Inference of genomic network dynamics with non-linear ODEs

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Joint work with Mahdi Mahmoudi, Itai Dattner and Ivan Vujačić
Motivation: dynamic genomic processes

The amount of RNA of a particular gene depends on
- the rate of DNA decay (depends on temperature,...)
- the amount of transcription factor
- the rate of transcription (depends on other factors,...)
Law of Mass Action

Given a basic reaction

$$A + B \xrightleftharpoons[k_{-1}, k_1]{k_{-1}, k_1} C$$

the rate of forward and backward and backward reactions is \textbf{linearly} proportional with concentration $A$, $B$ and $C$ respectively:

$$\frac{d[A]}{dt} = k_{-1}[C] - k_1[A][B].$$
Enzymes

- (typically) large proteins
- catalysts that change the *speed* of reaction, but not its *extent*.
- highly specific: speed up (typically) a single biochemical reaction.
- are highly regulated themselves by:
  - phosphorylation
  - calcium
  - ATP
  - their own products,
  - etc.
Basic problem of enzyme kinetics

Let \( S \) be a substrate which together with enzyme \( E \) results in a product \( P \):

\[
S + E \rightleftharpoons P + E.
\]

**Problem:**
according to *Law of Mass Action* rate increases linearly with \([S]\), but it actually saturates.

**Michaelis and Menten (1913):** an intermediate complex \( C \)

\[
S + E \xrightleftharpoons[\kappa_1]{\kappa_{-1}} C \xrightarrow{\kappa_2} P + E.
\]
Pseudo steady-state approximation

Mass action equilibrium:

\[(k_{-1} + k_2)[C] = k_1[S][E]\]

combined with the finite amount of enzyme \(m\):

\[m = [C] + [E],\]

We get

\[K[C] = [S](m - [C]), \text{ with } K = (k_{-1} + k_2)/k_1\]

so

\[[C](K + [S]) = [S]m.\]

The rate of production of product \(P\)

\[v_P = k_2[C] = \frac{k_2[S]}{K + [S]}\]
E-Coli SOS system

- 30 genes controlled by protein called LexA.
- 1 ODE per gene, particular kinetics parameters.
Modelling transcription kinetics in a SIM

Mean transcription dynamics follow Michaelis-Menten kinetics:

\[ \dot{\mu}_i(t) = p_i(t, \eta) - \delta_i \mu_i(t), \]

where

- \( p_i(t, \eta) = \) RNA production term of gene \( i \) by protein \( \eta \),
- \( \delta_i \mu_i(t) = \) natural RNA decay of gene \( i \).

If gene \( i \) only depends on activity \( \eta \) of one protein:

\[ p_i(t, \eta) = \alpha_i + \beta_i \frac{\eta(t)}{\gamma_i + \eta(t)} \]

**Activator**

or

\[ p_i(t, \eta) = \alpha_i + \beta_i \frac{1}{\gamma_i + \eta(t)} \]

**Repressor**

where

- \( \alpha_i = \) basal transcription rate of gene \( i \),
- \( \beta_i = \) maximal reaction-rate for gene \( i \),
- \( \gamma_i = \) half-saturation constant.
Cooperativity

Enzyme can bind two substrate molecules at different binding sites:

\[
S + E \overset{k_1}{\underset{k_{1}}{\rightleftharpoons}} C_1 \overset{k_2}{\rightarrow} P + E \\
S + C_1 \overset{k_3}{\underset{k_3}{\rightleftharpoons}} C_2 \overset{k_4}{\rightarrow} P + E
\]

or

\[
E \rightleftharpoons C_1 \rightleftharpoons C_2
\]

which results in the following rate of production of \( P \)

\[
\nu_P = \frac{(k_2K_2 + k_4[S])m[S]}{K_1K_2 + K_2[S] + [S]^2}.
\]
Independent binding sites

If there are 2 independent binding sites to which enzyme can bind:

\[ k_1 = 2k_3 = k_+ \]
\[ 2k_1 = k_3 = k_- \]
\[ 2k_2 = k_4 \]

which results in doubling the rate of production of \( P \)

\[ v_P = k_2[C] = \frac{2k_2[S]}{K + [S]} \]
... or more complex systems: fatty acid $\beta$-oxidation

$$v_{\text{vlcad}} = \frac{k_{\text{cat}} \cdot E \left( \frac{S_{\text{C}_{18} \text{AcylCoA}} \cdot S_{\text{FAD}}}{K_{m_{\text{C}_{18} \text{AcylCoA}}} \cdot K_{m_{\text{FAD}}}} - \frac{P_{\text{C}_{18} \text{EnoylCoA}} \cdot P_{\text{FADH}}}{K_{m_{\text{C}_{18} \text{EnoylCoA}}} \cdot K_{m_{\text{FAD}}} \cdot K_{eq}} \right)}{1 + \frac{S_{\text{C}_{18} \text{AcylCoA}}}{K_{m_{\text{C}_{18} \text{AcylCoA}}}} + \frac{P_{\text{C}_{18} \text{EnoylCoA}}}{K_{m_{\text{C}_{18} \text{EnoylCoA}}}} + \sum_{C_{12} - C_{16}} \left( \frac{S_{\text{C}_{n} \text{AcylCoA}}}{K_{m_{\text{C}_{n} \text{AcylCoA}}}} + \frac{P_{\text{C}_{n} \text{EnoylCoA}}}{K_{m_{\text{C}_{n} \text{EnoylCoA}}}} \right)} \cdot \left( 1 + \frac{S_{\text{FAD}}}{K_{m_{\text{FAD}}}} + \frac{P_{\text{FADH}}}{K_{m_{\text{FADH}}}} \right)$$

Differential equations play crucial role in science
Consider
\[ x'(t) = f(x(t), t; \theta), \quad t \in [0, T], \]

Noisy observations of solution \( x(t; \theta, \xi) \) are available:
\[ y(t_i) = x(t_i; \theta, \xi) + \varepsilon(t_i), \quad i = 1, \ldots, n, \]

where \( 0 \leq t_1 \leq \cdots \leq t_n \leq T \) and with initial values
\[ x_j(0) = \xi_j. \]

**Goal: inverse ODE problem**

1. Estimate the vector of parameters \( \theta \).
2. When initial values \( \xi \) are unknown, they are also estimated.
Possible approaches

1. Maximum likelihood (Khanin et al. 2007, Xue et al. 2010):
   - PRO: has guaranteed properties of estimator.
   - CON: requires solution of ODE.

2. Reproducing Kernel Hilbert Space (Gonzalez et al. 2014):
   - CON: has no guaranteed properties of estimator.
   - PRO: does not require solution of ODE.

3. Collocation Method (Ramsay et al. 2007):
   - PRO: consistency.
   - PRO: does not require solution of ODE.
   - CON: Iterative procedure with tuning parameters
Let $\theta = (\theta^*, \xi)$, including initial state.

**Our proposed framework:** iteration of

1. $\hat{x}(\theta) = \arg\min_{x \in \mathcal{X}} \mathcal{T}_{\alpha, \gamma}(x | \theta)$,
2. $\hat{\theta}_n = \arg\min_{\theta \in \Theta} M_n(\theta | \hat{x}(\theta), Y)$.

**Aim**

Define $\mathcal{T}_{\alpha, \gamma}$ and $M_n$ such that:

- It yields asymptotically efficient estimator.
- It can handle partially observed systems.
Well-posedness in sense of Hadamard

Let $F : \mathcal{X} \rightarrow \mathcal{Y}$ where $\mathcal{X}, \mathcal{Y}$ are linear normed spaces and solve:

$$F(x) = y,$$

(1)

$x \in \mathcal{X}, \ y \in \mathcal{Y}$.

The problem (1) is **well-posed in the sense of Hadamard** on $(\mathcal{X}, \mathcal{Y})$ if:

1. The solution of (1) exists.
2. It is unique.
3. It is continuous with respect to $y$.

The problem (1) is **ill-posed** on $(\mathcal{X}, \mathcal{Y})$ if it is not well-posed.
Solving ODEs

Consider

\[ x'(t) = f(x(t), t; \theta), \quad t \in [0, T], \]

for fixed \( \theta \).

Suppressing dependence on \( \theta \), define

\[ F(x) = x' - f(x, \cdot, \theta), \]

then ODE is equivalent to

\[ F(x) = 0_d. \]

In principle, this is an ill-posed problem: lack of uniqueness
“Quasi-solution” for ill-posed problems

Equation

\[ F(x) = y, \]

can be “solved” on a set \( S \subset X \) by minimizing objective functional

\[ J(x) = \| F(x) - y \|^2. \]

Quasi-solution
Minimizer of \( J \) on \( S \) is called quasi-solution, pseudo solution or least squares solution of \( F(x) = y \).

Remark:
Idea dates back to beginning of 19th century: Gauss, Legendre.
Given \( F(x) = x' - f(x, \cdot, \theta) \),

the corresponding ODE objective functional is

\[
\mathcal{J}(x) = \| x' - f(x, \cdot, \theta) \|_{2,w}^2.
\]

where

- \( L_2 \) norm, \( \| x \|_{2,w} = \sqrt{\sum_{i=1}^{d} w_i \int_0^T x_i^2(t) dt} \).
- with possible weights \( w = (w_1, \ldots, w_d) \).

Minimizing \( \mathcal{J} = \text{solving ODE} \)
Consider finite-dimensional subspaces $X_m \subset X$ ($m = 1, 2, \ldots$), such that

1. $X_1 \subset X_2 \subset \ldots$
2. $\bigcup_{m=1}^{\infty} X_m$ is dense in $X$.

$X_m \subset \mathcal{C}^1[0, T]$ linear subspace with basis $\{h_1, \ldots, h_m\}$, i.e. $x \in X_m$

$$x_i(t) = \sum_{k=1}^{m} \beta_{ik} h_k(t) = \beta_i^\top h(t),$$

such as cubic splines with increasing knots.

**Approximate ODE solution**

The solution

$$\hat{x} = \arg\min_{x \in X_m} ||x' - f(x, \cdot, \theta)||_{2,w},$$

is quasi-solution of ODE.

**Remarks:** in statistics literature $X_m$s are called *sieves.*
What subset $S$? Finite-dimensional approximation

Approximate ODE solution

The solution

$$\hat{x} = \arg\min_{x \in \mathcal{X}_m} \|x' - f(x, \cdot, \theta)\|_{2,w},$$

is quasi-solution of ODE.

Remarks: in statistics literature $\mathcal{X}_m$ are called sieves.
Tikhonov regularization

Ω is *stabilizing functional*, which
- incorporates a priori information on smoothness of solution \( x \).
- is usually given by norm or semi-norm on \( \mathcal{X} \).

**Tikhonov regularization**

involves minimization of *Tikhonov functional*

\[
T_\alpha(x) = J(x) + \alpha \Omega(x),
\]

where \( \alpha \geq 0 \) is *regularization parameter*

For ODE we consider norm in Sobolev space \( \{H^2[0, T]\}^d \)

\[
\Omega(x) = \sum_{i=1}^{d} v_i \int_0^T \{x_i''(t)\}^2 dt.
\]
Generalized Tikhonov regularization

$S$ is *similarity functional*, which
- incorporates a priori information on values of $x$.

Generalized Tikhonov regularization involves minimization of *generalized Tikhonov functional*

$$T_{\alpha,\gamma}(x) = J(x) + \alpha \Omega(x) + \gamma S(x),$$

where $\gamma \geq 0$ is *penalty parameter*.

We consider (for example) minus log-likelihood:

$$S(x) = -\sum_{i=1}^{d_1} \sum_{j=1}^{n} \log p(y_i(t_j)|x_i(t_j)).$$
General outline of our method

Consider \( x_\beta = \beta^t h \), such that

\[
  x_i(t) = \sum_{k=1}^{m} \beta_{ik} h_k(t) = \beta_i^\top h(t).
\]

1. For fixed \( \theta \), GTR solution \( \hat{x} = x_{\hat{\beta}} \) is given by

\[
  \hat{\beta} = \arg\min_{\beta \in \mathbb{R}^{dm}} T_{\alpha,\gamma}(x_{\beta}).
\]

2. For fixed \( \hat{x} \), obtain solution

\[
  \hat{\theta}_n = \arg\min_{\theta \in \Theta} M_n(\theta | \hat{x}(\theta), Y).
\]

Then iterate steps 1 and 2.
Consistency and asymptotic efficiency

Theorem (Consistency)

Let Assumptions 1-5 from Qi and Zhao (2010) hold. If as $n \rightarrow \infty$

1. $\alpha_n \rightarrow 0$
2. $\gamma_n \rightarrow 0$

then

$$\hat{\theta}_n - \theta_0 = o_P(1).$$

Theorem (Asymptotic normality & efficiency)

Let Assumptions 1-6 from Qi and Zhao (2010) hold. If $\alpha_n = o(n^{-2})$ and $\gamma_n = o(n^{-2})$, then

$$\hat{\theta}_n \text{ is asymptotically normal and efficient.}$$
ODE Systems linear in parameters

Nonlinear ODE systems that are linear in parameters $\theta$

$x'(t) = g(x(t))\theta,$

where $g : \mathbb{R}^d \to \mathbb{R}^{d \times p}$.

**Example 1.** Lotka-Volterra system

\[
\begin{align*}
    x'_1(t) &= \theta_1 x_1(t) - \theta_2 x_1(t)x_2(t), \\
    x'_2(t) &= -\theta_3 x_2(t) + \theta_4 x_1(t)x_2(t),
\end{align*}
\]

is linear in parameter $\theta = (\theta_1, \theta_2, \theta_3, \theta_4)^\top$ where

\[
g(x(t)) = \begin{pmatrix}
    x_1(t) & -x_1 x_2(t) & 0 & 0 \\
    0 & 0 & -x_2(t) & x_1 x_2(t)
\end{pmatrix},
\]

i.e. $(x'_1(t), x'_2(t))^\top = g(x(t))\theta$. 
Example 2. FitzHugh-Nagumo

FitzHugh-Nagumo system

\[
\begin{align*}
    x_1'(t) &= c\{x_1(t) - x_1(t)^3/3 + x_2(t)\}, \\
    x_2'(t) &= -\frac{1}{c}\{x_1(t) - a + bx_2(t)\},
\end{align*}
\]

is linear in parameter \( \theta = (c, 1/c, a/c, b/c)^\top \) where

\[
g(x(t)) = \begin{pmatrix}
    x_1(t) - x_1^3(t)/3 + x_2(t) & 0 & 0 & 0 \\
    0 & -x_1(t) & 1 & -x_2(t)
\end{pmatrix},
\]

i.e. \( (x_1'(t), x_2'(t))^\top = g(x(t))\theta. \)
We consider

$$X_m = \text{piecewise constant functions on } \lfloor \sqrt{m} \rfloor \text{ levels.}$$

and consider Tikhonov functional (non-dependent on $\theta$!)

$$T_{0,\infty}(x) = S(x).$$

Obtain

$$\hat{x} = \arg\min_{x \in X_m} T_{\infty,0}(x)$$

and estimate $\theta$ with objective function

$$M_n(\theta) = \int_0^T \| \dot{\hat{x}}(t) - f(\hat{x}(t), \theta) \|^q w(t) dt,$$

1-step GTR estimator!
STEP 1. Window smoother for $x$

The window smoother of $x$ is defined as

$$\hat{x}_n(t) = \frac{1}{|S(t)|} \sum_{t_j \in S(t)} y(t_j), \quad t \in S(t).$$
STEP 2. Objective function for $\theta$

Estimate parameters $\theta$ and $\xi$ by minimizing

$$M_n(\xi, \theta) = \int_0^T \left\| \hat{x}_n(t) - \xi - \int_0^t g(\hat{x}_n(s)) \, ds \theta \right\|^2 \, dt$$
STEP 2. Objective function for $\theta$

Estimate parameters $\theta$ and $\xi$ by minimizing

$$
M_n(\xi, \theta) = \int_0^T \left\| \hat{x}_n(t) - \xi - \int_0^t g(\hat{x}_n(s)) \, ds \theta \right\|^2 \, dt
$$

$$
= (\xi, \theta) \begin{pmatrix}
T \mathbb{I}_d & \hat{A}_n \\
\hat{A}_n^\top & \hat{B}_n
\end{pmatrix}
\begin{pmatrix}
\xi \\
\theta
\end{pmatrix}
+ 2 \left( \int_0^T \hat{x}_n(t) \, dt \int_0^T \hat{G}_n(t)^\top \hat{x}_n(t) \, dt \right) \begin{pmatrix}
\xi \\
\theta
\end{pmatrix}.
$$

where

$$
\hat{G}_n(t) = \int_0^t g(\hat{x}_n(s)) \, ds,
$$

$$
\hat{A}_n = \int_0^T \hat{G}_n(t) \, dt,
$$

$$
\hat{B}_n = \int_0^T \hat{G}_n(t)^\top \hat{G}_n(t) \, dt.
$$
STEP 2. Standard least squares form of estimators

Minimizing previous quadratic form yields

\[
(\hat{\xi}, \hat{\theta}) = \left( \begin{array}{c}
T \mathbf{I}_d \\
\hat{A}_n^\top \\
\hat{A}_n \\
\hat{B}_n
\end{array} \right)^{-1} \left( \begin{array}{c}
\int_0^T \hat{x}_n(t) dt \\
\int_0^T \hat{G}_n(t)^\top \hat{x}_n(t) dt
\end{array} \right).
\]

The estimators of \( \xi \) and \( \theta \) have the form:

\[
\hat{\xi}_n = (T \mathbf{I}_d - \hat{A}_n \hat{B}_n^{-1} \hat{A}_n^\top)^{-1} \int_0^T \{ \mathbf{I}_d - \hat{A}_n \hat{B}_n^{-1} \hat{G}_n(t)^\top \} \hat{x}_n(t) dt,
\]
\[
\hat{\theta}_n = \hat{B}_n^{-1} \int_0^T \hat{G}_n(t)^\top \{ \hat{x}_n(t) - \hat{\xi}_n \} dt,
\]
STEP2. ... even better! Explicit forms

\[ \hat{G}_n(t_i) = \sum_{m=1}^{i} g(\hat{x}_n(S_m))\Delta, \]

\[ \hat{A}_n(i) = t \hat{G}_n(t)\big|_{t_{i-1}}^{t_i} - \frac{1}{2}(2i - 1)\Delta^2 g(\hat{x}_n(S_i)), \]

\[ \hat{A}_n = T \hat{G}_n(T) - \frac{\Delta^2}{2} \sum_{i=1}^{l} (2i - 1)g(\hat{x}_n(S_i)). \]

\[ \hat{B}_n = \hat{G}_n(T)^\top \hat{A}_n - \frac{1}{2} \sum_{i=1}^{l} g(\hat{x}_n(S_i))^\top t^2 \hat{G}_n(t)\big|_{t_{i-1}}^{t_i} \]

\[ + \frac{\Delta^3}{6} \sum_{i=1}^{l} (3i^2 - 1)g(\hat{x}_n(S_i))^\top g(\hat{x}_n(S_i))) + \frac{\Delta^3}{2} \sum_{i=1}^{l-1} (2i - 1)R_i^\top g(\hat{x}_n(S_i)) \]

where \( R_i = \sum_{m=i+1}^{l} g(\hat{x}_n(S_m)). \)
Computational complexity

- Computing $\hat{\chi}_n$:
  \[ O(nd). \]
- Computing $\hat{\xi}_n$ and $\hat{\theta}_n$:
  \[ O(p^3 + d^3 + \sqrt{nd}p^2). \]

Consistency:
Window-based estimators of $\hat{\theta}_n$ and $\hat{\xi}_n$ are $\sqrt{n}$ - consistent.

A reviewer’s comment (Statistics and Computing):
“It’s an unusual idea and at first glance it is surprising that it works.”
A simulation example

FitzHugh-Nagumo system, given by the equations,

\[ x_1'(t) = c\{x_1(t) - x_1(t)^3/3 + x_2(t)\}, \]
\[ x_2'(t) = -\frac{1}{c}\{x_1(t) - a + bx_2(t)\}, \]

is linear in the parameter

\[ \theta = (\theta_1, \theta_2, \theta_3, \theta_4) = (c, 1/c, a/c, b/c). \]

*FhNdata* consist of

- 41 equally spaced observations on the interval \([0,20]\)
- obtained from the above model with noise levels \(\sigma_1 = \sigma_2 = 0.5\)
- and parameters \(a = 0.2, b = 0.2\) and \(c = 3\).
FhNdata from R package 'CollocInfer'

Inference of genomic network dynamics with non-linear ODEs
Window estimator provides a sensible initial guess

Window, Window + Generalized Profiling, Generalized Profiling $u = (1, 1, 1)$.

<table>
<thead>
<tr>
<th>Method</th>
<th>Ini guess</th>
<th>$a = 0.2$</th>
<th>$b = 0.2$</th>
<th>$c = 3$</th>
<th>Rel. error</th>
<th>Time(sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Window</td>
<td>NO</td>
<td>0.318</td>
<td>-0.140</td>
<td>3.003</td>
<td>2.291</td>
<td>0.011</td>
</tr>
<tr>
<td>Window</td>
<td>$0.1u$</td>
<td>0.237</td>
<td>0.144</td>
<td>3.050</td>
<td>0.483</td>
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<td>Window</td>
<td>$1u$</td>
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<td>0.009</td>
<td>0.069</td>
<td>2.130</td>
<td>8.687</td>
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<td>$10u$</td>
<td>0.144</td>
<td>0.567</td>
<td>3.461</td>
<td>2.266</td>
<td>55.725</td>
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<tr>
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<tr>
<td>Ramsay</td>
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<td>0.237</td>
<td>0.144</td>
<td>3.050</td>
<td>0.483</td>
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<td>Ramsay</td>
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<td>$6e+100$</td>
<td>681.449</td>
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</tbody>
</table>
Application 1. E-Coli SOS system

- 30 genes controlled by protein called LexA.
- 1 ODE per gene, particular kinetics parameters.
m genes $x_1(t), \ldots, x_m(t)$ and one transcription factor $\eta(t)$.

Model:

$$\dot{x}_k(t) = \beta_k \frac{1}{\gamma_k + \eta(t)} + \varphi_k - \delta_k x_k(t),$$

for $k = 1, \ldots, m$, $t \in [0, T]$.

Gene-dependent kinetics parameters: $\beta_k, \gamma_k, \delta_k$ and $\varphi_k$. 
Application 1. SOS system in Ecoli: Data

- Data set of 14 expression genes.

- The genes are targets of the master repressor LexA and their expression is studied under UV exposure (40J/m²).

- Abundance of the mRNA molecules measured at 0, 5, 10, 20, 40 and 60 minutes.

- The LexA abundance is unobserved.
Reconstructed the **activity of the repressor** $\eta(t)$ gene dinI.

Confidence intervals for the parameters $CI_{95\%}(\beta) = (0.56, 1.39)$

$CI_{95\%}(\delta) = (0.15, 0.27)$  
$CI_{95\%}(\gamma) = (1.71, 2.83)$, $\sigma = 0.04$. 

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Application 1. But not just that...

The aim of statistics is not just to fit data, but also
- compare scientific hypotheses
- draw scientific conclusions from the models

For example, the following **scientific conclusions** can be drawn:
- *recA* has the fastest effective production rate, \( r = \frac{\beta}{\gamma + \bar{\eta}} \), *uvrB* the slowest.
- *recA* and *umuC* are heavily affected by RNA decay.
- *recN*, *uvrB* and *umuC* has a non-negligible basal production rate \( \alpha \), so they are possibly also transcribed by another TF.
Streptomyces Coelicolor produces antibiotics. CdaR is a crucial regulator. TF CdaR also activates its own transcription.
Application 2. Generalized MM formulation

\[
\begin{align*}
\dot{x} &= \beta \frac{z^m}{\gamma + z^m} + \varphi - \delta x \\
\dot{z} &= \rho x - \tau z
\end{align*}
\]
**Application 2. CdaR data and inference**

**A** AIC selects Hill coefficient 2.

**B** Data for autoregulated gene SCO3217 (cdaR) and obtained smooth functions after estimating parameters.

Parameter estimates lie in bistable region, which may provoke bimodal effects at populational level.
Strange that Statistics has not dealt with ODEs until now!

- Ramsay et al. (2007) has been an important first step.
- In this talk,
  - We extended it via Generalized Tikhonov Regularization
  - We simplified it via Window Smoothing approach.
- Genetics may be quite static, *genomics is about dynamics.*

More good work is needed!