# Synbio 2007: Literature Review

## A Synthetic Oscillatory Network of Transcriptional Regulators

## Foundations of Engineering Biology

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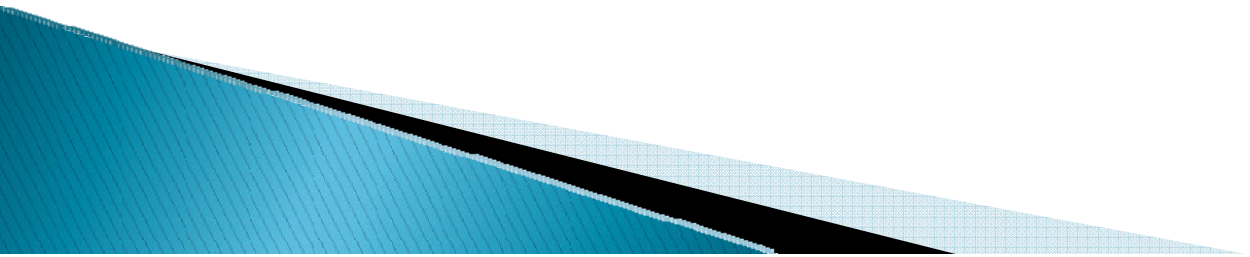
# Topics of Discussion

- Foundations of Engineering Biology
  - Synthetic Biology: What & Why?
  - Standardization
  - Decoupling
  - Abstraction
  - Evolution
  - Ethics
- A Synthetic Oscillatory Network of Transcriptional Regulators
  - Timecourse of fluorescence
  - Synthetic Oscillator
  - Sibling Comparison

# Foundations of Engineering Biology



# Synthetic biology: what and why?

- Engineering technology based on ‘living systems’
- Many different perspectives:
  biology, chemistry, engineers, ‘re-writers’

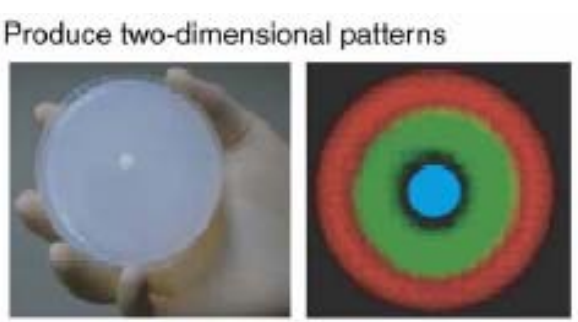
**Challenges**

Complexity
Tedious construction
Spontaneous variation
Evolution

**Strategies**

Standardization
Decoupling
Abstraction

need new strategies to tackle

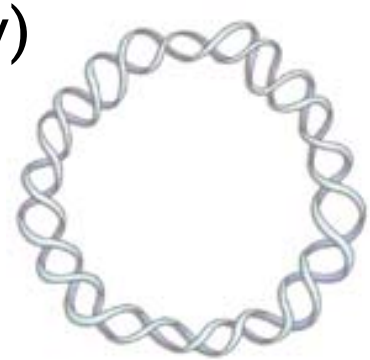





Voigt, C. Genetic parts to program bacteria. Current Opinion in Biotechnology 2006, 17:548–557

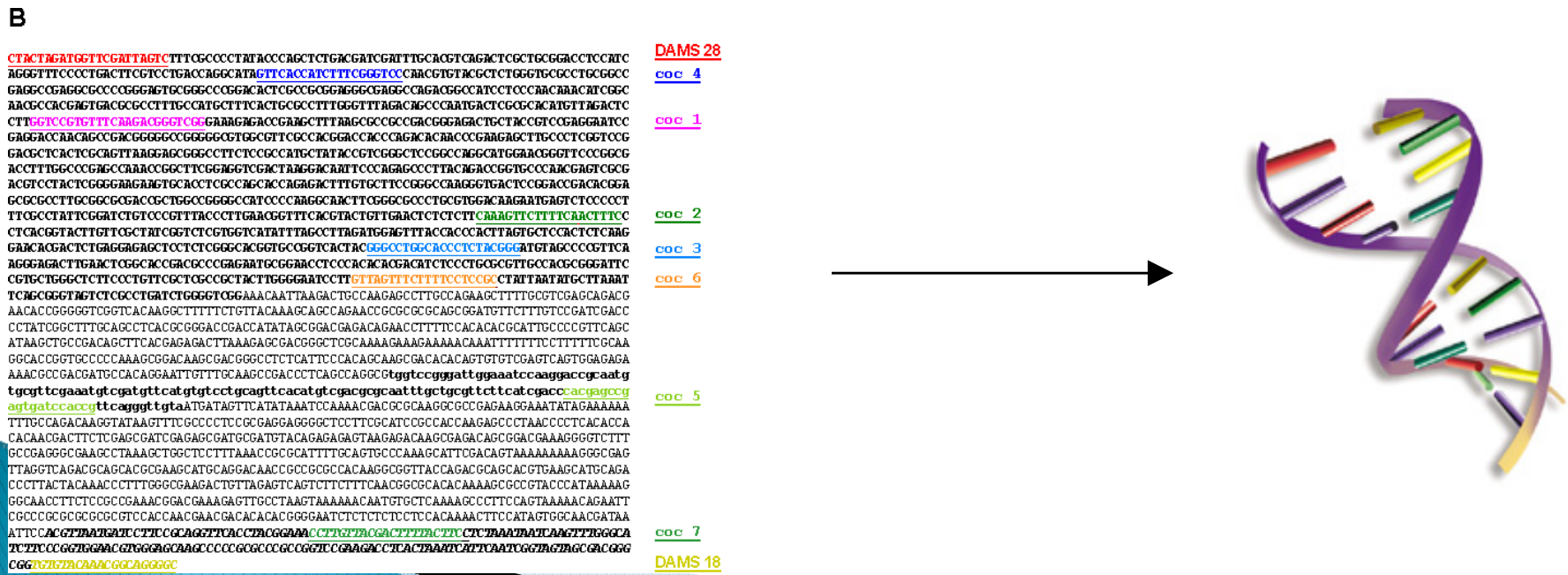
# Standardization


- Technical standards:
  - System operation(genetic background, media, env.)
  - Measurements (e.g. protein concentrations)
  - Biological function (e.g. promoter activity)
  - Registry of Standard Biological Parts
- Legal standards:
  - Intellectual property
  - Barcoding/watermarking for easy identification?




# Decoupling

- Separating complex problems into simpler ones that can be worked on individually then later combined
- Most immediate uses in decoupling of design and fabrication of DNA synthesis (some individuals design DNA while others build the sequence)
- Future applications: One engineer develops resource delivering system while another develops “devices” for the system

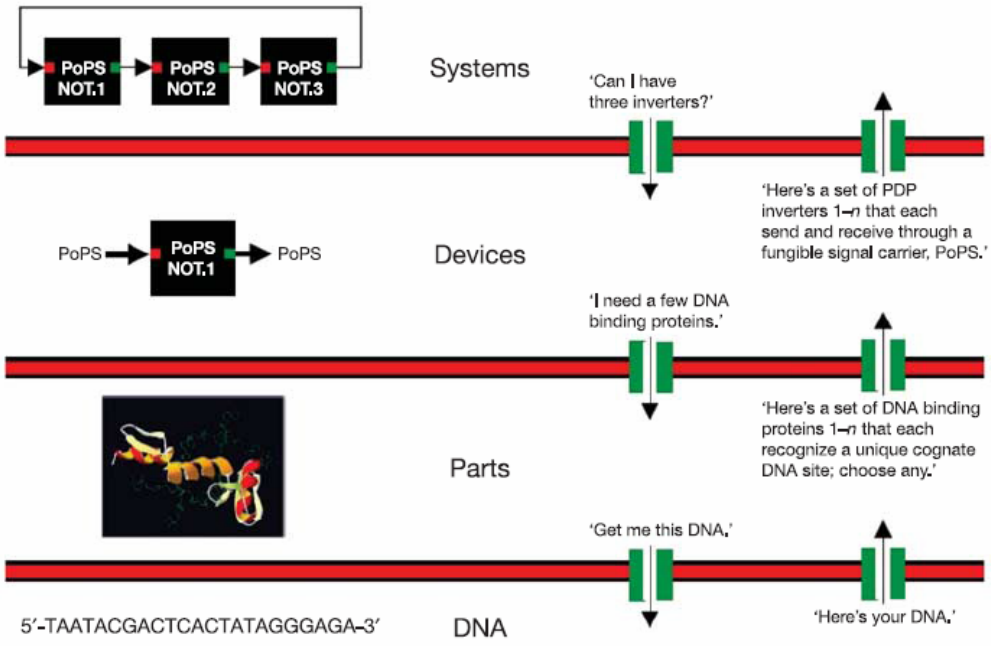


# Abstraction

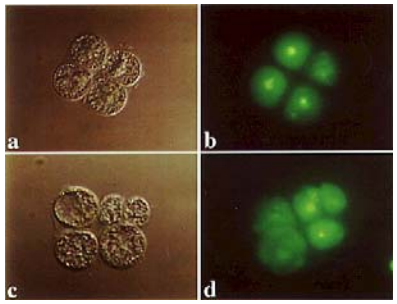
- Biological systems are complex and have many components–abstraction attempts to decompose these systems to more manageable concepts
- 2 Forms useful in biologic engineering:

i) Abstraction hierarchies–make describing biological systems simpler

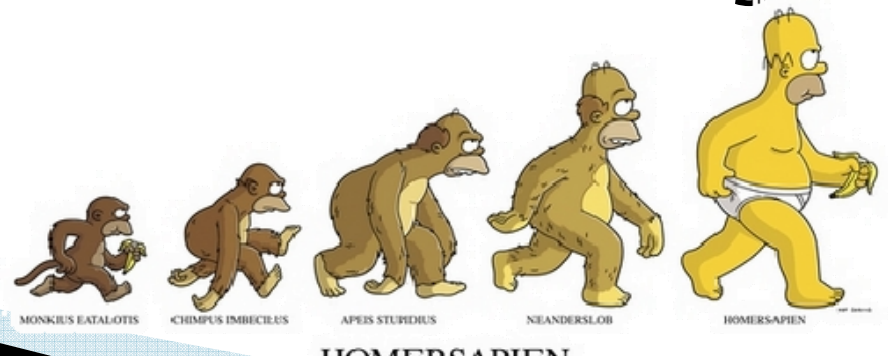
ii) Parts and devices used in engineered biological systems should be redesigned so they can more easily be used in combination

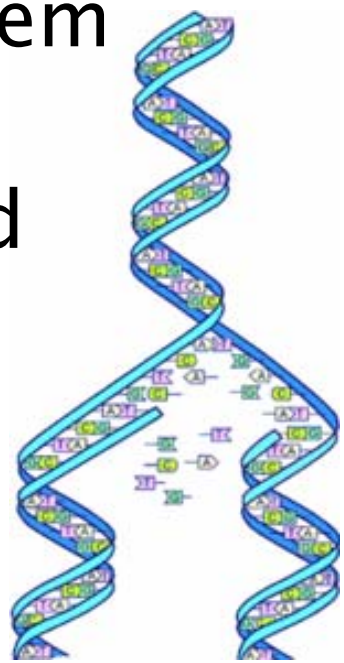


# Evolution




- Currently no practical theory supporting design of reproducing biological machines
- Biological systems are replicating machines that make mistakes during the replication process
- The greater the manipulation of a biological system, the greater the stress on the system and possibility of mutants
- Better control and understanding required

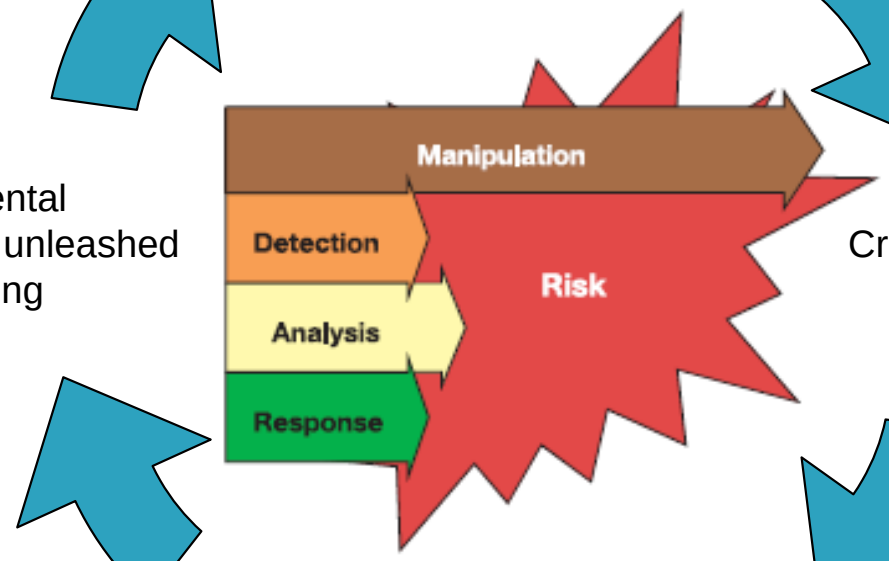


# Ethics

Possible mutation of synthetic organism/biomaterial in non-controlled environment
**(Evolution)**

Library of biological parts and well-defined processes for detection and characterization of biological threats leads to modularized and more efficient threat identification and assessment
**(Decoupling)**



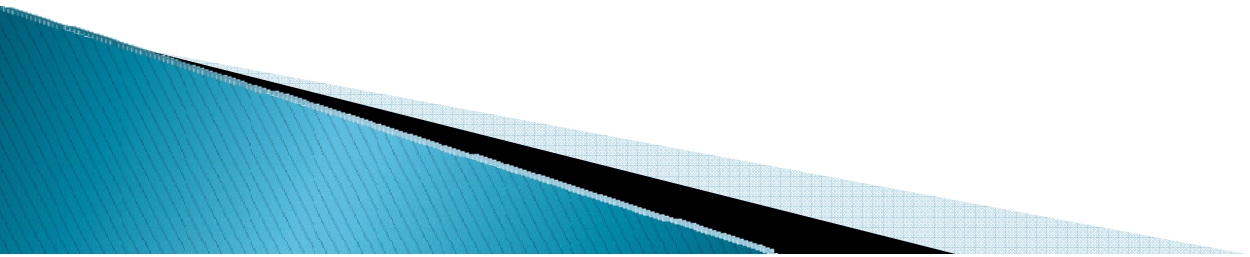

Bioweapons or experimental strains/organisms accidentally unleashed into environment causing detrimental effects

Creation, testing and deployment of biological counter to threat

Creation of standardized biological parts – proteins, plasmids, genes, etc. with well-defined function
**(Standardization)**

New measurement techniques and equipment developed in response to threat. Ideas on possible new parts and combinations inspired by threat
**(Abstraction)**

# A Synthetic Oscillatory Network of Transcriptional Regulators



# Timecourse of fluorescence

- Temporal Oscillations occurring with period of ~ 150 minutes

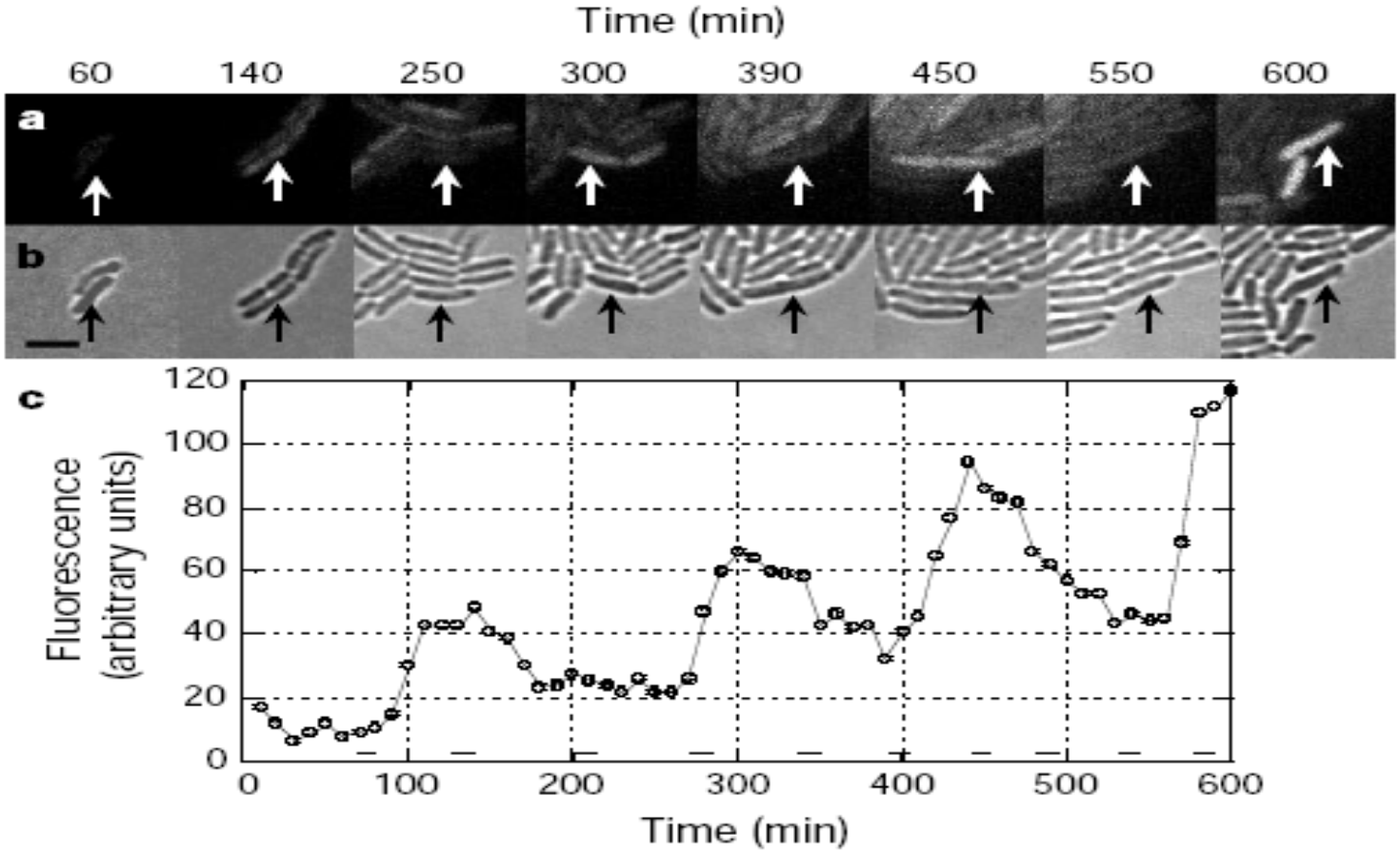
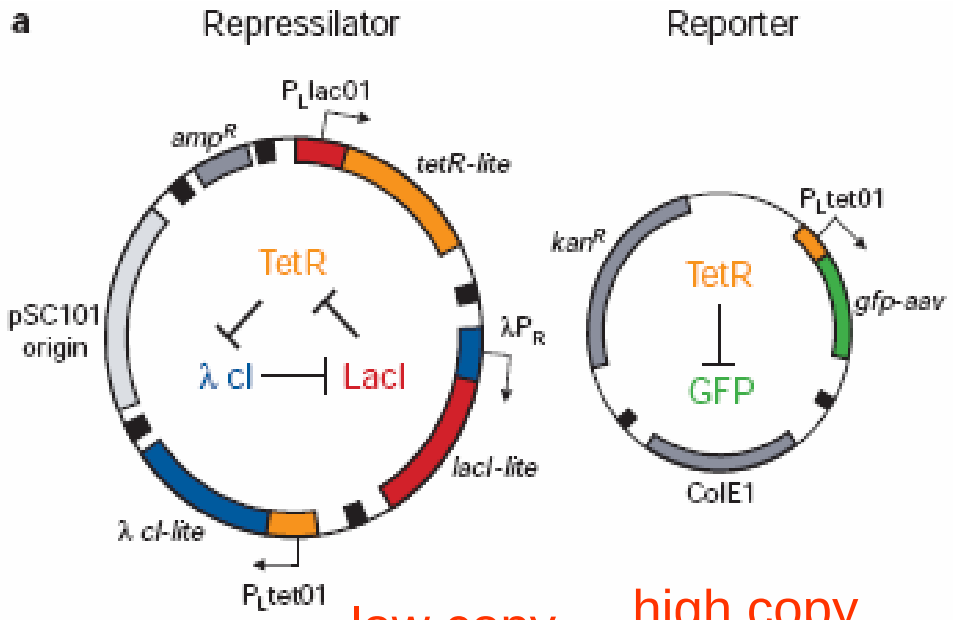



**Figure 2** Repressilation in living bacteria. **a**, **b**, The growth and timecourse of GFP expression for a single cell of *E. coli* host strain MC4100 containing the repressilator plasmids (Fig. 1a). Snapshots of a growing microcolony were taken periodically both in fluorescence (**a**) and bright-field (**b**). **c**, The pictures in **a** and **b** correspond to peaks and troughs in the timecourse of GFP fluorescence density of the selected cell. Scale bar, 4 μm. Bars at the bottom of **c** indicate the timing of septation events, as estimated from bright-field images.

# Synthetic Oscillator

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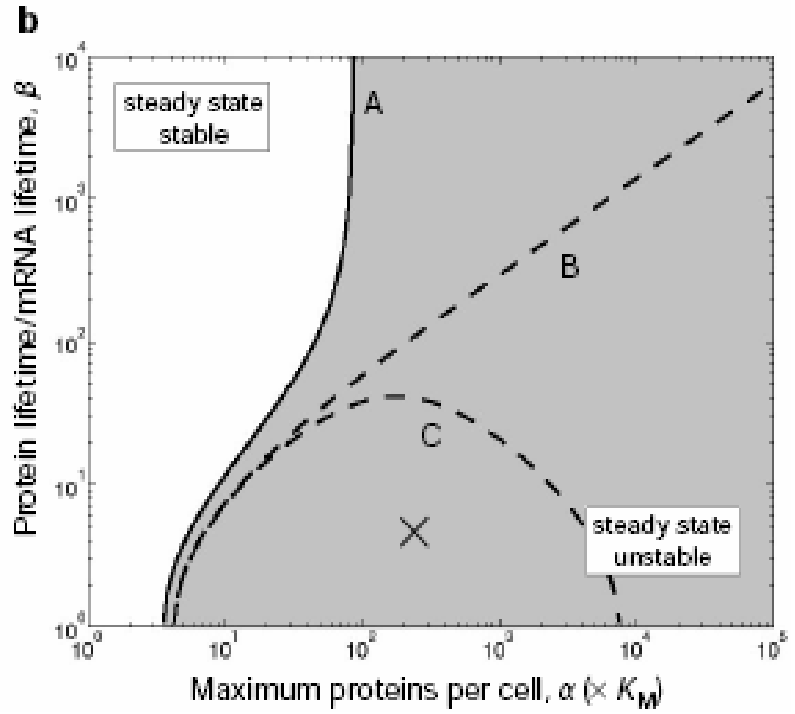
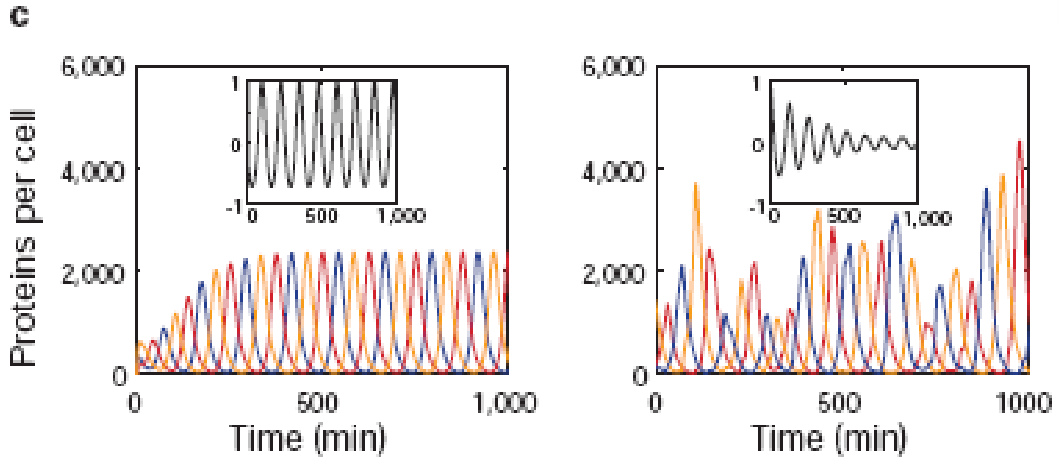



stability diagram




deterministic

stochastic

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{(1+p_j^n)} + \alpha_0 \qquad \begin{pmatrix} i = lacI, tetR, cI \\ j = cI, lacI, tetR \end{pmatrix}$$

$$\frac{dp_i}{dt} = -\beta(p_i - m_i)$$

Elowitz MB, Leibler S. A synthetic oscillatory network of transcriptional regulators. Nature. 2000 Jan 20;403(6767):335-8.

# Sibling Comparison

- Wide variability in performance between siblings demonstrating impact of mutations, interaction between siblings and influence of environment on repressilator dynamics

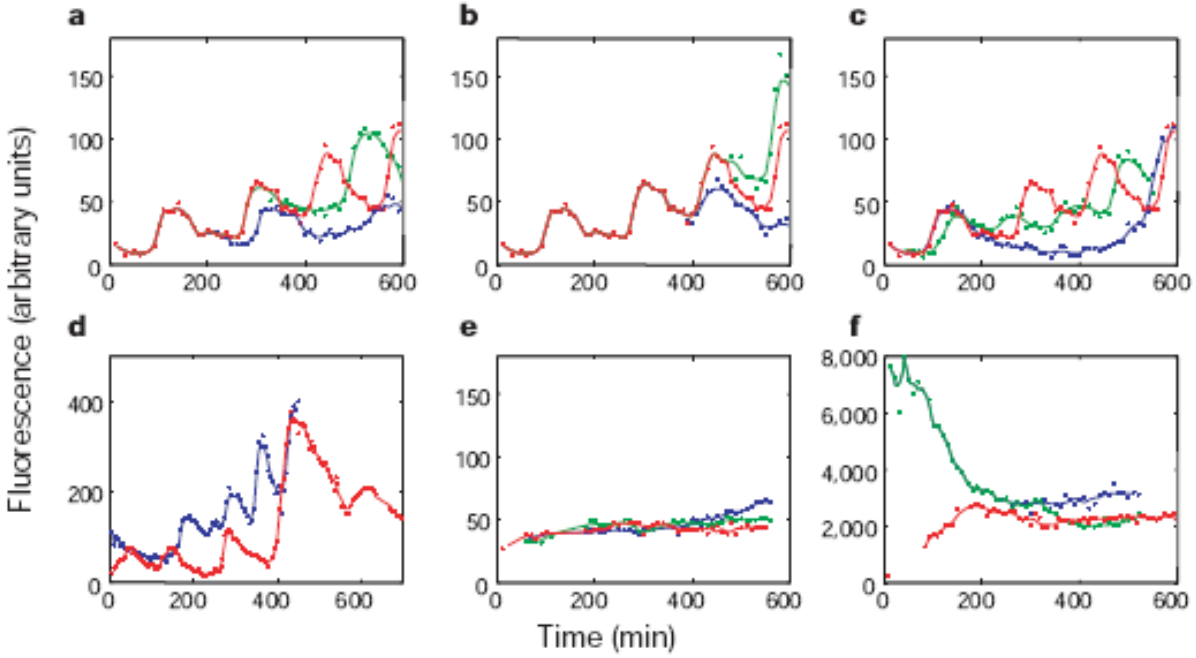



**Figure 3** Examples of oscillatory behaviour and of negative controls. **a**–**c**, Comparison of the repressilator dynamics exhibited by sibling cells. In each case, the fluorescence timecourse of the cell depicted in Fig. 2 is redrawn in red as a reference, and two of its siblings are shown in blue and green. **a**, Siblings exhibiting post-septation phase delays relative to the reference cell. **b**, Examples where phase is approximately maintained but amplitude varies significantly after division. **c**, Examples of reduced period (green) and long delay (blue). **d**, Two other examples of oscillatory cells from data obtained in different experiments, under conditions similar to those of **a**–**c**. There is a large variability in period and amplitude of oscillations. **e**, **f**, Examples of negative control experiments. **e**, Cells containing the repressilator were disrupted by growth in media containing 50 μM IPTG. **f**, Cells containing only the reporter plasmid.