

Electronic Supplementary Information

Tuning the load in gold and magnetic nanoparticles in nanogels through their design for enhanced dual magneto-photo-thermia

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Materials. Chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, 99.9%), sodium borohydride , (NaBH_4 , 99%), silver nitrate (AgNO_3 , 99+%), cetyltrimethylammonium bromide (CTAB, 99%, Sigma Ultra), ascorbic acid (99+%), Iron (II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), iron (III) nitrate nonahydrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$), ammonia solution (NH_3 , 20 %), nitric acid (HNO_3 , 52.5 %) and hydrochloric acid (HCl , 37 %), diethyl ether and technical acetone were purchased from VWR for the preparation of superparamagnetic nanoparticles of maghemite ($\gamma\text{-Fe}_2\text{O}_3$). Poly(ethylene glycol) methyl ether methacrylate (OEGMA, $M_n = 500 \text{ g}\cdot\text{mol}^{-1}$), di(ethylene glycol) methyl ether methacrylate (MEO₂MA, $M_n = 188.22 \text{ g}\cdot\text{mol}^{-1}$), poly(ethylene glycol) diacrylate (OEGDA, $M_n = 250 \text{ g}\cdot\text{mol}^{-1}$), methacrylic acid (MAA), potassium persulfate (KPS), potassium hydroxide (KOH, flakes), glycidyl methacrylate (GMA $M = 142.2 \text{ g}\cdot\text{mol}^{-1}$), 1,4-dithiothreitol (DTT, $M_n 154.2 \text{ g}\cdot\text{mol}^{-1}$) were purchased from Sigma-Aldrich for the synthesis of bare and magneto-plasmonic nanogels.

Nanogels characterization

Transmission Electron Microscopy (TEM). MNPs, AuNRs and Plasmagnanogels were observed using a Jeol-100 CX. A droplet of diluted suspension in water was deposited on a carbon coated copper grid and the excess was drained using filter paper. Size analysis was achieved on TEM images using ImageJ software.

Dynamic light scattering (DLS). Hydrodynamic diameter (d_h) and zeta potential measurements were recorded with a Malvern Instruments Nanosizer.

Fourier Transform Infra-Red (FT-IR) spectra were recorded on a Bruker Tensor 27 spectrometer on pressed KBr pellets. Spectra were obtained at regular time intervals in the MIR region of $4000 - 400 \text{ cm}^{-1}$ at a resolution of 4 cm^{-1} and analyzed using OPUS software.

UV-Vis-NIR. Absorbance measurements were done with an Avantes UV-visible spectrophotometer, with $100 \mu\text{m}$ optical fibers. UV/VIS measurements were configured with a range from 200 to 1100 nm. A combined deuterium-halogen light source was used.

Iron Titration. The total iron concentration (M) was determined by atomic absorption spectroscopy (AAS) with a Perkin-Elmer Analyst 100 apparatus after degradation of Fe_2O_3 NPs and Au in boiling HCl (35%).

Methods

1. Synthesis of MNPs

Magnetic nanoparticles (MNPs) of maghemite ($\gamma\text{-Fe}_2\text{O}_3$) were synthesized by coprecipitation of metallic salts (FeCl_2 and FeCl_3) according to Massart's procedure. A stable magnetic aqueous solution (ferrofluid, pH around 2), containing polydisperse, positively charged magnetic nanoparticles was obtained. Then, in order to reduce polydispersity, MNPs (with NO_3^- as counter ion) were sized-sorted, as stated by S. Lefebure et al.¹ This procedure is based on the destabilization of positively charged MNPs dispersion by increasing the ionic strength of the medium. For this purpose, a concentrated nitric acid solution (HNO_3 , 52.2 %) was added to the MNPs dispersion. When the destabilization was observed, the flocculate was separated from the supernatant by magnetic sedimentation and washed with acetone and ether. The precedent step was repeated until having a phase with the desired size and dispersity. Only the ferrofluid fraction containing the biggest MNPs was used afterwards. ($[\text{Fe}] = 1.24 \text{ mol/L}$, volume fraction $\phi = 1.98 \%$ determined by AAS).

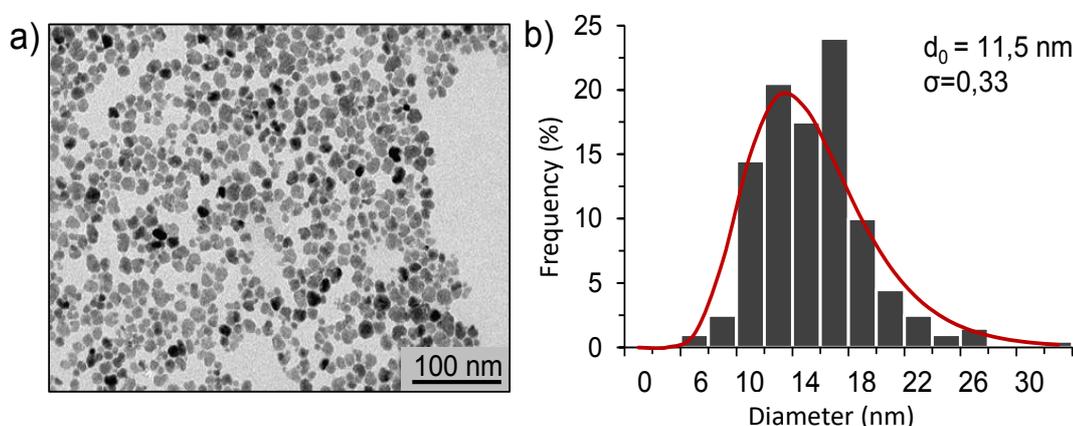


Fig. S1 (a) TEM image of maghemite NPs obtained after size sorting. (b) Size distribution of the MNPs obtained by analysis of several TEM images ($n = 200$ particles) and adjusted by a log-normal law (red curve). The NPMs have an average physical diameter $d_0 = 11.5$ nm and a standard deviation of $\sigma = 0.33$

2. Synthesis of AuNRs

Gold NRs were prepared according to the protocol of El Sayed et al.² For seed synthesis, 2.5 mL of 0.2 M CTAB solution (CTAB must be completely dissolved), 1.5 mL of 0.001 M HAuCl₄ solution, 2.5 mL of water and 0.6 mL of an ice-cold 0.01 M NaBH₄ solution were added to a beaker. The mixture was then stirred vigorously at 1000 rpm with a magnetic bar. When the color of the mixture turns pale yellow to brown-yellow, stirring is continued for a further 2 min. Then, the solution was stored at 25 ° C. 80 mL of 0.2 M CTAB solution were added to a 250 mL beaker, previously immersed in a 30 ° C water bath. After complete solubilization of CTAB, 12 mL of 0.01 M HAuCl₄ solution; 4.5 mL of a solution of AgNO₃ and 68 mL of ultra-pure Milli-Q water were added successively to the beaker with stirring at 500 rpm. Then 2 mL of a 0.079 M ascorbic acid solution were added dropwise to the mixed. Then, the bar magnet was removed from the growth solution which is left to settle for 5 min in the water bath at 30 ° C. Then 1.6 mL of the gold seed solution was added to it. The magnetic bar was returned to the beaker and the mixture was stirred at 500 rpm for 5 min. After 20 min of reaction, the solution turns red-violet. The bar magnet was again removed and the solution was covered with parafilm and left to stand overnight at 35 ° C. Finally, leave to stand for another 12 hours at room temperature. At the end of the synthesis, the dispersion of the AuNRs was centrifuged twice at 36,000 g, 20 ° C for 30 min, then twice at 15,000 g at 20 ° C for 30 min with a centrifuge (Thermo Fisher Scientific) to remove excess CTAB. At each centrifugation, the supernatant was removed and replaced with ultra-pure Milli-Q water. Finally, the AuNRs are redispersed in water.

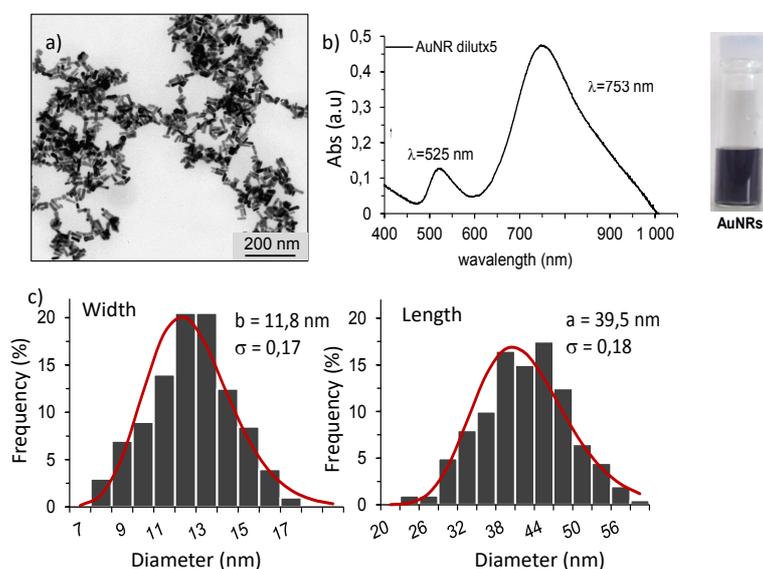


Fig. S2 a) Transmission electron microscopy (TEM) image of gold nanorods (AuNRs) after purification. (b) UV-Vis-NIR-I absorption spectrum showing the presence of two plasmonic modes in the visible (525 nm) and the NIR (753 nm). (c) Size distributions of the average length (a) and width (b) of the AuNRs (histograms) obtained by analysis of several TEM images ($n = 200$ particles) and fitted by a log-normal law (red curve). The AuNRs have an average length and width of 39.5 and 11.8 nm respectively, the respective standard deviations of which are = 0.18 and 0.17.

3. Simple core nanogels synthesis

OEGMA nanogels were prepared in a batch reactor by conventional precipitation radical copolymerization in water as previously reported.³

3.1 MNPs + AuNRs nanogels synthesis

The NPMs and AuNRs were added post-synthesis and bound to the polymer matrix, from the stock solutions of concentration $[\text{Fe}] = 1.14 \text{ mol.L}^{-1}$ and $[\text{Au}] = 5.4 \cdot 10^{-4} \text{ mol.L}^{-1}$ respectively. To assemble these inorganic nanoparticles with the nanogels, a two-stage protocol was set-up. The first step consists in synthesizing plasmonic nanogels (PlasmNanoGels).

In brief, 1 mL of the nanogel dispersion (PEG-co-MAA) were added to 2 mL of the AuNRs dispersion ($[\text{Au}] = 5.4 \cdot 10^{-4} \text{ mol.L}^{-1}$) and then the mixture was left under agitation at 25°C for 24 hours. Prior to mixing, for the PEG-co-MAA nanogels with carboxylate functions, the nanogels were dispersed in a pH 7.5 0.1 M HEPES buffer solution in order to form the carboxylate functions -COO- and promote electrostatic interactions between the negatively charged nanogels and the positively charged AuNRs. The hybrid nanogels were then produced from the PEG-co-MAA nanogels (second step). Three centrifugation cycles were performed (10,000 rpm, 30 min) to remove the HEPES buffer solution (supernatant) and replace it with distilled water. To ensure a good complexation of the MNPs, the initial pH of the nanogels was adjusted from 3 to 7. The amount of AuNRs was fixed to 3.1% (mass ratio) but different preparations were tested by adding a variable amount of MNPs 4.4%, 15.5 % 26.9% (mass ratio).

3.2 MNPs@AuNRs nanogels synthesis

The protocol set up for this synthesis was made of five stages described in figure 1 of the manuscript.

Step 1. Core formation was achieved by radical copolymerization in water. The functionalization of the core with carboxylic groups was carried out by adding methacrylic acid (MAA) before starting the polymerization.

Step 2. MNPs were incorporated by complexing the carboxylic groups (-COOH/-COO-) of the polymers on the surface of the MNPs.

Step 3. Shell formation 5 mL of the colloidal dispersion containing the core nanogels, as well as 5 mL of distilled water are placed in a three-necked flask equipped with a condenser, a magnetic stirrer and a hot plate. The dispersion is heated to 70 ° C and degassed under N₂ (g), for 30 min. Then, 2 mL of a solution containing MEO₂MA, OEGMA₅₀₀, OEGDA₂₅₀ and GMA are added to the reaction flask. As example, for a dispersion of cores whose dry extract is equal to [cores] = 3 mg.mL⁻¹, m core (in 5 mL) = total shell reagents = 0.015 g, the 2 mL solution added to the flask is composed of 7.44 mg (40 μmol) of MEO₂MA, 2.16 mg (4.3 μmol) of OEGMA₅₀₀, 5.16 mg (1.7 μmol) of OEGDA₂₅₀ and 0.42 mg (36 μmol) of GMA.

After 10 min, 150 μL of a 6 mol% KPS / solution (MEO₂MA + OEGMA) are added to the flask to start the polymerization, and the reaction is continued for 5 hours at 70 ° C under N₂ (g). The molar percentage of KPS is identical to that used for the development of the cores. Therefore, for a dispersion at [core] = 3 mg.mL⁻¹, the mass of KPS is 0.073 mg (0.27 μmol). At the end of the polymerization, the core-shell nanogels obtained are purified by several cycles of centrifugation (10,000 rpm, 30 min).

Step 4. Functionalization of the post-polymerization shell. The functionalization of the shell with thiol groups is carried out by following the procedure indicated by D. Suzuki and H. Kawaguchi.^{4, 5} First, a mixture containing 50 mg of the PEG-co-MAA @ PEG-co-GMA nanogels; 0.079 g (0.51 mmol) of DTT and 10 mL of water is placed in a flask and stirred at 400 rpm, at room temperature. Thus, for example, for a colloidal dispersion of core @ shell nanogels of dry extract equal to [core @ shell] = 6.25 mg.mL⁻¹, a volume of 8 mL of this dispersion (corresponding to 50 mg of nanogels) is withdrawn and introduced into the reaction flask. Then 0.079 g (0.51 mmol) of DTT, as well as 2 mL of distilled water are also added. The total volume of the reaction medium is 10 mL. The pH of the solution is adjusted to 11.0 by adding a few drops of a 6 M NaOH solution. The reaction is continued for 24 hours at room temperature. Finally, the PEG-co-MAA @ PEG-co-SH nanogels obtained are purified by several cycles of centrifugation (10,000 rpm; 30 min) until the conductivity of the water wash is close to that of distilled water (σ water ≈ 2 μS.cm⁻¹). After purification, the dispersion of nanogels is left stirring overnight to properly redisperse the nanogels in water.

Step 5. Addition of AuNRs by formation of covalent bonds between thiol groups (-SH) and the surface of AuNRs. The concentration of gold and iron in the PlasmMagNanoGels were determined by ICP-AES.

4. Characterizations

4.1 Dynamic light scattering (varying the size of the core-shell nanogels)

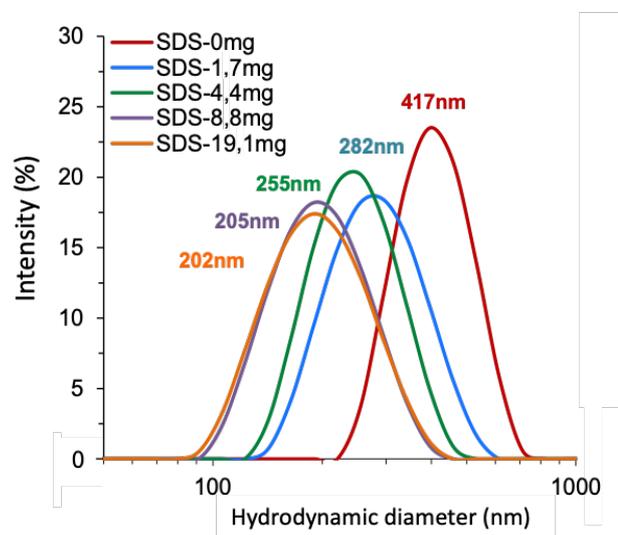


Figure S3. DLS measurement of core-nanogels after addition during the core synthesis of variable amount of SDS.

4.2 Magnetic manipulation of PlasMagNanogels and UV-Vis-NIR spectroscopy

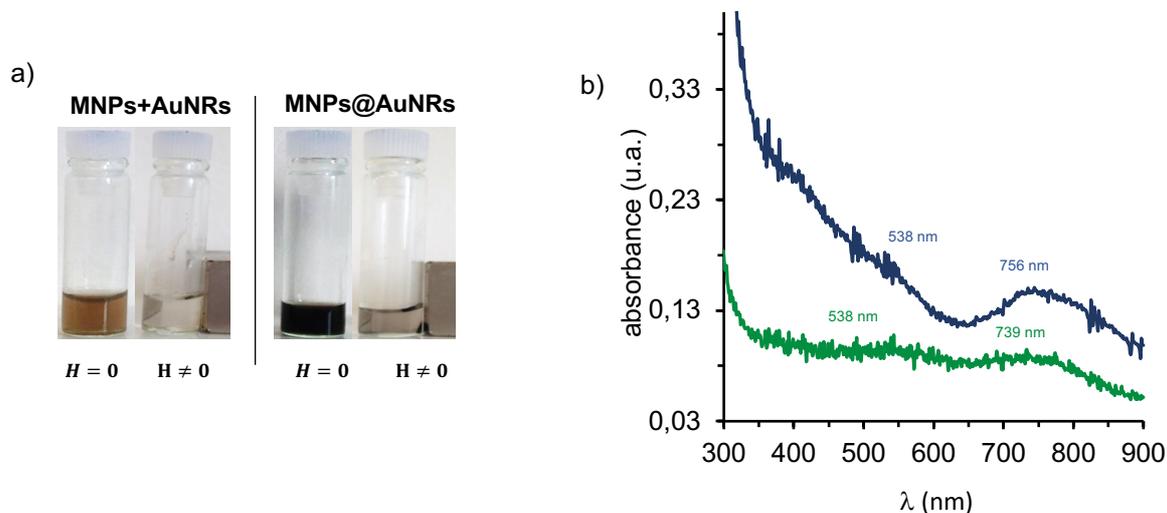


Figure S4. a) Magnetic manipulation of PlasMagNanogels with simple core structure a) and core-shell structure. A Nd-Fe-B magnet (1.2 T) is placed on the right side. b) UV-Vis-NIR spectra of PlasMagNanogels, green curve MNP+AuNRs, blue curve MNPs@AuNRs.

4.3 Thermal characterization

Heating potential of the nanogels was investigated upon magnetic hyperthermia (MHT) and photothermia (PT). The nanogels were dispersed in 50 μL and were submitted to MHT only, PT only, or both (DUAL). MHT was achieved using a NanoScale Biomagnetics device operating at 18 mT and 470 kHz. A 1064 nm laser (Laser Components S.A.S) was used for PT, with tuneable power up to 5W, set up at the two power density of 0.3 or 1 W/cm^2 (4.5 cm distance). For DUAL modality, sample was place inside the MHT device, and laser was applied on top of it, the 4.5 cm distance being enough to have no exposure of the laser fibre to the magnetic field. The temperature increase was recorded over 5 min, by infra-red camera (FLIR SC7000). The SLP (specific loss power), used as an indicator of heating potential, and expressed as Watt dissipation per unit mass, in $\text{W}\cdot\text{g}^{-1}$, was calculated according to:

$$\text{SLP} = CV/m \cdot DT/dt$$

with m the total mass of iron in the sample (either of total inorganic material, or Au and Fe only), C the specific heat capacity of the sample ($C=4185 \text{ J}\cdot\text{L}^{-1}\cdot\text{K}^{-1}$), V the sample volume, and DT/dt the initial temperature increase slope.

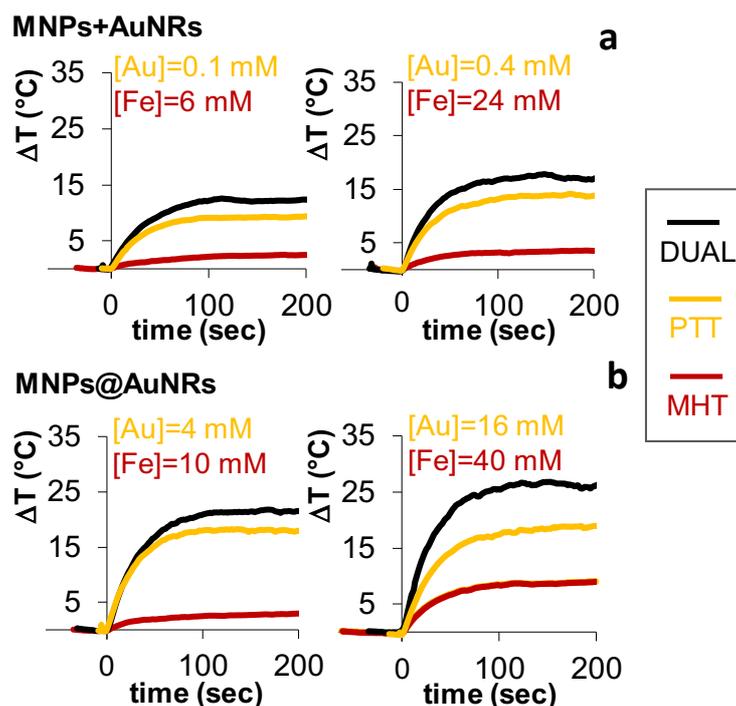


Figure S5. Heating curves for (a) MNPs+AuNRs and (b) MNPs@AuNRs dispersions (50 μ L) acquired by an IR camera on samples at different iron and gold concentration, as specified in the figure, and after 5 min exposure to an alternating magnetic field (MHT) or laser (PTT) or both (DUAL). MHT was performed at 470 kHz and 18 mT; PTT was performed at 808 nm wavelength, for a laser power density of 0.3 W/cm².

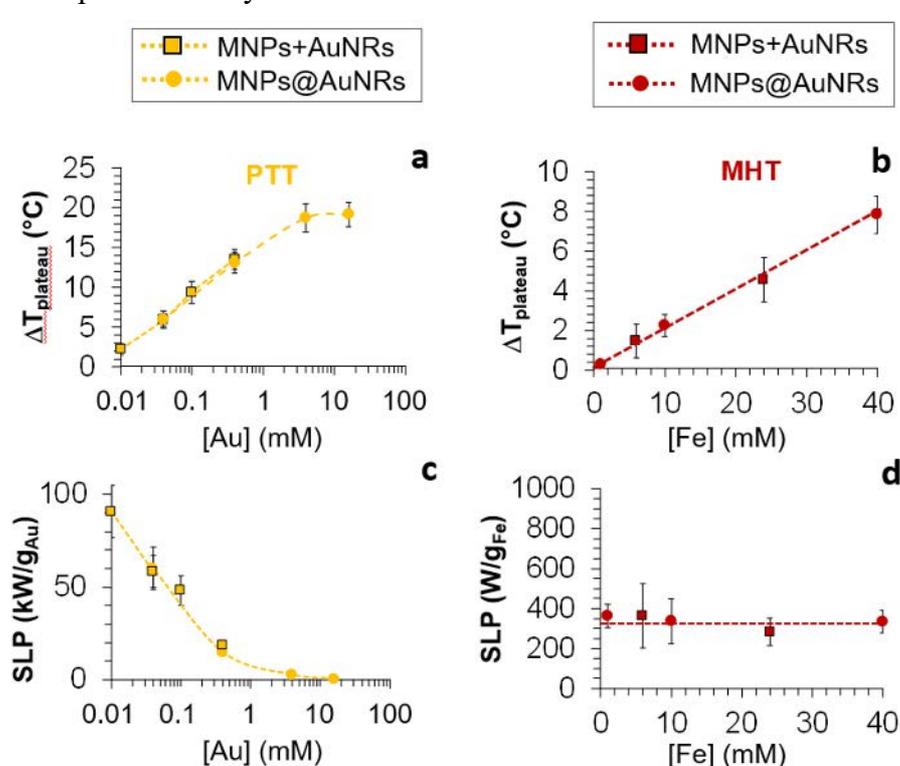


Figure S6. Temperature increase upon PTT (a) or MHT (b) for both MNPs+AuNRs (circles) and MNPs@AuNRs (squares) as a function of the solution concentration. SLP (specific loss

power) expressed in Watts per total grams of Au for PTT (c), and per total grams of Fe for MHT (d), also shown for both materials.

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