

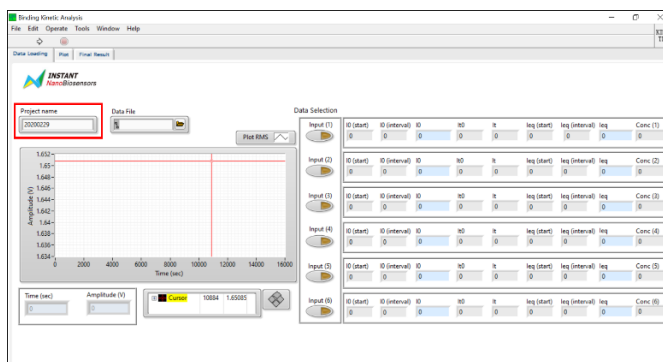
Determining the Kinetic & Affinity Constants of an Analyte

A Practical Guide to the FOPPR Binding Kinetics Software with IgG

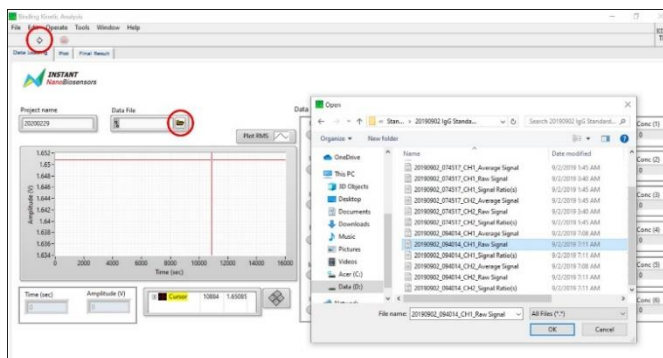
Binding kinetics and affinity of a ligand-analyte complex describe the reaction rate and formation strength, respectively, of the ligand-analyte pair. Determination of association rate constant k_a and dissociation rate constant k_d with FOPPR™ does not require repeated binding and regeneration cycles; these constants are analyzed from the raw data of an analyte standard curve. In this technical note, calculation of binding kinetics and affinity data with the FOPPR™ Binding Kinetics software is demonstrated with IgG.

Step 1. Launch the FOPPR™ Binding Kinetics software.

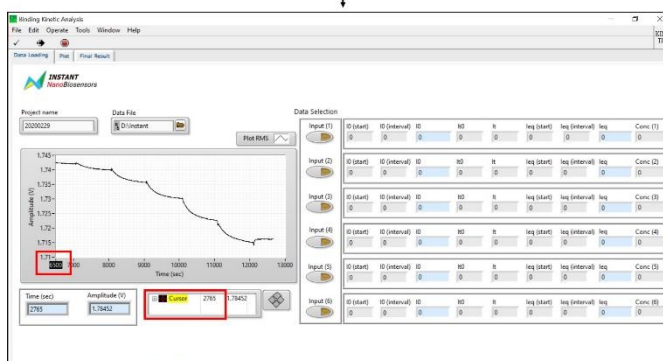
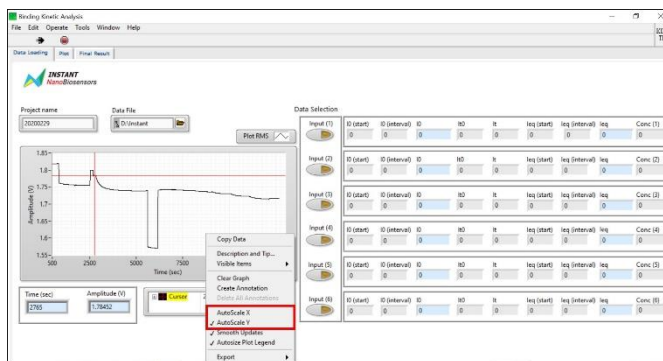
Step 2. Under the “Data Loading” tab, enter the project name.



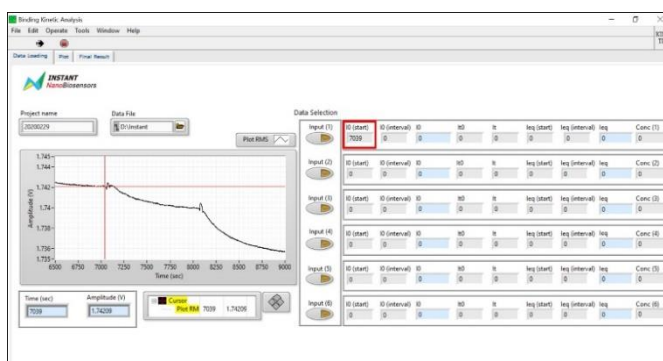
Step 3. Browse and import the raw data of a previously established standard curve by selecting the “Raw Signal” file. Click on the arrow to begin data analysis. Sensorgram of the imported experiment is displayed.



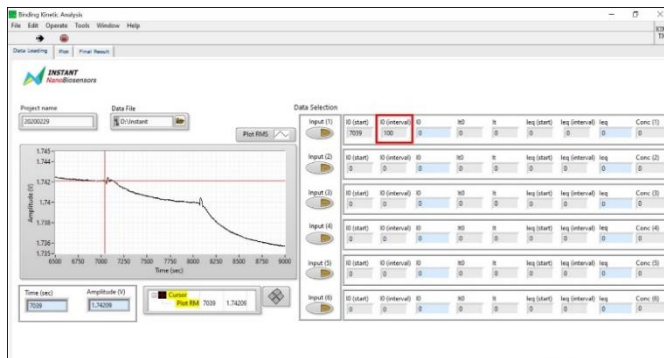
The sensorgram window can be adjusted to better view the data of interest. Make sure autoscale is off for the x-axis and on for the y-axis then enter the time span (x-axis) of interest. Signal amplitude (y-axis) will be automatically scaled to fit the time span (x-axis). Data points can be read with the red cursor and the cursor position (x, y = time, amplitude) is presented below the sensorgram. If the cursor is out of view, enter the x position of the cursor to be within the time span of interest to bring the cursor back into view.



Step 4. Adjust the cursor in the x direction to read the baseline time point. Enter the baseline time point t₀ (start). For the first concentration [Input (1)], the baseline time point is the final equilibrium time point of the buffer solution. For all subsequent concentrations [Input (2) to Input (6)], the baseline time point is the final equilibrium time point [leq (start)] of the previous concentration.



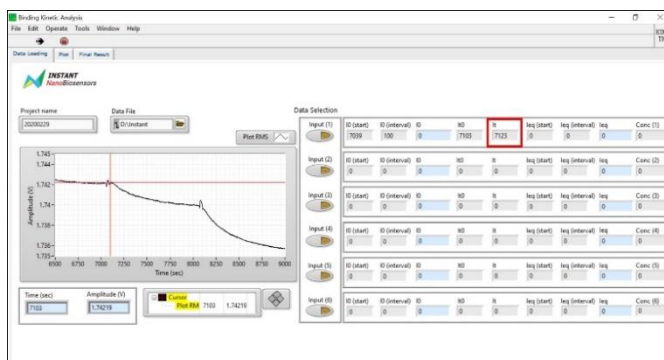
Step 5. Enter the number of data points to be used for calculation of the baseline I_0 (interval). An interval of 100 points is recommended.



Step 6. Adjust the cursor in the x direction to read the initial binding time point I_{t0} . Enter the initial binding time point I_{t0} . The initial binding time point I_{t0} is the time at which binding of the analyte with the ligand begins and is visualized in the sensorgram as the point of highest signal amplitude after introduction of the sample. This appears after a downward and upward signal.



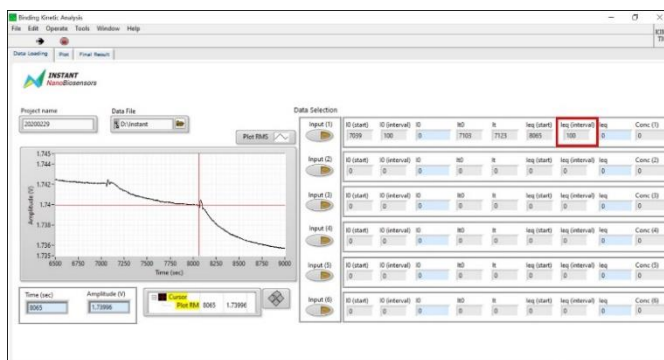
Step 7. Enter the analyte binding time point I_t , the time point 20 seconds after the initial binding time point I_{t0} . In other words, $I_t = I_{t0} + 20$.



Step 8. Adjust the cursor in the x direction to read the final equilibrium time point t_{eq} (start). Enter the final equilibrium time point t_{eq} (start).



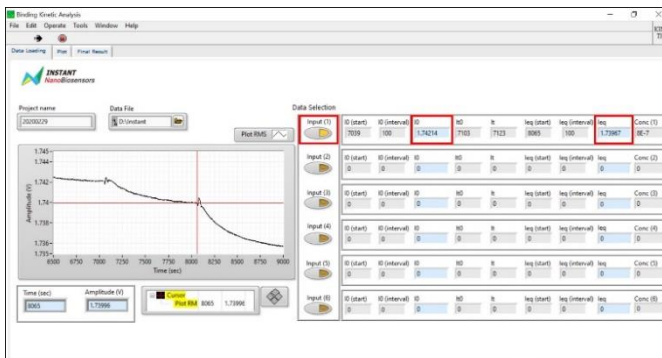
Step 9. Enter the number of data points to be used for calculation of the equilibrium interval t_{eq} (interval). An interval of 100 points is recommended.



Step 10. Enter the concentration of the analyte in scientific notation. The unit is g/mL.



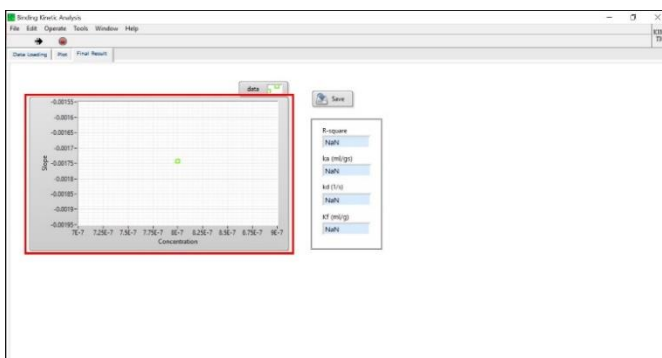
Step 11. Click on Input to calculate data. The calculated baseline and equilibrium amplitudes are presented in the “Data Loading” tab.



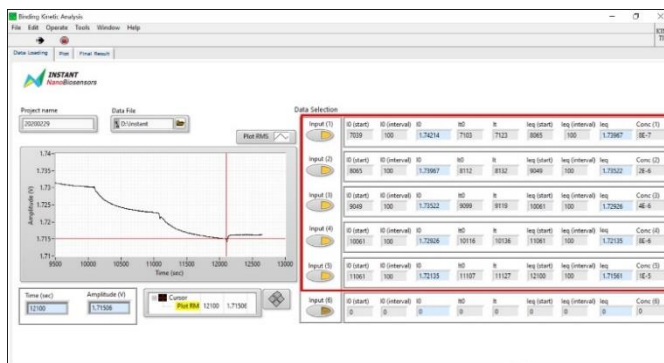
The fitted data are shown in the “Plot” tab. The standard error represents the data distribution in the analyte binding interval (from It_0 to It). To ensure the validity of the calculated data, the standard error of the chosen data points should be small ($< 2\%$). Adjust the chosen analyte binding initial time point It_0 and analyte binding time point It in the “Data Loading” tab to obtain a small standard error.



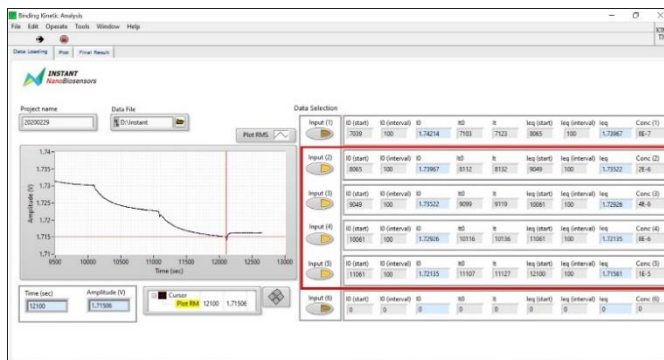
The fitted slope from the “Plot” tab is also shown in the “Final Results” tab.



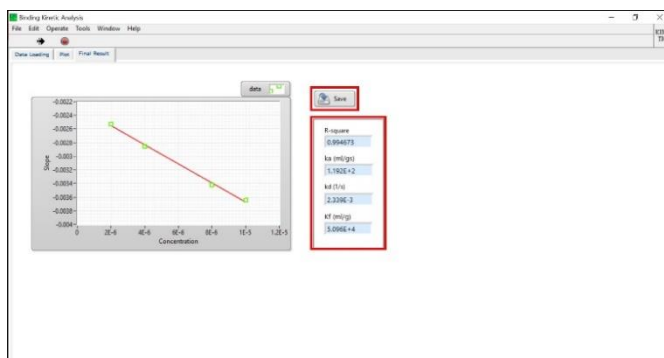
Step 12. Repeat steps 4 to 11 for the remaining concentrations.



Step 13. After all data points for the five (or six) concentrations have been optimally adjusted (standard error <2% for each concentration in the “Plot” tab), choose at least three concentrations in the “Data Loading” tab to achieve a linear correlation of R-squared >0.90 to calculate the binding kinetics and affinity data in the “Final Results” tab.



The association rate constant k_a , dissociation rate constant k_d , and formation constant K_f are presented in the “Final Results” tab. Export the analysis as a PDF file for future reference.



Reference:

[1] Chang et al. *Analytical Chemistry* 2013, 85, 245–250.