Zeta potential based Colorimetric Immunoassay for the direct detection of Diabetic marker HbA1c using Gold Nanoprobes

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Supporting Information

Experimental Procedure
Generation and Purification of anti HbA1c antibodies

Anti-HbA1c antibodies were raised by immunizing young white New Zealand rabbits using standard immunization protocol (Fig. S1). Rabbits were immunized with 300 μg of each serum proteins (HbA1c) emulsified in CFA for first injection and in IFA for booster doses which were repeated 3-4 times every 21 days and sera were collected on 5th day after each booster. After de-complementation at 56°C for 30 minutes, it was stored in aliquots at -20°C. The sera were pooled and precipitated under constant stirring at 4°C at 50% saturated ammonium sulfate and centrifuged. The precipitate was dissolved in minimum volume of PBS and was extensively dialyzed against PBS (pH 8.0) at 4°C. It was then passed through the protein-A sepharose column and bound antibody was eluted with 0.1M glycine-HCl buffer (pH 2.5). Fractions were neutralized immediately with 1M Tris (pH 8.0) and dialyzed extensively against PBS pH 7.4 at 4°C, and stored in aliquots at –20°C. Concentration was determined by taking absorbance at 280 nm and by taking 1.35 as extinction coefficient.

Critical Flocculation Concentration Assay and Synthesis of Antibody-GNP conjugate

In this assay, 100 μL of antibody solution prepared at different concentrations in phosphate buffer (20mM, pH 7.4) was added to tubes containing 1 mL of the colloidal gold solution. After 15 min, flocculation was induced by adding 100 μL of 10% NaCl and absorbance was measured at 580 nm. The amount of protein necessary to prevent flocculation was deduced graphically from the concentration at which the absorbance becomes nearly constant. For preparing Ab-GNPs conjugates, anti-HbA1c antibodies prepared in phosphate buffer were added separately into 1 mL colloidal gold solution under mild stirring conditions. The pH of the colloidal gold solution was maintained at 7.4 by addition of dilute 0.1M K2CO3 before adding the antibody concentration derived from CFC measurements. The mixture was incubated overnight at 4°C and centrifuged at 12,000 rpm for 30 min. The pellet obtained was further washed twice with 10 mM Tris (pH 8.0) under centrifugation at 12,000 rpm for 30 min to remove traces of unconjugated antibody.

Nanoprobe based Immunoassay

The homogeneous immunoassay was conducted in solution using the antibody-conjugated nanoparticles (50 μL) into each well of micro titre strip (Nunc, USA). Serial
dilutions of the antigen were prepared in carbonate buffer and 50 µL of sample was added to each well followed by the addition of 50 µL of NaCl (5M) after 5 min. The absorbance at 520 nm and 620 nm were measured using an ELISA plate reader (Biotech, USA). All experiments were performed at room temperature.
**Figures for ESI**

**Figure S1:** UV–Vis spectra recorded from the colloidal gold solutions reduced with glutamic acid of different concentrations. Inset shows the corresponding TEM images of the GNPs solutions. Scale bar =100 nm.

**Figure S2:** Binding of anti-HbA1c antibody with BSA (Bovine Serum Albumin), OVA (Ovalbumin), HSA (Human serum albumin) and GHb (Glycated Haemoglobin)
**Zeta Potential Measurements**

*Figure S4:* Zeta potential measurements for (a) GNP, (b) GNP-Ab, (c)-(i) variation in zeta potential of 40 nm GNPs aggregates with increasing amount of the antigen HbA1c.
Dynamic (DLS) measurements

Figure S5: DLS measurements for (a) GNP, (b) GNP-Ab, (c)-(i) variation in size of 40 nm GNPs aggregates with increasing amount of the antigen (HbA1c).