Kinetics –

or the importance of rate constants in biological and pharmaceutical research

When investigating molecular interactions, equilibrium dissociation constants (K_D) are commonly used as a measure of the affinity of two molecules for each other. In other words, the K_D is a direct measure of the strength of an interaction. Determining the K_D is very important to evaluate the biological relevance of an interaction, such as in the study of fusion proteins, DNA-protein binding and for standard protein analysis.

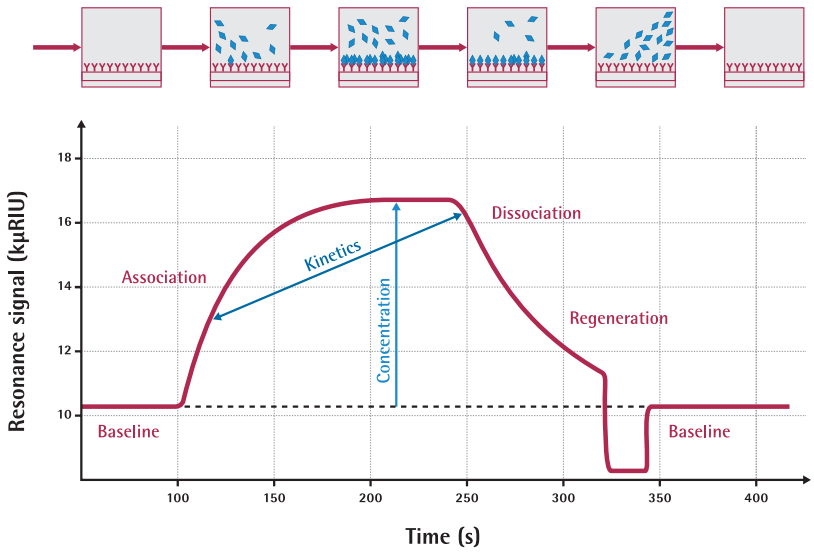


Fig. 1: Sensorgram of a typical surface plasmon resonance (SPR) measurement.
One of the interactants is immobilized on the sensor surface, while the others are free in solution and pass over the surface. A typical cycle of measurement contains association, steady-state and dissociation phases, eventually followed by regeneration; these phases are displayed in the sensorgram.

However, obtaining only a K_D value does not provide independent information on the kinetics of the molecular interaction. In other words, K_D does not tell you how quickly the two molecules bind (i.e., the association rate constant or "on-rate" of the interaction), or how quickly the molecules dissociate (i.e., the dissociation rate constant or "off-rate" of the interaction). Indeed, molecular interactions that have the same K_D can have widely different on- and off-rates (Fig. 2).

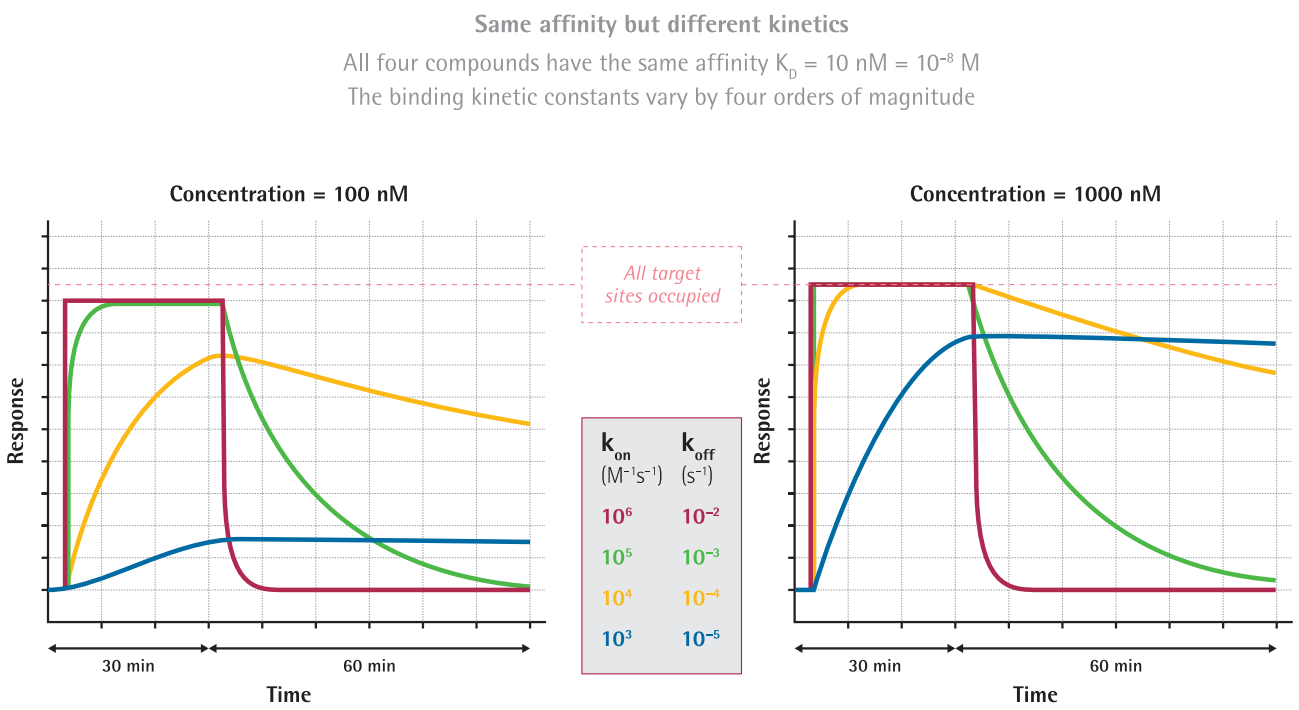
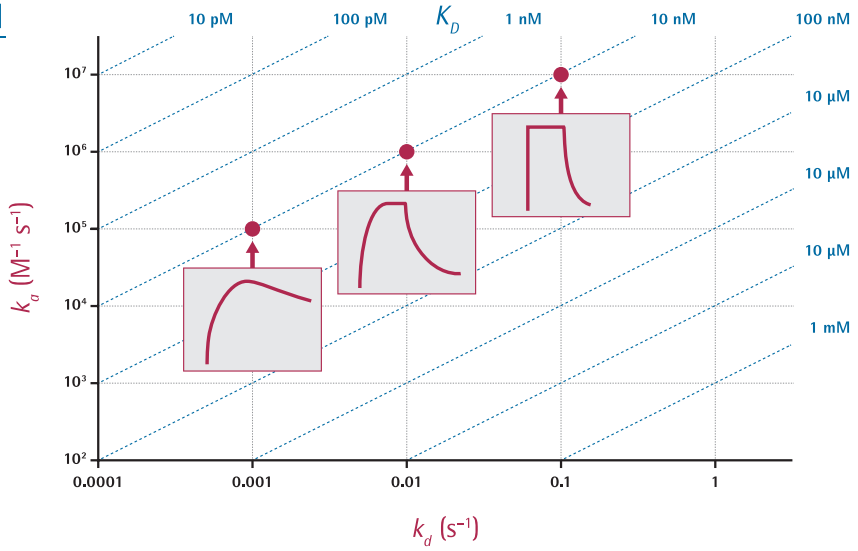


Fig. 2: Comparison of 4 different compounds with same affinity but different kinetics.

The kinetics of a molecular interaction are very important for drug discovery and development[1, 2, 3, 4]. The off-rate is especially important, because it tells you how long the compound binds to the ligand or target. A low off-rate would mean the drug could be formulated for less frequent dosing, whereas a high off-rate might require multiples doses per day. Given how important patient compliance is for treating many diseases—such as chronic diseases[5] and acute infections—ensuring a compound can be used in a convenient dosage regimen is vital to drug development. Many studies have shown that patients are less likely to miss a dose when they are taking a drug once a day compared with twice a day, and a once-weekly dose results in even better compliance.

Kinetic data can also help reveal a compound's mechanism of action[6, 7, 8] by determining whether the compound is interacting with, for example, one or multiple binding sites on the target[9]. This information is important for a number of reasons. For example, while most enzyme targets have a unique active site, they typically share other structural features with other enzymes. Interacting with such an allosteric site[10] could lead to unwanted reactions with other enzymes. Alternatively, a researcher might be looking for an allosteric interaction to activate or modulate the target.

$$K_D = \frac{k_d}{k_a} = \frac{[A] \cdot [L]}{[AL]}$$



Surface plasmon resonance (SPR) can be used to determine both kinetic/thermodynamic and equilibrium data, as well as the concentration of active compounds, to:

- Select protein-interacting therapeutic candidates according to their on/off-rates and not only by their affinity values.
- Identify potential drug targets and diagnostic markers in the early stages of pharmaceutical/biological research.
- Elucidate disease and cell mechanisms by characterizing protein interactions.
- Select the best antibodies as research tools, assay components or therapeutics by fully defining their interaction behavior.
- Develop assays to characterize proteins in drug development - with fragments, low-molecular-weight compounds, peptides, DNA, or other proteins.
- Detect and characterize immune responses during preclinical and clinical development.
- Characterize protein therapeutics and implement methods for quality control and quality management.

No other biophysical technique provides such comprehensive information in real time in only one system.

We hope you found this white paper helpful and informative. You can read more about SPR instrument hardware and sensor chips elsewhere on **XanTec's website.**

If you would like advice on how to best determine equilibrium constants and association and dissociation rate constants, please do not hesitate to contact us. Also, please contact us if you would like to suggest topics for future white papers, or if you have any suggestions to make our white papers even better.

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