SUPPLEMENTARY INFORMATION

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REGULAR ARTICLE

Synthesis and *In Vitro* Photobiological Studies of Porphyrin Capped Gold Nanoparticles[§]

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[§]Dedicated to Professor M V George on the occasion of his 90th Birth Anniversary

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Experimental methods for biological protocols

1. Determination of Cytotoxicity by Measuring Cellular Proliferation

The assay of 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) is a standard colorimetric assay for measuring cellular proliferation (cell growth). In the present investigation, human breast cancer cells (MDA MB 231 cells, 5 x 10^3 cells per well) were added to wells of two 96 well microliter plate. One for dark cytotoxicity and another for light cytotoxicity with 150 μL Dulbecco's Modified Eagle Medium (DMEM) with 10% serum and incubated for 24 h. Then the porphyrin derivatives were added in serial dilution up to 5 μM (stock 100 mM diluted using DMEM for test, 0.025% dimethyl sulphoxide, DMSO) for control and incubated for 18 h and washed with PBS and then fresh DMEM was added. After, photoirradiation was done in one plate using sodium vapor lamp with a total fluence of 100 J cm^{-2}, while the other plate was kept in dark. After 24 h of incubation, the plates were removed from the incubator and 10 μL of MTT (5 mg/mL stock) was added to each well of the plate. After 4 h, the supernatant was carefully removed taking care that the formazan crystals formed were not being removed and 100 μL of isopropyl alcohol was added to each well. The plates were covered with aluminium foil and kept

on a shaker until crystals were dissolved. The absorbance was monitored at 570 nm and the percentage growth inhibition was calculated using equation S1.

$$\%\text{Growth inhibition} = (\text{control-test})/\text{control}) \times 100 \qquad \text{(Eq. S1)}$$

2. Flow Cytometric Analysis with Annexin V-FITC/PI

The photoirradiation experiments were done 60 mm cell culture dish with approximately 5×10^5 MDA-MB-231 cells as mentioned as earlier in cell proliferation assay (MTT ASSAY). Annexin-V/PI staining was done using Sigma-Aldrich (USA) kit, following manufacturer's protocol. Flow cytometry analysis was carried out using FACS Aria, Special order system, BD, USA.

3. Tetramethylrhodamine Methyl Ester (TMRM) Assay

The photoirradiation experiments were carried out as mentioned in Annexin V-FITC/PI assay. Then cells were treated with 10 nM TMRM dye (Molecular Probes USA) dissolved in PBS followed by flow cytometry analysis.

4. Transmission Electron Microscopy (TEM) Analysis

The TEM analyses were performed on JEOL 100 kV high resolution transmission electron microscope. The **POPNPs** (10 μM) were drop casted on the top of carbon-coated Cu grid. The grids were mounted on reverse tweezers and the samples were dried at room temperature. The accelerating voltage of the transmission electron microscope was 100 kV and the beam current was 65 A. Samples were imaged using a Hamamatsu ORCA CCD camera.

5. Dynamic Light Scattering (DLS) Measurements

DLS was used to measure the diameter and polydispersity index of the **POPNPs**. Zeta potential was measured using Nano Zeta Sizer, Malvern instruments. The samples were prepared in water at a concentration of 5 x 10^{-6} M.

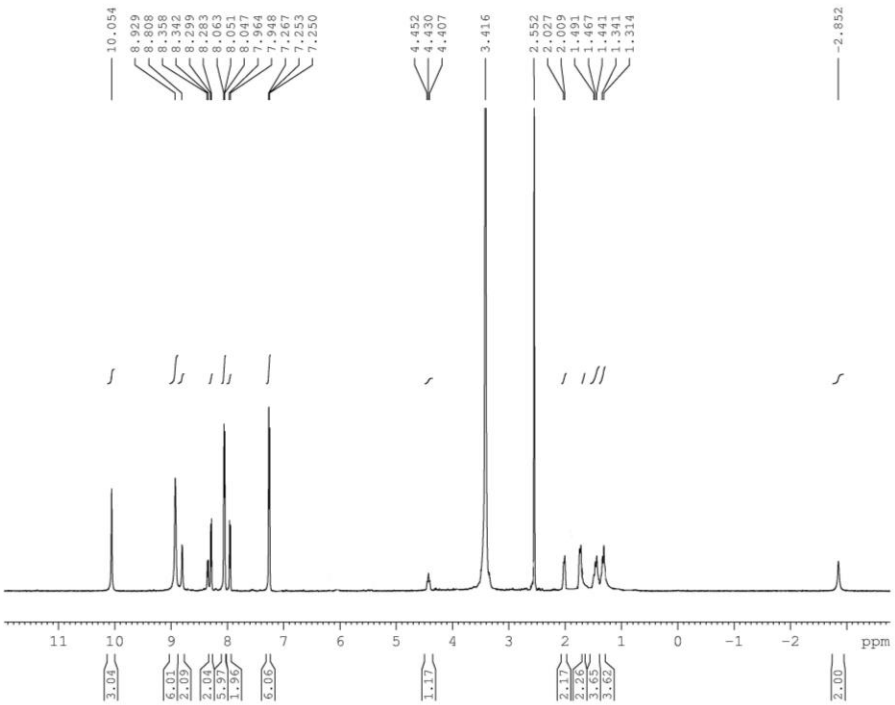


Figure S1. ^{1}H NMR spectrum of **2** in DMSO.

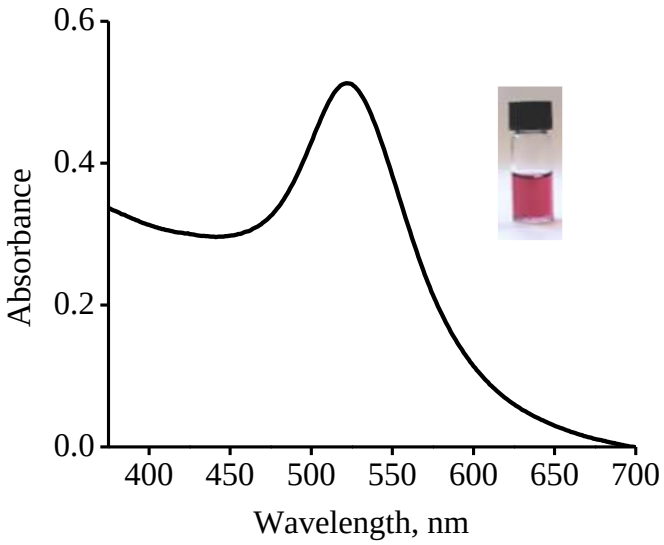
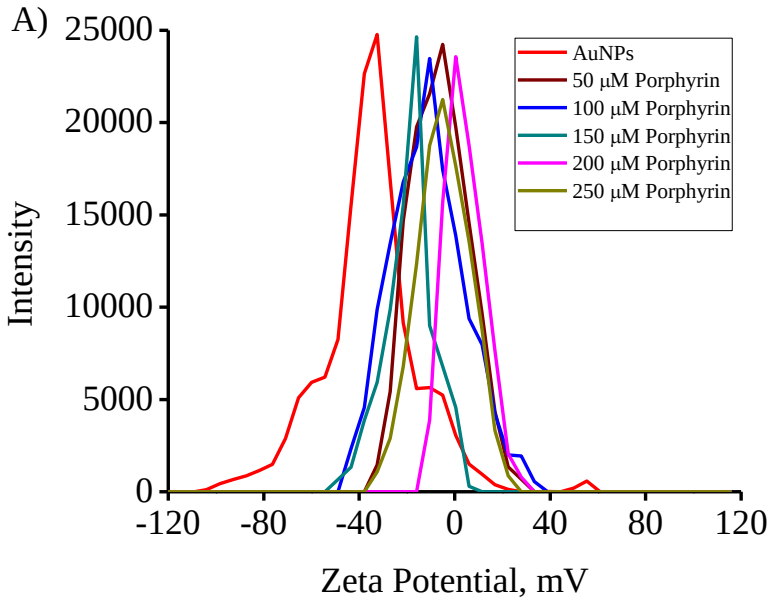


Figure S2. Surface plasmon resonance at 522 nm of the citrate capped gold nanoparticles (**AuNPS**).



B)

Description	Zeta Potential (m V)
AuNPs	–32.7
POPNPs with 50µM of 2	–4.75
POPNPs with 100µM of 2	–10.83
POPNPs with 150µM of 2	–16.0
POPNPs with 200µM of 2	0.82
POPNPs with 250µM of 2	–5.25

Figure S3. A) Graph showing the zeta potential measurements of AuNPS and **POPNPs** (50-250 µM of **2**); B) Table indicating the zeta potential values of various **POPNPs** (50-250 µM of **2**).

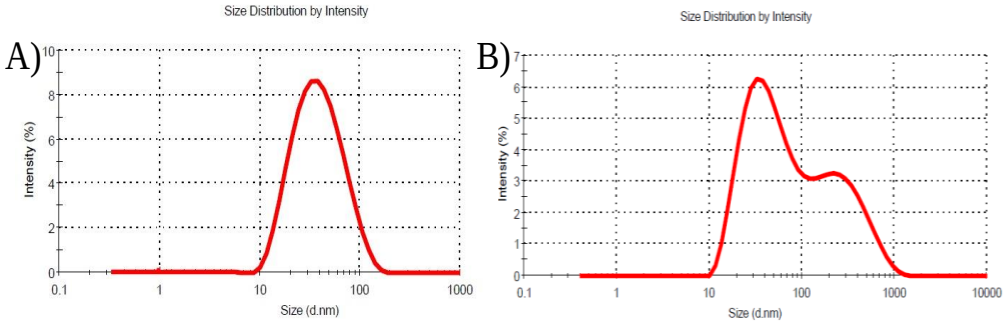


Figure S4. Size distribution of the AuNPs in aqueous media measured by DLS. B) Size distribution of the **POPNPs** in aqueous media measured by DLS experiments.

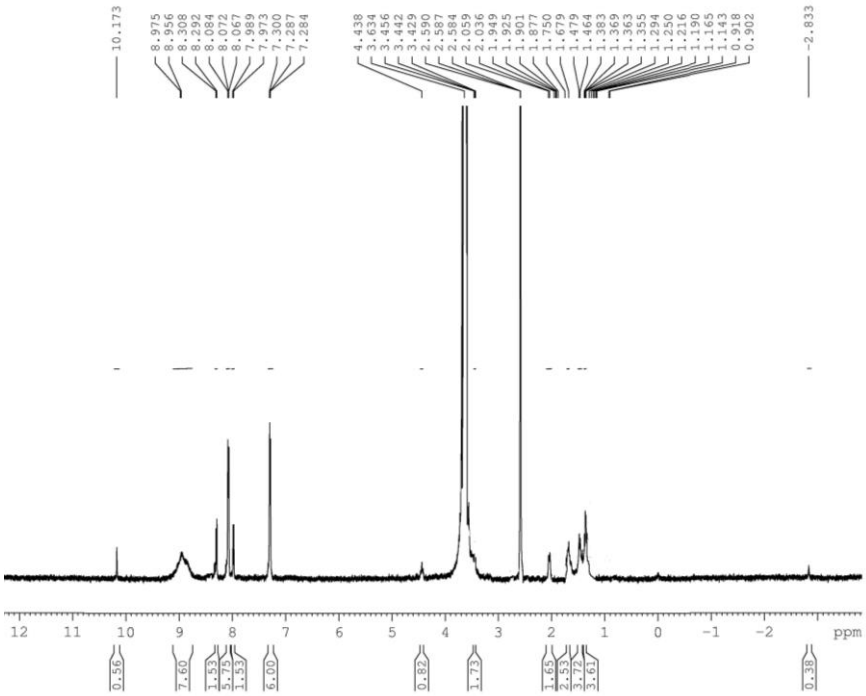


Figure S5. ^{1}H NMR spectrum of **POPNPs** in DMSO.

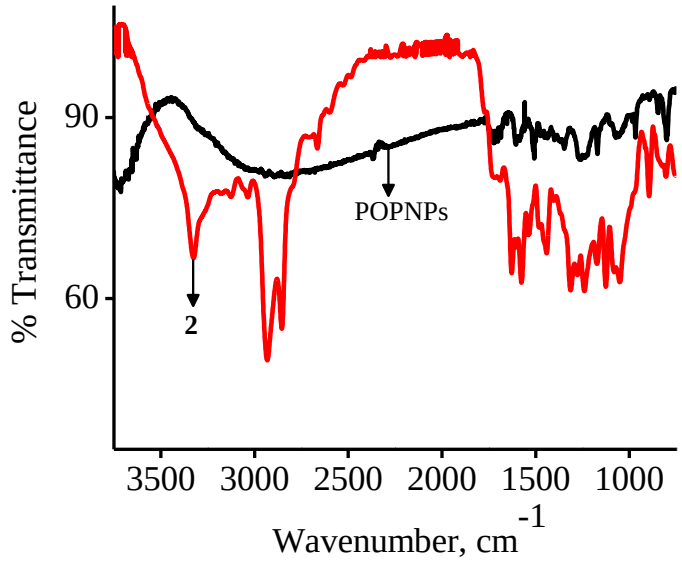


Figure S6. IR spectra of the porphyrin derivative **2** (red trace) and **POPNPs** (black trace).