Rapid and Efficient Determination of Kinetic Rate Constants Using the ProteOn™ XPR36 Protein Interaction Array System

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Introduction

Surface plasmon resonance (SPR) optical biosensors are being used increasingly in a wide range of applications in basic biological research and pharmaceutical product development (Rich and Myszka 2005). We present here a significant advance in SPR biosensor technology: the ProteOn XPR36 protein interaction array system. The ProteOn XPR36 system incorporates a multichannel module and interaction array sensor chip for analysis of up to 36 independent protein interactions in a single injection step.

In a typical SPR biosensor experiment, a ligand is first immobilized onto a sensor chip surface and is then presented with an analyte in solution. The SPR biosensor detects the binding of the analyte to the ligand in real time and produces data on the association and dissociation kinetic rate constants of the reaction. When determining kinetic rate constants of a biomolecular interaction, a range of analyte concentrations is required to provide sufficient data for analysis. At the end of the binding step for a single analyte concentration, the ligand surface is normally regenerated before running the next analyte concentration.

In the ProteOn XPR36 protein interaction array system, there is no need to regenerate the ligand surfaces between samples because up to six analyte samples can be injected in parallel and in a single injection step. To do this, the multichannel module in the ProteOn XPR36 instrument first directs flow of six ligands (or one ligand under six different immobilization conditions) into six parallel channels across the sensor chip surface, and the ligands are then immobilized onto the chip surface. The multichannel module then directs the flow of analyte into another set of six parallel channels, which are orthogonal to the six ligand channels, to create a 6 x 6 ligand-analyte interaction array (Figure 1). Six sets of six sensorgrams are rapidly generated in a single analyte injection step. From these sensorgrams, detailed kinetic data can be obtained on the interaction of up to six analytes with up to six different ligands.

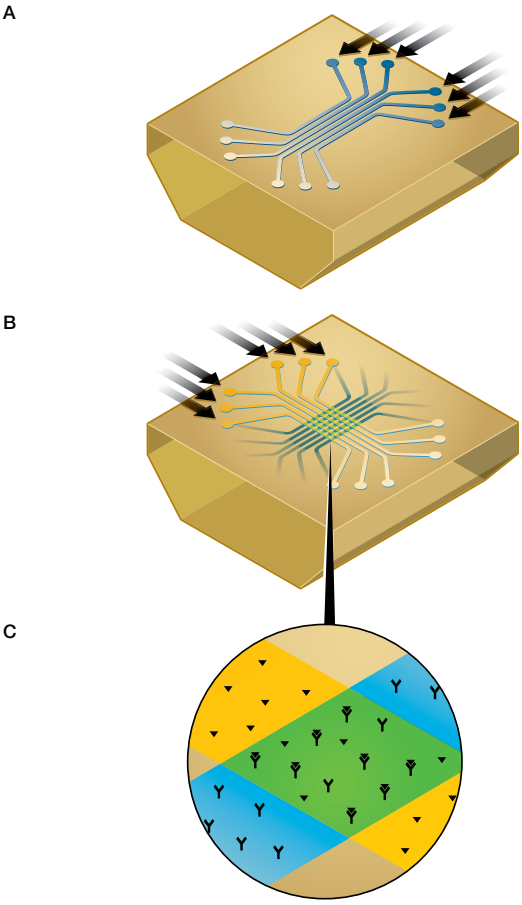

Fig. 1. Generation of the 6 x 6 ligand-analyte interaction array. A, six ligands are immobilized in six parallel ligand channels; **B**, six analyte samples are injected into six analyte channels orthogonal to the six ligand channels; **C**, detail of a single ligand-analyte interaction spot (green) showing the positions of the two interspot references (yellow).

In this tech note, we report the use of the ProteOn XPR36 system to determine the kinetic rate constants of the interaction between the human cytokine IL-2 and IL-2 antibody. Using "one-shot kinetics", six concentrations of IL-2 were presented in parallel across five different levels of immobilized IL-2 antibody (and one reference channel) to generate six sets of six sensorgrams that were then analyzed globally to yield kinetic constants in "one shot".

Methods

Instrumentation and Reagents

Experiments were performed using the ProteOn XPR36 protein interaction array system and one ProteOn GLC sensor chip. ProteOn phosphate buffered saline with 0.005% Tween 20, pH 7.4 (PBS/Tween) was used as running buffer throughout the experiments, and all experiments were performed at 25°C.

Immobilization of IL-2 Antibody

Mouse anti-human IL-2 antibody (ProteOn IL-2/IL-2 antibody pair) was immobilized on the ProteOn GLC sensor chip using the amine coupling reagents 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) and N-hydroxysulfosuccinimide (sulfo-NHS) (ProteOn amine coupling kit). Five ligand channels on the carboxylated sensor chip surface were activated using five concentrations of EDAC and sulfo-NHS as follows: channel 1, 133 mM EDAC and 33 mM sulfo-NHS; channel 2, 88 mM EDAC and 22 mM sulfo-NHS; channel 3, 60 mM EDAC and 15 mM sulfo-NHS; channel 4, 40 mM EDAC and 10 mM sulfo-NHS; channel 5, 26 mM EDAC and 6.5 mM sulfo-NHS. Then, 180 µl IL-2 antibody (25 µg/ml in ProteOn acetate buffer, pH 4.5) was injected at a flow rate of 30 µl/min into the five activated channels and immobilized to five different levels dependent on the degree of surface activation in each channel. To deactivate remaining carboxyl groups, 1 M ethanolamine HCl, pH 8.5 (ProteOn amine coupling kit) was then injected into each channel. A sample of running buffer was included in each step for injection into the sixth channel, which was used as a reference channel.

IL-2 Binding

Human cytokine IL-2 (ProteOn IL-2/IL-2 antibody pair) samples were prepared at concentrations of 80, 40, 20, 10, 5, and 2.5 nM by serial dilution in PBS/Tween. Samples of each concentration (100 µl) were injected into the six analyte flow channels at a flow rate of 100 µl/min. The analyte injection step included a 1 min association phase followed by an 11.7 min dissociation phase in running buffer.

Sensorgram Analysis

The 36 sensorgrams were grouped into six sets of six, with each set corresponding to the interaction of the six IL-2 concentrations with each ligand (IL-2 antibody) density and reference channel. Each sensorgram set was processed for baseline alignment and referencing. Both a reference channel and interspot references were used for referencing.

The response unit (RU) measured with the ProteOn XPR36 system is equal to a change in refractive index of one part in 10^6 by use of a set of solutions with known refractive indices over the range of 1.33–1.37.

Results and Discussion

Uniformity of IL-2 Antibody Immobilization

The immobilization level of IL-2 antibody along the six interaction spots in a single ligand channel was uniform, with a CV of <3% for each of the five ligand channels (Table 1, column 1). This indicates that each protein interaction spot within a ligand channel is an equivalent immobilization surface. Therefore, the sensorgrams required for a detailed kinetic analysis can be generated from the interactions of six analyte concentrations with the six interaction spots along a single ligand channel.

Determination of Kinetic Rate Constants

Analytical curves describing a homogeneous 1:1 bimolecular reaction model were fit globally to each set of six sensorgrams (Figure 2). The residual error (χ^2) for each fit was <3% of the associated R_{max} value. The adjustable kinetic parameters for

Table 1. One-shot kinetic values for the IL-2/IL-2 antibody interaction. The equilibrium dissociation constant, K_D, was calculated from k_d/k_a. Values for ligand density are averages ± coefficient of variation (CV).

		IL-2 Cytokine (Analyte)							
	IL-2 Antibody (Ligand)	Interspot Reference Subtraction				Reference Channel Subtraction			
Channel	Ligand Density (RU)	k_a ($M^{-1}sec^{-1}$)	k_d (sec^{-1})	K_D (M)	R_{max} (RU)	k_a ($M^{-1}sec^{-1}$)	k_d (sec^{-1})	K_D (M)	R_{max} (RU)
1	2,823 ± 2.2%	7.73×10^5	1.19×10^{-4}	1.54×10^{-10}	147	7.98×10^5	1.30×10^{-4}	1.63×10^{-10}	148
2	2,053 ± 2.8%	7.57×10^5	1.32×10^{-4}	1.74×10^{-10}	119	7.96×10^5	1.36×10^{-4}	1.71×10^{-10}	120
3	1,702 ± 2.6%	8.08×10^5	1.33×10^{-4}	1.65×10^{-10}	102	8.87×10^5	1.30×10^{-4}	1.47×10^{-10}	102
4	1,468 ± 2.7%	8.00×10^5	1.34×10^{-4}	1.68×10^{-10}	90	7.86×10^5	1.36×10^{-4}	1.73×10^{-10}	91
5	1,368 ± 2.6%	7.78×10^5	1.33×10^{-4}	1.71×10^{-10}	84	8.84×10^5	1.31×10^{-4}	1.48×10^{-10}	85
6	Reference channel	—	—	—	—	—	—	—	—
Average	—	7.83×10^5	1.30×10^{-4}	1.66×10^{-10}	—	8.31×10^5	1.33×10^{-4}	1.60×10^{-10}	—
SD	—	2.07×10^4	6.30×10^{-6}	7.70×10^{-12}	—	5.00×10^4	3.13×10^{-6}	1.07×10^{-11}	—
CV (%)	—	2.64	4.84	4.63	—	6.02	2.36	6.66	—

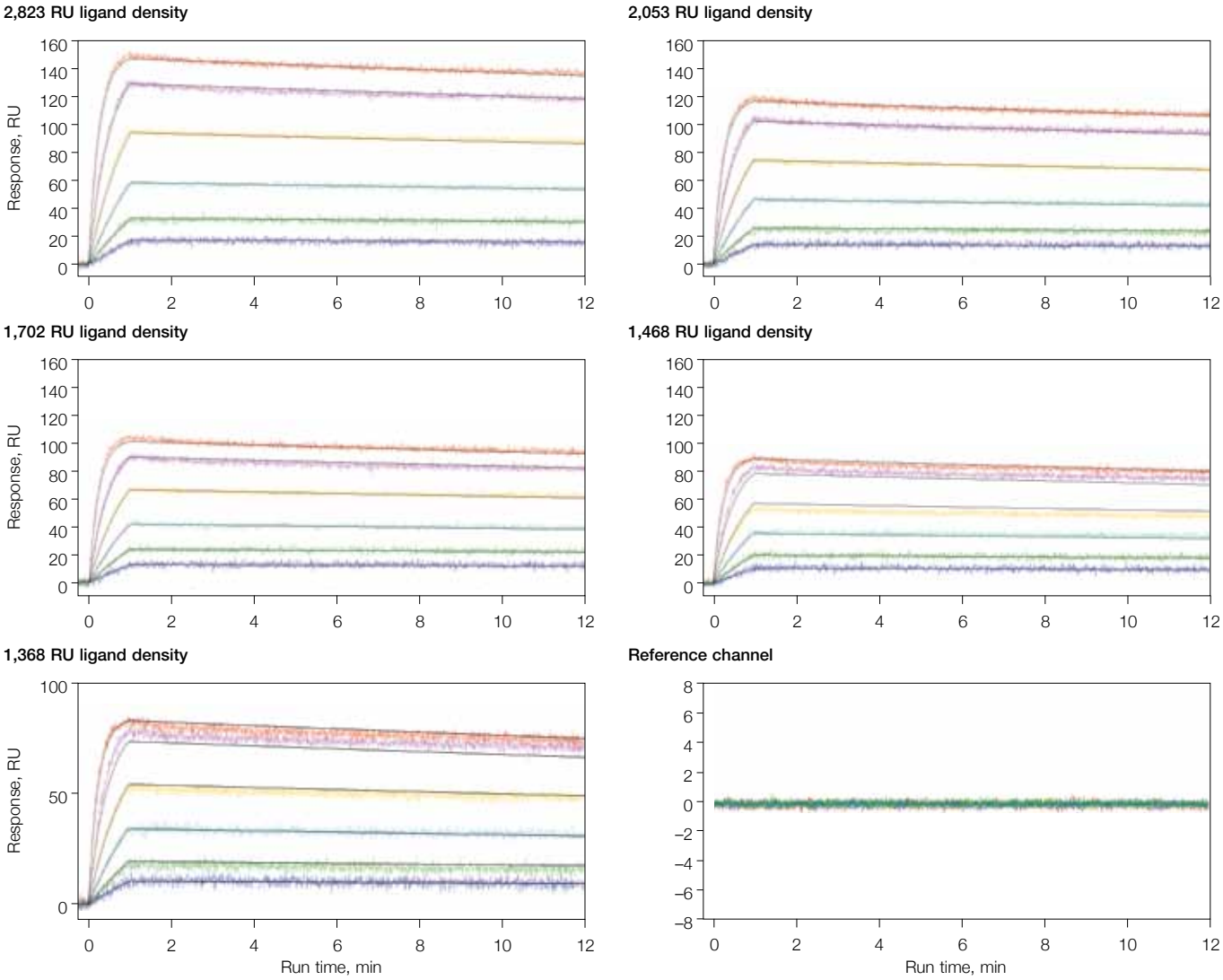

Fig. 2. One-shot kinetics for the IL-2 cytokine/IL-2 antibody interaction. Shown are the six sets of six sensorgrams generated in a single analyte injection step. Each set of six sensorgrams displays the responses from the six IL-2 cytokine concentrations (—, 80 nM; —, 40 nM; —, 20 nM; —, 10 nM; —, 5 nM; —, 2.5 nM) interacting with one immobilization level of IL-2 antibody. Sensorgrams are shown for the five levels of IL-2 antibody immobilization (ligand density) and the reference channel. Black lines represent the global fit of the sensorgrams to a 1:1 kinetic interaction model. See Table 1 for the kinetic constants derived from these data.

association (k_a), dissociation (k_d), and R_{max} for each IL-2 and IL-2 antibody sensorgram set were derived from the fitted curves and are shown in Table 1. The R_{max} values correlated well with the ligand immobilization levels (r = 99%).

Because each of the five experimental sensorgram sets provided acceptable fits, and because the interaction conditions were comparable, coefficients of variation (CVs) were calculated among these sets and were found to be in the range of 2–7% for the kinetic constants. Mass transport effects were estimated to be a minor contribution to the interaction by the consistency of the association rate constant at each ligand density (Table 1), which permitted the use of the five IL-2 antibody immobilization levels as replicates.

Reference Subtraction

In SPR experiments, referencing is needed to remove any contribution to the interaction response arising from differences in the refractive index of the sample solution vs. the refractive index of the running buffer (referred to as the "bulk effect"), and also from contributions due to drift and nonspecific binding. Referencing can be performed in two ways: by using a dedicated reference channel (for example, a channel that does not contain bound ligand) and by using interspot references.

A novel feature of the ProteOn XPR36 interaction array system is the ability to measure the SPR response in 42 interspot references. Interspot references are regions on the sensor chip

situated between flow channels, and thus adjacent to both sides of every interaction spot in the direction of analyte flow (Figure 1). During ligand immobilization, these interspot reference regions are not exposed to the activation or ligand solutions. However, during analyte binding, the interspot references are exposed to analyte flow, and because the interspot references do not have bound ligand, they can be used in place of a reference channel. The response of each interaction spot can be corrected by the average response from its two adjacent interspot references. Though any flow channel on the sensor chip may be used as a ligand or analyte reference channel, the inclusion of interspot references in the design of the ProteOn XPR36 system greatly increases its throughput and flexibility by allowing each flow channel to be used directly for interaction analysis.

Both referencing methods were used for the purpose of comparison, and the kinetic rate constants determined by both methods were nearly identical (Table 1). This demonstrates that results obtained using interspot references are equivalent to those obtained using a reference flow channel.

Finally, the %CV of the kinetic constants combined from samples prepared and analyzed independently on separate chips were in the range of <10%.

Conclusions

The capability of the ProteOn XPR36 protein interaction array system has been demonstrated for rapid and efficient determination of kinetic rate constants for protein interactions. Using one-shot kinetics, kinetic rate constants for the IL-2/IL-2 antibody interaction were determined from five replicate kinetic analyses in just 2 hr total instrument run time, including baseline stabilization and both the ligand immobilization and analyte binding injection steps.

A number of features of the ProteOn XPR36 protein interaction array system greatly increase the throughput, flexibility, and versatility of experimental design for protein-protein interaction analysis. In this experiment, the 6 x 6 interaction array was used to gain statistical confidence by treating the multiple interaction sets as replicates. In experimental situations where the interaction conditions are less known, the increased throughput can be used to immobilize ligand at different levels and to run analyte at different concentrations or in different buffers to rapidly find the optimal ligand immobilization and analyte binding conditions for the interaction under investigation.

In addition to the 6 x 6 interaction array, a number of other features enable the ProteOn XPR36 system to maximize throughput and flexibility for those experimental designs in which multiple ligand-analyte interactions are under investigation. The use of interspot references for sensorgram correction, which was validated in this study, lessens the need to dedicate a channel to referencing, and there is no need for regeneration (although the ligand surfaces can be regenerated for additional analytes, if needed). Because six analytes are run in parallel, kinetic analysis, K_D ranking, stability comparisons, epitope mapping, and other applications can be accomplished rapidly. The ProteOn XPR36 system represents a significant advance in SPR biosensor technology, and provides increased throughput and flexibility in experimental design.

Reference

Rich RL and Myszka DG, Survey of the year 2004 commercial optical biosensor literature, J Mol Recognit 18, 431–478 (2005)

This tech note was current as of the date of writing (2005) and not necessarily the date this version (rev B, 2013) was published.

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