Rapid and Detailed Analysis of Multiple Antigen-Antibody Pairs Using the ProteOn™ XPR36 Protein Interaction Array System

Vered Bronner, Tsafrir Bravman, and Kobi Lavie, Bio-Rad Laboratories, Inc., Gutwirth Park, Technion, Haifa 32000, Israel

Introduction

The ProteOn XPR36 protein interaction array system is a surface plasmon resonance (SPR) biosensor with a multichannel module and interaction array sensor chip for analysis of up to 36 protein interactions in a single injection step. The capability of the ProteOn XPR36 system to rapidly generate a 6 x 6 interaction array between six ligands and six analytes greatly increases the throughput, flexibility, and versatility of experimental design for a wide range of biomolecular interaction studies (Figure 1). Additional details on this array-format system are provided in Bronner et al. (2006).

We describe here the rapid and detailed kinetic analyses of four antigen-antibody interactions. Briefly, the interaction of each antibody (ligand) with a concentration series of its respective antigen (analyte) was analyzed in a sequence of a single immobilization cycle followed by four analyte injection cycles. Four different antibodies and a nonreactive negative control protein were immobilized in five parallel ligand channels on a single ProteOn sensor chip; the sixth ligand channel was left blank for use as a reference channel. For each antibody, a concentration series of antigen samples (six samples spanning a wide concentration range) was then injected into the set of six parallel analyte channels orthogonal to the ligand channels. The ligand channels were then regenerated for analysis of the next antigen concentration series, until all four antigen-antibody interactions were analyzed.

Each of the four antigen injections produced a set of 36 sensorgrams. These contained data not only on the interaction of an antigen concentration series with its respective antibody, but also on its interaction with each of the three other antibodies, the negative control protein, and the reference channel. Thus, data sufficient to perform a detailed kinetic analysis of each antigen-antibody interaction — as well as a determination of any potential cross-reactivity among the antigens and antibodies — were obtained in a minimal number of injection steps.

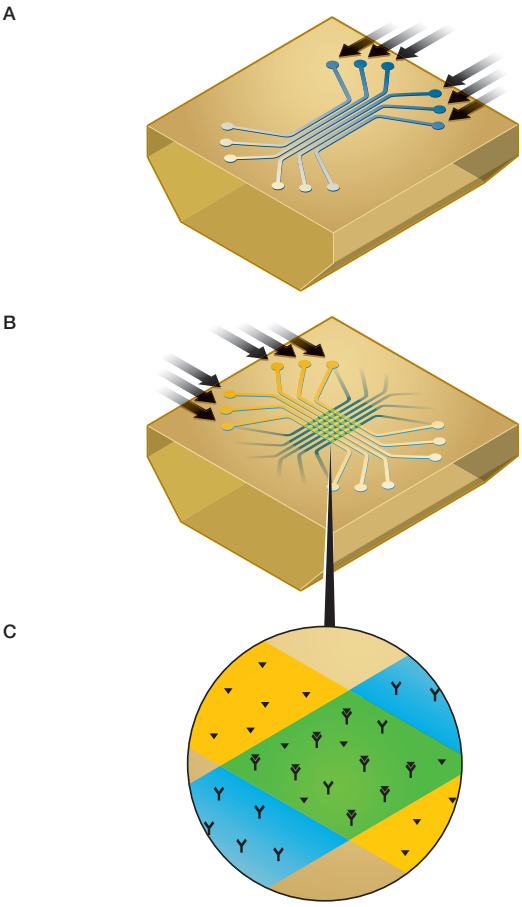

Fig. 1. Generation of the 6 x 6 ligand-analyte interaction array. A, six ligands are immobilized in six parallel ligand channels; **B**, six analyte samples are injected into six analyte channels orthogonal to the six ligand channels; **C**, detail of a single ligand-analyte interaction spot (green)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

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 Antibodies

Using the parallel sample processing capability of the ProteOn XPR36 system, the immobilization cycle of the four antibodies and negative control protein was accomplished in only three injection steps for surface activation, ligand coupling, and surface deactivation.

First, the ProteOn GLC sensor chip surface was activated in five of the six ligand channels by injection of the amine coupling reagents 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC, 100 mM) and *N*-hydroxysulfosuccinimide (sulfo-NHS, 25 mM) (components of the ProteOn amine coupling kit). The sixth channel was not modified and served as a reference channel.

Next, the four antibodies were diluted to the following concentrations in 10 mM ProteOn acetate buffer, pH 4.5: IL-2 antibody, 25 µg/ml (mouse anti-human IL-2, ProteOn IL-2/IL-2 antibody pair); IL-4 antibody, 50 µg/ml (mouse anti-human IL-4, Biosource International); IL-6 antibody, 12.5 µg/ml (mouse anti-rat IL-6, BD Biosciences Pharmingen); and IL-18 antibody, 12.5 µg/ml (hamster anti-mouse, Medical and Biological Laboratories Co., Ltd.). The four antibody samples (180 µl) and a nonreactive protein (TEM1 β-lactamase, 180 µl) were injected into the five activated ligand channels at a flow rate of 30 µl/min for immobilization. Lastly, to deactivate remaining carboxyl groups, 1 M ethanolamine HCl, pH 8.5 (ProteOn amine coupling kit) was then injected into the five activated channels. A buffer sample was included in each step for injection into the reference channel.

Antigen Interaction Analysis

Antigen samples were all prepared by serial dilution in PBS/Tween. Human cytokine IL-2 (ProteOn IL-2/IL-2 antibody pair) and rat cytokine IL-6 (BD Biosciences Pharmingen) samples were prepared at concentrations of 80, 40, 20, 10, 5, and 2.5 nM. Human cytokine IL-4 (Research Diagnostics, Inc.) samples were prepared at 32, 16, 8, 4, 2, and 1 nM, and mouse cytokine IL-18 (Medical and Biological Laboratories Co., Ltd.) samples at 60, 30, 15, 7.5, 3.8, and 1.9 nM.

Each concentration series of antigen was injected into the six analyte channels orthogonal to the ligand channels at a flow rate of 100 µl/min. The durations of these injections were 60 sec for 100 µl of IL-2 or IL-4, 120 sec for 200 µl of 1L-18, and 240 sec for 400 µl of IL-6. The durations of the IL-6 and IL-18 injections were increased to produce sufficient curvature in the association phase of the sensorgrams for kinetic analysis. The ligand channels were regenerated between injections of each antigen concentration series by a short pulse (30 µl) of 0.85% phosphoric acid.

Sensorgram Analysis

The 36 sensorgrams produced from each antigen injection were grouped into six sets of six, with each set corresponding to the interaction of an antigen concentration series with the four immobilized antibodies, negative control protein, and reference channel. The sensorgrams were processed for baseline alignment and referencing.

In addition to the reference channel, interspot references were used for background subtraction. Interspot references are exposed to analyte flow but do not have bound ligand, and so can be used in place of a reference channel (Bronner et al. 2006). When the response of each interaction spot was corrected by the average response from its two adjacent interspot references, the results were identical to those obtained using the reference channel (Figure 2).

Results and Discussion

The four sets of six sensorgrams were globally fit to a 1:1 bimolecular interaction model (Figure 2). The adjustable kinetic parameters for association (k_a), dissociation (k_d), and R_{max} for each interaction were derived from the fitted curves (Table 1).

The coefficients of variation of the kinetic constants obtained from samples analyzed at different times were in the range of 10%. The percent differences for ligand density in Table 1 were computed from the six interaction spots along a single ligand channel.

The kinetic properties of the different antigen-antibody interactions are readily compared. Table 1 summarizes the association and dissociation rates obtained from the fitted curves for each of the four antigen-antibody interactions, and the calculated equilibrium dissociation constant (K_D). The slower association rate (k_a) of IL-6/IL-6 antibody binding (3.72×10^4 $M^{-1}sec^{-1}$) was notable in comparison to the association rates of the other antigen-antibody pairs ($0.6–2.4 \times 10^6$ $M^{-1}sec^{-1}$). The dissociation constants (k_d) were similar for all pairs ($1.84–2.93 \times 10^{-4}$ sec^{-1}). This comparison of kinetic constants indicates a difference of 2 orders of magnitude in the equilibrium dissociation constants of IL-6 ($K_D = 7.89 \times 10^{-9}$ M) and IL-4 ($K_D = 9.29 \times 10^{-11}$ M).

In this experiment, no interaction between antigens and unrelated antibodies was detected, indicating no cross-reactivity (data not shown).

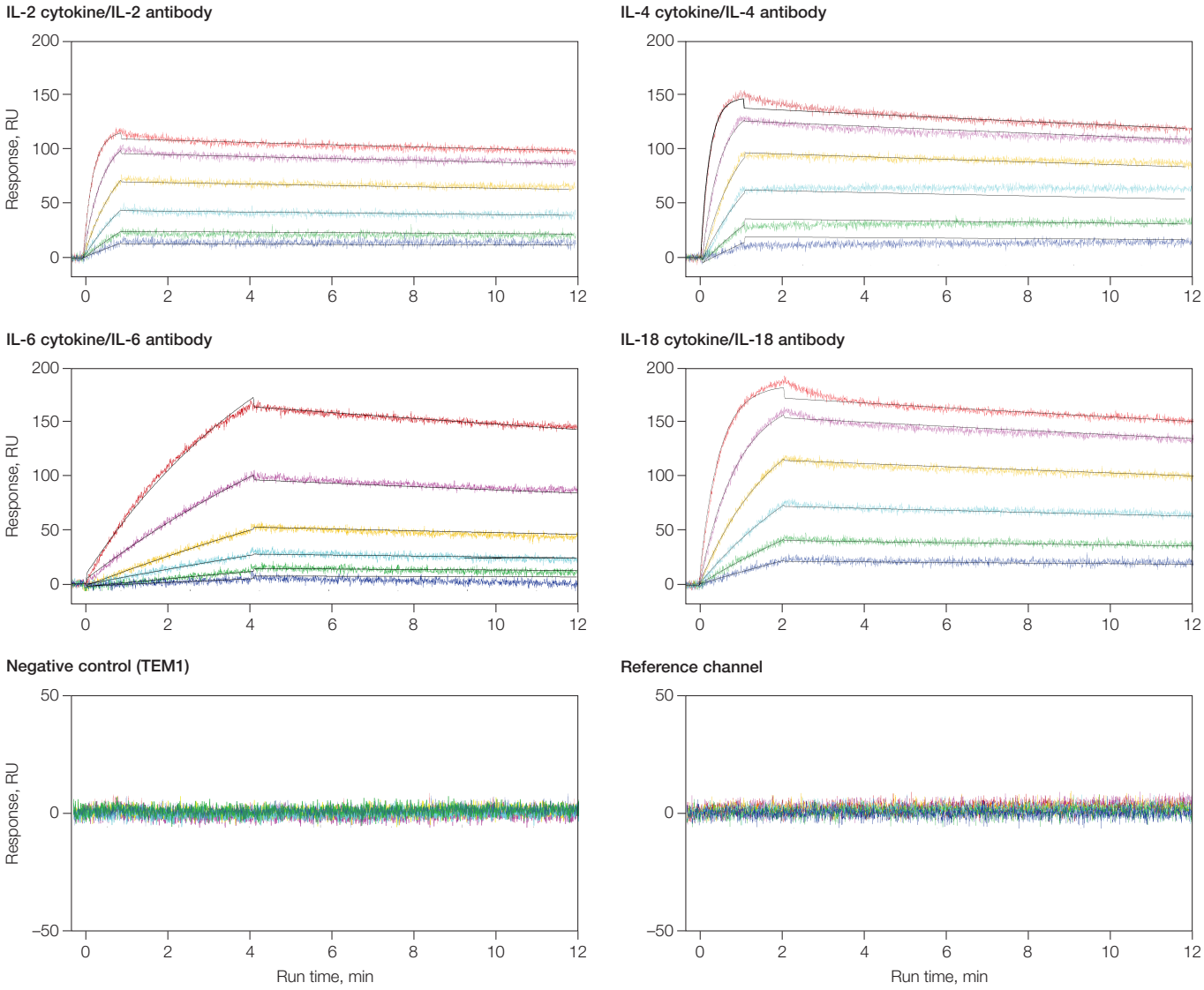

Fig. 2. Kinetic analysis of four antigen-antibody interactions. Shown are the four sets of sensorgrams generated for each specific antibody-antigen pair, as well as sensorgrams from the interaction of the IL-2 concentration series with the negative control and reference channel. The interactions of the four cytokines are shown from the highest cytokine concentrations (top traces) to the lowest concentrations (bottom traces). Black lines represent the global fit of the sensorgrams to a 1:1 interaction kinetic model. See Table 1 for the kinetic constants derived from these data.

Table 1. Kinetic constants for four antigen-antibody interactions. The equilibrium dissociation constant, K_D, was calculated from k_d/k_a.

	Antibody (Ligand)	Antigen (Analyte)			
Channel	Ligand Density (RU)	k_a ($M^{-1}sec^{-1}$)	k_d (sec^{-1})	K_D (M)	R_{max} (RU)
(1) IL-2	1,470 ± 2.65%	8.68×10^5	1.84×10^{-4}	2.12×10^{-10}	112
(2) IL-4	1,097 ± 4.64%	2.38×10^6	2.21×10^{-4}	9.29×10^{-11}	139
(3) IL-6	1,530 ± 1.86%	3.72×10^4	2.93×10^{-4}	7.89×10^{-9}	336
(4) IL-18	2,588 ± 1.28%	6.15×10^5	2.33×10^{-4}	3.78×10^{-10}	175

Conclusions

The ProteOn XPR36 protein interaction array system is well-suited for experimental designs in which a number of interacting protein pairs are to be analyzed and compared. Data can be rapidly obtained for protein interface characterization, affinity ranking, and epitope mapping of antibodies, as well as for selecting lead compounds in drug development.

The capability of the ProteOn XPR36 system to rapidly perform a detailed kinetic analysis of multiple protein-protein interactions in a 6 x 6 interaction array format is especially useful for antibody development and characterization. Here the kinetic rate constants were determined for four antigen-antibody pairs using just one ProteOn sensor chip and a minimum number of injection cycles. This throughput, together with the fact that the interactions between each antigen and the other immobilized antibodies are also measured (which offers information on cross-reactivity), makes the ProteOn XPR36 system a powerful tool for protein interaction analysis.

Reference

Bronner V et al., Rapid and efficient determination of kinetic rate constants using the ProteOn XPR36 protein interaction array system, Bio-Rad bulletin 3172 (2006)

This tech note was current as of the date of writing (2005) and not necessarily the date this version (rev A, 2006) was published.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD* ***Australia*** *02 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 21 3237 9400* ***Canada*** *905 712 2771* ***China*** *86 21 6426 0808* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84 0* ***Greece*** *30 210 777 4396* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 455 8800* ***India*** *91 124 4029300/5013478* ***Israel*** *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 5811 6270* ***Korea*** *82 2 3473 4460* ***Mexico*** *55 5200 05 20* ***The Netherlands*** *0318 540666* ***New Zealand*** *64 9415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 095 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *27 0861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55* ***Taiwan*** *886 2 2578 7189/2578 7241* ***United Kingdom*** *020 8328 2000*