BIAsimulation Software
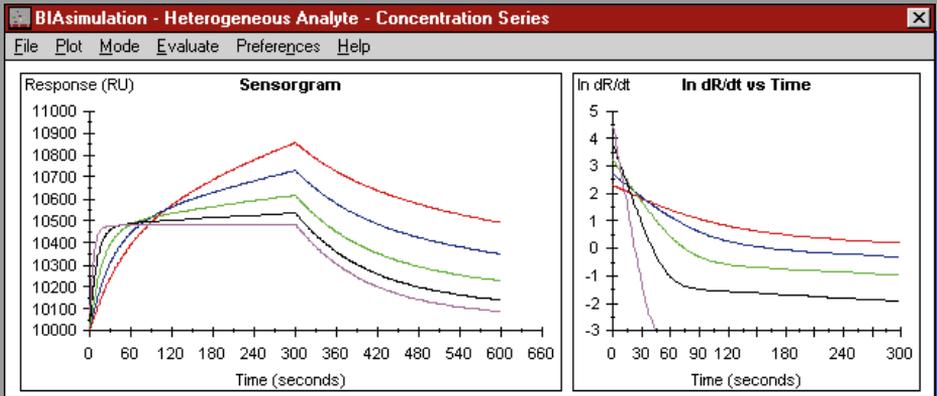
Handbook

BIAsimulation Software Handbook

BIAsimulation Software Handbook, Edition October 1996.

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1. Introduction to BIAsimulation

1.1 Scope of BIAsimulation

BIAsimulation software is a Windows-based software package which generates simulated sensorgrams for a range of real-time BIA applications. The package includes modules for simulating the following applications:

- *basic kinetic analysis*, including cooperativity, steric hindrance and mass transport limitations as well as experimental parameters such as signal noise and baseline drift. Facilities for simulating the effect of mass transport limitations are also provided.

- kinetics obtained with *heterogeneous ligands.*

- kinetics obtained with *heterogeneous analytes.*

- *epitope mapping*, allowing user-defined affinity constants for the various epitope probes.

- *multi-site binding experiments.*

- *concentration analysis*.

BIAsimulation software serves three main purposes:

- It is a valuable learning aid in getting acquainted with the concepts and range of applications of BIA. By running BIAsimulation, new users can familiarize themselves with the sort of results that may be expected from BIA experiments.

- It provides help in designing suitable experimental conditions for practical investigations. In some cases, the feasibility of an experimental project can be tested with BIAsimulation (e.g. the ability to distinguish between kinetic models for an interaction or to perform epitope mapping with antibodies of a given affinity range).

- It can provide help in testing the validity of kinetic evaluation of BIA measurements. In kinetic analysis with BIAevaluation, kinetic constants are normally derived from application of an analysis model to selected parts of the experimental sensorgrams. BIAsimulation can be used to generate whole sensorgrams using the experimentally obtained constants, and comparison of the actual and simulated data can reveal whether the model is valid over the whole time span of the sensorgrams. In addition, BIAsimulation can be used to explore the effects of varying experimental parameters, and can thus help to decide which experiments might be crucial in distinguishing between alternative kinetic models.

Note: BIAsimulation does not take account of steric hindrance effects at high surface binding levels, nor of the dynamic range of current BIA instrumentation (maximum response approximately 35,000 RU). Simulations performed with high concentrations of large molecules may for this reason not correspond to the results that would be obtained in practice under the same conditions. This consideration is particularly relevant to simulation multi-site binding and concentration analysis using sandwich techniques.

1.2   General interaction techniques

BIAsimulation software runs under Microsoft Windows and follows the standard Windows user interface (see the Microsoft Windows User's Guide). Starting BIAsimulation presents a menu screen for the simulation modules:
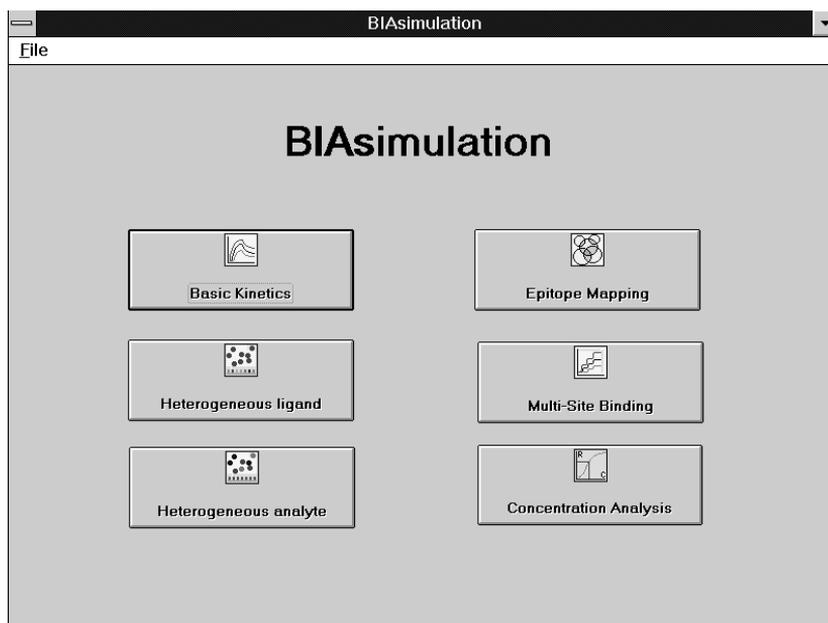
Choose a module by clicking on the appropriate button.

For each module, the BIAsimulation screen has a menu bar and a number of regions which varies according to the simulation module (Figure 1-1).
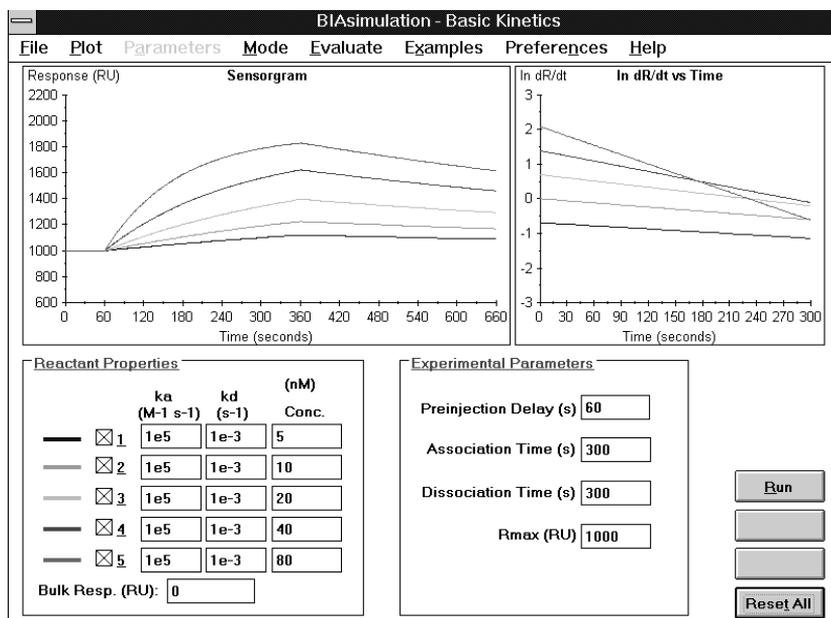
Figure 1-1. *Example of a BIAsimulation screen (example for basic kinetics), showing the common screen components.*

Common to all modules is a sensorgram window, input fields for experimental parameters and control buttons for running the simulation. Other components are specific to the different modules (e.g. data plotting windows for kinetics, interaction matrix for epitope mapping). In addition to standard Windows techniques, the following features apply:

• in a sensorgram or plot window, *double-click* with the *left* mouse button to open a dialogue box for setting the display scale:
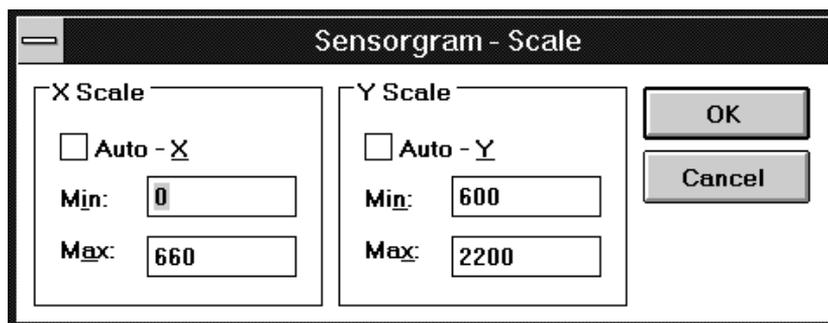
• in a sensorgram or plot window, *double-click* with the *right* mouse button to enlarge the sensorgram or plot to fill the BIAsimulation window. Double-click again with the right mouse button to restore the original display.

- in a sensorgram or plot window, zoom a selected area by holding down the *left* mouse button and dragging over the area to be enlarged. *Single-click* with the *right* mouse button anywhere in the window to return to the original display.

- in data input areas, click with the *right* mouse button on text elements for short help on the respective element. Help is only implemented on selected elements.

- all times are entered and displayed in simulated seconds. The simulation speed depends on the performance of your computer.

- numerical values may be specified in exponential or ordinary decimal notation: "5e-4" is equivalent to 0.0005 (=5 x 10^{-4}).

Only one module can be run at one time. To close a module, double-click on the system menu button at the top left of the module menu bar or choose **File:Close**. To close BIAsimulation, double-click on the system menu button at the top left of the menu bar or choose **File:Quit** from the start-up screen.

Note: The size of the BIAsimulation screen is fixed. You can reduce BIAsimulation to an icon on the Windows desktop from the start-up screen, but not from any of the module windows. You must close a module before you can use the menu bar on the start-up screen.

Printer setup
BIAsimulation software uses the current printer as defined in the Windows Control Panel. To change printer settings, open the Control Panel in Windows Program Manager and double-click on the **Printers** icon. Refer to the Windows User Guide and your printer documentation for setup facilities.

Choose **Preferences:All Curves Black** in the current BIAsimulation module if your printer does not reproduce coloured lines satisfactorily. This will display and print all simulated curves in black. (Most monochrome printers render colours as shades of grey, but some printers may produce unsatisfactory results for light colours.)

2.   Basic kinetics

The basic kinetics module simulates up to 5 sensorgrams per session, with the following features:

- user-defined association and dissociation rate constants

- user-defined bulk contribution to the signal

- user-defined analyte concentration, R_{max} and injection times

- random error generator within a user-defined interval for analyte concentration

- adjustable signal noise, averaging window and baseline drift

- independent cooperativity factors for association and dissociation

- steric hindrance

- simulation of mass transport limitations

- display of sensorgram and plots of dR/dt against R, ln(dR/dt) against t and dissociation plot

- evaluation of simulated results in terms of kinetic constants

The basic kinetics module can be used in basic or extended mode. Extended mode allows parameters for noise, drift, concentration error etc to be set by the user, and allows simulation of mass-transport controlled binding.

2.1   Theoretical background

The theory of kinetic analysis with real-time BIA is discussed in detail in the BIAtechnology Handbook, Chapter 8. Equations used in the basic kinetics simulation are as follows (these equations are also summarized under **Help:Formulae** in BIAsimulation):

2.1.1 Rate equations

Association phase

$$\frac{dR}{dt} = k_a C \left(R_{max} - R\right) - k_d R$$

where   dR/dt is the rate of change of the SPR signal
C is the concentration of analyte
R_{max} is the maximum analyte binding capacity in RU
and R_t is the SPR signal in RU at time t

Integrating with respect to time gives

$$R_t = \frac{k_a C R_{max}\left(1 - e^{-(k_a C + k_d)t}\right)}{k_a C + k_d} + R_0$$

where R_0 is an integration constant representing the baseline signal.

The association phase is analyzed in terms of two plots (see the BIAtechnology Handbook, Chapter 8 for details):

dR/dt against R gives   slope $-(k_a C + k_d)$
intercept $k_a C R_{max}$ at dR/dt=0

ln(dR/dt) against t gives   slope $-(k_a C + k_d)$
intercept $\ln(k_a C R_{max})$ at ln(dR/dt)=0.

Dissociation phase

$$\frac{dR}{dt} = -k_d R$$

Integrating with respect to time gives

$$\ln\frac{R_1}{R_t} = k_d\left(t - t_1\right)$$

where R_t is the response at time t
and R_1 is the response at an arbitrary starting time t_1

The dissociation phase is analyzed in terms of a plot of -dR/dt against R (the "dissociation plot") which has a slope of k.

BIAsimulation does not take account of possible rebinding of analyte during the dissociation phase unless mass transport limitations are included (rebinding only occurs if the free analyte concentration at the surface is not zero).

2.1.2 Cooperativity and hindrance
Cooperativity in association or dissociation is simulated by modifying the respective rate constant according to

$$k^{'} = k\left(1 + A\frac{R}{R_{max}}\right)$$

where A is the cooperativity factor (A>0 for positive cooperativity and A<0 for negative cooperativity). A value of zero indicates no cooperativity.

Steric hindrance is simulated by reducing the number of available ligand sites as association progresses. With no hindrance, the amount of free ligand on the surface at any time is given by $(R_{max}-R)$. To simulate steric hindrance, this term is replaced by $(R_{max}-B\cdot R)$, where B is the hindrance factor (B≥0). A value of 1 indicates no hindrance. Negative values indicate enhancement ("negative hindrance"). Hindrance is not relevant during dissociation.

The rate equation incorporating cooperativity in both association and dissociation and steric hindrance in association is given by

$$\frac{dR}{dt} = k_a\left(1 + A_a\frac{R}{R_{max}}\right) C\left(R_{max} - B\cdot R\right) - k_d\left(1 + A_d\frac{R}{R_{max}}\right)R$$

2.1.3 Mass transport
Under most conditions, the rate of formation of surface-bound complex will be limited by mass transport of analyte to the surface. An approximation to the rate equation which takes mass transport limitations into account is given by

$$\frac{dR}{dt} = \frac{k_aC(R_{max} - R) - k_dR}{1 + k_a\dfrac{G(R_{max} - R)}{MTC}}$$

where   G is a factor for converting response to surface concentration
and   MTC is the mass transport coefficient, given by

$$\mathrm{MTC} = \sqrt[3]{\frac{D^2 f}{0.3h^2 wl}}$$

where   D is the analyte diffusion coefficient (m^2/s)
          f is the volumetric flow rate (m^3/s)
and   h, w, l are the flow cell dimensions (height, width, length in m)

Note that the mass transport-limited rate equation, in contrast to the interaction-limited equations, requires a factor for converting response in RU to surface concentration. This is because the rate of mass transport is related to the absolute analyte concentration. In BIAsimulation, this factor is assumed to be constant at 1 RU = 1 pg/mm^2.

Mass transport is also governed by the diffusion constant of the analyte. Since this value is often unknown for biomolecules, BIAsimulation calculates an approximate diffusion constant based on entered values for the molecular weight, friction factor (asymmetry) and solvent viscosity.

2.2 Running basic kinetics

To run a basic kinetics simulation, choose the **Basic Kinetics** button from the BIAsimulation main menu. This starts the basic kinetics application with default values for all parameters.

The instructions below summarize briefly how to use the main features of basic kinetics simulation. Refer to the menu descriptions below for details.

- To run the simulation with default parameters, simply click on **Run**. The sensorgrams for the five reactant properties will be generated in the sensorgram window. During association, the plot window shows ln(dR/dt) against t by default: you can change this to dR/dt against R by selecting the appropriate plot from the **Plot** menu. During dissociation, the plot window shows the dissociation plot by default. Choose **Plot:Show all** to view the sensorgrams and all plots simultaneously.

- During a simulation, click **Pause** to pause the simulation or **Stop** to end the simulation. If a simulation is paused, the **Pause** button changes to **Continue:** click on this button to resume simulation.

- To change reactant properties (rate constants and/or analyte concentration), enter the appropriate values in the **Reactant Properties** frame. Default values for some typical situations can be chosen from the **Examples** menu.

- To change the duration of the simulation, enter the appropriate values under **Experimental Parameters**.

- To change the other experimental parameters, first choose **Mode:Extended Mode** and then select the appropriate option from the **Parameters** menu. See below (Section 2.5) for details.

- To simulate mass transport-limited kinetics, first choose **Mode:Extended Mode** then select **Mode:Mass Transport**. You can set the parameters for mass transport limitation with the **Parameters:Mass Transport** option (see Section 2.5.1 for details). BIAsimulation will calculate both interaction-limited and mass transport-limited kinetics, generating the latter as overlay curves in all plots. Note that you set mass transport parameters and choose curve generation from separate menus. Mass transport-limited curves will not be generated unless **Mode:Mass Transport** is checked.

- To restore all parameters to default values, click on **Reset all**. This will also restore the mode from **Extended Mode** to **Basic Mode**.

- To ignore any user settings for mass transport and other parameters, choose **Mode:Basic Mode**. BIAsimulation will then calculate sensorgrams for default parameter settings with no mass transport limitations.

- When a simulation is complete, you may choose **Evaluation** from the menu to calculate rate constants from the simulated curves. (These may differ from the constants entered under **Reactant Properties** if

for example mass transport limitations, noise, baseline drift or cooperativity are included.)

- To print the simulation, choose **File:Print**. You may choose to print a 2-page summary or selected parts of the simulation.

- If you want to use the simulated sensorgram data in another application, choose **File:Export Sensorgrams**. The data will be exported as RU-time coordinate pairs to a tab-separated text file.

2.2.1 Reactant properties

Reactant Properties

		ka (M-1 s-1)	kd (s-1)	(nM) Conc.
▬	⊠ **1**	1e5	1e-3	5
▬	⊠ **2**	1e5	1e-3	10
▬	⊠ **3**	1e5	1e-3	20
▬	⊠ **4**	1e5	1e-3	40
▬	⊠ **5**	1e5	1e-3	80

Bulk Resp. (RU): 0

In the **Reactant properties** frame, enter association and dissociation rate constants and analyte concentration and check the boxes for all simulations you wish to perform (up to 5 simulations per run). The following units are used for the parameters:

association rate constant	$M^{-1}s^{-1}$
dissociation rate constant	s^{-1}
analyte concentration	according to **Preferences:Concentration Units**

Bulk Resp. (RU)
Enter a value for bulk refractive index contribution in RU if desired. The same contribution will be used for all simulations. The bulk contribution is simulated as an instantaneous shift in the sensorgram which does not affect the evaluation of kinetic constants. In real experiments, a significant bulk contribution distorts the sensorgram for a few seconds at the beginning and end of the injection.

Note: In extended mode (see Section 2.6.2), the reactant properties frame also displays the actual analyte concentrations used for simulation. These may deviate from the entered concentrations if a concentration error is used (see Section 2.5.4).

2.2.2 Experimental Parameters

┌─ Experimental Parameters ──────────────┐
│   │
│   Preinjection Delay (s) [60   ]   │
│   │
│   Association Time (s) [300   ]   │
│   │
│   Dissociation Time (s) [300   ]   │
│   │
│   Rmax (RU) [1000]   │
│   │
│   │
└──┘

Preinjection Delay
Enter the time between the start of the sensorgram and injection of
sample. Use this delay to displace the time of injection from the
response axis.

Association Time
Enter the duration of the injection.

Dissociation Time
Enter the time for which dissociation will be simulated (i.e. the time
from the end of the injection to the end of the sensorgram).

Rmax
Enter the analyte binding capacity of the surface in RU.

Other parameters
In extended mode (see Section 2.6.2), the experimental parameters
frame also displays values for other parameters (noise, drift,
cooperativity etc). These parameters are set using commands in the
Parameters menu (see Section 2.5).

Note: All times are entered in simulated seconds.

Basic kinetics _____

2.3   The File menu

File
Export Sensorgrams...
Print...
Close

2.3.1   Export Sensorgrams

Exports data from simulated sensorgrams as tab-separated text files containing RU-time coordinate pairs. Data in tab-separated text files can be imported to BIAevaluation software and to many other software packages such as spreadsheets and data analysis programs.

Enter a file name for the exported data in the dialogue box.

Export dialog box

File Name: kinsim.txt
Directories: c:\biasim
Save File as Type: Text Files (*.txt)
Drives: c:
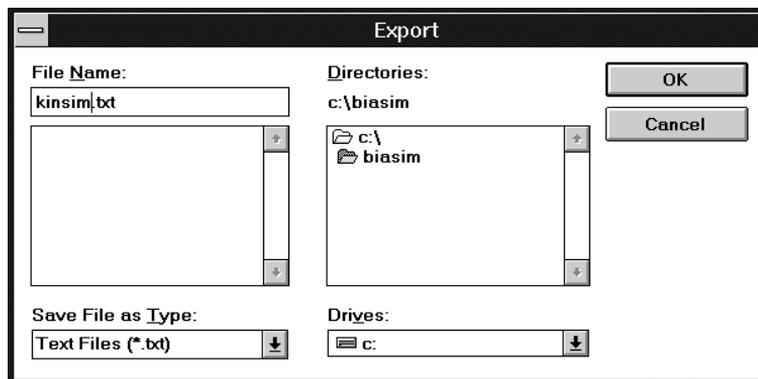
Notes: Reactant properties and experimental parameters are not exported with the sensorgram data. If you want to use the export function for full documentation of your simulation, make a separate note of the parameter values. The easiest way to do this is to use **File:Print** to print all relevant data.

2.3.2   Print

Prints a summary or selected parts of the simulation.
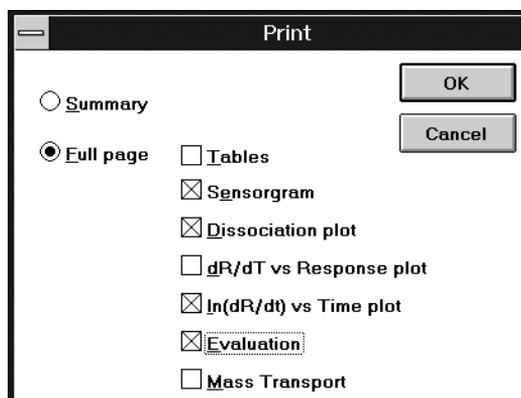
Print dialog box

○ Summary
◉ Full page
☐ Tables
☒ Sensorgram
☒ Dissociation plot
☐ dR/dT vs Response plot
☒ ln(dR/dt) vs Time plot
☒ Evaluation
☐ Mass Transport

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Summary
If the **Summary** button is checked, BIAsimulation will print a 2-page summary of the simulation.

Full page
If the **Full page** button is checked, BIAsimulation will print one page for each of the items marked. The **Evaluation** item cannot be printed unless a simulation has been performed.

2.3.3   Close
Closes the basic kinetics application and returns to the BIAsimulation main menu. You cannot close the application if a simulation is running or paused.

2.4   The Plot menu

Plot
Show <u>A</u>ll
l<u>n</u> dR/dt vs Time
<u>d</u>R/dt vs Response
<u>D</u>issociation Plot

Use options in this menu to control which plots are displayed. By default, BIAsimulation displays a plot of ln(dR/dt) against t during association and a dissociation plot during dissociation.

The sensorgram and plot panels can be enlarged to fill the whole BIAsimulation window by double-clicking in the respective panel with the right mouse button. Double-click again in the enlarged panel to restore the original display.

2.4.1   Show all
Displays four panels simultaneously with sensorgram, dR/dt against R, ln(dR/dt) against t and dissociation plot. Reactant properties and experimental parameter frames are not shown.
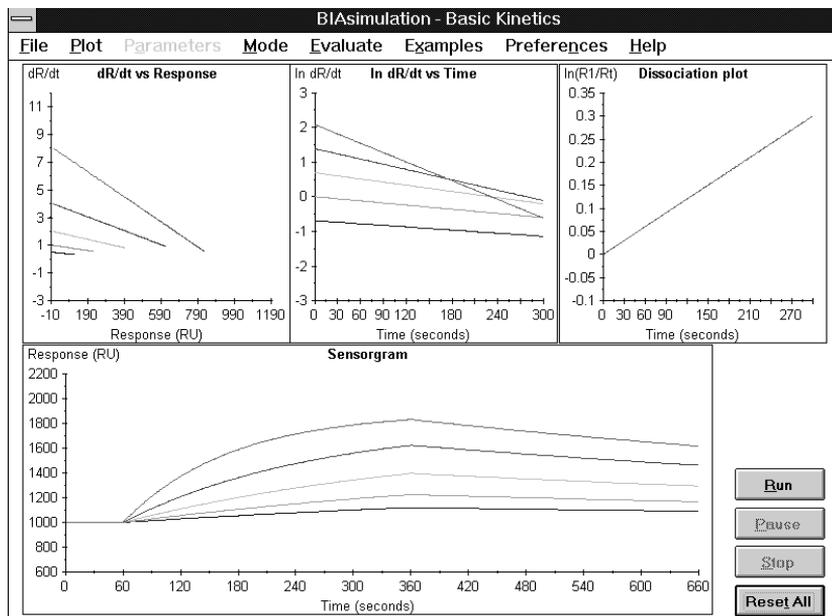
To restore the original display, choose **Plot:Show all** again.

2.4.2   In(dR/dt) vs Time
Displays a plot of ln(dR/dt) against t for the association phase.

2.4.3   dR/dt vs Response
Displays a plot of ln(dR/dt) against t for the association phase.

2.4.4   Dissociation plot
Displays a plot of $\ln(R_1/R_t)$ against t for the dissociation phase.

2.5   The Parameters menu

Parameters
Mass Transport...
Noise & Drift...
Cooperativity & Hindrance...
Concentration Error...

Use options in this menu to set values for determining mass transport limitations and for the experimental parameters instrument noise, baseline drift, baseline drift, cooperativity, steric hindrance and concentration error.

This menu is only available in extended mode (see Section 2.6.2).

2.5.1   Mass transport
Choose this option to set parameters which determine mass transport. To simulate kinetics with mass transport limitations, check **Mode:Mass transport**.

Instrument parameters
Enter the flow cell dimensions in mm in the appropriate fields. The default values are the dimensions of the standard flow cell in BIAcore and BIAlite. If you change the dimensions, you can reset the default by clicking on the **Set default** button.

Enter the volumetric flow in the **Flow** field. BIAsimulation will calculate the flow in terms of linear flow and cell volumes/s when you click on **Calculate**.

Solvent properties

Enter the viscosity of the bulk solution relative to that of water at
20°C. The default value of 0.89 applies for water at 25°C.

The viscosity factor is necessarily an approximation, since the viscosity
of the surface matrix on the sensor chip is unknown and will probably
vary with the amount of ligand and analyte bound. You may wish to
experiment with high viscosity values (e.g. 2-5) in the simulation to see
the effect on mass transport limitations in media like blood plasma
and other biological fluids.

Analyte properties

Enter the analyte molecular weight and friction factor (asymmetry
factor) in the appropriate fields. If you do not know the friction factor,
the default value 1.2 is suitable for most globular proteins.
Asymmetrical molecules have a higher friction factor.

BIAsimulation will calculate an approximate diffusion constant based
on the analyte and solvent properties when you click on **Calculate**.
Click with the right mouse button (help function) on the text
"Diffusion constant" to see the formula used for calculating the
diffusion constant.

Note: If you change the analyte molecular weight, you should
change the value of R_{max} accordingly in the **Experimental
parameters** frame (see Section 2.2.2 above) to retain the same
number of surface binding sites. The value of R_{max} in relation
to the molecular weight reflects the amount of immobilized
ligand, which is one of the experimental factors influencing
mass transport limitation (see BIAtechnology Handbook,
Chapter 5). For example, if you change the molecular weight
from 50,000 to 100,000, you should double the value of R_{max}.

Calculate

Click on **Calculate** to apply any changes you have made. The
following parameters are calculated and displayed:

Linear flow	from flow cell dimensions and volumetric flow rate
Cell volumes/s	from flow cell dimensions and volumetric flow rate
Diffusion constant	from analyte molecular weight and friction factor and solvent viscosity
Mass transport coefficient (see Section 2.1.3)	from cell dimensions, diffusion constant and flow rate

If you have made changes to any of the parameters, you must click on **Calculate** to use the changes in kinetic simulation. If you do not want to use the changes, click on **Cancel**.

2.5.2 *Noise and Drift*
Choose this option to simulate noise and baseline drift in the sensorgram.

```
┌─────────────────────────────────────────────────────────┐
│ ▭     BIAsimulation - Extended Mode - Noise and Drift    │
├─────────────────────────────────────────────────────────┤
│ Noise                                                    │
│ ┌──────────┐   Enter a value in the range 0 to 3 RU.     │
│ │1│        │   Noise will be generated randomly in       │
│ └──────────┘   the range +/- the value entered.          │
│                                                          │
│ Averaging window                                         │
│ ┌──────────┐   Enter a time interval >= 1 second         │
│ │1         │   over which response averaging             │
│ └──────────┘   will be done.                             │
│                                                          │
│ Drift                                                    │
│ ┌──────────┐   Enter a drift value in in the range       │
│ │0         │   -0.1 to 0.1 RU/second.                    │
│ └──────────┘                                             │
│                            ┌────────┐   ┌────────┐       │
│                            │  OK    │   │ Cancel │       │
│                            └────────┘   └────────┘       │
└─────────────────────────────────────────────────────────┘
```

Noise
Noise is simulated as random fluctuations within the interval you specify in the dialogue box (up to ±3 RU). Noise may not be very apparent on the sensorgram, but can have a dramatic effect on plots of dR/dt against R and ln(dR/dt) against t, particularly as the sensorgram flattens out. Noise can affect the accuracy of simulated evaluation.

To turn noise off, set a value of 0 in the dialogue box.

Note: Typical noise values for experimental sensorgrams are < ±2 RU for BIAcore and BIAlite and < ±0.3 RU for BIAcore 2000.

Averaging window
The averaging window can be set to a value higher than 1 to smooth noise fluctuations. A suitable value is in the range 1-10 s. The averaging window affects only noise, not the simulated signal.

The averaging window setting is irrelevant if noise is set to 0.

Drift
Enter a value for the baseline drift in RU/s (range -0.1 to 0.1). The baseline drift will be applied as a constant value throughout the simulated sensorgrams.

Note: The drift is specified in RU/s. Baseline drift in experimental sensorgrams is typically <0.3 RU/min (<0.005 RU/s) for BIAcore, BIAcore 2000 and BIAlite with active temperature control, and <1 RU/min (<0.017 RU/s) for BIAlite with passive temperature control.

2.5.3 Cooperativity and Hindrance
These parameters are used to simulate cooperativity and hindrance during association and dissociation:

• Cooperativity alters the binding affinity according to the number of ligand sites occupied.

• Hindrance alters the number of available sites in relation to the number of ligand sites occupied.

```
┌─────────────────────────────────────────────────────────────────────┐
│ ▭    BIAsimulation - Extended Mode - Cooperativity and Hindrance     │
├─────────────────────────────────────────────────────────────────────┤
│  Association cooperativity                                           │
│                                                                     │
│  Enter a value (Aa) to indicate whether the association rate constant│
│  will increase or decrease with an increasing response. kass will now│
│  depend on the response according to:                               │
│                                                                     │
│              ka (app) = ka ( 1 + Aa * R/Rmax )                      │
│                                                                     │
│  Aa = │0        │    The default value is 0.                        │
│                                                                     │
│  Dissociation cooperativity                                         │
│                                                                     │
│  Enter a value (Ad) to indicate whether the dissociation rate constant will│
│  increase or decrease with an increasing response.kdiss will now depend on│
│  the response according to:                                         │
│              kd (app) = kd ( 1 + Ad * R/Rmax )                      │
│                                                                     │
│  Ad = │0        │    The default value is 0.                        │
│                                                                     │
│  Hindrance                                                          │
│                                                                     │
│  Enter a value >=0 to indicate whether each binding event will block more│
│  than one site.Remaining free binding sites will now depend on the  │
│  response according to:                                             │
│            ( Rmax - R)  (app) = ( Rmax - B * R )                    │
│                                                                     │
│  B = │1        │    The default value is 1.                         │
│                                                                     │
│                                           ┌────────┐  ┌────────┐    │
│                                           │   OK   │  │ Cancel │    │
│                                           └────────┘  └────────┘    │
└─────────────────────────────────────────────────────────────────────┘
```

Association cooperativity
Enter a factor to represent cooperativity in analyte-ligand association.
The rate equation will be modified accordingly (see Section 2.1.2).

A value of 0 indicates no cooperativity. Positive values indicate
positive cooperativity (binding is tighter as more sites are occupied)
and negative values indicate negative cooperativity (binding is weaker
as more sites are occupied).

The association cooperativity factor modifies the association rate
constant in relation to the fraction of sites occupied. Do not confuse
negative cooperativity with steric hindrance (see below), which
modifies the number of sites available in relation to the fraction of
sites occupied.

Dissociation cooperativity
Enter a factor to represent cooperativity in analyte-ligand dissociation.
The rate equation will be modified accordingly (see Section 2.1.2).

A value of 0 indicates no cooperativity. Positive values indicate
positive cooperativity (dissociation is faster as more sites become free)
and negative values indicate negative cooperativity (dissociation is
slower as more sites become free).

Hindrance
Enter a factor to represent steric hindrance during association. The
number of remaining free sites will be modified accordingly (see
Section 2.1.2).

A value of 1 indicates no hindrance. Values above 1 indicate steric
hindrance: e.g. if a value of 2 is used, the number of available ligand
sites is reduced by 2 for each analyte molecule bound. Values less than
1 mean that each analyte molecule bound occupies effectively less than
one ligand site (i.e. binding one analyte molecule exposes additional
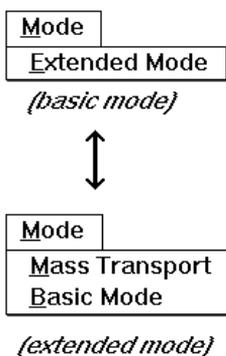ligand sites).

Hindrance affects only the association phase.

2.5.4 Concentration Error
Enter a value for random error in analyte concentration, up to 20%.
The analyte concentration used for simulation will be that specified in
under **Conc** in the **Reactant properties** frame (see Section 2.2.1) plus
or minus a random error up to the percentage specified (e.g. if the
concentration is specified as 10 nM and the error as 20%, a random
value in the range 8-12 nM will be used). The concentration used is
shown under **Reactant properties** when the simulation starts.

```
┌────────────────────────────────────────────────────────────────┐
│ ▬   BIAsimulation - Extended Mode - Concentration Error          │
├────────────────────────────────────────────────────────────────┤
│                                                                  │
│  Concentration error                                             │
│  ┌──────────────────┐   Enter a value in the range 0 to 20. A    │
│  │ 5                │   random concentration error in %          │
│  └──────────────────┘   within +/- the entered value will be     │
│                         generated for each concentration.        │
│                                                                  │
│                              ┌──────────┐   ┌──────────┐          │
│                              │   OK     │   │  Cancel  │          │
│                              └──────────┘   └──────────┘          │
└────────────────────────────────────────────────────────────────┘
```
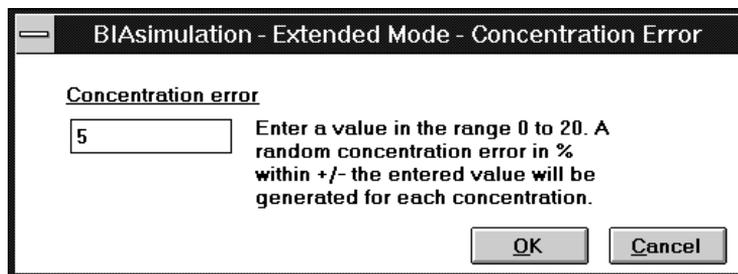
2.6   The Mode menu

The **Mode** menu switches between basic and extended mode and controls which parameters are used in simulating sensorgrams.

```
┌─────────────────┐
│ Mode            │
├─────────────────┤
│ Extended Mode   │
└─────────────────┘
   (basic mode)

        ↕

┌─────────────────┐
│ Mode            │
├─────────────────┤
│ Mass Transport  │
│ Basic Mode      │
└─────────────────┘
  (extended mode)
```

2.6.1   Basic Mode

In basic mode, any user settings for mass transport, noise and drift, cooperativity and hindrance and concentration error are ignored. Sensorgrams are simulated using the following settings, with no mass transport limitations:

- noise   0
  averaging window   irrelevant
  drift   0
  association cooperativity   0
  dissociation cooperativity   0
  hindrance   1
  concentration error   0

BIAsimulation starts by default in basic mode. If the mode is set to extended, choose **Mode:Basic Mode** to return to basic mode. Any extended mode parameter settings will be retained. You can also click on the **Reset All** button, which will reset all extended mode parameters to default values and switch to basic mode.

2.6.2   Extended Mode

In extended mode, simulation will take account of noise and drift, cooperativity and hindrance and concentration error parameters. Mass transport limitations can also be simulated.

When BIAsimulation is in extended mode, the **Parameters** settings and the effect of concentration error are shown in the experimental parameters and reagent properties panels respectively:

Reactant Properties				
	ka (M-1 s-1)	kd (s-1)	(nM) Conc.	Error Conc.
☒1	1e5	1e-3	5	4.814
☒2	1e5	1e-3	10	9.811
☒3	1e5	1e-3	20	20.09
☒4	1e5	1e-3	40	38.86
☒5	1e5	5e-3	80	77.688

Bulk Resp. (RU): 0   Max Err. (%)   5

Experimental Parameters	
Preinjection Delay (s)	60
Association Time (s)	300
Dissociation Time (s)	300
Rmax (RU)	1000

Noise Level (+/-) RU: 1.5
Averaging Window (s): 10
Drift (RU/s): 0.005
Ass. Coop. (A): .3
Hindrance (B): 2
Diss. Coop. (C): 0

BIAsimulation starts by default in basic mode. Choose **Mode:Extended Mode** to select extended mode.

To simulate mass transport limitations, choose **Mode:Extended Mode** and then **Mode:Mass Transport**. When the option is checked, each interaction is simulated both with and without mass transport limitations. The sensorgram without mass transport (interaction-limited kinetics) is shown in colour and the mass transport-limited sensorgram as an overlay in black. This is illustrated below for a single sensorgram, with the coloured (interaction-limited) sensorgram shown as a dashed line:

We recommend that you run mass transport overlays on one or two interactions at a time. If several simulations are performed in one run, it may be difficult to relate the mass transport overlays to their respective interaction-limited sensorgrams.

During simulation, the effect of mass transport limitations is displayed to the right of the **Experimental Parameters** frame as the mass transport-influenced binding rate expressed relative to the rate that would be obtained in the absence of mass transport:

```
Mass transp.
influenced
binding rate
relative to
pure kinetics
    0.858
```

This ratio is always less than or equal to one (mass transport
limitations reduce the observed binding rate). The ratio is updated
continuously as the simulation progresses. The value shown at the end
of the simulation applies to the last point in the last sensorgram.

If you want to change the mass transport limitation parameters,
choose **Parameters:Mass Transport** and enter the required settings (see
Section 2.5.1). Remember to click on **Calculate** in the mass transport
parameters dialogue box to implement the changed settings.

2.7   The Evaluate menu

```
Evaluate
  Evaluate...
```

Use the option in this menu to calculate kinetic constants from the
simulated sensorgrams. As long as mass transport limitations, noise,
cooperativity etc are not included, the results of evaluation should be
identical to the constants entered under **Reactant properties**. The
evaluation facility is however valuable in assessing the impact of the
various experimental parameters on the final result of a kinetic
determination.

The evaluation facility can only be used after completion of a
simulation.

2.7.1 Evaluate

	C (nM)	Start Time	End Time	Slope	Intercept
━1	5	1	500	-1.7305E-03	9.6419E-01
━2	10	1	500	-2.6255E-03	1.6137E+00
━3	20	1	500	-5.113E-03	2.4082E+00
━4	40	1	500	-1.187E-02	3.3915E+00
━5	80	1	500	-1.5248E-02	3.1007E+00
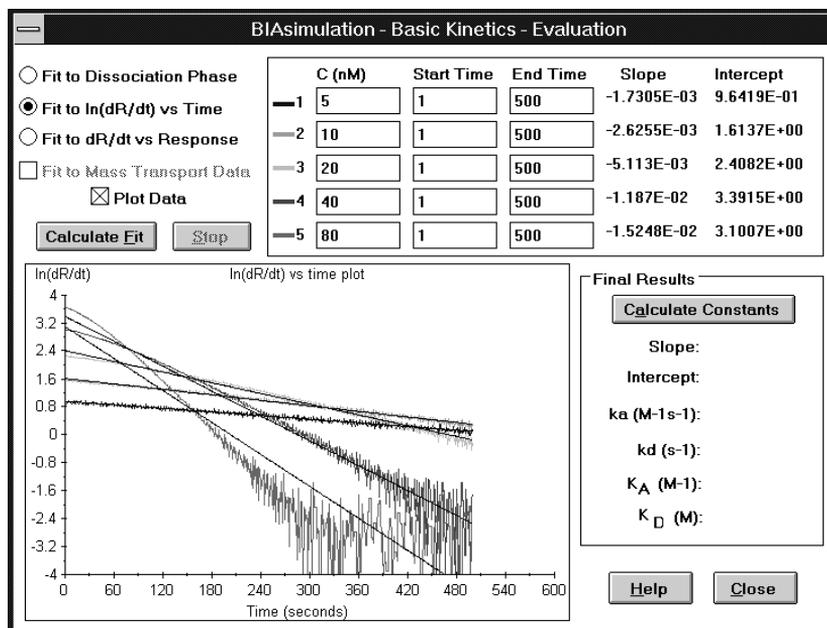
The **Evaluate** dialogue box is filled in by default with the nominal concentrations and start/end times for the association phase of the most recent simulation. To calculate rate constants:

1.   Choose the curve to fit. Both association and dissociation rate constants are derived from the association phase, using either dR/dt vs R or ln(dR/dt) vs t (see the BIAtechnology Handbook, Appendix A, for a discussion of the relative merits of these plots). Only dissociation constants are derived from the dissociation phase.

2.   Check **Fit to Mass Transport Data** if you have included mass transport in the simulation and wish to evaluate on the basis of the mass transport data.

3.   Check **Plot data** if you want to display the curve fitting graphically. This will slow down the evaluation process, but can give valuable information concerning deviations from ideal behaviour (e.g. noise, drift and mass transport limitations).

4.   Click on **Calculate Fit**. BIAsimulation will calculate a linear least squares regression fit for the selected plots. If **Plot Data** is checked, the fit is displayed directly in the graph frame. You can enlarge and zoom this frame using the same techniques as for other graph frames (see Section 1.2).

For association plots, the slope (= $-(k_aC+k_d)$) and intercept (= k_aCR_{max} or $\ln(k_aCR_{max})$) are listed as each plot is fitted. When the curve fitting is complete, click on **Calculate Constants** in the **Final Results** frame. (At least two concentrations must be simulated to use this function.) The calculated constants are displayed together with a plot of slope against C.
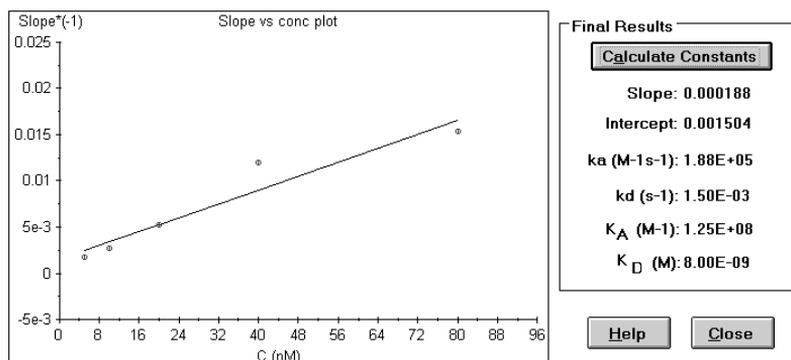
For dissociation plots, the slope of the plot is $-k_d$ and the intercept is theoretically zero. These values are displayed as each plot is fitted. The **Calculate Constants** button is not available for dissociation plots, since only k_d can be obtained.

5. If you wish to use only part of the association or dissociation phase to calculate constants, change the values under **Start Time** and **End Time**. These times apply to the association or dissociation phase according to which fit is selected, and are measured from the beginning of the phase (not in absolute simulated seconds). This is particularly useful in analysing mass transport-limited kinetics, where interaction-limited association may only be observed in the later phases of association.

Printing evaluation results
If you choose **File:Print** after performing an evaluation, you may choose to print the evaluation results under the **Full page** option (see Section Section 2.3.2). The printout will contain the results for the most recent evaluation. To print evaluation for both association and dissociation phases, evaluate one phase, print the results, then evaluate the other phase and print again.

2.8   The Examples menu

Examples
√ Same kass/kdiss Different Concentrations
Same kass/Different kdiss
Same kdiss/Different kass
Same Affinity - Different kass and kdiss

Use the options in the **Examples** menu to set default reactant property values for typical scenarios as specified below. The examples can only be used in basic mode. To run an example set of values in extended mode, choose the example in basic mode and then switch to extended mode.

2.8.1   Same kass/kdiss, different Concentrations

This is a typical concentration series for determining kinetic constants for a single interaction pair.

Run	k_a (M^{-1}s^{-1})	k_d (s^{-1})	K (M)	Conc (nM)
1	10^5	10^{-3}	10^{-8}	5
2	10^5	10^{-3}	10^{-8}	10
3	10^5	10^{-3}	10^{-8}	20
4	10^5	10^{-3}	10^{-8}	40
5	10^5	10^{-3}	10^{-8}	80

2.8.2   Same kass, Different kdiss
The concentrations in this series are equal to the K_D values, which give steady state saturation levels equal to $R_{max}/2$.

Run	k_a (M^{-1}s^{-1})	k_d (s^{-1})	K (M)	Conc (nM)
1	10^5	10^{-2}	10^{-7}	100
2	10^5	$5 \cdot 10^{-3}$	$5 \cdot 10^{-8}$	50
3	10^5	10^{-3}	10^{-8}	10
4	10^5	$5 \cdot 10^{-4}$	$5 \cdot 10^{-8}$	5
5	10^5	10^{-4}	10^{-9}	1

2.8.3   Same kdiss, Different kass
The concentrations in this series are equal to the K_D values, which give steady state saturation levels equal to $R_{max}/2$.

Run	k_a (M^{-1}s^{-1})	k_d (s^{-1})	K (M)	Conc (nM)
1	10^4	10^{-3}	10^{-7}	100
2	$5 \cdot 10^4$	10^{-3}	$2 \cdot 10^{-8}$	20
3	10^5	10^{-3}	10^{-8}	10
4	$5 \cdot 10^5$	10^{-3}	$2 \cdot 10^{-9}$	2
5	10^6	10^{-3}	10^{-9}	1

2.8.4   Same Affinity, Different kass/kdiss
The concentrations in this series are equal to the K_D values, which give steady state saturation levels equal to $R_{max}/2$.

Run	k_a (M^{-1}s^{-1})	k_d (s^{-1})	K (M)	Conc (nM)
1	10^4	10^{-4}	10^{-8}	10
2	$5 \cdot 10^4$	$5 \cdot 10^{-4}$	10^{-8}	10
3	10^5	10^{-3}	10^{-8}	10
4	$5 \cdot 10^5$	$5 \cdot 10^{-3}$	10^{-8}	10
5	10^6	10^{-2}	10^{-8}	10

2.9   The Preferences menu

Preferences
Concentration Unit...
All Curves Black

2.9.1   Concentration Unit
This setting determines the unit in which concentrations are to be
entered in BIAsimulation.
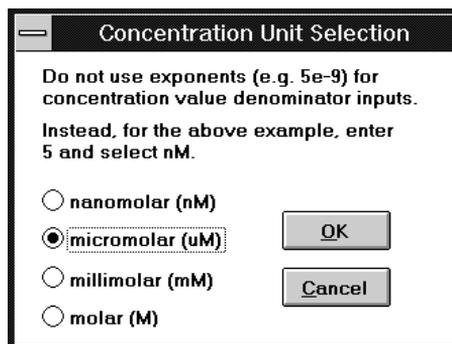
Choose the concentration unit you prefer to use. You can enter
concentration values using exponent notation if you wish (e.g. 5e-3
means 5×10^{-3}): the values will be interpreted in terms of the current
concentration unit setting. The following entries are thus equivalent
(and represent 5 μM):

Concentration unit	*Value*
nanomolar (nM)	5000 (or 5e3)
micromolar (uM)	5
millimolar (mM)	0.005 (or 5e-3)
molar (M)	0.000005 (or 5e-6)

The concentration unit setting is global and applies to all
BIAsimulation modules.

Note: The concentration unit setting applies *only* to entry of
concentration data. Rate and equilibrium constants are always
specified on a molar basis.

2.9.2   All Curves Black

Choosing this setting displays all curves in black. Use this setting if you want to print simulation results on a printer which does not handle colours satisfactorily.

Note: Changing this setting affects subsequent simulations but does not change the display of the current simulation.

2.10 The Help menu

Help
Help...
Mouse Functions...
Formulae...

2.10.1 Help

In general, click with the right mouse button on a text area to display help for the item (e.g. click on **Bulk Resp. (RU)** in the **Reactant properties** frame for help on this parameter). This works on all text which serves to identify an input or display field.

2.10.2 Mouse Functions

This command displays a summary screen of mouse operations, including a small sensorgram display for practice.

2.10.3 Formulae

This command displays a summary screen showing the formulae used in simulating basic kinetics (see Section 2.1).

3.   Heterogeneous ligand kinetics

This application simulates binding kinetics for a single analyte and a heterogeneous ligand, with up to 3 different ligand species for which association and dissociation constants are specified separately. Simulation may be run for a single analyte concentration, a series of analyte concentrations or as an overlay of two simulations with different analyte concentrations and/or ligand properties.

Heterogeneous ligand kinetics are simulated without mass transport limitations.

3.1   Theoretical background

When the immobilized ligand is heterogeneous, the observed kinetics are in principle the sum of independent kinetics for the separate ligands. BIAsimulation generates sensorgrams and plots for heterogeneous ligands on this basis, using the equations described in Section 2.1.

3.2   Running heterogeneous ligand kinetics

To run a heterogeneous ligand kinetics simulation, choose the **Heterogeneous ligand** button from the BIAsimulation main menu. This starts the heterogeneous ligand application with default values for all parameters.
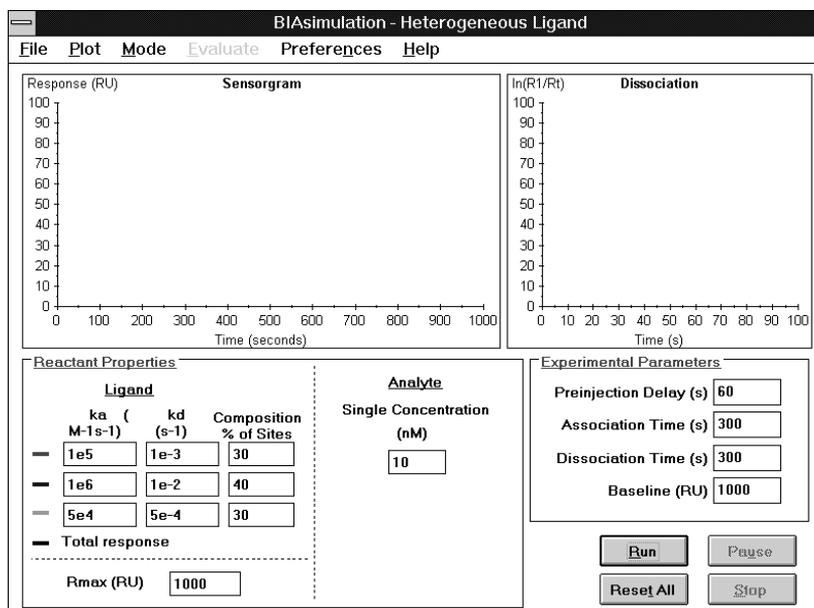
The instructions below summarize briefly how to use the main features of heterogeneous ligand simulation. Refer to the menu descriptions below for details.

- To run the simulation with default parameters, simply click on **Run**. Sensorgrams will be generated to represent the three separate ligand components and the total response (i.e. the observed sensorgram). During association, the plot window shows ln(dR/dt) against t by default: you can change this to dR/dt against R by selecting the appropriate plot from the **Plot** menu. During dissociation, the plot window shows the dissociation plot by default. Choose **Plot:Show All** to view the sensorgrams and all plots simultaneously.

- During a simulation, click **Pause** to pause the simulation or **Stop** to end the simulation. If a simulation is paused, the **Pause** button changes to **Continue**: click on this button to resume simulation.

- To change ligand properties (rate constants and/or proportions of the component ligands), enter the appropriate values in the **Reactant Properties** frame. Note that the amount of each ligand component is specified as a percentage of the total ligand. Simulation cannot be performed if the ligand components do not add up to 100%.

- To change the analyte concentration, enter the required value under **Analyte**. See Section 3.3 for generating an analyte concentration series.

- To change the duration of the simulation, enter the appropriate values under **Experimental Parameters**.

- To restore all parameters to default values, click on **Reset All**.

- When a simulation is complete, you may choose **Evaluation** from the menu to calculate rate constants from the simulated curves.

- To print the simulation, choose **File:Print**. You may choose to print a 2-page summary or selected parts of the simulation.

- If you want to use the simulated sensorgram data in another application, choose **File:Export Sensorgrams**. The data will be exported as RU-time coordinate pairs to a tab-separated text file.

3.2.1 Reactant properties

Under **Ligand**, enter association and dissociation rate constants and composition of total ligand for up to 3 ligand components. If you only want to use two ligand components, set the third component to 0%. In the **Rmax** field, enter the maximum analyte binding capacity of the surface. The following units are used for the parameters:

association rate constant	$M^{-1}s^{-1}$
dissociation rate constant	s^{-1}
composition	% of total (the sum must be 100%)
Rmax	RU

Under **Analyte**, enter the analyte concentration (units according to **Preferences:Concentration Units**) for a single concentration simulation. See Section 3.3 for generating an analyte concentration series.

3.2.2 Experimental parameters

Experimental Parameters	
Preinjection Delay (s)	60
Association Time (s)	300
Dissociation Time (s)	300
Baseline (RU)	1000

Preinjection Delay
Enter the time in (simulated) seconds between the start of the
sensorgram and injection of sample. Use this delay to displace the time
of injection from the response axis.

Association Time
Enter the duration of the injection in (simulated) seconds.

Dissociation Time
Enter the time in (simulated) seconds for which dissociation will be
simulated (i.e. the time from the end of the injection to the end of the
sensorgram).

Baseline
Enter a baseline value in RU to displace the sensorgram from the time
axis.

3.3   Concentration series

You can simulate a set of up to five sensorgrams for a series of analyte
concentrations with heterogeneous ligand, using concentrations either
specified individually or generated in geometric progression from a
starting concentration. This simulates the commonest approach to
analysing heterogeneous ligand kinetics in experimental situations.

Choose **Mode:Concentration Series** and enter the analyte
concentrations in the dialogue box.

The default setting is a concentration series of 5 steps with a factor of 2 between steps, starting at the concentration currently used for single concentration simulation. If you change any of the fields **Number of Steps**, **Start Conc** or **Change Per Step**, you will automatically generate new concentration values according to the changed settings. You can also make changes directly in the **Conc** fields, in which case the settings for generating the concentration values will be ignored.

In this mode, only the total sensorgram is shown for each analyte concentration. You can evaluate the results when the simulation is complete (see Section 3.8).
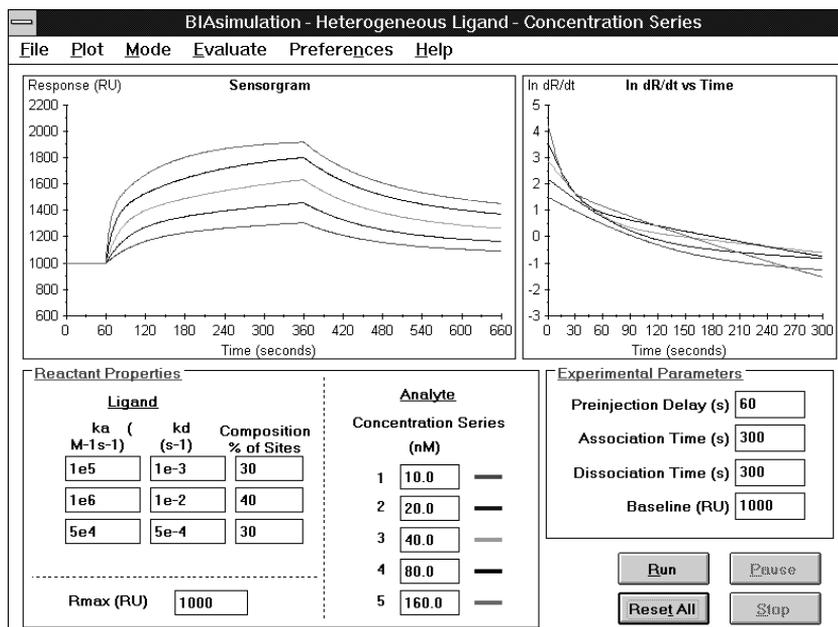
3.4 Overlay plots

You can generate an overlay plot of two simulations with different values for analyte concentration and/or ligand mixture, for ease of comparison between the sensorgrams and plots.

To run an overlay plot, first perform a simulation for a single analyte concentration. The choose **Mode:Overlay Plot** and enter the parameters for the second plot in the dialogue box:
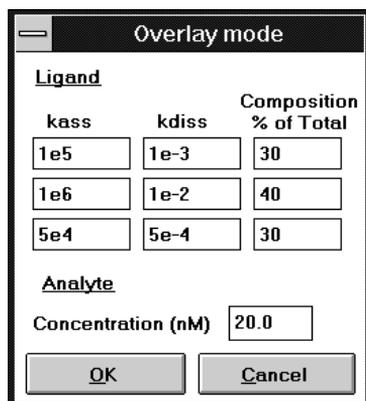
Click on **OK** in the dialogue box, then on **Overlay** in the main window to generate the overlay plot. The component sensorgrams and plots from the single concentration simulation are cleared, and only the total curves are shown.
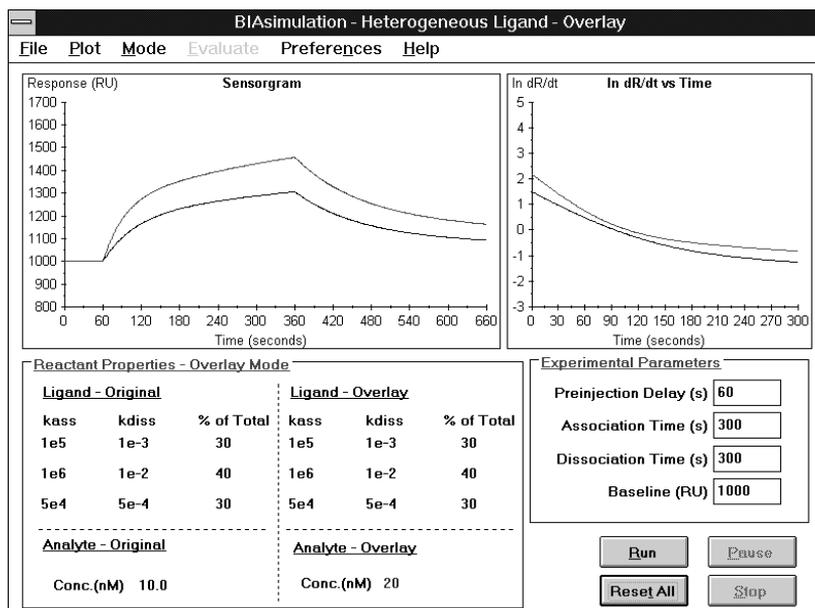
3.5   The File menu

File
Export Sensorgrams...
Print...
Close

Commands in this menu are the same as in the **File** menu for basic kinetics simulation (Section 2.3).

3.6   The Plot menu

Plot
Show All
In dR/dt vs Time
dR/dt vs Response
Dissociation Plot

Commands in this menu are the same as in the **Plot** menu for basic kinetics simulation (Section 2.4).

3.7   The Mode menu

Mode
Single concentration
Concentration Series...
Overlay Plot...

3.7.1   *Single concentration*

Simulates heterogeneous ligand interaction kinetics for a single analyte concentration (default mode). Sensorgrams and plots are shown separately for the component ligands and the total (observed) response.

3.7.2   *Concentration Series*

Generates heterogeneous ligand interaction kinetics for a series of up to 5 analyte concentrations (see Section 3.3). Only the total (observed) response is shown for each analyte concentration. The simulated results can be evaluated for apparent rate constants in terms of a homogeneous interaction model (see Section 3.8).

3.7.3   *Overlay plot*

Generates an overlay plot of two simulations with different values for analyte concentration and/or ligand mixture (see Section 3.4). Only the total (observed) response is shown for each analyte concentration.

3.8 The Evaluate menu
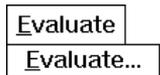
Evaluate

Evaluate...

3.8.1 Evaluate

This function evaluates a concentration series simulation in terms of apparent rate constants. The command is only available when a concentration series simulation has been performed.

The **Evaluate** function operates in the same way as for basic kinetics simulation. See Section 2.7 for a description.

Important
Heterogeneous ligand simulations are evaluated as though the interaction was homogeneous. The function gives the apparent rate and affinity constants that would be obtained if the data were analysed according to a homogeneous interaction model.

3.9 The Preferences menu
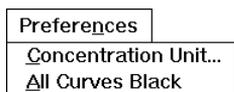
Preferences

Concentration Unit...
All Curves Black

Commands in this menu are the same as in the **Preferences** menu for basic kinetics simulation (Section 2.9).

3.10 The Help menu
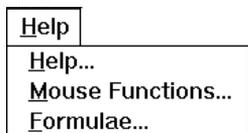
Help

Help...
Mouse Functions...
Formulae...

Commands in this menu are the same as in the **Help** menu for basic kinetics simulation (Section 2.10).

4. Heterogeneous analyte kinetics

This application simulates binding kinetics for a mixture of up to 3 analytes that compete for a single ligand. Association and dissociation constants are specified individually for the different analytes. Simulation may be run for a single analyte concentration or for a series of total analyte concentrations where the proportions of component analytes are kept constant.

Heterogeneous analyte kinetics are simulated without mass transport limitations.

4.1 Theoretical background

When the analyte is heterogeneous, the observed kinetics result from competition of the different analyte species for the same ligand. BIAsimulation generates sensorgrams and plots for heterogeneous ligands on this basis. See the BIAtechnology Handbook, Appendix A for equations.

4.2 Running heterogeneous analyte kinetics

To run a heterogeneous analyte kinetics simulation, choose the **Heterogeneous analyte** button from the BIAsimulation main menu. This starts the heterogeneous analyte application with default values for all parameters.
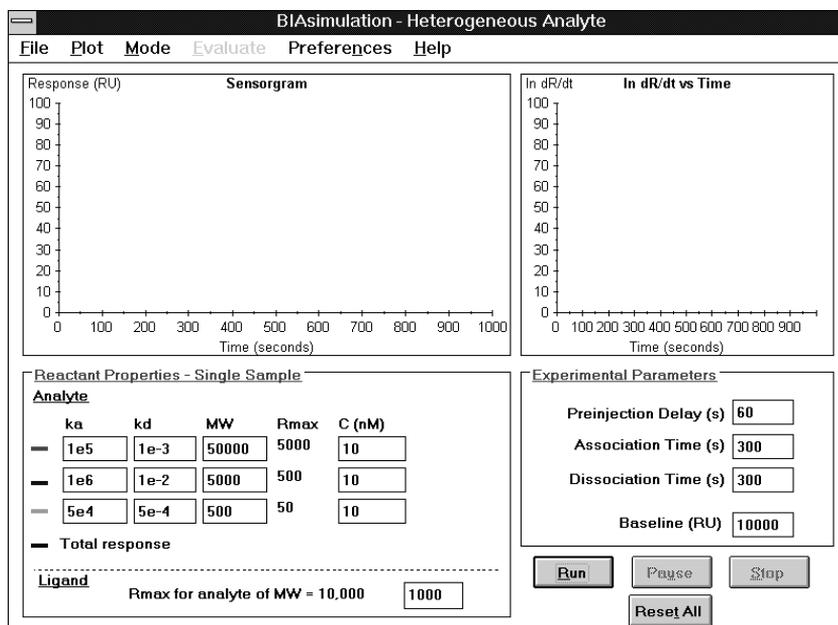
The instructions below summarize briefly how to use the main features of heterogeneous analyte simulation. Refer to the menu descriptions for details.

- To run the simulation with default parameters, simply click on **Run**. Sensorgrams will be generated to represent the three separate analyte components and the total response (i.e. the observed sensorgram). During association, the plot window shows ln(dR/dt) against t by default: you can change this to dR/dt against R by selecting the appropriate plot from the **Plot** menu. During dissociation, the plot window shows the dissociation plot by default. Choose **Plot:Show All** to view the sensorgrams and all plots simultaneously.

- During a simulation, click **Pause** to pause the simulation or **Stop** to end the simulation. If a simulation is paused, the **Pause** button changes to **Continue**: click on this button to resume simulation.

- To change analyte properties (rate constants, molecular weights and/or concentrations of the component analytes), enter the appropriate values in the **Reactant Properties** frame.

- To change the total analyte binding capacity of the surface (representing the amount of immobilized ligand), enter the required value for R_{max} for an analyte of MW 10,000. Values for R_{max} for the individual analytes are derived from this value together with the respective analyte molecular weights. Note that the *actual* R_{max} for the analyte mixture will be a function of the concentrations, affinities and individual R_{max} values for the separate analytes.

- To change the duration of the simulation, enter the appropriate values under **Experimental Parameters**.

- To restore all parameters to default values, click on **Reset All**.

- When a simulation is complete, you may choose **Evaluation** from the menu to calculate rate constants from the simulated curves.

- To print the simulation, choose **File:Print**. You may choose to print a 2-page summary or selected parts of the simulation.

- If you want to use the simulated sensorgram data in another application, choose **File:Export Sensorgrams**. The data will be exported as RU-time coordinate pairs to a tab-separated text file.

4.2.1   Reactant properties

Under **Analyte**, enter association and dissociation rate constants, molecular weights and concentrations for the analyte components. If you only want to use two analyte components, set the concentration of third component to 0. The following units are used for the parameters:

association rate constant	$M^{-1}s^{-1}$
dissociation rate constant	s^{-1}
molecular weight	daltons
concentration	according to **Preferences:Concentration Units**

Under **Ligand**, enter the total analyte binding capacity for an analyte with molecular weight 10,000. BIAsimulation will display the corresponding R_{max} values for the individual analytes based on their molecular weights.

4.2.2 Experimental parameters

```
┌─Experimental Parameters─────────┐
│   Preinjection Delay (s) │ 60  │  │
│     Association Time (s) │ 300 │  │
│    Dissociation Time (s) │ 300 │  │
│          Baseline (RU) │ 1000 │  │
│                                 │
└─────────────────────────────────┘
```

Preinjection Delay
Enter the time in (simulated) seconds between the start of the
sensorgram and injection of sample. Use this delay to displace the time
of injection from the response axis.

Association Time
Enter the duration of the injection in (simulated) seconds.

Dissociation Time
Enter the time in (simulated) seconds for which dissociation will be
simulated (i.e. the time from the end of the injection to the end of the
sensorgram).

Baseline
Enter a baseline value in RU to displace the sensorgram from the time
axis.

4.3 Concentration series

You can simulate a set of up to five sensorgrams for a series of mixed
analyte concentrations. Concentrations are generated in geometric
progression from a starting concentration. If desired, the concentration
of one component can be kept constant while the other components
are varied. This simulates a valuable approach to analysing
heterogeneous analyte kinetics in experimental situations, by adding a
fixed amount of a known (homogeneous) analyte to a concentration
series of the unknown analyte mixture.

Note: If you want to simulate the effect on binding a mixture of two
        analytes of adding a fixed amount of one of the analytes,
        define A and B in BIAsimulation with identical reactant. You
        can then keep A constant and generate a concentration series
        for B and C.

Choose **Mode:Concentration Series** and enter the analyte
concentrations in the dialogue box.

```
┌──────────────────────────────────────────┐
│ ▬   Concentration Series Values          │
├──────────────────────────────────────────┤
│                                          │
│     Change Per Step (-fold):  [2   ]     │
│     Number of Steps (Max. 5): [5   ]     │
│                                          │
│     ☐ Keep concentration of A constant   │
│                                          │
│                  Component               │
│              A        B        C         │
│  Start (nM) [10  ]  [10  ]  [10  ]       │
│  Conc.                                   │
│                                          │
│  Step 1  10.0     10.0     10.0          │
│       2  20.0     20.0     20.0          │
│       3  40.0     40.0     40.0          │
│       4  80.0     80.0     80.0          │
│       5 160.0    160.0    160.0          │
│                                          │
│     [  OK  ]        [ Cancel ]           │
└──────────────────────────────────────────┘
```

The default setting is a concentration series of 5 steps with a factor of
2 between steps, starting at the concentration currently used for single
concentration simulation. If you change any of the fields **Number of
Steps**, **Start Conc** or **Change Per Step**, you will automatically generate
new concentration values according to the changed settings.

In this mode, only the total sensorgram is shown for each analyte
mixture. You can evaluate the results (Section 4.7) when the
simulation is complete.
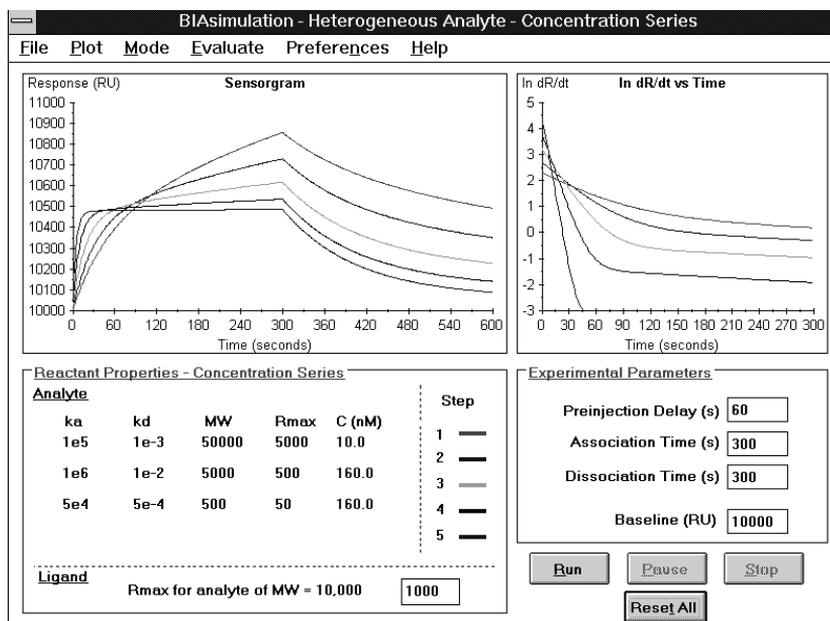
Note: During the simulation, analyte concentrations are displayed under **Reactant Properties** for the sensorgram currently being simulated. When the simulation is complete, this information applies to the last sensorgram generated. To review the concentrations for the other sensorgrams, choose **Mode:Concentration Series** and inspect the values.

4.4 The File menu

Commands in this menu are the same as in the **File** menu for basic kinetics simulation (Section 2.3).

4.5 The Plot menu

Commands in this menu are the same as in the **Plot** menu for basic kinetics simulation (Section 2.4).

4.6   The Mode menu

Mode
Single sample
Concentration Series...

4.6.1   Single sample
Simulates heterogeneous analyte interaction kinetics for a single analyte mixture (default mode). Sensorgrams and plots are shown separately for the component analytes and the total (observed) response.

4.6.2   Concentration Series
Generates heterogeneous analyte interaction kinetics for a series of up to 5 analyte mixtures (see Section 4.3). One of the analytes can if desired be maintained at a constant concentration while the concentration of the others is varied. Ony the total (observed) response is shown for each analyte concentration. The simulated results can be evaluated for apparent rate constants in terms of a homogeneous interaction model (see Section 4.7).

4.7   The Evaluate menu

Evaluate
Evaluate...

4.7.1   Evaluate
This function evaluates a concentration series simulation in terms of apparent rate constants. The sum of the individual analyte concentrations is used in the evaluation. The command is only available when a concentration series simulation has been performed.

The **Evaluate** function operates in the same way as for basic kinetics simulation. See Section 2.7 for a description.

> *Important*
> Heterogeneous analyte simulations are evaluated as though the interaction was homogeneous. The function gives the apparent rate and affinity constants that would be obtained if the data were analysed according to a homogeneous interaction model.

4.8   The Preferences menu
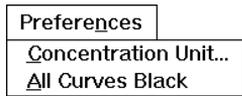
Preferences
<u>C</u>oncentration Unit...
<u>A</u>ll Curves Black

Commands in this menu are the same as in the **Preferences** menu for basic kinetics simulation (Section 2.9).

4.9   The Help menu
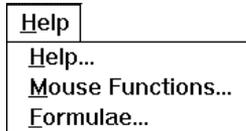
<u>H</u>elp
<u>H</u>elp...
<u>M</u>ouse Functions...
<u>F</u>ormulae...

Commands in this menu are the same as in the **Help** menu for basic kinetics simulation (Section 2.10).

5. Epitope mapping

5.1 Background

This application simulates epitope mapping by pairwise binding of monoclonal antibodies (Mabs) to an antigen. Rabbit anti-mouse Fc (RAMFc) is immobilized to the sensor chip surface and used to capture the first Mab. Antigen is then bound to the first Mab and challenged with the second Mab. If simultaneous binding is observed, the two Mabs define separate epitopes on the antigen. The principle of epitope mapping with this technique is discussed in more detail in the BIAapplication Handbook, Chapter 7.

Simulation of epitope mapping includes the following features:

- mapping with up to 6 Mabs in a 6x6 binding matrix

- user-defined association and dissociation rate constants for the separate Mab-antigen interactions (these values affect the theoretical steady state level for Mab binding)

- user-defined Mab concentrations

- user-defined association and dissociation rate constants for capturing the Mabs by RAMFc (these values affect the amount of first Mab captured by the surface)

- bulk contribution to the response for the separate Mab solutions

- random variation in the response, to simulate experimental variation

- user-defined matrix of interfering pairs, not necessarily symmetrical

- evaluation of results in terms of pairs which show positive binding

- overlay plot for comparing selected sensorgrams

Note: The terminology used in the epitope mapping application relates directly to immunological epitope studies. The application can however be used to simulate other kinds of multi-binding site mapping experiments, e.g. mapping multiple ligand sites on a receptor molecule.

5.2   Running epitope mapping simulation

To start epitope mapping, click on the **Epitope Mapping** button in the
BIAsimulation main menu.

5.2.1   Basic run

To run a mapping simulation with the default parameters, simply click
on **Run**. As each sensorgram is simulated, the check box in the **Pairs to
Run** matrix is greyed to show that the pair has been run, and values
are entered in the **Response Levels** matrix for first antibody, antigen
and second antibody. These values represent the response reached at
the end of the respective injections, modified by the random error
factor (which is 0 by default, see Section 5.3 below). A 3x3 matrix of
pairs to run generates 9 sensorgrams (several of which will frequently
overlap for the injection of first antibody and antigen):
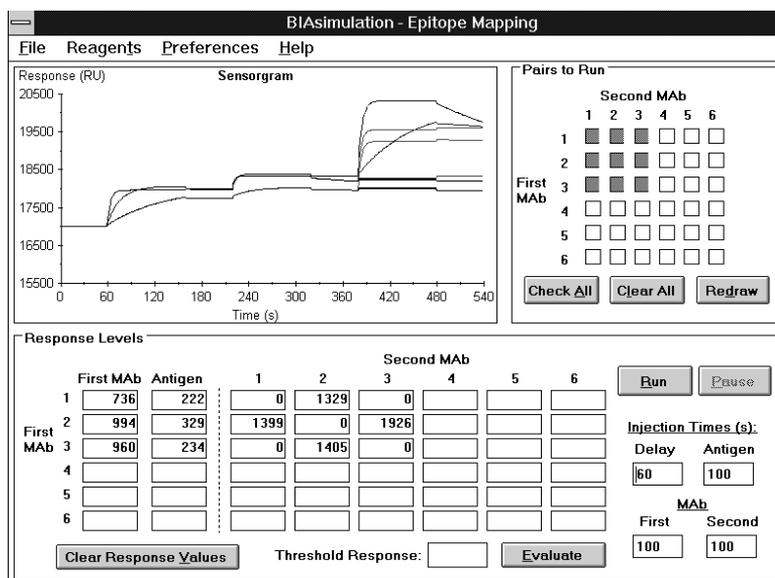
You may change the times of injection and delay between injections by
entering values in the appropriate fields. Using longer injections may
improve the results if binding does not reach a steady state (this is
determined by reagent properties, see Section 5.3).

To perform a new simulation, click on **Clear All** in the **Pairs to Run**
frame to clear the grey markings in the matrix, then check the pairs to
be run in the new simulation. You can also click on **Check All** to check
all pairs, then uncheck the pairs you do not want to run. The
sensorgrams and response levels will be cleared when you start the
new simulation.

You can print the results of the simulation with **File:Print** (Section 5.4).

5.2.2 Evaluating response levels

When the simulation is complete, examine the response levels in the matrix under **Second Mab**. Enter a threshold value above which responses are to be regarded as positive and click on **Evaluate**. BIAsimulation will mark values above the threshold with a grey background.

```
┌─Response Levels────────────────────────────────────────────────────┐
│                                              Second MAb             │
│           First MAb Antigen    1       2       3       4     5     6 │
│        1   [ 736]  [ 222]   [   0] [ 1329] [   0] [    ] [   ] [   ] │
│ First  2   [ 994]  [ 329]   [1399] [    0] [1926] [    ] [   ] [   ] │
│ MAb    3   [ 960]  [ 234]   [   0] [ 1405] [   0] [    ] [   ] [   ] │
│        4   [    ]  [    ]   [    ] [     ] [    ] [    ] [   ] [   ] │
│        5   [    ]  [    ]   [    ] [     ] [    ] [    ] [   ] [   ] │
│        6   [    ]  [    ]   [    ] [     ] [    ] [    ] [   ] [   ] │
│                                                                     │
│   [ Clear Response Values ]    Threshold Response: [100]  [Evaluate]│
└─────────────────────────────────────────────────────────────────────┘
```

The response levels are influenced by a series of factors (all responses may be influenced by injection times if steady state is not reached during the injection):

• The response for the first Mab is determined by the Mab concentration and kinetics of binding to RAMFc.

• The response for antigen is determined by the amount of first Mab bound, the antigen concentration, molecular weight and kinetics of binding to the first Mab.

• The response for the second Mab is determined by the amount of antigen bound, the Mab concentration and kinetics of binding to the antigen, and the random error value (see Section 5.3).

• Response levels for Mabs are influenced by the bulk RI contribution.

All response values are simulated as the difference in SPR signal between the start and end of the respective injection.

5.2.3 Examining individual sensorgrams

When many pairs are run in an epitope mapping matrix, it can be difficult to distinguish the individual sensorgrams in the simulation. The **Redraw** function allows you to examine selected sensorgrams.

When the simulation is complete, the pairs that have been run are marked in grey in the **Pairs to Run** matrix. To display one or more selected sensorgrams:

1. Click on **Clear all** to clear all pairs, then click on the pairs you wish to display to reselect them. (Alternatively, click on individual pairs that you do not want to display.)

2. Press **Redraw**. The sensorgram window will be redrawn, displaying only the selected pairs.

Each time you change the set of selected pairs, press **Redraw** to redraw the sensorgram window. To redraw sensorgrams for all pairs that have been run, press **Check All** and then **Redraw**.

5.3   Reagent properties

To set parameters for reagent properties, choose **Reagents:Reagent Properties**.

```
┌─────────────────────────────────────────────────────────────────────────────┐
│ ⊖          BIAsimulation - Epitope Mapping - Reagent Properties              │
├─────────────────────────────────────────────────────────────────────────────┤
│ ┌─MAb Properties────────────────────────────────────────────────────────┐   │
│                Conc.   BulkRI  RAMFc  RAMFc  ┌Pairs That Interfere In Binding┐│
│      ka    kd  (ug/ml)  (RU)    ka     kd         1  2  3  4  5  6            │
│  1  1e5  1e-3    20      40    1e5    1e-5    1  ☒  ☐  ☒  ☐  ☒  ☐            │
│  2  5e5  1e-4    80     -40    5e5    1e-4    2  ☐  ☒  ☐  ☐  ☒  ☐            │
│  3  1e6  5e-3    30      70    3e5    5e-4    3  ☒  ☐  ☒  ☐  ☐  ☐            │
│  4  4e4  1e-2   100      15    7e4    5e-4    4  ☐  ☐  ☐  ☒  ☐  ☒            │
│  5  7e5  5e-4   120      30    1e6    7e-4    5  ☒  ☒  ☐  ☐  ☒  ☐            │
│  6  2e6  3e-3    60     -10    5e4    1e-5    6  ☐  ☐  ☐  ☒  ☐  ☒            │
│                                                          [Clear]             │
│  MAb Valency: 2          RAMFc Rmax: 1000                                    │
│ ┌─Antigen Properties──┐                                                      │
│   Mol. Weight   25000      Start Baseline: 17000                             │
│   Conc (ug/ml)  10         Random Error (RU): 0          [  OK  ]            │
│   Bulk RI (RU)  60         [Reset All Values]            [Cancel]            │
└─────────────────────────────────────────────────────────────────────────────┘
```

Mab Properties

Under **ka** and **kd**, enter association and dissociation rate constants respectively for binding of the Mab to antigen.

Under **Conc**, enter the Mab concentration in μg/ml.

Under **Bulk RI**, enter the bulk refractive index contribution of the Mab solution to the SPR signal in RU.

Under **RAMFc ka** and **kd**, enter association and dissociation rate constants respectively for binding of the Mab to the capturing agent (immobilized RAMFc).

Note: The default kinetic constants for Mab binding to RAMFc represent a wide spread of affinities. This is instructive in demnostrating the effect of varying capture affinities, but in practice the affinities of capturing agent for the various Mabs should be relatively constant.

In **Mab Valency**, enter the number of antigen molecules which can bind to one Mab. This is normally 2. The same value is used for all Mabs. The valency only affects the amount of antigen that can bind to the first Mab (valency is ignored for binding of the second Mab to antigen).

In **RAMFc Rmax**, enter the maximum binding capacity of the RAMFc surface for Mab in RU.

Pairs That Interfere in Binding
Check the appropriate boxes in this matrix for pairs of Mabs that interfere in binding. Normally, the diagonal line from top left to bottom right will be checked (i.e. any one Mab interferes with its own binding), and the matrix will be symmetrical about this diagonal (i.e. interference between pairs is not dependent on the order of binding). You may however check or uncheck any boxes in the matrix. Click on **Clear** if you want to clear all boxes.

Antigen Properties
Enter the molecular weight, concentration (µg/ml) and bulk refractive index contribution (in RU) for the analyte solution.

Baseline
In the **Start Baseline** field, enter a baseline value in RU to displace the sensorgrams from the time axis.

Random Error
In the **Random Error** field, enter a value in RU to simulate experimental variations in the measurements. A random value up to this error will be added to or subtracted from the second Mab calculated response levels before the results are entered in the **Response levels** matrix. This error can help to simulate experimental uncertainty in evaluating positive binding results.

Click on **Reset All Values** to reset all parameters in the **Reagent Properties** dialogue box to their default values.

5.4   Printing results
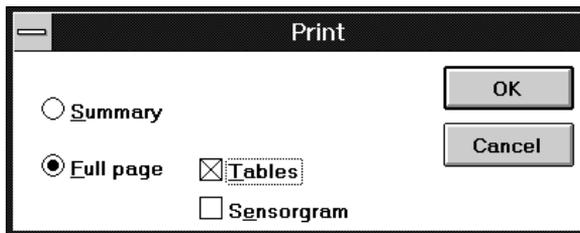
To print the results of a simulation, choose **File:Print**.

```
┌─────────────────────────────────────────────────┐
│ ▭                     Print                       │
├─────────────────────────────────────────────────┤
│                                    ┌──────────┐   │
│                                    │    OK    │   │
│  ○ Summary                         └──────────┘   │
│                                    ┌──────────┐   │
│  ◉ Full page      ⊠ Tables         │  Cancel  │   │
│                                    └──────────┘   │
│                   □ Sensorgram                    │
└─────────────────────────────────────────────────┘
```

Summary
Choose this option to print a one-page summary of the simulation.
Items printed are the main window (sensorgram with **Pairs to Run**
matrix, response levels and experimental conditions) and the reagent
properties window.

Full page
Choose this option to print tables (reagent properties and response
levels) and/or sensorgrams on a full page. The tables are printed
together on one page. Sensorgrams are enlarged to fill one page in
landscape orientation.

Notes: Only the sensorgrams currently displayed in the sensorgram
window (see Section 5.2.3) will be printed.

6. Multi-site binding

6.1 Background

This application simulates sequential injections of different analytes to test simultaneous binding to an immobilized ligand. The following features are included:

- user-defined properties for up to 6 analytes (referred to as probes) and one ligand (referred to as the target molecule)

- user-defined interference matrix between probe pairs

- free choice of injection sequence

- evaluation of binding in terms of response level for each probe

6.2 Running multi-site binding simulation

To start the multi-site binding application, click on **Multi-site Binding** in the BIAsimulation main menu.

To run a simulation with the default parameters, simply click on **Run**. One sensorgram is generated with probe injections in the sequence specified in the **Sequence To Run** matrix. The response for each injection is shown as the difference in SPR signal between the start and end of the injection. For comparison, the maximum independent binding capacity of the surface for each probe is also shown (derived from the reagent properties, see Section 6.3).
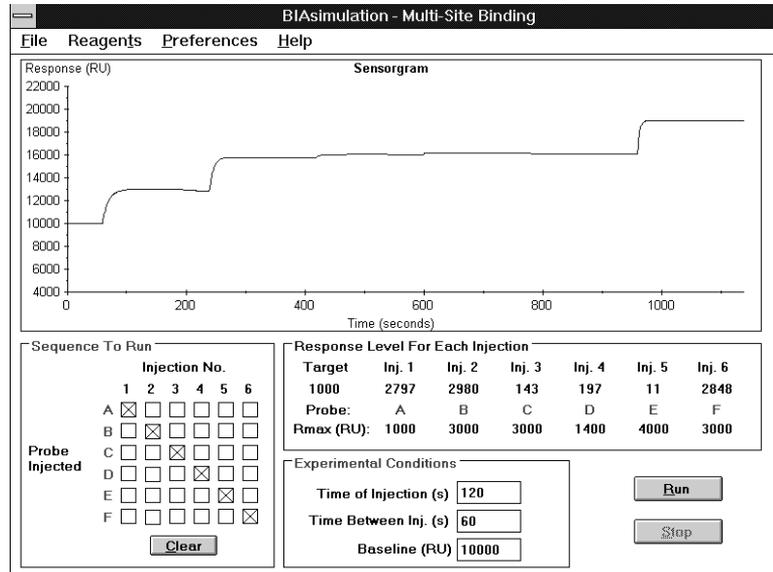
Probe injection sequence

To change the order of probe injections, check the appropriate boxes in the **Sequence to run** matrix. Vertical columns in this matrix represent injections and horizontal rows represent probes. You can only check one box in each vertical column (i.e. you can only inject one probe for each injection), but the same probe may be used in several injections. For example, the sequence illustrated below would inject probe A followed by B followed by A again:

Click on **Clear** to clear the whole matrix.

Injection parameters

The time of injection (in simulated seconds) and the time between injections can be changed by entering the required values in the appropriate fields. The time between injections is particularly relevant for probes with a relatively high dissociation constant, since probe is lost from the surface during the delay between injections.

You can also set a baseline value in RU to displace the sensorgram from the time axis.

Results
The results of multi-site binding simulation are displayed in the main window as the response level for each injection, measured from the beginning of the respective injection and corrected for any bulk refractive index contributions:

```
┌─Response Level For Each Injection─────────────────────────┐
│   Target    Inj. 1   Inj. 2   Inj. 3   Inj. 4   Inj. 5   Inj. 6 │
│    1000      2797     2980     143      197       11      2848 │
│   Probe:       A        B        C        D        E        F   │
│  Rmax (RU):  1000     3000     3000     1400     4000     3000 │
└────────────────────────────────────────────────────────────┘
```

6.3 Reagent properties

To set parameters for reagent properties, choose **Reagents:Reagent Properties**.

BIAsimulation - Multi-Site Binding - Reagent Properties

Probe Properties

Property	Reagent A	Reagent B	Reagent C	Reagent D	Reagent E	Reagent F
Mol. Weight:	50000	100000	150000	70000	200000	150000
Conc. (ug/ml):	50	30	300	21	120	60
ka (M-1 s-1):	1.0E+5	5.0E+5	5.0E+4	5.0E+6	1.0E+6	7.0E+5
kd (s-1):	1.0E-3	1.0E-4	5.0E-3	5.0E-4	1.0E-2	5.0E-5
Bulk RI (RU):	20	-10	60	35	-20	10

Interference Matrix
```
    A  B  C  D  E  F
A   ⊠  □  ⊠  ⊠  □  □
B   □  ⊠  □  □  ⊠  □
C   ⊠  □  ⊠  □  □  ⊠
D   ⊠  □  □  ⊠  □  □
E   □  ⊠  □  □  ⊠  □
F   □  □  ⊠  □  □  ⊠
```
Clear All

Target Molecule Properties

Mol. Weight: 50000

Immobilised (RU): 1000

OK

Cancel

Reset All To Default

Probe Properties
For each probe used, enter the molecular weight, concentration in µg/ml, association and dissociation rate constants and bulk refractive index contribution.

Interference Matrix
Check the appropriate boxes in this matrix for interference between
pairs of probes. Rows represent the first probe bound and columns
represent the second. Thus a checked box in row B, column D means
that binding of probe B interferes with subsequent binding of probe D.
Normally, the diagonal line from top left to bottom right will be
checked (i.e. any one probe interferes with its own binding), and the
matrix will be symmetrical about this diagonal (i.e. interference
between pairs is not dependent on the order of binding). You may
however check or uncheck any boxes in the matrix. Click on **Clear All**
if you want to clear all boxes.

Target Molecule Properties
Enter the molecular weight of the target molecule and amount
immobilized on the surface. For each probe, the independent binding
capacity of the surface is calculated from these figures and the
molecular weight of the probe, assuming 1:1 binding stoichiometry.
The binding capacity for a probe is related to the amount of target
molecule immobilized as follows:

$$R_{max(probe)} = R_{target} \times \frac{MW_{probe}}{MW_{target}}$$

Thus if the molecular weight of the target molecule is 50,000 and
1,000 RU are immobilized, the binding capacity for a probe of
molecular weight 100,000 is 2,000 RU.

Click on **Reset All To Default** to restore all reagent properties to their
default values.

6.4 Printing results

To print the results of a simulation, choose **File:Print**. The print destination is determined by the current printer setting in Windows Control Panel.
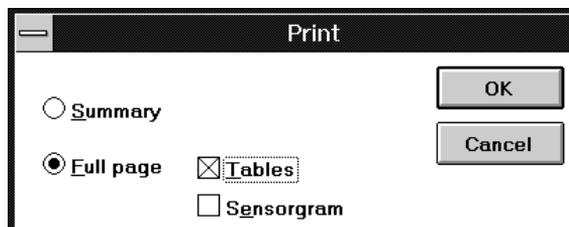
Summary
Choose this option to print a one-page summary of the simulation. Items printed are the main window (sensorgram, probe sequence, response level and experimental conditions) and the reagent properties window.

Full page
Choose this option to print tables (reagent properties and response levels) and/or sensorgrams on a full page. The tables are printed together on one page. Sensorgrams are enlarged to fill one page in landscape orientation.

BIAsimulation Software Handbook

7. Concentration analysis

7.1 Background

This application simulates standard curves for concentration analysis with BIA based on the response obtained in direct or sandwich assay protocols. Features include:

- replicate measurements with up to 20 concentrations and 10 injections per concentration

- variation between samples based on random system noise, baseline drift and regeneration artefacts

- sequential or random injection of samples to simulate the effects of systematic variations

- mass transport limitations for analyte binding

- $CV_{response}$ and CV_{dose} values and plots

- sensorgram display

- mixtures of up to 5 secondary reagents for sandwich assays

- estimation of detection limit

7.2 Running concentration analysis simulation

This section describes how to perform and evaluate a basic concentration analysis simulation. Refer to the detailed command descriptions (Sections 7.4-7.9) for more information.

7.2.1 Simulating a direct assay

1. Choose **Concentration Analysis** from the main BIAsimulation menu. An empty main window is displayed:
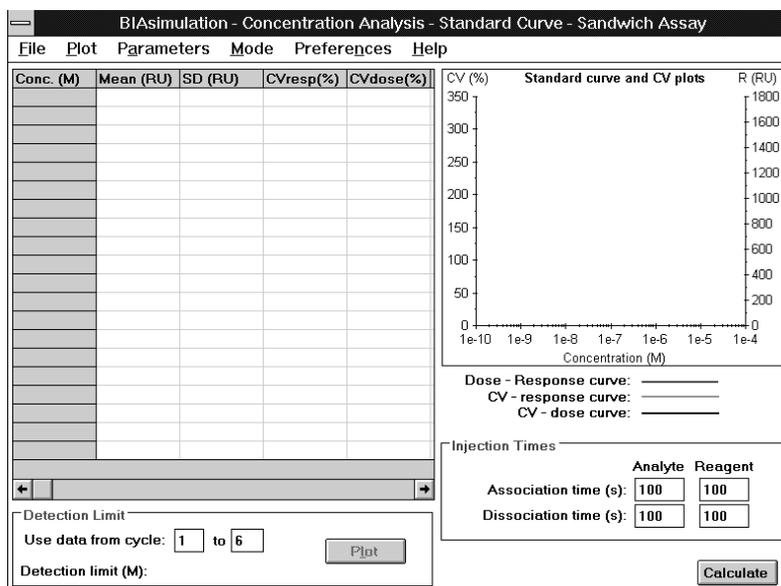
2. Choose **Mode:Direct Assay**.

3. Choose **Parameters:Analyte Properties**. These properties are filled in by default. Change the settings if you require:
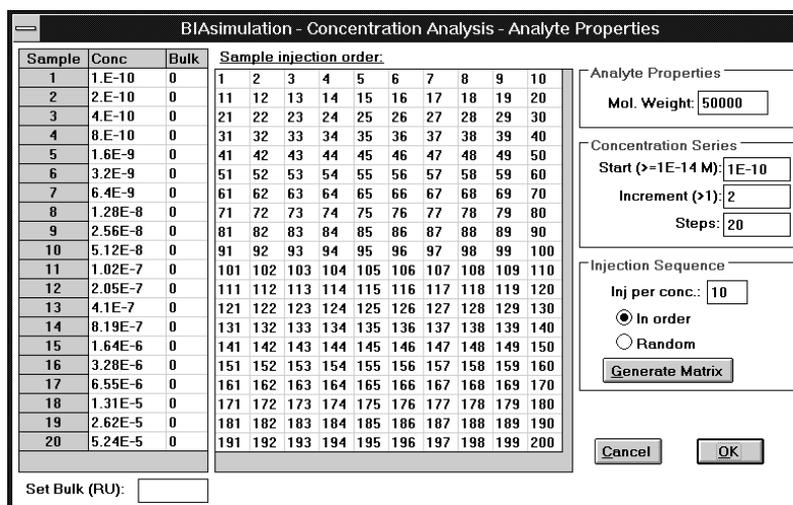
Enter the analyte molecular weight.

Under **Concentration Series,** enter the starting concentration, increment factor and number of steps (maximum 20).

Under **Injection Sequence**, enter the number of replicates and choose sequential or random order.

Click on **Generate Matrix** to generate the injection sequence. The numbers in the generated matrix represent the sequence numbers of the injections. If random order is chosen, a new sequence will be generated each time you click on **Generate Matrix**.

In the table to the left of the matrix, enter bulk refractive index contributions for the samples if required. This will only affect the appearance of simulated sensorgrams. You must enter the bulk contribution separately for each concentration.

Click on **OK** to return to the main window.

4. Under **Parameters:Reagent Properties** enter the molecular weight, kinetic parameters, ligand valency and amount immobilized for the surface ligand. For direct assays, the values under **Secondary Reagent Properties** are ignored.

BIAsimulation - Concentration Analysis - Reagent Properties					
Surface Ligand Properties					
Ligand MW: 150000			ka (M-1s-1): 1.E+5		
Ligand valency: 2			kd (s-1): 1.E-3		
Amount immobilised (RU): 5000					
Secondary Reagent Properties					
Enter value: 150000					
	Reagent	Mol wt	kass	kdiss	Bulk RI	Conc
☒	1	150000	1.E+5	5.E-4	0	1.E-7
☐	2	150000	5.E+4	5.E-4	0	1.E-6
Select ☐	3	150000	5.E+5	5.E-4	0	1.E-5
reagent ☐	4	150000	1.E+6	1.E-3	0	1.E-4
mixture ☐	5	150000	2.E+5	2.E-4	0	1.E-3
					Cancel	OK

Click on **OK** to return to the main window.

5. Additional settings that will affect the simulation are:
Parameters:Regeneration
Parameters:Noise and Drift
Parameters:Mass Transport Parameters together with **Mode:Mass Transport**
See the detailed descriptions in Sections 7.6 and 7.7 for more information.

6. Adjust the association and dissociation times in the main window if required. The response is measured at the end of the specified dissociation phase. For direct assay, the settings for **Reagent** are ignored.

7. Click on **Calculate** to simulate the concentration analysis. The results are presented numerically and graphically:

BIAsimulation - Concentration Analysis - Standard Curve - Sandwich Assay
File   Plot   Parameters   Mode   Preferences   Help

Conc. (M)	Mean (RU)	SD (RU)	CVresp(%)	CVdose(%)
1.E-10	5.22	5.14E-1	9.861	10.156
2.E-10	10.32	5.49E-1	5.324	5.428
4.E-10	20.24	7.52E-1	3.716	3.722
8.E-10	40.63	5.33E-1	1.311	1.324
1.6E-9	80.65	5.99E-1	0.743	0.75
3.2E-9	159.98	6.4E-1	0.4	0.407
6.4E-9	314.9	5.79E-1	0.184	0.19
1.28E-8	609.77	6.14E-1	0.101	0.107
2.56E-8	1147.87	6.14E-1	0.054	0.061
5.12E-8	2040.37	7.63E-1	0.037	0.049
1.02E-7	3269.02	3.6E-1	0.011	0.019
2.05E-7	4489.13	5.46E-1	0.012	0.038
4.1E-7	5123.61	6.73E-1	0.013	0.149
8.19E-7	5263.46	3.19E-1	0.006	0.419
1.64E-6	5296.28	3.47E-1	0.007	1.07
3.28E-6	5312.51	5.15E-1	0.01	3.16
6.55E-6	5320.67	7.16E-1	0.013	8.737
1.31E-5	5324.49	7.29E-1	0.014	17.778
2.62E-5	5326.72	5.67E-1	0.011	27.617
5.24E-5	5327.71	6.87E-1	0.013	66.953

Standard curve and CV plots

Dose - Response curve: ————
CV - response curve: ————
CV - dose curve: ————

Detection Limit
Use data from cycle: 1 to 6   [Plot]
Detection limit (M): 1.14E-11

Injection Times

	Analyte	Reagent
Association time (s):	100	100
Dissociation time (s):	100	100

[Calculate]

7.2.2   Simulating a sandwich assay

To simulate a sandwich assay, choose **Mode:Sandwich Assay**. Sandwich assays are simulated in the same way as direct assays with the following additions:

- The association and dissociation times for the secondary reagent can be set under **Injection Times** in the main window.

- A mixture of up to 5 secondary reagents can be simulated, with different binding kinetics and concentrations for each reagent. Secondary reagent properties are set under **Parameters:Reagent Properties** (see point 4 in Section 7.2.1).

The results of sandwich assay simulation are expressed in terms of secondary reagent responses. The analyte responses can be examined together with the sensorgrams (Section 7.2.4).

7.2.3 Examining the results

Numerical data
The table of numerical results from a simulation presents the mean response, SD, $CV_{response}$ and CV_{dose} values for each concentration. Use the scroll bar at the bottom of this panel to display the slope of the standard curve at each concentration point. For a definition of SD and CV, choose **Help:Concentration Analysis Formulae**.

Graphical data
The graphical panel displays the standard curve, $CV_{response}$ and CV_{dose} plots as a function of concentration. Drag over an area with the mouse to zoom the display of that area. Single-click with the right mouse button to restore the un-zoomed display. Double-click with the left mouse button in the graph area to display a dialogue box for setting the axis scales. Double-click with the right nouse button to switch between full-screen and panel display of the graphs.

In the **Plot** menu, choose which of the CV plots to display. The plot will be displayed when the corresponding choice is check-marked.

Note: The concentration axis is fixed as a logarithmic scale in the graphical display. For an indication of how far the standard curve is linear, examine the slope values in the numerical table.

7.2.4 Examining the sensorgrams
To examine the sensorgrams for a simulation, choose **Mode:Show Sensorgrams** then click on **Run**.
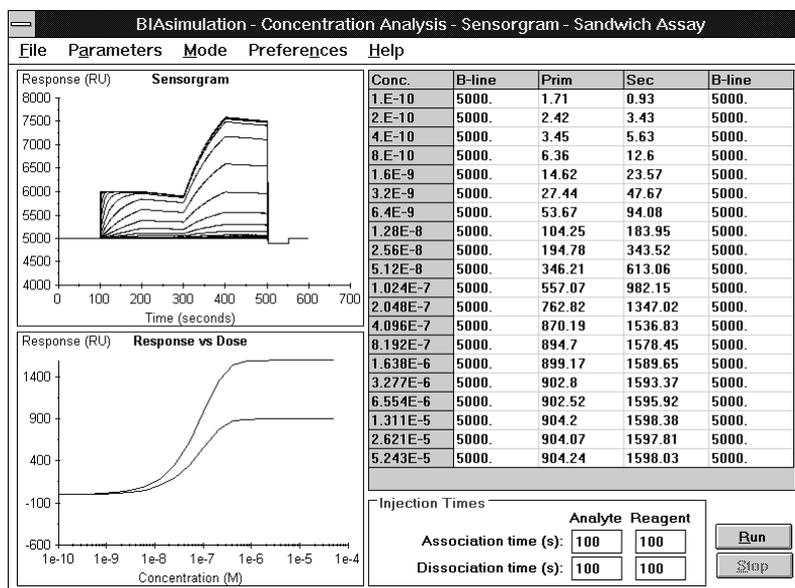
The sensorgram panel shows one representative sensorgram for each concentration. The table shows response values for baseline before injection, primary response (analyte), secondary response where applicable and baseline after regeneration. The response vs dose panel shows the standard curve on a logarithmic concentration scale for primary and (where applicable) secondary responses.

Systematic variations between cycles (system drift and regeneration artefacts) are simulated for one cycle per sensorgram, regardless of the number of replicates requested under **Parameters:Analyte Properties**. If random injection order is chosen, the concentrations will be listed and the sensorgrams generated in random order.

To display a single sensorgram, click with the left mouse button on the corresponding line in the table of concentrations. To display additional sensorgrams in an overlay plot, hold down the Ctrl key and click the required additional lines in the table of concentrations. To revert to display of all sensorgrams, click with the right mouse button anywhere in the table area.

Most parameters for the simulation can be changed from the sensorgram window menu in the same way as from the main window menu. Click on **Run** after changing parameter settings to recalculate the sensorgrams.

To return to the main window, choose **File:Close** or double-click on the window control box (at the top left corner of the window) to close the sensorgram window. Changes that you have made to parameter settings in the sensorgram window are not implemented in the main window until you click on **Calculate** in the main window.

7.3   Detection limit

The detection limit of an assay is defined as the standard deviation of the blank sample response expressed as analyte concentration ($SD_{dose(0)}$). Frequently, the practical detection limit is defined as $3 \times SD_{dose(0)}$.
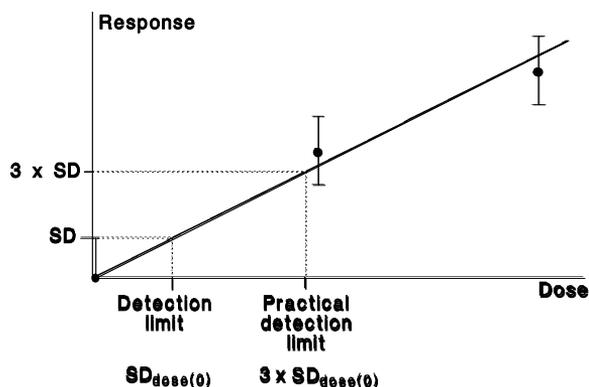
BIAsimulation does not simulate a blank sample (zero analyte concentration), and the detection limit is estimated by extrapolating SD_{dose} values to zero concentration. Usually, extrapolation from the first 6 concentrations (spanning a factor of 10 times the lowest concentration) are adequate for estimating the detection limit.

The detection limit for an assay estimated in this way is shown in the main window:

Note: The value shown here is 1 $SD_{dose(0)}$. Multiply by 3 to obtain the practical limit of $3 \times SD_{dose(0)}$.

If you want to estimate the detection limit from a different range of concentrations, change the settings in **Use data from cycle**. The detection limit is recalculated automatically.

Click on **Plot** to display the extrapolation of SD_{dose} to zero analyte concentration. This can indicate whether the choice of cycles to use for extrapolation is suitable (e.g. if the SD_{dose} values increase dramatically at higher concentrations, you should use fewer cycles).
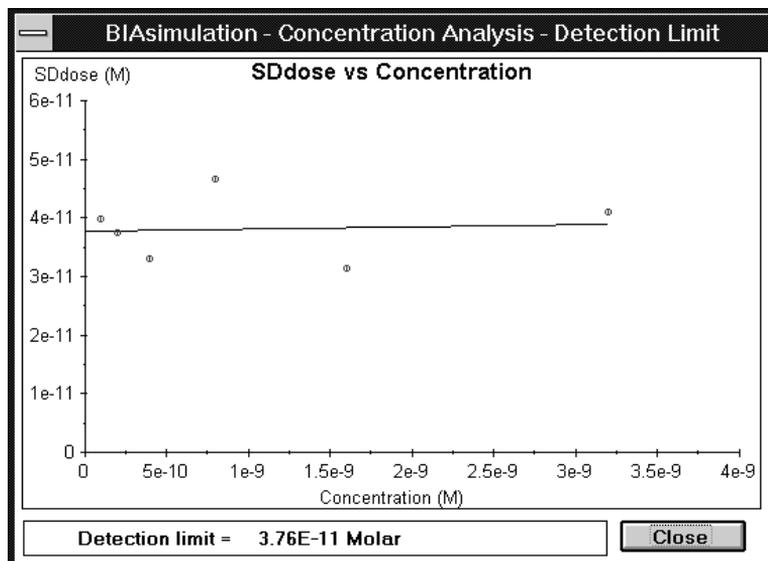
7.4   The File menu
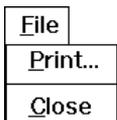
7.4.1   *Export Sensorgrams*

Exports data from simulated sensorgrams as tab-separated text files containing RU-time coordinate pairs. This command is only available in **Show Sensorgram** mode.

Enter a file name for the exported data in the dialogue box.
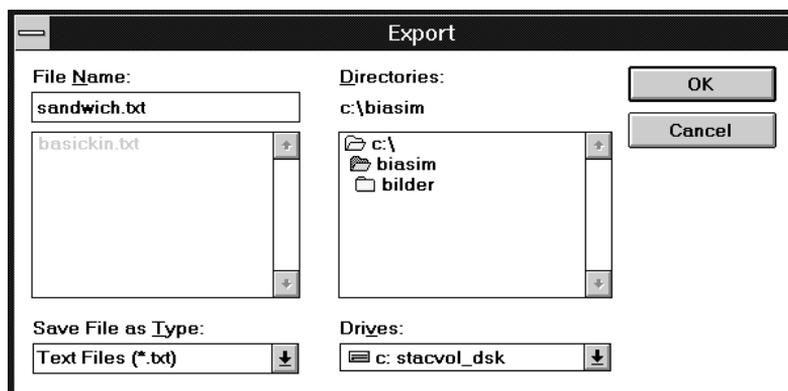
Note: Analyte and reagent properties and analyte concentrations are not included in the exported file.

7.4.2   Print
Prints a summary or selected parts of the simulation. Available choices differ in the main and sensorgram windows:

Main window
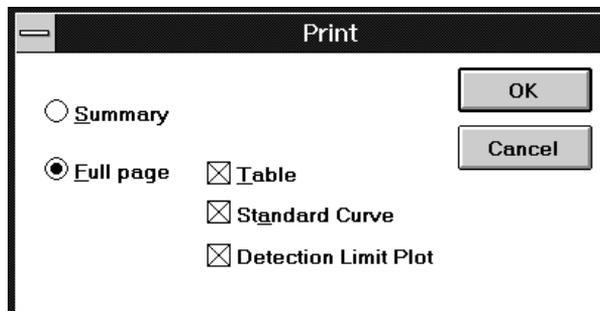
Sensorgram window
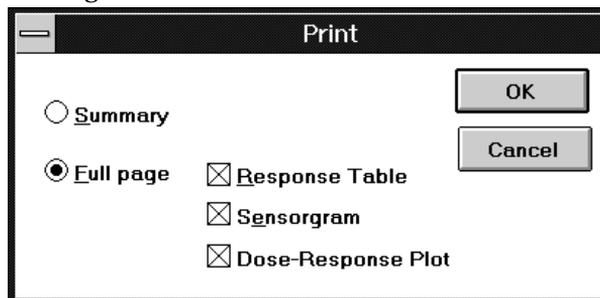
Summary
If the **Summary** button is checked, BIAsimulation will print a 2-page summary of the simulation.

Full page
If the **Full page** button is checked, BIAsimulation will print one page for each of the items marked. The **Evaluation** item cannot be printed unless a simulation has been performed.

Note: Only the currently displayed sensorgrams are printed from the sensorgram window.

7.4.3   Close
Closes the concentration analysis application and returns to the BIAsimulation main menu. You cannot close the application if a simulation is running or paused.

7.5   The Plot menu

The plot menu is only available in the main window.

Plot
√ Plot CVrespons
√ Plot CVdose

7.5.1   Plot CVresponse

The $CV_{response}$ plot is displayed in the main window when this option is checked.

7.5.2   Plot CVdose

The CV_{dose} plot is displayed in the main window when this option is checked.

7.6   The Parameters menu

Parameters
Analyte Properties...
Reagent Properties...
Regeneration...
Noise & Drift...
Mass Transport Parameters...
Parameters Summary...

7.6.1   Analyte Properties

Sets the properties of the analyte, analyte concentrations and injection sequence.

Analyte Properties
Enter the analyte molecular weight in daltons.

Concentration Series
Enter a starting concentration (M), increment factor and number of steps. Use exponential notation for the concentration (e.g. enter 5 nM

as 5E-9). The concentrations will be generated accordingly in the concentration table at the left of the dialogue box.

Injection Sequence
Enter the number of replicates for each concentration and choose whether to simulate injections in order or in a random sequence (this can affect the consequences of systematic variations such as regeneration artefacts, see Section 7.6.3). Click on **Generate Matrix** to generate the corresponding sample injection matrix in the panel to the left. Numbers in this matrix represent the sequence number of the injections.

Set Bulk (RU)
To simulate a bulk refractive index contribution from the analyte, click on the **Bulk** cell for the appropriate concentration in the concentration table and enter a bulk reponse in RU.

7.6.2 Reagent Properties
Sets the properties of the surface ligand and of the secondary reagent for sandwich assays.

```
┌─────────────────────────────────────────────────────────────────────────┐
│ ▭   BIAsimulation - Concentration Analysis - Reagent Properties          │
├─────────────────────────────────────────────────────────────────────────┤
│ ┌─Surface Ligand Properties──────────────────────────────────────────┐  │
│ │         Ligand MW: │150000│      ka (M-1s-1): │1.E+5│                │  │
│ │     Ligand valency: │2│           kd (s-1): │1.E-3│                  │  │
│ │  Amount immobilised (RU): │5000│                                    │  │
│ └─────────────────────────────────────────────────────────────────────┘  │
│ ┌─Secondary Reagent Properties───────────────────────────────────────┐  │
│ │                  Enter value: │150000│                              │  │
│ │                                                                     │  │
│ └─────────────────────────────────────────────────────────────────────┘  │
└─────────────────────────────────────────────────────────────────────────┘
```

	Reagent	Mol wt	kass	kdiss	Bulk RI	Conc
☒	1	150000	1.E+5	5.E-4	0	1.E-7
☐	2	150000	5.E+4	5.E-4	0	1.E-6
☐	3	150000	5.E+5	5.E-4	0	1.E-5
☐	4	150000	1.E+6	1.E-3	0	1.E-4
☐	5	150000	2.E+5	2.E-4	0	1.E-3

Select reagent mixture

Cancel   OK

Surface Ligand Properties
Enter the ligand molecular weight, the ligand valency, the amount of immobilized ligand and the kinetic constants for ligand-analyte binding. A ligand valency of 2 means that one ligand molecule can bind two analyte molecules. Fractional valencies can be entered to simulate partial ligand activity.

The maximum analyte binding capacity of the surface is determined by the amount of surface ligand, the ligand valency and the ligand and analyte molecular weights according to the equation

$$R_{max(analyte)} = R_{ligand} \times \frac{MW_{analyte}}{MW_{ligand}} \times ligand\,valency$$

Secondary Reagent Properties
Mixtures of up to 5 different secondary reagents can be simulated. Check the **Select reagent mixture** boxes for the reagents to be used, and enter the molecular weight, kinetic constants for reagent-analyte binding, bulk response and reagent concentration. The total bulk response for the secondary reagent will be the sum of the bulk contributions from the individual reagents used.

7.6.3   Regeneration
Sets the type and extent of regeneration artefacts that will be simulated.
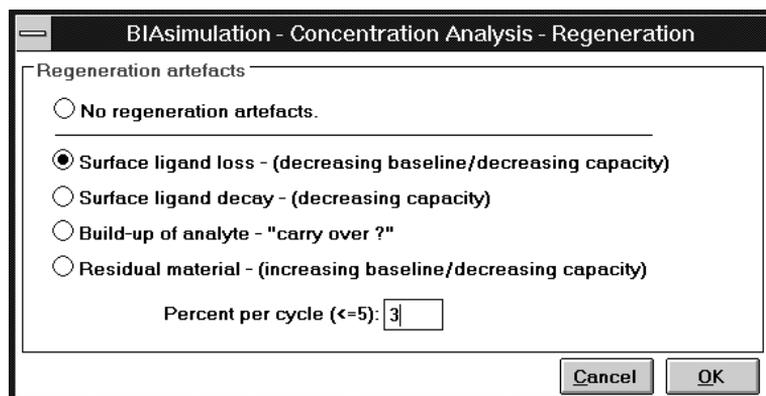
Choose the required type of artefact and enter the extent of the artefact in percent. During simulation, the extent is calculated as a percentage of the appropriate value from the previous cycle (e.g. 1% surface ligand decay will reduce the analyte binding capacity by 1% for each cycle without reducing the baseline).

Regeneration artefacts are applied after each cycle. In the sensorgram window, replicate samples are not simulated, so that regeneration artefacts are applied once for each concentration.

Regeneration artefacts introduce systematic variation between cycles. The effect of a regeneration artefact on the standard curve will therefore differ according to whether the injection sequence is random or in order.

7.6.4 *Noise and Drift*
Sets the level of system noise and baselinedrift.

System Baseline Drift
Enter a value for the baseline drift in RU/s. Baseline drift will affect the simulated response within each cycle, and will accumulate between cycles in sensorgram mode.

Note: The drift is specified in RU/s. Baseline drift in experimental sensorgrams is typically <0.3 RU/min (<0.005 RU/s) for BIAcore, BIAcore 2000 and BIAlite with active temperature control, and <1 RU/min (<0.017 RU/s) for BIAlite with passive temperature control.

System Noise
Enter a value for random noise in the simulated signal. This noise is the only source of variation between replicate samples. You may wish to set the noise higher than the typical instrument signal noise to simulate variations in sample preparation etc. If the noise is set to 0, replicate samples will be identical: CV values will be 0 and the detection limit will be meaningless.

7.6.5 *Mass Transport Parameters*
Sets mass transport parameters for the analyte.

This dialogue box is described in detail in Section 2.5.1.

Notes: The analyte molecular weight values in **Mass Transport Parameters** and **Parameters:Analyte Properties** are linked, so that a change in one is automatically reflected in the other. The mass transport coefficient is recalculated automatically if the analyte molecular weight is changed in the **Parameters:Analyte Properties** dialogue box.

To simulate mass transport effects, **Mode:Mass Transport On** must be checked.

Mass transport limitations are only simulated for analyte binding. Binding of secondary reagents in a sandwich assay is not affected by mass transport settings.

7.6.6 Parameters Summary

Displays a summary of the parameters settings for the current simulation.

7.7   The Mode menu

Mode
| Mass Transport On |
| √ Sandwich Assay |
| Direct Assay |
| Show Sensorgram... |

7.7.1   Mass Transport On

Determines whether mass transport limitations (see Section 7.6.5) will be simulated for analyte binding. Mass transport effects are considered when this command is checkmarked.

7.7.2   Direct Assay

Selects direct assay mode. In this mode, the response is based on binding of the analyte. Secondary reagent settings (see Section 7.6.2) are ignored.

7.7.3   Sandwich Assay

Selects sandwich assay mode. In this mode, the response is based on binding of the secondary reagent. Both primary (analyte) and secondary responses are shown in sensorgram mode.

7.7.4   Show Sensorgram

Displays the sensorgrams for the simulation (see Section 7.2.4). In this mode, one cycle is simulated for each concentration used, regardless of the number of replicates set under **Parameters:Analyte Properties**.

7.8   The Preferences menu

Preferences
| All Curves Black |

7.8.1   All Curves Black

Displays all curves in black. Use this setting if you want to print simulation results on a printer which does not handle colours satisfactorily. (Most monochrome printers render colours as shades of grey, but some printers may produce unsatisfactory results for light colours).

Note: Changing this setting affects subsequent simulations but does not change the display of the current simulation.

7.9   The Help menu

Help
| Help... |
| Mouse Functions... |
| Formulae... |
| Concentration Analysis Formulae... |

7.9.1   Help
In general, click with the right mouse button on a text area to display help for the item (e.g. click on **Bulk Resp (RU)** in the **Reactant Properties** frame for help on this parameter). This works on all text which serves to identify an input or display field.

7.9.2   Mouse functions
Displays a summary screen of mouse operations, including a small sensorgram display for practice.

7.9.3   Formulae
Displays a summary screen showing the formulae used in simulating basic kinetics (see Section 2.1). These equations are used to generate the sensorgrams from which the simulated responses are measured.

7.9.4   Concentration analysis formulae
Displays a summary screen showing the formulae and definitions used in concentration analysis simulation

BIAsimulation - Concentration Analysis - Formulae

SDresponse - Standard deviation of the response.

$$SDresponse = \frac{\sqrt{\Sigma(x_i - x)^2}}{n - 1} \quad \text{Unit: RU}$$

x_i = response value number i
x = mean value for all observations
n = total number of observations

CVresponse - Coefficient of variation for the response.

$$CVresponse = \frac{SDresponse}{x} \cdot 100 \quad \text{Unit: \%}$$

SDdose - Standard deviation of the dose.
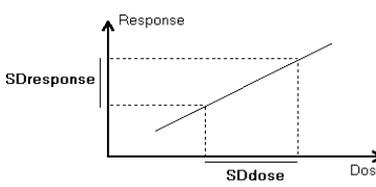
$$SDdose = \frac{SDresponse}{\text{Slope of the Standard Curve}}$$
Unit: Molar

CVdose - Coefficient of variation for the dose.

$$CVdose = \frac{SDdose}{Dose} \cdot 100 \quad \text{Unit: \%}$$

Detection limit.

Detection limit = SDdose when the Dose is zero.

Unit: Molar

Close

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BIACORE