Methionine-stabilized nonclassical growth within self-assembled *de novo* gold nanoparticles in conjunction with secondary nucleation inhibition

Jitendra K. Sahu, Shahbaz Ahmad Lone and Kalyan K. Sadhu*
Department of Chemistry, Indian Institute of Technology Roorkee, Roorkee – 247667, Uttarakhand, India
Email: sadhu@cy.iitr.ac.in

Supporting Information

EXPERIMENTAL SECTION

Materials.

Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), 6-mercaptohexanoic acid, 3-mercaptopropanoic acid and ethyl 4-amino-2-(methylthio)pyrimidine-5-carboxylate were purchased from Sigma-Aldrich. α-lipoic acid was purchased from TCI India. Hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) and glutathione (oxidized and reduced forms) were purchased from SISCO Research Laboratories. Methionine and sodium thiosulfate pentahydrate was purchased from Himedia Laboratories Pvt. Ltd. All the peptides used for this study were purchased from the GL Biochem (Shanghai) Ltd. Sodium sulfate was purchased from Rankem India. Sodium hydroxide was purchased from Thomas Baker and trisodium citrate dehydrate was purchased from Merck chemicals. All the chemicals were used without any further purification or modification. All the stock solutions of amino acids, $NH_2OH.HCl$, $HAuCl_4.3H_2O$ and the sulfur containing compounds except 1, 3 and 4 were prepared in pure Millipore water. The sulfur containing compounds 1, 3 and 4 were prepared in 50:50 water:ethanol mixture. The stock solutions of peptides were prepared in molecular biology grade DMSO obtained from SISCO Research Laboratories.

Characterizations.

The absorbance of GNPs solution was measured using Synergy microplate reader (Biotek, USA). Other absorbance measurements were performed by the Agilent Cary UV−Vis Multicell Peltier. The absorbance data were taken over mostly 350−800 nm wavelength range. The TEM images were obtained from the FEI, Technai G2 20 STWIN instrument. The redispersed solid sample in aqueous medium was drop-casted on the carbon-coated TEM grid (200 mesh). The HRTEM images were obtained either from the JEM 3200FS or JEM 2200FS Electron Microscope. The average size of the particles was calculated with the help of Image J software. A Zetasizer Nano ZS90 (Malvern Instruments) was used to measure the ζ-potential of the resultant particles with three measurements, each constituting 15 runs. The emission studies were performed through an Agilent Cary Eclipse Fluorescence Spectrophotometer by maintaining the similar detector voltage in all the cases. The excitation spectrum was obtained using the same instrument within the range of 300–420 nm with λ_{em} = 430 nm. X-ray photoelectron spectroscopy (XPS) data was obtained from a PHI 5000 Versa Prob II, FEI Inc. For characterization purpose a C60 sputter gun was used. The oxidation states of the Au were

analyzed by XPS with monochromatized Al K(α) excitation (h =1486.6 eV). The XPS peaks were calibrated by setting the C 1s value to 284.8 eV and Auger electron spectroscopy (AES) module the position of the elements. IR spectra were obtained in the mid IR range of 4000–450 cm^{-1} in a PerkinElmer spectrophotometer with the wavenumber expressed in cm^{-1}. The KBr was dried overnight and the samples were mixed, grinded and made into pellets form.

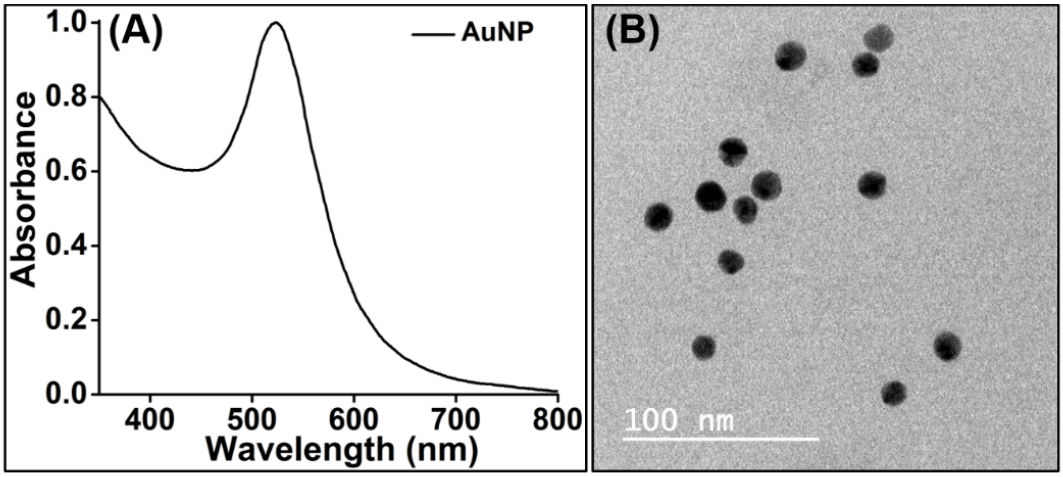


Figure S1. (A) Absorption spectrum of AuNP with SPR peak at 522 nm and (B) TEM image of AuNP (avg. size: 15.0 ± 2.0 nm), scale bar: 100 nm.

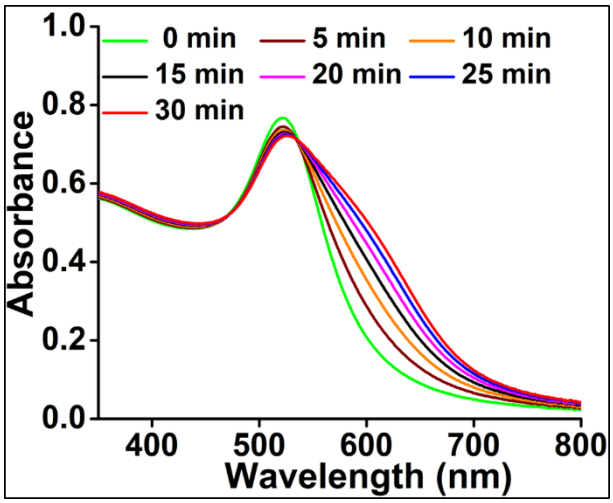


Figure S2: Absorption spectra of 1.2 nM AuNP incubated with 9 mM Met for 30 minutes before the growth reaction.

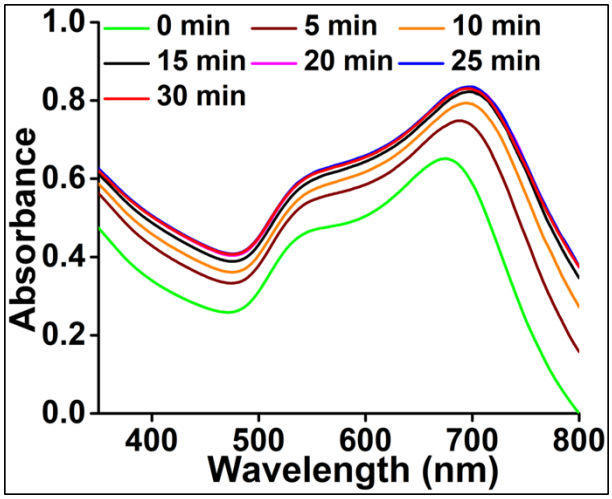


Figure S3: Time dependent absorption spectra of 1.2 nM AuNP incubated with 9 mM Met and 300 μM Au^{3+}.

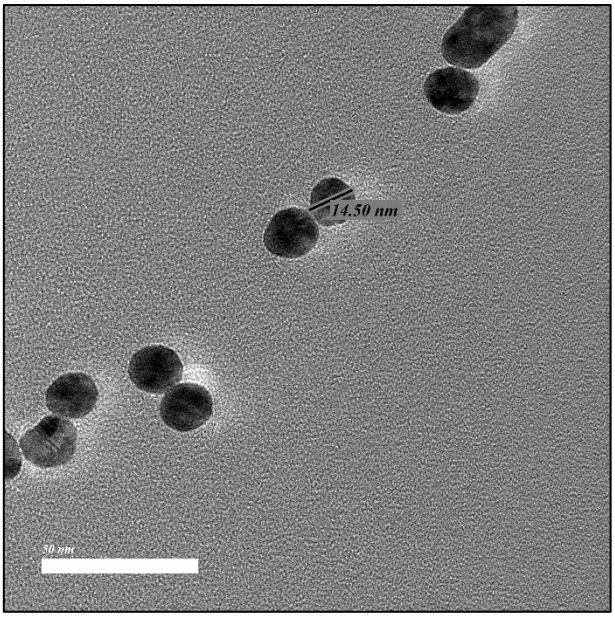


Figure S4. TEM image of the spectator seed after the growth reaction having identical size to the parent AuNP mentioned in Figure S1, scale bar: 50 nm.

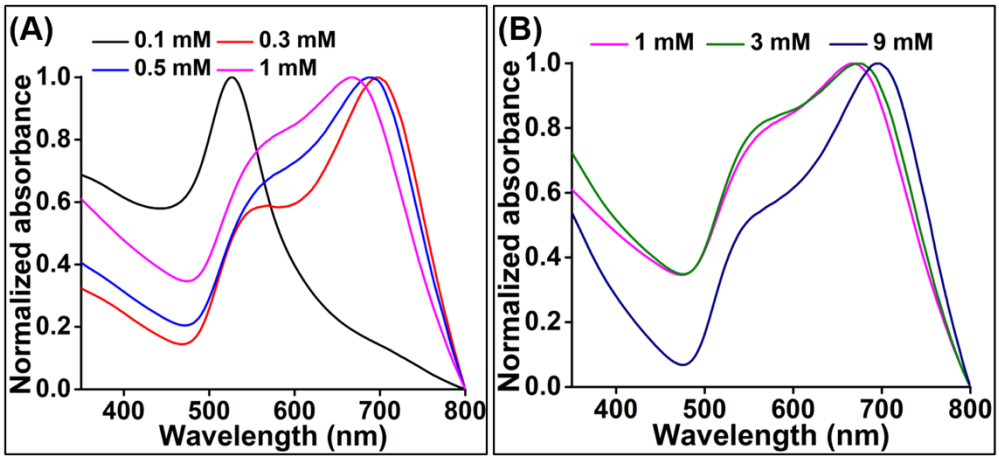


Figure S5: Normalized absorption spectra after 30 minutes growth reaction of 1.2 nM AuNP incubated with variable Met concentration and 300 μM Au^{3+}.

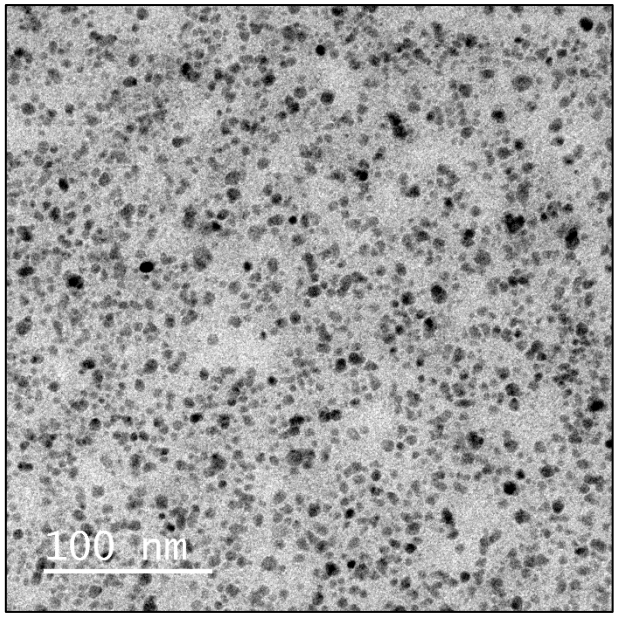


Figure S6. TEM image of the segregated nAuNPs formed after 30 min growth reaction of 1.2 nM AuNP in presence of 1 mM Met and 300 μM Au^{3+}, scale bar: 100 nm.

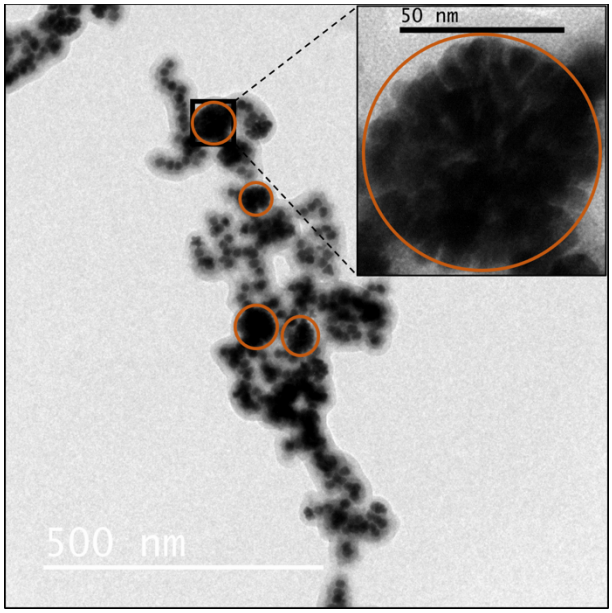


Figure S7. TEM image of the assembly of nAuNPs formed (orange circle) after 30 min growth reaction of 1.2 nM AuNP in presence of 3 mM Met and 300 μM Au^{3+}, scale bar: 500 nm. Inset shows the enlarged image of self-assembled nAuNPs, scale bar: 50 nm.

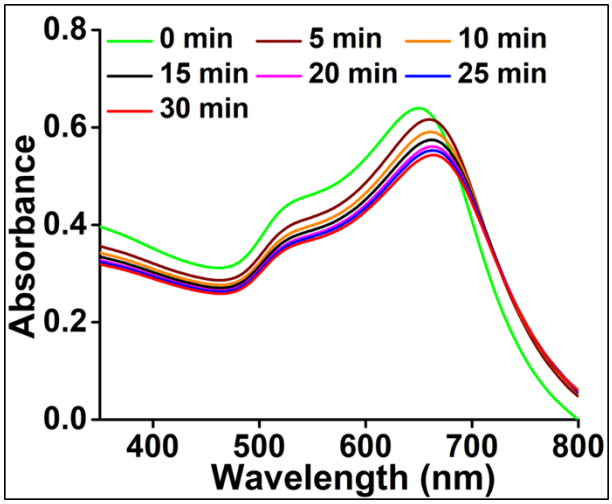


Figure S8: Time dependent absorption spectra during growth reaction of 1.2 nM AuNP incubated with 9 mM Met concentration and 10 μM Au^{3+}.

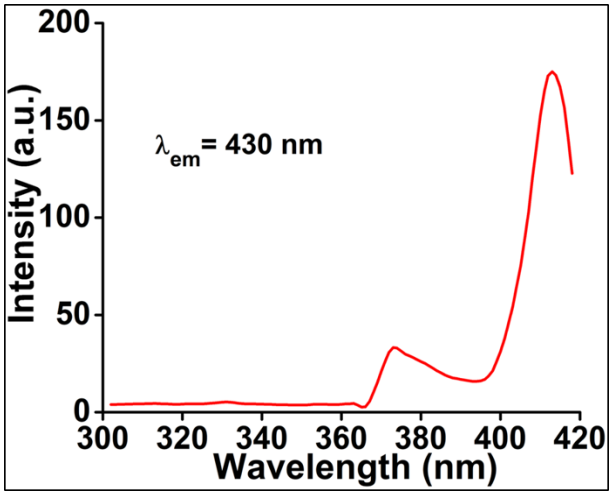


Figure S9. Excitation spectrum (λ_{em} = 430 nm) of self-assembled nAuNPs.

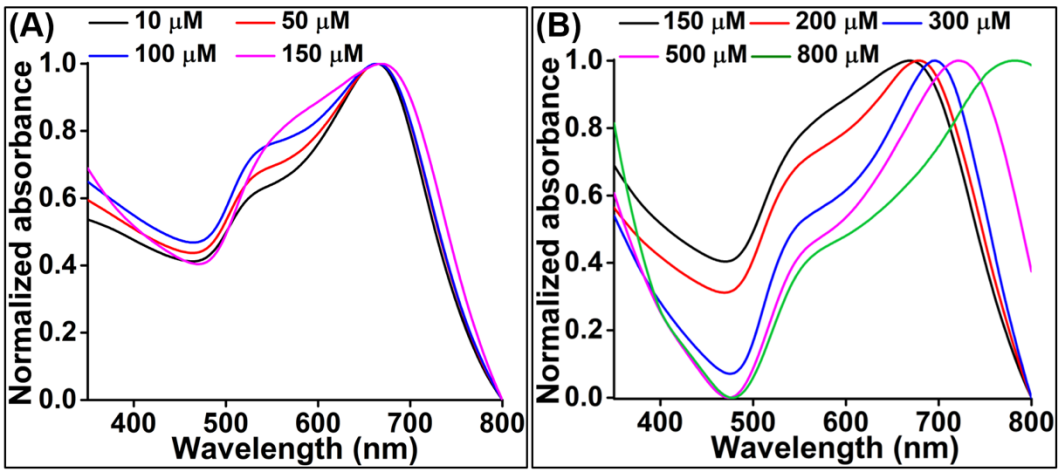


Figure S10: Normalized absorption spectra after 30 minutes growth reaction of 1.2 nM AuNP incubated with 9 mM Met and variable Au^{3+} concentration.

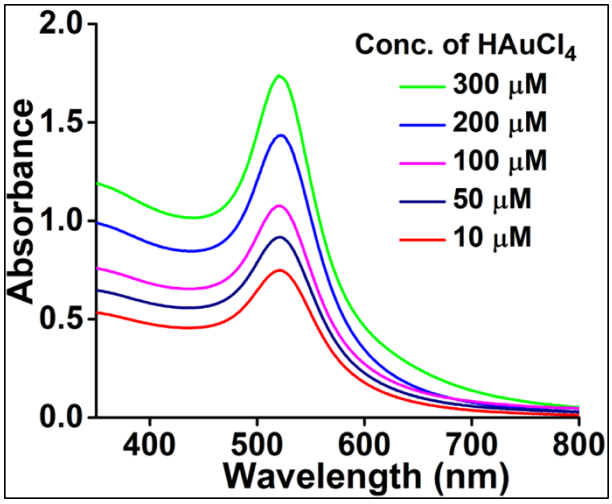


Figure S11: Absorption spectra after 30 minutes growth reaction of 1.2 nM AuNP with variable Au^{3+} concentration in absence of Met.

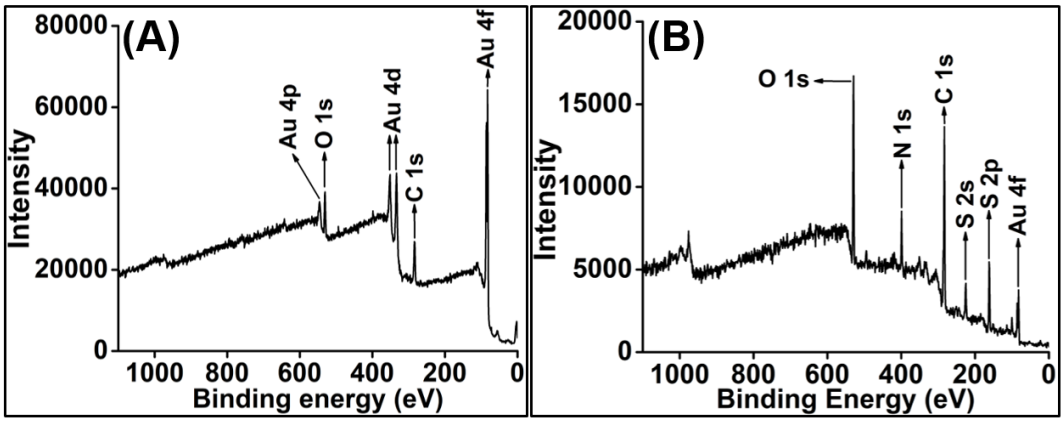


Figure S12. XPS survey spectra analysis of (A) AuNPs (B) self-assembled nAuNPs.

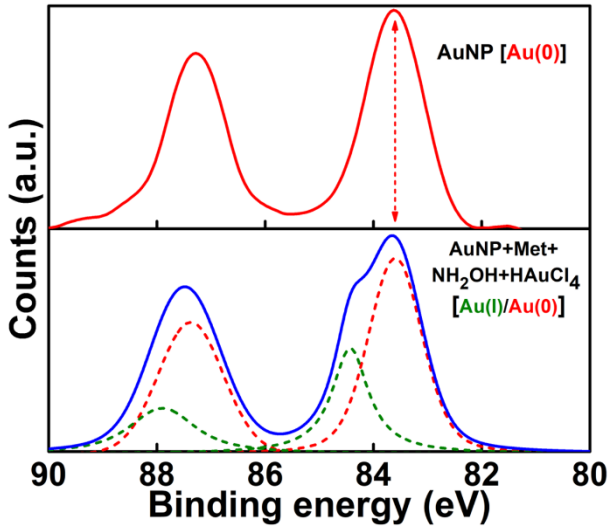


Figure S13. XPS spectra analysis Au $4f_{7/2}$ (right peak) and $4f_{5/2}$ (left peak) in case of (top) AuNPs having Au^0 (red) and (bottom) combination of self-assembled nAuNPs and spectator seed (blue) showing the generation of Au^+ (Olive).

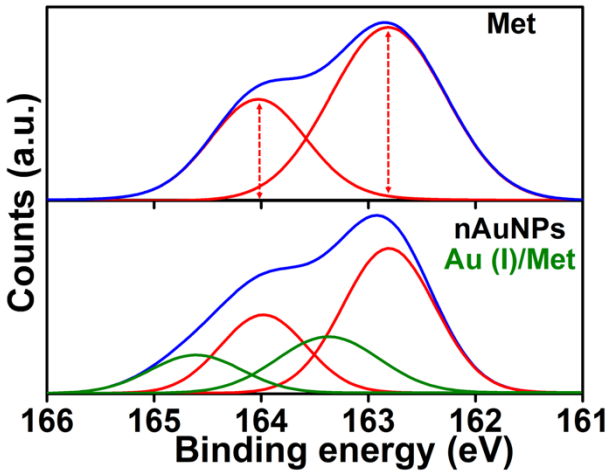


Figure S14. XPS spectra analysis of S $2p_{3/2}$ (right peak) and $2p_{1/2}$ (left peak) in case of (top) Met having free thio-ether (red) and (bottom) combination of self-assembled nAuNPs and spectator seed showing interacting thio-ether with Au^{+} (olive).

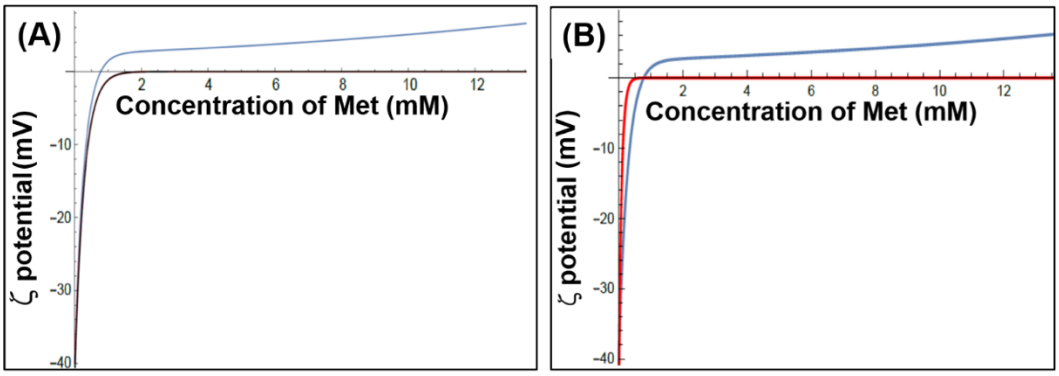


Figure S15. Blue fittings in (A) and (B) are based on $\zeta = \zeta_1 e^{-a(m)[Met]} + \zeta_2 e^{-b(1-m)[Met]}$ equation (1). In comparison, $\zeta = \zeta_1 e^{-a(m)[Met]}$ has been used for the black fitting with m = 0.32 in (A) and red fitting with m = 1 in (B) assuming ζ potential trend is solely dependent on Au^{+}-Met interaction. The coefficient ζ_1 (–42.3 mV) and constant *a* (11.3) from blue curves for equation (1) have been used in black and red curves.

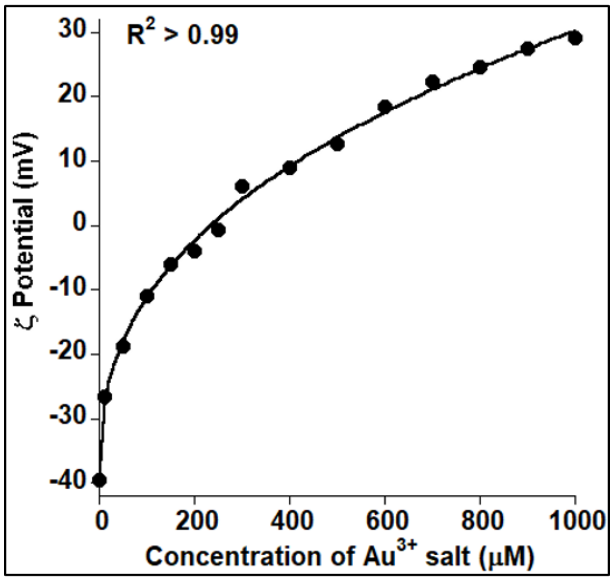


Figure S16: ζ potential after 30 minutes growth reaction of 1.2 nM AuNP incubated with 9 mM Met and variable Au^{3+} concentration.

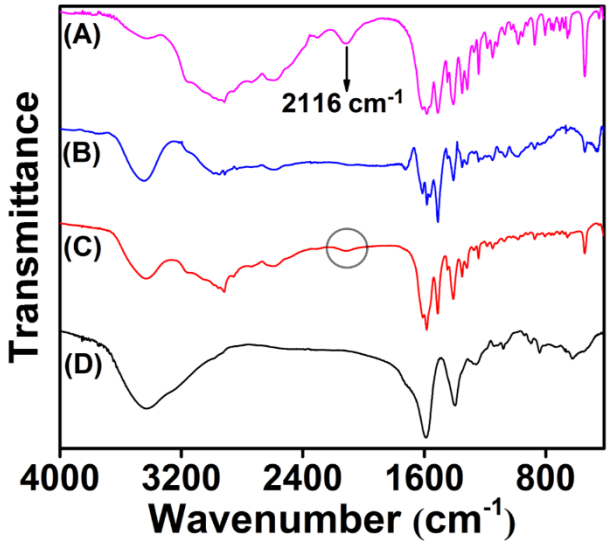


Figure S17: IR spectra of (A) Methionine, (B) self-assembled nAuNPs, (C) AuNP incubated with Met only and (D) AuNP.

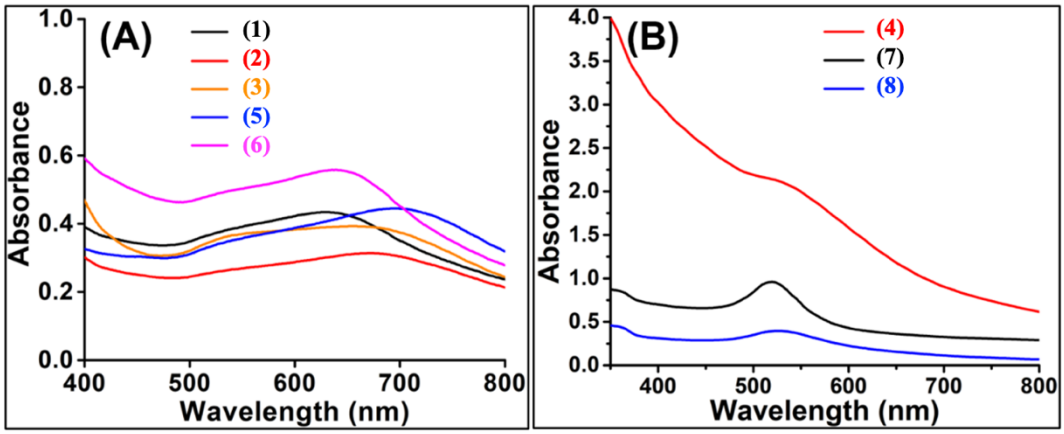


Figure S18: Absorption spectra after 30 minutes growth reaction of 1.2 nM AuNP incubated with (A) 6-mercaptohexanoic acid (1), 3-mercaptopropionic acid (2), ethyl 4-amino-2-(methylthio)pyrimidine-5-carboxylate (3), glutathione reduced (5), glutathione oxidized (6) and (B) Lipoic acid (4), sodium sulfate (7), sodium thiosulfate (8) (9 mM 1–8) and 300 μM Au^{3+} in each case.

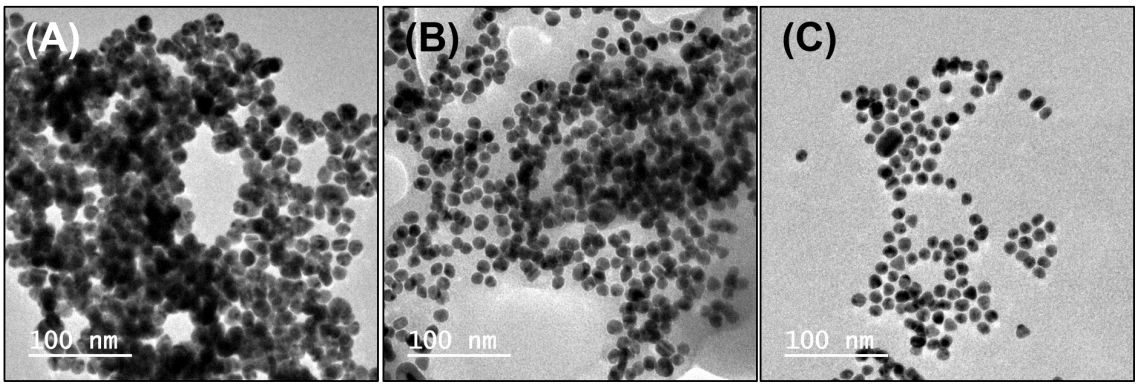


Figure S19. TEM images after 30 minutes growth reaction of 1.2 nM AuNP incubated with (A) glutathione oxidized, (B) glutathione reduced, (C) lipoic acid and 300 μM Au^{3+} in each case, scale bar: 100 nm.

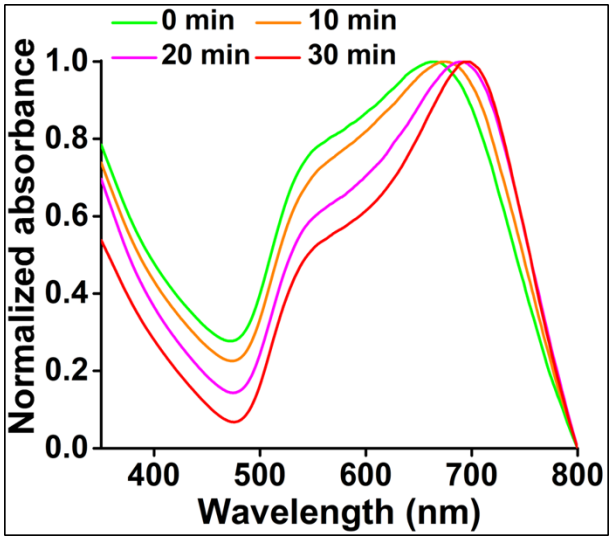
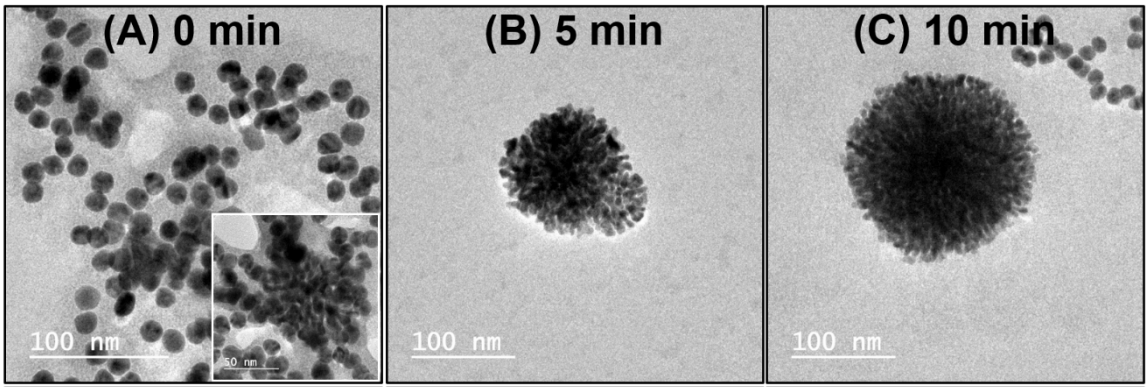


Figure S20: Normalized absorption spectra after 30 minutes growth reaction of 1.2 nM AuNP incubated with 9 mM Met concentration for variable time and 300 μM Au^{3+}. The incubation times of 1.2 nM AuNP with 9 mM Met have been varied for 0–30 minutes.



FigureS21: (A-C) TEM images after 30 minutes growth reaction of 1.2 nM AuNP incubated with 9 mM Met concentration for variable time and 300 μM Au^{3+}. The incubation times of 1.2 nM AuNP with 9 mM Met have been varied for (A) 0 minute, (B) 5 minutes and (C) 10 minutes.

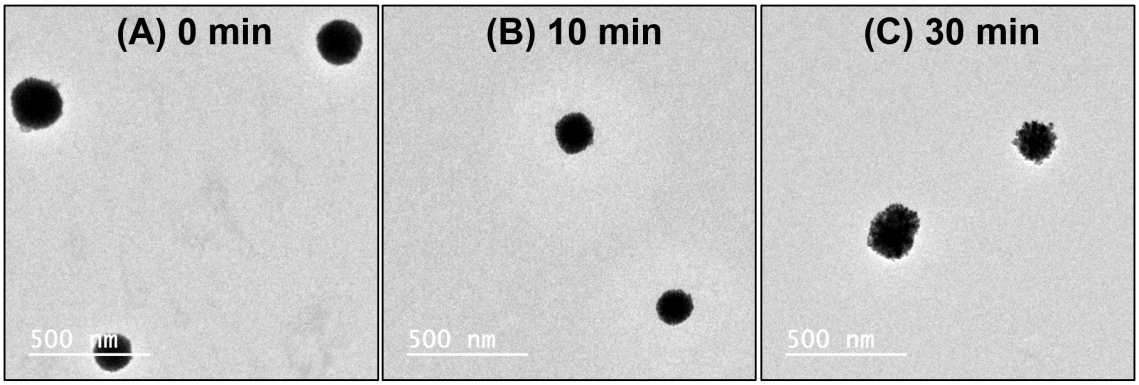


Figure S22: TEM images after variable growth reaction time (A) 0 min, (B) 10 min and (C) 30 min for 1.2 nM AuNP incubated with 9 mM Met concentration and 300 μM Au^{3+}. The incubation time of 1.2 nM AuNP with 9 mM Met have been kept constant for 30 minutes for all the cases.

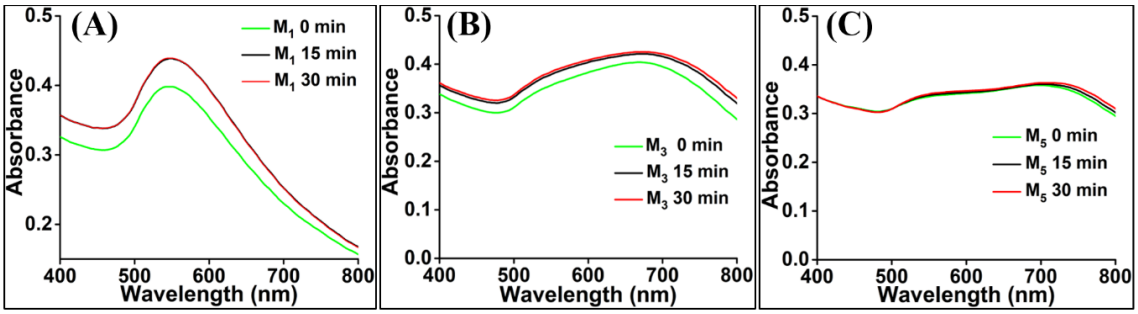


Figure S23: Absorption spectra after 30 minutes growth reaction of 1.2 nM AuNP incubated with 300 μM (A) M_1, (B) M_3, and (C) M_5 and 300 μM Au^{3+}.