What strategies can be used to correct the complex binding behavior of my kinetics data?

While an interaction displaying non-ideal behavior may be easily fit using a more complex binding model such as 2:1 or 1:2 binding, it is best not to choose a more complex curve fitting model without significant justification. Unless the interaction is known to be complex, the best approach is to optimize assay conditions until the profile displays 1:1 binding characteristics. Refer to How do I recognize the heterogeneous binding curve shape? for symptoms of heterogeneous binding.

A complex, non-ideal binding profile in a kinetic assay can be caused by a number of factors. Proper assay optimization can often alleviate the apparent heterogeneity and bring the interaction back to its expected binding behavior. One major component in complex binding behavior may be non-specific binding to the biosensor. When analyte concentrations far above the K_D are used, and/or ligand density on the surface is high, weaker non-specific interactions tend to come into play. Lowering the analyte concentration range in the assay or eliminating higher concentration data traces from the analysis can bring the interaction closer to an ideal curve fit. Lowering the ligand density on the surface, either by reducing ligand concentration during loading or by decreasing the loading step time, can have a similar effect.

Strategies for Correcting Complex Binding Behavior:

- Use a capture-based approach
- Lower the ligand density
- Lower the analyte concentration to around 0.1 to 10 times the K_D of the interaction
- Check the reagent stability and quality
- Modify the assay buffer by adding blocking agents, detergent
- Shorten association step time times to allow only as much time as needed to see some saturation behavior (“plateauing”) of the highest analyte concentration.

For more information on assay development for kinetics assays, refer to Application Note 14: Biomolecular Binding Kinetics Assays on the Octet Platform.

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