

Design of a Novel Plasmonic Nanoconjugated Analytical Tool for Ultrasensitive Antigens Quantification.

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SUPPLEMENTARY FIGURES

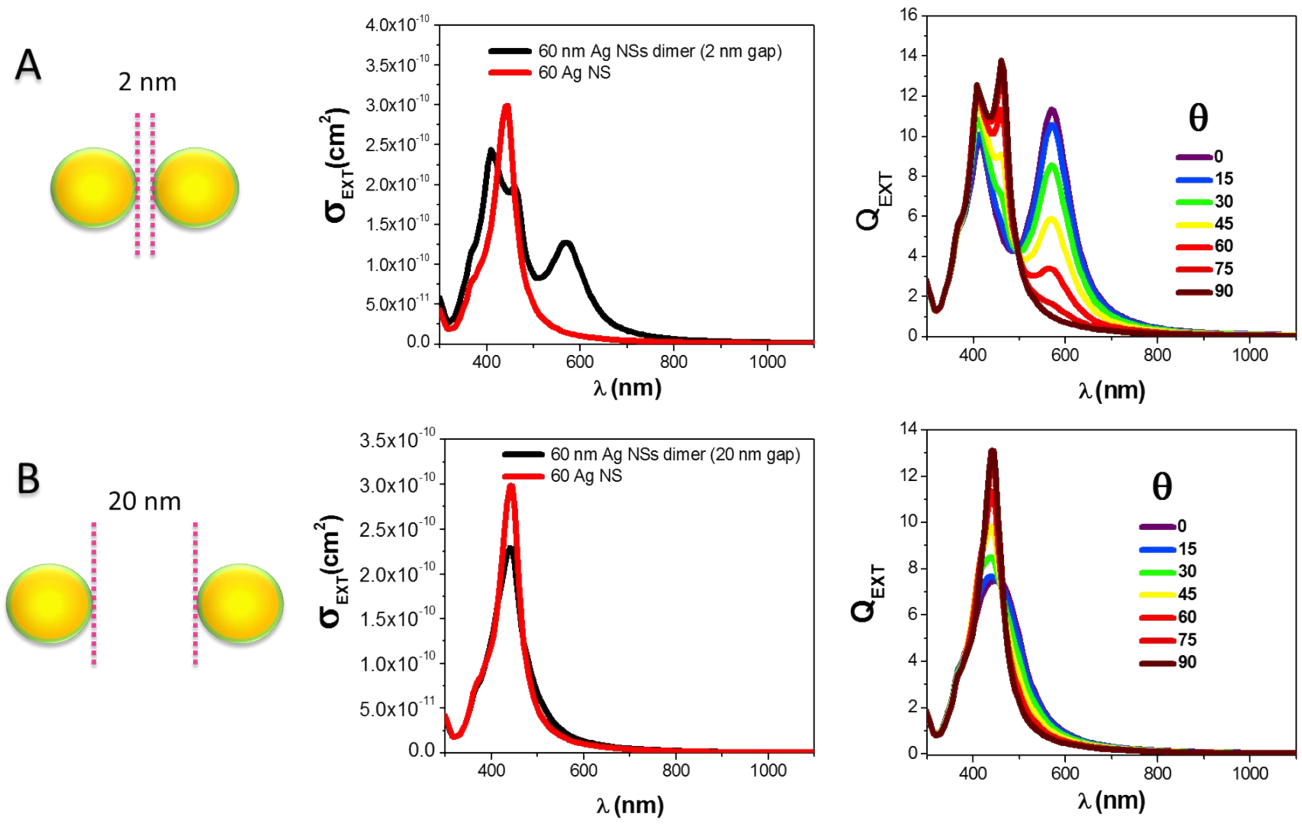


Figure S1: Theoretical simulations of the far-field optical response of Ag NS dimers. Average extinction cross section of the dimers (left panel), and extinction efficiency of the NS dimer at different orientations (averaged over 8 different orientations) (right panel). Simulations using GMM were performed for 60 nm Ag NS dimer with: a) 2 nm and b) 20 nm gap.

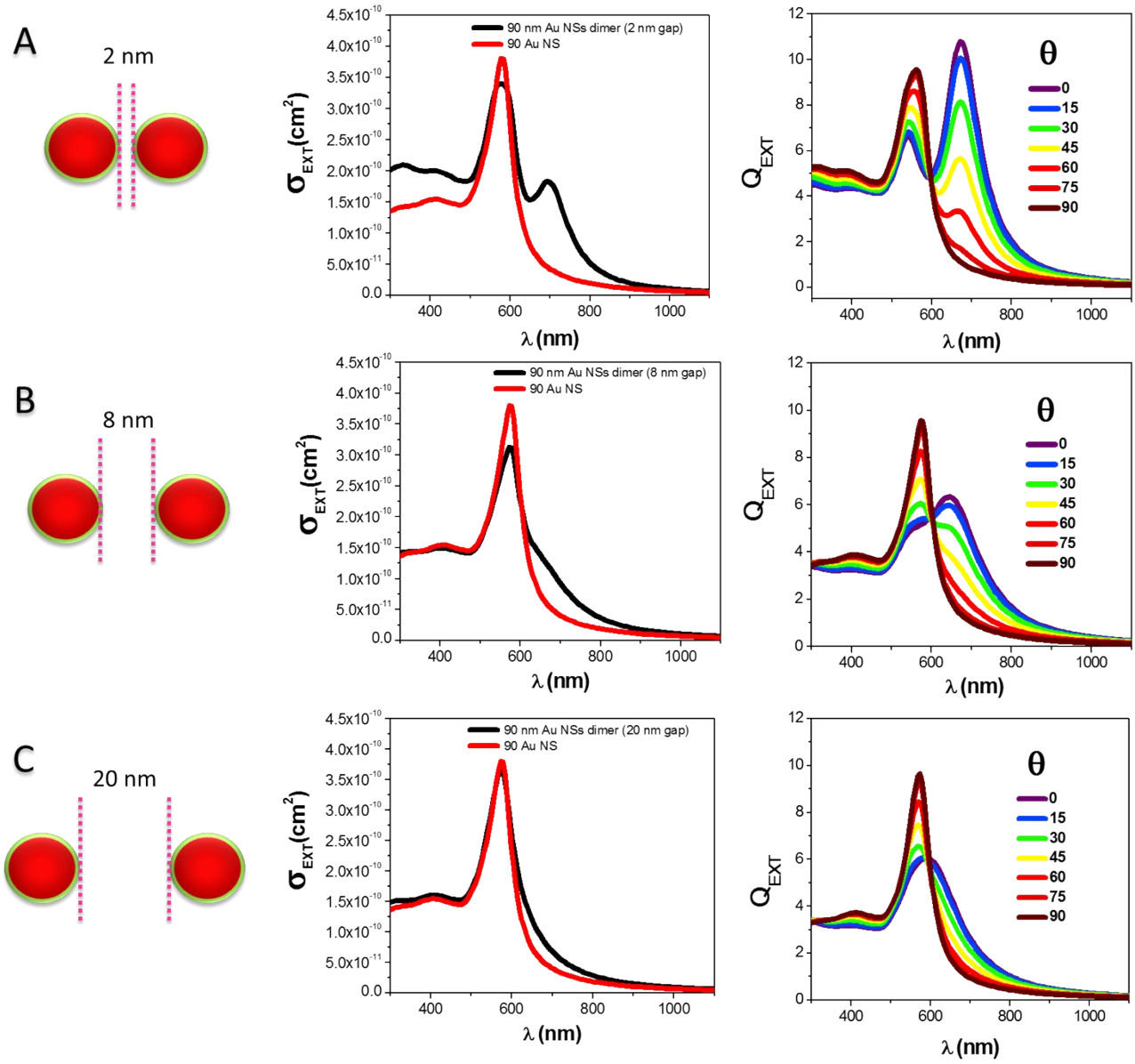
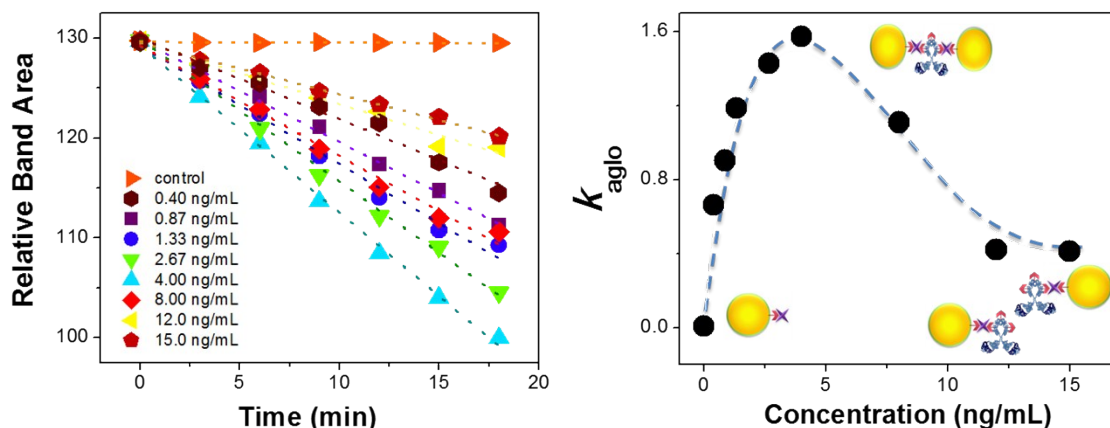
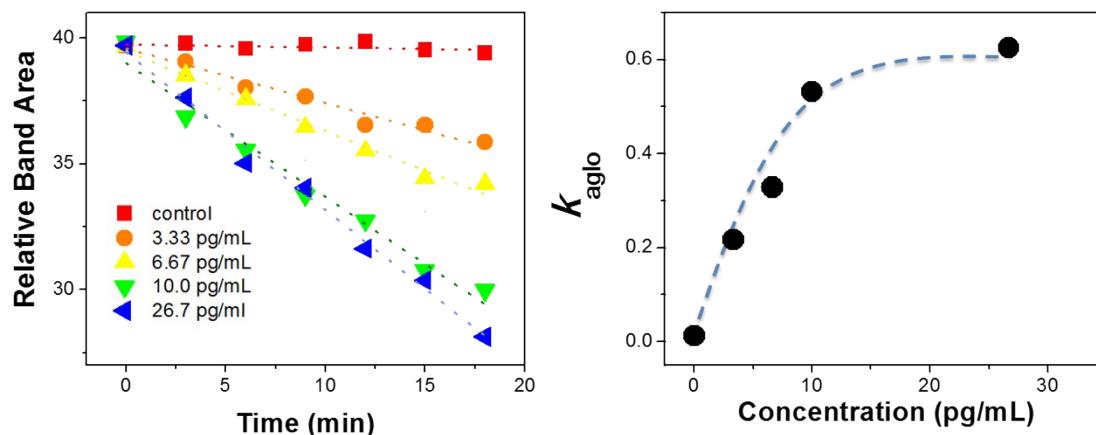


Figure S2: Theoretical simulations of the far-field optical response of Au NS dimers. Average extinction cross section of the dimers (left panel), and extinction efficiency of the NS dimer at different orientations (averaged over 8 different orientations) (right panel). Simulations using GMM were performed for 60 nm Ag NS dimer with: a) 2 nm, b) 8 nm and c) 20 nm gap.

A



B



C

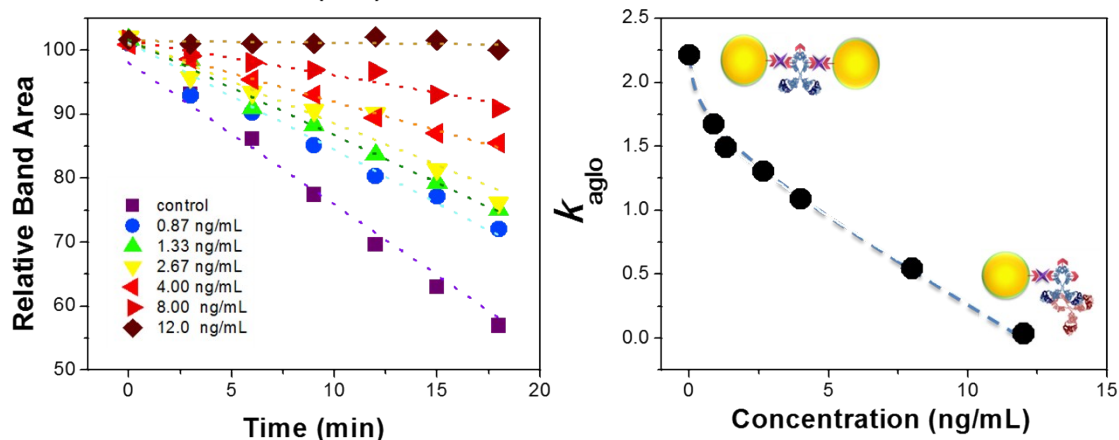


Figure S3: Kinetics measurements of the dimer formation using a biotinylated Goat Anti Rabbit IgG Antibody (Biot-IgG). **a**, Linear fitting of the integral of the extinction spectra (left panel), and trend of the agglomeration rate constant (k_{Aglo}) for dimers formation both as a function of the concentration of Biot-IgG. **b**, Effect of the dilution of the initial Ag NPs solution that leads to a lower detection limit: Linear fitting of the integral of the extinction spectra (left panel), and trend of k_{Aglo} for dimers formation both as a function of the concentration of Biot-IgG. **c**, Avoiding dimers formation due to the addition of purified rabbit IgG (antigen) to a solution of Ag NPs with Biot-IgG: Linear fitting of the integral of the extinction spectra (left panel), and trend of k_{Aglo} for dimers formation both as a function of the concentration of antigen. In every experiment the integral of the extinction spectrum was performed between 340 and 800 nm, and the blue dotted lines are a guide to the eye.

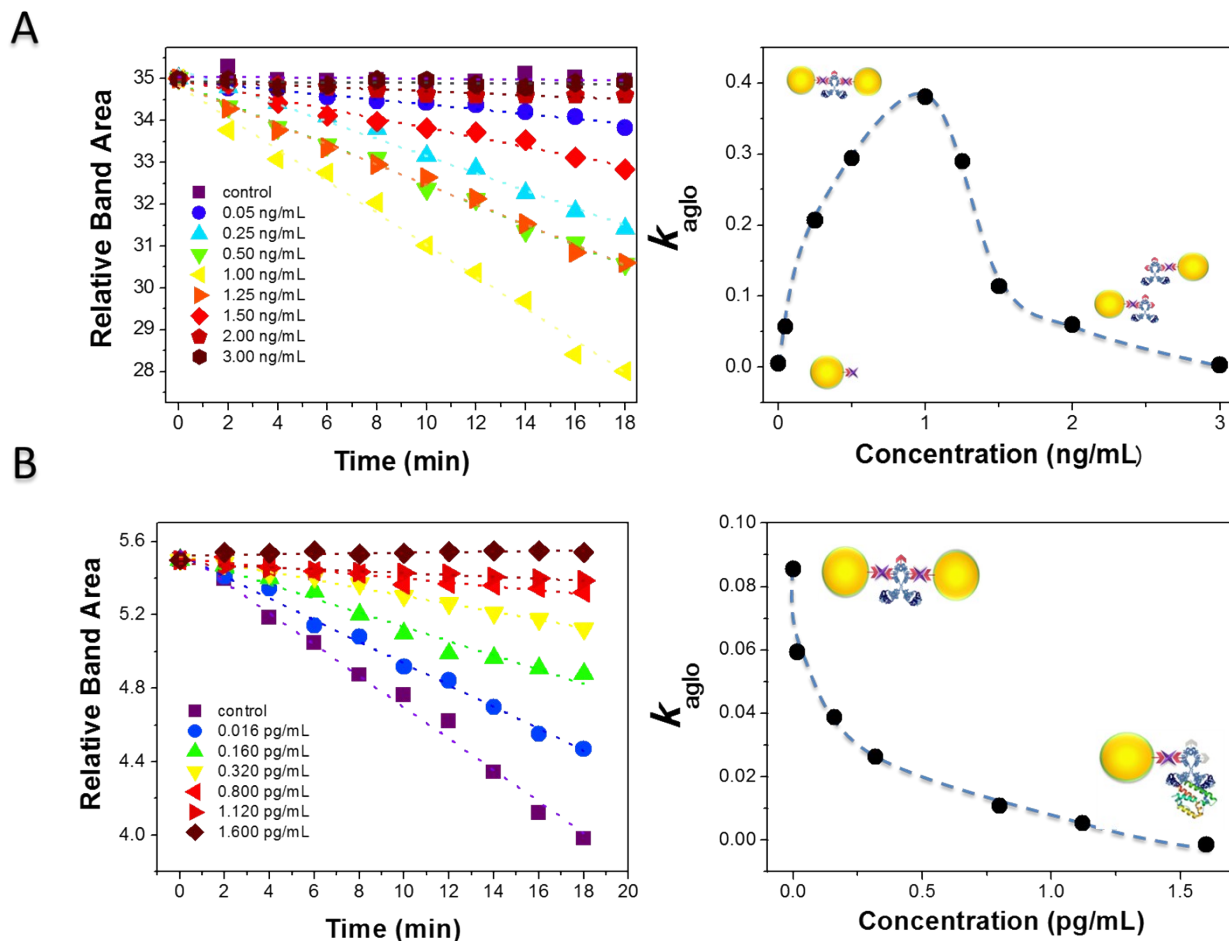
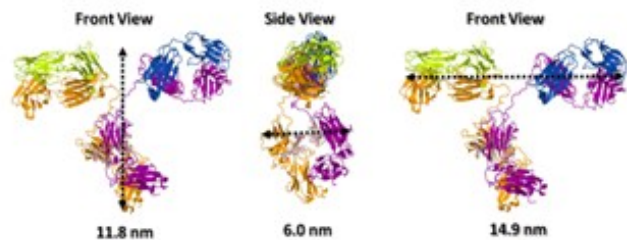
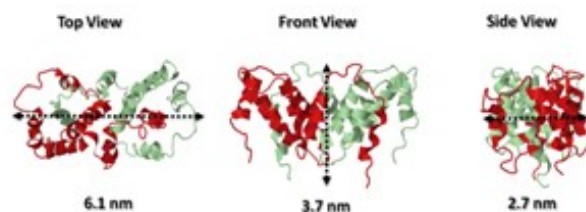


Figure S4: Kinetics measurements of the dimer formation using a biotinylated Anti IL-10 IgG Antibody (Biot-IgG). **a**, Linear fitting of the integral of the extinction spectra (left panel), and trend of the agglomeration rate constant (k_{Aglo}) for dimers formation both as a function of the concentration of Biot-IgG. **b**, Avoiding dimers formation due to the addition of recombinant IL-10 (antigen) to a solution of Ag NPs with Biot-IgG: Linear fitting of the integral of the extinction spectra (left panel), and trend of k_{Aglo} for dimers formation both as a function of the concentration of antigen. In every experiment the integral of the extinction spectrum was performed between 340 and 800 nm, and the blue dotted lines are a guide to the eye.

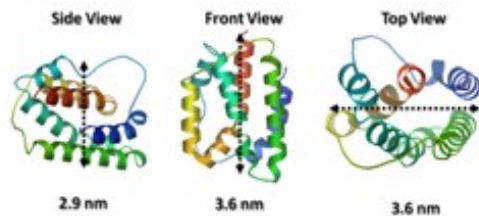
■ Immunoglobulin G



■ Interferon Gamma (IFN γ)



■ Interleukin 10 (IL-10)



■ Tumor Necrosis Factor Alpha (TNF α)

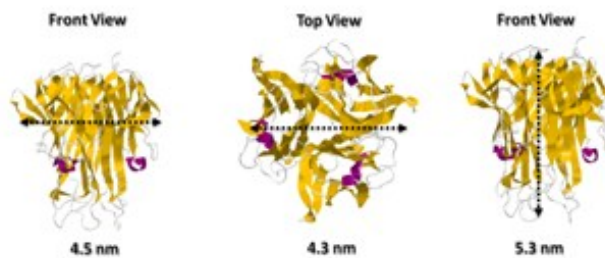
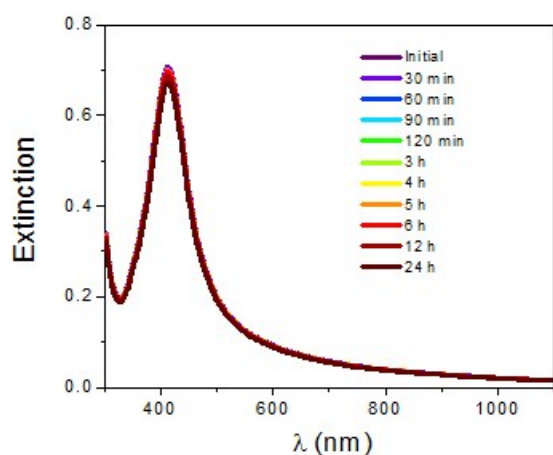
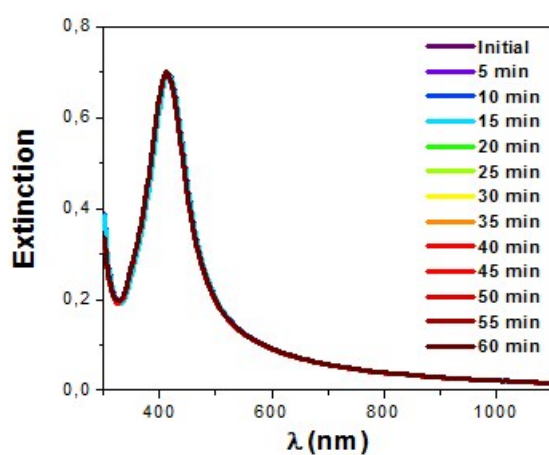


Figure S5: Structural parameters of the antigens used in this study. The differences guarantee that the impediment to the dimer formation is not a result of a steric repulsion; instead this could be attributed to a conformational change that “hides” the other biotins of the Biot-IgG.

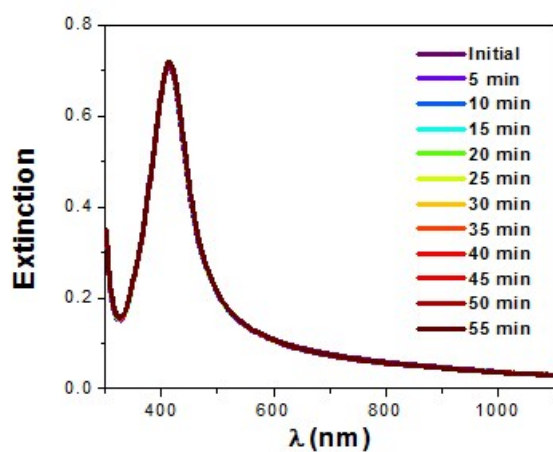
**PBS 1X (pH 7.2 – 7.4)
5% P/P BSA**



**Normal Human
Blood Serum**



**Lipemic Human Blood
Serum**



**Hemolyzed Human Blood
Serum**

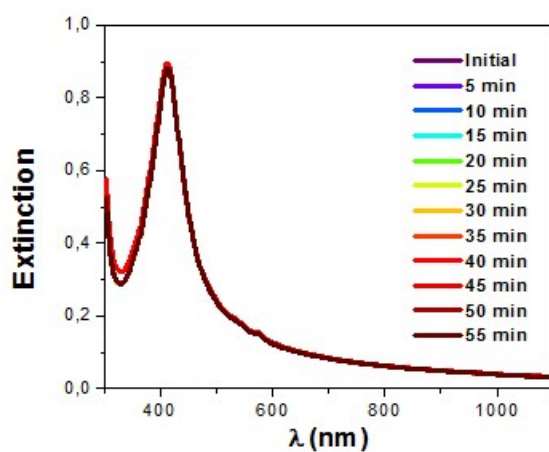


Figure S6: Spectral stability of the Ag NPs colloidal dispersion. The different figures depict the spectral stability of the functionalized Ag NPs after the addition of 50 μ L of buffer or human blood serum.

		IDILA	ELISA
Assay time (sample processing to detection)		< 2 h	> 5 h
Cost	Instrument	Can be performed using ELISA Microplate Readers	ELISA Microplate Readers
	Per Assay	~US\$10 (Ag nanoparticles, biotin HPDP, streptavidin, microplates, detection antibodies)	~US\$65 (microplates, capture and detection antibodies, recombinant standards, avidin-HRP, assay diluent, coating buffer, TMB substrate, stop solution)
POC use		Easily applicable	Currently most wide spread used technique

Supplementary Table S1. Comparison of methods for antigen quantification.