Electronic Supplementary Information

A three-component supramolecular nanocomposite as a heavy-atom-free photosensitizer

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Experimental Section

General Techniques: All experiments were carried out at room temperature (25 ± 1 ºC) unless otherwise mentioned. NMR spectra were measured on a 400 MHz Bruker Avance II 400 or 400 MHz JEOL JNM ECS400 NMR spectrometer. Chemical shifts are reported in parts per million (δ) calibrated using tetramethylsilane as an internal standard for samples in $CDCl_3$ and [D_6]DMSO and to the residual solvent signals at δ 4.79 ppm for samples in D_2O. ^{19}F NMR spectra were referenced by inserting a sealed capillary containing 5% trifluroacetic acid (s, δ –76.55 ppm) in D_2O into the NMR tube.[1] Mass spectra were measured on a Shimadzu GC coupled with a GCMS-QP 2010 plus mass detector and a single-quadrupole mass spectrometer Quantum (Shimadzu) with 100% dimethyl polysiloxane (Restek Rxi-1 ms; 30 mm × 0.25 mm, 0.25 µm film thickness) column. AFM measurements were performed on Nanoscope Multimode AFM operating in tapping mode in air. The images were taken in air at room temperature and data analysis was performed using Nanoscope 5.31r1 software. HR-TEM images were acquired on a Jeol 2100 HR operating at 120 kV. Samples were prepared by depositing a drop of diluted nanoparticle suspension on 300 mesh TEM grid and dried under vacuum for 2 hours, and were stained using 2% phosphotungstic acid. Dynamic light scattering experiment was performed using Malvern Zetasizer 2000 DLS spectrometer with 633 nm CW laser. The particles were dispersed in Milli Q water before analysis. Photosensitization experiments were carried out using a 400 W Xenon arc lamp (Oriel instruments) with a 475 nm cut-off filter (Newport Corporation). Absorption spectra were recorded on a Shimadzu UV-Vis spectrophotometer in 3 mL quartz cuvettes having a path length of 1 cm. Luminescence spectra were recorded on an Edinburgh Instruments FS5 or Edinburgh Instruments FLSP 920 spectrometer. For recording luminescence from singlet oxygen, samples were excited using 377 nm pulsed laser diode. Nanosecond laser flash photolysis experiment was carried out in an Applied Photophysics LKS-60 laser kinetic spectrometer using the third harmonic (355 nm, pulse duration ≈10 ns) of a Quanta Ray INDI-40-10 series pulsed Nd:YAG laser as the excitation source.

Materials: Pyrrole, boron trifluoride diethyletherate, $HAuCl_4$, and 1,3-diphenylisobenzofuran (DPBF) were purchased from Sigma-Aldrich and used as received. Acetyl chloride, L-tryptophan, L-cysteine, silica gel (60-120 mesh), acetone, dichloromethane, hexane and triethylamine were purchased locally. Solvents were distilled before use. Distilled water was used for all experiments.

Synthesis of 1: Freshly distilled pyrrole (0.48 mL, 7 mmol) was dissolved in 100 mL dry dichloromethane and acetyl chloride (0.24 mL, 3.5 mmol) was added drop-wise under an

atmosphere of N_2 at 25 °C. The reaction mixture was then stirred for 12 h at 25 °C. After cooling to 0 °C in an ice-bath, triethylamine (2.5 mL, 17.5 mmol) was added and stirred for 30 minutes. $BF_3.OEt_2$ (1.29 mL, 10.5 mmol) was then added and stirred for another 12 h at 25 °C. The reaction mixture was washed with water (3 × 25 mL) and brine solution (20 mL), the organic layers were collected, dried over Na_2SO_4, filtered and evaporated to get a dark residue. The crude product was purified by column chromatography over silica gel using a mixture of dichloromethane and hexane to afford compound **1** in 30% yield. ^{1}H NMR (DMSO-d_6, 298 K, 400 MHz): δ (ppm) 2.71 (s, 3H), 6.62-6.64 (m, 2H), 7.63-7.64 (d, 2H), 7.99 (s, 2H). GC-MS: m/z calculated for $C_{10}H_{10}BF_2N_2$ $(M+H)^+$: 207.09, found: 207.10.

Synthesis of nanocomposites 3: To a stirred solution of L-tryptophan (25 mM) in 10 mL water at 25 °C was added an aqueous solution of $HAuCl_4$ (1 mL from a 5 mM stock solution) followed by a solution of **1** in acetone (1 mL from a 5 mM stock solution). The solution which turned brown-orange upon mixing was stirred for 16 h at 25 °C. The precipitated residue was collected after centrifugation, washed with water (3 × 1 mL), dried, and re-dispersed in water. The formation of nanocomposites was monitored through UV-Vis absorption measurements and were characterized by DLS and microscopy measurements.

Synthesis of organic nanoparticles (ONPs): ONPs **4** was synthesized by the re-precipitation method. To 10 mL of distilled water, 1 mL from a 5 mM stock solution of **1** in acetone was added, and the resulting suspension was stirred for 12 h at 25 ºC. The reaction mixture was then centrifuged, the residue was collected, washed with water (3 × 1 mL), dried and re-dispersed in water. The formation of nanoparticles was monitored through UV-Vis measurements and were characterized by DLS and microscopy measurements.

Synthesis of 5: To a stirred solution of L-tryptophan (25 mM) in 10 mL water at 25 °C was added an aqueous solution of $HAuCl_4$ (1 mL from a 5 mM stock solution). The solution was observed to turn into pale pink upon mixing. The reaction mixture was stirred for 10 minutes at 25 °C, centrifuged, and the residue was collected, washed with water (3 × 1 mL), dried, and dispersed in water. The formation of nanocomposites was monitored through UV-Vis absorption measurements and were characterized by DLS and microscopy measurements.

Cysteine exchange studies: To a stirred solution of 1 mL of the nanocomposite **3**, L-cysteine (1 mL of 25 mM stock solution in aqueous sodium acetate, pH 9) was added and solution was stirred for 30

minutes at room temperature. The reaction mixture was then extracted with DCM (3 × 1 mL), the organic layers combined and dried over anhydrous sodium sulphate and solvent evaporated off to get a residue. The residue was analyzed by UV-Vis absorption spectroscopy which revealed the concentration of BODIPY (**1**) to be 0.96 mM. This translates to 15% loading of BODIPY in the nanocomposite.

Investigation of singlet oxygen generation: Stock solutions of the photosensitizers and the reference standard methylene blue (MB) were prepared in water whereas a stock solution of DPBF was prepared in ethanol. 1.6 mL of an aqueous solution of the photosensitizer was taken in a cuvette to which 0.4 mL DPBF in ethanol was added. After recording the absorbance, the solution was irradiated using a Xenon lamp with a 475 nm cut-off filter. The decrease in absorbance of DPBF at 420 nm was monitored at regular intervals.

Determination of singlet oxygen quantum yield: Singlet oxygen quantum yield was determined by following a reported procedure.[2] The quantum yield was calculated with reference to MB in water which is reported to have a quantum yield of 0.52.[3]
Singlet oxygen quantum yield was calculated according to the equation:

$$\Phi_{\Delta}(\text{sample}) = \Phi_{\Delta}(\text{ref}) \times \frac{\text{m(sample)} \times \text{F(ref)}}{\text{m(ref)} \times \text{F(sample)}}$$

where m is the slope of difference in change in absorbance of DPBF (at 420 nm) with the irradiation time, and *F* is the absorption correction factor, which is given by $F = 1–10^{-OD}$.

Cell culture: A rat origin glioblastoma cell line, C6 cells was obtained from National Centre for Cell Sciences, Pune, India. The cells were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM, Himedia) supplemented with 10% fetal bovine serum (FBS, Himedia) and 1% antibiotics antimycotic solution (Himedia) at 37 °C in a humidified incubator containing 5% CO_2.

Detection of Reactive Oxygen Species (ROS)*:* ROS was measured utilizing 2,7-dichlorofluorescein diacetate (DCFDA), a non-fluorescent dye which can readily diffuse into cells and gets cleaved by the intracellular esterases to form 2,7-dichlorofluorescein (DCF) by the ROS generated in cells. The amount of ROS is directly correlated with the excitation wavelength of 488 nm and emission wavelength of 530 nm, respectively. Nanocomposite **3** was tested against glioblastoma C6 cells in presence and absence of white light (36 W while LED lamp). After 12 hours incubation of particles with the cells, they were exposed to white light for 20 minutes. After 24 hours, cells were washed

with Hank's buffer and incubated with DCFDA (50 µg in 2 mL serum free media) for 1 hour. Then the cells were washed again with Hank's buffer to remove any excess dye. Then the cells were treated with lysis buffer (0.1M Tris HCl containing 1% Tween 20). The supernatant of lysed cells was evaluated for fluorescence intensity. The amount of ROS produced was expressed as percentage over control.

***In vitro* cytotoxicity and cell viability study**: The *in vitro* cytotoxicity of different concentrations of nanocomposite was assessed against C6 cells using the MTT method. Specifically, cells were seeded in a 96-well flat culture plate at a density of 2.5×10^4 cells per well and incubated at 37 °C under 5% CO_2 for 24 hours. Next day, different concentrations of nanocomposite was prepared in media and added to each group. After 6 hours of incubation, the cells were exposed to white light for 20 minutes followed by incubation for 48 hours in the CO_2 humidified incubator. The cells were washed with pre-warmed PBS buffer thrice to remove traces of sample. 20 µL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Aldrich) solution (5 mg/mL in PBS buffer) diluted with 180 µL media was added to the wells and incubated for 4 hours. After 4 hours, the plates were centrifuged at 1500 rpm for 5 minutes at room temperature. 150 µL DMSO was added to each well to dissolve the formazan crystals and all the wells were aspirated well before taking absorbance. The absorbance of the suspension was measured at 510 nm on an ELISA reader.

Cell viability was calculated by means of the following formula:

$$\text{Cell viability (\%)} = \frac{OD_{510\,(sample)} - OD_{510\,(blank)}}{OD_{510\,(control)} - OD_{510\,(blank)}} \times 100\ \%$$

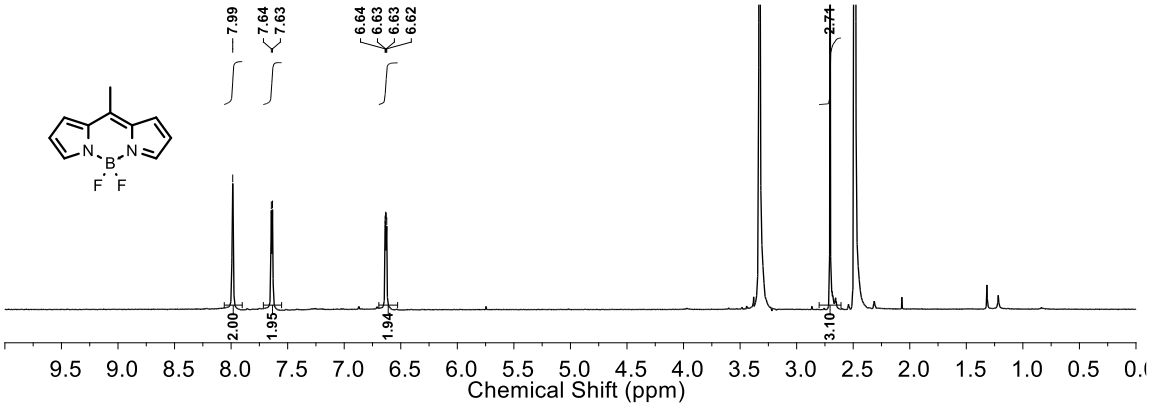

Figure S1. ^{1}H NMR spectrum of **1** in DMSO-d_6.

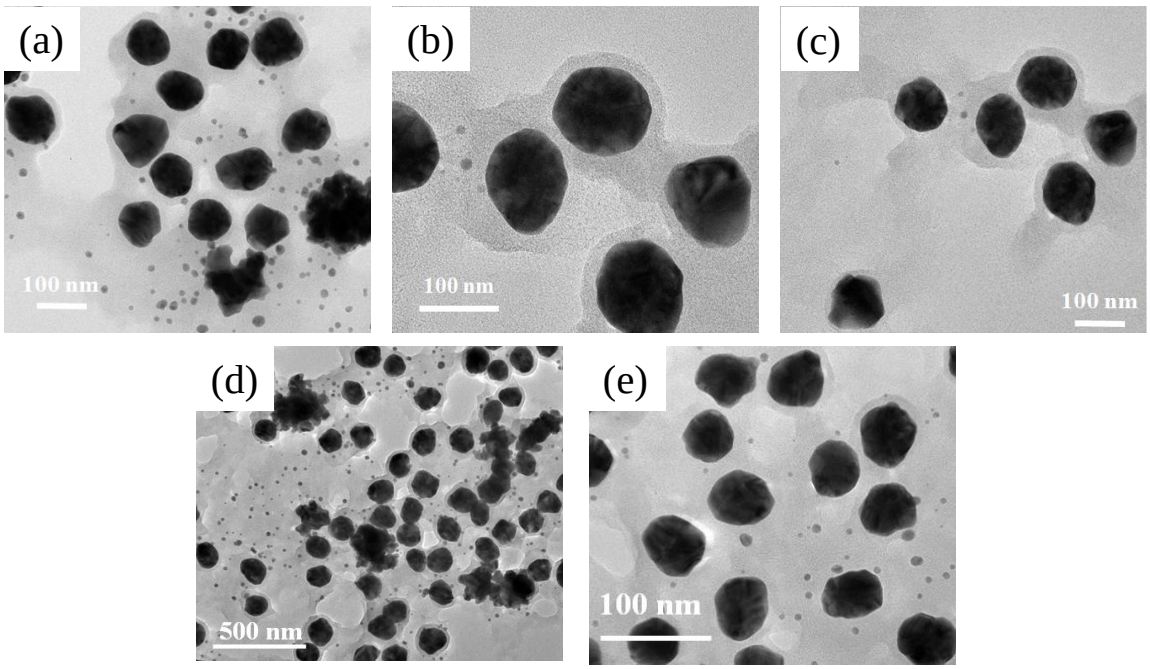

Figure S2. HR-TEM images of (a-c) **3**, (d) **4** and (e) **5**.

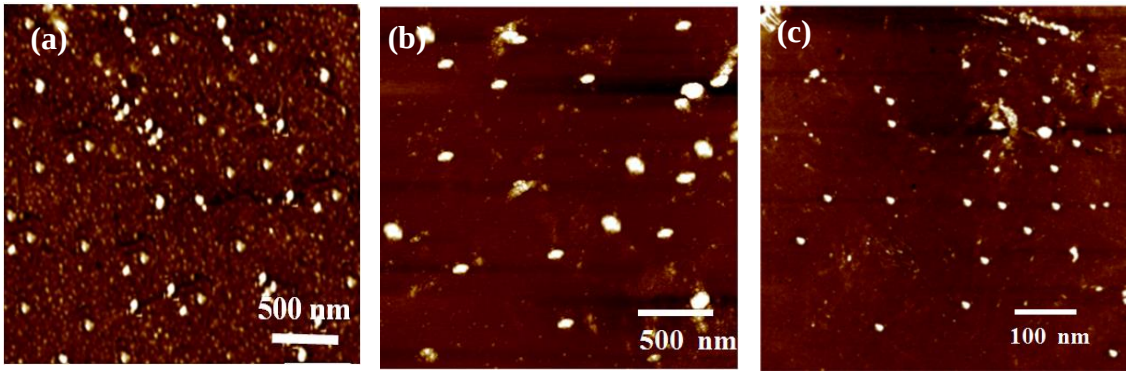

Figure S3. AFM images of (a) **3**, (b) **4** and (c) **5**.

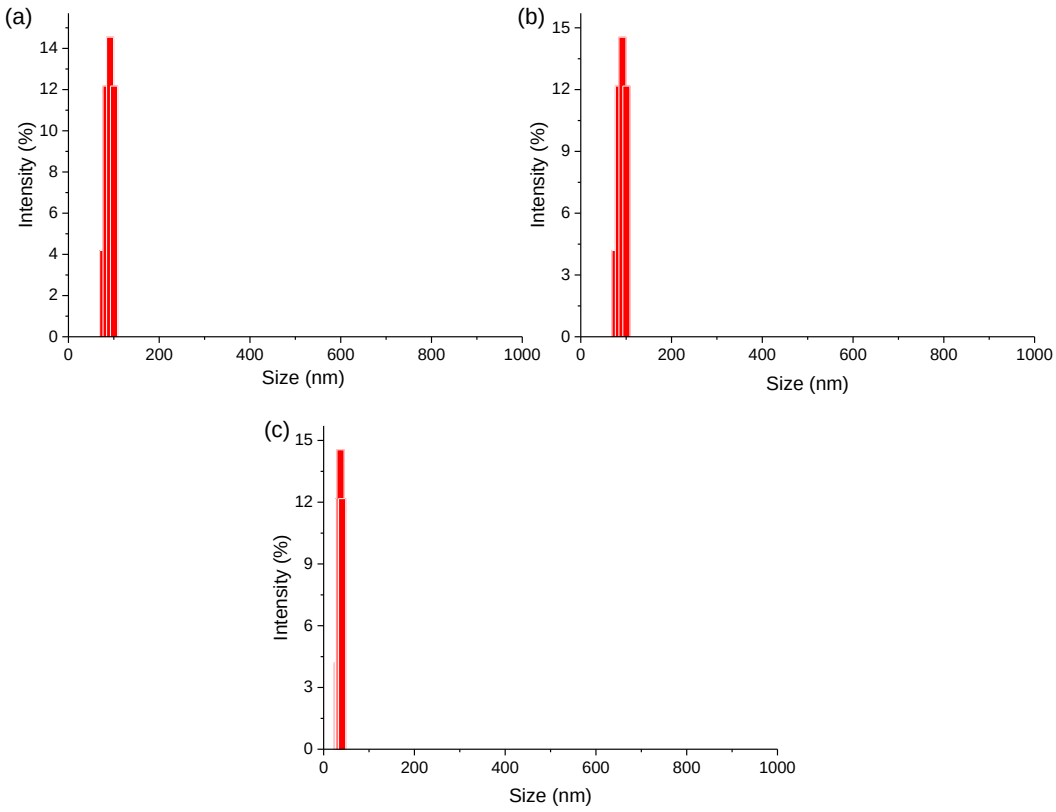

Figure S4. Particle size analysis of **3-5** by dynamic light scattering in water. The observed particles sizes were 98±2, 92±2 and 35±3 nm for **3-5**, respectively.

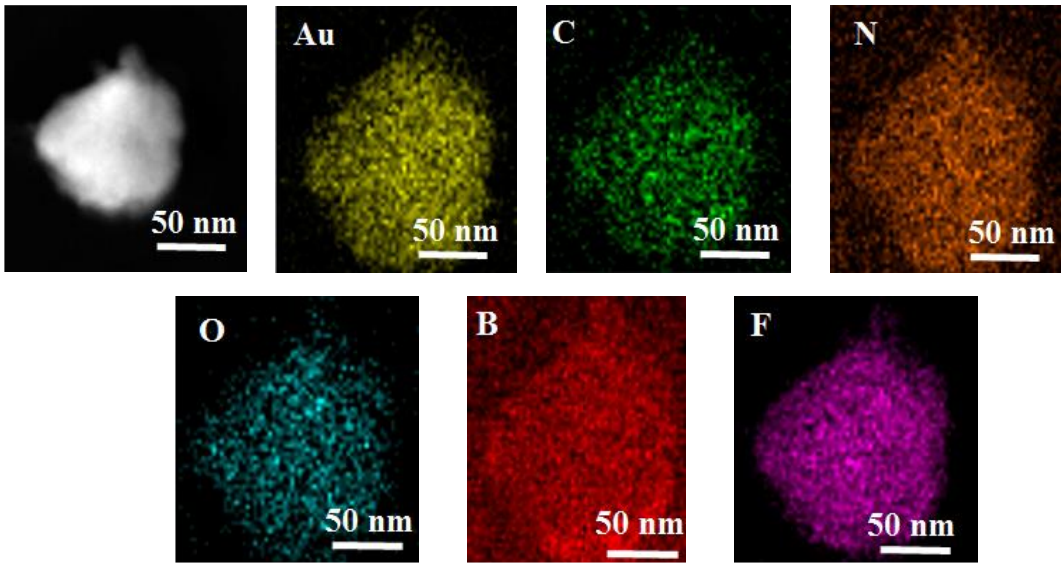

Figure S5. Bright-field TEM image and elemental maps of the nanocomposite **3**.

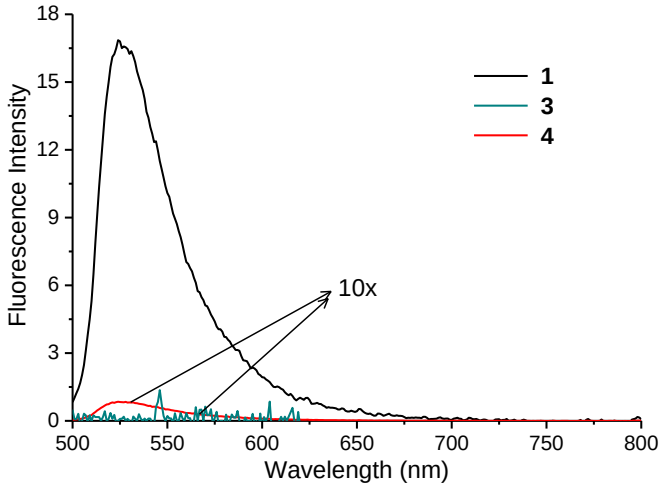

Figure S6. Fluorescence spectra of **1** (11 µM) in acetone, and **3** (100 µg/mL) and **4** (93 µg/mL) in water. Excitation wavelength, 490 nm.

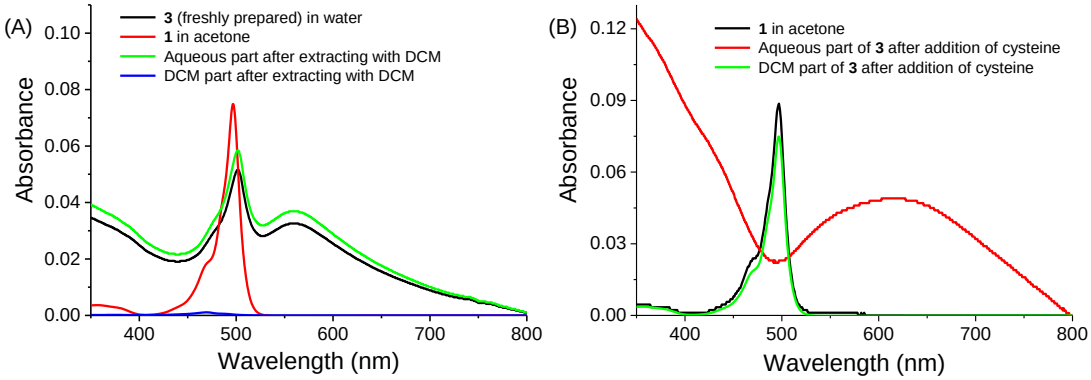

Figure S7. UV-Vis absorption spectra of freshly prepared nanocomposite **3**, and the DCM layer and aqueous layer (A) after extraction by stirring for 16 hours and (B) after reaction with cysteine.

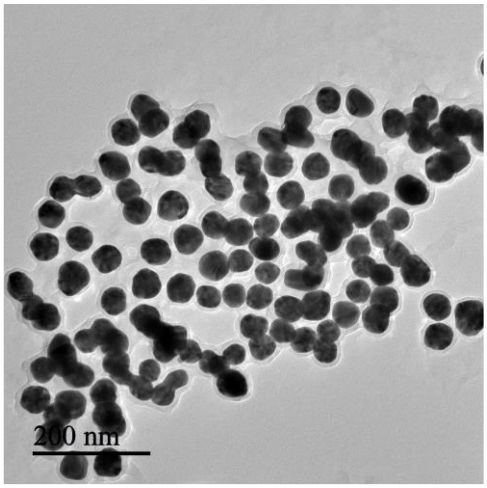

Figure S8. HR-TEM image of the aqueous layer of nanocomposite **3** after reaction with cysteine.

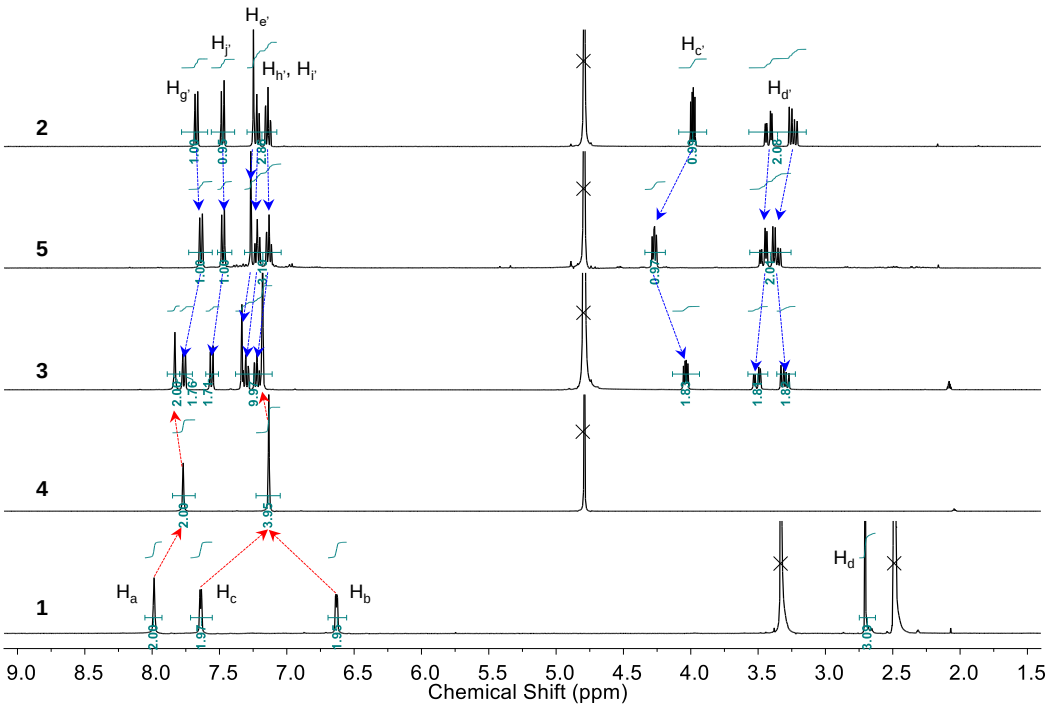

Figure S9. ^{1}H NMR spectrum of **1** in [D_6]DMSO and **2**-**5** in D_2O. Blue dotted arrows indicate the sequential changes in the chemical shift of the protons of the tryptophan moiety upon the formation of Au NPs **5** and the nanocomposite **3**. Red dotted arrows indicate similar changes in the chemical shift of the protons of the BODIPY moiety upon the formation of ONPs **4** and the nanocomposite **3**.

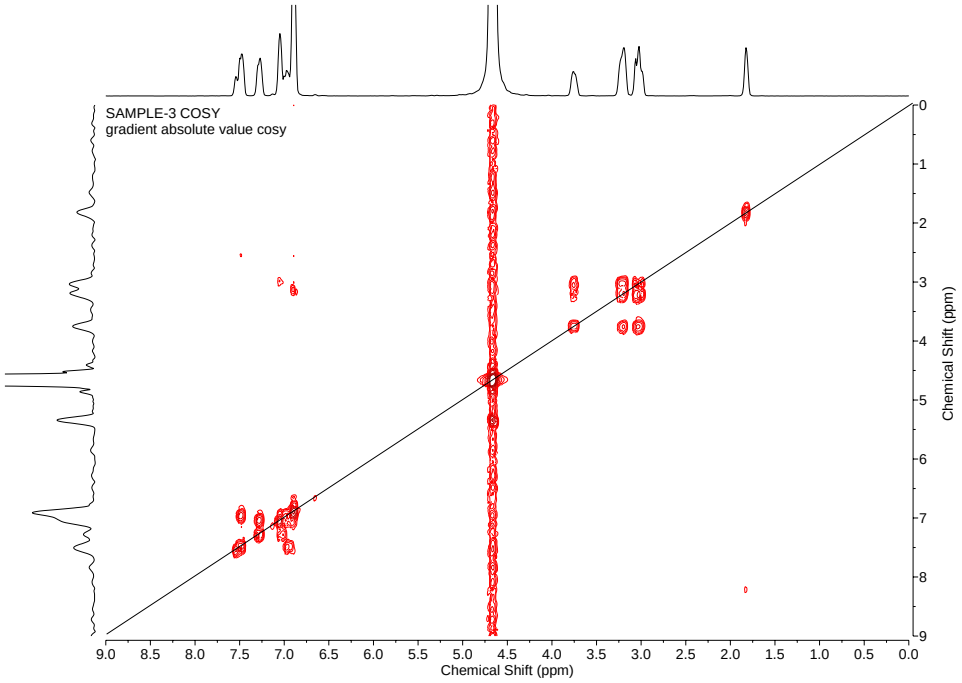

Figure S10. ^{1}H-^{1}H COSY spectrum of the nanocomposite **3** in D_2O.

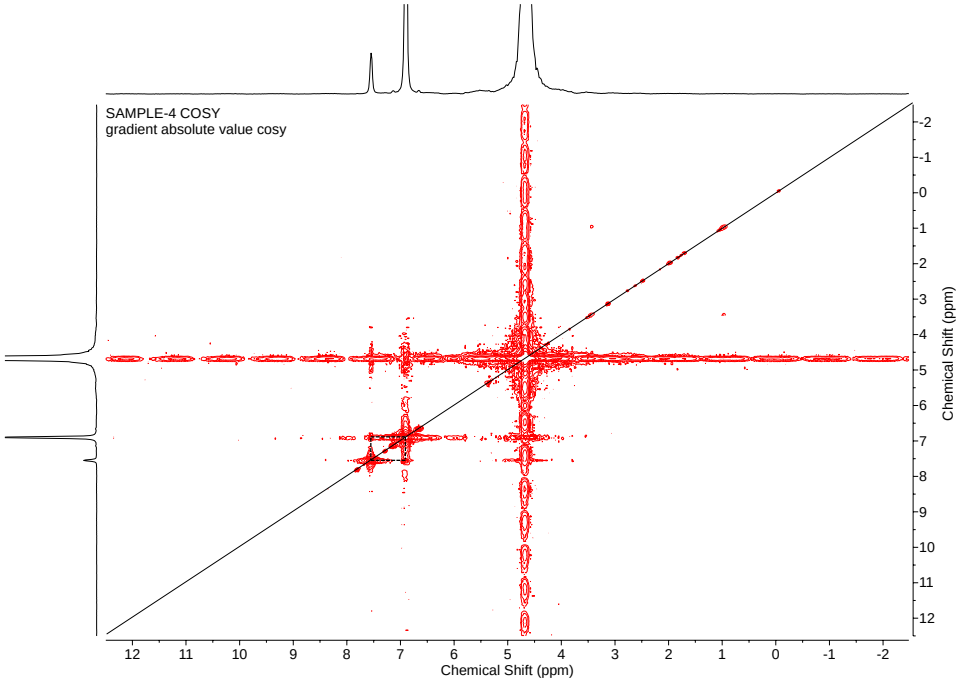

Figure S11. ^{1}H-^{1}H COSY spectrum of the organic nanoparticles **4** in D_2O.

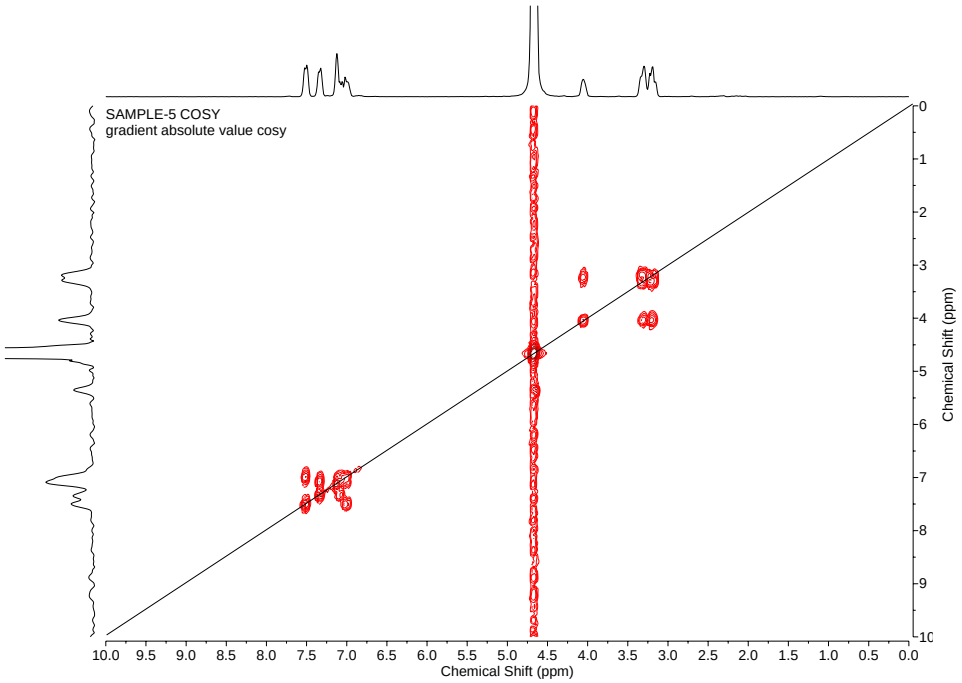

Figure S12. ^{1}H-^{1}H COSY spectrum of the gold nanoparticles **5** in D_2O.

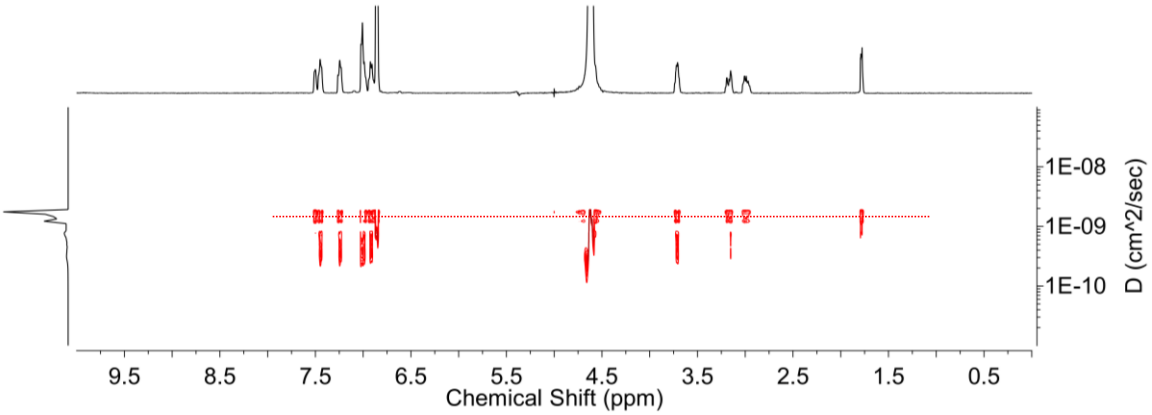

Figure S13. ^{1}H-^{1}H DOSY spectrum of the nanocomposite **3** in D_2O.

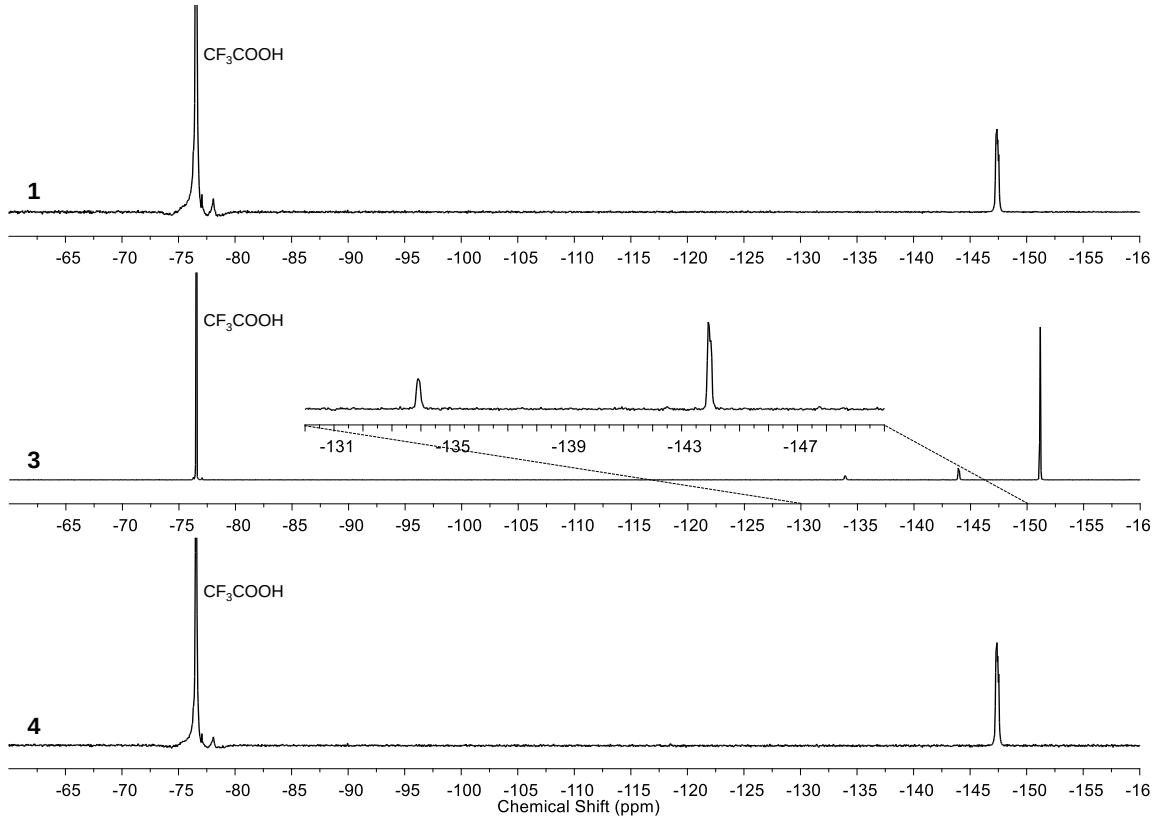

Figure S14. ^{19}F NMR spectrum of **1** in $CDCl_3$, and **3** and **4** in D_2O, respectively. Inset shows the region between δ –130 to –150 ppm in the spectrum of **3** in D_2O.

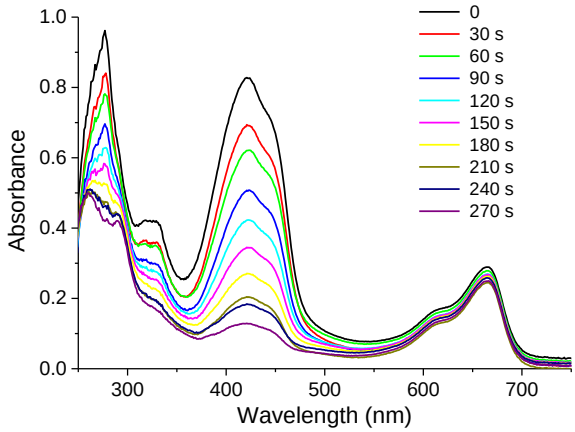

Figure S15. The decrease in the absorbance of DPBF (90 μM) in the presence of methylene blue (12 μM) in water.

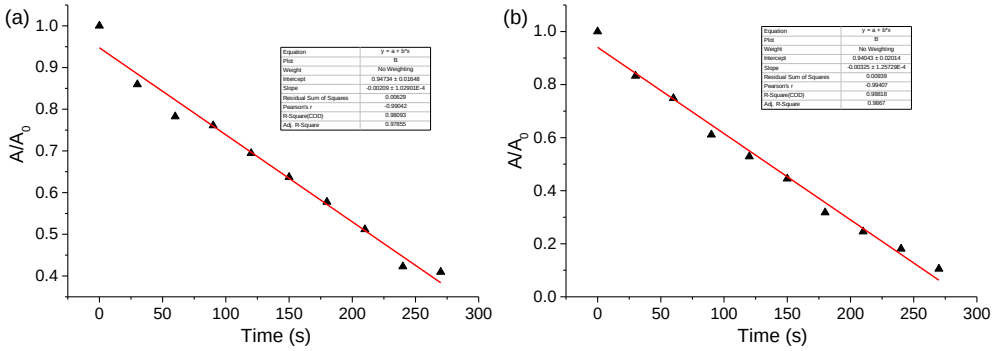

Figure S16. Curve-fitting data for the decrease in the absorbance of DPBF (90 μM) at 420 nm in the presence of (a) **3** (100 μg/mL) and (b) methylene blue (12 μM).

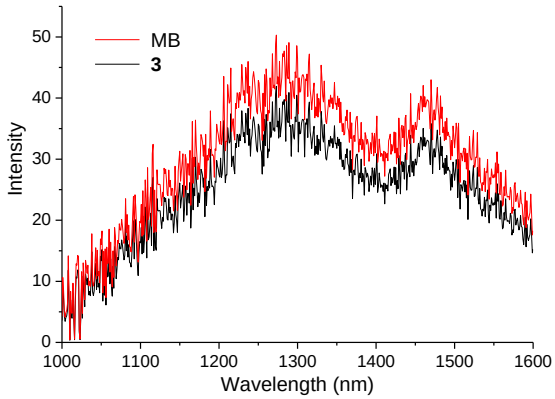

Figure S17. Luminescence spectra of a solution of **3** (100 μg/mL) and methylene blue (12 μM) in water. Excitation wavelength, 377 nm.

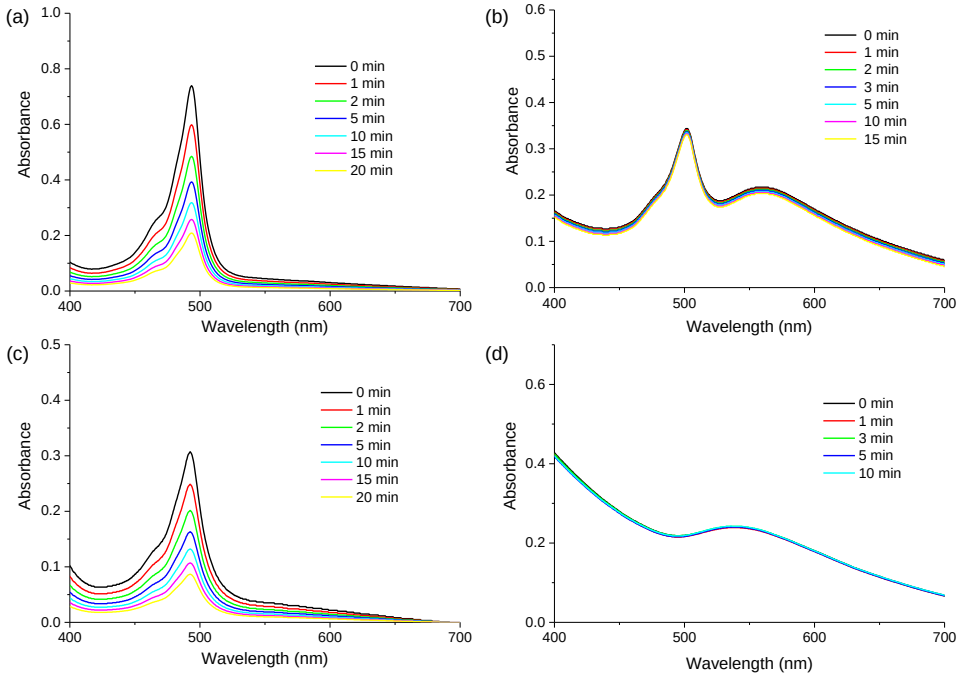

Figure S18. Changes in the absorption spectra of (a) **1** (11 µM) in acetone, (b) **3** (100 µg/mL), (c) **4** (93 µg/mL) and (d) **5** (100 µg/mL) in water.

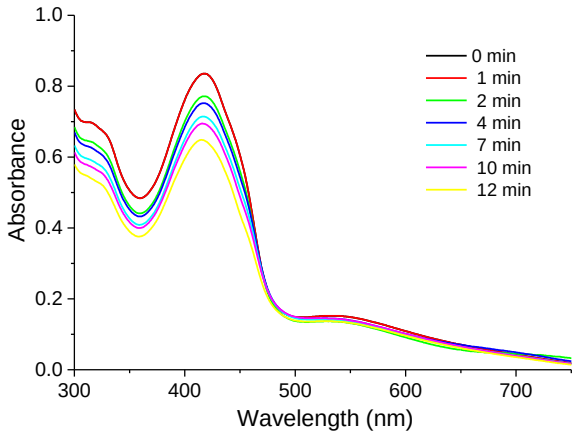

Figure S19. The decrease in the absorbance of DPBF (90 µM) in the presence of **5** (100 µg/mL) in water.

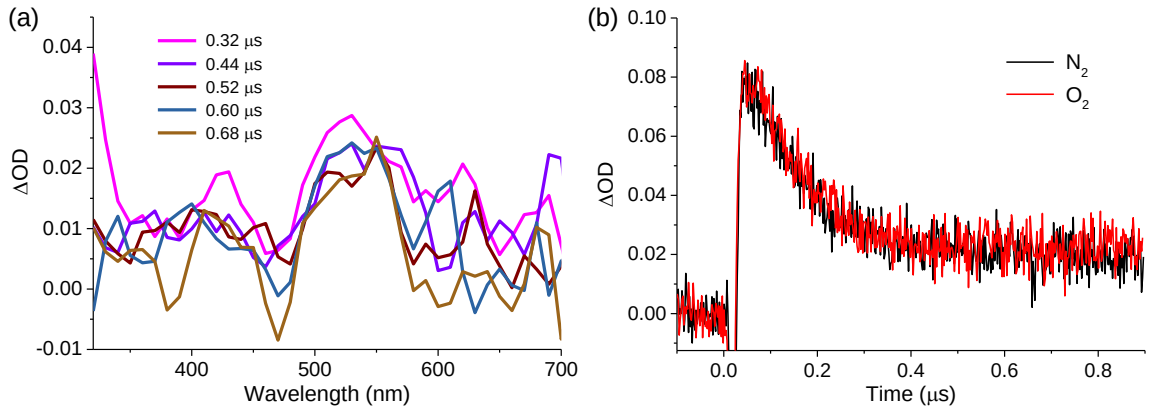

Figure S20. (a) Transient absorption spectra of **3** following 355 nm laser pulse excitation. (b) Transient decay of **3** at 530 nm under an atmosphere of nitrogen and oxygen.

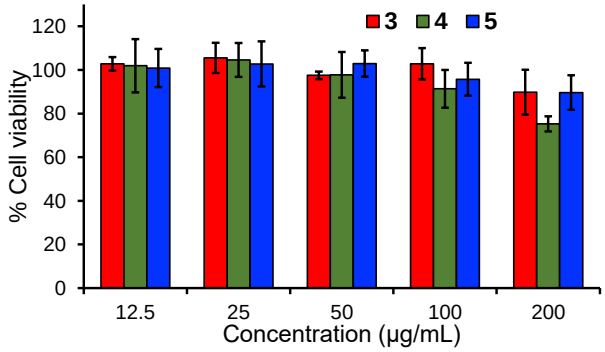

Figure S21. Concentration dependent viability assessment of nanocomposite **3**, ONPs **4** and AuNPs **5** against L929 mouse fibroblast cells.

References

[1] Z.-X. Jiang, Y. Feng, Y. B. Yu, *Chem. Commun.* **2011**, *47*, 7233–7235.

[2] S. Shah, A. Bajaj, A. Shibu, M. E. Ali, P. P. Neelakandan, *Chem. Eur. J.* **2018**, *24*, 18788–18794.

[3] W. Li, L. Li, H. Xiao, R. Qi, Y. Huang, Z. Xie, X. Jing, H. Zhang, *RSC Adv.* **2013**, *3*, 13417–13421.