

Step 1 : Input the file 'AtoB_6_1e-06s.txt'

Step 2 : enter A,B in the field which indicate the name of the species participating

Step 3 : enter the Value 1 microseconds

Step 4 : click the button Event driven directory

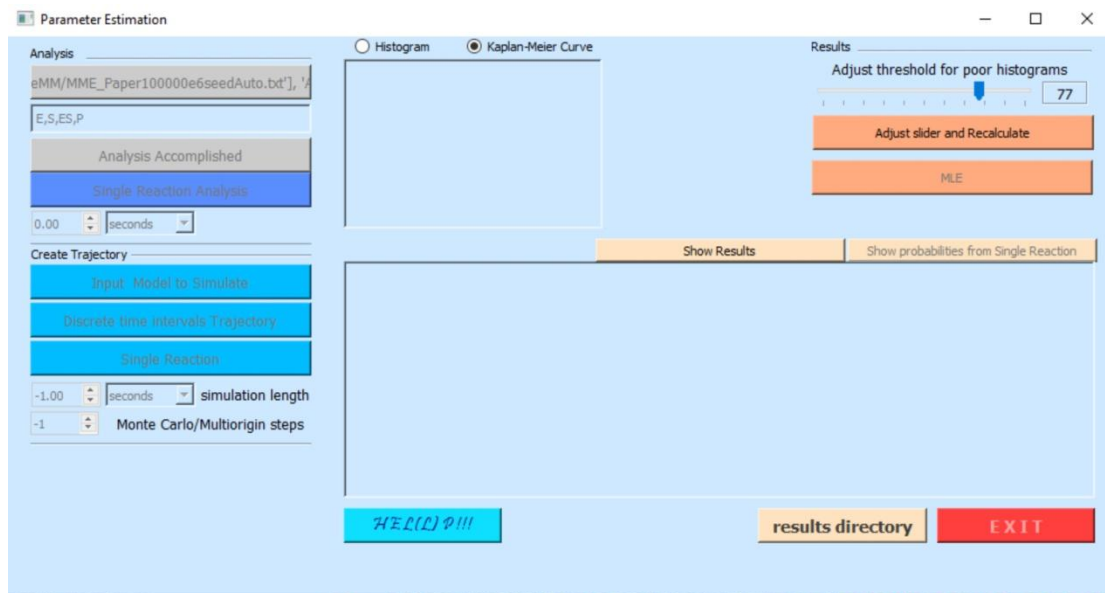
The screenshot shows the 'Parameter Estimation' window with the following annotations:

- Step 1:** Clicking on this button imports the file with the timeline (it may also be in more than one file). Points to the 'Input File' button.
- Step 2:** In this field the species names should be noted in the same order they appear in the file. Points to the 'Species' text box containing 'E,D,Ea,Eb,E3'.
- Step 3:** If the trajectory does not contain an entry with the time points of when the events are recorded the value should be set to the size of the time step used and the units can be set in the drop down list next to it. If it does contain an entry with the time each event occurred then then the value of box should be set to zero. Points to the time step input field showing '-1.00' and the unit dropdown showing 'seconds'.
- Step 4:** If the file(s) contains one long timeline of events, then this option should be used. Points to the 'Event Driven Trajectory' button.
- Alternative Step 4:** Alternatively, if multiple trajectories are to be analyzed that begin from the same state and are terminated at the first event (enrichment), then this button should be clicked. All other options remain the same. Points to the 'Single Reaction Analysis' button.

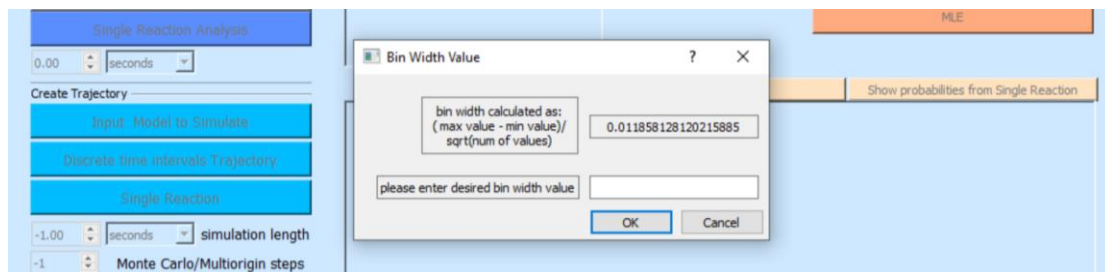
At some point this popup window appears, if the compartment volume is $1e-12$, if not fill in that value then check the lamda button if it is not already checked.

The screenshot shows a 'Select method for mean calculation' popup window. It contains the following elements:

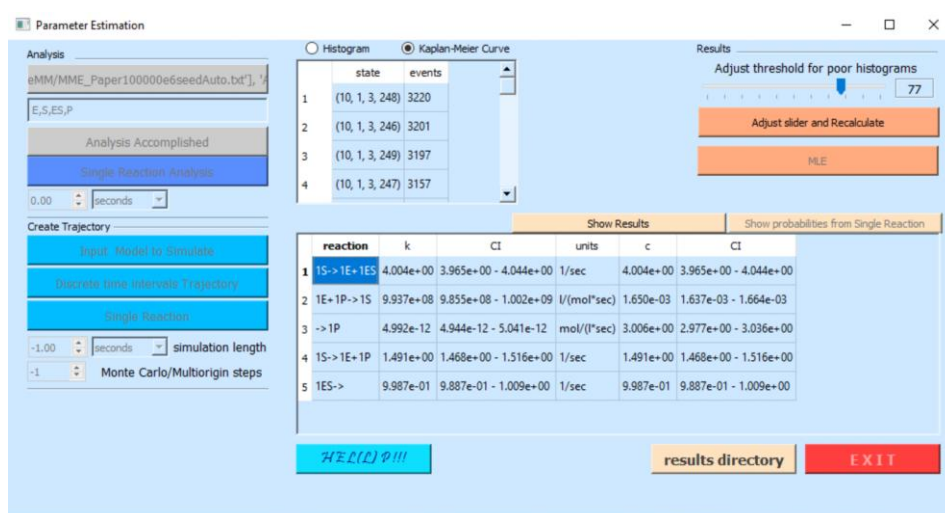
- Compartment Volume:** A text box containing '1e-12' with the unit 'in Liters e.g. 4.574645l'.
- Mean Survival Time:** Four radio button options:
 - ☐ Kaplan Meier for Survival Analysis
 - ☐ Trapezoid Rule for Histogram (for debug purposes only)
 - ☒ lamda
 - ☐ Arithmetic mean of survival times (for debug purposes only)
- Buttons:** 'OK' and 'Cancel' buttons at the bottom.



When the timeline analysis is finished the button changes to grey colour and the orange button on the right is activated. To proceed, the slider that sets the threshold cut off should be adjusted (preferably to 20). Then the button Adjust slider should be clicked. This popup window will appear, no action is required because its function is related to debugging purposes. Clicking OK is all that is needed to proceed.



After that the “show results” button is active and by clicking it the results are displayed in the box as it is shown in the following picture



On the top appears a table with the states the system has visited on the first column and the number of times the system was in that state on the second column.