Electronic Supplementary Material (ESI) for RSC Advances

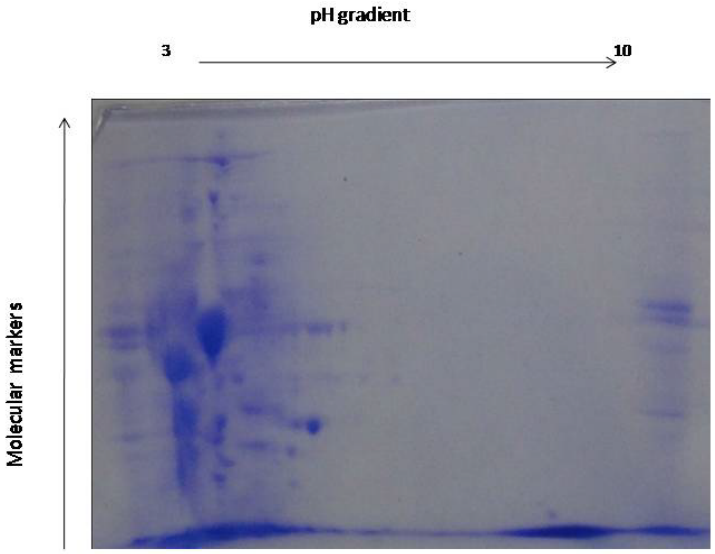
Enzyme mediated synthesis of water-dispersible, naturally protein capped, monodispersed gold nanoparticles; characterization and mechanistic aspects.

Shadab Ali Khan and Absar Ahmad*

Supporting Information

Two dimensional electrophoresis (2-DE)

Protein samples were precipitated on ice with 100%TCA and the final concentration was adjusted to 10%. Precipitate was recovered by centrifugation at 10,000 rpm at 4°C for 10min and washed twice with chilled acetone and dried in air. Protein precipitate (125 µg) was resuspended in 2D-rehydration buffer (8M urea, 2% CHAPS, 50mM dithiothretiol (DTT) 0.2% (w/v) Bio-Lyte 3/10 ampholytes and trace amount of Bromophenol blue). Protein samples were loaded by in-gel rehydration method onto a 7cm precast immobilized pH gradient IPG strip (pH 3–10) and iso-electrofocusing (IEF) was performed on a PROTEAN IEF Cell from Bio-Rad at 20°C for 10,000V-hr with the end voltage of 4000V. After IEF was achieved, the strip was reduced in equilibration buffer. The strip was then loaded on a 10% Polyacrylamide gel and resolved by SDS-PAGE. Protein spots were visualized by staining with Coomassie brilliant blue R-250.



Supporting figure 1: Preliminary Two Dimensional Electrophoresis of extracellular broth of *Thermomonospora* sp. showing number of spots corresponding to different proteins. A broad range IPG strip (3-10) was used and IEF was carried out horizontally from left to right which was followed by one dimensional SDS-PAGE (10%) vertically from top to bottom using different molecular weight markers.

Determination of sulfite reductase enzyme activity

The total reaction mixture of 1.5 ml contained 1.0 mM of freshly prepared sodium sulfite in 100 mM MES buffer, pH 6.0, 1.0 mM EDTA, 0.15 mM NADPH and appropriately diluted enzyme. The reaction was initiated by the addition of NADPH followed by incubation at 50^{o}C. The oxidation of NADPH was monitored spectrophotometrically at 340 nm. Samples containing sulfite reductase enzyme incubated in the absence of sodium sulfite served as blank. One unit of sulfite reductase enzyme activity is defined as the amount of enzyme required to oxidize 1 mole of NADPH/min under the assay conditions.

Fraction	**Total Protein (mg)**	**Total Activity (U)**	**Yield (%)**	**Specific activity milliunits / (mg) protein**	**Purification fold**
Crude	315	25.25	100	0.215	1
FPLC (mono Q) Chromatography	23	18.25	73	2.58	12
SDS-PAGE Elution	8	14.2	56	10.1	47

Supporting table 1: Sulfite reductase enzyme assay in different fractions

Amino acid analysis

The amino acid composition was determined on an AccQ-Fluo (Waters Corporation, USA) equipped with a fluorescent detector. Salt free enzyme sample (50µg) was hydrolyzed in 6 N HCl at constant boiling, in a vacuum sealed hydrolyzing tube for 24h at 110^{o}C. After hydrolysis, the sample was again lyophilized, dissolved in 100 µl of borate buffer (0.5M, pH9.0) and derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (AQC). 1µl of the hydrolysate was subjected to analysis on an AccQ-Tag Column equipped with a fluorescent detector.

Amino acid	Number of residues/mol
Ala	27
Met	19
Pro	16
Phe	17
Gly	21
Ile	18
Val	16
Trp	14
Leu	18
Cys	15
Asn	14
Gln	09
Ser	13
Thr	26
Tyr	11
Asp	14
Glu	39
Lys	23
Arg	13
His	07
Total	350

Supporting table 2: Amino acid composition of sulfite reductase enzyme