Electronic Supplementary Information - ESI

Carboxymethylcellulose-mediated Aqueous Colloidal Process for Building Plasmonic-Excitonic Supramolecular Nanoarchitectures Based on Gold Nanoparticles/ZnS Quantum Emitters for Cancer Theranostics

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Materials and methods

Chemicals

Sodium carboxymethylcellulose (CMC, Sigma-Aldrich, USA) with the degree of substitution (DS = 0.77, average molar mass M_M = 250 kDa, and viscosity of 735 cps, 2% in H_2O at 25 °C), tetrachloroauric (III) acid trihydrate ($HAuCl_4 \cdot 3H_2O$, ≥ 99.9 %, Sigma-Aldrich, USA), zinc chloride ($ZnCl_2$, ≥ 98 %, Sigma-Aldrich, USA), sodium sulfide nonahydrate ($Na_2S \cdot 9H_2O$, > 98 %, Sigma-Aldrich, USA), and sodium hydroxide (NaOH, ≥ 99 %, Merck, USA) were used as received, without any further preparation. Deionized water (DI water, Millipore Simplicity, USA) with a resistivity of 18 MΩ·cm was used to prepare all solutions, and the procedures were performed at room temperature (RT, 23 ± 2 °C) unless specified otherwise.

Green synthesis of AuNP@CMC conjugates

The synthesis of AuNPs was performed in an aqueous medium using *in situ* reduction by carboxymethyl cellulose simultaneously as a ligand to stabilize the AuNP@CMC plasmonic nanostructures as previously reported by our group.[1] Briefly, 600 µL of tetrachloroauric acid solution ($HAuCl_4$, 0.05 M) was poured into 200 mL of CMC solution (0.2 % w/v) in a one-neck flat-bottom flask under stirring, and the pH of the medium was gradually adjusted to 7.2 ± 0.2 (NaOH, 0.5 M). In the sequence, the temperature was raised to 95 ± 5 ˚C and maintained under reflux (3 h). After cooling, the suspension was purified by centrifugation (6 cycles × 5 min, 4,000 rpm), resuspended, and stored at 6 ± 2 ˚C.

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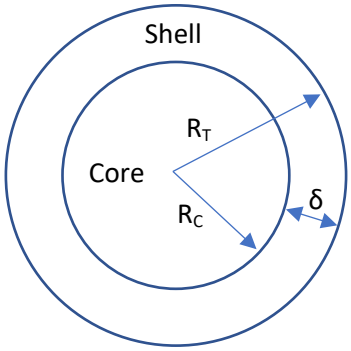
Design of AuNP-ZnS@CMC heterostructure

The amounts of $Zn^{2+}_{(aq)}$ and $S^{2-}_{(aq)}$ precursors for growing a layer of ZnS quantum emitter onto the AuNP@CMC nanoparticles were calculated following Scheme 1, where R_C is the radius of AuNP core, R_T is the radius of the AuNP-ZnS@CMC nanostructure, and δ is the thickness of ZnS shell.



Scheme 1 – Nanoparticle core-shell structure identification of dimensions.

The number of AuNP@CMC nanoparticles (N_{AuNPs}) in the suspension was calculated using the average value of R_C obtained by transmission electron microscopy (TEM) analysis, and the synthesis yield = 80 %. The density of the AuNP was considered 59 atoms/nm^3, which corresponds to 19.3 g/cm^3.[2]

The amount of Zn^{2+} and S^{2-} mass required for the synthesis was calculated using Equations S1-S5, and considering that the shell of ZnS was grown onto Zn:S molar ratio of 1.0:1.0.

$$m_{ZnS}\ (\text{one shell}) = 4/3\ \pi \times \rho_{ZnS} \times [(R_C + \delta)^3 - R_C^3] \qquad \text{Eq. S1}$$

$$m_{ZnS}\ (\text{total}) = m_{ZnS}\ (\text{one shell}) \times N_{AuNPs} \qquad \text{Eq. S2}$$

$$m_{ZnS}\ (\text{total}) = m_{Zn^{2+}} + m_{S^{2-}} \qquad \text{Eq. S3}$$

$$n_{mol\_Zn^{2+}} = m_{Zn^{2+}} / M_{M\ Zn^{2+}} \qquad \text{Eq. S4}$$

$$n_{mol\_S^{2-}} = m_{S^{2-}} / M_{M\ S^{2-}} \qquad \text{Eq. S5.}$$

where m is mass, M_M is molar mass, ρ_{ZnS} is the density of the ZnS (4.1 g/cm^3), R_C is the radius of AuNP core, and δ is the thickness of ZnS shell (δ = 2.5 nm).

Characterization of the AuNP@CMC and AuNP-ZnS@CMC nanostructures

Ultraviolet-visible (UV–vis) analysis of AuNP@CMC and AuNP-ZnS@CMC suspensions was performed using Lambda EZ-210 equipment (PerkinElmer, USA; transmission mode; quartz cuvette; wavelength, λ = 700 nm-200 nm; replicates, n = 3).

Photoluminescence (PL) characterization of ZnS quantum emitter (AuNP-ZnS@CMC suspension) was acquired using a FluoroMax-Plus-CP equipment (Horiba Scientific, Japan; RT; quartz cuvette) based on

steady state mode (excitation wavelength, λ_{exc} = 310 nm; n = 3), and 3D excitation-emission mapping (λ_{exc} = 250 to 400 nm; emission wavelength, λ_{em} = 300 to 700 nm, n = 2).

Morphological analysis of nanostructures was conducted using high-resolution transmission electron microscopy (HR-TEM; Tecnai G2-20-FEI, FEI Company, USA; 200 kV; sample preparation: droplets of the suspension onto porous carbon copper grids) and atomic force microscopy (AFM; XE-70, Parker Systems, USA; non-contact tapping mode; frequency = 325 Hz; scanning rate = 1.0 Hz; pixel resolution = 256 × 256; sample preparation: droplets of the suspension onto a mica plate). The particle size distribution was evaluated based on the TEM images (> 100 random measurements of nanostructures; DigitalMicrograph®, version 3.4, Gatan Microscopy Suite Software, Gatan, Inc., USA).

Zeta potential (ZP) and dynamic light scattering (DLS) analyses of CMC polymer and colloidal suspensions were performed using ZetaPlus equipment (Brookhaven Instruments Corporation, USA; laser of 660 nm, 35-mW; 90 °; temperature, T = 26 ºC; n ≥ 10).

Fourier transformed infrared spectroscopy (FTIR) spectra were obtained using attenuated total reflectance method (ATR; Nicolet 6700, Thermo Fisher Scientific Inc., USA; range: 4000 to 650 cm^{-1}; scans: 32; resolution: 4 cm^{-1}; sample preparation: concentrated dried films from CMC polymer and suspensions; n ≥ 2) and with background subtraction.

High-resolution x-ray photoelectron spectroscopy (HR-XPS; Amicus spectrometer, Kratos Analytical Ltd., Japan; Mg-Kα, 1253.6 eV; step size: 0.1 eV; sample: concentrated dried films from CMC polymer and suspensions; n ≥ 2) was performed for surface characterization and underneath surface analysis (Ar^+ ions; 3 s). The positions of peaks were adjusted using the C 1s binding energy (at 284.6 eV), and peak fitting was performed in the Vision Processing software (Kratos Analytical Ltd., Japan).

Experiments for applications in nanotheranostics

Cell and cell culture

Human brain glioblastoma cells (U-87, ATCC HTB-14) were purchased from the Brazilian cell repository (Banco de Células do Rio de Janeiro: BCRJ, Brazil; cell line authentication by molecular technique, Short tandem repeat DNA (STR); quality assurance and certification based on international standard NBR ISO/IEC 17025:2005).

U-87 cells (passage 35) cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco BRL, USA) with 10 % fetal bovine serum (FBS, Gibco, USA), streptomycin sulfate (10 mg/mL, Gibco BRL, USA), and antibiotic antimycotic solution (amphotericin-b: 0.025 mg/mL; penicillin G sodium:10 units/mL; Gibco, USA) in a humidified atmosphere at 5 % CO_2 and temperature of 37 ˚C.

Biological experiments

The *in vitro* evaluation of AuNP-ZnS@CMC as fluorescent imaging biological nanoprobes was performed using fluorescence microscopy after incubation with U-87 cell line for 24 h, using the protocol previously described by our group.[3] The digital images were captured with an Eclipse Ti-U

microscope (Nikon Instruments, USA) using the 10× and 40× objectives and TRITC (Tetramethylrhodamine Isothiocyanate) filter cube.

Cytotoxicity of nanoparticles and nanostructures were evaluated using 3-(4,5-dimethylthiazol-2yl-) 2,5-diphenyl tetrazolium bromide (MTT) protocols after incubation with U-87 cells for 24 h as previously reported[3] using the iMark™ Microplate Absorbance Reader (Bio-Rad, USA; λ = 595 nm). AuNP@CMC and AuNP-ZnS@CMC were added to each well at final concentrations of 0.25, 1.0, 2.5, 5.0, 12.5, 31.25, 62.5, and 125 µg/mL of AuNP. Control samples were designed for the experimental procedure as follows: control group (cell cultured with medium and 10 % FBS); positive control (cell cultured with phosphate saline buffer and 1.0 % v/v Triton™ X-100; and negative control (cell cultured with medium, 10 % FBS and chips of sterile polypropylene Eppendorf® (Germany), 1 mg/mL). The half-maximal effective concentrations (EC50) were calculated from dose-response curves (cell viability *versus* log concentration) fitted using the equation for a sigmoidal model. Statistical significance was evaluated using One-way ANOVA followed by Bonferroni's method ($\alpha = 0.05$; $n \geq 6$).

Evaluation of intracellular reactive oxygen species (ROS) was conducted using the protocol previously described by our group.[4] U-87 cells were treated with 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) fluorogenic probe and nanostructures (AuNP-ZnS@CMC, 2.5 µg/mL of AuNP, and AuNP-ZnS@CMC, 2.5 µg/mL and 31.5 µg/mL of AuNP) for 30 min. The intensity of fluorescence was measured using Varioskan™ LUX multimode microplate reader (Thermo Fisher Scientific Inc., USA; λ_{exc} = 485 nm and at λ_{em} = 528 nm).

The effect of plasmonic photothermal therapy (PTT) after incubation with AuNP-ZnS@CMC nanostructures was assessed using propidium iodide (PI) staining of dead cells. Cells were plated (1×10^4 cells/well) in 96-well microplates and synchronized in a serum-free medium for 24 h. In the sequence, AuNP-ZnS@CMC nanoconjugates were added to individual wells at a final concentration of 5.0 µg/mL of AuNP for 24 h. The Control group was cell culture with DMEM and 10 % FBS. After 24 h, the medium was aspirated and rinsed with phosphate-buffered saline (PBS, Gibco BRL, USA). The medium was replaced by 100 µL of PBS and cell cultures for the samples with and without the incubation of nanoparticles, and they were irradiated at λ=545 ± 15 nm for 30 minutes. Then, the medium was aspirated, and cells were treated for 20 min with 100 µL of PI staining (Invitrogen™, USA), according to the manufacturer's protocols. Fluorescence microscopy images were captured with an inverted-optical microscope (Eclipse Ti-U microscope, Nikon Instruments, USA) using a Texas Red filter cube. For positive control, cells were treated with 70 % ethanol in PBS (100 µL) for 30 min.

Figure

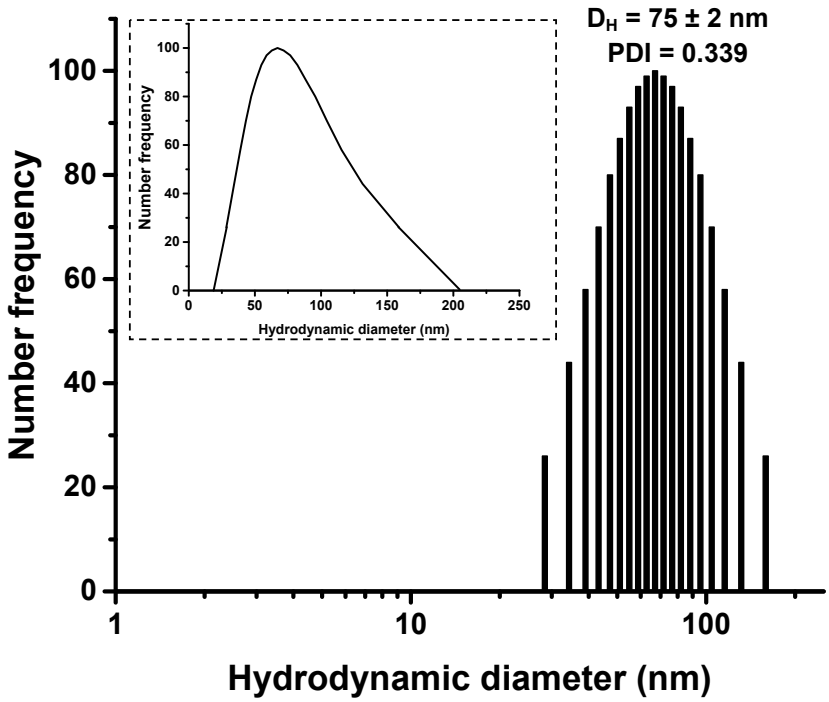


Fig. S1 Number-weighted size distribution data of AuNP-ZnS@CMC fitted with a lognormal function.

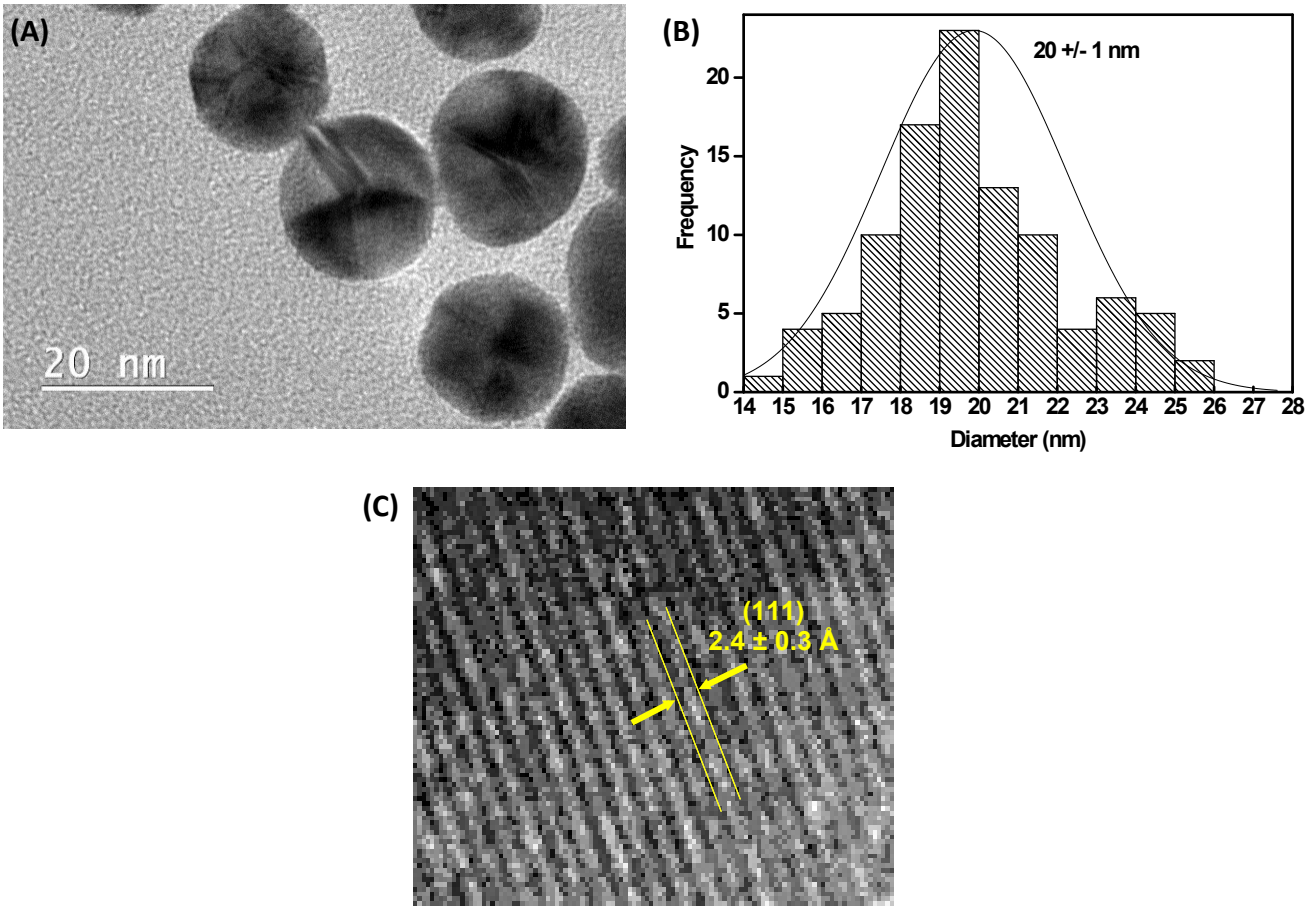


Fig. S2 (A) Transmission electron microscopy (HR-TEM) image and (B) histogram of the size distribution of AuNP@CMC. (C) Lattice fringes with interplanar distance in a high-resolution TEM image of AuNP@CMC.

References

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