Real-time control of gene expression

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INRIA Junior Seminar
What is gene expression?

Promoter  Coding sequence  Terminator

DNA

transcription

mRNA

translation

Protein
What is gene expression?

Promoter → Coding sequence → Terminator

DNA → transcription → mRNA → translation → Protein
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- Coding sequence
- Terminator
- RNA polymerase
- transcription factor
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DNA ➔ transcription ➔ mRNA ➔ translation ➔ Protein

transcription factor

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DNA → transcription → mRNA → translation → Protein
Regulational steps in gene expression

Regulated steps:
- transcription
- mRNA degradation
- protein stability
- DNA accessibility
- translation rate
Regulational steps in gene expression

- gene expression is a very complex process with many regulatory steps
- not an easy control problem
Motivation

- Classical approach to understand the dynamics of a cellular process
  - perturb the system (e.g. protein level) and monitor time response to perturbation
- Current methods for applying protein perturbations are very limited
  - remove protein, over-express protein
- Need for precise and time varying perturbations

- Goal: control precisely the level of a given protein over time
- Solution: develop integrated experimental platform for closed-loop control
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A closed loop control platform

Main features

1. real-time observation
2. real-time change of cellular stimulus
3. real-time control
Saccharomyces Cerevisiae

- Yeast used for baking and brewing
- Very simple eucaryotic organism
- Model organism in biology
  - easy and fast to grow
  - not toxic
  - simple genetic modification
Fluorescent proteins

- Green fluorescent protein (GFP)
  - isolated from jellyfish (*Aequoria victoria*) in 1962
  - exhibits green fluorescence when exposed to UV light
- Can be used to observe proteins in live cells
  - quantification and localization
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**promoter** coding sequence **terminator**

protein
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Activation of gene expression

- Cells respond to external conditions
  - crucial for survival (e.g. nutrient change, osmotic shock)

![Diagram of signal transduction]

- Example: increase in osmolarity
Activation of gene expression

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  - crucial for survival (e.g. nutrient change, osmotic shock)

Example: increase in osmolarity

- osmotic balance
- osmotic shock
Activation of gene expression

- Osmolarity triggers high osmolarity glycerol (HOG) pathway
  - activation of osmotic stress genes
  - increased production of glycerol

- One of the best studied signaling pathways

- Challenging to control
  - feedback mechanisms (step shock leads to transient protein expression)
  - solution: repeated short pulses separated by at least 20 minutes
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![Gene expression response to osmotic shock](image-url)
Activation of gene expression

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Main features

1. real-time observation (fluorescent protein)
2. real-time change of cellular stimulus (osmolarity)
3. real-time control (computer)
Model predictive control (MPC)

- MPC finds optimal input by simulating a model of the system
  1. search for control minimizing deviation between model prediction and target profile
  2. apply found control strategy for a short while
  3. observer systems response
  4. GOTO 1
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Model predictive control (MPC)

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4. GOTO 1
Model predictive control (MPC)

- Requirement: mathematical model of the controlled system
  - state estimation problem: extended Kalman filter

- Simple integration of constraints
  - osmotic shock length limited (5-8 min)
  - minimum 20 minutes between successive pulses
Modeling

- Requirements for a model:
  - predict gene expression response for different inputs
  - simple (allows for state estimation)
- Different models of the Hog1 pathway have been published
  - but not suited for controlling purposes
    (too complex or do not consider gene expression)
- We propose a simple two dimensional ODE model

Model equations

\[
\begin{align*}
\frac{dx_1}{dt} &= u(t - \tau) - \gamma_1 x_1(t) \\
\frac{dx_2}{dt} &= k_1 x_1(t) - \gamma_2 \frac{x_2(t)}{K + x_2(t)}
\end{align*}
\]
Results

- Apply the MPC controller to real cells
  1. constant target value
  2. varying target value
  3. control single cells
Results - constant target

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Results - varying target

[pSTL1-YFP [a.u.]]

0 200 400 600 800
0 2000 4000 6000 8000

pSTL1-YFP [a.u.]

0 200 400 600 800
0 2000 4000 6000 8000

mean all cells
std all cells
MPC predictions

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Conclusions and applications

- We can control gene expression in living cells!
  - works well although feedback in controlled system
  - works even well for single cells

- Control targets can vary with time

Applications

- understand biology by perturbing cells (reverse engineering)
- control the mass production of biomolecules
- alternative to synthetic biology
  (external rather than internal implementation of functionality)