Numerical Enzymology

Generalized Treatment of Kinetics & Equilibria

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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**

BioKin L t d.

DYNAFIT software

NUMERICAL ENZYME KINETICS AND LIGAND BINDING

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

ANALYTICAL BIOCHEMISTRY 237, 260–273 (1996)
ARTICLE NO. 0238

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

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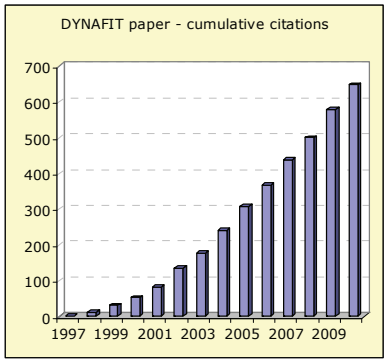
Received January 26, 1996

http://www.**biokin**.com/**dynafit**

DynaFit: Citation analysis

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



Biochemistry (USA)	~65%
J. Biol. Chem.	~20%

A "Kinetic Compiler"

HOW DYNAFIT PROCESSES YOUR BIOCHEMICAL EQUATIONS

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E.S \xrightarrow{k_3} E + P$$

Input (plain text file):

```
E + S ---> ES  :  k1
ES ---> E + S  :  k2
ES ---> E + P  :  k3
```

Rate terms:

$k_1 \times [E] \times [S]$

$k_2 \times [ES]$

$k_3 \times [ES]$

Rate equations:

$$d[\boldsymbol{E}] / dt = - k_1 \times [E] \times [S] + k_2 \times [ES] + k_3 \times [ES]$$

$$d[\boldsymbol{ES}] / dt = + k_1 \times [E] \times [S] - k_2 \times [ES] - k_3 \times [ES]$$

Similarly for other species...

System of Simple, Simultaneous Equations

HOW DYNAFIT PROCESSES YOUR BIOCHEMICAL EQUATIONS

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E.S \xrightarrow{k_3} E + P$$

"The LEGO method" of deriving rate equations

Input (plain text file):	Rate terms:	Rate equations:

`E + S ---> ES : k1`
`ES ---> E + S : k2`
`ES ---> E + P : k3`
	$k_1 \times [E] \times [S]$
	$k_2 \times [ES]$
	$k_3 \times [ES]$
		

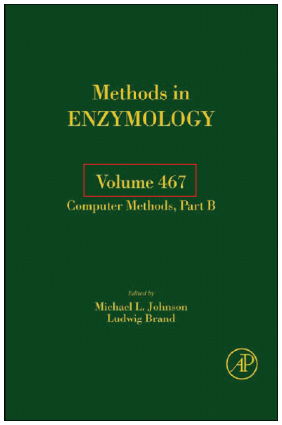
DynaFit can analyze many types of experiments

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

DynaFit: Recent enhancements

REVIEW (**2009**)



CHAPTER TEN

DynaFit—A Software Package for Enzymology

Petr Kuzmič

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

DynaFit Example 1: Trap1 ATPase cycle

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

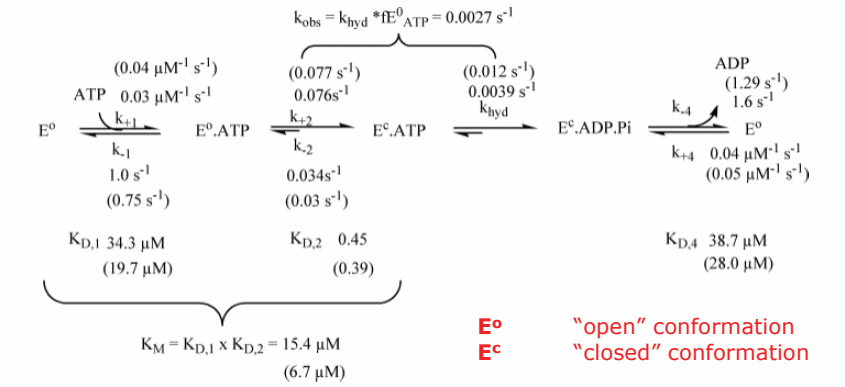
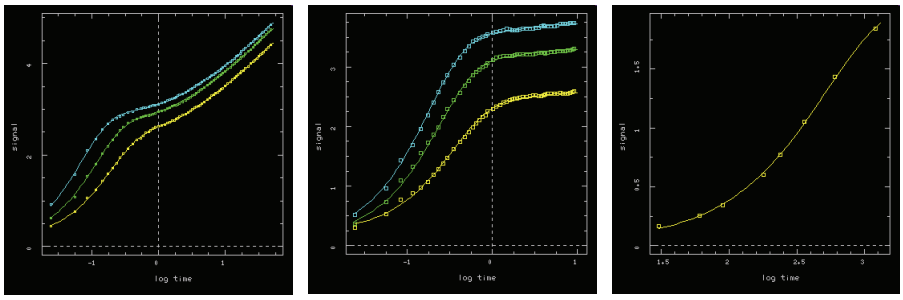


FIGURE 7. **Kinetic model of Trap1 ATPase cycle at 25 °C**

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

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)

```
[task]

   data = progress
   task = fit

[mechanism]

   Eo + ATP  <===> Eo.ATP    :   kat    kdt
      Eo.ATP <===> Ec.ATP    :   koc    kco
      Ec.ATP ----> Ec.ADP    :   khy
      Ec.ADP <===> Eo + ADP  :   kdd    kad

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

[data]

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

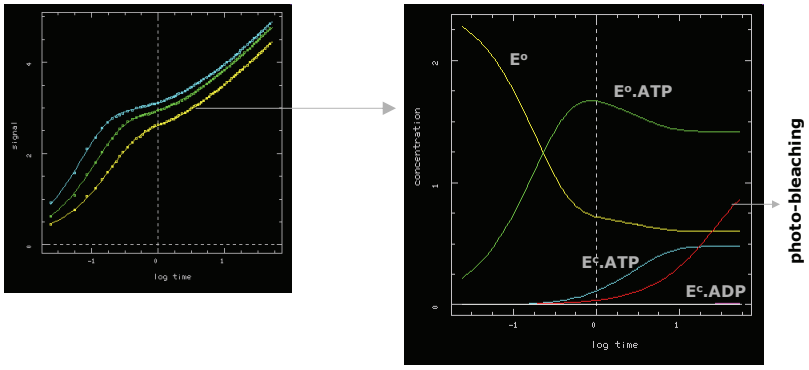
photo-bleaching is a first-order process

show concentrations of these species over time

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

DynaFit Example 1: Trap1 – species concentrations

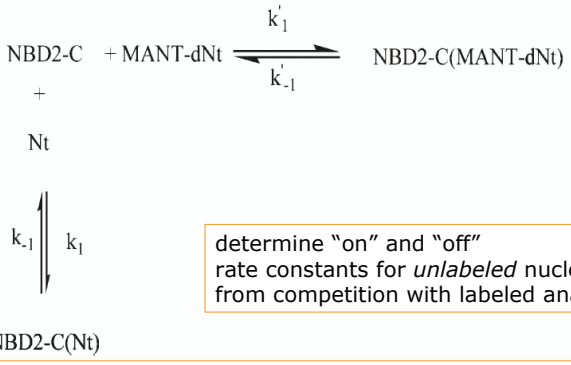
USEFUL WAY TO GAIN INSIGHT INTO THE MECHANISM



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)



NBD2-C = protein

MANT-dNt = labeled nucleotide

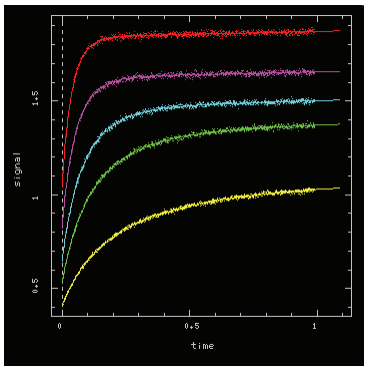
Nt = unlabeled nucleotide

determine "on" and "off"
rate constants for *unlabeled* nucleotides
from competition with labeled analogs

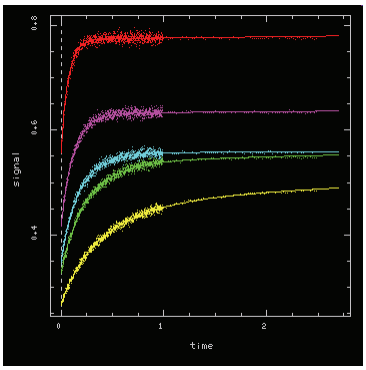
Werbeck *et al.* (2009)
"Nucleotide binding and allosteric modulation of the second AAA+ domain of ClpB probed by transient kinetic studies"
Biochemistry **48**, 7240-7250

DynaFit Example 2: Nucleotide binding to ClpB - data

AGAIN COMBINE TWO DIFFERENT EXPERIMENTS (ONLY "LABELED" NUCLEOTIDE HERE)



- variable [ADP*]
- constant [ClpB]



- constant [ADP*]
- variable [ClpB]

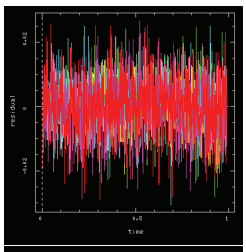
Werbeck *et al.* (2009) *Biochemistry* **48**, 7240-7250

DynaFit Example 2: Nucleotide binding to ClpB script

THE DEVIL IS ALWAYS IN THE DETAIL

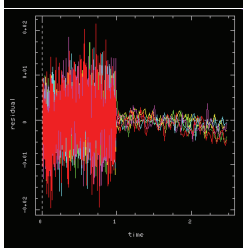
```
[task]
   data = progress
   task = fit
   model = simplest

[mechanism]
   P + mADP <==> P.mADP    : k1   k-1
            ---> drift     : v

[constants]
   k1   =   5 ?
   k-1  = 0.1 ?
   v    = 0.1 ?
```

"drift in the machine"



Residuals

- variable [ADP*]
- constant [ClpB]

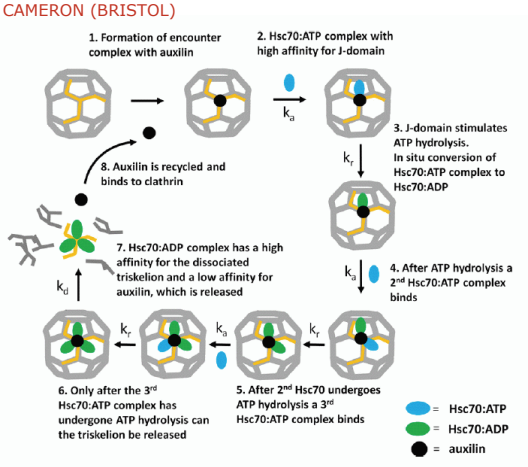


- constant [ADP*]
- variable [ClpB]

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"
Proc. Natl. Acad. Sci USA **108**, 6927–6932

DynaFit Example 3: Clathrin uncoating - script

MODEL DISCRIMINATION ANALYSIS

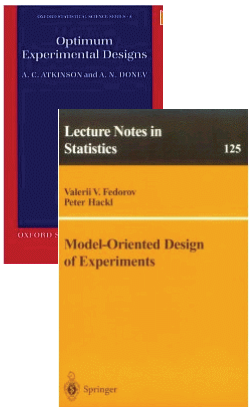
```
[task]
   task = fit
   data = progress
   model = AAAH ?
[mechanism]
   CA + T ---> CAT : ka
   CAT + T ---> CATT : ka
   CATT + T ---> CATTT : ka
   CATTT ---> CADDD : kr
   CADDD ---> Prods : kd
...
[task]
   task = fit
   data = progress
   model = AHAHAH ?
[mechanism]
   CA + T ---> CAT : ka
   CAT ---> CAD + Pi : kr
   CAD + T ---> CADT : ka
   CADT ---> CADD + Pi : kr
   CADD + T ---> 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*

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

Optimal Experimental Design: Books

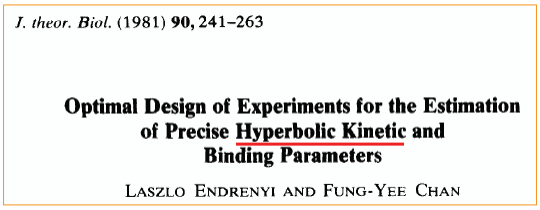
DOZENS OF **BOOKS**



- *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"

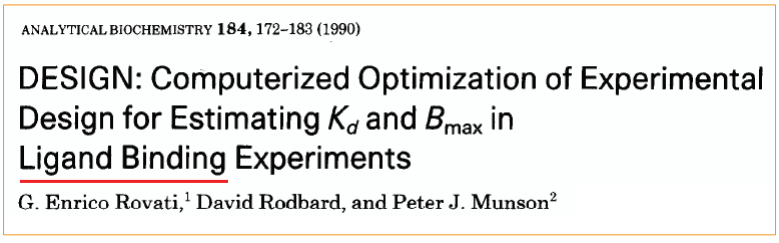
Optimal Experimental Design: Articles

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

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

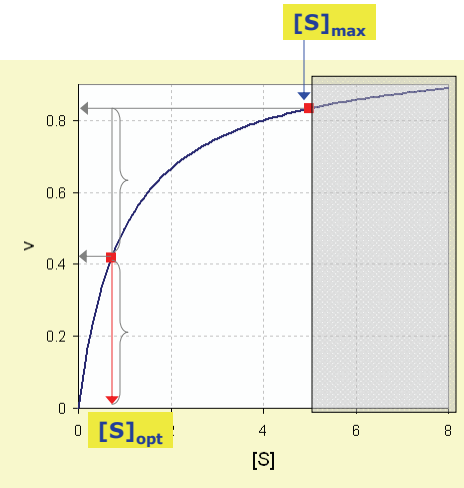
$$\det\mathbf{F} = F_{11}F_{22} - F_{12}F_{21}$$ **determinant**

"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 ?
```

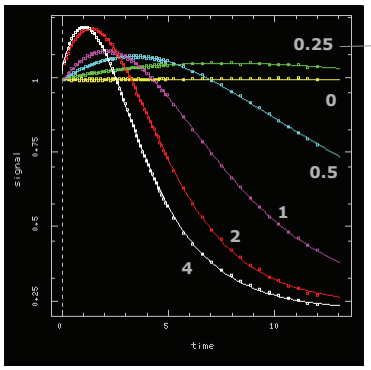
"Choose eight initial concentration of **T** such that the rate constants k_a, k_r, k_d are determined most precisely."

```
[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

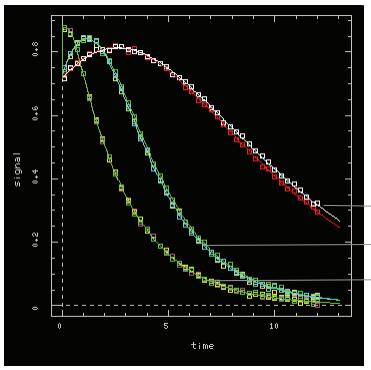


Actual concentrations of **[T]** (μM)

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)