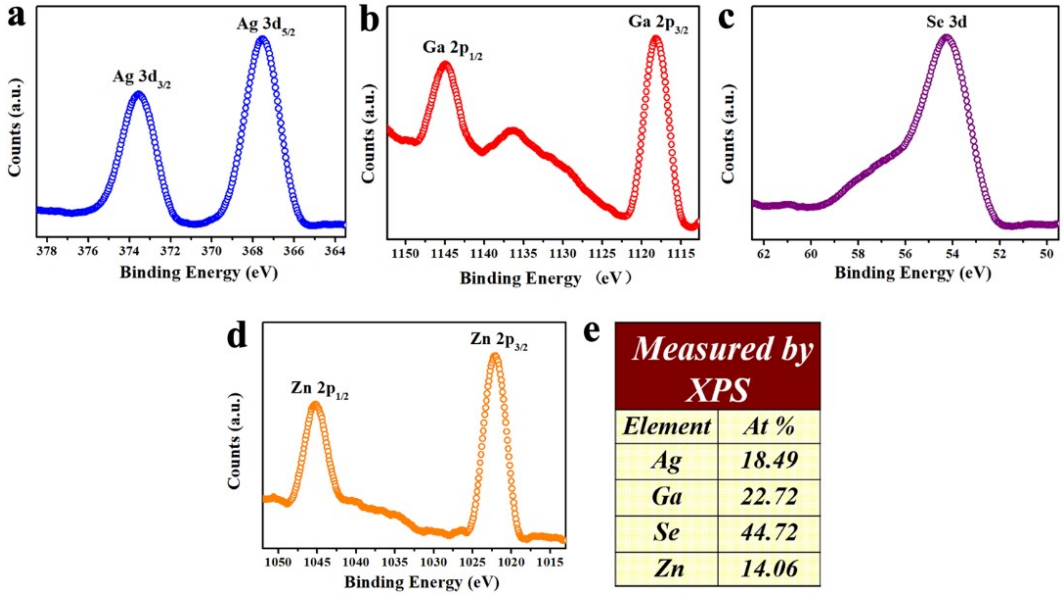

Fig. S2 XPS spectrum of the as-synthesized $AgGaSe_2$/ZnSe NCs. (a) Ag 3d, (b) Ga 2p, (c)

Se 3d, (d) Zn 2p core levels, and (e) resulting the average Ag/Ga/Se/Zn composition in particles.

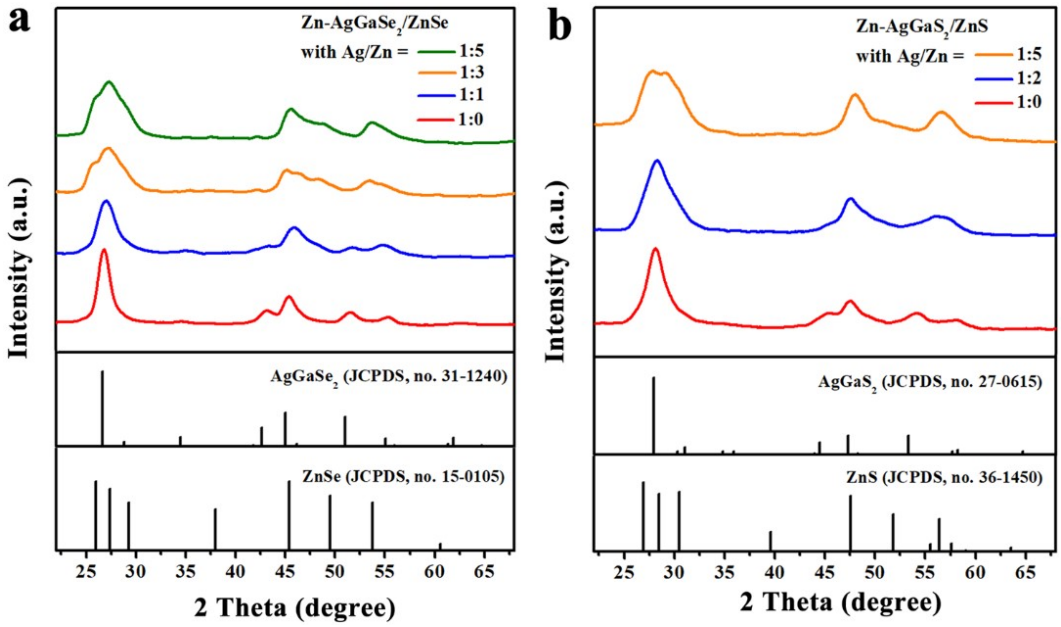

Fig. S3 XRD patterns of (a) as-synthesized $AgGaSe_2$/ZnSe NCs, and (b) $AgGaS_2$/ZnS NCs prepared with different molar ratios of Ag/Zn reactants.

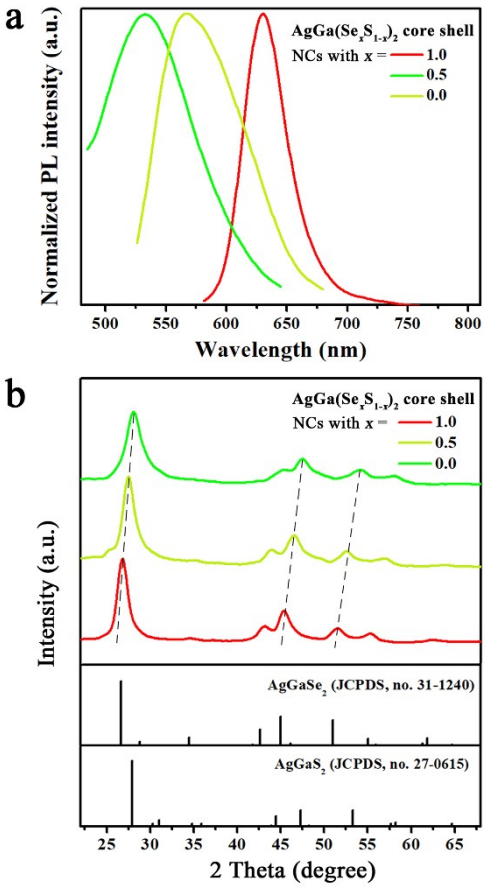

Fig. S4 (a) PL spectra, and (b) XRD patterns of as-synthesized $AgGa(Se_xS_{1-x})_2$/ZnSeS solid

solution NCs with *x* = 1.0, 0.5 and 0.0.

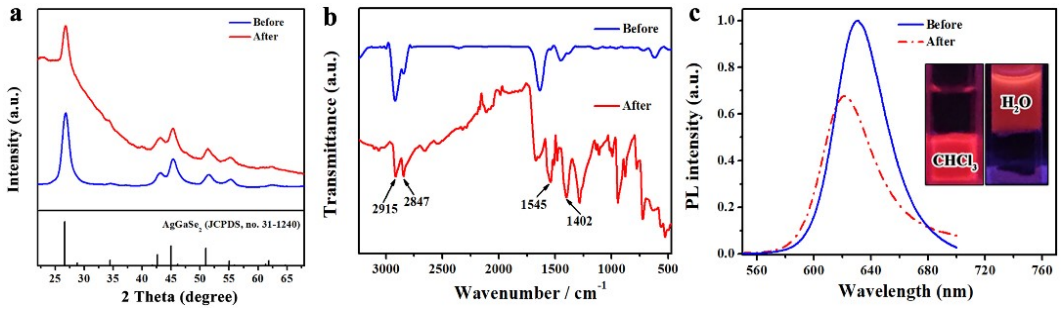

Fig. S5 (a) XRD patterns, (b) FTIR spectra, and (c) PL spectra of the $AgGaSe_2$/ZnSe NCs before (blue line) and after (red line) ligand exchange with MUA. The inset of (c) is the corresponding digital photographs of the samples under excitation at 365 nm. The upper layer is water, and the lower one is chloroform.

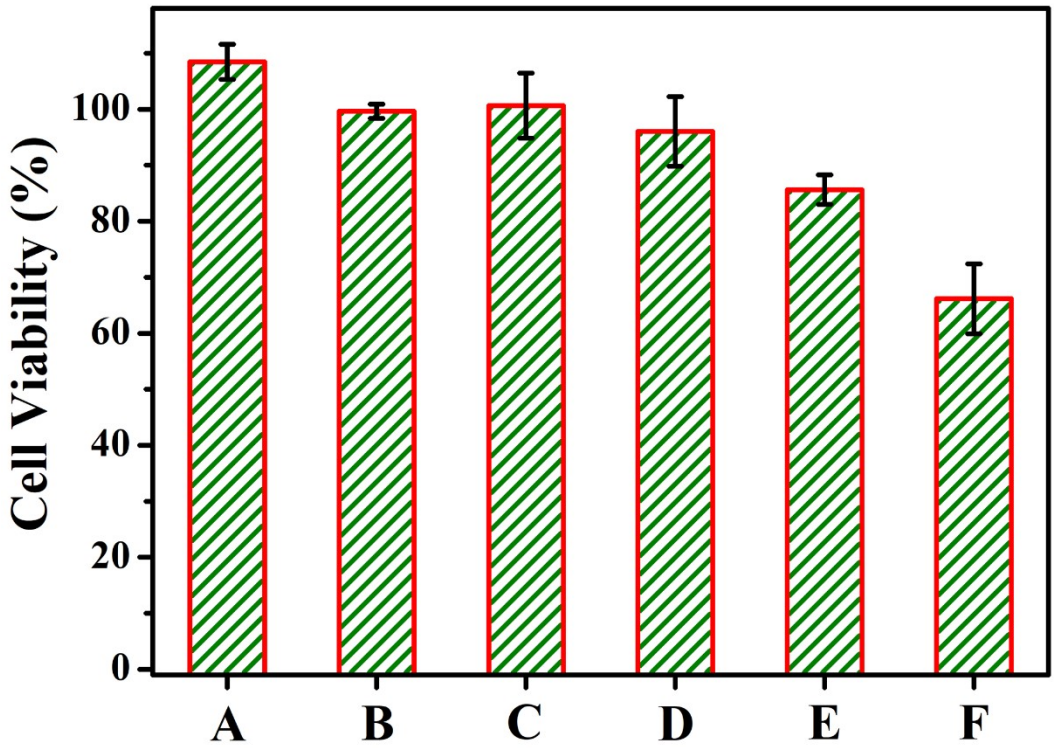

Fig. S6 The cell viability of MUA-capped $AgGaSe_2$/ZnSe NCs in the presence of different concentrations: A 20 μg/mL, B 40 μg/mL, C 60 μg/mL, D 80 μg/mL, E 100 μg/mL, and F 120 μg/mL.

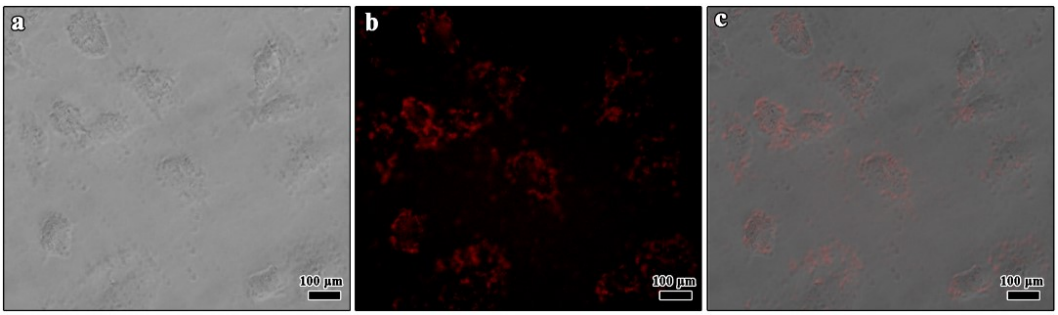

Fig. S7 (a) Bright-field image, (b) fluorescence image, and (c) merged image of live Hela cells incubated with $AgGaSe_2$/ZnSe NCs.

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