Real-time control of gene expression

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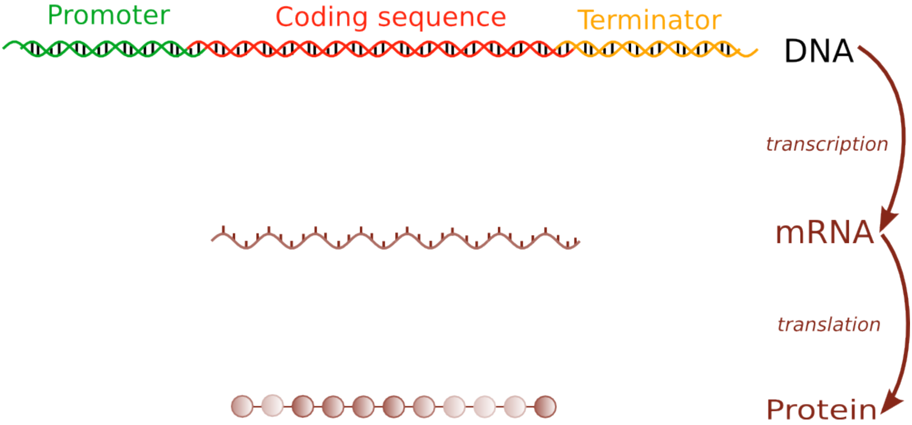
INRIA Junior Seminar

What is gene expression?



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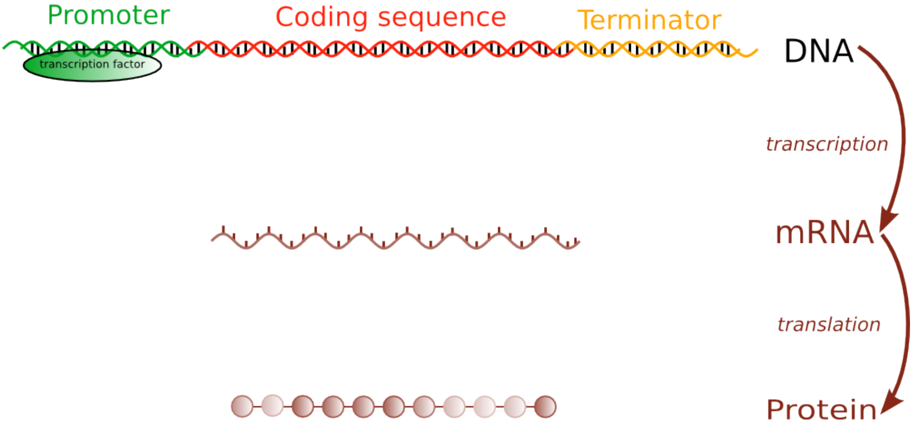

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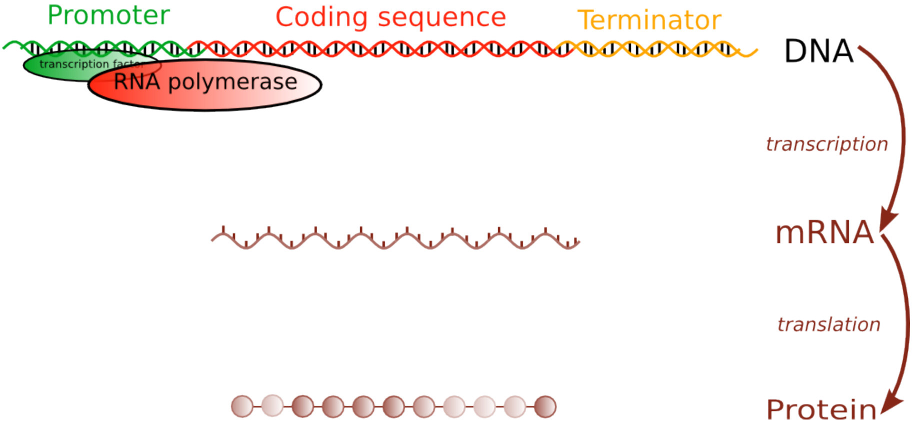

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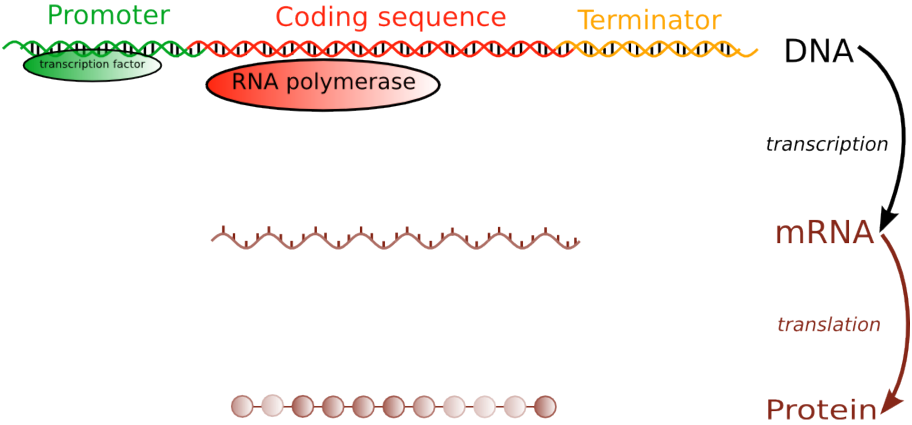

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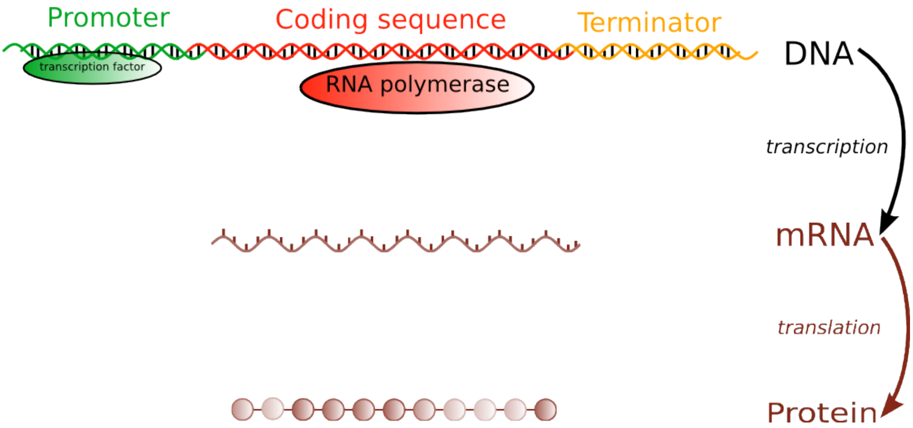

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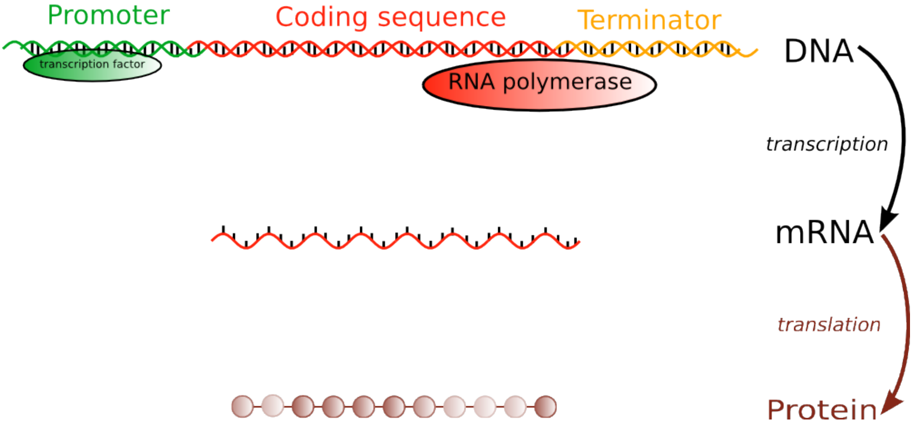

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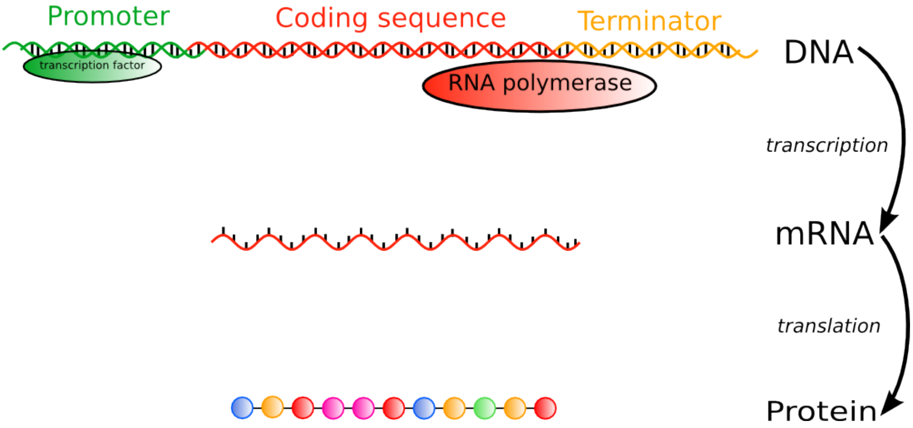

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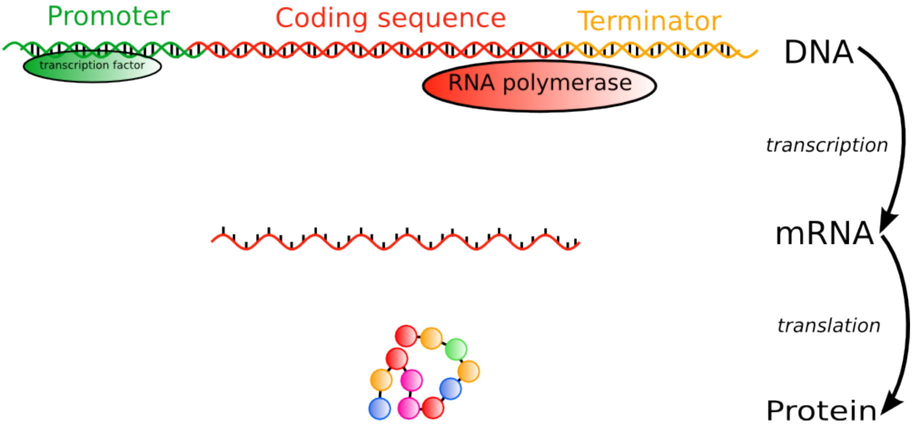
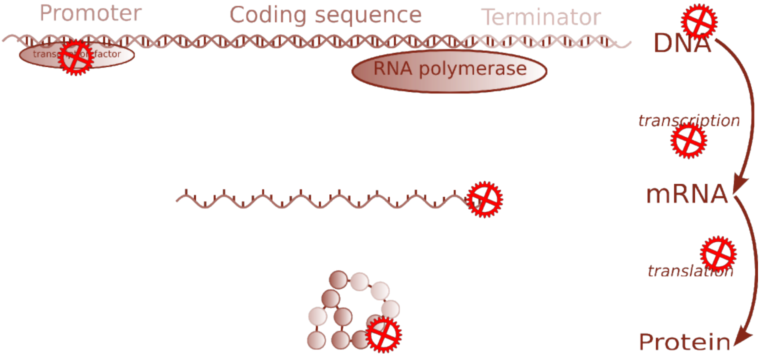


Regulational steps in gene expression

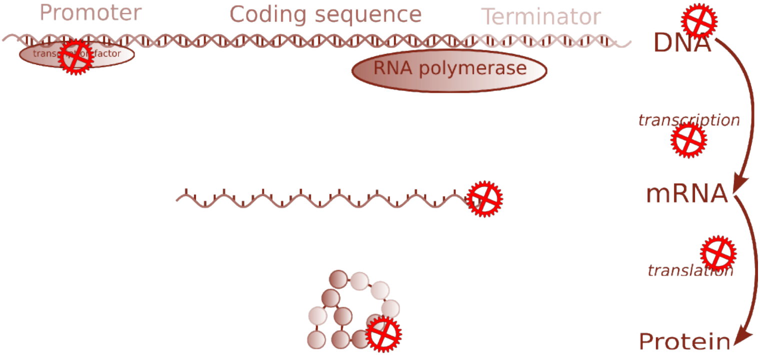


Regulated steps:

- 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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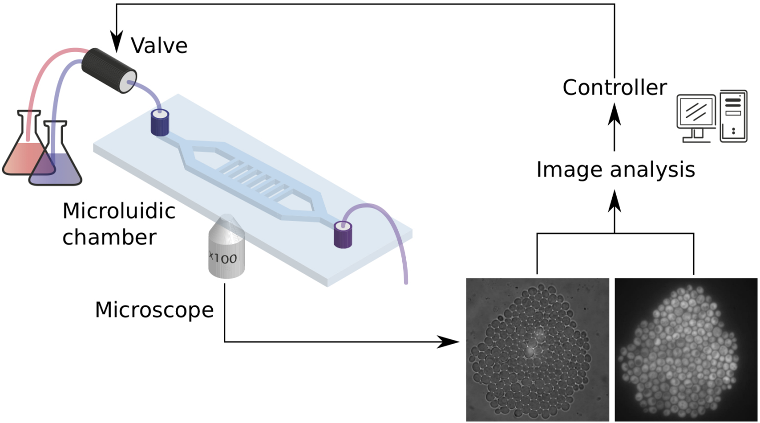
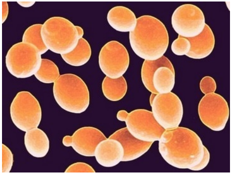
A closed loop control platform

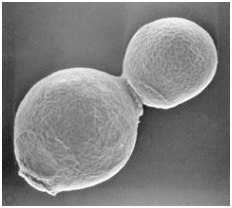


- Main features
  1. real-time observation
  2. real-time change of cellular stimulus
  3. real-time control

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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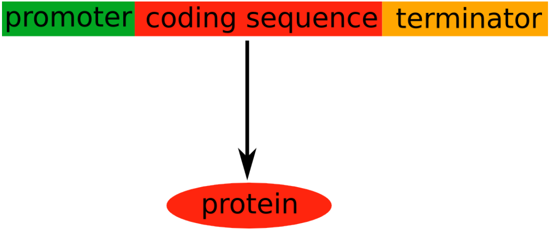

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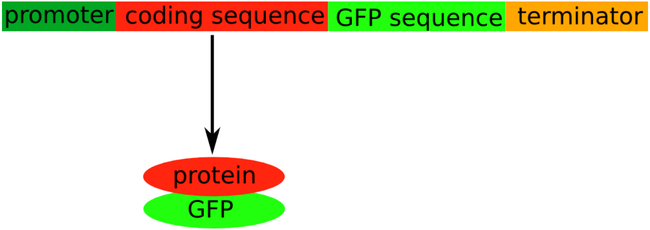

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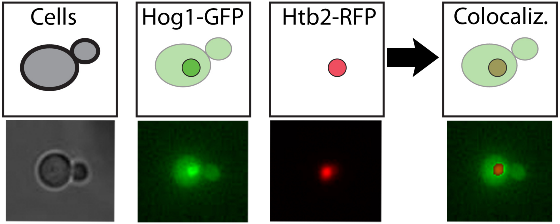
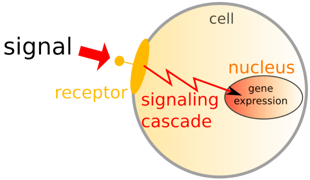


Activation of gene expression

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



- Example: increase in osmolarity

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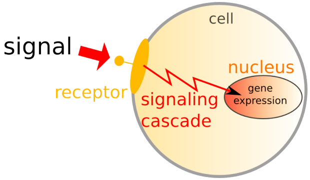

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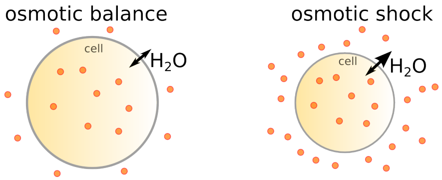

Activation of gene expression

- 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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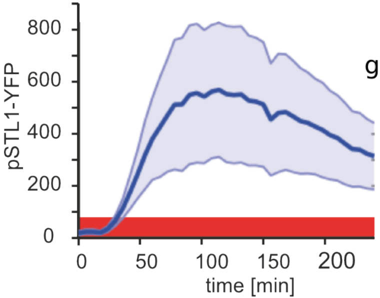

gene expression response
to osmotic shock

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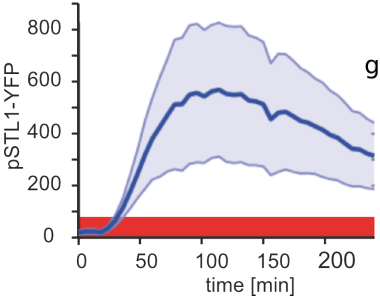

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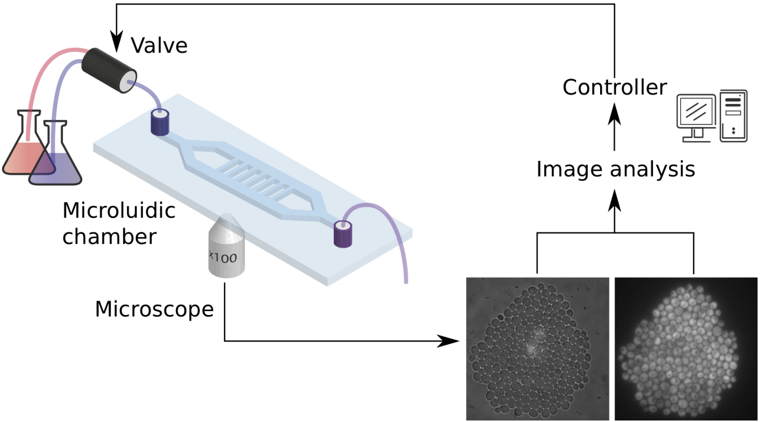
A closed loop control platform



- 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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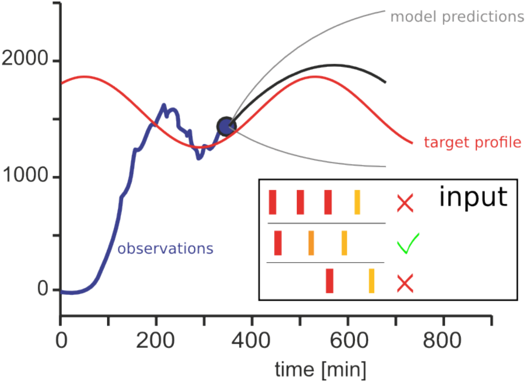

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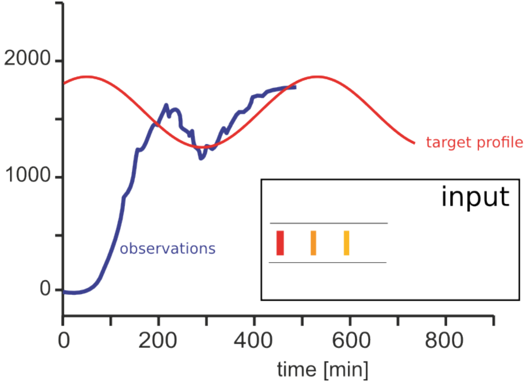

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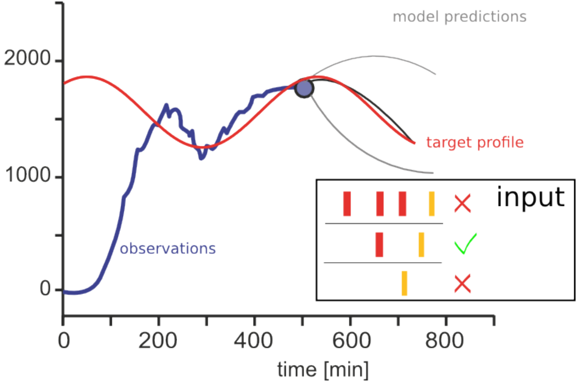

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Model predictive control (MPC)

- 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

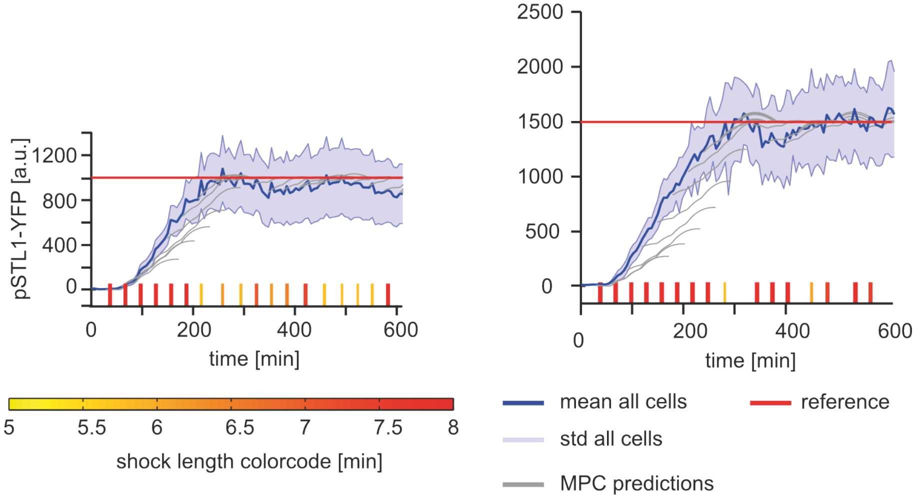
Model equations

$$\frac{dx_1}{dt} = u(t-\tau) - \gamma_1 x_1(t)$$
$$\frac{dx_2}{dt} = k_1 x_1(t) - \gamma_2 \frac{x_2(t)}{K + x_2(t)}$$

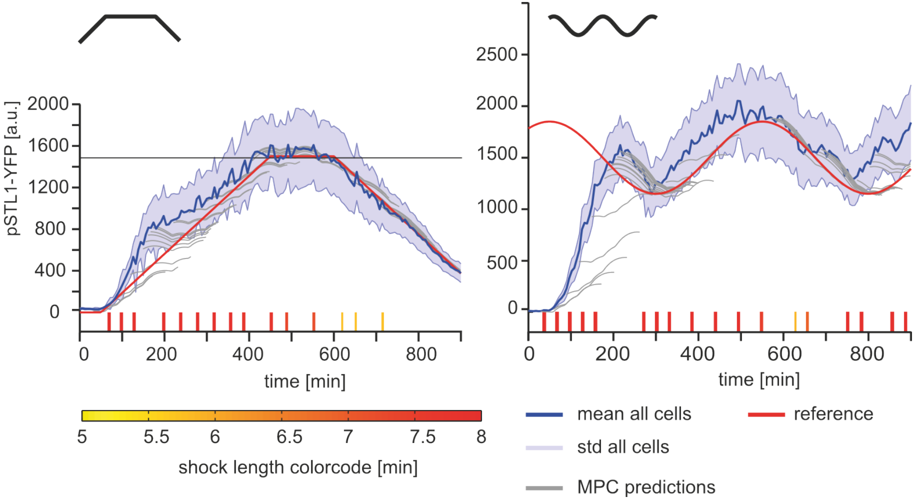
Results

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

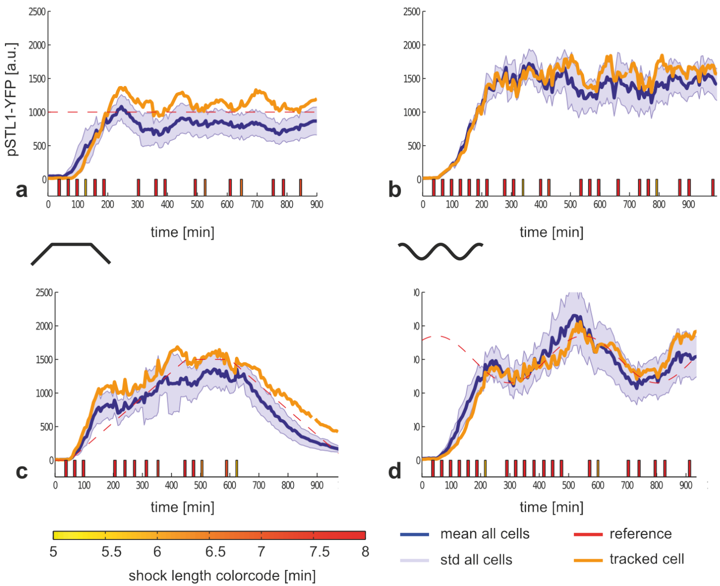
Results - constant target



Results - varying target



Results - single cell control



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)