Enzyme-free Immunoassay Using Silver Nanoparticles for Detection of Gliadin at ultra-low concentrations

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FUNCTIONALIZATION OF Ag NPs WITH BIOTIN AND STREPTAVIDIN.

Figure S1. A) Chemical structure of Biotin HPDP, Biotin (SH) and Pyridine-2-thione.
B) Schematic representation of the functionalization of an Ag NP with Biotin (SH) and Pyridine-2-thione.
STABILITY OF Ag NPs WITH BIOTIN AND STREPTAVIDIN.

Figure S2. A) Scheme showing the formation of Biotin-STV functionalized Ag NP when the reaction is performed with a strict stoichiometric control (with a 1: 1: 1 Ag NPs, Biotin and STV molar ratio). B) Stability of the colloidal dispersion of 58 nm diameter Ag NPs functionalized with STV-Biot produced under stoichiometric control.

The stability of the functionalized Ag NPs with STV-Biot in the absence of target has been also checked by TEM (Figure S3). It can be observed that any degree of NP aggregation is evidenced.

Figure S3. TEM image of 58 nm diameter Ag NPs synthesized by Turkevich method, after functionalization with biotin HPDP.
STABILITY OF Ag NPs WITH ETANHOL AND PBS1X-BSA5%

The stability of Ag NPs in the presence of 60% alcohol and PBS1X-BSA5% was tested by mixing 0.5 mL of previously functionalized Ag NPs, 0.01 mL of a 60% alcohol solution in water and supplemented with 0.49 mL of PBS-BSA5% to reach a final volume of 1 mL. Figure S3 shows that the extinction spectra remains the same (there is not any shift nor any change on its) indicating that the Ag NPs are stable in the presence of alcohol and buffer. The results of these tests are of crucial importance since the experiments carried out on microplates for the analysis of problem samples requires the use of both compounds in amounts which are proportionally similar to those used in these tests.

![Extinction spectra](image)

Figure S4. UV-Vis spectra of Ag NPs of 58 nm of diameter stability in the presence of a 60% alcohol solution. A volume of 0.5 ml of particles 0.49 ml of PBS1X-BSA5% and 0.01 ml of alcohol was used in a plastic cuvette.
Figure S5. Some representative TEM images of the dimeric structures obtained for two different average NP diameters after the addition of 1 ng/mL of IgG- Biot A) 58 nm B) 78 nm

THEORETICAL SIMULATIONS

Figure S6. A) Theoretical modelling using Mie theory and GMM theory for the cross-section of 1 monomer, 2 monomers and 1 dimer (averaged over x, y and z polarizations) for 58 nm diameter Ag NPs B) The same as in A) but for 78 nm diameter Ag NPs.
VARIATION OF THE DEGREE OF DEPLETION WITH Ag NP SIZE

The degree of depletion \( \frac{E_t}{E_0} \), at any time \( t \) and for any NP size, is obtained dividing eq.2 of the present manuscript by the initial extinction \( E_0 = [NPs]_t \sigma_m \)

\[
\frac{E_t}{E_0} = \left( \frac{1-f_d}{1+f_d} \right) + \left( \frac{f_d}{1+f_d} \right) \frac{\sigma_d}{\sigma_m} \quad \text{(3)}
\]

Taking the derivative of this expression with respect to \( f_d \), it can be clearly realized that the sensitivity of the method, i.e. the infinitesimal change of the degree of depletion when an infinitesimal change of the fractions of dimers formed, depends on the ratio between the cross section of the monomer and the cross section of the dimer, i.e. on \( \frac{\sigma_d}{\sigma_m} \) as well as on the fraction of dimers \( f_d \)

\[
\frac{d\left( \frac{E_t}{E_0} \right)}{df_d} = \frac{1}{(1+f_d)^2} \left( -2 + \frac{\sigma_d}{\sigma_m} \right) \quad \text{(4)}
\]

The value of \( \frac{\sigma_d}{\sigma_m} \) for Ag NPs diameters in the range 48-58 nm is almost the same, around 1.49 and 1.5, as shown in Figure S7 above, therefore the sensitivity is expected to be the same. For larger Ag NPs, around 78 nm, the ratio \( \frac{\sigma_d}{\sigma_m} \) increases to a value of 1.61.

Assuming an equal fraction of dimers \( f_d = 0.9 \) the derivative is 0.138 and 0.11 for Ag NPs in the range 48-58 nm and for 78 nm, respectively, that is there is only a slight increase on the sensitivity for the largest NP size. For \( f_d = 0.2 \), the derivative increases to 0.347 and 0.417. For this dimer fraction, in the absence of antigen, the change on the derivative is greater by a factor of 2.5 (for 48-58 nm Ag NPs) and 4.3 (for 78 nm Ag NPs). Therefore, it makes sense to use Ag NP sizes having different values of \( \frac{\sigma_d}{\sigma_m} \) such as those selected in the present work and, more importantly, we have worked under conditions where the dimer fraction is quite different (0.2 and 0.9) since \( f_d \) is the other crucial quantity controlling the sensitivity of the IDILA method.

Another issue to be considered is the fact that the almost negligible difference in the degree of depletion for Ag NPs sizes in the range 48-58 nm constitutes an advantage of the method. As it is well known, the fabrication of highly monodisperse Ag NPs is very difficult. The fact that the degree of depletion is almost the same in a wide range of Ag NP sizes somehow ensures the reproducibility of the IDILA method. The experiments performed using larger NPs are expected to give rise to a smaller degree of depletion for any dimer fraction, as shown in the pink curve of Figure S7. However, the sensitivity of the IDILA method depends on the change of the degree of depletion when an infinitesimal change on the fractions of dimers is produced at a given dimer fraction (the slope of each curve in Figure S7), which is significantly larger for 78 nm NPs than for 58 nm NPs, as the slope of the curve for \( f_d=0.2 \) is higher than the slope for \( f_d=0.9 \)
Figure S7: Variation of the degree of depletion on the dimer fraction for three representative NP diameters.

GIADIN EXTRACTION

Figure S8.A Shows the image obtained from the radiographic plates of the immunoblot. The left column corresponds to the reference molecular weight marker (in KDa) and the remaining columns correspond to the gliadin fraction extracted from oat, wheat, corn flour and corn starch. As expected the columns corresponding to oat and wheat, have strong marks corresponding to all the gliadin proteins complexes (molecular weight between 25-50 kDa) while corn flour and starch which are almost gluten free samples depicts a very week signal, being for corn starch not detectable. However, the IR-ATR spectrum of the different food samples where gliadin was extracted depicts the characteristic amide 1, amide 2 and amide 3 bands of the gliadin proteins only for oat and wheat flour while as the gliadin content of corn flour and corn starch is so low, these signals are not detectable.
Figure S8. A) Western blot with antibodies specific for gliadin, the first column is the molecular step marker (gliadin range 25-50 KDa), the second column represents the extracted content of gliadin for wheat flour, the third for oats and the fourth for maize flour, the content of maize Starch is so low that through this technique it has not been able to be revealed. B) FT-IR ATR spectra for wheat flour, maize flour, oats and corn starch where the amide 1° (1650 cm⁻¹), 2° (1550 cm⁻¹), 3° (1450 cm⁻¹) corresponding to protein structures and the starch band (1050 cm⁻¹).

MICROPLATE READER MEASUREMENTS.

The required amount (in order to reach a final concentration of 1 ng/mL) of the IgG-Biot antibody was added to 100 µL of 58 nm or 78 nm colloidal dispersion of the functionalized Ag NPs in the presence of PBS1X-BSA5% buffer to reach a final volume of 0.3 mL. Using this amount of antibody give rise, after 27 minutes to a very different fraction of dimers formed depending on the size of the Ag NPs. For building the calibration curve, a specific amount of IgG-Biot (final concentration 1 ng/mL), the buffer and different amounts of antigen were added directly to the mixture of functionalized Ag NPs. The intensity was monitored with a microplate reader spectrophotometer using a 450 nm filter. The variation of the intensity (buffer corrected) was plotted as a function of antigen concentration. The calibration curve in the whole
concentration range was adjusted to an exponential function \( I=-A_0e^{-bc} + I_0 \) where \( I \) = intensity and \( c \) = antigen concentration. The fitting gave a \( R^2 \) value above 0.97 for 58nm and 78 nm of diameter, alternatively the lineal range of the calibration curve can be used for quantifying and the optimization gave a \( R^2 \) value above 0.88 for 58 nm and 0.98 for 78 nm. For real samples measurements, dilutions of the real samples were performed: 1 µL of a 10:1000 dilution for Ag NPs of 58 nm diameter and 1:1000 for 78 nm of diameter, the gliadin extraction was added to the mixture of Ag NPs, IgG-Biot and buffer (final volume 0.3 mL). The extinction intensity (after corrected according to buffer intensity), was used to calculate the antigen concentration in the real samples using the calibration curve.