Strong Photoluminescence of Shortwave Infrared-Emitting Anisotropic Surface Charged Gold Nanoclusters

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1. Synthesis

Chemical products were purchased in Sigma-Aldrich (France) and deionized water was used for all synthesis.

We slightly modified a protocol described by Xie et al.[1] to produce the different Au NCs. 500 µL of $HAuCl_4.3H_20$ (20mM) was added to 4.8 mL water followed by 4 mL of the thiolated ligand mixture mercaptohexanoic acid (MHA, 5 mM)) / hexa(ethyleneglycol) dithiol (HDT, 5 mM) changing color from yellowish to slightly pale cloudy. Several volume ratio MHA/HDT were chosen from 4 mL/0 mL to 2 mL/2 mL; higher amount of HDT was leading to irreversible precipitation. **AuMHA, AuMHA/HDT type A, AuMHA/HDT type B, AuMHA/HDT type C, AuMHA/HDT type D** were obtained with the ratio: 4 mL/0 mL, 3.5 mL/0.5 mL, 3 mL/1 mL, 2.75 mL/1.25 mL, 2 mL/2 mL, respectively. After 1 min, 500 µL of NaOH (1M) was added dropwise leading to almost colorless sols. After 5 min, 150 µL $NaBH_4$ (20 mM in 0.2 M NaOH) was introduced dropwise under mild stirring at 350rpm for 8 hours.

Purification of the AuMHA/HDT on 3 kDa cut-off filter column (Amicon) were repeated 3 times to stop the reaction and sols were kept stored in the fridge before characterization.

Same protocol was used with ethylenedithiol (EDT) or tetra(ethyleneglycol) dithiol (TDT) instead of HDT to produce AuMHA/EDT and AuMHA/TDT.

To determine the impact of pH of the absorbance spectra of the series of AuMHA/HDT, 50 µL of concentrated solutions were diluted in 950µL of PBS solution (10 mM) at pH 9, 7.5, 6, and 4.5.

The Au NCs, $AuZwMe_2$ was synthesized following the protocol described in the literature[2].

2. Instruments

Metal core sizes were determined by high resolution transmission electron microscopy with an 200 kV monochromated TEM using dispersed Au NCs on ultra-fine carbon films. The hydrodynamic diameters of the Au NCs were measured in D_2O by DOSY-NMR experiments at 500 MHz on a Bruker AVANCE III spectrometer equipped with a cryo-probe Prodigy. The samples were around 2mM in 500 µL D_2O and the pH ~7. The 2D-DOSY experiments were run using the standard "ledbpges2s" Bruker sequence with linear gradient stepped between 2% and 98%. 64 scans were recorded for each gradient step. Data processing was performed using maximum entropy algorithm from Dynamics Center, a Bruker's NMR software, to obtain the diffusion coefficient D. An average value of D was used for the hydrodynamic diameter calculation according to the Stokes-Einstein equation $\emptyset HD = 2kBT/ 6\pi\eta$ where kB is the

Boltzmann constant, T is the temperature, η is the viscosity of the solvent (ηD_2O = 1.232 10^{-3} Pa.s at 298K).

XPS investigation was performed in a K-Alpha+ spectrometer (ThermoFisher Scientific) using a microfocused, monochromated Al Kα X-ray source (400 μm spot size). Data acquisition and processing using the Thermo Avantage software is described elsewhere[3]. The spectra were fitted with one or more Voigt profiles (BE uncertainty: ±0.2 eV) and Scofield sensitivity factors were applied for quantification[4]. All spectra were referenced to the C 1s peak at 285.0 eV binding energy (C–C, C–H) and controlled by means of the well-known photoelectron peaks of Cu, Ag, and Au, respectively. Measurements were performed on two different locations of dried samples.

Evolution of the Au NC size as a function of the ratio MHA/HDT was determined by electrospray ionization (ESI) on a commercial quadrupole time-of-flight (micro-qTOF, Bruker-Daltonics, Bremen, Germany, mass resolution ~10,000). The samples were prepared by diluting mother solution in water, to a final concentration of 0.25-0.5 mg.mL^{-1} in 50/50% water/meOH v/v. The samples were analyzed in negative ion mode: each data point was the summation of spectra over 5 min. External calibration was carried out with a set of synthetic peptides.

We have neglected the contribution of the contamination and based on the XPS results we obtained the following ratio HDT/MHA.

Type A: ratio HDT/MHA ~1/3 (see XPS (C-O)/C-C,C-H))

Type B: ratio HDT/MHA ~1/2 (see XPS (C-O)/C-C,C-H))

Considering gold (Au) were composed of Au(0) and Au(I) in the nanocluster and for all nAu+I, nS-I were consumed.

Therefore, we can propose the following formula:

Type A: $Au_n^{(0)}Au_m^{(+I)}SMHA_{q/2}^{(-I)}SMHA_{p/2}SHDT_{q/6}^{(-I)}SMHA_{p/6}$

Type B: $Au_n^{(0)}Au_m^{(+I)}SMHA_{m/2}^{(-I)}SMHA_{p/2}SHDT_{m/4}^{(-I)}SMHA_{p/4}$

For AuMHA/HDT type A:

m $Au^{(+1)}$ for q/2 SMHA and 2q/6 SHDT (considering that 2 sulphurs were oxidized for HDT at the interface).

q/2+2q/6 = m

q = 6m/5

Then, $Au_n^{(0)}Au_m^{(+I)}SMHA_{6m/10}^{(-I)}SMHA_{p/2}SHDT_{m/5}^{(-I)}SMHA_{p/6}$

By XPS measurement, we determined S-Au/S_{total} = 0.3 therefore:

(6m/10+m/5+p/2+p/6)/(6m/10+m/5) = 3.333

(0.83m+0.667p)/0.83m=3.33 $\rightarrow$1+0.803p/m = 3.33

p/m=2.9

By XPS measurement, we determined S-Au/Au_{total} = 0.4 therefore:

m/(n+m) = 0.4 therefore **m = 0.667n**

and **p = 1.9n**

We can express as a function of n:

n*197+0.667*n*197+0.4*n*148+0.85*n*148+0.133*n*314+0.32*n*314=$M_W$total (9400 Da)

n(197*1.667+148*1.25+314*0.46)=9400

n=14

To give a general formula for AuHDT/MHA type A: $Au_{23}MHA_{18}HDT_6$

For AuMHA/HDT type B:

m $Au^{(+1)}$ for m/2 SMHA and 2m/4 SHDT (considering that 2 sulphurs were oxidized for HDT at the interface).

By XPS measurement, we determined S-Au/S_{total} = 0.4 therefore:

(m/2+m/4+p/2+p/4)/(m/2+m/4)=2.5

1+p/m= 2.5

p/m=1.5

By XPS measurement, we determined S-Au/Au_{total}= 0.6 therefore:

m/(n+m)= 0.6 then **m=1.5n**

and **p=2.25n**

We can express as a function of n:

n*197+1.5*n*197+1.5/2*n*148+2.25/2*n*148+1.5/4*n*314+2.25/4*n*314=$M_W$total (9400 Da)

n(197*2.5+148*1.875+314*0.9375)=9400

n=8.8 ~ 9

To give a general formula for AuHDT/MHA type B: **$Au_{23}MHA_{17}HDT_9$**

- For M_W=10500 Da (second distribution):

Then n=10 giving a second formula **$Au_{25}MHA_{19}HDT_{10}$**

Absorption spectra of diluted AuNC samples were recorded on an Safas Monaco SP2000 UV-vis spectrophotometer between 350 and 1100 nm. Steady-state photoluminescence spectra were measured from 600 – 1750 nm with a calibrated FSP 920 (Edinburgh Instruments, Edinburgh, United Kingdom) spectrofluorometer equipped with a nitrogen-cooled PMT R5509P. Time-resolved measurements were performed inrthe wavelength region of 300 – 1100 nm using a FLS 920 (Edinburgh Instruments, Edinburgh, United Kingdom) lifetime spectrofluorometer equipped with an EPL-510 (Edinburgh Instruments, Edinburgh, United Kingdom) picosecond pulsed diode laser (excitation wavelength of 510±10 nm; power of 5mW) and a fast PMT R2658P from Hamamatsu , respectively.

Relative measurements of photoluminescence QYs ($\Phi_{f,x}$) were performed using the dye IR125 dissolved in dimethylsulfoxide (DMSO as reference. The QY of this dye was previously detrmined absolutely to $\Phi_{f,st} = 0.23$)[5]. The relative QY were calculated according to the formula of Demas and Crosby[6], see equation below.

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

The subscripts x, st, and ex denote sample, standard, and excitation respectively. $f(\lambda_{ex})$ is the absorption factor, F the integrated spectral fluorescence photon flux, and n the refractive index of the solvents used (DMSO for IR125; water for Au nanoclusters).

All spectroscopic measurements were done in a 1 cm quartz cuvettes from Hellma GmbH at room temperature using air-saturated solutions.

SWIR imaging was performed using a Princeton camera 640ST (900-1700nm) coupled with LED excitation source at λ= 830 nm (60mW/cm^2). We use short-pass excitation filter at 1000 nm (Thorlabs) and long pass filters (Thorlabs) on the SWIR camera at 1000 nm or 1250 nm. A 25 mm lens with 1.4 aperture (Navitar) was used to focus on the samples. Tubes containing AuNC solution or 10µL drops of each samples were put in front of the camera before acquisition and analyses were performed using FIJI software.

3. Physico-chemical characterizations

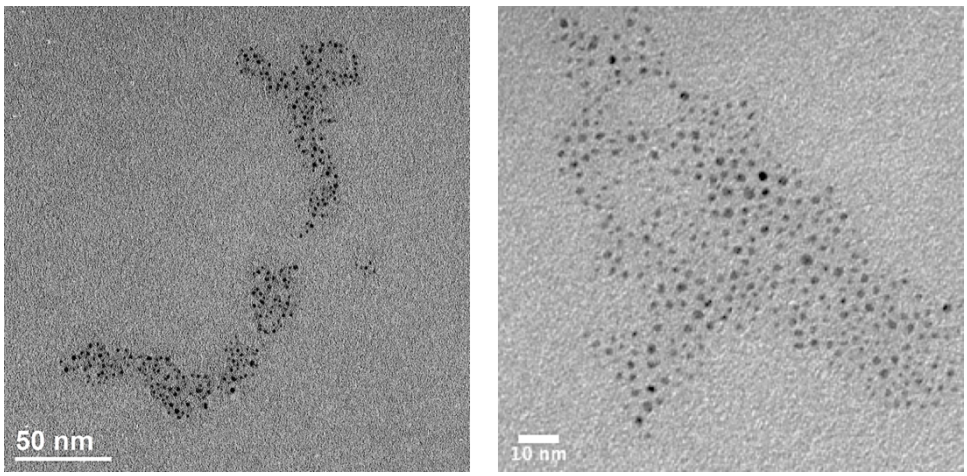


Fig. S1. TEM images of AuMHA.

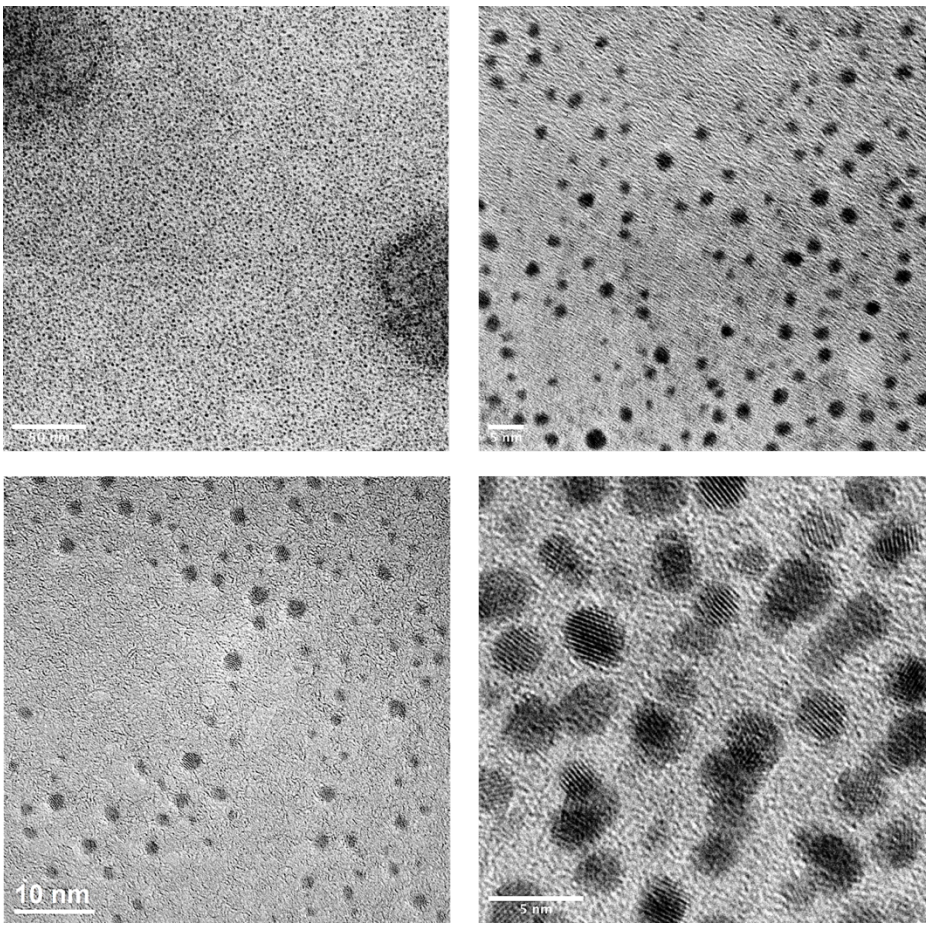


Fig. S2. TEM images of AuMHA/HDT type A.

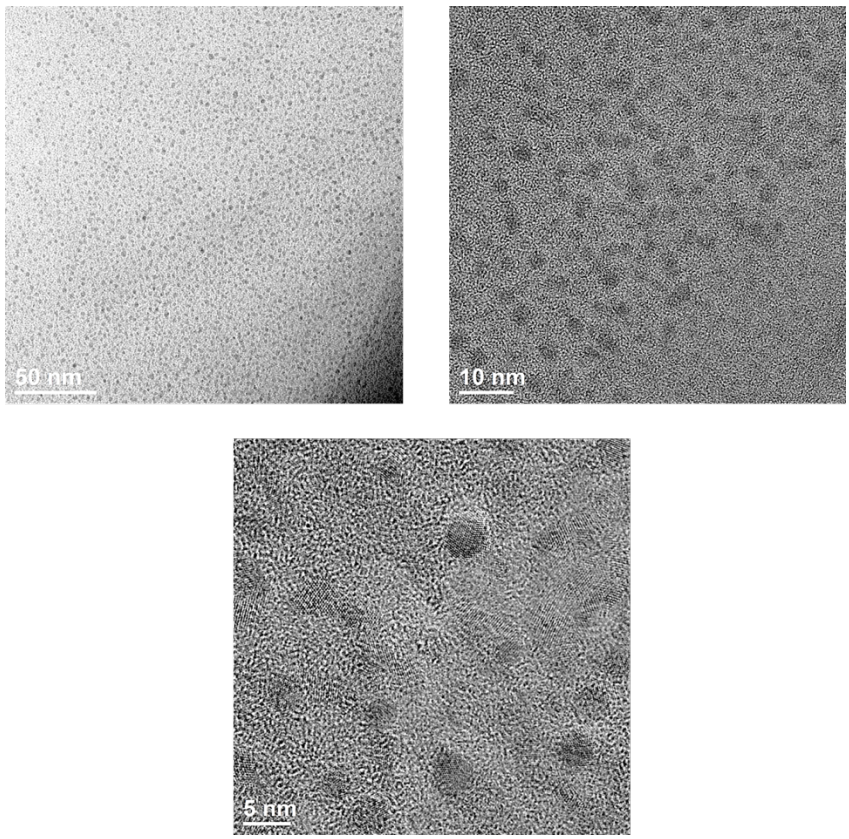


Fig. S3. TEM images of AuMHA/HDT type B.

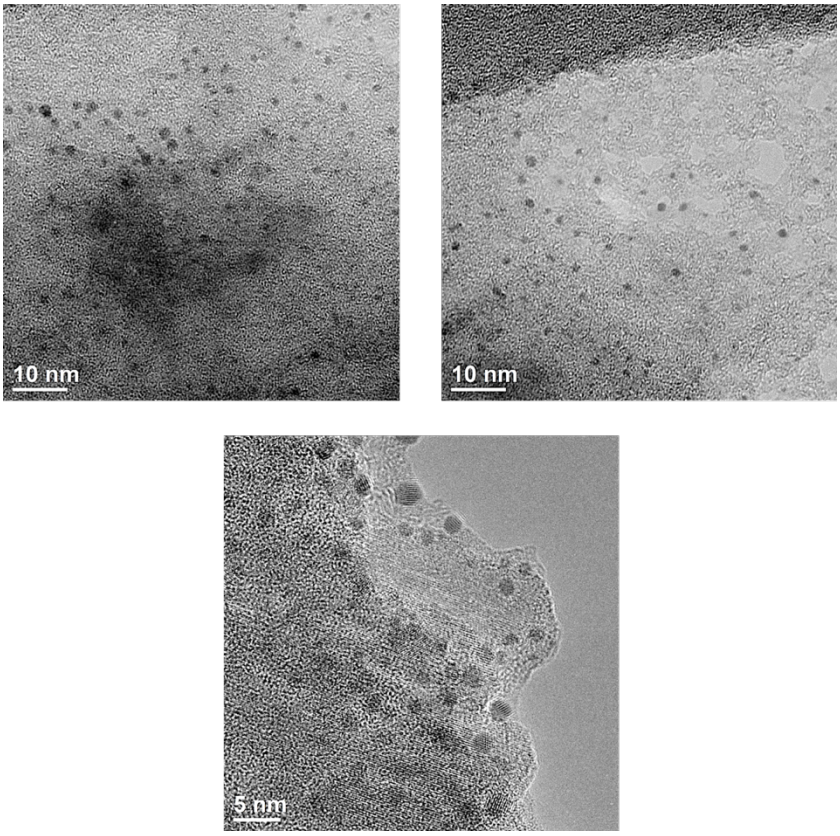


Fig. S4. TEM images of AuMHA/HDT type C.

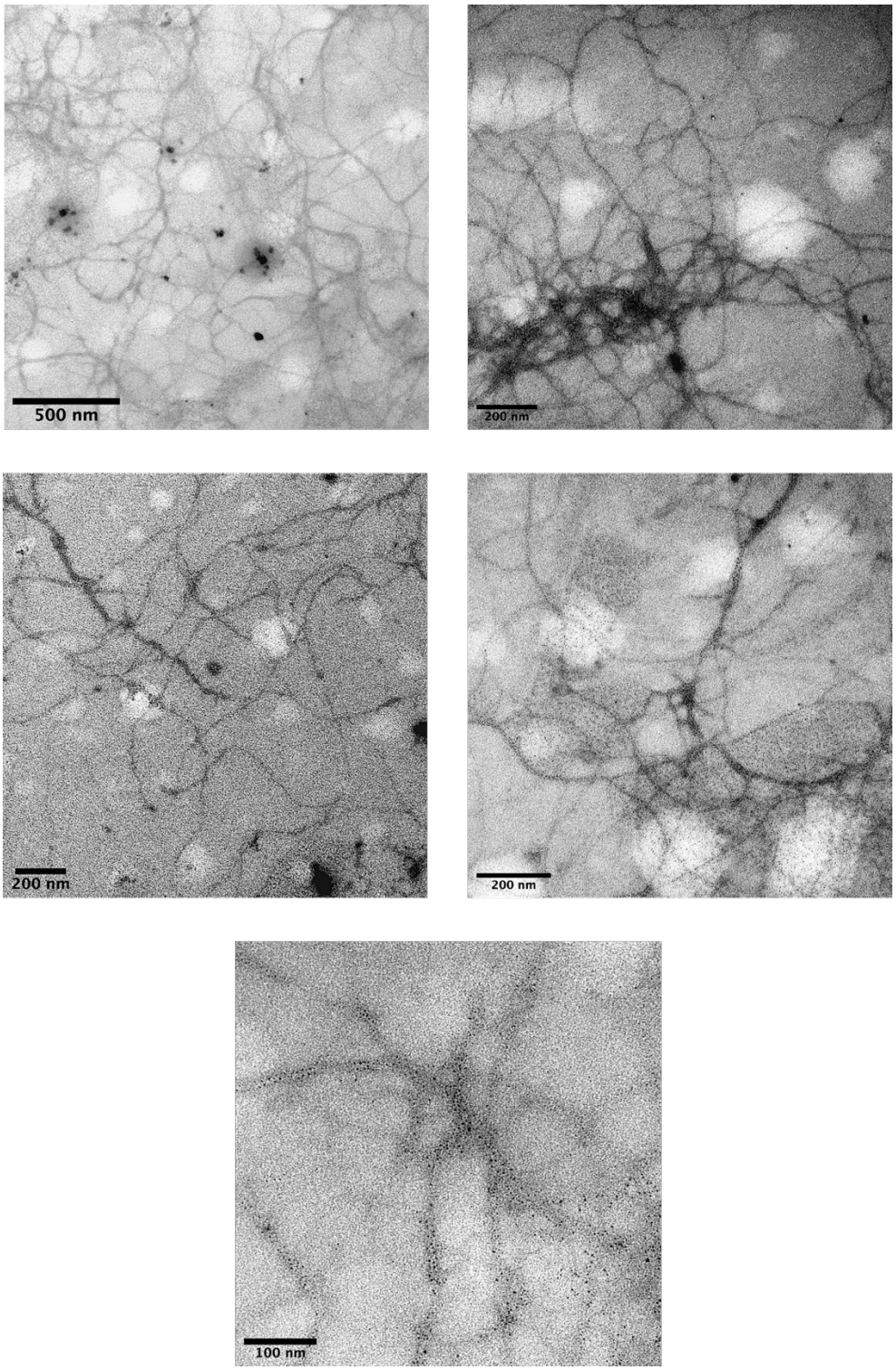
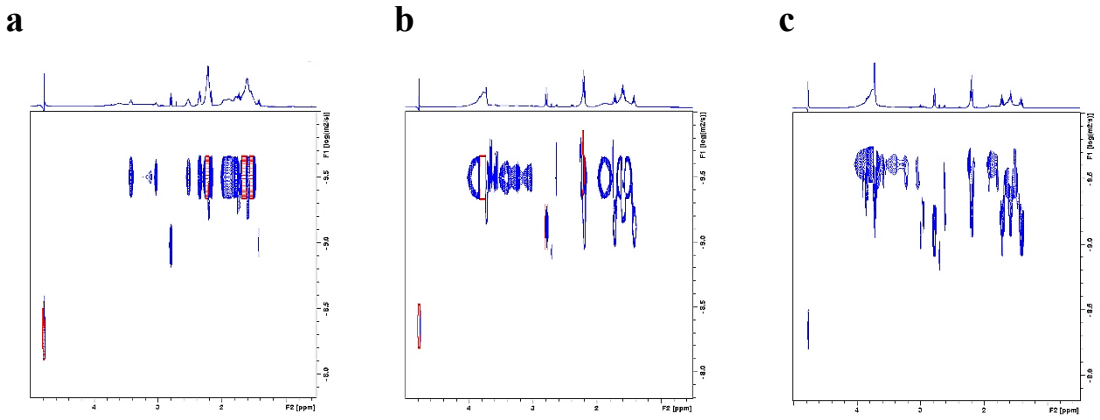


Fig. S5. TEM images of AuMHA/HDT type D.



	Diffusion coefficient D (10^{-10} m^2 s^{-1})	Hydrodynamic diameter (nm)	D_{D20}(10^{-9} m^2 s^{-1})
AuMHA	2.28 ± 0.05	1.54±0.04 nm	3.01
AuMHA/HDT type A	1.55 ± 0.10	2.28±0.10 nm	3.05
AuMHA/HDT type B	1.44± 0.10	2.46±0.20 nm	3.03

Fig. S6. DOSY-NMR spectra of (a) AuMHA, (b) AuMHA/HDT type A, (c) AuMHA/HDT type B diluted in D_20.

Samples	Core size[a]	Hydrodynamic diameter[b]	Molecular weight[c]
AuMHA	1.37±0.33 nm	1.54±0.04 nm	7.6 kDa
AuMHA/HDT type A	1.67±0.17 nm	2.28±0.10 nm	9-11 kDa
AuMHA/HDT type B	1.82±0.27 nm	2.46±0.20 nm	9-11 Da

Table S1. Physico-chemical properties of AuMHA, AuMHA/HDT type A and type B. [a]determined by HRTEM on at least 100 particles. [b]obtained by NMR-DOSY. [c]estimated by ESI-MS.

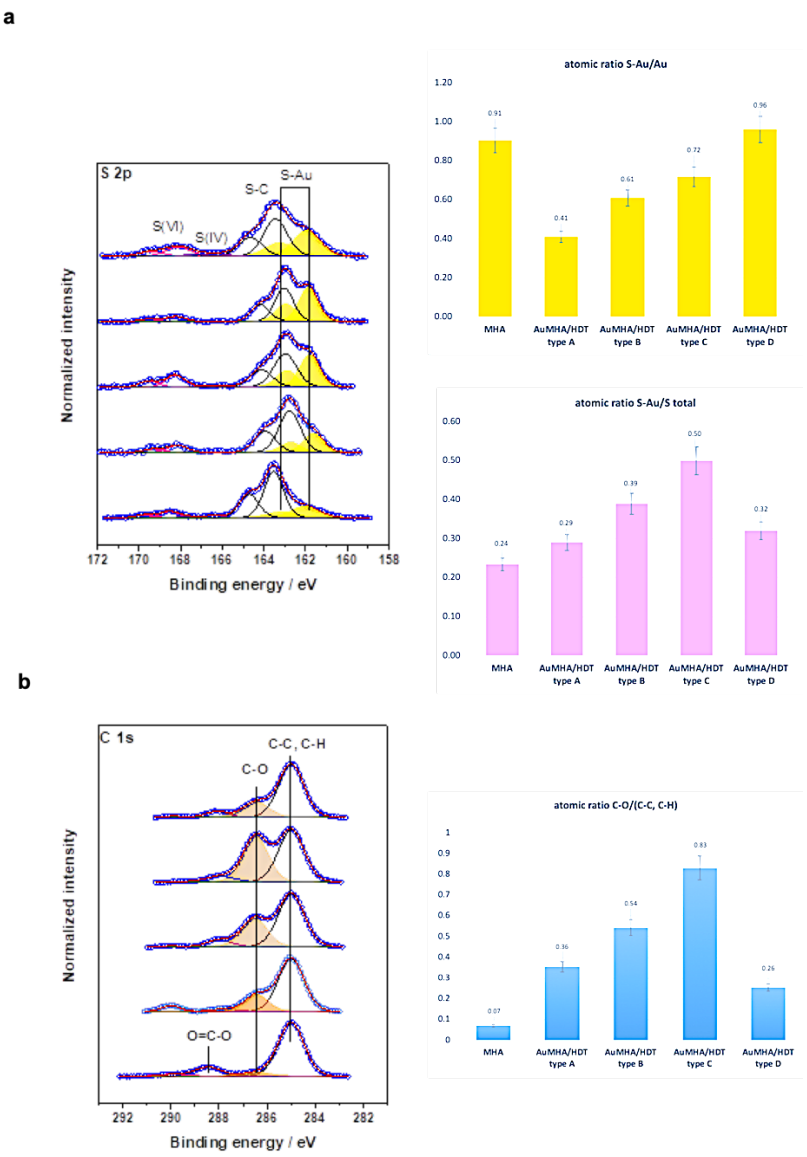


Fig. S7. (a) S 2p XP spectra of AuMHA and AuMHA/HDT type A, B, C, and D (from the bottom to the top). For S 2p, we can observe 3 different doublets which can be attributed to oxidized sulphur (+VI) at 168,2 eV and (+IV) at 166,2 eV, to sulphur bound to carbon at 163.5 eV and sulphur bound to gold at 161.8 eV. The quantitative analysis leads to the calculation of the atomic ratio S-Au/Au and S-Au/S total for the different samples (bar charts). (b) C 1s XP spectra of AuMHA and AuMHA/HDT type A, B, C, and D (from the bottom to the top). The peak at 286.5 eV is attributed to C-O and can be used as a marker to prove the presence of HDT. The evolution of the atomic ratio (C-O)/(C-C, C-H) is represented by the bar chart and shows the increasing proportion of HDT for the different synthesis with a maximum for C.

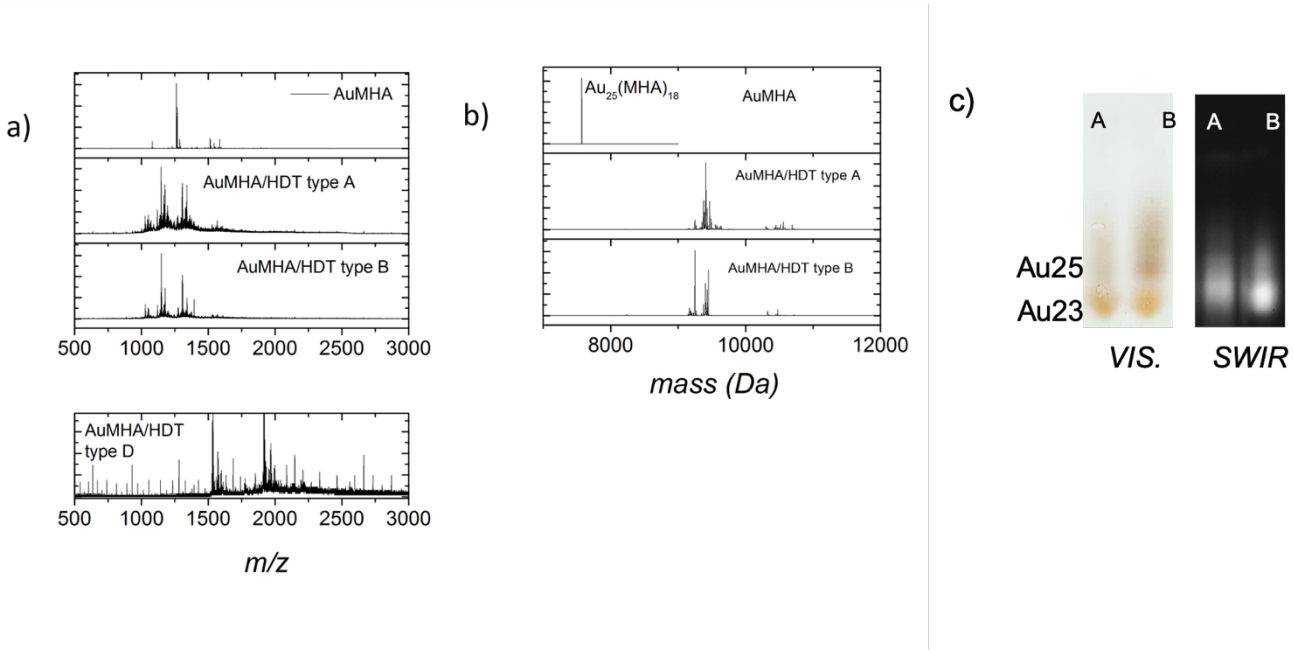


Fig. S8. a) ESI-MS in negative mode of electrospray of AuMHA, AuMHA/HDT type A, type B and type D nanoclusters, and b) deconvoluted ESI-MS of AuMHA, AuMHA/HDT type A and type B nanoclusters. c) PAGE electrophoresis of AuMHA/HDT type A and type B nanoclusters under Visible and SWIR cameras ($\lambda_{exc.}$ = 830nm; $\lambda_{em.}$ = 1000-1700 nm).

4. Optical characterizations

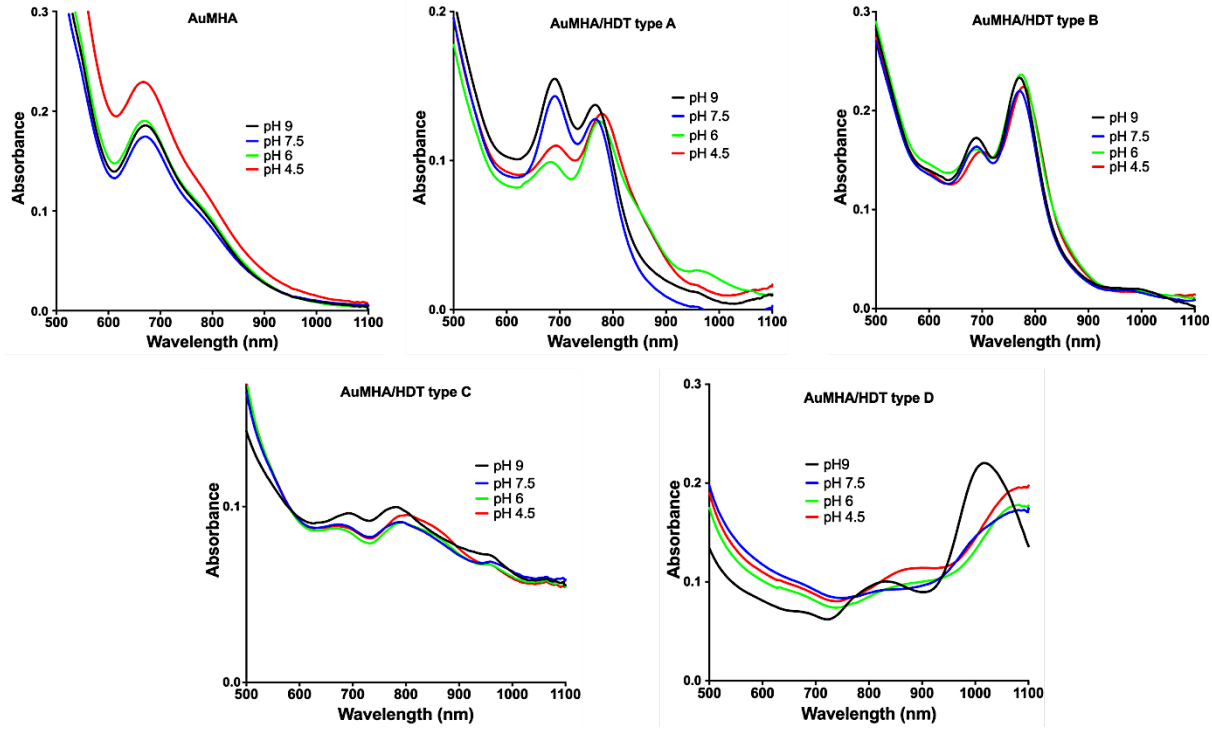


Fig. S9. Absorption spectra of AuMHA and AuMHA/HDT type A, B, C, and D in PBS buffer (10mM) at pH values of 9, 7.5, 6, and 4.5, respectively.

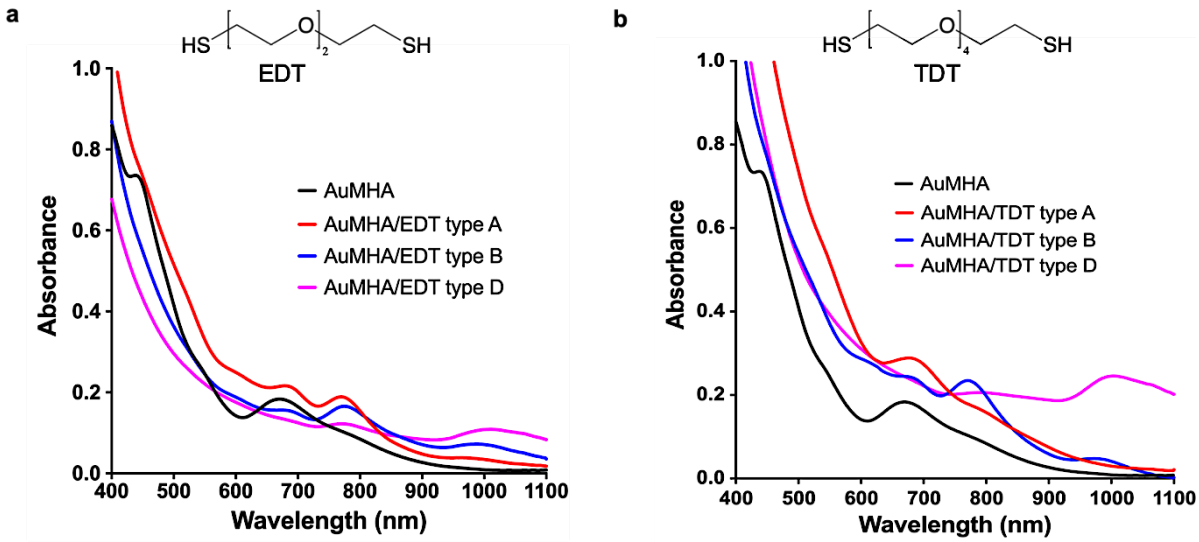


Fig. S10. Absorption spectra of AuMHA, (a) AuMHA/EDT type A, B, D and (b) AuMHA/TDT type A, B, D in water.

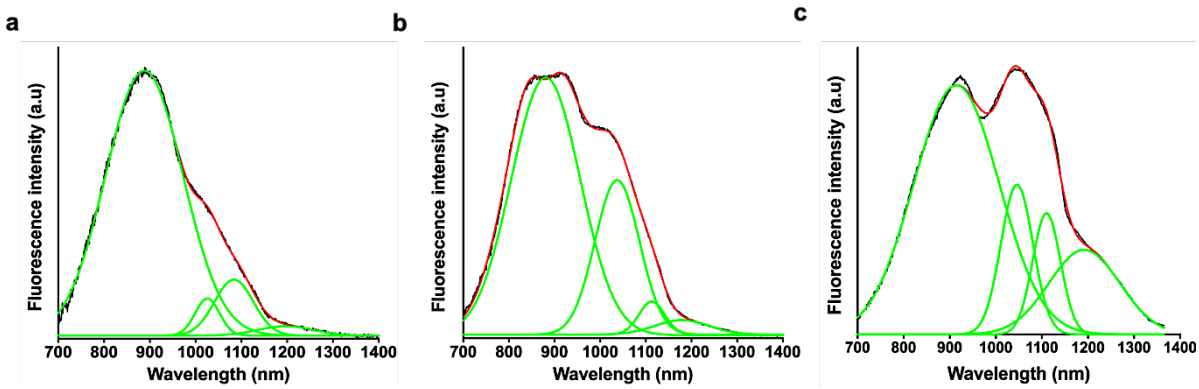


Fig. S11. Deconvoluted photoluminescent spectra of (a) AuMHA, (b) AuMHA/HDT type A, (c) AuMHA/HDT type B. ($\lambda_{exc.}$ 400 nm). Red curve represents the fitted spectra and green curves the multiple deconvoluted gaussian fits.

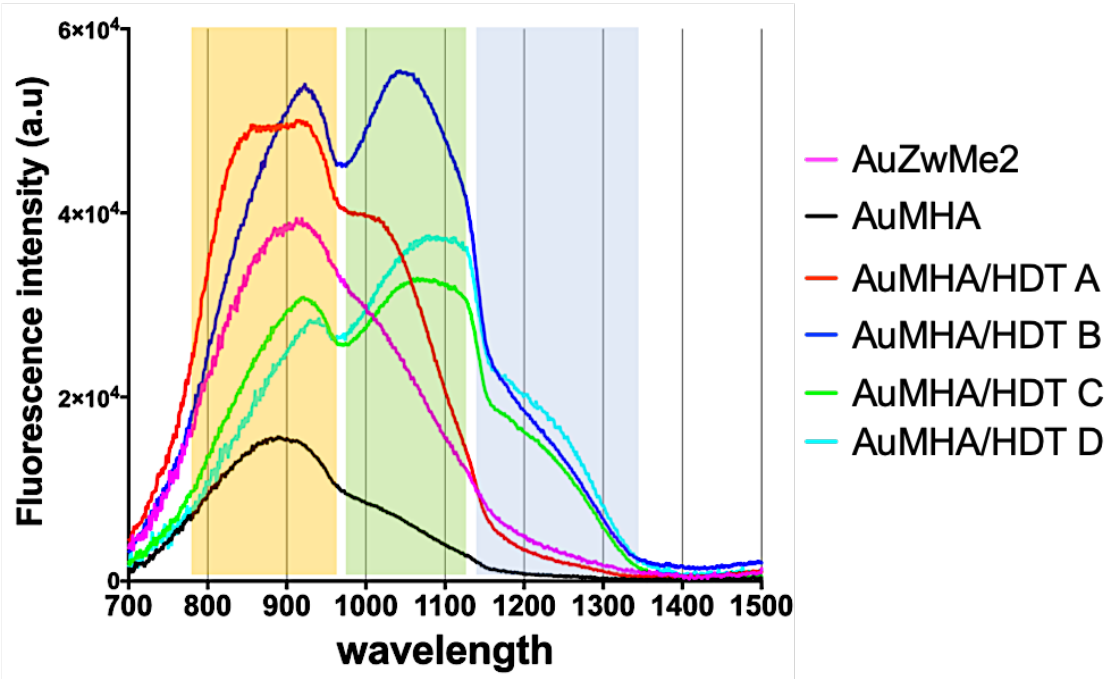


Fig. S12. Fluorescence excitation spectra of AuMHA, AuMHA/HDT type A (red line) and type B (blue line) in water at different emission wavelengths

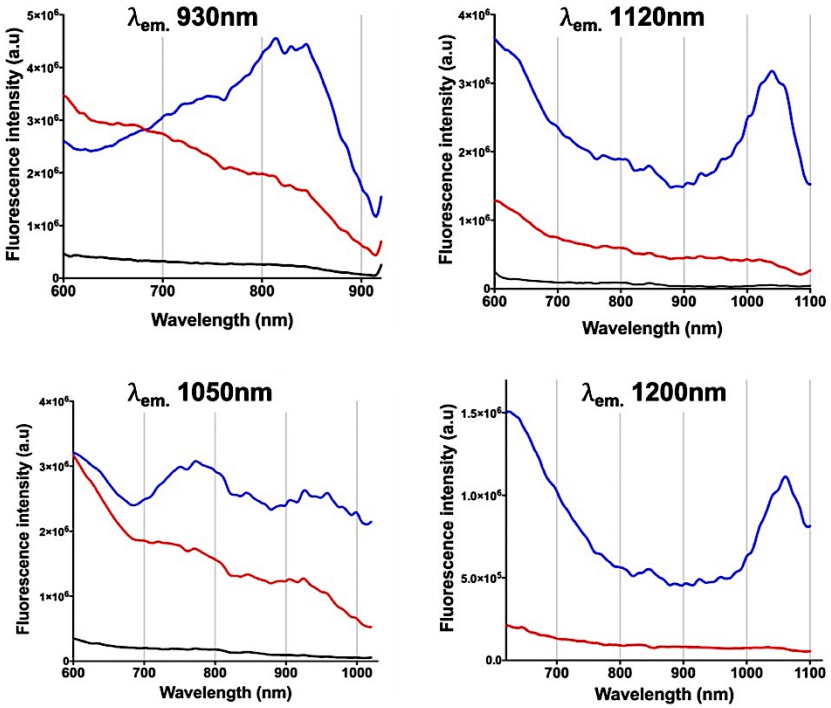
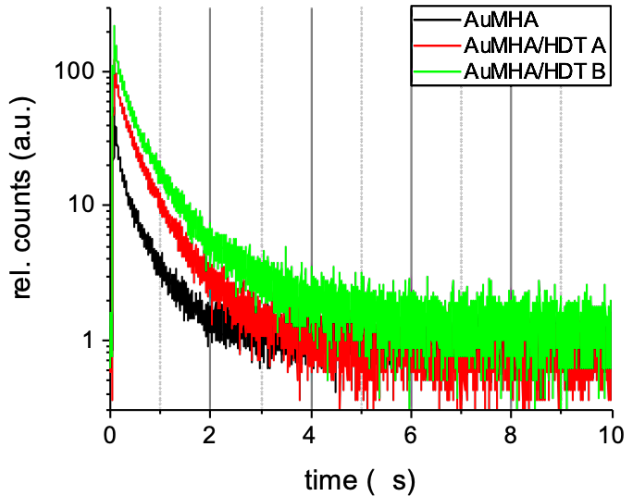


Fig. S13. Emission spectra of AuMHA, AuMHA/HDT type A, B, C, D and $AuZwMe_2$ in water; excitation (λ_{exc} 400nm).



Sample	B_1	τ_1 (ns)	B_2	τ_2 (ns)	B_3	τ_3 (ns)	B_4	τ_4 (ns)	**<τ> int. (ns)**
AuMHA			0.08	31.5	0.05	165.2	0.03	563.8	**403.9**
AuMHA_HDT_A			0.06	53.6	0.08	331.8	0.02	876.3	**525.2**
AuMHA_HDT_B	2.80	2.6	0.14	79.9	0.19	385.0	0.029	1321.6	**618.1**

Fig. S14. Fluorescence lifetime measurements of AuMHA, AuMHA/HDT type A and type B in water; excitation ($\lambda_{exc.}$) was at 510 nm, the emission ($\lambda_{em.}$) was detected at 930±30nm.

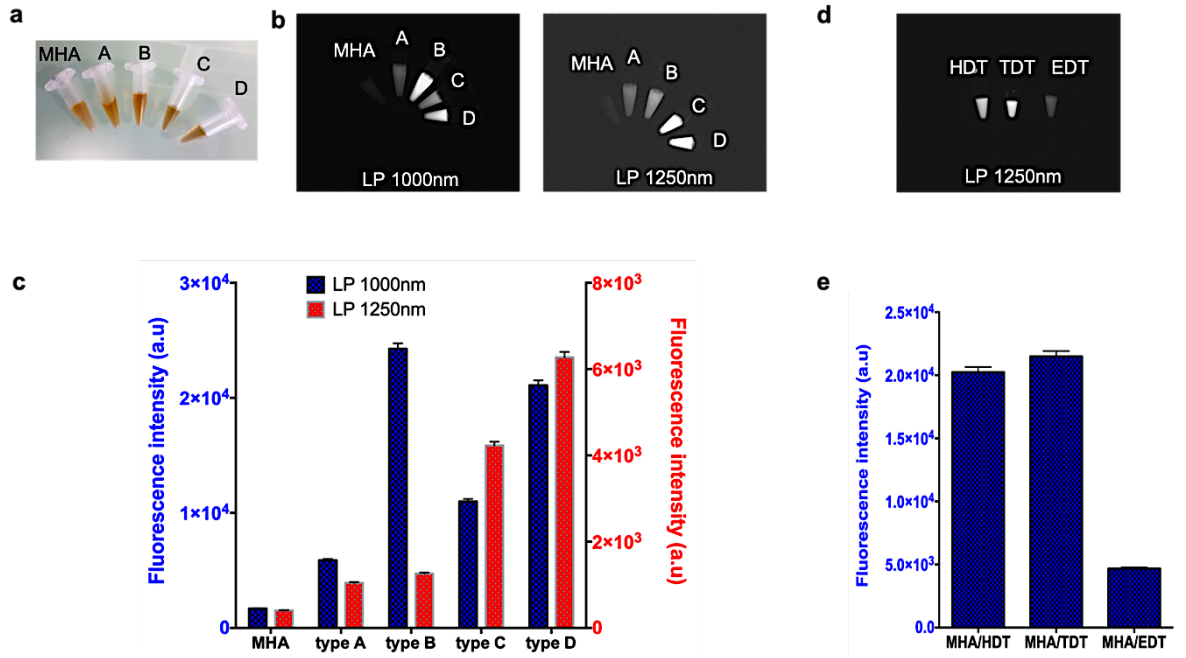


Fig. S15. AuMHA, AuMHA/HDT type A, B, C, D sols under visible (a) and SWIR cameras using long-pass filters LP1000 nm and LP1250 nm ($\lambda_{exc.}$ 830nm) (b,c). (d,e) Type D of AuMHA/HDT, AuMHA/TDT, AuMHA/EDT sols under SWIR camera using long-pass filter LP1250 nm ($\lambda_{exc.}$ 830nm).

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