Sapidyne Instruments

2017 Newsletter

KinExA® 4000

The next generation of KinExA instruments arrives in the 4th quarter of 2017. The KinExA 4000 will be able to charaterize molecules with high affinity and slow off rates, measure native cells, engineered cells, small molecules, unmodified molecules, and molecules in unpurified sample matrices with femtomolar sensitivity.

Features

- LED lamp with a 10 year warranty
- Smaller footprint
- An enclosed sample area that prevents outside contamination and collisions with the sipper tube
- Liquid level sensors that alert you to low buffer volume and high waste levels
- Simplified plumbing with lower maintenance costs
- Updated KinExA Pro software that makes analysis simple with one click giving you access to FDA 21 CFR Part 11 compliant data

Including Measured NSB in your Analysis

When making measurements to whole cells, a low cellular expression and a weak K_d it may be impossible to fully inhibit the constant binding partner (CBP). This results in a failure to be able to resolve the K_d and expression level (EL). In KinExA Pro software version 4.2.12 and newer we have added the ability to include a measured NSB point in the analysis, which dramatically improves the range of K_d and expression levels that can be resolved. In order for this extrapolation to work, you must: 1) have at least two curves 2) inhibit at least 20% of the higher ratio curve (**Figure 1**), and 3) measure an NSB point using just sample buffer or sample buffer with only cells, no CBP. Once two or more curves have been measured and added to an n-curve analysis, make sure the NSB points are specified as NSB in the Cells/mL column on the Binding Signals tab, and click analyze.

It is important to note that the two curves need to be separated, not laying on top of one another. As a general rule, CBP values 5 fold apart should be adequate and easily seen in the data. Figure 1 shows an example of a dual curve analysis that displays all of the desired qualities for the extrapolated analysis to be valid.

Our in-house research found that using the NSB signal in place of a saturating cell concentration gives comparable results, although with somewhat greater uncertainty. Slight variations in the NSB signal also reported insignificant changes to the measured K_d and EL (always within the true 95% confidence interval bounds).

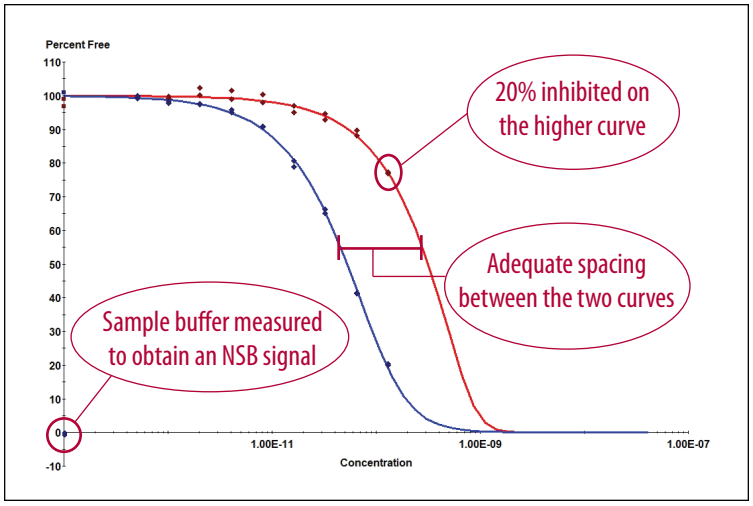

Figure 1. *A simulated system that resolved the K_d and EL, even though the amount of cells required to fully inhibit the CBP were not present. This example shows the minimum inhibition required for the analysis to be valid. The data point on the x-axis is the signal from just sample buffer and label flowing over the solid phase.*

Reference Spotlight

In a recent 2017 publication , researchers used the KinExA platform to accurately determine the equilbrium dissociation constants for SIP (lipid) binding to an antibody and to serum albumin simultaneously.

This paper showcases a new KinExA technique to overcome challenges in measuring and interpreting data from protein-lipid interactions. As is mentioned in the paper, **"the kinetic exclusion assay (KinExA®) provides a compelling alternative to SPR-based techniques for determining equilibrium dissociation constants of protein-ligand interactions"** and **" It is especially useful for observing protein-lipid interactions, as binding of native lipids occurs entirely in solution, and monoclonal antibodies can be used to directly compete with a protein of interest for lipid binding. "**

For a detailed description of the method, refer to the reference below.

Fleming JK, Wojciak JM. 2017. Measuring Sphingosine-1-Phosphate: Protein Interactions with the Kinetic Exclusion Assay. Methods Mol Biol. 10.1007/7651_2017_5. https://www.ncbi.nlm.nih.gov/pubmed/28349502

Ask the Inventor

Question: Let's say I have a K_d controlled curve with an adequately resolved K_d. Why should I go to the trouble to perform a second experiment and use the n-curve analysis?

Answer: The short answer is: because you don't want to be wrong. An n-curve analysis gives you greater confidence that the reported K_d truly reflects the binding pair and that it has not been affected by complex binding. Having the correct answer is important, since the result will likely be used to guide further work, or will be reported to a "customer" who is taking your number at face value. A single curve may fit the theory quite well and have narrow bounds, but still be incorrect because of complex binding or a concentration error. A surprising number of molecules exhibit some form of complex binding such as cooperativity or heterogeneity in the CBP (Constant Binding Partner) which can affect the shape of the binding curves. A heterogeneous mixture of CBP will make the binding curve less steep, make the ratio lower than it should be, and result in the K_d to appear weaker, as well as the CBP activity to appear lower. Negative cooperativity will affect the curve in the same way as heterogeneity, but positive cooperativity will cause the ratio to appear higher than it actually is, making the K_d appear tighter and the CBP activity higher. For more information regarding cooperativity refer to Tech Note 213 *Cooperativity* (**TN213**).

An n-curve analysis can reveal complex binding and give the correct answer in a way a single curve does not. This is because information for the K_d and activity in single curve analysis comes solely from the shape of the single binding curve whereas an n-curve uses the spacing, as well as the curve shape. To understand why the curve spacing is so important, it is helpful to use our Theory Curve as described in Tech Note 220 *Theory Curve* (**TN220**). Specifically, note the exercise described under Multiple Curves. If the K_d is near or between the CBP concentrations then the spacing is determined by the K_d. If both curves have a high ratio then the spacing is equal to the difference in CBP concentration. The spacing between the curves is not affected by complex binding.

Stepping back a little, the other big draw of an n-curve is to improve resolution in cases of unknown Titrant concentration, such as in the whole cell analysis. When using the CBP as the reference concentration for the analysis, a single K_d controlled curve typically won't resolve either the K_d or the Titrant concentration. To understand why, take a look at Tech Note 229 *KinExA Analysis* (**TN229**).

Another strong reason for an n-curve analysis is that it supports a directly measured Non Specific Binding (NSB) point. Including a measured NSB point enables resolution of whole cell K_d in cases where it's not possible to get full saturation of the CBP due to cell availability, low expression or the like. This is further explained by the Including Measured NSB article elsewhere in this newsletter and in TechNote 211 *Whole Cell Analysis* (**TN211**).

European KinExA Seminar 2017

In an effort to expand KinExA knowledge throughout the scientific community, we are excited to announce our European KinExA Seminar taking place on **December 14th** near the lovely Bavarian city of **Munich**. This one day event will begin with an introduction to the technology and move on to more advanced topics as the day progresses. We will have a guest speaker discuss their current research in complex cell binding using KinExA Technology and Sapidyne scientists will answer your questions and discuss applications.

During the seminar you will be able to access the new KinExA 4000 and see our technology in action. Please visit our website, sapidyne.com or sapidyne.eu, for more information about the free seminar.

Exporting Charts (Geek Corner)

Have you ever found yourself needing to copy charts created from KinExA Pro into a presentation or publication? We have a solution. The KinExA Pro software provides a couple of different techniques for doing just that.

From within KinExA Pro go to the File menu, select Export, and then select Charts. You will be prompted for a directory where images of the charts will be placed. These images will appear exactly as they do within KinExA Pro and can be easily imported or copied into your publication.

If the images produced do not meet your publication standards there is no need to worry. The KinExA Pro software allows you to export the analysis data that it uses to generate the charts. The raw data will also be included in the file.

From within KinExA Pro go to the File menu, select Export, and select Analysis Data. This will generate a tab delimited data file that can be imported into Excel or similar software where you can recreate the charts from the data to meet your requirements.

For more information, see How To Guide 262 *Exporting Charts* (**HG262**).

Tips & Tricks

Queue Cleaning Templates

Obtaining clean data is a constant goal when running experiments. Working with sticky systems, however, can result in material buildup in the instrument lines leading to less than desirable results. To combat this issue, typically the buffer would need to be replaced with cleaning solution and rinsed multiple times, or a custom experiment template would need to be made to rinse the instrument without replacing the buffer. To streamline the process of cleaning, software versions 4.2.13 and newer now incorporate a cleaning file template that can be used between or after experiments (**Figure 2**). This is particularly useful when multiple experiments need to be run consecutively. Adding cleaning experiments into the queue can significantly reduce build up of sticky materials in the flow cell and lines. The template defaults to [5] cycles of a 300 second soak in cleaning solution (**Figure 3**). This is a reasonable starting point though users may find they need more or can get by with less depending on the particular system under study. This new template can be used with the KinExA instrument or Autosampler.

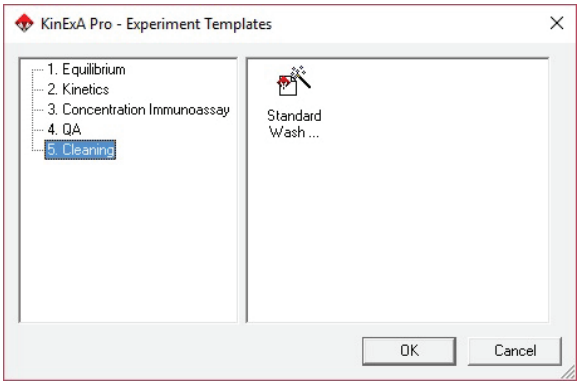

Figure 1. *"New from Template" cleaning file*

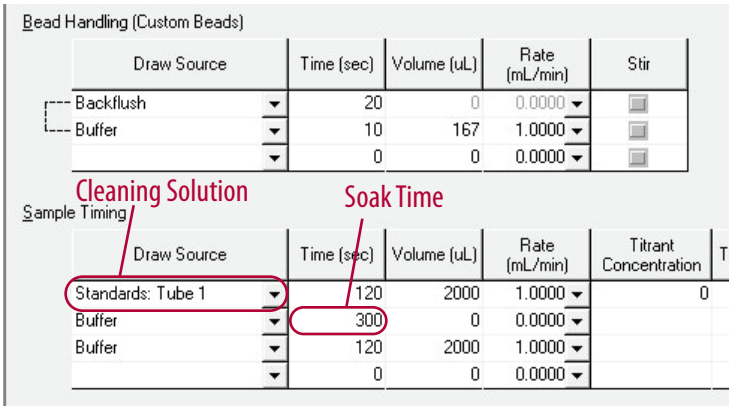

Figure 2. *Location to adjust the desired soak time in the timing file.*

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