Introduction to Bioengineering
BIOE/ENGR.80
Stanford University

Spring 2020 Class Slides

Day 9
24 April 2020

Letter | Published: 20 January 2000

A synthetic oscillatory network of transcriptional regulators

Michael B. Elowitz ✉ & Stanislas Leibler

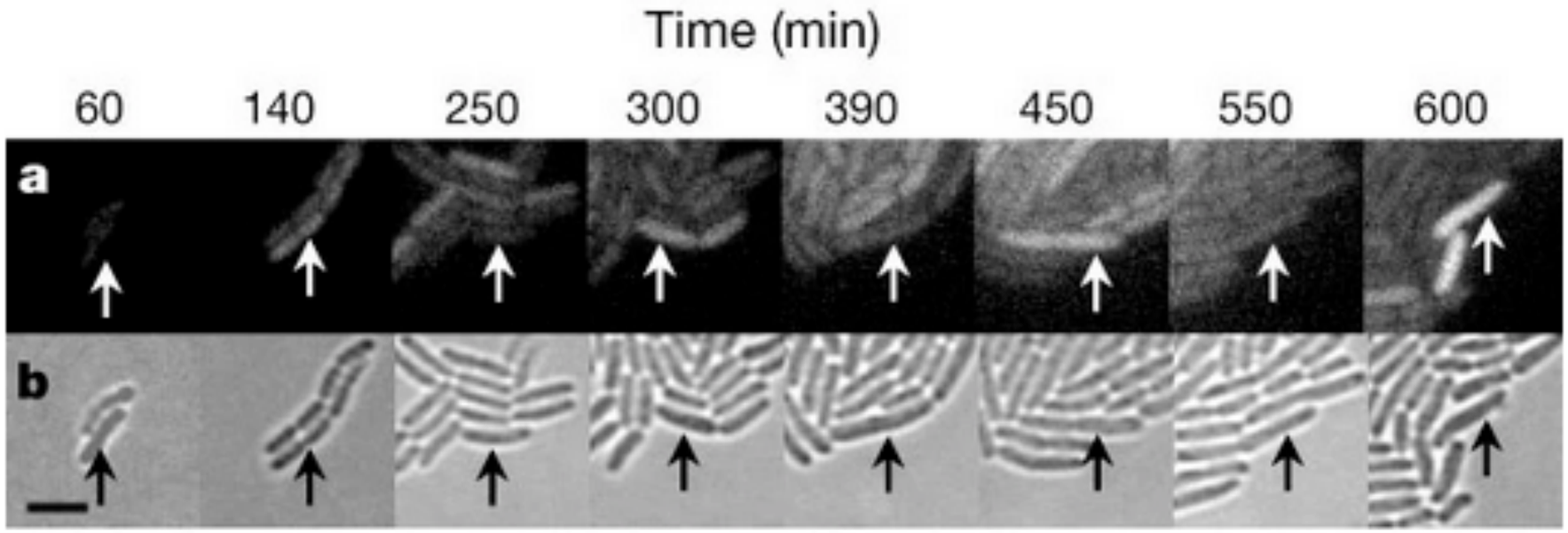
Nature **403**, 335–338(2000) | Cite this article

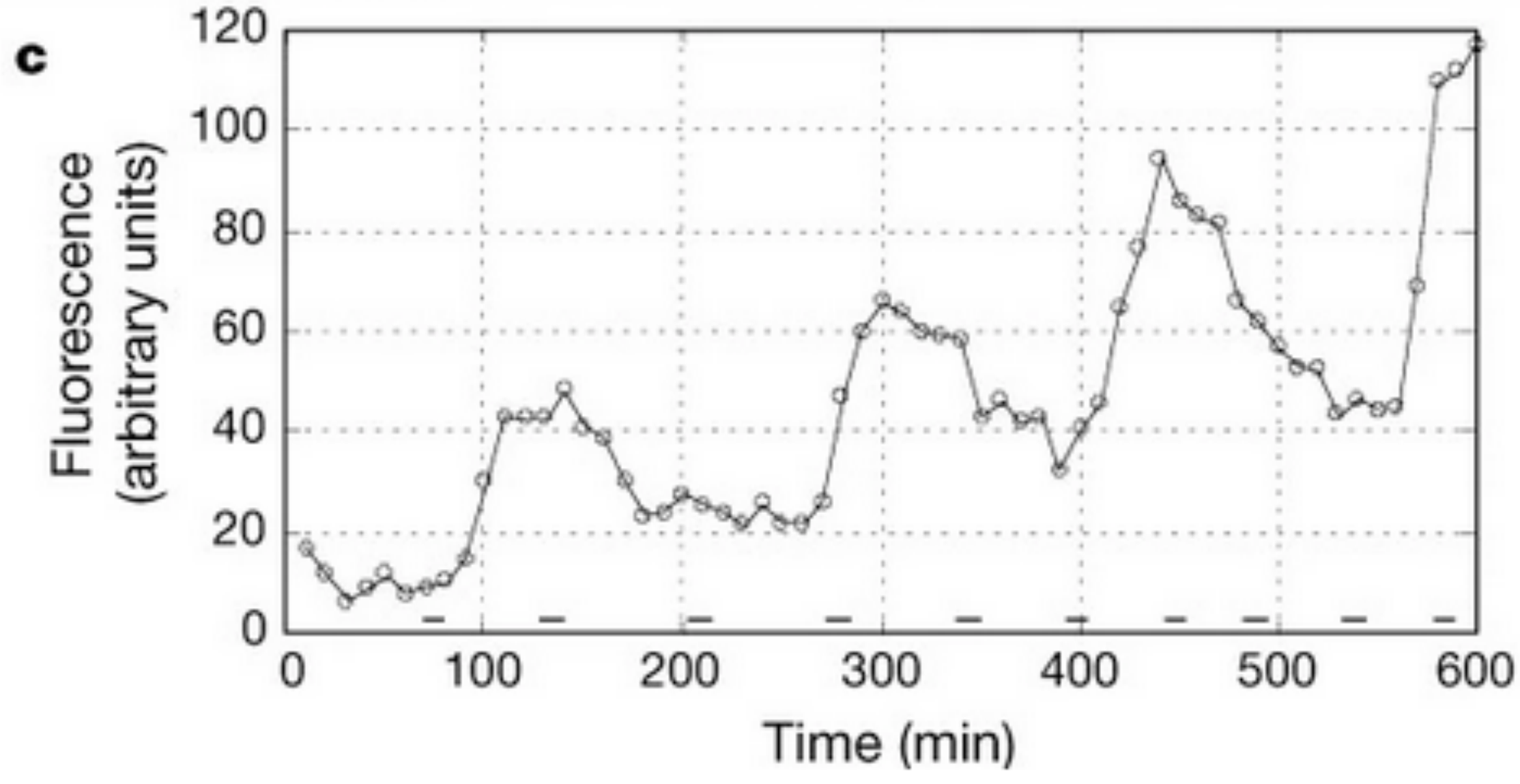
Abstract

Networks of interacting biomolecules carry out many essential functions in living cells[1], but the 'design principles' underlying the functioning of such intracellular networks remain poorly understood, despite intensive efforts including quantitative analysis of relatively simple systems[2]. Here we present a complementary approach to this problem: the design and construction of a synthetic network to implement a particular function. We used three transcriptional repressor systems that are not part of any natural biological clock[3,4,5] to build an oscillating network, termed the repressilator, in *Escherichia coli*. The network periodically induces the synthesis of green fluorescent protein as a readout of its state in individual cells. The resulting oscillations, with typical periods of hours, are slower than the cell-division cycle, so the state of the oscillator has to be transmitted from generation to generation. This artificial clock displays noisy behaviour, possibly because of stochastic fluctuations of its components. Such 'rational network design' may lead both to the engineering of new cellular behaviours and to an improved understanding of naturally occurring networks.

Main claim →

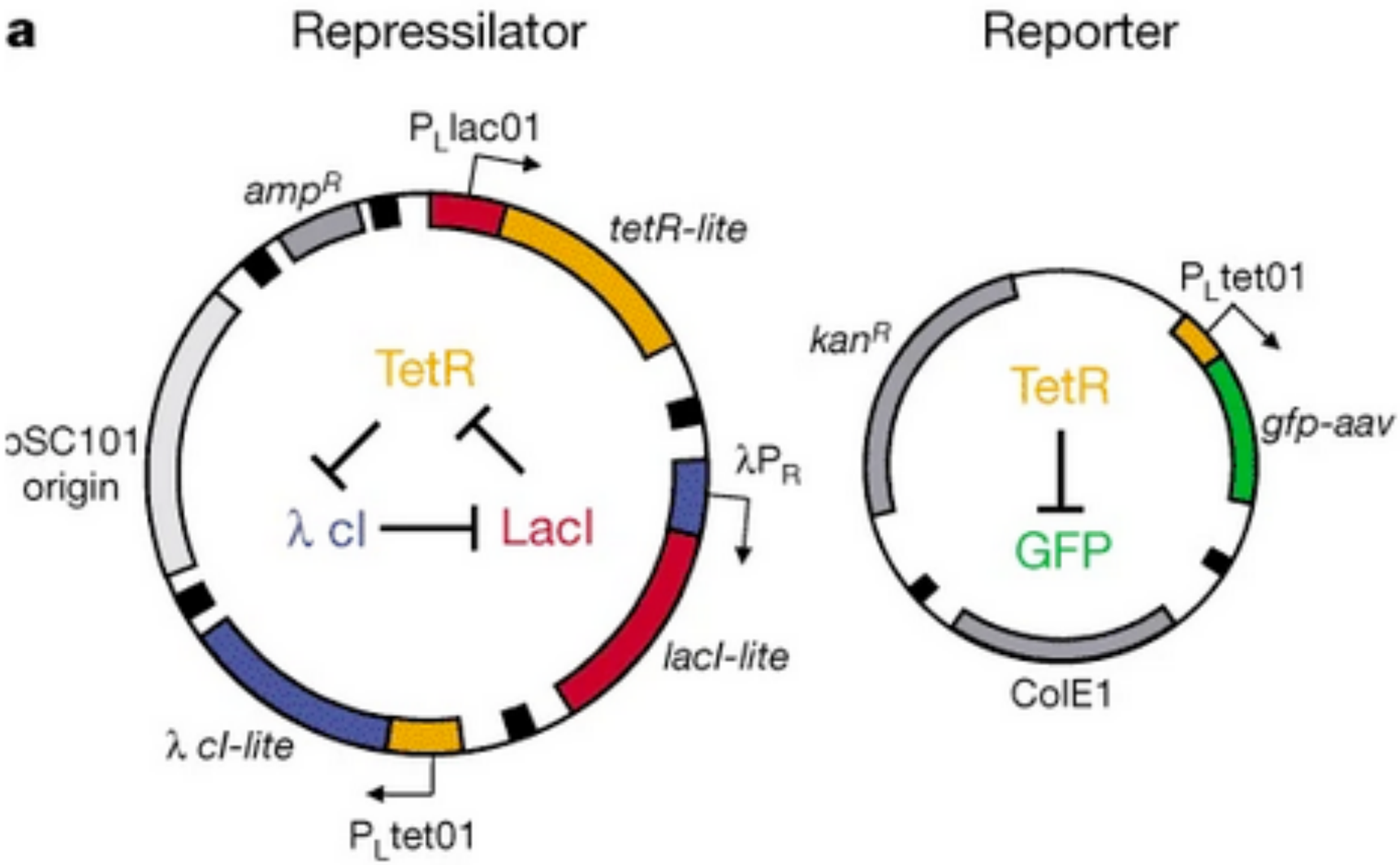
Main Evidence





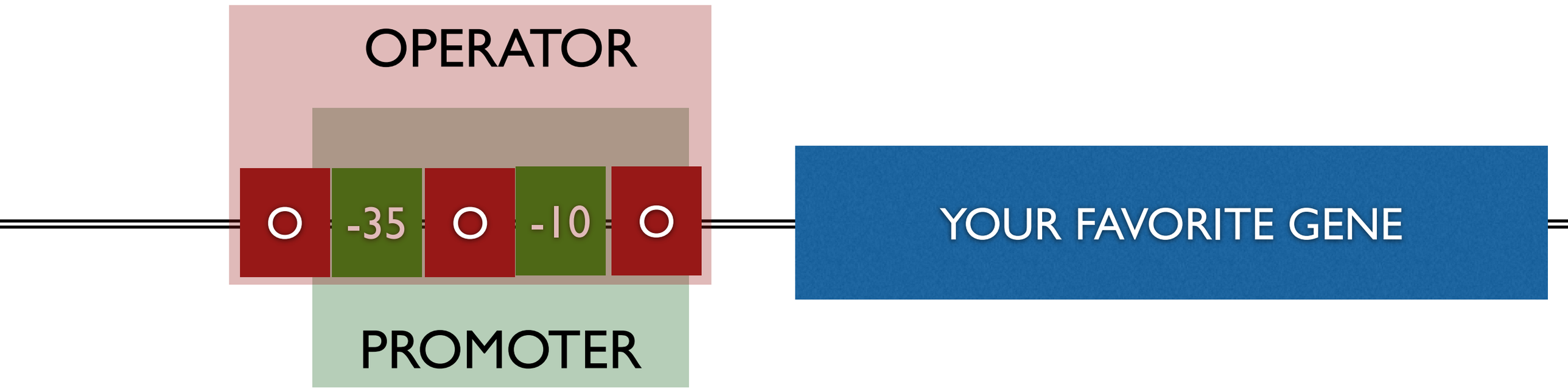
How does this genetic system work?

(oscillator is made of four proteins encoded by four open reading frames; three of the proteins regulate transcription w/in the system)



Regulation of gene expression via repressors

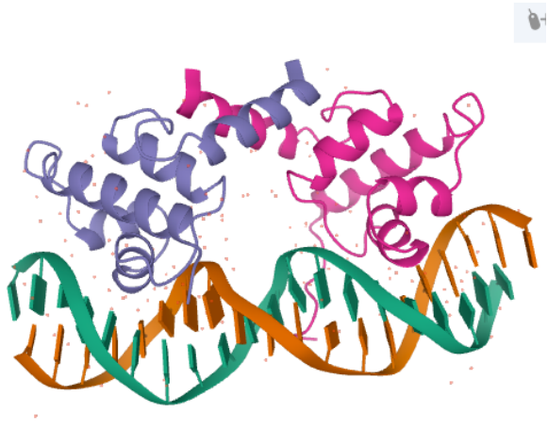
(for a gene to be expressed an RNA polymerase molecule must bind DNA at a specific promoter to start transcription; repressors block binding at specific operator sites and inhibit transcription)



(Repressors most-simply work by the principle of two objects cannot occupy the same space at the same time; if a repressor protein is bound to operator sequences interspersed within a promoter, then the RNA polymerase cannot bind the promoter. Note, activator proteins can turn on gene expression by binding to operator sites next to promoters, helping the RNA polymerase to start transcription)

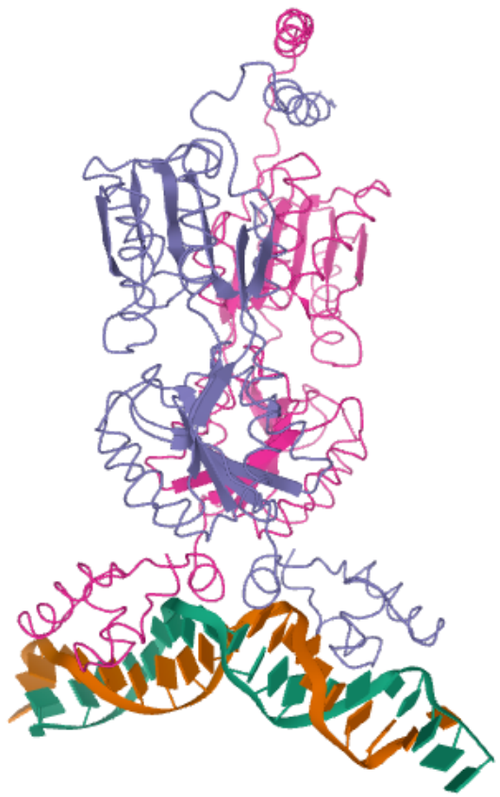
Repressor are proteins that bind DNA

(we can use biomolecule analysis and design tools to analyze and design DNA binding proteins, as per last class)



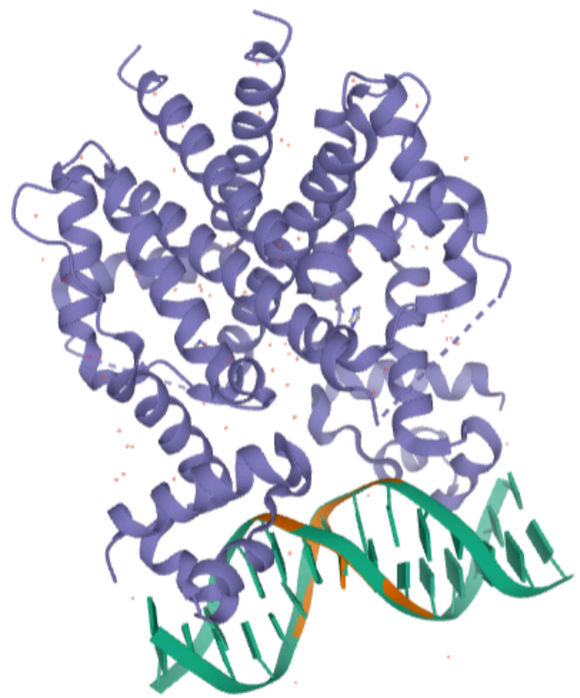
"lambda" repressor

https://www.rcsb.org/3d-view/1LMB/1



"lactose" repressor

https://www.rcsb.org/3d-view/1LBG/1



"tetracycline" repressor

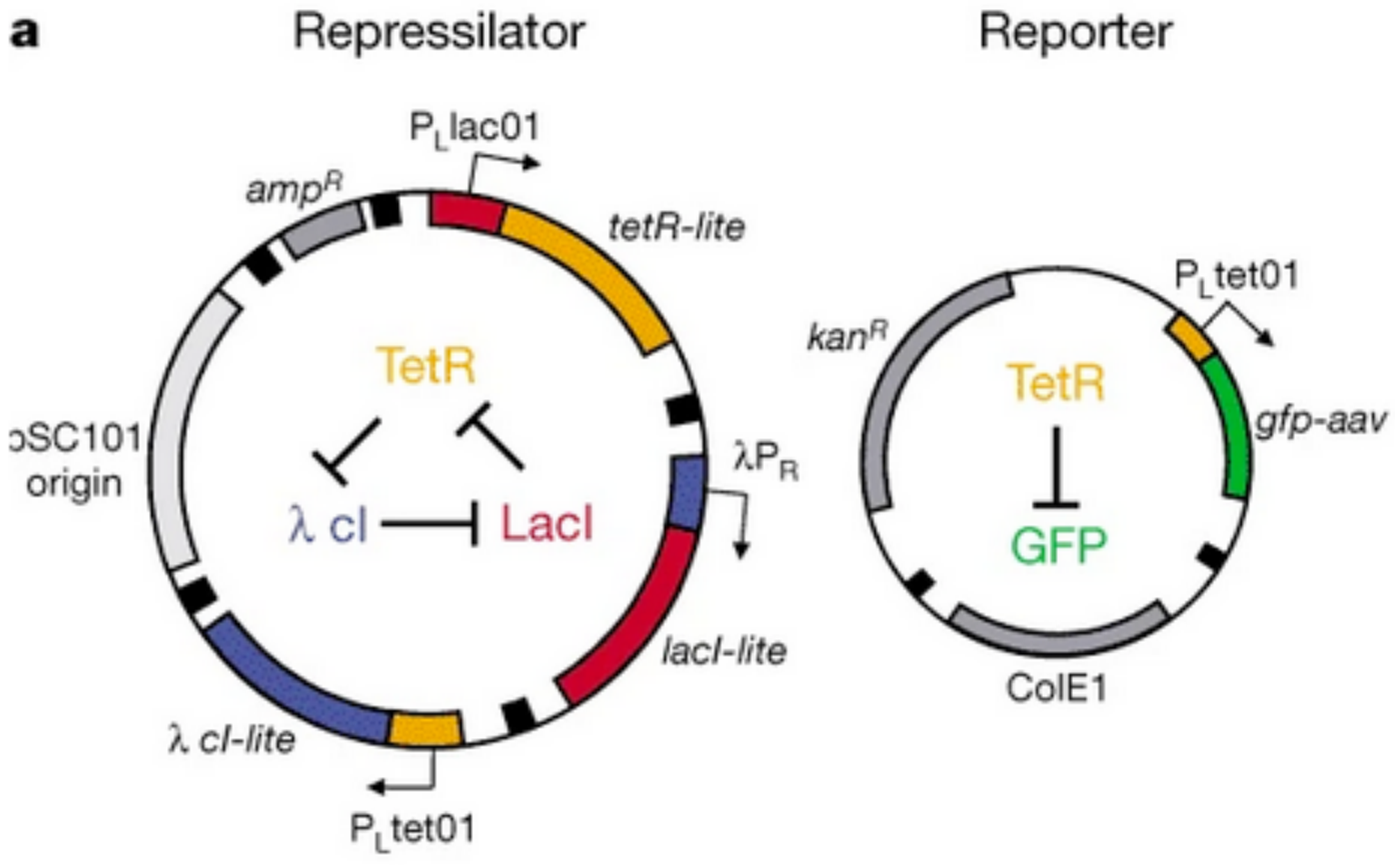
https://www.rcsb.org/3d-view/1QPI/1

green fluorescent protein

https://www.rcsb.org/3d-view/1GFL/1

How does this genetic system work?

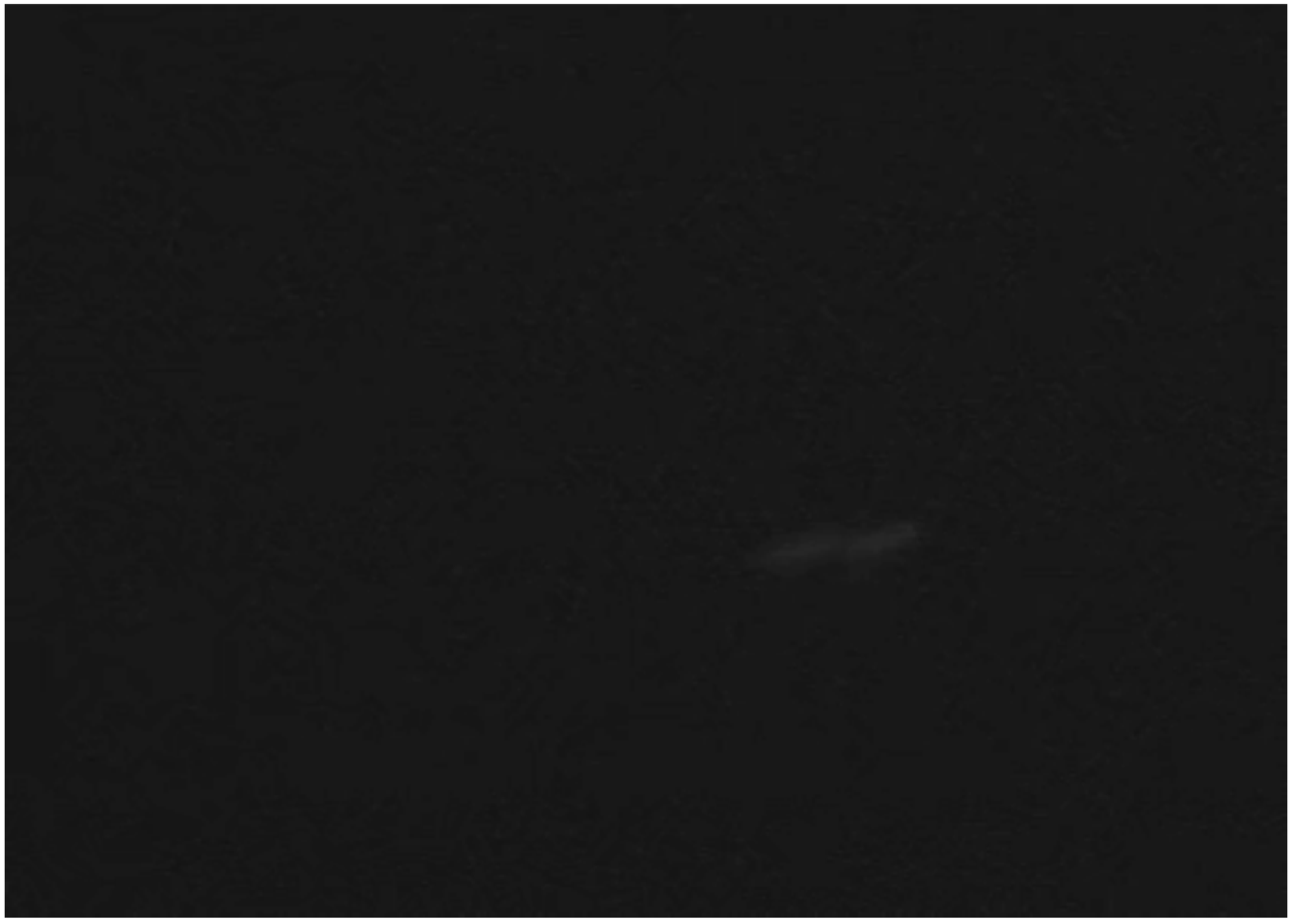
(when one repressor is highly abundant w/in a cell, the expression of the next repressor will be lowered, and so on. Because an odd number of repressors have been engineered to form a closed cycle, the system feeds back on itself resulting in an oscillation)



(what would happen if the system had two or four repressors in a cycle?)

Oscillations via a movie…

(how soon the cells start behaving differently! why?)



https://vimeo.com/291608242

Determination of cell fate selection during phage lambda infection

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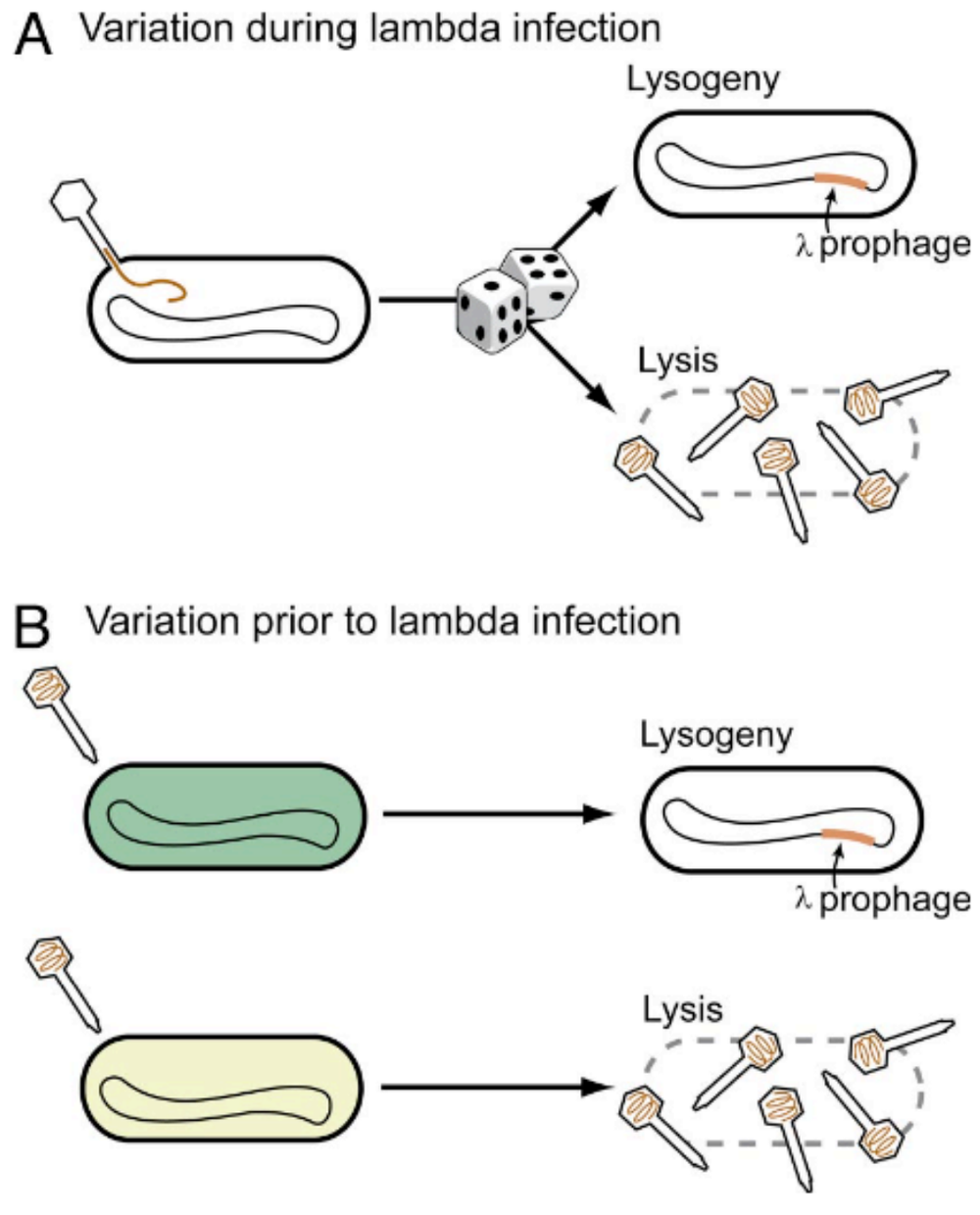
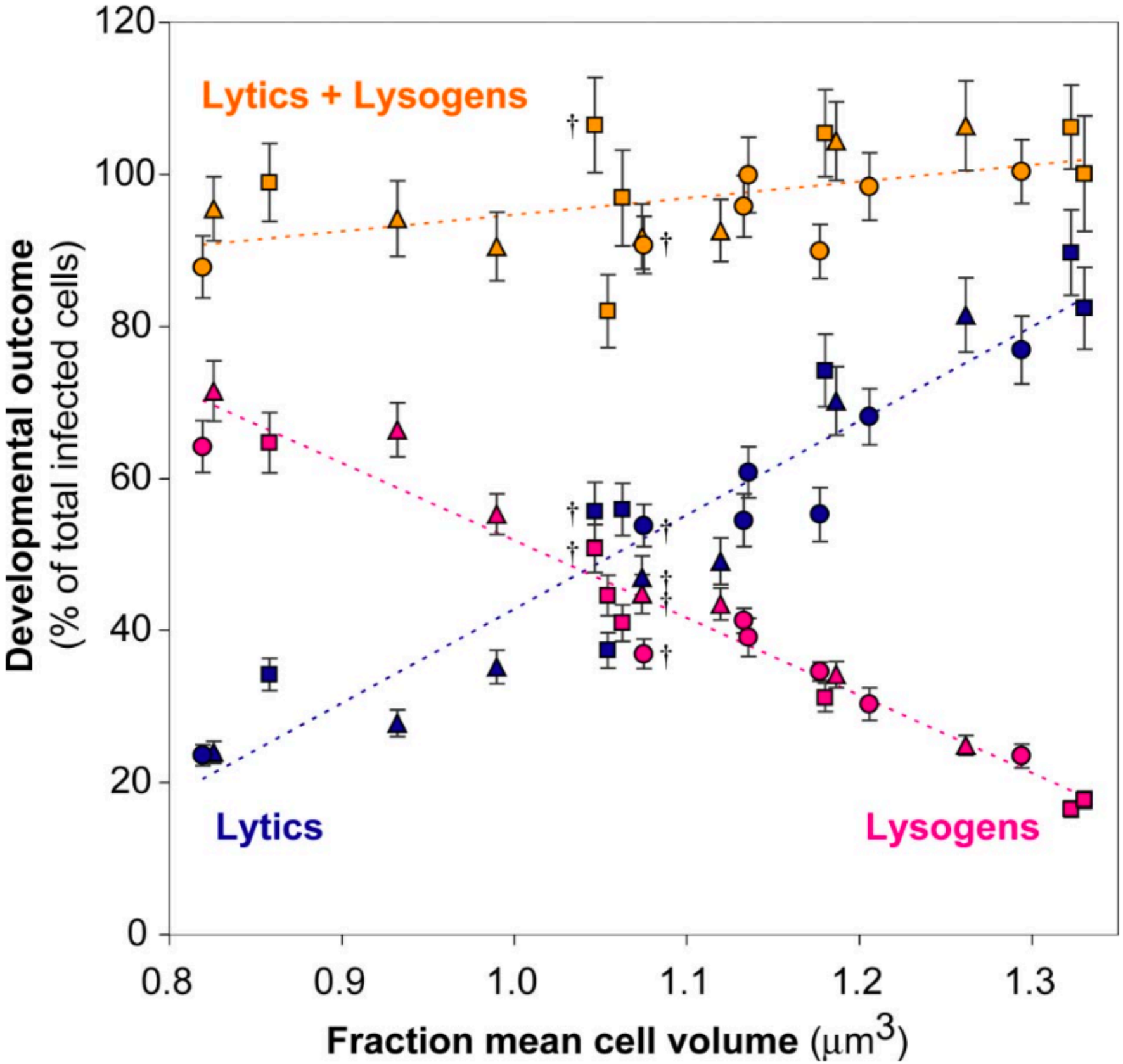
Edited by Mark Ptashne, Memorial Sloan–Kettering Cancer Center, New York, NY, and approved October 8, 2008 (received for review September 5, 2008)

Bacteriophage lambda infection of *Escherichia coli* can result in distinct cell fate outcomes. For example, some cells lyse whereas others survive as lysogens. A quantitative biophysical model of lambda infection supports the hypothesis that spontaneous differences in the timing of individual molecular events during lambda infection leads to variation in the selection of cell fates. Building from this analysis, the lambda lysis–lysogeny decision now serves as a paradigm for how intrinsic molecular noise can influence cellular behavior, drive developmental processes, and produce population heterogeneity. Here, we report experimental evidence that warrants reconsidering this framework. By using cell fractioning, plating, and single-cell fluorescent microscopy, we find that physical differences among cells present before infection bias lambda developmental outcomes. Specifically, variation in cell volume at the time of infection can be used to help predict cell fate: a ≈2-fold increase in cell volume results in a 4- to 5-fold decrease in the probability of lysogeny. Other cell fate decisions now thought to be stochastic might also be determined by pre-existing variation.

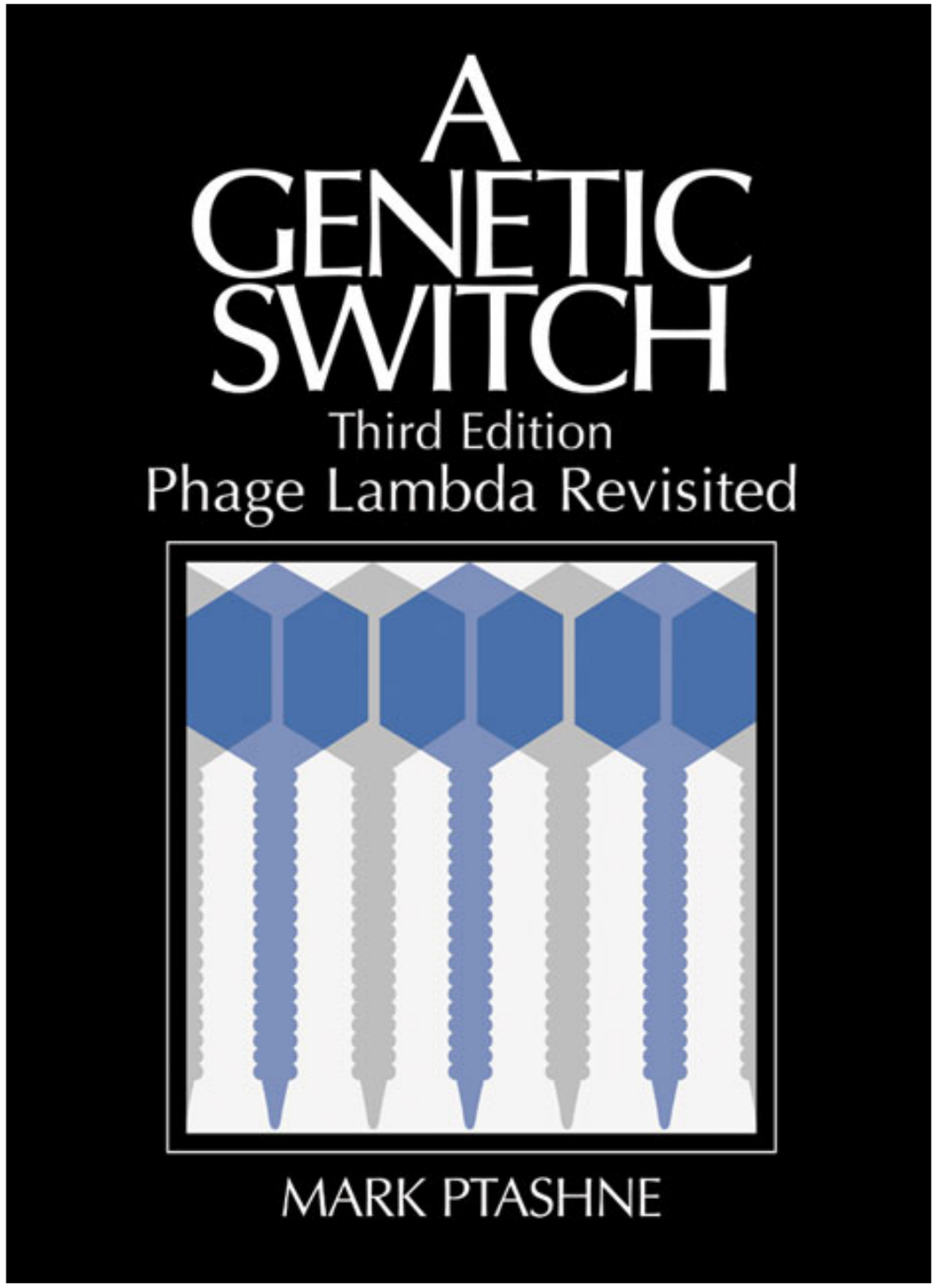


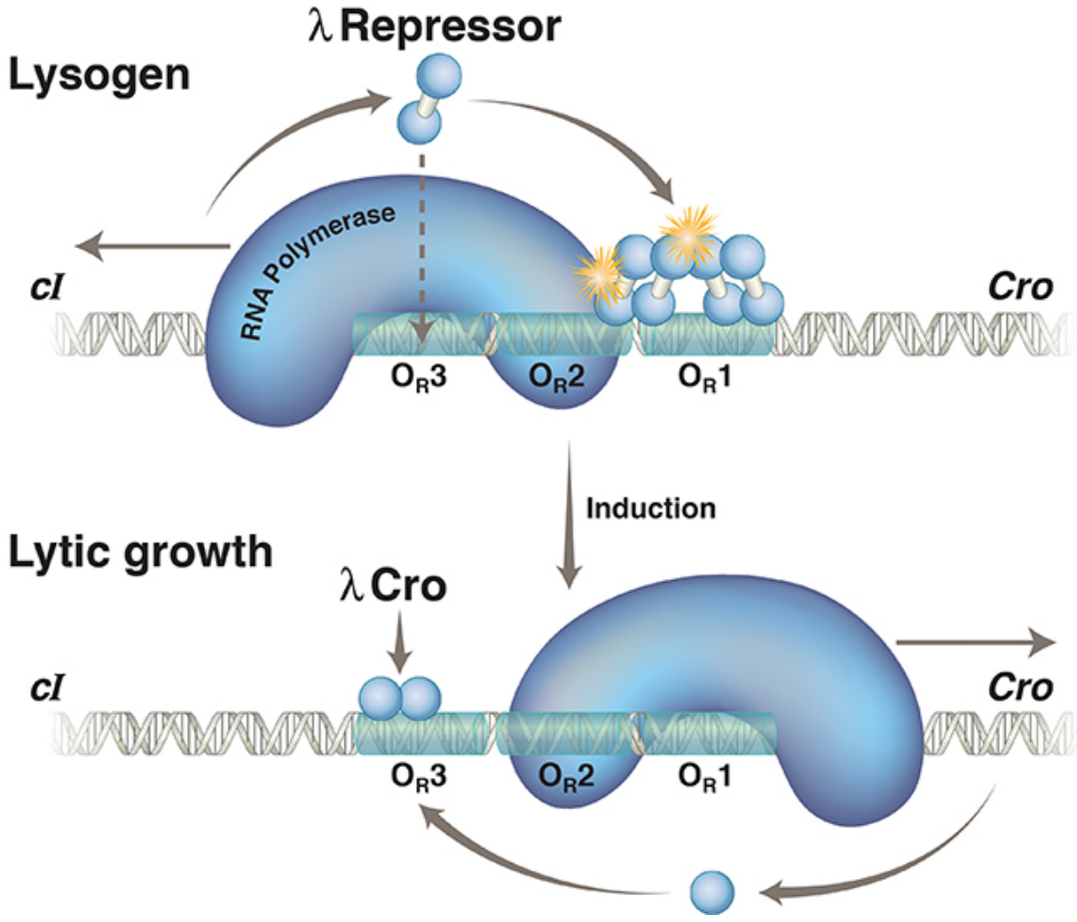
Main claim

Main Evidence



Is this switch digital? Life = 1; death = 0?





Cold Spring Harbor Laboratory Press; 3rd edition (April 8, 2004)

Stochastic Kinetic Analysis of Developmental Pathway Bifurcation in Phage λ-Infected *Escherichia coli* Cells

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Manuscript received March 5, 1998
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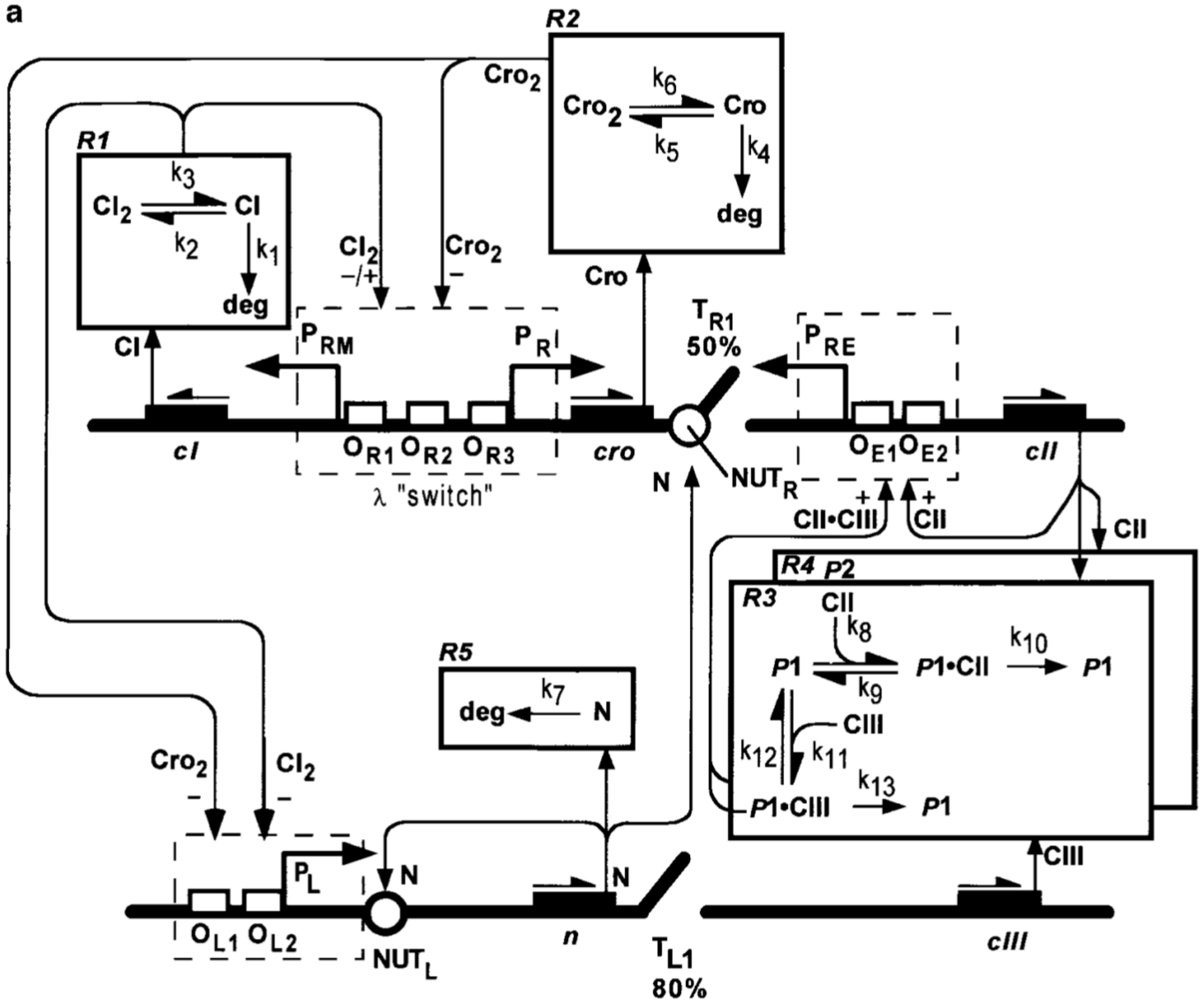
ABSTRACT

Fluctuations in rates of gene expression can produce highly erratic time patterns of protein production in individual cells and wide diversity in instantaneous protein concentrations across cell populations. When two independently produced regulatory proteins acting at low cellular concentrations competitively control a switch point in a pathway, stochastic variations in their concentrations can produce probabilistic pathway selection, so that an initially homogeneous cell population partitions into distinct phenotypic subpopulations. Many pathogenic organisms, for example, use this mechanism to randomly switch surface features to evade host responses. This coupling between molecular-level fluctuations and macroscopic phenotype selection is analyzed using the phage λ lysis-lysogeny decision circuit as a model system. The fraction of infected cells selecting the lysogenic pathway at different phage:cell ratios, predicted using a molecular-level stochastic kinetic model of the genetic regulatory circuit, is consistent with experimental observations. The kinetic model of the decision circuit uses the stochastic formulation of chemical kinetics, stochastic mechanisms of gene expression, and a statistical-thermodynamic model of promoter regulation. Conventional deterministic kinetics cannot be used to predict statistics of regulatory systems that produce probabilistic outcomes. Rather, a stochastic kinetic analysis must be used to predict statistics of regulatory outcomes for such stochastically regulated systems.

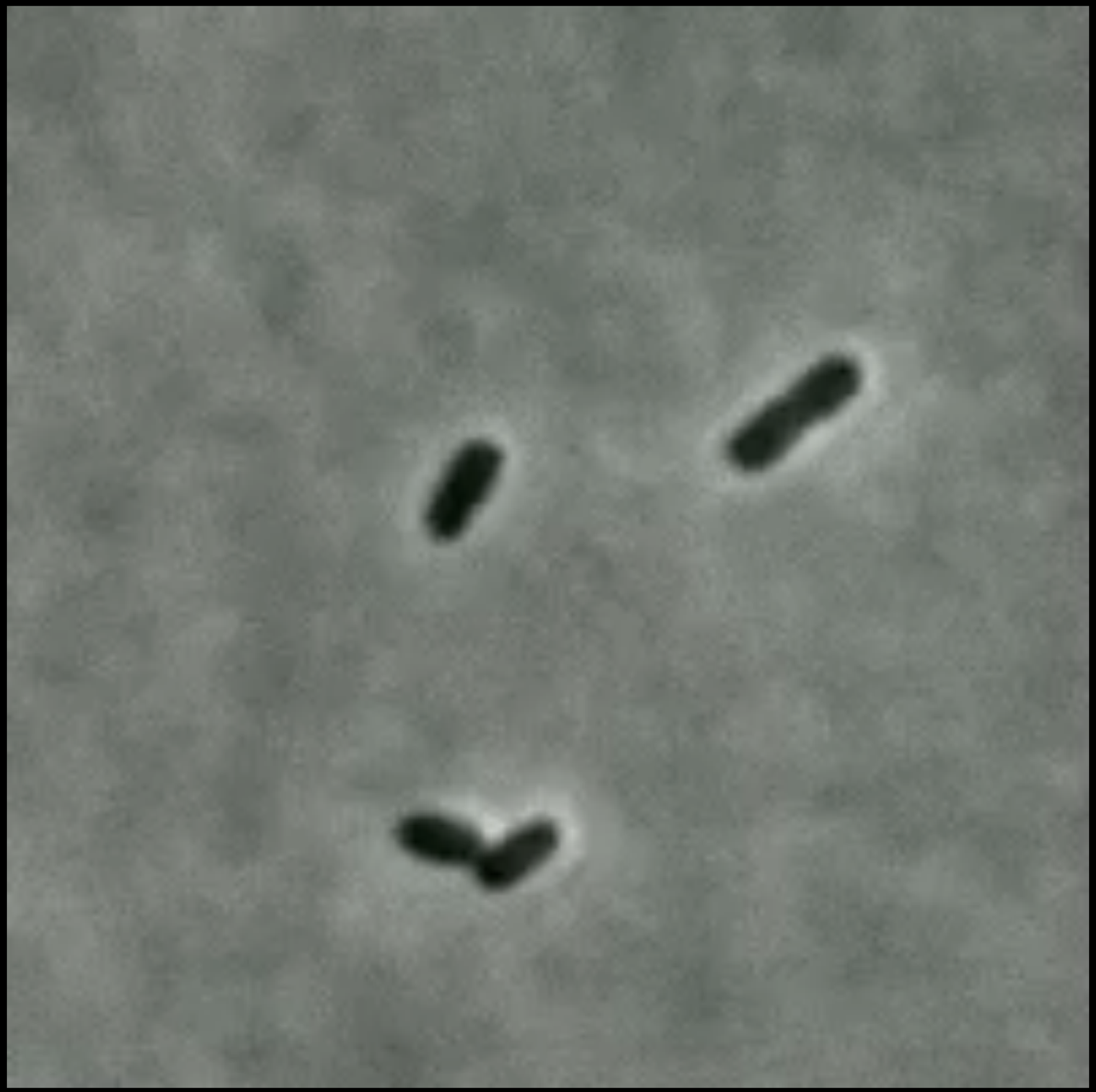
Arkin et al., Genetics. 1998 Aug;149(4):1633-48.



Arkin et al., Genetics. 1998 Aug;149(4):1633-48.

Is life (1) or death (0) random?

(answering this question, in general, matters greatly for bioengineers. why?)



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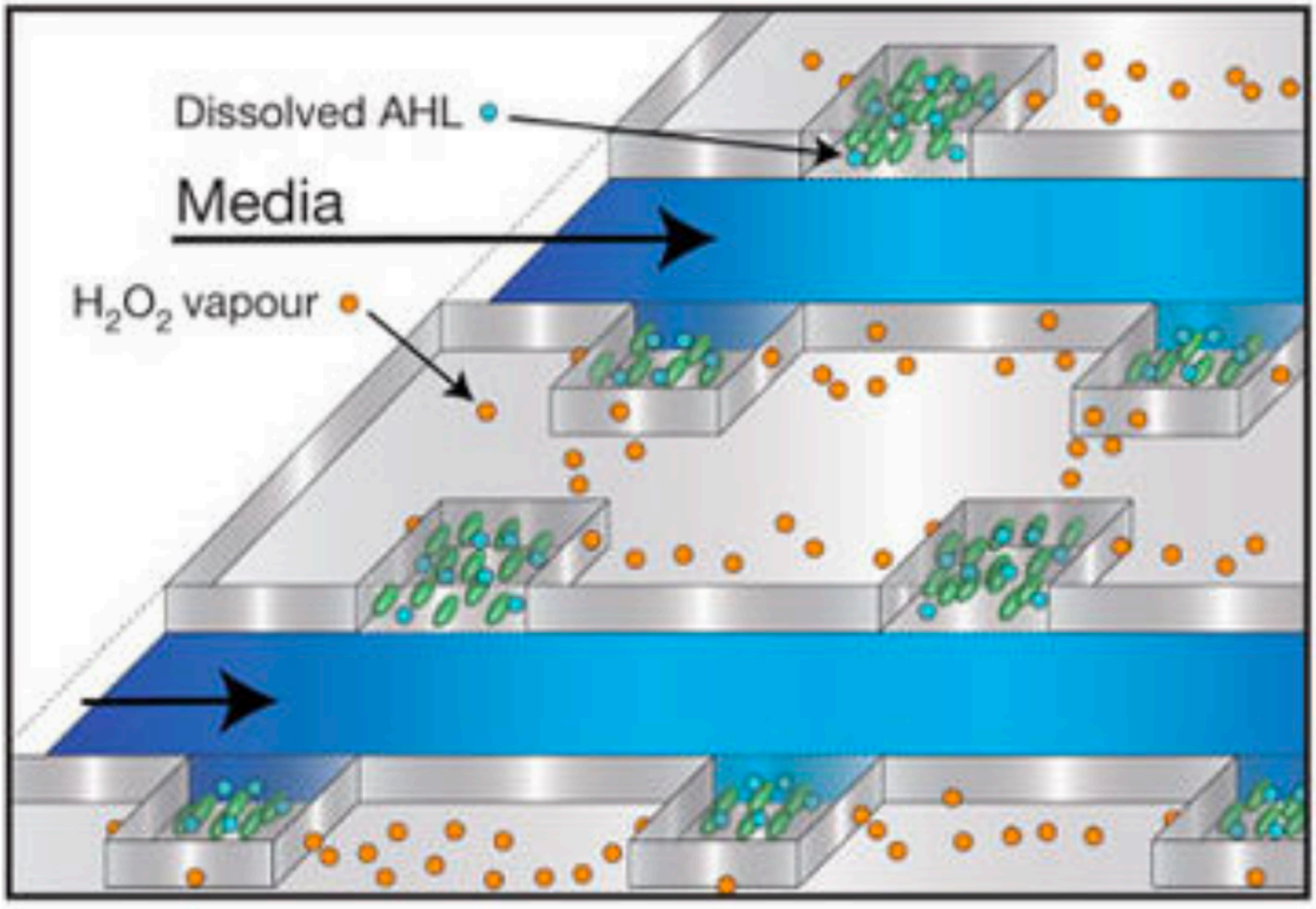
0 min

http://biodynamics.ucsd.edu/



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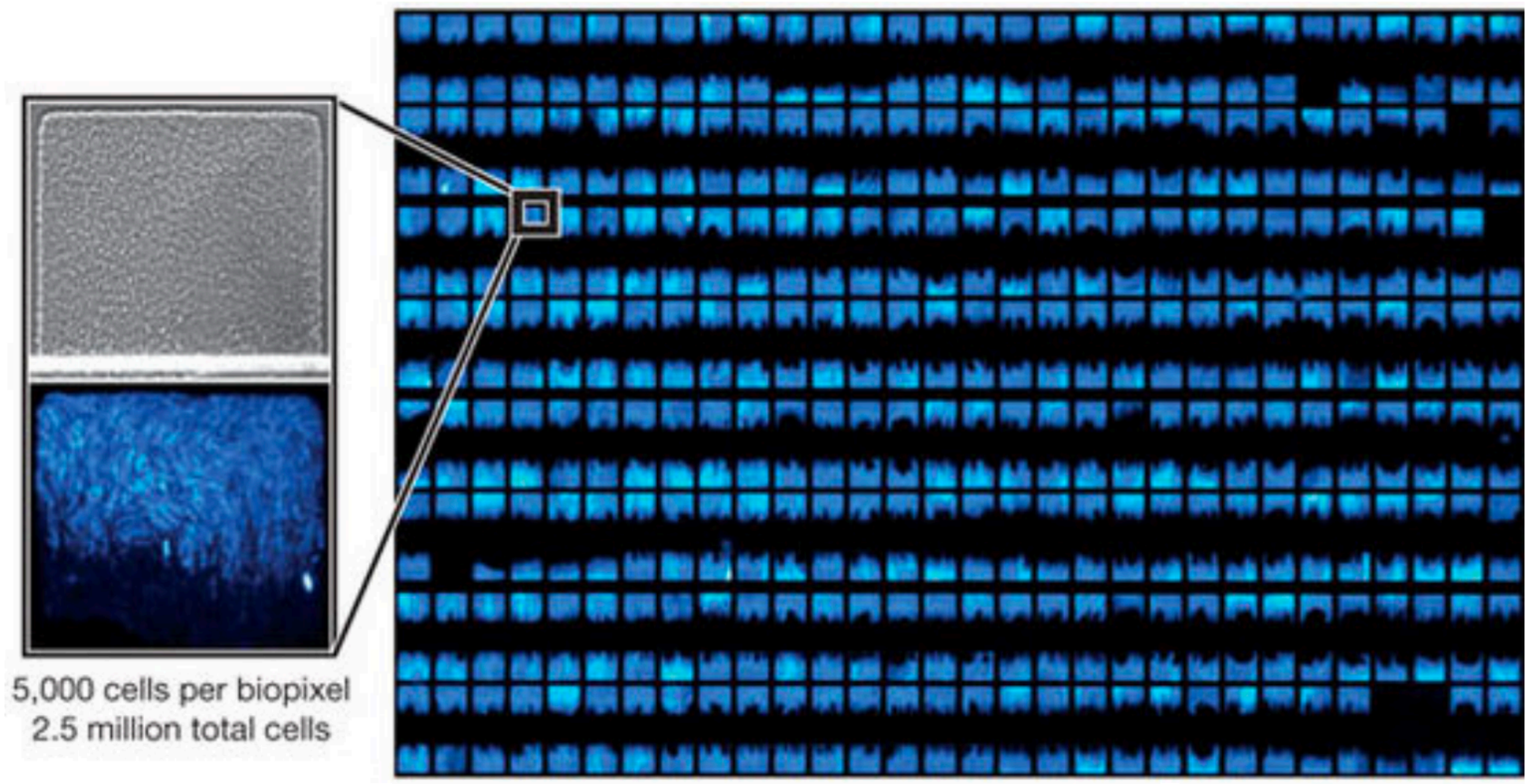

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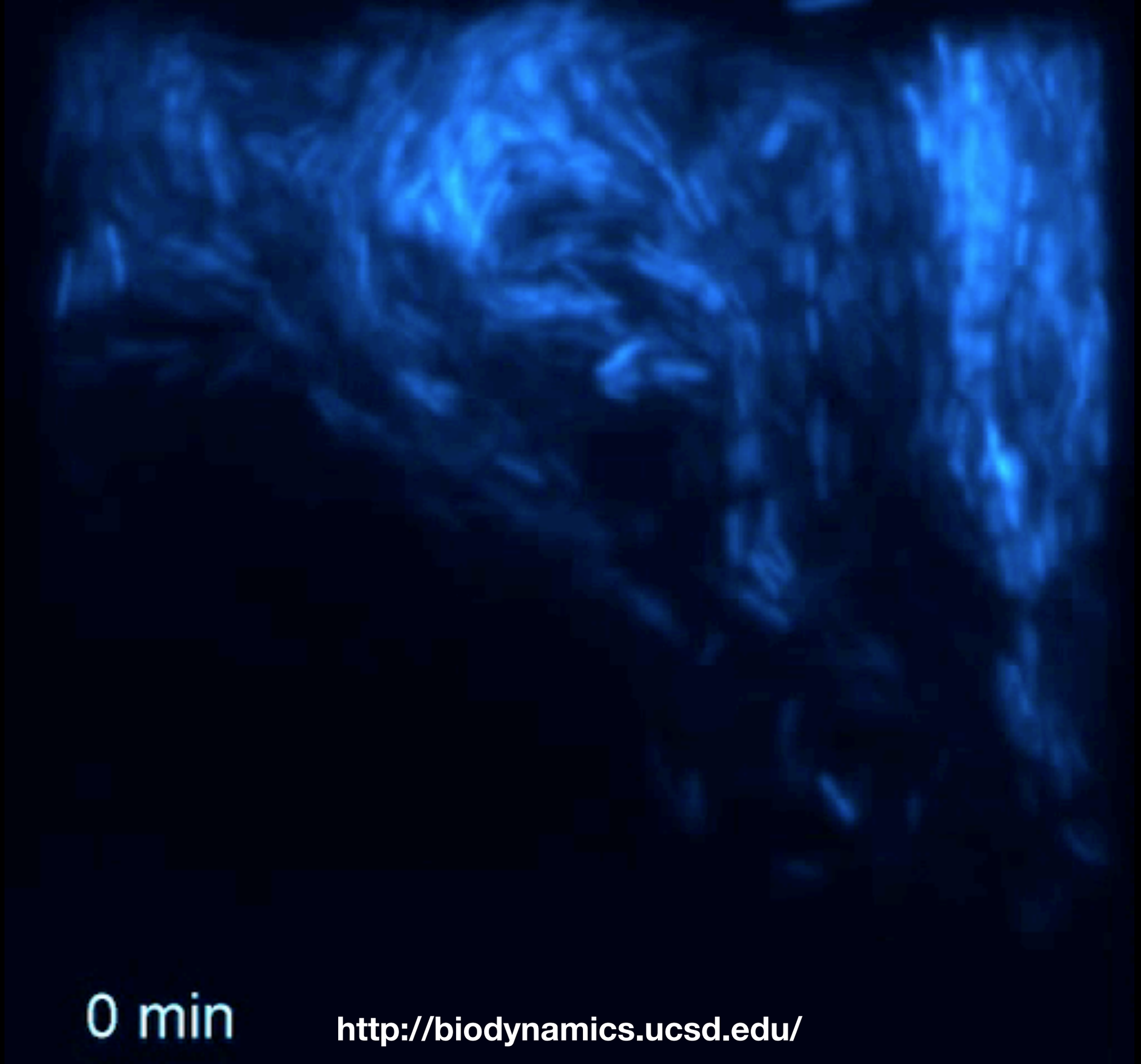
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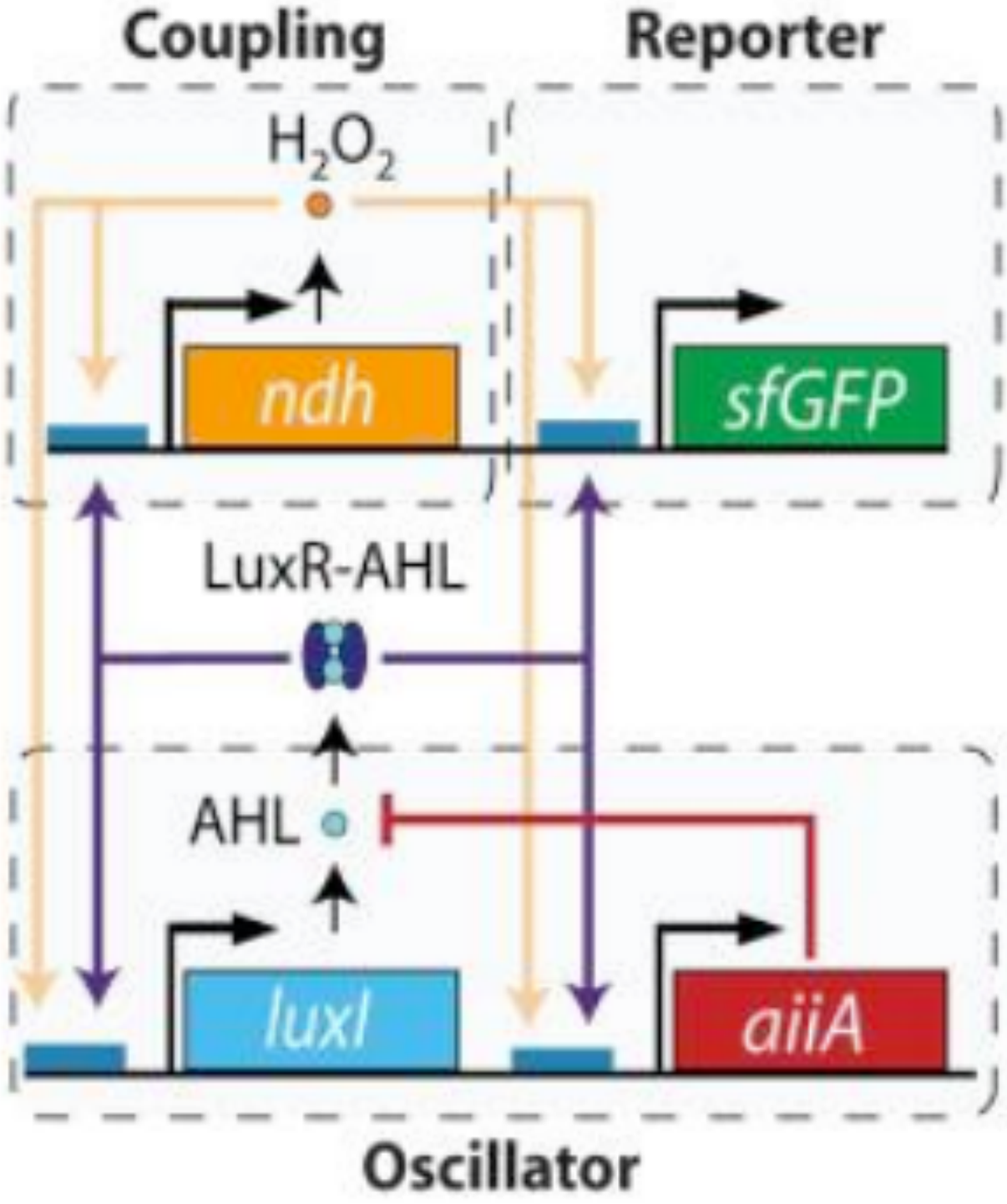
How can we manage how we think about systems like this? Abstraction! (Week 4)



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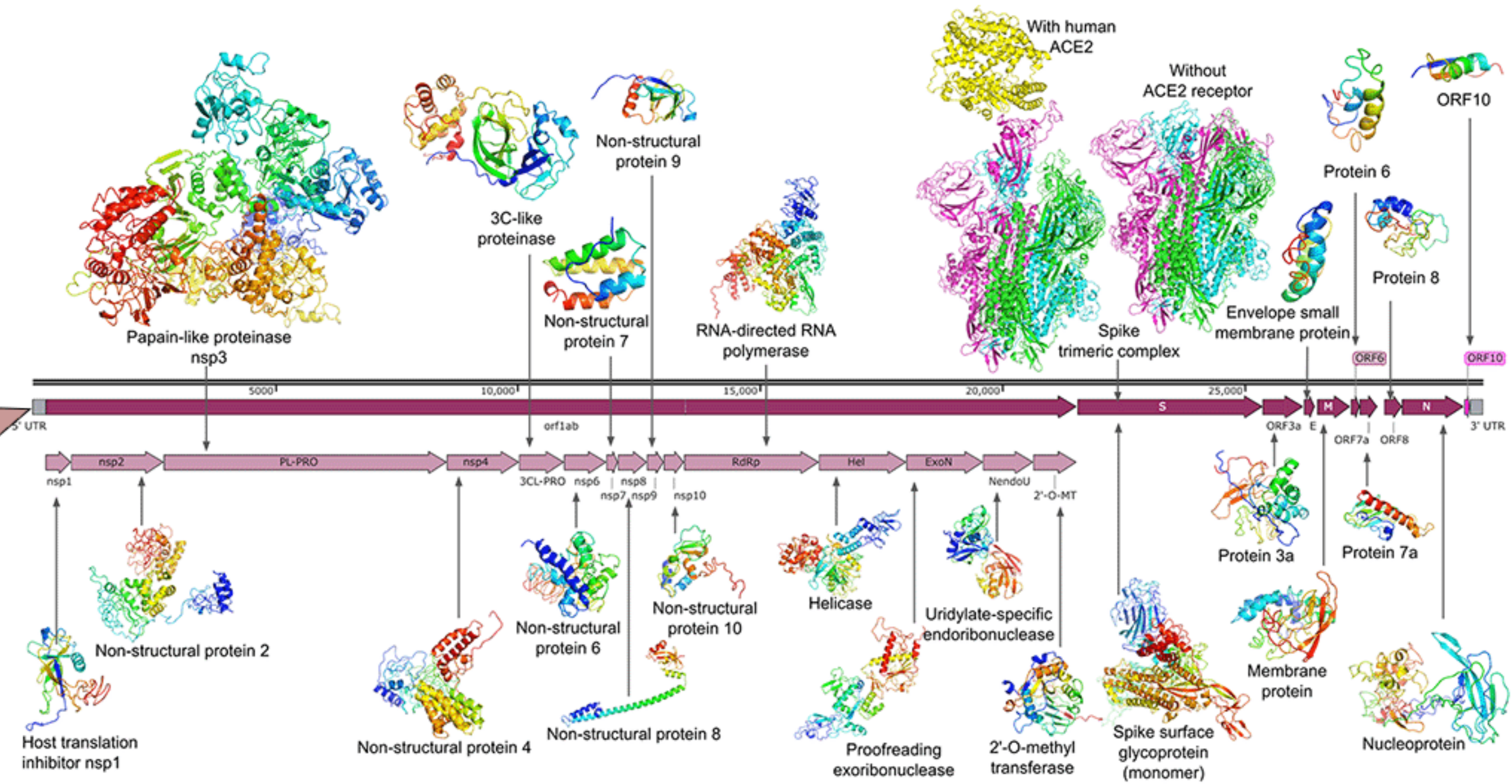
How to use tools to analyze natural systems? Bioinformatics! (Week 5)



https://zhanglab.ccmb.med.umich.edu/COVID-19/