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Supporting Information

Dog-bone Shaped Gold Nanoparticle-Mediated Chemo-Photothermal Therapy Impairs Powerhouse to Trigger Apoptosis in Cancer Cells

Jaypalsing Ingle,[a] Bhawna Uttam,[a,b] Reha Panigrahi,[a] Saumyakanti Khatua,[a]* Sudipta Basu [a]*

a. Discipline of Chemistry, Indian Institute of Technology (IIT) Gandhinagar, Palaj, Gandhinagar, Gujarat, 382355, India.

b. Current address: Department of Chemistry, J.C. Bose University of Science and Technology, YMCA, Faridabad, Haryana 121006, India.

* Corresponding author. Email: Sudipta.basu@iitgn.ac.in, khatuask@iitgn.ac.in

Materials and Instruments:

All the materials required in the experiments are purchased from suppliers. Reagents required for the reactions was purchased from Sigma Aldrich, TCI, Chem scene, Avra Synthesis, BLD Pharm and GLR innovation. All the reactions were set up in clean and oven-dried Borosil or Asco glassware. For the reactions requiring inert condition, was performed in presence of nitrogen or argon atmosphere. Analytical TLC was used, where silica gel 60 F254 is coated on aluminium sheet (made in Germany). Heidolph Rotary evaporator was used to evaporate the solvent. Crude compounds were purified by column chromatography using silica 100-200 mesh size, 230-400 mesh size or neutral aluminium oxide as a stationary phase. Hexane, ethyl acetate, dichloromethane, chloroform and methanol are the common mobile phase solvents used as a mobile phase and are purchased from Finar. UV spectra were recorded on a V-750 JASCO spectrophotometer. Fluorescence experiments were done on Fluorolog HORIBA JOBIN YVON. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded in either $CDCl_3$ or DMSO-d6 solvents and recorded on Ascend NMR-500 MHz (Bruker) NMR spectrometer. Chemical shifts are indicated in parts per million (PPM). Residual protons in the deuterated solvents are considered as a reference ppm value. FE-SEM experiment was done using JeOL JSM-7600F (Japanese company). DLS is performed on Multi angle DLS instrument by Malvern Panalytical. Transmission electron microscopy (TEM) is performed on TEM instrument by Thermo Scientific, Themis 300 G3. ICP-OES experiment is performed on Nexion 2000B ICP-MS/OES by Perkin Elmer. For cell biology experiments, DMEM (Gibco) with 10% FBS (Gibco) and 1% Peicilin-Streptomycin (Gibco) is used to prepare complete media. 3-(4, 5- dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide (MTT) from Sigma Aldrich was used for the cytotoxicity experiment. MitoTracker red, JC-1, MitoSox red, FITC Annexin-V and Propidium iodide was purchased from Themo Fischer Scientific. TMRM and H2DCFDA are purchased from TCI chemical and Sigma Aldrich respectively. All required reagents are purchased from Himedia. Antibodies required in immunofluorescence and western blot experiment are purchased from Cell signaling, Santa Cruz and Thermo fischer scientific. Absorbance for MTT assay is recorded on Perkin Elmer multimode plate reader. Confocal imaging is performed on Leica (TCS SP8). Data analysis is performed by GraphPad prism or Origin software and confocal images were analysed by Image J software.

Reaction procedure:

Synthesis of (S)-4-ethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl 5-(1,2-dithiolan-3-yl)pentanoate (3):
In a clean, oven dried 10 mL round bottom flask, containing Teflon coated magnetic stirrer bar. Take Lipoic acid **(1)** (15.7 mg, 0.076 mmol), 10 hydroxycamptothecin **(2)** (30 mg, 0.076 mmol), EDC.HCl (17.5 mg, 0.091 mmol), HOBt (17.5 mg, 0.091 mmol) DMAP (2 mg, 0.016 mmol), and 1 mL anhydrous DMF and stir reaction mixture at room temperature for 36h. Inert atmosphere using N_2 is maintained thought the reaction. Completion of reaction is monitored by TLC. After completion of reaction, reaction mixture was dissolved in ice cold brine solution and extracted by ethyl acetate twice. Combined ethyl acetate is dried by Na_2SO_4, solvent was evaporated on rota evaporator. Purification was done by silica gel chromatography with hexane and ethyl acetate as mobile phase.

Yield: 67%

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.24 (d, *J* = 9.2 Hz, 1H), 7.68 (d, *J* = 2.4 Hz, 1H), 7.67 (s, 1H), 7.56 (dd, *J* = 9.2, 2.5 Hz, 1H), 5.75 (d, *J* = 16.3 Hz, 1H), 5.31 (d, *J* = 14.4 Hz, 3H), 3.85 (s, 1H), 3.63 (dq, *J* = 12.5, 6.3 Hz, 1H), 3.25 – 3.18 (m, 1H), 3.14 (dt, *J* = 11.1, 6.9 Hz, 1H), 2.68 (s, 2H), 2.50 (tt, *J* = 13.1, 6.5 Hz, 1H), 1.95 – 1.68 (m, 8H), 1.04 (t, *J* = 7.4 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 173.7 (s), 171.6 (s), 157.5 (s), 152.3 (s), 150.0 (s), 149.5 (s), 146.8 (s), 146.1 (s), 131.2 (s), 130.5 (s), 129.0 (s), 128.4 (s), 125.8 (s), 118.7 (s), 118.6 (s), 97.9 (s), 72.6 (s), 66.2 (s), 56.2 (s), 49.9 (s), 40.2 (s), 38.4 (s), 34.5 (s), 34.1 (s), 31.5 (s), 28.6 (s), 24.5 (s), 7.7 (s)

HRMS (ESI-TOF) (*m/z*): $[M + H]^+$ calculated for $C_{28}H_{29}N_2O_6S_2$ = 553.1462 Observed (M+H) = 553.1467

Synthesis of (3-(5-(1,2-dithiolan-3-yl)pentanamido)propyl)triphenylphosphonium (5):
In a clean, oven dried 10 mL round bottom flask, containing Teflon coated magnetic stirrer bar. Take Lipoic acid **(1)** (123 mg, 0.5 mmol), (3-aminopropyl)triphenylphosphonium **(4)** (200 mg, 0.5 mmol), EDC.HCl (115 mg, 0.6 mmol), HOBt (81 mg, 0.6 mmol) DMAP (10 mg, 0.08 mmol), and 2 mL anhydrous DMF and stir reaction mixture at room

temperature for 36h. Inert atmosphere using N_2 is maintained thought the reaction. Completion of reaction is monitored by TLC. After completion of reaction, reaction mixture was dissolved in ice cold brine solution and extracted by ethyl acetate twice. Combined ethyl acetate is dried by Na_2SO_4, solvent was evaporated on rota evaporator. Purification was done by silica gel chromatography with dichloromethane and methanol as mobile phase.

Yield: 56%

^{1}H NMR: (500 MHz, $CDCl_3$) δ 8.68 (t, *J* = 5.9 Hz, 1H), 7.83 – 7.73 (m, 9H), 7.73 – 7.64 (m, 6H), 3.78 (ddd, *J* = 13.2, 11.6, 5.6 Hz, 2H), 3.55 (dq, *J* = 8.3, 6.3 Hz, 1H), 3.47 (dd, *J* = 10.1, 4.9 Hz, 2H), 3.15 (ddd, *J* = 11.0, 7.0, 5.5 Hz, 1H), 3.08 (dt, *J* = 11.0, 6.8 Hz, 1H), 2.49 – 2.37 (m, 3H), 1.95 – 1.88 (m, 1H), 1.67 (dt, *J* = 18.1, 7.3 Hz, 5H), 1.53 – 1.42 (m, 2H), 1.25 (s, 1H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 174.2 (s), 135.1 (s), 133.5 (s), 130.5 (s), 118.3 (s), 56.5 (s), 40.1 (s), 38.7 (s), 38.3 (s), 35.9 (s), 34.4 (s), 28.7 (s), 25.4 (s), 22.6 (s), 21.4 (s).

^{31}P NMR (202 MHz, $CDCl_3$) δ 24.29 (s).

HRMS (ESI-TOF) (*m/z*): $[M + H]^+$ calculated for $C_{29}H_{35}NOPS_2$ = 508.1896 Observed (M+H) = 508.1892

Drug loading:

Amount of gold and platinum in Mito-AuDB-NP were analyzed by ICP-OES and were found to be 48 µg/mL and 15.2 µg/mL respectively. Drug loading for lipoic acid conjugated 10-hydroxy camptothecin was calculated by UV at λ_{max}= 357 nm and was found out to be 18.9 µg/mL.

ICP-OES Sample preparation:

For the digestion process of AuNP-final, 50 µL of Mito-AuDB-NP from the 1 mL stock is dissolved in 1 mL HNO_3 and HCl (9:1) mixture of acid. 1 mL H_2O_2 was added in the solution and carefully heat in water bath at 60 °C for 1 hour. Then kept at room temperature for next 24 h. Diluted with distilled water to make 10 mL final volume. Finally, the solution was pass through 2 µM syringe filter to remove if any contaminants are present. Experiment is performed on Nexion 2000B ICP-MS/OES by Perkin Elmer.

Photo-thermal effect:

2 mL solution of 4 different concentration for Mito-AuDB-NPs were used in beaker, as Mito-AuDB-NP (Au = 48 µg), Mito-AuDB-NP in water (3:1) (Au = 36 µg), Mito-AuDB-NP in water (1:1) (Au = 24 µg), and Mito-AuDB-NP (1:3) (Au = 12 µg). All these 4 solutions were independently irradiated by 740 nm LED at 0.9 W/cm^2 of power. At each minute of time point image was captured by IR thermal imaging camera to measure temperature of solution, exposure was continued till 18 min. Experiment was performed in triplicate and data was plotted by GraphPad Prism.

Photo-thermal stability:

Mito-AuDB-NP solution in beaker was irradiated by 740 nm LED (0.9 W/cm^2) and rise in temperature at each minute of time point till 18 minutes was measured. LED irradiation was stopped to allow cooling of solution to 25–27 °C, measuring the temperature in every minute was continued during the cooling step. Same solution was again irradiated by LED for 18 min followed by cooling. Experiment was continued for 3 cycles. Complete experiment was performed in triplicate and data was plotted by GraphPad Prism.

Photothermal conversion efficiency calculation:

The photothermal conversion efficiency (η) of Mito-AuDB-NPs was calculated using the following equation[4]:

$$\eta = \frac{hs(T_{max} - T_{min}) - Q_{solvent}}{I\,(1 - 10^{-A_\lambda})} \quad \text{---------- (1)}$$

Where, T_{max} and T_{min} are the maximum and minimum temperature of the solution irradiated with 740 nm LED with power density of 0.97 W/cm^2, respectively, h is the heat transfer coefficient, which is calculated as follows:

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \quad \text{---------- (2)}$$

$$t = -\tau_s \, ln(\theta) \quad \text{---------- (3)}$$

$$hs = \frac{m \, c_p}{\tau_s} \quad \text{---------- (4)}$$

here, T is the real–time temperature, T_{max} is the maximum steady temperature attained by the system and T_{surr} is the ambient temperature taken as 23 °C, t is the time corresponding to T during the cooling period, τ_s is the sample system time constant which is obtained from 'time *versus* –Ln (θ)' graph, m is the mass of the sample, c_p is the heat capacity of the sample, and s is the surface area of the container. Furthermore, $Q_{solvent}$ is heat dissipated by the container, which was measured independently by irradiating the container filled with DI water, I is the power of the light irradiated and A_λ is the optical density at 740 nm.

In our case, T_{max} and T_{min} are 54.2 °C and 25.1 °C, respectively. T_{max} with only DI water is 36.4 °C. m is taken 2g and C_p is taken 4.2 J/s °C.

On putting all the values in equation (1), the photothermal conversion efficiency, η was deduced to be 49.7%.

Cell Biology experiments:

Cell viability assay in Dark:
Nearly 5000 HeLa cells per well, were seeded in 96 well plates in 100 µL complete media and incubated for 18h for attachment. Treated with the Mito-AuDB-NP in dose dependent manner and incubated further for 24h. Aspirated media and MTT reagent solution was prepared (0.5 mg/mL) in complete media containing 10% PBS, was added and incubated for 4h to generate formazan crystals. Media was removed carefully without disturbing the crystals, 100 µL DMSO was added and after gentle shaking absorbance for each well was recorded at 570 nm, on Perkin Elmer multimode plate reader. Graph was plotted with GraphPad Prizm.

Cell viability assay in light:
Nearly 5000 HeLa cells per well, were seeded in 96 well plates in 100 µL complete media and incubated for 18h for attachment. Treated with the Mito-AuDB-NP in dose dependent manner, and incubated at 37 °C for 20h, all the well of plate are irradiated by 740 nm LED with 0.9 W/cm^2 of power for 10 minutes then continue the incubation at 37 °C for next 4 hour. Aspirated media and MTT reagent solution prepared (0.5 mg/mL) in complete media containing 10% PBS, is added and incubated for 4h to generate formazan crystals. Media was removed carefully without disturbing the crystals, 100 µL DMSO was added and after gentle shaking absorbance for each well is recorded at 570 nm, on Perkin Elmer multimode plate reader. Graph is plotted with GraphPad Prizm.

ATP Assay:
2500 HeLa cells per well were seeded in 96 well plate and incubated for 16h. Cells are then treated with Mito-AuDB-NP in dose dependent manner and incubated for 24h. Mito-AuDB-NP + LED group cells were then irradiated by 740 nm LED with 0.9 W/cm^2 of power for 10 min, whereas Mito-AuDB-NP group of cells were kept without exposer by LED. Further all the cells were treated with 5 µL of CellTiter-Glo (Promega) per well and shaken on rocker for 10 min. Finally, luminescence for each well was recorded on Perkin Elmer multimode plate reader. Graph was plotted by Graph pad Prizm.

Live Cell Colocalization:
Nearly 25,000 HeLa cells per plate were seeded in 2 live cell plates and incubated overnight for attachment, treated with the Mito-AuDB-NP and incubated for 1h (plate-1) and 3h (Plate 2). Remove media from both the plates and gently washed with PBS. Followed by staining with MitoTracker Red (100 nM) in DMEM, incubated for 20 min. Gently washed twice with PBS, fresh DMEM was added and live cell imaging is performed on Leica confocal instrument. 5% CO_2 and 37 °C temperature is maintained during the experiment.

Mito-morphology:
Nearly 25,000 HeLa cells per plate were seeded in 4 live cell plates (Plate 1: Control, Plate 2: only LED, Plate 3:Au-DB-NP, and Plate 4: Au-DB-NP + LED) and incubated overnight for attachment, Plate 3: Au-DB-NP and Plate 4: Au-

DB-NP + LED are treated with the Mito-AuDB-NP (Au = 4.8 ng/µL, Pt = 1.5 ng/µL and 10-OH-Campto-LA = 1.9 ng/µL), whereas control and only LED are kept untreated, incubate again for 24 hours at 37 °C. After incubation Plate 2: Only LED and Plate 4: Au-DB-NP + LED are irradiated by 740 nm LED with 0.9 W/cm^2 of power for 10 minutes. Remove media from both the plates and gently washed with PBS. Followed by staining with MitoTracker Red (100 nM) in DMEM, incubated for 20 min. Gently washed twice with PBS, fresh DMEM was added and live cell imaging is performed on Leica confocal instrument. 5% CO_2 and 37 °C temperature is maintained during the experiment.

H2DCFDA Assay:
Nearly 25,000 HeLa cells per plate were seeded in 4 live cell plates (Plate 1: Control, Plate 2: only LED, Plate 3:Au-DB-NP, and Plate 4: Au-DB-NP + LED) and incubated overnight for attachment, Plate 3: Au-DB-NP and Plate 4: Au-DB-NP + LED are treated with the Mito-AuDB-NP (Au = 4.8 ng/µL, Pt = 1.5 ng/µL and 10-OH-Campto-LA = 1.9 ng/µL), whereas control and only LED are kept untreated, incubate again for 24 hours at 37 °C. After incubation Plate 2: Only LED and Plate 4: Au-DB-NP + LED are irradiated by 740 nm LED with 0.9 W/cm^2 of power for 10 minutes. Remove media from both the plates and gently washed with PBS. Followed by staining with H2DCFDA (2 µM) in DMEM, incubated for 30 min. Gently washed twice with PBS, fresh DMEM was added and live cell imaging is performed on Leica confocal instrument. 5% CO_2 and 37 °C temperature is maintained during the experiment.

MitoSox Assay:
Nearly 25,000 HeLa cells per plate were seeded in 4 live cell plates (Plate 1: Control, Plate 2: only LED, Plate 3:Au-DB-NP, and Plate 4: Au-DB-NP + LED) and incubated overnight for attachment, Plate 3: Au-DB-NP and Plate 4: Au-DB-NP + LED are treated with the Mito-AuDB-NP (Au = 4.8 ng/µL, Pt = 1.5 ng/µL and 10-OH-Campto-LA = 1.9 ng/µL), whereas control and only LED are kept untreated, incubate again for 24 hours at 37 °C. After incubation Plate 2: Only LED and Plate 4: Au-DB-NP + LED are irradiated by 740 nm LED with 0.9 W/cm^2 of power for 10 minutes. Remove media from both the plates and gently washed with PBS. Followed by staining with MitoSOX™ Red Mitochondrial Superoxide Indicators (500 nM) in DMEM, incubated for 30 min. Gently washed twice with PBS, fresh DMEM was added and live cell imaging is performed on Leica confocal instrument. 5% CO_2 and 37 °C temperature is maintained during the experiment.

TMRM Assay:
Nearly 25,000 HeLa cells per plate were seeded in 4 live cell plates (Plate 1: Control, Plate 2: only LED, Plate 3:Au-DB-NP, and Plate 4: Au-DB-NP + LED) and incubated overnight for attachment, Plate 3: Au-DB-NP and Plate 4: Au-DB-NP + LED are treated with the Mito-AuDB-NP (Au = 4.8 ng/µL, Pt = 1.5 ng/µL and 10-OH-Campto-LA = 1.9 ng/µL), whereas control and only LED are kept untreated, incubate again for 24 hours at 37 °C. After incubation Plate 2: Only LED and Plate 4: Au-DB-NP + LED are irradiated by 740 nm LED with 0.9 W/cm^2 of power for 10 minutes. Remove media from both the plates and gently washed with PBS. Followed by staining with TMRM dye (100 nM) in DMEM, incubated for 20 min. Gently washed twice with PBS, fresh DMEM was added and live cell imaging is performed on Leica confocal instrument. 5% CO_2 and 37 °C temperature is maintained during the experiment.

JC1 Assay:
Nearly 25,000 HeLa cells per plate were seeded in 4 live cell plates (Plate 1: Control, Plate 2:only LED, Plate 3:Au-DB-NP, and Plate 4: Au-DB-NP + LED) and incubated overnight for attachment, Plate 3: Au-DB-NP and Plate 4: Au-DB-NP + LED are treated with the Mito-AuDB-NP (Au = 4.8 ng/µL, Pt = 1.5 ng/µL and 10-OH-Campto-LA = 1.9 ng/µL), whereas control and only LED are kept untreated, incubate again for 24 hours at 37 °C. After incubation Plate 2: Only LED and Plate 4: Au-DB-NP + LED are irradiated by 740 nm LED with 0.9 W/cm^2 of power for 10 minutes. Remove media from both the plates and gently washed with PBS. Followed by staining with JC1 dye (10 µg/mL) in PBS, incubated for 15 min. Gently washed twice with PBS, fresh DMEM was added and live cell imaging is performed on Leica confocal instrument. 5% CO_2 and 37 °C temperature is maintained during the experiment.

FITC Annexin-V assay:
Nearly 25,000 HeLa cells per plate were seeded in 4 live cell plates (Plate 1: Control, Plate 2:only LED, Plate 3:Au-DB-NP, and Plate 4: Au-DB-NP + LED) and incubated overnight for attachment, Plate 3: Au-DB-NP and Plate 4: Au-DB-NP + LED are treated with the Mito-AuDB-NP (Au = 4.8 ng/µL, Pt = 1.5 ng/µL and 10-OH-Campto-LA = 1.9 ng/µL), whereas control and only LED are kept untreated, incubate again for 24 hours at 37 °C. After incubation Plate 2: Only LED and Plate 4: Au-DB-NP + LED are irradiated by 740 nm LED with 0.9 W/cm^2 of power for 10 minutes. Remove media from both the plates and gently washed with PBS. Followed by staining with FITC-conjugated Annexin-V solution (5 µL) from ThermoFischer scientific in DMEM, incubated for 25 min. Gently washed twice with

PBS, fresh DMEM was added and live cell imaging is performed on Leica confocal instrument. 5% CO_2 and 37 °C temperature is maintained during the experiment.

PI Staining:
Nearly 25,000 HeLa cells per plate were seeded in 4 live cell plates (Plate 1: Control, Plate 2:only LED, Plate 3:Au-DB-NP, and Plate 4: Au-DB-NP + LED) and incubated overnight for attachment, Plate 3: Au-DB-NP and Plate 4: Au-DB-NP + LED are treated with the Mito-AuDB-NP (Au = 4.8 ng/µL, Pt = 1.5 ng/µL and 10-OH-Campto-LA = 1.9 ng/µL), whereas control and only LED are kept untreated, incubate again for 24 hours at 37 °C. After incubation Plate 2: Only LED and Plate 4: Au-DB-NP + LED are irradiated by 740 nm LED with 0.9 W/cm^2 of power for 10 minutes. Remove media from both the plates and gently washed with PBS. Followed by staining with Propidium iodide (500 nM) in DMEM, incubated for 15 min. Gently washed twice with PBS, fresh DMEM was added and live cell imaging is performed on Leica confocal instrument. 5% CO_2 and 37 °C temperature is maintained during the experiment. All the confocal images are analysed by image J software and data is plotted using GraphPad prism software.

Immunostaining analysis:
Nearly 50,000 HeLa cells per well seeded in 6 well plate (Group 1: Control, Group 2: only LED, Group 3: Au-DB-NP, and Group 4: Au-DB-NP + LED) on the top of glass coverslip and incubate at 37 °C overnight for attachment. Group 3: Au-DB-NP and Group 4: Au-DB-NP + LED are treated with the Mito-AuDB-NP (Au = 4.8 ng/µL, Pt = 1.5 ng/µL and 10-OH-Campto-LA = 1.9 ng/µL), whereas Group 1: control and Group 2: only LED are kept untreated, incubate again for 24 hours at 37 °C. Group 2: Only LED and Group 4: Au-DB-NP + LED are then irradiated by 740 nm LED with 0.9 W/cm^2 of power for 10 minutes. Cells from all the well were then washed with PBS and permeabilized with PBST (0.2% Tween-20 in PBS) for 15 minutes at room temperature. Cells were then blocked with 1% BSA in PBST (0.1% Tween-20) for 30 minutes at 37 °C. Then washed with PBST and incubated with primary antibody diluted in 1% BSA in PBST for 4 hours at 37 °C. Next washing with PBST and were incubated with Alexa fluor 633 conjugated secondary antibody diluted in 1% BSA in PBST for about 1 hour under dark condition at room temperature. Cells were then washed thrice with PBS and glass coverslip were mounted on clean glass side using SlowFade Gold antifade with DAPI reagent and incubated overnight at 4 °C. Images are captured on Laica confocal instrument. Intensity calculation analysis of done using Image J software.

Western Blot analysis:

1 million cells were seeded in three 6 cm tissue culture plate (Plate 1: Control, Plate 2: Au-DB-NP, and Plate 3: Au-DB-NP + LED) and incubate overnight for attachment. Plate 2: Au-DB-NP and Plate 3: Au-DB-NP + LED are treated with the Mito-AuDB-NP (Au = 4.8 ng/µL, Pt = 1.5 ng/µL and 10-OH-Campto-LA = 1.9 ng/µL), whereas Plate 1: control cells are kept untreated, incubate again for 24 hours at 37 °C. Plate 3: Au-DB-NP + LED treated cells are then irradiated by 740 nm LED with 0.9 W/cm^2 of power for 10 minutes. Cells were then washed with PBS, trypsinised and then lysed by lysis buffer. Protein concentration is evaluated by Bradford assay. Whole proteins are then resolved using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE), and transfer to nitrocellulose membrane. Block with 5 % milk in TBST and incubated overnight at 4 °C by primary antibody (concentration and solution are used as recommended on supplier's web page), followed by washing with TBST and incubated by HRP-Conjugated secondary antibody for 2-3 hours. The blot was developed using clarity Enhanced Chemiluminescence (ECL) substrate and images were captured on Gel Doc instrument. Analysis is performed using image lab software.

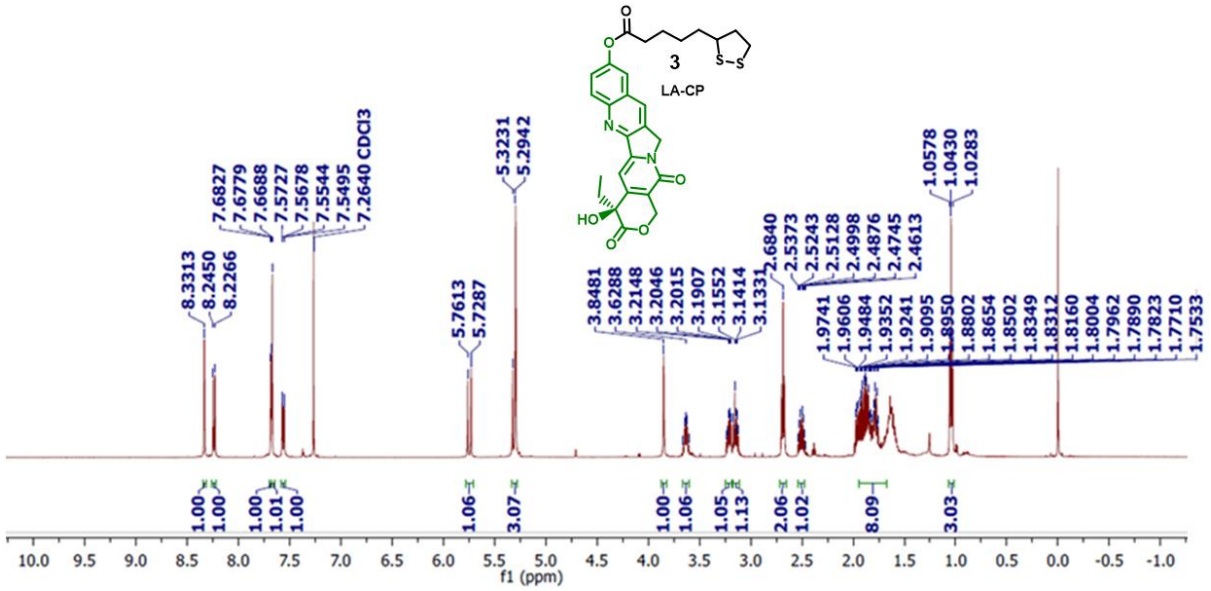


Fig. S1: ^{1}H NMR spectra of compound 3.

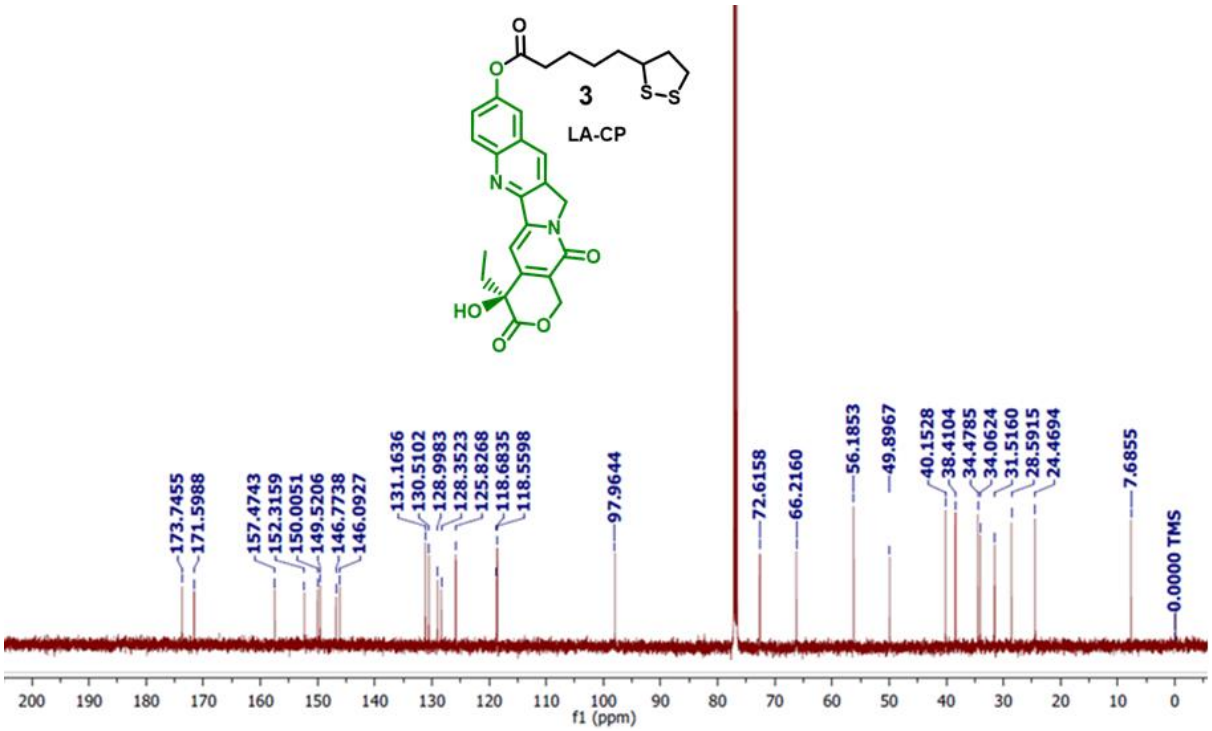


Fig. S2: ^{13}C NMR spectra of compound 3.

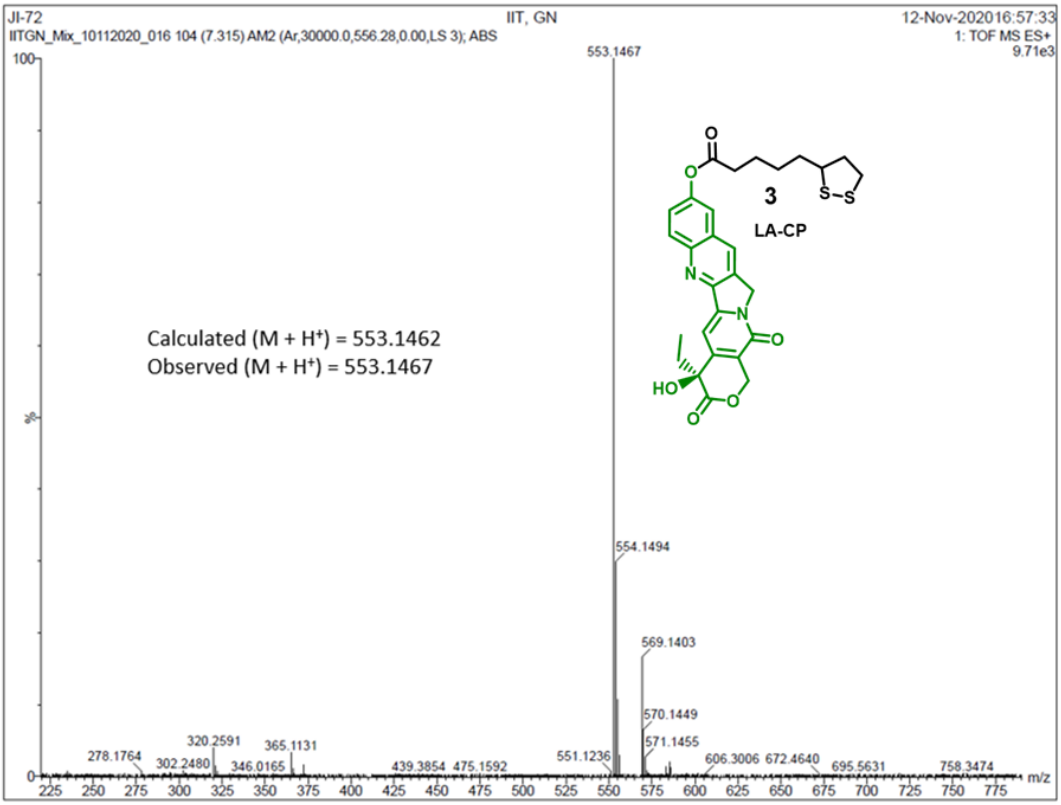


Fig. S3: HR-MS spectra of compound 3.

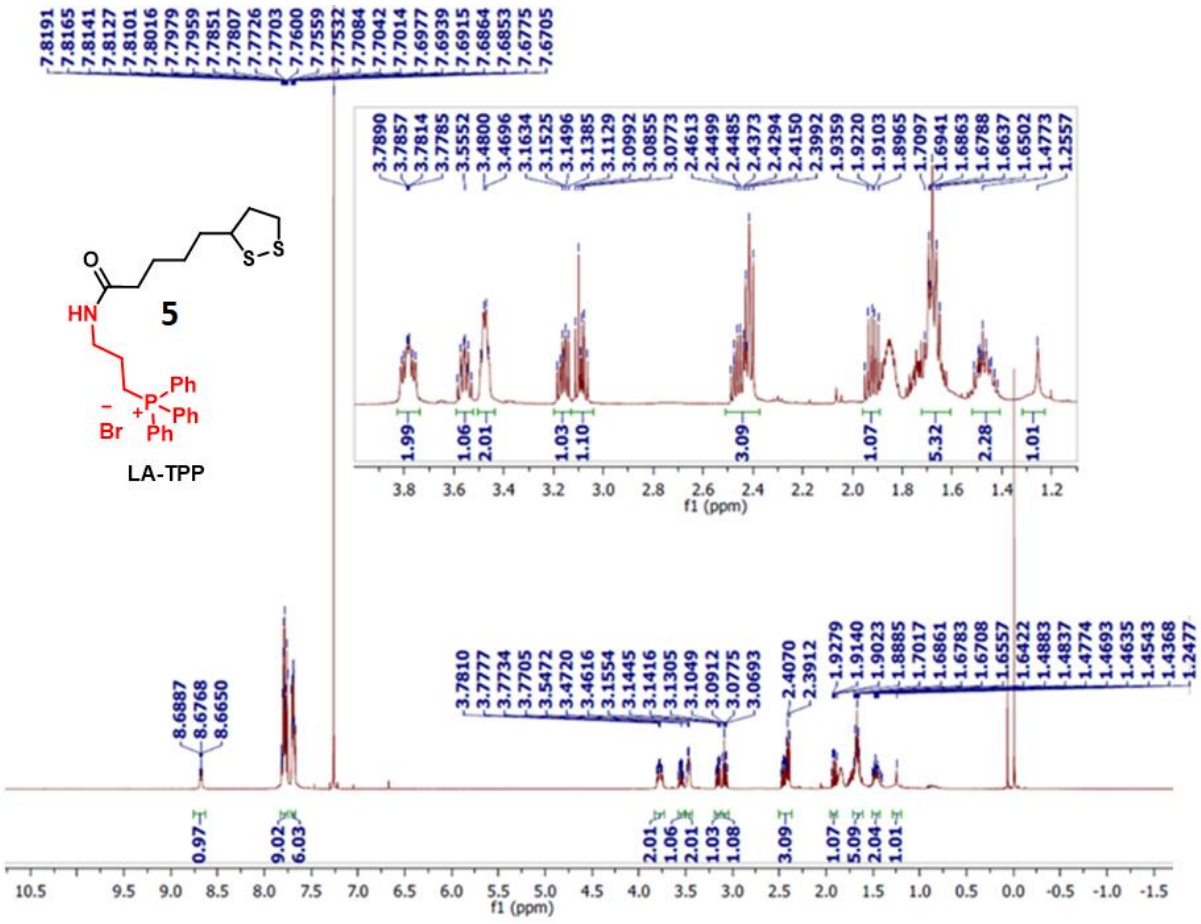


Fig. S4: ^{1}H NMR spectra of compound 5.

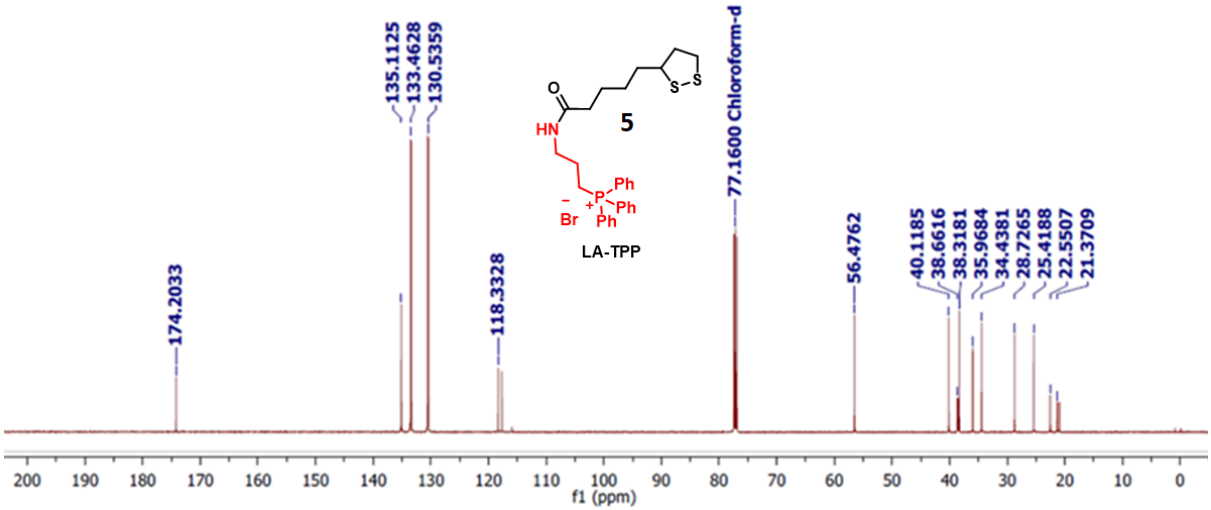


Fig. S5: ^{13}C NMR spectra of compound 5.

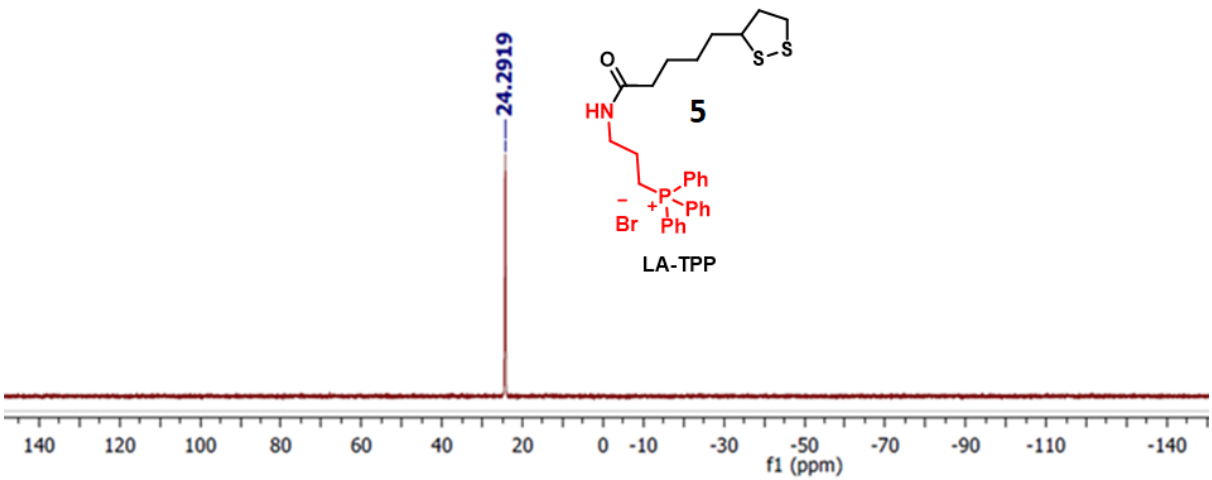


Fig. S6: ^{31}P NMR spectra of compound 5.

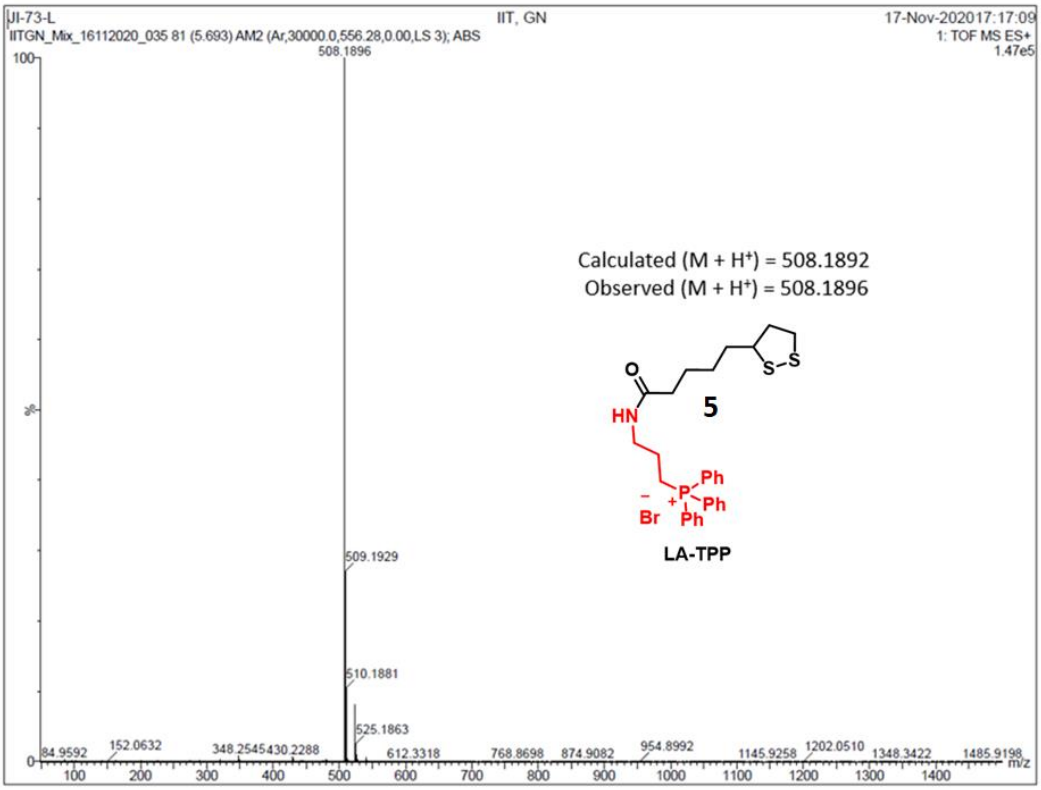


Fig. S7: HR-MS spectra of compound 5.

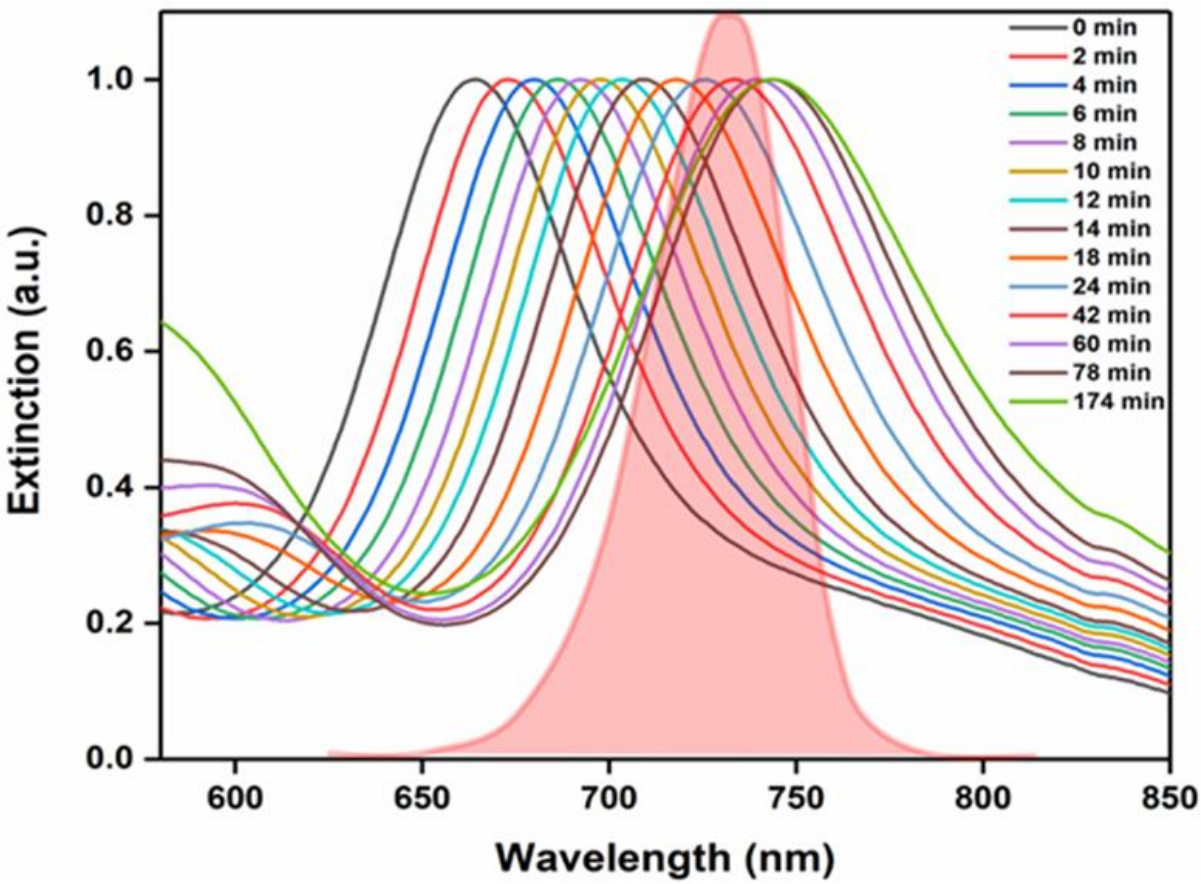


Fig. S8: Change of the longitudinal surface plasmon resonance of gold dog-bone-shaped nanoparticles (Au-DB) during their synthesis. The growth process could be stopped at the desired LSPR wavelength to match the LED excitation wavelength (shown as the red coloured shaded area).

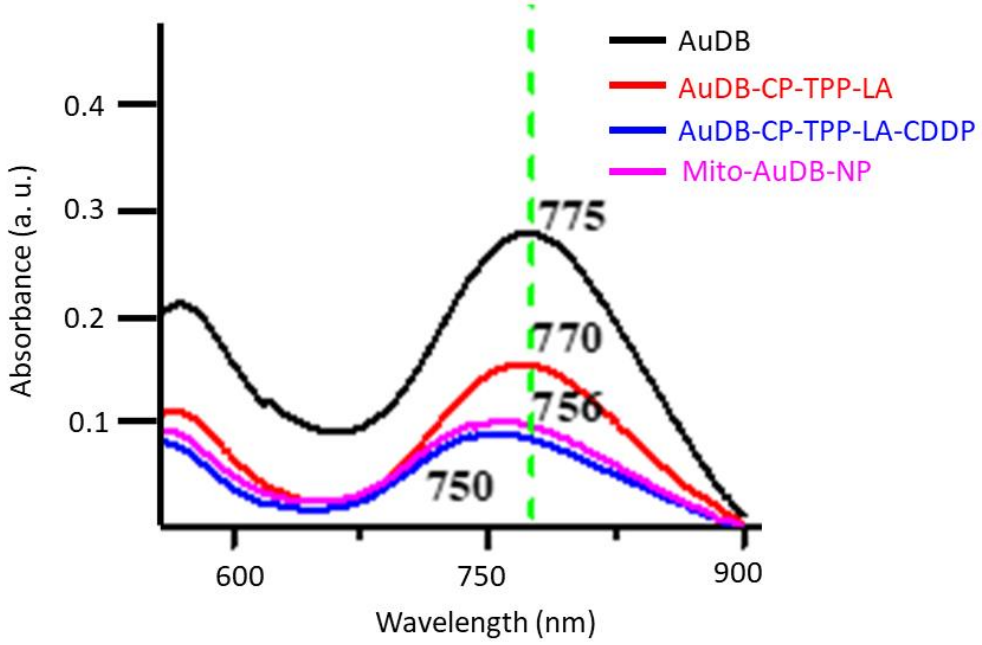


Fig. S9: UV-Vis spectra of AuDB, AuDB-CP-TPP-LA, AuDB-CP-TPP-LA-CDDP and Mito-AuDB-NPs.

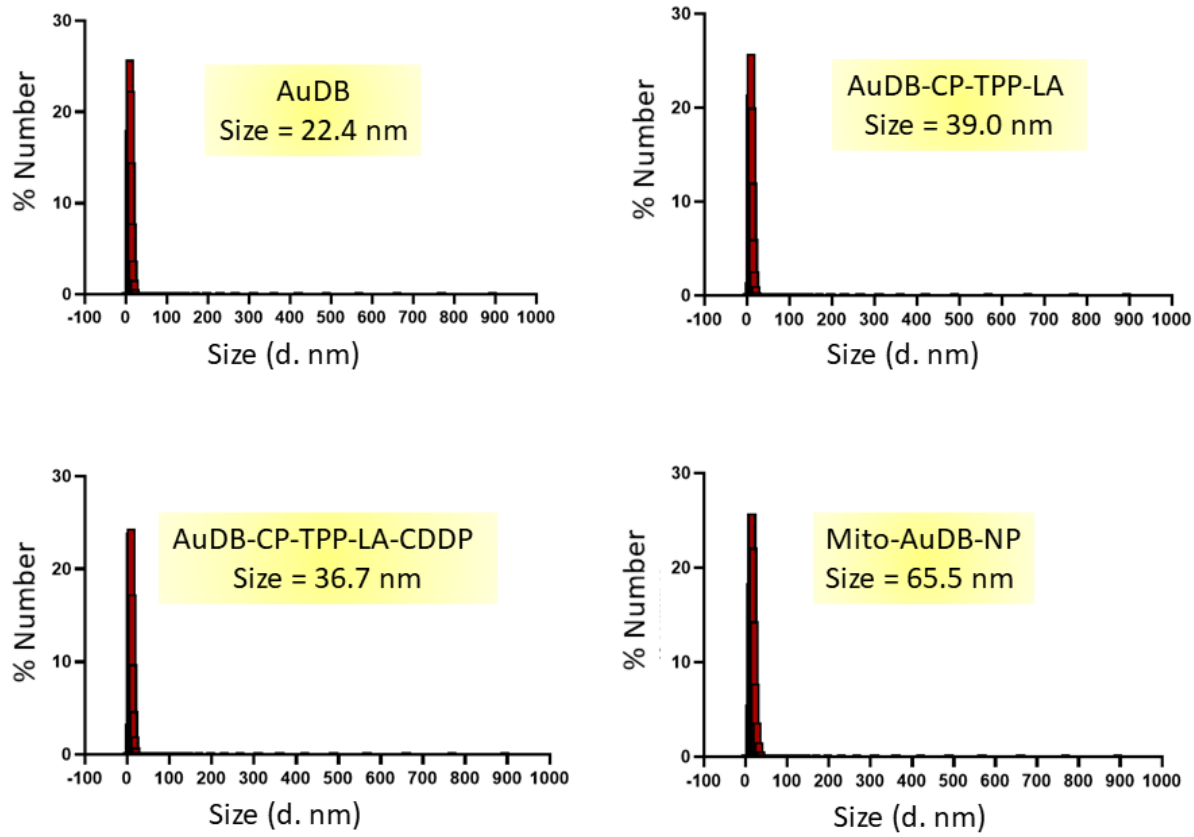
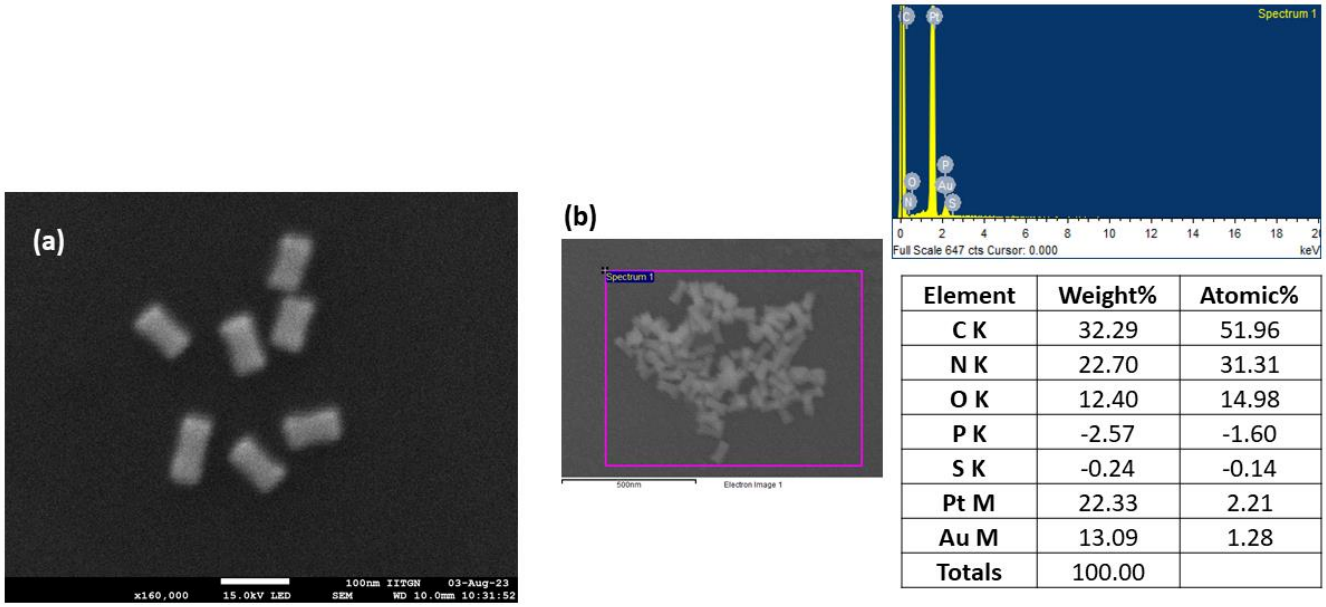


Fig. S10: Size distribution of AuDB, AuDB-CP-TPP-LA, AuDB-CP-TPP-LA-CDDP and Mito-AuDB-NP by dynamic light scattering (DLS).



Element	Weight%	Atomic%
C K	32.29	51.96
N K	22.70	31.31
O K	12.40	14.98
P K	-2.57	-1.60
S K	-0.24	-0.14
Pt M	22.33	2.21
Au M	13.09	1.28
Totals	100.00	

Fig. S11: (a) FESEM image of Mito-AuDB-NPs. (b) Determination of Pt and Au content in Mito-AuDB-NPs by EDAX.

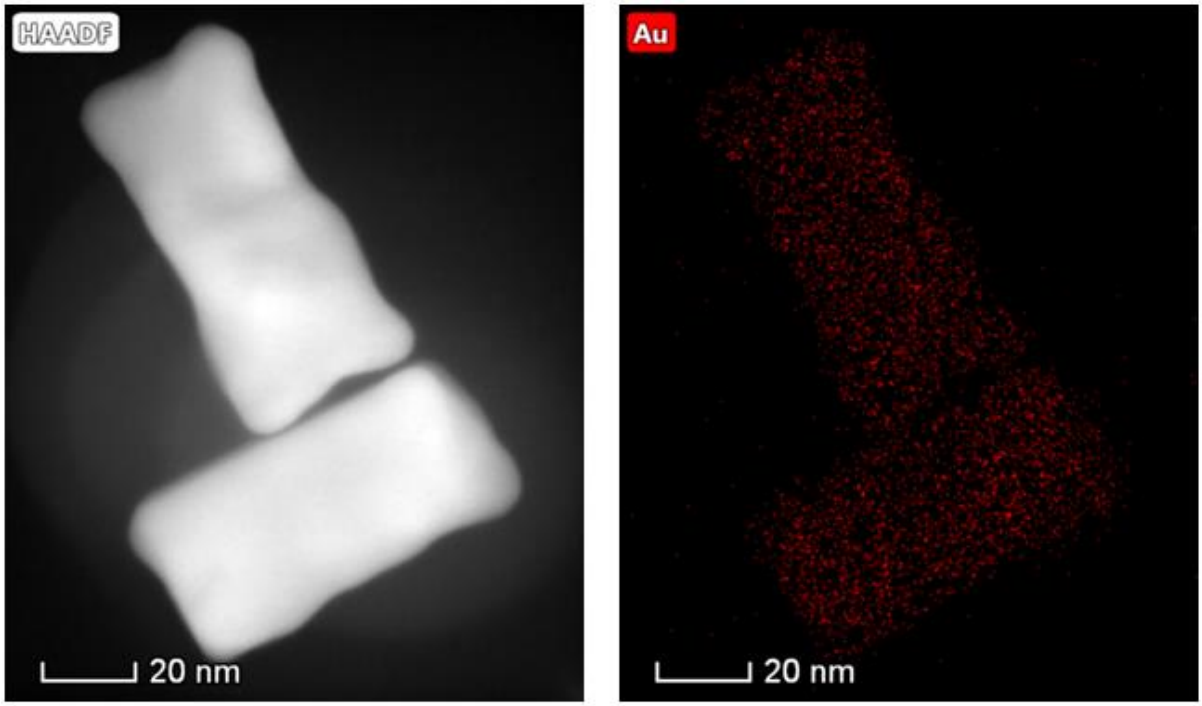


Fig. S12: Elemental mapping of Au in Mito-AuDB-NPs from TEM.

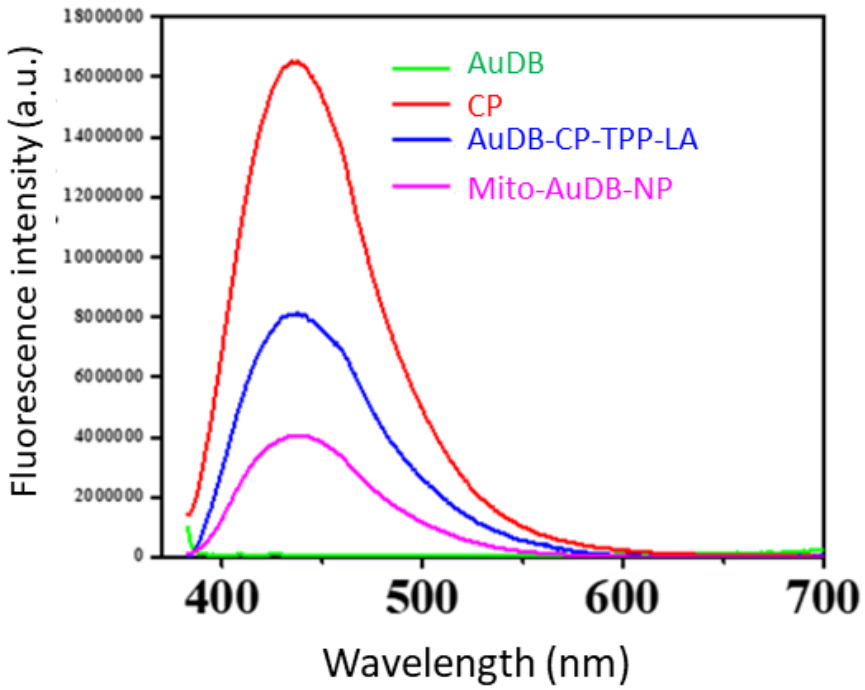


Fig. S13: Fluorescence emission spectra of AuDB, 10-hydroxycamptothecin (CP), AuDB-CP-TPP-LA and Mito-AuDB-NPs to confirm the presence of CP.

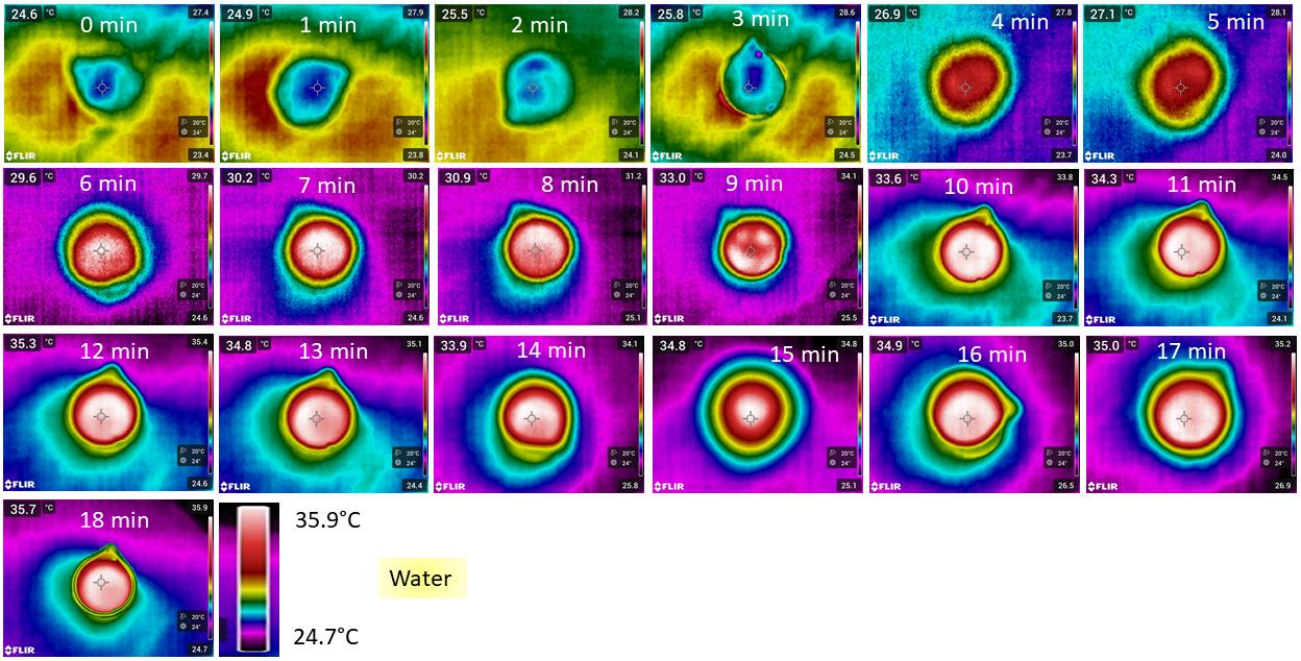


Fig. S14: Time dependent temperature change by the infrared thermal imaging instrument of water as control solvent.

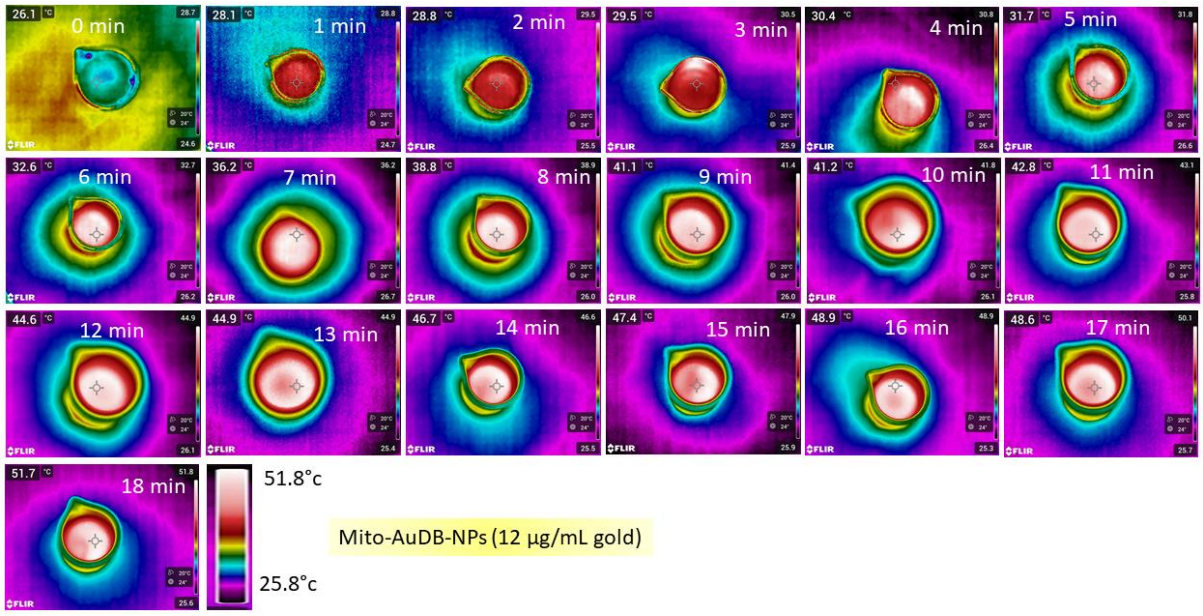


Fig. S15: Time dependent temperature change by the infrared thermal imaging instrument of Mito-AuDB-NPs at 12 µg/mL gold concentration.

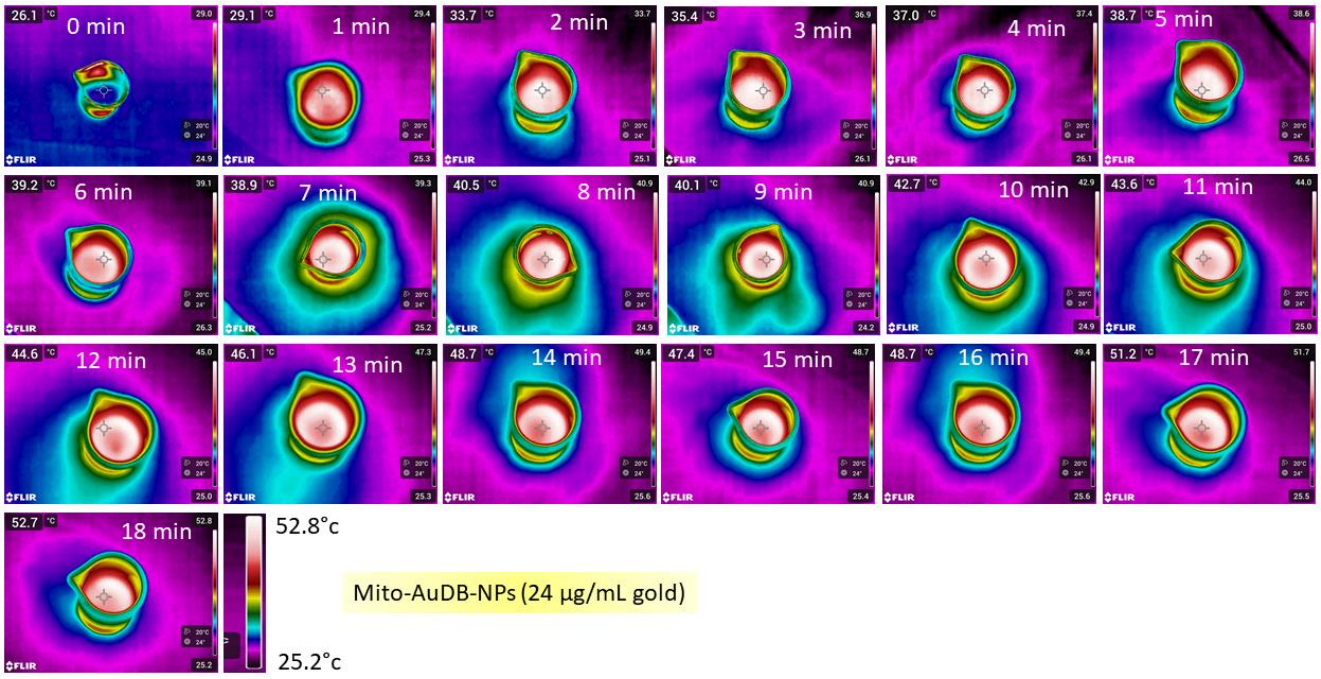


Fig. S16: Time dependent temperature change by the infrared thermal imaging instrument of Mito-AuDB-NPs at 24 µg/mL gold concentration.

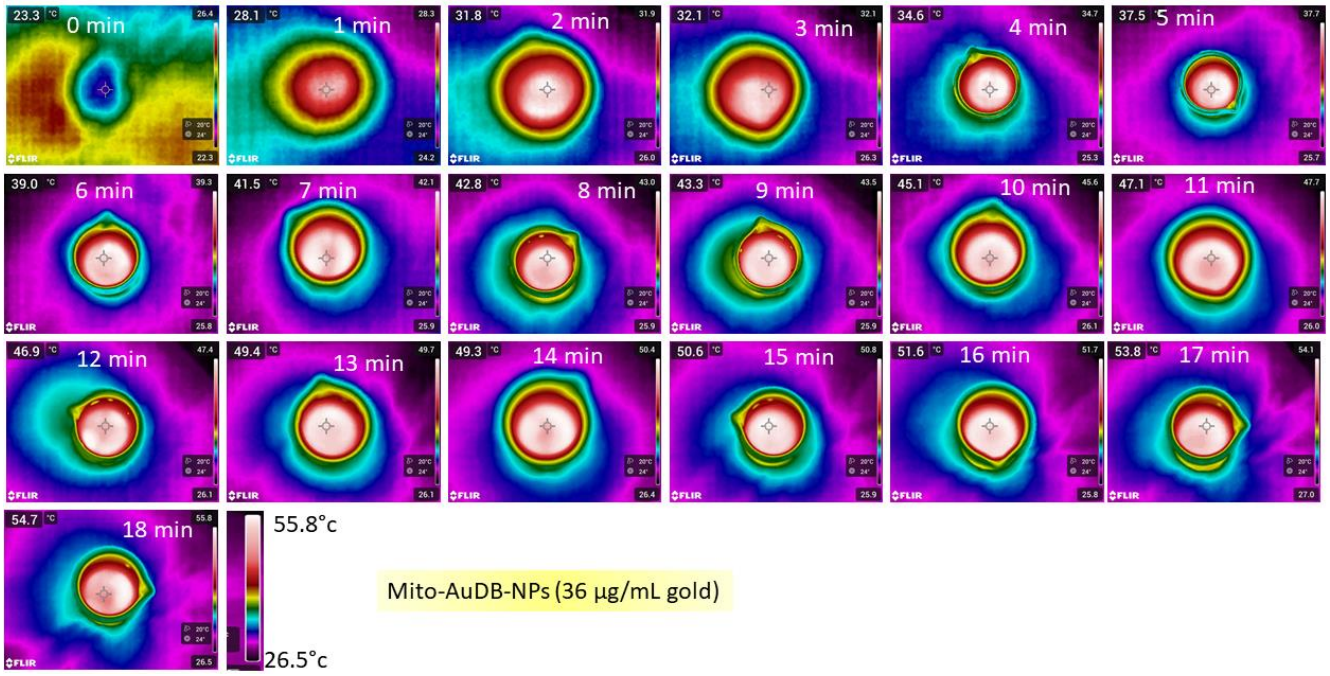


Fig. S17: Time dependent temperature change by the infrared thermal imaging instrument of Mito-AuDB-NPs at 36 µg/mL gold concentration.

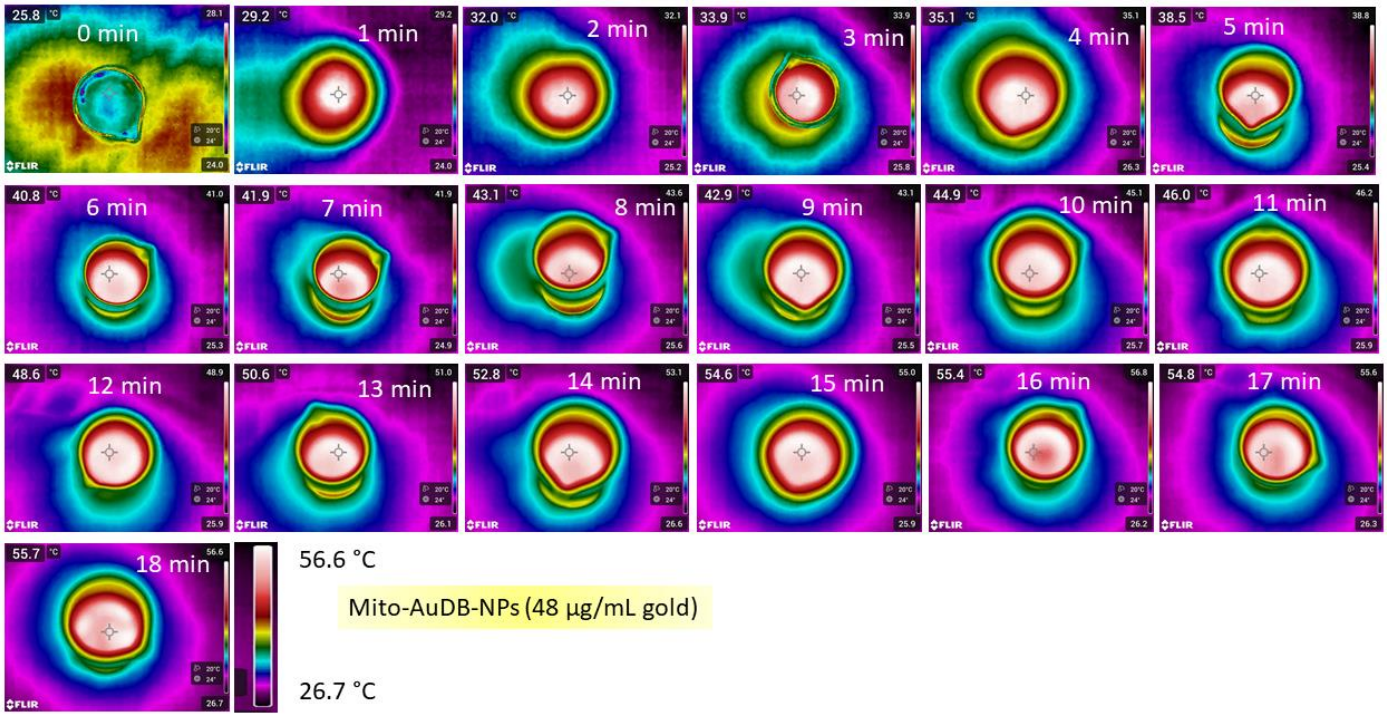


Fig. S18: Time dependent temperature change by the infrared thermal imaging instrument of Mito-AuDB-NPs at 48 µg/mL gold concentration.

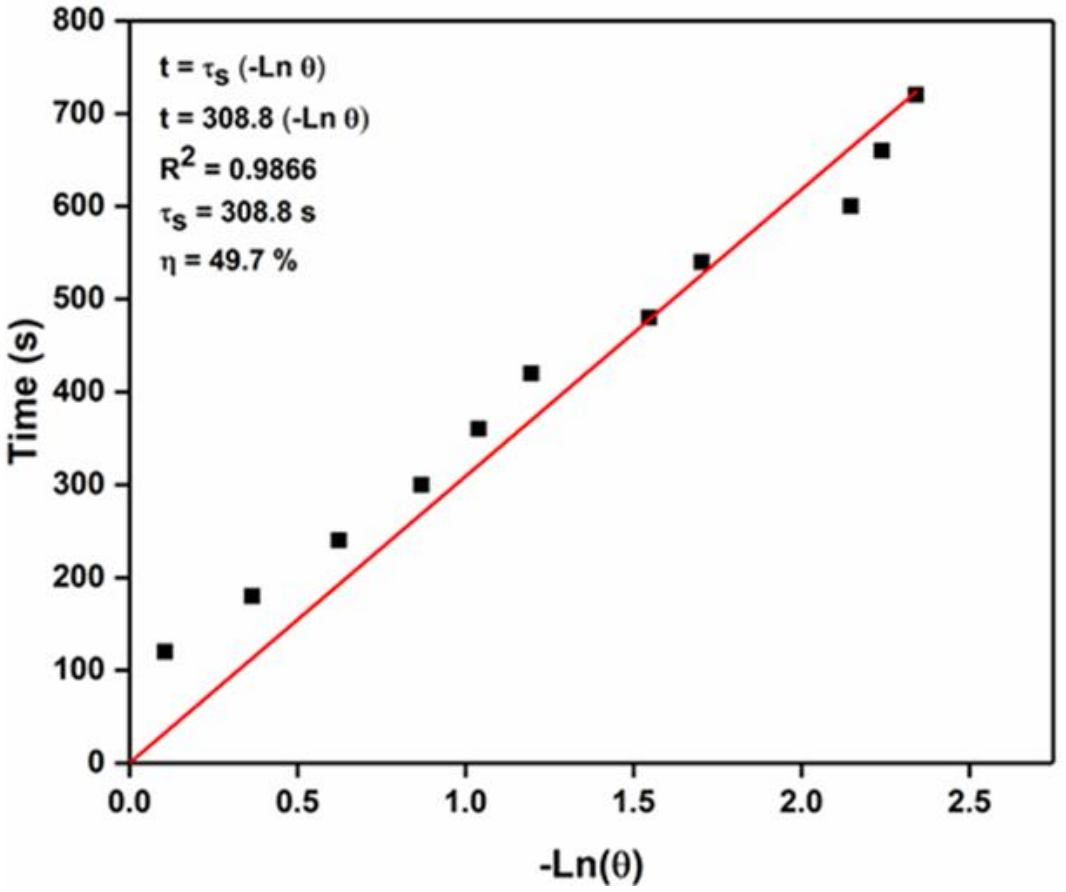


Fig. S19: Sample system time constant τ_s was determined to be 308.8s, from the slope of 'time *versus* –Ln (θ)' graph, plotted for the cooling period (after 840 s) of AuDB NPs.

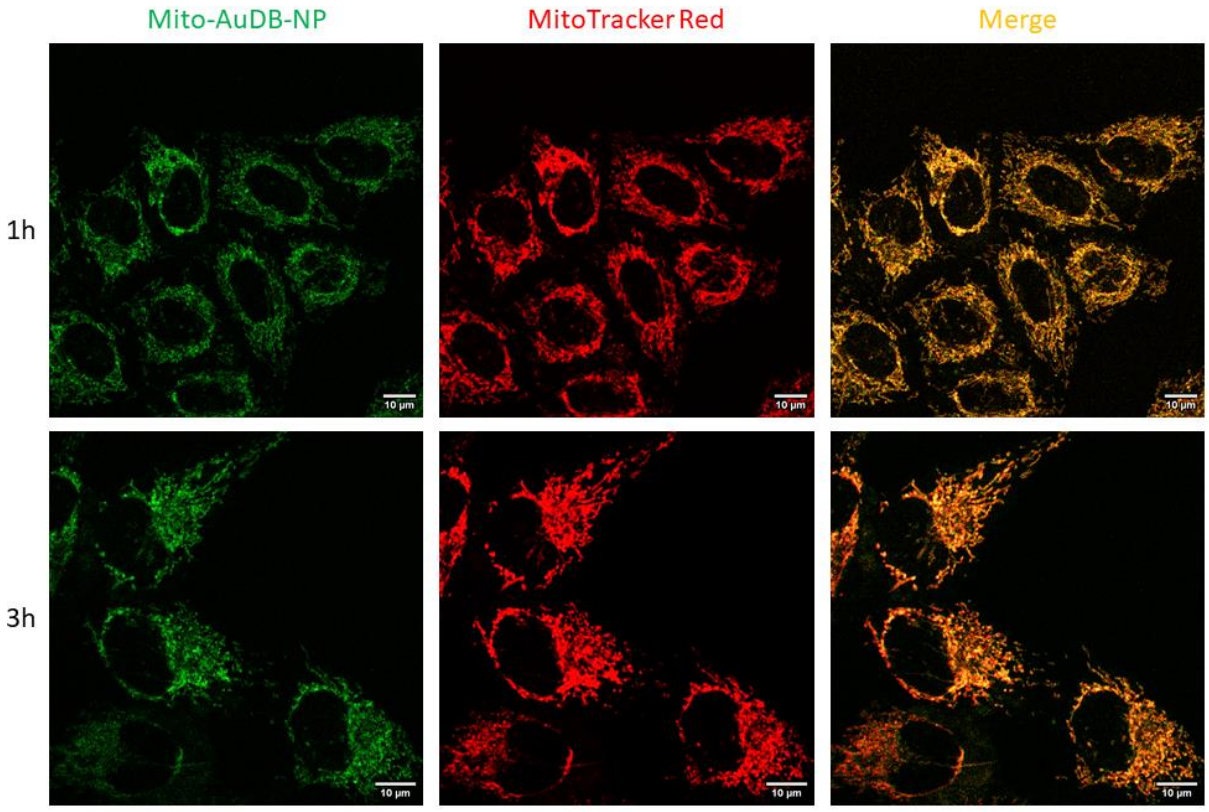


Fig. S20: Confocal laser scanning microscopy images of HeLa cells after incubating with the Mito-AuDB-NPs for 1h and 3h followed by staining the mitochondrial with MitoTracker Red dye. Scale bar = 10 μm.

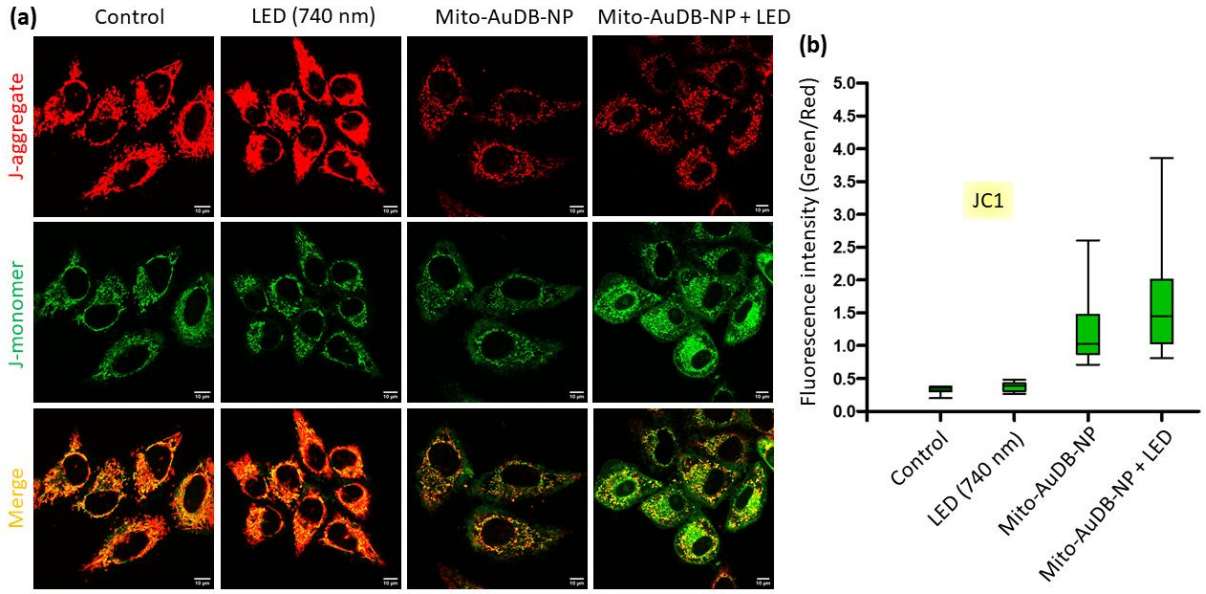


Fig. S21: (a) Confocal laser scanning microscopy images of the HeLa cells after incubating with Mito-AuDB-NPs for 24h followed by irradiating with or without 740 nm NIR LED. The cells were stained with JC1 dye. Scale bar = 10 μm. (b) Quantification of the green: red fluorescence intensity from the confocal images for different treatment groups.

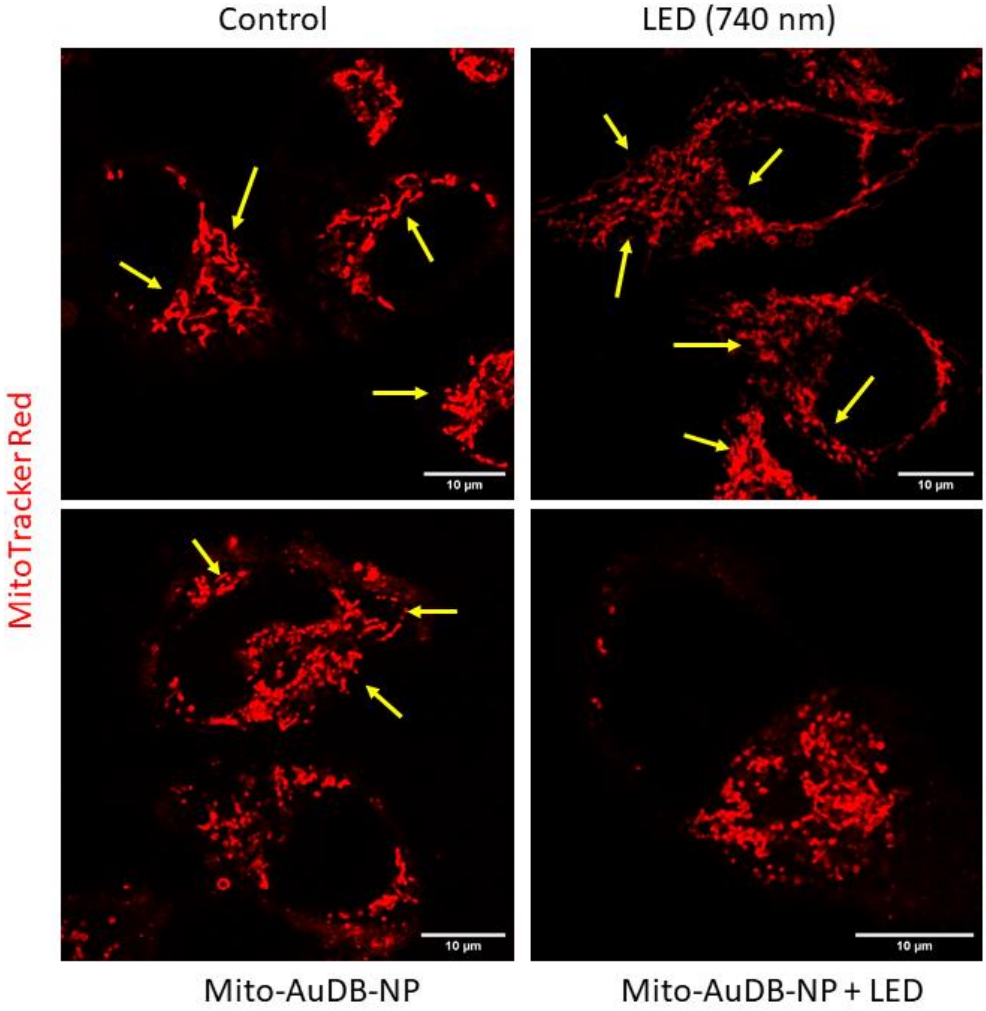


Fig. S22: Confocal laser scanning microscopy images of the HeLa cells after treatment with Mito-AuDB-NPs for 24h followed by irradiating with or without 740 nm NIR LED for 10 min. The mitochondria were stained with MitoTracker Red dye to visualize the mitochondrial morphology damage. Scale bar = 10 µm.

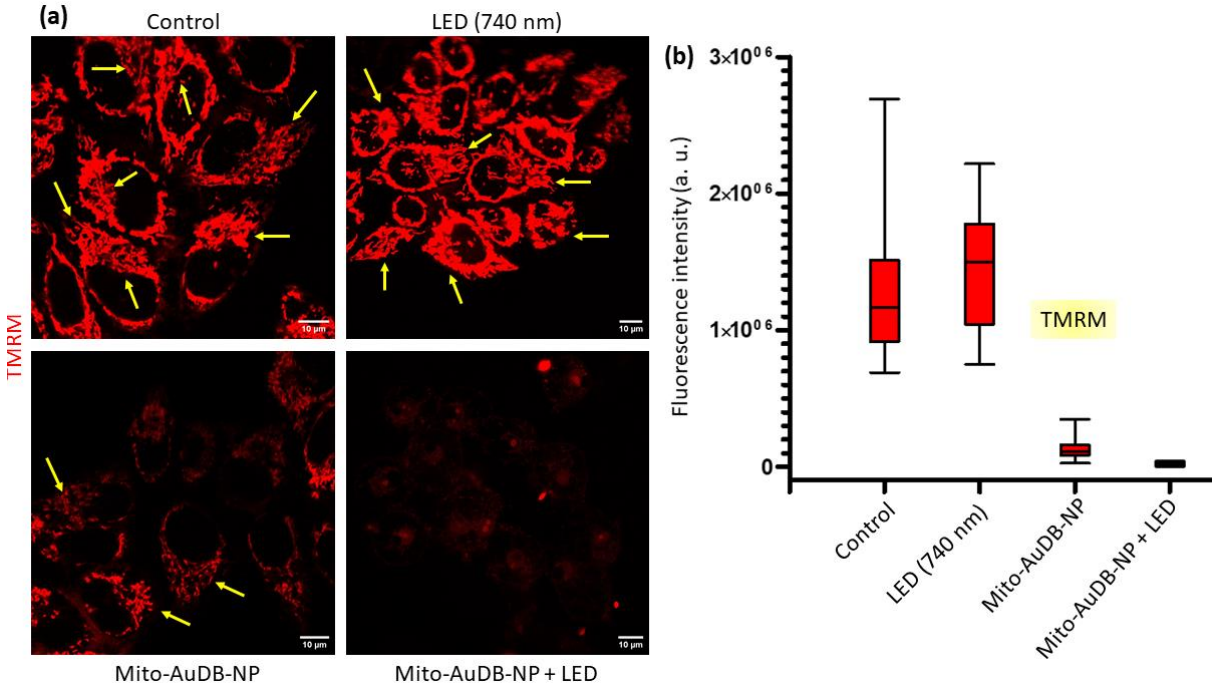


Fig. S23: (a) Confocal laser scanning microscopy of HeLa cells treated with Mito-AuDB-NPs for 24h followed by irradiation with/without 740 nm NIR LED for 10 min. The cells were then stained with TMRM dye. Scale bar = 10 µm. (b) Quantification of the red fluorescence intensity from the confocal images in different treatment groups.

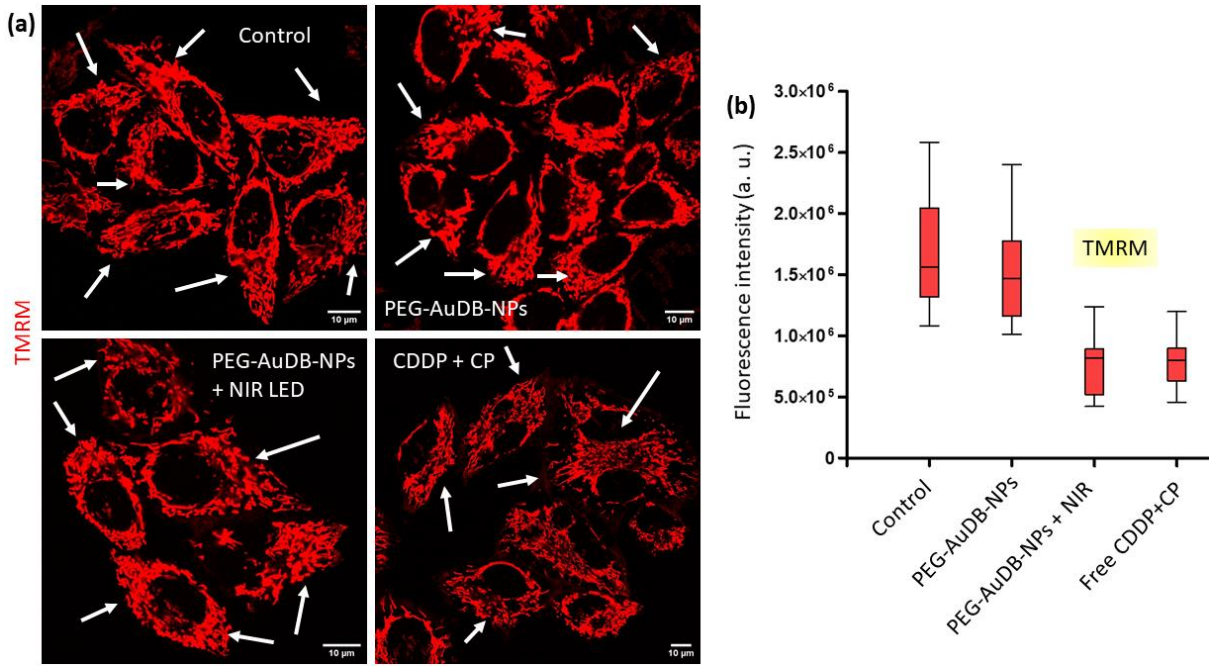


Fig. S24: (a) Confocal laser scanning microscopy of HeLa cells treated with PEG-AuDB-NPs for 24h followed by irradiation with/without 740 nm NIR LED for 10 min. As control, HeLa cells were treated with CDDP and CP drug combinations for 24h. The cells were then stained with TMRM dye. Scale bar = 10 µm. (b) Quantification of the red fluorescence intensity from the confocal images in different treatment groups.

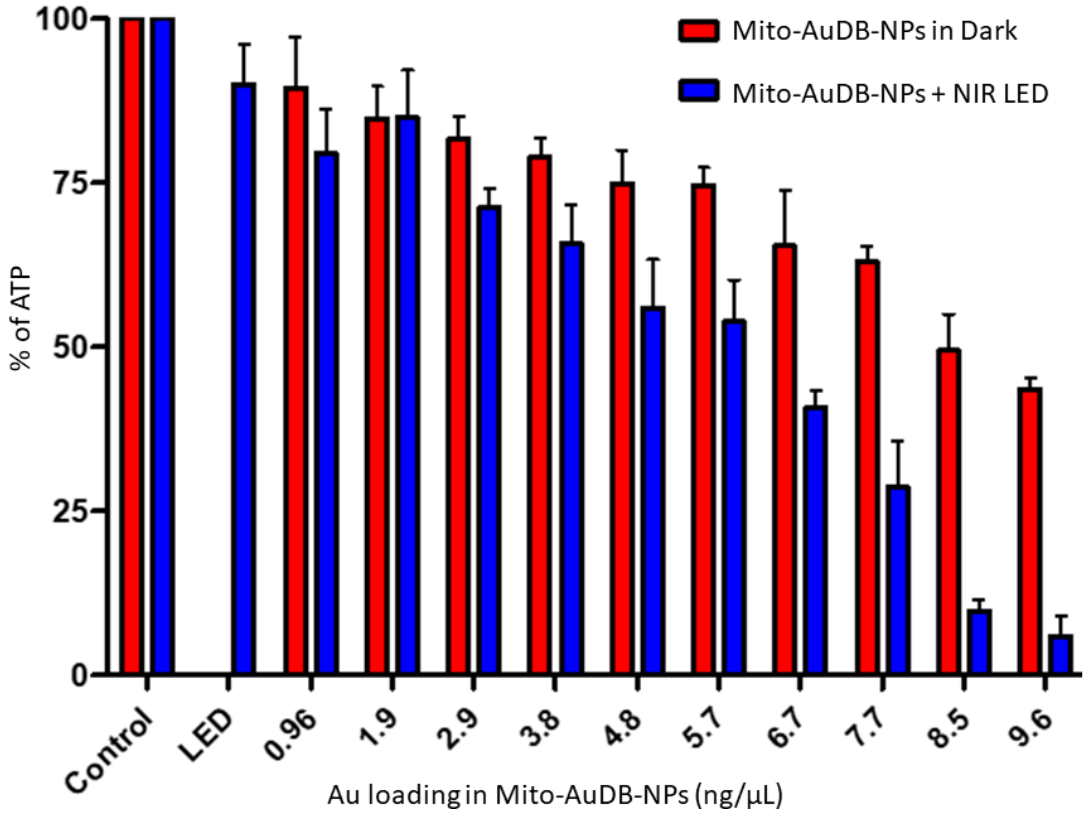


Fig. S25: Quantification of sub-cellular ATP by CellTiter-Glo assay in HeLa cells after treatment with dose dependent Mito-AuDB-NPs for 24h followed by irradiating them with or without 740 nm NIR LED.

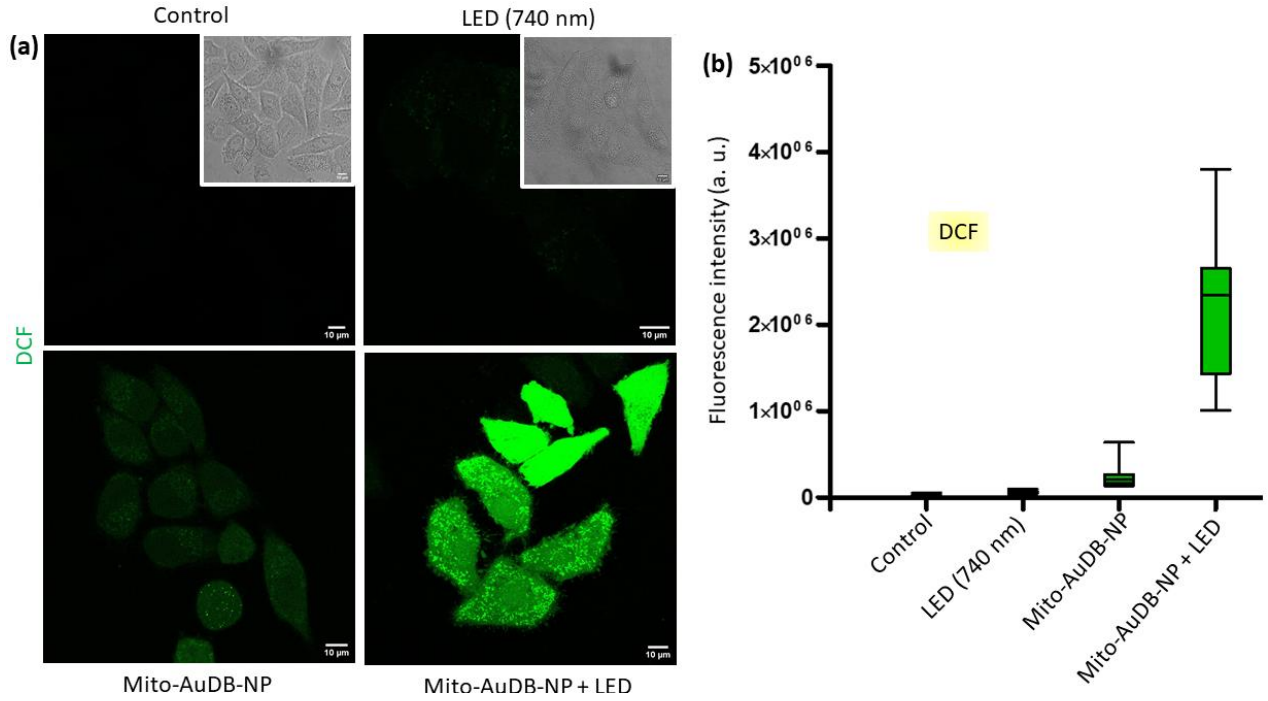


Fig. S26: (a) Confocal laser scanning microscopy of HeLa cells treated with Mito-AuDB-NPs for 24h followed by irradiation with/without 740 nm NIR LED for 10 min. The cells were then incubated with the H2DCFDA dye to visualize ROS generation. Scale bar = 10 µm. Inset: Bright field images of the HeLa cells, Scale bar = 10 µm (b) Quantification of the green fluorescence intensity from the confocal images in different treatment groups.

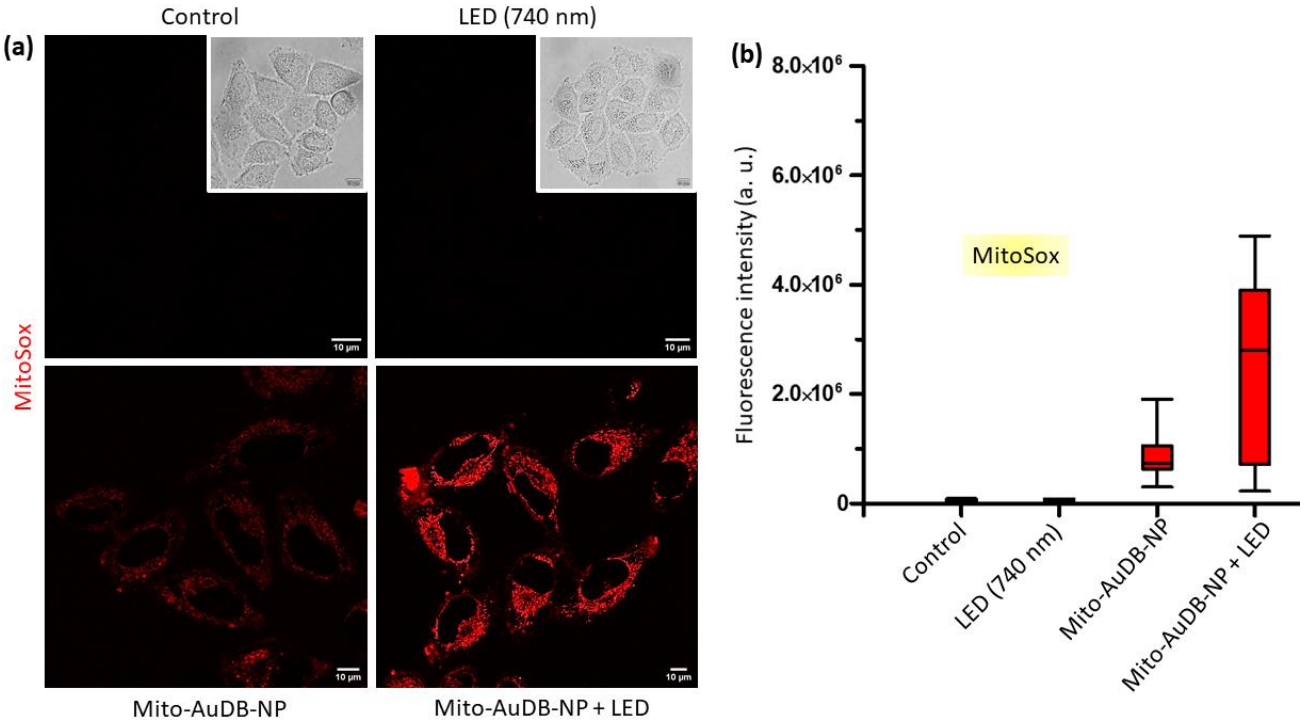


Fig. S27: (a) Confocal laser scanning microscopy of HeLa cells treated with Mito-AuDB-NPs for 24h followed by irradiation with/without 740 nm NIR LED for 10 min. The cells were then incubated with MitoSox dye to visualize the mitochondrial superoxide generation. Scale bar = 10 µm. Inset: Bright field images of the HeLa cells, Scale bar = 10 µm (b) Quantification of the red fluorescence intensity from the confocal images in different treatment groups.

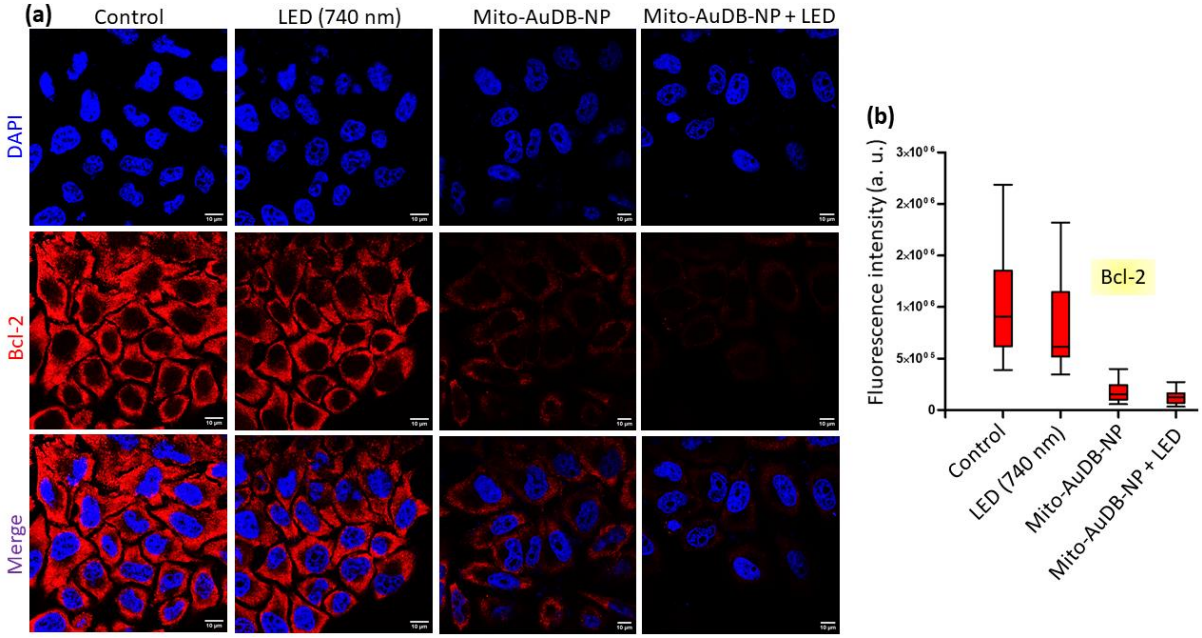


Fig. S28: (a) Confocal laser scanning microscopy of HeLa cells treated with Mito-AuDB-NPs for 24h followed by irradiation with/without 740 nm NIR LED for 10 min. The cells were then treated with Bcl-2 specific primary antibody followed by Alexa Fluor 633-labelled secondary antibody (red) to visualize sub-cellular Bcl-2. Scale bar = 10 µm. (b) Quantification of the red fluorescence intensity from the confocal images in different treatment groups.

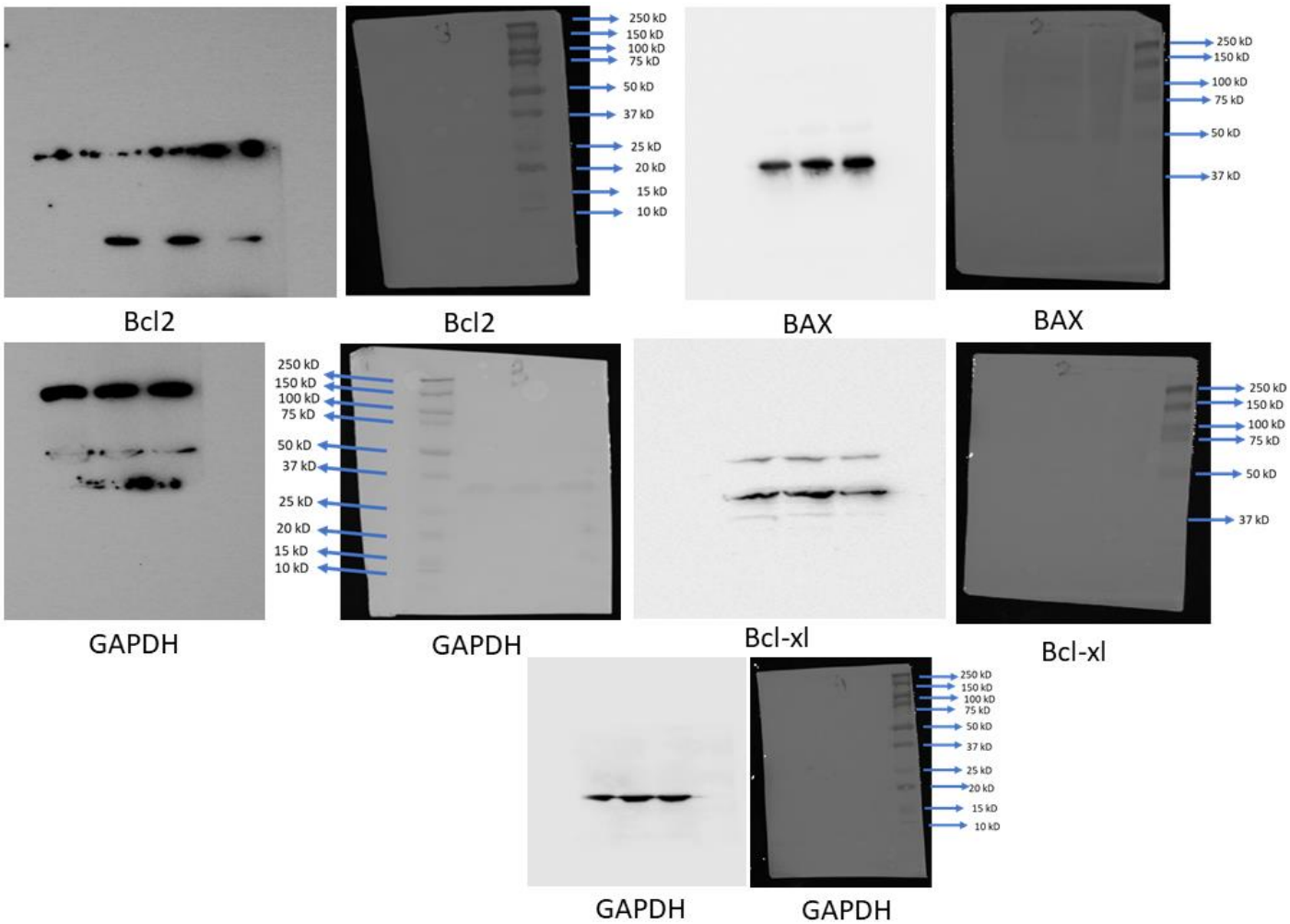


Fig. S29: Uncropped gel electrophoresis images of blots of Bcl-2, Bcl-xl, BAX and GAPDH.

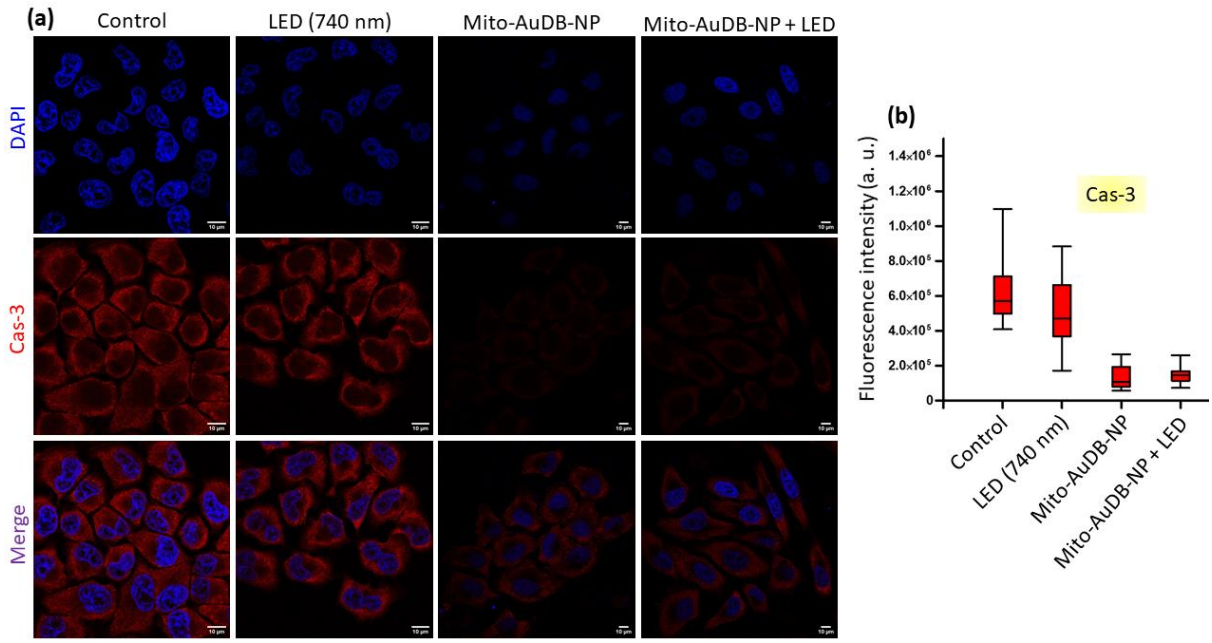


Fig. S30: (a) Confocal laser scanning microscopy of HeLa cells treated with Mito-AuDB-NPs for 24h followed by irradiation with/without 740 nm NIR LED for 10 min. The cells were then treated with Caspase-3 specific primary antibody followed by Alexa Fluor 633-labelled secondary antibody (red) to visualize sub-cellular Caspase-3. Scale bar = 10 µm. (b) Quantification of the red fluorescence intensity from the confocal images in different treatment groups.

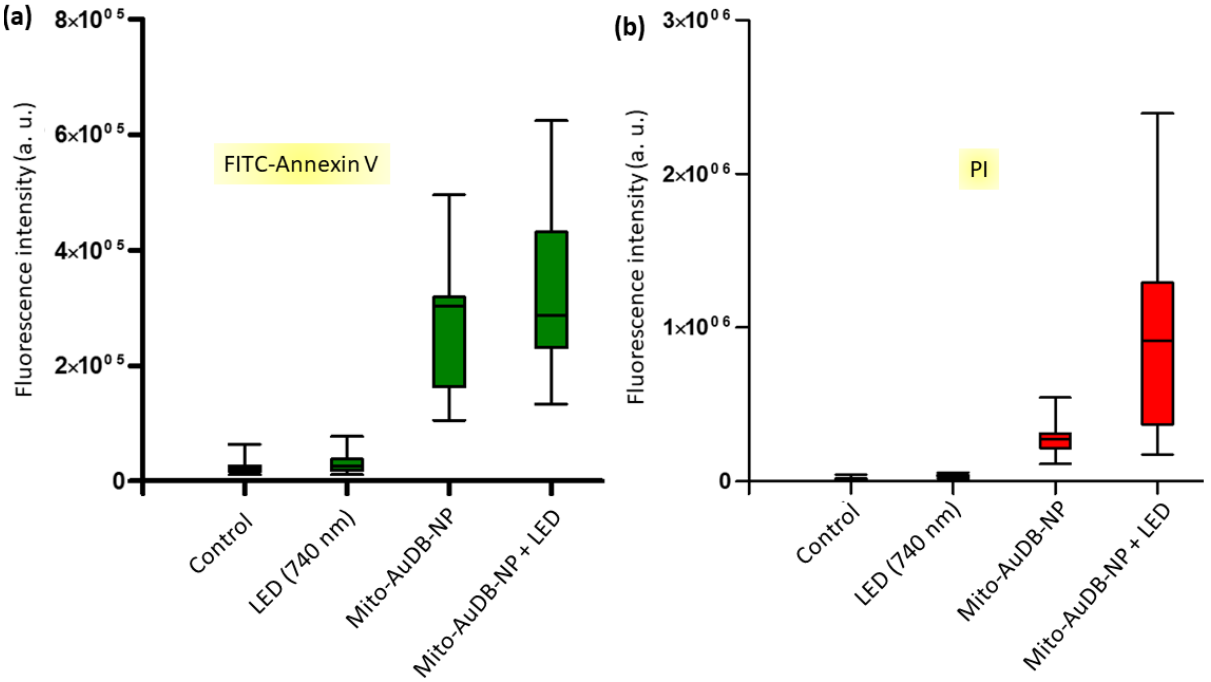


Fig. S31: Quantification of the (a) green fluorescence and (b) Red fluorescence intensity from the confocal images of the HeLa cells after treatment with Mito-AuDB-NPs for 24h followed by irradiation with/without 740 nm NIR LED for 10 min. The cells were then stained with Annexin-V-FITC and PI respectively.

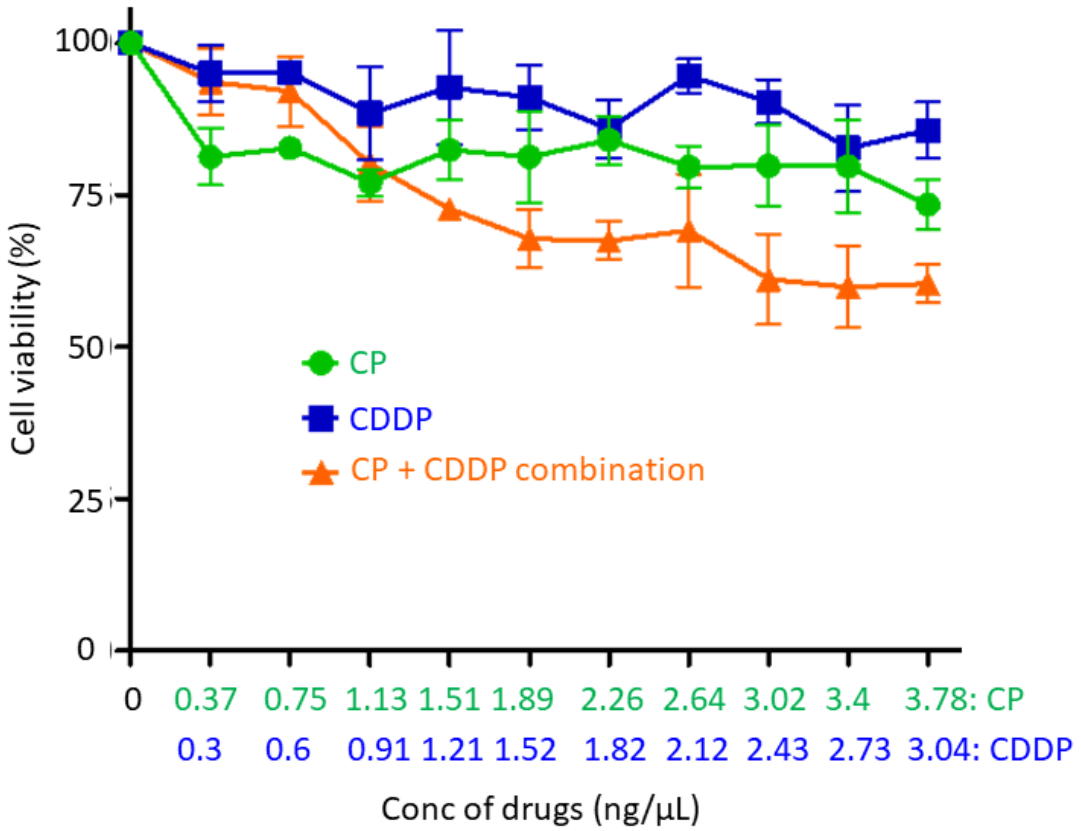


Fig. S32: Viability of the HeLa cells after dose-dependent treatment with CDDP, CP and drug combinations for 24h, measured by MTT assay.

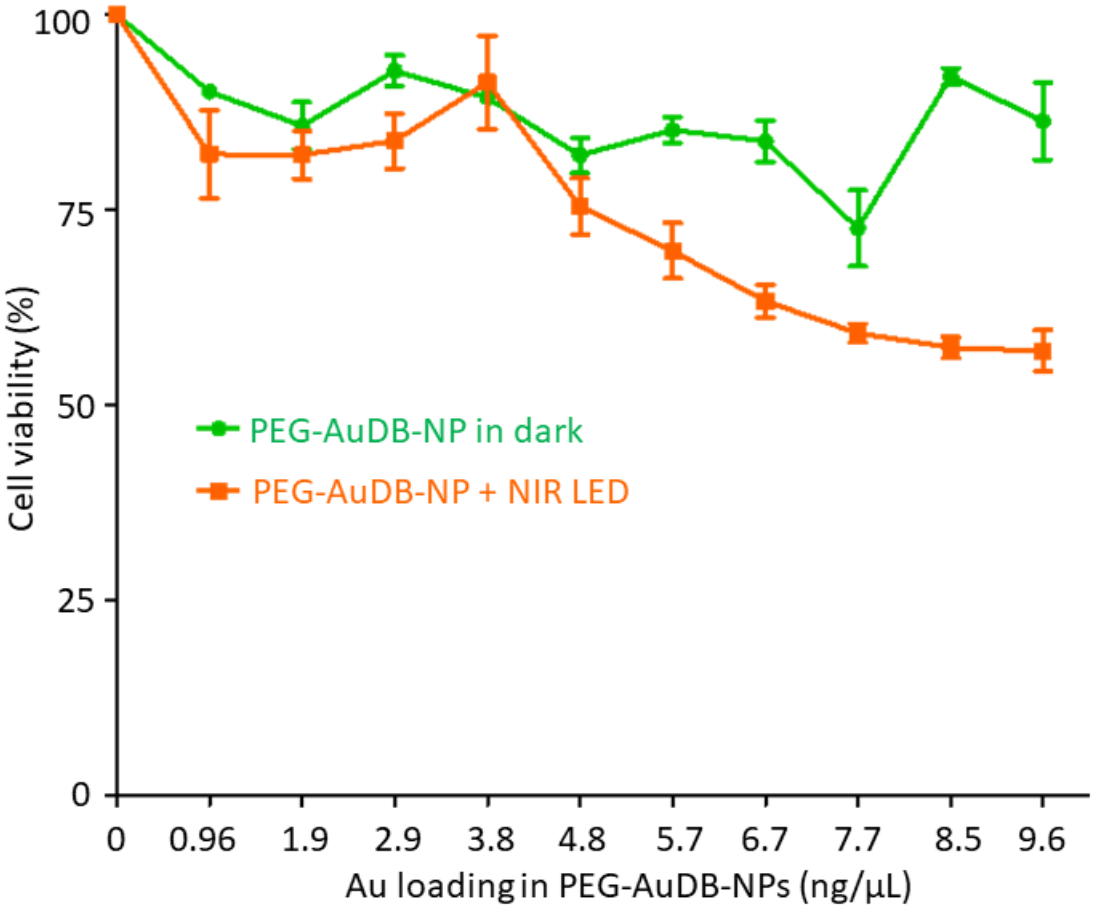


Fig. S33: Viability of the HeLa cells after dose-dependent treatment with PEG-AuDB-NPs for 24h followed by irradiating them with or without 740 nm NIR LED.

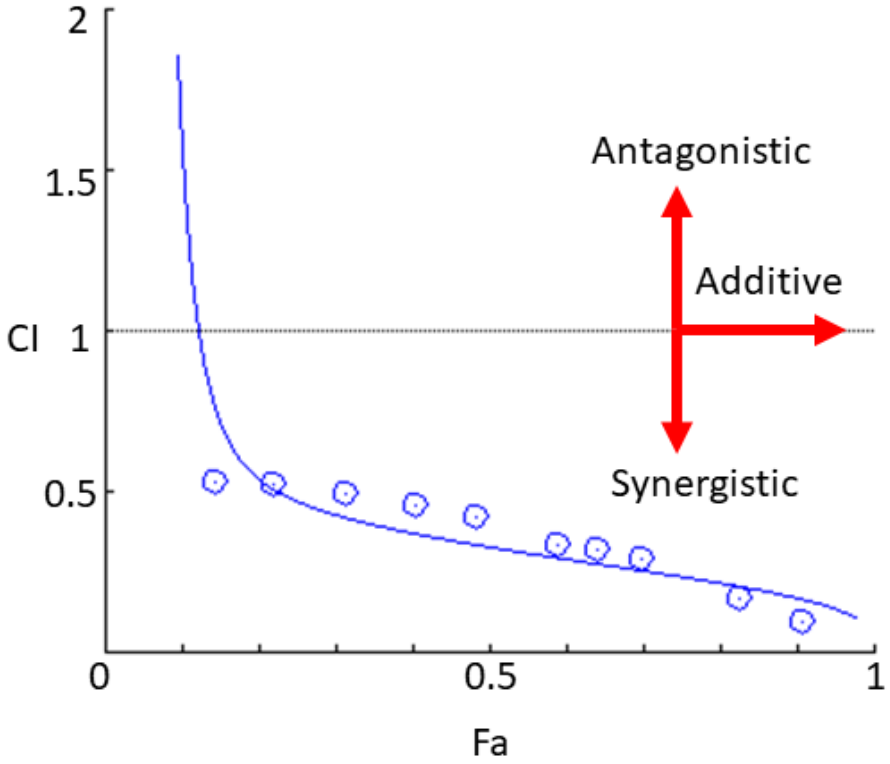


Fig. S34: Chou-Talaly analysis of Mito-AuDB-NPs under irradiation of 740 nm NIR LED in HeLa cells to determine synergistic, additive and antagonistic effects.

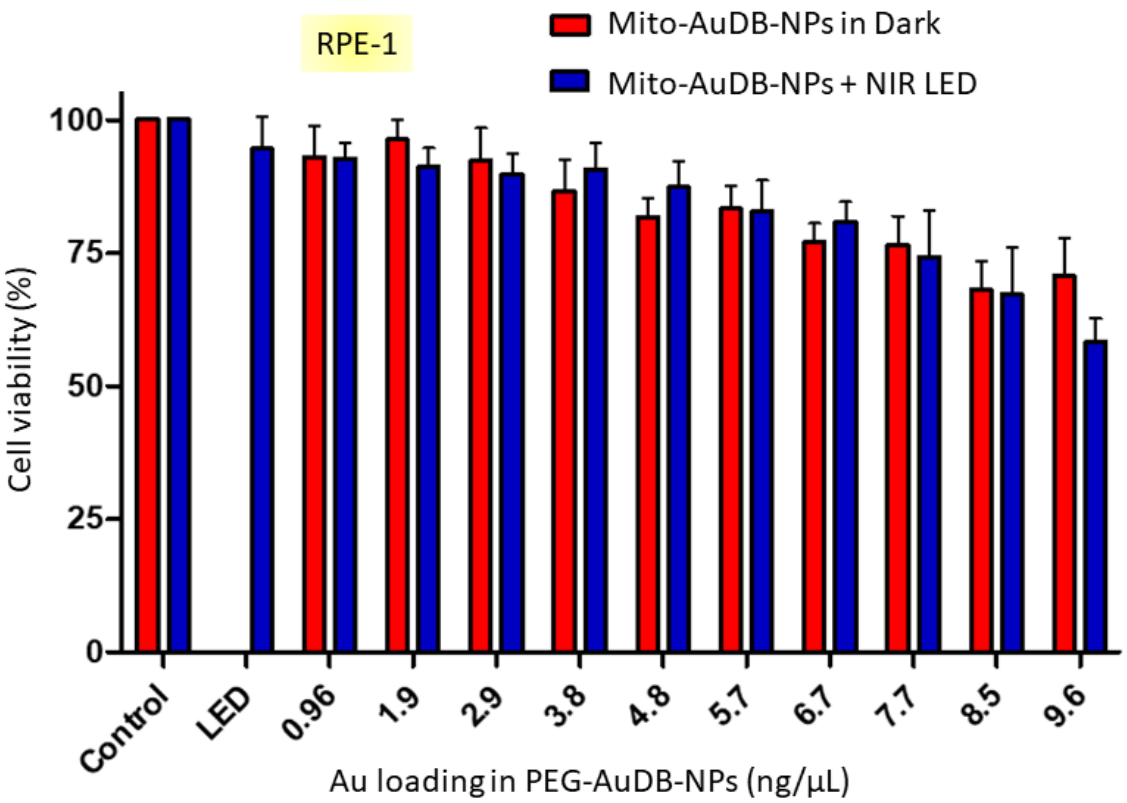


Fig. S35: Viability of the RPE-1 cells after dose dependent treatment with Mito-AuDB-NPs for 24h followed by irradiating them with or without 740 nm NIR LED.