SPR-Simulation

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Content

1. Introduction

The simulation of kinetic curves can help in the design and understanding of interaction experiments. One of the aims of the simulations is to find the best interactions conditions (response, analyte concentrations, and injection and dissociation times) and thereby save ligand, analyte and experimental time. In addition, simulations can be used to understand the results of an interaction by inspecting the differences between the simulated curves and the real data.

Several studies show the benefits of kinetic simulations. Palau and Di Primo show that simulated single cycle kinetics can improve the design of the surface plasmon resonance assay and thereby saving experimental time and sample (3). De Crescenzo et al. uses online optimization to improve sample throughput and enhance the confidence in the results (1). And Muller et al. show that by comparing simulated curves with real measured sensorgrams can be helpful in understanding the type of interaction (2).

The SPR-Simulation program is a standalone application to simulate kinetic interaction curves. The program draws the curves after the input of the kinetic rate constants, the analyte concentration and the association and dissociation times. Other features are the simulation of equilibrium sensorgrams and plotting an overlay over an imported sensorgram figure.

Ideally the SPR-Simulation program is used after some initial analyte injections and fittings. The simulation of interaction curves based on the fitting results can give extra information to improve the experimental set-up. For instance, use the program to determine that the analyte concentration is such that it saturates 10 to 90% of the ligand (approximately 0.1 to 10 times the K_D). In addition, simulations can show what the optimal association and dissociation times should be to have enough curvature and decay in the curves.

The program can be found on the SPR-Pages (www.sprpages.nl/downloads/software).

2. Installation

First unzip the SPR-Simulation.zip file to a location of your choosing. It is recommended to create a separate directory to store the SPR-Simulation program and helper files. After unzipping two files are present.

SPRSimulaton.exe	The SPR simulation program
SPRSimulation.pdf	This Help file

SPR-Simulation is ready to be used. No installation or activation. SPR-Simulation does not write anything to the registry.

The following files are a part of the SPR-Simulation program. Depending on the settings some files are added or removed in the same directory as where the SPR-Simulation program is located. If the program is not starting, these files can be deleted.

SPRSimulation.ini	Program settings
SPRSimulation.dat	File with last used kinetic values
GridValuesFile.ini	File with last used kinetic settings

2.1. Error in converting value

This error occurs sometimes and has something to do with the decimal and number grouping symbols.

In Windows 10 go to configuration – clock and region
Then select Land/region – more settings to change.
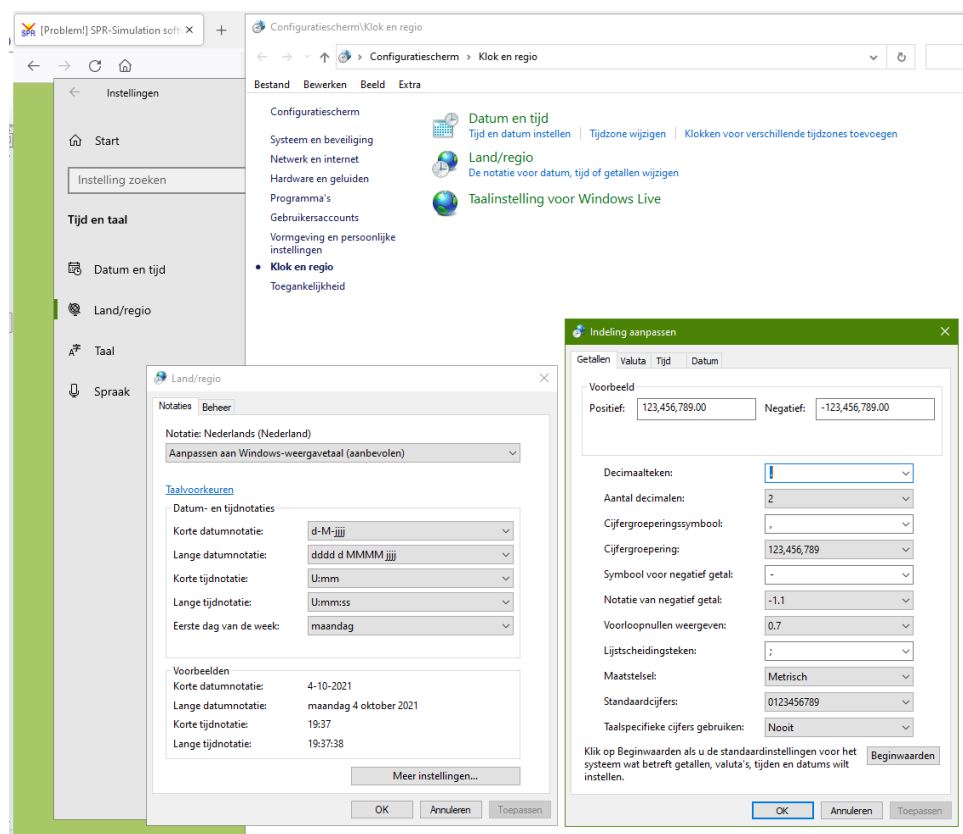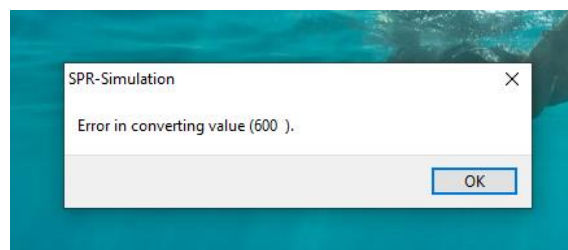
3. General components in the program

Because the program is still in development, some of the figures can differ from the actual program.

3.1. Menu

The menu gives access to some general features.

- With File menu the program can be closed although the close button at the right side is more convenient.
- With the examples menu four pre-defined kinetic examples can be chosen for the 1:1 kinetic graph. Clicking on the menu items will set the example after which it can be modified. This menu is disabled when the other tabs are selected.
- The settings menu opens the settings dialog. See separate paragraph.
- The help menu gives access to this help file and the About box with the program information.
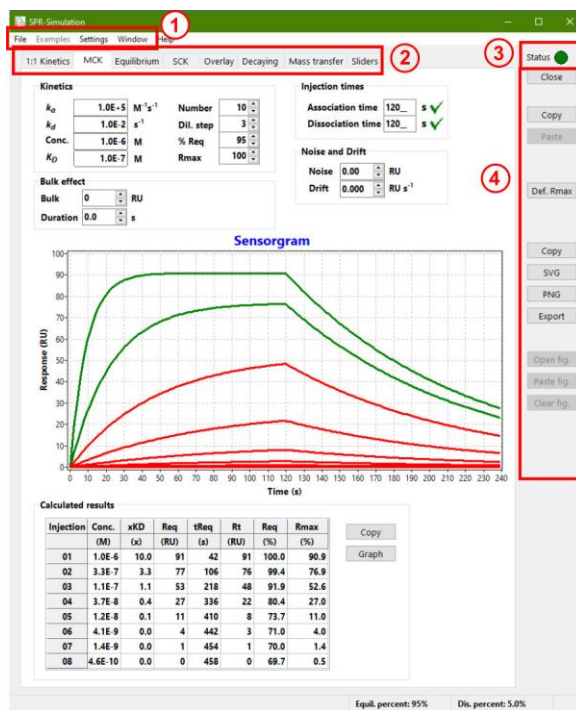
3.2. Tabs

The main window contains several tabs, which display different types of kinetic curves.

- **1:1 kinetics** displays four curves that can be individually set. Change the kinetic parameters in the table and compare the results in the sensorgram and calculated results page.
- **MCK** displays a multi cycle kinetics sensorgram.
- **Equilibrium** displays a set of curves and the equilibrium plot. The purpose is to model the parameters (kinetic rate constants, analyte concentration and injection times) such that at the end of the analyte injection all the curves are at equilibrium.
- **SCK** displays a single cycle kinetics sensorgram.
- **Overlay**. Use the overlay tab to load a sensorgram figure from an article or book. Then adjust the kinetic parameters of the simulation and try to overlay the simulation over the loaded figure.
- **Decaying**. Use this tab to simulate a ligand capture and subsequent analyte injection.
- **Mass transfer**. Simulate curves without and with mass transfer limitation and compare.

3.3. Status indicator

The status indicator signals if the simulation is updated with the latest changes. Updating is automatic but has a slight delay. If you start editing during the delay, updating is halted until new values are accepted.

3.4. Buttons

The close button closes the program.

The upper Copy and Paste button can be used to copy and paste the values between the different tabs. The restriction here is that when the copied value is not present in the destination tab it is ignored. Also, values which where undefined when the copy button was pressed are ignored in the destination tab.

With the Def.Rmax a new Rmax can be defined based on the ligand and analyte properties. This button opens a new dialog. In this dialog the theoretical Rmax can be calculated. Provide the values for the ligand and analyte and the new Rmax will be calculated. If OK is pressed the new Rmax value is copied to the SPR-simulation program.

There are several buttons to copy, save and export the simulated sensorgrams or curve data.

- Copy   will copy the sensorgram to clipboard as a bitmap

- SVG   will save the sensorgram to a SVG-file
- PNG   will save the sensorgram to a PNG-file
- Export   will export the curves to a Text-file (refer to the settings dialog)

3.5. Settings

The settings menu opens a new dialog with several tabs.

- **Start-Up** will define some start-up behaviour. Choose if the program should start with the default values or with the last used values.
- **Calculations** will define some parameters used in the calculations. The baseline time is the time before the sensorgram begins. Equilibrium defines the percentage equilibrium that should be reached and is used in the time to reach equilibrium calculations (tReq) in the calculated results table.
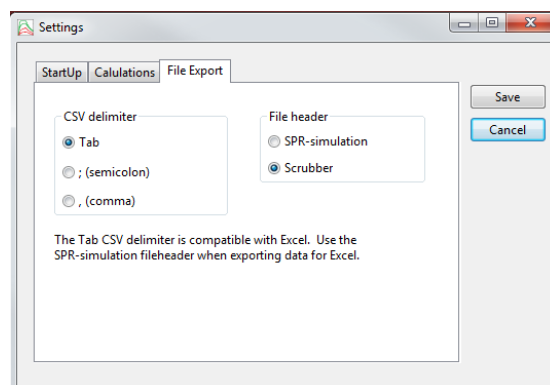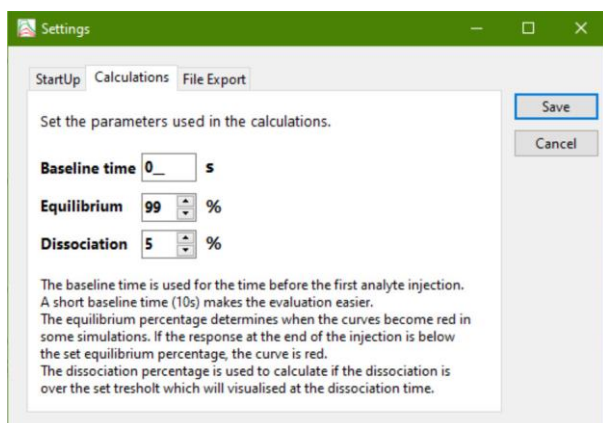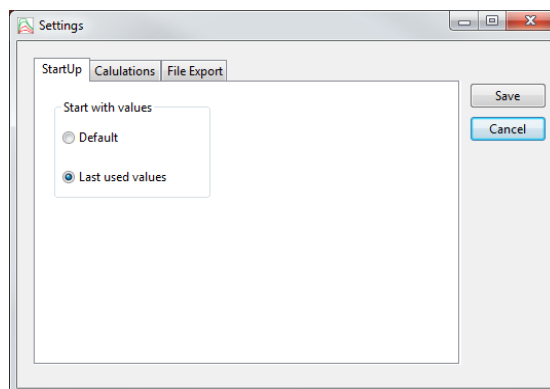
- **Export** defines the method the curves are exported. The CSV delimiter defines the method of storing the data in the file. The Tab is compatible with Excel and Scrubber files. Use SPR-simulation setting if you want to make your own graphs (in Excel) and the Scrubber setting to import the data in Scrubber or BiaEvaluation (old versions).

The values from the Equilibrium percentage and Dissociation percentage are visible in the statusbar on the bottom of the program.

	Equil. percent: 95%	Dis. percent: 5.0%
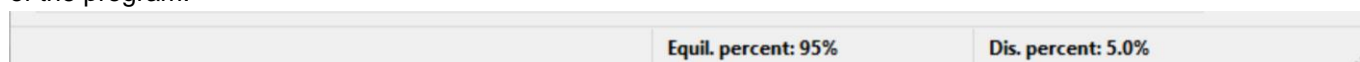
3.6. Limits

The program has some limits to the values that can be entered.

Table 3.1.

Parameter	Lowest value	Highest value
k_a (M^{-1}s^{-1})	1	10^8
k_d (s^{-1})	10^{-6}	0.9
Concentration (M)	10^{-9}	1
Time (s)	60	86400 (24 hours)
Rmax (RU)	1	10^5
K_m (m s^{-1})	10^{-9}	10^{-4}
K_t (RU M^{-1}s^{-1})	10^4	9.99 10^9
Mw (Da)	100	10^9
%Req	1	99

4. Tools

4.1. Zooming

Zooming is very easy -- You just have to drag a rectangle with the left mouse button down around the feature of interest that you want to see in detail. Keep in mind that you have to drag the mouse from the top-left to the right-bottom corner of the rectangle to get the zoom effect. After you release the mouse button, the region is blown up to fill the entire chart.
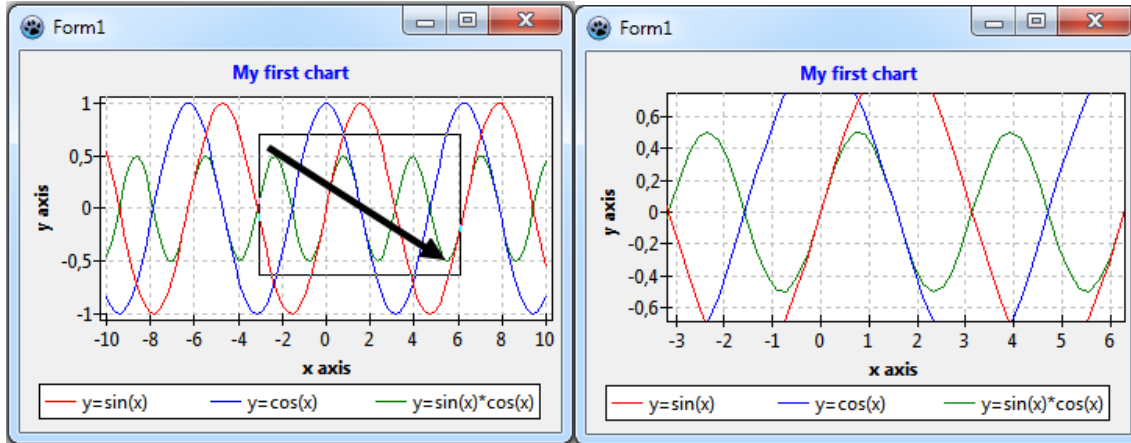
You can repeat this procedure to zoom into the chart deeper and deeper. If you want to restore the original viewport just click into the chart with the left button, or left-drag a rectangle in any direction, except of course from top-left to right-bottom -- this would zoom in even further.

4.2. Panning

After you have zoomed into a chart you may want to shift the viewport a bit to find a better view. This operation is called "panning". Pan by holding the right mouse button down and drag the viewport into the desired direction.
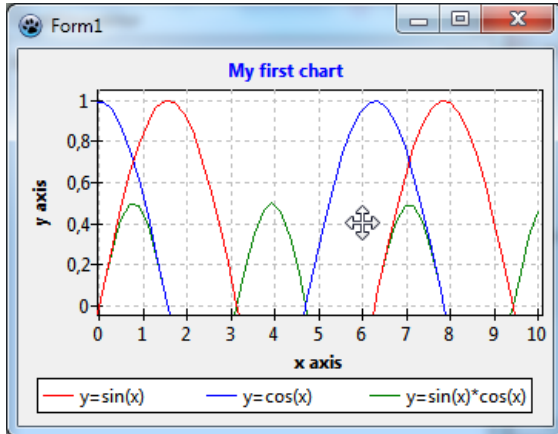
As with zooming you can restore the original viewport by clicking into the chart, or by dragging a rectangle in any direction, except from top-left to bottom-right. Note that you use the left mouse button for "un-panning" although you had used the right button for panning.

Zooming and panning work a little different on the 'Overlay-tab'. Choose the zooming direction and use the scroll-wheel of your mouse to zoom. Zooming starts at the origin of the sensorgram. Panning can be done by holding the right-mouse button down and moving the mouse.

5. TABS

Each tab has a slightly different layout but there are also common areas.

1. **Kinetics:** here are the association and dissociation rate constants defined. In addition, define the highest analyte concentration. Depending on the tab, define the number of curves, analyte dilution step and the maximal response.
2. **Injection times:** define the association and dissociation times. The time before the injection start is defined in the settings – calculations.
   At most tabs there is a tick besides the times. When the tick is green the time is long enough to have the association reaching the set equilibrium percentage and the dissociation is long enough to reach the dissociation percent.
3. **Sensorgrams and graphs:** here the sensorgrams and graphs are displayed.
4. **Calculated results:** here the calculated results based on the input are displayed. The columns may differ depending on the tab selected
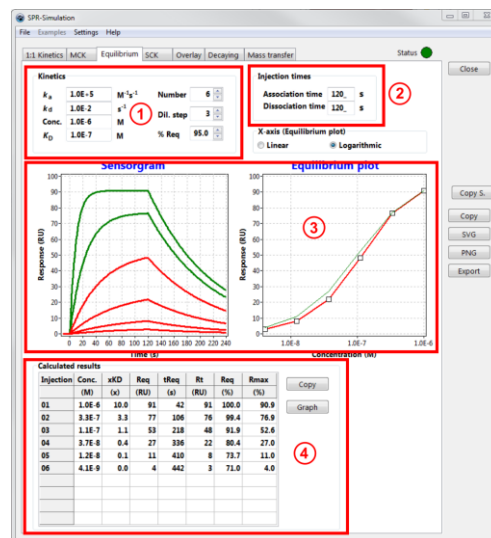
At some tabs more options are available. See below for more information.

5.1. 1:1 kinetics

Use the 1:1 kinetics to simulate four curves. For each curve, individual kinetic parameters can be set (k_a, k_d, analyte concentration and Rmax). In addition, set association and dissociation time and add noise and drift if desired. The menu item 'Examples' contains four pre-defined settings that can be copied to the input grid by selecting the option.
In the 'calculated results' the input values and calculated parameters are given.

Calculated results

Conc. (M)	the set analyte concentration
k_a (M^{-1}s^{-1})	the set association rate constant
k_d (s^{-1})	the set dissociation rate constant
K_D (M)	the calculated dissociation constant
xK_D (x)	the ratio of the analyte concentration and the K_D
Rmax (RU)	the set Rmax
Req (RU)	the calculated response at equilibrium when the injection time is long enough to reach equilibrium
tReq (s)	the time to reach (99%) equilibrium
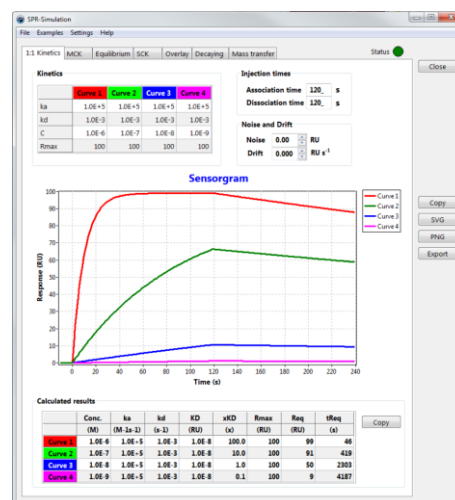
5.2. Multi cycle kinetics

Use the multi cycle kinetics (MCK) tab to simulate an analyte concentration series. One set of kinetic parameters (k_a, k_d, analyte concentration and Rmax) can be defined. Set the number of curves and dilutions step of the concentration series. The %Req defines the colour of the curves. All curves reaching at least the set value in %Req will become green indicating that they reached the minimal set equilibrium response at the end of the injection time. When the curves are red, they are below the set value. Compare with the Equilibrium tab. Behind the association and dissociation rate constants are ticks. When they are green then the time is long enough to reach the percentage steady state (as give in the settings) or the time is long enough to give the percentage dissociation.

In the MCK it is also possible to add the bulk effect during the association. The bulk value in RU is for the highest concentrations and for the dilutions the bulk value is calculated accordingly. The duration of the bulk affect can be set between 1 – 6 seconds.

Calculated results

Conc. (M)	the set analyte concentration
xK_D (x)	the ratio of the analyte concentration and the K_D
Req (RU)	the calculated response at equilibrium when the injection time is long enough to reach equilibrium
tReq (s)	the time to reach (99%) equilibrium
Rt (RU)	the response at the end of the injection time
Req (%)	the percentage of reached Req at the end of the injection time
Rmax (%)	the percentage of Rmax reached at the end of the injection time

5.3. Equilibrium

The equilibrium tab shows two graphs. On the left the kinetic sensorgram and on the right the equilibrium plot which plots the response values from the end of the analyte injection time. In the sensorgram the curves which reach less than the set %Req are red and the curves above this value are green. Use the X-axis options to change the x-axis of the equilibrium plot.

Calculated results

|	
Conc. (M)	the set analyte concentration
xK_D (x)	the ratio of the analyte concentration and the K_D
Req (RU)	the calculated response at equilibrium when the injection time is long enough to reach equilibrium
tReq (s)	the time to reach (99%) equilibrium
Rt	the response at the end of the injection
Req (%)	the percentage of reached Req at the end of the analyte injection time
Rmax (%)	the percentage of reached Rmax when steady state is reached

Use the graph button to make a graph of the Req, xK_D and the tReq versus the analyte concentration. This graph shows how the Req, xKD and tReq varies with the concentration of the analyte.

5.4. Single cycle kinetics

Use the single cycle kinetics tab to optimize for analyte concentration, dilution step and injection times. The sensorgram will calculate five dilutions and an extra dissociation time is calculated by multiplying the dissociation time by the Ext. diss. factor.

Calculated results

Conc. (M)	the set analyte concentration
xK_D (x)	the ratio of the analyte concentration and the K_D
Req (RU)	the calculated response at equilibrium when the Injection time is long enough to reach equilibrium
tReq (s)	the time to reach (99%) equilibrium
Rt	the response at the end of the injection
Req (%)	the percentage of reached Req at the end of the analyte injection time
Rmax (%)	the percentage of reached Rmax when steady state is reached
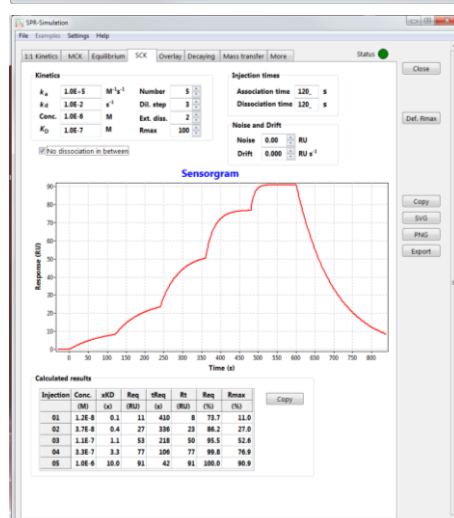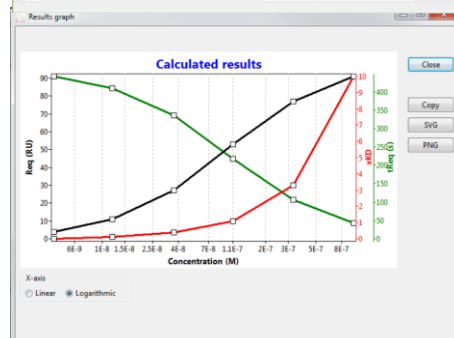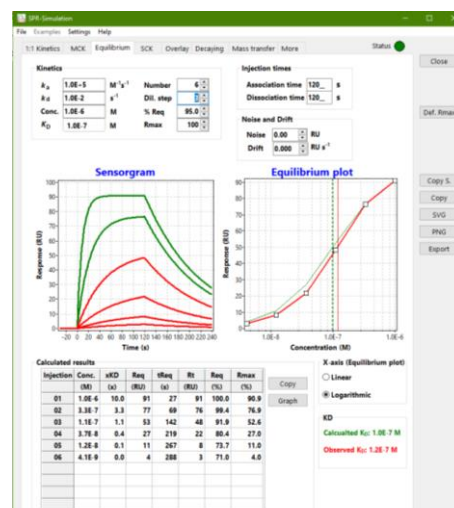
If the box "No dissociation in between" the dissociation between the analyte injections is omitted (see figure). Some instruments allow injecting subsequent higher analyte concentrations without dissociation.

5.5. Overlay

Use the overlay tab to load a sensorgram from another source. This can be a bitmap, GIF or PNG file. Choose the type of simulation: Multi cycle or single cycle kinetics. With the single cycle kinetics use the box "No dissociation in between" to remove dissociation in between injections. An extra dissociation time is calculated by multiplying the dissociation time by the Ext. diss. factor. Adjust the kinetic constants, injection times and sensorgram size to overlap with the loaded figure.

Use the mouse wheel to resize and set the zoom-direction to resize in one direction. Use the right mouse button to pan the sensorgram.
The overlay sensorgram can be exported but the loaded figure is not incorporated in the export.

Calculated results

Conc. (M)	the set analyte concentration
xK_D (x)	the ratio of the analyte concentration and the K_D
Req (RU)	the calculated response at equilibrium when the injection time is long enough to reach equilibrium
tReq (s)	the time to reach (99%) equilibrium
Rt	the response at the end of the injection
Req (%)	the percentage of reached Req at the end of the analyte injection time
Rmax (%)	the percentage of reached Rmax at the end of the analyte injection time
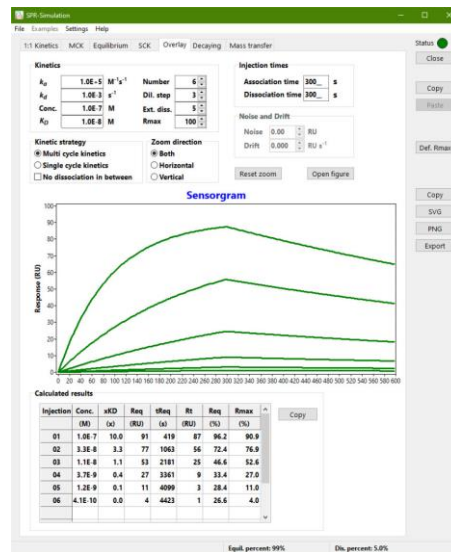
5.6. Decaying

Use the decaying tab to simulate the possible outcome of an interaction on a decaying surface. Set in the ligand capture, the kinetic parameters for the ligand capturing. Then set the kinetics for the analyte interaction.

Press subtract to get the analyte curves without the ligand decaying. Press calculate to return.

5.7. Mass transfer

Use the mass transfer tab to simulate mass transfer limited curves. In addition to the kinetic constants defining a 1:1 interaction the mass transfer constants k_m and k_t can be defined. In the graph there are two coloured graphs. The green is the 1:1 interaction without mass transfer and the red is the 1:1 interaction with mass transfer. This gives a good insight in how the mass transfer is affecting the shape of the curve.

The mass transfer curves are calculated with the following formulas.

Reaction equation

$$A_{bulk} \; \underset{k_m}{\overset{k_m}{\rightleftharpoons}} \; A_{surface} + L \; \underset{k_d}{\overset{k_a}{\rightleftharpoons}} \; LA$$

Differential equation

$$\frac{d[LA]}{dt} = \left(\frac{k_a}{1 + \dfrac{k_a \cdot [L]}{10^9 \cdot Mr \cdot k_m}} \right) \cdot [L] \cdot [A] - \left(\frac{k_d}{1 + \dfrac{k_a \cdot [L]}{10^9 \cdot Mr \cdot k_m}} \right) \cdot [LA]$$

[A]	= $C_{analyte}$
$[L]_0$	= R_{max}
$[LA]_0$	= 0

Parameters
[L]   = concentration of free ligand in RU
[A]   = concentration of free analyte in M
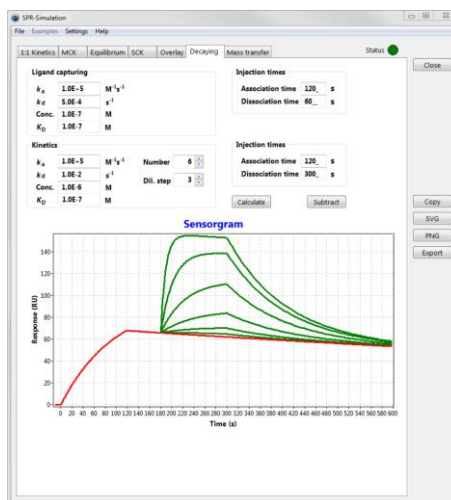
[LA]	= concentration of ligand-analyte complex in RU	
k_a	= association rate constant in M^{-1} s^{-1}	
k_d	= dissociation rate constant in s^{-1}	
k_m	= mass transfer constant in m s^{-1}	
Mr	= molecular mass of analyte in Dalton	
10^9	= conversion factor between molar concentration and RU	

When the values are changed in either of the mass transfer constants the other are recalculated with the formula:

$$k_t = k_m \cdot M_r \cdot 10^6$$

Calculated results

Conc. (M)	the set analyte concentration
xK_D (x)	the ratio of the analyte concentration and the K_D
Req (RU)	the calculated response at equilibrium when the injection time is long enough to reach equilibrium
tReq (s)	the time to reach (99%) equilibrium
Rt (RU)	the response at the end of the injection time
Req (%)	the percentage of reached Rmax when steady state is reached

Note: when comparing BiaSimulation with the Evaluation software of the BC2000/3000 the Mass transfer equations with k_t the molecular weight of the analyte to use to convert to k_m is 1000 Da.

5.7.1. Mass transfer calculations

The mass transfer calculations dialog box contains four tabs that calculate each a different step in the mass transfer. Two buttons are present which act on the calculated values.

Get Prev. will copy the calculated value of the previous tab to the next
Get Value will copy the value of the k_m or k_t to the SPR-simulation part.

Viscosity

The first tab calculates the viscosity of water at a defined temperature (in °K).

Diffusion

This tab calculates the diffusion of a molecule based on the molecular mass, friction factor (globularity of the molecule) and the solution viscosity.

Mass transfer coefficient (km)

The mass transfer coefficient is calculated based on the diffusion factor of the molecule, flow rate and the geometry of the flow cell. Default values are based on the Biacore 2000/3000 instruments.

Mass transfer coefficient (kt)

This mass transfer coefficient is based on the k_m and the molecular size of the molecule.
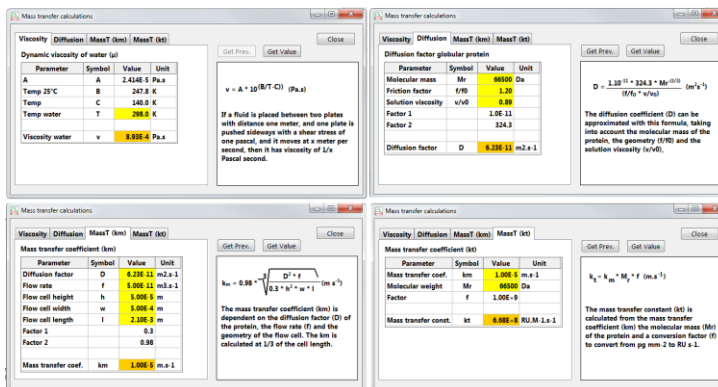
5.8.   Sliders

This is a whole new way to show the kinetics. By moving the sliders and adding the numbers, you can see almost real time the changes in the curves.

Use the boxes to input your starting values. Each slider is will make the value maximal 10 times smaller or larger.

By fixing the Rmax, the Y-axis of the sensorgram will stay the same during the simulation. Otherwise, it will scale to the curves.

By fixing the K_D both k_a and k_d are changed to give the same K_D.

Work in progress….
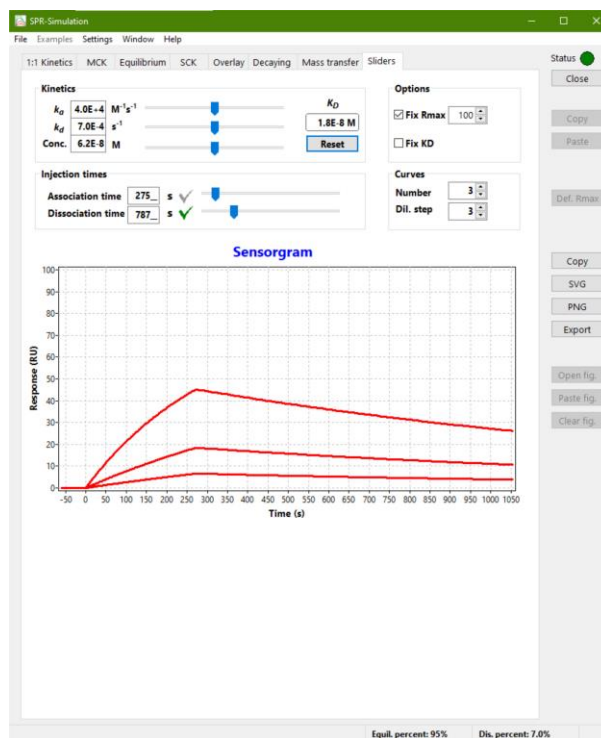
6. Calculated results

The formulas used to calculate the results are as follows

6.1. Association part of the curves

$$R_t = R_{eq} \cdot (1 - e^{-(k_a \cdot C + k_d)(t - t_0)}) \qquad (1)$$

6.2. Dissociation part of the curves

$$R_t = R_0 \cdot e^{-(k_d \cdot (t - t))} \qquad (2)$$

6.3. KD

The equilibrium dissociation constant (K_D) is calculated by the division of the dissociation and association rate constant.

$$K_D = \frac{k_d}{k_a} \quad (M) \qquad (3)$$

6.4. xKD

The xKD is the ratio between the analyte concentration and the equilibrium dissociation constant. This value gives a good estimation of the practical range of the analyte concentration. It is recommended to inject an analyte concentration between 0.1 en 10 x K_D.

$$xK_D = \frac{C_{analyte}}{K_D} \quad (x) \qquad (4)$$

6.5. tReq - Time to reach equilibrium

This formula calculates the time to reach equilibrium based on the association and dissociation rate constants and the analyte concentration. The default percentage of Req to reach is set to 95% of the Rmax and can be changed in the settings (10 – 99%).

$$t_\Theta = \frac{-\ln(1 - \Theta)}{k_a \cdot C + k_d} (s) \qquad (5)$$

6.6. Rt – The response at end of injection time

Rt in RU is the calculated response at the end of the injection time.

6.7. Req (RU) or (%)

The Req in RU is the response at equilibrium and calculated from the kinetic rate constants, the analyte concentration and the Rmax. The Req in percentage is calculating how much of the Req is reached at the end of the injection time.

$$R_{eq} = \frac{k_a \cdot C}{k_a \cdot C + k_d} R_{max} \qquad (RU) \qquad (6)$$

$$R_{eq} = \frac{R_t}{R_{eq}} \cdot 100\% \qquad (\%) \qquad (7)$$

6.8. Rmax (%)

The Rmax in percent is calculated from the Req and Rmax (both in RU) and shows the percentage of saturation of the ligand when the injection time is long enough.

$$R_{max} = \frac{R_{eq}}{R_{max}} \quad (\%) \tag{8}$$

7. References

1.   **De Crescenzo, G., Woodward, L. and Srinivasan, B.**; Online optimization of surface plasmon resonance-based biosensor experiments for improved throughput and confidence. *Journal of Molecular Recognition* **(21)** 4: 256-266; 2008.
2.   **Muller, K. M., Arndt, K. M. and Pluckthun, A.**; Model and simulation of multivalent binding to fixed ligands. *Analytical Biochemistry* **(261)** 2: 149-158; 1998.
3.   **Palau, W. and Di Primo, C.**; Simulated single-cycle kinetics improves the design of surface plasmon resonance assays. *Talanta* **(114)** 0: 211-216; 2013.