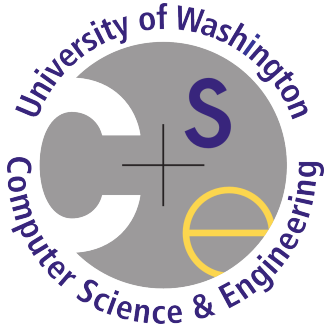


Module 12: Synthetic gene circuits and noise

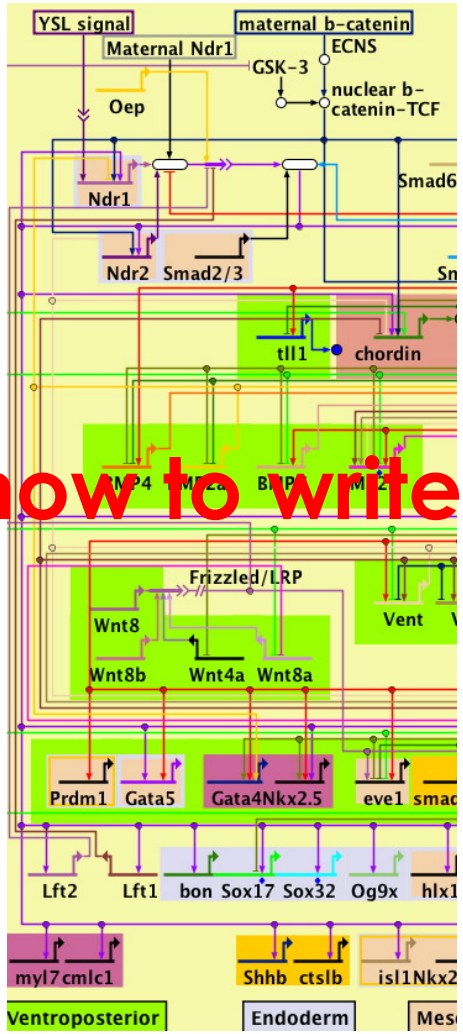
CSE590: Molecular programming and neural computation.

Biological inspiration

DNA Genome

...GTGGTACAGGTG
AATTTGGGTAGGCTA
AATTGTCCATAGTTT
ATGTGTGTGAATGAG
GGTGTATGGATGTTT
CTCAGAGATGGGTTG
CAGCTGGAAGGGCGT
CC[illegible]
TATGCTGGAGAAGTT
GCCGGTTCATTCTGC
TGTGGCGACCCCAGA
TTAATAAAAGGACTA
AGCCGAAAAGAAAAT
GAAACATATATATAT
ATATATATATATATA
TATATATATA...

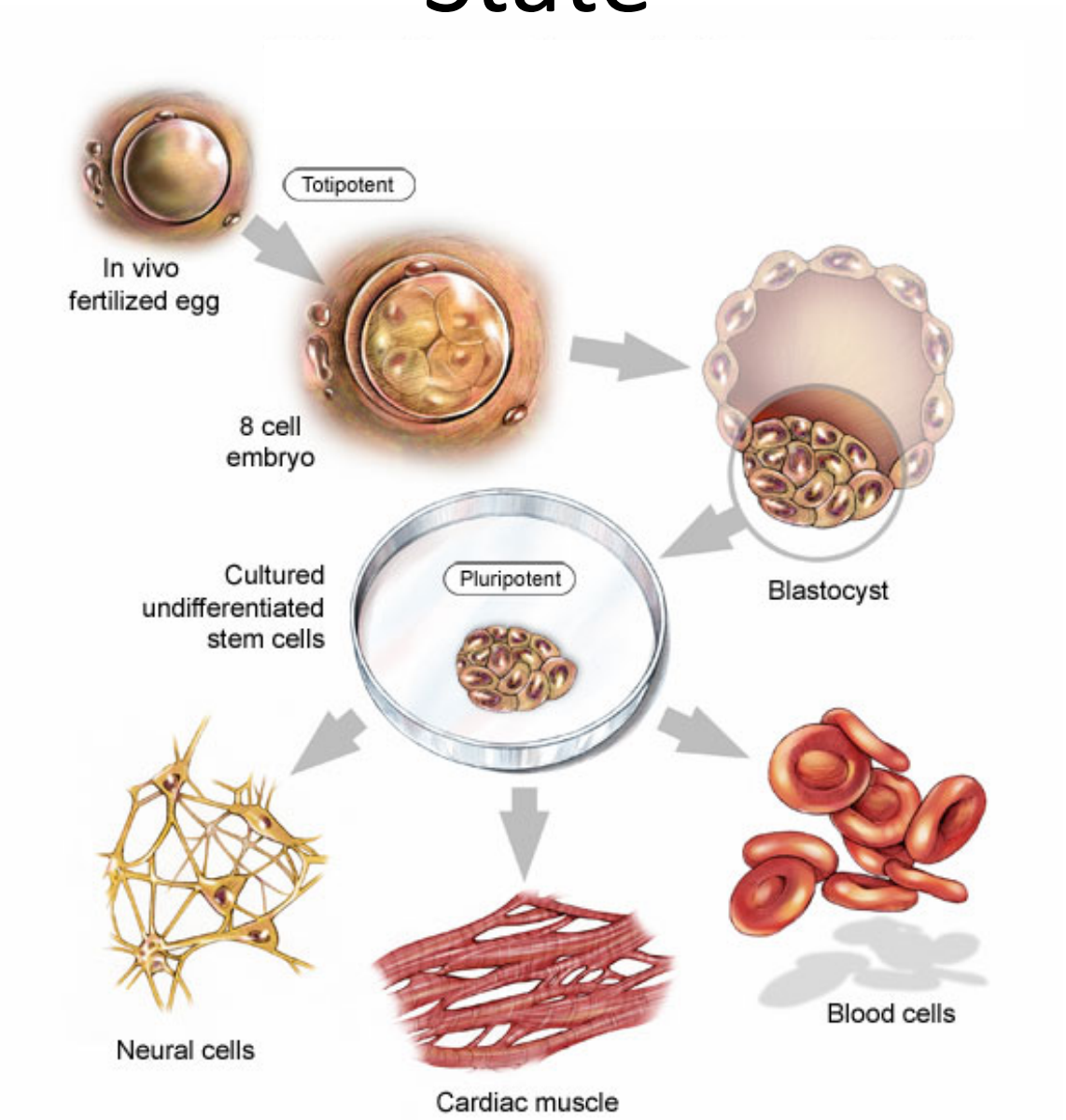
Regulatory Circuitry



Zebrafish Development

Can we learn how to write such a program?

State

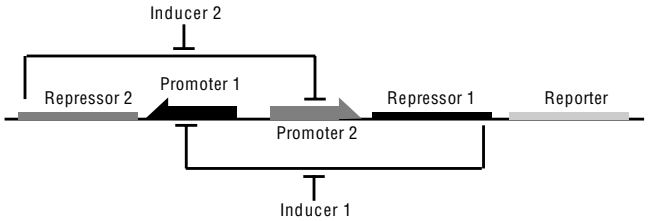

Totipotent
In vivo
fertilized egg
8 cell
embryo
Blastocyst
Cultured
undifferentiated
stem cells
Pluripotent
Neural cells
Cardiac muscle
Blood cells

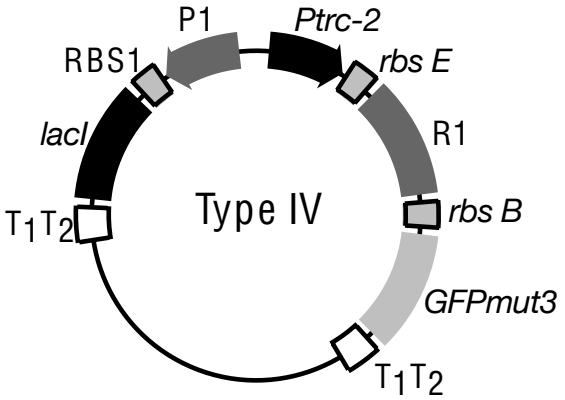
A bistable switch

Construction of a genetic toggle switch in *Escherichia coli*

Timothy S. Gardner*†, Charles R. Cantor* & James J. Collins*†

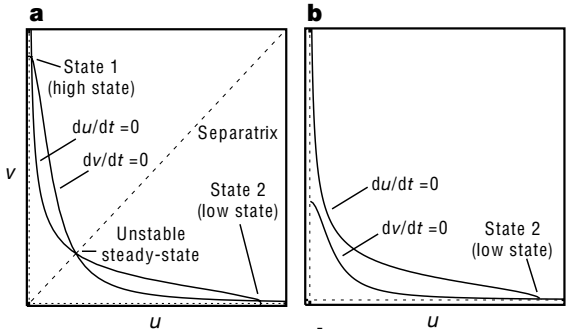
** Department of Biomedical Engineering, † Center for BioDynamics and ‡ Center for Advanced Biotechnology, Boston University, 44 Cummington Street, Boston, Massachusetts 02215, USA*





$$\frac{du}{dt} = \frac{\alpha_1}{1 + v^{\beta}} - u$$

$$\frac{dv}{dt} = \frac{\alpha_2}{1 + u^{\gamma}} - v$$



Nature 403, 339-342 (20 January 2000) | doi:10.1038/35002131; Received 15 September 1999; Accepted 23 November 1999

A bistable switch

Construction of a genetic toggle switch in *Escherichia coli*

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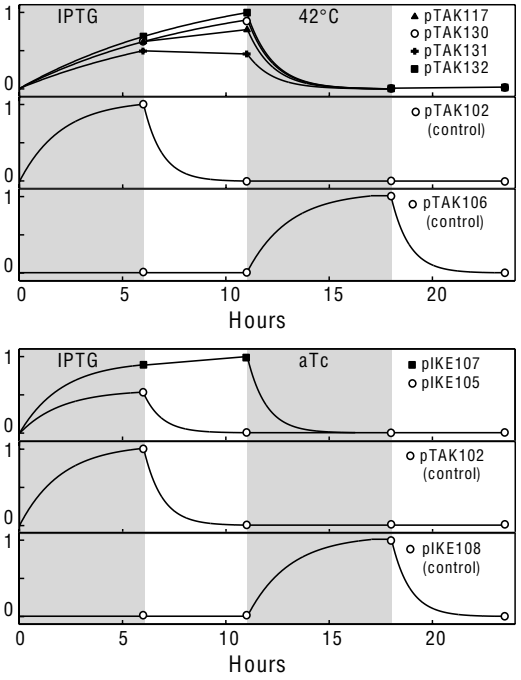
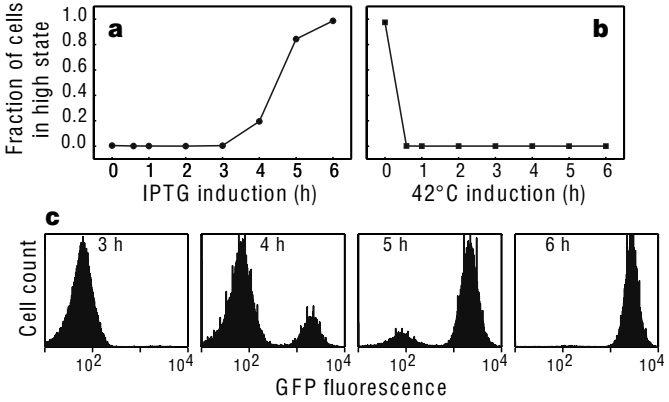


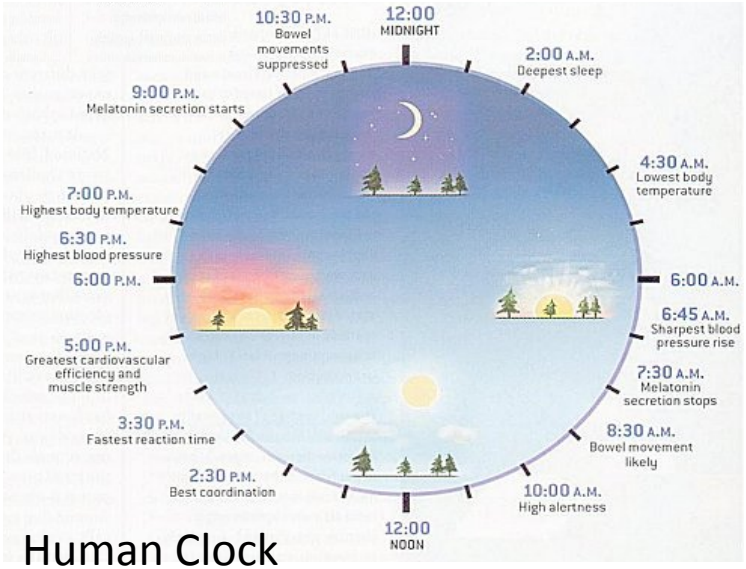


Problems:

- Growth rate hit
- Leak
- Balance

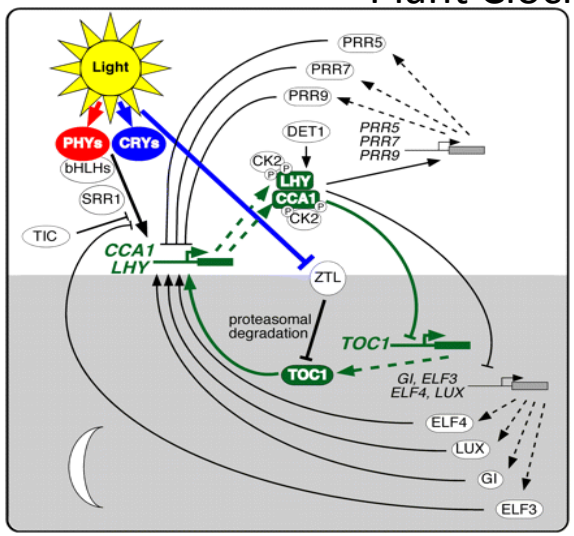
Nature 403, 339-342 (20 January 2000) | doi:10.1038/35002131; Received 15 September 1999; Accepted 23 November 1999

Rythm

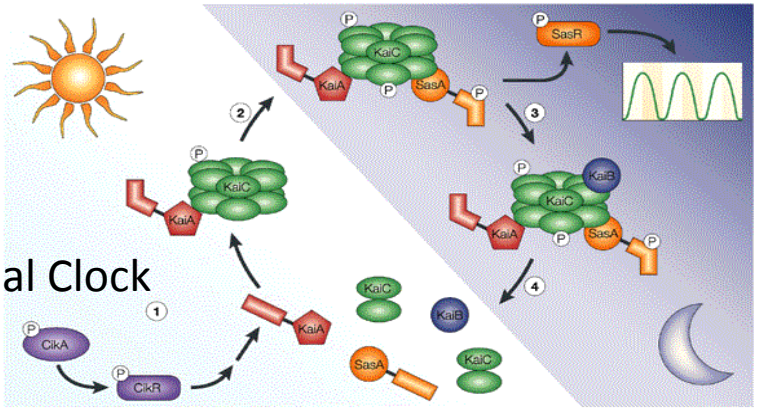


Human Clock

Plant Clock



Cyanobacterial Clock

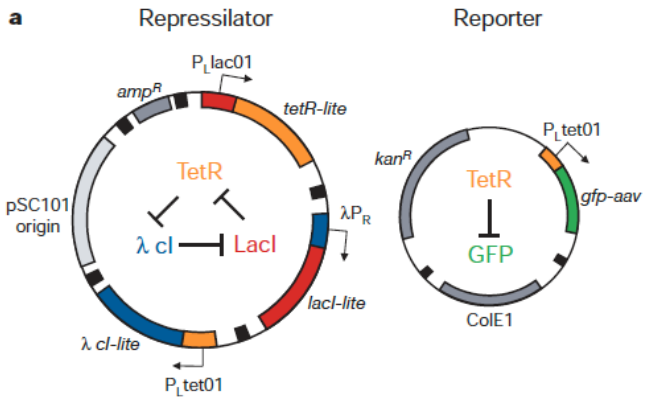


The repressilator

A synthetic oscillatory network of transcriptional regulators

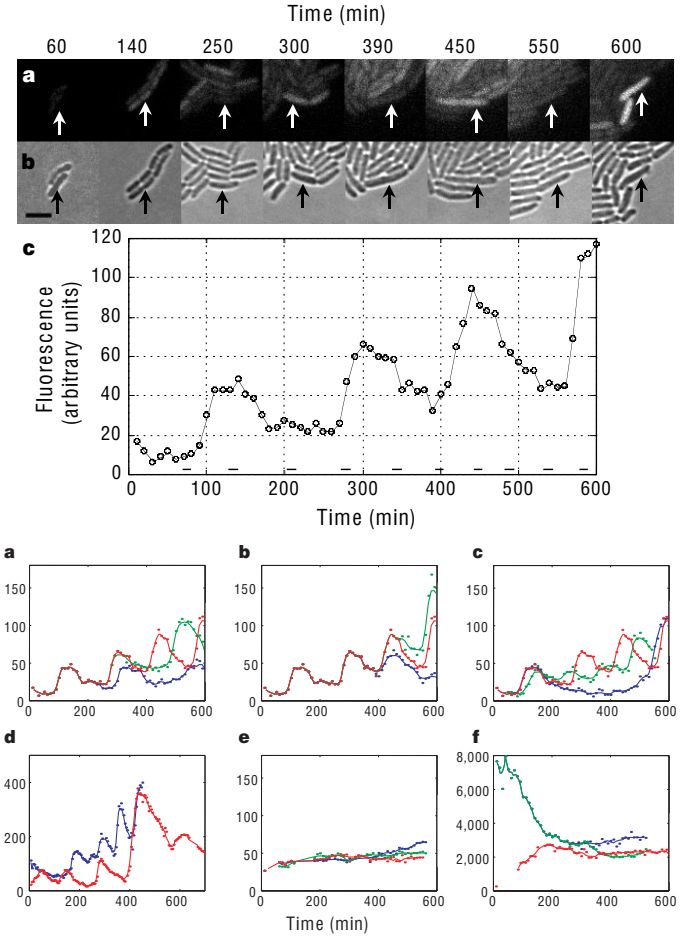
Michael B. Elowitz & Stanislas Leibler

Departments of Molecular Biology and Physics, Princeton University, Princeton, New Jersey 08544, USA



$$\frac{dm_i}{dt} = -m_i + \frac{\alpha}{(1+p_j^n)} + \alpha_0 \qquad \frac{dp_i}{dt} = -\beta(p_i - m_i)$$

$$\begin{pmatrix} i = lacI, tetR, cI \\ j = cI, lacI, tetR \end{pmatrix}$$



Nature 403, 335-338 (20 January 2000) | doi:10.1038/35002125; Received 6 July 1999; Accepted 9 November 1999

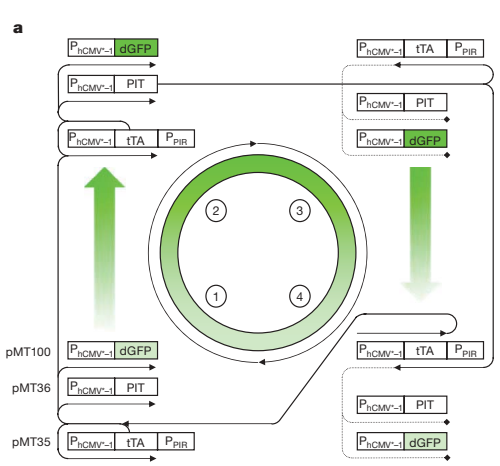
The Repressilator

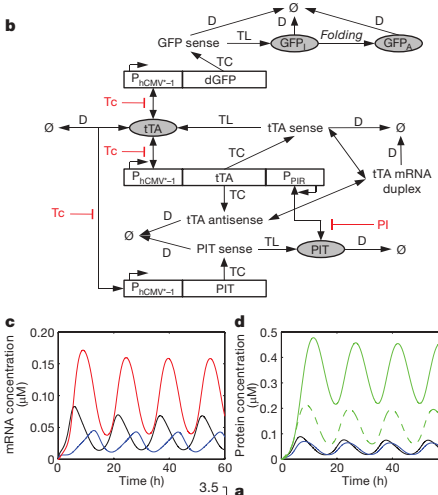
A biological oscillator
(Elowitz, 2001)

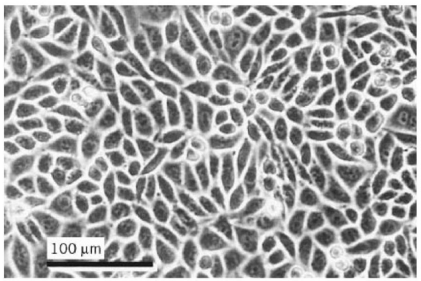
An mammalian synthetic oscillator

A tunable synthetic mammalian oscillator

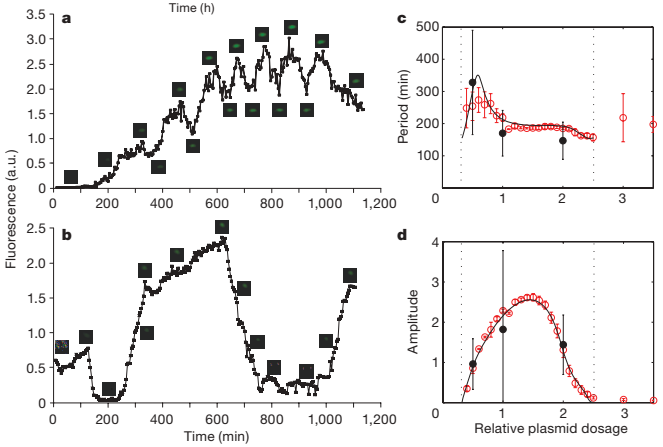
Marcel Tigges[1], Tatiana T. Marquez-Lago[1,2,3], Jörg Stelling[1,2,3] & Martin Fussenegger[1]





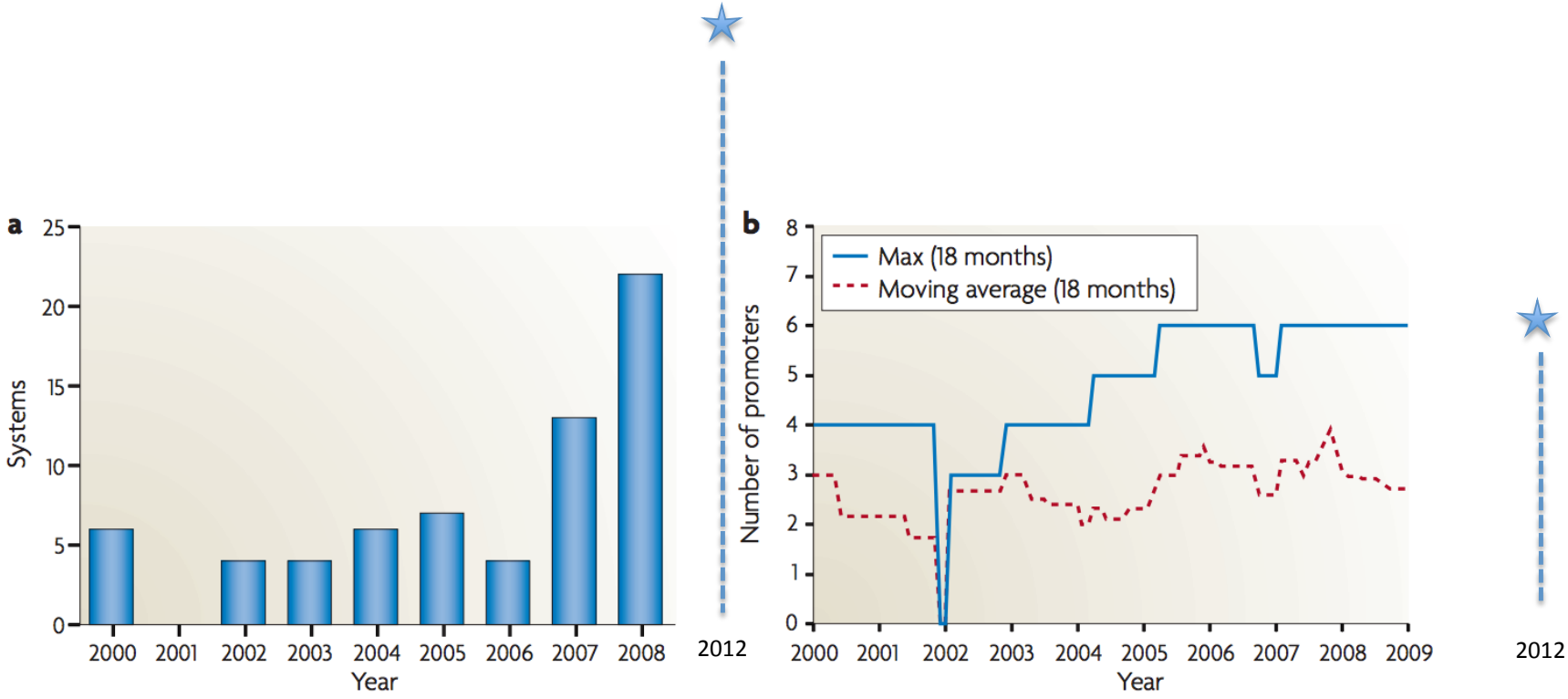


in Chinese Hamster Ovary Cells!



Vol 457|15 January 2009|doi:10.1038/nature07616

The complexity brake?



Priscilla E. M. Purnick & Ron Weiss
Nature Reviews Molecular Cell Biology, 2009

FIVE HARD TRUTHS FOR SYNTHETIC BIOLOGY

Can engineering approaches tame the complexity of living systems? **Roberta Kwok** explores five challenges for the field and how they might be resolved.

Many of the parts are undefined

A biological part can be anything from a DNA sequence that encodes a specific protein to a promoter, a sequence that facilitates the expression of a gene. The problem is

The circuitry is unpredictable

Even if the function of each part is known, the parts may not work as expected when put together, says Keasling.

The complexity is unwieldy

As circuits get larger, the process of constructing and testing them becomes more daunting. A system developed by Keas-

Many parts are incompatible

Once constructed and placed into cells, synthetic genetic circuits can have unintended effects on their host. Chris

Variability crashes the system

Synthetic biologists must also ensure that circuits function reliably. Molecular activities inside cells are prone to random fluctuations, or noise. Variation in growth con-

"The field has had its hype phase. Now it needs to deliver."
— Martin Fussenegger

http://www.nature.com/nature/focus/syntheticsytemsbiology/index.html

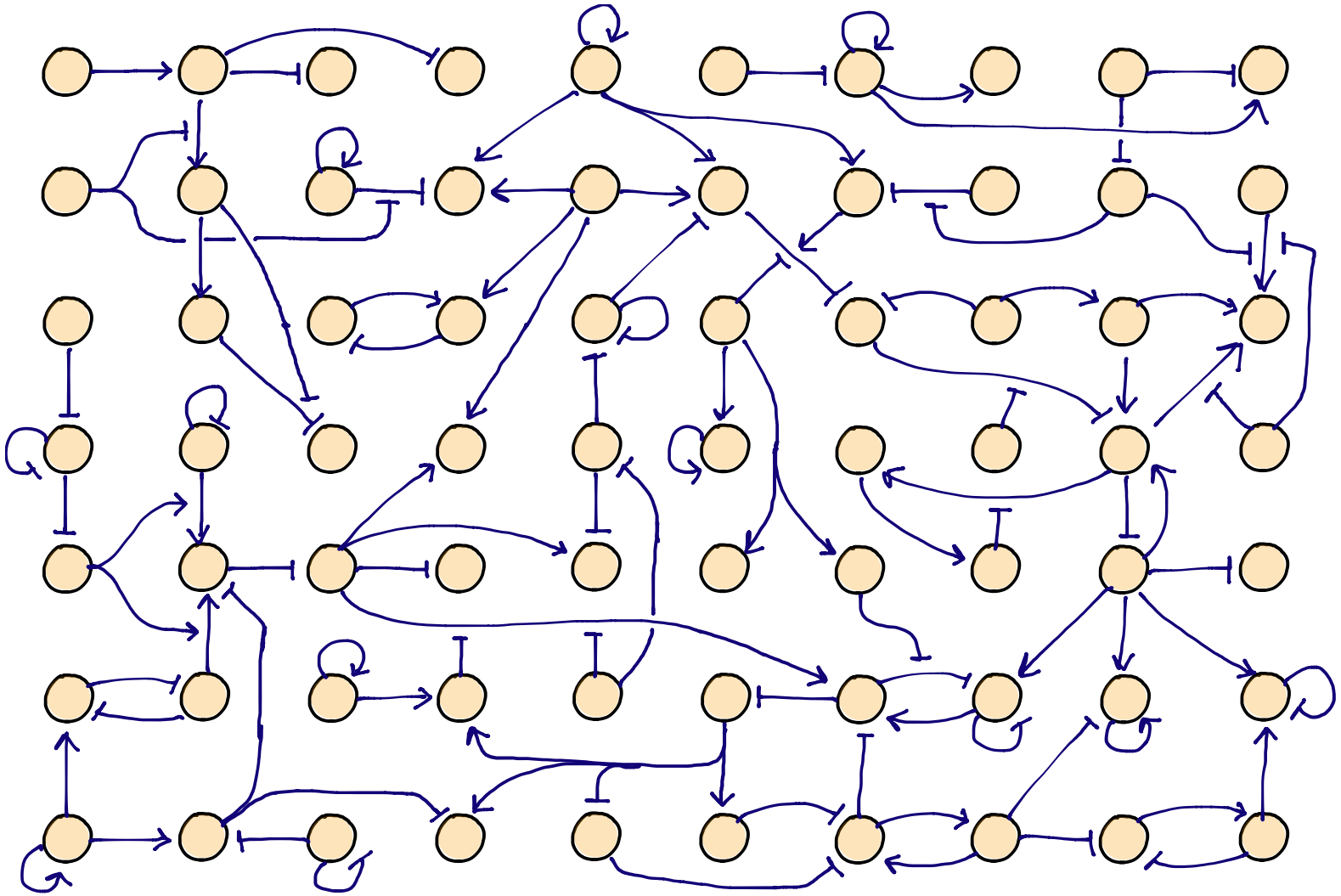
Why is it difficult to engineer synthetic gene circuits?

1. Synthetic gene circuits have to operate in a complex biological environment

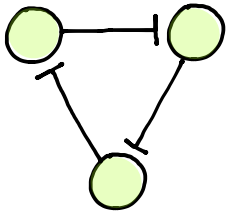
2. Biology is "noisy" (small copy numbers of many molecules,…)

3. Existing parts aren't modular or well characterized

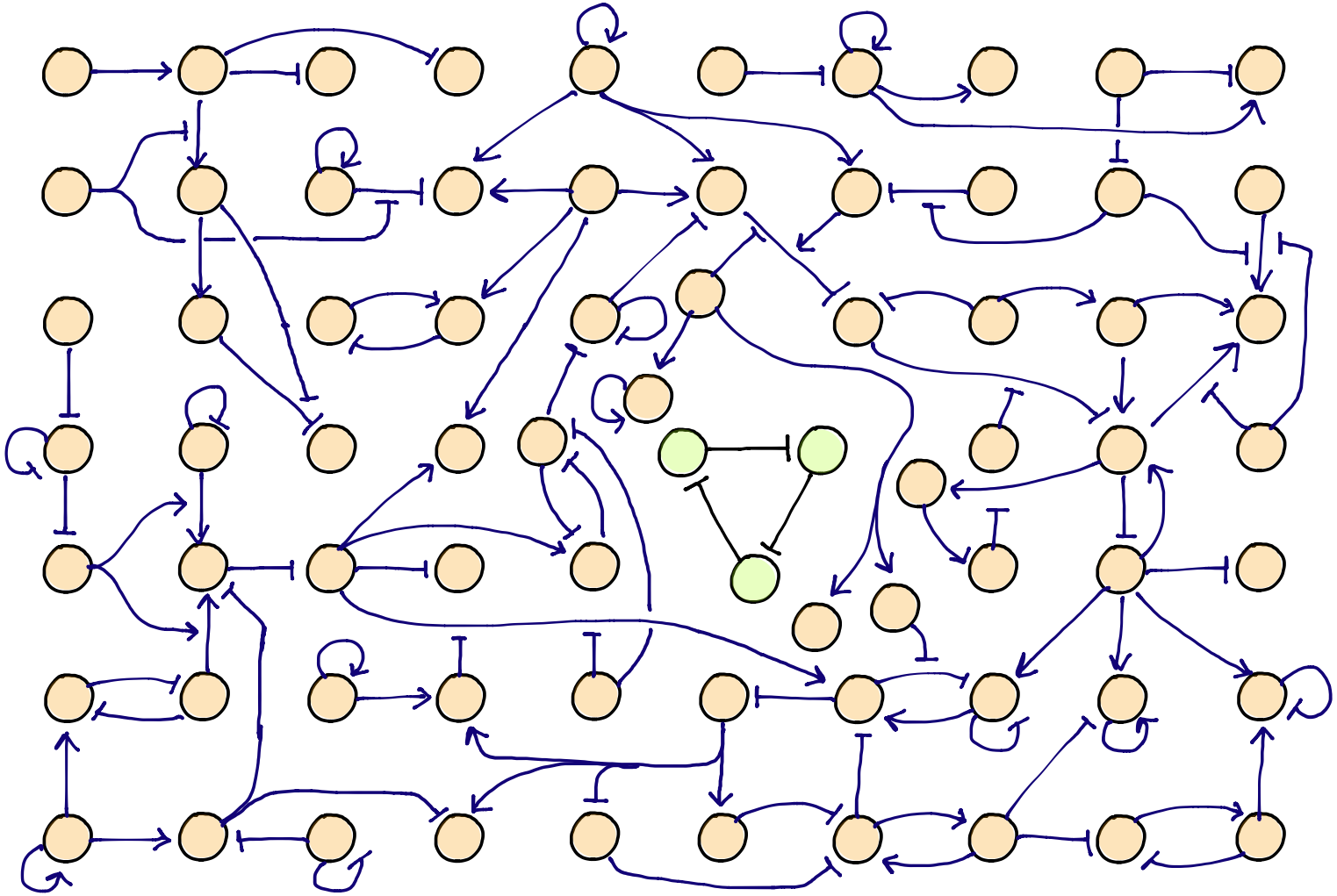
A simple biological network



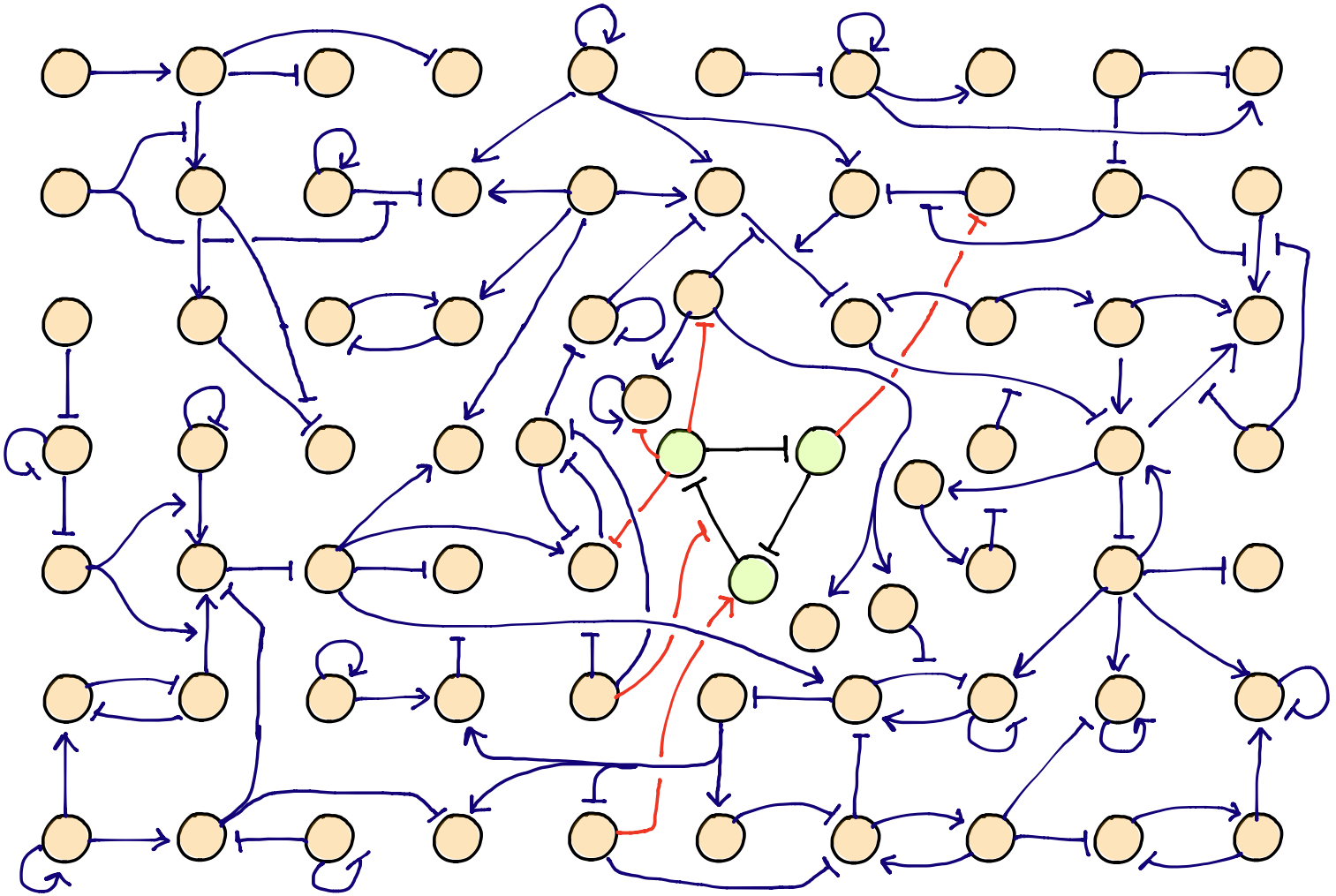
A complex synthetic network



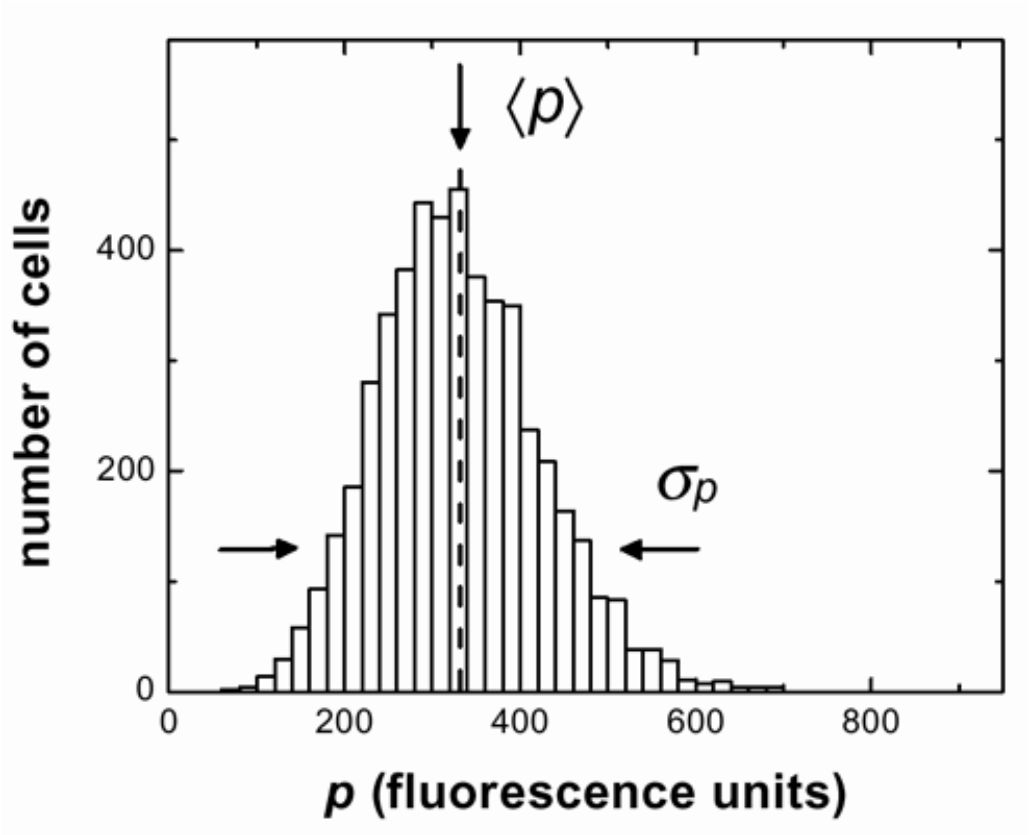
A complex synthetic network



An complex synthetic network



Noise in gene expression

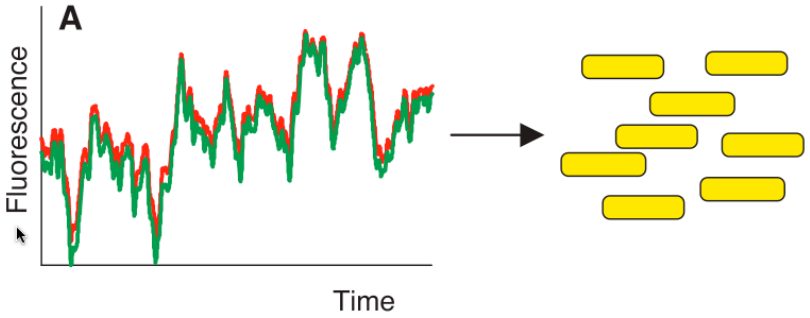


Noise in gene expression

Consider genes for two fluorescent proteins controlled by identical promoters in *E. coli*

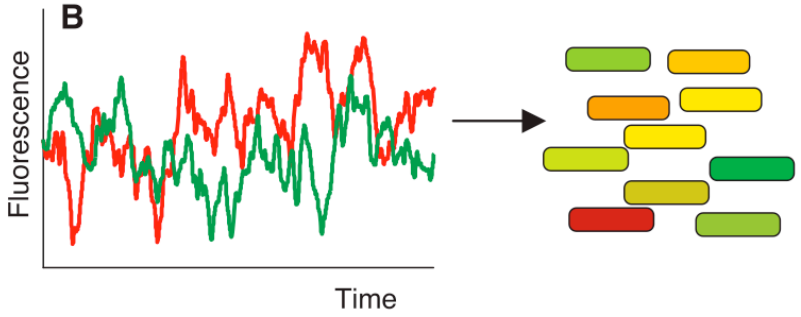
Monitor time-varying fluorescence within a single cell and across cell populations:
cells with the same amount of each fluorescent protein species appear yellow,
cells with differing amounts of the two species appear red or green

Without intrinsic noise, red and green signals are correlated within a single cell



Fluorescence intensities vary between cells due to extrensic noise

With intrinsic noise, the red and green signals become uncorrelated within a single cell

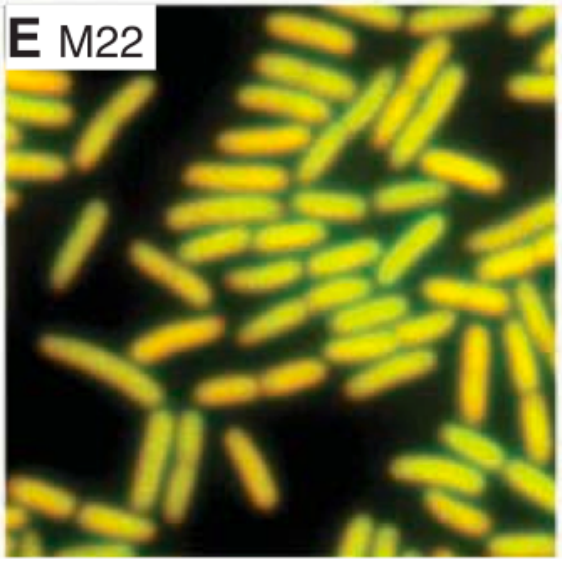


Fluorescence color varies between cells due to intrinsic noise

M.B. Elowitz, A.J. Levine, E.D. Siggia, P.S. Swain, Science, 2002

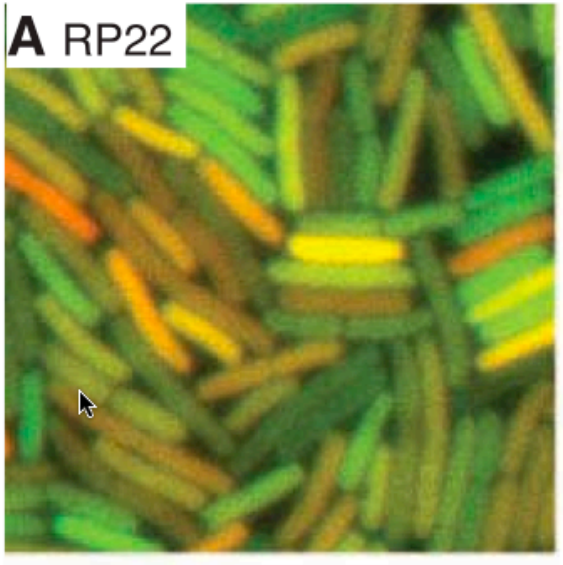
Noise in gene expression

Express reporter genes under lac-repressible promoters in *E. coli* strain (M22) lacking repressor protein lac



Minimal intrinsic noise without lac repression of reporter genes

Express reporter genes under lac-repressible promoters in wild-type *E. coli* strain (RP22) expressing repressor protein lac



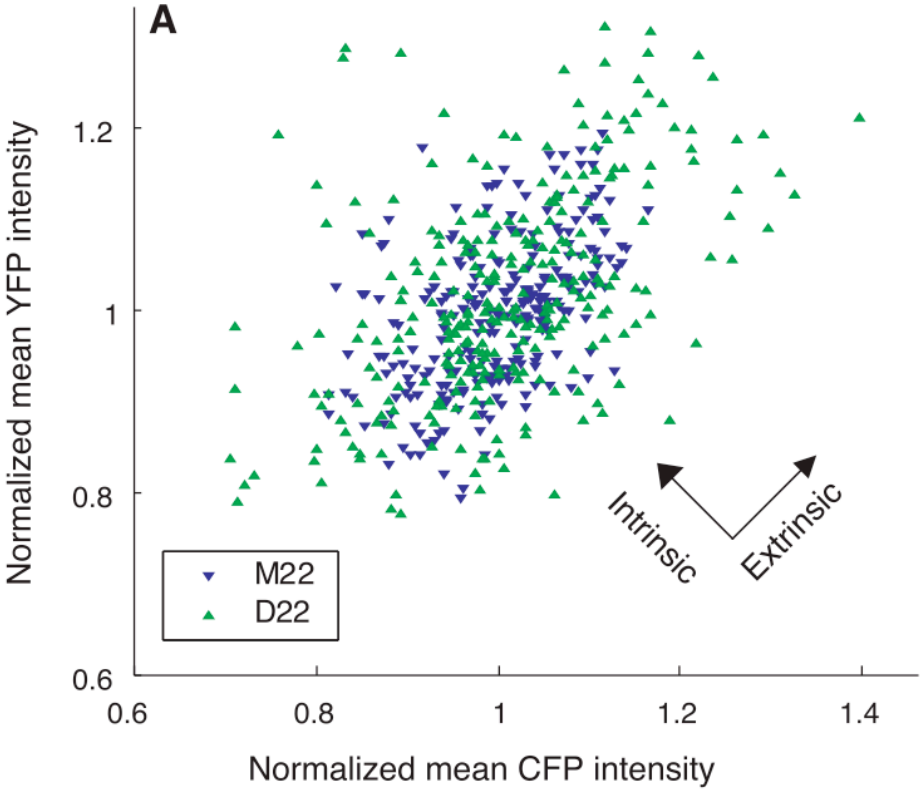
Increased intrinsic and extrensic noise due to lac repression

Extrensic noise increase suggests cell-cell variation in lac expression

M.B. Elowitz, A.J. Levine, E.D. Siggia, P.S. Swain, Science, 2002

Noise in gene expression

Quantification of intrinsic and extrensic noise for populations of two strains of *E. coli*



Each point represents mean red and green fluorescence in a single cell

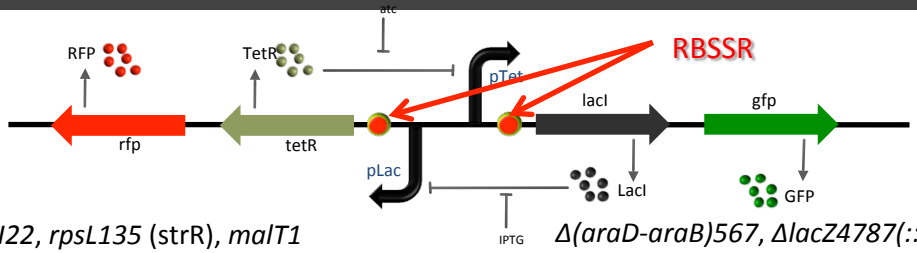
Strain M22 is less noisy
Strain D22 is more noisy

M.B. Elowitz, A.J. Levine, E.D. Siggia, P.S. Swain, Science, 2002

What can we do to make better gene circuits?

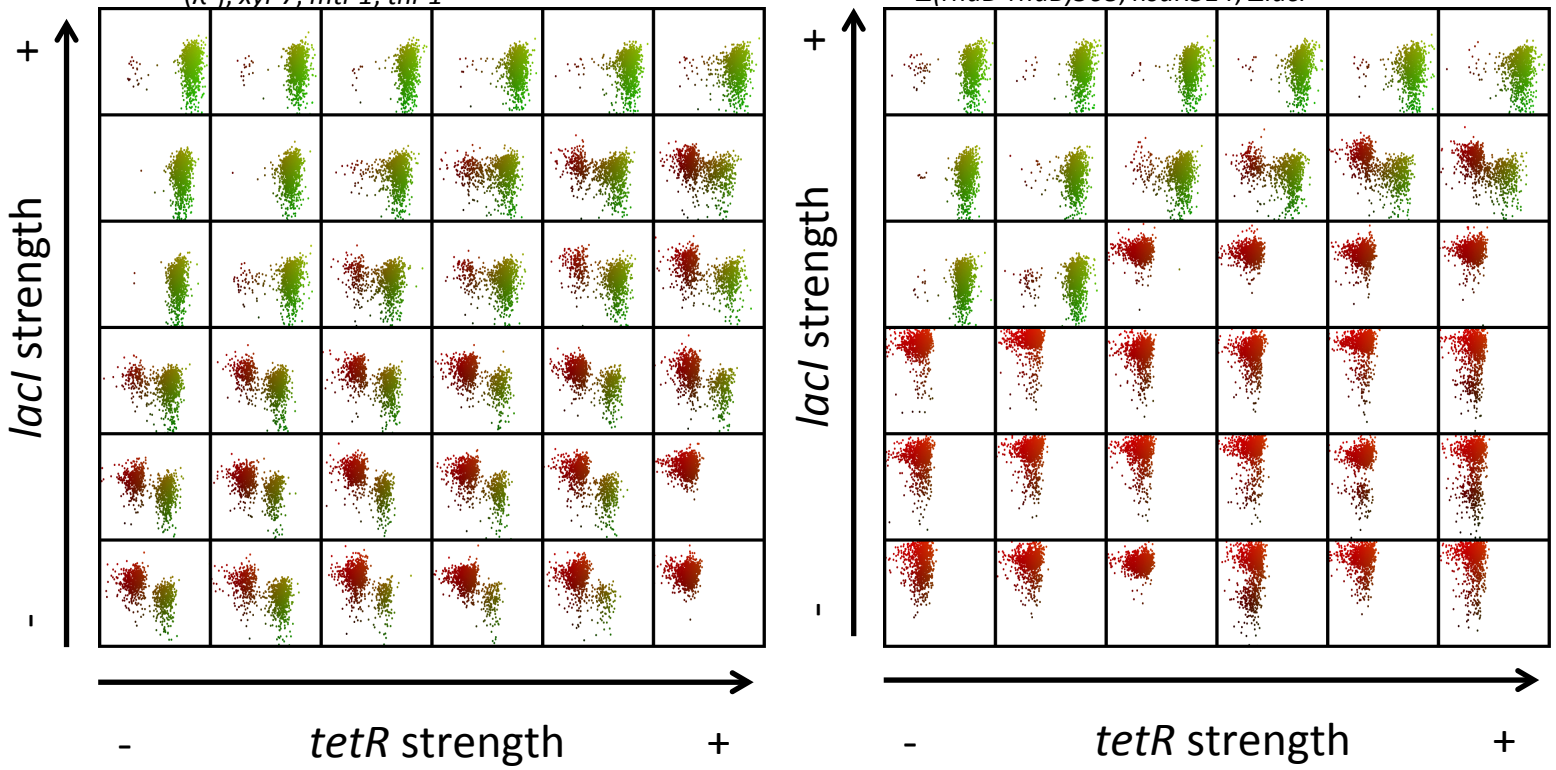
1. Create new parts and characterize them better
2. Design network architectures that are more robust to perturbations
3. …

An improved and tunable bistable switch



lacZ13(Oc), *lacI22*, *rpsL135* (strR), *malT1* (λ^R), *xyl-7*, *mtl-1*, *thi-1*

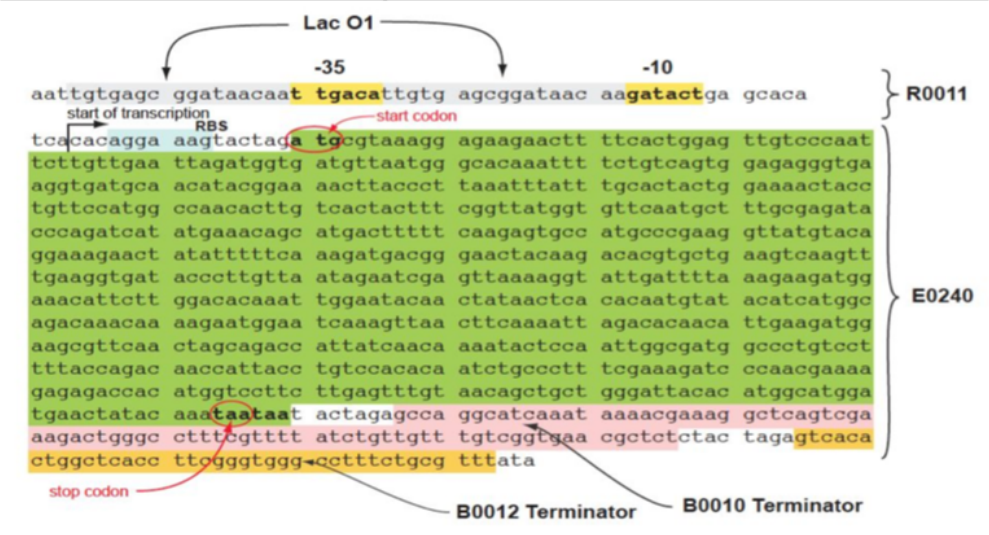
Δ(araD-araB)567, ΔlacZ4787(::rrnB-3), λ-, rph-1, Δ(rhaD-rhaB)568, hsdR514, ΔlacI



Egbert and Klavins, **Fine Tuning with Simple Sequence Repeats**, *Proceedings of the National Academy of Science*, Aug. 2012.

An improved and tunable bistable switch

- Tuning via Changing the DNA:



This is what a gene for GFP production looks like. You can tune via

- Modifying the promotor so that RNAP sticks more or less efficiently.
- Changing the concentration of active transcription factor (e.g. LacI) → later.
- Changing the RBS (eg. longer spacers)
- Tweaking the RNA or Protein degradation rate by adding "degradation tags"

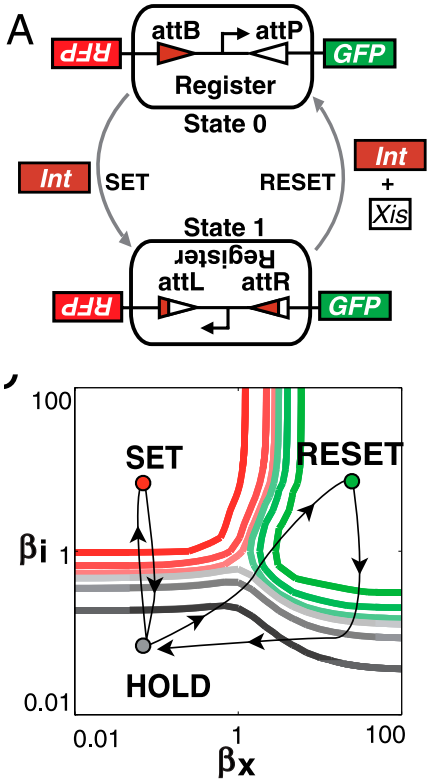
An improved and tunable bistable switch

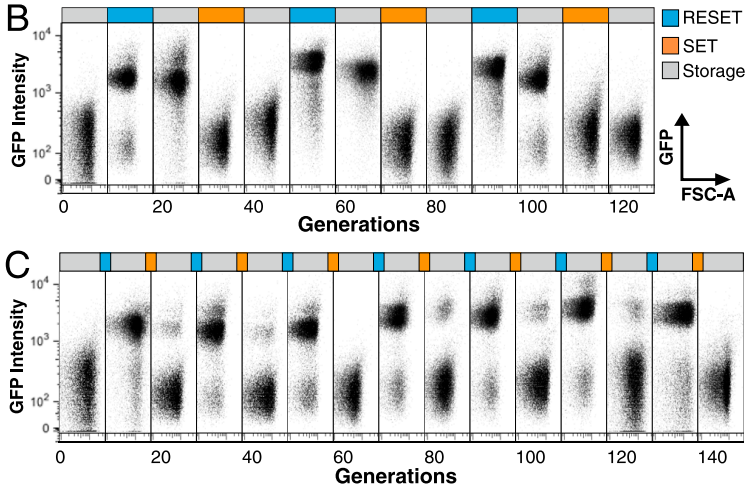
Rewritable digital data storage in live cells via engineered control of recombination directionality

Jerome Bonnet, Pakpoom Subsoontorn, and Drew Endy[1]

Department of Bioengineering, Room 269B, Y2E2 Building, 473 Via Ortega, Stanford University, Stanford, CA 94305

Edited by David Baker, University of Washington, Seattle, WA, and approved April 6, 2012 (received for review February 8, 2012)





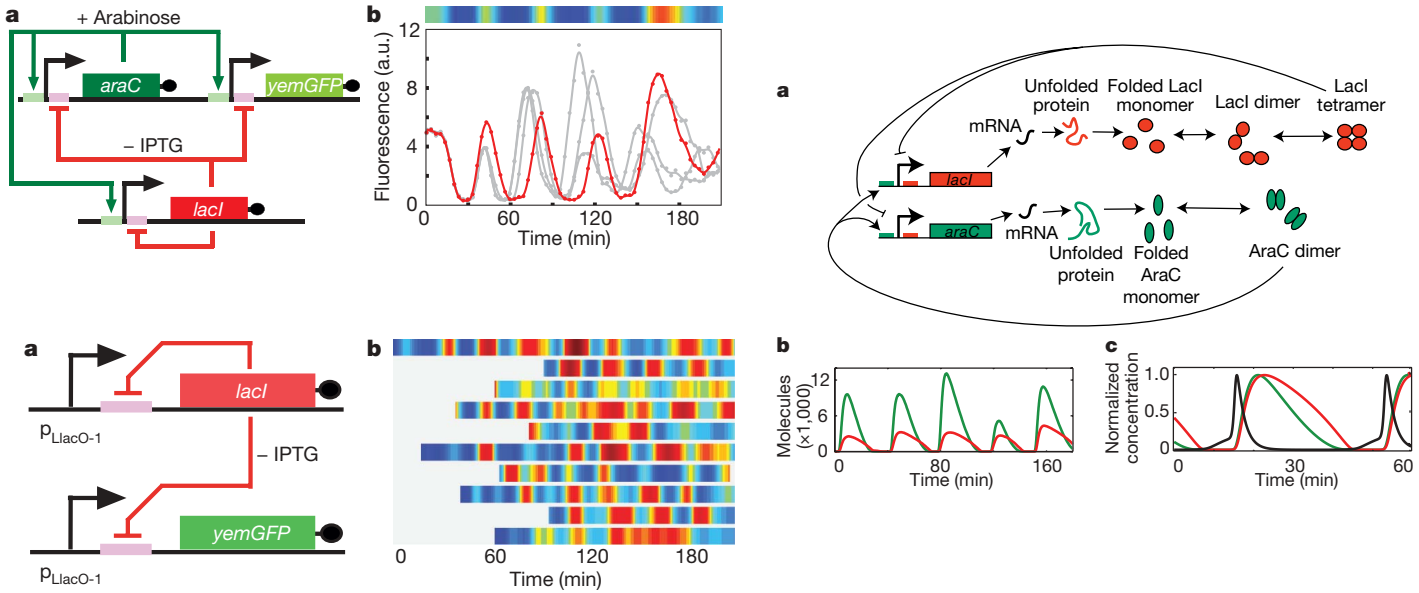
Published online before print May 21, 2012, doi: 10.1073/pnas.1202344109
PNAS June 5, 2012 vol. 109 no. 23 8884-8889

An improved synthetic oscillator

LETTERS

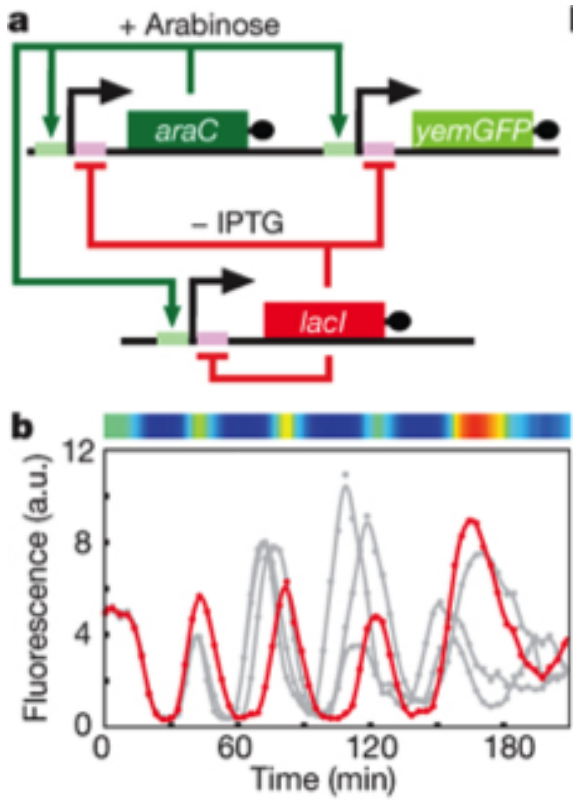
A fast, robust and tunable synthetic gene oscillator

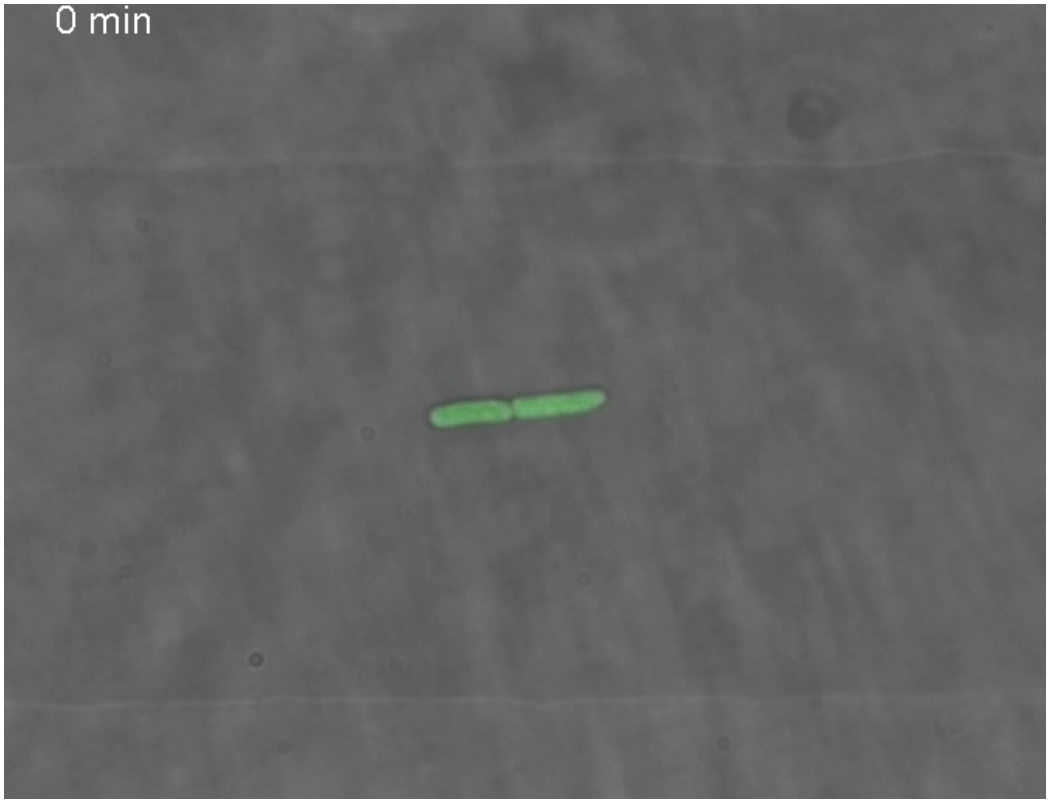
Jesse Stricker[1]*, Scott Cookson[1]*, Matthew R. Bennett[1,2]*, William H. Mather[1], Lev S. Tsimring[2] & Jeff Hasty[1,2]



Nature. 2008 Nov 27;456(7221):516-9. Epub 2008 Oct 29.

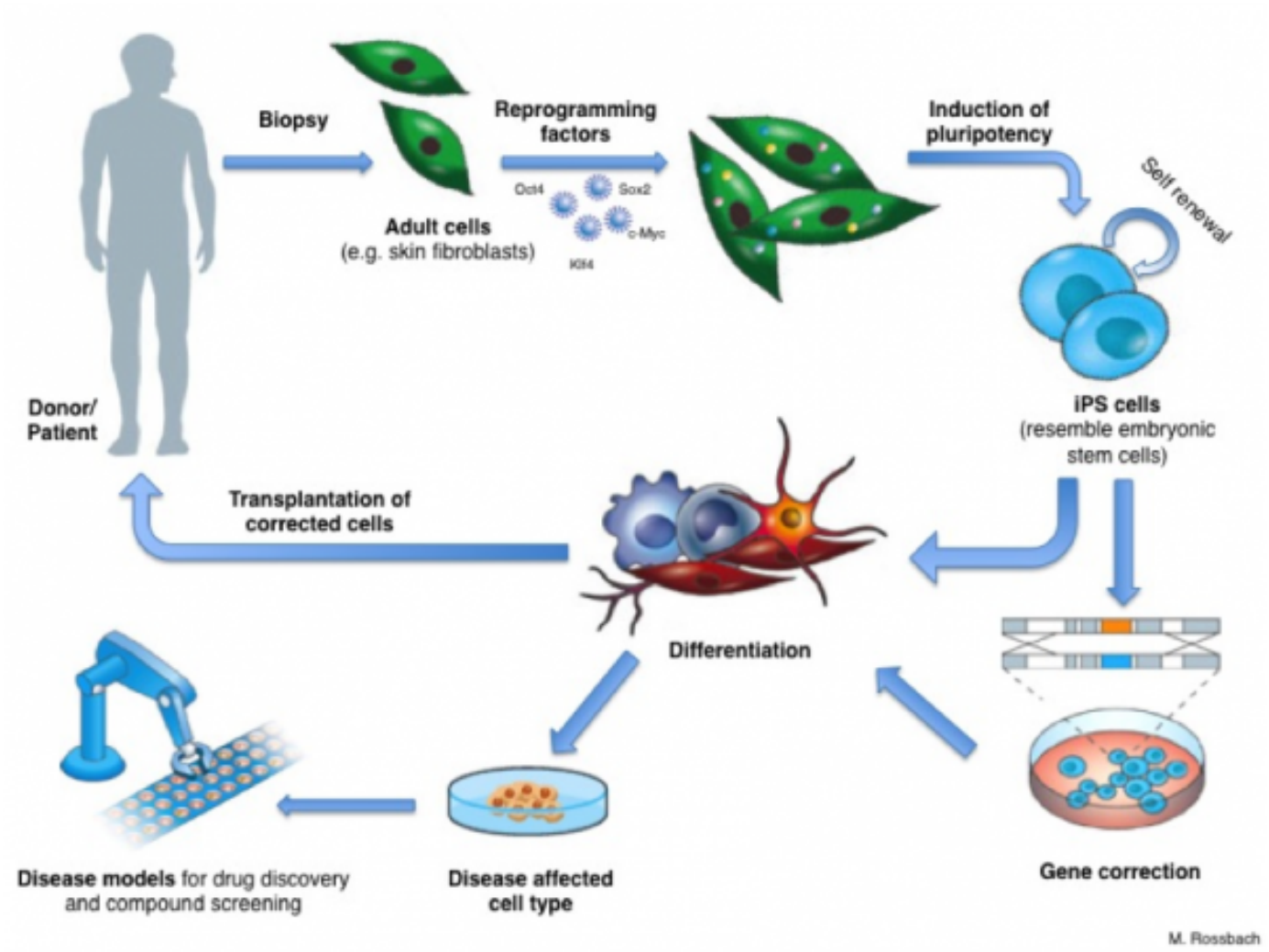
An improved synthetic oscillator





A biological oscillator
(Hasty lab)

Cellular reprogramming



Production of the antimalarial drug precursor artemisinic acid in engineered yeast

Dae-Kyun Ro[1]*, Eric M. Paradise[2]*, Mario Ouellet[1], Karl J. Fisher[6], Karyn L. Newman[1], John M. Ndungu[3], Kimberly A. Ho[1], Rachel A. Eachus[1], Timothy S. Ham[4], James Kirby[2], Michelle C. Y. Chang[1], Sydnor T. Withers[2], Yoichiro Shiba[2], Richmond Sarpong[3] & Jay D. Keasling[1,2,4,5]

Malaria is a global health problem that threatens 300–500 million people and kills more than one million people annually[1]. Disease control is hampered by the occurrence of multi-drug-resistant strains of the malaria parasite *Plasmodium falciparum*[2,3]. Synthetic antimalarial drugs and malarial vaccines are currently being developed, but their efficacy against malaria awaits rigorous clinical testing[4,5]. Artemisinin, a sesquiterpene lactone endoperoxide extracted from *Artemisia annua* L (family Asteraceae; commonly known as sweet wormwood), is highly effective against multi-drug-resistant *Plasmodium* spp., but is in short supply and unaffordable to most malaria sufferers[6]. Although total synthesis of artemisinin is difficult and costly[7], the semi-synthesis of artemisinin or any derivative from microbially sourced artemisinic acid, its immediate precursor, could be a cost-effective, environmentally friendly, high-quality and reliable source of artemisinin[8,9]. Here we report the engineering of *Saccharomyces cerevisiae* to produce high titres (up to 100 mg l^{-1}) of artemisinic acid using an engineered mevalonate pathway, amorphadiene synthase, and a novel cytochrome P450 monooxygenase (*CYP71AV1*) from *A. annua* that performs a three-step oxidation of amorpha-4,11-diene to artemisinic acid. The synthesized arte-

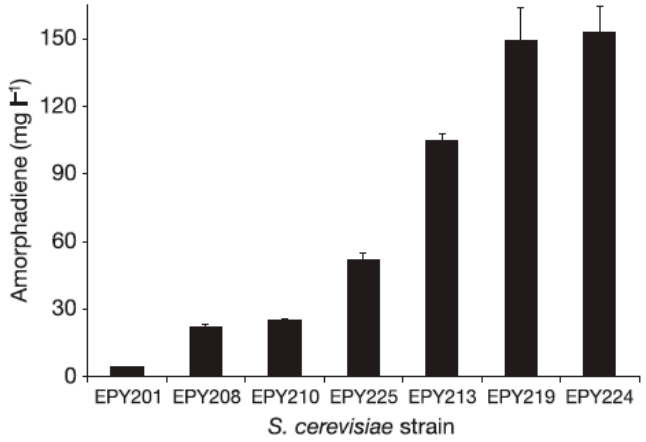
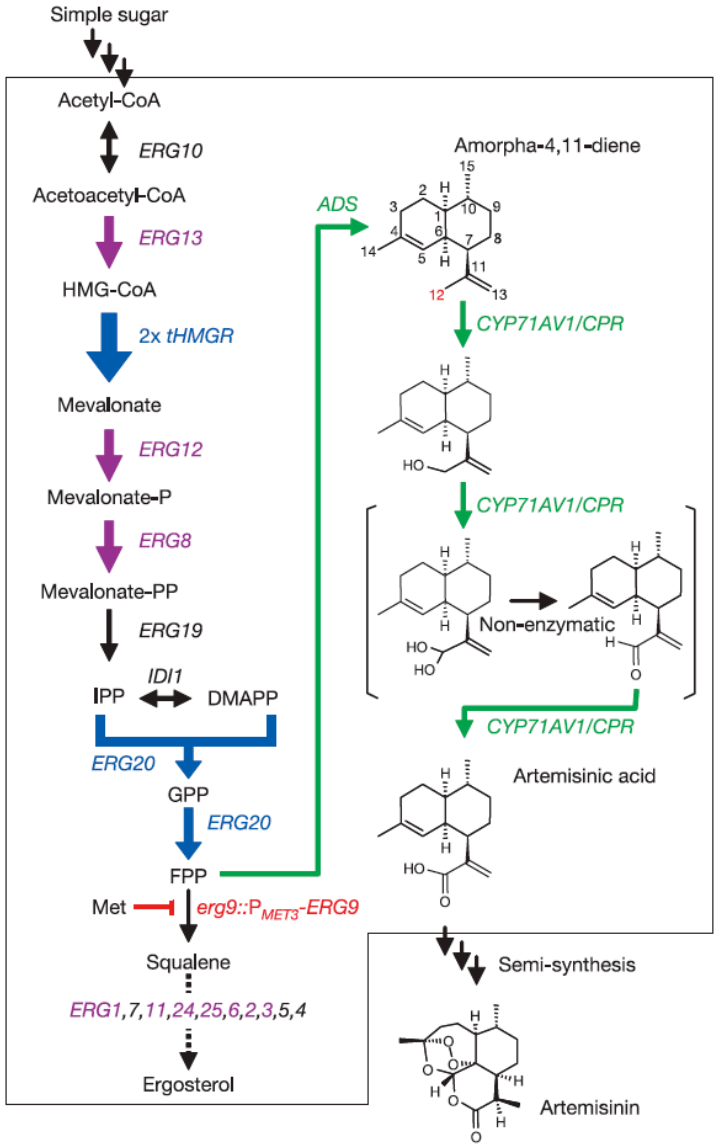


Figure 2 | Production of amorphadiene by *S. cerevisiae* strains. The various *S. cerevisiae* strains are described in the text. Cultures were sampled after 144 h of growth, and amorphadiene levels were quantified. Data, shown as total production, are mean ± s.d. ($n = 3$).



Not much about metabolic engineering in this course.

Applications

- Tissue Engineering
- Diagnostics
- Therapeutics
- Chemical Synthesis
- Materials