How to use *KdCalc*

Short answer: open the GUI, choose options, enter experimental data and concentrations, press Analyze.

1. Terms
   1. Receptor: the labeled species that is visible by NMR
   2. Ligand: the unlabeled species that is added
2. All protein concentrations in μM, then Kd will be in μM.
3. Test data
   1. Files labeled “#.txt” contain the peak lists for each experimental point. The number in the file name is the approximate ligand/protein ratio. So 0.txt is the first point (free receptor), then 0.25.txt is next. 4.txt is the final point.
   2. File “concentrations.txt” contains the receptor and ligand concentrations at each experimental point. Note how the concentrations match the ligand ratios that are specified in the files names of the peaks lists (e.g. 0.txt, 4txt).
4. Preparation of data – peak picking and dependency on user
   1. Each line in the experimental peak lists (e.g. 0.txt, 4.txt) must correspond to the same residue as the identical line in other peak lists. In other words, the first line of each peak lists must correspond to the same residue; the second line in each peak list must correspond to the same residue; the final line in each peak list must correspond to the same residue.
   2. Practically, this is pretty simple to get correct.
      1. Open all spectra together
      2. Open the dialog box which shows how many peaks are in each peak list.
      3. Pick peaks one residue at a time
      4. Important: observe that after peaking each residue, the number of peaks in each peak list is consistant. In other words, after picking the first residue, each peak list should have 1 peak. After picking the second residue, each peak list has two peaks.
      5. Can be no other text above or below. Just a two column list of chemical shift values
5. Using KdCalc
   1. Launch program using “kdcalc” in command line or from icon.
   2. Using the menu to open a new Fast Exchange GUI
6. Using GUI
   1. Choose which nuclei were using in the experiment. This performs some validation related to chemical shift value ranges
   2. Choose which order the nuclei appear in the peak lists.
      1. 127.44 9.83 would be “Nitrogen Proton”
      2. 9.82 127.44 would be “Proton Nitrogen”
   3. Choose directory where results will be written. If the same directory is already created, then it will be overwritten
   4. Enter experimental data
      1. Choose a peak list and then enter the corresponding receptor and ligand concentrations. Note that protein concentrations can’t be entered unless a file is chosen.
      2. Continue enter data (preferably in the order the experiments were done, with free receptor as the first point and the highest ligand/protein ratio as the final point)
   5. Press Analyze or Save
      1. Analyze: will run the program and produce output (takes a few seconds because its writing many images of fits to disk). If the program thinks it was successful (fair amount of validation is included), then a dialog box will appear indicating results were written to disk.
      2. Save: will save data in binary format. Only valid data can be saved
      3. Issues: if exception dialog appear, try to follow the instructions.
      4. If nothing happens when Analyze is pressed, then an exception that wasn’t caught might have been thrown. This is something bad that was not anticipated by developer. If you launch the program from the command line, and run the program again, then the stack trace from the exception will be printed to the terminal window. Would be helpful if you saved this stack trace and emailed the developer ([alex.rizzo2000@gmail.com)](mailto:alex.rizzo2000@gmail.com)).
7. Results
   1. finalResults.txt
      1. Kd from cumulative fit (global)
      2. Δω for each residue that was fit with the global Kd
      3. Presentation fit: a way to plot the experimental data vs the model which would be appropriate for a publication figure.
   2. finalFit.png – the data from the “presentation fit” in a line graph
   3. resultsByResidueTwoParamFit directory - contains a .txt file and image for each residue that fits both Kd and Δω individually for each residue.
   4. resultsByResidueKdFixed directory - contains a .txt file and image for each residue that fits only Δω individually for each residue using the Kd from the global fit.
   5. twoParamFitByResidue.txt – contains the Kd and Δω for each residue when fit individual. Both Kd and Δω are fit for each residue.
   6. sortedPeakLists.txt
      1. Contains the raw data sorted by residue (data came in sorted by experimental point.
      2. Inspecting this should give some idea if the program worked (or at least if sorting worked). For each residue, the chemical shift values gradually change in one direction.
   7. identifierNumberList.txt– matches the arbitrary identifier (number that was attached to each residue when the data was read in) to the location of the peak for free receptor. This will help to figure out which assigned residue this corresponds too.