

Supplementary Information S1

The role of temperature in the photoluminescence quantum yield (PLQY) of Ag₂S-based nanocrystals

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S1 – Supplementary information on procedural aspects

Chemicals

Silver (I) diethyldithiocarbamate (AgDDTC, 99%), 1-dodecanethiol (DDT, >98%), toluene (TOL, 99.8%), acetone (technical grade), oleylamine (OLA, 70%), sulphur powder (S, synthesis grade), chloroform (CHCl₃, >99.8%), tetrachloroethylene (TCE, >99%), ethanol (EtOH, 96%), selenium powder (Se, 99.99%), trioctylphosphine (TOP, 97%), dodecylamine (98%, Sigma-Aldrich), poly (isobutylene-alt-maleic anhydride) (MW ~6,000, 12-200 mesh, 85%, Sigma-Aldrich), and sodium hydroxide (NaOH, 97%), nitric acid (HNO₃, 68%, Sigma-Aldrich). All chemicals were used without further purification

Synthesis of Ag₂S NCs by hot injection (Core Ag₂S NCs)

0.2 mmol of AgDDTC, 40 mmol of DDT and 10 ml of TOL were mixed in a three-neck round-bottom flask, connected to a Schlenk line. The mixture was heated to 100 °C until the complete dissolution of the silver salt. At this point 100 µL of a solution of 0.4 mmol of S-powder in 1 ml of OLA (S@OLA) is swiftly injected. This solution corresponds to 0.012 ± 0.001 g of sulfur powder dissolved in 0.84 ± 0.01 g.

If just Ag₂S cores are desired, the solution is then left to react for 5 minutes and then cooled down to room temperature. When the temperature reaches approximately 60 °C, 3 mL of CHCl₃ is added to the sample to stop the reaction. The sample is transferred into two Falcon

tubes (50 mL) and precipitated by adding ethanol, sonicating for 5 min, followed by centrifugation at 9000 rpm for 10 min (one cycle). After this cycle, the sample is re-dispersed in 10 mL of CHCl_3 .

Synthesis of $\text{Ag}_2\text{S}/\text{Ag}_2(\text{S},\text{Se})$ NCs (CS NCs)

In this case, after the injection of sulphur in OLA and after 5 minutes of reaction, 100 μL of a 1 M solution of selenium, in the form of Se-powder, in TOP (prepared by dissolving 0.7894 ± 0.0001 g of Se-powder in 1.0 ± 0.1 mL of TOP) is injected into the NCs dispersion. The solution is left to react for 10 minutes, and is then cooled down to room temperature. When the temperature reaches approximately 60°C , 3 mL of CHCl_3 is added to the sample to stop the reaction. For purification, the NC solution is divided into 3 equal parts and added into 3 Falcon tubes (50 mL), using ethanol as non-solvent. The volume of ethanol used is approximately 2/3 of the volume of the nanoparticle dispersion in each Falcon tube. The Falcon tubes are centrifuged (9000 rpm, 10 min), and the supernatant discarded. The precipitates are collected in a total volume of 10 mL of CHCl_3 . The average concentration of Ag measured by ICP is ~ 1 mg/mL of Ag.

Preparation of the Zinc-oleate stock solution

A supersaturated Zinc stock solution was prepared by mixing in a 3-neck flask 0.219 g of $\text{Zn}(\text{Ac})_2 \cdot 2\text{H}_2\text{O}$, 4.4 g OA and 4.5 g of ODE. The mixture was heated up to 130°C for 1.5 h under vacuum. This reaction forms Zn-oleate, and the vacuum removes the produced acetic acid from the solution.^{1,2} With the help of a syringe, this solution is extracted from the 3-neck flask and transferred to a vial, with a rubber cap in the cap, where air has been displaced by previously flowing N_2 . The preparation of a fresh solution for each synthesis is recommended, to obtain optimized PL results of the final NCs solutions.

Synthesis of $\text{Ag}_2\text{S}/\text{Ag}_2(\text{S},\text{Se})$ treated with the Zn-oleate solution. Formation of a gradated shell with $\text{Ag}_2(\text{S},\text{Se})$ and ZnS (CSS NCs)

5 mL of the previously prepared $\text{Ag}_2\text{S}/\text{Ag}_2(\text{S},\text{Se})$ ~ 1 mg/mL NCs in CHCl_3 were added into a 3-neck flask, connected to the Schlenk line and heated to 60°C . Upon reaching this temperature, 0.25 mL of the Zn-solution (previously heated to $\sim 80^\circ\text{C}$) were added, and left to react for 4 minutes. After this short time, the mixture was left to cool down to room temperature (RT); thereafter, it was washed in a cycle of ethanol/chloroform, as mentioned before.

The S and Se injections, performed under N_2 or in air, produce Ag_2S NCs cores or CS NCs; these do not drastically influence the final PLQY of the NCs. However, the Zn-treatment step is crucially influencing the final PL properties. Thus, to obtain CSS NCs with high PLQY, this Zn-treatment must be performed in air (for comparative studies see Figures S3 and S4 in the Supplementary Information document S2).

Preparation of N-PMA

The preparation of this polymer was performed according to previous work.³

Aqueous transfer with N-PMA

0.5 mL of $\text{Ag}_2\text{S}/\text{Ag}_2(\text{S},\text{Se})$ NCs or $\text{Ag}_2\text{S}/\text{ZnS}/\text{Ag}_2(\text{S},\text{Se})$ NCs in CHCl_3 (~ 1 mg/mL) were mixed with 60 μL of 0.5 M N-PMA solution. The mixture was shaken for 1 minute and the CHCl_3 removed under a soft N_2 flow. To the dried deposit, 4 mL of a 0.03M NaOH solution is added under continuous sonication (the flask is placed inside the sonicating bath while the NaOH

solution is added) for 10 minutes. After this time, two washing cycles including centrifugation (5 minutes at 10.000 rpm) and re-dispersion in water were performed.

Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES).

A Plasma Emission Spectrometer ICP PERKIN ELMER mod. OPTIMA 2100 DV CNHS Elemental Analyzer PERKIN ELMER 2400 was used to determine the amount of Ag in the different samples. To digest the sample, to 10 μ L of the NCs solution, 10 μ L of HNO₃ 68% (w/w) are added and left overnight. Afterwards, 990 μ L of double deionized water was added. Once the concentration of the Ag₂S cores, CS and CSS NCs solutions were obtained, the three concentrations were adjusted to 0.04 \pm 0.01 mg/mL. These solutions with similar concentration were used to fill the cuvettes and to take the images shown in Figure 2c.

Optical characterization

Absorption spectra of diluted NC samples were recorded, using a Cary5000 (Agilent) UV-Vis-NIR spectrophotometer in the range 250 - 1600 nm. Steady-state photoluminescence spectra were measured in the range 700 - 1600 nm, using a calibrated FSP 920 (Edinburgh Instruments, UK) spectrofluorometer, equipped with a nitrogen-cooled PMT R5509P. Relative measurements of photoluminescence QYs ($\Phi_{f,x}$) were performed using the dye IR125 dissolved in ethanol as a reference. The absolute QY of this dye was previously determined as $\Phi_{f,st} = 0.13$.⁴ The relative QYs were calculated according to the formula of Demas and Crosby, namely

$$\Phi_{f,x} = \Phi_{f,st} \frac{F_x f_{st}(\lambda_{ex,st}) n_x^2(\lambda_{ex,x})}{F_{st} f_x(\lambda_{ex,x}) n_{st}^2(\lambda_{ex,st})}$$

The subscripts x, st, and ex denote sample, standard, and excitation, respectively; $f(\lambda_{ex})$ is the absorption factor; F represents the integrated spectral fluorescence photon flux, and n is the refractive index of the solvents used. All spectroscopic measurements were performed in a 1 cm quartz cuvettes (Hellma GmbH, Germany) at room temperature, using air-saturated solutions.

Steady-state NIR and UV optical absorption measurements were carried out in solution using a UV-Vis-NIR spectrophotometer (Cary 500, Varian). Photoluminescence spectra were recorded using a NIR spectrofluorometer (Fluorolog TCSPC, Horiba), equipped with an InGaAs detector (Symphony II, Horiba).

Heating-cooling cycles of NCs solutions. The NCs solutions were placed in a 1 cm optical path cuvette and inserted in a temperature controlled cuvette holder with imaging lens that allows for excitation and detection of fluorescence emission at right angles ((qpod 3e from Quantum Northwest). A 808 nm laser was used for the excitation and a Andor NIR detector iDus InGaAs for detection.

Transient absorption spectroscopy

Transient Absorption Spectroscopy (TAS) was carried out using a femtosecond Clark-MXR CPA210 regenerative amplifier, delivering 120 fs pulses at 775 nm, with 1 kHz repetition rate. The primary beam was split into pump and probe beams. The pump pulse beam was sent to a computer-controlled delay line to shift it temporally in relation to the probe pulse; it was focused onto the sample and could be adjusted to different fluences. The probe beam was focused on a sapphire plate, to generate a supercontinuum pulse; this was spatially overlapped with the pump beam on the sample. Before impinging on the sample, a portion of the sample probe beam was split off, serving as a reference.

The sample and reference probe beams were then sent to a prism spectrometer (Entwicklungsbüro Stressing, Germany) and monitored independently with two linear CCD array detectors (VIS-Enhanced InGaAs, Hamamatsu Photonics). A bespoke software synchronized the delay line setting with the signal acquisition system and recorded the normalized difference in probe intensities $((T^*-T)/T)$ for each individual laser pulse. Measurements were performed with pump and probe beams polarized at the so-called “magic angle” (for a brief description of the magic angle use in time-resolved fluorescence imaging; see, e.g., Buehler *et al*⁵). The samples in solution were enclosed in nitrogen purged cuvettes and stir in-situ with a micro stirrer to refresh the photo-pumped volume.

High-resolution transmission electron microscopy

High-resolution transmission electron microscopy (HRTEM) images were acquired on a JEOL-JEM Grand ARM 300cF microscope (Madrid, Spain), operating at 60 kV, and equipped with a Cs Corrector (ETA-JEOL) and CMOS camera (4096×4096 pixels, Gatan OneView).

Energy Dispersive Spectroscopy (EDS) for local chemical analysis was carried out on a JEOL-JSM ARM200F (Cold Emission Gun), probe spherical aberration corrected microscope, operating at 80 kV.

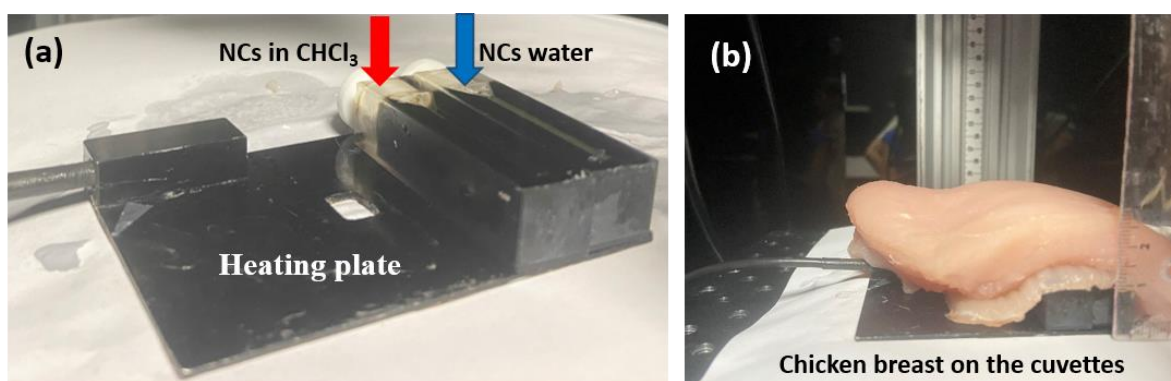
Synchrotron X-Ray absorption

X-ray absorption spectroscopy (XAS) measurements at the Ag K-edge (25514 eV) were performed at the BM30 beamline (ESRF synchrotron, Grenoble, France) in fluorescence mode. The monochromator energy was calibrated with Ag metallic foil. Data were processed with Athena software⁶ for polynomial pre- and post-edge background subtraction.

EXAFS fits were carried out on the k^2 -weighted signal Fourier-transformed to obtain the radial distribution around 1.0–5.0 Å from absorbing atoms.

Experiments of PL-imaging with the chicken breast tissue

The boneless, skinless chicken breast was obtained commercially from Mercadona supermarkets (Madrid, Spain). The chicken breast was placed on top of two cuvettes, each containing CSS NCs in CHCl_3 or in water. These cuvettes were placed on a heating element (see Figure a, left). The image on the right (b) corresponds to the tissue with thickness of 2 cm on top of the cuvettes. Figure 6e in the main text is acquired in this situation.



(a) Configuration for the experiment shows in Figure 6, main text. (b) Picture of the 2cm-thick chicken breast tissue on the cuvettes.

To perform the experiments gathered for the data presented in Figure 6 of the main text, hyperspectral PL-imaging was employed. The system (IR-VIVO 20219, Photon etc, Canada) was composed of a filter wheel for multispectral filtering, a HyperCube™ for hyperspectral filtering, and an InGaAs camera (ZephIR™ 1.7, Photon etc, Canada) for detection in the range 900-1600 nm. The cuvettes containing the NCs solutions are in contact with a thermoelectrical Peltier plate (SEGA INVEX 2018-0110, UAM, Spain) at the appropriate distance to obtain the NIR images (at around 16 cm); an image of the equipment was published elsewhere.^{7,8}

Externally, a LIMO 808 nm laser (Aerodiode, France) was used as the illumination source, at a power density in the range 0.4-50 mW/cm². The luminescence was then collected and spectrally analysed by the HyperCube™ (spectral resolution: $\delta\lambda = 6$ nm) before reaching the InGaAs camera. The integration time was varied between 5 ms and 100 ms. For the recording of the luminescence images and spectra, the PHySpec™ software (Photon etc, Canada) was utilized.

X-Ray Photoelectron Spectroscopy (XPS)

X-ray photoelectron spectroscopy (XPS) has been used to characterize the surface chemical composition of the samples. These experiments were performed in an UHV chamber with a base pressure of 10⁻¹⁰ mbar; the system was equipped with a hemispherical electron energy analyzer (Phoibos 150, SPECS Surface Nano Analysis GmbH, Germany) and a 2D delay-line detector (Surface Concept GmbH, Germany), using an x-ray source of Al-K α (1486.6 eV).⁹

XPS spectra were acquired at normal emission take-off angle, using an energy step of 0.50 and 0.10 eV and a pass-energy of 40 and 20 eV for survey spectra and detailed core level regions, respectively. The absolute binding energies of the photoelectron spectra were referenced to the C 1s photoelectron peak at 285.0 eV. The XPS spectra were analyzed with the CasaXPS program (Casa Software Ltd., Cheshire, UK), using a Shirley method for background subtraction and data processing. Spectra are displayed after the subtraction of the contribution of the Al K α satellite emission; in some cases, the spectra were normalized to the maximum intensity to highlight line shape differences. This approach provides direct, valuable insight regarding the presence of different energy-shifted components which frequently confirm that various chemical species are present.

The overall sample composition was studied from the survey spectra in which emission from Ag 3d, S 2p, Zn 2p core levels is detected as main constituents of the samples, with different peak intensities. Apart from these, intense C 1s and O 1s signals originated from spurious contamination rather than oxide materials; this is expected, due to the exposure of the samples to air at atmospheric pressure, prior to the XPS experiments.

In order to extract information not only from the outermost surface of the samples but also from deeper regions, samples were bombarded with low-energy Ar ions (1 keV), under “mild” conditions (1 μ A/cm², 10 minutes), in two consecutive steps. In some sense, the associated “erosion” process allows one to monitor the depth-dependent chemical nature of the graded nanoparticles. Note that for these experiments the samples were drop-casted on dedicated sample holders, directly from chloroform solutions.

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