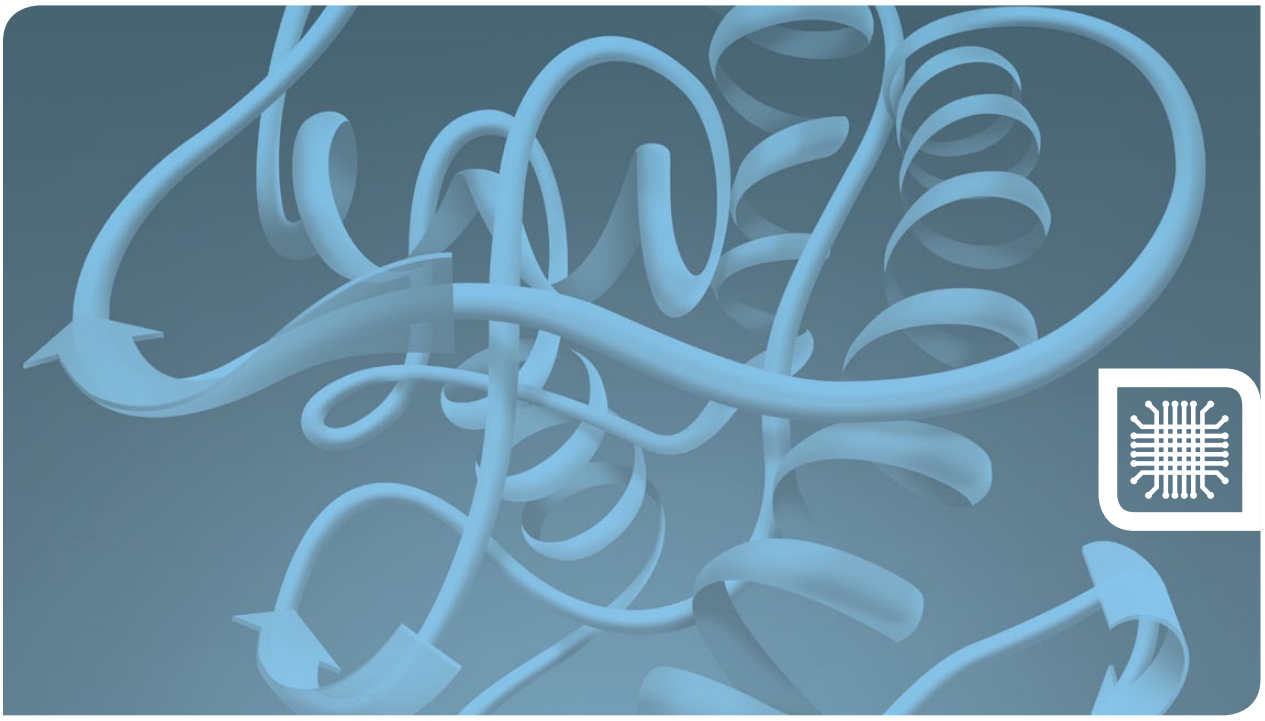


ProteOn™ XPR36

Protein Interaction Array System

The Power of Parallel Analysis

BIO-RAD

Explore the World of Parallel Analysis with XPR36

The ProteOn XPR36 protein interaction array system is a surface plasmon resonance (SPR) biosensor platform that provides real-time label-free analysis of the specificity, affinity, and kinetics of biomolecular interactions. Using the XPR36 configuration, this system generates a 6 x 6 interaction array for the simultaneous analysis of up to six ligands with up to six analytes. The ProteOn XPR36 system increases the versatility of experiment design and the productivity of experimental workflow, enabling the completion of high-quality SPR experiments very efficiently. The parallel-flow SPR biosensor platform:

- Analyzes up to 36 different protein interactions in a single run on a single chip
- Measures a variety of experimental conditions simultaneously using parallel-flow fluidics
- Screens multiple panels of analytes
- Acquires the resonance angle shift as SPR response units (RU) for accurate kinetics
- Employs One-shot Kinetics™ technology, which enables a complete kinetic analysis in a single run

Biomolecular Interaction in a New Light

Two biomolecules, **A** and **B**, interact with each other to form a complex **AB**. Using an SPR biosensor, besides the equilibrium constant $\mathbf{K_D}$, the association rate constant $\mathbf{k_a}$ and the dissociation rate constant $\mathbf{k_d}$ can be measured, determining more details of the interaction compared to other methods.

$$A + B \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AB$$

ProteOn XPR36 System

Key Applications in Research and Discovery

Basic Research

- Identification of biomolecular interactions
- Characterization of biomolecular structures

Applied Research

- Identification and validation of drug targets
- Rapid assay design

Screening

- Monoclonal antibody screening
- Small molecule and fragment screening
- Affinity ranking
- Off-rate screening

Lead Optimization

- Antibody epitope binning
- Antibody epitope mapping
- Kinetic and thermodynamic characterization of drug-target interactions

Preclinical/Clinical

- Serum sample analysis
- Vaccine validation
- Clinical assay design

Process/Quality Control

- Active concentration analysis
- Quantitation and kinetics in 1 workflow
- 21 CFR Part 11 compliance

ProteOn XPR36 System Advantages

Versatility

- Flexible experimental configuration
- Efficient experimental optimization
- Kinetic, affinity, and thermodynamic analysis in 1 platform
- Compatible with crude samples
- Compatible with both qualitative and quantitative assays
- Compatible with direct and indirect binding assays

Productivity

- Analysis of 36 interactions in a single injection
- Up to 6 full kinetics measured in a single injection in 1.1 hr
- Full kinetics of 96 antibody supernatant samples in 11 hr
- Unattended running by system automation
- Batch data processing and analysis by software

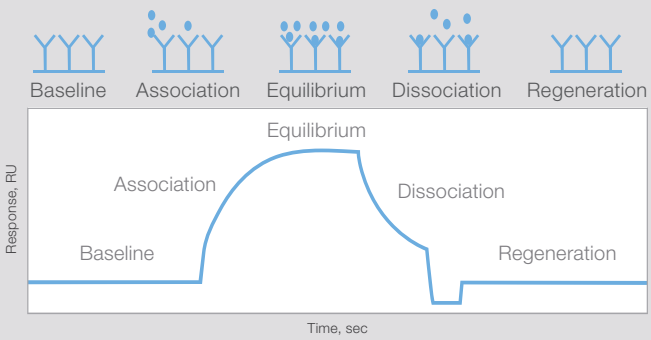
High Quality

- Label-free analysis with native protein structures
- Novel referencing options for accurate kinetics and affinity
- Excellent sensitivity
- Multiple surface chemistries for optimal assay conditions
- Parallel-flow fluidics allowing for real-time comparison of multiple interactions

Biomolecular interaction analysis does not only mean measuring the binding affinity. The ProteOn XPR36 system characterizes the following aspects of a biomolecular interaction:

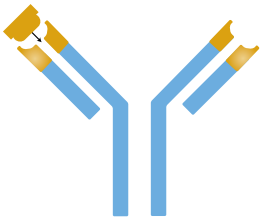
- How specific is the interaction?
- How fast is the interaction (k_a)?
- How stable is the complex (k_d)?
- How strong is the interaction (K_D [$K_D = k_d / k_a$])

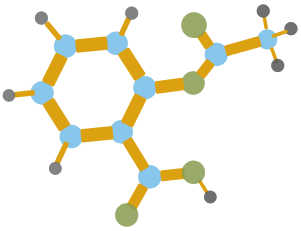
The parameters are obtained from the data fitting of the association, equilibrium (optional), and dissociation phases of a sensorgram.

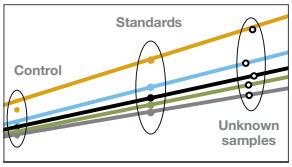


SPR sensorgram. —, surface; Y, ligand; ●, analyte. RU, response units.

ProteOn XPR36 System Applications







	Antibody Characterization and Profiling	Drug Discovery Screening	Protein-Protein Interactions	Protein Quantitation and Kinetics
Applications	Screen antibody-antigen interactions, including kinetics, epitope mapping, and epitope binning.	Analyze protein–small molecule interactions, such as screening compounds for drug discovery.	Analyze protein-protein interactions to pinpoint structures on proteins that are responsible for binding.	Analyze the active concentration of a protein sample by the initial binding rate.
Traditional Methods	▪ Enzyme-linked immunosorbent assay (ELISA) ▪ Isothermal calorimetry (ITC) ▪ Conventional serial flow SPR	▪ Competitive ELISA ▪ ITC ▪ Conventional serial flow SPR	▪ X-ray crystallography ▪ ITC ▪ Conventional serial flow SPR	▪ ELISA ▪ Biolayer interferometry ▪ Conventional serial flow SPR
Advantages of ProteOn XPR36 System	**Anitbody Kinetic Screening** ▪ Efficient experimental optimization ▪ Accurate kinetics ▪ Compatible with crude samples ▪ High-throughput screening **Epitope Mapping/ Epitope Binning** ▪ Flexible experimental configurations ▪ Available for various types of assays ▪ High-throughput screening	▪ High sensitivity ▪ High-throughput screening ▪ Available for fragment screening ▪ Accurate kinetics	▪ Efficient experimental optimization ▪ HTG and HTE sensor chips based on tris-NTA (3 x NTA) surface chemistry for stable and regenerable capture of histidine-tagged proteins ▪ Efficient online purification process using tris-NTA (3 x NTA) or other surface chemistries for antibody screening or mutagenesis in structural biology ▪ Compatible with crude samples ▪ Accurate kinetics	▪ Accurate quantitation and kinetics ▪ Wide dynamic range in quantitation ▪ High throughput for rapid sample processing

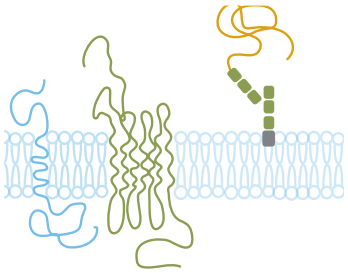
For more details, visit **www.bio-rad.com/proteon/app**.

Assay Design

Discover the optimal design and experimental conditions for high-quality biological assays.

- ELISA
- Conventional serial flow SPR

- Rapid label-free screening for assay components and conditions
- Rapid epitope binning for designing sandwich immunoassays
- Versatile assay configurations
- Multiple surface chemistries for different types of assays
- Unattended running for assay validation

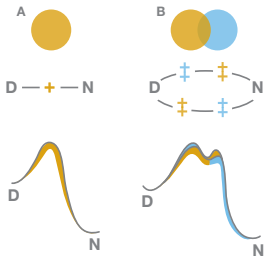


Lipid-Based Interactions

Analyze interactions of lipid bilayer membranes or membrane proteins with other biomolecules using SPR.

- Lipophilic surface chemistry

- ProteOn liposome capturing kit based on hydrophilic surface chemistry using DNA hybridization
- Hydrophilic surface chemistry for easy regeneration and high performance when capturing lipid assemblies
- ProteOn GLC lipid kit based on traditional lipophilic surface chemistry using alkyl modification; provides customized surface lipophilicity for optimal performance
- Real-time referencing for reliable experimental results

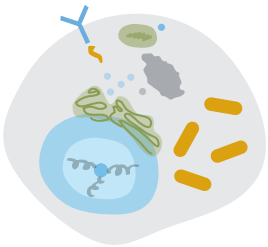


Thermodynamics and Energetics

Analyze thermodynamics to further characterize biomolecular interactions.

- ITC
- Conventional serial flow SPR

- Highly efficient thermodynamic analysis workflow for structural biology
- Experimental repeats in a single run for accurate thermodynamics and energetics



Cell Surface Interactions

Analyze the interaction between a target and a cell, including bacterial and mammalian cells.

- Flow cytometry
- Label-free cell morphology sensing
- Quartz crystal microbalance
- Conventional serial flow SPR

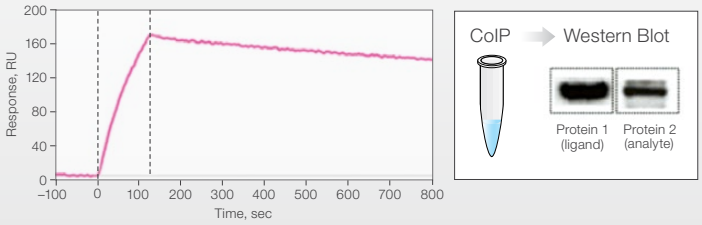
- Rapid label-free assays
- Efficient experimental optimization
- Flow channels compatible with cell samples to avoid clogging in the system

SPR–the Key Technology for Biomolecular Interaction Analysis

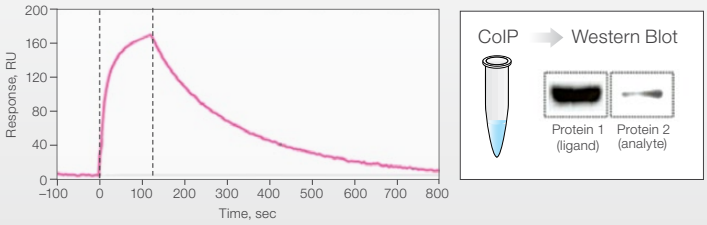
Interaction between biomolecules is of great interest in biological research. Understanding the whole set of biomolecular interactions in a cell, known as an interactome, lays the foundation of molecular and cell biology. There are different technologies available for biomolecular interaction analysis, which can be categorized as real-time analysis and end-point analysis.

- Real-time analysis technologies provide a complete time trace and kinetics of a biomolecular interaction
- End-point analysis technologies provide a readout after a biomolecular interaction takes place

A. Slow Off-Rate Biomolecular Interaction



B. Fast Off-Rate Biomolecular Interaction

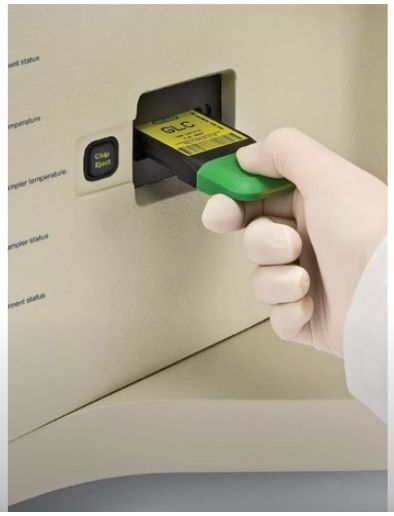


Real-time vs. end-point analysis. SPR technology analyzes all types of biomolecular interactions, including those with slow and fast off-rates. **A**, slow off-rate biomolecular interactions, which typically occur with strong binding affinity, can be measured by both SPR and end-point analysis methods, such as coimmunoprecipitation (CoIP)–western blot. In addition, SPR provides kinetics for further characterization. **B**, fast off-rate biomolecular interactions, which typically occur with weak binding affinity, can also be measured by SPR. However, these interactions are difficult to measure or even detect using end-point analysis methods because of their dissociation in rinsing steps. RU, response units.

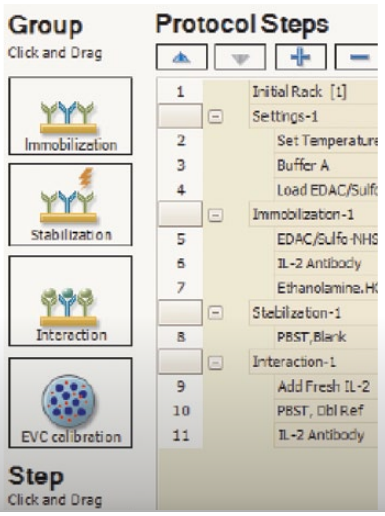
Workflow Using the ProteOn XPR36 System for an SPR Experiment

1 Start up system

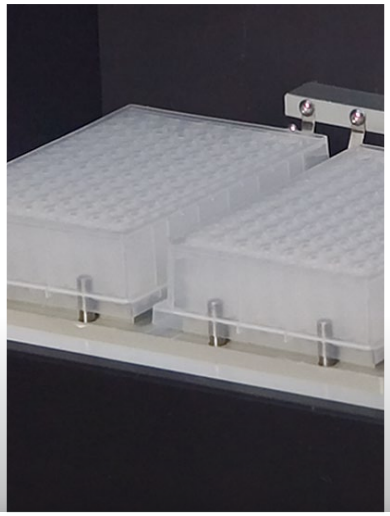
2 Load a sensor chip



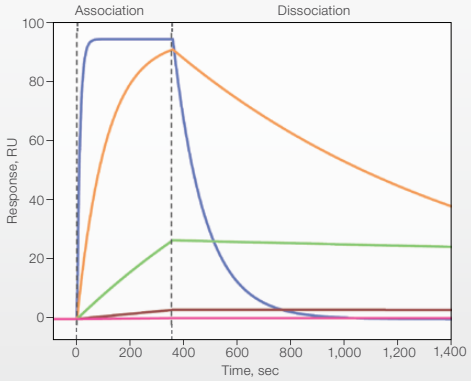
3 Create a protocol



4 Load samples

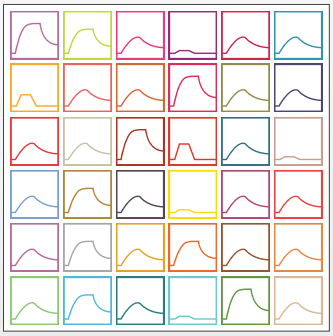


SPR, as a key technology in this field providing real-time biomolecular interaction analysis in a label-free manner, offers unique benefits that are not available with other technologies.



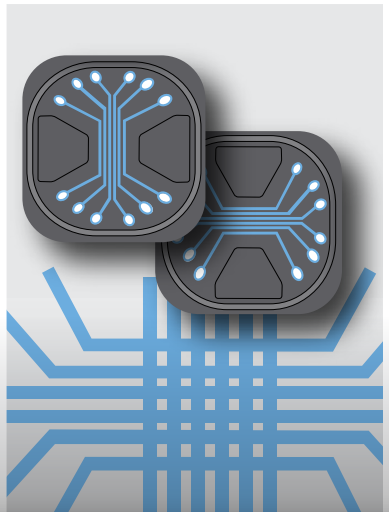
K_D = 1.0 nM [A] = 10 nM	
k_a ($M^{-1}sec^{-1}$)	k_d (sec^{-1})
1 x 10^3	1 x 10^{-6}
1 x 10^4	1 x 10^{-5}
1 x 10^5	1 x 10^{-4}
1 x 10^6	1 x 10^{-3}
1 x 10^7	1 x 10^{-2}

Kinetic analysis. Different biomolecular interactions with the same affinity (K_D) may have diversified binding kinetics (k_a and k_d). RU, response units.

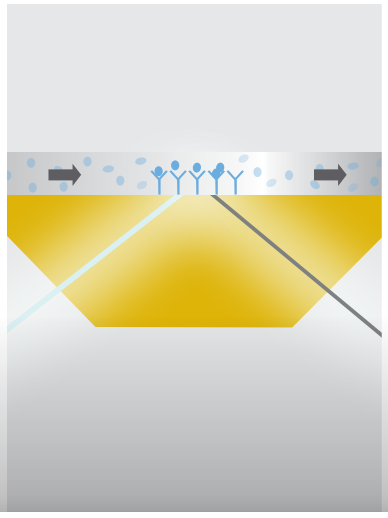


SPR technology is amenable to high-throughput platforms. The ProteOn XPR36 system features a 6 x 6 interaction array available for high-throughput applications.

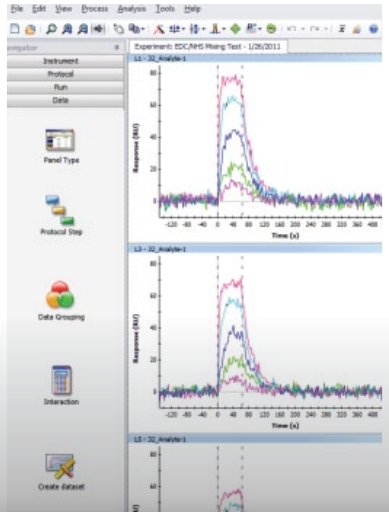
5 Run an experiment



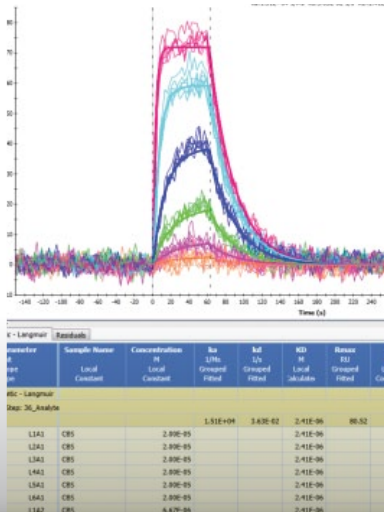
6 Collect data



7 Process data

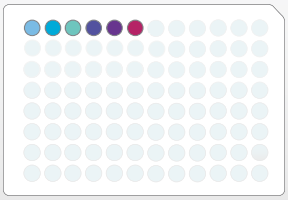


8 Analyze data

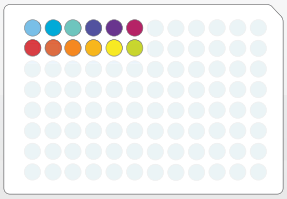


ProteOn XPR36 Protein Interaction Array System

SPR-based biosensors determine binding kinetics of protein-protein interactions by measuring refractive index changes on an optical surface. The ProteOn XPR36 system is a parallel-flow SPR biosensor platform featuring 36 interaction spots on a 6 x 6 array.



Apply up to 6 unique target molecules, such as mutant or wild-type proteins



Evaluate binding against 6 analytes, such as small inhibitor molecules

Benefits of 6 x 6 Array

Versatility – Multiple Experimental Configurations and Fast Qualitative and Quantitative Assays

Kinetic Characterization (1-to-1)

In kinetic characterization experiments, the optimization of experimental protocols is usually the most labor-intensive and time-consuming step. Probing at one time six ligand immobilization conditions together with six analyte injection conditions, the ProteOn XPR36 system allows for full optimization in a single run. This ensures the optimal experimental conditions for the interaction between the ligand and the analyte. The method of using a single run of 6 x 6 injections for a complete kinetic analysis is called One-shot Kinetics.

6 variations of a target

6 concentrations of the same analyte

Kinetic Screening (6-to-1)

In kinetic screening experiments, each of the six ligand channels gives a full kinetic analysis in a single run. This high throughput enables fast processing of a large number of samples while accurate kinetics is maintained. The ProteOn XPR36 system provides the best balance between throughput and accuracy of kinetic screening.

6 different targets

6 concentrations of the same analyte

Multiplex Screening (6-to-6) and Array Screening (36-to-1)

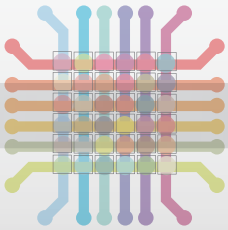
In multiplex or array screening experiments, the 6 x 6 interaction array of the ProteOn XPR36 system is fully utilized for high throughput, which enables multiplex or 36-ligand screening.

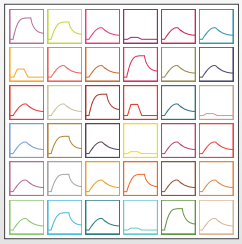
6 different targets

6 different analytes

36 different targets

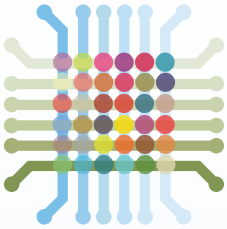
1 analyte





Productivity – Fast Protocol Optimization and High Throughput

Sensor chip surface



Kinetic Characterization

- ProteOn XPR36 system: 1.1 hr, 6 full kinetics (36 data points, surface regeneration not required)
- Conventional serial flow SPR system: 3.5 hr, 3 full kinetics (18 data points, surface regeneration required)

Kinetic Screening

Captured Ligand Screening (for mAb supernatants)

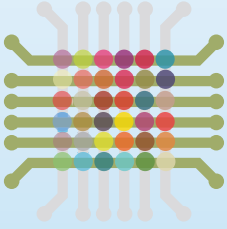
- ProteOn XPR36 system: 11 hr, 96 full kinetics (576 data points)
- Conventional serial flow SPR system: 65 hr, 96 full kinetics (576 data points)

Analyte Screening

- ProteOn XPR36 system: 25 hr, 96 x 6 full kinetics (3,456 data points)
- Conventional serial flow SPR system: 5.7 days, 96 x 3 full kinetics (1,728 data points)

Multiplex Screening

- ProteOn XPR36 system: 0.7 hr, 6 x 6 binning matrix (36 data points)
- Conventional serial flow SPR system: 2.5 hr, 3 x 6 binning matrix (18 data points)



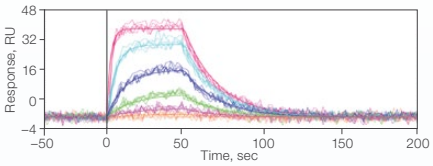
Array Screening

- ProteOn XPR36 system: 12 hr, 36 x 36 binning matrix (1,296 data points)
- Conventional serial flow SPR system: no equivalent

Four Factors for High-Quality SPR Results

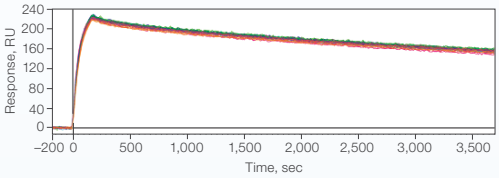
1 SPR System

Sufficient Signal to Noise Ratio



ProteOn XPR36 system signal-to-noise ratio. ProteOn XPR36 system noise is 1 RU and ~2 RU after double referencing. SPR responses over three times signal-to-noise ratio (3 x SNR) are detectable. RU, response units.

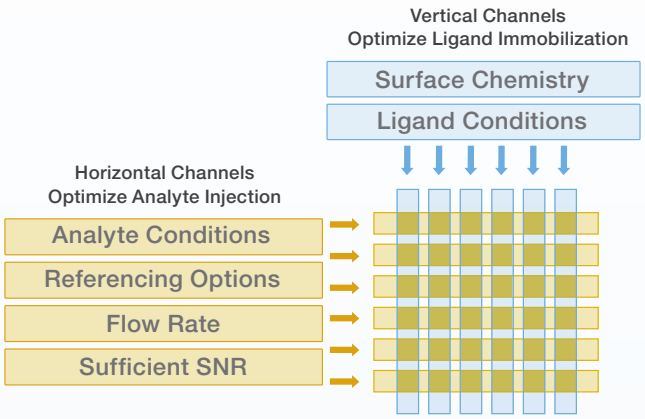
Instrument Stability



Evaluation of k_d value reproducibility using the ProteOn One-shot Kinetics kit. 2 systems x 3 chips x 6 ligand channels x 6 analyte channels = 216 sensorgrams. CV = 6.1% (over 2 systems and 6 sensor chips). RU, response units.

2 Experiment Design

XPR36 Configuration Optimizes Multiple Factors Simultaneously



Optimal experimental conditions are obtained in a single run.

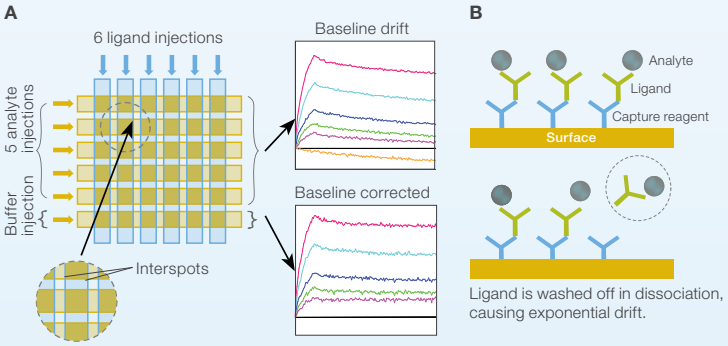
3 Data Processing

Data Referencing

The key step in data processing is data referencing. Data referencing corrects for the artifacts in SPR experimental results.

The ProteOn XPR36 system has two novel advantageous referencing modes that no other SPR system provides: an interspot reference to correct for refractive index change (bulk effect) and nonspecific binding, and a real-time injection reference to correct for baseline drift resulting from the changes of the ligand surface.

Note: For additional information about the referencing options in the ProteOn XPR36 system, watch **www.bio-rad.com/proteon/reference**.



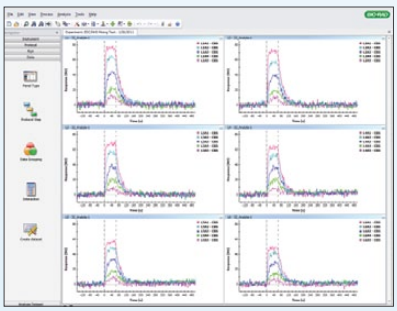
Novel ProteOn XPR36 system references. The ProteOn XPR36 system provides **A**, an interspot blank surface reference to save interaction spots and provide immediate approximate referencing and **B**, a real-time injection reference to correct the exponential baseline drift when using ligand-capture surface chemistry.

4 Data Analysis

Software Advantages

ProteOn Manager™ software is a comprehensive, user-friendly tool for the analysis of biomolecular interactions.

- Ease of use
- Integration of data acquisition, data processing, and data analysis
- Powerful graphic user interface
- Intuitive protocol writing interface
- Fast and accurate data processing
- Accurate fitting with 8 models
- Rapid data analysis
- Concise analysis reports
- Export functions for further data processing in Excel or other software

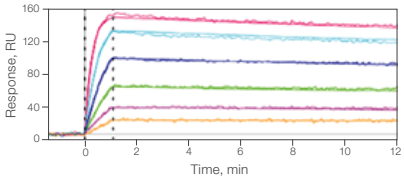


ProteOn Manager software data analysis window.

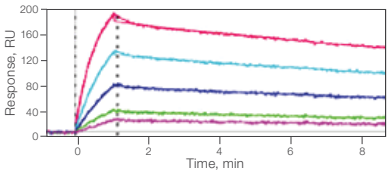
Surface Chemistries

Direct Coupling of Targets

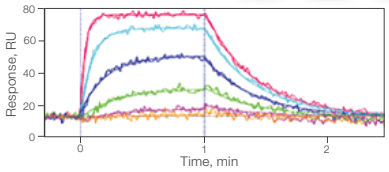
The ProteOn GLC, GLM, and GLH sensor chips are designed for direct coupling of proteins and petides, offering compact, medium, and high surface capacity levels, respectively.



ProteOn GLC sensor chip. The interaction analysis between cytokine IL2 and an anti-IL2 antibody was achieved using the compact-capacity GLC chip. RU, response units.



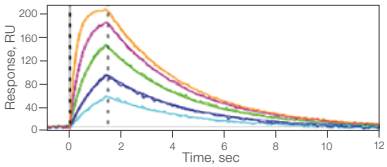
ProteOn GLM sensor chip. The interaction analysis between a TEM1 β-lactamase mutant and the β-lactamase inhibitor protein (BLIP) was achieved using the medium-capacity GLM chip. RU, response units.



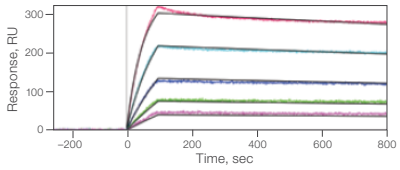
ProteOn GLH sensor chip. The interaction analysis between carbonic anhydrase II and an inhibitor carboxybenzenesulfonamide (MW 201) was achieved using the high-capacity GLH chip. RU, response units.

Capturing of Targets

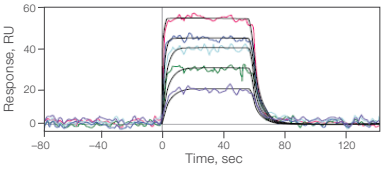
The ProteOn NLC sensor chip is designed for site-specific capturing of biotinylated biomolecules. ProteOn HTG and HTE sensor chips are designed for site-specific capturing of histidine-tagged proteins.



ProteOn NLC sensor chip. The interaction analysis between an antibody Fab fragment and biotinylated MHC I/Tyr was achieved using the NLC chip. RU, response units.



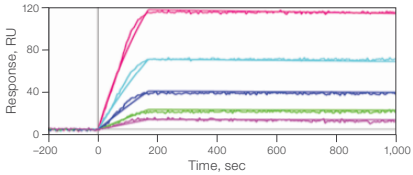
ProteOn HTG sensor chip. The interaction analysis between histidine-tagged protein A and human IgG was achieved using the HTG chip, showing its capability to resolve high-affinity kinetics requiring long dissociation time. RU, response units.



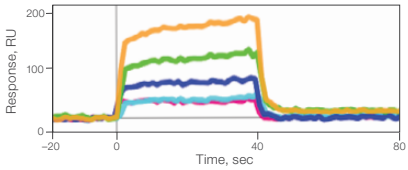
ProteOn HTE sensor chip. The interaction analysis between histidine-tagged ERK2 (a MAP kinase) and the inhibitor purvalanol B (MW 433) was achieved using the HTE chip, showing its capability to screen small molecules. RU, response units.

Capturing of Lipid Assemblies

The ProteOn LCP sensor chip together with the ProteOn LCP capturing reagent kit, and the ProteOn GLC sensor chip together with the ProteOn lipid modification kit are designed for capturing lipid assemblies for the analysis of lipid-bilayer membranes or membrane proteins.



ProteOn LCP sensor chip. The interaction analysis between FITC-labeled DSPC liposomes captured on the LCP sensor chip and an anti-FITC antibody was achieved using the LCP chip. RU, response units.



Modified ProteOn GLC sensor chip. The interaction analysis between POPC liposome and a small molecule drug, tetracaine (MW 264), was achieved using the modified GLC chip. RU, response units.

Specifications

Hardware	
Number of interaction spots	36
Experiment temperature range	15–40ºC
Dynamic range	1–40,000 RU absolute for all types of sensor chips, regardless of surface chemistry
Uniformity of response	>98% (CV <2%)
Baseline drift	<1 RU/min
Baseline noise	<1 RU
Sample flow rate	25–200 µl/min
Sample flow rate uniformity in 6 parallel channels	>99% (CV <1%)
Autosampler	Temperature-controlled sample rack for 72 x 2 ml sample vials or two 96-well plates
Syringe pumps	6 sample and 6 buffer syringes with each set operated in unison for uniform and stable flow rate
Sensor chip detection	Automatic bar code recognition of sensor chip type, expiration date, and previous experiment
Software	
PC operating system	Windows XP or Windows 7
Program	ProteOn Manager software
Data-fitting models	Langmuir, Langmuir off-rate analysis, Langmuir with mass transfer, heterogeneous analyte, bivalent analyte, heterogeneous ligand, two states, Langmuir with drift
GXP (optional)	21 CFR Part 11; IQ/OQ software tools
Typical Working Ranges	
Molecular mass detection limit	Typically >95 Da
Sample concentration	Typically >10 pM
Association rate constant (k_a)	Typically 3 x 10^3–3 x 10^6 $M^{-1}sec^{-1}$
Dissociation rate constant (k_d)	Typically 1 x 10^{-6}–6 x 10^{-1} sec^{-1}
Equilibrium constant (K_D)	Typically 2 x 10^{-4}–1 x 10^{-12} M

Ordering Information

Catalog #	Description
ProteOn XPR36 System and Software	
176-0100	**ProteOn XPR36 Protein Interaction Array System**, 100–240 V, includes ProteOn XPR36 instrument, 2 licensed copies of ProteOn Manager software, controller and display, communication cable, sample rack, rack needle set, microplate needle set, collection tank, choice of 2 sensor chips, One-shot Kinetics kit, maintenance kit, 2 bottles of PBS/Tween running buffer, chip normalization solution, 100 sample vials, 25 microplates with standard wells, 50 sheets of microplate sealing film, instruction manual
176-0200	**ProteOn Manager Software**, 1-user license, includes 1 HASP key
176-0210	**ProteOn Manager Software, Security Edition**, allows U.S. FDA 21 CFR Part 11 compliance, 1-user license, includes 1 HASP key
Sensor Chips	
176-5011	**ProteOn GLC Sensor Chip**, for general amine coupling, compact polymer matrix layer with binding capacity of approximately 1 protein monolayer
176-5012	**ProteOn GLM Sensor Chip**, for general amine coupling, polymer matrix layer with intermediate binding capacity
176-5013	**ProteOn GLH Sensor Chip**, for general amine coupling, polymer matrix layer with highest binding capacity
176-5021	**ProteOn NLC Sensor Chip**, for binding of biotinylated molecules, contains NeutrAvidin immobilized to GLC layer
176-5031	**ProteOn HTG Sensor Chip**, for capturing histidine-tagged proteins, polymer matrix layer contains tris-NTA complexes with compact binding capacity
176-5033	**ProteOn HTE Sensor Chip**, for capturing histidine-tagged proteins, polymer matrix layer contains tris-NTA complexes with higher binding capacity
176-5041	**ProteOn LCP Sensor Chip**, for capturing lipid assemblies such as liposomes, for use with ProteOn LCP capturing reagent kit
Sensor Chip Application Kits	
176-2300	**ProteOn Liposome Capturing Kit**, includes 1 ProteOn LCP sensor chip, 1 ProteOn LCP capturing reagent kit, and ProteOn lipid modification conditioning solution
176-2350	**ProteOn GLC Lipid Kit**, includes 1 ProteOn GLC sensor chip and 1 ProteOn lipid modification kit
176-2500	**ProteOn HTG Capturing Kit**, includes 1 ProteOn HTG sensor chip and 1 ProteOn HTG and HTE reagent kit
176-2600	**ProteOn HTE Capturing Kit**, includes 1 ProteOn HTE sensor chip and 1 ProteOn HTG and HTE reagent kit
Reagent Kits	
176-2110	**ProteOn Immobilization Buffer Kit**, includes 1 each sodium acetate buffer (pH 4.0, 4.5, 5.0, 5.5)
176-2210	**ProteOn Regeneration and Conditioning Kit**, includes 1 each glycine buffer (pH 1.5, 2.0, 2.5, 3.0), and NaOH, SDS, HCl, phosphoric acid, NaCl; 50 ml solution each
176-2310	**ProteOn LCP Capturing Reagent Kit**, for capturing lipid assemblies such as liposomes, for use with ProteOn LCP sensor chip
176-2360	**ProteOn Lipid Modification Kit**, includes ProteOn lipid modification conditioning solution and ProteOn lipid modification solution
176-2410	**ProteOn Amine Coupling Kit**, includes EDAC (EDC), sulfo-NHS, and ethanolamine HCl
176-2510	**ProteOn HTG and HTE Reagent Kit**, includes reagents for activation and regeneration of HTG and HTE sensor chips

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