Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Self-assembled Star-shaped Chiroplasmonic Gold Nanoparticles for Ultrasensitive Chiro-immunosensor of Viruses

Syed Rahin Ahmed[1], Éva Nagy[2], Suresh Neethirajan[1*]

[1]BioNano Laboratory, School of Engineering, University of Guelph, Guelph, Ontario, Canada N1G 2W1

[2]Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1

[*]Corresponding author*:* sneethir@uoguelph.ca (SN)

Characterisation of QDs

CdTe NPs was chosen to make nanohybrids with CAu NPs because of its strong emission properties and energy overlap possibility between excitonic and plasmonic states of nanohybrids. Photoluminance spectra (PL) and ultra-violet (UV) spectra of QDs is shown in Fig.S1A; emission peak was located at 710 nm. Particles size and concentration were 6.5 nm and $3.7x10^{-7}$ M respectively.

Specificity of antibodies towards target virus

The specificity of anti-influenza A (H5N1) virus hemagglutinin (HA) antibody Ab 135382 against recombinant influenza virus A (Avian/Vietnam/1203/04) (H5N1) was confirmed using a conventional ELISA method. Figure S1B shows that the optical density obtained due to enzymatic activity for the target virus/Ab 135382 HA/HRP-conjugated secondary antibody/ TMBZ–H_2O_2 complex and the target virus/anti-H5N1 NA/HRP-conjugated secondary antibody/ TMBZ–H_2O_2 complex was higher than those of anti-H5N2 HA and anti-H7N9 HA Ab, implying the specificity of Ab 135382 towards recombinant influenza virus A (Avian/Vietnam/1203/04) (H5N1).

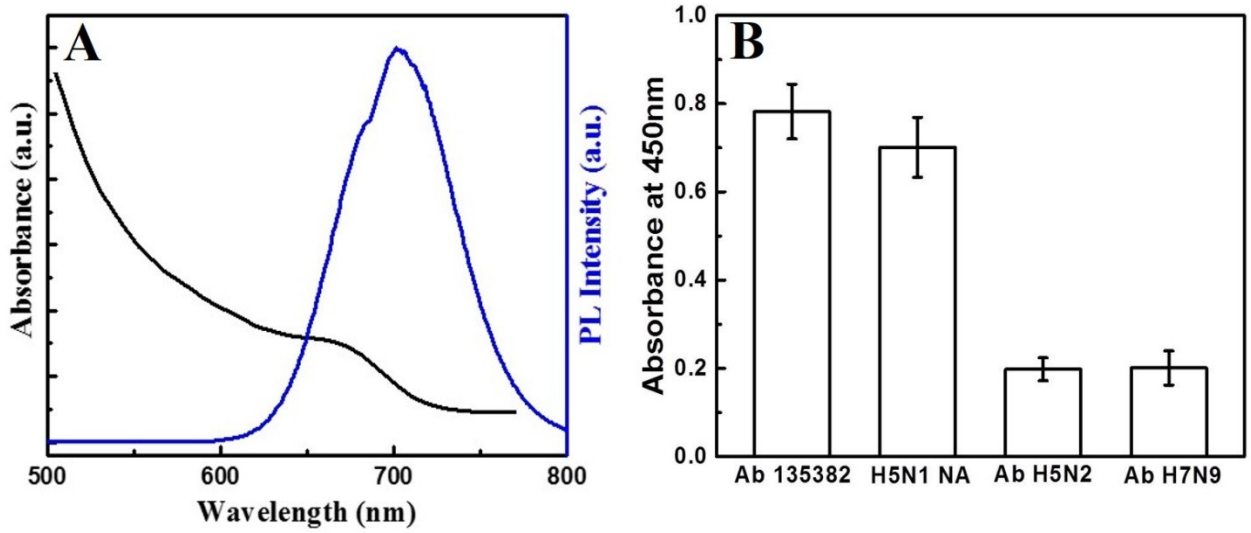

Figure S1: Characterisation of QDs and antibody-antigen specificity; (A) PL & UV-spectra of CdTe QDs, (B) ELISA results of antibodies specificity towards target virus.

Binding confirmation of anti-H5N1 Ab 135382 with CAu nanostructures

The binding of anti-H5N1 Ab 135382 and CAu nanostructures was confirmed by ELISA. In Figure S2A, higher optical density obtained for anti-H5N1 Ab 135382 and CAu nanostructures complex in ELISA results confirmed the successful binding between anti-H5N1 Ab 135382 and CAu nanostructures.

Binding confirmation of anti-H5N1 NA antibodies with CdTe QDs

The binding between anti-H5N1 Ab 135382 and CdTe QDs was confirmed by FTIR spectrum. As shown in Fig. S2B, FTIR bands found at 3700–3500 cm^{-1} for amide N–H stretching represents the chemical binding between Ab 135382 and CdTe QDs, whereas only carboxylic acid O-H stretching band appeared for CdTe QDs alone.

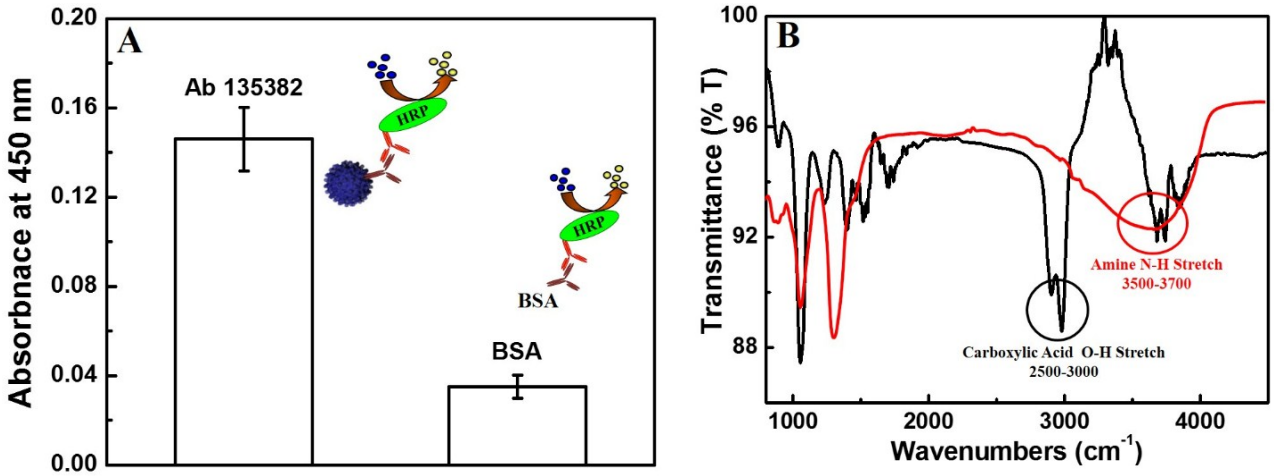

Figure S2: Binding confirmation of antibodies; (A) ELISA results of anti-HA antibodies binding with CAu nanostructures, (B) FTIR spectra of anti-NA antibodies and QDs binding.

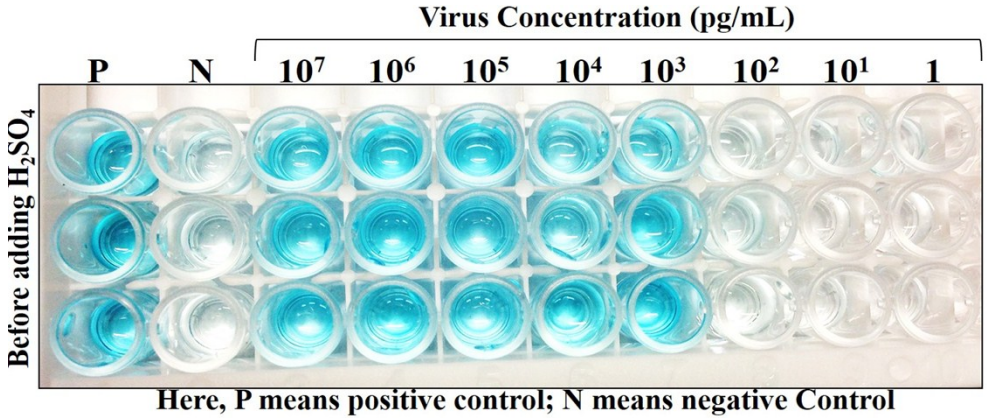

Figure S3: Naked eye image of influenza A (H5N1) virus detection using commercial kit.

Specificity of Anti-H4 (A/environment/Maryland/1101/06) (H4N6) polyclonal antibody towards avian influenza A (H4N6) virus

The specificity of anti-influenza A (H4N6) virus against avian influenza A (H4N6) virus was confirmed using a conventional ELISA method. Figure S4 shows that the optical density obtained due to enzymatic activity for the target virus/ anti-influenza A (H4N6) /HRP-conjugated secondary antibody/ TMBZ–H_2O_2 complex was higher than those of H5N1, H5N2 HA and BSA implying the specificity of anti-H4 (A/environment/Maryland/1101/06)(H4N6) polyclonal antibody towards avian influenza A (H4N6) virus.

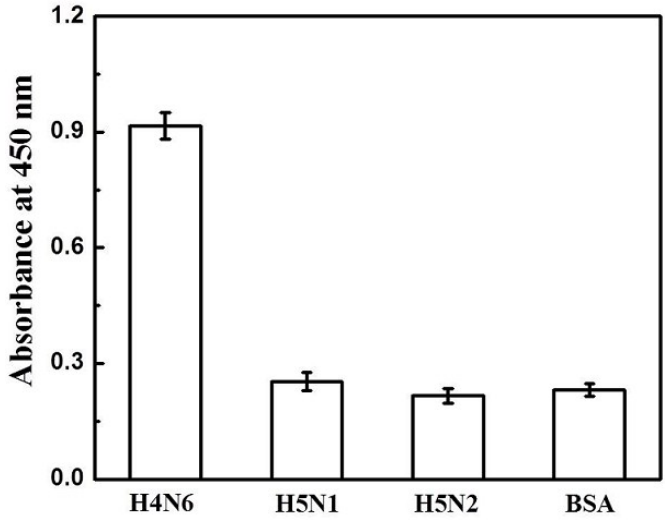

Figure S4: Specificity of anti-H4N6 antibody towards influenza A (H4N6) virus.

Binding confirmation of anti-H4 (A/environment/Maryland/1101/06) (H4N6) polyclonal antibody with CdTe QDs and CAu nanostructure

The binding of anti-H4N6 antibody with QDs and CAu nanostructures was confirmed by ELISA. In Figure S5, higher optical density obtained for anti-H4N6 antibody conjugated QDs and CAu nanostructures in ELISA results confirmed the successful binding of anti-H4N6 antibody with QDs and CAu nanostructures.

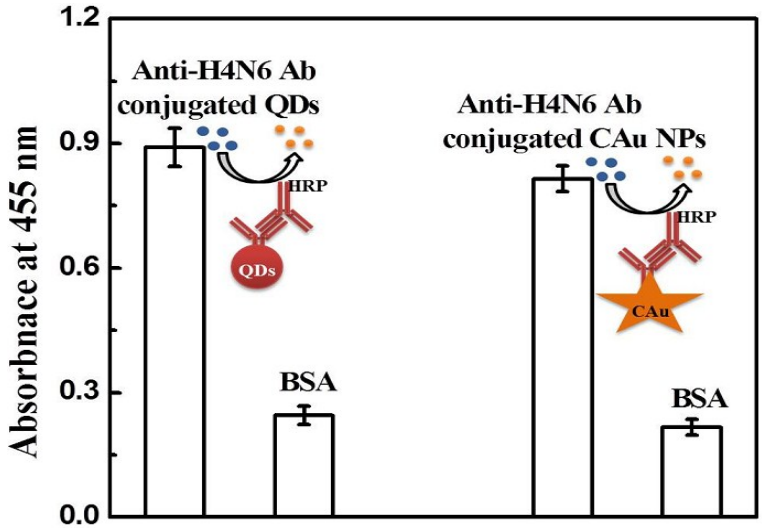

Figure S5: Specificity of anti-H4N6 antibody towards influenza A (H4N6) virus.

ELISA for binding confirmation of Chicken Adenovirus, Group II Polyclonal Antibody with CdTe QDs and CAu nanostructure

ELISA experiment was performed to check the binding of anti-adenovirus antibody with QDs and CAu nanostructures. As shown in figure S6, higher optical density obtained for anti-adenovirus antibody conjugated QDs and CAu nanostructures in compare to control experiment BSA confirmed the successful binding of anti-adenovirus antibody with QDs and CAu nanostructures.

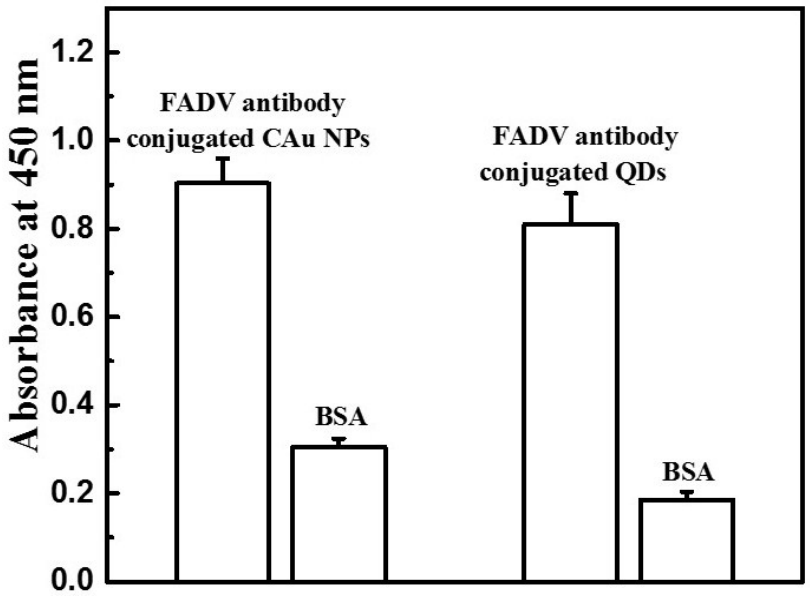

Figure S6: ELISA results for binding confirmation of fowl adenovirus (FADV) antibody with CAu nanostructures and QDs.

Specificity of proposed method for fowl adenovirus (FADV) detection

To validate the specificity of the proposed method for detection of FADV compared to others similar avian virus, we have analyzed with several viruses i.e., infectious bronchitis virus (IBV), avian influenza A (H5N1), avian influenza A (H4N6), avian influenza A (H9N2) and avian influenza A (H1N1) using anti-FADV antibody conjugated CAu nanostructure and QDs. As shown in Figure S7, the chiroptical responses for other viruses are negligible compared to the FADV result. These results demonstrate that the proposed method is highly selective for fowl adenovirus (FADV) from other viruses.

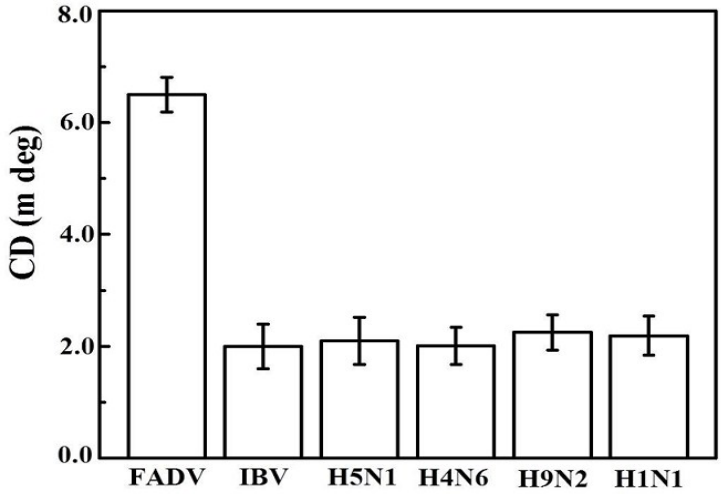

Figure S7: Selectivity results of fowl adenovirus (FADV) detection.

ELISA for binding confirmation of Mouse Infectious Bronchitis Virus Monoclonal Antibody with CdTe QDs and CAu nanostructure

An experiment was performed using conventional ELISA method to check the binding of anti-infectious bronchitis virus (IBV) antibody with QDs and CAu nanostructures. As shown in figure S8, higher optical density obtained for anti-IBV antibody conjugated QDs and CAu nanostructures in compare to BSA confirmed the successful binding of anti-IBV antibody with QDs and CAu nanostructures.

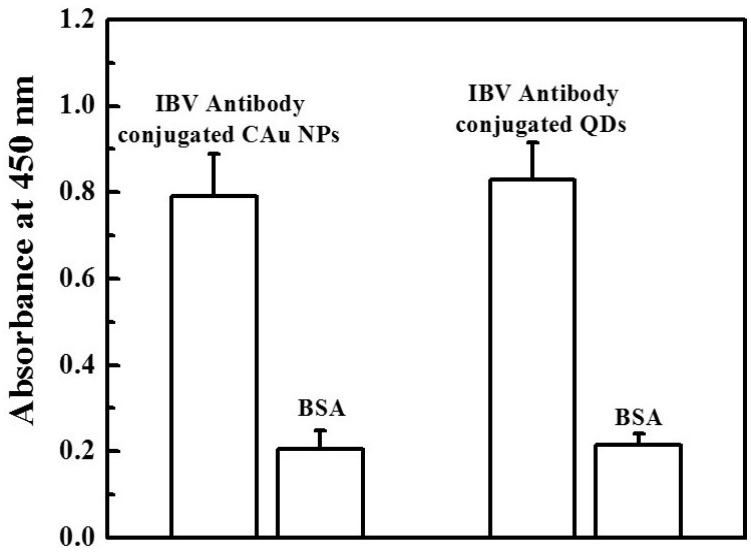

Figure S8: ELISA results for binding confirmation of infectious bronchitis virus (IBV) antibody with CAu nanostructures and QDs.

Specificity of proposed method for infectious bronchitis virus (IBV) detection

The specificity of the proposed method for the detection of FADV was validated with others similar avian viruses. As shown in figure S9, fowl adenovirus (FADV), avian influenza A (H5N1), avian influenza A (H4N6), avian influenza A (H9N2) and avian influenza A (H1N1) were analyzed along with target infectious bronchitis virus (IBV) using anti-IBV antibody conjugated CAu nanostructure and QDs. As shown in Figure S9, the chiroptical responses for other viruses are negligible compared to the IBV demonstrates that the proposed method is highly selective for infectious bronchitis virus (IBV) from other viruses.

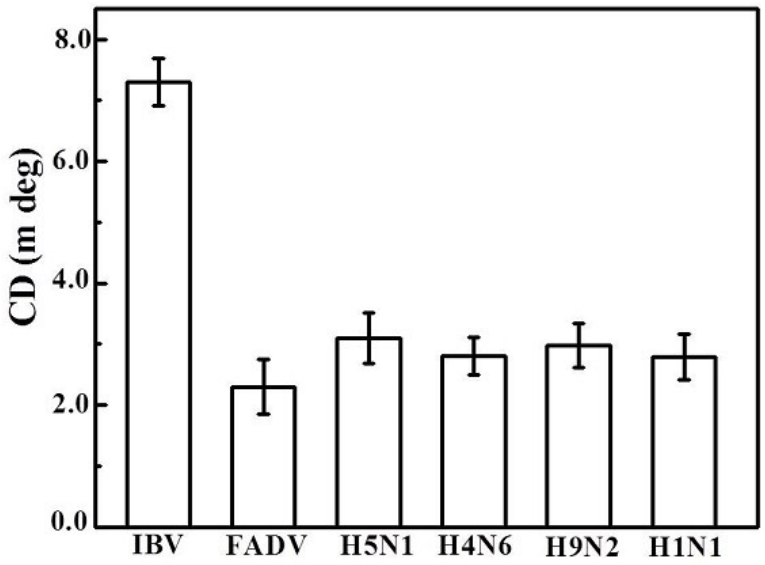

Figure S9: Selectivity results of infectious bronchitis virus (IBV) detection.

Table S1: Comparison study of avian influenza A (H4H6) virus detection

Detection method	Virus concentration (HAU/ 50 µL)					
	128	12.8	1.28	0.128	0.0128	0.00128
This study	+	+	+	+	+	–
Commercial kit	+	+	+	+	–	–