Engineering self-sustaining networks: resolving the paradox

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Synthetic biology

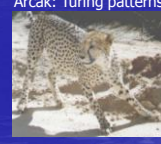
Making Life
Changing Life
for a purpose

Always: stick molecules into sufficient networks

What is Life?

- A self-sustaining (stubborn) system
  - Repair or duplication
  - Gibbs energy dissipation
  - Labile structure
  - Heterogeneity & symmetry breaking beyond statistical thermodynamics

Arcak: Turing patterns

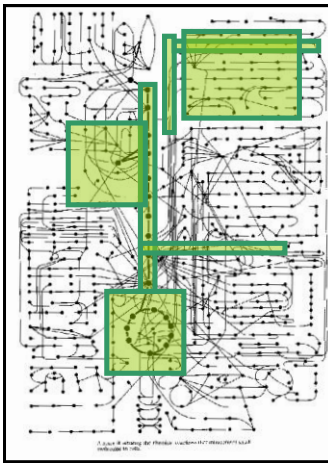


Engineering the self-sustaining networks

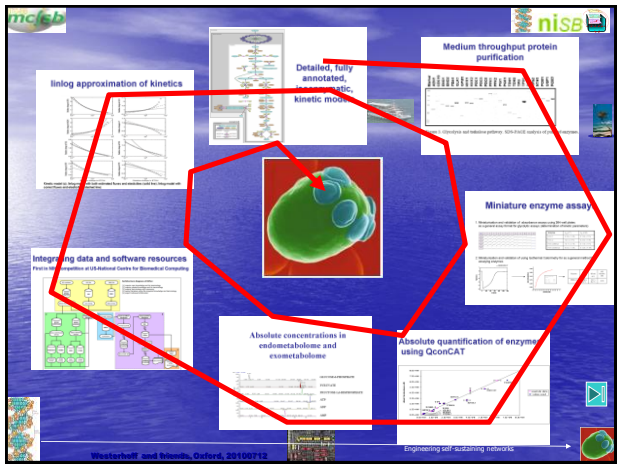
- Self-sustaining cell may not worry about efficiency
- How does the cell engineer itself
- Synthetic biology: microsurgery
- Noise and heterogeneity beyond the thermodynamic limit
- Towards synthetic biology of heterogeneous cross-talk

Our first objective for synthetic biology:

a dynamic model
of a metabolic chassis
to facilitate synthetic biology



- **Chassis**
  - **Pathways that manage the large fluxes essential for the conserved properties and Gibbs energy:**
  - **First: carbon and energy metabolism**

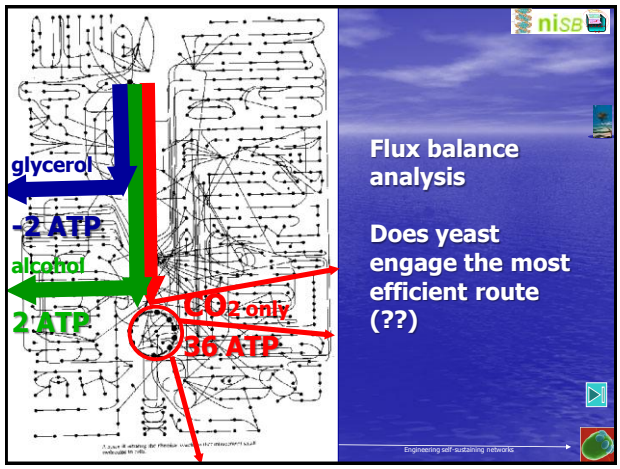


Michael hecht: Life is special

It is, hence:

An example of self-sustaining network-based complexity:

lack of interest in efficiency



Summary of exometabolomics: **Not** the maximal efficiency route

Flux		Flux percentage of input
$J_{Biomass}$		6
J_{CO2} (offgas)		20
$J_{ethanol}$ (exometabolome plus off gas)		74
$J_{Acetate}$ (exometabolome)		0
$J_{Glycerol}$ (exometabolome)		2
$J_{Acetaldehyde}$ (exometabolome)		0
$J_{Trehalose}$ (exometabolome)		1

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Engineering self-sustaining networks

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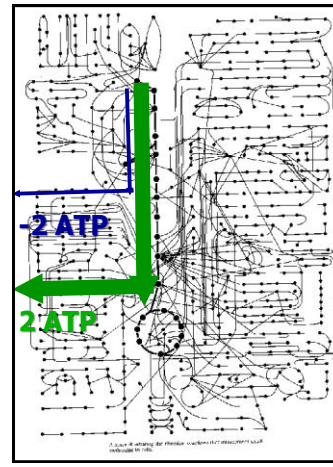
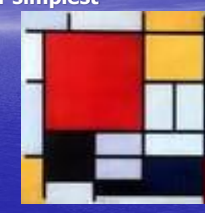
Engineering self-sustaining networks



Neither maximally efficient
Nor simplest



Engineering self-sustaining networks

Engineering the self-sustaining networks

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Engineering strategy for a network

- Engineer the most controlling step?
- Or
- Do what the organism does? Or at least learn from this.

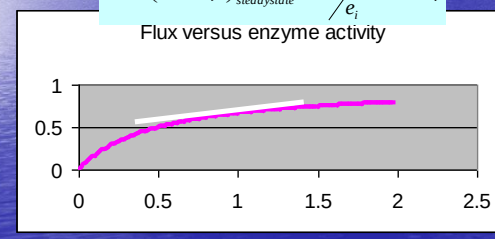
How to identify the strongest controller?

experimentally or

in silico

The Flux Control Coefficient

$$C_i^J = \left(\frac{d \ln |J|}{d \ln e_i} \right)_{steadystate} = \frac{dJ/J}{de_i/e_i} = \frac{\%dJ}{\%de_i}$$



Flux control distribution *ex silico*:

	C^J
GLT	0.97
HK	0.15
PGI	0.00
PFK	0.00
ALD	0.00
TPI	
GAPDH	0.01
PGK	0.00

High control confirmed experimentally, though indirectly)

Should we just engineer this step, or should we perhaps try to learn from the Biology?

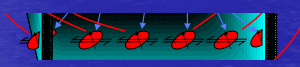
Does the organism re-engineer itself by modulating the step with the highest control on the flux?

For: Control ≠ Regulation

Control: what limits a flux

Regulation: what the cell actually does to change the flux

The cell invokes gene expression: adaptation



Straighforward strategies for regulation of **pathway flux**

Does the cell only regulate the step with highest control?

or

Does the cell regulate flux by reducing all enzyme levels equally?

Regulation analysis:
How much of function is regulated by gene expression, how much metabolically?

$$v = v(e, X) = e \cdot \upsilon(X)$$

Gene expression

Metabolic/direct

$$\rho_h \equiv \frac{\Delta \ln e}{\Delta \ln J} \cong \frac{\textit{fold change in amount of enzyme}}{\textit{fold change of flux through it}}$$

Average over a larger number of studies

Enzyme	ρ_h	Standard deviation
HXT	0.0	0.9
HXK	0.3	0.6
PGI		
PFK		
ALD		
TPI		
GAPDH		
PGK		
GPM		
ENO		
PYK		
PDC		
ADH		
Overall		

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Not the high control step!

	C^J
GLT	0.97
HK	0.15
PGI	0.00
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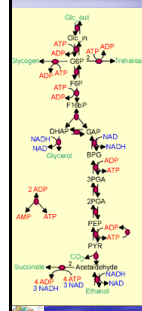
Strategies of regulation of **pathway flux**

Does the cell only regulate the step with highest control?

or

Does the cell regulate flux by reducing all enzyme levels equally?

If homogeneous regulation



- System Biology laws

$$C_1^J + C_2^J + C_3^J + ... + C_n^J = 1$$

- If all ρ_h's equal to each other

$$\left(C_1^J + C_2^J + C_3^J + ... + C_n^J\right) \cdot \rho_h = 1$$

- **then:**

$$\rho_h = 1$$

Is Regulation homogeneous (the entire network equally)?

Nitrogen starvation	ρ_h
HK	1.0
PGI	0.8
PFK	0.4
ALD	1.1
TPI	0.1
GAPDH	0.7
PGK	0.0
PGM	1.0
ENO	0.4
PK	1.4
PDC	2.3
ADH	1.7

No:!

Does the living cell use the straighforward strategies for regulation of **pathway flux?**

Does the cell only regulate the step with highest control?

or

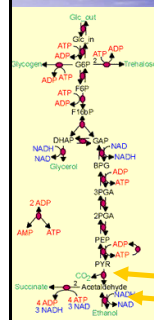
Does the cell regulate flux by reducing all enzyme levels equally?

Neither of the obvious strategies is used by the cell

Rather: a more sophisticated strategy

Propeller enzymes/genes: $\rho_{h,i} > 1 \Rightarrow \rho_{m,i} < 0$

Regulation understood: propeller enzymes and followers



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ADH	1.7

New engineering strategy?

Identify and then activate the propeller enzymes, i.e. use the regulation of the cell itself:

A different kind of Control Theory (cf. Murat Arcak):

Engineering self-sustaining systems

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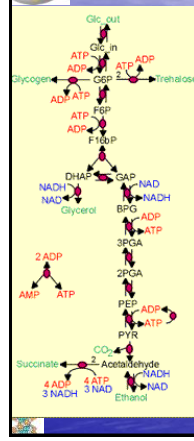
Engineering self-sustaining networks

Synthetic Biology

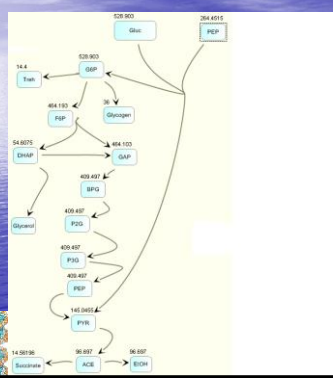
The microsurgery method



Engineering self-sustaining systems

Such that they do not notice:

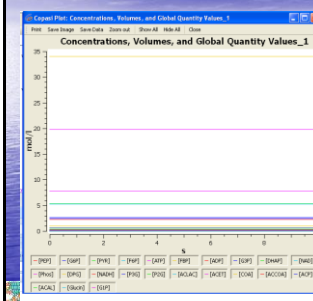
– Keep metabolite concentrations the same



The principle *in silico*: balance the fluxes with expression levels

Engineering self-sustaining networks

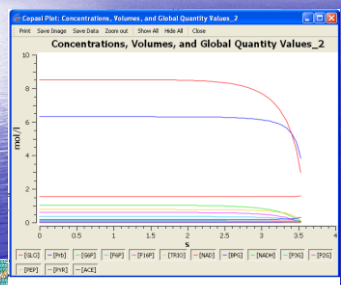
Yeast HXT+HXK into *L. lactis* (*in silico*)



A steady state indeed

Engineering self-sustaining networks

L. lactis PTS into yeast (*in silico*)



Steady state, but....

An unstable one

In silico microsurgery for synthetic biology:

- Stability requires additional regulatory loops
- Criterion: real part of eigenvalue with positive real part

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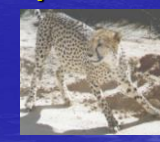


The steady consequences of dynamic noise

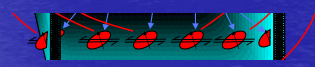
Cell-cell heterogeneity

Pattern formation

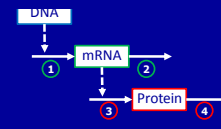
Living systems may expend Gibbs free energy to stay heterogeneous

but how?

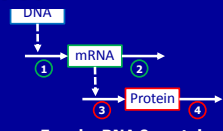
Non-equilibrium metabolic noise might be generated by gene expression



Dependence of Protein Noise on Rates



Dependence of Protein Noise on Gene expression noise



Fast mRNA reactions

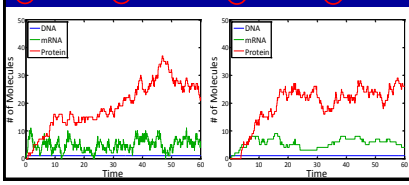
1 = 5*DNA 2 = 1*mRNA
3 = 0.5*mRNA 4 = 0.1*prot

Equal mRNA & protein reactions

1 = 0.5*DNA 2 = 0.1*mRNA
3 = 0.5*mRNA 4 = 0.1*prot

Fast protein reactions

1 = 0.5*DNA 2 = 0.1*mRNA
3 = 5*mRNA 4 = 5*prot



Thermodynamic view

Noise can contain Gibbs free energy

Equilibrium noise is limited in magnitude

$$\sigma^2(N) = \bar{N}$$

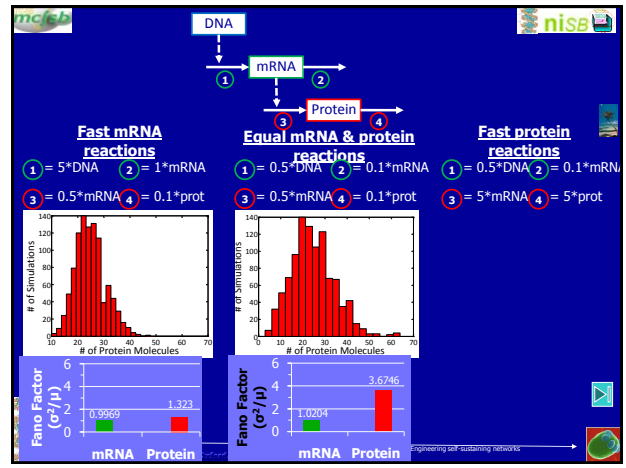
$$\frac{\sigma(N)}{\bar{N}} = \frac{1}{\sqrt{\bar{N}}}$$

$$\frac{\sigma(4)}{4} = 0.5$$

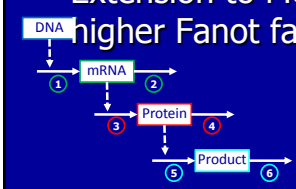
$$\frac{\sigma(1000)}{1000} = 0.01$$

$$Fano\ factor = \varphi = \frac{\sigma^2(N)}{\bar{N}} = 1\ at\ equilibrium$$

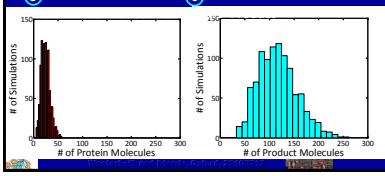
$$\varphi \gg 1?$$



Extension to Metabolites: even higher Fanot factor



1 = 0.5*DNA
2 = 0.1*mRNA
3 = 0.5*mRNA
4 =
5 =
6 = 0.1*Protein



Metabolic noise exceeding the thermodynamic limit through gene expression hierarchy

Step towards diversity and robustness?

Implications for synthetic biology of patterns

– Non-Poisson distribution proves mechanism to reach heterogeneity beyond triviality

– Engineering the required noise characteristics

See also poster by Vicky Jackson

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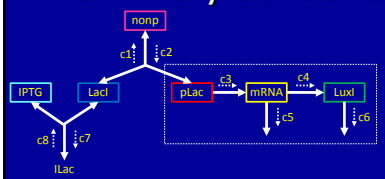
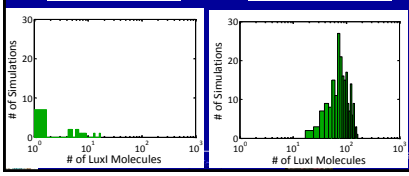
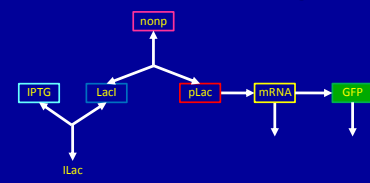
Preliminary Sender Cell Model



5 IPTG Molecules **35 IPTG Molecules** **200 IPTG Molecules**



Preliminary Sender Cell Experiments (Fluorescent Protein Version)



Higher diversity than with the simple model

Self-sustaining networks are amplifying?

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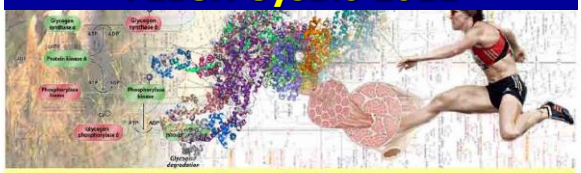

We cordially invite you to the

Joint FEBS/Systems X Advanced Lecture Course on

Systems Biology – From Molecules to Function

26 February 2011 – 3 March 2011: Innsbruck, Austria, EU

http://www.febssysbio.net/

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