KinTek Global Kinetic Explorer Student Version 3.0.2508. Copyright Kenneth A. Johnson and KinTek Corporation

Project

New... Open... Save Save As...

Filename /Users/kajohnson/Desktop/Exam 2 2013/Exam2_2013_data.mec

Model Fit FitSpace Experiment Data

Model Editor

Edit Model... Clear Model

Reactions <none>

- [x] Visualize Model
- [x] Display Model Values
- [x] Continuous Simulation
- [ ] Display Optimization

Reset Layout

Reactions k+ k-

Data Fit Editor

Data Fit Options

- [x] Normalize residuals using available Sigma values

Fit Active Exp Fit All Exp Batch Fit

FitSpace Editor

FitSpace Options

Chi2 Threshold Limit	1.6
Resolution of Grid	10
Param Multiple Min (Lower Bound)	0.0001
Param Multiple Max (Upper Bound)	100

- [ ] Include Non-Rate Parameters in FitSpace
- [ ] Use Individual Parameter Bounds

Compute FitSpace 1D Set Individual Bounds...

Compute FitSpace 2D

Delete Plot

Experiment Editor

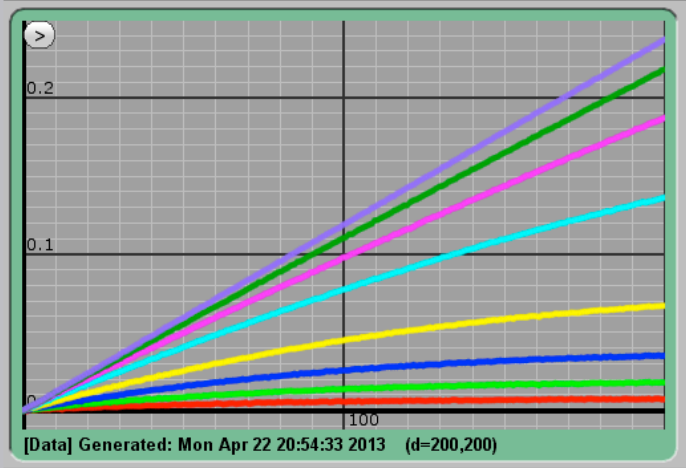
New Copy Delete

- [ ] View Single Plot

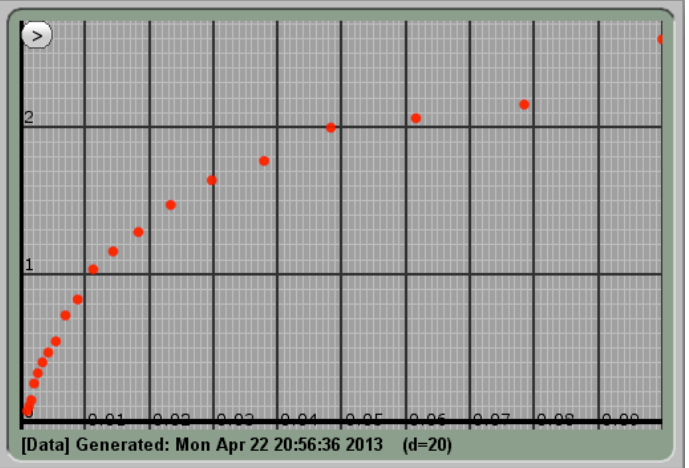
Data Repository

- [ ] View Single Plot
- [ ] List Individual Traces

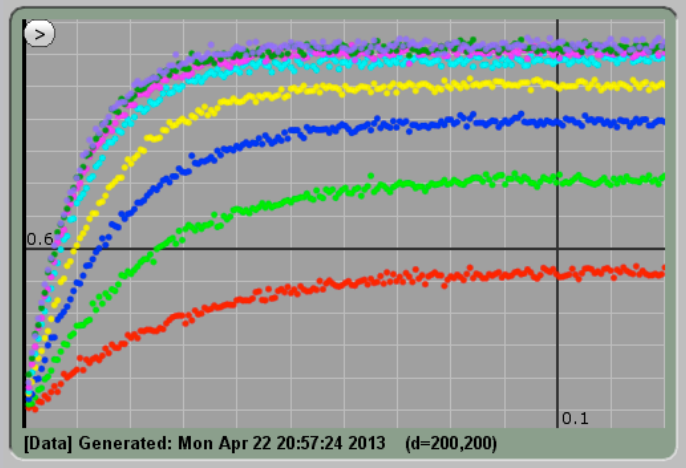
- [x] Generated: Mon Apr 22 20:54:33 2013 N 200, Sigma 0.0002, Deadtime 0.001
- [x] Generated: Mon Apr 22 20:56:36 2013 N 20, Sigma 0.05, Deadtime 0.001
- [x] Generated: Mon Apr 22 20:57:24 2013 N 200, Sigma 0.001, Deadtime 0.001
- [x] Generated: Mon Apr 22 20:57:58 2013 N 200, Sigma 0.0001, Deadtime 0.001
- [x] Generated: Mon Apr 22 21:00:24 2013 N 500, Sigma 0.001, Deadtime 0.001



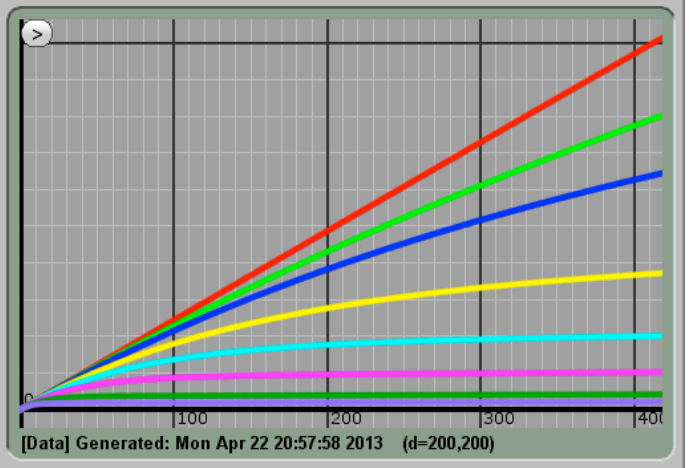
[Data] Generated: Mon Apr 22 20:54:33 2013 (d=200,200)



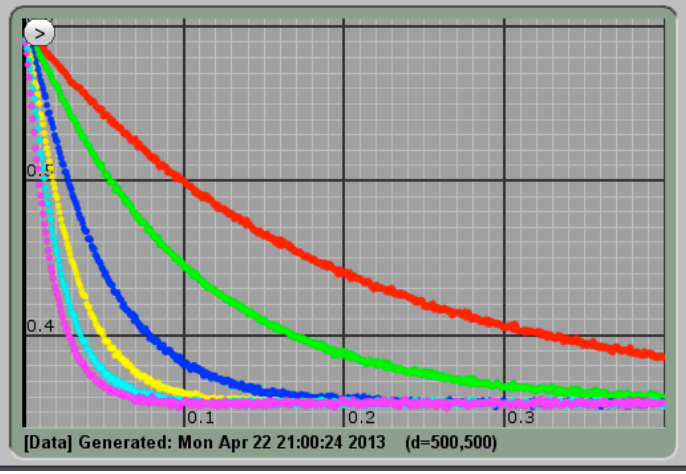
[Data] Generated: Mon Apr 22 20:56:36 2013 (d=20)



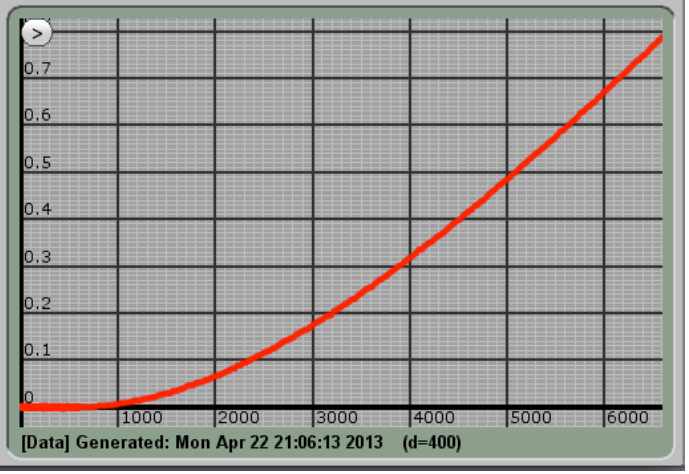
[Data] Generated: Mon Apr 22 20:57:24 2013 (d=200,200)



[Data] Generated: Mon Apr 22 20:57:58 2013 (d=200,200)

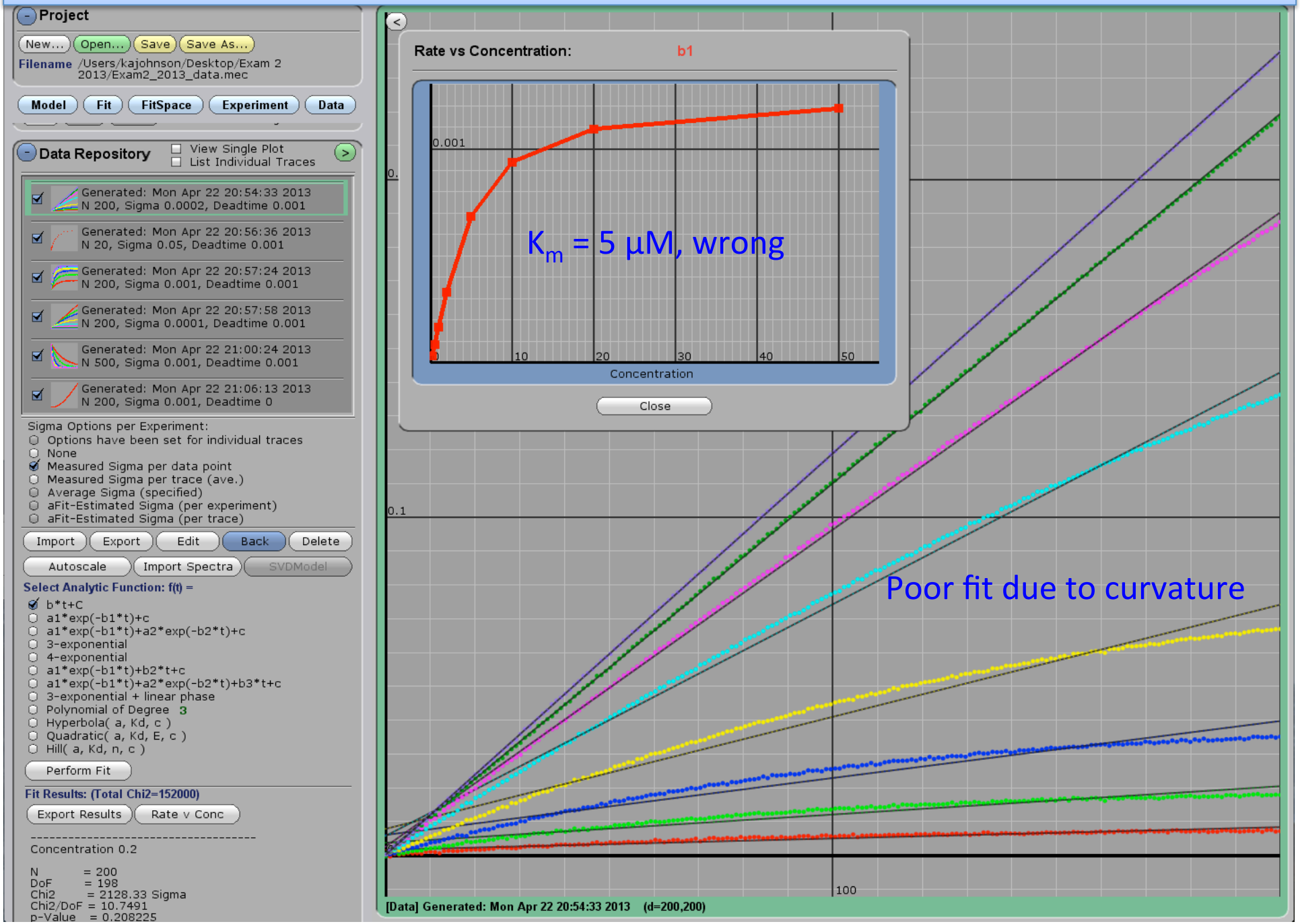


[Data] Generated: Mon Apr 22 21:00:24 2013 (d=500,500)

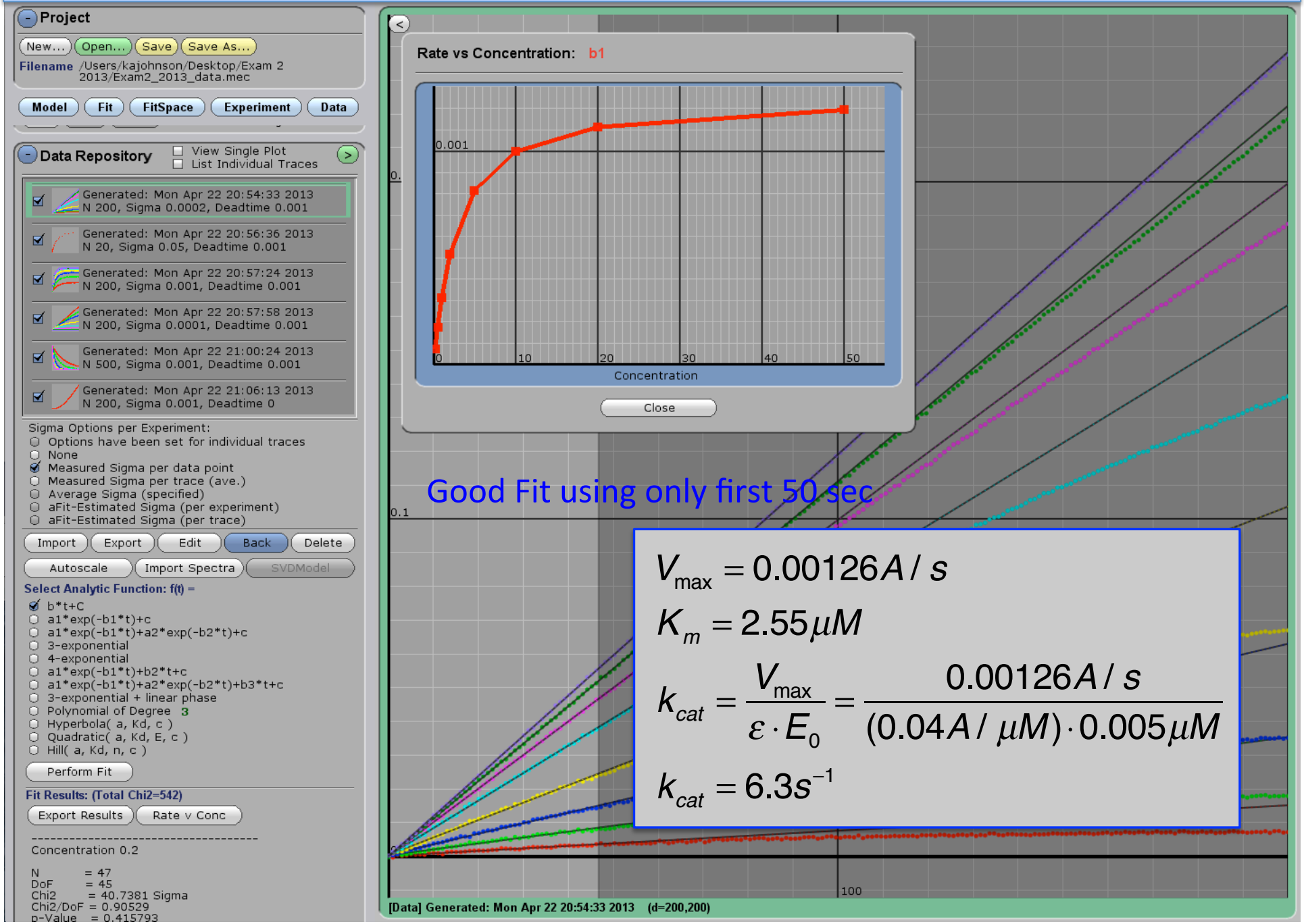


[Data] Generated: Mon Apr 22 21:06:13 2013 (d=400)

Conventional fit for Experiment 1



Conventional fit for Experiment 3



$$V_{max} = 0.00126 A/s$$

$$K_m = 2.55 \mu M$$

$$k_{cat} = \frac{V_{max}}{\varepsilon \cdot E_0} = \frac{0.00126 A/s}{(0.04 A/\mu M) \cdot 0.005 \mu M}$$

$$k_{cat} = 6.3 s^{-1}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E \bullet S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E \bullet P \xrightarrow{k_3} E + P$$

$$E + S \underset{k_{-1}}{\overset{100\mu M^{-1}s^{-1}}{\rightleftharpoons}} E \bullet S \underset{0}{\overset{k_2}{\rightleftharpoons}} E \bullet P \xrightarrow{fast} E + P$$

Simplifying approximation for global fitting

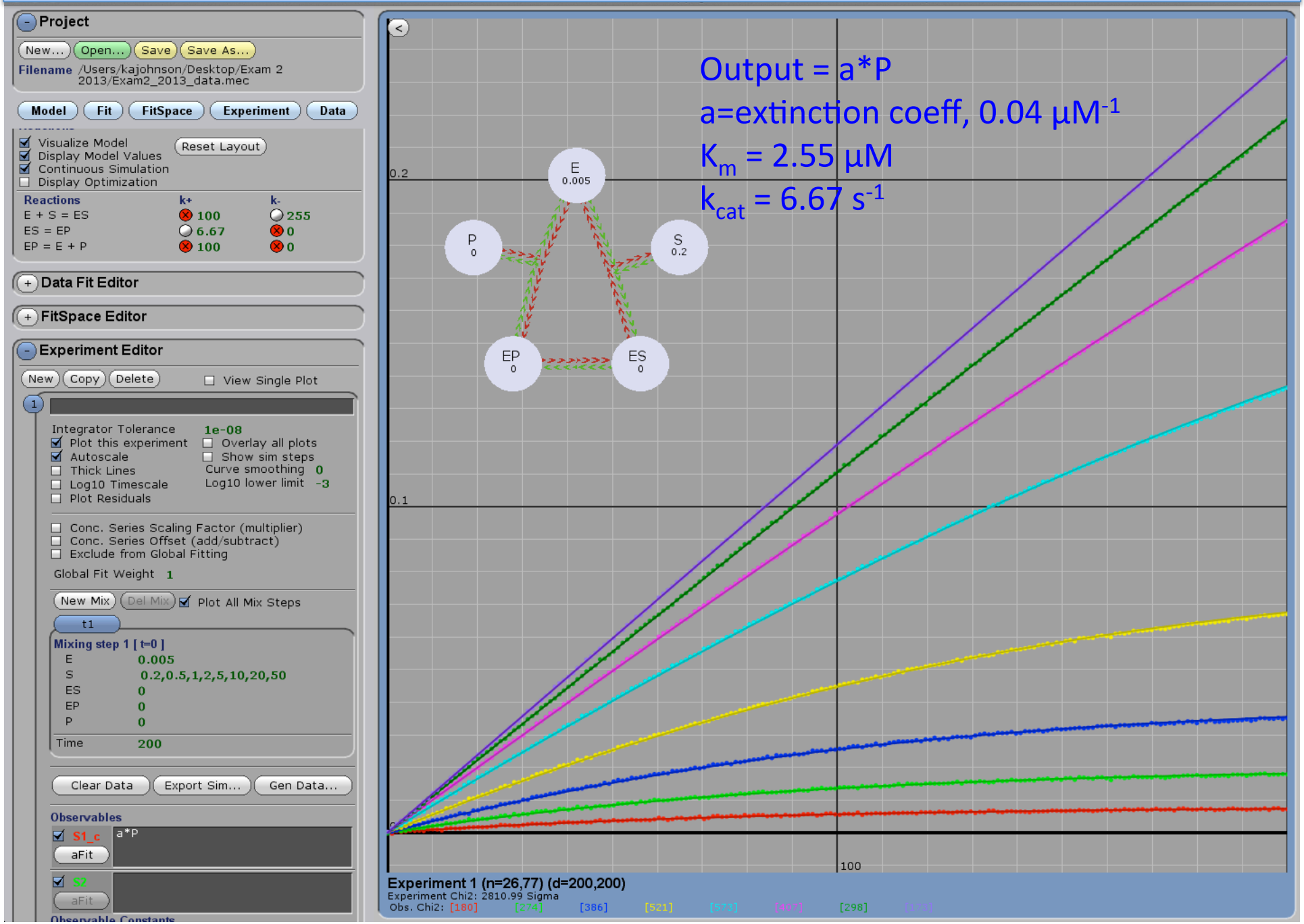
$$v = \frac{V}{[E]_0} = \frac{k_{cat}[S]}{K_m + [S]}$$

$$k_{cat} = k_2$$

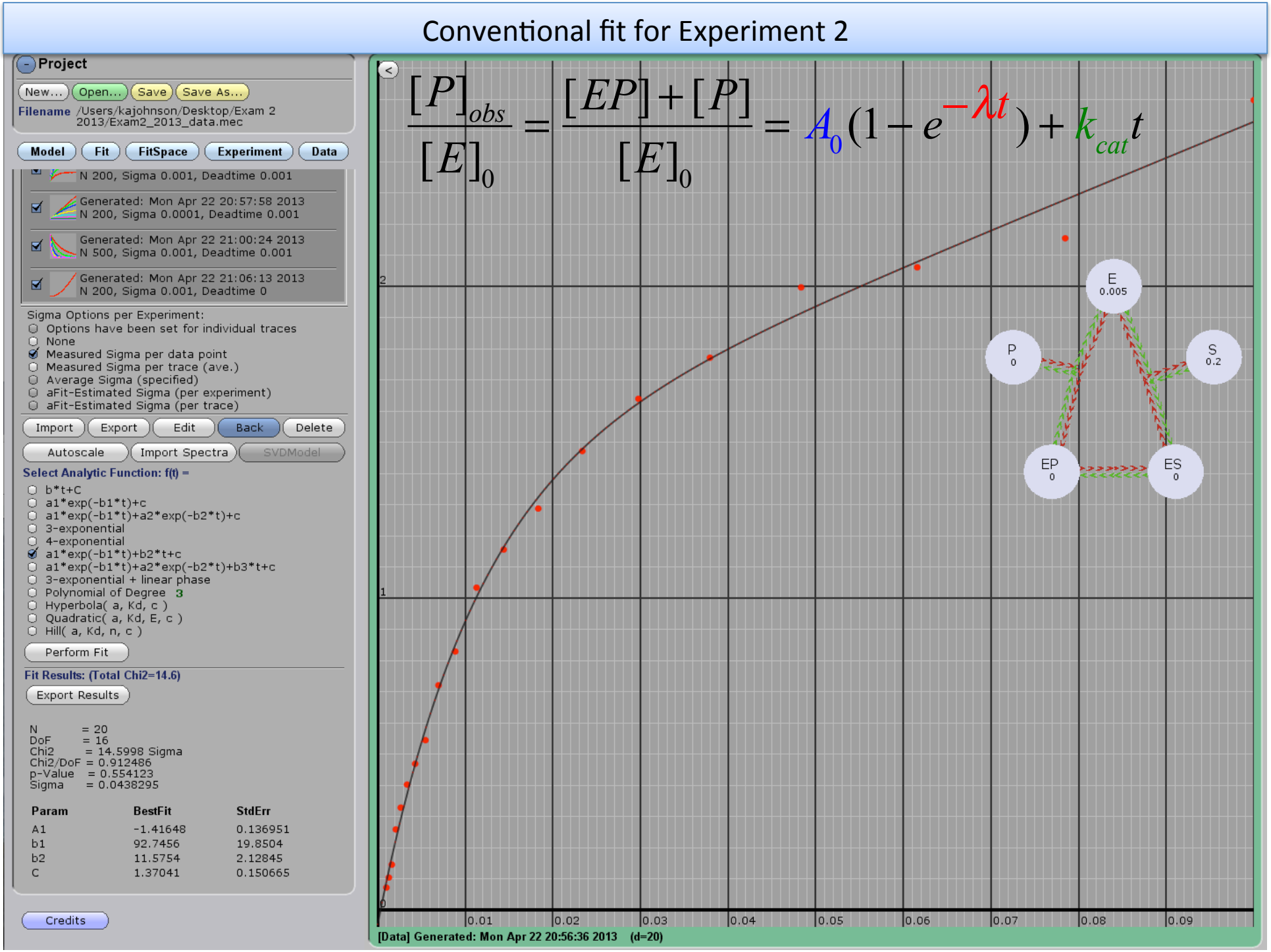
$$K_m = \frac{k_{-1}}{k_1}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Global fit for Experiment 1



Conventional fit for Experiment 2



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\bullet S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E\bullet P \xrightarrow{k_3} E + P$$

$$\frac{[P]_{obs}}{[E]_0} = \frac{[EP]+[P]}{[E]_0} = A_0(1 - e^{-\lambda t}) + k_{cat} t$$

$$\lambda = k_2 + k_{-2} + k_3 = 92.7 s^{-1}$$

$$A_0 = \frac{k_2(k_2 + k_{-2})}{(k_2 + k_{-2} + k_3)^2} = \frac{1.42 \mu M}{2 \mu M} = 0.71$$

$$k_{cat} = \frac{k_2 k_3}{k_2 + k_{-2} + k_3} = \frac{11.6 \mu M / s}{2 \mu M} = 5.8 s^{-1}$$

$$k_2 = 71.5 s^{-1}$$

$$k_{-2} = 13.7 s^{-1}$$

$$k_3 = 7.5 s^{-1}$$

Fit Experiment 1 and 2 Simultaneously, so fit is consistent with steady state parameters

Project

New... Open... Save Save As...

Filename /Users/kajohnson/Desktop/Exam 2 2013/Exam2_2013_data.mec

Model Fit FitSpace Experiment Data

Param	BestFit	StdErr
k-1	1148.36	32.0193
k+2	81.139	0.993819
k-2	13.4288	0.820644
k+3	7.89447	0.0601155

☐ Plot observable traces at best fit values
☐ Plot observable traces at StdErr bounds

FitSpace Editor

Experiment Editor

New Copy Delete ☐ View Single Plot

1
2

Integrator Tolerance 1e-08
☑ Plot this experiment ☐ Overlay all plots
☑ Autoscale ☐ Show sim steps
☐ Thick Lines Curve smoothing 0
☐ Log10 Timescale Log10 lower limit -3
☐ Plot Residuals

☐ Conc. Series Scaling Factor (multiplier)
☐ Conc. Series Offset (add/subtract)
☐ Exclude from Global Fitting

Global Fit Weight 50

New Mix Del Mix ☑ Plot All Mix Steps

t1

Mixing step 1 [t=0]

E	2
S	50
ES	0
EP	0
P	0
Time	0.1

Clear Data Export Sim... Gen Data...

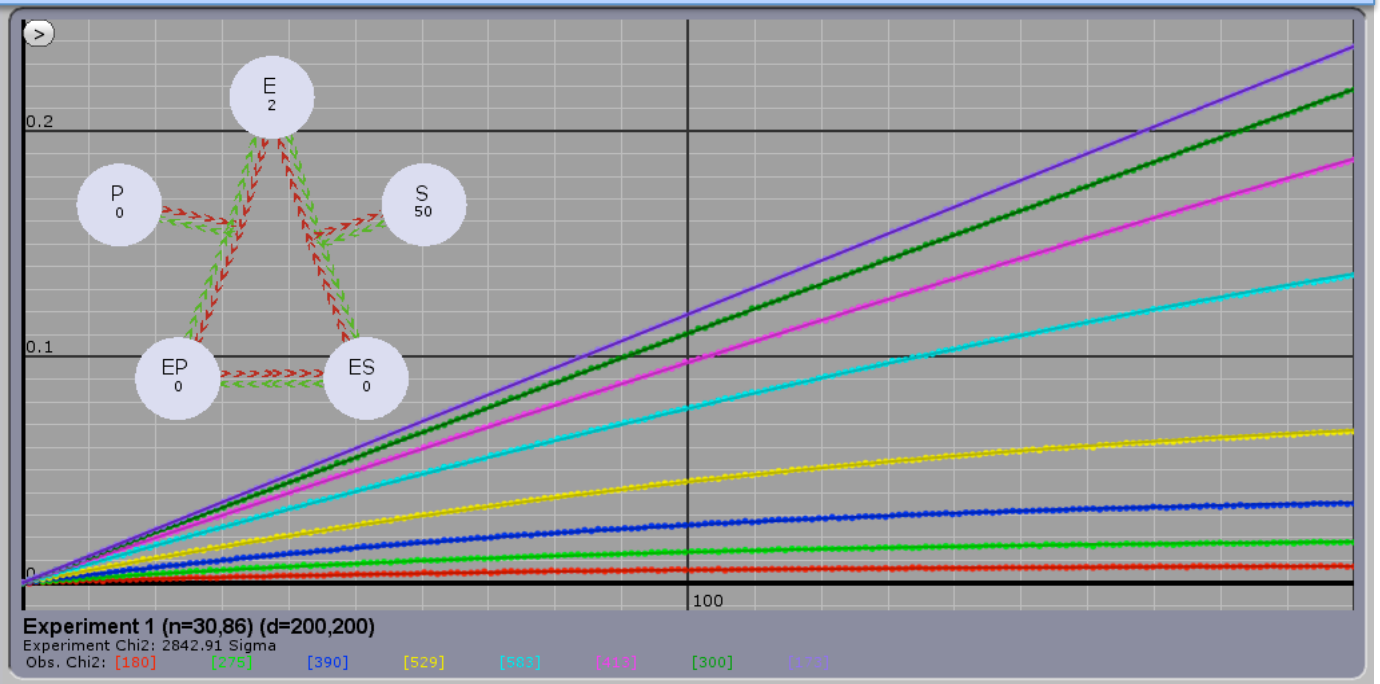
Observables

☑ S1_c EP + P
aFit

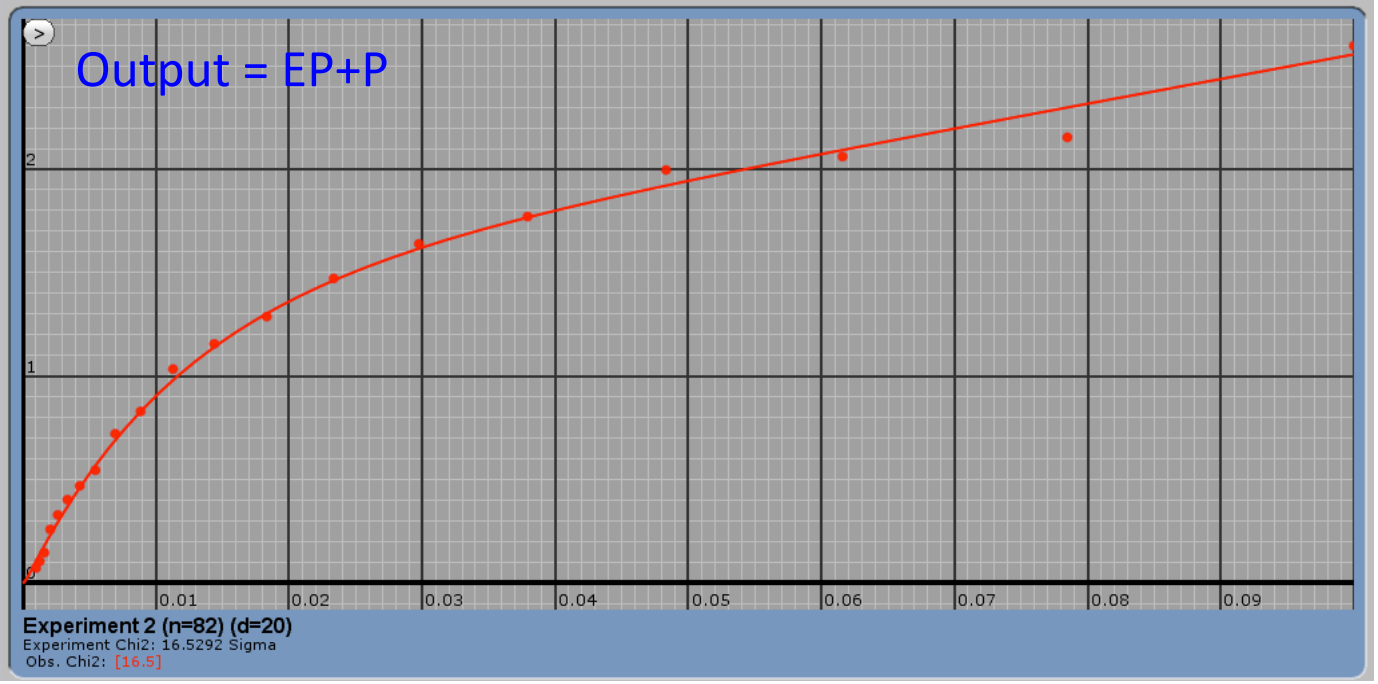
☑ S2
aFit

Observable Constants

a 0.04

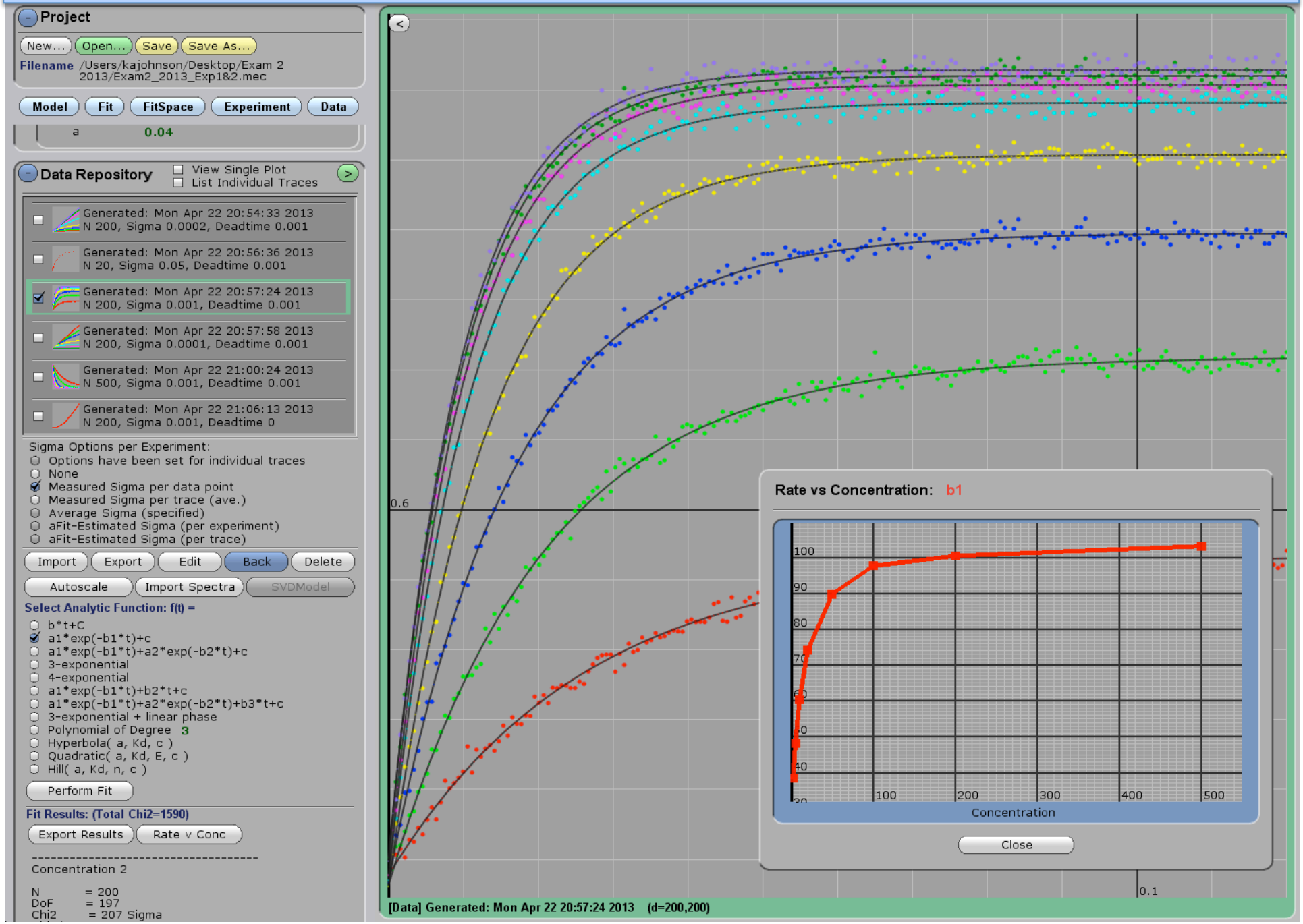


Experiment 1 (n=30,86) (d=200,200)
Experiment Chi2: 2842.91 Sigma
Obs. Chi2: [180] [275] [390] [529] [583] [413] [300] [272]



Experiment 2 (n=82) (d=20)
Experiment Chi2: 16.5292 Sigma
Obs. Chi2: [16.5]

Conventional fit for Experiment 3

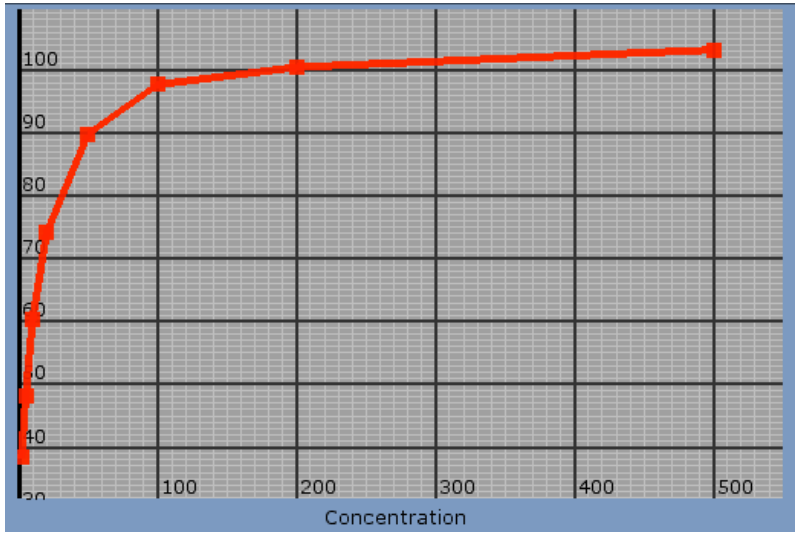


Fit time course to single exponential

$$Y = A_1 \cdot e^{-\lambda_1 \cdot t} + c$$

$$E + S \underset{k_{-2}}{\overset{K_1}{\rightleftharpoons}} E\bullet S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E\bullet X \qquad \lambda_1 = k_2 \frac{K_1[S]}{K_1[S]+1} + k_{-2}$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\bullet S \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E\bullet P \xrightarrow{k_3} E + P$$



$$\lambda_1 \approx k_2 \frac{K_1[S]}{K_1[S]+1} + k_{-2} + k_3$$

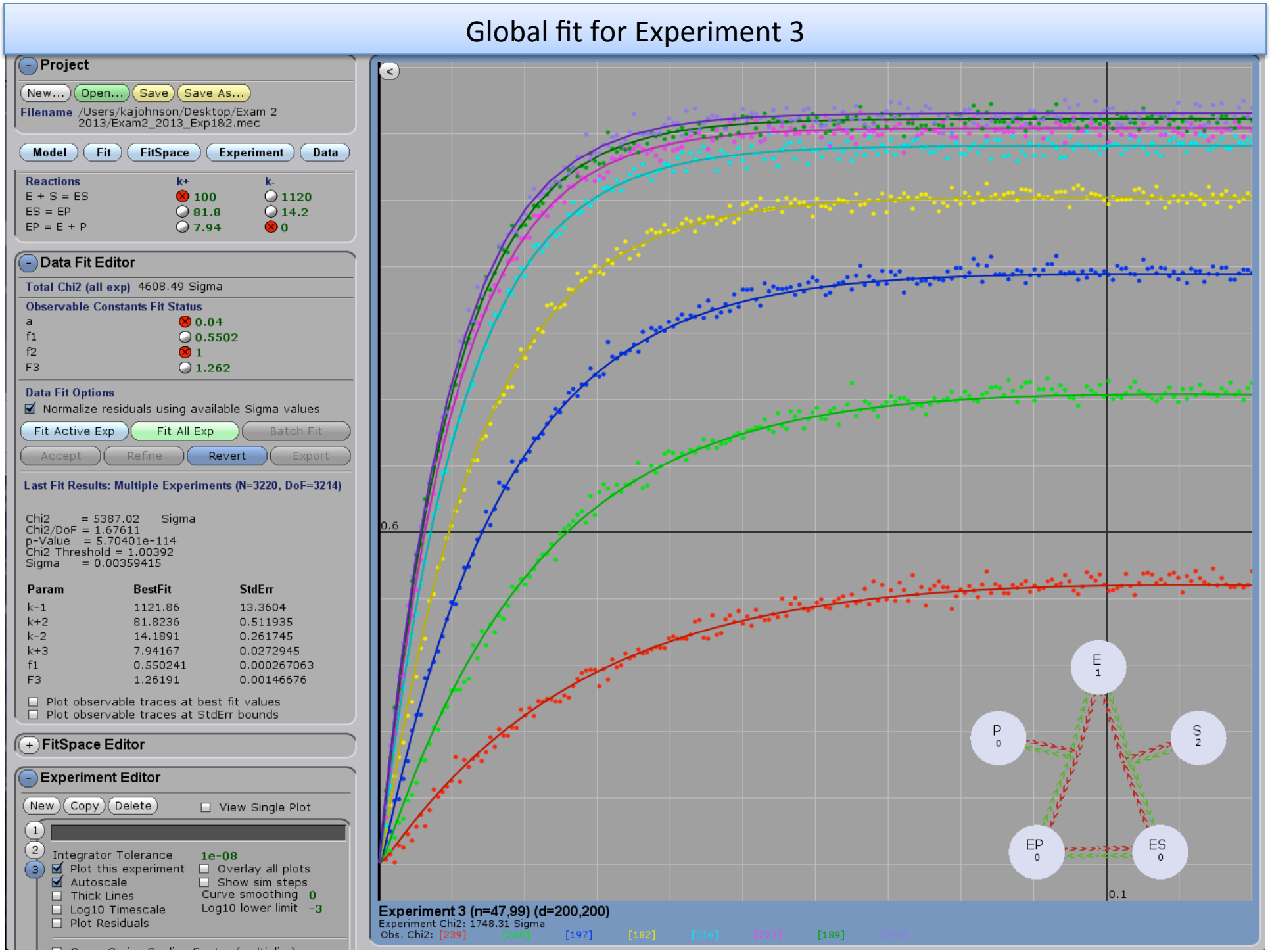
$$k_2 = 78 s^{-1}$$

$$k_{-2} + k_3 = 27.9 s^{-1}$$

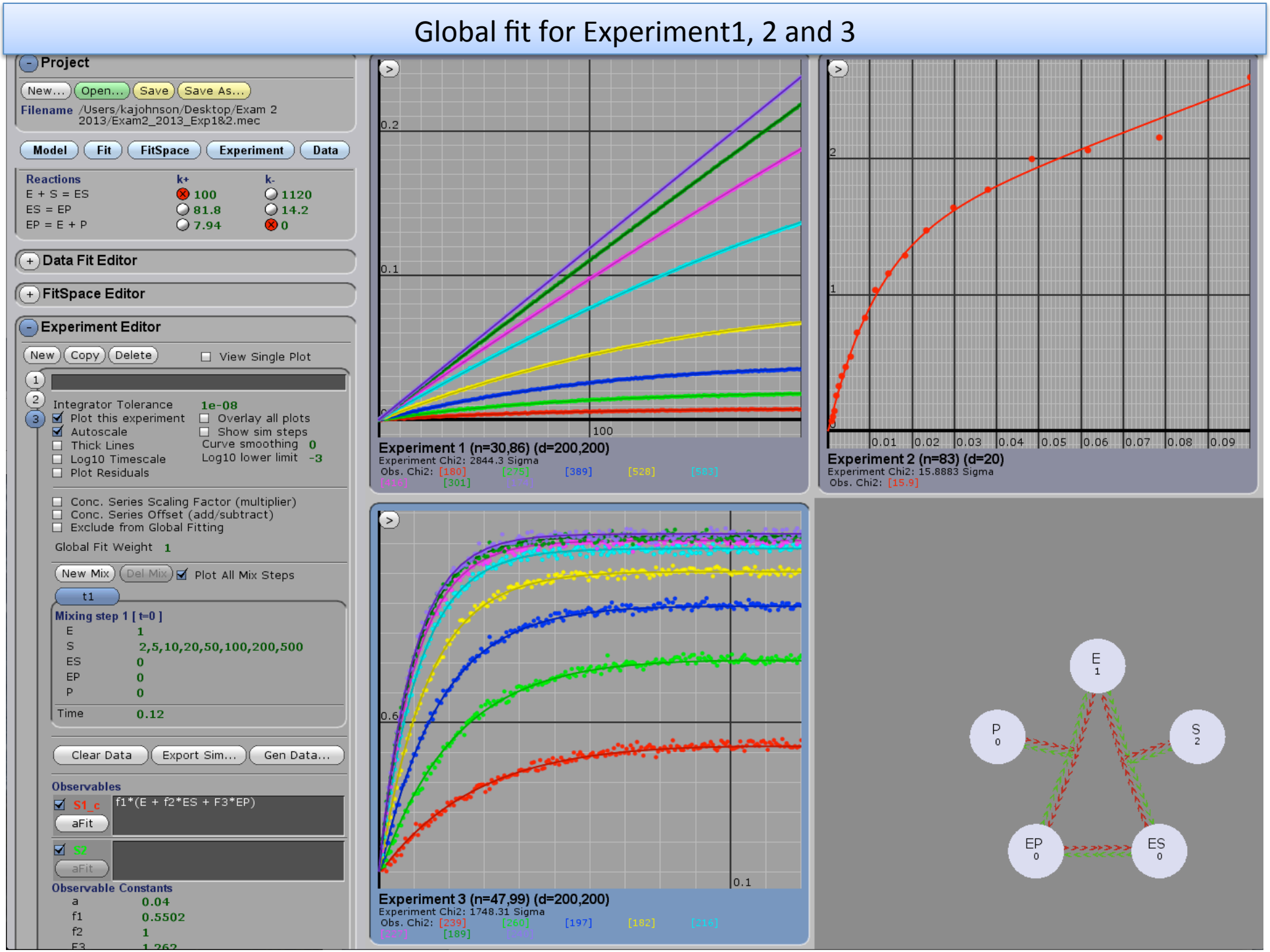
$$K_1 = 0.073 \mu M^{-1}$$

$$1/K_1 = 13.7 \mu M$$

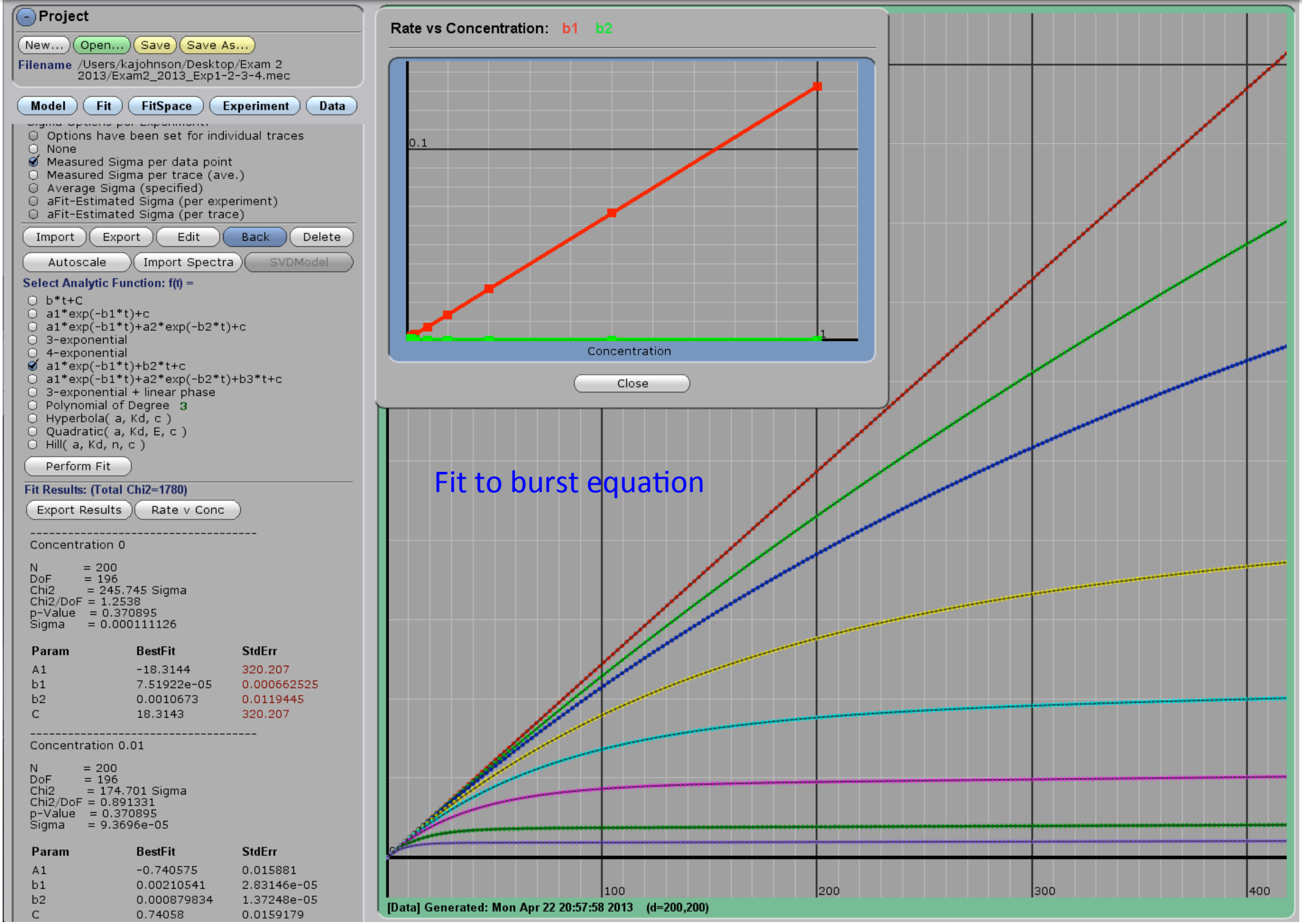
Global fit for Experiment 3



Global fit for Experiment1, 2 and 3



Conventional fit for Experiment 4



Conventional fit for Experiment 4

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EI$$

$$[P]/[E_0] = \frac{v_i - v_f}{k_{obs}} \cdot (1 - e^{-k_{obs} t}) + v_f \cdot t$$

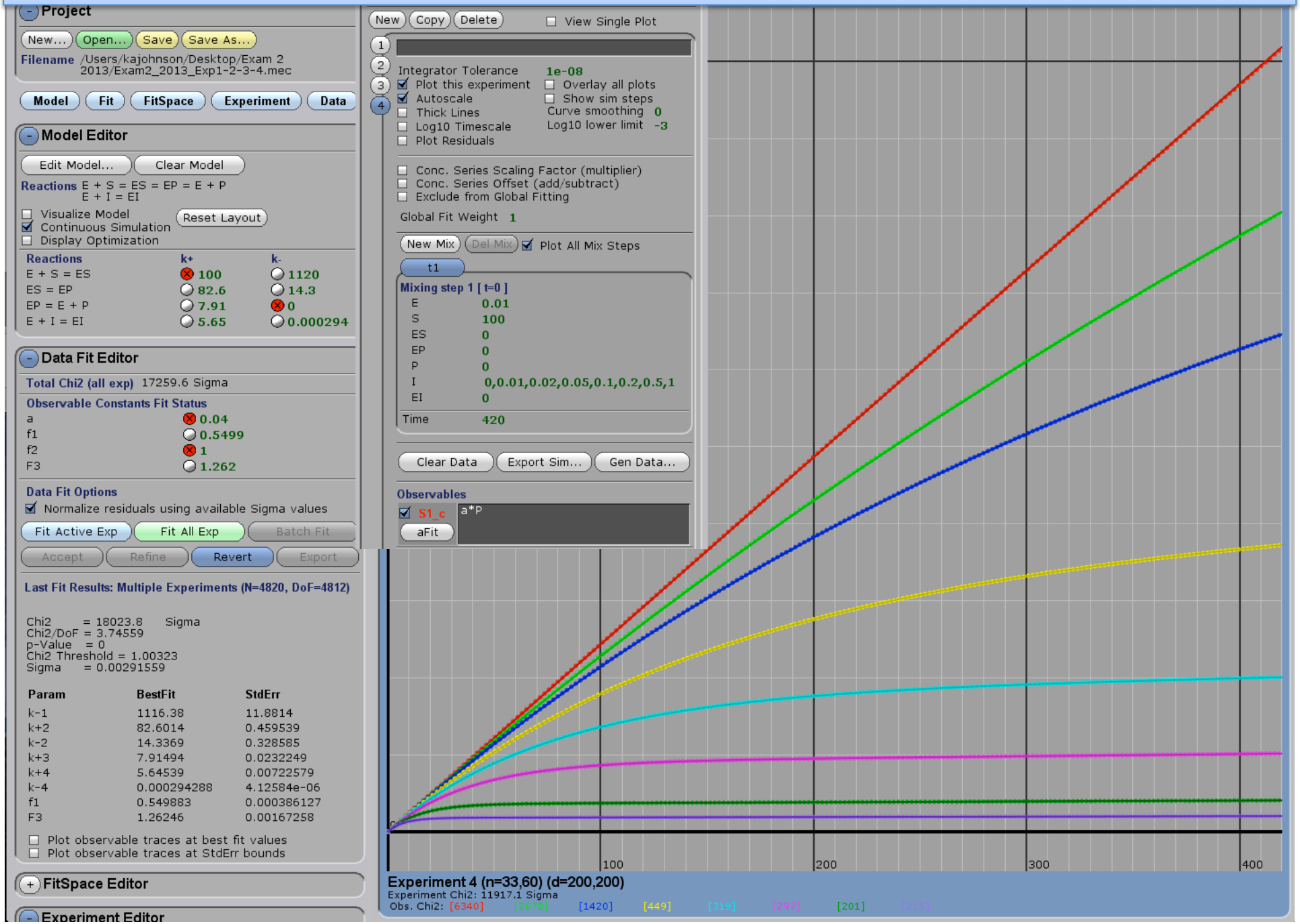
$$k_{obs} = \frac{k_3 [I]}{1 + [S]/K_m} + k_{-3}$$

$$slope = \frac{k_3}{1 + [S]/K_m} = 0.132 \mu M^{-1} s^{-1}$$

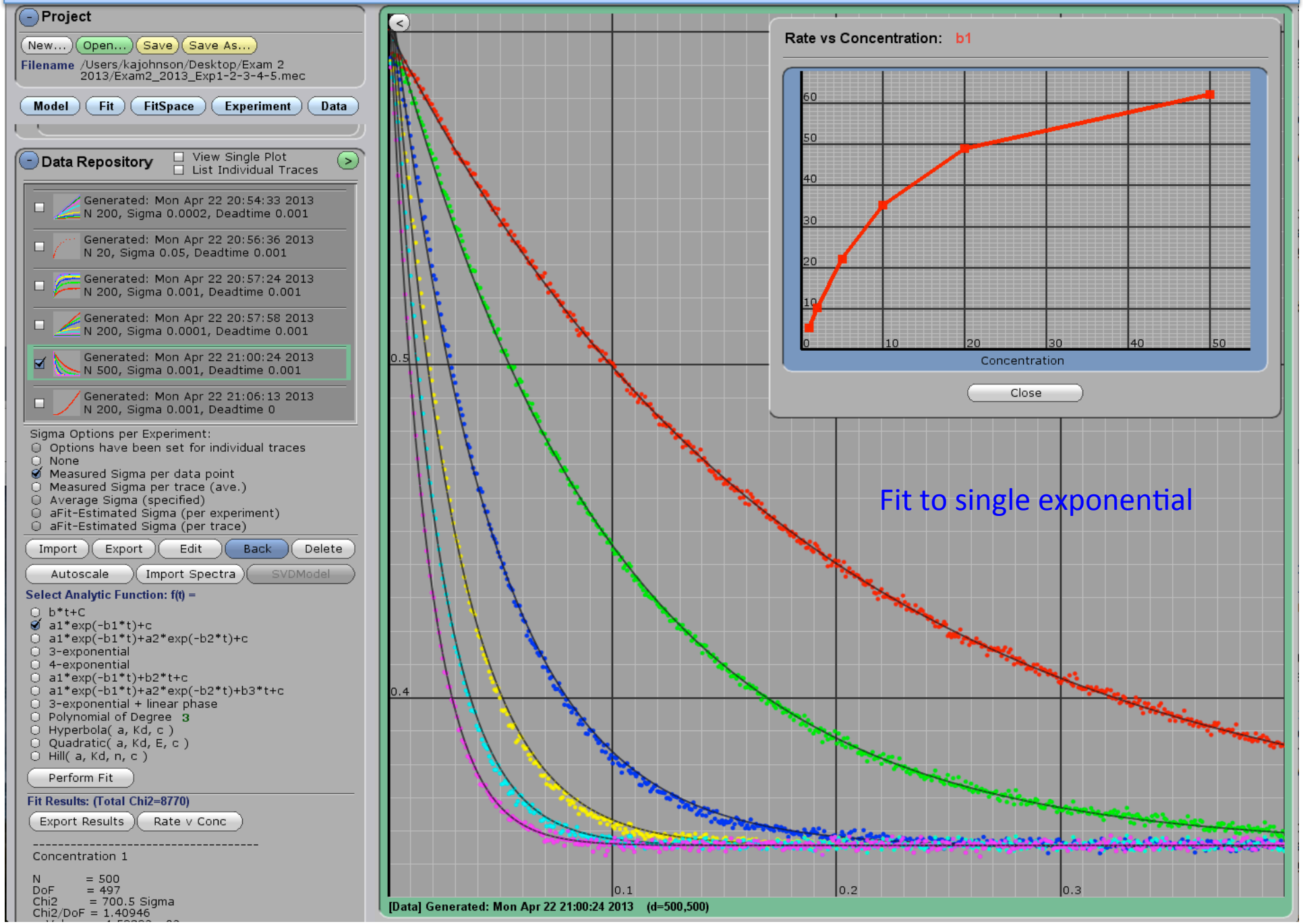
$$k_3 = slope \cdot (1 + [S]/K_m) = 0.132 \cdot (1 + 100 \mu M / 2.55 \mu M)$$

$$k_3 = 5.3 \mu M^{-1} s^{-1}$$

Global fit for Experiment 4



Conventional fit for Experiment 5



Experiment 5: Two-step inhibitor binding

Fit time course to single exponential: $Y = A_1 \cdot e^{-\lambda_1 \cdot t} + c$

$$E + I \underset{k_{-2}}{\overset{K_1}{\rightleftharpoons}} EI \overset{k_2}{\rightleftharpoons} FI$$

$$\lambda_1 = k_2 \frac{K_1[S]}{K_1[S]+1} + k_{-2}$$

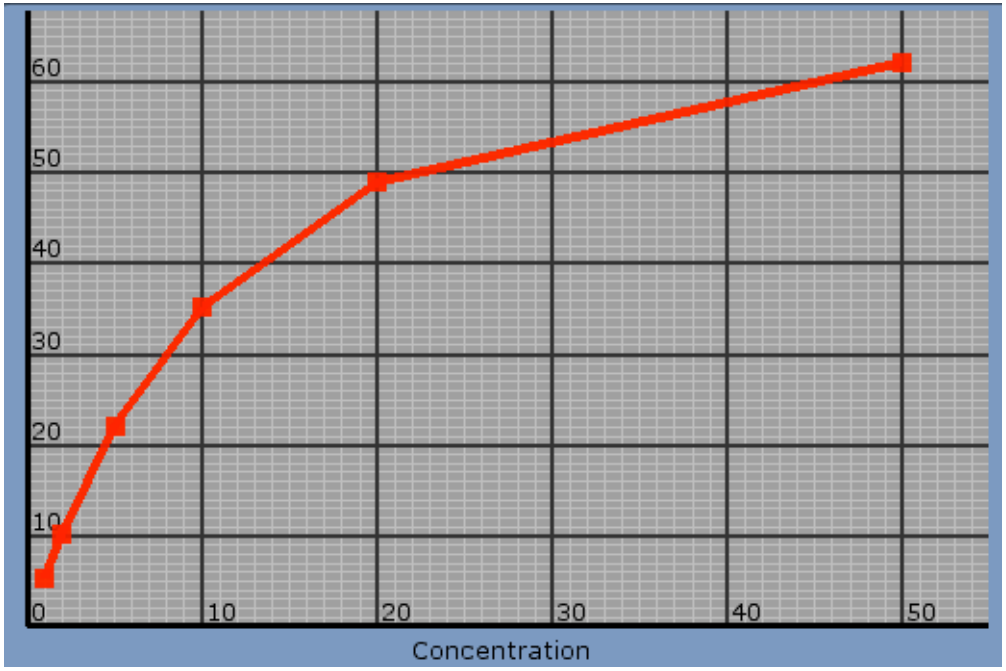
$k_2 = 78.3 s^{-1}$

$K_1 = 0.0866 \mu M^{-1}$

$1/K_1 = 11.5 \mu M$

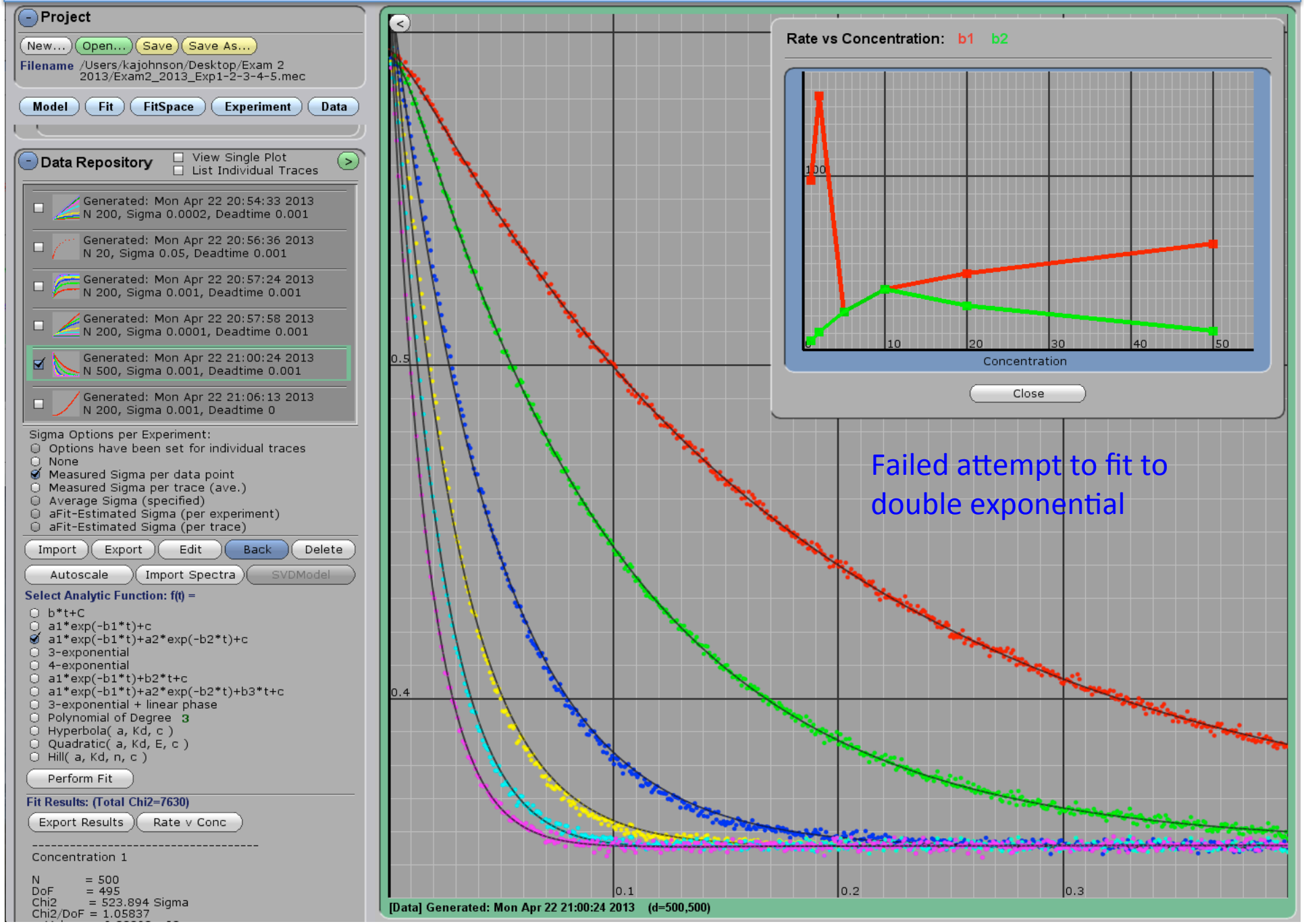
$k_{-2} \sim 0$

$K_1 k_2 = 6.8 \mu M^{-1} s^{-1}$

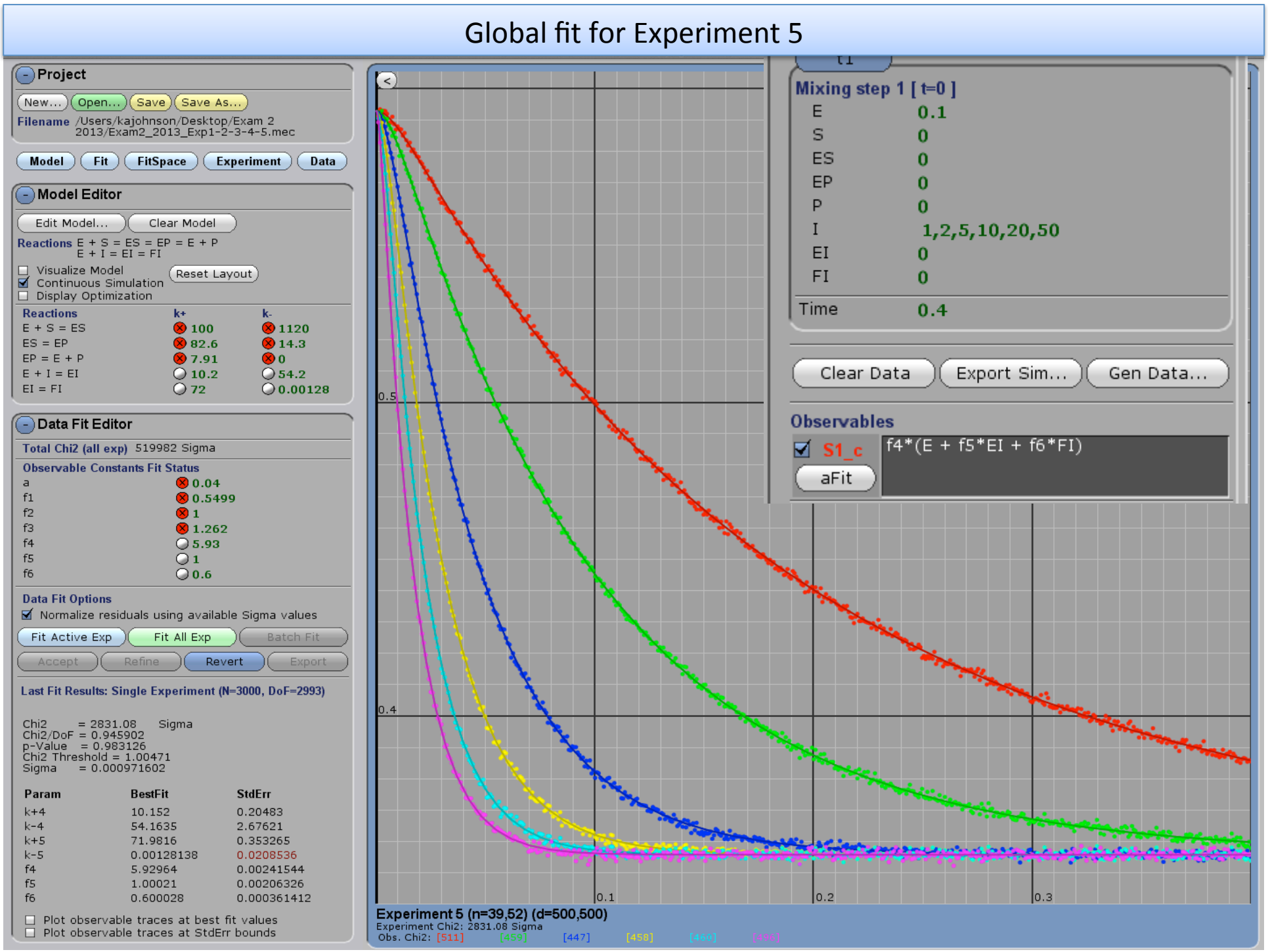


The slow onset inhibition experiment only went to 1 µM, so it was still in the linear portion of the inhibitor concentration dependence. The apparent second order rate constant is defined by $K_1 k_2$ = 6.8 $\mu M^{-1} s^{-1}$.

Conventional fit for Experiment 5



Global fit for Experiment 5



Global fit for Experiment 1, 2, 3, 4 and 5

Project

New... Open... Save Save As...

Filename /Users/kajohnson/Desktop/Exam 2 2013/Exam2_2013_Exp1-2-3-4-5.mec

Model Fit FitSpace Experiment Data

Model Editor

Edit Model... Clear Model

Reactions E + S = ES = EP = E + P
E + I = EI = FI

☐ Visualize Model ☑ Continuous Simulation ☐ Display Optimization Reset Layout

Reactions	k+	k-
E + S = ES	⊗ 100	○ 1120
ES = EP	○ 82.6	○ 14.1
EP = E + P	○ 7.89	⊗ 0
E + I = EI	○ 9.14	○ 44.8
EI = FI	○ 72.9	○ 0.000769

Data Fit Editor

Total Chi2 (all exp) 20995.3 Sigma

Observable Constants Fit Status

a	⊗ 0.04
f1	⊗ 0.5499
f2	⊗ 1
f3	⊗ 1.262
f4	○ 5.906
f5	○ 1.002
f6	○ 0.6022

Data Fit Options

☑ Normalize residuals using available Sigma values

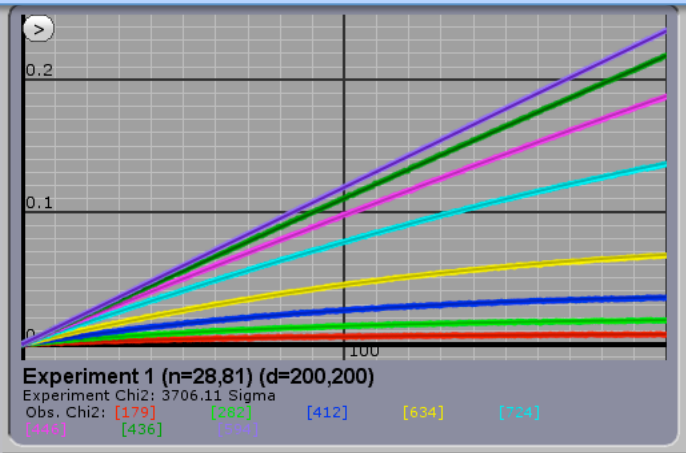
Fit Active Exp Fit All Exp Batch Fit

Accept Refine Revert Export

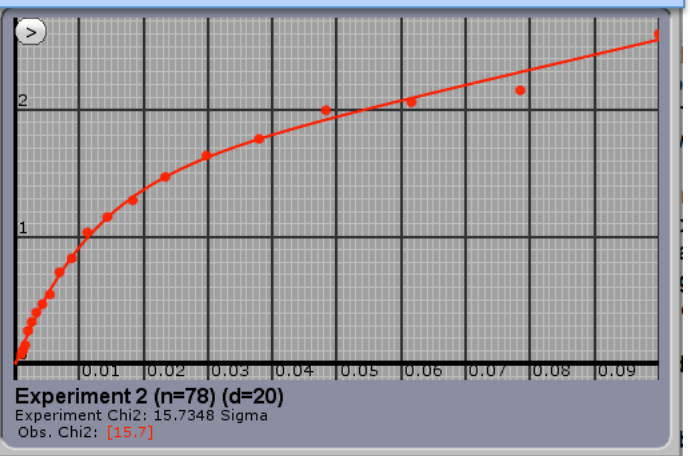
Last Fit Results: Multiple Experiments (N=7820, DoF=7809)

Chi2 = 21766.3 Sigma
Chi2/DoF = 2.78733
p-Value = 0
Chi2 Threshold = 1.00252
Sigma = 0.00239683

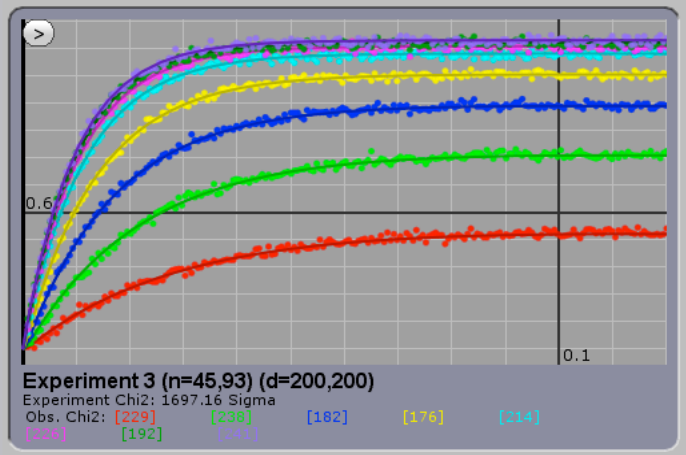
Param	BestFit	StdErr
k-1	1123.41	4.61212
k+2	82.5775	0.392391
k-2	14.0745	0.164304
k+3	7.89328	0.0083673
k+4	9.14284	0.0410335
k-4	44.7801	0.505643
k+5	72.8875	0.487039
k-5	0.000768581	7.28284e-06
f4	5.90594	0.00298008
f5	1.00195	0.00245457



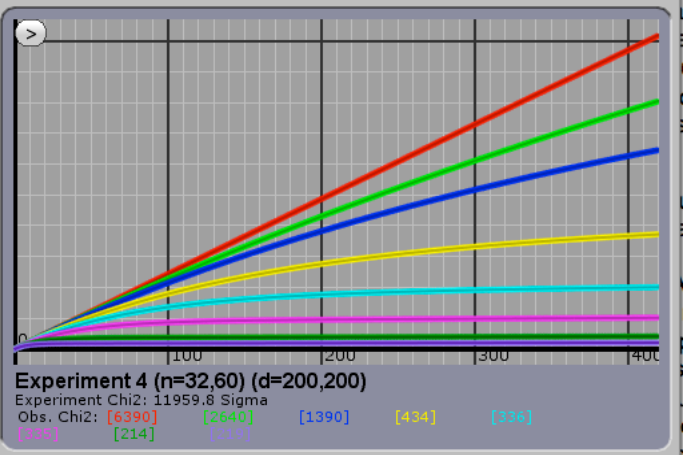
Experiment 1 (n=28,81) (d=200,200)
Experiment Chi2: 3706.11 Sigma
Obs. Chi2: [179] [282] [412] [634] [724]
[446] [436]



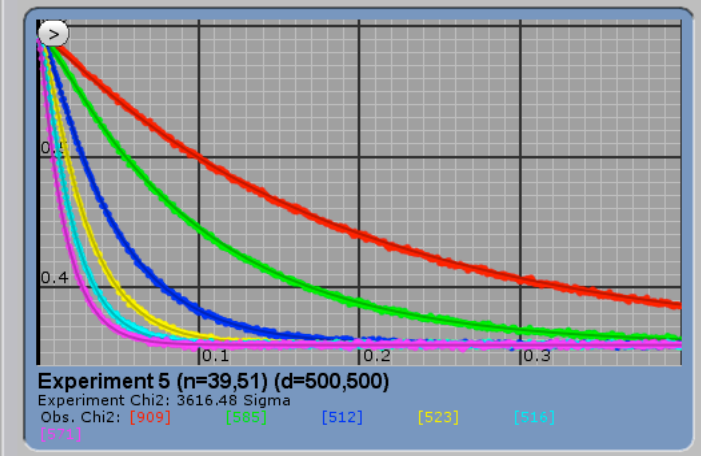
Experiment 2 (n=78) (d=20)
Experiment Chi2: 15.7348 Sigma
Obs. Chi2: [15.7]



Experiment 3 (n=45,93) (d=200,200)
Experiment Chi2: 1697.16 Sigma
Obs. Chi2: [229] [238] [182] [176] [214]
[226] [192]



Experiment 4 (n=32,60) (d=200,200)
Experiment Chi2: 11959.8 Sigma
Obs. Chi2: [6390] [2640] [1390] [434] [336]
[335] [214]



Experiment 5 (n=39,51) (d=500,500)
Experiment Chi2: 3616.48 Sigma
Obs. Chi2: [909] [585] [512] [523] [516]
[571]

Conventional fit for Experiment 6

Project

New... Open... Save Save As...

Filename /Volumes/Disk_2/Chem_394/Exam2/Exam 2 2013/Global_Fit_answers.mec

Model Fit FitSpace Experiment Data

Generated: Mon Apr 22 20:57:58 2013
N 200, Sigma 0.0001, Deadtime 0.001

Generated: Mon Apr 22 21:00:24 2013
N 500, Sigma 0.001, Deadtime 0.001

Generated: Thu May 2 12:56:05 2013
N 200, Sigma 0.001, Deadtime 0

Sigma Options per Experiment:
- Options have been set for individual traces
- None
- Measured Sigma per data point
- Measured Sigma per trace (ave.)
- Average Sigma (specified)
- aFit-Estimated Sigma (per experiment)
- aFit-Estimated Sigma (per trace)

Import Export Edit Back Delete

Autoscale Import Spectra SVDModel

Select Analytic Function: f(t) =
- b*t+C
- a1*exp(-b1*t)+c
- a1*exp(-b1*t)+a2*exp(-b2*t)+c
- 3-exponential
- 4-exponential
- a1*exp(-b1*t)+b2*t+c
- a1*exp(-b1*t)+a2*exp(-b2*t)+b3*t+c
- 3-exponential + linear phase
- Polynomial of Degree 3
- Hyperbola(a, Kd, c)
- Quadratic(a, Kd, E, c)
- Hill(a, Kd, n, c)

Perform Fit

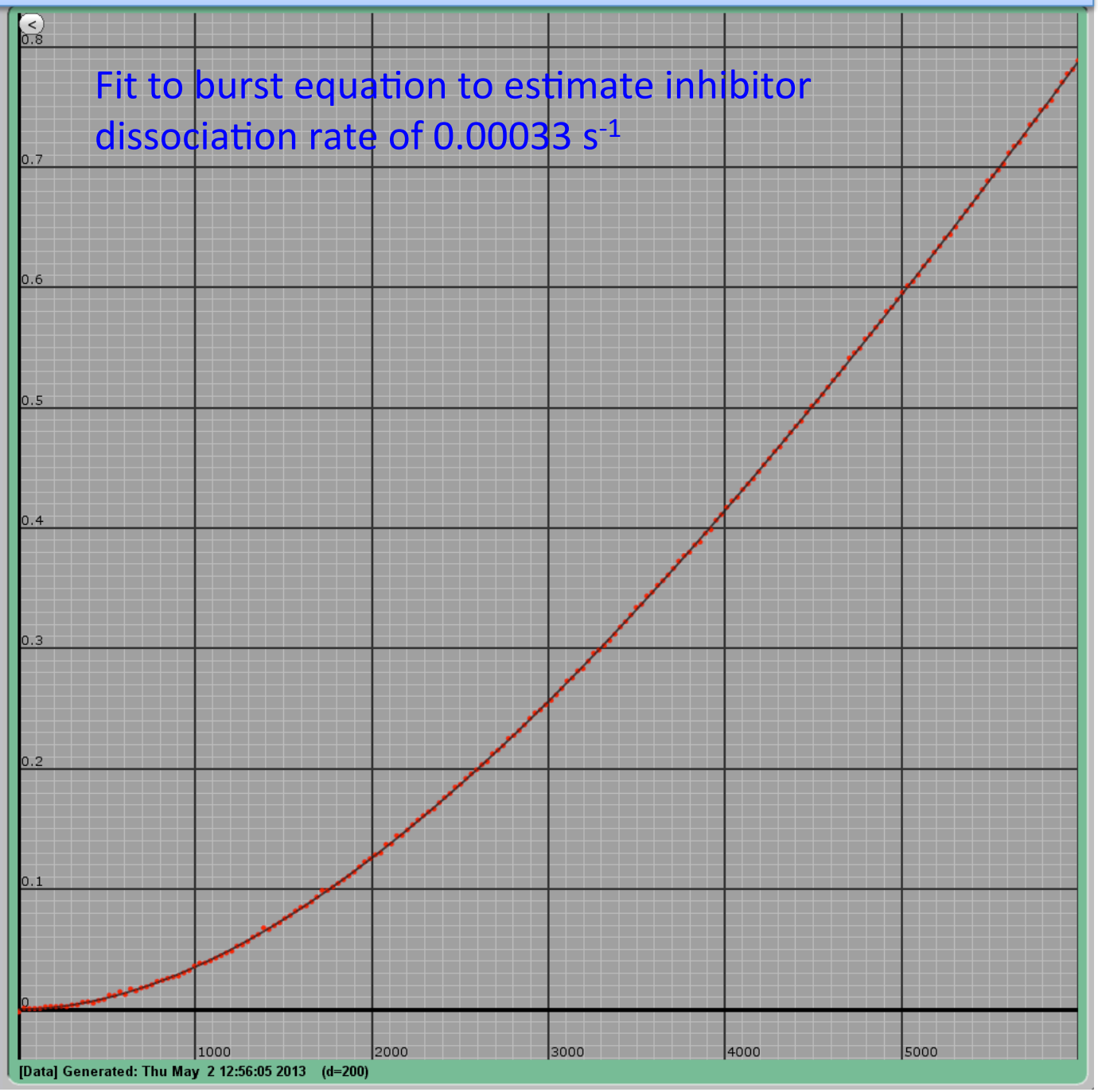
Fit Results: (Total Chi2=228)

Export Results

N = 200
DoF = 196
Chi2 = 227.536 Sigma
Chi2/DoF = 1.1609
p-Value = 0.0608301
Sigma = 0.0010693

Param	BestFit	StdErr
A1	0.695181	0.0186797
b1	0.000330947	7.50383e-06
b2	0.000231215	2.10524e-06
C	-0.695128	0.019072

Credits



Global fit for Experiment 6

Project

New... Open... Save Save As...

Filename /Volumes/Disk_2/Chem_394/Exam2/Exam 2 2013/Global_Fit_answers.mec

Model | Fit | FitSpace | Experiment | Data

Edit Model... Clear Model

Reactions E + S = ES = EP = E + P
E + I = EI = FI

☐ Visualize Model Reset Layout
☑ Continuous Simulation
☐ Display Optimization

Reactions	k+	k-
E + S = ES	56.7	601
ES = EP	82.4	14.5
EP = E + P	7.96	0.0862
E + I = EI	10	52.8
EI = FI	72	0.00072

Data Fit Editor

Total Chi2 (all exp) 7890.75 Sigma

Observable Constants Fit Status

a	0.04
f0	0.5501
f1	1
f2	1.262
g1	5.928
g2	0.6002

Data Fit Options

☑ Normalize residuals using available Sigma values

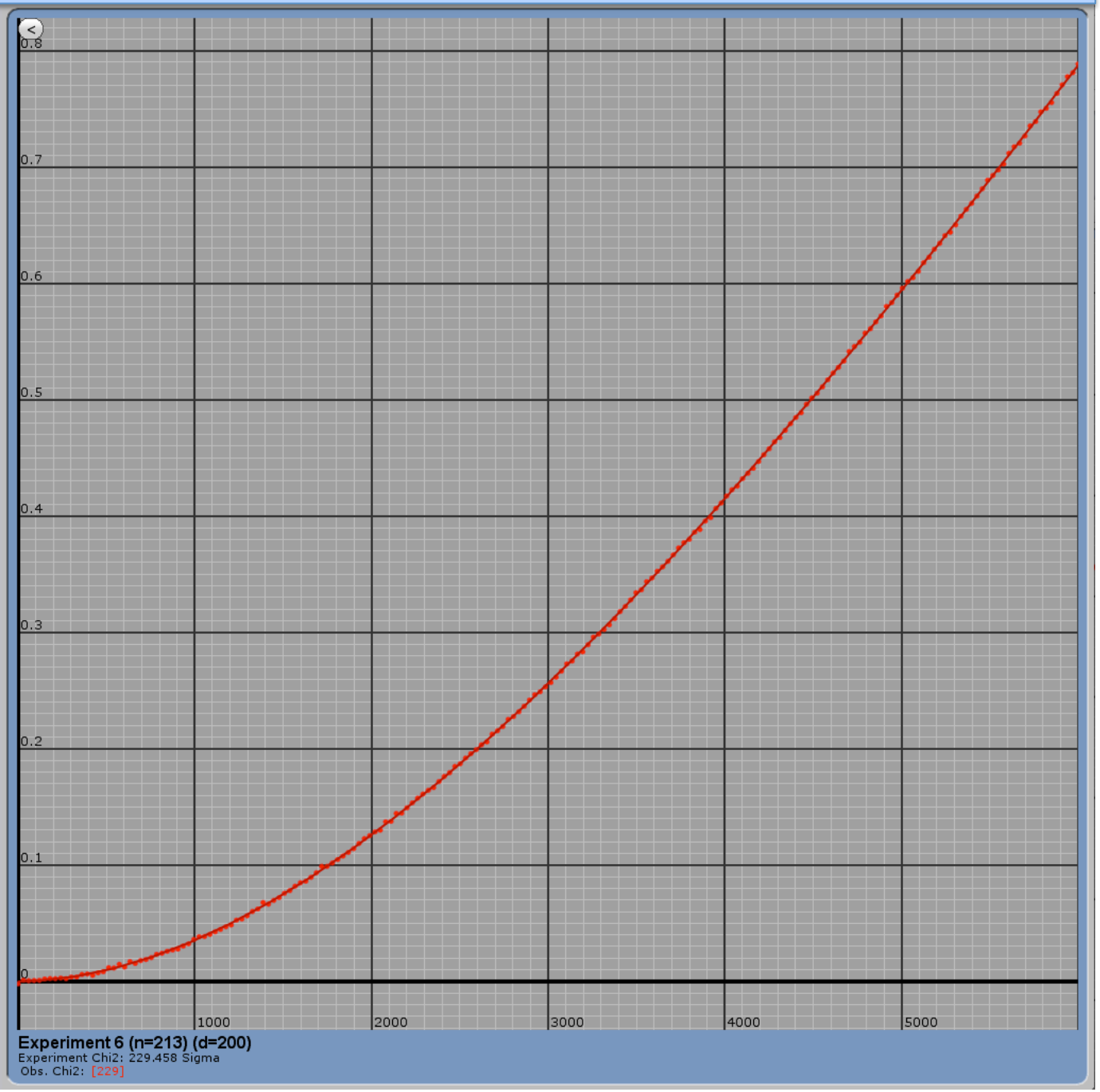
Fit Active Exp | Fit All Exp | Batch Fit

Last Fit Results: Multiple Experiments (N=8020, DoF=8007)
Settings have changed since this fit was computed.

Chi2 = 7890.75 Mixed Sigma
Chi2/DoF = 0.985482
p-Value = 0
Chi2 Threshold = 1.00279
Sigma = 0.00233758

Param	BestFit	StdErr
k+1	56.7471	1.02661
k-1	600.955	12.2941
k+2	82.4011	0.664723
k-2	14.5357	0.271535
k+3	7.958	0.0327142
k+4	10.0348	0.0373336
k-4	52.7965	0.491297
k+5	71.9988	0.22064
k-5	0.000719538	5.80544e-06
f0	0.550091	0.000222698
f2	1.26217	0.00155288
g1	5.92848	0.00155873
g2	0.600166	0.000178663

☐ Plot observable traces at best fit values



Global fit for Experiments 1 - 6

Project

New... Open... Save Save As...

Filename /Volumes/Disk_2/Chem_394/Exam2/Exam 2 2013/Global_Fit_answers.mec

Model Fit FitSpace Experiment Data

Model Editor

Edit Model... Clear Model

Reactions E + S = ES = EP = E + P
E + I = EI = FI

☐ Visualize Model Reset Layout
☑ Continuous Simulation
☐ Display Optimization

Reactions	k+	k-
E + S = ES	56.8	601
ES = EP	82.4	14.6
EP = E + P	7.96	0.0862
E + I = EI	10	52.7
EI = FI	72.1	0.000721

Data Fit Editor

Total Chi2 (all exp) 7892.92 Sigma

Observable Constants Fit Status

a	0.04
f1	0.5501
f2	1
f3	1.262
f4	5.929
f5	1
f6	0.6002

Data Fit Options

☑ Normalize residuals using available Sigma values

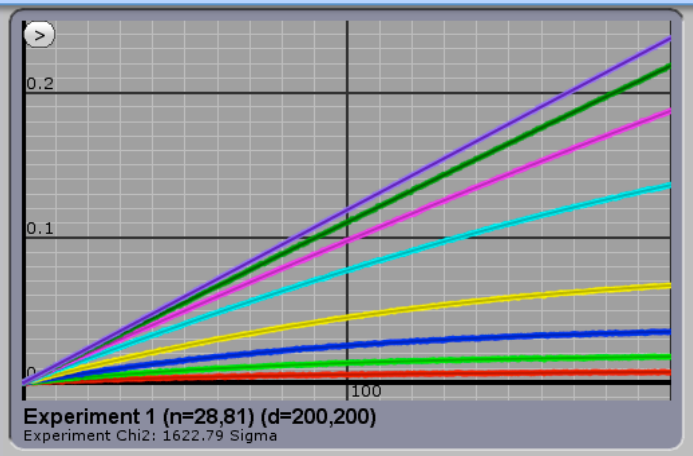
Fit Active Exp Fit All Exp Batch Fit

Accept Refine Revert Export

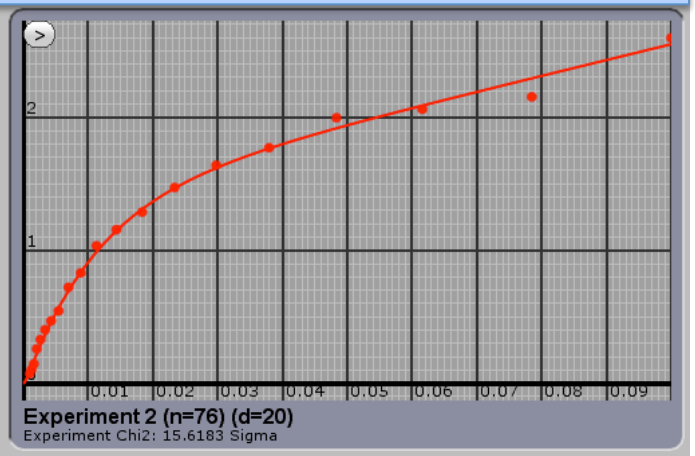
Last Fit Results: Multiple Experiments (N=8020, DoF=8007)

Chi2 = 7892.92 Sigma
Chi2/DoF = 0.985752
p-Value = 0.816083
Chi2 Threshold = 1.00279
Sigma = 0.00233666

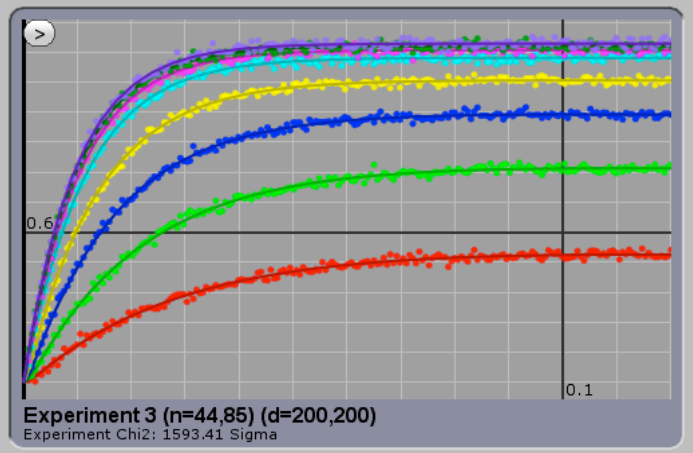
Param	BestFit	StdErr
k+1	56.785	0.380638
k-1	601.038	4.99913
k+2	82.4136	0.643113
k-2	14.5527	0.260023
k+3	7.9591	0.032268
k+4	10.0206	0.00903301
k-4	52.6674	0.169156
k+5	72.0674	0.204701
k-5	0.000720997	4.64321e-06
f1	0.550076	0.000220153



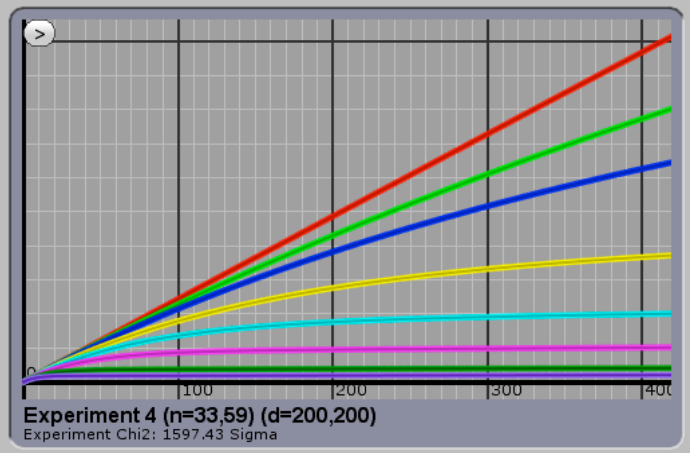
Experiment 1 (n=28,81) (d=200,200)
Experiment Chi2: 1622.79 Sigma



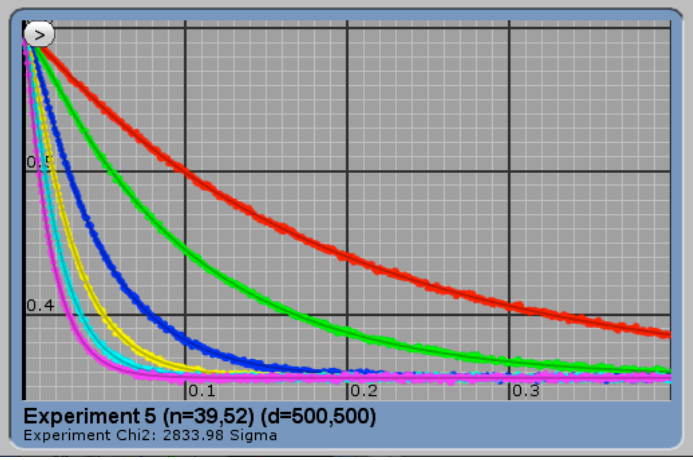
Experiment 2 (n=76) (d=20)
Experiment Chi2: 15.6183 Sigma



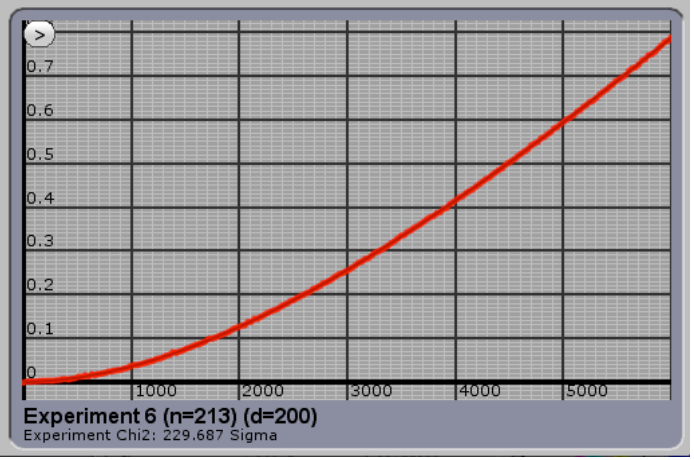
Experiment 3 (n=44,85) (d=200,200)
Experiment Chi2: 1593.41 Sigma



Experiment 4 (n=33,59) (d=200,200)
Experiment Chi2: 1597.43 Sigma



Experiment 5 (n=39,52) (d=500,500)
Experiment Chi2: 2833.98 Sigma



Experiment 6 (n=213) (d=200)
Experiment Chi2: 229.687 Sigma

Global fit for Experiments 1 - 6

$$E + S \underset{601 s^{-1}}{\overset{56.7 \mu M^{-1} s^{-1}}{\rightleftharpoons}} E \bullet S \underset{14.5 s^{-1}}{\overset{82.4 s^{-1}}{\rightleftharpoons}} E \bullet P \xrightarrow{8 s^{-1}} E + P$$

$$E + I \underset{52.8 s^{-1}}{\overset{10 \mu M^{-1} s^{-1}}{\rightleftharpoons}} E \bullet I \underset{0.00072 s^{-1}}{\overset{72 s^{-1}}{\rightleftharpoons}} F \bullet I$$

$$K_m = 2.4 \mu M$$

$$k_{cat} = 6.25 s^{-1}$$

$$K_I = \frac{1}{K_4 (1 + K_5)} = 0.000053 \mu M$$

The fluorescence changes occur on forming EP and FI only.

Note that $1/K_4$=5.28 µM, and Experiment 4 only went up to 1 µM, thus explaining the linear dependence of rate on inhibitor concentration, even though there is a two-step binding reaction.