

# BOTTOM-UP EXPERIMENTAL MODELS OF CELLULAR NETWORKS

YANNICK RONDELEZ  
CNRS-ESPCI-PSL

MULTISCALE INTEGRATION IN BIOLOGICAL  
SYSTEMS NOV 2021

Yannick Rondelez  
ESPCI, FR



PS  
Pouzet Sylvain

AC  
Alexander CHAM...

F  
Francesco (Guest)



# Information management across biological scales



PS

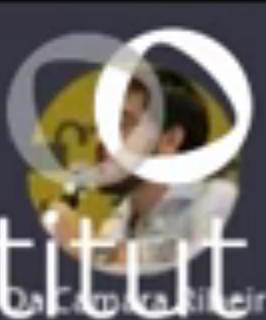
Pouzet Sylvain

AC

Alexander CHAM...

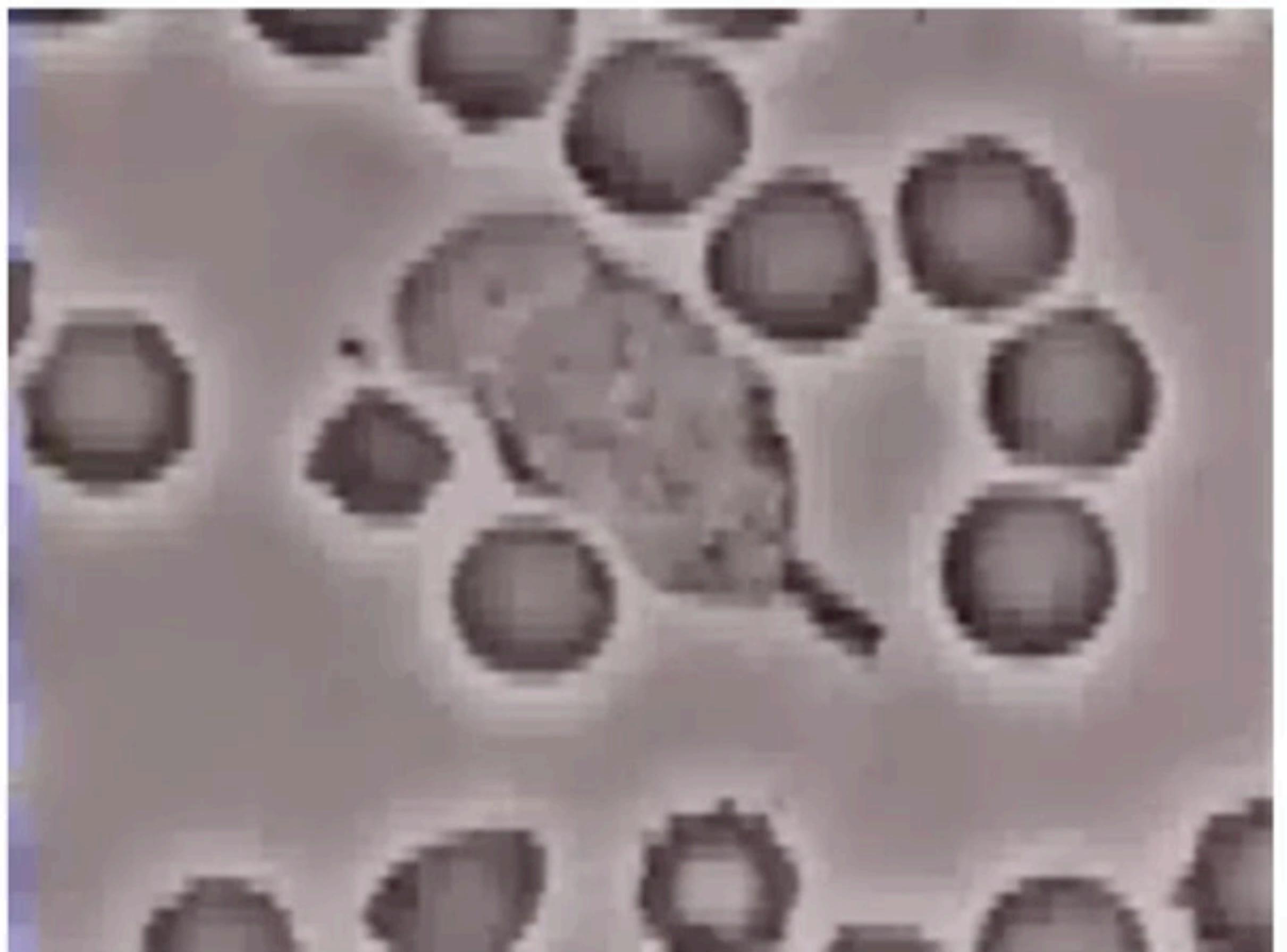
F

Francesco (Guest)



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# Information management across biological scales



Neutrophil chasing a bacteria (D. Roger 1950)

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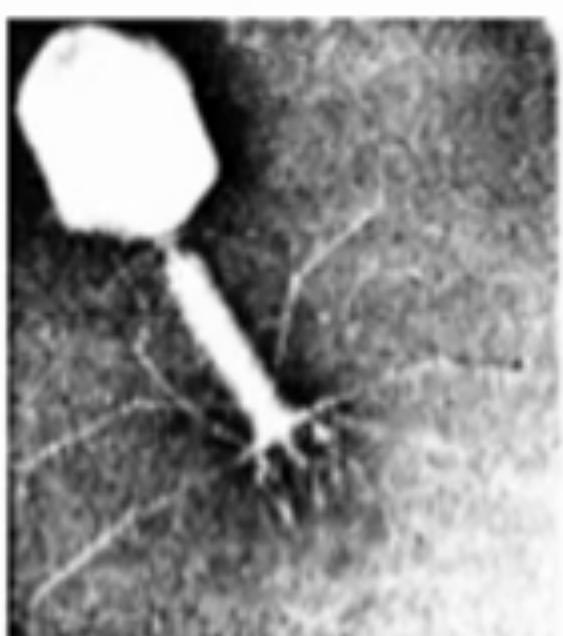
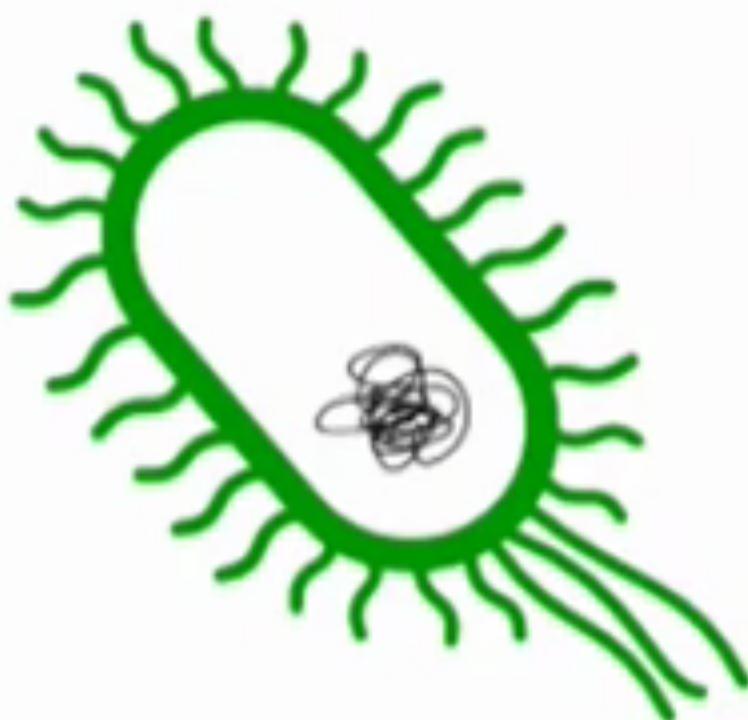
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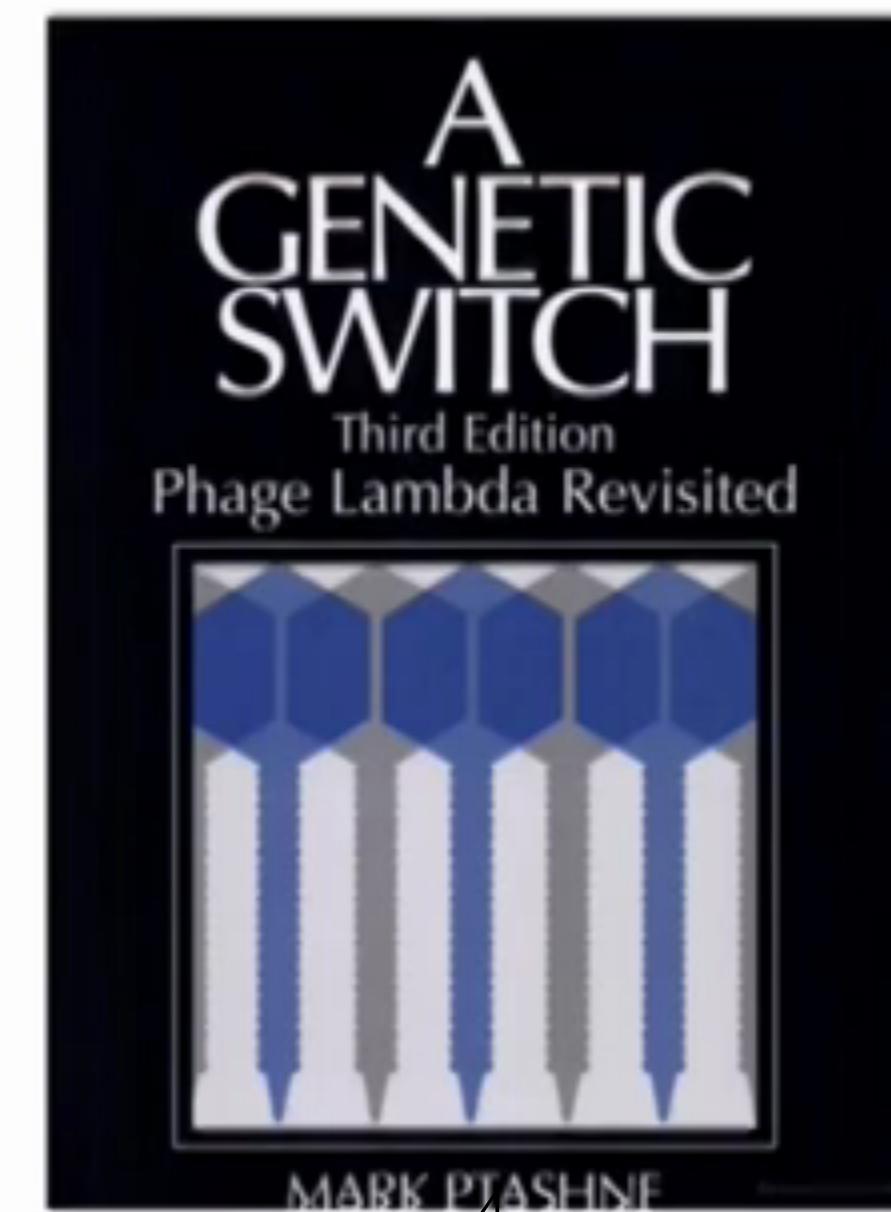


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Raman Sh

# Information management across biological scales



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



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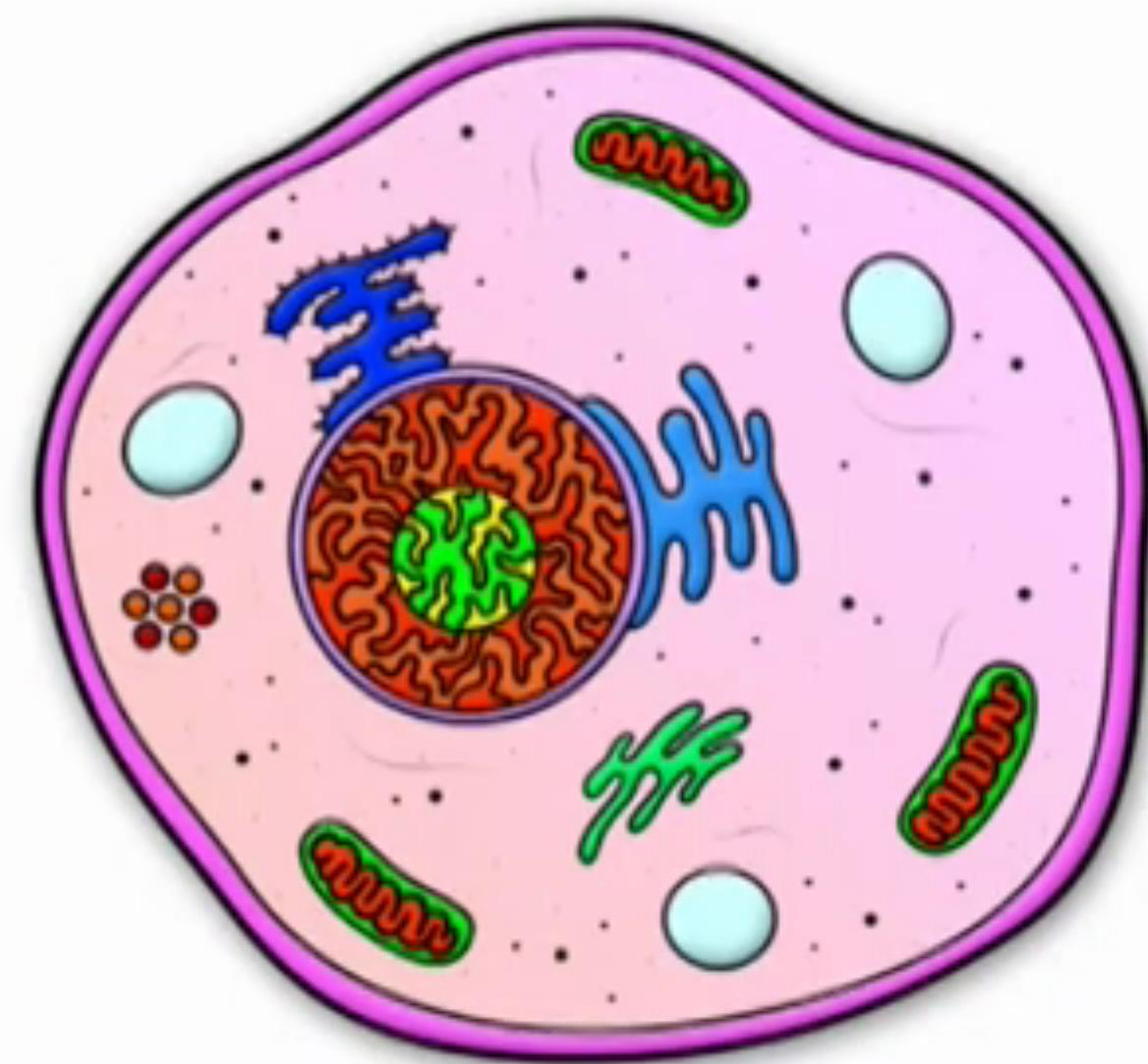
Da Cunha Ribeir...

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# How does it work ?

Where/what is the computer inside the microscopic bags of chemical that we call cells?



PS

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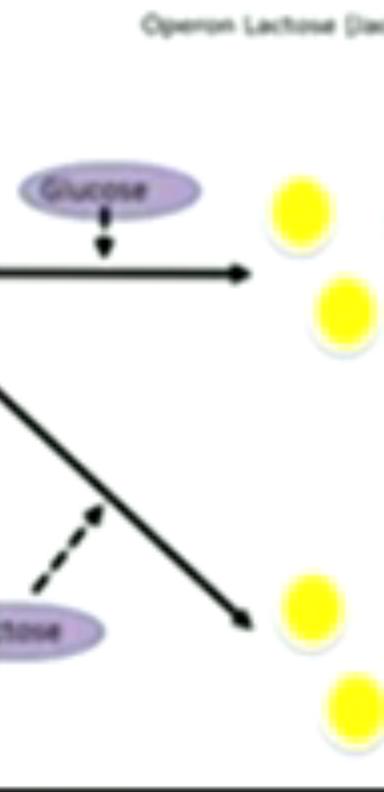
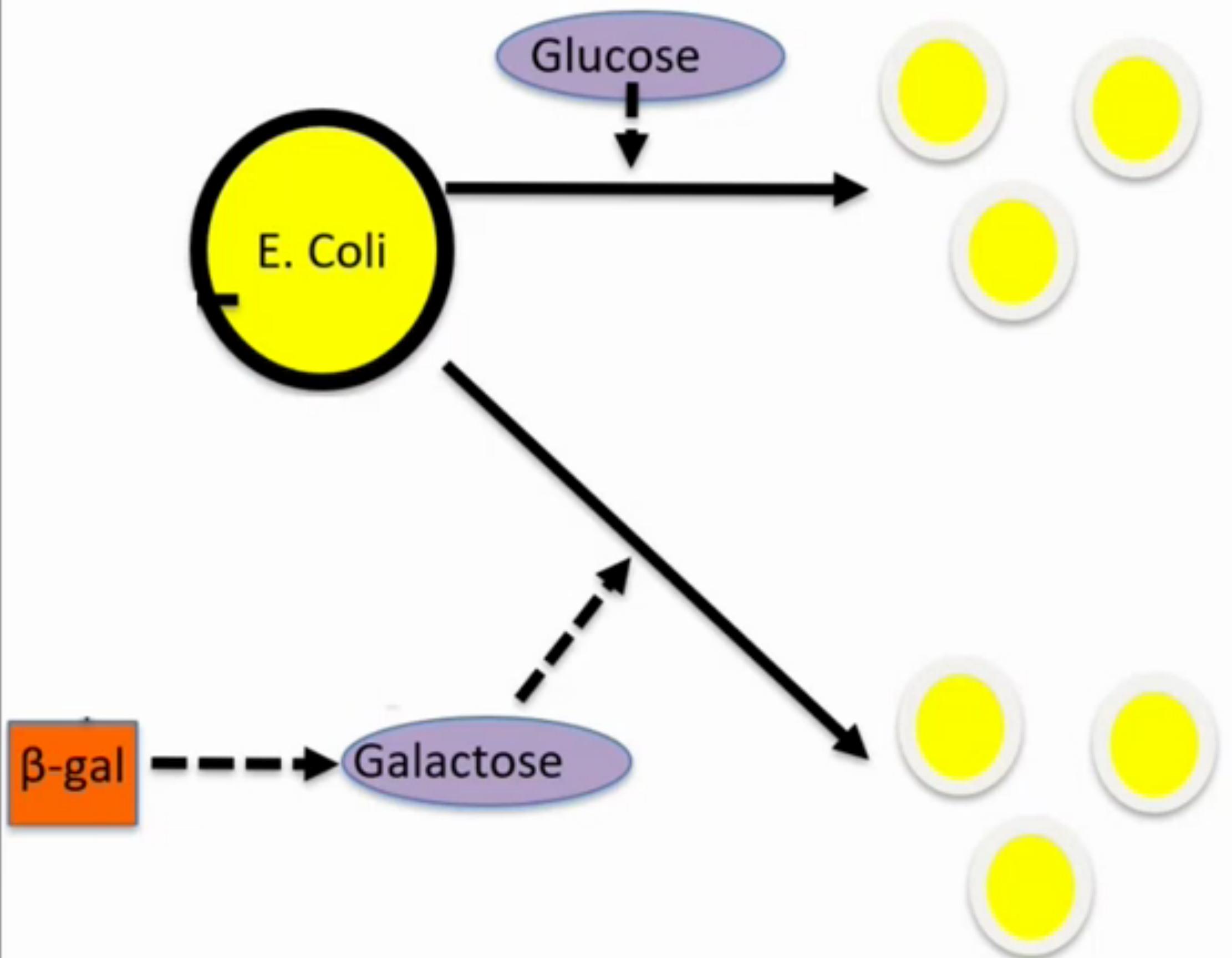
MC

Maria Isabel NOS...

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# Molecular Systems&Circuits

Operon Lactose (Jacob and Monod)



PS

Pouzet Sylvain

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Alexander CHAM...

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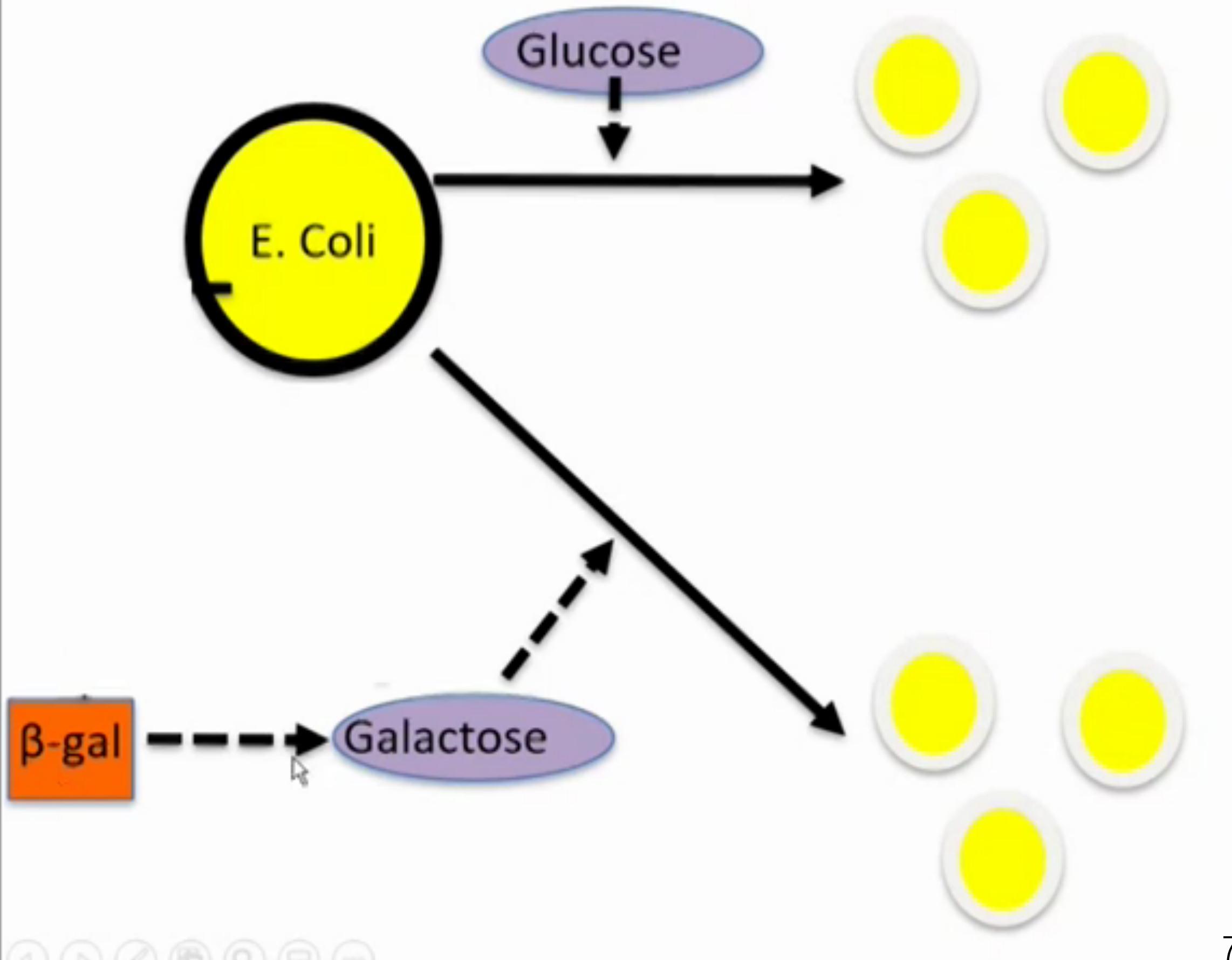
Francesco (Guest)

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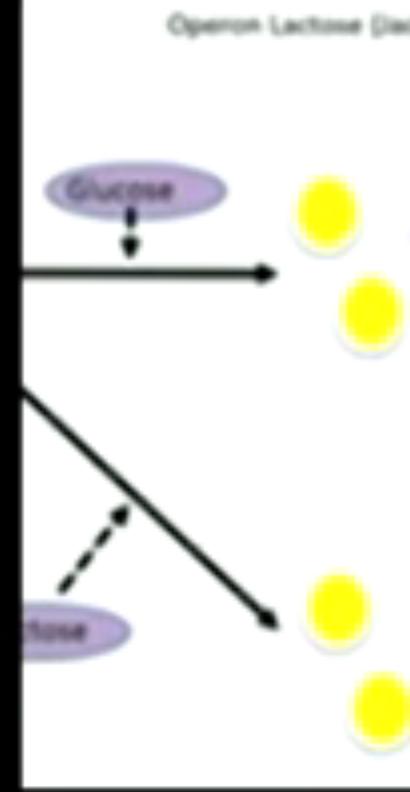
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# Molecular Systems&Circuits

Operon Lactose (Jacob and Monod)



Glucose	Lactose	Bacteria decision
+	-	Do not activate
+	+	Do not activate
-	+	activate
-	-	( die )



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Deanna Shea

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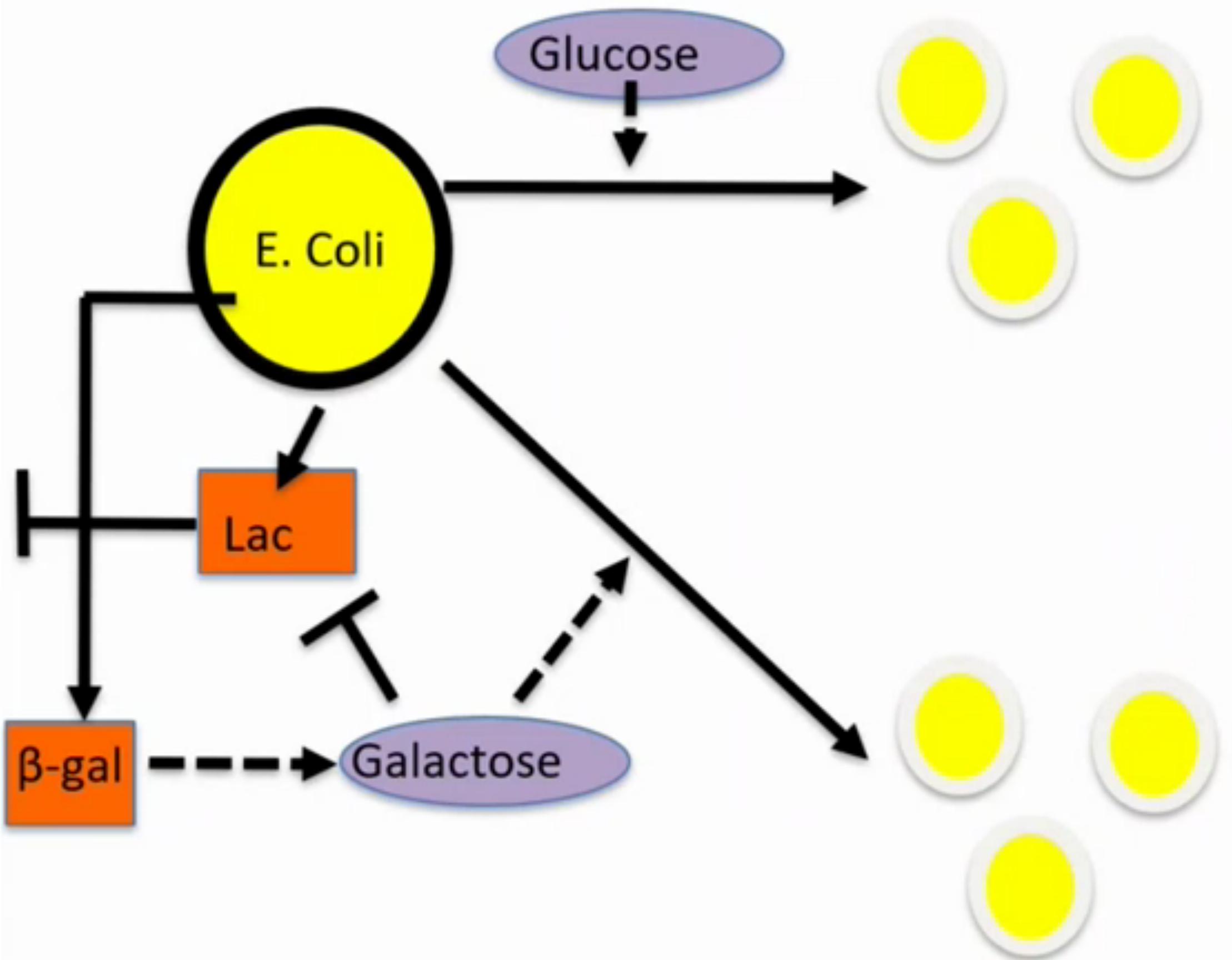
tra.mignacco



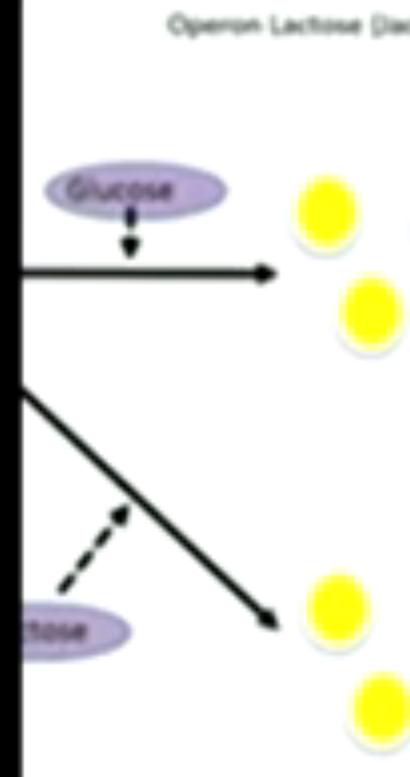
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# Molecular Systems & Circuits

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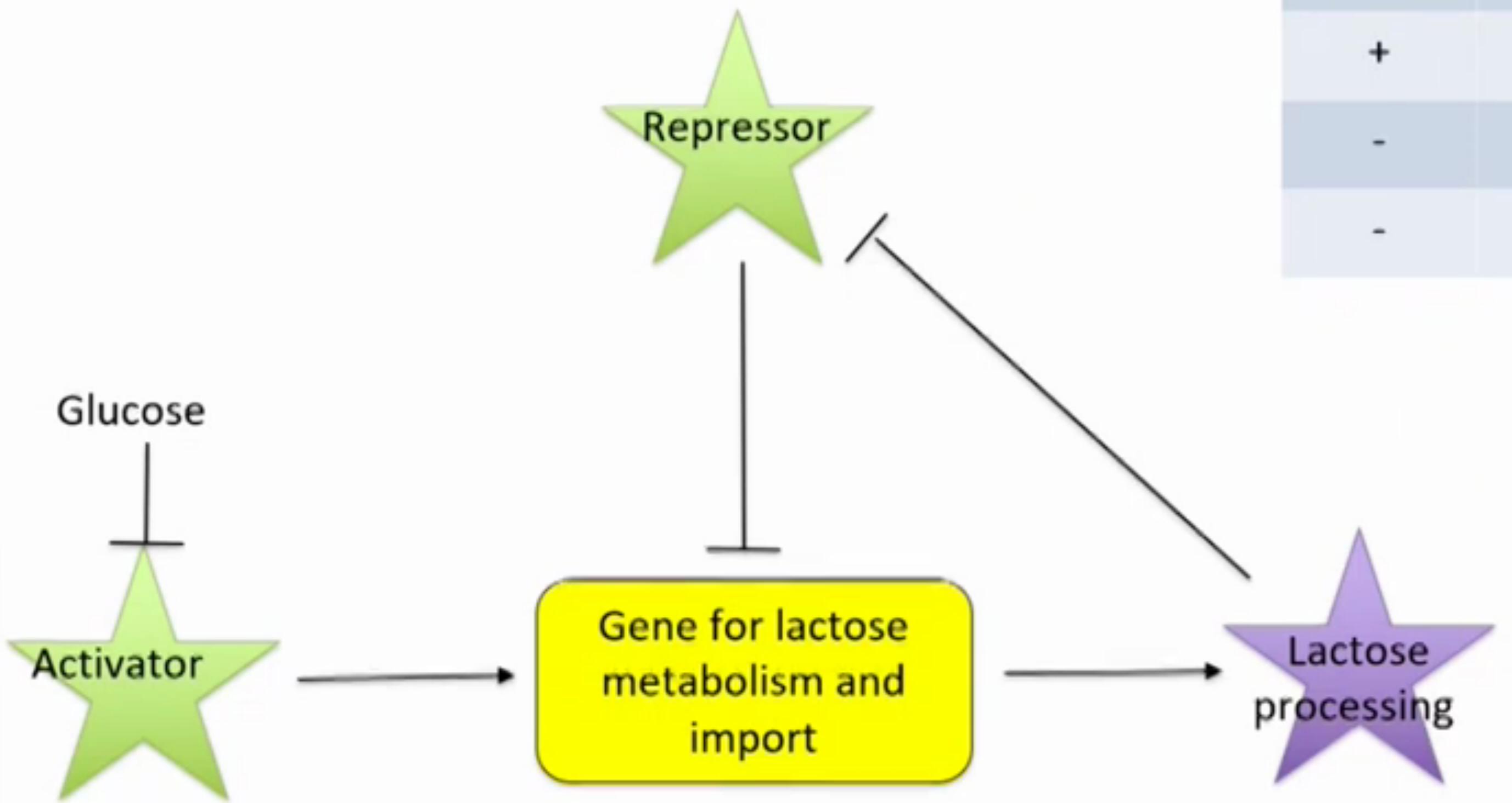
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Deanna Shea

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fria.mignacco

  
Da Camara Ribeir...

RS  
Raman Sheshk...

# Molecular Systems & Circuits



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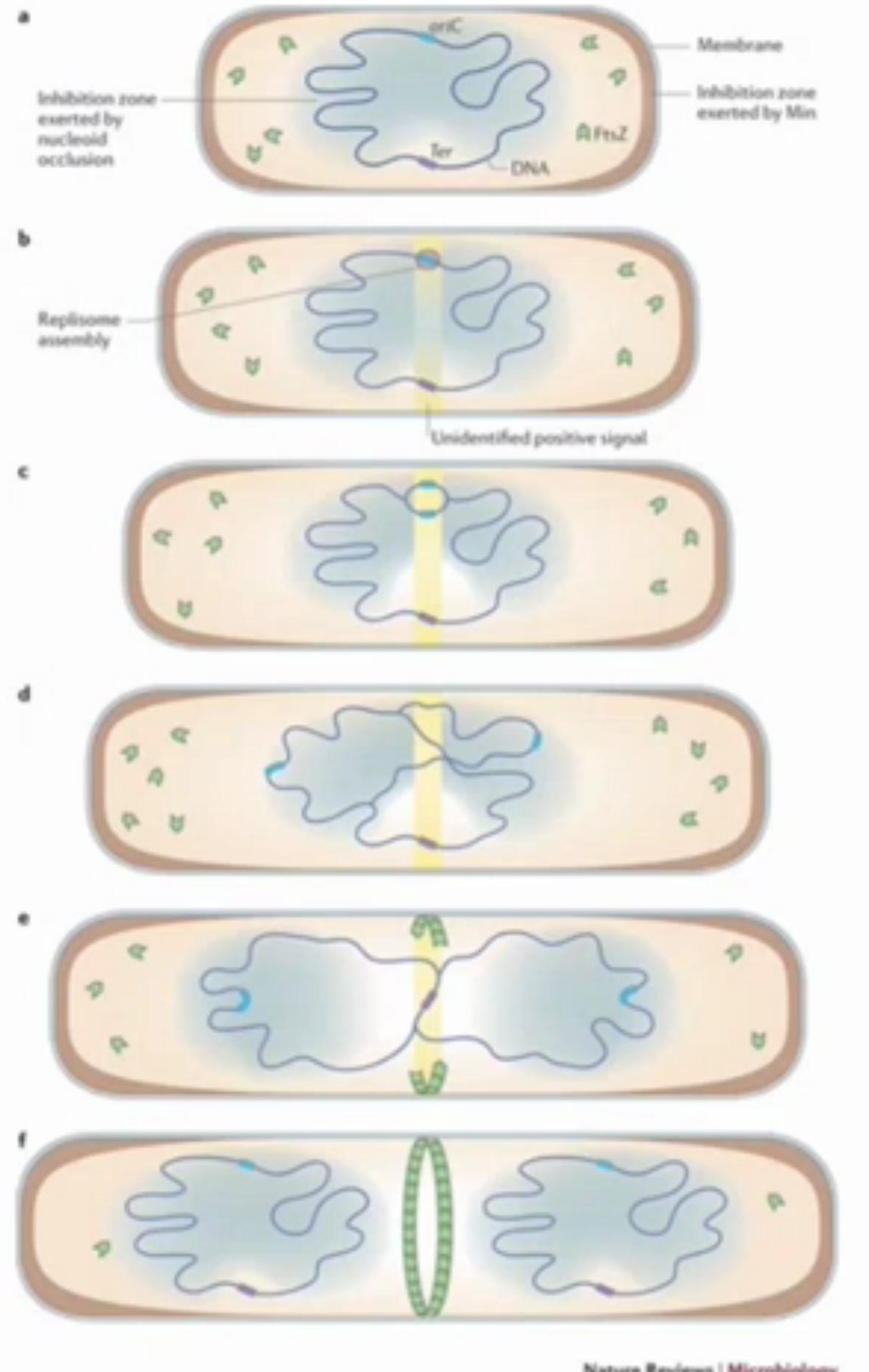
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# How does a bacteria finds its middle?



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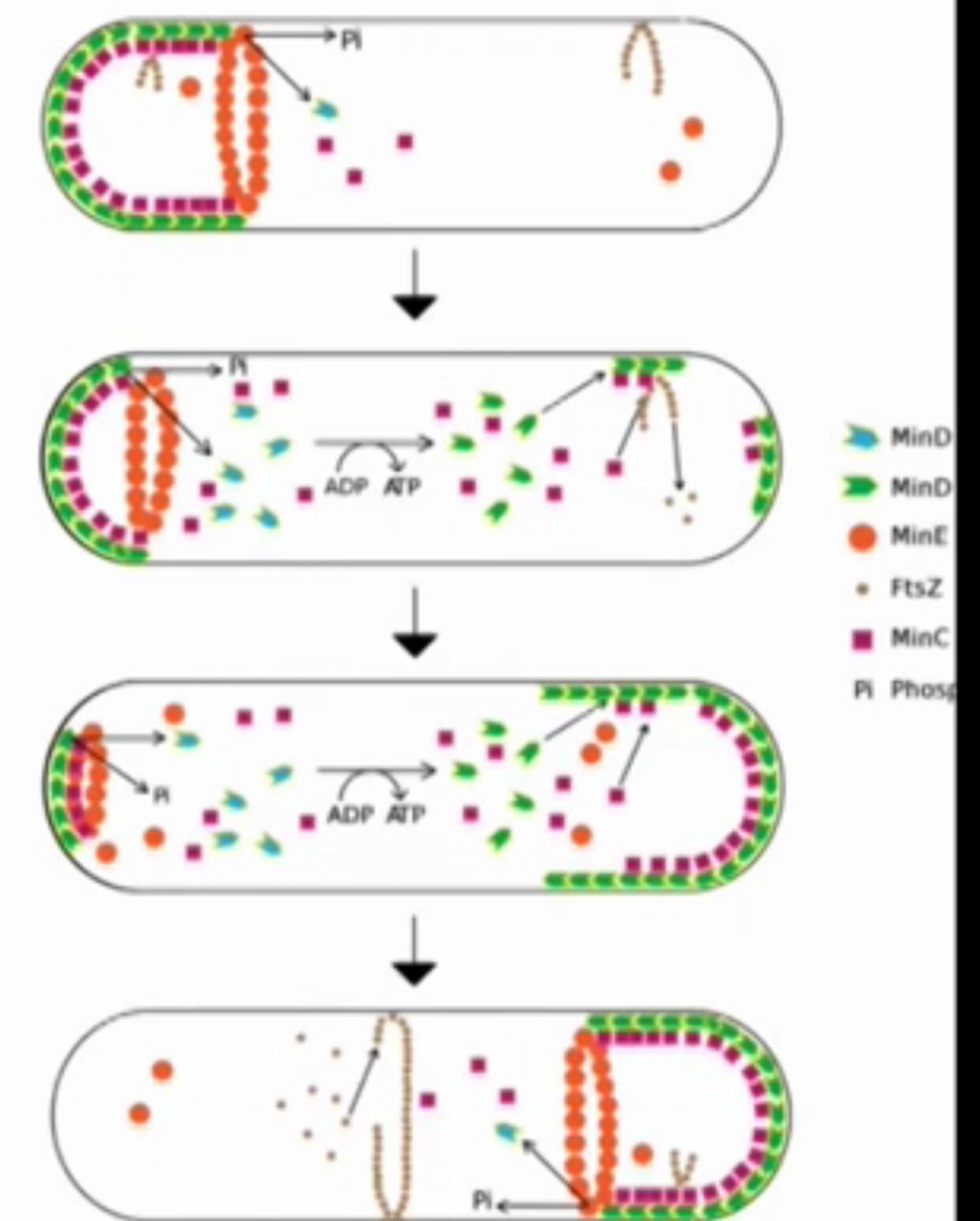
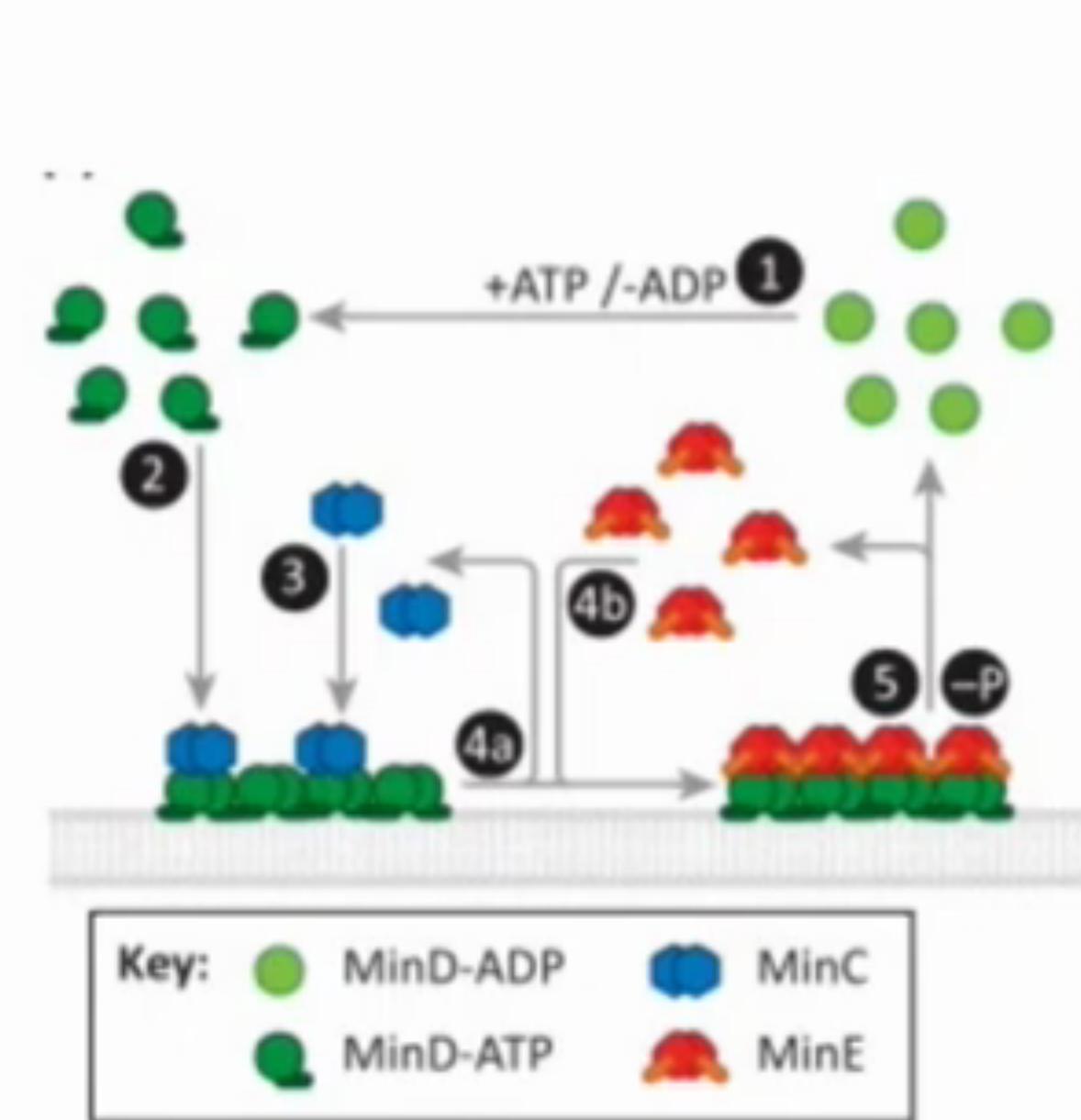
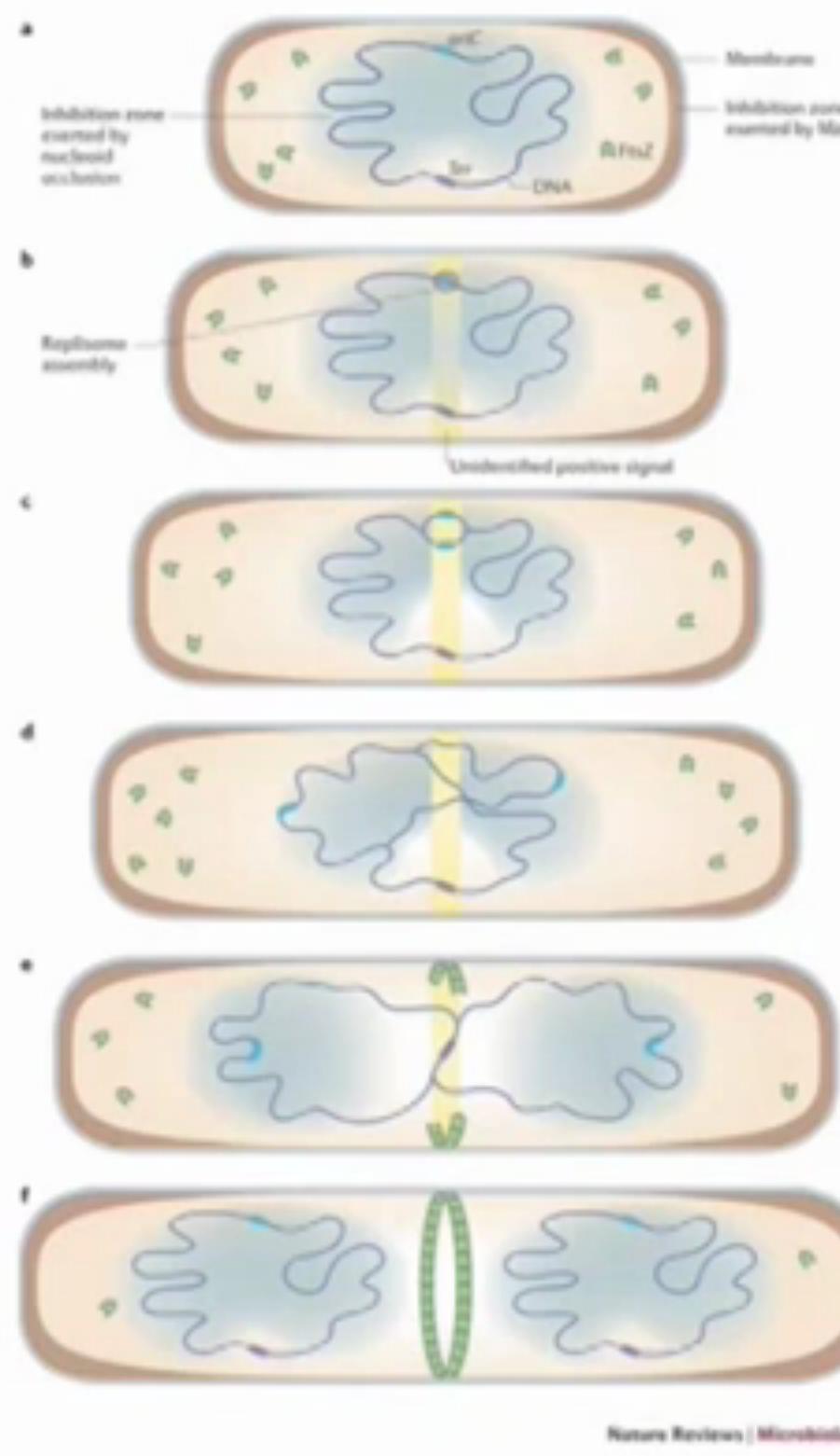
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# How does a bacteria finds its middle?

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



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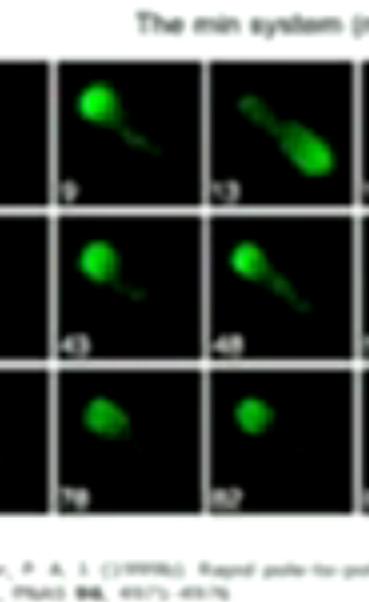
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# How does a bacteria finds its middle?

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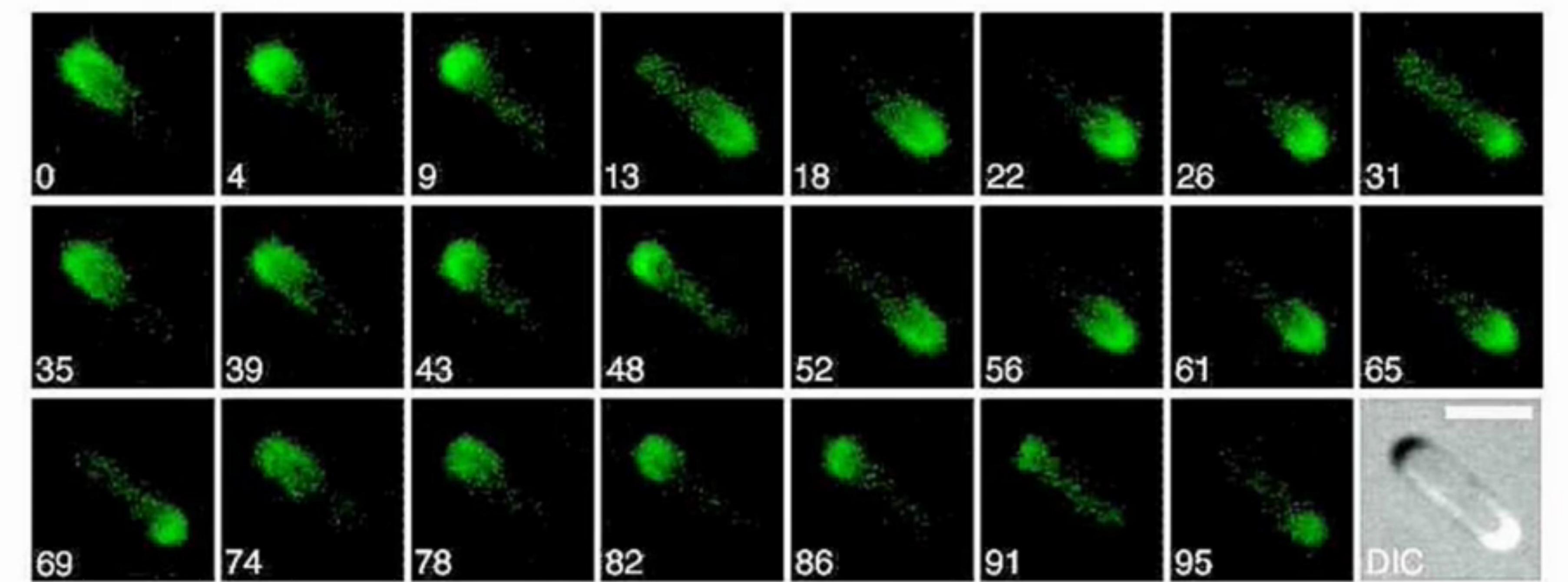
Da Camara Ribeir...

RS  
Raman Sheshka (...)

PS  
Pozzi Sardella

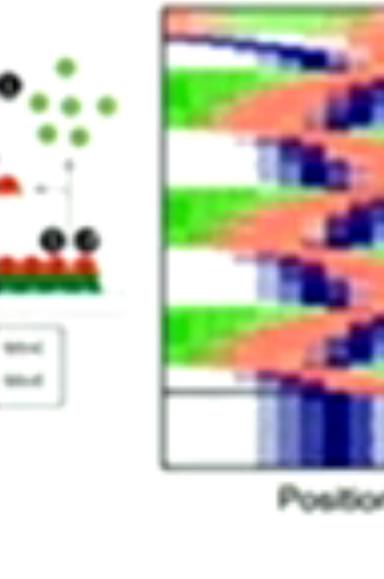
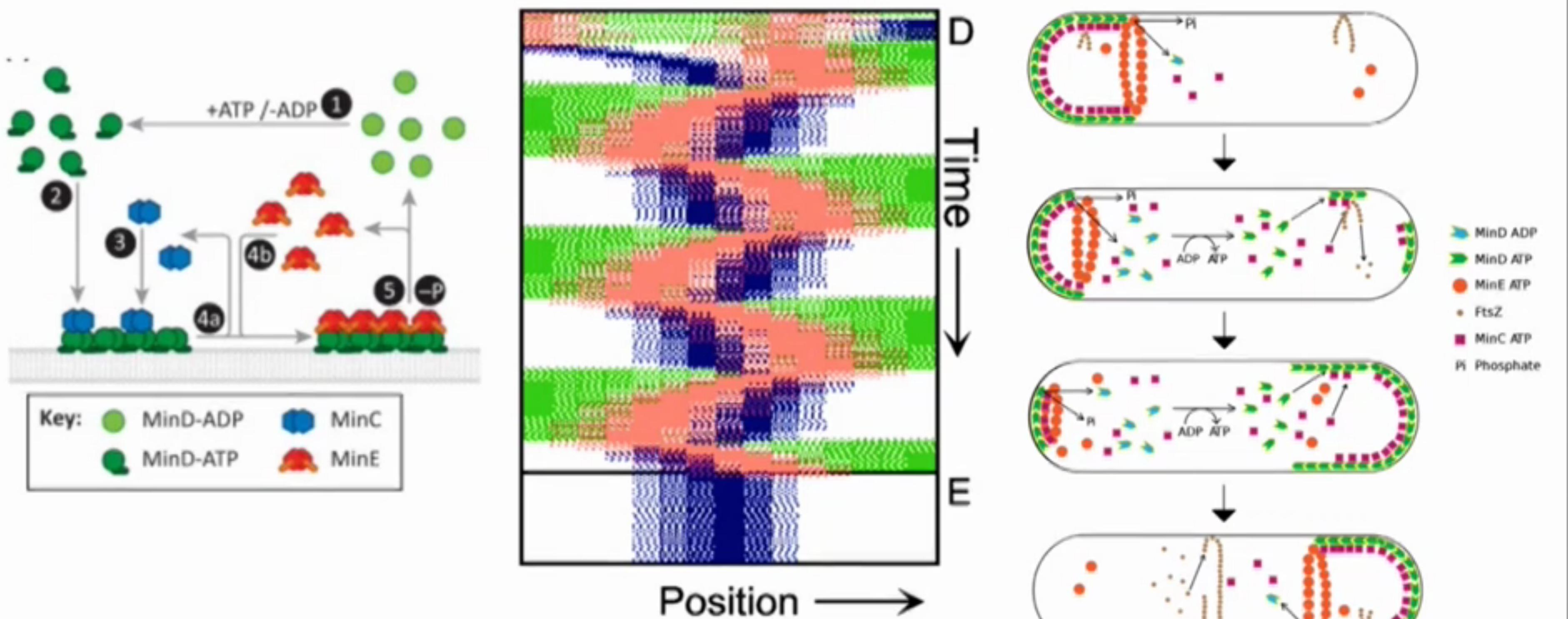
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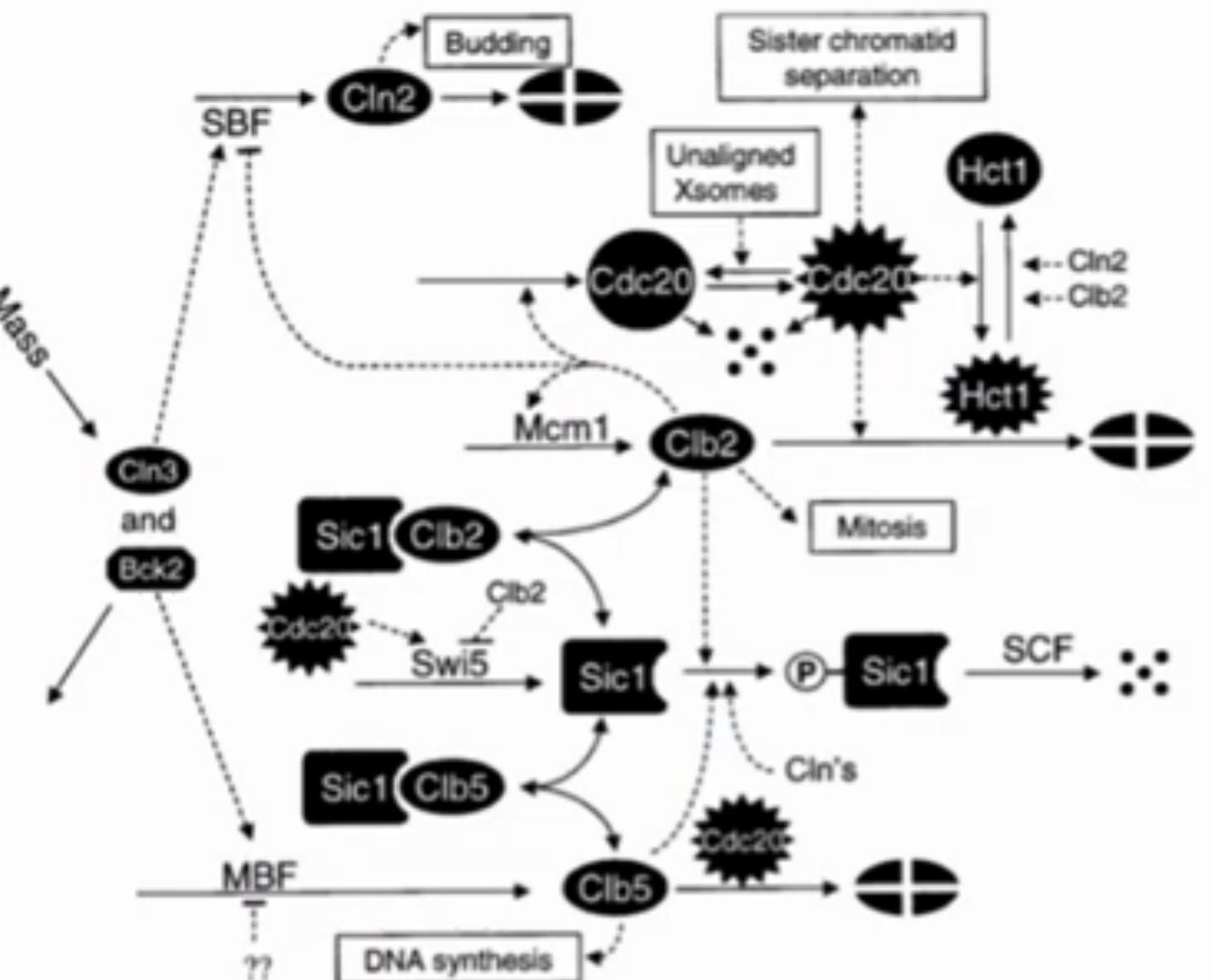
RS

Raman Shrikha (...)

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# 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?

The image shows a vertical list of user profiles from a mobile application. Each profile consists of a colored circular icon with a letter, the user's name, and a small thumbnail image.

- DS Deanna Shea
- F fra.mignacco
- Da Camara Ribeir...
- RS Raman Sheshka (...)
- PS

At the bottom right, there is a logo for "institut Curie" with "IH" below it.

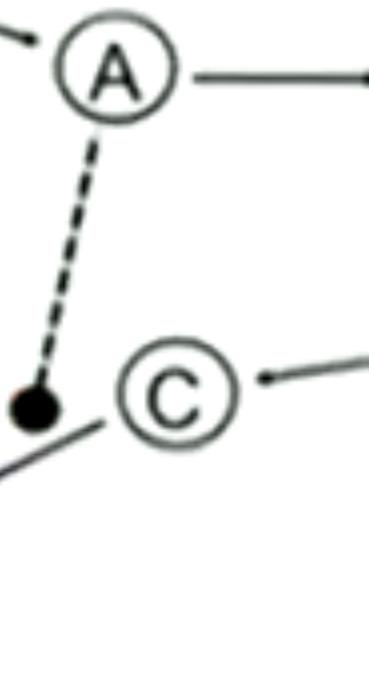
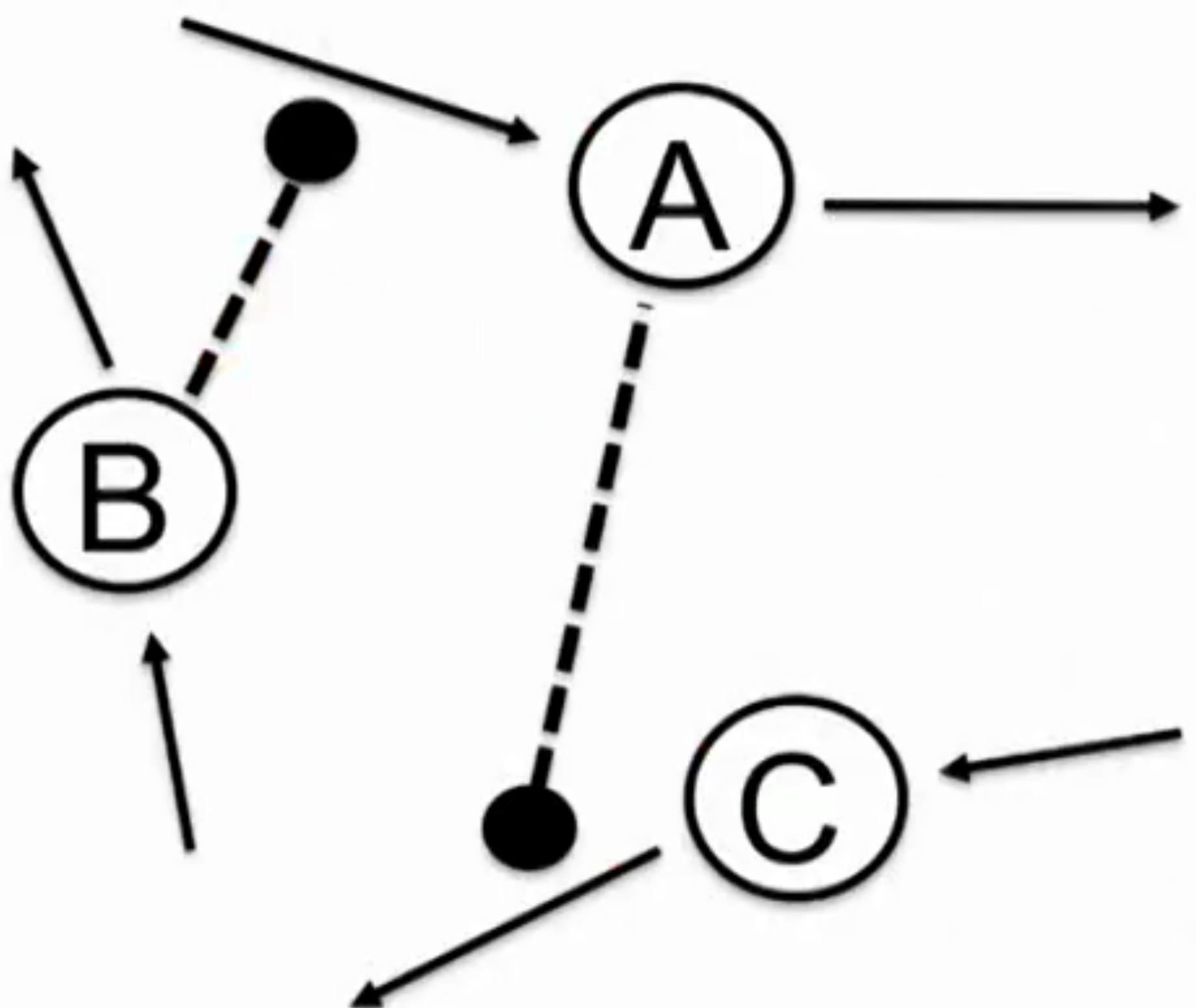
# Are molecular networks special?

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



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Da Camara Ribeir...

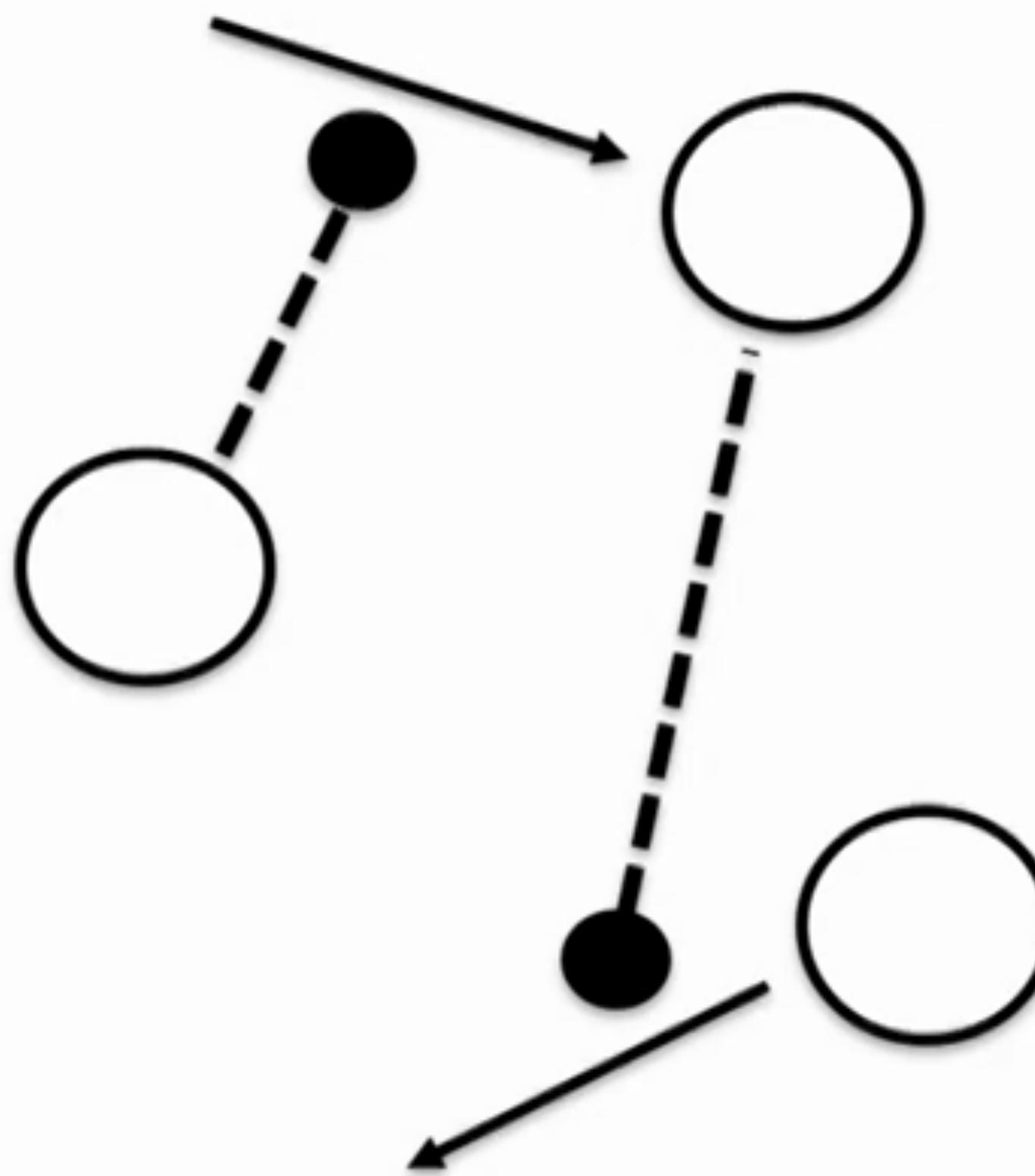
RS

Raman Shekhar (...)

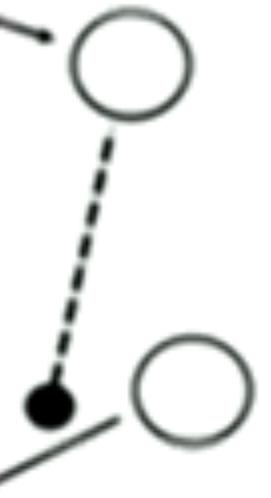
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Boyle, S...

# Universality of networks



Higher level properties of interaction networks emerge largely from their **topological structure** regardless of the **identity of the nodes**



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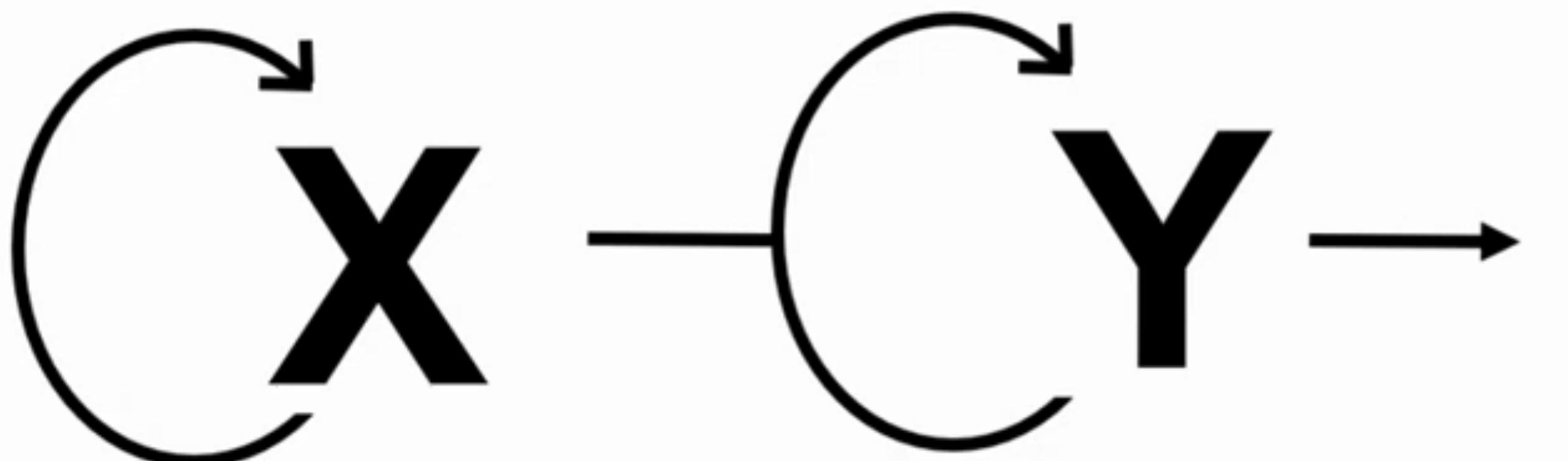
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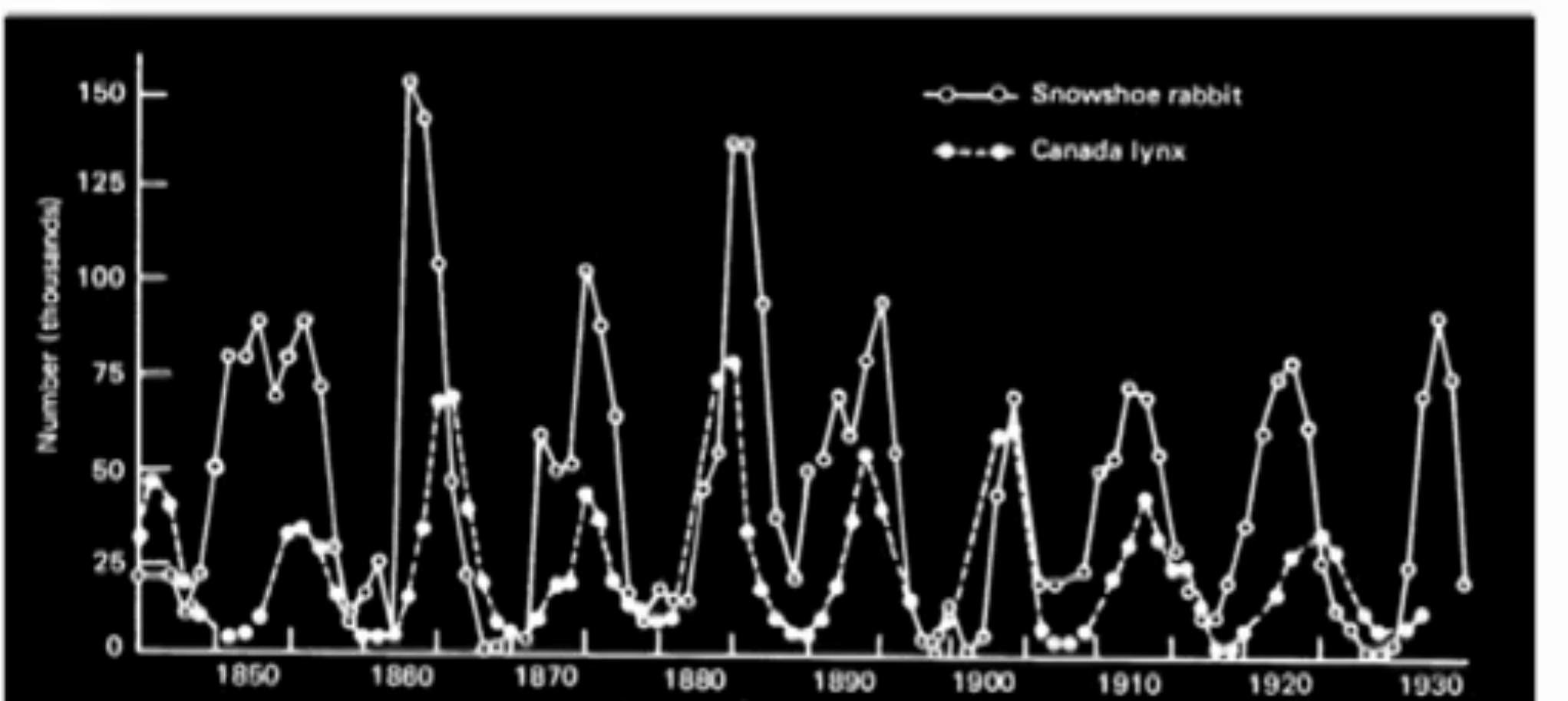
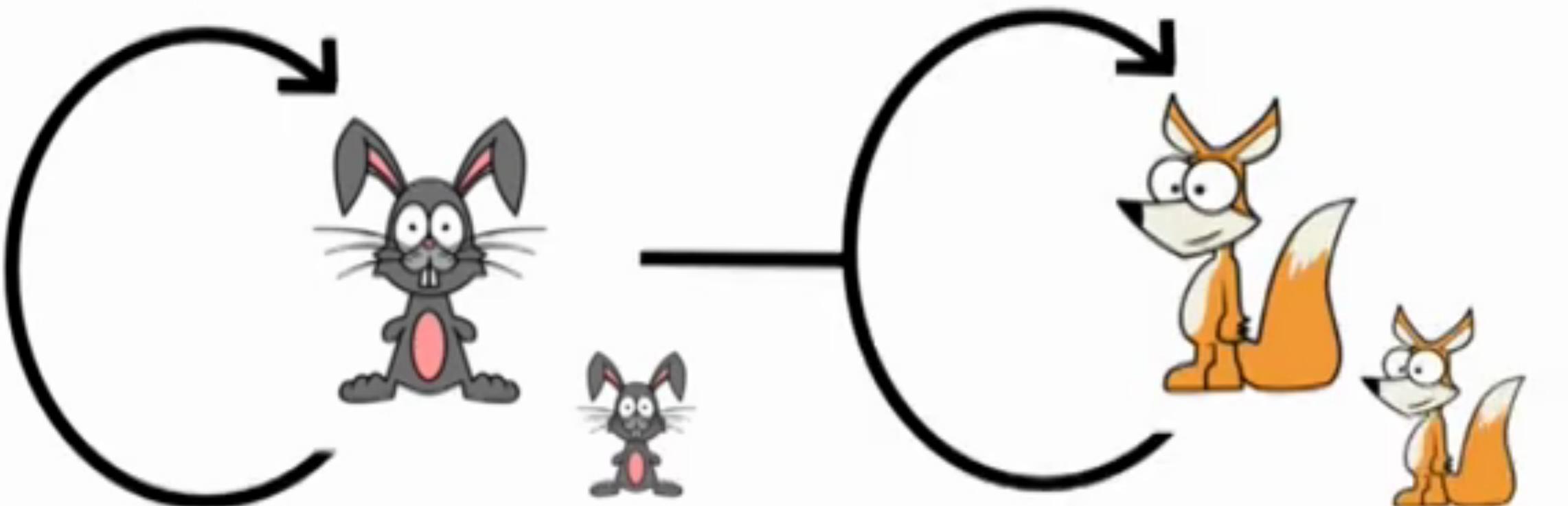
# Lotka-Volterra equations

$$\dot{X} = aX - bXY$$

$$\dot{Y} = cXY - dY$$



# Lotka-Volterra equations



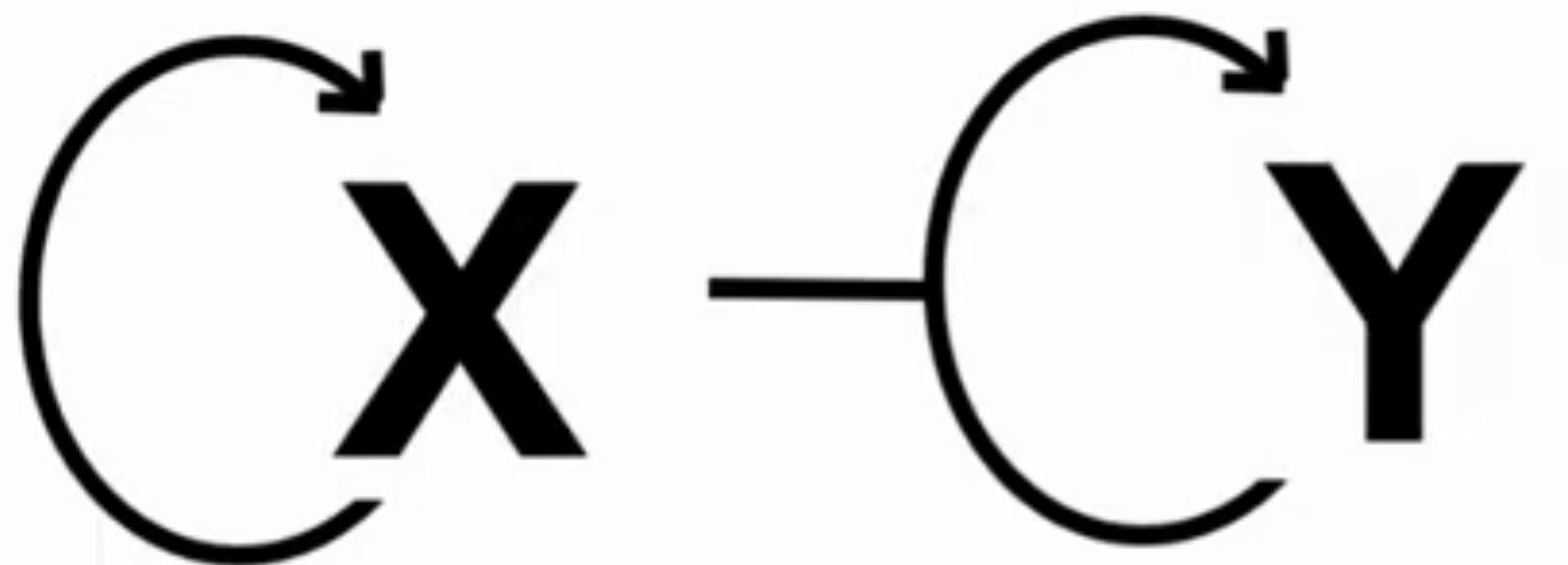
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# Lotka-Volterra equations



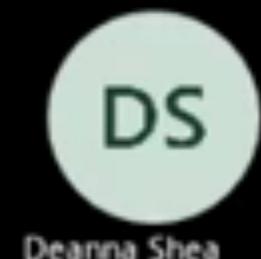
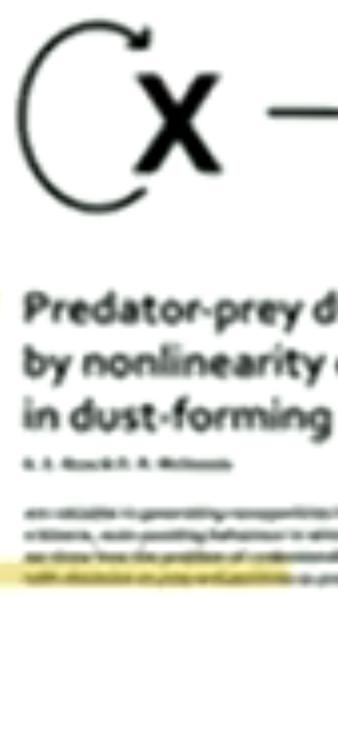
OPEN

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

Received: 01 October 2015

A. E. Ross & D. R. McKenzie

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



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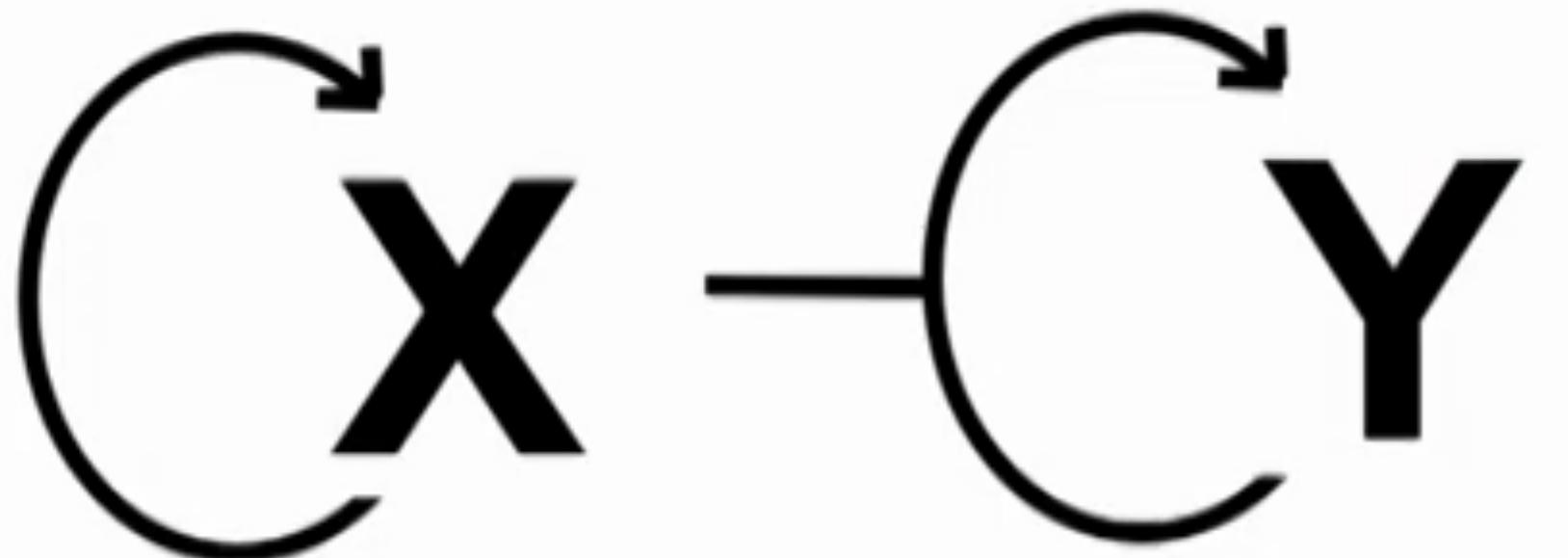


RS

Da Camara Ribeiro...



# Lotka-Volterra equations



Gen Relativ Gravit (2014) 46:1753  
DOI 10.1007/s10714-014-1753-8

Jérôme Perez · André Füzfa · Timoteo Carletti ·  
Laurence Mélot · Lazare Guédézounme

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RESEARCH ARTICLE

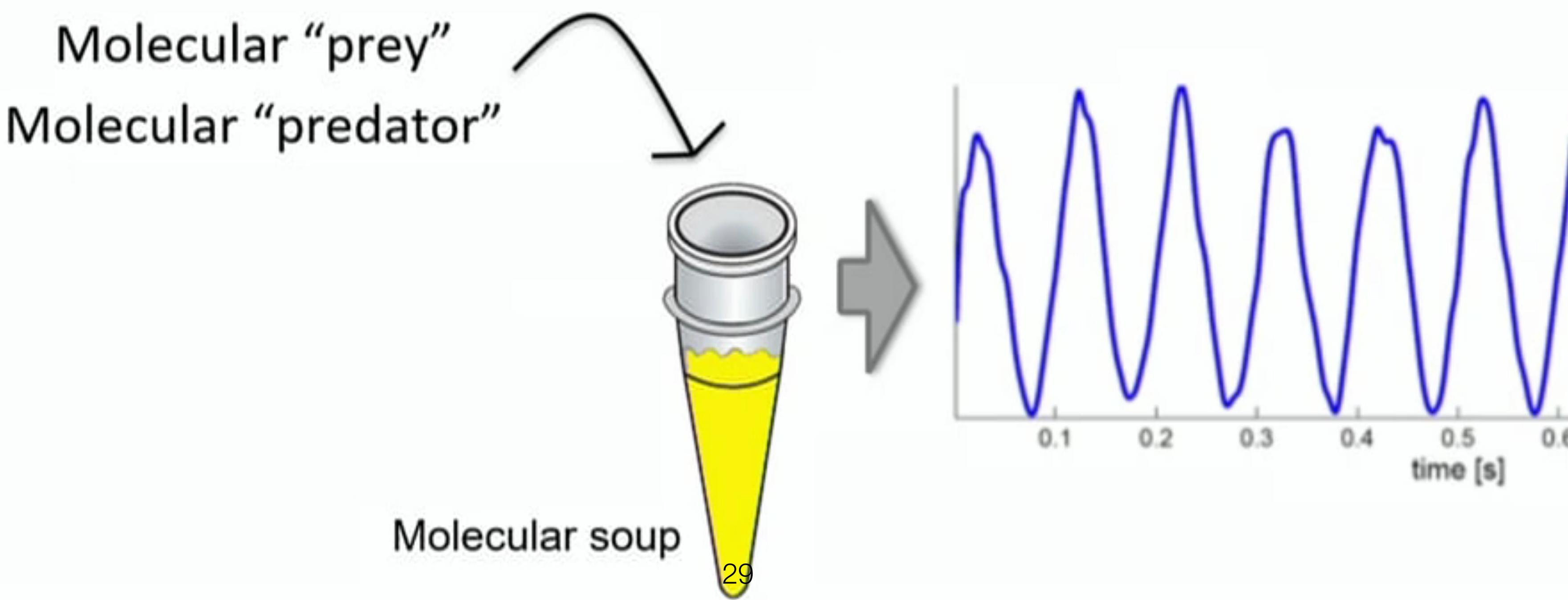
**The Jungle Universe: coupled cosmological models  
in a Lotka–Volterra framework**



# Lotka-Volterra systems in chemistry?

Alfred Lotka original suggestion concerned chemical kinetics:

He suggested that a chemical system with two imbricated auto-catalytic chemicals could oscillate

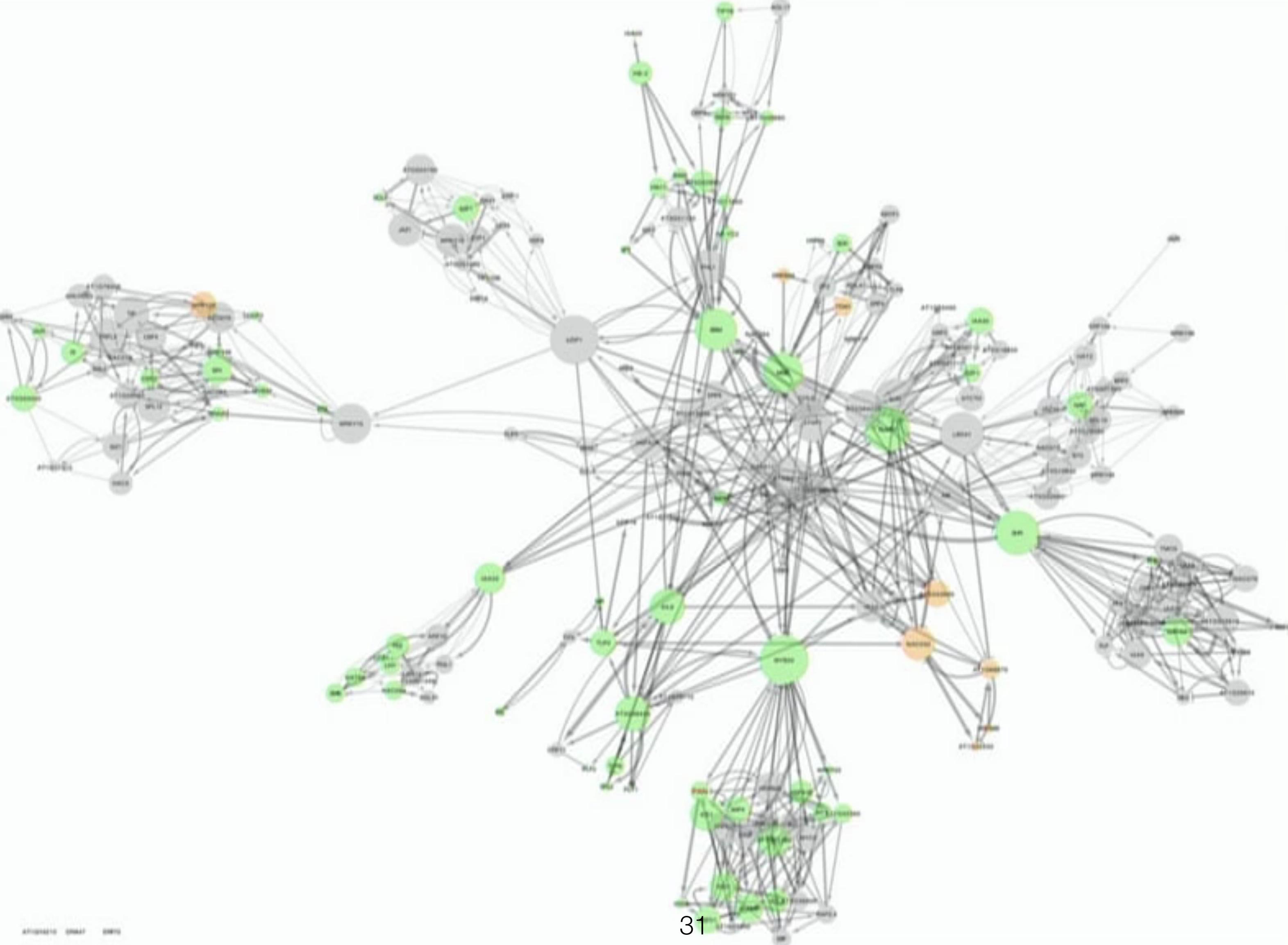


Various flavors of networks exist  
within cells

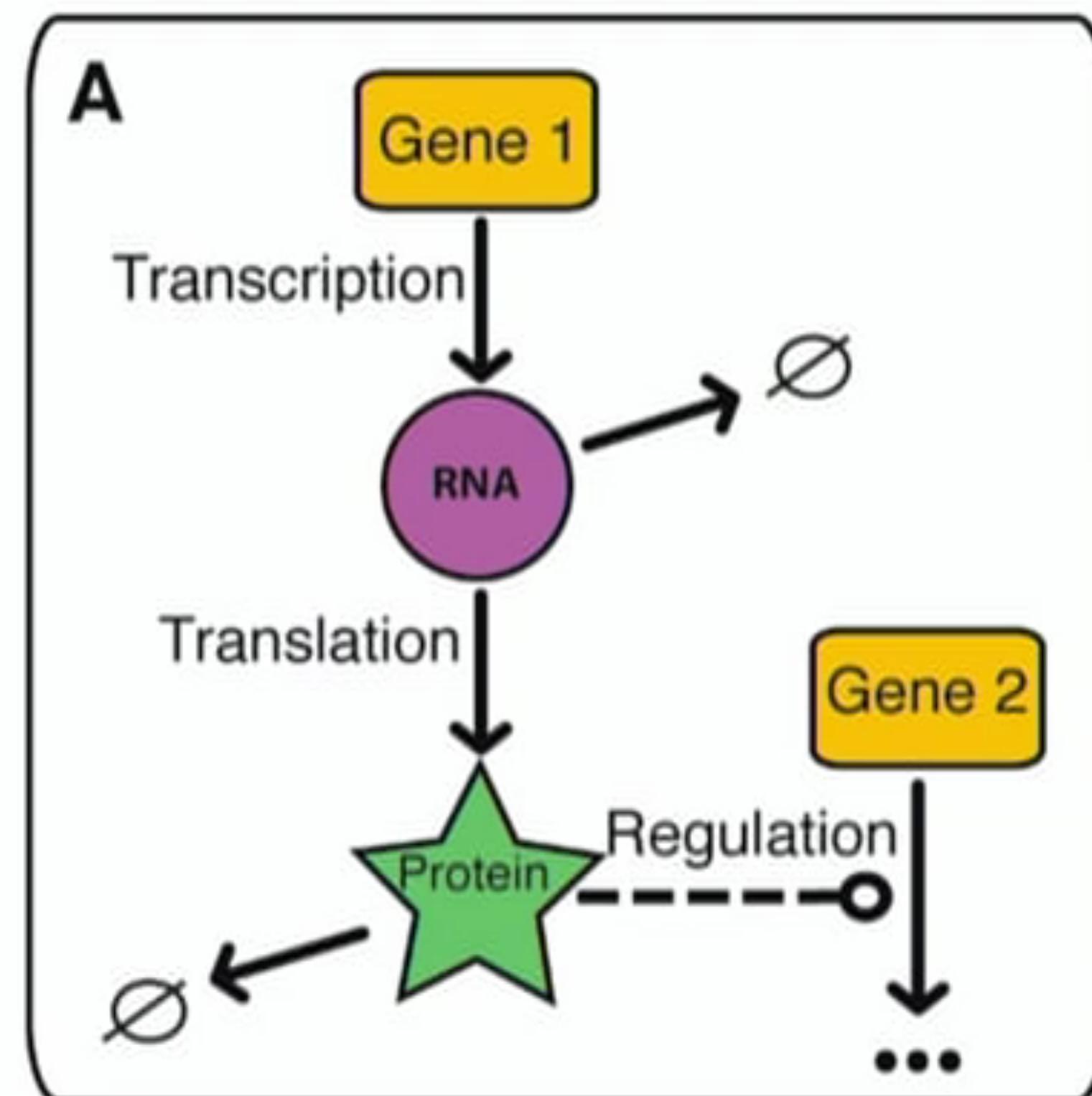
- Gene regulatory networks
- Signalling cascades
- Enzymatic networks
- Regulated metabolic networks



# Gene regulatory networks can be very complex but they are conceptually simple

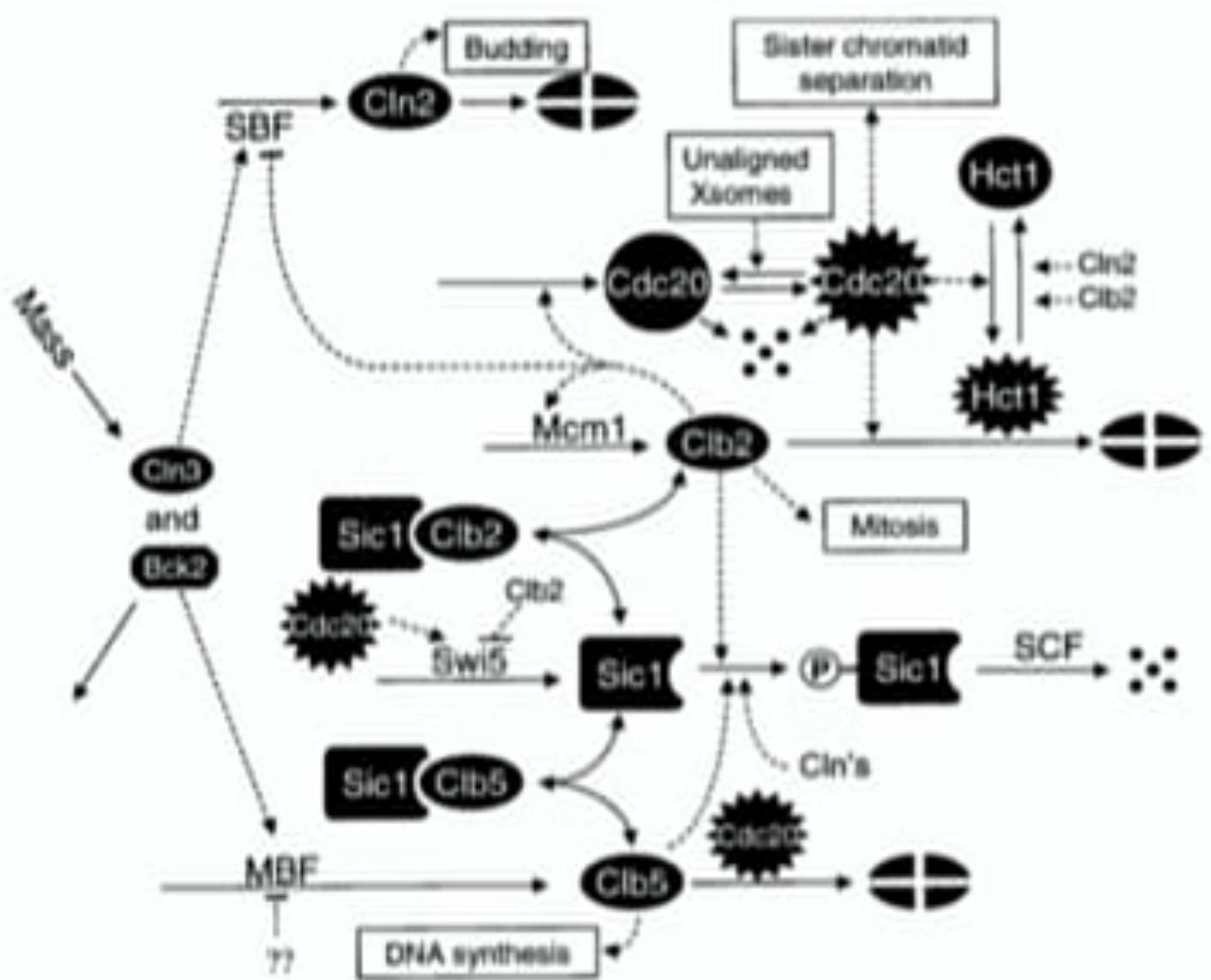
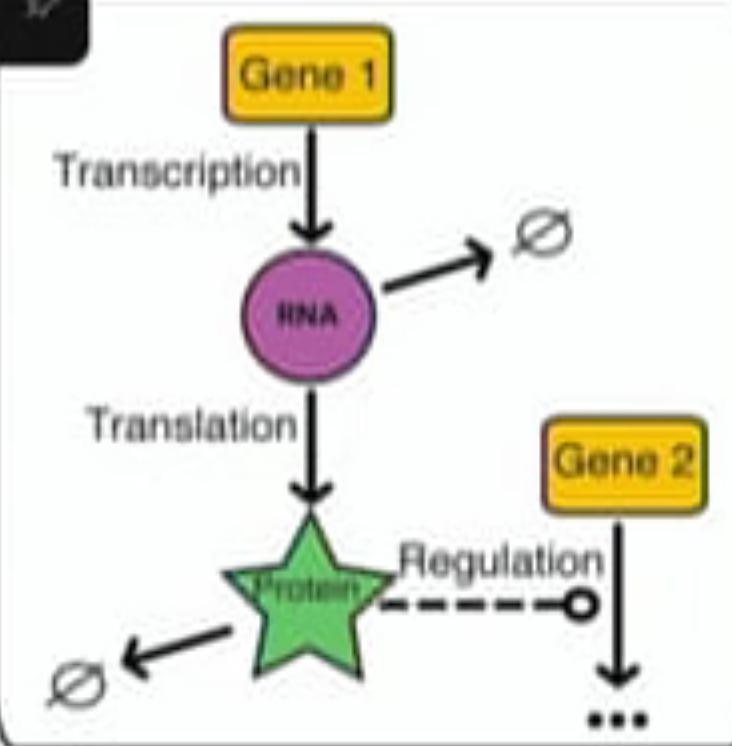


# Gene regulatory networks: A modular way to build reaction networks, *in vivo*, using the protein expression machinery



- ✓ 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



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

**TOPOLOGY**

In the case of spatially distributed systems

**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



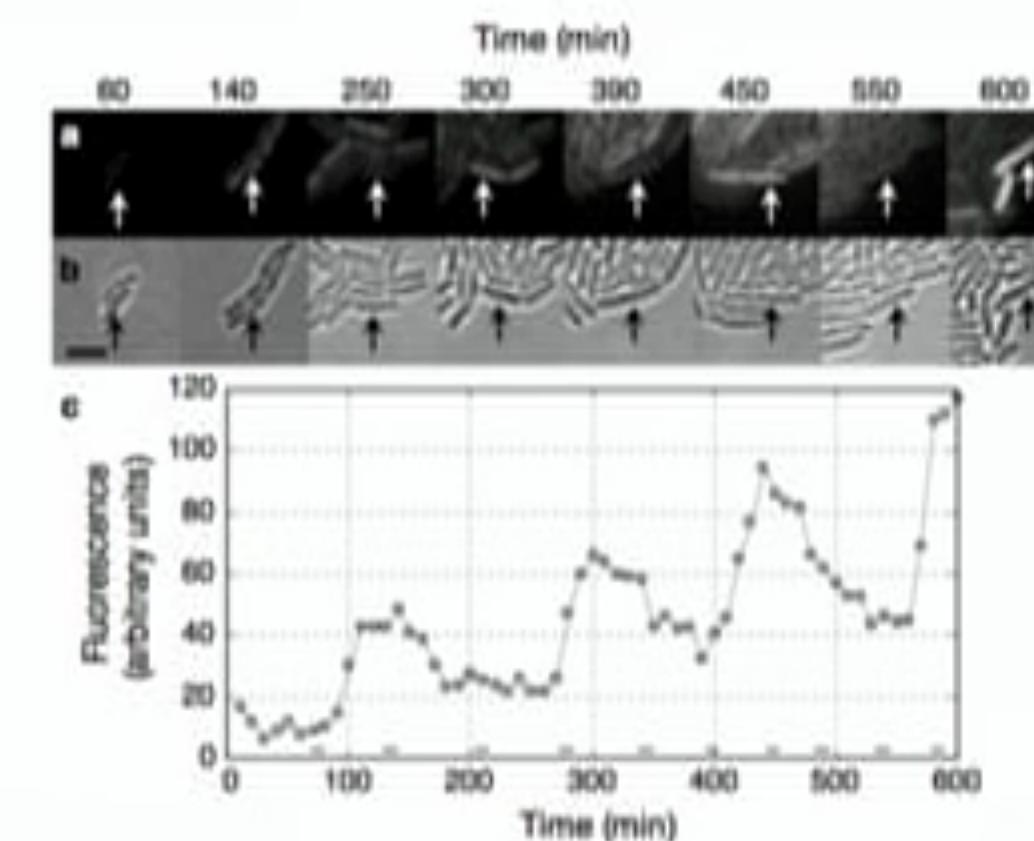
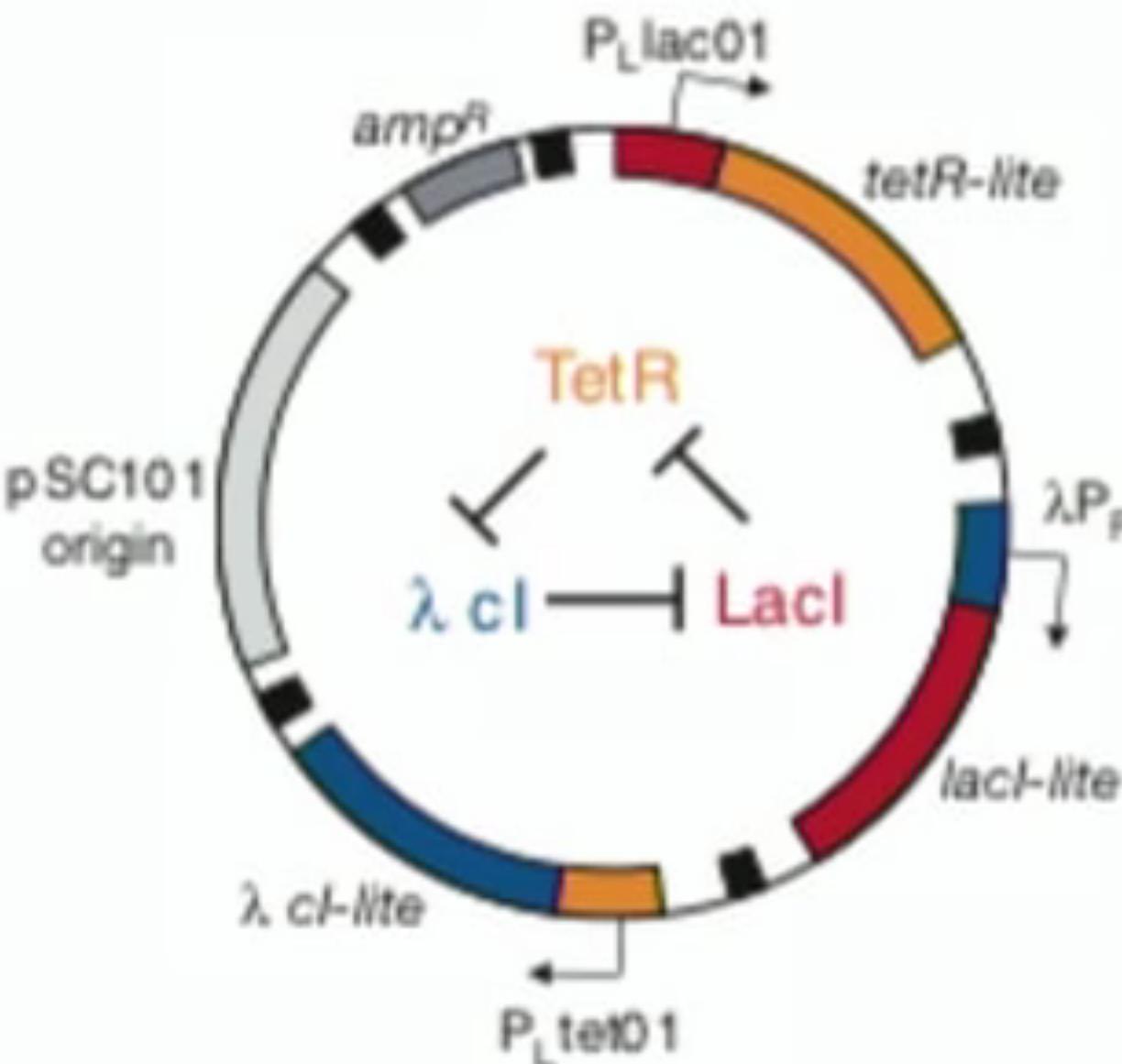
# Synthetic biology

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 available

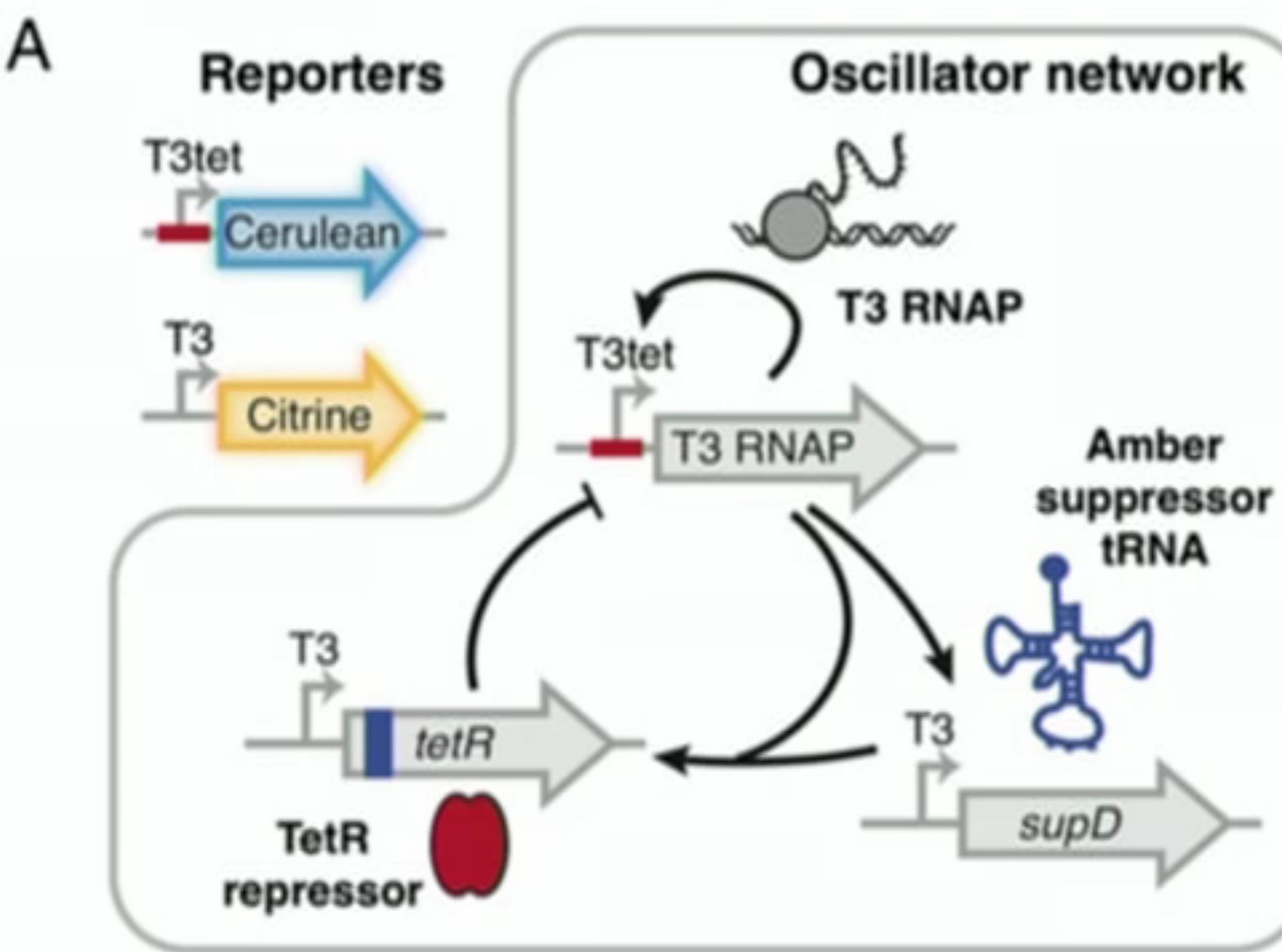
**Lambda-cl** is a phage regulator that controls the switching between the two life style of a phage (a virus)

in vitro circuits based on full cellular machinery (protein expression and gene regulation)  
i.e. in cell extracts

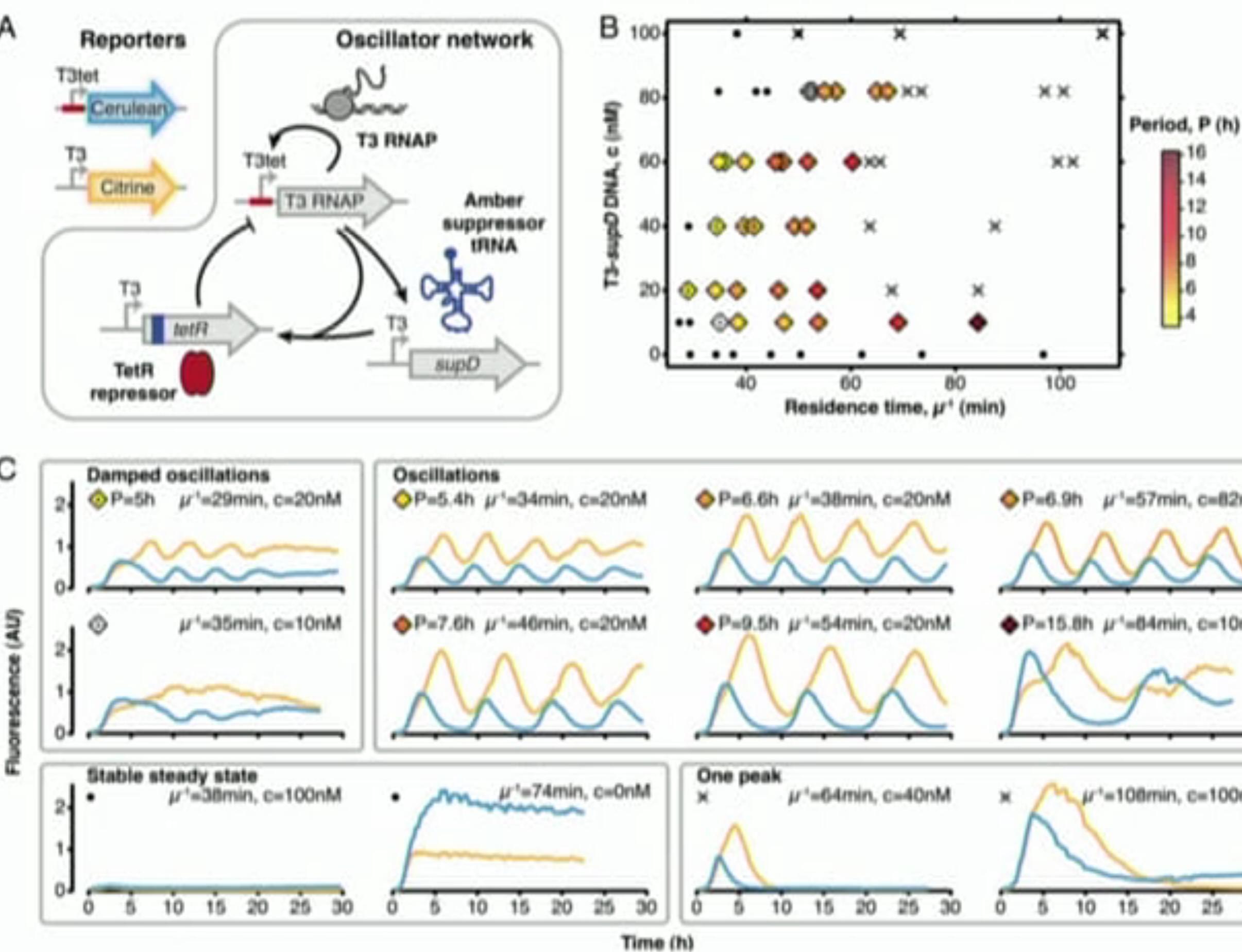
### Implementation of cell-free biological networks at steady state

Henrike Niederholtmeyer, Viktoria Stepanova, and Sebastian J. Maerkl<sup>1</sup>

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

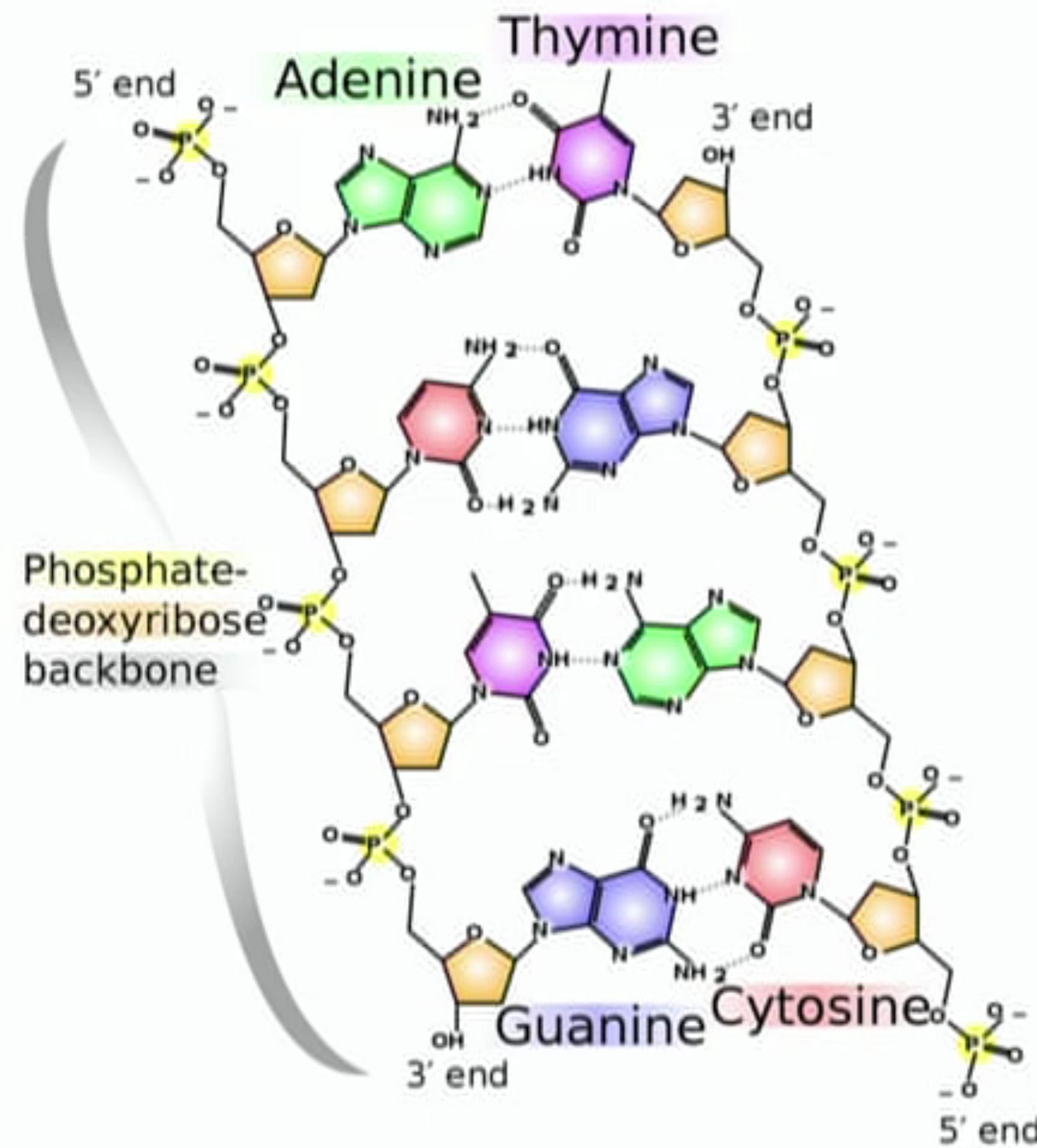
# Harnessing synthetic DNA and purified enzymes to construct *in vitro* reaction networks

As models of cellular networks  
For a chemistry of information processing

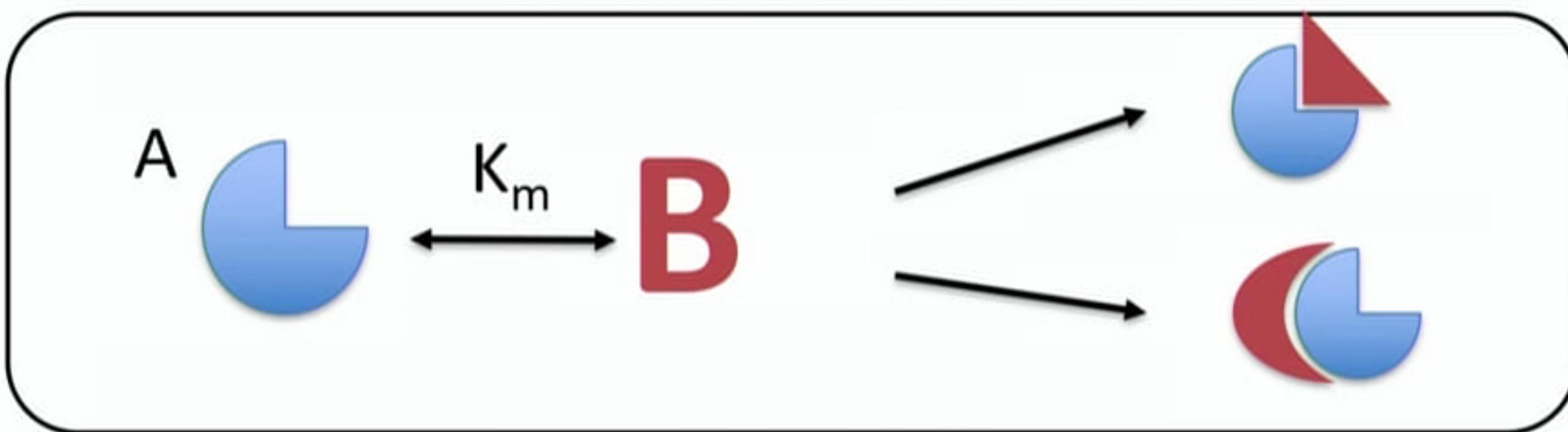


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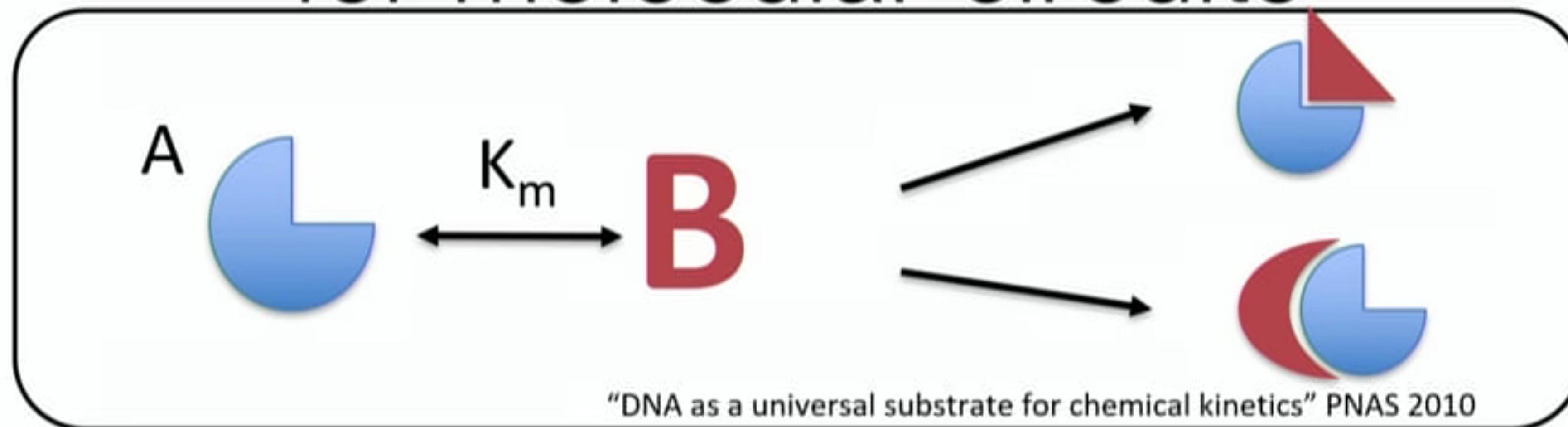
# DNA as a *synthetic* material for molecular circuits



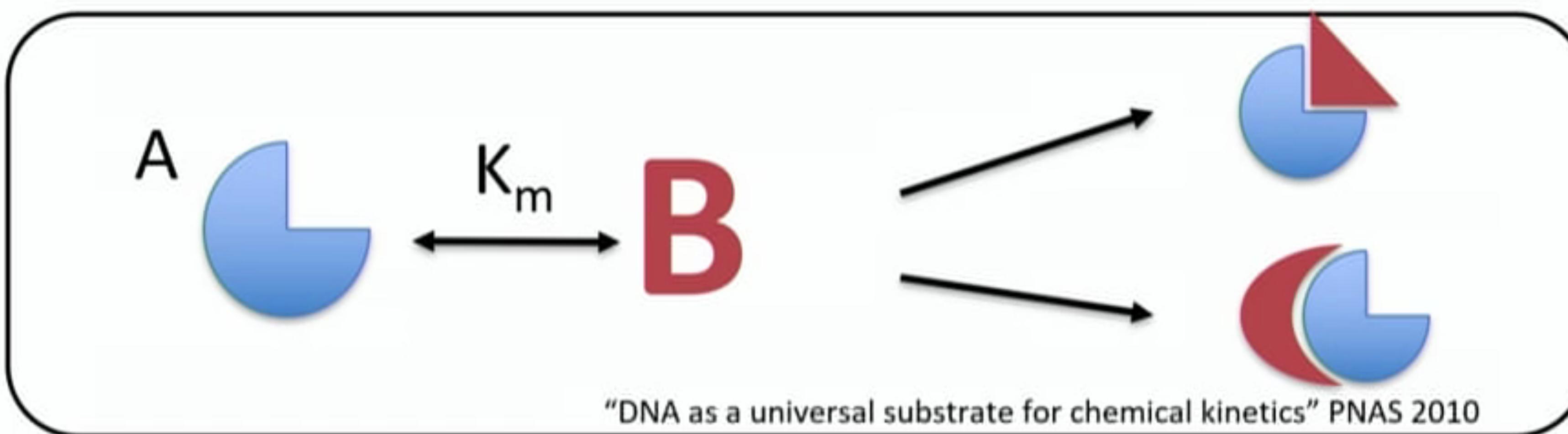
# DNA as a *synthetic* material for molecular circuits



# DNA as a *synthetic* material for molecular circuits



# DNA as a synthetic raw material for molecular circuits



## Enzymes:

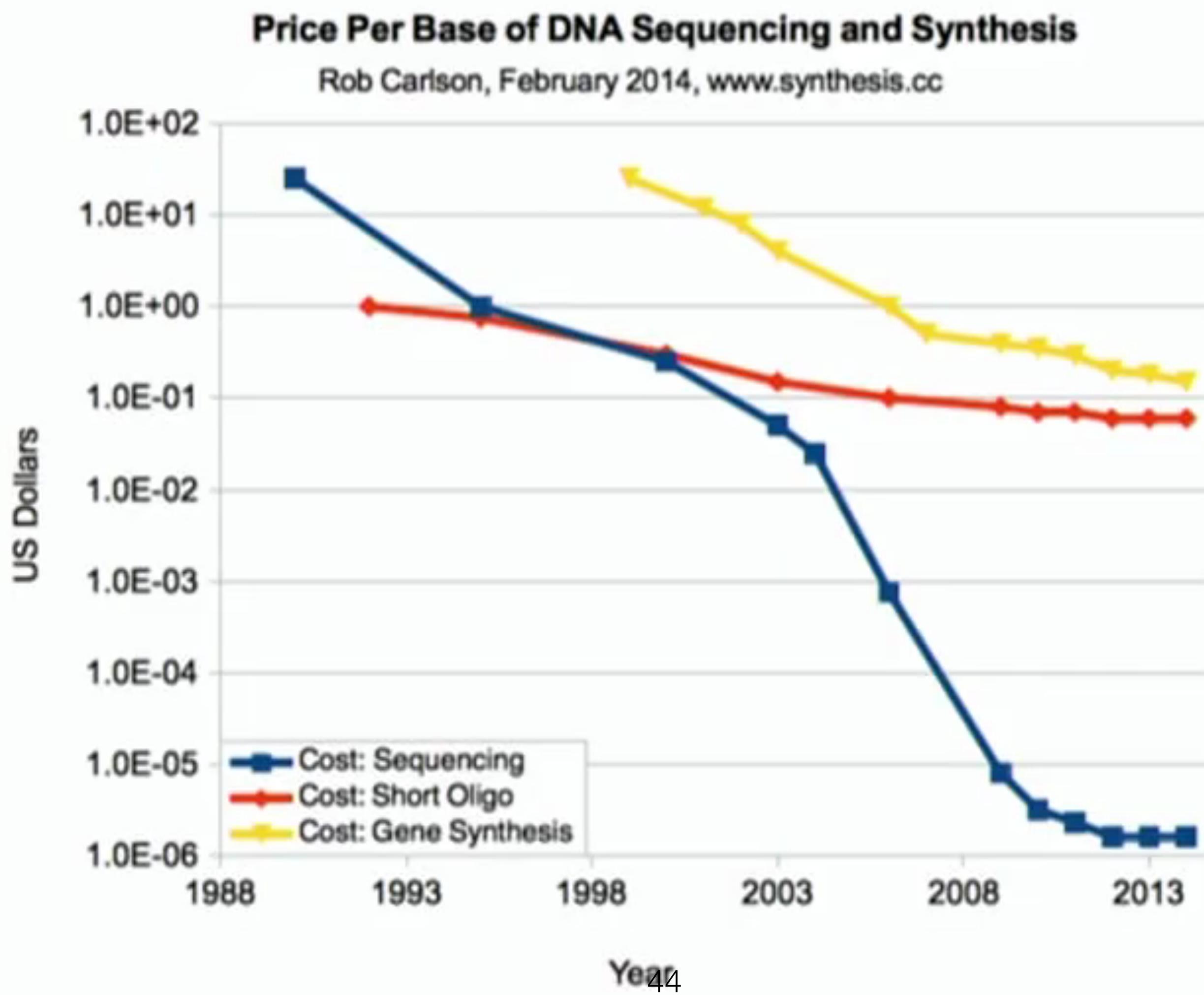
Copy: Polymerases

Cut-paste: Restriction enzymes and Ligases

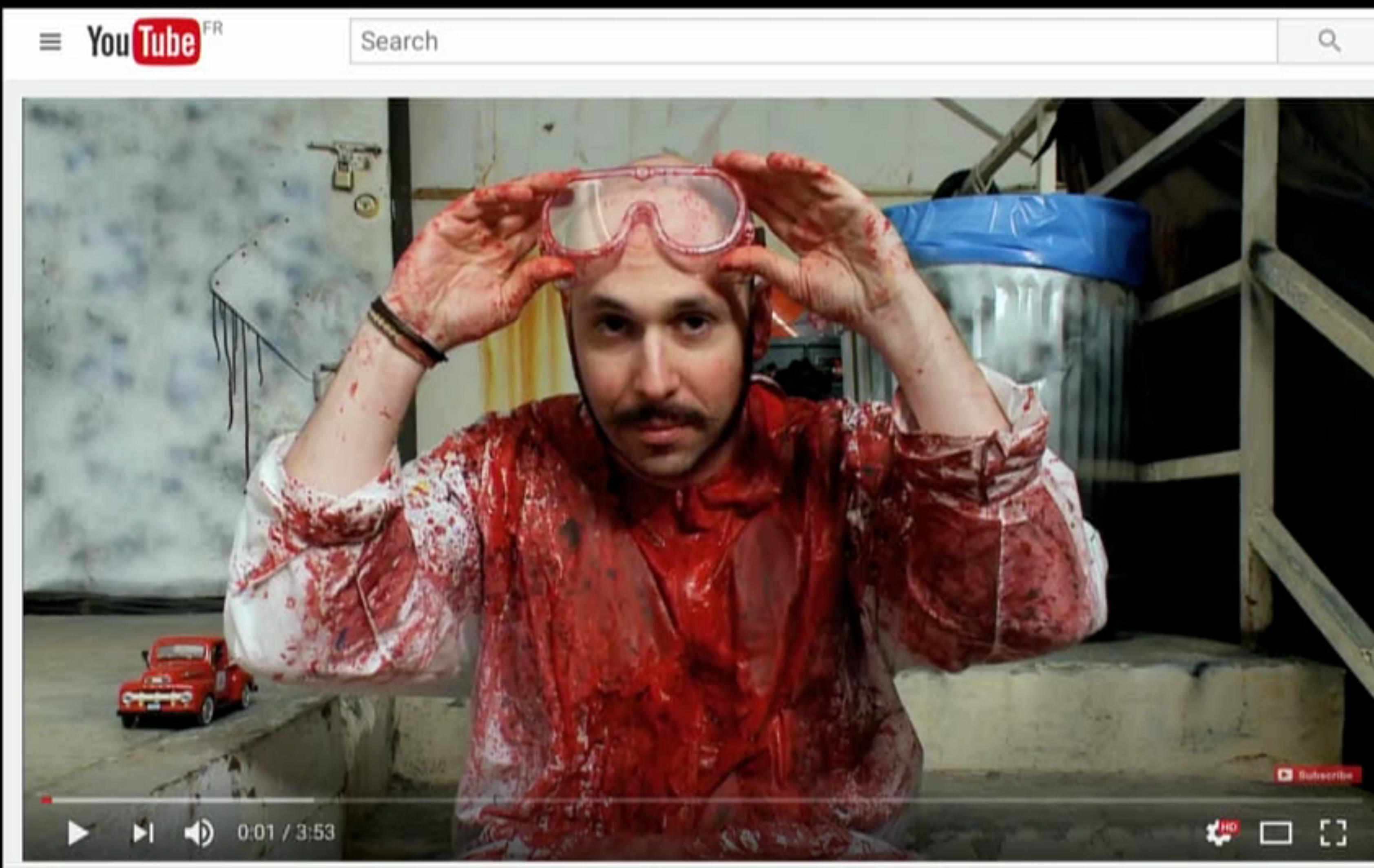
Delete: Nucleases



# Low cost DNA synthesis



# DNA data storage: 200 MB written in, stored and read from DNA

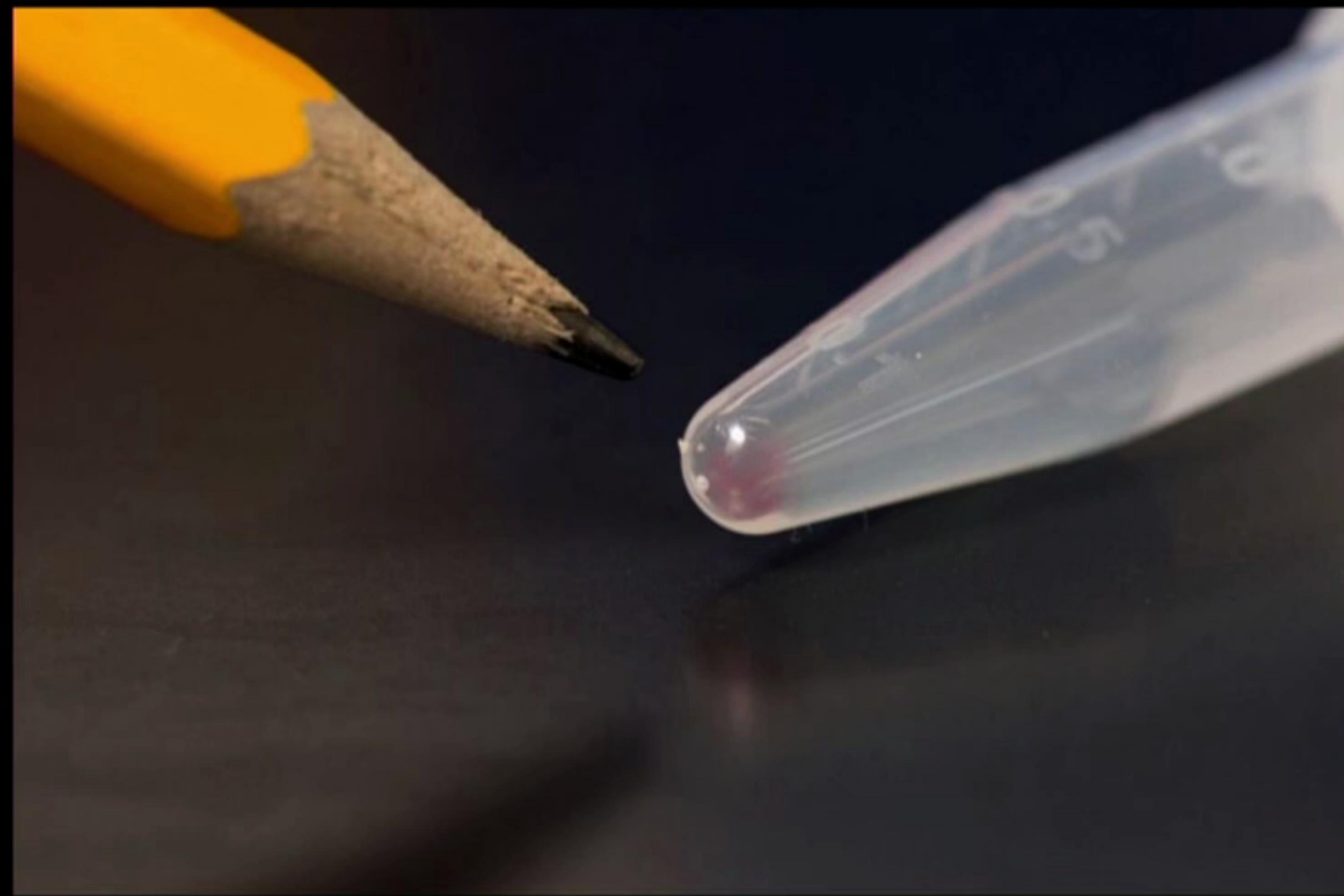


(OK go video) Microsoft, U. Washington, Twist



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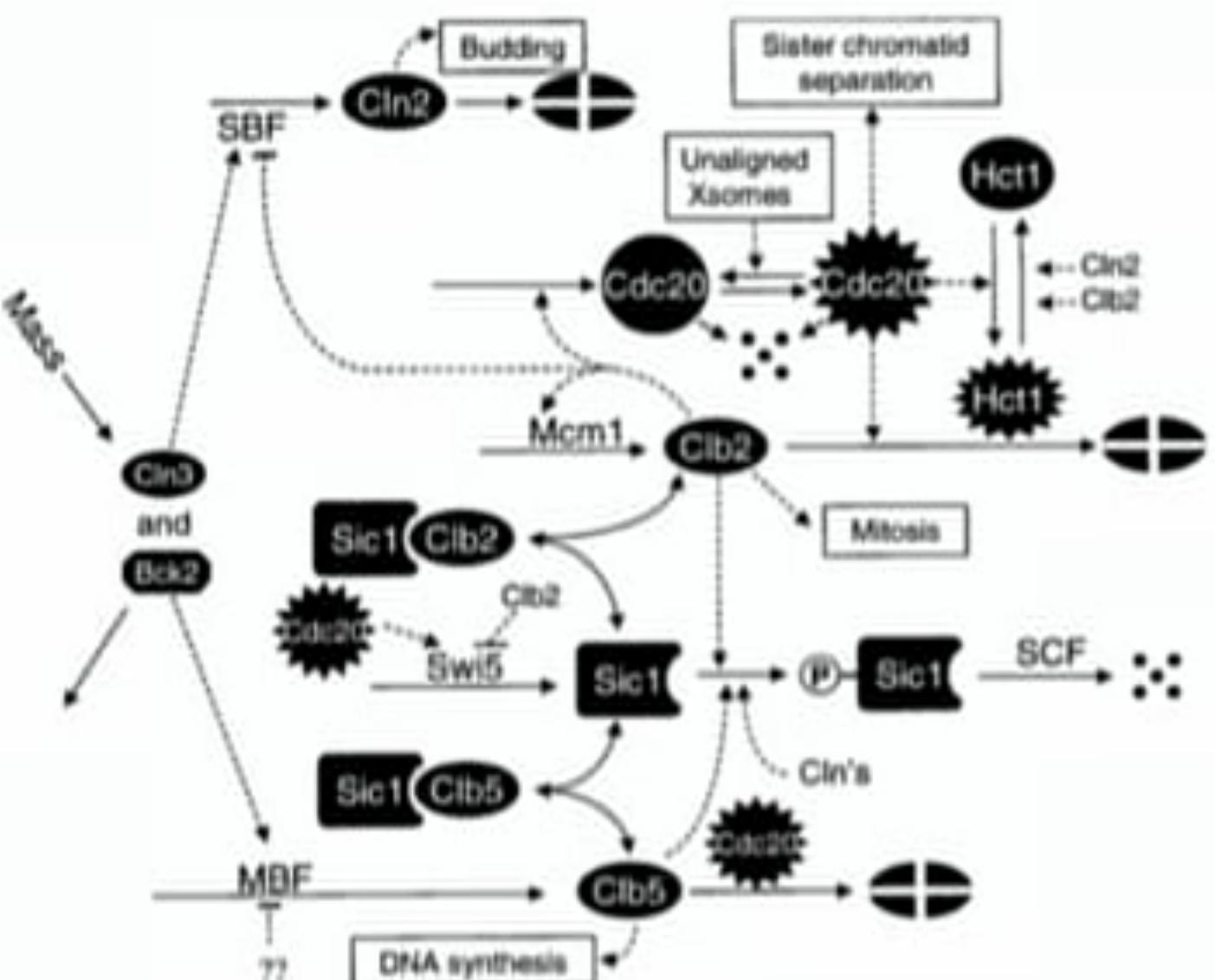
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# Molecular networking approaches

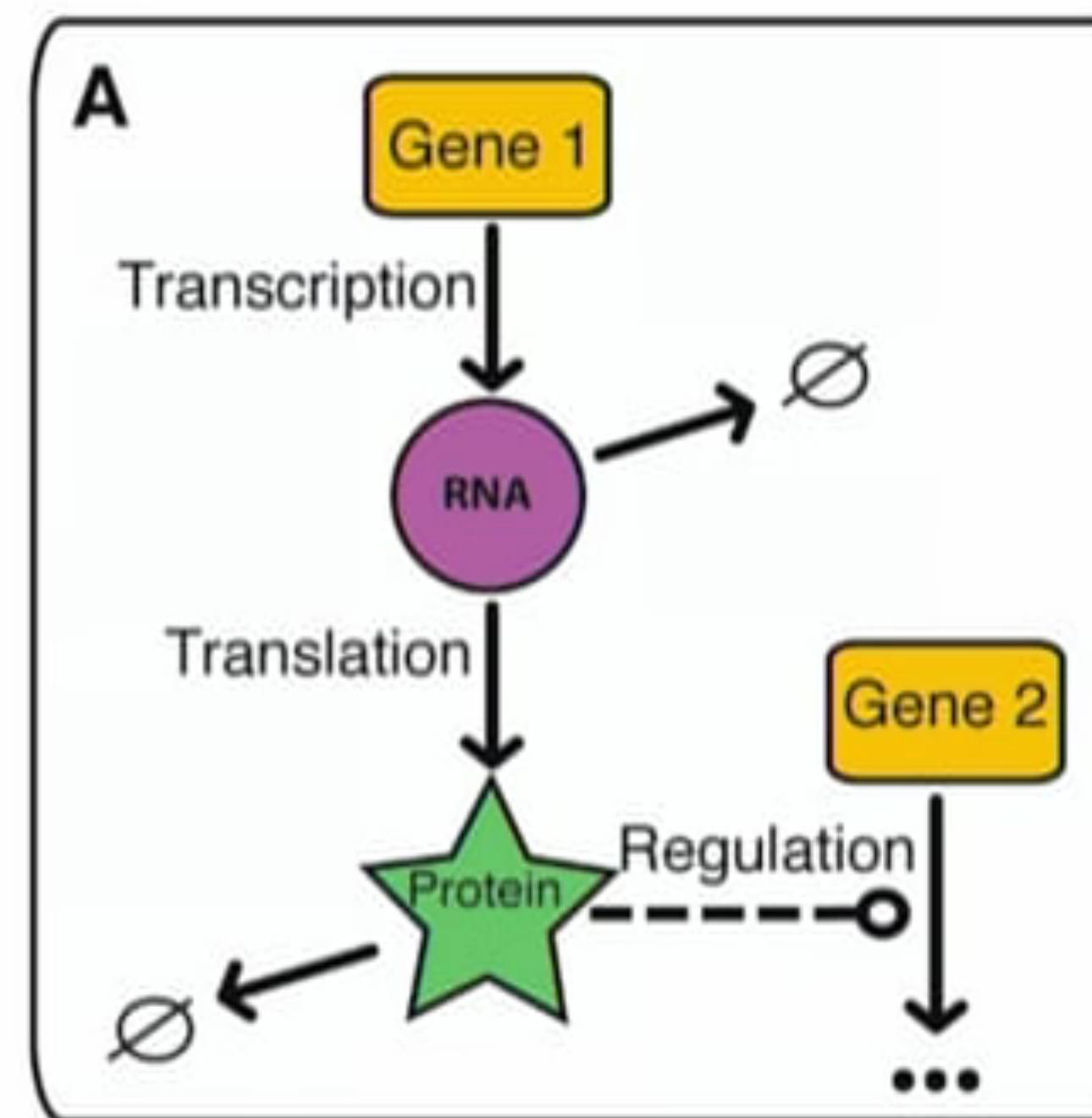


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# Simplified models of biological regulatory networks



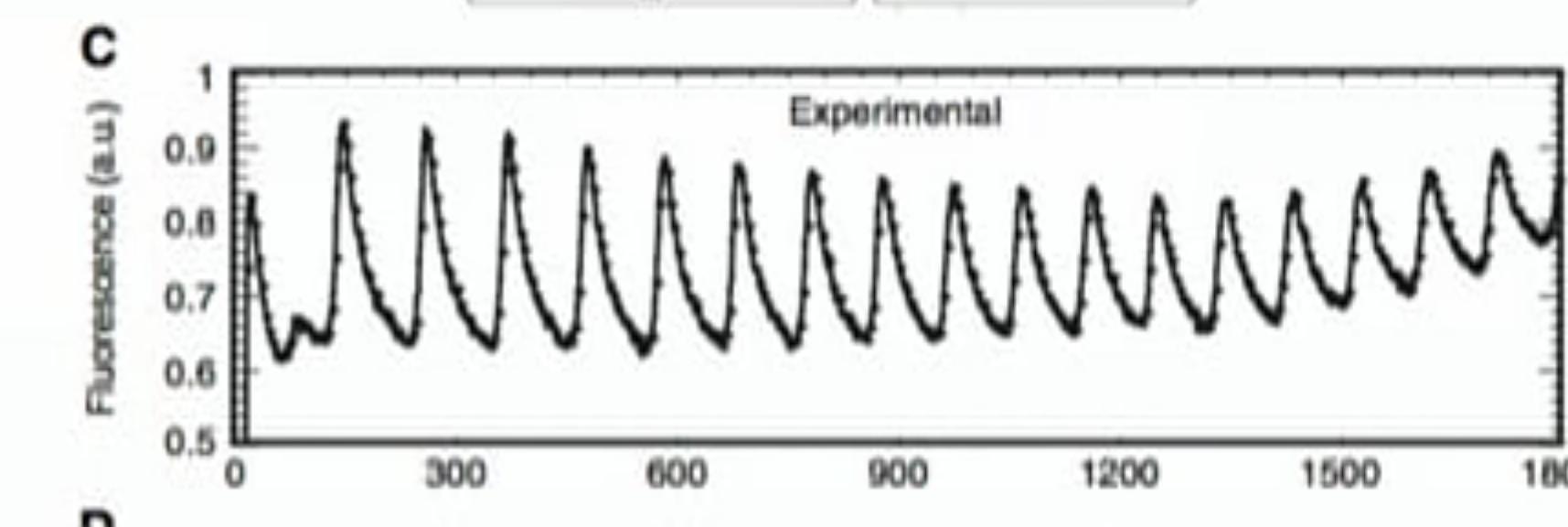
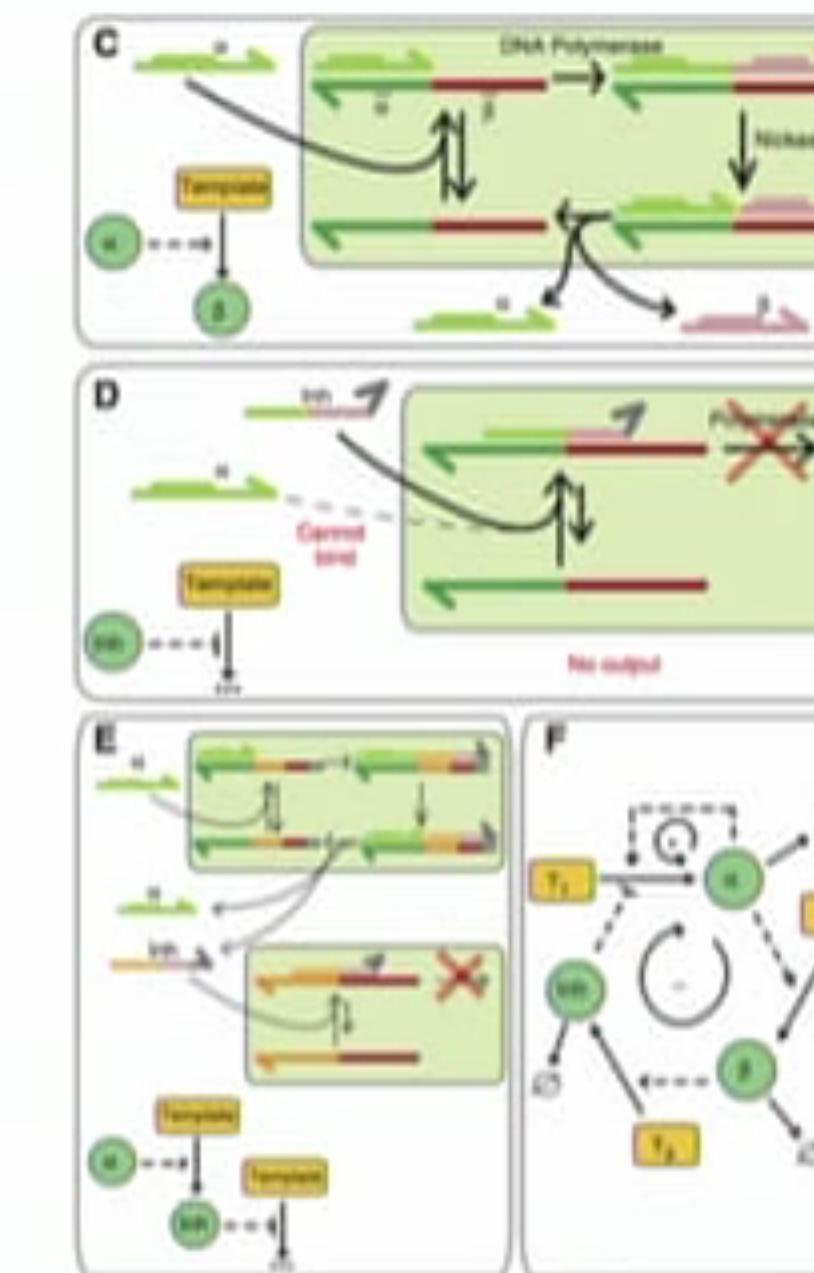
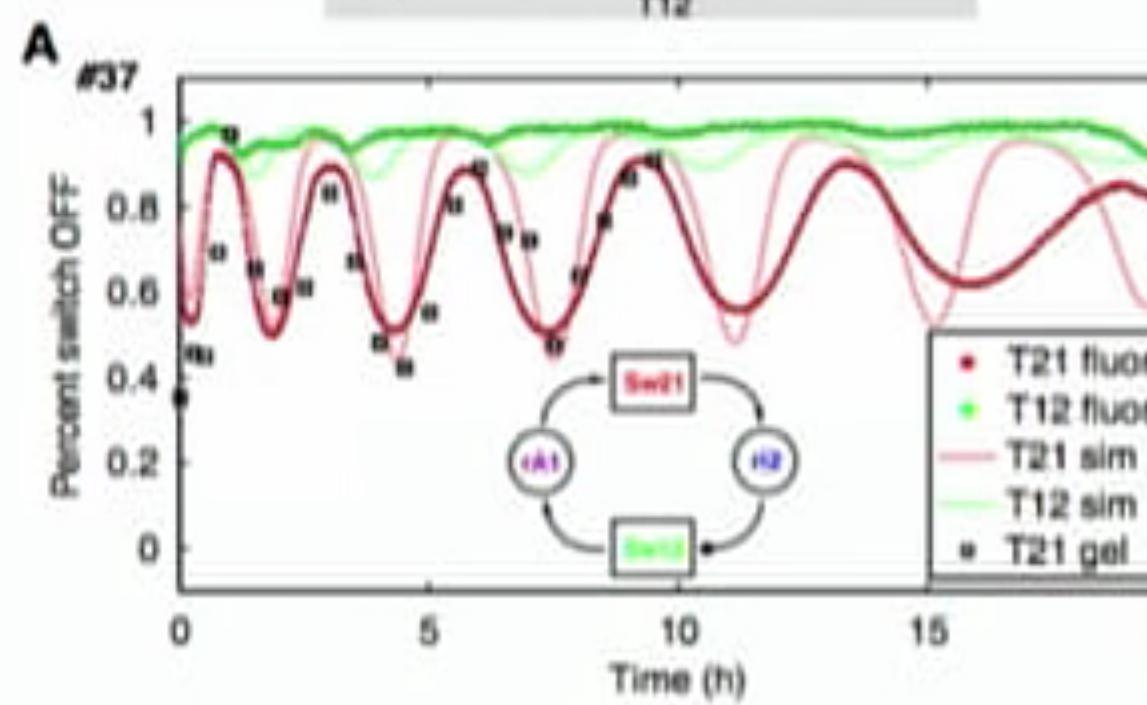
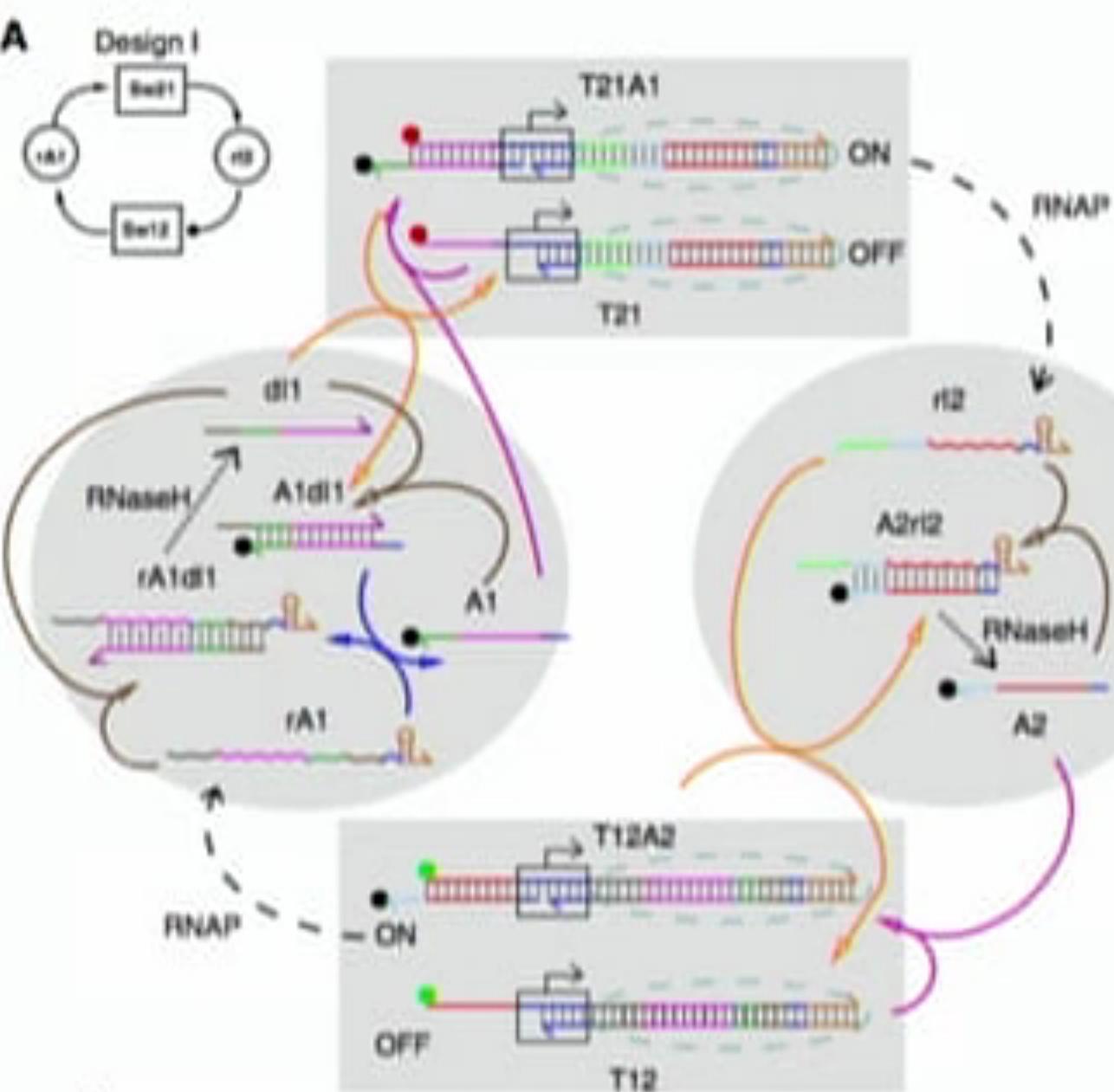
Regulatory network of the budding yeast cell cycle (Tyson 2006)



- We want to maintain the modularity and cascadability
- But simplify the chemistry as much as possible

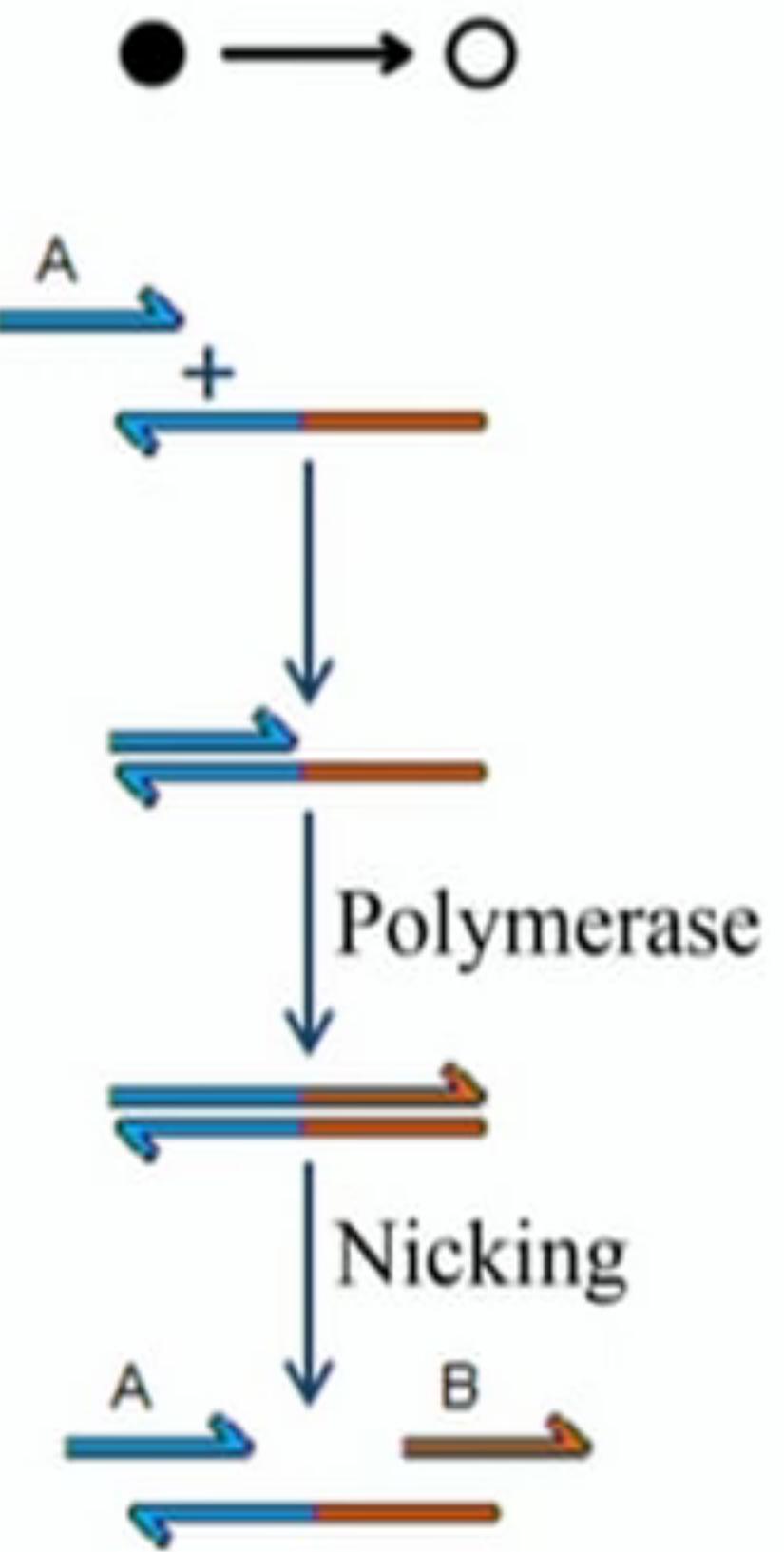


# Artificial equivalent of regulatory networks

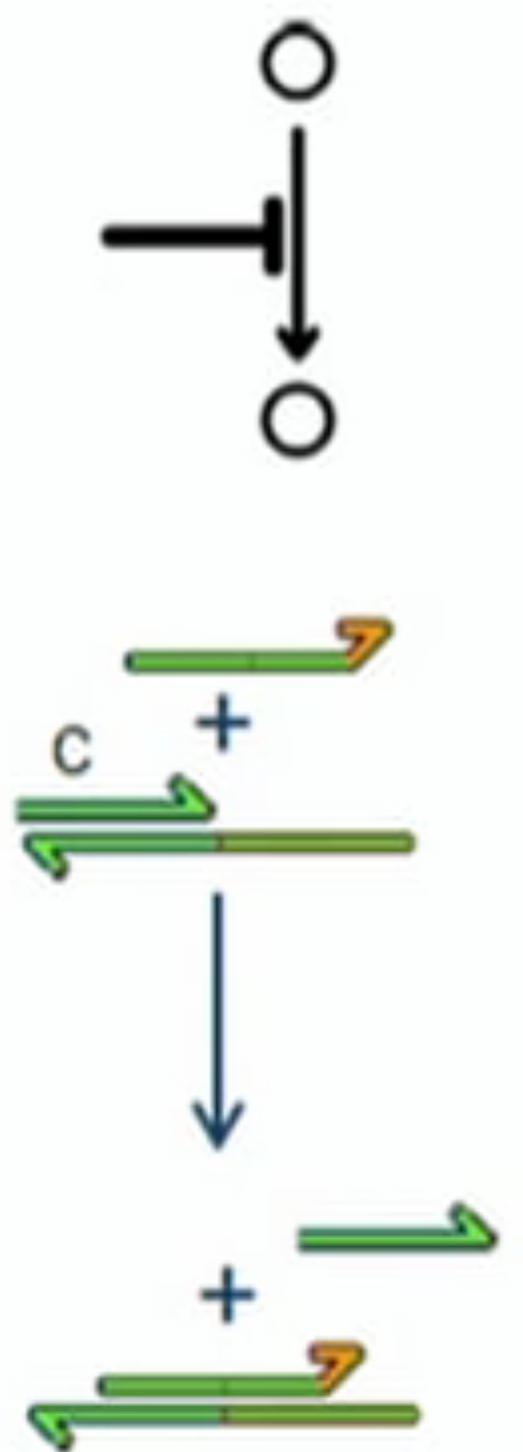


# PEN DNA toolbox: an *in vitro* “universal” reaction set

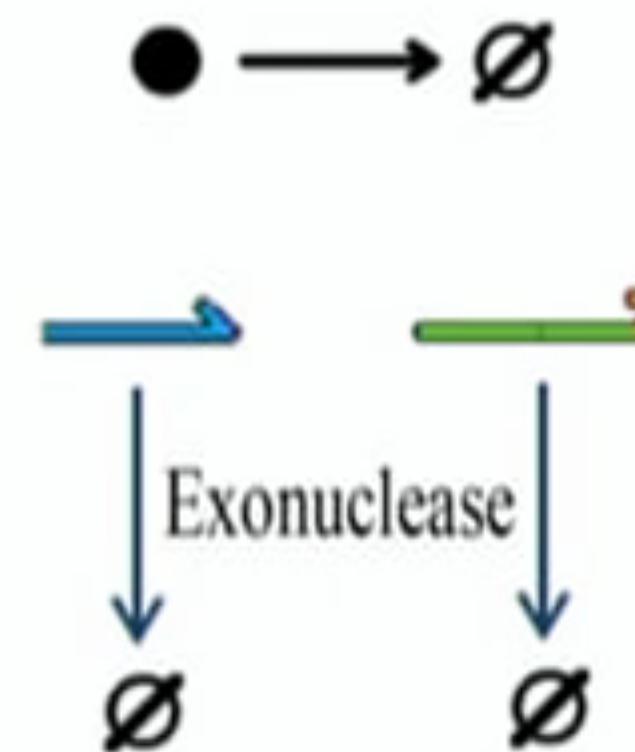
(a) Activation



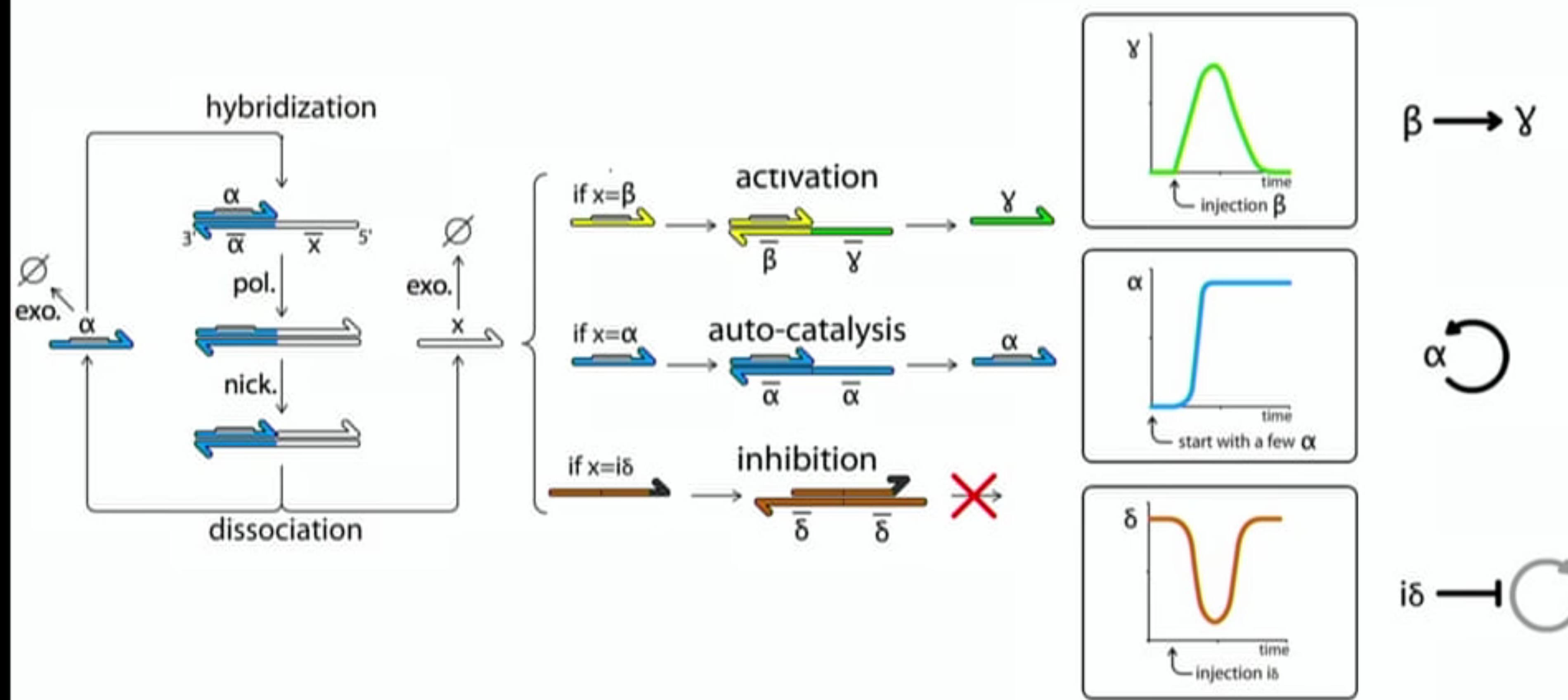
(b) Inhibition



(c) Degradation



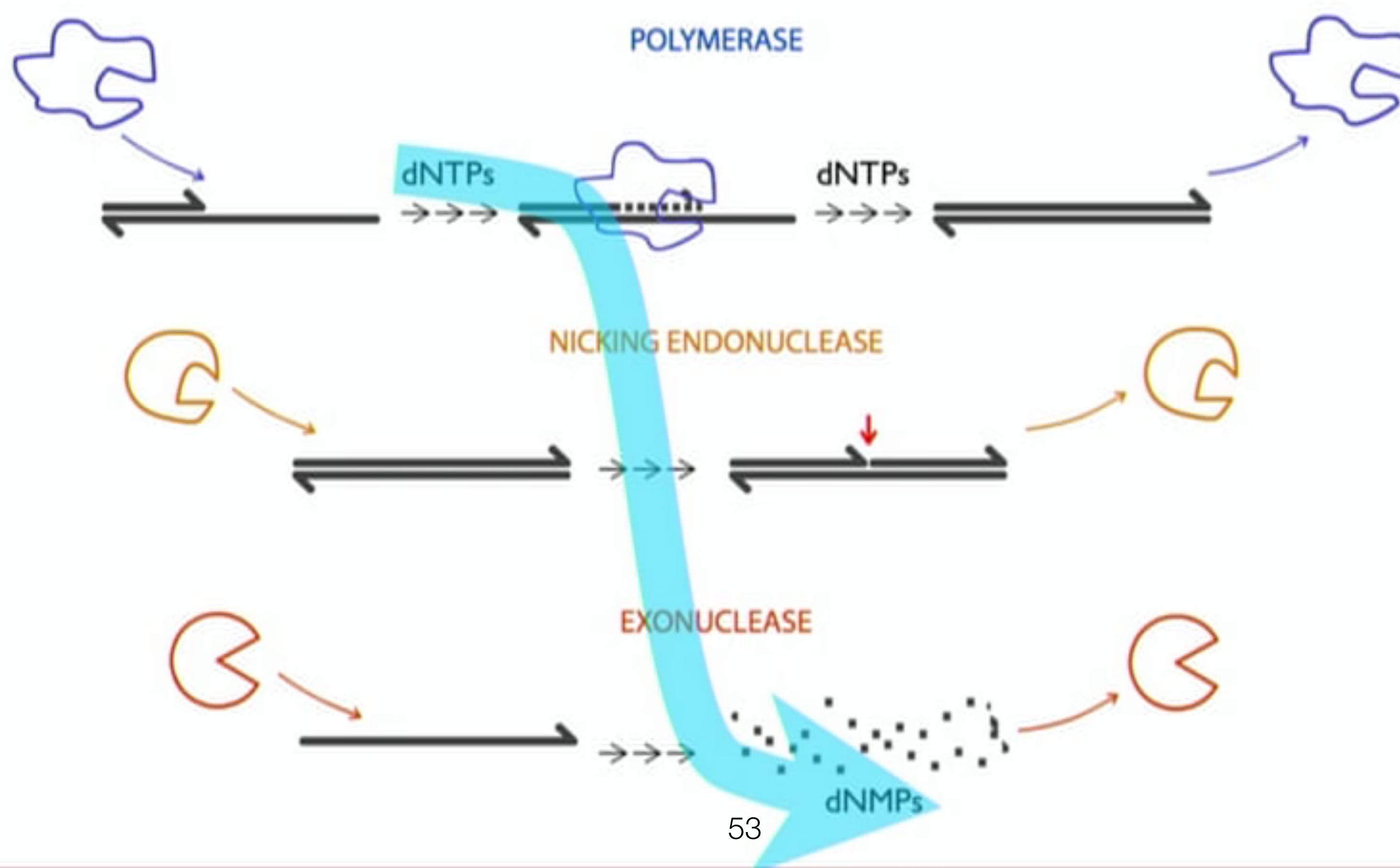
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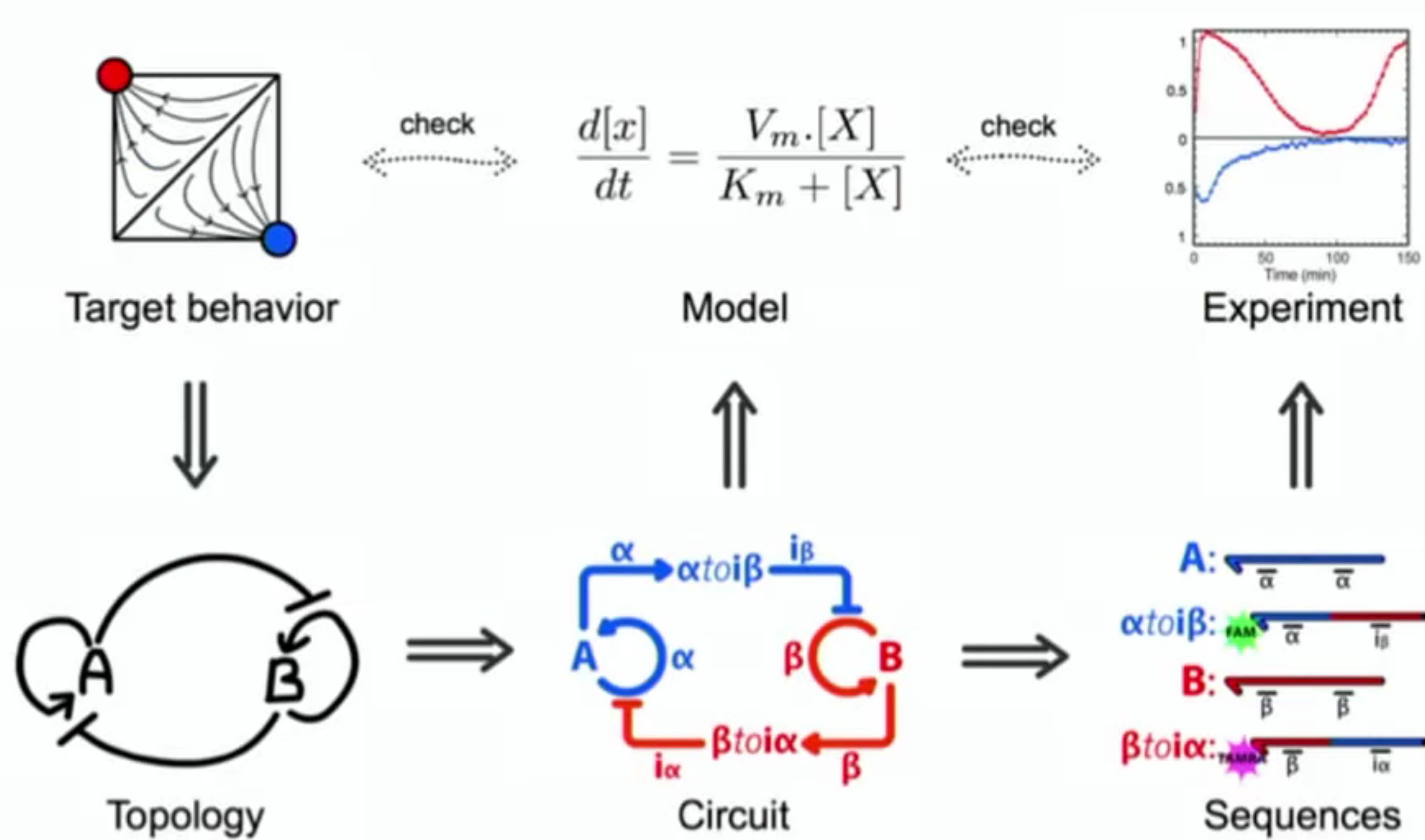
- Cascadable
- Dissipative (Global reaction and energy flux: dNTP  $\rightarrow$  Oligonucleotides  $\rightarrow$  dNMP)

# Energy source and flow

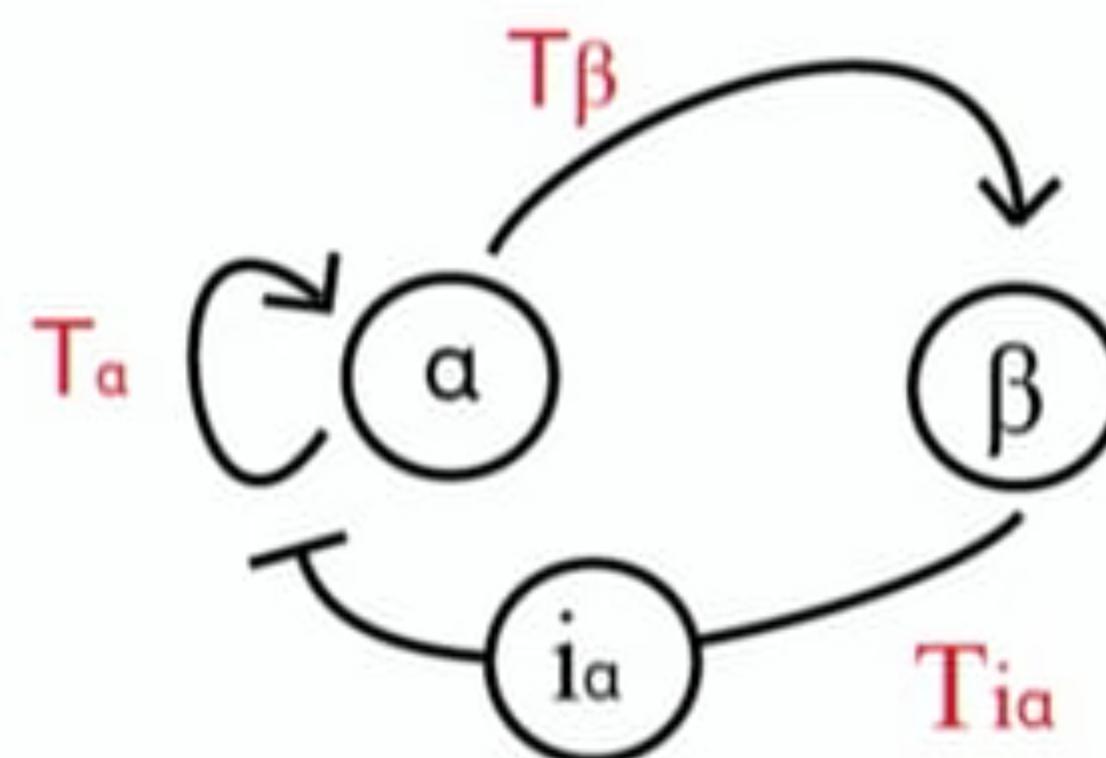
## Enzymes



# Synthesizing dynamics

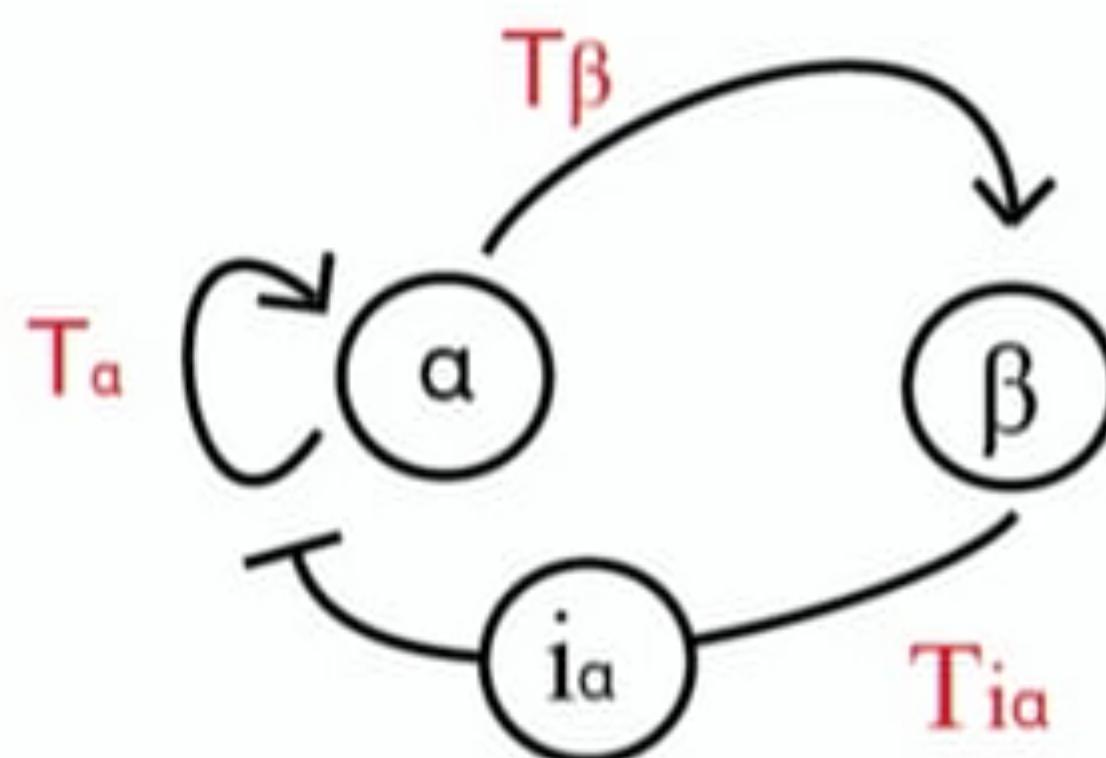


# Building a DNA-encoded oscillator

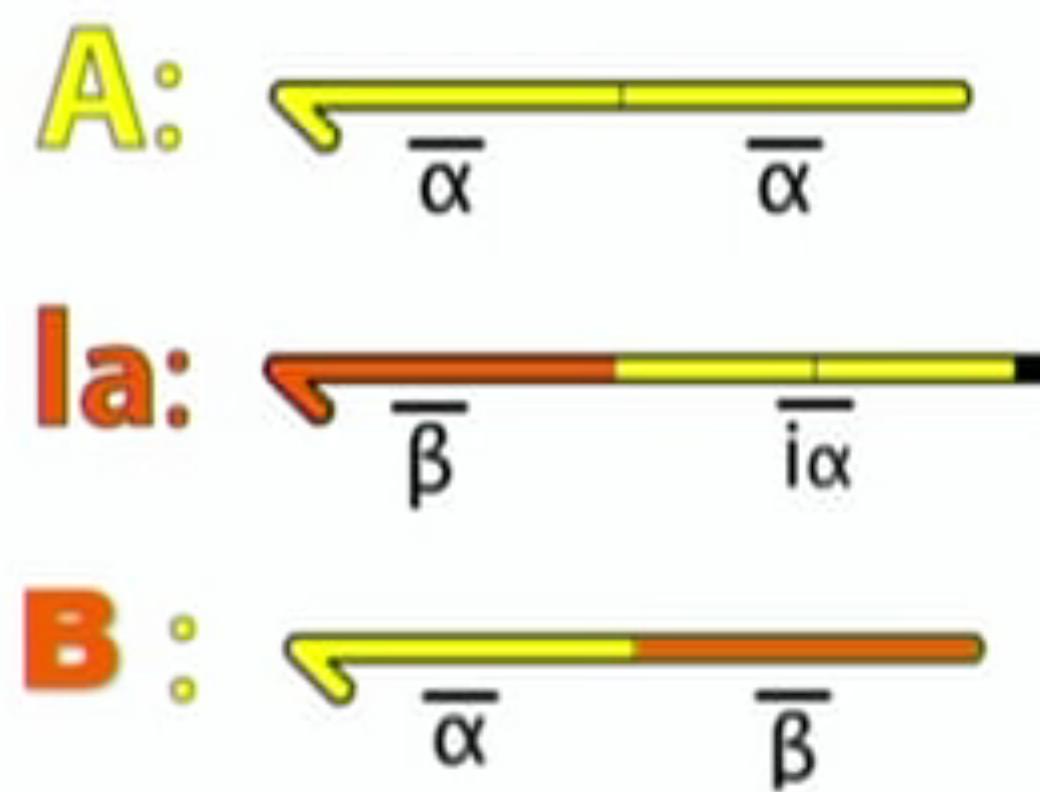


Relaxation oscillator

# Building a DNA-encoded oscillator



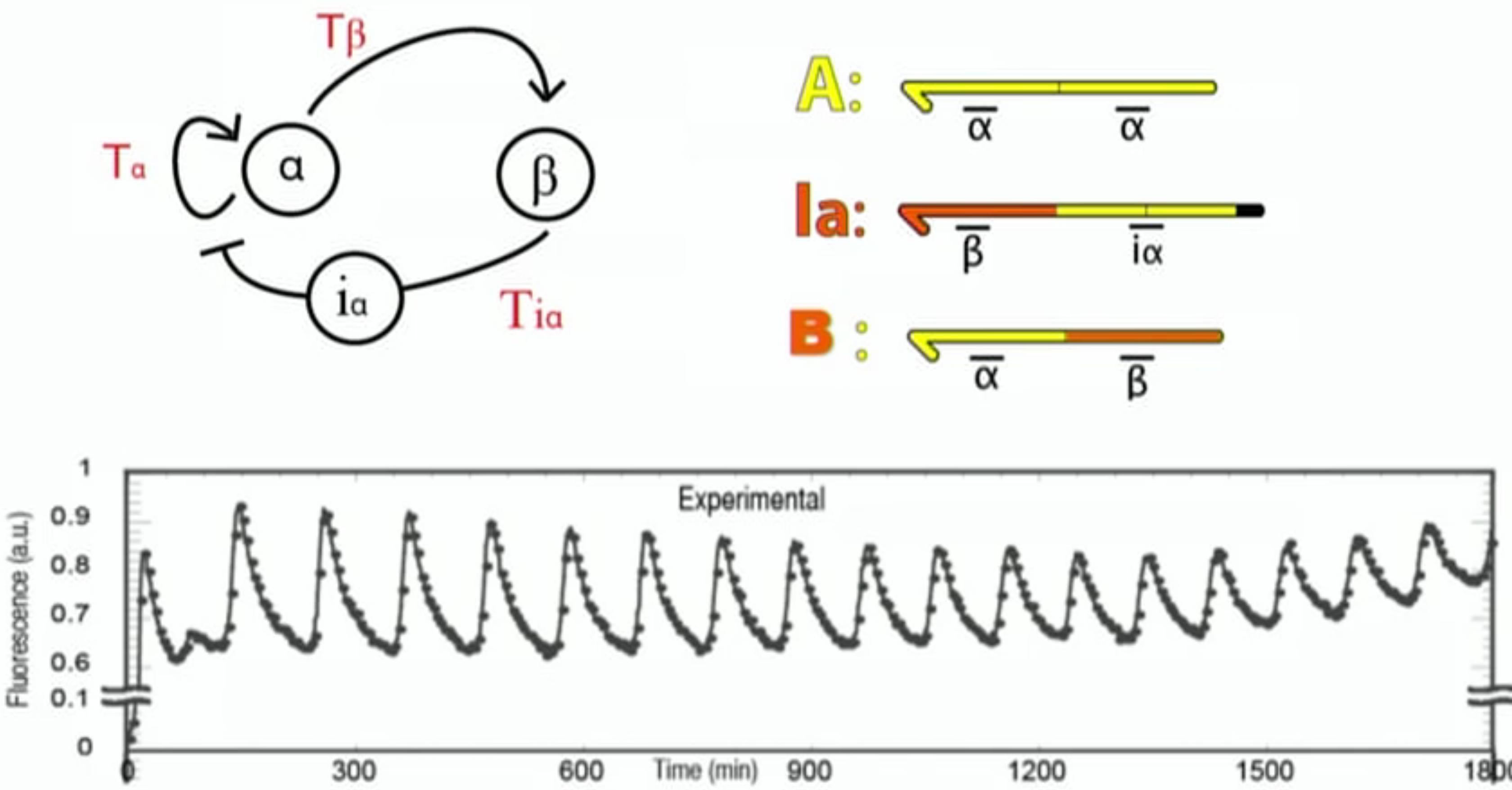
Relaxation oscillator



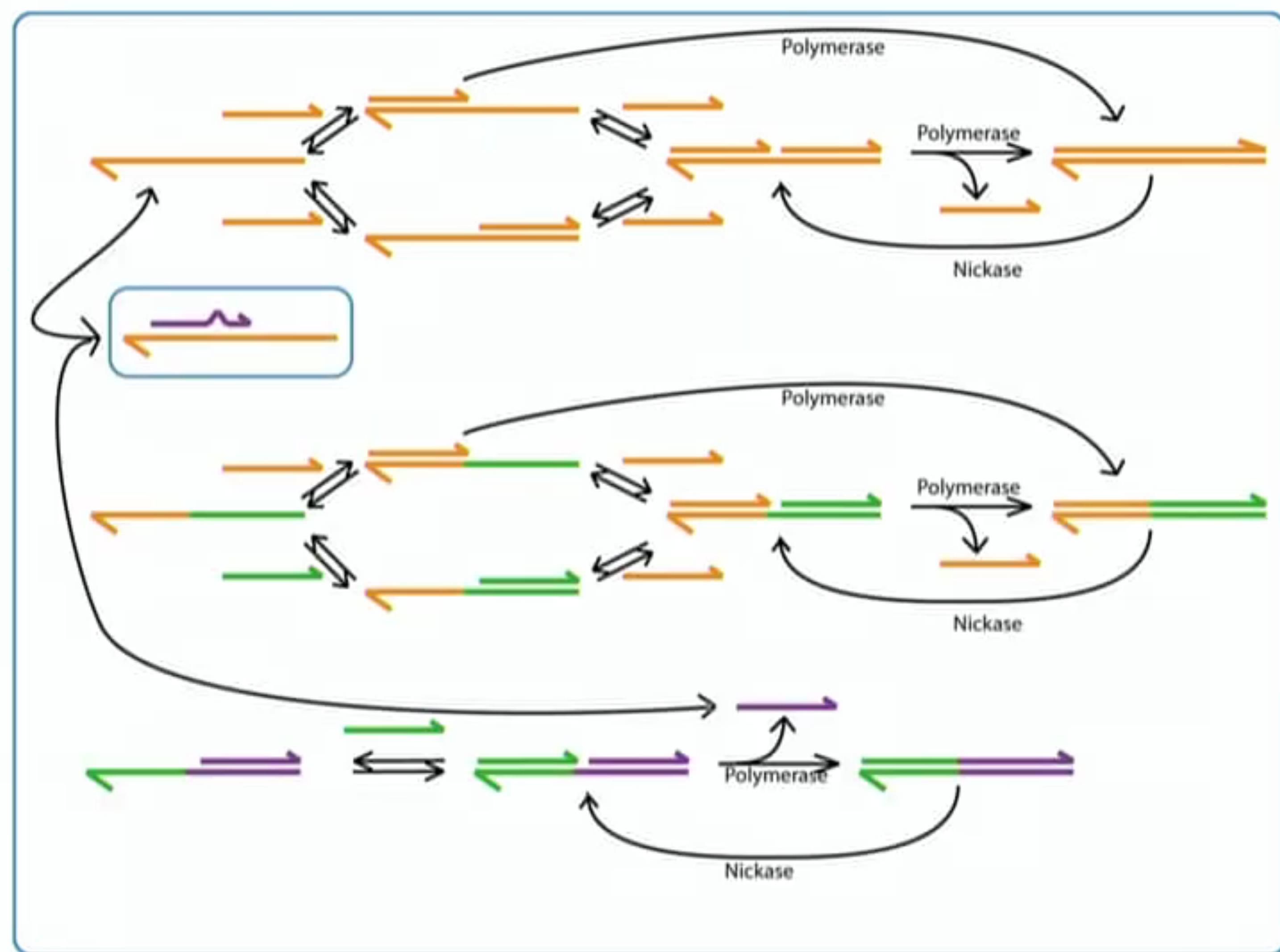
5'-AACAG**ACTCGA**-AACAG**ACTCGA**-3'  
5'-TTACTCGAAACAGACT-GGAT**GACTCCA**-3'  
5'-GGAT**GACTCCA**-AACAG**ACTCGA**-3'



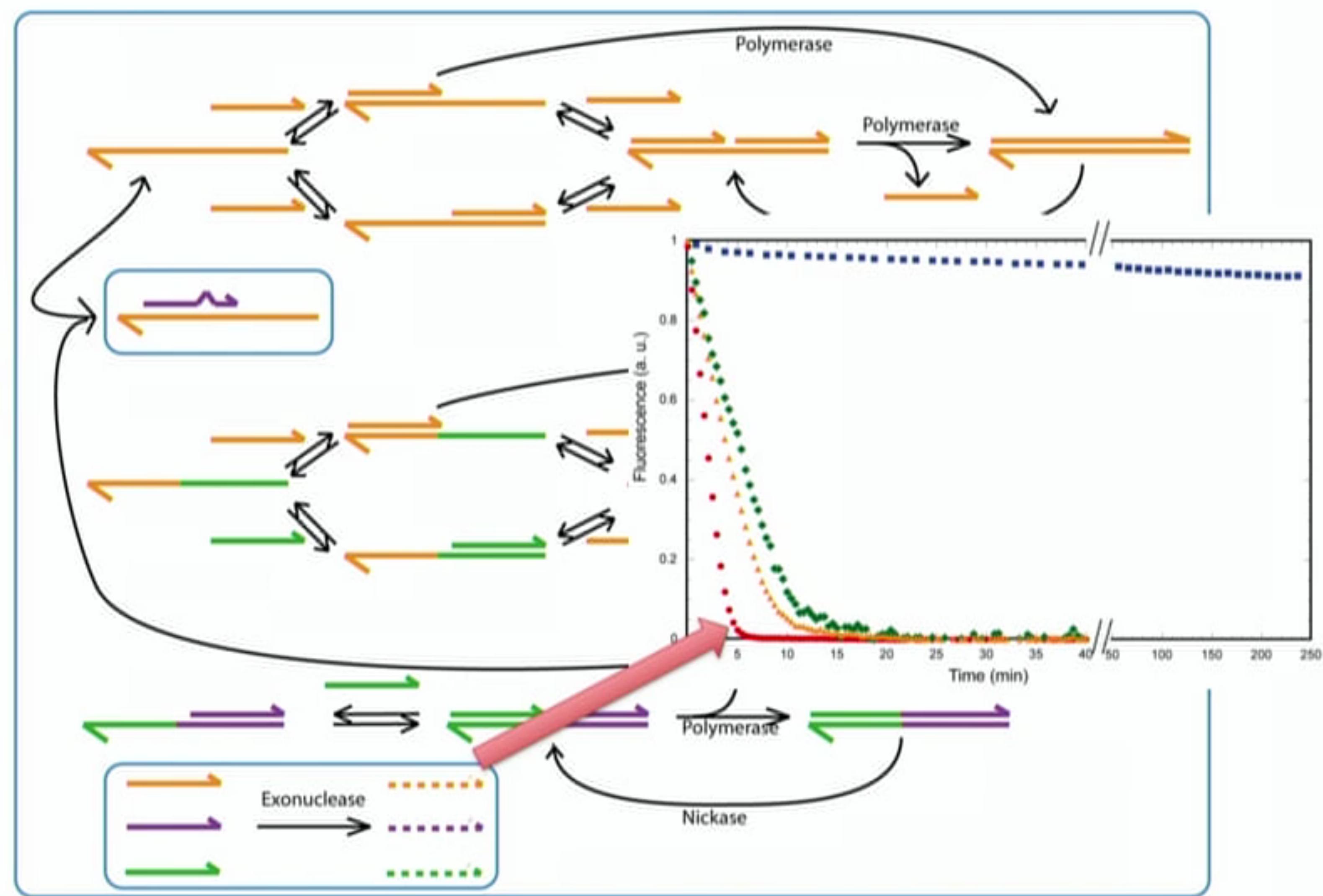
# Building a DNA-encoded oscillator



# Modeling (detailed kinetics)



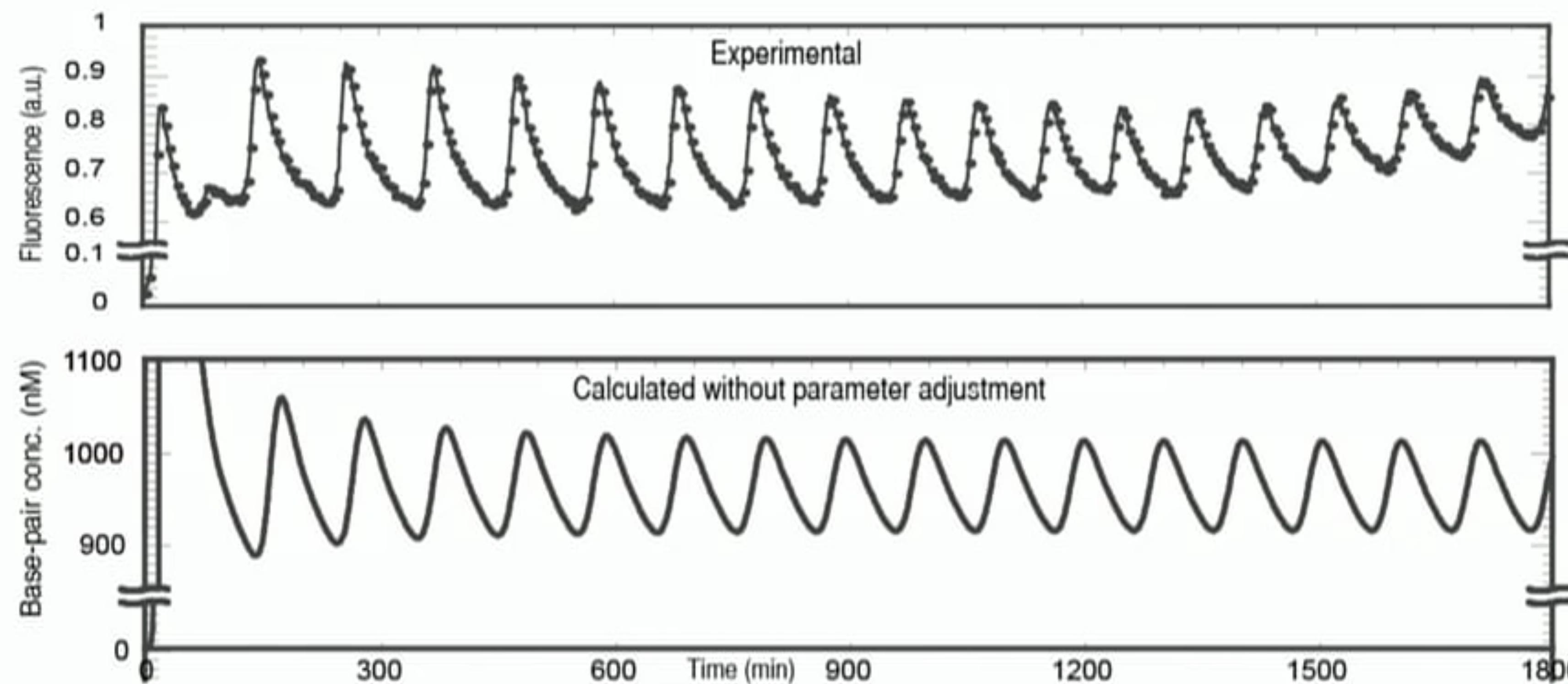
# Modeling (detailed kinetics)



# ODE system

$$\begin{aligned}
 x'(t) &= \frac{\text{cpol kpsd } x\text{Tx}(t)}{\text{Kmsd MMf}(t)} + \text{cpol leak kp } T(t) - k1 \ x(t) (\text{Dr}(t) + 2 \ T(t) + \text{Tx}(t) + \text{Txa}(t) + \text{xT}(t) + \text{xTx}(t)) + k1 \left( \frac{x\text{T}(t)}{\text{K}1\text{l}} + \frac{x\text{Tx}(t) + x\text{Txa}(t)}{\text{K}1\text{l}} + \frac{\text{Tx}(t)}{\text{K}1\text{o}} + \frac{\text{xTxa}(t) + \text{xTx}(t)}{\text{K}1\text{o}} \right) - \frac{\text{crec krec } x(t)}{\text{Kmr MMr}(t)}, \\
 T'(t) &= k1 \left( \frac{\text{xAT}(t) + \text{xT}(t)}{\text{K}1\text{l}} + \frac{\text{Tx}(t) + \text{Txa}(t)}{\text{K}1\text{o}} \right) - k1 \ T(t) (2 \ x(t) + 2 \ \text{xT}(t)), \\
 x\text{T}'(t) &= - \frac{\text{cpol kp } x\text{T}(t)}{\text{Km MMf}(t)} - \frac{k1 \ x\text{T}(t)}{\text{K}1\text{l}} + \frac{k1 \ (x\text{Tx}(t) + x\text{Txa}(t))}{\text{K}1\text{o}} + k1 \ T(t) \ x(t) - k1 \ x\text{T}(t) (x(t) + \text{xT}(t)), \\
 \text{Tx}'(t) &= \frac{k1 \ (\text{xATx}(t) + \text{xTx}(t))}{\text{K}1\text{l}} - \frac{k1 \ \text{Tx}(t)}{\text{K}1\text{o}} + k1 \ T(t) \ x(t) - k1 \ \text{Tx}(t) (x(t) + \text{xT}(t)), \\
 \text{Tx2}'(t) &= \text{cpol} \left( \frac{\text{kp } x\text{T}(t)}{\text{Km MMf}(t)} + \frac{\text{kpsd } x\text{Tx}(t)}{\text{Kmsd MMf}(t)} \right) - \frac{\text{Cn kn Tx2}(t)}{\text{Kmn MMm}(t)}, \\
 x\text{Tx}'(t) &= \frac{\text{Cn kn Tx2}(t)}{\text{Kmn MMm}(t)} - \frac{\text{cpol kpsd } x\text{Tx}(t)}{\text{Kmsd MMf}(t)} - k1 \left( \frac{1}{\text{K}1\text{l}} + \frac{1}{\text{K}1\text{o}} \right) \text{xTx}(t) + k1 \ x(t) (\text{Tx}(t) + \text{xT}(t)), \\
 \text{xAT}'(t) &= - \frac{k1 \ \text{xAT}(t)}{\text{K}1\text{l}} + \frac{k1 \ (\text{xATx}(t) + \text{xTx}(t))}{\text{K}1\text{o}} + k1 \ T(t) \ \text{xT}(t) - k1 \ \text{xAT}(t) (x(t) + \text{xT}(t)), \\
 \text{Txa}'(t) &= \frac{k1 \ (\text{xATxa}(t) + \text{xTx}(t))}{\text{K}1\text{l}} - \frac{k1 \ \text{Tx}(t)}{\text{K}1\text{o}} + k1 \ T(t) \ \text{xT}(t) - k1 \ \text{Txa}(t) (x(t) + \text{xT}(t)), \\
 \text{xTx}'(t) &= - k1 \left( \frac{1}{\text{K}1\text{l}} + \frac{1}{\text{K}1\text{o}} \right) \text{xTx}(t) + k1 \ \text{Txa}(t) \ x(t) + k1 \ \text{xT}(t) \ \text{xT}(t), \\
 \text{xATx}'(t) &= - k1 \left( \frac{1}{\text{K}1\text{l}} + \frac{1}{\text{K}1\text{o}} \right) \text{xATx}(t) + k1 \ \text{Tx}(t) \ \text{xT}(t) + k1 \ x(t) \ \text{xAT}(t), \\
 \text{xATx}'(t) &= k1 \ \text{xT}(t) (\text{Tx}(t) + \text{xT}(t)) - k1 \left( \frac{1}{\text{K}1\text{l}} + \frac{1}{\text{K}1\text{o}} \right) \text{xATx}(t), \\
 \text{Dr}'(t) &= - k1 \ \text{Dr}(t) (x(t) + \text{xT}(t)) + \frac{k1 \ \text{Drxa}(t)}{\text{K}2} + \frac{k1 \ x\text{Dr}(t)}{\text{K}1\text{o}}, \\
 \text{xDr}'(t) &= - \frac{\text{cpol kp } x\text{Dr}(t)}{\text{Km MMf}(t)} + k1 \ \text{Dr}(t) \ x(t) - \frac{k1 \ x\text{Dr}(t)}{\text{K}1\text{o}}, \\
 \text{Drxa}'(t) &= \frac{\text{cpol kp } x\text{Dr}(t)}{\text{Km MMf}(t)} + k1 \ \text{Dr}(t) \ \text{xT}(t) - \frac{k1 \ \text{Drxa}(t)}{\text{K}2}, \\
 \text{xT}'(t) &= - k1 \ \text{xT}(t) (\text{Dr}(t) + 2 \ T(t) + \text{Tx}(t) + \text{Txa}(t) + \text{xAT}(t) + \text{xT}(t)) + k1 \left( \frac{\text{Drxa}(t)}{\text{K}2} + \left( \frac{1}{\text{K}1\text{l}} + \frac{1}{\text{K}1\text{o}} \right) \text{xATx}(t) + \frac{\text{xAT}(t) + \text{xTx}(t)}{\text{K}1\text{l}} + \frac{\text{Tx}(t) + \text{xTxa}(t)}{\text{K}1\text{o}} \right) - \frac{\text{crec krec } \text{xT}(t)}{\text{Kmr MMr}(t)}, \\
 \text{MMf}(t) &= \frac{x\text{T}(t)}{\text{Km}} + \frac{x\text{Tx}(t)}{\text{Kmsd}} + 1, \\
 \text{MMn}(t) &= \frac{\text{Tx2}(t)}{\text{Kmn}} + 1, \\
 \text{MMr}(t) &= \frac{\text{Dr}(t)}{\text{Kmxe}} + \frac{T(t)}{\text{Kmxe}} + \frac{x(t)}{\text{Kmr}} + \frac{\text{xT}(t)}{\text{Kmr}} + 1,
 \end{aligned}$$

# Numerical integration

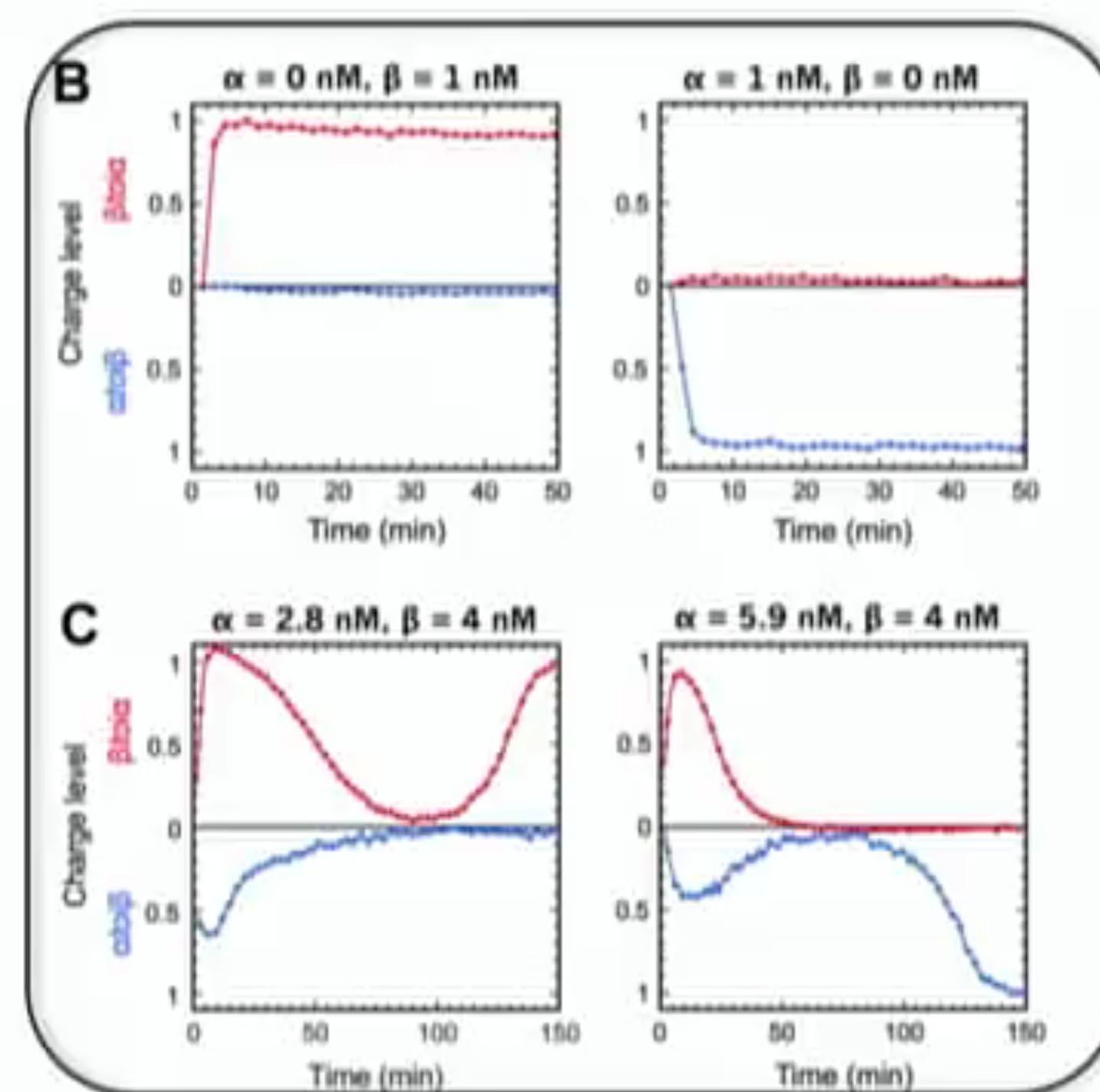
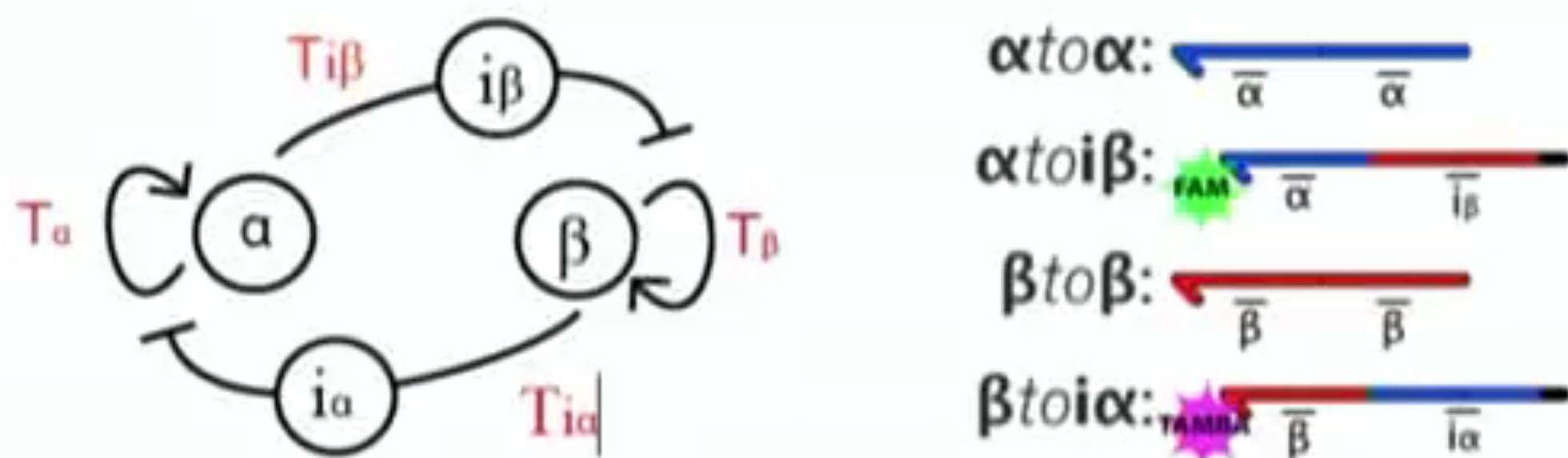


# Building a bistable system (1bit memory)



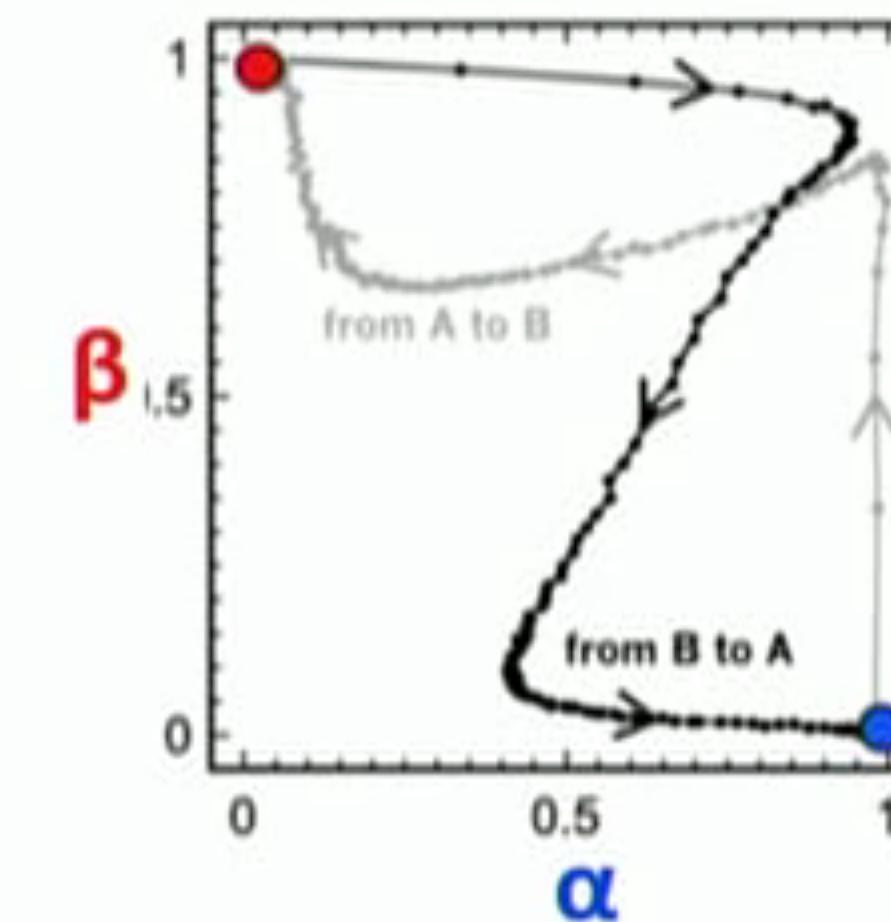
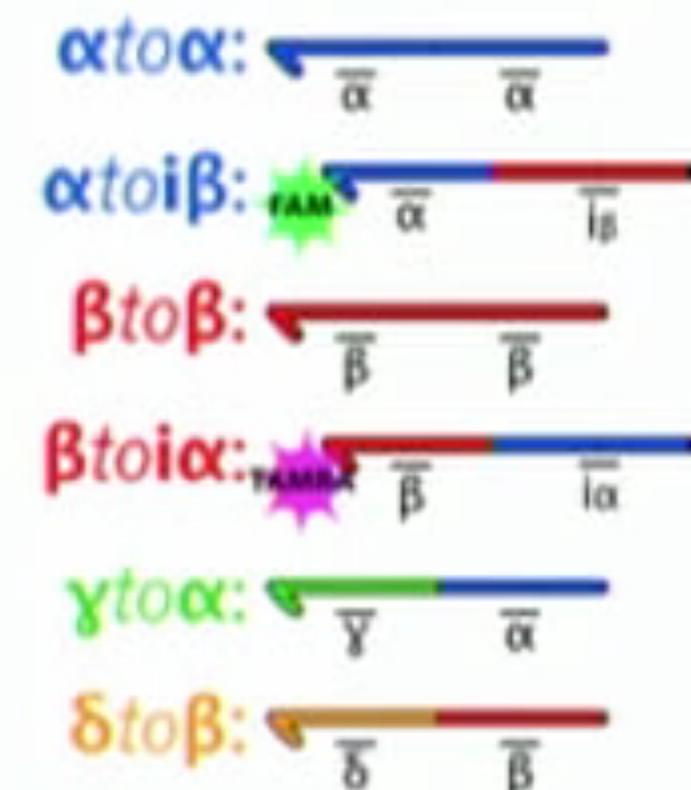
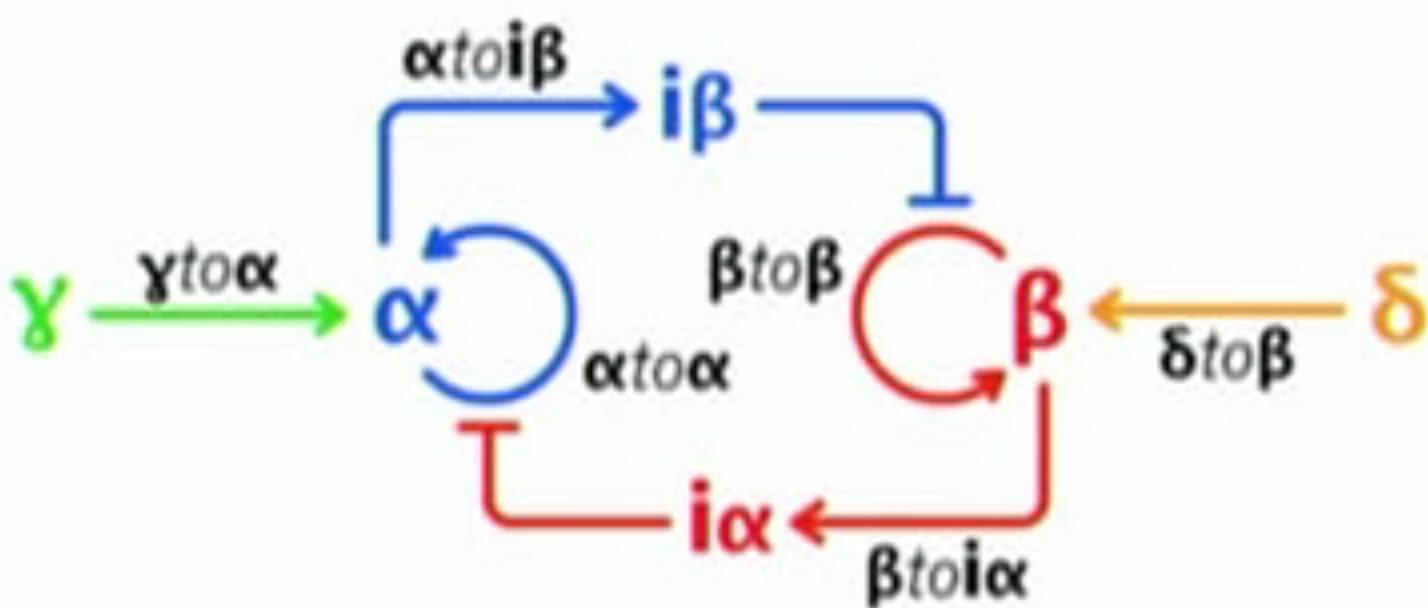
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# An *in vitro* Bistable switch

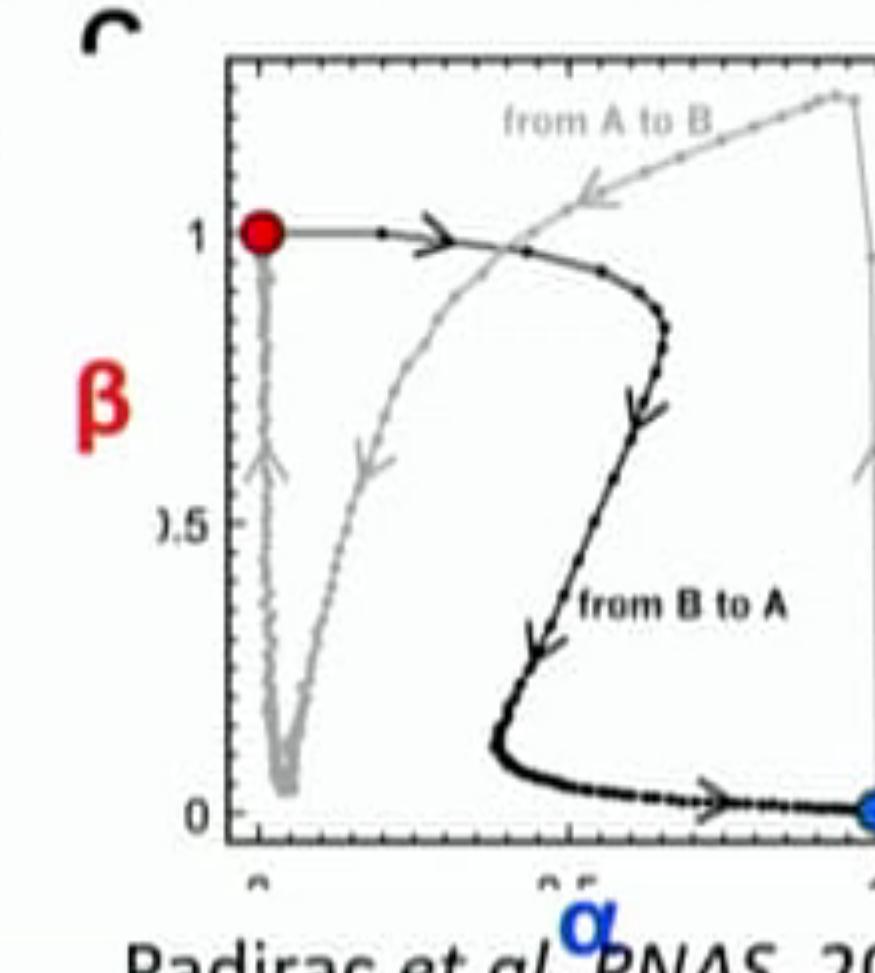
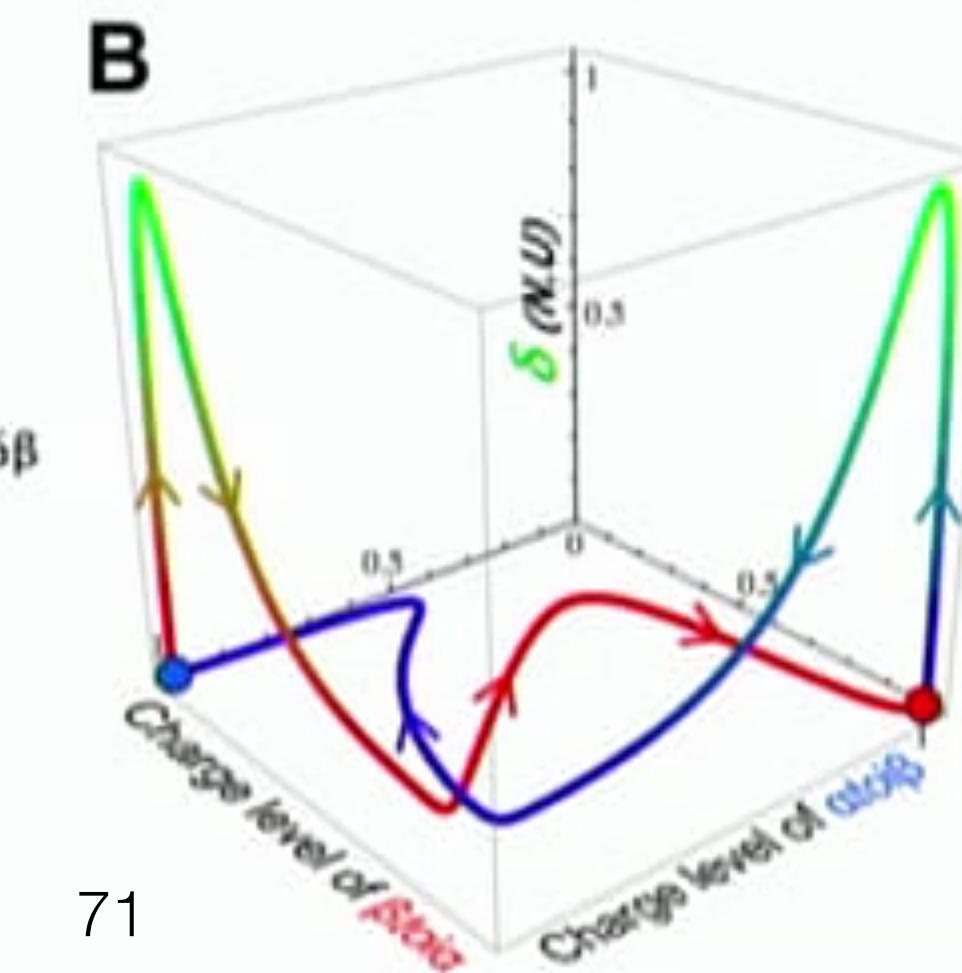
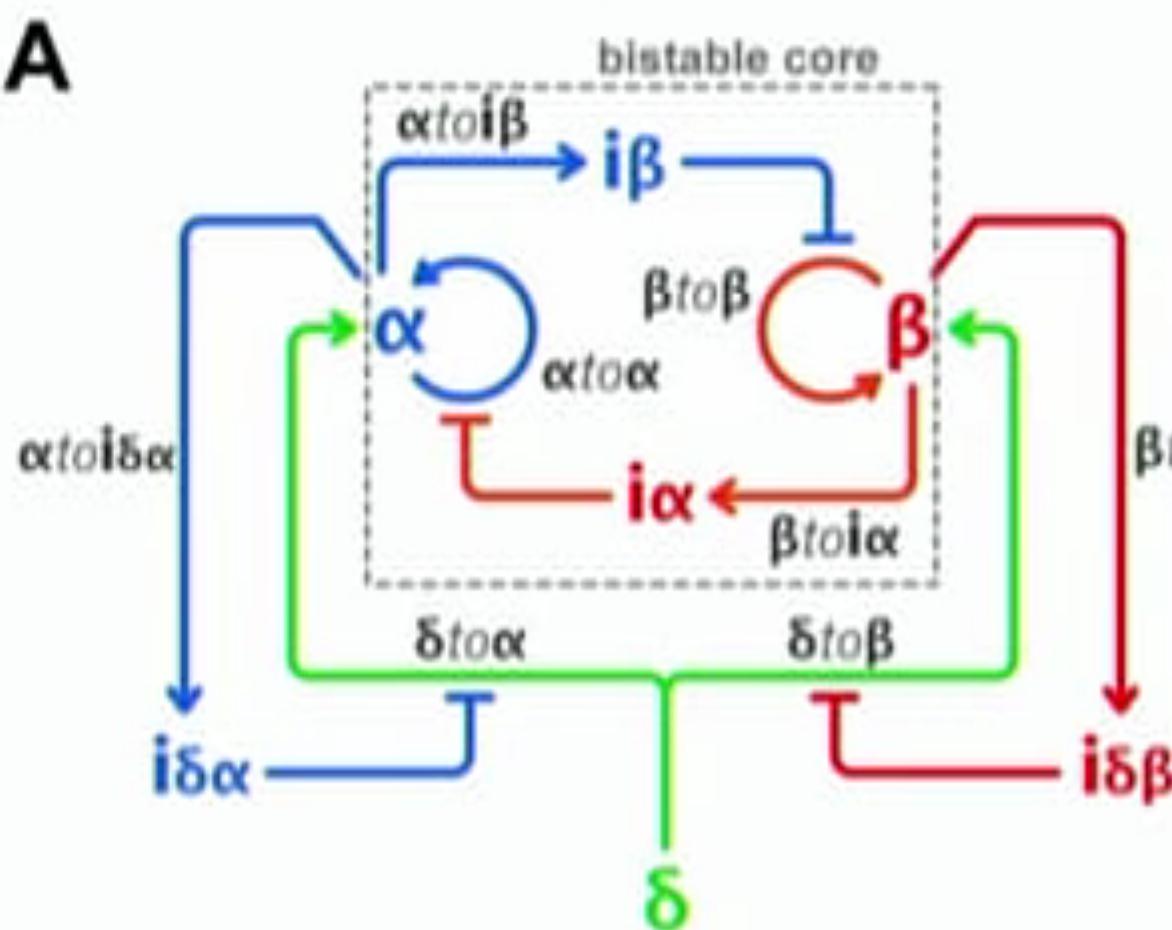


# Bistable switch => Push-push button

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)

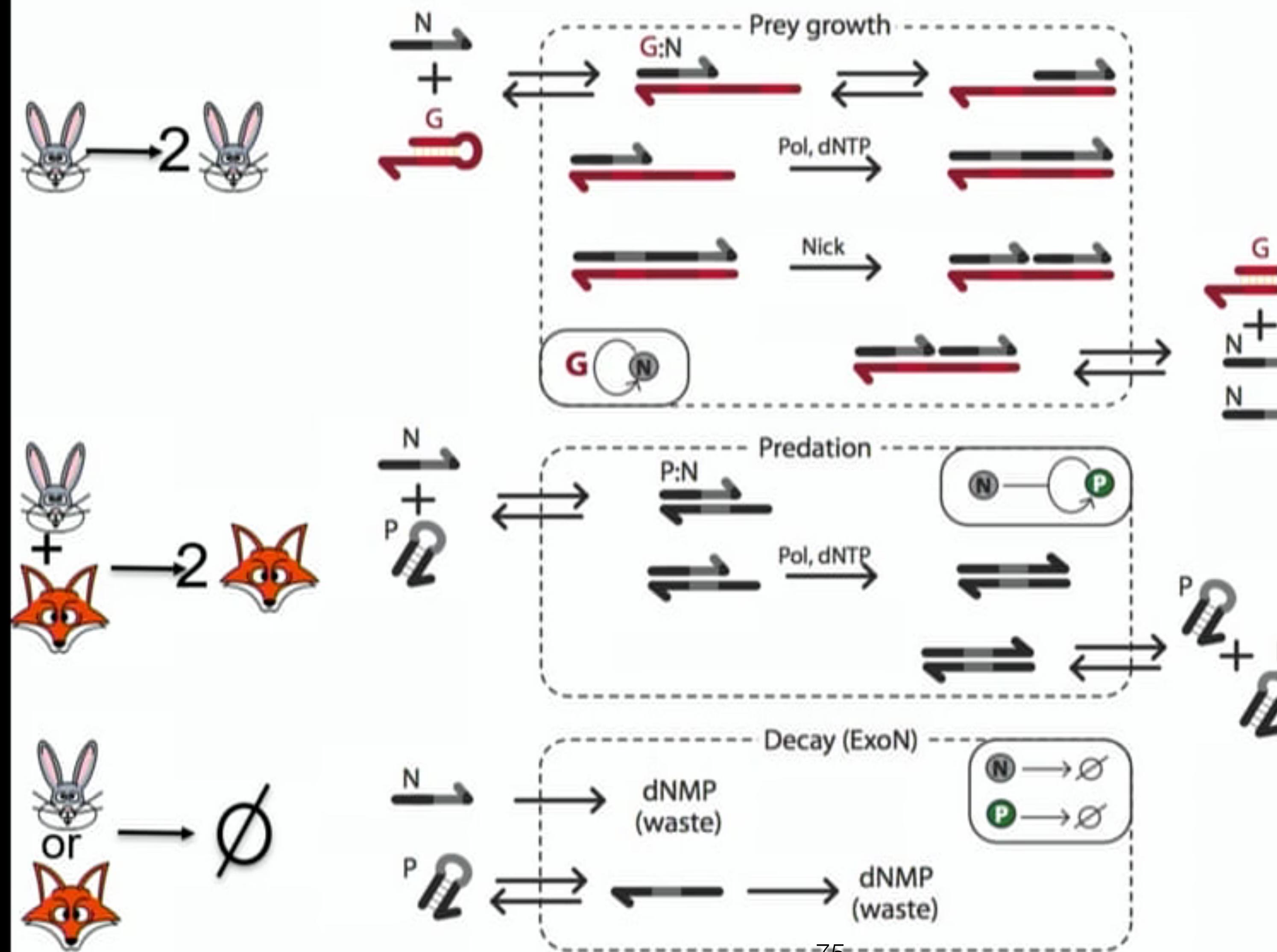


# Predator Prey network

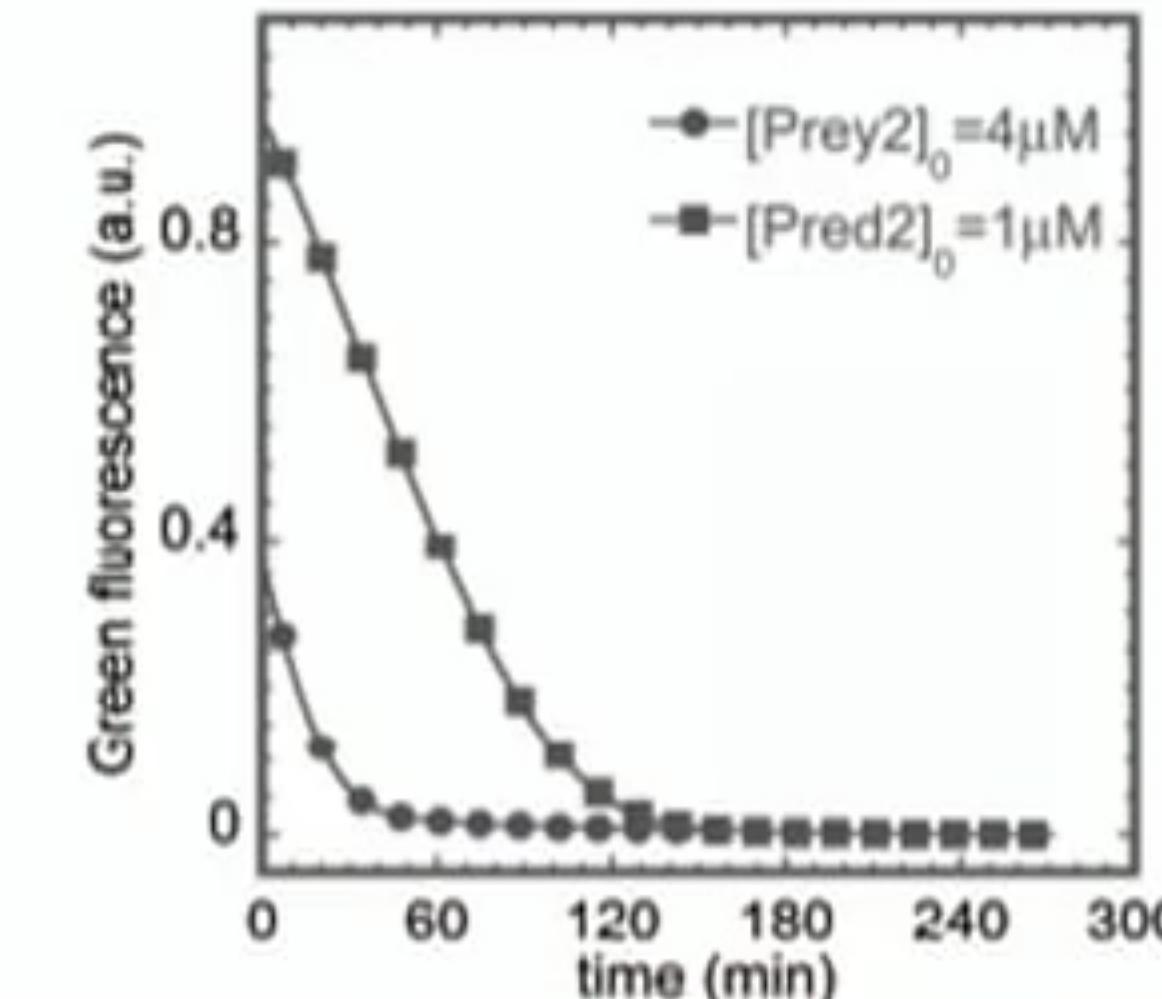
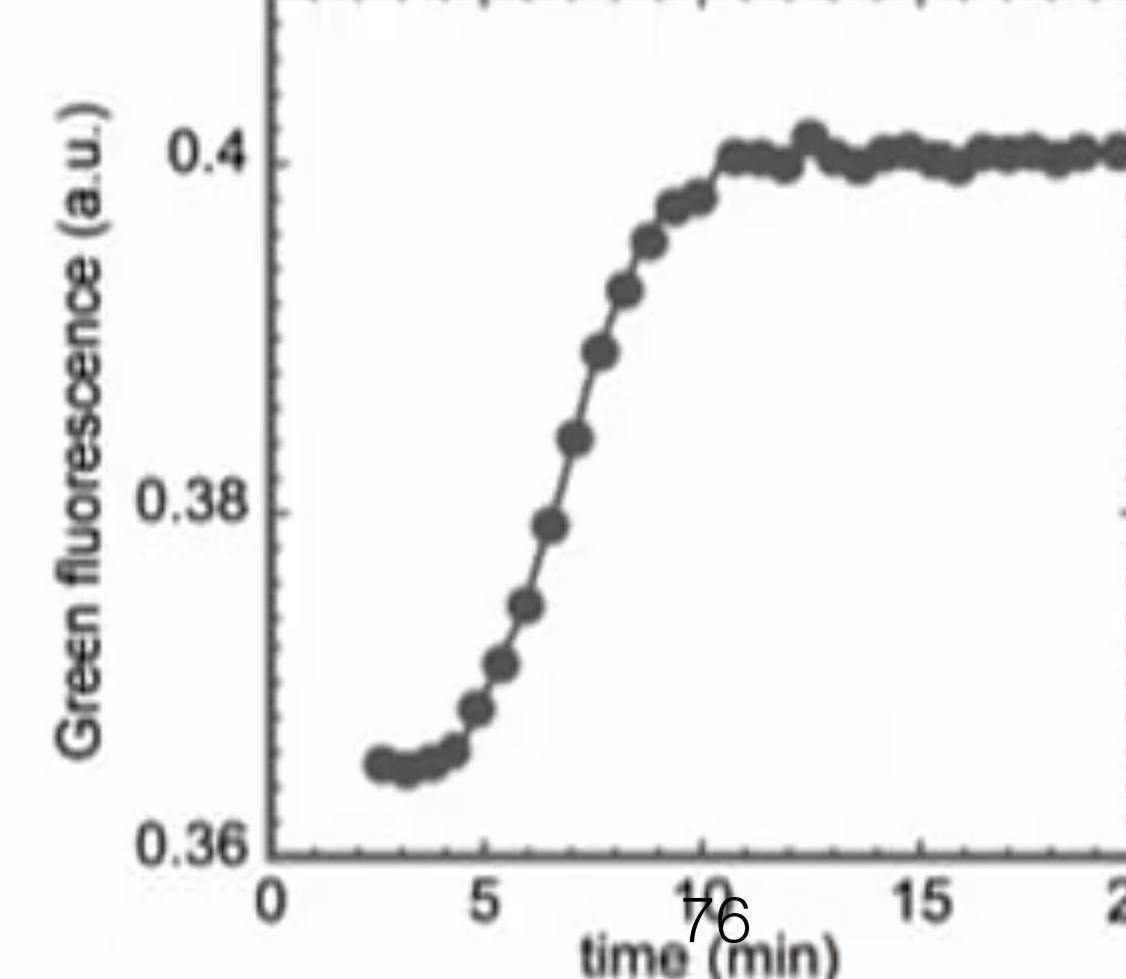
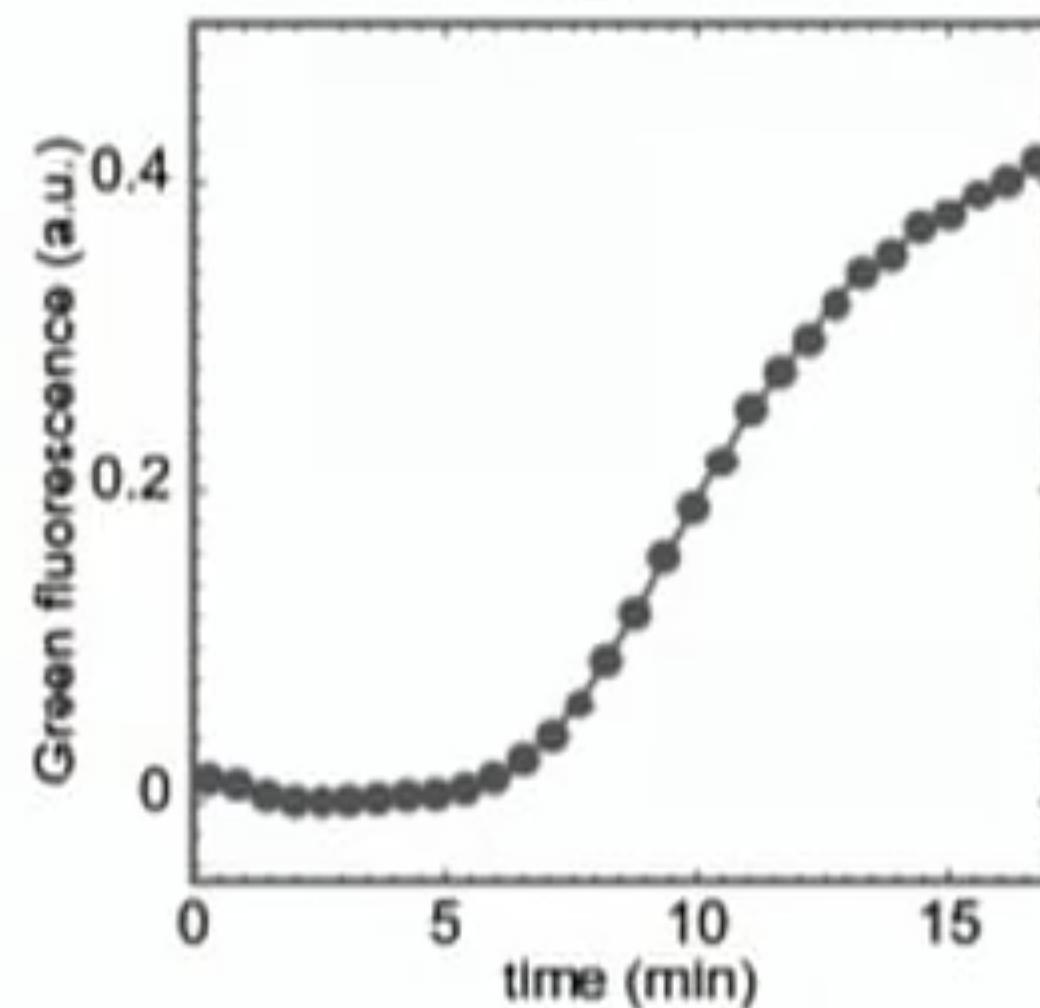
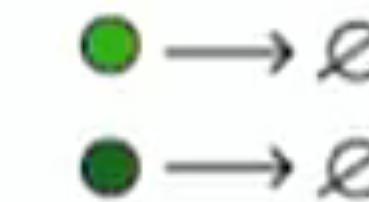
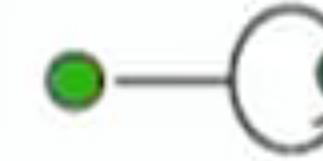
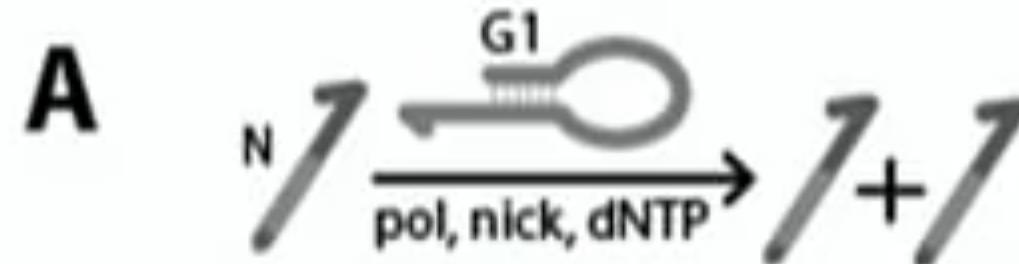
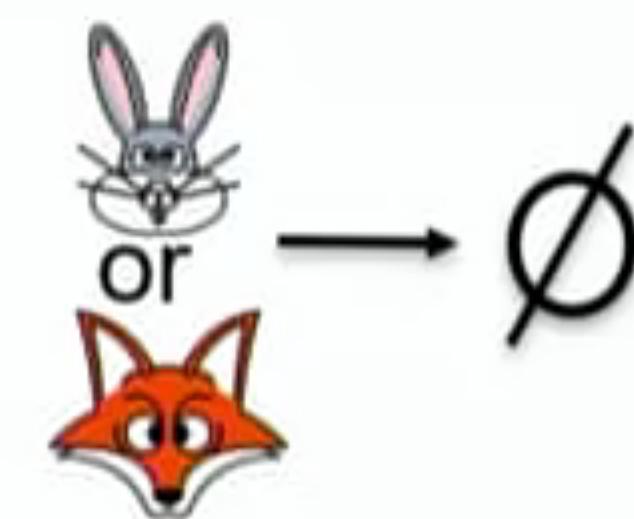
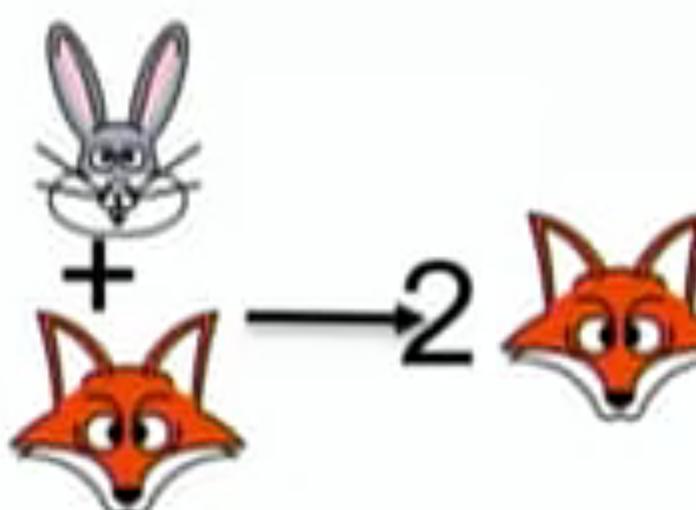
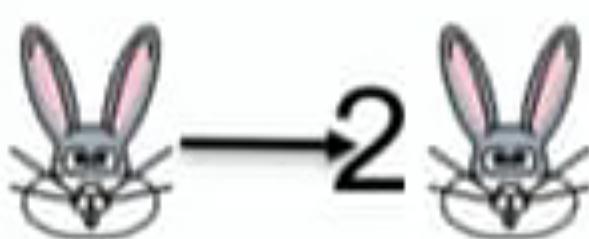


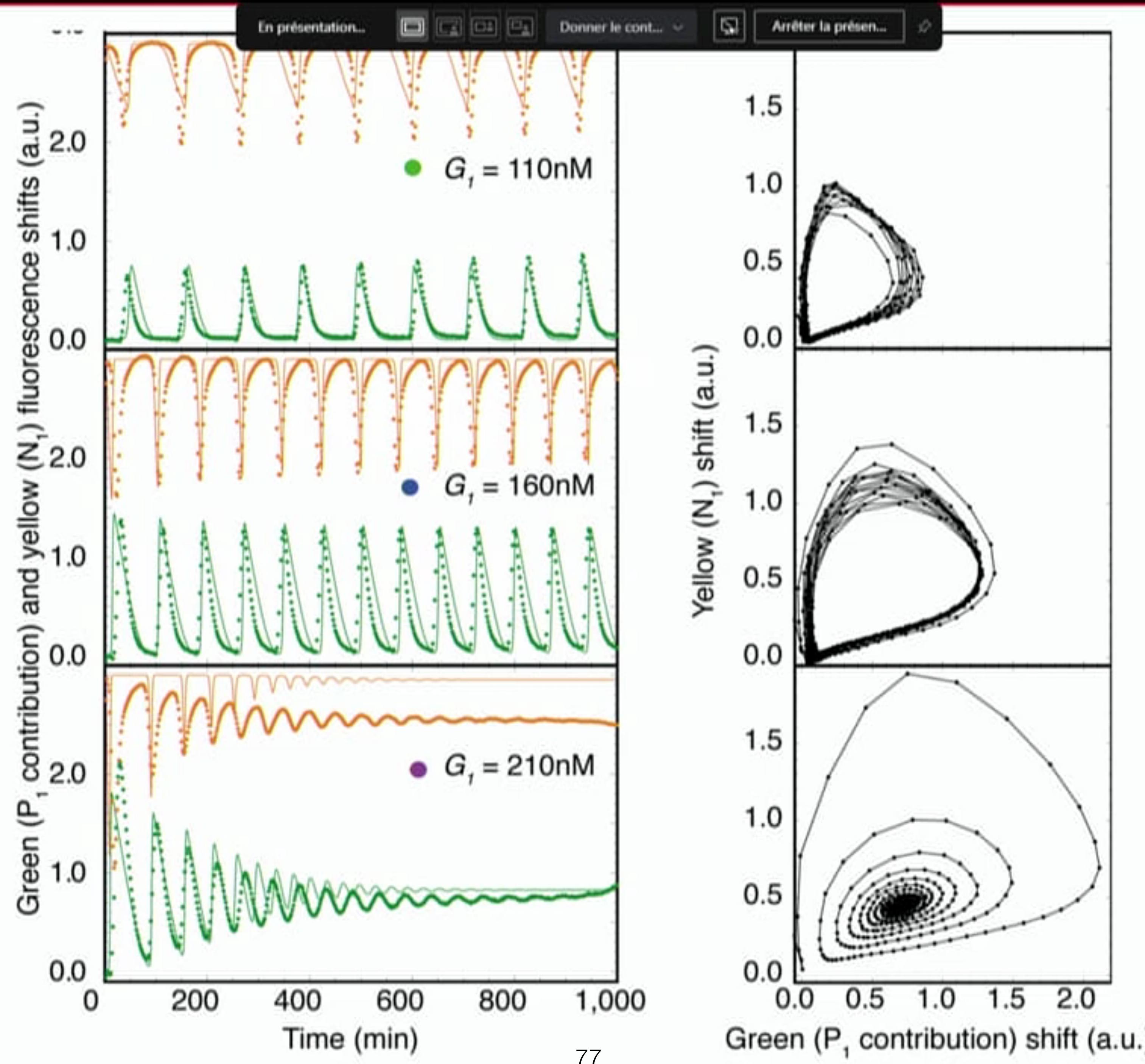
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# Predator-Prey molecular ecosystem

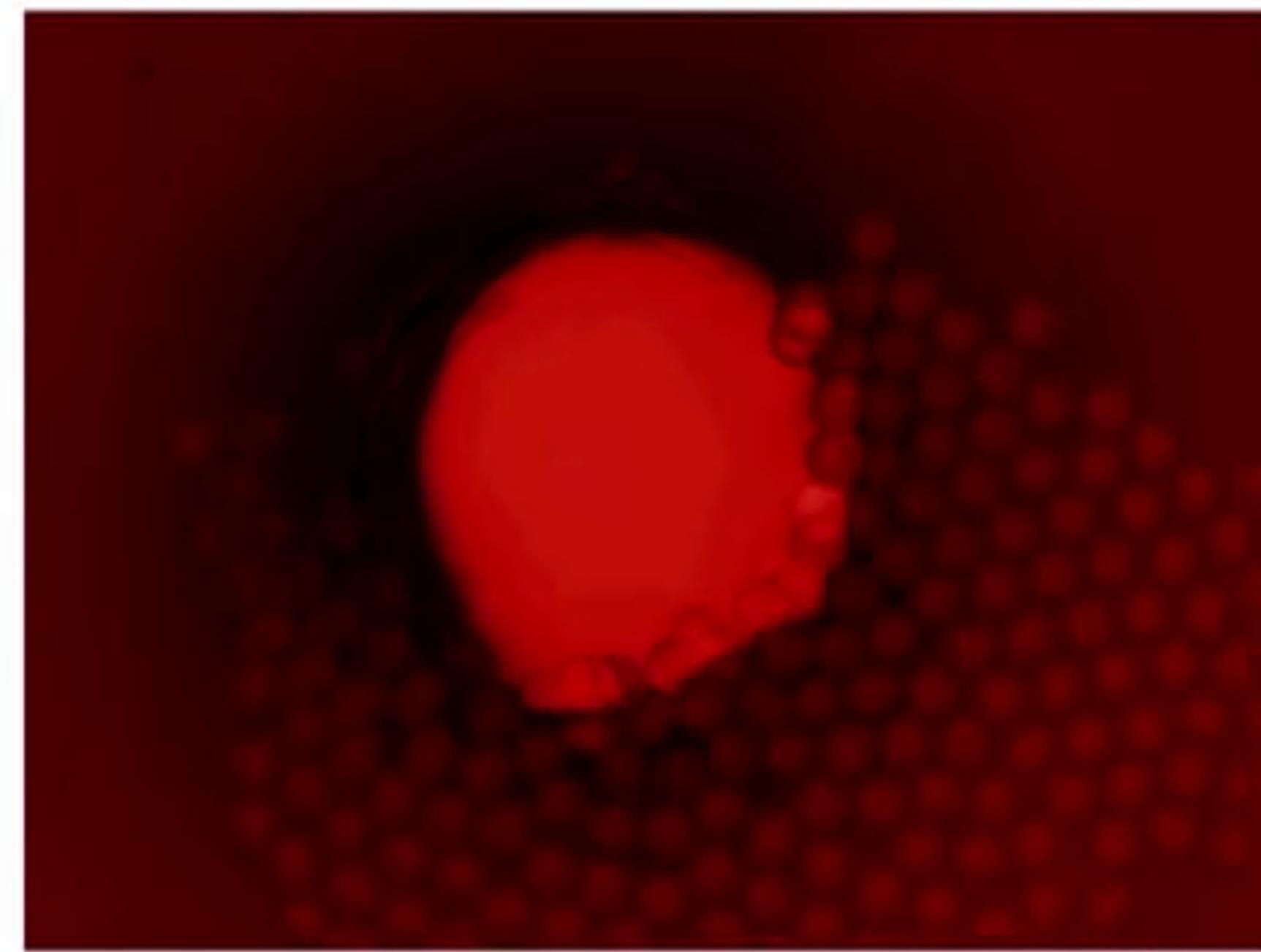
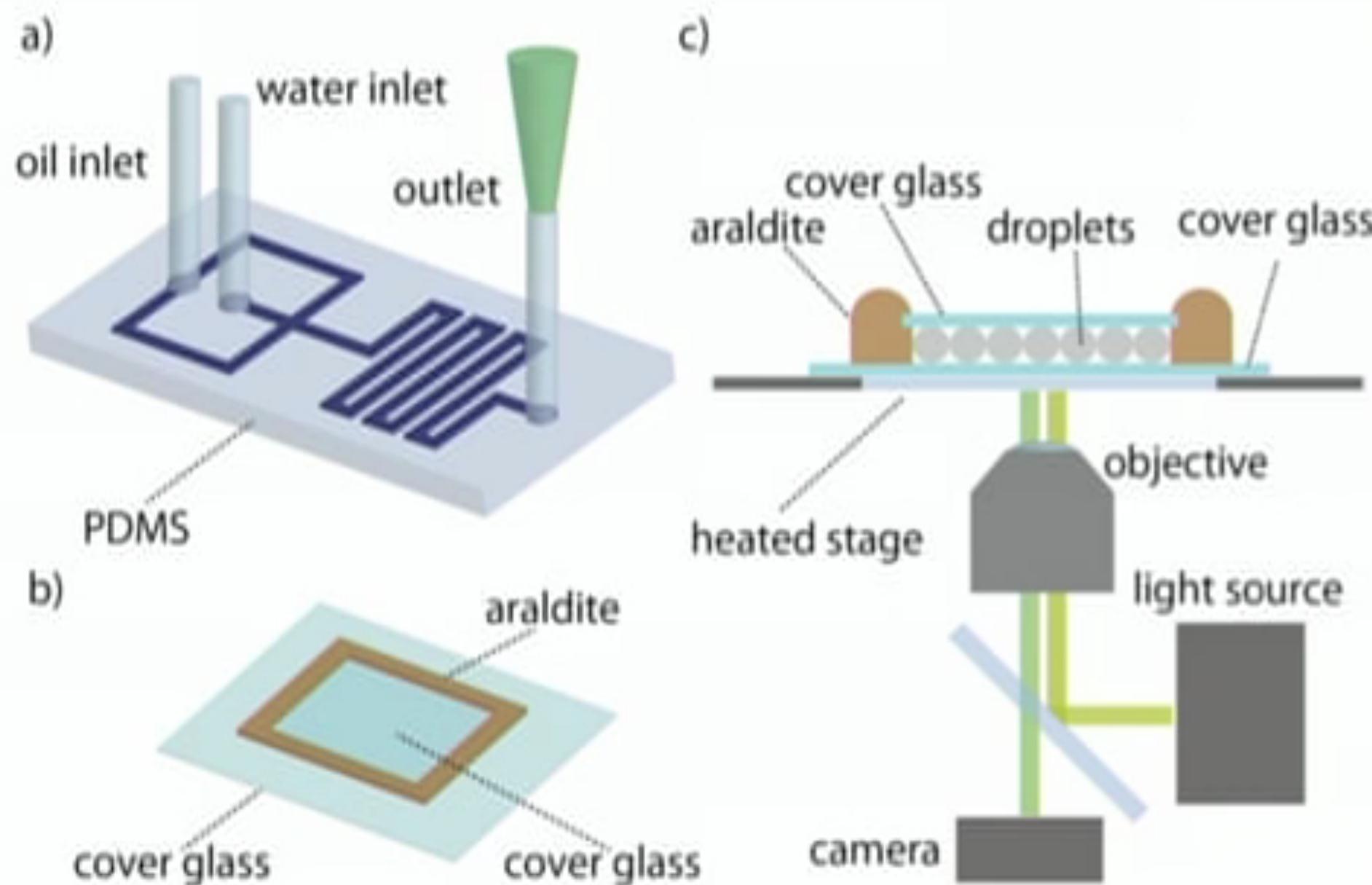


# Predator-Prey molecular ecosystem

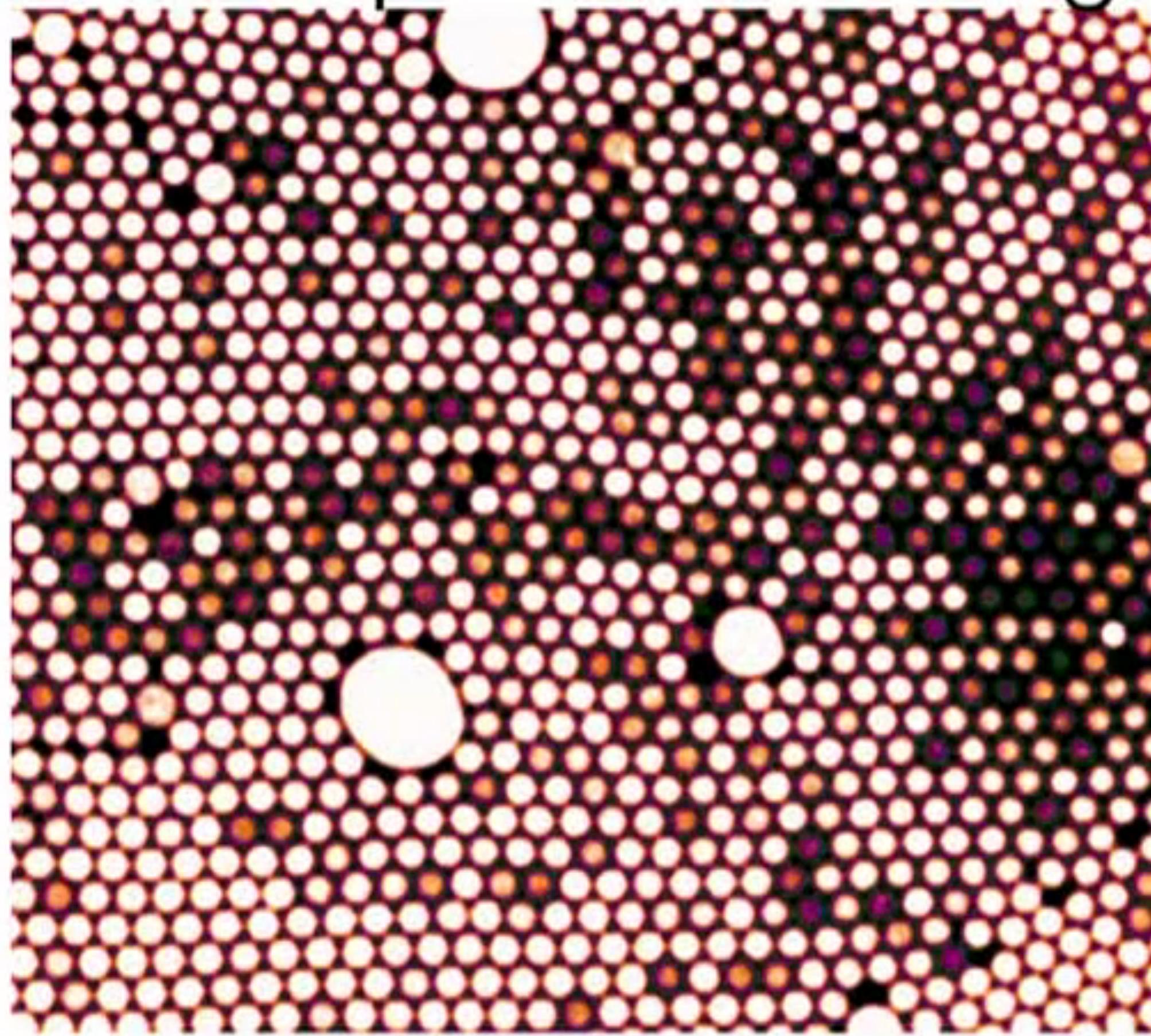




# Partitioning a molecular oscillator in a microscopic emulsion



# Partitioning a molecular oscillator in a microscopic emulsion gives...

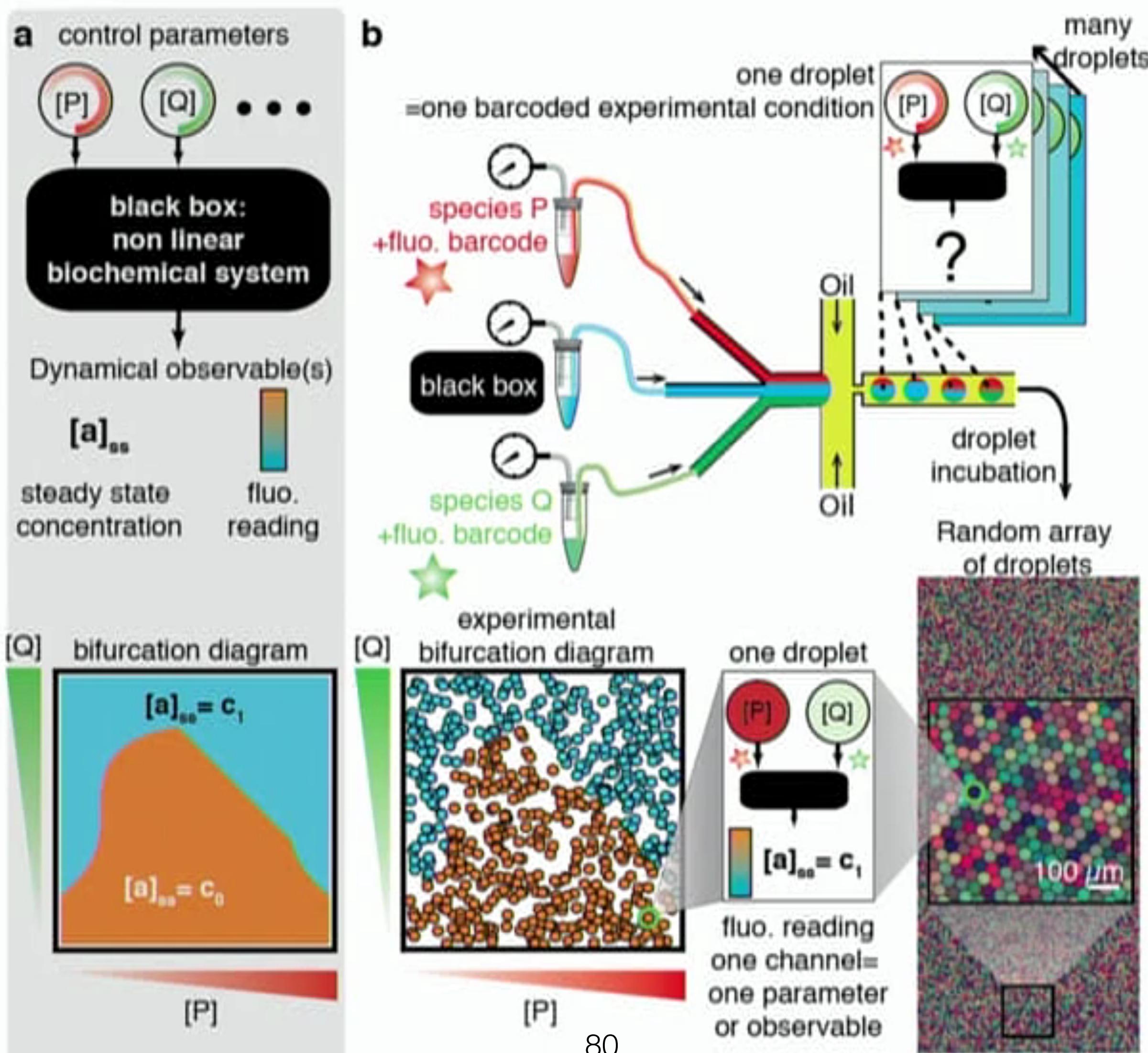


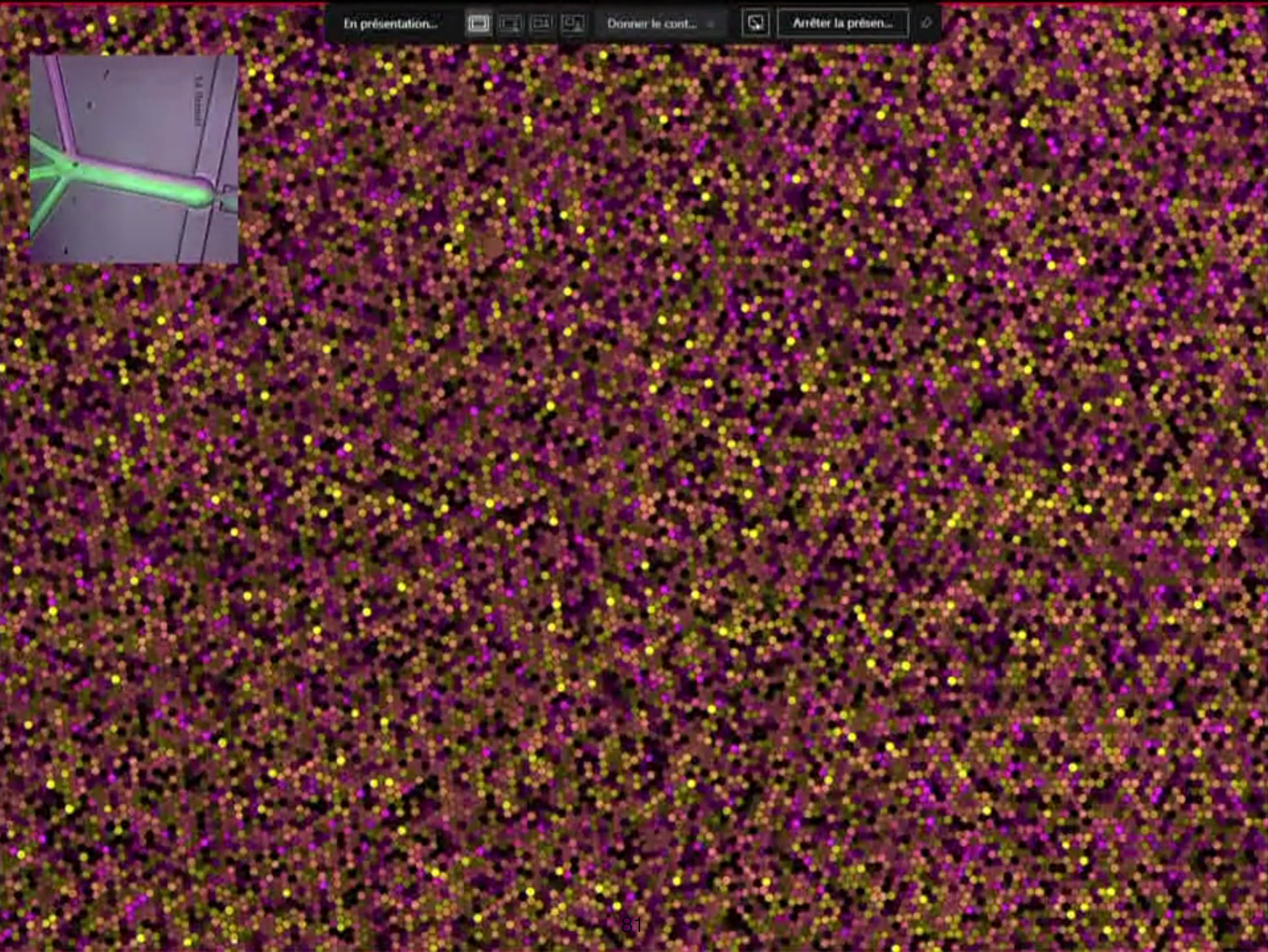
...many identical oscillators.



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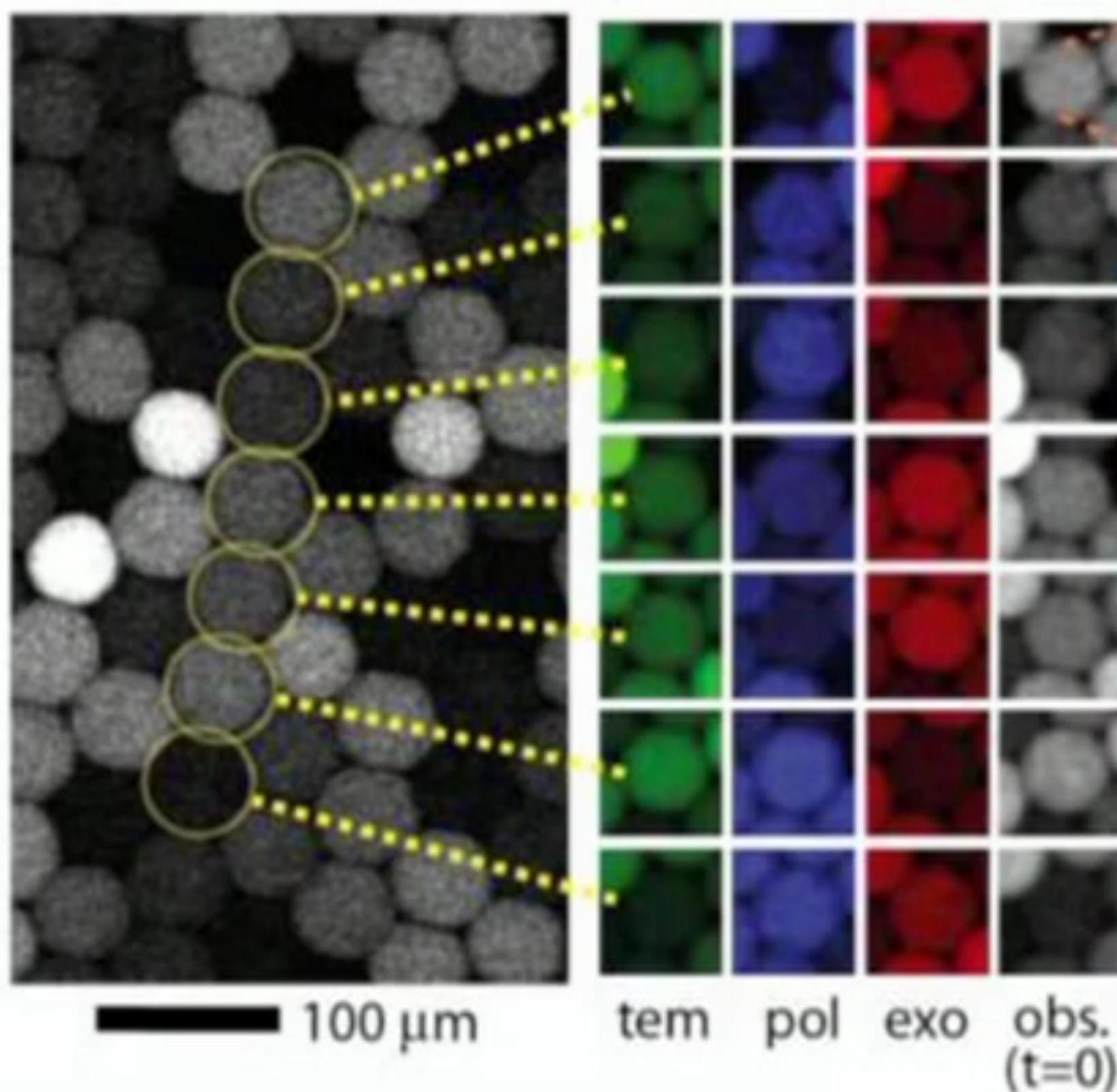
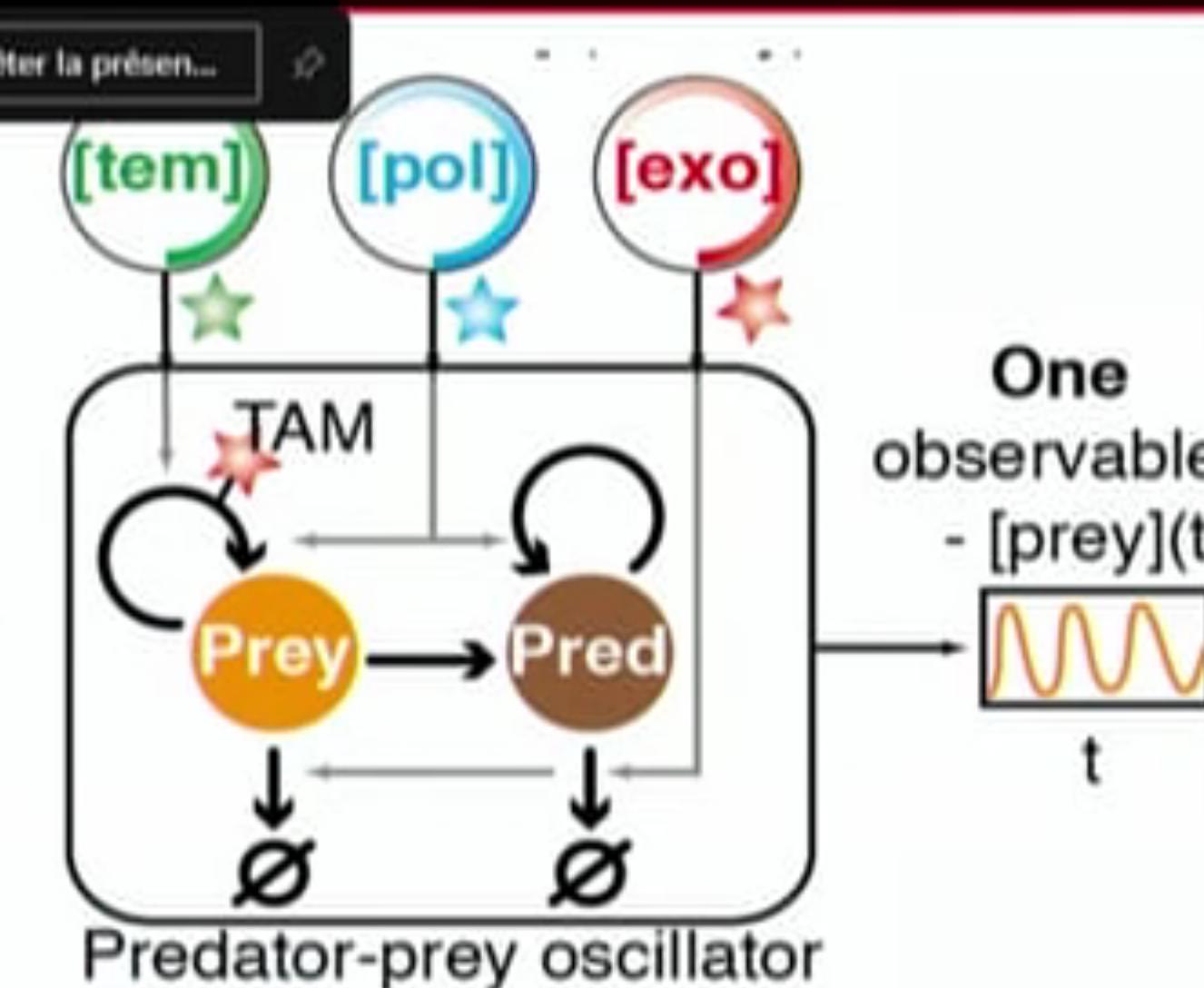
# A platform for analysis of $\sim 10^4$ different systems simultaneously

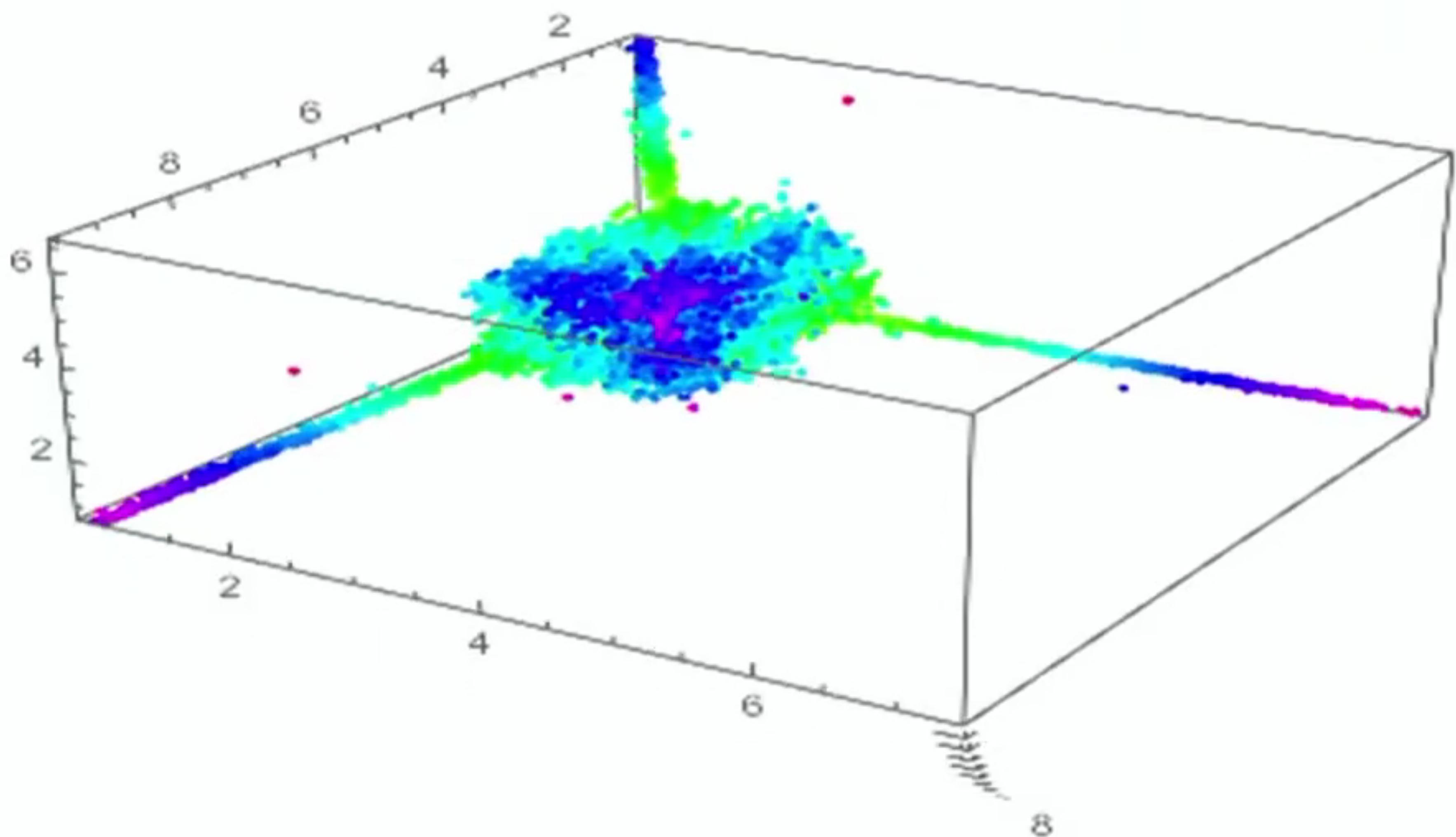




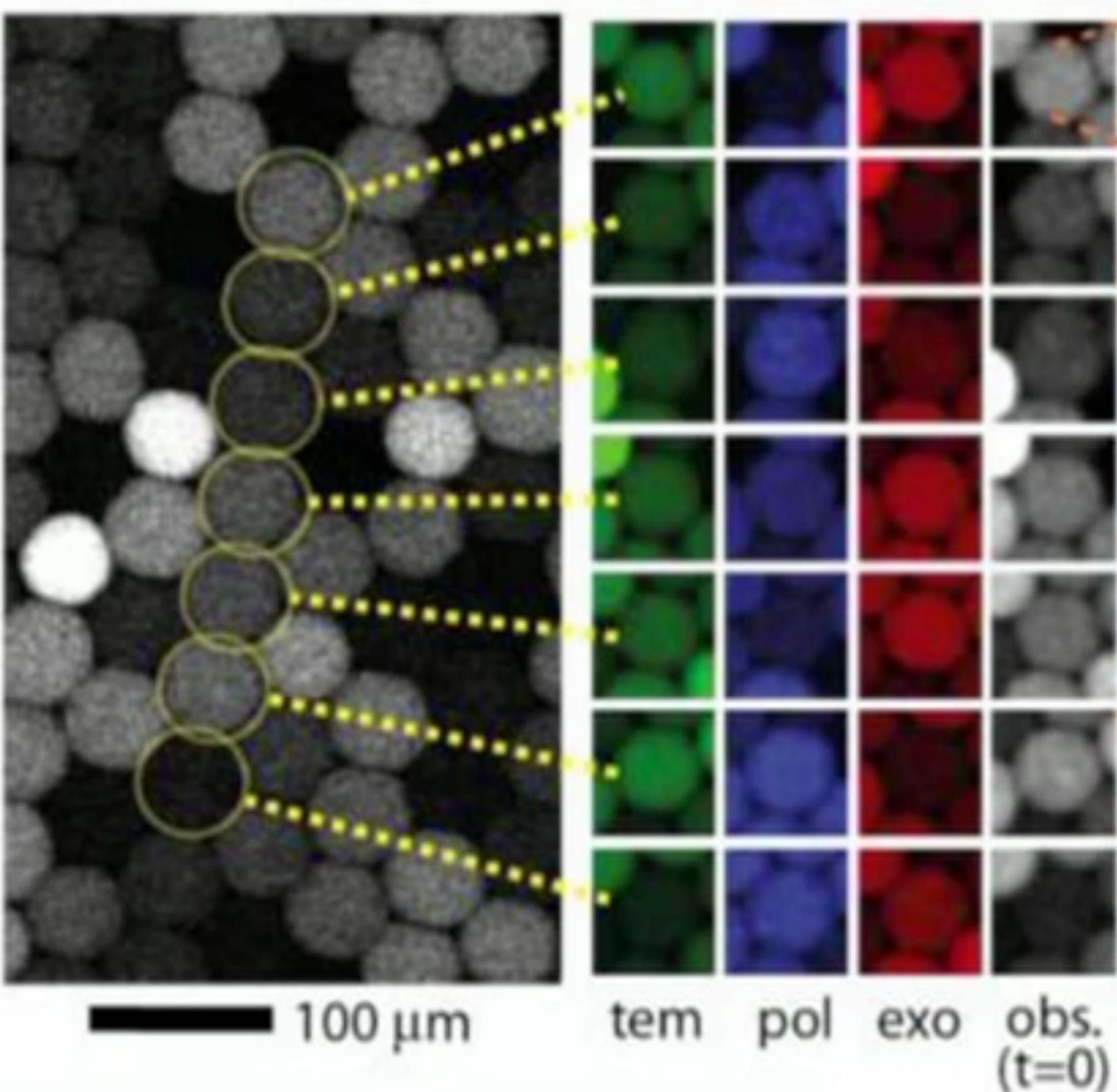
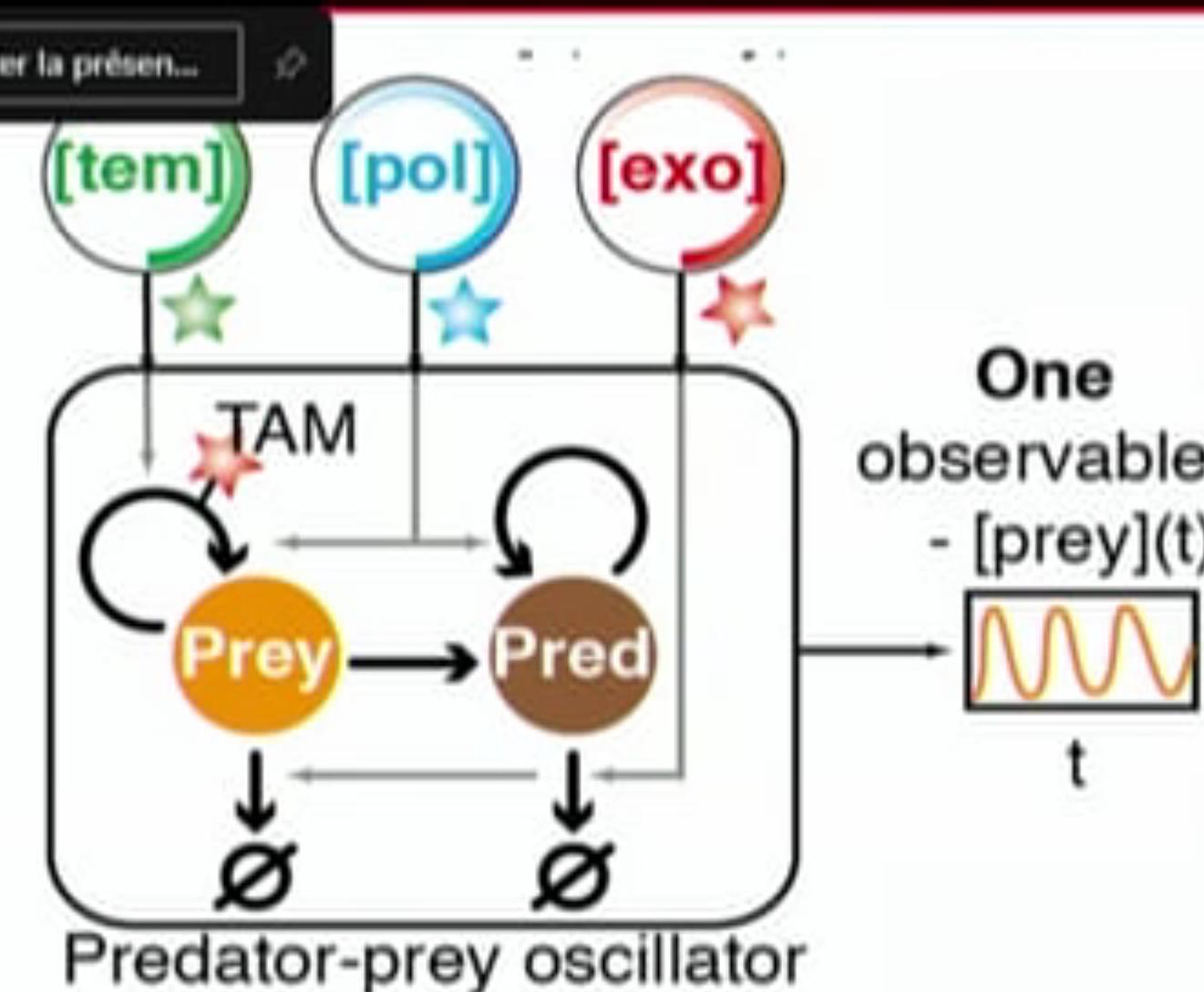
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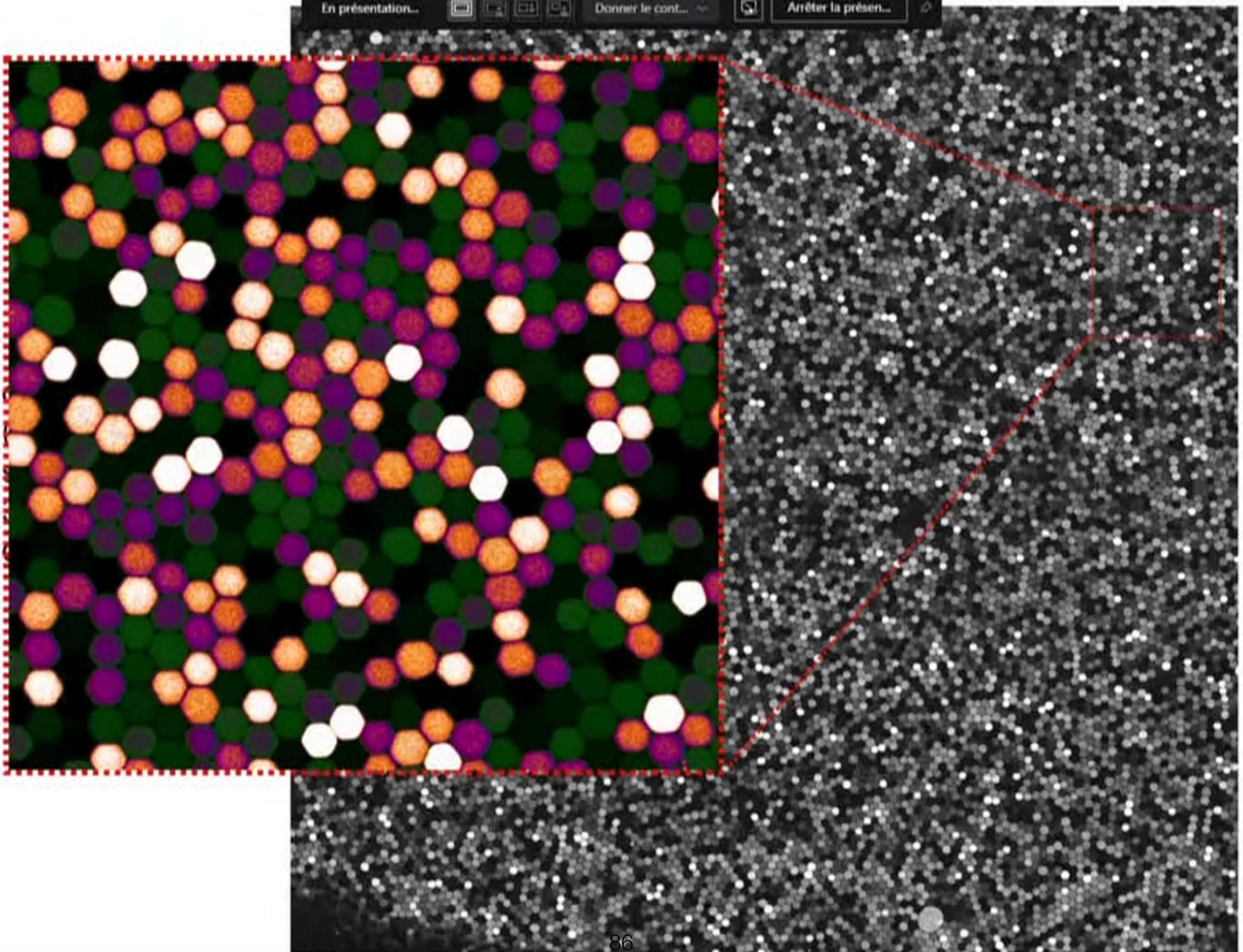
# Bifurcations of oscillator using 3D Parameter scanning



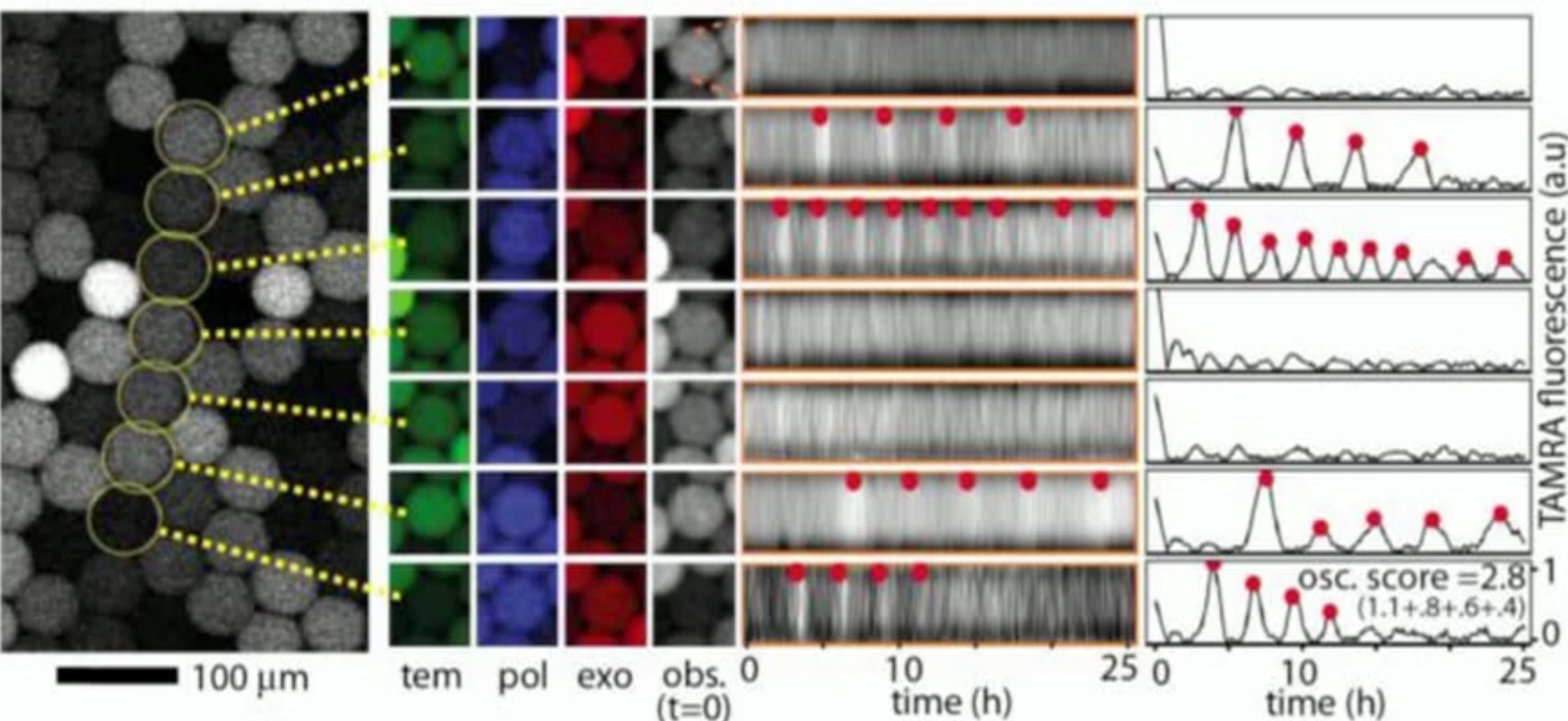
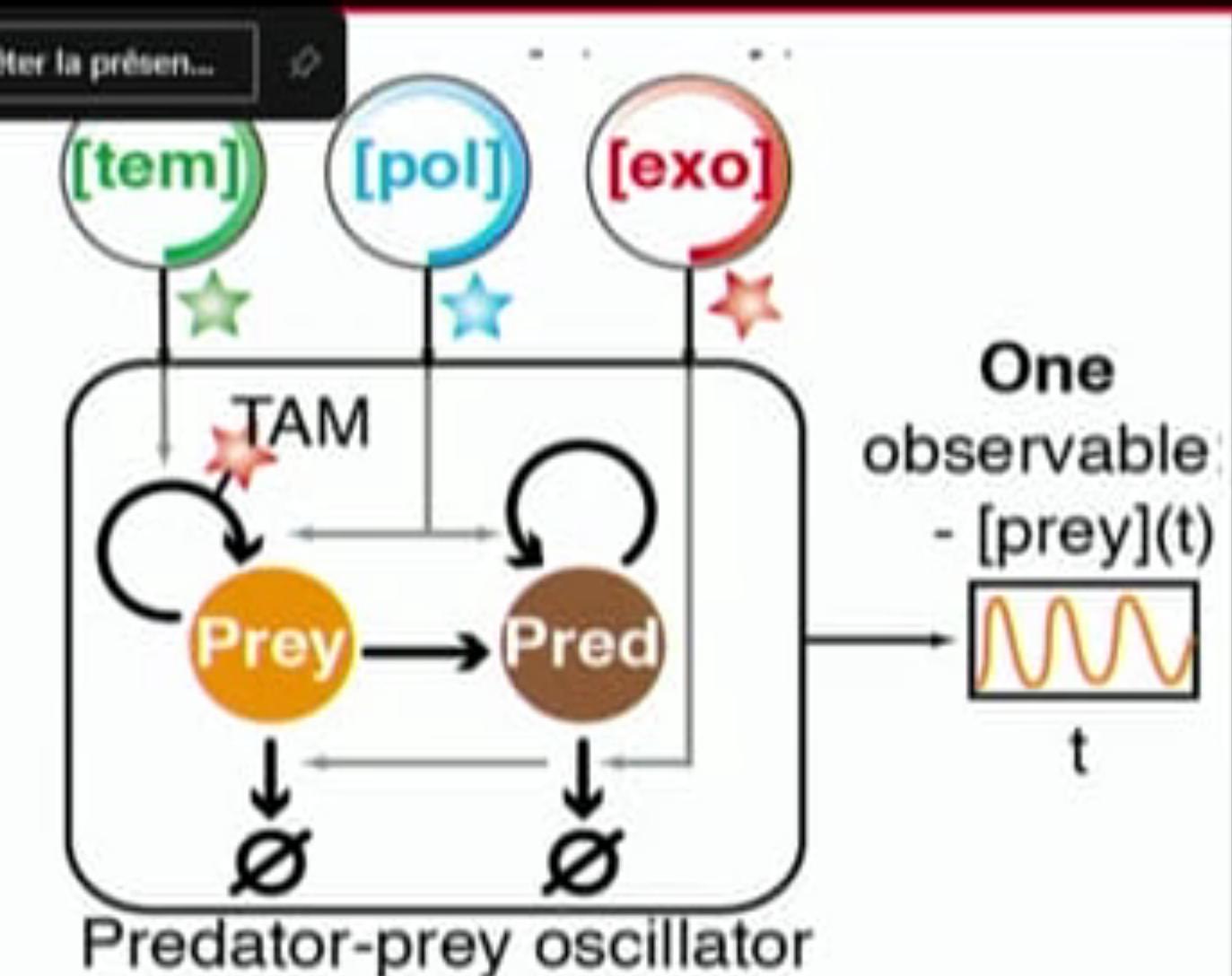


# Bifurcations of oscillator using 3D Parameter scanning

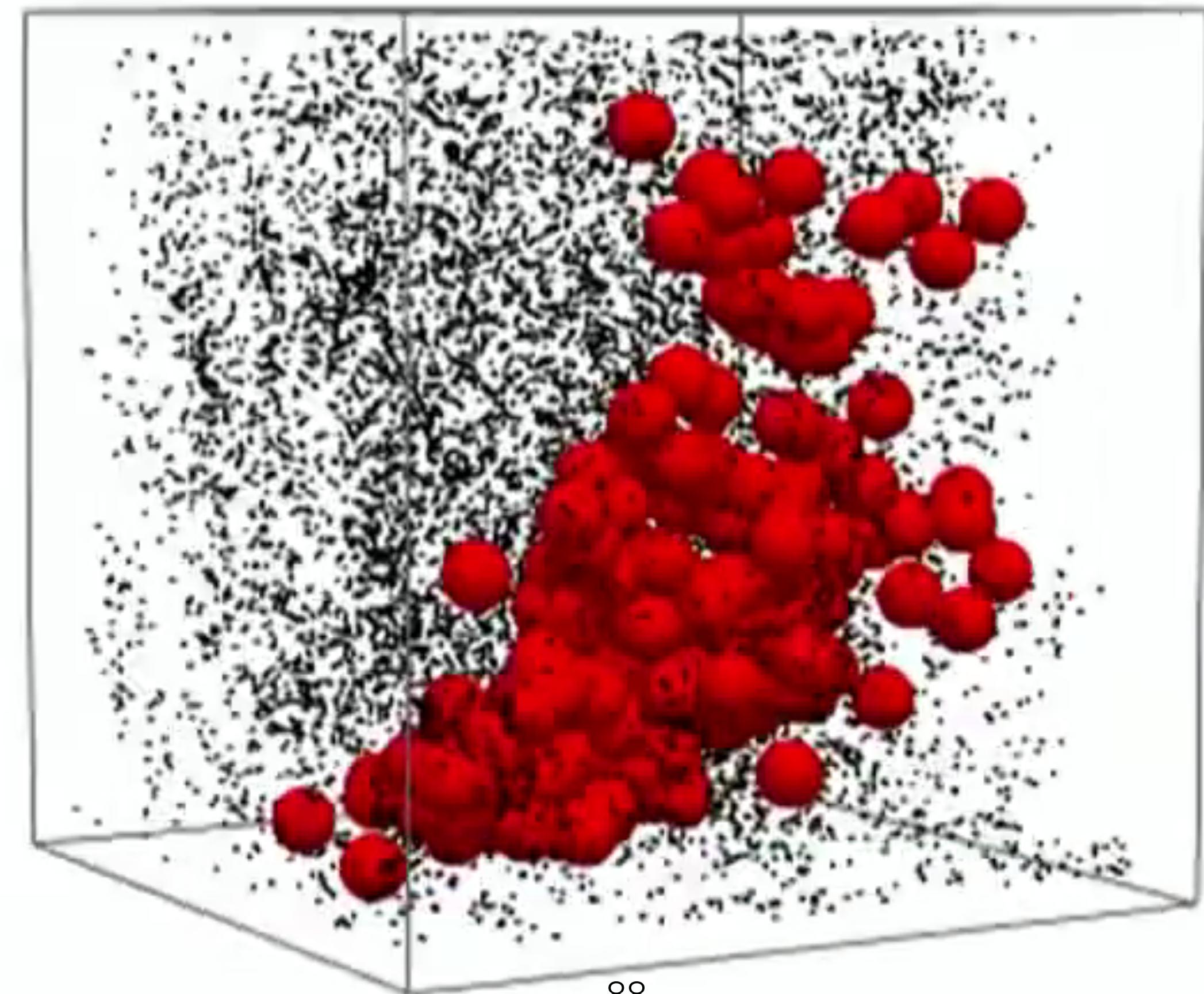




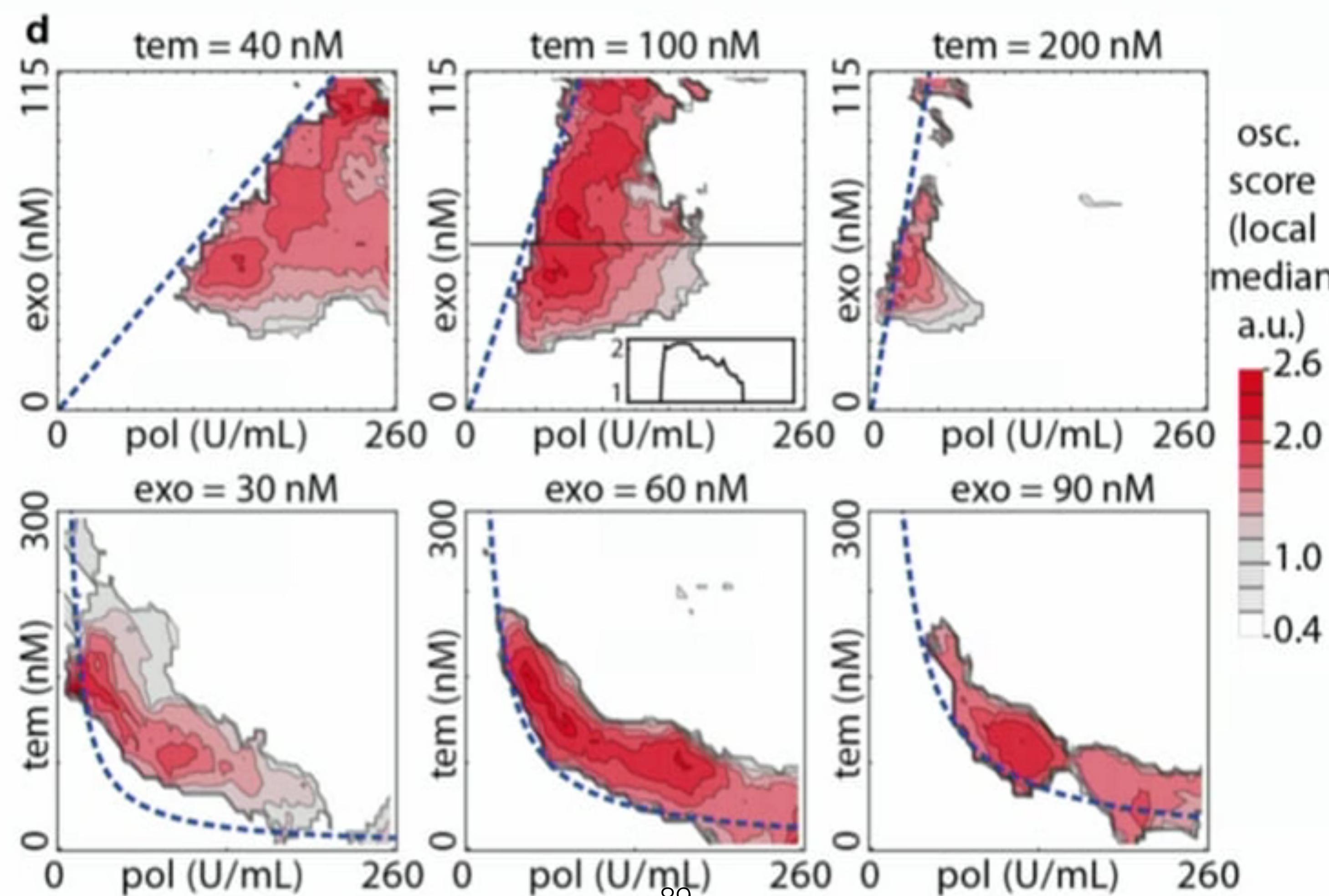
# Bifurcations of oscillator using 3D parameter scanning



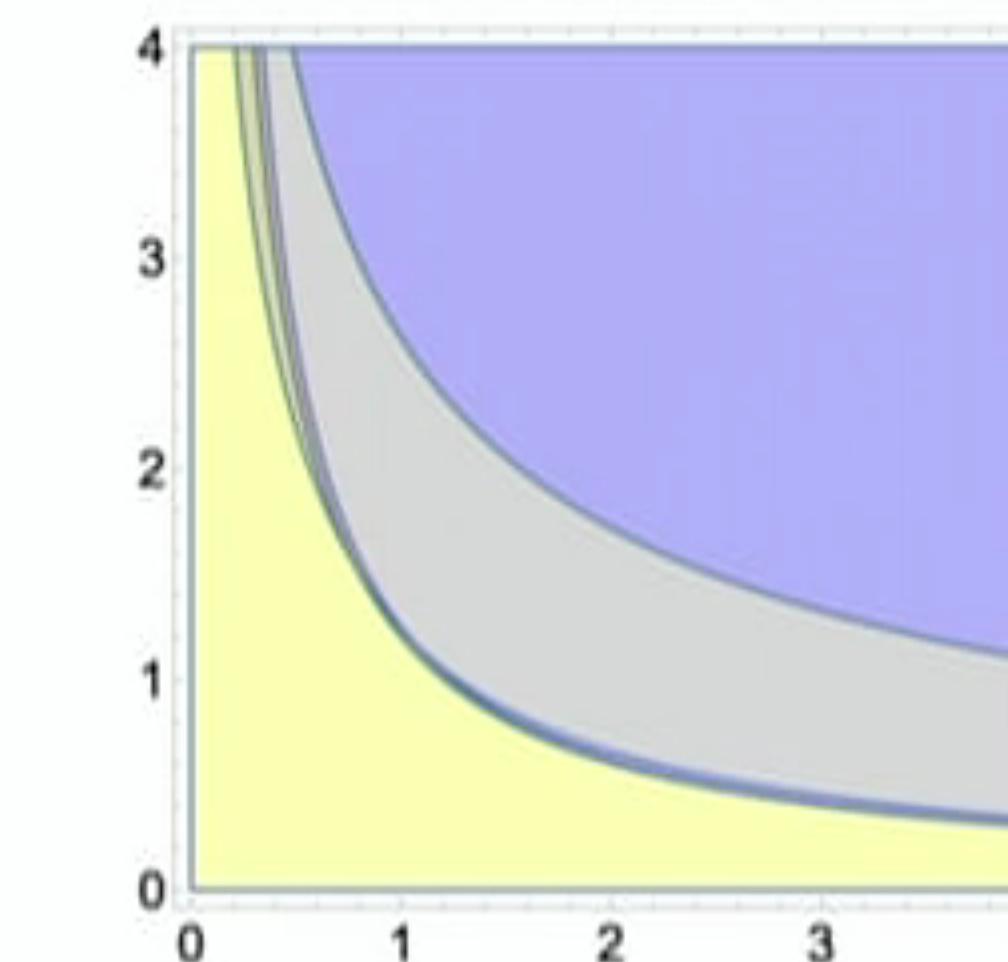
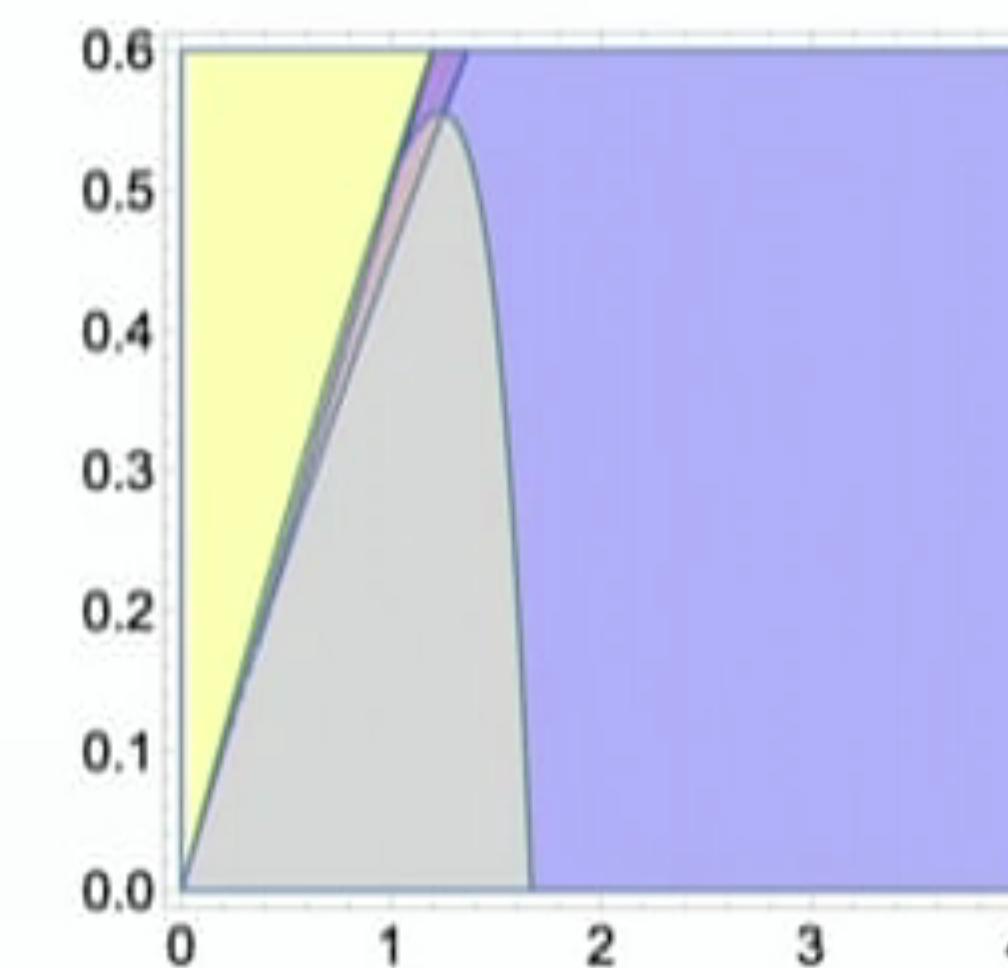
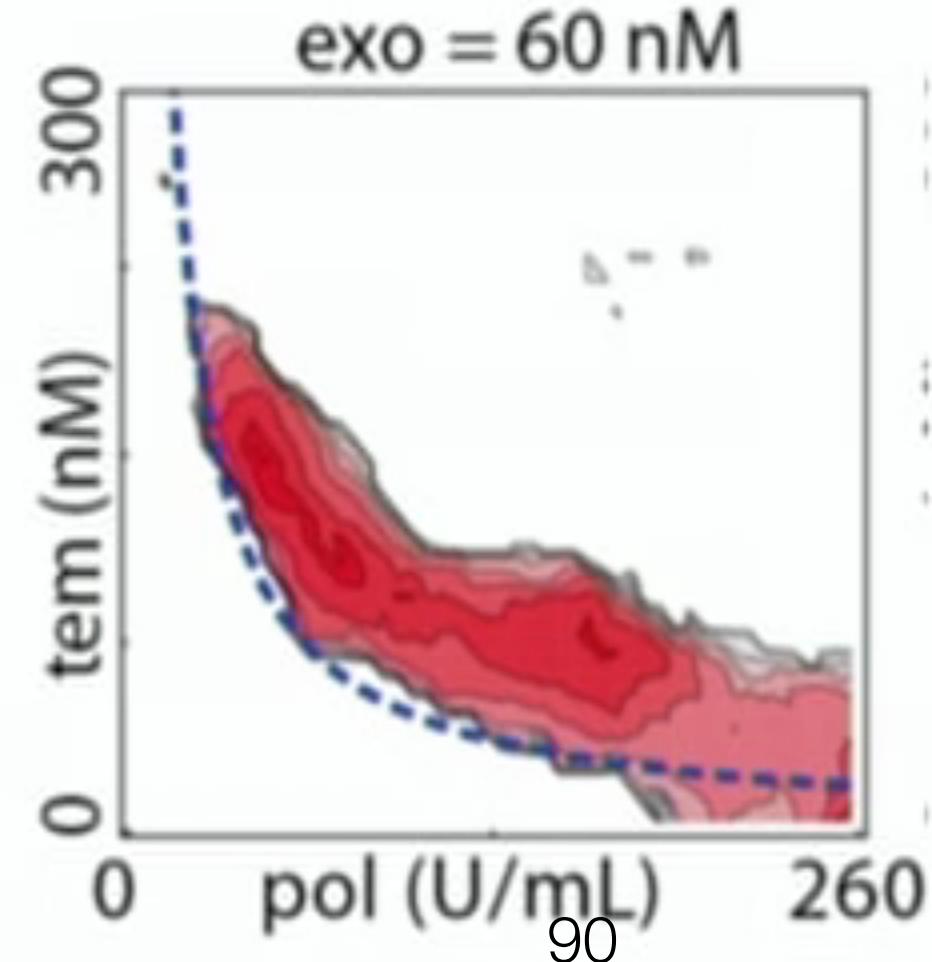
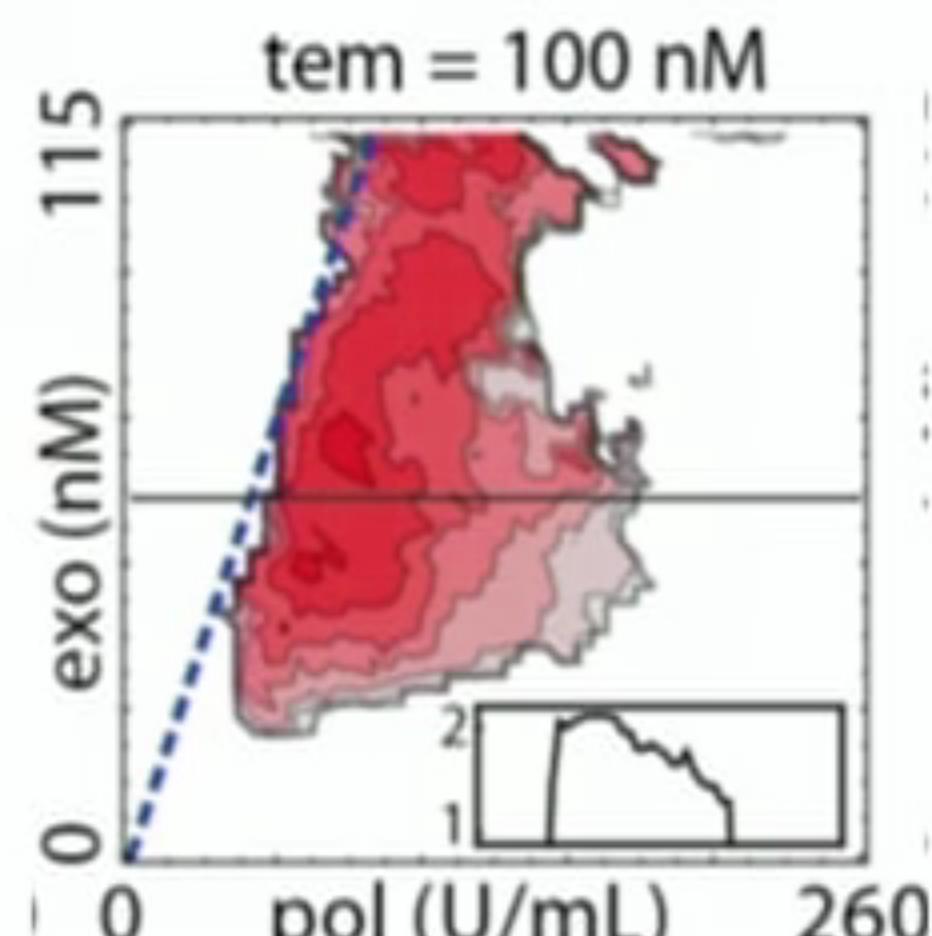
# 3-dimensional bifurcation diagram of an oscillator



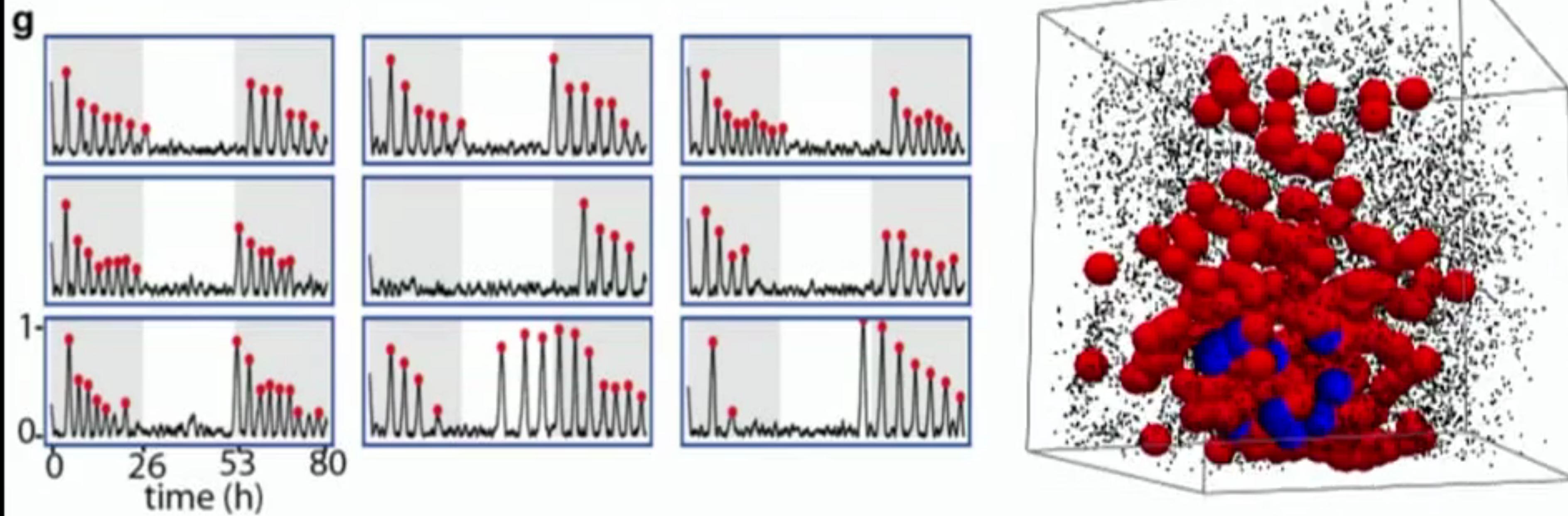
# 3-dimensional bifurcation diagram of an oscillator



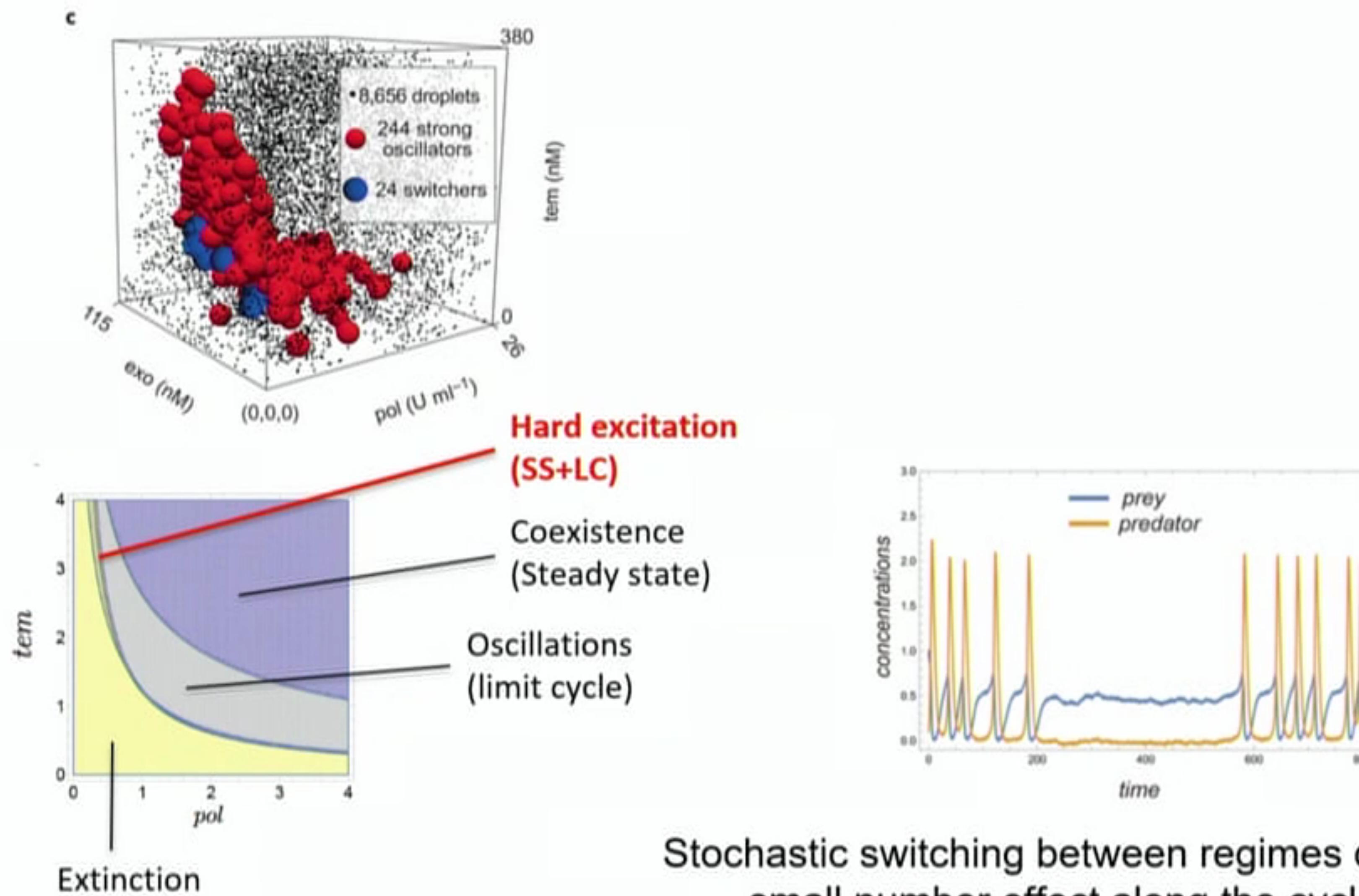
# 3-dimensional bifurcation diagram of an oscillator



# Stochastic bursters located at the Hopf bifurcation

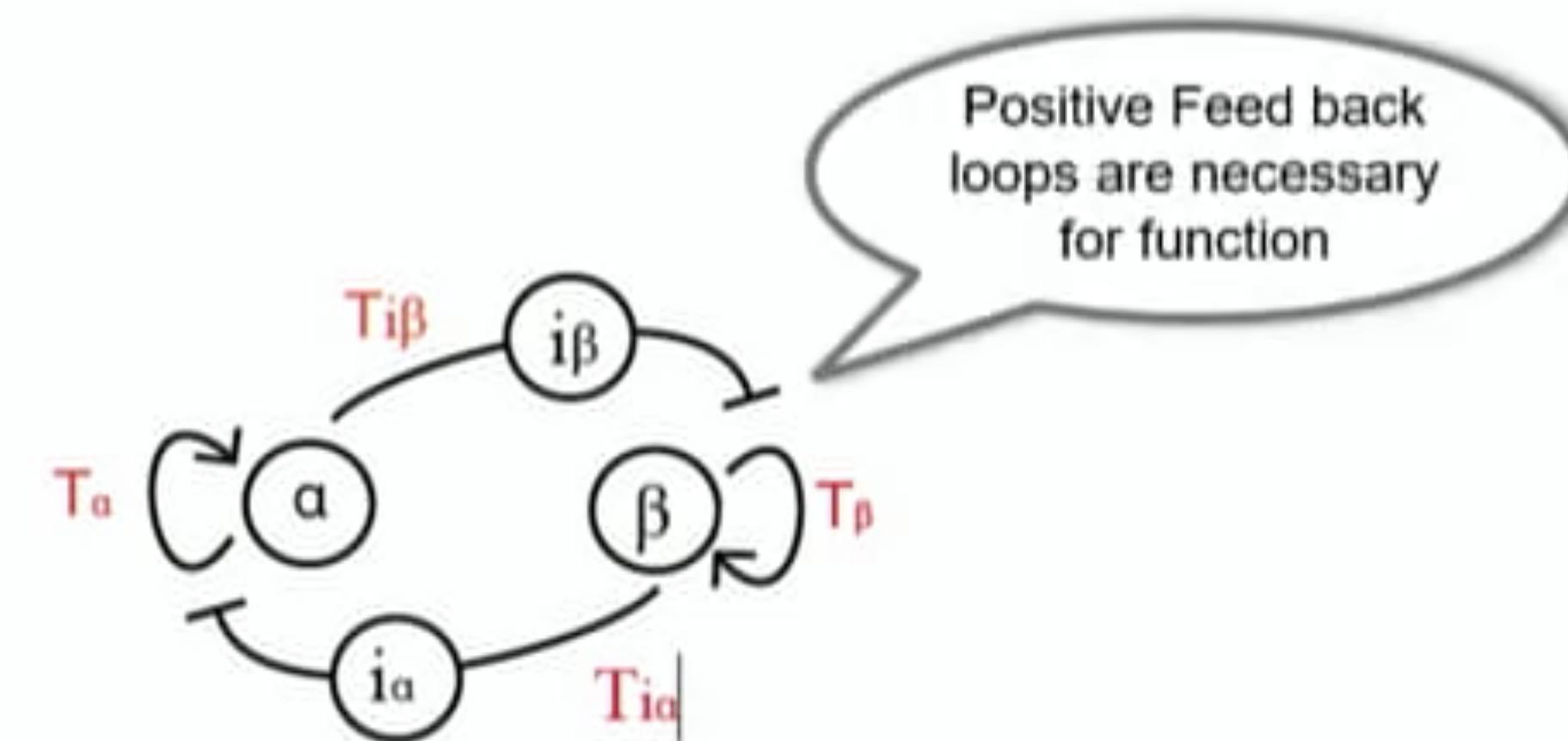
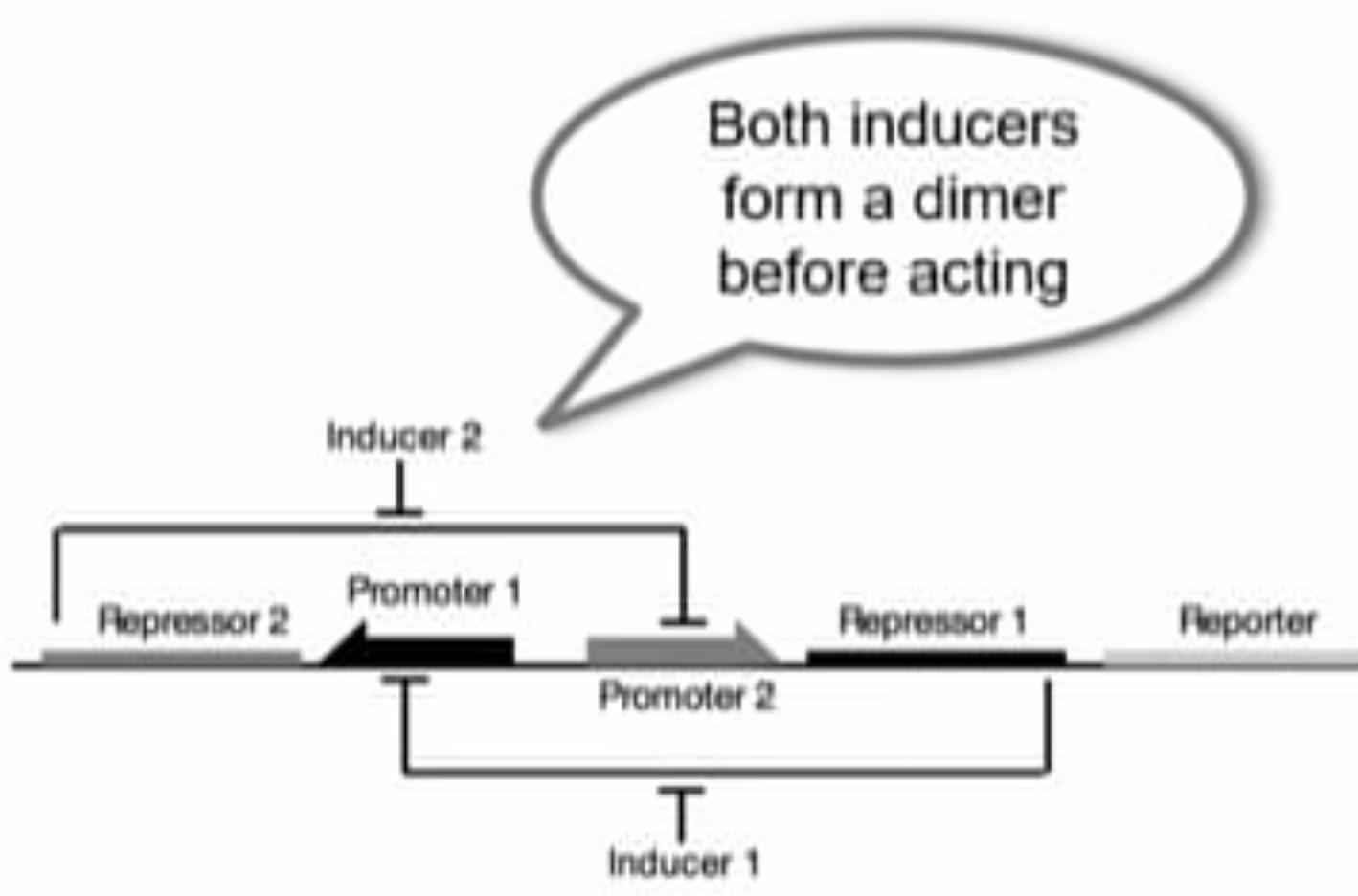


# Bursting droplets



# Learning by building

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



# Learning by building

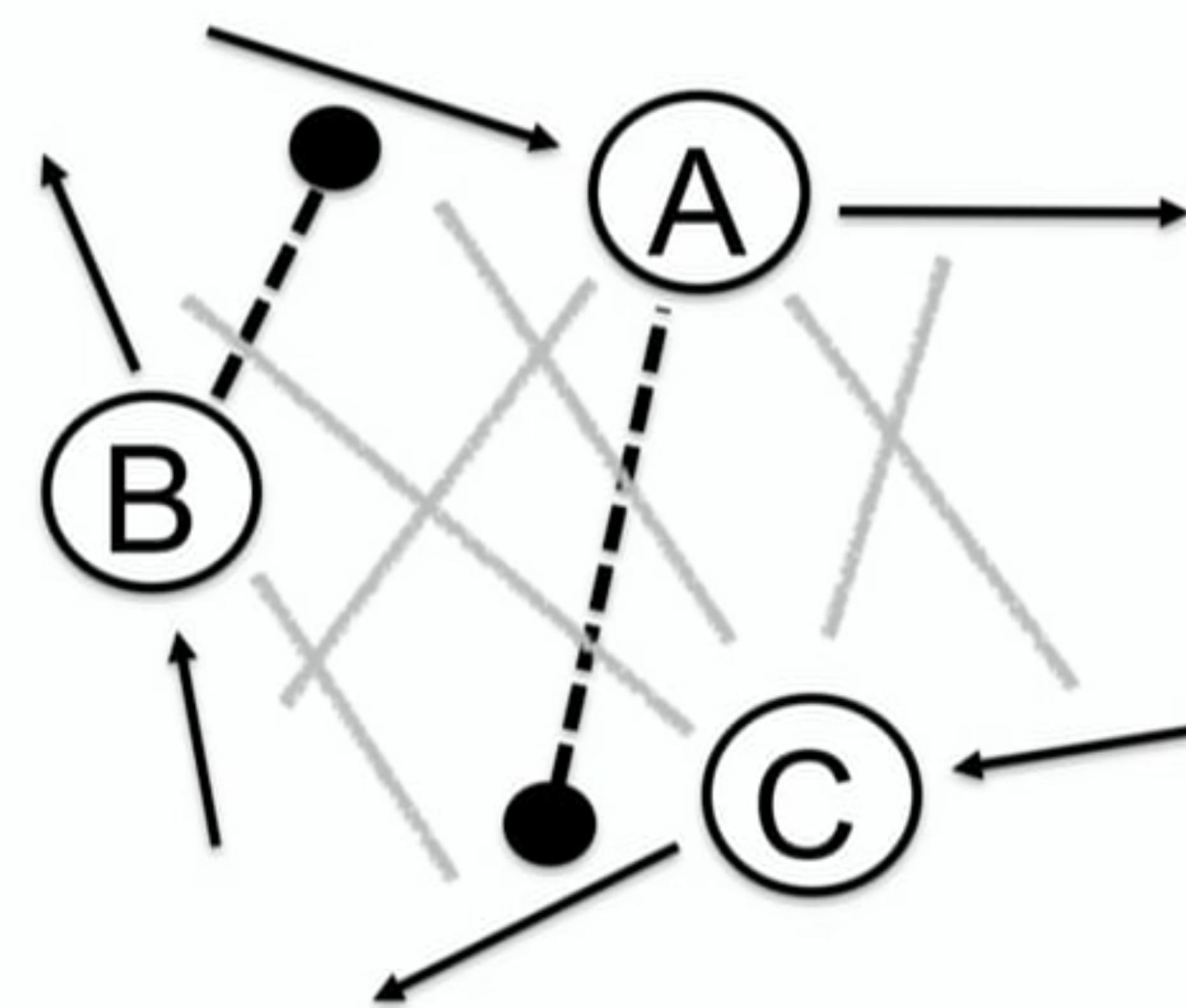
The edges hide a lot: non linear behaviours are essential, and provided by higher orders kinetics, delays or feedback loops

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

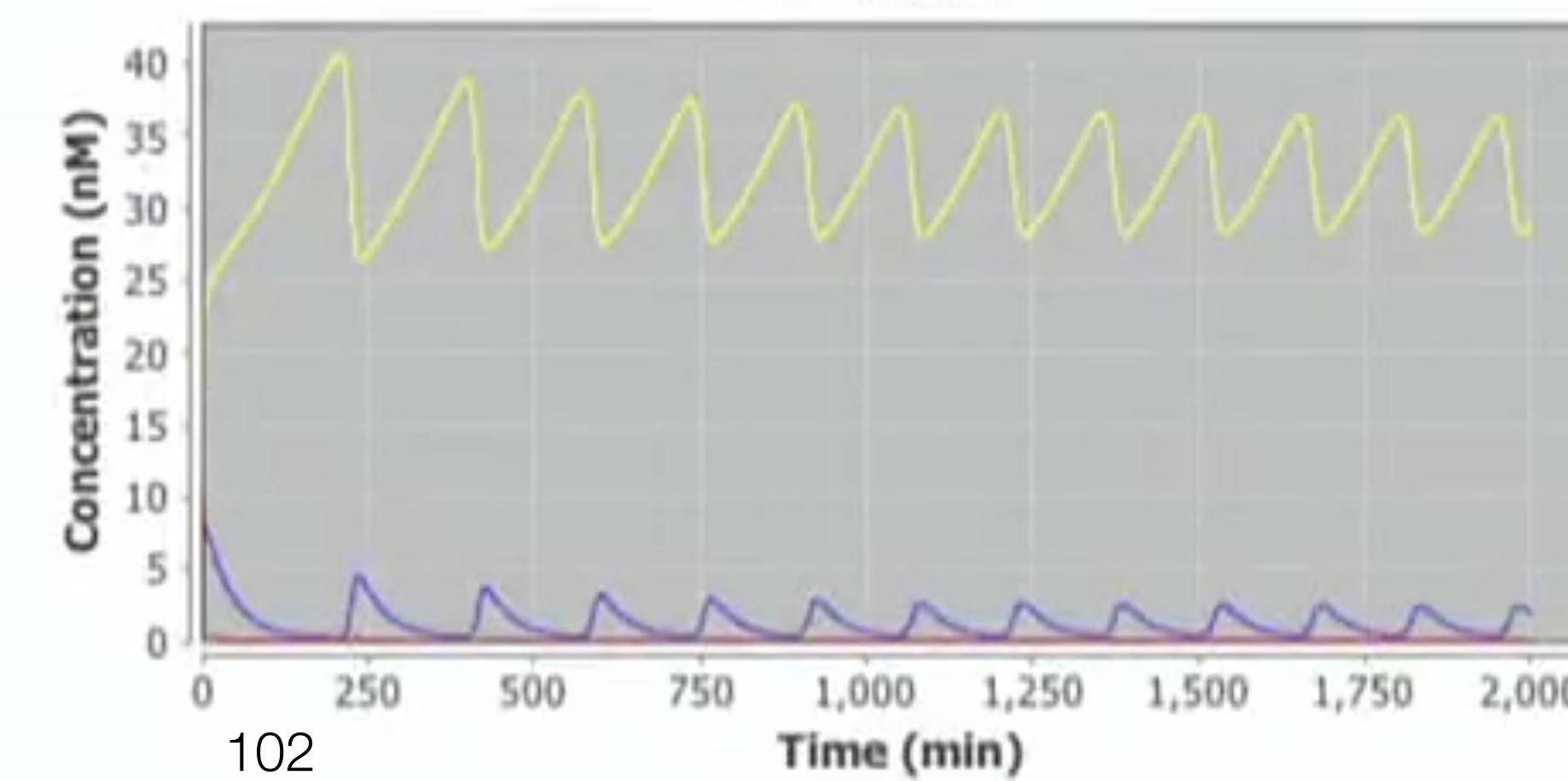
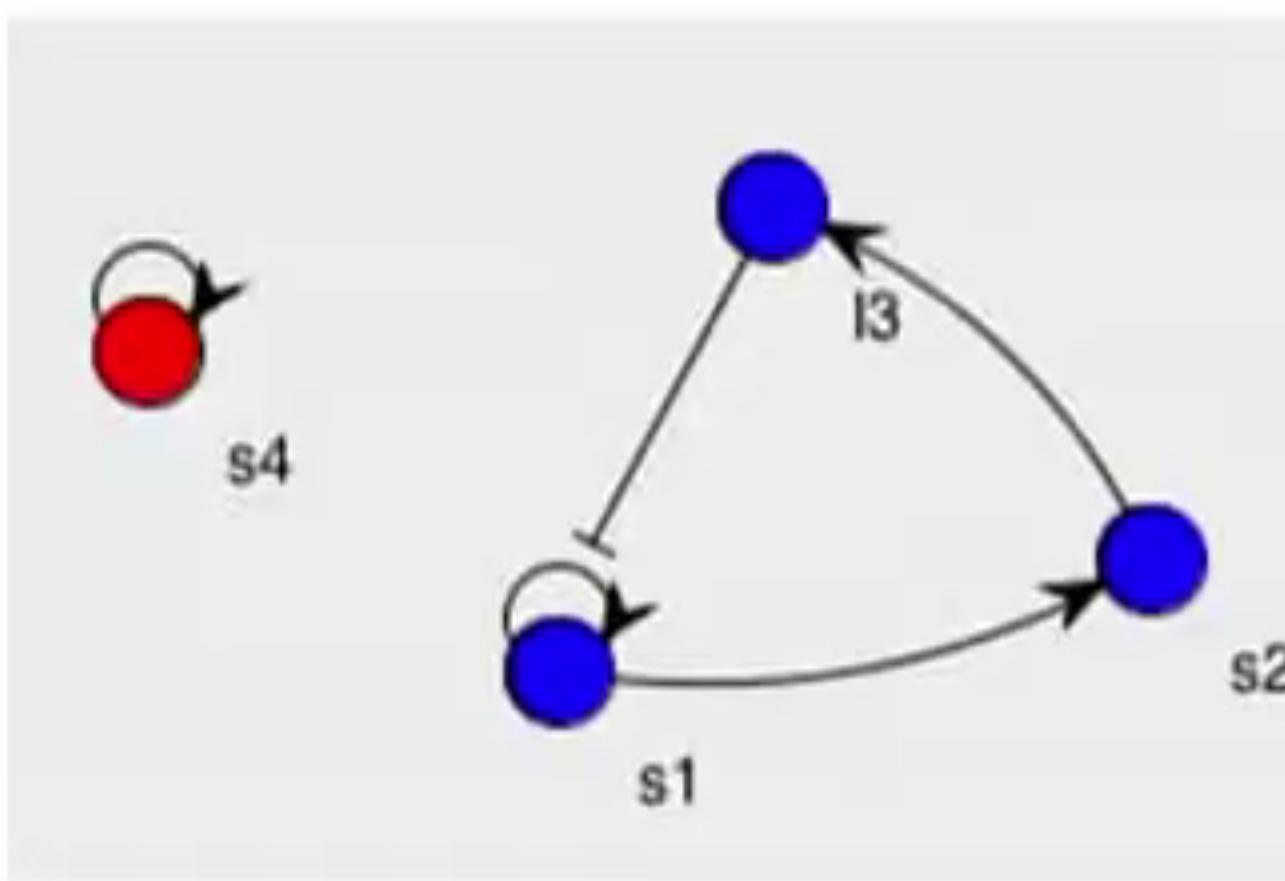
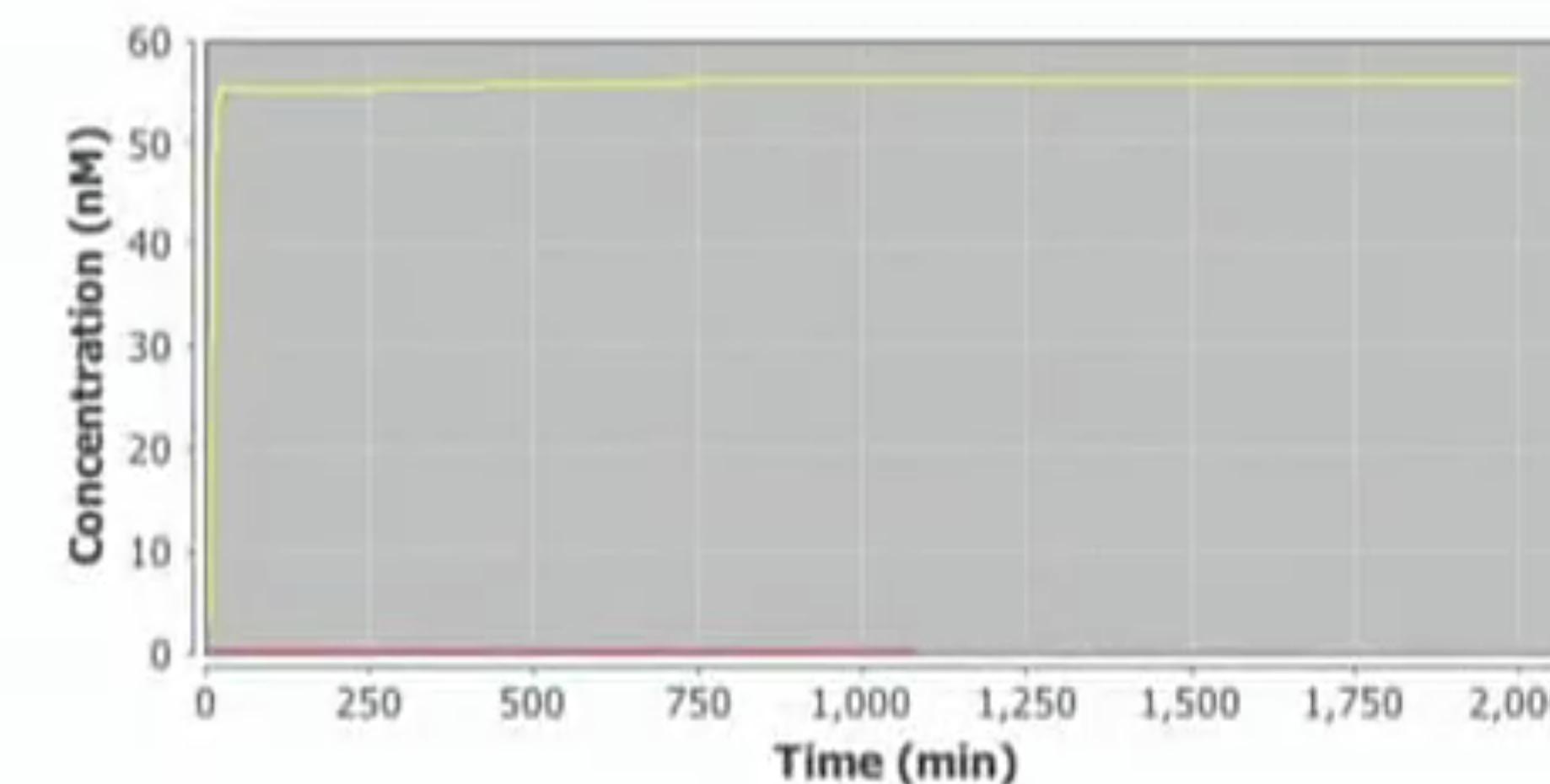
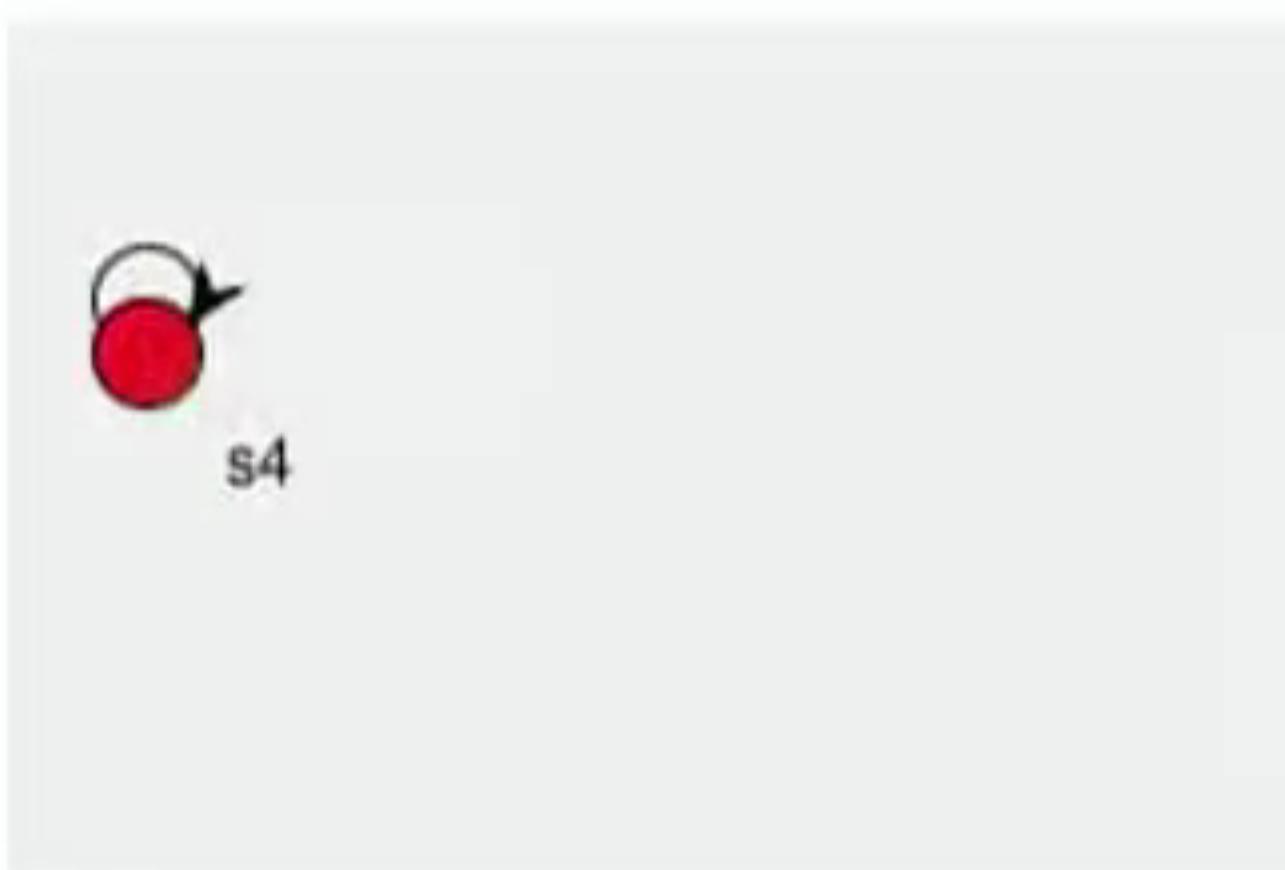
The role of global couplings: Ideal versus real molecular networks



In chemistry, networks with a “discrete” structure are an idealisation

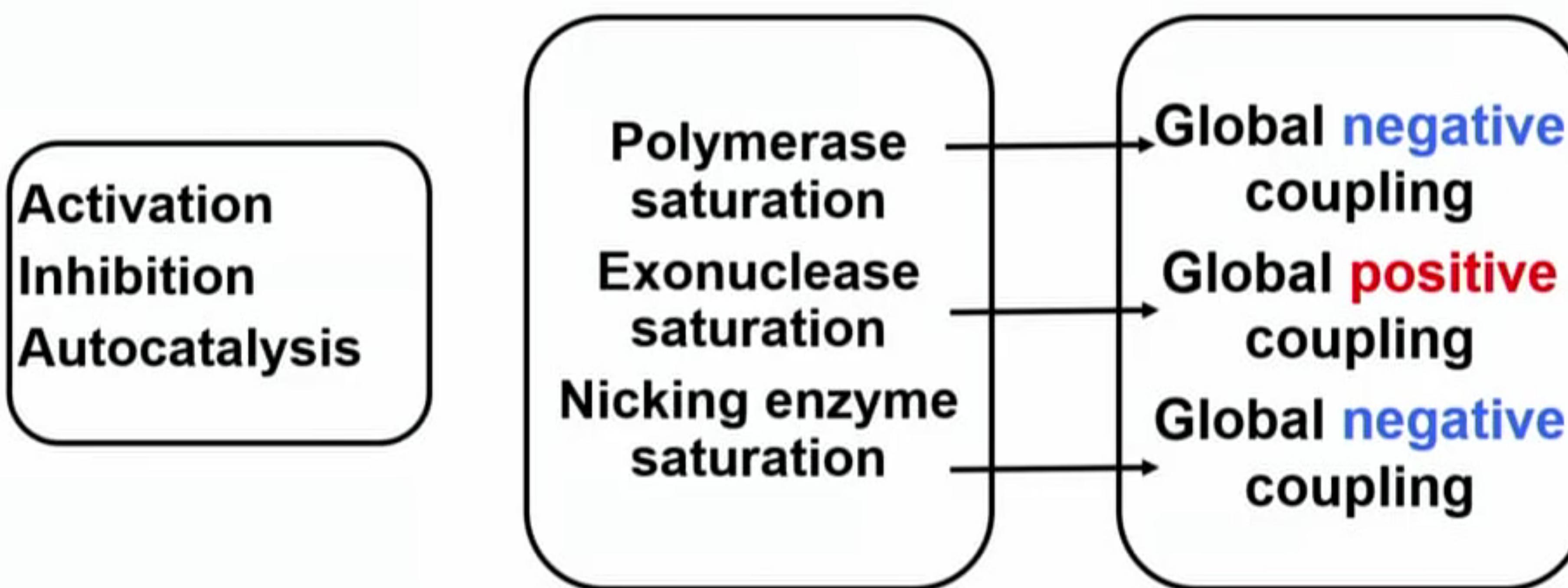


In (bio)-chemistry, networks with a “discrete” structure are an idealisation

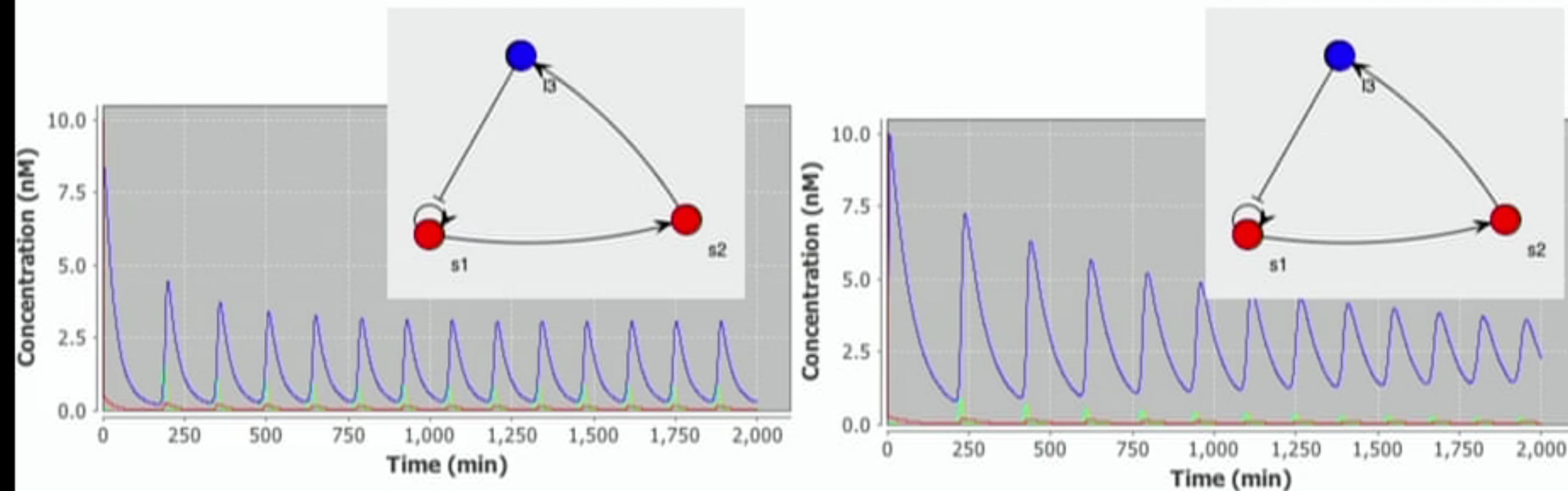


In chemistry, networks with a “discrete” structure are an idealisation

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

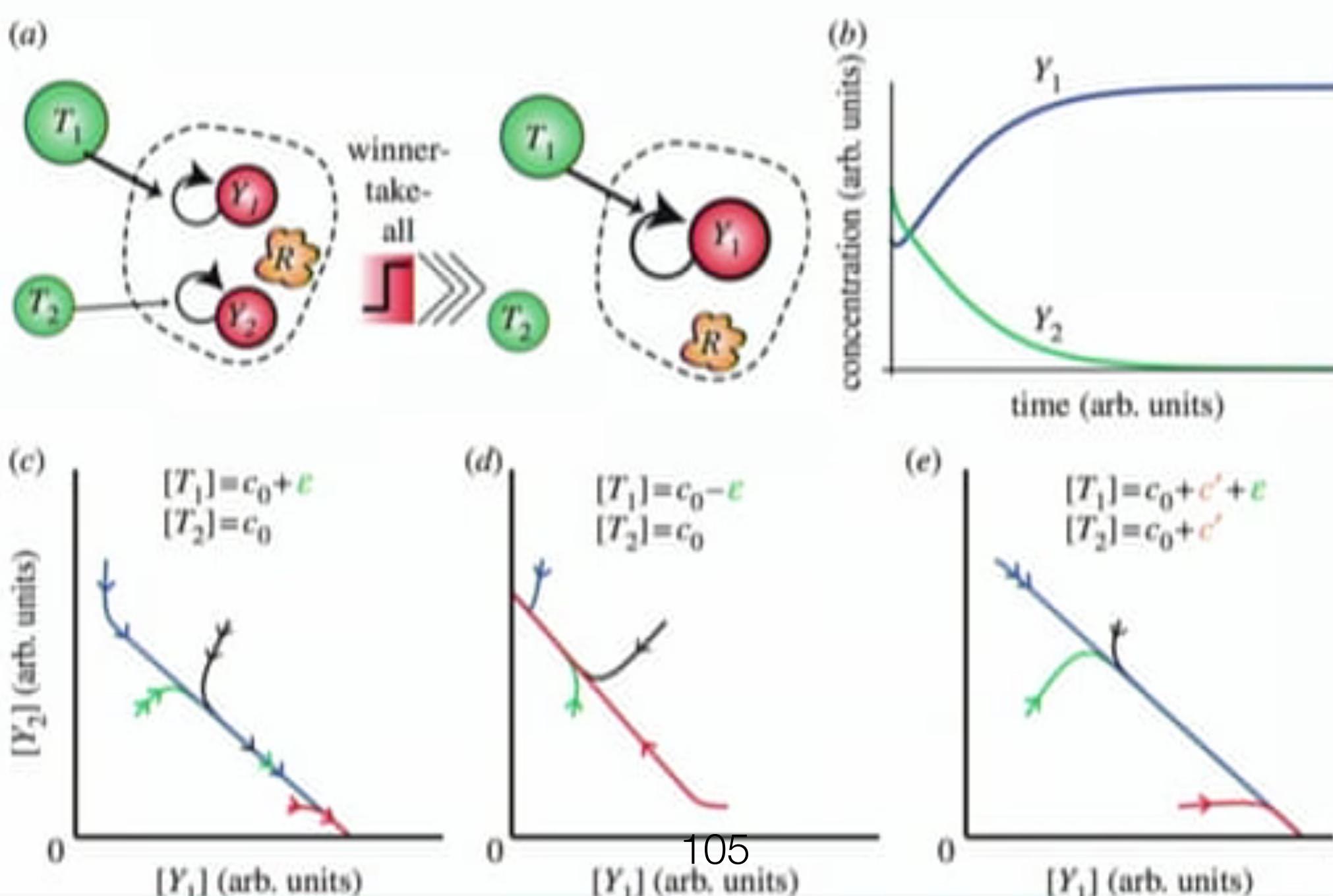


# Global loads can negatively affect function



# 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 *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.

