Receptor Valency Effects in KinExA Measurements

Kinetic Exclusion Assay (KinExA®) experiments determine the free fraction of a receptor in a sample containing receptor, ligand, and receptor-ligand complex. Using this information the concentration of one of the analytes or the K_d of a binding pair can be derived, depending on the experimental conditions. The theoretical models used for the determination assumes 1:1 binding, while in fact most of the time the valency is greater than one. Does this cause an error in the experimental results? This tech note will explore the effect of receptor valency on experimental results, including when and how valency will affect the measurements.

Source of Multivalency Measurement Errors

In any assay that relies on capturing a free binding site (this includes ELISA, SPR, and KinExA), the signal is related to the amount of receptor that is captured. Once captured, a receptor gives the same signal whether it had one, two, or more free binding sites in solution. Therefore, the signal does not directly reflect the number of free binding sites when the receptor is bivalent (or more generally multivalent). Although the signal from a captured receptor is independent of the number of free binding sites, the probability of being captured can be affected by the number of free binding sites.

If the overall capture probability is low, the capture probability converges to being proportional to the number of free binding sites, so that the signal once again is proportional to the free binding sites in the sample. A complete mathematical treatment of this has been published[1], concluding that for low capture probabilities, the error can be ignored.

In KinExA measurements the contact of the sample to the solid phase is kept intentionally short, unlike other capture assays. The short contact time is to kinetically prevent competition between the solution ligand and the solid phase ligand for the receptor. While this works well in allowing samples to be measured without perturbing the bound fraction, it also tends to keep the capture probability low, giving a more accurate measurement of free binding sites when using multivalent receptors.

Capture Probability and Measurement Error

The original mathematical treatment of capture probability and measurement error[2] only discussed the error in the free binding sites estimated from the measured signal as a function of the overall capture probability. Of more relevancy is the resulting error in K_d measurement. To get a visual representation of the effect on a K_d measurement, a theoretical case of a bivalent receptor (e.g. an antibody) was analyzed at different capture probabilities to generate a graph of calculated K_d values. In ***Figure 1***, the X axis is the capture probability for a bivalent receptor. The Y axis is the K_d value expressed in units of picomolar (pM). Each data point is the average of 1000 independent analyses, using 3% random noise and run through the standard KinExA analysis software. The error bars indicate a range encompassing 95% of the individual K_d values.

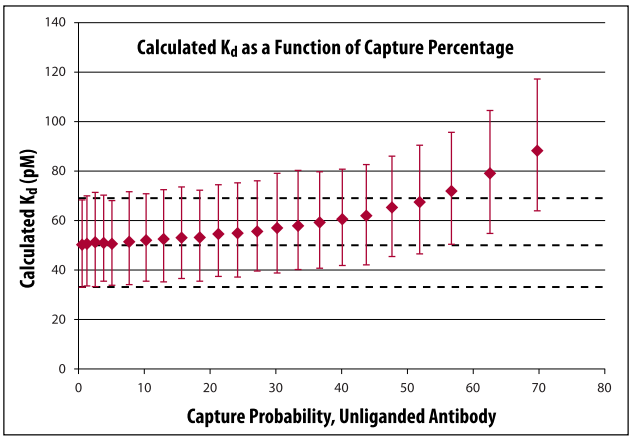

Figure 1. *Graph of the K_d calculated from a theoretical case analyzed at different capture probabilities.*

Conclusions

As long as the capture probability is below 40-50%, the error caused by the bivalency of the receptor is well within the normal measurement error. While the capture probability is not normally measured as part of an analysis, it has been reported in publications that the instrument has a "typical capture efficiency of less than 10%"[3] or, more specifically, 1.2% to 5.9%[2]. Although this is within the desired range, it does vary from system to system. Since the usual goal is to get a highly active solid phase (high capture probability), it may be higher than 50% for some systems. If there is a concern, a simple test can be performed to be sure the capture probability is in a range that is not causing a perceptible measurement error. See How to Guide 250 *Capture Precent Test* (**HG250**) for information on how to perform the test.

References

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2. Ohmura, N., Lackie, S.J., et. al. **2001**. An immunoassay for small analytes with theoretical detection limits. *Anal Chem* 73(14): 3392-9.
3. Blake, R.C., Pavlov, A.R., and Blake, D.A. **1999**. Automated kinetic exclusion assays to quantify protein binding interactions in homogeneous solution. *Anal Biochem* 272: 123-134.