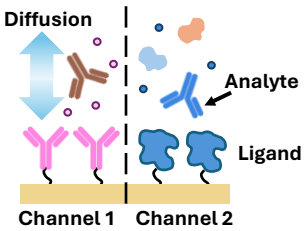
Cheat Sheet

# Static and Kinetic SPR

## Main Differences

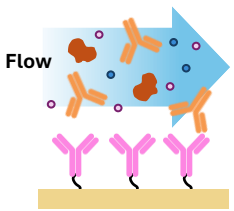
### Static SPR




**Purpose:** Scouting or screening prior to Kinetic SPR, yes/no binding

- Like label-free ELISA: ligand-analyte interaction depends on **diffusion, concentration,** and binding **strength** is measured as **Apparent $K_D$**
- **Concentration determination** of active analyte in complex media (e.g. crude lysate)
- **Quick verification** of protein expression
- **Contact time ~ seconds to hours** so **slow interactions** can be measured with Static SPR
- Low viscosity buffers and biomolecules **10-500 kDa** are recommended[2]
- Up to 4 independent experiments (4 channels) for P4 SPR device[1]

### Kinetic SPR

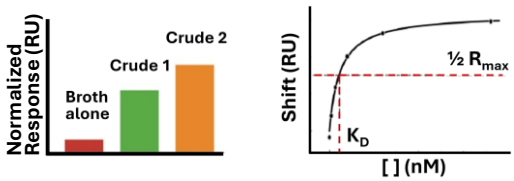



**Purpose:** Sensitive, quantitative binding characterization

- **Constant flow** of analyte enables ligand-analyte interaction so both **strength** and **rate** of binding are measured as **Quantitative $K_D$**
- Can transfer Static SPR conditions to Kinetic SPR
- Due to controlled flow, Kinetic SPR can accommodate a broader range of molecule sizes (100+ Da)
- Suitable for **low analyte concentrations**, better real-time sensitivity

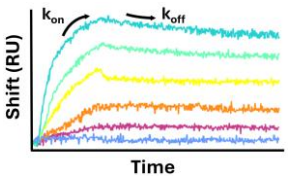
## Data Output Differences

### Static SPR




- Association without dissociation
- **End-point** response units are analyzed
- Like ELISA, **Apparent $K_D$** can be estimated at ½ $RU_{max}$ determined by concentration titration

### Kinetic SPR




- Both association and dissociation
- **Quantitative $K_D$** calculated by $k_{off}/k_{on}$
- Steeper upward curve → faster association
- Steeper downward curve → faster dissociation

| | Static | Kinetic |
|---|---|---|
| **Use Case** | Screening, end-points | Real-time binding |
| **Binding parameters** | Apparent $K_D$ | $k_{on}$, $k_{off}$, $K_D$ |
| **Concentration determination** | Yes | Yes |
| **Analyte types & average size range** | Peptides, antibodies, protein complexes (10 kDa – 500 kDa)[2] | Small Molecules, peptides, antibodies, nanoparticles, vesicles (100 Da – 500+ kDa) |
| **No. of experiments** | Up to 4 independent experiments[1] | 4 (Up to 2 independent experiments) |
| **Amount of ligand** | > 10 µg | > 10 µg |
| **Sample injection type** | Manual | Semi-automated |
| **Average assay time** | 5 – 15 mins | 5 – 20 mins |
| **Methods used alongside SPR** | Western blot, ELISA, immunoprecipitation assay, isothermal calorimetry, biolayer interferometry, fluorimetry, atomic force microscopy | |

[1]Four different conditions (media, surface chemistries, ligands, etc.) in one setup using Affinité's P4SPR device
[2]In some cases, small molecules and large nanoparticles can also be used - contact us for details