Four Orders-of-Magnitude Enhancement in Two-Photon Excited

Photoluminescence of Homoleptic Gold Thiolate Nanoclusters following

Zinc-Ion-Induced Aggregation

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supplementary information

EXPERIMENTAL DETAILS

Synthesis of Au10MPA10 and their zinc-induced assembly.

1 mL of HAuCl₄ of 10mM is added to 10 mL of Millipore water under stirring condition, then 0.5mL of 134mM (3-mercapto-propanoic acid from Alfa Aesar) is added to the solution. After two minutes the dispersion is centrifuged at 6000rpm for 10 min to obtain Au₁₀MPA₁₀ NCs. The resulting precipitate was collected and again re dispersed in 5 mL of Millipore water with the adding of 20 μ L of NaOH of 1M. Based on gold atom, the yield of synthesis was estimated to ~80%. For Zn stabilized Au NCs Assemblies, the above solution of Au NCs (before centrifugation), 100 mg of zinc acetate dihydrate was added. The reaction was continuously stirred for 2hrs. Then the reaction mixture was centrifuged at 12000 rpm for 10mins at 22°C. The resulting precipitate was collected and again re dispersed in 5 mL of Millipore water. The resultant solution was centrifuged at 12000 rpm and the precipitate is collected and re dispersed in 5 mL of Millipore water.

Of note, both the samples were dispersible in Millipore water when fresh. All experiments (mass spectrometry, optical and nonlinear optical measurements) were done on fresh solutions. However, on one-week old sample, upon dispersion in water, yielded a milky consistency which could be due to degradation.

Mass measurements. For AuMPA nanoclusters, mass analysis was determined by electrospray ionization (ESI) on a commercial quadrupole time-of-flight (micro-qTOF, Bruker-Daltonics, Bremen, Germany, mass resolution ~10 000). For self-assembly of AuMPA nanoclusters, mass analysis was performed on a custom-built charge detection mass spectrometer with an electrospray ionisation (ESI) source. The injected samples and entered the electrospray chamber through a 0.1 mm internal diameter stainless steel capillary tube located inside the needle tip. Dry Nitrogen gas was injected between the end cap and the transfer glass capillary and passed through a heater typically set at 200 °C. The vacuum interface was composed of a glass transfer capillary that passes the ions into the first stage of the vacuum system, an end cap, a skimmer

between the first and second vacuum stages, an hexapole ion guide and an exit lens. The charge detection device was used in a single pass mode. The signal induced on the tube was picked up by a JFET transistor and was amplified by a low-noise, charge-sensitive preamplifier and then shaped and differentiated by a home-built amplifier. The signal was recorded with a waveform digitizer card that recorded the entire waveform for each ion passing through the detector tube at a sampling rate of 10 MHz. The data were transferred to a desk-top computer where they were analyzed to compute the charge and mass of each ion. Internal calibration in charge was performed using a test capacitor that allowed a known amount of charge to be pulsed onto the pick-up tube.

Sizing nanoparticles by Fluorescence time-resolved fluorescence anisotropy. The anisotropy of fluorescence is an important tool to measure the size of fluorescence materials, transition dipole moment is the main parameter in this technic. The photon will be absorbed by the coupling with the dipole transition dipole moment of the fluorophore. The absorption is more efficient when the light and the transition dipole moment are parallel. Since the transition dipole of the fluorophore is also responsible for the emission, the emitted light will have the same direction of the excitation light. In this case we could have the same orientation if the fluorophore is motionless during excitation, but in must materials and due to the loss of the energy, the fluorophore will move during the life time of its excited state (rotational Brownian motion) and the emitted light polarization will be different. To quantify this loss of polarization, the fluorescence anisotropy r(t) is introduced, and, in the time-resolved experiment, is defined as

$$r(t) = \frac{I_{VV}(t) - GI_{VH}(t)}{I_{VV}(t) + 2GI_{VH}(t)}$$

where I_{VV} corresponds to the fluorescence intensity of vertically polarized excitation and vertically polarized emission, I_{VH} to the vertically polarized and horizontally polarized emission and *G* to the correction factor, re-evaluated for each fluorescence anisotropy measurement, with

$$G = \frac{I_{HV}(t)}{I_{HH}(t)}$$

where I_{HV} corresponds to the fluorescence intensity of horizontally polarized excitation and vertically polarized emission and I_{HH} to the horizontally polarized excitation and horizontally polarized emission. Anisotropy decays were analyzed with a mono-exponential fitting model using the following equation:

$$r(t) = r_0 e^{\frac{-t}{\theta}}$$

where r_0 is the anisotropy at time t = 0 and θ is the rotational correlation time defined by the Stokes-Einstein relation:

$$\theta = \frac{\eta V}{kT}$$

where η is the viscosity of the solution, k the Boltzmann constant, T the temperature and V the volume of the rotating object where here we considerate that the NC's have a spherical geometry so

$$V = \frac{4}{3}\pi R^3$$

where R is the radius of the sphere and so the size is equal to the diameter 2R.

Gold BSA (Bovine Serum Albumin) with the size of $9,4 \pm 0.9$ nm was used as calibrant (mixture of water/Glycerol (40:60%, v/v), η =11 mPa.s at 25 °C). Pure water was used for gold assemblies. Fluorescence anisotropy decay curves were obtained by averaging 4 replicates, where every replicates corresponded to I_{VV} I_{VH} I_{HV} I_{HH}.

A picosecond laser (PGx03 series Optical Parametric Generators (OPG) from EKSPLA) pumped by a 1kHz mode-locked laser was used for excitation, the optical design was optimized to produce low divergence at a pulse duration of approximation 35ps, the excitation wavelength used is 500nm. The fluorescence decay signal was preamplified by a fast preamplifier 350 MHz Preamplifier SR445A, recorded as a function of time with a fast digital oscilloscope (Lecroy LT322). For time resolved fluorescence anisotropy measurement, a polarizing beam splitter cube and a half-wave plate were used to tune the vertical or horizontal linear polarity of the light on the excitation line. A linear broadband in the range 500-720 nm (for the 500 nm excitation wavelength). To study the statistic of the system, we made 8 replicates for each measurement. Fluorescence anisotropy decay curves for the 8 replicates are given in the figure A. The samples were tested directly after the synthesis. Standard deviations on size determined with the 8 decay curves were used to estimate the error (e.g. ± 1.2 nm).



Figure A : Fluorescence anisotropy decay curves for the 8 replicates.

Optics. UV–vis spectra in solution were recorded using an AvaSpec-2048 fiber optic spectrometer and an AvaLight-DH-S deuterium-halogen light source. The luminescence (emission and excitation) spectra of all samples were acquired using a Horiba Jobin Yvon Fluoromax-4 spectrofluorometer.

Two-photon fluorescence measurements were performed with a customized confocal microscope (TE2000-U, Nikon Inc.) in which the excitation light entrance has been modified

to allow free-space laser beam input, instead of the original optical-fiber light input. The luminescence was excited at 780 nm with a mode-locked frequency-doubled femtosecond Er-doped fiber laser (C-Fiber 780, MenloSystems GmbH). The output power of the femtosecond laser was 62 mW. The laser spectrum was bounded by two filters (FELH0750 and FESH0800, Thorlabs Inc.). The laser beam was focused by a Nikon CFI Plan Apo VC objective (20x/0.75 NA) on a sample containing NCs (see Figure B). The emitted signal was collected in epifluorescence illumination mode at 10-50 µm from bottom of the sample holder. The two-photon fluorescence emission was separated from the incident light through a dichroic mirror (NFD01-785, IDEX Health & Science LLC) and a FESH0750 filter. The two-photon fluorescence emission spectra were recorded using an iHR320 spectrometer equipped with a 51013 grating from Horiba Jobin Yvon, and detected by a -20°C cooled photomultiplier tube (R943-02, Hamamatsu Photonics).



Figure B : cuvette containing nanomaterials in water used for biphotonic confocal measurements.



Figure S1 : Fluorescence anisotropy decay of zinc mediated assembly of homoleptic gold nanoclusters in water. Monoexponential fitting model curve is shown in red.



Figure S2: Representative TEM images of Zn Au NCs acquired on TEM grids and analyzed by a Jeol 2100 TEM machine (operated at a maximum voltage of 200 kV).



Figure S3: (a) absorption spectra of nanoclusters and their zinc-induced assembly. (b) one-photon (vs two-photon) excited emission of zinc-induced aggregates excited at 390 nm (vs 780 nm).

We attempted to measure QY for AuNCs assemblies with the reference. The quantum yield is determined using the comparative method, which relies on the use of fluorescence standards with known fluorescence quantum yields . We use the equation:

QYx=QYs* [As/Ax]*[Fx/Fs]*[nx/ns]²

where Ax and As are the absorbance at excitation wavelength of the sample x and the standard s. Fx and Fs are integrated intensity of emission of the sample x and the standard s. The n's are the refractive indices with nx=1.33 for water and ns=1.36 for ethanol. Rhodamine B in ethanol as reference dye. We found a QY of 7 ± 5 % for AuNC assemblies.



Figure S4: Excitation spectra of zinc-added Au NCs. The wavelength of emission was set at 500 nm.

Concentration

Concentration of AuMPA NCs (Au₁₀MPA₁₀). 6.2 mg of AuMPA NCs were diluted in 11.5 mL giving a concentration of 0.18 mM, using a molar mass of Au₁₀MPA₁₀ of 3040 g/mol.

Concentration of zinc mediated assembly of AuMPA NCs. Using the average mass of assemblies found by CDMS measurements (e.g. \sim 300 MDa), and assuming that every carboxylic group of AuMPA NCs are bound to one zinc ion, the total number of nanoclusters in one zinc mediated assembly will be MW(assembly)/MW(NCs)= 300 \cdot 10⁶/3690= 81300. An initial concentration of NCs of 0.18 mM will correspond to a total number of nanoclusters of 1.08 \cdot 10²⁰ NC per liter. For one zinc mediated assembly, there are 81300 NC. Then, it gives a final concentration of 2.21 nM for zinc mediated assembly of AuMPA NCs.

TPPL cross section

Two photon excited photoluminescence is an important optical characterization for bio-imaging domain. To study TPPL cross section, one needs to referee to a common fluorophore with a known TPPL cross section. Fluorescein is used to calculate TPPL cross section of the NC's and their assemblies and so TPPL cross section is calculated using this equation :

$$\sigma_{TPPL} = \frac{\eta^{ref} \sigma_2^{ref} c^{ref}}{c} \frac{l}{l_{ref}}$$
(S1)

where η is the quantum yield of fluorescein is 0.9, σ_2^{ref} is the fluorescein cross section 37 GM (see https://doi.org/10.1364/JOSAB.13.000481), *c* is the concentration in particles per liter and *I* is the TPPL intensity.

We used a concentration of 10 μ M for the fluorescein and 1.8 mM for AuMPA NCs. For assemblies, we used 2.21 nM. TPPL areas for fluorescein, AuMPA Ncs and their zinc-induced assemblies were determined with TPPL spectra displayed in Fig. S3.

 $C_{\text{fluorescein}} = 6.02 \cdot 10^{18} \text{ particles/L and Area} = 19189 \text{ a.u.}$

 $C_{NCs} = 1.08 \cdot 10^{21}$ particles/L and Area = 39,59 a.u.

 $C_{assemblies} = 1.33 \cdot 10^{15}$ particles/L and Area = 73976 a.u.

Then, using Equation (S1):

TPPL Cross section for zinc mediated assembly = $5.8 \ 10^5 \ \text{GM}$

TPPL Cross section for AuMPA NCs = $3.8 \cdot 10^{-4}$ GM



Figure S5 : Two-photon excited emission spectra of AuMPA Ncs and zinc-induced aggregates excited at 780 nm and compared with fluorescein TPPL spectrum.