Numerical Enzymology

Generalized Treatment of Kinetics & Equilibria

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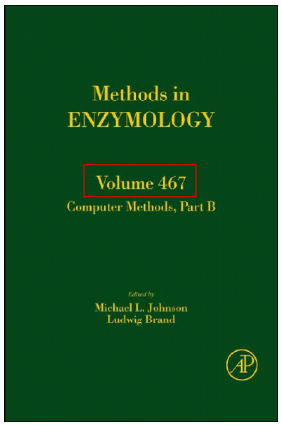
DYNAFIT SOFTWARE PACKAGE

BioKin L t d.

DynaFit software

"NUMERICAL" ENZYME KINETICS AND LIGAND BINDING



CHAPTER TEN

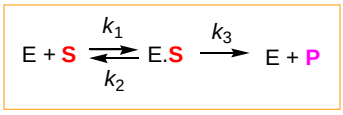
DYNAFIT—A SOFTWARE PACKAGE FOR ENZYMOLOGY

Petr Kuzmič

Kuzmic, P. (2009) *Meth. Enzymol.* **467**, 248-280

A "Kinetic Compiler"

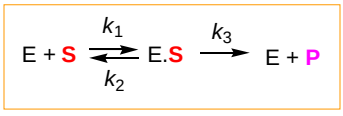
HOW DYNAFIT PROCESSES YOUR BIOCHEMICAL EQUATIONS



Input (plain text file):	Rate terms:	Rate equations:
E + S ---> ES : k1	$k_1 \times [E] \times [S]$	d[***E***] / dt = - $k_1 \times [E] \times [S]$ + $k_2 \times [ES]$ + $k_3 \times [ES]$
ES ---> E + S : k2	$k_2 \times [ES]$	d[***ES***] / dt = + $k_1 \times [E] \times [S]$ - $k_2 \times [ES]$ - $k_3 \times [ES]$
ES ---> E + P : k3	$k_3 \times [ES]$	Similarly for other species...

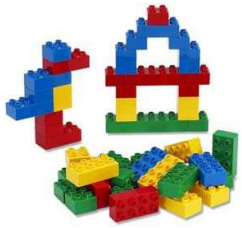
System of Simple, Simultaneous Equations

HOW DYNAFIT PROCESSES YOUR BIOCHEMICAL EQUATIONS



"The LEGO method" of deriving rate equations

Input (plain text file):	Rate terms:	Rate equations:
E + S ---> ES : k1	$k_1 \times [E] \times [S]$	
ES ---> E + S : k2	$k_2 \times [ES]$	
ES ---> E + P : k3	$k_3 \times [ES]$	



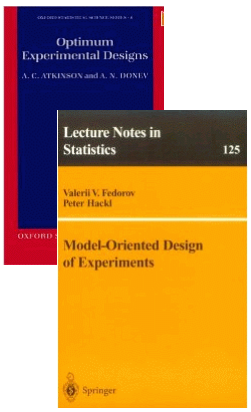
DynaFit can analyze many types of experiments

MASS ACTION LAW AND **MASS CONSERVATION LAW** IS APPLIED TO DERIVE DIFFERENT MODELS

EXPERIMENT	DYNAFIT DERIVES A SYSTEM OF …
Reaction progress	First-order ordinary differential equations
Initial rates	Nonlinear algebraic equations
Equilibrium binding	Nonlinear algebraic equations

Optimal Experimental Design: Books

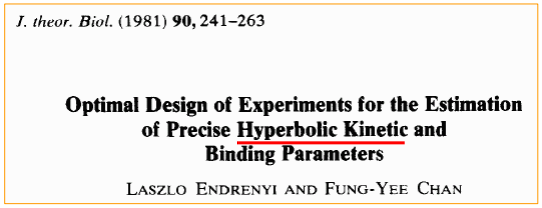
DOZENS OF **BOOKS**



- *Fedorov, V.V. (1972)*
  "Theory of Optimal Experiments"
- *Fedorov, V.V. & Hackl, P. (1997)*
  "Model-Oriented **Design** of Experiments"
- *Atkinson, A.C & Donev, A.N. (1992)*
  "Optimum Experimental **Designs**"
- *Endrenyi, L., Ed. (1981)*
  "**Design** and Analysis of **Enzyme** and Pharmacokinetics Experiments"

Optimal Experimental Design: Articles

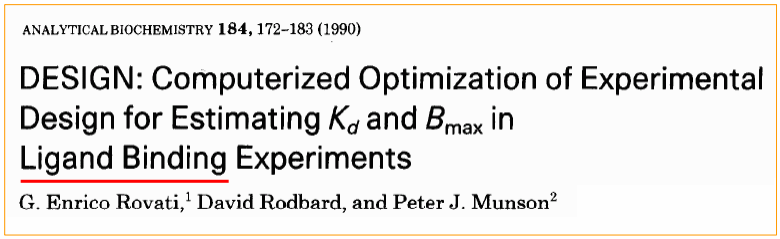
HUNDREDS OF **ARTICLES**, INCLUDING IN **ENZYMOLOGY**



J. theor. Biol. (1981) **90**, 241–263

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

LASZLO 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,[1] David Rodbard, and Peter J. Munson[2]

Some theory: Fisher information matrix

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

Fisher information matrix: $$(\mathcal{I}(\theta))_{i,j} = -\mathrm{E}\left[\frac{\partial^2}{\partial\theta_i\,\partial\theta_j}\ln f(X;\theta)\,\middle|\,\theta\right]$$

EXAMPLE: Michaelis-Menten kinetics

Model:

$$v = \frac{V[S]}{[S]+K}$$

two parameters (***M***=2)

Design: four concentrations (***N***=4)

$[S]_1$, $[S]_2$, $[S]_3$, $[S]_4$

Derivatives: ("sensitivities")

$$s_V \equiv \frac{\partial v}{\partial V} = \frac{[S]}{[S]+K}$$

$$s_K \equiv \frac{\partial v}{\partial K} = -V\frac{[S]}{([S]+K)^2}$$

Some theory: Fisher information matrix (contd.)

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

Approximate Fisher information matrix (***M*** × ***M***):

$$F_{i,j} = \sum_{k=1}^{N} s_i([S]_k) s_j([S]_k)$$

EXAMPLE: Michaelis-Menten kinetics

$$\mathbf{F} = \begin{pmatrix} \sum_{k=1}^{N} \left(\frac{[S]_k}{[S]_k + K} \right)^2 & \sum_{k=1}^{N} \left(-V \frac{[S]_k}{([S]_k + K)^2} \right) \left(\frac{[S]_k}{[S]_k + K} \right) \\ \sum_{k=1}^{N} \left(-V \frac{[S]_k}{([S]_k + K)^2} \right) \left(\frac{[S]_k}{[S]_k + K} \right) & \sum_{k=1}^{N} \left(-V \frac{[S]_k}{([S]_k + K)^2} \right)^2 \end{pmatrix}$$

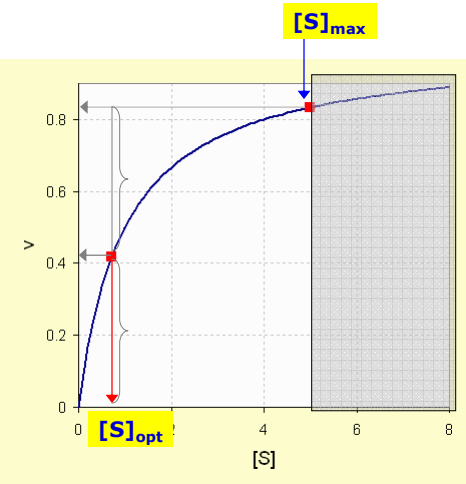
$$\det \mathbf{F} = F_{11}F_{22} - F_{12}F_{21}$$ **determinant**

"D-Optimal" Design:

Maximize determinant of **F** over design points $[S]_1$, ... $[S]_4$.

Optimal Design for Michaelis-Menten kinetics

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



Model:

$$v = V \frac{[S]}{[S] + K}$$

$V = 1$
$K = 1$

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

***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 –> CADDD + Pi     : kr
   CADDD –> Prods          : kd

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

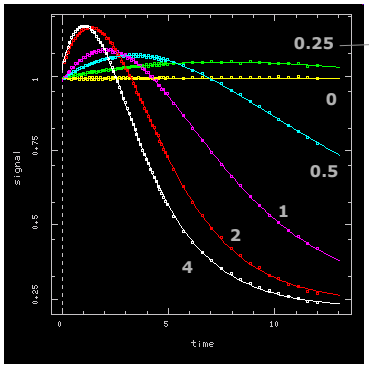
"Choose eight initial concentration of **T** such that the rate constants k_a, k_r, k_d are determined most precisely."

```
[data]
   file run01 | concentration CA = 0.1, T = 1 ?? (0.001 .. 100)
   file run02 | concentration CA = 0.1, T = 1 ?? (0.001 .. 100)
   file run03 | concentration CA = 0.1, T = 1 ?? (0.001 .. 100)
   file run04 | concentration CA = 0.1, T = 1 ?? (0.001 .. 100)
   file run05 | concentration CA = 0.1, T = 1 ?? (0.001 .. 100)
   file run06 | concentration CA = 0.1, T = 1 ?? (0.001 .. 100)
   file run07 | concentration CA = 0.1, T = 1 ?? (0.001 .. 100)
   file run08 | concentration CA = 0.1, T = 1 ?? (0.001 .. 100)
```


Optimal Experimental Design: Preliminary experiment

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

Rothnie *et al.* (2011) *Proc. Natl. Acad. Sci USA* **108**, 6927–6932

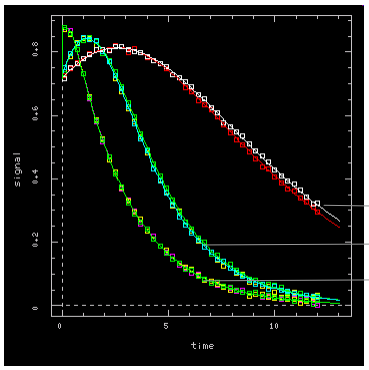


Actual concentrations of **[T]** (μM)

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"
upswing 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)