Sāpidyne
Instruments

## Summer 2015 Newsletter

KinExA Mode

In KinExA® experiments, samples containing a mixture of two binding partners (along with their complex) are briefly exposed to a solid phase coated with one of the binding partners.

Kinetic Exclusion Assay refers to the underlying principle that contact time between any portion of the sample to the solid phase is short enough that dissociation of the sample's molecular complex is insignificant. Experiments run under these conditions are said to be in "KinExA Mode." This article will briefly outline how to determine if your experiments are in KinExA Mode.

When a system is out of KinExA Mode there is significant dissociation of the sample complex and the solid phase captures not only free constant binding partner (CBP) but CBP that has dissociated from the titrant. This results in an elevated signal and a falsely high estimate of the percent free CBP. An easy test can be conducted to determine if a system is in KinExA Mode. The test compares a single point of inhibition (ideally ~ 50% free CBP) to determine if the percent free CBP decreases as flow rate increases. Because signals vary with flow rate, an uninhibited point (Sig 100%) and a point near full inhibition (NSB) is needed to calculate the percent free CBP.

Figure 1 displays a comparison between a weak system that was not in KinExA Mode and a tight system that was in KinExA Mode. For the weak system the percent free CBP drops from ~ 58% free at 0.25 mL/min to ~ 39% free at 1.5 mL/min (the flow rate at which the weak system has finally entered KinExA Mode). The tight system shows multiple flow rates in KinExA Mode for illustrative purposes only. If a significant change in percent free CBP is not observed as the flow rate is increased further testing is not necessary.

KinExA Mode tests can be performed prior to running equilibrium experiments if preliminary range find results indicate a K_d weaker than single digit nanomolar. When running Kinetic Exclusion Assays at the default 0.25 mL/min flow rate the contact time of the sample to the solid phase is ~ 0.5 seconds. Most systems with a single digit nM K_d or tighter will be in KinExA Mode at the default flow rate. Weak systems can be brought back into KinExA Mode by increasing the flow rate, which shortens the contact time. See Tech Note 221 *KinExA Mode* (**TN221**) for more information.
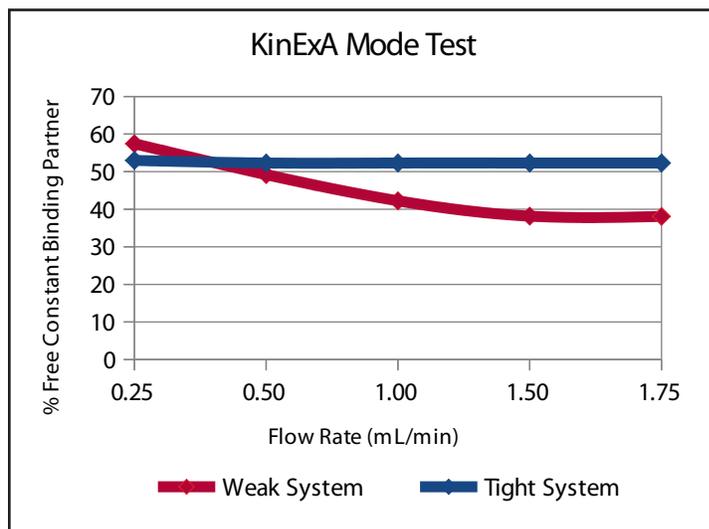
Figure 1. *A comparison of a weak and tight binding system. The weak system experiences significant dissociation at slower flow rates while the tight system does not.*

Hints & Tricks

Reducing NSB

Specific binding is the binding of a constant binding partner's (CBP) active site to its target on the titrated binding partner. Non specific binding (NSB) is any other binding that occurs in the system that interferes or adds to the specific binding and complicates the measurement (*Figure 2*).

Although NSB is accounted for in the analysis and subtracted from the signals, noise still contributes to greater uncertainty in the measurements. If NSB is a significant percent of the total signal, it is worth the effort to reduce it before continuing.

The first step in reducing NSB is to identify and understand the source of NSB. *Table 1* summarizes common sources and strategies to reduce NSB. For more information, refer to Tech Note 219 *Reducing Non Specific Binding* (**TN219**).

Note: Some NSB strategies may interfere with one another so it is best to try them individually.

Source of NSB	Strategies to reduce NSB
Solid phase material	One of the most effective ways of reducing NSB is to change the solid phase. Refer to Tech Note 222 *Solid Phase Selection Guide* (**TN222**) for more information.
Secondary fluorescent molecule	Try a different label. There are a variety of different labels available that are subclass specific, heavy or light chain specific, or cross adsorbed. We have had success with labels from Jackson Labs (www.jacksonimmuno.com). See Tech Note 223 *Labeling Strategies* (**TN223**) for more information.
Titrant	Titrant NSB has a characteristic shape on the binding curve. If high concentrations are needed to saturate the binding curve, it may be necessary to reverse the assay. In this case, the titrant would then become the CBP and can be used at a lower concentration. See Tech Note 210 *Titrant Related NSB* (**TN210**) for more information.
Tagged molecules	HIS-tags and Biotin tags can increase NSB. Using the tagged molecule as the CBP reduces the amount needed. Also, adding imidazole to the sample buffer can help with HIS-tagged molecules.
Other sources	Different additives such as blocking proteins, salt, detergents, heparin, or imidazole in the sample buffer or running buffer can help.

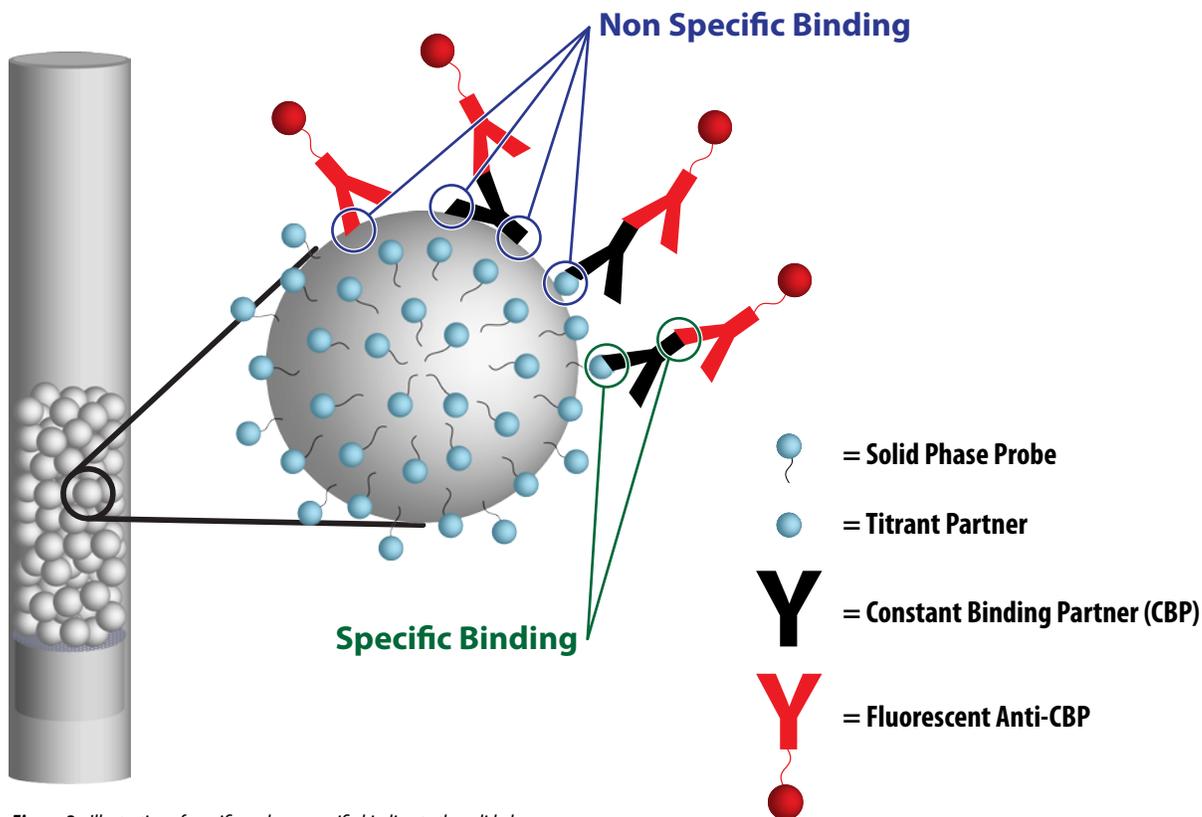**Table 1.** *Summary of common NSB sources and strategies to reduce NSB.*

Figure 2. *Illustration of specific and non specific binding to the solid phase.*

- = Solid Phase Probe
- = Titrant Partner
- Y = Constant Binding Partner (CBP)
- Y = Fluorescent Anti-CBP

? Ask the Inventor

Question: The only way I can increase my signal is to run very high volumes. The large samples make painfully long experiment run times, is there anything else I can do to optimize my signal?

Answer: Increase your signal to what? Everyone, including me, likes big signals but what actually impacts data quality is not the signal level but the signal to noise ratio. My suggestion is don't increase your signal. You may significantly increase material needed and run time without any gain. To demonstrate the practicality of this approach, I ran a series of experiments in which I prepared very large volumes of titrated ligand-antibody mixtures. I split each of the 16 titrations into eight separate sets of tubes and then ran duplicate volumes of 20 mL, 5 mL, 1 mL, and 0.5 mL at 0.5 mL/min and 0.25 mL/min. The antibody I used was a commercially available mouse anti-insulin and the solid phase was azlactone coated with insulin. The results are summarized in *Figure 3*.

As you can see, the confidence intervals for the K_d values all show substantial overlap even though net signals vary from 0.09 to 2.8 volts, run times range from 6.8 to 50.2 hours, and sample material usage varies 40 fold.
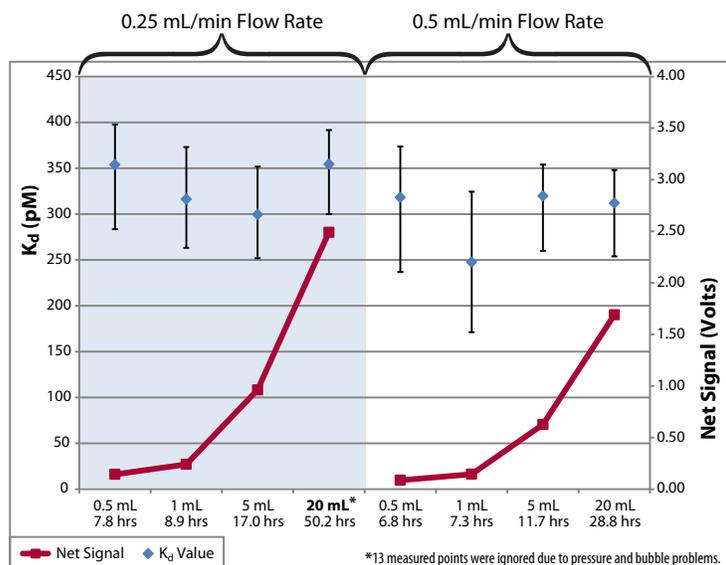
A further investigation of 370 experiments found that over half (52%) had a net signal of less than 1 volt and 24% had a net signal of less than 0.5 volts. The %Error had more variability at the smallest signal levels, but many had a low %Error (2% or less). In *Figure 4* I've grouped the signals into ranges and show the average %Error and the standard deviation in the %Error for each group.

The average %Error for the lowest signal range is significantly higher, statistically, than the other signal ranges. Differences between the other ranges are marginal or insignificant. Sample volume information was not available in this data extraction but it is likely that many of the small signal experiments also included large volumes which may have contributed to the higher %Error values.

My suggestion is that if your signal is less than 0.5 volts and your sample volume is less than 5 mL, go ahead and increase your volume up to 5 mL or until your signal is over 0.5 volts. As a rule, don't go over 5 mL sample volumes. Larger volumes frequently lead to clogging related pressure problems (I had to ignore 13 points in one of my 20 mL experiments and I changed the flow cell after both 20 mL experiments due to pressure increases). Overall, I believe your chance of success is higher with a smaller signal than with 10 or 20 mL sample volumes.

Note: If you are interested in seeing the experiment files behind *Figure 3* you can download them from www.sapidyne.com under the *Downloads* section.
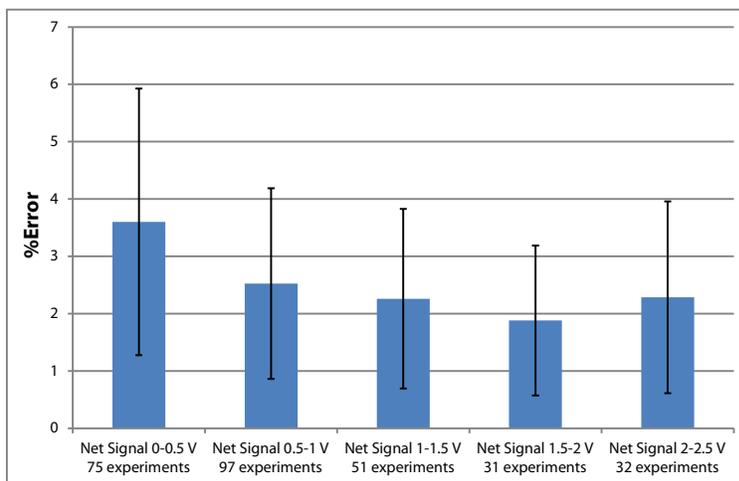

Figure 3. The K_d values and confidence intervals (blue diamonds and error bars) for different volumes and rates of the same sample. Red squares indicate the net signal (Sig 100%-NSB) for each measurement. The x-axis shows the volume of sample used and the total elapsed time for the measurement (16 points in duplicate).

Figure 4. The residual %Error from 370 experiments, covering a wide range of molecules and K_d values, grouped by net signal level. The lowest net signal range (0 to 0.5 volts) is slightly higher than the other ranges, however this may simply be due to the larger sample volumes used in these experiments.

Software (Geek Corner)

KinExA Pro Product Activation

The purchase of a KinExA Instrument includes two licenses of the KinExA Pro software. The software needs to be activated prior to use. Activation requires a serial number supplied by Sapidyne, a unique computer ID automatically generated by the software, and access to the Internet.

In the event that the computer does not have access to the Internet, an activation code can be provided by Sapidyne. To create a code, the computer ID generated by the KinExA Pro software must be provided to Sapidyne. The computer ID can be found on the product activation screen when attempting to run the software after installation and prior to activation.

Should you ever need to transfer a license from one computer to another, you will need to contact Sapidyne so that we may free up a license prior to the activation of the software on the new computer. If possible, please provide the computer ID for the computer that will be removed from use. To find the computer ID access the tool bar within the software and select *Help > About KinExA Pro*. A new screen from within the software will appear and the computer ID number will be designated as the *Serial No*.

Additional licenses may be purchased from Sapidyne with price breaks at various quantities of licenses purchased.

Windows Application Data Folder Permissions

The KinExA Pro software stores instrument configuration information as well as license/authentication information in the common Windows application data folder. This requires that KinExA users be granted permission to write to the Sapidyne folder. Administrative privileges are required to adjust security settings. This may require the IT department to be involved. If the security settings don't give the user permission, newer versions of Windows will attempt to virtualize the Sapidyne folder causing KinExA Pro software to behave incorrectly. If virtualization occurs you will need to close KinExA Pro, navigate to the Sapidyne folder within the program data virtual store folder, delete it, and fix the application data folder permissions before executing KinExA Pro again. Please see How to Guide 212 *Computer Setup Checklist* (**HG212**) for version specific instructions.

Proxy Server Settings

If your company network uses a proxy server and you will be connecting the KinExA computer to that network, you will need to have the IT department add an exception in the LAN settings. Without this exception, communication to the KinExA Flow Cell Camera will potentially be blocked by the proxy server. Please see How to Guide 212 *Computer Setup Checklist* (**HG212**) for version specific instructions.

Web Browser Requirements

Several of the tools within the KinExA Pro software (including the Theoretical Binding Curve, Flow Cell Camera Alignment, and Autosampler MAT Calibration) have been rewritten to use modern browser-based technology. In order to use these tools an updated browser needs to be installed. Current versions of Mozilla Firefox, Google Chrome, and Internet Explorer (version 9 and later) have all been tested and currently work with the software. **Note:** *an Internet connection is not needed since everything is run locally on the computer.*

Spotlight

Expansion

Sapidyne is excited to announce that we're expanding! We are enlarging our headquarters in Boise, ID and have opened a new office in Hannover, Germany.

In Boise, the larger space will allow us to expand our laboratory and cell culture capabilities to facilitate more in-house research and a larger staff. We will also have more space for inventory and shipping; allowing for more stock of our products while keeping the processing time for shipments as short as possible.

Our new office in Hannover will further decrease the response time to customers in Europe both for inventory items and customer service. The location will likewise allow us to have a greater presence at international trade shows and events.

As we grow, we are striving to enhance our level of service and support for our customers. To Affinity and Beyond!!!