Electronic Supplementary Information

Impact of ligand-protected gold nanoclusters on liposomal morphology: fission to semi-gel formation in aqueous medium

Mallika Mukherjee,[a] Asmita Das [b] and Pradipta Purkayastha*[a]

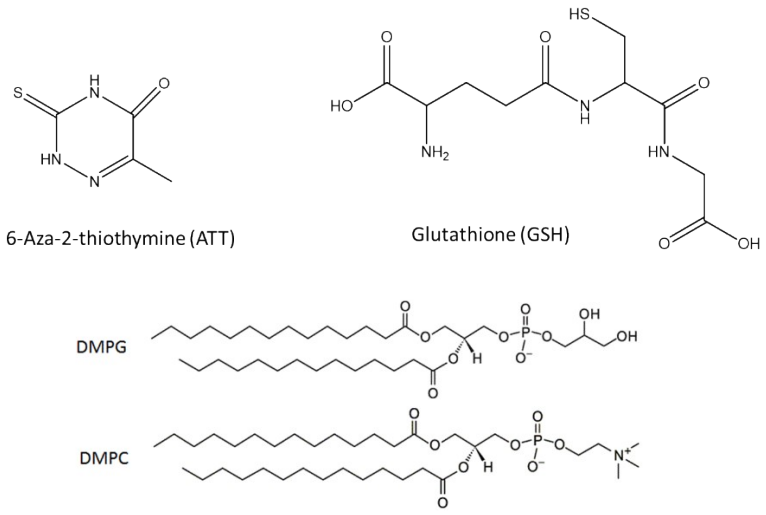
[a]Department of Chemical Sciences and Centre for Advanced Functional Materials, Indian Institute of Science Education and Research (IISER) Kolkata, Mohanpur 741246, WB, India. Email: ppurkayastha@iiserkol.ac.in

[b]Department of Chemistry, Rabindranath Tagore University, Hojai, Assam 782436, India.

Experimental section:

Materials

Gold(III) chloride hydrate, silver nitrate, methanol, sodium hydroxide, formic acid, 6-aza-2-thiothymine (ATT), L-glutathione (GSH) and cholesterol were purchased from Sigma Aldrich. Chloroform was purchased from Spectrochem, Pvt. Ltd., India. DMPC was purchased from Avanti Polar Lipids, Inc. DMPG was purchased from TCI. Throughout the experiment we used ultrapure water and spectroscopy grade solvents from FINAR. All the experiments were done at 25 °C. The chemical structures of the ligands and the lipids are given in Scheme S1.



Scheme S1: Representative structures of the ligands and the lipids used in the present work.

Methods

Steady state fluorescence and absorption spectroscopy

The UV-vis absorption spectra were collected on a U-2900 UV−visible double-beam spectrophotometer from Hitachi. A QM-40 spectrofluorometer from PTI, Inc. was used to collect all the steady-state fluorescence spectra at room temperature. The excitation and emission slits were kept at 2 nm throughout all the experiments.

Time-resolved emission decays

Time-resolved fluorescence measurements have been conducted using a DeltaFlex TCSPC/MCS fluorescence lifetime system, equipped with a picosecond photon counting detector capable of measuring fluorescence lifetimes ranging from picoseconds to seconds. Data analysis was done using the HORIBA EzTime software. A diode laser emitting at 459 nm has been utilized as the excitation source. The average fluorescence lifetime values, $<\tau>$, were calculated using the equation: $<\tau> = \Sigma_i A_i\tau_i^2 / \Sigma_i A_i\tau_i$, where, τ_i is the excited-state lifetime component of the decay curve and A_i is the corresponding amplitude of that component.

Dynamic light scattering (DLS) measurement

The dynamic light scattering (DLS) data were obtained on a Malvern Zetasizer Nano equipped with a 4.0 mW HeNe laser operating at λ = 633 nm. All the samples were measured in an aqueous system at room temperature with a scattering angle of 173°. Nano software using non-negative least-squares analysis (NNLS) calculated the size distribution.

Atomic force microscopy (AFM)

Atomic force microscopy (AFM) was performed using a Di INNOVA AFM machine. A silicon cantilever (RTESPA-300, BRUKER) was used to measure the samples. All the data were collected in tapping mode to avoid damage to the sample surface.

Zeta potential measurement

A Nano Particle Analyzer SZ-100 from Horiba Scientific was used for the zeta-potential measurements aided with a 633 nm He–Ne laser beam.

X-ray photoelectron spectroscopy (XPS)

The XPS experiments were performed on an X-ray photoelectron spectrometer (PHI 5000 versa probe III) working at ultrahigh vacuum (1×10^{-9} bar) and equipped with an AlK α X-ray source and a monochromator. The X-ray beam size was 100 μm. The survey spectra were recorded with pass energy (PE) 280 eV, and the high-energy resolution spectra were recorded with pass energy (PE) 55 eV. An aqueous solution of NCs was drop-casted on a silicon wafer, dried, and subjected to XPS analysis.

Calculation of the binding constants

The respective binding constants (K_b) were calculated from the collected emission spectra due to the interactions of the ATT-AuNCs with the lipid vesicles using the Benesi-Hildebrand equation[3] and using the double-reciprocal plot.

$$\frac{1}{I - I_0} = \frac{1}{I_m - I_0} + \frac{1}{(I_m - I_0)K_b[Q]}$$

where, I_0, I_m and I are the PL intensities without, at the maximum and at varying concentrations of the quencher Q.

Synthesis of green-emitting ATT-AuNCs

The ATT-protected green-emitting AuNCs were synthesized following a previously reported method.[1] An aqueous solution of $HAuCl_4.3H_2O$ (1 mL, 20 mM) was added to the ATT (63 mM) solution containing 0.2 M NaOH under vigorous stirring in the dark at room temperature for 6 h. The pH of the solution was maintained at 10 throughout the reaction. The resulting ATT-AuNC solution was centrifuged at 13000 rpm for 30 min. The ATT-AuNCs were stored at low temperature before further experiments and characterization.

Synthesis of large unilamellar vesicles (LUVs)

The liposomes were synthesized following a previously reported method.[2] DMPC and DMPG lipids were chosen for the preparation of lipid vesicles. Stock solutions were prepared by dissolving DMPG, DMPG and cholesterol (Chol) at a molar ratio 2:1:1 in chloroform. The required amounts of lipids were solubilized in 4:1 (v/v) chloroform-methanol mixture. The solvent was evaporated using a rotary evaporator, and the resultant lipid film was kept overnight in vacuum to ensure complete evaporation of the solvent. Tris-HCl buffer (2 mL) at pH 7.4 was added into the dried film to have 1.64 mM lipid solution. The dispersion was briefly vortexed. A clear solution of LUVs (20–30 nm) was obtained on sonication for 45 min at 35 °C in a bath sonicator. Fresh lipid vesicles were used for each experiment.

Characterization of ATT-coated AuNCs

The green emitting ATT-AuNCs were characterized by absorption (Fig. S1a), excitation (Fig. S1b), steady state (Fig. S1c) and time-resolved fluorescence spectroscopy (Fig. S1d). The absorption spectrum of the ATT-AuNCs showed bands at 400 nm and 470 nm, which match well with the maxima in the excitation spectrum. The emission maximum of the ATT-AuNCs, obtained at 520 nm, is independent of excitation wavelength (Fig. S1c). The fluorescence

quantum yield of the ATT-AuNCs in water was calculated to be 1.45% with respect to that of Rhodamine 6G (95% in ethanol). The average lifetime (<τ>) of the ATT-AuNCs was found to be 23.15 ns. The sufficiently photostable AuNCs (Fig. S1e) showed bright fluorescence in solution on excitation (Fig. S1f). The particle size (0.62 nm hydrodynamic diameter) was measured by DLS (Fig. S1g) and the dispersity was monitored through zeta potential distribution (-28.9 mV at pH 6.8) (Fig. S1h). Constitution of the AuNCs was verified using XPS (Fig. S1i), which revealed that the valence states of the Au and S atoms in the ATT-AuNCs. The binding energies of Au $4f^{7/2}$ and $4f^{5/2}$ are at 86 eV and 88 eV, which indicate the presence of mixed valence states of Au(I) and Au(0) in the NCs. Furthermore, XPS spectrum of S 2p reveals the binding energies of S $2p^{3/2}$ are 160 eV and 165 eV, where the dominating peak at 162 eV is attributed to the Au–S bond in the NCs. The other smaller peak may be due to the unbound S present on the surface.

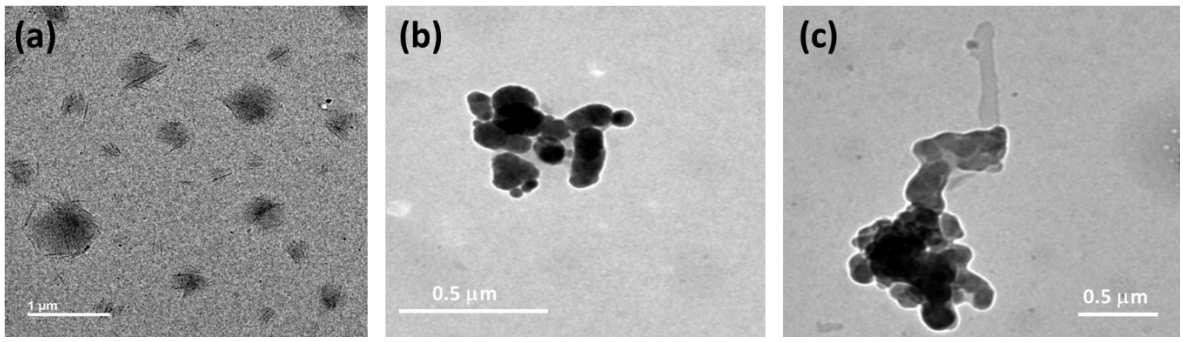


Fig. S1 Action of ATT (63 mM) on (a) DMPG LUVs, which are partly converted to fibrils, (b) DMPC LUVs and (c) DMPC/DMPG/Chol(2:1:1) LUVs converted to rod shaped morphology by liposomal deformation. The liposomes did not show progressive fission.

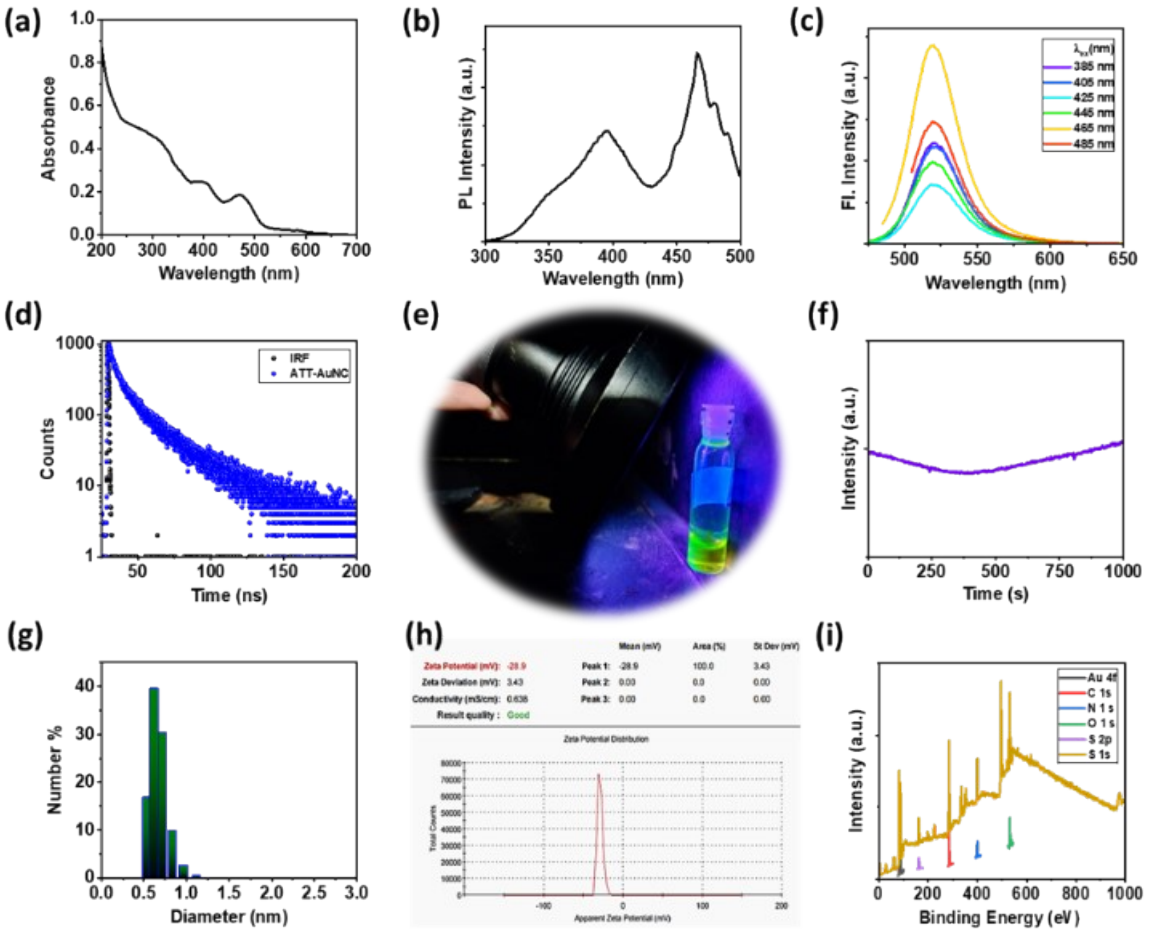


Fig. S2 (a) Absorption, (b) excitation and (c) emission spectra of the ATT-AuNCs along with their (d) fluorescence decay, (e) a photograph, (f) photostability, (g) DLS, (h) zeta potential measurements and (i) XPS spectra.

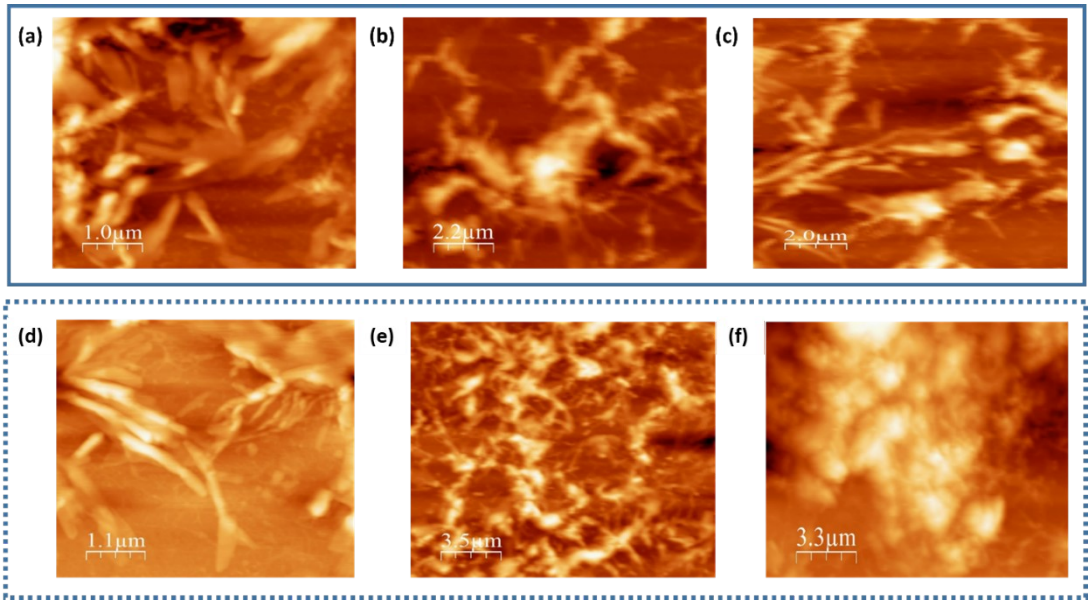


Fig. S3 AFM images of (a-c) DMPC lipid fibrils (d-f) DMPG semi-gel formation due to the interaction of the respective LUVs with the ATT-AuNCs.

Table S1. Time-resolved emission decay of the ATT-AuNCs, monitored at the 520 nm, upon binding with the DMPC LUVs. Values in parentheses are the percentage contribution to the decay component and the χ^2 values indicate the goodness of fit to the raw data. All the data are within an acceptable ±5 % error limit.

[DMPC] (μM)	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	τ_{av} (ns)	χ^2
0	6.33 (36)	22.75 (51)	1.63 (13)	19.74	1.01
5	6.31 (38)	21.95 (52)	1.70 (10)	19.00	1.15
10	5.58 (40)	22.55 (50)	1.90 (10)	19.86	1.09
15	5.49 (30)	23.51 (49)	1.85 (21)	20.70	1.14
25	6.20 (41)	27.78 (41)	1.10 (18)	23.56	1.11
35	6.66 (39)	28.85 (42)	1.23 (19)	24.25	1.10

Table S2. Time-resolved emission decay of the ATT-AuNCs, monitored at the 520 nm, upon binding with the DMPG LUVs. Values in parentheses are the percentage contribution to the decay component and the χ^2 values indicate the goodness of fit to the raw data. All the data are within an acceptable ±5 % error limit.

[DMPG] (μM)	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	τ_{av} (ns)	χ^2
0	6.33 (36)	22.75 (51)	1.63 (13)	19.74	1.01
5	6.33 (42)	23.55 (44)	1.33 (14)	19.77	1.05
10	5.47 (40)	21.75 (47)	1.33 (13)	19.76	1.06
15	7.28 (40)	28.31 (39)	1.73 (21)	23.52	1.09
25	6.18 (41)	28.40 (40)	1.49 (19)	23.95	1.12
35	6.30 (39)	29.25 (42)	1.33 (19)	25.06	1.10

Table S3. Time-resolved emission decay of the ATT-AuNCs, monitored at the 520 nm, upon binding with the DMPC/DMPG/Chol (2:1:1) LUVs. Values in parentheses are the percentage contribution to the decay component and the χ^2 values indicate the goodness of fit to the raw data. All the data are within an acceptable ±5 % error limit.

[DMPC/DMPG/ Chol] (μM)	τ_1 (ns)	τ_2 (ns)	τ_3 (ns)	τ_{av} (ns)	χ^2
0	6.33 (36)	22.75 (51)	1.63 (13)	19.74	1.01
5	6.30 (42)	24.85 (44)	1.38 (14)	20.95	1.05
10	5.58 (40)	23.75 (47)	1.35 (13)	20.47	1.09
15	5.28 (40)	28.51 (39)	1.29 (21)	24.25	1.06
25	6.20 (40)	28.68 (41)	1.25 (19)	24.35	1.15
35	7.26 (39)	30.35 (42)	1.23 (19)	25.83	1.01

References

1 Y. Tian, W. Zheng, X. Zhang, Y. Wang, Y. Xiao, D. Yao and H. Zhang, *Nano Lett.*, 2023, **23**, 4423-4430.

2 D. Mukherjee, D. Paul, S. Sarker, Md. N. Hasan, R. Ghosh, S. E. Prasad, P. K. Vemula, R. Das, A. Adhikary, S. K. Pal and T. Rakshit, *ACS Appl. Bio Mater.*, 2021, **4**, 8259-8266.

3 S. Das and P. Purkayastha, *ACS Omega*, 2017, **2**, 5036-5043.