Electronic Supplementary Information

Nanoscale size impact of nanoparticle interaction and activity studies with urease

Materials and instruments

The materials were obtained from Sigma Aldrich (UK): GOPS (3-glycidyloxypropyl) trimethoxysilane, Urea AR grade 98%, Tri Sodium citrate dihydrate, Tannic acid, Silver nitrate, and urease from Jack beans U1500-20KU(Canavalia ensiformis). Milli-Q water (18 $M\Omega.cm^{-1}$ resistivity) was used to prepare aqueous solutions. All the chemicals were analytical grade.

Ultraviolet-visible (UV-Vis) spectroscopy measurements were assessed using a spectrophotometer made by BioTek Synergy H1 reader UK. Using a JEOL 2100 Transmission electron microscope (JEOL, USA), the transmission electron micrographs were captured with excess concentrations of both components. The Zetasizer NanoZS (Malvern, UK) was utilized to measure the size and charge distribution of AgNPs. The Jasco J-815 CD spectropolarimeter device was used to record the circular dichroism spectrum, run at a scan rate of 100 nm/minute and response time of 16 seconds over a wavelength range of 260 to 190 nm. A Malvern VP-ITC microcalorimeter was used for isothermal calorimetry experiments at 25°C. Monolith NT115 was used for microscale thermophoresis experiments.

S1 Synthesis of different size AgNPs

All glassware was cleaned for two hours with aqua regia solution, followed by treatment with GOPS (3-Glycidyloxypropyl) trimethoxysilane for one hour. A fresh 50 mL solution of tannic acid and sodium citrate was prepared, and the mixture was heated to 180 °C for 30 minutes while being vigorously stirred in the dark. At the end of the 30 minutes duration, 1 mL of $AgNO_3$ solution was added. The color changes to yellow when the nucleation process begins. The solution was kept to boiling and constantly stirred for 20 minutes to finish the nucleation process. Tannic acid, sodium citrate, and silver nitrate concentrations were changed to regulate the size of the AgNPs mentioned in Table S1. For later application, the produced AgNPs were kept in storage at 4° C.

Table S1: Composition of different silver nanoparticles.

Components	5 nm	20 nm	50 nm	100 nm
Tri Sodium Citrate	4.28×10^{-3} M	3.55×10^{-3} M	2×10^{-3} M	17.7×10^{-3} M
Tannic Acid	2×10^{-3} M	1×10^{-3} M	5×10^{-4} M	5×10^{-6} M
Silver Nitrate	1×10^{-3} M	1×10^{-3} M	1.22×10^{-3} M	2×10^{-3} M
Temperature	180 °C	180 °C	180 °C	180 °C

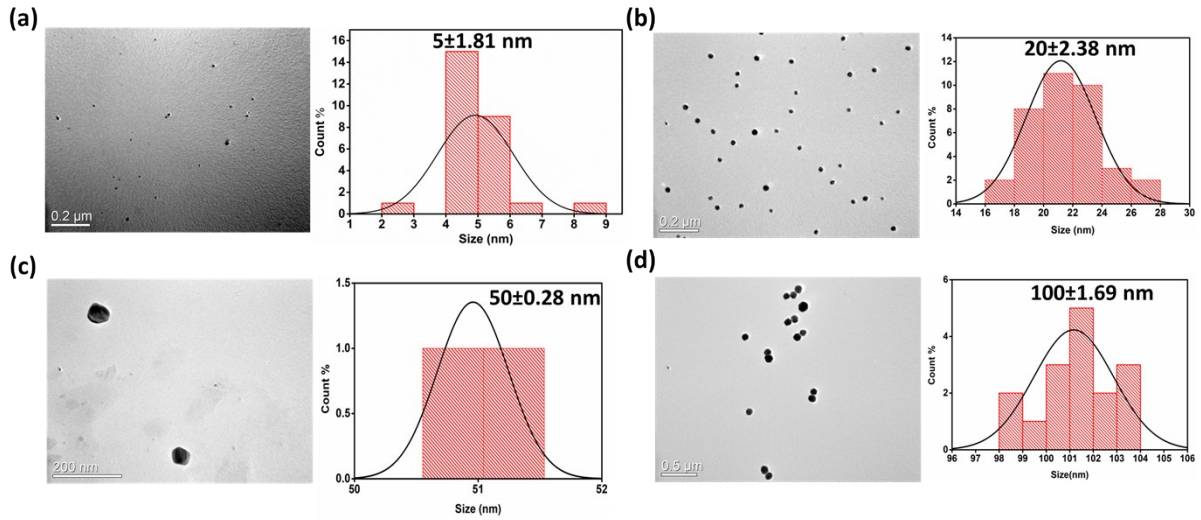

Fig S1 TEM graph of different sizes of AgNPs along with their size distribution. (a) AgNP5, (b) AgNP20, (c) AgNP50, and (d) AgNP100

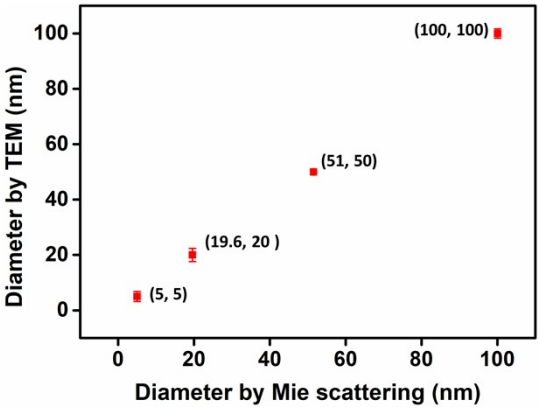

Fig S2 Theoretical versus experimental sizes of synthesized AgNPs

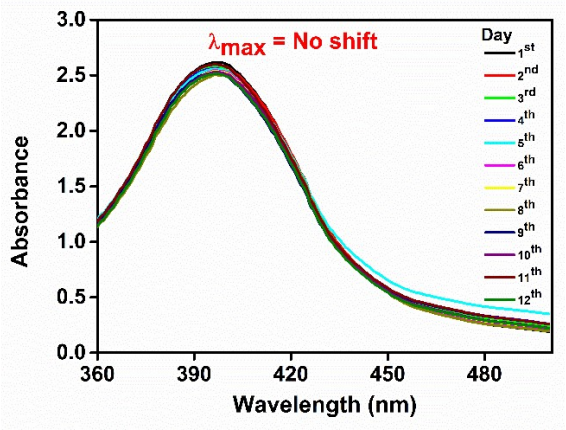

Fig S3 Day-wise UV-Vis spectra study

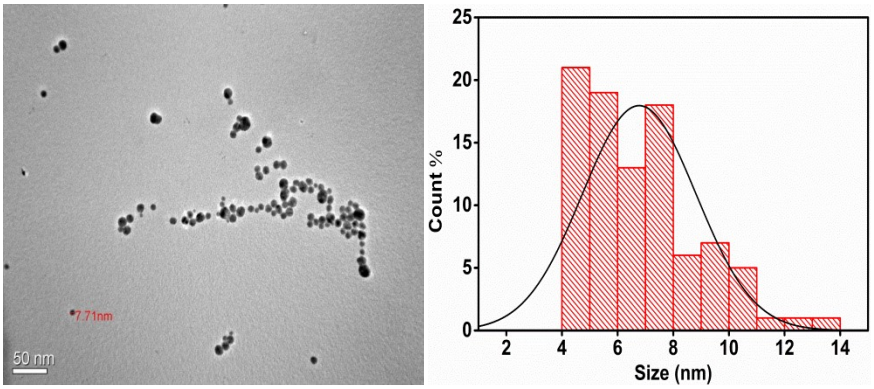

Fig S4: TEM image and size distribution graph of AgNP5 stored in 4°C for 5 months

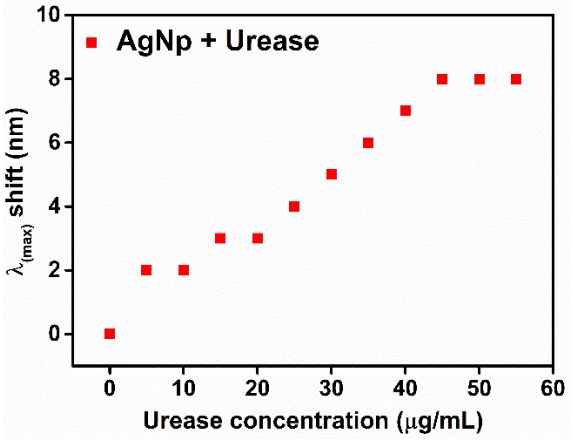

Fig S5: λmax shift of AgNP + Urease , observed after 10 min incubation

S2 FTIR

The lyophilized samples of AgNP, Urease, and AgNP + Urease were used for FTIR analysis. The peak at 2925 cm^{-1} was observed for the asymmetric stretching of the carboxylate ($–COO^-$) group[1]. Moreover, the characteristic carboxylic peak at 1382 cm^{-1} was attributed to the symmetric vibration of the carboxylate ($–COO^-$) group that refers to the citrate functionality of AgNPs[2]. The peak at 2979-2916 cm^{-1} indicates the asymmetric/symmetric stretching of the methyl (–C–H) group in urease[3]. The peak at 1642 cm^{-1} corresponds to the vibration of the amine (–N–H) group in urease. The broad peak ranging from 3400–3380 cm^{-1} is attributed to the stretching vibrations of amine (–NH) groups, as observed in the spectrographs of urease and AgNPs + Urease. The peaks for both AgNP and urease were found in the AgNP + Urease spectrograph, confirming their successful interaction.

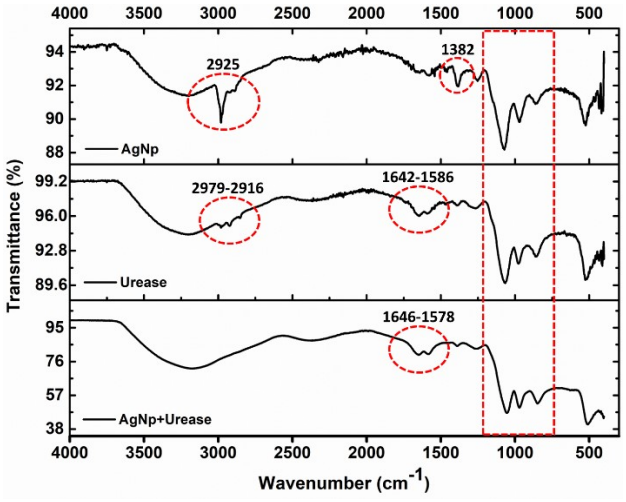

Fig S6 FTIR spectrograph of AgNP, Urease and AgNP + Urease using the KBr method at a best resolution of 0.5 cm^{-1}

Table S2: Hydrodynamic size of AgNP and AgNP + Urease

S.No	AgNP	Size before urease interaction (nm)	Size after urease interaction (nm)	% Increase in size
1	AgNP5	16.30±2.75	45.50±3.96	179.14%
2	AgNP20	35.95±2.67	79.28±2.56	120.52%
3	AgNP50	58.03±3.18	89.43±1.86	54.10%
4	AgNP100	117.33±3.01	170.03±8.55	45.17%

Table S3: Secondary structure content of Urease and AgNPs + Urease obtained from the CD spectra. The CD data was analyzed using BESTSEL.

S. No.	AgNPs + Urease	α-helix (%)	β-sheet (%)	Turn
1	only Urease	29.2	19.8	12.1
2	AgNP5 + Urease	2	40.8	9.9
3	AgNP20 + Urease	31.1	20.2	13.2
4	AgNP50 + Urease	21.2	21.6	13.2
5	AgNP100 + Urease	29.3	23.2	13.6

S3 Isothermal calorimetry

The urease was extensively dialyzed against MQ water. The urease (1 nM) was placed in a sample cell. The 20 nM of AgNPs (5-100 nm) were used as injectants. We followed standard manufacturer's protocol the calorimetric experiment employed 20 to 35 successive injections of 1.16 µL/injection of AgNPs at 150-second intervals. Two methods were used to measure the heat changes caused by the dilution effect: titrating protein with water and titrating water with an injectant. The experiment was performed with all sizes of AgNPs. Prior to data processing, these reference curve data were subtracted from the experimental curve. Using Microcal Origin 7 analysis software provided by Microcal Inc, a sequential model was fitted to the subtracted data, and the chi-square value, along with the thermodynamic parameters Ks, ΔH, and ΔS, were estimated. The molar Gibbs free energy changes (ΔG) of the reaction were calculated from the experimentally determined ΔS and ΔH values.

Table S4: Thermodynamic parameter estimation using ITC

NPs	K_a M^{-1}	-ΔG kJ mol^{-1}	ΔH Cal mol^{-1}	ΔS Cal mol^{-1} deg^{-1}
AgNP5	1.57×10^{10}	58.15	-2.30×10^{17}	-7.73×10^{14}
AgNP20	5.32×10^{8}	49.77	2.14×10^{6}	7.27×10^{3}
AgNP50	4.31×10^{9}	54.95	1.26×10^{17}	1.72×10^{15}
AgNP100	2.53×10^{5}	30.82	-7.68×10^{16}	-6.00×10^{14}

S4 Urease inhibition by AgNPs

A stock solution of urease enzyme (1 mg/mL) was prepared in milli-Q water. The substrate stock solution is 200 µM phenol red, 10 mM HCl, and 1.6 mM urea.
To evaluate the inhibitory effect of AgNPs on enzyme activity, a stock solution of AgNPs was prepared. Varying volumes of this solution were added to a fixed concentration of urease enzyme and incubated for 10 minutes. After incubation, a substrate solution was introduced, as mentioned in Table S5(B), and the reaction was monitored using UV-Vis spectroscopy at 570 nm over a period of 30 minutes. A similar reaction was performed with 0-50 nM of all sizes of AgNPs. Changes in absorbance were used to determine the extent of enzyme inhibition by the AgNPs, providing insights into the efficiency of different AgNP sizes in inhibiting urease activity.

Table S5 (A): Urease optimization

S.No.	H_2O (µL)	Urease enzyme (µL)	Substrate (µL)	Total reaction set to 250 µL	Results (Read after 10 minutes of incubation)
1	150	**0**	100		No color change (**Yellow**)
2	150	**0.2**	100		Color appeared very slow
3	150	**0.5**	100		Color changes to orange
4	150	**0.8**	100		Light pink
5	150	**1.0**	100		**Dark Pink color**

Table S5 (B): Urease inhibition by different size of AgNPs

S.No	AgNPs (5 – 100 nm) (nM)	H_2O (µL)	Urease (µL)	Substrate (µL)	10 minutes Incubation	Results (Read at 570 nm)
1	**0-50**	The volume set to 150	1	100		The color changes from yellow to pink.

References:

1 A. E. Segneanu, G. Vlase, A. T. Lukinich-Gruia, D. D. Herea and I. Grozescu, *Antioxidants*, DOI:10.3390/antiox11112261.

2 P. Wulandari, T. Nagahiro, N. Fukada, Y. Kimura, M. Niwano and K. Tamada, *J Colloid Interface Sci*, 2015, **438**, 244–248.

3 J. Coates, in *Encyclopedia of Analytical Chemistry*, Wiley, 2000.