Supporting Information

Effect of Surface Chemistry and Morphology of Gold Nanoparticle on the Structure and Activity of Common Blood Proteins

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Material and methods:

Materials: All glass wares were washed with aqua regia (3 HCl: 1 HNO3), followed by rinsing several times with double distilled water. All the Chemicals Gold (III) Chloride hydrate ($HAuCl_4$, 99.99%), Sodium Citrate tribasic hydrate (> 99%), Sodium Borohydride ($NaBH_4$, 99%), L- Ascorbic Acid were purchased from Sigma Aldrich. CTAB (N-Cetyl-N,N,N-trimethyl-ammonium bromide), Silver Nitrate ($AgNO_3$,99.5%), Sodium Hydroxide Pellet purified (NaOH, 97%), were purchased from Merck. Sodium Iodide (NaI, 99%), p-nitro-phenol were purchased from Fisher Scientific. Double distilled 18.3 mΩ deionized water (Elga Purelab Ultra) was used throughout the preparation of solutions. Human serum albumin (HSA A9511, 99.99%) was purchased from Sigma Aldrich.

Synthesis of TGNP:

TGNP was synthesized by the established seed mediated method [1]. Approximately 5 nm spherical seed was prepared by mixing 0.5 mL of a 20mM aqueous $HAuCl_4 \cdot 3H_2O$ solution, 1 mL of a 10mM aqueous solution of sodium citrate and 1 mL of a 100mM aqueous $NaBH_4$ (Ice-cold) solution in 36.5 mL of deionized water. The solution was vigorous stirred till color of the solution turned into red. To prepare TGNP, three labeled (namely 1, 2 and 3) flasks were prepared. A mixture of 108 mL of 0.05 M aqueous CTAB solution and 54μL of 0.1 M aqueous NaI solution was divided into the above three containers. 9 ml of mixture was added in each container 1 and 2. The remaining mixture 90 ml was added in container 3. Finally, a mixture of 125μL of a 20mM aqueous $HAuCl_4 \cdot 3H_2O$ solution, 50μL of 100mMNaOH, and 80μL of 100mM ascorbic acid was added to each container 1 and 2. A mixture of 1.25 mL of 20mM $HAuCl_4 \cdot 3H_2O$, 0.5 mL of 100mMNaOH, and 0.5 mL of 100mM ascorbic acid was added to

container 3. 1 ml of the seed solution was added to container 1 with mild shaking, followed by addition of 1 ml of container 1 solution into container 2. After gentle shaking, the whole solution of container 2 was added to container 3. The solution was kept overnight and extra amount of CTAB was removed from the solution by centrifugation (5000 rpm for 10 minutes) followed by re-suspension of the TGNP in water.

Synthesis of GNR:

GNR was also synthesized using the seed mediated method [2]. Seeds were prepared by adding an aqueous ice-cold $NaBH_4$ solution (0.600 mL, 0.01 M) to a solution obtained by adding aqueous $HAuCl_4 \cdot 3H_2O$ (0.250 mL, 0.01 M) to an aqueous solution of CTAB (7.5 mL, 0.10 M). The seed growth was allowed to growth for 2 hours. Next, an aliquot of the seed solution was added to a solution containing aqueous CTAB (95 mL, 0.10 M), $HAuCl_4 \cdot 3H2O$ (4 mL, 0.01 M), aqueous $AgNO_3$ (0.6 mL, 0.01 M) and an aqueous ascorbic acid solution (0.64 mL, 0.10 M). Immediately after the addition of seed solution, the mixture was stirred gently for 10 s. Finally, the solution was kept at 27 °C (in a water bath) undisturbed for at least 3 hours. The extra CTAB was removed by centrifugation (5000 rpm for 10 minutes) followed by resuspension in water.

Synthesis of CTAB Coated spherical GNP:

CTAB coated spherical GNP was synthesized by using a seed-mediated growth method [3].The container for seed synthesis held 5 mL of 0.50mM $HAuCl_4 \cdot 3H_2O$ and 5 mL of 0.20 M CTAB. The solution was reduced by addition of 600μL of ice-cold $NaBH_4$ (0.010 M). Next, the container was shaken vigorously for 2 min and occasionally opened to vent for any evolved hydrogen gas. The seed solution was brown suspensions and was allowed to age for 2 hours. 12μL of seed solution was to a solution already containing 9.50 mL of 0.10 M CTAB, 80μL of

0.010 M $AgNO_3$, 500μL of 0.010 M $HAuCl_4 \cdot 3H_2O$ and 55μL of 0.10 M Ascorbic Acid. The mixture was stirred for 10min. This resulted in a red suspension that was again left undisturbed for 24 hour to increase the yield. The extra CTAB was removed by centrifugation and re-suspension of the nanoparticle into equal amount of water.

Synthesis of Citrate coated spherical GNP:

Citrate stabilized spherical GNP of 40 nm diameter was prepared by seed mediated method [4]. 36.5ml deionized water taken in a conical flask and 0.5 ml of 10mM $HAuCl_4 . 3H_2O$ was added into it. The temperature was maintained at 40 °C. The mixture was vigorously stirred on a hot magnetic stirrer. 1 ml of 5mM aqueous solution of sodium citrate and 1 ml of 50mM $NaBH_4$ (Cold) solutions were added to the solution. The solution was then kept at room temperature with additional stirring for 10 minutes until the color of the solution turned from pale yellow to light Red. The resulting mixture was aged for 2-4 hour to allow the hydrolysis of unreacted $NaBH_4$.

Characterization of synthesized GNPs:

Particle size and dispersity of the synthesized nanoparticles were characterized by using a TECNAI 200 kV TEM (FEI, Electron Optics). The hydrodynamic diameter (D_H) of the nanoparticles and their protein complexes were measured by DLS using Zeta Sizer Nano, equipped with a He Ne laser illumination at 633 nm in a single photon counting mode using avalanche photodiode for signal detection (Malvern Instrument). The CD spectra were measured using Jasco spectrophotometer equipped with a thermostatically controlled cell holder. The far UV region was scanned in between 200 to 260 nm with an average of three scanning with a band width of 5nm. The final spectra were obtained by subtracting the buffer contribution from the

original sample spectra. UV-vis absorbance spectra of nanoparticles were measured by UV-2576 series from Schimadzu.

Protein adsorption on the surface of GNP:

The major blood proteins adsorbed on the nanoparticles surface by electrostatic interaction or by the formation of salt bridges. In the present case, adsorption of protein on to citrate coated spherical GNP occurred most probably with the formation of salt bridges of the carboxylate ammonium type, between the citrate and the lysine on the protein surface. On the other hand, electrostatic interaction should favor the protein adsorption onto the CTAB stabilized TGNP, GNR and spherical GNP.[12] After surface functionalization with PEDOT:PSS, proteins are adsorbed on the PSS terminating surface and adsorption takes place mainly through end-on configurations. The proteins can fully cover the adsorption surface and even lead to a quite dense layer [5]. All experiment was carried out at pH 7.4.

Cell culture:

A human breast cancer cell line MDA-MB 231, was obtained from NCCS (national centre for cell science), Pune, India. All cell culture related reagents, such as DMEM (dulbecco's modified eagle medium), FBS (fetal bovine serum), penicillin, MTT (methyl thiazolyltetrazolium) were purchased from Sigma Aldrich. MDA-MB-231 cells were grown in normal DMEM culture medium with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin.

Cytotoxicity assay:

The in-vitro cytotoxicity was measured using a standard MTT assay. MDA-MB 231 cells were seeded into 96-well cell culture plate at 10^4/well and then incubated for 24 h at 37°C under 5% CO_2. The cells were incubated with fixed concentration of all nanoparticles (with and without

modification of PEDOT:PSS) for 72 h followed by 1 h incubation with the MTT reagent. Then the optical density of the sample was recorded using multimode plate reader (TECAN).

Theoretical simulation

Surface modifications of GNP (111). The surface was modified by simply changing the coordinates in the PDBQT files [6-8]. The different surfactant molecules were added by adding their PDBQT coordinates and parameters to the PRBQT file of the gold surface. Molecules were aligned to the intended region with proper orientations. MATLAB7.10.0 and NOTEPAD++ were used to carry out all the coordinate transformation. The ligand was treated as a rigid entity. These molecules along with all other surfactant molecules were optimized by MGL Tools 1.5.4 by adding Marsilli–Gasteiger partial charges on each constituent atom.

Docking experiments. The MGL Tools 1.5.4 was used as a user interface to prepare the docking files and AutoGrid4 and AutoDock4 was used to carry out the grid and energy calculations respectively [9,10]. The new parameter library was used in both cases. The grid was set to the maximum size allowed by Auto Dock which is 126 points in each three directions with grid spacing of 1 Angstrom. This much of grid volume was to ensure that the gold surface can move freely around the small protein at the center. The standard Auto Dock force fields were used for energy calculations while the best conformation was optimized by Lamarckian Genetic Algorithm (GA). With an initial population size of 150 and number of evaluation of 250 000, the GA was allowed to run up to 27 000 generations to find one best individual. Each of our experiments was set for 100 GA runs. The gene mutation and crossover rate was considered as 2%and 80% respectively. Auto Dock Clustering were analyzed to know the nature of the

simulation result, i.e., how well they were converging and what was the population size for each conformation.

CD spectrometry: The CD spectra were measured using a Chirascan Cd/2T spectrophotometer equipped with a thermostatically controlled cell holder. Protein concentration was used as 10μM for all the measurements. The far UV region was scanned in between 200 to 260 nm with an average of three scanning with a band width of 5nm. The final spectra were obtained by subtracting the buffer contribution from the original sample spectra. The ellipticity MRE was calculated as follows.

$$\text{MRE (deg cm}^2\text{ dmol}^{-1}) = \frac{\theta_{obs}}{C_p n l \times 10}$$

Where, θ_{obs} is the observed ellipticity in mdeg, Cp is the molar concentration of the protein, n is the number of amino acid residues, l is the cell path-length.

The change in % helicity was then determined from the following equation [11]

$$\%\ \alpha\ \text{Helix} = \frac{-(\text{MRE} - 2340)}{30300} \times 100$$

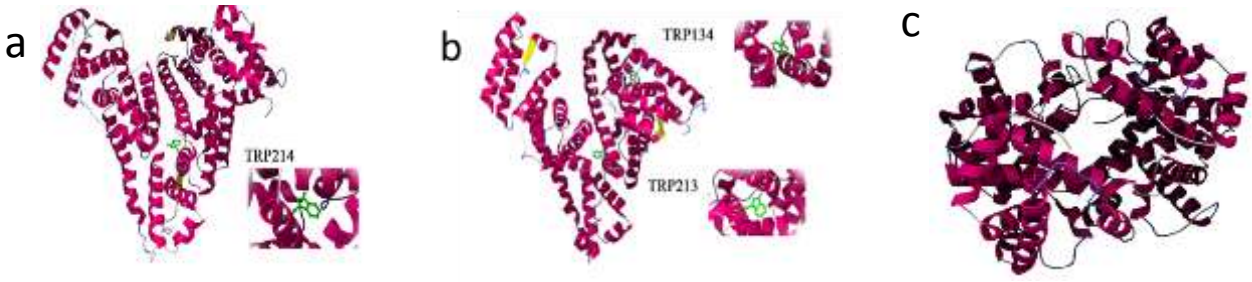

Fig. S1: Three dimensional structures of (a) HSA (b) BSA and (c) HB. HSA and BSA have closely similar in structure, whereas the structure of HB is completely different.

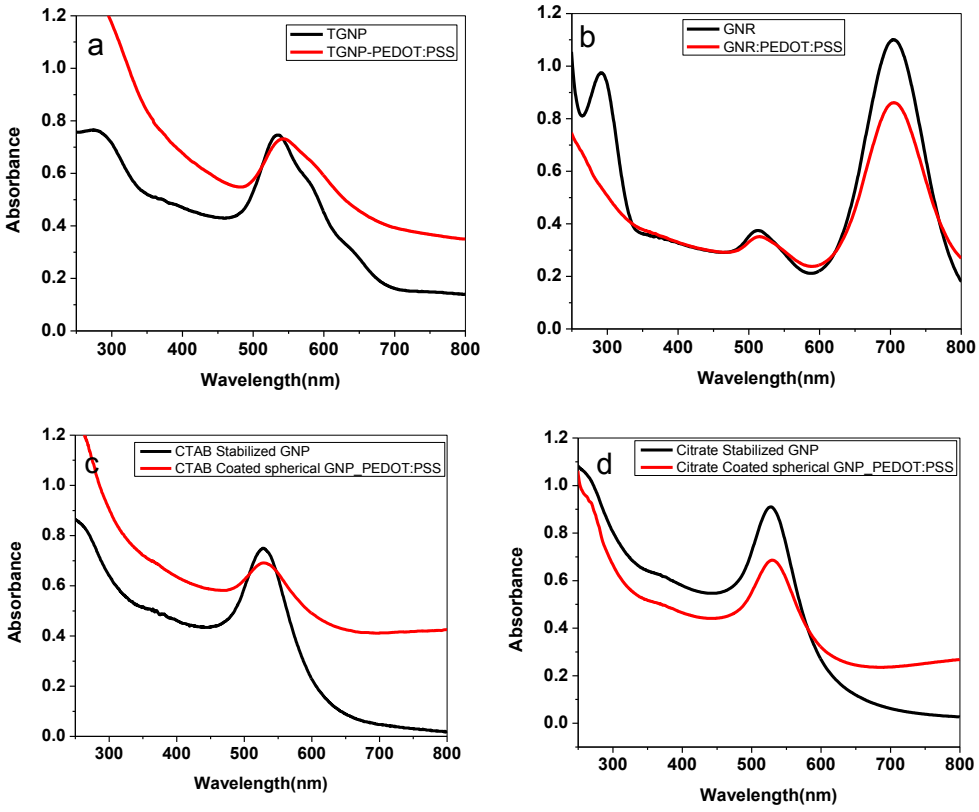

Fig. S2: SPR absorption bands for the as synthesized (Black) and PEDOT: PSS (red) functionalized (a) TGNP (b) GNR (c) CTAB coated spherical GNPs and (d) citrate coated spherical GNPs.

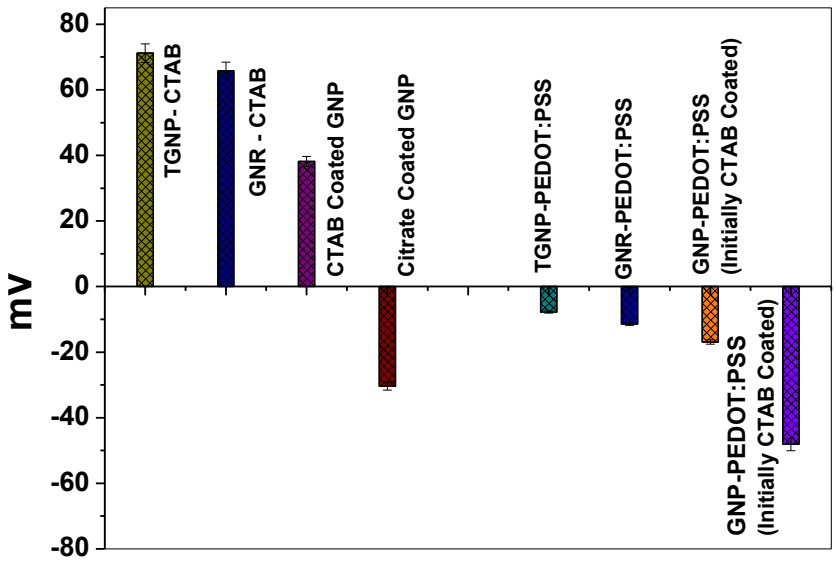

Fig. S3: Zeta potential of the GNPs without and with surface functionalization by PEDOT:PSS.

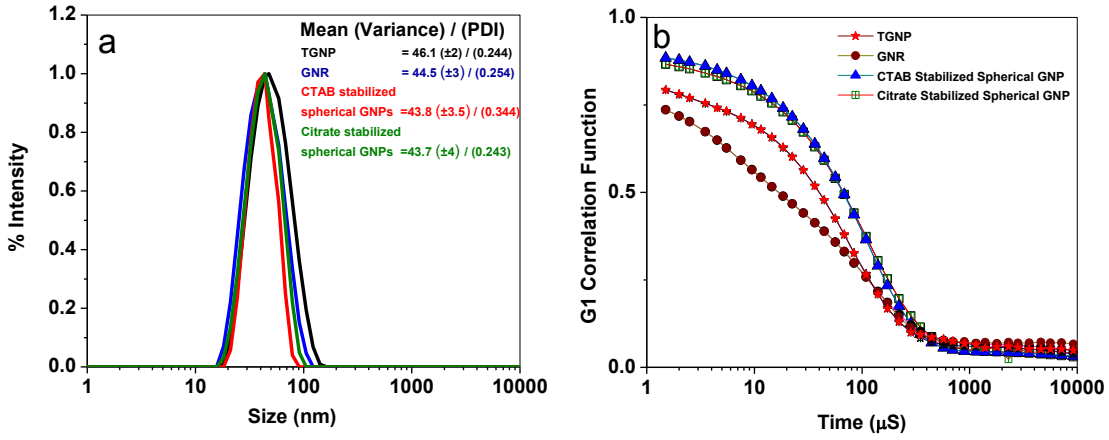

Fig. S4: (a) Dynamic Light scattering graph of the as synthesized different GNPs. Mean, variance and PDI (polydispersity index) are shown in the inset captions. (b) Distribution fit curve of the GNPs.

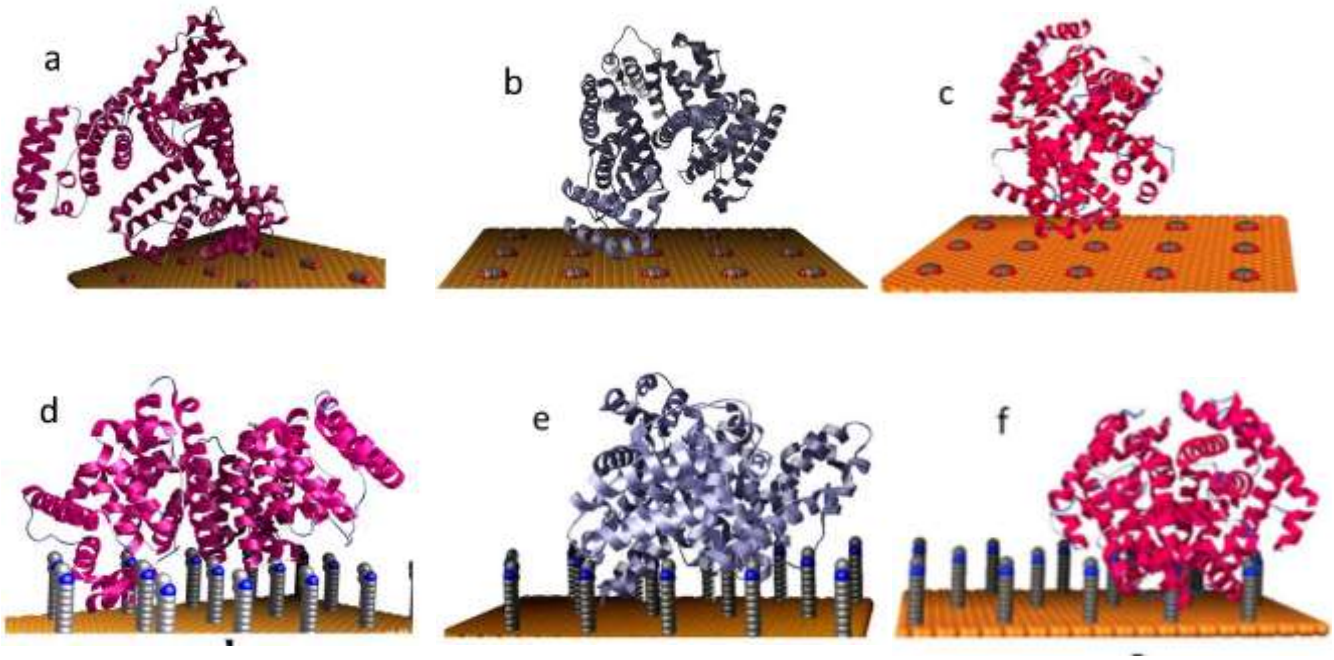

Fig. S5: Simulated orientation of (a) HSA (b) BSA and (c) HB on the surface of citrate coated GNP while (d) to (f) showing the same on a CTAB coated GNP.

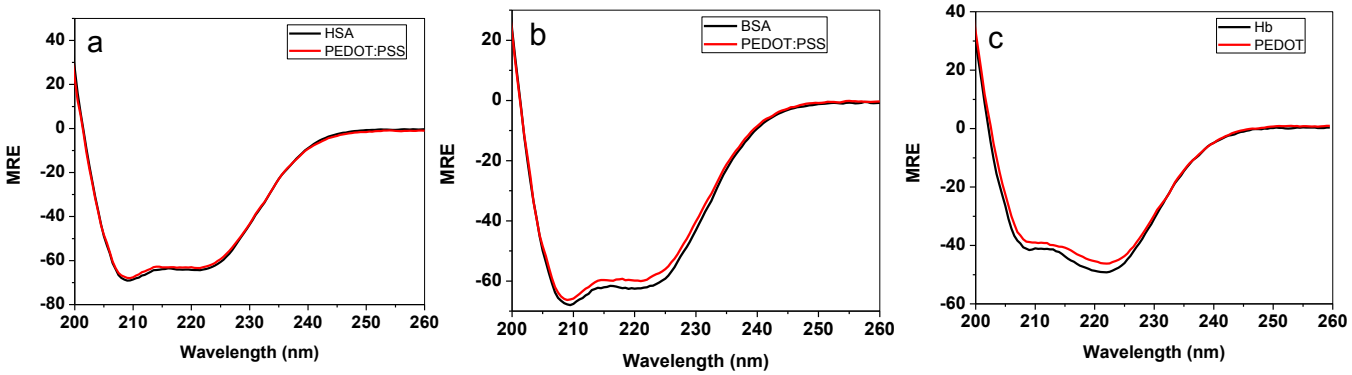

Fig. S6: (a) CD spectra of (a) HSA (b) BSA and (c) HB in presence of PEDOT:PSS. Secondary structure changes in presence of PEDOT:PSS is very less.

Table S1: The Zeta potential of all four nanoparticle without modification and after modification with PEDOT:PSS.

S.N	Nanoparticles	Zeta potential (without PEDOT:PSS)	Zeta potential (with PEDOT:PSS)
1	TGNP	+70	-8
2	GNR	+60	-11
3	CTAB Coated spherical GNP	+38	-16
4	Citrate Coated spherical GNP	-30	-48

Table S2: Surface coverage calculation of nanoparticles by foot print method [11].

Type of GNP	Surface area	Number Density (NP/ml)
TGNP	2152 nm^2	2.3 x 10^{16}
GNR	2368 nm^2	1.1 x 10^{16}
CTAB Coated spherical GNP	5074 nm^2	9.66 x10^{14}
Citrate Coated spherical GNP	5253 nm^2	8.46 x10^{14}

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