AnglerFish

Limit ionic mobilities and *pKa* constants fitting tool

User guide

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Overview

AnglerFish intended use:

- Fit pH dependence of effective electrophoretic mobility of a chemical compound with a theoretical function.
- Derive thermodynamic *pKa*s and *limit ionic mobilities* (μ_{lim}) of the chemical compound.
- Correct the derived thermodynamic constants for ionic effects using both *Debye-Hückel* and *Onsager-Fuoss* models.

Overview

Required input data:

- Experimentally obtained effective mobilities of the compound of interest (=analyte) in a "reasonable" pH range.
- Composition of buffers that were used in experiments.
- Estimated pKas and μ_{lim}s.
- Reasonable pH range is such a range that contains at least three data points where the analyte is fully charged.
- If the analyte is an ampholyte, both positive and negative form at full charge is required.
- Number of analyte's dissociation states is theoretically unlimited but analytes with a lot of dissociation states may be impossible to fit with sufficient precision.

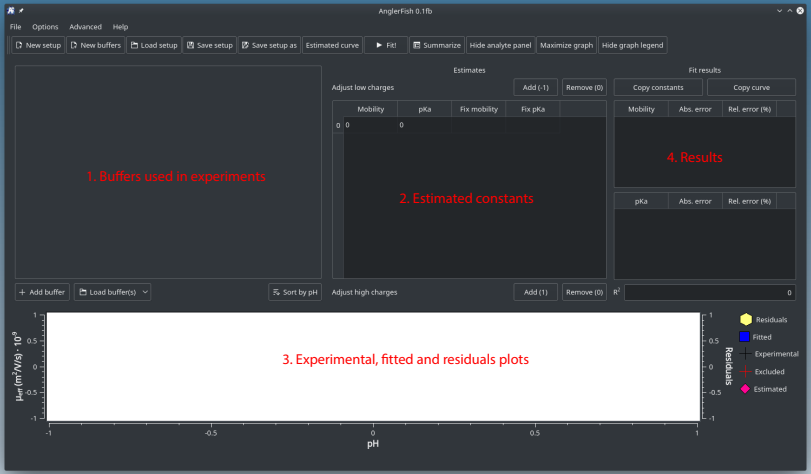
Overview

A few things to keep in mind:

- The fit results will be only as good as your experimental data.
- Buffers with different composition but same pH may affect your analyte's mobility differently.
- Even though the included database of common compounds contains *pKa* values of excellent quality, you should always check that the calculated and measured pH of your buffers match. If there is a considerable mismatch, you may find some tips in the troubleshooting section.

Overview

AnglerFish main window

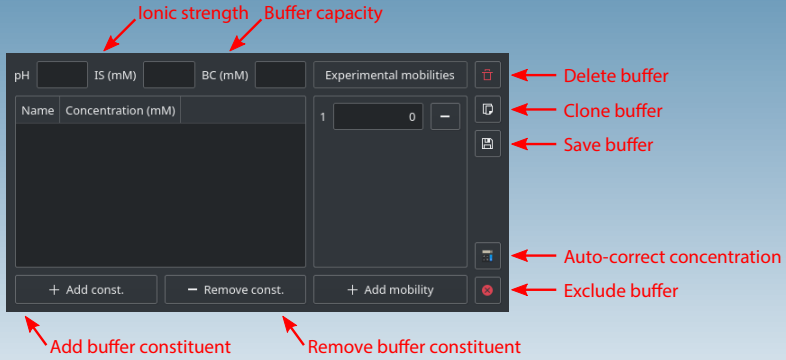


Adding buffers

There are two ways how to add a buffer to *AnglerFish* setup. One possibility is to define a buffer manually. In order to do so, click

button in the middle left part of the main window.

A new section with an empty buffer will appear in the left part of the main window.



Adding buffers

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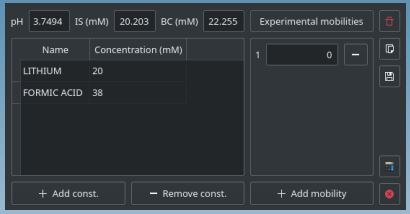
Add or remove buffer constituents. The process of adding buffer components is identical to *PeakMaster 6*. Please refer to *PeakMaster 6* user guide for details.
- Removes the buffer from the list.
- Clones a buffer. This creates a new buffer with the same composition as the current one. Values of experimental mobilities are not copied.
- Exports the buffer into *PeakMaster 6* JSON file.
- Automatic concentration correction. This is explained further in the manual.
- Excludes buffer from fit. This is explained further in the manual.

Adding buffers

A sample buffer containing 38 mmol dm^{-3} formic acid and 20 mmol dm^{-3} lithium.



Click on *Concentration (mM)* cells to adjust concentrations. pH and ionic strength is recalculated immediately.

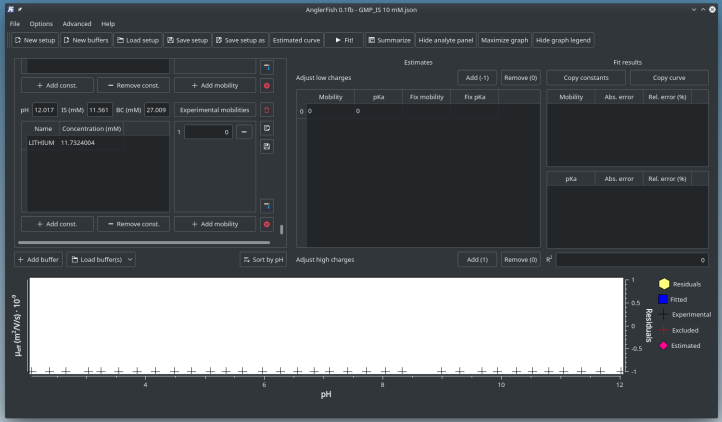
Adding buffers

- Alternatively, a buffer can be loaded from a *PeakMaster 6* JSON file. To do so, click

button and pick a file form the dialog.
- If you receive an error message, the file you selected is probably not a *PeakMaster 6* JSON file.
- Composition of analyte zone and CE system settings are ignored by *AnglerFish*.
- It is possible to pick multiple files simultaneously.

Adding buffers

AnglerFish window with a series of buffers.

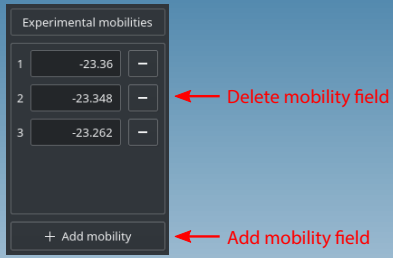


Adding experimental mobilities

- Each buffer must be accompanied by effective mobilities of the analyte that were measured experimentally in that buffer.
- Experimental effective mobilities are *signed*. Mobility of analyte that migrates towards negatively charged electrode is positive and vice versa. In other words, dissociation states with positive net charge have positive mobility and vice versa.
- If you conduct multiple measurements in one buffer, it is recommended to enter all measured mobilities instead of an average value.

Adding experimental mobilities

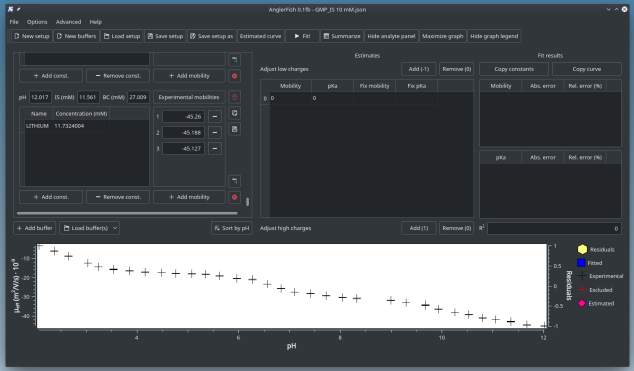
Experimental mobilities panel with three mobilities entered



- Use the respective buttons to add or remove mobility fields. There must always be at least one mobility field.
- Mind that the expected mobility unit is $m^2/V/s \cdot 10^{-9}$.

Adding experimental mobilities

AnglerFish window with fully defined experimental conditions



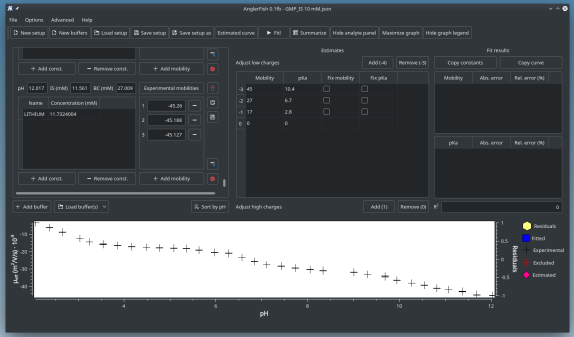
Notice the plotted black crosses in the bottom section of the window. Black crosses represent the experimental mobility curve.

Adding estimated parameters

- *Levenberg-Marquardt* nonlinear fit algorithm requires estimated values of the parameters of the fitted function to start with.
- The estimates shall be reasonably close to the actual values. Insufficiently good estimates may cause the fit to fail or converge to bogus values.
- There is no universal way how to come up with "good enough" estimates.
- A few tips how to derive reasonable estimates of *pKa*s and μ_{lim}s are listed further in this guide.

Adding estimated parameters

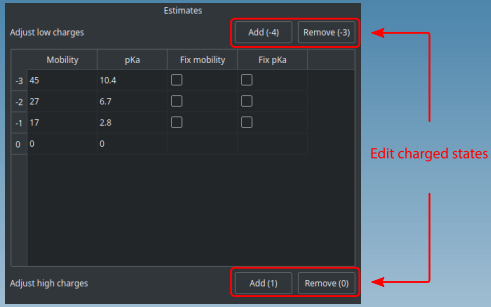
Use the center panel to specify dissociation states of your analyte and the initial estimates.



- Beware that if the dissociation states are not entered correctly, the fitting algorithm will not detect that. This will lead to bogus results.

Adding estimated parameters

Example initial estimates for *guanosine monophosphate*.



- You may use the *Fix* checkboxes to exclude a parameter from the fit.
- Value of a fixed parameter will remain at the initial estimated value.

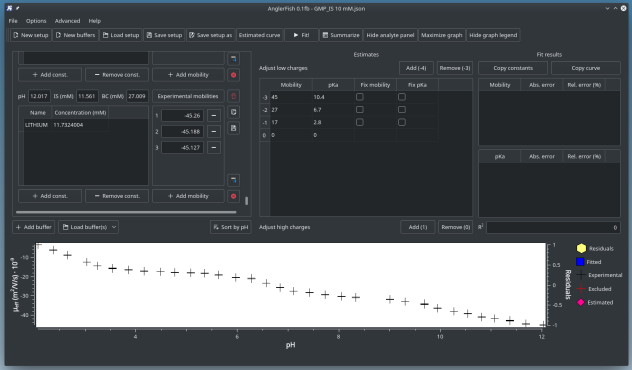
Adding estimated parameters

AnglerFish applies some constraints on the values of the estimated parameters, specifically:

- *pKa* values must be descending most negatively charged species toward the most positively charged species.
- μ_{lim} value of a form with higher absolute charge must be within certain interval based on the μ_{lim} of the previous form. This limitation is optional and is discussed in more detail further in the guide.

Getting results

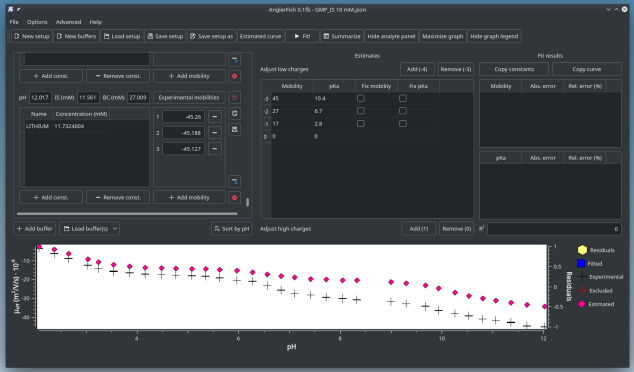
Fully set up *AnglerFish*



Before you perform the actual fit you may wish to check what would the expected mobility curve look like with the estimated parameters. Do to so, click Estimated curve.

Getting results

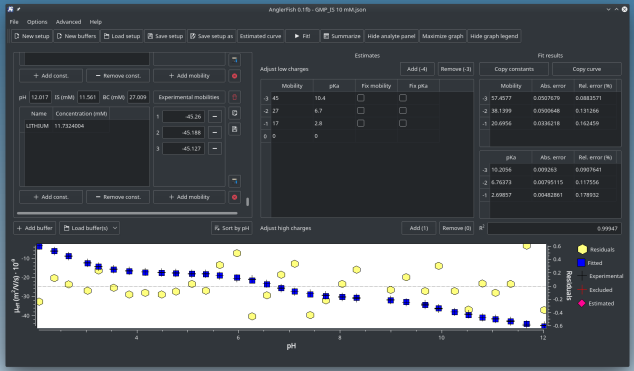
AnglerFish with the estimated mobility curve displayed in purple.



You can perform the fit by clicking the ▶ Fit! button.

Getting results

AnglerFish with results displayed



- Blue squares mark the theoretical mobility curve
- Yellow hexagons mark the residuals. Residuals scale is on the secondary (right) Y-axis

Toggling ionic effects compensation

Experimentally obtained mobilities are affected by electrostatic interactions between the ions in a solution. Algorithm used by *AnglerFish* provides two models that allow to compensate for these ionic effects and enable calculation of true thermodynamic *pKa*s and limit mobilities.

- **Debye-Hückel** – Compensation of *pKa* shifts by using activities instead of concentrations.
- **Onsager-Fuoss** – Compensation of reduced mobilities due to electrostatic interaction.

Both corrections can be switched on or off in *Options* $\rightarrow$ *Ionic effects corrections* menu. Both corrections are switched on by default.

Toggling limit mobility constraints

In order to stabilize the nonlinear fit algorithm and reduce the possibility of calculating physically senseless results, *AnglerFish*'s algorithm applies constraints on the minimum and maximum values of computed limit mobilities. These constraints are based on the idea that e.g. $+2$ charged state has approximately twice the mobility of the $+1$ charged state etc.
Although it is highly recommended to use these constraints, the constraining can be disabled in *Options* $\rightarrow$ *Limit mobility constraints* menu. Information about currently used constraints can be also found there.

Excluding buffers from fit

If you suspect that some points of your experimental data set are highly deviated from the expected value, you may temporarily exclude (mask) them from the fit. This can be done by clicking on the exclude ( ) button. Doing so will exclude the entire buffer from fit. Excluded data points are marked with a red cross in the plot.

Automatic concentration correction

AnglerFish provides an option to calculate concentration of weak electrolyte in a buffer automatically. This functionality calculates appropriate concentration of one buffer constituent based on concentrations of other constituents and the requested pH.

To calculate concentration of one constituent, first click on the constituent whose concentration is to be adjusted. Then click on the *Automatic correction* ( ) button and enter the desired *pH*.

- Use this function cautiously! Always verify that the auto-corrected concentration of the selected constituent is sensible.

Generating summary file

AnglerFish provides the option to create a summary file with all input parameters and calculated results. To create a summary file, click Summarize button.

You may choose what data to include in the summary by checking the respective checkboxes.

- Include buffers – Includes composition of all buffers and the respective measured mobilities
  - Abbreviate buffers – Uses shorter names for buffer constituents
- Include estimates – Includes the used estimated parameters
- Include ionic effects – Lists which ionic effect corrections were enabled during calculation
- Include curve – Includes the experimental and theoretical mobility plot

Mismatching buffer pH

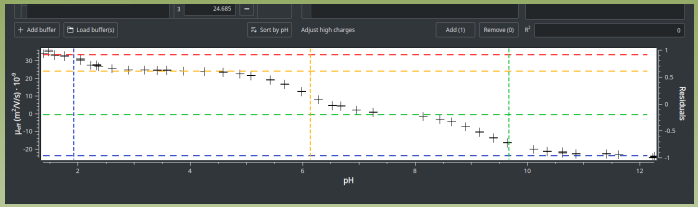
Common cases for mismatching predicted and measured pH include

- Miscalibrated pH-metering device
  - Check the device's calibration range
  - Check calibration temperature
- Incorrect constituent concentrations
  - Check purity of the stock compounds used to prepare the buffers. E.g. some compounds may be in a form of hydrate salts etc.
  - Database *pKa*s of some less commonly used compounds may be inaccurate

Gross pH mismatch is usually indicative of incorrect composition of the real buffer. Keep in mind that imprecisely prepared buffers may have a huge impact on the accuracy of the calculated values.

Getting estimates from experimental data

The image below shows an experimental mobility curve of histidine measured in buffers of ionic strength $= 20\,\mathrm{mmol\,dm^{-3}}$.



Horizontal lines: approx. limit mobilities – straight sections
Vertical lines: approx. *pKa* values – inflexes of the curve
Use the respective x or y axis value to get the approx. value

Red line	+2 charge	(32)	Blue line	+2 *pKa*	(1.9)
Yellow line	+1 charge	(22)	Yellow line	+1 *pKa*	(6.1)
Green line	0 charge	(0)	Green line	−1 *pKa*	(9.6)
Blue line	−1 charge	(−22)			

System requirements

- OS: Microsoft® Windows® XP™ with Service Pack 3 or above
  - 64-bit version requires Vista™ 64bit or above
- SSE2-capable x86 CPU