

Supporting Information

A fast and highly sensitive method for the detection of canine distemper virus using gold nanoparticles

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Experimental Part

Chemicals. 11-mercaptopundecanoic acid, 95% (MUA), N-hydroxysuccinimide (NHS) 98% and N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), phosphate-buffered saline (PBS), sodium citrate dehydrate 99%, absolute ethanol 99%, bovine serum albumin solution (BSA) 98% (1 mg/mL⁻¹ in 10 mM PBS, pH 7.4), 0.2 mol L⁻¹ glycine-HCl (pH 3.0), 99% and gold (III) chloride trihydrate (HAuCl₄) 99.99%, were purchased from Sigma-Aldrich (USA). The water used in all solution preparations was obtained from a Millipore unit (USA), all working solutions were prepared with analytical grade chemicals.

Instruments. UV-Vis absorption spectra are obtained using a Biochrom Lira S11 spectrophotometer (Biochrom Ltd, England) with a 1 cm glass cell. The spectra were from 400-800 nm wavelengths with 1 nm step and speed of 500 nm min⁻¹.

The aspect ratios of the gold nanoparticles and modification nanoparticles were determined from transmission electron microscopy (TEM) images are acquired with a Tecnai Spirit (Fei Company-USA).

Antibody. Canine distemper virus was obtained by collecting viral urine samples from animals with histories and clinical signs compatible with distemper. Samples were extracted using an RNAspin Mini kit (GE Healthcare). The presence of CDV was confirmed using nested-PCR according to a published procedure. The material was inoculated into the Madin-Darby Canine Kidney (MDCK) cell monolayer showing 80% confluence in Minimum Essential Medium (MEM) with 1% fetal bovine serum. The fetal bovine serum was stored at -70 °C until use. The virus suspension was purified using a 20-60% sucrose gradient. The material was aliquoted, and each fraction was

read at 260 nm and 280 nm wavelengths. Fractions corresponding to a density of 1.18gcm^{-3} were separated and stored at -70°C for later quantification of the protein using the BCA method. Antibodies against CDV were obtained by subcutaneous inoculation of a suspension of purified virus containing 50 gmL^{-1} of protein into guinea pigs. Preparations of the antigen solutions were emulsified with Freund's complete adjuvant (v/v), $500\mu\text{L}$ were inoculated, and the procedure was repeated after 20 days. Thirty days after the initial inoculation, the animals were bled, and serum was inactivated at 56°C for thirty minutes, divided into 2 mL aliquots and stored at -20°C until use.

Samples. Fourteen positives urines with CDV virus and four negatives urines samples were obtained from the laboratory for molecular Diagnostics, Department of Microbiology and Immunology, Institute of Bioscience, UNESP, Botucatu campus. The samples had previously tested positive in traditional RT-qPCR. All experiments were performed in compliance with the relevant laws and institutional guidelines, and the appropriate authorities kindly approved the experimental work.

AuNP preparation and surface modification. To prepare gold nanoparticles, was first diluted 0.004g HAuCl_4 in 10 mL of Milli-Q water and 0.1 g of sodium citrate in 10 mL of Milli-Q water. Add on a balloon 3.2 mL of de gold solution and 16.7 mL of the Milli-Q water, heat the solution to boiling on a stir/hot plate while stirring with the magnetic stir bar.²⁸ After the solution begins to boil, add 2 mL of sodium citrate. Continue to boil and stir solution until it is a deep red color. When the solution is a deep red color, turn off the hot place, but remain stirring for another 5 min. Cool the solution to room temperature before using and store in dark glass in the refrigerator. The final solution of gold nanoparticles showed a concentration 0.09 gL^{-1} . The characterization of gold nanoparticles using TEM was performed using an N:400, presenting an average diameter of $26.7\pm 3.8\text{ nm}$ and polydispersity of 14.3% .

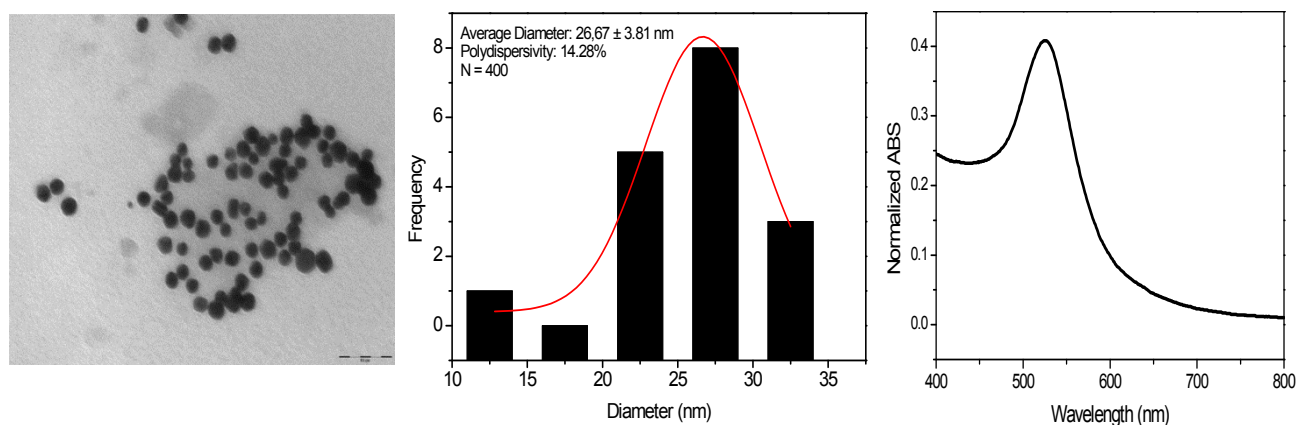
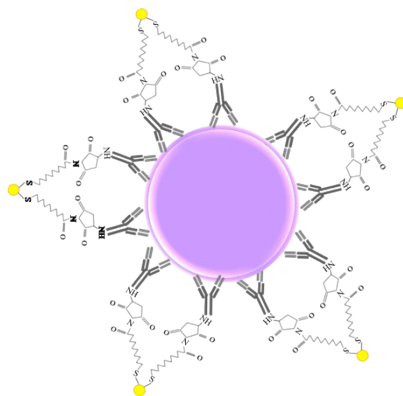


Figure S1: A) TEM gold nanoparticles with a scale of one 0.1 μm . B) Distribution of nanoparticles with an average diameter of 26 ± 3.8 nm and C) UV-Vis spectrum showing absorbance and wavelength of 525 nm measurement of gold nanoparticles.

Procedure. In a falcon tube were added 2 mL of the gold nanoparticles solution. The gold nanoparticles were modified by deposition of self-assembled monolayer (SAM) using alkanethiols, in which the irreversible adsorption of thiol selected of the nanoparticles occurred. The SAM solution was prepared by dissolving 0.02 g MUA in 100 μL of absolute ethanol. This solution, 5 μL were added in 5 mL of absolute ethanol, yielding the final solution. 200 μL of MUA were added in the falcon tube with the gold nanoparticles and remained for 3 hours. The terminal carboxylic groups of the linkers were activated with 200 μL of a 1:1 mixture of 0.4 mol L^{-1} EDC and 0.1 mol L^{-1} NHS remained for 1 hour that created covalent bonds with the amines of antibodies. Subsequently, 100 μL of antibody was added in the falcon tube and finally, 100 μL positive urine sample was added to make the detection of CDV. To perform experimental control all steps were performed using urine sample negative.



Scheme S1: Wiring diagram CDV virus with antibody modified by deposition of self-assembled monolayers and gold nanoparticles

SUPPLEMENTARY RESULTS AND DISCUSSIONS

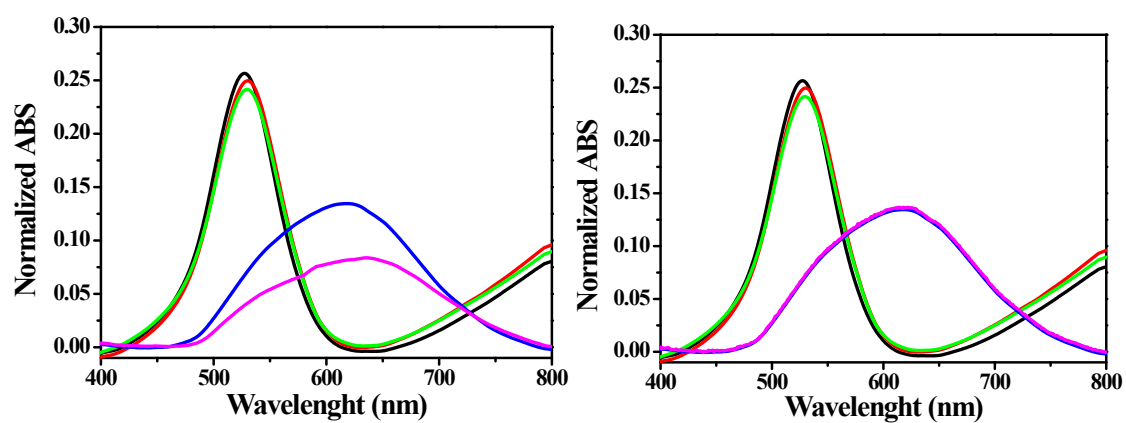


Figure S2: Comparative study between positive and negative samples. A) gold nanoparticles (black line), MUA (red line), EDC/NHS (green line), antibody (blue line) and positive urine sample (pink line). B) gold nanoparticles (black line), MUA (red line), EDC/NHS (green line), antibody (blue line) and negative urine sample (pink line).

The tests were performed with the following dilutions: 1 mL of antibody sample to 0.0, 200.0, 500.0 and 1000.0 mL PBS (10 mM pH 7.4) (Figure S2). It can be observed that as the decreases concentration of antibody, larger the peak absorbance and smaller its wavelength. It is worth noting that the LSPR bands only decrease in intensity after the recognition event between nanoparticles and anti-CDV antibody attachment. However, the decrease in intensity reduction and the blue shift appear at higher concentrations (10 mL PBS). This shift is most likely due to interaction between the gold nanoparticles attached of anti-CDV either in small or larger numbers can be detected by changes in the corresponding LSPR bands are not obvious. The black line shows the lowest concentration of 1/1000 mL PBS with absorbance of 0.10 and a wavelength of 601 nm; and the blue line shows the highest concentration of 10 mL PBS with absorbance of 0.06 and wavelength of 612 nm (Figure S2A). In Figure S2B, we have the calibration curve for IgG. One can see that the absorbance intensity decreases as the IgG concentration increases and at concentration 1 mL_{sample} to 200 mL_{PBS} the system becomes saturated. On the insert it is shown the linear range of IgG concentration as well as the linear fit; the gold nanoparticles shown to be very sensitive to the presence of IgG; by rising one unit of IgG concentration the absorbance decreases 8.92 units (Figure 5B).

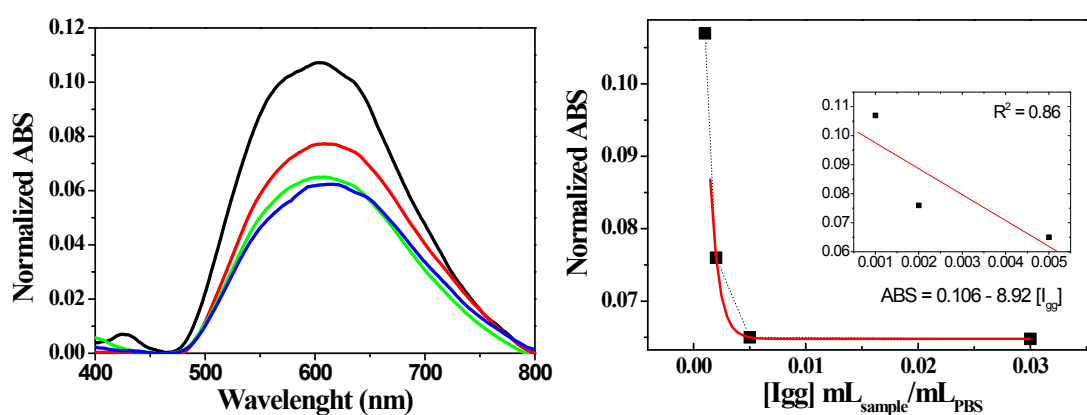


Figure S3: Dilutions of the antibody's concentrations. A) sample of antibody diluted in 1 ml of 2.0 µg L-1 in 10 mM PBS to 0.0 ml PBS (blue line), 200.0 ml PBS (green line), 500.0 ml PBS (red line) and 1000.0 ml PBS (black line). B) Calibration curve for IgG. 1 mL of IgG x mg/mL were diluted at 1000, 500, 200 and 0 mL of PBS.