Shape dependent protein induced stabilization of gold nanoparticles: from protein corona perspective.

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Abstract

Gold nanoparticles (GNPs) are promising materials for many bioapplications. However, upon contacting with biological media, GNPs undergo changes. The interaction with proteins results in the so-called protein corona (PC) around GNPs, leading to the new bioidentity and optical properties. Understanding the mechanisms of PC formation and its functions can help us to utilise its benefits and avoid its drawbacks. To date, most of the previous works aimed to understand the mechanisms governing PC formation and focused on the spherical nanoparticles although non-spherical nanoparticles are designed for a wide range of applications in biosensing. In this work, we investigated the differences in PC formation on spherical and anisotropic GNPs (nanostars in particular) from the joint experimental (extinction spectroscopy, zeta potential and surface enhanced Raman scattering [SERS]) and computational methods (the finite element method and molecular dynamics [MD] simulations). We discovered that protein does not fully cover the surface of anisotropic nanoparticles, leaving SERS hot-spots at the tips and high curvature edges “available” for analyte binding (no SERS signal after pre-incubation with protein) while providing protein-induced stabilization (indicated by extinction spectroscopy) of the GNPs by providing a protein layer around the particle’s core. The findings are confirmed from our MD simulations, the adsorption energy significantly decreases with the increased radius of curvature, so that tips (adsorption energy: 2762.334 kJ/mol) would be the least preferential binding site compared to core (adsorption energy: 11819.263 kJ/mol). These observations will help the development of new nanostructures with improved sensing and targeting ability.
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Gold nanoparticles (GNPs) are promising materials for many bioapplications. However, upon contacting with biological media, GNPs undergo changes. The interaction with proteins results in the so-called protein corona (PC) around GNPs, leading to the new bioidentity and optical properties. Understanding the mechanisms of PC formation and its functions can help us to utilise its benefits and avoid its drawbacks. To date, most of the previous works aimed to understand the mechanisms governing PC formation and focused on the spherical nanoparticles although non-spherical nanoparticles are designed for a wide range of applications in biosensing. In this work, we investigated the differences in PC formation on spherical and anisotropic GNPs (nanostars in particular) from the joint experimental (extinction spectroscopy, zeta potential and surface enhanced Raman scattering [SERS]) and computational methods (the finite element method and molecular dynamics [MD] simulations). We discovered that protein does not fully cover the surface of anisotropic nanoparticles, leaving SERS hot-spots at the tips and high curvature edges “available” for analyte binding (no SERS signal after pre-incubation with protein) while providing protein-induced stabilization (indicated by extinction spectroscopy) of the GNPs by providing a protein layer around the particle’s core. The findings are confirmed from our MD simulations, the adsorption energy significantly decreases with the increased radius of curvature, so that tips (adsorption energy: 2762.334 kJ/mol) would be the least preferential binding site compared to core (adsorption energy: 11819.263 kJ/mol). These observations will help the development of new nanostructures with improved sensing and targeting ability.  
Introduction  
Gold nanoparticle (GNPs) possesses unique optoelectronic properties, which originate from their localized surface plasmon resonance (LSPR).[1–3] LSPR is a collective oscillation of conduction electrons caused by interaction with an electromagnetic wave (light) of specific (resonant) wavelength.[3–4] The optoelectronic
properties of GNPs can be fine-tuned through colloidal chemistry, via engineering nanostructures’ size and shape.\textsuperscript{[5,6]} For instance, anisotropic nanoparticles such as gold nanostars and nanorods have strong extinction coefficient in the near-infrared region where light shows deeper tissue penetration whereas spherical GNPs have distinct bright colour, that is due to light extinction in the visible region and sensitivity to change of their size.\textsuperscript{[7,8]} As such, GNPs have the potential for extensive medical applications in diagnosis and treatment. In pre-clinical settings, the use of GNPs includes point-of-care diagnostics\textsuperscript{[9,10]} (lateral flow assays such as pregnancy tests and rapid COVID-19 tests), in vivo imaging\textsuperscript{[11,12]} (photoacoustic and computerised tomoscopy), and therapeutics\textsuperscript{[13–15]} (photothermal therapy, radiotherapy, catalytic therapy, and drug delivery). Although GNPs treatment designs and applications are rapidly developing with some having reached clinical trials,\textsuperscript{[16–18]} none has yet been approved for clinical use primarily because it is hard to predict the changes that nanoparticles undergo and how they behave in complex biological systems. Upon exposure to biological media (e.g. plasma or serum), GNPs interact with large amounts of biomolecules which form layers around the nanoparticles. The main component of the layer is protein and it is referred to as the protein corona (PC).\textsuperscript{[19,20]} The PC characteristically consists of two layers: the “hard corona” – a layer of tightly bound proteins attached through covalent bonds, hydrophobic and electrostatic interactions; and the “soft corona” – a layer of loosely bound proteins outside the “hard corona” that are attached via weak electrostatic forces. The “soft corona” can easily be removed and thus exists in a dynamic state of constantly exchanging proteins, depending on the environment. Contrary to this, the “hard corona” tends to remain unchanged. Even after several centrifugation-resuspension cycles, tightly bound proteins remain on the surface of nanoparticles.\textsuperscript{[19,20]} The PC formation on GNPs induces high variations in their optical properties, targeting and sensing abilities, as well as their bio-recognition by cells (caused by aggregation, morphology changes and surface fouling).\textsuperscript{[21,22]} Some of the effects of a PC can also be beneficial to GNPs applications.\textsuperscript{[23,24]} Exposure of naked GNPs to high ionic strength liquids (biological fluids like blood and urine; buffers, such as phosphate buffered saline [PBS] or isotonic saline solutions) results in their aggregation due to the ion-nanoparticle interactions and loss of electrostatic repulsion.\textsuperscript{[25,26]} PC formation, however, prevents GNPs aggregation, preserving nanoparticles stability in high ionic strength liquids thus reducing cytotoxicity, create immunological stealthiness, increasing blood-circulation time, and preserving optical properties.\textsuperscript{[25]} To date, we have not fully explored the mechanisms underlying the formation of the PC on GNPs, and most of the GNP-protein interaction studies that have been carried out have been on spherical particles.\textsuperscript{[25,27,28]} Whilst spherical particles are often easiest to work with, it has been previously demonstrated that GNPs with different shapes and surface roughness interact with proteins differently from spherical nanoparticles.\textsuperscript{[29,30]} For example, in our previous study, we have demonstrated that ascorbate-capped gold nanostars reduce protein absorption resulting in enhanced sensing ability in protein-contained media.\textsuperscript{[31]} Further, non-spherical GNPs have been developed for a wide range of sensing applications. In order to use them with biological samples, we need to understand how anisotropic particles interact with proteins. With the increased demand for new nanostructures with enhanced optical properties, it is important to account for shape effects when considering biomedical applications. In this work, we revisit GNP-protein interaction with different shapes of gold nanoparticles (spherical, ellipsoidal and star-shaped) and discuss possible differences in protein-induced stabilization of spherical and anisotropic nanoparticles. We utilize computational methods including Finite Element Method (FEM) and molecular dynamics (MD) simulations to support our experimental observations. Our goal is to understand GNP-protein interactions and the PC mechanisms of GNPs stabilization with non-spherical particles to help create nanostructures that optimise the benefits and mitigate the flaws of PC formation.

2. Results and Discussion

2.1 GNPs synthesis and characterization

For this study, we prepared citrate-capped spherical and elongated gold nanoparticles of 50 and 70 nm diameter respectively (AuNP50 and AuNP70, Figure 1 A and B ) and ascorbate-capped gold nanostars (AuNS) of tip to tip diameter about 80 nm (Figure 1 C ). The synthesis methods are described in the
Supporting Information (Table S1). AuNP50 and AuNP70 have distinctive LSPR bands at 531 and 551 nm respectively. AuNS, with sharp tips, have a distinct LSPR peak in the NIR region at 751 nm and a shoulder in the same wavelength region as the spherical nanoparticles, which is attributed to the extinction (sum of all light that is not transmitted through a sample, absorbed and scattered) mode of the AuNS core (Figure 1 D). As synthesized AuNP50, AuNP70 and AuNS were monodispersed due to protective capping agents (citrate for AuNP and ascorbate for AuNS), that preserve electrostatic repulsion between the particles (Figure 1 E).

![Figure 1](image-url)

**Figure 1** A-C) Transmission electron microscopy images of AuNP50, AuNP70 and AuNS respectively; D) Extinction spectra of GNPs normalised to 1 at their maximum; E) Size distribution measured by Nanoparticles Tracking Analysis (NTA)

2.2 Protein-induced stabilization of GNPs in media with high ionic strength

To test the nanoparticles’ stability in high ionic strength media, GNPs (concentration adjusted to $1 \times 10^{10}$ particles/mL for all samples) were either centrifuged and resuspended in $1 \times$ PBS (NaCl: 137 mM, KCl: 2.7 mM, Na$_2$HPO$_4$: 10 mM, KH$_2$PO$_4$: 1.8 mM) or incubated for 30 min with (9 mg/mL) bovine serum albumin (BSA) and then centrifuged and resuspended in PBS. The presence of a large amount of salts in the PBS caused GNPs (without protein) aggregation as indicated in the extinction spectra by a loss of peak sharpness and extinction intensity (Figure 2 A, B and C). By way of contrast, nanoparticles coated with protein prior to resuspension in PBS, remained stable. The LSPR bands of GNPs (with protein) remained sharp with extinction intensities similar to those of the original GNPs. A small red-shift of the LSPR bands of GNPs (with protein) is observed due to the change of the refractive index of the media near the nanoparticles’ surface upon protein absorption. GNPs salt-induced aggregation or protein-induced stabilization were also confirmed by nanoparticles size analysis – the mean size and size standard deviation of nanoparticles increased 2 times (see Supporting Information Table S1).

It is accepted that the high ionic strength medium reduces the nanoparticle citrate electrostatic repulsion leading to aggregation whereas the PC displaces the citrate but stabilizes nanoparticles by acting as a “complex” surfactant and protecting the GNPs. That knowledge is, however, based on tests only with spherical particles. In other publications that discuss PC formation on anisotropic nanoparticles, the adsorption sites and protein-induced stabilization are either not discussed or assumed to be the same as for spherical nanoparticles – that is uniform protein coverage. However, we had previously observed that AuNS samples had reduced BSA fouling – i.e. not full PC coverage – and retained the ability of AuNS to enhance Raman intensities of small molecules. We, therefore, expected to see some effect of ionic strength (aggregation).
even in the presence of BSA and were surprised to see the nanostar data of **Figure 2** which imply that the nanostars and spherical particles behave in the same way (retaining their stability after pre-incubation with BSA). We are left with the question of how GNPs with complex shapes have a protein-mediated stabilization mechanism which prevents aggregation, while having plasmonic “hot-spots”, areas of high curvature with tightly confined plasmons, that create large electric field enhancement, accessible to analytes (**Figure 2 D**).[34]

![Figure 2](image)

**Figure 2** Extinction spectra of GNPs, GNPs in PBS and GNPs pre-treated with BSA prior to resuspension in PBS: A) AuNP50, B) AuNP70 and C) AuNS. D) Scheme of protein-induced stabilization of GNPs in high ionic strength liquid.

### 2.2.1 Stability as a function of protein concentration

To study further the mechanism of protein-induced stabilization of GNPs of different shapes, we incubated AuNP50, AuNP70, and AuNS with aqueous BSA solutions of different concentrations (0.1, 2, 9 and 15 mg/mL) without any salts present. Extinction spectra of GNPs samples incubated with BSA without removal of unbound proteins (“unwashed”) are shown in **Figure 3 A–C**. Extinction spectra of the same samples after one cycle of centrifugation and resuspension in water (“washed”) are shown in **Figure 3 D–F**. The AuNS show little change as a function of the protein concentration, but spherical particles intriguingly aggregated at intermediate protein concentrations and do not at low or high concentrations.
After the “washing” cycle to remove unbound proteins, all three types of GNPs demonstrated similar behaviour patterns – at least some aggregation, when the GNPs were preincubated with BSA at concentrations \([?] \geq 2\) mg/mL and stabilization, when preincubated with BSA at concentrations \([?] \geq 9\) mg/mL. It could be due to only very tightly bound BSA molecules remaining on the GNPS during the “washing”, while weakly bound proteins were lost.

These observations are supported by zeta potential measurements of the “washed” samples (Figure 4). Aqueous-media exposed –COOH groups of the capping agents are ionized, creating negative surface charges and an acidic pH of the colloid GNPs solutions (pH \(\approx 3\) for all three solutions after synthesis). At a pH lower than its isoelectric point, BSA molecules have a net positive charge. Thus, after preincubation with a low concentration of BSA and removal of any unbound protein, the absolute surface charge of the GNPs@BSA nanoparticles is much lower than the as-synthesized samples – almost neutral causing aggregation due to the reduction of interparticle electrostatic repulsion. We speculate that with the increase of protein bound to the nanoparticles, the solution pH increases, and GNPs@BSA charge stabilizes at around \(-15\) mV. It is generally accepted that absolute values of surface charge lower than 30 mV are not high enough to prevent NPs from aggregation due to a lack of electrostatic repulsion. However, the stability of the GNPs@BSA particles is enhanced by the BSA layer, presumably by its steric repulsion.

**Figure 3** A–C) Extinction spectra of GNPs incubated with BSA without removal of unbound proteins; D–F) Extinction spectra of GNPs samples incubated with BSA after removal of unbound proteins (by centrifugation and resuspension).
Figure 4 Zeta potential (surface charge) of GNPs as prepared and of GNPs incubated with BSA after removal of unbound proteins and resuspended in water.

To understand the differences in the LSPR peak shift and influence of adsorbed and free BSA molecules on the extinction of GNPs we performed FEM simulations of extinction spectra of AuNP50, AuNP70 and AuNS, with different BSA concentrations (unbound proteins) and with different thicknesses of PC around them (bound proteins) (see Supporting Information for more details). The approximate thickness of PC was calculated by evaluating the size of AuNP50 and AuNS70 before and after incubation with BSA (estimated to be about ~ 6.5 nm see Supporting Information Table S1 ). According to the simulation results, the LSPR peak position is not affected much by the presence of free protein in the solution, while the thickness of PC has a significant impact, creating a larger red shift with the increase of its thickness (Figure 5 ).

Figure 5 FEM simulated LSPR peak shift with the change of A) protein concentration in the solution and B) protein corona thickness around nanoparticles (for AuNS the PC is designed around the core only).
Based on the experimental (Figure 3 A–C) and simulation results, the PC on spherical and anisotropic nanoparticles seems to form quite differently. The PC on spherical and ellipsoidal nanoparticles (AuNP50 and AuNP70) reaches a certain thickness (about \( \sim 6.5 \) nm see Supporting Information Table S1) with the lowest concentrations of BSA (which is more than enough to form a monolayer, considering the number of molecules per particle 156 to 1119, depending on BSA orientation), after which further increase of protein amount does not contribute to the hard BSA-layer formation.\cite{38} However, the LSPR shift of AuNS is more pronounced and changes with the increase of protein concentration in the media, which indicates an increase of the PC thickness.

2.3 Protein interaction with spherical and anisotropic nanoparticles

Surface enhanced Raman spectroscopy (SERS) signal by GNPs requires close proximity (up to 5 nm) between the analyte and the particle surface.\cite{39,40} preferably near “hot spots”. We, therefore, used SERS measurements to confirm the BSA adsorption onto the AuNP50 and AuNP70. As can be seen from Figure 6 A and B, there are multiple high intensity peaks in the SERS spectra of spherical particles incubated with BSA (see Supporting Information Table S2 for peaks assignment). By way of contrast, the SERS spectrum of AuNS incubated with BSA (Figure 6 C) does not have any distinct peaks above the noise level. We know from the extinction spectroscopy and zeta potential analysis given above that the protein does adsorb on the AuNS particles, but it seems that nanoparticles “hot-spots” are protein-free.
Figure 6 SERS spectra of GNPs, GNPs in PBS, GNPs incubated with 9 mg/mL BSA and GNPs preincubated with 9 mg/mL BSA and resuspended in PBS: A) AuNP50, B) AuNP70 and C) AuNS.

To explain the observed SERS results, the electric field distribution around the AuNS was simulated via FEM calculation (Figure 7 A). The electric field around tips has a dramatic enhancement compared to the region around the core so protein molecules, adsorbed onto the core would not have a significant SERS enhancement, though they do contribute to the redshift of the LSPR (Figure 7 B). Spherical nanoparticles do not have areas with high curvature, which makes enhancement homogeneous on the surface and much weaker, compared to AuNS “hot-spots” enhancement (Supporting Information Figure S1).

Figure 7 A) The simulated enhanced electric field distribution near the tips (top row) and around the core (bottom row) of AuNS. E is the polarisation direction and K is the propagation direction. The wavelength of the incident light is 785 nm; B) The simulated LSPR peaks of AuNS@BSA with different thicknesses of protein layers around the core (PC – protein corona and its thickness).

To further study this phenomenon we performed MD simulations to reveal the potential link between gold surface curvature (shape of GNPs) and the adsorption of BSA protein. Four types of gold surface models were constructed (Figures 8 A–D): plane (P), truncated cone large (TCL), truncated cone small (TCS), and spherical tip (ST). For other details regarding the simulation please see Supporting Information.

Initially, the protein was placed away from the gold surface with a minimum distance of 0.8 nm. The protein was adsorbed on the gold surface within a 1 ns simulation. The energy of the system was averaged over a 1 ns simulation after adsorption under a canonical ensemble. The temperature was set to be 300 K. To quantify the adsorption strength between BSA and the gold surface, the adsorption energy $E_{\text{ad}}$ was calculated as (Equation 1):

$$E_{\text{ad}} = -(E_{\text{Gold@BSA}} - E_{\text{BSA}} - E_{\text{Gold}})$$

where $E_{\text{Gold@BSA}}$, $E_{\text{BSA}}$ and $E_{\text{Gold}}$ are energies of Gold@BSA, BSA and gold surface structures respectively. A larger adsorption energy indicates a stronger interaction between BSA and the gold surface, so the BSA is more likely to remain adsorbed and less likely to desorb under the local thermal fluctuations. For each gold surface model, the extreme and average adsorption energies along 10 directions are presented in Figure 8 E. The complete results including each orientation can be found in the Supporting Information Table S3. When the curvature of the gold surface increases, the number of gold atoms within the force cut-off distance of BSA decreases, and the van der Waals force decreases. Thus, with the increase of the radius of curvature, the adsorption energy decreases significantly. Such results illustrate that BSA is less likely to be adsorbed on the surface with large curvatures, such as the spikes on the gold nano star surface, which supports the experimental finding that BSA is seldom found on the AuNS tips.
Figure 8 The adsorption configurations of the maximum adsorption energy for A) the P (plane) model; B) the ST (spherical tip) model (0°_270° BSA orientation); C) the TCL (truncated cone large) model (0°_180° BSA orientation); D) the TCS (truncated cone small) model (90°_0° BSA orientation). E) The average adsorption energy for each model. The error bar shows the maximum and the minimum value in 10 orientations.

The adsorbed amino acids, as defined by being less than 0.5 nm from the gold surface, were also counted. For the same type of gold surface, the adsorbed amino acid number had a positive correlation with the adsorption energy (Supporting Information Table S3). However, for different surfaces, the average adsorbed amino acid number is similar but the adsorption energy per amino acid for the smaller curvature surfaces is significantly higher. The types of the adsorbed amino acids were counted, and the results are shown in Supporting Information Figure S2.

Conclusion

In this work, we have studied GNP-protein interactions and found some notable differences between BSA adsorption to spherical and anisotropic (star-shaped) nanoparticles. In particular, we have considered the effects of high ionic strength salt and varying protein concentrations (as represented by the main protein in most biological media, BSA). We found salt and proteins stabilise or destabilise the particles depending on their concentrations and the particle shape. The protein layers strongly adsorbed onto the GNPs surfaces, also known as the hard protein corona, affect the biological identity and alter their function and properties.

We have found that the PC is not always the “enemy”. Formation of protein layers around nanoparticles can increase their stability in presence of salts. To utilize the benefits and omit the drawbacks of PC formation, it requires a thorough understanding of nanoparticle-protein interaction patterns. For spherical nanoparticles, the PC formation was confirmed by extinction spectroscopy, zeta potential and SERS measurements. However, in the case of anisotropic nanostars while extinction spectroscopy and zeta potential analysis indicated the presence of a PC, SERS analysis, did not show the presence of protein on the surface of nanostars. With FEM simulations we have shown that the electric field enhancement around different parts of nanostars varies with the strongest enhancement at the tips and almost no enhancement at the core. MD simulations, conversely, showed more proteins would bind near the more planar core of the nanostars than at the tips. Thus we see evidence of a PC but a lack of SERS enhancement of the proteins on nanostars. Overall, this fascinating result means that anisotropic nanoparticles might enjoy protein-mediated stabilization and other benefits of PC, while having their plasmonic properties preserved for small molecule analytes to bind to their “hot-spots” which are free from protein binding.

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anisotropic gold nanoparticles, protein corona, stability, SERS, molecular dynamics simulation

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Shape dependent protein induced stabilization of gold nanoparticles: from protein corona perspective

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1. Materials and Methods

Gold spherical nanoparticles preparation (AuNP)

1.1.1 Reagents

1% Chloroauric acid (HAuCl₄), trisodium citrate dehydrate (Na₃C₆H₅O₇), hydrochloric acid (HCl) and nitric acid (HNO₃) were purchased from Sigma-Aldrich (Sydney, Australia). Milli-Q water (18.2 MΩ [Ω cm] at 25 °C) was used for all aqueous solutions.

1.1.2 Synthesis

Concentrated HCl and concentrated HNO₃ were combined in a 3:1 volume ratio to create aqua regia, which was used to clean all glass flasks before the synthesis.

A stock aqueous solution of 0.5 mL 1% HAuCl₄ was added into a flask containing 49.5 mL Milli-Q water. The mixture was vigorously stirred (600 rpm) while being heated to 250 °C on a hotplate. Aluminium foil was utilised to cover the flask during the synthesis to prevent contamination and solvent evaporation. 0.45 mL of 1% Na₃C₆H₅O₇ was rapidly added into the HAuCl₄ solution flask after it had boiled under atmospheric pressure. The mixture was continuously heated and stirred for 20 min. After the heat was turned off, it was stirred for an additional 10 min. The main variable controlled to achieve the required particle size is the molar ratio (MR) of Na₃C₆H₅O₇ to HAuCl₄. The sample then was cooled naturally to room temperature and stored in the fridge at 4 °C.

Gold nanostars preparation (AuNS)

1.2.1 Reagents

1% HAuCl₄, silver nitrate (AgNO₃), and L-Ascorbic acid (C₆H₈O₆), HCl and HNO₃ solutions were purchased from Sigma-Aldrich (Sydney, Australia). Milli-Q water (18.2 MΩ [Ω cm] at 25 °C) was used for all aqueous solutions.

1.2.2 Synthesis

Concentrated HCl and concentrated HNO₃ were combined in a 3:1 volume ratio to create aqua regia, which was used to clean all glass beakers before the synthesis.

AgNO₃ and C₆H₅O₆ solutions were freshly prepared before synthesis. Briefly, 400 μL of 1% HAuCl₄ were mixed with 600 μl of Milli-Q water to make 1 mL of 10 mM HAuCl₄. 10 mL of 10 mM AgNO₃ solution and 1 mL of 100 mM C₆H₅O₆ solution were prepared by dissolving 17.2 mg of solid AgNO₃ in 10 mL of Milli-Q water and 17.6 mg of solid C₆H₅O₆ in 1 mL of Milli-Q water, respectively.

In a glass beaker 10 mM AgNO₃ was mixed with 10 mM HAuCl₄ in 1:18 ratio for 30 sec. The solution colour was slightly yellow, due to presence of yellow coloured gold salt ions. This was followed by the slow dropwise addition of 100 mM C₆H₅O₆ with a 1:6 ratio (AgNO₃: C₆H₅O₆) and then stirring for another 30 sec, during which the colour of the solution turned blue. The synthesized particles were stored in the refrigerator at 4 °C until further use.

Gold nanoparticle characterization

1.3.1 Morphology: Transmission electron microscopy (TEM)

TEM (Philips CM10 TEM, Eindhoven, The Netherlands) was used to estimate the size and morphology of nanoparticles. TEM samples were prepared by the following method: a 10 μL drop of nanoparticle suspension
was placed on top of the carbon side of the carbon-coated copper grid (Zhongjingkeyi Film Technology, Beijing, China), the drop was then removed with filter paper after it had settled for 3 minutes. This was then repeated three times, after which sample was then allowed to dry overnight.

1.3.2 Size: Nanoparticle tracking analysis (NTA)

NTA on the NanoSight NS300 device was used to measure nanoparticles concentrations and size. The GNPS samples were diluted 10 or 100 times from their original concentration. They were then put through the detector at a speed of 100 units of syringe pump at a temperature of 21 °C. A 405 nm laser was used to irradiate the particles.

1.3.3 Optical properties: Extinction spectroscopy

Extinction spectroscopy was carried out in 400–900 nm wavelength scanning range on the JASCO V-760 UV-Vis Spectrometer (Jasco, Hachioji, Japan). 500 μL of each sample were utilised in the Starna 18B/Q/10 cuvette.

1.3.4 Surface charge: Zeta potential

Surface charges of nanoparticles were measured with Zetasizer ZS (Malvern Panalytica, Malvern, UK). 1000 μL of each sample were place in the disposable folded capillary cells for the measurement of zeta potential (DTS1070). Each sample was tested 3 times over the course of 15 runs.

1.3.5 Surface enhanced Raman spectroscopy: SERS

SERS spectra were collected with a portable IM-52 Raman microscope (Snowy Range Instruments, USA) with the 785 nm incident laser power of 100 mW and integration time of 1 second. 100 μL of each sample was placed in the quarts cuvette Starna 18B/Q/10 and 15 spectra measurements of each sample was averaged and used as a final result. The spectra were baseline-corrected using the Peak software (Snowy Range Instruments, Laramie, WY, USA).

Gold nanoparticles protein interactions

The following samples were prepared to determine GNPs-protein interactions. To test GNPs stability in high ionic strength liquid GNPs samples (concentration, determined with NTA, was adjusted to $10^{10}$ particles/mL for all samples) have been either centrifuged and resuspended in 1X PBS or incubated for 30 min with 9 mg/mL bovine serum albumin (BSA, heat shock fraction, pH 7, [?][98%], purchased from Sigma-Aldrich) and then centrifuged and resuspended in PBS. To prepare “washed” and “unwashed” samples of GNPs@BSA, AuNP50, AuNP70 and AuNS particles were incubated for 30 min with varying concentrations BSA, including 0.1 mg/mL, 2 mg/mL, 9 mg/mL, and 15 mg/mL. The “unwashed” samples were incubated with BSA without removal of unbound proteins. The “washed” samples were incubated were incubated with BSA for 30 minutes, after which they were centrifuged at 6,000 rpm for 10 minutes and resuspended in Milli-Q water.

Further analysis of GNPs@BSA samples via extinction spectroscopy, zeta potential and SERS measurements were performed as indicated earlier.

Finite-element method (FEM) simulation

To simulate the extinction spectra of different nanostructures and enhanced electric field distribution around them, Wave Optics Module from COMSOL Multiphysics software package (see www.comsol.com) was used. Spherical nanoparticle and ellipsoidal nanoparticle were built in the software while nanostars were built in Fusion 360 (Autodesk, USA, CA) and then was imported to COMSOL Multiphysics. Sizes and geometries of nanoparticles were measured from TEM images and analysed in ImageJ software (National Institutes of Health, MD, USA). The refractive index of bovine serum albumin was taken to be as $n = 1.6021^{[1]}$ and water ($n = 1.33$) was used as surrounding medium. The real part and imaginary part of refractive index for gold was taken from Johnson and Christy.$^{[2]}$ All simulations were performed for the interaction of a linearly polarized plane wave with the nanostructures. For spherical and ellipsoidal nanoparticles, the wavelength of incident light was tuned from 300 nm to 800 nm. For nanostars, the wavelength of incident light was tuned
from 400 nm to 850 nm. All enhanced electric field distributions were calculated for 785 nm incident light, equal to the experimental laser used for Raman measurements.

Molecular dynamics (MD) simulation

We performed MD simulations to reveal the potential link between gold surface curvature and the adsorption of BSA protein. Four types of gold models are constructed: plane (P), truncated cone large (TCL), truncated cone small (TCS), and spherical tip (ST). The adsorption surface of P in the model is the Au (111) facet, presenting an infinitely large radius of curvature. Sizes and geometries of nanoparticles were measured from TEM images and analysed in ImageJ software (National Institutes of Health, MD, USA). The geometries of the truncated cones are constructed according to the TEM sizes for the first type of spikes. The axial direction (growing direction) of the truncated cone is aligned in the [111] direction. The starting radii of the TCL and TCS are 7 nm and 4.5 nm, respectively. The radii narrows linearly, and the descending ratio is around 0.11, mimicking the first type of spike. The axial length of both models is 20 nm. The ST model was constructed by a hemisphere with a 2.25 nm radius and a truncated cone (axial length 2.25 nm). The structure of BSA was acquired from Protein Data Bank, ID 3V03. Because of the complex geometry of BSA, the protein was rotated and allowed to approach the gold surface at different angles. For each gold model, 10 directions were tested with an angle interval of 90°, which are named according to the rotation angle about the x, y, and z BSA molecule position axis (R_x, R_y, R_z).

To accelerate the simulations, a coarse-grained (CG) model of BSA was adopted with the MARTINI potential. A full atomic model, described by a Lennard-Jones (LJ) interaction was fixed for the simulation of the gold surfaces. The interactions between gold and protein was described by LJ interactions. Initially, the protein was placed away from the gold surface with a minimum distance of 0.8 nm. The protein adsorbed onto the gold surface within 1 ns of simulation. The energy of the system was determined by averaging over 1 ns simulation after adsorption under a canonical ensemble. The temperature was controlled at 300 K. GROMACS was used to perform MD simulation. The timestep were 0.02 ps. The cut-off distances for the van der Waals interaction and electrostatic force were 1.2 nm.

Supplementary data

Table S1 Size of gold nanoparticles evaluated with NTA.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Size: Mean ± SD (Mode), nm</th>
<th>Size: Mean ± SD (Mode), nm</th>
<th>Size: Mean ± SD (Mode), nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>GNP</td>
<td>53 ± 27 (44.4)</td>
<td>185 ± 105 (110.3)</td>
<td>55 ± 13 (50.9)</td>
</tr>
<tr>
<td>AuNP70</td>
<td>72 ± 18 (68.9)</td>
<td>172 ± 110 (79.6)</td>
<td>89 ± 2 (75.7)</td>
</tr>
<tr>
<td>AuNS</td>
<td>100 ± 30 (81.9)</td>
<td>138 ± 67 (84.6)</td>
<td>134 ± 44 (110.1)</td>
</tr>
</tbody>
</table>

Table S2 Characteristic SERS peaks of BSA on GNP.

<table>
<thead>
<tr>
<th>Wavelength, cm⁻¹</th>
<th>Assignment[7,8,9]</th>
</tr>
</thead>
<tbody>
<tr>
<td>490</td>
<td>v(S–S)</td>
</tr>
<tr>
<td>562</td>
<td>v(S–S)</td>
</tr>
<tr>
<td>602</td>
<td>δ(COO–)</td>
</tr>
<tr>
<td>753</td>
<td>Trp, v(C–S)</td>
</tr>
<tr>
<td>836</td>
<td>Tyr</td>
</tr>
<tr>
<td>941</td>
<td>δ(C–C–N) symmetric, α-helical skeletal</td>
</tr>
<tr>
<td>996</td>
<td>R breathing</td>
</tr>
<tr>
<td>1003</td>
<td>Phe: asymmetric ring breathing</td>
</tr>
<tr>
<td>1183</td>
<td>Tyr, v(–C–N)</td>
</tr>
<tr>
<td>1200–1300</td>
<td>Trp, Phe: δ(R), Amide III – region</td>
</tr>
<tr>
<td>Wavelength, cm⁻¹</td>
<td>Assignment[^7,8,9]</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>1309</td>
<td>Wagging (CH₂)</td>
</tr>
<tr>
<td>1383</td>
<td>δ(CH₃), ν(COO⁻)</td>
</tr>
<tr>
<td>1437</td>
<td>asymmetric δ(CH₃), bend (CH₂)</td>
</tr>
<tr>
<td>1560</td>
<td>Trp: ν(R), ν(r), amide II</td>
</tr>
<tr>
<td>1610</td>
<td>NH₂ in amino acids</td>
</tr>
</tbody>
</table>

Figure S1 FEM simulations of local electric field distribution of A) AuNP50, B) AuNP70 C) AuNS.

Table S3 The adsorption energy for each orientation on different gold surfaces: P (plane), ST (spherical tip), TCL (truncated cone large), and TCS (truncated cone small).

<table>
<thead>
<tr>
<th>P Orientation (Rₓ,Rᵧ,Rz)</th>
<th>Adsorption Energy (kJ/mol)</th>
<th>Adsorption Energy (kJ/mol)</th>
<th>Adsorbed amino acid count</th>
<th>ST Orientation (Rₓ,Rᵧ,Rz)</th>
<th>Adsorption Energy (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0_0_0</td>
<td>11506.61</td>
<td>9</td>
<td>0_0_0</td>
<td>2323.83</td>
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<tr>
<td>0_0_90</td>
<td>12426.27</td>
<td>22</td>
<td>0_0_90</td>
<td>2582.15</td>
<td>12</td>
</tr>
<tr>
<td>0_0_180</td>
<td>10875.28</td>
<td>8</td>
<td>0_0_180</td>
<td>2806.22</td>
<td>9</td>
</tr>
<tr>
<td>0_0_270</td>
<td>12439.67</td>
<td>34</td>
<td>0_0_270</td>
<td>3087.55</td>
<td>15</td>
</tr>
<tr>
<td>0_90_0</td>
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<td>40</td>
<td>0_90_0</td>
<td>3214.42</td>
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</tr>
<tr>
<td>0_180_0</td>
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<td>7</td>
<td>0_180_0</td>
<td>2268.25</td>
<td>8</td>
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<tr>
<td>0_270_0</td>
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<td>0_270_0</td>
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</tr>
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<td>90_0_0</td>
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<tr>
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<td>2841.13</td>
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<tr>
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<td>11465.64</td>
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<td>270_0_0</td>
<td>2619.71</td>
<td>14</td>
</tr>
<tr>
<td>TCL Orientation (Rₓ,Rᵧ,Rz)</td>
<td>Orientation (Rₓ,Rᵧ,Rz)</td>
<td>Adsorption Energy (kJ/mol)</td>
<td>Adsorbed amino acid count</td>
<td>TCS Orientation (Rₓ,Rᵧ,Rz)</td>
<td>Adsorption Energy (kJ/mol)</td>
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<tr>
<td>0_0_0</td>
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<tr>
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<td>5864.51</td>
<td>5</td>
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<tr>
<td>0_0_180</td>
<td>8825.72</td>
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<td>30</td>
</tr>
<tr>
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</tr>
<tr>
<td>0_90_0</td>
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<td>23</td>
<td>0_90_0</td>
<td>6368.40</td>
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<tr>
<td>0_180_0</td>
<td>9823.76</td>
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</tbody>
</table>
Figure S2. The adsorbed amino acid count by residue type in all orientations on different gold surfaces: P (plane), ST (spherical tip), TCL (truncated cone large), and TCS (truncated cone small).

References