Applications of the ProteOn™ NLC Sensor Chip: Antibody-Antigen, DNA-Protein, and Protein-Protein Interactions

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Introduction

The ProteOn NLC sensor chip is one of several types of sensor chips designed for use with the ProteOn XPR36 protein interaction array system. The chip is coated with a layer of immobilized NeutrAvidin used for single-step immobilization of various biotinylated biomolecules (for example, peptides, proteins, oligonucleotides, and carbohydrates). The neutral charge of this NeutrAvidin layer minimizes nonspecific binding, and also enhances immobilization of oligonucleotides because their negative charge does not interfere with surface immobilization. Terminal labeling of immobilized oligonucleotides with biotin ensures high accessibility to analyte molecules, and no surface activation or deactivation steps are required, making the NLC immobilization protocol both rapid and effective.

In this tech note we demonstrate the use of the ProteOn NLC sensor chip and surface plasmon resonance (SPR) with the ProteOn XPR36 system (Figure 1) to determine the kinetic rate constants for three bimolecular model interactions: 1) cytokine IL-2 with anti-IL-2 antibody (antigen-antibody interaction), 2) *E. coli* tryptophan (*trp*) operator sequence with *trp* repressor protein (DNA-protein interaction), and 3) interferon-α2 (IFNA2) with interferon receptor IFNAR2 (protein-protein interaction). For each model interaction, six concentrations of analyte were injected across five channels of immobilized biotin-labeled ligand in a single automated step, generating six sets of five analysis sensorgrams. These sensorgrams were fitted with a global 1:1 interaction model to yield kinetic constants using the One-shot Kinetics™ technique (Bronner et al. 2006).

Methods

Instrumentation and Reagents

The ProteOn XPR36 protein interaction array system (Bio-Rad Laboratories, Inc.) was used for all experiments, and each of the three model interactions was studied on a separate NLC sensor chip. Biotinylated ligand was immobilized at a constant concentration in five of the six ligand channels; the sixth channel was used as a reference channel. ProteOn phosphate buffered saline, pH 7.4, with 0.005% Tween 20 (PBS/Tween) was used as diluent and running buffer. Experiments were performed at 25°C.

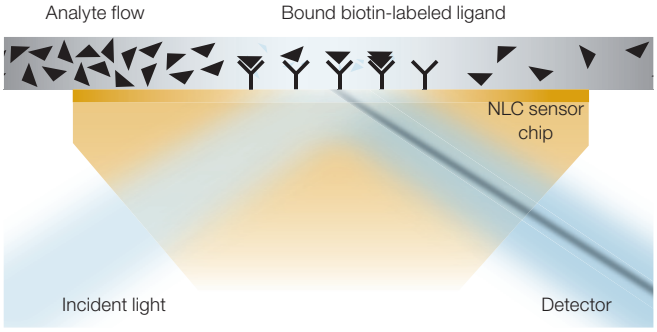

Fig. 1. Surface plasmon resonance and the ProteOn NLC sensor chip. Analyte molecules bind to biotin-labeled ligands at the surface of the ProteOn NLC sensor chip, causing a shift in the SPR response curve. The shift is proportional to the mass change near the NLC sensor chip surface.

Antigen-Antibody Interaction

Ligand — Mouse anti-human IL-2 antibody (BD Pharmingen) was biotinylated with biotin-NHS ester (Sigma) and immobilized in five channels of a ProteOn NLC sensor chip at 25 µg/ml (167 nM) in PBS, pH 7.4, at a flow rate of 30 µl/min. The sixth channel was used as a reference channel.

Analyte — Analyte samples of human cytokine IL-2 (BD Pharmingen) were prepared at concentrations of 80, 40, 20, 10, 5, and 2.5 nM by serial dilution in PBS. Following initial preconditioning of the sensor chip surface with a short injection of 0.85% phosphoric acid solution, the six IL-2 samples were injected into the six analyte channels at a flow rate of 100 µl/min. The injection step included a 1 min association phase followed by an 11 min 40 sec dissociation phase in PBS, which was used as the continuous-flow buffer throughout the experiment.

DNA-Protein Interaction

Ligand — A biotinylated, single-stranded 3-mer oligonucleotide containing the *trp* operator sequence (5'-biotin-TTTTTTTTTAT-GCTATCGTACTCTTTAGCGAGTACAACCGGGG, Sigma) was immobilized in five channels of a ProteOn NLC sensor chip at a concentration of 0.5 µM in PBS, pH 7.4, at a flow rate of 30 µl/min. The unlabeled complementary strand (0.5 µM) was injected into the same channel, and hybridization was monitored (~35% hybridization was achieved). Running buffer was injected into the sixth channel, which was used as a reference channel.

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Analyte — Samples of *trp* repressor protein (Panvera) were prepared at concentrations of 8, 6, 4, 2, 1, and 0.5 nM by dilution in PBS/Tween containing 4 mM tryptophan. Samples of each concentration were injected into the six analyte channels at a flow rate of 40 µl/min. The injection step included a 1 min 30 sec association phase followed by a 3 min 20 sec dissociation phase in PBS with 4 mM tryptophan, which was used as the continuous-flow buffer throughout the analyte interaction step.

Protein-Protein Interaction

Ligand — The interferon receptor IFNAR2-EC (25 kD) (provided by G Schreiber, Weizmann Institute) was labeled with *N*-biotinyl-6-aminocaproic acid-*N*-succinimidyl ester (Fluka). The biotinylated IFNAR2-EC (0.2 µM) was immobilized on an NLC sensor chip in five ligand channels in PBS, pH 7.4, at a flow rate of 30 µl/min. The sixth channel was used as a reference channel.

Analyte — Samples of IFNA2 (provided by G Schreiber) were prepared at concentrations of 100, 50, 25, 12.5, 6.25, and 3.12 nM by serial dilution in PBS. Samples of each concentration were injected into the six analyte channels at a flow rate of 30 µl/min. The injection step included a 4 min association phase followed by a 5 min dissociation phase in PBS, which was used as the continuous-flow buffer throughout the experiment.

Sensorgram Acquisition and Data Analysis

Each of the three model interactions was studied on a separate ProteOn NLC sensor chip. The interactions of the six analyte concentrations with one concentration of immobilized ligand were monitored in parallel, generating six sets of five analysis sensorgrams. Two different types of referencing were used for each of the model interactions: interspot referencing (measurement of two inactive spots adjacent to each of the 36 interaction spots) and a reference channel (measurement along the sixth channel, without bound ligand) (Figure 2). Sensorgram correction and kinetic model fitting were applied separately for each of these two types of referencing. Each set of six reference-subtracted sensorgrams was fitted globally to curves describing a homogeneous 1:1 bimolecular reaction model. Global kinetic rate constants (k_a and k_d) were derived from this reaction model.

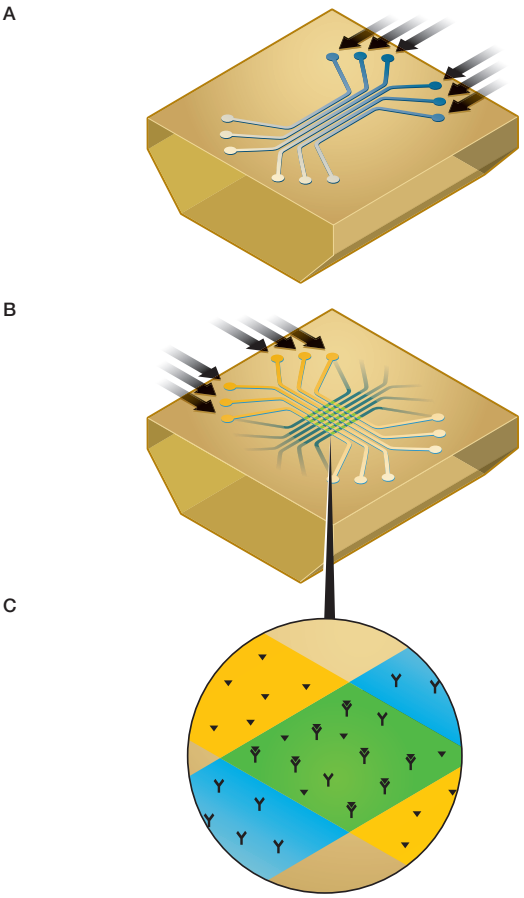


Fig. 2. The ProteOn NLC sensor chip's 6 x 6 interaction array. **A**, biotinylated ligand is immobilized in five parallel channels, with the sixth channel used as a reference channel; **B**, six different concentrations of analyte are injected into the six orthogonal channels, generating the 36-element interaction array; **C**, detail of a single ligand-analyte interaction spot (green) showing the positions of the two interspot references (yellow).

Results and Discussion

Uniformity of Ligand Immobilization

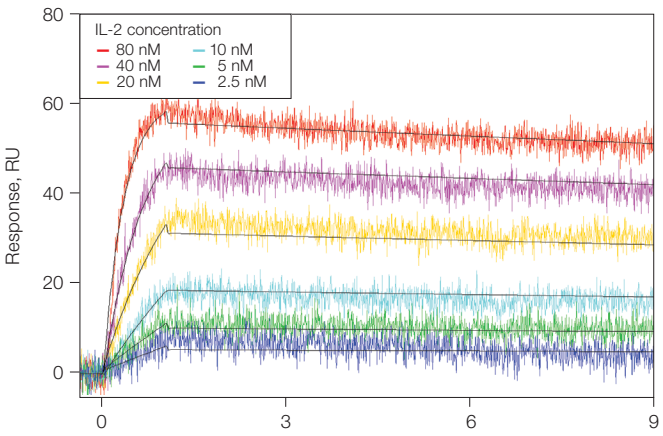
The immobilization level (ligand density) of each biotinylated ligand was uniform at the six protein interaction spots in each of the six ligand channels (CV ≤3%) (Table 1). These results indicate that each protein interaction spot within each ligand channel acts as an equivalent immobilization surface, ensuring accurate measurement of ligand density and interaction kinetics.

Table 1. Summary of kinetic constants derived for the three model interactions.

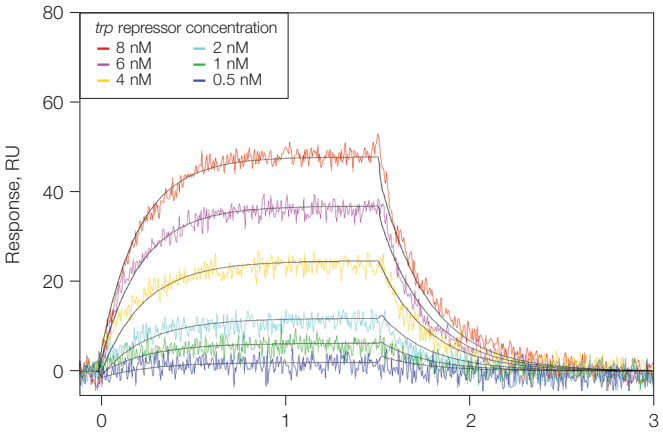
		Interspot Reference Subtraction			Reference Channel Subtraction		
Model	Ligand Density, RU	k_a, $M^{-1}sec^{-1}$	k_d, sec^{-1}	K_D,* M	k_a, $M^{-1}sec^{-1}$	k_d, sec^{-1}	K_D, M
IL-2	1,685 ± 1.8%	5.1×10^5	1.1×10^{-4}	2.2×10^{-10}	6.6×10^5	1.9×10^{-4}	2.9×10^{-10}
trp repressor	293 ± 3.0%	1.4×10^6	5.8×10^{-2}	4.1×10^{-8}	1.5×10^6	6.6×10^{-2}	4.4×10^{-8}
IFNAR2	234 ± 2.7%	2.7×10^6	1.4×10^{-2}	5.2×10^{-9}	2.3×10^6	1.2×10^{-2}	5.2×10^{-9}

*The equilibrium dissociation constant, K_D, was calculated from k_d/k_a.

A. IL-2 and IL-2 antibody



B. *trp* repressor and *trp* operator



C. Interferon-α2 (IFNA2) and interferon receptor IFNAR2

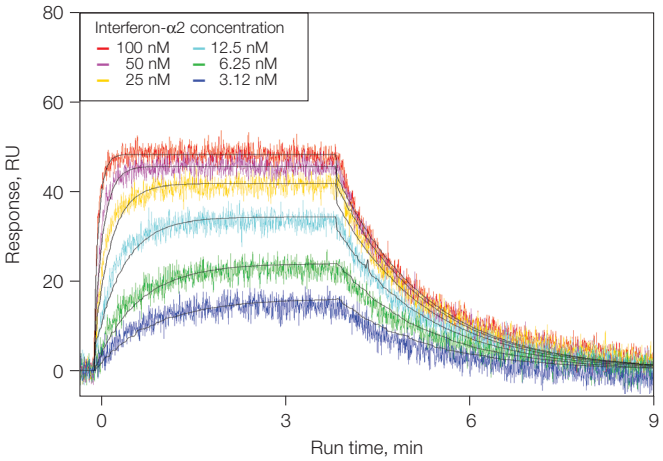


Fig. 3. Three sets of sensorgrams showing three model interactions. Each set of six sensorgrams corresponds to the responses of six analyte concentrations interacting with immobilized ligand. **A**, cytokine IL-2 interaction with anti-IL-2 antibody; **B**, *trp* repressor protein interaction with the *trp* operator sequence; **C**, interferon-α2 (IFNA2) with the interferon receptor IFNAR2. The black lines depict global 1:1 interaction curve-fitting models for each of the three interactions.

Determination of Kinetic Rate Constants

Table 1 summarizes the results for kinetic constants of association (k_a) and dissociation (k_d) derived from each of the three model interactions. Figure 3 shows representative sensorgrams fitted to 1:1 global curves for each model interaction. The residual error for each fit was <10% of the associated R_{max} value (data not shown).

Reference Subtraction

As described in Methods, two different types of reference sensorgrams were used for each interaction. Sensorgram correction and kinetic model fitting were performed separately for each reference type. Kinetic constants derived from each referencing method were similar (Table 1), demonstrating the equivalence of the two reference models and validating the use of interspot references as a rigorous empirical tool. The use of interspot references is especially useful in experimental protocols where high throughput and greater experimental flexibility are desired (Bronner et al. 2006).

Conclusions

In this tech note we demonstrate the determination of kinetic rate constants for three typical bimolecular model interactions using the One-shot Kinetics approach with the ProteOn NLC sensor chip. Each of the three models studied used a simple two-step protocol: immobilization of one concentration of biotinylated ligand, followed by injection of six different concentrations of analyte. The results demonstrate the efficiency of the One-shot Kinetics technique described in detail by Bronner et al. (2006), and because interspot references eliminate the need for using a reference channel, throughput and flexibility are maximized for those experimental designs in which multiple ligands are under investigation.

Acknowledgements

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Reference

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

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