

Electronic Supplementary information

Glucosamine functionalized gold nanoparticles for specific detection and colorimetric assay of Glutathione from real samples

Anurag Kumar Singh^a, Saumya Singh^a, Raksha Singh^a, Manish Sharma^b, Ida Tiwari^a and K. K. Upadhyay^{a*}

*Corresponding Author

^aDepartment of chemistry (Centre of Advanced Study), Institute of Science, Banaras Hindu University, Varanasi-221005, India.

^bDefence Institute of Physiology and Allied Sciences (DIPAS), DRDO, Lucknow Road, Timarpur, Delhi 110054, India.

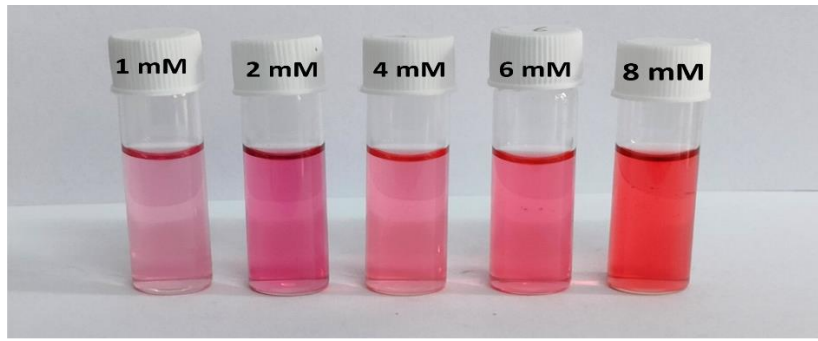
Email: drkaushalbhu@yahoo.co.in

Table of content

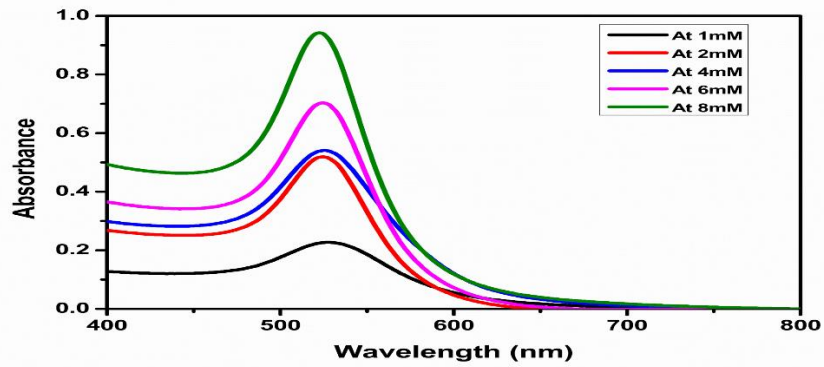
S. No.	Fig. No.	Content	Page No.
1.	Fig.S1	(a) Visual response of GNPs synthesised using different concentrations of glucosamine (1mM, 2Mm, 4mM, 6mM) keeping other reaction parameters constant (b) Corresponding UV-Visible spectra	4
2.	Fig.S2	(a) Visual response of GNPs with varying reaction time (1/2, 1, 1.5, 2, 3 hr) (b) Corresponding UV-Visible spectra	4
3.	Fig.S3	(a) Visual response of GNPs with varying reaction temperature (40, 50, 60, 70 and 80) (b) Corresponding UV-Visible spectra	5
4.	Fig.S4	Comparison between controlled preparations and GNPs	6
5.	Fig.S5	UV-Visible spectra of various controlled preparation and GNPs	6
6.	Fig.S6	Image showing Tyndell effect	7
7.	Fig.S7	Stability check through electrolyte (0.1 M NaCl) addition and its corresponding UV-Visible spectra at the interval of 5 min	7
8.	Fig.S8	Stability check through electrolyte (1 M NaCl) addition and its corresponding UV-Visible spectra at the interval of 5 min	8
9.	Fig.S9	Stability check through electrolyte (2 M NaCl) addition and its corresponding UV-Visible spectra at the interval of 5 min	8
10.	Fig.S10	pH Study: Visual response of pH metric titration of GNPs from 1-14 pH	9
11.	Fig.S11	pH Study: UV-Visible response of pH metric titration of GNPs from 1-14 pH	9

12.	Fig.S12	Practical applicability of GNPs on cotton balls	10
13.	Fig.S13	Interference studies of GNPs at 521 nm with different essential amino acids	10
14.	Fig.S14	Matrix study	11
15.	Fig.S15	Comparison table for Limit of Detection	11
16.	Fig.S16	FT-IR spectrum of GNPs	12
17.	Fig.S17	XPS studies for GNPs, (a) Spectrum of Au 4f (b) Spectrum of C 1s (c) Spectrum of N 1s, (d) Spectrum of O 1s, (e) Survey spectrum of GNPs	13
18.	Fig.S18	Dynamic Light Scattering (DLS) study showing hydrodynamic diameter of GNPs	14
19.	Fig.S19	Zeta Potential of GNPs	14
20.	Fig.S20	EDAX spectrum of GNPs	15
21.	Fig.S21	SAED pattern of GNPs	15
22.	Fig.S22	(a) TEM image of GNPs used for preparation of histogram chart [at 50 nm scale bar] (b) Histogram showing size distribution of GNPs, (c) Concentration of GNPs	16
23.	Fig.S23	FT-IR spectrum of GNPs after interaction with Glutathione	17
24.	Fig.S24	FT-IR spectrum of GSH in experimental condition	17
25.	Fig.S25	Comparison table for IR spectra	18
26.	Fig.S26	XPS studies of GNPs after addition of GSH, (a) Spectrum for Au 4f, (b) Spectrum for C 1s, (c) Spectrum for N 1s, (d) Spectrum for O 1s, (e) Spectrum for S 2p, (f) Survey spectrum of GNPs after addition of GSH	18
27.	Fig.S27	Dynamic Light Scattering (DLS) study showing hydrodynamic diameter of GNPs after addition of Glutathione	19
28.	Fig.S28	Zeta Potential of GNPs after addition of Glutathione	19
29.	Fig.S29	EDAX spectrum of GNPs after interaction with Glutathione	20
30.	Fig.S30	SAED pattern of GNPs after addition with Glutathione	20
31.	Fig.S31	pH Study: Visual response of pH metric titration of GNPs with Glutathione in the pH range (1-14)	21
32.	Fig.S32	pH Study: UV-Visible spectra of corresponding pH metric titration of GNPs after with Glutathione in the pH range (1-14)	21
33.	Fig.S33	Tentative mechanistic pathway for GNPs synthesis	22

34.	Fig.S34	Mechanistic pathway for GNPs interaction with GSH	23
35.	Fig.S35	TEM image of GNPs (From low to high resolution)	23
36.	Fig.S36	TEM image of GNPs with Glutathione (from low to high resolution)	24
37.	Fig.S37	SEM image of GNPs (from low to high resolution)	24
38.	Fig.S38	SEM image of GNPs with Glutathione (from low to high resolution)	25
39.	Fig.S39	(a) Visual response of (1) GNPs (2) GNPs after addition of Maxiliv (without boiling) (3) GNPs after addition of Glutaderm (without boiling) (b) Corresponding UV-Visible spectra	25
40.	Fig.S40	(a) Visual response of GNPs after addition of Maxilliv at various time intervals (Intra-day repeatability) (b) Corresponding UV-Visible spectra	26
41.	Fig.S41	(a) Visual response of GNPs after addition of Maxilliv over a course of five days intervals (Inter-day repeatability) (b) Corresponding UV-Visible spectra	26
42.	Fig.S42	(a) Visual response of GNPs after addition of Glutaderm at various time intervals (Intra-day repeatability) (b) Corresponding UV-Visible spectra	27
43.	Fig.S43	(a)Visual response of GNPs after addition of Maxilliv over a course of five days (Inter day repeatability (b) Corresponding UV-Visible spectra	27
44.	Fig.S44	(a) Image showing the amount of various constituents present in Glutaderm (b) Image showing Alpha lipoic acid used for selectivity study (c) Image showing Vitamin C used for selectivity study	28
45.	Fig.S45	(a) Visual response of GNPs, GNPs+Glutaderm, GNPs+Alpha Lipoic acid, GNPs+Vitamin C (b) Corresponding UV-Visible spectra	28
46.	Fig.S46	UV-Visible spectrum of glutathione (GSH) in experimental conditions	29

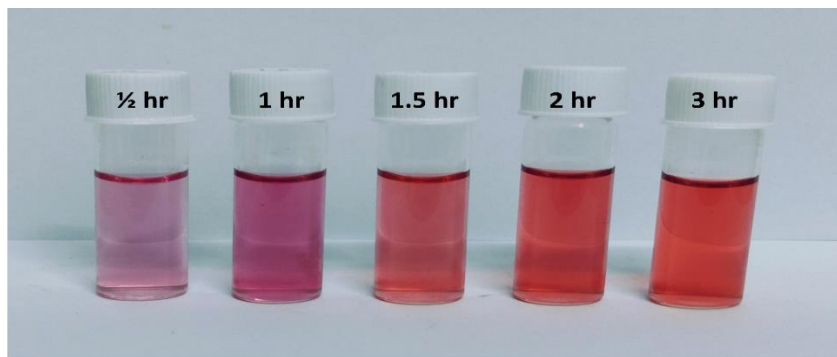


(a)

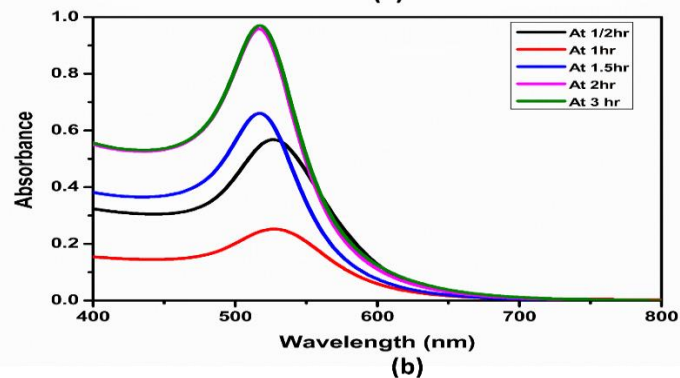


(b)

Fig.S1: (a) Visual response of GNPs synthesised using different concentrations of glucosamine (1mM, 2Mm, 4mM, 6mM) keeping other reaction parameters constant (b) Corresponding UV-Visible spectra

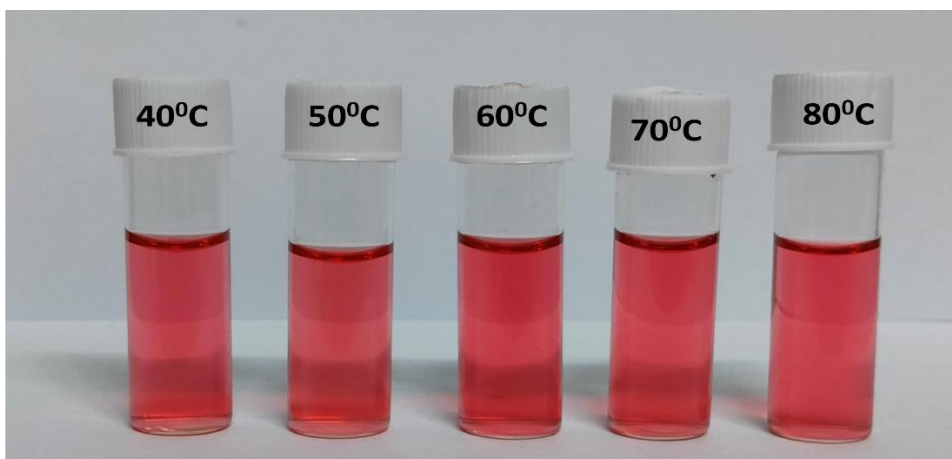


(a)

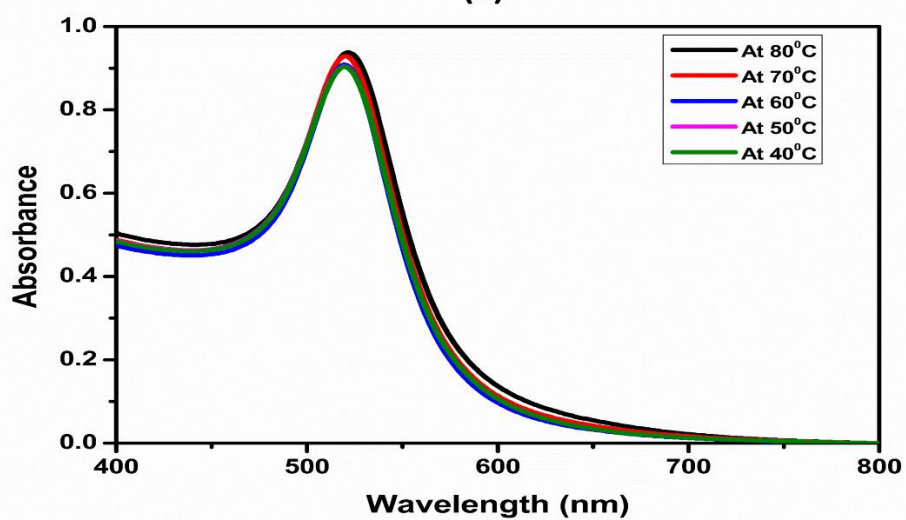


(b)

Fig.S2: (a) Visual response of GNPs with varying reaction time (1/2, 1, 1.5, 2, 3 hr) (b) Corresponding UV-Visible spectra



(a)



(b)

Fig.S3: (a) Visual response of GNPs with varying reaction temperature (40, 50, 60, 70 and 80) (b) Corresponding UV-Visible spectra

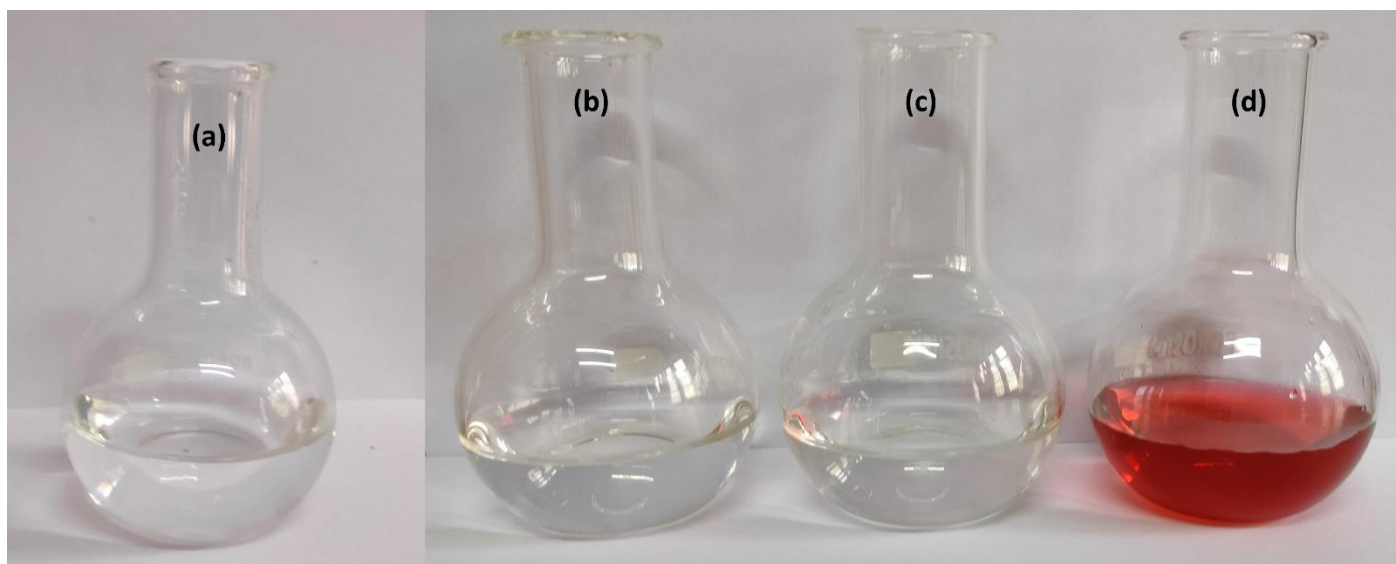


Fig.S4: Comparison between controlled preparations and GNPs: (a) Gold (III)chloride trihydrate + NaOH (b) Glucosamine + NaOH (c) Gold (III) chloride trihydrate + Glucosamine (d) GNPs (Gold (III) chloride trihydrate + Glucosamine + NaOH)

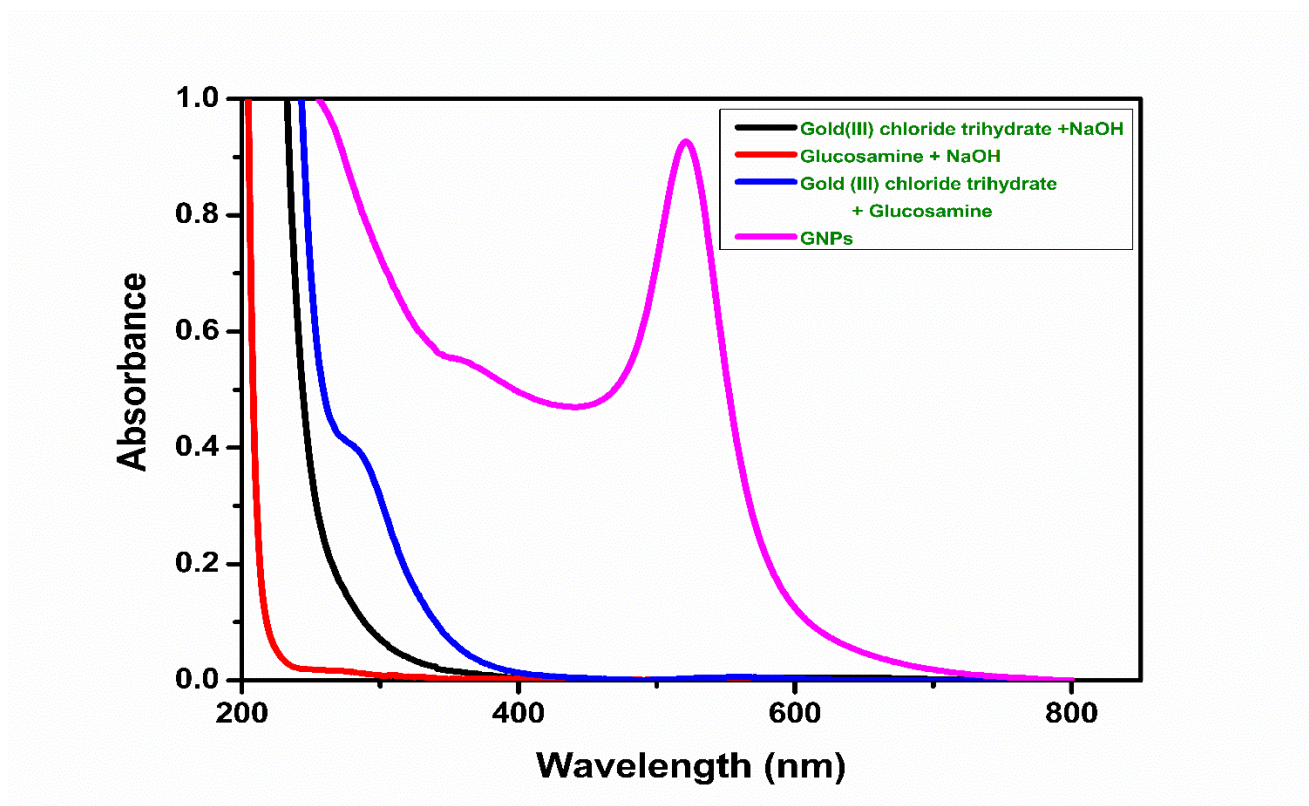


Fig.S5: UV-Visible spectra of various controlled preparation and GNPs

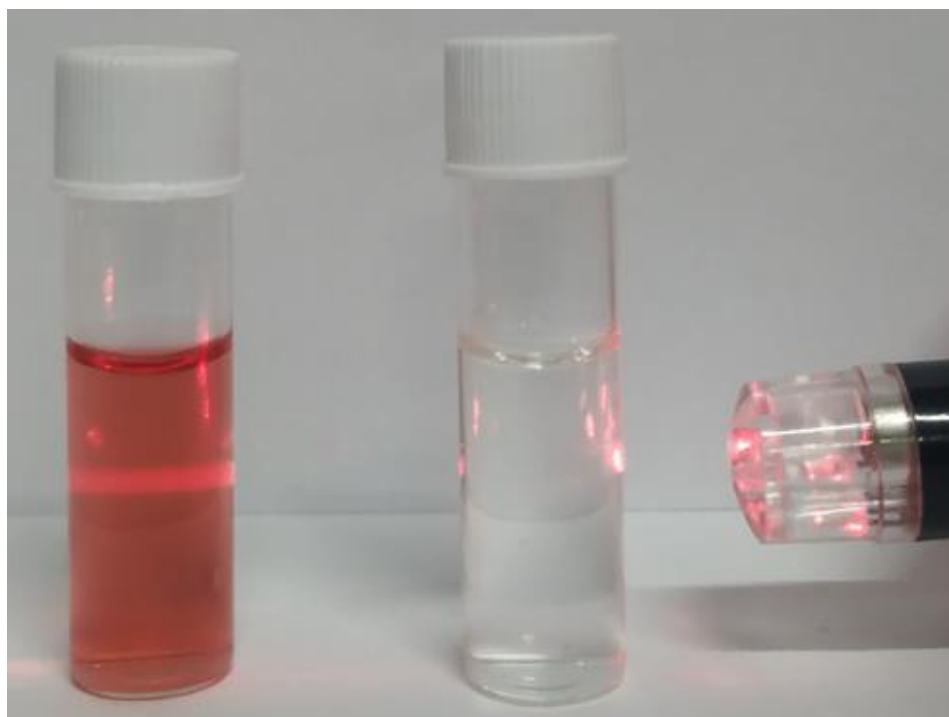


Fig.S6: Image showing Tyndell effect [left side: GNPs, right side: Milli-Q water]

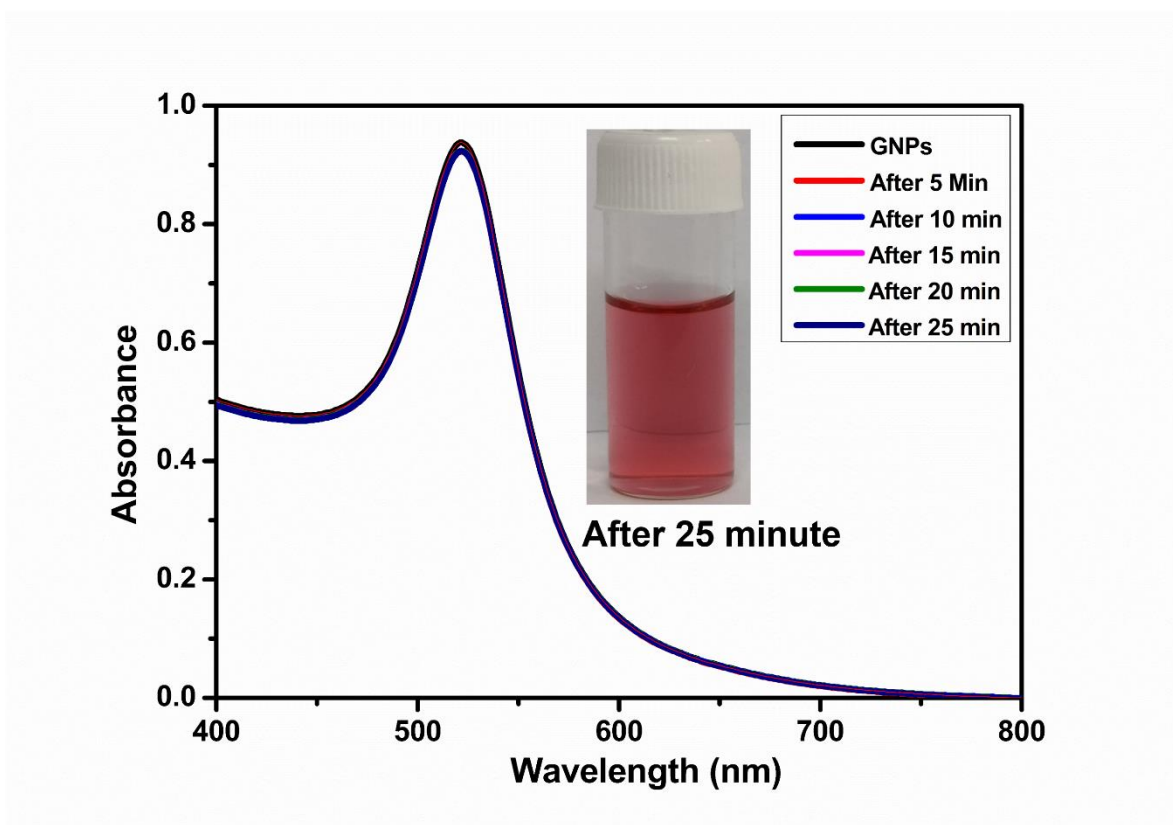


Fig.S7: Stability check through electrolyte (0.1 M NaCl) addition and its corresponding UV-Visible spectra at the interval of 5 min

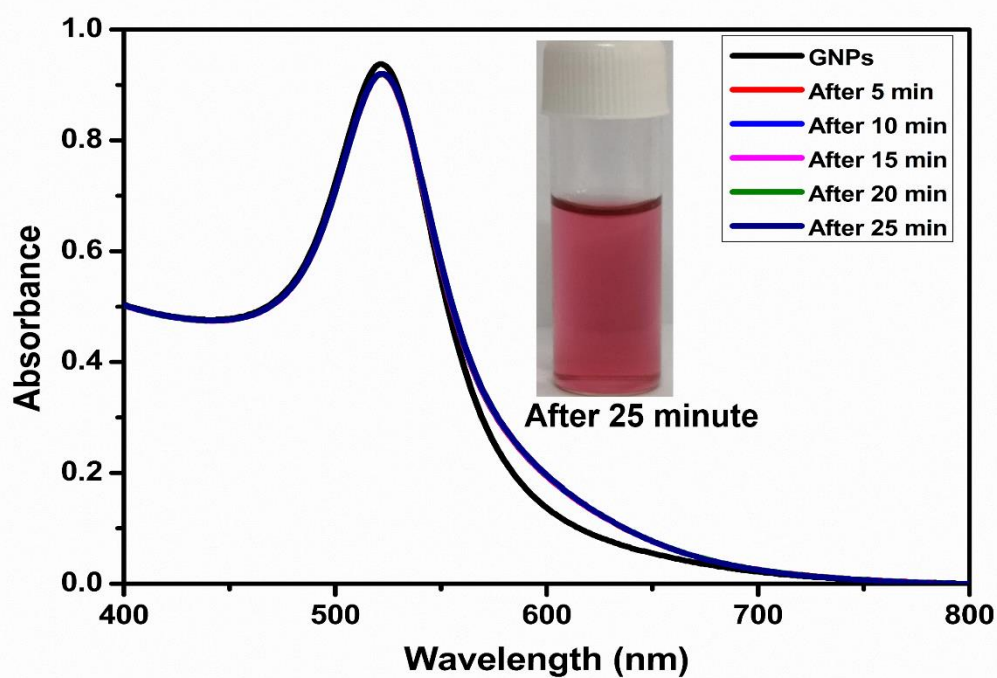


Fig.S8: Stability check through electrolyte (1 M NaCl) addition and its corresponding UV-Visible spectra at the interval of 5 min

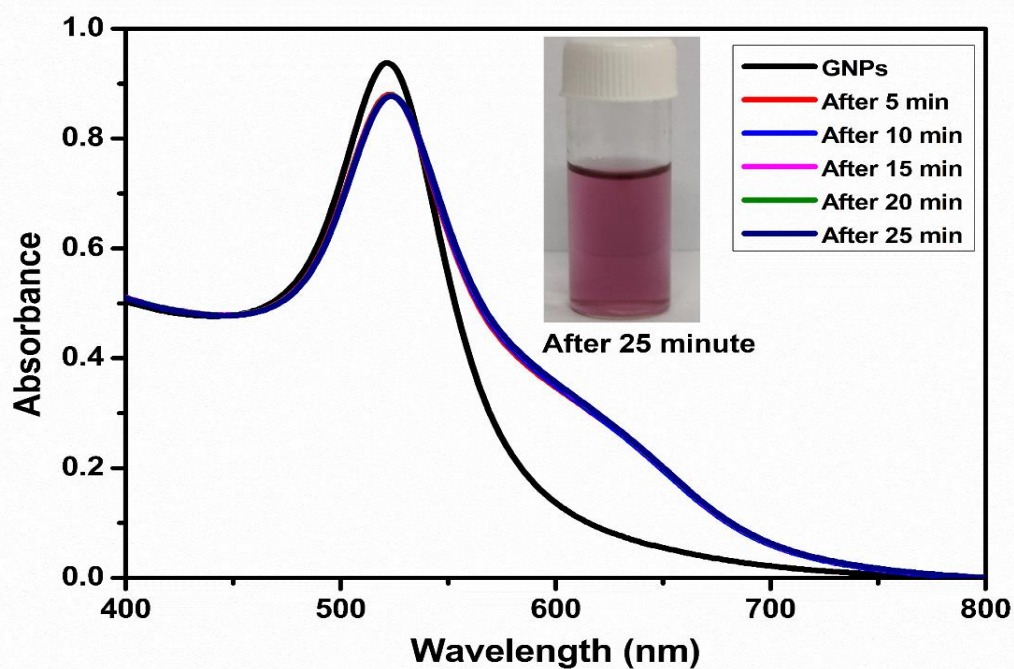


Fig.S9: Stability check through electrolyte (2 M NaCl) addition and its corresponding UV-Visible spectra at the interval of 5 min

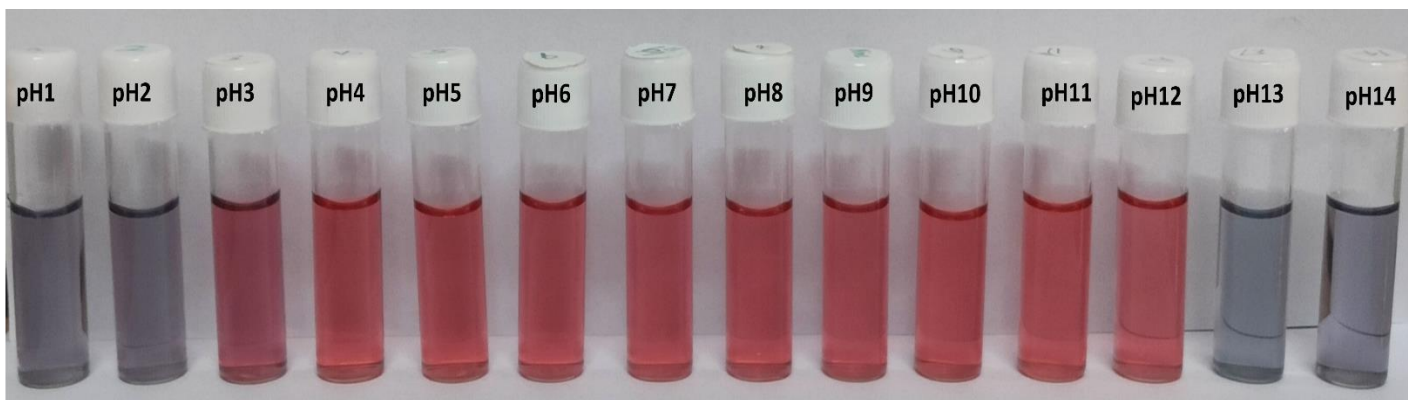


Fig.S10: pH Study: Visual response of pH metric titration of GNPs from 1-14 pH

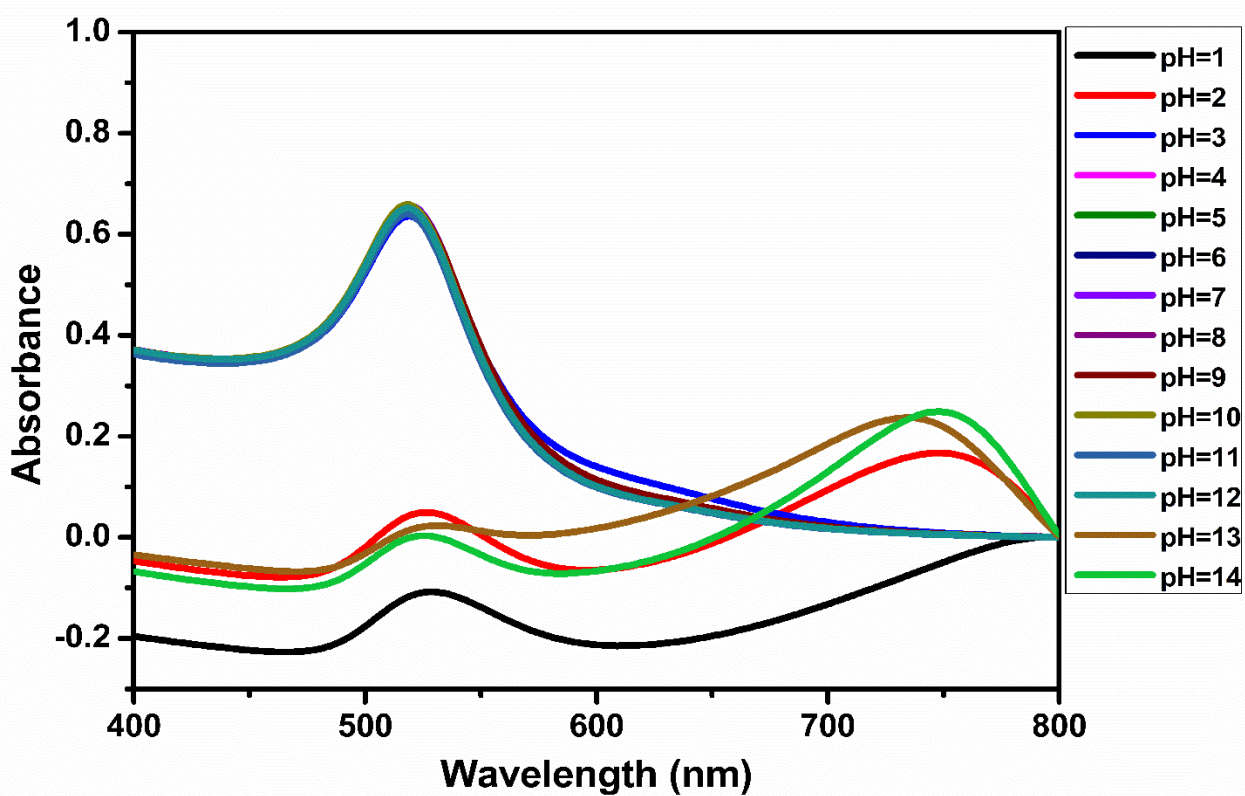


Fig.S11: pH Study: UV-Visible response of pH metric titration of GNPs from 1-14 pH

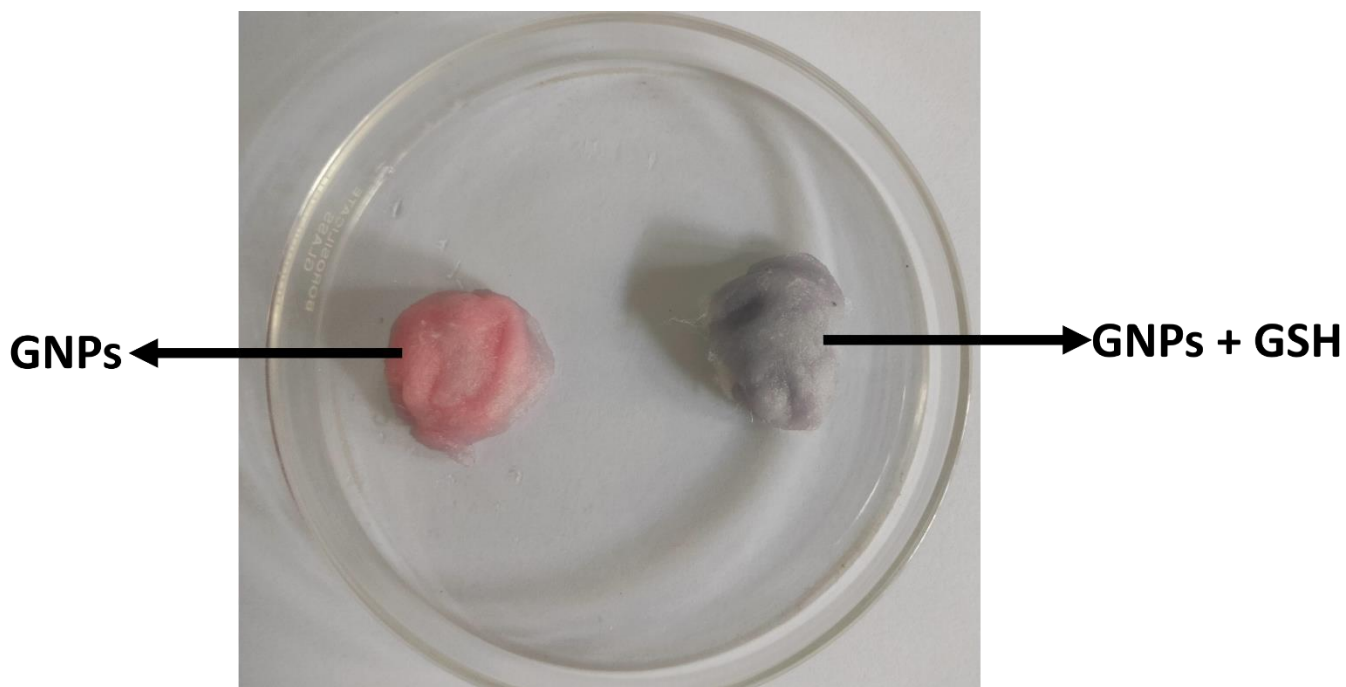


Fig.S12: Practical applicability of GNPs on cotton balls

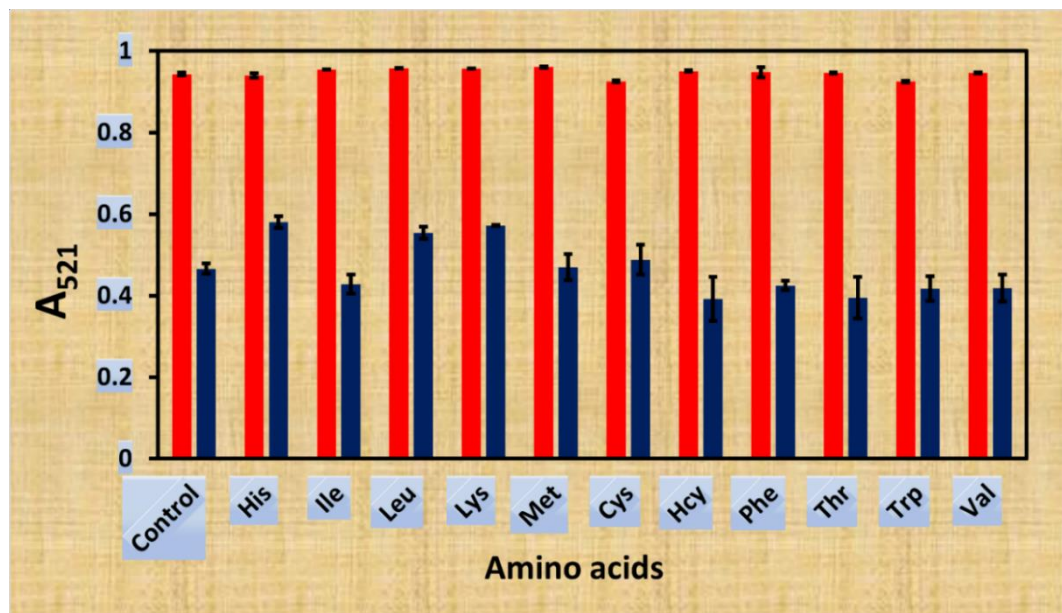


Fig.S13: Interference studies of GNPs at 521 nm with different essential amino acids. Red Bar: GNPs + Amino acids, Blue Bar: GNPs + Amino acids + Glutathione, control: Red Bar: only GNPs, Blue bar: GNPs + GSH

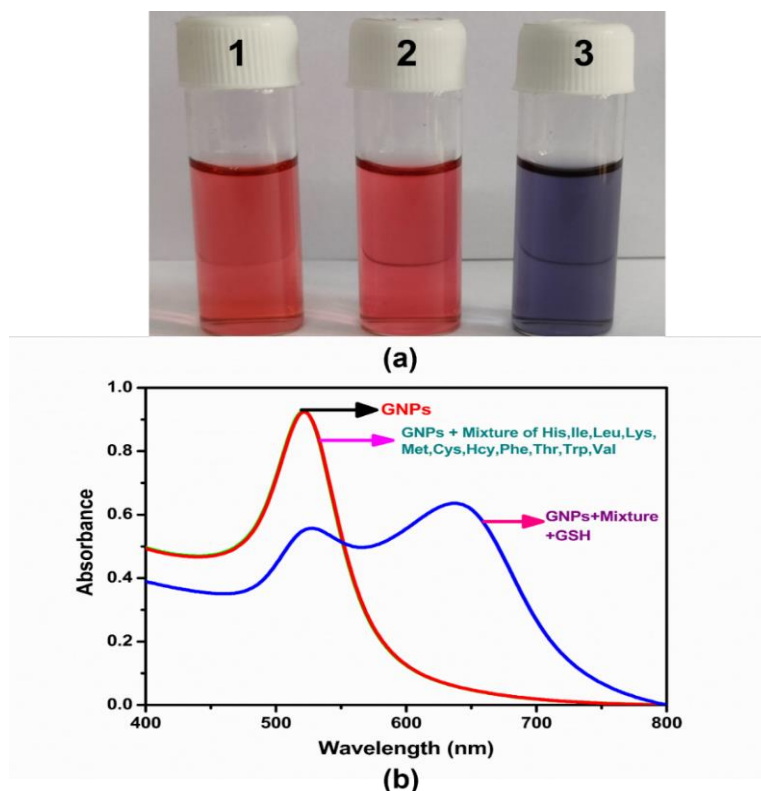


Fig.S14: Matrix study: (a) Visual responses of (1) GNPs (2) GNPs + Mixture (solution) containing different amino acids i.e. His, Ile, Leu, Lys, Met, Cys, Hcy, Phe, Thr, Trp, Val, (3) GNPs + Mixture + Glutathione, (b) Corresponding UV-Visible response

Fig.S15: Comparison table for Limit of Detection;

Detection System	Methods	LOD	References
Citrate capped AuNPs	Colorimetric	0.5 μM	1
Diazo capped AgNPs	Colorimetric	4.11 μM	2
FeS ₂ nanoparticles	Colorimetric	0.15 μM	3
graphene nanoribbons/silver nanoparticles (GNR/Ag NPs) hybrid	Colorimetric	0.23 μM	4
Phloroglucinol capped AgNPs	Colorimetric	3.1 μM	5
Silica nanoparticle–gold nanocluster (MSN–AuNC)	Colorimetric	0.34 μM	6
Glucosamine capped GNPs	Colorimetric	0.26 μM	Present study

1. B. Hu, X. Cao and P. Zhang, *New J. Chem.*, 2013, **37**, 3853.
2. I. Sanskriti and K. K. Upadhyay, *New J. Chem.*, 2017,**41**, 4316-4321.
3. C. Song, W. Ding, W. Zhao, H. Liu, J. Wang, Y. Yao, C. Yao, *Biosens. and Bioelectron.*, 2020, **151**, 111983,
4. S. Rostami, A. Mehdinia, R. Niroumand, A. Jabbari, *Anal. Chim. Act.*, 2020, **1120**, 11-23.
5. A. K. Singh, R. Singh, M. Yadav, M. Sharma, I. Tiwari, and K. K. Upadhyay, *New J. Chem.*, 2023,**47**, 10842-10848
6. G. Zhang, M. Xiang R. M. Kong and F. Qu, *Analyst*, 2020,**145**, 6254-6261

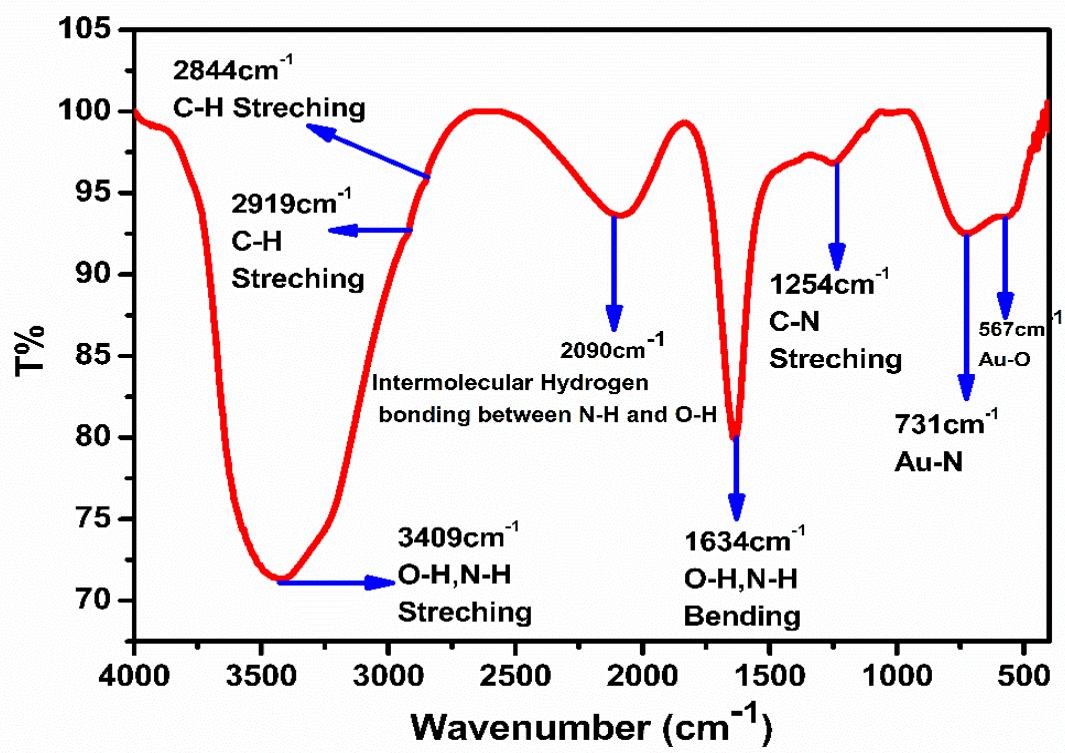
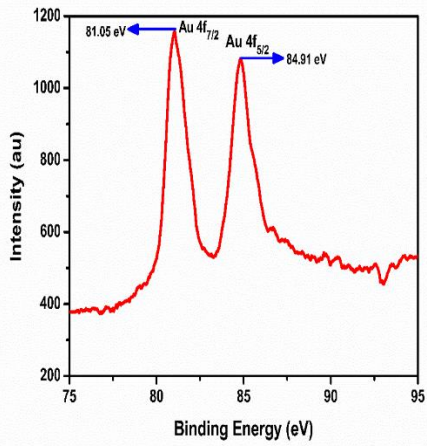
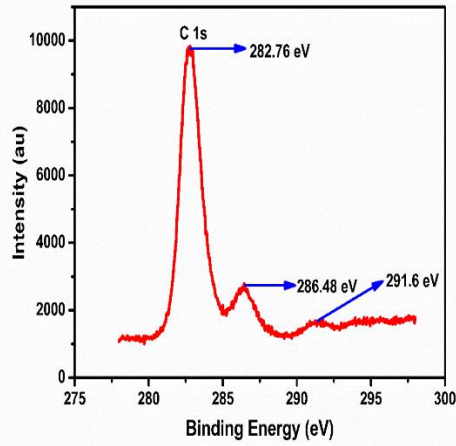


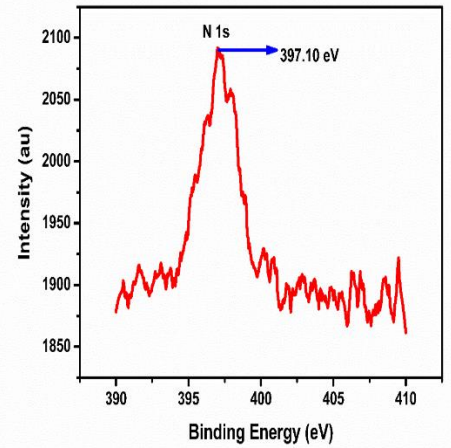
Fig.S16: FT-IR spectrum of GNPs



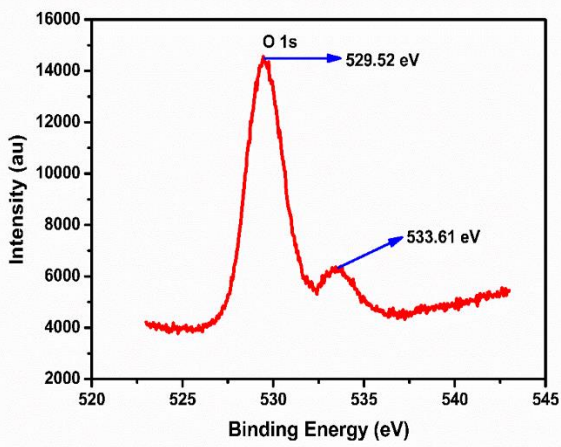
(a)



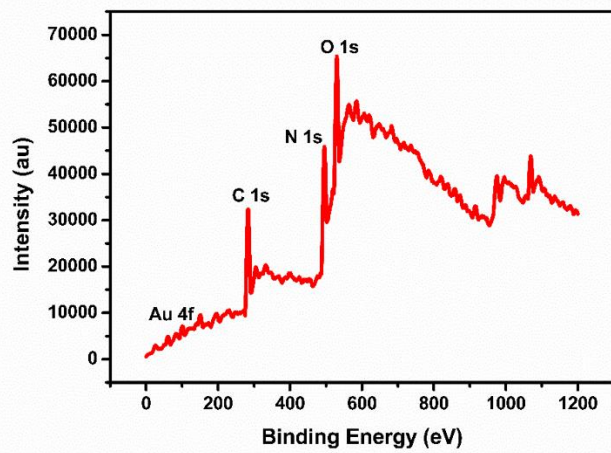
(b)



(c)



(d)



(e)

Fig.S17: XPS studies for GNPs, (a) Spectrum of Au 4f, (b) Spectrum of C 1s, (c) Spectrum of N 1s, (d) Spectrum of O 1s, (e) Survey spectrum of GNPs

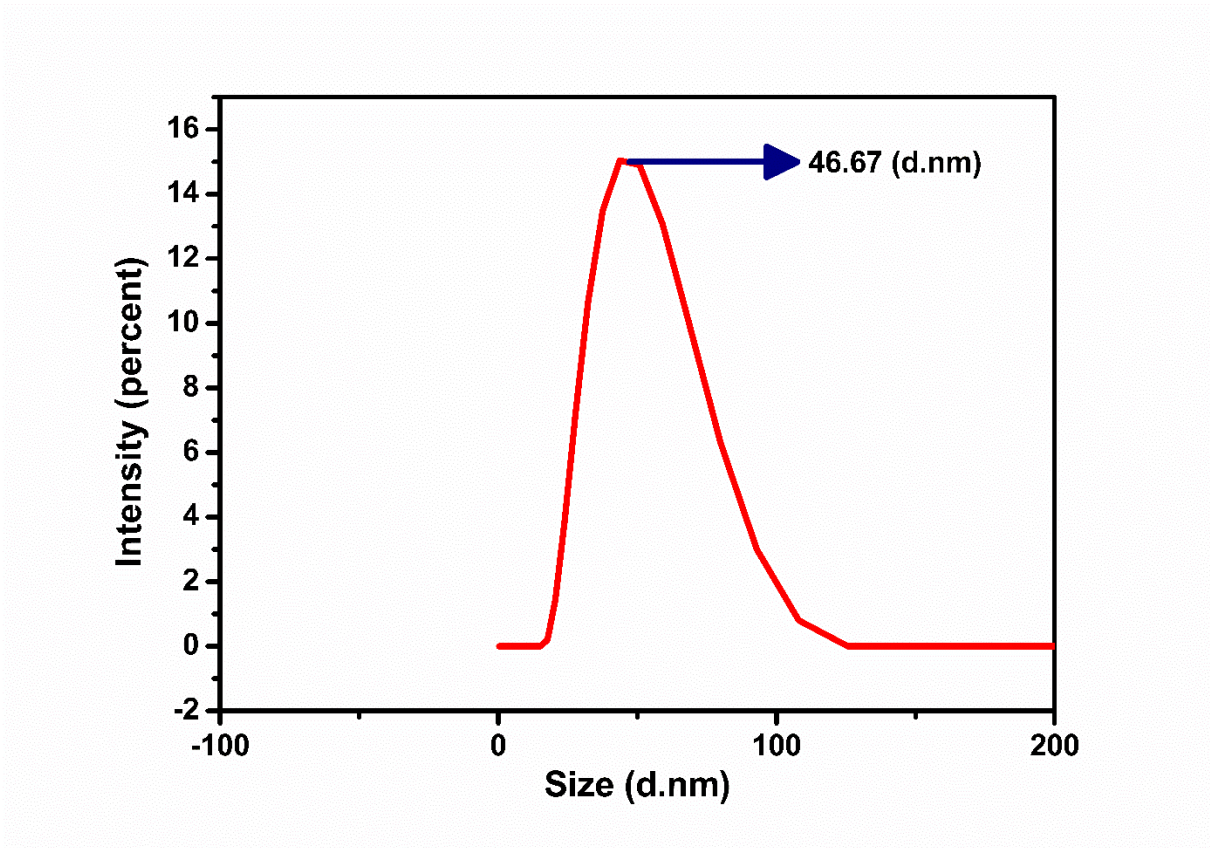


Fig.S18: Dynamic Light Scattering (DLS) study showing hydrodynamic diameter of GNPs

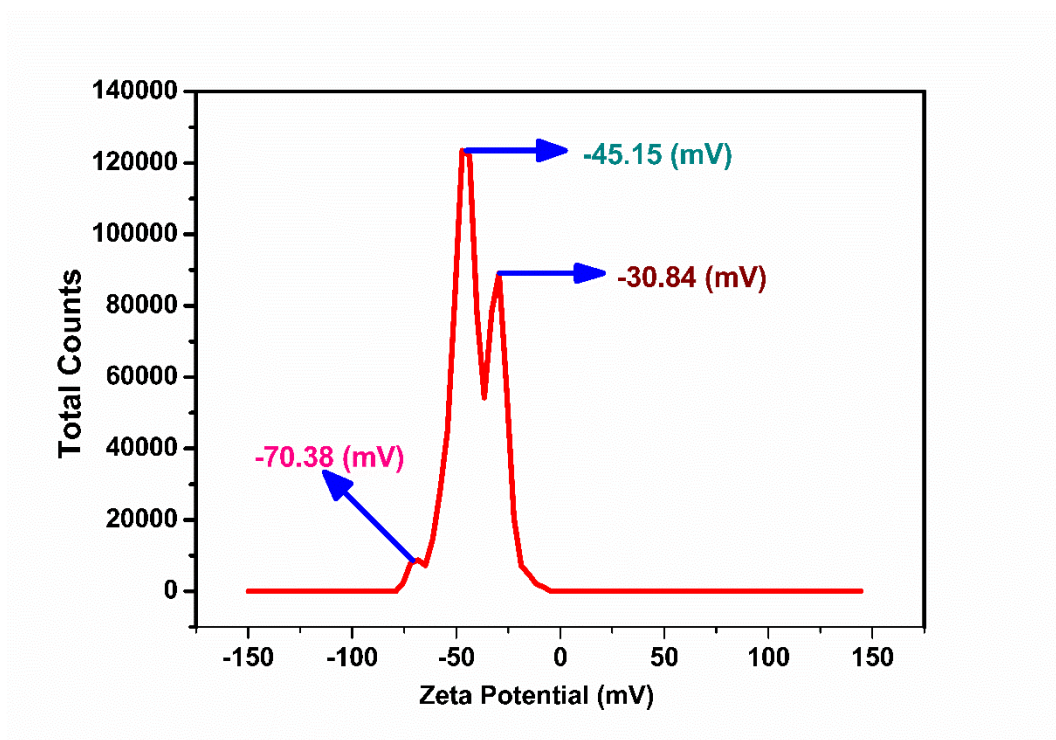


Fig.S19: Zeta Potential of GNPs

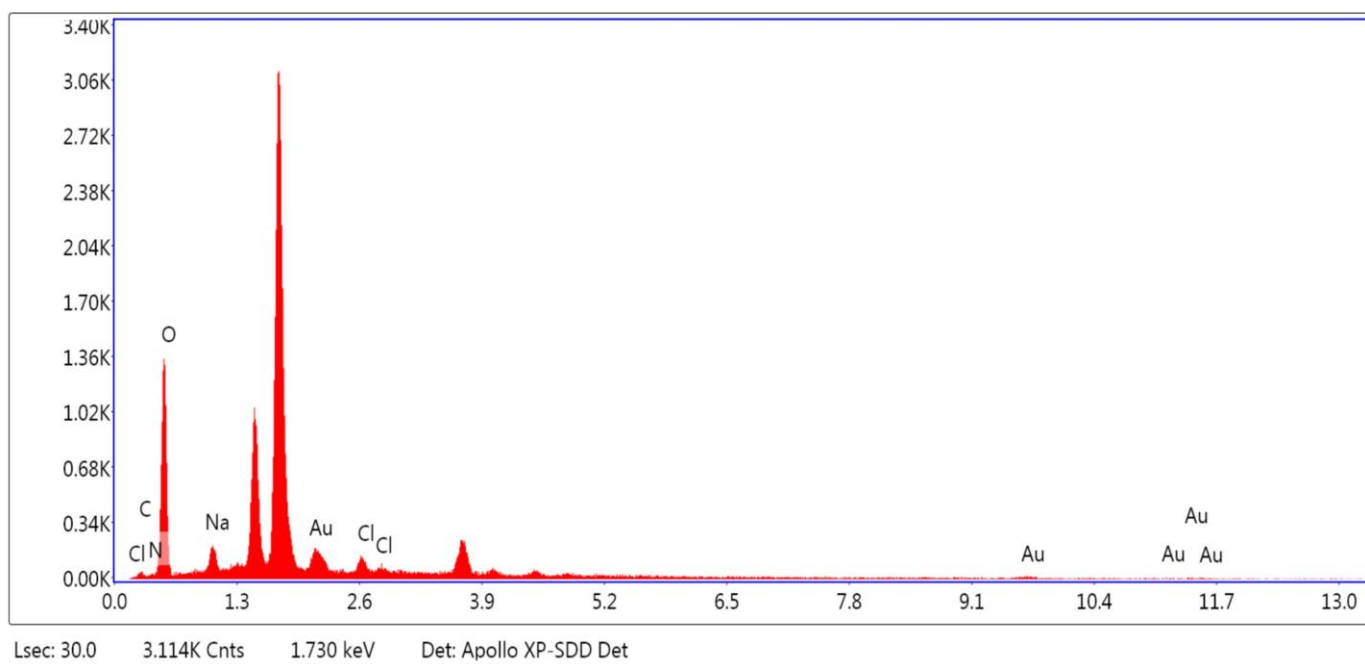


Fig.S20: EDAX spectrum of GNPs

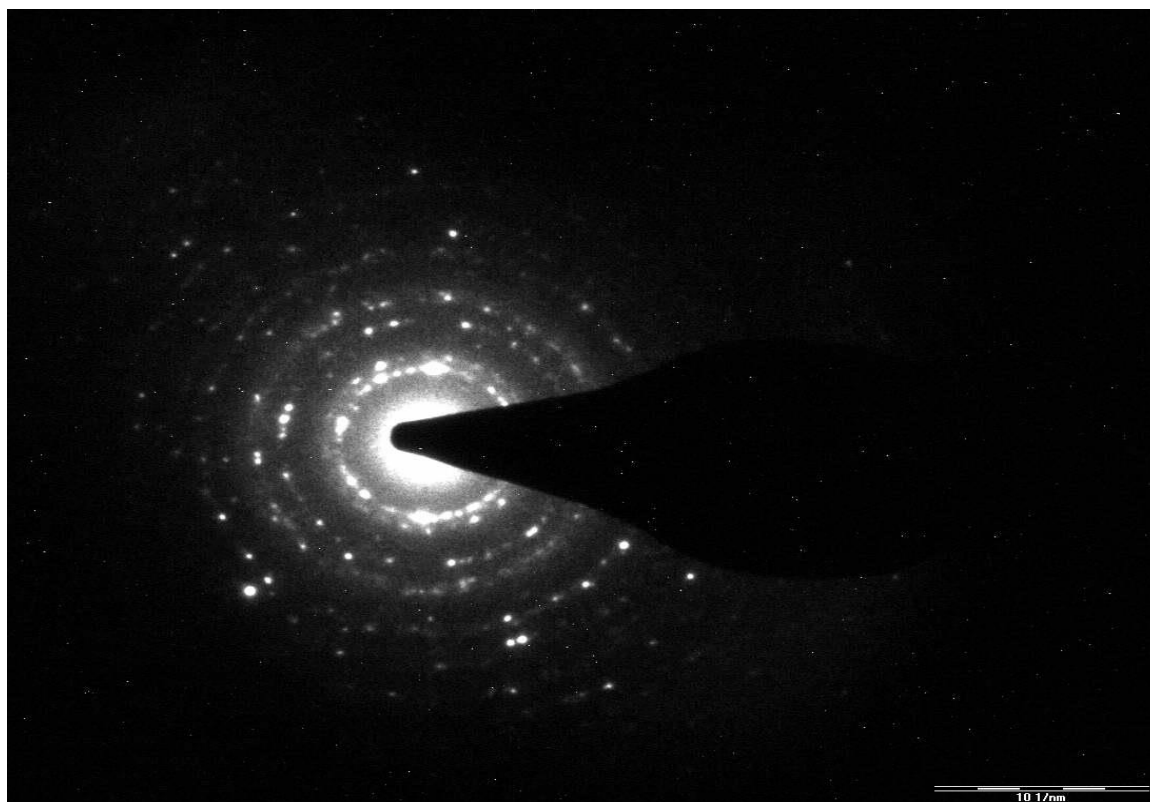
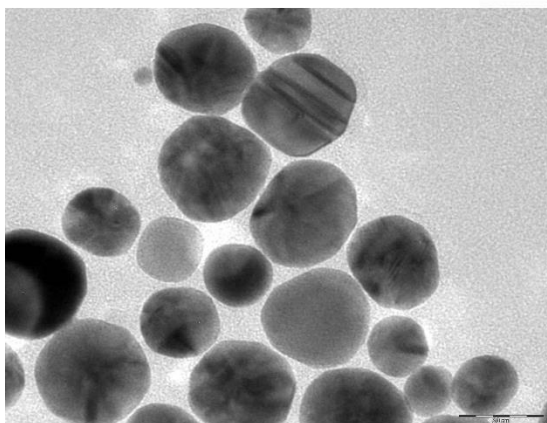
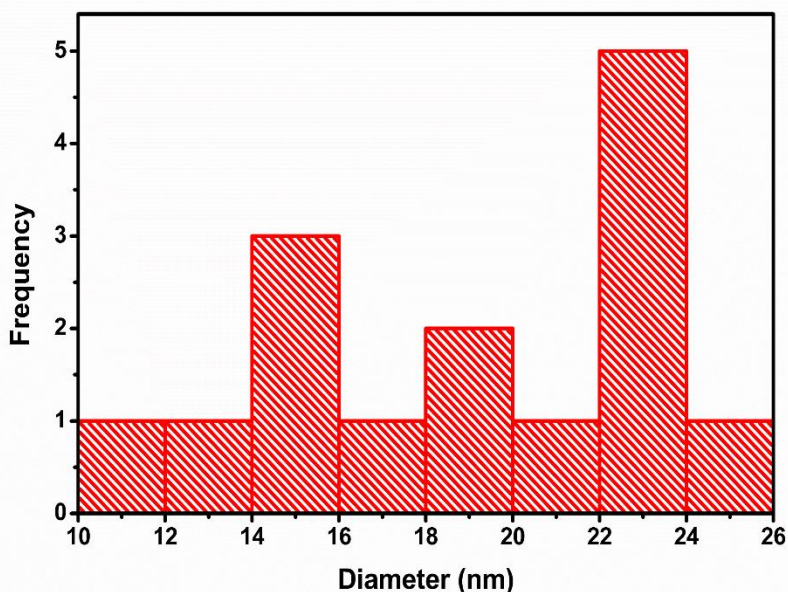


Fig.S21: SAED pattern of GNPs



(a)



(b)

(c) Concentration of GNPs

Concentration of spherical gold nanoparticles (GNPs) can be established by using the equation $C = N_{\text{Total}} / NVN_A$

where N_{Total}/NVN_A where N_{Total} is the total number of gold atoms in the reaction solution, N is the number of gold atoms present in each nanoparticle, V is volume of solution in litres and N_A is Avogadro constant. Herein, we have used 2mL of 8mM Gold (III) chloride trihydrate in a total volume of 50mL.

d= 23 nm GNPs

$$N = 31d^3 = 31 \times (23)^3 = 377,177$$

$$\text{Concentration of GNPs} = N_{\text{Total}} / NVN_A$$

$$= \frac{0.016 \times 10^{-3} \times 6.02 \times 10^{23}}{377,177 \times 0.05 \times 6.02 \times 10^{23}}$$

$$= 8.4 \times 10^{-9} \text{ M}$$

$$= 8.4 \text{ nM}$$

Fig.S22: (a) TEM image of GNPs used for preparation of histogram chart [at 50 nm scale bar] (b) Histogram showing size distribution of GNPs, (c) Concentration of GNPs

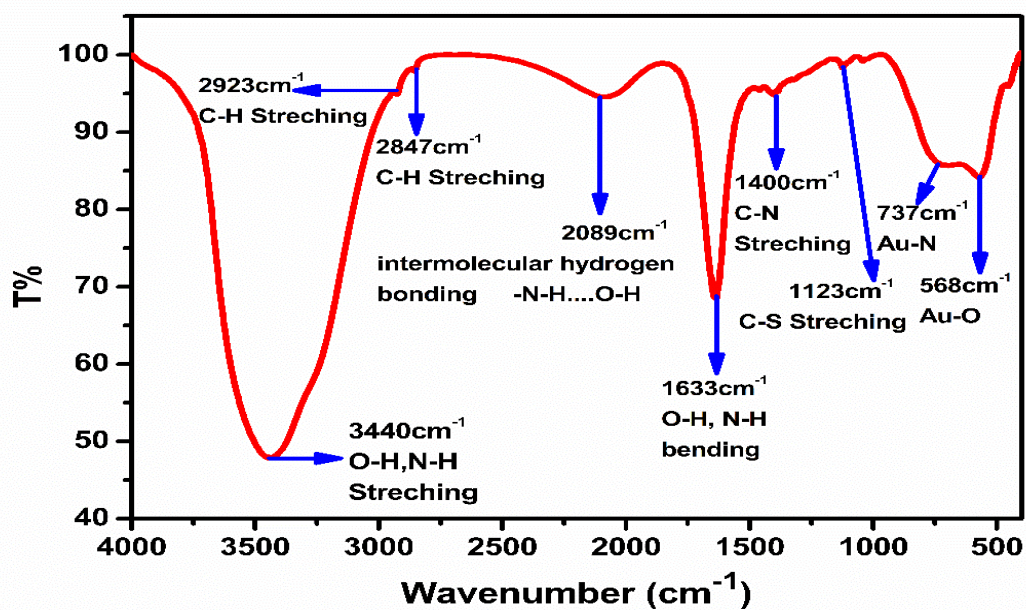


Fig.S23: FT-IR spectrum of GNPs after interaction with Glutathione

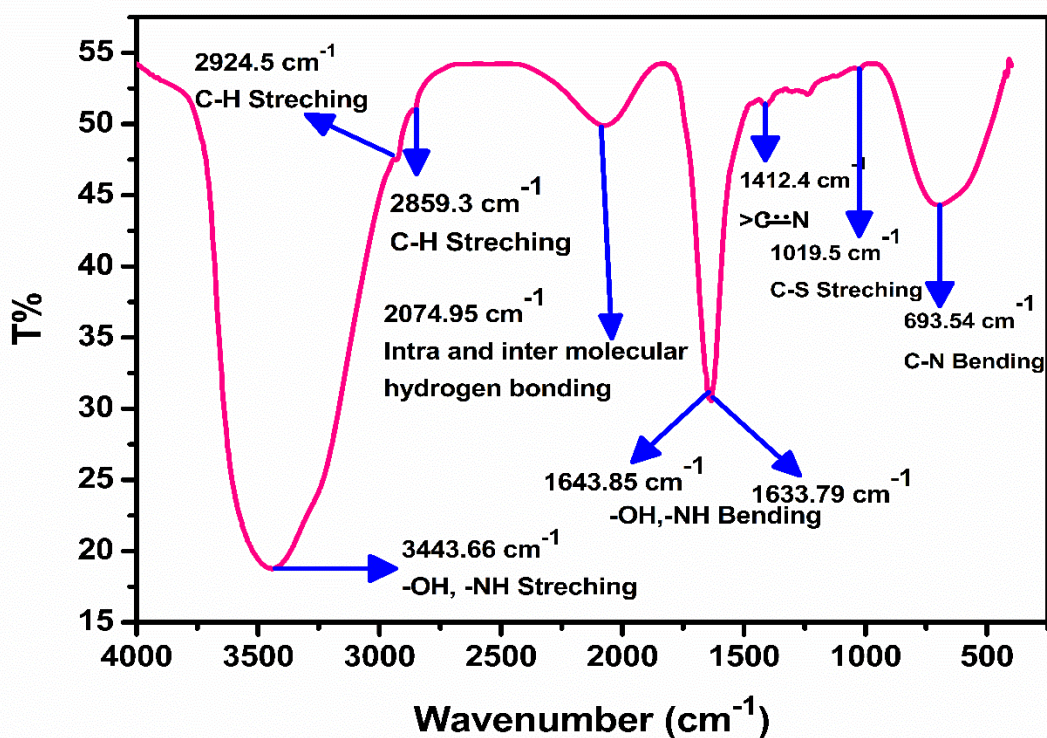


Fig.S24: FT-IR spectrum of GSH in experimental condition

Fig. S25: Comparison table for IR spectra;

S. No.	Samples	Peaks (cm ⁻¹)
1.	GNPs	3409, 2919, 2844, 2090, 1634, 1254, 731, 567
2.	GNPs with GSH	3440, 2923, 2847, 2089, 1633, 1400, 1123, 737, 568
3.	Only GSH	3443.66, 2924.5, 2859.3, 2074.95, 1643.85, 1633.79, 1412.4, 1019.5, 693.54

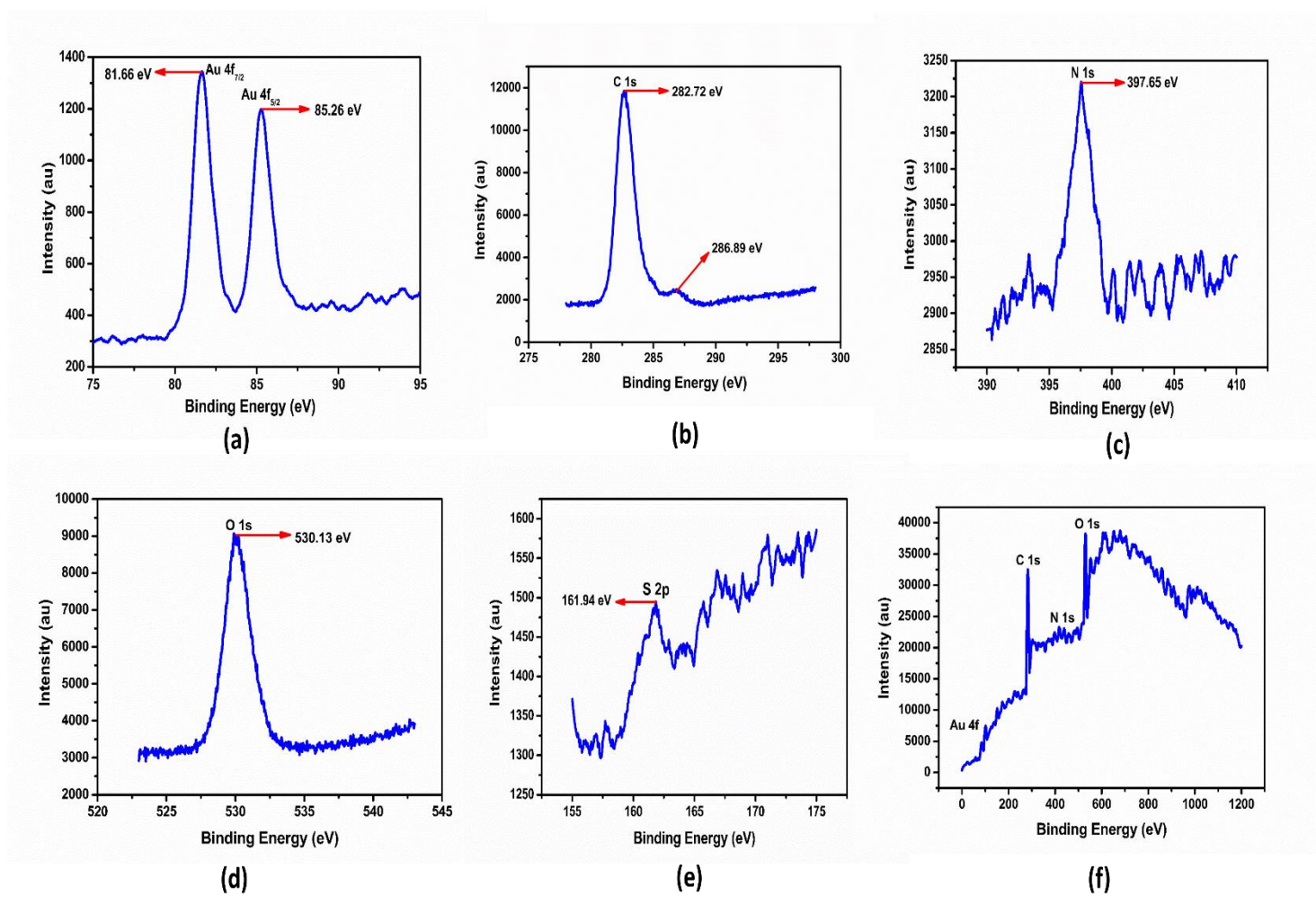


Fig.S26: XPS studies of GNPs after addition of GSH, (a) Spectrum for Au 4f, (b) Spectrum for C 1s, (c) Spectrum for N 1s, (d) Spectrum for O 1s, (e) Spectrum for S 2p, (f) Survey spectrum of GNPs after addition of GSH

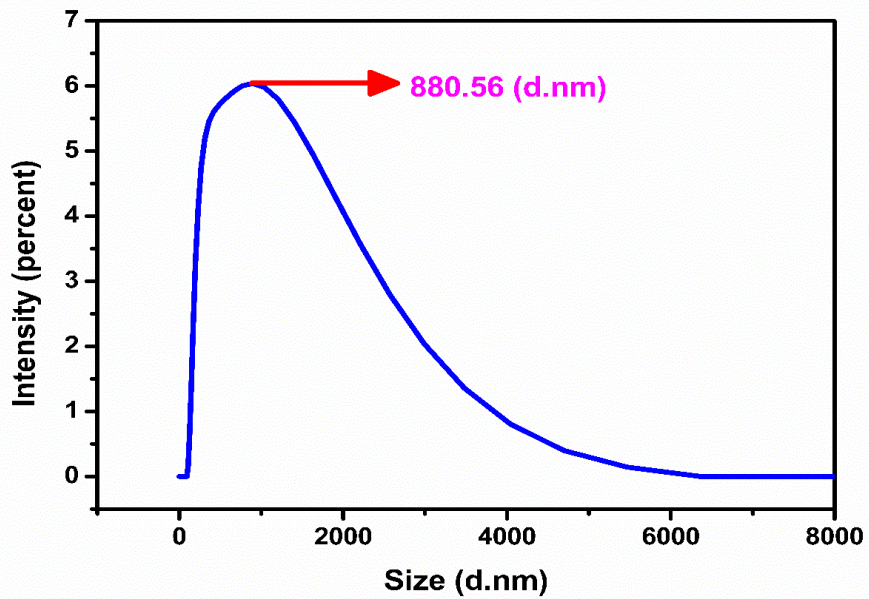


Fig.S27: Dynamic Light Scattering (DLS) study showing hydrodynamic diameter of GNPs after addition of Glutathione

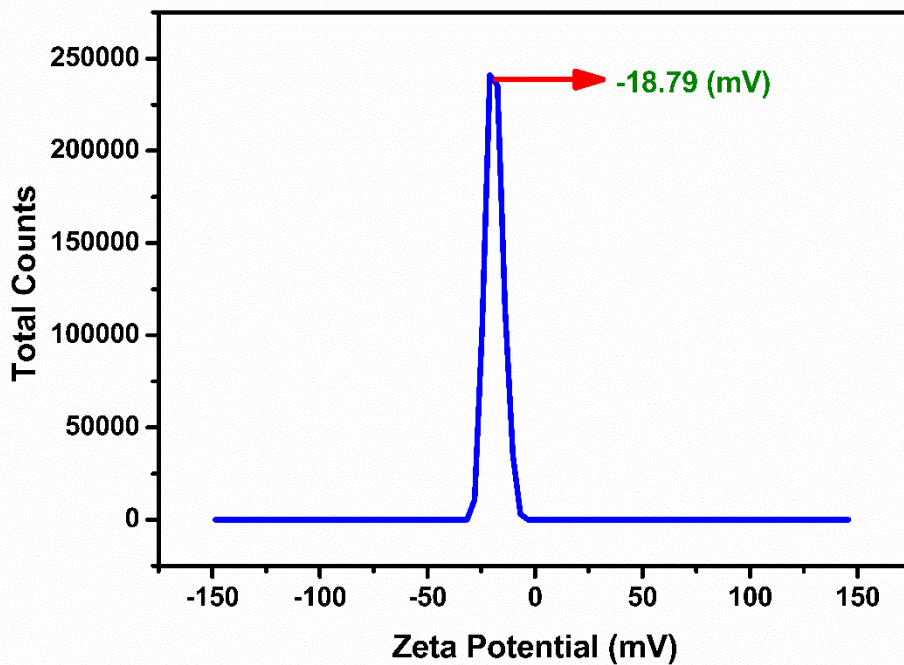


Fig.S28: Zeta Potential of GNPs after addition of Glutathione

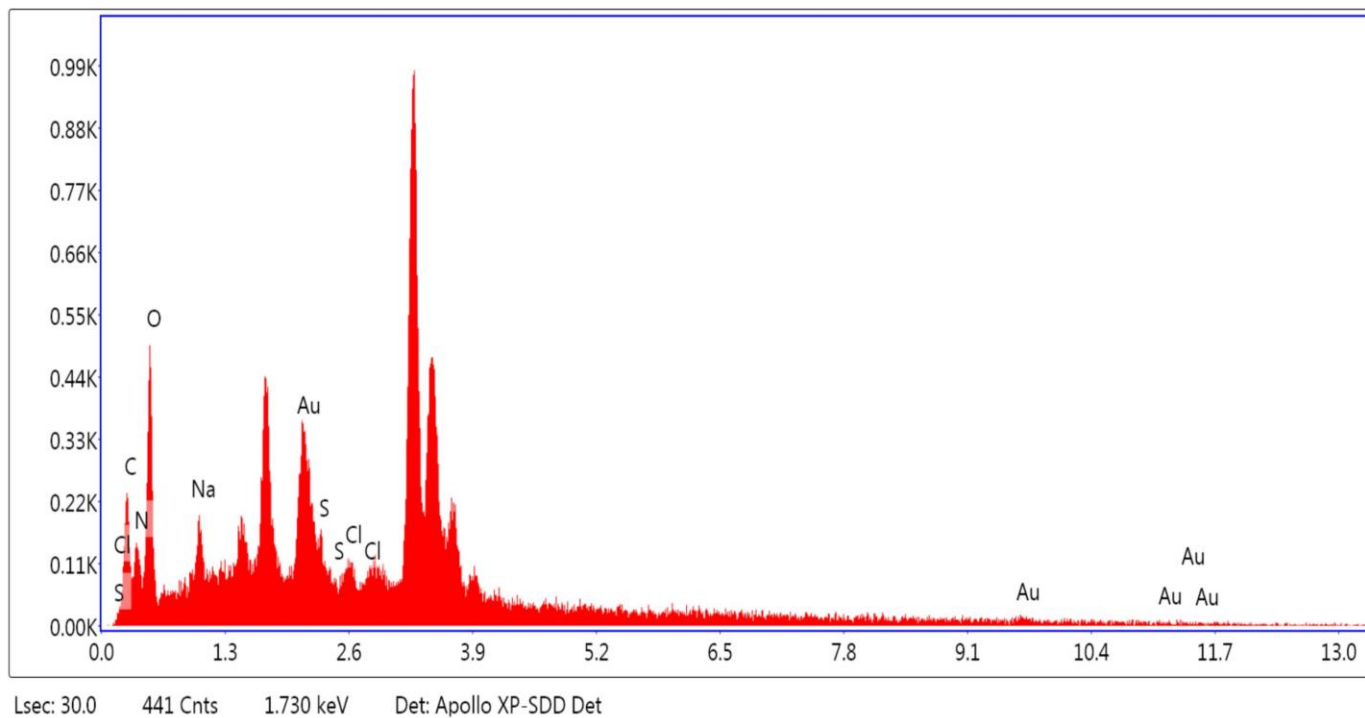


Fig.S29: EDAX spectrum of GNPs after intraction with Glutathione

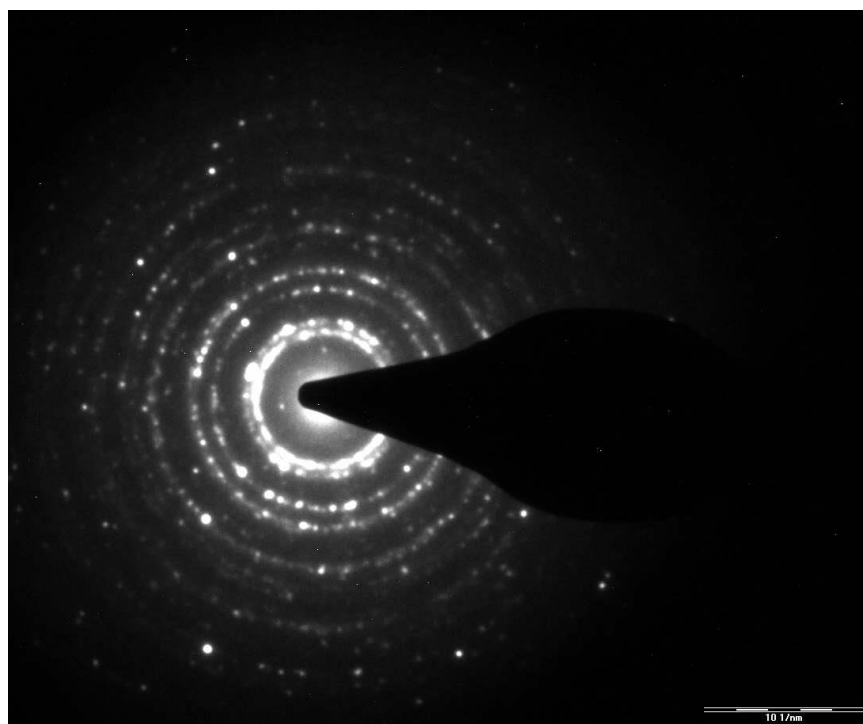


Fig.S30: SAED pattern of GNPs after addition with Glutathione

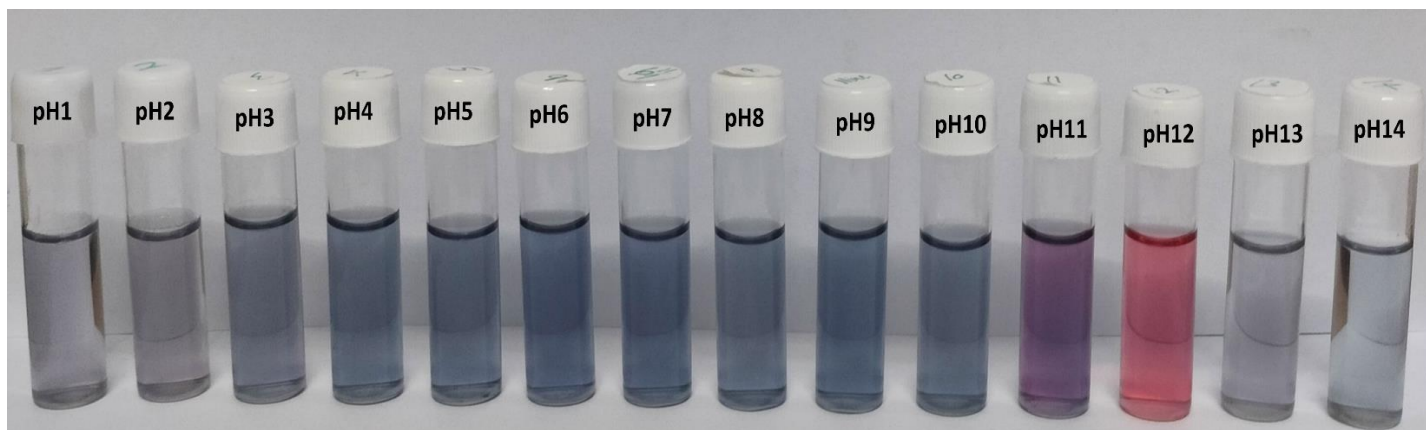


Fig.S31: pH Study: Visual response of pH metric titration of GNPs with Glutathione in the pH range (1-14)

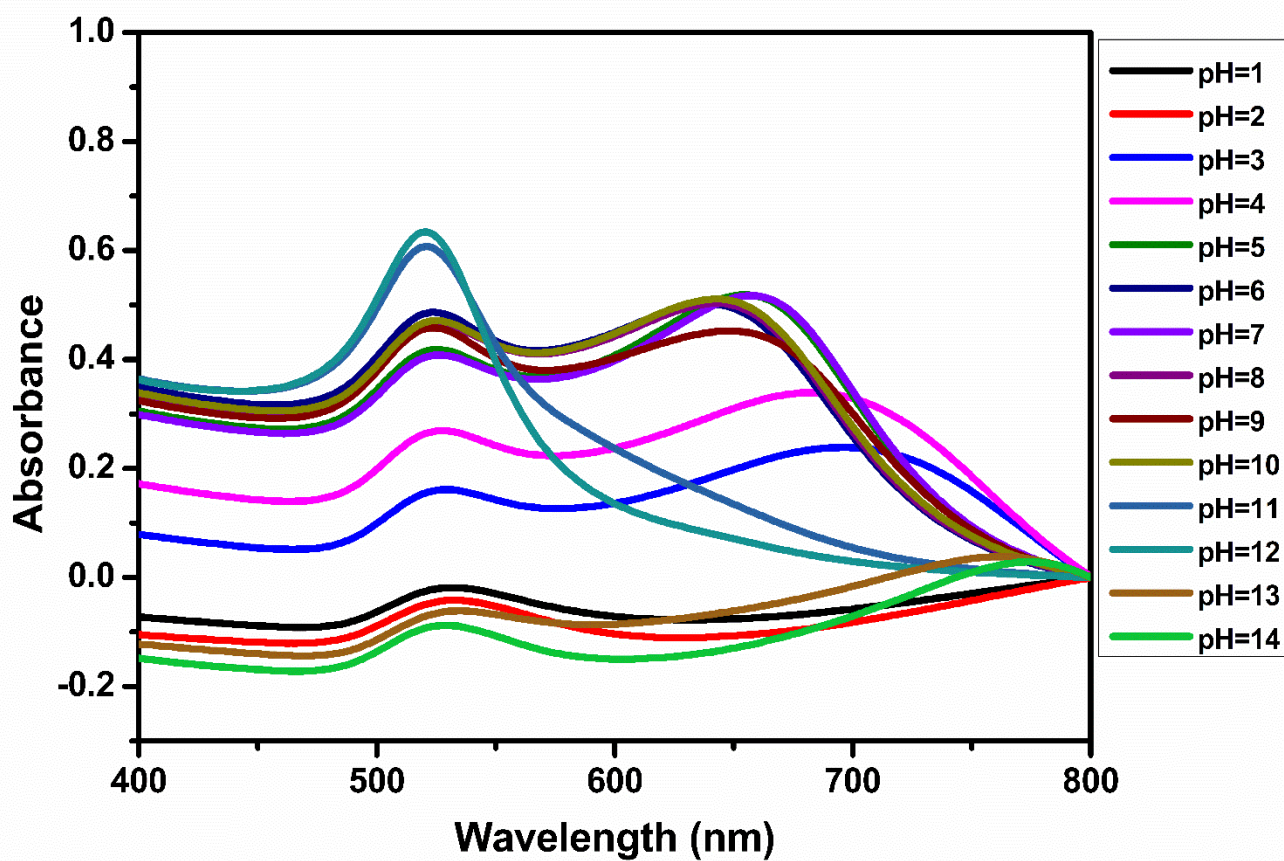


Fig.S32: pH Study: UV-Visible spectra of corresponding pH metric titration of GNPs after with Glutathione in the pH range (1-14)

Fig.S33: Tentative mechanistic pathway for GNPs synthesis;

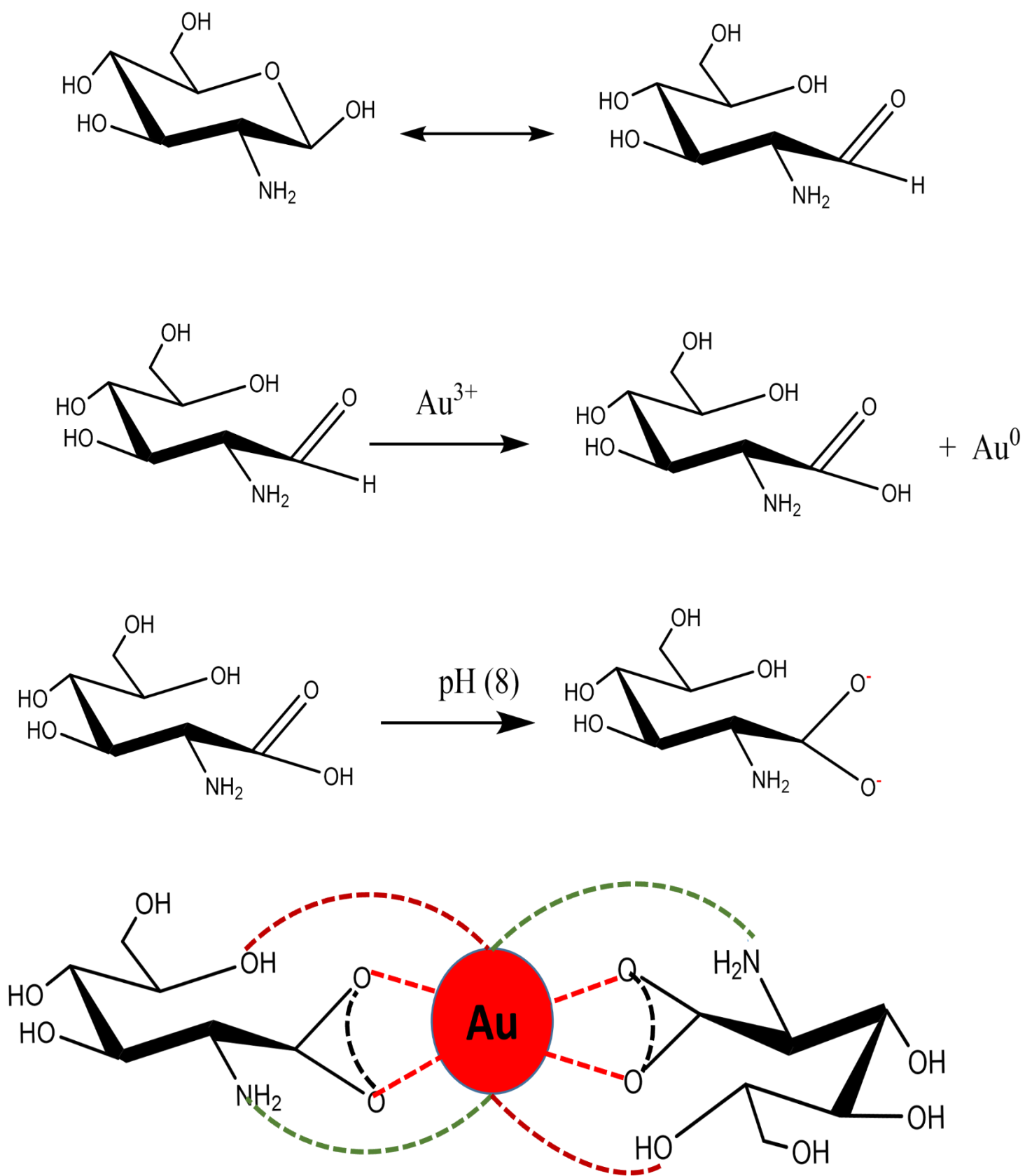


Fig.S34: Mechanistic pathway for GNPs interaction with GSH

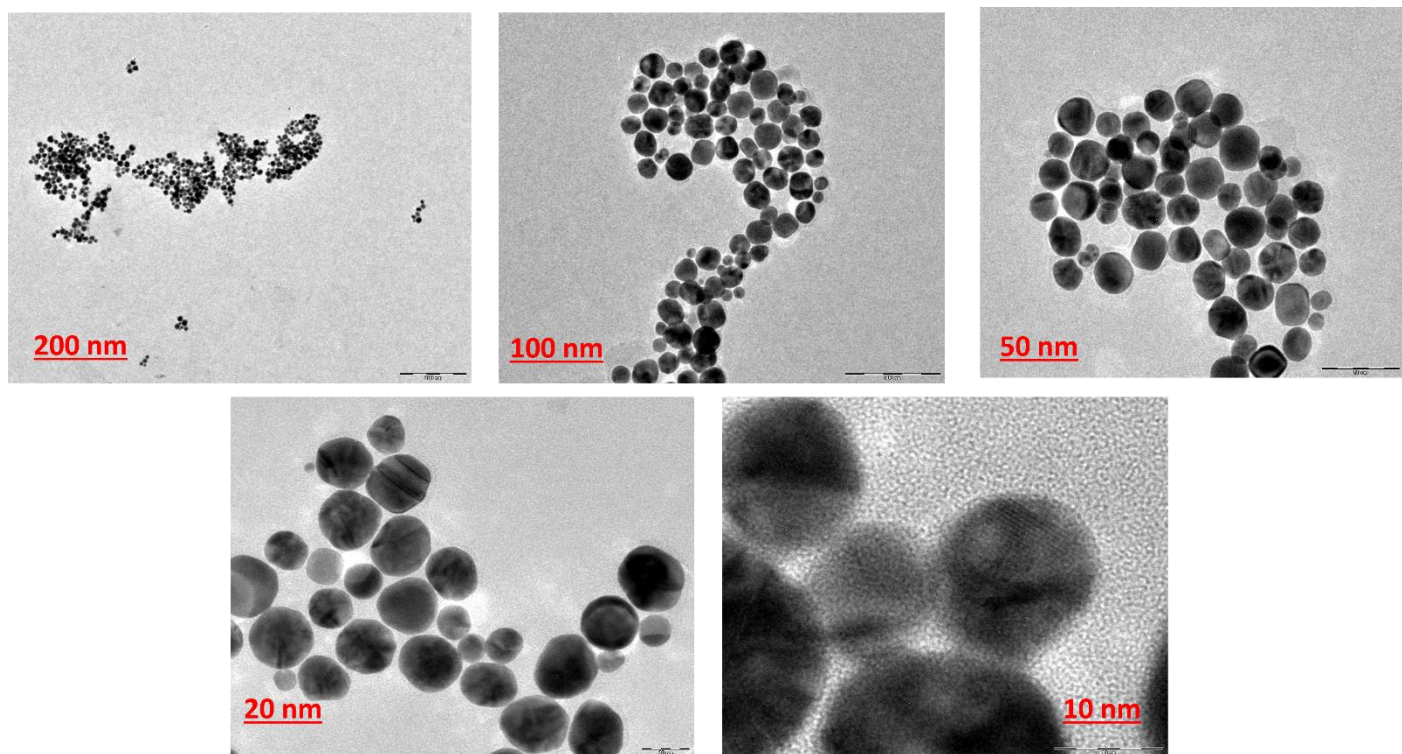
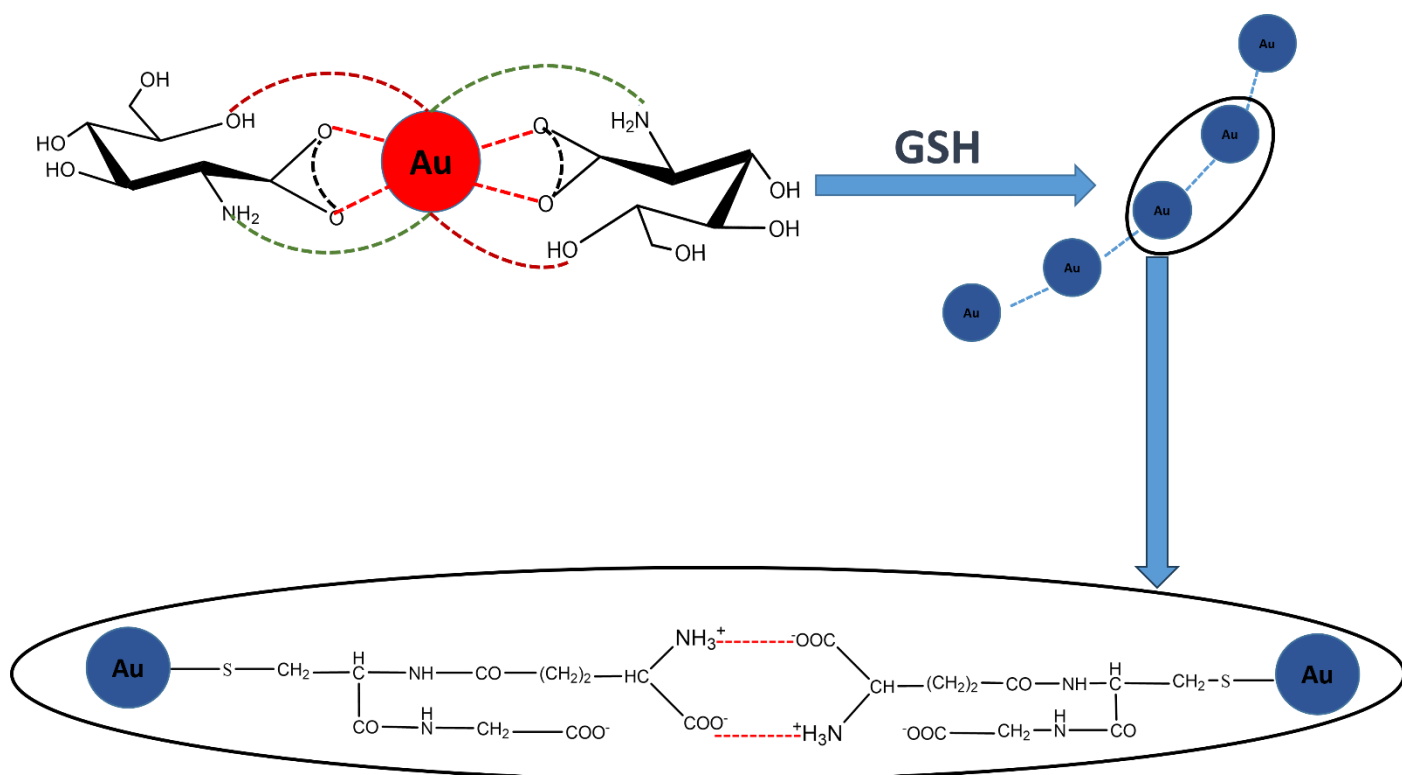


Fig.S35: TEM image of GNPs (From low to high resolution)

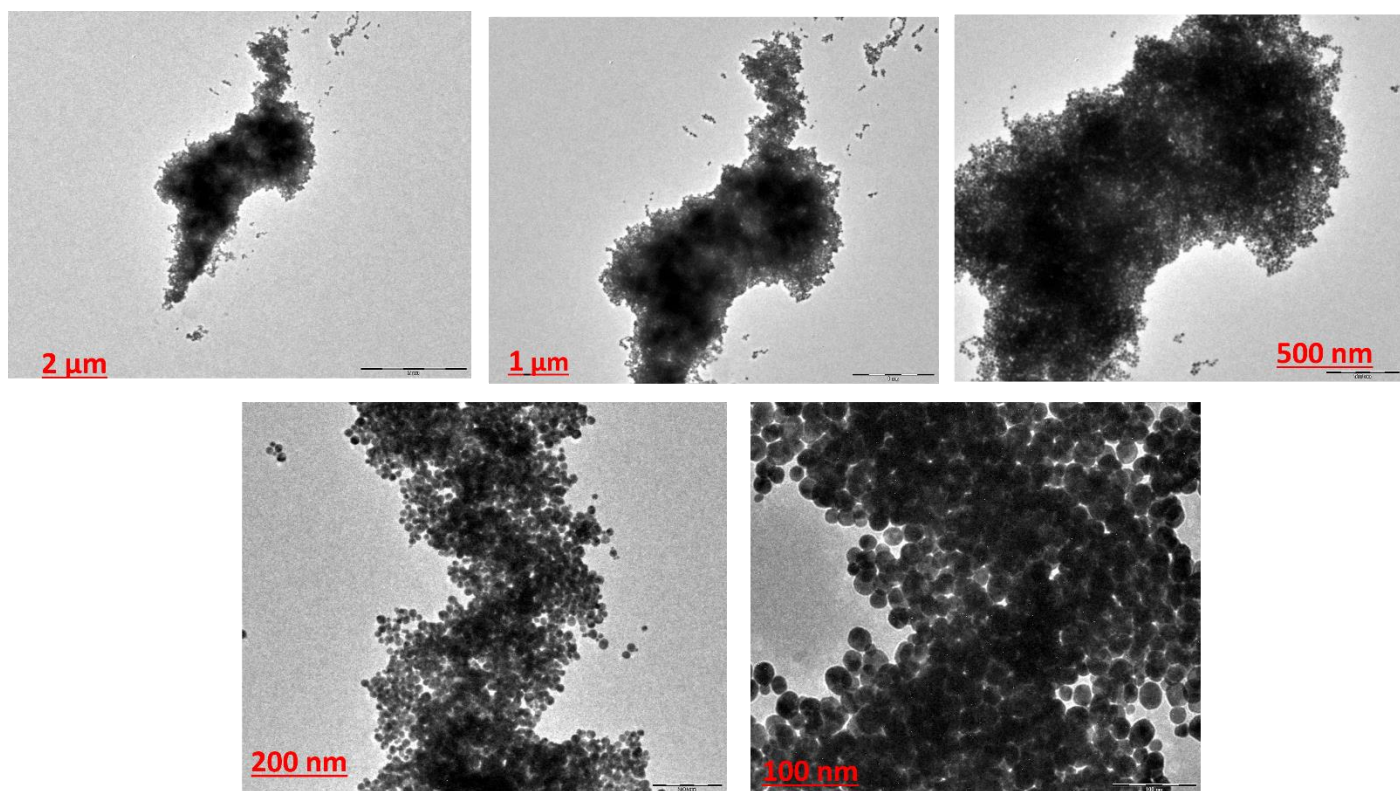


Fig.S36: TEM image of GNPs with Glutathione (from low to high resolution)

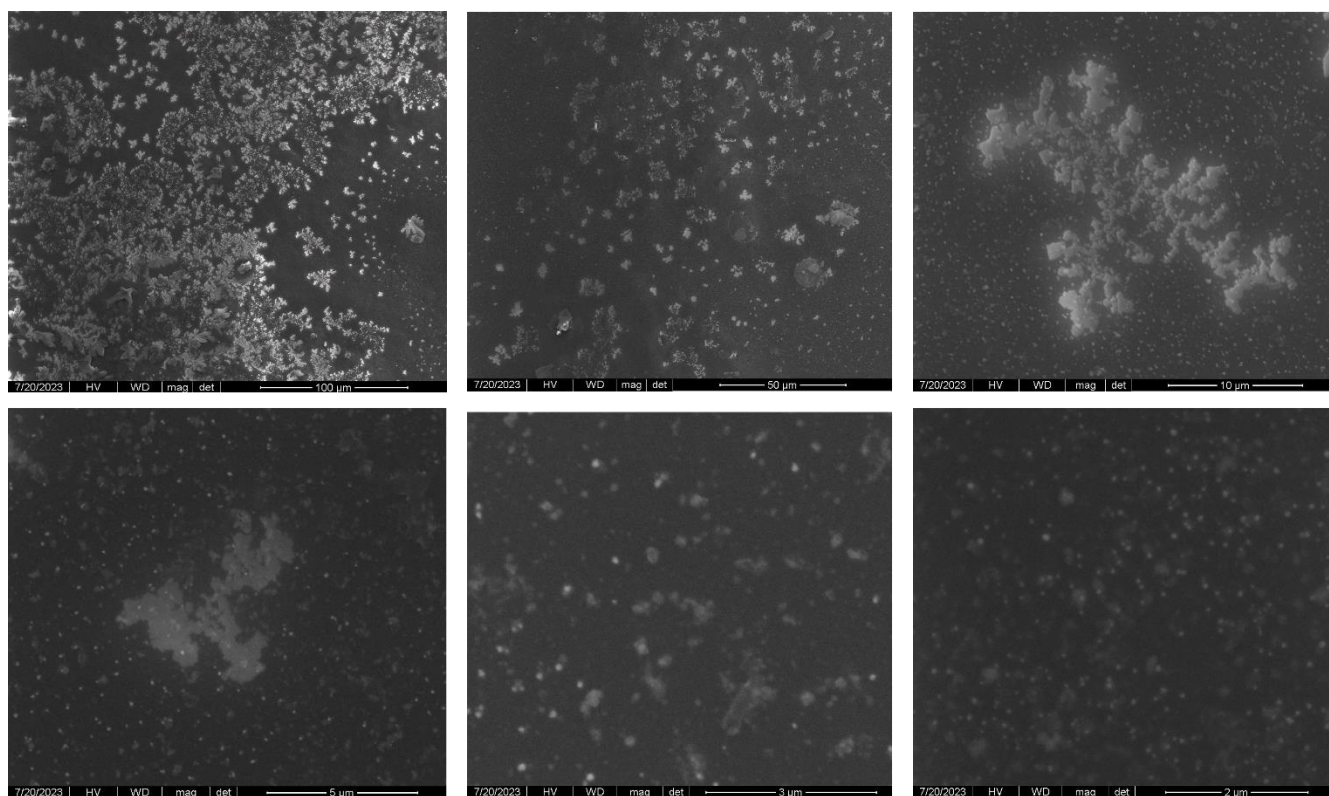


Fig.S37: SEM image of GNPs (from low to high resolution)

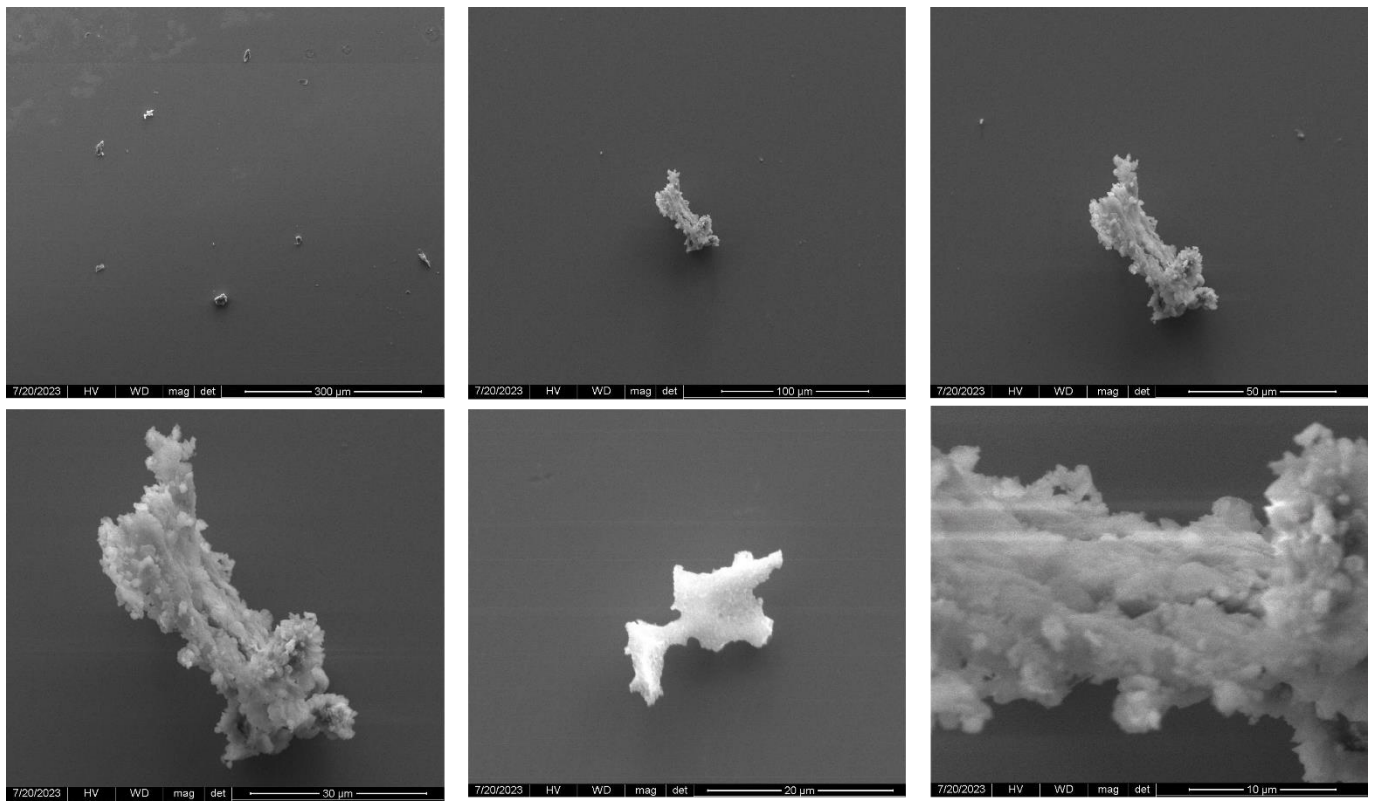
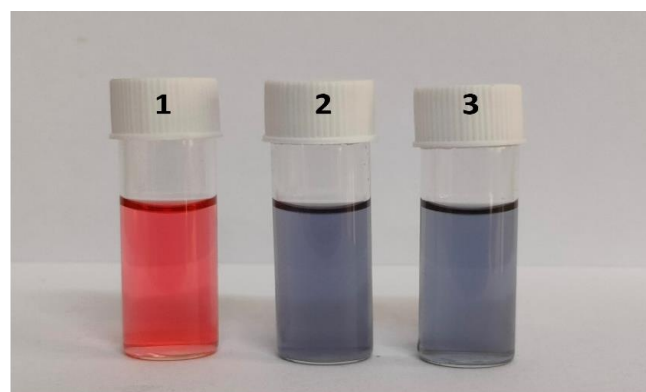
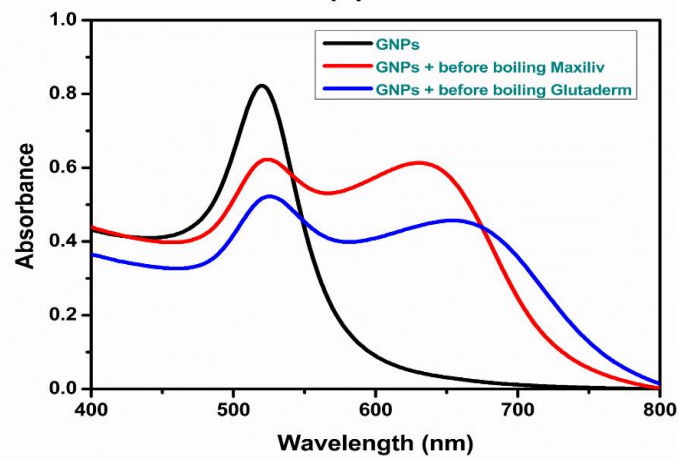


Fig.S38: SEM image of GNPs with Glutathione (from low to high resolution)



(a)



(b)

Fig.S39: (a) Visual response of (1) GNPs (2) GNPs after addition of Maxiliv (without boiling) (3) GNPs after addition of Glutaderm (without boiling) (b) Corresponding UV-Visible spectra

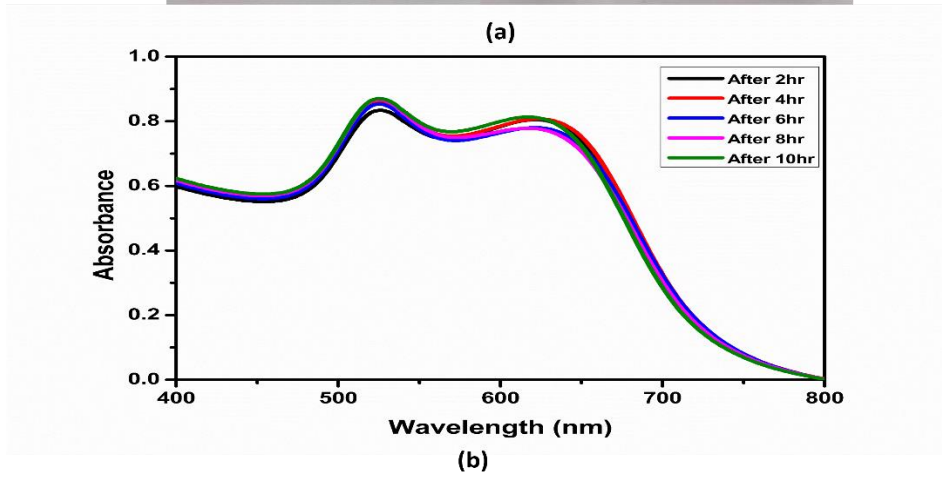
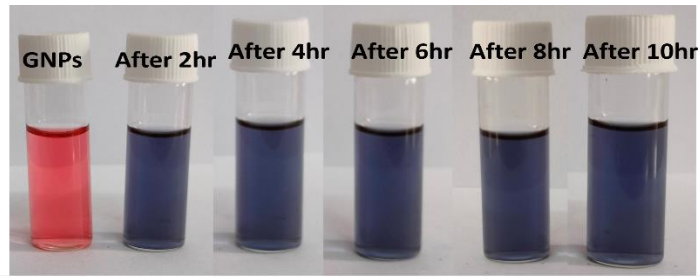


Fig.S40: (a) Visual response of GNPs after addition of Maxilliv at various time intervals (Intra-day repeatability) (b) Corresponding UV-Visible spectra

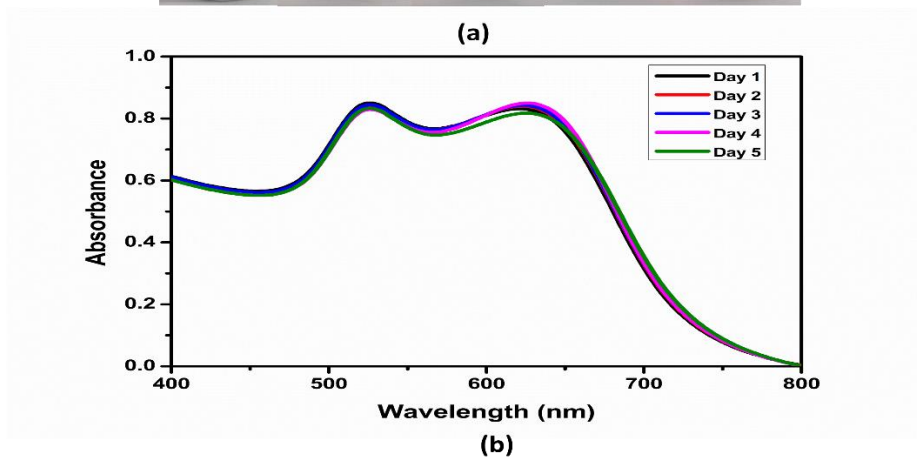
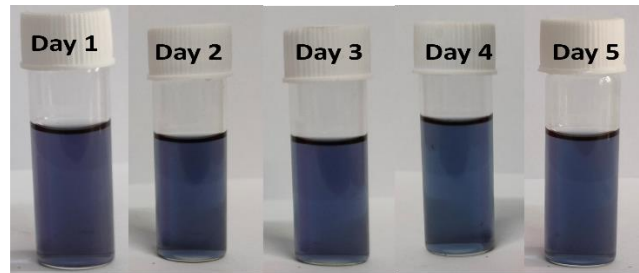
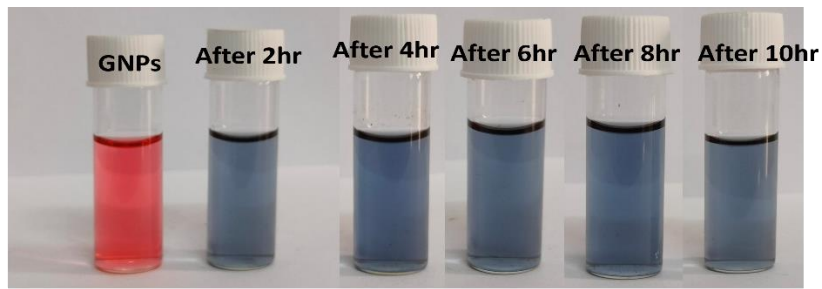
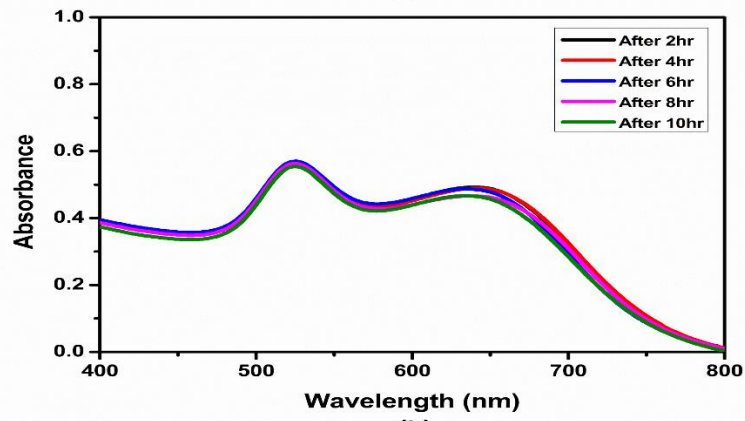


Fig.S41: (a) Visual response of GNPs after addition of Maxilliv over a course of five days intervals (Inter-day repeatability) (b) Corresponding UV-Visible spectra

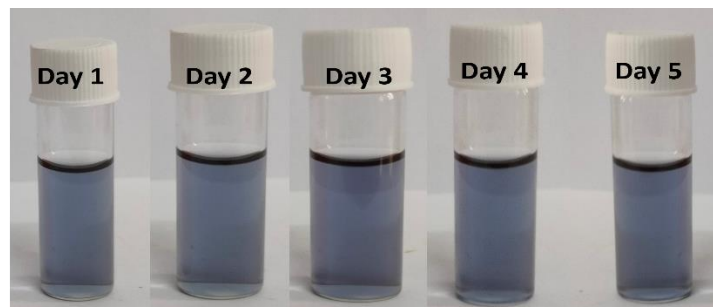


(a)

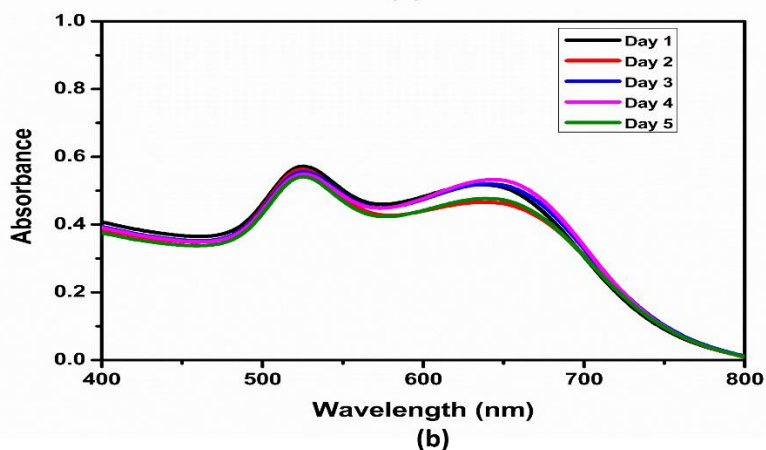


(b)

Fig.S42: (a) Visual response of GNPs after addition of Glutaderm at various time intervals (Intra-day repeatability) (b) Corresponding UV-Visible spectra



(a)



(b)

Fig.S43: (a) Visual response of GNPs after addition of Maxilliv over a course of five days (Inter day repeatability) (b) Corresponding UV-Visible spectra

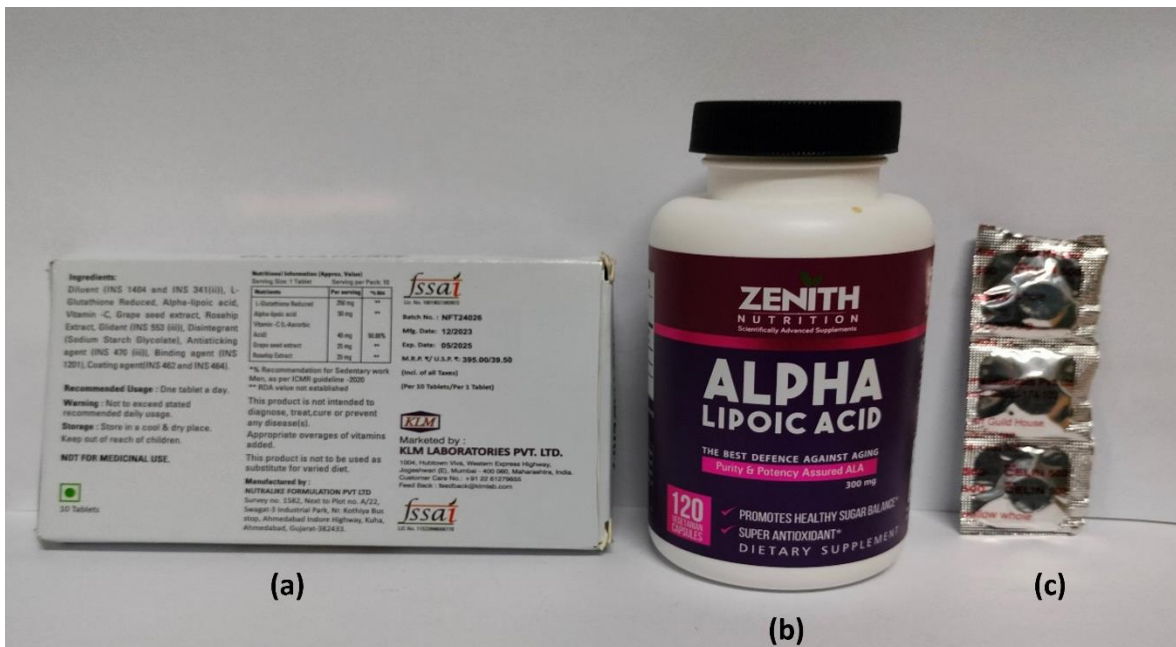


Fig.S44: (a) Image showing the amount of various constituents present in Glutaderm (b) Image showing Alpha lipoic acid used for selectivity study (c) Image showing Vitamin C used for selectivity study

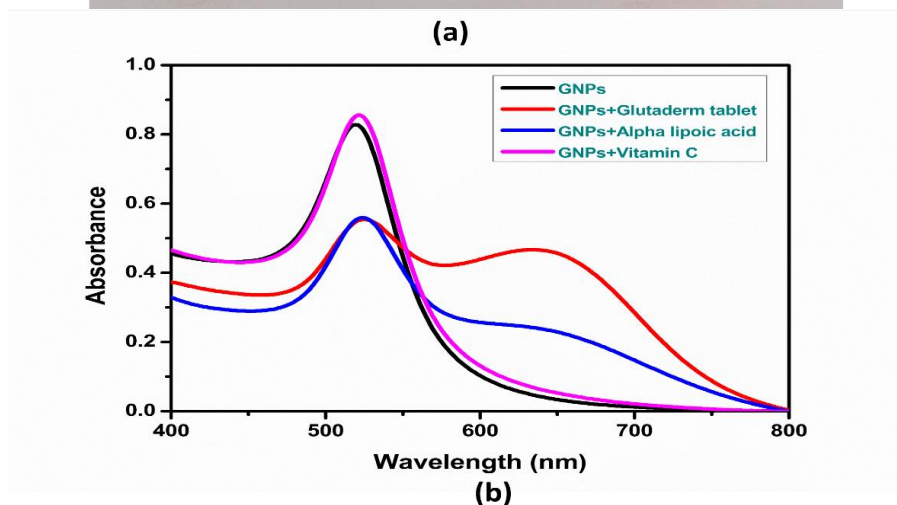
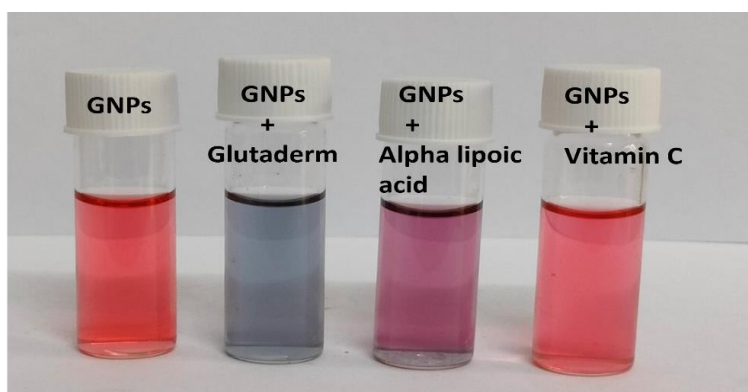


Fig.S45: (a) Visual response of GNP, GNP+Glutaderm, GNP+Alpha Lipoic acid, GNP+Vitamin C (b) Corresponding UV-Visible spectra

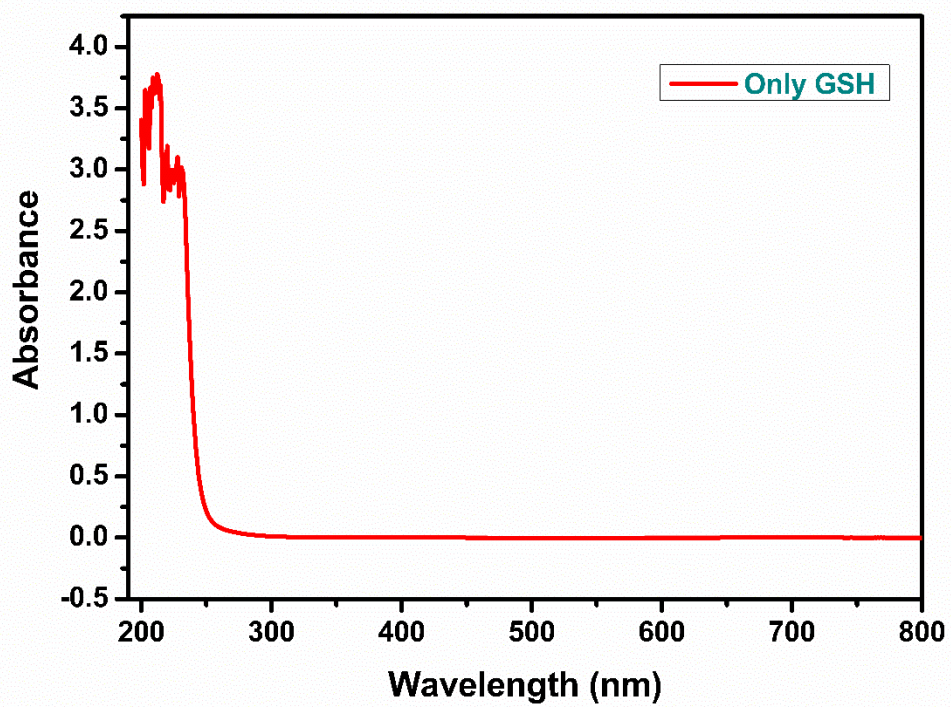


Fig.S46: UV-Visible spectrum of glutathione (GSH) in experimental conditions