

**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>

- Visualize Model
- Display Model Values
- Continuous Simulation
- Display Optimization

Reactions k+ k-

**Data Fit Editor**

**Data Fit Options**

- 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

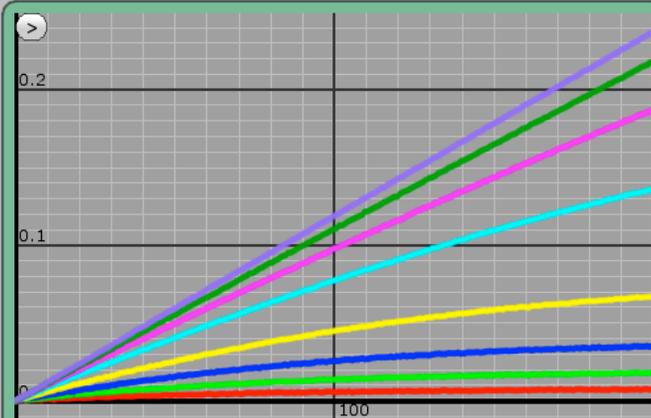
**Experiment Editor**

New Copy Delete  View Single Plot

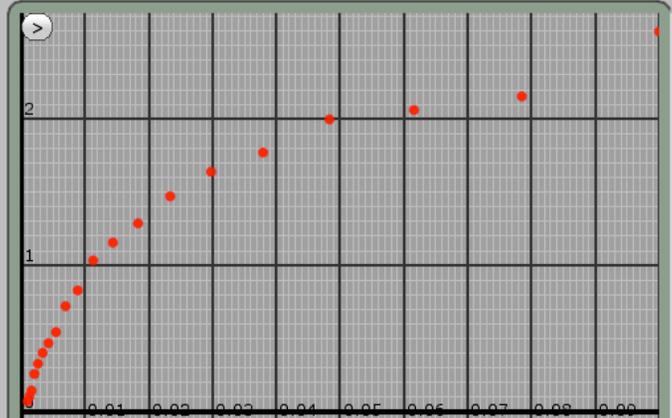
**Data Repository**

- View Single Plot
- List Individual Traces

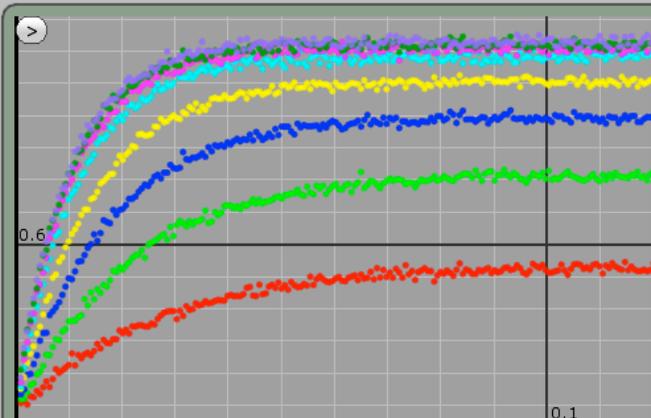
- Generated: Mon Apr 22 20:54:33 2013  
N 200, Sigma 0.0002, Deadtime 0.001
- Generated: Mon Apr 22 20:56:36 2013  
N 20, Sigma 0.05, Deadtime 0.001
- Generated: Mon Apr 22 20:57:24 2013  
N 200, Sigma 0.001, Deadtime 0.001
- Generated: Mon Apr 22 20:57:58 2013  
N 200, Sigma 0.0001, Deadtime 0.001
-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



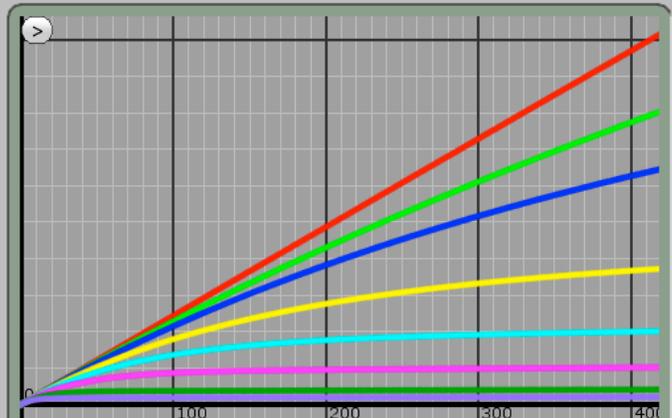
[Data] Generated: Mon Apr 22 20:54:33 2013 (d=200,200)



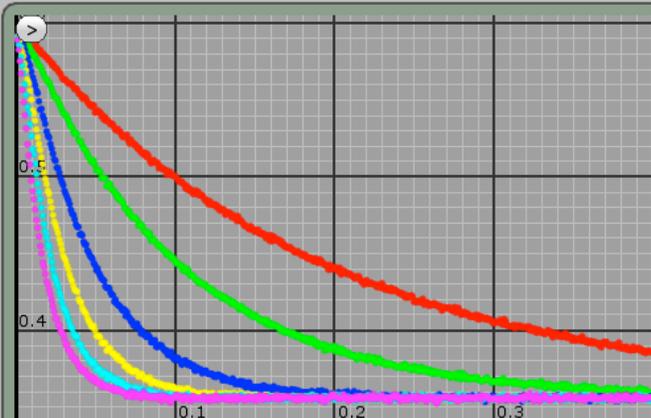
[Data] Generated: Mon Apr 22 20:56:36 2013 (d=20)



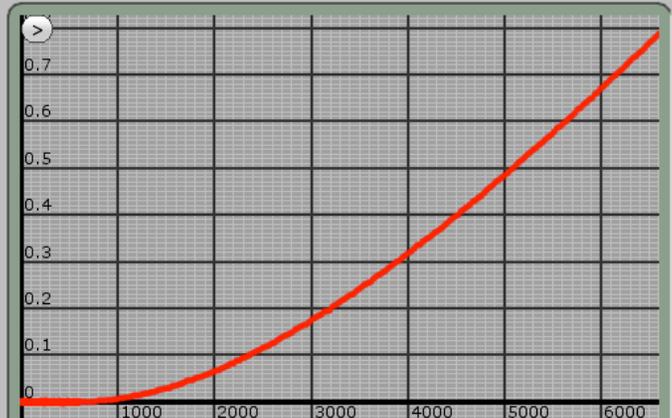
[Data] Generated: Mon Apr 22 20:57:24 2013 (d=200,200)



[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

## Project

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

Filename /Users/kajohnson/Desktop/Exam 2  
2013/Exam2\_2013\_data.mec

Model Fit FitSpace Experiment Data

## Data Repository

View Single Plot  
 List Individual Traces

- Generated: Mon Apr 22 20:54:33 2013  
N 200, Sigma 0.0002, Deadtime 0.001
- Generated: Mon Apr 22 20:56:36 2013  
N 20, Sigma 0.05, Deadtime 0.001
- Generated: Mon Apr 22 20:57:24 2013  
N 200, Sigma 0.001, Deadtime 0.001
- 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: Mon Apr 22 21:06:13 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 )

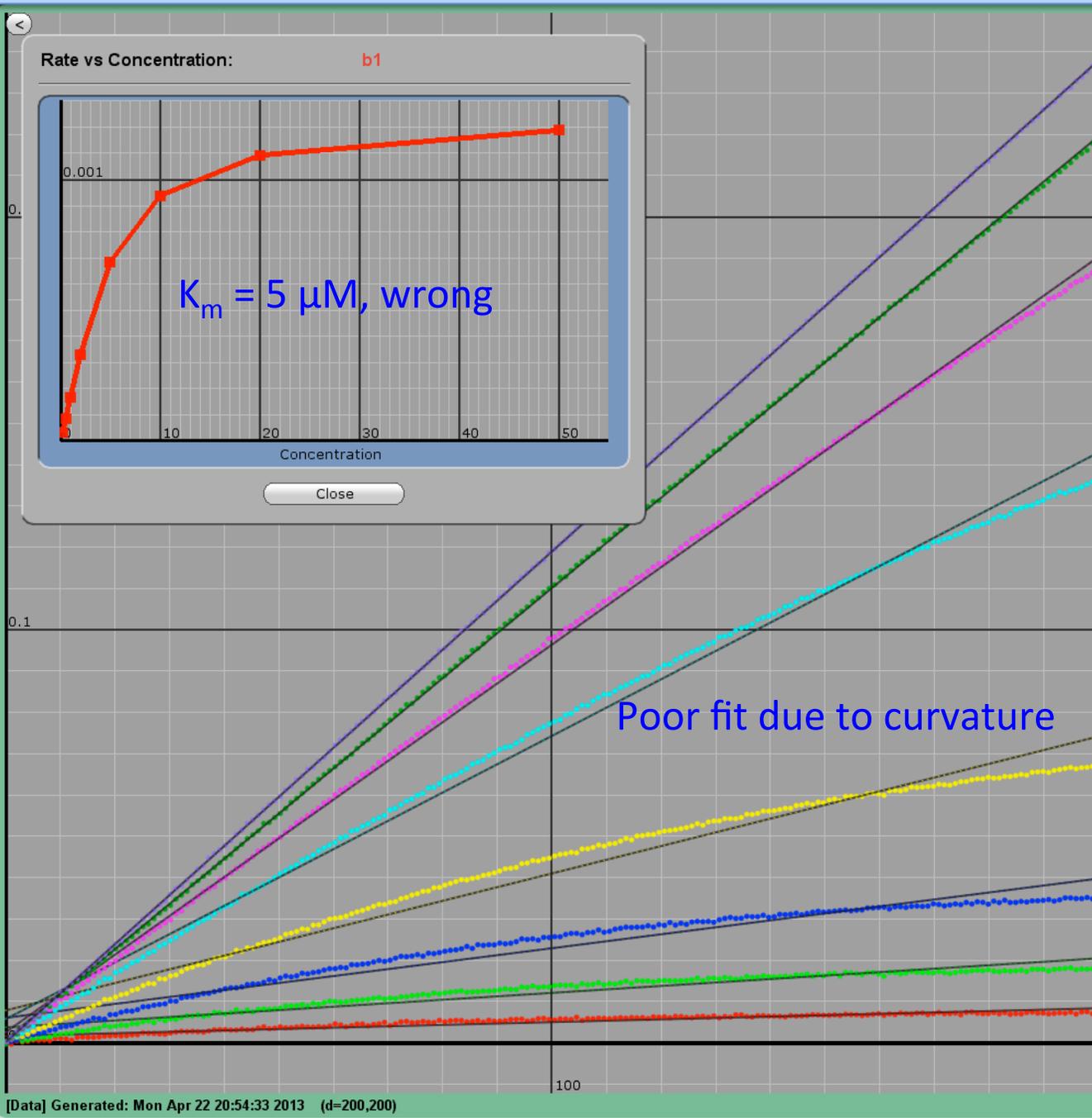
Perform Fit

### Fit Results: (Total Chi2=152000)

Export Results Rate v Conc

Concentration 0.2

N = 200  
DoF = 198  
Chi2 = 2128.33 Sigma  
Chi2/DoF = 10.7491  
p-Value = 0.208225



# Conventional fit for Experiment 3

**Project**

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

Filename /Users/kajohnson/Desktop/Exam 2 2013/Exam2\_2013\_data.mec

Model Fit FitSpace Experiment Data

---

**Data Repository**  View Single Plot  List Individual Traces

- Generated: Mon Apr 22 20:54:33 2013  
N 200, Sigma 0.0002, Deadtime 0.001
- Generated: Mon Apr 22 20:56:36 2013  
N 20, Sigma 0.05, Deadtime 0.001
- Generated: Mon Apr 22 20:57:24 2013  
N 200, Sigma 0.001, Deadtime 0.001
- 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: Mon Apr 22 21:06:13 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

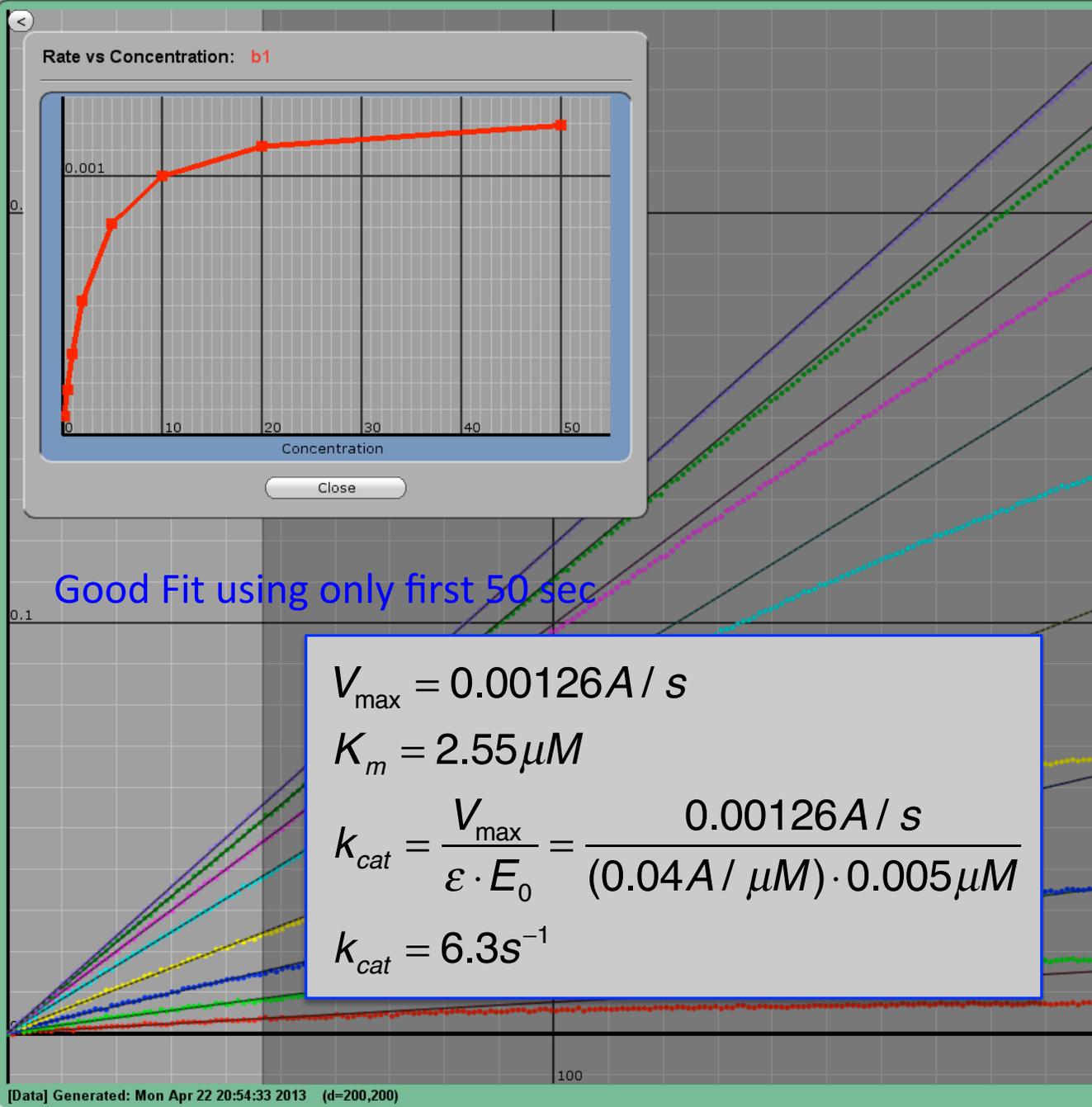
Fit Results: (Total Chi2=542)

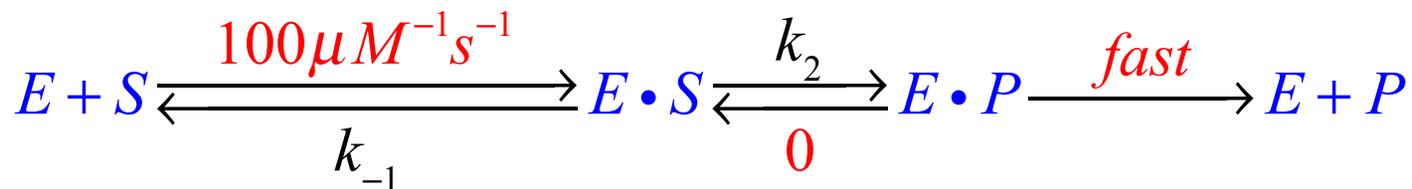
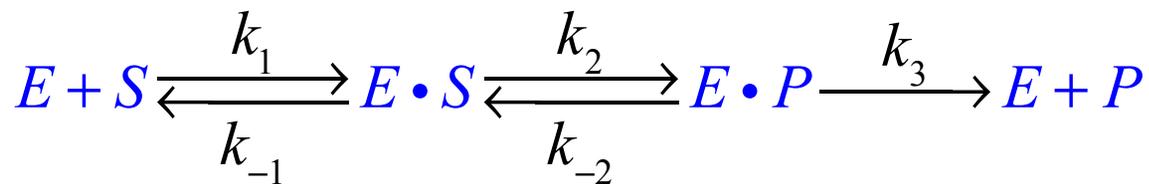
Export Results Rate v Conc

---

Concentration 0.2

N = 47  
DoF = 45  
Chi2 = 40.7381 Sigma  
Chi2/DoF = 0.90529  
p-Value = 0.415793



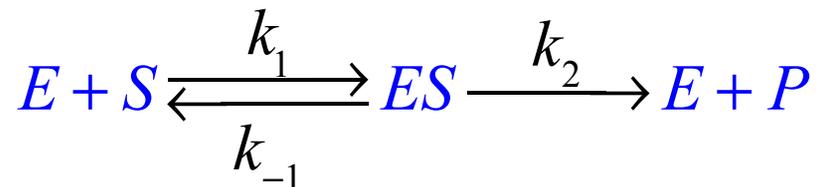


Simplifying approximation for global fitting

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

$$k_{cat} = k_2$$

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



# Global fit for Experiment 1

**Project**

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

Filename /Users/kajohnson/Desktop/Exam 2 2013/Exam2\_2013\_data.mec

Model Fit FitSpace Experiment Data

Visualize Model

Display Model Values

Continuous Simulation

Display Optimization

Reactions	k+	k-
E + S = ES	100	255
ES = EP	6.67	0
EP = E + P	100	0

**Data Fit Editor**

**FitSpace Editor**

**Experiment Editor**

New Copy Delete  View Single Plot

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 1

New Mix Del Mix  Plot All Mix Steps

t1

Mixing step 1 [t=0]

E 0.005

S 0.2, 0.5, 1, 2, 5, 10, 20, 50

ES 0

EP 0

P 0

Time 200

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

**Observables**

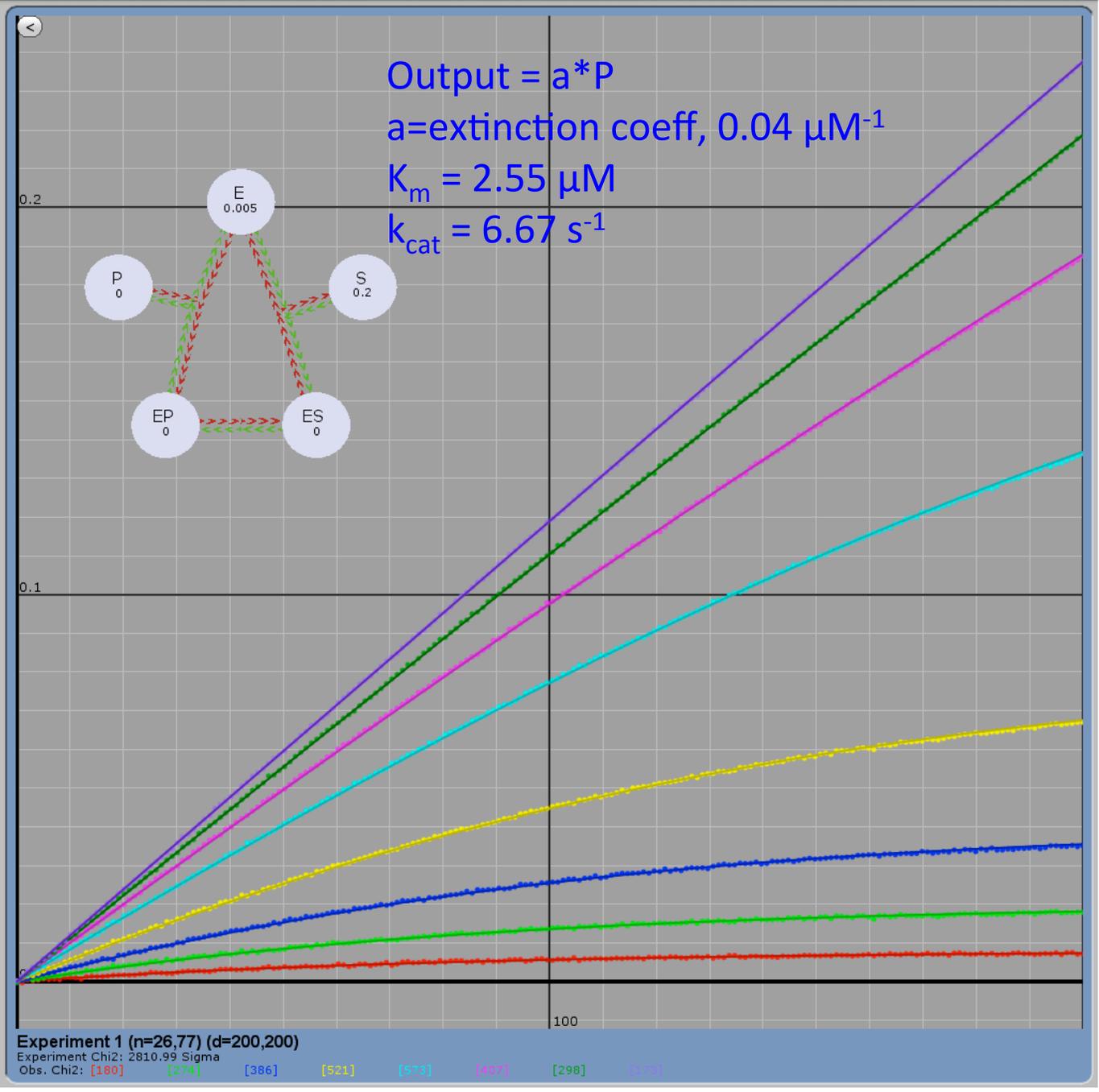
S1\_c a\*P

aFit

S2

aFit

Observable Constants



# Conventional fit for Experiment 2

## Project

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

Filename /Users/kajohnson/Desktop/Exam 2  
2013/Exam2\_2013\_data.mec

Model Fit FitSpace Experiment Data

- N 200, Sigma 0.001, Deadtime 0.001
- 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: Mon Apr 22 21:06:13 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

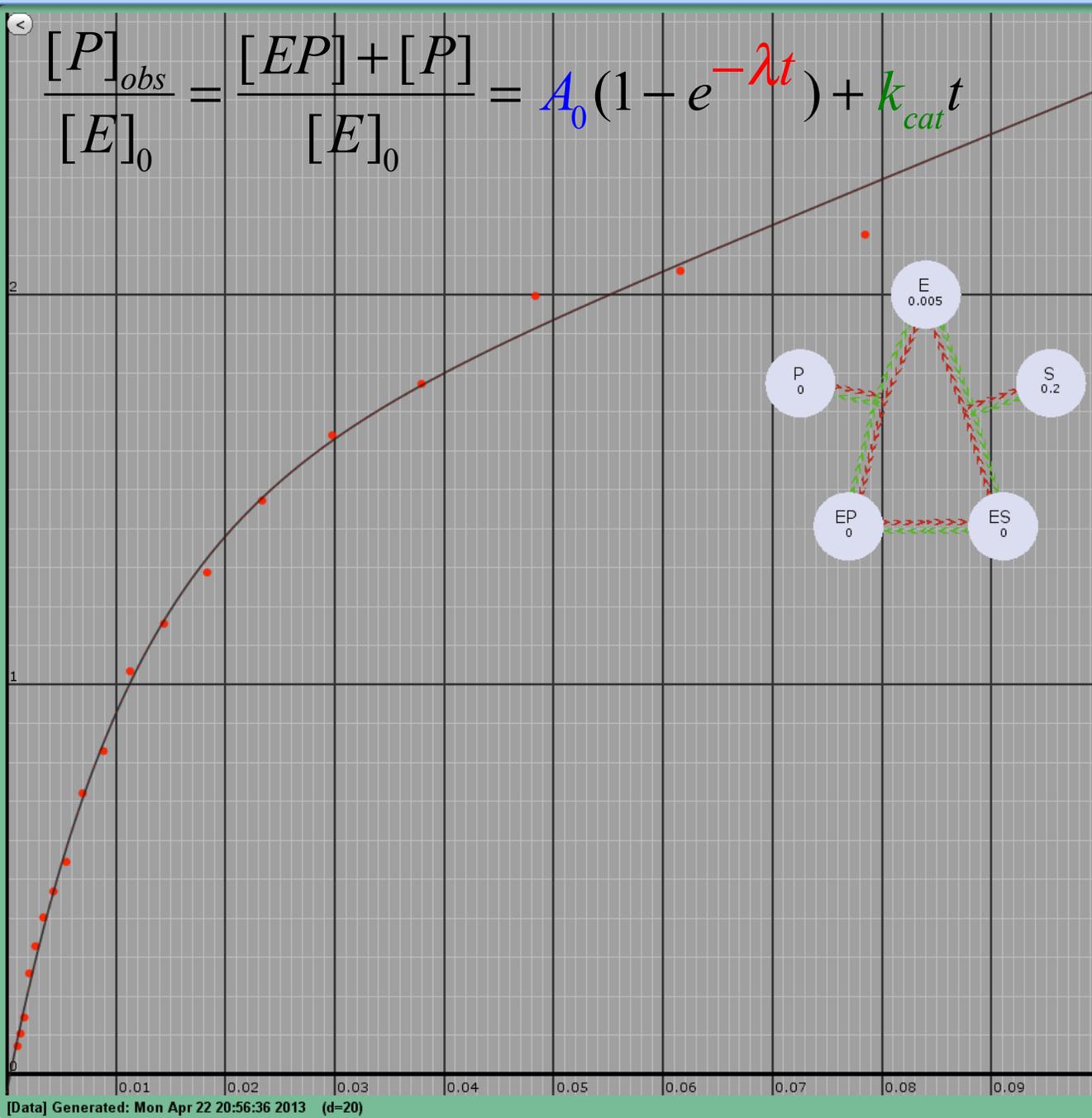
### Fit Results: (Total Chi2=14.6)

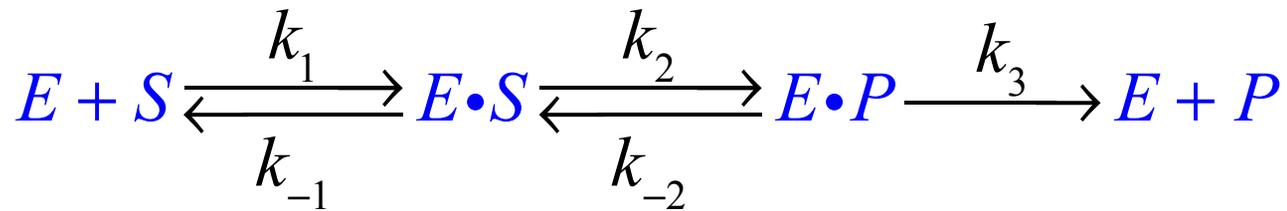
Export Results

N = 20  
DoF = 16  
Chi2 = 14.5998 Sigma  
Chi2/DoF = 0.912486  
p-Value = 0.554123  
Sigma = 0.0438295

Param	BestFit	StdErr
A1	-1.41648	0.136951
b1	92.7456	19.8504
b2	11.5754	2.12845
C	1.37041	0.150665

Credits





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

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

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

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

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

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

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

# 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...

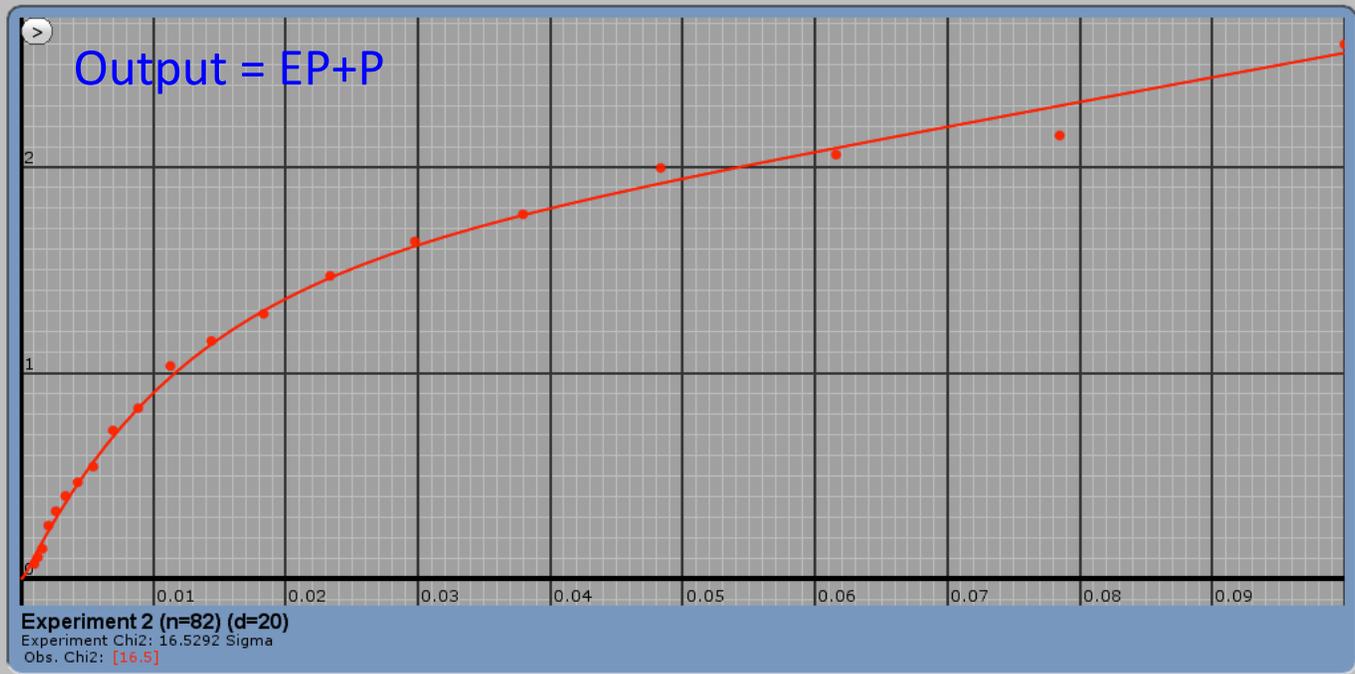
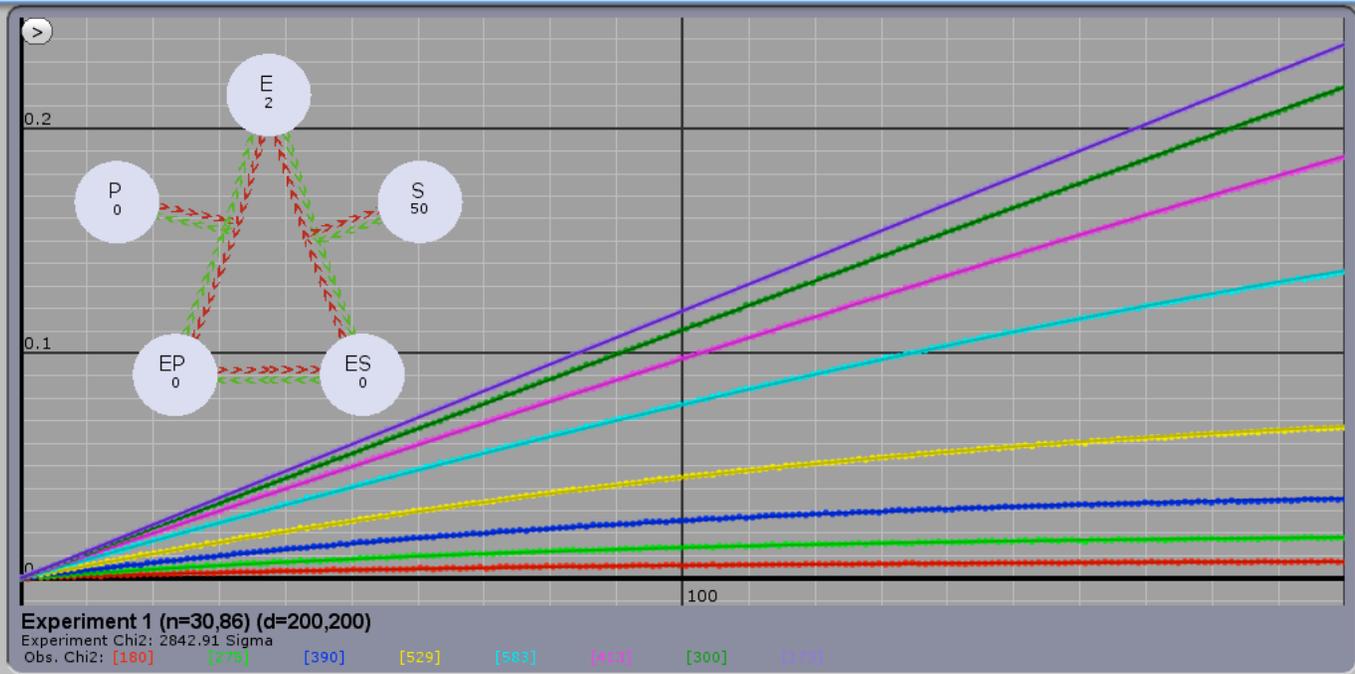
**Observables**

S1\_c EP + P  
aFit

S2  
aFit

**Observable Constants**

a **0.04**



# Conventional fit for Experiment 3

## Project

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

Filename /Users/kajohnson/Desktop/Exam 2  
2013/Exam2\_2013\_Exp1&2.mec

Model Fit FitSpace Experiment Data

a 0.04

## Data Repository

View Single Plot  
 List Individual Traces

- Generated: Mon Apr 22 20:54:33 2013  
N 200, Sigma 0.0002, Deadtime 0.001
- Generated: Mon Apr 22 20:56:36 2013  
N 20, Sigma 0.05, Deadtime 0.001
- Generated: Mon Apr 22 20:57:24 2013  
N 200, Sigma 0.001, Deadtime 0.001
- 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: Mon Apr 22 21:06:13 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 )

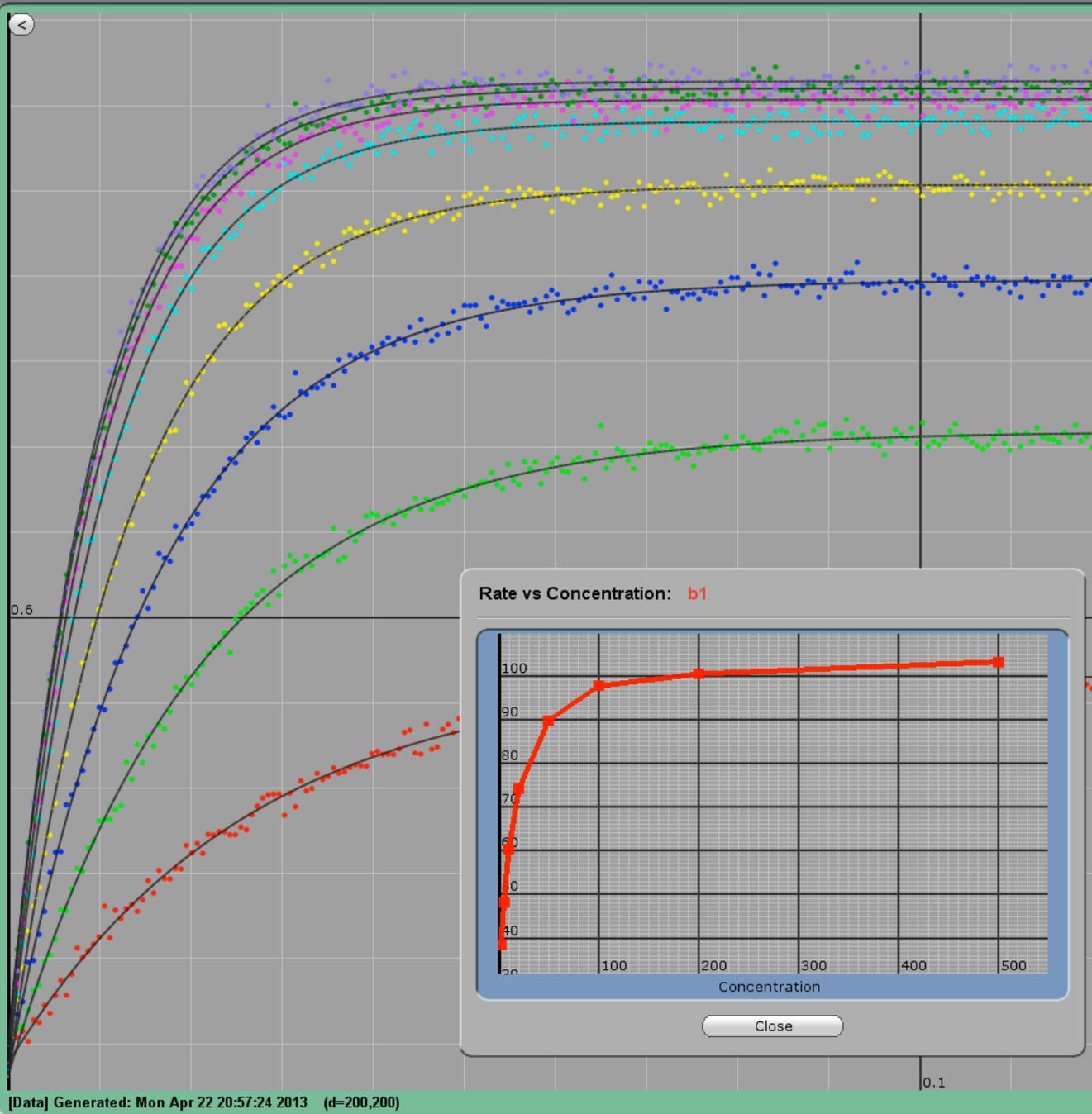
Perform Fit

### Fit Results: (Total Chi2=1590)

Export Results Rate v Conc

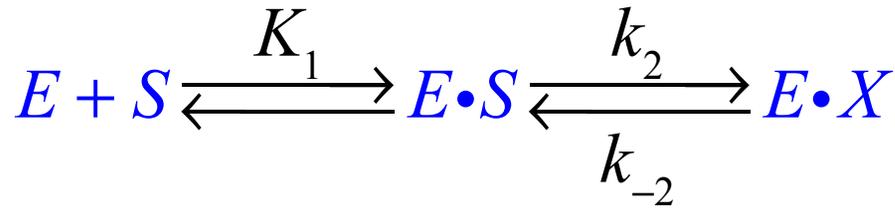
Concentration 2

N = 200  
DoF = 197  
Chi2 = 207 Sigma

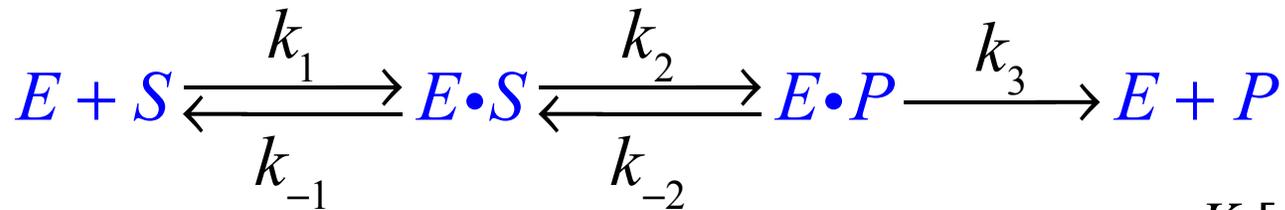


Fit time course to single exponential

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



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



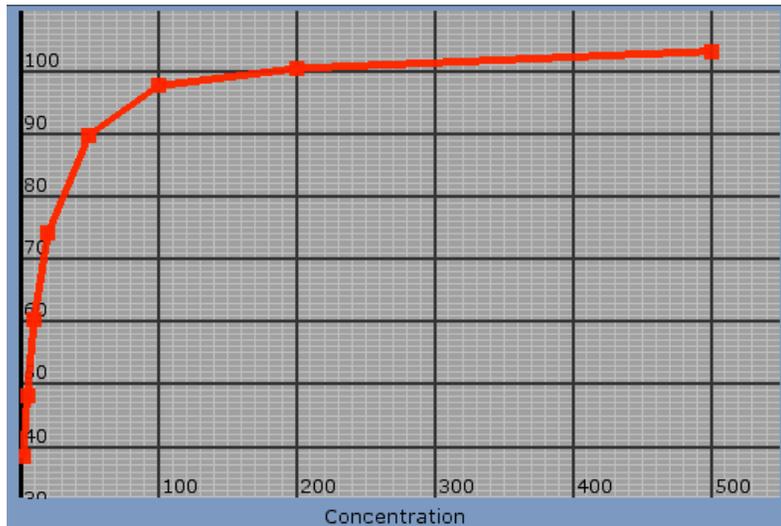
$$\lambda_1 \approx k_2 \frac{K_1[S]}{K_1[S] + 1} + k_{-2} + k_3$$

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

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

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

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



# Global fit for Experiment 3

## Project

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

Filename /Users/kajohnson/Desktop/Exam 2013/Exam2\_2013\_Exp1&2.mec

Model Fit FitSpace Experiment Data

Reactions  $k^+$   $k^-$   
 E + S = ES  100  1120  
 ES = EP  81.8  14.2  
 EP = E + P  7.94  0

## Data Fit Editor

Total Chi2 (all exp) 4608.49 Sigma

### Observable Constants Fit Status

a  0.04  
 f1  0.5502  
 f2  1  
 F3  1.262

### 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=3220, DoF=3214)

Chi2 = 5387.02 Sigma  
 Chi2/DoF = 1.67611  
 p-Value = 5.70401e-114  
 Chi2 Threshold = 1.00392  
 Sigma = 0.00359415

Param	BestFit	StdErr
k-1	1121.86	13.3604
k+2	81.8236	0.511935
k-2	14.1891	0.261745
k+3	7.94167	0.0272945
f1	0.550241	0.000267063
F3	1.26191	0.00146676

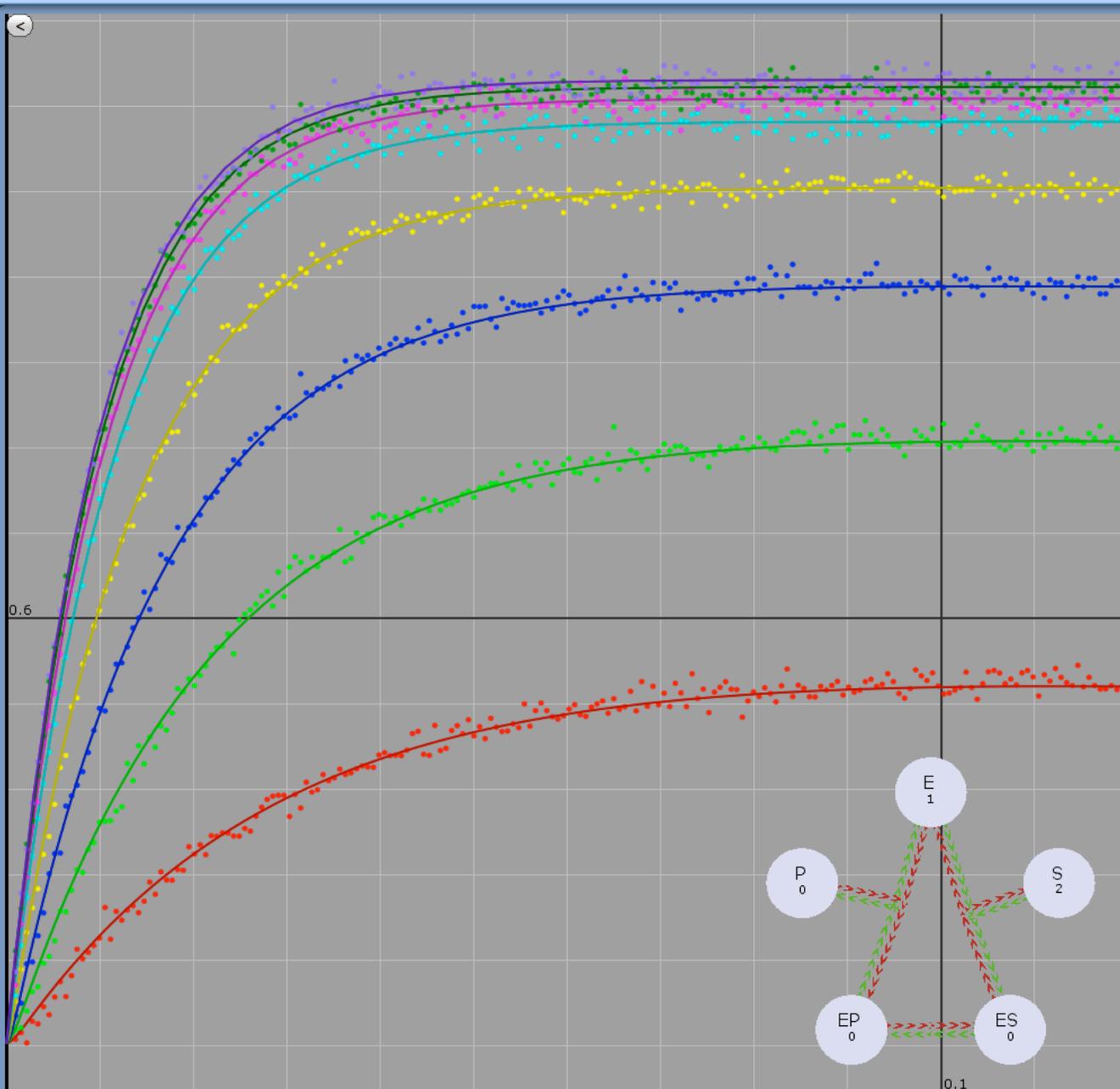
- Plot observable traces at best fit values
- Plot observable traces at StdErr bounds

## FitSpace Editor

## Experiment Editor

New Copy Delete  View Single Plot

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



Experiment 3 (n=47,99) (d=200,200)

Experiment Chi2: 1748.31 Sigma

Obs. Chi2: [239] [260] [197] [182] [214] [227] [189] [240]

# Global fit for Experiment1, 2 and 3

**Project**

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

Filename /Users/kajohnson/Desktop/Exam 2  
2013/Exam2\_2013\_Exp1&2.mec

Model Fit FitSpace Experiment Data

Reactions

	k+	k-
E + S = ES	<input checked="" type="radio"/> 100	<input type="radio"/> 1120
ES = EP	<input checked="" type="radio"/> 81.8	<input type="radio"/> 14.2
EP = E + P	<input type="radio"/> 7.94	<input checked="" type="radio"/> 0

**Data Fit Editor**

**FitSpace Editor**

**Experiment Editor**

New Copy Delete  View Single Plot

- 
- Integrator Tolerance **1e-08**
  - Overlay all plots
  - Show sim steps
- Plot this experiment  Thick Lines
  - Autoscale  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 1

New Mix Del Mix  Plot All Mix Steps

t1

Mixing step 1 [ t=0 ]

E	1
S	2,5,10,20,50,100,200,500
ES	0
EP	0
P	0
Time	0.12

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

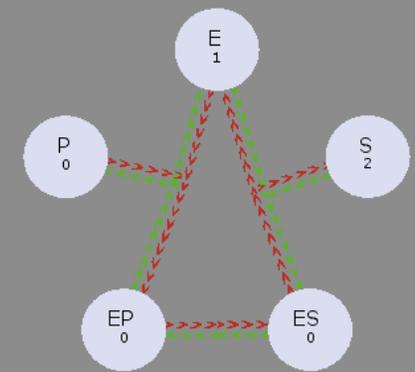
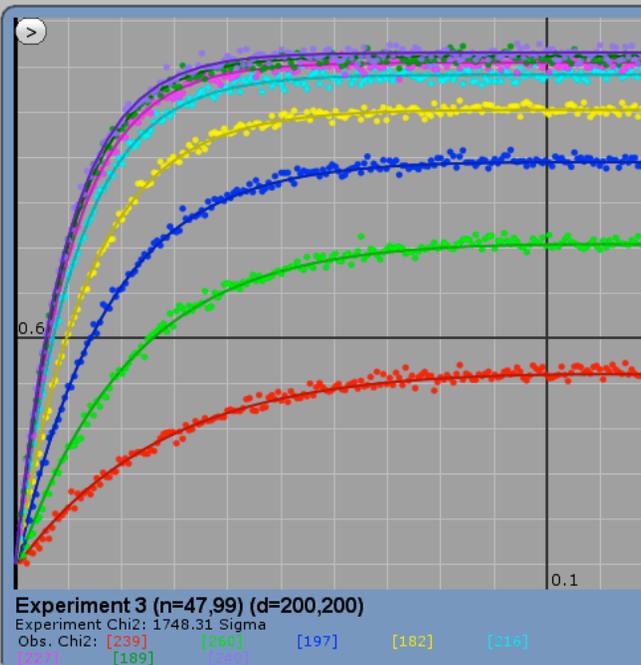
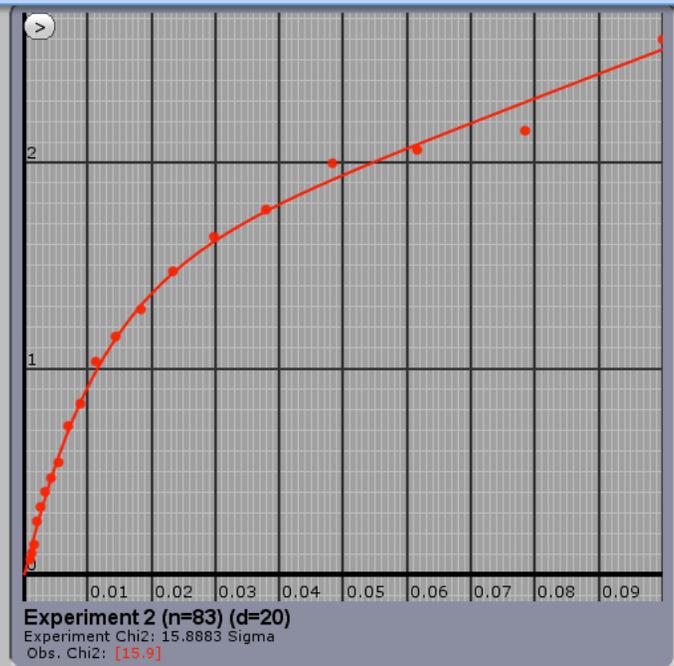
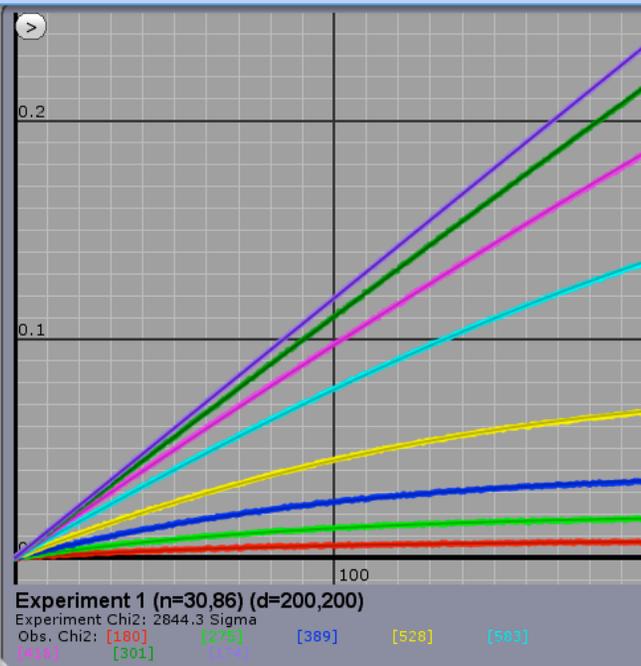
**Observables**

S1\_c f1\*(E + f2\*ES + F3\*EP)  
aFit

S2  
aFit

**Observable Constants**

a	0.04
f1	0.5502
f2	1
F3	1.262



# Conventional fit for Experiment 4

## Project

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

Filename /Users/kajohnson/Desktop/Exam 2  
2013/Exam2\_2013\_Exp1-2-3-4.mec

Model Fit FitSpace Experiment Data

- 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=1780)

Export Results Rate v Conc

Concentration 0

N = 200  
DoF = 196  
Chi2 = 245.745 Sigma  
Chi2/DoF = 1.2538  
p-Value = 0.370895  
Sigma = 0.000111126

Param	BestFit	StdErr
A1	-18.3144	320.207
b1	7.51922e-05	0.000662525
b2	0.0010673	0.0119445
C	18.3143	320.207

Concentration 0.01

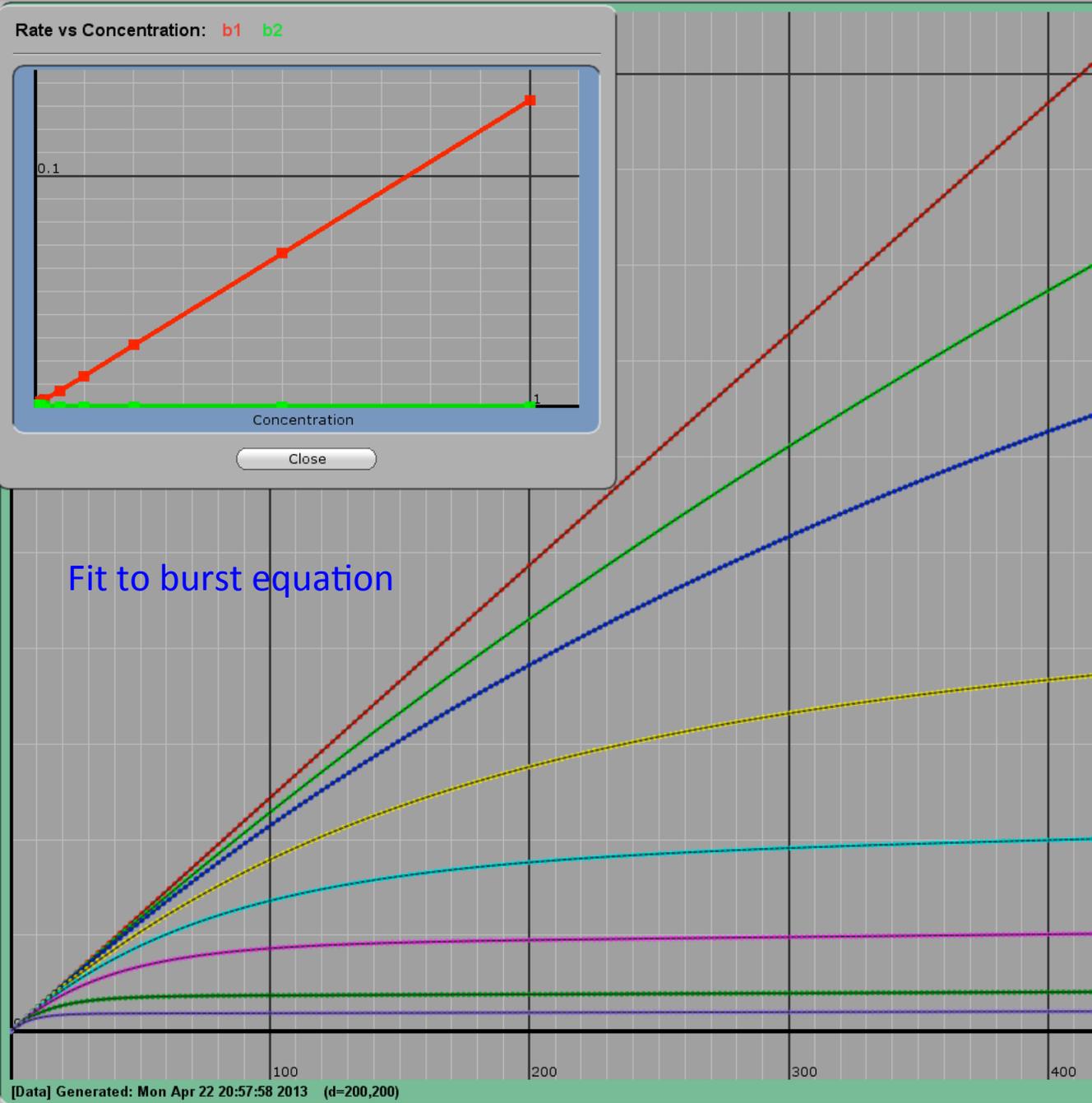
N = 200  
DoF = 196  
Chi2 = 174.701 Sigma  
Chi2/DoF = 0.891331  
p-Value = 0.370895  
Sigma = 9.3696e-05

Param	BestFit	StdErr
A1	-0.740575	0.015881
b1	0.00210541	2.83146e-05
b2	0.000879834	1.37248e-05
C	0.74058	0.0159179

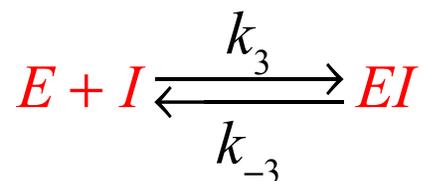
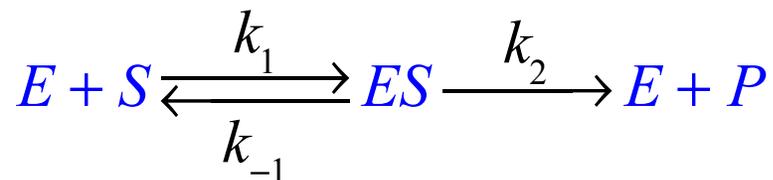
Rate vs Concentration: b1 b2



Fit to burst equation



## Conventional fit for Experiment 4



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

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

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

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

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

# Global fit for Experiment 4

**Project**

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

Filename /Users/kajohnson/Desktop/Exam 2  
2013/Exam2\_2013\_Exp1-2-3-4.mec

Model Fit FitSpace Experiment Data

**Model Editor**

Edit Model... Clear Model

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

Visualize Model  Continuous Simulation  Display Optimization

Reactions

$E + S = ES$	k+ <input checked="" type="radio"/> 100	k- <input type="radio"/> 1120
$ES = EP$	<input type="radio"/> 82.6	<input type="radio"/> 14.3
$EP = E + P$	<input type="radio"/> 7.91	<input checked="" type="radio"/> 0
$E + I = EI$	<input type="radio"/> 5.65	<input type="radio"/> 0.000294

**Data Fit Editor**

Total Chi2 (all exp) 17259.6 Sigma

Observable Constants Fit Status

a	<input checked="" type="radio"/> 0.04
f1	<input type="radio"/> 0.5499
f2	<input checked="" type="radio"/> 1
F3	<input type="radio"/> 1.262

**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=4820, DoF=4812)**

Chi2 = 18023.8 Sigma  
Chi2/DoF = 3.74559  
p-Value = 0  
Chi2 Threshold = 1.00323  
Sigma = 0.00291559

Param	BestFit	StdErr
k-1	1116.38	11.8814
k+2	82.6014	0.459539
k-2	14.3369	0.328585
k+3	7.91494	0.0232249
k+4	5.64539	0.00722579
k-4	0.000294288	4.12584e-06
f1	0.549883	0.000386127
F3	1.26246	0.00167258

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  
3  Plot this experiment  Overlay all plots  
4  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 1

New Mix Del Mix  Plot All Mix Steps

t1

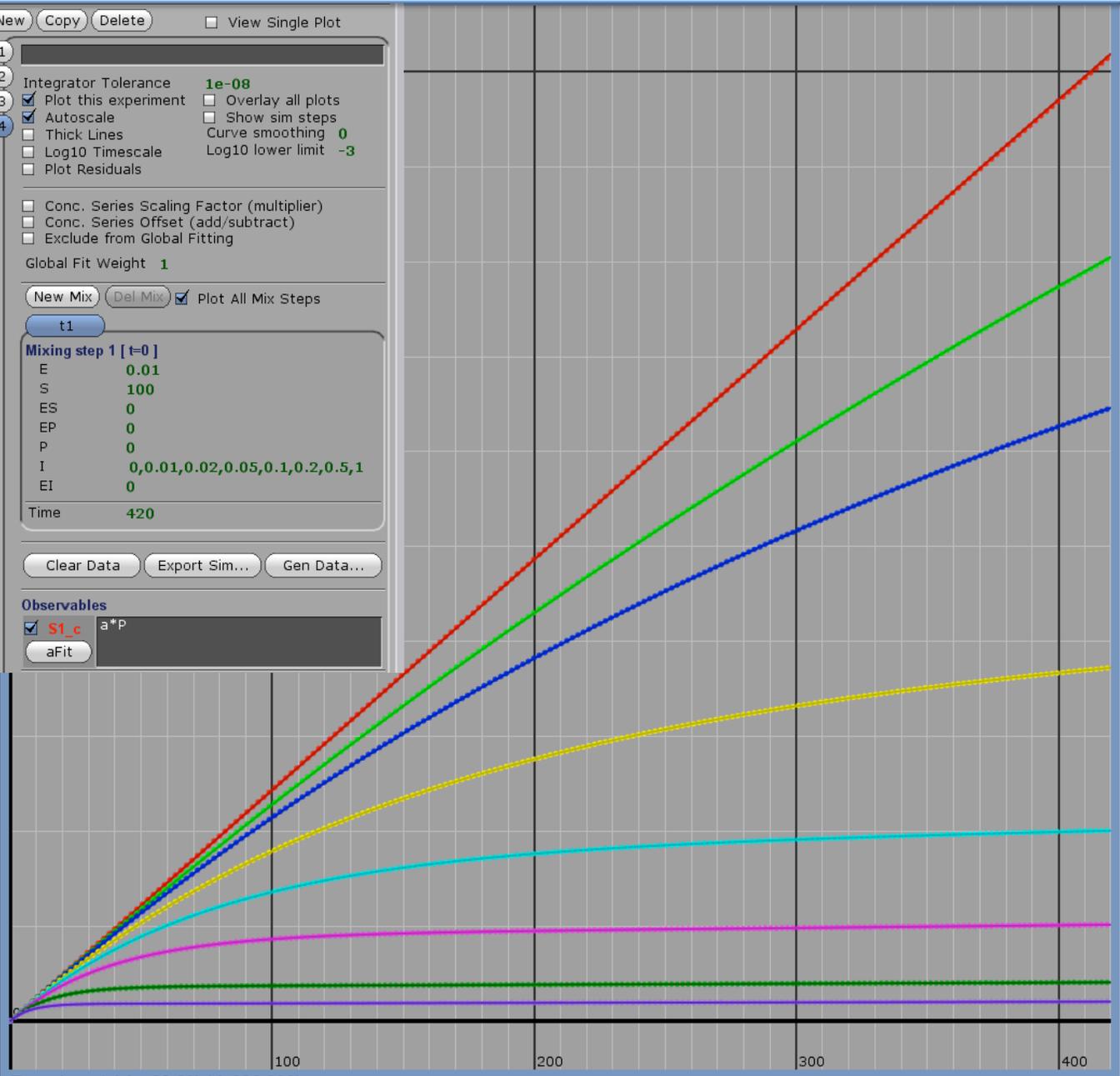
Mixing step 1 [ t=0 ]

E	0.01
S	100
ES	0
EP	0
P	0
I	0,0.01,0.02,0.05,0.1,0.2,0.5,1
EI	0
Time	420

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

Observables

S1\_c a\*P  
aFit



Experiment 4 (n=33,60) (d=200,200)  
Experiment Chi2: 11917.1 Sigma  
Obs. Chi2: [6340] [2670] [1420] [449] [319] [297] [201] [113]

# Conventional fit for Experiment 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

**Data Repository**  View Single Plot  List Individual Traces

- Generated: Mon Apr 22 20:54:33 2013  
N 200, Sigma 0.0002, Deadtime 0.001
- Generated: Mon Apr 22 20:56:36 2013  
N 20, Sigma 0.05, Deadtime 0.001
- Generated: Mon Apr 22 20:57:24 2013  
N 200, Sigma 0.001, Deadtime 0.001
- 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: Mon Apr 22 21:06:13 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 )

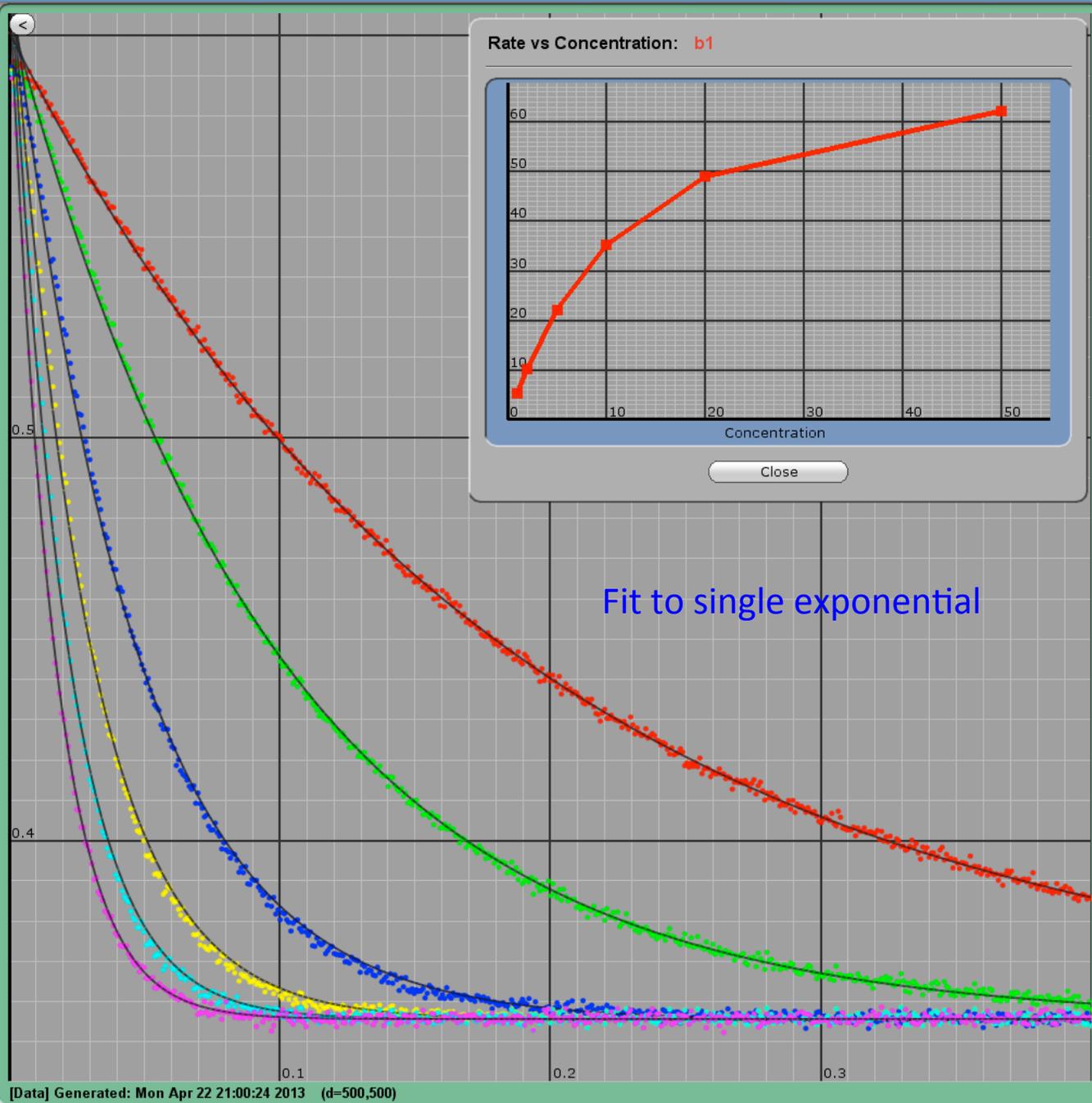
Perform Fit

Fit Results: (Total Chi2=8770)

Export Results Rate v Conc

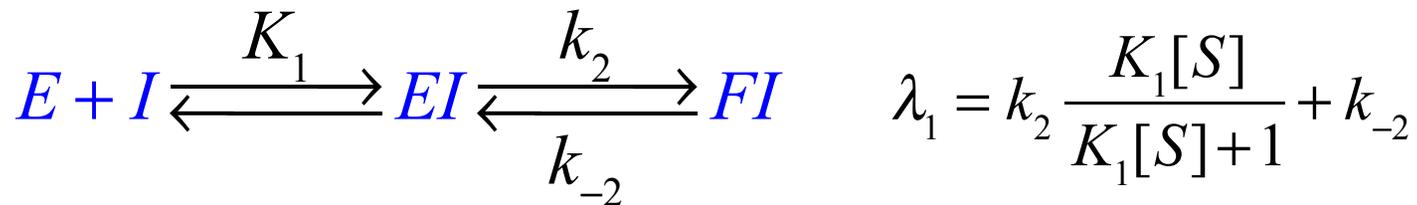
Concentration 1

N = 500  
DoF = 497  
Chi2 = 700.5 Sigma  
Chi2/DoF = 1.40946



## Experiment 5: Two-step inhibitor binding

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



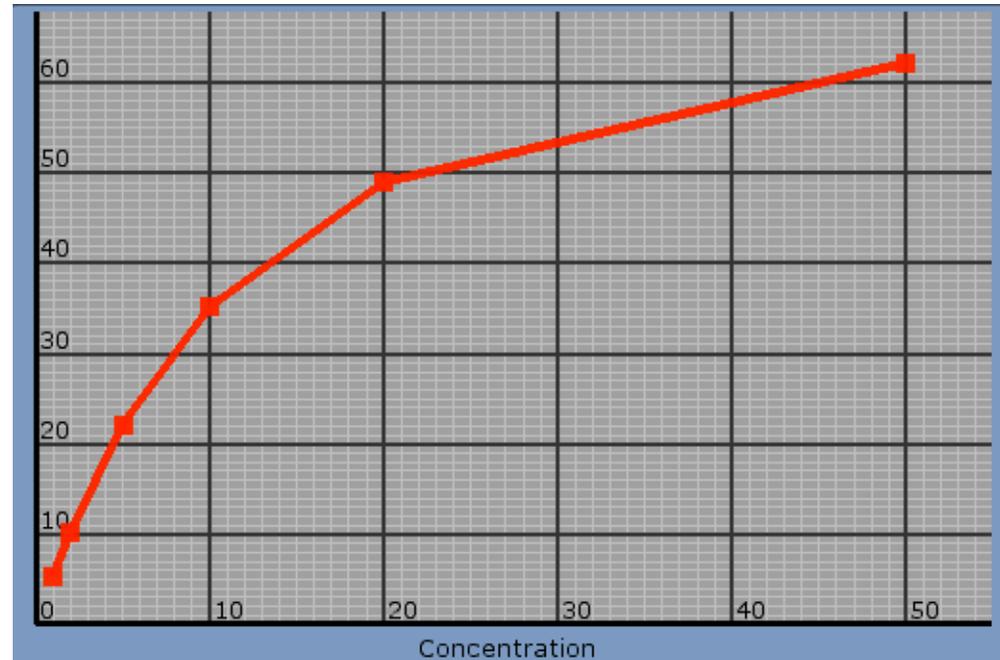
$$k_2 = 78.3s^{-1}$$

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

$$1/K_1 = 11.5\mu M$$

$$k_{-2} \sim 0$$

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



The slow onset inhibition experiment only went to 1  $\mu M$ , 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

**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

**Data Repository**  View Single Plot  List Individual Traces

- Generated: Mon Apr 22 20:54:33 2013  
N 200, Sigma 0.0002, Deadtime 0.001
- Generated: Mon Apr 22 20:56:36 2013  
N 20, Sigma 0.05, Deadtime 0.001
- Generated: Mon Apr 22 20:57:24 2013  
N 200, Sigma 0.001, Deadtime 0.001
- 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: Mon Apr 22 21:06:13 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 )

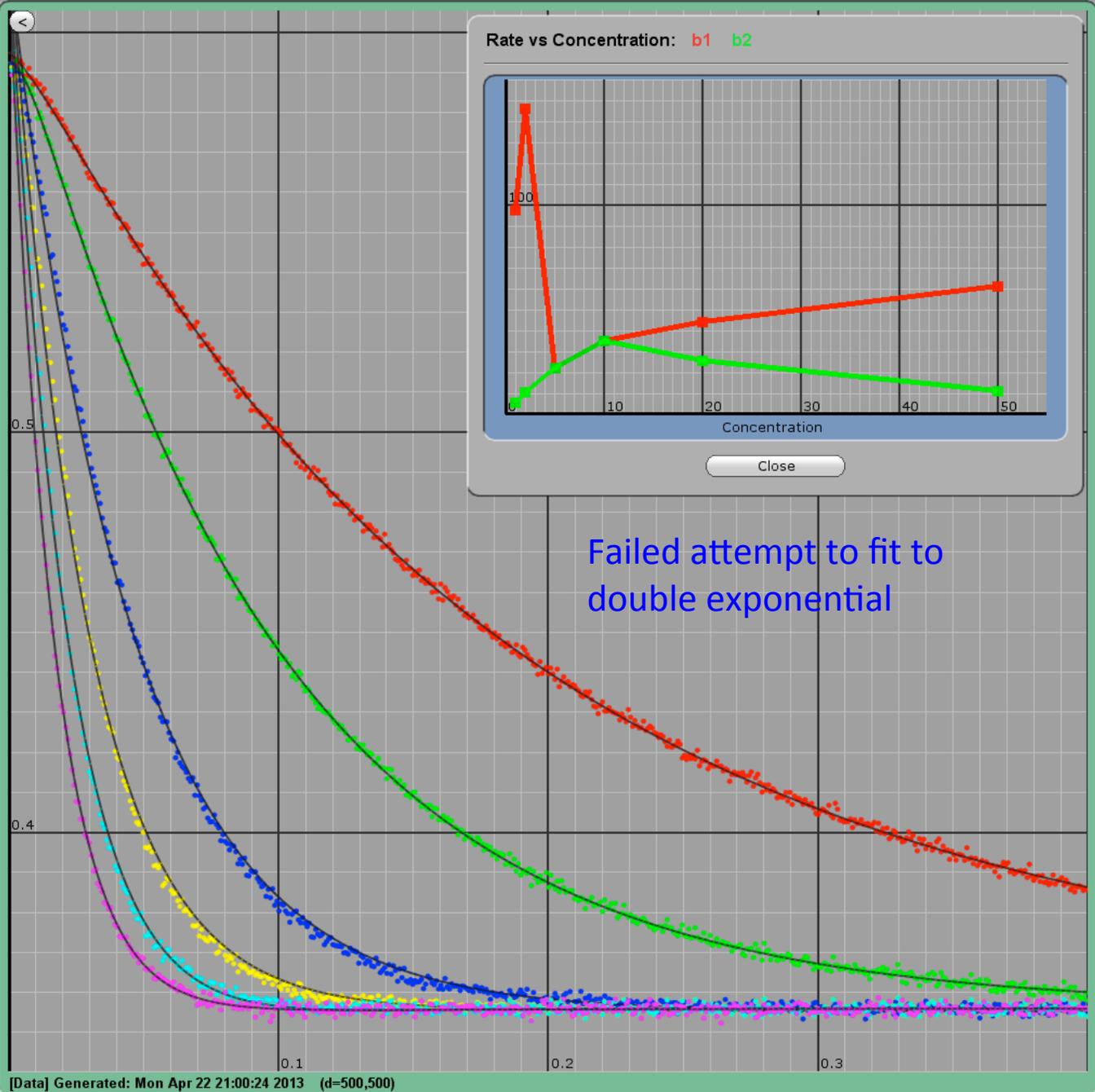
Perform Fit

Fit Results: (Total Chi2=7630)

Export Results Rate v Conc

Concentration 1

N = 500  
DoF = 495  
Chi2 = 523.894 Sigma  
Chi2/DoF = 1.05837



Failed attempt to fit to double exponential

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

# Global fit for Experiment 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	<input checked="" type="checkbox"/> 100	<input checked="" type="checkbox"/> 1120
ES = EP	<input checked="" type="checkbox"/> 82.6	<input checked="" type="checkbox"/> 14.3
EP = E + P	<input checked="" type="checkbox"/> 7.91	<input checked="" type="checkbox"/> 0
E + I = EI	<input type="checkbox"/> 10.2	<input type="checkbox"/> 54.2
EI = FI	<input type="checkbox"/> 72	<input type="checkbox"/> 0.00128

**Data Fit Editor**

Total Chi2 (all exp) 519982 Sigma

**Observable Constants Fit Status**

a	<input checked="" type="checkbox"/> 0.04
f1	<input checked="" type="checkbox"/> 0.5499
f2	<input checked="" type="checkbox"/> 1
f3	<input checked="" type="checkbox"/> 1.262
f4	<input type="checkbox"/> 5.93
f5	<input type="checkbox"/> 1
f6	<input type="checkbox"/> 0.6

**Data Fit Options**

Normalize residuals using available Sigma values

Fit Active Exp Fit All Exp Batch Fit

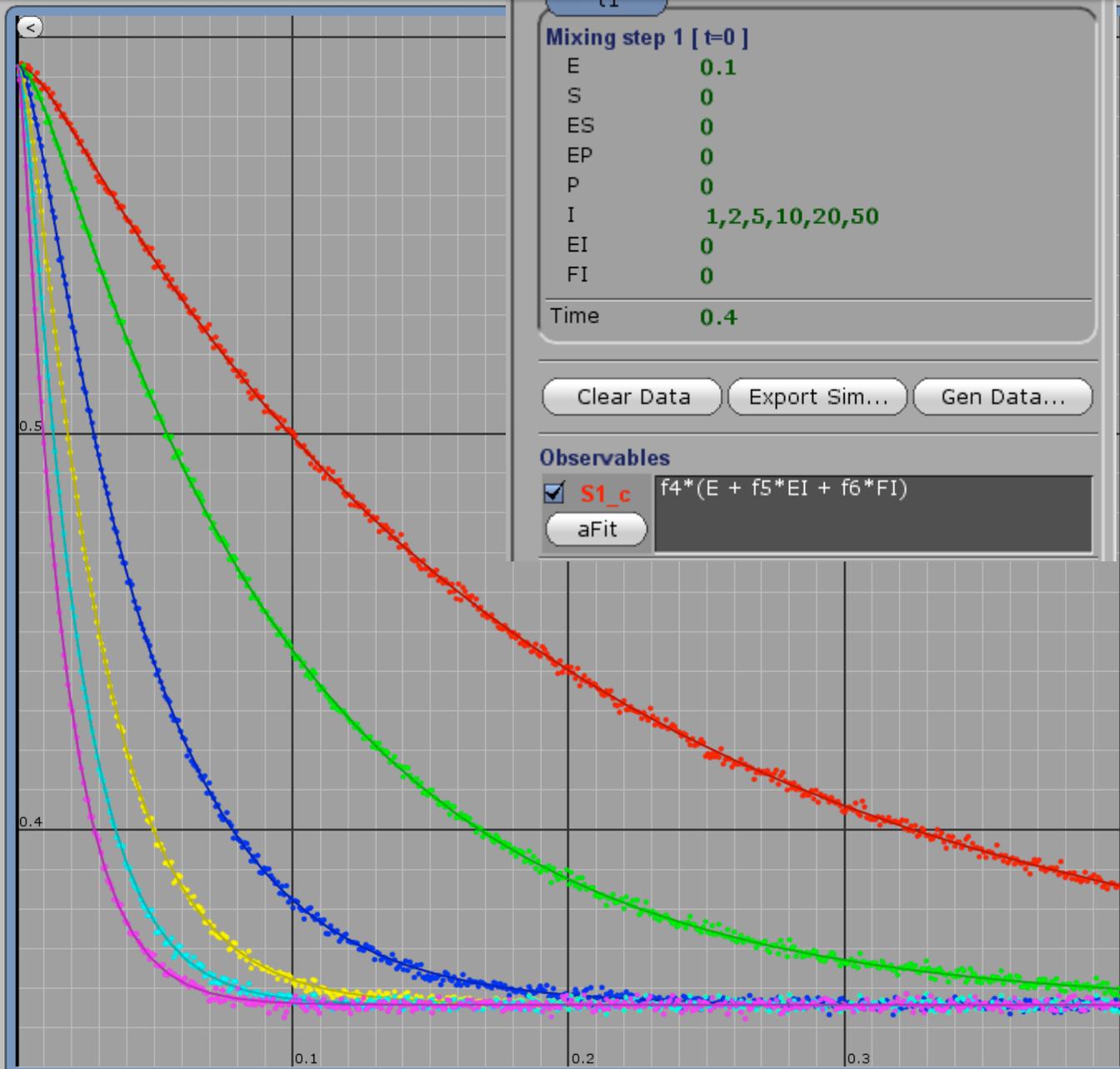
Accept Refine Revert Export

**Last Fit Results: Single Experiment (N=3000, DoF=2993)**

Chi2 = 2831.08 Sigma  
Chi2/DoF = 0.945902  
p-Value = 0.983126  
Chi2 Threshold = 1.00471  
Sigma = 0.000971602

Param	BestFit	StdErr
k+4	10.152	0.20483
k-4	54.1635	2.67621
k+5	71.9816	0.353265
k-5	0.00128138	0.0208536
f4	5.92964	0.00241544
f5	1.00021	0.00206326
f6	0.600028	0.000361412

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



**Mixing step 1 [ t=0 ]**

E	0.1
S	0
ES	0
EP	0
P	0
I	1,2,5,10,20,50
EI	0
FI	0
Time	0.4

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

**Observables**

S1\_c f4\*(E + f5\*EI + f6\*FI)

aFit

**Experiment 5 (n=39,52) (d=500,500)**  
Experiment Chi2: 2831.08 Sigma  
Obs. Chi2: [511] [459] [447] [458] [486] [496]

# 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

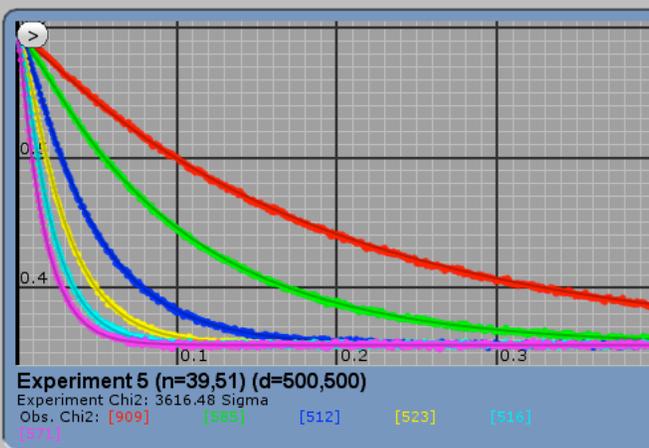
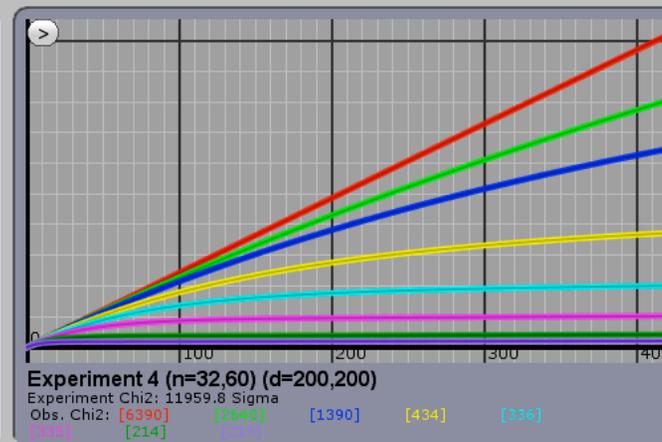
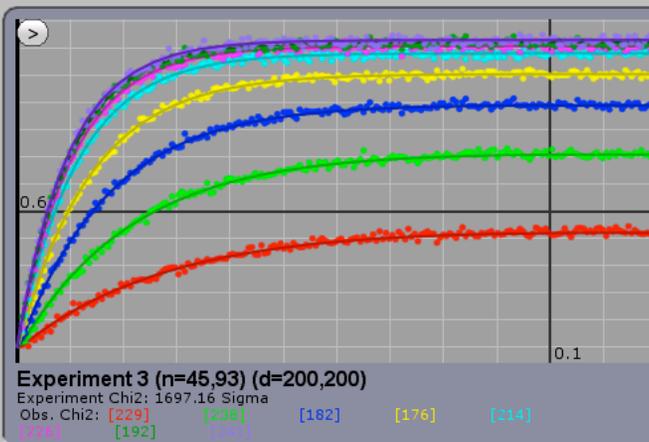
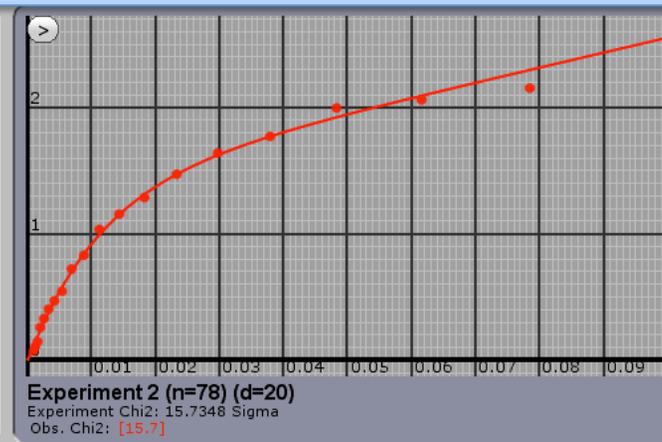
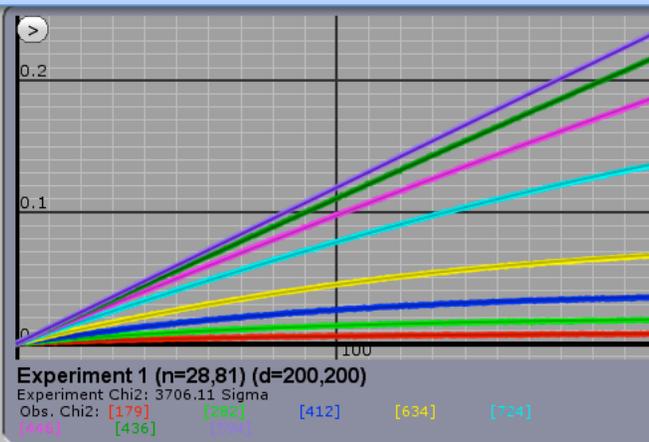
Fit Active Exp Fit All Exp Batch Fit

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



# 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

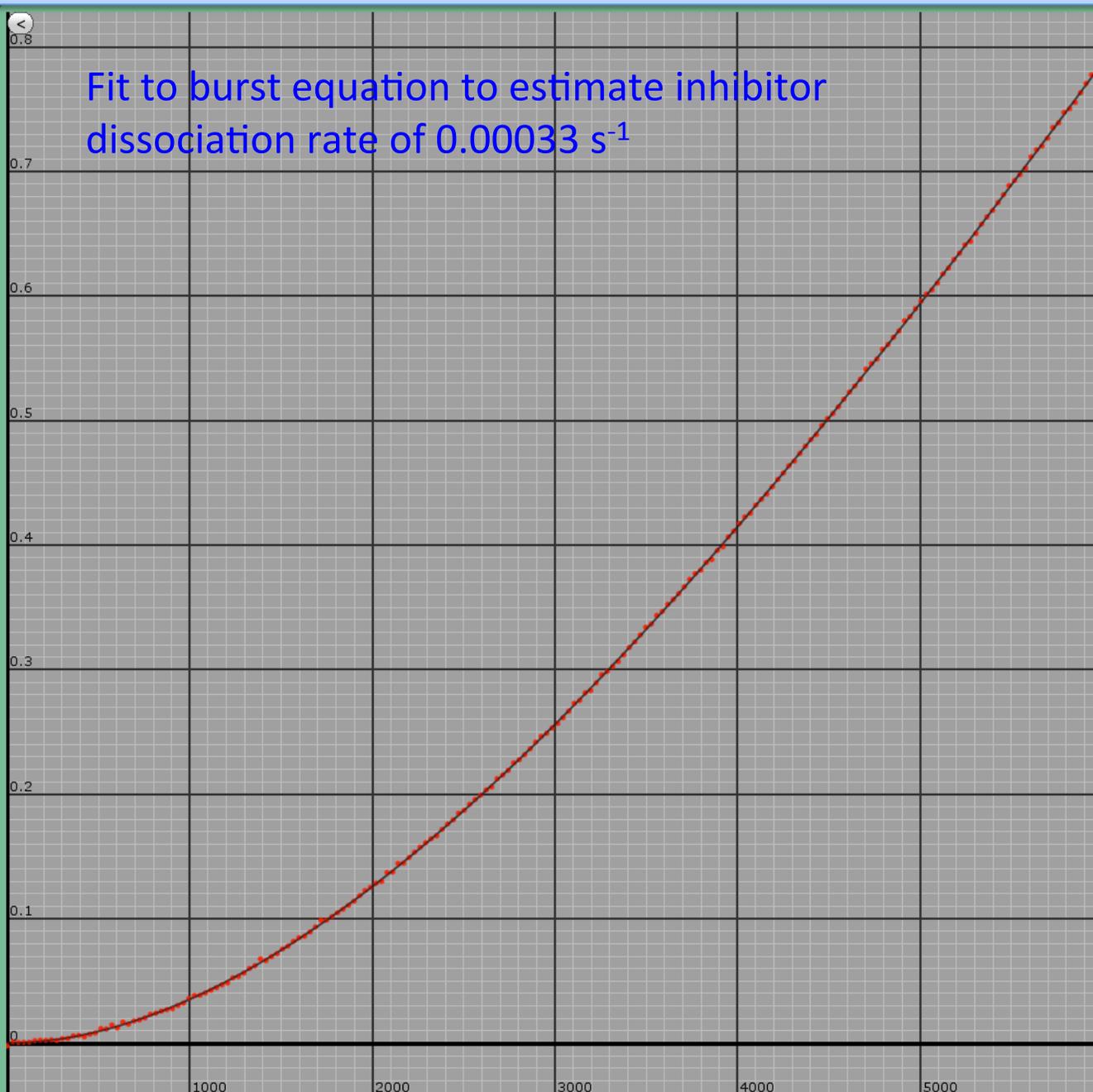
### Fit Results: (Total Chi2=228)

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



[Data] Generated: Thu May 2 12:56:05 2013 (d=200)

# 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  
 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

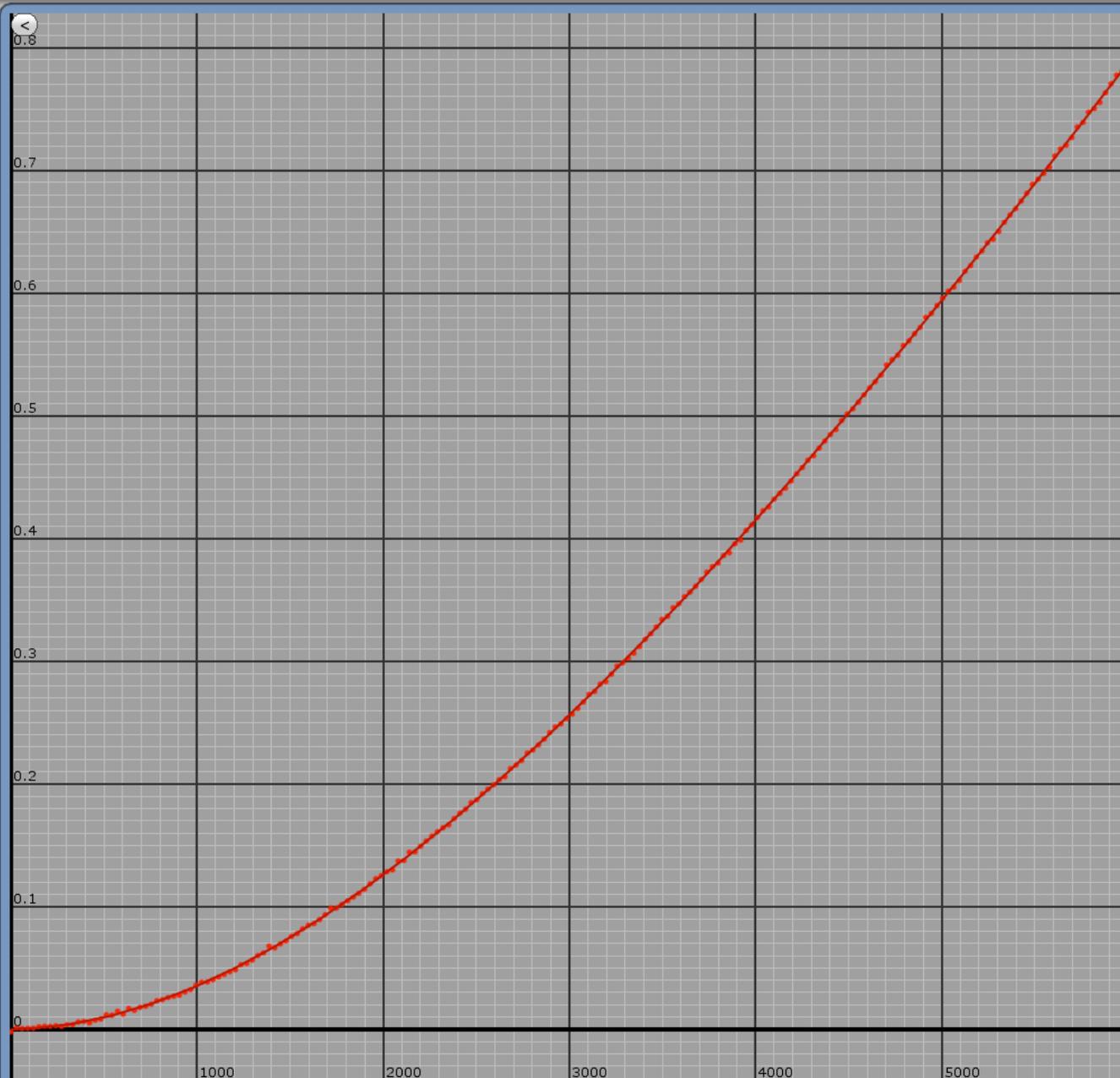
### Last Fit Results: Multiple Experiments (N=8020, DoF=8007)

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



Experiment 6 (n=213) (d=200)  
Experiment Chi2: 229.458 Sigma  
Obs. Chi2: [229]

# 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  
 Continuous Simulation  
 Display Optimization

Reactions	k+	k-
$E + S = ES$	<input type="radio"/> 56.8	<input type="radio"/> 601
$ES = EP$	<input type="radio"/> 82.4	<input type="radio"/> 14.6
$EP = E + P$	<input type="radio"/> 7.96	<input checked="" type="radio"/> 0.0862
$E + I = EI$	<input type="radio"/> 10	<input type="radio"/> 52.7
$EI = FI$	<input type="radio"/> 72.1	<input type="radio"/> 0.000721

## Data Fit Editor

Total Chi2 (all exp) 7892.92 Sigma

### Observable Constants Fit Status

a	<input checked="" type="radio"/> 0.04
f1	<input type="radio"/> 0.5501
f2	<input checked="" type="radio"/> 1
f3	<input type="radio"/> 1.262
f4	<input type="radio"/> 5.929
f5	<input checked="" type="radio"/> 1
f6	<input type="radio"/> 0.6002

### Data Fit Options

Normalize residuals using available Sigma values

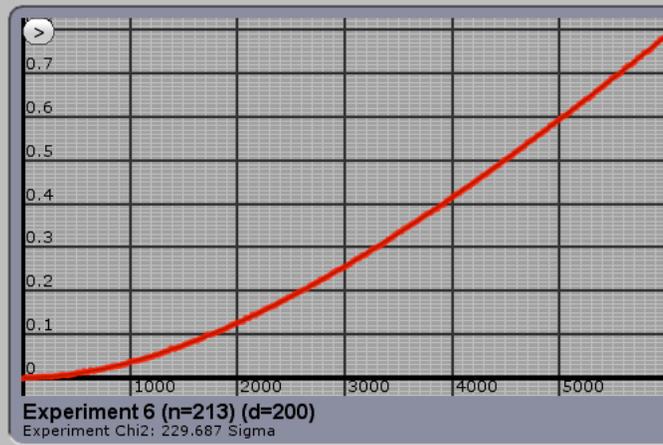
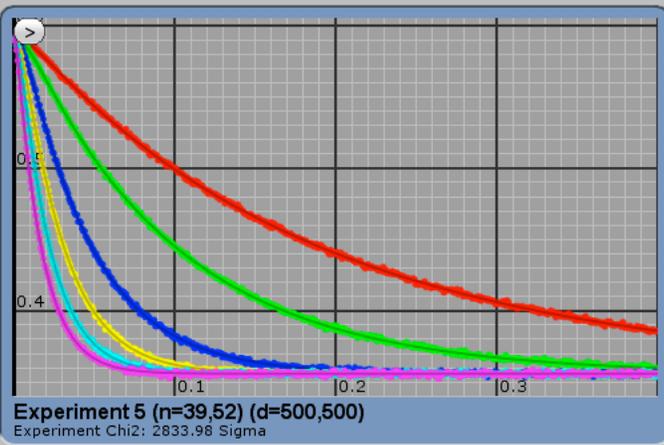
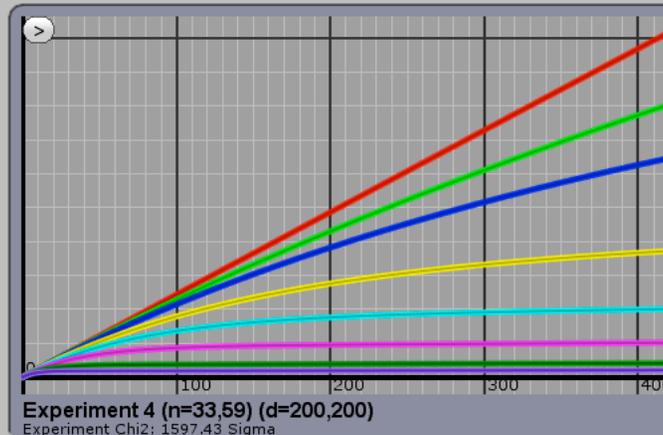
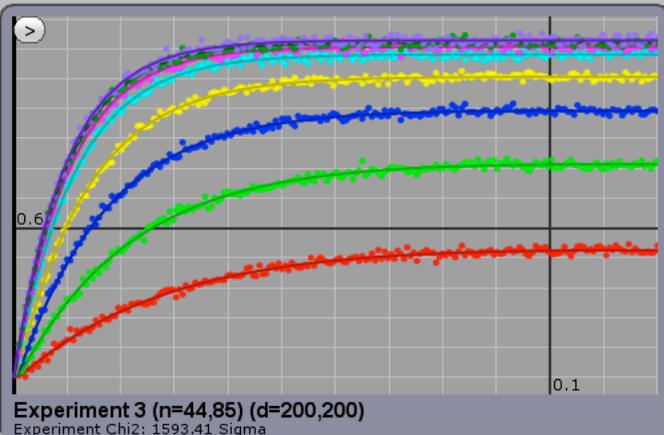
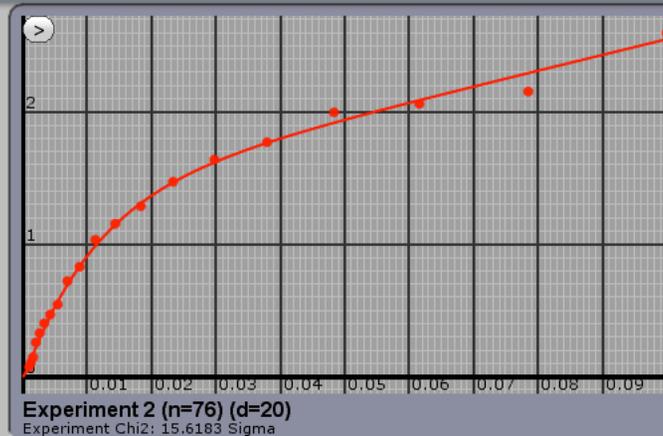
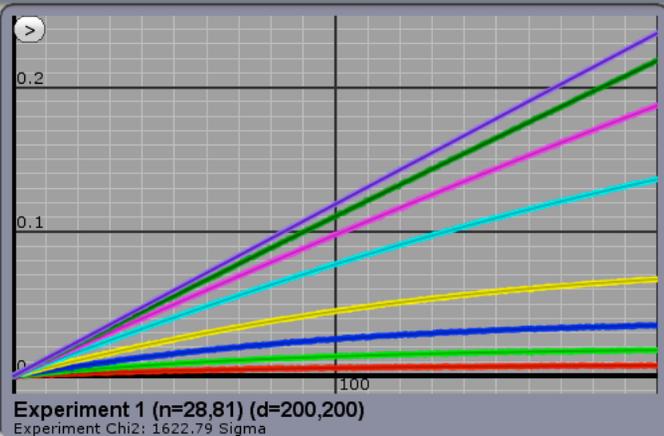
Fit Active Exp Fit All Exp Batch Fit

Accept Refine Revert Export

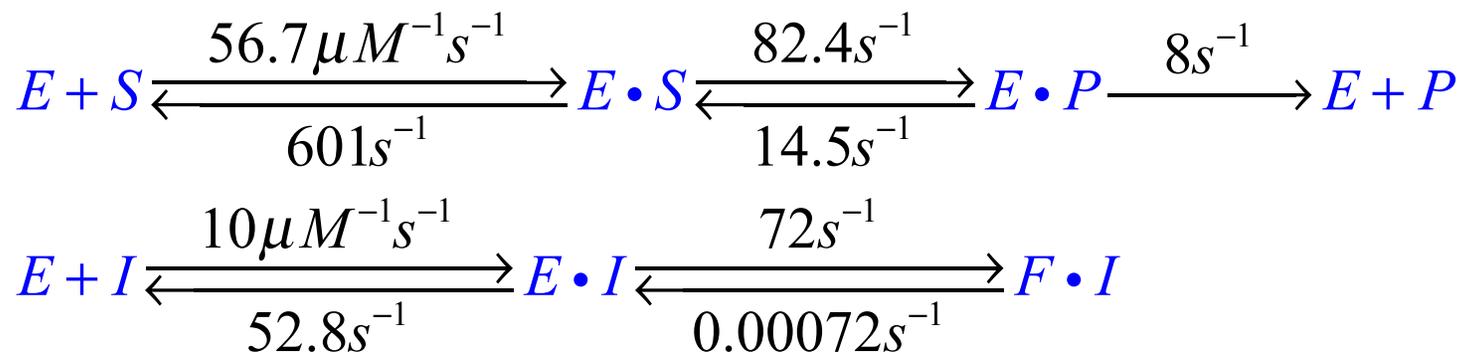
### Last Fit Results: Multiple Experiments (N=8020, DoF=8007)

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



## Global fit for Experiments 1 - 6



$$K_m = 2.4\mu M$$

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

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

The fluorescence changes occur on forming EP and FI only.

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