

Signal Versus Signal to Noise

Everyone, including us, likes big signals but what actually impacts data quality is not the signal level but the signal to noise ratio. You may be better off not increasing the signal, since this can often significantly increase material usage and run time without any gain in data quality.

To demonstrate the practicality of this approach, we ran a series of experiments in which very large volumes of titrated ligand-antibody mixtures were prepared. Each of the 16 titrations were split into eight separate sets of tubes and then each ran in duplicate, but with different volumes and flow rates (20 mL, 5 mL, 1 mL, and 0.5 mL at both 0.5 mL/min and 0.25 mL/min). The antibody was a commercially available mouse anti-insulin and the solid phase was azlactone coated with insulin. The results are summarized in **Figure 1**.

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. This is very compelling data, but it is from only one system. Does this same conclusion hold true generally for other systems?

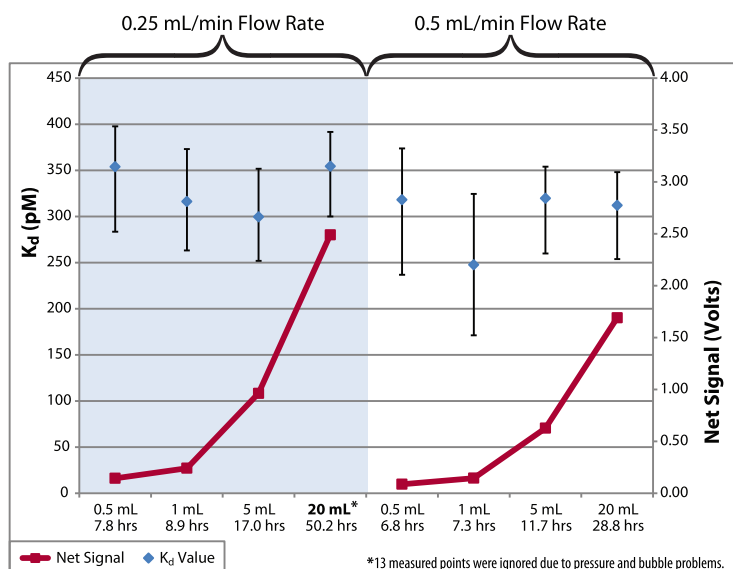


Figure 1. The K_d values and confidence intervals (blue diamonds and error bars) for different volumes and rates of the same sample. Red squares indicate the net signal (Sig 100%-NSB) for each measurement. The x-axis shows the volume of sample used and the total elapsed time for the measurement (16 points in duplicate).

To find out we compiled the recent experiments done in our lab for a variety of systems. This 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 2** the signals are grouped 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.

Our 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 (we had to ignore 13 points in one of the 20 mL experiments and change the flow cell after both 20 mL experiments due to pressure increases). Overall, your chance of success is better with a smaller signal than with 10 or 20 mL sample volumes.

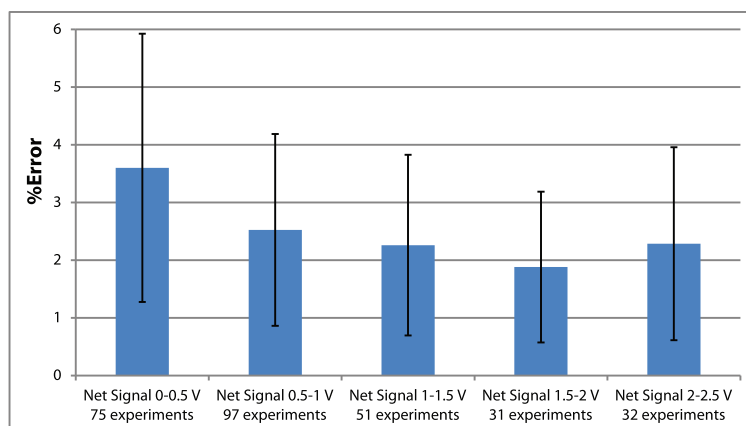


Figure 2. The residual %Error from 370 experiments, covering a wide range of molecules and K_d values, grouped by net signal level. The lowest net signal range (0 to 0.5 volts) is slightly higher than the other ranges, however this may simply be due to the larger sample volumes used in these experiments.