Electronic Supplementary Material (ESI) for RSC Advances

Conformation aspect in the alpha-amylase induced agglomeration of citrate – stabilized Gold Nanoparticle

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Electronic Supporting Information (ESI)

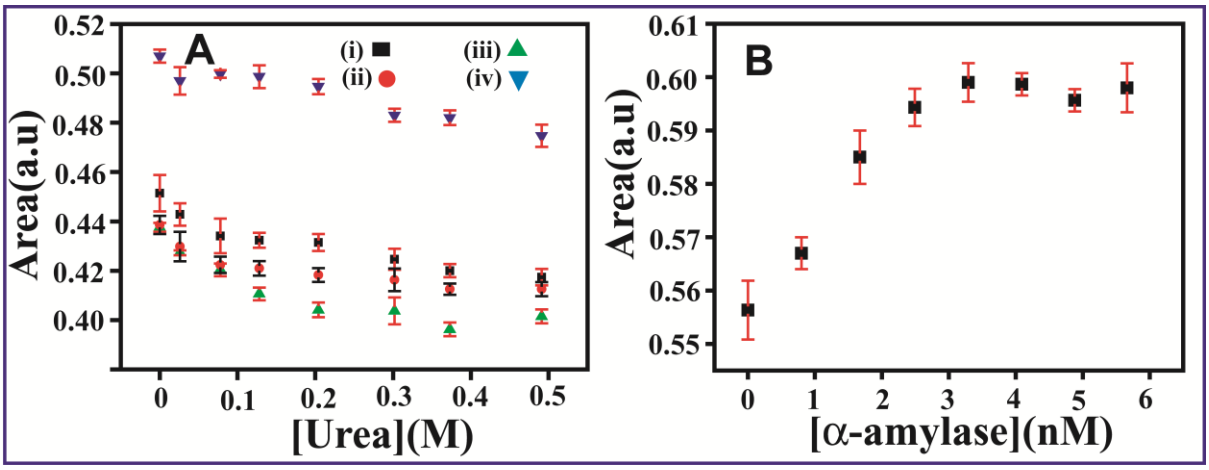

Figure S1: Normalised area under the UV–Vis spectra versus the corresponding urea concentration in mol/L, keeping the α-amylase concentrations constant (i) 0.02 mL (ii) 0.04 mL and (iii) 0.06 mL (iv) 0.00 mL of α-amylase.(B) Normalised area under UV–Vis spectra versus corresponding concentrations of α-amylase to cit–Au NPs. The stock concentration of α-amylase was 127 nM. The error bar were calculated from three sets of experiments.

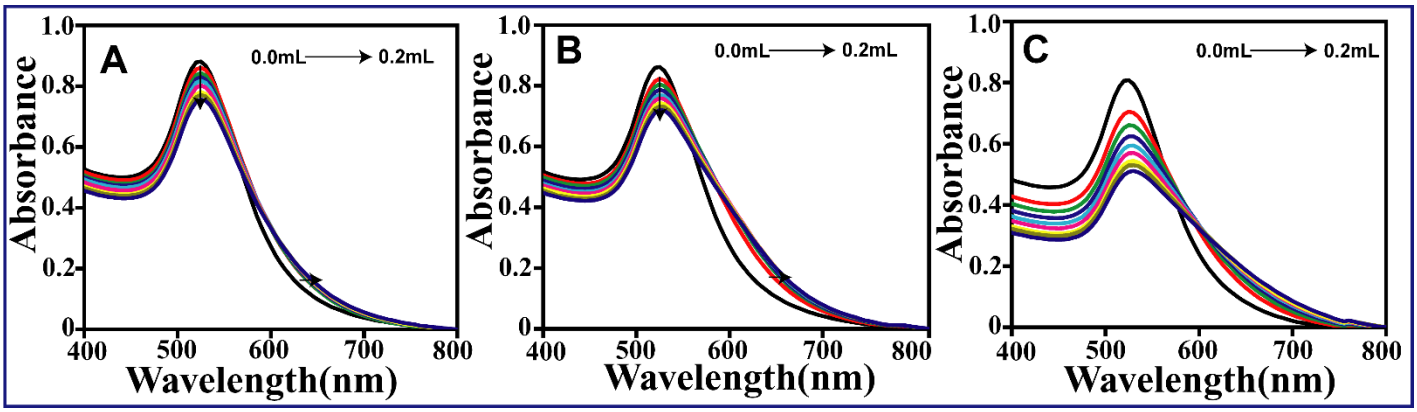

Figure S2: UV–Vis spectra of 3.0 mL of cit–Au NPs solution on successive addition (from 0.01 mL to 0.2 mL) of 4 M stock urea solution, already containing **(A)** 0.02 mL **(B)** 0.04 mL α-amylase and **(C)** 0.06 mL of α-amylase. The stock concentration of α-amylase was 127 nM. All the UV–Vis spectra were recorded after 5 min incubation. Cit -Au NPs mixture with α-amylase was kept for 5 min, urea added to the composite, kept further for 5 min and then the spectra recorded.

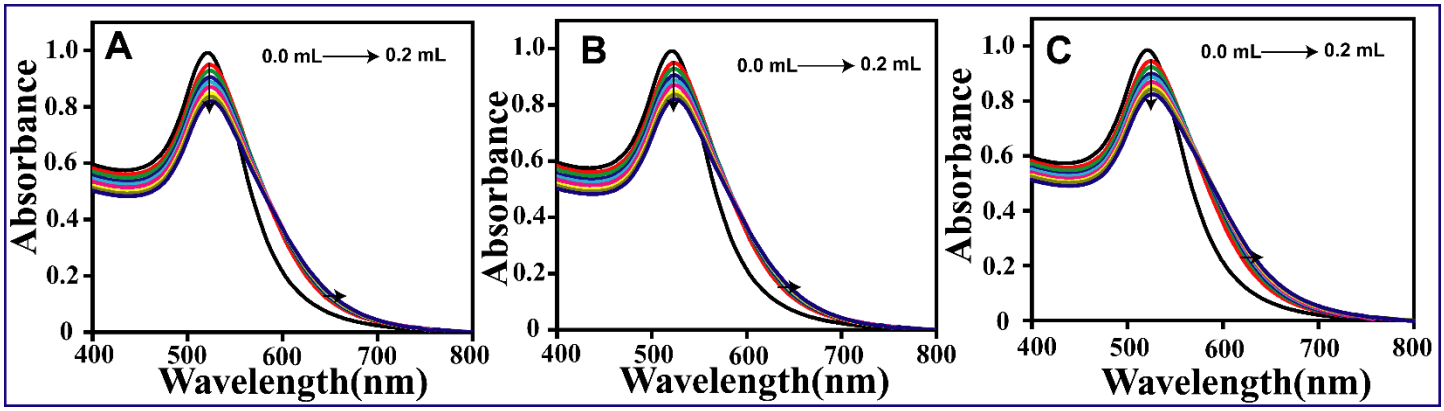

Figure S3: UV–Vis spectra of cit–Au NPs with **(A)** 0.02 mL **(B)** 0.04 mL **(C)** 0.06 mL of α-amylase with successive addition of urea (6 M) solution (from 0.01 mL to 0.2 mL). The stock concentration of α-amylase was 127 nM. All the UV–Vis spectra were recorded after 5 min incubation. Cit–Au NPs mixture with α-amylase was kept for 5 min, urea added to the composite, kept further for 5 min and then the spectra recorded.

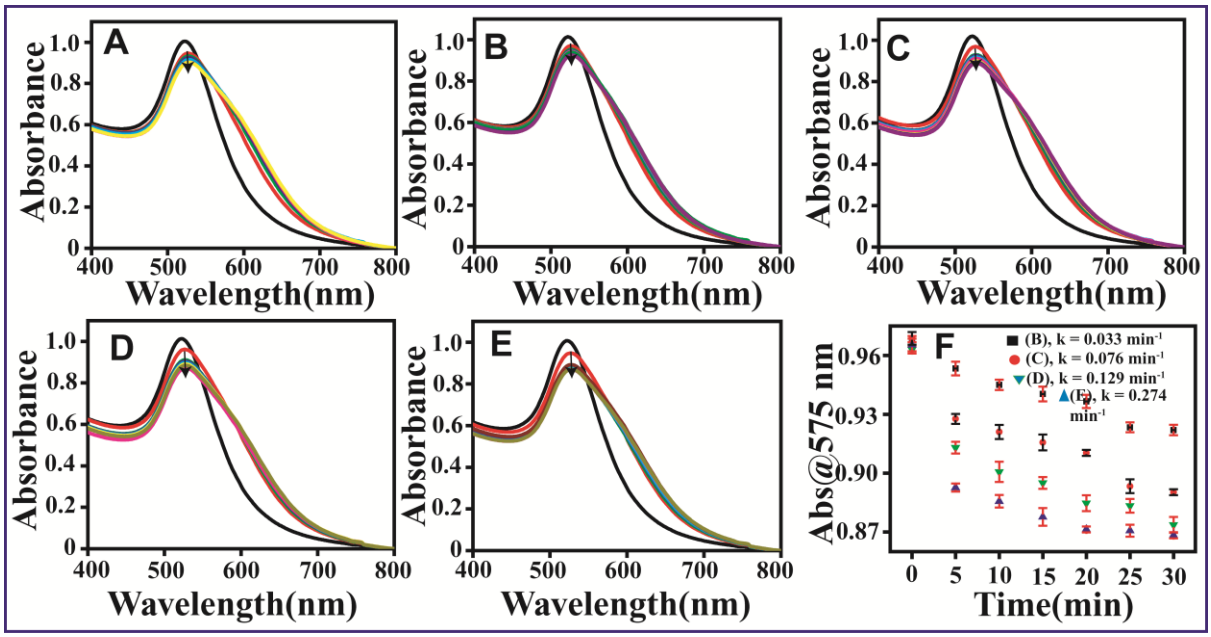

Figure S4: Time dependent UV–Vis spectra for cit–Au NPs NPs with 0.06 mL α-amylase in presence of **(A)** 0.00 mL **(B)** 0.05 mL **(C)** 0.10 mL **(D)** 0.15 mL **(E)** 0.20 mL of urea solution. All samples were kept for 5 min before recording the spectra**.** The stock concentration of α-amylase and urea were 127 nM and 8 M, respectively**. (F)** Absorbance at 575 nm of (B), (C), (D) and (E) with time interval of 5 min. The error bar were calculated from three sets of experiments.

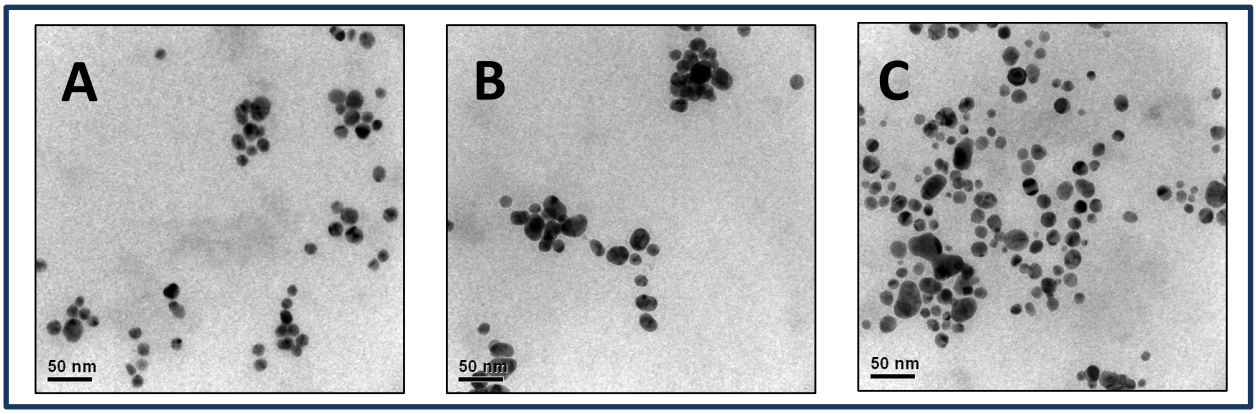

Figure S5: TEM images (50 nm scale bar) of 3.0 mL of cit–Au NPs in presence of **(A)** 0.01 mL (**B)** 0.05 mL **(C)** 0.12 mL of 8 M urea solution.

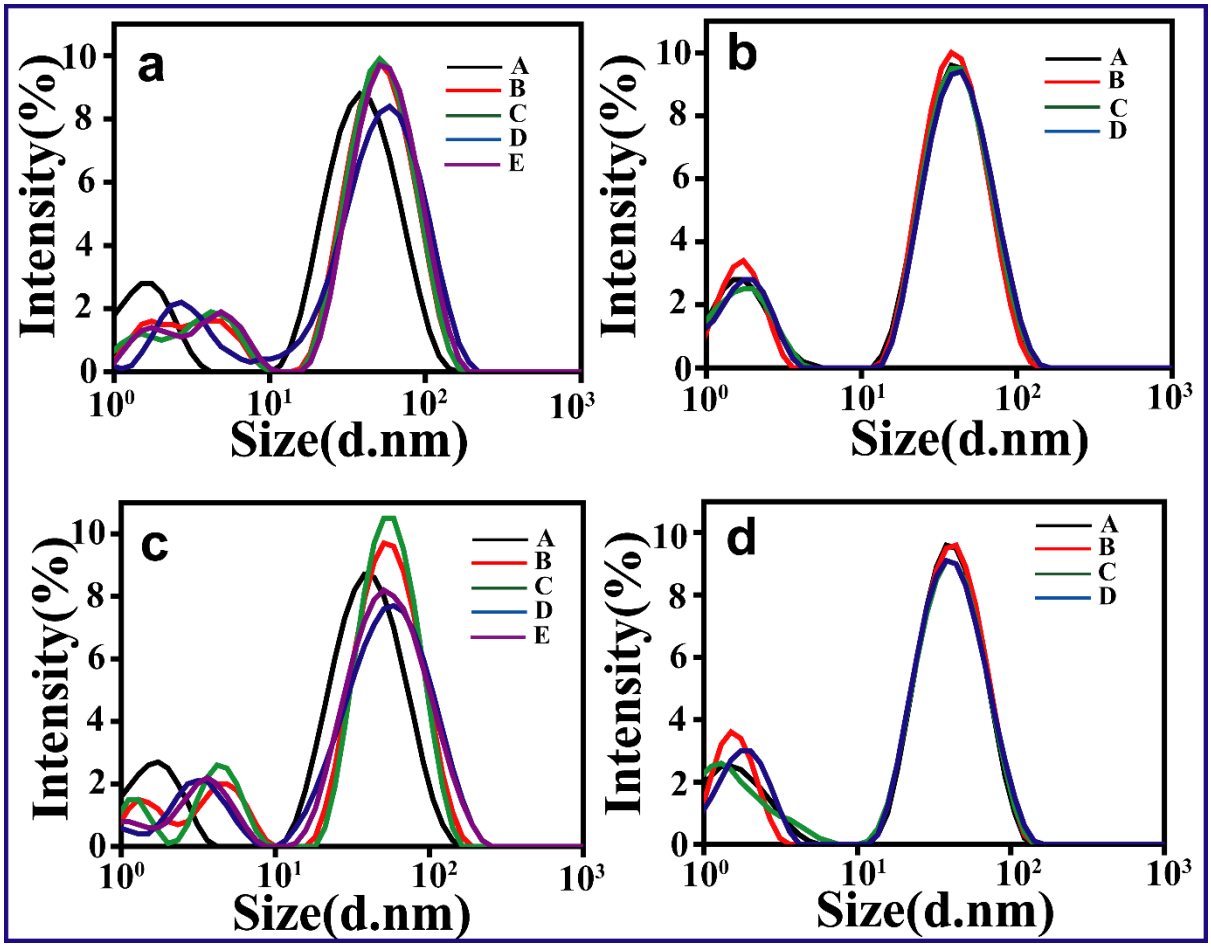

Figure S6: Particle size distribution curves of **(a)**: **(A)** 3.0 mL cit–Au NPs dispersion **(B)** 3.0 mL cit–Au NPs + 0.06 mL α-amylase solution **(C)** 3.0 mL cit–Au NPs + 0.06 mL α-amylase solution + 0.05 mL urea solution **(D)** 3.0 mL cit–Au NPs + 0.06 mL α-amylase solution + 0.150 mL urea solution **(E)** 3.0 mL cit–Au NPs + 0.06 mL α-amylase solution + 0.250 mL urea solution (stock concentration of α-amylase and urea are 127 nM and 4 M, respectively)

(b): (A) 3.0 mL of Cit–Au NPs dispersion **(B)** 3.0 mL cit–Au NPs + 0.50 mL urea solution **(C)** 3.0 mL cit–Au NPs + 0.15 mL urea solution **(D)** 3.0 mL cit–Au NPs + 0.25 mL urea solution (stock concentration of urea solution is 4 M)

(c): (A) 3.0 mL of cit–Au NPs dispersion **(B)** 3.0 mL cit–Au NPs + 0.06 mL α-amylase solution **(C)** 3.0 mL cit–Au NPs + 0.06 mL α-amylase solution + 0.05 mL urea solution **(D)** 3.0 mL cit–Au NPs + 0.06 mL α-amylase solution + 0.150 mL urea solution **(E)** 3.0 mL cit-

Au NPs + 0.06 mL α-amylase solution + 0.250 mL urea solution (stock concentration of α-amylase and urea are 127 nM and 6 M, respectively)

(d): (A) 3.0 mL cit–Au NPs dispersion **(B)** 3.0 mL cit–Au NPs + 0.50 mL urea solution **(C)** 3.0 mL Cit–Au NPs + 0.15 mL urea solution **(D)** 3.0 mL cit–Au NPs + 0.25 mL urea solution (stock concentration of urea solution is 6 M).

TABLE S1 Particle size distribution from DLS measurements using 4 M urea stock solution All the samples were kept for 5 min before recording the particle size distribution. Urea was added to cit–Au NPs –amylase composite every 5 min interval**.**

Sample	Size (d.nm)	Peak 1 size (d.nm)	Peak 1 area (%)	Peak 2 size (d.nm)	Peak 2 area (%)
3.0 mL cit–Au NPs	12.4	43.9	78.8	1.5	21.2
3.0 mL cit–Au NPs + 0.06 mL of α-amylase	20.6	56	80	4.4	10.5
3.0 mL cit–Au NPs + 0.06 mL of α-amylase + 0.05 mL of 4 M urea solution	21	57	80	4.2	13.6
3.0 mL cit–Au NPs + 0.06 mL of α-amylase + 0.15 mL of 4 M urea solution	21.7	60	83	3.1	15
3.0 mL cit–Au NPs + 0.06 mL of α-amylase + 0.25 mL of 4 M urea solution	23	60	80	4.8	11.3

DLS measurments of cit-Au NPs in presence of 4 M of urea solution only. All the samples were kept for 5 min before recording the particle size distribution. Urea was added to cit–Au NPs –amylase composite every 5 min interval

Sample	Size (d.nm)	Peak 1 size (d.nm)	Peak 1 area (%)	Peak 2 size (d.nm)	Peak 2 area (%)
3.0mL cit–Au NPs	12.54	47	79	1.7	21
3.0ml cit–Au NPs + 0.01mL of 8M urea solution	12.17	43	80	1.7	20
3.0ml cit–Au NPs + 0.05 mL of 8 M urea solution	12.39	46	79	1.7	21
3.0ml cit–Au NPs + 0.120 mL of 8 M urea solution	12.54	47	79	1.7	20.9

TABLE S2: Particle size distribution from DLS measurements using 6 M urea stock solution. All the samples were kept for 5 min before recording the particle size distribution. Urea was added to cit–Au NPs –amylase composite every 5 min interval.

Sample	Size (d.nm)	Peak 1 size (d.nm)	Peak 1 area (%)	Peak 2 size (d.nm)	Peak 2 area (%)
3.0 mL cit–Au NPs	12.54	43.6	77.6	2	17.9
3.0 mL cit–Au NPs + 0.06 mL of α-amylase	21.4	59.4	80	4.6	12.3
3.0 mL cit–Au NPs + 0.06 mL of α-amylase + 0.05 mL of 6 M urea solution	21.7	59	80	4.5	13.6
3.0 mL cit–Au NPs + 0.06 mL of α-amylase + 0.15 mL of 6 M urea solution	23.4	63	82.5	3.3	14.6
3.0 mL cit–Au NPs + 0.06 mL of α-amylase + 0.25 mL of 6 M urea solution	23.7	63.2	82	3.6	14.3

DLS measurments of cit-Au NPs in presence of 6 M of urea solution only. All the samples were kept for 5 min before recording the particle size distribution. Urea was added to cit–Au NPs every 5 min interval**.**

Sample	Size (d.nm)	Peak 1 size (d.nm)	Peak 1 area (%)	Peak 2 size (d.nm)	Peak 2 area (%)
3.0mL cit-Au NPs	12.17	44	78	1.7	21
3.0ml cit-Au NPs + 0.01mL of 8M urea solution	12.04	45	80	1.6	19
3.0ml cit-Au NPs + 0.05 mL of 8 M urea solution	12.12	45	78	1.7	22
3.0ml cit-Au NPs + 0.120 mL of 8 M urea solution	12.05	45	78	1.8	20

Table S3: Measurement of zeta potentials of cit–Au NPs in presence of 8M urea only. All the samples were kept for 5 min before recording the zeta potential. Urea was added to cit–Au NPs every 5 min interval**.**

Sample	Zeta potential (mV)	Size (d.nm)
3.0 mL cit–Au NPs	-49.7	12.48
3.0 mL cit–Au NPs + 0.01 mL of 8 M urea solution.	-47.9	12.48
3.0 mL cit–Au NPs + 0.05 mL of 8 M urea solution.	-45.8	12.56
3.0 mL cit–Au NPs + 0.12 mL of 8 M urea solution.	-44.1	12.66

Table S4: DLS measurement of cit–Au NPs in presence of 8M of urea solution. All the samples were kept for 5 min before recording the particle size distribution. Urea was added to cit–Au NPs every 5 min interval.

Sample	Size (d.nm)	Peak 1 size (d.nm)	Peak 1 area (%)	Peak 2 size (d.nm)	Peak 2 area (%)
3.0mL cit–Au NPs	19	45.84	78	1.69	21
3.0ml cit–Au NPs + 0.01mL of 8M urea solution	19.05	45	78.6	1.7	22
3.0ml cit–Au NPs + 0.05 mL of 8 M urea solution	19	46	77	2.2	14
3.0ml cit–Au NPs + 0.120 mL of 8 M urea solution	19	48	79	2.04	21

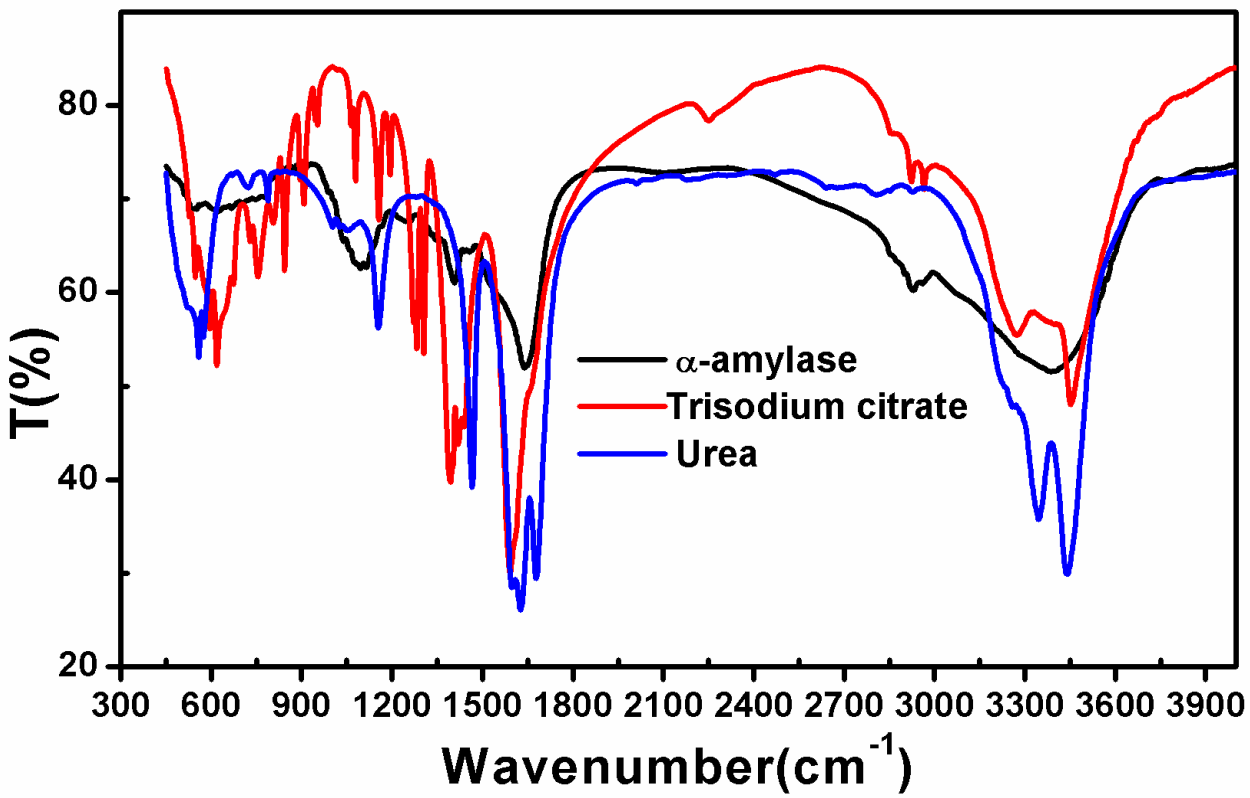

Figure S7: IR spectra of native α-amylase, trisodium citrate and urea.

Enzymatic Activity of α-amylase:

Preparation of Au NP-α-amylase composite. A 60.0 mL dispersion of cit–Au NPs was taken in a 250 mL conical flask. To this 0.06 mL of 1270 nM of α-amylase (10 times more than the UV–Vis study) was added which was then shaken well and kept for 15 min. The final concentration of α-amylase was found to be 24.5 nM. The Au NP–protein composite was then centrifuged at 25,000 rpm and 4°C for 30 min to remove excess α-amylase and free Au NPs. The supernatant was discarded, and the pellet was re-suspended in phosphate buffer (pH 7.0). Similarly, to 60.0 mL of cit–Au NPs and 0.06 mL of 1270 nM of protein composite, 1.5 mL of 8 M stock urea solution was added, shaken well and kept for 15 min. The Au NP–protein–urea composite was then by centrifuged at 25,000 rpm for 30 min and 4°C to remove excess amounts of Au NPs, urea and protein.

Enzymatic starch digestion studies. For the starch digestion kinetic studies, a stock solution of starch was prepared in phosphate buffer (pH 7.0) with a concentration of 0.5 mg/mL. 2.0 mL of Au NP–protein composite was added to the 30.0 mL of above starch solution and was incubated at 37 ºC. Similarly, an equivalent amount of Au NP–protein containing urea was taken to compare the digestion of starch. Aliquots (3.0 mL) from each of the reaction mixtures were withdrawn at regular interval of time, 0.2 mL of iodine solution (Gram's iodine, HiMedia) was added and the UV–Vis spectra recorded. For kinetics, the area under the curves was plotted as a function of time.

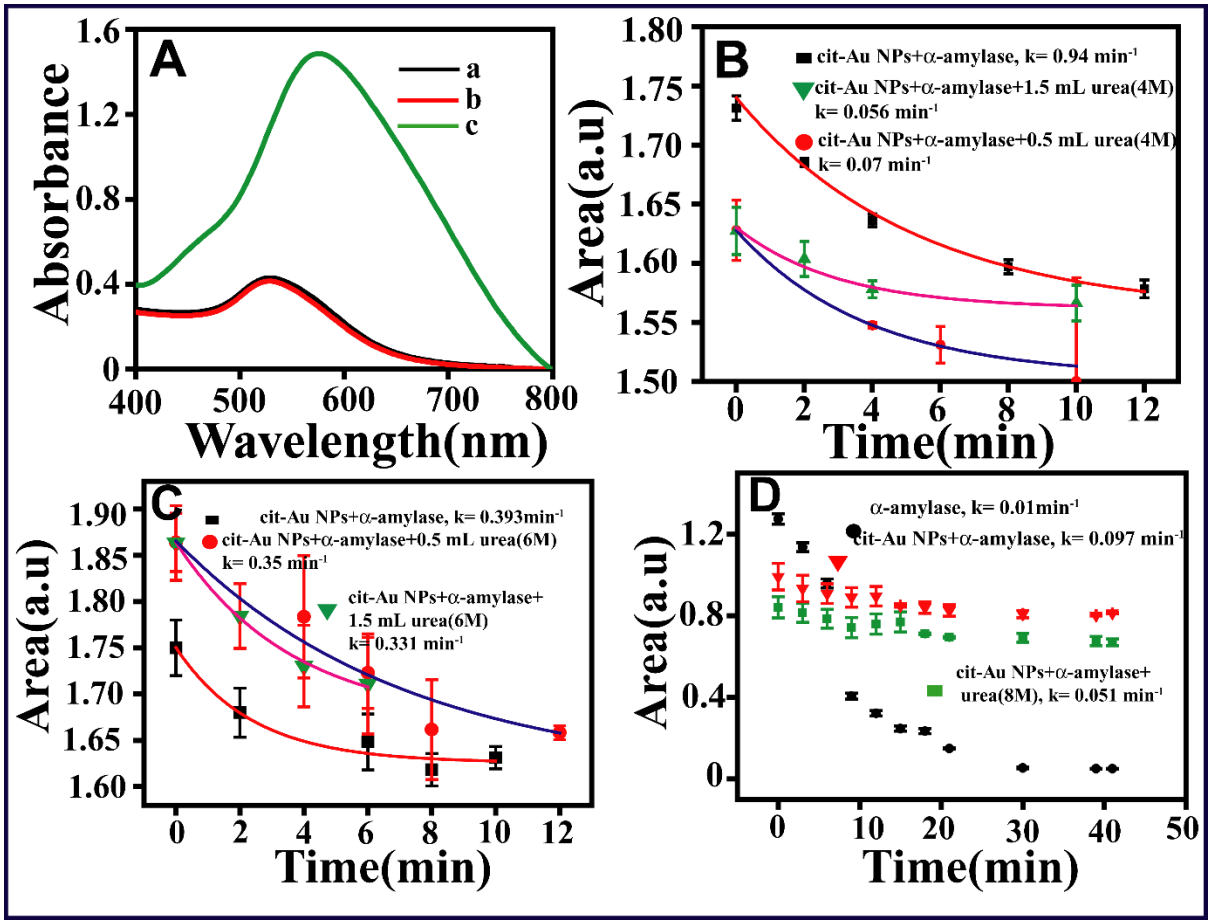

Figure S8. **(A)** UV–Vis spectra of the starch-Au NPs composite (black line, a), starch–Au NPs–urea composite (red line, b) compared to the starch–Au NPs–urea in the presence of iodine (green line, c). 1.5 mL of 8 M urea was added to 3.0 mL of cit–Au NPs and 0.06mL of α-amylase of 1270 nM. Comparative kinetic studies of starch digestion in the starch-Au NPs in absence and presence of (**B)** 4 M. **(C)** 6 M and **(D)** 8 M of urea solution. The stock concentration of α–amylase was 1270 nM. The error bar were calculated from three set of experiments.

Enzymatic activity of α-amylase was measured by following the digestion of starch in absence of, and in presence of the α-amylase–Au NP composite, as well as urea treated α-amylase–Au NP composite. The amount of starch left was followed by the iodine test method by an earlier adopted procedure.[1] When iodine is added to the mixture of Au NP–

protein and starch a new UV–vis band emerged at 565 nm, which is the characteristics peak for the starch–iodine complex (Figure **S8B**). UV–Vis spectra were recorded at regular intervals of time and the area under the curves were determined and plotted as a function of time as shown in Figure **S8A**. In all cases the digestion of starch was found to follow first order kinetics (Figure **S8A)**. Previous studies have shown that the enzyme activity of the extensively agglomerated cit–Au NPs composites is reduced while the activity of mildly agglomerated cit–Au NPs composites is enhanced.[2]The marginal reduction in the enzymatic activity of cit–Au NPs protein composite from the native protein in this study.

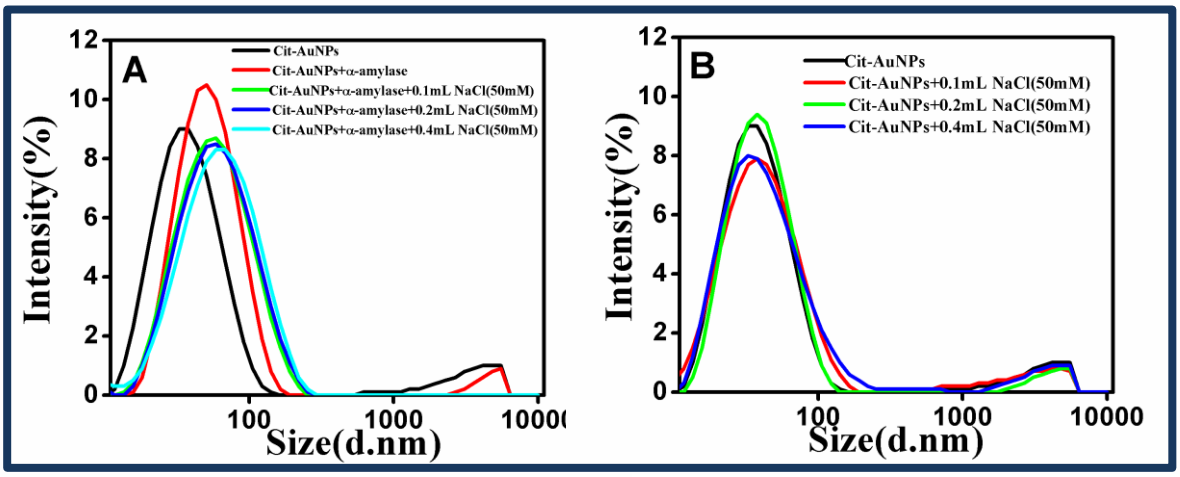

Figure S9: Particle size distribution curves of 3.0 mL cit–Au NPs in presence of 50 mM NaCl and: **(A)** 0.06 mL **(B)** 0.0 mL of α-amylase solution. All the samples were kept for 5 min before recording the particle size distribution. NaCl was added to cit–Au NPs-amylase composite every 5 min interval.

Table S5: Particle size distribution of 3.0 mL cit–Au NPs in presence of 50 mM NaCl. All the samples were kept for 5 min before recording the particle size distribution. NaCl was added to cit–Au NPs every 5 min interval.

Sl No.	Sample	Size (d.nm)
1	3.0 mL of cit–Au NPs	16
2	3.0 mL of cit–Au NPs + 0.1 mL of 50 mM NaCl	18
3	3.0 mL of cit–Au NPs + 0.2 mL of 50 mM NaCl	19.8
4	3.0 mL of cit–Au NPs + 0.4 mL of 50 mM NaCl	20.7

Table S6: Particle size distribution of 3.0 mL cit–Au NPs in presence of 0.06 mL of 127 nM α-amylase solution and 50 mM NaCl. All the samples were kept for 5 min before recording the particle size distribution. NaCl was added to cit–Au NPs-amylase composite every 5 min interval.

Sl No.	Sample	Size (d.nm)
1	3.0 mL of cit–Au NPs	16
2	3.0 mL of cit–Au NPs + 0.06 mL α-amylase	29
3	3.0 mL of cit–Au NPs + 0.06 mL α-amylase + 0.1 mL 50 mM NaCl	30
4	3.0 mL of cit–Au NPs + 0.06 mL α-amylase + 0.2 mL 50 mM NaCl	31
5	3.0 mL of cit–Au NPs + 0.06 mL α-amylase + 0.4 mL 50 mM NaCl	32

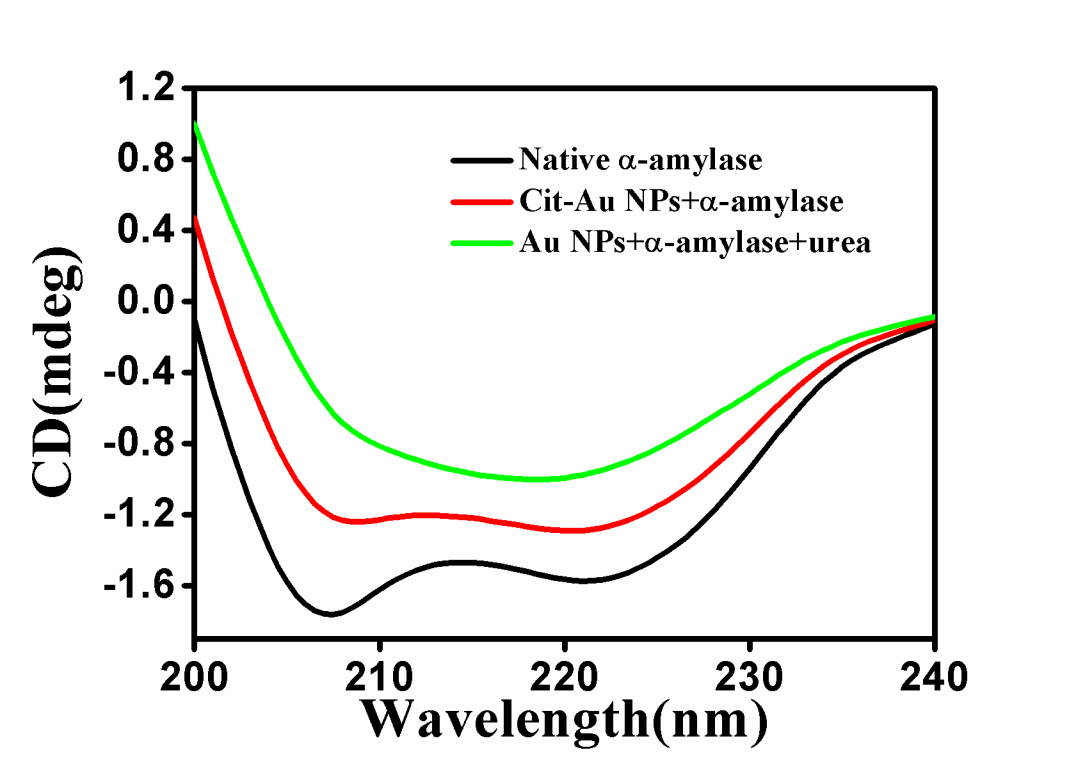

Figure S10: CD spectra of 0.06 mL of α-amylase (black line), 3.0 mL of cit–Au NPs + 0.06 mL of α-amylase (red line) and 3.0 mL of cit–Au NPs + 0.06 mL of α-amylase + 0.4 mL of urea (green line). Spectra were recorded in the wavelength region 200–250 nm. The stock concentrations of α-amylase and urea solution were 1270 nM and 8 M, respectively, and the samples were kept for 30 min.

Table S7: Determination of helicity of α-amylase from UV- CD spectroscopy

Sample	α-helix (%)	β-sheet (%)
Native α-amylase	82	6
3.0 mL cit–Au NPs + 0.06 mL of 1270 nM of α-amylase	70	26
3.0 mL cit–Au NPs + 0.06 mL of 1270 nM of α-amylase + 0.4 mL of 8 M urea solution	32	67

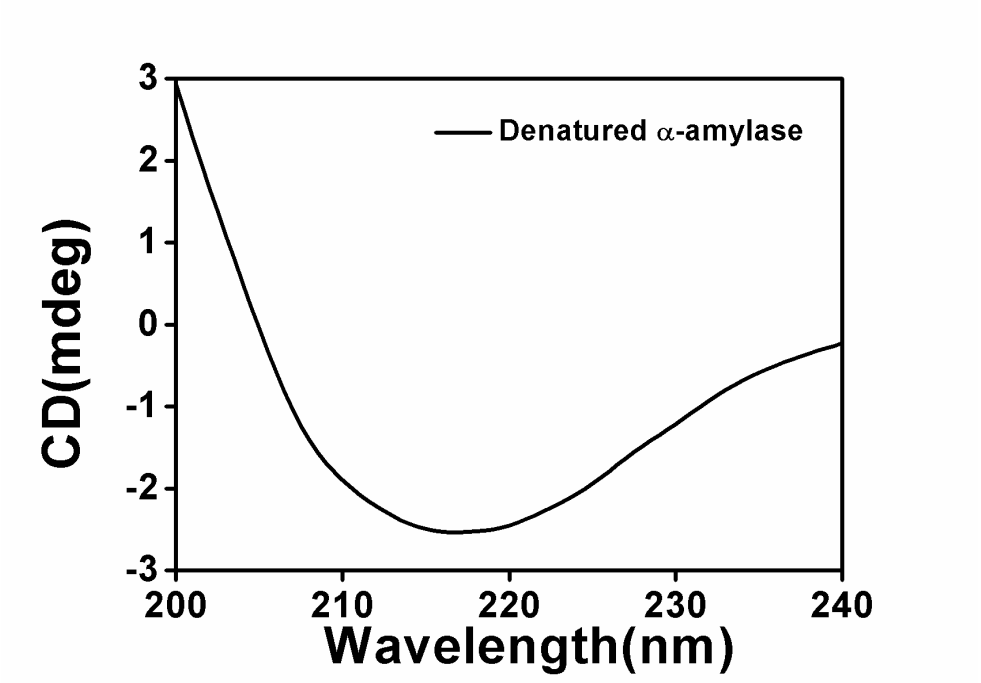

Figure S11: CD spectra of 0.06 mL of α-amylase+ 0.4 mL of 8 M urea solution. Spectra were recorded in the wavelength region 200–250 nm. The concentration of α-amylase in solution was found to be 24.9 nM. The α-amylase and urea mixture was kept for 3 hours at 4°C.

Table S8: Determination of helicity of α-amylase from UV- CD spectroscopy

Sample	α-helix (%)	β-sheet (%)
Native α-amylase	82	6
0.06 mL of α-amylase+ 0.4 mL of 8 M urea solution	10	73

Reference

1. J. Deka, A. Paul, A. Ramesh, A. Chattopadhyay. *Langmuir*, 2008, **24**, 9945–9951.
2. J. Deka, A. Paul, A. Chattopadhyay, *RSC Advances*, 2012, **2**, 4736–4745