Numerical Enzymology
Generalized Treatment of Kinetics & Equilibria
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BioKin, Ltd.

DYNAFIT SOFTWARE PACKAGE

1. Overview of recent applications
2. Selected examples
   - ATPase cycle of Hsp90 Analog Trap1 (Leskovar et al., 2008)
   - Nucleotide binding to ClpB (Werbeck et al., 2009)
   - Clathrin uncoating (Rothnie et al., 2011)
3. Recent enhancements
   - Optimal Experimental Design

DYNAFIT software

NUMERICAL ENZYME KINETICS AND LIGAND BINDING

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

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Received January 26, 1996

http://www.biokin.com/dynafit
DynaFit: Citation analysis

JULY 2011: 683 BIBLIOGRAPHIC REFERENCES (*WEB OF SCIENCE*)

### A "Kinetic Compiler"

**HOW DYNAFIT PROCESSES YOUR BIOCHEMICAL EQUATIONS**

<table>
<thead>
<tr>
<th>Input (plain text file):</th>
<th>Rate terms:</th>
<th>Rate equations:</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ E + S \rightarrow ES : k_1 ]</td>
<td>[ E\times[S]]</td>
<td>[ d[ES] / dt = + [E] \times [S] + k_2 \times [ES] + k_3 \times [ES] ]</td>
</tr>
<tr>
<td>[ ES \rightarrow E + S : k_2 ]</td>
<td>[ k_2 \times [ES] ]</td>
<td>[ d[ES] / dt = + [E] \times [S] - k_2 \times [ES] - k_3 \times [ES] ]</td>
</tr>
<tr>
<td>[ ES \rightarrow E + P : k_3 ]</td>
<td>[ k_3 \times [ES] ]</td>
<td></td>
</tr>
</tbody>
</table>
**System of Simple, Simultaneous Equations**

How DynaFit processes your biochemical equations

```
E + S ⇌ E.S ⇌ E + P
```

"The LEGO method" of deriving rate equations

<table>
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</thead>
<tbody>
<tr>
<td>E + S (\rightarrow) ES : (k_1)</td>
<td><img src="image1" alt="Rate terms" /></td>
<td><img src="image2" alt="Rate equations" /></td>
</tr>
<tr>
<td>ES (\rightarrow) E + S : (k_2)</td>
<td></td>
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</tr>
<tr>
<td>ES (\rightarrow) E + P : (k_3)</td>
<td></td>
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</tr>
</tbody>
</table>

**DynaFit can analyze many types of experiments**

Mass action law and mass conservation law is applied to derive different models

<table>
<thead>
<tr>
<th>Experiment</th>
<th>DynaFit derives a system of...</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction progress</td>
<td>First-order ordinary differential equations</td>
</tr>
<tr>
<td>Initial rates</td>
<td>Nonlinear algebraic equations</td>
</tr>
<tr>
<td>Equilibrium binding</td>
<td>Nonlinear algebraic equations</td>
</tr>
</tbody>
</table>
DynaFit: Recent enhancements


DynaFit Example 1: Trap1 ATPase cycle

EXCELLENT EXAMPLE OF COMBINING "TRADITIONAL" (ALGEBRAIC) AND NUMERICAL (DYNAFIT) MODELS

FIGURE 7. Kinetic model of Trap1 ATPase cycle at 25 ºC

Leskovar et al. (2008)
"The ATPase cycle of the mitochondrial Hsp90 analog Trap1"
J. Biol. Chem. 283, 11677-688

Eo "open" conformation
Ec "closed" conformation
**DynaFit Example 1: Trap1 ATPase cycle - experiments**

**THREE DIFFERENT TYPES OF EXPERIMENTS COMBINED**

- varied ATP analog
- stopped flow fluorescence
- varied ADP analog
- stopped flow fluorescence
- single-turnover ATPase assay

**Leskovar et al. (2008) J. Biol. Chem. 283, 11677-688**

**DynaFit Example 1: Trap1 ATPase cycle - script**

**MECHANISM INCLUDES PHOTO-BLEACHING (ARTIFACT)**

```plaintext
[task]
data = progress
    task = fit

[mechanism]
Eo + ATP <=> Eo.ATP : kat kdt
Eo.ATP <=> Ec.ATP : koc koo
Ec.ATP ----> Ec.ADP : khy
Ec.ADP <=> Eo + ADP : kdd kad

PbT ---> PbT* : kbt
PbD ---> PbD* : kbd

photo-bleaching is a first-order process
```

**[data]**

directory ./users/EDU/DE/MPImF/Leskovar_A/...
extension txt
plot logarithmic
monitor Eo, Ec.ATP, Ec.ADP, PbT*, PbD*

show concentrations of these species over time

**Leskovar et al. (2008) J. Biol. Chem. 283, 11677-688**
DynaFit Example 1: Trap1 – species concentrations

USEFUL WAY TO GAIN INSIGHT INTO THE MECHANISM

Leskovar et al. (2008) J. Biol. Chem. 283, 11677-688

DynaFit Example 2: Nucleotide binding to ClpB

FROM THE SAME LAB (MAX-PLANCK INSTITUTE FOR MEDICAL RESEARCH, HEIDELBERG)

Werbeck et al. (2009)
"Nucleotide binding and allosteric modulation of the second AAA+ domain of ClpB probed by transient kinetic studies"
Biochemistry 48, 7240-7250
DynaFit Example 2: Nucleotide binding to ClpB - data

Again combine two different experiments (only "labeled" nucleotide here)

Werbeck et al. (2009) Biochemistry 48, 7240-7250

DynaFit Example 2: Nucleotide binding to ClpB script

The devil is always in the detail

\[
\text{task} = \begin{cases}
data = \text{progress} \\
task = \text{fit} \\
model = \text{simplest}
\end{cases}
\]

\[
\text{mechanism} = \begin{cases}
P + mADP \leftrightarrow P.mADP : k_1 \ k_{-1} \\
\rightarrow \text{drift} : v
\end{cases}
\]

\[
\text{constants} = \begin{cases}
k_1 = 5 ? \\
k_{-1} = 0.1 ? \\
v = 0.1 ?
\end{cases}
\]

"drift in the machine"

Werbeck et al. (2009) Biochemistry 48, 7240-7250
DynaFit Example 3: Clathrin uncoating kinetics

IN COLLABORATION WITH GUS CAMERON (BRISTOL)

Rothnie et al. (2011)
"A sequential mechanism for clathrin cage disassembly by 70-kDa heat-shock cognate protein (Hsc70) and auxilin"

MODEL DISCRIMINATION ANALYSIS

[task]
task = fit
data = progress
model = AAAH ?

[mechanism]
CA + T ---> CAT : ka
CAT + T ---> CATT : ka
CATT + T ---> CATT : ka
CATT ---> CADDD : kr
CADDD ---> Prods : kd
...

[task]
task = fit
data = progress
model = AHAHAH ?

[mechanism]
CA + T ---> CAT : ka
CAT ---> CAD + Pi : kr
CAD + T ---> CADT : ka
CADT ---> CADDD + Pi : kr
CADDD ---> CADDT : ka
CADDT ---> CADDD + Pi : kr
CADDD ---> Prods : kd
...

• Arbitrary number of models to compare
• Model selection based on two criteria:
  - Akaike Information Criterion (AIC)
  - F-test for nested models
• Extreme caution is required for interpretation
  - Both AIC and F-test are far from perfect
  - Both are based on many assumptions
  - One must use common sense
  - Look at the results only for guidance

Optimal Experimental Design: Books

**DOZENS OF BOOKS**

- *Fedorov, V.V. (1972)*
  “Theory of Optimal Experiments”

- *Fedorov, V.V. & Hackl, P. (1997)*
  “Model-Oriented Design of Experiments”

  “Optimum Experimental Designs”

- *Endrenyi, L., Ed. (1981)*
  “Design and Analysis of Enzyme and Pharmacokinetics Experiments”

Optimal Experimental Design: Articles

**HUNDREDS OF ARTICLES, INCLUDING IN ENZYMOLOGY**


**Optimal Design of Experiments for the Estimation of Precise Hyperbolic Kinetic and Binding Parameters**

László Endrenyi and Fung-Yee Chan

*Analytical Biochemistry 184, 172-183 (1990)*

**DESIGN: Computerized Optimization of Experimental Design for Estimating $K_d$ and $B_{max}$ in Ligand Binding Experiments**

G. Enrico Rovati, David Rodbard, and Peter J. Munson
Some theory: Fisher information matrix

"D-OPTIMAL" DESIGN: MAXIMIZE DETERMINANT OF THE FISHER INFORMATION MATRIX

Fisher information matrix: 
\[ (I(\theta))_{ij} = -E \frac{\partial^2}{\partial \theta_i \partial \theta_j} \ln f(X; \theta) \]

EXAMPLE: Michaelis-Menten kinetics

Model: 
\[ v = \frac{[S]}{[S] + K} \]

Design: four concentrations \((N=4)\) 
\([S]_1, [S]_2, [S]_3, [S]_4\)

Derivatives: ("sensitivities")
\[ s_r = \frac{\partial v}{\partial V} = \frac{[S]}{[S] + K} \]
\[ s_k = \frac{\partial v}{\partial K} = -V \frac{[S]}{([S] + K)^2} \]

Some theory: Fisher information matrix (contd.)

"D-OPTIMAL" DESIGN: MAXIMIZE DETERMINANT OF THE FISHER INFORMATION MATRIX

Approximate Fisher information matrix \((M \times M)\):
\[ F_{i,j} = \sum_{k=1}^{N} s_i([S]_k) s_j([S]_k) \]

EXAMPLE: Michaelis-Menten kinetics

\[ F = \begin{pmatrix} 
\sum_{i=1}^{N} \left( \frac{[S]_i}{([S]_i + K)^4} \right)^2 & \sum_{i=1}^{N} \left( -V \frac{[S]_i}{([S]_i + K)^4} \right) \left( \frac{[S]_i}{([S]_i + K)^2} \right) \\
\sum_{i=2}^{N} \left( -V \frac{[S]_i}{([S]_i + K)^4} \right) \left( \frac{[S]_i}{([S]_i + K)^2} \right) & \sum_{i=2}^{N} \left( -V \frac{[S]_i}{([S]_i + K)^4} \right) \left( \frac{[S]_i}{([S]_i + K)^2} \right)^2 
\end{pmatrix} \]

\[ \det F = F_{11}F_{22} - F_{12}F_{21} \] determinant

"D-Optimal" Design:
Maximize determinant of \( F \) over design points \([S]_1, \ldots, [S]_4\).
Optimal Design for Michaelis-Menten kinetics

DUGGLEBY, R. (1979) J. THEOR. BIOL. 81, 671-684

Model:
\[ v = V \frac{[S]}{[S] + K} \]
\[ v = 1 \]
\[ K = 1 \]

\[ [S]_{opt} = \frac{[S]_{max} K}{[S]_{max} + 2K} \]

KS

SVv

+=[S]

max

max

opt

KS

K is assumed to be known!

Optimal Design: Basic assumptions

OPTIMAL DESIGN FOR ESTIMATING PARAMETERS IN THE GIVEN MODEL

TWO FAIRLY STRONG ASSUMPTIONS:

1. Assumed mathematical model is correct for the experiment
2. A fairly good estimate already exists for the model parameters

“Designed” experiments are most suitable for follow-up (verification) experiments.
Optimal Experimental Design: Initial conditions

In many kinetic experiments the observation time cannot be chosen.

Conventional Experimental Design:

- Make an optimal choice of the independent variable:
  - Equilibrium experiments: concentrations of varied species
  - Kinetic experiments: observation time

DynaFit Modification:

- Make an optimal choice of the initial conditions:
  - Kinetic experiments: initial concentrations of reactants

Assume that the time points are given by instrument setup.

Optimal Experimental Design: DynaFit input file

Example: CLATHRIN UNCOATING KINETICS

```
[task]
task = design
data = progress

[mechanism]
CA + T -> CAT   : ka
CAT -> CAD + Pi : kr
CAD + T -> CADT : ka
CADT -> CADD + Pi : kr
CADD + T -> CADDT : ka
CADDT -> CADD + Pi : kr
CADD -> Prods   : kd

[constants]
ka = 0.69
kr = 6.51
kd = 0.38

“Choose eight initial concentration of T such that the rate constants $k_a$, $k_r$, $k_d$ are determined most precisely.”
```

```
Optimal Experimental Design: Preliminary experiment

**EXAMPLE: CLATHRIN UNCOATING KINETICS – ACTUAL DATA**


Actual concentrations of $[T]$ (µM)

![Graph showing actual concentrations of $[T]$](image)

Six different experiments

Optimal Experimental Design: DynaFit results

**EXAMPLE: CLATHRIN UNCOATING KINETICS**

D-Optimal initial concentrations:

- $[T] = 0.70$ µM, $0.73$ µM
- $[T] = 2.4$ µM, $2.5$ µM, $2.5$ µM
- $[T] = 76$ µM, $81$ µM, $90$ µM

"maximum feasible concentration"

upsing phase no longer seen

Just three experiments would be sufficient for follow-up
**Optimal Experimental Design in DynaFit: Summary**

**NOT A SILVER BULLET!**

- **Useful for follow-up (verification) experiments only**
  - Mechanistic model must be known already
  - Parameter estimates must also be known

- **Takes a very long time to compute**
  - Constrained global optimization: "Differential Evolution" algorithm
  - Clathrin design took 30-90 minutes
  - Many design problems take multiple hours of computation

- **Critically depends on assumptions about variance**
  - Usually we assume *constant variance* ("noise") of the signal
  - Must verify this by plotting *residuals against signal* (not the usual way)