Electronic Supporting information

Ru(II)-polypyridyl surface functionalised gold nano-particles as DNA targeting supramolecular structures and luminescent cellular imaging agents

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General

All NMR spectra were recorded using either a 400 MHz Bruker Spectrospin DPX-400 or AV-600 spectrometer, operating at 400.1/ 600.1 MHz for ^{1}H NMR and 100.2/150.2 MHz for ^{13}C NMR respectively. Shifts are referenced relative to the internal solvent signals. Electrospray mass spectra were recorded on a Micromass LCT spectrometer, running Mass Lynx NT V 3.4 on a Waters 600 controller connected to a 996 photodiode array detector with HPLC-grade methanol or acetonitrile. High resolution mass spectra were determined by a peak matching method, using leucine Enkephalin, (Tyr-Gly-Gly-Phe-Leu), as the standard reference (m/z = 556.2771). All accurate mass were reported within ±5 ppm. Melting points were determined using an IA9000 digital melting point apparatus. Infrared spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer fitted with a Universal ATR Sampling Accessory. Elemental analysis was conducted at the Microanalytical Laboratory, School of Chemistry and Chemical Biology, University College Dublin. All TEM images were collected on the Jeol 2100 microscope operating at 200 kV with a Lanthanum Hexaborise emission source placed on the CMA, Trinity College Dublin.

UV-visible absorption spectra were recorded on a Varian CARY 50 spectrophotometer with a wavelength range of 200-800 nm and a scan rate of 600 nm min^{-1}. Baseline correction measurements were used for all spectra. Fluorescence and luminescence measurements were made with a Varian Carey Eclipse Fluorimeter in 3 cm quartz cuvettes The concentration of titration solutions was calculated according to the extinction coefficients of the free Ru(II) complexes to enable direct comparison of free complexes with AuNP bound complexes. The Ru(II) concentration was 4.0×10^{-6} M, 2.2×10^{-6} M and 7.7×10^{-6} M for **1-3.AuNP** respectively which corresponded to AuNP concentrations of 8.68×10^{-11} M, 1.17×10^{-10} M and $1.33 \cdot 10^{-10}$ M for the corresponding conjugates **1-3.AuNP**.[i] Titrations were carried out at ambient temperature by monitoring changes in the absorption and emission spectra, at pH 7.4 in 10 mM phosphate buffer upon successive additions of aliquots of st-DNA. The results are quoted using the concentration of st-DNA expressed as a nucleotide phosphate to dye ratio (P/D ratio). Circular dichroism (CD) spectra were recorded at a concentration corresponding to an optical density of approximately 2.5, in buffered solutions, on a Jasco J-810-150S spectropolarimeter.

Solutions of Salmon testes (st) DNA in 10 mM phosphate buffer (pH 7.4) gave a ratio of UV absorbance at 260 and 280 nm of 1.86:1, indicating that the DNA was sufficiently free of protein. Its concentration was determined spectrophotometrically using the molar absorptivity of 6600 M^{-1} cm^{-1} (260 nm). Thermal denaturation experiments were performed on a thermoelectrically coupled Perkin Elmer LAMBDA 25 UV/Vis Spectrophotometer. The temperature in the cell was ramped from 30 to 90 ºC, at a rate of 1 ºC min^{-1} and the absorbance at 260 nm was measured every 0.2 ºC. All other reagents and solvents were purchased commercially and used without further purification. Phosphate buffer was made from two 1 M stock solutions of NaH_2PO_4 and Na_2HPO_4 (using 10 mL volumetric flasks) in Millipore water. Portions of each solution were diluted together to achieve 10 mM phosphate buffer and the pH was adjusted to pH 7.4 by addition of NaOH.

The DNA photocleavage studies were carried out by treating pBR322 plasmid DNA (1 mg/mL) with each of the complexes at varying ratios. The samples were then subjected to 2 J/cm^2 (30 mins irradiation) using an Hamamatsu L2570 200 Watt HgXe Arc Lamp equipped with a $NaNO_2$ filter before being separated using horizontal agarose gel electrophoresis in a TBE (8.9 mM Tris-borate, 2 mM EDTA, pH 8.0) buffer. A 0.8% (w/v) agarose solution was prepared by dissolving 0.8 g of agarose in

100 mL of TBE buffer. The agarose was melted by boiling, and the gel was poured while warm and left to cool. Electrophoresis was carried out at ca. 5 V/cm (40 mA, 90 V) to separate covalently closed circular (Form I), open circular (Form II), and linear (Form III) forms of the plasmid DNA. A loading dye solution composed of sucrose (40%), xylene-cyanol (0.25%), and bromophenol (0.25%) in TBE was added to the samples to help them sink in the wells of the gel. Visualization of the DNA after electrophoresis was achieved by staining the gel for 90 mins with an aqueous solution of SYBR Green. The dye within the gel was illuminated with a transilluminator (Bioblock 254 UV illuminator) and the gel photographed to provide a record of the distances migrated by the various DNA fragments. The ratio of the different forms was estimated using ImageJ Gel analysis software.

Cell Culture

HeLa cells were cultured in DMEM medium with phenol red indicator supplemented with 10% (v/v) Foetal Calf serum (FCS), Penicillin and Streptomycin (200 μg/ml), at 37 °C in 75 cm² tissue culture flasks and incubated at 37 °C in a humidified atmosphere of 95% oxygen and 5% carbon dioxide. RAW 264.7 cells were cultured as for HeLa but using RPMI media supplemented 10% (v/v) Foetal Calf serum (FCS), Penicillin and Streptomycin (200 μg/ml).

Alamar Blue Viability assay

Alamar Blue (AB) is a safe, non-toxic aqueous dye that is used to asses cell viability and cell proliferation. 0.5 x 10^5 cells were seeded in 200 μL/well of a 96-well plate and treated with the relevant compound for the required time and incubated at 37 °C. The cells were exposed to a xenon lamp for 1 h at a dose of 4 J/m² and re-incubated. Cells were treated with 10 μL of AB pre-warmed to 37 °C, covered in tin foil and re-incubated at 37 °C. Once the control untreated cells turned light purple (3-6 h depending on cell type) each plate was read on a fluorescent plate reader with emission 590 nm, excitation 544 nm. The background fluorescence of the media without cells but with Alamar blue was taken away from each group and the control cells represented 100% viability.

Confocal Microscopy (Live cell Imaging)

1.7 x 10^5 cells/well were plated in glass bottom wells (10 mm), incubated overnight at 37 °C in a humidified atmosphere of 95% oxygen and 5% carbon dioxide. The wells were treated with the relevant compound for the required time. The cells were then washed with PBS and fresh media, DAPI was then added before imaging. DAPI is a blue fluorescent nucleic acid stain which associates with AT clusters in the minor groove of DNA. A 488 nm argon laser was used to excite the compounds (emission 620 nm) and a 405 nm laser to excite the DAPI (emission 620 nm). An Olympus FV1000 point scanning microscope fitted with a 60x oil immersion lens with an NA (numerical aperture) of 1.42 was used to visualize the samples. And the software used to collect images was FluoView Version 7.1 software.

Transmission Electron Microscopy on cells (TEM)

7.5 x 10^5 cells were seeded in 10 mL of media and incubated overnight at 37 °C in a humidified atmosphere of 95% oxygen and 5% carbon dioxide. The cells were treated with the relevant compound and re-incubated for the required time. The cells were treated with trypsin (1x) for 5 min at 37 °C, washed with PBS, centrifuged at 1200

rpm for 5 min and re-suspended in filtered PBS. One part of fix solution (16% paraformaldehyde, 25% Em Grade Glutaraaldehyde, 0.666 M Hepes pH 7.5) was mixed with one part cell suspension at room temperature (RT). After 30 min incubation at RT the cells were collected by centrifugation and washed with PBS. The final pellet was re-suspended in 500 μL PBS in an eppendorf and passed on to the Centre for Microscopy and Analysis (CMA) TCD for TEM processing. A sample of the compound alone was also passed on to the CMA for analysis.

Synthesis

The Ru(II) precursors cis-$[Ru(bpy)_2Cl_2]$,[ii] cis-$[Ru(TAP)_2Cl_2]$,[iii] $[RuCl_2(\eta^4\text{-COD})]$,[iv] were synthesized according to a literature procedure.[v]

11-Mercapto-N-(1,10-phenanthrolin-5-yl)undecanamide (4): 5-Amino-1,10-phenanthroline (0.50 g, 2.56 mmol, 1 eq.) was dissolved in distilled CH_2Cl_2 (100 mL) before the solution was cooled to 0 °C. 11-Mercaptoundecanoic acid (0.56 g, 2.56 mmol, 1 eq.) was added followed by 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) (1.23 g, 6.4 mmol, 2.5 eq.) and finally 4-dimethylaminopyridine (DMAP) (0.31 g, 2.56 mmol, 1 eq.) before the reaction mixture was stirred at 0 °C for a further 1 h. The mixture was allowed to reach room temperature before being stirred for a further 24 h. The solvent was removed under reduced pressure before H_2O (50 mL) was added causing precipitation of a white solid which was isolated by centrifugation. The resulting solid was re-dispersed in MeCN (10 mL) and again centrifuged before being collected by suction filtration and dried under high vacuum to yield a beige/white solid. (0.8 g, 78%). m.p. 88-90 °C. δ_H (400 MHz, $[D_6]$ DMSO): 10.12 (s, 1H, N-H), 9.13 (dd, 1H, J = 1.56, J = 4.24, CH-15), 9.04 (dd, 1H, J = 1.72, J = 4.24, CH-14), 8.61 (dd, H, J = 1.48, J = 8.4, CH-17), 8.44 (dd, 1H, J = 1.72, J = 8.16, CH-12), 8.18 (s, 1H, CH-11), 7.83 (dd, 1H, J = 4.28, J = 8.4, CH-16), 7.74 (dd, 1H, J = 4.32, J = 8.08, CH-13), 2.53 (from CH COSY) (t, 2H, CH2-1), 2.43 (t, 2H, J =7.2, CH2-10), 1.69 (t, 2H, J =6.52, CH2-9), 1.47 (t, 2H, J = 6.44, CH2-2), 1.42 – 1.20 (br s, 12H, CH2-3, 4, 5, 6, 7 and 8). δ_C (100 MHz, $[D_6]$ DMSO): 6132.25 (CH, 17), 132.05 (qC), 128.57 (qC), 125.09 (qC) 124.03 (CH, 13), 123.25 (CH, 16), 120.40 (CH, 11), 36.43 (CH2, 1), 31.54 (CH2, 10), 29.56 (CH2, 2), 29.38 (CH2), 29.36 (CH2), 29.26 (CH2), 29.20 (CH2), 29.04 (CH2), 28.64 (CH2), 25.69 (CH2, 2). ν_{max} (film)/cm^{-1}: 3253 (amide-N-H stretch), 2919 (alkane-C-H stretch), 2849 (alkane-C-H stretch), 1658 (C=O), 1533 (alkane-C-H bend), 721 (C-S).

General procedure for the synthesis of the Ru(II) polypyridyl complexes[v]

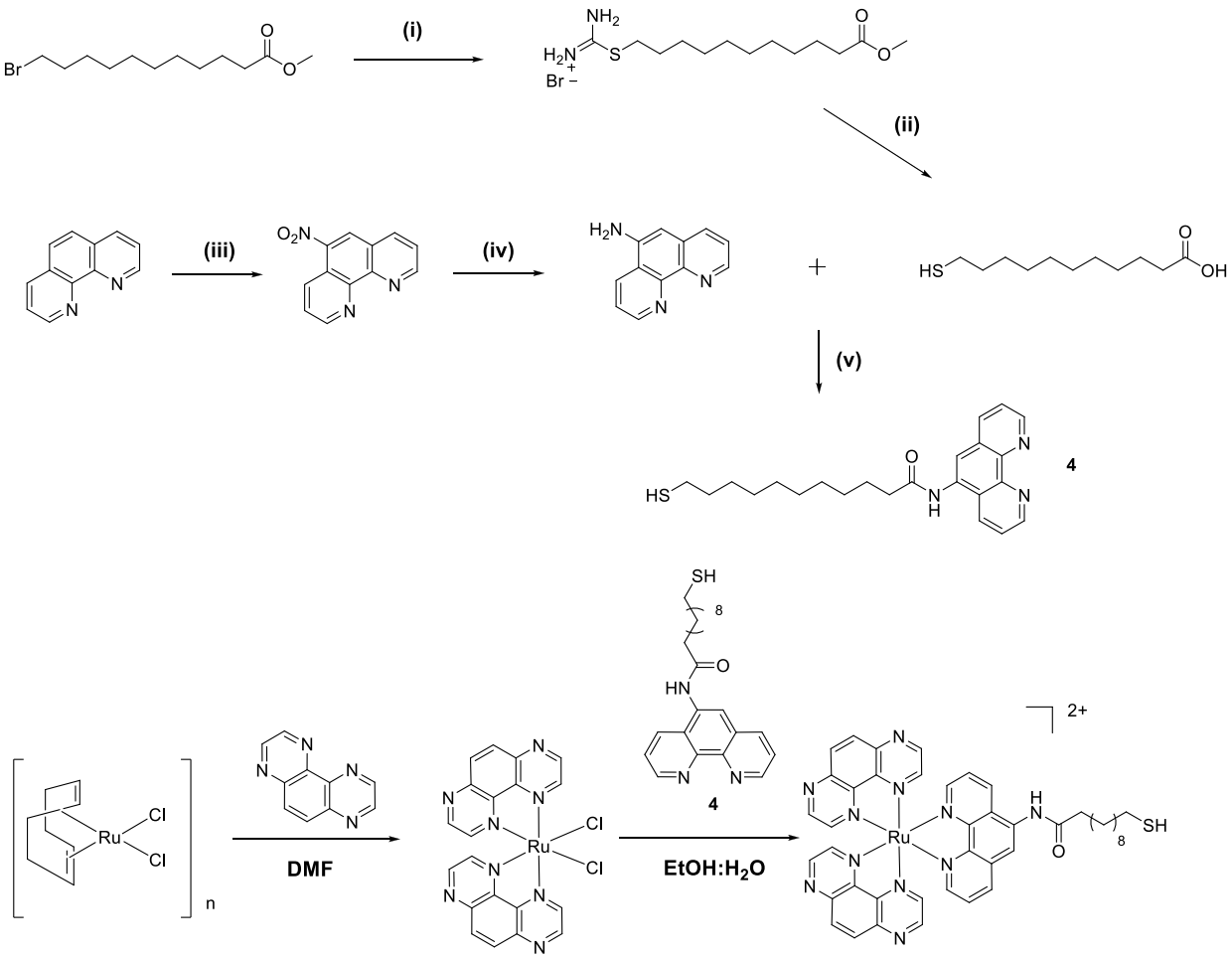
Ligand 11-mercapto-N-(1,10-phenanthrolin-5-yl)-undecanamide (1 eq.) and the appropriate bis polypyridyl Ru dichloride (1 eq.) were suspended in ethanol:H_2O (8:2) and the suspension was degassed by bubbling with argon for 15 mins. The reaction mixture was heated at 120 °C for 40 minutes using microwave irradiation before being filtered. As example a representation of the synthesis of the Ru(II) polypyridyl complex **3** is shown in Scheme S1. The PF_6 salt was formed by addition of a concentrated aqueous solution of NH_4PF_6 with the resulting precipitate being collected by centrifugation. The dried solid was redissolved in MeCN before purification by silica flash column chromatography eluting with MeCN:H_2O:Aq. $NaNO_3$(sat) (40:4:1). The chloride form of the complex was reformed by stirring a solution of the PF_6 salt in MeOH with Amberlite anion exchange resin (Cl^- form) for one hour.

Ru-(11-mercapto-N-(1,10-phenanthrolin-5-yl)undecanamide) (bipyridine)$_2$Cl$_2$ (1): Complex **1** was synthesised according to general procedure indicated above using 11-mercapto-N-(1,10-phenanthrolin-5-yl)undecanamide (0.10 g, 0.253 mmol, 1 eq.) and Ru(bpy)$_2$Cl$_2$ (0.123 g, 0.253 mmol, 1 eq.) giving the product as a red/brown solid (0.167 g, 75%). Calculated for $C_{43}H_{45}Cl_2N_7ORuS•H_2O•1.25NaCl$: C, 53.20; H, 4.88; N, 10.09. Found: C, 53.00; H, 4.99; N, 9.60. m.p. 115-120 °C. δ_H (600 MHz, MeOD): 8.77 (d, 1H, J = 7.68, H-15), 8.74 (d, 2H, J = 8.22, bpy-H), 8.70 (d, 2H, J = 8.16, bpy-H), 8.64 (d, 1H, J = 7.5, H-14), 8.48 (s, 1H, H-11), 8.22 (d, 1H, J = 4.38, H-17), 8.17 (t, 2H, J = 7.92, bpy-H), 8.13 (d, 1H, J = 5.16, H-12), 8.07 (t, 2H, J = 7.98, bpy-H), 7.93 (t, 2H, J = 5.82, bpy-H), 7.86 (dd, 1H, J = 5.28, J = 8.52, H-16), 7.79 (dd, 1H, J = 5.28, J = 8.28, H-13), 7.64 (d, 2H, J = 5.52, bpy-H), 7.55 (m, 2H, bpy-H), 7.33 (m, 2H, bpy-H), 2.76 (m, 2H, CH2-1), 2.66 (t, 2H, J = 7.44, CH2-10), 1.79 (m, 4H, CH2-9 and 2), 1.49 (m, 2H, CH2-8), 1.37 (s, 10H, CH2-7, 6, 5, 4, 3). δ_C (150 MHz, MeOD): 174.66, 157.25, 157.23, 157.01, 157.00, 152.03 (C-H, 17), 151.33 (C-H, 12), 151.31 (C-H, bpy), 151.21 (C-H, bpy), 151.19, 147.78, 145.51, 137.79 (C-H, bpy), 137.67 (C-H, bpy), 136.30 (C-H, 14), 133.89, 132.58 (C-H, 15), 130.58, 127.44 (C-H, bpy), 127.39 (C-H, bpy), 127.36, 127.31, 127.28, 126.01 (C-H, 13), 125.43 (C-H, 16), 124.11 (C-H, bpy), 124.09 (C-H, bpy), 124.05, 124.03, 121.29, 51.30 (CH2, 1), 35.94 (CH2, 10), 28.99 (CH2), 28.93 (CH2), 28.91 (CH2), 28.87 (CH2), 28.77 (CH2), 28.23 (CH2, 8), 25.20 (CH2, 9), 22.26 (CH2, 2).νmax (film)/cm^{-1}: 3366 (Aromatic C-H stretch), 2922 (alkane-C-H stretch), 2851 (alkane-C-H stretch), 1627 (C=O), 1421 (C-N stretch), 769 (C-S). HRMS (m/z -ES): Found: 954. 2139 ($(M+PF_6)^+$; $C_{43}H_{45}F_6N_7OPRuS$, Requires: 954.2139).

Ru(11-mercapto-N-(1,10-phenanthrolin-5-yl)undecanamide) (phenanthroline)$_2$Cl$_2$ (2): Complex **2** was synthesized according to the general procedure using 11-mercapto-N-(1,10-phenanthrolin-5-yl)undecanamide (0.10 g, 0.253 mmol, 1 eq.) and Ru(phen)$_2$Cl$_2$ (0.135 g, 0.253 mmol, 1 eq.) giving the product as a red/brown solid (0.152 g, 65%). Calculated for $C_{47}H_{45}Cl_2N_7ORuS•-H_2O•1.25NaCl$: C, 55.40; H, 4.65; N, 9.62. Found: C, 55.55; H, 4.71; N, 9.27. m.p. 115-120 °C. δ_H (600 MHz, MeOD): 8.74 (d, 1H, J = 8.4, H-15), 8.69 (t, 4H, J = 8.04, Phen-H), 8.61 (d, 1H, J = 8.22, H 14), 8.49 (s, 1H, H-11), 8.32 (s, 4H, Phen-H), 8.14 (m, 3H, Phen-H and H-17), 8.10 (dd, 2H, J = 5.22, J = 8.16, Phen-H), 8.06 (d, 1H, J = 5.1, H-12), 7.74 (m, 5H, Phen-H and H-16), 7.68 (dd, 1H, J = 5.28, J = 8.28, H-13), 2.76 (m, 2H, CH2-1), 2.65 (t, 2H, J = 7.32, CH2-10), 1.76 (m, 4H, CH2-9 and 2), 1.48 (m, 2H, CH2-8), 1.36 (s, 10H, CH2-7, 6, 5, 4, 3). δ_C (150 MHz, MeOD): 174.67 (qC, amide), 152.38 (C-H, Phen), 152.36 (C-H, Phen), 152.26 (C-H, Phen), 152.23 (C-H, Phen), 151.58 (C-H, 12), 148.16, 147.66, 147.64, 147.63, 145.91, 136.75 (C-H, Phen), 136.27 (C-H, 14), 133.87, 132.55 (C-H, 15), 130.96, 130.54, 127.88 (C-H, Phen), 127.43, 125.88 (C-H, 13), 125.80, 125.78 (C-H, Phen), 125.75, 125.30 (C-H, 16), 121.30 (C-H, 11), 51.30 (CH2, 1), 35.93 (CH2, 10), 28.98 (CH2), 28.92 (CH2), 28.91 (CH2), 28.87 (CH2), 28.76 (CH2), 28.23 (CH2, 8), 25.20 (CH2, 9), 22.26 (CH2, 2). ν_{max} (film)/cm^{-1}: 3362 (Aromatic C-H stretch), 2922 (alkane-C-H stretch), 2850 (alkane-C-H stretch), 1627 (C=O), 1424 (C-N stretch), 720 (C-S). HRMS (m/z -ES): Found: 2147.3765 $(2M+3PF_6)^+$; $C_{94}H_{88}F_{18}N_{14}O_2P_3Ru_2S_2$, Requires: 2147.3669.

Ru(11-mercapto-N-(1,10-phenanthrolin-5-yl)undecanamide) (tetraazaphenanthrene)$_2$Cl$_2$ (3): Complex **3** was synthesized according to the general procedure using 11-mercapto-N-(1,10-phenanthrolin-5-yl)undecanamide (0.10 g, 0.253 mmol, 1 eq.) and Ru(TAP)$_2$Cl$_2$ (0.135 g, 0.253 mmol, 1 eq.) giving the product

as a red/brown solid (0.127 g, 54%). Calculated for $C_{43}H_{41}Cl_2N_{11}ORuS$•NaCl: C, 54.47; H, 5.90; N, 12.70. Found: C, 54.78; H, 5.03; N, 12.78. m.p. 115-120 °C. δ_H (600 MHz, MeOD): 9.03 (d, 4H, J = 2.58, TAP-H), 8.86 (dd, 1H, J = 0.96, J = 8.58, H-15), 8.71 (dd, 1H, J = 0.90, J = 8.28, H-14), 8.65 (s, 4H, TAP-H), 8.53 (s, 1H, H-11), 8.45 (dd, 2H, J = 2.64, J = 6.42, TAP-H), 8.30 (m, 3H, TAP-H and H-17), 8.20 (dd, 1H, J = 0.96, J = 5.1, H-12), 7.83 (dd, 1H, J = 5.28, J = 8.58, H-16), 7.76 (dd, 1H, J = 5.28, J = 8.28, H-13), 2.73 (m, 2H, CH2-1), 2.66 (t, 2H, J = 7.38, CH2-10), 1.80 (m, 2H, CH2-9), 1.74 (m, 2H, CH2-2), 1.47 (m, 2H, CH2-8), 1.37 (s, 10H, CH2-7, 6, 5, 4, 3). δ_C (150 MHz, MeOD): 174.61 (qC, amide), 153.32 (C-H, 17), 152.43 (C-H, 12), 149.40 (C-H, TAP), 149.37 (C-H, TAP), 149.25 (C-H, TAP), 149.23 (C-H, TAP), 148.57 (C-H, TAP), 148.55 (C-H, TAP), 148.05 (C-H, TAP), 148.02 (C-H, TAP), 147.27, 145.36, 145.34, 144.95, 142.16, 142.14, 142.12, 142.10, 137.65 (C-H, 14), 134.01 (C-H, 15), 132.58 (C-H, TAP), 132.51 (C-H, TAP), 130.79, 127.55, 126.31 (C-H, 13), 125.74 (C-H, 16), 121.15 (C-H, 11), 51.29 (C-H2, 1), 35.94 (C-H2, 10), 28.96 (C-H2), 28.90 (C-H2), 28.88 (C-H2), 28.83(C-H2), 28.74 (C-H2), 28.21 (C-H2, 8), 25.18 (C-H2, 9), 22.23 (C-H2, 2). ν_{max} (film)/cm^{-1}: 3368 (Aromatic C-H stretch), 2922 (alkane-C-H stretch), 2851 (alkane-C-H stretch), 1629 (C=O), 1485 (C-N stretch), 723 (C-S). HRMS (m/z -ES): Found: 2155.3379 $(2M+3PF_6)^+$; $C_{86}H_{80}F_{18}N_{22}O_2P_3Ru_2S_2$, Requires: 2155.3288.



Scheme S1 *: (i) EtOH:H_2O, thiourea; (ii)(a) aq. NaOH; (b) 6N H_2SO_4; (iii) H_2SO_4, HNO_3, H_2O; (iv) N_2H_2, 10% Pd/C, EtOH; (v) DCM, EDCI, DMAP; (vi) $Ru(bpy)_2Cl_2$, EtOH:H_2O, µW; (vii) $Ru(phen)_2Cl_2$, EtOH:H_2O, µW; (viii) $Ru(TAP)_2Cl_2$, EtOH:H_2O, µW.*

Figure S1. ^{1}H (400 MHz) and ^{13}C NMR of **4** in [D_6] DMSO (100 MHz)

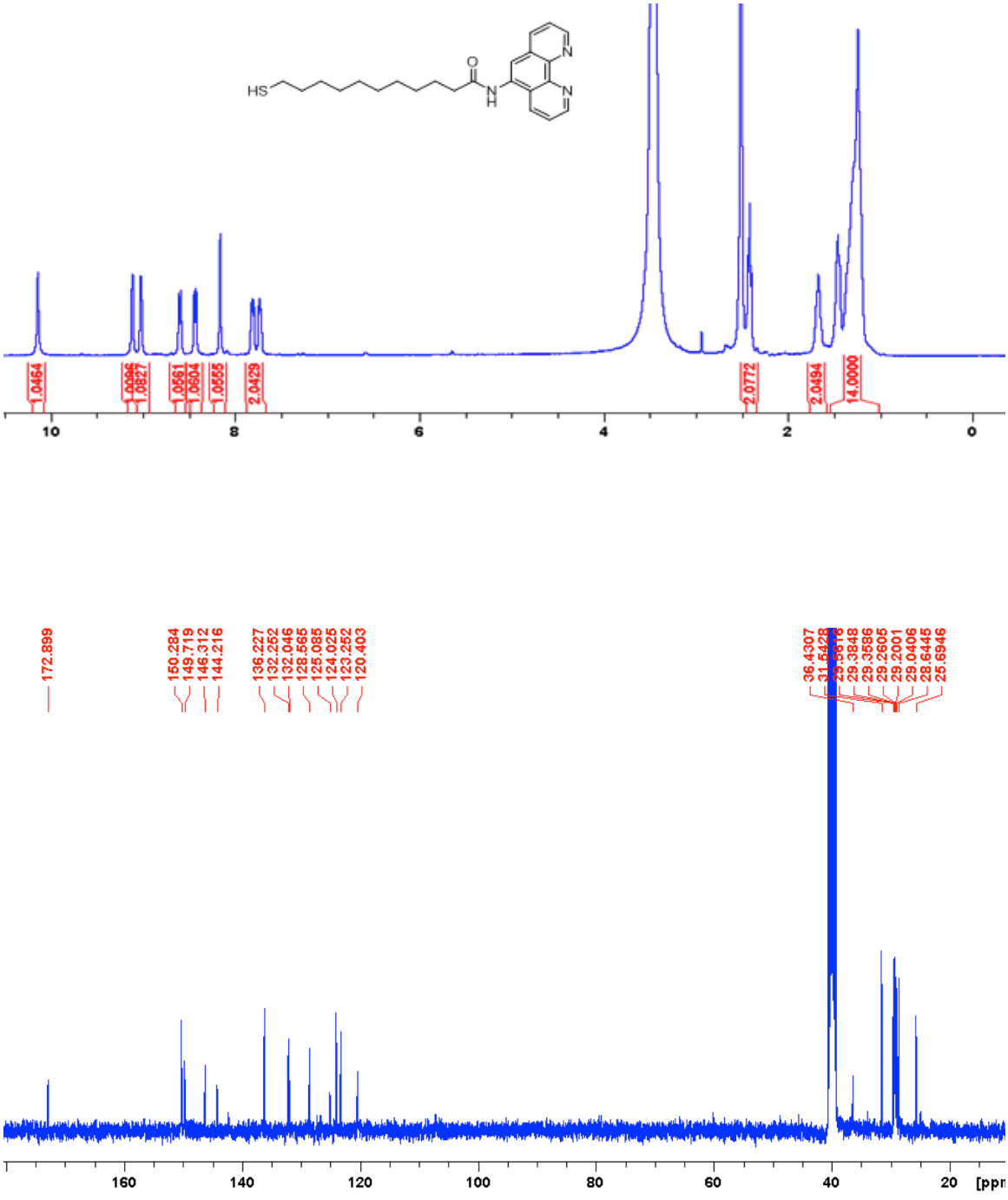


Figure S2. ^{1}H (600 MHz) and ^{13}C NMR of **1** in MeOD (150 MHz)

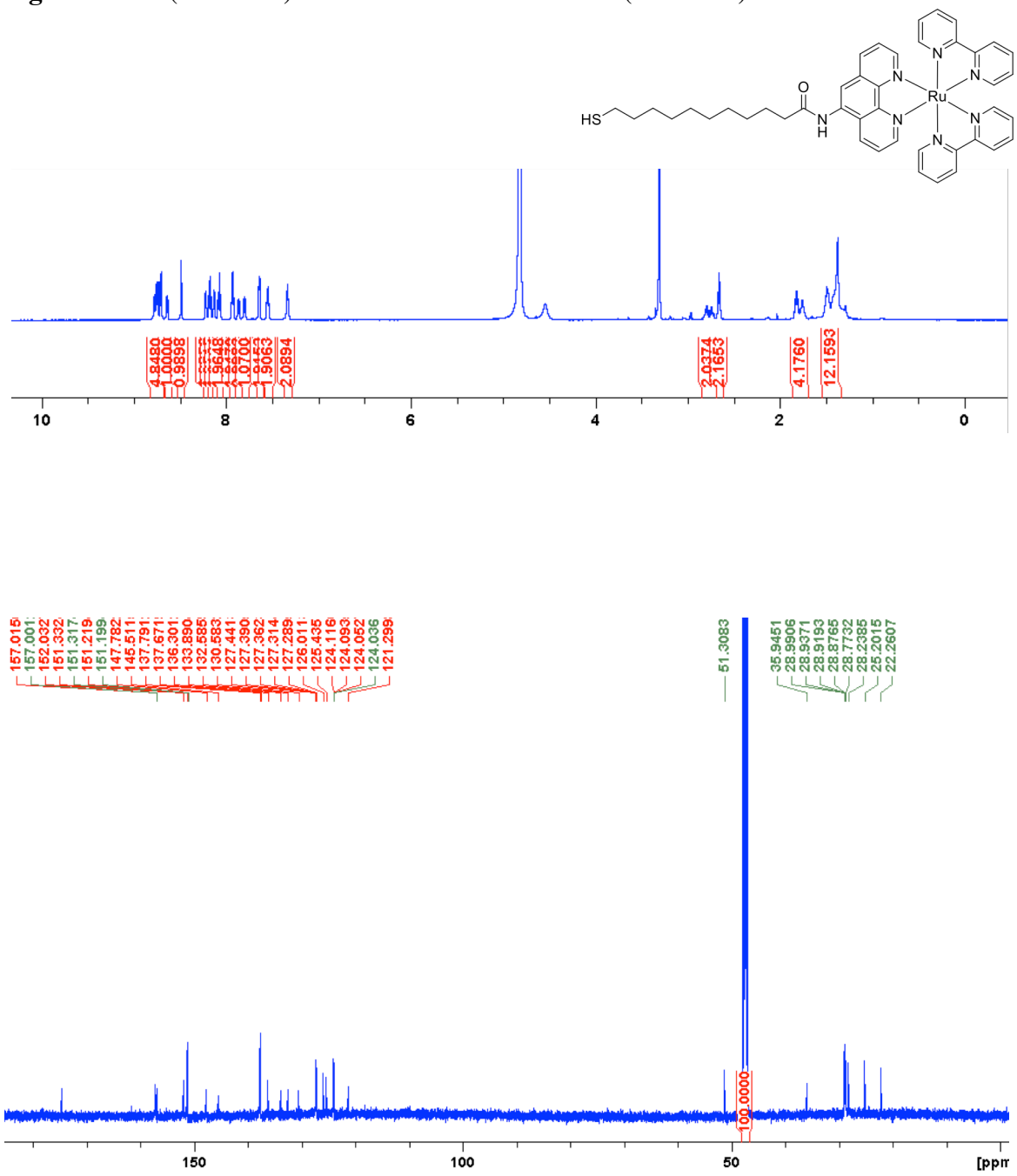


Figure S3. ^{1}H (600 MHz) and ^{13}C NMR of **2** in MeOD (150 MHz)

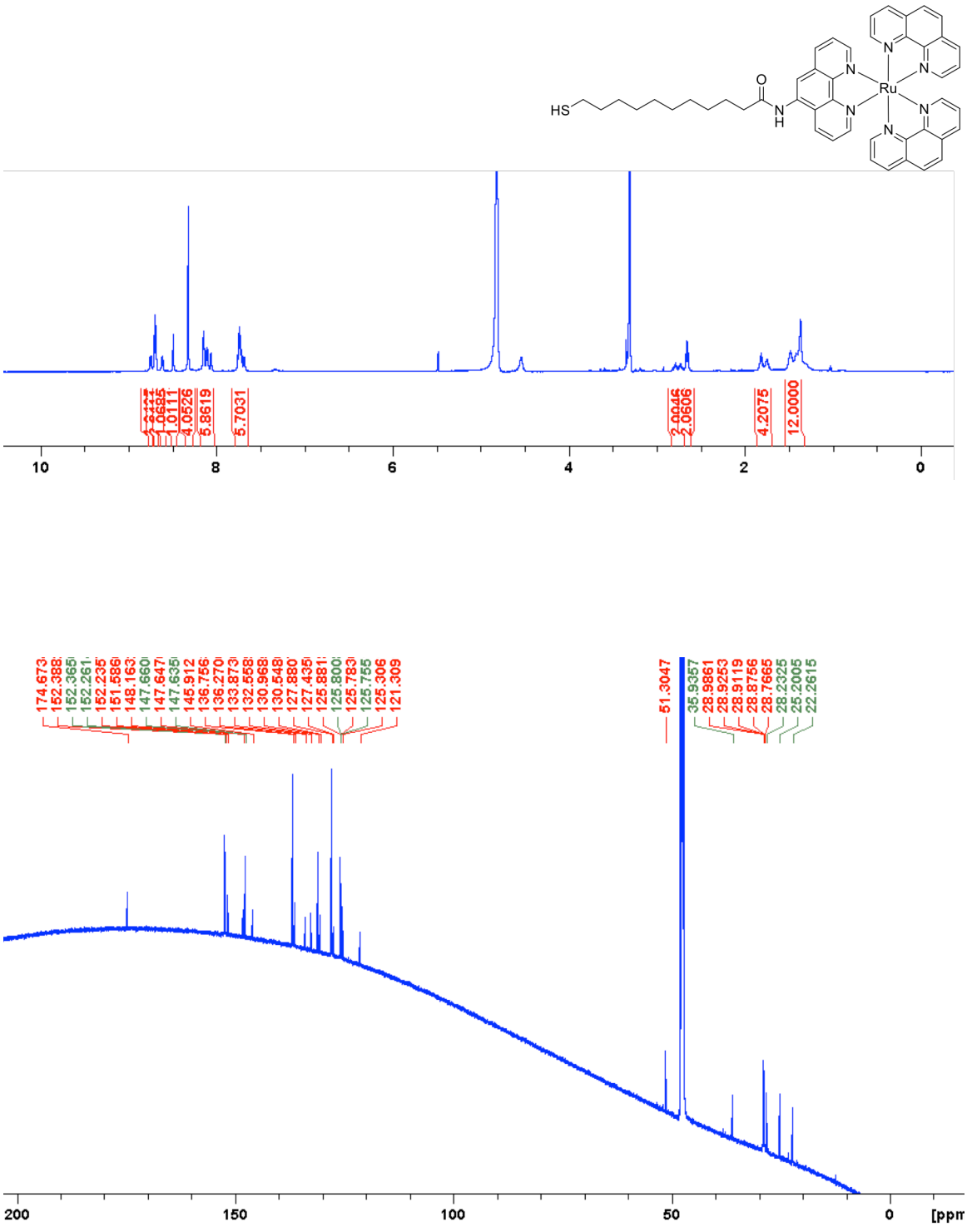


Figure S4. ^{1}H (600 MHz) and ^{13}C NMR of **3** in MeOD (150 MHz)

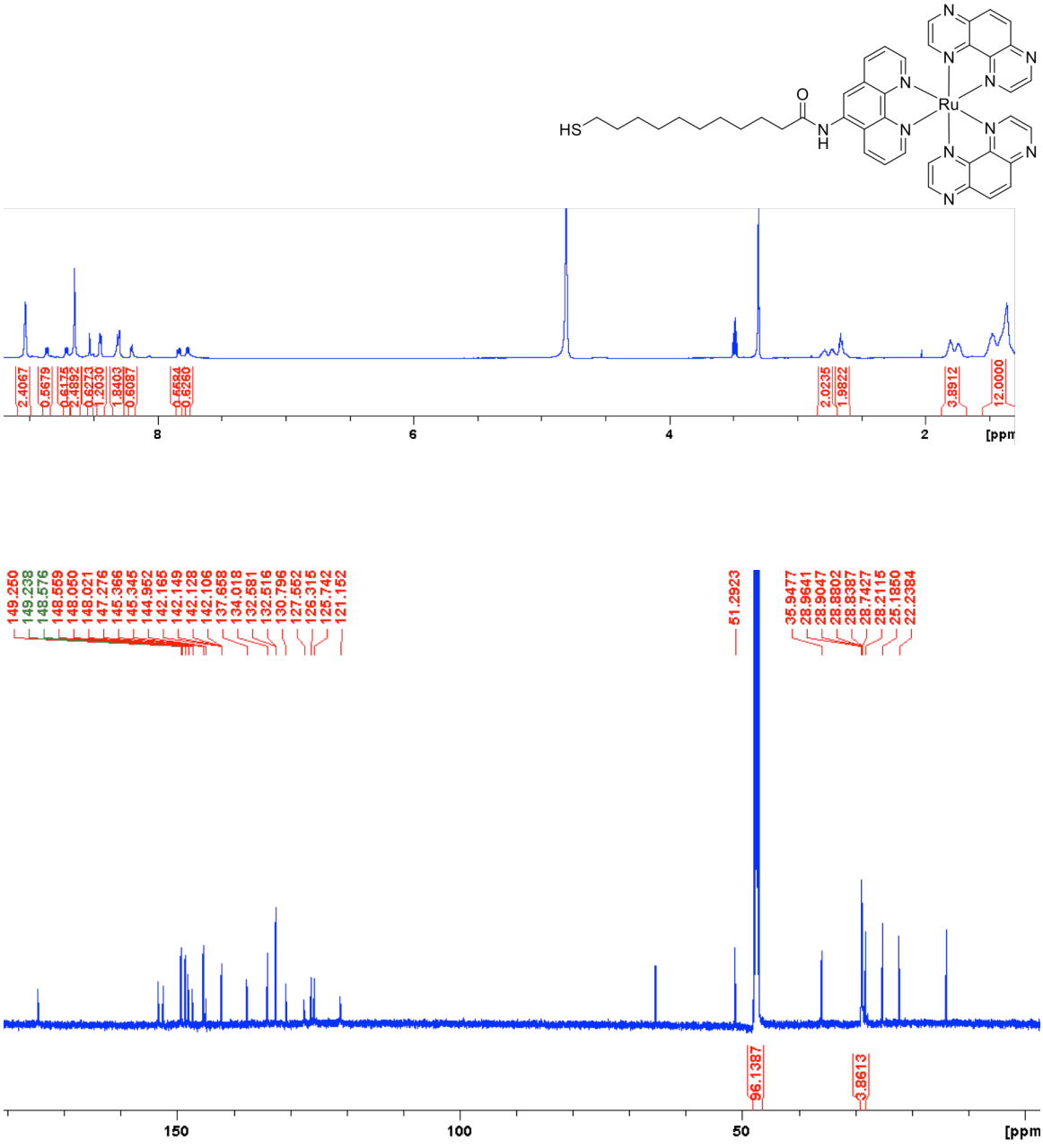
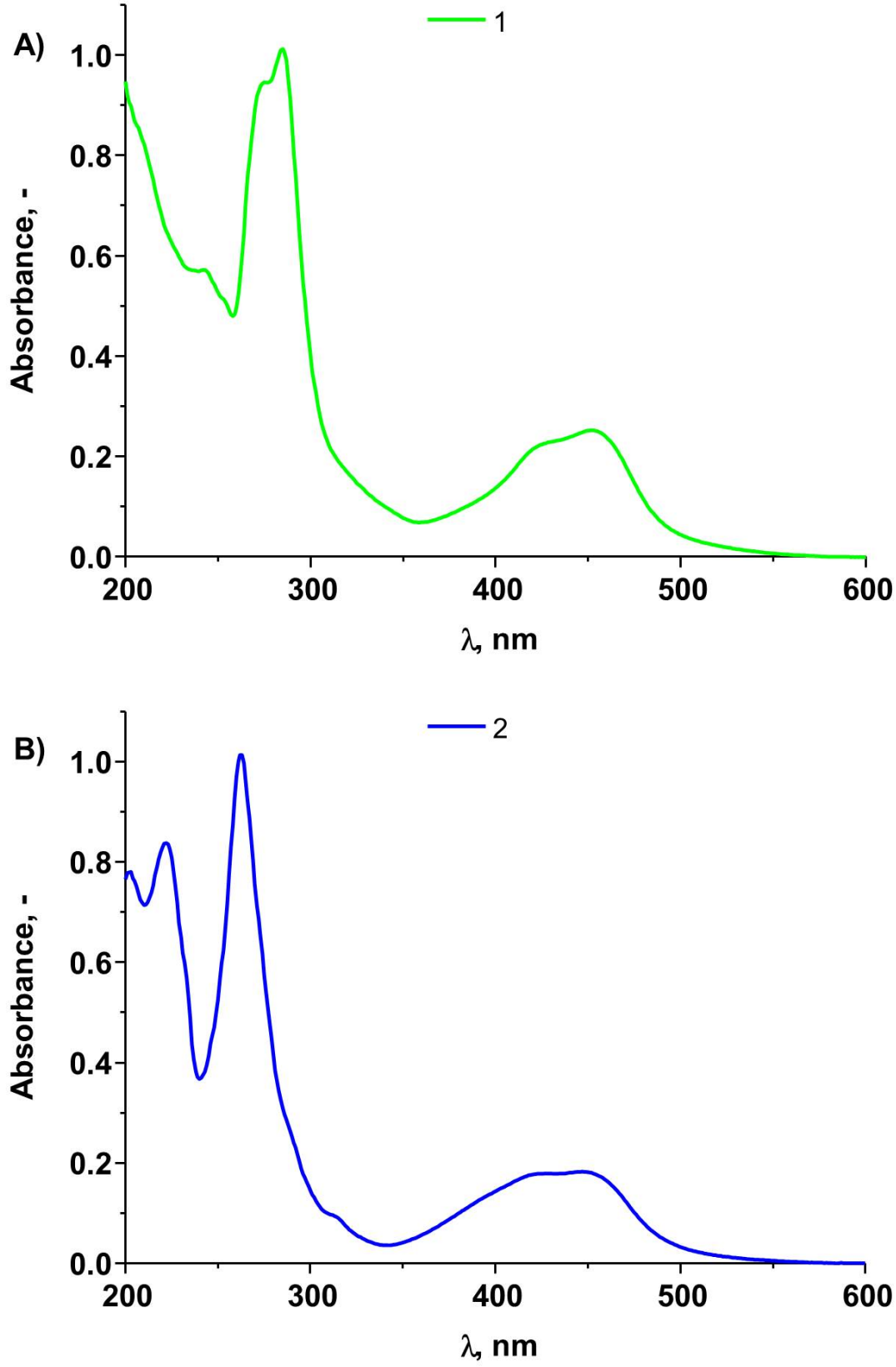


Figure S5. Absorption spectra of the Ru(II)-polypyridyl complexes **A) 1**, **B) 2** and **C) 3**.



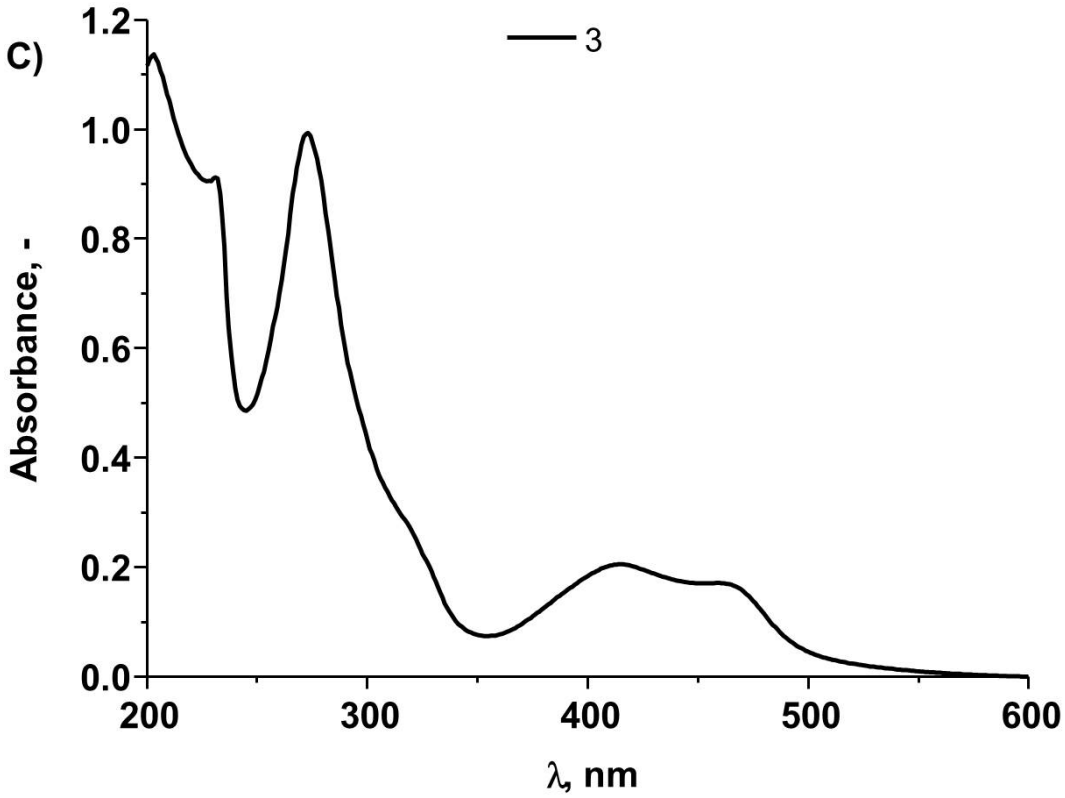
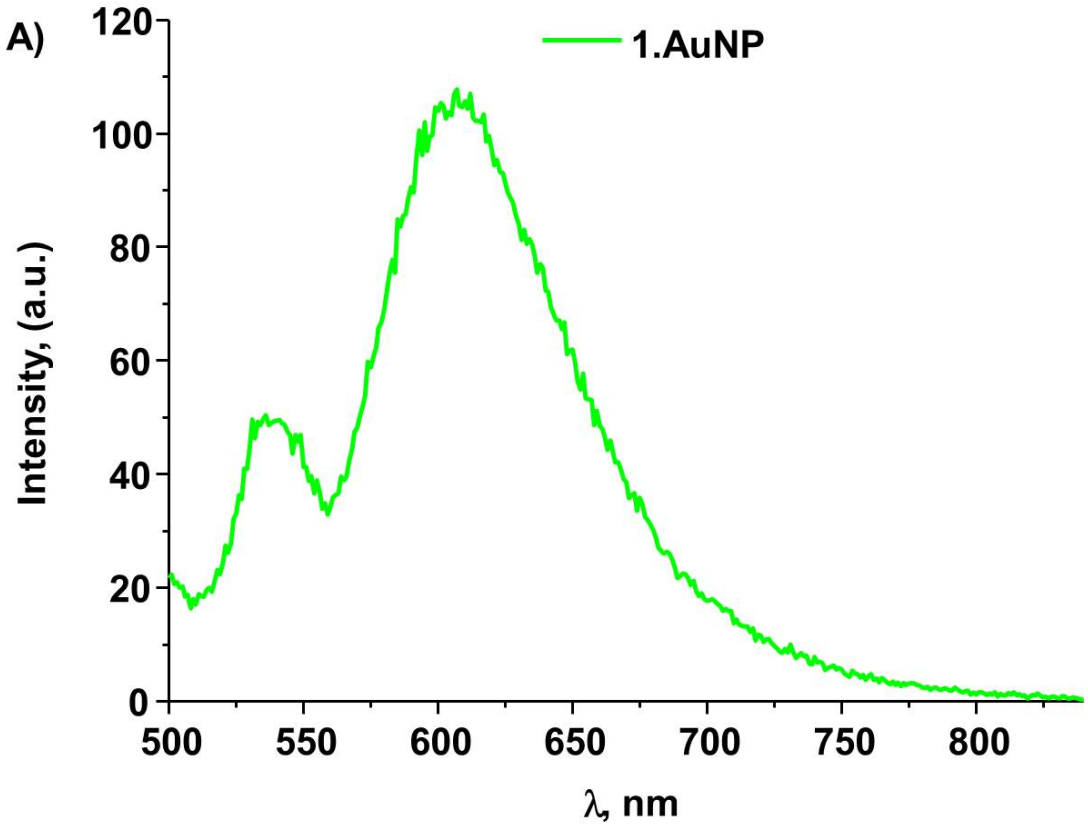
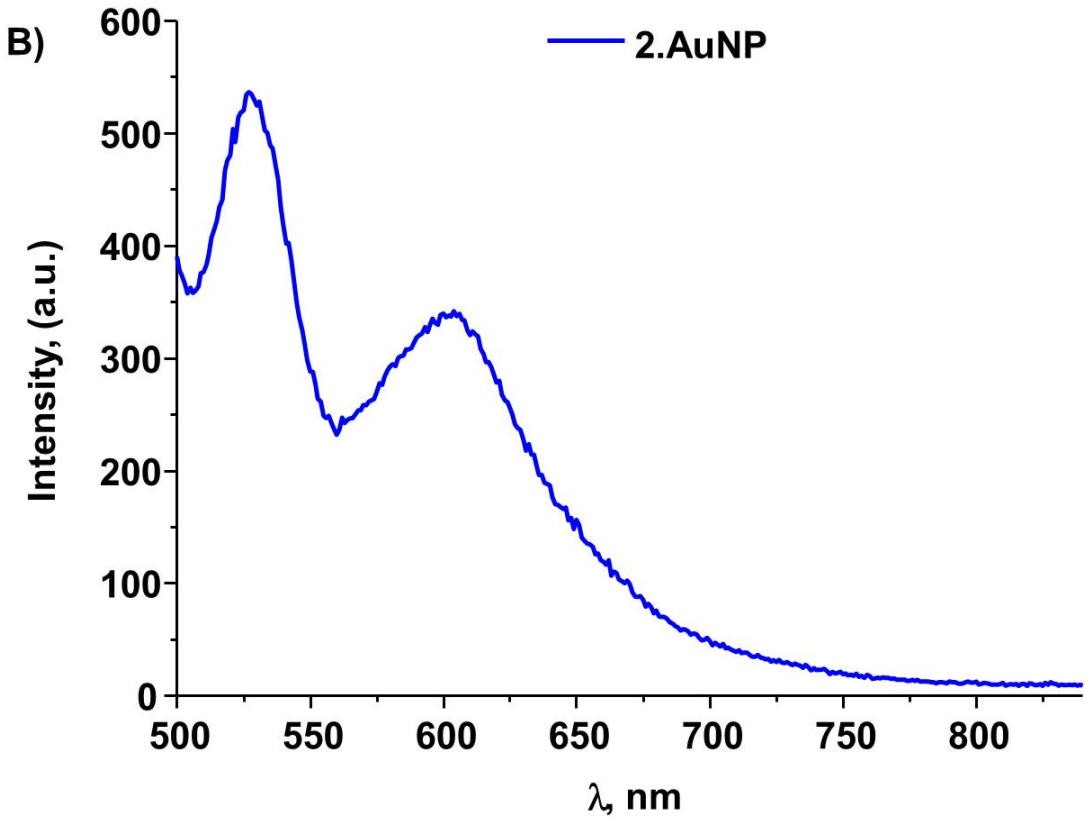


Figure S6. Luminescence spectra of the MLCT emission bands corresponding to **A) 1.AuNP**, **B) 2.AuNP** and **C) 3.AuNP** recorded from water solution.




B)
2.AuNP
Intensity, (a.u.)
λ, nm
0
100
200
300
400
500
600
500
550
600
650
700
750
800

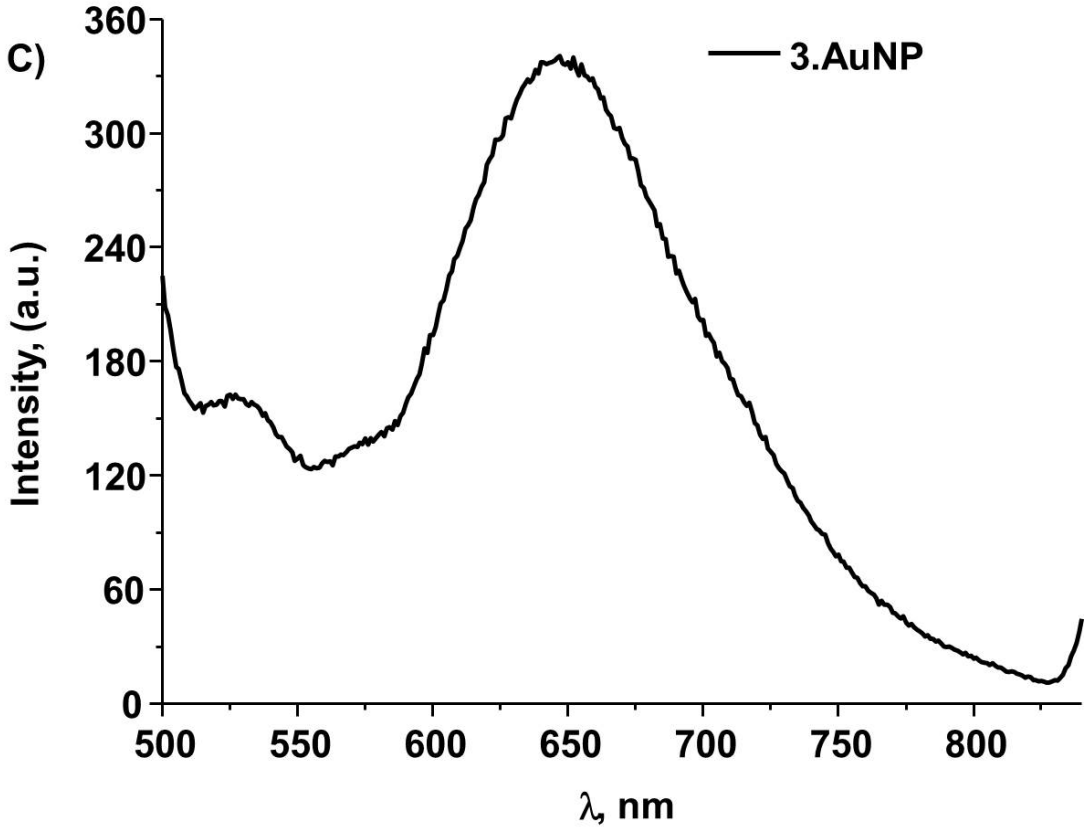
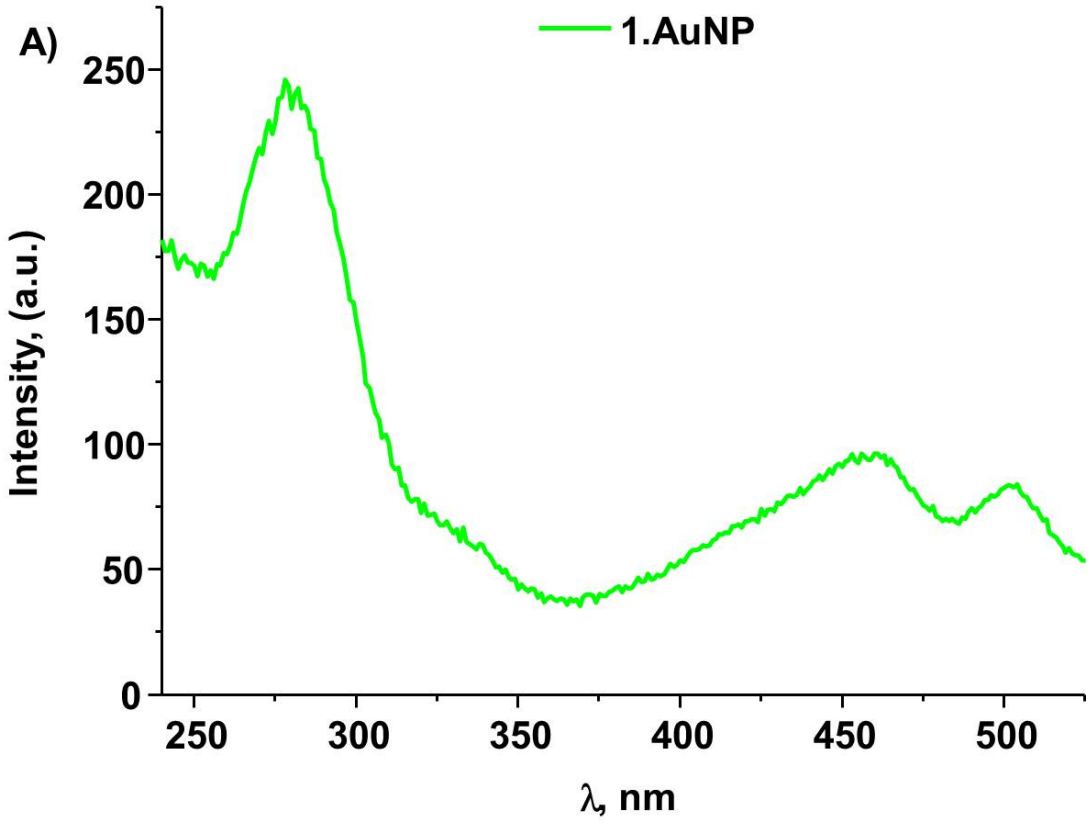
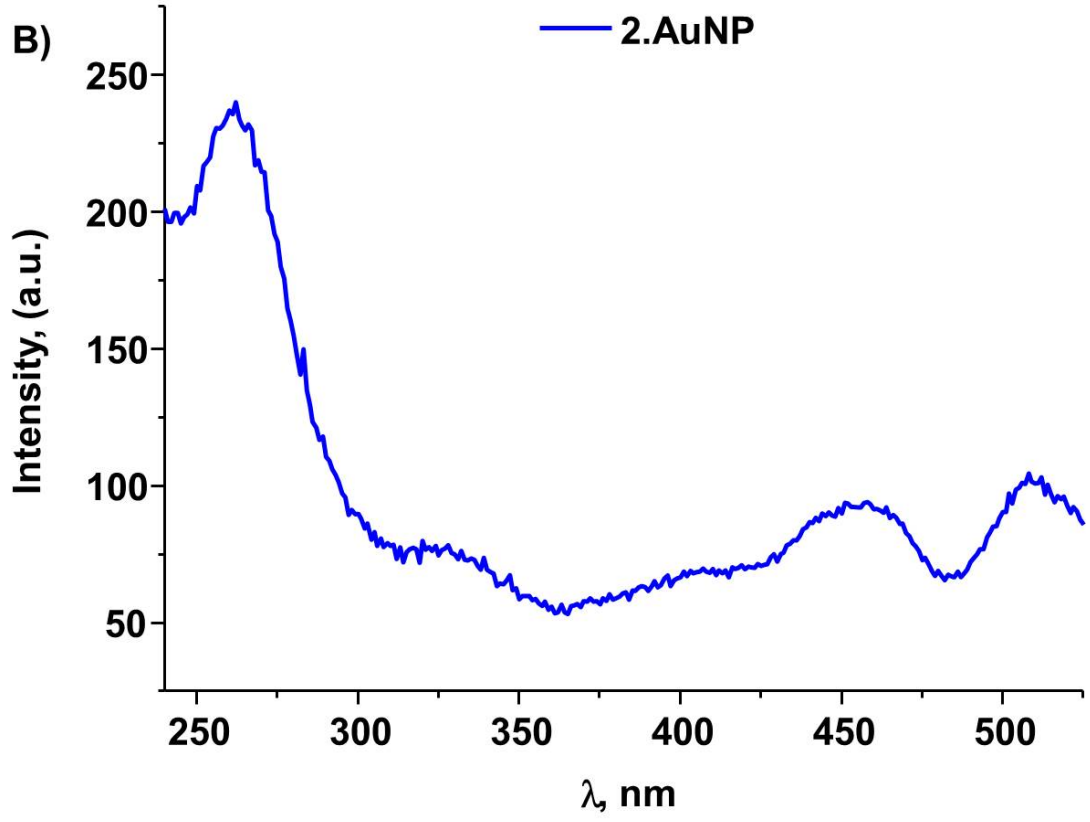

C)
3.AuNP
Intensity, (a.u.)
λ, nm
0
60
120
180
240
300
360
500
550
600
650
700
750
800

Figure S7. Spectra obtained from the excitation of the **A) 1.AuNP**s, **B) 2.AuNP** and **3.AuNP** (at the λ_{max} for all the MLCT bands as determined for **1-3**).





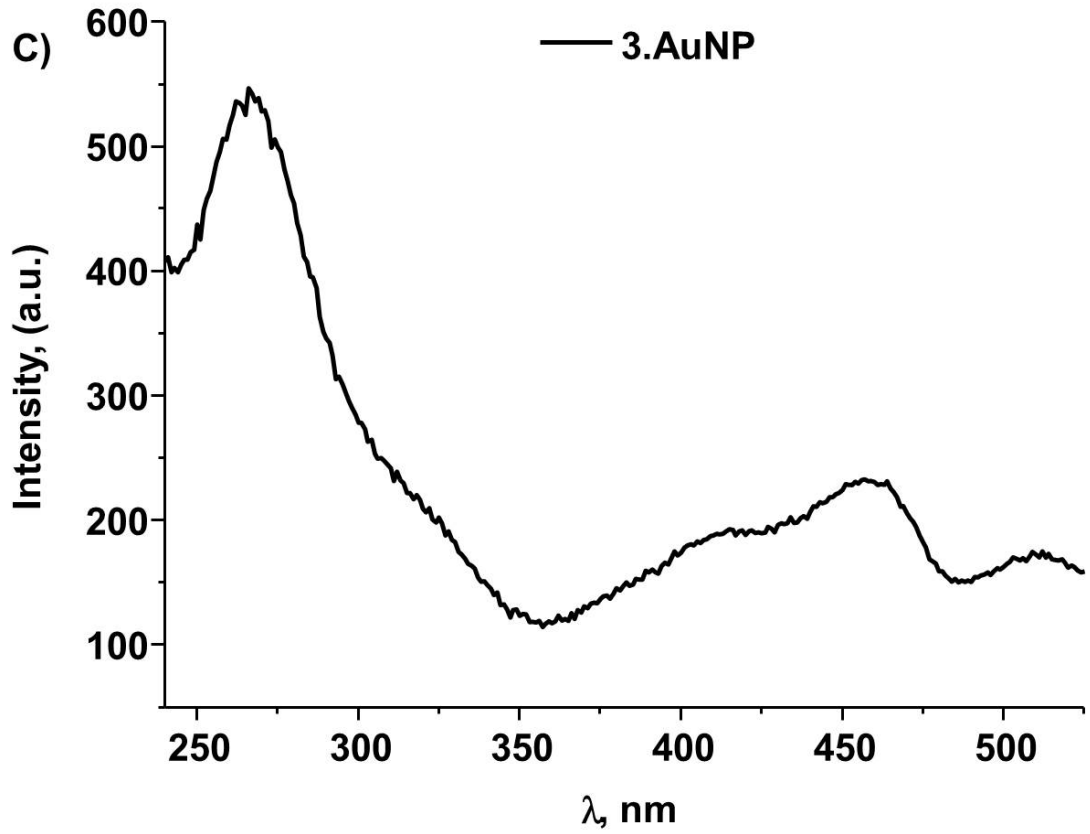


Figure S8. The DLS studies performed in deionised water for compounds **A) 1.AuNP, B) 2AuNP** and **C) 3.AuNP**.

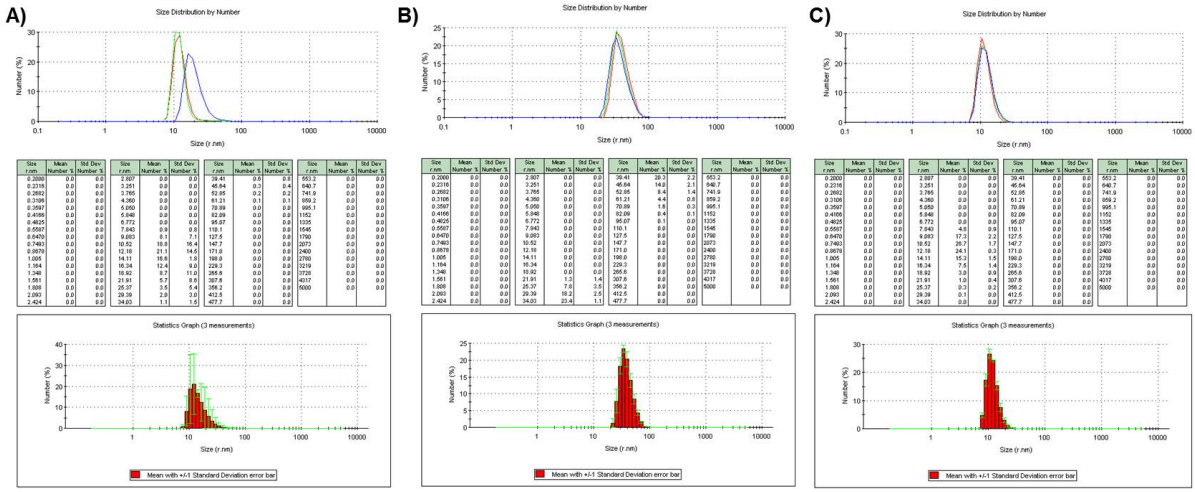


Figure S9. The DLS studies performed in 10mM phosphate buffer at pH 7.4 for compounds **A) 1.AuNP, B) 2AuNP** and **C) 3.AuNP**.

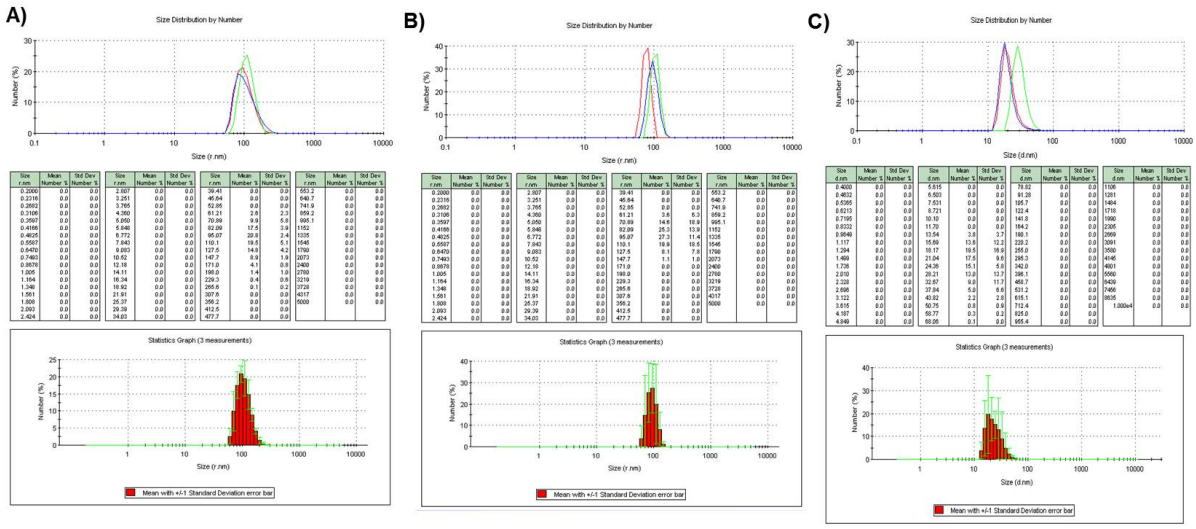


Figure S10. Frequency diagrams obtained from the TEM images of the functionalized gold nanoparticles: a) **1.AuNP**, b) **2AuNP** c) **3.AuNP**.

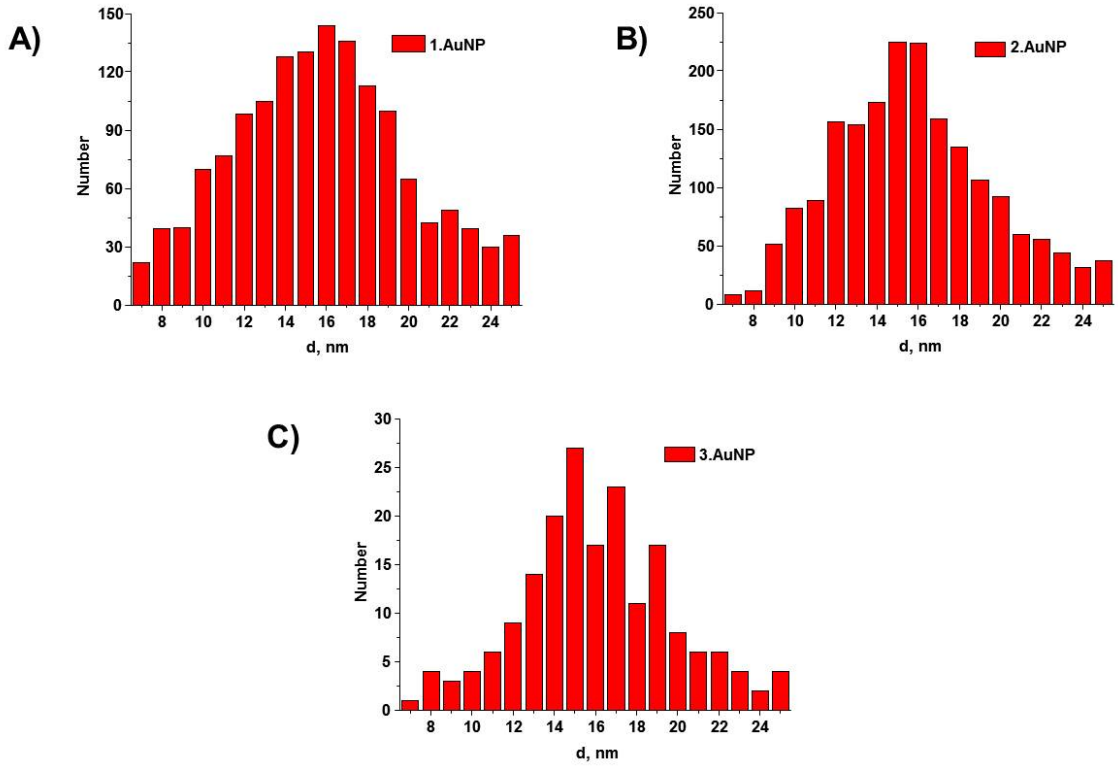


Figure S11. A) UV-vis and **B)** fluorescence titration of **1.AuNP** with stDNA in 10mM phosphate buffered solution.

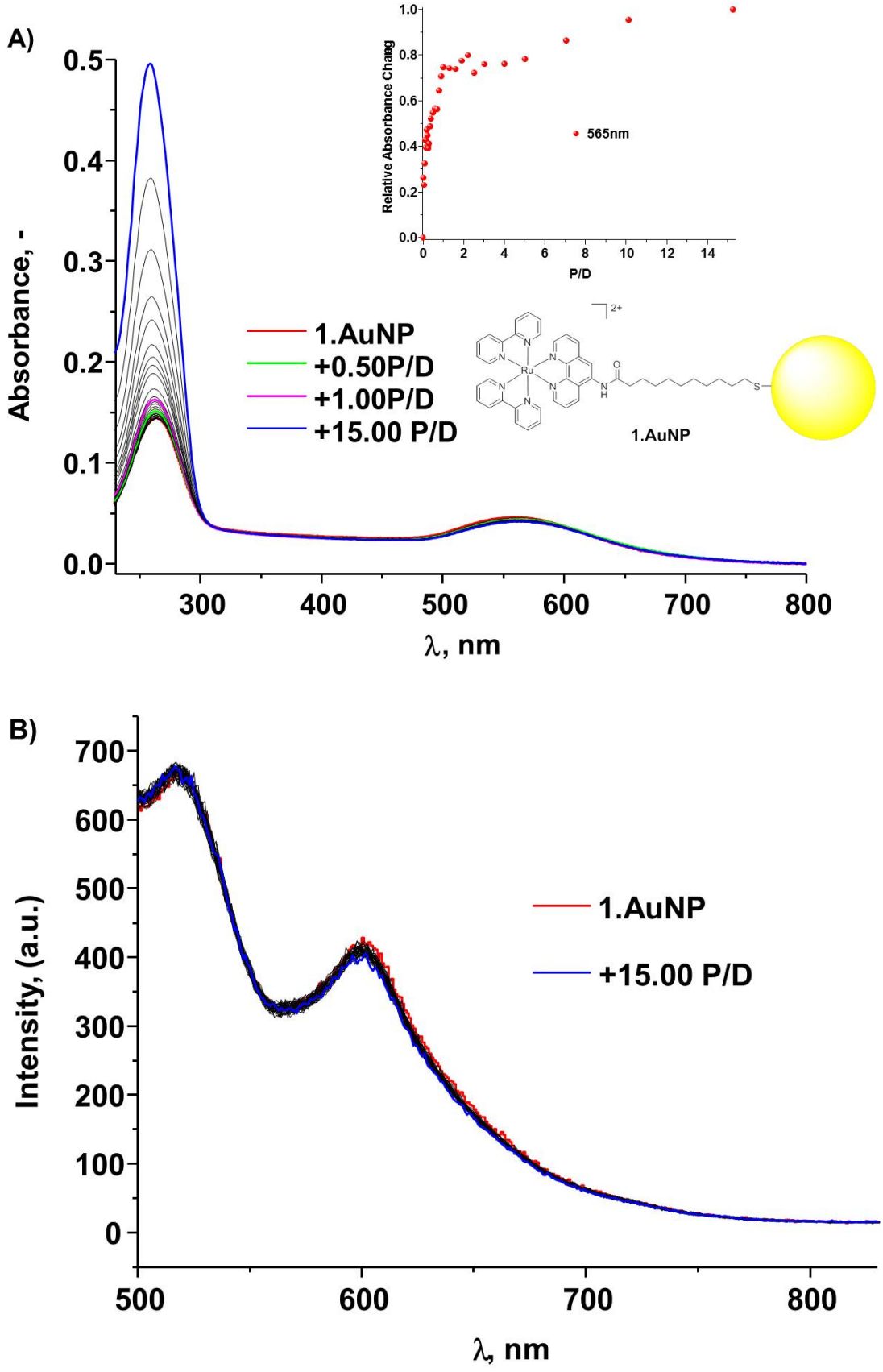


Figure S12. A) UV-vis and **B)** fluorescence titration of **2.AuNP** with stDNA in 10mM phosphate buffered solution.

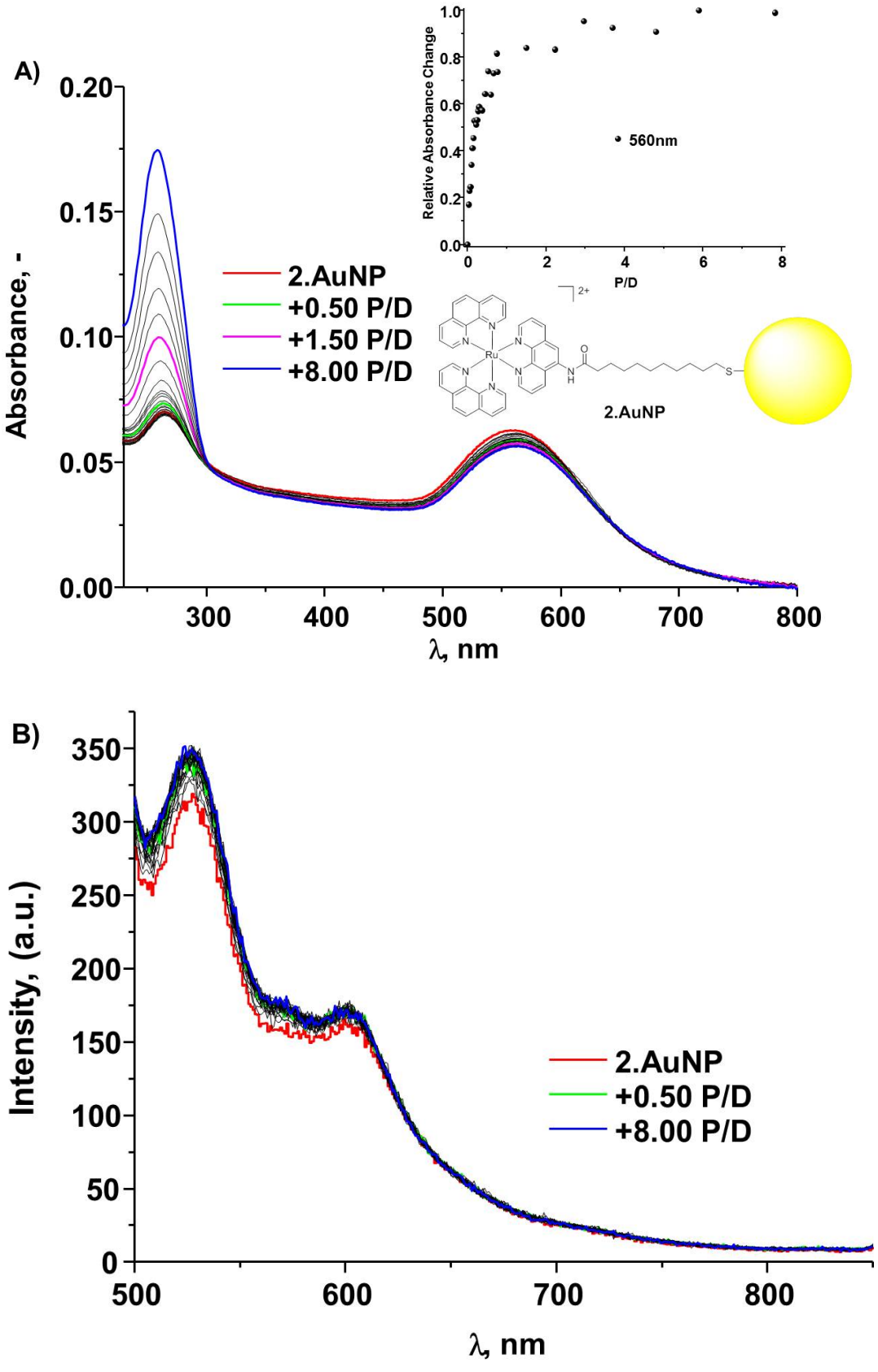
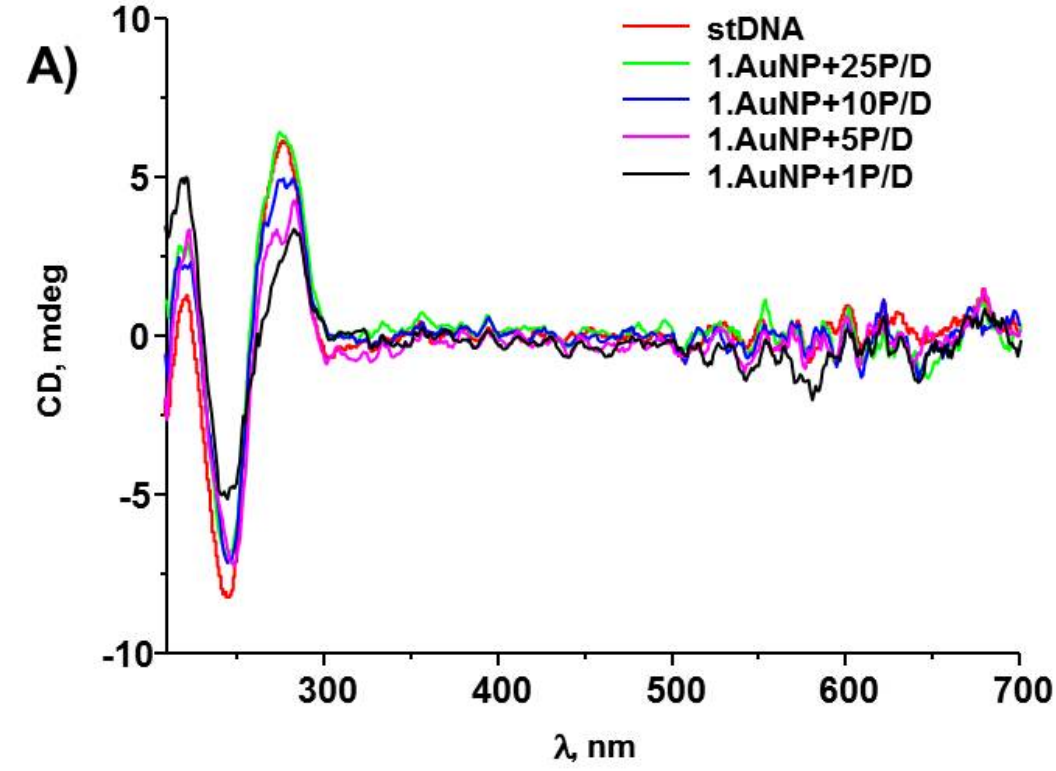
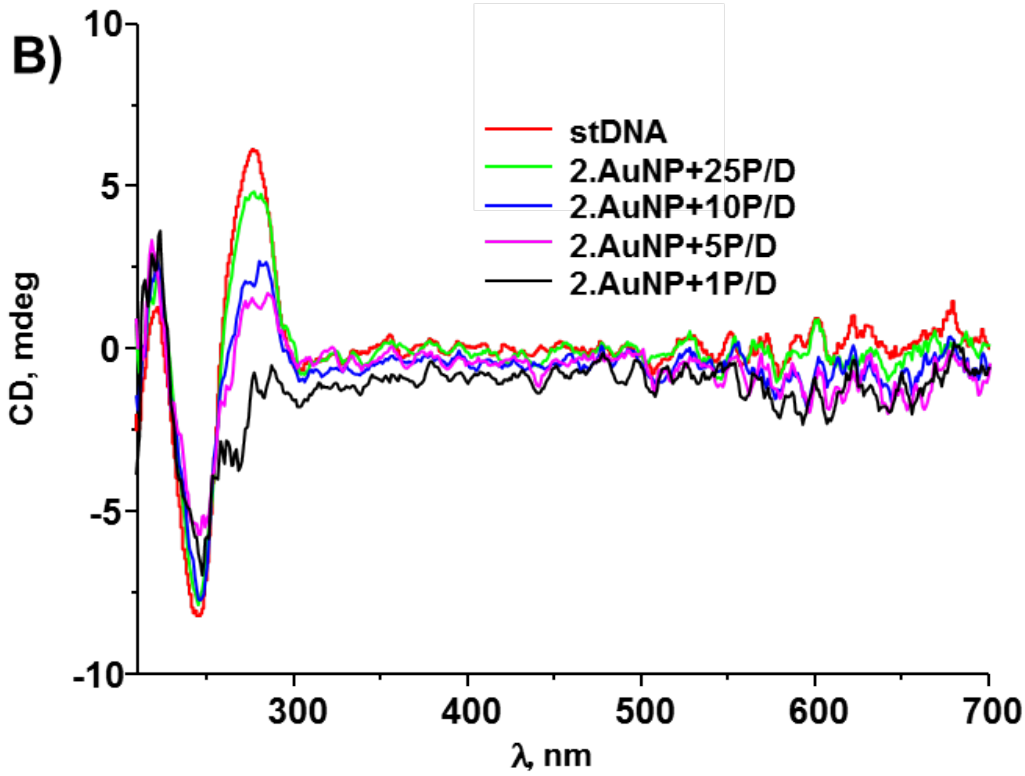


Figure S13. CD spectra performed at different P/D ratios with **A) 1.AuNP**, **B) 2.AuNP** and **3.AuNP.**





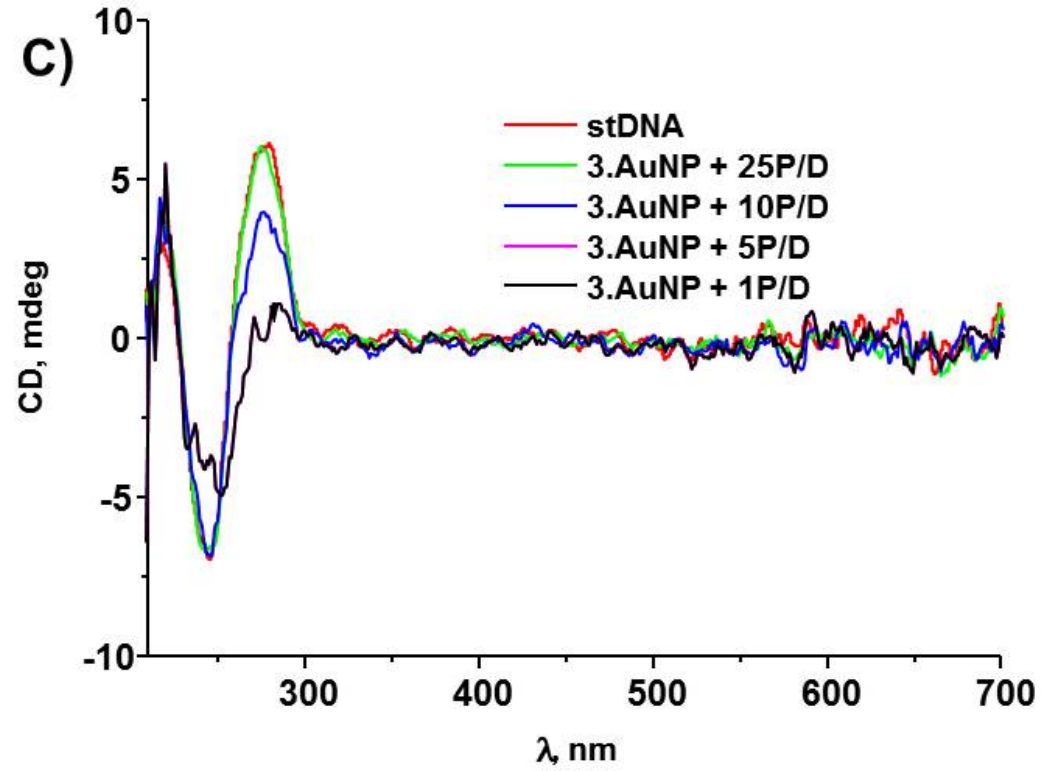


Figure S14. Thermal denaturation curves of st-DNA (150 μM) in 10 mM phosphate buffer, pH 7.4.

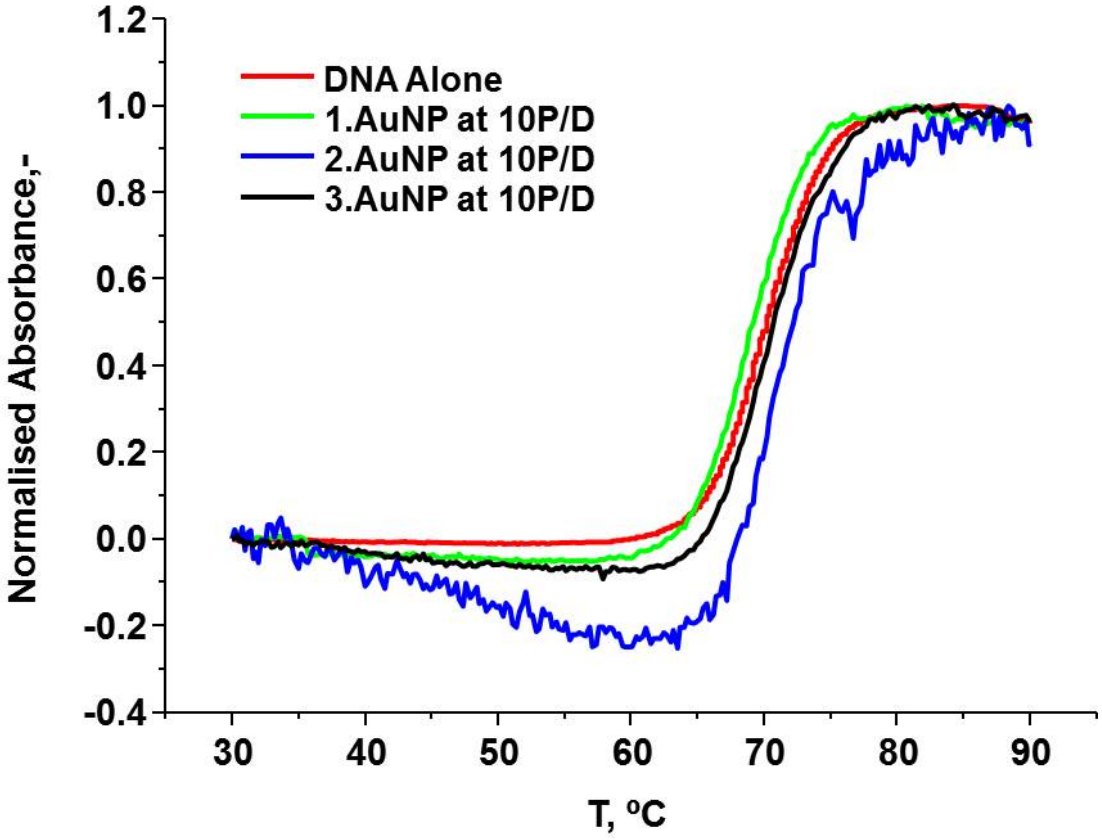


Figure S15. Agarose gel electrophoresis of pBR322 DNA (1mg/mL) after 60 min irradiation (2 J/cm^2) in 10 mM phosphate buffer, pH 7.4; Lane 1: Plasmid DNA control ;Lane 2: $Ru(bpy)_3^{2+}$ (P/D 10); Lane 3: **3.AuNP** (P/D 20); Lane 4: **3.AuNP** (P/D 10); Lane 5: **3.AuNP** (P/D 5); Lanes 6: **3.AuNP** + 10mM NaN_3 (P/D 10); Lane 7: **3.AuNP** in the dark (10 P/D) and Lane 8: **3.AuNP** in the dark (5 P/D).

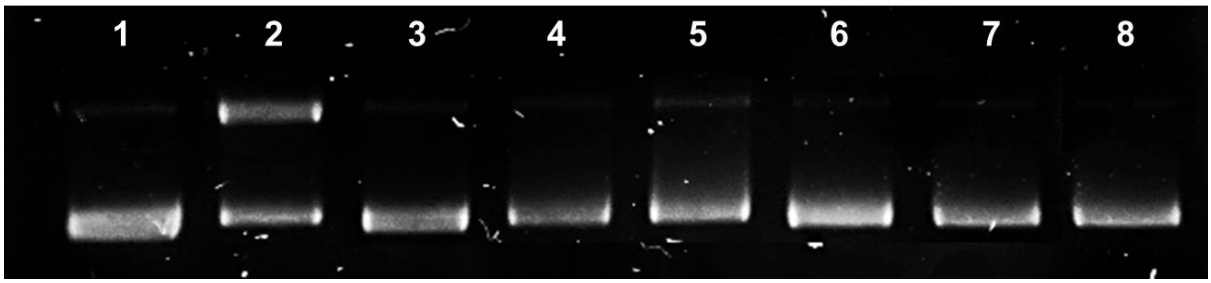


Table S1. Summary of percentages of supercoiled vs. open form pBR322 obtained from Figure S14.

Lane	% Open	% Supercoiled	Lane	% Open	% Supercoiled
1	2.7	97.3	5	5.4	94.6
2	52.3	47.7	6	1.2	98.8
3	2.1	97.9	7	1.9	98.1
4	4.6	95.4	8	1.3	98.7

Figure S16. DLS Study of the interaction of **1.AuNP** after addition of **A)** 0.00 to 0.21 P/D, **B)** 0.21 to 1.00 P/D and **C)** 1.00 to 20.00 P/D of st DNA in 10mM phosphate buffered solution

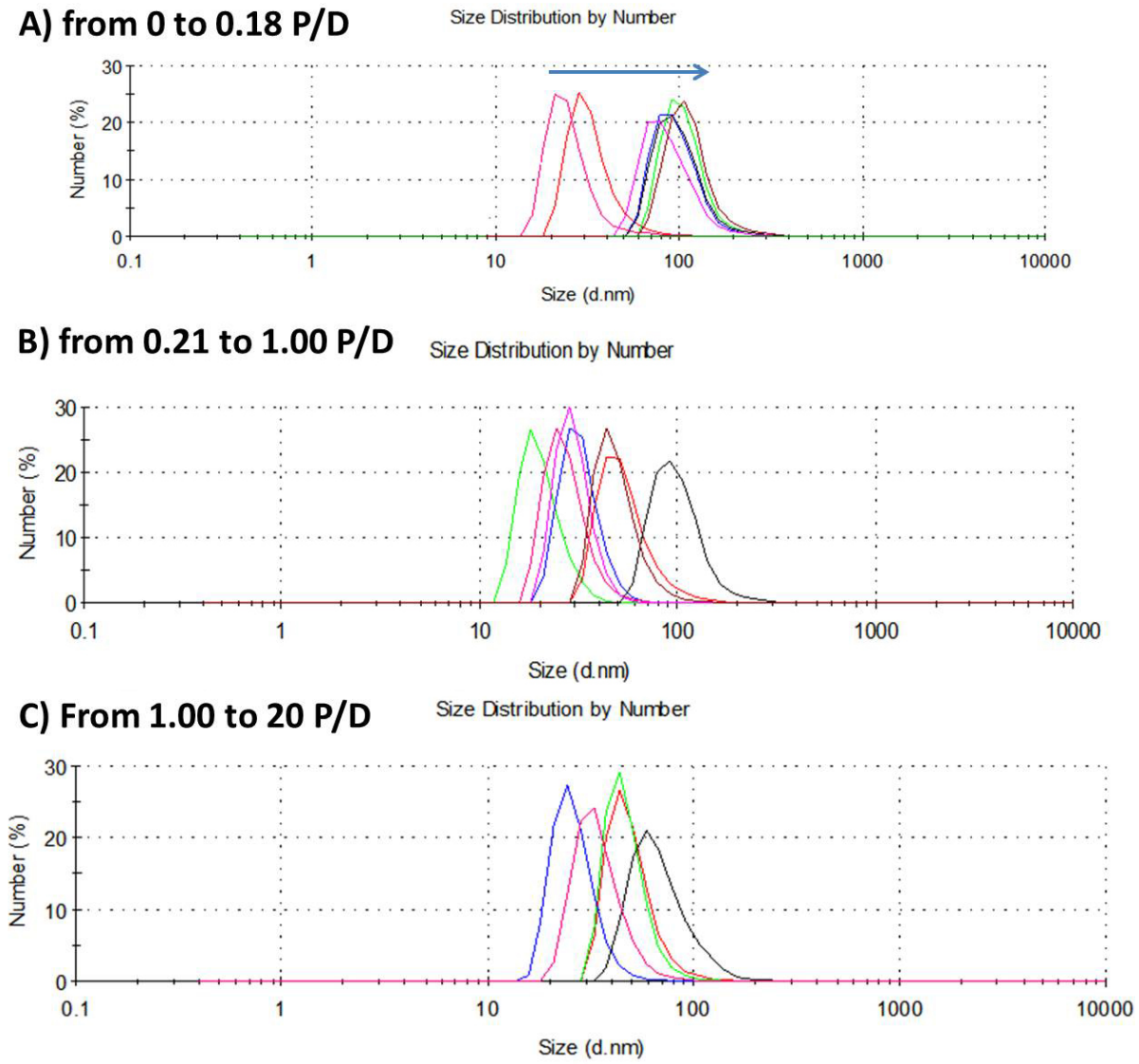


Figure S17. DLS Study of the interaction of **2.AuNP** after addition of **A)** 0.00 to 0.21 P/D, **B)** 0.21 to 1.00 P/D and **C)** 1.00 to 40.00 P/D of st DNA in 10mM phosphate buffered solution

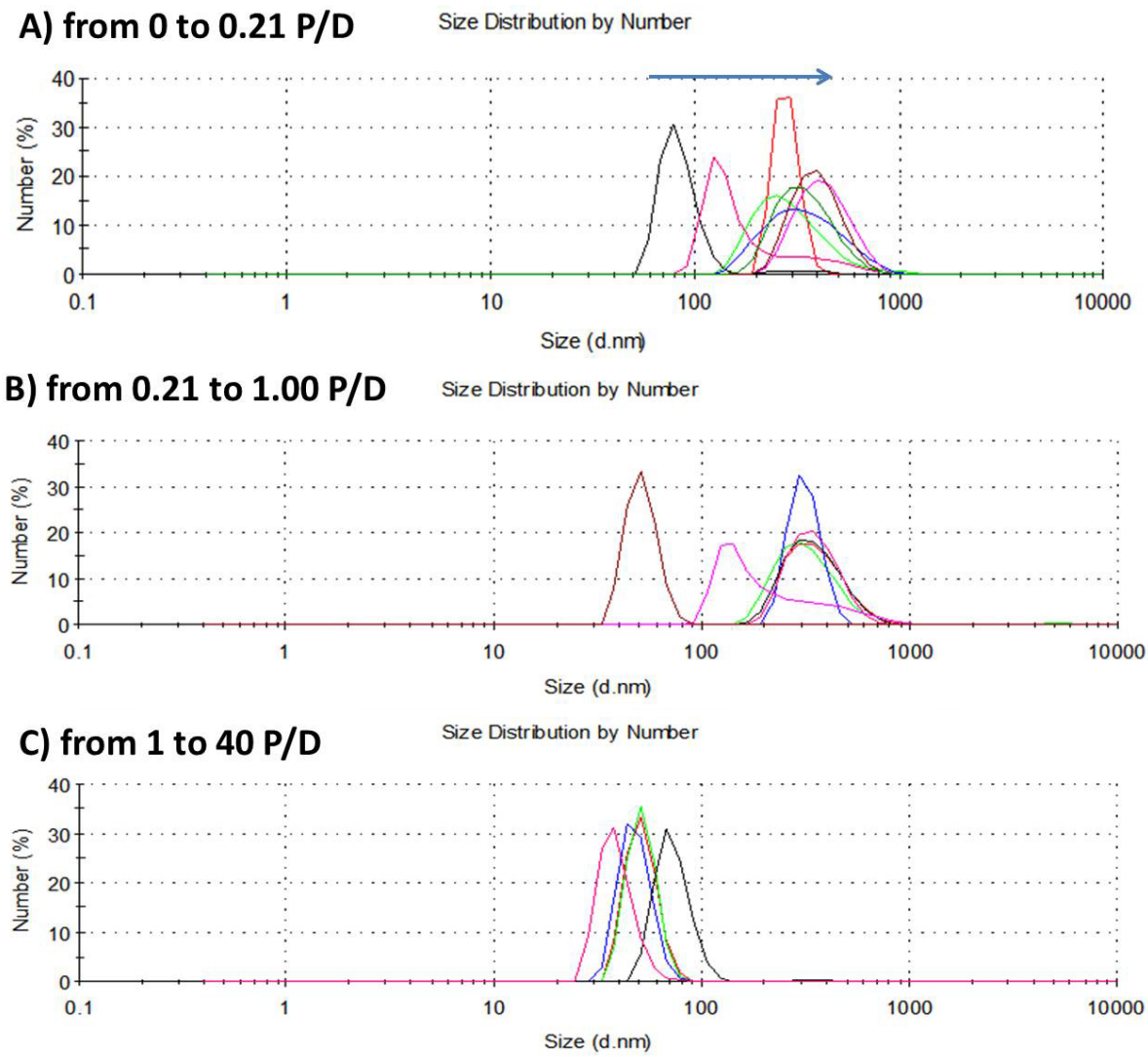


Figure S18. Images obtained by TEM **1.AuNP** at different ratios P/D: **A)** 0; **B)** 0.23and **C)** 1.00.

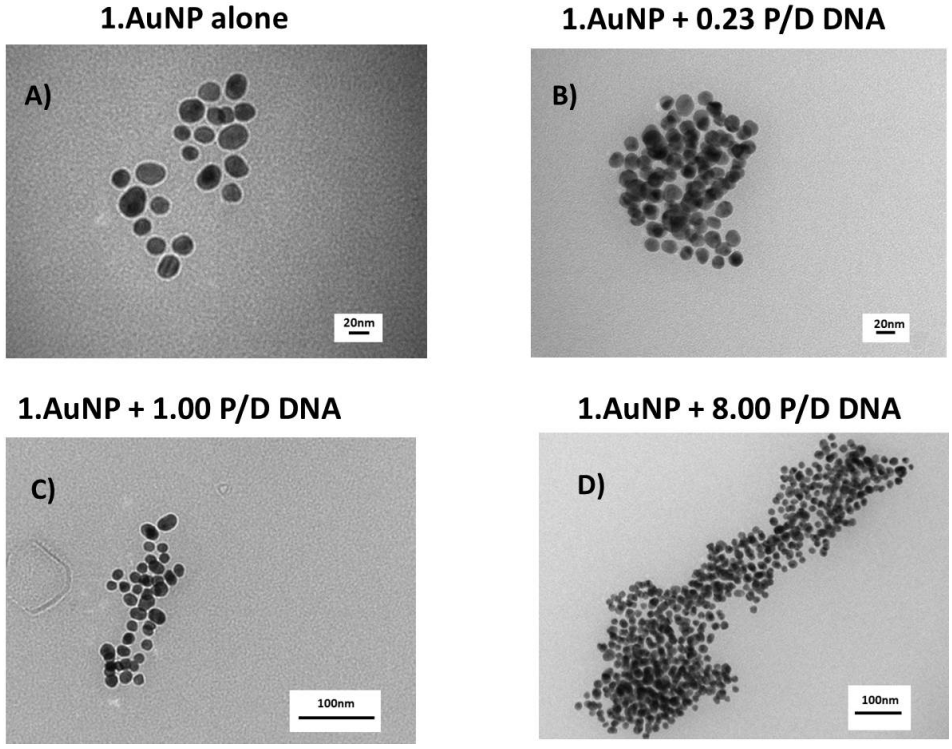


Figure S19. Images obtained by TEM **2AuNP** at different ratios P/D: **A)** 0; **B)** 0.23and **C)** 1.00.

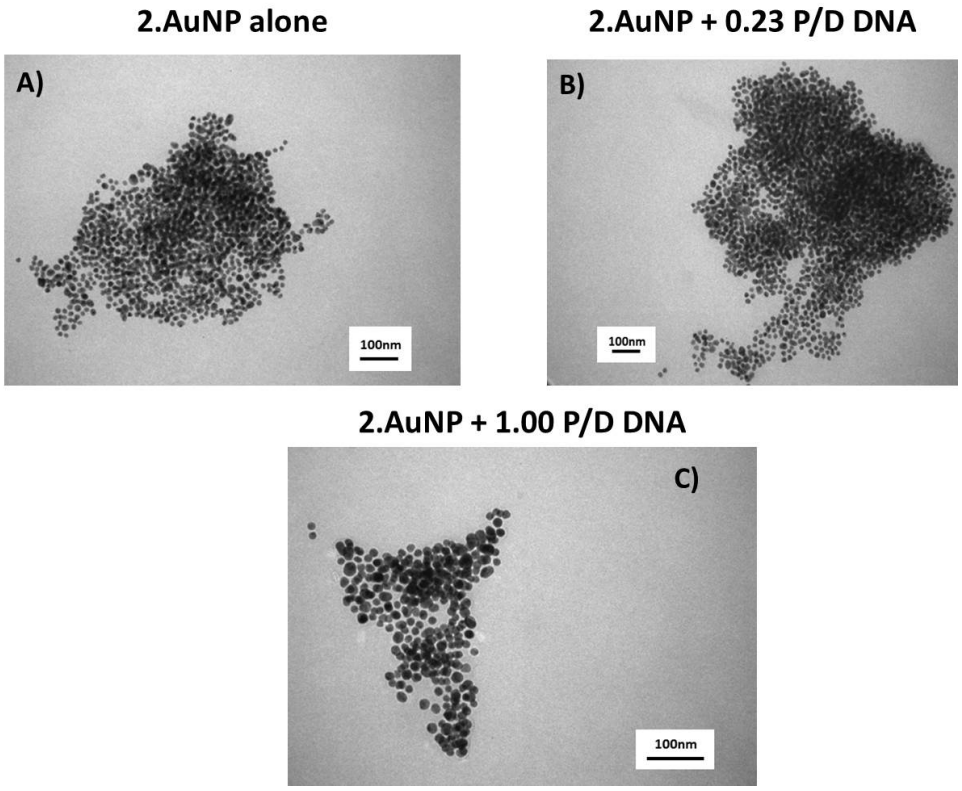
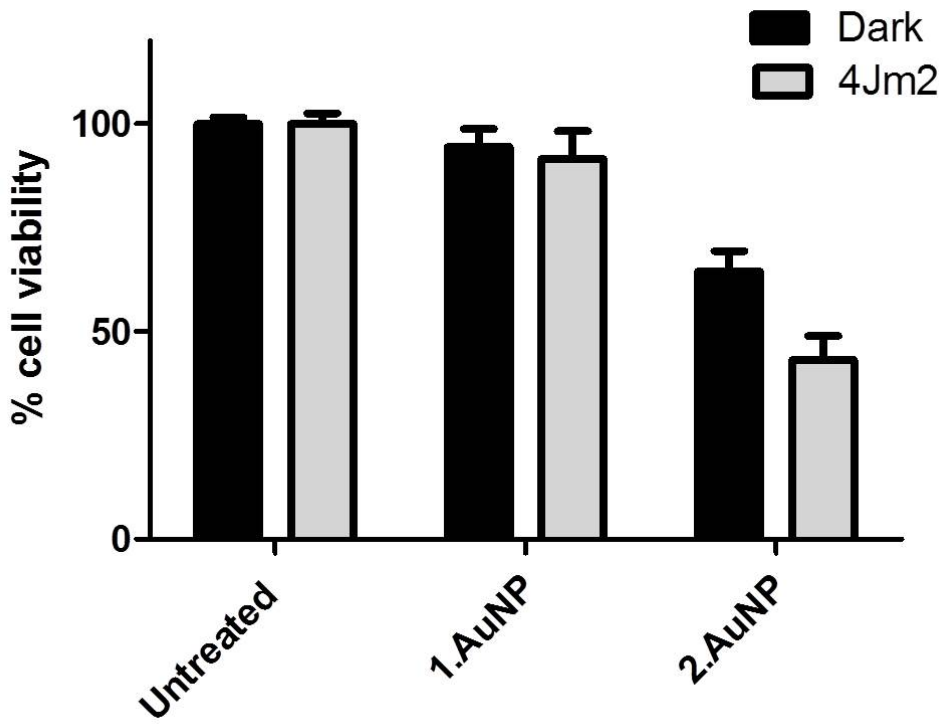
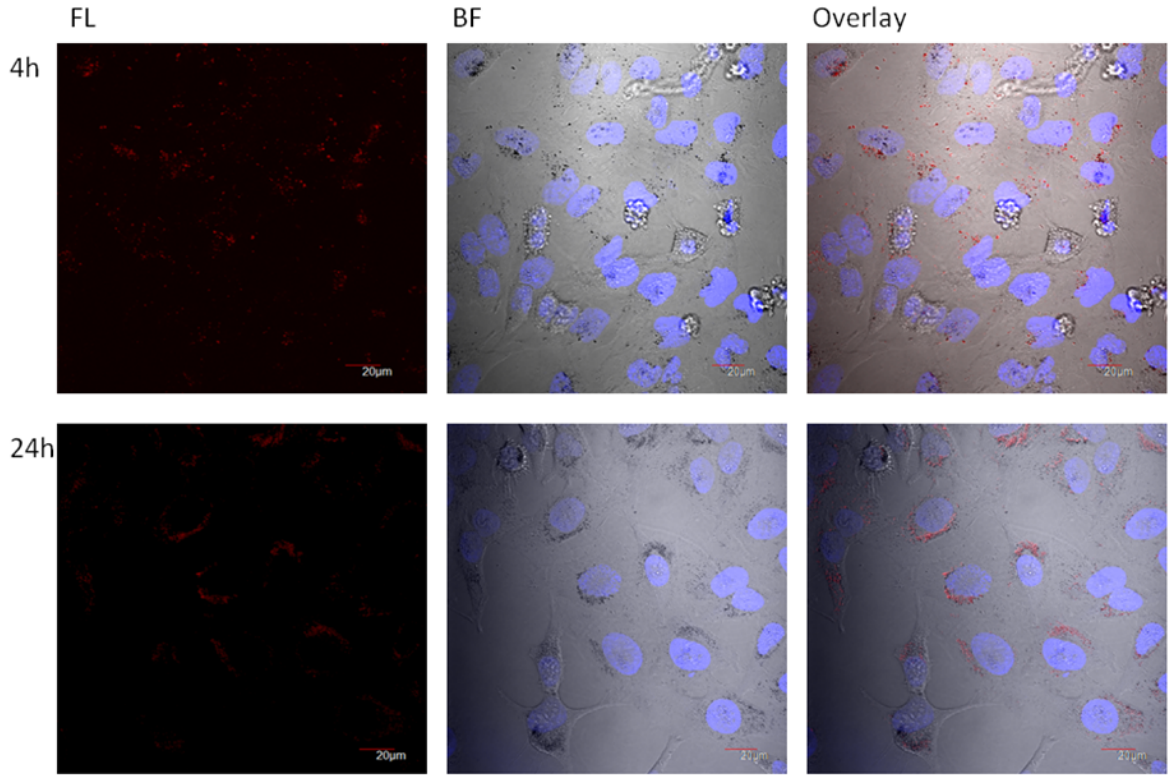


Figure S20. HeLa cells were treated with **1.AuNP** and **2.AuNP** (20μM) for 24h, irradiated or left in the dark. Cell viability was assessed 24h later using Alamar Blue assay



FigureS21. Confocal Microscopy images of HeLa cells treated with 20μM **1.AuNP**.



FigureS22. Confocal Microscopy images of HeLa cells treated 20µM **2.AuNP**.

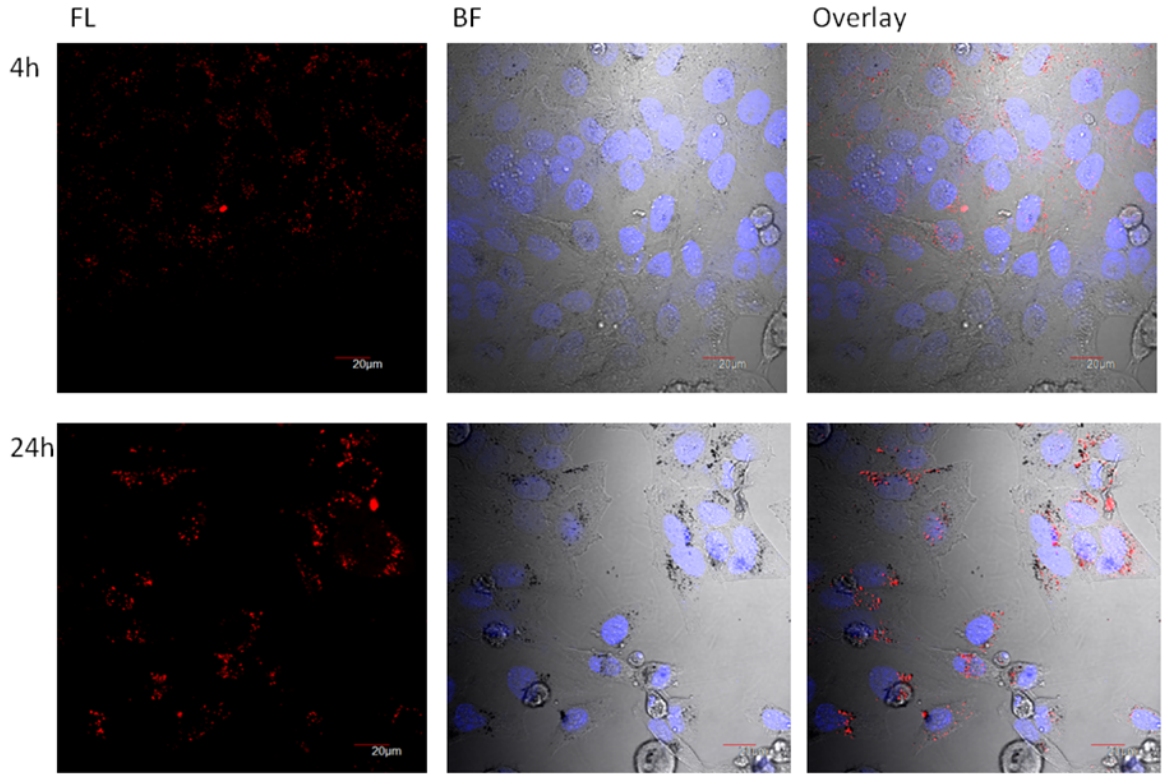
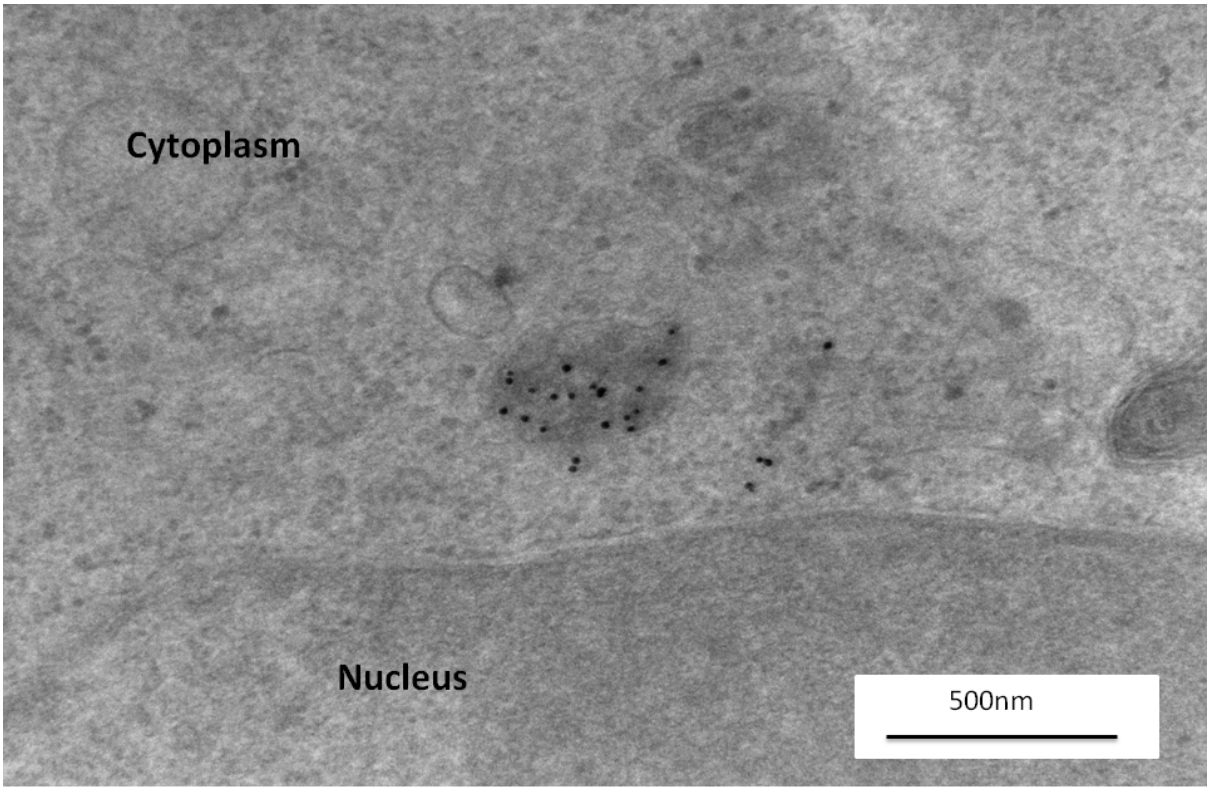
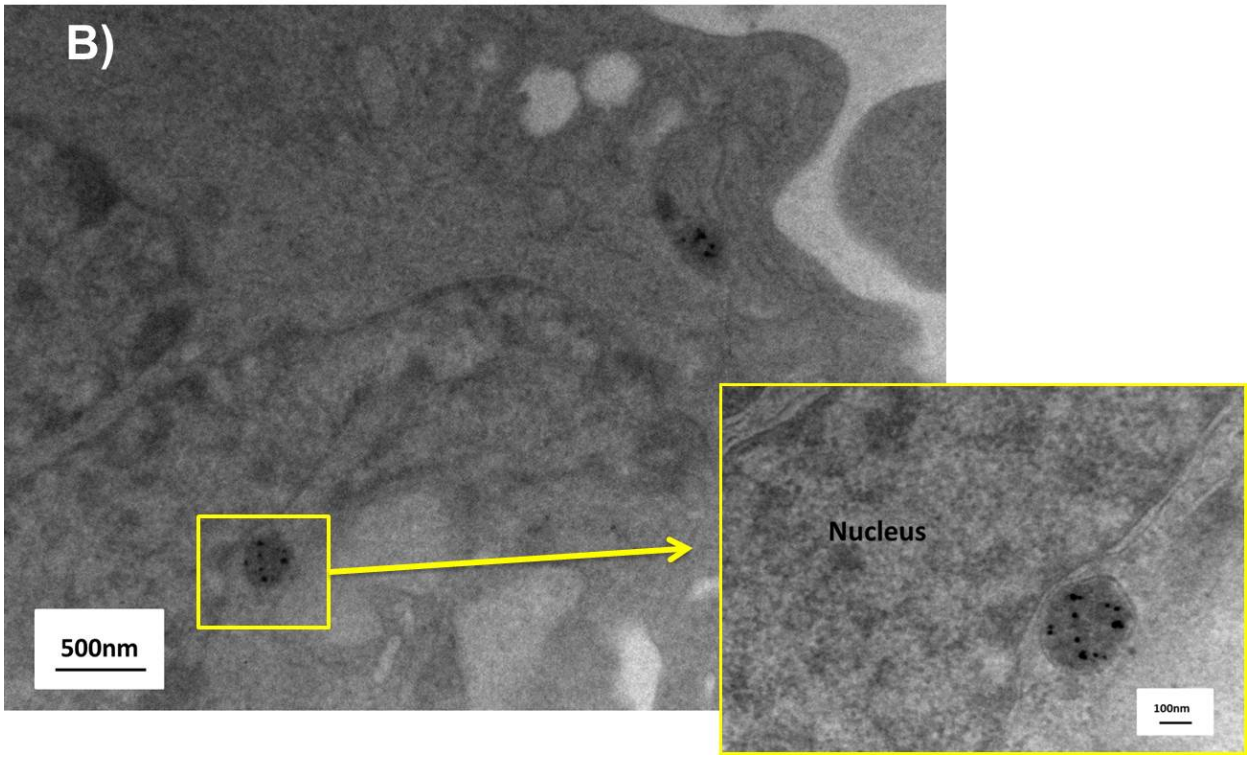


Figure S23. TEM images of HeLa cells treated for 4h with 20µM **3.AuNP.**




B)
500nm
Nucleus
100nm

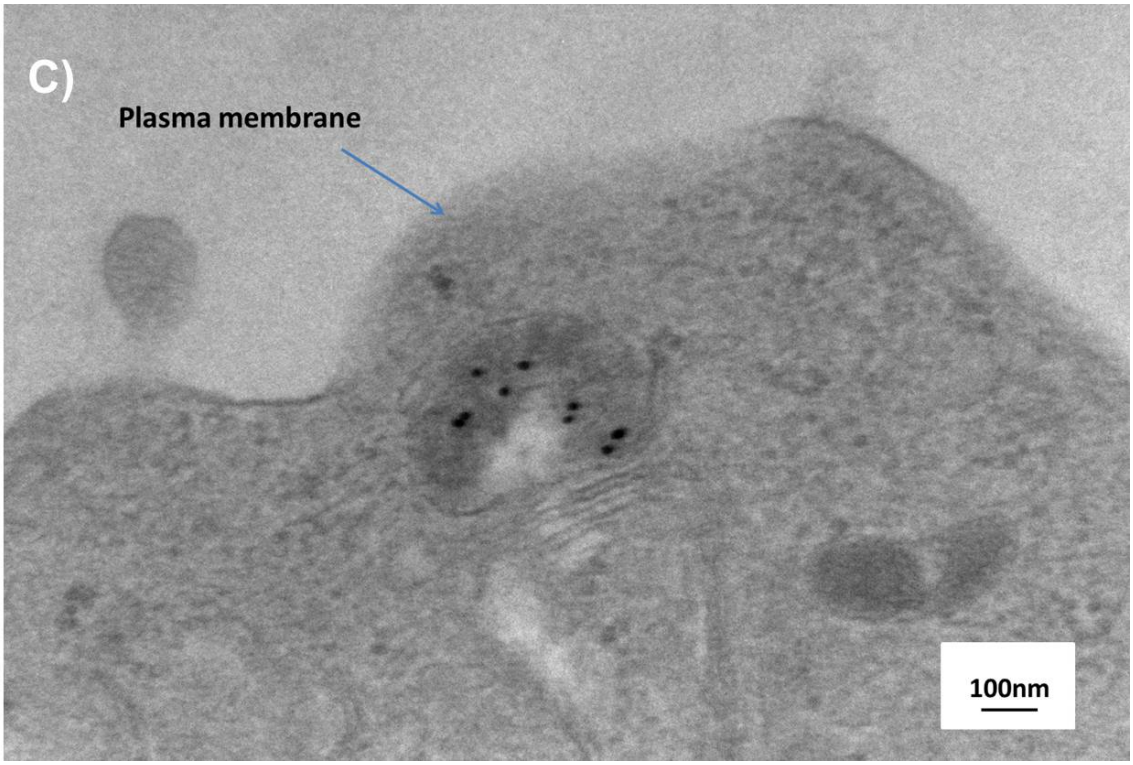

C)
Plasma membrane
100nm

Figure S24. TEM image of a HeLa cell treated for 6 h with 20μM **3.AuNP.**

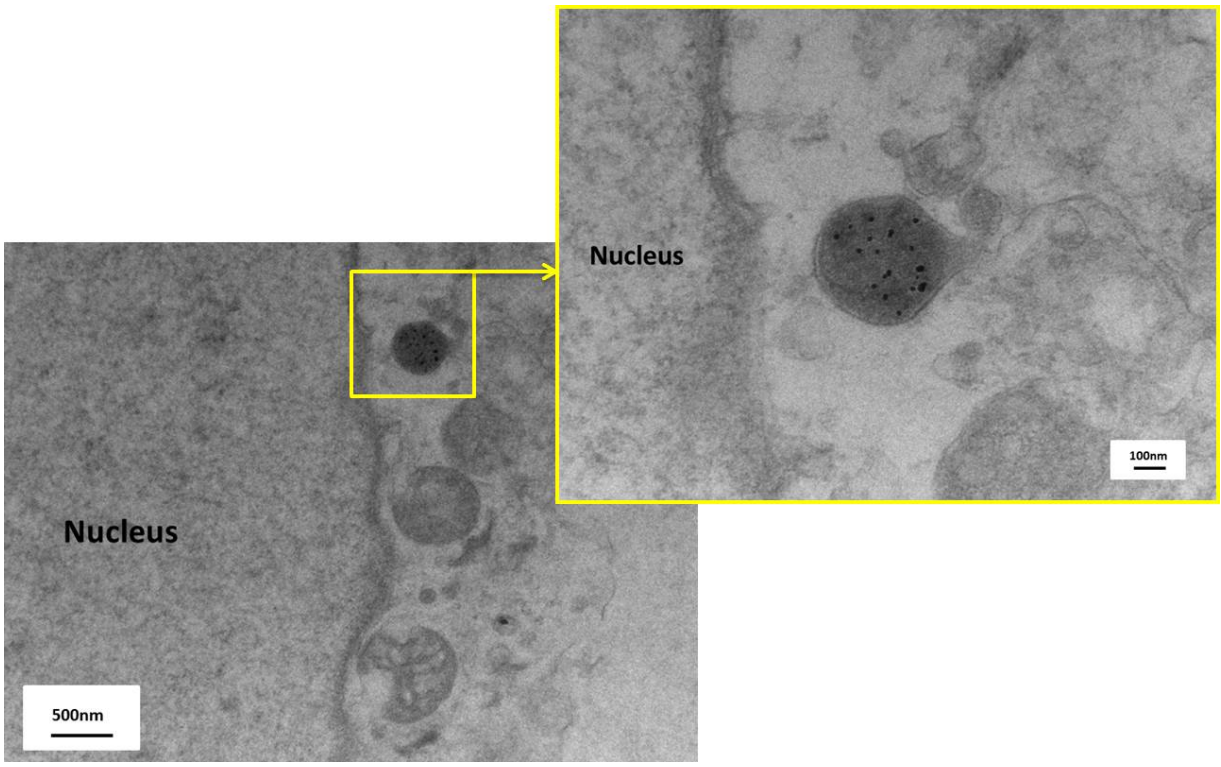


Figure S25. TEM image of a HeLa cell treated for 24h with 20µM **3.AuNP.**

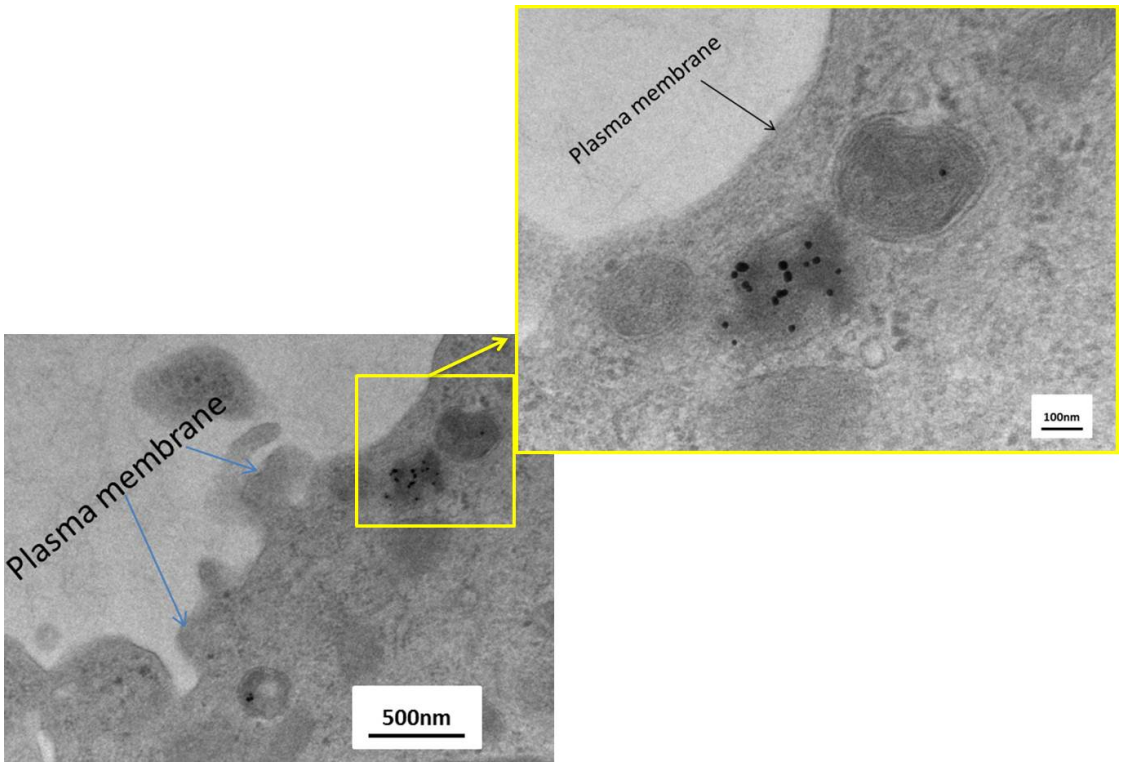
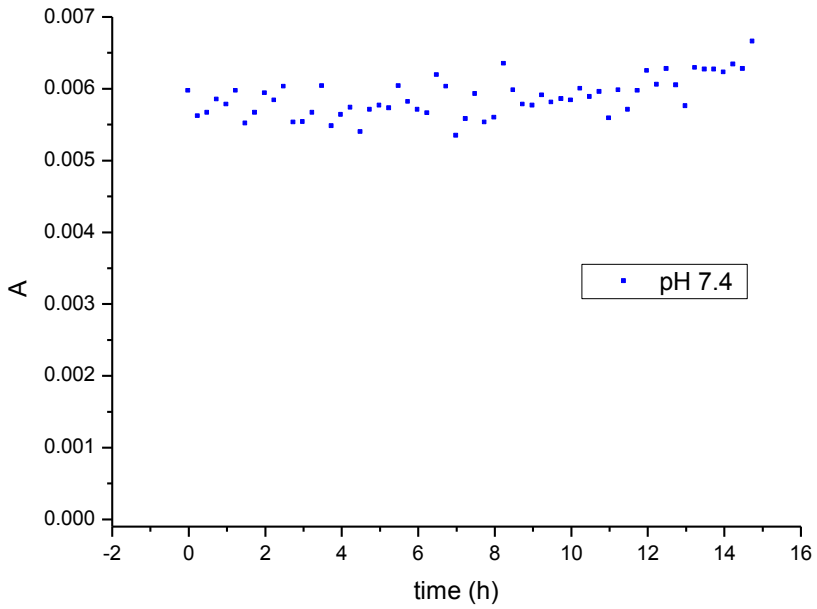


Figure S26. Stability of **3.AuNP** in buffered 7.4 solution as a function of time.



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