MASARYKOVA UNIVERZITA
INNOVATION LECTURES (INNOIEC)
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Binding and Kinetics for Experimental Biologists

Lecture 8

Optimal design of experiments

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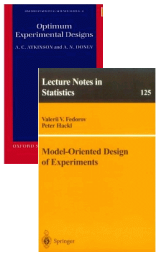
Tento projekt je spolufinancován Evropským sociálním fondem a státním rozpočtem České republiky.
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Lecture outline

- The problem:
  - How should we **plan an experiment** such we learn the most from it?
- The solution:
  - Use the **Optimal Design Theory** of statistics
- An implementation:
  - Software ***DynaFit***
- An example:
  - Kinetics of clathrin cage disassembly

Optimal Experimental Design: Where to find basic information

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"

Theory of D-optimal design

MAXIMIZE THE **DETERMINANT** ("D") OF **FISHER INFORMATION MATRIX**

$y_i = f(x_i, \mathbf{p})$ — f algebraic fitting function; x_i independent variable, ith data point (i = 1, 2, ..., N); y_i dependent variable, ith data point; $\mathbf{p}$ vector of M model parameters

$s_{i,j} = \frac{\partial f(x_i, \mathbf{p})}{\partial p_j}$ — $s_{i,j}$ sensitivity of f with respect to jth parameter, ith data point

$F_{j,k} = \sum_{i=1}^{N} s_{i,j} s_{i,k}$ — $F_{i,j}$ (i,j)th element of the **Fisher information matrix** $\mathbf{F} = \begin{pmatrix} F_{1,1} & F_{1,2} & \cdots & F_{1,M} \\ F_{2,1} & F_{2,2} & \cdots & F_{2,M} \\ \vdots & \vdots & \ddots & \vdots \\ F_{M,1} & F_{M,2} & \cdots & F_{M,M} \end{pmatrix}$

D-Optimal Design:

$$\max_{x_1, x_2, \ldots, x_N} |\det \mathbf{F}|$$

Choose the independent variable x_1, ..., x_N (e.g., total or initial concentrations of reagents) such that the determinant of **F** is maximized.

D-Optimal design example: Michaelis-Menten equation

RONALD **DUGGLEBY** – UNIVERSITY OF QUEENSLAND, AUSTRALIA (**1979**)
J. Theor. Biol. **81**, 671-684 (1979)

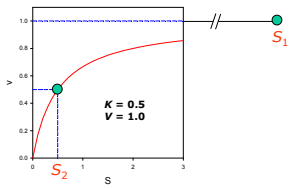
$v_i = V \frac{S_i}{S_i + K}$ — v_i initial rate of enzyme reaction, ith data point; S_i substrate concentration, ith data point (i = 1, 2, ..., N); V, K vector of model parameters; K ... Michaelis constant, V ... maximum rate

$s_{i,V} \equiv \frac{\partial v_i}{\partial V} = \frac{S_i}{S_i + K}$ $\quad s_{i,K} \equiv \frac{\partial v_i}{\partial K} = -V \frac{S_i}{(S_i + K)^2}$ sensitivity functions

Box-Lucas **two-point** design:

$$\max_{S_1, S_2} \det \begin{pmatrix} s_{1,V} & s_{1,K} \\ s_{2,V} & s_{2,K} \end{pmatrix}$$

$S_1 \to \infty$
$S_2 = K$



Realistic design for the Michaelis-Menten equation

"INFINITE" SUBSTRATE CONCENTRATION (TO GET V_{max}) IS IMPOSSIBLE TO ACHIEVE

Box-Lucas **two-point** design with one point (S_{max}) already given: **assume K = 0.5, S_{max} = 2.0**

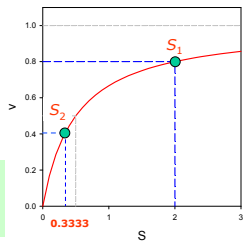
$$\max_{S_2} \det \begin{pmatrix} s_{\max,V} & s_{\max,K} \\ s_{2,V} & s_{2,K} \end{pmatrix}$$

$S_1 = S_{max}$

$S_2 = \frac{S_{max}\, K}{S_{max} + 2\, K}$

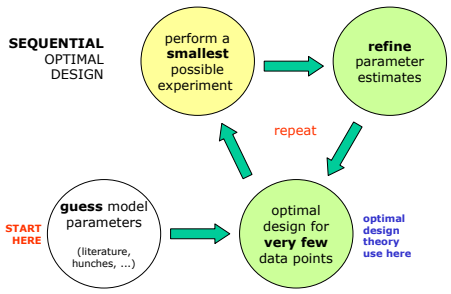
RECIPE

In the determination of K_M, always **include a substrate concentration** that corresponds to a reaction rate approximately **one half of maximum *achievable* rate.**



Theory of D-optimal design: The chicken-and-egg problem

PROBLEM: TO DESIGN AN EXPERIMENT WE MUST FIRST GUESS THE FINAL ANSWER!



Special case: Time-course experiments with fixed time-points

A typical kinetics experiment: Fixed mesh of time-points

PROBLEM: WE DO NOT ALWAYS HAVE A CHOICE OF INDEPENDENT VARIABLE VALUES

Rothnie, Kuzmic, *et al.* (2011) *Proc. Natl. Acad. Sci. USA* **108**, 6927-35

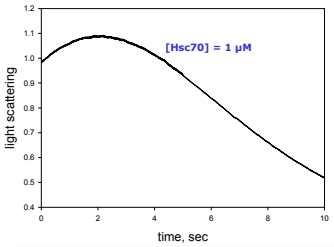
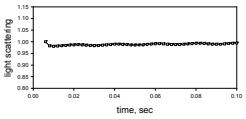


Fig. 4. Stopped-flow measurements of light scattering to examine the early stages of clathrin cage disassembly. Clathrin cages (0.09 μM triskelia) pre-mixed with 0.1 μM auxilin were mixed with Hsc70 (concentrations in μM shown on graph) and 500 μM ATP, and perpendicular light scattering was measured using stopped-flow techniques.

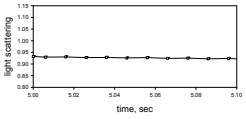
Stopped flow experimentation: Fixed step size

AT BEST, WE CAN CHANGE THE (FIXED) STEP SIZE FOR A GIVEN INTERVAL

Rothnie, Kuzmic, *et al.* (2011) *Proc. Natl. Acad. Sci. USA* **108**, 6927-35, *Fig. 4*



t = 0 to 5 sec:
step size 20 msec



t = 5 to 10 sec:
step size 100 msec

Variation of the design problem: Optimize initial conditions

IF WE CAN'T CHOOSE OBSERVATION TIME, AT LEAST WE CAN CHOOSE **INITIAL CONCENTRATIONS**

BASIC PRINCIPLE:

- In *kinetic* studies
  - the **independent variable** is **time**;
  - **initial concentrations** are considered "**parameters**" of the model.
- D-Optimal design theory is concerned with optimal choice of **independent variable**
  - in this case the observation time.
- Unfortunately, in the real-world we cannot choose particular observation times:
  - usual instruments are offering us only a fixed mesh of output points.
- But we can turn things around and
  - **treat initial concentrations as "independent variables"**.
  - Then we can optimize the choice of initial concentrations, using the usual formalism of the D-Optimal Design theory.

Theory of D-optimal design: Initial conditions in ODE systems

MAXIMIZE THE **DETERMINANT** ("D") OF **FISHER INFORMATION MATRIX**

$$dc_i/dt = f_i(\mathbf{c},\mathbf{k}) \qquad t=0 \;:\; \mathbf{c}=\mathbf{c}_0$$

initial value problem (first-order ordinary differential equations)
c … vector of concentrations
k … vector of rate constants
$\mathbf{c}_0$ … concentrations at time zero

$$y_i = g(\mathbf{c}(t_i),\mathbf{r})$$

y_i experimental signal at *i*th data point (time t_i)
$\mathbf{c}$ concentrations at time t_i
$\mathbf{r}$ vector of molar responses and/or offset on signal axis

$$s_{i,j} = \frac{\partial g(\mathbf{c}(\mathbf{k},t_i),\mathbf{r})}{\partial p_j}$$

$s_{i,j}$ sensitivity of *f* with respect to *j*th parameter, *i*th data point
p … model parameters: vectors ***k*** and ***r*** combined

$$F_{j,k} = \sum_{i=1}^{N} s_{i,j} s_{i,k}$$

D-Optimal Design: $\max_{\mathbf{c}_0} |\det \mathbf{F}|$

Optimize initial conditions: DynaFit notation

THE SOFTWARE TAKES CARE OF ALL THE MATH

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

[mechanism]
...

[data]
   set ...
   concentration X = 1 ?? (0.01 .. 100)
```

syntax otherwise used for **confidence intervals**

lower and upper bounds must be given

this value is ignored
(present for syntactical reasons only)

Optimize initial conditions: Algorithm and *DynaFit* settings

THE **DIFFERENTIAL EVOLUTION** ALGORITHM REQUIRES SPECIAL SETTINGS

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

[mechanism]
...

[settings]

{DifferentialEvolution}

   PopulationSizeFixed              = 300
   MaximumEvolutions                = 1
   MinimumEvolutions                = 1
   TestParameterRange               = n
   TestParameterRangeAll            = n
   TestParameterRangeFull           = n
   StopParameterRange               = 0.1
   TestCostFunctionRange            = y
   StopCostFunctionRange            = 0.01
   TestCostFunctionChange           = y
   StopCostFunctionChange           = 0.00001
   TestCostFunctionChangeCount      = 5
```

copy these settings from one of the distributed example problems

population **not too large**

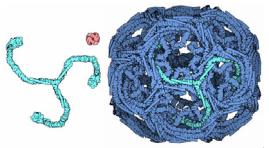
perform the optimization **only once**

relatively weak **convergence criteria**

Case study: Kinetics of clathrin cage disassembly

Clathrin structure: triskelions and cages

CLATHRIN CAGES ARE LARGE ENOUGH TO VISIBLE IN **MICROSCOPY** AND **LIGHT SCATTERING**

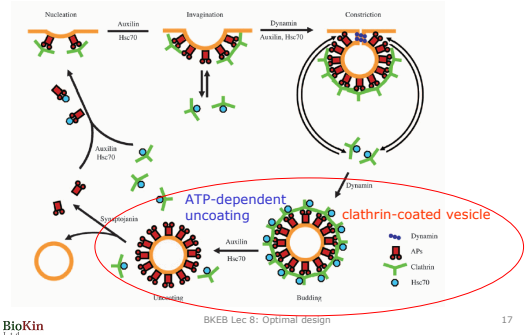


clathrin *triskelion* **clathrin cage**

Clathrin biology: Role in endocytosis

CLATHRIN IS INVOLVED IN INTRACELLULAR TRAFICKING

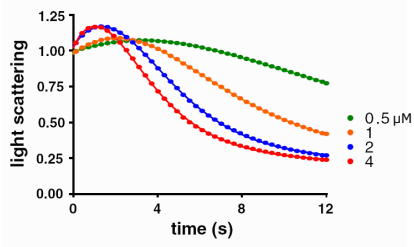
Eisenberg *et al.* (2007) *Traffic* **8**, 640-646



In vitro kinetics of clathrin disassembly: Experimental data

WATCHING CLATHRIN CAGES TO FALL APART BY **PERPENDICULAR LIGHT SCATTERING**

Rothnie, Kuzmic, *et al.* (2011) *Proc. Natl. Acad. Sci. USA* **108**, 6927-35, *Fig. 4*

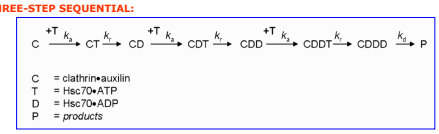


"**Clathrin** cages (0.09 µM **triskelia**) premixed with 0.1 µM **auxilin** were mixed with **Hsc70** (concentrations in µM shown on graph) and 500 µM **ATP**, and perpendicular light scattering was measured using stopped-flow…"

In vitro kinetics of clathrin disassembly: Theoretical models

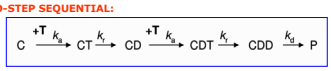
MODEL SELECTION USING THE **AKAIKE INFORMATION CRITERION (DYNAFIT)**
Rothnie, Kuzmic, *et al.* (2011) *Proc. Natl. Acad. Sci. USA* **108**, 6927-35

THREE-STEP SEQUENTIAL:

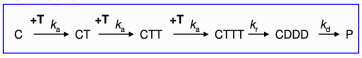


C = clathrin•auxilin
T = Hsc70•ATP
D = Hsc70•ADP
P = *products*

TWO-STEP SEQUENTIAL:



THREE-STEP CONCERTED:



Etc. In total *five* different models were evaluated.

In vitro kinetics of clathrin disassembly: DynaFit notation

THE MOST PLAUSIBLE MODEL: THREE STEP SEQUENTIAL
Rothnie, Kuzmic, *et al.* (2011) *Proc. Natl. Acad. Sci. USA* **108**, 6927-35

DYNAFIT INPUT:

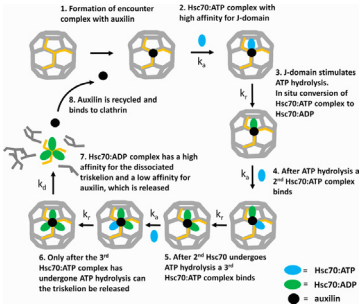
```
[task]
   task = fit
   data = progress
   model = AHAHAH ?
[mechanism]
      CA + T -> CAT          : ka
         CAT -> CAD + Pi     : kr
    CAD + T -> CADT          : ka
         CADT -> CADD + Pi   : kr
    CADD + T -> CADDT        : ka
      CADDT -> CADDD + Pi    : kr
       CADDD -> Prods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

AUTOMATICALLY GENERATED MATH MODEL:

d[CA]/*dt* = - k_a[CA][T]
d[T]/*dt* = - k_a[CA][T] - k_a[CAD][T] - k_a[CADD][T]
d[CAT]/*dt* = + k_a[CA][T] - k_r[CAT]
d[CAD]/*dt* = + k_r[CAT] - k_a[CAD][T]
d[Pi]/*dt* = + k_r[CAT] + k_r[CADT] + k_r[CADDT]
d[CADT]/*dt* = + k_a[CAD][T] - k_r[CADT]
d[CADD]/*dt* = + k_r[CADT] - k_a[CADD][T]
d[CADDT]/*dt* = + k_a[CADD][T] - k_r[CADDT]
d[CADDD]/*dt* = + k_r[CADDT] - k_d[CADDD]
d[Prods]/*dt* = + k_d[CADDD]

In vitro kinetics of clathrin disassembly: Preferred mechanism

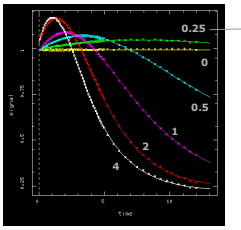
CONCLUSIONS: **THREE ATP MOLECULES** MUST BE HYDROLYZED BEFORE THE CAGE FALLS APART
Rothnie, Kuzmic, *et al.* (2011) *Proc. Natl. Acad. Sci. USA* **108**, 6927-35



In vitro kinetics of clathrin disassembly: Raw data

THIS WAS A **VERY EXPENSIVE EXPERIMENT** TO PERFORM
Rothnie, Kuzmic, *et al.* (2011) *Proc. Natl. Acad. Sci. USA* **108**, 6927-35

ACTUAL EXPERIMENTAL DATA:



- **six** assays / experiment
- 90 nM clathrin
- 100 nM auxilin
- up to 4 μM Hsc70

a lot of material

expensive and/or time consuming to obtain

How many assays are actually needed?

D-OPTIMAL DESIGN IN DYNAFIT

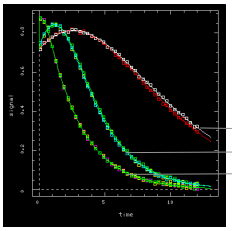
```
[task]
   task = design
   data = progress

[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 ?

[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)
```

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

Optimal Experimental Design: DynaFit results

SURPRISE: WE DID TOO MUCH WORK FOR THE INFORMATION GAINED

SIMULATED DATA – OPTIMAL EXPERIMENT:



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

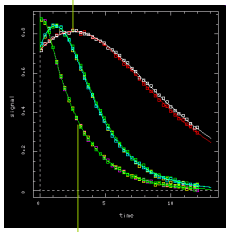
Just **three** experiments would be sufficient for follow-up!

One half of the material compared to the original experiment.

Optimal Experimental Design: DynaFit results - discussion

EACH OF THE **THREE** UNIQUE ASSAYS TELLS A DIFFERENT "STORY"

mostly
ATP association (k_a)



mostly
disassembly (k_d)

association ("upswing") is no longer visible

```
[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
```


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)