Supporting Information

Magnetite-Quantum Dot Immunoarray for Plasmon-coupled-Fluorescence Imaging of Blood Insulin and Glycated Hemoglobin

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Table S1. DLS and zeta potential measurements of insulin and HbA1c conjugates (mean ± standard deviation, N = 3 replicates).

Sample	Hydrodynamic size (nm)	Zeta potential (mV)
MNP (amine functionalized)	115 ± 2	45 ± 7
1.MNP-QD_{800}	143 ± 3	–24 ± 1
MNP-QD_{800}-aptamer	186 ± 11	–10 ± 2
MNP-QD_{800}-aptamer-control (unspiked with insulin)	480 ± 22	–11 ± 1
MNP-QD_{800}-aptamer-spiked with 50 pM insulin	681 ± 22	–14 ± 1
2. MNP-QD_{565}	127 ± 1	–22 ± 4
MNP-QD_{565}-aptamer	223 ± 7	–9 ± 2
MNP-QD_{565}-aptamer-control (unspiked with HbA1c)	674 ± 19	–10 ± 1
MNP-QD_{565}-aptamer-spiked with 6% HbA1c	759 ± 25	–12 ± 1

Table S2. Different strategies for insulin and HbA1c detection in clinical matrices.

Strategies	Matrix	Limit of Detection (LOD)	Reference
Insulin:			
1. ELISA from Mercodia Inc.	Serum or plasma	0.9 pM	-
2. Solid phase extraction liquid chromatography mass spectrometry (SPE-LCMS) quantitation of endogenous insulin levels	Whole blood hemolyzed to extract plasma; plasma diluted 1:1 with 50 mM acetic acid	Limit of quantitation (LOQ) was 172 pM	[1]
3. Bloodspot chemiluminescent insulin assay	Insulin standards prepared in 0.55 haematocrit	5.9 pM	[2]
4. Chemiluminescence bead based sandwich immunoassay	Plasma	0.3 pM	[3]
5. Gold coated crystal-antibody (Au-$Ab_{insulin}$) modified mass and impedimetric sensor	50% serum	5 pM	[4]
6. Nanotube-pyrene-$Ab_{insulin}$ voltammetric sensor	50% serum	5 pM	[5]
7. Au-$Ab_{insulin}$ modified SPRi sensor (sandwich assay)	50% serum	4 pM	[6]
8. MNP-QD-aptamer - insulin conjugation (sandwich assay with Au-dendrimer-$Ab_{insulin}$ modified surface)	Unprocessed whole blood diluted 20-times in PBS	4 pM	This study

HbA1c:			
9. Zirconium dioxide nanoparticles ferrocene boronic acid (ZrO_2/ FcBA) modified amperometric HbA1c biosensor	Real blood samples diluted with PBS to give 20 μM total hemoglobin concentration	HbA1c control samples of concentrations 6, 8, 10, and 14% were purchased	[7]
10. Thiophene-3-boronic acid (T3BA) self-assembled monolayer (SAM) covered Au impedimetric HbA1c sensor	HbA1c reference solutions were pretreated	HbA1c reference solutions of concentrations 4.54, 5.27, 6.96, 9.24, and 11.58% were purchased	[8]
11. Fluorinated polydimethyl siloxane (PDMS) modified antibody microarrays using enhanced chemiluminescent luminol reagent kit (ECL)	No sample pretreatment. Whole blood diluted 5 orders of magnitude	3.58 ng/mL of total hemoglobin and 0.20 ng/mL of HbA1c (5.6%)	[9]
12. Gold nanoparticle modified amino phenyl boronic acid modified ampero-metric HbA1c sensor	Lyophilized HbA1c in pretreated hemoglobin with 10-fold dilution	0.052%	[10]
13. Luminol chemi-luminescence HbA1c biosensor	HbA1c in PBS buffer pH 8. Real blood samples diluted 50-fold	10 ng/mL (2.9%)	[11]
14. MNP-QD-aptamer -HbA1c conjugation (sandwich assay with Au-dendrimer-Ab_{HbA1c} modified surface)	Unprocessed whole blood diluted 20-times in PBS	1%	This study

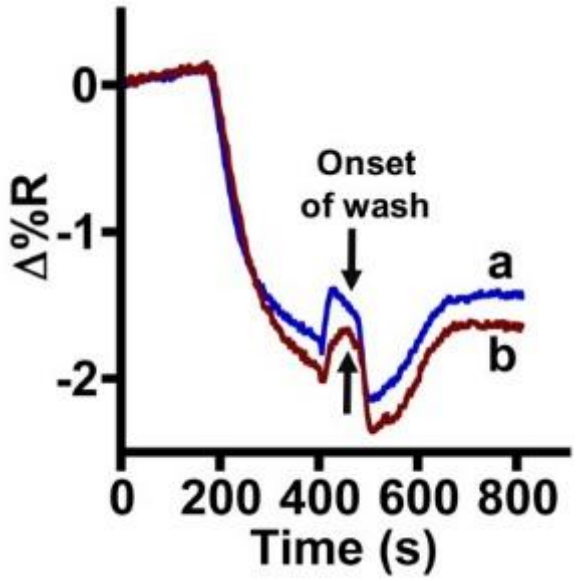

Figure S1. Negative SPR reflectivity upon binding of mixed thrombin and PDGF conjugates on surface immobilized (a) anti-insulin or (b) anti-HbA1c antibody.

Cyanmethemoglobin assay for quantitation of total hemoglobin in HbA1c standards.

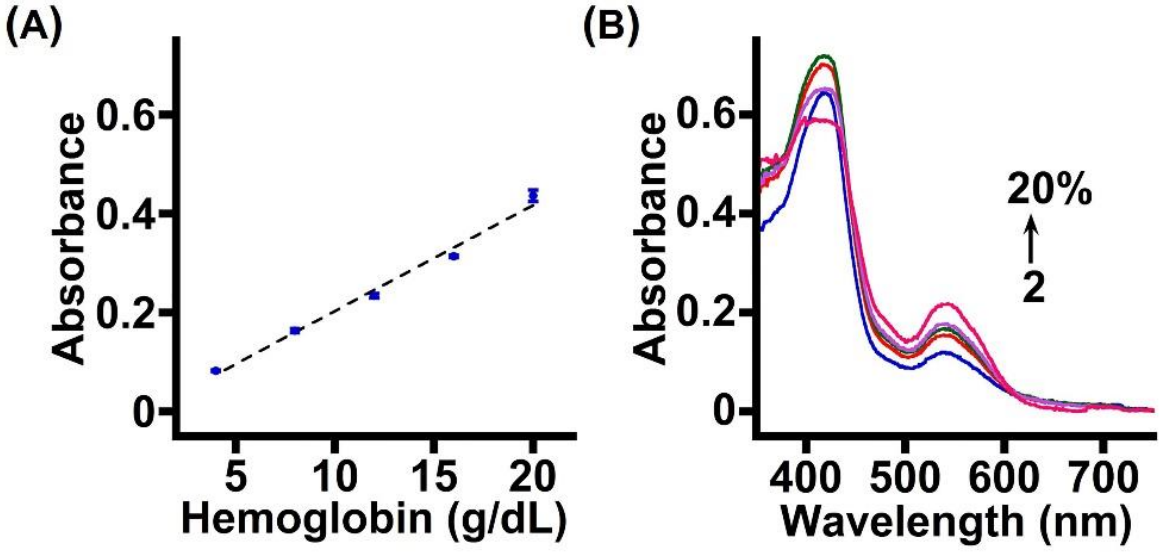

Figure S2. (A) Standard curve of cyanmethemoglobin standards and (B) UV-vis spectra of prepared HbA1c standards.

The kinetic analysis was performed using the kinetic equations shown below.

The interaction between the two macromolecules follows a one-to-one binding reaction and is written as:

$$A + B \overset{k}{\leftrightarrow} AB$$

Association phase: $\frac{d[AB]}{dt} = k_a[A][B] - k_d[AB]$ (1)

Dissociation phase: $\frac{d[AB]}{dt} = - k_d[AB]$ (2)

where A is the antibody of insulin or HbA1c immobilized on the SPR chip, B is the concentration of insulin or HbA1c conjugated to the MNP-QD-aptamer, and AB is the complex formed between insulin (or HbA1c) and surface antibody. Because the concentration of A is constant, the complex formation has been considered to follow a pseudo first order kinetics.[12-14] The SPR response of the interaction with time can be written as:

$$\frac{dR}{dt} = k_a C R_{max} - (k_a C + k_d) R_t \quad (3)$$

The integrated form of (3) is,[15]

$$R_t = \frac{k_a C R_{max}[1 - e^{-(k_a C + k_d)t}]}{k_a C + k_d} + R_0 \quad (4)$$

$$k_{obs} = k_a C + k_d \quad (5)$$

$$K_D = \frac{k_d}{k_a} \quad (6)$$

where R_t is the SPR response at time t, R_{max} is the maximum reflectivity change, R_o is the SPR response at time t = 0, C is the concentration of the analyte (insulin or HbA1c), k_a is the association rate constant, k_d is the dissociation rate constant, and K_D is the binding constant.

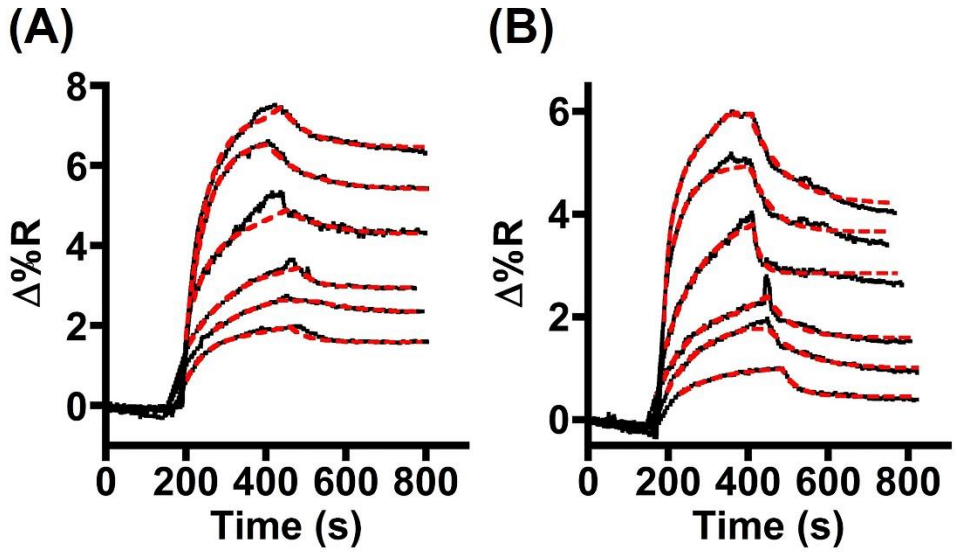

Figure S3. Simulated (red) and experimental SPR sensograms (black, data of Fig. 4A-B in main Ms.) of (A) insulin and (B) HbA1c.

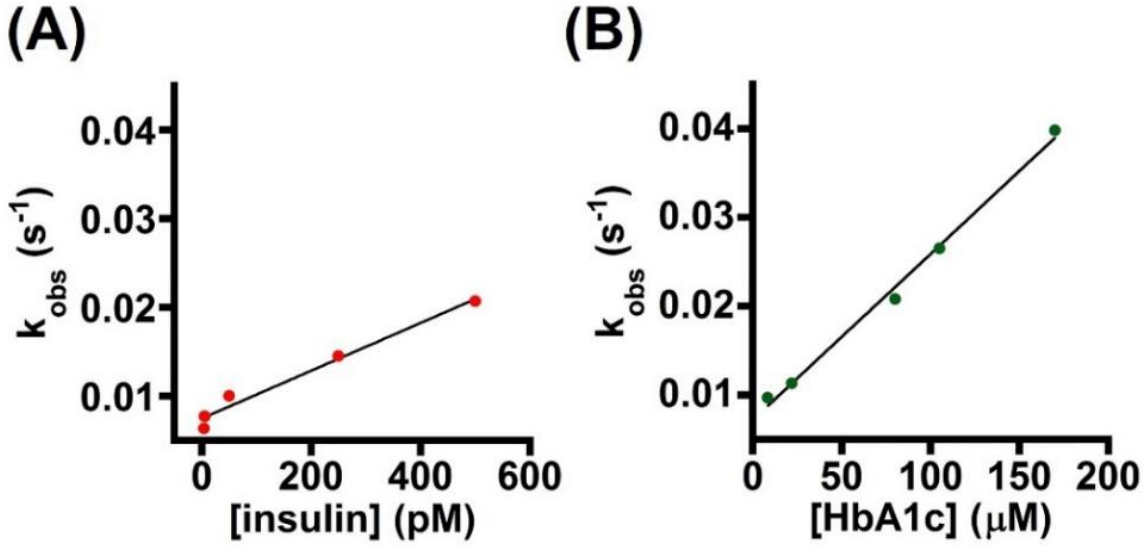

Figure S4. Plot of k_{obs} and concentration of (A) insulin and (B) HbA1c.

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