Nonlinear physics in the study of synthetic genetic oscillators

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COMMENT

TECHNOLOGY Lessons for future of the Internet in history of communications **p.892**

DRUGS Opium dominates show on legal and illegal highs **p.896**

ECONOMICS Beware, politicians will exploit any indicator **p.897**

OBITUARY Allan Sandage, who measured the Universe's expansion, remembered **p.898**

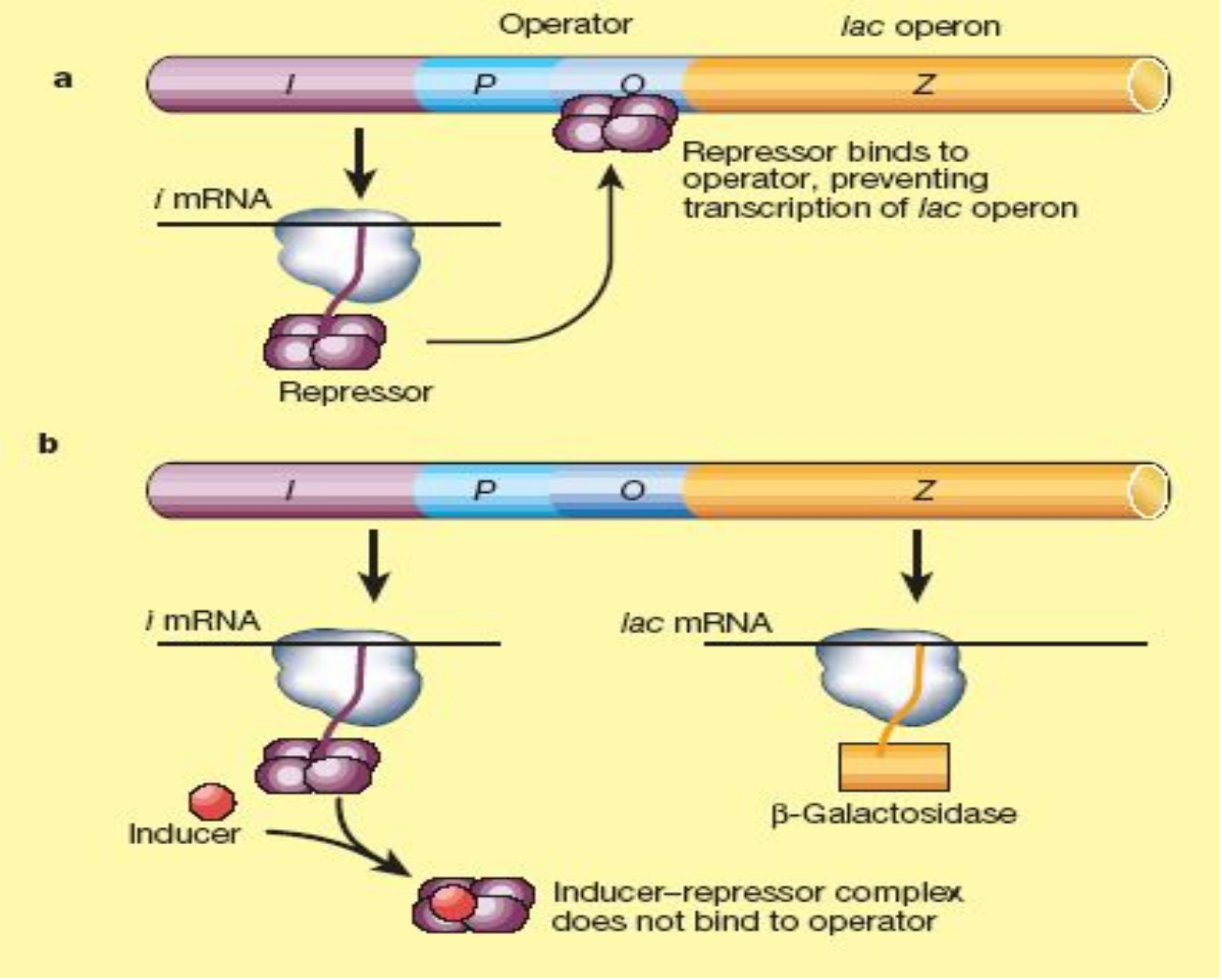
Build life to understand it

Biologists and engineers should work together: synthetic biology reveals how organisms develop and function, argue **Michael Elowitz** and **Wendell A. Lim**.

Content

- **Genetic Switch: starting point**
- **Repressilator:** circuit, equations and reality
- **How to couple genetic elements: Quorum Sensing**
- **QS in single Repressilator**

Qualitative pictures for Lac-operon functioning



Two mutual repressors: Nature 2000

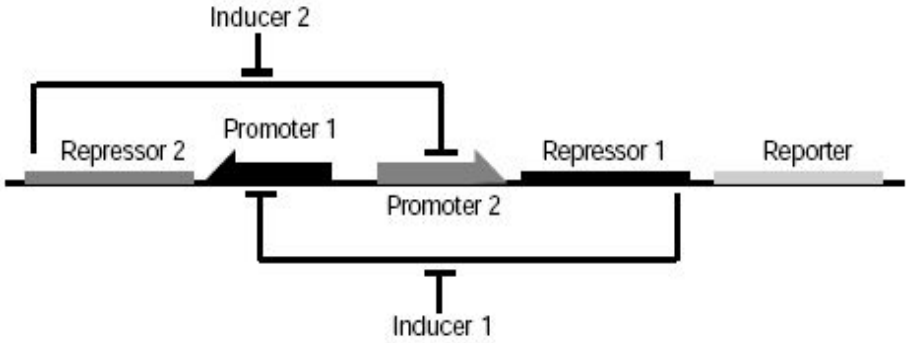


Figure 1 Toggle switch desition from Promoter 1 and is induced by Inducer 1. Repressor 2 inhibits transcription from Promoter 2 and is induced by Inducer 2.

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*

Model of genetic toggle – Nature 2000

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

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

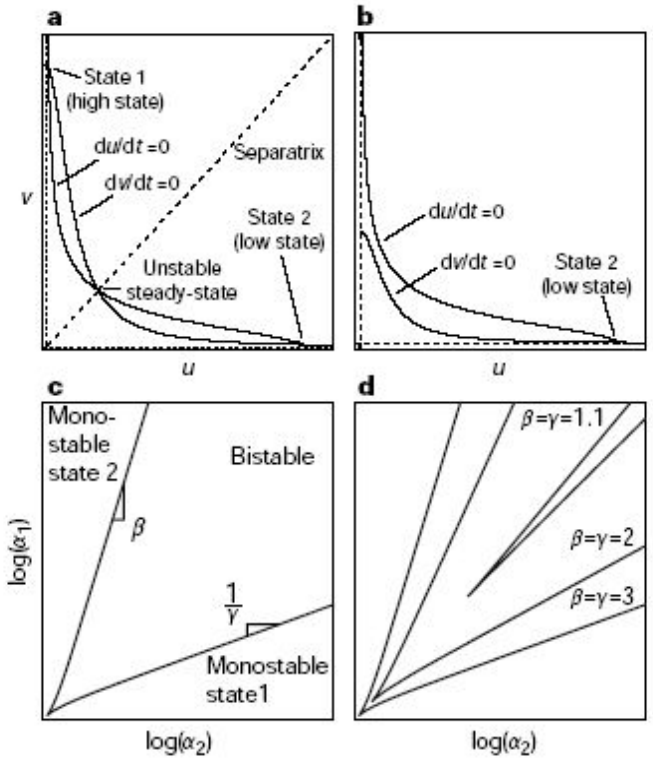


Figure 2 Geometric structure of the toggle equations. **a**, A bistable toggle network with balanced promoter strengths. **b**, A monostable toggle network with imbalanced promoter strengths. **c**, The bistable region. The lines mark the transition (bifurcation) between bistability and monostability. The slopes of the bifurcation lines are determined by the exponents β and γ for large α_1 and α_2. **d**, Reducing the cooperativity of repression (β and γ) reduces the size of the bistable region. Bifurcation lines are illustrated for three different values of β and γ. The bistable region lies inside of each pair of curves.

Switch → Limit Cycle

- The synthetic oscillators may help in the understanding of mechanism of such basic phenomena as Circadian Rhythm and Cell Cycle regulation.
- There are many mechanisms for oscillations in gene expression but the switch is a good starting point.

Repressilator

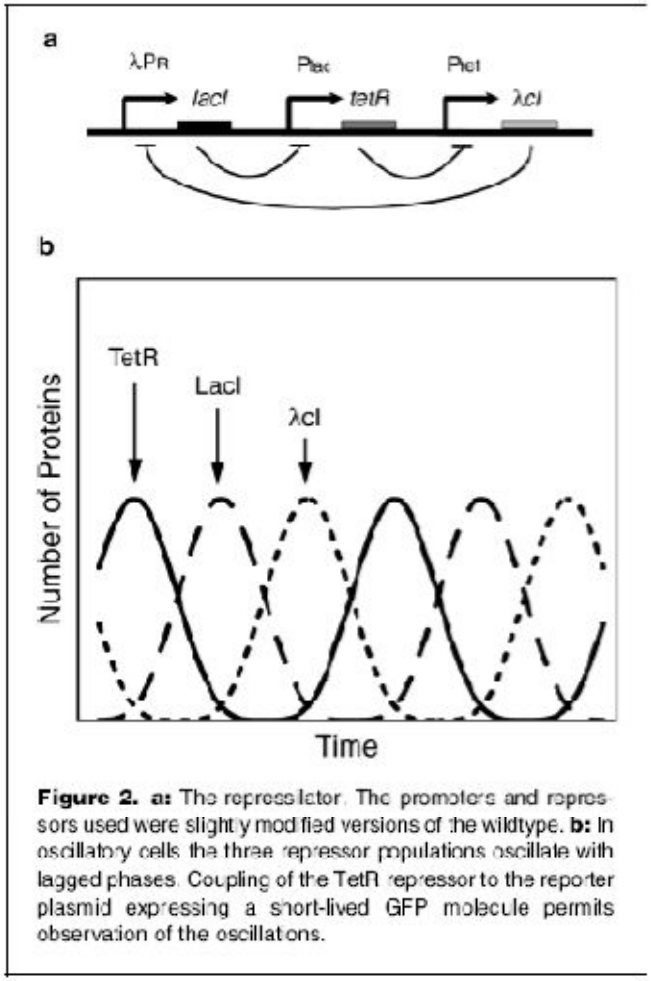
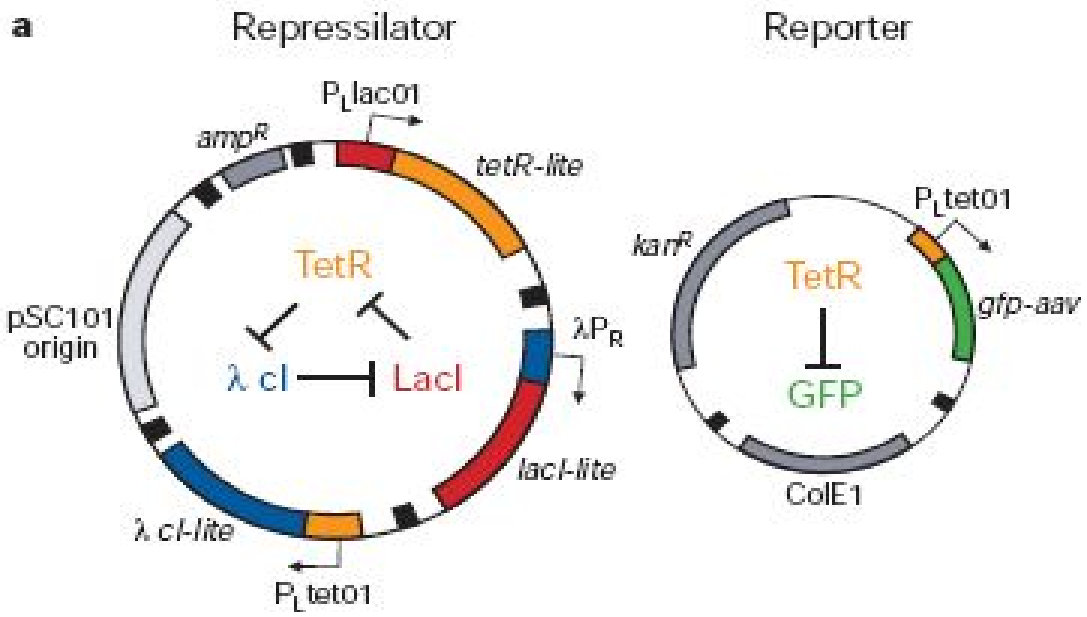


Figure 2. a: The repressilator. The promoters and repressors used were slightly modified versions of the wildtype. **b:** In oscillatory cells the three repressor populations oscillate with lagged phases. Coupling of the TetR repressor to the reporter plasmid expressing a short-lived GFP molecule permits observation of the oscillations.

Nature, 403, 2000

A synthetic oscillatory network of transcriptional regulators

Michael B. Elowitz & Stanislas Leibler



Repressilator works – Elowitz, Leibler: Nature, 2000

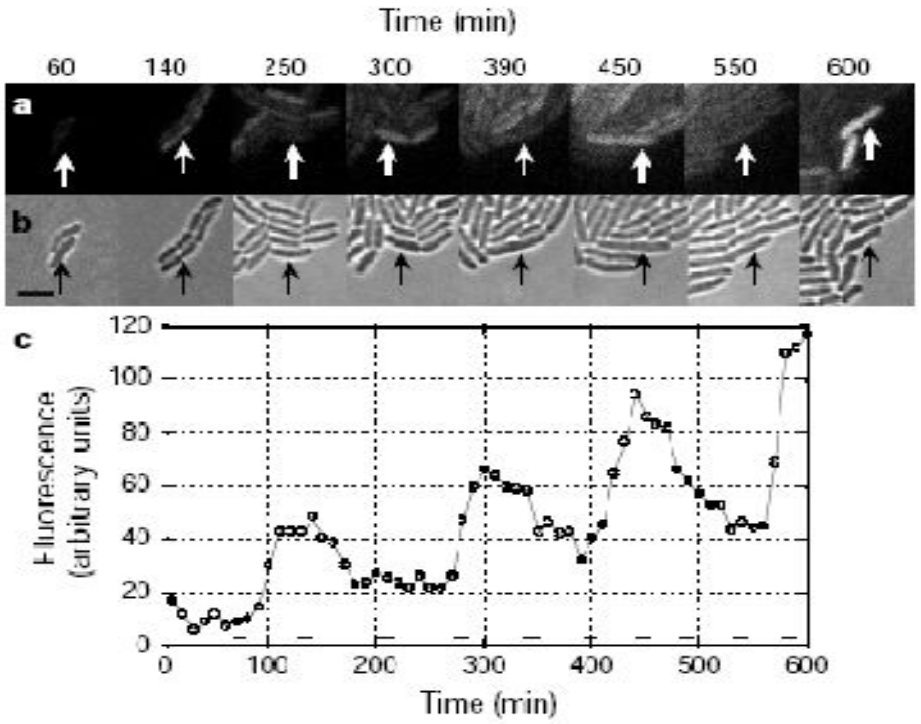


Figure 2 Repressilation in living bacteria. **a**, **b**, The growth and timecourse of GFP expression for a single cell of *E. coli* host strain MC4100 containing the repressilator plasmids (Fig. 1a'). Snapshots of a growing microcolony were taken periodically both in fluorescence (**a**) and bright-field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

Isolated Repressilator – Nature 2000

Three repressor-protein concentrations, p_i, and their corresponding mRNA concentrations, m_i (where i is *lacI*, *tetR* or *cI*) were treated as continuous dynamical variables. Each of these six molecular species participates in transcription, translation and degradation reactions. Here we consider only the symmetrical case in which all three repressors are identical except for their DNA-binding specificities. The kinetics of the system are determined by six coupled first-order differential equations:

The

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

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

Isolated Repressilator has a smooth trajectory and is stable in the large interval of parameters.

Coupling by : J.Garcia-Ojalvo, M. Elowitz, S. Strogatz, PNAS, 2004

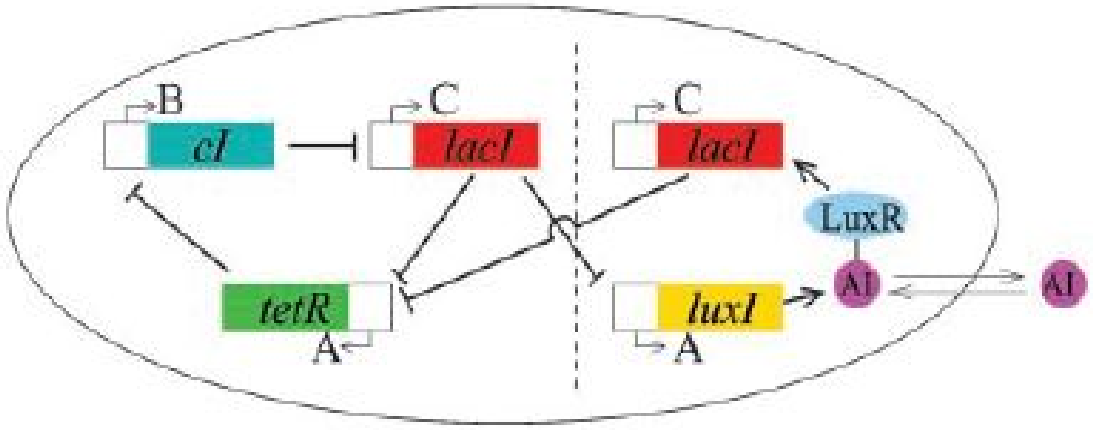
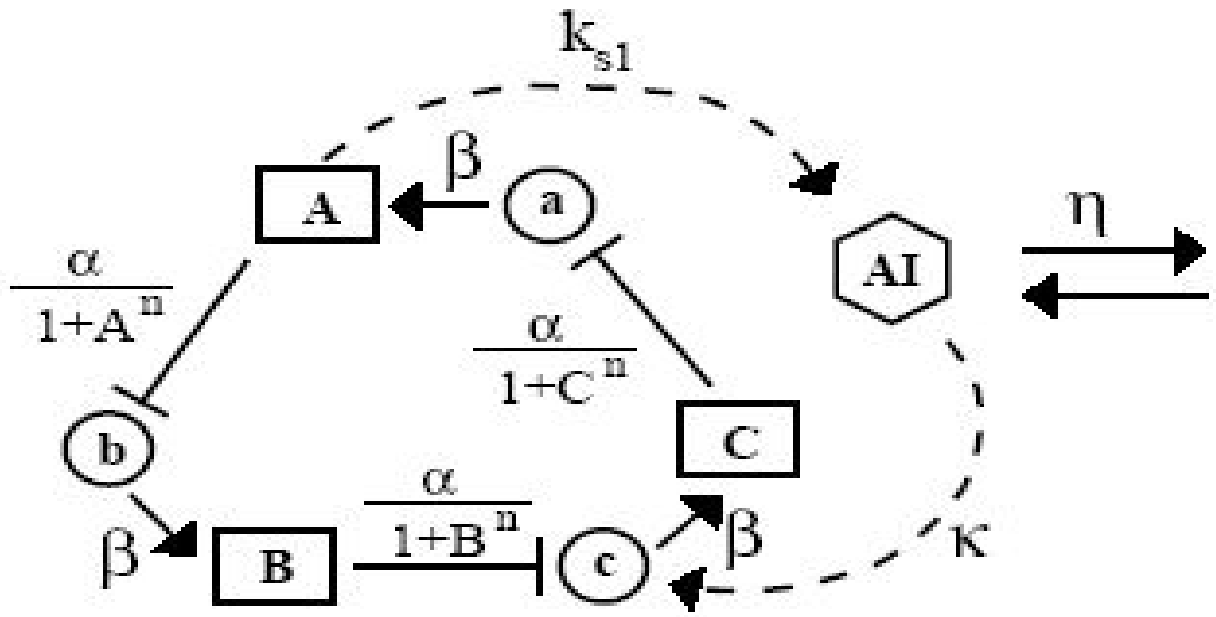


Fig. 1. Scheme of the repressilator network coupled to a quorum-sensing mechanism. The original repressilator module is located at the left of the vertical dashed line, and the new coupling module appears at the right. The letters A, B, and C correspond to the notation used in the text. The coupling module can be added to existing repressilator strains.

The equation-oriented scheme of Repressilator



: The minimal scheme of repressilator with AI production [15].

globally coupled repressilators 2004

mRNA

$$\frac{da_i}{dt} = -a_i + \frac{\alpha}{1 + C_i^n},$$

$$\frac{db_i}{dt} = -b_i + \frac{\alpha}{1 + A_i^n},$$

$$\frac{dc_i}{dt} = -c_i + \frac{\alpha}{1 + B_i^n} + \frac{\kappa S_i}{1 + S_i}$$

proteins

$$\frac{dA_i}{dt} = \beta(a_i - A_i),$$

autoinducer

$$\frac{dS_i}{dt} = -k_{s0}S_i + k_{s1}A_i - \eta(S_i - S_e),$$

$$S_e = \frac{k_{diff}}{k_{se} + k_{diff}}\bar{S} \equiv Q\bar{S}.$$

$$Q = \frac{\delta N/V_{ext}}{k_{se} + \delta N/V_{ext}}$$

bifurcation parameter, which is absent in standard all-to-all coupling

In-phase synchronization due to cell density: PNAS 2004

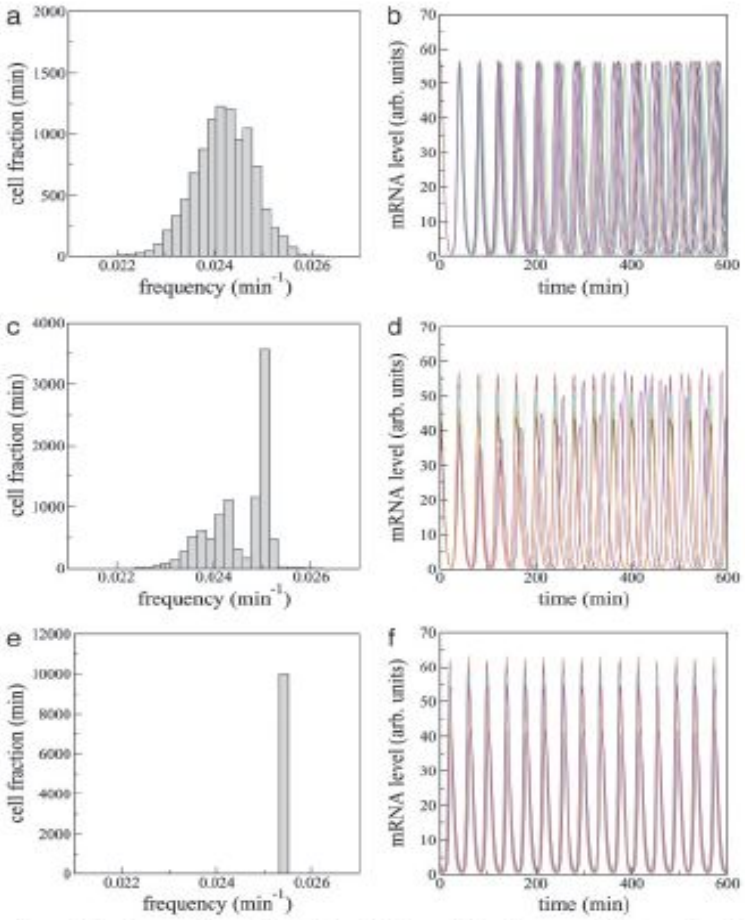


Fig. 2. Frequency histogram (*a*, *c*, and *e*) and time evolution of $b_i(t)$ (*b*, *d*, and *f*) for 10 cells and increasing cell density. (*a* and *b*) $Q = 0.4$. (*c* and *d*) $Q = 0.63$. (*e* and *f*) $Q = 0.8$. Other parameters are $n = 10^4$, $\alpha = 216$, $\kappa = 20$, $n = 2.0$, $k_{b0} = 1$, $\eta = 2.0$, and $k_{d1} = 0.01$. The lifetime ratio β in the different cells is chosen from a random Gaussian distribution of mean $\beta = 1.0$ and standard deviation $\Delta\beta = 0.05$. In the plots (*b*, *d*, and *f*), the oscillators are allowed to evolve from an initially synchronous state. We note, however, that synchronization also arises from initially unsynchronized cultures. arb., Arbitrary.

Typical “question” for synthetic genomics

BMC Genomics

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Research article

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Network motifs: structure does not determine function

Piers J Ingram*[1], Michael PH Stumpf[2] and Jaroslav Stark[1]

Multistability and Clustering in a Population of Synthetic Genetic Oscillators via Phase-Repulsive Cell-to-Cell Communication

Ekkehard Ullner,[1] Alexei Zaikin,[2] Evgenii I. Volkov,[3] and Jordi García-Ojalvo[1]

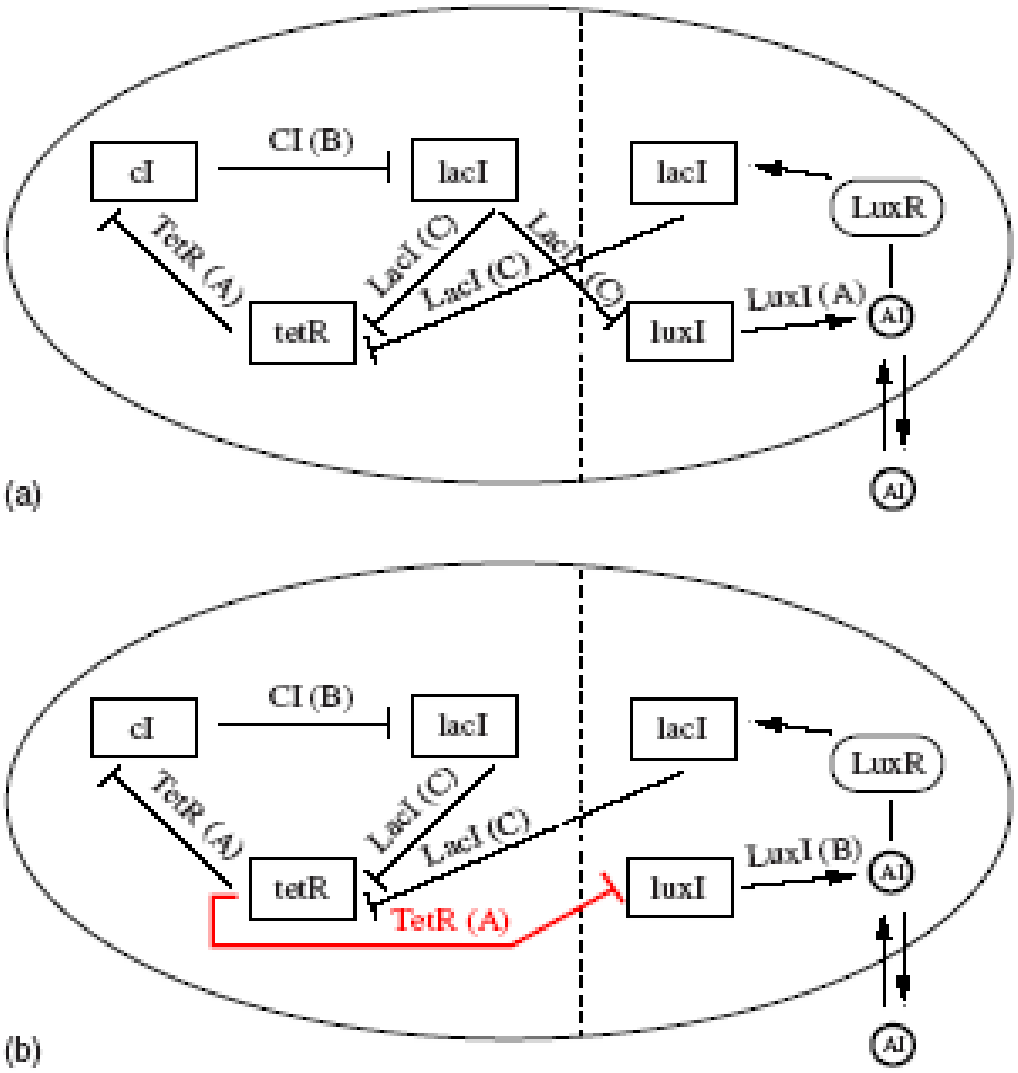
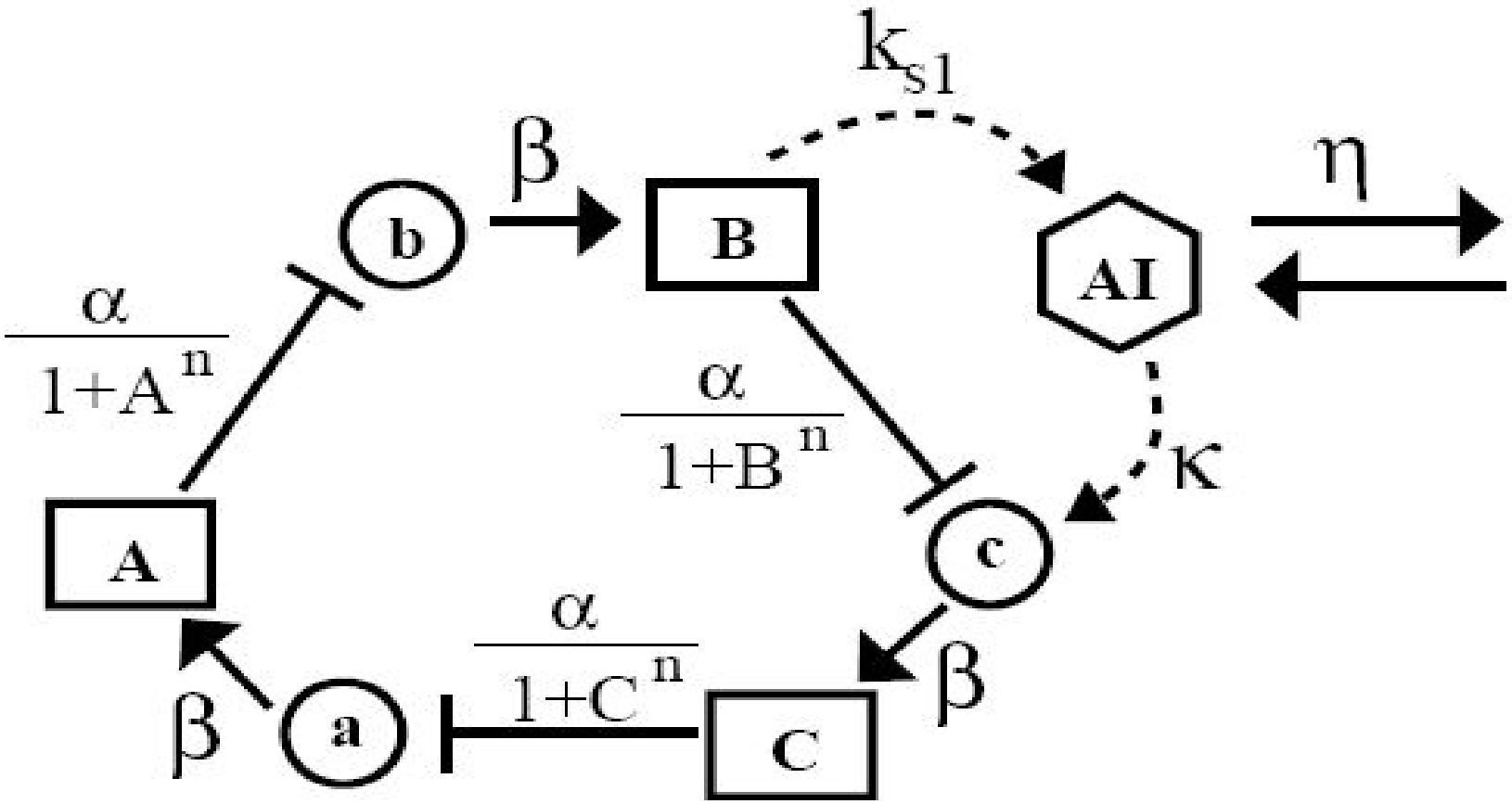


FIG. 1. (Color online) Scheme of the repressilator with quorum sensing cell-to-cell communication: with reinforcing coupling [5]

The equation-oriented scheme of Repressilator – version B



Modified equations of Repressilator – version B

RNA

$$\dot{a}_i = -a_i + \frac{\alpha}{1 + C_i^n}$$

$$\dot{b}_i = -b_i + \frac{\alpha}{1 + A_i^n}$$

$$\dot{c}_i = -c_i + \frac{\alpha}{1 + B_i^n} + \kappa \frac{S_i}{1 + S_i}$$

proteins

$$\dot{A}_i = \beta_a (a_i - A_i)$$

$$\dot{B}_i = \beta_b (b_i - B_i)$$

$$\dot{C}_i = \beta_c (c_i - C_i)$$

AI-production and coupling

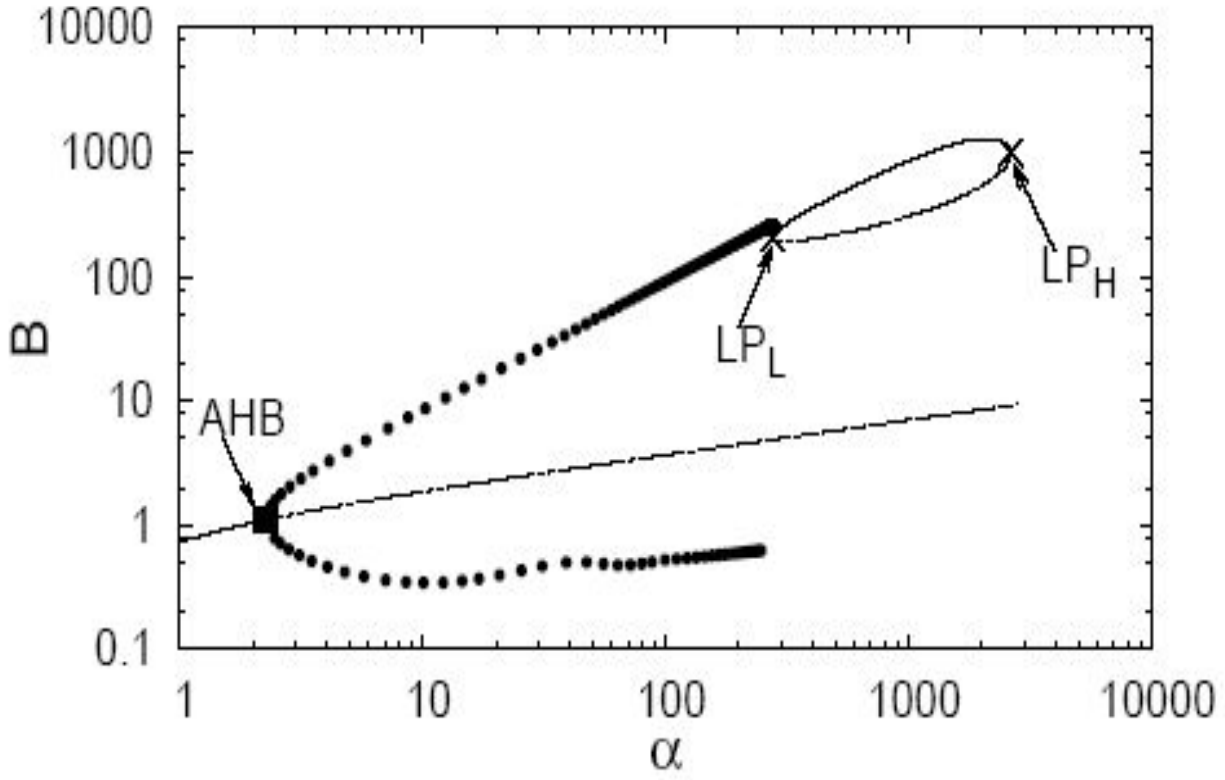
$$\dot{S}_i = -k_{s0} S_i + k_{s1} B_i - \eta (S_i - S_e)$$

$$S_e = Q \bar{S}$$

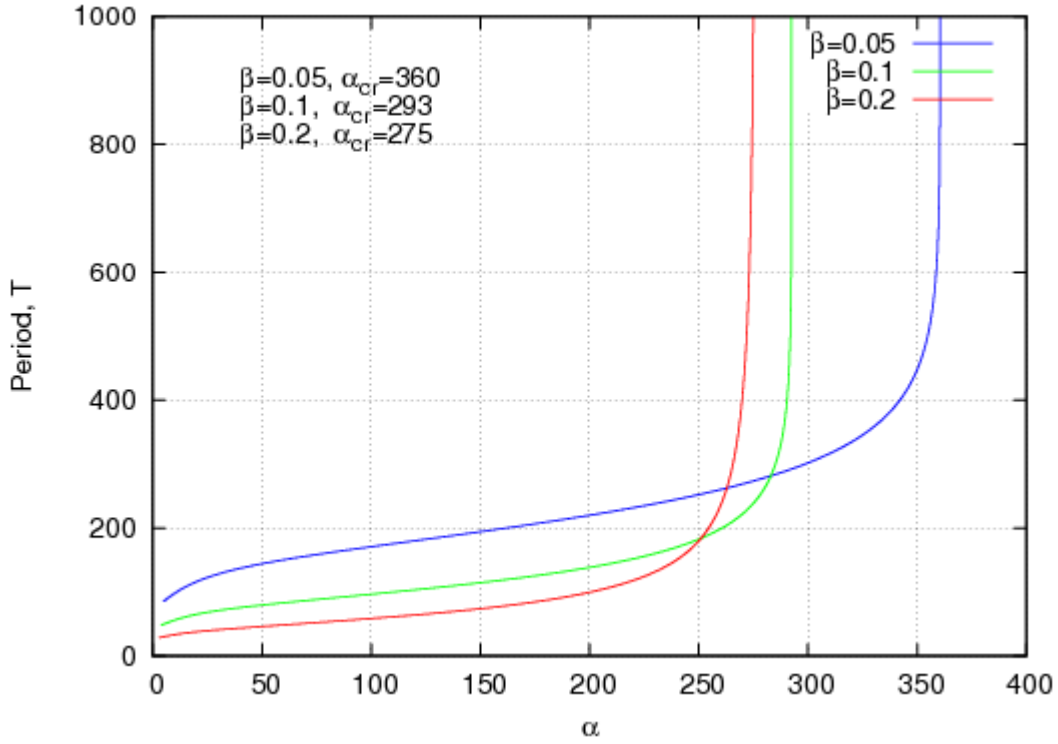
$$\bar{S} = \frac{1}{N} \sum_{i=1}^{N} S_i$$

The isolated Repressilator version B: all **β(i)** =1 – the same timescales for RNA and proteins kinetics.

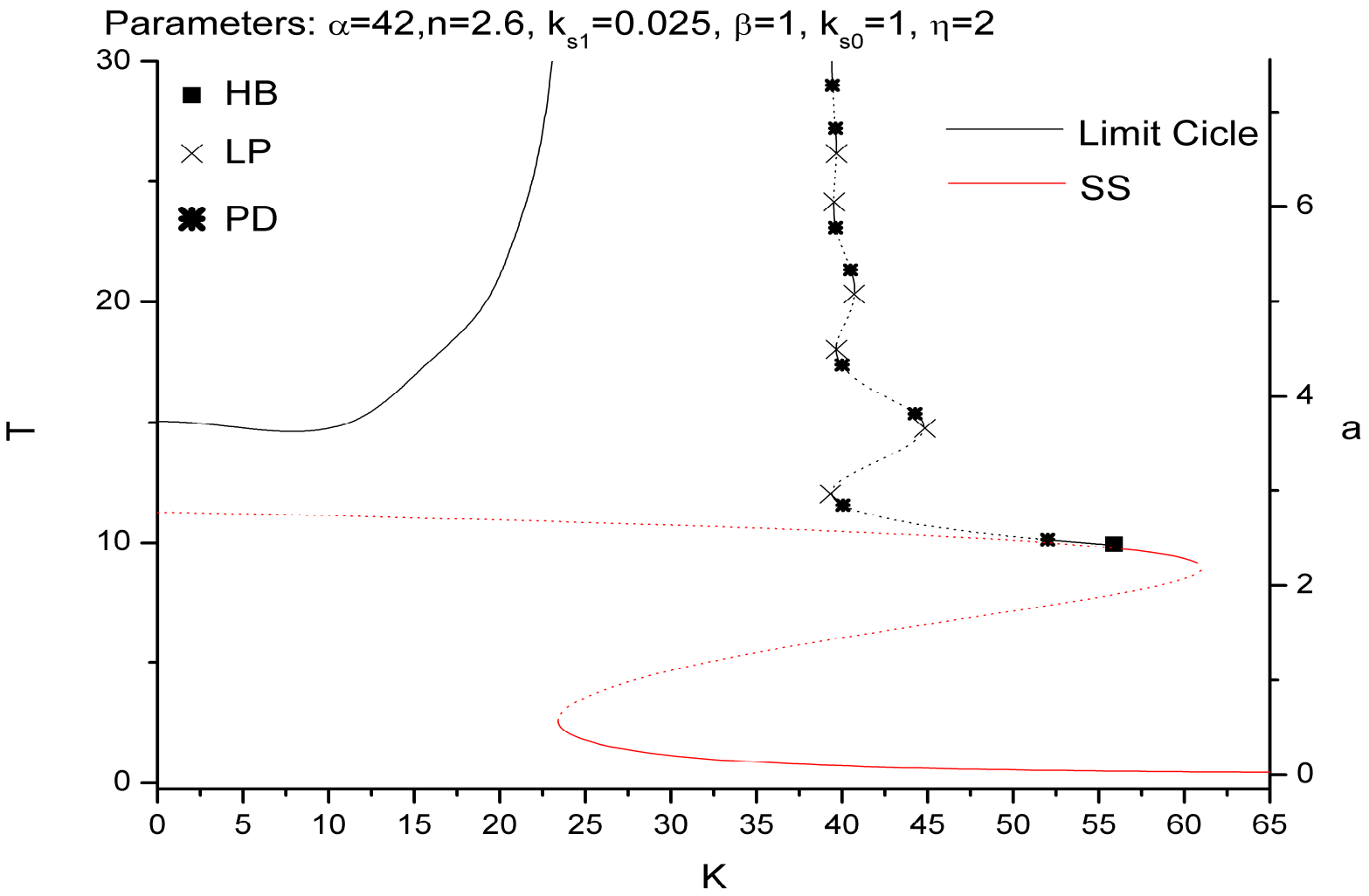
The appearance of Steady State as alpha increases



Period of single Repressilator B as a function **α** under different time scales of protein synthesis and weak AI activity: **eta=2.,k=25.,ks0=1.,ks1=0.01, n=2.6**



Bifurcations of SS and LC as a function of k – the role of autoinducer activity



Bifurcations of LC as a function of α – rate of RNA production, β=1.0

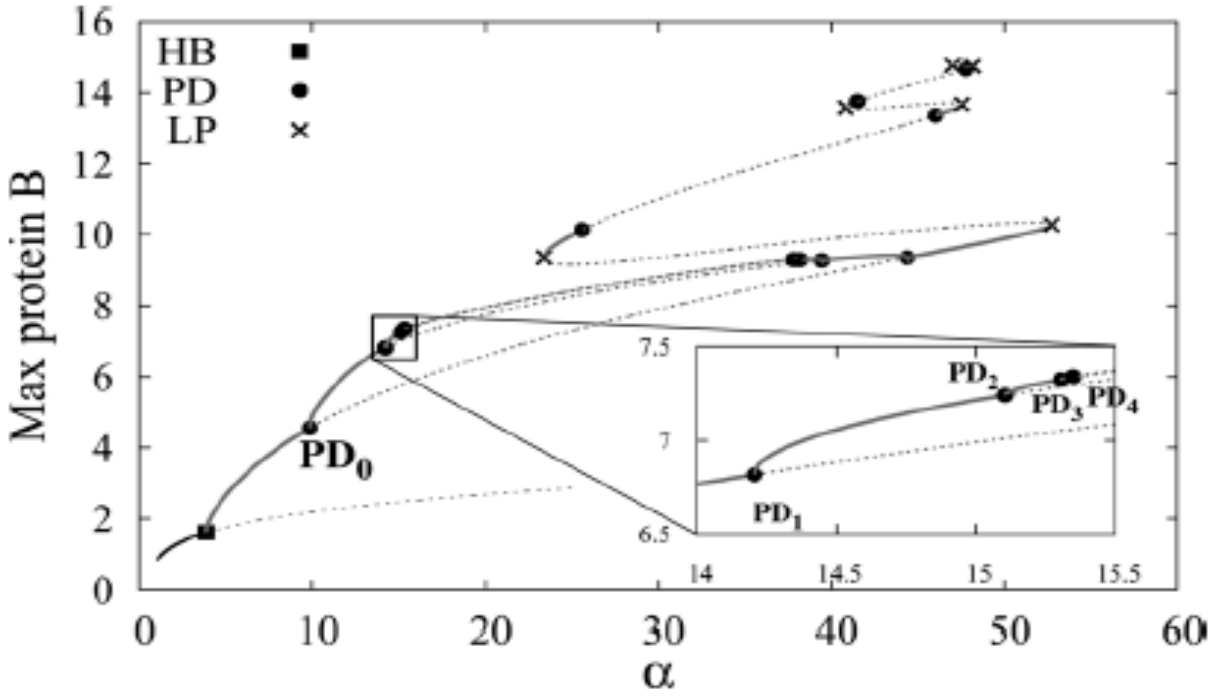


FIG. 9. The detailed continuation of the limit cycle over transcriptional rate α for fixed $\kappa = 40$, $\beta = 1.0$, $k_{s1} = 0.025$ and other parameters: $n = 2.6, k_{s0} = 1, \eta = 2$. The HB gives rise to the limit cycle, which undergoes sequentially two PD and two LP bifurcations. This sequence is repeated several times. Each PD from the main branch (PD_0) gives a cascade of PD (PD_i, where $i = 1, 2, \ldots$) bifurcations leading to chaos.

Main Lyapunov exponent vs α

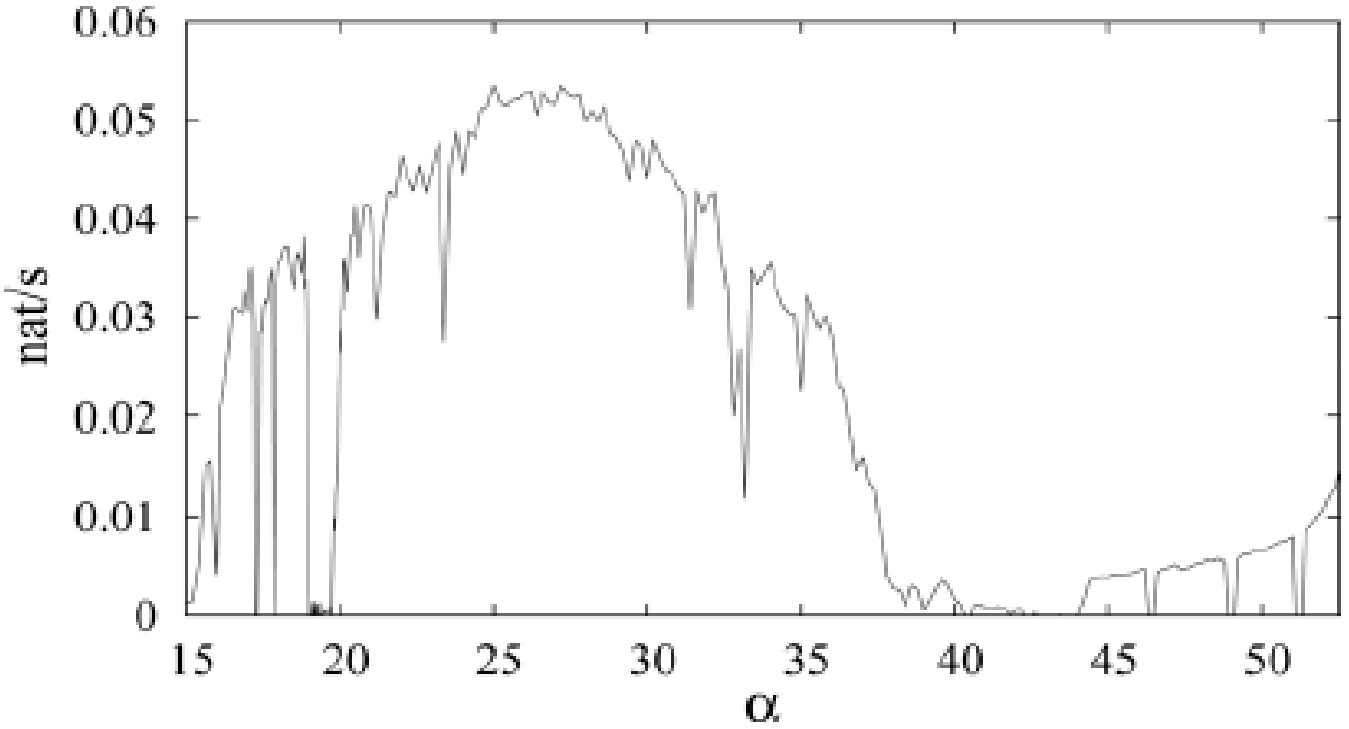


FIG. 10. The main Lyapunov exponent changes along the continuation over the transcriptional rate α for $\kappa = 40$, $\beta = 1.0, k_{s1} = 0.025$ and other parameters: $n = 2.6, k_{s0} = 1, \eta = 2$.

The role of time scales ratio

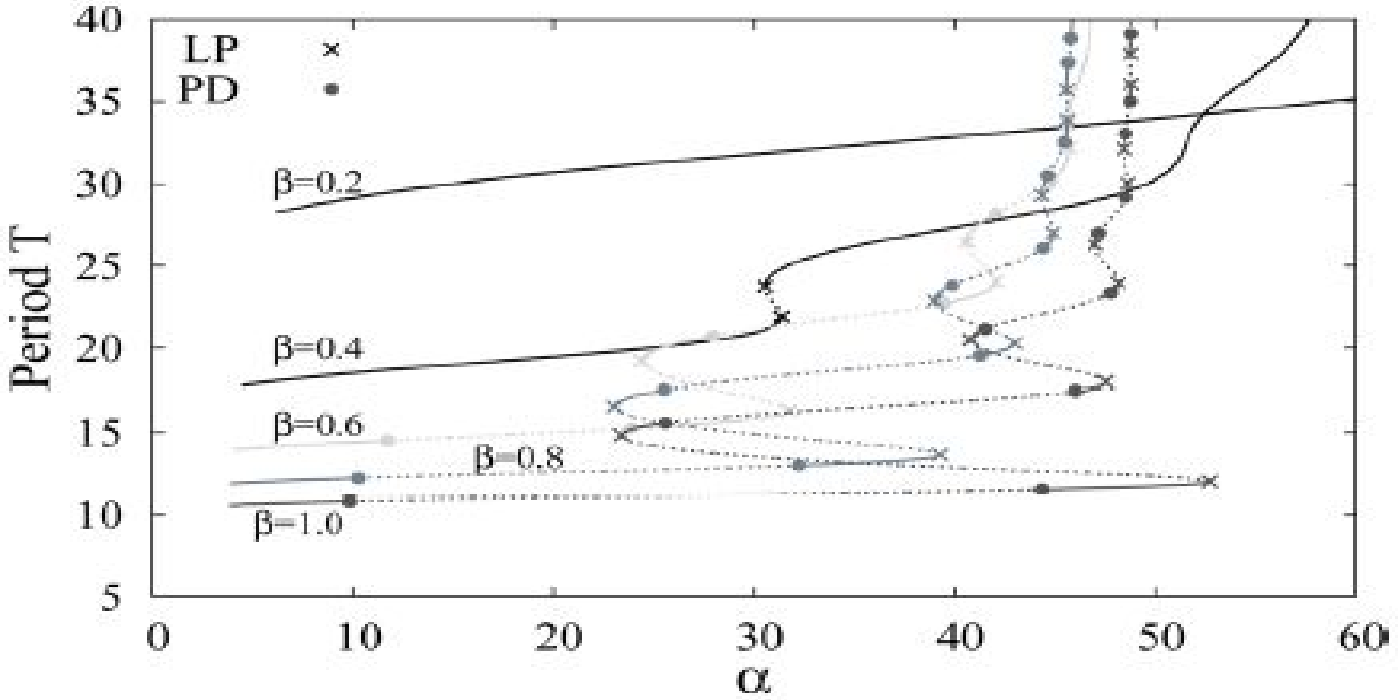


FIG. 12. The mRNA-to-protein lifetime ratio β determines the dynamic properties of the limit cycle (only period T shown): changing both the period-doubling cascades, leading to chaos, and the size of the hysteretic region. PD is a period-doubling bifurcation and LP is a limit point. Parameters: $\kappa = 40, k_{s1} = 0.025, n = 2.6, k_{s0} = 1, \eta = 2$.

Coexistence SS/LC for β=0.1

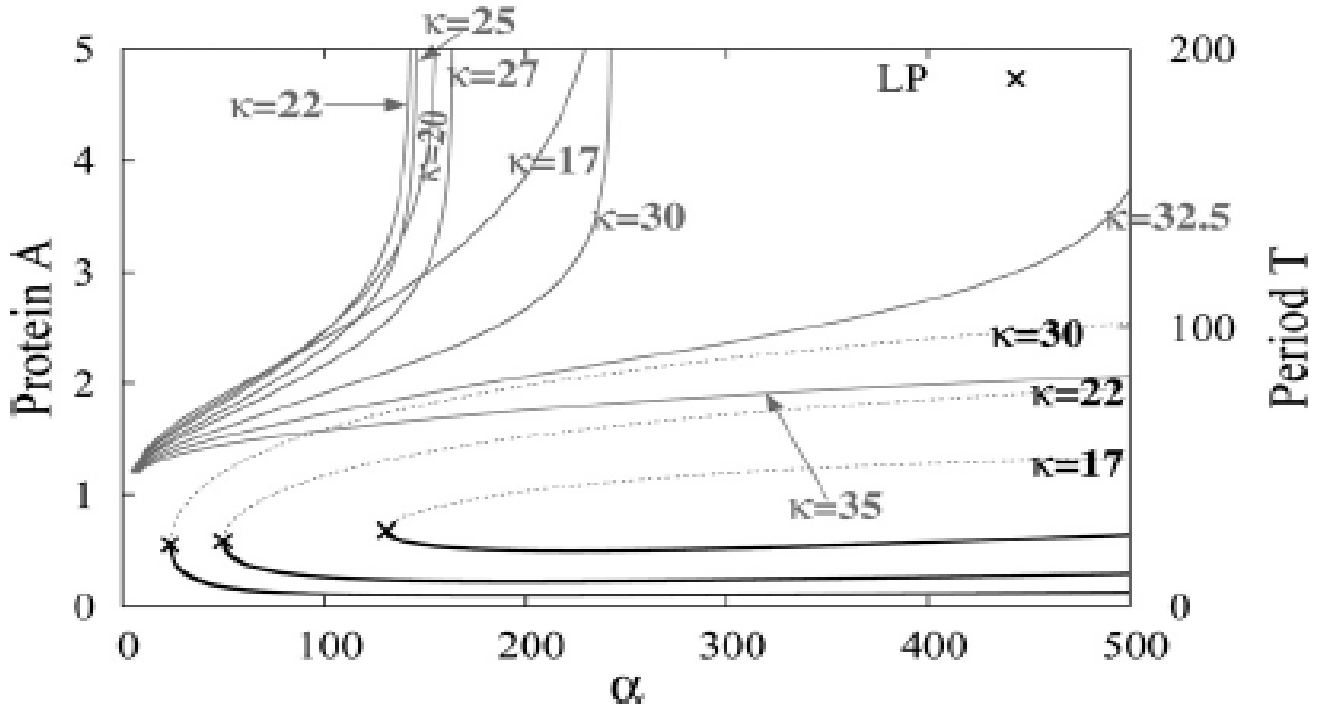


FIG. 7. Coexistence of the limit cycle (right y-axis, gray) and the SSS (left y-axis, black) for different values of κ. LP is a limit point. The bigger values of κ provide bigger region of the SSS. Hysteresis region depends on κ in a nonlinear way. Parameters: $\beta = 0.1, k_{s1} = 0.025, n = 2.6, k_{s0} = 1, \eta = 2$.

Problems: Noise

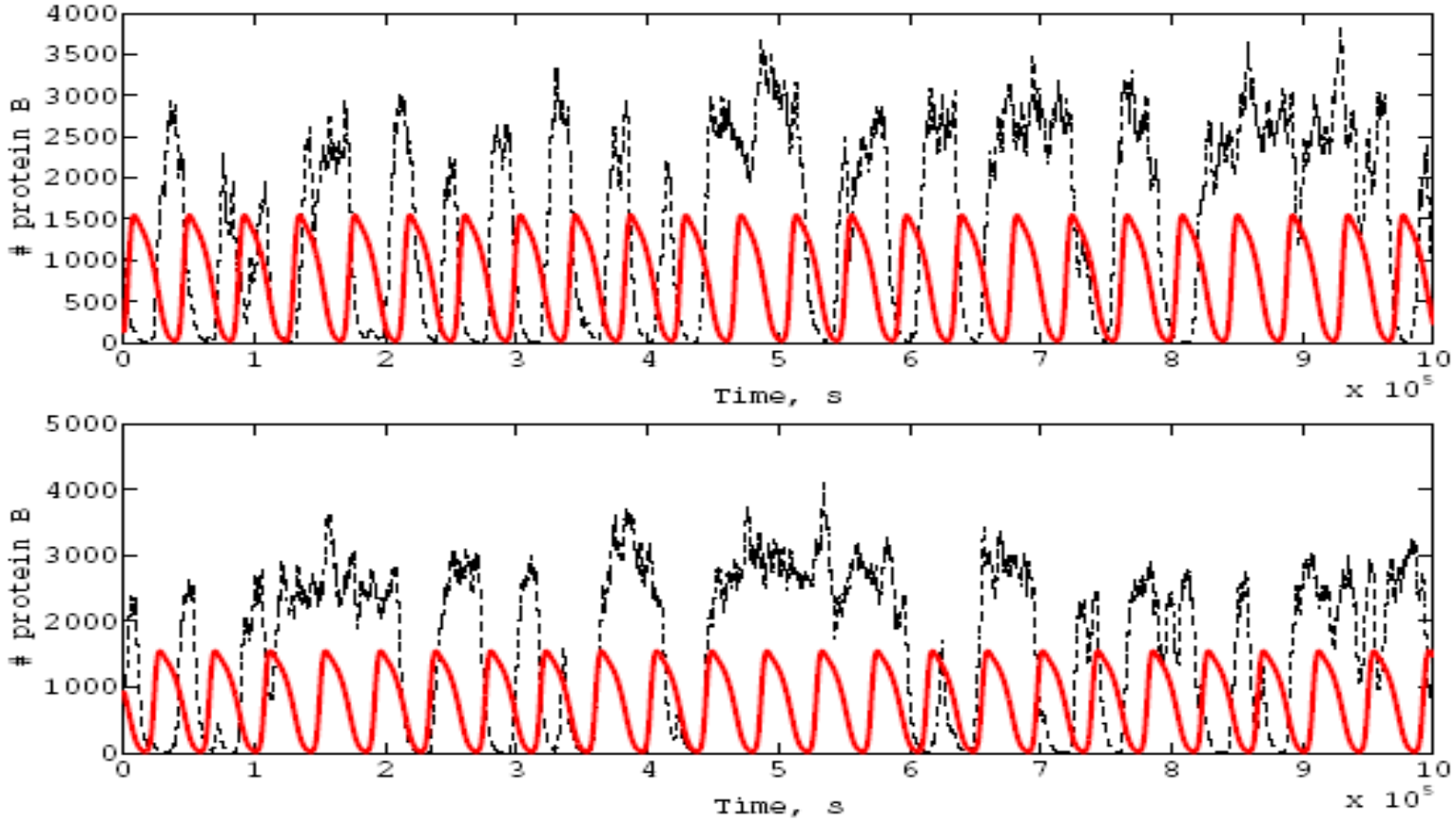


Fig. 7. Time series of the system in the hysteresis region: two values of transcription activity were taken, namely, $\alpha_d = 0.026$ and $\alpha_d = 0.03$ s. The system is in either of two attractors. The deterministic time series is shown in solid red and one stochastic realization as black dashed line.

Conclusions

1. Repressilator, which have been developed as simple robust oscillator, demonstrates interesting collective regimes if the production of the appropriate signal molecule, e.g. Quorum Sensing autoinducer, is taking into account.
2. The version of Repressilator (AI by cI) is developed which manifests the phase-repulsive interaction resulting in the existence and coexistence of many attractors: stable homogeneous steady state, anti-phase limit cycle, stable inhomogeneous steady state (oscillation death), inhomogeneous limit cycle and chaos.
3. This work was motivated by the study of genetic circuits and the rich dynamical behavior may be interpreted as the background of "dynamical differentiation" in synthetic "cell". But the core of Repressilator which is simple sequence of odd number of inhibitory reactions is not specific for genetic reactions that makes the presented mechanism of regimes generation actual for similar circuits in other fields if coupling is properly designed.