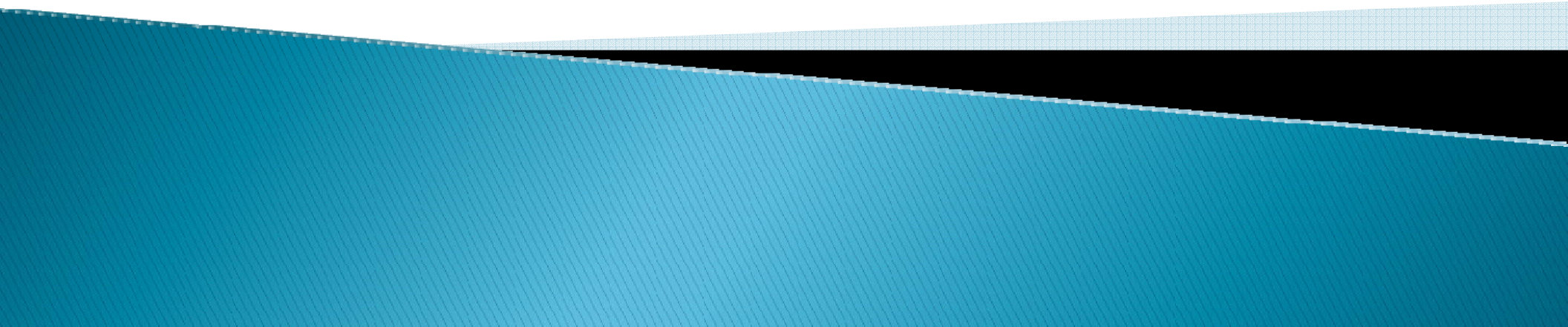


Synbio 2007: Literature Review

A Synthetic Oscillatory Network of
Transcriptional Regulators

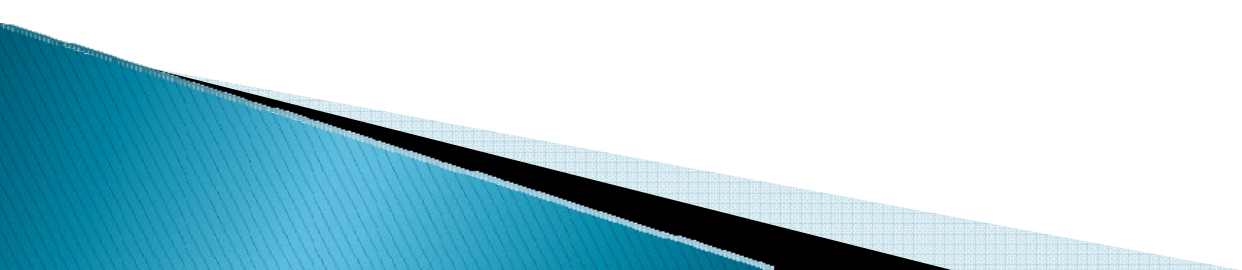
Foundations of Engineering Biology

Sisi Chen, Jim Cheng, Arash Calafi

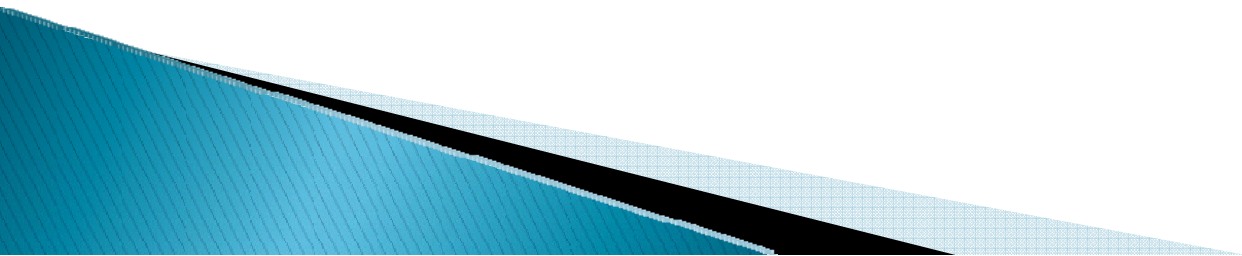


Topics of Discussion

- ▶ 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

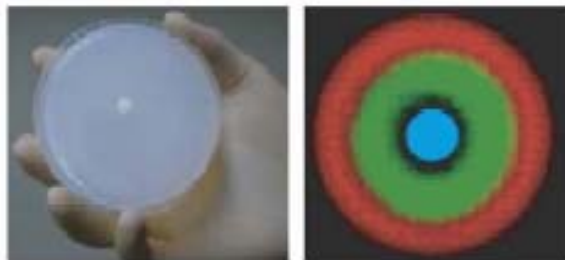
Complexity
Tedious construction
Spontaneous variation
Evolution

Strategies

Standardization
Decoupling
Abstraction

need new strategies to tackle

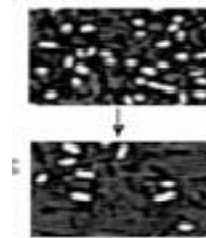
Produce two-dimensional patterns



Take your picture



Commit suicide or kill malignant cells



Standardization

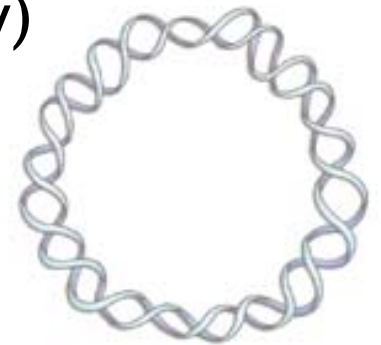


► Technical standards:

- 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?



"They don't trust each other to share research."

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

B

CTCTAGTGTGGTTCGGTTAGTCTTTGCCCTATTACCGAGCTCTGAGCATCGATTTCGAGTCCGAGTCTGTCGGAGCATCTCCCTC
AGGTTGCTCCCTGACCTTCTGCTCTGACACGAGCTGATGTTCCACCTCTTTCCGGTCC **CACGCTGTTACGCTTGGGTTGGCTCCGGCCG**
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DAMS 28
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coc 1
coc 2
coc 3
coc 6
coc 5
coc 7
DAMS 18

DAMS 28

coc 4

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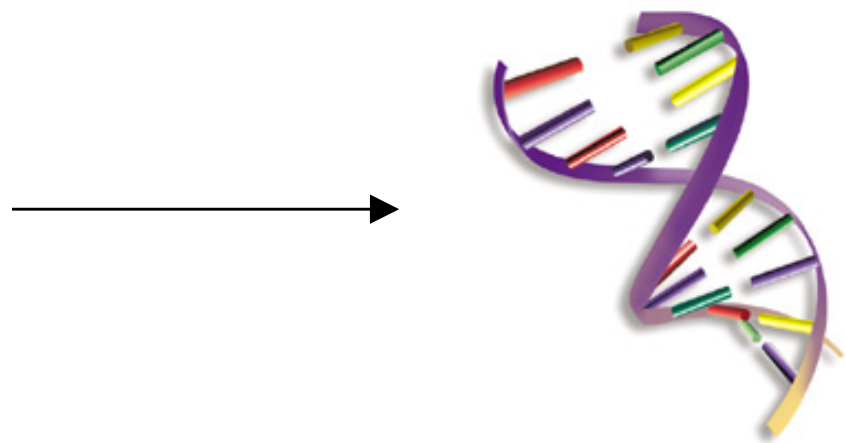
coe 3

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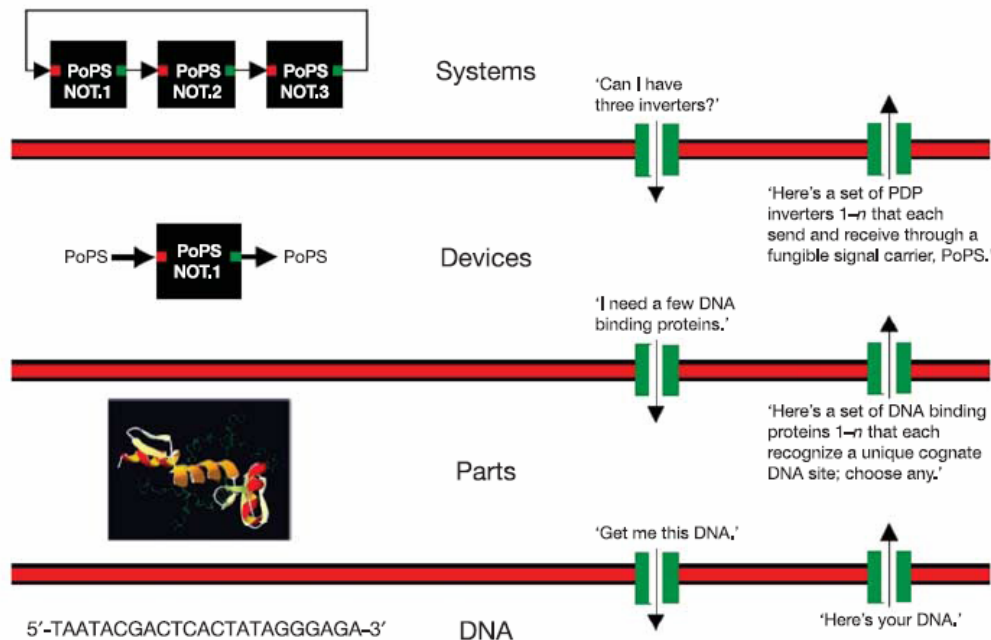
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DAMS 18



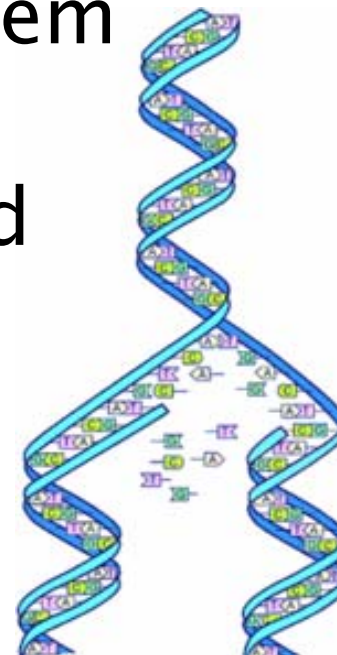
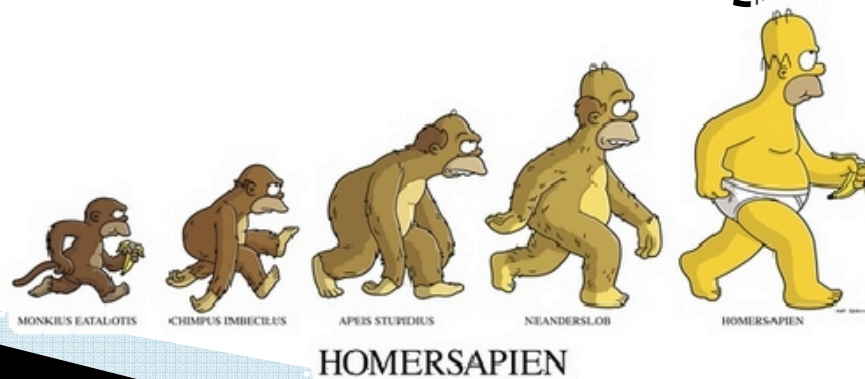
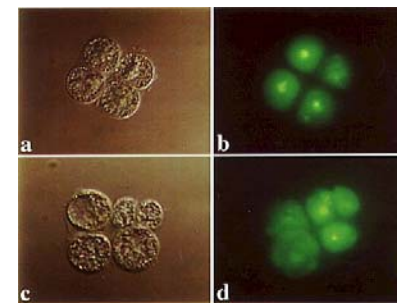
Abstraction

- ▶ 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)

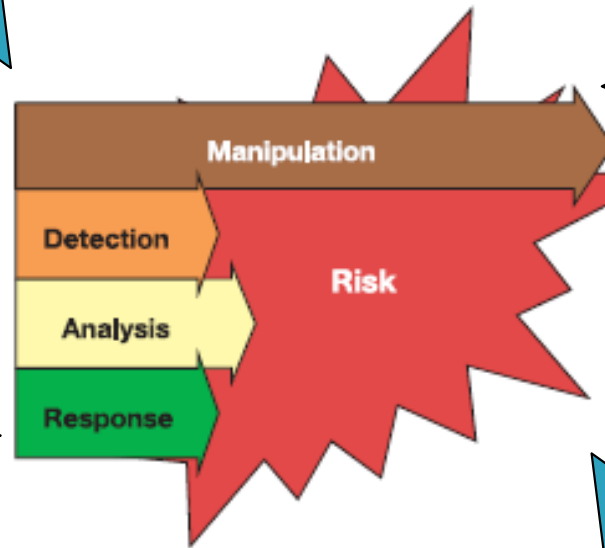
Library of biological parts and well-defined processes for detection and characterization of biological threats leads to modularized and more efficient threat identification and assessment
(Decoupling)

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

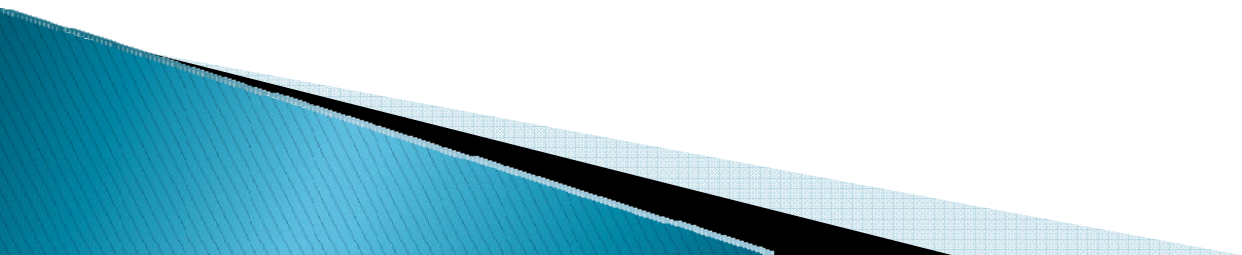
Creation, testing and deployment of biological counter to threat

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

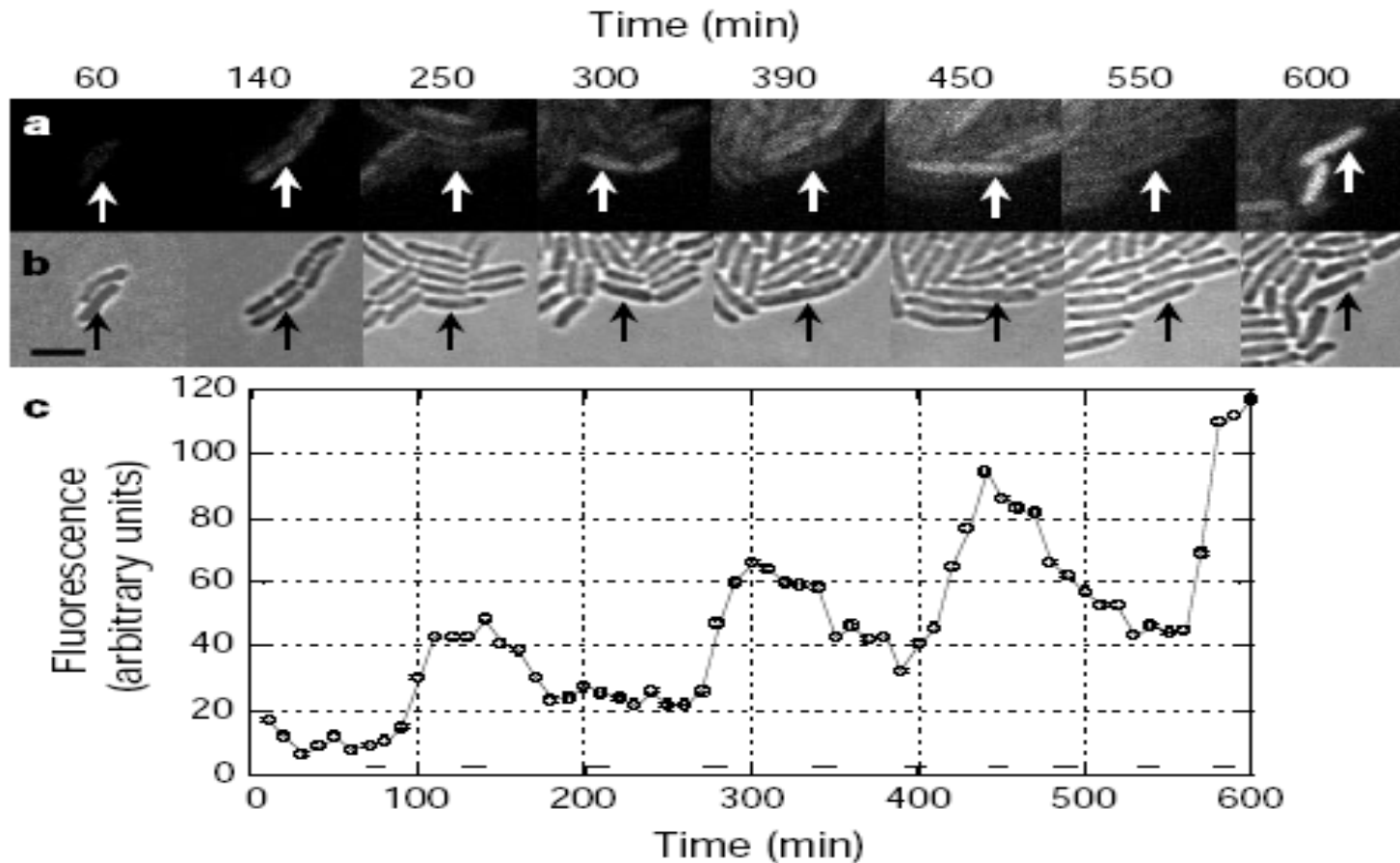
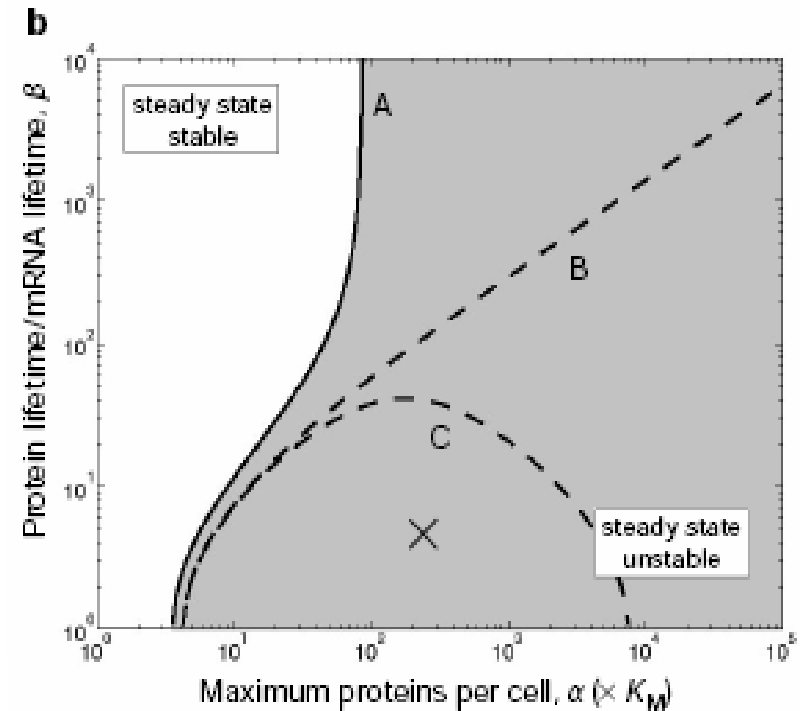
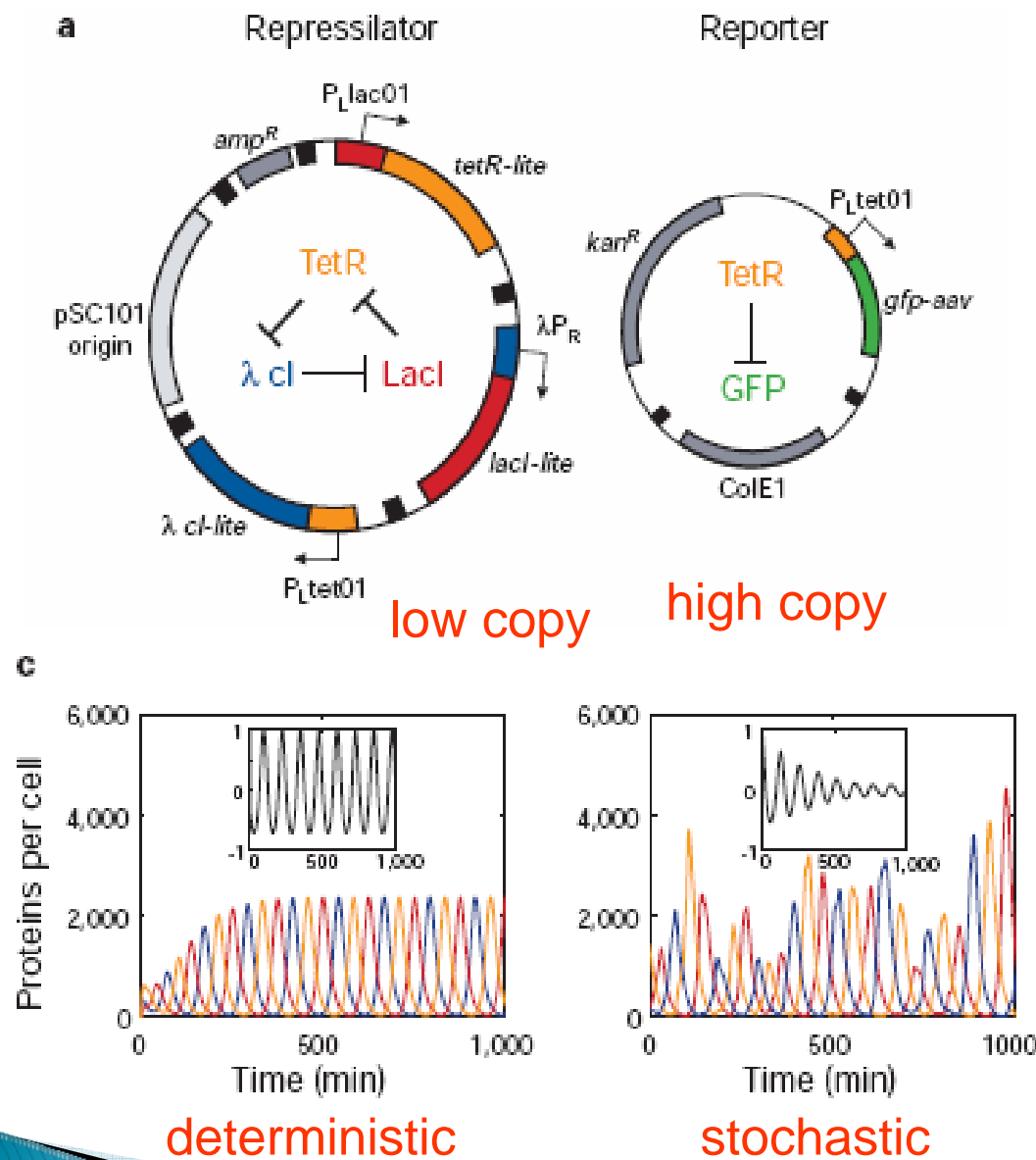


Figure 2 Repressilation in living bacteria. **a**, **b**, The growth and timecourse of GFP expression for a single cell of *E. coli* host strain MC4100 containing the repressilator plasmids (Fig. 1a). Snapshots of a growing microcolony were taken periodically both in fluorescence (**a**) and bright-field (**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



stability diagram

$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{(1 + p_i^n)} + \alpha_0 \quad \left(\begin{array}{l} i = lacI, tetR, cl \\ j = cl, lacI, tetR \end{array} \right)$$

$$\frac{dp_i}{dt} = -\beta p_i - m_j$$

Sibling Comparison

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

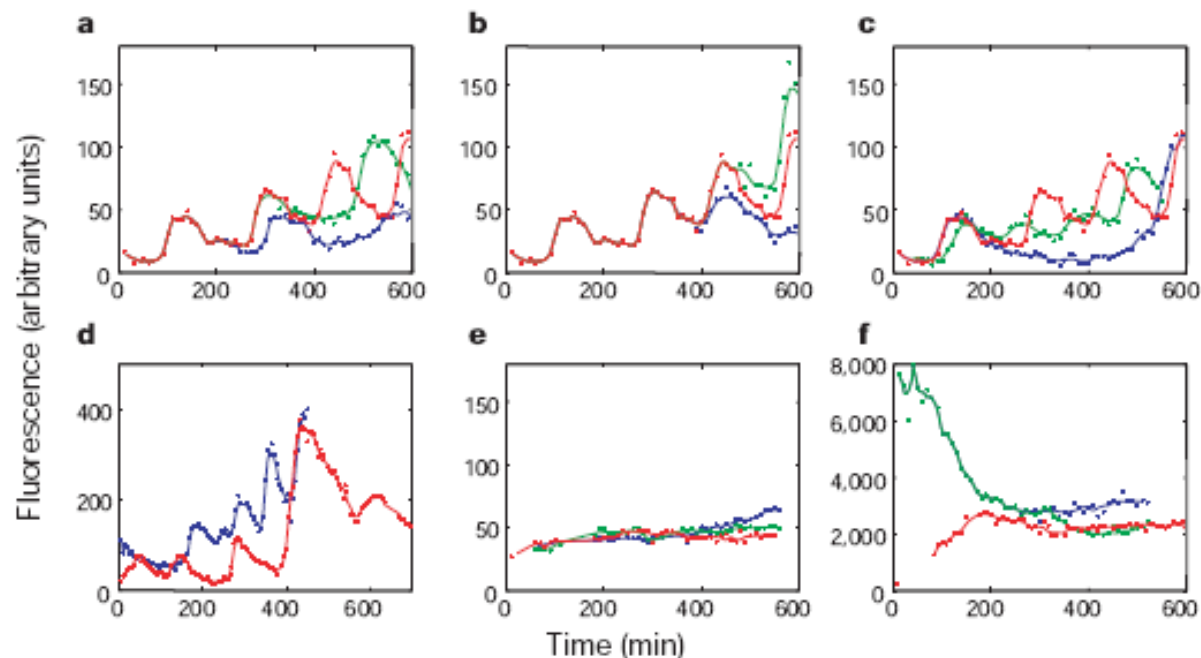


Figure 3 Examples of oscillatory behaviour and of negative controls. **a–c**, Comparison of the repressilator dynamics exhibited by sibling cells. In each case, the fluorescence timecourse of the cell depicted in Fig. 2 is redrawn in red as a reference, and two of its siblings are shown in blue and green. **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 μ M IPTG. **f**, Cells containing only the reporter plasmid.