Achieving data-driven decisions with real-time interaction analyses

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Biacore™ systems for label-free interaction analysis

Achieving data-driven decisions with real-time interaction analyses

Introduction

Biological processes are "real-time" events, driven and regulated by dynamic interactions between key molecules.

End-point techniques such as ELISA offer a snapshot view of interactions providing only basic information, such as overall binding strength (affinity). The affinity depends on the ratio of on- and off-rates so that equal affinity interactions can have very different kinetic properties, resulting in different biological responses.

Biacore SPR systems can provide key data in real-time to discriminate these crucial differences, even for interactions where challenging targets are involved.

Biacore systems are designed to help you to generate decisive, information-rich data in real time. This information will help answer the following key questions concerning the nature of the binding.

How strong?

Affinity is a steady-state measurement made at equilibrium of a binding event and reflects the strength of an attraction between molecules.

How fast?

Binding kinetics determine how fast/slow a complex forms or dissociates within a given time span and allow calculation of association and dissociation rate constants.

How much?

Qualitative and quantitative determination of active analyte binding to a target protein.

How specific?

Is the molecule specific for its target? Does the antibody recognize multiple derivatives? The flexibility in the Biacore assay design allows rapid assessment of cross-reactivity and specificity.

Data-driven understanding

The high information data provided by label-free interaction analysis enables scientists to fully understand binding events between almost any types of biologically relevant interactants.

- Understand the relationship between molecular interaction and function
- Screen for hits and optimize leads based on selectivity, affinity, and kinetics
- Examine interactions of ions, small molecules, and multidomain proteins or viruses with targets
- Screen and characterize antibodies and proteins based on yes/no binding, affinity, and kinetics from the fastest on-rates to the slowest off-rates
- Quantitate protein by measuring the concentration of active protein with retained biological function

SPR principle

During SPR analysis, one of the interacting molecules is immobilized on a sensor surface, while the potential interacting partner flows over the sensor surface in solution. Interactions between the two are detected in real-time through changes in mass concentration close to the sensor surface. Binding data is presented in a sensorgram, where SPR responses in resonance units (RU) are plotted versus time (Fig 1).

Advantages of kinetics

Interactions characterized by similar affinities can have very different kinetic properties, resulting in different biological responses. By resolving affinity into on- and off-rates, comprehensive information is obtained on how the dynamics of molecular interactions relate to protein function. On-rates reflect recognition between interacting partners while off-rates indicate stability of the complex.

This information provides an extra dimension which can be crucial in supporting hit-to-lead development (Fig 2).

Kinetic properties play an important role in pharmacokinetics for drug development. A compound that shows rapid binding will have a quick effect, but if dissociation is also fast the effect will be short-lived. On the other hand, a compound with slower binding kinetics might need more time to reach full effect but will not need to be given as often.

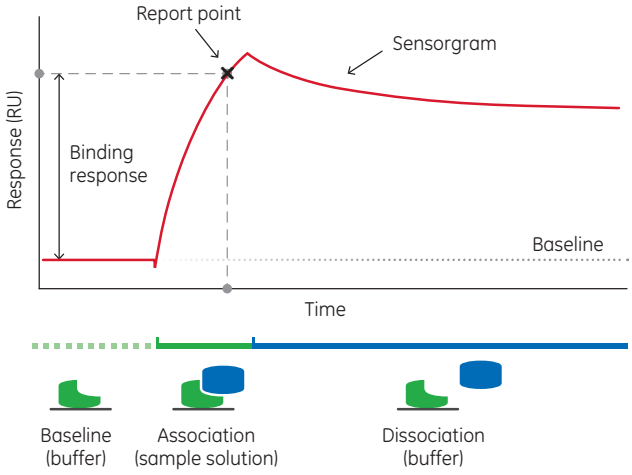

Fig 1. The sensorgram provides real-time information about binding profiles with binding responses measured in resonance units (RU). Association: interactions in solution bind to molecules on sensor chip surface. Dissociation: Binders allowed to dissociate from molecule on sensor chip surface. Any remaining bound sample molecules may be removed in a regeneration step that prepares the surface for the next sample injection.

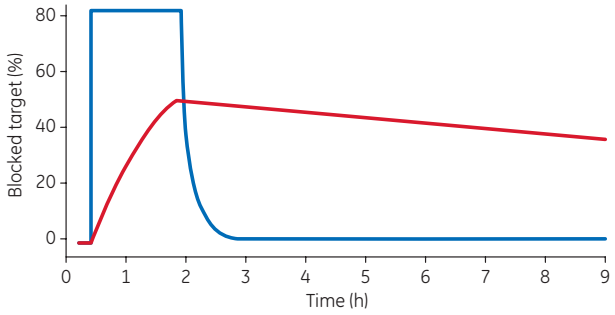

Fig 2. Blue sensorgram illustrates rapid kinetics. Frequent administration of low dose is required to block target. Red sensorgram illustrates slow kinetics. Infrequent administration of high dose blocks target long after injection.

Importance of outstanding SPR sensitivity for reliable binding results

Full characterization of antigen-antibody interactions is of great importance when assessing the suitability of antibodies as therapeutic or diagnostic tools. Ranking of strong binders can be complicated by avidity effects, and the dissociation rate will appear slower than in reality. To clearly differentiate strong-binding antibodies in terms of dissociation rates, it is necessary to use low levels of immobilized binding partner to obtain clean, avidity-free interaction studies. Obtaining accurate data from the low immobilization levels requires a highly sensitive SPR sensor for the analysis. Sensitivity and low baseline noise are equally important to reliably detect and profile interactions involving very small compounds or targets with low activity levels. High sensitivity with low noise enables resolution of sensorgrams at sub-resonance unit levels, exemplified in Figure 3.

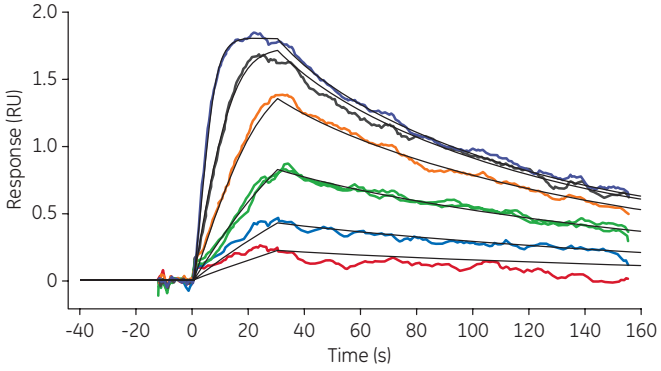

Fig 3. The high sensitivity of Biacore 8K enables confident analysis of fast on-rates. Sensorgram showing binding of melagatran to thrombin: k_a 4.0 × 10^7 M^{-1} s^{-1}; k_d 0.014 s^{-1}.

Conclusion

Understanding the nature of interactions between molecules is fundamentally important for increased understanding of biological processes. The sensitivity and performance of Biacore systems provide high-quality molecular interactions data in a range of fields, including research, drug discovery, development, and quality control.

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