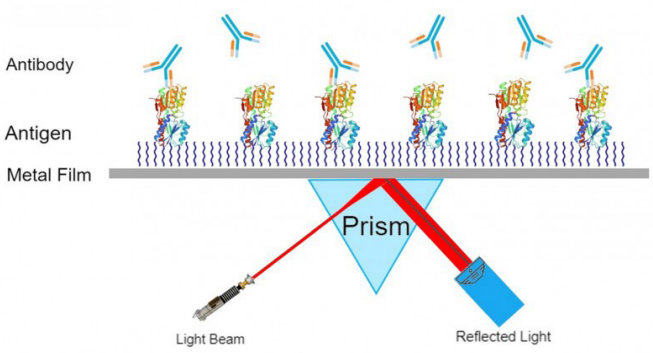
Affinity Measurements by Surface Plasmon Resonance (SPR)

Surface Plasmon Resonance (SPR) is the process of using surface plasmons (an electromagnetic surface wave) to sensitively measure the angle of minimum reflection of absorbed molecules such as proteins on a detector surface. This angle changes measurably based upon the molecules absorbed onto the surface changing the resonance conditions of the surface plasmon waves. Due to this highly sensitive measurement the number of molecules bound can be accurately characterized. CovalX utilizes SPR for high-throughput, real-time, label-free affinity and kinetic characterizations on a range of sample types including monoclonal antibodies, proteins, and small molecules.



Analysis Services offered with SPR:

Drug Discovery

CovalX provides the following antibody related services via SPR analysis:

- Monoclonal antibody pairing by "epitope binning" to identify and group different mAbs
- Full kinetic analysis/ ranking by on-rates (k_a), off-rates (k_d) and affinity constants (K_D)
- Antibody screening against label-free antigen directly in solution

Common Ligand to Analyte Assay Formats:

- Antigen and Antibody Fragment Characterization
- Protein to Protein or Peptide Interactions
- Protein to DNA or RNA Interactions
- Protein to Lipid Interactions
- Protein to Small Molecule Interactions

Type of analyzed data available:

- Kinetics/affinity characterization or screening (k_a/k_d)
- LMW interaction analysis
- Fragment screening
- Immunogenicity
- Concentration analysis
- Comparability

Why is CovalX's SPR Services the Best Option?

- **Expertise:** Experienced team analyzing protein interactions in the industry for more than 15 years.
- **Latest Equipment:** CovalX uses only the latest SPR instrumentation and software.
- **Quality Work:** Advanced initial intact screening of proteins helps provide the most reliable rate of success.
- **Affordable:** Competitive cost compared with other technologies.
- **Low Sample Consumption:** Only 75µg of each protein is required.

Additionally, CovalX offers unique mass spectrometric based verification of individual proteins and protein interactions as part of our quality control of sample received for SPR analysis.

Lead Optimization

A lead compound's pharmacokinetics and pharmacodynamics (PK/PD) are directly affected by the compound-target on-rate (k_a) and off-rate (k_d). Kinetic evaluation of a lead molecule by SPR ensures in vivo selection of compounds on conditions that are related to target binding and target selectivity. Characterization of biotherapeutic candidates and label-free screening using SPR are able to adjust early in vitro ADME analyses.

Additionally, CovalX offers a more in-depth characterization of lead compound binding sites through our HDX-MS analytical service.

Target and Compound Characterization

Drugs targeting proteins and protein interactions continue to be a large focus for research and development. SPR can offer a unique solution to measure these challenging proteins in their native states. Rapid characterization of the interactions for drug compounds with a target protein is crucial before optimization when numerous potential 'lead compounds' must be selected. SPR offers an approach that enables the direct determination of the interactions between unlabeled compounds and the targets, providing a trusted and rapid method for compounds characterization.

How SPR Works

Unlike mass spectrometry which measures the mass of molecules, SPR measures molecular adsorption on a surface. This adsorption can be due to binding or interaction of protein interactions such as ligand binding, or in the case of antibodies, binding their target antigen. SPR characterization has become

an established analysis technique within the biotechnology and pharmaceutical industries.

This technology can be applied to identify the interactions of almost any molecular system. SPR can detect minute changes in the refractive index of plasmon waves close to the surface through which they pass. SPR provides a real-time analysis with no sample labeling required. The absence of sample labeling decreases the time demanded to prepare samples for determination and eliminates the concern that a tag may influence the reaction. Real-time monitoring makes it feasible to measure detailed information about binding events, including the association and dissociation reaction kinetics.

Timeline, Sample Requirements and Resolution

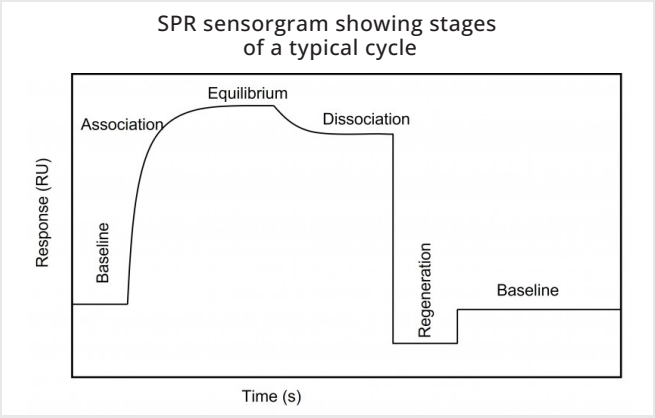
- **Sample Consumption:** 75µg of each binding protein.
- **Cost:** CovalX offers upfront competitive pricing on a per-project basis.
- **Affinity range:** fM to mM binding
- **Dissociation rate constant (k_d):** 10-5 to 1 s-1
- **Sample type:** Small-molecule drug candidates to high-molecular weight proteins (also DNA, RNA, polysaccharides, lipids, VLPs) in various sample environments (e.g., in DMSO-containing buffers, plasma, and serum)

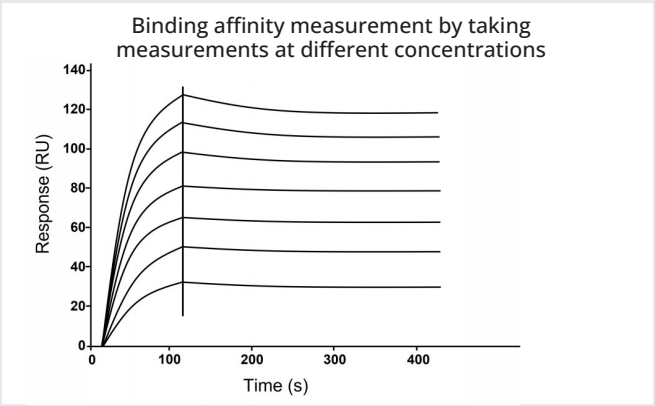
Notable Benefits with CovalX SPR Services

1. Initial Mass Spectrometric characterization of intact protein provides robust screening before beginning interaction experiments.
2. Expertise: Experienced team analyzing protein interactions in the industry for more than 15 years.
3. Latest Equipment: CovalX utilizes the latest SPR instrumentation and analysis software available.
4. The interaction is analyzed in solution in native conditions, with the accuracy of SPR.

Related CovalX Services for Protein Interaction:

- Epitope Mapping
- Biosimilar characterization
- Conformational/Protein dynamics
- Protein-Protein interactions
- Protein folding characterization





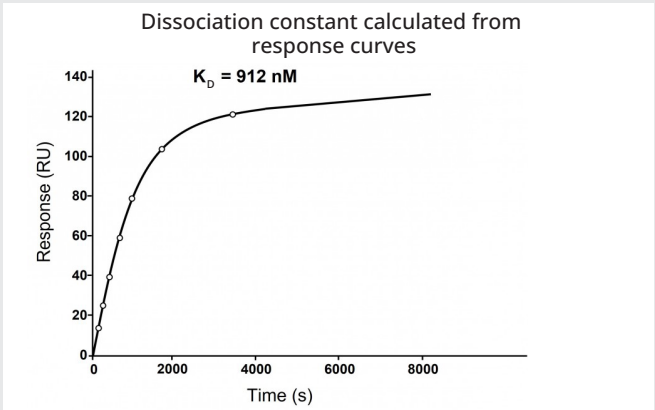


Figure 1. How Surface Plasnom Resonance Works
By varying the concentration of the analyte being measured, accurate affinity measurements can be determined from multiple sensorgrams. Using multiple sensorgrams, an accurate dissociation constant (K_D) can be calculated for a given reaction.

CovalX
Solutions for Protein Complex Analysis

Switzerland | Schützengasse 23, Zürich CH-8001 | +41 44 585 3964
United States | 12 Gill Street, Suite 4850, Woburn, MA 01801 | +1 (617) 297 2263
France | 2, Allée Ulysse Gayon, 33650 Martillac | +33 7 66 86 59 50
www.covalx.com | info@covalx.com