

BLI Data analysis

Open the Data Analysis software. (but not while an acquisition is in progress)

Go to File and Load folder. Find your experiment and click Load. You have the chance here to redefine any of the assay steps in case you made an error earlier.

Click **tab 2** for **Processing**.

Step 1 is Sensor selection: choose reference well(s) to have it/them subtracted from data. R click on only the R plate map to select it/them. Choose Change Well Type and select Reference. Then check off the box to the left of the reference well.

Step 2, check Subtraction, choose Reference wells.

Step 3, check Align to Y axis: use Baseline, selecting the last 5 sec. of the baseline before the Association step for the vast majority of cases.

Step 4, check off Inter-step correction and choose Align to Dissociation. (If dissociation is really fast, try analyzing the data without inter-step correction first and see how bad the jumps look.)

Step 5, choose Savitzky-Golay Filtering. Click on Process Data! button.

Step 6 can be ignored.

Step 7 gives you the chance to Save Raw Data into an Excel spreadsheet.

Click **tab 3** for **Analysis**.

Be sure View: Stacked is chosen.

For analysis, under Curve fitting, choose Association/Dissociation. The Model button has different ratios. Start with 1:1 which usually works for general use. You can try different ratios on to the data without changing the raw data.

For the first fit, choose both Local and Full, then click Fit Curves! You need at least 4 different concentrations to get a good K_D fit.

The chart at the bottom will show you k_{on} , k_{off} and K_D as well as χ^2 and R^2 values.

k_{on} and k_{err} should be within an order of magnitude. With local fitting, that might not occur.

To get a single K_D for the whole experiment, go back to Fitting and choose Global. Group sensors by color. R click on the first value and a menu comes up. Choose Set Color By and choose Sample ID. Click the Fit Curves! button again. Read the K_D off the chart.

At the front of the chart, you can uncheck points you don't like. Make a change and then hit Fit Curves! again.

To export the data, click on Save Report. It exports everything to an Excel spreadsheet.

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