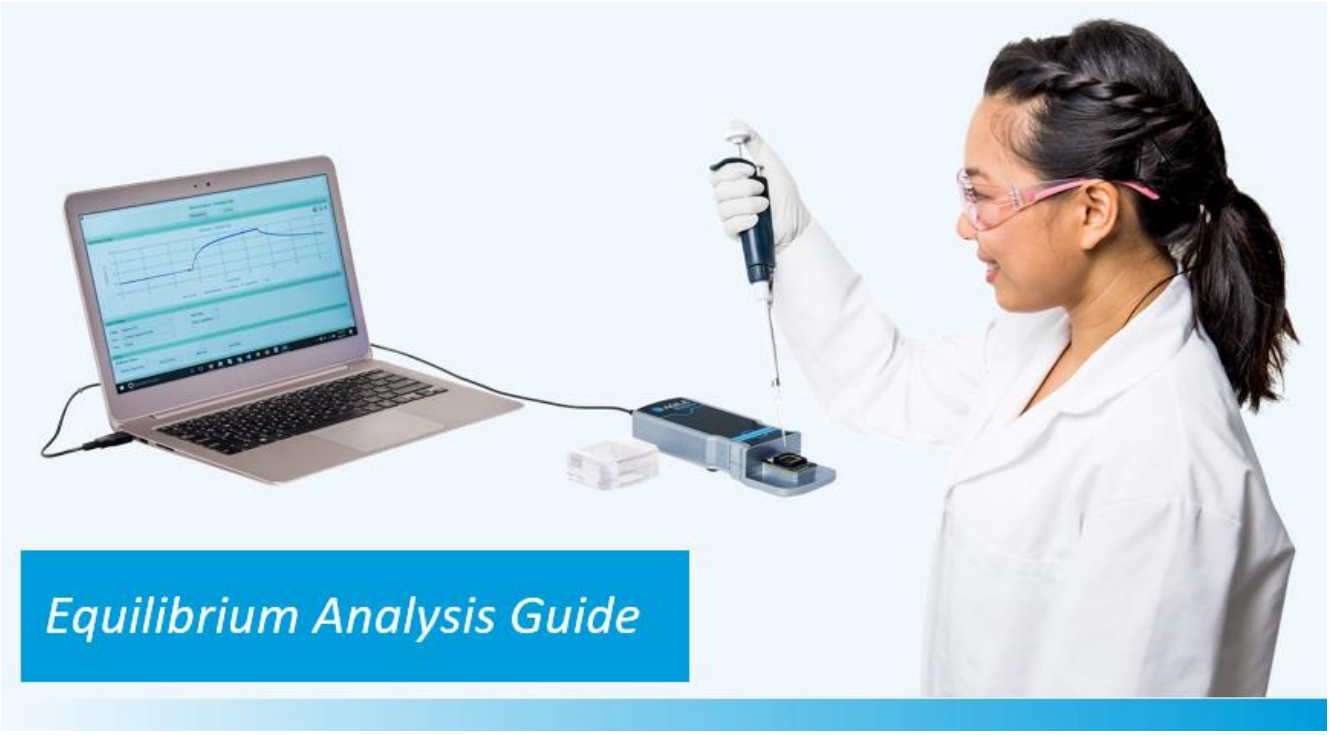


See Deeper

Know more.

Basic Binding, Equilibrium, and Affinity

In a basic, reversible binding interaction, analyte A and target T bind to form complex AT:

$$A + T \rightleftharpoons AT$$

The rate at which A and T associate to form AT is dependent on the association rate constant k_{on} and the concentrations of A and T. The rate at which AT dissociates into A and T is dependent on the dissociation rate constant k_{off} and the concentration of complex AT.

$$\text{rate of complex AT association} = k_{on}[A][T]$$

$$\text{rate of complex AT dissociation} = k_{off}[AT]$$

When the concentrations of A, T, and complex AT are no longer changing in the binding interaction, the system is said to be at equilibrium.

$$k_{on}[A][T] = k_{off}[AT]$$

A number called the equilibrium dissociation constant (K_D) quantifies the strength, or 'affinity', of the interaction between A and T. By definition, K_D is the dissociation rate constant divided by the association rate constant, which is equivalent to the equilibrium concentrations of A and T divided by AT.

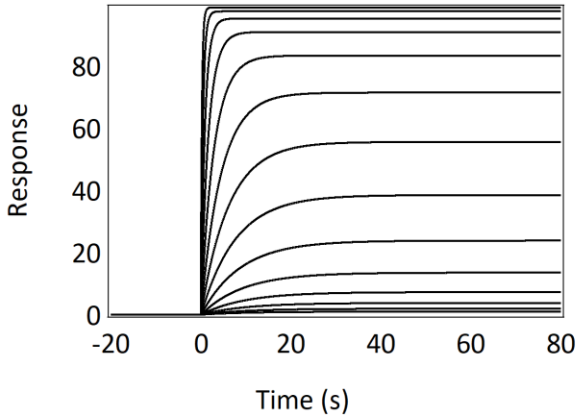
$$K_D = k_{off}/k_{on}$$

$$K_D = [A][T]/[AT]$$

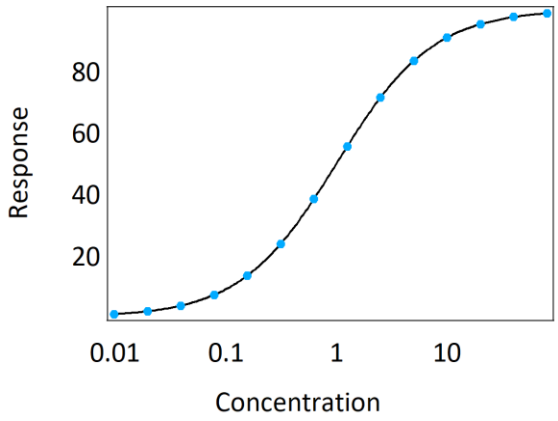
K_D is a key metric in the world of drug discovery and is often used to quantify the affinity of drug-target interactions. A small K_D implies a strong interaction between drug and target, while a large K_D implies a weaker interaction.

Equilibrium Analysis Method to Determine Affinity

In equilibrium analysis, the concentration of T is held constant and the concentration of A is varied over a series of equilibrium binding experiments. Equilibrium binding experiments with increasing concentrations of A are repeated until the plateau value of the binding curve no longer increases, indicating that T has been saturated (i.e. no more AT can form). To illustrate, the figure below shows 14 concentrations of analyte A binding to a target T.



The data from these curves are then combined into a single curve by plotting the concentration of A in each binding experiment versus the plateau value of each binding experiment, yielding an equilibrium affinity plot. The plateau values represented on the y-axis can be interpreted as 'percent T bound', with the upper values being 100%.



By rearranging the equation K_D = [A][T]/[AT], one can determine that K_D = [A] when 50% of T is bound by A, or the concentration of AT has reached ½ of it's maximum value. This allows K_D to be directly calculated from the equilibrium affinity plot.

When designing an experiment to determine affinity through equilibrium analysis, it's important to: 1) start with a zero-concentration sample, and 2) increase concentrations of analyte until the sensor response no longer changes (i.e. plateau is reached).

We hope you enjoyed this guide! We're here to help you see deeper, know more, and make new discoveries. For any additional questions, please contact us at techsupport@nanomedicaldiagnostics.com.