Antibody binding analysis to compare static and kinetic SPR characteristics

Introduction

Surface plasmon resonance instruments provide a wealth of information such as kinetics, affinity, and concentration. Two types of setups allow SPR analysis static and kinetic. In a static system such as the P4SPR, the sample solution is introduced manually by syringe into the flow cell and interacts with the surface through diffusion. In a kinetic system, a sample loop is loaded (by manual syringe or autosampler), and the sample is introduced in the flow cell by flowing running buffer through the sample loop via a syringe pump.

This tech notes characterizes biomolecular interactions between immobilized polyclonal mouse anti-human IgG (MAH IgG) and whole human IgG (Hm IgG) with static and kinetic Affinite Instruments SPR devices.

Let us start by defining basic affinity and kinetic parameters. Using a basic Langmuir binding model assuming a 1:1 interaction where immobilized ligand (L) interacts with an analyte (A).

$$A + L \rightleftharpoons AL \qquad (1),$$

the rate constants (k_{on} and k_{off}) represent the forward (association) and backward (dissociation) reactions, respectively. The dissociation equilibrium constant, KD, which indicates when half of the surface-immobilized ligands are bound to analytes, can be expressed in terms of the concentrations of free analyte [A], ligand [L], and analyte-ligand complex [AL] (Eq. 2). Alternatively, K_D can be defined using the rate constants k_{on} and k_{off} (alternatively, k_a and k_d) (Eq. 2):

$$K_D = \frac{[A][L]}{[AL]} = \frac{k_{off}}{k_{on}} \qquad (2)$$

KD is often preferred as a parameter to solve for, as it is expressed in molarity (M), while the association equilibrium constant KA is expressed in M-1.

P4 Static vs Kinetic

Both kinetic and static analyses provide valuable information in different ways.

Kinetic analysis allows us to understand the dynamics of molecular interactions by providing insights into the speed and strength of binding. It helps study the mechanisms of biological processes and develop targeted therapies. By monitoring interactions in real-time, we can evaluate association and dissociation rates and derive affinity constants.

On the other hand, static analysis is well-suited for screening and quantification of analytes. It provides a snapshot of binding at a specific time point, allowing for rapid analysis of large sample sets. Assay times in static SPR can be up to half the time as kinetic SPR due to the simplified sample introduction process, quicker baseline establishment, and efficient system flushing. This makes static SPR a time-efficient choice for specificity screening, analyte quantification, and basic affinity characterization.

By utilizing both kinetic and static analyses, we can obtain a comprehensive understanding of molecular interactions. Kinetic analysis helps us uncover the dynamic aspects of binding, while static analysis allows for efficient screening and quantification. Together, they provide a holistic view of the binding process, from the initial association to the steady-state binding levels.

Table. 1. Comparison of features between static and kinetic modes of SPR experiment

SPR Applications	Static	Kinetic
Live binding data	☑	☑
Specificity Screening/ ranking	☑	☑
Concentration Analysis	☑	☑
Affinity characterization (K_D)	☑	☑
Kinetics characterization (k_{on} k_{off})		☑
Simultaneous analysis of 4 samples	☑	
Single and multi cycle analysis	☑	☑

Instrumentation

Figures 1 and 2 display Affinité instrument devices used in kinetic and static analysis. These instruments are designed for simplicity and ease of use in conducting various assays. With intuitive controls and user-friendly interfaces, researchers can efficiently perform screening, quantification, and basic affinity characterization of analytes. The instruments streamline the process, enabling researchers to obtain valuable data quickly and accurately.

In both Affinité instrument devices, the sample solution is introduced into the device through manual syringe injection. In the static P4SPR 2.0 system, the sample is directly introduced from the syringe inlet into the flow cell (Figure 1). In the P4PRO kinetic system, the sample solution is manually injected into a sample loop and can be flowed through the flow cell using the Affipump (Figure 2).

Assay Design

Two analogous assays were run under the same conditions in static and kinetic systems. The Affinité Instruments MHDA sensors were first activated by injecting a 1:1 (v/v) mixture of 30mM EDC and 100mM NHS for 4 min. The capture reagent mouse anti-human IgG was prepared at 5ug/mL in 10mM sodium acetate adjusted to pH 4.5 and injected manually for a period of 30 seconds in sample channels. Both activated surfaces were then deactivated by a 6-minute injection of 1M ethanolamine pH 8.0.

A similar level of MAH IgG was immobilized over 2 MHDA surfaces (114RU, 61RU). Sample solutions of the same concentrations were processed by the P4SPR 2.0 for the static analysis and by the P4PRO for the kinetic analysis.

Fig. 1. P4SPR 2.0 Diagram

Different concentrations of human IgG (0.889-72nM, three-fold serial dilution) were prepared in buffer containing PBS 1x + 0.1% Tween-20, pH 7.4 and injected for 600s in the P4SPR 2.0 at an approximate flow rate of 100uL/s and for 150s in the P4PRO at a flow rate of 50uL/min (0.833uL/s). Sensor surfaces were regenerated between sample injections with 10 second injections of 10mM glycine hydrochloride adjusted to pH 2.0. The control used in both static and kinetic assays were a surface activated and deactivated under the same conditions where the same analyte solutions were flowed.

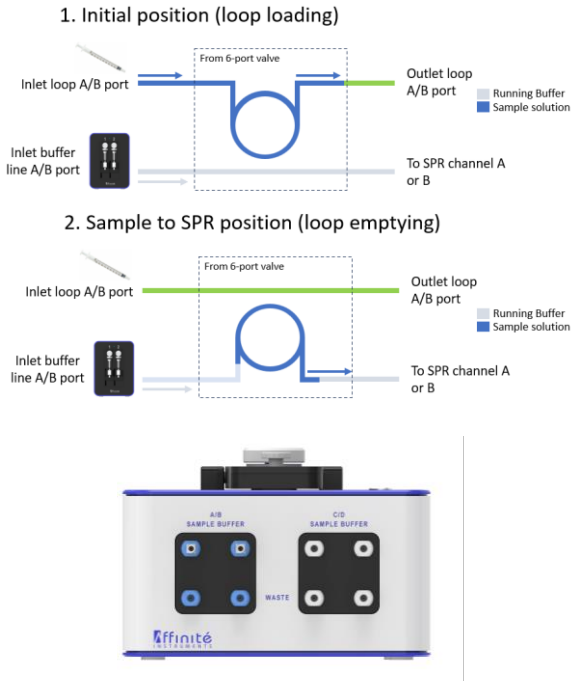


Fig. 2. P4PRO Diagram

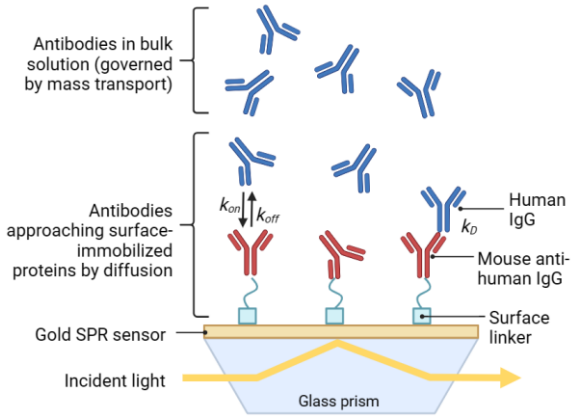


Fig. 3. Scheme of binding and molecular events.

Results

In static SPR only the association phase is observable. A typical graphical overlay is represented in figure 4. The signal gradually increased as the analyte binds to the ligand immobilized on the surface. Once most of the available ligand sites are occupied, the signal reaches a plateau called the steady state where the is equilibrium between ligand and analyte association and dissociation. The shape and kinetics of the curve can also provide qualitative information about the binding affinity, association rate and stoichiometry of the analyte-ligand interaction. SPR shift values were plotted as function of concentration in figure 5 and a curve fitting was obtained via Affinité's ezControl software. With this curve fitting Rmax was evaluated at 63RU and the concentration at ½ R_{max} (K_D) as evaluated at 4.9nM.

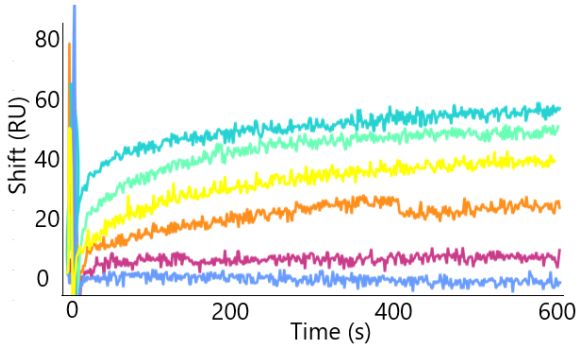


Fig. 4. Compilation of static SPR sensorgram of Hm IgG of different concentrations (0, 0.89, 2.7, 8.0, 24, 72nM) binding with immobilized MAH IgG adjusted with reference channel.

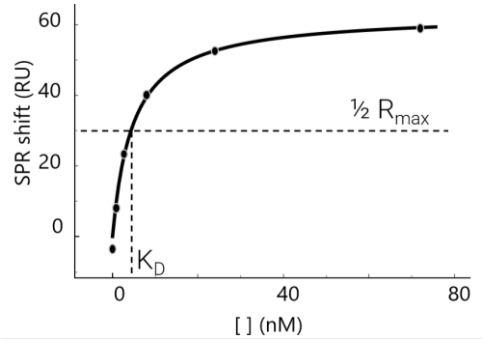


Fig. 5. Static evaluation of K_D in ezControl software obtained by plotting SPR shift in RU vs concentration in nanomolar.

In kinetic SPR both association and dissociation phases are observable. A graphical overlay of kinetic injections of Hm IgG is presented in figure 6. During the association phase, the analyte is injected and allowed to bind to the ligand immobilized on the surface. As the binding occurs, the signal on the SPR sensorgram gradually increases. The rate at which the signal increases reflects the association rate constant (ka), which indicates how quickly the binding occurs. The association phase allows us to monitor the formation of the analyte-ligand complex in real-time.

Following the association phase, the dissociation phase begins. In this phase, the analyte is replaced by the running buffer, and the signal on the sensorgram starts to decrease. The rate at which the signal decreases reflects the dissociation rate constant (kd), which indicates how quickly the analyte-ligand complex dissociates. The dissociation phase allows us to observe the stability of the binding and measure the dissociation rate.

The shape of the curves in SPR depends on the binding kinetics and the affinity between the analyte and ligand. In a typical binding scenario, during the association phase, the signal on the sensorgram increases rapidly until it reaches a plateau, indicating that most of the available ligand sites are occupied. The rate at which the signal reaches the plateau depends on the association rate constant (ka) and the concentration of the analyte.

A tracedrawer fitting of the kinetic data is represented I figure 7: it denotes two joined segments representing on-rates and off-rates. A relatively steep on-rate slope suggests quick association and a flatter dissociation slope suggests a slower off-rate, indicating strong antibody binding. Conversely, a steeper downside would indicate a faster off-rate and weaker antibody binding.

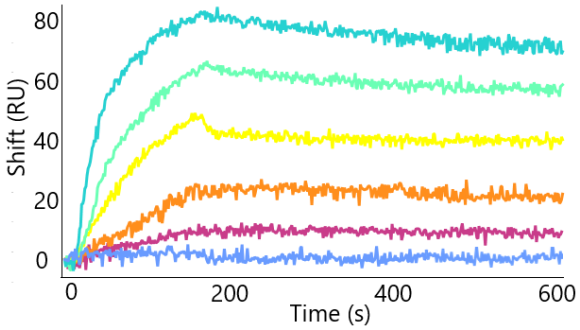
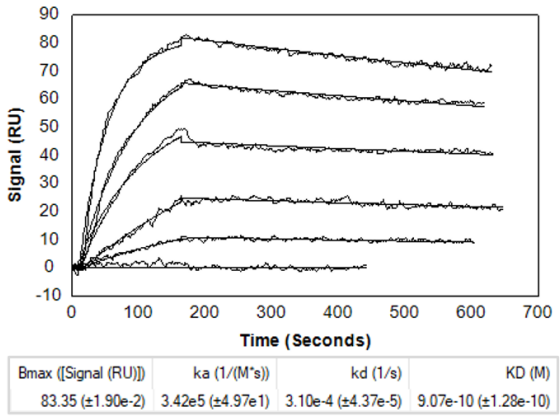


Fig. 6. Compilation of kinetic SPR sensorgram of Hm IgG of different concentrations (0, 0.89, 2.7, 8.0, 24, 72nM) binding with immobilized MAH IgG adjusted with reference channel and blank.



Bmax ([Signal (RU)])	ka (1/(M*s))	kd (1/s)	KD (M)
83.35 (±1.90e-2)	3.42e5 (±4.97e1)	3.10e-4 (±4.37e-5)	9.07e-10 (±1.28e-10)

Fig. 7. Evaluation of kinetic parameters via TraceDrawer

Parameters	***Static SPR***	***Kinetic SPR***
K_D *(nM)*	5	1
K_{on} *($M^{-1}*s^{-1}$)*	NA	3.42×10^5
K_{off} *(s^{-1})*	NA	3.10×10^{-4}
R_{max} *(RU)*	63	83

Table 2. Evaluation of kinetic parameters via TraceDrawer (NA: non-applicable)

Table 1 provides a comparison of various parameters between static SPR and kinetic SPR. In kinetic SPR, the analyte-ligand interaction shows an association rate (kon) of 3.42 x 10^5 M-1*s-1, and a dissociation rate (koff) of 3.10 x 10^-4 s-1 resulting in an affinity (KD) value of 1 nM and a maximum binding response (Rmax) of 83. In static SPR, kon and koff rates are not observable, the Rmax was evaluated at 63RU resulting in a KD evaluation of 5nM. A longer analyte on-time in the static system would likely result in a higher Rmax projection and therefore a lower, more correlated KD value relative to the kinetic analysis.

Summary

These parameters obtained from kinetic SPR provide valuable insights into the biomolecular interactions. On the other hand, static SPR offers a simpler and more cost-effective approach for screening and quantification of analytes.

While static SPR provides fewer parameters compared to kinetic SPR, it can still yield similar results in a shorter amount of time. The simplified sample introduction process, quicker baseline establishment, and efficient system flushing in static SPR contribute to its time efficiency. Additionally, the simplified system design and lower cost of static SPR instruments make them more accessible and affordable for many researchers.

In summary, kinetic SPR provides a comprehensive set of parameters to characterize biomolecular interactions, including affinity, association rate, dissociation rate, and maximum binding response. On the other hand, static SPR can provide some of these parameters, such as affinity, with similar results in a quicker, simpler, and more affordable system. The choice between kinetic and static SPR depends on the specific experimental requirements and the information sought from the analysis.

About Affinité Instruments

Established in 2015 as a spin-off of the Université de Montréal, Affinité instruments' foundation is built on deep knowledge accrued throughout more than a decade of research in SPR. The commercialization of promising innovations is spearheaded by a leadership team experienced in business, science and engineering.