Supplementary information

Collagen – A Newly Discovered Major Player in Protein Corona Formation on Nanoparticles

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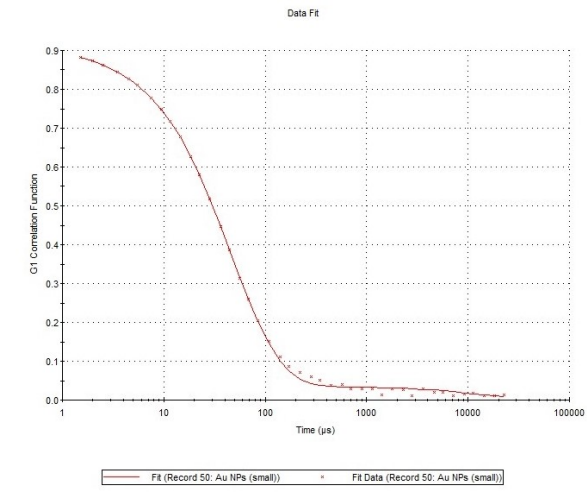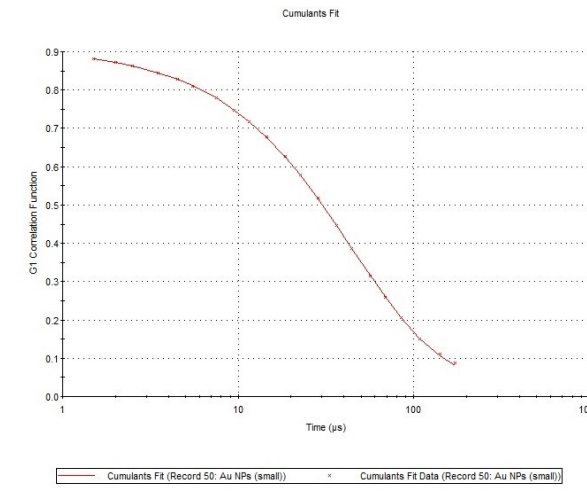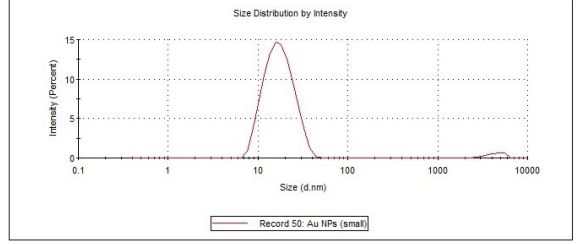
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A

Sample Name: Au NPs (small)
SOP Name: gold np.sop
File Name: Jan 28.dts
Record Number: 50
Material RI: 0.20
Material Absorbtion: 3.320

Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: January 28, 2022 2:26:39 PM

Temperature (°C): 25.0
Count Rate (kcps): 416.6
Cell Description: Disposable sizing cuvette

Duration Used (s): 150
Measurement Position (mm): 4.65
Attenuator: 9

		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 16.31	Peak 1:	17.94	97.4	6.516
PdI: 0.184	Peak 2:	4331	2.6	951.3
Intercept: 0.906	Peak 3:	0.000	0.0	0.000
Result quality : Good				

B

Sample Name: Au NPs (large)
SOP Name: gold np.sop
File Name: Jan 28.dts
Record Number: 51
Material RI: 0.20
Material Absorbtion: 3.320

Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Measurement Date and Time: January 28, 2022 4:53:06 PM

Temperature (°C): 25.0
Count Rate (kcps): 264.5
Cell Description: Disposable sizing cuvette

Duration Used (s): 150
Measurement Position (mm): 1.05
Attenuator: 7

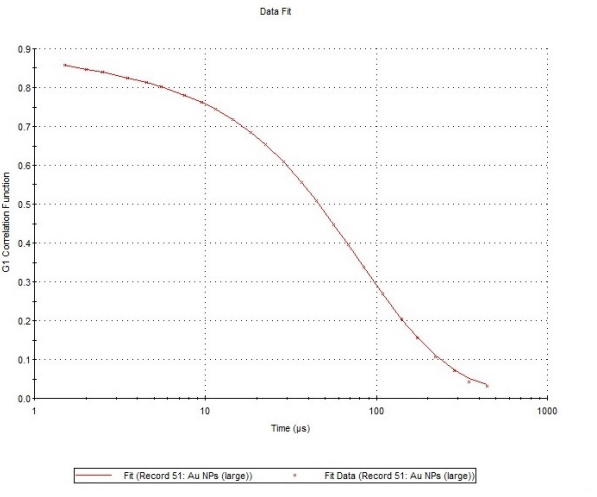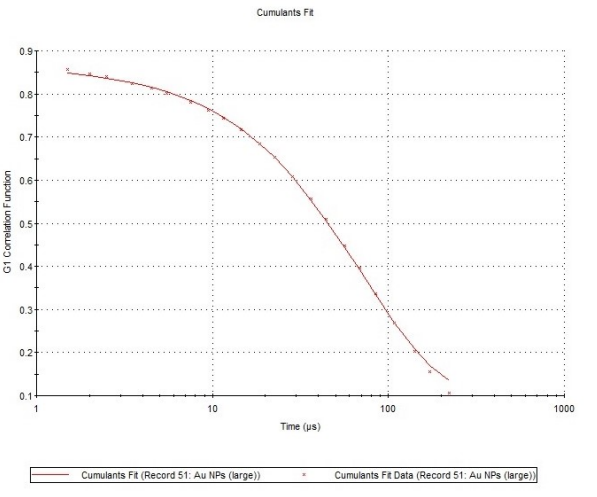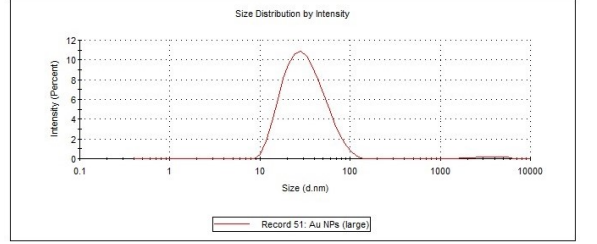|		Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 26.32	Peak 1:	34.32	98.7	18.33
PdI: 0.246	Peak 2:	3614	1.3	1205
Intercept: 0.870	Peak 3:	0.000	0.0	0.000
Result quality : Good				

Figure S1. Dynamic light scattering (DLS) data on the batches of small (A) and large (B) Au NPs employed in the study. Prior to DLS measurements, samples were sonicated for 10 min and centrifuged for 5 min at 2000 rpm in order to remove large aggregates.
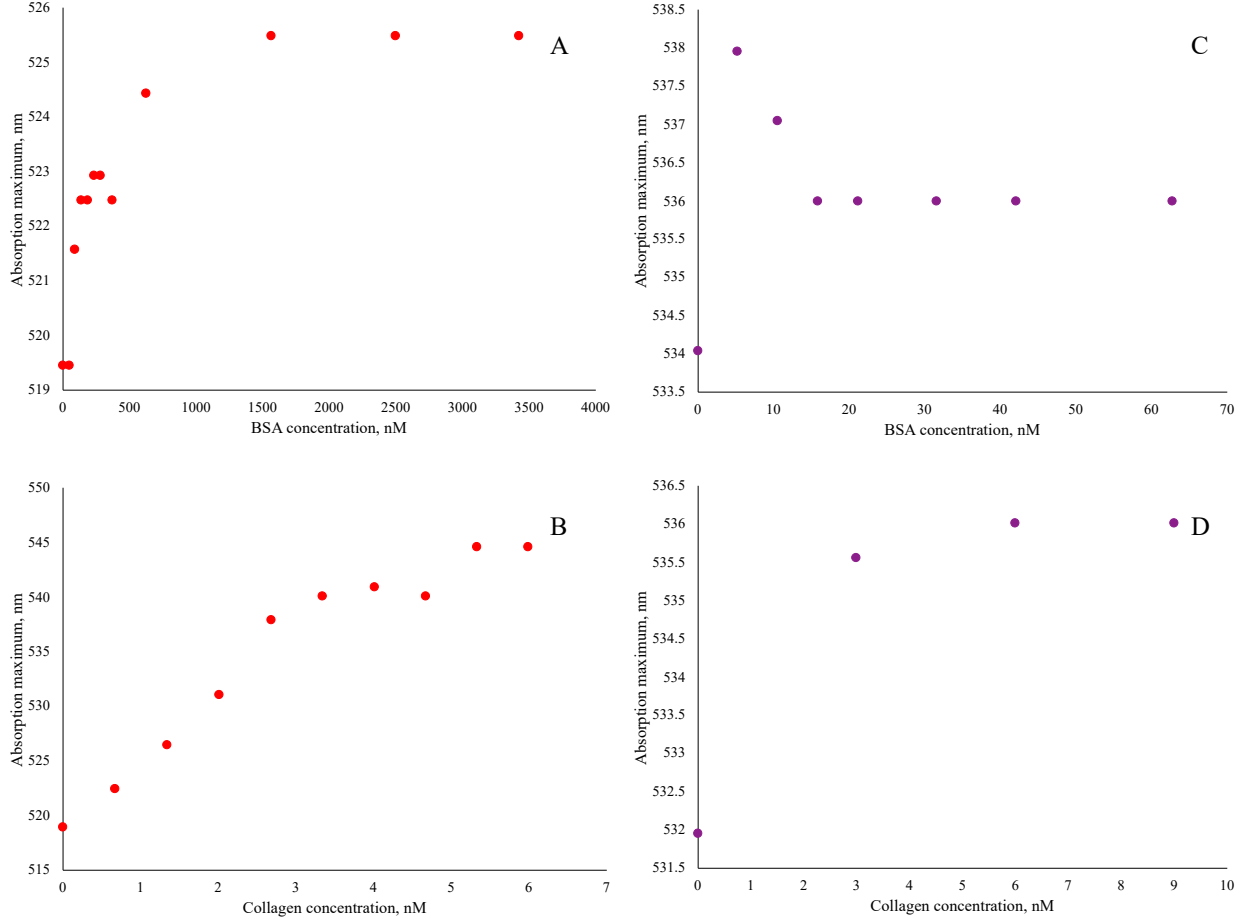
Figure S2. Spectral evolution of the LSPR maxima as a function of protein concentration for the 15 nm AuNPs (A and B) and the 30 nm AuNPs (C and D).

Refractive indices of proteins and their solutions

In order to evaluate whether the observed spectroscopic changes occurred as a result of an increased refractive index upon protein addition, the calculations of the refractive indices were performed using the method by McMeekin et al.[1] Briefly, the molar refractivity $[cm^3/mol]$ is defined by the Lorenz-Lorentz equation:

$$R_p = \frac{M}{\rho}\frac{n^2 - 1}{n^2 + 2} = V_p\frac{n^2 - 1}{n^2 + 2}$$

(S1)

where M is the molecular weight, ρ is the density, V_p is the partial specific volume, and n is the refractive index.

Rearranging the equation, the protein refractive index is found to be

$$n = \sqrt{\frac{2R_p + V_p}{V_p - R}}$$

(S2)

The protein molar refractivity R_p and the partial volume V_p may be written as the weighted contributions of the amino acids constituting the protein:

$$R_p = \frac{\sum\limits_{n} R_n M_n}{\sum\limits_{n} M_n}$$

(S3)

$$V_p = \frac{\sum\limits_{n} V_n M_n}{\sum\limits_{n} M_n}$$

(S4)

where R_n, V_n, and M_n are the molar refractivity, partial specific volume, and molecular weight of the nth amino acid in the protein sequence, respectively.

A change in the refractive index is given by: [2]

$$\frac{dn}{dc} = \frac{3}{2} V_p n_0 \frac{n^2 - n_0^2}{n^2 + 2n_0^2}$$

(S5)

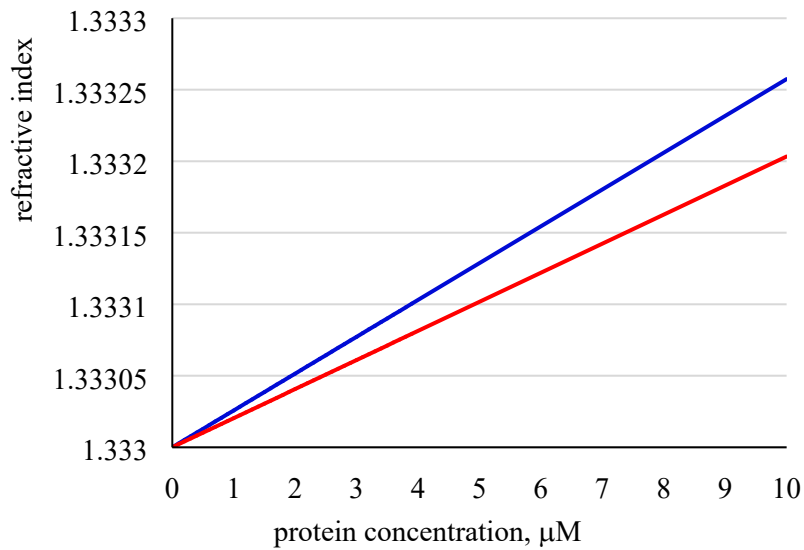where n_0 is the refractive index of the medium (1.333 for water).

Figure S3. Changes in the refractive indices of BSA (blue) and collagen (red) solutions as a function of protein concentration. The plot was generated using (S5).
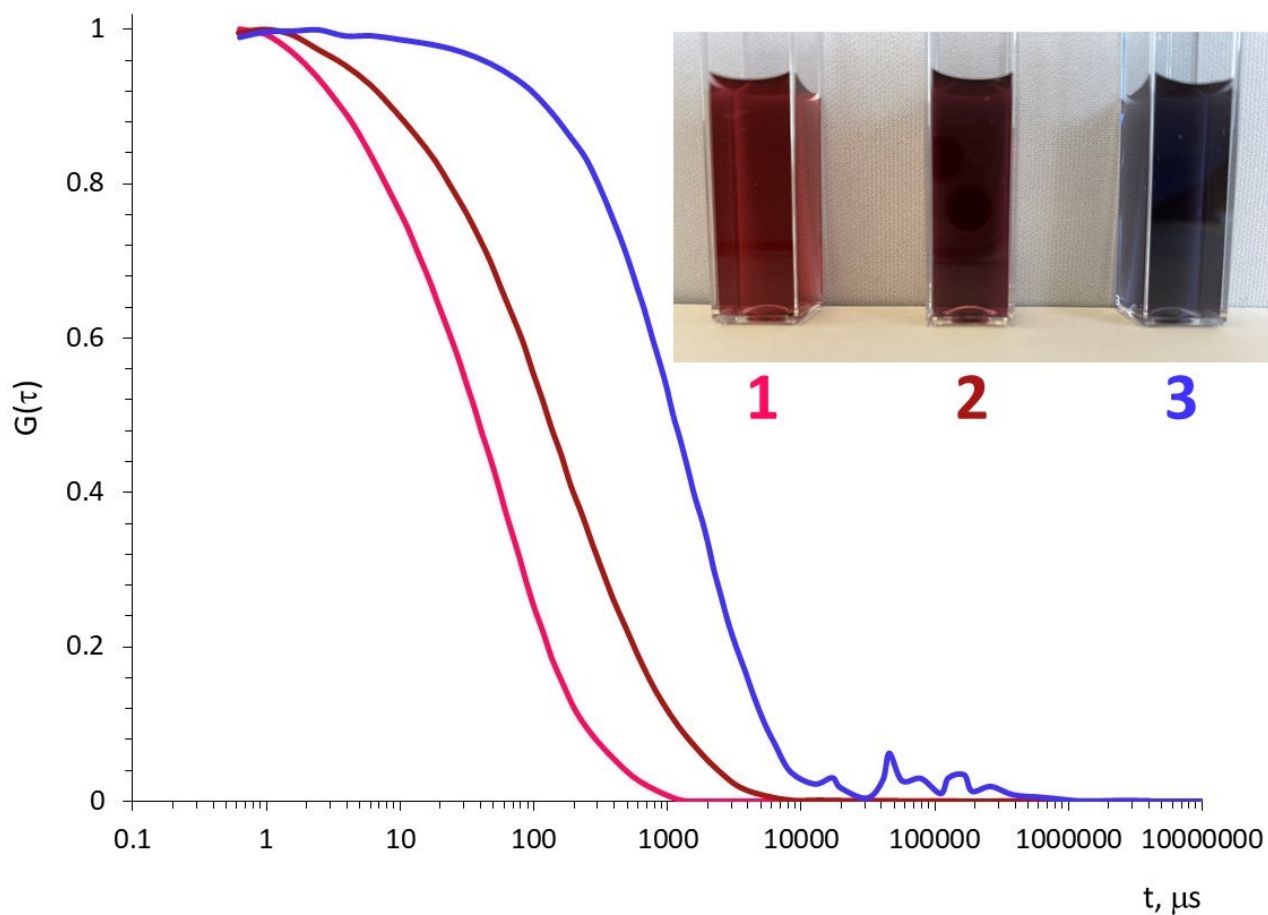
Figure S4. Autocorrelation functions obtained in the DLS experiment for the 30 nm AuNPs in the presence of 0 (left curve, cuvette 1), 0.3 nM (middle curve, cuvette 2), and 0.6 nM (right curve, cuvette 3) of collagen. As the protein concentration increases, the autocorrelation functions broaden indicating the formation of diffusing species with greater molecular weights, which is consistent with aggregation of NPs. The inset shows the NP suspensions and the colour change caused by adding the proteins, which is also consistent with NP aggregation. A similar behavior of the autocorrelation functions was observed when the 30 nm AuNPs were exposed to BSA at concentrations below 10 nM (data not shown).

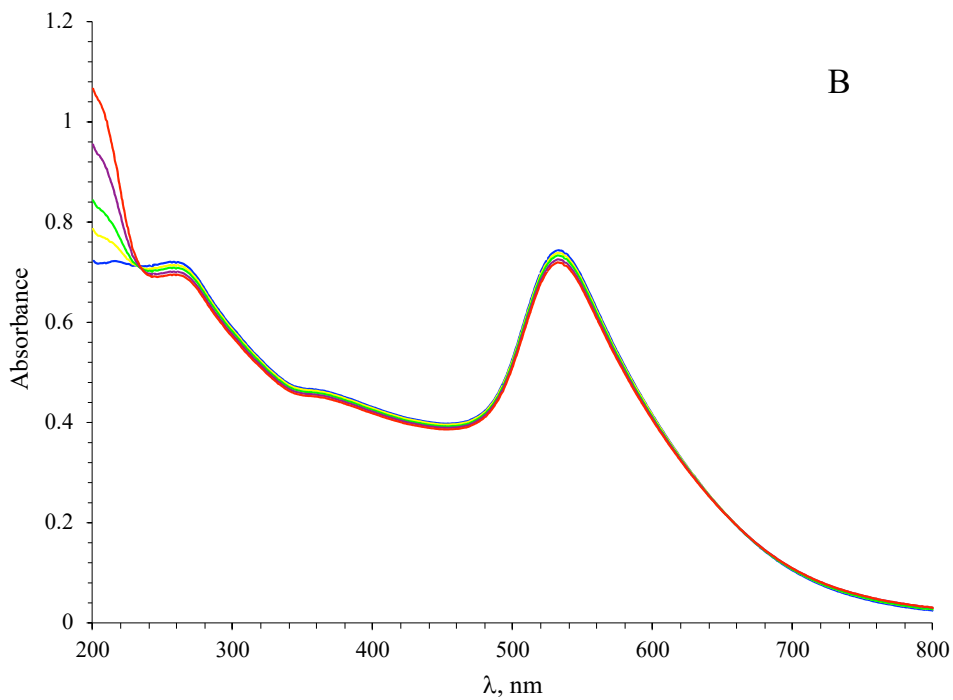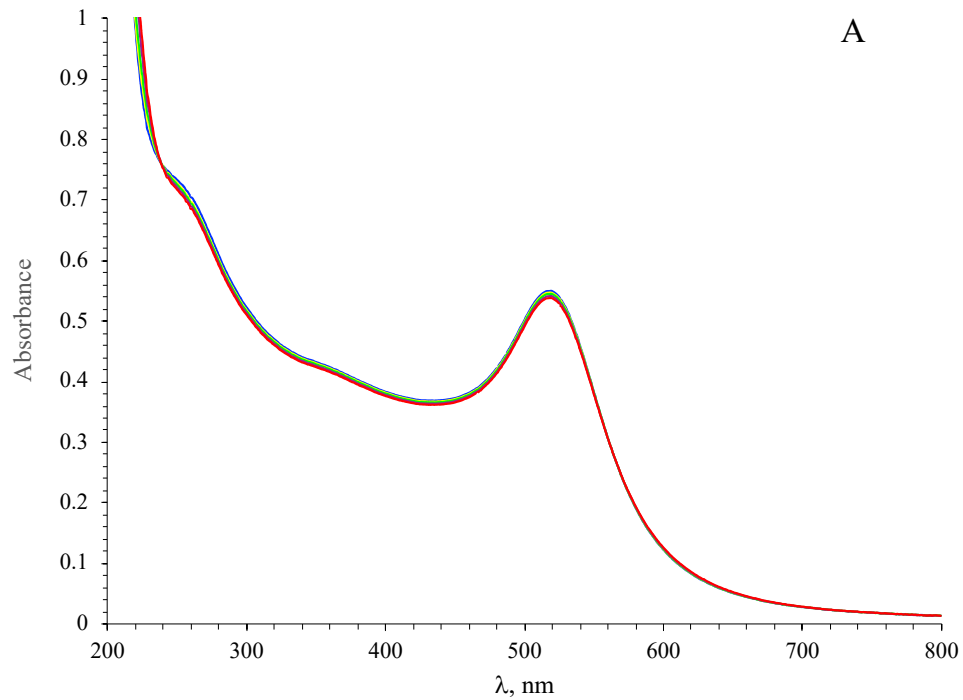**Figure S5.** The absorption spectra of the 15 nm (A) and the 30 nm (B) AuNPs upon titration with different amounts of acetic acid. The spectra were measured at neutral pH (blue), pH3.8 (yellow), pH3.65 (green), pH3.5 (purple), and pH3.4 (red). The pH range was selected to match the conditions when the NPs were titrated with collagen. A slight decrease in the absorption maxima is attributed to dilution upon titration.

Position of LSPR as a function of the refractive index

The considerations below follow Bohren and Huffman.[3]

When the nanoparticle is smaller than the wavelength of the incident light, its entire core experiences almost a constant field, and, consequently, the electrostatic approximation may be applied.

The electrical component E of the incident light induces a dipole moment p in the NP with the magnitude proportional to the field:

$$p = \epsilon_m \alpha E_0 \tag{S6}$$

where ϵ_m is the real dielectric function of the surrounding medium and the polarizability α is a coefficient showing how easily the NP becomes polarized.

Approximating the NP as a perfect dipole, the cross sections for extinction and scattering is calculated as:

$$C_{ext} = k\,Im(\alpha) \tag{S7}$$

$$C_{sca} = \frac{k^4}{6\pi}|\alpha|^2 \tag{S8}$$

where k is the wavenumber in the dispersion medium.

The equation for the extinction cross section is only correct if scattering is small compared to absorption. Therefore, it is possible to write that

$$C_{abs} \approx C_{ext} = k\,Im(\alpha) \tag{S9}$$

The polarizability α for a bare NP is given by:

$$\alpha_c = 4\pi R^3 \frac{\epsilon_c - \epsilon_m}{\epsilon_c + 2\epsilon_m} \tag{S10}$$

where R is the radius of the NP and ϵ_c is the complex dielectric function of the core.

For a special case of a coated sphere, the expression becomes more complicated and is given by

$$\alpha_{c@s} = 4\pi(R + \Delta r)^3 \frac{(\epsilon_s - \epsilon_m)(\epsilon_c + 2\epsilon_s) + (1 - g)(\epsilon_c - \epsilon_s)(\epsilon_m + 2\epsilon_s)}{(\epsilon_s + 2\epsilon_m)(\epsilon_c + 2\epsilon_s) + (1 - g)(2\epsilon_s - 2\epsilon_m)(\epsilon_c - \epsilon_s)} \tag{S11}$$

where Δr and ϵ_s are the thickness and the complex dielectric function of the shell, respectively, and g is the volume fraction of the shell layer:

$$g = 1 - \frac{R^3}{(R + \Delta r)^3} \tag{S12}$$

The resonance observed in AuNPs corresponds to the condition when the electron displacement by the electric component of the incident light is the largest, i.e. the polarizability is the largest. The condition is achieved when the denominators in the equations for α_c and $\alpha_{c@s}$ approach zero:

$$\epsilon_c + 2\epsilon_m = 0 \tag{S13}$$

$$(\epsilon_s + 2\epsilon_m)(\epsilon_c + 2\epsilon_s) + (1 - g)(2\epsilon_s - 2\epsilon_m)(\epsilon_c - \epsilon_s) = 0 \qquad \text{(S14)}$$

Solving the above equations for ϵ_c provides the conditions of resonance for bare and coated NP, respectively:

$$\epsilon_c = -2\epsilon_m \qquad \text{(S15)}$$

$$\epsilon_c = -2\epsilon_s \frac{[\epsilon_s g + \epsilon_m(3 - g)]}{[\epsilon_s(3 - 2g) + 2\epsilon_m g]}$$
(S16)

Considering that both the medium and the shell do not absorb light, only the real part of the dielectric function needs to be considered. This part is given by the Drude model which is applicable to the systems in which light absorption is governed by free electrons:[4]

$$\epsilon_c = \epsilon^\infty - \frac{\omega_p^2}{\omega^2 + \omega_d^2} \qquad \text{(S17)}$$

where ϵ^∞ is the high frequency dielectric constant for interband and core transitions, ω_d is the relaxation frequency that can be neglected in this approximation, and ω_p is the bulk plasma frequency given by

$$\omega_p^2 = \frac{Ne^2}{m\epsilon_0}$$
(S18)

where N is the concentration of free electrons in the metal and m is the effective mass of an electron.

$$\epsilon_c = \epsilon^\infty - \frac{\omega_p^2}{\omega^2} = \epsilon^\infty - \frac{\lambda^2}{\lambda_p^2} \qquad \text{(S19)}$$

For gold, the previous equation gives results that are in good agreement with the experiment, when the values $\epsilon^\infty = 12.2$ and $\lambda_p = 131\ nm$ are used.

Rearranging and substituting equations for ϵ_c obtained in Part 1, the expressions for the resonance wavelengths of bare and coated NPs are obtained:

$$\lambda_{max}^{bare} = \lambda_p \sqrt{\epsilon^\infty - \epsilon_c} = \lambda_p \sqrt{\epsilon^\infty + 2\epsilon_m} \qquad \text{(S20)}$$

$$\lambda_{max}^{coated} = \lambda_p \sqrt{\epsilon^\infty - \epsilon_c} = \lambda_p \sqrt{\epsilon^\infty + 2\epsilon_s \frac{[\epsilon_s g + \epsilon_m(3 - g)]}{[\epsilon_s(3 - 2g) + 2\epsilon_m g]}} \qquad \text{(S21)}$$

The last two equation predict the position of the LSPR maxima in bare and coated AuNPs as a function of dielectric constant of the medium, dielectric constant of the shell, and shell thickness represented by g.

Rearranging the last equation for g:

$$g = \frac{6\epsilon_s\epsilon_m - 3\epsilon_s\left(\left[\dfrac{\lambda_{max}^{coated}}{\lambda_p}\right]^2 - \epsilon^\infty\right)}{(2\epsilon_m - 2\epsilon_s)\left(\left[\dfrac{\lambda_{max}^{coated}}{\lambda_p}\right]^2 - \epsilon^\infty\right) - \left(2\epsilon_s^2 - 2\epsilon_s\epsilon_m\right)}$$

(S22)

From pure geometrical considerations, the thickness of the shell may be expressed as

$$\Delta r = R\left(\frac{1}{\sqrt[3]{1-g}} - 1\right)$$

(S23)

We have calculated the shell thickness as a function of the refractive index of the protein coating the NPs. The data is given in Table S1 and S2.

Table S1. BSA

λ_{max}, nm	g	$\Delta r_{(R\,=\,12\,nm)}$	$\Delta r_{(R\,=\,19\,nm)}$
520	0.00	0.00	0.00
521	0.04	0.18	0.28
522	0.08	0.36	0.57
523	0.13	0.55	0.87
524	0.17	0.75	1.19
525	0.21	0.96	1.52
526	0.24	1.18	1.86
527	0.28	1.41	2.23
529	0.36	1.91	3.03
531	0.43	2.48	3.93
532	0.47	2.80	4.43
533	0.50	3.13	4.96
534	0.54	3.50	5.54
535	0.57	3.89	6.16
536	0.60	4.32	6.85
537	0.64	4.80	7.60

538	0.67	5.33	8.44
539	0.70	5.92	9.37
540	0.73	6.59	10.43
541	0.76	7.36	11.65
542	0.79	8.26	13.08
543	0.82	9.34	14.79
544	0.85	10.67	16.90
545	0.88	12.39	19.61

Table S2. Collagen

λ_{max}, nm	g	$\Delta r_{(R\,=\,12\;nm)}$	$\Delta r_{(R\,=\,19\;nm)}$
520	0.00	0.00	0.00
521	0.04	0.19	0.31
522	0.08	0.39	0.63
523	0.13	0.61	0.96
524	0.17	0.83	1.31
525	0.21	1.07	1.69
526	0.24	1.32	2.08
527	0.28	1.58	2.50
529	0.36	2.17	3.43
531	0.43	2.85	4.50
532	0.47	3.23	5.11
533	0.50	3.64	5.77
534	0.54	4.10	6.50
535	0.57	4.61	7.30
536	0.60	5.18	8.21

537	0.64	5.83	9.23
538	0.67	6.58	10.41
539	0.70	7.45	11.80
540	0.73	8.50	13.45
541	0.76	9.79	15.50
542	0.79	11.46	18.14
543	0.82	13.75	21.77
544	0.85	17.25	27.32
545	0.88	23.91	37.86

References

1. T. L. McMeekin, M. Wilensky and M. L. Groves, *Biochemical and Biophysical Research Communications*, 1962, **7**, 151-156.
2. W. Heller, *The Journal of Physical Chemistry*, 1965, **69**, 1123-1129.
3. C. F. Bohren and D. R. Huffman, *Absorption and scattering of light by small particles*, Wiley, New York, 1983.
4. C. Kittel, *Introduction to solid state physics*, Wiley, New York, 1956.