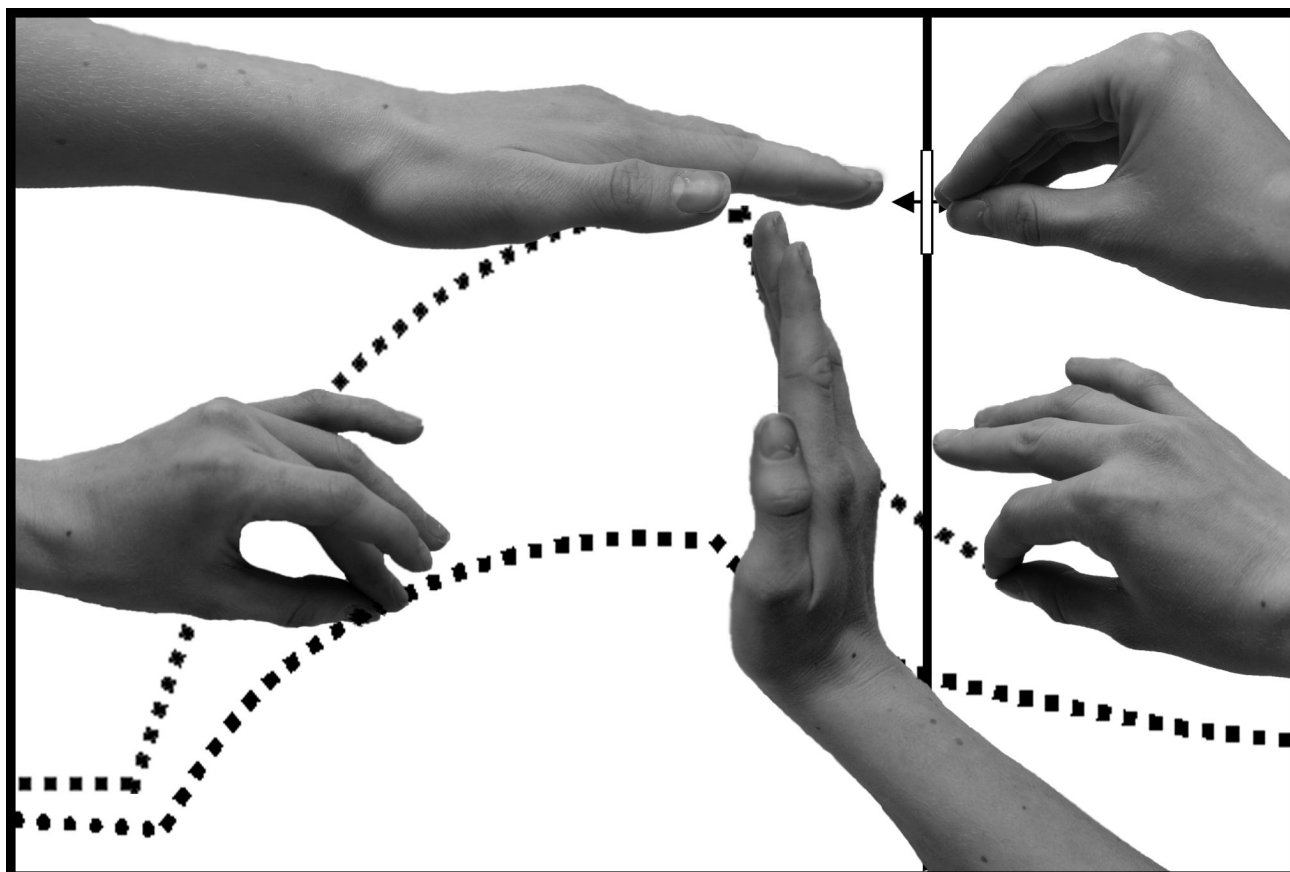


TraceDrawer 1.5

Handbook



TraceDrawer 1.5

Handbook

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Contents

Introduction	- 6 -
Collect	- 6 -
Evaluate	- 6 -
Compare	- 6 -
Compile reports	- 7 -
Glossary	- 8 -
Compatibility	- 9 -
Overview of the software	- 10 -
Getting started	- 11 -
Step-by-step example A	- 11 -
1. Add a file.....	- 11 -
2. Change run properties.....	- 11 -
3. Use the properties window	- 12 -
4. Change name	- 14 -
5. Create an overlay	- 14 -
6. Move the curves.....	- 14 -
7. Cut the curves	- 16 -
8. Zoom	- 16 -
9. Change appearance, curves.....	- 16 -
10. Change appearance, graph	- 17 -
12. Print overlay.....	- 19 -
13. Print the runs	- 19 -
14. Save	- 20 -
Step-by-step example B	- 21 -
1. Add files.....	- 21 -
2. Create the overlay.....	- 21 -
3. Create curve intervals.....	- 21 -
4. Move curves using curve intervals	- 22 -
5. Normalize	- 22 -
6. Copy	- 23 -
7. Save	- 23 -
Step-by-step example C: Kinetic evaluation	- 24 -
1. Add runs	- 24 -
2. Create an overlay	- 24 -
3. Enter concentrations	- 24 -
4. Set baseline to zero	- 25 -
5. Create an evaluation item.....	- 25 -
6. Add concentration changes	- 26 -
7. Cut spikes.....	- 28 -
8. Alter fit arguments	- 28 -
9. Create a OneToOne fit.....	- 30 -

10. Using the data	- 30 -
Step-by-step example D: Affinity estimation	- 31 -
1. Add run.....	- 31 -
2. Create an affinity item.....	- 31 -
3. Enter concentrations.....	- 32 -
4. Create a curve interval	- 32 -
5. Choose curve interval for signal.....	- 32 -
6. Signal vs concentration plot.....	- 33 -
7. Fit curve and optimize	- 33 -
8. Finalize.....	- 33 -
9. Summary.....	- 33 -
Summary of modification tools	- 34 -
Modification tab	- 34 -
Curve move.....	- 34 -
Curve offset	- 34 -
Curve X axis extents	- 35 -
Curve subtraction.....	- 35 -
Curve split.....	- 35 -
Curve concatenation.....	- 35 -
Cut spikes	- 35 -
Scaling tab.....	- 35 -
Normalize	- 35 -
Time scale.....	- 36 -
Curve scale	- 36 -
Data reduction.....	- 36 -
Differentiate	- 36 -
Curve Intervals tab.....	- 36 -
Summary of graph appearance tools	- 38 -
Axis settings	- 38 -
Font settings	- 39 -
Textbox.....	- 39 -
Line/arrow	- 39 -
Export	- 40 -
Export graph image.....	- 40 -
Export data	- 40 -
Summary of evaluation tools and models	- 41 -
Affinity/EC50 estimation.....	- 41 -
Affinity fit models	- 41 -
Kinetic evaluation	- 43 -
Change concentration.....	- 43 -
Cut data.....	- 43 -

Fit settings	- 43 -
Evaluation fit models	- 45 -
Kinetics competition	- 52 -
Competition fit models	- 53 -
Interaction Maps.....	- 56 -
Recommended reading.....	- 60 -
Summary items	- 61 -
On/off rate map.....	- 61 -
Interaction Map summary.....	- 61 -
General	- 62 -
Fonts and graph	- 62 -
<i>Add simulated data</i>	<i>- 63 -</i>
<i>Summary of menu-based and plot-based functions</i>	<i>- 65 -</i>

Introduction

Welcome to TraceDrawer, the quick and easy way to summarize and evaluate your real-time data! The many possibilities in TraceDrawer are found in four main functions: **Collect**, **Evaluate**, **Compare** and **Compile reports**.

Collect

TraceDrawer collects data from different runs into one file. This helps the user to keep track of the results and to grasp the overall picture of the investigation. Furthermore, all critical information about each curve is defined by the instrument vendor and stored in the TraceDrawer file.

Evaluate

Real-time interaction data is information rich. It is often possible to estimate important interaction characteristics like kinetics and affinity based on only a few curves. TraceDrawer contains tools for fitting common interaction models to either real-time binding traces (kinetic analysis) or steady-state results (Affinity/EC50 analysis). If your assay analysis only requires an end-point value, TraceDrawer can provide that as well.

Compare

The ability to browse through a large set of curves gives the user context and confidence in the results. Therefore, TraceDrawer provides overview through use of thumbnails and user-designed overlay plots. Details about each thumbnail object are at immediate hand – a detail area is activated by selecting any of the thumbnails.

Comparison of data is the key for generating knowledge and TraceDrawer is tailored for data comparisons. Many data manipulation tools are available, for example:

- Adjustment in X and Y direction
- Curve normalization
- Curve scaling, needed for comparison of curves with e.g. different timescale
- Curve split
- Curve subtraction, when using references
- Curve intervals from which signal levels can be extracted

Compile reports

Short reports can easily be compiled, summarizing sub-steps in a larger investigation. The graphs can be copy-pasted into other documents, or printed together with the experimental description and properties (ligand, concentration, temperature etc.) as a summarizing single sheet report.

Glossary

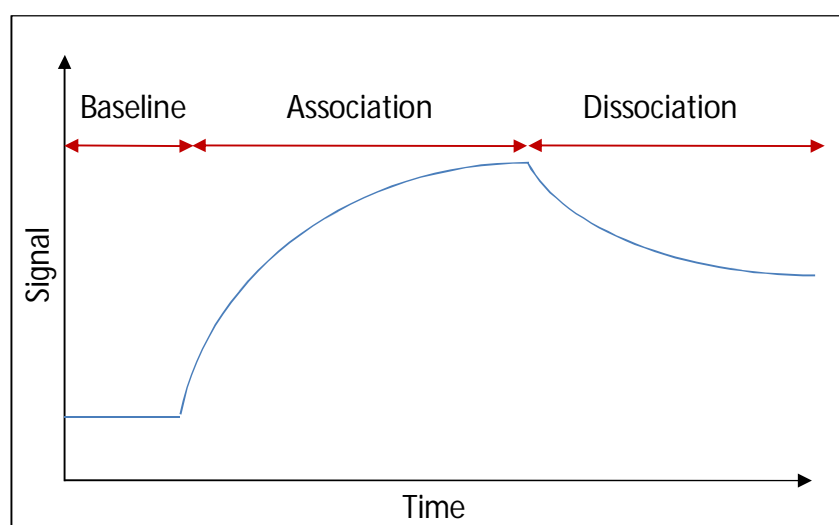
Investigation:	The scientific question under study
Run:	Measurements performed at one point in time stored in one file. An investigation typically comprises several runs.
Curve:	A single binding curve depicting e.g. how an antibody interacts with an antigen. One run may contain several curves.

Many real-time measurements consist of three common phases:

Baseline: The start of a run, when measuring background signal.

Association phase: The part of the run when the signal increases due to complex formation. Typically, a molecule A in solution binds/associate to an immobilized molecule B on the surface. Association phase is sometimes referred to as the uptake phase.

Dissociation phase: The part of the run when the concentration of free molecule in solution is zero. A will dissociate from the immobilized molecule B on the surface, resulting in a decreasing signal. Dissociation phase is sometimes referred to as the retention phase.



Software Installation

Insert the TraceDrawer Installation CD. This should automatically start the installation. If not, right-click on the CD icon in the file explorer and choose Install. The software requires .Net Framework 2.0. The installation program will check if this is already installed in the computer. If not, install it and rerun the TraceDrawer installation. The .Net installation file is included in the TraceDrawer Installation CD.

If problems emerge: Uninstall any previous versions of TraceDrawer installed on your computer and try again.

Compatibility

The compatibility of TraceDrawer depends on the license code received from the vendor. At the time of print, the compatibility list was the following:

Instrument type	Dedicated license code required
Attana® instruments (*.att)	yes
BioNavis® instruments (*.bne)	yes
LigandTracer® instruments (*.ltr)	yes
Sierra Sensors® instruments (*.sdf)	yes
Plain text files (*.txt)	no, always possible

If you use open plain text files, make sure to format them according to the file Example_textformat.txt available in the installation folder of TraceDrawer (typically C:\Program Files\Ridgeview Instruments AB\TraceDrawer).

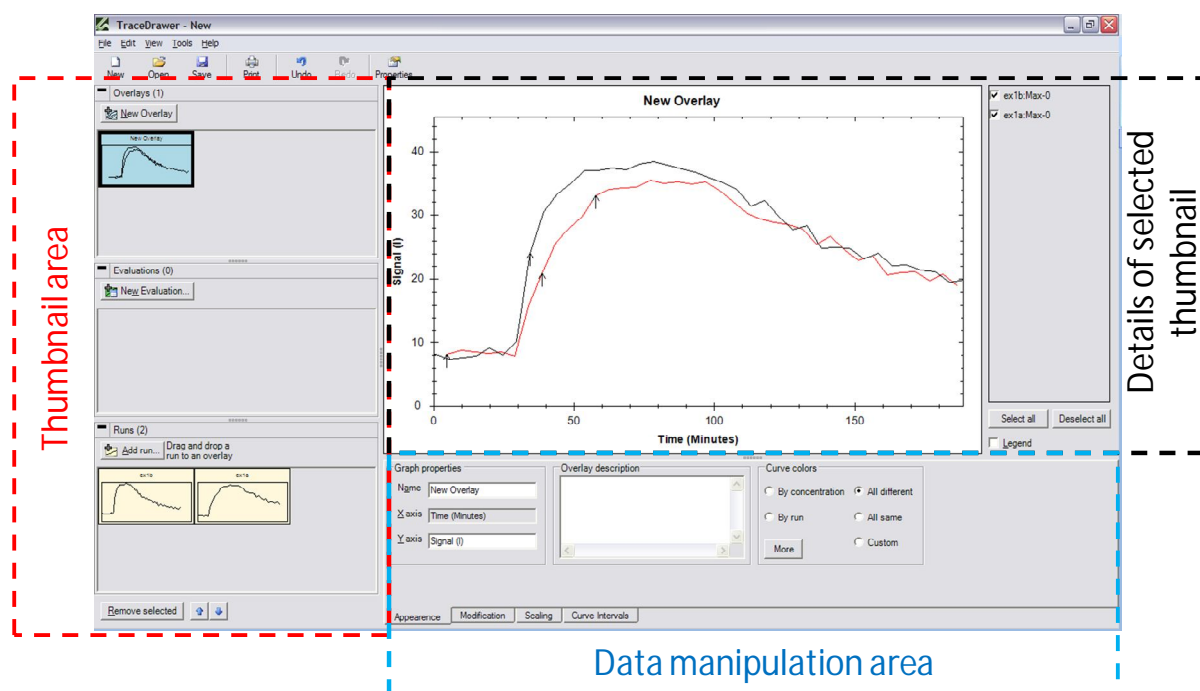
TraceDrawer can have several dedicated license codes installed, thus being compatible with several instruments. The default configuration values (for e.g. kinetic fitting) differ between are determined at installation.

Overview of the software

TraceDrawer has three main areas for providing the curve information needed to make decisions. Most of the detailed information is kept hidden until the user requests it, and this is what makes TraceDrawer a powerful tool for making summaries in large investigations.

The collected runs are shown as thumbnails in the left hand pane. The thumbnail area consists of three separated fields, one for opened runs (lower part), one for evaluation items (middle part) and one for created overlays (upper part). When selecting a thumbnail, details of it are depicted as a plot in the middle area, together with a list to the right to keep track of the curves in the plot. Other properties, such as ligand concentration and type of instrument in the run, can be found as a separate properties window, accessed through the right-click-menu of a thumbnail.

Curves can be manipulated in forms of appearance, data modification and scaling. This is done in the lower part of the window, where it is also possible to create curve intervals for extracting signal levels and calculating the relationship between curves. The different tasks are separated with tab buttons and normally apply to selected curves only.



Getting started

This section contains four examples with step-by-step instructions for creating comparisons of runs in TraceDrawer. You can follow the instructions and use the *.txt files in the Example Files folder under the TraceDrawer installation folder (typically C:\Program Files\Ridgeview Instruments AB\TraceDrawer\Example Files).

Step-by-step example A

1. Add a file

TraceDrawer is operated by adding files to the work area and then comparing results in an overlay plot. Start by pressing **Add run** in the left panel, and add the file ex1a.txt.

2. Change run properties

When the file is added, an **Open run window** will appear summarizing the experiment. Note that this window will appear differently depending on the format of the file added. For more information on the Open run window of your file format, press Help in the lower left corner.

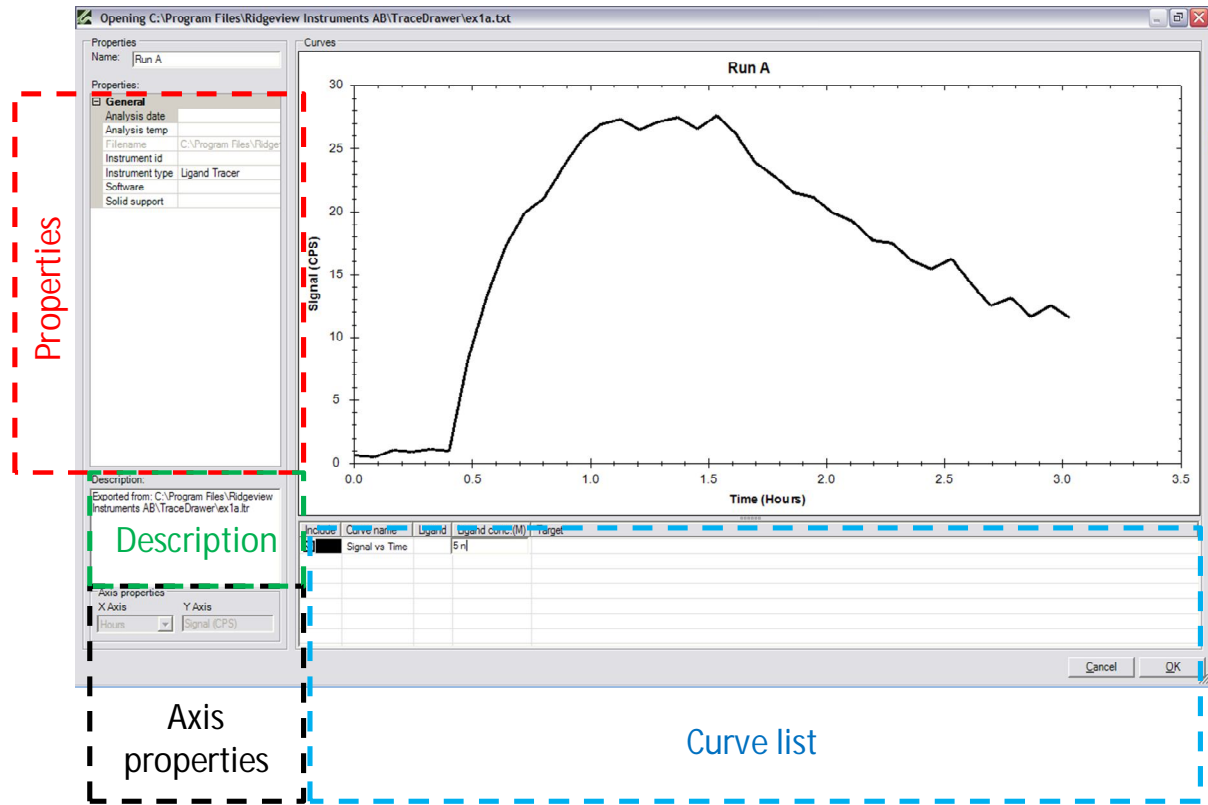
Change the **name** of the run in the upper left corner, e. g. to *Run A*.

Depending on file format, TraceDrawer can sometimes extract experimental specific data, which is automatically stored in the **properties** field to the left. Some of the cells in the properties table can also be modified by the user and supplementary information can be gathered in the **description** area. Feel free to explore these areas and change some of the properties.

The **axis properties** are found in the lower left corner. If the file contains information about the axis properties these fields are write-protected. This is the case here.

The **plot** shows the binding traces from the run. A list of the curves included is found below the plot, where more information about the curves can be added. In this case there is only one curve in the run. Click on the cell describing the ligand to activate it and change the name to *Ligand A*. Also, change the

concentration to 5 nM. The concentration is written in molar with the abbreviations m for mM, u for μM , n for nM or p for pM.

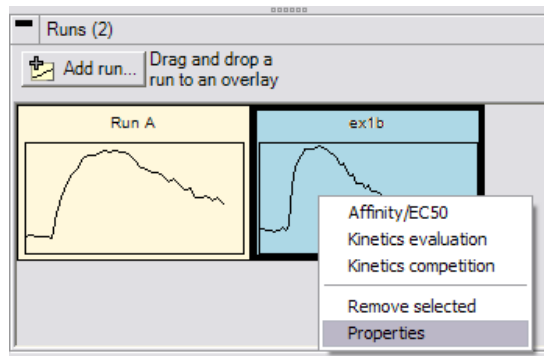


When finished, press **OK** in the lower right corner. A thumbnail representing the run will be created in the lower left area of the main window.



3. Use the properties window

Add the file ex1b.txt as described in step 1. Click OK without any modification. A thumbnail representing run ex1b.txt is now found next to the previous run.



Right-click on the ex1b-thumbnail and select **Properties**. The same information as found in the Open run window will now appear in a separate window. Change the concentration to 10 nM (e.g. *10 n* or *1E-8*) and click OK.

ex1b properties

Run: ex1b

Description:
Exported from: C:\Program Files\Ridgeview Instruments AB\TraceDrawer\ex1b.ltr

General

Analysis date	
Analysis temp	
Filename	C:\Program Files\Ridgeview Instruments AB\TraceDrawer\Example
Instrument id	
Instrument type	LigandTracer
Software	
Solid support	

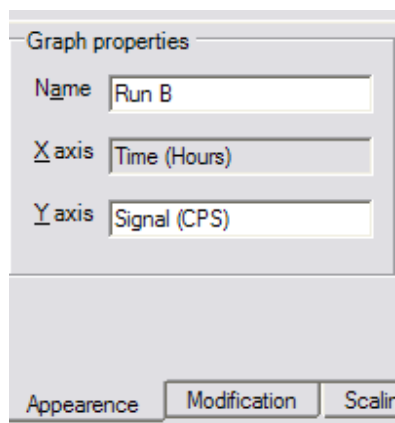
Curves:

Name	Ligand	Ligand conc.(M)	Target	Source	Curve description
Signal vs Time		1E-8		C:\Progra...	

Copy to clipboard Cancel OK

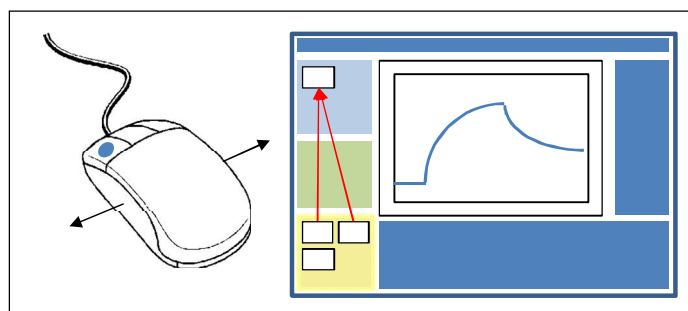
4. Change name

In the lower part of the main window you will find the data manipulation area, with manipulation tools separated with tab buttons. Type *Run B* as a new Name in the **Appearance tab**.



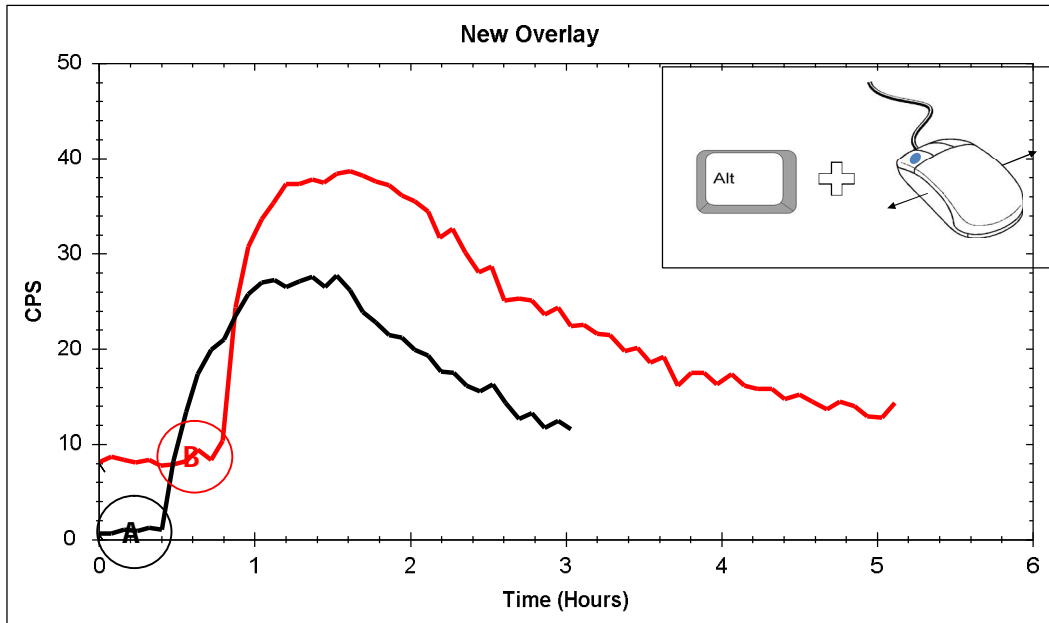
5. Create an overlay

Click on **New overlay** in the upper left area of TraceDrawer. This will create a plot where you get a good overview of curves from several runs simultaneously. Drag and drop your Run thumbnails to the overlay plot to include the data in the overlay. Note that data added to TraceDrawer are only copies of the runs. If binding traces are modified or curve properties altered, the original data is not affected. This way, you do not have to worry about original data accidentally getting lost.

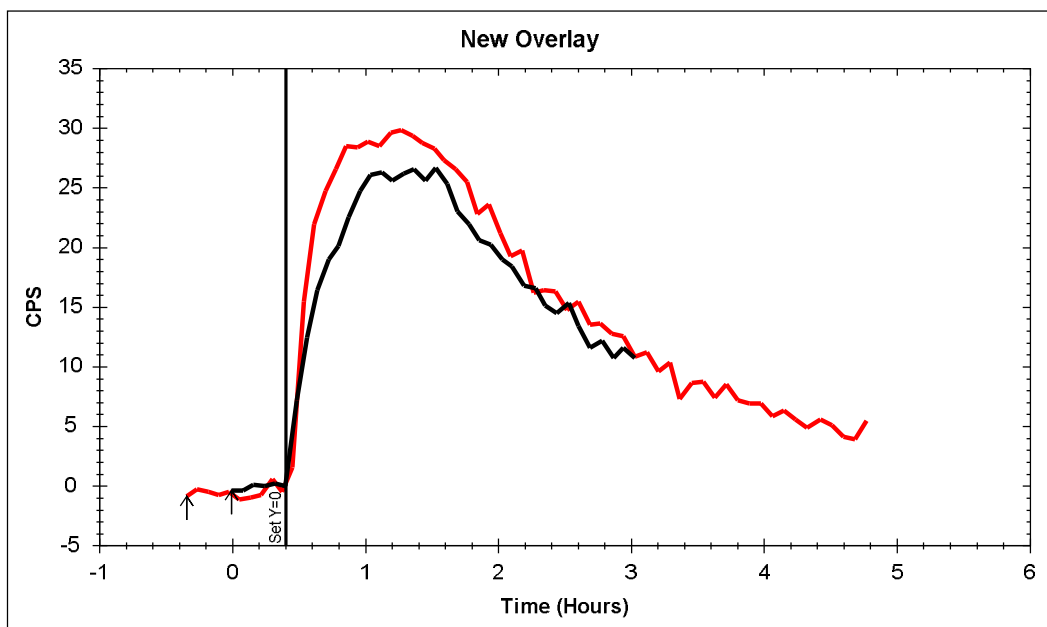


6. Move the curves

There are several ways of moving curves in TraceDrawer. Moving curves can be of use when comparing curvature and signal level. To manually move a curve, hold down the **Alt button**, click on the curve and drag it so that point A and point B intersect.

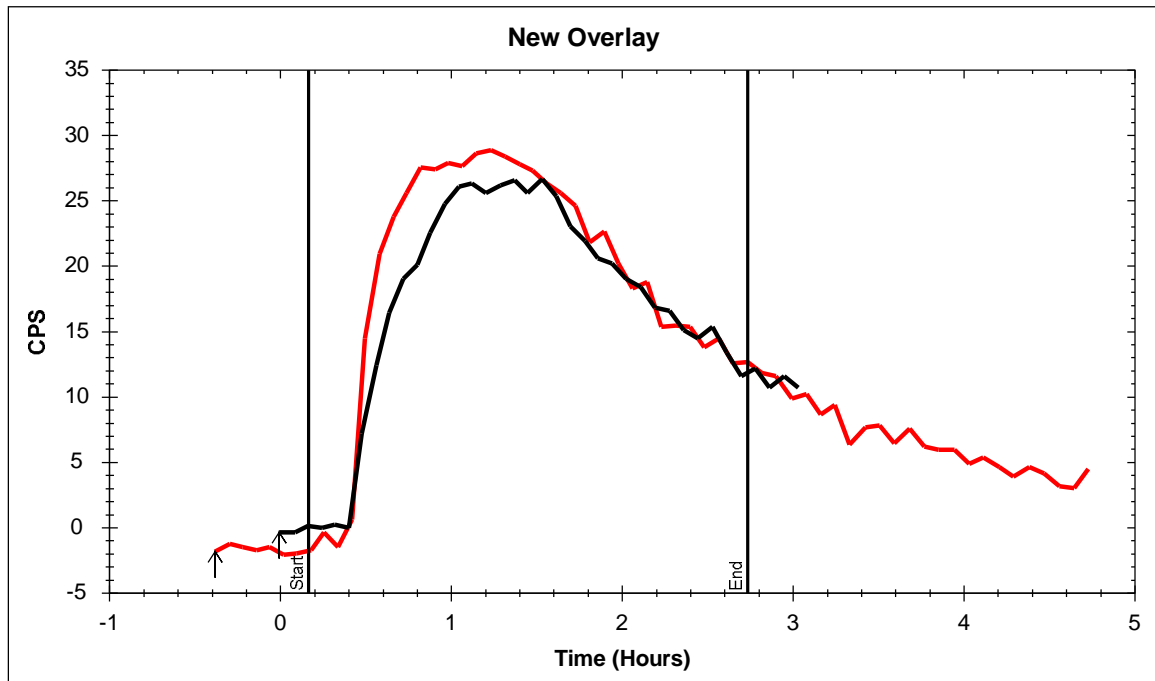


As the points A and B represent a background signal we want this value to be set to zero. Click on the **Modification tab** in the lower part of the main window and select **Curve offset**. This tool will only affect selected curves. Select the two curves by pressing the **Select all button** to the right, or left-click on the curves in the plot while holding down **Ctrl**. In this example we choose not to move the curves in X direction. This is done by unticking the **X=0** box. Move the cursor to the position of A and B and press **Apply**. The Y values for A and B are now set to zero.



7. Cut the curves

To make the curves equally long, choose **Curve X axis extents** in the **Modification tab**. Make sure that both curves are selected as described in step 6. Move the Start and End cursor to a suitable length, e.g. approximately as seen in the figure below, and press **Apply**.



8. Zoom

To get a better view of what is left of the curves, choose **Zoom to extents** in the **right-click menu** of the plot.

There are other zooming tools available:

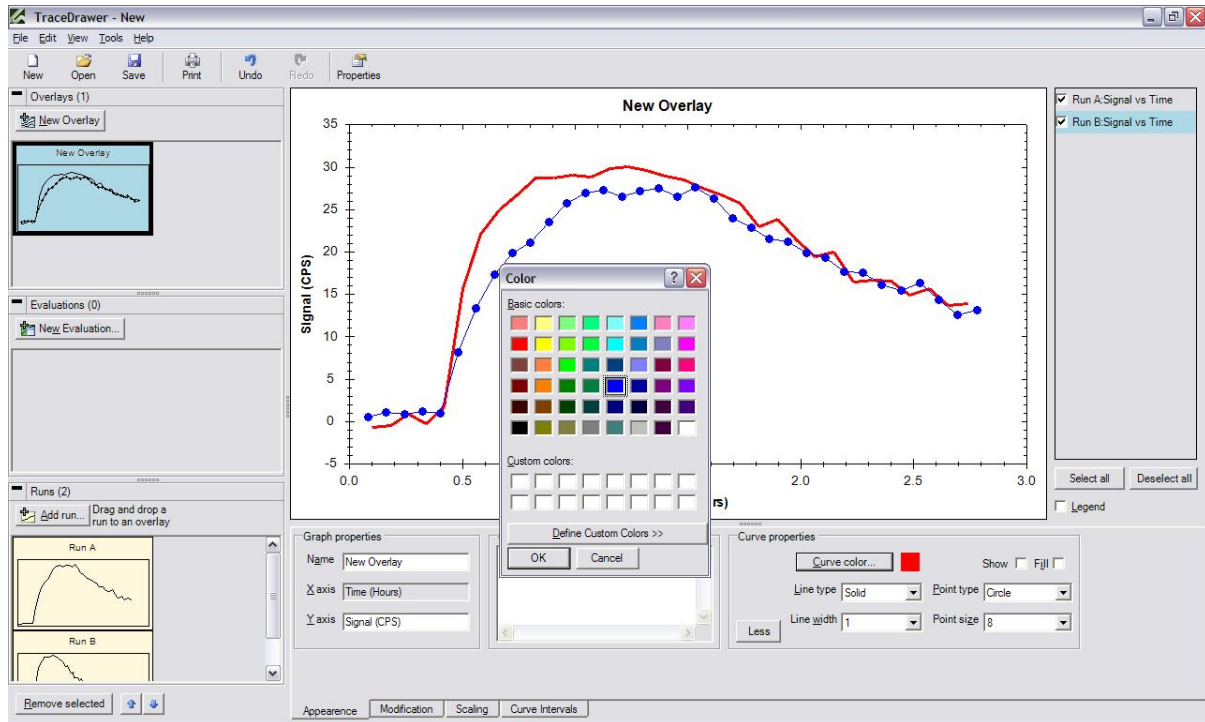
- To zoom in: Hold down the left mouse button and drag it to create a rubber band box in the plot.
- To zoom out (after zooming in): Double click with the left mouse button.

9. Change appearance, curves

The **Appearance tab** provides tools to control the look of your curves. In the **Overlay description field**, type *This is an overlay of Runs A and B*.

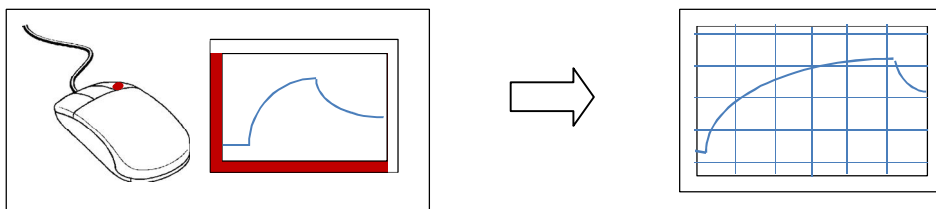
The area for changing curve appearance is found to the right of the Description field. The radio buttons enables you to quickly change the colors in a suitable

way. Click on **More**. This opens a new area where the curves can be modified in detail. Select one of the curves and change color and line width. Select the other curve and tick the box **Show**. This makes the data points of the curve appear. The points can be modified in type and size. Feel free to explore and modify as you wish.

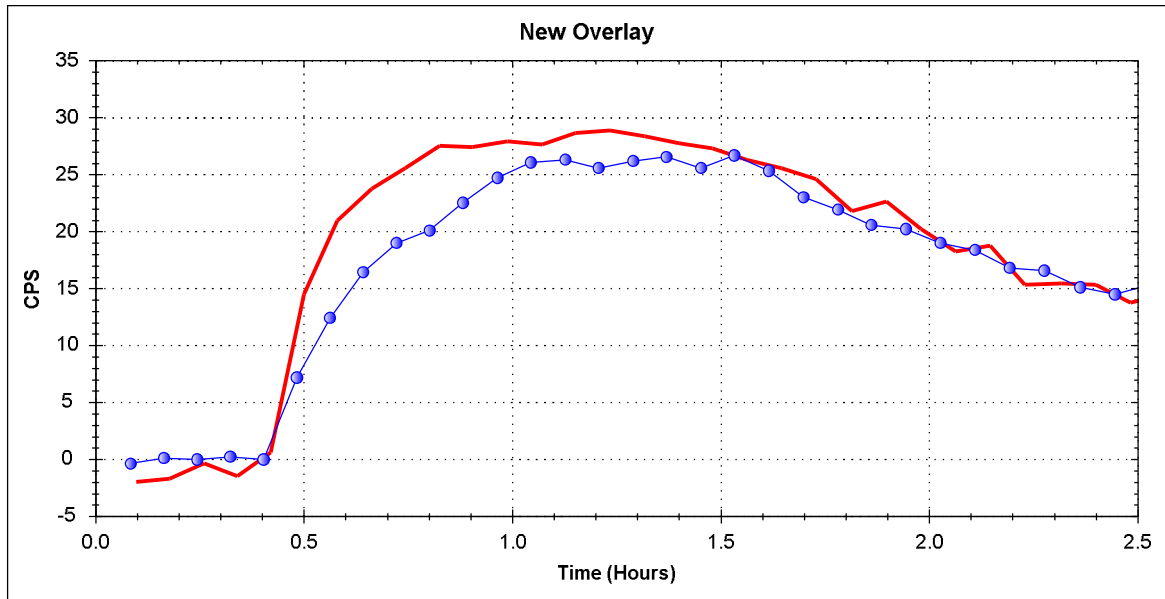


10. Change appearance, graph

The appearance of the graph, e. g. grid lines and the font of the title, can also be modified. Right click on the X or Y axis and choose **Axis settings**. Type 2.5 in the **Max** field under **X axis**. Tick the **Lock** box. This will adjust the zoom to always have a maximum value of 2.5 (hours) in X direction. Unticking the Lock box will make the zoom temporary.

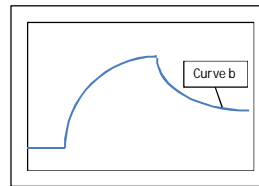
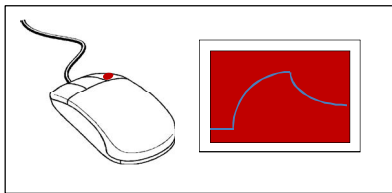


Tick the **Grid line** boxes associated with the major ticks of both X and Y axis.



11. Add arrow and textbox

Right-click in the plot and choose **Add textbox**. A **Create text box** window will appear. Type "This is curve B" and press OK. Hold down Alt and drag the textbox close to curve B.



Create text box

Text:

Left
 Center
 Right

Font... ■ Arial, 12 pt

Angle

Textbox border
 Textbox fill ■

Set on axis

On X axis
 Inside graph
 On Y axis
 Outside graph
 On Axis

Axis value:

Create line

Line width: ■

Move the line using Alt+Left click in the graph.

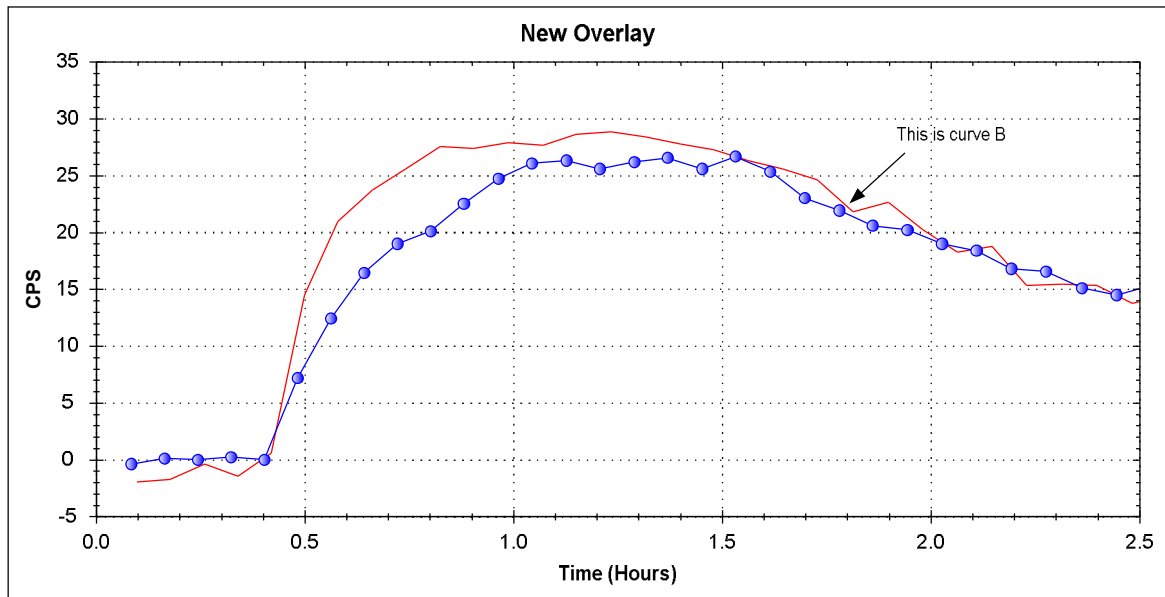
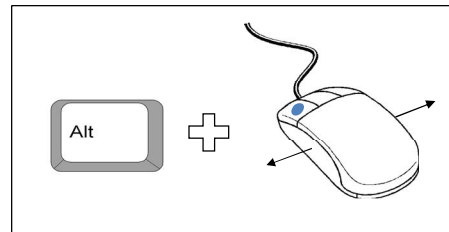
Guide line

Vertical
 Horizontal

Axis value:

Add arrow head
 Size:

Right-click in the plot and choose **Add line/arrow**. Press OK. Hold down Alt and drag the arrow so that the head is close to curve B. The arrow can be altered in angle and length by dragging any of the ends (holding down Alt). Move the non-head end of the arrow close to the textbox.



12. Print overlay

When all adjustments have been done, it is time to compile a report of the overlay. Click on the **Print icon** in the tool bar. A new window opens where you can select what your report should include. The report consists of the plot, the run table and the curve table by default, if this is the first time you print in TraceDrawer. Tick the box **Description** and click on **Print preview**. A preview of the first page in the report will appear. You can make more pages visible using the buttons found in the upper left corner:

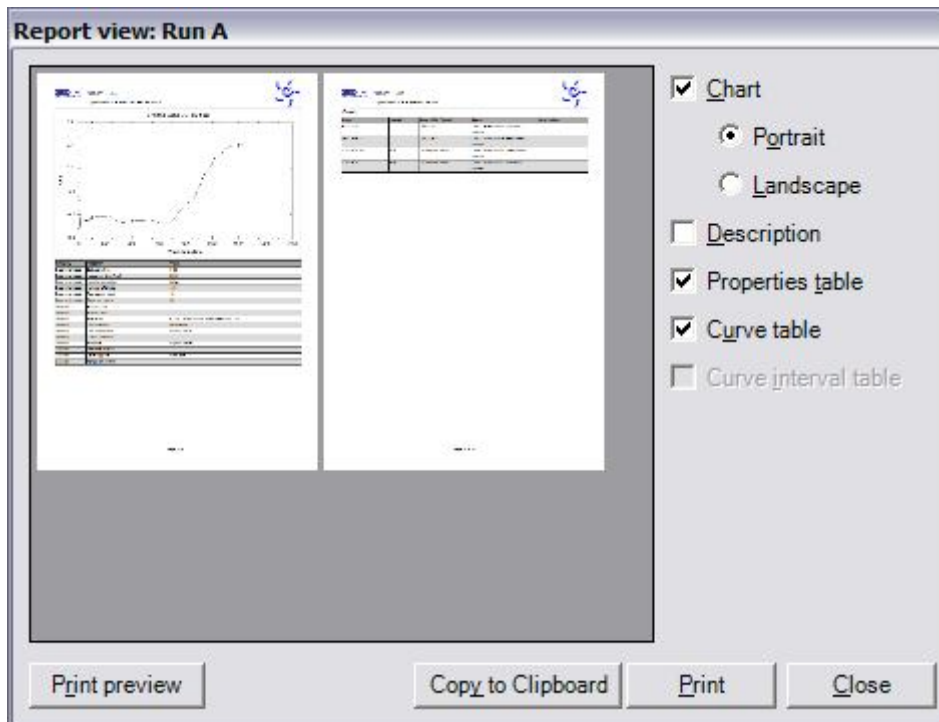


If satisfied, press the Print icon to get a paper copy of your report.

13. Print the runs

Reports describing a specific run can also be created. Select a run thumbnail and press the Print icon. Tick the box **Properties table** to include the properties

of the run in the report. Press **Print** and repeat the procedure with the other run.



14. Save

Click on the **Save icon** in the upper left corner. Choose a name for your project and press **Save**. This will save your entire investigation, with the runs, color settings, properties etc.

Step-by-step example B

1. Add files

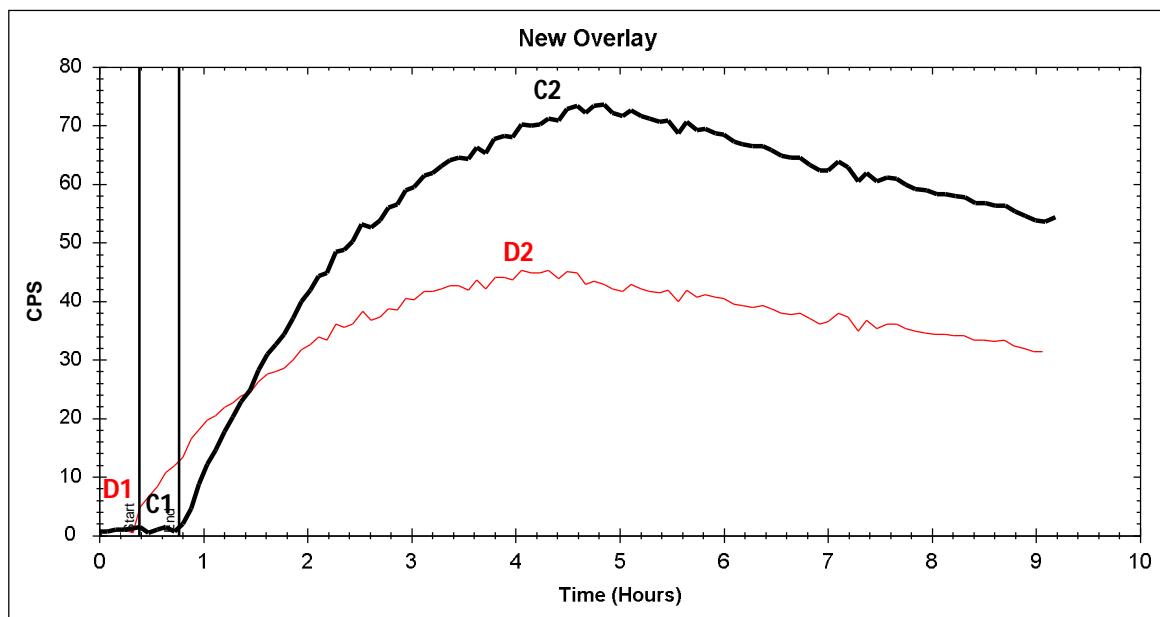
Add file ex2c.txt and ex2d.txt as described in Example A: Step 1. Press OK in the Open run window without any changes.

2. Create the overlay

Open Tools->Preference. Make sure that the "Add run name..." box is checked. Create an overlay and add the two runs, as described in Example A: Step 5.

3. Create curve intervals

Curve intervals are markers suitable for extracting signal levels, slopes and relative values from the curves in an overlay or in a run plot. Choose the **Curve Intervals tab** in the data manipulation area. Select the Run C curve by clicking on it. Type the name *C Start*. Drag the cursors close to point C1 and click **Add**.



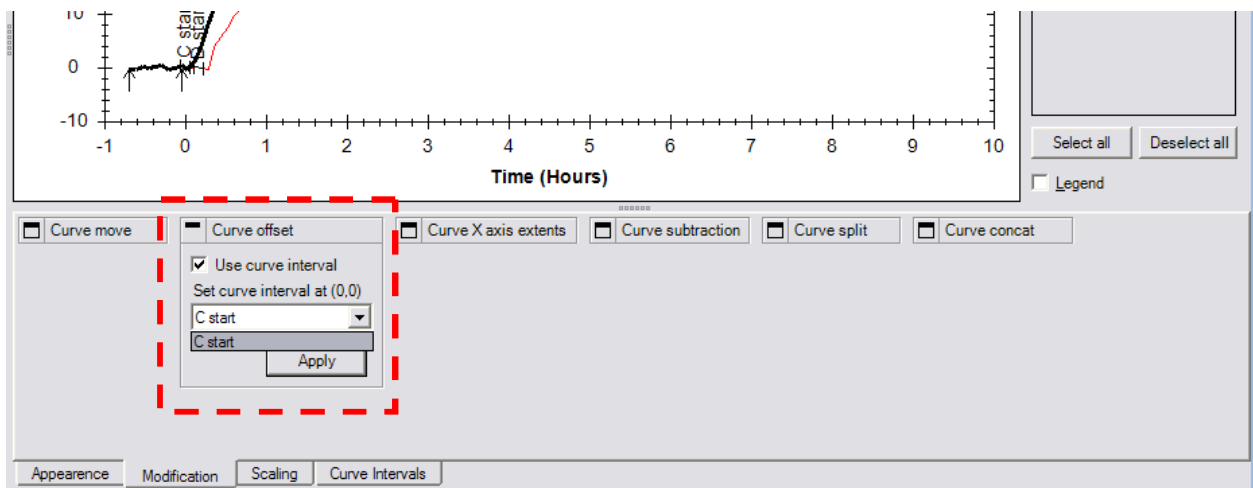
This will create a curve interval for the start of the Run C curve, with information about position, mean and slope. Create another interval for the end of the association phase, by dragging the cursors close to point C2. Type the name *C End* and select the new overlay to be relative to *C start* (found in the list **Relative**). Press Add. This interval will also contain information about the mean relative to the mean of *C start*.

Name:	Relative:
C end	C start
Start:	End:
4.47	4.82
<input type="checkbox"/> Use width:	
0.35	<input type="button" value="Add"/> <input type="button" value="Remove"/>

Repeat the procedure for the other curve, setting *D start* and *D end* near the points D1 and D2 and choosing *D end* to be relative *D start*.

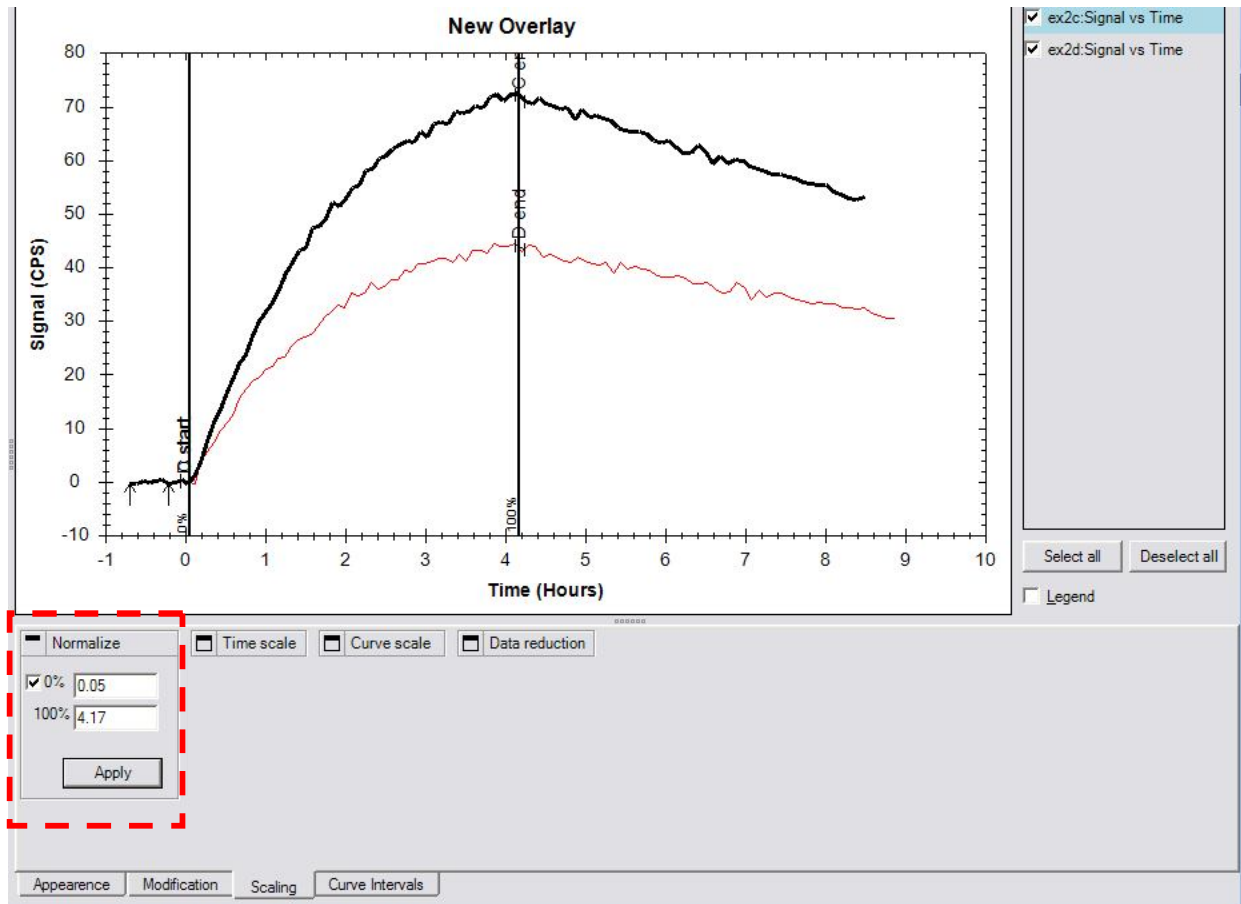
4. Move curves using curve intervals

Curves can be moved using curve intervals. In the Modification tab, choose **Curve offset**. Tick the **Use curve interval** box. Click on the C curve, select *C start* in the pull-down list and press Apply. This will move the *C start* interval to the origin (0.0). Do the same for the D curve, by selecting it and choosing *D start* in the list.



5. Normalize

Curves in an overlay can be normalized to easier compare the curvature. In the **Scaling** tab, select **Normalize**. Drag the **0% cursor** to *C start/D start* and the **100% cursor** to the end of the association, i.e. to *C end/D end* and press Apply.



6. Copy

You can copy your overlay as a report and paste it into other documents. This can be done as described in Example A: Step 10. Instead of choosing Print or Print preview, click on **Copy to clipboard** in the Report view.

7. Save

Save, as described in Example A: step 11.

Step-by-step example C: Kinetic evaluation

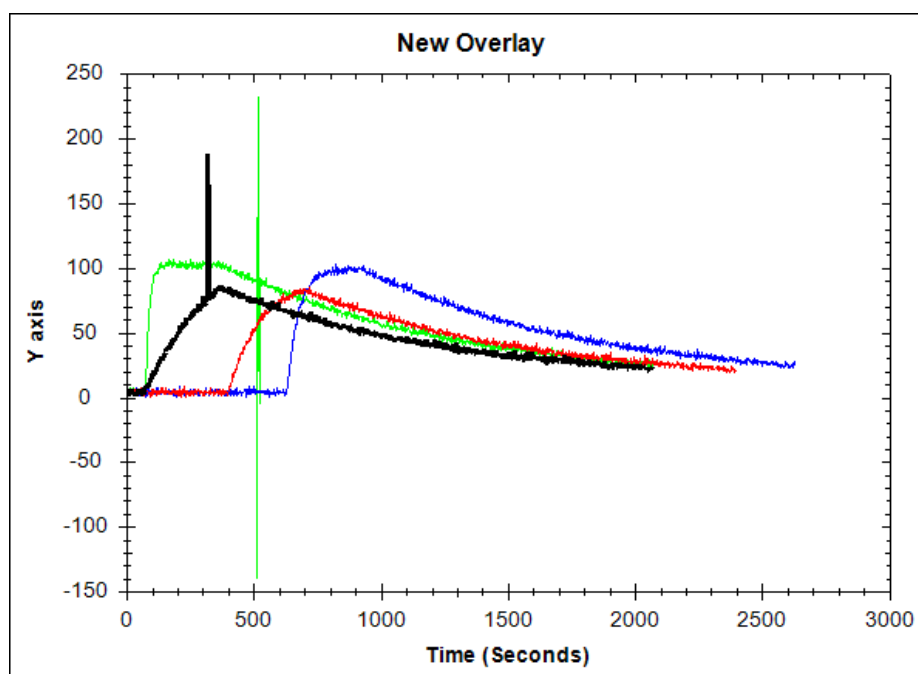
Example C describes the evaluation of real-time data using a kinetic fit. It is advisable to go through the step-by-step examples A and B prior to C and D, because explanations of the basic functions of TraceDrawer are omitted in examples C and D.

1. Add runs

Add runs C_curve1.txt, C_curve2.txt, C_curve3.txt, C_curve4.txt found in the TraceDrawer installation folder.

2. Create an overlay

Create a new overlay and drag and drop the four curves into it.



3. Enter concentrations

Right-click on the overlay thumbnail and select **Properties**. In the curve table, enter the concentrations:

Curve 1: 30 nM (enter e.g "30 n" or "30e-9")

Curve 2: 100 nM

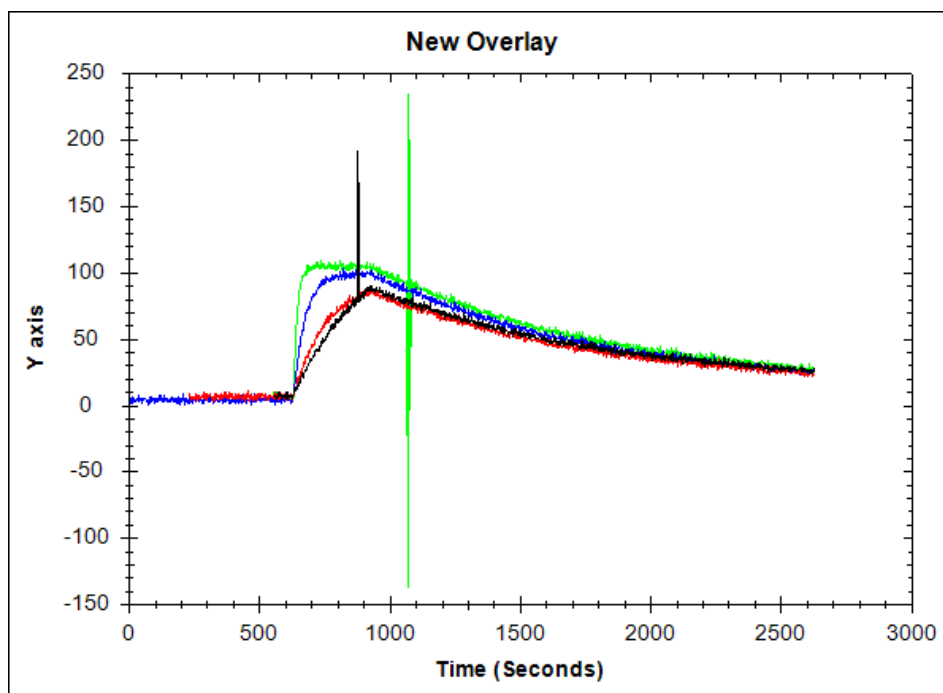
Curve 3: 300 nM

Curve 4: 1 μ M ("1 u")

4. Set baseline to zero

Curves can be moved in either X or Y direction by dragging them when pressing down Alt+Shift. Move the curves so that the start of the association phases intersects.

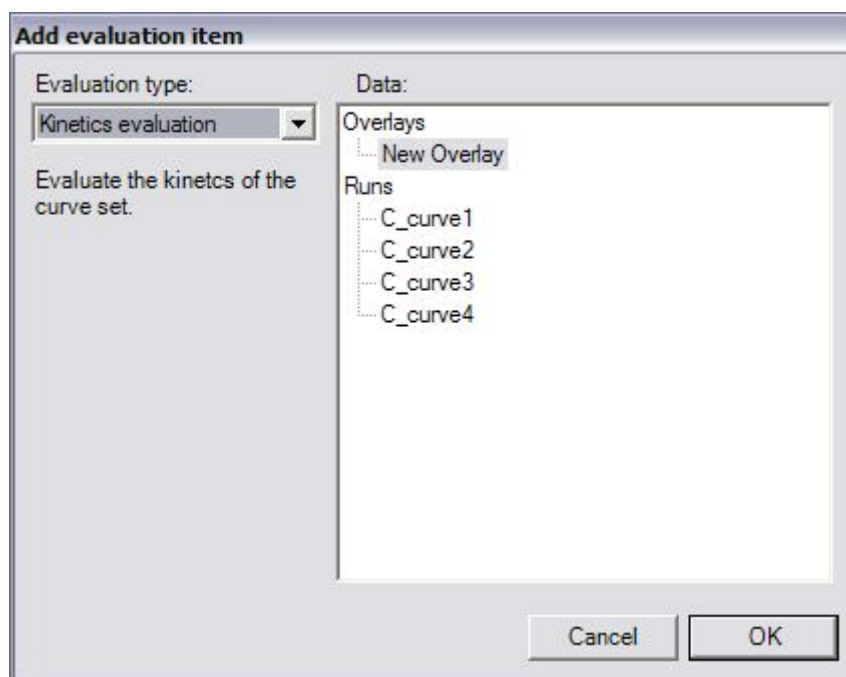
In kinetic evaluations, the signal corresponding to when no receptors are bound must be set to 0. This can be done in **Curve offset**, in the modification tab. Select all curves. Move the cursor to the end of the baseline (corresponding to the signal immediately before addition of protein) and press **Apply**. It is also possible to set the X value to zero, by ticking the box.



5. Create an evaluation item

To create a kinetic evaluation item, press **New Evaluation** found in the Evaluations thumbnail area to the left. Choose **Kinetics evaluation** as Evaluation type and select the name of your overlay, **New Overlay**, as Data.

An evaluation item can also be created from the right-click menu of an overlay or run.



6. Add concentration changes

The kinetics evaluation item consists of an Appearance and a Kinetics evaluation tab. The latter contains two steps: **Concentrations** and **Cut and fit**. For any 1:1 kinetic fit (describing one monovalent ligand binding to one target), the curves usually contain:

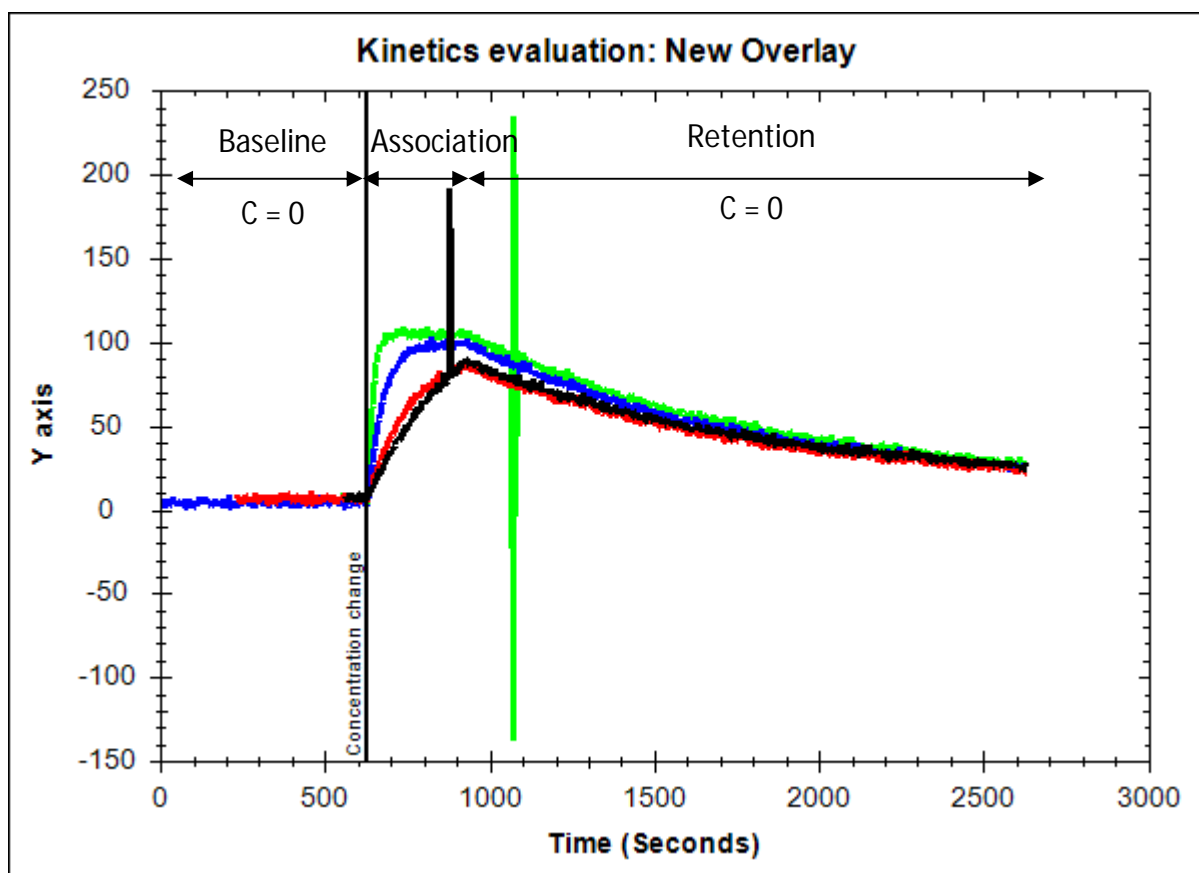
- A baseline, where the concentration of ligand (the molecule in solution) is zero.
- An association phase, where the concentration is nonzero.
- A dissociation phase, where the concentration is zero

The start of the baseline is set as the start of the curves by default. The start of the association and dissociation, as well as the concentration, must be entered manually.

Create a concentration change by dragging the cursor to the start of the association, as described in the figure. Select all curves. Press **Add change**. A concentration change is added for all selected curves, seen as a new row in the concentrations table. Right-click on any of the cells in the row and choose **Use curve property concentrations**. This will add the concentrations previously entered in the overlay properties window.

If no concentrations had been added in the properties, three other strategies for entering the concentrations in the kinetic evaluation item are:

- Enter the concentrations manually in the table, once a concentration change has been added. Click on a cell to activate it.
- Select one curve at a time and enter the concentration in the **Conc. (M)** field.
- Step through the Concentration change **Wizard**, accessed via a button to the right. See page 43 for more information.

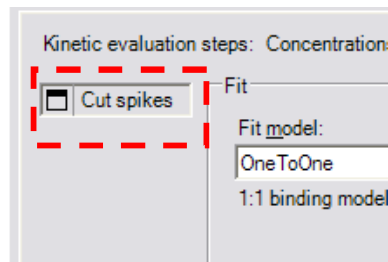


Move the cursor to the end of the association phase (see figure) and create a new concentration change with zero concentration for all of the curves.

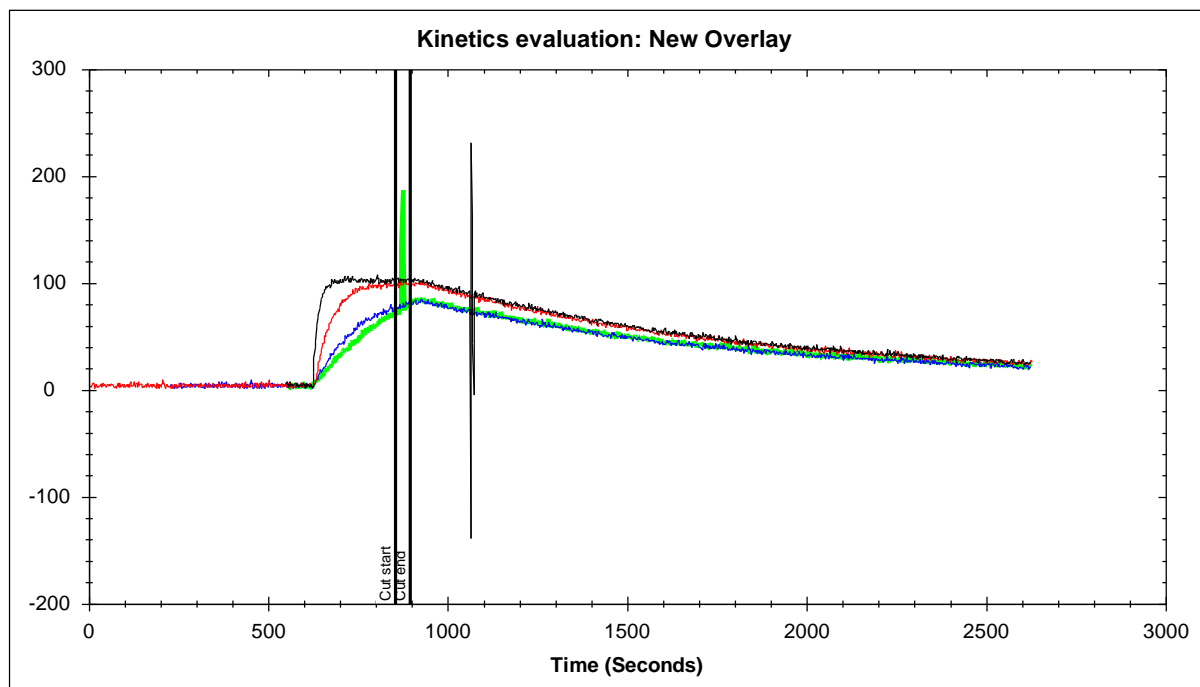
When the concentrations have been added, press **Next** in the lower right corner.

7. Cut spikes

In some cases, there may be disturbances in the data that could affect the kinetic fit, e.g. air spikes or transient electronic noise. Such data should be removed before the fit. Cutting poor data is possible in the **Cut and Fit** part of the Kinetics evaluation tab. Maximize the **Cut spikes** tool.



Select Curve 1. Drag the **Cut start** and **Cut end** cursors close to the spike on curve 1 and press the **Cut data** button. In the same way, remove the spike on curve 4. Minimize Cut spikes.



8. Alter fit arguments

Make sure that **OneToOne** is chosen in the **Fit model** list. Click on **Settings**, which will open the **Fit settings** window. The Fit arguments window is simply a mean to define how the curves in the overlay are related to each other. The values shown in the window describes the start guesses of the parameters.

During the fit, these will change to create an accurate fit describing the real data. Dependent on how close the start values are to the final result, the fitting time will vary.

Typically, the interaction is the same for all curves, which means that the kinetic rate constants, k_a and k_d are set to global. **BI** describes the bulk effect and is often local, meaning that each curve has an individual BI value. Some detection technologies do not result in bulk effect signals, and for these BI can be set to constant = 0. This is the case in this particular example. **Bmax** is the maximum signal possible to obtain, which is dependent on e.g. the amount of targets on the surface and the labeling of the ligand (i.e. the molecule in solution). If the target surface does not change between the curves and if the same batch of ligand is used, Bmax is most likely global.

In this case, two different sets of target surfaces was used, one for curve 1 and one for curves 2, 3 and 4. The target molecule and the ligand was the same for all curves, meaning that k_a and k_d is **global**. However, since the target surface changed between the runs, Bmax must be set to **Per Set**. Click on the **Fit type** of Bmax in the **Scope area** and change to PerSet.

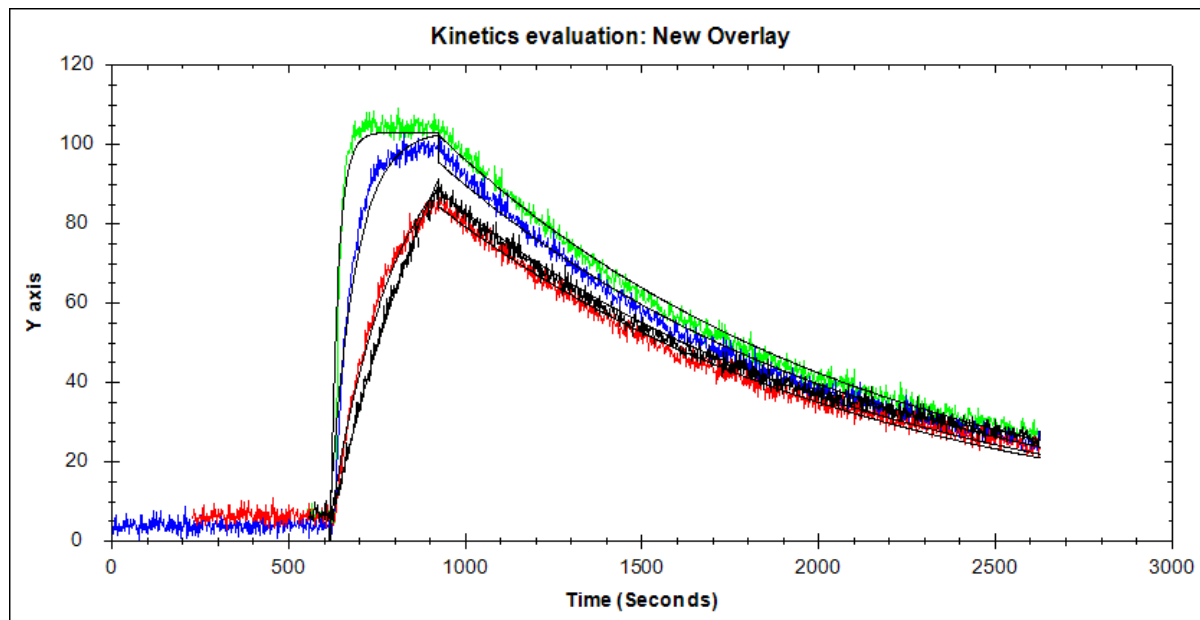
In the **Per set area** (the lower area of the Fit settings window), click on the value of C_curve1: CURVE1. Press **Define set** in the lower left corner. The other three curves will automatically create another set. Press **Ok**.

Argument name	C_curve1:CURVE1	C_curve4:CURVE4	C_curve2:CURVE2	C_curve3:CURVE3
Set ID	1	0	0	0
Bmax	187.34	187.34	187.34	187.34
k_a				
k_d				
BI				

For more information about the fit settings, see the "Fit settings" chapter found in the "Summary of evaluation tools: Kinetic evaluation" part of this manual.

9. Create a OneToOne fit

When the fit model is chosen and the fit arguments are set to describe the curves of the overlay, press Fit. Typically, an OneToOne fit will take a couple of seconds, resulting in a fit close to the real data.



10. Using the data

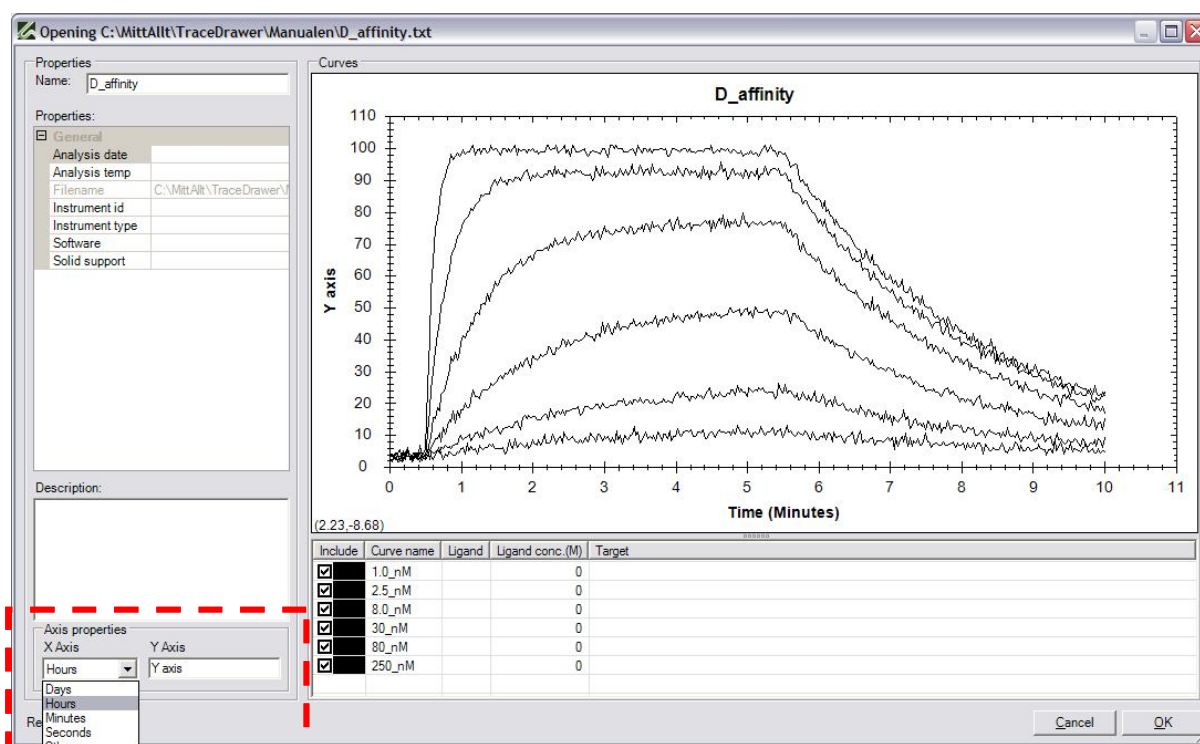
The calculated values of the parameters are found in the **Result list**. These can be printed together with the plot or, if finalized, compared with results from other evaluations in the **Summary** tool. The Summary will be explained in step-by-step example D.

Step-by-step example D: Affinity estimation

Example D describes the evaluation of real-time data using an affinity estimation. It is advisable to go through the step-by-step examples A and B prior to C and D, because explanations of the basic functions of TraceDrawer are omitted in examples C and D.

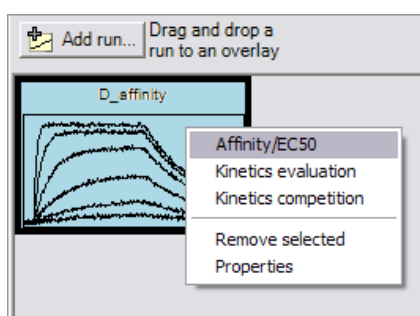
1. Add run

Add the run D_affinity.txt. TraceDrawer will assume the time scale to be seconds if nothing else is specified. For the curves in D_affinity.txt the time format was hours when created. Change the X axis properties to **Hours** in the lower left corner of the Open run window.



2. Create an affinity item

Right-click on the run thumbnail and choose **Affinity/EC50** to create a new affinity item.



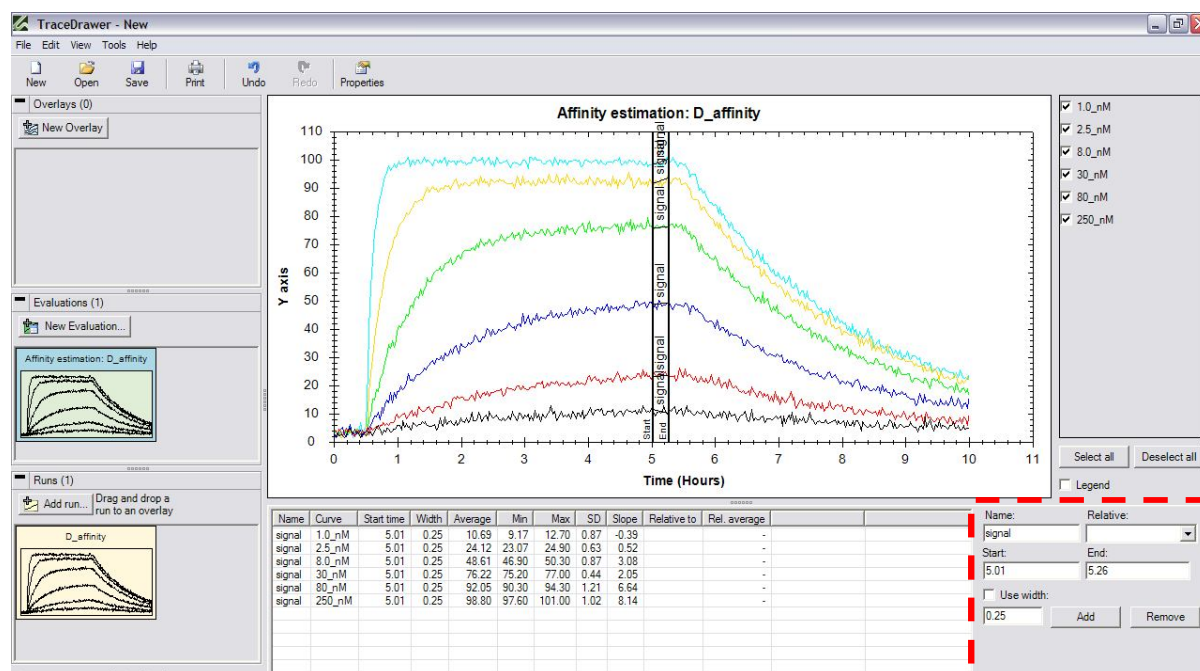
3. Enter concentrations

The first step of the **Affinity/EC50 evaluation** tab in the affinity item is adding the **Concentrations**. Enter the concentrations manually in the list. The name of the curves describes what concentrations to use. For example: enter 2.5 n for the second curve in the list.

4. Create a curve interval

Open the **Curve Intervals** tab in the affinity item. Select all curves. Drag the **Start** and the **End** to form a short segment at the end of the association phase. At that segment, the signal corresponds to the steady-state value of the interaction for each concentration. It is required that all curves have reached steady-state, i.e. shown evidence of saturation, for the affinity fit.

Choose a **Name**, i.e. "Signal", and press **Add**.



The curve interval Signal is created for all curves.

5. Choose curve interval for signal

Go back to the Affinity/EC50 evaluation tab and press **Next** in the lower right corner. The **Select and fit** part appears. To the left, in the **Curve interval** list, choose the name of your interval.

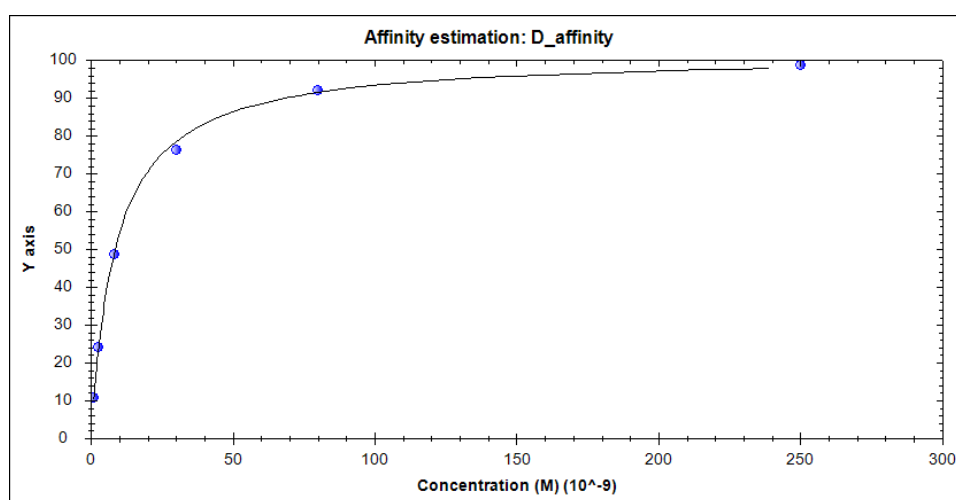
In the **Value** list it is possible to choose which value of the intervals to use: the minimum, maximum or average value. Keep it on **Average**.

6. Signal vs concentration plot

A signal vs concentration plot will automatically appear when a curve interval is selected. Otherwise, click the **Show plot** button. The overlay curves can easily be made visible again using the **Show curves** button.

7. Fit curve and optimize

When having the signal vs concentration plot visible, press **Fit curve**. A fit based on non-linear regression appears. The calculated values of the equilibrium dissociation constant, K_D , and the maximum signal, B_{max} , is found in the Result list.



If any of the data points seems poor for the fit it may be excluded by hiding them in the plot. Simply **untick** the curve/point in the curve list to the right. Press **Fit curve** to create a new fit. Feel free to include or exclude points and recalculate the result to study the effect.

8. Finalize

When everything is calculated and fitted, an evaluation item can be marked as finalized. No more alterations can be done to the item from that on. Press **Finalize** in the upper right corner of the Affinity evaluation tab.

9. Summary

A summary can be created of all finalized evaluation items. Right-click on the thumbnail for your affinity evaluation item and choose **Summary**. Other evaluation items can be dragged and dropped into it to gather more results. Only evaluation items of the same type (e.g. Kinetics or Affinity item) and using the same parameters (e.g. fit models) are allowed in the same summary.

Summary of modification tools

TraceDrawer provides you with several functions for modifying your curves to quickly create comprehensible overviews. It is advisable to look through the software by yourself and explore the tools that seem necessary for your needs. Some of the tools are explained briefly below. A majority of these are found in the Modification tab in the Data manipulation area, in the lower part of the main window.

In some cases, the modification only affects the selected curves. Curves are selected by **left-clicking** on them in the plot or in the curve list. To select more than one, hold down the **Ctrl** button at the same time. It is also possible to select or unselect all curves using the buttons below the curve list.

Modification tab

Curve move

To move selected curves, type how much you want to move them in X and Y direction, and press **Apply**.

Curves can also be moved manually by holding down the **Alt** button, clicking on a curve and dragging in to the wished position. This maneuver is independent from Curve move and can be done any time when the plot is visible. A variant of this is to hold down **Alt+Shift**. This limits the movement to either X or Y direction.

Curve offset

It can sometimes be suitable to move curves according to specific parts of them. In Curve offset, selected curves can be adjusted to $Y = 0$ (and $X=0$ if the checkbox is checked) at a specific X position. Drag the cursor and press **Apply**.

Curves can also be moved according to curve intervals. Tick the **Use curve interval box**. Choose an interval from the pull down list to be set to (0,0) and press Apply. Only the selected curves containing the curve interval will be moved. For more information on curve intervals, see the part **Curve intervals** below.

Curve X axis extents

Curves can be cut in the X direction. Move the cursors to a suitable start and end position for the selected curves and press **Apply**. It is also possible to type the exact positions in the text fields.

Curve subtraction

To subtract a reference curve from all curves in a plot, select the reference curve in the plot and press **Apply**. This may e.g. be of use when eliminating background signal or when comparing signal levels between curves. If more than one curve is selected when pressing Apply, the average of the selected curves will be subtracted.

Curve split

Sometimes a run consists of several important fragments. In these cases it may be of use to separate these segments and treat them as different curves. Move the cursor to an appropriate splitting position and press **Apply** to split selected curves. The fragment(s) to the right of the cursor will automatically become new curves.

Note that this function is only allowed in overlay mode.

Curve concatenation

Curves can be ligated in Curve concat. Select two curves and press **Apply** to create a new one. The two selected curves must not overlap.

Cut spikes

To remove segments of transient disturbances in the curve data, move the cursors to the start and end of the segment that you want to remove and press **Apply**. Only data on selected curves will be removed.

Scaling tab

Normalize

Curves in an overlay can be normalized to easier compare them. Select X values representing 0 % and 100 % and press **Apply**. All curves in the plot will be affected.

If there are no points in the curve that could represent 0% bound target molecules, untick the **0%** box. This results in the curves being adjusted only according to the position of the 100% cursor and can be of particular use when analyzing dissociation measurements.

Time scale

With a single button click, the time scale of the graph can be changed. All curves will be affected. Runs summarized in an overlay are automatically adjusted to be in the same time scale.

Curve scale

Selected curves can be modified in Y direction by changing curve scale. Type the value with which the curves should be multiplied and press **Apply**. If necessary, change the name of the Y axis in the Appearance tab.

Data reduction

This is a tool to reduce noise for selected curves. The reduction factor defines the number of data points that is averaged for each new point.

Differentiate

The Differentiate scaling tool exchanges the curves in an overlay with the corresponding derivative curves.

If **Create new overlay** is checked, the tool can be applied on either a run or an overlay. This results in a new overlay with the differentiated curves.

Curve Intervals tab

Curve intervals are markers suitable for extracting signal levels, slopes and relative values from the curves in an overlay or in a run plot. Furthermore, other tools may use curve intervals as markers (e.g. Curve offset). The Curve interval function is found as a separate tab in the Data manipulation area.

Choose a **name** and drag the cursors to appropriate start and end positions. Tick the **Width** box to fix the width to the current. This width can be redefined in the text field. **Add** or **remove** a curve interval by the buttons to the right.

Data describing the interval is found in the table. Information about the signal level relative to a previously defined interval can be provided by selecting an interval from the Relative list when creating a new curve interval.

Summary of graph appearance tools

The appearance of a specific graph can be modified, regarding e.g. axis properties, textboxes and arrows. The information is saved in the Itv-file and applies only to the specific item. The general appearance of all runs, overlays or evaluation items can be modified in Tools->Preferences->Fonts/Graph. For more information, see "Preferences" below.

Axis settings

Right-click on the X or Y axis and choose **Axis settings**.

The thickness of the axis can be selected and you can choose whether to keep the right and top axis.

Other modifications will affect either X or Y axis:

- The axes can be fixed in length by defining minimum and maximum values. This adjustment will not alter the data, only describe the zoom of the plot. Check the **Lock** box to save the chosen zoom. This means that the chosen minimum and maximum values will be remembered and used when e.g. zooming to extents. Uncheck the Lock box will make the zoom temporary.
- **Major and minor ticks** can be added and altered in position and length. The distances between the ticks is set to Automatic by default, but can be chosen manually.
- **Grid lines** can be added and will be locked in position according to the ticks.

All modifications can be saved ("Copy to preferences") and applied as preferences for all new objects.

Font settings

Right-click on the X or Y axis and choose **Font settings**. Click on any of the Font buttons to alter font, font style, size, effects and color.

All modifications can be saved ("Copy to preferences") and applied as preferences for all new objects.

Textbox

Right-click inside the plot and choose **Add textbox**. Type your text and modify the box according to your wishes. Click on the **Font** button to alter font, font style, size, effects and color. The changes will appear when the box is created. The box can also be fixed to the X or Y axis, when defining e.g. maximum level or time events. Press **OK** to add the box.

Once a box is created, it can be moved manually. Hold down the **Alt-button** and drag it to desired position.

A textbox can be edited or removed. Right-click on a box and select command from the menu.

Line/arrow

Right-click inside the plot and choose **Add line/arrow**. It can be modified in width, color and arrow head. A line can also be fixed to the X or Y axis, just as a text box.

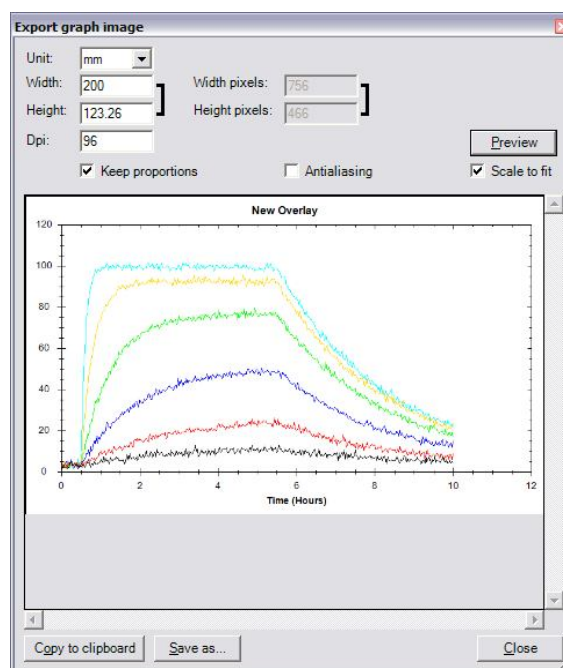
Once a line is created, it can be moved manually. Hold down the **Alt-button** and drag it to desired position. Dragging the end of the line (visible as a red dot) enables changes in length and angle.

A line can be edited or removed. Right-click on it and select command from the menu.

Export

Export graph image

Graphs of binding curves are often suitable for inclusion in reports and scientific publications. TraceDrawer contains a tool for exporting the binding curve graph into a high-resolution image file. In this tool, the size and resolution of a plot image can be modified. This is an important step in composing publication-ready figures. The tool is found in File (top menu) -> Export graph. The combination of width, height and dpi (the resolution, dots per inch) will affect the width and the height of the image. Press Preview to see the plot in its new size.



Tick the **Scale to fit** box and press Preview to create an image of the plot that fits the Export graph image window. This will be a preview of the proportions, not regarding to the correct pixel size.

Antialiasing is a filter to remove disturbances in the image, which essentially means to blur the image slightly. Tick the Antialiasing box and press **Preview** to study the results.

The image can be copied to clipboard (creating an Enhanced metafile) or saved as a BMP, PNG, JPG or TIFF file.

Export data

All visible curves in a plot can be exported as a tab-separated text file, to be opened in e.g. Microsoft Excel. Right-click in a plot and choose **Export curves**.

Summary of evaluation tools and models

Affinity/EC50 estimation

Before a fit in the Affinity Estimation, curve intervals corresponding to the steady state signal must be created and the concentrations entered. Curve intervals can be created in the overlay prior to the affinity estimation, or from the Curve Intervals tab in the Affinity tool. The concentration list is found in step 1 in the Affinity evaluation tab.

Fit settings

The initial values of e.g. K_D and B_{max} can manually be selected by pressing the **Settings** button. If not defined by the user, TraceDrawer automatically estimates starting values based on curve concentrations and the selected curve interval. These are presented in the Fit Settings window as Default values.

Affinity fit models

Affinity model

The Affinity fit calculates the equilibrium dissociation constant K_D from a Signal vs Concentration plot, using non-linear regression. B_{max} , the maximum signal, is also calculated. The equation used in Affinity modeling is:

$$Y = B_{max} * c / (c + K_D)$$

Affinity OneToTwo model

The Affinity OneToTwo fit calculates the equilibrium dissociation constants of two parallel interactions (K_{D1} , K_{D2}) from a Signal vs Concentration plot, using non-linear regression. B_{max1} and B_{max2} , the maximum signals, are also calculated. The equation used in Affinity OneToTwo modeling is:

$$Y = B_{max1} * c / (c + K_{D1}) + B_{max2} * c / (c + K_{D2})$$

AffinityOffset model

AffinityOffset is an ordinary affinity fit to which an offset term has been added. The equation used in AffinityOffset modeling is:

$$Y = B_{\max} * c / (c + K_D) + \text{Offset}$$

AffinityAggregation model

AffinityAggregation is an ordinary affinity fit where deviations due to protein aggregation is taken into account and corrected for. Apart from the standard affinity parameters, the aggregation constant AggC is also calculated. The equation used in AffinityAggregation modeling is:

$$Y = B_{\max} * c / (c + K_D) + \text{AggC} * c$$

EC50HiLo and EC50lohi models

The half maximal effective concentration, EC₅₀, can be calculated from a Signal vs Concentration plot, using non-linear regression. EC50HiLo is for signals that are decreasing with increasing concentration and EC50LoHi is used for signals that are increasing with increasing concentration. The parameters Top and Bottom define the maximum and minimum signal in the assay, and Hill Slope controls the slope of the sigmoidal curve of the fitted function. The equations used in EC₅₀ modeling are:

$$\text{EC50hilo: } Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + (c/\text{EC}_{50})^{\text{HillSlope}})$$

$$\text{EC50lohi: } Y = \text{Top} - (\text{Top} - \text{Bottom}) / (1 + (c/\text{EC}_{50})^{\text{HillSlope}})$$

Linear

Linear creates a linear Signal to Concentration fit generating the slope k and the signal at zero concentration, m. The equation used in linear modeling is:

$$Y = k * c + m$$

Kinetic evaluation

Change concentration

Before a kinetic fit, the concentrations of the ligand must be entered in the Concentrations step of the Kinetics evaluation.

The concentration change wizard

Press the **Wizard** button. A new window appears, where the concentrations can be added one curve at a time.

Drag the cursor to set the start of a new concentration, or enter a specific timepoint in the **Time** input field. Enter the concentration value in molar in the **Conc.** input field and click **Add concentration change**. If there is more than one curve in the plot, press **Next** to continue with another curve. Press **Done** once changes for all curves have been added.

A concentration can be changed from the corresponding cell in the table. Furthermore, a change can be removed using the **Remove** button below the cell.

Cut data

In some cases, there may be turbulence in the data that could affect the kinetic fit, e.g. air spikes or electronic disturbances. This data should therefore be removed before the fit. Cutting poor data is possible in the **Cut and Fit** part of the Kinetics evaluation tab. Maximize the **Cut spikes** tool, drag the cursors and press **Cut data** to remove unwanted data of selected curves.

Fit settings

All parameters for the kinetic fits can be defined as Global, Local, Constant or Per Set. This can be selected in the Scope area, in the upper part of the Fit settings window. Simply click on the Fit type of any argument and use the arrow keys to select a suitable setting. Start values of the parameters can also be altered in Fit Settings.

The button Set defaults will change any modifications back to default values. Redefining which curves to be included in the fit (Hide/Unhide) will also reset the values to default.

Global fit type

A global parameter will be the same for all curves, optimized to give the best fit in total. All interaction characteristics (k_a , k_d , K_D , etc) are typically set global.

Local fit type

A local parameter will be different for each curve. An example of a local parameter is using different receptor amounts for different curves. In this case, Bmax must be chosen local.

The more local parameters, the longer the calculation time. If possible, decrease the number of curves to make the data set more homogenous. This may decrease the number of parameters needed as local.

Constant fit type

Some parameters may be constant (i.e. have a value known in advance) and should therefore not be altered by the fitting mechanism. An example of this may be the volume of the detection vial in a OneToOneDepletionCorrected fit.

Per set fit type

If there is a subset of curves in a data set that should share parameters, these can be grouped into a set. This can happen when one set of curves, obtained from a first measurement and having one number of targets, is merged with another set of curves obtained from a second measurement with a different number of targets. This is an alternative to the Local fit type, decreasing the calculation time.

In the Per set area (the lower area of the Fit settings window), select the curves to be defined as a set by clicking on the name of the curves, holding down Ctrl. Press Define set in the lower left corner.

Evaluation fit models

OneToOne model

OneToOne creates an ordinary 1:1-fit, describing one monovalent ligand binding to one target. This is the simplest model of an interaction, but is in many cases close to reality. The fit generates:

Bmax [Signal units]: The maximum signal, dependent on e.g. the number of receptors

k_a [1/(M*s)]: The association rate constant

k_d [1/s]: The dissociation rate constant

BI [Signal units]: Possible bulk effect

For a OneToOne fit, the curves must contain:

- A baseline, when no ligand has been added. The baseline level must be adjusted to 0 prior to the kinetic evaluation.
- An association phase, where ligand has been added.
- Optionally (but preferably) a dissociation phase, where the detachment of ligand from the target is studied, i.e. where the ligand concentration in solution is zero.

The interaction model of OneToOne is a differential equation:

$$dY/dt = (k_a * c - k_d) * Y$$

$$Y(t=0) = 0, \text{ signal from saturated target} = B_{\max}$$

Y is the recorded signal and c is the concentration of ligand in the bulk liquid. This equation is solved using analytical tools or by numerical integration, depending on how the assay was conducted.

OneToOneDiffusionCorrected model (Mass transport limitation)

OneToOneDiffusionCorrected is an ordinary 1:1-fit where the ligand concentration near the target area may be lower than the bulk ligand

concentration due to diffusion limitation. This effect is prominent when an interaction with high association rate is quantified at too low flow rate in flow based instruments (or the stirring in cuvette based systems) on solid supports with large amounts of targets. Whenever possible, try to avoid diffusion limitation by using high flow rates and low target density on the solid support of your instrument. As a rule of thumb, the vast majority of antibody-antigen interactions do not suffer from diffusion limitation, but drug-receptor interactions may require the OneToOneDiffusionCorrected model for accurate estimates of kinetic parameters. Diffusion limitation is also known as mass transport limitation (MTL). The fit generates the parameters found in an ordinary OneToOne fit, as well as:

k_t ([Signal unit/(M*s)] : mass transfer coefficient

For a OneToOneDiffusionCorrected fit, the curves must contain the same regions as for a OneToOne fit.

The interaction model of OneToOneDiffusionCorrected is a differential equation:

$$dY/dt = (k_a * L - k_d) * Y$$

$$dL/dt = k_t * (c-L) - dY$$

$$Y(t=0) = 0, \text{ signal from saturated target} = B_{\max}$$

Y is the recorded signal, L is the concentration of ligand near the target area, and c is the concentration of ligand in the bulk liquid. This equation is solved by numerical integration.

OneToOneDepletionCorrected model

OneToOneDepletionCorrected is an ordinary 1:1-fit where the depletion of ligand due to the formation of ligand-target complexes is taken into account. This effect is sometimes prominent in cuvette based instruments, and rarely appears in flow based instruments. The risk for ligand depletion is high when using low concentrations of ligand, where the number of ligands is in the same

order of the amount of receptors. The fit generates the parameters found in an ordinary OneToOne fit, as well as:

nB: The number of receptors

vol: The volume of the vial used during detection

For a OneToOneDepletionCorrected fit, the curves must contain the same regions as for a OneToOne fit.

The interaction model of OneToOneDepletionCorrected is a differential equation:

$$dY/dt = +k_a * L/N_A/vol * (Bmax - Y) - k_d * Y$$

$$dL/dt = - dY*nB/Bmax;$$

$$Y(t=0) = 0 , L(t=0)=c, \text{ signal from saturated target} = Bmax$$

Y is the recorded signal, L is the concentration of ligand at time t, c is the concentration of ligand at the time t=0, nB is the number of monovalent target molecules and N_A is Avogrados number. This equation is solved by numerical integration.

OneToOne TwoState model

OneToOne TwoState is an interaction model representing an initial interaction event, which after some time changes into a different interaction. The typical hypothesis is that the initial binding is weak, but when the binding partners have been in complex for some time they rearrange into a stronger interaction.

The fit generates:

Bmax [Signal units]: The maximum signal, dependent on e.g. the number of receptors

k_{a1} [1/(M*s)]: The association rate constant for the first binding state

k_{d1} [1/s]: The dissociation rate constant for the first binding state

k_{a2} [1/s]: The formation rate constant for the second binding state

k_{d2} [1/s]: The disruption rate constant for the second binding state

BI [Signal units]: Possible bulk effect

Since the formation of the second binding state does not cause any change in signal, it is sometimes difficult to estimate the kinetic constants of an OneToOne TwoState interaction. Using several interaction measurements with different lengths of association (different injection times or incubation times) normally increases the chances to obtain reliable results. Also remember that the two association rate constants have different units and hence cannot be compared.

The interaction model of OneToOne TwoState is a differential equation:

$$dB/dt = -(k_{a1} * B * c - k_{d1} * A1B)$$

$$dA1B/dt = (k_{a1} * B * c - k_{d1} * A1B) - (k_{a2} * A1B - k_{d2} * A2B)$$

$$dA2B/dt = (k_{a2} * A1B - k_{d2} * A2B)$$

$$Y = A1B + A2B$$

$$A1B(t=0) = A2B(t=0) = 0, B(t=0) = B_{max}$$

Y is the recorded signal, A1B is the primary complex, which after some time transforms into the secondary complex A2B. B is the unbound target and c is the concentration of ligand. This equation is solved by numerical integration.

BivalentInteraction model

Bivalent is an interaction model designed for measurements where a bivalent molecule (e.g. an antibody) in solution is binding to a target (e.g. an antigen) on a solid support. **Do not use this model in any other assays**, because it will produce inaccurate results. The fit generates the following parameters:

Bmax [Signal units]: The maximum signal, dependent on e.g. the number of receptors

k_{a1} [1/(M*s)]: The first association rate constant

k_{d1} [1/s]: The first dissociation rate constant

k_{a2} [1/([SignalUnit]*s)]: The second association rate constant

k_{d2} [1/s]: The second dissociation rate constant

BI [Signal units]: Possible bulk effect

IMPORTANT: The second association parameter has an odd unit, which includes the signal unit of the measurement device. This makes the interpretation of the second association rate parameter difficult. In many cases, the magnitude of the second association rate constant will be 5-7 orders of magnitude lower than the first association parameter, even though this is strongly dependent on the type of instrument used.

For a Bivalent fit, the curves must contain the same regions as for a OneToOne fit. The interaction model of Bivalent is a differential equation:

$$dB/dt = - (ka1*L*B - kd1*AB) - (ka2*AB*B - kd2*ABB)$$

$$dAB/dt = (ka1*L*B - kd1*AB) - (ka2*AB*B - kd2*ABB)$$

$$dABB/dt = (ka2*AB*B - kd2*ABB)$$

$$B(t=0)=Bmax, AB(t=0)=0, ABB(t=0)=0$$

B is the number of free targets on the solid support, L is the concentration of ligand at time t, AB is the number of A bound with one epitope, and ABB is the number of A bound with two epitopes. The measured signal is equal to AB. This equation is solved by numerical integration.

OneToTwo model

OneToTwo is an interaction model designed for measurements where a ligand in solution is binding to two independent targets on a solid support. Use this model **only** in situations where it is known that there are two independent targets on the solid support. OneToTwo will always produce a "better" fit than the OneToOne model because it has greater flexibility. The fit generates the following parameters:

Bmax1 [Signal units]: The maximum signal from target 1, dependent on e.g. the number of receptors

k_{a1} [1/(M*s)]: The association rate constant for target 1

k_{d1} [1/s]: The dissociation rate constant for target 1

B_{max2} [Signal units]: The maximum signal from target 2, dependent on e.g. the number of receptors

k_{a2} [1/(M*s)]: The association rate constant for target 2

k_{d2} [1/s]: The dissociation rate constant for target 2

BI [Signal units]: Possible bulk effect

For a OneToTwo fit, the curves must contain the same regions as for a OneToOne fit. The interaction model of OneToTwo is a differential equation:

$$dAB/dt = (k_a * c - k_d) * AB$$

$$dAC/dt = (k_a * c - k_d) * AC$$

$$Y = AB + AC$$

$$AB(t=0) = 0, AC(t=0) = 0, \text{ signal from saturated target} = B_{max} \text{ and } C_{max}$$

Y is the recorded signal, c is the concentration of ligand in the bulk liquid, B and B_{max} relates to target 1 and C and C_{max} relates to target 2. This equation is solved by numerical integration.

DissociationRate model

DissociationRate studies the dissociation of the ligand, i.e. how the complex dissociates. The fit generates:

B_{zero} [Signal unit]: The signal when starting the dissociation

k_d [1/s]: The dissociation rate constant

The curves must contain nothing but the dissociation phase, when the ligand concentrations are zero. If there are more data in the run, cut the curves before creating a Kinetic evaluation object. The equation used in the DissociationRate model is

$$Y = B_{\text{zero}} * e^{(-kd*t)}$$

Where Y is the recorded signal.

Kinetics competition

The kinetics competition module evaluates experiments where two ligands bind to the same receptor. It can also evaluate self-competition, i.e. when labeled and unlabeled variants of the same ligand compete. The module is similar to the Kinetics evaluation module, so for a quick understanding the Step-by-step Example C is recommended.

A kinetic competition item can be created from the right-click menu of an overlay or run thumbnail. The fit procedure is then divided into three steps:

- Concentrations ligand 1&2, concentrations: Just like an ordinary kinetic fit, the concentrations of the ligand must be entered for each curve. Drag the **Concentration change** cursor to the time of a concentration change and enter the concentration in the **Conc.** field. Note that the concentration is in molar. Abbreviate n for nM, u for μM and m for mM, or use scientific numbers (1E-9 for 1 nM).
- Concentrations ligand 1&2, Signal property value: The **Signal property value** (s1 and s2) is a way of correcting for differences in signal between the two molecules. For SPR and QCM data the signal is relative the molecular weights so the weight of the molecules should be entered. For labeled molecules the signal will depend on the degree of labeling, thus enter the ratio of labeling instead. Example:

	MW	Labeling
A	1 000 Da	1 MBq/ug
B	10 000 Da	2 MBq/ug
C		unlabeled

Experiment	s1 (A)	s2 (B or C or unlabeled A)
SPR/QCM, competition between A and B	1000	10000
LigandTracer, competition between 2 different labeled compounds (A and B)	1	2
LigandTracer, competition between labeled compound A and unlabeled compound C	1	0
LigandTracer, competition between labeled and unlabeled compound A	1	0

As it is only the ratio between the competing molecules that is relevant, s1 and s2 could also be chosen 1 and 10 in the SPR/QCM case.

- Cut and fit: The Cut and fit step is similar to the fit step of a kinetic evaluation:
 - Remove poor data: Possible disturbances in the data can be removed using **Cut spikes**. Minimize the tool after the data has been cut.
 - Choose model: The two models to choose from describe competition between either two different molecules (OneOneCompetition) or the same molecule (OneOneSelfCompetition). If OneOneSelfCompetition is chosen, s1 and s2 is automatically redefined to s1 = 1 and s2 = 0.

Competition fit models

OneOneCompetition

OneOneCompetition describes when two different ligands in solution bind to one target on a solid support. The fit generates the following parameters:

Bmax [Signal units]: The maximum signal from target, dependent on e.g. the number of receptors.

k_{a1} [1/(M*s)]: The association rate constant for ligand 1

k_{d1} [1/s]: The dissociation rate constant for ligand 1

k_{a2} [1/(M*s)]: The association rate constant for ligand 2

k_{d2} [1/s]: The dissociation rate constant for ligand 2

BI [Signal units]: Possible bulk effect

The interaction model of OneOneCompetition is a differential equation:

$$dAB/dt = k_{a1} * conc1 * B - k_{d1} * AB$$

$$dCB/dt = k_{a2} * conc2 * B - k_{d2} * CB$$

$$B = B_{\max} - AB - CB$$

$$Y = s_1 * AB + s_2 * CB$$

$$B(t=0) = B_{\max}$$

Y is the recorded signal, conc1 is the concentration of ligand 1 in the bulk liquid, conc2 is the concentration of ligand 2 in the bulk liquid, B is the number of free targets, AB the number of targets in complex with ligand 1, AC the number of targets in complex with ligand 2. The composition of the recorded signal is controlled by the constants s1 and s2, which define the relative signal contribution of the two ligands. This equation is solved by numerical integration.

OneOneSelfCompetition

OneOneSelfCompetition describes when a labeled ligand in solution competes with the unlabeled ligand. It is assumed that the interaction properties of the labeled and the unlabeled ligand are identical. The fit generates the following parameters:

Bmax [Signal units]: The maximum signal from target, dependent on e.g. the number of receptors.

ka [1/(M*s)]: The association rate constant

kd [1/s]: The dissociation rate constant

BI [Signal units]: Possible bulk effect

The interaction model of OneOneCompetition is a differential equation:

$$dAB/dt = k_{a1} * \text{conc1} * B - k_{d1} * AB$$

$$dCB/dt = k_{a2} * \text{conc2} * B - k_{d2} * CB$$

$$B = B_{\max} - AB - CB$$

$$Y = AB$$

$$B(t=0) = B_{\max}$$

Y is the recorded signal, conc1 is the concentration of labeled ligand in the bulk liquid, conc2 is the concentration of unlabeled ligand in the bulk liquid, B is the number of free targets, AB the number of targets in complex with labeled ligand, AC the number of targets in complex with unlabeled ligand. The recorded signal is set to the labeled component only. This equation is solved by numerical integration.

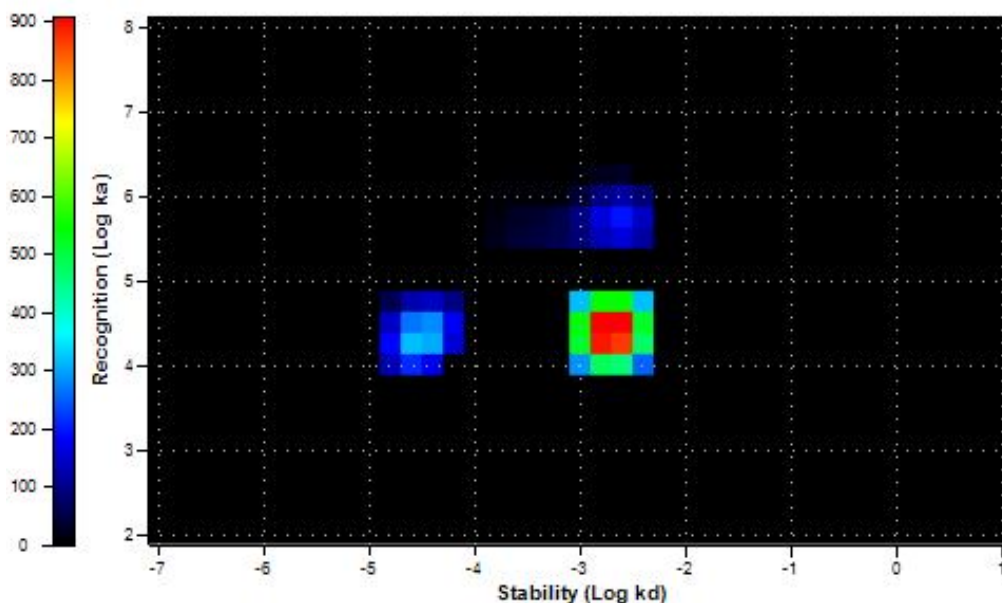
Interaction Maps

Interaction Map (IM) analysis is an add-on evaluation function provided by Ridgeview Diagnostics AB which is charged for separately. IM is compatible with all types of real-time interaction data. To get access to IM, contact Ridgeview Instruments AB (info@ridgeview.eu) or Ridgeview Diagnostics AB (info@ridgeviewdiagnostics.com). The IM tool in TraceDrawer is visible only if an IM compatible license code is entered.

Interaction Map is a mathematical tool to evaluate heterogeneous interaction data, i.e. when a monovalent ligand binds to a set of different targets simultaneously. The method deciphers a measured real-time interaction curve into a number of contributing 1:1 interactions – each with a unique combination of k_a and k_d :

$$MeasuredCurve = \sum_{i=1}^n \sum_{j=1}^m [W_{ij} \cdot CurveComponent(conc, k_a^i, k_d^j)]$$

These interactions are presented in a modified on/off plot, where each of the colored peaks represents one of the curve components (i.e. a contributing interaction). The color represents the weighing factors W_{ij} – the warmer the color, the stronger the contribution.



Interaction Maps display the number and magnitudes of parallel 1:1 binding events that in concert reproduces the measured binding curve. It is a method for elucidating how homogenous an interaction is. Importantly, it is not a proof of which binding model is the accurate, but rather a tool for formulating or rejecting hypotheses on the binding mechanism of a particular interaction. When combined with other types of analyses, IM is a powerful tool for biological research as shown by Björkelund et al (2011).

Create Interaction Maps from curve data

Real-time curve data opened in TraceDrawer can be used for IM calculations. The calculations are performed using an external web based software to which the raw data is sent by e-mail.

A kinetic evaluation object can be saved in a text file format compatible with the IM calculator. After all concentration changes in the object have been defined, proceed to the Cut and fit step of the kinetic evaluation and press **Interaction Map data**. If you choose to let TraceDrawer create the e-mail, you only need to send the mail. If you choose to save the Interaction Map raw data file and send it from a different computer, use the following address details:

Recipient: im@ridgeviewdiagnostics.com

Subject: Interaction Map

Attachment: The file with raw data.

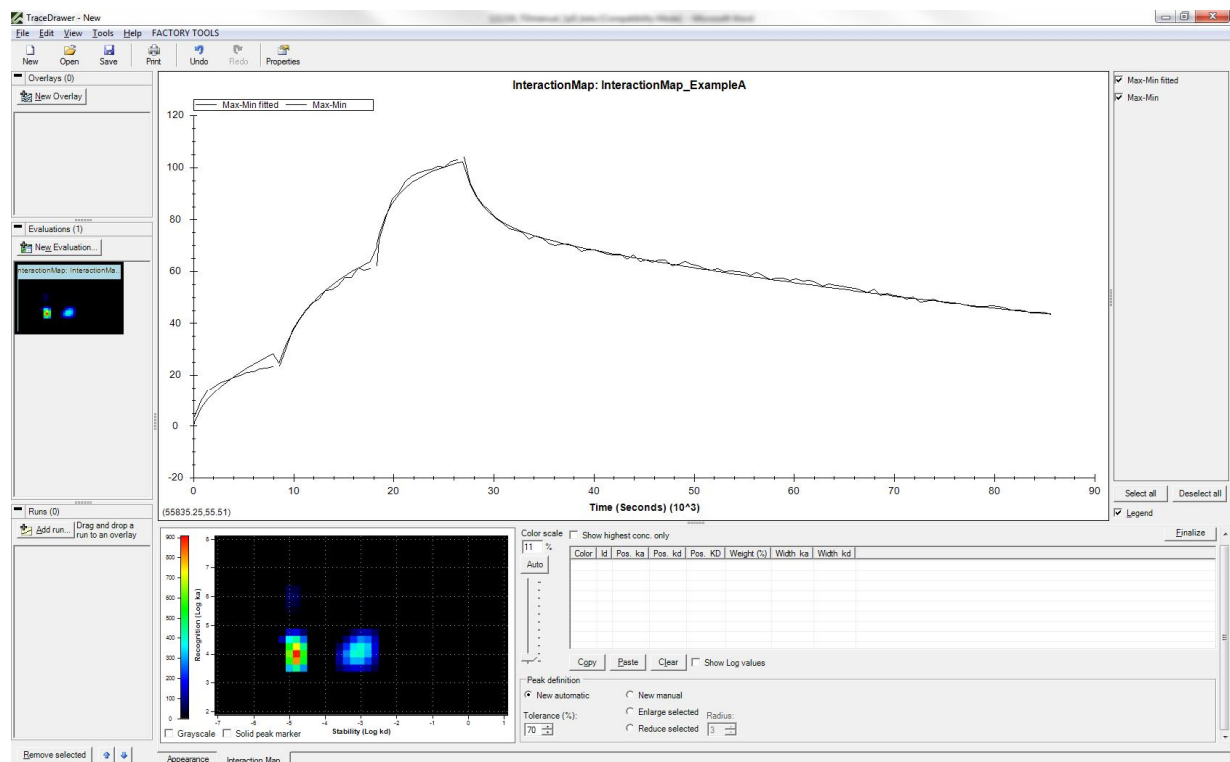
The raw data file is a plain text file but it contains a check sum. Hence, you can open and review the contents of the file. If you edit the file, the Interaction Map server will reject it because the file has been tampered with.

Shortly after the email has been sent, a confirmation message is returned to your inbox. The resulting interaction map is returned when ready, normally within 24h.

Open Interaction Maps

Interaction Map (.imp) files is opened through the **Add run** button. Make sure that either All files (*.*) or Interaction map file (*.imp) is selected in the **Files of type** list. There are two exemplary imp files available in the installation folder, (typically C:\Program Files\Ridgeview Instruments AB\TraceDrawer\Example Files).

Opened IM files are found as thumbnails in the Evaluation section. The data consist of both the Interaction Map (lower area) and a graph presenting the original curve data and its IM fit (upper area).



Evaluate Interaction Maps

There are several tools for evaluating Interaction Map data and modifying the appearance of the maps:

- Color scale can be shifted using the tool to the right of the map. The **Auto** button sets the scale so that the pixels with largest contribution become red.

- Selection of peaks:
 - Automatic selection: Choose **New automatic**. Click on a peak in the map. The sensitivity of the peak definition tool can be modified by altering the value of Tolerance (%).
 - Manual selection: Choose **New manual**. The size of the marker can be modified using the **Radius** list. **Enlarge** or **reduce** the selected peak using the radiobuttons. Only adjacent areas can be added to an existing peak.

The kinetic properties of the peak, such as k_a and k_d , is presented in the table, as well as how much the interaction contributed to the measured interaction (%).

The corresponding curve of the selected peak is presented in the graph above. The weight defines the amplitude of the curve.

The **Clear** button removes all area definitions from the map and table.

- To compare peaks in different IMs, click the **Copy** button below the table. This copies the area definitions of the selected IM. Choose another IM and click **Paste**. The peak definitions of the first IM appear.
- Check the **Grayscale** box to shift to a b/w mode. May facilitate IM interpretation and is suitable for publication.

Recommended reading

Kinetic analysis and fitting has been described by numerous authors. The following papers are recommended for those that want to learn more about kinetic analysis:

- Myszka DG, Morton TA, Doyle ML, Chaiken IM. *Kinetic analysis of a protein antigen-antibody interaction limited by mass transport on an optical biosensor*. Biophys Chem. 1997 Feb 28;64(1-3):127-37
- Karlsson R, Fält, A, *Experimental design for kinetic analysis of protein-protein interactions with surface Plasmon resonance biosensors*, J. Imm. Meth 1997;200:121-133
- Karlsson, R. *Affinity analysis of non-steady-state data obtained under mass transport limited conditions using BIAcore technology*, J. Mol. Recognit. 1999;12:285–292
- Önell, A, Andersson K. *Kinetic determinations of molecular interactions using Biacore—minimum data requirements for efficient experimental design*, J. Mol. Recognit. 2005; 18: 307–317
- Khomyakova E et al, *On-chip hybridization kinetics for optimization of gene expression experiments*, BioTechniques 2008; 44:109-117
- Björkelund H, Gedda L, Andersson K, *Avoiding false negative results in specificity analysis of protein-protein interactions*, J. Mol. Recognit. 2010, DOI: 10.1002/jmr.1026
- Björkelund H, Gedda L, Barta P, Malmqvist M, Andersson K. *Gefitinib Induces Epidermal Growth Factor Receptor Dimers Which Alters the Interaction Characteristics with ¹²⁵I-EGF*. 2011, PLoS ONE 6(9): e24739.

Summary items

The generated results from the evaluation items can be gathered in a summary item. Only finalized evaluations can be summarized. The Finalize button is found in the upper right corner of the Kinetics evaluation or Affinity evaluation tab.

Create a new summary by right-clicking on a finalized evaluation item and choose **Summary**. It is also possible to press the New Evaluation button and choose from the finalized evaluations in the list. When one Summary item is created, other finalized evaluations can be dragged and dropped into it to produce a list of results from several evaluations. Only evaluation items of the same type (e.g. Kinetics or Affinity item) and using the same parameters (e.g. fit models) are allowed in the same summary.

On/off rate map

Summaries of kinetic evaluation items can be presented as on/off plots by clicking the **On/off rate map** button in the summary tool. Each curve is then presented as a coordinate in the k_a/k_d -space. New data points can be added to this space, e.g. using data from literature for comparison. Press **Enter data** and enter a name and the k_a/k_d values.

Interaction Map summary

Finalized Interaction Map objects can be compared in a Summary. All defined peaks are presented in the table and it is possible to shift between **Curve data** and **Map data**.

Preferences

Preferences is found in Tools->Preferences. All modifications in the Preferences dialogue are saved on the computer and will be remembered when opening a new instance of TraceDrawer.

The **Default Values** button in the lower left corner reset all values of the active tab.

General

The **General tab** provides tools for altering the appearances of thumbnails (size and color).

The **number of decimals** concerns all numbers shown in TraceDrawer, e.g. in the Modification tab and the calculated results in evaluation items.

A new logo can be added to give a personal touch to your print-out report.

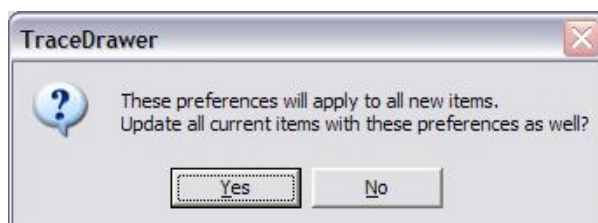
The **Add run name to curve in overlays** box defines the curve names in overlays. If you have a "Curve 1" in Run A and drag the run to an overlay, the curve will either be named "Run A: Curve 1" or just "Curve 1", depending on if the box is checked or not. Information about the run of a curve can always be found in the Overlay properties, under "Source" in the curve list.

The **Undo stack size** selection defines the number of operations possible to undo. The Unlimited option makes it possible to reverse all actions done in TraceDrawer, but uses more internal memory (RAM), thus decreasing the performance of the software.

Fonts and graph

Any modifications of fonts and graph in Preferences is general and will apply to all new runs, overlays or evaluation items. When modified, there is an option to change all current items as well.

For more information about these tools, see Summary of graph appearance tools above.



Add simulated data

Data can be simulated to improve the understanding of kinetics. It also makes it possible to compare simulated and real data in order to better comprehend results from experiments.

The simulation tool is found in the Tools top menu. A series of curves with different properties can be created according to user-defined settings:

1. Data model

- a. **Frequency:** The data point frequency can be set to 1 or 10 points/second (typical for SPR and QCM measurements) or one point each 10 seconds (typical LigandTracer measurements).
- b. **Uptake and retention time:** The simulated measurement normally consists of a short baseline, followed by an uptake (association) phase and a retention (dissociation) phase. The baseline is automatically set to suit the chosen time scale.
- c. **Interaction model:** Choose between four kinetic models: An ordinary 1:1 interaction, a 1:1 interaction with depletion or diffusion or a 1:1 dissociation (without the uptake phase).
- d. **Interaction parameters** (for more information: See Kinetic evaluation):
 - i. B_{\max} : The maximum possible signal, representing full saturation of the receptors
 - ii. k_a = association rate constant (uptake is defined by $(dY/dt = (k_a * c - k_d) * Y)$ in an ordinary 1:1 interaction, where Y = number of complexes and c = concentration)
 - iii. k_d = dissociation rate constant (retention is defined by $(dY/dt = - k_d * Y)$ in an ordinary 1:1 interaction)
 - iv. Bl = possible bulk effect, in signal units
 - v. k_t = mass transport coefficient

vi. n_B = Number of receptors on the surface

vii. Vol = Volume, in litre

2. **Concentrations:** Define the concentration span of the ligand by choosing the number of curves and how the concentrations are increased.

Example:

1:st conc: 1 nM

Factor: 5

curves: 4

will generate four curves simulating the interaction between the receptors and the ligand of concentrations 1 nM, 5 nM ($5 * 1$ nM), 25 nM ($5 * 5$ nM) and 125 nM ($5 * 25$ nM).

3. **Noise:** Add noise to create curves that are more similar to reality.

Summary of menu-based and plot-based functions

Operation	Command (different alternatives)
Select curves in a plot	<ul style="list-style-type: none"> • Left-click on the curve names in the curve list • Hold the Ctrl key while left-clicking on the curve names to multi select. • Hold the Shift key and left-click on the first and last name of a group of adjacent curves in the list to stretch the selection. Click on the Select/Unselect all buttons below the curve list
Hide curves in a plot	<ul style="list-style-type: none"> • Untick the boxes in the curve list • Select curves (see above), right-click in the plot and choose Hide selected • Select curves (see above), right-click in the curve list and choose Hide selected
Remove curves in a plot	Select curves (see above), right-click in the curve list and choose Remove selected
Rename curves	Right click on a name in the curve list and choose Rename ...
Zoom in	<ul style="list-style-type: none"> • Hold down the left mouse button and drag it to create a rubber band box in the plot • Scroll with the middle mouse wheel • Right-click in the plot and choose Zoom to extents • If zoomed out: Right-click in the plot and choose Un-zoom
Zoom out	<ul style="list-style-type: none"> • Double-left-click in the plot • Scroll with the middle mouse wheel • Right-click in the plot and choose Zoom to extents • If zoomed in: Right-click in the plot and choose Un-Zoom

Move curves	<ul style="list-style-type: none"> • Drag a curve in the plot while pressing Alt • Drag a curve in the plot while pressing Alt+Shift to move it either in X or Y direction • Choose dX and dY in Curve move • Move curve intervals or positions of selected curves in Curve offset
Show Legend	Tick the box Legend below the curve list to the right
Move Legend	Change settings in View(top menu)->Legend position
Reorder items in the thumbnail area	Drag and drop selected thumbnails to other parts of the area
Reorder curve list items	Drag and drop the name of the curves in the curve list.
Open Properties	<ul style="list-style-type: none"> • Right-click on a thumbnail, choose Properties • The Properties icon below the top menu
Create Evaluation	<ul style="list-style-type: none"> • The New Evaluation button in the Evaluations thumbnail field – choose an overlay from the list • Right-click on an overlay and choose either Affinity/EC50, Kinetics Evaluation or Kinetics Competition
Simulate data	<ul style="list-style-type: none"> • Tools -> Add simulated data
Modify axis	<ul style="list-style-type: none"> • Tools -> Preferences -> Graph (applies to all new items) • Right-click on an axis (applies to the specific item)
Modify fonts	<ul style="list-style-type: none"> • Tools -> Preferences -> Fonts (applies to all new items) • Right-click on an axis (applies to the specific item)
Add textbox	Right-click inside a plot, choose Add textbox
Add line/arrow	Right-click inside a plot, choose Add line/arrow
Compile reports/Print	<ul style="list-style-type: none"> • File-Print • The Print icon below the top menu
Concentration abbreviations, 1 nM	<ul style="list-style-type: none"> • Letter format: n (p for pM, u for μM, m for mM) • Scientific format: 1e-9 • Number format: 0.000000001

