Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

The corona of protein - gold nanoparticle systems: role of ionic strength

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ELECTRONIC SUPPLEMENTARY INFORMATION

Determination of AuNP surface occupancy by β2m

β2m is a globular protein whose three-dimensional structure can be assimilated to a cylindroid with longitudinal and transverse axes of 4.3-3.8 and 2.5-2.0 nm, respectively.
Through simple geometrical calculations, it is possible to estimate the number of proteins that can be adsorbed on the surface of a single nanoparticle. If the ratio between the volumes is considered as proposed by Calzolai *et al.*,[1] it is possible to obtain the number of proteins (N) from this equation

$$N = 0.65 \times \frac{(R_{complex}^3 - R_{NP}^3)}{R_{protein}^3} \qquad \text{Eq. S1}$$

where $R_{complex}$ is given by the sum of the NP radius and the diameter of β2m, R_{NP} is the radius of the nanoparticle and $R_{protein}$ is the radius of β2m. Considering the crystallographic cylindrical shape of β2m, the previous equation (Eq. S1) becomes

$$N = 0.65 \times \frac{\frac{4}{3}(R_{complex}^3 - R_{NP}^3)}{h_{cyl} \times r_{cyl}^2} \qquad \text{Eq. S2}$$

where $R_{complex}$ is given by the sum of the NP radius and the height of the β2m cylindroid, R_{NP} is the radius of the nanoparticle and h_{cyl} and r_{cyl} are the height and the base radius of β2m cylindroid. Following this equation, a spherical Cit-AuNP with average diameter of 7.5 nm can accommodate 55-95 β2m monomers. This estimation uses the maximum packing density factor possible for spherical proteins. Since the β2m cylindroid base dimension is a half with respect to height, considering symmetric tetramers instead of cylindroids can improve the geometrical model, leading to 15-30 tetramers per NP that means 60-120 monomers. Both cylindrical and tetrameric volume-based packing densities, however, appear unrealistic. Instead, the number of protein monomers per NP is reduced to 45-56 if the ratio of Cit-AuNP surface area and protein cylindroid base is considered.[2] This last calculation was experimentally confirmed by UV-Vis experiments in which the shift of the AuNP surface plasmon band (SPB) was followed by increasing β2m concentration (Figure S1). The UV-Vis titration experiment showed that a plateau in the redshift started when β2m/AuNP ratio was around 50. Based on this experimental assessment done with 7.5 nm Cit-AuNPs, we determined the occupancy of the 5 nm Cit-AuNPs used in this work by calculating the ratio of NP surface area and protein cylindroid base leading to an estimation of 14-20 proteins per NP.

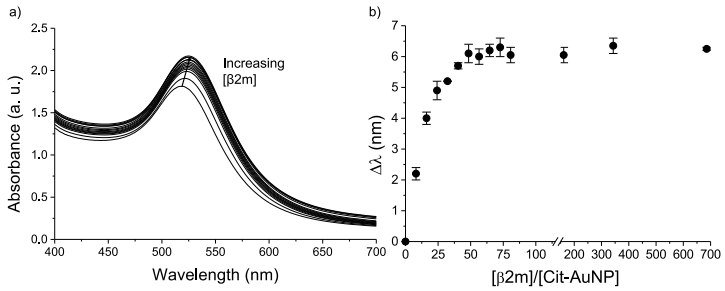

Figure S1. a) UV-Vis spectra of 25 nM Cit-AuNPs (7.5 nm diameter) after the progressive addition of β2m (0-20 μM). b) Plot of SPB shift as a function of [β2m]/[Cit-AuNP] ratio. The experiment was done in triplicate and the error is the standard deviation

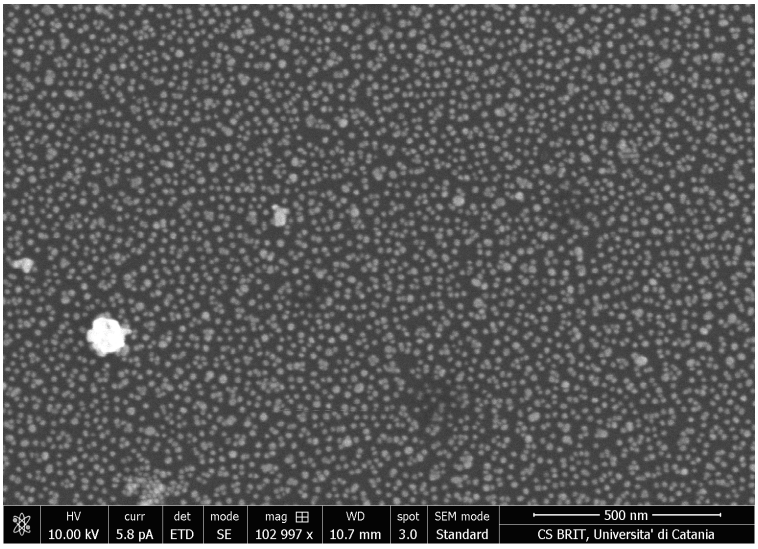

Figure S2. SEM image of Cit-AuNP monolayer used as an active SERS substrate.

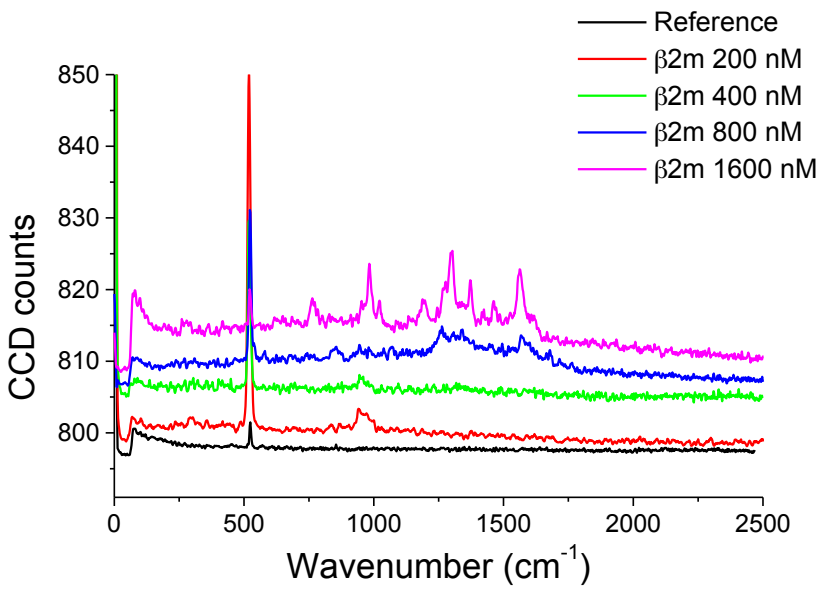

Figure S3. SERS spectra of 0 nM (black), 200 nM (red), 400 nM (green), 800 nM (blue) and 1600 nM (magenta) β2m dissolved in 50 mM HEPES (pH 7) and deposited on a layer of Cit-AuNP solution. The main bands observed in the highest concentration trace can be tentatively assigned to β-sheet Amide II (1550-1600 cm^{-1}), random coil (~1250 cm^{-1}), helix-like (from turns and loops) Amide I and III (~1620 and ~1300 cm^{-1}, respectively) and aromatic side chains (Trp, at ~750 and ~1400 cm^{-1}, and Phe, around 950 cm^{-1} and 1150 cm^{-1}). An analogous ensemble of bands, albeit with a different intensity pattern and an additional β-sheet Amide I (~1700 cm^{-1}) band, can be recognized also in the spectrum reported in Figure 1 of the main text and relative to 10nM β2m dissolved in 0.12 mM HEPES.

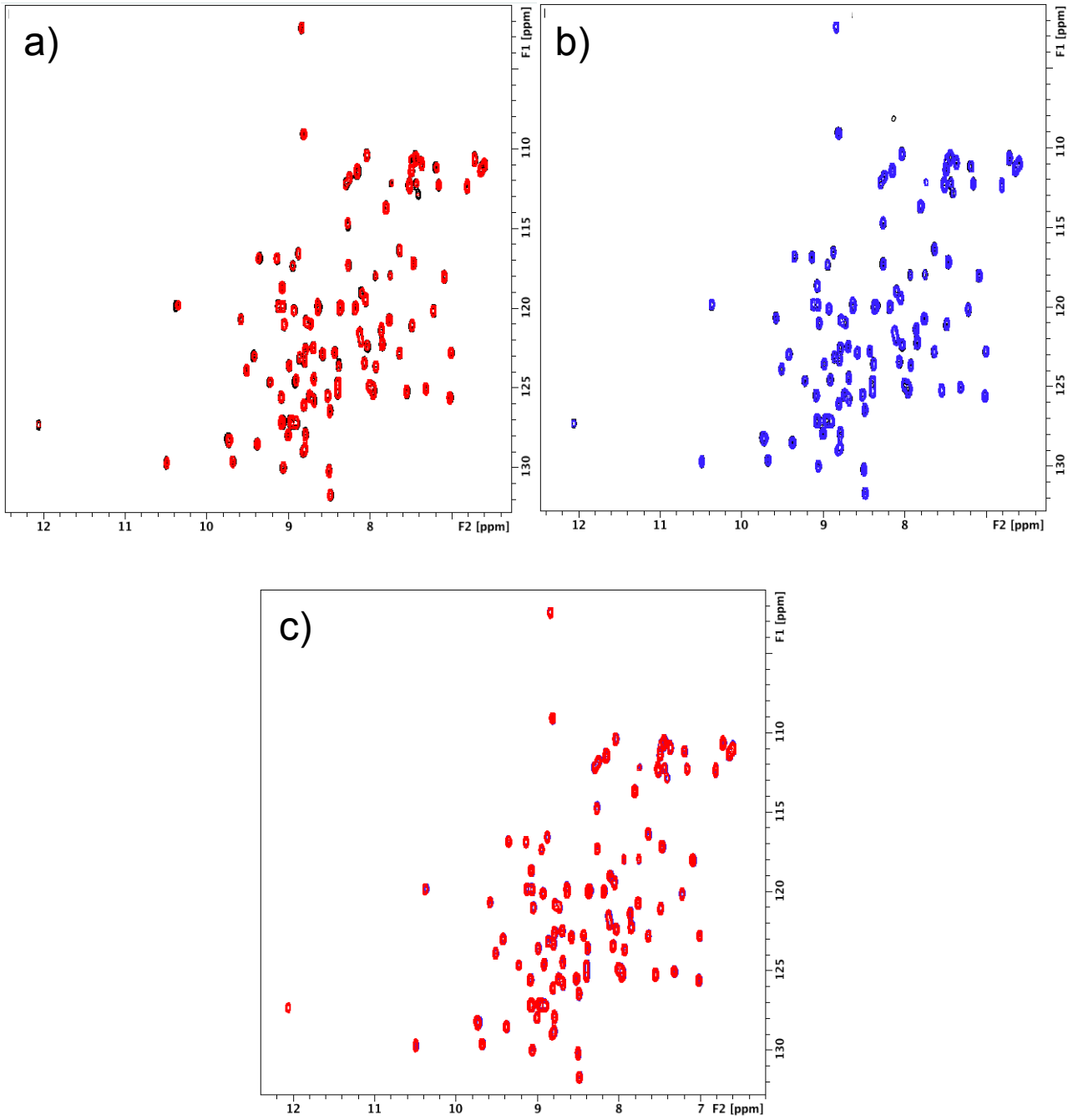

Figure S4. Chemical shift invariance of β2m NMR spectra with Cit-AuNPs at high and low ionic strength. a) Overlay of ^{15}N-^{1}H HSQC maps of 8μM β2m in 0.1 mM HEPES without (black contours) and with (red contours) 60 nM Cit-AuNPs. b) Overlay of ^{15}N-^{1}H HSQC maps of 8μM β2m in 50 mM HEPES without (black contours) and with (blue contours) 60 nM Cit-AuNPs. a) Overlay of ^{15}N-^{1}H HSQC maps of 8μM β2m with 60 nM Cit-AuNPs at 50 mM (blue contours) and 0.1 mM (red contours) HEPES. The chemical shift invariance indicates that the secondary and tertiary structure of the protein is preserved in the presence of NPs at low and high ionic strength.

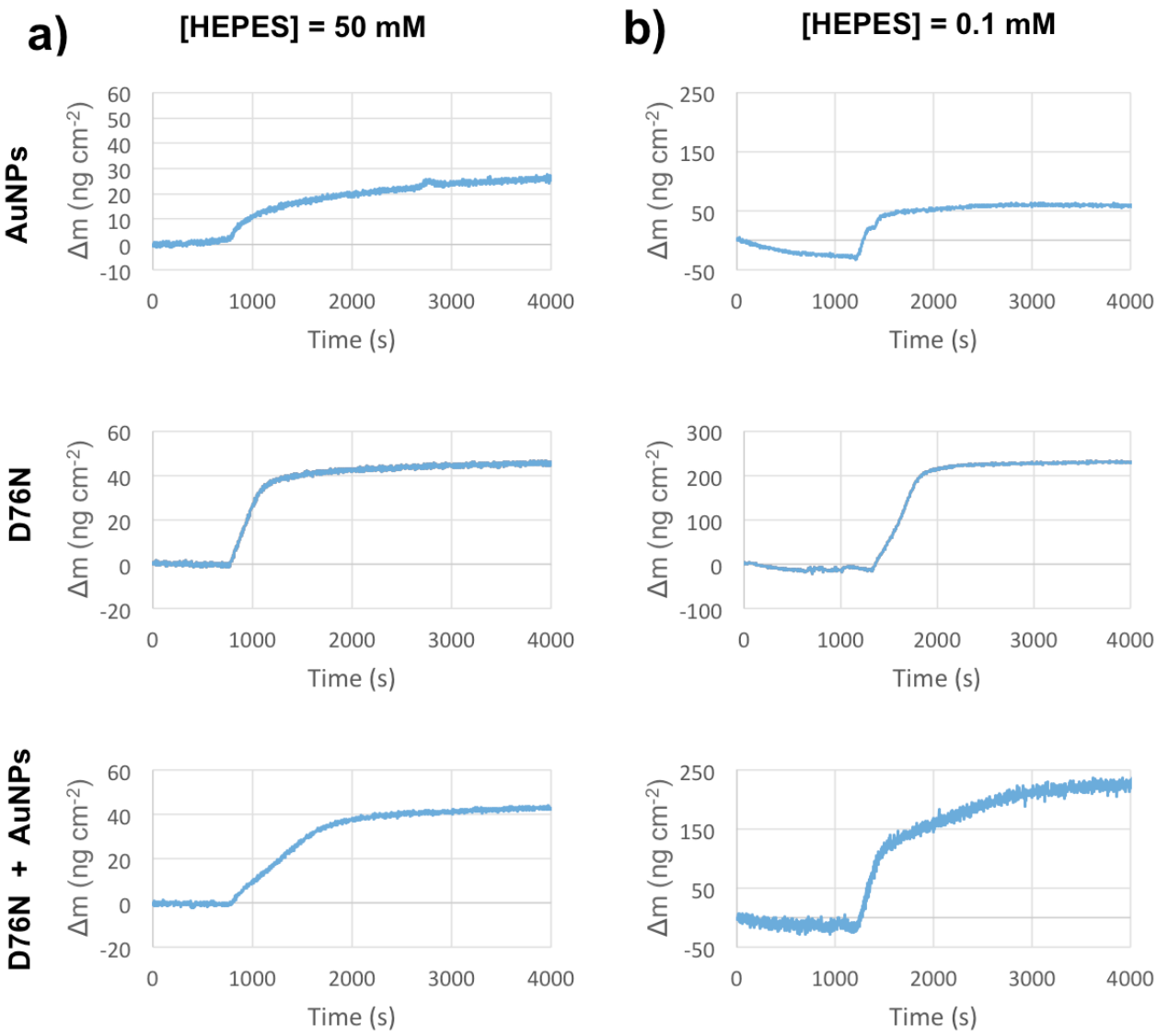

Figure S5. Time course of the QCMD Δ*m* changes for Cit-AuNPs, D76N β2m without and with Cit-AuNPs at 50 mM (a) and 0.1 mM (b) HEPES. The Δ*m* values were calculated using the Sauerbrey equation (see main text) from the total Δ*f* values measured over 4,000 s flow.

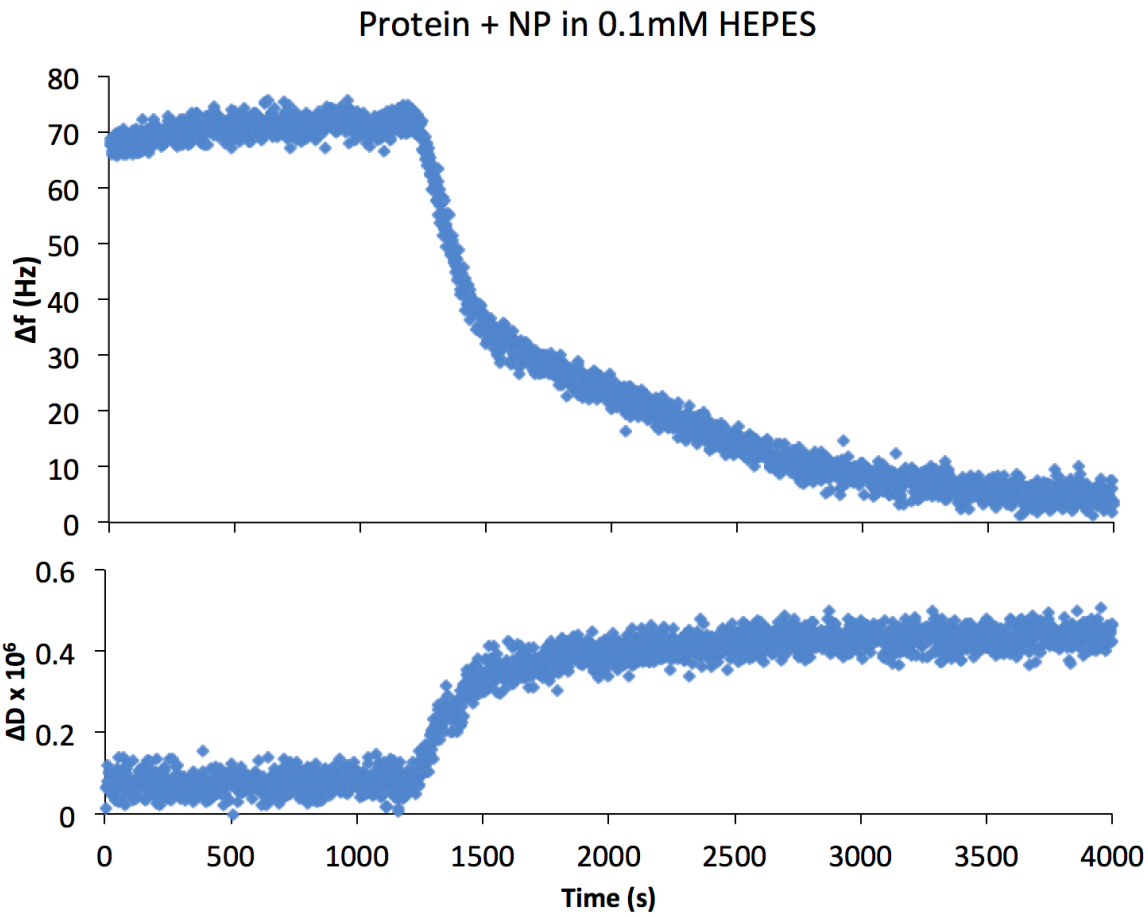

Figure S6. Time courses of QCMD Δ*f* and Δ*D* obtained from 0.5 mM D76N β2m with 25 nM Cit-AuNPs at low ionic strength. The sampled experimental points are shown without decimation. The enlargement highlights the coincidence of the initial deposition phase with the single dissipation step.

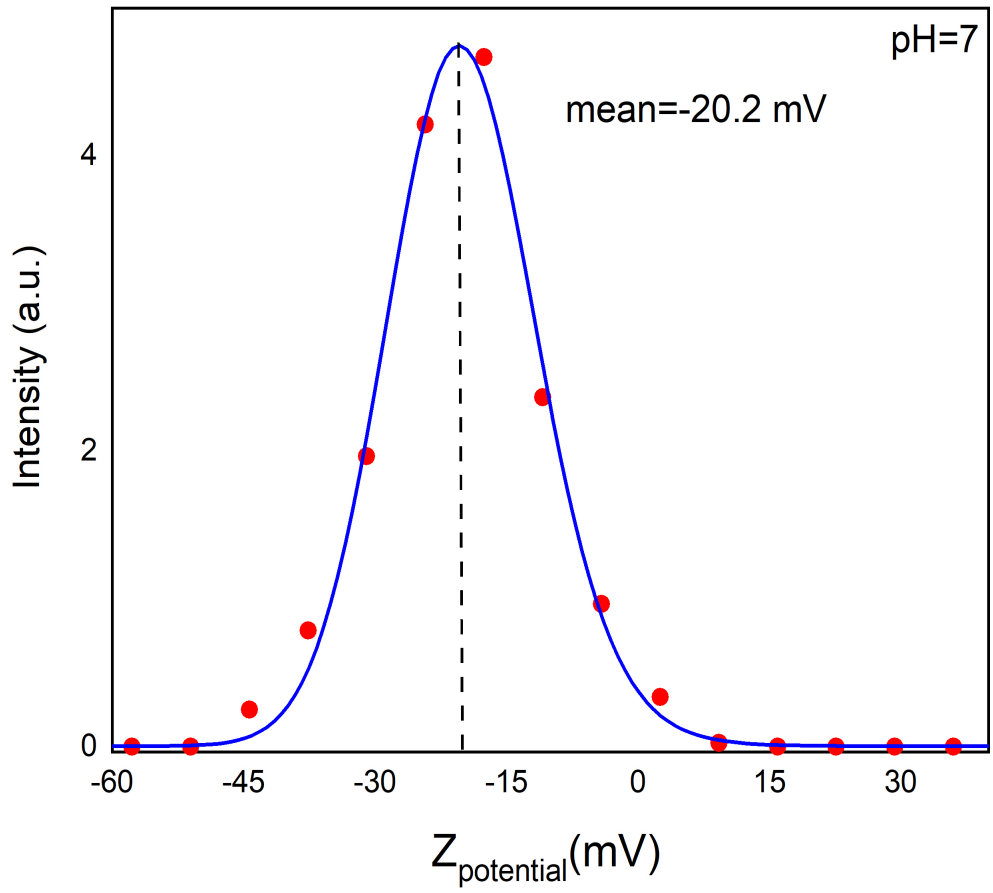

Figure S7. Zeta potential for citrate-coated AuNPs with 5 nm diameter. The determination was carried out on a ~ 5 nM NP sample in 0.12 mM, HEPES at 25°C and pH 7 (the same condition as used in SERS spectra), using a Horiba Scientific nanoPartica SZ100 instrument.

References

1 L. Calzolai, F. Franchini, D. Gilliland and F. Rossi, *Nano Lett.*, 2010, **10**, 3101–3105.
2 Y. Teshima and T. Ogawa, *Forma*, 2001, **15**, 347–364.