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dill@zimm.ucsf.edu

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angel@t10.lanl.gov

Newsletter Editor
Sonya Bahar
bahar@neurodyn.umsl.edu

Website Coordinator
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dan.gauthier@duke.edu

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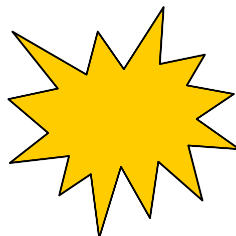
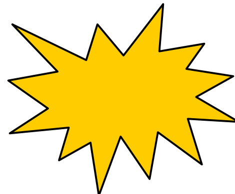


Upcoming Conference!

First SIAM Conference on Life Sciences.

September 24-26, 2001, Boston Park Plaza Hotel, Boston Mass. For more information see the conference website http://www.siam.org/meetings/ls01/



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Check out the *Virtual Journal of Biological Physics Research* **at**

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Molecular Biophysics at NEST-INFM

The extraordinary growth of the electronics industry in the past several decades has prompted an unprecedented development in technological tools designed to probe and manipulate systems at the nanoscale. This body of knowledge is currently being transferred to different areas with the notable inclusion of the newly-born field of proteomics. Protein identification and the investigation of their function require a truly multidisciplinary approach that is opening new avenues and opportunities in biomolecular and biomedical sciences.

This scenario motivated the creation of the Molecular Biophysics laboratory at NEST, the National Enterprise in Nanoscience and Nanotechnology with the joint sponsorship of Istituto Nazionale per la Fisica della Materia and Scuola Normale Superiore in Pisa, Italy (www.nest.sns.it). Current research focuses on the investigation of biological processes *in vivo* with the ambitious goal of reaching the ultimate limit of monitoring single biomolecular events. Much of the activity is based on optical techniques. Computer-aided design and molecular engineering of optimized fluorescent tags of the green-fluorescent-protein (GFP) class represent an important aspect of the ongoing activity. On this note, we should like to describe recent results on the direct visualization of protein-protein interaction by fluorescence resonant energy transfer (FRET)[1] and we discuss the observation of optically-controlled bistability in individual GFP mutants[2].

FRET allows the direct observation of protein interaction following labeling with optically-matched fluorophores[3]. Such complementary fluorophores are characterized by overlapping absorption (for the acceptor tag) and emission (for the donor tag) spectra. The GFP family offers several mutated pairs suitable for FRET experiments. In our studies, in particular, we use the enhanced GFP (EGFP) and the blue fluorescent protein (BFP) mutants.

FRET exploits radiationless energy transfer driven by dipole-dipole interaction occurring from the donor in the excited state to the acceptor, when in close proximity. Energy transfer is followed by acceptor fluorescence. The presence of FRET indicates direct protein-protein interaction since it is observable only for nanometer-scale fluorophore distance (see Fig. 1). This implies in particular that simple colocalization of two proteins is not sufficient to yield energy transfer. FRET is a non-invasive technique that can give clear, unambiguous answers to questions about protein-protein interaction and can do so in live cells.

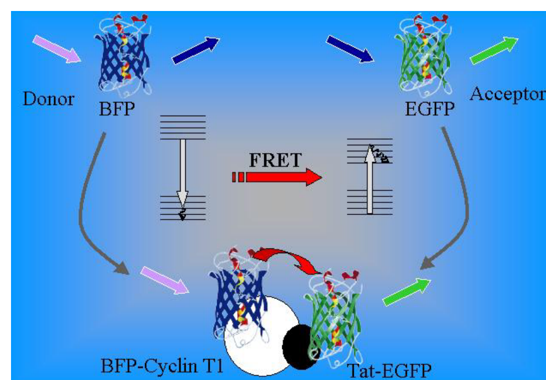


Fig. 1. Schematic description of Fluorescence Resonant Energy Transfer (FRET) process between a blue and a green GFP mutant. These mutants were used to label Human cyclin T1 and HIV1 Tat proteins.

Figure 2 shows an example of a FRET experiment in HeLa cells. Here, the objective is to test the existence of

interaction between HIV-1 Tat and human CyT1[4] and to determine the subcellular compartments where this interaction takes place. These proteins play a crucial role in human immunodeficiency virus (HIV-1) trascriptional activation and were fused to BFP and EGFP. Following the demonstration of the preservation of the biological activity of the labeled proteins, FRET analysis was performed in two steps. First, EGFP fluorescence at 520 nm was collected after direct excitation at 480 nm, i.e. resonantly with the EGFP absorption band (panels in column 2). Second, EGFP emission at 520 nm was measured following excitation at 350 nm, i.e. resonantly with BFP absorption (panels in column 3). In the latter case EGFP emission mainly originates from FRET. The ratio between the two signals provides FRET efficiency and directly relates to Tat-CyT1 interaction. High-resolution FRET intensity maps can provide much information on protein cooperative functions and subcellular localization[1].

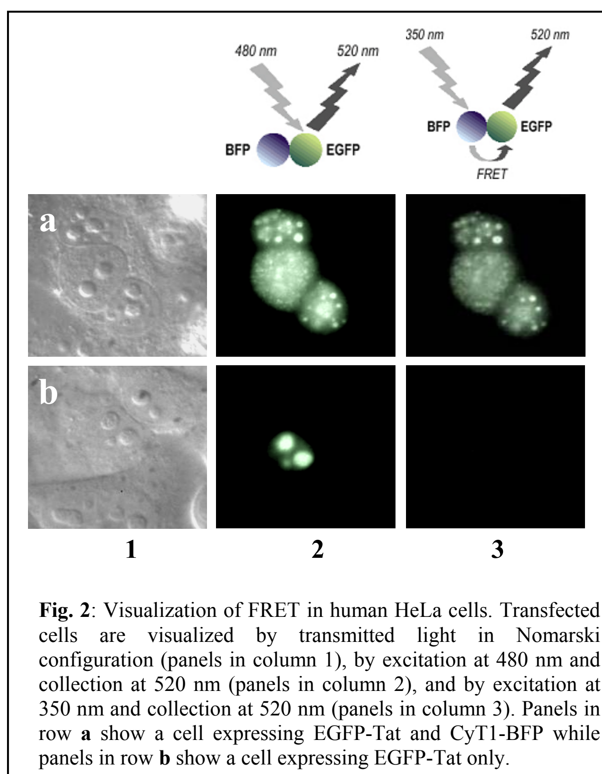


Fig. 2: Visualization of FRET in human HeLa cells. Transfected cells are visualized by transmitted light in Nomarski configuration (panels in column 1), by excitation at 480 nm and collection at 520 nm (panels in column 2), and by excitation at 350 nm and collection at 520 nm (panels in column 3). Panels in row **a** show a cell expressing EGFP-Tat and CyT1-BFP while panels in row **b** show a cell expressing EGFP-Tat only.

GFP labeling can in principle be used to monitor single-molecule trafficking and interactions. To this end specific actions must be taken to avoid photobleaching[5] which at the single-molecule level yields an irreversible switching off of the emission after few seconds of excitation following transition from the bright to a dark state[6]. A specific point substitution at position 203 of EGFP amminoacid sequence, however, induces optically-controllable conversion from the dark back to the bright states as demonstrated in Fig. 3.

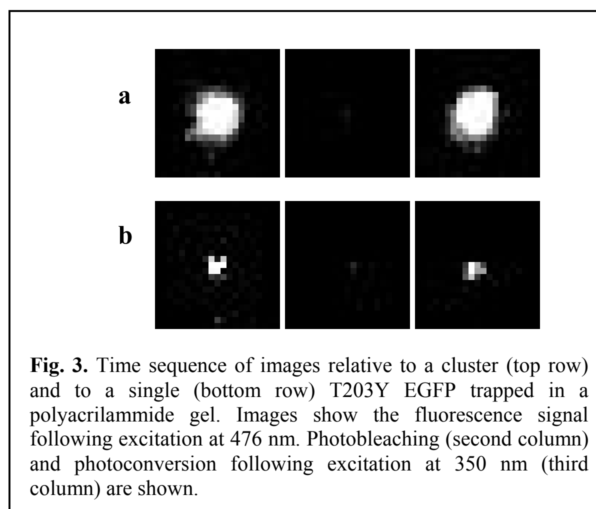


Fig. 3. Time sequence of images relative to a cluster (top row) and to a single (bottom row) T203Y EGFP trapped in a polyacrilammide gel. Images show the fluorescence signal following excitation at 476 nm. Photobleaching (second column) and photoconversion following excitation at 350 nm (third column) are shown.

Article contributed by Aldo Ferrari, Vittorio Pellegrini, Riccardo A. G. Cinelli, Mauro Giacca, and Fabio Beltram. *NEST-INFM, Scuola Normale Superiore, Pisa (Italy).*

References

1. Marcello, A. et al. to be published in *J Biol Chem*.
2. Cinelli R.A.G. et al. submitted to *Applied Physics Letters*; cond-mat/ 0107576
3. Selvin, P. R. *Nat Struct Biol* **7**, 730 (2000).
4. Zhang, J. et al. *J Biol Chem* **275**, 3431 (2000).
5. Dickson, R.M. et al. *Nature* **388**, 355 (1997).
6. Cinelli, R. A. G. et al. *Photochem and Photobiol* **71**, 771 (2000); *Phys Rev. Lett.* **86**, 3439 (2001).

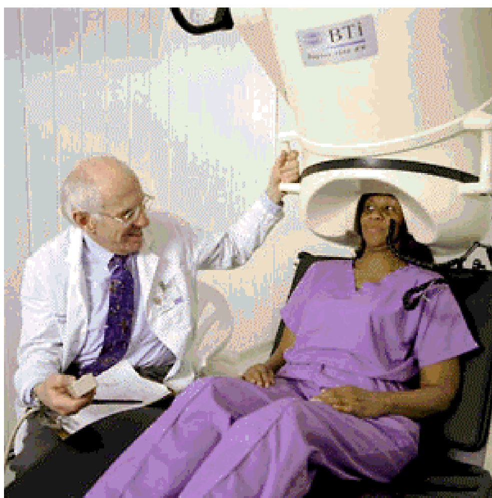
Contact info: Vittorio Pellegrini, NEST-INFM, vp@sns.it

Biological Physics at Oakland University

by Dr. Brad Roth

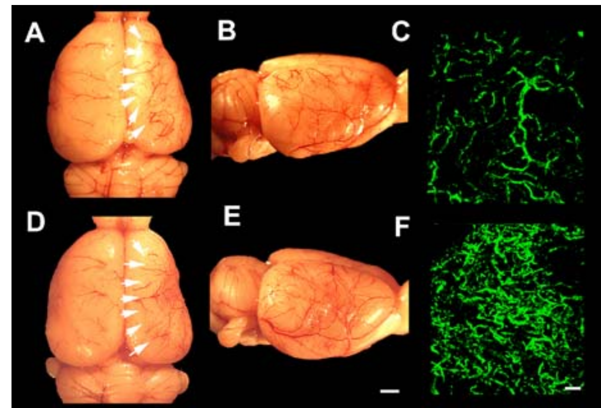
The Department of Physics at Oakland University has an active graduate program in Biological and Medical Physics. Oakland is a public university in Rochester, Michigan, 30 miles north of Detroit, with a student population of about 15,000. In 1982, the department instituted a Ph.D. program in Medical Physics. The program contains elements of both traditional medical physics (such as radiation oncology, and imaging) and the more research-oriented fields of Biological Physics. The program at Oakland is administered by the Physics Department, so graduate students get a solid education in physics as well as the opportunity to do research at the cutting edge of biology and medicine.

All the faculty members engaged in biological physics research are currently funded by the National Institutes of Health. Dr. Norman Tepley, the coordinator of the Medical Physics Graduate Program, is a leading researcher in magnetoencephalography (MEG): the measurement of the magnetic field produced by electrical activity in the brain. MEG is useful both for basic studies in neurophysics and for clinical diagnosis, particularly pre-surgical mapping. In his laboratory at Henry Ford Hospital, Tepley has one of only a small number of whole-head large-array neuromagnetometers in North America. His group has become recognized as the world's leader in the measurement of very slowly varying neuromagnetic activity, such as that associated with stroke, head trauma, migraine headache, and epilepsy.



Dr. Norman Tepley using a neuromagnetometer to record an MEG.

Dr. Michael Chopp is an internationally recognized expert in the development and treatment of stroke, and is the Director of the Stroke Center at Henry Ford Hospital. His laboratory has developed novel methods using magnetic resonance imaging (MRI) and sophisticated image reconstruction algorithms that permit the non-invasive evaluation of brain tissue. These techniques allow them to identify whether brain cells are simply affected and compromised by a stroke, are in the process of dying, or are already dead.

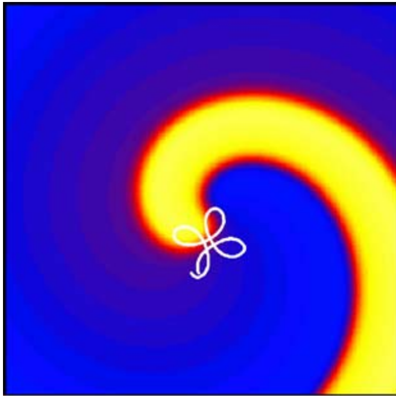


Administration of vascular endothelial growth factor (VEGF) to rats subjected to stroke significantly increases cerebral blood vessels (D-F) compared with control (A-C). Images C and F obtained using laser scanning confocal microscopy. Dr. Michael Chopp.

Dr. Yang Xia uses high spatial resolution MRI (NMR microscopy) to study a number of biological problems. He is able to resolve structures as small as tens of microns. Of particular interest to Xia and his students is the molecular structure of and activities in articular cartilage, whose degradation plays a major role in the early development of osteoarthritis. His laboratory is unique in combining and correlating a number of tissue-specific signatures using high-resolution MRI (Bruker AMX 300), optical microscopy (digitized Leica polarized microscope), biomechanical instrumentation (EnduraTEC ELF3200), biochemical assays, and histology.

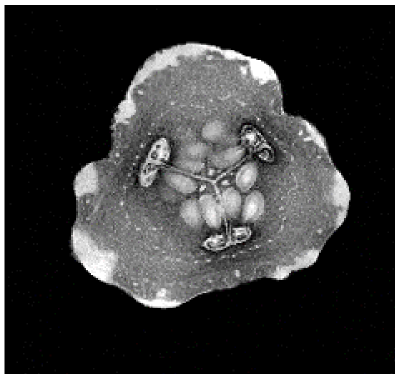
The research of Dr. Bradley Roth focuses on bioelectric phenomena, such as the electrical activity of nerve and muscle. A particular interest of his is electrical stimulation of the heart. Electric fields can cause the heart to contract, and can even induce abnormal behavior such as cardiac arrhythmias and fibrillation. Moreover, strong electrical shocks are

applied in defibrillation in order to stop abnormal and potentially fatal electrical behavior. Dr. Roth's goal is to understand the fundamental physics that underlies electrical stimulation and defibrillation of the heart. To study the heart, he uses mathematical modeling and numerical simulations.



Computer simulation of a spiral wave of electrical activity in the heart. The white curve indicates the path of the tip of the spiral wave as it meanders through the cardiac muscle. Dr. Bradley Roth.

In addition to the department's full-time faculty members, Emeritus Professor Abe Liboff maintains an active research program studying the biological effects of weak electromagnetic fields. Several adjunct faculty lead active medical physics research programs at nearby hospitals, such as Henry Ford Hospital in Detroit and William Beaumont Hospital in Royal Oak. Physics faculty also collaborate with members of other departments at Oakland. Biological Physics research is part of a University-wide focus spearheaded by the Center for Biomedical Research.



A high-resolution MRI of a pickle (!!), with a pixel size of 49 microns. Dr. Yang Xia

In its nearly twenty years of operation, the program has produced a number of graduates who express fond memories of their experience at Oakland. "My time at OU was one of the most creative and rewarding periods of my life," recalls Dr. Joseph Helpern, who received his PhD in 1988 and is currently on the faculty at the New York School of Medicine. Adds Craig Branch (PhD 1989), currently a Program Director for the Center for Advanced Brain Imaging at the Nathan S. Kline Institute, "The faculty of the department of Physics encouraged me to pursue the PhD in Biomedical Physics. I only wish I could attract a few of their recent graduates, particularly those with MR experience, toward New York, because the need is still growing!"

Some recent publications from Oakland faculty:

Bray MA, Lin SF, Aliev RR, **Roth BJ**, Wikswo JP Jr, 2001, Experimental and theoretical analysis of phase singularity dynamics in cardiac tissue. *J. Cardiovasc Electrophysiol*, **12**:716-722.

Roth BJ, 2001, Meandering of spiral waves in anisotropic cardiac tissue. *Physica D*, **150**:127-136.

Jacobs MA, Knight RA, Zhang ZG, Windham JP, Soltanian-Zadeh H, Peck DJ, Goussev AV, **Chopp M**, 2000, Unsupervised segmentation of multiparameter MRI in experimental cerebral ischemia with comparison to T2, diffusion, and ADC MRI parameters and histopathological validation. *J Magn Reson Imaging*, **11**:425-437.

Jiang Q, Zhang RL, Zhang ZG, Ewing JR, Jiang P, Divine GW, Knight RA, **Chopp M**, 2000, Magnetic resonance imaging indexes of therapeutic efficacy of recombinant tissue plasminogen activator treatment of rat at 1 and 4 hours after embolic stroke. *J Cereb Blood Flow Metab*, **20**:21-27.

Liboff AR, Jenrow K, 2000, Cell sensitivity to magnetic fields. *Electro- and MagnetoBiology*, **19**:223-236.

Moran JE, **Tepley N**, 2000, Two dimensional inverse imaging for current sources in magnetoencephalography. *Brain Topography*, **12**:201-217.

Wijesinghe R, **Roth BJ**, **Tepley N**, 1998, Modeling of spreading cortical depression using a realistic head model. *Brain Topography* **11**: 3-12. 1998.

Xia Y, 2000, Magic angle effect in MRI of articular cartilage - A review. *Investigative Radiology*, **35**:602-621.

Xia Y, J Moody, N Burton-Wurster, and G Lust, 2001, Quantitative in situ correlation between microscopic MRI and polarized light microscopy studies of articular cartilage, *Osteoarthritis and Cartilage*, **9**:393-406.

For more information, contact Norman Tepley at tepley@oakland.edu, Tel. (248) 370-3408, or visit http://www.oakland.edu/physics for more detail about the department and about the specific research projects discussed in this article.

The Applied Biodynamics Laboratory at Boston University

by Drs. Jeff Hasty and James J. Collins

Our work on gene regulation in the Applied Biodynamics Lab **(http://cbd.bu.edu/abl/)** is directed towards the development of theoretical, computational and experimental tools for modeling, designing and constructing synthetic gene networks. From an engineering perspective, the control of cellular function through the design and manipulation of gene regulatory networks is an intriguing possibility. Current examples of potential applicability range from the use of genetically engineered microorganisms for environmental cleanup purposes [8], to the flipping of genetic switches in mammalian neuronal cells [3]. While these studies are certainly impressive, it is clear that the systematic development of a biocomputing toolbox of applications would be of enormous value. Such a toolbox could potentially reduce the degree of "trial-and-error" experimentation, and lead to testable predictions regarding the current understanding of complex biological networks. In addition, the ability to design synthetic gene networks offers the exciting prospect of extracting carefully chosen subsystems from natural organisms, and focusing both modeling and experimental effort on determination of the behavior of the subsystems in isolation. The long-range goal of such work is to assemble increasingly complete models of the behavior of natural systems, while maintaining at each stage the ability to test models in a tractable experimental system.

Importantly, synthetic gene networks also represent a first step towards logical cellular control, whereby biological processes can be manipulated or monitored at the DNA level. From the construction of a simple set of genetic building-block circuits (e.g., toggle switches, oscillators, etc.), one can imagine the design and construction of integrated biological circuits capable of performing increasingly elaborate functions An integrated biological circuit could, like electronic control circuits, possess data-processing and storage circuitry, as well as input/output components necessary for sensing and affecting its environment. Ultimately, synthetic gene circuits encoded into DNA, might be "downloaded" to cells creating, in effect, a "wet" nano-robot. These cellular robots could be utilized for a variety of functions, including *in vivo* biosensing, autonomously synthesizing complex biomaterials, executing programmed cell death, and interfacing with microelectronic circuits by transducing biochemical events to and from the electronics.

A Genetic Toggle Switch

Our group has designed and constructed a fundamental unit of biocomputing memory storage, a genetic toggle switch or flip flop, in *E.coli* [2]. This study involved a synthetic gene network where each of two proteins negatively regulates the synthesis of the other (Fig. **1a**); protein *A* turns off the promoter for gene *B*, and protein *B* turns off the promoter for gene *B* [2]. In this work, it was shown how certain biochemical parameters lead to two

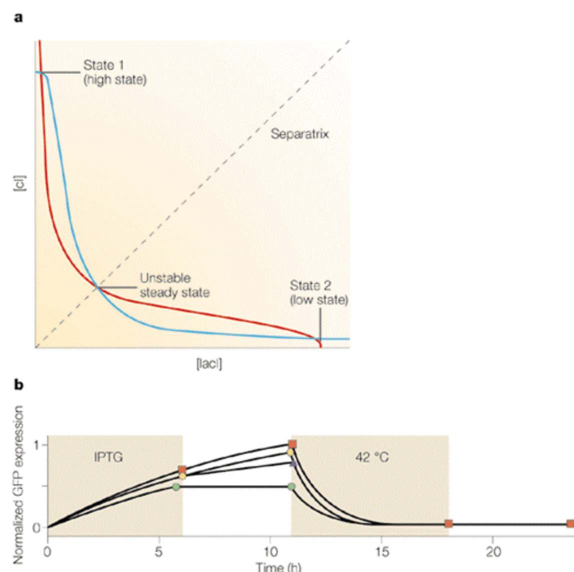


Fig 1. a*. Analysis of a bistable toggle network with equal promoter strengths driving the expression of lacI and cI proteins. Plots show two stable steady states (low lacI, high cI (state 1) and high lacI, low cI (state 2)) and one unstable steady state.* ***b.*** *Experimental demonstration of bistability in a genetic toggle switch. The response of green fluorescent protein (GFP) is shown, which corresponds to expression of the cI gene and is inversely proportional to expression of the lacI gene. Upon induction with isopropyl-β-D-thiogalactopyranoside (IPTG), lac repressor protein is removed from its operator site and allows expression of cI and the reporter protein, GFP. As observed, after six hours, the system exists in a high steady state. The system remains in a high state after removal of IPTG. Upon induction at high temperature (42 °C), the system returns to the low state and remains there after a return to low temperature. Shaded areas indicate periods of chemical or thermal induction. The symbols represent four toggle plasmids.*

stable steady states, with either a high concentration of *A* (low *B*), or a high concentration of *B* (low *A*).

One version of the co-repressive toggle switch utilized the cI and Lac proteins, where each protein shuts off transcription from the other protein's promoter region. The experimental design was guided by the model equations

$$\dot{u} = \frac{\alpha_1}{1+v^{\delta}} - u$$
$$\dot{v} = \frac{\alpha_2}{1+u^{\gamma}} - v \qquad (1)$$

where u and v are dimensionless concentrations of the Lac and cI proteins, respectively The cI protein used in the experiments is temperature-sensitive, changing its rate of degradation with temperature. Switching is induced either by changing the temperature or by adjusting the concentration of isopropyl-β-D-thiogalactopyranoside (IPTG). Over a wide range of parameter values, the system has two stable fixed points.

One counter-intuitive finding in the toggle work was that not all co-repressive systems will exhibit bistability. In fact, a central feature of this work was the use of mathematical and computational tools in deducing *a priori* the criteria for a robust toggle switch. The feasibility of a toggle switch is manifest in the existence of two stable fixed points, and the design of an operating toggle thus depended on parameter choices that lead to bistability. These criteria included the use of strong and balanced constitutive promoters, effective transcriptional repression, the formation of protein multimers, and similar protein degradation rates.

The reliable toggling between states was induced experimentally through the transient introduction of either a chemical or a thermal stimulus, and shown to be significantly sharper than for that of a network designed without co-repression. Specifically, IPTG, which binds to Lac repressor tetramers, was used to render the Lac repressor unable to repress its promoter. Likewise, a temperature-sensitive cI protein was used, so that its degradation rate was an increasing function of temperature. For detection, the green fluorescent protein (GFP) gene was transcribed polycistronically with the cI gene, so that GFP concentrations were proportional to the concentration of the λ–repressor (encoded by cI). Results for one of the toggles are presented in Fig. **1b**. The system, beginning in the high-Lac repressor/low-cI state, was toggled to the high-cI/low-Lac repressor state with IPTG. As expected, the system remained in the high-cI state after removal of the IPTG stimulus. Toggling to the low-cI state was then accomplished by tuning the temperature to 42 °C, and this state was subsequently stable upon the return of the system to 32 °C.

In spite of its simplistic description of the events underlying gene expression, the toggle model was extremely effective in predicting the principal qualitative features of the experimental gene network. The reasonable agreement between the toggle theory and experiment suggests that the theoretical design of complex and practical gene networks is a realistic and achievable goal [2].

An Autocatalytic Single-Gene Network

Following our model work, we have recently designed and constructed an autocatalytic single-gene network. Modeling results have provided insight into two important issues pertaining to such a positive feedback network [6,7]. First, it has been shown that activation should decrease the stability of the equilibrium state, thus leading to variations characterized by wider distributions as compared with networks without feedback. Second, a single-gene network with positive regulation is capable of bistability.

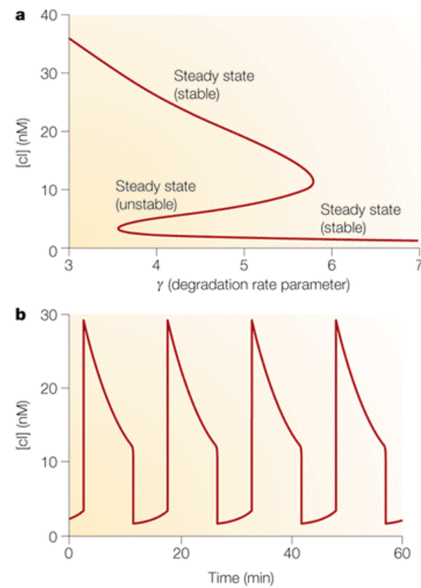


Fig 2. a. *Bifurcation plot for the steady-state concentration of repressor versus the model parameter γ, representing the degradation rate. For values of γ between 3.6 and 5.8, there are three possible steady-state concentrations (arrows). The top and bottom branch values are stable (concentrations near these values will remain nearby despite small fluctuations), whereas the middle branch value is unstable (any tiny fluctuation will be amplified, driving the protein concentration towards one of the two stable states on the upper and lower branches).* ***b.*** *Results from a relaxation oscillator network in which two identical promoters each drive the expression of different genes. Promoter PRM drives the expression of -repressor protein, which activates its own production as well as the production of a second protein, RcsA (regulator of capsule synthesis A), driven by a second copy of the PRM promoter. Protein RcsA cleaves the -repressor protein, which prevents -repressor from activating the PRM promoters that transcribe cI and rcsA. Oscillations arise as the RcsA-induced degradation of repressor causes a traversal of the hysteresis diagram.*

This implies that a single-gene switch can be constructed as an alternative to the co-repressive toggle discussed above. Additionally, such a switch can be used as the basic element for an oscillator [1,4].

As a concrete example of an activating system, we have constructed a synthetic network consisting of the P_{RM} promoter of λ phage and the cI gene encoding the protein λ repressor. The governing equation for this system is

$$\dot{x} = \frac{m(1+x^2+\alpha\sigma_1 x^4)}{1+x^2+\sigma_1 x^4+\sigma_1\sigma_2 x^6} - \gamma x \qquad (2)$$

where α is the degree to which transcription is enhanced by dimer-occupation of the operator site and γ is the degradation parameter [4]. The first term on the right-hand side of Eq (2) represents production of repressor due to transcription. The even polynomials in *x* occur due to dimerization and subsequent binding to the promoter region. The σ_i prefactors denote the relative affinities for

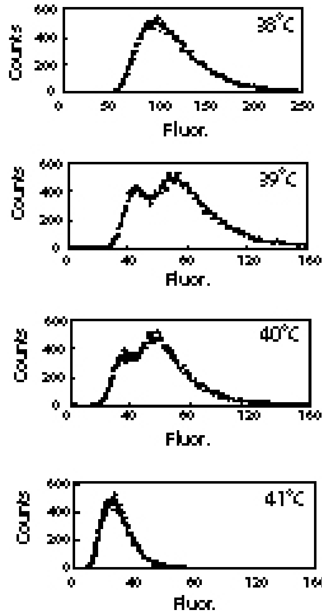


Fig 3 *Experimental confirmation of bistability. When the initial temperature of 38°C is increased to 39°C and 40°C (middle plots), the temperature-sensitive CI857 protein degrades more readily and bimodal distributions emerge. A further increase to 41°C results in a return to a unimodal distribution characterized by a low mean concentration. The experiments, which confirm our theoretical predictions, were done by Farren Isaacs and have been submitted for publication.*

dimer binding to OR1 versus that of binding to OR2 (σ_1) and OR3 (σ_2). The prefactor $\alpha>1$ on the x^4 term is present because transcription is enhanced when the two operator sites OR1 and OR2 are occupied (x^2x^2). The x^6 term represents the occupation of all three operator sites, and arises in the denominator because dimer occupation of OR3 inhibits polymerase binding and shuts off transcription.

For the operator region of λ phage, the known parameter values are $\sigma_1 \sim 2$, $\sigma_2 \sim 0.08$, and $\alpha \sim 11$, so that the parameters γ and *m* in Eq. (2) determine the steady-state concentration of repressor. The parameter γ is directly proportional to the protein degradation rate, and in the construction of synthetic gene networks, it can be utilized as a tunable parameter. The integer parameter *m* represents the number of plasmids per cell. While this parameter is not accessible during an experiment, it is possible to design a plasmid with a given copy number, with typical values in the range of 1-100.

The nonlinearity of Eq. (2) leads to a bistable regime in the steady-state concentration of repressor, and in Figure **2a** we plot the predicted steady-state concentration of repressor as a function of the parameter γ. The bistability arises as a consequence of the competition between the production of *x* along with dimerization and its degradation. For certain parameter values, the initial concentration is irrelevant, but for those that more closely balance production and loss, the final concentration is determined by the initial value.

Experimental results, validating the theory by demonstrating bistability in a positive feedback circuit, are shown in Fig. **3**. At a temperature of 38 °C, there is one distinct population of cells, corresponding to the monostable region predicted by the theory. When the temperature is increased to 39 to 40 °C, bistability emerges as the temperature-sensitive cI857 protein degrades more readily and a population of cells are found in a lower state corresponding to very little cI protein. Finally, a further increase to 41 °C results in a transition to monostability characterized by a low mean concentration. Theoretically, this corresponds to falling off the upper branch depicted in Fig. **2a**. Current work is focused on the development of a stochastic model that correctly describes the distributions seen in Fig. **3**.

1. Barkai N and Liebler S. *Nature* **403**:267, 2000.
2. Gardner TS, Cantor CR and Collins JJ. *Nature* **403**:339, 2000.
3. Harding TC, Geddes BJ, Murphy D, Knight D and Uney JB. *Nature Biotech* **16**:553, 1998.
4. Hasty J, Isaacs F, Dolnik M, McMillen D and Collins JJ. *Chaos* **11**:207, 2001a.
5. Hasty J, McMillen D, Isaacs F and Collins JJ. *Nature Reviews Genetics* **2**:268, 2001b.
6. Hasty J, Pradines J, Dolnik M and Collins JJ. *Proc. Natl. Acad. Sci.* **97**:2075, 2000.
7. Savageau MA. *Nature* **252**:546, 1974.
8. Szafranski P, Mello C, Sano T, Smith CL, Kaplan DL and Cantor CR. *Proc Natl Acad Sci* **94**:1059,1997.

The authors may be contaced at hasty@bu.edu and jcollins@bu.edu.