Kinetic models of gene regulation

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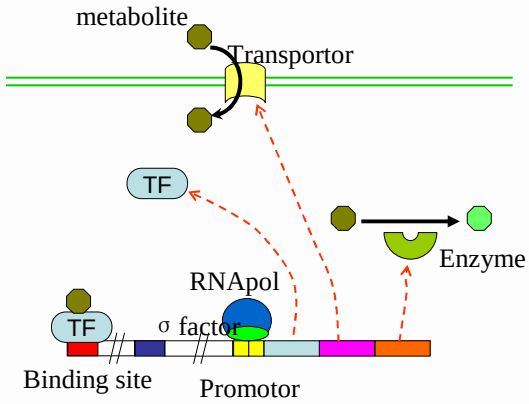
computational systems biology

Synthetic biology

- Design of artificial circuits which mimic functions of living cells
- Try to find common design principles of small gene regulatory circuit
- Artificial life: studies natural life by attempting to recreate biological phenomena from scratch within computers and other artificial media
- Design based on modelling (difference between genetic engineering, metabolic engineering and synthetic biology: rewiring regulatory circuit)
- Examples: toggle switch, oscillation, logical gate, etc

Biological Processes



Transport

Metabolite-protein binding

DNA-protein binding

Transcription and translation

Enzyme catalyzed metabolic reaction

Protein degradation

Two component signal transduction

……

Kinetic models needed to describe the processes!

Represent processes as reactions

- Transport: m_{out} à m_{in}
- M-P binding: m+TFà mTF
- Transcription: mTF+DNAà mTF+mRNA (?)
- Translation: mRNAà mRNA+protein (?)
- Metabolic reaction: m1à m2
- Degradation: proteinà null, mRNAà null(?)

In gene regulation process the mass balance is not important

Most important: determine the rate of these reactions?

$v = f(x)$ Which factors affect reaction rate? In which function?

Kinetic equations

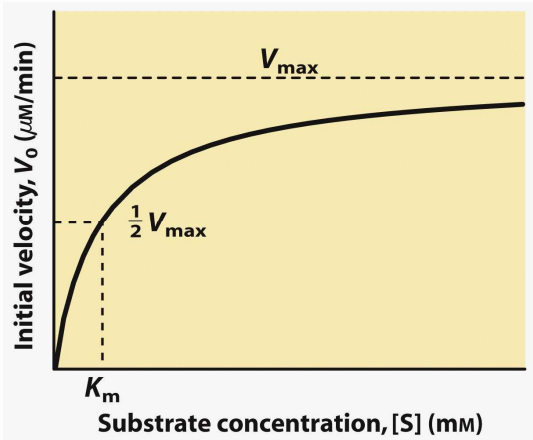
Mass action kinetics $$v = k * S_1 * S_2 * \cdots * S_n$$

Michaelis-Menten Kinetics $$v = \frac{v_m S}{K_s + S}$$

Hill equation $$v = \frac{v_m S^h}{K^h + S^h} = v_m \frac{\left(\frac{S}{K}\right)^h}{1 + \left(\frac{S}{K}\right)^h}$$

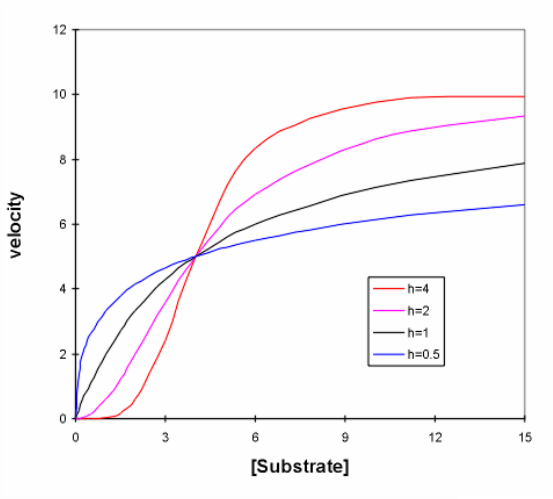
h: Hill coefficient

Michaelis-Menten



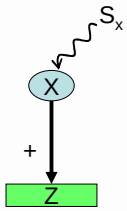
Saturated kinetics

Hill equation



Sigmoid function, a switch mechanism

A simple gene regulation model



- M-TF binding: Sx+Xà XSx
- Transcription: XSx +DNAà XSx +$mRNA_z$
- Translation: $mRNA_z$à $mRNA_z$+Z
- Degradation: Zà null (degradation tag)

Simplification (time scale)

- M-TF binding is a switch process:

$$XS_x = \begin{cases} 0 \text{ if } S_x = 0 \\ X \text{ if } S_x = 1 \end{cases}$$

- Transcription and Translation combined:
  XSx +DNAà XSx +Z

$$v = B_z + \frac{v_m * XS_x^h}{K^h + XS_x^h}$$

Bz=0

- Degradation:

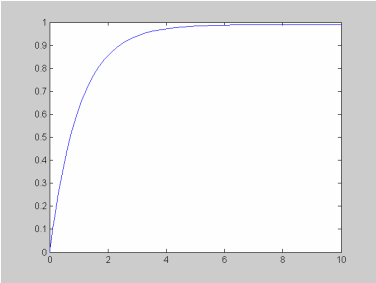
$$v = \alpha_z Z$$

Simulation (switch on Sx=1)

- Which are the variables in the system?
- regulator X is not controlled by other TFs, therefore assume X constant: X=1
- Only one variable Z

$$\frac{dZ}{dt} = \frac{v_m * XS_x^h}{K^h + XS_x^h} - \alpha_z Z = \frac{v_m}{K^h + 1} - \alpha_z Z$$

Production Degradation



Initial concentration Z_0=0

Vm=1, K=0.1, h=2, az=1

Types of regulatory interactions

- EcoTFs database: 50 TFs and their cofactors
- Four types of interactions
  - bind to active an activator (coactivator, AraC+Arabinose): 20
  - bind to deactive a repressor (inducer, LacI+IPTG): 14
  - Bind to deactive an activator (FadR+long-chain acyl-CoA): 5
  - Bind to active a repressor (corepressor, ArgR+arginine): 11

other models

- X bind to DNA to active transcription while XSx not: X +DNAà X +mRNAz

$$X = \begin{cases} 0 \text{ if } S_x = 1 \\ X \text{ if } S_x = 0 \end{cases} \qquad v = \frac{v_m * X^h}{K^h + X^h}$$

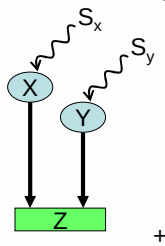
- X bind to DNA to repress transcription while XSx not (ArsR, lac operon, inducer)

$$v = \frac{v_m K^h}{K^h + X^h}$$

- XSx bind to DNA to repress transcription (ArgR+arginine, corepressor)

$$v = \frac{v_m K^h}{K^h + XS_x^h}$$

Combinatorial regulation



AND relationship

$$v = f(X) * f(Y)$$

OR relationship

++

$$v = \frac{v_m\left(\left(X/Kx\right)^h + \left(Y/Ky\right)^h\right)}{1 + \left(X/Kx\right)^h + \left(Y/Ky\right)^h}$$

+-

$$v = \frac{v_m\left(\left(X/Kx\right)^h + 1\right)}{1 + \left(X/Kx\right)^h + \left(Y/Ky\right)^h}$$

-+

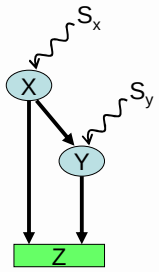
$$v = \frac{v_m\left(1 + \left(Y/Ky\right)^h\right)}{1 + \left(X/Kx\right)^h + \left(Y/Ky\right)^h}$$

--

$$v = \frac{v_m}{1 + \left(X/Kx\right)^h + \left(Y/Ky\right)^h}$$

Mangan and Alon, (2003) PNAS, 100:11980

Feed forward loop



FFL is one of the most important network motifs in the transcriptional regulatory network (Shen-Oorr et al., Nature Genetics, 31:64).

The regulator Y is also regulated by X, therefore the concentration of Y is also changed but not constant

$$\frac{dY}{dt} = \frac{v_{my} * XS_x^h}{K_{XY}^h + XS_x^h} - \alpha_y Y$$

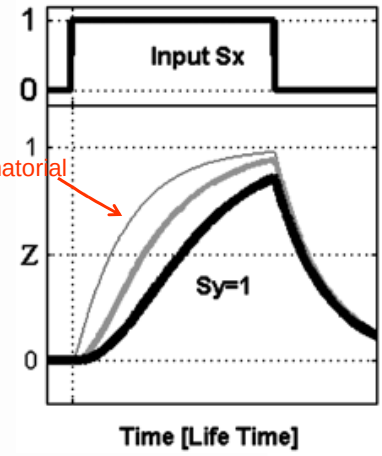
AND

$$\frac{dZ}{dt} = \frac{v_m * XS_x^h * YS_y^h}{\left(K_{xz}^h + XS_x^h\right)\left(K_{yz}^h + YS_y^h\right)} - \alpha_z Z$$

OR

$$\frac{dZ}{dt} = \frac{v_m\left(\left(XS_x/Kxz\right)^h + \left(YS_y/Kyz\right)^h\right)}{1 + \left(XS_x/Kxz\right)^h + \left(YS_y/Kyz\right)^h} - \alpha_z Z$$

Dynamics of FFL

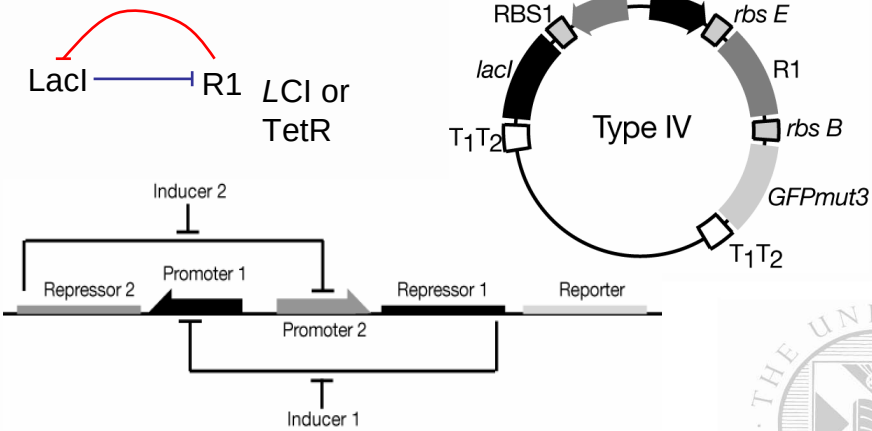


Simple combinatorial regulation

The response to Sx switch on is delayed for FFL controlled genes. The target gene only respond to persistent signal but not noise

Mangan and Alon, (2003) PNAS, 100:11980

Toggle Switch



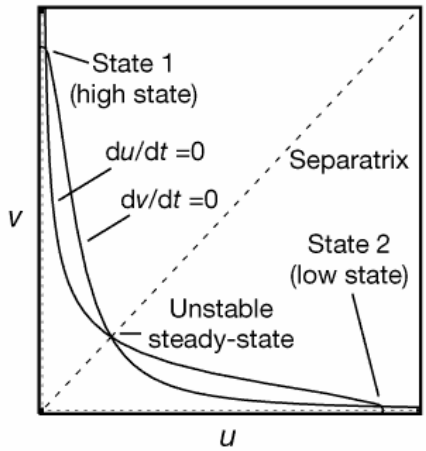
Gardner et al, Construction of a genetic toggle switch in *Escherichia coli*, Nature, *403-339, 2000*

Model

Dimensionless model

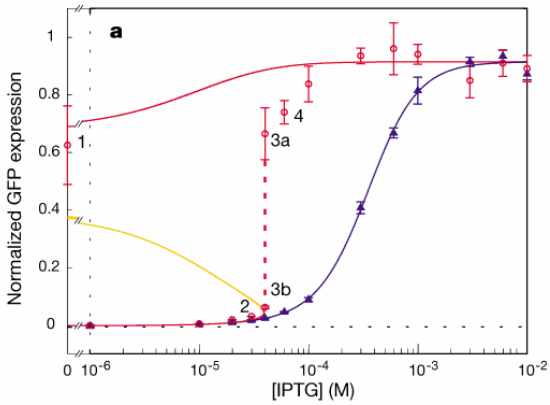
$$\frac{du}{dt} = \frac{\alpha_1}{1+v^{\beta}} - u$$

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

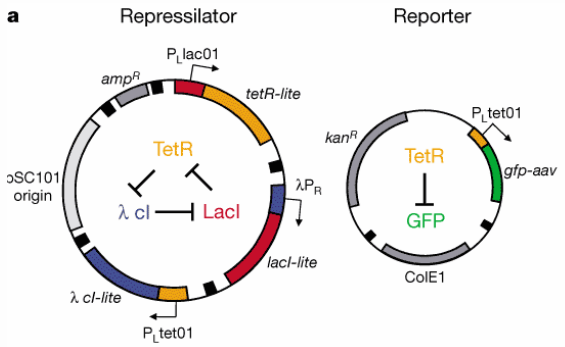


$$\beta = \gamma = 2$$

IPTG Induction



Repressilator



Link the repressor genes to promoters controlled by another repressor.

Elwitz et al, A synthetic oscillatory network of transcriptional regulators, Nature, 403-335, 2000

Model

- 12 reactions
- Transcription of *lacI, tetR* and *cI* mRNA
- Translation of LacI, TetR and CI protein
- Degradation of *lacI, tetR* and *cI* mRNA
- Degradation of LacI, TetR and CI protein
- Degradation: mass action kinetics
- Transcription: Hill equation with basal level transcription
- Translation: mass action kinetics

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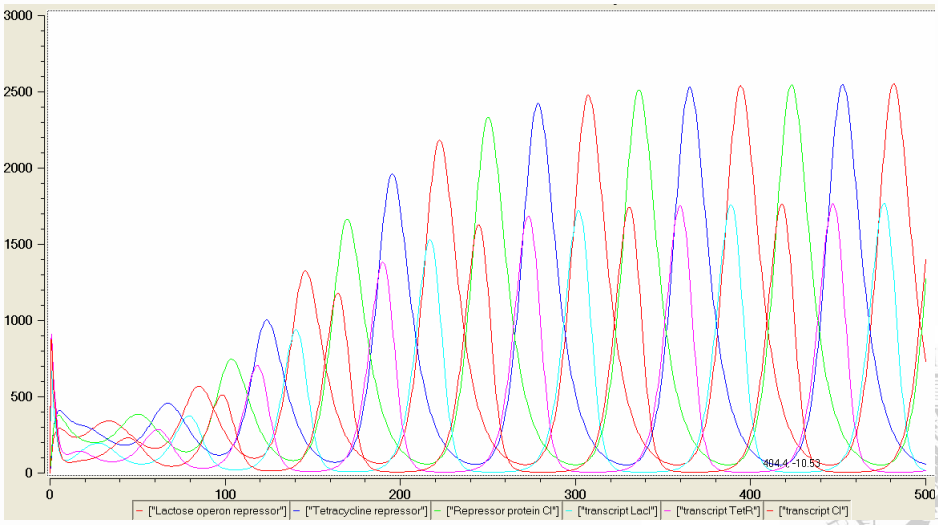
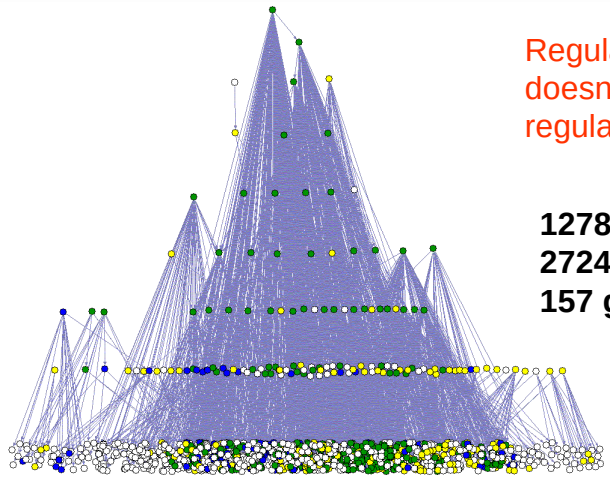
Simulation result



Questions

- If combine transcription and translation
- Can not get oscillation behavior?
- Anyone want to do a stability analysis?
- Can we use activators rather than repressors for circuit design? (more activators than repressors in the network). Any new dynamic behavior?

Unfortunately



Regulatory loop doesn't exist in E. coli regulatory network

1278 genes
2724 interactions
157 genes for TFs

Can you design a new circuit showing oscillation behavior?

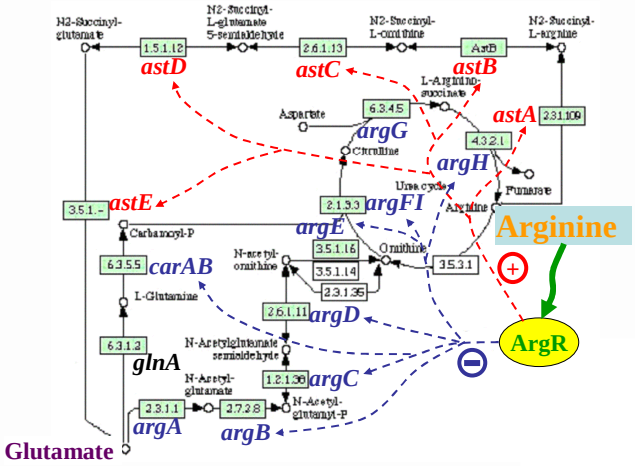
In real network

	Coherent FFLs: 330				Incoherent FFLs: 152			
type								
number	265	28	7	30	119	9	8	16
examples	*flhC-fliA-fliGH*	*cpxR-csgD-csgA*	*fis-hns-cysG*	*fnr-narL-dcuB*	*crp-nagC-manXYZ*	*arcA-betI-betAB*	*ihf-flhD-nrfA*	*fnr-narL-moeAB*

Dynamic behavior

Mangan and Alon, (2003) PNAS, 100:11980

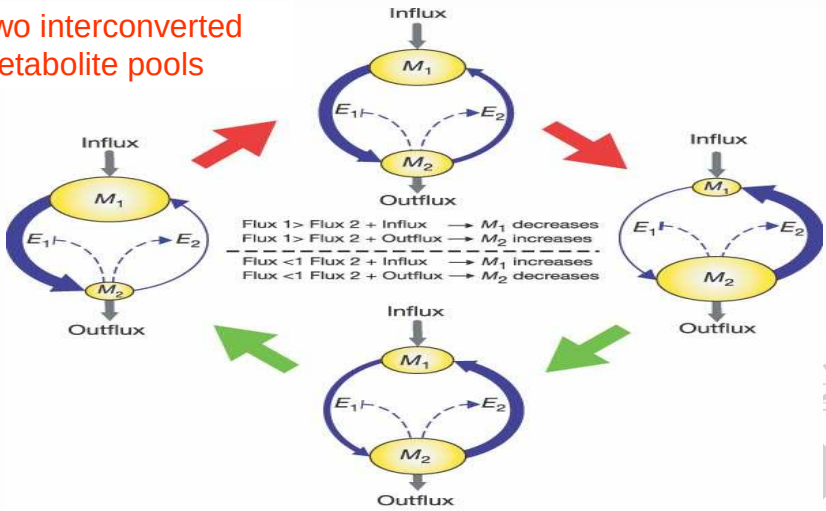
Feedback through metabolites



A circuit with feed back loops through metabolite-TF interaction?

Metabolator: concepts

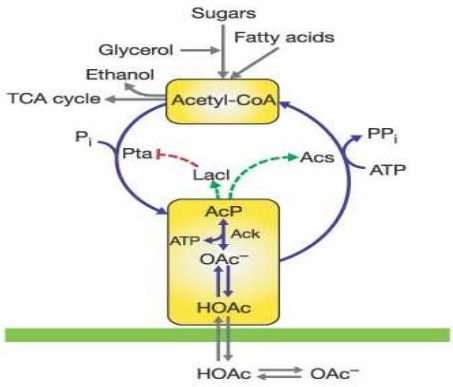
Two interconverted metabolite pools



Fung et al, A synthetic gene-metabolic oscillator, Nature, 435-118, 2005

Metabolator: real design

Design a gene circuit so that Pta and Acs are controlled by AcP



AcP active NtrC which binds to glnAp2 promoter

A plasmid where acs is linked with glnAp2

Also link lac repressor (LacI) with glnAp2

LacI binds to lacO-1 promoter, pta linked with that promoter

GFP controlled by LacI through tac promoter for readout

Single cell culture on thin agar gel

Open questions

- Many other promoters controlled by NtrC and LacI, How about use other promoters, how is the effect of other genes regulated by NtrC
- How about use other repressors rather than LacI
- How about other pathways, for example between F6P and FDP

Modelling

- Glycolytic flux, V_gly: nil -> AcCoA fixed
- Flux to TCA cycle/ETOH, V_TCA: AcCoA -> TCA/EtOH
- HOAc ex/import,reversible, V_out: HOAc -> HOAc_E
- Acetic acid-base equillibrium, reversible, V_Ace: OAC + H -> HOAc
- reversible, V_Ack: AcP + ADP -> OAc + ATP

Mass action

- V_Pta: AcCoA + Pi -> AcP + CoA
- V_Acs: OAC + ATP -> AcCoA +PPi

M-M kinetics

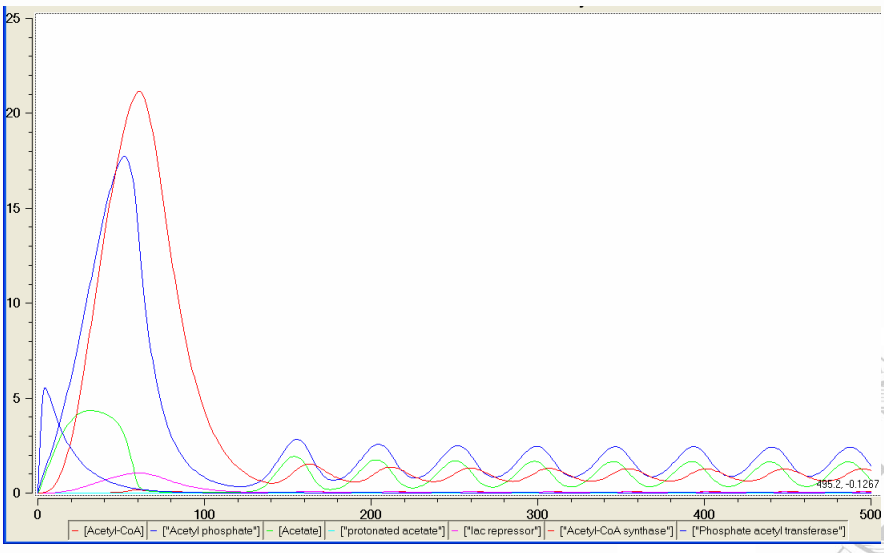
- Expression of LacI, R_LacI: nil -> LacI
- Expression of Acs, R_Acs: nil -> Acs
- Expression of Pta, R_Pta: nil -> Pta

Hill equation +basal level

- LacI degradation, R_dLacI: LacI -> nil
- Acs degradation, R_dAcs: Acs -> nil
- Pta degradation, R_dPta: Pta -> nil

Mass action

Results



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Questions

- How If the in flux is also related with metabolite concentration but not constant
- How about if we group the AcP pool
- How about if we have more detail models of gene transcription
- Will the model show the same qualitative behavior?

Databases on models

- Biomodels database http://www.ebi.ac.uk/biomodels/
- Nearly 100 Curated models from literature on metabolic pathways, gene regulatory circuits and signal transduction pathways
- SBML files can be directly imported by many softwares for simulation
- Graph visualization for easy checking

Softwares for modelling

- Copasi: www.copasi.org, very good software for kinetic model analysis but not for visualization
- Jdesigner/Jarnec: sys-bio.org, diagram+simulation
- CellDesigner: automatic layout+simulation
- Simbiology: by mathworks, powerful and expensive, only tool to deal with the currency metabolites in visualization

Alves, et al: Tools for kinetic modeling of biochemical networks, Nature Biotechnology, v24:667, 2006

Copasi Demo

- Add reactions, select rate law and set parameters
- Check species, give initial concentration and set constant species
- Check generated ODE
- Set plots and run simulation
- User defined kinetic equation
- Import sbml file, sbml files from biomodels