Electronic Supplementary Material (ESI) for RSC Advances

Modulating Enzymatic Activity in the Presence of Gold Nanoparticles

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Electronic Supplementary Information

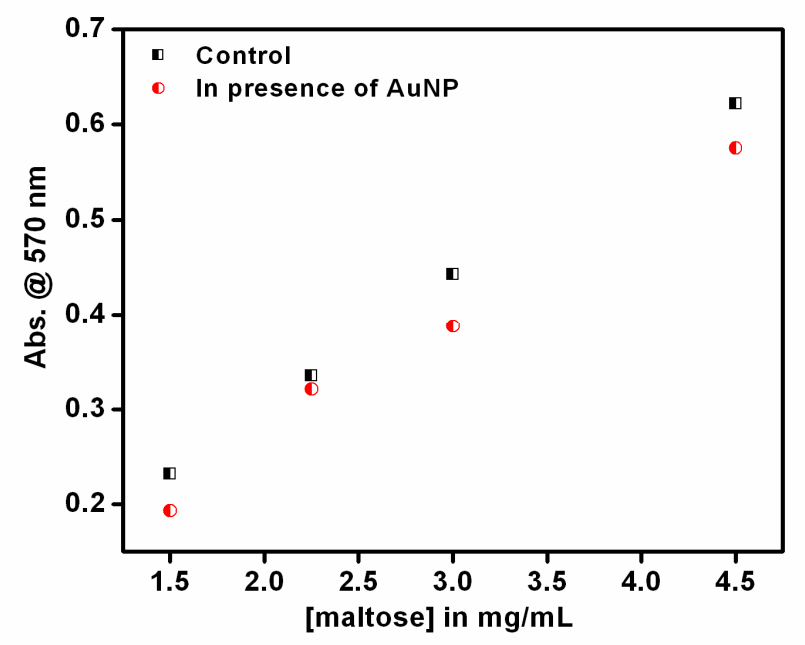


Figure S1: Control experiment performed in absence and presence of cit-Au NP to check whether cit-Au NP interferes with the DNS test, which was negative. The graph shows the absorbance at 570 nm of the UV-vis spectrum of product formed (maltose) after treating with DNS.

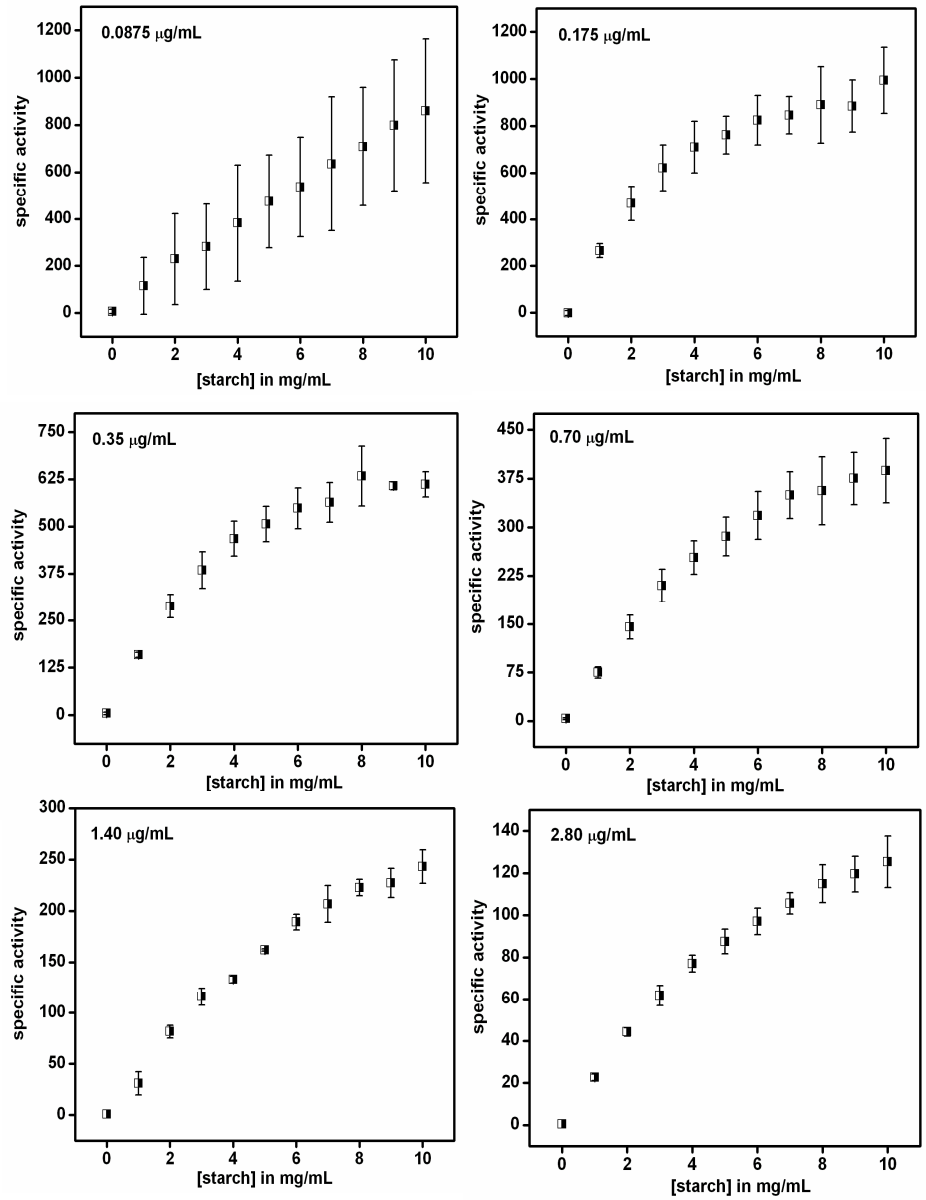
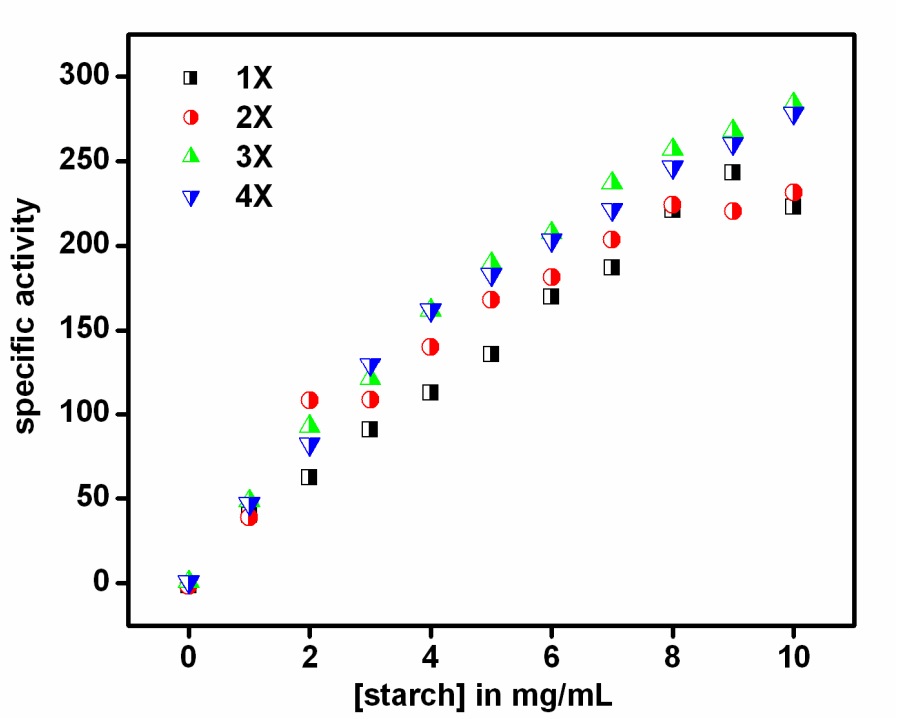


Figure S2: Specific activity of α-amylase (μmol mg^{-1} min^{-1}) in the presence of cit-Au NPs. Legend represents the respective concentration of enzyme at which the experiments were carried out. The results are average of three sets.



	No. of Au NPs in the reaction mixture	V^a_{max}/ (10^2 µmol mg^{-1} min^{-1})	K^a_m / (mg mL^{-1})	k^a_{cat} / (10^4 min^{-1})
1X	8.0×10^{12}	4.00	10.00	2.00
2X	4.0×10^{12}	8.00	10.00	4.00
3X	2.7×10^{12}	8.00	10.00	4.00
4X	2.0×10^{12}	7.69	10.00	3.84

Figure S3: The graph shows specific activity of α-amylase (1.40 µg/mL) (µmol mg^{-1} min^{-1}) in the presence of different concentrations of cit-Au NPs. 1X refers to undiluted cit-Au NP, 2X, 3X and 4X refers to two times, three times and four times diluted (with buffer) cit-Au NPs respectively. The table represents the $V^a_{max,}$ K^a_m and k^a_{cat} values obtained for the various concentrations of cit-Au NPs.

Calculation of number of nanoparticles and fraction of surface atom using a spherical cluster approximation model:[5]

$$W = V \times M \times A.\,wt/\,1000 \qquad [1]$$

Where, W is the weight of the Au particles in the medium, V is the final volume of the reaction in mL, M is the molar concentration of Au^{3+} ion and A. wt is the atomic weight of Au atom.

Here an excess of citrate was used, so assumed that all of $HAuCl_4$ was converted to Au NPs.

750.0 µL of 1.7262×10^{-2} M $HAuCl_4$ was reduced. Hence the moles of $Au^{3+} = 1.295 \times 10^{-5}$.

Therefore weight of $Au^{3+} = 1.295 \times 10^{-5}$ mol $\times$ 196.97 g $mol^{-1} = 2.55 \times 10^{-3}$ g.

Since the total volume in which $HAuCl_4$ was reduced = 30.0 mL hence concentration of Au^{3+} = Au(0) = 8.5×10^{-5} g/mL.

In our experiments we had diluted cit-Au NPs to two times, hence [Au] = 4.25×10^{-5} g/mL.

We had taken 2.0 mL of this solution and added to 3.0 mL starch solution. Hence final [Au] = 1.7×10^{-5} g/mL i.e. 8.5×10^{-5} g of Au was present in the medium (5 mL).

From the TEM results, we observed that Au NPs were spherical in shape, and we can roughly write,

$$V_{cluster} = NV_{atom} \qquad [2]$$

$$\frac{4}{3}\pi (R_{cluster})^3 = N \frac{4}{3}\pi (R_{atom})^3 \qquad [3]$$

Here, $V_{cluster}$ is the volume of a cluster (NP) and V_{atom} is the volume of an atom, $R_{cluster}$ is the radius of a cluster and R_{atom} atomic radius and N is the total number of atoms within the cluster. From eqn. 1 & 2,

$$R_{cluster} = N^{1/3} R_{atom.} \qquad [4]$$

Calculation of amount of gold nanoparticles formed (N_{NP}) when 8.5 x 10^{-5} g of Au particles were used for catalysis:

From the TEM results we know that average diameter of cit-Au NP was 11 nm. Therefore $R_{cluster}$ = 5.5 nm. Also radius of gold atom i.e. R_{atom} = 0.137 nm. Therefore the number of gold atoms per nanoparticle was estimated using eqn. [4],

$N = (R_{cluster} / R_{atom})^3 = (5.5 / 0.137)^3 = 64703$ gold atoms per nanoparticle.

Amount of gold particles used, W = 8.5 x 10^{-5} g = 8.5 x 10^{-5} g / 196.97 g mol^{-1} = 4.315 x 10^{-7} moles.

No. of gold atoms,

N_{atom} = 4.315 x 10^{-7} x 6.023 x 10^{23} = 2.599 x 10^{17}

No. of nanoparticles in 8.5 x 10^{-5} g of gold particles,

$N_{NP} = N_{atom} / N$ = 2.599 x 10^{17} / 64703 = 4.02 x 10^{12}

= 4.02 x 10^{12} nanoparticles were formed per 8.5 x 10^{-5} g of Au.

Reference:

1. Lewis, J. D.; Day, M. T.; Mac Pherson, V. J.; Pikeramenou, Z. *Chem Commun*. 2006, 1433.

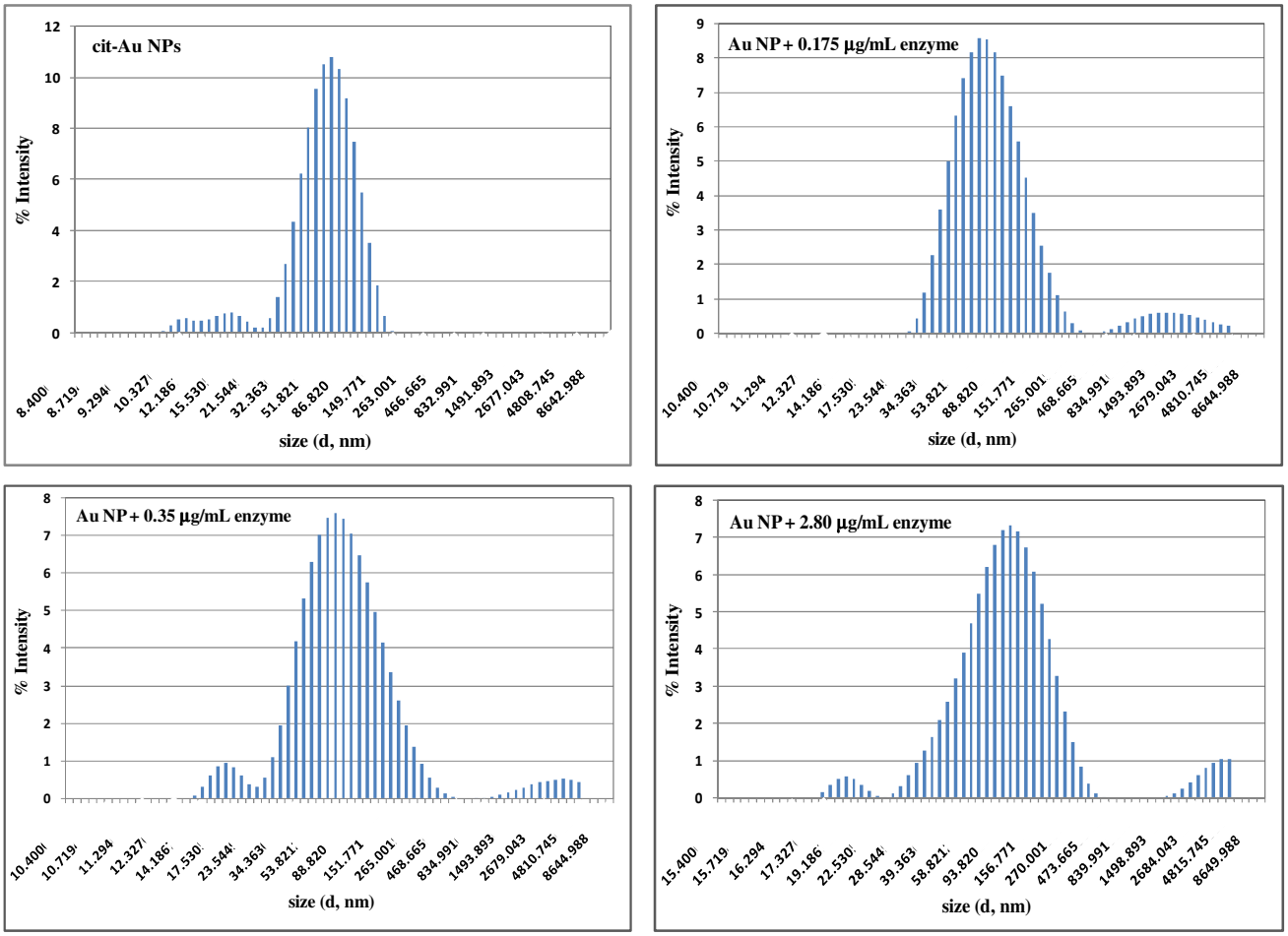


Figure S4: Dynamic Light Scattering (DLS) based particle size distribution of cit-Au NPs and cit-Au NPs in the presence of three different concentrations of enzyme as mentioned in the legends (also same as used in the enzymatic activity studies).

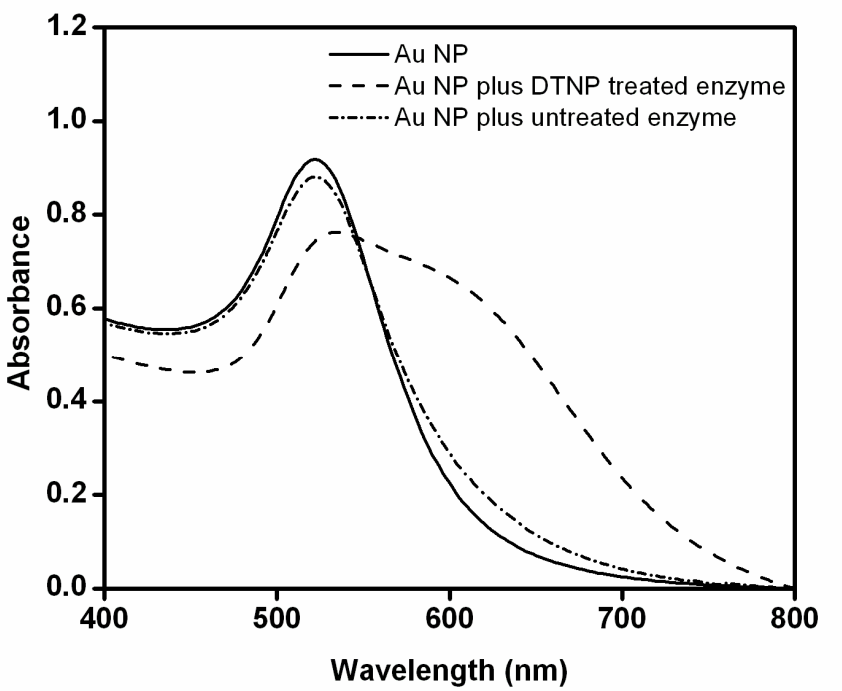


Figure S5: UV-visible spectrum of cit-Au NPs and that in the presence of DTNP treated enzyme as well as unmodified enzyme.

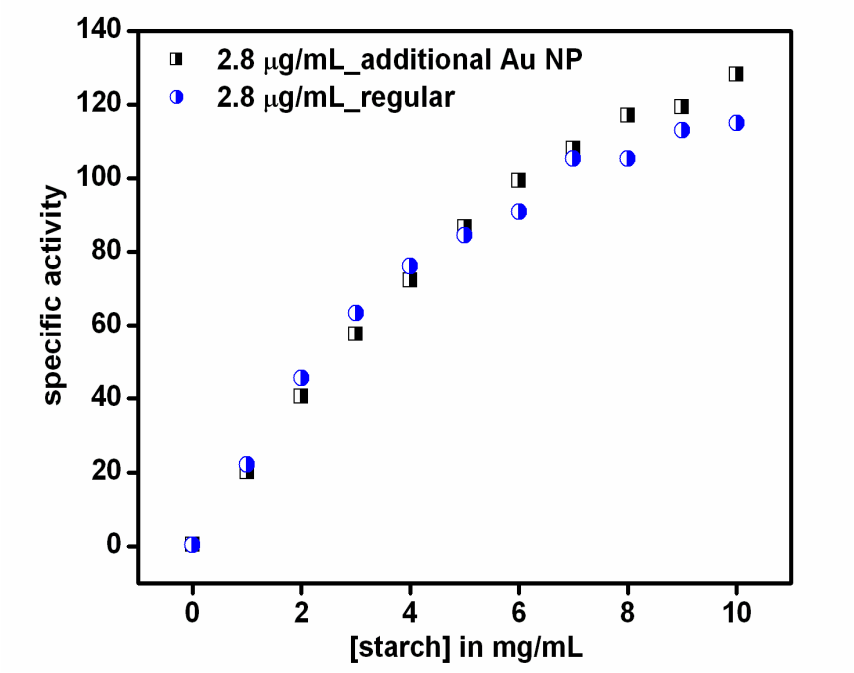


Figure S6: Specific activity of α-amylase (2.80 μg/mL) (μmol mg^{-1} min^{-1}) in the presence of cit-Au NPs as compared to the one with same concentration of enzyme and cit-Au NP followed by additional cit-Au NPs to the Au NP-enzyme composite.

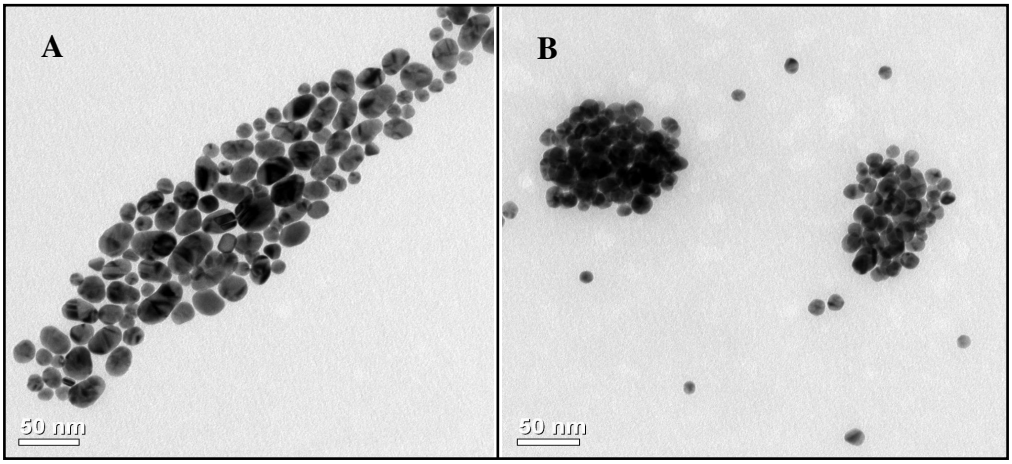


Figure S7: TEM images of Au NPs from the media (after starch digestion) with 2.80 μg/mL of α-amylase and **(A)** regular amount of cit-Au NPs and **(B)** additional cit-Au NPs added to the as formed Au NP-enzyme composite.

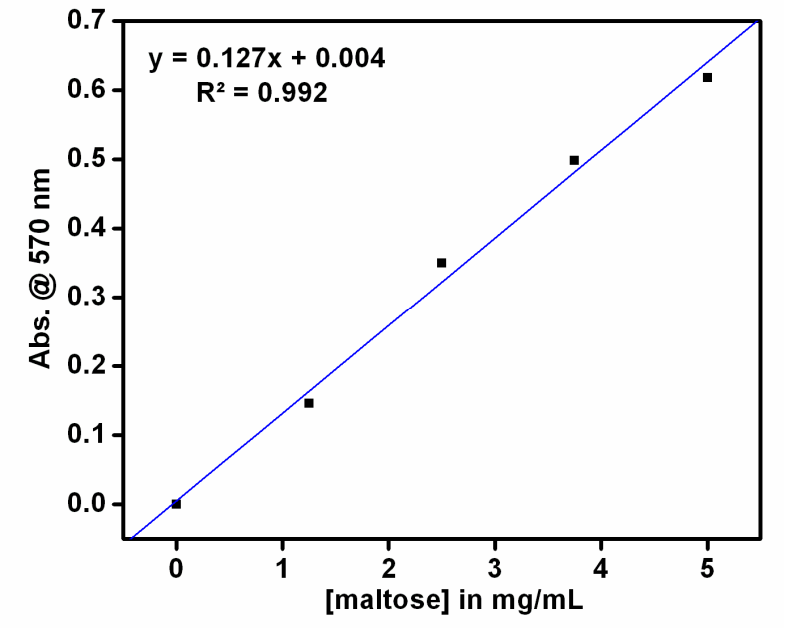


Figure S8: Calibration graph for DNS method of maltose estimation, as prepared with known concentrations of maltose. The graph shows absorbance at 570 nm of the UV-vis spectrum of the known concentration of maltose after treating with DNS.

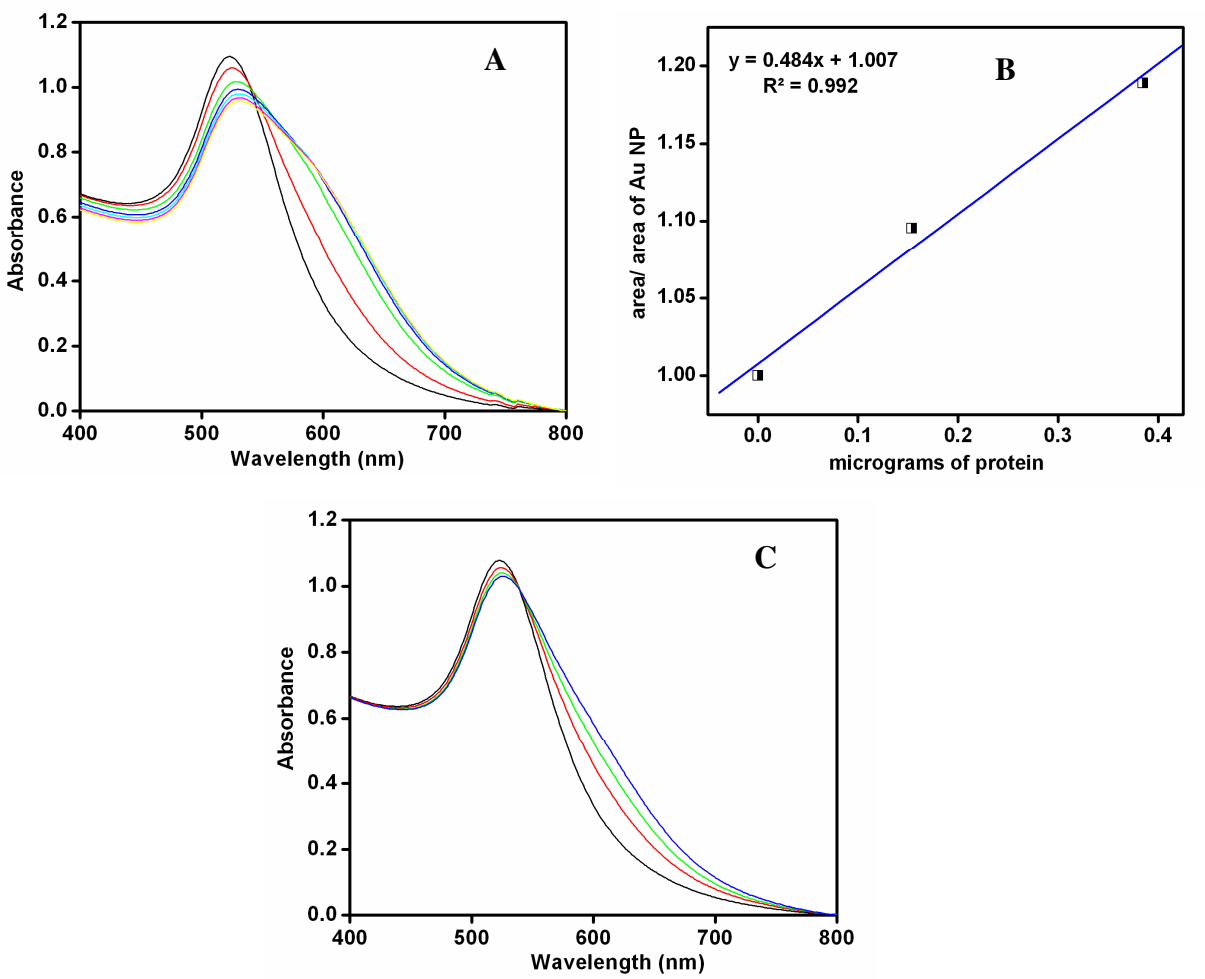


Figure S9: **(A)** UV-vis spectra of cit-Au NPs in presence of increasing amount of α-amylase. **(B)** Calibration graph for calculating micrograms of protein as generated by plotting the ratio of area under graphs (in '**A**') for cit-Au NP in presence of definite amount of protein to that of cit-Au NP only. **(C)** UV-vis spectra of cit-Au NPs in presence of different amount of the supernatant (of the same solution) after separating from the Au NP bound enzyme by centrifugation. Refer to the text in the manuscript for details of the process of estimation.