

**Manual
HJCFIT for Windows**
Current version 0.8 beta

HJCFIT
for Windows

A program for maximum likelihood fitting of putative reaction mechanisms to single ion channel recordings. The calculation of likelihood is based on the exact solution of the missed events problem by [Hawkes, Jalali., & Colquhoun \(1990. 1992\).](#)

Acrobat 8.0 Note: the links jump to the top of the page where the entry starts

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Installation

The *instalshield* program, winsetup.exe, installs ancillary files that are needed to run the programs, in a directory of your choice (the default is C:\winprogs). A separate zip file contains all the files that are needed to run the three demonstrations with minimum effort. The registry is not altered by the installation.

Input files

Three sorts of files are needed to run HJCFIT.

- (1) An ***initialisation file, e.g. hjcfit.ini***. This file contains almost all the settings needed to run the fit, so little effort is needed to run the fit once it is set up, as shown by the demonstrations (once a fit is set up, keep the ini file under a suitable name so you can then repeat the fit easily). You can run the program without an initialisation file, but this means setting every single input, and it will usually be easier to change an existing ini file rather than make a new one.
- (2) ***The data: *.scn file(s)***. This takes the form of an idealised list of open times, shut times and amplitudes, produced by our time-course fitting program, SCAN. Other options will be added as time permits.
- (3) ***A mechanisms file (*.mec)***. This file contains predefined reaction mechanisms. The file contains several records, and each record contains both a mechanism and a set of values for the rate constants for the mechanism. The program allows definition of new mechanisms if the one you want is not already there, and the mechanism can be stored in a .mec file. Each mechanism may appear several times in the file, accompanied by different values of the rate constants, a convenient way to store different sets of initial guesses, or the final results of a fit.

Output files

The results of the fitting are recorded in a text file (by default, hjcfit.txt). This, together with the graphs, is your permanent record of what was done. Graphs can be kept as wmf files. Or, more conveniently, stored as a plot queue file (.plq) for plotting later with AUTPLOT.

Running the demonstrations

The demonstrations are as follows. Almost all you need to do to run them is to keep clicking ‘continue’ (or ‘OK’ or ‘All done’). All the settings are taken from the ini file.

(1) Nicotinic acetylcholine receptor demonstration.

This uses a single set of idealised data (a single scn file, scansim.scn), which in this case is simulated data for a single channel recording from nicotinic channels at 50 nM acetylcholine (the simulation was done with the SCSIM

program). The data were simulated as part of the study of the performance of HJCFIT ({Colquhoun, 2003 8585 /id}) and resemble the experimental records shown by {Hatton, 2003 8586 /id}. Because 50 nM is a very low concentration, and it cannot be assumed that only one channel is present, there is not enough information to identify all the rate constants, so one rate constant is fixed (see {Colquhoun, 2003 8585 /id})

Try this one first because it converges in about 1 minute, much faster than the second demonstration

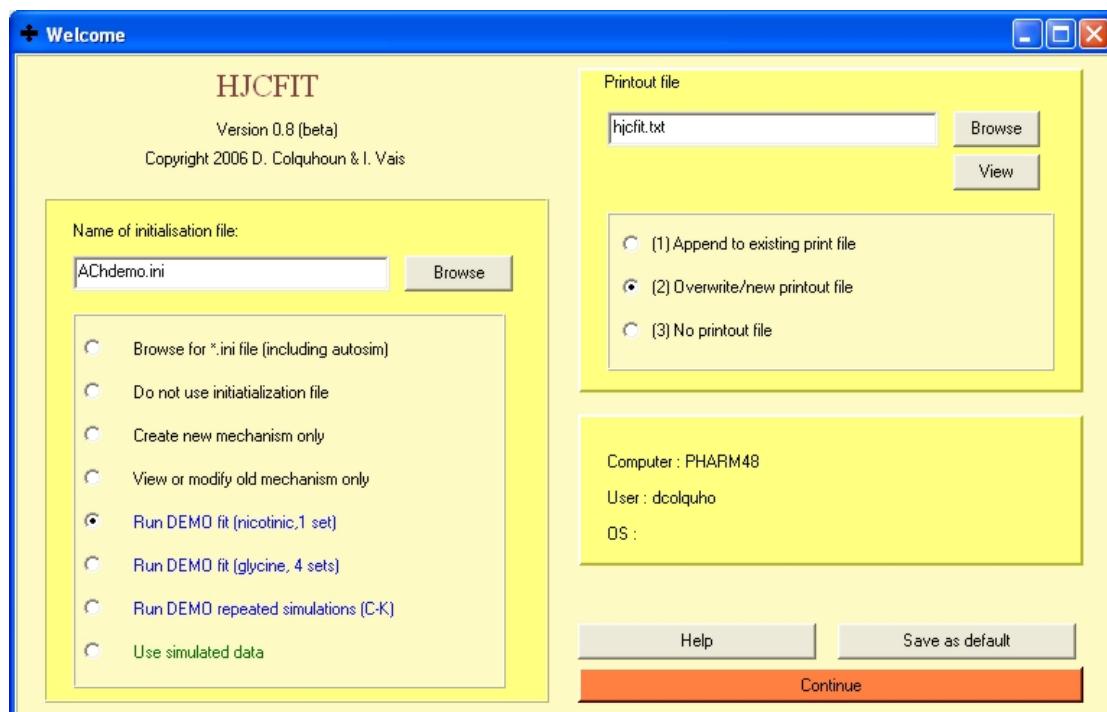
(2) Glycine receptor demonstration

The four data files (*.scn) are derived from idealisation (using SCAN) of experimental recordings of the single channel activity of glycine channels obtained at four different concentrations of glycine (10, 30, 100 and 1000 μM). The experiments, and the idealisation of the raw records in SCAN, were done by Valeria Burzomato and they are shown in {Burzomato, 2004 8688 /id}). All four sets are fitted simultaneously with a single set of rate constants as the free parameters. Because of the amount of data, the number of free parameters (14) and the poor initial guesses, this takes longer to converge than the first demonstration (about 1 hour).

(3) Repeated simulations demonstrations

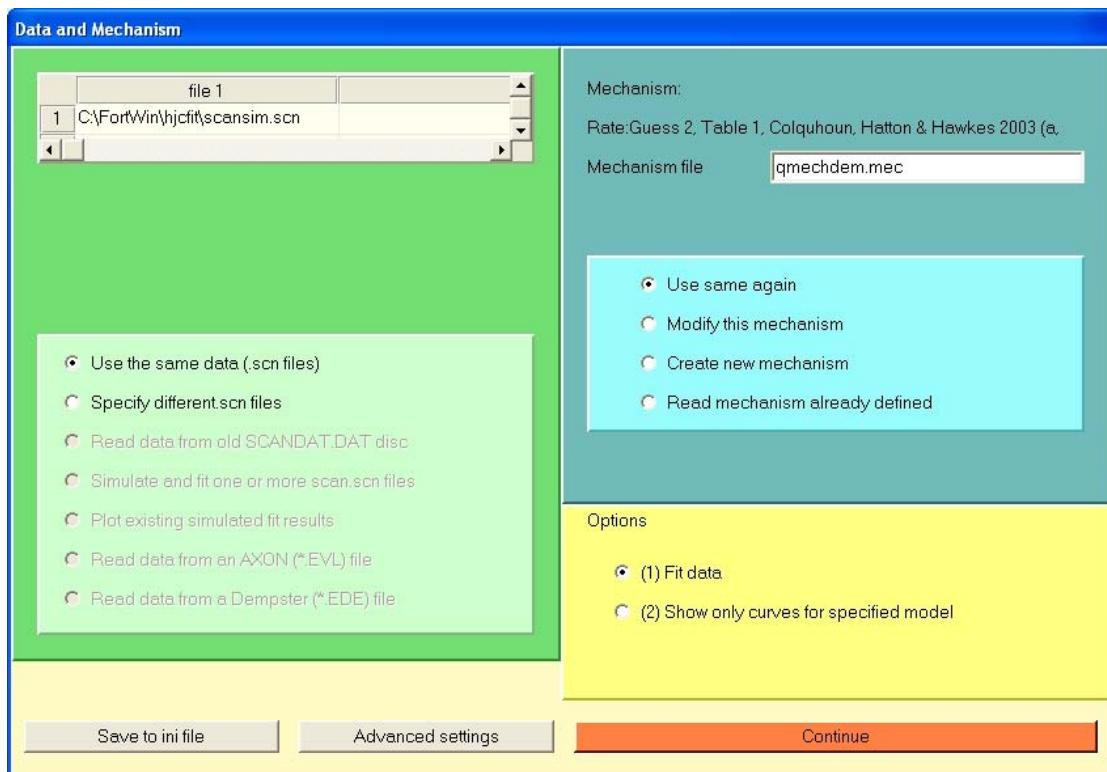
Running the demonstrations

The following instructions are kept as brief as possible so as to allow you to produce a result as quickly as possible. The meanings of the various inputs will be explained later.

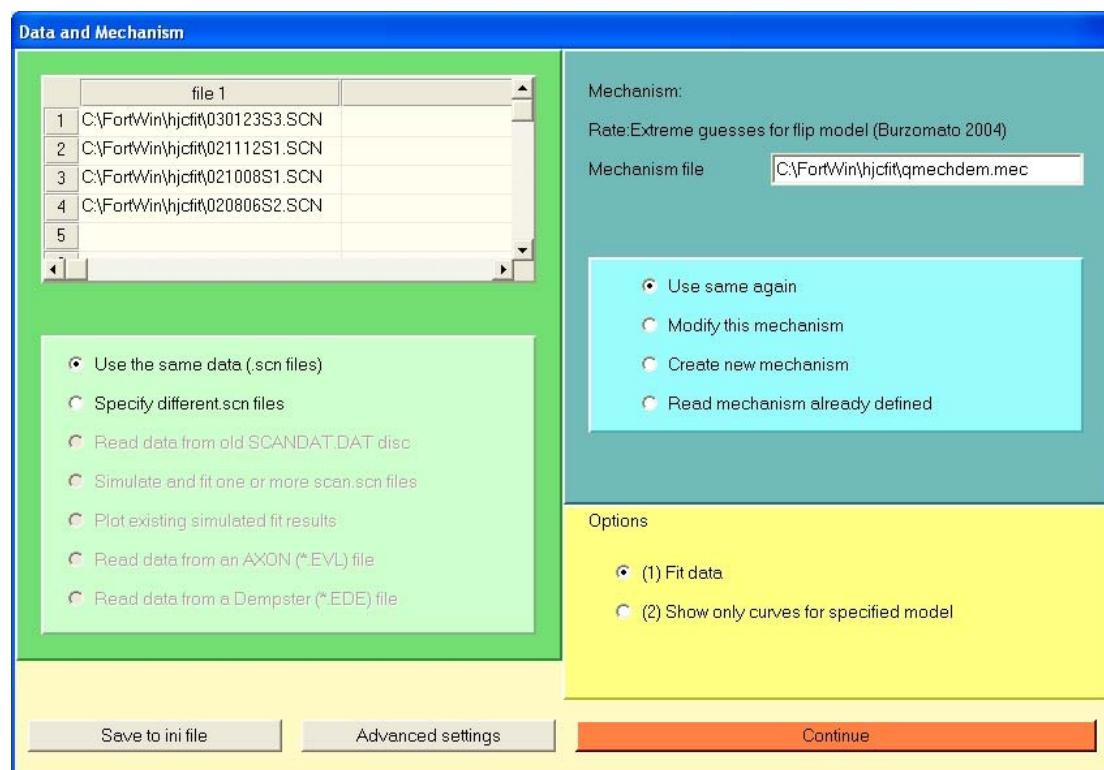


(1) On the initial yellow welcome screen, left-click one of the ‘Run DEMO’ radio-buttons (as below for the nicotinic receptor demonstration). The appropriate ini file name will appear in the window, so then click ‘continue’. The right hand side controls where the printout goes (hjcfit.txt in this case) and whether the printout should be appended to a previous file). **Click ‘continue’.**

(2) The Rates and Mechanisms window then appears. For the nicotinic demo it looks like this.

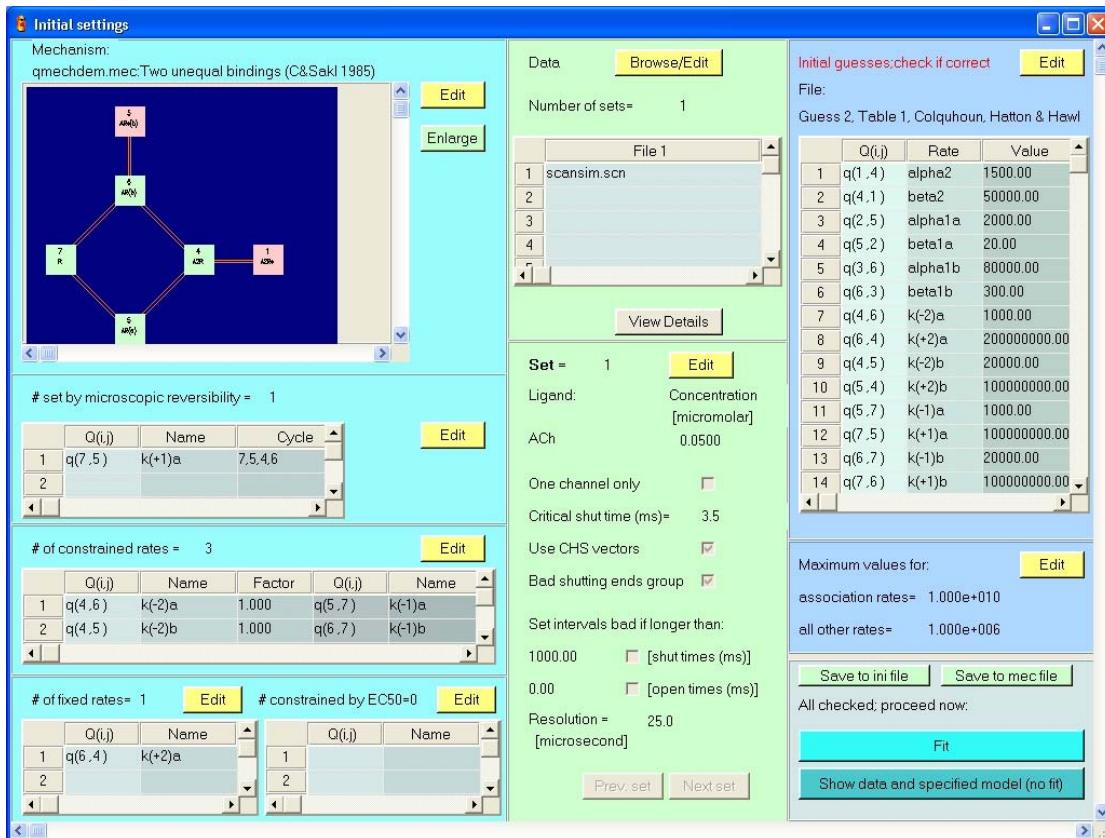


And for the glycine demo it looks like this.



The data files appear in the top section, and in this case we want ‘use the same data files’. The mechanism and rates are taken from the .mec file specified at the top right. **All you have to do is click ‘continue’ again.**

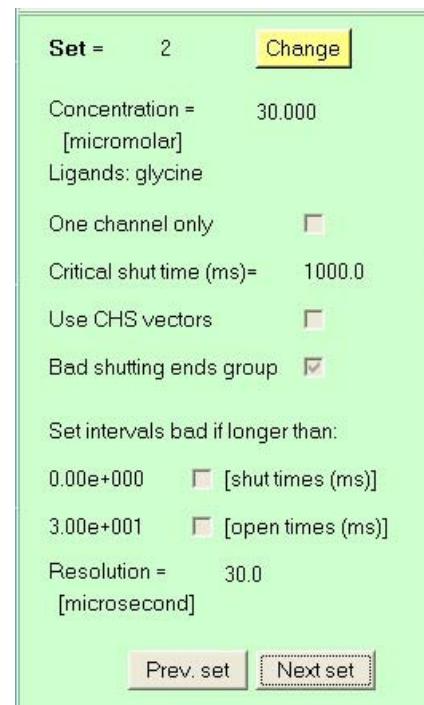
(3) The **large main window ('initial settings')** now appears. This shows the model and constraints on the left, the data to be fitted in the middle and initial guesses for the fit on the right. For the nicotinic demo it looks like this.



Not all of the model is showing at the top left. To see it all, use the scroll bar, or click '[enlarge](#)'

The idea of this window is to make visible almost all the settings that are needed for the fit, so they can be checked before the fit starts (if you are fitting more than one set, as in the glycine demonstration, you'll need the click the ‘next set’ button (centre panel bottom) to see the settings for each data set. For the second set of glycine data, this part of the screen looks as shown on the right.

For the demonstrations, all the values should be filled in correctly from the .ini file, so **all you have to do for the demonstration is to click ‘Fit data’ (bottom right)**.



(4) A window appears that shows the initial guesses for the fit. Just **click 'continue'**. A summary window appears that shows how many free parameters (rate constants) will be fitted. Again click 'OK'. You are asked whether you want to save the settings in an .ini file –choose 'No' (if any settings had been changed you might want to save the new settings for use in another run).

(5) The a window appears that contains default settings for the simplex optimisation routine. Again just click 'OK'. The fitting then starts, and you'll see the values of the parameters in the black 'teletype' window. Now just wait for convergence. The fit takes around 1 minute for the one-set demonstration (9 free parameters) but for the 4-set glycine example, convergence will take 1 – 2 hours. It is much slower because of the larger amount of data, because there are 14 free parameters to be fitted, and because the initial guess for β_3 is much too small in this example.

(6) At the end of the fit a small blue window offers the chance to print the final **Q** matrix. The default is 'no' so just click 'continue'. [*** soon this will be replaced with a button to print Q matrix on the 'results of fit' window'.]

(7) The 'results of fit' window appears. This shows the final values for the rate constants (as well as initial guesses). Normally the next step is to 'calculate errors' so click that option (it is also possible to go straight on to do plots here). When the calculation is finished (it can take a while) the '*results of fit with errors*' window appears. This shows for each parameter the estimated coefficient of variation (CV) expressed as a percentage, and standard deviation (the corresponding correlation matrix is in the print out, but not displayed at present). The final column columns show which parameters were constrained or fixed; these will not have errors (apart from parameters that were constrained by microscopic reversibility, for which it is sometimes possible to calculate an error).

Click 'continue' to do the plots.

(8) Now do the plots to see how well the estimated rate constants describe the observations. The 'Select Plot' window appears. There are 6 types of plot, and if more than one set was fitted, each sort of plot should be done for every set. After each plot is done, the defaults in the 'select plot' window automatically increment to the next data set (if more than one set was fitted) and then to the next plot type. Click 'continue', and then 'continue' again in the '*plot options*' window, and the '*histogram settings*' windows that appear. The graph then appears, and can be printed, saved or queued by the buttons at the top of the graph (which have bubble help). The graphs can be re-scaled by the buttons on the upper menu bar. Details are given below.

Running real problems

A guide to how to set the inputs and run the program

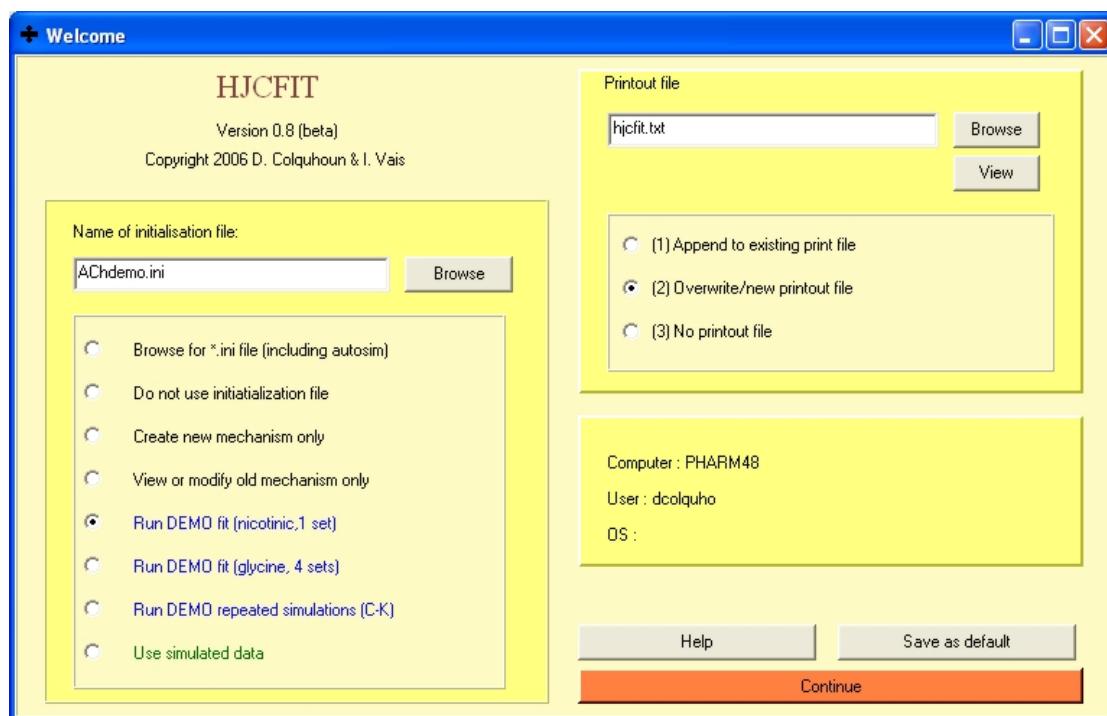
(1) When you run HJCFIT, the first thing you see is the yellow welcome window. The left hand panel controls what is to be done.

Welcome window

Choose the .ini file

As seen while running the demonstrations, the *.ini file contains all the settings that were used for the last fit. If you click do not use ini file, you will have to enter everything.

Often it will be easier to change an existing ini file (and then save it under a different name). Choose an ini file for a problem that is as similar as possible for the problem you want to solve (e.g. same data, or same model).



Click the *Browse for initialisation file* button, the *Browse* to select the ini file that you want.

Options in the welcome window (left panel) The details are described in separate sections (click the links).

- *Browse for initialisation file* Take all the settings from an .ini file
- *Do not use initialisation file* If this is clicked everything has to be specified manually. Detailed instructions are given below, in the section '[Starting without an ini file](#)'.

- *Create new mechanism only*, This leads to the [model designer](#), to allow you to define a new kinetic mechanism. This can be stored in a .mec file and used later for fitting to data.
- *View or modify and old mechanism only*. Choose this if you want to see what mechanisms are defined in an existing .mec file, and, if you wish, [modify an existing mechanism](#), e.g by adding or deleting states.

Demonstration options. Run these when you first try the program, and whenever you want to experiment with something.

- *Run DEMO fit (nicotinic, 1 set)* ([notes above](#))
- *Run DEMO fit (glycine, 4 sets)* ([notes above](#))
- *Run DEMO repeated simulations (CK)*. This option is not quite finished, It uses the simple Del Castillo-Katz mechanism so the fits run quite fast and many simulations run in a reasonable time. See [Simulate repeated experiments](#) for details.

Simulation option

- *Use simulated data.* This allows simulation of data for any specified number of 'experiments' (e.g. 1000). Each set of simulated data is then fitted and the distributions of the 1000 parameter estimates can then be plotted in various ways. (see [details below](#)).

Printout files: the hard copy. The right hand side of the Welcome page controls the hard copy that records the result of the fit. It is important to keep this, along with the plots, as a record of what was done. The printout file is plain ASCII text file, kept as a .txt file. Give the file a name, or choose an existing .txt file. If you want the output to be appended to an existing .txt file, click the appropriate button. If you don't choose *append* any existing file of the same name will be overwritten and lost.

The *save as default* button will make the settings in the Welcome window appear the same the next time HJCFIT is started.

Printout control.

On the right hand side of the welcome window, choose the name for the file that contains the printed output. It is the only record (apart from the graphs) of what was done. This is a plain ASCII file that can be read into Notepad, Word or any other program that handles text.

Reading the printout file into Word

The reaction mechanism is drawn out in the printout, when it is possible. This needs line drawing characters which will not usually appear correctly in Word or Notepad (it depends on how your text program interprets ASCII characters with codes above 128). You can make them appear correctly in Word if you go to Tools/Options/general tab, and check the box next to "confirm conversion at Open". Then, when you open a .txt (or anything but a .doc) file, a window appears in Word asking "Convert text from" Highlight the second line, *encoded text*. In the file conversion box that appears, choose the MS DOS option.

If a file of the same name already exists you can choose whether to overwrite it with a new printout file, or whether to append the output to the existing file.

The name of the file is specified (by default it is hjcfit.txt, but that should be changed to something appropriate for the fit you are doing either here, or when you view it at the end).

You can append the printout to an existing file, but the default is to start a new one (note that this will overwrite any existing file with the same name)

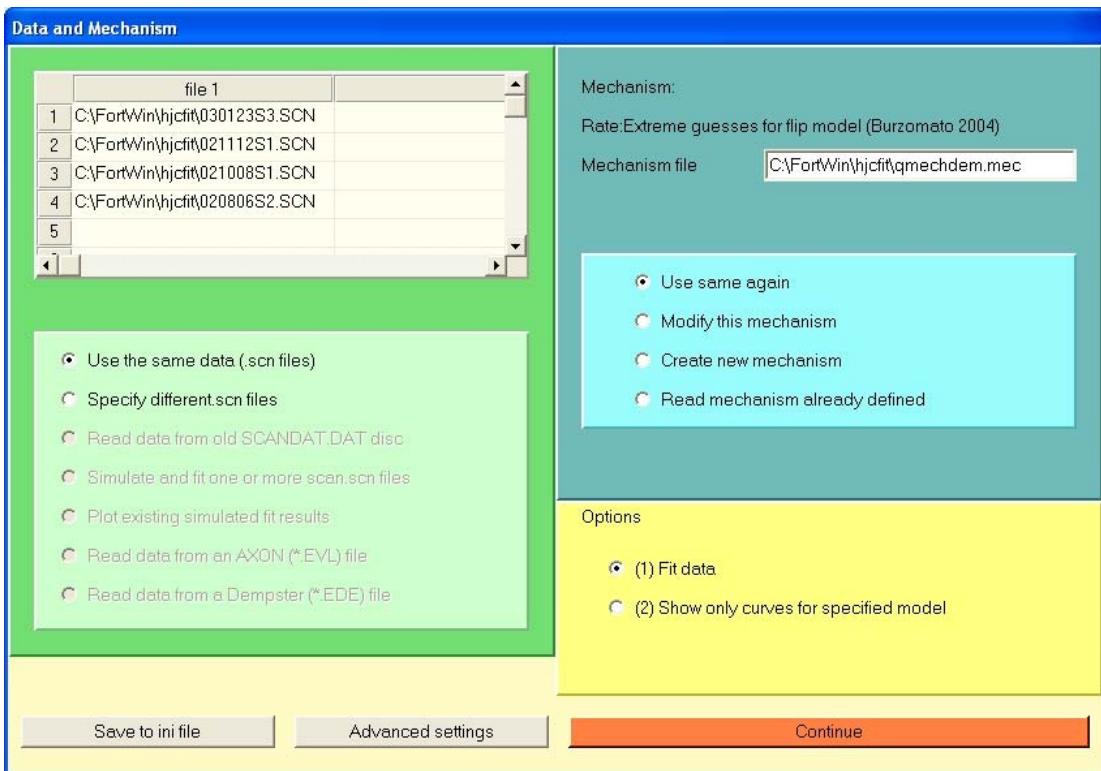
Save as default. Clicking this keeps the settings in this window as the defaults for the next time the program is run

The ini file contains almost all the settings needed to do a fit, as seen when you run the demonstrations. Now the meaning of the various inputs will be discussed in more detail (with separate sections below for topics that need longer discussions). And how to change the settings that come from the ini file will be described. The example to be described in more detail now is that used for the glycine receptor demonstration.

Data and mechanism window

When the Welcome window is set correctly, click continue. The ‘Data and mechanism’ window appears.

The bottom right radio button (*Show only curves for specified model*) allows the predicted HJC distributions (with allowance for missed events) to be plotted without any data or fit, using the rate constants specified as initial guesses.



If the data (left) and mechanism (right) files are OK then just click continue and you go straight to the [initial settings window](#).

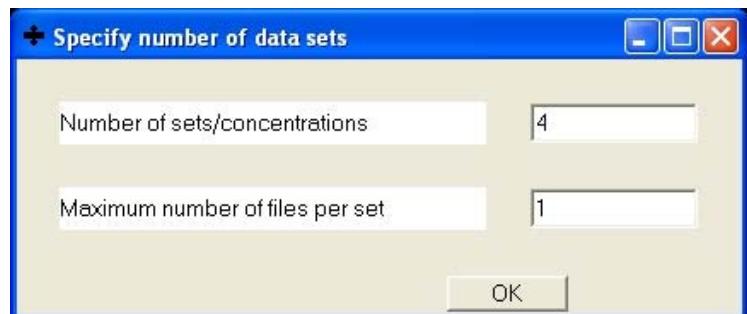
Both data and mechanism can be changed now, and they can also be changed later, from the initial settings window.

Changing the mechanism

If the mechanism file shown in the Rates and Mechanism window is wrong, click one of the radio buttons on the right to choose another mechanism. If you have a different .mec file in which the mechanism has already been defined, choose the bottom button, 'Change to/read mechanism already defined'. More details are given below, under [What to do if the ini file specifies the wrong mechanism file](#).

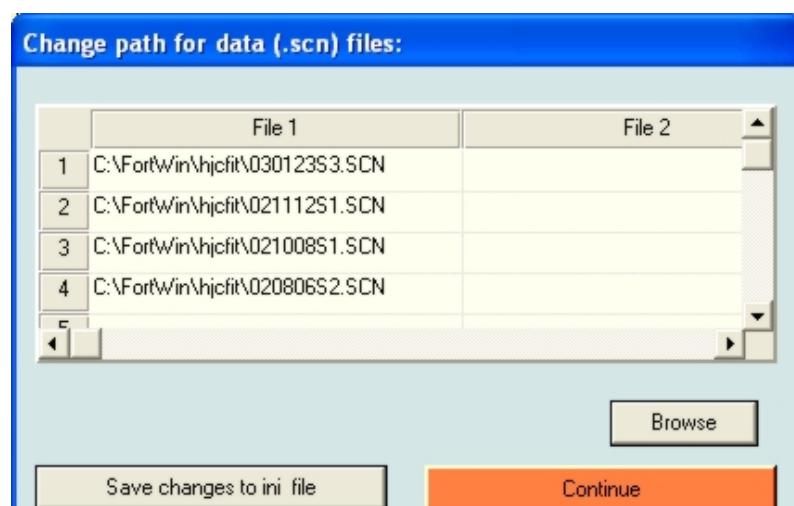
Changing the data files

If the data (.scn) file(s) that are shown at the top left are *not* right, then click the radio button 'Specify different .scn file(s)'. First specify how many sets of data are to be fitted simultaneously. Each set will usually correspond to a different concentration (they could also correspond to different membrane potentials but HJCFIT is not really designed for that –it easily could be). Several data (.scn) files can be pooled within each set as long as they are



homogeneous –specify the maximum number here.

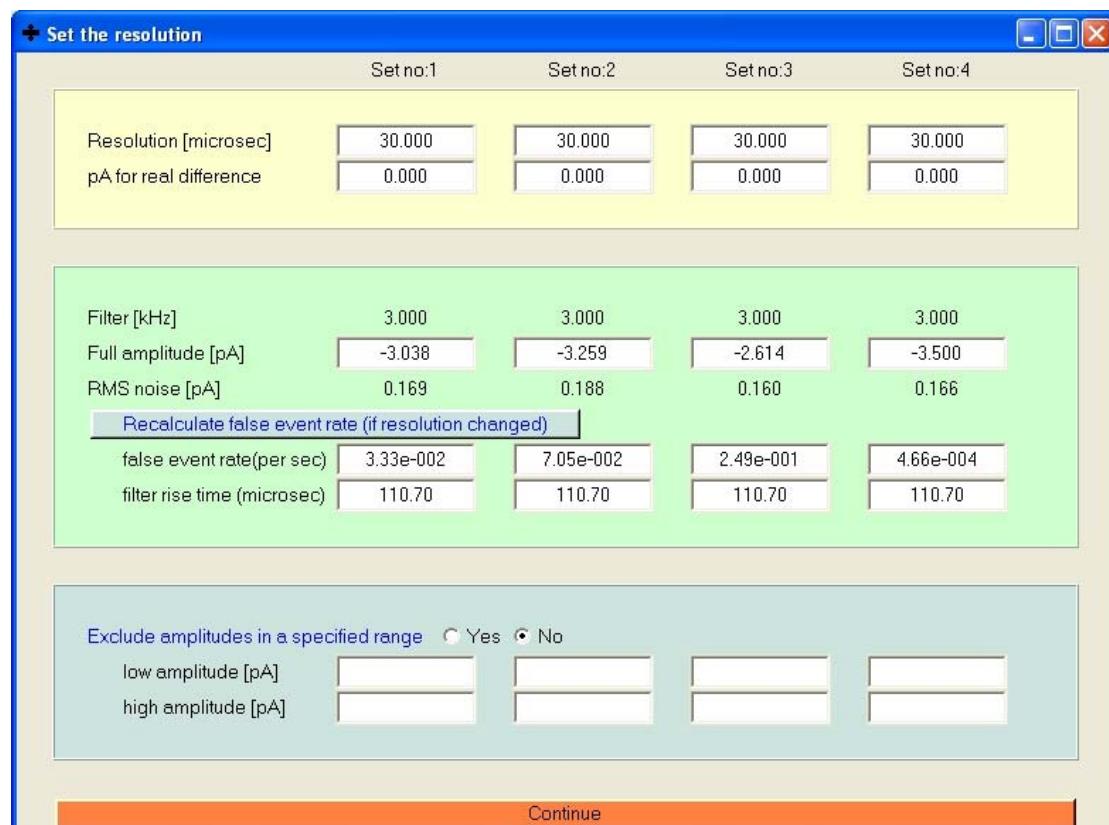
Clicking ‘*continue*’ produces the ‘*change path*’ window, which will initially show the default data files. Then choose the files you want. The file browser appears automatically, until the data files have been chosen for each set.



When the data files are shown correctly, click *continue*. This displays the *experiment details* window. This displays, for each experiment, the information in the header of the .scn file (only the latest version has the ligand concentration in the header, so check the values and correct if necessary).

Experiment details: check the concentration(s)				
	Set:1	Set:2	Set:3	Set:4
File	E:\FortWin\hjcfit\0	E:\FortWin\hjcfit\0	E:\FortWin\hjcfit\0	E:\FortWin\hjcfit\0
Date	23-Jan-2003	12-Nov-2002	08-Oct-2002	06-Aug-2002
Title	Rat Gly alpha1 Bet	Rat Gly alpha1Beta	100 microM gly	1000 micromol gly
Concentration (μM)	10.000	30.000	100.000	1000.000
Full amplitude (pA)	-3.038	-3.258	-2.614	-3.500
Tape details	VB datsomething	DAT VB	100 microM gly	vb5
Nr of transitions	15786	17576	17447	12510
Patch number	3	1	1	2
Patch type	cell-attached	cell-attached	cell-attached	cell-attached
Membrane potential [mV]	100.00	100.00	100.00	100.00
Temperature [o C]	20.00	20.00	22.00	21.00
Filter (kHz, -3 dB)	3.000	3.000	3.000	3.000
RMS noise (pA)	0.169	0.188	0.160	0.166
Calibration	0.002	0.002	0.002	0.004
Risetime setting:				
Nominal filter setting	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>
Effect of prefilter+tap	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Specify value here	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Total nr of transitions	63319			
Temperature (mean)	20.750			
Full amplitude (mean)	-3.103			
RMS noise (mean)	0.171			
Potential (mV) (mean)	100.000			
<input type="button" value="Check concentration(s) and save"/> <input type="button" value="OK"/>				

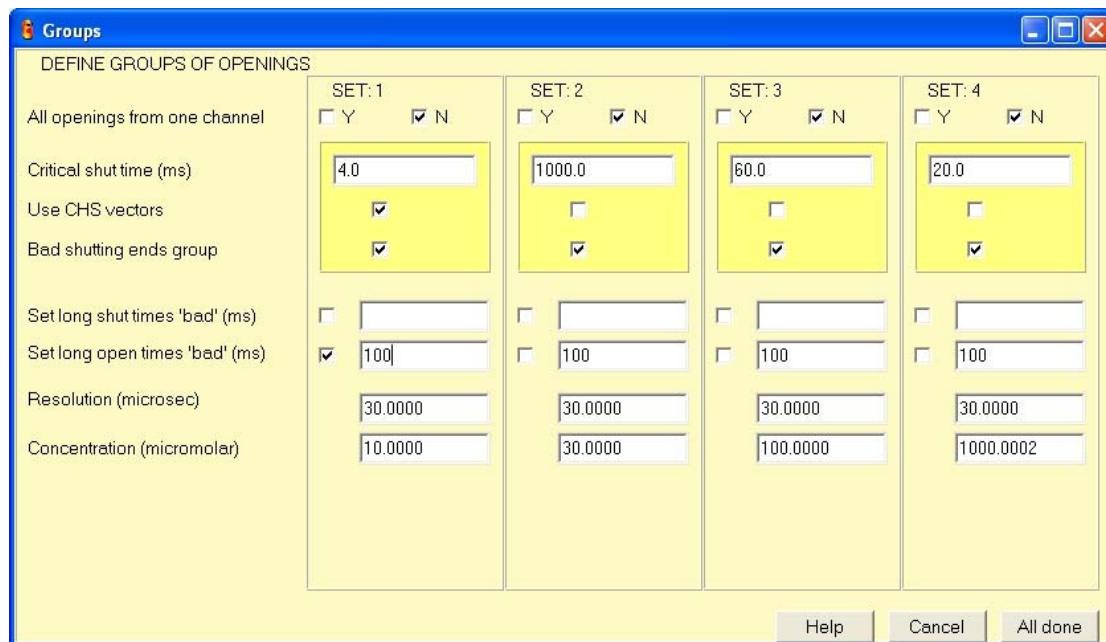
Once checked, click OK to bring up the *Set the resolution* window.



The main thing to be set here is the resolution that is to be imposed on each data set. In the case of the glycine demonstration it is 30 μ s for each of the four sets. The false event rate is shown. If you change the resolution, then click the button to recalculate (refresh) the false event rate. This is the number of false events (arising from random noise) per second that would be expected to appear to be longer than the resolution. The other values in this table normally have their default values, as shown.

Click *continue* when ready, and then define the groups of openings.

Define groups of openings.



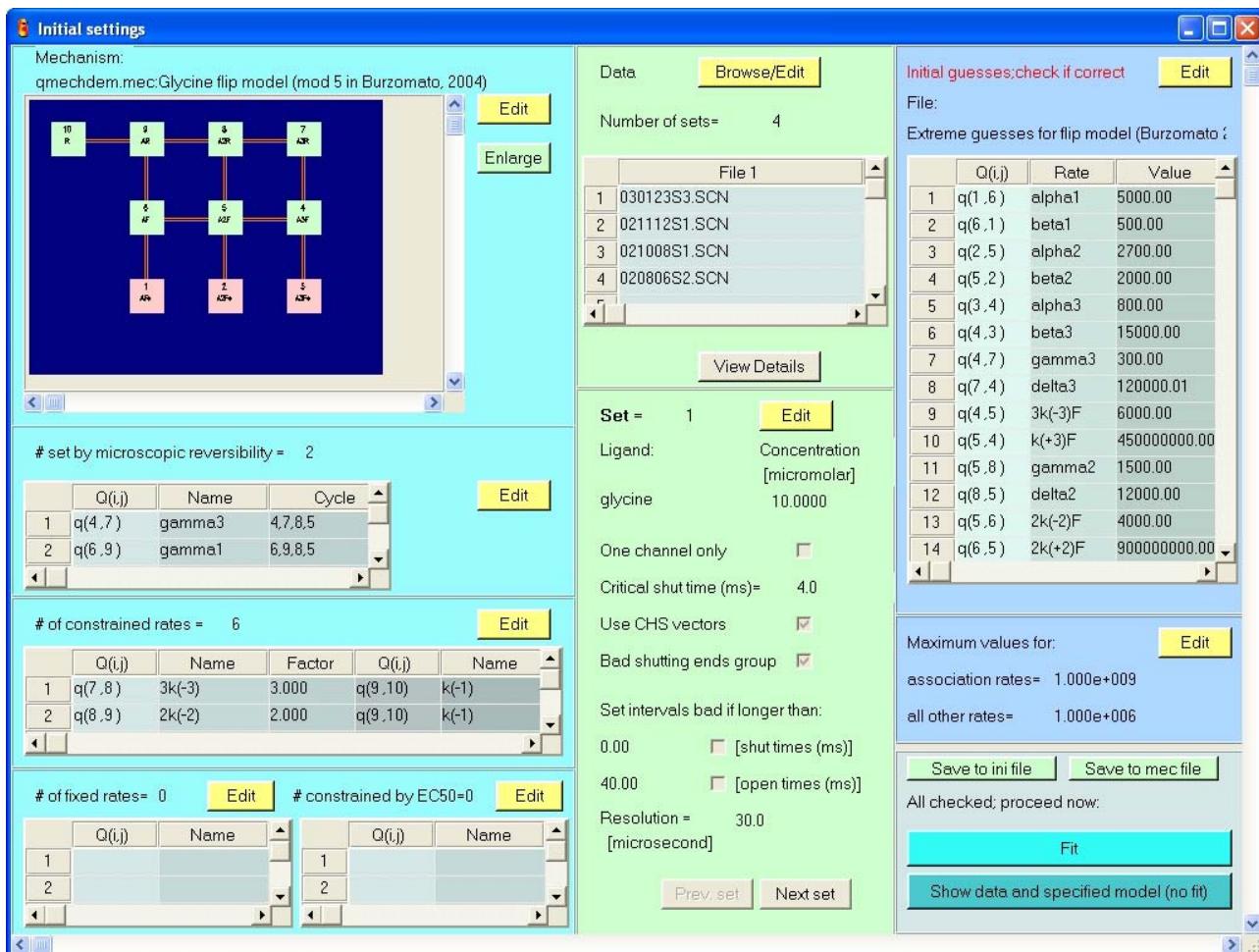
In most cases it won't be known that the patch contained only one channel , so *No* is checked on the top line. In this case, define on the next line the critical shut time that is used to divide the record into groups of openings such that all the openings in each group arise, almost certainly, from the same individual channel. For details see notes on [*The problem of an unknown number of channels*](#) and [*Notes on the choice of \$t_{crit}\$*](#) .

Check the box on the next line if CHS vectors are to be used for the start and end of each group for calculation of the likelihood (see [*Notes on CHS vectors*](#))

Clicking *All done* brings you to the main *Initial Settings* window (if you used the default data and mechanism files, you jump straight to here)

The main initial settings window

Once data and mechanism files have been chosen, you get to the main *Initial settings* window, which will now be described in greater detail. This is what it looks like for the glycine demonstration.



For the demonstrations, the various settings in this window should be correct so all you have to do is to click *Fit*. Alternatively you can click *Show data and specified model (no fit)*: this skips the fitting and shows the predictions using the rate constants you specified as initial guesses superimposed on the data.

If anything is changed in the *Initial settings* window, you may want to save the new settings in an ini file so they can be used again later without re-entering the changes. Click the *save to ini file* button just above the *Fit* button to do this. You may also want to save changes to the mechanism, if the same mechanism with a changed set of rate constants, in a mechanism (.mec) file. To do this click the *Save to .mec file* button, and choose an existing .mec file, or name a new one.

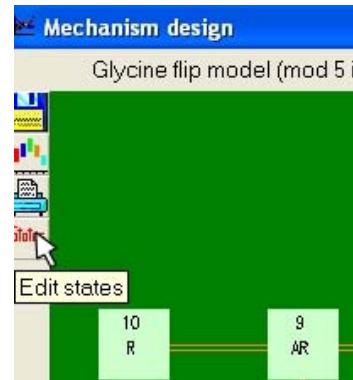
The various settings in this window will now be described in more detail, to show what should be done if they are *not* correct.

The mechanism

The mechanism is shown in the top left panel. If it is not all visible, click the *enlarge* button to show the mechanism enlarged, thus.



The icons on the left edge of this window (from top down) (a) store the mechanism (in a .mec file), (b) keep the mechanism as a .bmp or .wmf graphics file, (c) print the mechanism, and lastly (d) bring up a window showing the properties of the states in the mechanism. These icons have bubble help, which pops up when the cursor hovers over the icon,

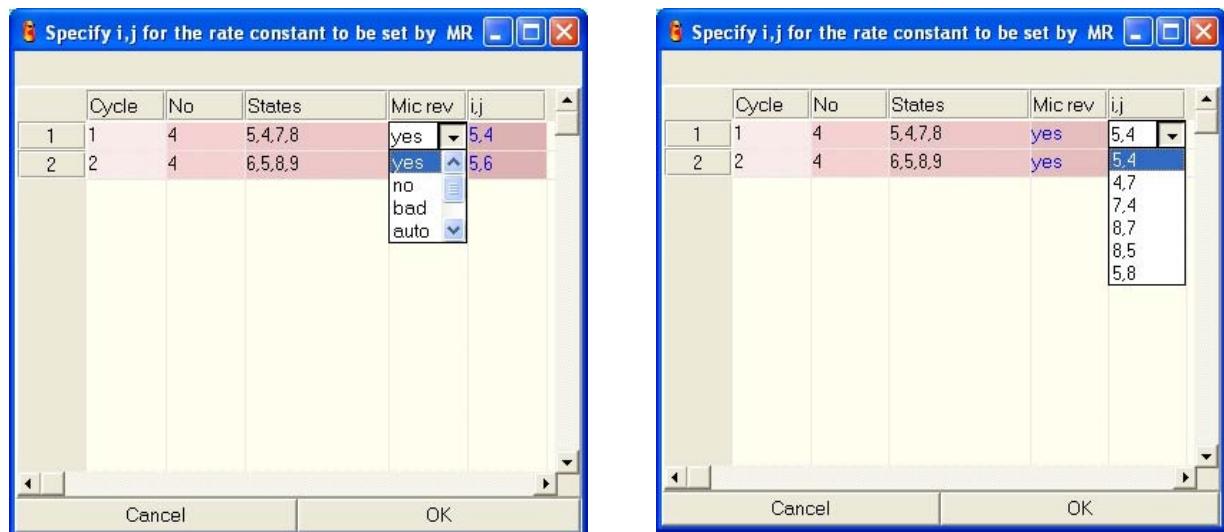


Clicking the 'edit states' icon shows the *properties of states* window (shown below). The settings can be edited in this box. If you change anything here, remember to click *store changes*

Microscopic reversibility constraints.

The second panel down on the left if the [initial settings window](#) shows that two rate constants are specified by microscopic reversibility constraints, so they are not free parameters (see *Notes on microscopic reversibility*, below). Scroll down the box if they are not all visible. In this case, for example, states 5, 4, 7 and 8 form a 4-state cycle. Since we usually wish the fitted mechanism to obey microscopic reversibility, one of the eight rate constants in this cycle will be calculated from the other seven. It should make no difference to the final estimates which is chosen: in this case the rate from state 5 to state 4,

' $k(+3)F$ ', is calculated from the others. Click the *Edit* button to alter the settings for microscopic reversibility constraints. The number of cycles is detected as two and the settings can be changed by altering the last two columns in the window that appears when a change is requested.



If you don't want a cycle to obey microscopic reversibility then choose 'No' in the drop down menu, as on the left (ignore 'bad' and 'auto' for now). To change which rate constant is to be calculated by microscopic reversibility, choose one from the drop down list, as on the right. For more details see [Notes on microscopic reversibility](#), below.

Constrained rate constants

In this case there are six constrained rate constants (scroll the window to see all of them). To change the values, click *Edit*. This produces a list of all the rate constants.

Scroll the box if the number of rate constants is too large to show all at once.

Rates available for constraint						
	Q(i,j)	Name	Constraint	Factor x/ Total	Q(i,j)	Name
1	q(1,6)	alpha1	None	0.00		
2	q(6,1)	beta1	None	0.00		
3	q(2,5)	alpha2	None	0.00		
4	q(5,2)	beta2	None	0.00		
5	q(3,4)	alpha3	None	0.00		
6	q(4,3)	beta3	None	0.00		
7	q(4,7)	gamma3	None	0.00		
8	q(7,4)	delta3	None	0.00		
9	q(4,5)	3k(-3)F	Multiplicative	1.50	q(5,6)	2k(-2)F
10	q(5,4)	k(+3)F	MR	0.00		
11	q(5,8)	gamma2	None	0.00		
12	q(8,5)	delta2	None	0.00		
13	q(5,6)	2k(-2)F	MR	0.00		
14	q(6,5)	2k(+2)F	Multiplicative	2.00	q(5,4)	k(+3)F
15	q(6,9)	gamma1	None	0.00		
16	q(9,6)	delta1	None	0.00		
17	q(7,8)	3k(-3)	Multiplicative	3.00	q(9,10)	k(-1)
18	q(8,7)	k(+3)	None	0.00		
19	q(8,9)	2k(-2)	Multiplicative	2.00	q(9,10)	k(-1)
20	q(9,8)	2k(+2)	Multiplicative	2.00	q(8,7)	k(+3)
21	q(9,10)	k(-1)	None	0.00		
22	q(10,9)	3k(+1)	Multiplicative	3.00	q(8,7)	k(+3)

This box lists all the rate constants, and shows which are constrained, which are fixed and which are set by microscopic reversibility (marked 'MR'). In this example, 6 are constrained, none are fixed and two are found by MR so there are $22 - 6 - 2 = 14$ free parameters to be estimated.

In the glycine receptor demonstration there are 6 constraints. They ensure that the binding (association and dissociation rate constants are identical for all binding steps for the resting conformation (R) and that they are equal for the flipped conformation (F). For example the last constraint shown (parameter 22) specifies that $q_{10,9}$ which is k_{+1} is not a free parameter but is calculated as a factor (3.00) times k_{+3} . Notice that the text name for the first association was specified when the model was created as "3 k_{+1} " rather than " k_{+1} "; the statistical factor, 3, was included as part of the name as a reminder (see section below, about "[Naming of rate constants](#)". This is a good idea even if the parameter is not constrained. '

The constraints can be changed via columns 3, 4 and 5 in the table shown above. Column 3 has a drop down menu that gives a choice of 'none' (no constraint), 'multiplicative' or 'additive'..

A 'multiplicative' constraint is like the one just described. The rate in the left columns is not free but is calculated as the factor that you type in column 4 times the rate constant specified in the right columns. The latter is specified by picking the appropriate $q(i,j)$ from the drop down list (shown above on the bottom line). The rate constant name in the last column doesn't (yet) change instantly when you change $q(i,j)$, but appears in the constraints window when you click 'continue' to return to the main '[initial settings](#)' window (and if you click 'change' again, the correct name appears).

An additive constraint allows the *sum* of two rate constants to be constrained to a specified value(e.g. the total dissociation rate from the two different binding sites in the nicotinic demonstration). The two rate constants are specified as above, and the fixed total of the two is typed into the factor/total column.

Fixed rate constants

At the bottom of the left hand panel the number of fixed rate constants is specified (none in this case, but one for the nicotinic demonstration). Click 'Edit', to fix or unfix any rate constant. The value of a fixed rate stays fixed, at the value set as the initial guess, throughout the fit.

EC50 constraint

It is possible to reduce the number of free rate constants by one, if we specify here that one rate constant is to be calculated so as to produce a specified EC50 (if you have a good value for the EC50 from other data). It is quite possible that no reasonable value for the calculated rate can produce the specified EC50 so you can specify limits on the value of the rate constant to prevent the fit becoming unstable. For detailed instructions, see [Using and EC50 constraint](#) .

Data files

At the top of the middle column of the [initial settings window](#) the data (.scn) files appear. These should have been specified already, but can be changed here if necessary. For the glycine demo, there are four data sets, each at a different concentration of glycine. Clicking *Edit* in this section takes you to the definition of data files already [described above](#).

Note that it is your responsibility to ensure that the ligand concentration is specified correctly for each set (in next section). This is automatic only with the latest versions of SCAN which incorporate the numerical value of the ligand concentration(s) in their header information of the .scn file. This greatly reduces the chance of getting the concentrations wrong.

You can update any old .scn files by using them as the restart file in SCAN, then going straight to write of .scn file, and supplying the ligand concentration so the .scn file can be resaved in the new format,

Values that must be set for each data set

The lower part of the middle column shows values that are specific for data set 1. There isn't room to show values for more than one set, so when, as in this case, there are four sets, it is essential to click next/previous set to check that all the values are correct before starting the fit. DO NOT FIT UNTIL ALL VALUES ARE CHECKED. The settings for set 1 are shown below. Click on '*Edit*' to adjust any of the settings.

This produces the 'groups window', Check that the ligand concentration is correct for the first data set, and if not, enter the correct value. The other

settings are explained under [Define groups of openings](#), above. [to be completed –see DOS manual for now]

Set =	1	Change
Concentration =	10.000	
[micromolar]		
Ligands:		
One channel only	<input type="checkbox"/>	
Critical shut time (ms)=	4.0	
Use CHS vectors	<input checked="" type="checkbox"/>	
Bad shutting ends group	<input checked="" type="checkbox"/>	
 Set intervals bad if longer than:		
0.00e+000	<input type="checkbox"/> [shut times (ms)]	
4.00e+001	<input type="checkbox"/> [open times (ms)]	
Resolution =	30.0	
[microsecond]		
 Prev. set Next set		

Groups

DEFINE GROUPS OF OPENINGS

SET: 1

<input type="checkbox"/> Y	<input checked="" type="checkbox"/> N	
4.000		
<input checked="" type="checkbox"/>		
<input checked="" type="checkbox"/>		
<input type="checkbox"/>		
<input type="checkbox"/>	40	
Concentration (micromolar)		
10.000000000		
Resolution (microsec)		
30.000		
Ligands	glycine	
Help	Cancel	All done

Then click 'next' to repeat this for the other three sets. This produces the following displays for sets 2, 3 and 4. Click ; *Edit* if necessary.

Limits on the maximum value of fitted rate constants

Set = 2	Change	Set = 3	Change	Set = 4	Change
Concentration = [micromolar]	30.000	Concentration = [micromolar]	100.000	Concentration = [micromolar]	1000.000
Ligands:		Ligands:		Ligands:	
One channel only	<input type="checkbox"/>	One channel only	<input type="checkbox"/>	One channel only	<input type="checkbox"/>
Critical shut time (ms)=	1000.0	Critical shut time (ms)=	60.0	Critical shut time (ms)=	20.0
Use CHS vectors	<input type="checkbox"/>	Use CHS vectors	<input type="checkbox"/>	Use CHS vectors	<input type="checkbox"/>
Bad shuttling ends group	<input checked="" type="checkbox"/>	Bad shuttling ends group	<input checked="" type="checkbox"/>	Bad shuttling ends group	<input checked="" type="checkbox"/>
Set intervals bad if longer than:					
0.00e+000	<input type="checkbox"/> [shut times (ms)]	0.00e+000	<input type="checkbox"/> [shut times (ms)]	0.00e+000	<input type="checkbox"/> [shut times (ms)]
3.00e+001	<input type="checkbox"/> [open times (ms)]	2.00e+001	<input type="checkbox"/> [open times (ms)]	5.00e+001	<input type="checkbox"/> [open times (ms)]
Resolution = [microsecond]	30.0	Resolution = [microsecond]	30.0	Resolution = [microsecond]	30.0
Prev. set		Next set		Prev. set	
				Prev. set	

In the right hand column of the main *Initial settings* window, you see maximum values for fitted rate constants. These can be specified to prevent the fit from producing very large values that are not realistic from a physical point of view. Separate values are specified for association rate constants (units $M^{-1} s^{-1}$) and for all other rate constants (units s^{-1}). In this case they are $10^9 M^{-1} s^{-1}$ and $10^6 s^{-1}$. Click *Edit* to set other values: in the example the upper limit for any association rate constant has been changed to $10^{10} M^{-1} s^{-1}$.

Of course, if the fit produces a result that is very close to the specified maximum value, that shows that the fit of the model is imperfect, because a better result would have been obtained with a still larger value/

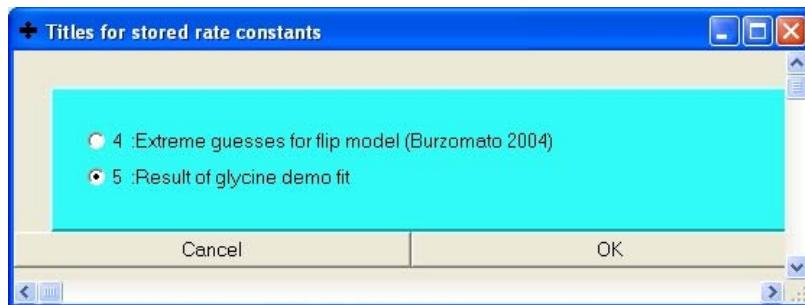
Maximum values for:	Change
association rates=	1.000e+009
all other rates=	1.000e+006

Max value for association rate constant	1.e10
Max value for any other fitted rate constant	1000000.0
Cancel	OK

Initial guesses

At top of the right hand column of the main ‘initial settings’ window, the initial guesses are listed for each of the rate constants.

Click on *Edit* to alter the initial guesses. This brings up a window (‘Titles for stored rate constants’) which lists all the sets of rate constants (for the model in use) that exist in the mechanism (.mec) file that you are using. In the case of the glycine demonstration, there are two such sets. Record 4 in qmechdem.mec contains the set of initial guesses used in the demonstration, and record 5 contains the stored results of doing the demonstration fit. If you choose the latter, then you can skip the fit, by clicking ‘Show data and specified model (no fit)’ (rather than ‘Fit’) in the main initial settings window, and go straight on to the plots. The calculated curves will show the predictions (with missed event correction) for the model, resolution and the rate constants specified as initial guesses.



Initial guesses			
File: Extreme guesses for flip model (Burzomato 2004)			
	Q(i,j)	Rate name	Value
1	q(1,6)	alpha1	5000.00
2	q(6,1)	beta1	500.00
3	q(2,5)	alpha2	2700.00
4	q(5,2)	beta2	2000.00
5	q(3,4)	alpha3	800.00
6	q(4,3)	beta3	15000.00
7	q(4,7)	gamma3	300.00
8	q(7,4)	delta3	120000.01
9	q(4,5)	3k(-3)F	6000.00
10	q(5,4)	k(+3)F	4500000000.00
11	q(5,8)	gamma2	1500.00
12	q(8,5)	delta2	12000.00
13	q(5,6)	2k(-2)F	4000.00
14	q(6,5)	2k(+2)F	900000000.00

When you click either *Fit* or *Show data and specified model (no fit)*, in the initial settings window, before the fit starts you see the *Initial guesses for:* window. The fixed and constrained values are indicated in the window (they can't be changed here) but at this point you can type in any value for the initial guess that you want. At the bottom of this window you see the EC50 and maximum P_{open} values that correspond to the rate constants shown. If you change the value of a rate constant, click on *Recalculate* to update these values. They serve as a guide to whether your initial guesses are reasonable. In the case of the glycine demonstration, the EC50 for the default initial guesses is 11.9 μM (see Fig), whereas the final fit gives an EC50 of 65.7 μM . In this case the initial guesses were poor (that is why they are named ‘extreme guesses’). In particular, the initial guess for the main, fully-liganded opening rate constant, β_3 , was purposely set low (15000 s^{-1}), to test whether the fit would nevertheless converge to the much higher value found the other initial guesses. It does in fact converge to 129160 s^{-1} .

The initial guesses window looks like this.

Initial guesses for: Glycine flip model (mod 5 in Burzomato, 2004)

	i,j	Rate name	Value	Constraint	Fix
1	q(1,6)	alpha1	5000.00000		no
2	q(6,1)	beta1	500.00000		no
3	q(2,5)	alpha2	2700.00000		no
4	q(5,2)	beta2	2000.00000		no
5	q(3,4)	alpha3	800.00000		no
6	q(4,3)	beta3	15000.00000		no
7	q(4,7)	gamma3	300.00000	MR	no
8	q(7,4)	delta3	120000.00781		no
9	q(4,5)	3k(-3)F	6000.00000		no
10	q(5,4)	k(+3)F	4.50000e+008		no
11	q(5,8)	gamma2	1500.00000		no
12	q(8,5)	delta2	12000.00000		no
13	q(5,6)	2k(-2)F	4000.00000		no
14	q(6,5)	2k(+2)F	9.00000e+008	constraint	no
15	q(6,9)	gamma1	7500.00000	MR	no
16	q(9,6)	delta1	1200.00000		no
17	q(7,8)	3k(-3)	3000.00000	constraint	no
18	q(8,7)	k(+3)	4.50000e+006		no
19	q(8,9)	2k(-2)	2000.00000	constraint	no
20	q(9,8)	2k(+2)	9.00000e+006	constraint	no
21	q(9,10)	k(-1)	1000.00000		no
22	q(10,9)	3k(+1)	1.35000e+007	constraint	no

Press this button after changing guesses to recalculate EC50

At zero concentration of glycine P(open) = -0.00000

Equilibrium response-concentration curve is monotonic, Maximum Popen = 0.94925

EC50 = Conc of glycine for 50% of this maximum (muM) = 11.86877

When you are happy with the values, click *continue* to start the fit.

The fitting process

Before the fitting starts, you get a chance to store any changed settings in a mechanism (.mec) file, so they can easily be recovered to use again.

Then you are asked about *risetime settings*. This specifies the rise time of your recording system in response to a square input. It is used in HJCFIT only so that the false event rate that corresponds with your chosen

Risetime setting:

<input checked="" type="checkbox"/> Nominal filter setting
<input type="checkbox"/> Effect of prefilter+tape
Fr rec [kHz] Famp [kHz]
1 0.00000 0.00000
2 0.00000 0.00000
3 0.00000 0.00000
4 0.00000 0.00000

Specify value here

Trise [us]
1 110.70000
2 110.70000
3 110.70000
4 110.70000

resolution can be printed. The value is not used in any of the fitting calculations. Usually the rise time is dominated by the final filter in your recording system, usually an 8-pole Bessel filter. In this case it is sufficient to specify the corner frequency for this filter (check top box). In the glycine demonstration the records at all for concentrations were filtered at 3 kHz, so the rise time is (e.g. Colquhoun & Sigworth, 1995)

$$t_{\text{rise}} = 10^6 \frac{0.3321}{3000} = 110.7 \mu\text{s}$$

for all four sets (as shown in the lower part of the display)

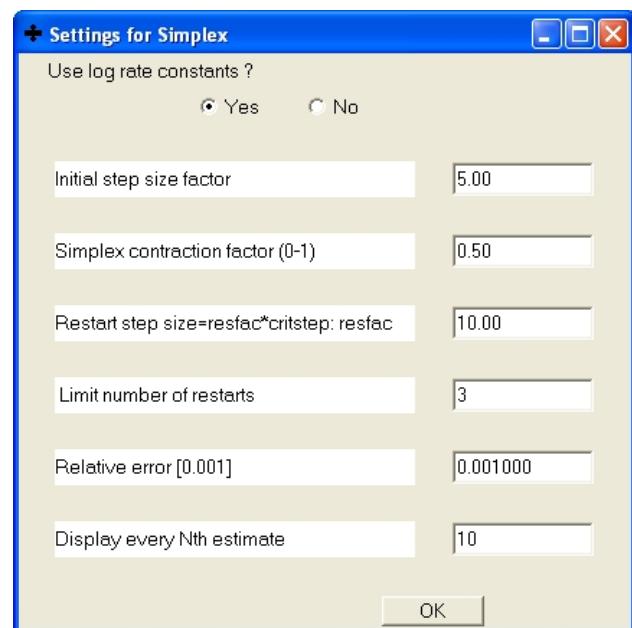
The simplex algorithm that is used to maximise the likelihood need certain settings to be defined,

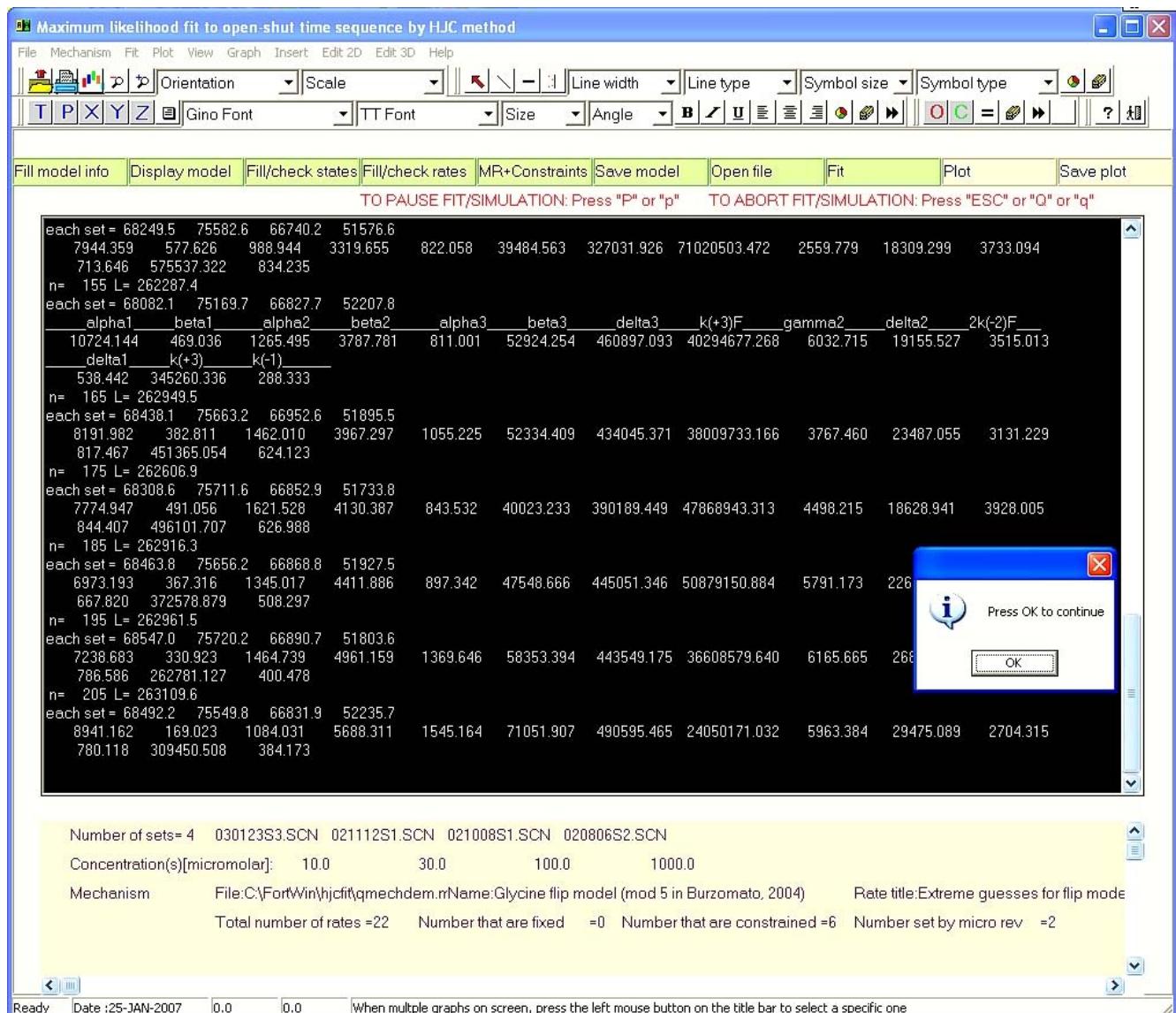
The default is to use the logs of the rate constants for the fitting process. This is usually faster than using the rate constants themselves and it assures that a rate constant estimate can't be negative.

The settings are discussed in more detail in a separate section, but usually the default values are quite satisfactory so you can just click OK.

Then the fitting starts. The progress is shown in the black 'teletype box', for every 10th iteration (or whatever you specify in the simplex settings window).

You can press 'p' or 'P' to pause the fitting to allow inspection of the progress. After 205 iterations the screen looks like this. The names of the free parameters are printed periodically as an aid to interpreting the numbers. You can see that after 155 iterations the total log(likelihood) has reached 266287.4 and is steadily increasing. The log(likelihood) is also shown separately for each of the four data sets, so you can see how much each set contributes to the total. The estimate of β_3 , for which the initial guess was 15000 s^{-1} has already risen to 52924 s^{-1} by iteration number 155, but looking down the page shows that the estimate is still far from steady





At the end of the fit (which takes about an hour) we see

```

n= 6015 L= 264473.3
each set= 68958.0 76043.7 67026.8 52444.8
 3685.214 5969.552 2468.693 32764.405 6871.543 129159.553 21047.207 166308258.458 20812.217 6306.015 2306.002
 145.517 611773.044 272.989
n= 6025 L= 264473.3
each set= 68958.0 76043.7 67026.8 52444.8
 3685.214 5969.552 2468.693 32764.405 6871.543 129159.553 21047.207 166308258.458 20812.217 6306.015 2306.002
Return with best vertex
and Simplex finished at 11:53:43
number of evaluations = 6028
Max log(likelihood) 264473.3

```

The fit has taken 6028 iterations and achieved a total log(likelihood) of 264473.3. The final estimate for β_3 is 129159.5 s^{-1} .

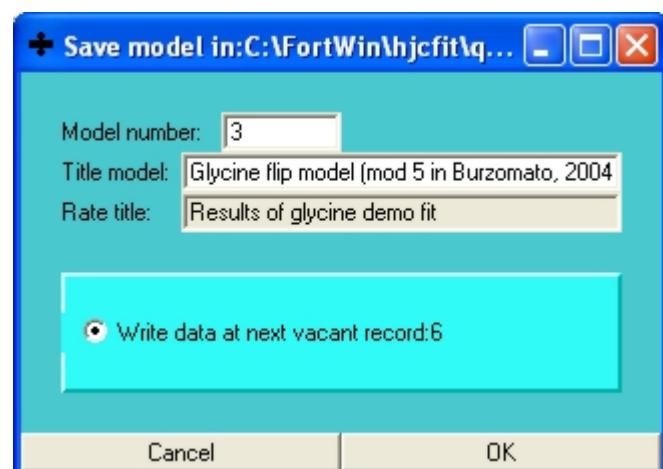
After a chance to print the entire final Q matrix you see the results of the fit as follows.

Results of fit:

	Q(i,j)	Name	Initial guess	Constrain/Fix	Final value
1	q(1,6)	alpha1	5000.00000		3685.21436
2	q(6,1)	beta1	500.00000		5969.55176
3	q(2,5)	alpha2	2700.00000		2468.69336
4	q(5,2)	beta2	2000.00000		32764.40430
5	q(3,4)	alpha3	800.00000		6871.54346
6	q(4,3)	beta3	15000.00000		129159.55469
7	q(4,7)	gamma3	300.00000	MR	1079.24365
8	q(7,4)	delta3	120000.00781		21047.20703
9	q(4,5)	3k(-3)F	6000.00000		3459.00342
10	q(5,4)	k(+3)F	4.50000e+008		1.66308e+008
11	q(5,8)	gamma2	1500.00000		20812.21680
12	q(8,5)	delta2	12000.00000		6306.01465
13	q(5,6)	2k(-2)F	4000.00000		2306.00220
14	q(6,5)	2k(+2)F	9.00000e+008	constraint	3.32617e+008
15	q(6,9)	gamma1	7500.00000	MR	30911.18359
16	q(9,6)	delta1	1200.00000		145.51706
17	q(7,8)	3k(-3)	3000.00000	constraint	818.96600
18	q(8,7)	k(+3)	4.50000e+006		611773.06250
19	q(8,9)	2k(-2)	2000.00000	constraint	545.97729
20	q(9,8)	2k(+2)	9.00000e+006	constraint	1.22355e+006
21	q(9,10)	k(-1)	1000.00000		272.98865
22	q(10,9)	3k(+1)	1.35000e+007	constraint	1.83532e+006

Redo fit Store fitted rates Estimate errors Plot now

At this stage you can store the values of the fitted rate constants as a record in a mechanisms (.mec) file if you want to keep them. If you do so, it means that you will later be able to recall these values as 'initial guesses' and then skip the fit to do more plots. Choose a .mec file, and type in a suitable name for the results. In this case they were called 'Results of glycine demo fit'.



Errors for fitted rate constants

Clicking *Estimate errors* allows internal estimates to be made of the standard deviations for each of the fitted parameter values, and of the correlations between all pairs of parameter estimates (the latter appears only on the printout file at the moment). This is done by numerical evaluation of the Hessian matrix at the maximum likelihood (see below ‘Notes on estimation of errors and correlations’, for explanation). In order to estimate the second derivatives accurately, the program first has to find a suitable increment in each parameter. The default is to find an increment in each parameter that decreases the log(likelihood) by 0.1 unit (if in doubt, try other values). After the increments are calculated the elements of each row of the information matrix (since it is a symmetrical matrix, only the lower triangle need be found do each row takes longer than the last). The results are then displayed, with standard deviations and, more conveniently, coefficients of variation (as percentage) for each free parameter.

Results of fit with errors:								
Q(i,j)	Name	Initial guess	Final value	CV(%)	SD	Constraint/Fix	Factor	
1	q(1,6)	alpha1	5000.00000	3685.21436	5.18325	191.01405		
2	q(6,1)	beta1	500.00000	5969.55176	13.56302	809.65137		
3	q(2,5)	alpha2	2700.00000	2468.69336	5.20013	128.37524		
4	q(5,2)	beta2	2000.00000	32764.40430	9.22054	3021.05469		
5	q(3,4)	alpha3	800.00000	6871.54346	13.24349	910.03229		
6	q(4,3)	beta3	15000.00000	129159.55469	3.18138	4109.05518		
7	q(4,7)	gamma3	300.00000	1079.24385			MR	
8	q(7,4)	delta3	120000.00781	21047.20703	6.47811	1363.46106		
9	q(4,5)	3k(-3)F	6000.00000	3459.00342			3.000	
10	q(5,4)	k(+3)F	4.50000e+008	1.66308e+008	6.14211	1.02148e+007		
11	q(5,8)	gamma2	1500.00000	20812.21680	4.11999	857.46063		
12	q(8,5)	delta2	12000.00000	6306.01465	3.09369	195.08881		
13	q(5,6)	2k(-2)F	4000.00000	2306.00220	10.10724	233.07307		
14	q(6,5)	2k(+2)F	9.00000e+008	3.32617e+008			constraint	3.000
15	q(6,9)	gamma1	7500.00000	30911.18359			MR	
16	q(9,6)	delta1	1200.00000	145.51706	12.82702	18.66551		
17	q(7,8)	3k(-3)	3000.00000	818.96600			constraint	3.000
18	q(8,7)	k(+3)	4.50000e+006	611773.06250	7.54547	46161.16406		
19	q(8,9)	2k(-2)	2000.00000	545.97729			constraint	3.000
20	q(9,8)	2k(+2)	9.00000e+006	1.22355e+006			constraint	3.000
21	q(9,10)	k(-1)	1000.00000	272.98865	7.71017	21.04788		
22	q(10,9)	3k(+1)	1.35000e+007	1.83532e+006			constraint	3.000

The complete correlation matrix appears too, on the printout file.

There is another chance at this stage to store the fitted rate constants. Then go on to *Do plots*

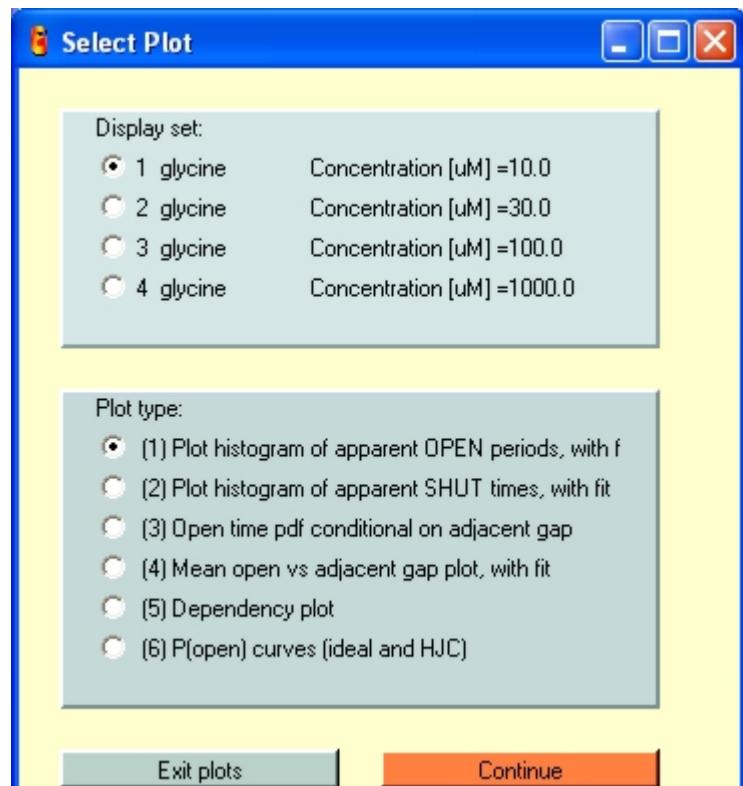
Plotting the results

Now the fit is completed, the next stage is to see how well it describes the observations. This can be done by six different sorts of plot of the data. In each case the observed data are plotted, and the prediction of the fit is superimposed on the observations. To the extent that they agree, the fit has been a success. Each sort of plot can be done for every data set, so in the case of the glycine demonstration (four data sets) 24 plots can be generated.

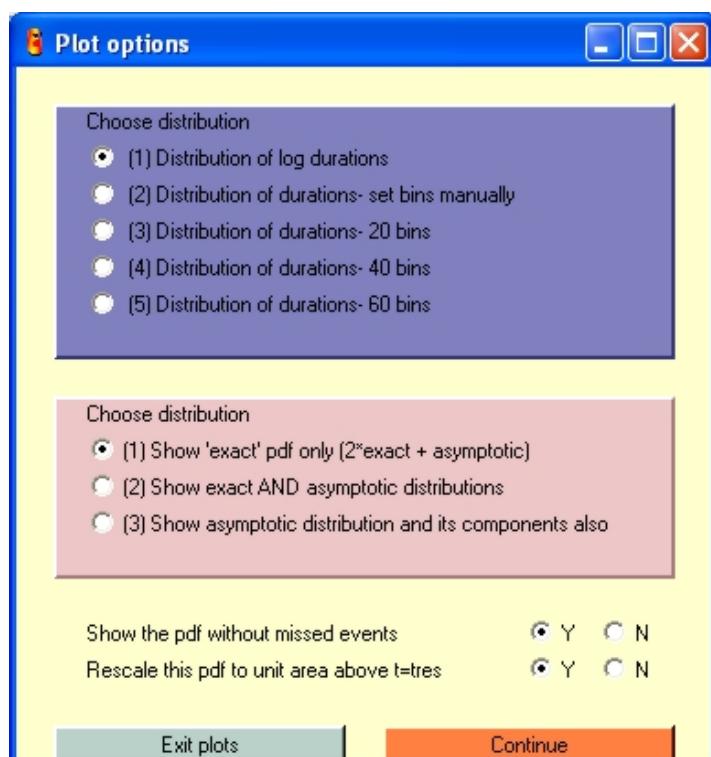
The selection of data set and plot type is done through the Select Plot window.

When this first appears, the default is to use set 1 and to do plot type 1, the distribution of apparent open times. When this plot is done, the defaults increment automatically, first through sets, and then through plot types,

Click continue on the screen shown here to plot the apparent open time distribution for data set 1.

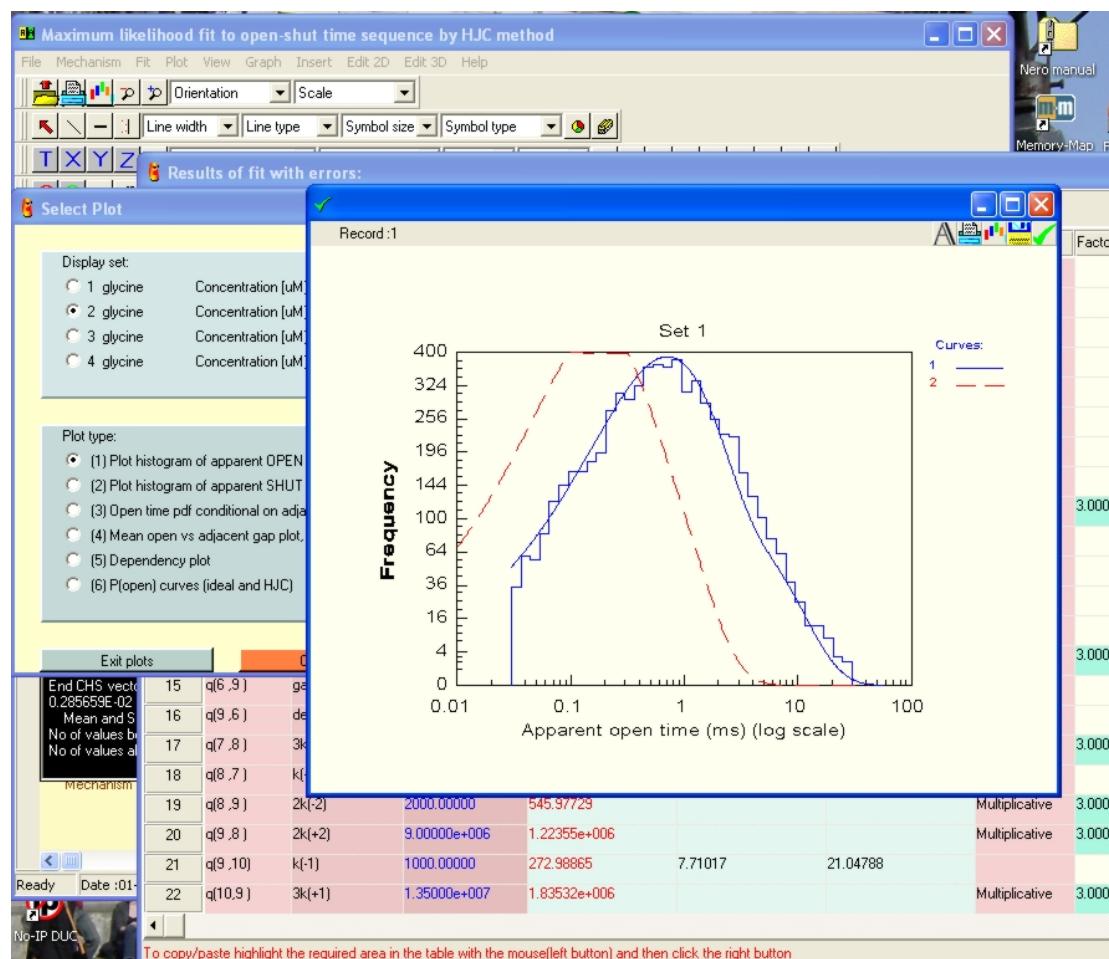
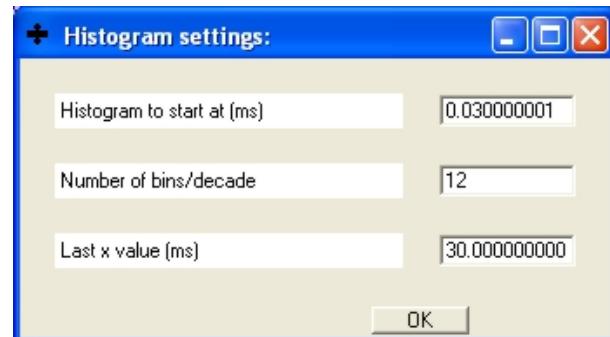


There are several options for plotting distributions of dwell times. These are set in the *Plot options* window. Mostly (but not always) the distribution of log(dwell time) is used (top panel). The density function for apparent dwell times (HJC distribution) that is predicted by the fit is superimposed on the histogram, as specified in the second panel down (the other two options are there only to test the adequacy of the asymptotic HJC distribution and will not normally be needed). There are two options at the bottom. The 'ideal' distribution that is predicted by the fit in the absence of missed events is plotted



(as a dashed red line) when the Y box is checked as shown. This is distribution (in this case, of open times) calculated from the fitted rate constants, as described, for example by Colquhoun & Hawkes (1982) with no allowance for missed events. The bottom option is always 'yes'.

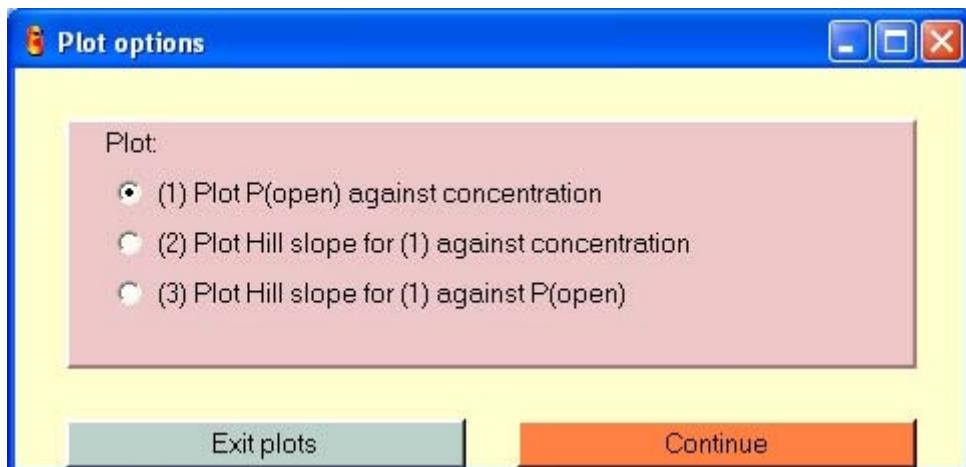
Next the settings for construction of the histogram of experimental observations are specified. Usually the default settings are adequate. For a distribution of log(dwell times) they are as shown on the right. They can be changed in the *Histo settings* window.



As soon as OK is hit, the graph appears, along with the Select plot window, ready for the next plot (in this case the default for the next plot will be the distribution of apparent open times for set 2). The whole screen looks like this (after clicking in the plot to bring it to the front)

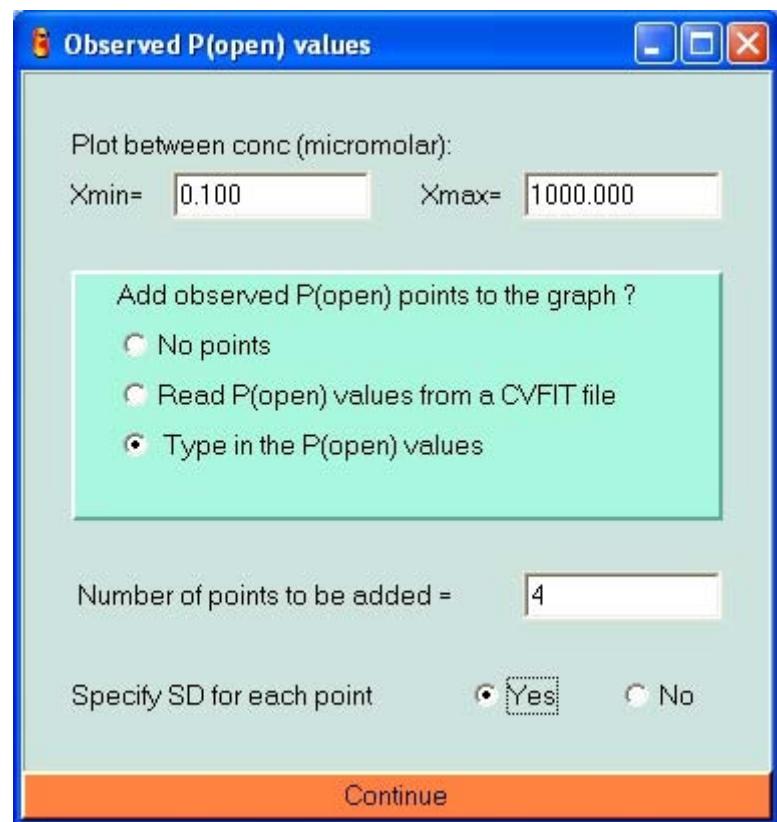
The P_{open} plots

The sixth way of evaluating the fit is to plot the predicted P_{open} graph. Obviously there is only one such graph, however many data sets were fitted. Two curves are plotted, the ideal curve calculated from the fitted rate constants and the model (red) and the HJC curve (blue). The latter uses the apparent (HJC) mean open and shut times, and so makes allowance for the fact that brief open and shut times will be missed when P_{open} is measured from single channel recordings. When option 6 is chosen in the [select plot window](#) you see this.



The second and third options are useful if you want to see how the Hill slope varies with concentration, but the first option is the one that can be compared with experimental data.

Only the predicted curves come from the fitting process. If you have experimental values for P_{open} which you want to compare with the curves, specify them now. Say how many points you want to add, and whether or not you have a standard deviation for the numbers. If you have, they will be plotted as error bars.



When 4 points are specified, a window appears for their entry.

You can type in values, or you can paste values from Excel, or from Word (if they are delimited with tabs). For the latte method, right click on the top left cell and chose paste, as shown..

	Concentration	P(open)	SD
1	0.000	0.000	0.000
2	0.000	0.000	0.000
3	0.000	0.000	0.000
4	0.000	0.000	0.000

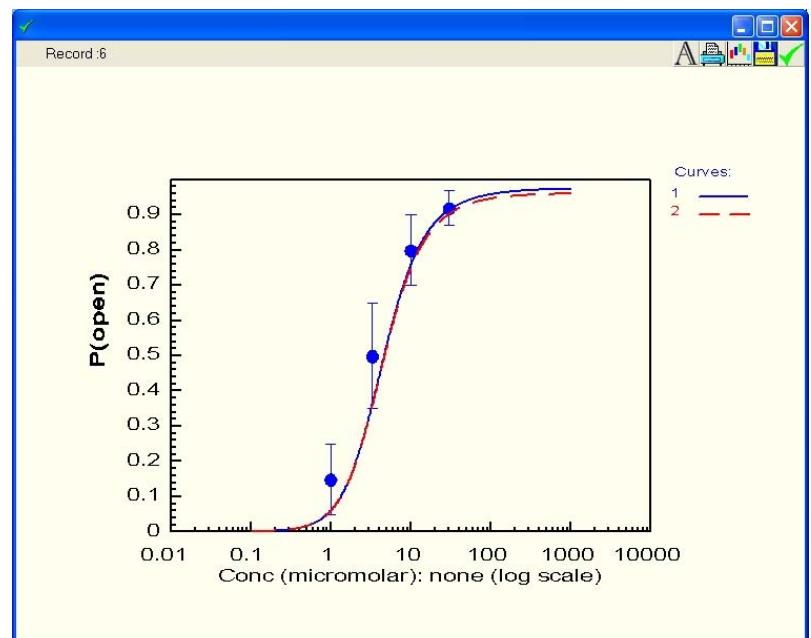
Help cut and paste | Save to ini | Continue

Once the numbers are entered, click continue

	Concentration	P(open)	SD
1	1.000	0.150	0.100
2	3.300	0.500	0.150
3	10.000	0.800	0.100
4	0.000	0.000	0.000

Help cut and paste | Save to ini | Continue

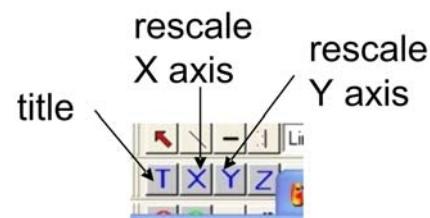
The graph then appears, with the entered points and their error bars superimposed on them.



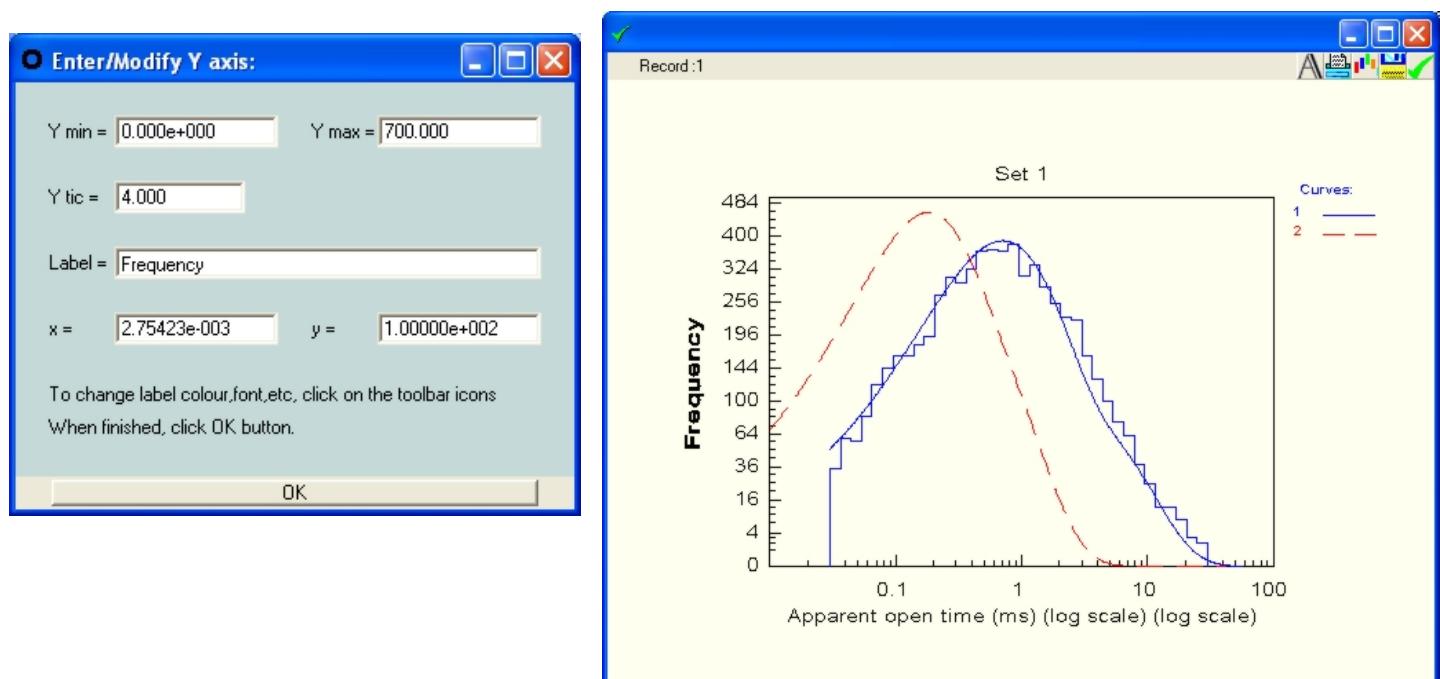
Editing and saving the plots

Rescaling the graph

In the open time distribution shown above, the data and the HJC distribution (solid blue line) predicted by the fit are on scale. But the predicted open time distribution in the absence of missed events (dashed red line) goes off scale. To see it properly, rescale the Y axis. Click on the 'Y' icon (top left). This brings up the Y axis window.



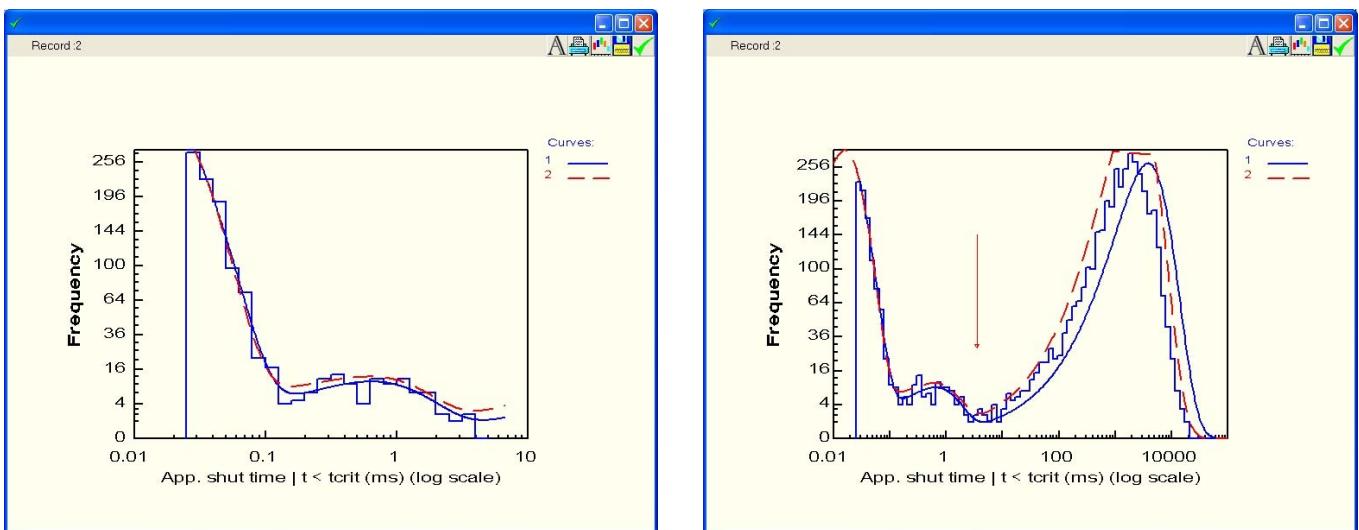
The maximum value for the Y axis has been changed from its value of 400 to 700. The label on the Y axis, and the tick length can also be changed here. (The x and y values at the bottom define the position of the Y axis label - eventually this will be made dragable). Click OK and the rescaled graph appears,



Rescaling shut time distributions

The shut time distribution is different from others because the default display shows only the shut times that were used for the fitting process, those up to t_{crit} . Sometimes, though, it will be interesting to see how well the fit predicts the distribution of longer shut times. It cannot be expected that the fit will necessarily be good, particularly if the patch contained more than one channel. The reason for defining t_{crit} was to define groups of openings all of which originate from the same individual channel. Longer shut times are

excluded from the fit because they may separate openings from different channels. Here is an example from the nicotinic receptor demonstration. The value of t_{crit} in this case was 3.5 ms (so the likelihood is calculated separately for every short burst of openings –every channel activation). The shut time distribution is shown only up to 3.5 ms, though the X axis extends to 10 ms (log scales, by default, are scaled to have an integer number of decades. If we click on the 'X' icon (top left) and insert a new value for X_{max} of 10000 ms, the graph appears as on the right.



The whole of the data are now shown, and a vertical red arrow appears to mark the value of t_{crit} (3.5 ms), as a warning that shut times longer than this were not used in the fitting process.

Notice that the prediction of the slowest component in the right hand graph is not very good, despite the fact that there is known to be only one channel in the record (that is known only because the data are not real, but are simulated). The poor prediction of the slow component is not, in this case, result of experimental error, but because the fit was done with one fixed rate constant, $q_{6,4} = k_{+1a}$ and this was fixed at a value of $1 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$. This is the wrong value because the simulation that generated the data used $k_{+1a} = 2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$. Unlike in real life, we know the true values for the rate constants. The effect of fixing a rate constant at the wrong value is investigated in Colquhoun Hatton & Hawkes (2003). A better result can be obtained by use of an EC50 constraint if you have an accurate value for the EC50, as described below ([Using an EC50 constraint](#)),

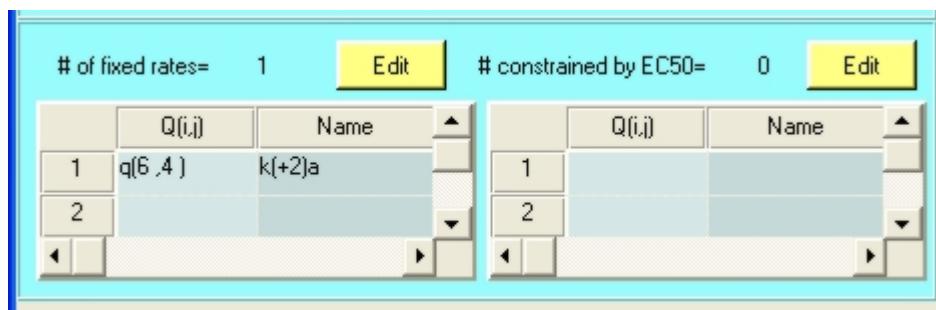
Using an EC50 constraint

The need to fix a parameter may result from the fact that when results are obtained at a single low concentration, as in this case, and it isn't known how many channels are present in the patch, then the results contain no reliable information about the frequency of channel activations, or about P_{open} , so it does not contain enough information to estimate all the rate constants. If you

have a reliable estimate of the EC50 from some other source then this can be used to provide the missing information, rather than resorting to fixing a rate constant to a value that is no more than a guess. For example an EC50 estimate could be found from a macroscopic concentration-response curve (if it is possible to get one that is not distorted excessively by desensitisation).

As an example, consider the nicotinic demonstration. The data are from a single run with a low agonist concentration. This means that isn't enough information to identify the values of all the rate constants, unless we know that there is only one channel in the patch. In this particular example we do know that there is only one channel because the data were simulated so this knowledge could have been used by checking the "one channel only" box (see [values that must be set for each data set](#)). If this were done, all the rate constants in the mechanism could be estimated accurately, with no need to fix any of them. However in real life, it is hardly ever known that there is only one channel present when the P_{open} is low, so for the demonstration fit, t_{crit} was set at 3.5 ms, because this is the longest length of record for which we can be sure that all openings come from the same channel. It encompasses only a single burst of openings (a single channel activation). This is what one would have to do in real life, but it means that there is not enough information to estimate all of the rate constants. There is no information about how often channel activations occur. For that reason, one of the rate constants had to be fixed. In the demonstration, $q_{6,4} = k_{+1a}$ and this was fixed at a value of $1 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, rather than the correct value, $2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$. However, if we knew that the EC50 was 3.3 μM , this information could be used to replace the missing information. This is done as follows.

Initially the bottom left part of the [initial settings window](#) looks like this



We wish now, rather than fixing the value of k_{+2a} , to have its value calculated from the other parameters so as to produce the specified EC50. Before this can be set, k_{+2a} must first be unfixed, so click *edit*. This produces a list of the rate constants, as shown. In the last column, headed *Fix* choose *no* from the drop-down menu for k_{+2a} , t

Then click *continue* to return to the initial settings window

Fix (or unfix) rate constants,

i,j	Rate name	Value	Constraint	Fix
1	q(1,4)	alpha2	1500.00000	no
2	q(4,1)	beta2	50000.00000	no
3	q(2,5)	alpha1a	2000.00000	no
4	q(5,2)	beta1a	20.00000	no
5	q(3,6)	alpha1b	80000.00000	no
6	q(6,3)	beta1b	300.00000	no
7	q(4,6)	k(-2)a	1000.00000	Multiplicative
8	q(6,4)	k(+2)a	1.00000e+008	fixed
9	q(4,5)	k(-2)b	20000.00000	Multiplicative
10	q(5,4)	k(+2)b	1.00000e+008	Multiplicative
11	q(5,7)	k(-1)a	1000.00000	no
12	q(7,5)	k(+1)a	1.00000e+008	MR
13	q(6,7)	k(-1)b	20000.00000	no
14	q(7,6)	k(+1)b	1.00000e+008	no
15				
16				

Cancel Continue

After unfixing k_{+2a} the bottom left portion of the initial settings window looks like this.

# of fixed rates=	0	Edit	# constrained by EC50=	0	Edit
Q(i,j)	Name		Q(i,j)	Name	
1			1		
2			2		

Now click on the right hand *Edit* button to set the EC50 constraint. Enter the known EC50 (3.3 mM), choose which rate constant to

Specify EC50

Ligand: ACh

EC50 (micromolar) =

Rate constant to be constrained by EC50 =

Lower limit for specific rate constant

Upper limit for specific rate constant

Cancel OK

constrain from the drop down list. $q_{6,4} = k_{+1a}$ in this case (choose *none* to remove an EC50 constraint), and specify limits for the value of $q_{6,4} = k_{+1a}$, to prevent crazy values being produced. In this case $q_{6,4} = k_{+1a}$ is an association rate constant so we set a lower limit of $10^6 \text{ M}^{-1} \text{ s}^{-1}$, and an upper limit of $10^{10} \text{ M}^{-1} \text{ s}^{-1}$.

The click OK to go back to the initial settings window, the bottom left part of which will now look like this.

# of fixed rates=	0	Edit	# constrained by EC50=	1	Edit
Q(i,j)	Name		Q(i,j)	Name	
1			1	$q(6,4)$	$k(+2)a$
2			2		

Now start the fit (the initial guess for $q_{6,4} = k_{+1a}$ no longer matters because it is calculated from the other rate constants. There are still 9 free parameters but the fit takes a bit longer (2 minutes 7 seconds, rather than 40 seconds, because of the need to find, at each iteration, the value of $q_{6,4} = k_{+1a}$ that produces an EC50 of 3.3 μM). The results look like this.

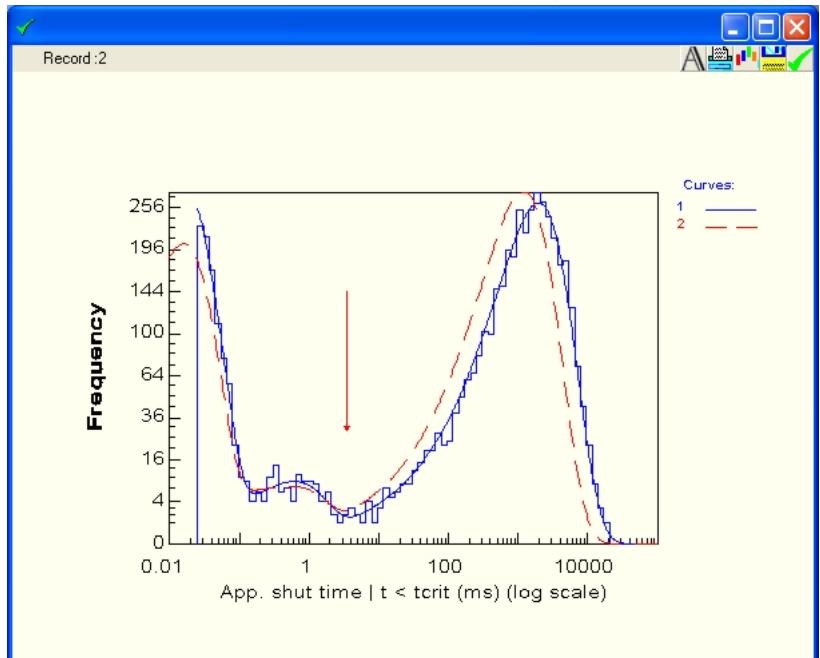
Results of fit:				
Q(i,j)	Name	Initial guess	Constrain/Fix	Final value
1	$q(1,4)$	alpha2	1500.00000	2127.53271
2	$q(4,1)$	beta2	50000.00000	52260.88281
3	$q(2,5)$	alpha1a	2000.00000	5939.67480
4	$q(5,2)$	beta1a	20.00000	57.07429
5	$q(3,6)$	alpha1b	80000.00000	56259.52344
6	$q(6,3)$	beta1b	300.00000	174.71588
7	$q(4,6)$	$k(-2)a$	1000.00000	Multiplicative 1585.66541
8	$q(6,4)$	$k(+2)a$	1.00000e+008	EC50 2.01728e+008
9	$q(4,5)$	$k(-2)b$	20000.00000	Multiplicative 9412.87695
10	$q(5,4)$	$k(+2)b$	1.00000e+008	Multiplicative 4.14432e+008
11	$q(5,7)$	$k(-1)a$	1000.00000	1585.66541
12	$q(7,5)$	$k(+1)a$	1.00000e+008	MR 2.01728e+008
13	$q(6,7)$	$k(-1)b$	20000.00000	9412.87695
14	$q(7,6)$	$k(+1)b$	1.00000e+008	4.14432e+008
15				
16				

To copy/paste highlight the required area in the table with the mouse(left button) and then click the right button

Redo fit Store fitted rates Estimate errors Plot now

Notice that the estimate of $q_{6,4} = k_{+1a}$ is $2.017 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, very close to the correct value of $2 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$, despite the fact that no shut time longer than 3.5 ms has been used in the fitting process.

Consequently, when the shut time distribution is rescaled to show the whole range of shut times, the predicted HJC distribution (solid blue line) is now a good fit over the entire range.



Adding text etc

Saving the graph

==Up to here –more to come in this section

Naming of rate constants

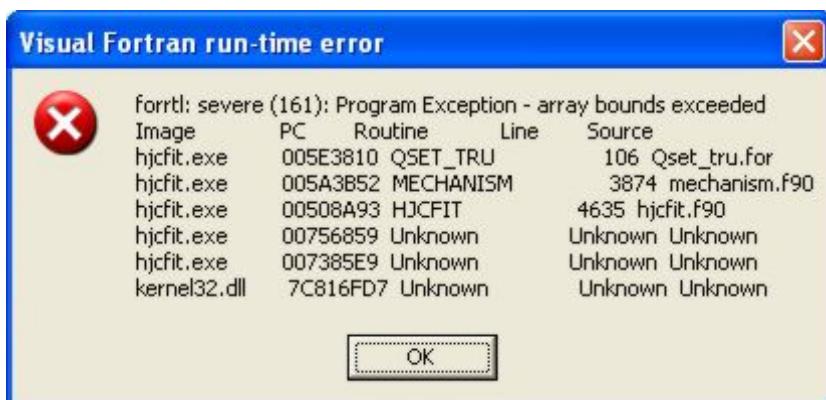
The names given to rate constants are defined when a mechanism is defined, and can be changed from the *Initial settings* window. For example, in the nicotinic mechanism shown in the *Initial settings* window on p. 5, the name given to the transition rate from state 1 to state 4, is 'alpha2'. This name is just an ASCII string that is used to make the results clearer. It plays no part in the computations. Nevertheless it is recommended strongly that 'statistical factors' should be included as part of the name. For example, in the *Initial settings* window for the glycine 'flip' mechanism (p. 9), consider the transition rate from state 4 to state 5. This represents the dissociation of one agonist molecule from the triliganded flip conformation (A_3F) to produce the diliganded flip conformation (A_2F). The microscopic transition rate for this dissociation is denoted k_{-3F} (represented as $k(-3)F$ in the rate name which does not allow subscripts). Because any one of the three binding sites can dissociate (and the sites are supposed to be identical) the actual transition rate, which appears in the \mathbf{Q} matrix is not k_{-3F} but is $3k_{-3F}$. (The factor of 3 is described as a 'statistical factor'). The initial guess is for $3k_{-3F}$ and the final estimate produced by the fit is for $3k_{-3F}$. Giving this transition rate the name ' $3k(-3)F$ ' provides a valuable reminder of the fact that you need to divide the final estimate of this rate by 3 before entering into a table of results as your estimate of k_{-3F} .

Error reporting

It isn't much help to say you 'got a crash'. I need the error message!

Unfortunately the message that appears when you crash does not seem to allow cut and paste of the text, so do this.

- (a) Click on the box with error message
- (b) press alt-print screen (this copies the window that is in focus to the clipboard as a graphic)
- (c) paste (ctrl-V) the picture into email, or save it as a jpg and attach it to an email. It might look like this



What to do if the ini file specifies the wrong mechanism file

The .mec file may contain many records. Each record in the file contains both a mechanism ('model') and a set of values for the rate constants in that model. Thus the same model may occur in several different records, with different sets of values for the rate constants.

The ini file specifies the mec file name and the record number that contains the model and rate constant values used in a previous run. It also contains the names and locations of the data (.scn) files.

In the demonstrations these are all set up correctly, but in real life there are two common problems.

- (1) The record number specified in the .mec file is wrong. This often happens because you stored in a new .mec file a set of rates (eg initial guesses, or results of a fit), and this will go into record 1 of a new .mec file.
- (2) You take the ini file and mec file from another computer. The ini files specifies that the data files are in a directory that doesn't exist on your computer.

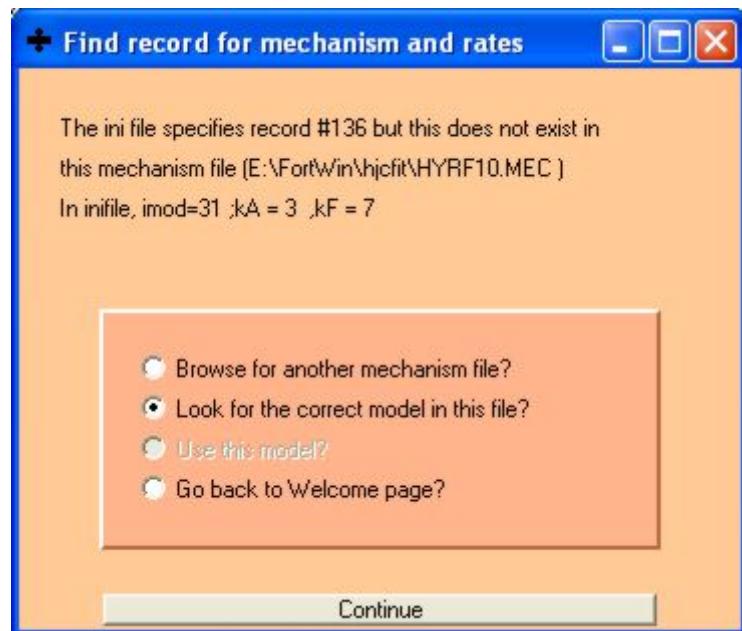
These problems are overcome as follows

If a problem with the record number is detected using the .mec specified in the ini file, you should get a window labelled "change path" which invites you to browse for a .mec file.



Browse to choose the .mec (it may or may not have the same name as the default, which is that specified in the ini file).

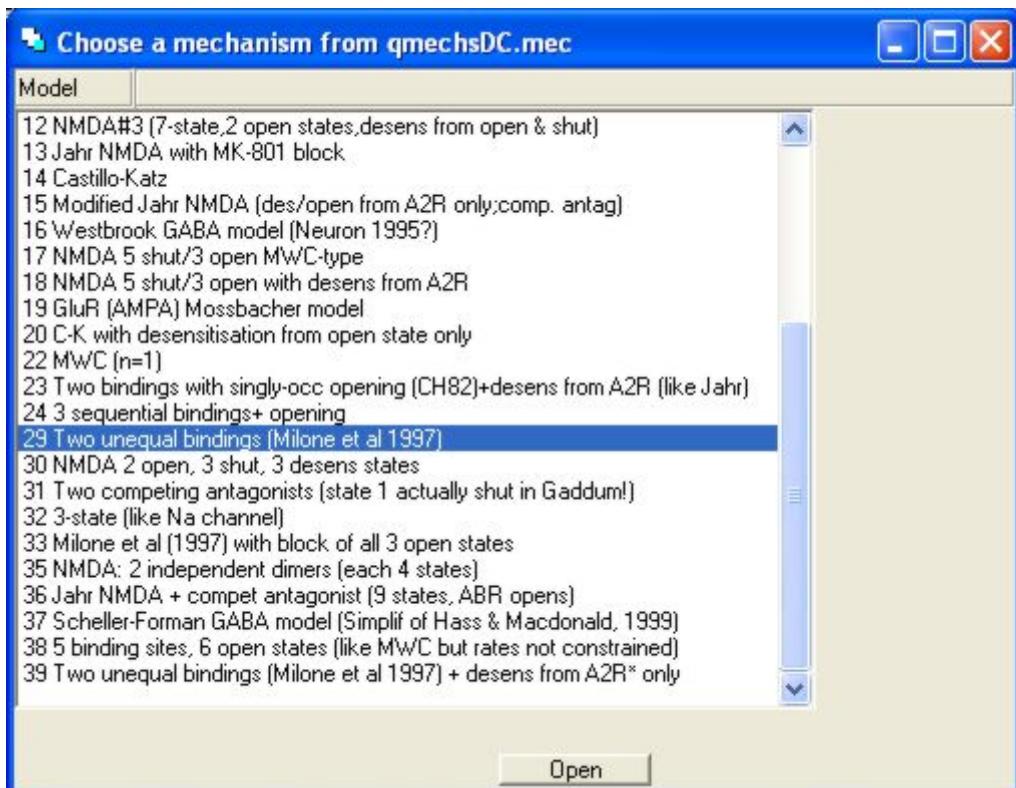
If the record number specified in the ini file is not present in the .mec file you specify you should get a window that looks like this. In this version the third option is greyed out because record 136 does not exist in the specified mec file (if it did option 3 would allow you to use whatever mechanism was in record 136).



In this case it is the 2nd option you want ('look for the correct model in this file'). In your case there is only one so you get.
If the mec file contains only one record you then get this window that shows the name of the mechanism.



More generally the mec file will contain definitions of several mechanisms, so you see something like this

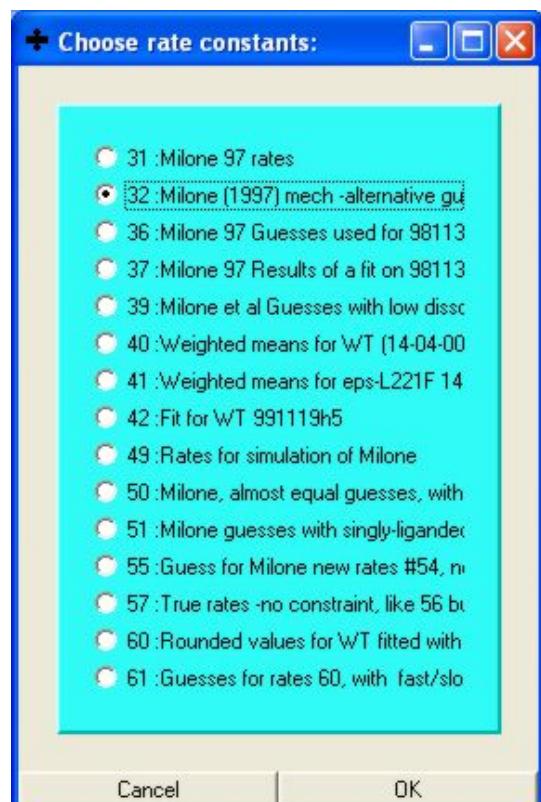


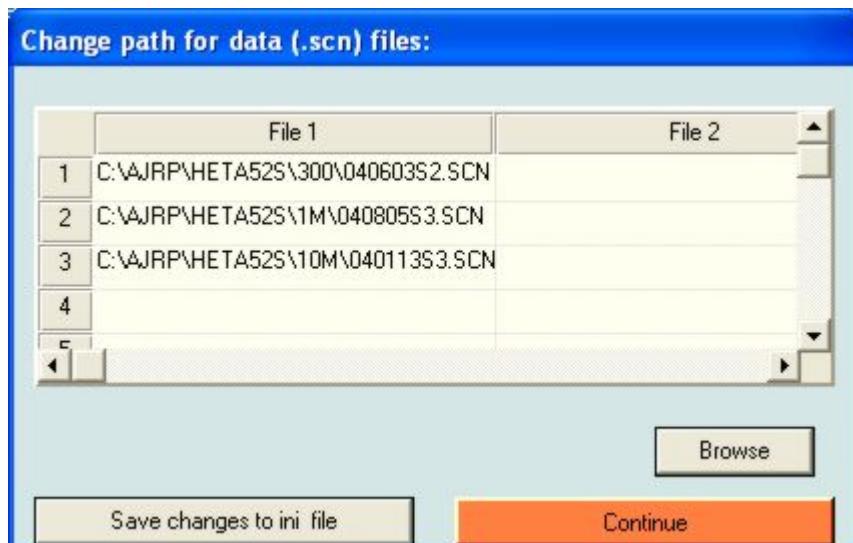
Select the mechanism that you want and click Open.

If the mec file contains more than one set of values for the rate constants for the chosen mechanism, then you now have to choose which set of rate constants to be used in the next window. These values are used as the initial guesses for the fit. The values can be changed before you start the fit

Select the record you want (#32 above) and then 'OK'.

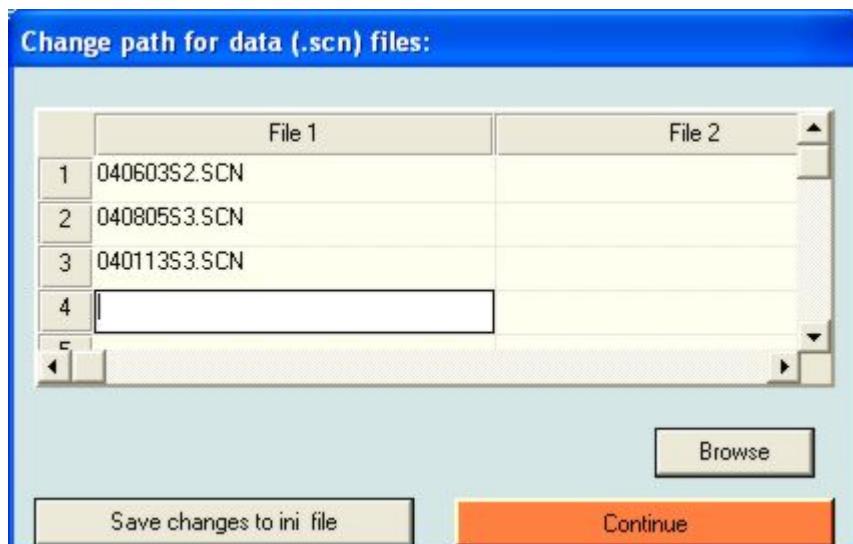
You may then come to another common problem when you move an ini from one computer to another. The data (.scn) files are not in the directory specified in the .ini. When this happens, you get something like this





The paths for the data files are different on your computer from those on which the ini file was made (or you have moved the data files).

In this example the names of the .scn files are write but the paths are wrong. Browse for the files or just enter the right path in the window. For example if the .scn files are in the same directory as HJCFIT then you can just edit the window to delete the paths, thus

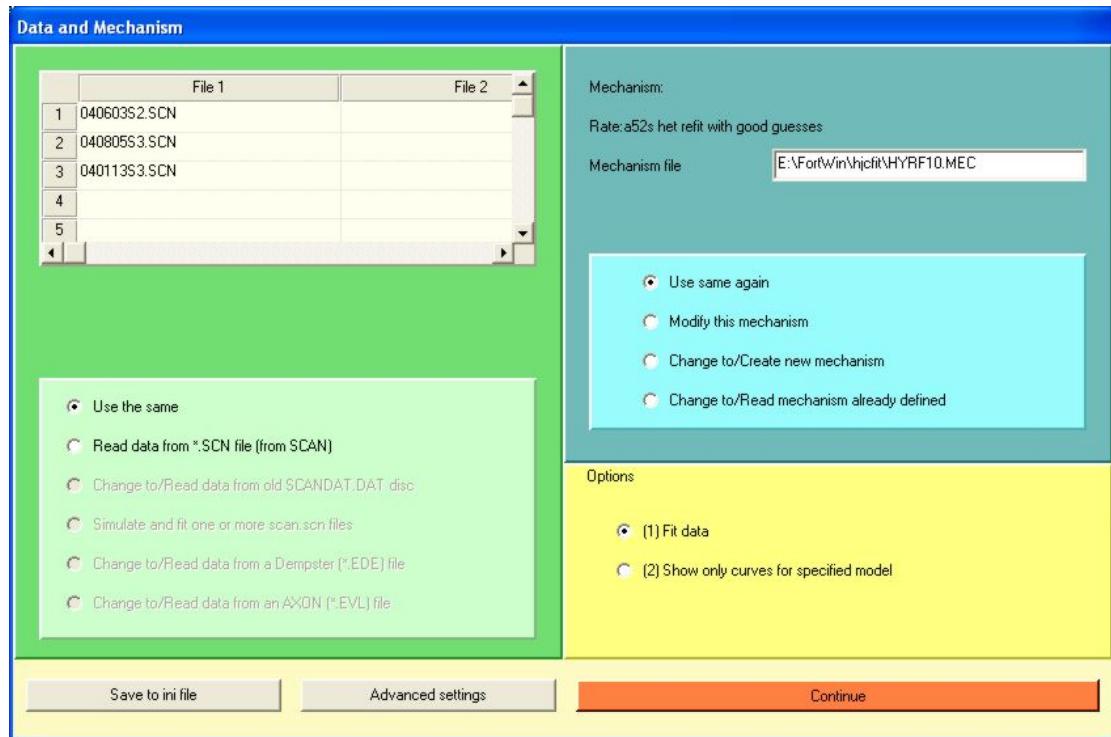


Once the paths are set correctly click *continue*

Caution. Remember that the files must be in the right order. In future the ligand concentration should be included in the header info of the scn file, but at present it isn't (only in the title as text) so it isn't possible to check whether the concentration of each scn file is right (you can change it manually in the main window if necessary)

(f) This should take you back to the "Data and mechanism" window which normally follows the welcome window. Remember to save the new settings in

an ini file (with same or different name). This can be done on the window above, on the main window (check the box above 'fit' button) or at the end



If all is OK then click *continue* to carry on to the main [Initial settings window](#), where everything can be checked before going on to fit.

Starting without an ini file

If you start with no initialisation file by choosing '*Do not use initialisation file*' on the [welcome window](#), the first thing to do is to specify which data files to use. This is done as [described above](#). After the data files have been specified you then choose a reaction mechanism to be fitted, as [described here](#). Once that has been done you get to the [initial settings window](#), and proceed as before.

Model designer

Design a mechanism by modifying an existing one

Notes on microscopic reversibility

Notes on CHS vectors

The problem of an unknown number of channels

Notes on choice of t_{crit}

The

Notes on CHS vectors

Notes on settings for simplex algorithm

Notes on estimation of errors and correlations

Simulate repeated experiments and look at the distributions of estimates of rate constants so obtained