Electronic Supplementary Material (ESI) for Analyst

Electrochemical Mass Sensor for Diagnosing Diabetes in Human Serum

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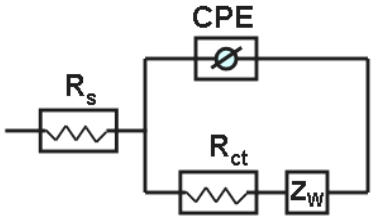
EXPERIMENTAL.

Materials. Insulin (recombinant human) and human serum were purchased from Sigma. A freshly prepared mixture of (1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC, 0.35 M) and N-Hydroxysuccinimide (NHS,0 .1 M) [Thermo Scientific] was used to activate the carboxylic acid groups of MPA on Au-MPA to immobilize the insulin antibody (0.25 mg mL^{-1}, pH 5.0). Similarly, the EDC/NHS mixture was used to activate the carboxylic acid groups of MNP to attach the Lys residues of insulin as detailed below. All other reagents used were high purity analytical grade. Gold coated quartz crystal resonators (10 MHz frequency) were purchased from the International Crystal Mfg. (OK, USA).

Methods. The frequency change and charge transfer resistance (R_{ct}) were simultaneously measured using an electrochemical quartz crystal microbalance (eQCM, Gamry Instruments) interfacing a QCM and a potentiostat (Model: Interface 1000).

Impedance. The faradic impedance measurements were performed to obtain R_{ct} for different concentrations of serum insulin in insulin-MNP bound to the antibody immobilized Au-MPA quartz resonators. The impedance acquisition parameters used were 0.22 V vs Ag/AgCl, amplitude 10 mV, and frequency range 0.1-100 kHz. The following equivalent circuit was used to fit the experimental impedance data into the Nyquist plot (provided by Gamry Instruments) to obtain the R_{ct} values.[1-3]

Equivalent circuit model.



Where CPE = constant phase element; R_s = solution resistance; R_{ct} = charge-transfer resistance; Z_W = Warburg impedance.

Preparation of insulin-magnetic nanoparticles (insulin-MNP) conjugate. The surface lysine residues of insulin (2 found on the surface, Figure S1 below) were covalently attached to the carboxylic acid groups of polyacrylic acid functionalized magnetic nanoparticles (MNP, 100 nm hydrodynamic diameter, Chemicell GmbH Inc.) following the manufacturer's protocol. In brief, a freshly prepared aqueous solution (150 µL) containing 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 0.35 M) and N-hydroxysuccinimide (NHS, 0.1 M) was added to 0.5 mg of MNP (~ 9×10^{11} particles, Chemicell Inc.) and incubated for 10 min to convert the carboxylic acid groups of MNP into easily leaving N-succinimidyl ester groups.

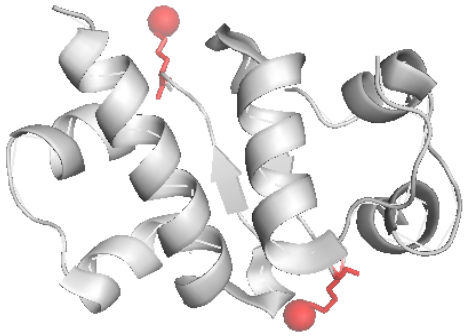


Figure S1. Representation of the crystal structure of human insulin (PDB 3V19) using Pymol. The two surface lysine (Lys) residues of insulin available for covalent attachment to carboxylic acid groups of magnetic nanoparticles are highlighted in red. The sphere in each Lys residue denotes the free amine group.

The MNP-succinimidyl ester was then separated from the unreacted EDC/NHS solution by applying a magnetic field, washed in water, and suspended in 250 µL of phosphate buffer saline (PBS). To the MNP-succinimidyl ester suspensions in separate vials, we added 250 µL of different 2x concentrations of insulin prepared in human serum. This provided the final insulin concentrations of 0, 5, 25, 50, 75, 100, 500, and 1000 pM. The final serum composition in the reaction solution with MNP was thus 50 %. After incubating for 2 h at room temperature with a continuous gentle mixing, the insulin conjugated MNP (insulin-MNP) were separated from the serum using a magnet, washed twice in PBS containing 0.1 % BSA (PBS-BSA), and resuspended in 100 µL of fresh PBS-BSA.

Immobilization of insulin-antibody on to a gold resonator surface. A stable self-assembled monolayer of 3-mercaptopropionic acid (MPA) was formed by immersing Au-coated quartz resonators in MPA (10 mM in ethanol) for overnight (12-14 h). Here, the thiol groups of MPA form a Au-S monolayer and provide free carboxylic acid surface groups. After the EDC/ NHS activation of the surface –COOH groups, the monoclonal anti insulin-antibody (0.25 mg mL^{-1} in 50 mM Na^{+}-acetate buffer, pH 5.0) was covalently attached for 1 h at 4 ^{o}C.

Insulin-MNP detection by a direct immunoassay. After the antibody attachment on to the Au-MPA surface was complete, the free sensor surface was blocked for 30 min at 4^{o}C using 2 % BSA in PBS, pH 7.4. This step minimizes the non-specific binding of insulin and false positive signals.[4] Then different concentrations of insulin in insulin-MNP conjugate were bound to the surface antibody on the Au-MPA resonators for 20 min. The frequency decrease at each step of the described immunoassay was monitored in PBS buffer as shown in Figure 1. For comparison, the free insulin samples in human serum were prepared and tested in the immunoassay (Figure 3A(a)).

Using Sauerbrey equation (eq S1 below), we estimated the amount of molecules coated on the sensor surface from the observed decrease in frequency at each step of the direct immunoassay (Table S1).

$$\Delta f = \frac{-2 f_0^{\,2} \Delta m}{A\sqrt{\mu\rho}} \qquad \text{(eq S1)}$$

Where Δf = frequency decrease due to mass adsorbed; f_o = fundamental oscillation frequency of the quartz crystal (10 MHz); A = geometric area of Au-surface (0.2 cm^2); ρ = density of quartz (2.648 g cm^{-3}), and μ = shear modulus of quartz (2.947 x 10^{11} dyn cm^{-2}).

Table S1. Average decrease in oscillation frequency at each step of the direct immunoassay and the corresponding mass coated on to the Au-surface of quartz resonators (N = 6 repeats).

Sensor surface	Frequency decrease (Hz)	Mass coated (μg cm^{-2})
Au (initial)		
Au-MPA	72 ± 23	0.065 ± 0.02
Au-MPA-Antibody	297 ± 65	0.27 ± 0.06
Au-MPA-Antibody-BSA	152 ± 54	0.14 ± 0.05
Au-MPA-Antibody-BSA-50 pM insulin-MNP (above control response)	246 ± 31	0.22 ± 0.03

(1) L. Alfonta and I. Willner, *Anal. Chem.,* 2001, **73**, 5287-5295.
(2) E. Katz and I. Willner, *Electroanalysis,* 2003, **15**, 913-947.
(3) H. Liu, R. Malhotra, M. W. Peczuh and J. F. Rusling, *Anal. Chem.,* 2010, **82**, 5865–5871.
(4) S. Krishnan, V. Mani, D. Wasalathanthri, C. V. Kumar and J. F. Rusling, *Angew. Chem. Int. Ed.,* 2011, **50**, 1175 –1178.