Supporting Information

to

Ultra-stable Silver Nanoplates: Efficient and Versatile Colorimetric Reporters for Dipstick Assays

Maurice Retout,[‡] Bryan Gosselin,[‡,§] Amina Adrovic,[‡,§] Pascale Blond,[§] Ivan Jabin,[,§] and Gilles Bruylants,*[,‡]*

[‡] Engineering of Molecular NanoSystems, Ecole Polytechnique de Bruxelles, Université libre de Bruxelles (ULB), avenue F. D. Roosevelt 50, CP165/64, B-1050 Brussels, Belgium.
[§] Laboratoire de Chimie Organique, Université libre de Bruxelles (ULB), avenue F. D. Roosevelt 50, CP160/06, B-1050 Brussels, Belgium.

*Corresponding authors: Gilles.Bruylants@ulb.be; Ivan.Jabin@ulb.be

Table of contents

1. Materials

Silver nitrate ($AgNO_3$), hydrogen peroxide (H_2O_2), trisodium citrate ($Na_3C_6H_5O_7$), L-Ascorbic acid, sodium borohydride ($NaBH_4$), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC.HCl), N-Hydroxysulfosuccinimide sodium salt (Sulfo-NHS), bovin serum albumin (BSA) and Absorbent Pad SureWick (1.7x 30 cm) were purchased from Sigma-Aldrich (St-Louis, MO). 1.5- and 0.5-mL Protein LoBind Eppendorf were purchased from VWR Chemical (Radnor, Pennsylvania). Calix[4]arenes C1 and C2 were synthesized according to literature procedures. Rabbit IgG (PP64), Anti-Rabbit IgG (SAB3700848) and Anti-Human IgG (I2136) were ordered from Sigma-Aldrich (St-Louis, MO). Recombinant SARS-Cov-2 spike protein was obtained from RayBiotech (Norcross, GA). SARS-Cov-2 IgG were purchased from GenenTech (San Diego, CA). NC membranes were obtained from Cytiva (Washingotn, DC) for HP170 and Sartorius (Wood Dale, IL) for CN140. Before use, all glassware and Teflon-coated stir bars were washed with aqua regia (3:1 volume ratio of concentrated HCl and HNO_3) and rinsed thoroughly with water. Caution! Although we have not encountered any problem, it is noted that diazonium salt derivatives are potentially explosive and should be handled with appropriate precautions. Aqua regia is highly toxic and corrosive and requires proper personal protective equipment. Aqua regia should only be handled in a fume hood.

2. Instrumentation

2.1 UV-Vis spectroscopy

UV-Vis absorption spectra were recorded from 1000 to 300 nm at a 120 nm/min scan speed with a UV-Vis-NIR spectrophotometer in disposable cuvettes (PMMA) with a 1 cm optical path length at room temperature.

2.2 Dynamic light scattering

Samples were characterized by dynamic light scattering (DLS) with back scattering (NIBS 173°). Measurements were performed at 25 °C and the AgNPls were dispersed in Lichrosolv water to obtain 1 mL of AgNPls (OD = 0.1) in disposable semi-micro cuvettes (PMMA) and multiple DLS measurements were performed. The reported values are the average hydrodynamic diameter obtained from three independent measurements using the Z average as calculated by the Zetasizer software.

2.3 Attenuated Total Reflection Fourier-transform Infrared

Attenuated Total Reflection Fourier-transform Infrared **(**ATR-FTIR) spectra were recorded at 22 °C on a FTIR spectrophotometer equipped with a liquid-nitrogen-cooled mercury−cadmium−telluride detector. The spectrophotometer was continuously purged with dried air. The AgNPls were deposited in solution on a germanium single-crystal internal reflection element (triangular prism of 6.8 × 45 mm, with an internal incidence angle of 45°), and the solvent was removed with a flow of nitrogen gas. Bare germanium was used for the background spectrum. Opus software (4.2.37) was used to record 128 scans with a nominal resolution of 2 cm^{-1}. Data were processed and analyzed using the home written Kinetics package in Matlab R2013a by subtraction of water vapor, baseline correction, and apodization at 4 cm^{-1}.

2.4 Transmission Electron Microscopy

Images of the AgNPs were obtained with a Philips CM20-UltraTWIN Transmission Electron Microscope (TEM) equipped with a lanthanum hexaboride (LaB6) crystal at a 200kV accelerating voltage. The average size and 95% confidence interval were determined by measuring the size of at least 100 AgNPls.

3. Characterization of calixarene-coated AgNPls

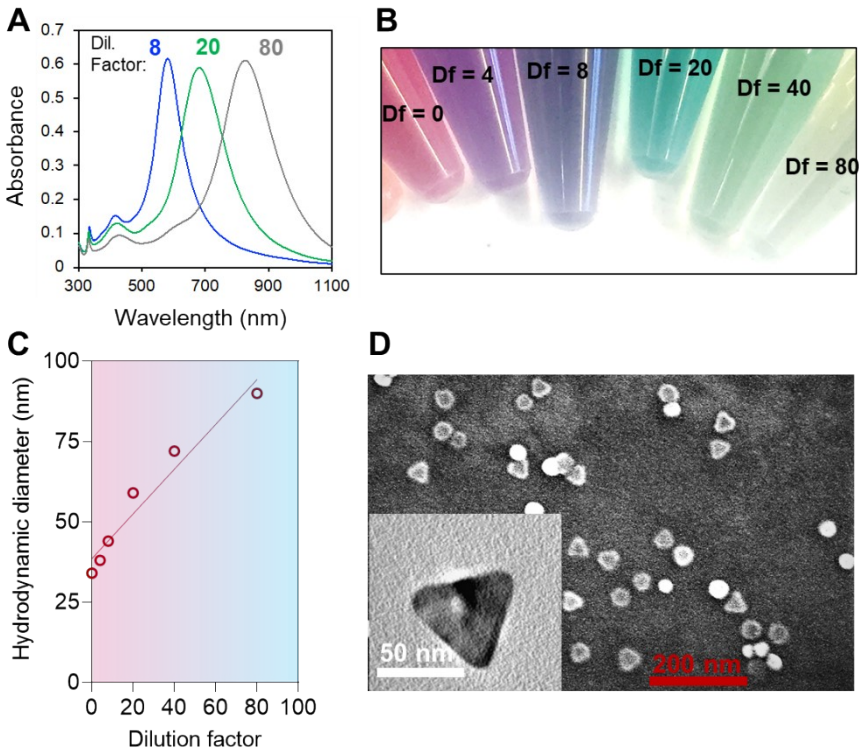

Figure S1. **(A)** UV-Vis spectra of AgNPls^{Df}-**C2** suspended in pure water with corresponding picture of the suspensions **(B)**. **(C)** Hydrodynamic diameters obtained by dynamic light scattering of AgNPls^{Df}-**C2** suspended in pure water. **(D)** SEM pictures of AgNPls^{20}-**C2**. Inset shows the corresponding TEM image.

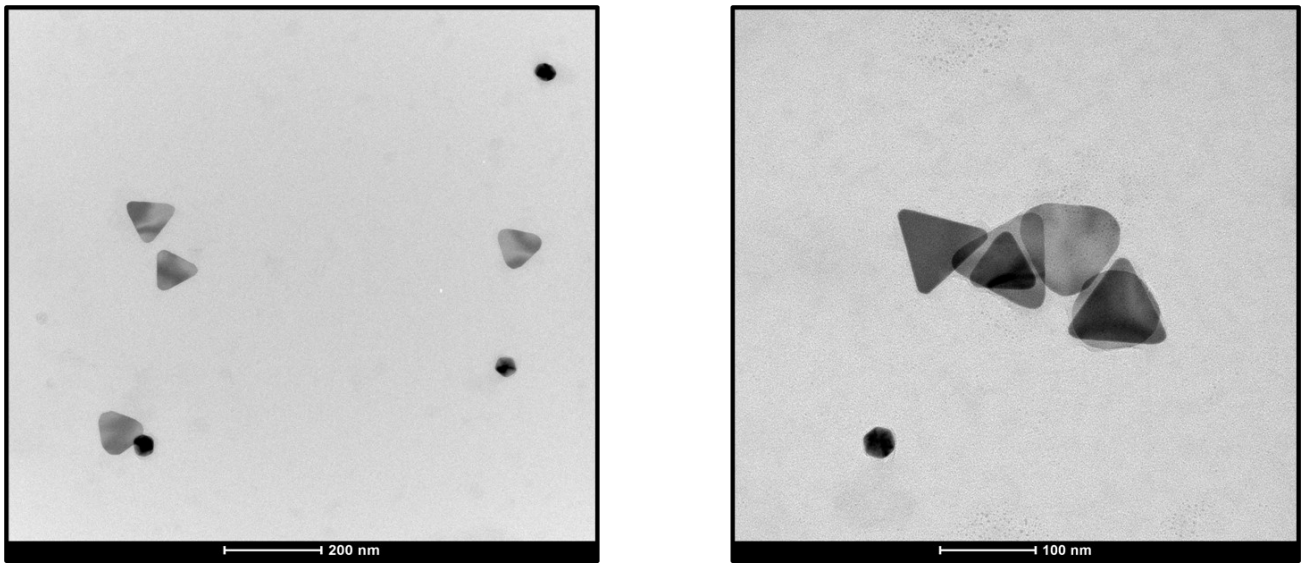

Figure S2. TEM images of AgNPls^{80}-**C1**.

4. IR characterization of C1, C2 and AgNPls^{8}-citrate

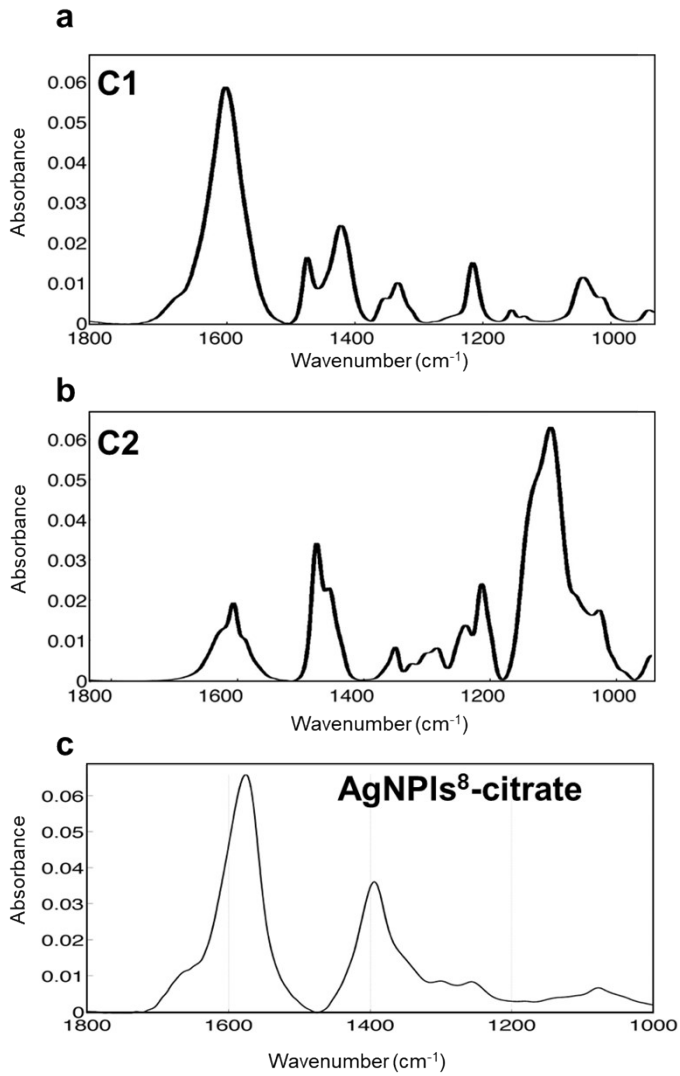

Figure S3. ATR-FTIR spectra of (a) **C1**, (b) **C2** and (c) AgNPls^{8}-citrate.

5. Stability of calixarene-coated AgNPls

A. Stability of AgNPls^{Df}-**C1** over time

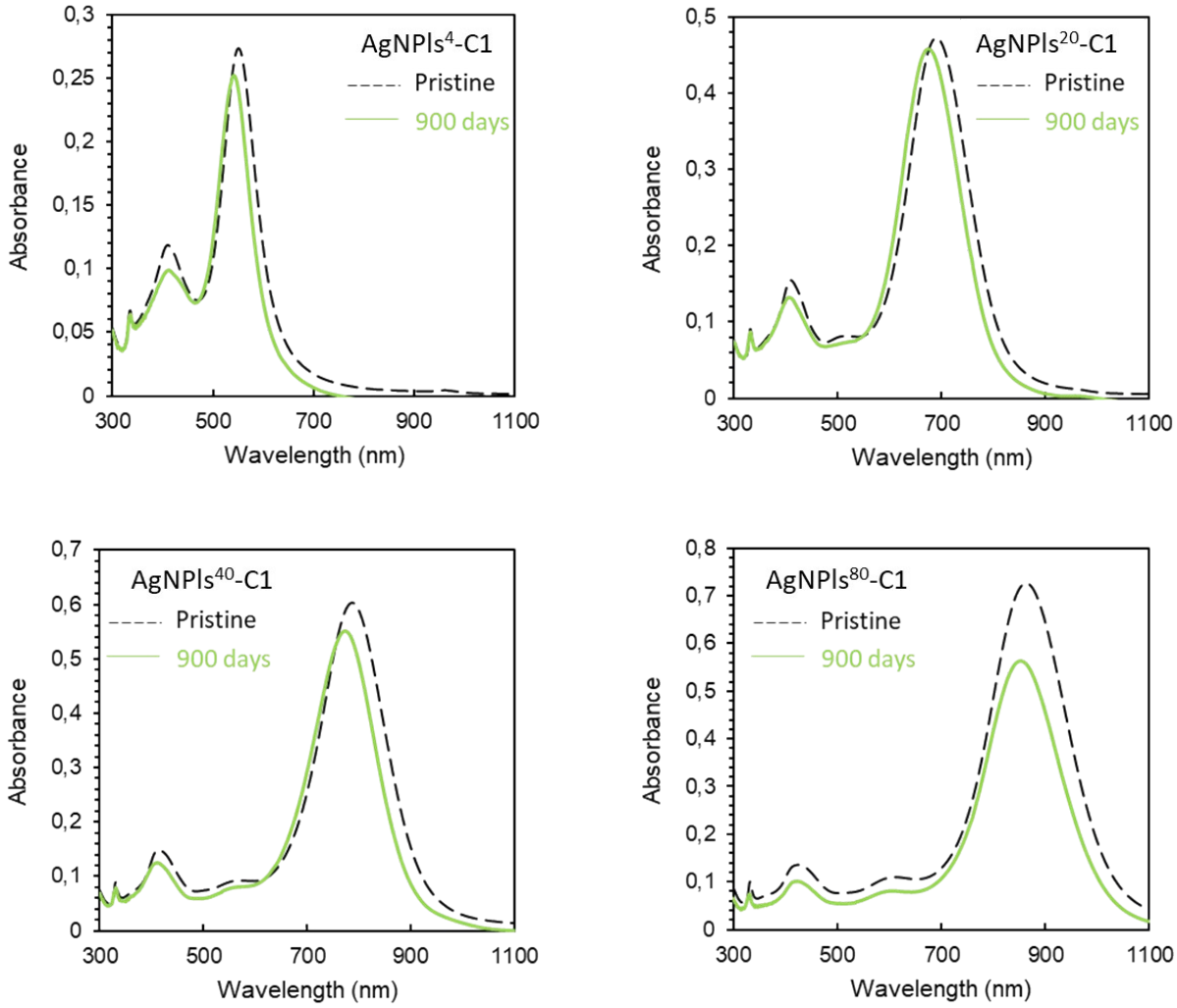

Figure S4. UV-Vis spectra of AgNPls^{Df}-**C1** right after the synthesis (pristine) or after 900 days of storage in pure water.

B. Stability of AgNPls^{Df}-**C1** against acidic medium

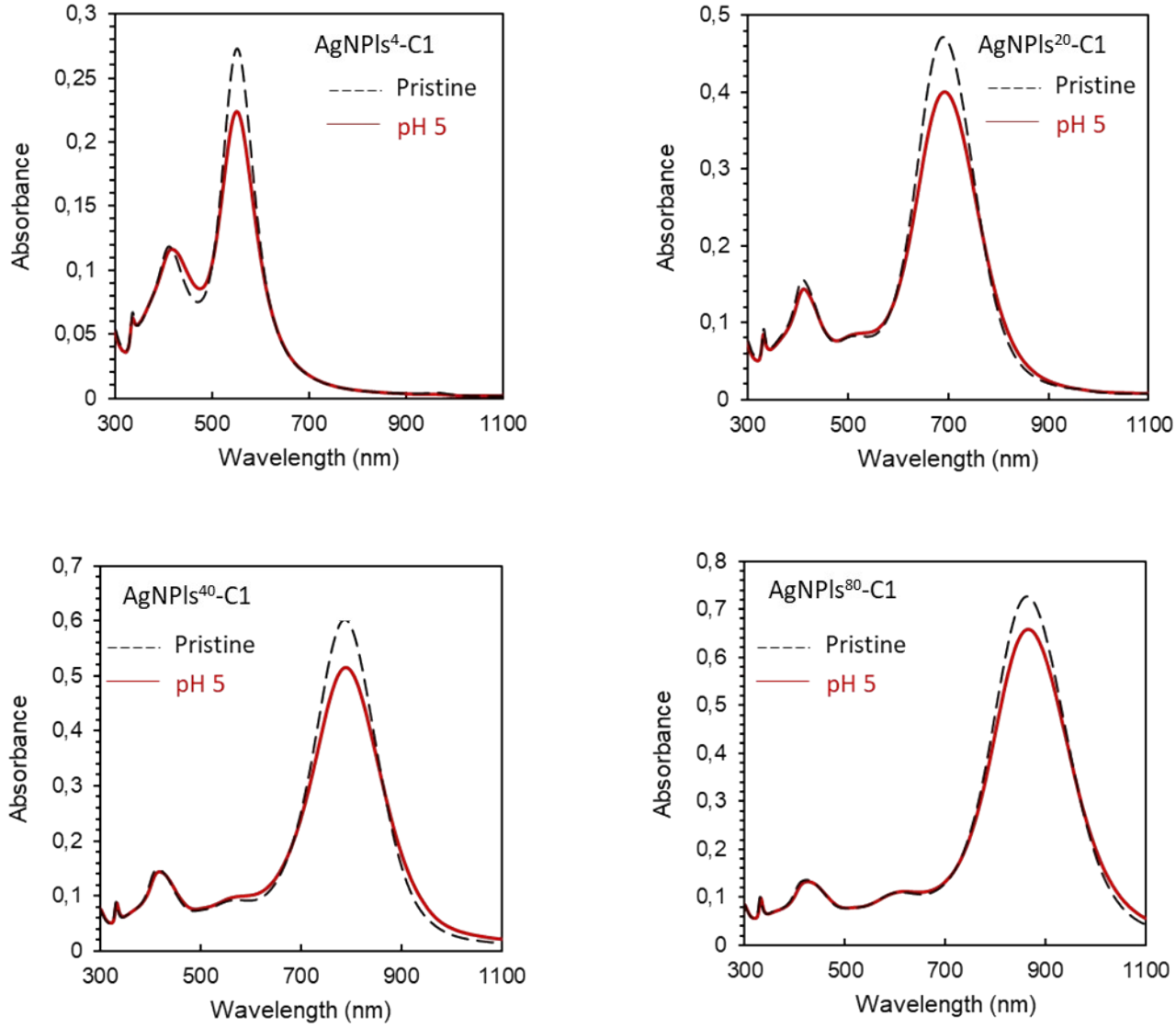

Figure S5. UV-Vis spectra of AgNPls^{Df}-**C1** after 2 hours of suspension, either in water or acidic condition (pH=5).

C. Stability of AgNPls^{Df}-**C1** in presence of PBS

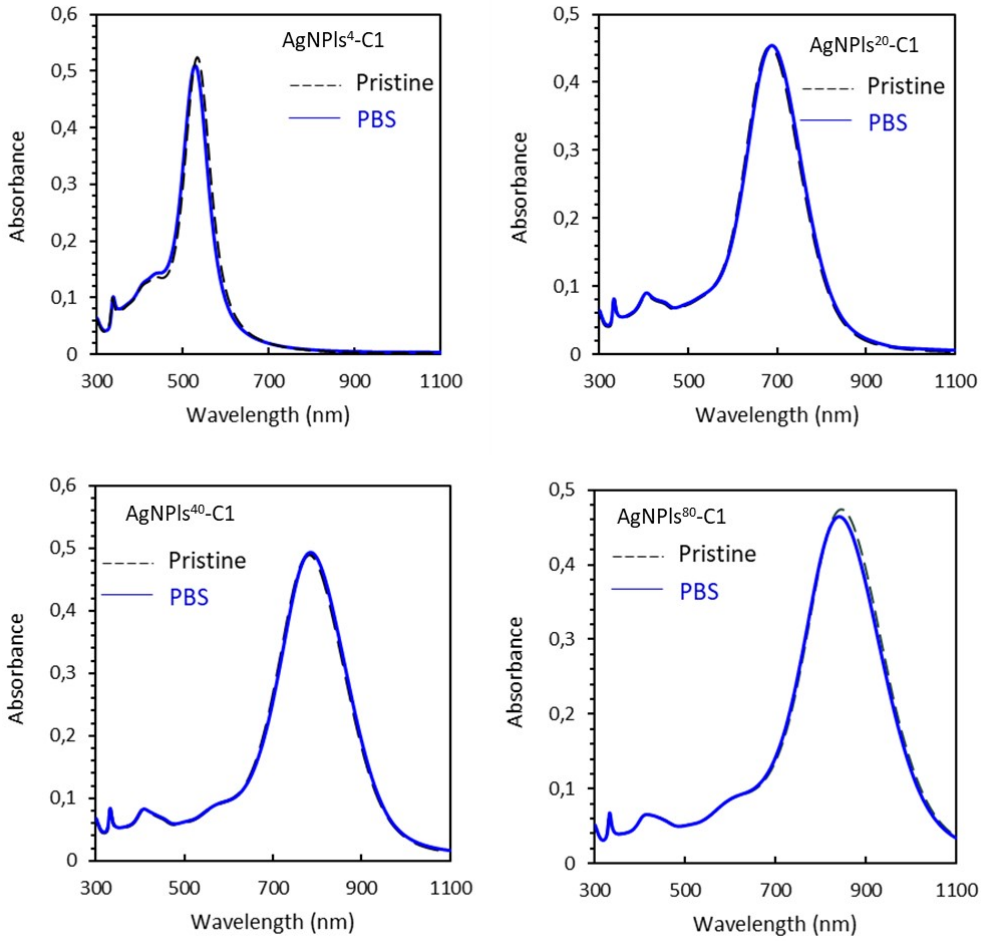

Figure S6. UV-Vis spectra of AgNPls^{Df}-**C1** after 2 hours of suspension either in water or 33% PBS.

D. Stability of of AgNPls^{Df}-**C2** in presence of PBS

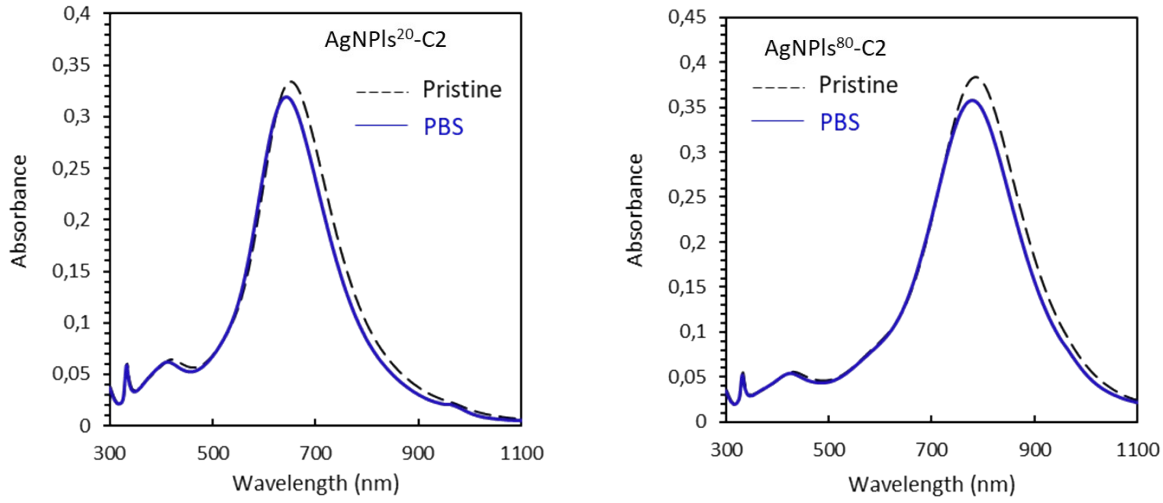

Figure S7. UV-Vis spectra of AgNPls^{Df}-**C2** after 2 hours of suspension either in water or 33% PBS.

Figure S8. Picture of the cuvette containing AgNPls^{Df}-**C2** dispersed in 33% PBS showing the particles sticking onto the cuvette walls.

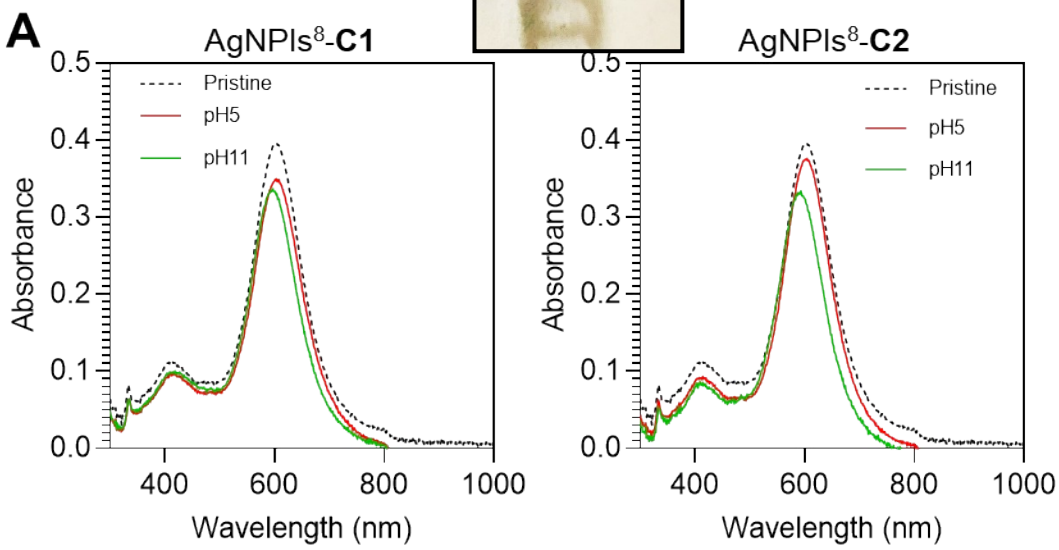

Figure S9. UV-Vis spectra of **(A)** AgNPls^{8}-**C1** and **(B)** AgNPls^{8}-**C2** suspended in water after pH variation from 7 (pristine, black) then 5 (red) and finally 11 (green).

6. Conjugation of Anti-Rabbit IgG to calixarene-coated AgNPls and use for dipstick assays

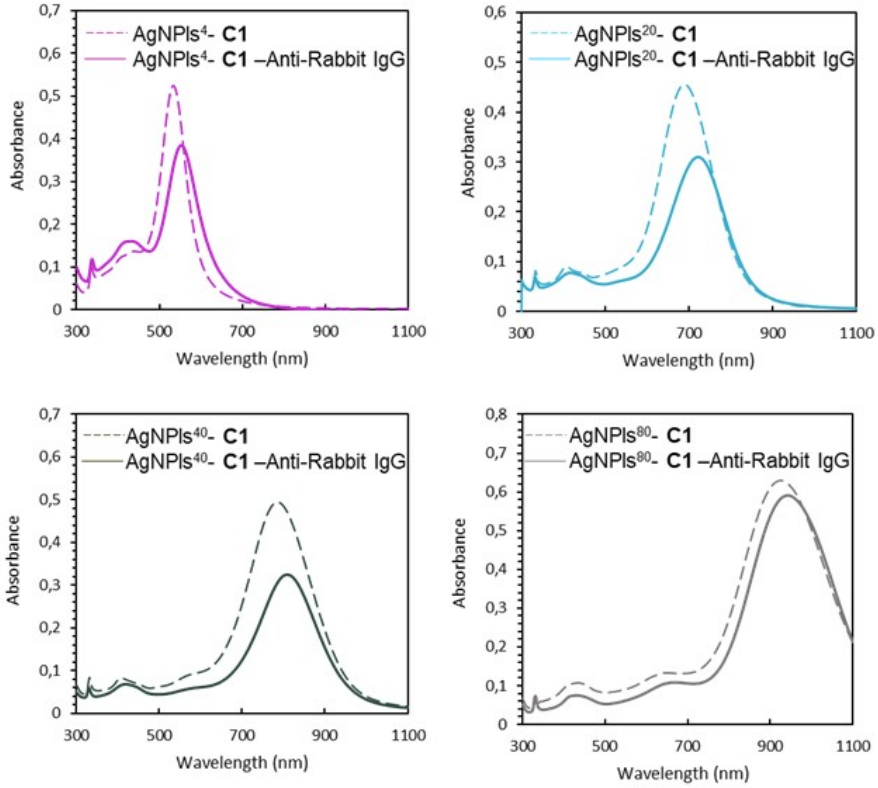

Figure S10. UV-Vis spectra of AgNPls^{Df}-**C1** before and after bioconjugation to anti-Rabbit-IgG antibodies via EDC/NHS chemistry.

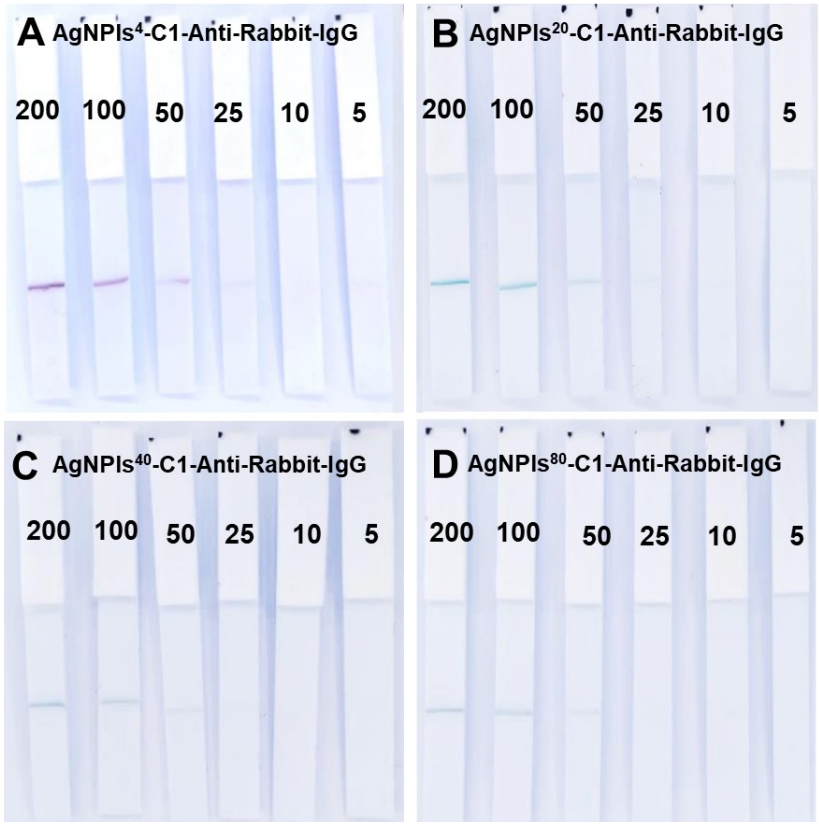

Figure S11. Pictures of the dipstick assays for the detection of various concentrations of Rabbit IgG with AgNPls^{Df}-**C1**-Anti-Rabbit-IgG of different sizes. Label values corresponds to the concentration of Rabbit IgG in ng/mL.

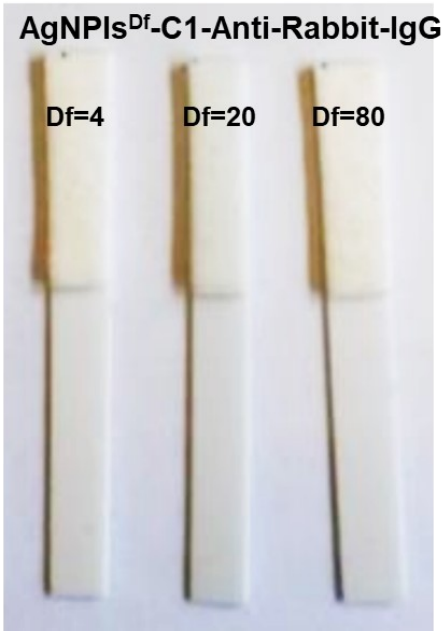

Figure S12. Pictures of the dipstick assays with AgNPls^{Df}-**C1**-Anti-Rabbit-IgG in the absence of rabbit IgG in human plasma.

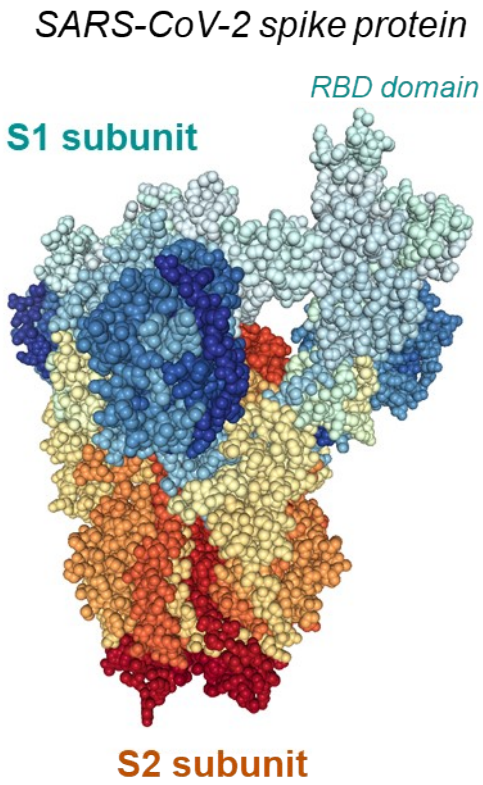

Figure S13. Protein model of SARS-CoV-2 spike glycoprotein (PDB: 5X5B)