A Matrix-insensitive Platform for Characterizing Protein-Protein Interactions

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ABSTRACT

Background: Ligand binding assay (LBA) instruments are sensitive to complex buffers. It is a major limitation for understanding protein binding behavior in biologically relevant solutions such as plasma or serum. Therefore, understanding ligand binding kinetics in a biologically relevant buffer has been, until now, a significant challenge. Here a novel platform is described that can measure protein binding kinetics precisely, reproducibly and with great sensitivity in biological matrices.

Objective: Demonstrate the capability of the MagArray platform to measure protein interactions in complex matrices.

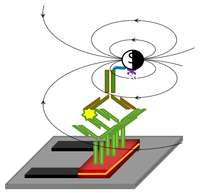
Methods and Results: TSH protein was fixed on the sensor surface and TSH antibody was conjugated to magnetic beads. A simple model for the curve fitting was developed to obtain the dissociation and rate constants. More importantly, we demonstrated the measurement of kinetic parameters of binding in complex matrices, such as buffers spiked with different amount of plasma.

Results: Representative graphs of binding curves are shown here. The calculated kinetic parameters are compared with the reported values.

Conclusions: A novel platform has been described for accurately monitoring protein binding kinetics in increasing concentrations of plasma, without being affected by the matrix. The results derived from Biacore X100 were deemed unreliable in buffers that contained more than 0.5% plasma. Studies such as these on the MagArray platform provide for the first time an easy measurement of protein binding behavior in a biologically relevant matrix, and thus promise broader applications in the field of ligand binding that were not feasible in the past.

INTRODUCTION

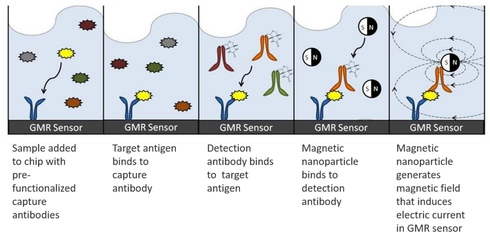
Fig. 1. GMR Sensors for MNPs



GMR sensors are intrinsically proximal in detecting magnetic nanoparticles (MNPs) bound to the sensors surface

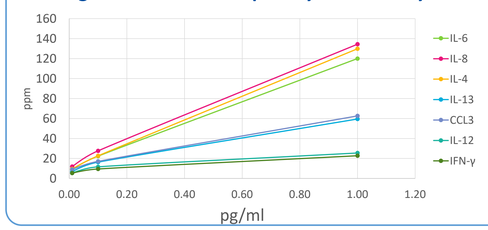
MagArray Assay Principles

Based on standard sandwich ELISA process, with magnetic nanoparticles used as labels



Applications: Magnetic biosensors are a versatile microarray-based platform for sensitive and multiplex detection of biomolecules.

Fig. 2. Sensitive Multiplex Cytokine Assays



Selected cytokines shown here have sensitivities at 0.1 pg/ml or lower concentration. In addition, the high-sensitivity is achieved in a multiplex assay format.

METHODS

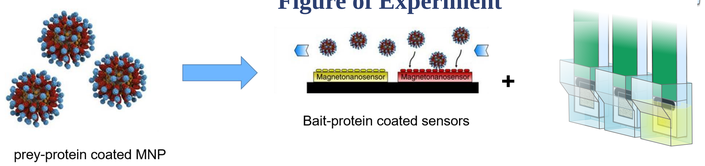
Sensor Surface Preparation: Native human TSH proteins are applied to the sensors at different concentrations, from which an optimal condition (concentration) will be selected for kinetic studies.

Magnetic Nanoparticle Preparation: Commercial TSH antibodies are individually conjugated to magnetic nanoparticles. Both the sensor surface and modified MNPs are blocked following conventional methods to prevent non-specific interactions.

Experiments: The real-time reading of the binding signals is realized by applying the modified MNPs to the sensors directly, at t = 3 minutes after recording start . Since only proximity signals are detected, the signal reflects the specific binding between MNPs and the surface proteins. The mechanism is shown in Figure below.

TSH protein and antibody interaction is studied in both simple buffer and complicated solutions including plasma and buffers with different amount of detergent of Tween 20 to probe the effect of matrix on the antigen-antibody interaction. Up to 80% of plasma or 2% Tween 20 in the binding buffers was evaluated .

Figure of Experiment



Data Processing: Conventional pseudo-Langmuir curve fittings are applied to the real-time signals, and k_{on}, k_{off}, and K_D are calculated from the following equations.

$$\text{Association Curve: } S_t = S_0 \times \left[1 - \exp\{-(c \cdot k_{on} + k_{off}) \cdot t\}\right] \quad (1)$$

$$\text{Dissociation Curve: } S_t = a \cdot \exp\{-k_{off} \cdot t\} \quad (2)$$

RESULTS

I: Measurement and Effect of Plasma for the Binding Two TSH antibodies (5405 and 5409) binding with target TSH protein in buffer with up to 80% plasma are studied. Conventional methods such as BIACORE platform would suffer greatly from non-specific binding in these conditions. The magnetic sensors however provide clear specific signals and binding curves that can probe their binding behaviors in this complicated matrix.

Fig. 3. Ab5405 buffer

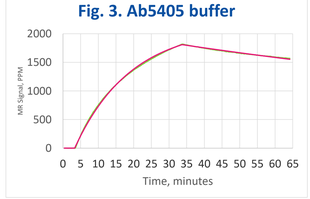


Fig. 4. ab5405-80% plasma

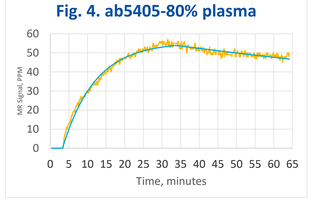
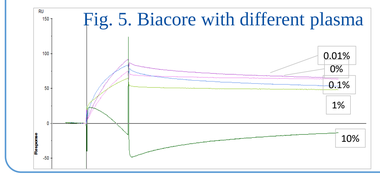


Fig. 5. Biacore with different plasma



The same binding pairs with plasma were used on Biacore X100

Biacore results are no longer reliable with plasma above 0.1%.

Table 1. Calculated Kinetic Parameters in Different Buffers

	5405-80% plasma	5405-50% plasma	5405-25% plasma	5405-buffer	5409-80% plasma	5409-50% plasma	5409-25% plasma	5409-buffer
k_{on}	1.8E+06	1.3E+06	1.6E+06	1.1E+06	1.6E+06	1.1E+06	1.6E+06	1.5E+06
k_{off}	1.6E-04	8.4E-05	9.1E-05	8.4E-05	8.1E-05	6.8E-05	6.9E-05	6.5E-05
K_D	9.1E-11	6.4E-11	5.7E-11	7.9E-11	5.1E-11	6.2E-11	4.3E-11	4.3E-11

Table 1. lists the kinetic parameters of two antibodies in buffers with up to 80% plasma. It is interesting to observe that even though the signals decrease with increased amount of plasma, both antibodies show similar k_{on}, k_{off}, and K_D values in different buffers. The values in pure buffer are also compared with SPR values (Table 2.), which shows good agreement.

Table 2. Comparison of Parameters from Both Magnetic sensors and SPR Methods

	5405-MagArray	SPR	5409-MagArray	SPR
k_{on}	1.1E+06	1.2E+06	1.5E+06	2.1E+06
k_{off}	8.4E-05	1.9E-04	6.5E-05	1.0E-04
K_D	7.9E-11	1.6E-10	4.3E-11	4.8E-11

II: **Measurement and Effect of Surfactant on Protein Bindings** The two antibodies in buffers with different amount of Tween 20 (up to 2%) are studied.

Fig. 6. Ab5405 in Buffer with 0.05% Tween 20

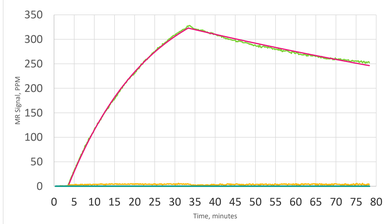
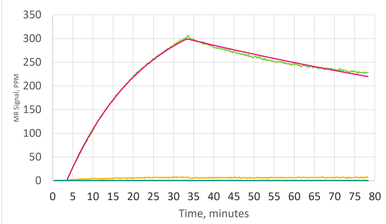


Fig. 7. Ab5405 Binding in Buffer with 2% Tween 20



Figures: Antibody 5405 binding with TSH proteins in buffers with different amount of Tween, the binding behavior is almost not affected by the presence of Tween 20 to up to 2%. Table 3. shows the curve fitting results confirming the observation.

Table 3. Studying the Effect of the Surfactant Tween20 on the Protein Binding

Tween 20 content	5405-0.05%	5405-0.5%	5405-1%	5405-2%
k_{on}	8.6E+05	8.8E+05	8.7E+05	8.2E+05
k_{off}	1.1E-04	1.1E-04	9.9E-05	1.0E-04
K_D	1.3E-10	1.2E-10	1.1E-10	1.2E-10

CONCLUSIONS

A matrix-insensitive platform has been described for monitoring protein binding kinetics in increasing concentrations of plasma and surfactant without being affected by the matrix. Studies such as these demonstrate that the MagArray platform is a powerful tool for analyzing protein binding behavior in a biologically relevant matrix. This enables a broad range of applications in the field of ligand binding which were not feasible in the past.