SUPPLEMENTARY FILE

Anodic stripping voltammetry of anti-Vi antibody functionalized CdTe quantum dots for the specific monitoring of *Salmonella enterica* serovar Typhi

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1. METHODS

1.1 Flocculation test for the optimization of antibody concentration

The minimum amount of IgG antibody required to stabilize the GNPs was optimized by employing a flocculation assay as reported earlier.[1] The aggregation of gold nanoparticles caused by NaCl could be characterized by the shift of maximum absorbance peak from 520 to 610 nm. The anti-Vi IgG antibody concentration required to prevent aggregation can be determined by visualising the shift in the absorbance peak from 520 to 610 nm. To determine the critical flocculation concentration (CFC) of anti Vi IgG -GNPs conjugate, increasing amount of NaCl (0-2.5 %) was added to GNPs-IgG conjugate and incubated for 1 hour at room temperature. The threshold NaCl concentration in the gold solution that caused the aggregation of the particles was determined as the CFC.

1.2 Agarose electrophoresis

Conjugate was confirmed by its mobility in gel electrophoresis using 1% agarose gel prepared in 0.5X TAE (tris acetate-EDTA) buffer.[1]

1.3 Generation of anti-Vi antibodies

Antibodies used in this study were generated against Vi polysaccharide antigen of *S.* Typhi. Initially, Vi antigen was purified from an over expressing culture of *S.* Typhi by employing well established protocol.[2] Gel permeation chromatography (GPC) was used to confirm the presence of Vi antigen in extracted samples. Hyperimmune sera were generated against the Vi antigen in white New Zealand rabbits of 8-10 weeks old. Before immunization, pre-immune sera were collected and non-specific IgG concentration was recorded. In brief, Vi antigen (200 μg mL^{-1}) was immunized subcutaneously with complete adjuvant (1:1 v/v) followed by three booster doses with an incomplete adjuvant at an intervals of 21 days. Sera

were drawn 7 days after each booster immunization separately and processed to isolate IgG as reported earlier by our group.[3, 4]

1.4 Antibody purification, characterization affinity estimation

Freshly collected blood was incubated at room temperature for 2 hours and was kept at 4 ˚C overnight. Serum was separated, and was kept at 56 ˚C in a water bath for 20 minutes to deactivate the complement system. Serum was diluted with saline (1:2) and was saturated with ammonium sulphate drop wise to a final concentration of 45%. The solution was kept on a stirrer at 4 ˚C for 2 hours followed by centrifugation at 7000 rpm for 20 minutes. The precipitates obtained were washed with 45% saturated ammonium sulphate and the solution was re-centrifuged at 7000 rpm for 20 minutes. The precipitates were then dissolved in 1/10th volume in PBS (pH 7.4) and dialyzed extensively against PBS. The dialyzed antibody solution was used for IgG antibody purification. Protein A column (GE Healthcare) was used for IgG antibody purification. The antibody solution was filtered through 0.45 µm syringe filter and loaded on Protein-A column pre-equilibrated with PBS (pH 7.0). The column was washed extensively with PBS (pH 7.0) till O.D reached 0.01. Antibody IgG was eluted with 100 mM glycine-HCL buffer (pH 2.5) and the collected fractions were immediately neutralized with 1M Tris-Cl (pH 8.0). Peak fractions were pooled and immediately dialyzed against PBS. Dialysed antibody was concentrated in 10 Kd filter membrane tubes (Spectra/Por, USA). Absorbance was read at 280 nm and the concentration was calculated.
Affinity constant of anti-Vi antibodies extracted from different boosters were estimated by using Scatchard plot separately.[5-7] A graph was plotted between the ratios of bound antigen to unbound antigen versus the molar concentrations of Vi bound to the antibody and the slope was calculated separately.

1.5 Determination of anti- Vi antibody titre

The anti-Vi antibody titre was determined by ELISA. ELISA plates were precoated with 100 µL poly-L-lysine (0.1M) in 50 mM phosphate buffer saline (PBS), pH 7.4 and incubated overnight at 4 ˚C. The plates were washed three times using 200 µL of PBS per well. 100µL of Vi antigen in PBS was added to each well. After washing with PBS, plates were blocked by skim-milk (10%) and incubated for 2 hour at 37 ˚C. Different dilutions of anti-Vi IgG antibody (1:1000-1:32000) were prepared in PBSM (phosphate buffer saline containing 0.1%

skimmed milk) and 100 µL was added in each well. The plate was then incubated for 1 hour at 37 °C. After washing three times with 200 µL of PBST, secondary antibody (alkaline phosphatase conjugated goat anti-rabbit IgG) was diluted (1:10000) in PBSM, and 100 µL was added to each well and again the plates were incubated for 1 hour at 37 °C. The plate was washed yet again, and 100 µL of p-nitrophenylphosphate was added to each well. The plate was incubated at 37 °C for 20 minutes and reaction was stopped by 0.1 N NaOH, and the absorbance was determined at 405 nm.[4]

1.6 Estimation of nanoparticle concentration in CdTe QD and anti-Vi IgG conjugates

The cadmium ion concentration of CdTe QD has been estimated electrochemically. The electrochemical response observed for 2.5 µL of CdTe QD was 29.2 µA that will corresponds to the concentration of 17.2816 nmoles of Cd ions /2.5 µL according to the standard graph provided in Fig. 3A (main manuscript) and will turnouts to be 3.1547 x 10^{13} QDs/2.5 µL. The atomic radius of Cd ions is 0.161 nm and the surface volume is 0.017 nm^3 whereas for 2.5 nm QD it will be 8.18 nm^3. The atomic radius of tellurium ion is 0.123 nm and its surface volume is 0.00779 nm^3. Therefore, in a QD molecule cadmium can have ~68.5% share and hence ~330 Cd ions per QD molecule. Accordingly, the electrochemical experimental observations of 3.1547 x 10^{13} QDs/2.5 µL will translates into a CdTe QD concentration of 20.95 µM used in this experiment. The electrochemical response observed for 2.5 µg of IgG-QD was 71.22 µA. This corresponds to 43.24 nmoles of Cd ion per 2.5 µg of IgG-QD or 17.29 nmoles of Cd per 1 µg of IgG-QD as per the standard graph provided in the Fig. 3A. Therefore, there will be 260.47 x 10^{14} Cd ions per 2.5 µg of IgG-QD which corresponds to 7.9 x 10^{13} QDs per 2.5 µg of IgG-QD. This ultimately corresponds to 7.86 QDs per 1 Ab molecule.

In the contrary, the response observed for IgG-GNP was 2.86 µA for 44 ng of IgG-GNP tested. This corresponds to 1.53 nmoles of Au according to the standard graph provided in the Fig. 3B. There were 9.21 x 10^{14} Au ions which correspond to 1.57 x 10^{10} GNPs of 13.5 nm size considering the atomic radius of Au as 0.174 nm with a surface volume of 0.022 nm^3. Accordingly, there will be 58580.36 Au/1 GNP (13.54 nm). Hence, the electrochemical estimated value was 0.089 GNPs per 1 Ab or ~11.2 Abs per GNP molecule. Theoretical values suggests that a GNP of 13.5 nm may accommodate ~12 IgG molecules by assuming that one antibody on the surface of a colloidal gold particle occupies 45 nm^2 area as reported by Harris et al., (1998).[8]

Reference

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Figure. S1

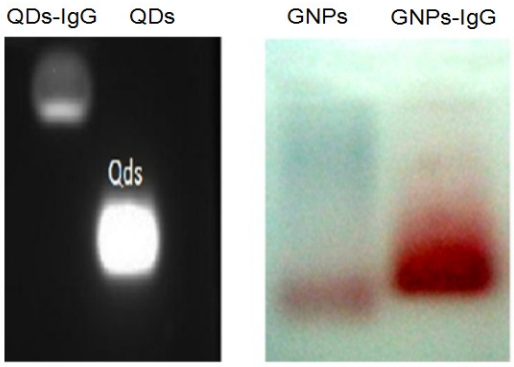
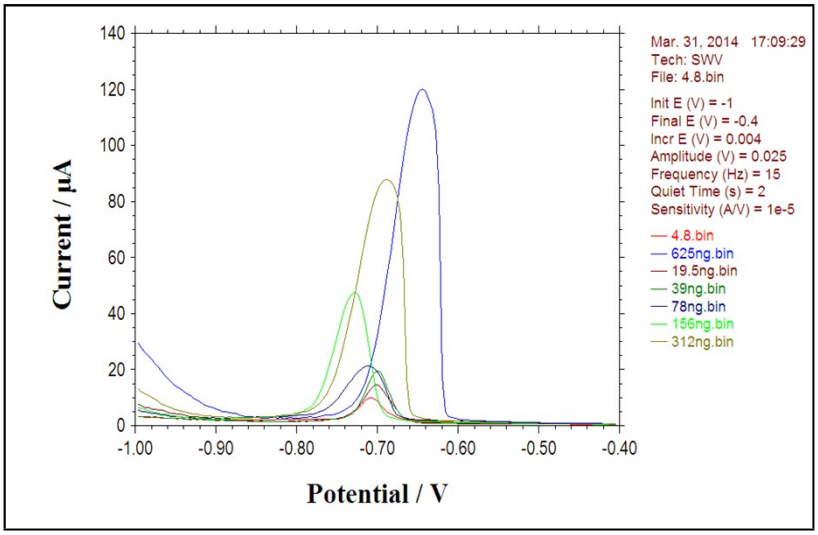


Figure S1 Representative 1% agarose gels photograph shows the bioconjugation of anti-Vi antibody with QDs and GNPs.

Figure. S2



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119 **Figure S2** Response stripping graph of IgG-CdTe nanobioprobe for monitoring Vi antigen of

120 *S.* Typhi.