

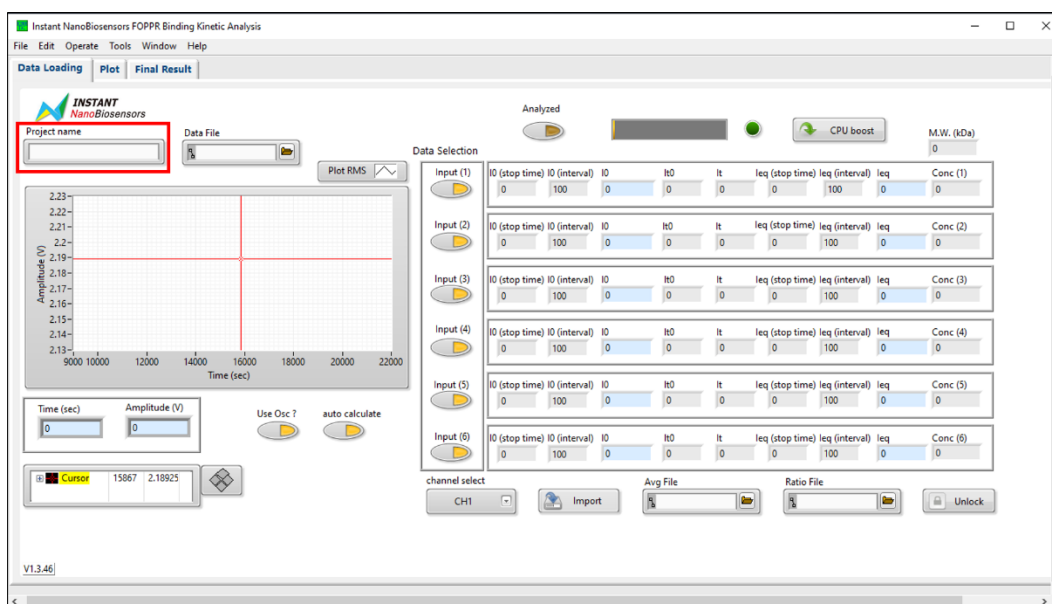
Determining the Kinetic & Affinity Constants of an Analyte

A Practical Guide to the FOPPR Binding Kinetics Software

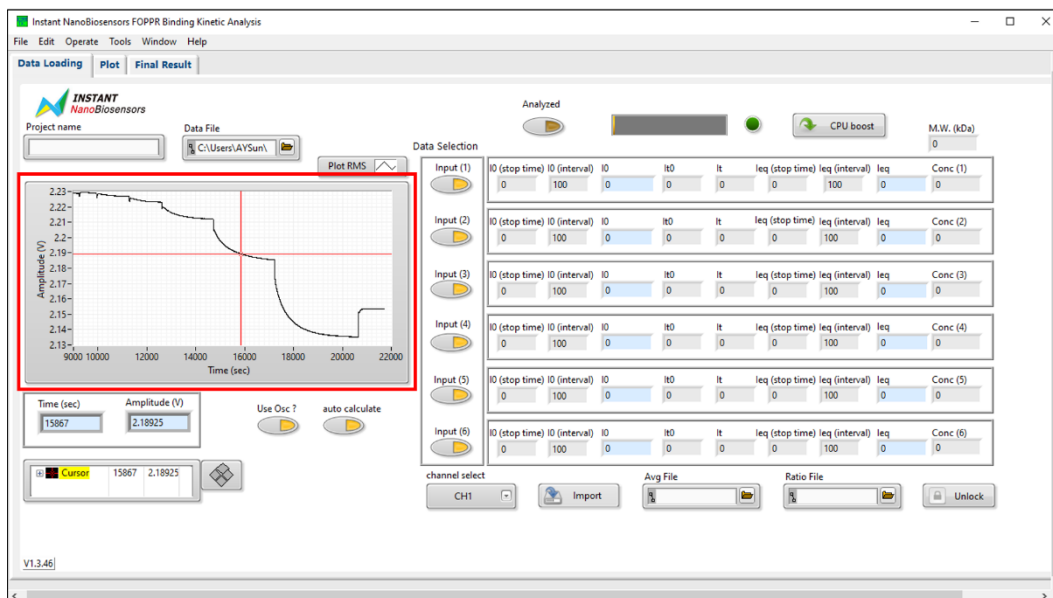
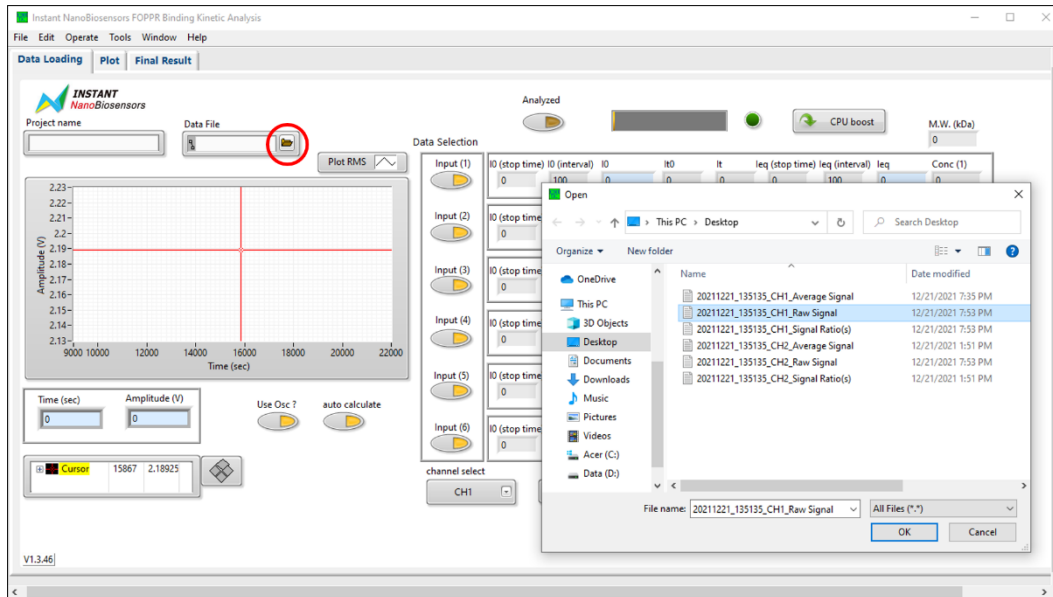
Binding kinetics and affinity of a ligand-analyte complex describe the reaction rate and formation strength, respectively, of the ligand-analyte pair. Determination of association rate constant k_a , dissociation rate constant k_d , and affinity constant K_D with FOPPR does not require repeated binding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

Step 1. Launch the FOPPR Binding Kinetics software.

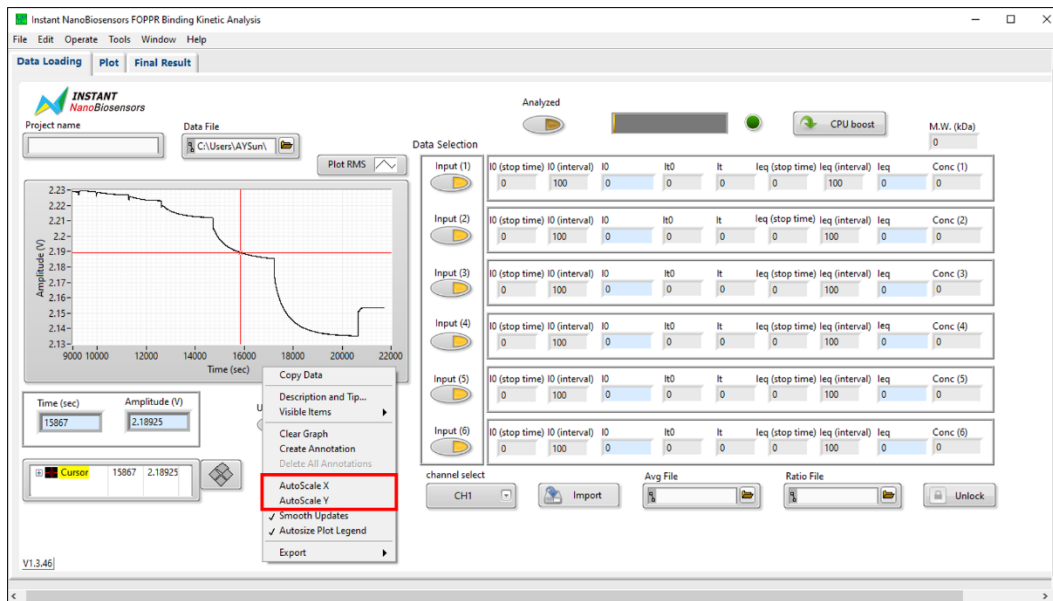
Step 2. Under the “Data Loading” tab, enter the Project Name.



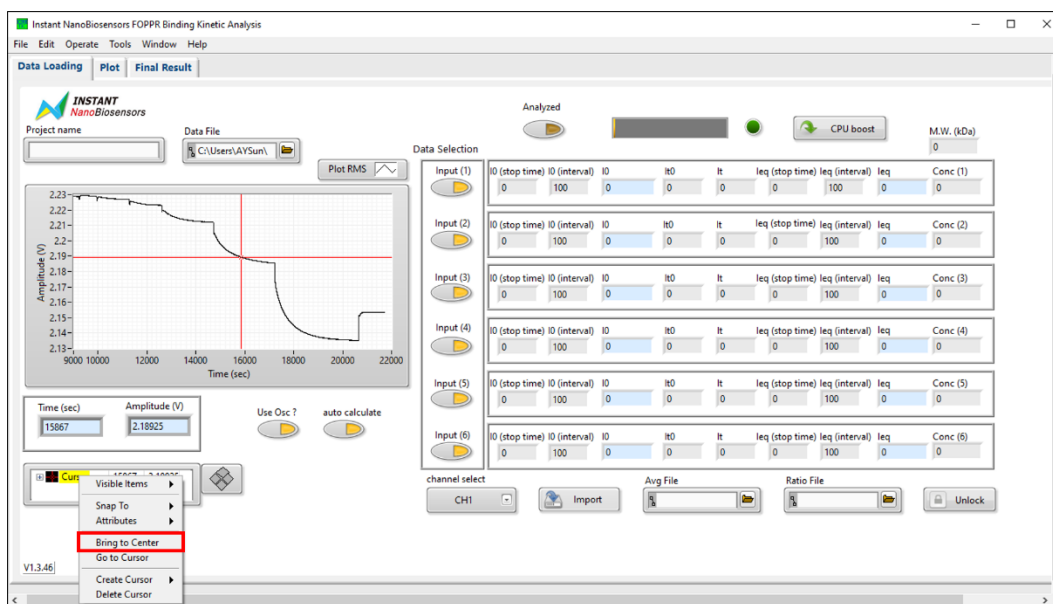
Step 3. Browse and import the raw data of a previously established standard curve by selecting the "Raw Signal" file. Sensorgram of the imported experiment will be displayed automatically.



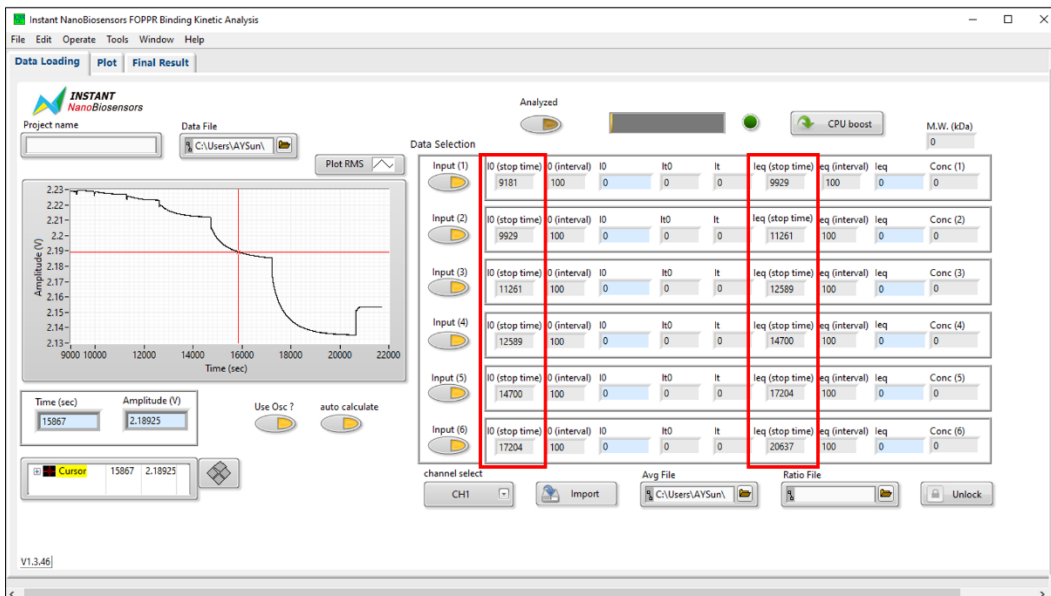
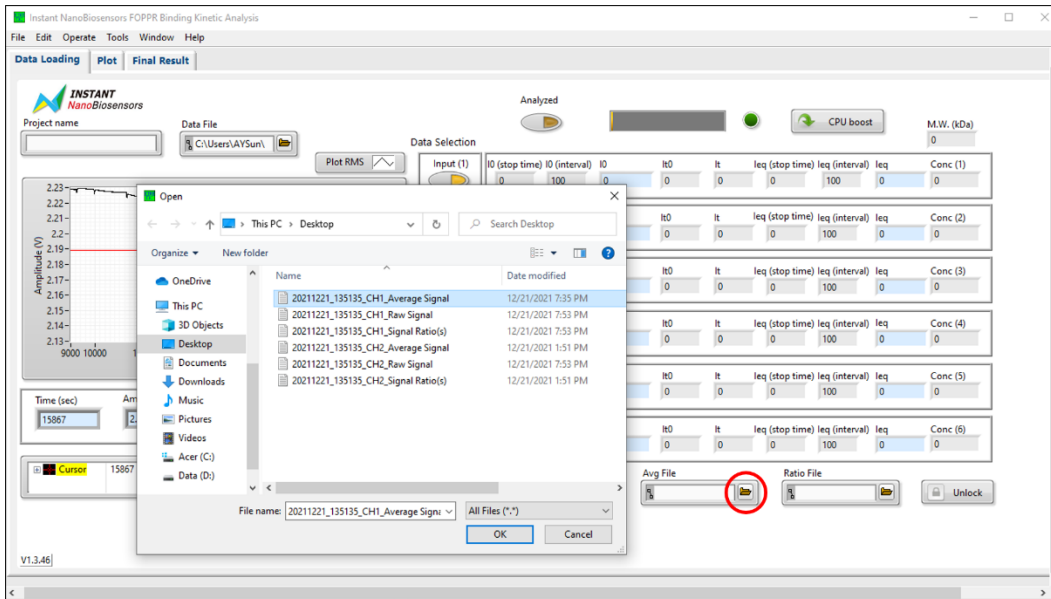
The sensorgram window can be adjusted to better view the data of interest. Right click the frame of the sensorgram to turn off autoscale for the x- and y- axes then enter the time range of interest.



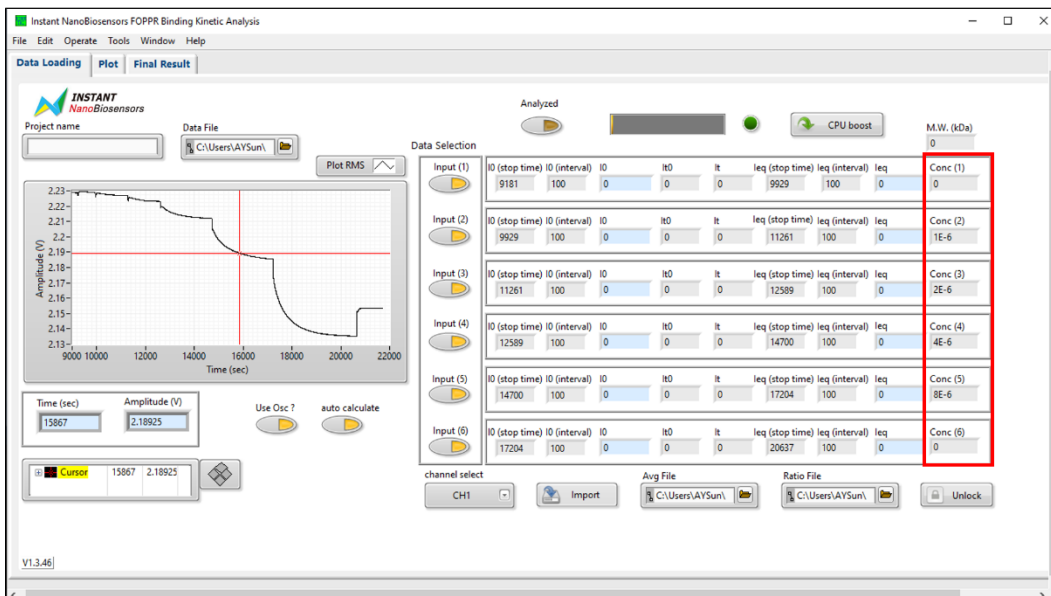
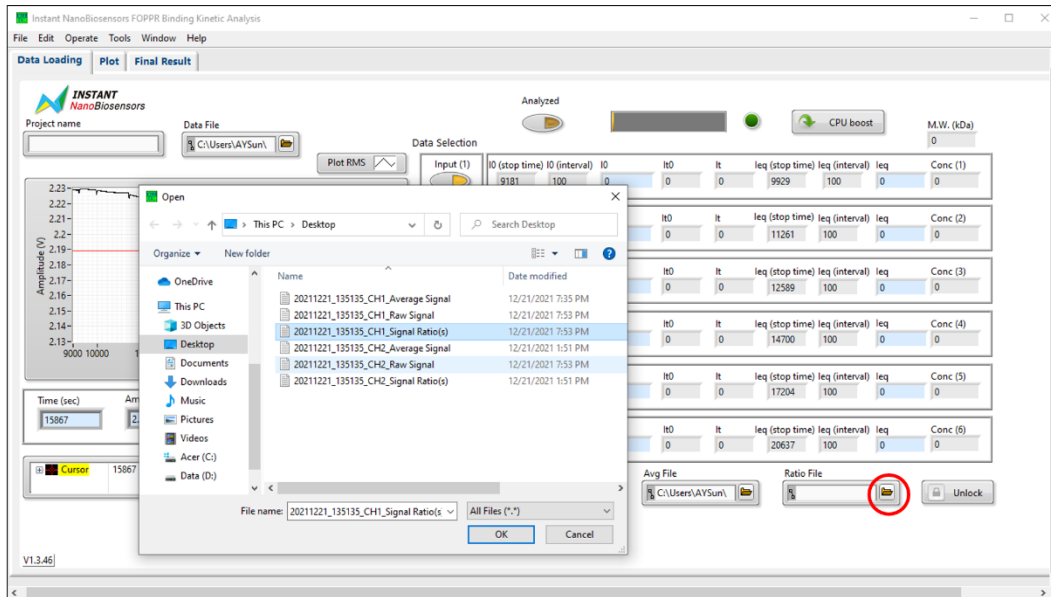
Data points can be read with the red cursor and the cursor position (x, y = time, amplitude) is presented below the sensorgram. If the cursor is out of view, enter the x position of the cursor to be within the time span of interest to bring the cursor back into view.



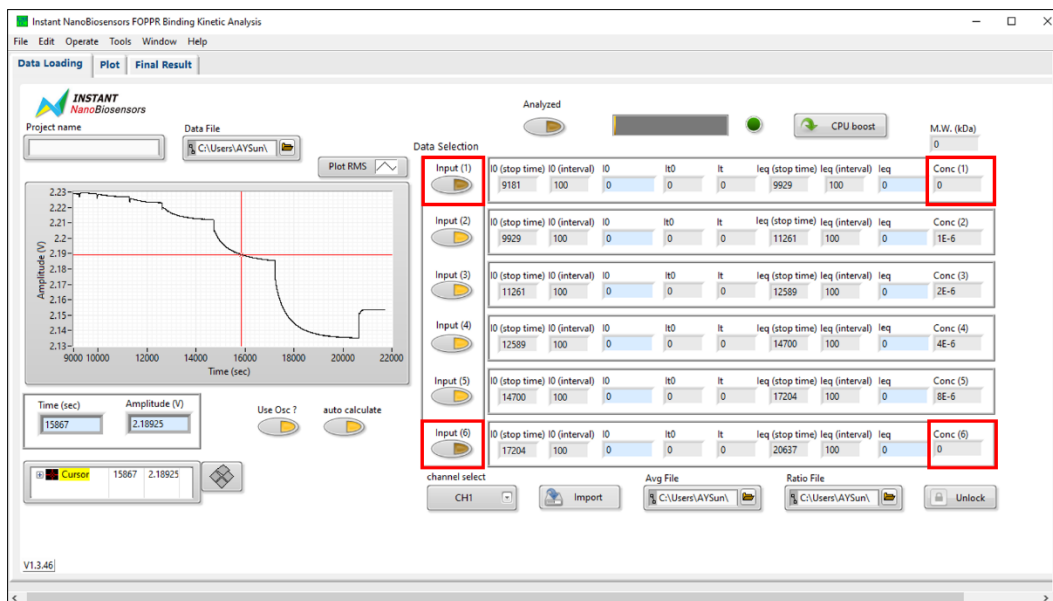
Step 4. Browse and import the signal ratio data of a previously established standard curve by selecting the “Average Signal” file. The time points for calculation (I_0 and I_{eq}) will be displayed automatically.



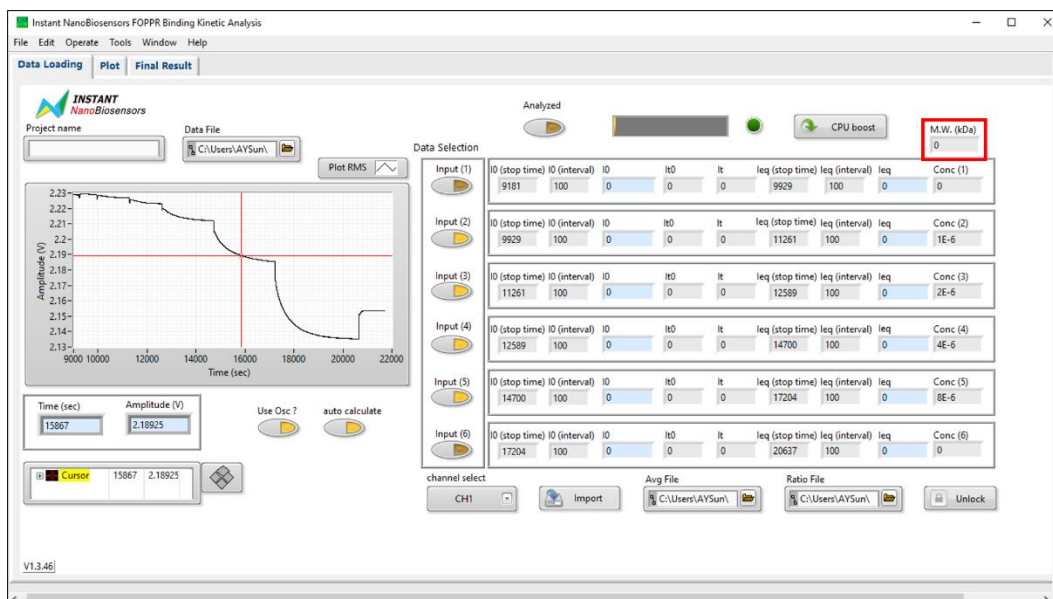
Step 5. Browse and import the concentration data of a previously established standard curve by selecting the “Signal Ratio(s)” file. The concentrations of the analytes will be displayed automatically.



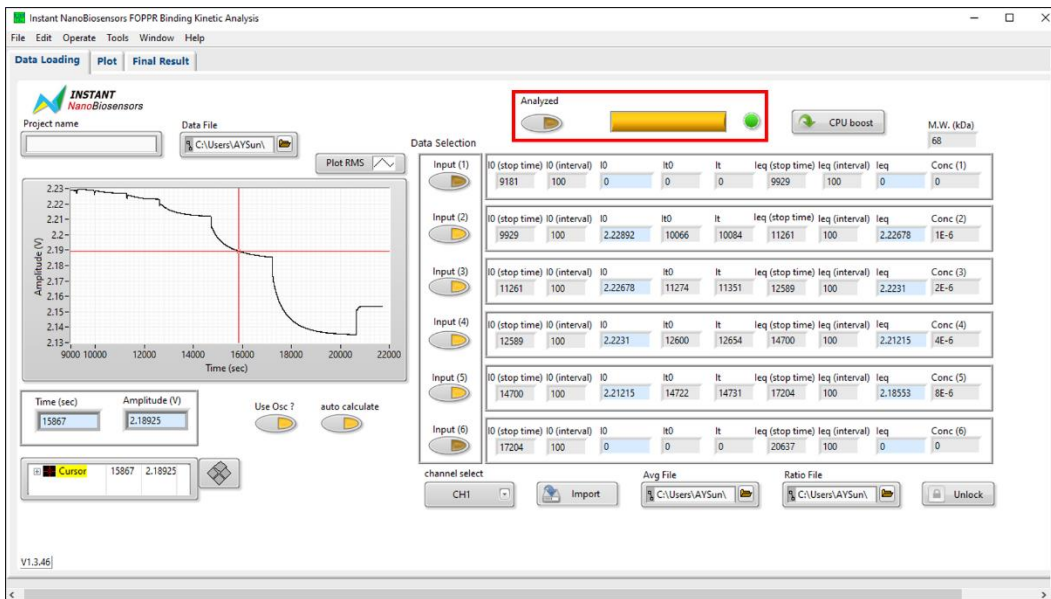
Turn off any data points unfit for analysis by unclicking the input.



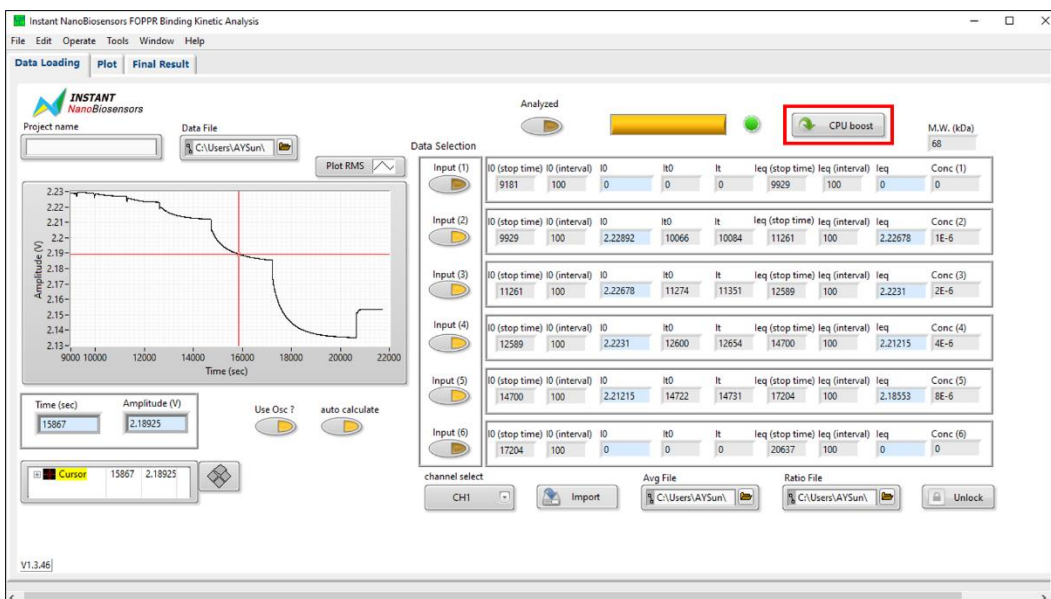
Step 6. Enter the molecular weight of the analyte.



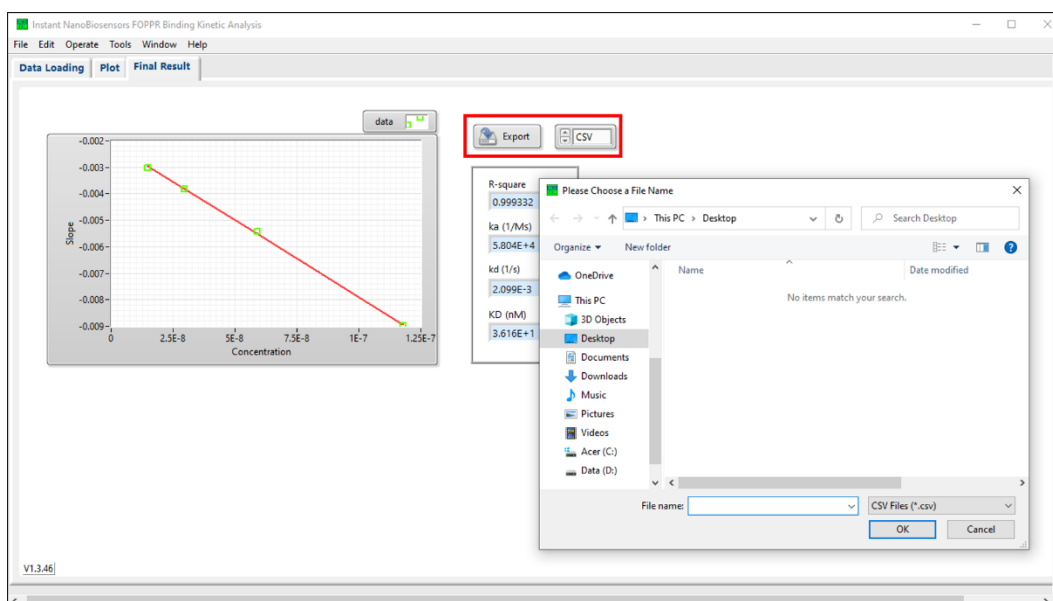
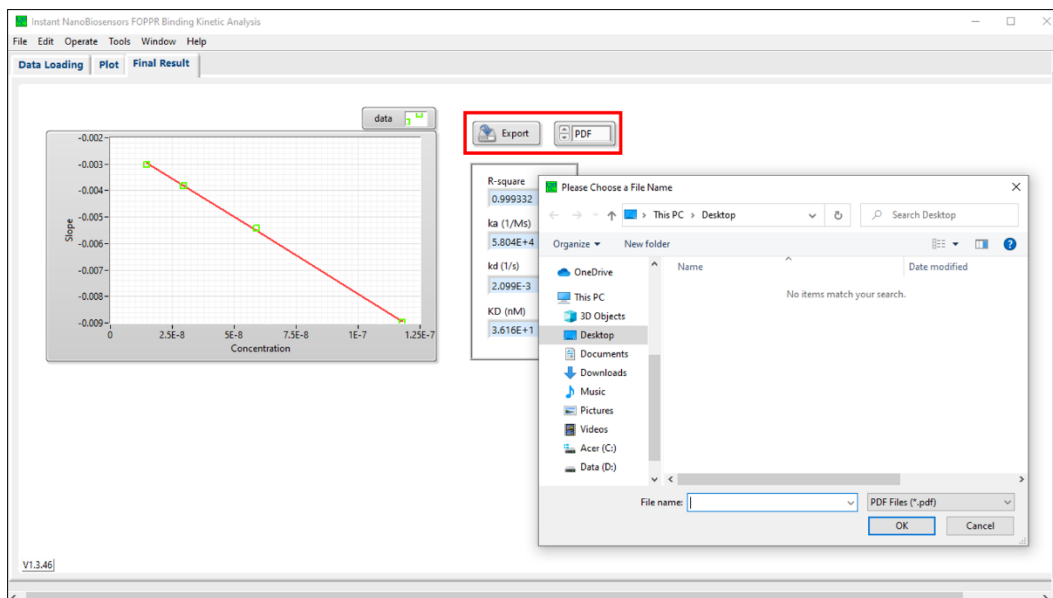
Step 7. Click on the “Analyze” button to begin data analysis. The yellow progress bar will run from left to right during analysis. A green light indicates completion of analysis.



If analysis takes a long time, click on the CPU boost button to speed up analysis.



Step 8. Analysis results are displayed in the “Final Result” tab. The association rate constant k_a , dissociation rate constant k_d , and affinity constant K_D data can be exported as a PDF report or as a CSV file.



Reference

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