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

1. 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.

В	C	D	E	F	G	н	1	J	K
Verapamil		Ethinylestradiol		Bergamottin		Raloxifene		Mifepristone	
time (min)	IC50 (µM)	time (min)	IC50 (µM)	time (min)	IC50 (µM)	time (min)	IC50 (µM)	time (min)	1C50 (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
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2. 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.

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- 3. Create a new model for Ki/kinact estimation
 - a. 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

- 4. 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.
- 5. Copy and Paste the following formula (corresponding to formula 23 in the paper)

(((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.

Type in the m g(y) for equa Alternatively models, an in	odel name, formula $f(x)$ for the equation $y=f(x)$ and optionally inverse form tion $x=g(y)$. type in the 3D formula $f(x,y)$ for the equation $z=f(x,y)$. Due to limitations with verse formula cannot be entered.	ula n 3D
Type in the m exp(), ln(), lo Integral().	odel name and formula: You can use operations +, -, *, /, ^, 0, sin(), cos(), g(), Min(), Max(), Size(), Sign(), Sun(), Step(), Ones(), Slope(), Rank() and	
Step() is step Ones() is arr Slope() is line integer.	function: 0 when argument is less than 0 and 1 otherwise. ay of 1s with the number of elements as nearest to argument integer, ar interpolation from 0 to 1 with number of elements as nearest to argument	nt
Integral() is a	counulative sum of elements of an argument data array.	
Name	kikinactestimation	
Comment		2 3
Comment Formula	[(49*(1+(S.Mm)))+y)))*x))-(2*exp((((-1)*kinact)*(y)((44*(1+(S.Mm)))+y)))*	×)))))
Comment Formula	(PO^(1+(S4Km)))+y)))*x))-(2*exp((((-1)*kinact)*(y)((R0^{r}(1+(S4Km)))+y)))*	×)))))
Comment Formula nverse Formu Parameters	(r0*(1+(S:Km)))+y))*x))-(2*exp((((-1)*kinact)*(y)((R0*(1+(S:Km)))+y)))* 10 S Kin Kin kin innet Locked	×))))).

- 6. 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.
- 7. In your Excel worksheet, click 3D Fit Wizard in the toolbar.

3D Fit Designer		×
Data Model Best Fit Se	earch <u>P</u> arameters	Chart Data Statistics Fit Statistics Parameter Statistics
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8. 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.

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9. Select the model inserted from the model Category by expanding your model category in the Models tree and clicking the model to select it as shown below.



Any comments associated with the category or equation are displayed in the Details pane when the category or equation are selected.



- 10. In the Category Parameters, enter a cell in the Output Cell box for KI and kinact to return the parameter values to the worksheet or click in the box and select a cell from the worksheet.
- 11. Click **Ok** to return to the worksheet and to display the estimated KI and kinact in the cell you chose.

Verapamil		Ethinylestradiol		Bergamottin		Raloxifene		Mifepristone	
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30	2.4	30	2.2	30	2.2	30	0.069	30	0.77
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