Supplemental Data

Title: A two-state model for the kinetics of competitive radioligand binding

Running title: a two-state model for kinetic investigation

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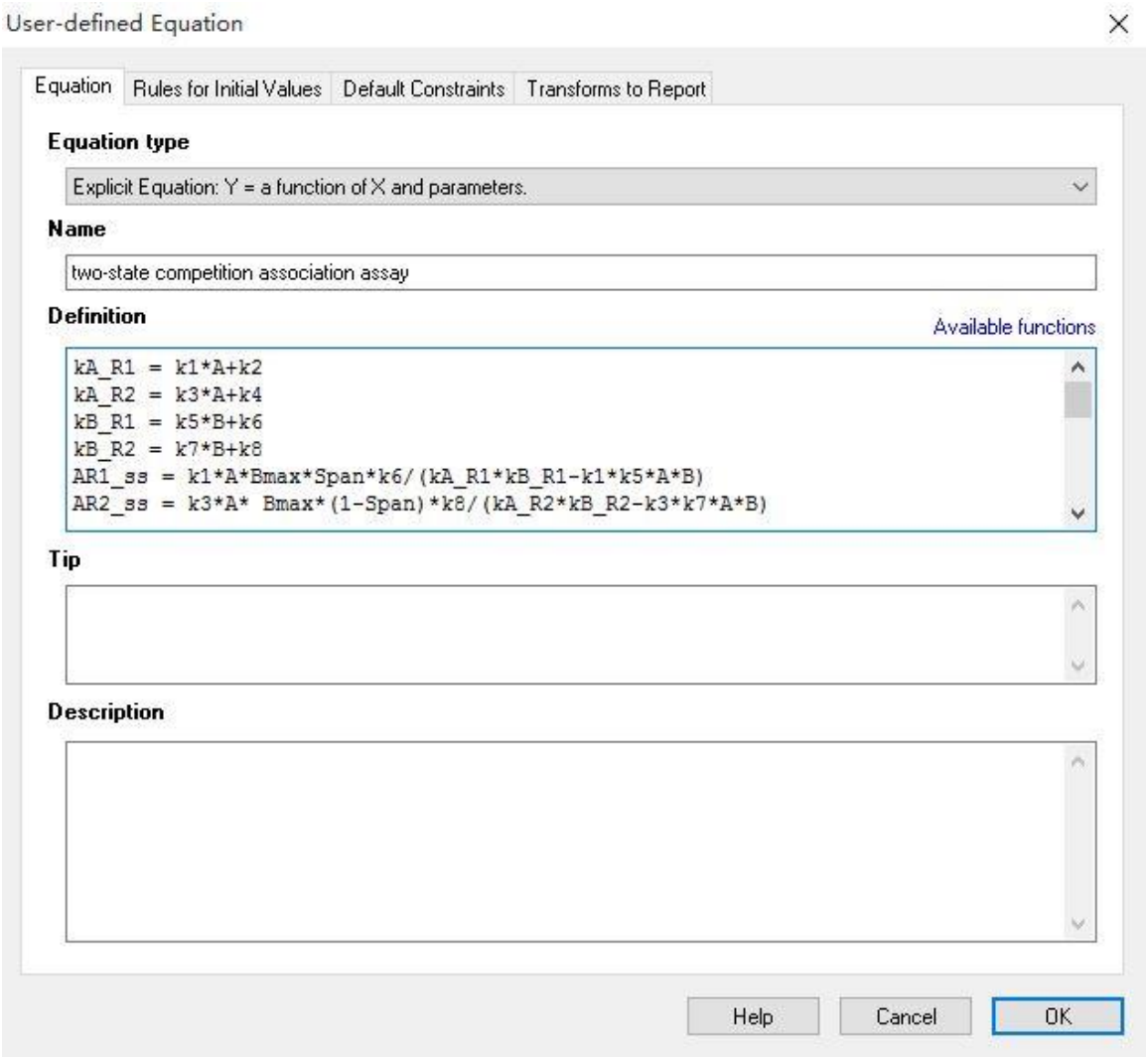
1. Enter equations for <u>unlabeled ligands</u> into Graphpad Prism 6.

Enter the data as below. The title for each group is the concentration of the competitor in the unit of M. For this example, Y values have already been corrected to baseline and normalized to the maximal binding of radioligand at 180 min. Values at each data point represent the mean of five individual experiments each performed in duplicate.

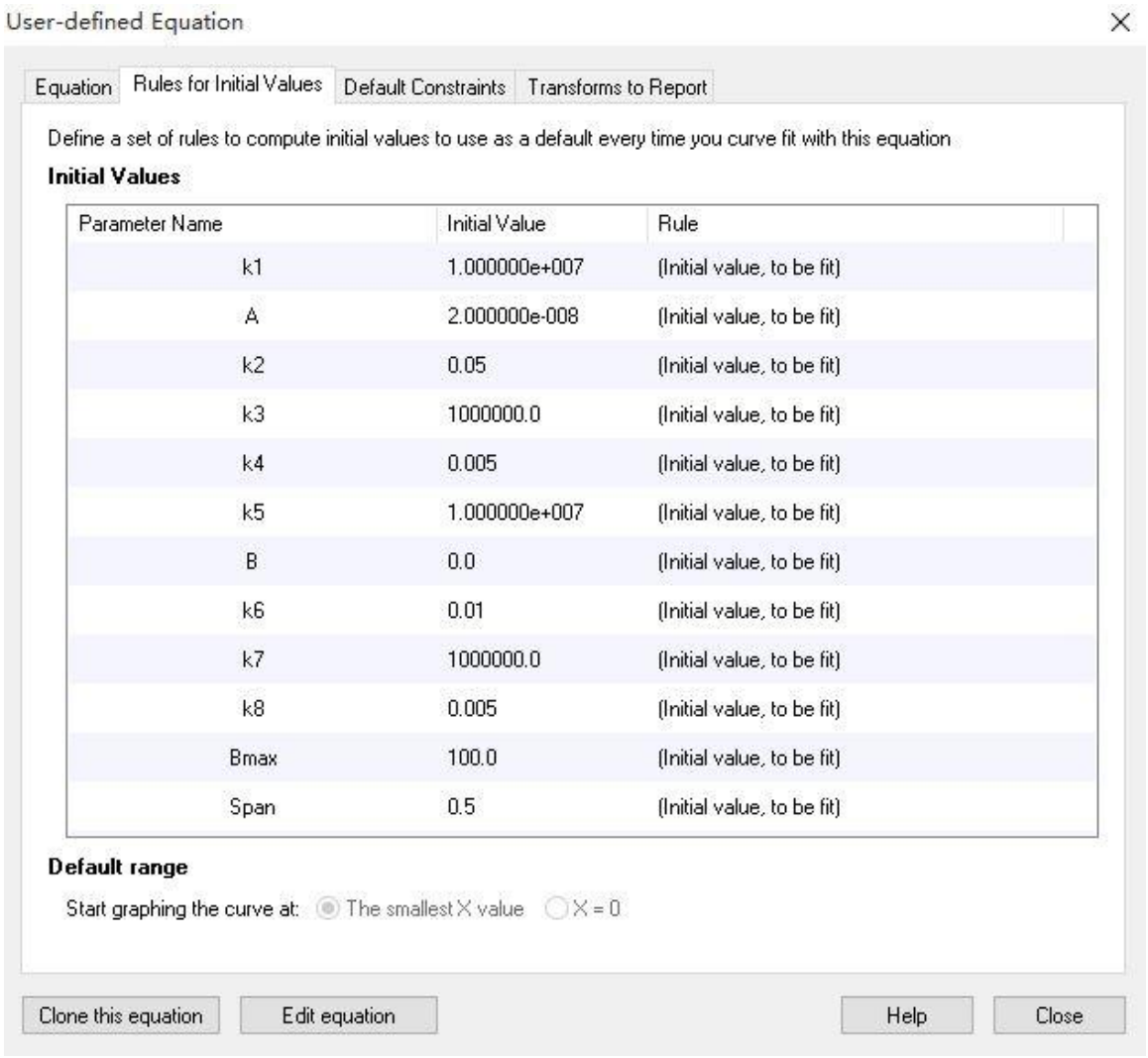
X	Group A					Group B					Group C					Group D				
time (min)	0					1e-8					3e-8					1e-7				
X	A:Y1	A:Y2	A:Y3	A:Y4	A:Y5	B:Y1	B:Y2	B:Y3	B:Y4	B:Y5	C:Y1	C:Y2	C:Y3	C:Y4	C:Y5	D:Y1	D:Y2	D:Y3	D:Y4	D:Y5
0	0.000000	0.00000	0.00000	0.000000	0.000000	0.00000	0.00000	0.00000	0.000000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.000000	0.000000	0.000000	0.000000	0.000000
1	9.894714	18.87369	19.31058	17.069660	21.093460	10.10958	14.62253	13.28648	9.921174	26.09900	11.02278	11.77620	13.70482	10.80457	11.10085	6.456811	6.596628	8.383534	5.966295	10.066490
3	32.939410	37.50306	37.44980	32.345750	37.439970	27.78255	31.49279	29.23360	20.250070	36.59032	19.72497	26.20327	26.67336	19.92389	16.54969	12.591320	12.338140	10.090360	10.179400	13.151090
7	49.462830	53.67701	56.62651	52.718130	46.250460	39.07391	44.28292	49.21352	33.283500	48.04211	30.35024	32.07916	32.81459	26.78717	24.52900	16.394500	16.393840	14.072960	13.549880	15.958630
14	60.582290	65.28219	64.97657	60.750200	59.456960	52.55694	54.49548	53.29662	39.725470	54.63613	32.14439	38.49255	37.86814	30.52460	31.41854	17.651480	20.290740	16.080990	16.188460	19.689690
22	71.153850	71.91547	67.80455	66.553410	57.739190	56.15599	58.36795	54.76908	45.392770	49.55671	38.07478	42.29172	40.69612	33.35146	41.52198	19.907610	21.915470	18.708170	16.526230	24.529000
30	73.205840	73.18593	74.39759	72.546890	65.773920	55.43618	61.20205	63.21954	46.412070	55.43037	35.05587	44.00195	45.96394	34.76488	44.08940	18.672110	22.843880	18.473900	17.205760	25.378650
40	80.092390	80.18568	82.29585	75.129110	72.201700	61.64590	66.56487	66.08099	54.294640	61.74732	40.81435	46.10310	45.49866	39.31775	41.44810	19.348950	21.646710	18.992640	19.271540	25.360180
60	84.561670	83.58173	85.24097	82.440880	76.209820	68.65062	67.40777	60.74297	63.563470	61.71038	37.96734	48.02101	50.51874	42.59310	56.20613	19.338200	23.137060	19.996650	19.978250	27.151830
90	89.589600	86.14708	86.19478	93.299810	91.263390	71.29351	71.85439	65.88019	68.143520	79.73772	44.90761	53.65258	49.73228	43.53085	55.06095	23.239080	24.138770	19.611780	22.329540	30.070190
120	101.310700	96.55509	95.19746	90.323460	92.371630	77.18092	74.67628	72.35609	82.386520	78.42630	42.81263	52.77303	49.59839	44.90351	60.04802	19.123330	23.808940	21.201470	21.377910	29.700780
180	100.000000	100.00000	100.00000	100.000000	100.000000	85.58230	78.10896	78.24632	77.806470	75.45253	48.29179	55.47276	48.81191	44.00652	63.46509	26.418130	29.196190	26.288490	25.604780	28.463240

With this, click on the **Analyze** button. From **XY analyses** choose **Nonlinear regression (curve fit)** and click **User-defined equations.** Click **New** and choose **Create new equation.** Enter the following equations under the tag **Equation** in **Definition** as illustrated below:

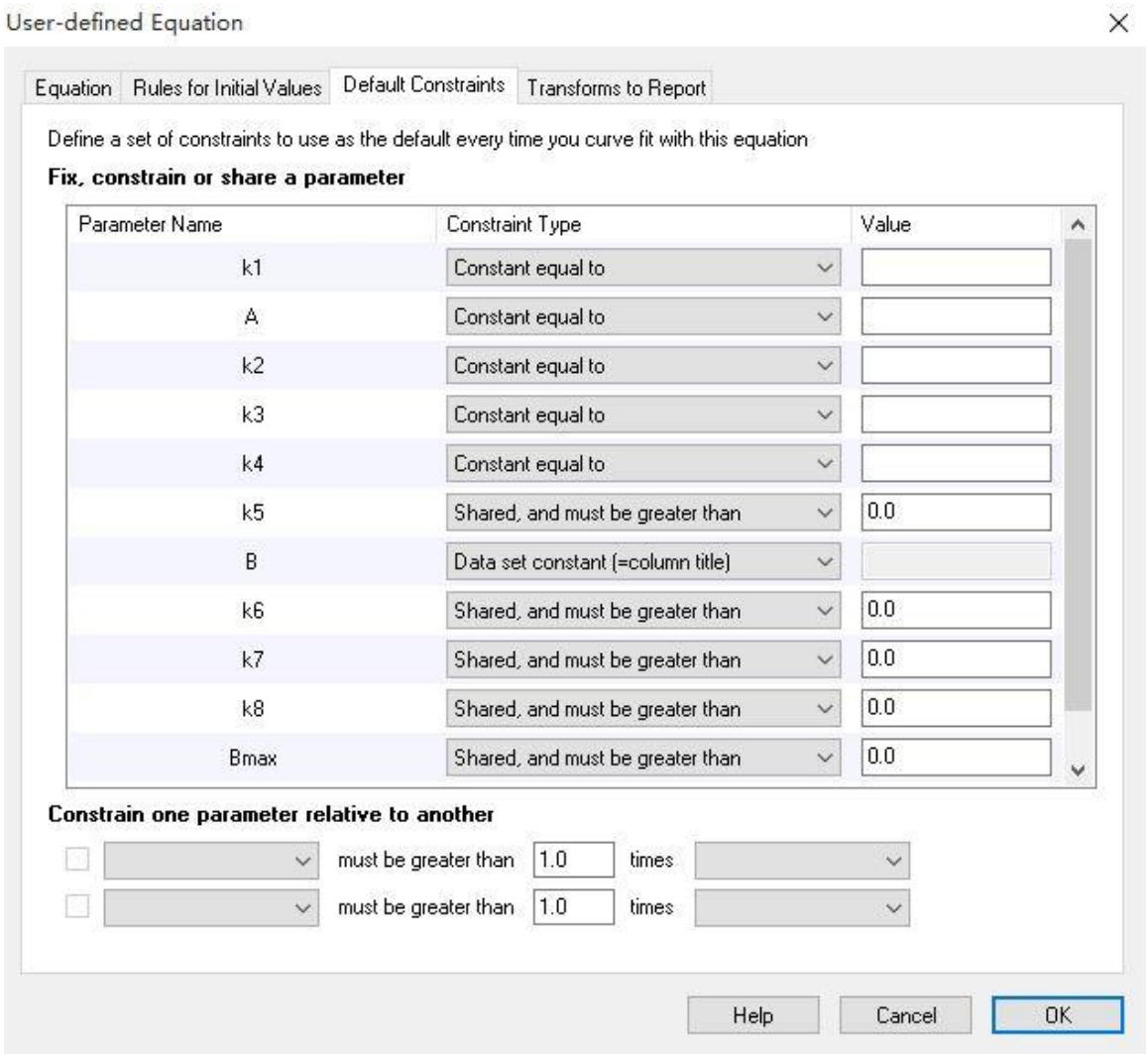
kA_R1 = k1*A+k2
kA_R2 = k3*A+k4
kB_R1 = k5*B+k6
kB_R2 = k7*B+k8
AR1_ss = k1*A*Bmax*Span*k6/(kA_R1*kB_R1-k1*k5*A*B)
AR2_ss = k3*A* Bmax*(1-Span)*k8/(kA_R2*kB_R2-k3*k7*A*B)
S_R1= SQRT((kA_R1-kB_R1)^2 + 4*k1*k5*A*B)
S_R2= SQRT((kA_R2-kB_R2)^2 + 4*k3*k7*A*B)
KF_R1 = 0.5*(kA_R1 + kB_R1+ S_R1)
KS_R1 = 0.5*(kA_R1 + kB_R1 - S_R1)
KF_R2 = 0.5*(kA_R2 + kB_R2 + S_R2)
KS_R2 = 0.5*(kA_R2 + kB_R2 - S_R2)
P1 = (k1*A* Bmax*Span- KS_R1*AR1_ss) /(KF_R1 - KS_R1)
P2 = (k3*A* Bmax*(1-Span) - KS_R2*AR2_ss) /(KF_R2 -KS_R2)
Q1 = (KF_R1*AR1_ss - k1*A*Bmax*Span) /(KF_R1 - KS_R1)
Q2 = (KF_R2*AR2_ss - k3*A*Bmax*(1-Span)) /(KF_R2 - KS_R2)
Y = AR1_ss - P1*exp(-KF_R1*x) - Q1* exp(-KS_R1*x) + AR2_ss - P2*exp(-KF_R2*x) - Q2*exp(-KS_R2*x)



Click OK and choose the tag “Rule for Initial Values”. Enter parameters as illustrated below

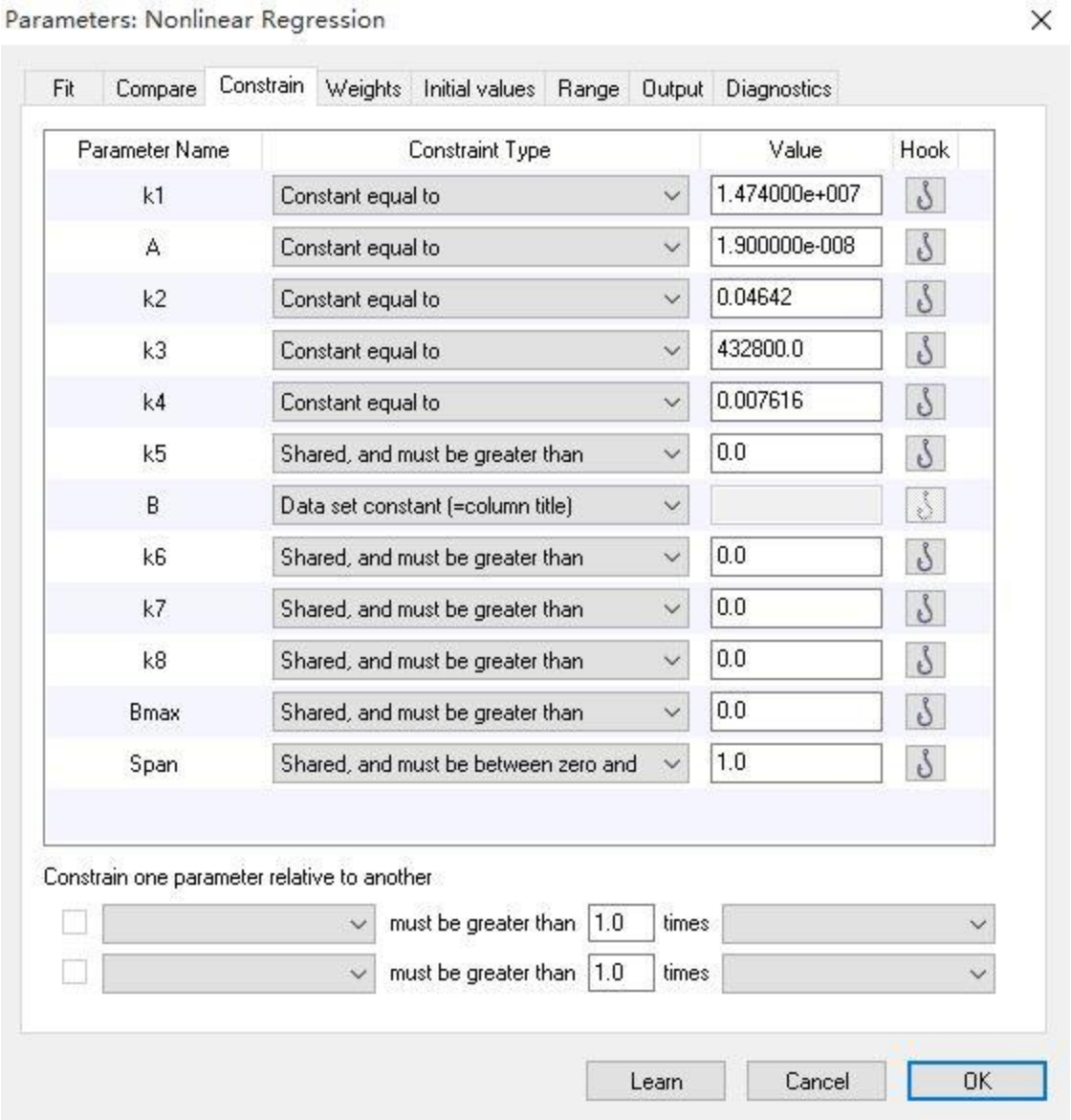


Click OK and choose the tag “Default Constrains”. Set parameters as illustrated below


User-defined Equation
Equation
Rules for Initial Values
Default Constraints
Transforms to Report
Define a set of constraints to use as the default every time you curve fit with this equation
Fix, constrain or share a parameter
Parameter Name
Constraint Type
Value
k1
Constant equal to
A
Constant equal to
k2
Constant equal to
k3
Constant equal to
k4
Constant equal to
k5
Shared, and must be greater than
0.0
B
Data set constant (=column title)
k6
Shared, and must be greater than
0.0
k7
Shared, and must be greater than
0.0
k8
Shared, and must be greater than
0.0
Bmax
Shared, and must be greater than
0.0
Constrain one parameter relative to another
must be greater than
1.0
times
must be greater than
1.0
times
Help
Cancel
OK

And click OK.

Enter k1, k2, k3 and k4 predetermined by kinetic radioligand binding experiments and the concentration of the radioligand (A) as below. Note: the units for A and B are in M.



Click OK and analyze the result.