Proteins enriched in charged amino acids control the formation and stabilization of selenium nanoparticles in *Comamonas testosteroni* S44

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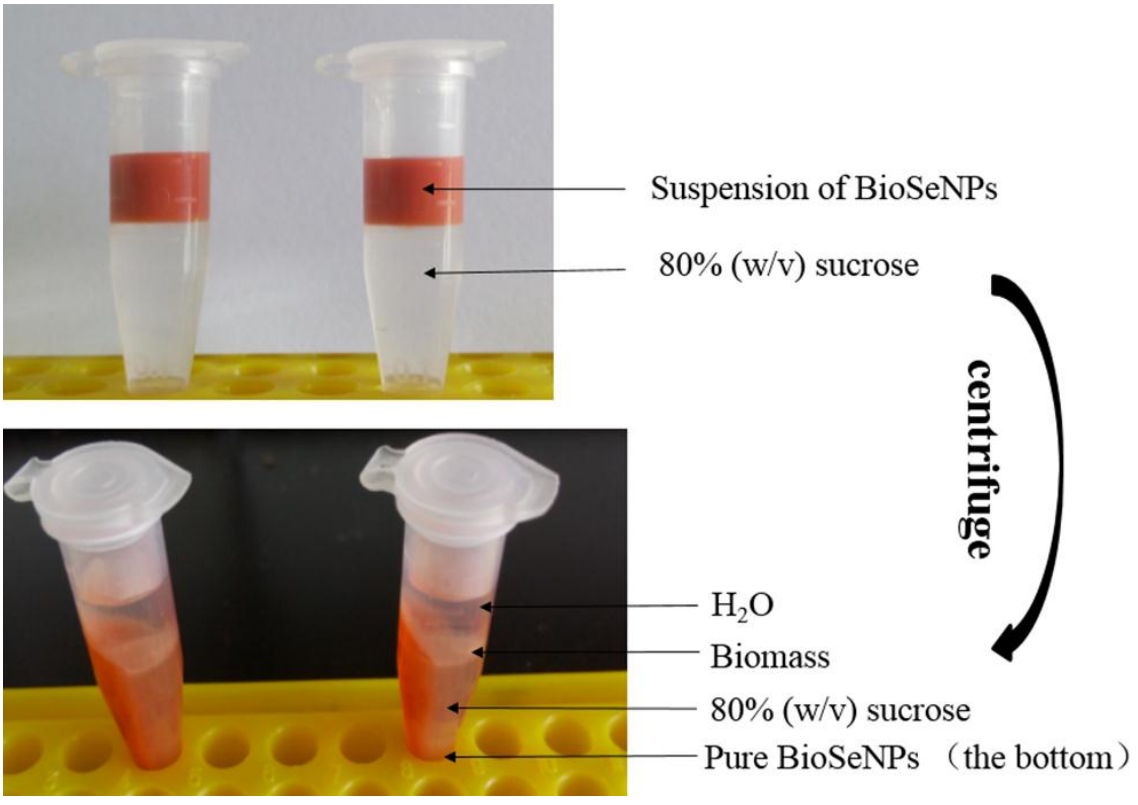

Fig. S1. Method of BioSeNPs purification

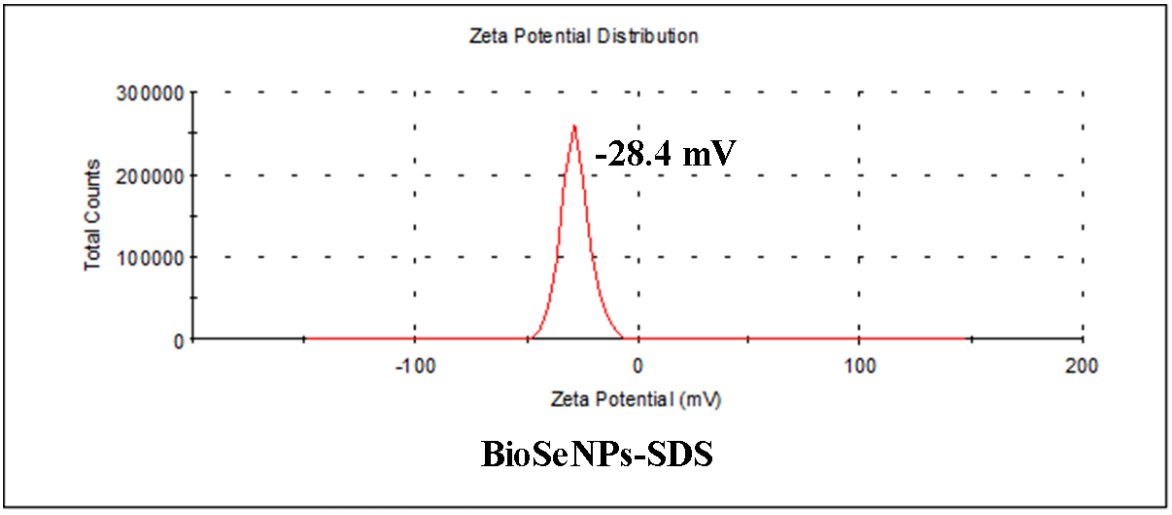

Fig. S2. The zeta potential of BioSeNPs after the coating agents were almost removed by 10 % SDS solution.

Method

LC-MS/MS Analysis

Samples were re-suspended with Nano-RPLC buffer A (Sangon, Shanghai, China). The online Nano-RPLC was employed on the Eksigent nanoLC-Ultra™ 2D System (AB SCIEX). The samples were loaded on C18 nanoLC trap column(100 μm ×3cm, C_{18}, 3 μm, 150 Å) and washed by Nano-RPLC Buffer A (0.1%FA, 2%ACN) at 2μL/min for 10 mins. An elution gradient of 5-35% acetonitrile (0.1%formic acid) in 90 mins gradient was used on an analytical ChromXP C18 column (75μm x 15 cm, C18, 3μm 120 Å) with spray tip. Data acquisition was performed with a Triple TOF 5600 System (AB SCIEX, USA) fitted with a Nanospray III source (AB SCIEX, USA) and a pulled quartz tip as the emitter (New

Objectives, USA). Data were acquired using an ion spray voltage of 2.5 kV, curtain gas of 30 PSI, nebulizer gas of 5 PSI, and an interface heater temperature of 150 ℃. For information dependant acquistion (IDA), survey scans were acquired in 250 ms and as many as 35 product ion scans were collected if they exceeded a threshold of 150 counts per second (counts/s) with a 2^+ to 5^+ charge-state. The total cycle time was fixed to 2.5s. A rolling collision energy setting was applied to all precursor ions for collision-induced dissociation (CID). Dynamic exclusion was set for ½ of peak width (18 s). And the precursor was then refreshed off the exclusion list. Based on combined MS and MS/MS spectra, proteins were successfully identified based on 95% or higher confidence interval of their scores in the MASCOT V2.3 search engine (Matrix Science Ltd., London, U.K.), using the following search parameters: cow-lacbobacillus casel mix database, trypsin as the digestion enzyme, two missed cleavage site, fixed modifications of Carbamidomethyl (C), partial modifications of Acetyl (Protein N-term), Deamidated (NQ), Dioxidation (W), Oxidation (M) Phospho (ST) and Phospho (Y). ±15ppm for precursor ion tolerance and ±0.15Da for fragment ion tolerance.