

Symbolic Analysis
of
Initial-Rate Kinase Kinetics

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Outline

- Difficulty with traditional steady-state kinetic analysis.
- Alternative to algebra: symbolic / numerical analysis.
- *Example 1:* EuATP inhibition of hexokinase.
- Equilibrium approximation: general numerical treatment.
- *Example 2:* Mechanism of p56^{lck} protein kinase inhibition.
- Beyond kinases: Analysis of highly complex mechanisms.
- Demonstration of computer program *DynaFit*.

Difficulty of Enzyme Kinetics
 Algebraic Complexity

“The traditional method for introducing enzyme kinetics is to lead the reader through a **tortuous maze of algebra**. We shall try instead to show how the equations are derived and manipulated. In this way, the **nonmathematical person** can learn enough of the subject [...] without having to bother with the actual detailed algebra.”

(W.W. Cleland, 1970)

L Thirty years later, can a “non-mathematical person” studying enzyme kinetics avoid “bothering with algebra”?

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W. W. Cleland (1970) in “*The Enzymes*” P. D. Boyer (Ed.), Vol. II

Steady-State Kinase Kinetics
 Part 1: Mechanism

Steady-state sequential ordered “Bi Bi” mechanism

$$\begin{array}{l}
 E + A \xrightleftharpoons[k_{-1}]{k_1} EA \\
 EA + B \xrightleftharpoons[k_{-2}]{k_2} EAB \xrightleftharpoons[k_{-p}]{k_p} EPQ \xrightleftharpoons[k_{-3}]{k_3} EQ + P \\
 EQ \xrightleftharpoons[k_{-4}]{k_4} E + Q
 \end{array}$$

A ... ATP
 B ... peptide
 Q ... ADP
 P ... phospho-peptide

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I. Segel (1975) “*Enzyme Kinetics*” Wiley, New York, p. 560

Steady-State Kinase Kinetics
Part 2: Algebraic Model

$$K_{iA} = \frac{k_{-1}}{k_1}$$

$$K_{mA} = \frac{k_3 k_4 k_p}{k_1(k_3 k_4 + k_3 k_p + k_4 k_p + k_4 k_{-p})}$$

$$K_{iB} = \frac{k_{-1} k_{-2} + k_{-1} k_p + k_{-1} k_{-p} + k_{-2} k_{-p}}{k_2(k_p + k_{-p})}$$

$$K_{mB} = \frac{k_4(k_2 k_3 + k_{-2} k_{-p} + k_3 k_p)}{k_2(k_3 k_4 + k_3 k_p + k_4 k_p + k_4 k_{-p})}$$

$$V_f = [E] \frac{k_3 k_4 k_p}{k_3 k_4 + k_3 k_p + k_4 k_p + k_4 k_{-p}}$$

plus five other constants:

$K_{iQ}, K_{mQ},$

$K_{iP}, K_{mP},$

V_r

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I. Segel (1975) "Enzyme Kinetics" Wiley, New York, p. 564

Steady-State Kinase Kinetics
Part 2: Algebraic Model (continued)

Rate equation:

$$v = \frac{V_f V_r \left([A][B] - \frac{[P][Q]}{K_{eq}} \right)}{V_r K_{iA} K_{mB} + V_r K_{mB} [A] + V_r K_{mA} [B] + \frac{V_f K_{mQ} [P]}{K_{eq}} + \frac{V_f K_{mP} [Q]}{K_{eq}} + V_r [A][B] + \frac{V_f K_{mQ} [A][P]}{K_{iB} K_{eq}} + \frac{V_f [P][Q]}{K_{eq}} + \frac{V_r K_{mA} [B][Q]}{K_{iQ}} + \frac{V_r [A][B][P]}{K_{iP}} + \frac{V_f [B][P][Q]}{K_{iB} K_{eq}}}$$

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I. Segel (1975) "Enzyme Kinetics" Wiley, New York, p. 563

Symbolic Modeling of Initial Rate Data
Program *DynaFit*

"Program DYNAFIT for the Analysis of Enzyme Kinetic Data:
Application to HIV Proteinase"

Kuzmic, P. (1996) *Anal. Biochem.* **237**, 260-273.

- Designed for the analysis of (a) reaction progress, (b) initial velocities, and (c) equilibrium binding data.
- 1998-2000: 48 journal articles cited *DynaFit* ; so far used exclusively for the analysis of *reaction progress* (see handout for list of references).
- Can be applied conveniently for the analysis of initial-rate kinase kinetics.

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Steady-State Kinase Kinetics
Part 3: Symbolic Model

Mechanism:

$$E + A \xrightleftharpoons[k_{-1}]{k_1} EA$$

$$EA + B \xrightleftharpoons[k_{-2}]{k_2} EAB \xrightleftharpoons[k_{-p}]{k_p} EPQ \xrightleftharpoons[k_{-3}]{k_3} EQ + P$$

DynaFit data:

$$EQ \xrightleftharpoons[k_{-4}]{k_4} E + Q$$

[mechanism]			
E + A <==> EA	:	k1	k-1
EA + B <==> EAB	:	k2	k-2
EAB <==> EPQ	:	kp	k-p
EPQ <==> EQ + P	:	k3	k-3
EQ <==> E + Q	:	k4	k-4

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Steady-State Kinase Kinetics
Part 4: Differential-Equation (DE) Model

Mathematical model internally derived by *DynaFit* :

d[E]/dt	=	$-k_1[E][A] + k_{-1}[EA] + k_4[EQ] - k_{-4}[E][Q]$
d[A]/dt	=	$-k_1[E][A] + k_{-1}[EA]$
d[EA]/dt	=	$+k_1[E][A] - k_{-1}[EA] - k_2[EA][B] + k_{-2}[EAB]$
d[B]/dt	=	$-k_2[EA][B] + k_{-2}[EAB]$
d[EAB]/dt	=	$+k_2[EA][B] - k_{-2}[EAB] - k_p[EAB] + k_{-p}[EPQ]$
d[EPQ]/dt	=	$+k_p[EAB] - k_{-p}[EPQ] - k_3[EPQ] + k_{-3}[EQ][P]$
d[EQ]/dt	=	$+k_3[EPQ] - k_{-3}[EQ][P] - k_4[EQ] + k_{-4}[E][Q]$
d[P]/dt	=	$+k_3[EPQ] - k_{-3}[EQ][P]$... overall rate
d[Q]/dt	=	$+k_4[EQ] - k_{-4}[E][Q]$

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Example 1: Hexokinase Inhibition by EuATP
1. Raw Data

Morrison & Cleland (1980) *Biochemistry* **19**, 3127-3131. Figure 3.

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Example 1: Hexokinase Inhibition by EuATP
2. Mechanism

$$\begin{array}{c}
 \text{Mg}^{2+} + \text{ATP} \xrightleftharpoons[k_{d(\text{Mg})}]{k_{a(\text{Mg})}} \text{S} \\
 \text{Eu}^{2+} + \text{ATP} \xrightleftharpoons[k_{d(\text{Eu})}]{k_{a(\text{Eu})}} \text{I} \\
 \hline
 \text{E} + \text{S} \xrightleftharpoons[k_{d(\text{S})}]{k_{a(\text{S})}} \text{ES} \xrightarrow{k_r} \text{E} + \text{P} \\
 \text{E} + \text{I} \xrightleftharpoons[k_{d(\text{I})}]{k_{a(\text{I})}} \text{EI}
 \end{array}$$

Morrison & Cleland (1980) *Biochemistry* **19**, 3127-3131. 10

Example 1: Hexokinase Inhibition by EuATP
3. Algebraic Model

$$v = V_{\max} \frac{b + 2c / K_m^{app} + \sqrt{b^2 - 4ac}}{2(aK_m^{app} + b + c / K_m^{app})}$$

where

$$a = \left(1 + \frac{[I]}{K_i} \right) \left(1 + \frac{[S] + [I]}{K_i} \right) + \frac{K_d}{K_i} \frac{[Mg^{2+}]}{K_{Mg,ATP}}$$

$$b = [S] \left(1 + \frac{[S] + [I]}{K_i} \right) + [Mg^{2+}] \frac{K_d}{K_{Mg,ATP}} \left(1 - \frac{[S] + [I]}{K_i} \right)$$

$$c = -[Mg^{2+}] \frac{K_d}{K_{Mg,ATP}} ([S] + [I])$$

Morrison & Cleland (1980) *Biochemistry* **19**, 3127-3131. Equation (11). 11

Example 1: Hexokinase Inhibition by EuATP
4. Symbolic Model

DynaFit input data (partial display):

```

[task]
data = velocities
task = fit

[mechanism]
Mg + ATP <====> S : ka(Mg) kd(Mg)
Eu + ATP <====> I : ka(Eu) kd(Eu)

E + S <====> E.S : ka(S) kd(S)
E.S ----> E + P : kr
E + I <====> E.I : ka(I) kd(I)

[constants] ...
    
```

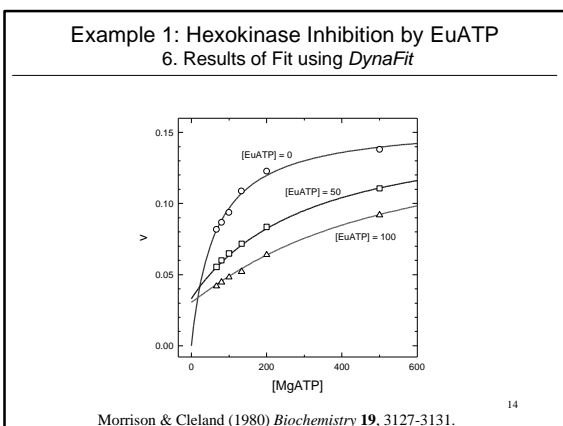
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Example 1: Hexokinase Inhibition by EuATP
 5. Differential Equation Model

Generated automatically by *DynaFit*:

$$\begin{aligned} d[\text{Mg}]/dt &= -k_{\text{amg}}[\text{Mg}][\text{ATP}] + k_{\text{dmg}}[\text{S}] \\ d[\text{ATP}]/dt &= -k_{\text{amg}}[\text{Mg}][\text{ATP}] + k_{\text{dmg}}[\text{S}] - k_{\text{aeu}}[\text{ATP}][\text{Eu}] + k_{\text{deu}}[\text{I}] \\ d[\text{S}]/dt &= +k_{\text{amg}}[\text{Mg}][\text{ATP}] - k_{\text{dmg}}[\text{S}] - k_{\text{as}}[\text{S}][\text{E}] + k_{\text{ds}}[\text{E.S}] \\ d[\text{Eu}]/dt &= -k_{\text{aeu}}[\text{ATP}][\text{Eu}] + k_{\text{deu}}[\text{I}] \\ d[\text{I}]/dt &= +k_{\text{aeu}}[\text{ATP}][\text{Eu}] - k_{\text{deu}}[\text{I}] - k_{\text{ai}}[\text{I}][\text{E}] + k_{\text{di}}[\text{E.I}] \\ d[\text{E}]/dt &= -k_{\text{as}}[\text{S}][\text{E}] + k_{\text{ds}}[\text{E.S}] + k_{\text{r}}[\text{E.S}] - k_{\text{ai}}[\text{I}][\text{E}] + k_{\text{di}}[\text{E.I}] \\ d[\text{E.S}]/dt &= +k_{\text{as}}[\text{S}][\text{E}] - k_{\text{ds}}[\text{E.S}] - k_{\text{r}}[\text{E.S}] \\ d[\text{P}]/dt &= +k_{\text{r}}[\text{E.S}] \\ d[\text{E.I}]/dt &= +k_{\text{ai}}[\text{I}][\text{E}] - k_{\text{di}}[\text{E.I}] \end{aligned}$$

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Example 1: Hexokinase Inhibition by EuATP
 6. Results of Fit (*continued*)

	Initial Fit	Error %	
k_{deu}	1	1.4	0.21
k_{ds}	10000	6040	300
k_{r}	200	157	2.3
k_{di}	1000	1880	110
k_{amg}	10		
k_{dmg}	12		
k_{as}	100		
k_{ai}	100		
$[\text{E}]$	0.001		

$K_{\text{m}} = (k_{\text{ds}} + k_{\text{r}}) / k_{\text{as}}$	62.0 μM
$K_{\text{d}} = k_{\text{deu}} / k_{\text{aeu}}$	0.14 μM
$K_{\text{i}} = k_{\text{di}} / k_{\text{ai}}$	18.8 μM
$V_{\text{max}} = [\text{E}] \times k_{\text{r}}$	0.157 $\mu\text{M}/\text{sec}$

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Example 1: Hexokinase Inhibition by EuATP
7. Comparison of Results

	<i>DynaFit</i>	Morrison & Cleland (1980)
K_m (μM)	62	63 ± 7
K_d (μM)	0.14	0.16 ± 0.04
K_i (μM)	19	18 ± 2
V_{\max} ($\mu\text{M}/\text{sec}$)	0.157	0.158 ± 0.006

Morrison & Cleland (1980) *Biochemistry* **19**, 3127-3131.

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Example 1: Hexokinase Inhibition by EuATP
8. Conclusions

- Algebraic method and the differential-equation method (used in *DynaFit*) give the same results.
- Algebraic model is tedious to derive and prone to error. *DynaFit* model is derived by the computer.
- Algebraic model cannot be extended to “tight binding”. *DynaFit* model is applicable to “tight binding” without change.

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Rapid Equilibrium Approximation
Quick Summary

- Applicable when the catalytic step in the mechanism is relatively slow compared to binding and dissociation.
- *DynaFit* solves multiple simultaneous equilibria by using a special numerical (iterative) method :
I & Nancollas (1972) *Anal. Chem.* **44**, 1940-1950.
- This numerical method is equally applicable to “tight-binding” and to classical enzyme inhibition.

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**Rapid Equilibrium Approximation
Example Script**

```

[task]
task = fit
data = velocities

[mechanism]
E + S <==> ES : Ks  dissoc.
ES ---> E + P : kcat
ES + S <==> ES2 : Ks2 dissoc.
E + I <==> EI : Ki  dissoc.
ES + I <==> ESI : Kiu dissoc.

[constants]
Ks = 5000 ?, kcat = 400000 ?
Ks2 = 2000 ?
Ki = 10 ?
Kiu = 10 ?
...
    
```

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**Example 2: p56^{lck} Tyrosine Kinase Inhibition
1. Raw Data - Peptide as Varied Substrate**

Lineweaver-Burk plot

NC(=O)c1ccc(cc1N2C=NC(=O)N2)c3ccc(cc3N4CCNCC4)N5CCNCC5

WIN-61651

Faltynek et al. (1995) *J. Enz. Inhib.* **9**, 111-122.

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**Example 2: p56^{lck} Tyrosine Kinase Inhibition
2. Peptide Kinetics: Michaelis-Menten Model**

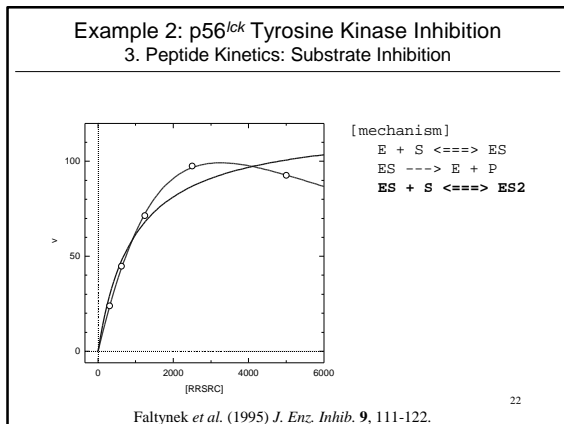
[mechanism]

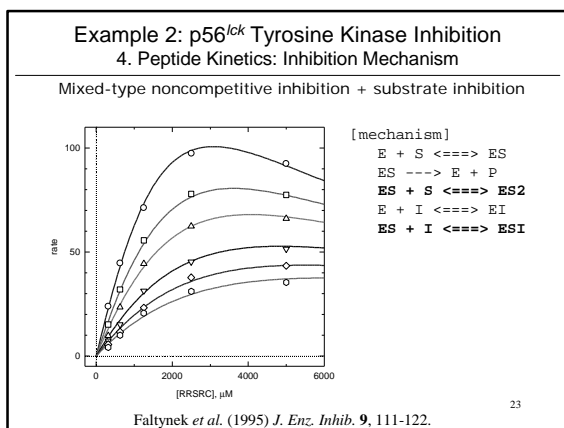
E + S <==> ES

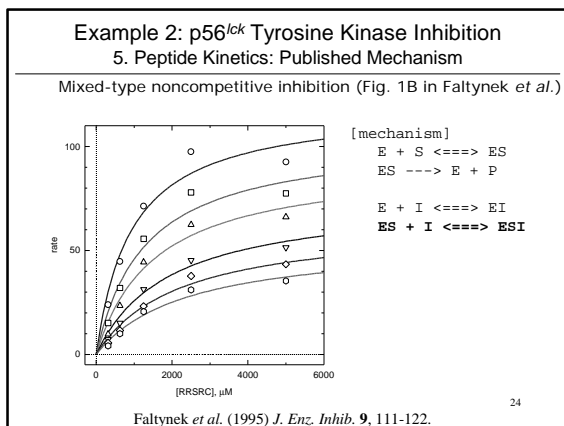
ES ---> E + P

Faltynek et al. (1995) *J. Enz. Inhib.* **9**, 111-122.

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Example 2: p56^{lck} Tyrosine Kinase Inhibition
6. Peptide Kinetics: Comparison of Results

	<i>DynaFit</i>	Faltynek <i>et al.</i> (1995)
K_s (μM)	9100 \pm 3700	990 \pm 140
K_{s2} (μM)	1100 \pm 450	—
K_i (μM)	28 \pm 2	18 \pm 4
K_{iu} (μM)	14 \pm 5	67 \pm 18
<i>squares</i>	2.1	19.5

Faltynek *et al.* (1995) *J. Enz. Inhib.* **9**, 111-122. 25

Example 2: p56^{lck} Tyrosine Kinase Inhibition
7. Peptide Kinetics: Conclusions

<i>DynaFit</i>	Faltynek <i>et al.</i> (1995)
<ul style="list-style-type: none"> • Peptide substrate of p56^{lck} kinase shows <i>substrate inhibition</i>. • WIN-61651 has greater affinity for <i>ATP site</i> than for peptide site. 	<ul style="list-style-type: none"> • Peptide substrate of p56^{lck} kinase follows pure <i>Michaelis-Menten</i> kinetics. • WIN-61651 has greater affinity for <i>peptide site</i>.
<ul style="list-style-type: none"> • These conclusions are <i>incorrect</i>. 	

Faltynek *et al.* (1995) *J. Enz. Inhib.* **9**, 111-122. 26

Beyond Kinases
Analysis of highly complex mechanisms

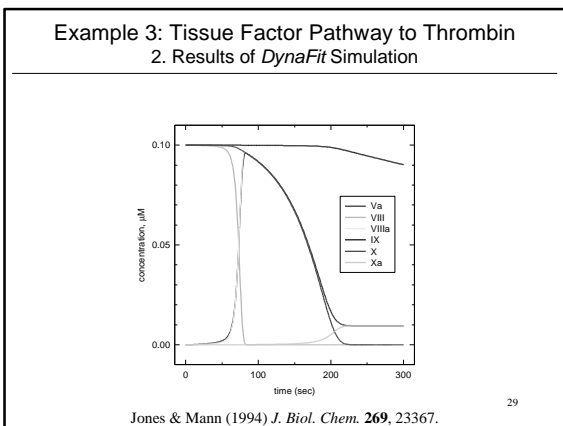
<ul style="list-style-type: none"> • At least in principle, mechanisms shown so far <i>can</i> be described by algebraic models. (Exception: <i>tight-binding</i> inhibition). • However, many biochemical mechanisms <i>cannot</i> be described by algebraic models. A single rate equation can never be derived. • In the latter case, tools such as <i>DynaFit</i> become a necessity, not just a convenience.

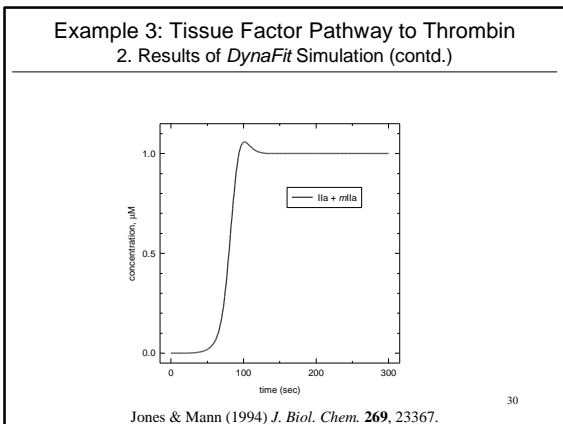
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Example 3: Tissue Factor Pathway to Thrombin
1. Symbolic Definition of Mechanism

```
[mechanism]
IX + TF.VIIa <=> IX.TF.VIIa      : k6   k16
IX.TF.VIIa --> TF.VIIa + IXa    : k11
X + TF.VIIa <=> X.TF.VIIa       : k6   k17
X.TF.VIIa --> TF.VIIa + Xa     : k12
X + VIIIa.IXa <=> X.VIIIa.IXa   : k6   k18
X.VIIIa.IXa --> VIIIa.IXa + Xa  : k13
IX + Xa --> Xa + IXa           : k15
V + Xa --> Va + Xa             : k1
VIII + Xa --> VIIIa + Xa       : k3
V + IIa --> IIa + Va           : k2
VIII + IIa --> VIIIa + IIa     : k4
II + Va.Xa <=> II.Va.Xa         : k6   k19
II.Va.Xa --> Va.Xa + mIIa      : k14
mIIa + Va.Xa --> Va.Xa + IIa   : k5
VIIIa + IXa <=> VIIIa.IXa      : k7   k9
Va + Xa <=> Va.Xa              : k8   k10
                                     28
```

Jones & Mann (1994) *J. Biol. Chem.* **269**, 23367.





Example 4: Fatty-Acid Biosynthesis Assay

1. Symbolic Definition of Mechanism

```

[mechanism]

; Malonyl transfer (fabD)
MalCoA + fabD <=> MalCoA.fabD      : k1 k-1
MalCoA.fabD + AcACP <=> MalCoA.fabD.AcACP : k2 k-2
MalCoA.fabD.AcACP --> MalAcACP + CoA + fabD : k3

; Condensation (fabF)
MalAcACP + fabF <=> MalAcACP.fabF    : k4 k-4
MalAcACP.fabF <=> KeBuACP.fabF       : k5 k-5
KeBuACP.fabF --> KeBuACP + fabF     : k6

; Reduction (fabG)
KeBuACP + fabG <=> KeBuACP.fabG      : k7 k-7
KeBuACP.fabG + NADPH <=> KeBuACP.fabG.NADPH : k8 k-8
KeBuACP.fabG.NADPH --> HyBuACP + fabG + NADP : k9

; Coupled reduction (FMN oxidoreductase)
NADP + E <=> NADP.E                  : k10 k-10
NADP.E + FMNH2 <=> NADP.E.FMNH2     : k11 k-11
NADP.E.FMNH2 --> NADPH + E + FMN   : k12
    
```

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Example 4: Fatty-Acid Biosynthesis Assay

2. Results of *DynaFit* Simulation

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Symbolic Analysis of Initial Rate Kinetics

Summary and Conclusions

- Program *DynaFit* can be used to analyze initial reaction velocities observed in enzyme assays.
- The two main advantages are (a) convenience and (b) general applicability to an arbitrarily complex mechanism.
- REMAINING PROBLEM : A major limitation on analysis of complex mechanisms is that sufficient information (*i.e.*, rate constants) must be available for individual steps.
- POSSIBLE SOLUTION : Customize *DynaFit* to simplify kinetic analysis where insufficient information is available about individual reaction steps (steady-state approximation; “differential-algebraic” systems).

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