KinExA® Error Curves and the 95% Confidence Interval

KinExA analysis is based on a least squares fitting of an exact bimolecular binding equation (the theory) to the measured signals. KinExA error graphs show a plot of the least squares residual error as a function of one of the parameters. 95% confidence intervals are derived from the error graph in the manner described below.

Raw KinExA signals are proportional to the concentration of free Constant Binding Partner (CBP) in the solution. An equation has been derived expressing the signal level as a function of the K_d, the Active CBP, the titrant concentration, the 100% signal (Sig100%), and the Non Specific Binding signal (NSB) (Ohmura, Lackie et al. 2001). In the standard analysis, titrant concentrations are treated as independent (i.e. known values) and the other four parameters are varied to find the smallest value of the sum of squares of the differences (errors) between the theory and the corresponding measured values. The result is a single set of four values that give the least squares best fit of the theory to the data.

Figure 1 shows a typical experimental analysis. The best fit of the theory (red line) to the data occurs when the theory uses the following parameters: K_d = 3.25 pM, CBP = 28.43 pM, Sig100% = 0.72 volts and NSB = 0.04 volts. When these parameters are used in the theory, the residual error is 1.51%. For the data in ***Figure 1***, all other combinations of the four fitted parameters give residual errors larger than 1.51%.

Explanation of error graphs and confidence intervals

The K_d error graph (***Figure 2***) for the experiment in ***Figure 1*** has a minimum residual error of 1.51% at 3.25 pM. The remainder of the graph is computed by fixing the K_d value at points across the X-axis range shown and then optimizing the remaining three parameters for each point. For example, when the K_d is fixed at 1 pM the best fit that can be found by varying the other three parameters has a residual error of 2.3% (***Figure 2***).

Computing the confidence interval from the error graph correctly includes the asymmetry that really exists in the uncertainty of the K_d value. In ***Figure 2*** there is a well defined minimum in the graph but sometimes the error graph will be flat, indicating essentially the same residual error for any value of K_d less than the one reported. This will usually arise in a higher ratio (more concentration controlled) experiment, as shown in ***Figure 3***. In such a case, the CI will be unbounded on the low end indicating that an upper bound has been put on the K_d but that the K_d has not been resolved.

Analysis approaches that report symmetric intervals, based on formal standard, errors are not valid when applied to K_d analysis. The reason is that the parameters of the analysis model (K_d, CBP, Sig100% and NSB) are not independent of each other (Kuzmic 1999).

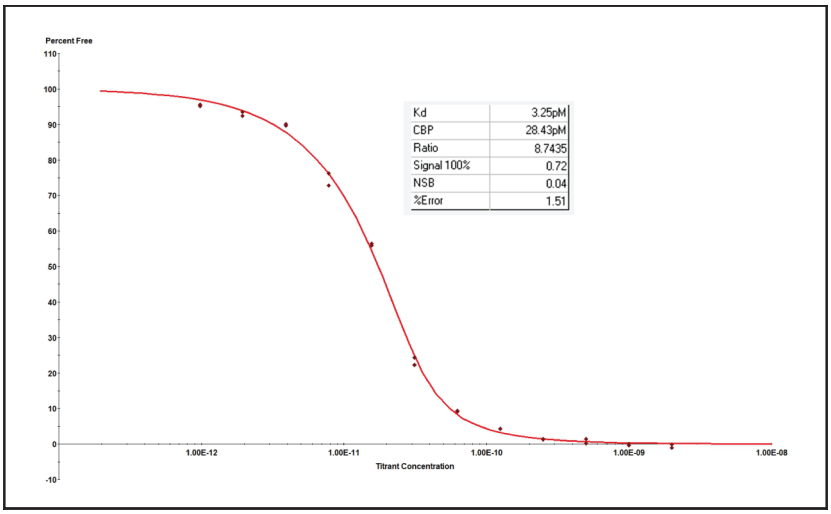

Figure 1. *Typical KinExA analysis results.*

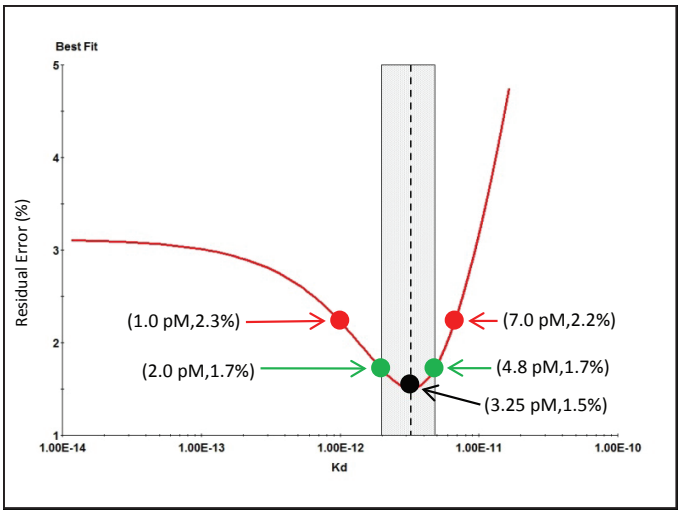

Figure 2. *A KinExA K_d Error curve with a well defined confidence interval.*

Does this analysis really work?

The confidence interval approach was developed and incorporated into the first KinExA analysis software. Several years later accumulation of QA testing data allowed a direct measure of its validity. The K_d of a reference system, a commercially available anti-digoxin antibody, was used. Data was compiled from 131 separate measurements obtained on 33 different instruments by multiple operators. Data was analyzed assuming the average value of the 131 measurements was the true K_d value. After examining the 131 confidence intervals, it was found that 125 or 95.5% included the true K_d value.

References

Ohmura, N., S. J. Lackie, et al. (2001). "An immunoassay for small analytes with theoretical detection limits" Anal Chem 73(14): 3392-9.

Kuzmic P. (1999). "General numerical treatment of competitive binding kinetics: application to thrombin-dehydrothrombin-hirudin" Anal Biochem 267: 17-23.

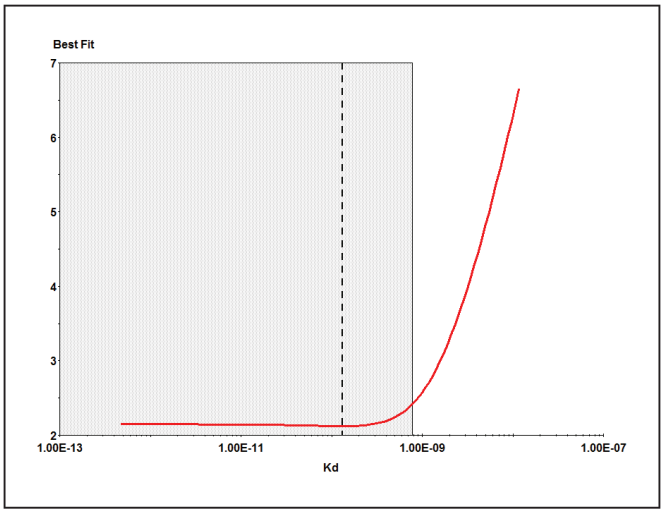

Figure 3. *A KinExA K_d Error curve illustrating an undefined confidence interval.*