Supplementary Information

Bifunctional cysteine gold nanocluster for β-amyloid fibril inhibition and fluorescence imaging: A distinctive approach to manage Alzheimer's disease

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S1. Materials and Methods

S1.1. Materials

Hydrogen tetrachloroaurate (III) trihydrate (99.9%), L-dopa, MTT, Thioflavin-T, Hoechst33342, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-Hydroxysuccinimide (NHS) were purchased from Sigma and L-Cysteine hydrochloride (98%) was purchasedfrom Alfa Aesar. bEnd.3 (cerebral endothelial cells), C8-D30 (Astrocyte type III clone) and Neuro-2a cells (N2A) were purchased from ATCC. DMEM, Fetal Bovine Serum (FBS),antibiotic-antimycotic solution, and cell culture insets were purchased from Himedia. $A\beta_{1-42}$ peptide was purchased from Abcam. All the glassware was cleaned using aqua regia (1:3 ratios of hydrochloric acid (HCl) and nitric acid (HNO_3)). The water used in all experiments was Millipore Milli Q grade (18 $M\Omega^{-cm}$).

S1.2. Measurements

UV−Vis absorption measurements were carried out by Shimadzu (UV-1800) UV-Vis spectrophotometer. Fluoro Max-spectrofluorometer (Horiba Instruments) was used for the Fluorescence measurements. Fourier transform infrared (FT-IR) spectra were measured using Agilent Cary 660 FTIR spectrometer. High-resolution transmission electron microscopy (HRTEM) images were collected using a JEOL-JEM0310 microscope with an accelerating voltage of 80 kV to characterize the size and size distribution. A diluted solution was spotted on the carbon-coated copper grid (300 mesh) and was dried at room temperature. Energy-

dispersive X-ray spectroscopy (EDAX) analyses were done with the same microscope. Zeta potential measurements were performed by nano ZS (Malvern, UK). The X-ray photoelectron spectroscopic data were recorded on an Omicron Nanotechnology X-ray photoelectron spectrometer (XPS) with an Al Kα = 280.00 eV excitation source. The binding energies of the core levels were calibrated with C 1s binding energy (BE) set at 284.0 eV. The quantum yield (QY) of fluorescence of Cy-AuNCs was measured using Rhodamine 6G as a standard reference (QY: 95% in ethanol). Fluorescence lifetime measurements were performed using a Fluoro Max-4C Spectrofluorometer (Horiba Instruments, USA) and the time-correlated single-photon counting (TCSPC) technique with an excitation wavelength of 344 nm. *In-vitro* fluorescence experiments were performed using an inverted fluorescence microscope (IX83; Olympus Corp., Tokyo, Japan) with a cooled CCD camera (XM10, monochrome, Olympus). *In-vivo* and the *ex-vivo* animal imaging was performed with a live animal optical imaging system (Xenogen, IVIS Spectrum).

S1.3. *In-vitro* cytotoxicity study

The MTT assay is used to quantify the percentage of viable cells. The MTT assay relies on the ability of metabolically active cells to convert the yellow, water-soluble tetrazolium salt (MTT) to purple, formazan crystals using the mitochondrial enzyme succinate dehydrogenase.[1,2] The intensity of the purple colour so formed is proportional to the number of viable cells. L929, (mouse fibroblast cells) were seeded in a 96-well tissue culture plate at 5 x10^3 cells/well in 100 µL DMEM with 10 % FBS and 1% antibiotic-antimycotic solution for determining the cell viability. After attaining 40-50% confluency, cells were treated with various concentrations of Cy-AuNCs and AuCs-LD (10, 20, 40, 80, 160, 320 µg/mL). After 24 h, the medium was removed and washed with phosphate-buffered solution, and then 100 µL MTT (5 mg/ml) was added to each well. After 3 hours of incubation at 37 ^oC, 100 µL DMSO was added to each well and was allowed to incubate at room temperature for 30 minutes. The absorbance of the resulting solution was recorded at 570 nm using an automated microplate reader. Cells treated with MTT solution without materials were used as control. The percentage viability was calculated as follows.

% Cell Viability = ([Abs] sample/ [Abs] Control) x 100 where Abs is the absorbance.

S1.4. *In-vitro* cellular uptake

Cell uptake study of both Cy-AuNCs and AuCs-LD were evaluated using bEnd.3 cells. 1 X 10^5 cells were seeded on a coverslip. When the cells formed a complete monolayer, 40µg/mL of Cy-AuNCs and AuCs-LD were added, in separate wells. After 3 h, cells were washed with PBS.

For fluorescence microscopic study, the nucleus was stained with Hoechst33342 as per the manufacturer's instructions. Then, it was washed with PBS and viewed under a fluorescence microscope using the filters DAPI and Hc RED. In all the cases, cells without material were used as control.

S1.5. Spontaneous alternation test

A Y-maze made of wood with dimensions 35cm x 5cm (walls 10cm high) was used for this purpose. Mice were placed in one arm and allowed to explore the arms.

For the spontaneous alternation test, mice were taken and dropped in arm and its movement across all the arms were recorded using a digital camera for 5 min. An entry into an arm is indicated only when all 4 limbs are into that arm. An alternation is defined as consecutive entries into all three arms. Percentage of alternation can be calculated using the formula: -

% Alternation = (Number of alternations) / (Total number of arm entries – 2) x 100.[3]

S1.6 Novel arm test

For the novel arm test, one of the arms of the maze was blocked using a removable door and the mice were dropped onto one side and allowed to move around. The movement was recorded using a digital camera for 15 min. Similar procedure was performed with the open door and the movement was recorded for 5 min. The number of entries into the novel arm is then compared to the entries into the other arms to assess the degree of spatial memory[3]. The difference in behavioural pattern was observed before and after ICV injection and nanocluster injection.

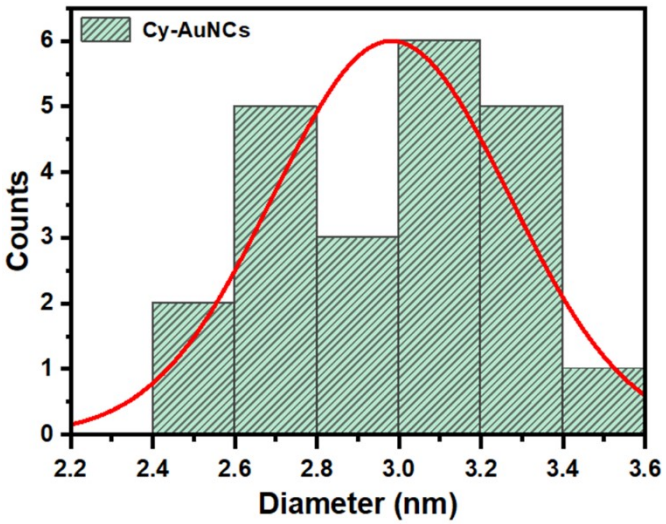

Fig. S1 The particle size distribution of Cy-AuNCs (average size distribution is ~3 nm)

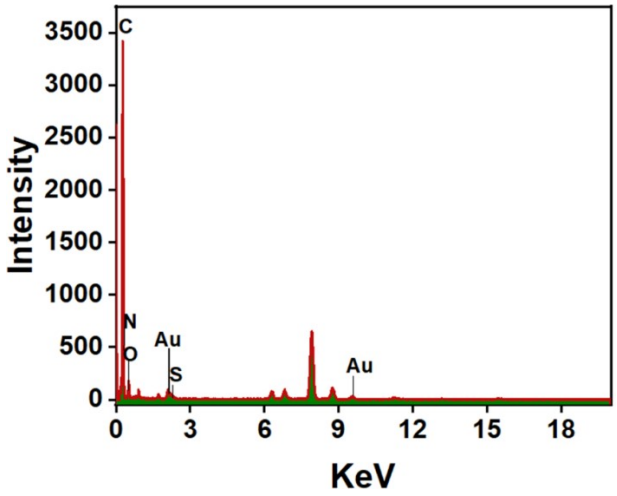

Fig. S2 EDAX spectrum of Cy-AuNCs

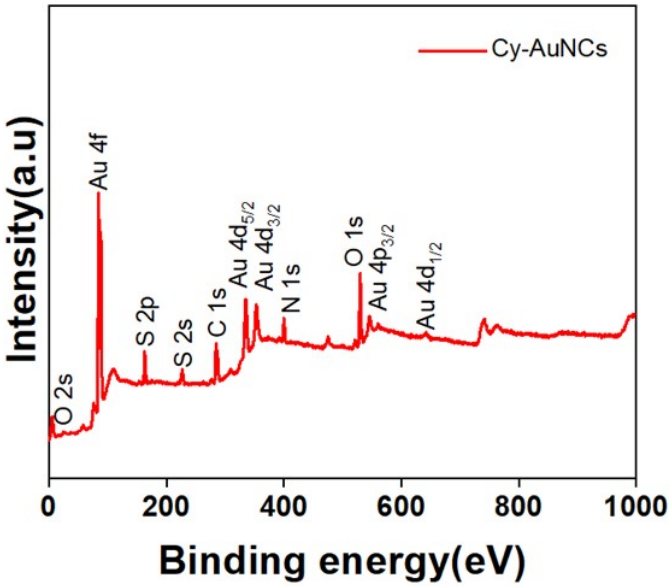

Fig. S3 XPS survey analysis of Cy-AuNCs

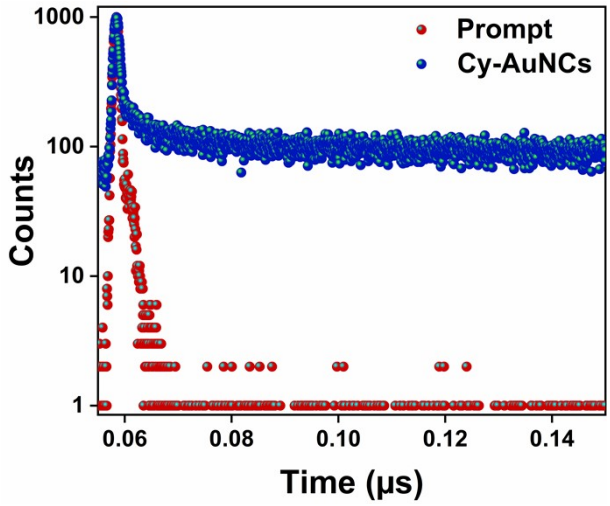

Fig. S4 Fluorescence lifetime measurement of Cy-AuNCs

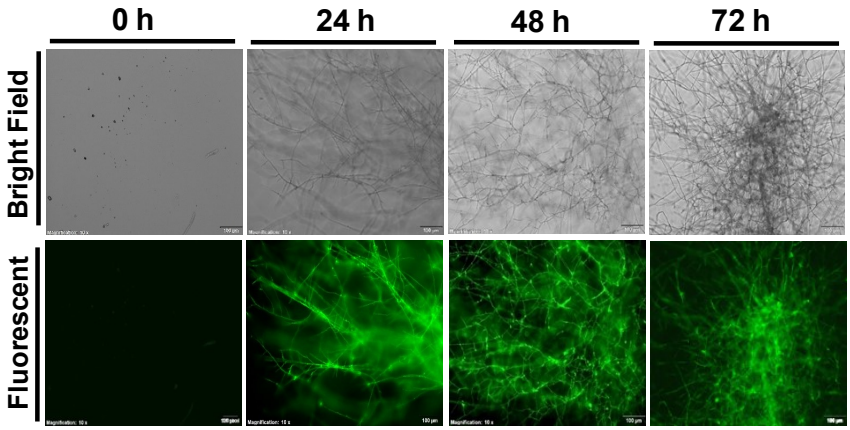

Fig. S5 Thioflavin T-stained fluorescence images of $A\beta_{1-42}$ samples at different time points. Scale bar is 100 µm in all cases

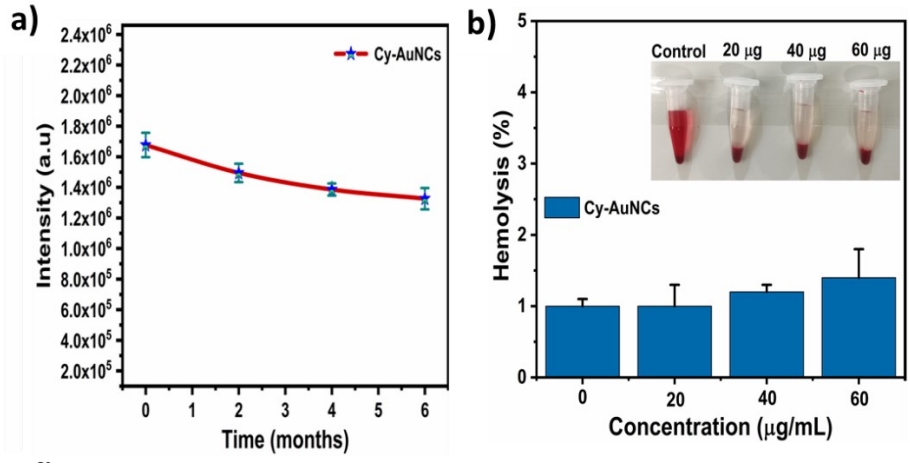

Fig. S6 Stability and biocompatibility of Cy-AuNCs. a) Fluorescence stability b) Hemocompatibility analysis of Cy-AuNCs

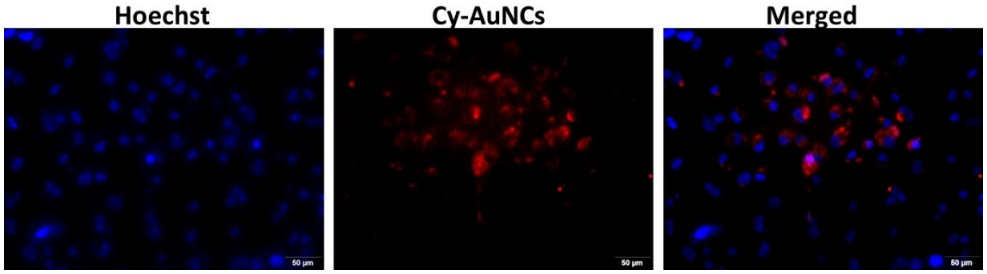

Fig. S7 Cellular uptake of Cy-AuNCs in N2A cells. Scale bar is 50 µm in all cases

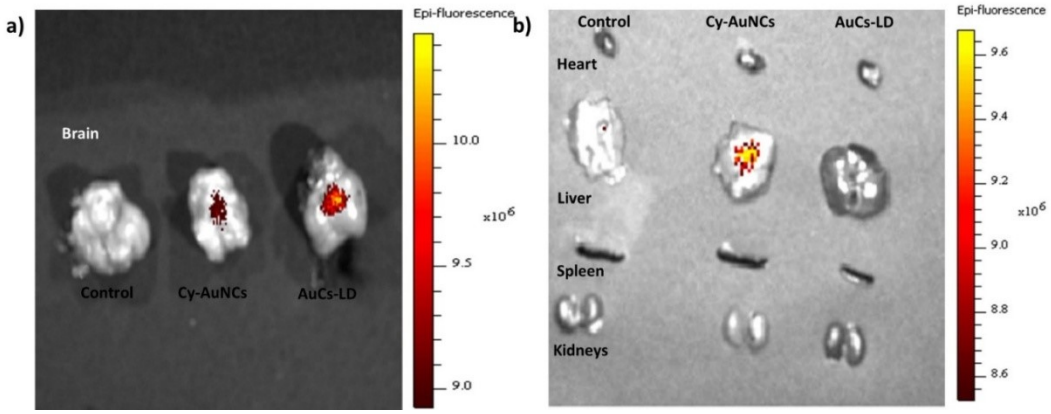

Fig.S8 Ex-vivo fluorescence imaging of a) brains and b) different organs of mice treated with Cy-AuNCs and AuCs-LD

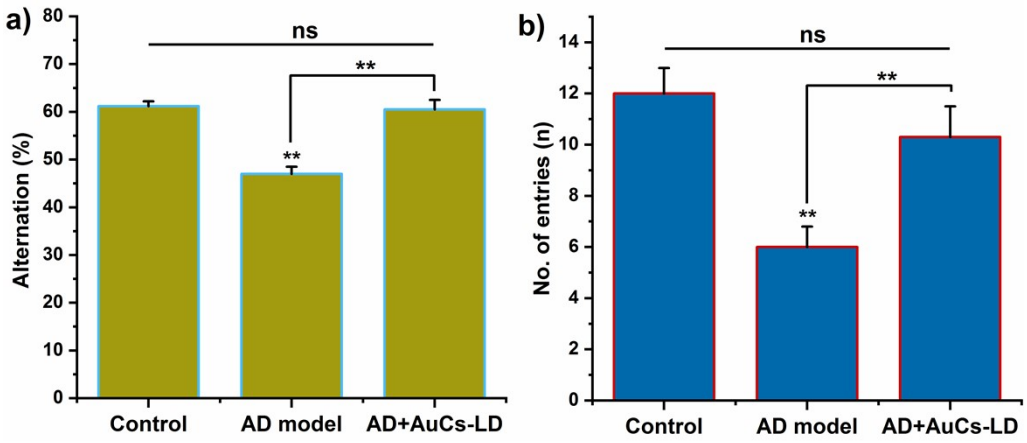

Fig. S9 Behavioural analysis of AD animal models and AuCs-LD treated animal models. a) Spontaneous alteration test and b) Novel arm test. Error bar indicates the SD of three indipendent experiment. ** $p<0.05$, ns- non significant

Table S1: Quantitative XPS results of **Cy-AuNCs** sample

	C 1s	**N 1s**	**O 1s**	**S 2p**	**Au 4f**
Cy-AuNCs (At%)	44.22	9.30	18.66	13.66	14.17

Table S2: Quantum yield measurement of **Cy-AuNCs**

Sample	Refractive index(η)	Quantum yield(Q)(%)
Rhodamine 6G	1.36 (ethanol)	95
Cy-AuNCs	1.33 (Water)	7.2

The quantum yield was calculated based on the following equation:

$$Q_x = Q_R \times I_x / I_R \times A_R / A_x \times \eta^2{}_x / \eta^2{}_R$$

where Q is quantum yield, I is integrated PL intensity of the sample, A is the absorbance intensity, η is the refractive index for the solvent, X means as-prepared **Cy-AuNCs**, and R refers to Rhodamine 6G as reference fluorophore.

Table S3: ICP-OES analysis

Label	Solution Concentration	Unit	SD	%RSD	Intensity	Calculated Concentration
Au (242.794 nm)	0.02	ppm	0.02	0.92	187.05	2.07 (ppm)

Reference

1 M. Ghasemi, T. Turnbull, S. Sebastian and I. Kempson, *International Journal of Molecular Sciences*, 2021, **22**, 12827

2 Y. Rai, R. Pathak, N. Kumari, D. K. Sah, S. Pandey, N. Kalra, R. Soni, B. S. Dwarakanath and A. N. Bhatt, *Scientific Reports*, 2018,**24**,1531

3 A. K. Kraeuter, P. C. Guest and Z. Sarnyai, *Methods in molecular biology (Clifton, N.J.)*, 2019, **1916**, 105–111.