Supplementary Data

Economical Gold Recovery Cycle from Bio-sensing AuNPs: An application for Nanowaste and Covid-19 Testing Kits

Amol V. Pansare[‡a]*, Shubham V. Pansare[‡b], Priyanka V. Pansare[‡c], Bhausaheb P. More[d], Amit A. Nagarkar[e], Michel Barbezat[a], Kamini J. Donde[c]*, Vishwanath R. Patil[b]* and Giovanni P. Terrasi[a]*
‡ Authors contributed equally

Experimental section:

Procedure of in vitro anticancer activities

Cell culture*:* The human breast cancer cell line MCF-7 and MDA-MB-468 were propagated in RPMI1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine at 37 °C under a humidified atmosphere of 5% CO_2 in air.

In vitro anti-cancer activity study*:* Sulforhodamine B (SRB) based in vitro cytotoxicity assay was performed to compare the anti-tumor effects of AuNPs and ADR against MCF-7 and MDA-MB-468 cell lines according to the previously established method. [V. Vanicha and K. Kanyawim, *Nat. Protoc.,* **2006**, 1, 1112– 1116] MCF-7 and MDA-MB-468 cells in a logarithmic phase were dispensed into 96 well microtiter plates in 90 µl at plating densities of 5×10^3 cells per well. Different concentrations of AuNP solutions and adriamycin (positive control drug) viz. 1×10^{-7} M, 1.0×10^{-6} M, 1.0×10^{-5} M and 1.0×10^{-4} M were prepared by serial dilution of the stock solution. The each cells were grown in plates for 24 hours and then compounds were added at different dilutions. The cultures were further incubated under standard conditions for 48 hours and the assay was terminated by the addition of cold TCA. The cells were fixed in situ by the gentle addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4°C. The supernatant was discarded. The plates were washed five times with double distilled water and dried in air.

SRB staining: SRB solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of the wells, and the plates were incubated for 20 minutes at room temperature. After staining, the unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. The bound stain was subsequently eluted with 10 mM Trizma base, and the absorbance was read on an ELISA plate reader at a wavelength of 540 nm with 690 nm as a reference wavelength. [F. Arjmand, F. Sayeed, S. Parveen, S. Tabassum, A. S. Juvekar and S. M. Zingde, *Dalton Trans.,* **2013**, 42, 3390–3401]

EQN S1. Sterm-Volmer equation

$$F_0/F = 1 + Ksv\,[Q] = 1 + Kq.\tau_0\,[Q]$$

Where F_0 and F denotes the fluorescence intensities of hsDNA in absence and in presence of AuNPs, [Q] is the concentration of the AuNPs, Kq is the bimolecular quenching rate constant, τ_0 is the average lifetime of the molecule in the absence of quencher which is equal to 10^{-8}s.

EQN S2. Double logarithmic Stern-Volmer equation .

$$\text{Log}\ [(F_0-F)/F] = \text{Log}K + n\log[Q]$$

EQN S3. Vant Hoff's equation

$$\ln K = (-\Delta H^0/RT) + (\Delta S^0/R)$$

Where, K, R and T are binding constant, gas constant and temperature respectively.

EQN S4. Gibbs free energy

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 = -RT \ln K$$

EQN S5. The CD results are expressed in terms of mean residue ellipticity (MRE) in deg cm^2 d mol^{-1}:

$$\text{MRE= Intensity of CD (m deg) / 10Cpnl}$$

Where Cp is molar concentration of the protein, n is the number of amino acid residues, l is the path length.

EQN S6. The equation used for calculating the α-helical content of free BSA as well as bound BSA from MRE values at 208 and 222 nm:

$$\alpha\text{-Helix (\%)} = [(-MRE_{208}-4000) / (33{,}000-4000)] \times 100$$

Where MRE_{208} and MRE_{222} were the observed values at 208 and 222 nm respectively, 4000 was the MRE for the B-form and random coil conformation cross at 208, 222 nm. 33,000 were the MRE value for pure α-helix at 208 nm. The MRE value of the pure α-helix at wavelength 222 nm was 36,000.

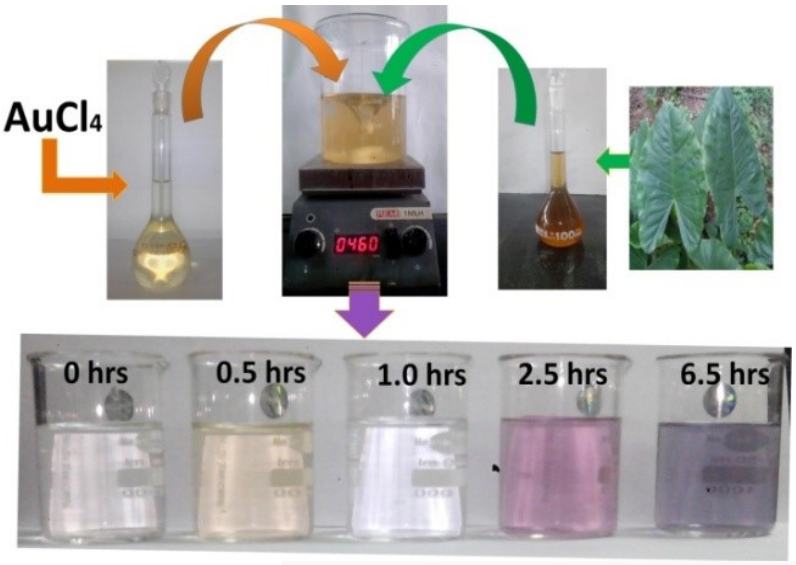

Fig.S1 Green synthesis of AuNPs from *Colocasia esculenta* leaves extract with gold salt.

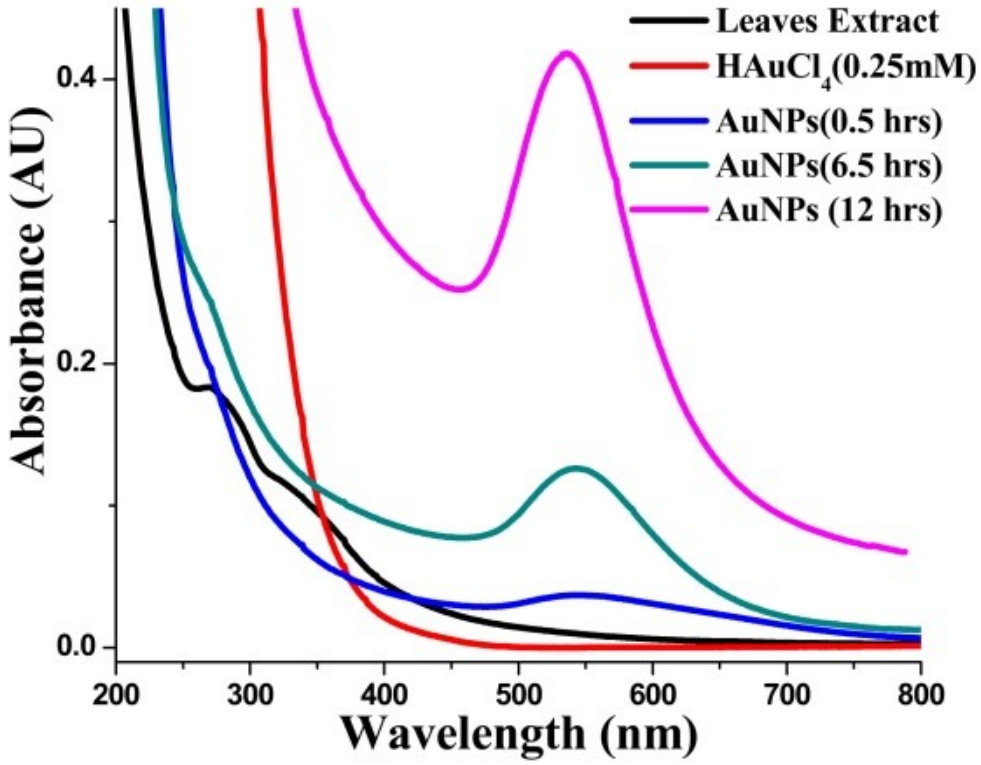

Fig.S2 UV- Visible spectra of AuNPs as a function of reaction time for the reaction of 0.25mM $HAuCl_4$ solution with *C. Esculenta* leave extract.

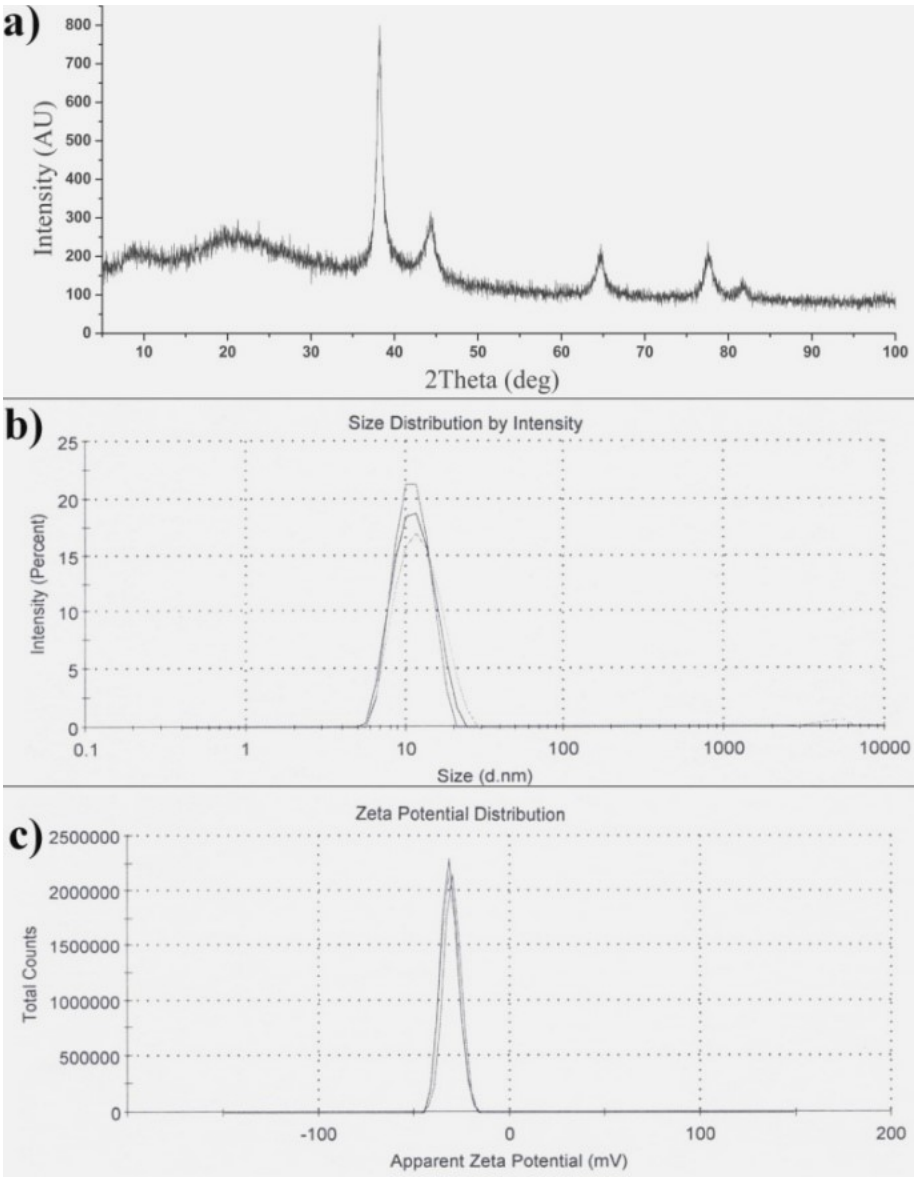

Fig.S3 (a) Diffraction pattern of AuNPs **(b)** Particle size distribution DLS analysis **(c)** Zeta potential.

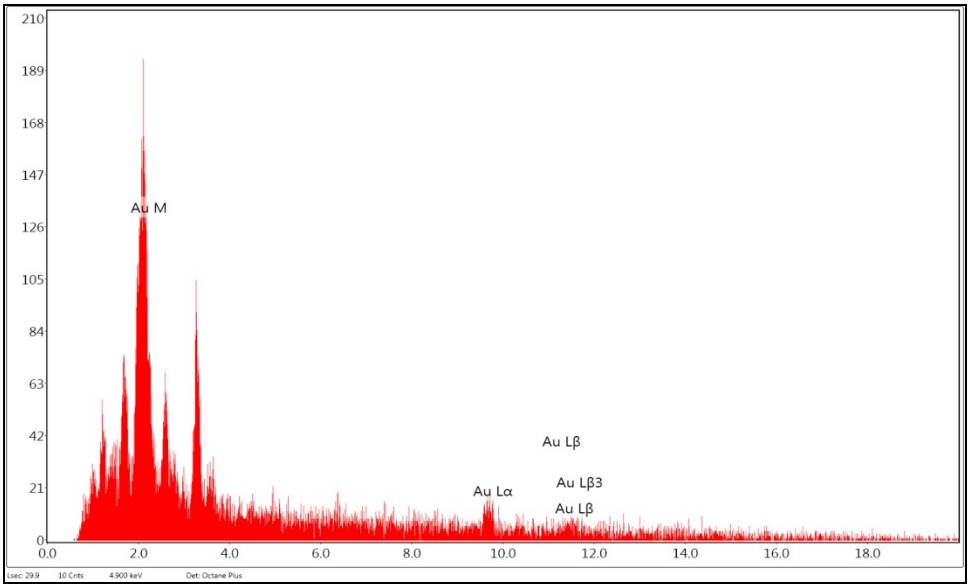

Fig. S4 EDAX spectrum of AuNPs

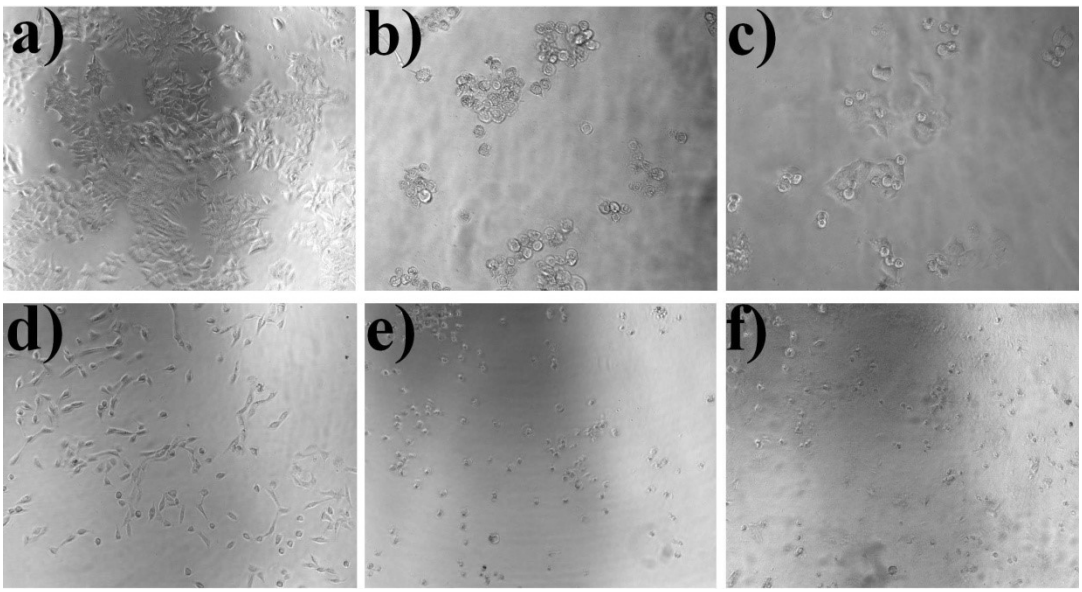

Fig. S5 Phase-contrast microscopic images obtained for morphological study of **(a)** breast cancer cells MCF-7 (control), **(b)** MCF-7 + ADR, **(c)** MCF-7 + AuNPs, **(d)** breast cancer cell MDA-MB-468 (control), **(e)** MDA-MB-468 + ADR and **(f)** MDA-MB-468 + AuNPs.

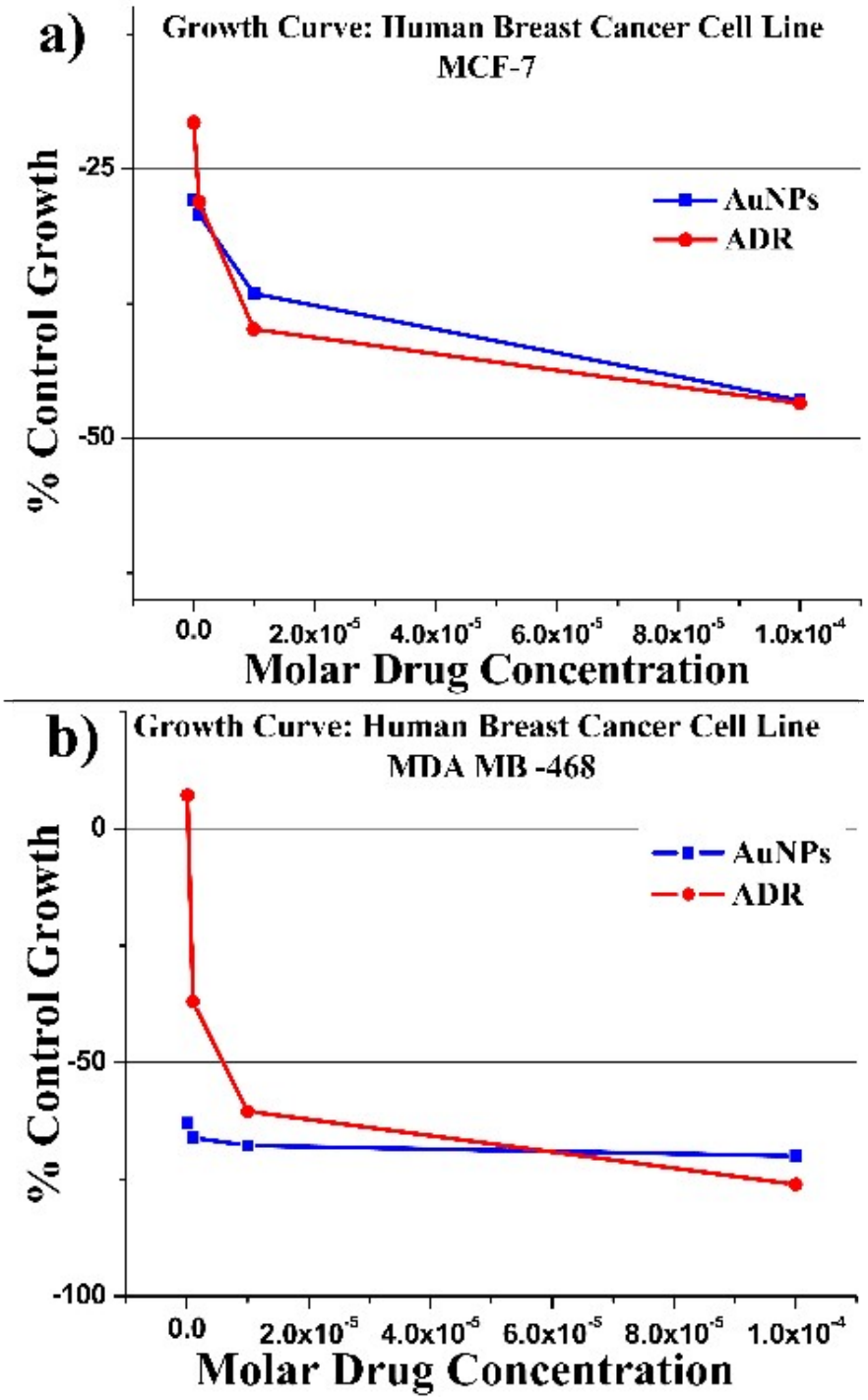

Fig.S6 % Control Growth curve of human breast cancer cell lines **(a)** MCF-7 with AuNPs and Adriamycin (ADR) Positive control compound in molar concentrations, **(b)** MDA-MB-468 with AuNPs and ADR Positive control compound in molar concentrations

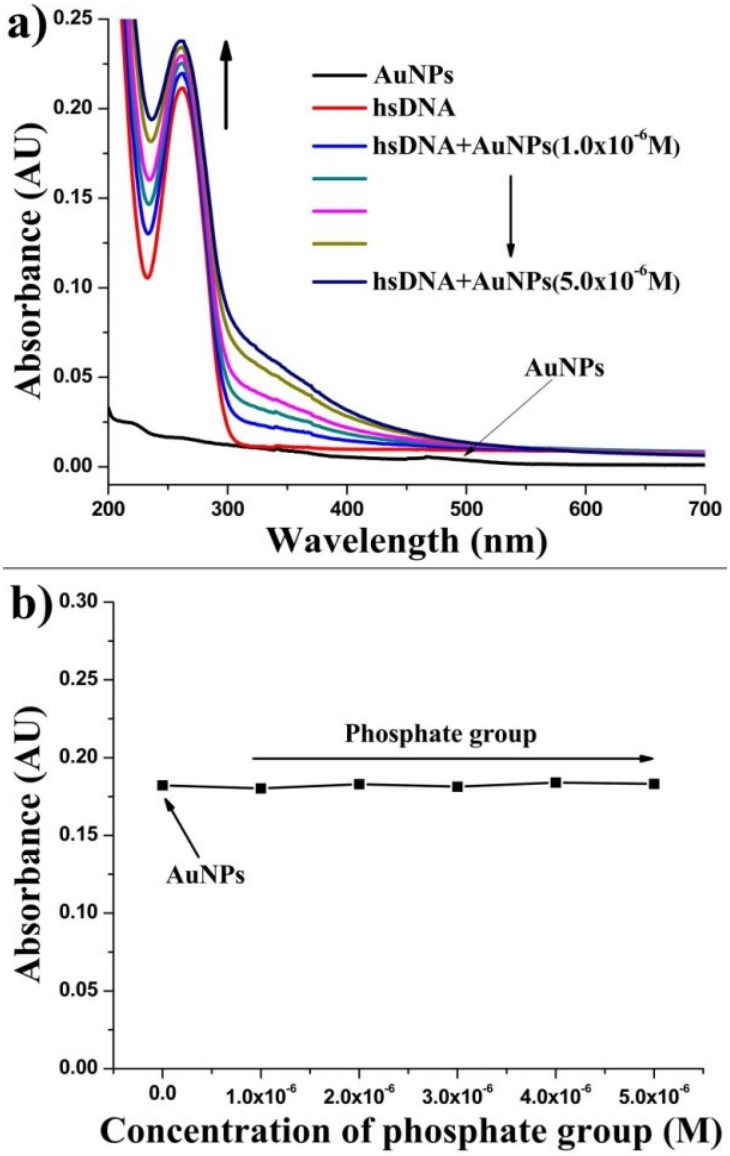

Fig.S7 (a) Overlies UV-visible spectra showing hsDNA-AuNPs complex formation for constant concentration hsDNA and increasing concentrations of AuNPs, **(b)** UV- visible absorption was performed by the addition of different concentrations of phosphate group to 1mM AuNPs solution

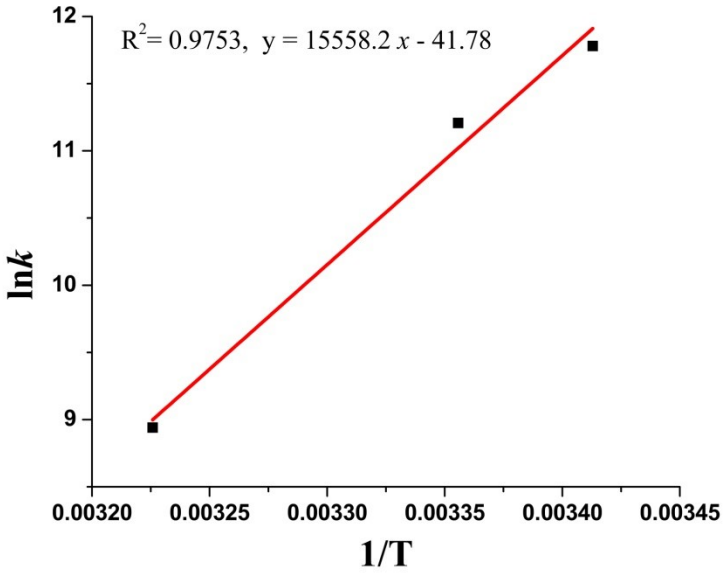

Fig. S8 Plot of ln*K* vs. 1/*T* for the interaction between hsDNA and AuNPs at different temperatures.

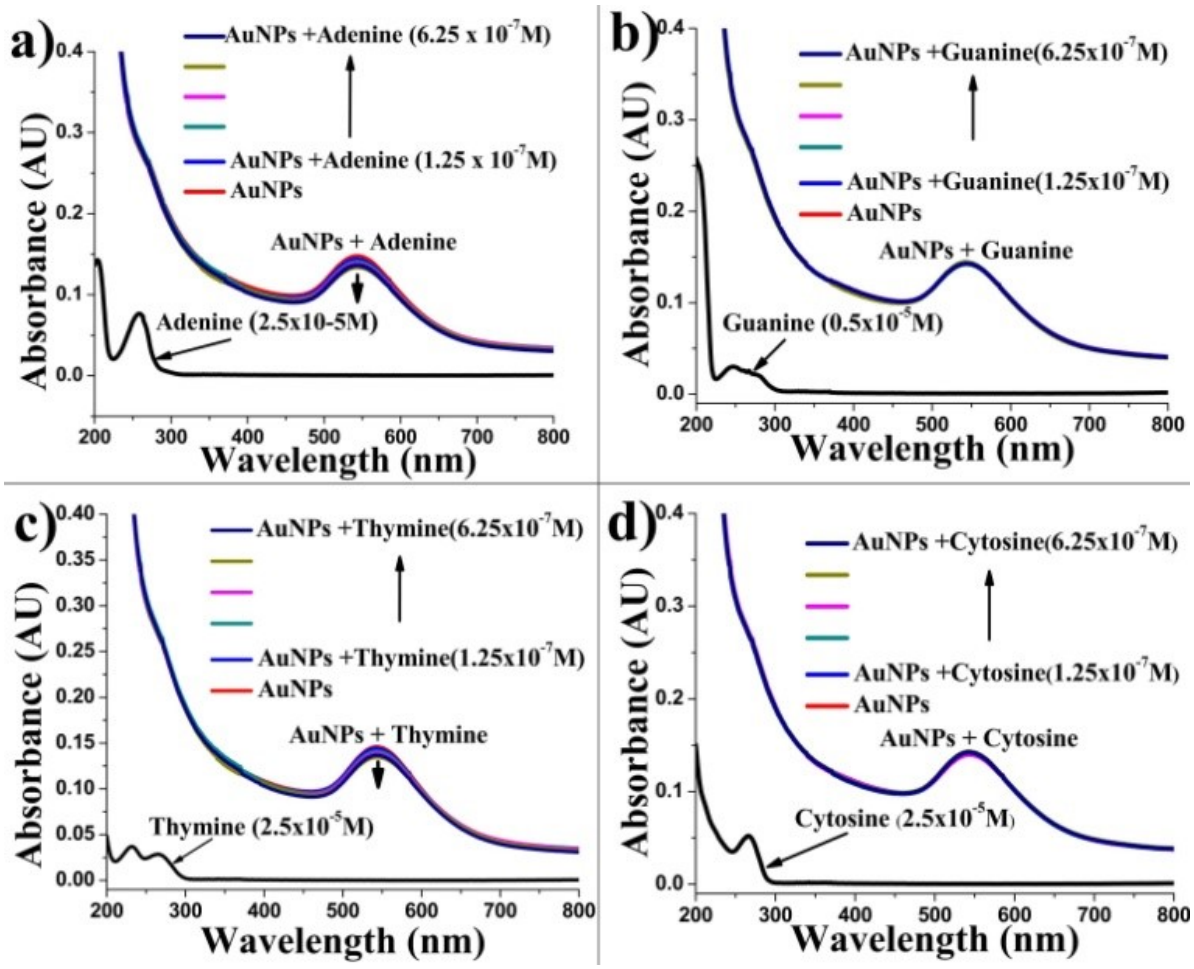

Fig. S9 UV- visible spectra of AuNPs (constant concentration 1.0mM) with different concentrations of **(a)**Adenine, **(b)** Thymine, **(c)** Cytosine and **(d)** Guanine. DNA bases ($2.5x10^{-6}$M) was demonstrated using red line in each figure.

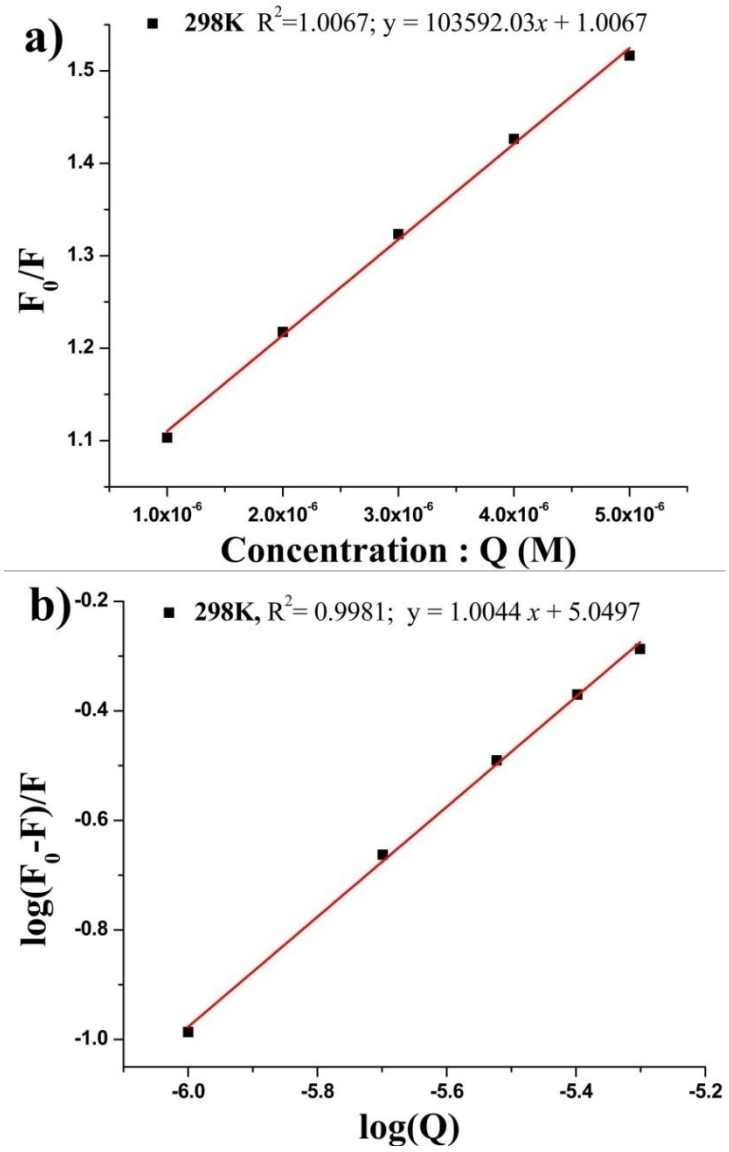

Fig. S10 (a) Stern Volmer plots for the quenching, **(b)** Double-logarithm plot for the quenching of BSA- Warfarin by AuNPs

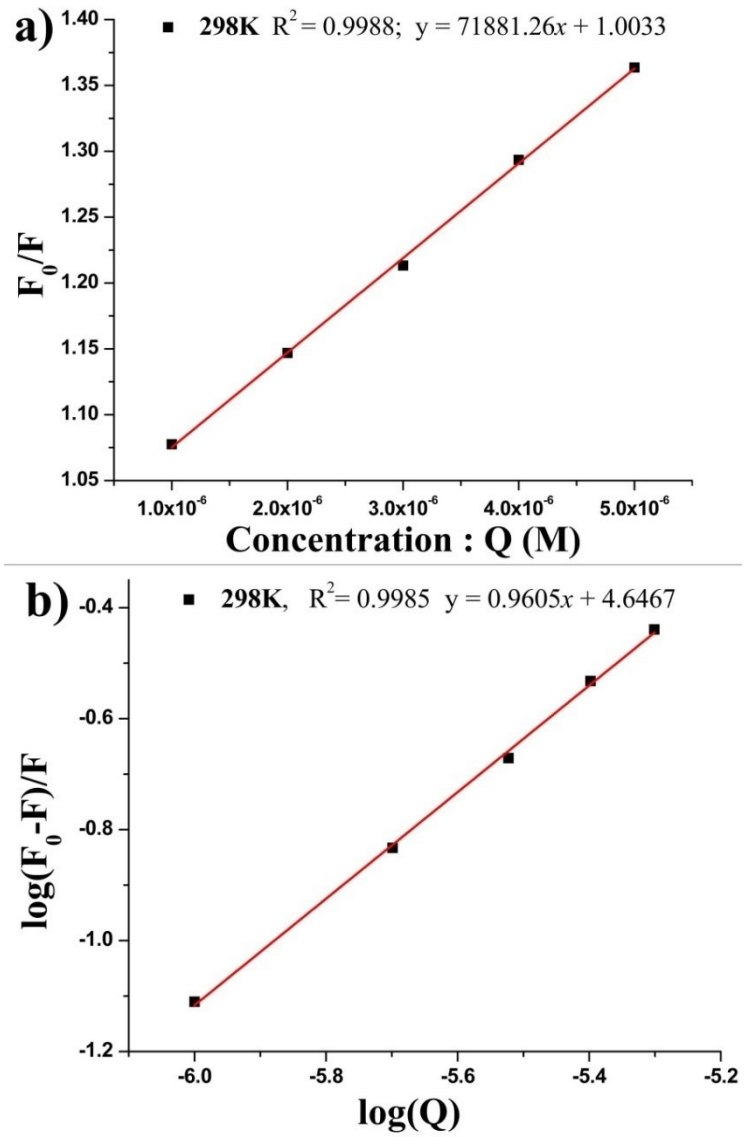

Fig. S11 (a) Stern Volmer plots for the quenching, **(b)** Double-logarithm plot for the quenching of BSA- Ibuprofen by AuNPs.

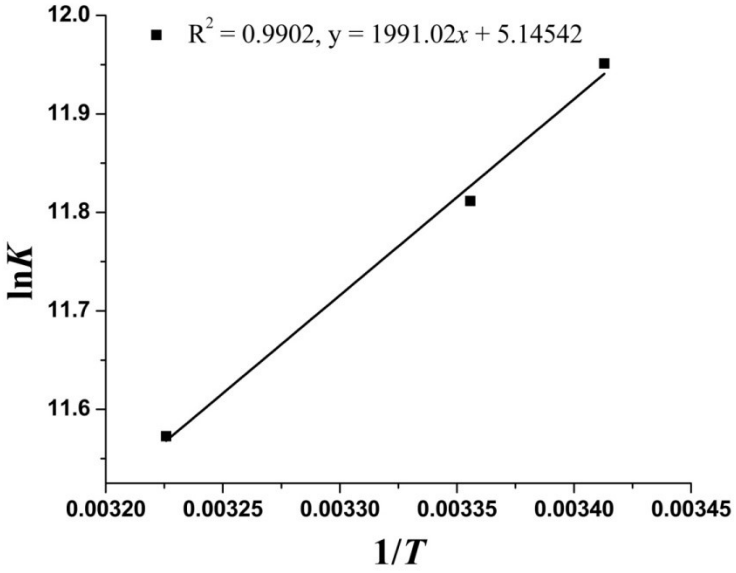

Fig. S12 Plot of ln*K* vs. 1/*T* for the interaction between BSA and green synthesized AuNPs at different temperatures

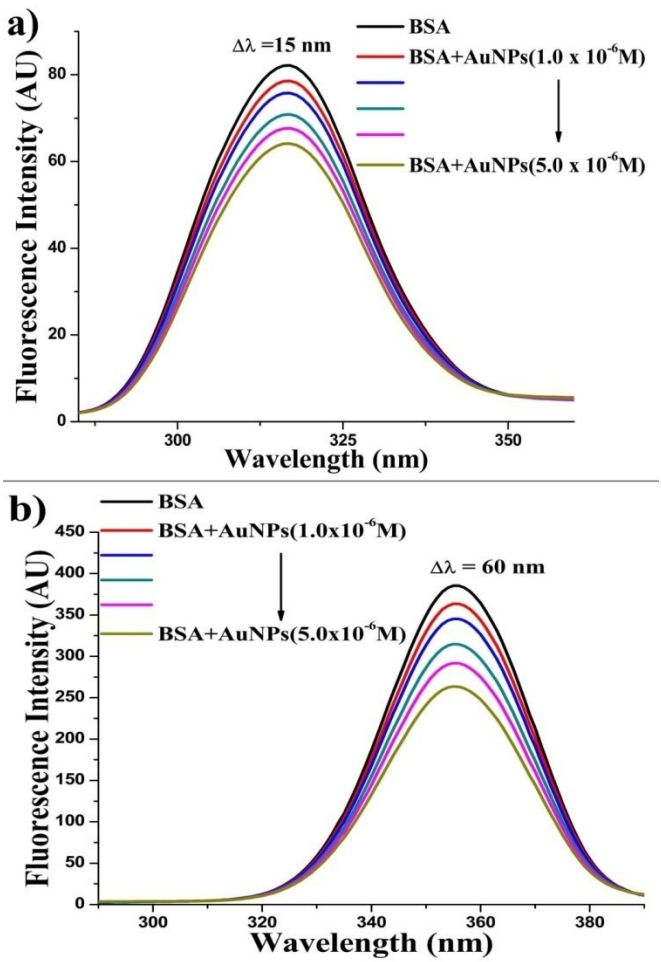

Fig. S13 (a) Synchronous fluorescence spectra at Δλ = 15nm showed shifting blue shift and **(b)** Δλ = 60nm showed shifting red shift of BSA in absence and in presence of AuNPs at different molar concentrations.

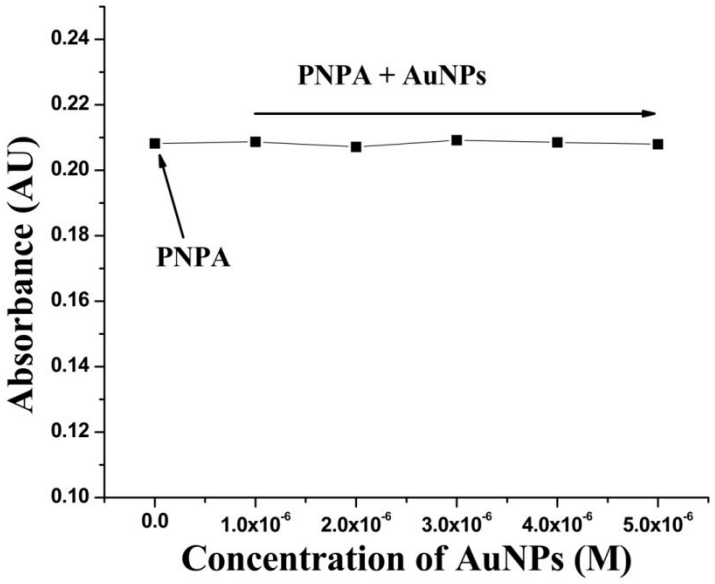

Fig. S14 Constant absorbance of PNPA with addition of various concentrations of AuNPs

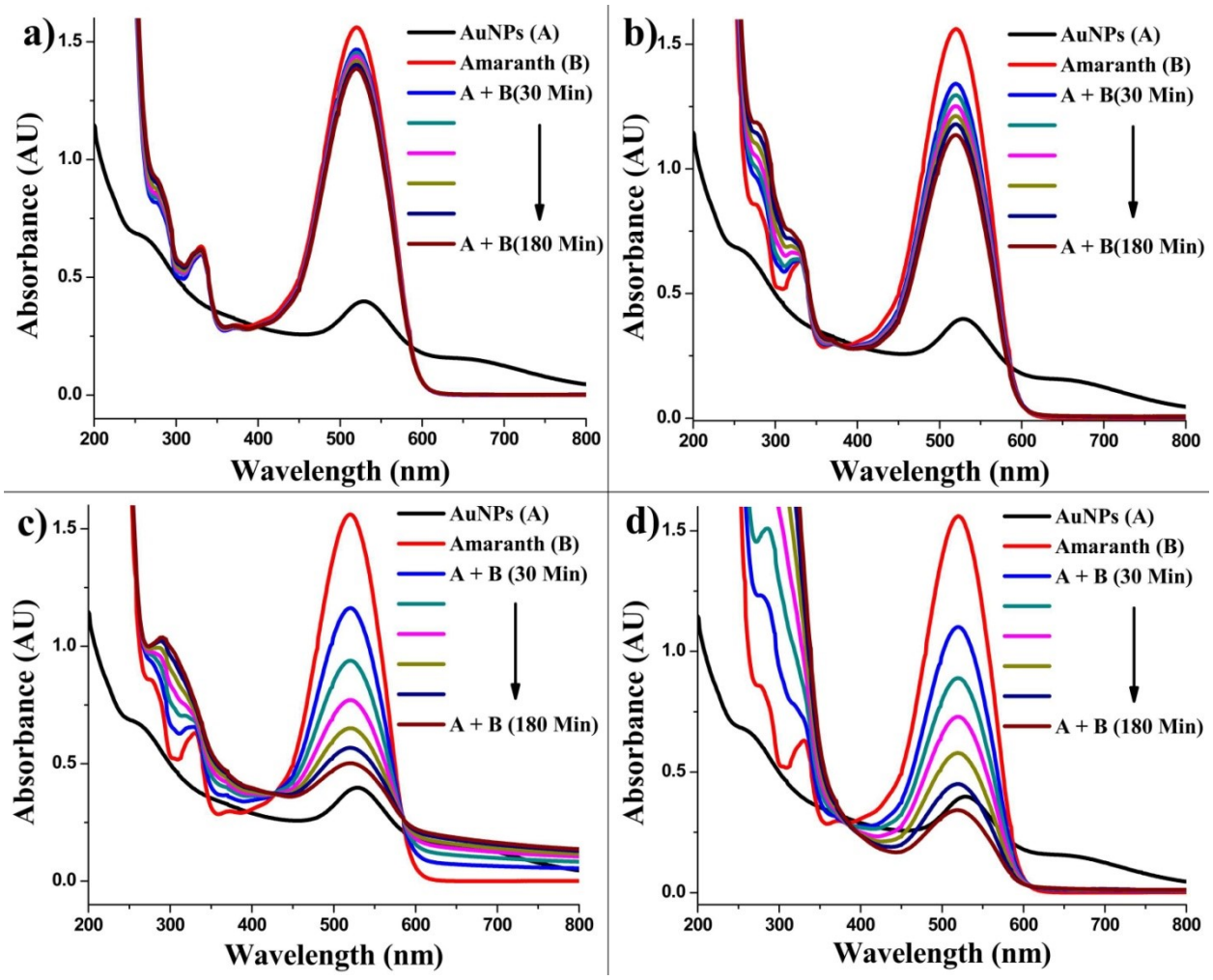

Fig. S15 UV–Vis spectra of amaranth dye (6.66×10^{-5}M) with AuNPs (5×10^{-6}M) at 30 min time intervals **(a)** on stirring, **(b)** irradiation with IR Light, **(c)** irradiation with Solar light and **(d)** irradiation with Ultraviolet-B light.

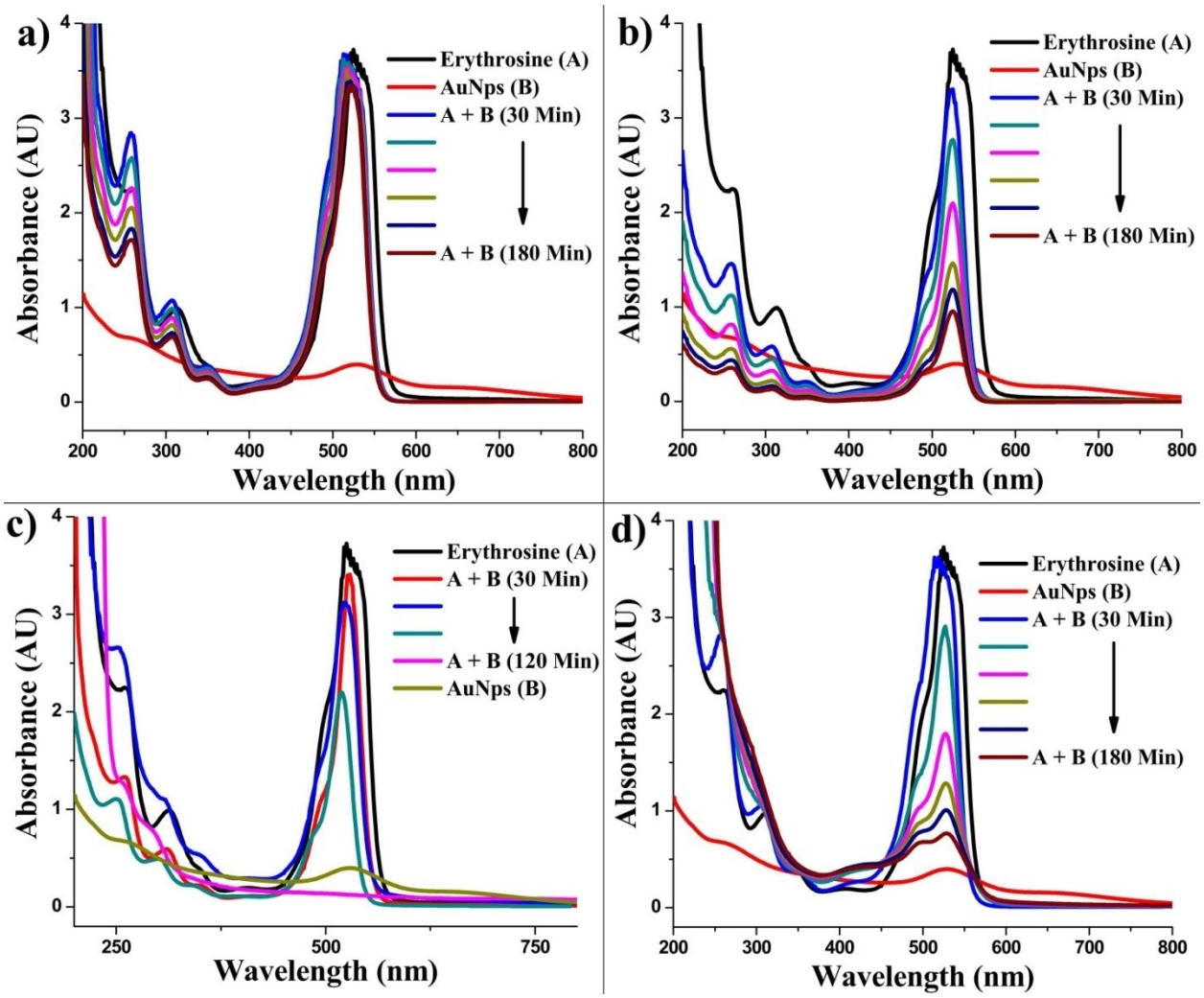

Fig. S16 UV–Vis spectra of erythrosine dye (5.98×10^{-5}M) with AuNPs (5×10^{-6}M) at 30 min time intervals **(a)** on stirring, **(b)** irradiation with IR Light, **(c)** irradiation with Solar light and **(d)** irradiation with Ultraviolet-B light.

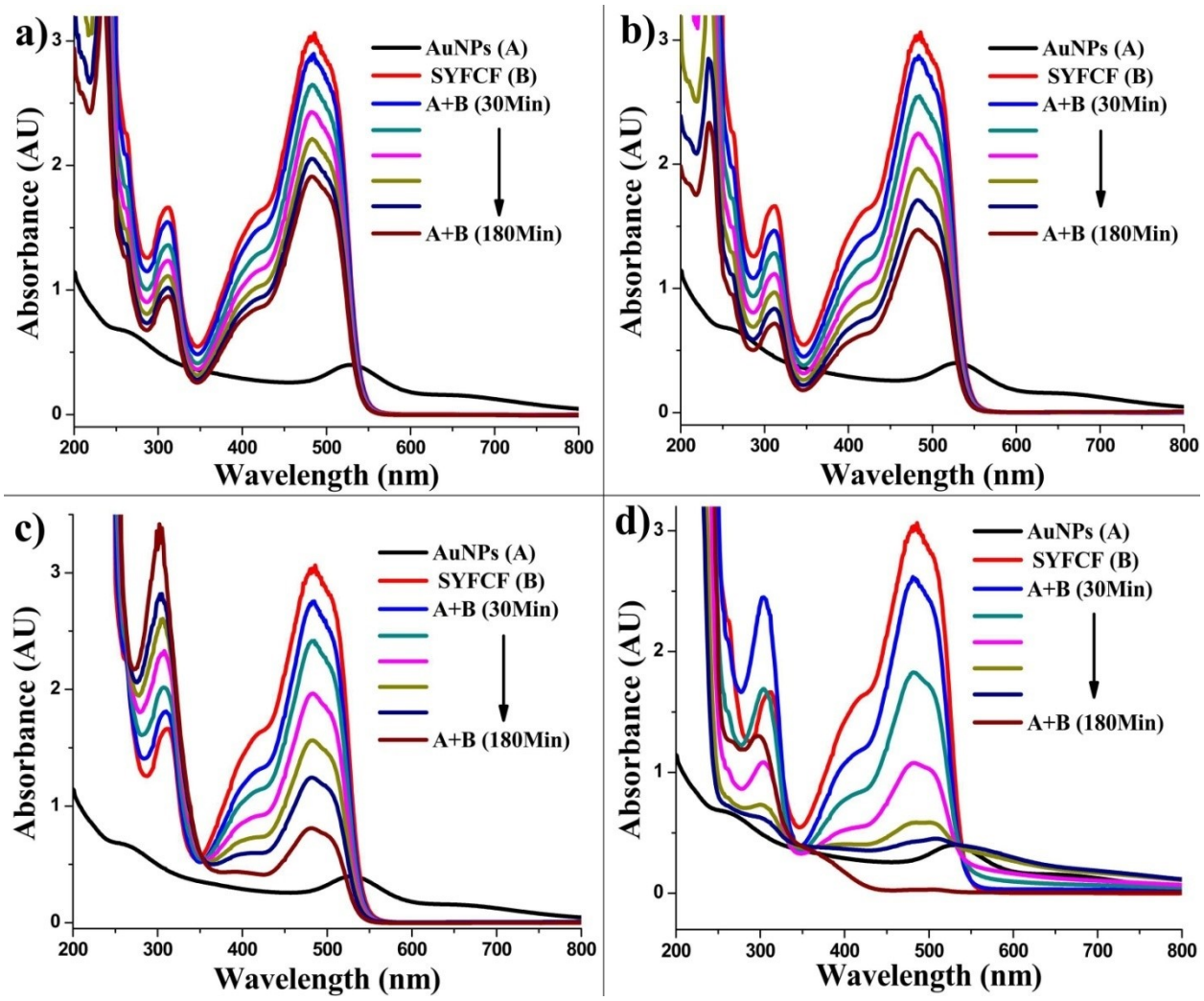

Fig. S17 UV–Vis spectra of SYFCF dye ($4.4x10^{-4}$M) with AuNPs ($5x10^{-6}$M) at 30 min time intervals **(a)** on stirring, **(b)** irradiation with IR Light, **(c)** irradiation with Solar light and **(d)** irradiation with Ultraviolet-B light.

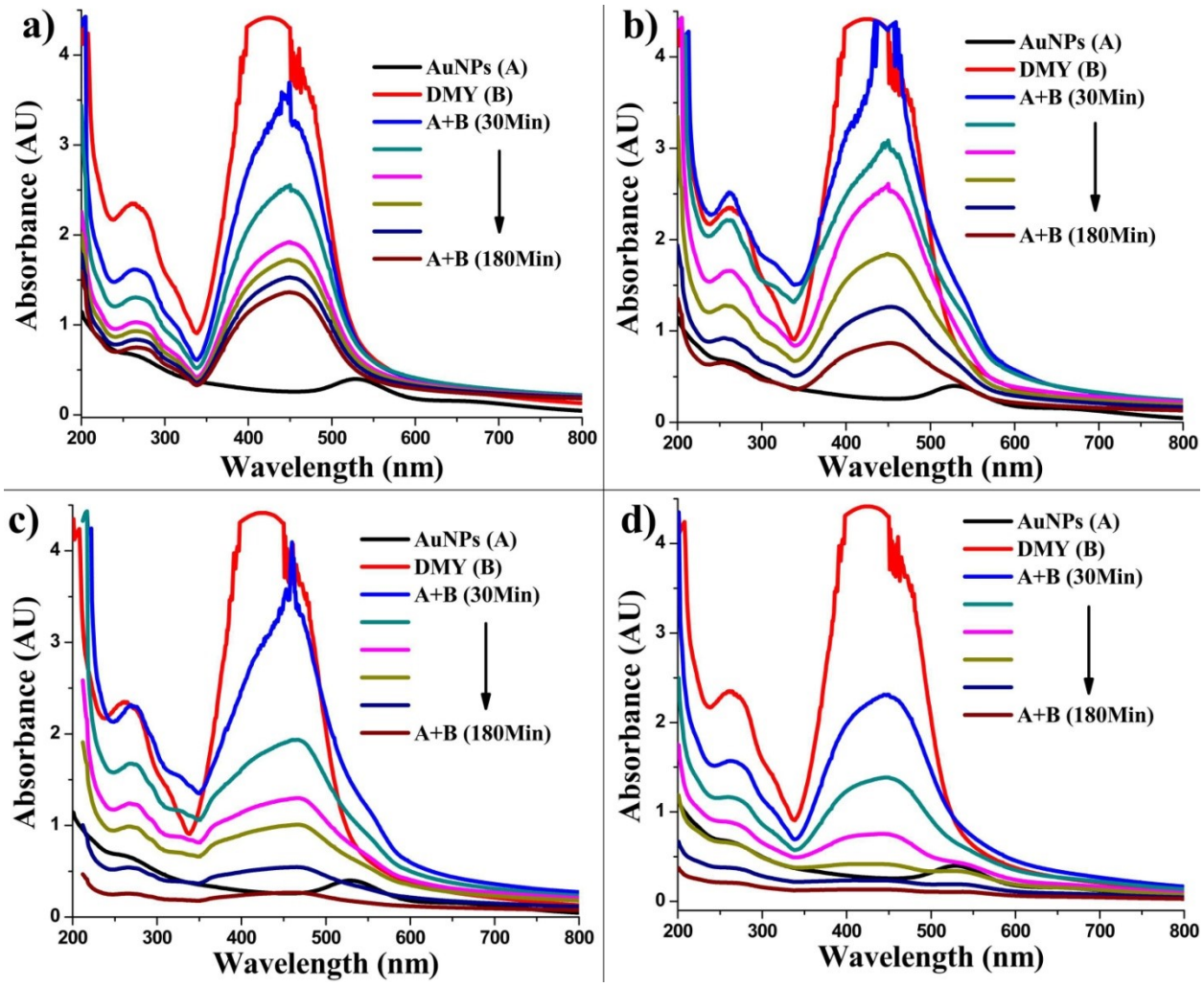

Fig. S18 UV–Vis spectra of DMY dye ($2.5x10^{-4}$M) with AuNPs ($5x10^{-6}$M) at 30 min time intervals **(a)** on stirring, **(b)** irradiation with IR Light, **(c)** irradiation with Solar light and **(d)** irradiation with Ultraviolet-B light.

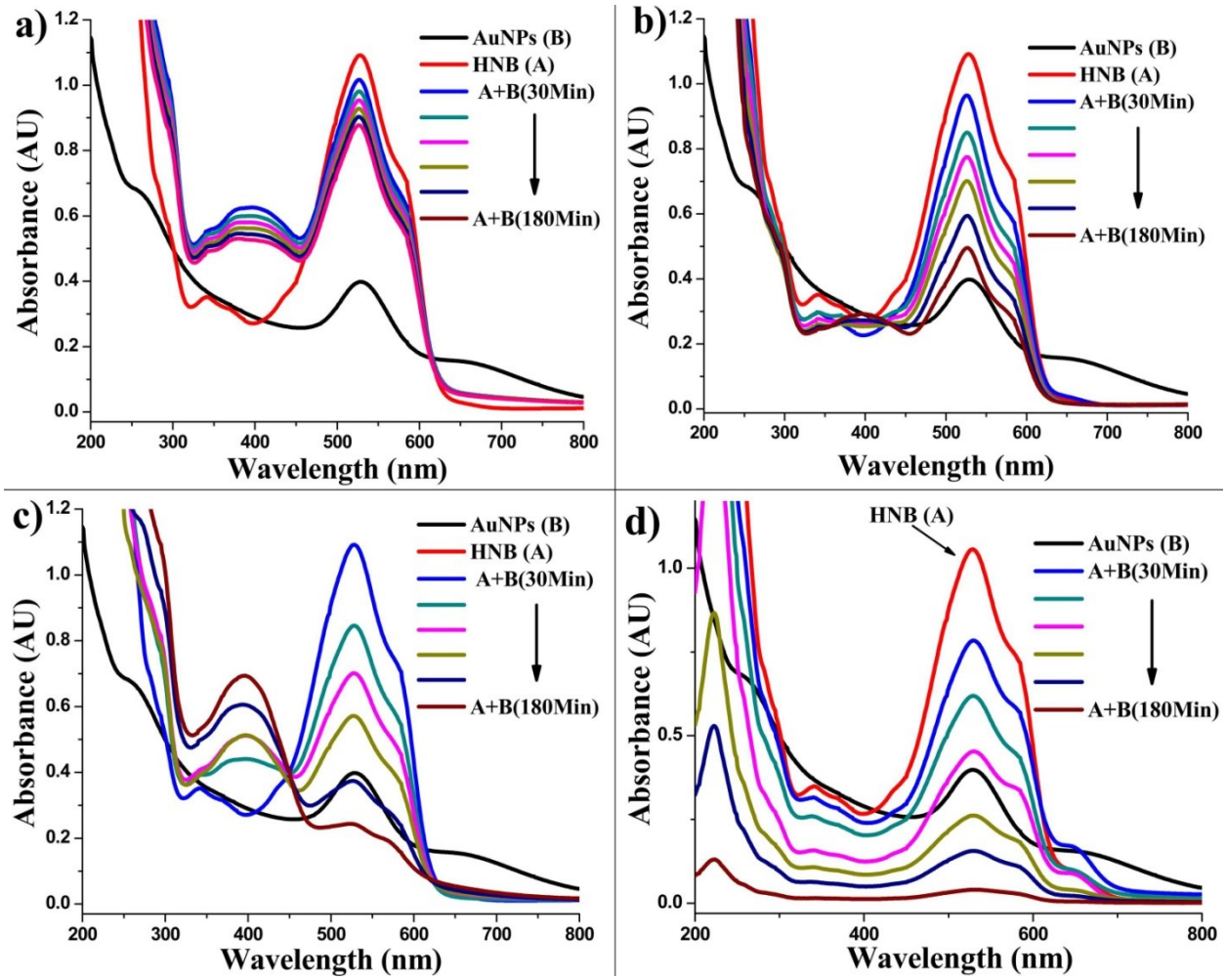

Fig. S19 UV–Vis spectra of HNB dye (8.33×10^{-5}M) with AuNPs (5×10^{-6}M) at 30 min time intervals **(a)** on stirring, **(b)** irradiation with IR Light, **(c)** irradiation with Solar light and **(d)** irradiation with Ultraviolet-B light.

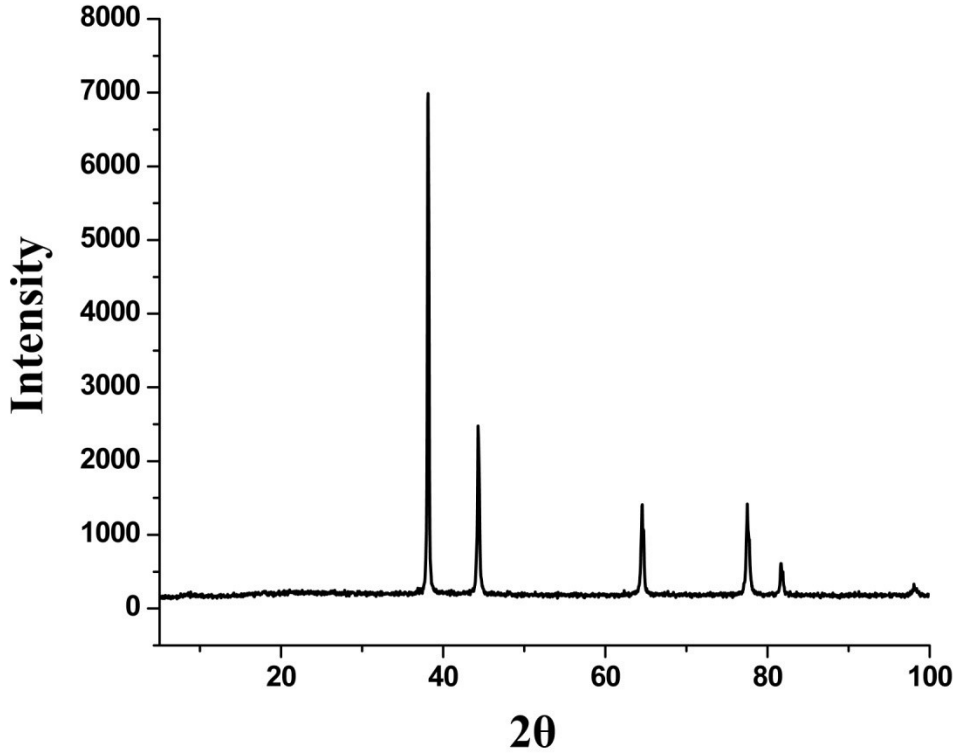

Fig. S20 X-ray diffractogram of recovered gold.

Table S1: Human Breast MCF-7 cancer cell line activity of AuNPs (incubation time: 48 hours).

Human Breast Cancer Cell Line MCF-7								
% Control Growth								
Molar Drug Concentrations								
	Experiment 1				Experiment 2			
Conc.	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
AuNPs	- 12.8	-20.5	-11.7	-38.9	-33.8	-31.8	-42.2	-42.1
ADR	-20.6	-27.9	-41.5	-44.9	-26.3	-35.5	-43.9	-42.9
	Experiment 3				Average Values			
Conc.	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
AuNPs	-37.0	-35.4	-55.8	-58.4	-27.9	-29.2	-36.6	-46.5
ADR	-15.1	-20.9	-34.3	-52.5	-20.7	-28.1	-39.9	-46.8

Table S2: Human Breast MDA MB 468 cancer cell line activity of AuNPs (incubation time: 48 hours).

Human Breast Cancer Cell Line MDA MB 468								
% Control Growth								
Molar Drug Concentrations								
	Experiment 1				Experiment 2			
Conc.	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
AuNPs	-61.4	-64.0	-64.1	-68.2	-65.6	-66.3	-68.3	-69.4
ADR	47.5	-17.1	-60.1	-76.5	-12.3	-37.0	-61.7	-72.8
	Experiment 3				Average Values			
Conc.	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
AuNPs	-62.1	-67.9	-71.0	-72.3	-63.0	-66.1	-67.8	-70.0
ADR	-13.6	-56.7	-59.3	-78.7	7.18	-36.9	-60.4	-76.0

Table S3: MCF-7 breast cancer cell line activity of AuNPs (incubation time: 48 hours).

	Drug concentrations (μMolar) calculated from graph		
MCF-7	**LC50**	**TGI**	**GI50***
AuNPs	NE	<10	<10
ADR	NE	<10	<10

LC_{50} = Concentration of drug causing 50% cell kill, GI_{50} = Concentration of drug causing 50% inhibition of cell growth, TGI = Concentration of drug causing total inhibition of cell growth, ADR = Adriamycin, Positive control compound, GI_{50} value of $\leq 10^{-6}$ molar (i.e. 1 μmolar) considered to demonstrate activity.

Table S4: MDA MB 468 breast cancer cell line activity of AuNPs (incubation time: 48 hours).

	Drug concentrations (μMolar) calculated from graph		
MDA-MB-468	**LC_{50}**	**TGI**	**GI_{50*}**
AuNPs	>100	<0.1	<10
ADR	45.0	<0.1	<10

Table S5: Binding parameters of green synthesized AuNPs with hsDNA at different temperatures.

Temperature (K)	*Ksv* (M^{-1})	*Kq* ($M^{-1}s^{-1}$)	*K* (LM^{-1})	*n*
293	2.69×10^{4}	2.69×10^{12}	7.36×10^{4}	0.9950
298	1.68×10^{4}	1.68×10^{12}	7.64×10^{3}	0.9943
310	1.31×10^{4}	1.31×10^{12}	1.30×10^{3}	0.9826

Table S6: Thermodynamic parameters of green synthesized AuNPs-hsDNA interaction

Temperature (K)	ΔH^0 (KJmol^{-1})	ΔS^0 (Jmol^{-1}K^{-1})	ΔG^0 (KJmol^{-1})
293	-129.35	-342.44	-29.01
298			-26.295
310			-19.822

Table S7: Binding parameters of green synthesized AuNPs with BSA at different temperatures.

Temperature (K)	*Ksv* (M^{-1})	*Kq* ($M^{-1}s^{-1}$)	*K* (LM^{-1})	*n*
293	1.10×10^{5}	1.10×10^{13}	1.06×10^{5}	0.9900
298	1.06×10^{5}	1.06×10^{13}	1.35×10^{5}	1.0005
310	1.02×10^{5}	1.02×10^{13}	1.55×10^{5}	0.9962

Table S8: Thermodynamic parameters of green synthesized AuNPs-BSA interaction

Temperature (K)	ΔH^0 (KJmol^{-1})	ΔS^0 (Jmol^{-1}K^{-1})	ΔG^0 (KJmol^{-1})
293	-16.55	42.77	-29.08
298			-29.29
310			-29.81