Supporting information

Synthesis, Characterization and Catalytic Activity of Gold Nanoparticles Biosynthesized with *Rhizopus oryzae* Protein Extract

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Preparation of fungal protein from *Rhizopus oryzae* mycelia: The *R. oryzae* (NCIM 1009) used in this study was obtained from National Chemical Laboratory, Pune, India and maintained in potato dextrose agar (20% potato extract, 2% dextrose and 2% agar) slants. The organism was grown in potato dextrose broth by inoculating mycelia into medium and then incubated at 30 °C under static condition for 72 h into stationary phase. Following 72 h of fermentation, mycelia were harvested from the culture medium thoroughly washed with phosphate buffer solution (50 mM, pH 7.2), crushed with sea sand with a mortar and pestle at 4 °C. Cell debris was removed by centrifugation at 15,000 rpm for 30 min. The supernatant fluid (protein extract) was collected and dialyzed (10 kDa molecular weight cut off) overnight against the same buffer at 4 °C. Following dialysis protein was collected and used for AuNPs synthesis. The protein content in the extract was measured by the Bradford method.[1]

Characterization of gold nanoparticles: The size, shapes, crystallinity and elemental analysis of the as-synthesized AuNBC were carried out through high resolution transmission electron microscope (HRTEM) equipped with EDXA system. The samples for HRTEM analysis were prepared by drop-casting methodology. In brief, AuNBC was separated from solution by centrifugation (19,000 rpm for 20 min) followed by repeated washing in ultrapure water to remove unbound proteins from gold nanoparticle surface. The pellet was then dissolved in

ultrapure water and drop-casted on a carbon coated copper grid and soaked for 5 min. The excess solution was removed by placing a filter paper below the grid. Following desiccation, the samples were analyzed through HRTEM (JEOL JEM 2010, Japan) at an accelerating voltage of 200 kV. Conjugation of proteins on nanoparticles surface was studied by Fourier Transform Infrared (FTIR) and X-ray photoelectron spectroscopy (XPS). The samples were dried by lyophilisation. FTIR spectroscopy was recorded at a resolution of 4 cm^{-1}. Dried AuNBC was crushed with KBr in a mortar with 1:100 ratios. The pressed pellet was recovered with a clip, and immediately analyzed in the region of 4000–400 cm^{-1} over 500 scan in a FTIR spectroscopy (Perkin Elmer Spectrum GX FTIR, USA).

For X-ray Photoelectron Spectroscopy (XPS) study, dried powder sample was mounted on stainless steel holder. XPS was performed in a Kratos AXIS 165 spectrometer using monochromatic Al Kα radiation of energy 1486.6 eV. High resolution spectra were taken at fixed pass energy of 20 eV. Core level binding energies were determined using C 1s peak at 284.8 eV as the charge reference. For peak synthesis of high resolution spectra, a mixed Gaussian-Lorenzian function with a Shirley type background subtraction were used. Relative sensitivity factors used are from CasaXPS library containing Scofield cross-sections. Surface charge was efficiently neutralised by flooding the sample surface with low energy electrons. Standard procedures and literatures were followed to assign the peaks.

Estimation of gold ion concentration in AuNBCs: On completion of the reaction, AuNBC were separated by centrifugation at 20,000 rpm for 30 min. The solid particles were collected and dissolved in aqua regia. The concentration of gold ions was measured in triplicate by flame atomic absorption spectrometer (Varian Spectra AA 55, USA) against a standard gold solution. Initial gold ions concentrations were also measured.

For catalytic activity, AuNBCs suspensions were separated by centrifugation. Pellets were collected, washed with ultra pure water to remove any unreacted gold ions and finally resuspended in ultra pure water to adjust Au concentration same as presented in **Table S1**, column 3. The AuNBC were then diluted to make the final concentration of Au at 0.0101 mM in reaction mixture of catalytic activity. The removal of unreacted gold ions from AuNBCs eliminated the possibility of catalytic activity of free gold ions.

Table S1: The gold content prior to reaction and as determined after digestion of the AuNBCs.

Size of AuNBCs	Au concentration in reaction mixture (mM)	Au concentration in AuNBC (mM)	Au remaining (mM)[1]
5 nm	0.50	0.48	0.02
8 nm	1.25	1.20	0.05
20 nm	1.80	1.75	0.05
32 nm	2.20	2.10	0.10
42 nm	2.25	2.15	0.10
65 nm	2.52	2.4	0.12

[1]The remaining Au is obtained by difference.

XPS study of AuNBC: The chemical compositions of AuNBC were confirmed by XPS measurement. **Figure S1** shows the wide scan XPS spectra of AuNBC which demonstrate the presence of Au along with C, N, O, and P in the AuNBC. The core-level spectra of C 1s, N 1s, O 1s, and P 2p are presented in **Figure S2**. The C 1s spectrum (**Figure S2A**) can be resolved into five components that can be assigned to proteins bound to nanoparticle surface. The peak at 284.8 eV can be attributed to C-C and C-H. Carbon bound to nitrogen (C–N) and hydroxyl groups (C–OH) are located at 285.5 and 286.4 eV, respectively. The peak at 287.9 eV corresponds to carbonyl groups (aldehydic or ketonic carbon). The high binding energy peak at 289.1 eV is attributed to carboxylate (O=C-OH) and amide carbon (N-C=O) of proteins molecules. The carboxylate (O=C-OH) and amide carbon (N-C=O) peaks were shifted by 0.6 eV to higher energy with respect to the control protein that show a peaks at 288.5 eV. The N 1s core levels are broad and centred at 399.9 and 401.4 eV (**Figure S2B**), which can be assigned to the unprotonated and protonated amine /amide species, respectively present in protein molecules. In the control protein, the protonated amine /amide species appeared at 0.5 eV higher energy i.e. 401.9 eV. Shifting of carboxylate and amine groups following binding with metal nanoparticles was previously reported.[2-4] Mukerjee et al.[5] also reported covalent binding of AuNPs with protein molecules through NH_2-functionalities. Non-specific encapsulation of proteins around AuNPs might also occur. Three distinct peaks at 531, 532.3 and 533.3 eV in the O 1s spectrum (**Figure S2C**) represent the incorporation of C=O, C–OH and O-C-O components of protein moiety, respectively. The P 2p spectrum (**Figure S2D**) is fitted with a doublet $2p_{3/2}$ and $2p_{1/2}$ at

133.7 eV and 134.6 eV corresponding to the phosphate groups of protein molecules attached to Au nanoparticles. The peak for oxygen atoms from P-O-C chemical bonding, due to binding of phosphate with protein, is reported to be centred at 533.5 eV in phosphate ester. This peak overlaps with O-C-O chemical bonding observed at 533.5 eV. This result indicates that chemical interaction between gold and different functional groups of protein molecules occurs in the conjugation process, which increase the stability of the biosynthesized gold nanoparticles.

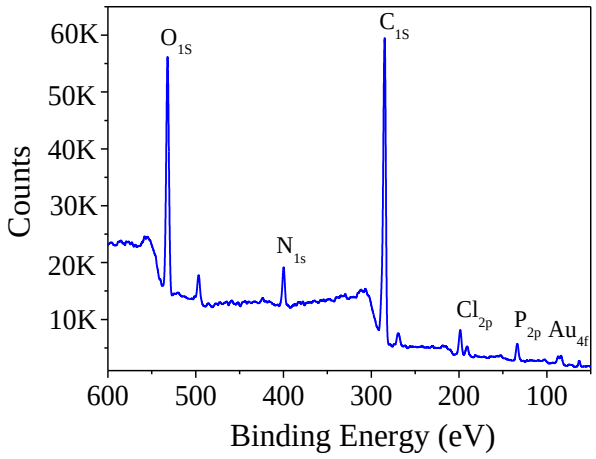

Figure S1: XPS survey spectra of AuNBC.

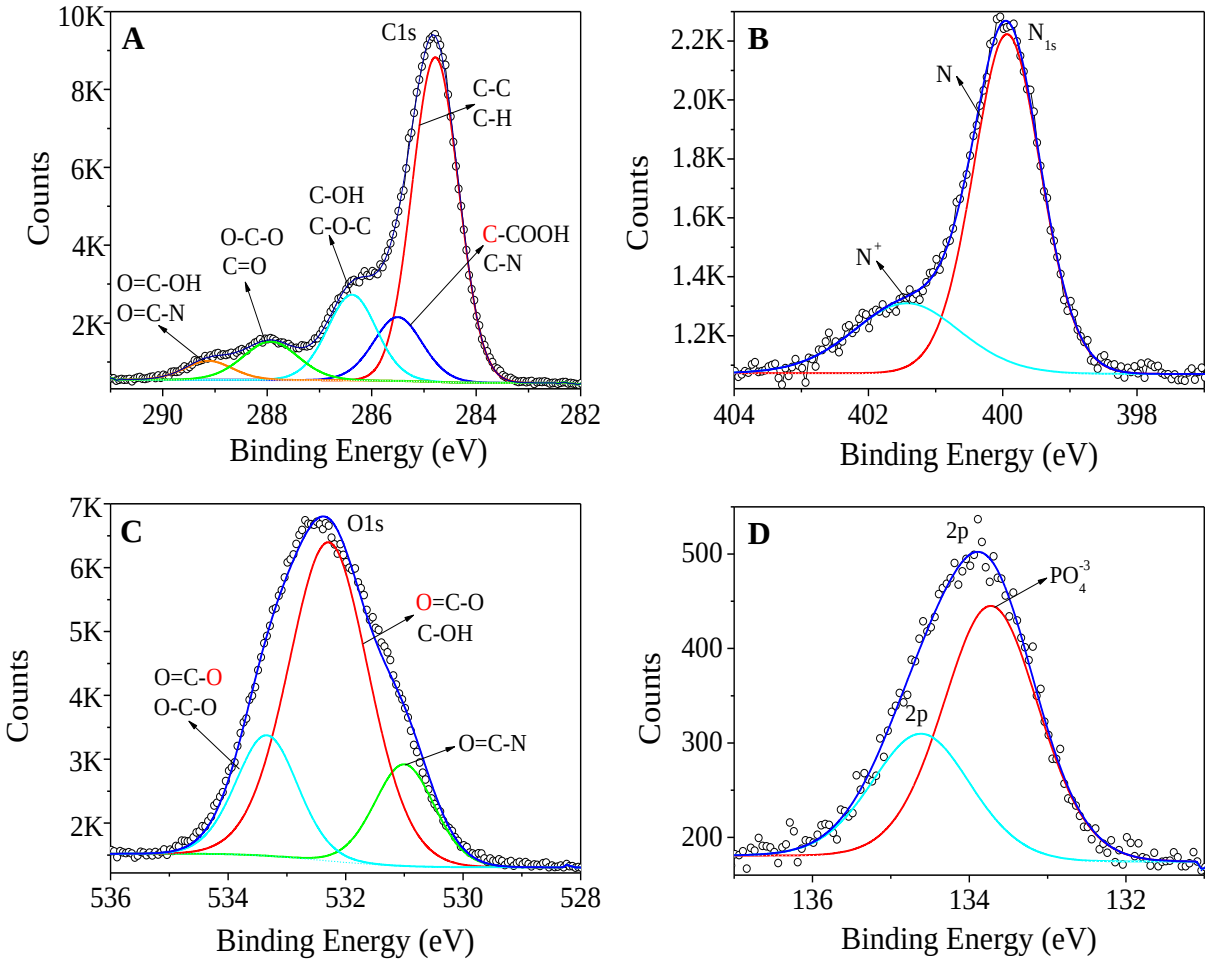

Figure S2: Core level XPS spectra C 1s (A), N 1S (B), O 1s (C) and P 2p (D) of AuNBC.

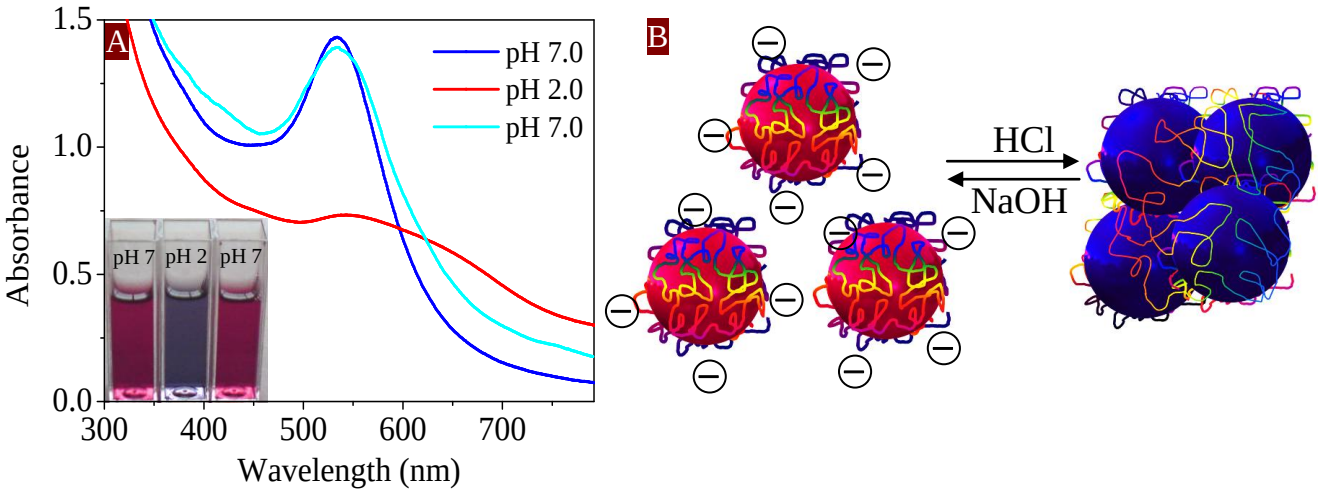

Figure S3: UV-vis absorption spectra (A) of pH mediated reversible cycling between aggregated and dispersed states of AuNBC. Inset picture (A) shows the aggregated (left) and disaggregated (right) cycle of AuNBCs. (B) Proposed model for the assembly and disassembly of negatively charged gold AuNBCs upon switching solution pH between 2.0 and 7.0.

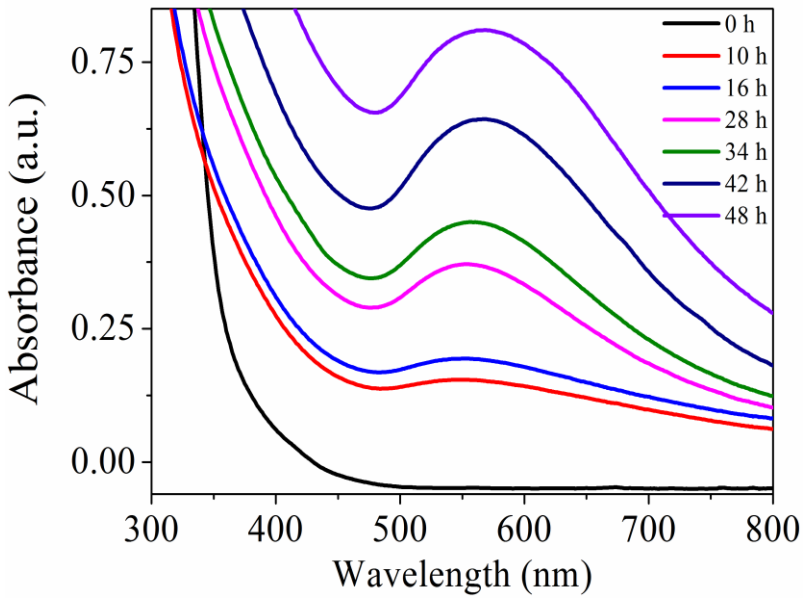

Figure S4: UV-vis spectra of gold nano-flower formation at different time intervals.

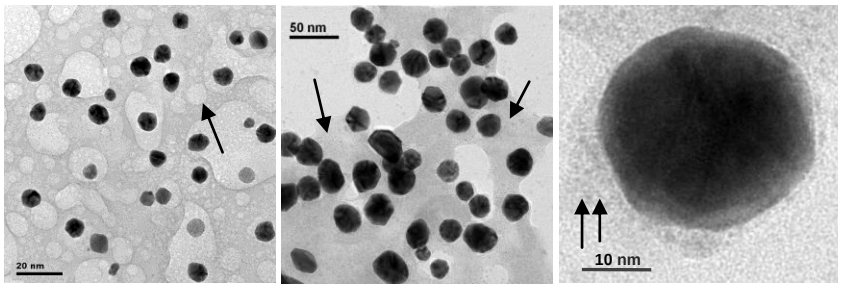

Figure S5: TEM images show capping of protein layer around the nanoparticles surface. Arrows indicate protein layer.

Table S2: Catalytic rate constant (k_{app} min^{-1}) of AuNBC at different temperatures

Temperature	AuNBC					
(^oC)	5 nm	8 nm	20 nm	32 nm	42 nm	65 nm
20	0.1±0.015	0.14±0.016	0.48±0.03	0.3±0.026	0.24±0.018	0.17 ±0.03
30	0.17±0.02	0.2 ±0.018	0.73±0.05	0.43±0.04	0.34±0.02	0.25 ±0.04
40	0.22±0.022	0.29±0.016	1.11±0.09	0.71 ±0.06	0.44±0.03	0.36 ±0.03
50	0.36±0.031	0.45±0.035	1.75±0.1	1.65 ±0.12	0.66±0.05	0.53 ±0.06
60	0.59±0.07	0.78±0.065	2.59± 0.17	1.95 ±0.16	1.3±0.11	0.97 ±0.1

References

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