Octet RED384: CMI Guide to Legacy Data Analysis Software

Most users should use the newer **Data Analysis HT** software for data processing, as it has many features and improvements. However, the legacy software is still supported and here is a brief guide.

Data Analysis 11.0 (legacy data analysis software)

1. Open the Octet Analysis Software (on the desktop).
2. Go to File → Load Folder.
3. Find the Octet Data Folder and click Load to add to the Loaded Data Directory.
4. In the Loaded Data Directory, click on your experiment name to open it.
5. In the Sensor Tray Tab, select the biosensors to be analyzed.
6. Go to the Processing Window.

Processing

STEP 1: Data Selection.

1. Click the Sensor Selection Button.
2. Assign Parallel Reference Sensors (sensors to which no ligand is immobilized).
    a. In the Sensor Tray map, the default assignment for all sensors is as Ligand sensors.
    b. Reassign reference sensors by selecting sensors, right-clicking, and changing sensor type to Reference Sensor.
3. Assign Reference Wells (zero concentration of analyte).
    a. In the Sample Plate, select the reference wells, right-click, and change well type to Reference Well.
    b. Check the box that appeared next to the row with reference wells to select the reference wells.

STEP 2: Subtraction.

1. Check the subtraction box when performing reference subtractions.
2. Chose subtraction options:
    a. Reference Well (subtracts a zero concentration of analyte to correct for drift).
    b. The Parallel Reference Sensor subtracts a reference sensor, to which no ligand has been immobilized, for each concentration of analyte.
    c. Double Reference subtracts both Reference Well and Parallel Reference.

STEP 3: Align Y-axis.

1. Select Baseline.
2. Choose a range for baseline correction (default is the full baseline before association).
    - May choose a truncated region closer to the start of association.

STEP 4: Inter-step Correction.

- NOTE: for most effective inter-step correction: baseline before association and dissociation steps must be performed from the same well of a sample plate.

1. This step corrects for system artifacts (optical artifacts from buffer mismatch, etc.).
    - Should not be performed with very fast on-rates.
2. Choose a step to align.

STEP 5: Process.

3. Select Savitzky-Golay Filtering if measuring protein/protein interactions or when the signal to noise is **only** high. This removes high-frequency noise with a smoothing function.
4. Click Process Data.
5. Examine the processed data.
6. Save Processed Data and Processing Parameters.
7. Go to Analysis Window.

Analysis

1. *Kinetic Fit.*
    b. Choose Steps to Analyze: Association and Dissociation.
    c. Choose Model: 1:1.
    d. Select Global Fitting (full).
        i. Group by Color, if each concentration is measured on a different sensor (a parallel experiment).
        - *If grouped by Color, select rows of data from the table, right-click and change color to a shared color for all data to be grouped.*
        ii. Group by Sensor if all concentrations are measured on the same sensor (a serial experiment).
    e. Fit Curves.
2. *Steady-State (Equilibrium) Fit.*
    a. Select rows in the table to include.
    b. In Steady-state dialog, choose Response as the mode of analysis.
    c. Select the Region of Analysis.
        i. This should be a region of the association curve that has reached equilibrium (or steady-state).
        ii. Default is a five second window, five seconds from the end of association.
        iii. To view, go to the graph window in the bottom right corner and select the steady-state tab.
    d. Save Analysis Parameters.
    e. Save Report (for a summary of the analysis). This does not include kinetic data in a graphable format.
    f. To export data curves:
        i. Right-click on the kinetic curves.
        ii. Go to the Export Dialog.
        iii. Choose Text/Data.
        iv. Choose File.
        v. Enter File Name.
        vi. Click Export.
        vii. Select Table format.
        viii. Select Points/Subsets

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