# Unknown Titrant Analysis

The KinExA® method computes K_d values by measuring a series of samples with a constant binding partner concentration and titrated binding partner concentrations. When samples reach equilibrium the free constant is quantified. For the standard analysis, the titrant concentration is specified and the constant concentration is optimized along with the K_d to effect a fit between the equilibrium binding equation and the measured data. On the other hand, the Unknown Titrant Analysis uses the specified constant concentration and optimizes the titrant concentration along with the K_d. Because the titrant concentrations differ from sample to sample, the fit parameter is a Titrant Concentration Multiplier (TCM). The TCM can be multiplied by each of the nominal titrant concentrations to get the optimized value.

Basic Set Up

1. Enter a nominal titrant concentration into the software at the start of the experiment. The initial nominal concentration will not influence the generated data, so it is only important that the correct dilution factor between the nominal concentrations are entered correctly.

2. Open the Binding Curve tab, and use the drop down menu under Analysis Method to choose "Affinity, Unknown Titrant".
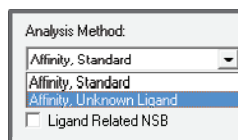
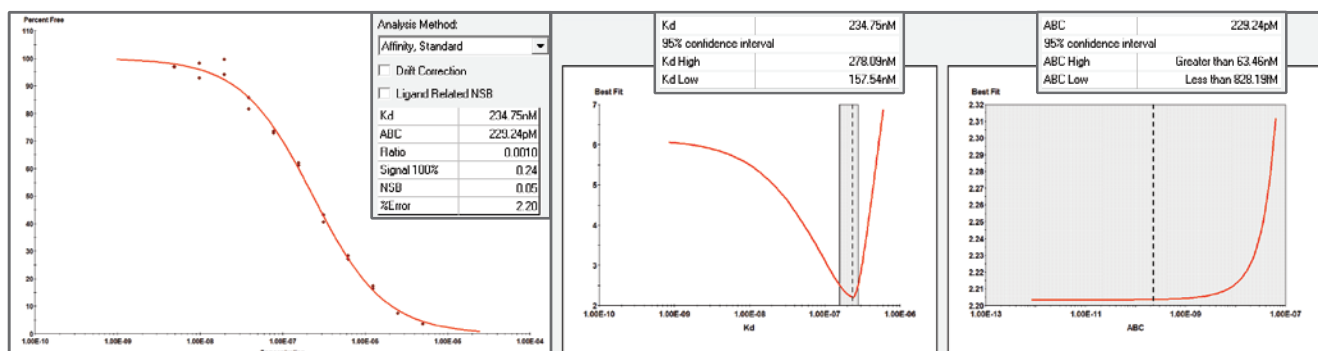3. Analyze the data by clicking the "Analyze Data" icon.

4. The software will perform a best fit of a 1:1 binding model to the data using the supplied starting values (constant concentration and nominal titrant concentration).

5. Based on the best fit, the software reports a K_d and TCM (Titrant Concentration Multiplier) along with error curves and confidence intervals. If the TCM confidence interval has tight bounds, the user can multiply the starting nominal titrant concentration by the calculated TCM to determine the actual concentration of titrant. In other words, a multiplier of "4" suggests that the initial nominal concentrations should be multiplied by "4" to fit the curve with minimal error.

Analysis Details

Unlike the standard analysis, the Unknown Titrant analysis will fail to give satisfactory bounds for the K_d value if the TCM cannot be resolved. This is true even if the data fits well in the standard analysis with little error. To understand why this occurs, consider the standard analysis results shown in **Figure 1**. Notice that the K_d value is resolved with upper and lower bounds. The ABC however is only resolved on one end indicating that the curve is K_d controlled and not receptor controlled.

Since the curve generated in **Figure 1** has little power to resolve the unknown concentration. The K_d can be resolved in the standard analysis even though the ABC is unresolved because the titrant concentration is known and an x-axis can be created.

Figure 1. *Experimental data indicates a K_d controlled curve when analyzed with the Standard Analysis.*

In the unknown titrant analysis, if the TCM is not resolved, then the x-axis is unknown. Without the correct scaling on the x-axis even a K_d controlled curve will not isolate whether 1 pM is actually 1 pM, 0.1 pM, or 100 pM. Notice the error curves and 95% confidence intervals for the same data analyzed with Unknown Titrant Analysis in *Figure 2*.

Figure 3 is the n-curve analysis which includes the curve from *Figure 2* and a second experiment using a higher constant concentration. Analyzed on the n-curve analysis, this resolves the TCM and gives tighter bounds for both the TCM and the K_d.
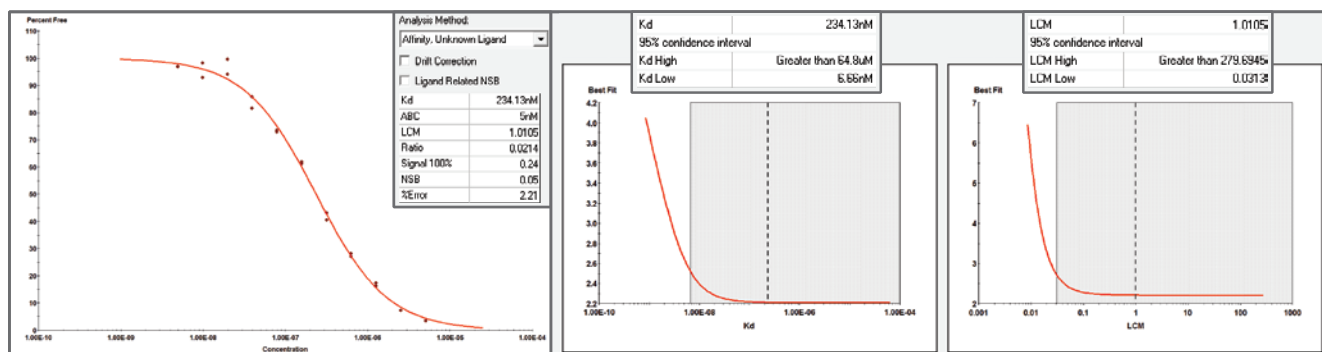
Unknown titrant analysis is useful for system where the constant concentration is known and trusted more than the titrant concentration. An important limitation to understand is that in order to resolve the K_d, the TCM must be resolved. Sometimes it is possible to resolve both from a single experiment in which the ABC between about 5 and 20 fold above the K_d, but if the analysis reports results with no bounds like *Figure 2*, then it is best to perform another curve with a higher constant concentration and use the n-curve analysis.

Figure 2. *The same experimental data from* **Figure 1** *measured with the Unknown Titrant Analysis.*
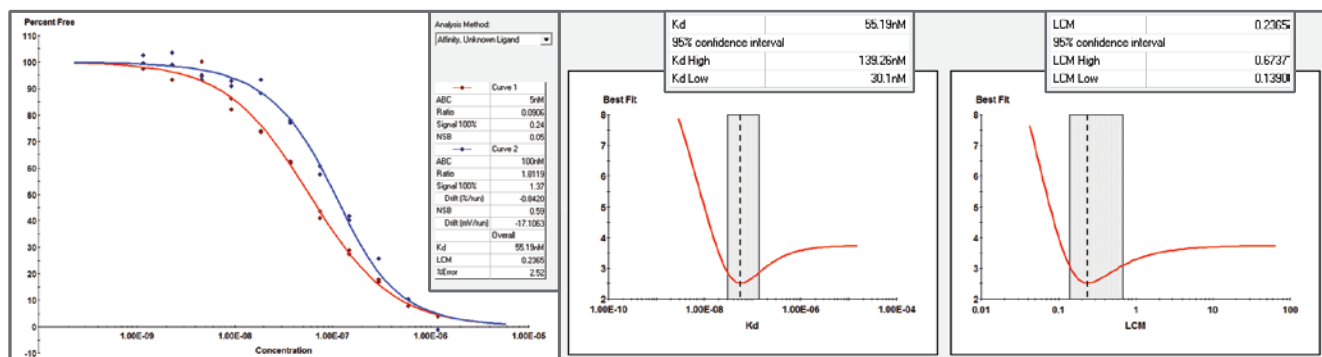

Figure 3. *N-curve analysis data with the curve from* **Figure 2** *and an additional experiment at a higher constant concentration.*