Dynafit

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1.整理数据

首先对 MCR-ALS 输出的数据 copt 进行整理。时间为 0-289.5s，每 0.5s 一个记录，生成物浓度为 copt 的第二列。将仅含时间和浓度的两列数据整合到一个表中，三个平行试验分开。R 语言代码如下。

```
copt<-read.table("F:/ComputationalBiochemistry/dynafit/copt.txt",header = F)
copt<-data.frame(copt)
time<-seq(0,298.5,0.5)
react1<-data.frame(cbind(time,copt[1:598,1]))
react2<-data.frame(cbind(time,copt[599:1196,1]))
react3<-data.frame(cbind(time,copt[1197:1794,1]))
write.table(react1,"F:/ComputationalBiochemistry/dynafit/react1.csv",
            row.names = F,col.names=F,sep =",")
write.table(react2,"F:/ComputationalBiochemistry/dynafit/react2.csv",
          row.names = F,col.names=F,sep =",")
write.table(react3,"F:/ComputationalBiochemistry/dynafit/react3.csv",
          row.names = F,col.names=F,sep =",")
product1<-data.frame(cbind(time,copt[1:598,2]))
product2<-data.frame(cbind(time,copt[599:1196,2]))
product3<-data.frame(cbind(time,copt[1197:1794,2]))
write.table(product1,"F:/ComputationalBiochemistry/dynafit/product1.csv",
            row.names = F,col.names=F,sep =",")
write.table(product2,"F:/ComputationalBiochemistry/dynafit/product2.csv",
            row.names = F,col.names=F,sep =",")
write.table(product3,"F:/ComputationalBiochemistry/dynafit/product3.csv",
            row.names = F,col.names=F,sep =",")

copt_t<-read.table("F:/ComputationalBiochemistry/dynafit/copt_t.txt",header = F)
copt_t<-data.frame(copt_t)
time<-seq(0,298.5,0.5)
react_t1<-data.frame(cbind(time,copt_t[1:598,1]))
react_t2<-data.frame(cbind(time,copt_t[599:1196,1]))
react_t3<-data.frame(cbind(time,copt_t[1197:1794,1]))
write.table(react_t1,"F:/ComputationalBiochemistry/dynafit/react_t1.csv",
            row.names = F,col.names=F,sep =",")
write.table(react_t2,"F:/ComputationalBiochemistry/dynafit/react_t2.csv",
            row.names = F,col.names=F,sep =",")
write.table(react_t3,"F:/ComputationalBiochemistry/dynafit/react_t3.csv",
            row.names = F,col.names=F,sep =",")
product_t1<-data.frame(cbind(time,copt_t[1:598,2],copt_t[1:598,3]))
product_t2<-data.frame(cbind(time,copt_t[599:1196,3]))
product_t3<-data.frame(cbind(time,copt_t[1197:1794,3]))
write.table(product_t1,"F:/ComputationalBiochemistry/dynafit/product_t1.csv",
            row.names = F,col.names=F,sep =",")
write.table(product_t2,"F:/ComputationalBiochemistry/dynafit/product_t2.csv",
            row.names = F,col.names=F,sep =",")
write.table(product_t3,"F:/ComputationalBiochemistry/dynafit/product_t3.csv",
            row.names = F,col.names=F,sep =",")
```

2.漆酶+底物体系（LAC_SUB）

对 Laccase + Substance 体系用以下模型通过 dynafit 进行拟合。结果得到三次平行实验的初始反应速率 0.0406341，0.0402161，0.0435739，求平均值得到 Initial reaction rate=0.0414747 μM / s =2.488482 μM / min。

从参数优化结果来看，第一、三次平行实验的优化参数标标准误较大，因此认为第二次的参数更符合实际结果，得到 k1+=3.3； k1-=9.5； k2+=0.348； r=0.0246。

Model

Mechanism

$$E + S \underset{k_{1-,S}}{\overset{k_{1+,S}}{\rightleftharpoons}} E.S \qquad E.S \xrightarrow{k_{2+,P}} E + P$$

Model equation

The fitting function (model equation) for each individual data set is

$S(t) = S_0 + \sum_{i=1}^{n} r_i\ c_i(t)$

where

$S(t)$	the experimental signal observed at time t
S_0	offset on the signal axis (a property of the instrument)
n	number of unique molecular species participating in the reaction mechanism
$c_i(t)$	the concentration of the ith species at time t
r_i	the molar response coefficient of the ith species

In this case, n= 4. The molecular species participating in the mechanism are: E, S, E.S, P. The concentrations of these molecular species at time $t$are computed from their initial concentrations (at time zero, t

= 0) by solving an initial-value problem defined by a system of simultaneous first-order Ordinary Differential Equations (ODEs) listed below.

ODE system

Initial value problem: Given the concentrations of molecular species at $t = 0$, compute the concentrations at an arbitrary later time $t > 0$ by solving the following system of ODEs. The solution is obtained by *numerical integration*.

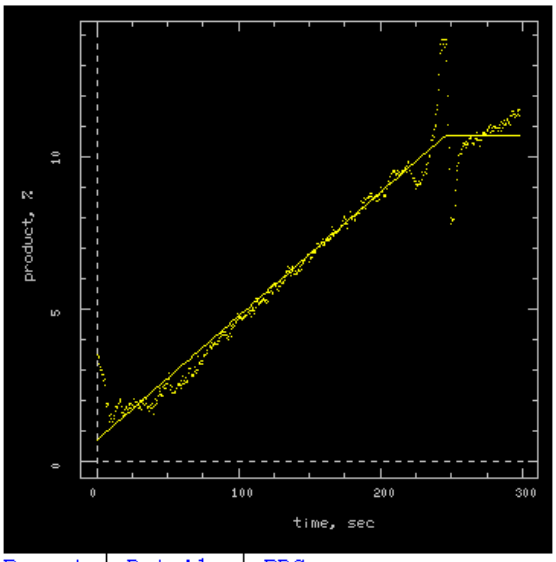
$$d[E]/dt = -k_{1+,S}[E][S] + k_{1-,S}[E.S] + k_{2+,P}[E.S]$$
$$d[S]/dt = -k_{1+,S}[E][S] + k_{1-,S}[E.S]$$
$$d[E.S]/dt = +k_{1+,S}[E][S] - k_{1-,S}[E.S] - k_{2+,P}[E.S]$$
$$d[P]/dt = +k_{2+,P}[E.S]$$

```
Welcome to DynaFit.  Please complete the script template be
To get started, choose menu Help :: Getting Started.
-------------------------------------------------------------
[task]
   task = fit
   data = progress discontinuous
[mechanism]
   E + S <===> E.S     :     k1+.S     k1-.S
   E.S ----> E + P     :     k2+.P
[constants]
   k1+.S = 1 ?
   k1-.S = 0.01 ?
   k2+.P = 1 ?
[concentrations]
   S = 47
   E = 0.5
[responses]
   P = 10 ?
[data]
   directory   F:/ComputationalBiochemistry/dynafit
   sheet       product1.csv
   column      2
   offset      auto ?
[output]
   directory   F:/ComputationalBiochemistry/dynafit/output
[settings]
{Output}
   XAxisLabel = time, sec
   YAxisLabel = product, %
[end]
```

平行实验一

Data and model

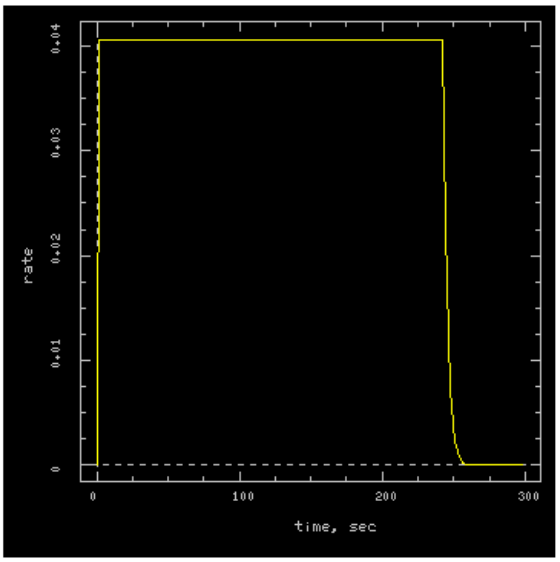
:



Export | Details | EPS

Derivatives (reaction rates)

:



EPS

Initial reaction rates

No.	Dataset	Time	Rate
1	F:/ComputationalBiochemistry/dynafit/product1.csv:col(2)	1	0.0406341

Parameters

Trust Region Algorithm

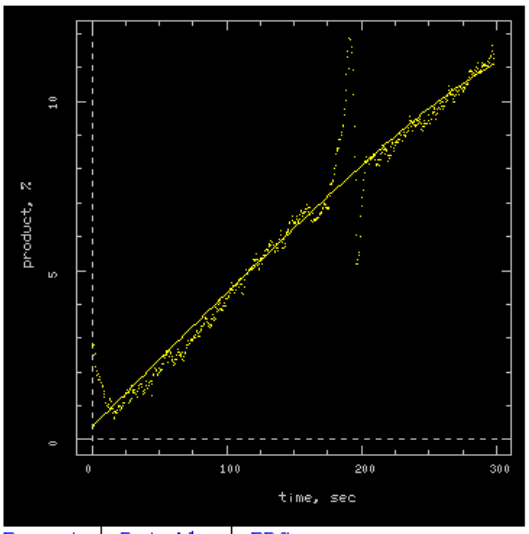
Optimized Parameters

No.	Par#Set	Initial	Final	Std. Error	CV (%)	Note
#1	k1+.S	1	15000	1.4e+008	901089.4	
#2	k1-.S	0.01	1.3e-008	0.00045	3458950.2	
#3	k2+.P	1	0.3824	0.0061	1.6	
#4	r(P)	10	0.2125	0.0021	1.0	
#5	offset#1	3.53082	0.714	0.07	9.8	

平行实验二

Data and model

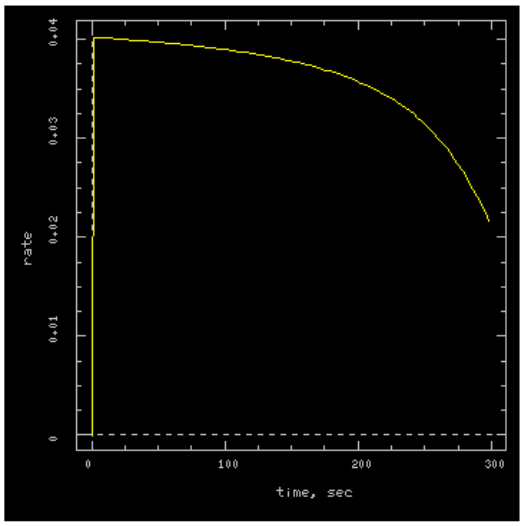
:



Export | Details | EPS

Derivatives (reaction rates)

:



EPS

Initial reaction rates

No.	Dataset	Time	Rate
1	F:/ComputationalBiochemistry/dynafit/product2.csv:col(2)	1	0.0402161

Parameters

Trust Region Algorithm

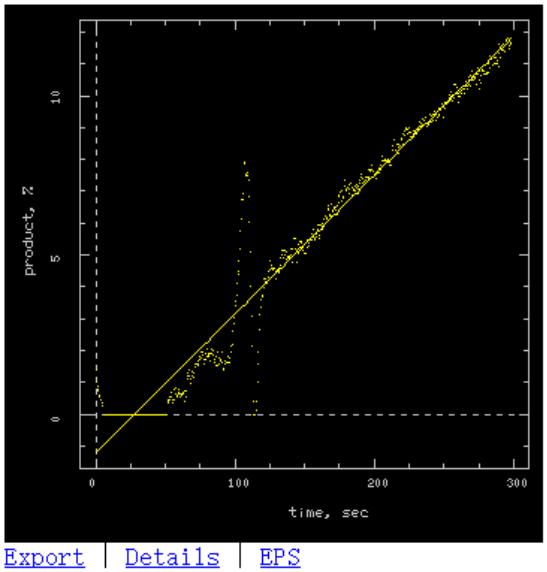
Optimized Parameters

No.	Par#Set	Initial	Final	Std. Error	CV (%)	Note
#1	k1+.S	1	3.3	1300	40683.1	
#2	k1-.S	0.01	9.5	4000	42331.0	
#3	k2+.P	1	0.348	0.039	11.1	
#4	r(P)	10	0.246	0.039	15.8	
#5	offset#1	2.75703	0.36	0.13	35.7	

平行实验三

Data and model

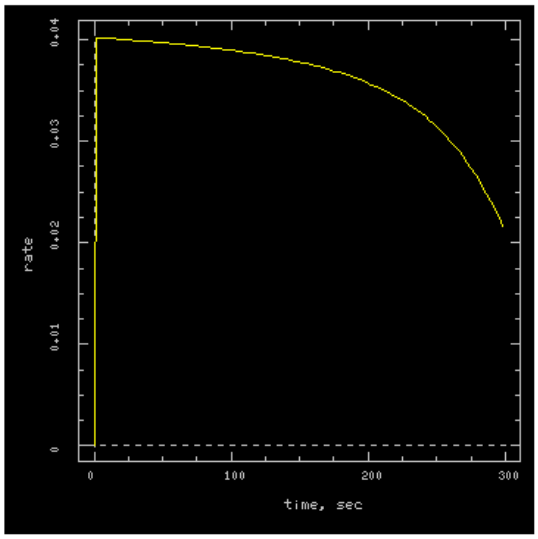
:



Export | Details | EPS

Derivatives (reaction rates)

:



EPS

Initial reaction rates

No.	Dataset	Time	Rate
1	F:/ComputationalBiochemistry/dynafit/product3.csv:col(2)	1	0.0435739

Parameters

Trust Region Algorithm

Optimized Parameters

No.	Par#Set	Initial	Final	Std. Error	CV (%)	Note
#1	k1+.S	1	8.8	59000	668967.2	
#2	k1-.S	0.01	0.79	200000	24877995.8	
#3	k2+.P	1	0.01	2300	23308981.5	
#4	r(P)	10	8.7	2e+006	23042252.0	
#5	offset#1	1.39777	-1.2	0.76	63.0	

3.漆酶+介体 TEMPO+底物体系（LAC_TEMPO_SUB）(model 1)

对 Laccase + TEMPO + Substance 体系用以下模型通过 dynafit 进行拟合。结果得到三次平行实验的初始反应速率 0.0645759，0.0816004，0.0932676，求平均值得到 Initial reaction rate=0.07981463 μM / s =4.788878 μM / min。

从参数优化结果来看，第一次平行实验的优化参数标标准误最小，因此认为第一次的参数更符合实际结果，得到 k1+=0.00073； k1-=0.00042； k2+=2.8；k3+=0.57；k4+=1e-008； k4-=0.002； k5+=1.1； r=4.8。

Model

Mechanism

$$
\begin{aligned}
&\xrightarrow{} \quad \xleftarrow{} \\
&E + S \leftrightarrows E.S \quad : k_{1+.S} \quad k_{1-.S} \\
&E.S \rightarrow E + P \quad : k_{2+.P} \\
&E + M \leftrightarrows E.M \quad : k_{3+.M} \quad k_{3-.M} \\
&E.M \rightarrow E + M^* \quad : k_{4+.M^*} \\
&S + M^* \rightarrow P + M \quad : k_{5+.P}
\end{aligned}
$$

Model equation

The fitting function (model equation) for each individual data set is

$$S(t) = S_0 + \sum_{i=1}^{n} r_i \, c_i(t)$$

where

$S(t)$	the experimental signal observed at time t
S_0	offset on the signal axis (a property of the instrument)
n	number of unique molecular species participating in the reaction mechanism
$c_i(t)$	the concentration of the ith species at time t
r_i	the molar response coefficient of the ith species

In this case, n= 7. The molecular species participating in the mechanism are: E, S, E.S, P, M, E.M, M*. The concentrations of these molecular species at time $t$are computed from their initial concentrations (at time zero, t

= 0) by solving an initial-value problem defined by a system of simultaneous first-order Ordinary Differential Equations (ODEs) listed below.

ODE system

Initial value problem: Given the concentrations of molecular species at $t = 0$, compute the concentrations at an arbitrary later time $t > 0$ by solving the following system of ODEs. The solution is obtained by *numerical integration*.

$$
\begin{aligned}
d[E]/dt &= -k_{1+.S}[E][S] + k_{1-.S}[E.S] + k_{2+.P}[E.S] - k_{3+.M}[E][M] + k_{3-.M}[E.M] + k_{4+.M^*}[E.M] \\
d[S]/dt &= -k_{1+.S}[E][S] + k_{1-.S}[E.S] - k_{5+.P}[M^*][S] \\
d[E.S]/dt &= +k_{1+.S}[E][S] - k_{1-.S}[E.S] - k_{2+.P}[E.S] \\
d[P]/dt &= +k_{2+.P}[E.S] + k_{5+.P}[M^*][S] \\
d[M]/dt &= -k_{3+.M}[E][M] + k_{3-.M}[E.M] + k_{5+.P}[M^*][S] \\
d[E.M]/dt &= +k_{3+.M}[E][M] - k_{3-.M}[E.M] - k_{4+.M^*}[E.M] \\
d[M^*]/dt &= +k_{4+.M^*}[E.M] - k_{5+.P}[M^*][S]
\end{aligned}
$$

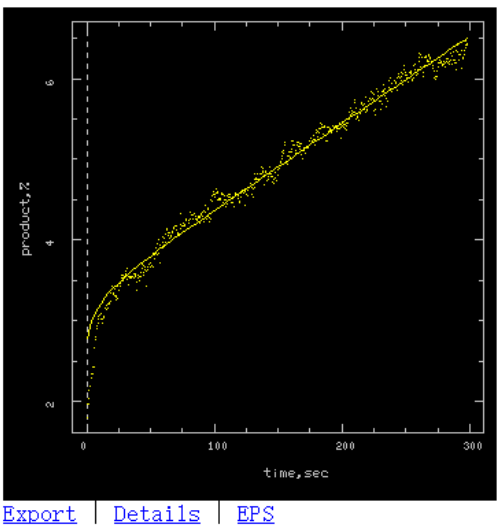
```
Welcome to DynaFit.  Please complete the script template be
To get started, choose menu Help :: Getting Started.

[task]
   task = fit
   data = progress discontinuous
[mechanism]
   E + S <===> E.S     :     k1+.S    k1-.S
   E.S ----> E + P     :     k2+.P
   E + M <===> E.M     :     k3+.M    k3-.M
   E.M ----> E + M*    :     k4+.M*
   M* + S ----> M + P     :     k5+.P
[constants]
   k1+.S = 1 ?
   k1-.S = 0.01 ?
   k2+.P = 1 ?
   k3+.M = 1 ?
   k3-.M = 0.01 ?
   k4+.M* = 1 ?
   k5+.P = 1 ?
[concentrations] ; nM
   S = 47
   E = 0.5
   M = 0.5
[responses]
   P = 15 ?
[data]
   directory   F:/ComputationalBiochemistry/dynafit
   sheet       product_t1.csv
   column      2
   offset      auto ?
[output]
   directory   F:/ComputationalBiochemistry/dynafit/output
[settings]
{Output}
   XAxisLabel = time,sec
   YAxisLabel = product,%
[end]
```

平行实验一

Data and model

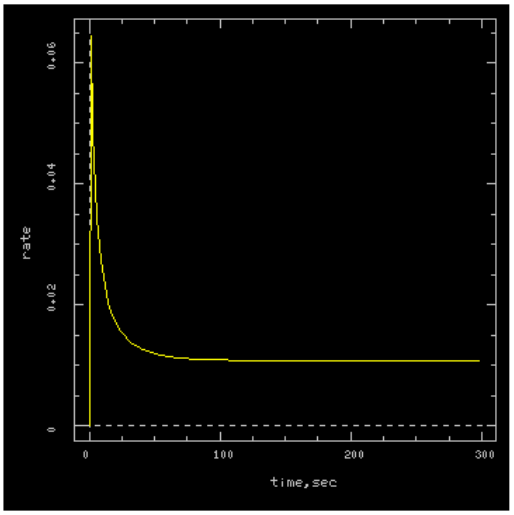
:



Export | Details | EPS

Derivatives (reaction rates)

:



EPS

Initial reaction rates

No.	Dataset	Time	Rate
1	F:/ComputationalBiochemistry/dynafit/product_t1.csv:col(2)	1	0.0645759

Parameters

Trust Region Algorithm

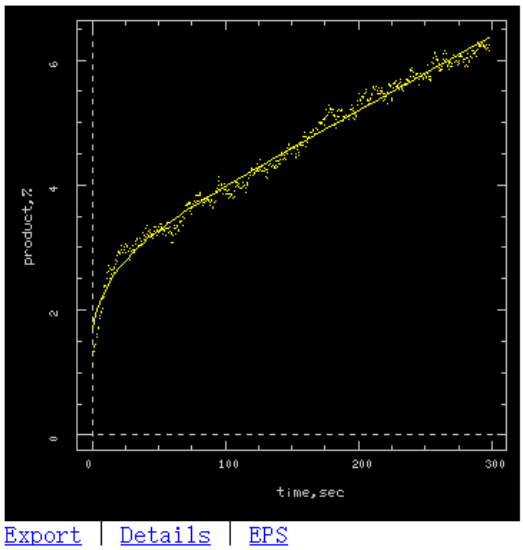
Optimized Parameters

No.	Par#Set	Initial	Final	Std. Error	CV (%)	Note
#1	k1+.S	1	0.00073	0.0058	791.5	
#2	k1-.S	0.01	0.00042	0.33	78369.5	
#3	k2+.P	1	2.8	23	831.6	
#4	k3+.M	1	0.57	1.4	245.7	
#5	k3-.M	0.01	1e-008	1.6e-006	16038.3	
#6	k4+.M*	1	0.002	0.01	501.6	
#7	k5+.P	1	1.1	540	49319.4	
#8	r(P)	15	4.8	41	858.4	
#9	offset#1	1.79074	2.78	0.13	4.8	

平行实验二

Data and model

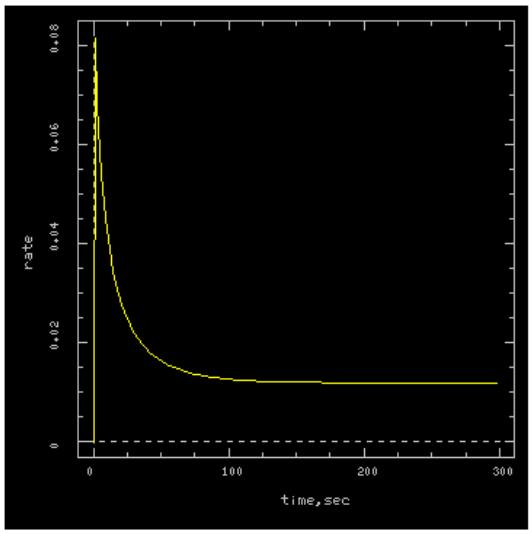
:



Export | Details | EPS

Derivatives (reaction rates)

:



EPS

Initial reaction rates

No.	Dataset	Time	Rate
1	F:/ComputationalBiochemistry/dynafit/product_t2.csv:col(2)	1	0.0816004

Parameters

Trust Region Algorithm

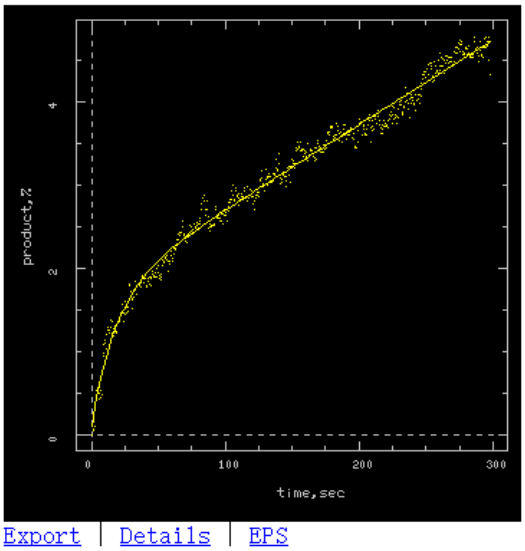
Optimized Parameters

No.	Par#Set	Initial	Final	Std. Error	CV (%)	Note
#1	k1+.S	1	0.86	77	8900.5	
#2	k1-.S	0.01	2.6	110	4147.0	
#3	k2+.P	1	0.016	1.1	6995.9	
#4	k3+.M	1	4.1	430	10481.4	
#5	k3-.M	0.01	0.0022	0.005	225.1	
#6	k4+.M*	1	3.1e-006	0.0012	40258.0	
#7	k5+.P	1	1	310	30853.7	
#8	r(P)	15	13	900	6913.0	
#9	offset#1	1.14014	1.7	0.14	8.0	

平行实验三

Data and model

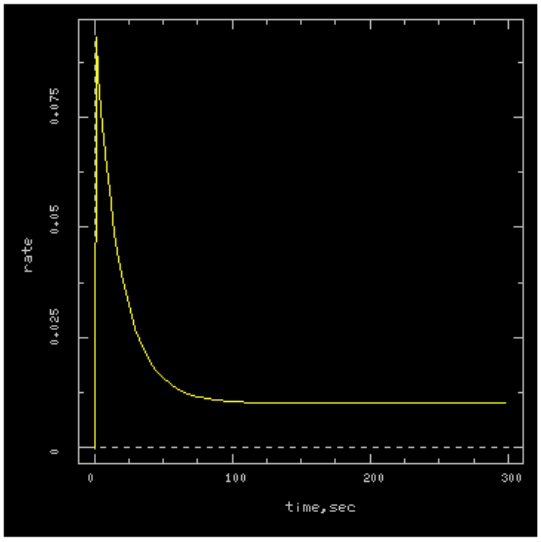
:



Export | Details | EPS

Derivatives (reaction rates)

:



EPS

Initial reaction rates

No.	Dataset	Time	Rate
1	F:/ComputationalBiochemistry/dynafit/product_t3.csv:col(2)	1	0.0932676

Parameters

Trust Region Algorithm

Optimized Parameters

No.	Par#Set	Initial	Final	Std. Error	CV (%)	Note
#1	k1+.S	1	0.49	150	30941.2	
#2	k1-.S	0.01	1e-008	2.1e-005	207942.4	
#3	k2+.P	1	0.001	0.029	2878.0	
#4	k3+.M	1	11	3500	31651.8	
#5	k3-.M	0.01	0.004	2.4	59363.9	
#6	k4+.M*	1	0.063	2.3	3657.1	
#7	k5+.P	1	1	540	54208.1	
#8	r(P)	15	16	570	3558.8	
#9	offset#1	0	0.1	0.12	120.7	

4.漆酶+介体 TEMPO+底物体系（LAC_TEMPO_SUB）(model 2)

对 Laccase + TEMPO + Substance 体系用以下模型通过 dynafit 进行拟合。结果得到三次平行实验的初始反应速率 0.018622，0.0195482，0.0221405，求平均值得到 Initial reaction rate=0.02010357 μM / s =1.206214 μM / min。

从参数优化结果来看，第二次平行实验的优化参数标标准误最小，因此认为第二次的参数更符合实际结果，得到 k1+=12； k1-=1.6e-008； k2+=9.6；k3+=0.0063； r=0.145。

已知介体能一定程度提升漆酶活性，因此 Laccase + TEMPO + Substance 体系反应速率应比 Laccase + Substance 体系反应速率快，可推断 Laccase + TEMPO + Substance 的 model1 更符合理想结果。

Model

Mechanism

$$E + M \leftrightarrows E.M \quad : k_{1+.M}\ k_{1-.M}$$
$$E.M \rightarrow E + M^* \quad : k_{2+.M^*}$$
$$M^* + S \rightarrow M + P \quad : k_{3+.P}$$

Model equation

The fitting function (model equation) for each individual data set is

$S(t) = S_0 + \sum_{i=1}^{n} r_i\ c_i(t)$

where

$S(t)$	the experimental signal observed at time t
S_0	offset on the signal axis (a property of the instrument)
n	number of unique molecular species participating in the reaction mechanism
$c_i(t)$	the concentration of the ith species at time t
r_i	the molar response coefficient of the ith species

In this case, n= 6. The molecular species participating in the mechanism are: E, M, E.M, M*, S, P. The concentrations of these molecular species at time $t$are computed from their initial concentrations (at time zero, t

= 0) by solving an initial-value problem defined by a system of simultaneous first-order Ordinary Differential Equations (ODEs) listed below.

ODE system

Initial value problem: Given the concentrations of molecular species at $t = 0$, compute the concentrations at an arbitrary later time $t > 0$ by solving the following system of ODEs. The solution is obtained by *numerical integration*.

$$d[E]/dt = -k_{1+.M}[E][M] + k_{1-.M}[E.M] + k_{2+.M^*}[E.M]$$
$$d[M]/dt = -k_{1+.M}[E][M] + k_{1-.M}[E.M] + k_{3+.P}[M^*][S]$$
$$d[E.M]/dt = +k_{1+.M}[E][M] - k_{1-.M}[E.M] - k_{2+.M^*}[E.M]$$
$$d[M^*]/dt = +k_{2+.M^*}[E.M] - k_{3+.P}[M^*][S]$$
$$d[S]/dt = -k_{3+.P}[M^*][S]$$
$$d[P]/dt = +k_{3+.P}[M^*][S]$$

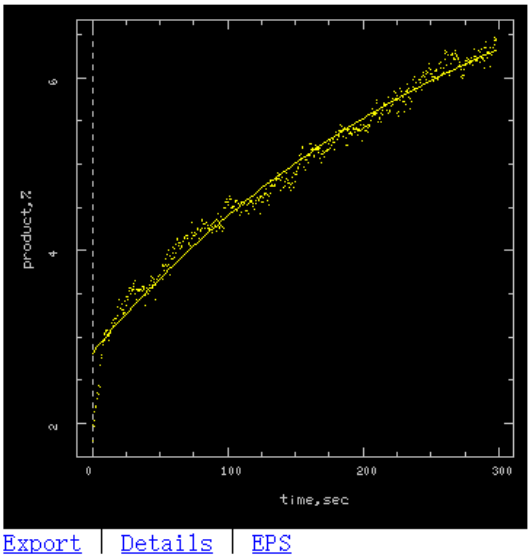
```
Welcome to DynaFit.  Please complete the script template be
To get started, choose menu Help :: Getting Started.
```

```
[task]
   task = fit
   data = progress discontinuous
[mechanism]
   E + M <===> E.M     :     k1+.M   k1-.M
   E.M ----> E + M*    :     k2+.M*
   M* + S ----> M + P    :     k3+.P
[constants]
   k1+.M = 1 ?
   k1-.M = 0.01 ?
   k2+.M* = 1 ?
   k3+.P = 1 ?
[concentrations] ; nM
   S = 47
   E = 0.5
   M = 0.5
[responses]
   P = 15 ?
[data]
   directory   F:/ComputationalBiochemistry/dynafit
   sheet       product_t1.csv
   column      2
   offset      auto ?
[output]
   directory   F:/ComputationalBiochemistry/dynafit/output
[settings]
{Output}
   XAxisLabel = time,sec
   YAxisLabel = product,%
[end]
```

平行实验一

Data and model

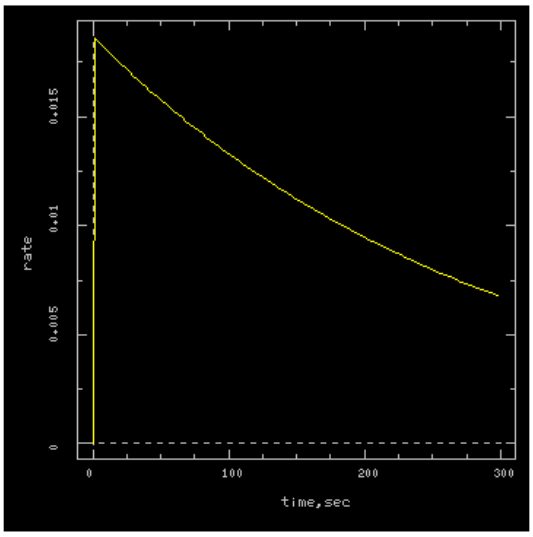
:



Export | Details | EPS

Derivatives (reaction rates)

:



EPS

Initial reaction rates

No.	Dataset	Time	Rate
1	F:/ComputationalBiochemistry/dynafit/product_t1.csv:col(2)	1	0.018622

Parameters

Trust Region Algorithm

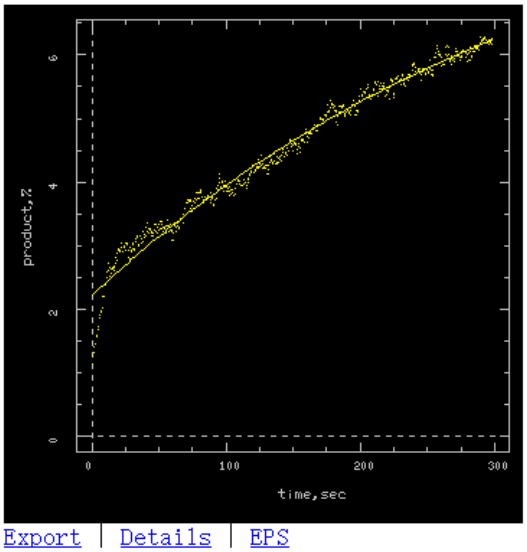
Optimized Parameters

No.	Par#Set	Initial	Final	Std. Error	CV (%)	Note
#1	k1+.M	1	630	9.8e+006	1550534.8	
#2	k1-.M	0.01	0.0002	34000	16984455116.8	
#3	k2+.M*	1	500	1.2e+007	2420547.2	
#4	k3+.P	1	0.0068	0.0041	60.7	
#5	r(P)	15	0.117	0.023	19.9	
#6	offset#1	1.79074	2.81	0.15	5.3	

平行实验二

Data and model

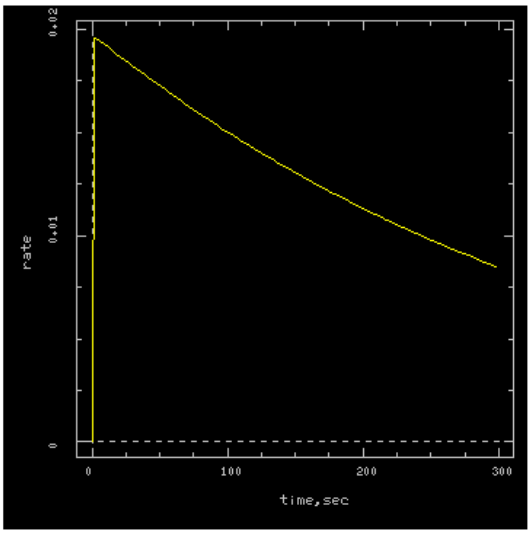
:



Export | Details | EPS

Derivatives (reaction rates)

:



EPS

Initial reaction rates

No.	Dataset	Time	Rate
1	F:/ComputationalBiochemistry/dynafit/product_t2.csv:col(2)	1	0.0195482

Parameters

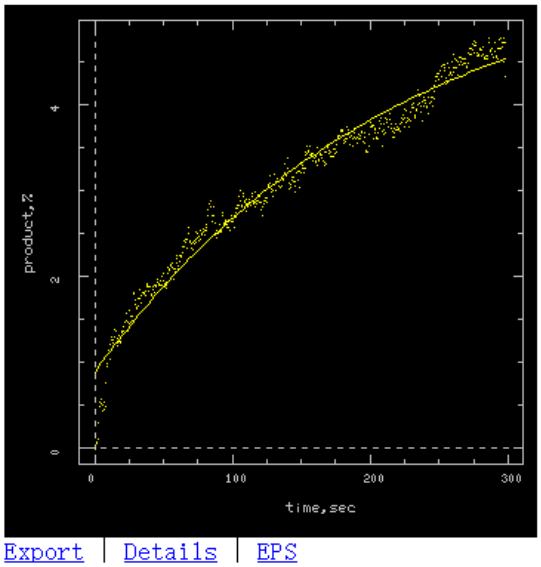
Trust Region Algorithm

Optimized Parameters

No.	Par#Set	Initial	Final	Std. Error	CV (%)	Note
#1	k1+.M	1	12	4300	35567.0	
#2	k1-.M	0.01	1.6e-008	2	12811791104.0	
#3	k2+.M*	1	9.6	5100	52612.1	
#4	k3+.P	1	0.0063	0.0079	125.3	
#5	r(P)	15	0.145	0.061	42.1	
#6	offset#1	1.14014	2.23	0.17	7.7	

Data and model

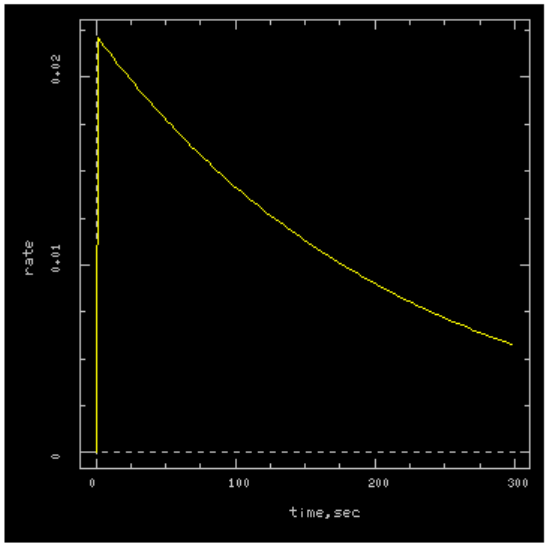
:



Export | Details | EPS

Derivatives (reaction rates)

:



EPS

Initial reaction rates

No.	Dataset	Time	Rate
1	F:/ComputationalBiochemistry/dynafit/product_t3.csv:col(2)	1	0.0221405

Parameters

Trust Region Algorithm

Optimized Parameters

No.	Par#Set	Initial	Final	Std. Error	CV (%)	Note
#1	k1+.M	1	2300	1.2e+008	5285979.6	
#2	k1-.M	0.01	0.00016	47000	29110485758.5	
#3	k2+.M*	1	1900	1.5e+008	7966478.1	
#4	k3+.P	1	0.009	0.0043	47.3	
#5	r(P)	15	0.105	0.013	12.3	
#6	offset#1	0	0.89	0.15	17.3	