Influence of particle size on the SARS-CoV-2 spike protein detection using IgG-capped gold nanoparticles and dynamic light scattering.

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Supplementary Data

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S1. Determination of nanoparticles concentration

The gold mass added in µg/mL is known from the reaction stoichiometry. We considered all the gold atoms equally distributed between identical spheres with the diameters determined by TEM (average Feret diameter). The volume (V, nm^3) of a sphere is dependent on its diameter (D, nm) by:

$$V = \frac{4\pi(D/2)^3}{3} \qquad \text{eqn. (1)}$$

From the relation $V = m/d$ (m = mass in µg ; d = density in µg cm^{-3}), we obtain:

$$\frac{m}{d} = \frac{\pi(D)^3}{6} \qquad \text{eqn. (2)}$$

Then, the number of particles (NP) in a given volume (mL) of a colloidal suspension is dependent on the gold mass (µg) and on the diameter of the particles (nm) by:

$$m = \frac{\pi d(D)^3(1 \times 10^{-21})NP}{6} \qquad \text{eqn. (3)}$$

S2. Size distributions (TEM)

The AuNP sizes were determined from TEM images using an amplification of 46,000 ×. The images are shown for each sample in Figures S2(a-e). The number of images and nanoparticles used to calculate the size distribution depended on the amount of nanoparticles found on their grids. We have used at least 450 nanoparticles to obtain the size distribution. After selecting the adequate images, they were measured using the software ImageJ. Then, the Feret diameters obtained were binned (bin width = 5 nm) and plotted as histograms using the software gnuplot (version 5.4). Data were fitted using a Gaussian equation (eqn. 4) with the software gnuplot. The average Feret diameter was given by μ (the center of gaussian, nm), the standard deviation by σ (nm) and the amplitude is given by a:

$$f(x) = \frac{a}{\sigma\sqrt{2\pi}} e^{\left(\frac{-(x-\mu)^2}{(2\sigma)^2}\right)} \qquad \text{eqn. (4)}$$

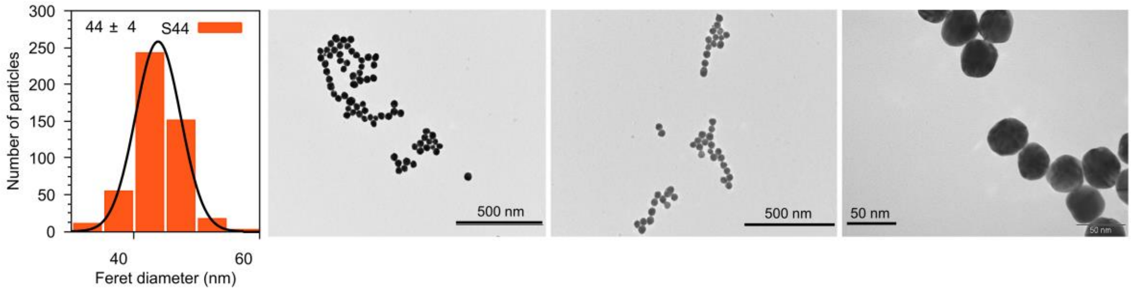

Figure S2a. AuNP size distribution for **S44** was calculated using 15 TEM images with a total of 520 nanoparticles.

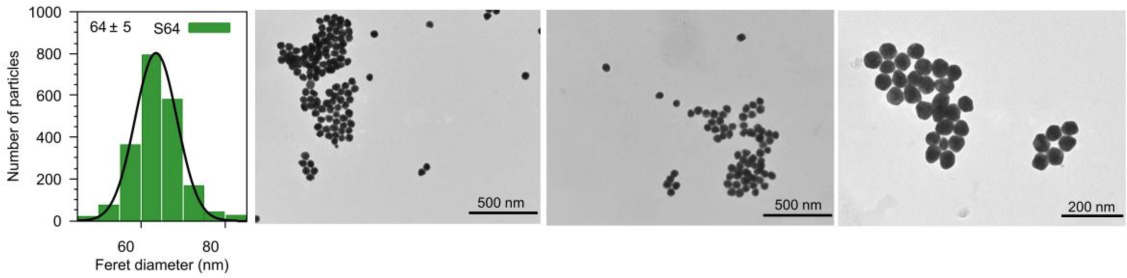

Figure S2b. AuNP size distribution for **S64** was calculated using 68 TEM images with a total of 2180 nanoparticles.

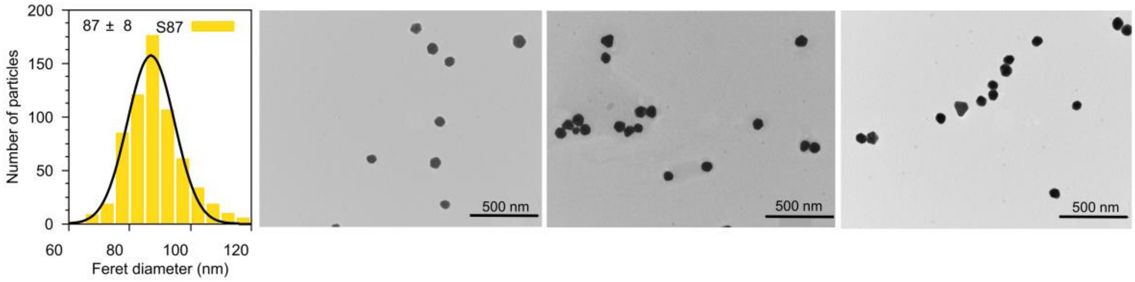

Figure S2c. AuNP size distribution for **S87** was calculated using 64 TEM images with a total of 772 nanoparticles.

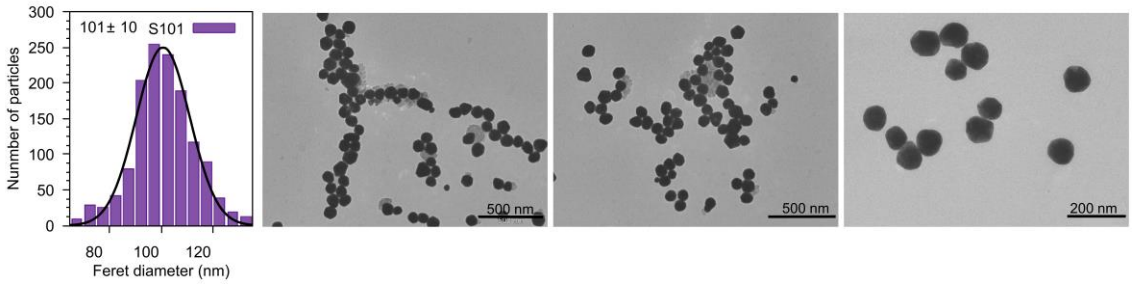

Figure S2d. AuNP size distribution for **S101** was calculated using 59 TEM images with a total of 1425 nanoparticles.

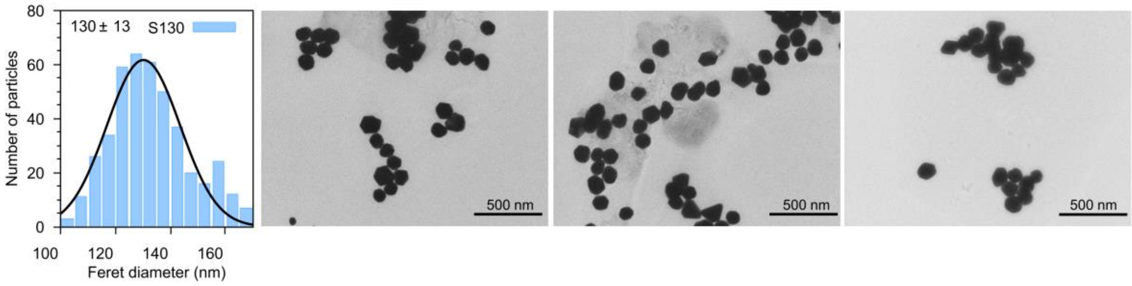

Figure S2e. AuNP size distribution for **S130** was calculated using 74 TEM images with a total of 454 nanoparticles.

S3. DLS results from AuNPs synthesized (S44 to S130)

Figures S3(a-e) show the hydrodynamic diameters (D_H) obtained from DLS for all citrate-coated AuNPs (**S44**, **S64**, **S87**, **S101** and **S130**) as function of (a) light scattering intensity, (b) particle number, and (c) particle volume. The particle distribution using intensity mode describes how much light is scattered by the particles in the different size bins. The number and volume-based distributions are calculated by the Malvern software using the Mie theory assuming spherical shape for the particles.[1] This conversion requires particle refractive index (RI) and absorbance. They show the number and volume of particles in the different size bins.[1] The distribution modes (intensity, number and volume) show all citrate-coated AuNPs synthesized in this work are monomodal without aggregates.

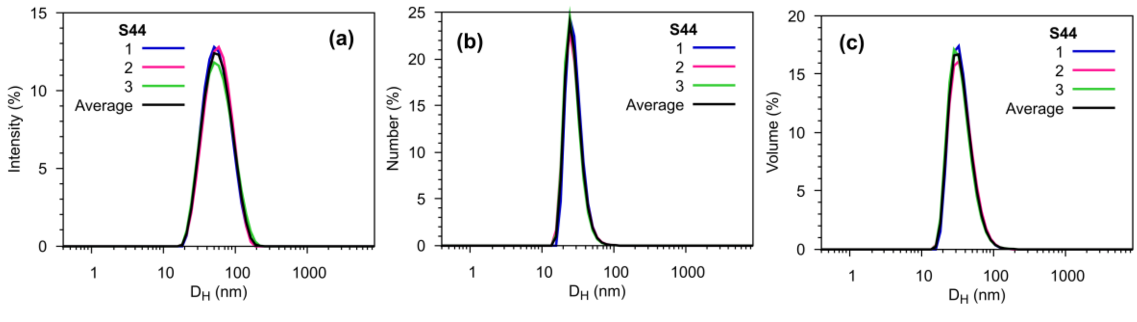

Figure S3a. D_H results for **S44** as function of (a) intensity, (b) number and (c) volume.

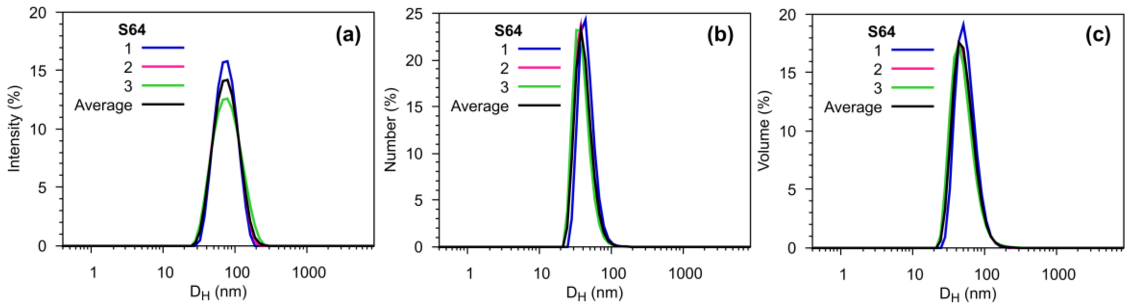

Figure S3b. D_H results for **S64** as function of (a) intensity, (b) number and (c) volume.

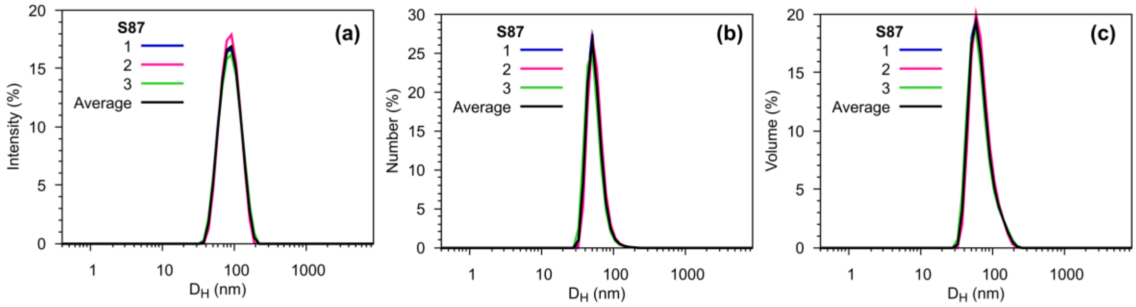

Figure S3c. D_H results for **S87** as function of (a) intensity, (b) number and (c) volume.

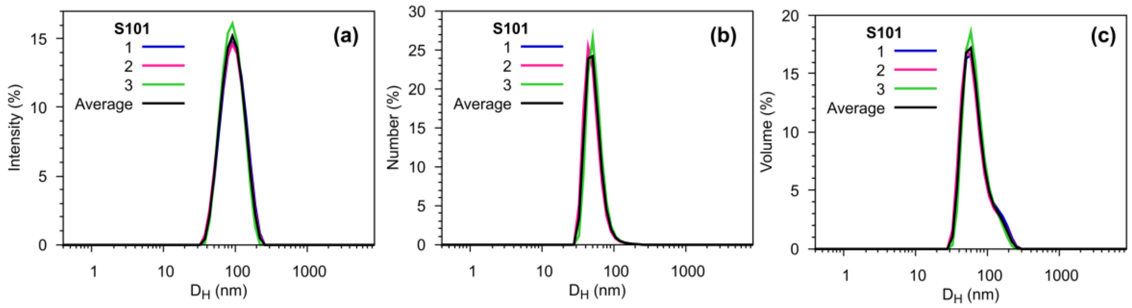

Figure S3d. D_H results for **S101** as function of (a) intensity, (b) number and (c) volume.

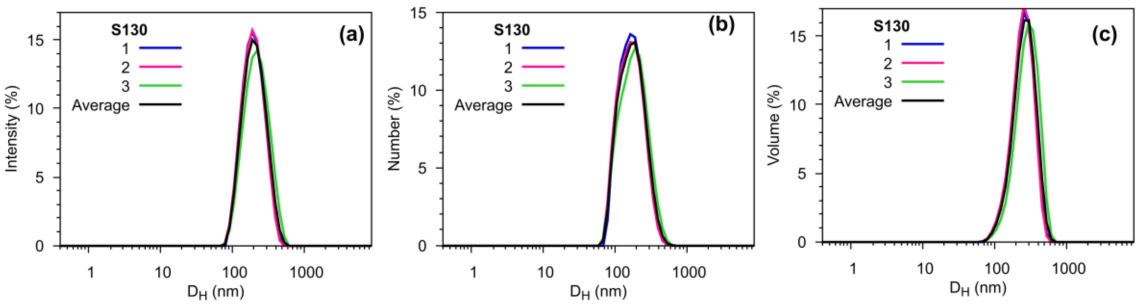

Figure S3e. D_H results for **S130** as function of (a) intensity, (b) number and (c) volume.

S4. DLS results from AuNPs dilution series

DLS results are shown in Figures S4(a-c) for the serial dilutions of the AuNPs S44 to S130.

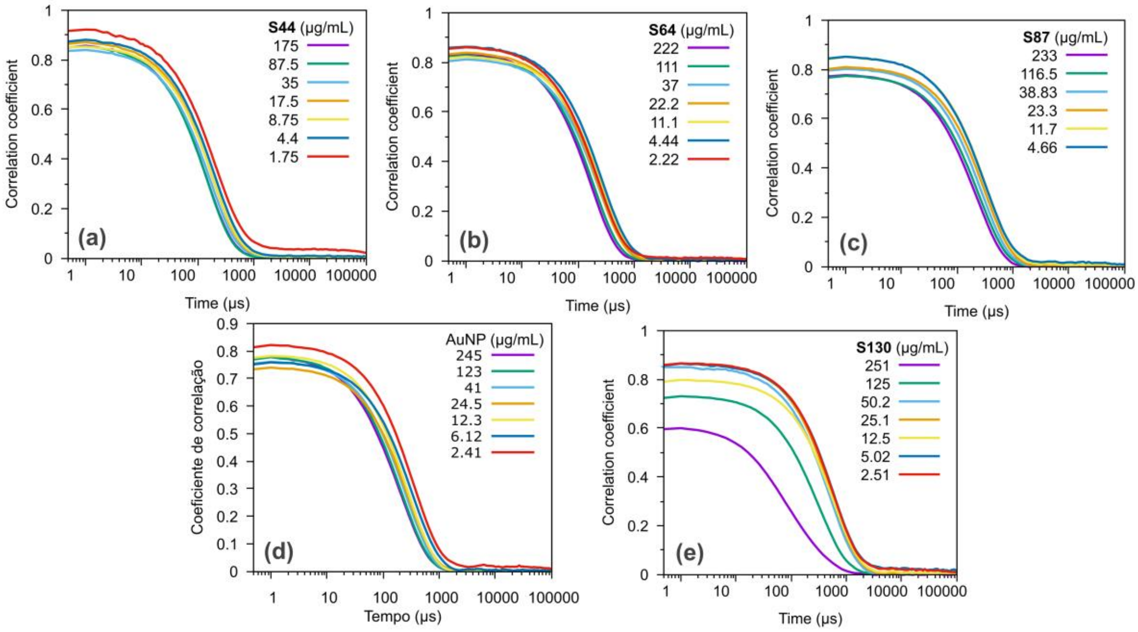

Figure S4a. Correlograms obtained by DLS for the dilution series of the synthesized AuNPs (**S44 to S130**).

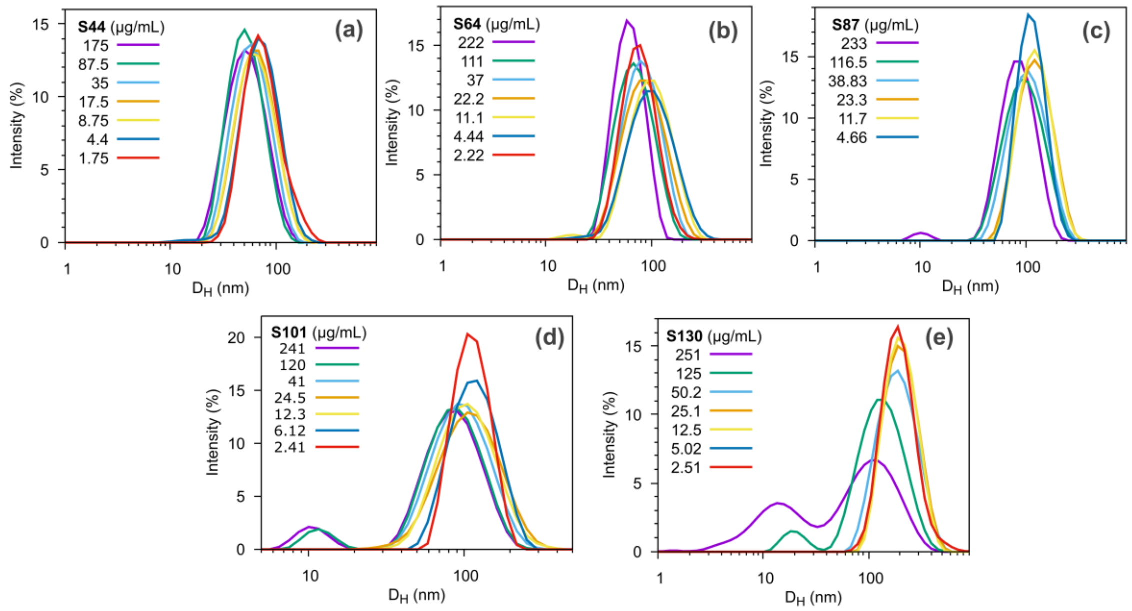

Figure S4b. D_H obtained by DLS as function of intensities for the dilution series of the synthesized AuNPs (**S44 to S130**).

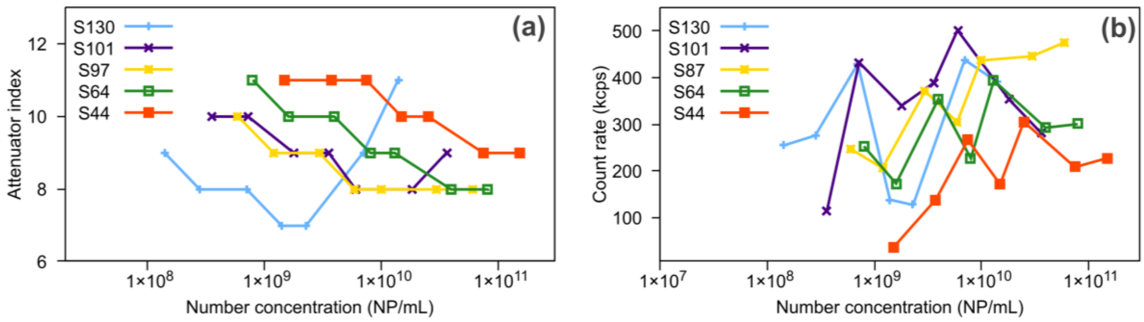

Figure S4c. (a) Attenuation Index and (b) count rate (kcps) obtained from DLS for the dilution series of the synthesized AuNPs (**S44 to S130**). Equivalence between attenuation index/laser transmission (%): 7: 1; 8: 3; 9: 10; 10: 30; 11:100.

S5. Reaction scheme for the bioconjugation

Figure S4 exhibits the reaction scheme for the two-steps bioconjugation. The binding occurs by the free amine onto IgG surface in a covalent manner.

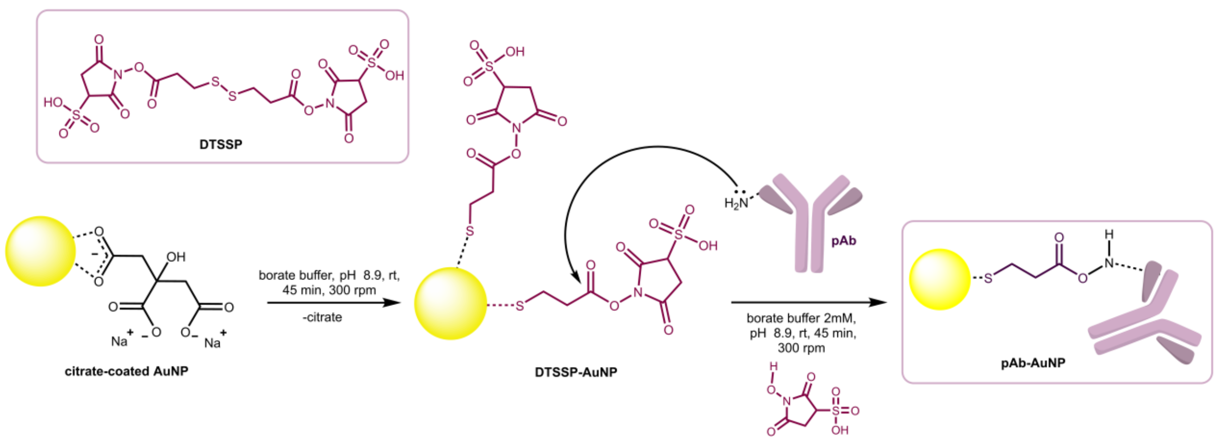

Figure S5. Reaction scheme for the preparation of the **pAb-AuNP** biosensor.

S6. FTIR analysis

In Figure S6 are shown the FTIR spectra for the AuNPs prepared at each step of the bioconjugation, as well as DTSSP and BSA (from solid samples). AuNPs samples were prepared by the concentration of the colloids in a centrifuge followed by the evaporation of the remaining water on air. The amide (I, II and III) bands, characteristic of proteins, can be observed in the samples before the BSA step ("without BSA" lines). After the BSA introduction, spectra are dominated by the BSA bands.

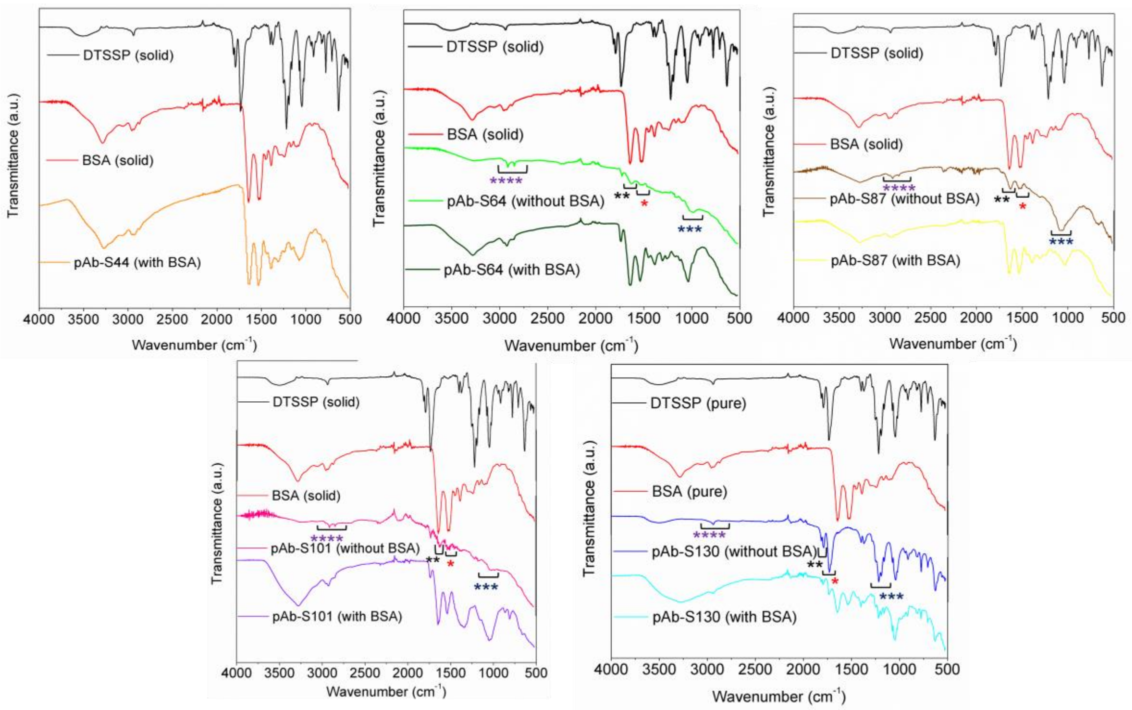

Figure S6. ATR-FTIR spectra of **DTSSP**, **BSA**, **pAb-S44, pAb-S64, pAb-S87** and **pAb-S101** with and without **BSA.** In the graphs the symbol * represents the amide I bands, the symbol ** represents the amide II bands, the symbol *** represents the amide III bands and **** represents the CH_2 and CH_3 stretching characteristic of proteins.

S7. DLS data for serial dilution of pAb-S130

Figure S7 presents some DLS data for the serial dilution of **pAb-S130** biosensor, such as (a) attenuation index and count rate, and (b) the correlograms.

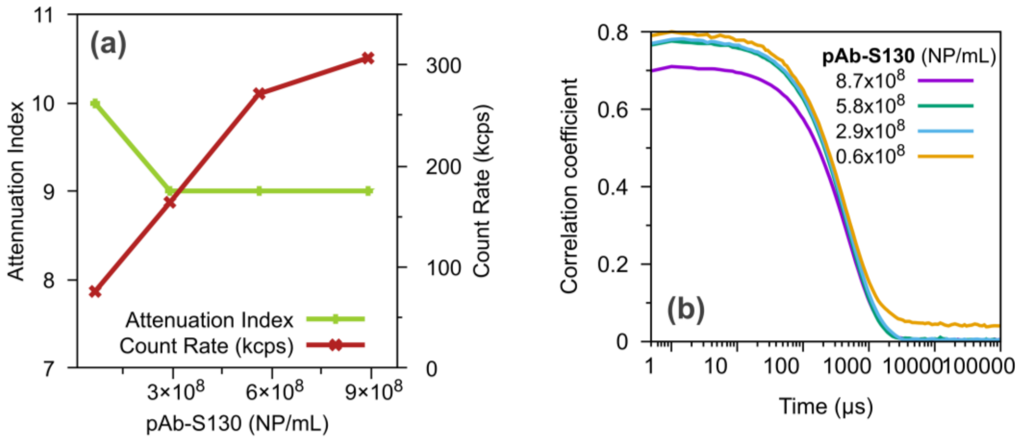

Figure S7. (a) Attenuation index and count rate and (b) correlograms obtained by DLS for the dilution series of **pAb-S130**.

Reference

1- Nobbmann, U. and Morfesis, A. Light scattering and nanoparticles. *Materials Today* **2009**, *12* (5) 52–54. https://doi.org/10.1016/S1369-7021(09)70164-6