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SUPPORTING INFORMATION

Gold nanoparticle-based two-photon fluorescent nanoprobe for monitoring intracellular nitric oxide levels

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1. Materials and methods

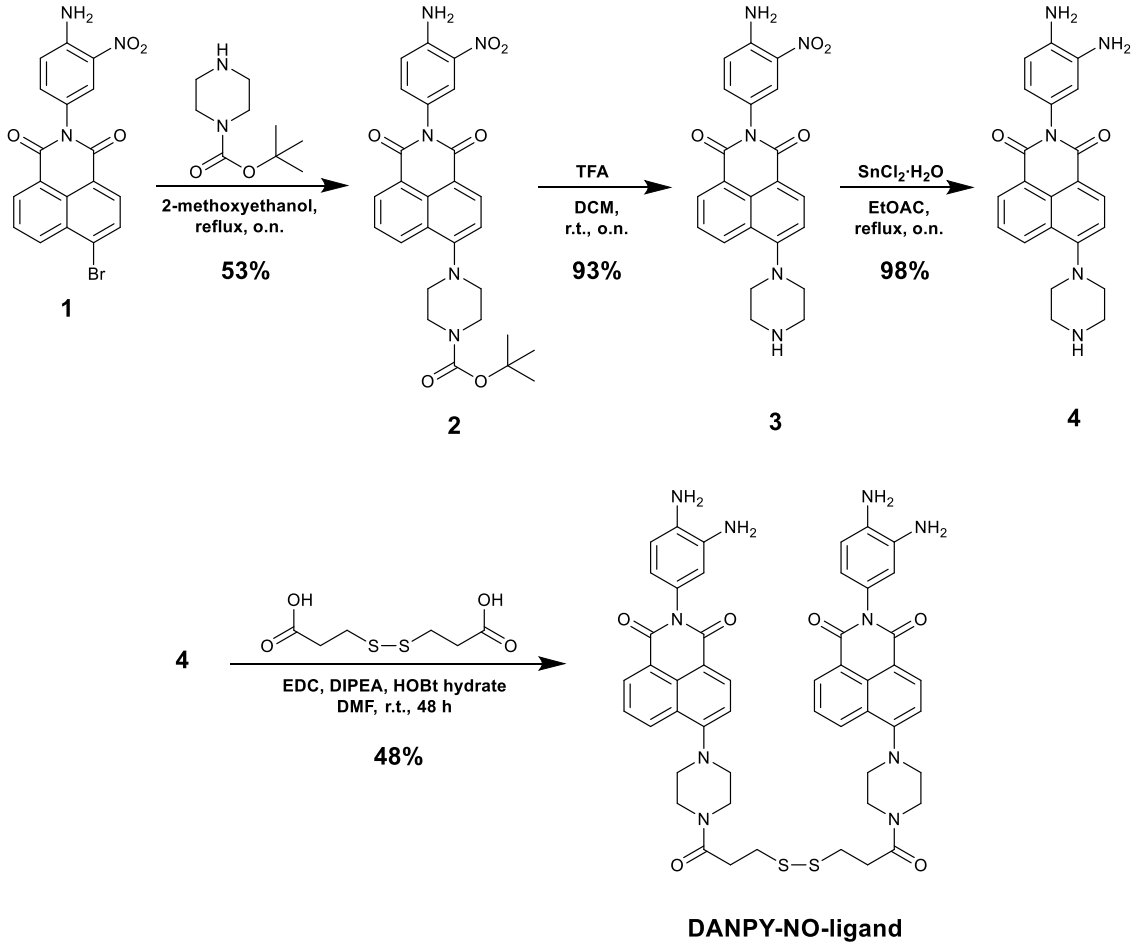
a. Materials and instruments

Commercially available reagents and high-performance liquid chromatography-grade solvents were used as received and purchased from Sigma-Aldrich or Fisher Scientific unless otherwise stated. MiliQ water was always used. CellTiter Blue reagent was purchased from Promega. The cell lines RAW264.7γ NO^- and MDA-MB-231 were purchased from ATCC (USA). The cell line THP-1 was kindly provided by Dr Anastasia Sobolewski (School of Pharmacy, University of East Anglia). Endothelial cells were kindly provided by Dr Derek Warren (School of Pharmacy, University of East Anglia).

Accurate weights were obtained with a Denver Instrument SI-234 balance (230 g x 0.1 mg) or a Mettler Toledo XS205 DualRange balance (81 g/220 g x 0.01 mg/0.1 mg). For thin layer chromatography (TLC) technique, commercially available aluminium sheets pre-coated with silica gel (0.20 mm with fluorescent indicator UV254, Grace GM BH & Co) were used. Column chromatography was performed using silica gel 60, 0.032-0.063 mm (230-450 mesh, Alfa Aesar). ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Ascend 500 or a Bruker Ultrashield Plus 400 spectrometers at room temperature using deuterated solvents. Chemical shifts (δ) are given in parts per million (ppm) and coupling constants values (*J*) are given in Hertz (Hz) and are approximated to the nearest 0.1 Hz. Calibration of the NMR spectra was made using DMSO solvent peaks (residual DMSO-d_5 (2.5 ppm) in ^{1}H NMR and deuterated DMSO-d_6 (40 ppm) in ^{13}C NMR). Abbreviations for multiplicities are as follows: s – singlet, d – doublet, dd – doublet of doublets, t – triplet, q – quartet and m – multiplet. High-resolution mass spectra were carried out using electro spray ionisation (ESI) in an Agilent 6210 time-of-flight mass spectrometer, with an Agilent 1200 HPLC. The column used was a Thermo Scientific Accucore C18 column, 2.1 mm x 50 mm with a 2.6 µm pore size. UV-Vis absorption spectra were recorded using a Hitachi U-3000 spectrophotometer at room temperature. Quartz cuvettes with a 1 cm path length were used. Fluorescence excitation and emission spectra, and quantum yield values were obtained using an Edinburgh Instrument FS5 fluorescence spectrometer. The spectra were recorded in right angle mode using quartz cuvettes with a 1 cm path length. The fluorescence quantum yield values were obtained using the integrating sphere sample cassette available for the FS5 spectrometer. The Fluoracle software was used to calculate the absolute quantum yield values by comparing the blank and the sample spectra. All the experiments were performed at room temperature. Infrared spectra were acquired using a Perkin Elmer System 400 FT-IR spectrophotometer. Solid samples were run as thin dried films of their solution in dichloromethane. pH measurements were performed in a Mettler Toledo Seven Easy pH meter. The fluorescence emission in 96-well plates was recorded using a CLARIOstar® (BMG Labtech) microplate reader. Transmission electron microscopy (TEM) images were obtained using a Jeol 2010 TEM operating at 200 kV. Samples (10 µL) were deposited on holey carbon film 300 mesh copper grids from Agar Scientific, UK; and the solvent was left evaporating overnight. Cellular images were collected either on a Zeiss LSM 980 with Airyscan 2 (pinhole adjusted to obtain an optical section of 1.5 µm) or on a LaVision Biotec TriM Scope II multiphoton microscope with a 63x or 40x

objective. Images acquired using the confocal LSM and the multiphoton microscope were analysed using the ImageJ software. Flow cytometry data were obtained in a BD Biosciences LSRFortessa flow cytometer and the results were analysed using the FlowJo software.

b. Synthesis of intermediates 1 – 4 and DANPY-NO-ligand



Scheme S1. Synthetic route for the preparation of the NO-sensitive ligand **DANPY-NO-ligand**. Percentages under the arrows correspond to the chemical yield for each reaction. r.t. stands for room temperature and o.n. stands for overnight.

Synthesis of intermediate 1

Intermediate **1** was synthesised following the protocol reported by Arnau del Valle *et al.*[1] 4-Bromo-1,8-naphthalic anhydride (1.10 g, 3.78 mmol) and 1,4-diamino-2-nitrobenzene (1.18 g, 7.29 mmol) were dissolved in 2-methoxyethanol (30 mL) and stirred under reflux (130 °C) for 24 h. Cooling of the reaction solution yielded a precipitate that was filtered and washed with ethanol to obtain intermediate **1** as a greenish solid (1.30 g, 83%). Spectroscopic data matches the reported.[1]

Synthesis of intermediate 2

Intermediate **1** (0.30 g, 0.73 mmol) and 1-Boc-piperazine (0.69 g, 3.7 mmol) were dissolved in 2-methoxyethanol (5 mL). The reaction mixture was refluxed at 130 °C overnight. The reaction mixture was cooled down at room temperature and poured into ice-water (*ca.* 120 mL) yielding a brown precipitate. The solid was filtrated, dissolved in chloroform and after a second filtration and evaporation of the solvent, a green-brown solid was obtained as intermediate **2** (0.26 g, 53%). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.54 (dd, *J* = 8.5, 1.2 Hz, 1H; **a/c**-H), 8.49 (dd, *J* = 7.3, 1.2 Hz, 1H; **a/c**-H), 8.41 (d, *J* = 8.1 Hz, 1H; **d/e**-H), 8.01 (d, *J* = 2.4 Hz, 1H; **h**-H), 7.85 (dd, *J* = 8.5, 7.3 Hz, 1H; **b**-H), 7.61 (s, 2H; **i**-H), 7.42 – 7.36 (m, 2H; **d/e**-H and **f**-H), 7.11 (d, *J* = 9.0 Hz, 1H; **g**-H), 3.69 – 3.65 (m, 4H; **j,j'/k,k'**-H), 3.23 – 3.65 (m, 4H; **j,j'/k,k'**-H), 1.45 (s, 9H; **l**-H). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 163.98 (C), 163.44 (C), 155.52 (C), 153.93 (C), 145.93 (C), 136.84 (CH), 132.13 (CH), 130.76 (CH), 130.62 (C), 129.72 (C), 129.56 (C), 126.21(CH), 125.83 (CH), 125.55 (C), 123.43 (C), 123.18 (C), 119.28 (CH), 116.53 (C), 115.46 (CH), 52.50 (CH_2), 28.08 (CH_3). HRMS (ESI+) calc. for $C_{27}H_{27}N_5O_6$ $[M+H]^+$: 518.1961; found: 518.1835.

Synthesis of intermediate 3

Intermediate **2** (0.11 g, 0.22 mmol) was mixed with trifluoroacetic acid (TFA, 3 mL) and dichloromethane (DCM, 3 mL). The reaction mixture was stirred at room temperature overnight. The volatiles were removed *in vacuo* and the crude was basified using 0.1 M aq. NaOH (80 mL). Following three extractions with DCM and evaporation of the solvent, a yellow solid was obtained as intermediate **3** (83 mg, 93%). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.54 – 8.45 (m, 2H; **a**-H and **c**-H), 8.41 (d, *J* = 8.1 Hz, 1H; **d/e**-H), 8.01 (d, *J* = 2.3 Hz, 1H; **h**-H), 7.84 (dd, *J* = 8.4, 7.3 Hz, 1H; **b**-H), 7.61 (s, 2H; **i**-H), 7.41 – 7.34 (m, 2H; **d/e**-H and **f**-H), 7.11 (d, *J* = 9.0 Hz, 1H; **g**-H), 3.25 – 3.17 (m, 4H; **j,j'/k,k'**-H), 3.12 – 3.03 (m, 4H; **j,j'/k,k'**-H). **i**-H and **m**-H could not be seen in the ^{1}H NMR spectrum. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 163.98 (C), 163.48 (C), 155.18 (C), 135.13 (CH), 134.84 (CH), 132.04 (CH), 130.68 (C), 130.37 (CH), 129.40 (C), 126.25 (C), 125.57 (CH), 125.25 (CH), 123.28 (C), 117.23 (CH), 116.72 (C), 115.48 (CH), 52.72 (CH_2), 45.76 (CH_2). HRMS (ESI+) calc. for $C_{22}H_{19}N_5O_4$ $[M+H]^+$: 418.1437; found: 418.1464.

Synthesis of intermediate 4

Intermediate **3** (0.11 g, 0.26 mmol) and $SnCl_2 \cdot H_2O$ (0.83 g, 3.6 mmol) were dissolved in EtOAc (6 mL) and stirred under reflux (80 °C) overnight. The mixture was poured into ice-water (150 mL) and the pH was adjusted to 10 using 1 M aq. NaOH. The product was extracted with DCM (3 times). The organic layers were collected, dried ($MgSO_4$), and concentrated *in vacuo* to provide the product as a yellow solid (96 mg, 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.50 – 8.43 (m, 2H; **a**-H and **c**-H), 8.38 (d, *J* = 8.2 Hz, 1H; **d/e**-H), 7.82 (dd, *J* = 8.5, 7.3 Hz, 1H; **b**-H), 7.33 (d, *J* = 8.2 Hz, 1H; **d/e**-H), 6.56 (d, *J* = 8.1 Hz, 1H; **g**-H), 6.38 (d, *J* = 2.3 Hz, 1H; **h**-H), 6.27 (dd, *J* = 8.1, 2.3 Hz, 1H; **f**-H), 4.62 – 4.47 (m, 4H; **i**-H and **n**-H), 3.19 – 3.13 (m, 4H; **j,j'/k,k'**-H), 3.04 – 2.99 (m, 4H; **j,j'/k,k'**-H). **m**-H could not be seen in the ^{1}H NMR spectrum. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 164.01 (C), 163.51 (C), 156.03 (C), 135.14 (C), 134.83 (C), 132.17 (CH), 130.61 (CH), 129.49 (CH), 128.71 (C), 126.48 (CH), 125.97 (CH), 125.47 (C), 125.31 (C), 123.23 (C), 117.25 (CH),

116.11 (CH), 114.69 (CH), 113.94 (C), 53.65 (CH_2), 45.41 (CH_2). HRMS (ESI+) calc. for $C_{22}H_{21}N_5O_2$ $[M+H]^+$: 388.1695; found: 388.1663.

Synthesis of DANPY-NO-ligand

Intermediate **4** (0.097 g, 0.25 mmol) was added to a solution containing 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (0.055 g, 0.29 mmol), hydroxybenzotriazole hydrate (HOBt) (0.040 g, 0.30 mmol) and 3,3'-dithiodipropionic acid (0.023 g, 0.11 mmol) in DMF (3.35 mL). Next, *N,N*-diisopropylethylamine (DIPEA, 250 µL, 1.4 mmol) was added to the previous mixture. The reaction mixture was stirred at room temperature for 48 h and then poured into a K_2CO_3 aqueous solution obtaining the product as a yellow precipitate. The solid was collected by filtration, washed with distilled water, dissolved in a DCM/MeOH mixture and dried ($MgSO_4$). After filtration and evaporation of the solvent, a solid was obtained as **DANPY-NO-ligand** (50 mg, 48%). NMR data is reported as the monomer. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.54 (d, *J* = 8.6 Hz, 1H; **a/c**-H), 8.46 (d, *J* = 7.8 Hz, 1H; **a/c**-H), 8.38 (d, *J* = 7.8 Hz, 1H; **d/e**-H), 7.83 (m, 1H; **b**-H), 7.40 – 7.35 (m, 1H; **d/e**-H), 6.56 (d, *J* = 8.1 Hz, 1H; **f/g**-H), 6.38 (s, 1H; **h**-H), 6.30 – 6.24 (m, 1H; **f/g**-H), 4.63 – 4.54 (m, 4H; **i**-H and **n**-H), 3.84 – 3.79 (m, 4H; **j**-H and **j'**-H), 3.28 – 3.24 (m, 2H; **k**-H), 3.21 – 3.14 (m, 2H; **k'**-H), 3.02 – 2.96 (m, 2H; **o**-H), 2.89 – 2.82 (m, 2H; **p**-H). ^{13}C NMR (101 MHz, DMSO-d_6) δ = 169.07 (C), 163.96 (C), 163.46 (C), 155.10 (C), 135.14 (C), 134.84 (C), 132.01 (CH), 130.67 (CH), 129.37 (CH), 126.24 (CH), 125.53 (C), 125.25 (C), 123.25 (C), 117.25 (CH), 116.73 (C), 115.45 (CH), 114.67 (CH), 113.94 (CH), 52.74 (CH_2), 52.51 (CH_2), 44.93 (CH_2), 41.32 (CH_2), 33.96 (CH_2), 32.32 (CH_2). HRMS (ESI+) calc. for $C_{50}H_{48}N_{10}O_6S_2$ $[M+H]^+$: 949.3278; found: 949.3275.

c. Synthesis of DANPY-NO@AuNPs

DANPY-NO@AuNPs were synthesised following a modified version of the protocol previously reported by Marín *et al.*[2] **DANPY-NO-ligand** (3.27 mL of a 0.37 mM stock solution in DMF, 1.2 µmol) and $AuCl_4 \cdot 3H_2O$ (1.1 mL of a 2.54 mM stock solution in DMF, 1.10 mg, 2.79 µmol) were mixed and stirred for 5 min. Next, a fresh solution of $NaBH_4$ (1.34 mg, 35.42 µmol) was prepared by dissolving 1.34 mg of $NaBH_4$ in H_2O (2.12 mL) and it was added to the previous mixture. The solution became brownish after the addition of $NaBH_4$. Following stirring at room temperature for 3 h, **DANPY-NO@AuNPs** were transferred into a centrifugal filter unit and centrifuged (3,000 rcf, 30 min). Next, H_2O (5 mL) was used to dilute the concentrated suspension of **DANPY-NO@AuNPs** and it was centrifuged again two more times at 4,000 rcf for 30 min. The final concentrated suspension of **DANPY-NO@AuNPs** was dispersed in a DMSO/H_2O (5:2) mixture (7 mL). The suspension of NPs was kept at room temperature until further use.

d. Electronic spectroscopic studies of DANPY-NO-ligand and DANPY-NO@AuNPs

Absorption and extinction properties

The UV-Vis absorption spectra of the intermediates and **DANPY-NO-ligand** were recorded in DMSO (62.5 µM). The UV-Vis extinction spectra of **DANPY-NO@AuNPs** were recorded in DMSO/H_2O (5:2) mixture.

Determination of extinction coefficient of DANPY-NO-ligand

DANPY-NO-ligand solutions ranging from 0 to 150 µM were prepared in DMSO using 1 mM stock solution. The solutions were analysed by UV-Vis spectroscopy between 350 and 700 nm starting with the lowest concentration. A calibration curve at 398 nm was created and a value for the extinction coefficient was obtained.

Fluorescence excitation and emission properties

The fluorescence excitation and emission spectra of intermediates and **DANPY-NO-ligand** were recorded in DMSO (62.5 µM) using λ_{em} = 525 nm and λ_{exc} = 397 nm, respectively. The fluorescence excitation and emission spectra of **DANPY-NO@AuNPs** were recorded in DMSO/H_2O (5:2) using λ_{em} = 550 nm and λ_{exc} = 397 nm, respectively.

e. Sensitivity and selectivity of DANPY-NO-ligand and DANPY-NO@AuNPs towards nitric oxide

Aqueous solution of **DANPY-NO-ligand** (11.7 µM, 1.4% DMSO) and **DANPY-NO@AuNPs** (20% DMSO) were prepared in multi-buffer medium and treated with NONOate (2.5 mM in NaOH 0.01 M) or with the same amount of NaOH 0.01 M for the control. The pH was adjusted to 7.42 using 0.01 M HCl. The absorbance and the fluorescence emission and excitation spectra of all the samples were recorded using λ_{em} = 550 nm and λ_{exc} = 397 nm.

A time-dependent NO detection experiment was performed using **DANPY-NO@AuNPs** (83 µg/mL, 7% DMSO). Upon addition of NONOate (0.5 mM in NaOH 0.01M), fluorescence emission spectra of **DANPY-NO@AuNPs** at different time points (from 0 to 24 h) were recorded at λ_{exc} = 405 nm.

Determination of the limit of detection of DANPY-NO@AuNPs towards nitric oxide

The LOD of **DANPY-NO@AuNPs** was calculated by mixing **DANPY-NO@AuNPs** (83 µg/mL, 7% DMSO) with different concentrations of NO (from 0 to 675 µM) released using NONOate. The fluorescence emission spectrum of each sample was recorded at λ_{exc} = 405 nm. Each experiment was repeated in triplicates. The fluorescence emission intensity at 547 nm was plotted against the NO concentration and the linear range was used to calculate the LOD using the mathematical definition of LOD = $3 \cdot \sigma_b / k$; where σ_b is the standard deviation of the blank signals and k is the slope of the calibration curve.

Acid-base studies: pH titration of DANPY-NO@AuNPs

The pH titration curve of **DANPY-NO@AuNPs** was obtained by measuring the fluorescence emission spectrum (λ_{exc} = 405 nm) of aqueous solutions containing multi-buffer, 100 mM NaCl and **DANPY-NO@AuNPs** (0.14 mg/mL, 12% DMSO) at different pHs (from 3.49 to 7.99). pH variations were achieved by adding small amounts of NaOH and HCl (0.01 – 1.00 M). The spectra were recorded 24 h following sample preparation.

Nitric oxide detection by DANPY-NO@AuNPs at different pH values

Aqueous multi-buffer solutions of **DANPY-NO@AuNPs** (86 μg/mL, 7% DMSO) were prepared at pH 4.12, pH 5.63 and pH 7.12 and treated with NONOate (0.5 mM in NaOH 0.01 M) or with the same amount of NaOH 0.01 M for the controls. The fluorescence emission spectra of all the samples and controls were recorded at λ_{exc} = 396 nm. Experiments were performed in triplicates and the average results were plotted.

Selectivity studies of DANPY-NO@AuNPs towards nitric oxide

Aqueous solutions of NO_2^-, NO_3^-, ClO^-, Na^+, K^+ and Ca^{2+} (100 μM) were prepared from stock solutions (1 mM) of $NaNO_2$, $NaNO_3$, NaOCl, NaCl, KCl and $CaCl_2$, respectively. An aqueous solution of singlet oxygen ($^1O_2$, 100 μM) was prepared by mixing H_2O_2 (100 μM) and NaClO (100 μM), and $^{\cdot}OH$ was prepared by mixing H_2O_2 (100 μM) and $FeSO_4$ (100 μM). To prepare the $O_2^{\cdot-}$, KO_2 (50 mM) solution was mixed with 18-crown-6 (0.15 M, DMSO) and sonicated to fully dissolve the KO_2. The UV-Vis spectrum of the sample was recorded and the concentration of $O_2^{\cdot-}$ was estimated to be 600 μM. An aqueous solution of $O_2^{\cdot-}$ (100 μM) was prepared using the previous stock solution. Concentrations of 100 μM were selected as an excess of each interference assuming that, at normal conditions and in general, cells produce lower concentrations of all these species.[3-8]

Solutions containing **DANPY-NO@AuNPs** (86 μg/mL) and the corresponding interferent (no-interferent, H_2O_2, NO_2^-, NO_3^-, $O_2^{\cdot-}$, $^{\cdot}OH$, $^1O_2$, Na^+, K^+, Ca^{2+}, L-Arg, AA, DHA, ClO^- or NO, 100 μM) were prepared and their fluorescence emission spectrum (λ_{exc} = 405 nm) was recorded after 24 h. Each experiment was repeated in triplicates. Following 24 h from the preparation of the mixtures, NO (100 μM) was added to each sample and the fluorescence emission spectra (λ_{exc} = 405 nm) were recorded following a further 24 h incubation.

f. Biological experiments

Imaging medium preparation

Imaging Medium (IM) was prepared by mixing NaCl (120 mM), KCl (5 mM), $CaCl_2{\cdot}2H_2O$ (2 mM), $MgCl_2{\cdot}6H_2O$ (1 mM), NaH_2PO_4 (1 mM), $NaHCO_3$ (1 mM), 4-(2-hydroxyethyl)piperazine-4-ethanesulfonic acid (HEPES, 25 mM), D-glucose (11 mM) and bovine serum albumin (BSA, 1mg/mL) in MiliQ H_2O and the pH was adjusted to 7.4 using an aqueous solution of NaOH (1 mM).

Culture RAW264.7Y NO^- macrophages

The macrophage cell line RAW264.7Y NO^- was cultured in a humidified atmosphere of 5% CO_2 at 37 ^oC in phenol red-free Dulbecco's Modified Eagle's Medium (DMEM) containing 4.5 mg/mL D-glucose and supplemented with 1% L-glutamine (200 mM), 1% penicillin-streptomycin (P-S) (100 U/mL and 100 µg/mL, respectively) and 10% foetal bovine serum (FBS).

A frozen cryotube containing RAW264.7Y NO^- was defrosted in a water bath at 37 ^oC. The cell suspension (1 mL) was transferred to a centrifuge tube containing 9 mL of supplemented DMEM media and the cells were centrifuged at 1,000 rcf for 5 min in a Heraeus Labofuge 400R Function Line centrifuge. The freezing medium was discarded, and the cells were resuspended in fresh supplemented DMEM (12 mL). The content was placed into a 75 cm^2 Nunc Easy Flask and the cells were incubated in an Heracell 150i CO_2 incubator at 37 ^oC in a 5% CO_2 atmosphere. Subcultures were obtained by dislodging the cells from the flask surface using a cell scraper (18 mm blade), centrifuging them at 800 rpm for 5 min and resuspending the cells in supplemented DMEM medium. Dilutions 1:12 were made every 3 days.

Culture and differentiation of THP-1 cells

The human monocytic THP-1 cell line derived from an acute monocytic leukaemia patient was cultured in phenol red-free RPMI 1640 medium containing L-glutamine (300 mg/mL) and supplemented with 1% pen/strep (100 U/mL and 100 µg/mL, respectively) and 20% FBS.

A frozen cryotube containing THP-1 cells was defrosted in a water bath at 37 ^oC. The cell suspension was transferred to a centrifuge tube and the cells were centrifuged at 1,000 rcf for 5 min in a Heraeus Labofuge 400R Function Line centrifuge. The freezing medium was discarded, and the cells were resuspended in fresh supplemented RPMI 1640 medium (12 mL). The content was placed in a 75 cm^2 Nunc Easy Flask and the flask was placed in an incubator at 37 ^oC in a 5% CO_2 atmosphere. Subcultures were obtained by centrifuging the cell suspension at 800 rpm for 5 min and resuspending the cells with fresh supplemented RPMI 1640 medium. Dilutions (1:12) were made every 2 days.

To generate inflammatory macrophages, THP-1 cells were placed in 6-well plates containing 18 mm diameter coverslips. The cells were treated overnight with 100 ng/mL phorbol 12-myristate 13-acetate (PMA) at 37 ^oC in a 5% CO_2 atmosphere. After this time, the cells were adhered to the coverslips and they were washed three times with phosphate-buffered saline (PBS, 0.5 mL). Finally, supplemented RPMI 1640 medium was added to the cells (2 mL).

Culture endothelial cells

Cells were kindly provided by Dr Derek Warren. Endothelial cells were cultured in Immortalised Mouse Lung Endothelial Cell (IMMLEC) Medium that was prepared by mixing 250 ml Ham's F12 and 250 mL DMEM (low glucose) and supplemented with 100 µg/mL Heparin, 1% P-S and 10% FBS.

Cells growing in 75 cm^2 Nunc Easy Flask were diluted every 4 days with a 1:3 dilution. To do this, the media was removed from the flask and the cells were washed with 5 mL PBS. After removing the PBS, the cells

were harvested from the flask by adding 2 mL of 0.25% Trypsin:EDTA and incubating at 37 ºC in a 5% CO_2 atmosphere for 3 min. Next, 7 mL of IMMLEC media were added to the flask and the content was diluted by placing 3 mL of suspended cells into a 75 cm^2 Nunc Easy Flask containing 9 mL of fresh IMMLEC media and pre-coated with 1% gelatin solution (prepared by dissolving 0.4 g gelatin in 400 mL distilled H_2O and autoclaved) for 1 h at 37 ºC in a 5% CO_2 atmosphere. The coating solution was removed prior to seeding cells.

Culture MDA-MB-231 human breast cancer cells

MDA-MB-231 human breast adenocarcinoma cells were routinely cultured in DMEM containing 4.5 mg/L D-glucose and supplemented with 1% L-glutamine (200 mM), sodium pyruvate (1 mM) and 10% FBS. The cells were grown at 37 °C in a 5% CO_2 atmosphere in Nunc Easy Flasks with porous caps.

A cryo tube containing the MDA-MB-231 cells (1 mL in freezing medium) was defrosted in a water bath at 37 °C. The cells were transferred into a 15 mL centrifuge tube containing DMEM (9 mL). The suspension was centrifuged at 1,000 rcf for 5 min, the supernatant was removed and the cell pellet was resuspended in DMEM (12 mL). The content of the centrifuge tube was transferred to a 75 cm^2 Nunc Easy Flask and the flask and placed in an incubator at 37 °C in a 5% CO_2 atmosphere. Subcultures (1:4) were made every 4 days by dislodging the cells from the flask surface by addition of trypsin 0.25% (1x) EDTA (5 mL) and incubation for 5 min at 37 °C in a 5% CO_2 atmosphere. Trypsin was deactivated by addition of DMEM (5 mL) and removed by centrifugation at 800 rcf for 5 min. The supernatant was removed and the cell pellet was resuspended in DMEM (12 mL) and transferred to 75 cm^2 Nunc Easy Flasks.

Culture of cells onto coverslips for imaging experiments

For imaging experiments, the cells were placed on 18 mm diameter glass coverslips in 6 well-plates and incubated at 37 °C in a 5% CO_2 atmosphere for 18 – 20 h before performing experiments. After this time, the cells were attached to the coverslips and the medium was replaced with the corresponding fresh culture medium (2 mL).

Nitric oxide detection in RAW264.7Y NO^- cells by DANPY-NO@AuNPs

For cellular experiments using **DANPY-NO@AuNPs**, RAW264.7Y NO^- cells were cultured on 18 mm coverslips in 6-well plates. Cells grown to confluence in 75 cm^2 Nunc Easy Flask were harvested from the surface using a cell scraper, centrifuged at 800 rpm for 5 min and resuspended in DMEM medium (9 mL). From the cell suspension, 0.5 mL were added to wells containing 2 mL of DMEM medium. The cells were incubated at 37 ºC in a 5% CO_2 atmosphere overnight. After this time, the cells were attached to the coverslips and the medium was replaced with fresh cell culture medium. RAW264.7Y NO^- cells were incubated with **DANPY-NO@AuNPs** (4.2 μg/mL) and stimulated following the addition of LPS (0.7 μg/mL) and IFN-Y (17 μg/mL). Unstimulated cells were only incubated with **DANPY-NO@AuNPs** (4.2 μg/mL). L-NAME treatment was performed by treating RAW264.7Y NO^- cells with L-NAME (2 mM, 30min) prior to stimulation with LPS (0.7 μg/mL) and IFN-Y (17 μg/mL) and incubation with **DANPY-NO** (5 μM). Control

cells that were not incubated with **DANPY-NO@AuNPs** were also prepared containing the same LPS and IFN-ϒ concentrations used for stimulated cells. Control cells treated with L-NAME (2 mM, 30 min) prior stimulation with LPS (0.7 μg/mL) and IFN-ϒ (17 μg/mL) were also prepared. The cells were incubated at 37 ^{o}C in a 5% CO_2 atmosphere overnight. Following incubation, the cells were imaged using the confocal LSM (λ_{exc} = 405 nm, $\Delta\lambda_{em}$ = 422 – 689 nm) and/or the multiphoton microscope (λ_{exc} = 800 nm).

Nitric oxide detection in THP-1 macrophages by DANPY-NO@AuNPs

For cellular experiments using **DANPY-NO@AuNPs**, PMA-activated inflammatory THP-1 macrophages cultured on 18 mm coverslips in 6-well plates were incubated with **DANPY-NO@AuNPs** (4.2 μg/mL) and stimulated by addition of LPS (5 μg/mL). Unstimulated cells were only incubated with **DANPY-NO@AuNPs** (4.2 μg/mL). L-NAME treatment was performed by treating PMA-differentiated THP-1 cells with L-NAME (0.1 mM, 30 min) prior stimulation with LPS (5 μg/mL) and incubation with **DANPY-NO@AuNPs** (4.2 μg/mL). Control cells that were not incubated with **DANPY-NO@AuNPs** were also prepared containing the same LPS concentration as stimulated cells incubated with NPs. Control cells treated with L-NAME (0.1 mM, 30 min) prior to stimulation with LPS (5 μg/mL) were also prepared. The cells were incubated at 37 ^{o}C in a 5% CO_2 atmosphere for 48 h. Following incubation, the cells were imaged using a confocal LSM (λ_{exc} = 405 nm, $\Delta\lambda_{em}$ = 422 – 689 nm).

Nitric oxide detection in endothelial cells by DANPY-NO@AuNPs

For intracellular experiments in endothelial cells with DANPY-NO@AuNPs, cells were cultured overnight on 18 mm coverslips in 6-well plates pre-coated with 1% gelatine solution. After this time, cells incubated with **DANPY-NO@AuNPs** (4.2 μg/mL) were prepared under different conditions: 1) without further treatment, 2) with Ca^{2+} ionophore A-23187 (1 μM), 3) pre-treated with LNAME (200 mM) 30 min prior incubated with **DANPY-NO@AuNPs** and 4) with *S*-Nitroso-*N*-acetylpenicillamine (SNAP, 220 μM). The corresponding control cells that were not loaded with **DANPY-NO@AuNPs** but that were incubated with Ca^{2+} ionophore A-23187, with L-NAME or with SNAP were also prepared. Following incubation overnight at 37 °C in a 5% CO_2 atmosphere, the cells were imaged using confocal LSM (λ_{exc} = 405 nm, $\Delta\lambda_{em}$ = 422 – 689 nm).

Nitric oxide detection in MDA-MB-231 human breast cancer cells by DANPY-NO@AuNPs

MDA-MB-231 cells were cultured overnight on 18 mm coverslips (in 6-well plates) at 37 °C in a 5% CO_2 atmosphere. After this time, cells were incubated overnight with **DANPY-NO@AuNPs** (4.2 μg/mL) and were treated with different concentrations of SNAP (from 0 to 440 μM) for 40 min. Corresponding control cells that were not incubated with **DANPY-NO@AuNPs**, without SNAP and with SNAP (440 μM) were also prepared. Following incubation, the cells were imaged using confocal LSM (λ_{exc} = 405 nm, $\Delta\lambda_{em}$ = 422 – 689 nm). The calibration curve was obtained by plotting the fluorescence emission intensity at 551 nm for each sample as a function of the concentration of nitrites (calculated based on the conversion of 1 mmol SNAP yielding 30.1 μmol nitrites after 1 h treatment).

Imaging live cells using a confocal laser scanning microscope and a multiphoton microscope

To image live cells in both confocal LSM and multiphoton microscope, the coverslips containing the cells were securely tightened into a Ludin chamber (Life Imaging Services, Reinach, Switzerland) for 18 mm coverslips. The cells were washed three times with IM and 1 mL of IM was added to conduct the imaging on live cells. The Ludin chamber was mounted on a heated stage of the microscope (37 ^{o}C). A Carl Zeiss Objective 63x with oil immersion (1.4 NA) was used for imaging RAW264.7Y NO^{-} cells, THP-1 cells and MDA-MB-231 cells. A Carl Zeiss Objective 40x with oil immersion (1.4 NA) was used to image endothelial cells. In the confocal LSM, **DANPY-NO@AuNPs** were excited using a 405 nm diode laser and the fluorescence emission was collected between 422 and 689 nm. Differential Interference Contrast (DIC) images were collected simultaneously with fluorescence images using the same laser. For spectral analysis on the LSM, the samples were excited at 405 nm and the fluorescence emission spectra were recorded between 430 and 690 nm. For imaging experiments in the multiphoton microscope, the samples were excited at 800 nm using a Vision II Ti:Sapphire laser (Coherent Ltd, Ely, UK) and the emission of **DANPY-NO@AuNPs** was collected in the green channel (500 - 550 nm); Dodt contrast images were acquired simultaneously with the same wavelength.

Note regarding data analysis: The display of the images within each figure was adjusted relative to the brightest sample in each experiment. When the cells were treated with **DANPY-NO@AuNPs** and to confirm their internalisation and colocalization (Figure 3, Figure S22 and Figure S25), the display of the images was adjusted to be maximum. On the other hand, when studying the intracellular detection of NO by the **DANPY-NO@AuNPs**, the display was maximum upon detection of NO and the other displays were normalised accordingly. Thus, in this case, the corresponding fluorescence emission intensity of the nanoprobe in the absence of NO is much lower and it cannot be compared to the emission obtained in previous experiments. Therefore, images *within* figures are comparable, but fluorescence brightness is not comparable *between* figures.

Colocalisation studies

LysoTracker Red DND-99 was used as a fluorescent marker of acidic organelles to study the location of **DANPY-NO@AuNPs** in those organelles. Cells cultured on 18 mm coverslips in 6-well plates incubated with **DANPY-NO@AuNPs** (4.2 µg/mL) at 37 ^{o}C in a 5% CO_2 atmosphere overnight were further incubated with LysoTracker Red DND-99 (5 µM) at 37 ^{o}C for 5 min. Following incubation, the cells were imaged using confocal LSM. **DANPY-NO@AuNPs** were excited using the 405 nm diode laser and the fluorescence emission was recorded between 500 and 580 nm. LysoTracker Red DND-99 was excited using the 561 nm diode laser and the fluorescence emission was recorded between 580 and 625 nm. Confocal microscopy images of cells were analysed in triplicates, each image containing an average of three cells. The Pearson's correlation coefficient (with coefficient 1 meaning perfect colocalisation) and the scatterplot were obtained using ImageJ.

CellTiter-Blue cell viability assay

To evaluate the cytotoxicity of **DANPY-NO@AuNPs** in RAW264.7Y NO^- macrophages, THP-1 cells and endothelial cells, the CellTiter-Blue cell viability Assay was conducted. The cells were seeded overnight on 96-well black-bottom microplates prior to treating the cells. Next, the cells were incubated with **DANPY-NO@AuNPs** at concentrations ranging from 0 to 8.3 μg/mL. Cells without **DANPY-NO@AuNPs** were used as a control. All samples were prepared in triplicate. The cells (100 μL/well) were incubated overnight at 37 ^oC in a 5% CO_2 atmosphere. After three washes with PBS (50 μL), the corresponding cell culture media was added (100 μL/well). At this point, CellTiter-Blue reagent (20μL/well) was added and the cells were incubated for 4 h at 37 ^oC in a 5% CO_2 atmosphere. The fluorescence emission of the CellTiter-Blue reagent was then measured at 594 nm following excitation at 561 nm using a CLARIOstar (BMG Labtech) microplate reader. Background fluorescence was corrected by subtracting fluorescence emission from DMEM phenol red-free medium. Cell viability was calculated as a percentage of non-treated. All the samples were analysed in triplicates. The statistical analysis (Student's t-test) was applied to study the significant difference to the control cells.

Experiments performed using flow cytometry

RAW264.7Y NO^- cells unstimulated and stimulated with LPS (0.7 μg/mL) and IFN-Y (17 μg/mL), both incubated with **DANPY-NO@AuNPs** (4.2 μg/mL) were prepared in a 6-well plate as previously explained but without coverslips in the wells. The corresponding control cells without **DANPY-NO@AuNPs** were also prepared. After overnight incubation, the cells were washed three times with HBSS and 1 mL of fresh HBSS was added per well. Cells were harvested from the surface of the wells using a cell scraper resulting in a cell suspension. The fluorescence emission intensity of each suspension of cells was recorded at λ_{exc} = 405 nm ($\Delta\lambda_{em}$ = 500 – 550 nm) and at λ_{exc} = 488 nm to record the forward scatter (FSC) and side scatter (SSC) signals.

2. ^{1}H NMR and ^{13}C NMR spectra of intermediates 2 – 4 and DANPY-NO-ligand

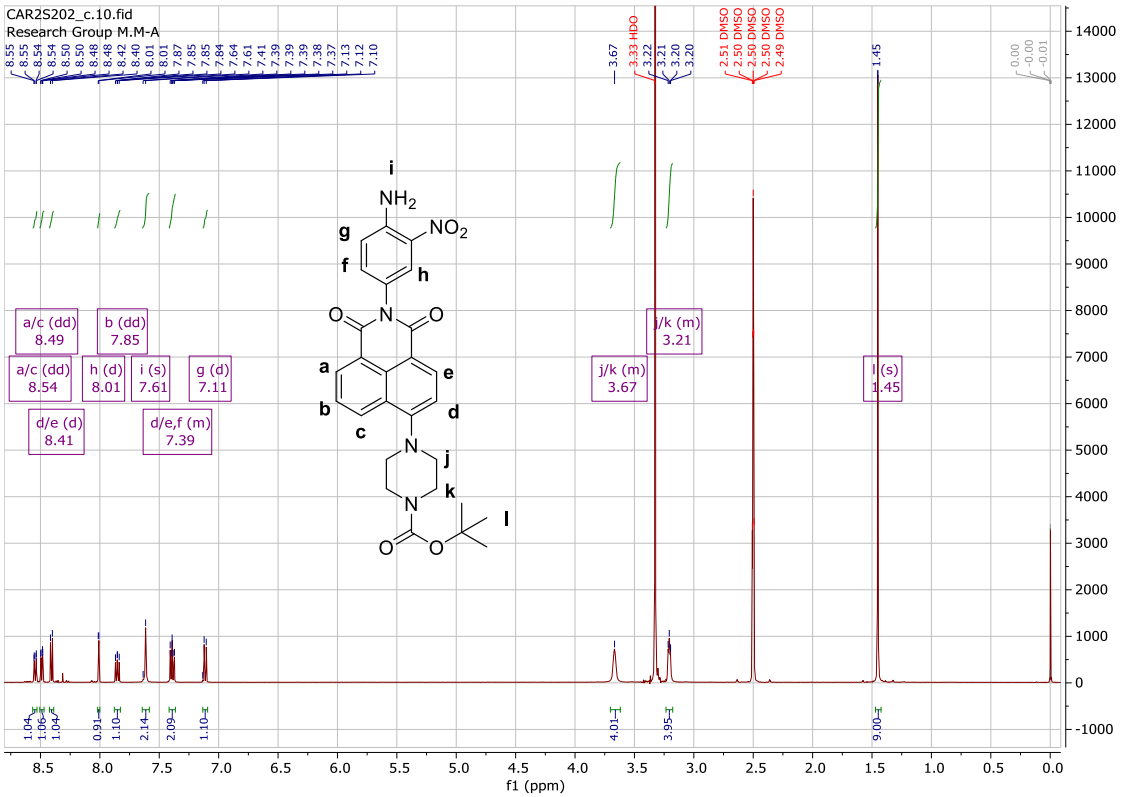


Figure S1. ^{1}H NMR spectrum of intermediate **2** in DMSO-d_6.

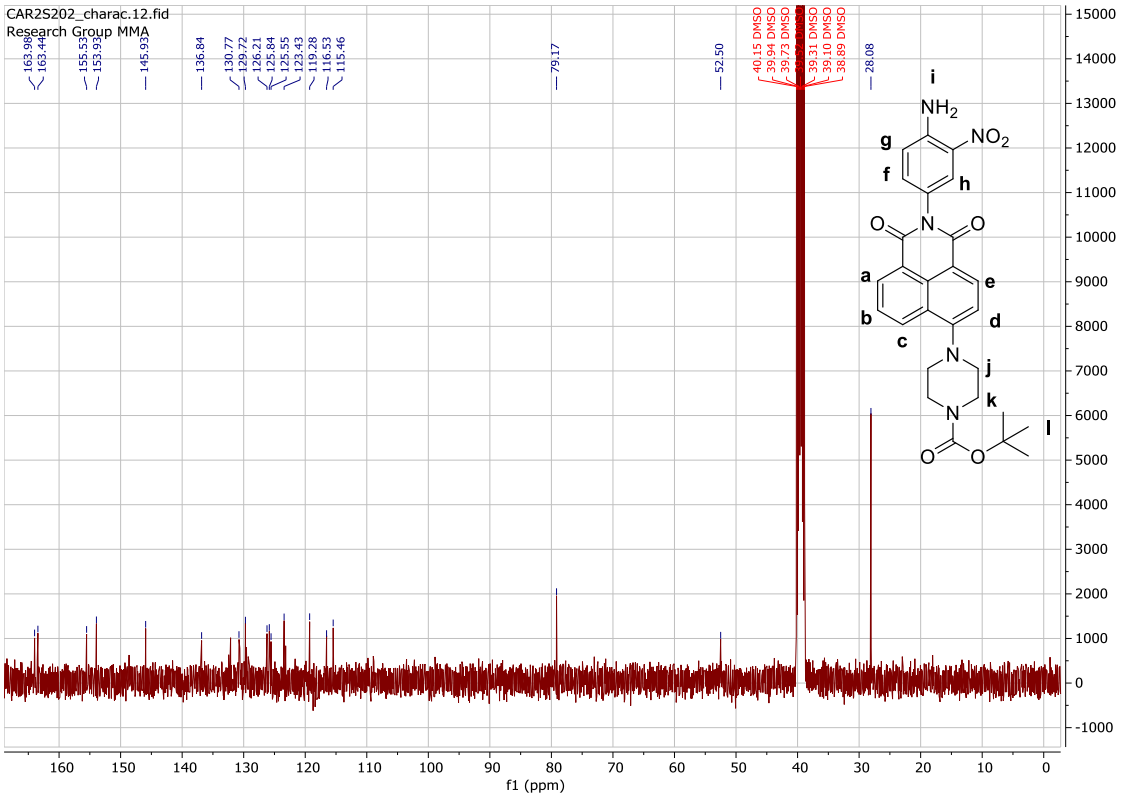


Figure S2. ^{13}C NMR spectrum of intermediate **2** in DMSO-d_6.

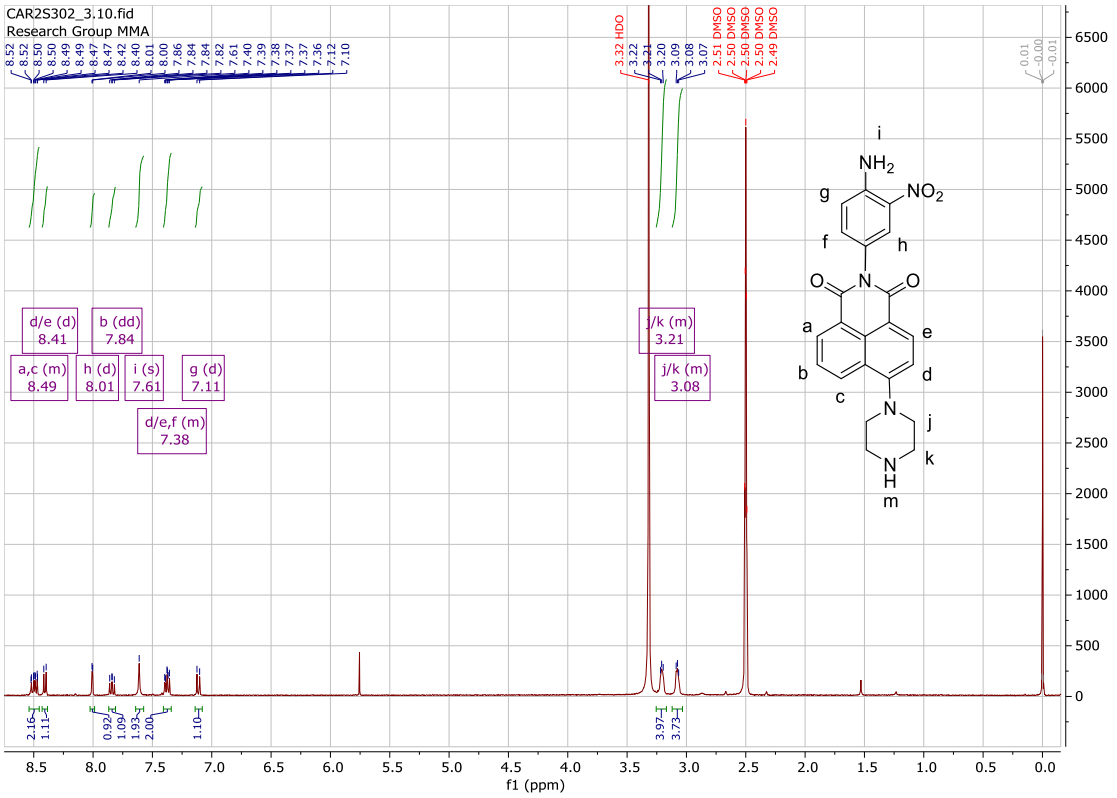


Figure S3. ^{1}H NMR spectrum of intermediate **3** in DMSO-d_6.

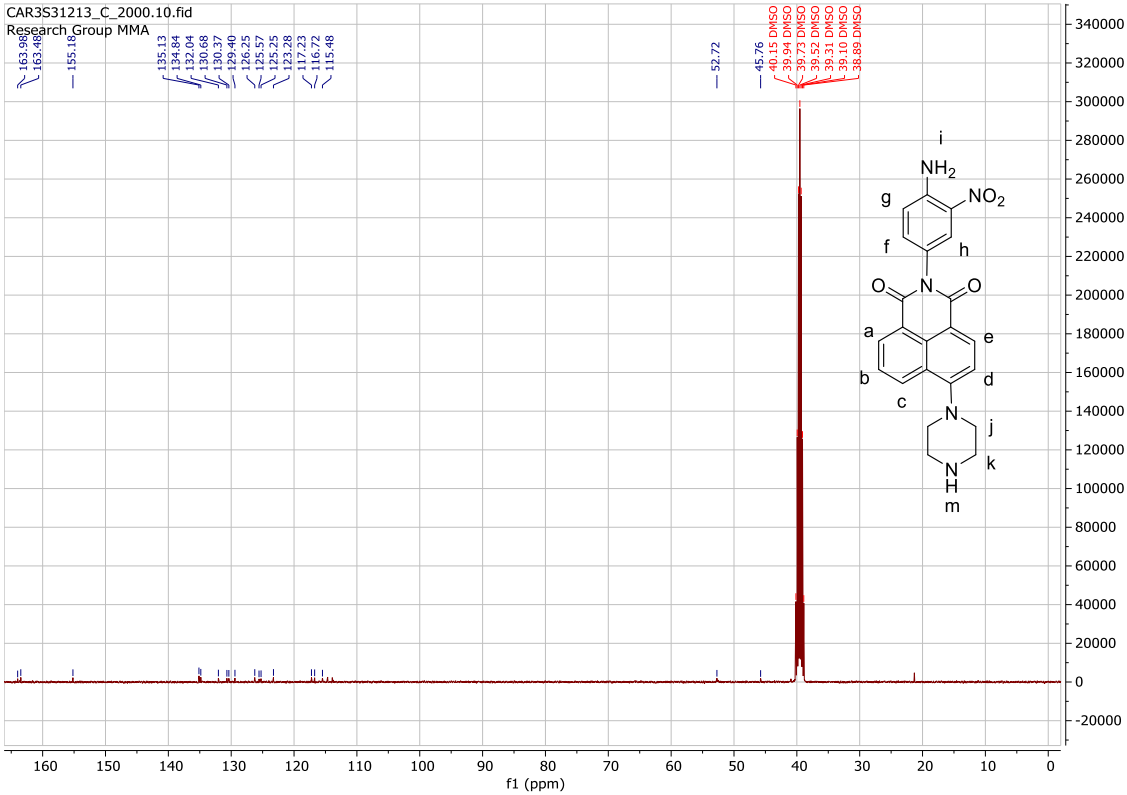


Figure S4. ^{13}C NMR spectrum of intermediate **3** in DMSO-d_6.

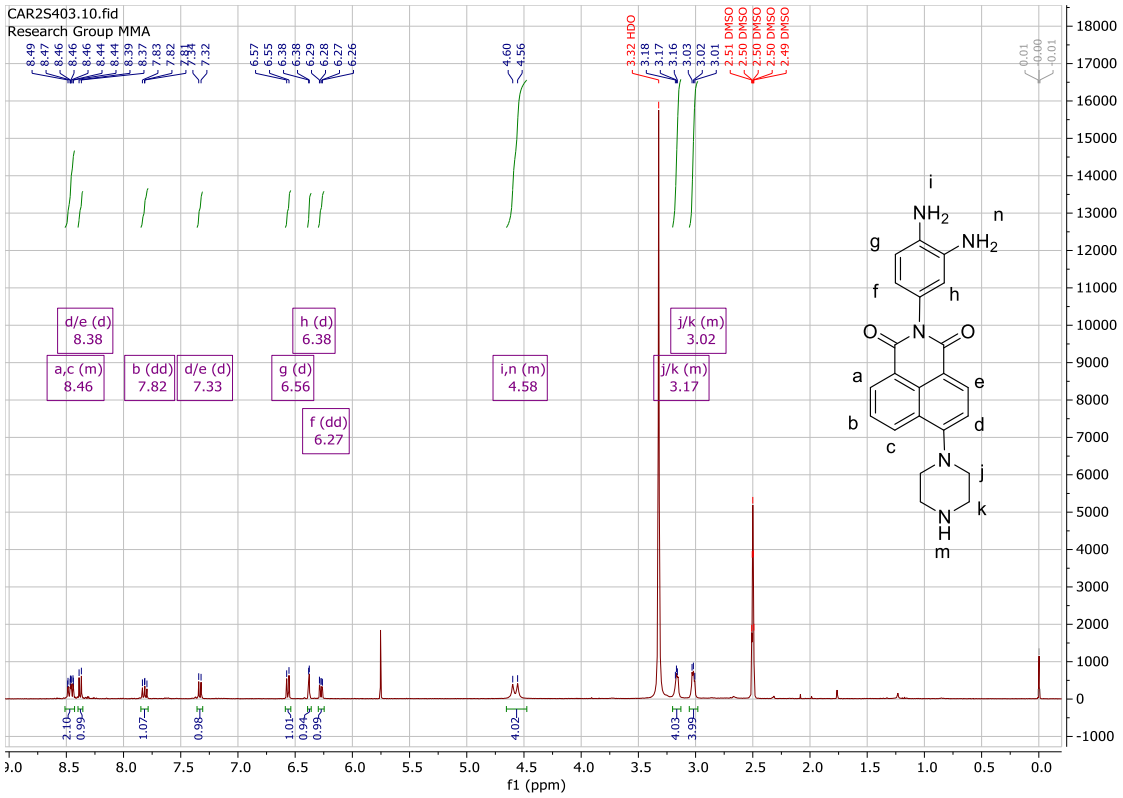


Figure S5. ^{1}H NMR spectrum of intermediate **4** in DMSO-d_6.

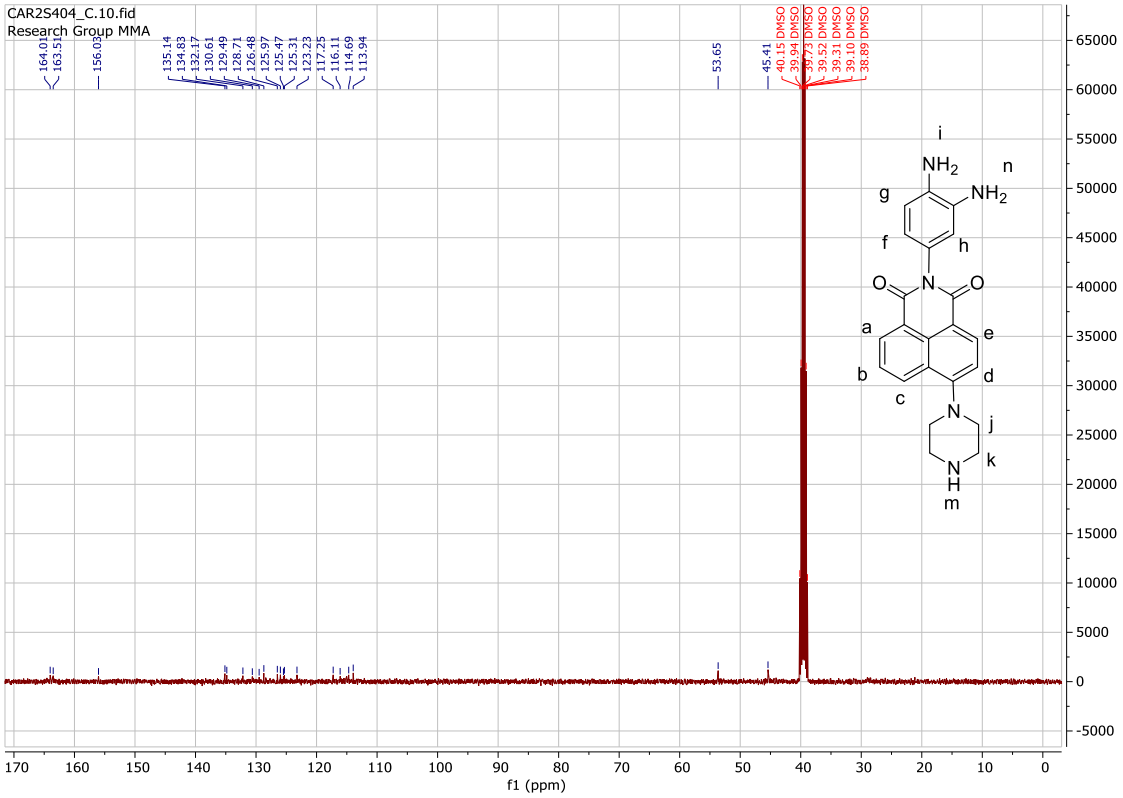


Figure S6. ^{13}C NMR spectrum of intermediate **4** in DMSO-d_6.

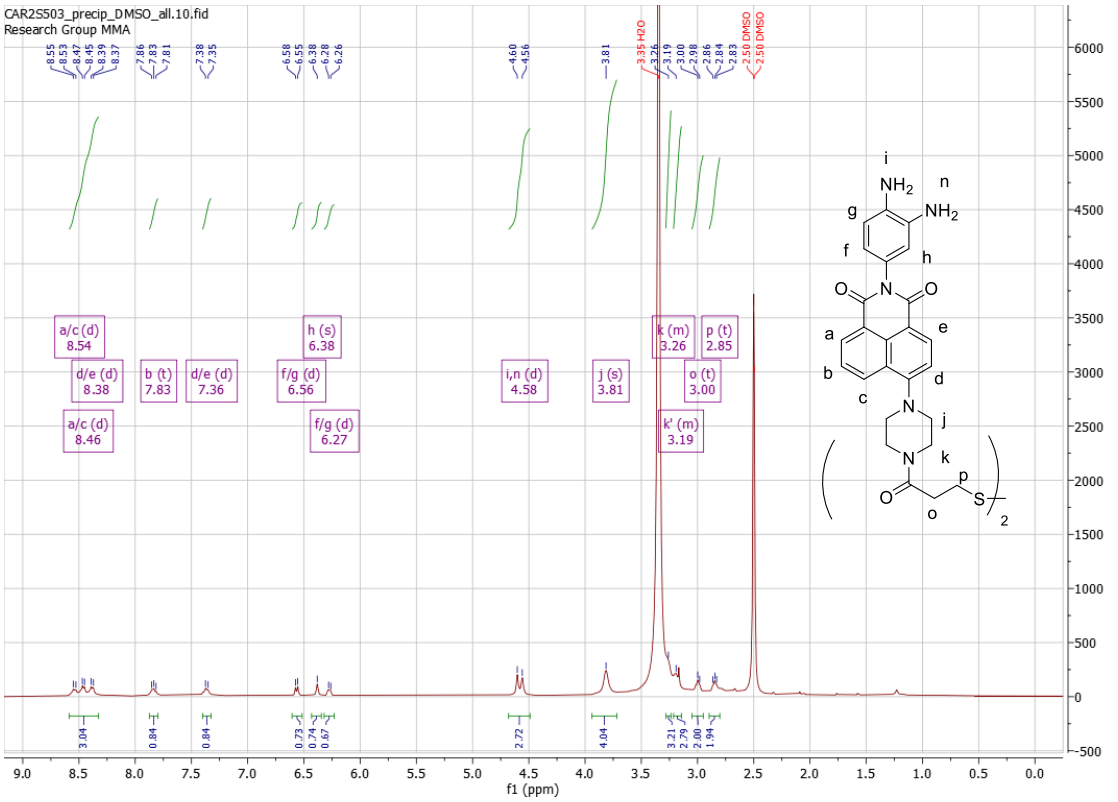


Figure S7. ^{1}H NMR spectrum of **DANPY-NO-ligand** in DMSO-d_6.

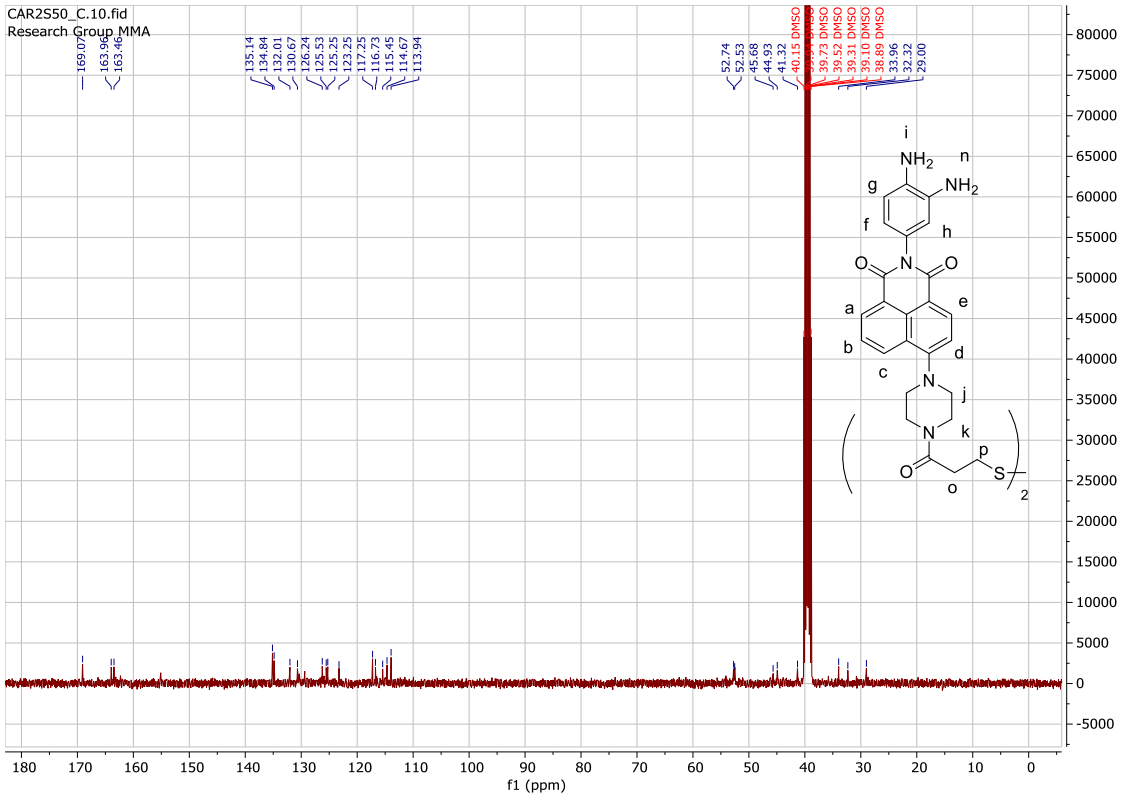


Figure S8. ^{13}C NMR spectrum of **DANPY-NO-ligand** in DMSO-d_6.

3. Transmission electron microscopy characterisation of DANPY-NO@AuNPs

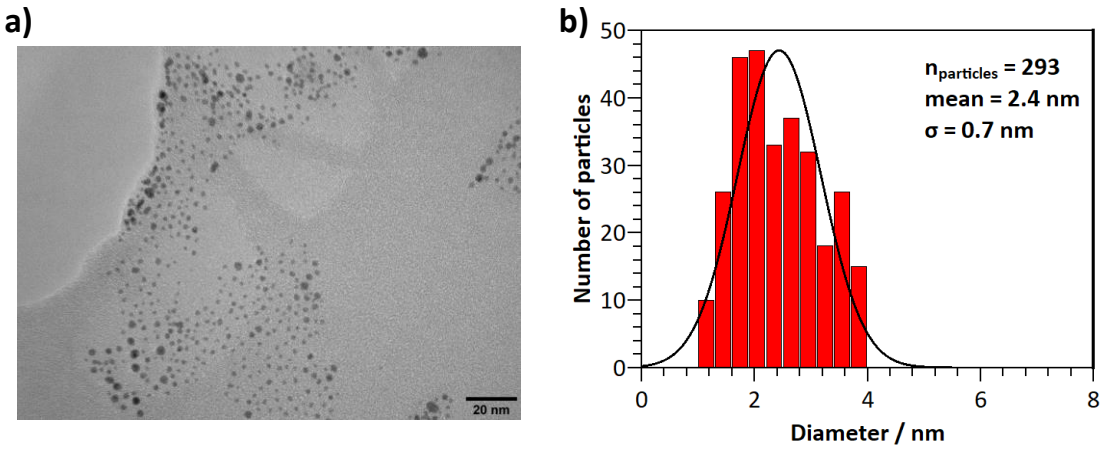


Figure S9. a) Transmission electron micrograph of a sample of **DANPY-NO@AuNPs**, the scale bar represents 20 nm (magnification of 100,000x); and **b)** size distribution of **DANPY-NO@AuNPs**.

4. Calibration curve DANPY-NO-ligand in DMSO – Extinction coefficient determination

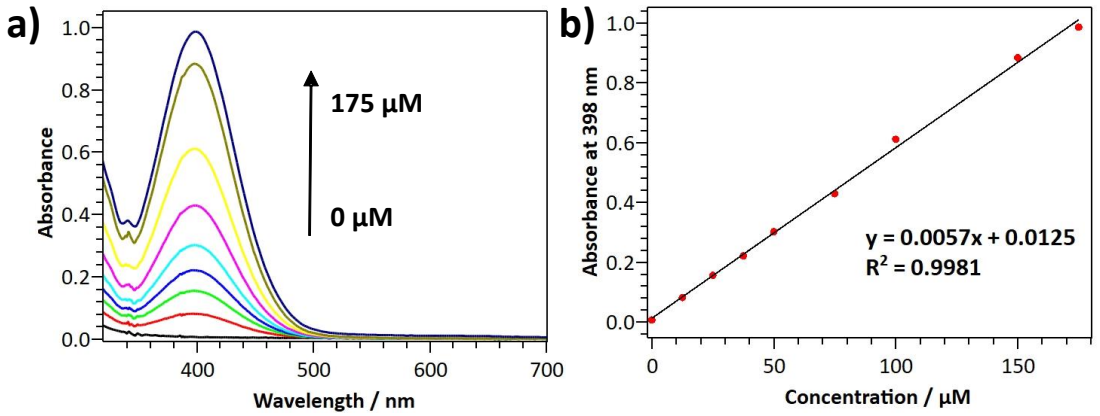


Figure S10. a) UV-vis absorption spectra of **DANPY-NO-ligand** in DMSO at various concentrations (from 0 to 175 µM); and **b)** the corresponding calibration curve measured using the absorbance intensity at 398 nm as a function of the concentration of **DANPY-NO-ligand**. Linear adjustment to obtain the linear regression equation.

5. Electronic spectroscopic properties of DANPY-NO-ligand and DANPY-NO@AuNPs

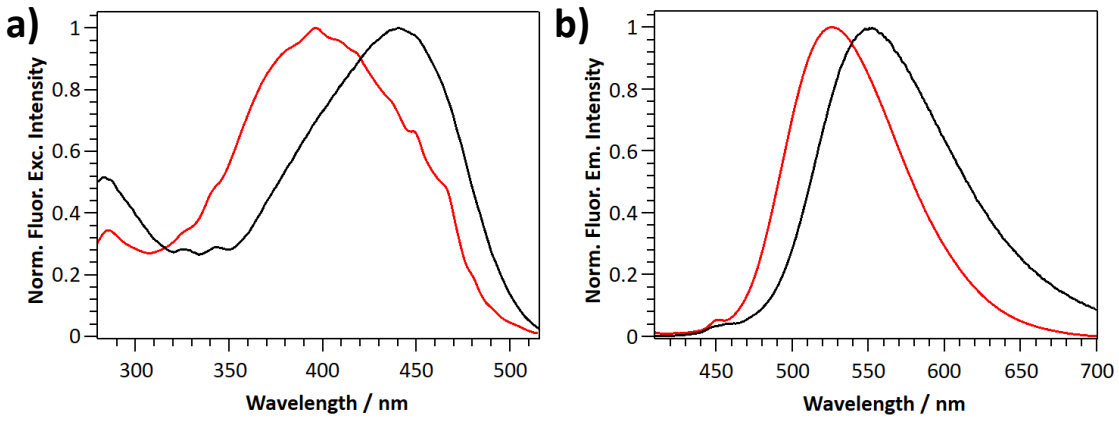


Figure S11. Normalised fluorescence **a)** excitation spectra (λ_{em} = 525 nm or λ_{em} = 550 nm) and **b)** emission spectra (λ_{exc} = 397 nm) of **DNAPY-NO-ligand** in DMSO (red) and **DNAPY-NO@AuNPs** in a DMSO/H_2O mixture (5:2) (black).

6. Characterisation of DANPY-NO@AuNPs in biologically relevant environments

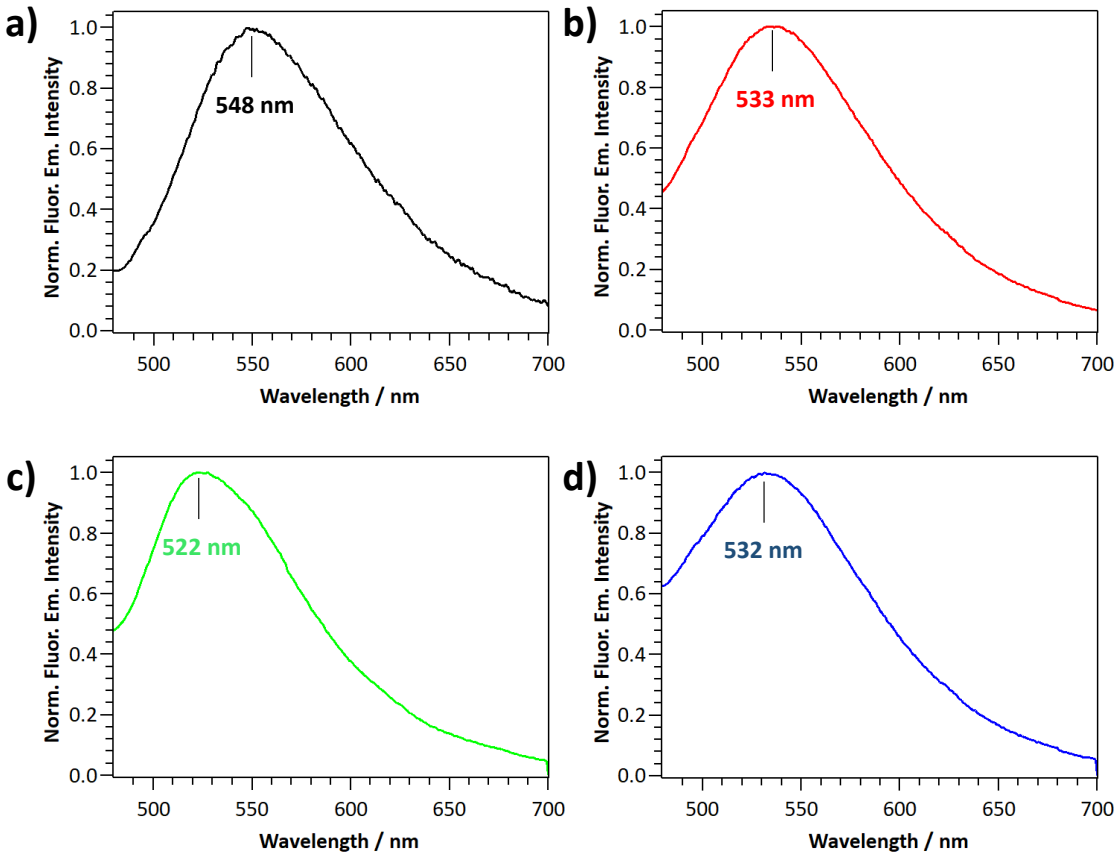


Figure S12. Normalised fluorescence emission spectra (λ_{exc} = 405 nm) of aqueous **DANPY-NO@AuNPs** (83 µg/mL, 7% DMSO) in **a)** H_2O, **b)** BSA 0.1% solution, **c)** DMEM cell culture medium and **d)** HBSS medium.

7. Reproducibility in the synthesis of DANPY-NO@AuNPs

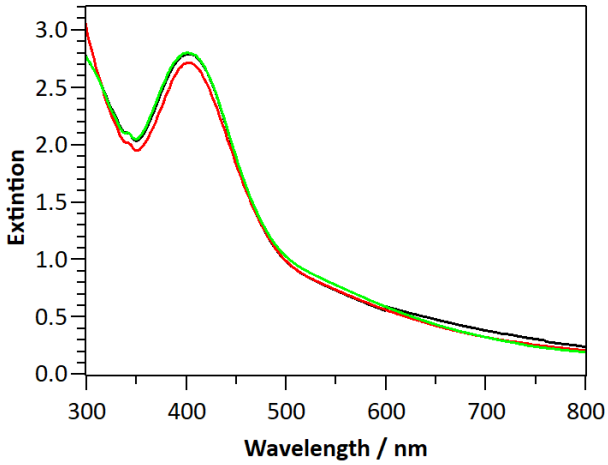


Figure S13. UV-Vis extinction spectra of **DNAPY-NO@AuNPs** (0.83 mg/mL, DMSO/H_2O (5:2)) synthesised on three different days.

8. Stability over time of DANPY-NO@AuNPs in solution

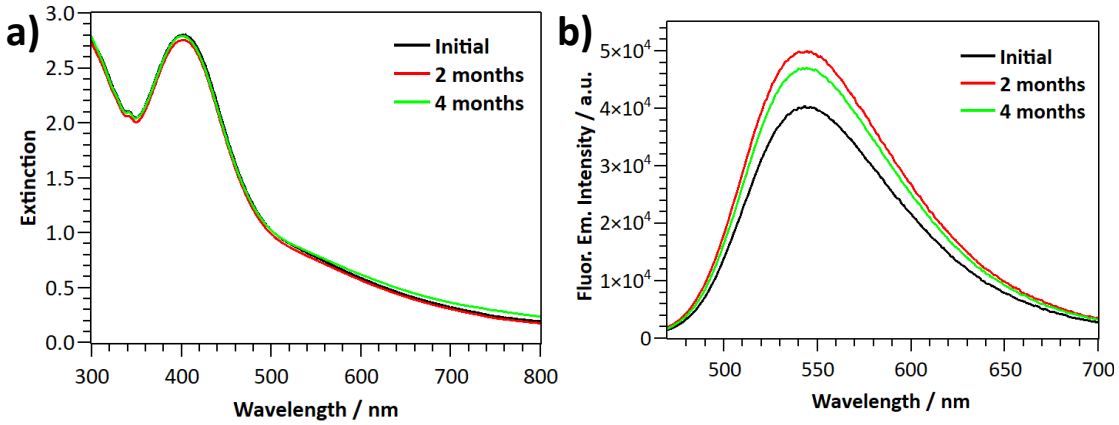


Figure S14. Variation in the **a)** UV-Vis extinction spectrum and **b)** fluorescence emission spectrum (λ_{exc} = 405 nm) of **DNAPY-NO@AuNPs** (0.83 mg/mL, DMSO/H_2O (5:2)) over time, from the day of the synthesis to four months following the synthesis. The particles were stored at room temperature.

9. Sensitivity of DANPY-NO@AuNPs towards nitric oxide

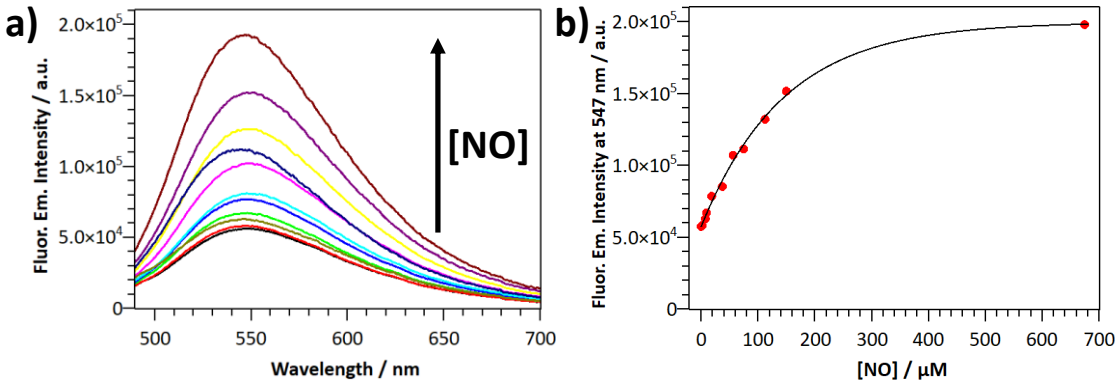


Figure S15. a) Fluorescence emission spectra (λ_{exc} = 405 nm) of aqueous **DANPY-NO@AuNPs** (83 µg/mL, 7% DMSO), following the addition of different concentrations of NO (from 0 to 675 µM); and **b)** corresponding fluorescence emission intensities at 547 nm *versus* the NO concentration with adjustment to a first-order exponential decay (R^2 = 0.995).

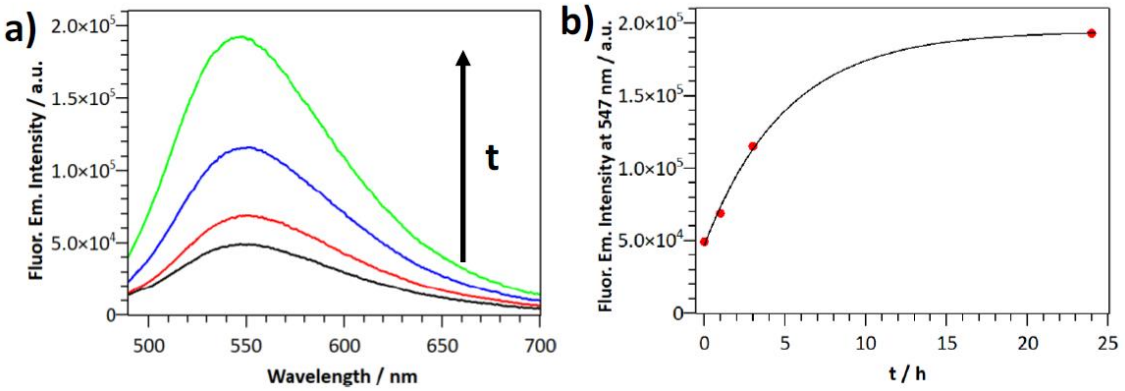


Figure S16. a) Fluorescence emission (λ_{exc} = 405 nm) spectra of aqueous **DANPY-NO@AuNPs** (83 µg/mL, 7% DMSO) over time following the addition of NONOate (500 µM in NaOH 0.01M) (from 0 to 24 h); and **b)** corresponding fluorescence emission intensities at 547 nm versus time with adjustment to a first-order exponential decay (R2 = 0.997).

10. Nitric oxide detection at different pH values by DANPY-NO@AuNPs

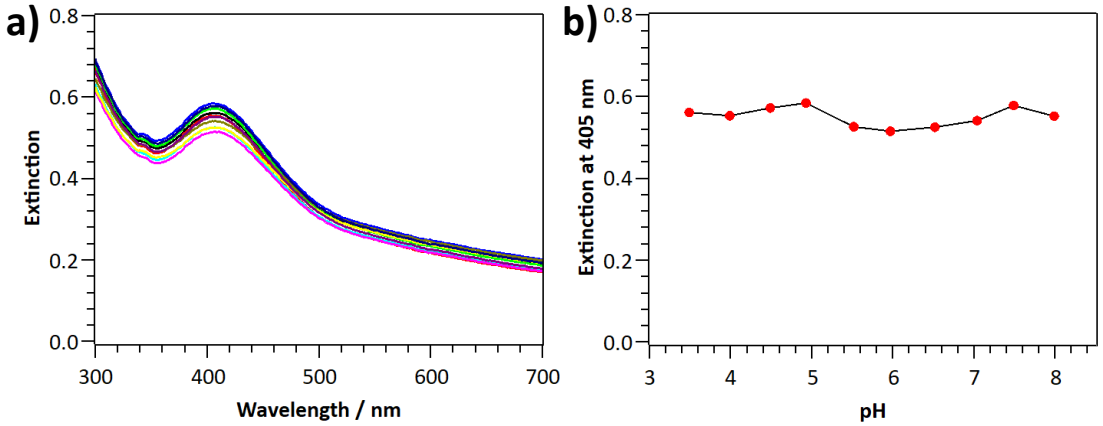


Figure S17. a) UV-Vis extinction spectra of aqueous **DANPY-NO@AuNPs** (0.14 mg/mL, 12% DMSO) at different values of pH (from 3.49 to 7.99); and b) pH titration curve of **DANPY-NO@AuNPs**, absorption at 405 nm as a function of the pH.

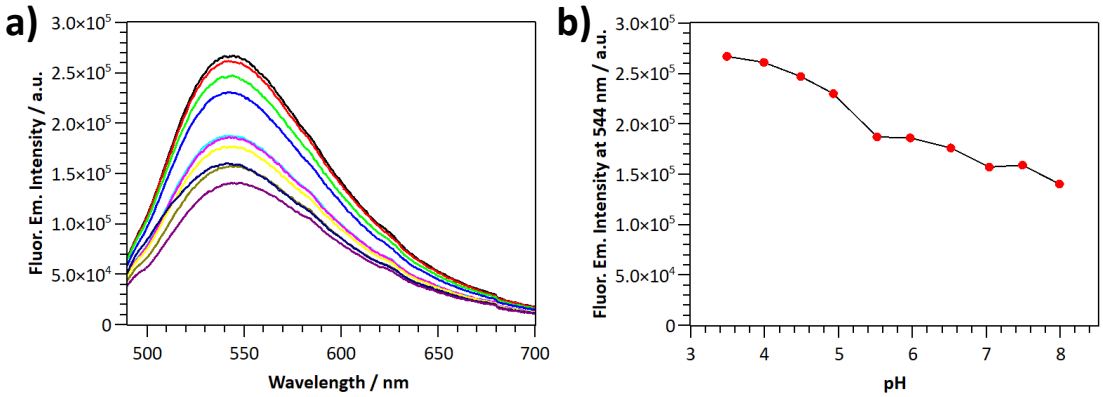


Figure S18. a) Fluoresce emission (λexc = 405 nm) spectra of aqueous **DANPY-NO@AuNPs** (0.14 mg/mL, 12% DMSO) at different values of pH (from 3.49 to 7.99); and **b)** pH titration curve of **DANPY-NO@AuNPs**, fluorescence emission intensity at 544 nm as a function of the pH.

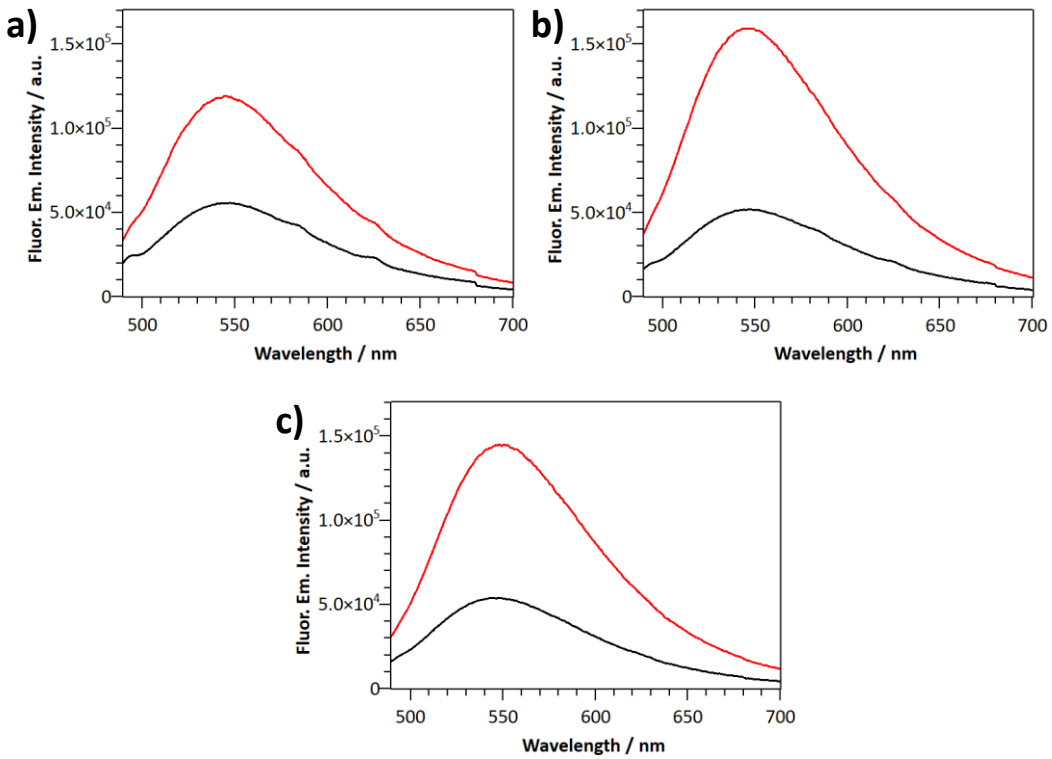


Figure S19. Fluorescence emission spectra (λ_{exc} = 396 nm) of aqueous **DANPY-NO@AuNPs** (86 µg/mL, 7% DMSO) before (black) and after (red) addition of NONOate (0.5 mM in NaOH 0.01 M) at different pH values: **a)** pH 4.14, **b)** pH 5.63 and **c)** pH 7.30. Average results of three samples in each case. The emission intensity increase at 544 nm is **a)** 118 ± 32%, **b)** 207 ± 31% and **c)** 168 ± 27%.

11. Selectivity of DANPY-NO@AuNPs towards nitric oxide

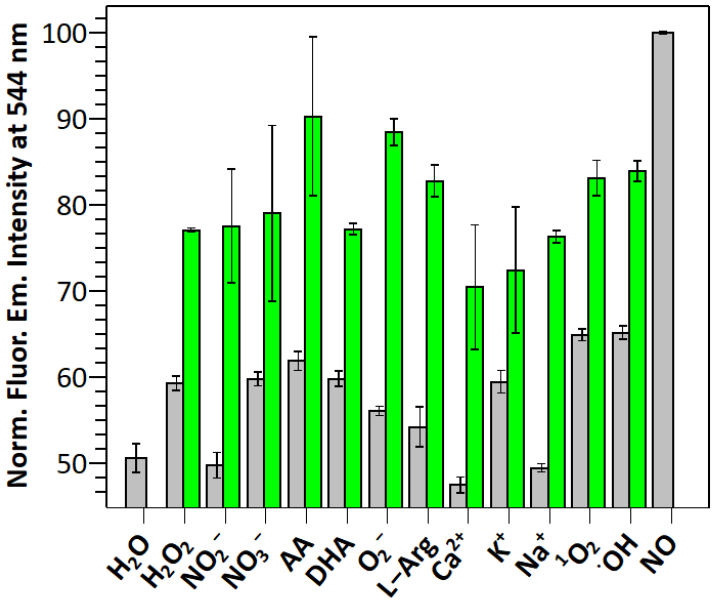


Figure S20. Normalised fluorescence emission (λ_{exc} = 405 nm) response of aqueous **DANPY-NO@AuNPs** (83 µg/mL, 7% DMSO) at 544 nm in the presence of various biologically relevant species (H_2O, H_2O_2, NO_2^-, NO_3^-, AA, DHA, O_2^-, L-Arg, Ca^{2+}, K^+, Na^+, $^1O_2$ and $^{\cdot}OH$) before (grey) and after the addition of NO (100 µM) to each sample (green). Each experiment was repeated in triplicate and the relative standard error is indicated by the error bars.

12. Cytotoxicity studies of DANPY-NO@AuNPs in RAW264.7Y NO$^-$ cells

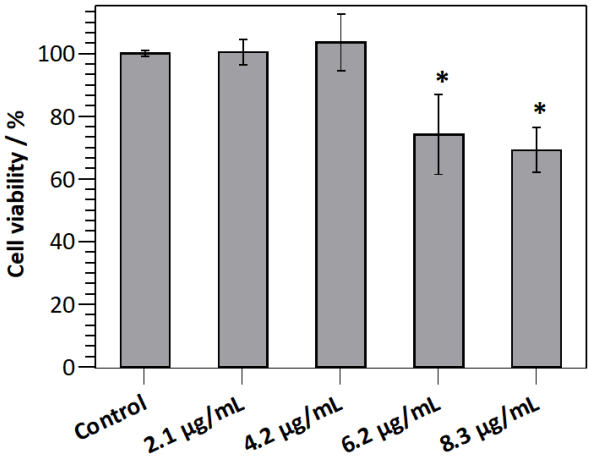


Figure S21. Cell viability studies of **DANPY-NO@AuNPs** (increasing concentrations shown in the x-axis, where control indicates 0 µg/mL) in RAW264.7Y NO$^-$ cells. CellTiter-Blue viability assay was used to determine the cytotoxicity of **DANPY-NO@AuNPs**. n = 3, error bars indicate the standard deviation of the three measurements. *Represents a statistically significant difference of $p<0.05$ (Student's t-test) comparing measurements.

13. Control experiments in RAW264.7Y NO$^-$ cells

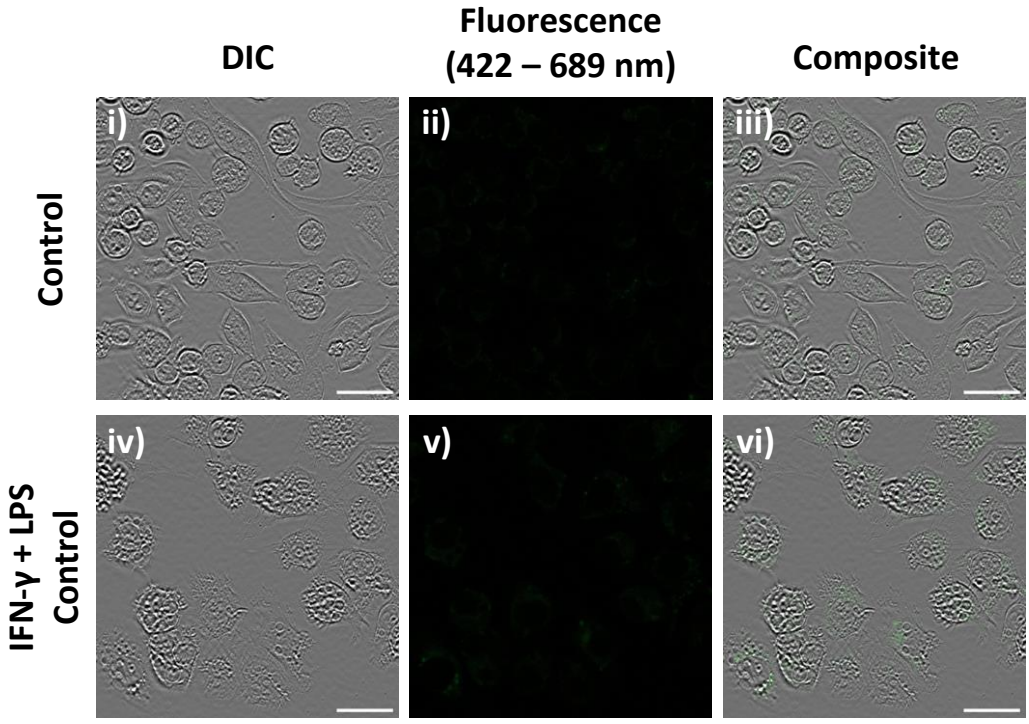


Figure S22. CLSM images of unstimulated (i – iii) and stimulated (iv – vi) control RAW264.7Y NO$^-$ cells. Stimulation was performed overnight with LPS (0.7 µg/mL) and IFN-Y (17 µg/mL). λ_{exc} = 405 nm and $\Delta\lambda_{em}$ = 422 – 689 nm. Scale bars = 25 µm.

14. Selectivity of DANPY-NO@AuNPs towards nitric oxide in RAW264.7Y NO^- cells

Although the fluorescence emission intensities observed in the CLSM images (**Figure S23**) for cells stimulated and incubated with **DANPY-NO@AuNPs** are not as bright as following 24 h incubation (**Figure 4**), the shorter incubation time required for this experiment was sufficient for the internalisation of **DANPY-NO@AuNPs** and the successful NO detection.

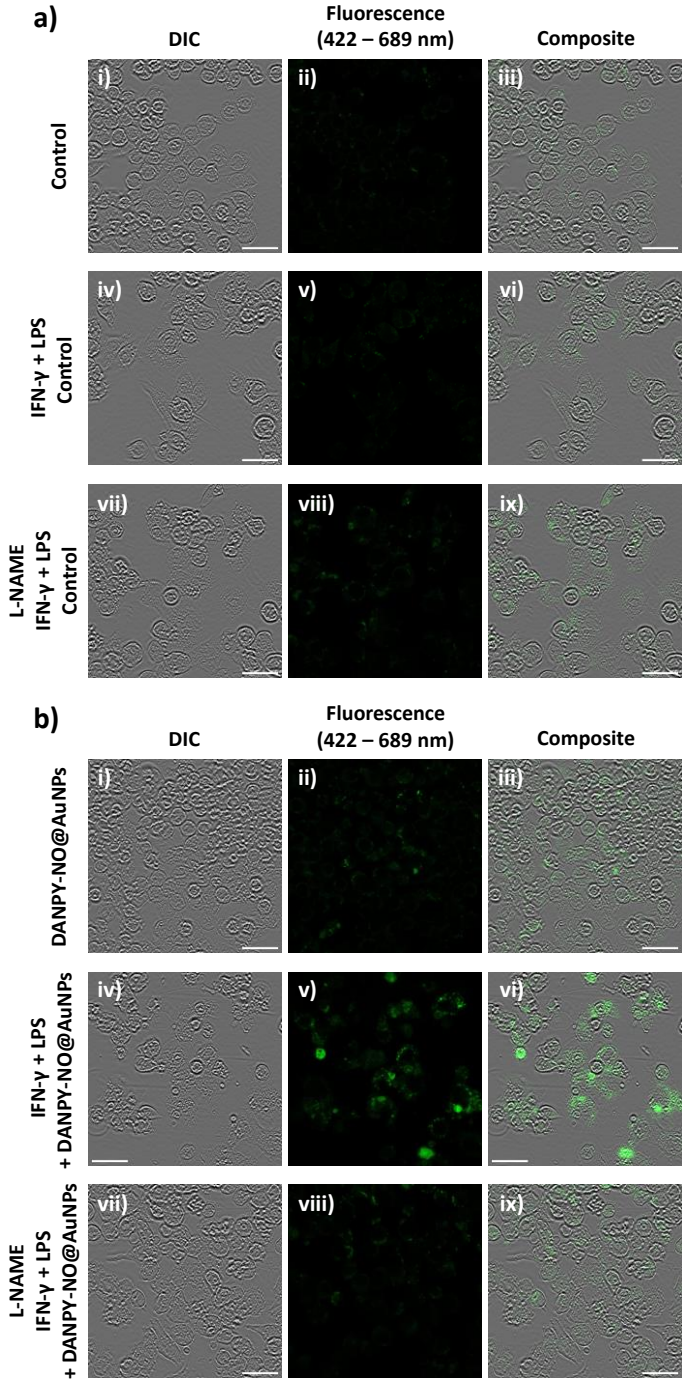


Figure S23. CLSM images of **a)** unstimulated (i – iii), stimulated (iv – vi) and pre-incubated with L-NAME and stimulated (vii – ix) control RAW264.7Y NO^- macrophages; and **b)** unstimulated and incubated with **DANPY-NO@AuNPs** (i – iii), stimulated and incubated with **DANPY-NO@AuNPs** (iv – vi) and pre-incubated with L-NAME, stimulated and incubated with **DANPY-NO@AuNPs** (vii – ix). Incubation with **DANPY-NO@AuNPs** (4.2 μg/mL) was done for 3 h. Stimulation was performed overnight using LPS (0.7 μg/mL) and IFN-Y (17 μg/mL) and the pre-treatment with L-NAME (2 mM) was done for 30 min. λ_{exc} = 405 nm and $\Delta\lambda_{em}$ = 422 – 689 nm. Scale bars = 25 μm.

15. Control experiments from multiphoton microscopy of RAW264.7Y NO$^-$ cells

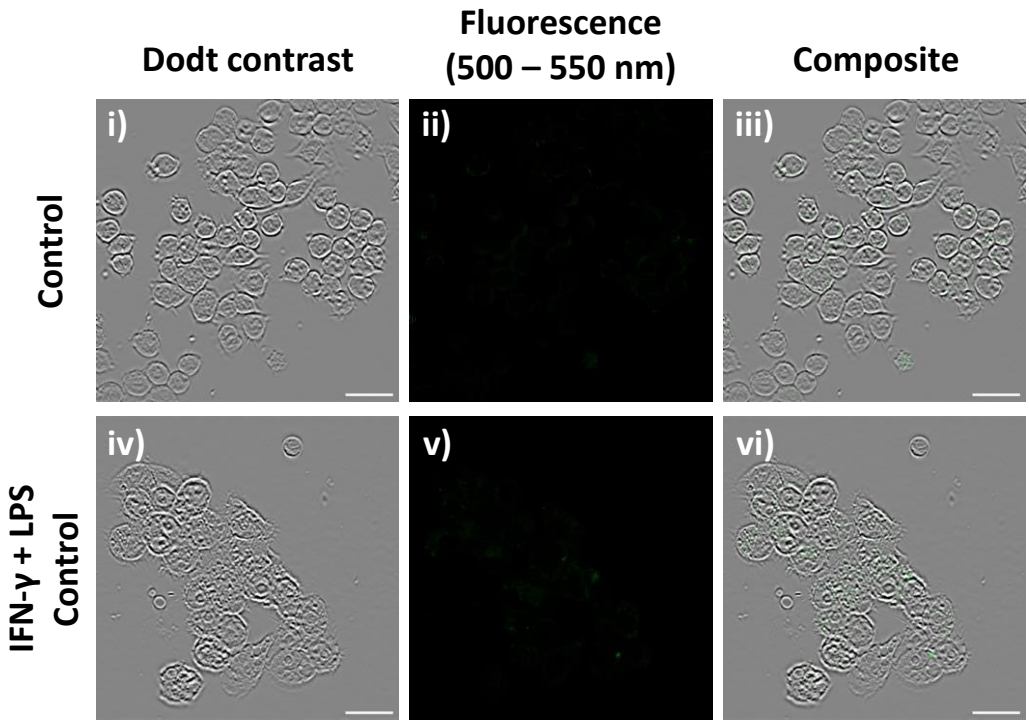


Figure S24. Multiphoton microscopy images of unstimulated (i – iii) and stimulated (iv – vi) control RAW264.7Y NO$^-$ cells; λ_{exc} = 800 nm and $\Delta\lambda_{em}$ = 500 – 550 nm. Scale bars = 25 µm.

16. Colocalisation studies of DANPY-NO@AuNPs in THP-1 macrophages

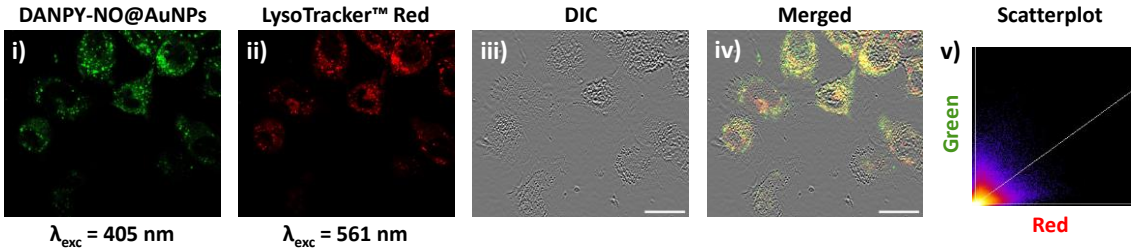


Figure S25. CLSM images of THP-1 macrophages incubated with **DANPY-NO@AuNPs** (4.2 µg/mL) and LysoTracker™ Red DND-99 (5 µM) (i – iv) and scatterplot showing the correlation between the green and red emission intensities (v). Images collected upon excitation at i) λ_{exc} = 405 nm, $\Delta\lambda_{em}$ = 500 – 580 nm and ii) λ_{exc} = 561 nm, $\Delta\lambda_{em}$ = 580 – 625 nm; iii) DIC channel; and iv) composite image of green, red and DIC channels. Scale bars = 25 µm.

17. Cytotoxicity studies of DANPY-NO@AuNPs in THP-1 cells

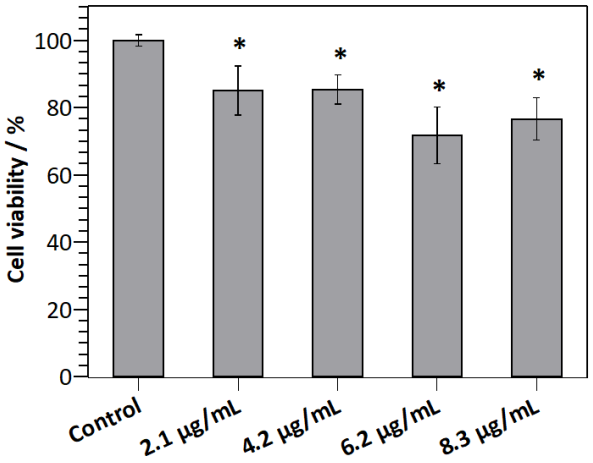


Figure S26. Cell viability studies of **DANPY-NO@AuNPs** (increasing concentrations shown in the x-axis, where control indicates 0 µg/mL) in THP-1 cells. CellTiter-Blue viability assay was used to determine the cytotoxicity of **DANPY-NO@AuNPs**. n = 3, error bars indicate the standard deviation of the three measurements. *Represents a statistically significant difference of $p<0.05$ (Student's t-test) comparing measurements.

18. Nitric oxide detection in THP-1 cells using DANPY-NO@AuNPs

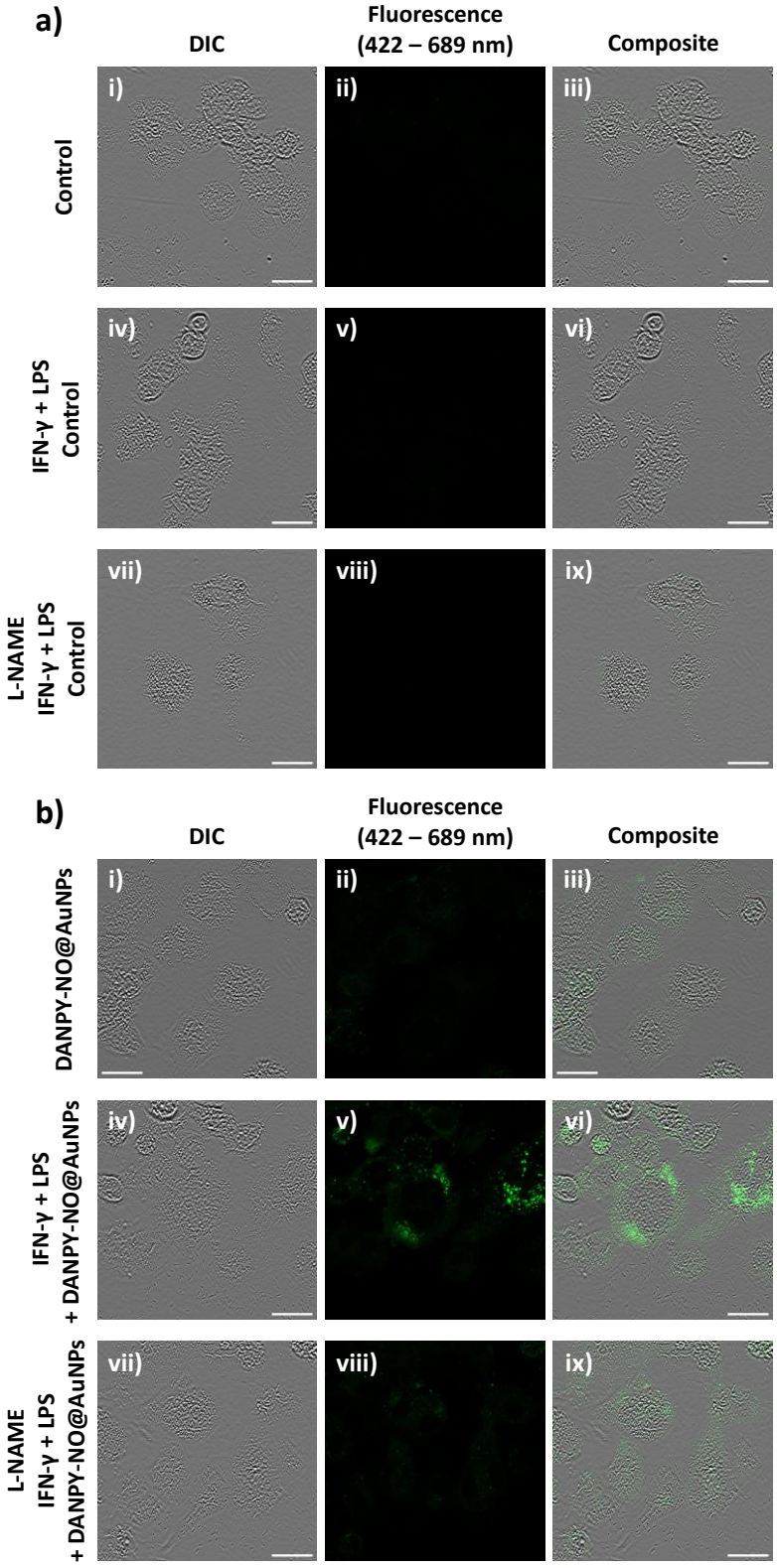


Figure S27. CLSM images of **a)** unstimulated (i – iii), stimulated (iv – v), and pre-treated with L-NAME and stimulated (vii – ix) control THP-1 macrophages; and **b)** unstimulated (i – iii), stimulated (iv – v), and pre-treated with L-NAME and stimulated (vii – ix) THP-1 macrophages all incubated overnight with **DANPY-NO@AuNPs** (4.2 μg/mL). Stimulation was performed overnight with LPS (5 μg/mL) and the treatment with L-NAME (0.1 mM) was done 30 min prior to stimulation. λ_{exc} = 405 nm and $\Delta\lambda_{em}$ = 422 – 689 nm. Scale bars = 25 μm.

19. Colocalisation studies of DANPY-NO@AuNPs in endothelial macrophages

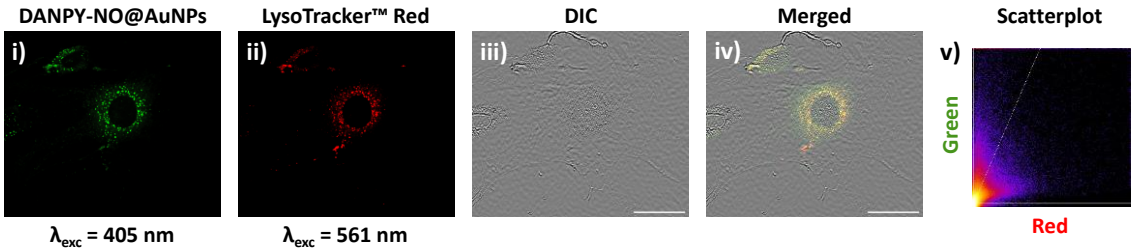


Figure S28. CLSM images of endothelial cells incubated with **DANPY-NO@AuNPs** (4.2 µg/mL) and LysoTracker™ Red DND-99 (5 µM) (i – iv) and scatterplot showing the correlation between the green and red emission intensities (v). Images collected upon excitation at i) λ_{exc} = 405 nm, $\Delta\lambda_{em}$ = 500 – 580 nm and ii) λ_{exc} = 561 nm, $\Delta\lambda_{em}$ = 580 – 625 nm; iii) DIC channel; and iv) composite image of green, red and DIC channels. Pearson's coefficient of 0.63 ± 0.02 (n = 3 images, *ca.* 5 cells). Scale bars = 50 µm.

20. Cytotoxicity studies of DANPY-NO@AuNPs in endothelial cells

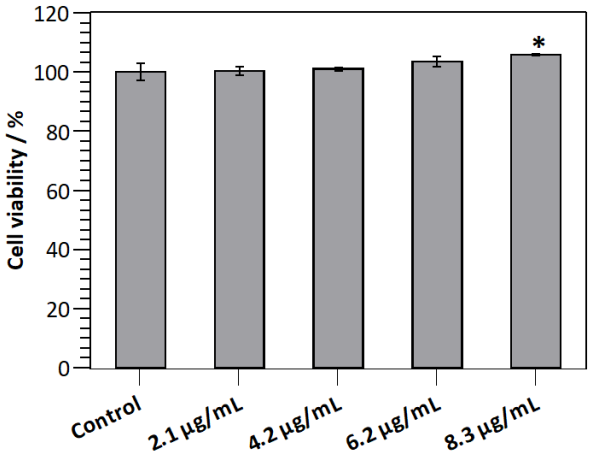


Figure S29. Cell viability studies of **DANPY-NO@AuNPs** (increasing concentrations shown in the x-axis, where control indicates 0 µg/mL) in endothelial cells. CellTiter-Blue viability assay was used to determine the cytotoxicity of **DANPY-NO@AuNPs**. n = 3, error bars indicate the standard deviation of the three measurements. *Represents a statistically significant difference of $p<0.05$ (Student's t-test) comparing measurements.

21. Control experiments in endothelial cells

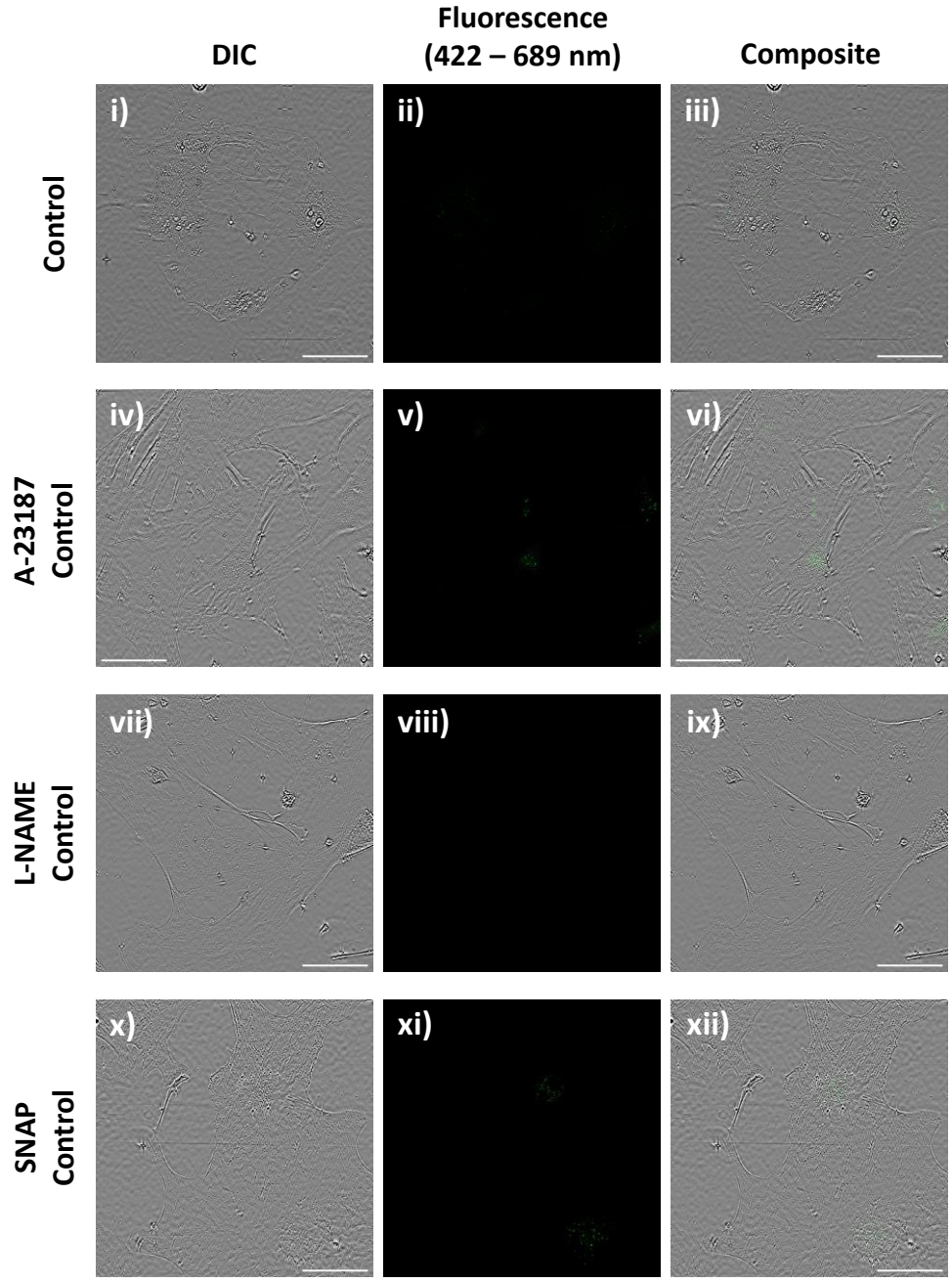


Figure S30. CLSM images of **a)** untreated (i – iii), treated with Ca^{2+} ionophore A-23187 (iv – vi), treated with L-NAME (vii – ix) and treated with SNAP (x – xii) control endothelial cells. Treatment with Ca^{2+} ionophore A-23187 (1 µM), with L-NAME (200 mM) and with SNAP (220 µM) were performed overnight. λ_{exc} = 405 nm and $\Delta\lambda_{em}$ = 422 – 689 nm. Scale bars = 50 µm.

22. Intracellular nitric oxide detection in breast cancer cells using DANPY-NO@AuNPs

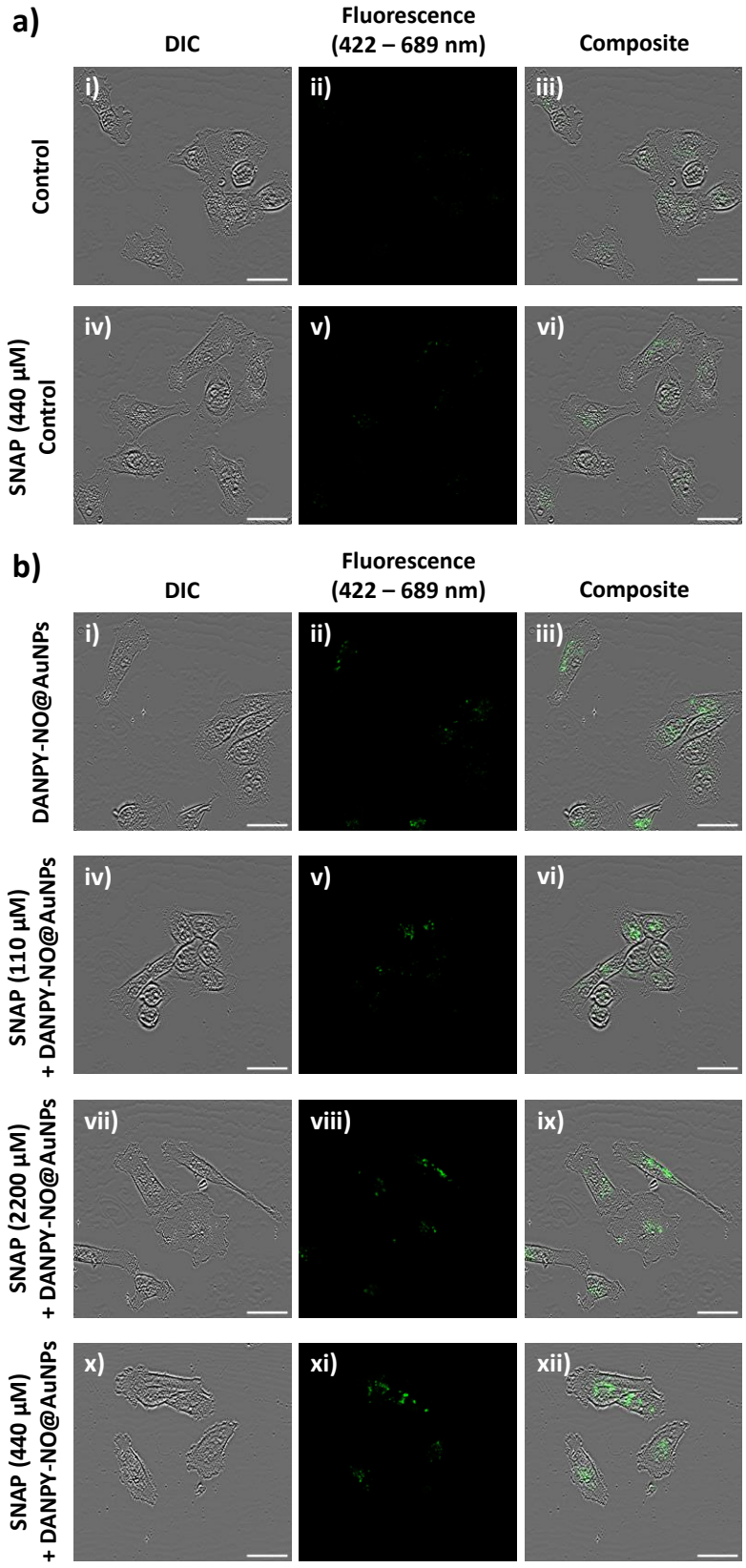


Figure S31. CLSM images of **a)** untreated (i – iii) and treated with SNAP (iv – vi) control MDA-MB-231 cells; and **b)** untreated (i – iii) and treated with different concentrations of SNAP (40 min): 110 µM (iv – vi), 220 µM (vii – ix) and 440 µM (x – xii) MDA-MB-231 cells all incubated overnight with **DANPY-NO@AuNPs** (4.2 µg/mL). λ_{exc} = 405 nm and $\Delta\lambda_{em}$ = 422 – 689 nm. Scale bars = 25 µm.

23. References

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