Octet RED Software Version 5.2

NOTE: The changes listed below are listed in this document only. No changes have been made to the Octet RED User Guide.

NEW FEATURES IN VERSION 5.2

Octet software version 5.2 offers a range of data analysis features to enable a more streamlined and more flexible data analysis workflow. These features provide more options to the end-user for analyzing and visualizing data produced on the Octet platform. New capabilities include:

- **Raw data viewing enhancements**: select, view and align raw data by any step in the data (baseline, association, dissociation etc.) prior to processing.
- **Processed data alignment options**: process data by aligning data on the y-axis using either the baseline, association or dissociation step as the reference step.
- **Window of interest**: analyze only the data in the relevant experimental time frame.
- **Data exclusion from fitting option**: exclude specific curves in the processed data set for more directed analysis.
- **Global fitting by color**: fit analyzed data by color; each color can represent biosensors, samples, compounds, concentrations or other relevant parameters.
- **Color-coding final results**: set color of final processed data by biosensor, sample ID, compound, or other relevant parameters.
- **Steady state analysis**: determine the $K_D$ of an interaction using equilibrium data.
- **Iso-affinity plots**: compare $K_D$ values from multiple interactions.
- **Report writing**: export data into Microsoft Excel spreadsheets.

RAW DATA VIEWING ENHANCEMENTS

In the Octet RED software version 5.2, acquisition data can be viewed before processing the raw data for analysis. Additionally, prior to performing any processing procedures, the raw data can be aligned by any experimental step shown in the data (ie baseline, load, association or dissociation).

**Directions for use**

In the Octet RED data analysis software, select a data set and then click the Processing tab. Acquisition data will be populated on the right side of the window in the raw data view. Within

![Figure 1: In this example, all traces have been aligned by the association step starting 87 minutes (5210 seconds) after the beginning of the experiment.](image-url)
Directions for use
In the Analysis tab of the Octet RED data analysis software, enter time values in the Association and/or Dissociation fields to select portions of the current data set for inclusion. During any subsequent data fitting, only the portions of the data curves included in the window of interest will be fit (Figure 3).

DATA EXCLUSION FROM FITTING OPTION
Octet RED software 5.2 gives the option of excluding curves from the current analysis when fitting data. This is particularly useful when there are obvious outliers or other types of unreliable data that can greatly affect the results of the data fitting.

Directions for use
In the Octet RED data analysis software, the first column of the Analysis tab results table allows the user to either include or exclude selected curves from the current analysis. Right-clicking a curve in the current analysis allows the choice of including or excluding the data from that well. After adding or removing a curve from the current analysis, clicking the Analyze Data button causes the data to be reanalyzed using the current selected data set (Figure 4).

GLOBAL FITTING BY COLOR
In Octet RED 5.2 software, the processed data can be fit globally across biosensors or across colors that the user defines. This allows global data fitting by specific parameters such as sample ID, compound ID, concentration, or other relevant parameters. Once the data is fit, the data can be re-colored without affecting the fit (see Color-Coding Final Results, below).

Directions for use
In the Analysis tab of the Octet RED data analysis software, click the appropriate Global Fitting parameter for the analysis: “By Color” or “By Sensor” (Figure 5). To define the color parameter, right-click any row or any selection of rows in the data table at the bottom and choose “Set Color By” (Figure 6).

COLOR-CODE FINAL RESULTS
Octet RED software 5.2 can assign different colors to data analysis results, to create a visual correlation between data in the results table and plotted data. Colors can be assigned to a specific biosensor or group of biosensors or other relevant parameters without affecting the fits.

Directions for use
On the Analysis tab of the Octet RED data analysis software, the second column of the results table allows the user to assign a color to the currently selected curve or group of curves. To define the color, right-click any row or any selection of rows...
in the data table at the bottom, then choose “Set Color By” (Figure 6). To change the color of the selected curve, right-click the current color and select “Set Color” to open the color palette selector. In the color palette, click the desired color, then click the OK button. The curve will now be displayed in the selected color.

STEADY STATE ANALYSIS
The steady state analysis option allows the user to determine the $K_d$ of an interaction using equilibrium data instead of on- and off-rates. This ability provides another type of analysis to confirm values determined through traditional kinetics analysis.

Directions for use
To start a steady state analysis in the data analysis software, check the Steady State Analysis check box on the Analysis tab. Once the steady state analysis is started, select which curves to include in the analysis by marking the corresponding check-box. Once the desired curves are selected, you have the option of entering in the time when equilibrium was reached and a response should be calculated. By default, the time selected for calculating the response at equilibrium is a ten-second window that starts 15 seconds prior to the dissociation step. When the appropriate time has been entered, click the Calculate Response button to update the values in the table and the displayed graph (Figure 7).
### ISO-AFFINITY PLOTS

The Iso-Affinity Plots feature allows comparison of \( K_D \) values from multiple interactions using the subordinate parameters \( k_{\text{a}} \) and \( k_{\text{d}} \). The resulting graph plots the data for easy evaluation. This feature also allows creation of a plot showing the relationship of any of the parameters available in the pull down boxes (e.g. Molar Concentration vs Req).

**Directions for use**

In the data analysis module of the Octet RED software, the lower right corner of the Analysis tab contains the interface for creating iso-affinity plots. Selecting the Iso-Affinity Graph checkbox will create a graph with \( k_{\text{a}} \) plotted on the x axis and \( k_{\text{d}} \) plotted on the y axis (Figure 8). Clicking the Export Report button will export the results table, including the iso-affinity graph, into a Microsoft Excel file.

### REPORT WRITING

Octet RED software 5.2 is able to export data directly into a Microsoft Excel spreadsheet.

**Directions for use**

Once results have been calculated in the data analysis software, click the Export Report button to generate a report. The resulting Excel spreadsheet contains the results table, a stacked graph with all analyzed curves and corresponding fits, and a sheet containing the individual curves and their corresponding fits (Figure 9).