Information management across biological scales
Information management across biological scales

Neutrophil chasing a bacteria (D. Roger 1950)
Information management across biological scales

- Monod and Jacob and *Gene Regulation* in bacterial species (1961)

![Image of a bacteriophage](image.png)

![Image of a genetic switch](image.png)
How does it work?
Where/what is the computer inside the microscopic bags of chemical that we call cells?
Molecular Systems & Circuits

Operon Lactose (Jacob and Monod)
Molecular Systems & Circuits

Operon Lactose (Jacob and Monod)

<table>
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<th>Glucose</th>
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<th>Bacteria decision</th>
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Molecular Systems & Circuits

Operon Lactose (Jacob and Monod)

E. Coli

Glucose

Lactose

Galactose

β-gal

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Glucose

Activator

Repressor

Gene for lactose metabolism and import

Lactose processing
How does a bacteria find its middle?
How does a bacteria find its middle?

Three proteins called the min system (minC,D,E)
How does a bacteria finds its middle?

Three proteins called the min system (minC,D,E)

The min system (minC,D,E)

How does a bacteria finds its middle?

Three proteins called the min system (minC,D,E)
Larger networks control more complex functions

Regulatory network of the budding yeast cell cycle (Tyson 2006)

- Molecular networks are fundamental building blocks of biological systems
- They are inevitable because of the very process of chemical replication, but they also provide opportunities for function, in particular computation
Are molecular networks special?
Are molecular networks special?

The answer is at the same time NO, YES and We don’t know

- NO, because all networks share some fundamental properties
- YES, because the edges of the network are instantiated by molecular interactions and reactions and this is quite specific
- We don’t know, because we don’t have much first-hand molecular networking experience. Most of the one we know were just discovered in nature.
Universality of networks
Universality of networks

Higher level properties of interaction networks emerge largely from their topological structure regardless of the identity of the nodes.
Lotka-Volterra equations

\[ \dot{X} = aX - bXY \]
\[ \dot{Y} = cXY - dY \]
Lotka-Volterra equations
Lotka-Volterra equations

\[ \frac{dx}{dt} = ax - bxy \]
\[ \frac{dy}{dt} = cy - dx \]

Predator-prey dynamics stabilised by nonlinearity explain oscillations in dust-forming plasmas

A. E. Ross & D. R. McKenzie

Received: 01 October 2015

are valuable in generating nanoparticles for medicine and electronics. Dust-forming plasmas exhibit a bizarre, even puzzling behaviour in which they oscillate with timescales of seconds to minutes. Here we show how the problem of understanding these oscillations may be cast as a predator-prey problem, with electrons as prey and particles as predators. The addition of a nonlinear loss term to the classic
Lotka-Volterra equations

The Jungle Universe: coupled cosmological models in a Lotka–Volterra framework
Lotka-Volterra systems in chemistry?

Alfred Lotka original suggestion concerned chemical kinetics:

He suggested that a chemical system with two imbricated autocatalytic chemicals could oscillate.

Molecular “prey”
Molecular “predator”

Molecular soup

Graph showing oscillations over time [s].
Various flavors of networks exist within cells

- Gene regulatory networks
- Signalling cascades
- Enzymatic networks
- Regulated metabolic networks
Gene regulatory networks can be very complex but they are conceptually simple.
Gene regulatory networks: A modular way to build reaction networks, in vivo, using the protein expression machinery

- A gene can activate another gene
- A gene can repress another gene
- This motif can be cascaded over and over
Key elements of molecular regulatory networks

TOPOLOGY

KINETICS (laws & rates)

DIFFUSION RATES

BOUNDARY CONDITIONS

CHEMICALS

There is no direct link between the physico-chemical nature/feature of the parts and the function

Regulatory network of the budding yeast cell cycle (Tyson 2000)
Synthetic Biology: Creating networks in cells, by connecting existing “parts” to obtain artificial behaviors

Elowitz & Leibler, 2000: The first synbio oscillatory network

**a**

Repressilator

- **tetR** is a regulator involved in antibiotic resistance in bacteria
- **LacI** is a metabolic regulator from E. Coli, that controls the expression of sugar-processing enzymes depending on availability
- **Lambda-cl** is a phage regulator that controls the switching between the two life style of a phage (a virus)
in vitro circuits based on full cellular machinery (protein expression and gene regulation)
i.e. in cell extracts

Implementation of cell-free biological networks at steady state

Henrike Niederholtmeyer, Viktoria Stepanova, and Sebastian J. Maerkl

PNAS 2013
in vitro circuits based on full cellular machinery (protein expression and gene regulation)

Implementation of cell-free biological networks at steady state

PNAS 2013
Harnessing synthetic DNA and purified enzymes to construct *in vitro* reaction networks

As models of cellular networks
For a chemistry of information processing
DNA as a *synthetic* material for molecular circuits
DNA as a **synthetic** material for molecular circuits

A \[\rightleftharpoons^K_m B\]
DNA as a *synthetic* material for molecular circuits

“A" \[ \xrightarrow{K_m} \] “B"

“DNA as a universal substrate for chemical kinetics” PNAS 2010
DNA as a synthetic raw material for molecular circuits

“DNA as a universal substrate for chemical kinetics” PNAS 2010

Enzymes:
Copy: Polymerases
Cut-paste: Restriction enzymes and Ligases
Delete: Nucleases
Low cost DNA synthesis
DNA data storage: 200 MB written in, stored and read from DNA

(OK go video) Microsoft, U. Washington, Twist
DNA data storage: 200 MB written in, stored and read from DNA

(OK go video) Microsoft, U. Washington, Twist
Molecular networking approaches
Simplified models of biological regulatory networks

- We want to maintain the modularity and cascadability
- But simplify the chemistry as much as possible
Artificial equivalent of regulatory networks

“genelets” Kim & Winfree MSB 2011

“PEN toolbox” Montagne & Rondelez MSB 2011
PEN DNA toolbox: an *in vitro* “universal” reaction set

(a) Activation

(b) Inhibition

(c) Degradation

Polymerase

Nicking

Exonuclease
PEN DNA toolbox: an *in vitro* “universal” reaction set

- Cascadable
- Dissipative (Global reaction and energy flux: dNTP -> Oligonucleotides -> dNMP)
Energy source and flow

Enzymes
Synthesizing dynamics

Target behavior

Model

Experiment

Topology

Circuit

Sequences
Building a DNA-encoded oscillator

Relaxation oscillator
Building a DNA-encoded oscillator

Relaxation oscillator

A:

\[
\begin{align*}
\alpha \quad \alpha \\
\beta \\
\end{align*}
\]

B:

\[
\begin{align*}
\alpha \quad \beta \\
\end{align*}
\]

5'-AACAGACTCGA-AACAGACTCGA-3'
5'-TTACTCGAACCAGACT-GGATGACTCCA-3'
5'-GGATGACTCCA-AACAGACTCGA-3'
Building a DNA-encoded oscillator

Montagne et al, MSB 2011
Modeling (detailed kinetics)
Modeling (detailed kinetics)
ODE system
Numerical integration

Experimental

Calculated without parameter adjustment

Fluorescence (a.u.)

Base-pair conc. (nM)

Time (min)
Building a bistable system
(1bit memory)
An *in vitro* Bistable switch

\[ T_\alpha \rightarrow i\beta \rightarrow T_i\beta \rightarrow i\alpha \rightarrow T_\alpha \]

\[ \alpha \to \alpha: \]
\[ \alpha \to i\beta: \]
\[ \beta \to i\beta: \]
\[ \beta \to \alpha: \]

![Graphs](image.png)

*PNAS 2012*
Bistable switch => Push-push button

We add input modules to force the bistable back and forth

We then expand the system to a push-push memory circuit (single input toggle)
Predator Prey network
Predator-Prey molecular ecosystem

Prey growth:
- N + G → G:N
- G:N → Pol, dNTP
- Pol, dNTP → Nick
- Nick → G

Predation:
- N + P → P:N
- P:N → Pol, dNTP
- Pol, dNTP → P

Decay (ExoN):
- N → dNMP (waste)
- P → dNMP (waste)
Predator-Prey molecular ecosystem

A. \( \text{pol, nick, dNTP} \)

B. \( \text{pol, dNTP} \)

C. \( \text{exoN, dNMP (waste)} \)

**Graphs:**
- **Graph 1:** Green fluorescence vs. time (min) for different conditions.
  - [Prey2] \(_{0} = 4\mu M\)
  - [Pred2] \(_{0} = 1\mu M\)
Partitioning a molecular oscillator in a microscopic emulsion
Partitioning a molecular oscillator in a microscopic emulsion gives...

...many identical oscillators.

A platform for analysis of $\sim 10^4$ different systems simultaneously.
Bifurcations of oscillator using 3D Parameter scanning
Bifurcations of oscillator using 3D Parameter scanning
Bifurcations of oscillator using 3D parameter scanning
3-dimensional bifurcation diagram of an oscillator
3-dimensional bifurcation diagram of an oscillator
3-dimensional bifurcation diagram of an oscillator
Stochastic bursters located at the Hopf bifurcation
Bursting droplets

- Hard excitation (SS+LC)
- Coexistence (Steady state)
- Oscillations (limit cycle)
- Extinction

Stochastic switching between regimes due to small number effect along the cycle

Learning by building

The edges hide a lot: non linear behaviours are essential, and provided by higher orders kinetics, delays or feedback loops.

Both inducers form a dimer before acting.

Positive feedback loops are necessary for function.
Learning by building

The edges hide a lot: non linear behaviours are essential, and provided by higher orders kinetics, delays or feedback loops.

Parasites (chemical virus) are ubiquitous free riders when a powerful molecular machine is provided.

The role of global couplings: Ideal versus real molecular networks.
In chemistry, networks with a “discrete” structure are an idealisation.
In (bio)-chemistry, networks with a "discrete" structure are an idealisation.
In chemistry, networks with a “discrete” structure are an idealisation.

In the case of the DNA toolbox, the enzymatic machine contains 3 enzymes:

- Activation
- Inhibition
- Autocatalysis

- Polymerase saturation
- Exonuclease saturation
- Nicking enzyme saturation

- Global negative coupling
- Global positive coupling
- Global negative coupling
Global loads can negatively affect function
Global competition can also provide **new** functions

- The **WINNER-TAKE-ALL** effect happens in collections of self-replicating agents when there is competition for growth
  - Exponential growth
  - Limited shared resource
  - Discretization: Elimination of all but one species
- It is called **competitive exclusion** in ecology but also applies to molecular systems
Conclusion

- Artificial Molecular Networking approaches provide versatile tools to explore questions related to networks and information processing in (bio)-chemical systems.
- There exist a range of platforms, going from \textit{in vitro synbio}, to DNA-only networks.
- They also open the way to new applications, where chemical systems are used not for their physic-chemical properties, but for their information-processing potential.