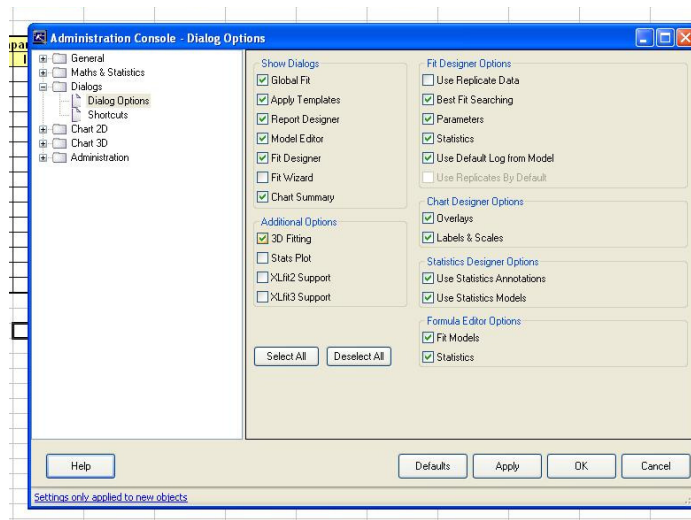


How to estimate KI and kinact from time-dependent IC50 measurements using XLfit

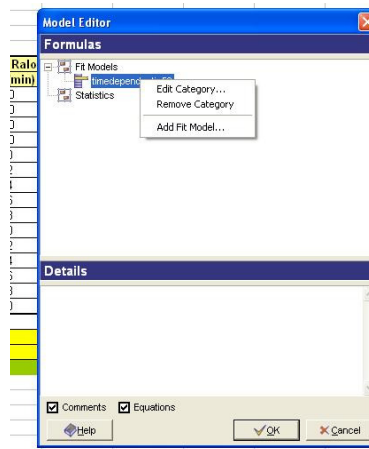
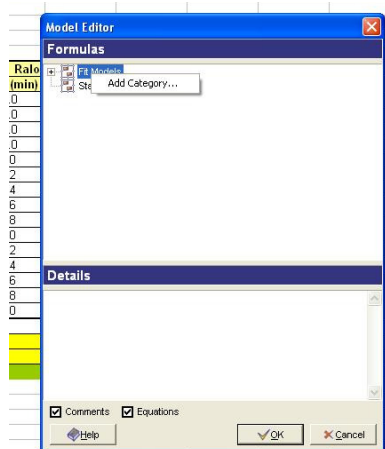
- For the following guide it is assumed that you have you measured IC50 values and the corresponding timepoints stored in a Excel sheet like shown below.

Verapamil		Ethinylestradiol		Berqamottin		Raloxifene		Mifepristone	
time (min)	IC50 μM	time (min)	IC50 μM	time (min)	IC50 μM	time (min)	IC50 μM	time (min)	IC50 μM
2	13	2.0	12	2.0	11	2.0	0.43	2.0	5.3
4	8.9	4.0	6.7	4.0	9.0	4.0	0.31	4.0	3.3
6	6.1	6.0	4.8	6.0	6.9	6.0	0.22	6.0	2.8
8	5.6	8.0	4.2	8.0	5.9	8.0	0.21	8.0	2.6
10	4.8	10	3.7	10	5.2	10	0.20	10	2.5
12	4.6	12	3.5	12	4.6	12	0.18	12	2.4
14	4.1	14	3.3	14	4.1	14	0.15	14	2.1
16	4.1	16	3.2	16	3.7	16	0.14	16	2.1
18	3.6	18	3.0	18	3.4	18	0.13	18	1.8
20	3.3	20	2.8	20	3.1	20	0.12	20	1.6
22	3.1	22	2.7	22	2.9	22	0.11	22	1.4
24	2.9	24	2.5	24	2.8	24	0.092	24	1.2
26	2.7	26	2.4	26	2.6	26	0.087	26	1.1
28	2.6	28	2.3	28	2.4	28	0.077	28	0.92
30	2.4	30	2.2	30	2.2	30	0.069	30	0.77

- Ensure that the option for 3D Fit models in the Administrator Console is activated. If it is activated the symbol for the 3D Fit Wizard appears in the toolbar.



- Create a new model for Ki/kinact estimation
 - Open the Model Editor and add a new Category by a right click on Fit Model

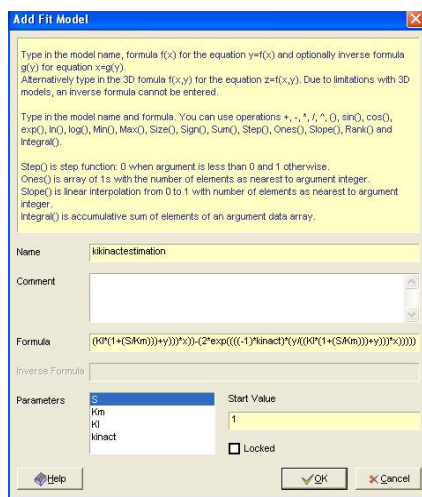


B.-F. Krippendorff, R. Neuhaus, P. Lienau, A. Reichel, W. Huisinga, ``Mechanism-based Inhibition: Deriving KI and kinact directly from Time-Dependent IC50 Values'', J Biomol Screen. 14 (2009), pp. 913-923

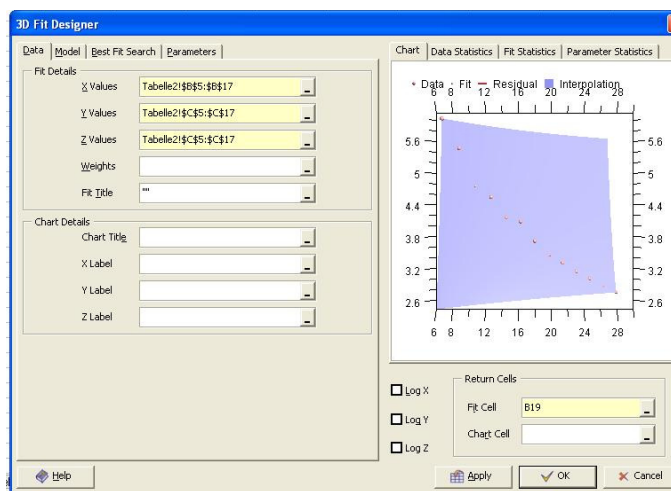
- Add a new Fit Model to the created Category. The Add Fit Model dialog is displayed. The pane at the top of the dialog displays instructions on using the Add Fit Model dialog. Enter a name for the new fit model in the Name box. Add a comment, if you like, in the Comment box.
- Copy and Paste the following formula (corresponding to formula 23 in the paper)

$$\frac{((1+(S/Km))*KI)/((kinact*(y/((KI*(1+(S/Km))))+y))*x)/((2-((kinact*(y/((KI*(1+(S/Km))))+y))*x))- (2*\exp(((1)*kinact)*(y/((KI*(1+(S/Km))))+y))*x))))$$

in the Formula box. The variable x represents the timepoints and y the measured IC50 values.



- The Parameters box is automatically updated with parameters extracted from the formula entered. Select S and Km in the Parameters box and enter the values for the substrate and isoenzyme. Select the Locked checkbox to lock the starting value for S and Km. Click OK to save the model. The model name is displayed below the category folder in the Fit Models cabinet.
- In your Excel worksheet, click 3D Fit Wizard in the toolbar.



- The panel of the 3D Fit Wizard allows you to specify the x, y and z variables. Click in the corresponding box and select for x the time, for y the measured IC50 values and for z also the same measured IC50 values from your measured data worksheet.

