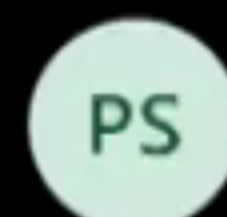


BOTTOM-UP EXPERIMENTAL MODELS OF CELLULAR NETWORKS

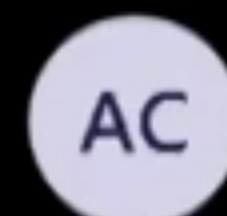
**YANNICK RONDELEZ
CNRS-ESPCI-PSL**

**MULTISCALE INTEGRATION IN BIOLOGICAL
SYSTEMS NOV 2021**

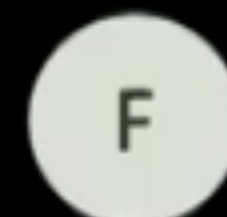
Yannick Rondelez
ESPCI, FR



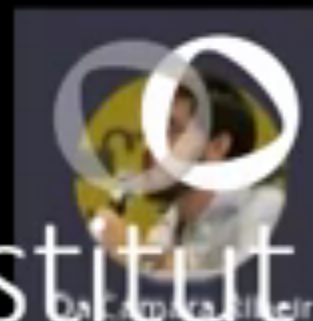
Pouzet Sylvain



Alexander CHAM...



Francesco (Guest)



**institut
Curie**

Information management across biological scales



PS

Pouzet Sylvain

AC

Alexander CHAM...

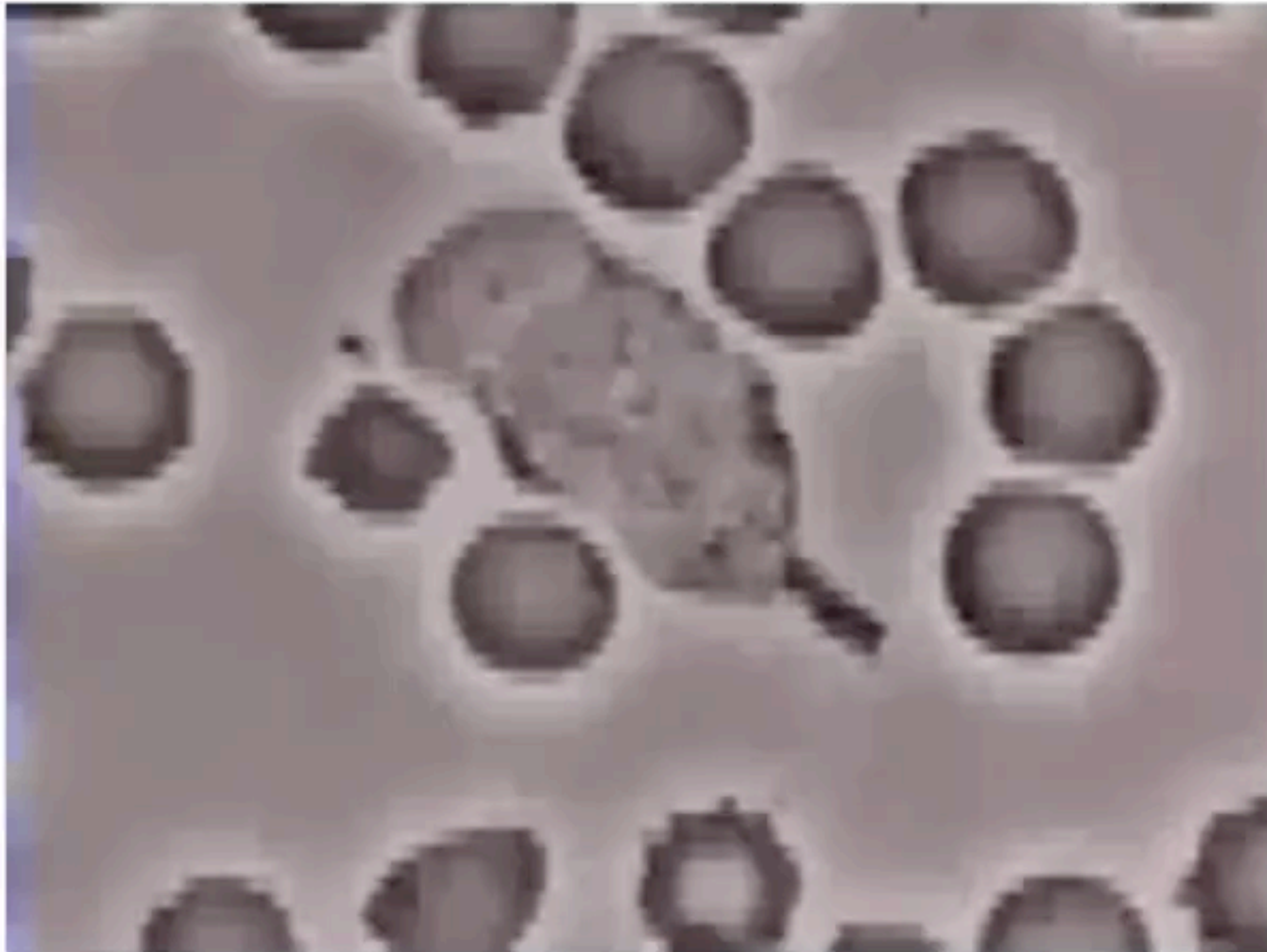
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Information management across biological scales



Neutrophil chasing a bacteria (D. Roger 1950)



PS

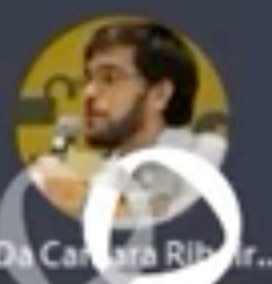
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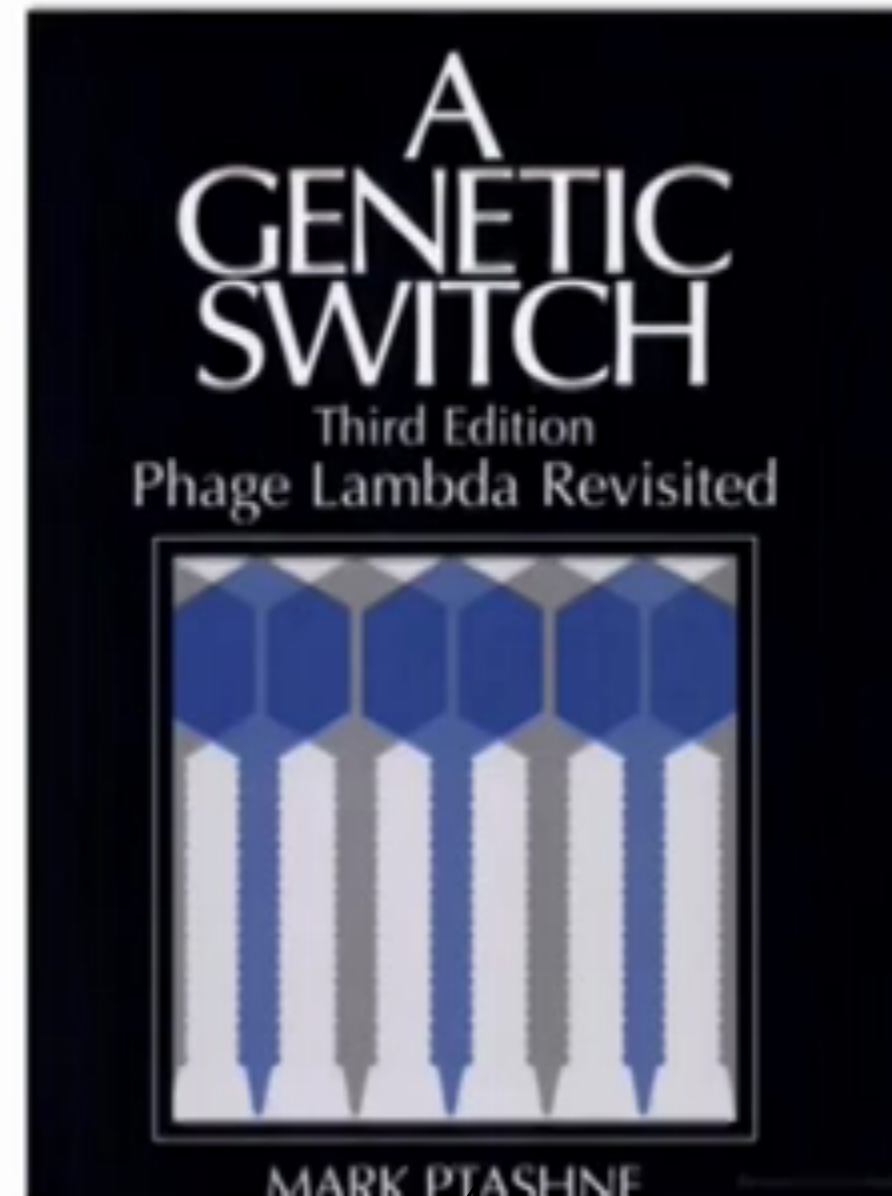
Da Camara Ribeiro...

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RS
Romain Sh

Information management across biological scales



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



PS

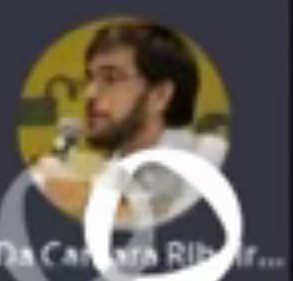
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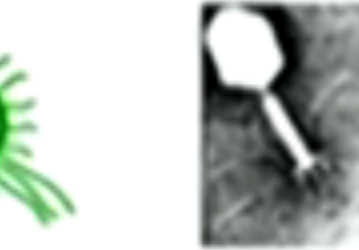
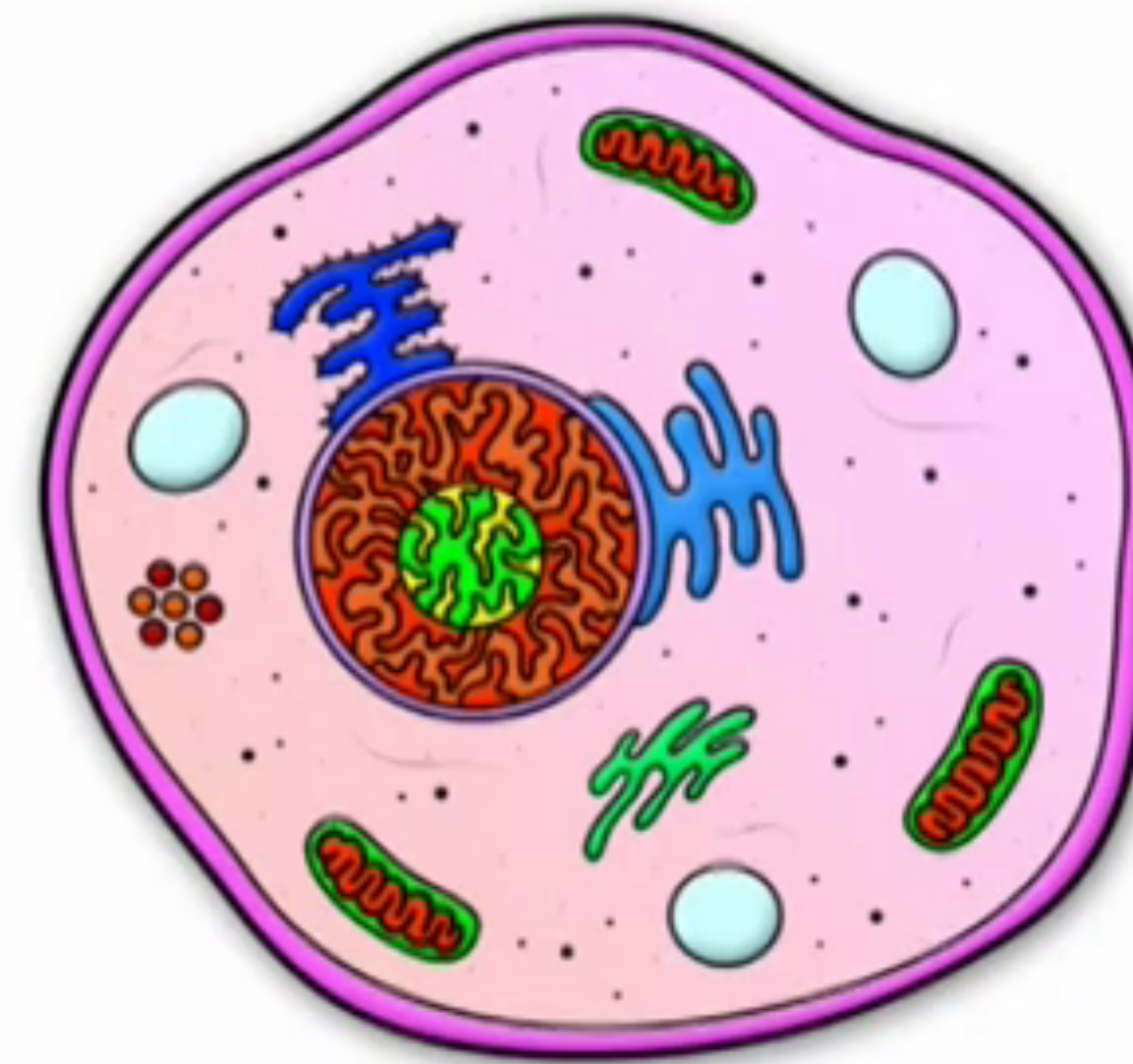
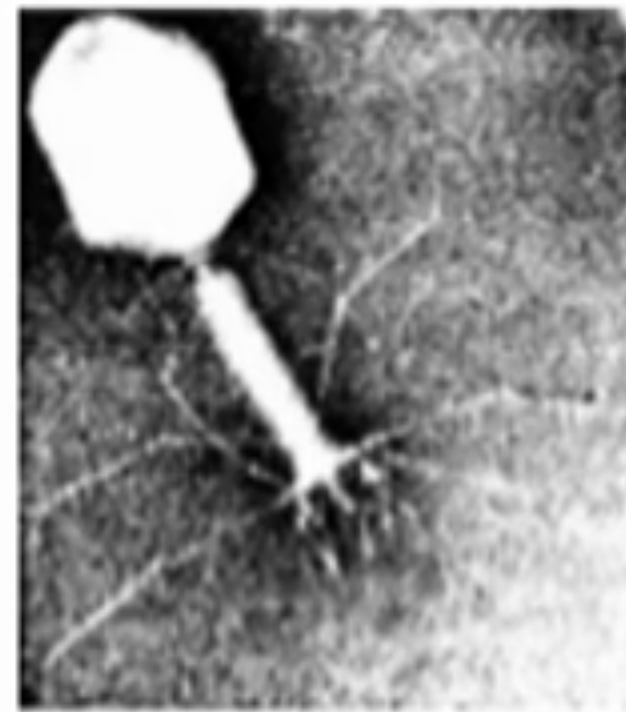
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Ramnan Sh...

How does it work ?

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



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Francesco (Guest)

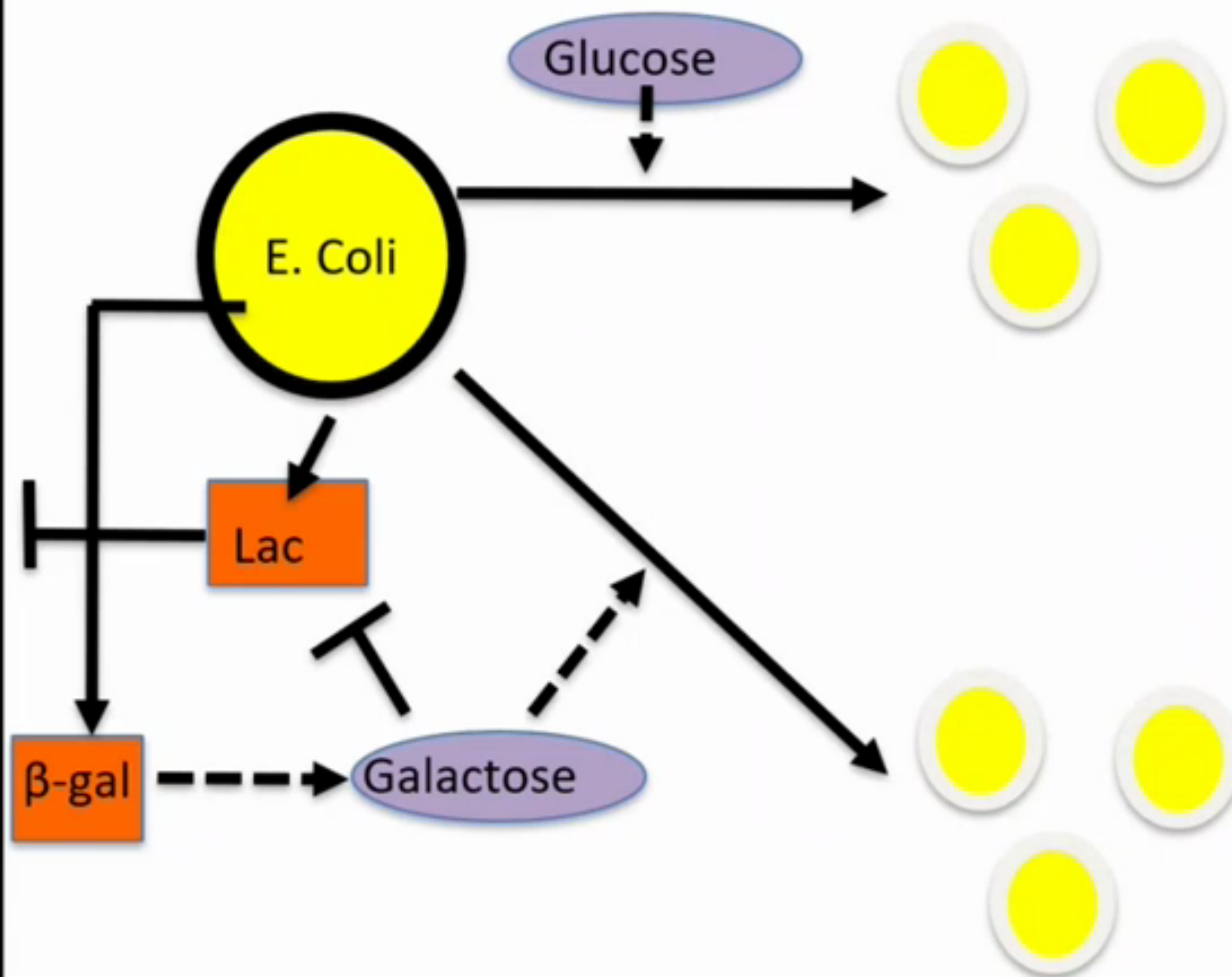
MC

Marion MOS...

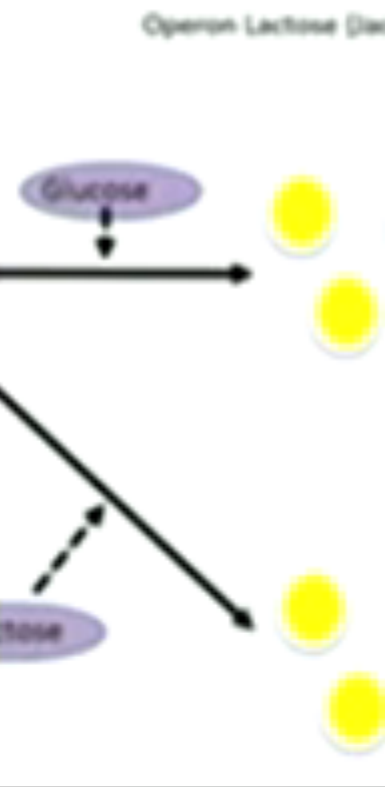
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Molecular Systems&Circuits

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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fra.mignacco

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RS

Raman Greshk (...)

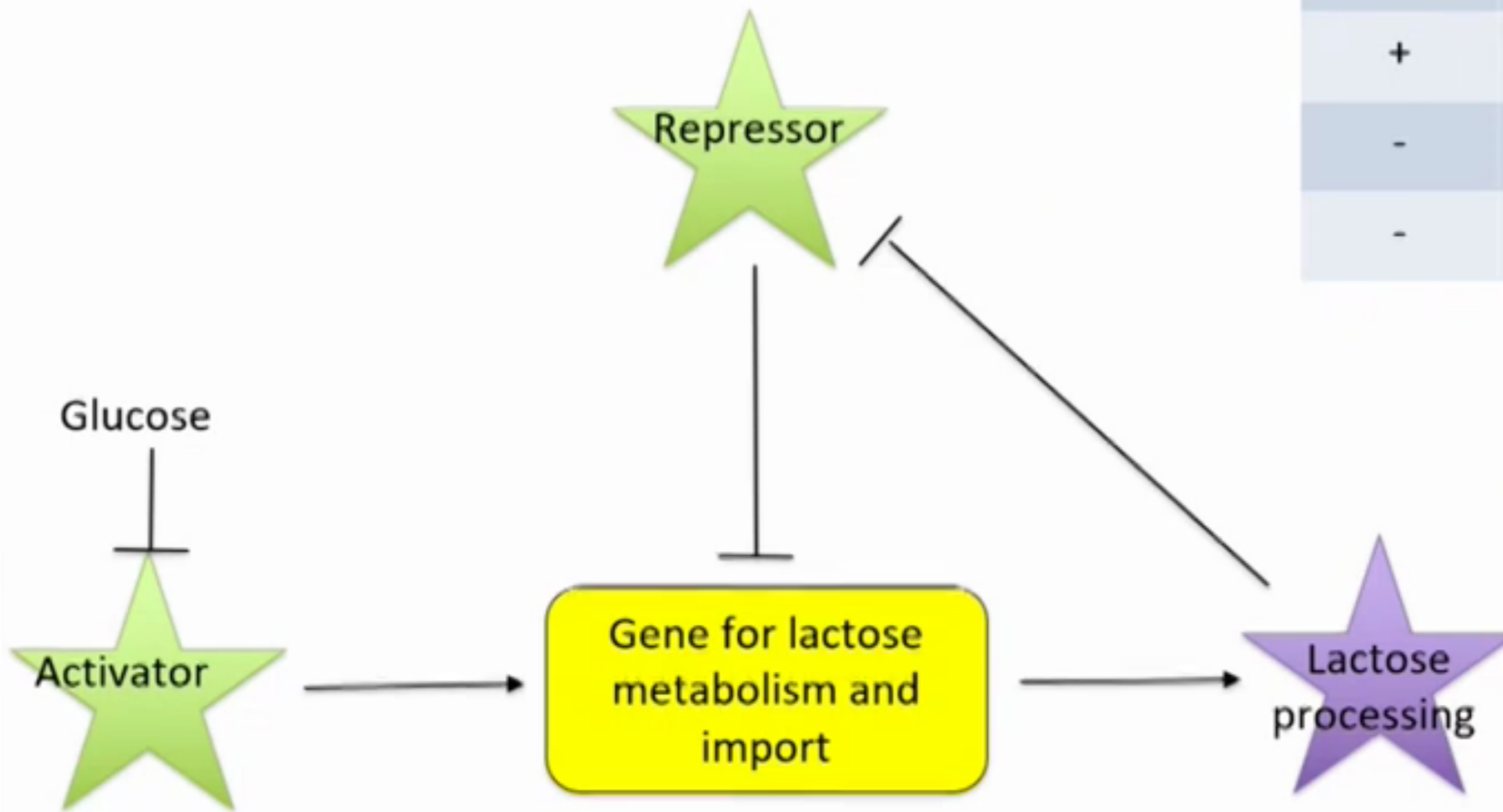
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Pouzet Sy

Molecular Systems&Circuits

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



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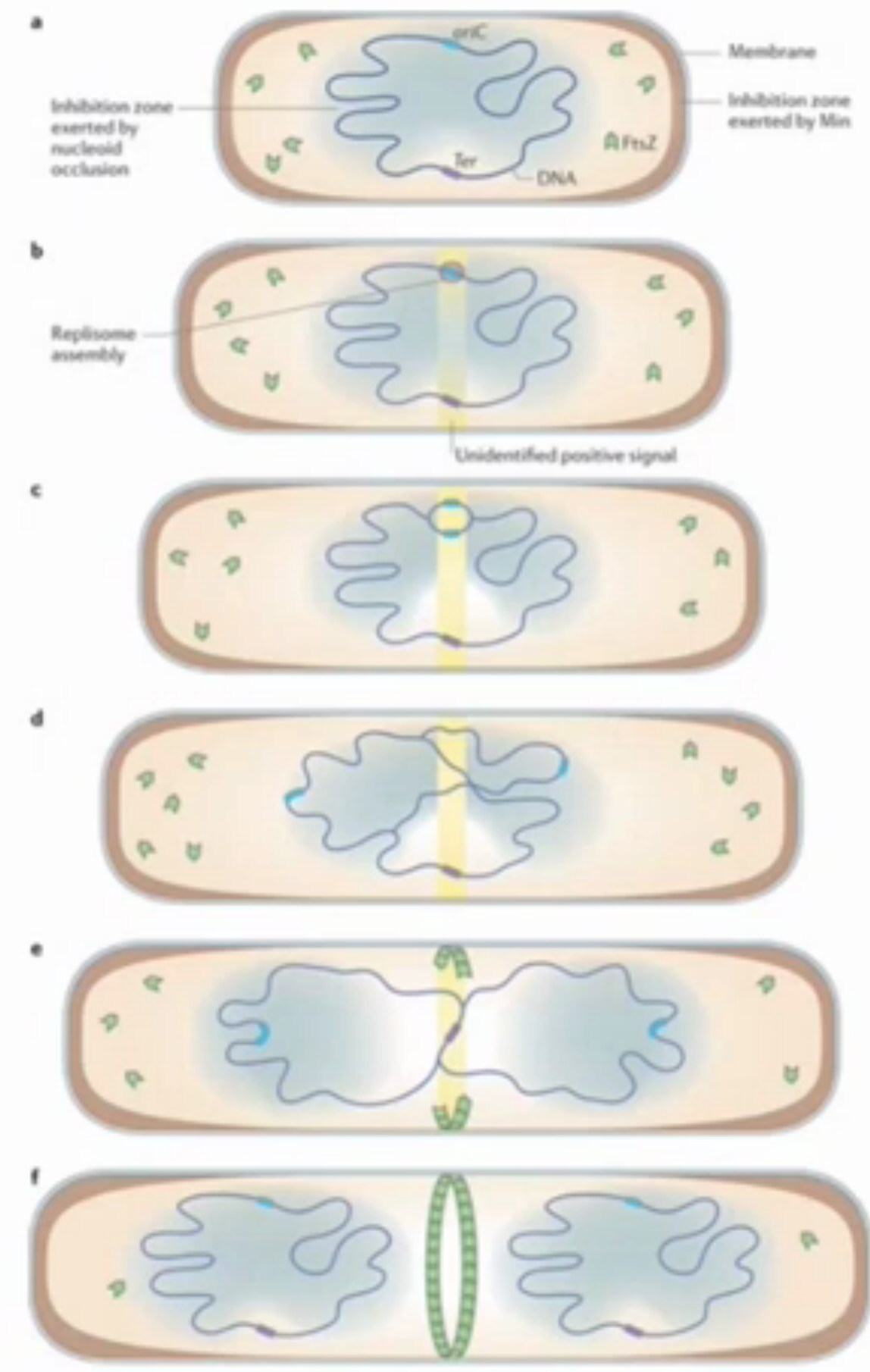
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Raman Gosh...

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How does a bacteria finds its middle?



Nature Reviews | Microbiology



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Da Camara Ribeir...

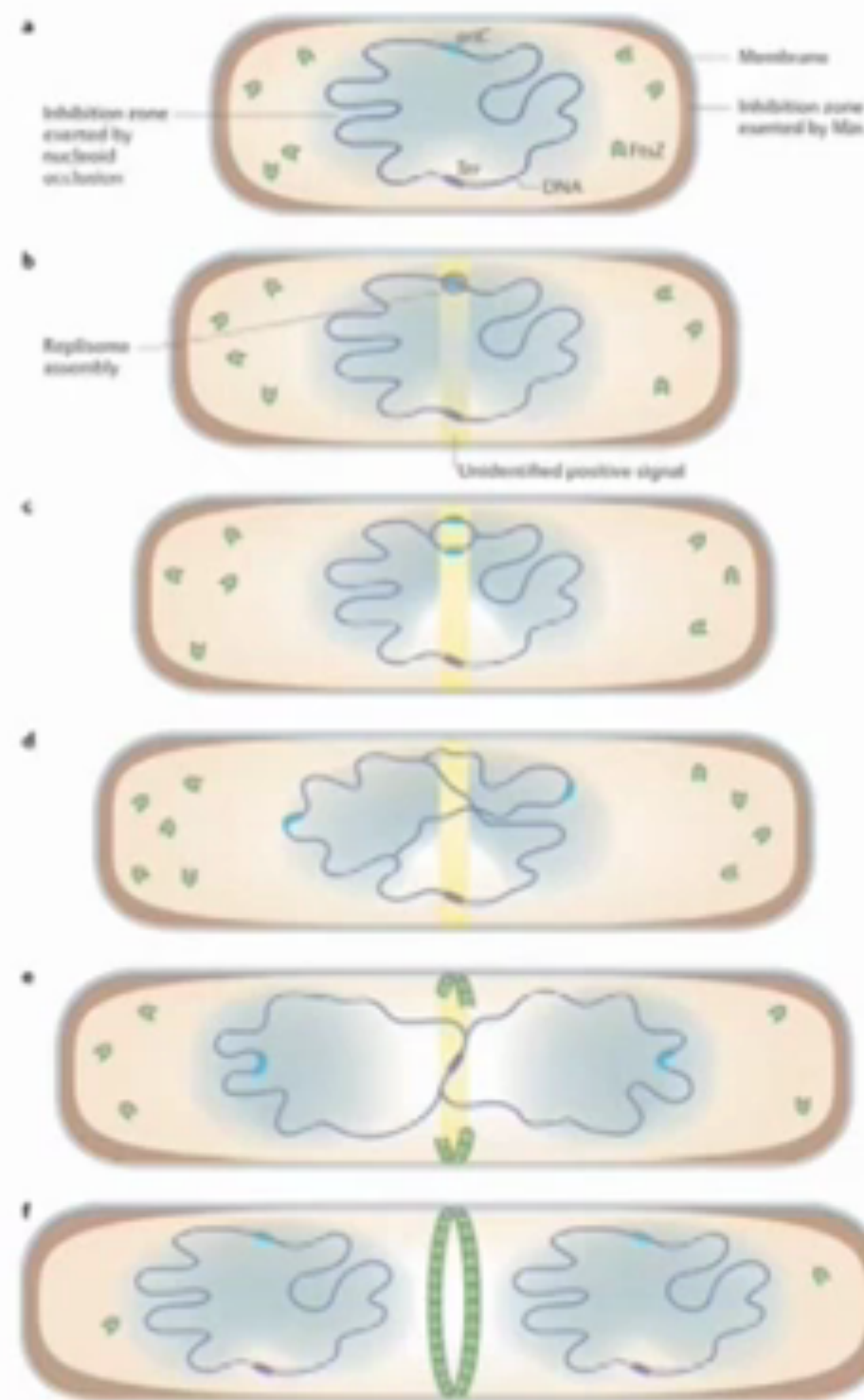
RS

Raman Greshk (...)

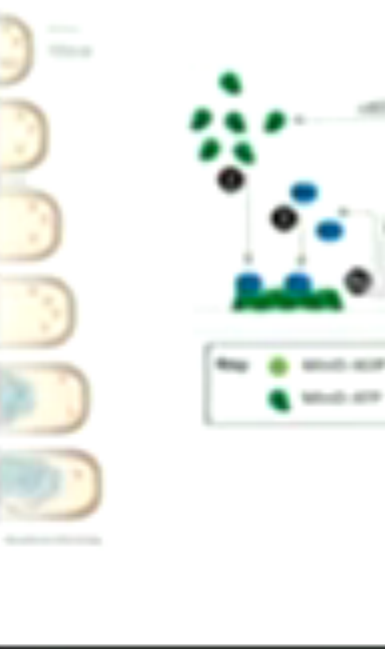
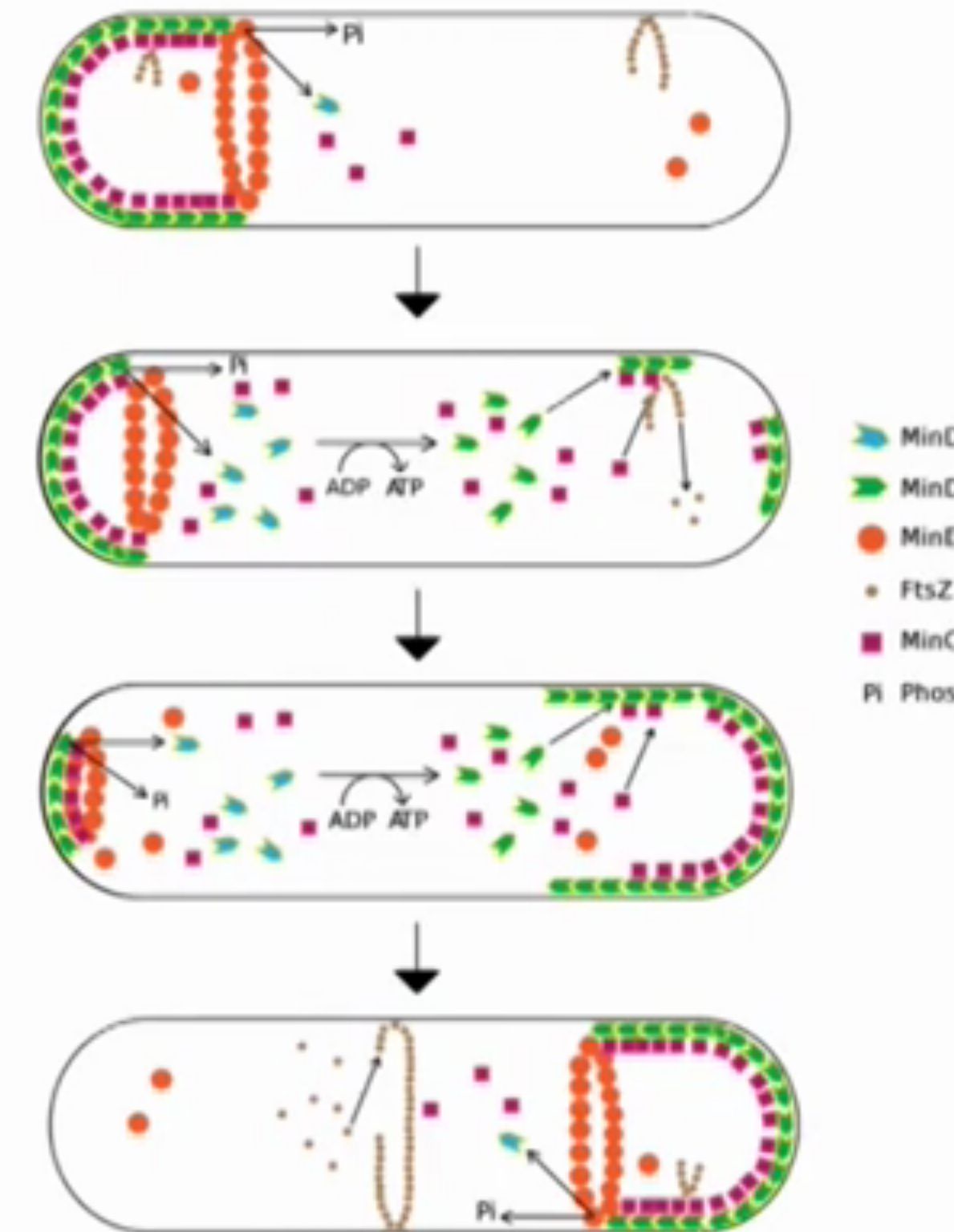
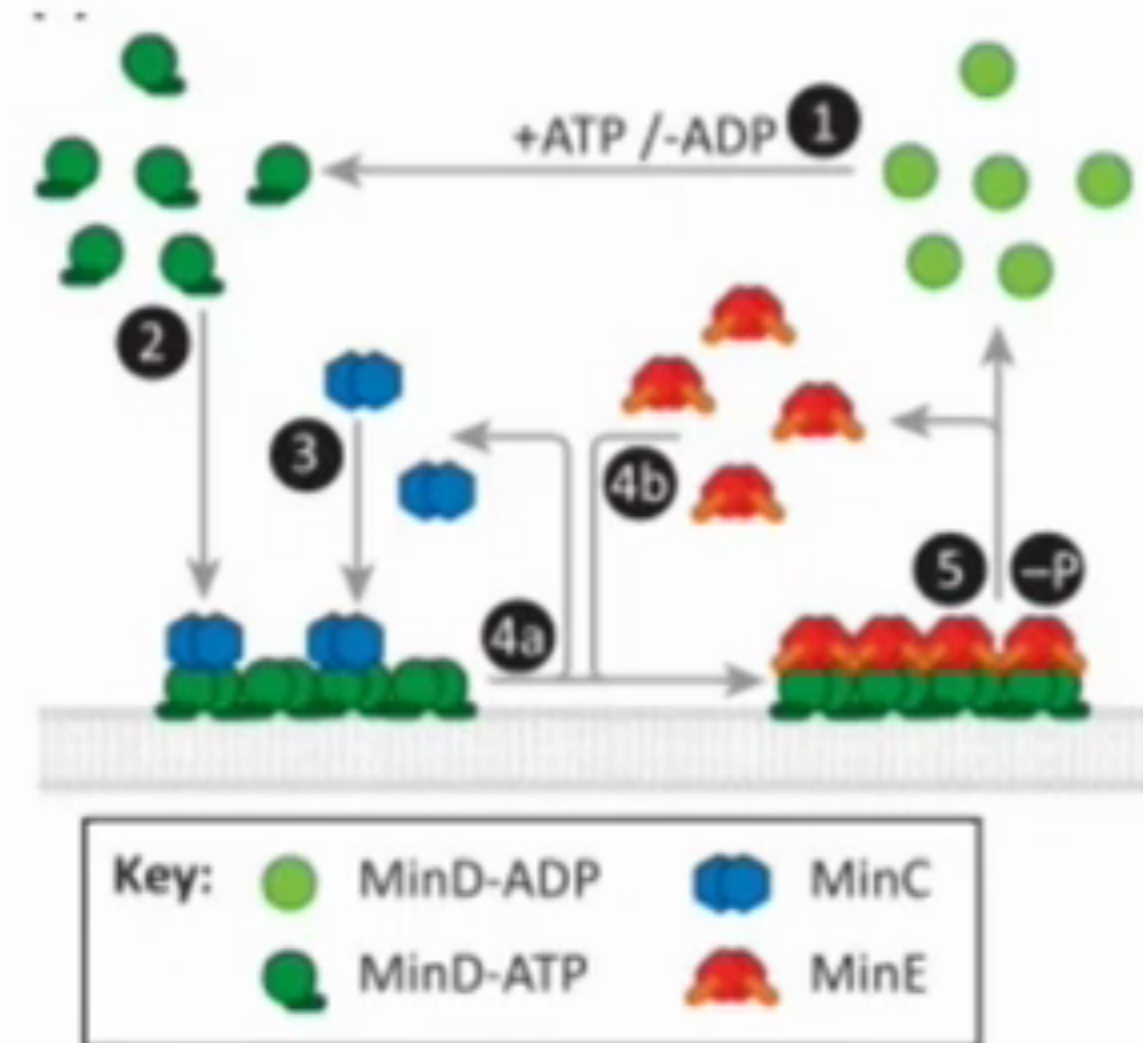
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How does a bacteria finds its middle?

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



Nature Reviews | Microbiology



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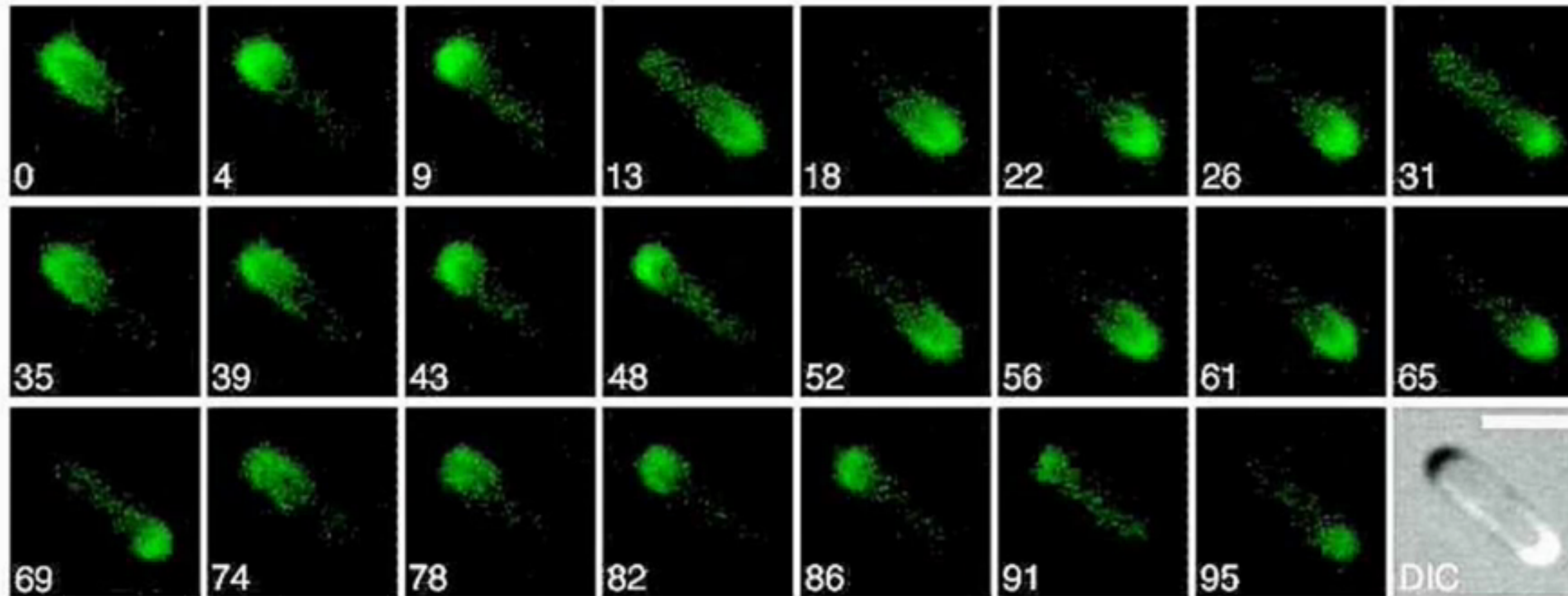
Ramona Greshko (...)

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Pouzet Sylvain

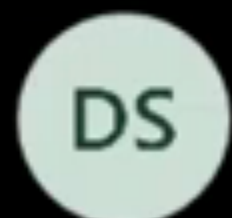
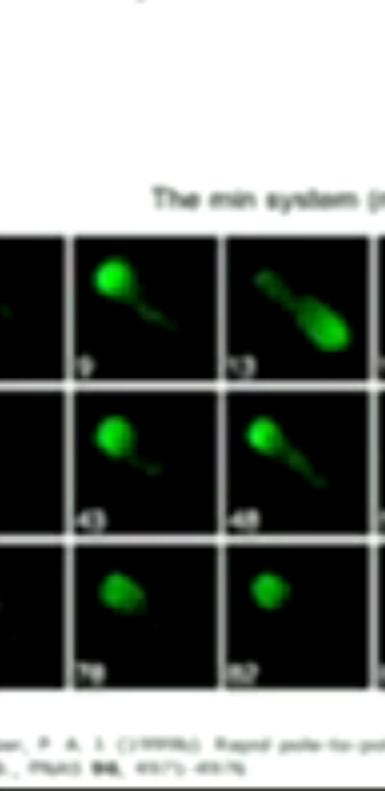
How does a bacteria finds its middle?

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

The min system (minC,D,E)



Raskin, D. M., and de Boer, P. A. J. (1999b). Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*, PNAS **96**, 4971-4976



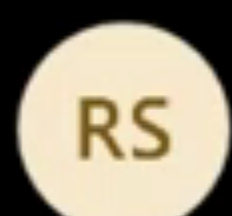
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Da Camara Ribeir...



Raman Sheshka (...)

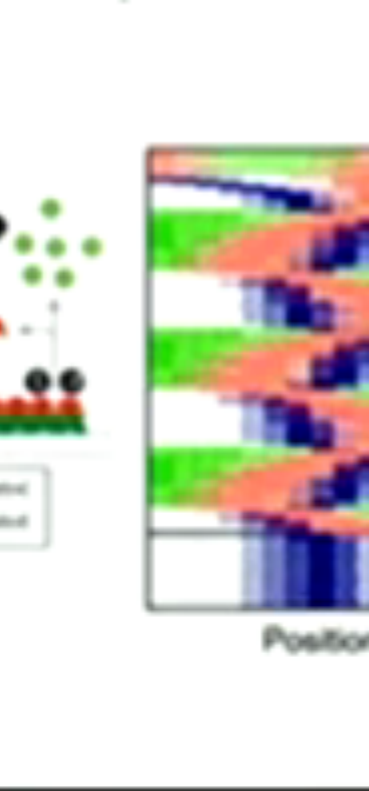
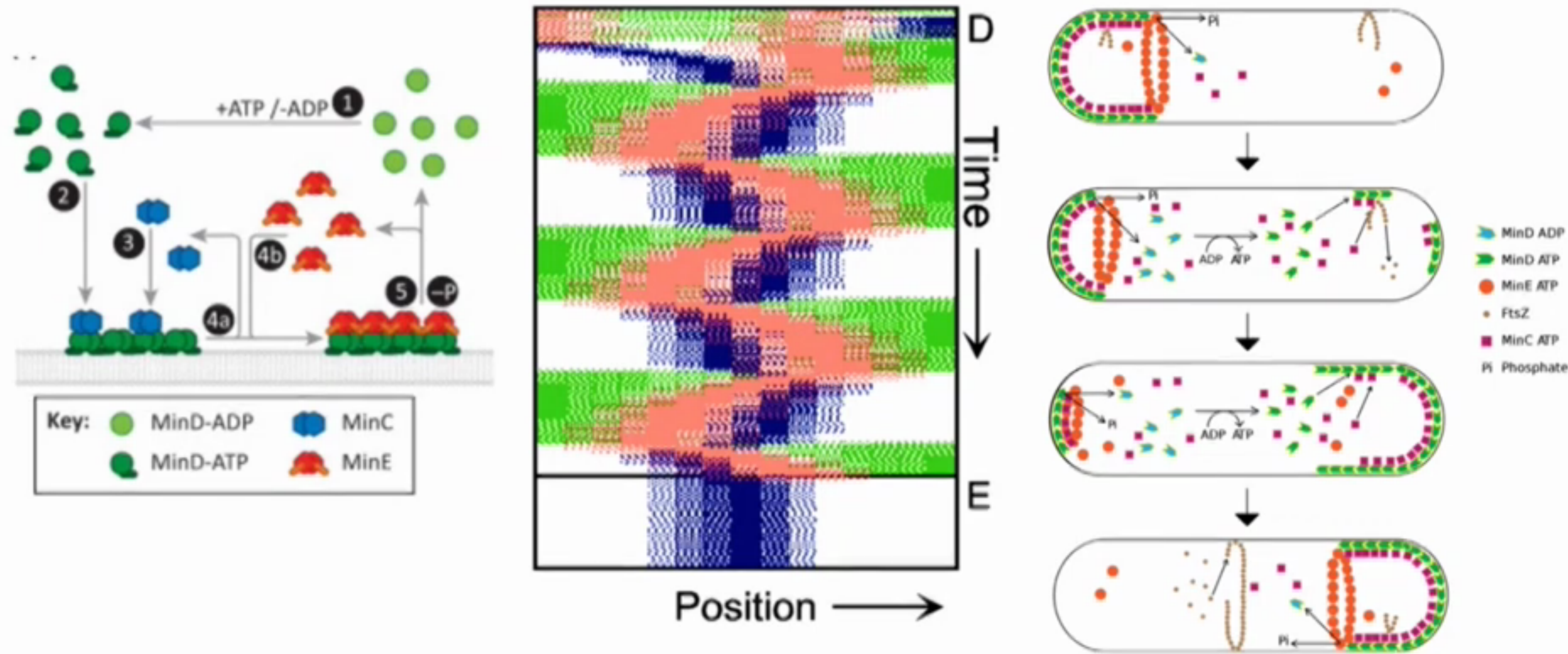


Bo Zet Schulz

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How does a bacteria finds its middle?

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



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Raman Sheetha (...)

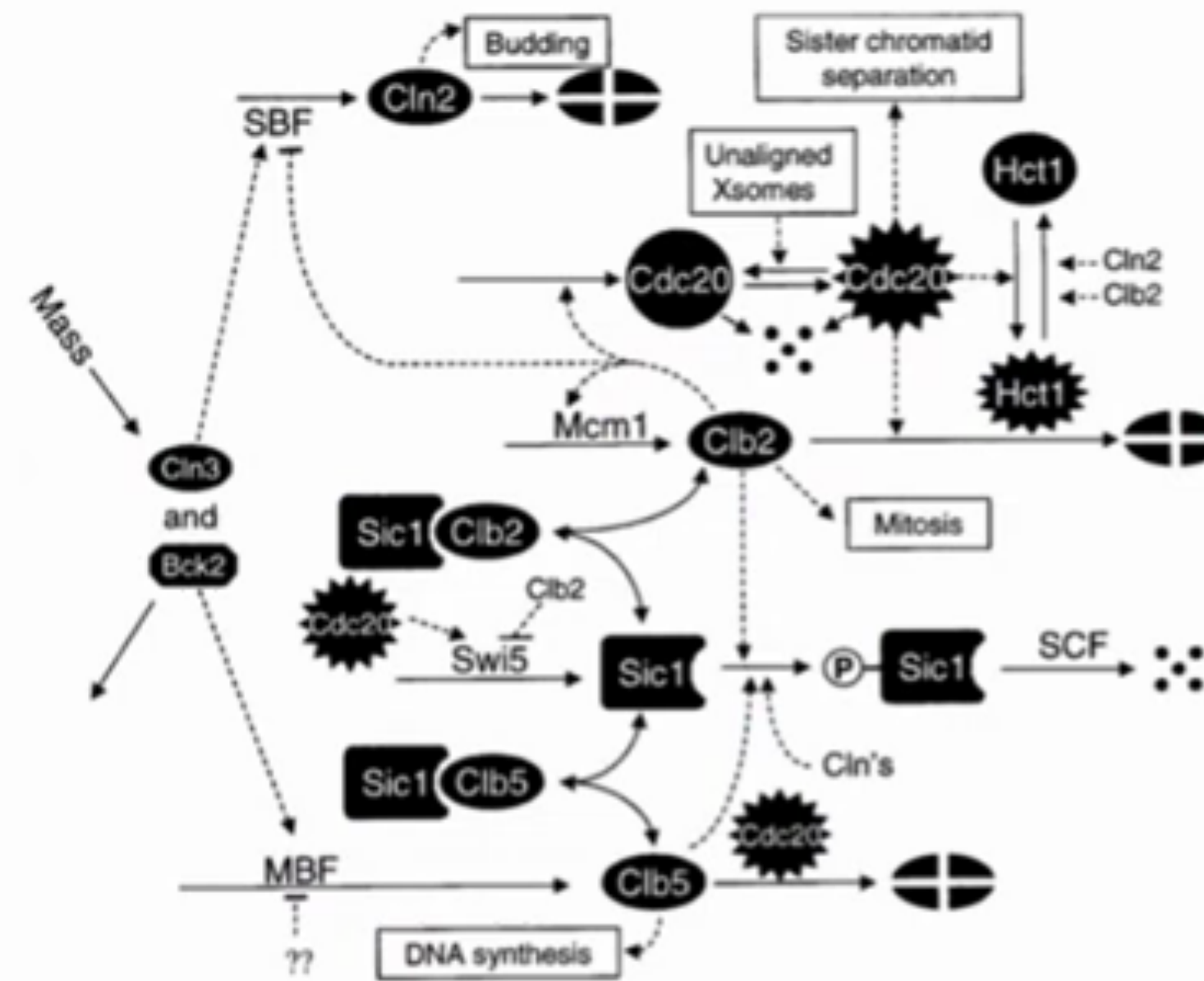
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So Z. Z. Z. Z. Z.

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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



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.

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Deanna Shea

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Da Camara Ribeir...

RS
Raman Sheshka (...)

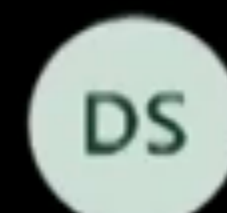
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Are molecular networks special?

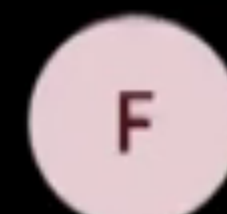
The answer is at the same time **NO**, **YES** and **We don't know**

- **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.**

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fundamen
YES, because the e
instantiated by mol
reactions and t
don't know, beca
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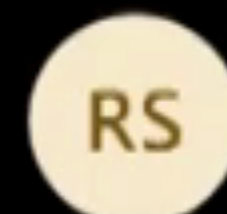
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Da Camara Ribeir...



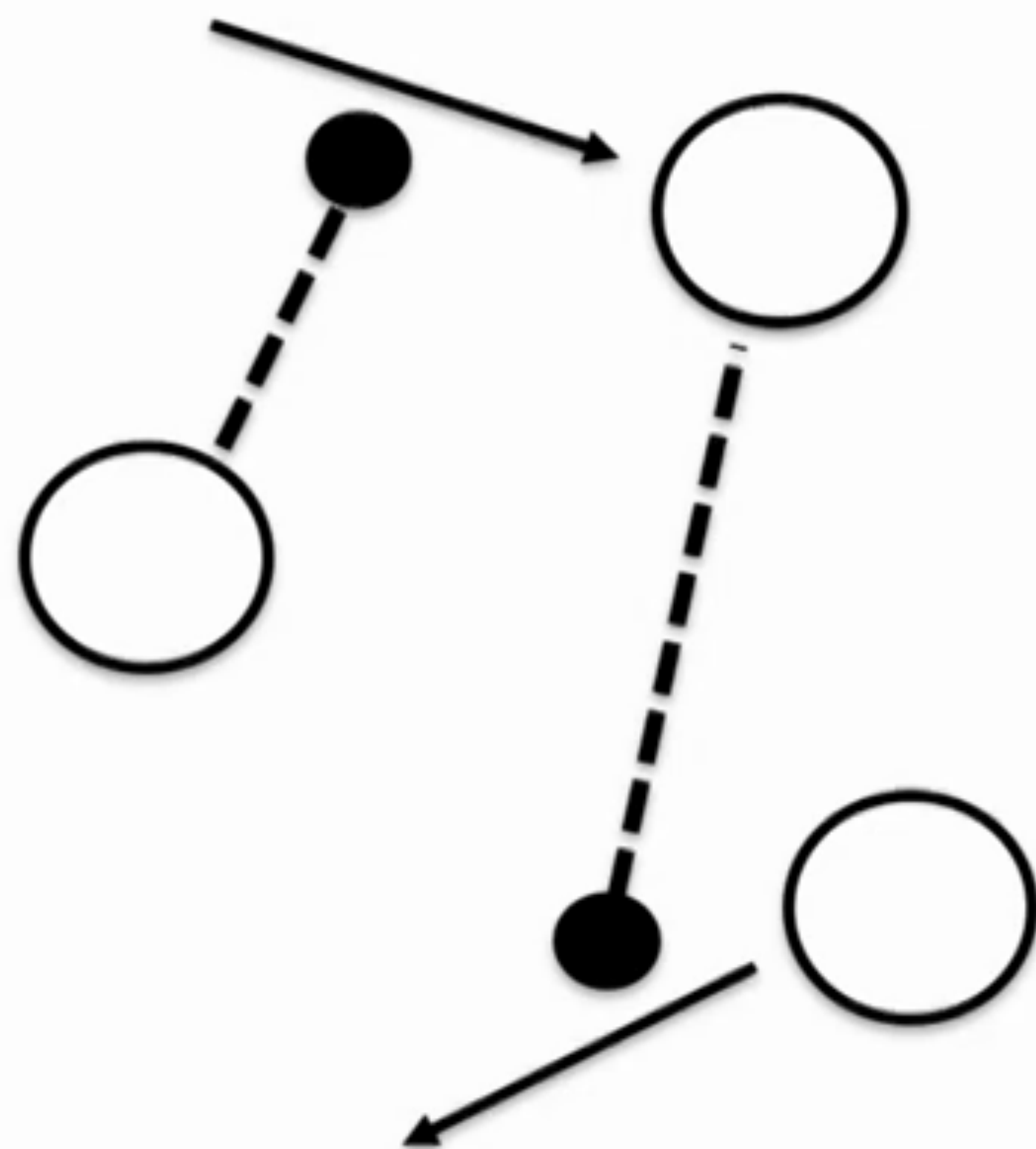
Raman Shethka (...)



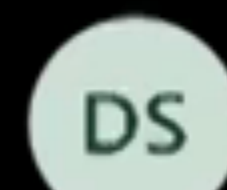
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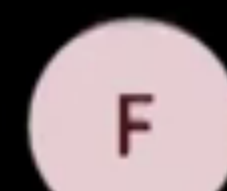
Universality of networks



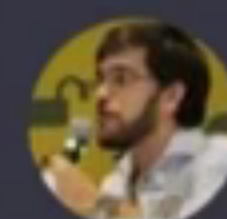
Higher level properties of
interaction networks
emerge largely from their
topological structure
regardless of the **identity**
of the nodes



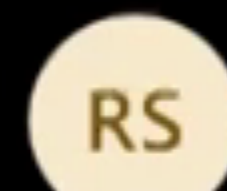
Deanna Shea



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Da Camara Ribeiro...



Raman Seshika (...)



Bo Zeng

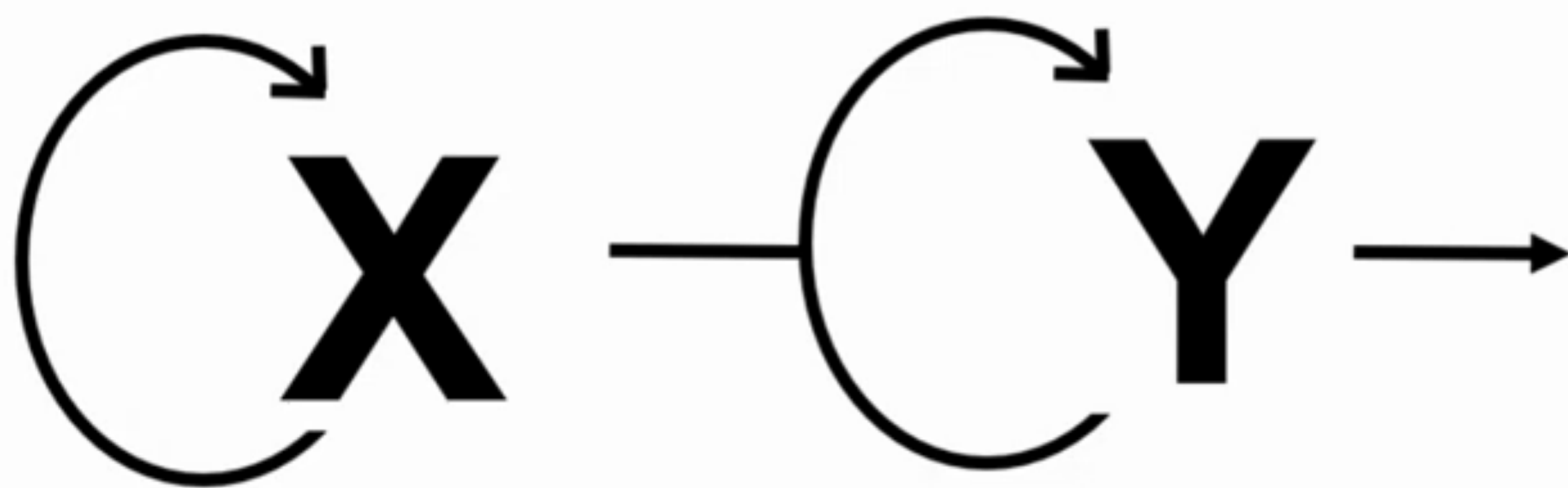
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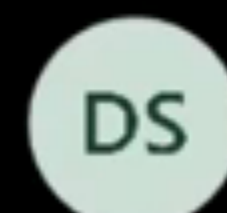
Lotka-Volterra equations

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

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



$$\begin{aligned}\dot{X} &= aX - \\ \dot{Y} &= cXY\end{aligned}$$



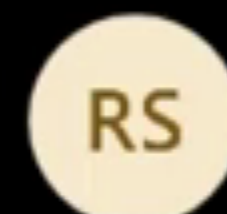
Deanna Shea



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Da Camara Ribeir...



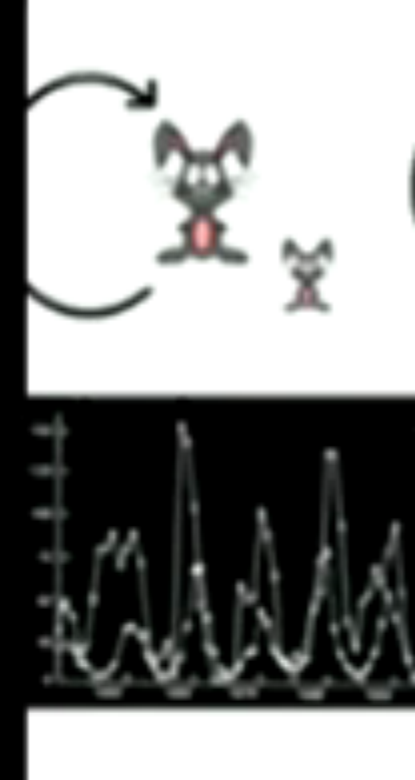
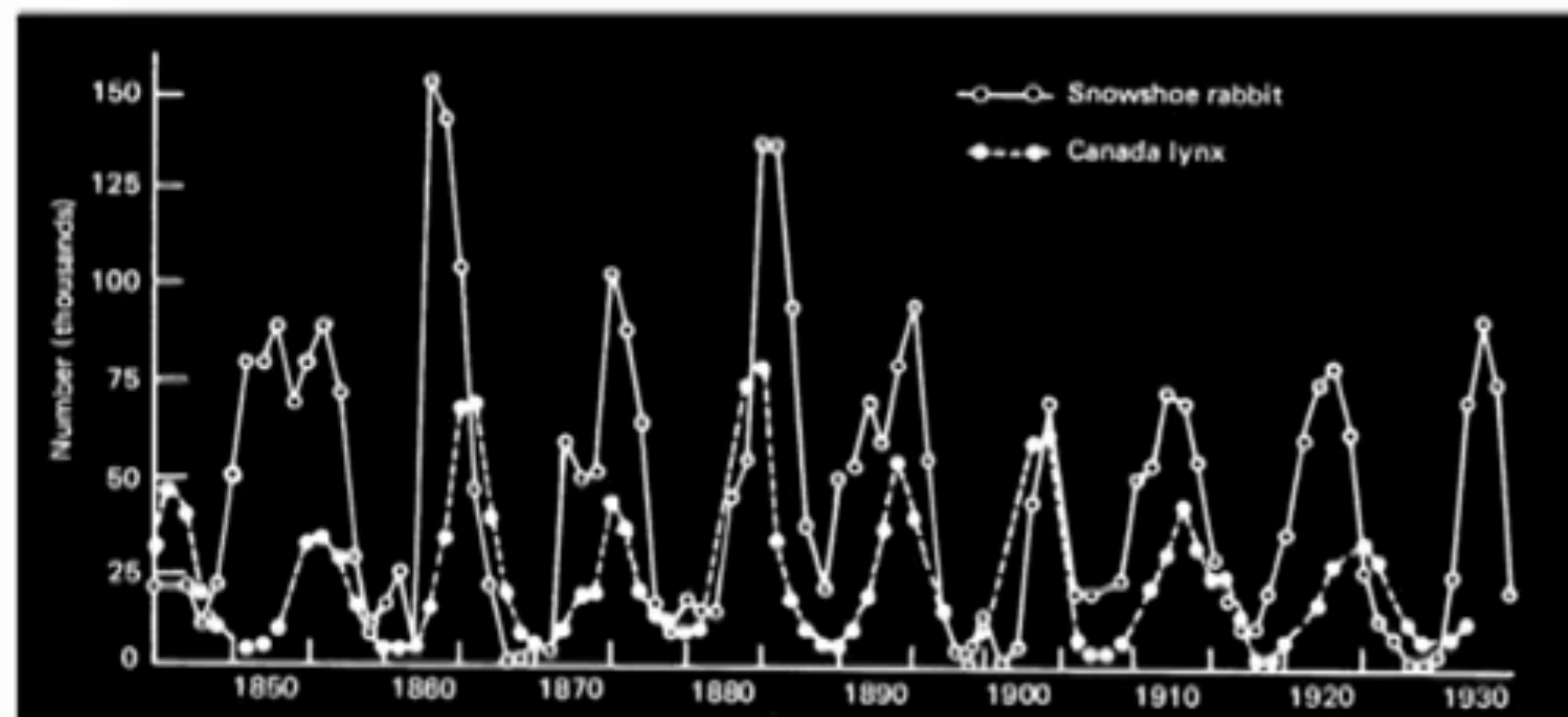
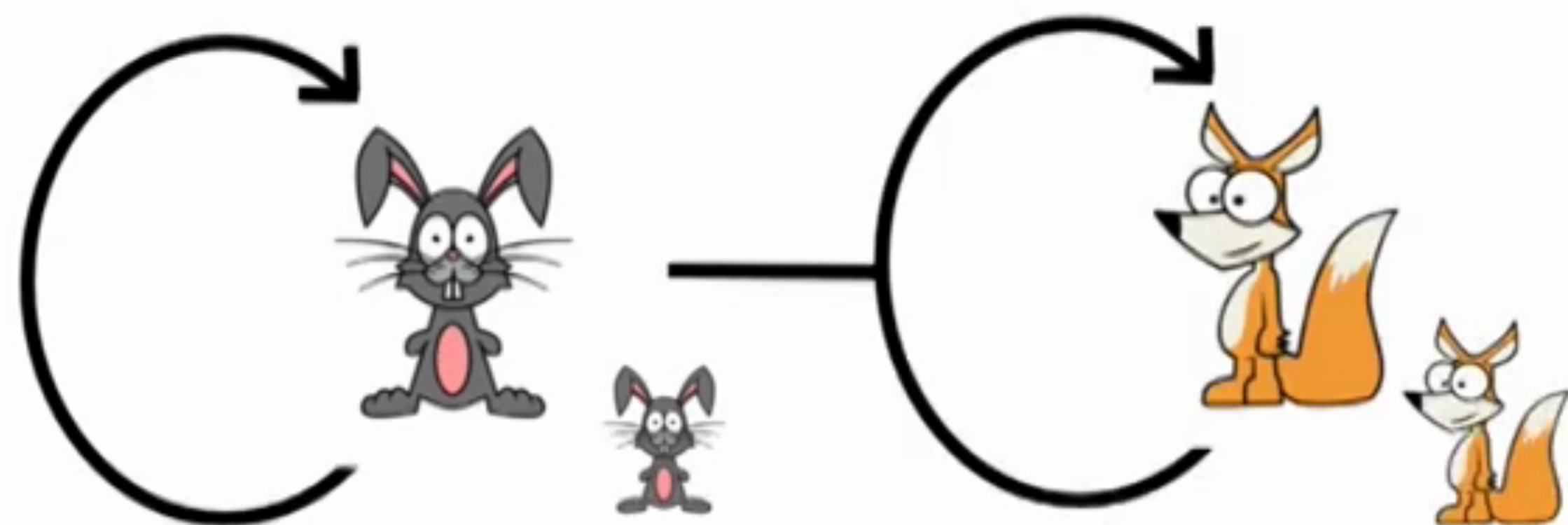
Raman Sheshka (...)



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Lotka-Volterra equations



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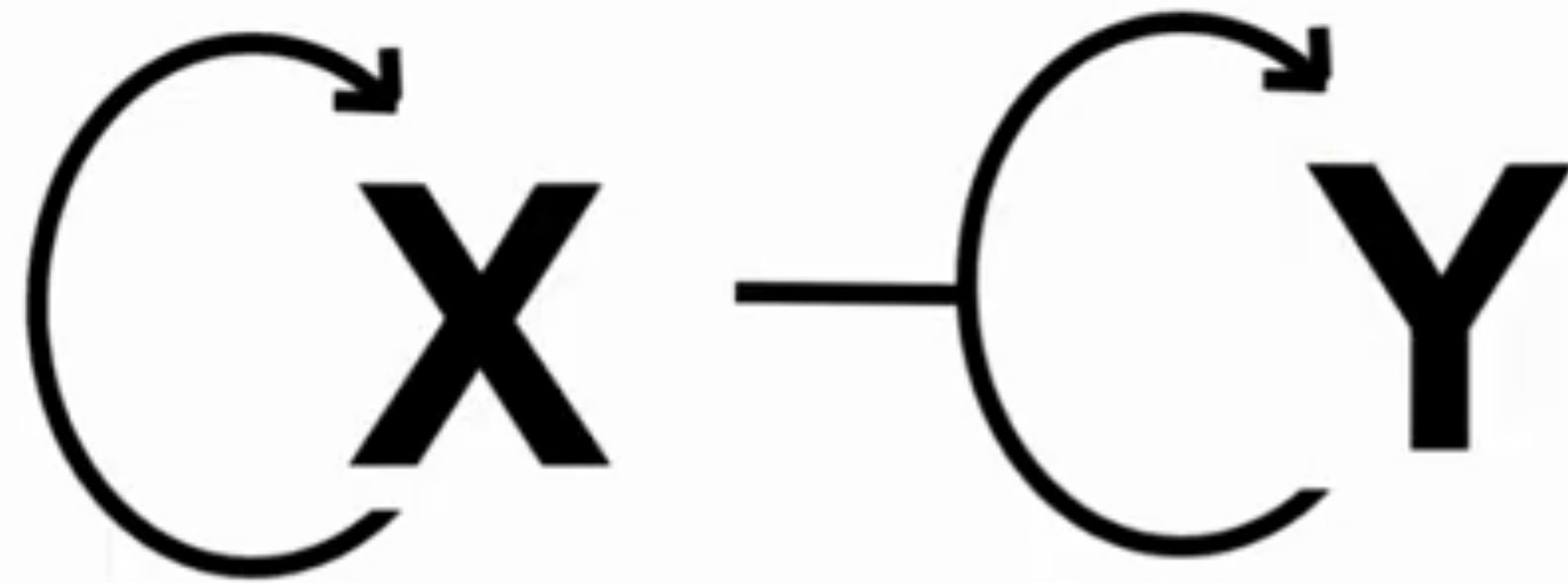
Raman Sreshka (...)

PC

Go to the...

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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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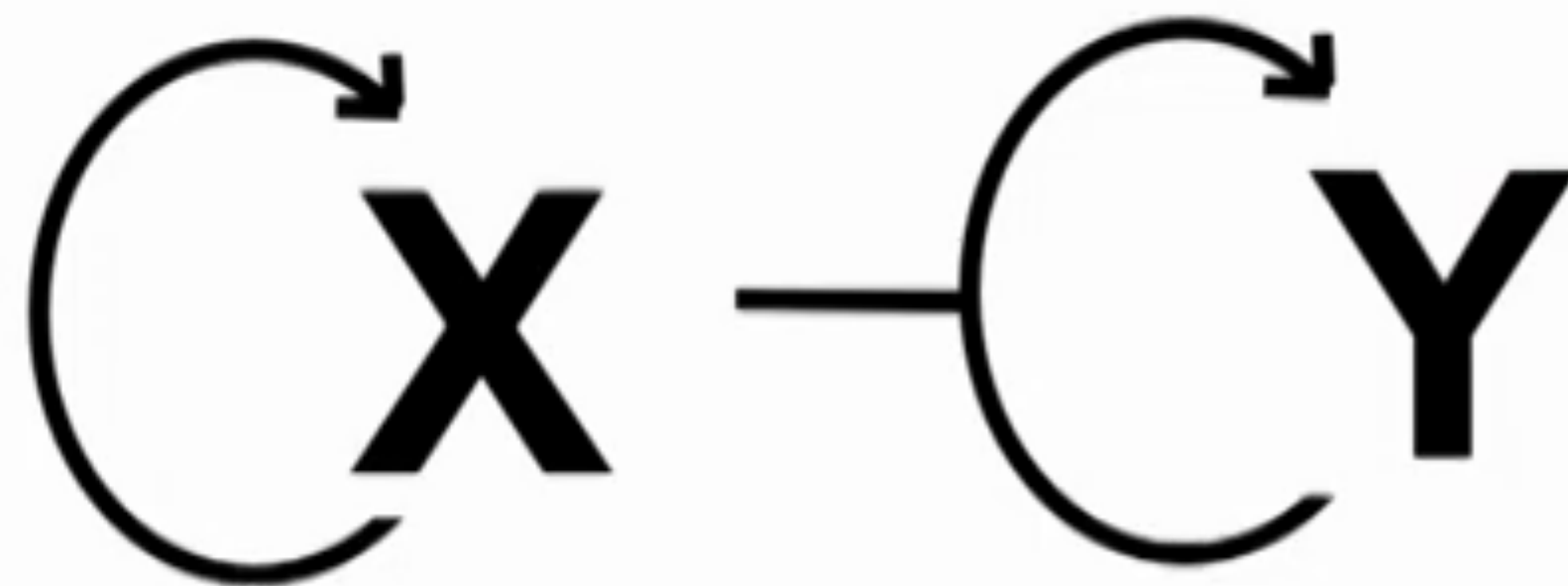
Raman Sheshka (...)

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Lotka-Volterra equations

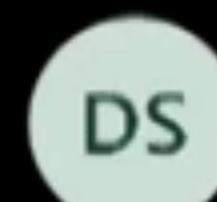


Gen Relativ Gravit (2014) 46:1753
DOI 10.1007/s10714-014-1753-8

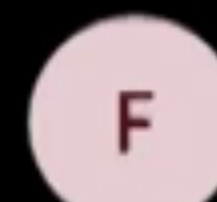
Jérôme Perez · André Füzfa · Timoteo Carletti ·
Laurence Mélot · Lazare Guedeounme

RESEARCH ARTICLE

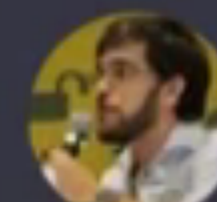
**The Jungle Universe: coupled cosmological models
in a Lotka–Volterra framework**



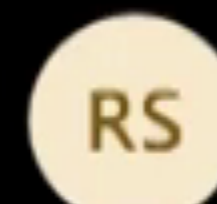
Deanna Shea



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Da Camara Ribel...



Raman Shethka (...)



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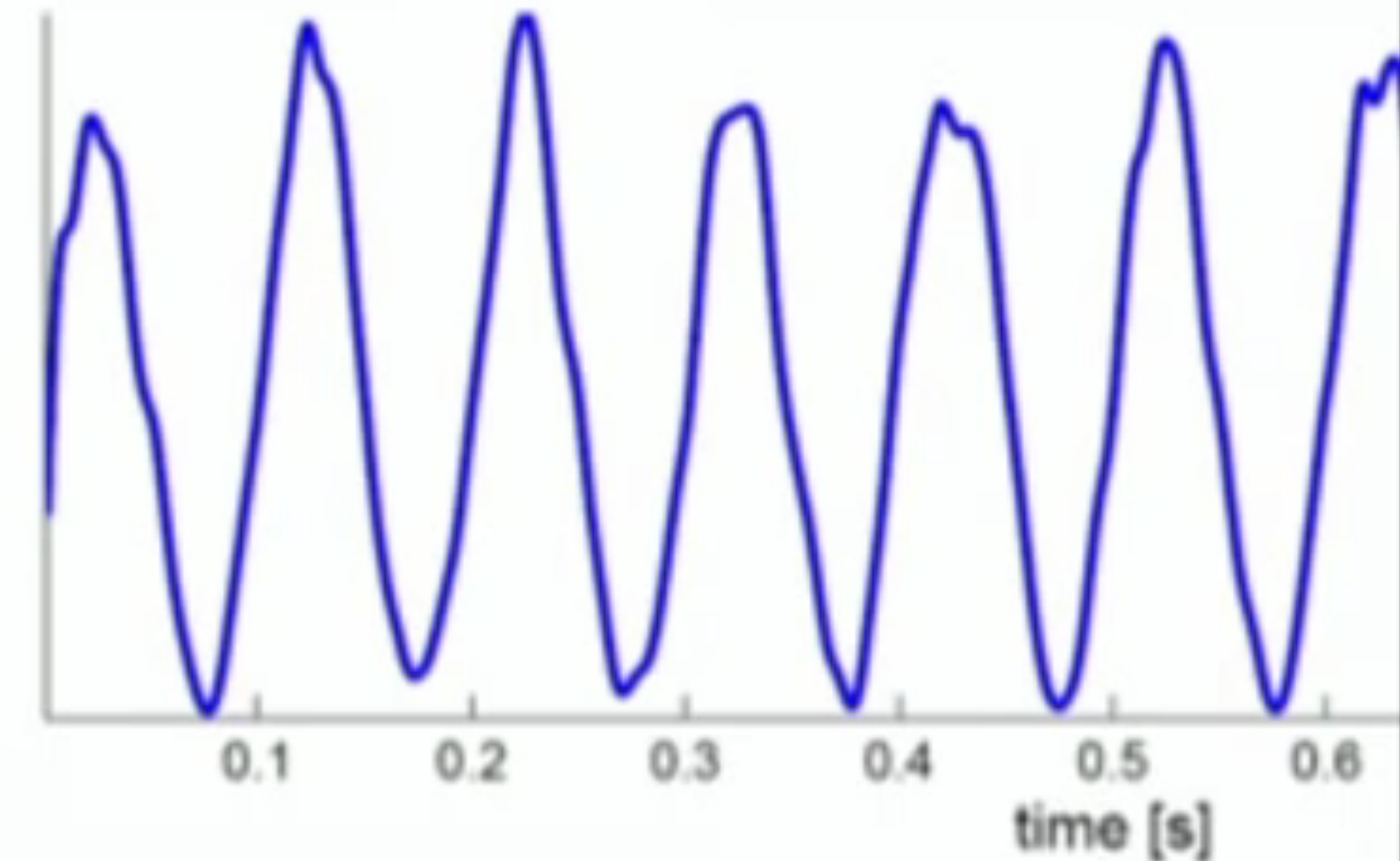
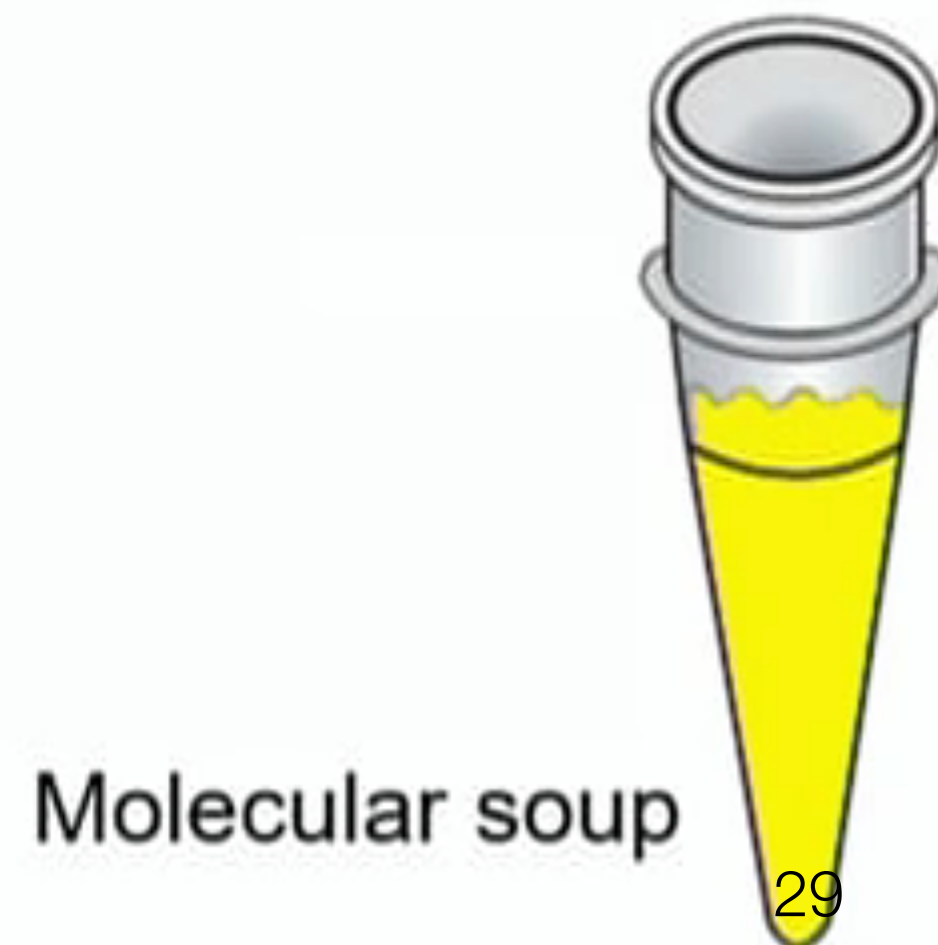


Lotka-Volterra systems in chemistry?

Alfred Lotka original suggestion concerned chemical kinetics:

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

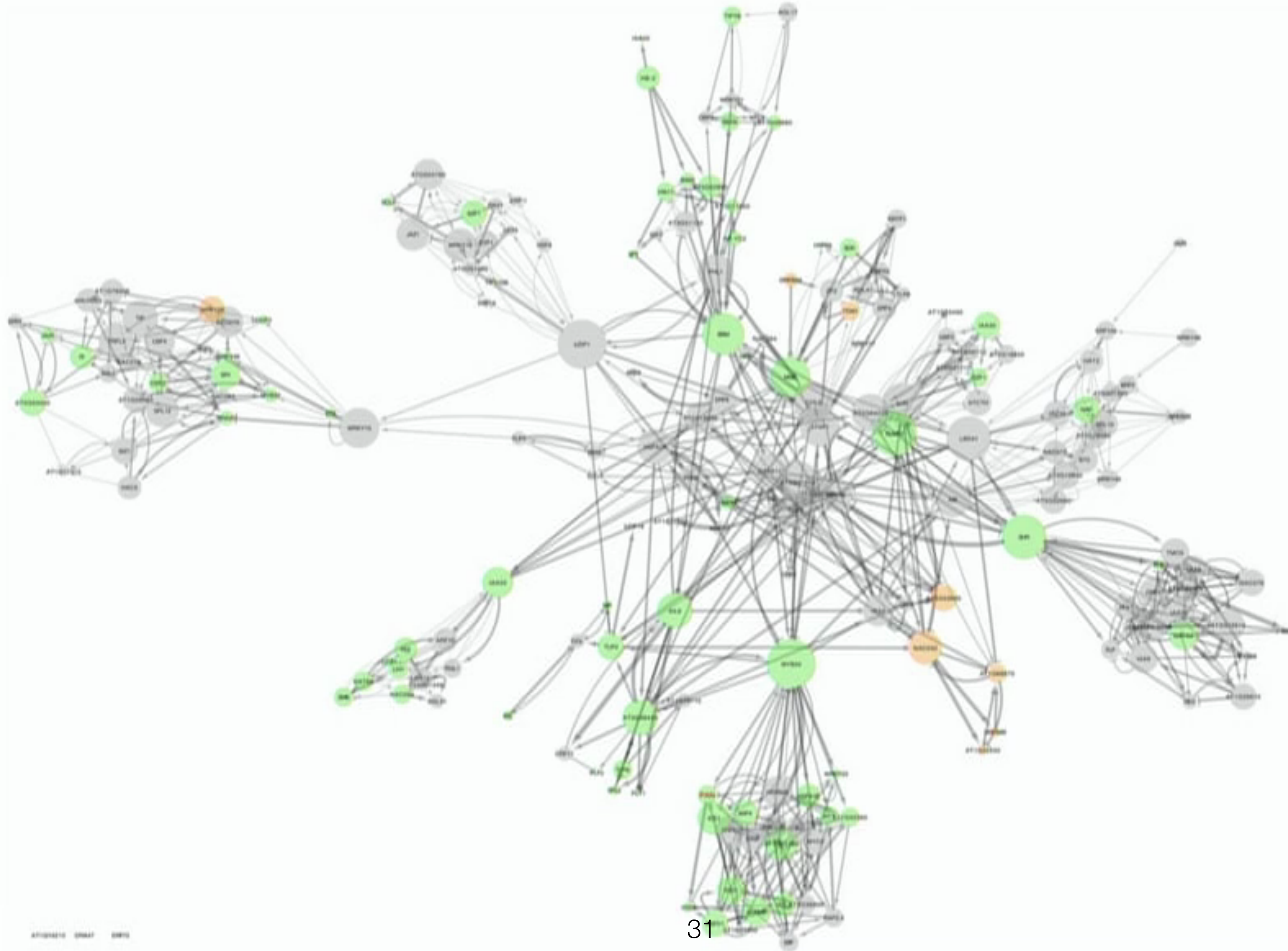
Molecular "prey"
Molecular "predator"



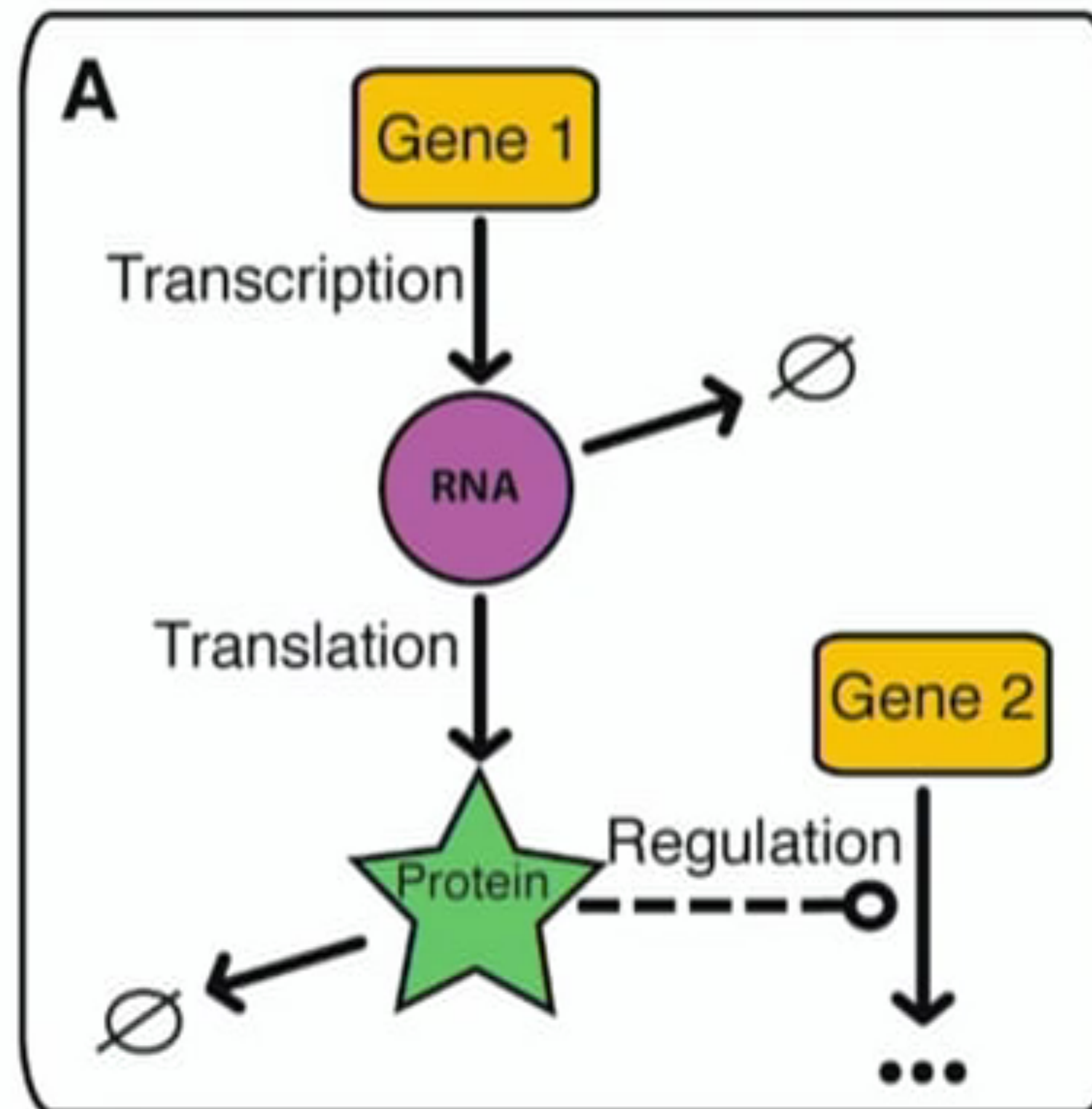
Various flavors of networks exist within cells

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

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

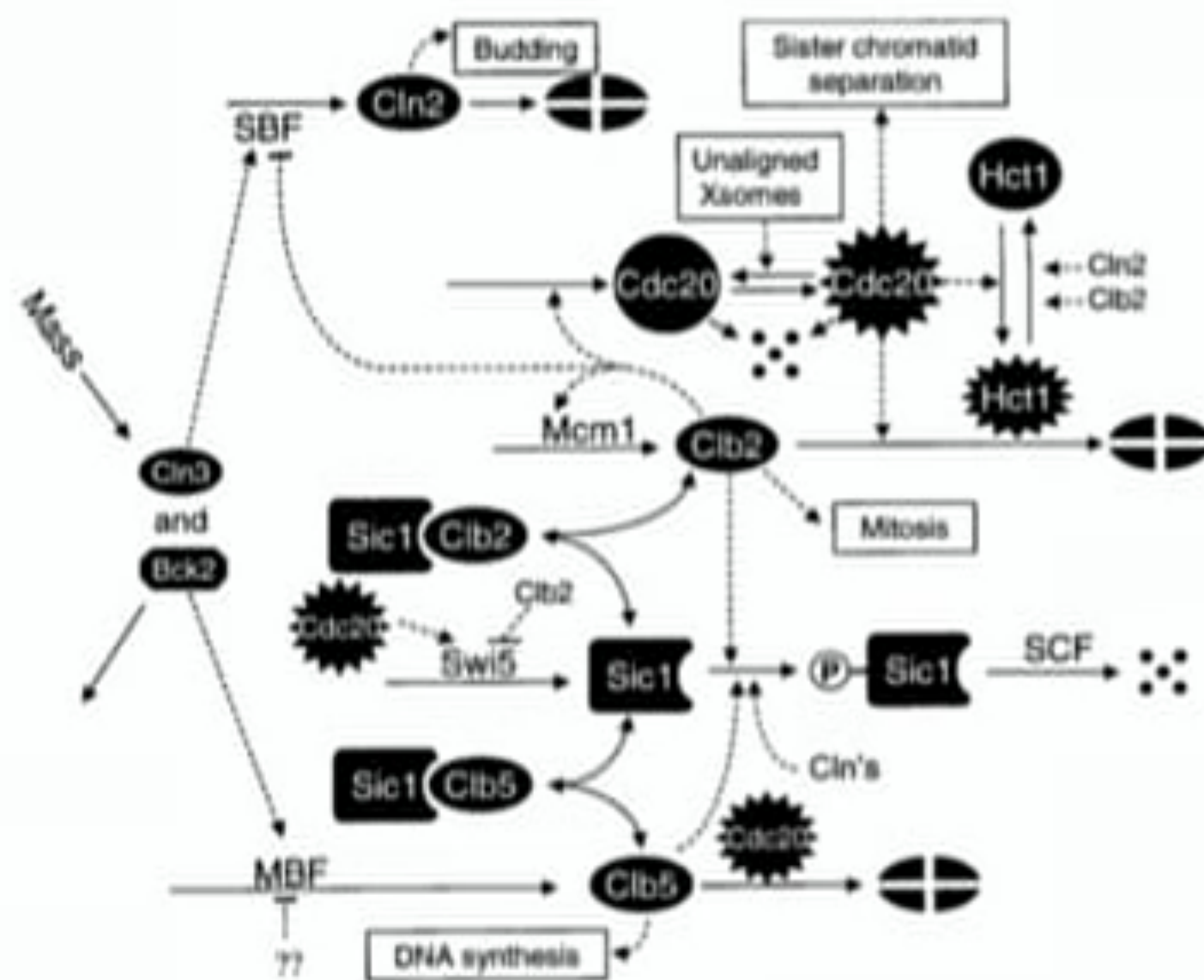
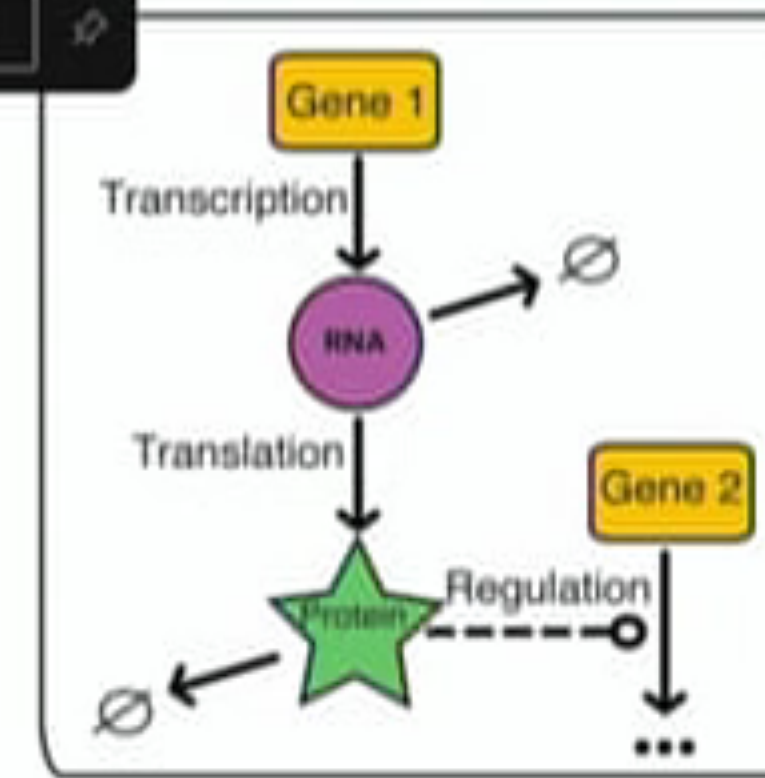


Gene regulatory networks: A modular way to build reaction networks, in vivo, using the protein expression machinery



- ✓ 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

KINETICS (laws & rates)

DIFFUSION RATES

BOUNDARY CONDITIONS

~~CHEMICALS~~

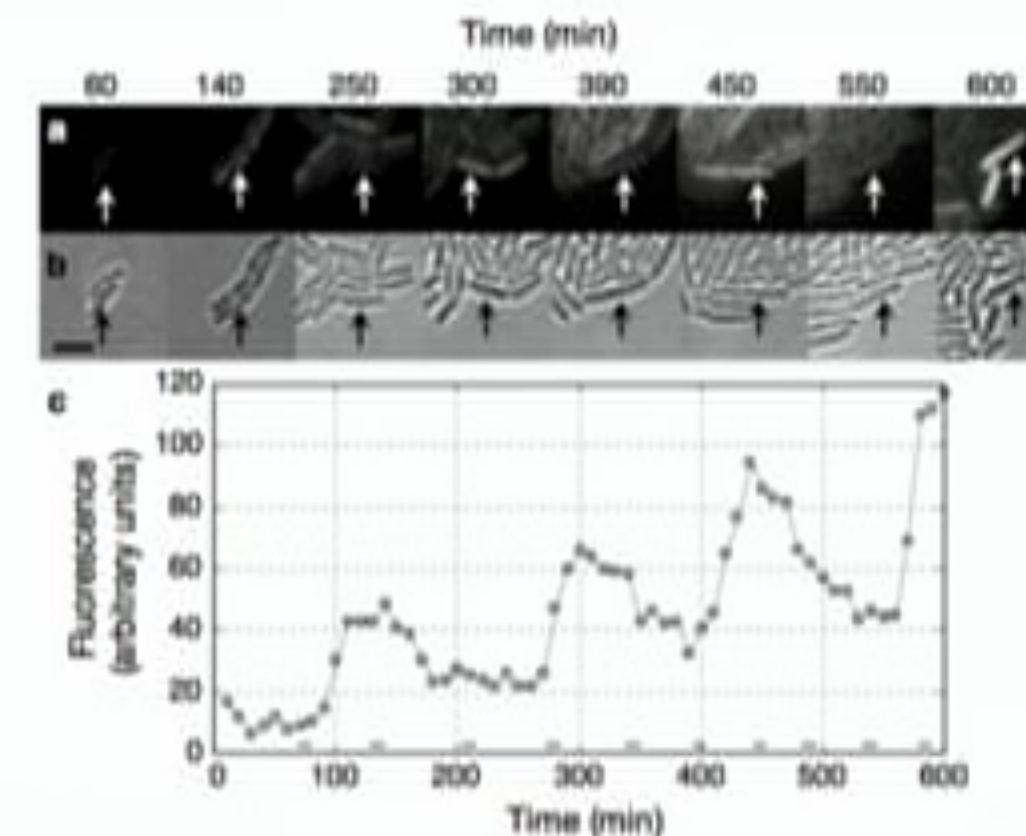
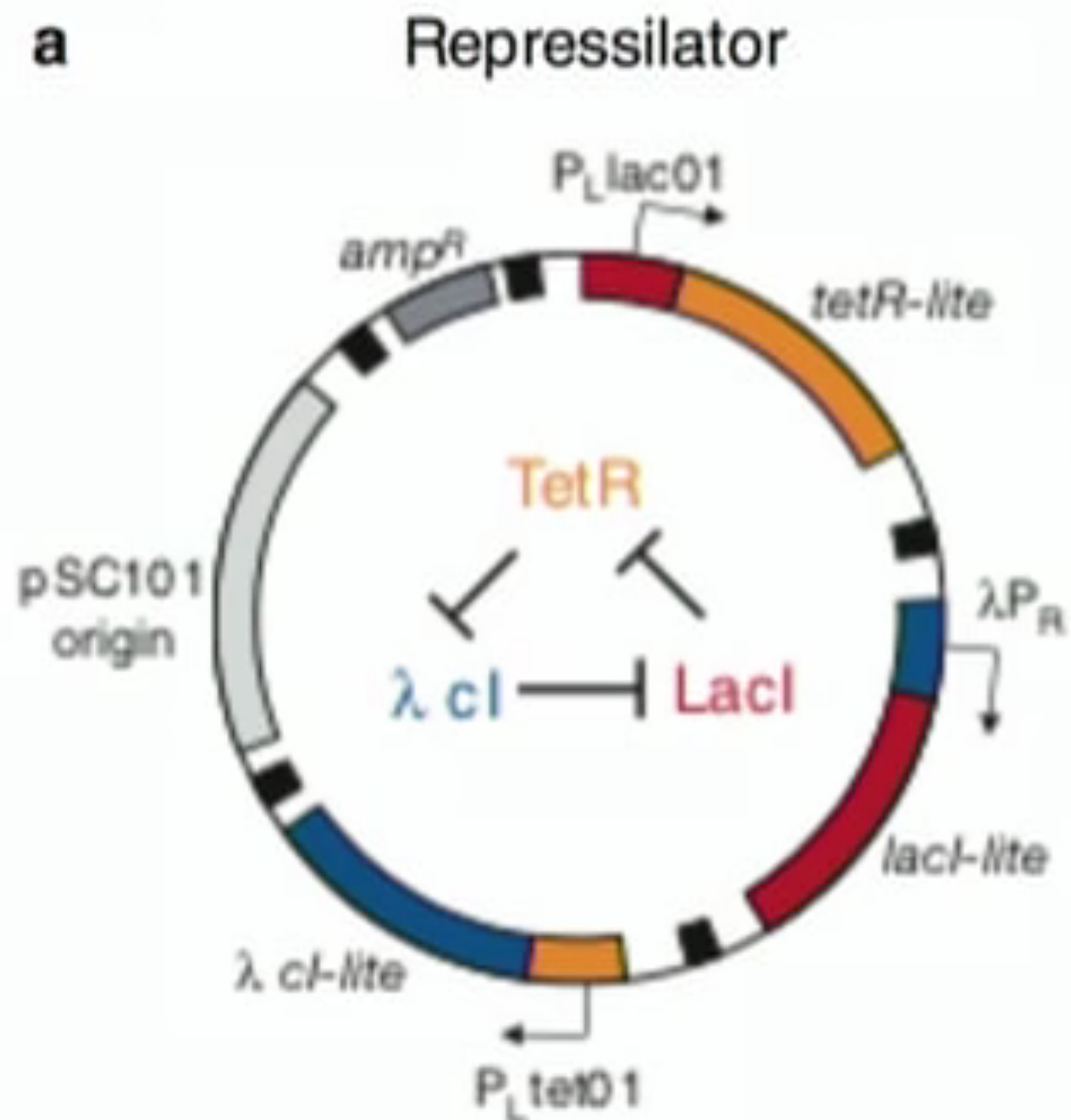
In the case of spatially distributed systems

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



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 lactose

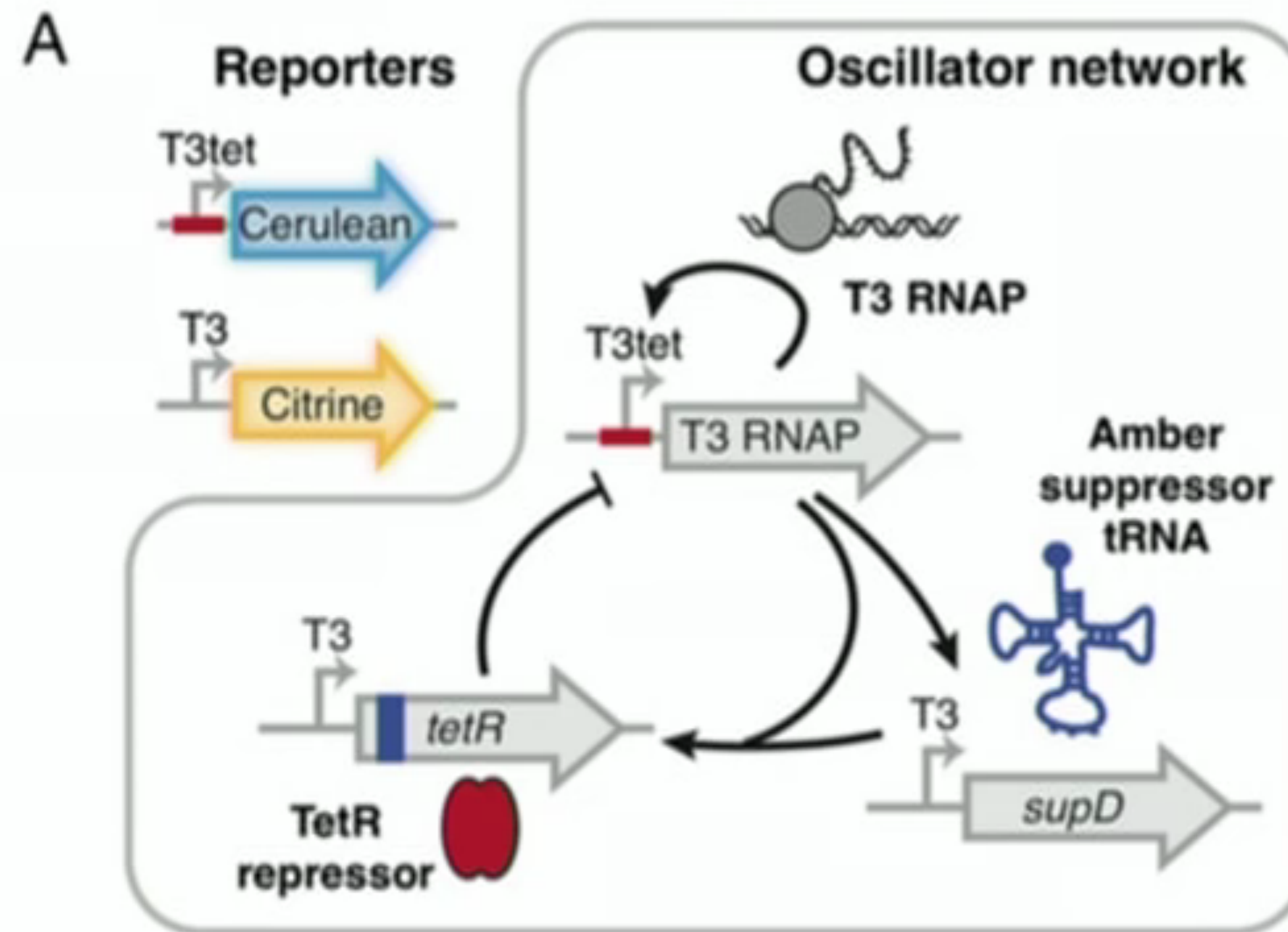
Lambda-cl is a phage regulator that controls the switching between the two life styles of a phage (a virus)

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

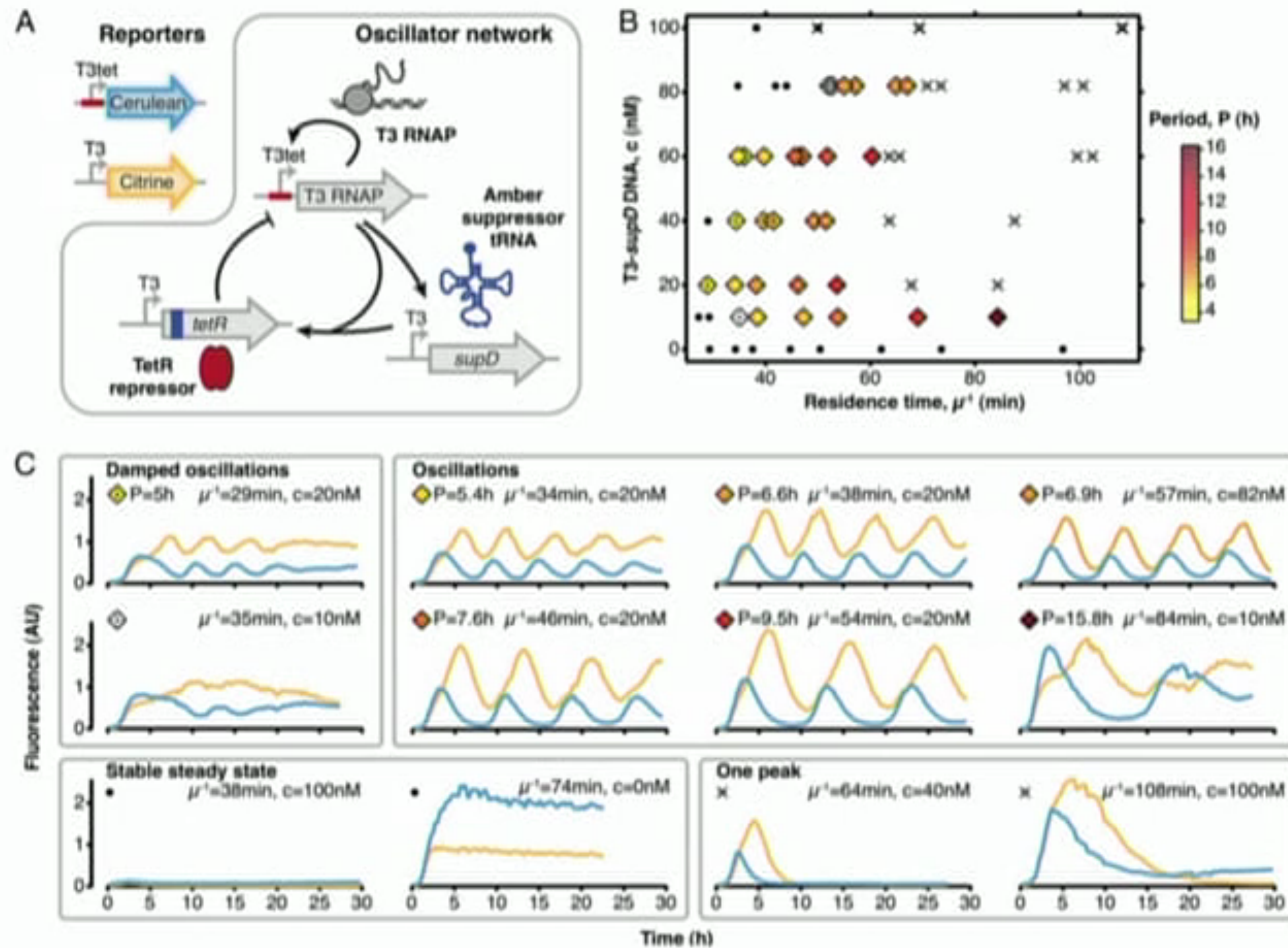
Implementation of cell-free biological networks at steady state

Henrike Niederholtmeyer, Viktoria Stepanova, and Sebastian J. Maerkl¹

PNAS 2013



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



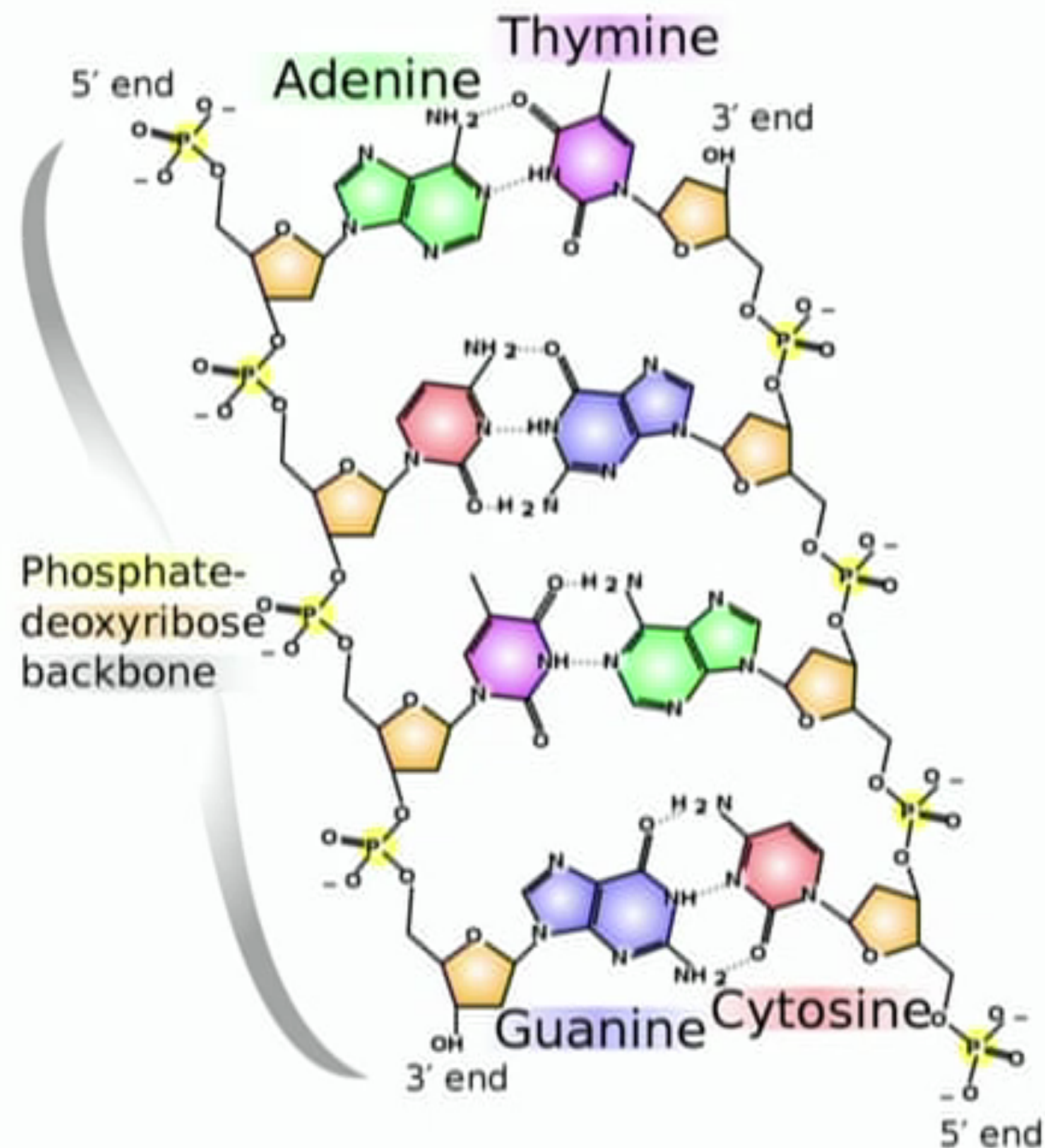
Implementation of cell-free biological networks at steady state

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

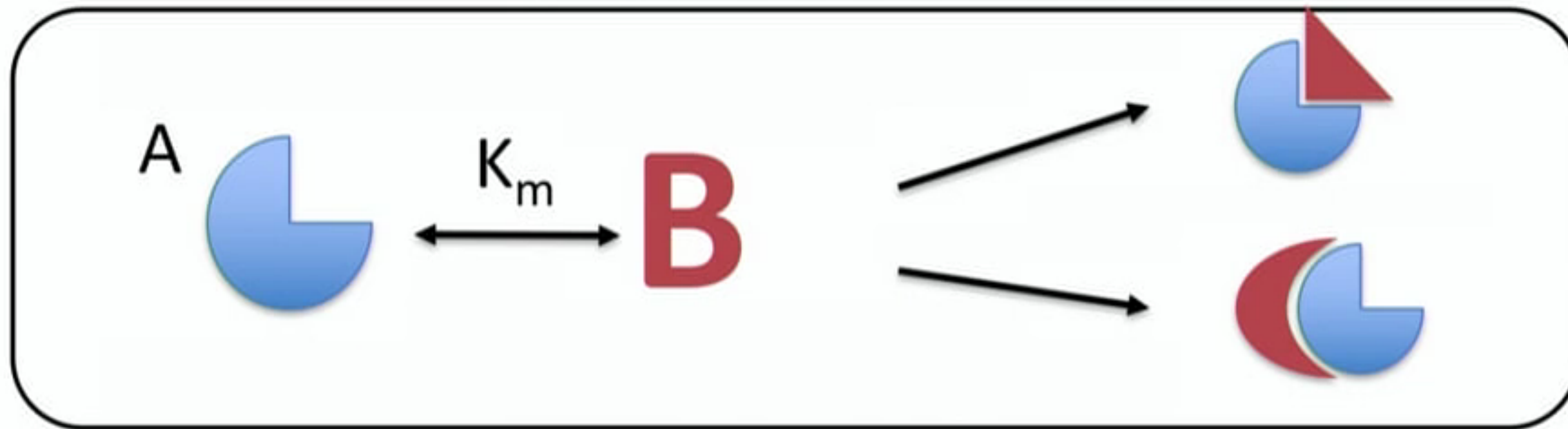
As models of cellular networks

For a chemistry of information processing

DNA as a *synthetic* material for molecular circuits



DNA as a *synthetic* material for molecular circuits



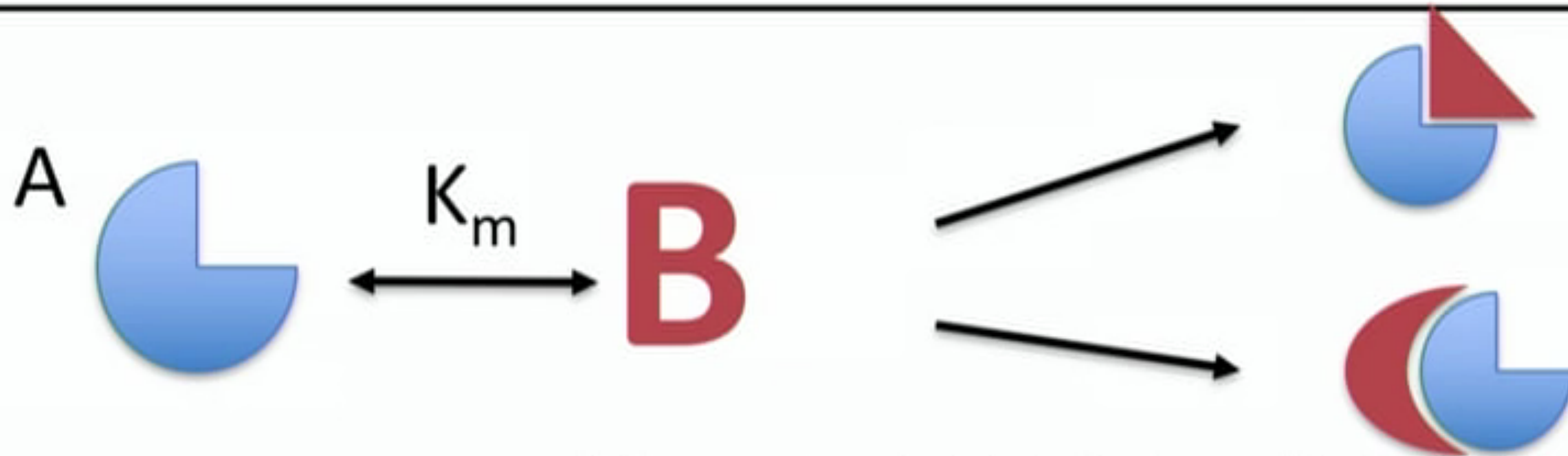
DNA as a *synthetic* material for molecular circuits



"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

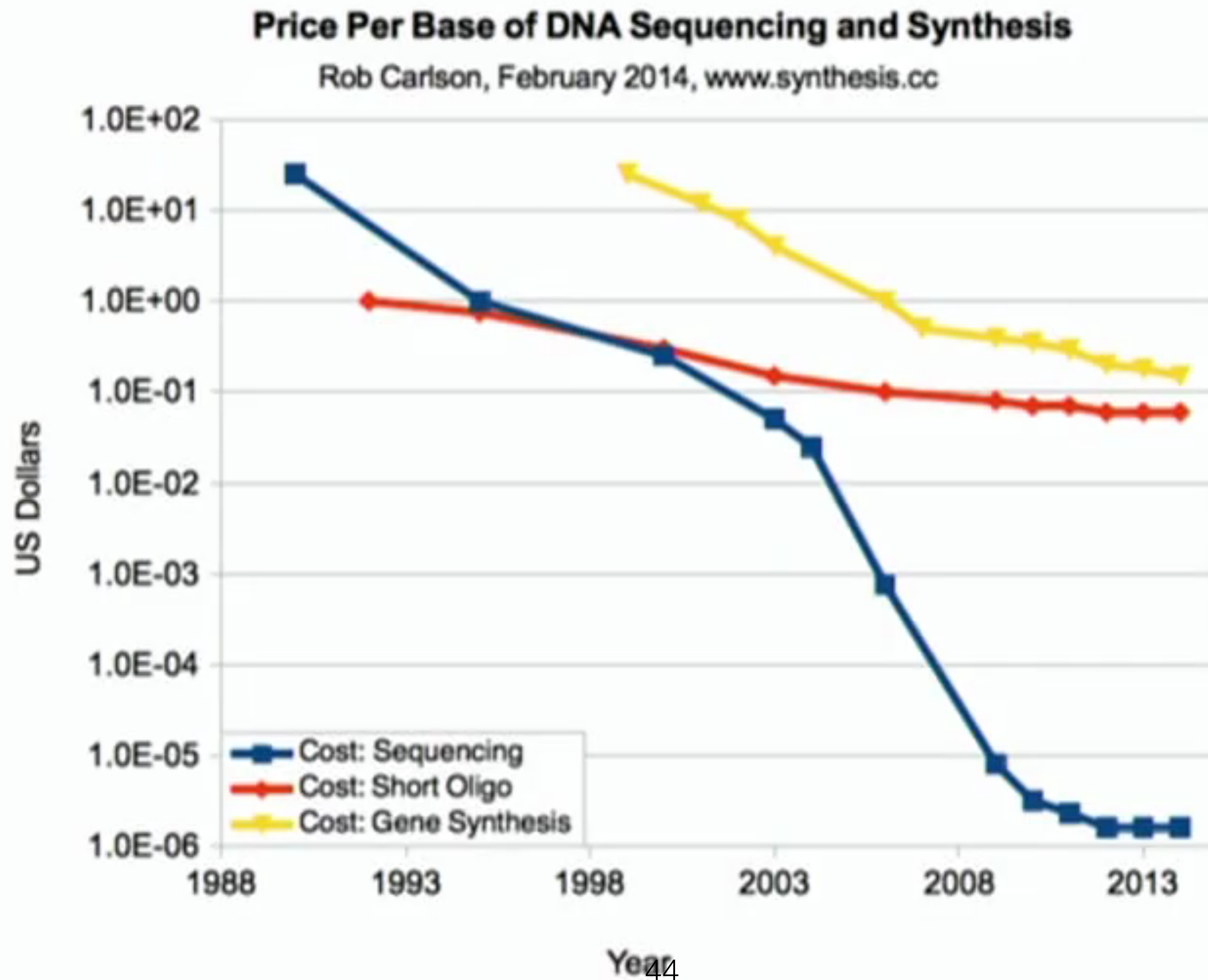
Enzymes:

Copy: Polymerases

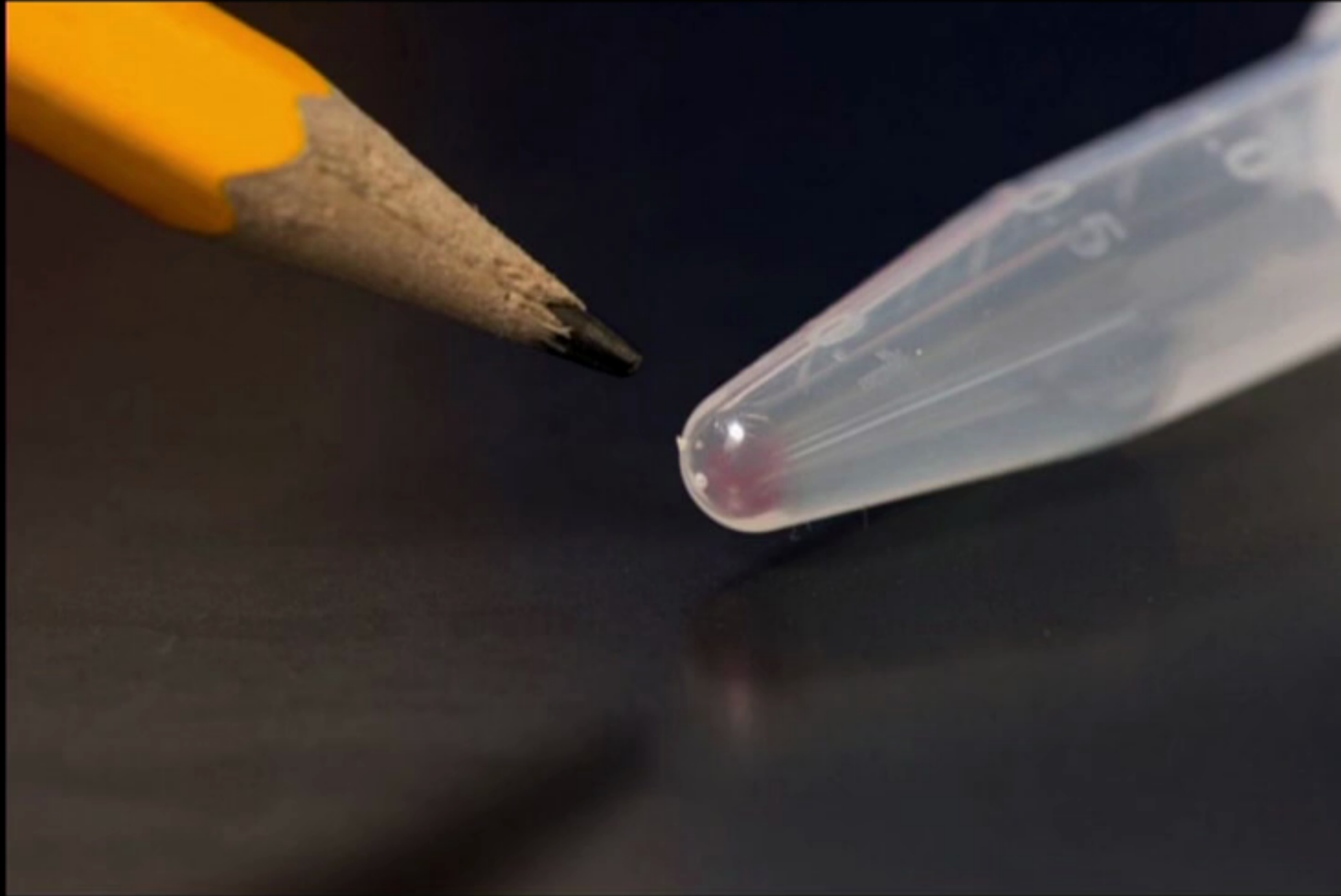
Cut-paste: Restriction enzymes and Ligases

Delete: Nucleases

Low cost DNA synthesis



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

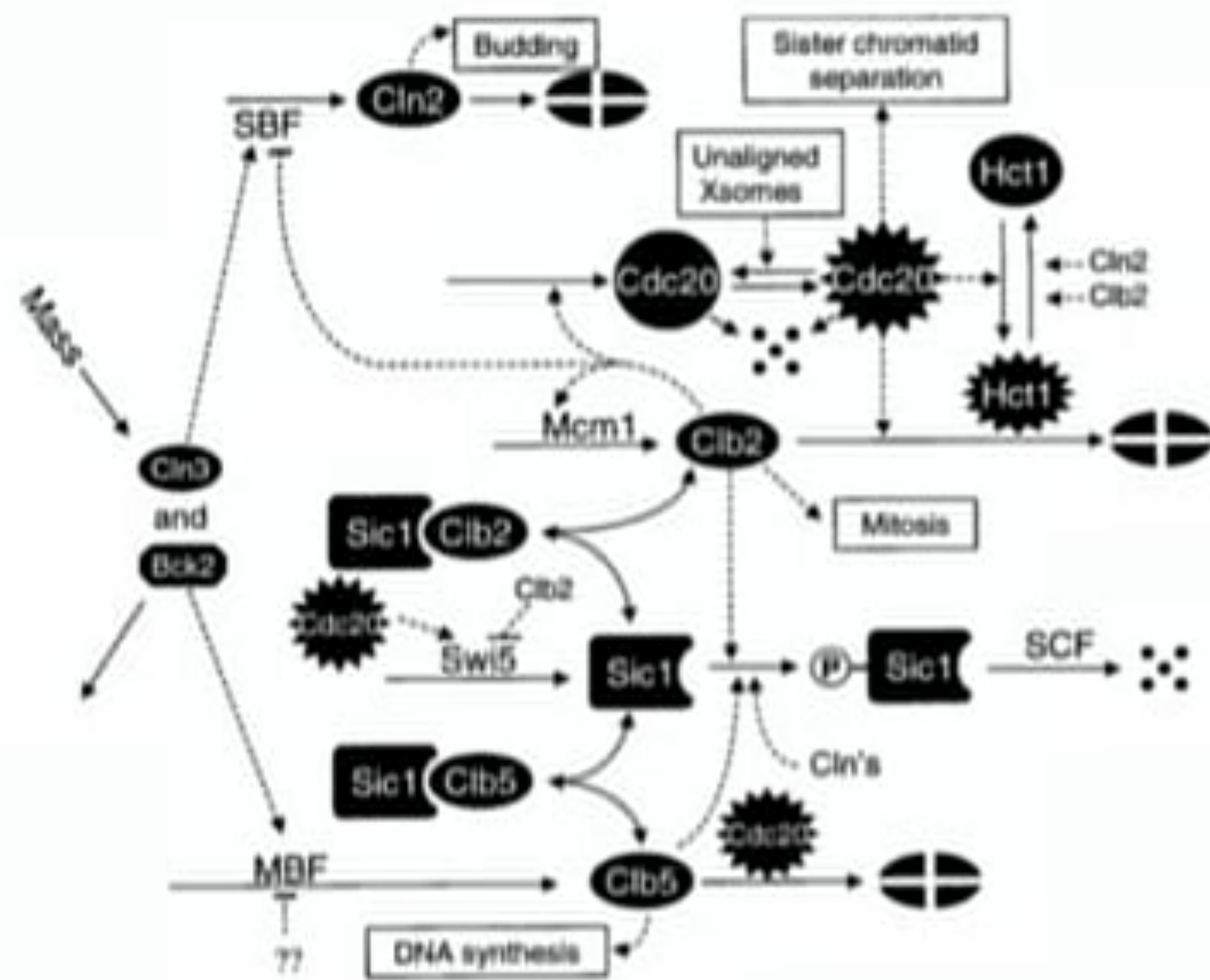


(OK go video) Microsoft, U. Washington, Twist

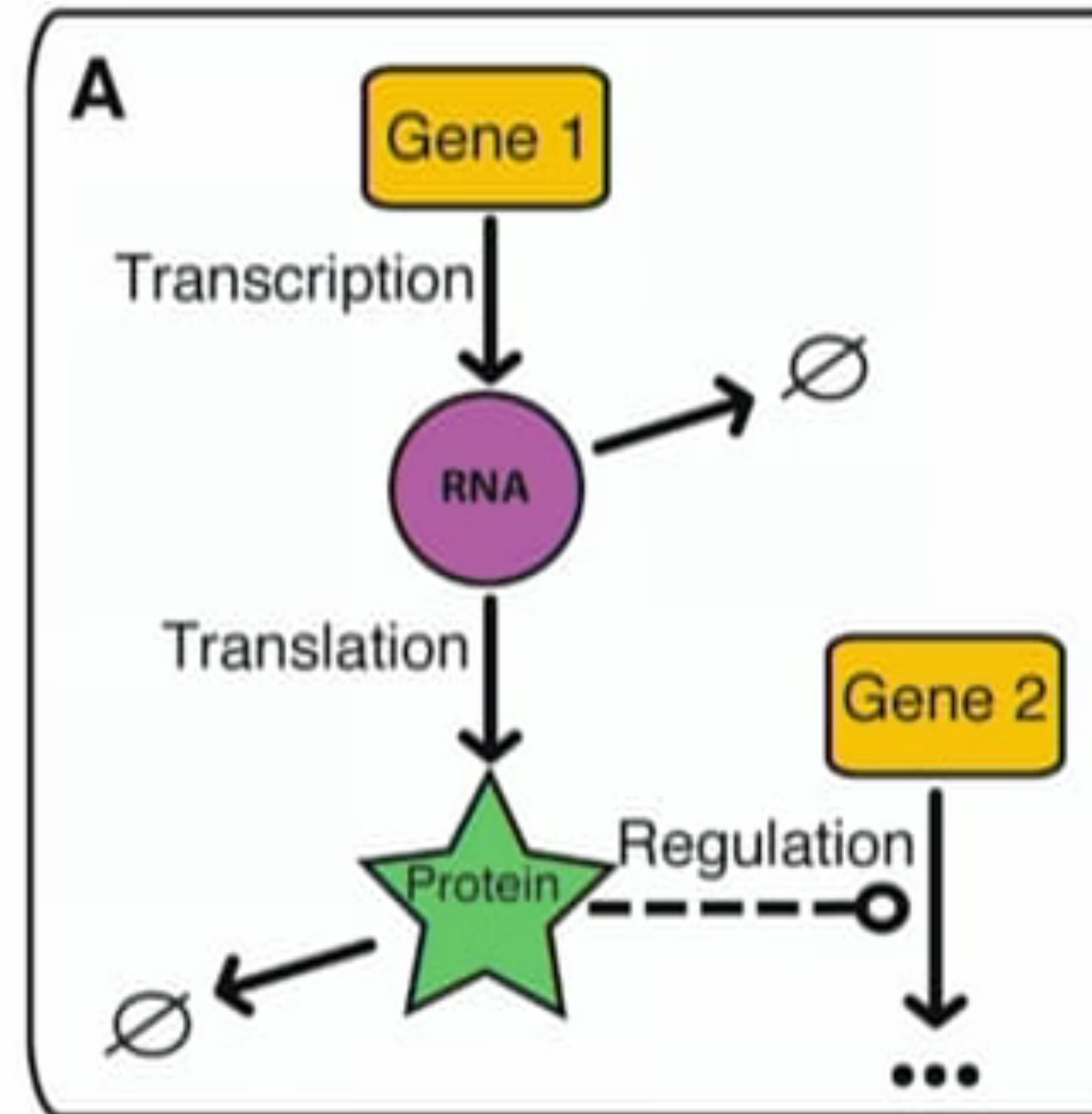


Molecular networking approaches

Simplified models of biological regulatory networks

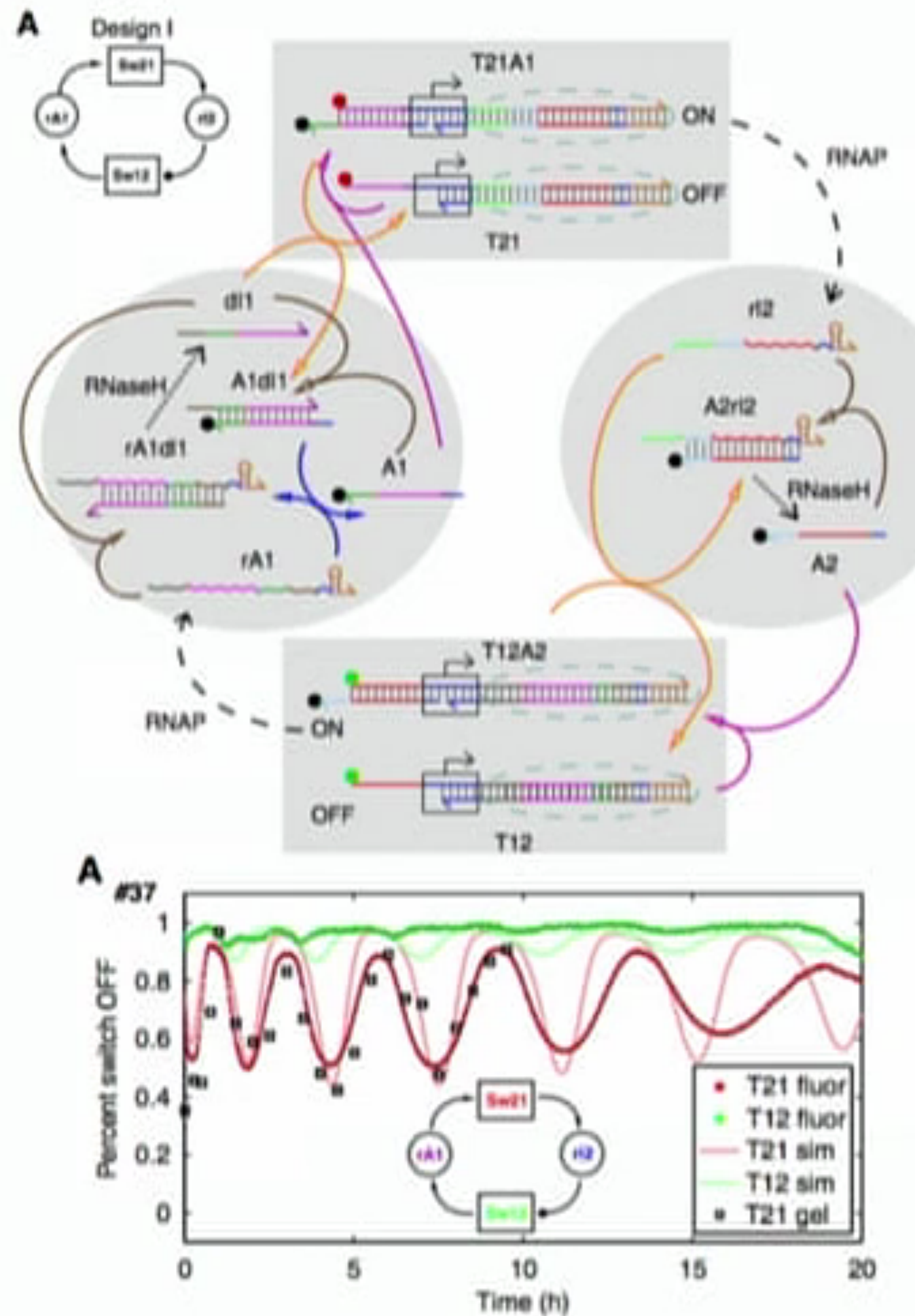


Regulatory network of the budding yeast cell cycle (Tyson 2006)

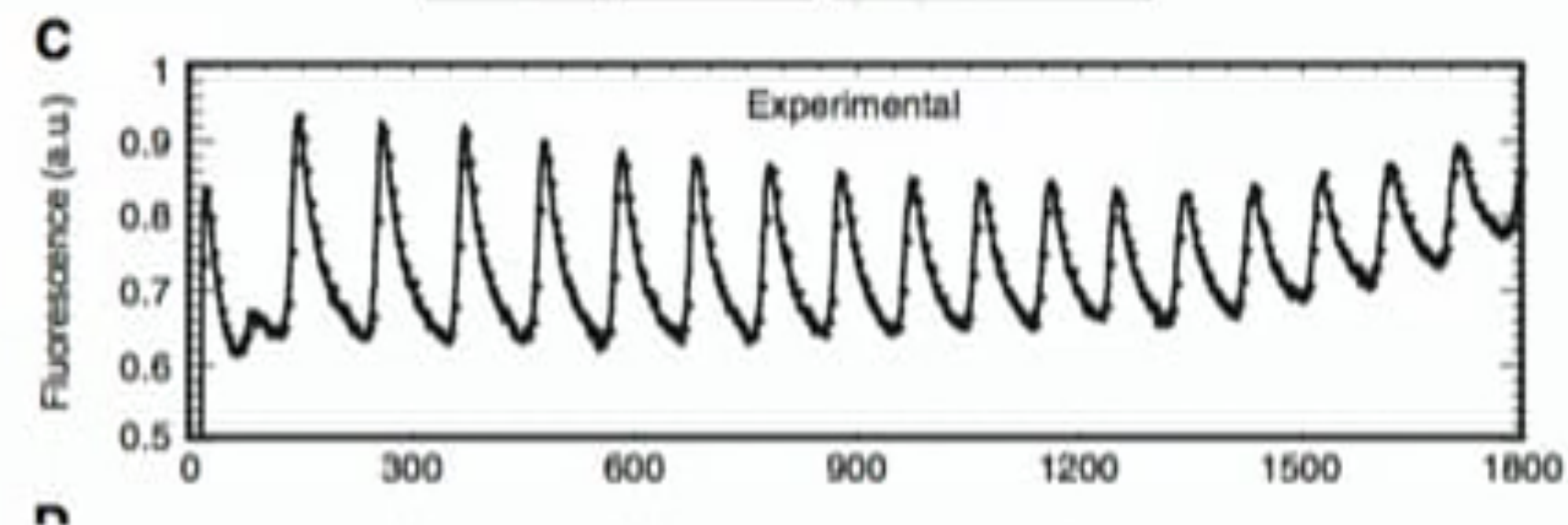
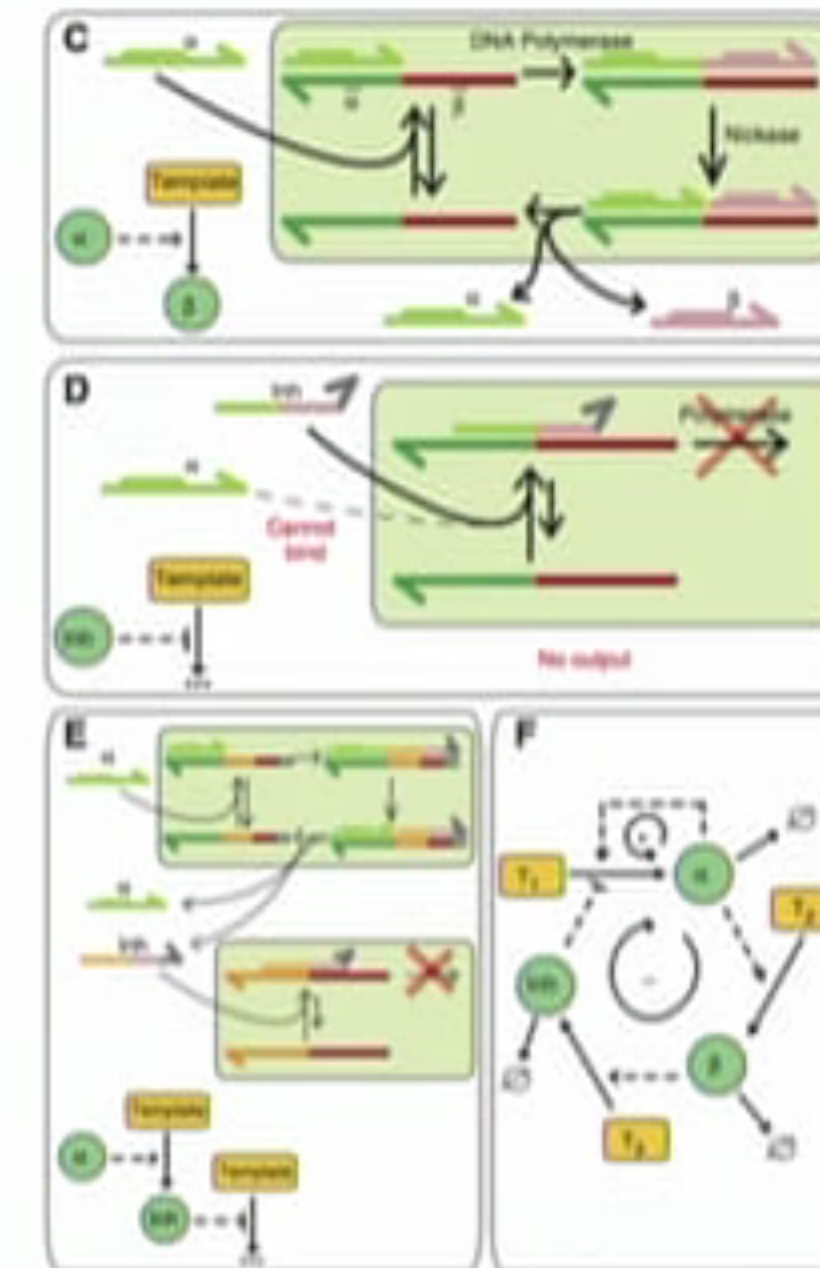


- We want to maintain the modularity and cascadability
- But simplify the chemistry as much as possible

Artificial equivalent of regulatory networks



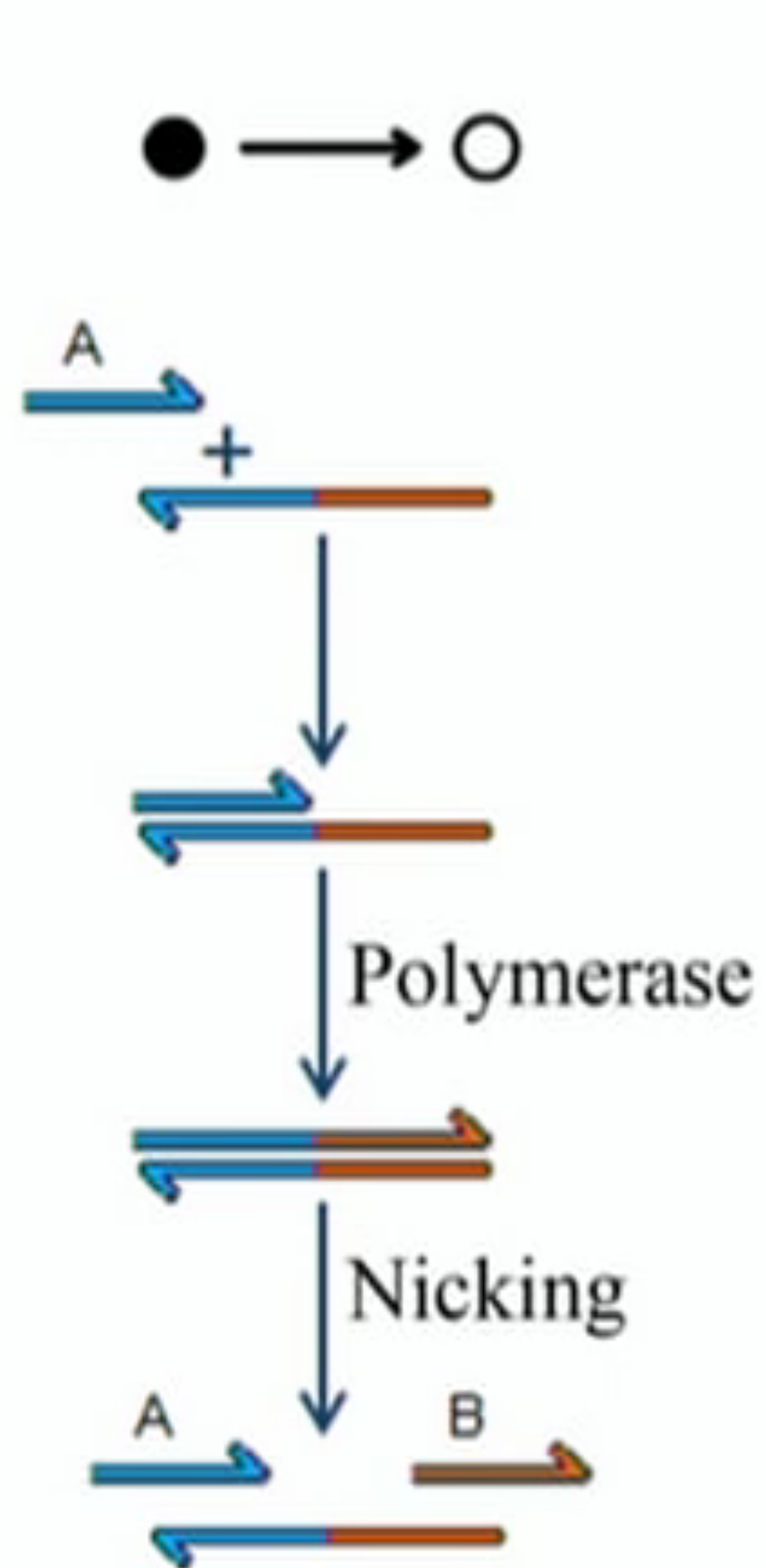
“genelets” Kim&Winfree MSB 2011



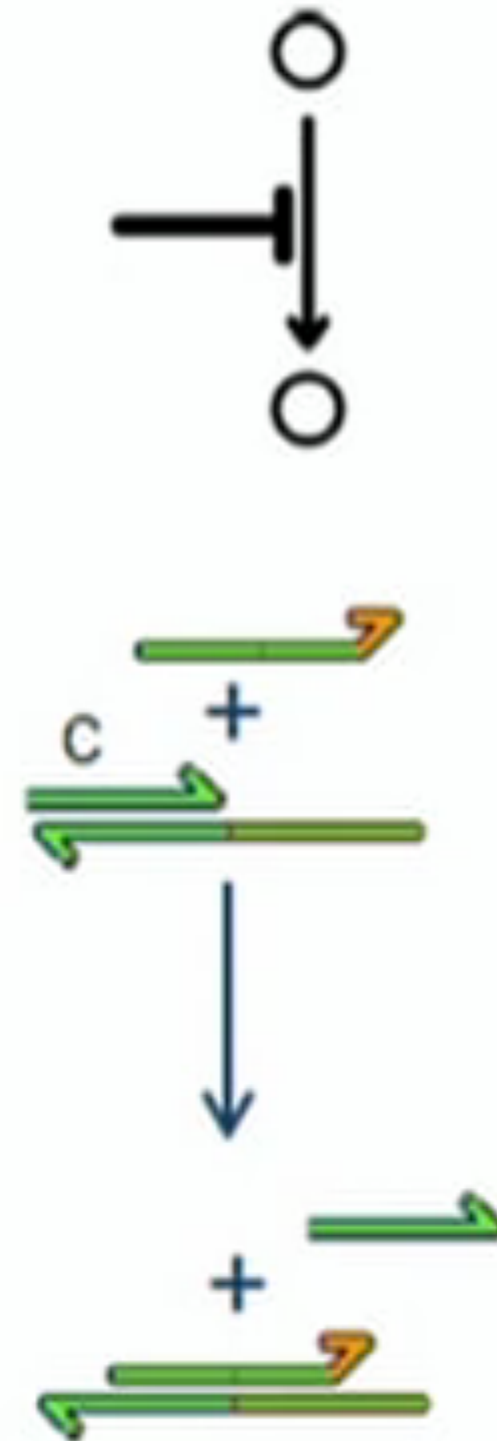
“PEN toolbox” Montagne&Rondelez MSB 2011

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

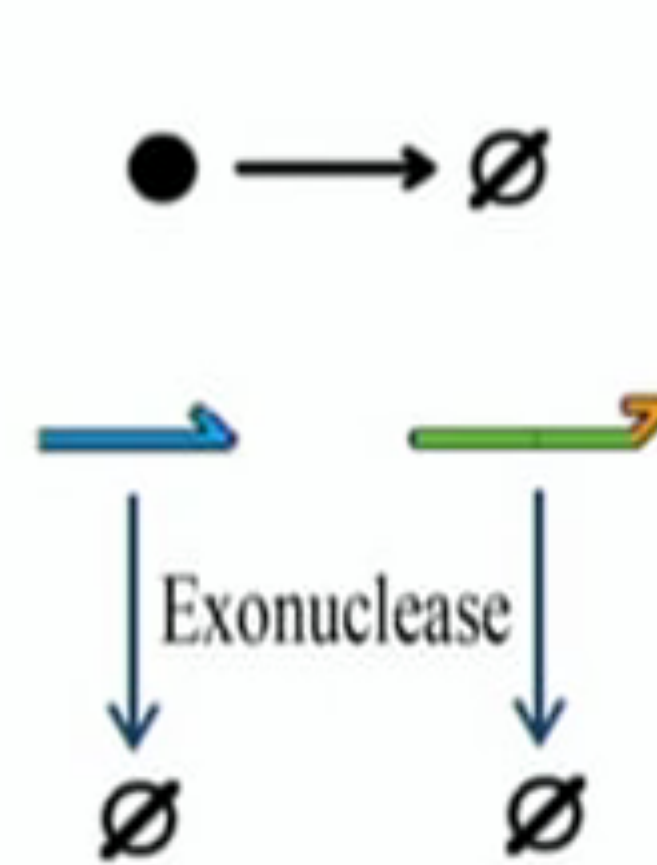
(a) Activation



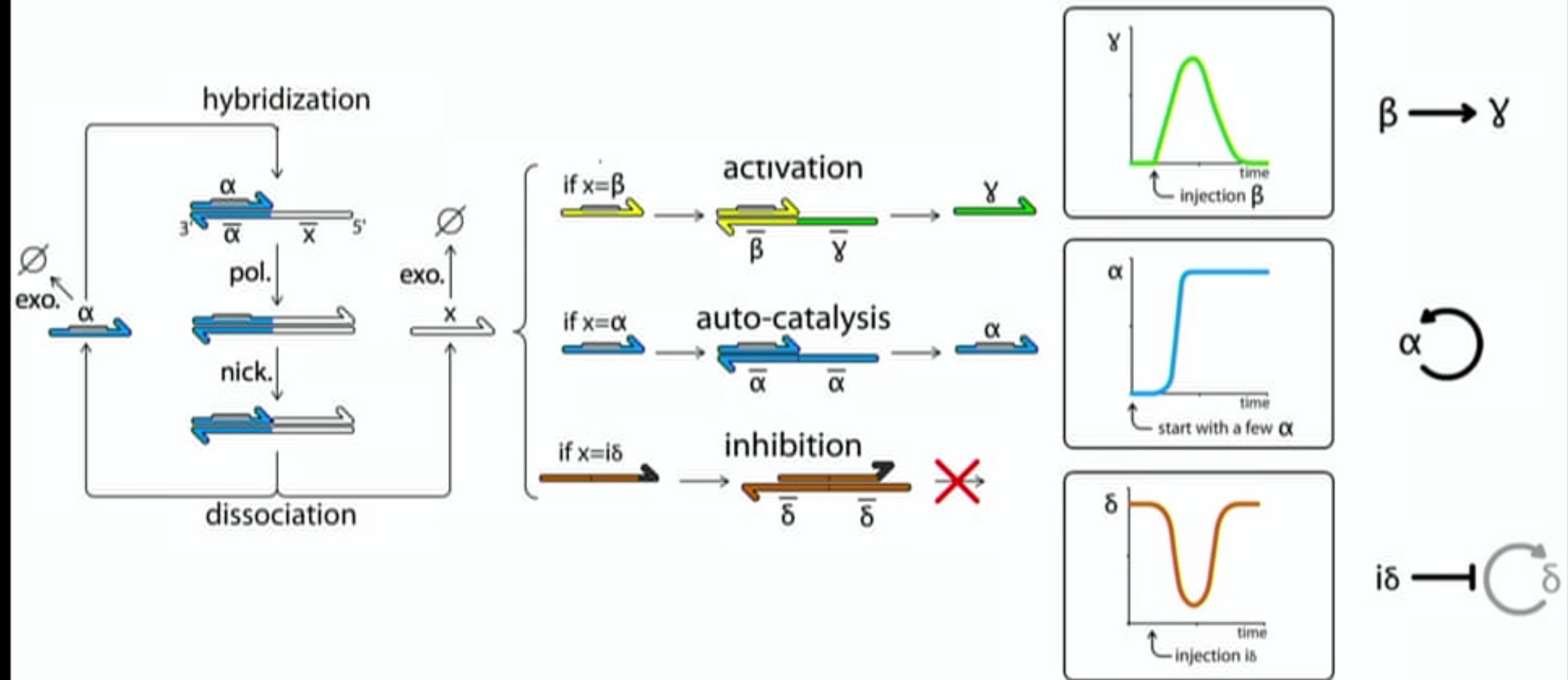
(b) Inhibition



(c) Degradation



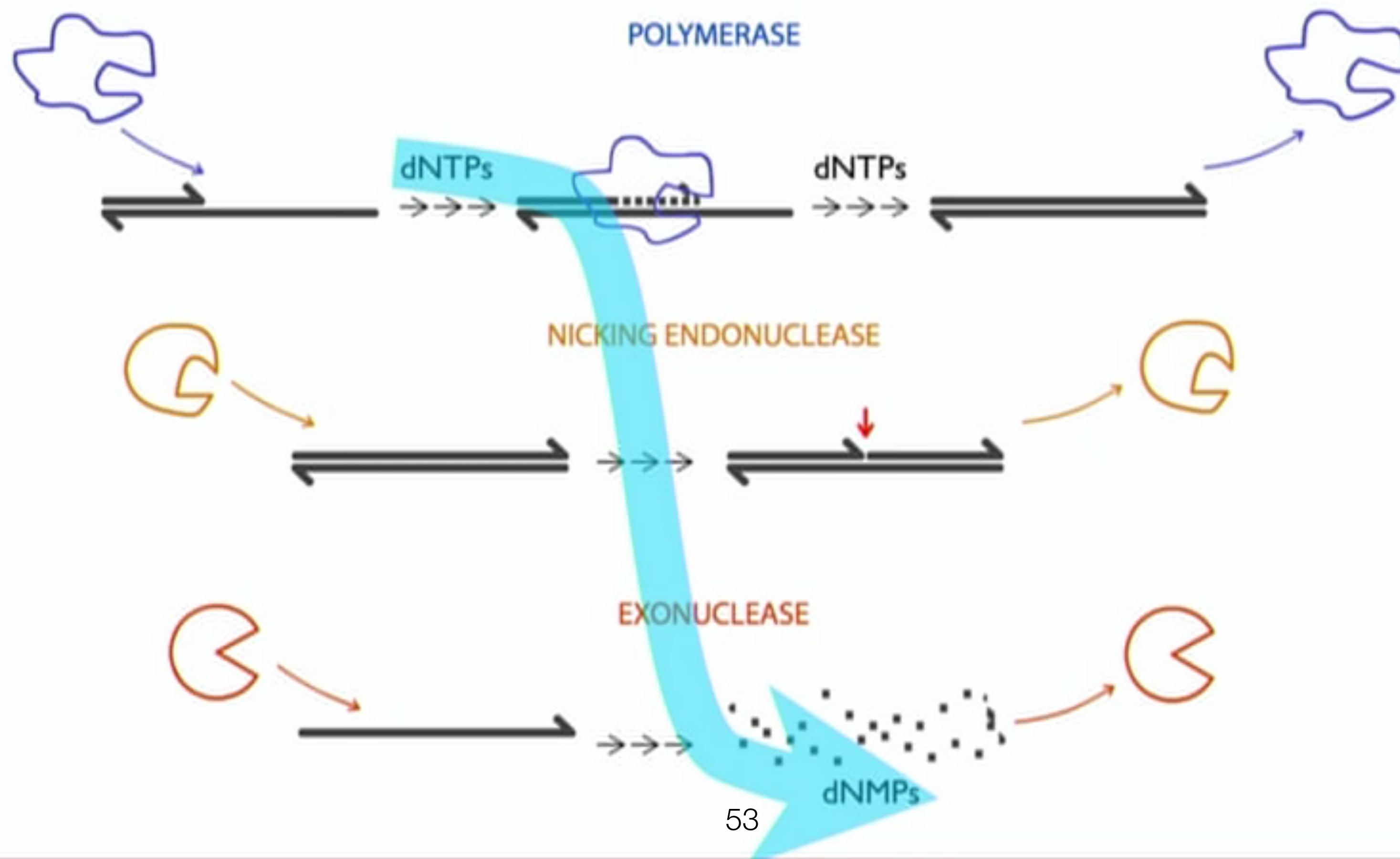
PEN DNA toolbox: an *in vitro* “universal” reaction set



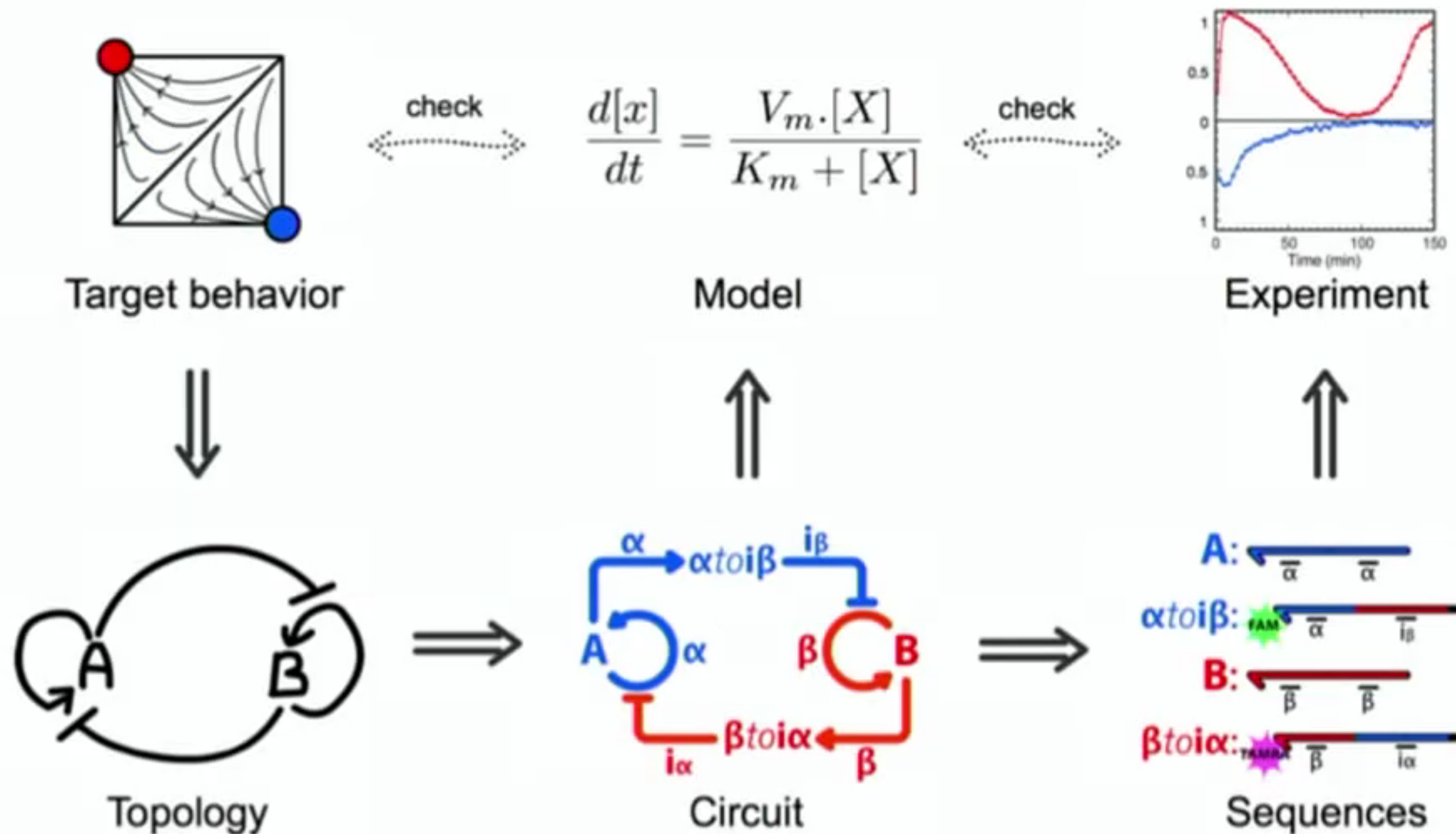
- Cascadable
- Dissipative (Global reaction and energy flux: dNTP $\rightarrow$ Oligonucleotides $\rightarrow$ dNMP)

Energy source and flow

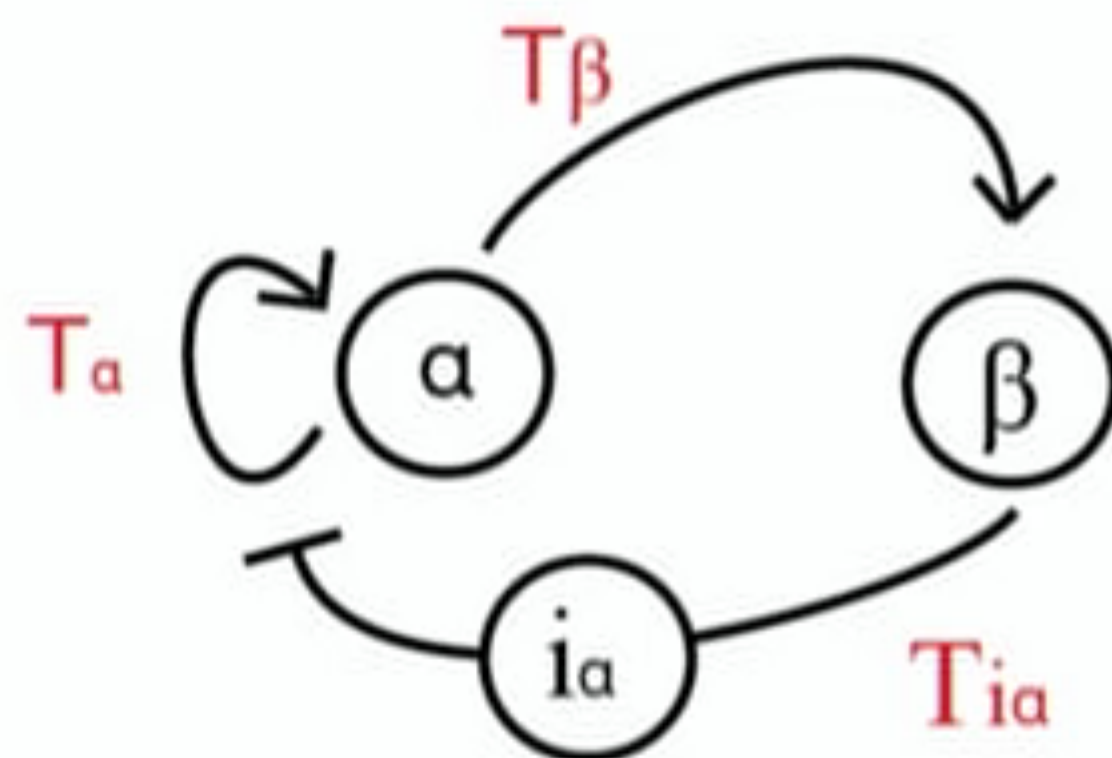
Enzymes



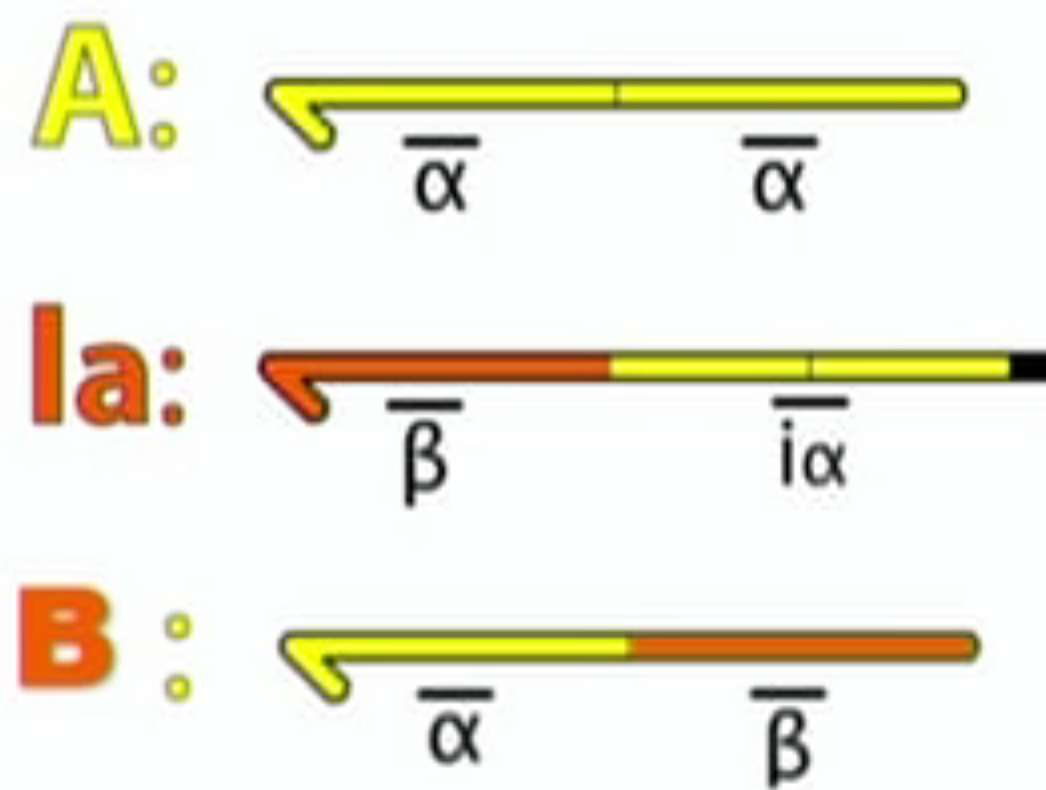
Synthesizing dynamics



Building a DNA-encoded oscillator

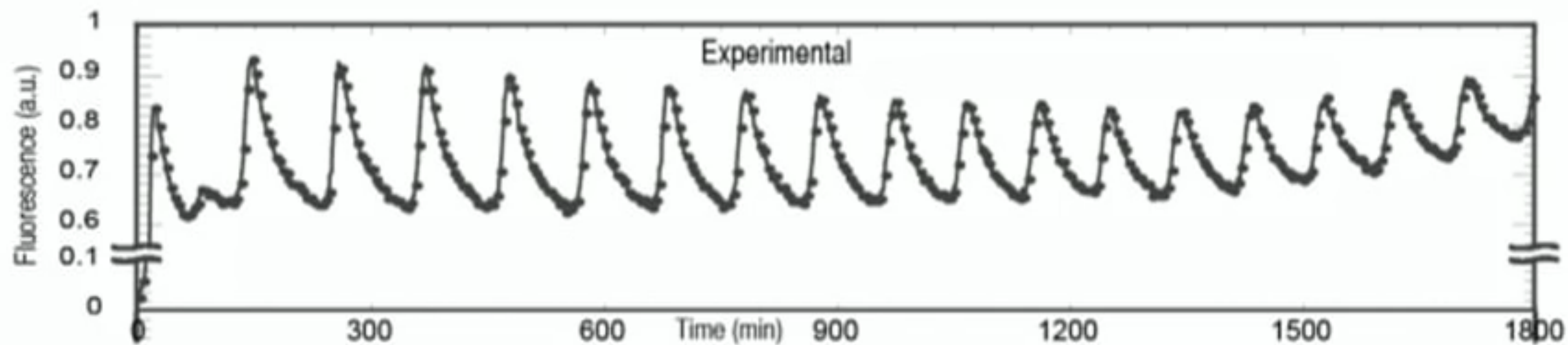
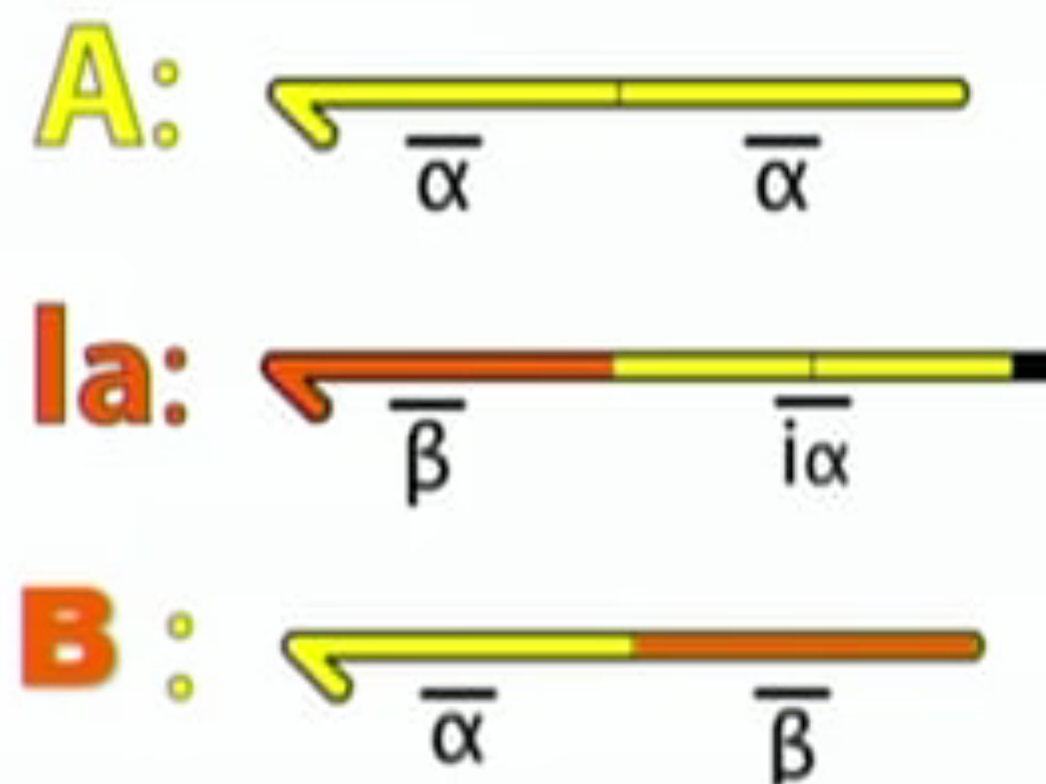
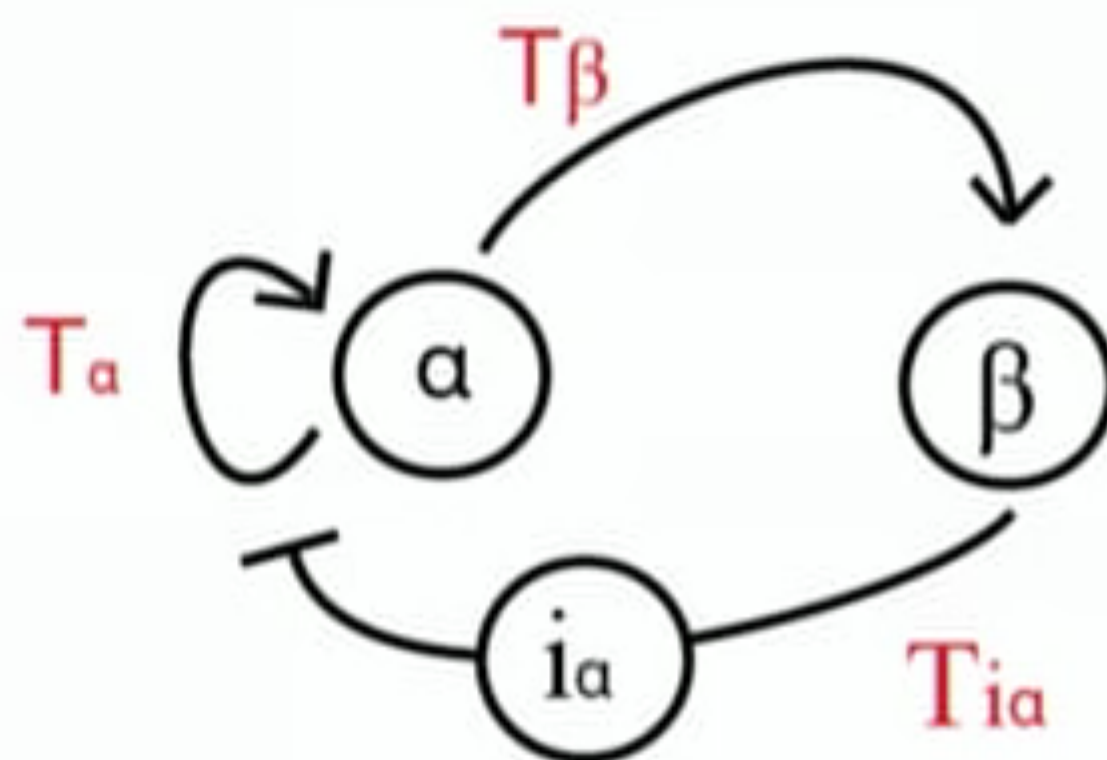


Relaxation oscillator

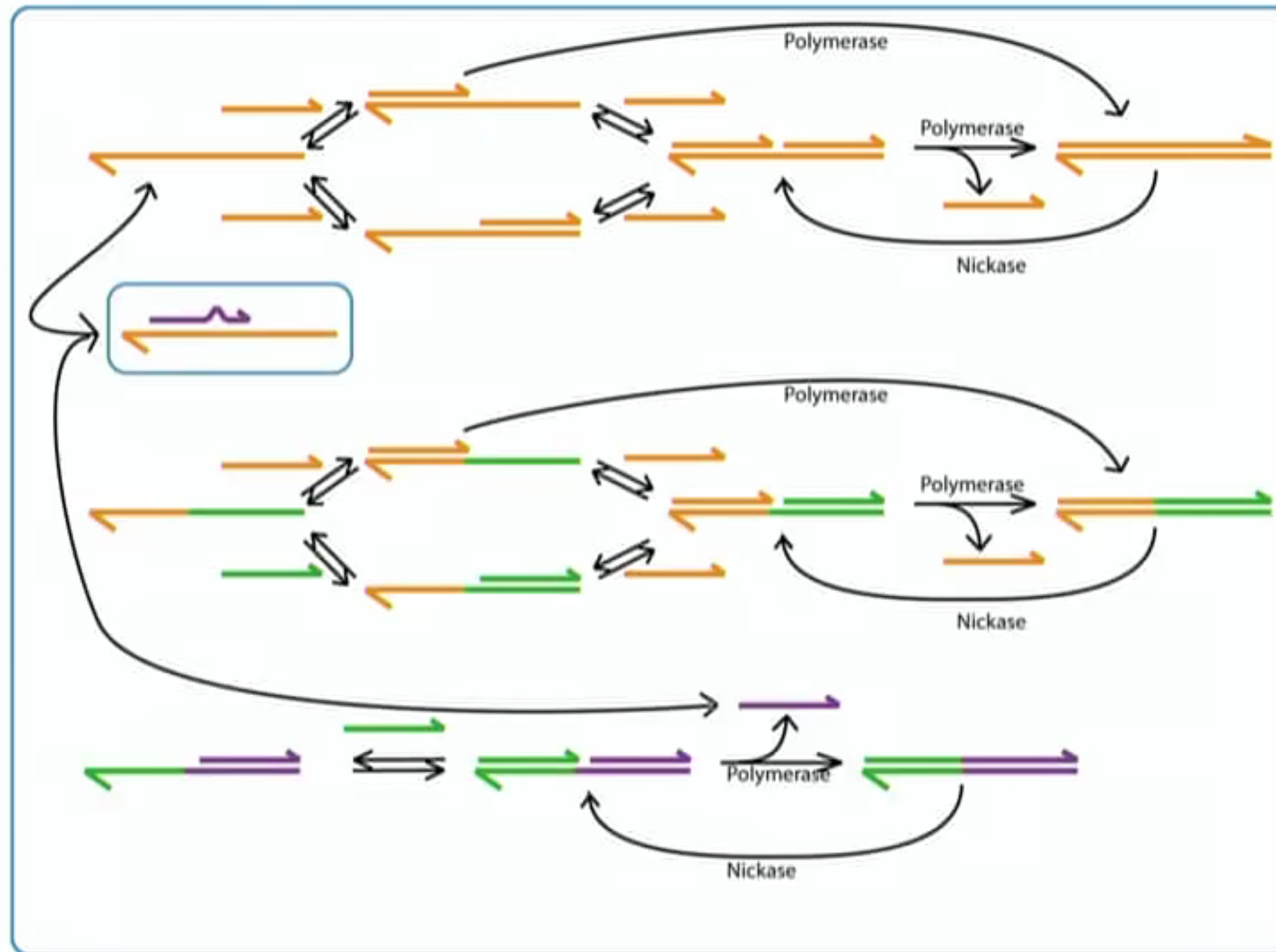


5'-AACAG**ACTCGA**-AACAG**ACTCGA**-3'
 5'-TTACTCGAAACAGACT-GGAT**GACTCCA**-3'
 5'-GGAT**GACTCCA**-AACAG**ACTCGA**-3'

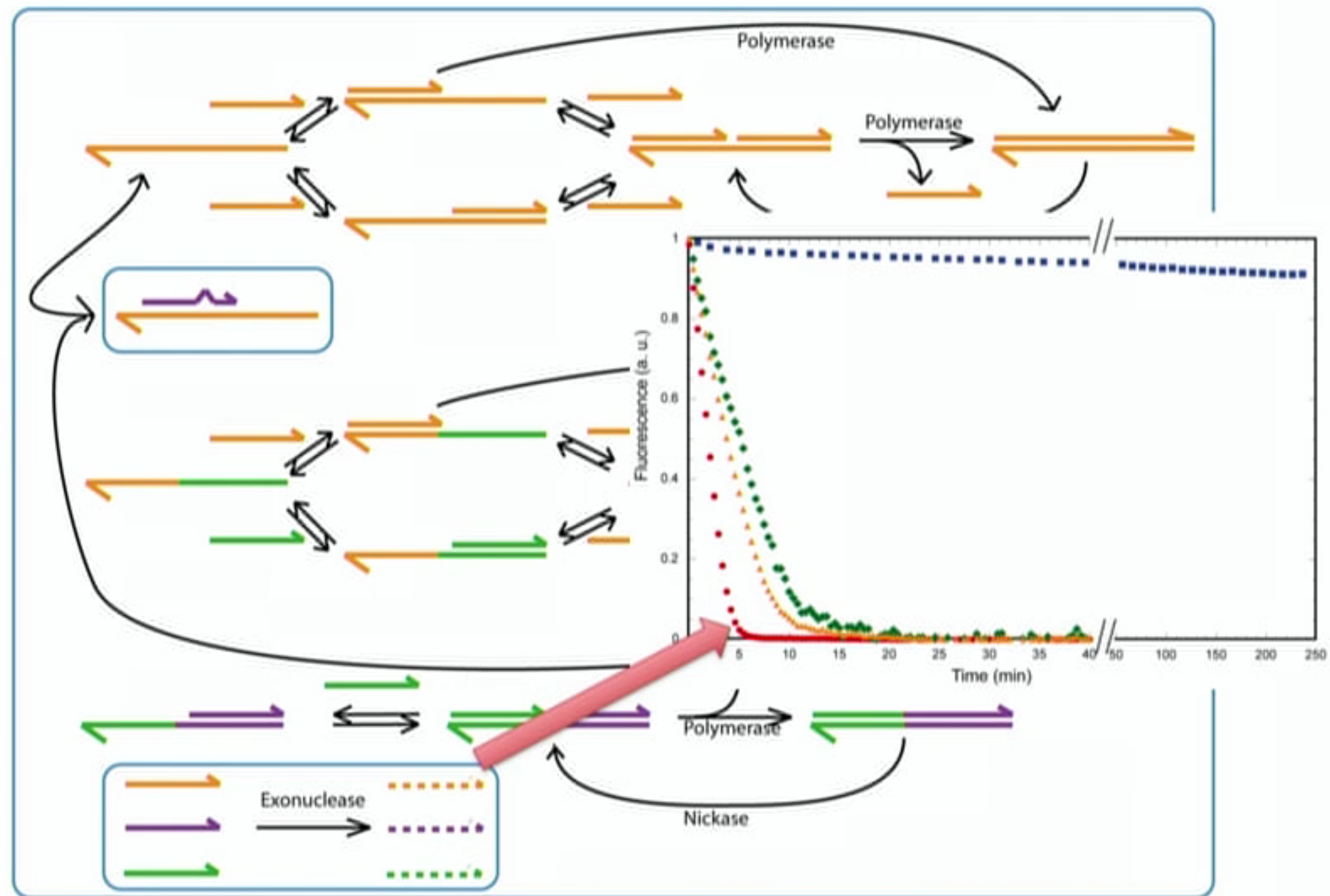
Building a DNA-encoded oscillator



Modeling (detailed kinetics)



Modeling (detailed kinetics)





ODE system

$$x'(t) = \frac{\text{cpol kpsd } xT_x(t)}{K_{msd} MM_f(t)} + \text{cpol leak kp } T(t) - k_1 x(t) (Dr(t) + 2 T(t) + Tx(t) + Txa(t) + xaT(t) + xT(t)) + k_1 \left(\frac{xT(t)}{K_{li}} + \frac{xTx(t) + xTxa(t)}{K_{li}} + \frac{Tx(t)}{K_{lo}} + \frac{xaTx(t) + xTx(t)}{K_{lo}} \right) - \frac{\text{crec krec } x(t)}{K_{mr} MMr(t)},$$

$$T'(t) = k_1 \left(\frac{xaT(t) + xT(t)}{K_{li}} + \frac{Tx(t) + Txa(t)}{K_{lo}} \right) - k_1 T(t) (2 x(t) + 2 xa(t)),$$

$$xT'(t) = -\frac{\text{cpol kp } xT(t)}{K_m MM_f(t)} - \frac{k_1 xT(t)}{K_{li}} + \frac{k_1 (xTx(t) + xTxa(t))}{K_{lo}} + k_1 T(t) x(t) - k_1 xT(t) (x(t) + xa(t)),$$

$$Tx'(t) = \frac{k_1 (xaTx(t) + xTx(t))}{K_{li}} - \frac{k_1 Tx(t)}{K_{lo}} + k_1 T(t) x(t) - k_1 Tx(t) (x(t) + xa(t)),$$

$$Tx2'(t) = \text{cpol} \left(\frac{\text{kp } xT(t)}{K_m MM_f(t)} + \frac{\text{kpsd } xTx(t)}{K_{msd} MM_f(t)} \right) - \frac{C_n k_n Tx2(t)}{K_{mn} MM_n(t)},$$

$$xTx'(t) = \frac{C_n k_n Tx2(t)}{K_{mn} MM_n(t)} - \frac{\text{cpol kpsd } xTx(t)}{K_{msd} MM_f(t)} - k_1 \left(\frac{1}{K_{li}} + \frac{1}{K_{lo}} \right) xTx(t) + k_1 x(t) (Tx(t) + xT(t)),$$

$$xaT'(t) = -\frac{k_1 xaT(t)}{K_{li}} + \frac{k_1 (xaTx(t) + xTxa(t))}{K_{lo}} + k_1 T(t) xa(t) - k_1 xaT(t) (x(t) + xa(t)),$$

$$Txa'(t) = \frac{k_1 (xaTxa(t) + xTxa(t))}{K_{li}} - \frac{k_1 Txa(t)}{K_{lo}} + k_1 T(t) xa(t) - k_1 Txa(t) (x(t) + xa(t)),$$

$$xTxa'(t) = -k_1 \left(\frac{1}{K_{li}} + \frac{1}{K_{lo}} \right) xTxa(t) + k_1 Txa(t) x(t) + k_1 xa(t) xT(t),$$

$$xaTx'(t) = -k_1 \left(\frac{1}{K_{li}} + \frac{1}{K_{lo}} \right) xaTx(t) + k_1 Tx(t) xa(t) + k_1 x(t) xaT(t),$$

$$xaTxa'(t) = k_1 xa(t) (Txa(t) + xaT(t)) - k_1 \left(\frac{1}{K_{li}} + \frac{1}{K_{lo}} \right) xaTxa(t),$$

$$Dr'(t) = -k_1 Dr(t) (x(t) + xa(t)) + \frac{k_1 Drxa(t)}{K_2} + \frac{k_1 xDr(t)}{K_{lo}},$$

$$xDr'(t) = -\frac{\text{cpol kp } xDr(t)}{K_m MM_f(t)} + k_1 Dr(t) x(t) - \frac{k_1 xDr(t)}{K_{lo}},$$

$$Drxa'(t) = \frac{\text{cpol kp } xDr(t)}{K_m MM_f(t)} + k_1 Dr(t) xa(t) - \frac{k_1 Drxa(t)}{K_2},$$

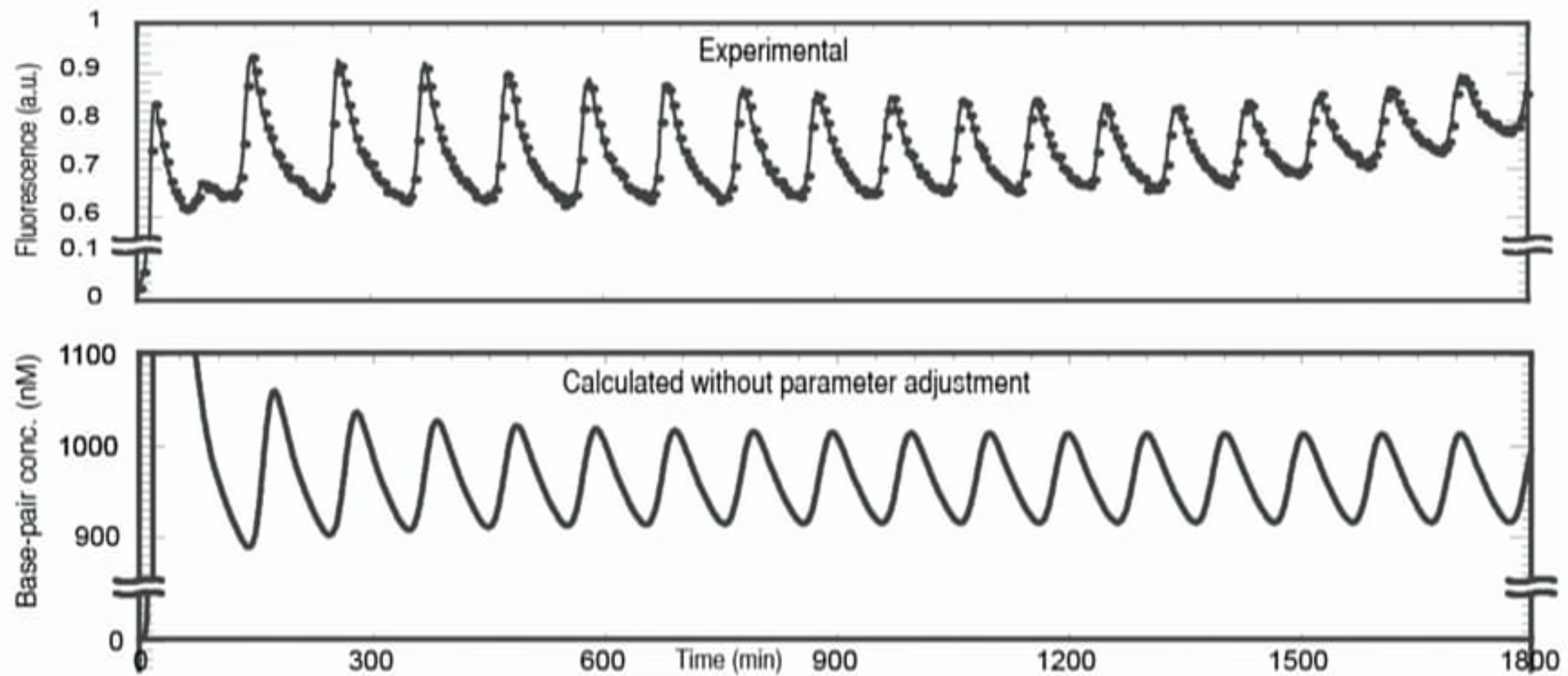
$$xa'(t) = -k_1 xa(t) (Dr(t) + 2 T(t) + Tx(t) + Txa(t) + xaT(t) + xT(t)) + k_1 \left(\frac{Drxa(t)}{K_2} + \left(\frac{1}{K_{li}} + \frac{1}{K_{lo}} \right) xaTxa(t) + \frac{xaT(t) + xaTx(t)}{K_{li}} + \frac{Tx(t) + xTxa(t)}{K_{lo}} \right) - \frac{\text{crec krec } xa(t)}{K_{mr} MMr(t)},$$

$$MM_f(t) = \frac{xT(t)}{K_m} + \frac{xTx(t)}{K_{msd}} + 1,$$

$$MM_n(t) = \frac{Tx2(t)}{K_{mn}} + 1,$$

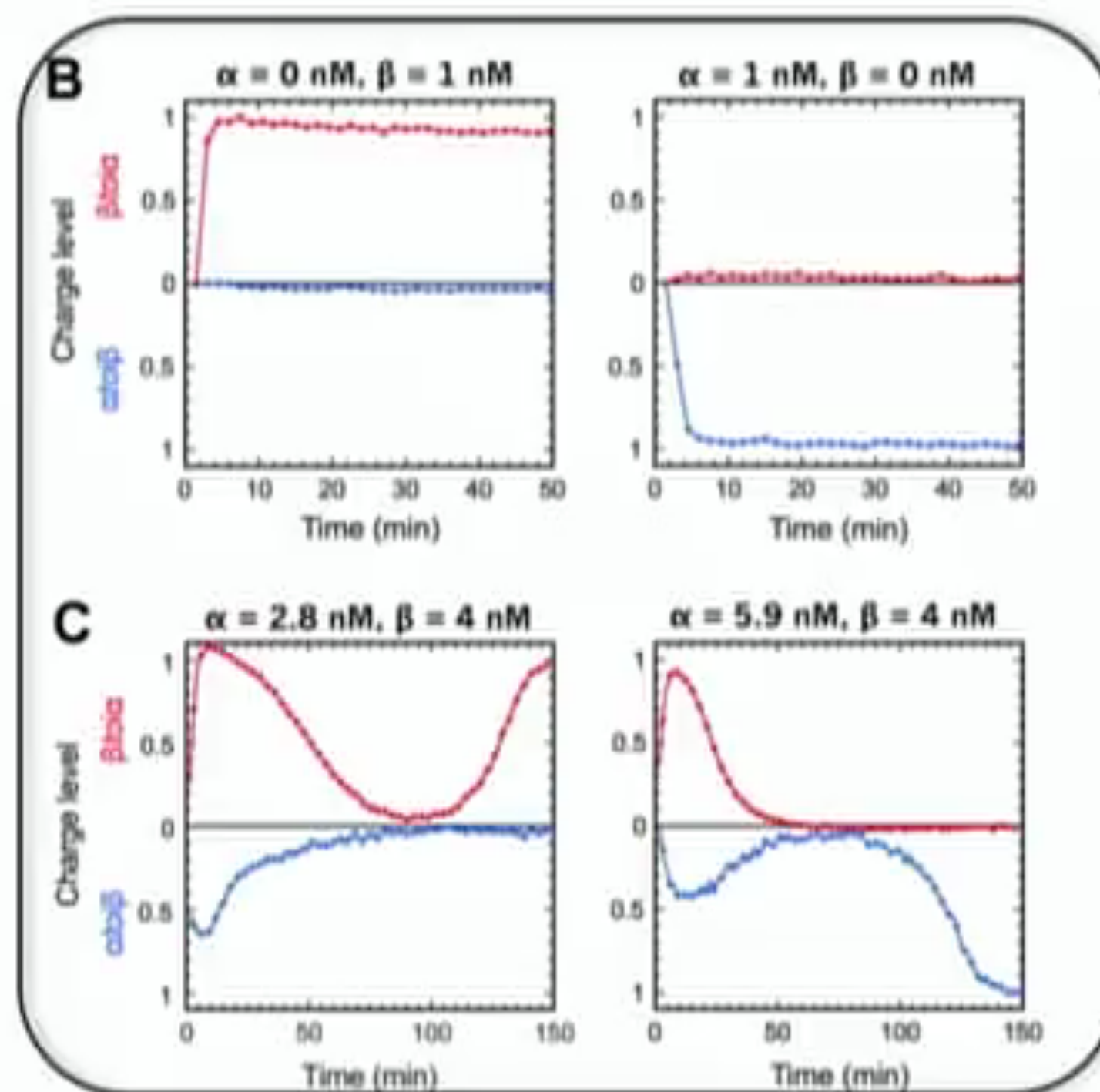
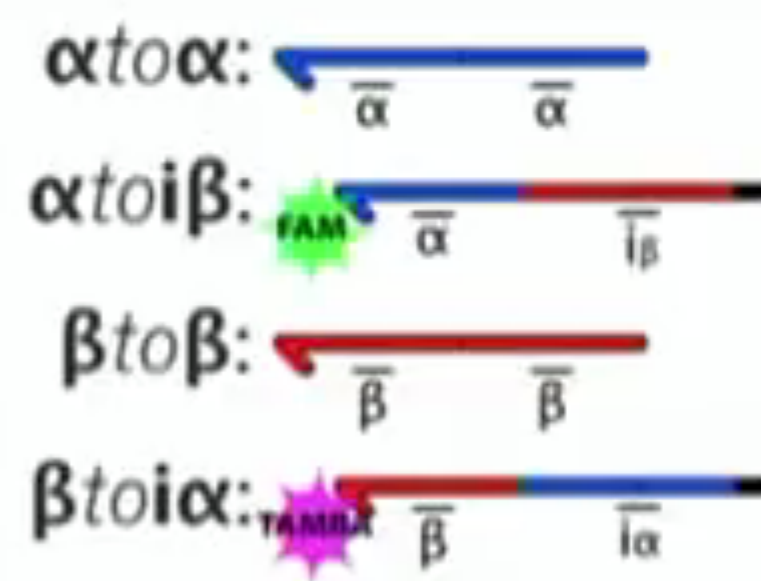
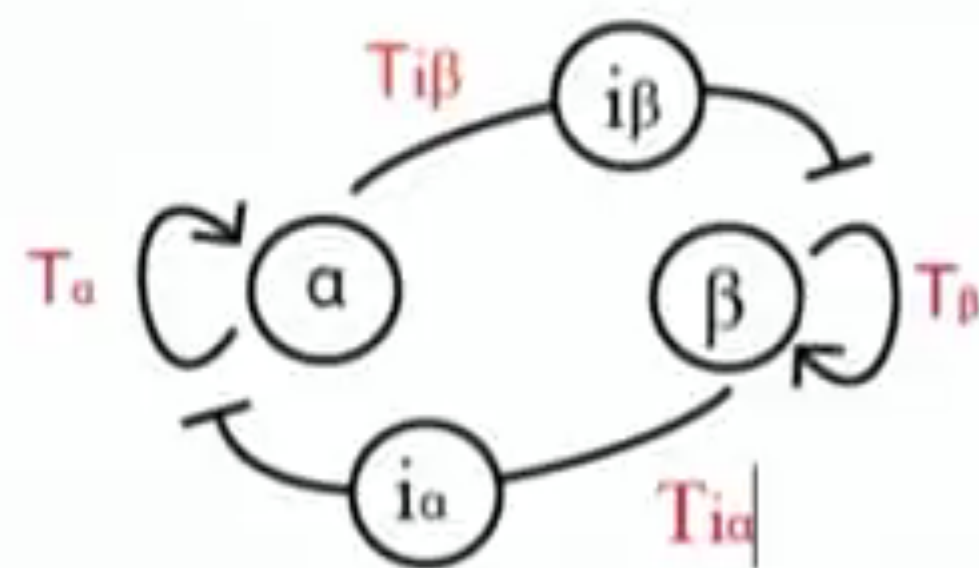
$$MM_r(t) = \frac{Dr(t)}{K_{lexo}} + \frac{T(t)}{K_{lexo}} + \frac{x(t)}{K_{mr}} + \frac{xa(t)}{K_{mr}} + 1,$$

Numerical integration



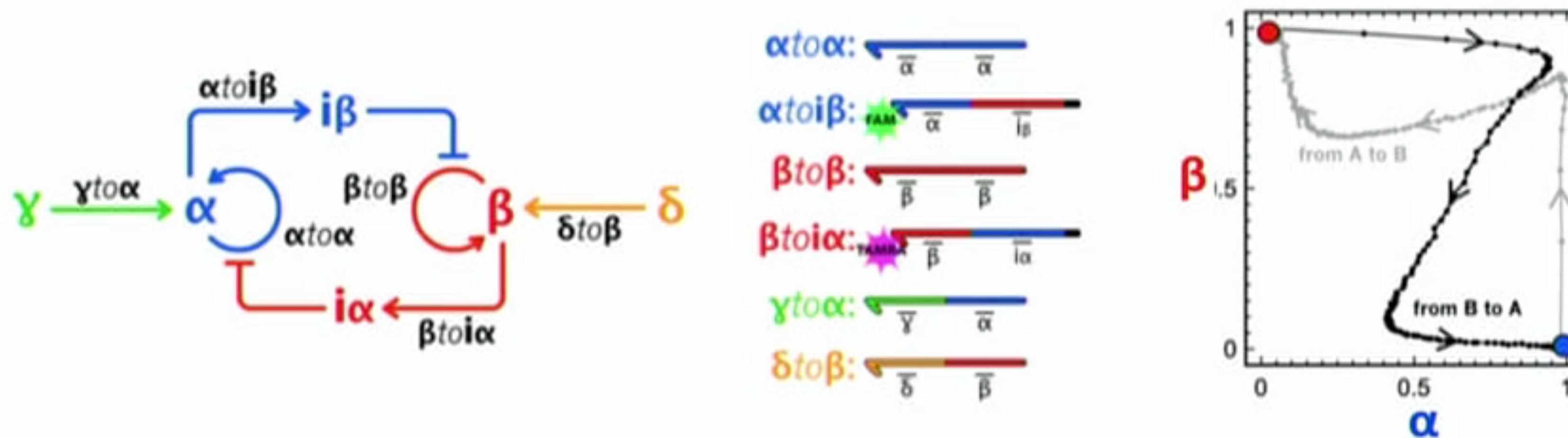
Building a bistable system (1 bit memory)

An *in vitro* Bistable switch

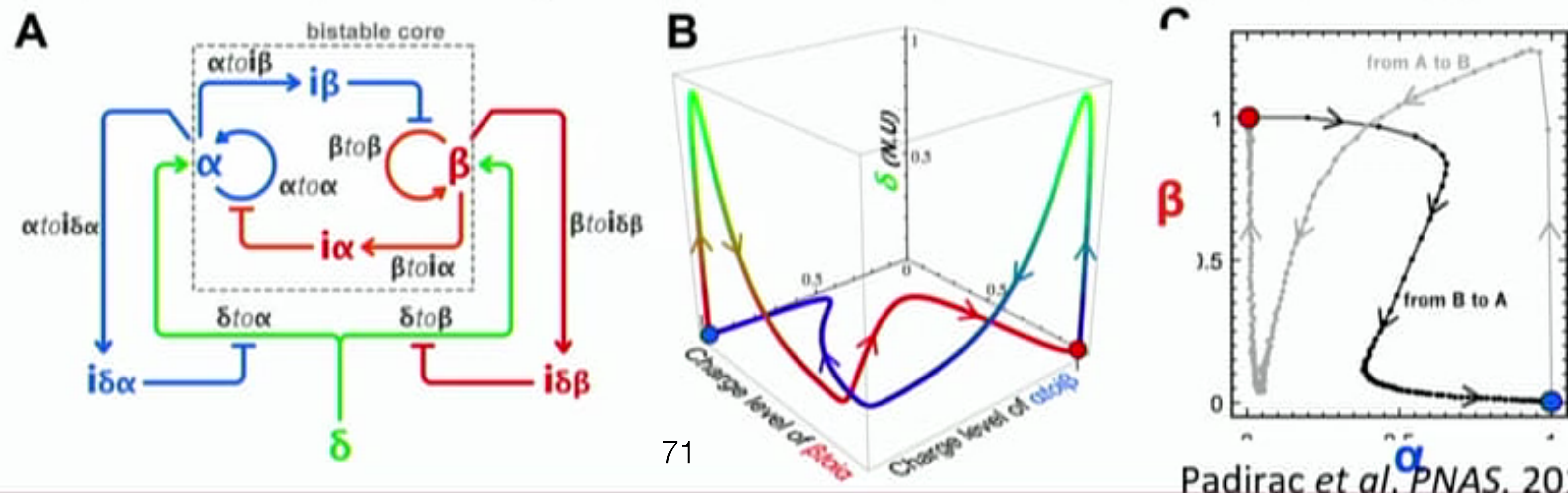


Bistable switch => Push-push button

We add input modules to force the bistable back and forth



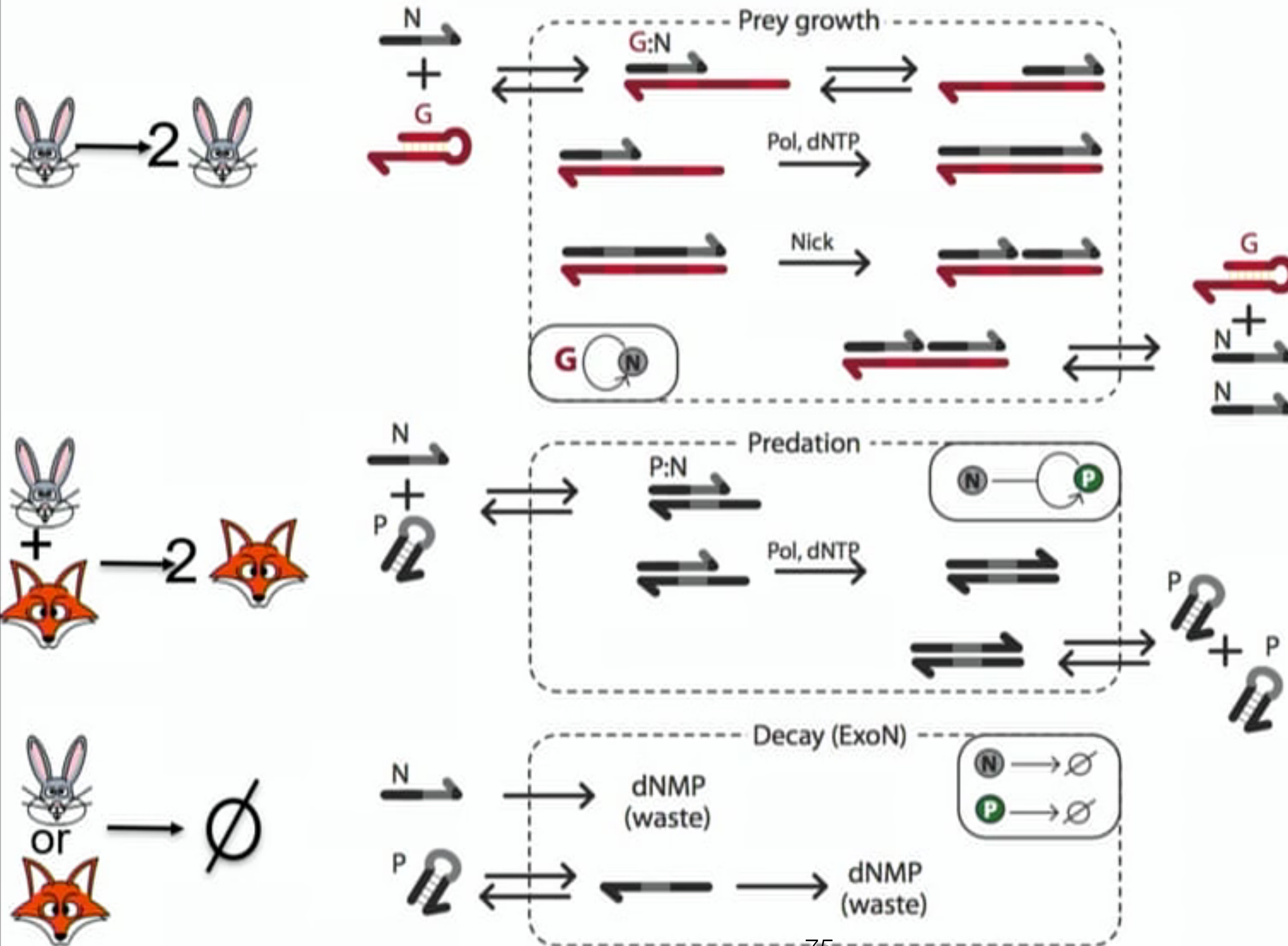
We then expand the system to a push-push memory circuit (single input toggle)



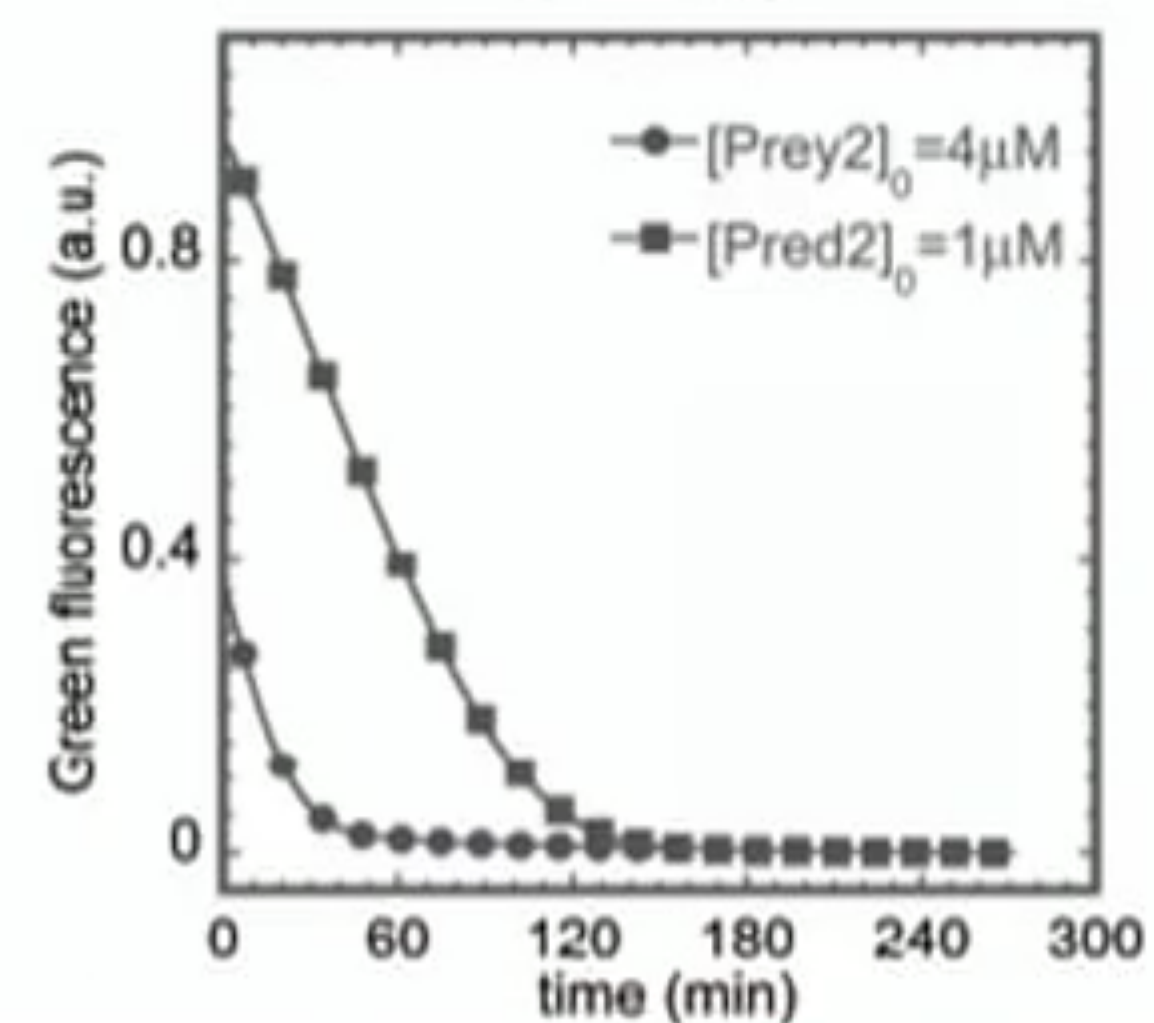
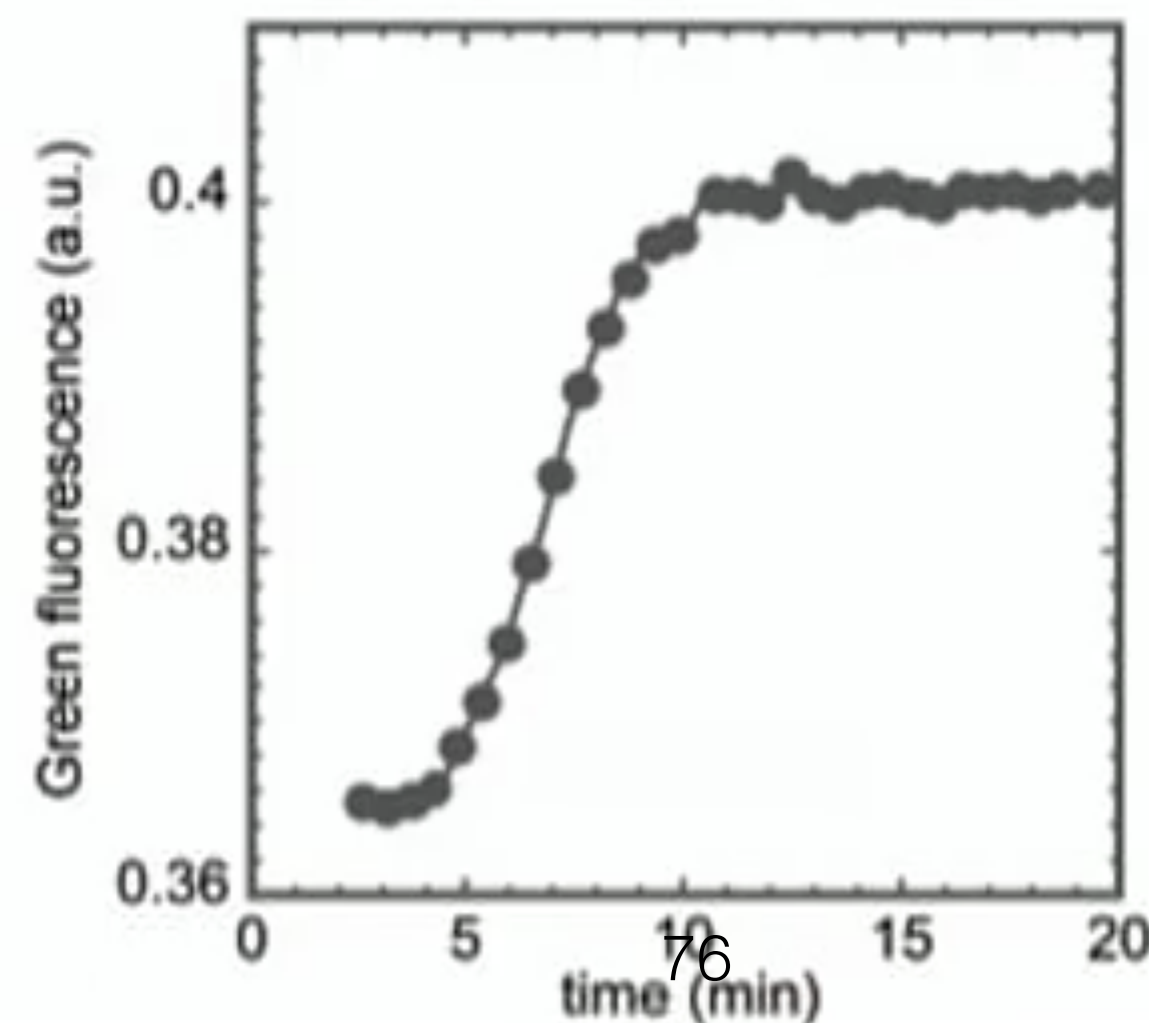
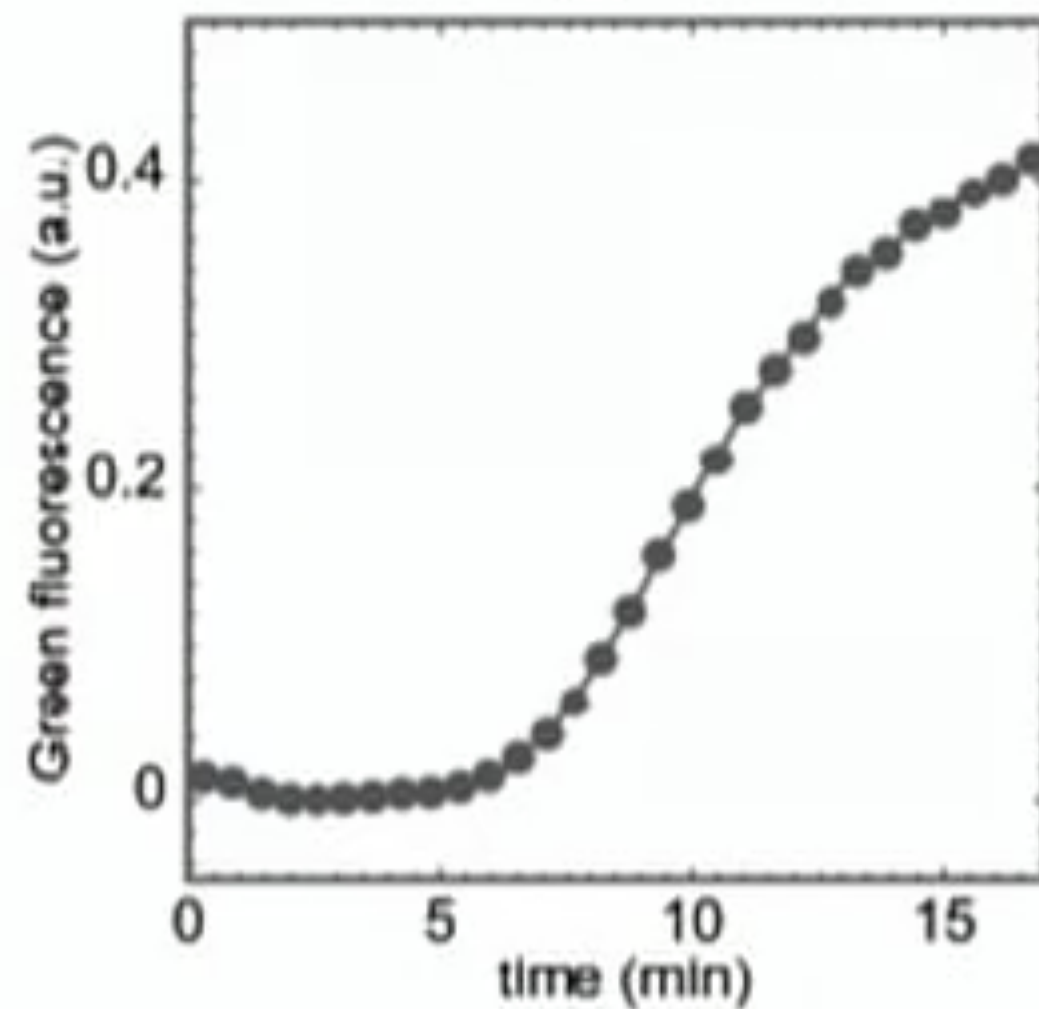
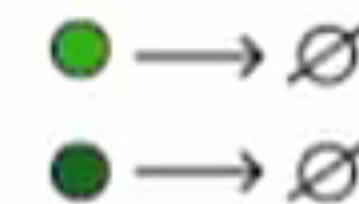
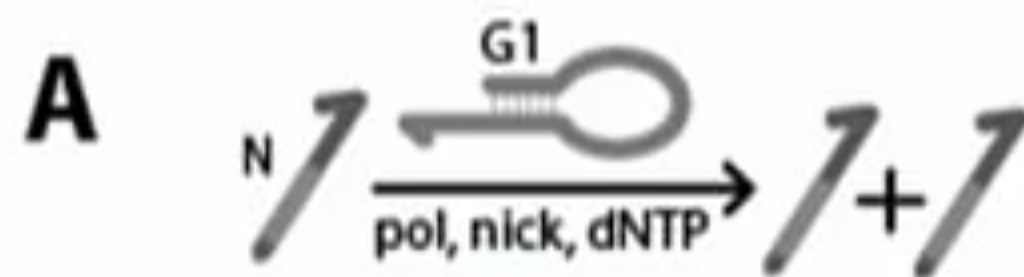
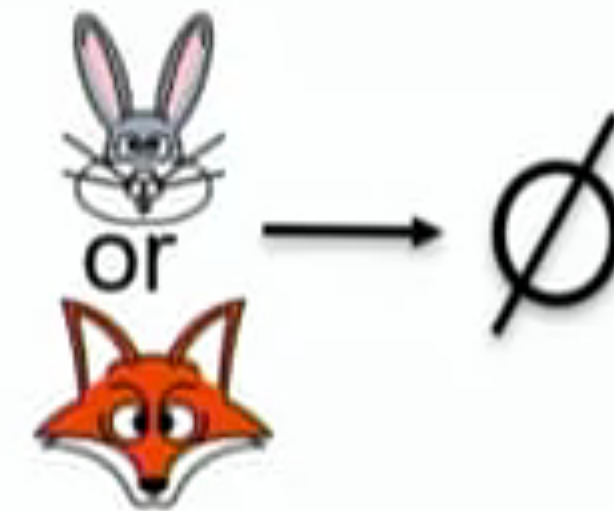
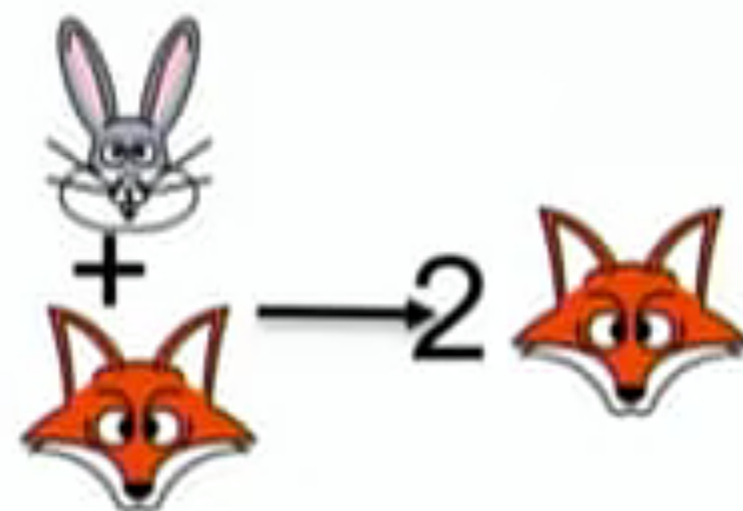
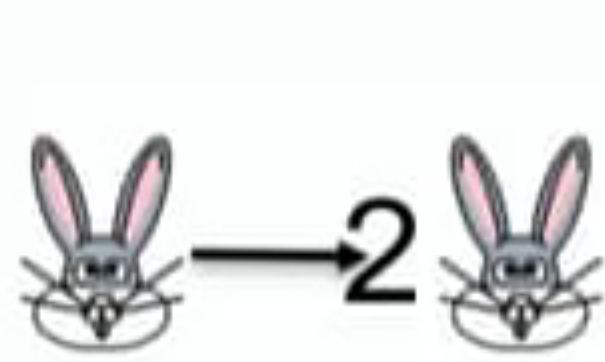


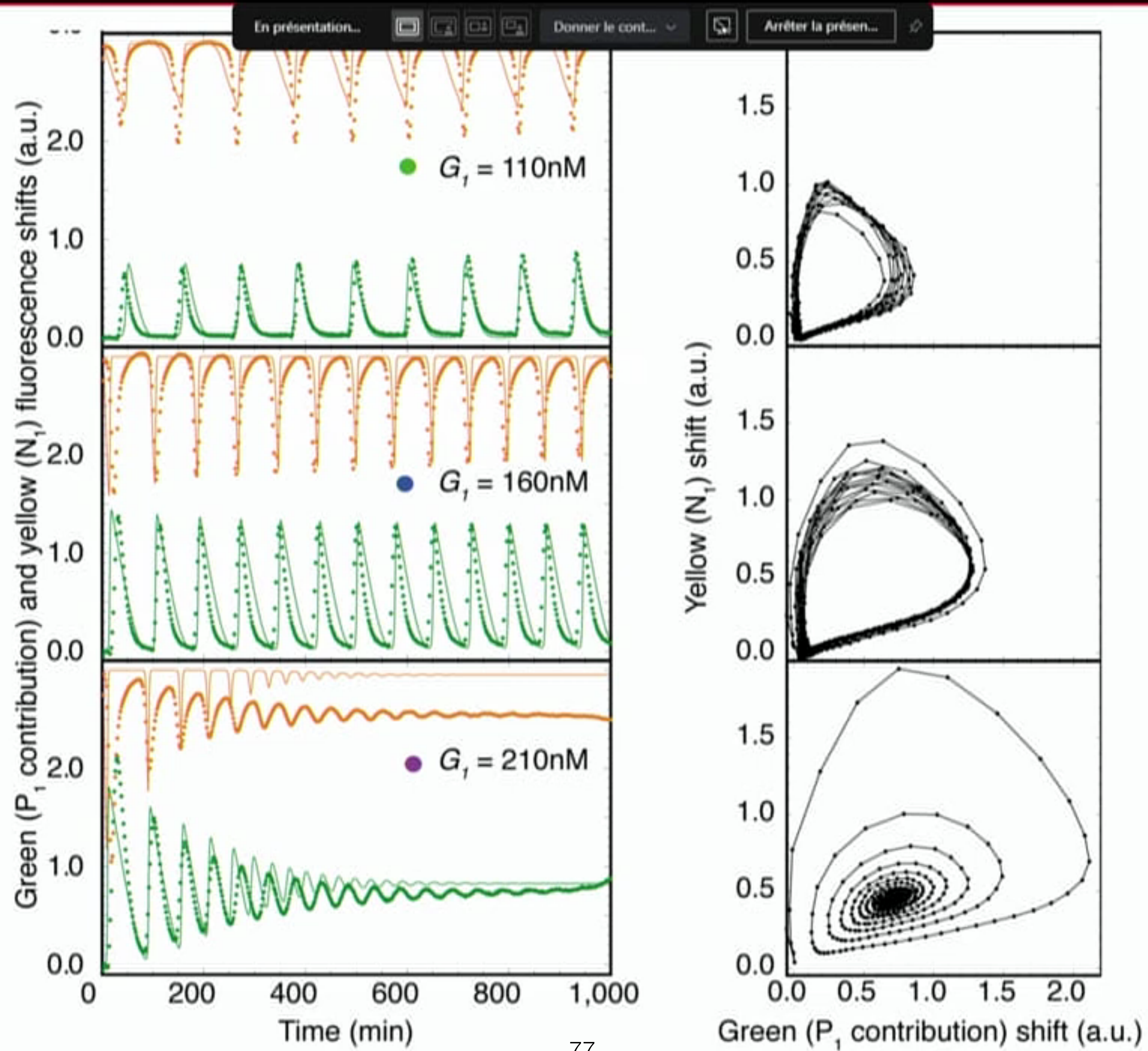
Predator Prey network

Predator-Prey molecular ecosystem

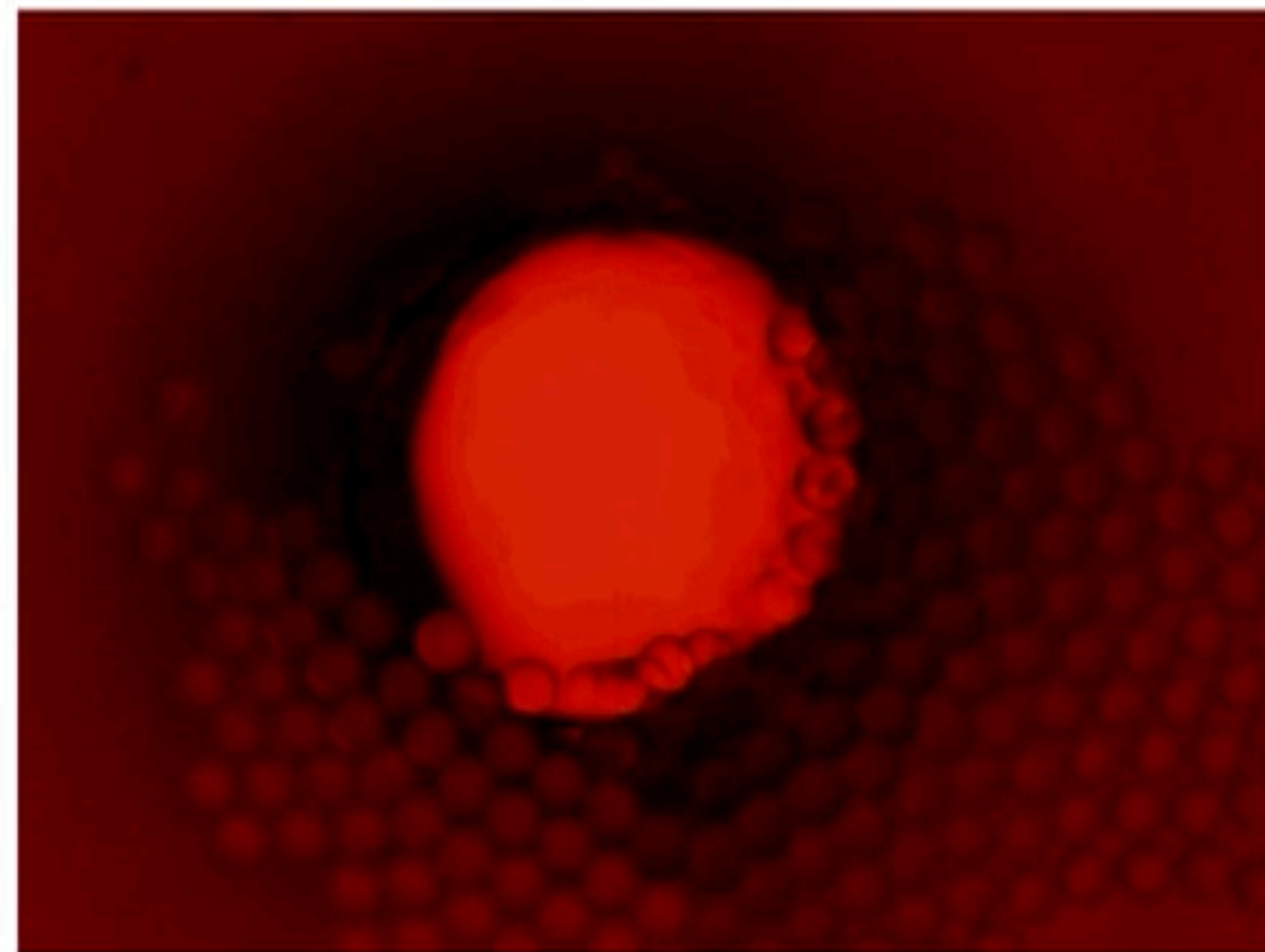
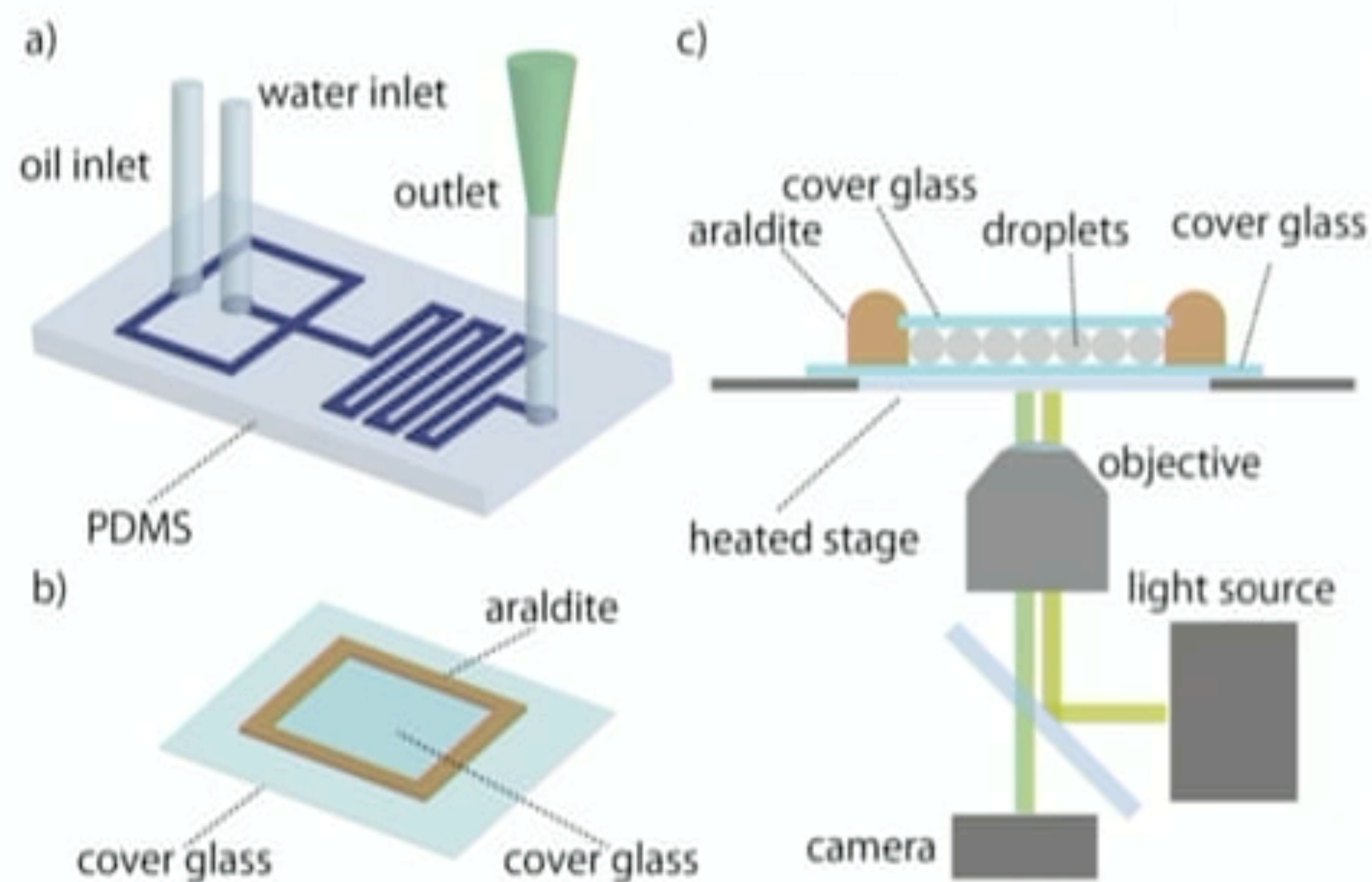


Predator-Prey molecular ecosystem

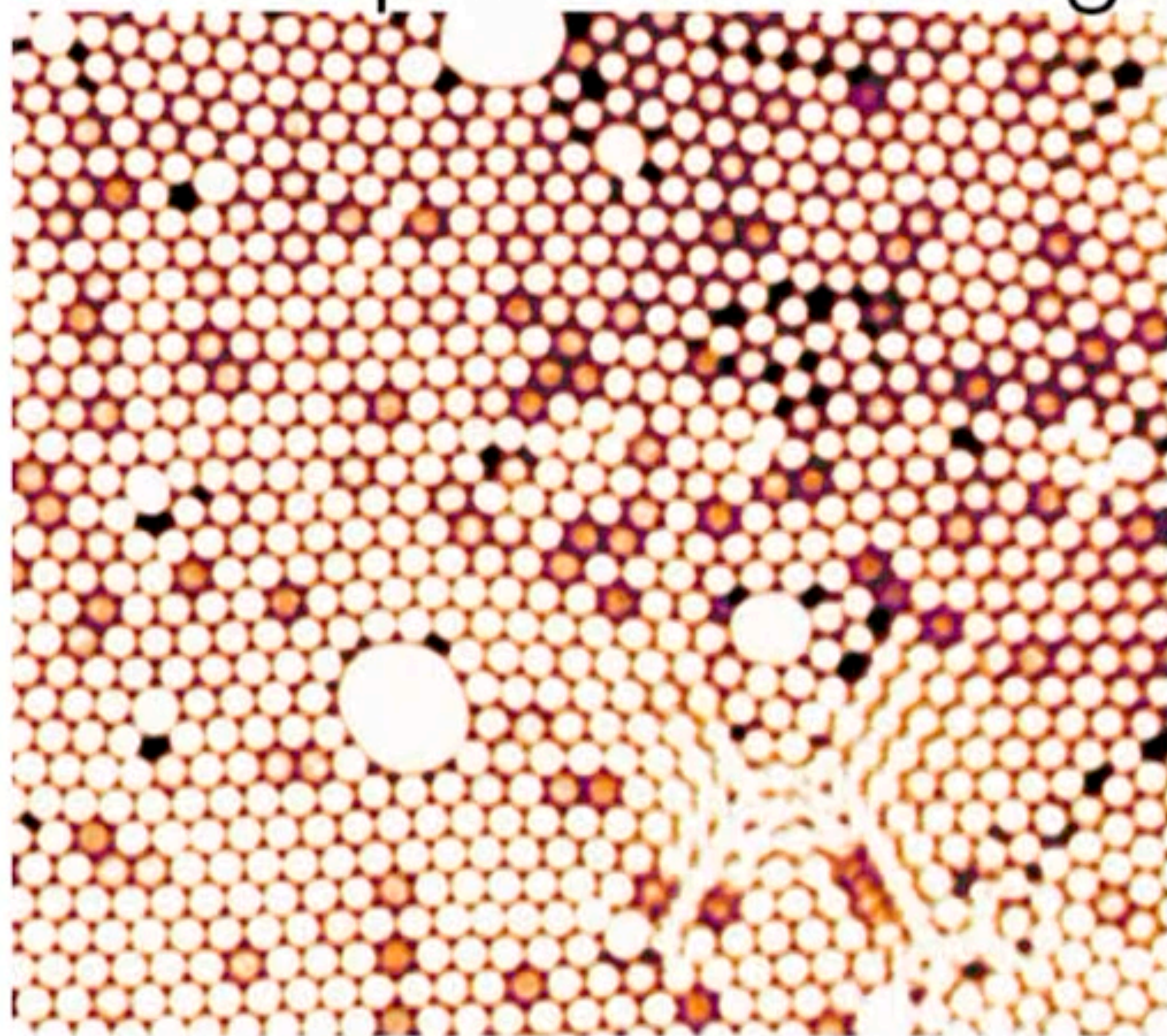




Partitioning a molecular oscillator in a microscopic emulsion

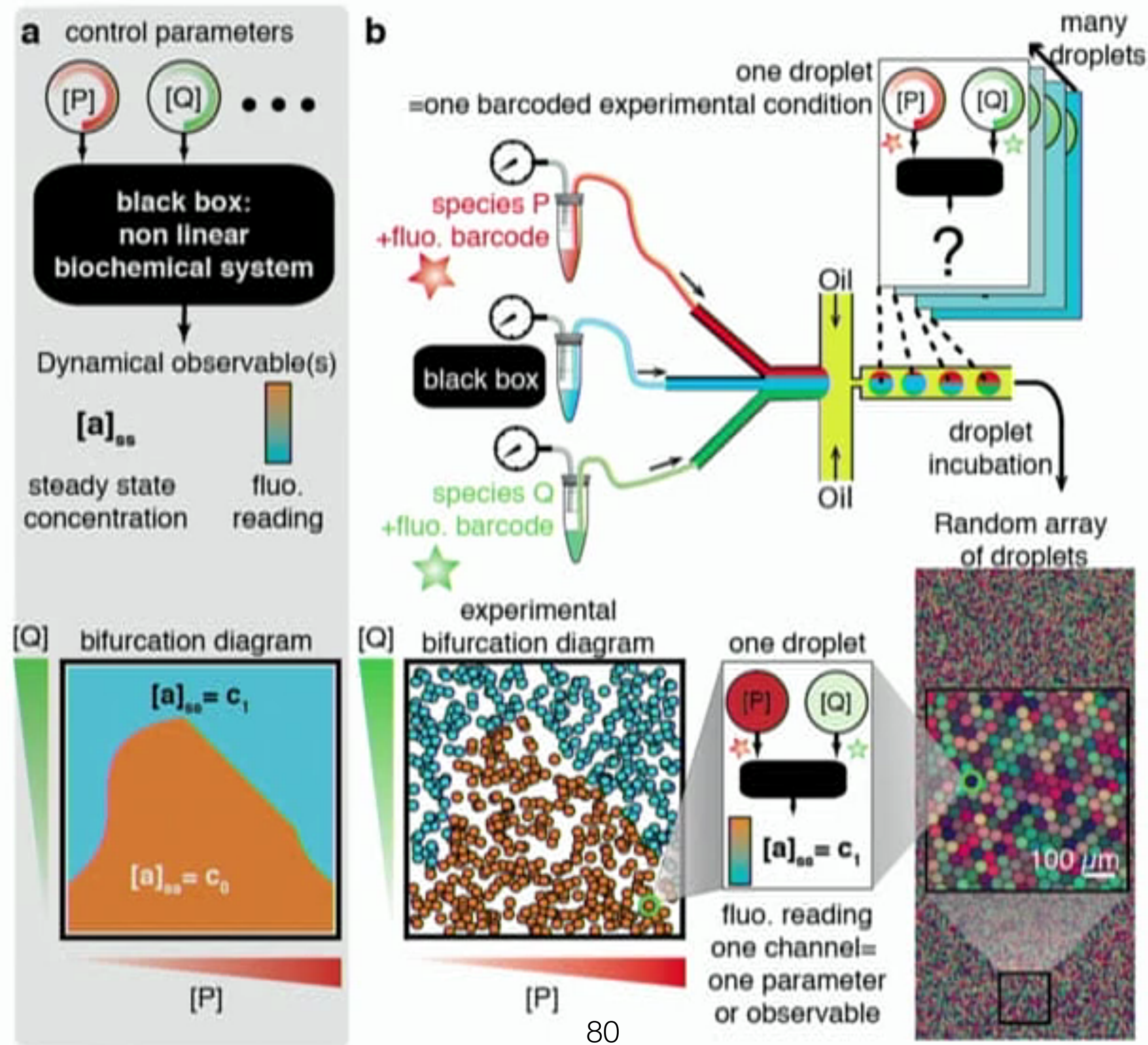


Partitioning a molecular oscillator in
a microscopic emulsion gives...



...many identical oscillators.

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



En présentation...



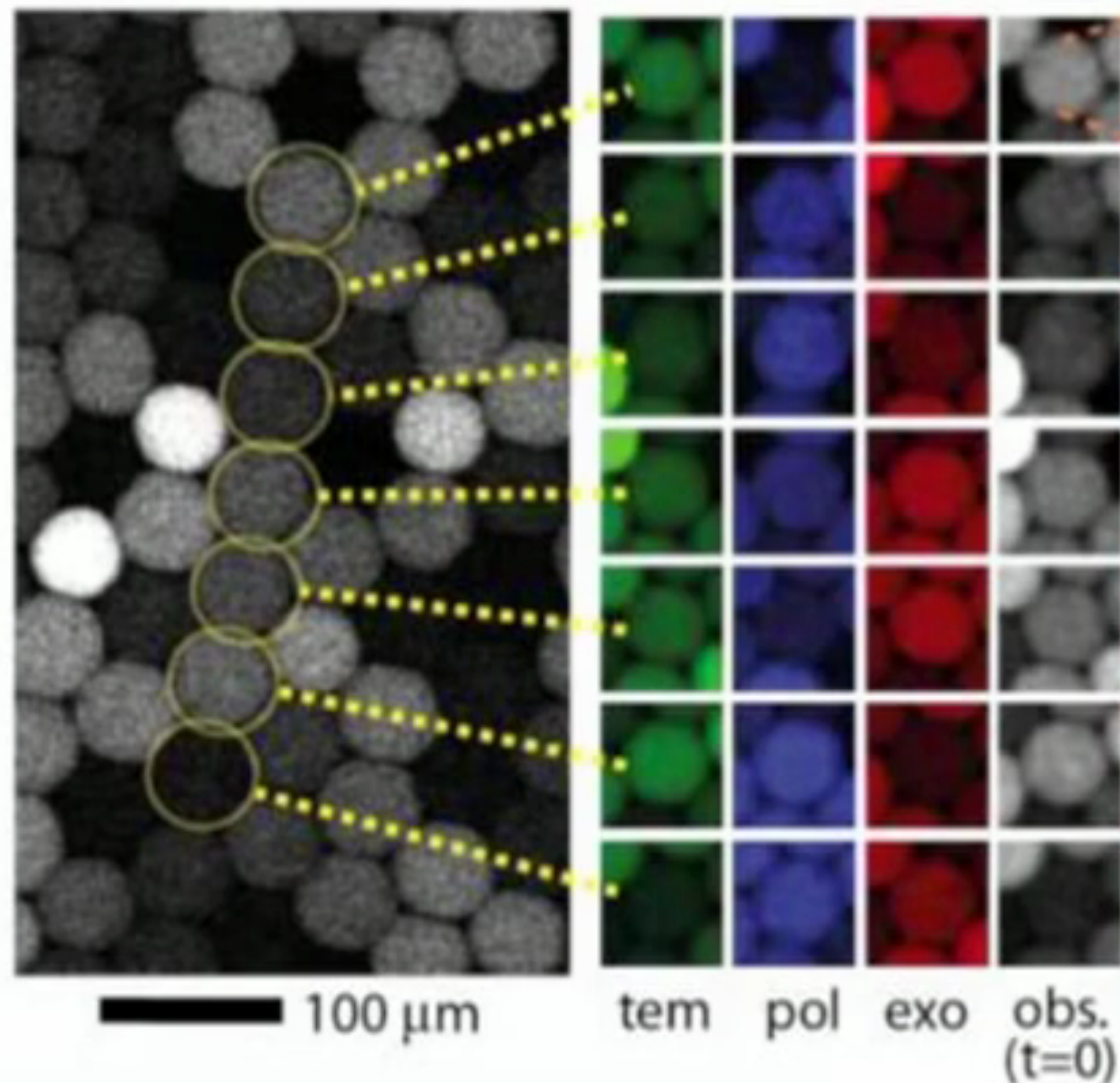
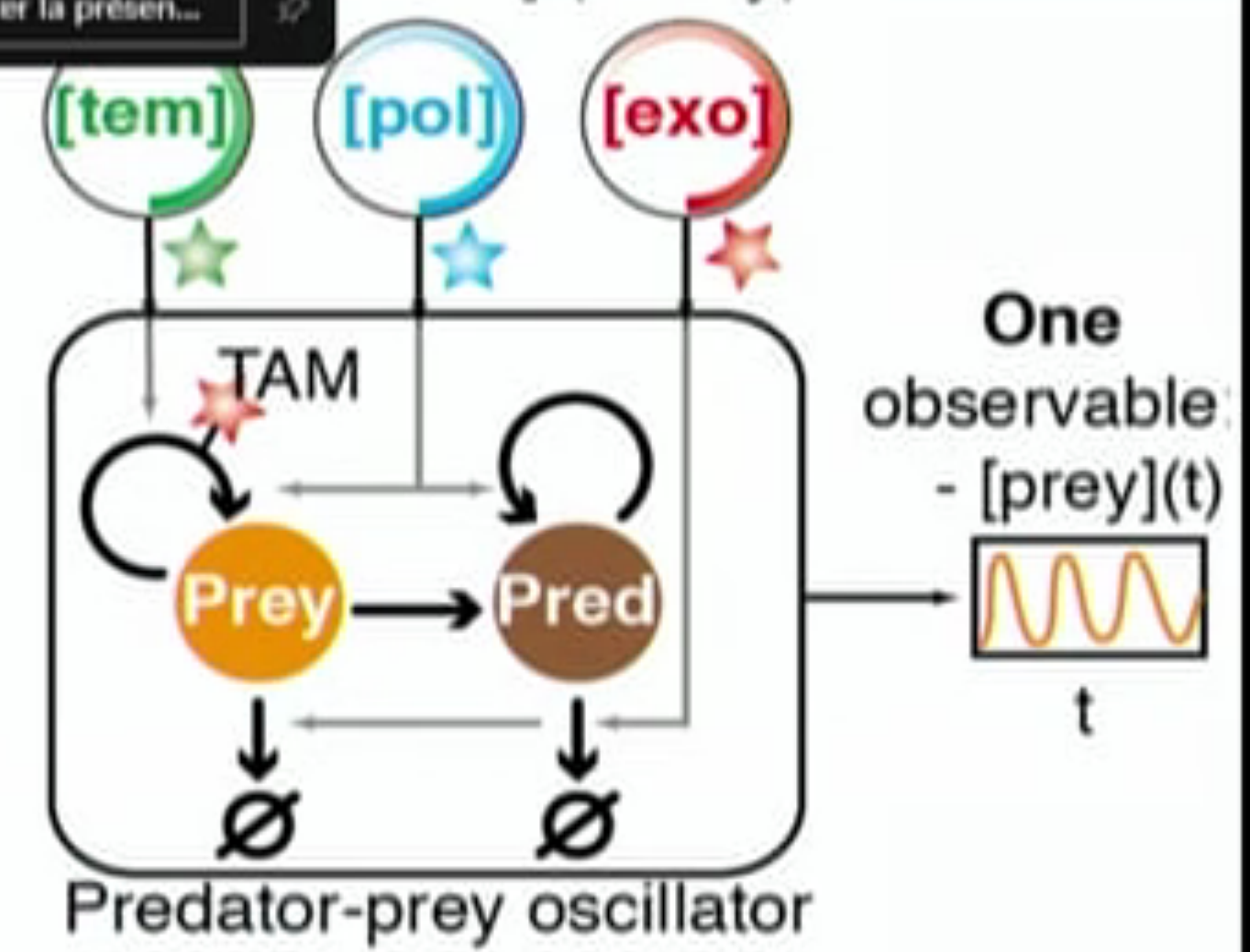
Donner le cont...

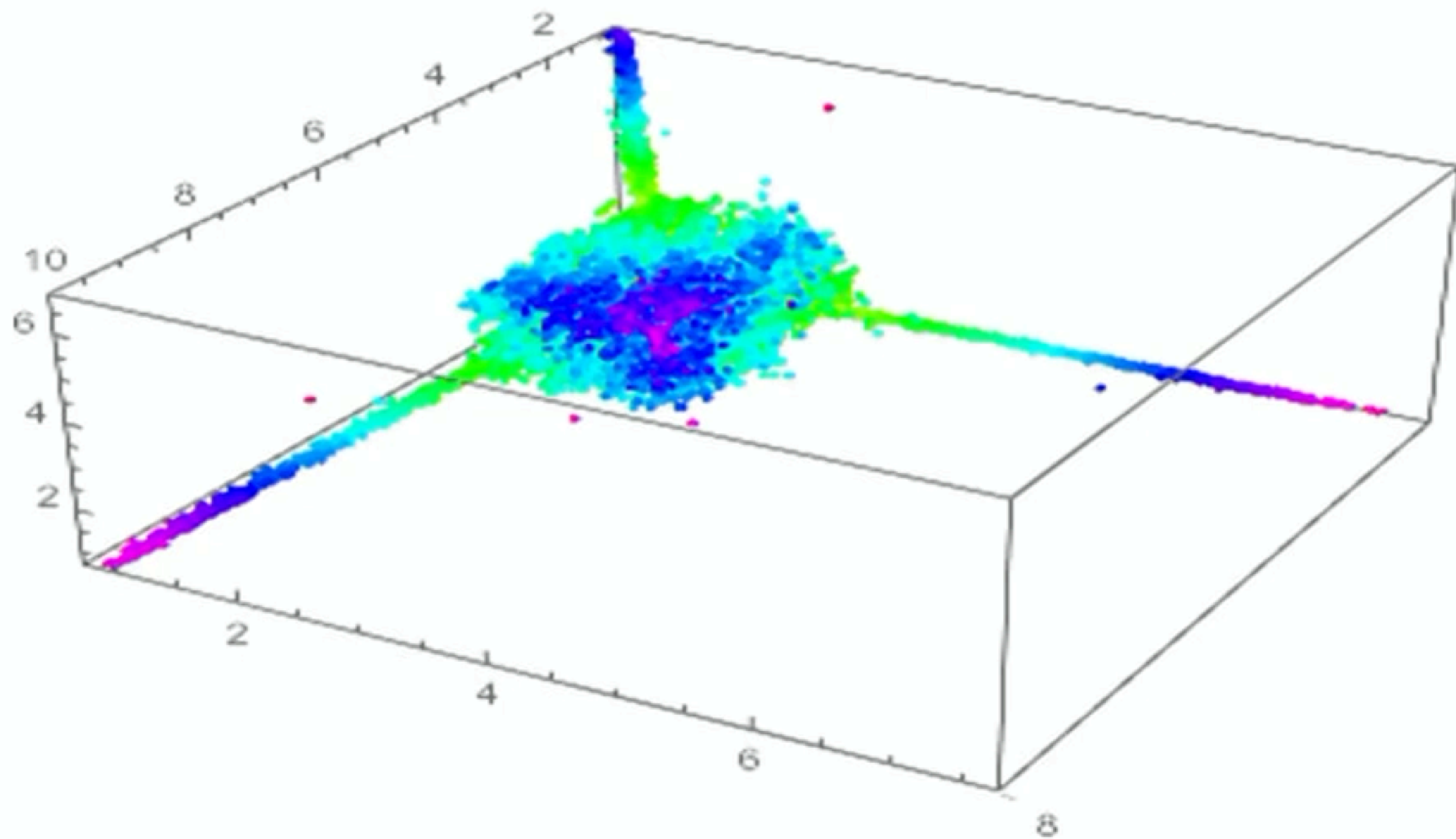


Arrêter la présen...

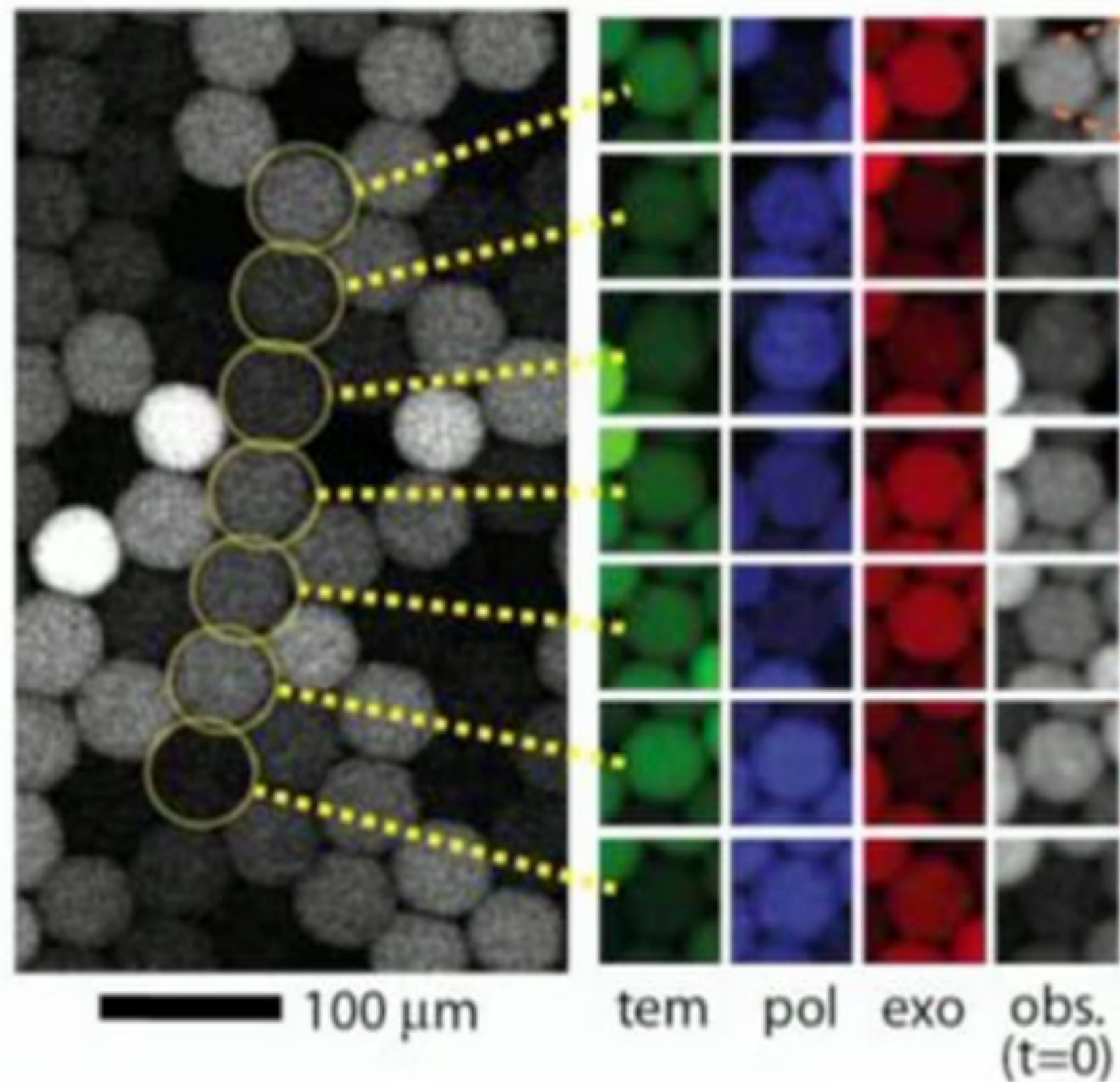
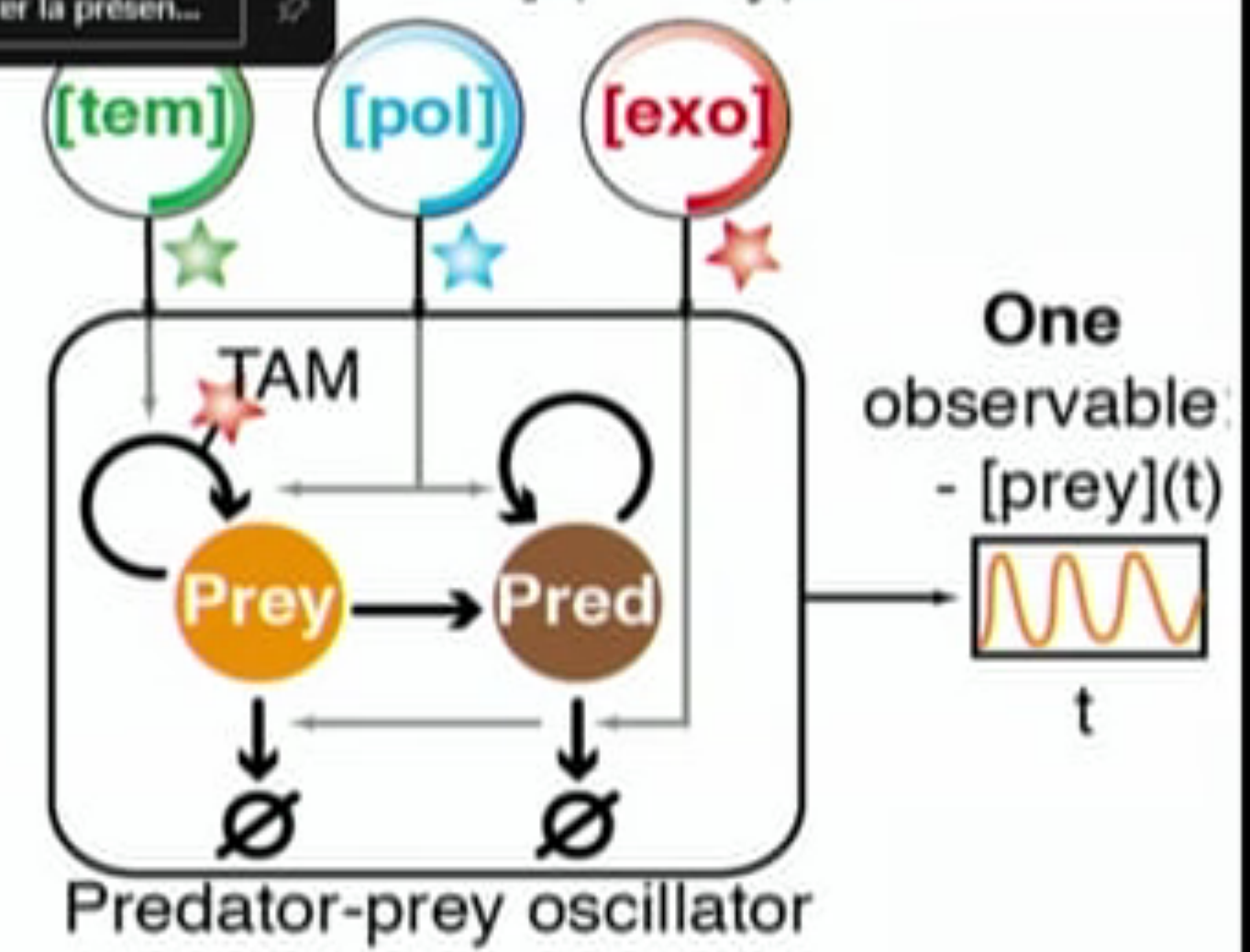


Bifurcations of oscillator using 3D Parameter scanning

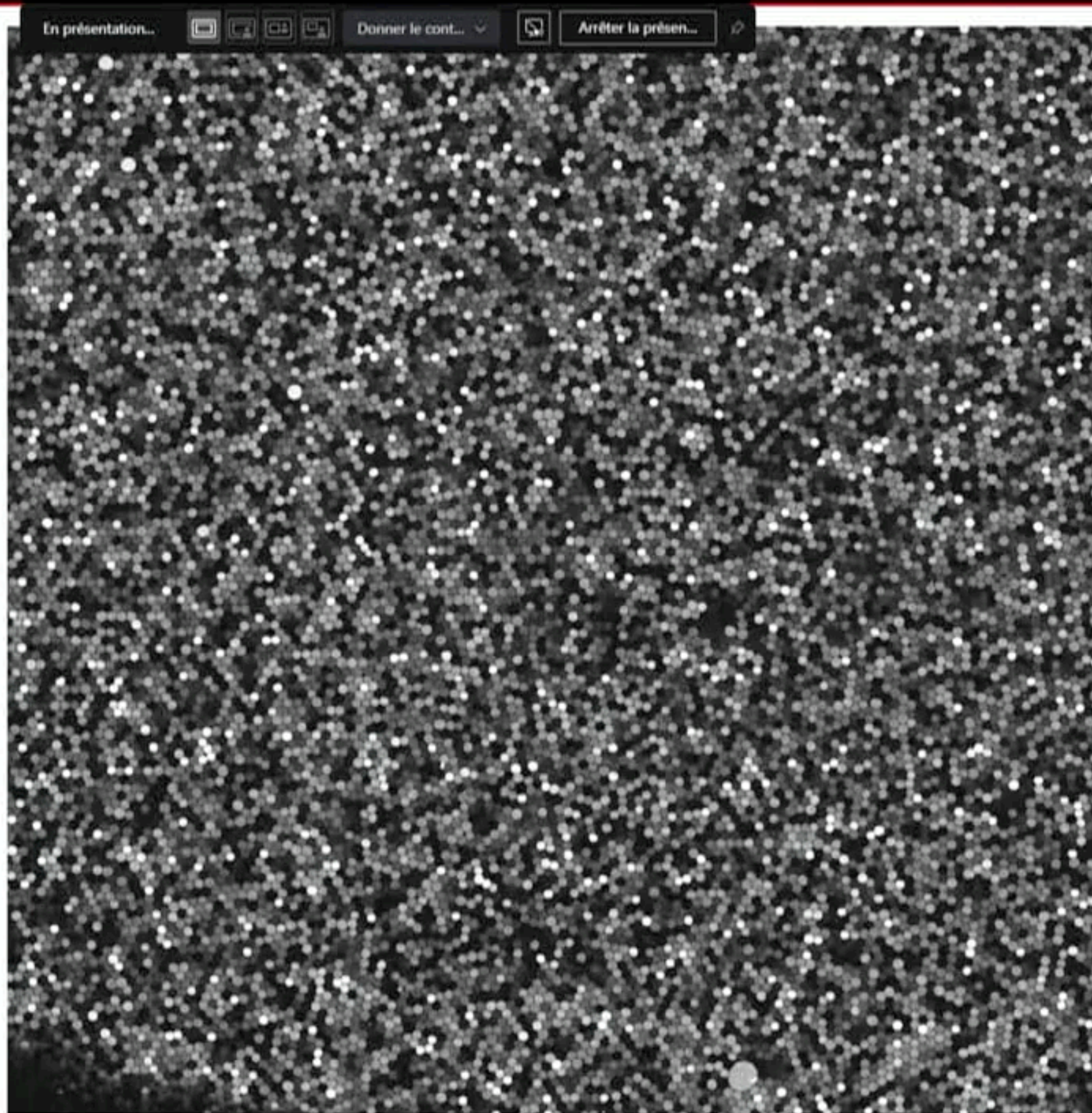




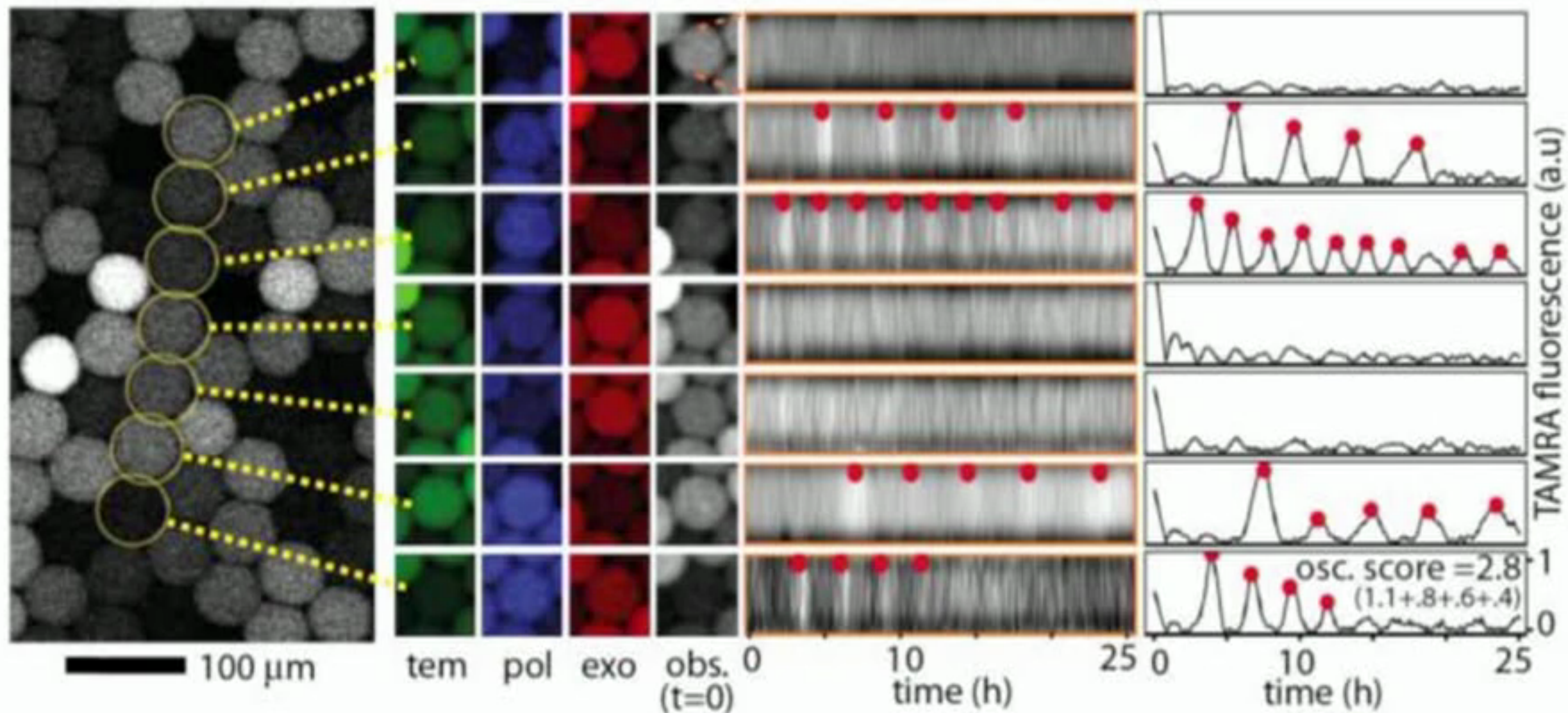
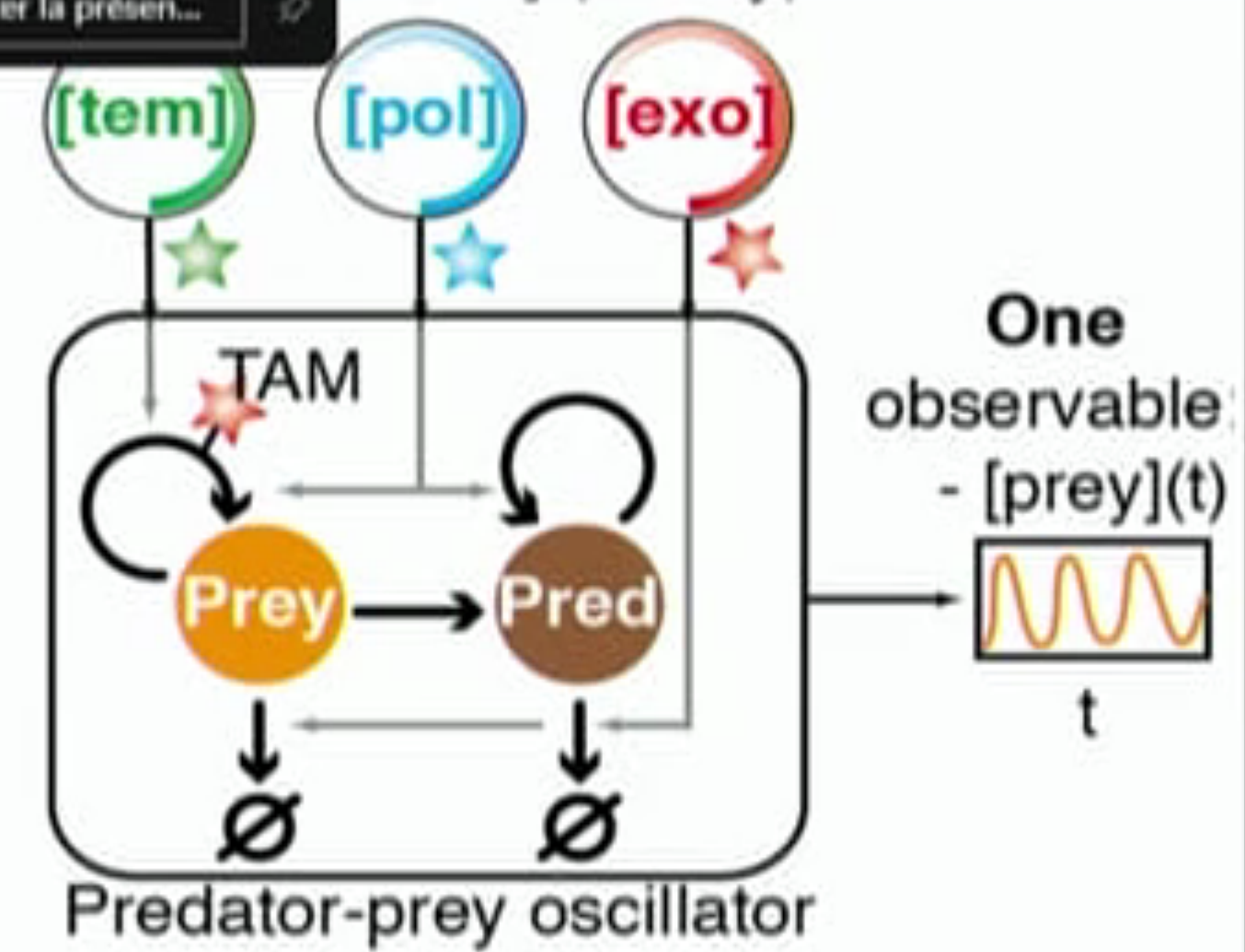
Bifurcations of oscillator using 3D Parameter scanning



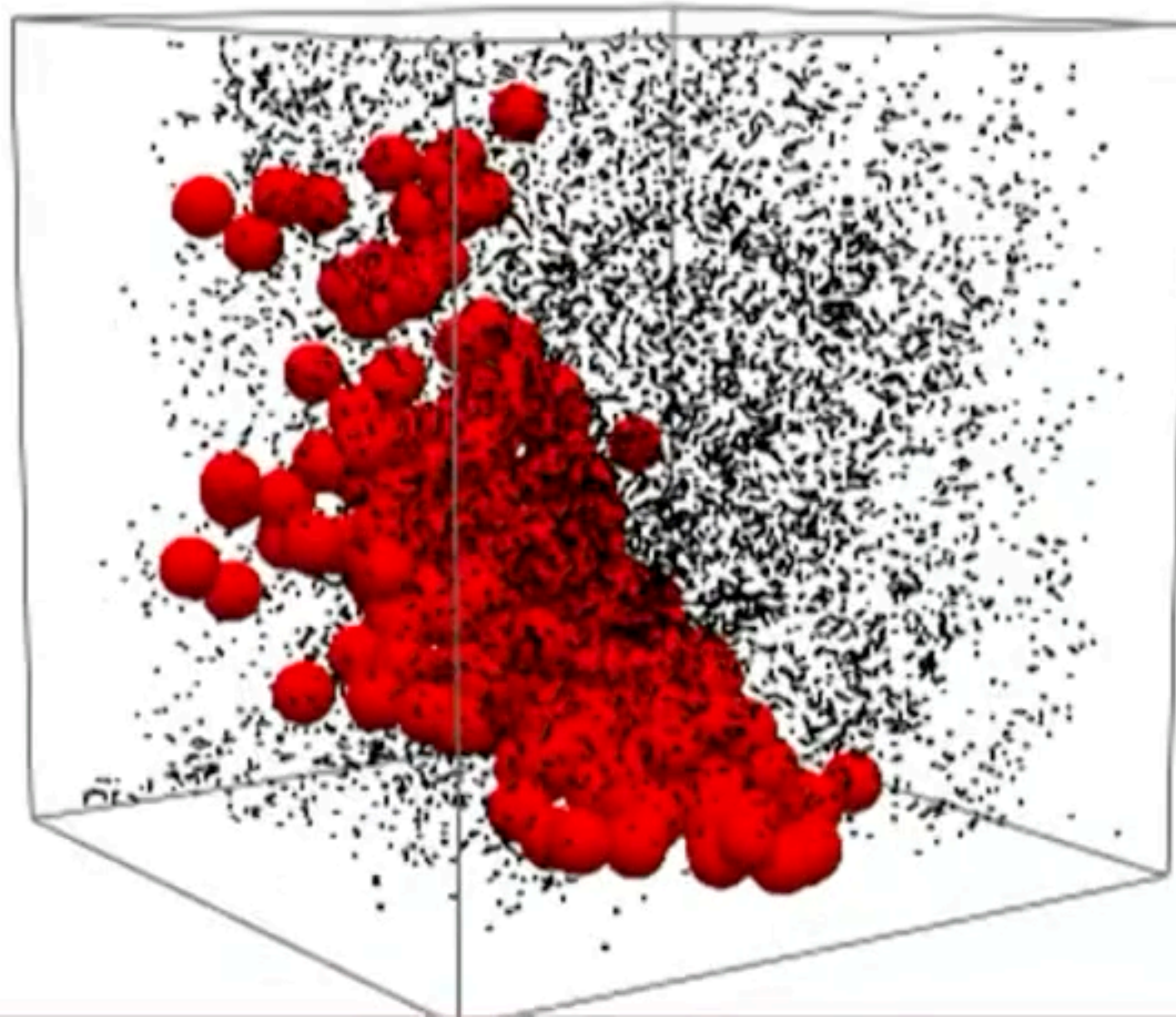
12000 individual
droplets
(i.e. a coverage of
search space of
3% in each
dimension)



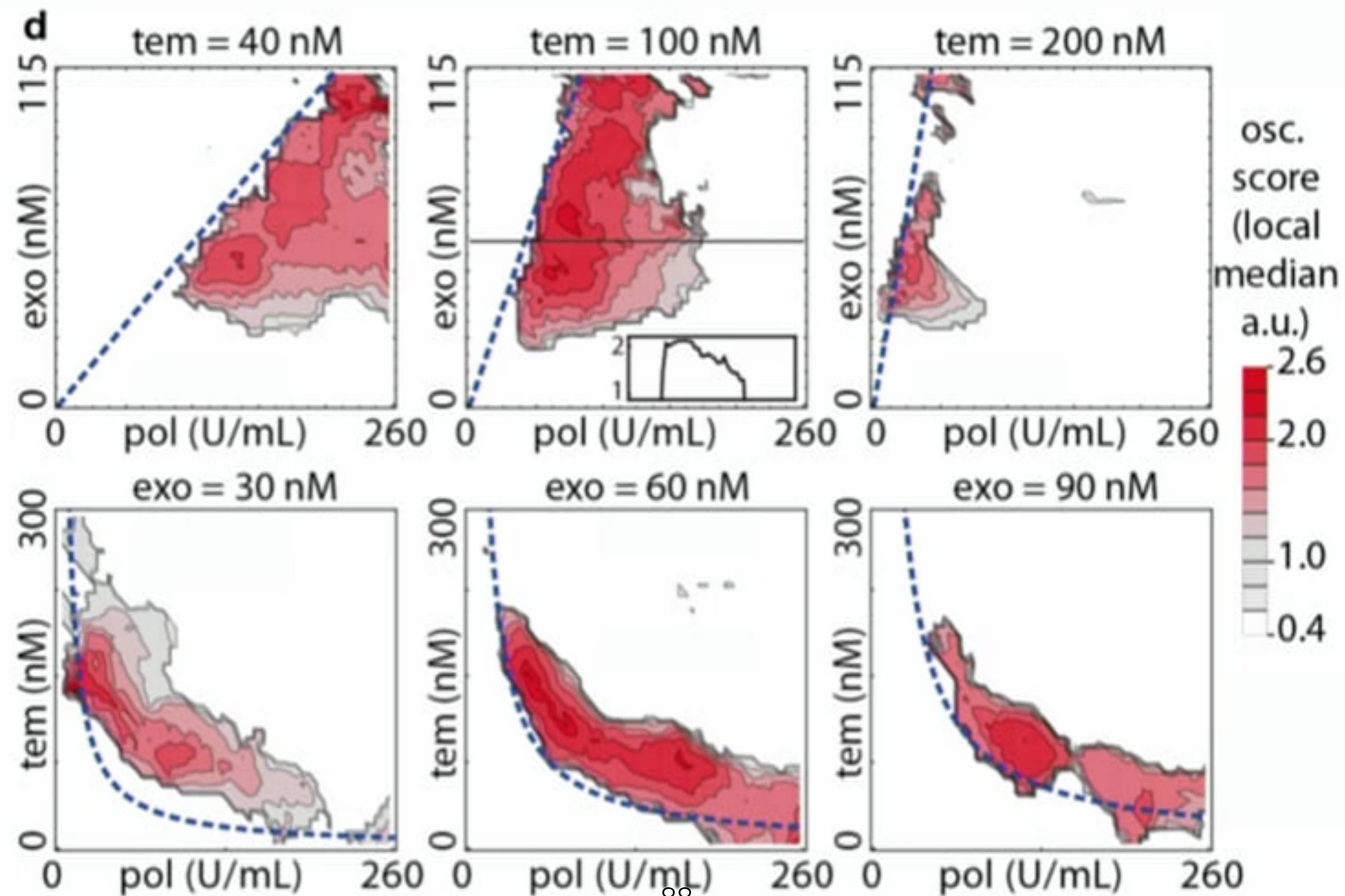
Bifurcations of oscillator using 3D parameter scanning



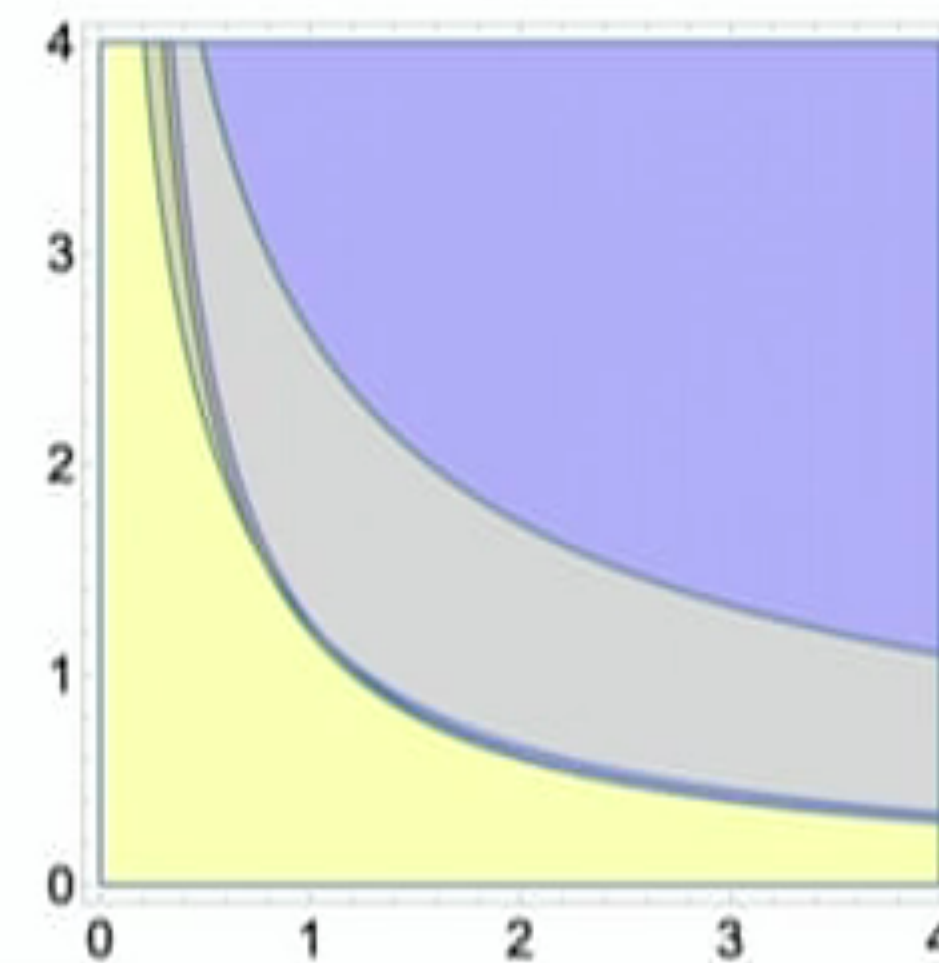
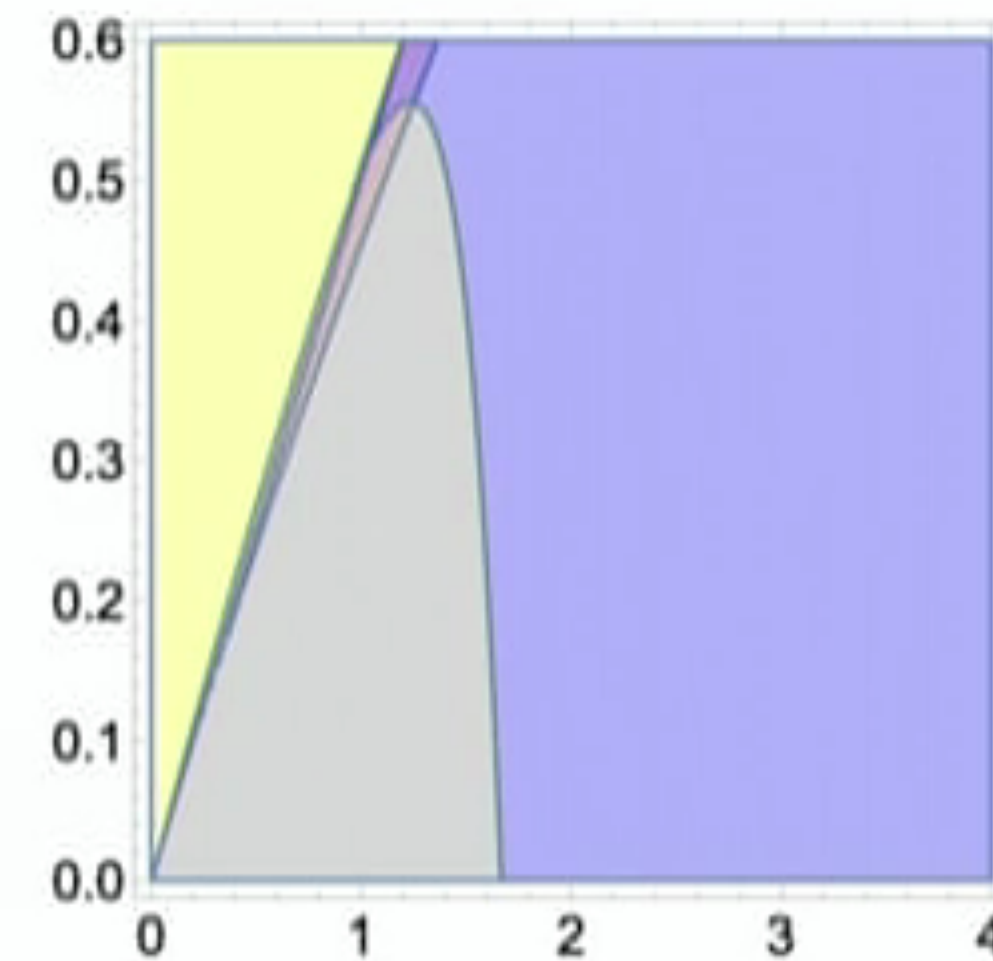
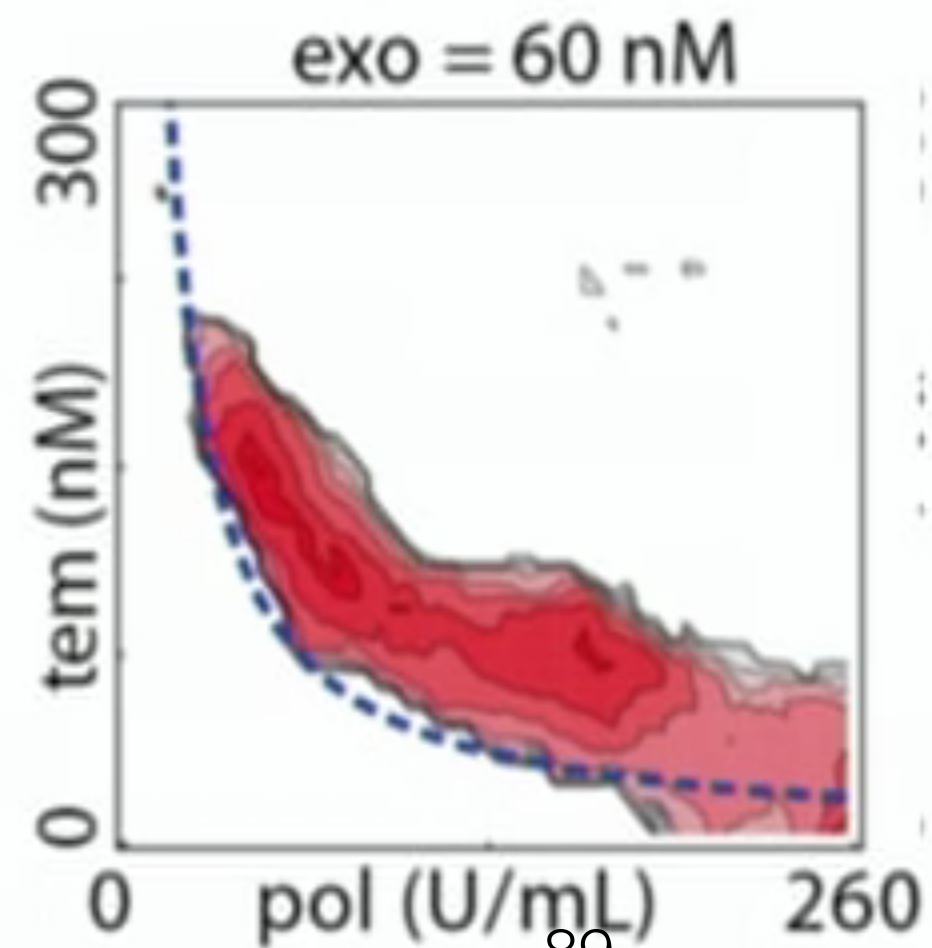
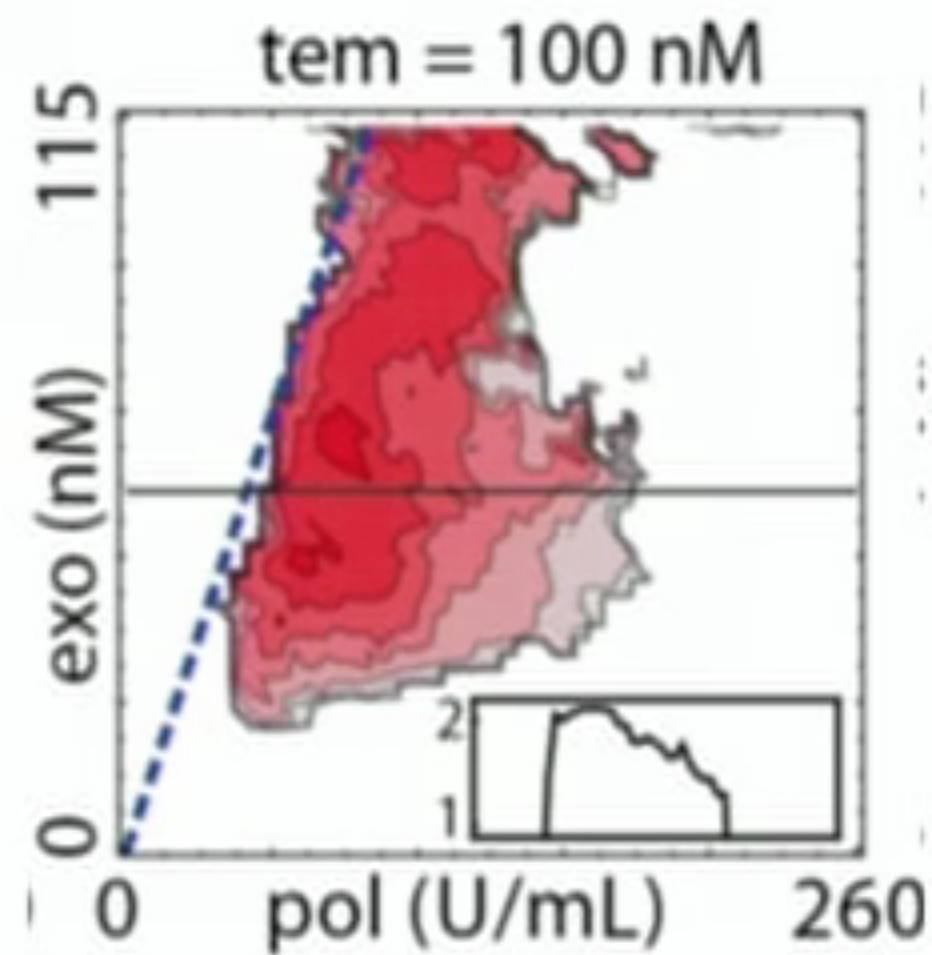
3-dimensional bifurcation diagram of an oscillator



3-dimensional bifurcation diagram of an oscillator

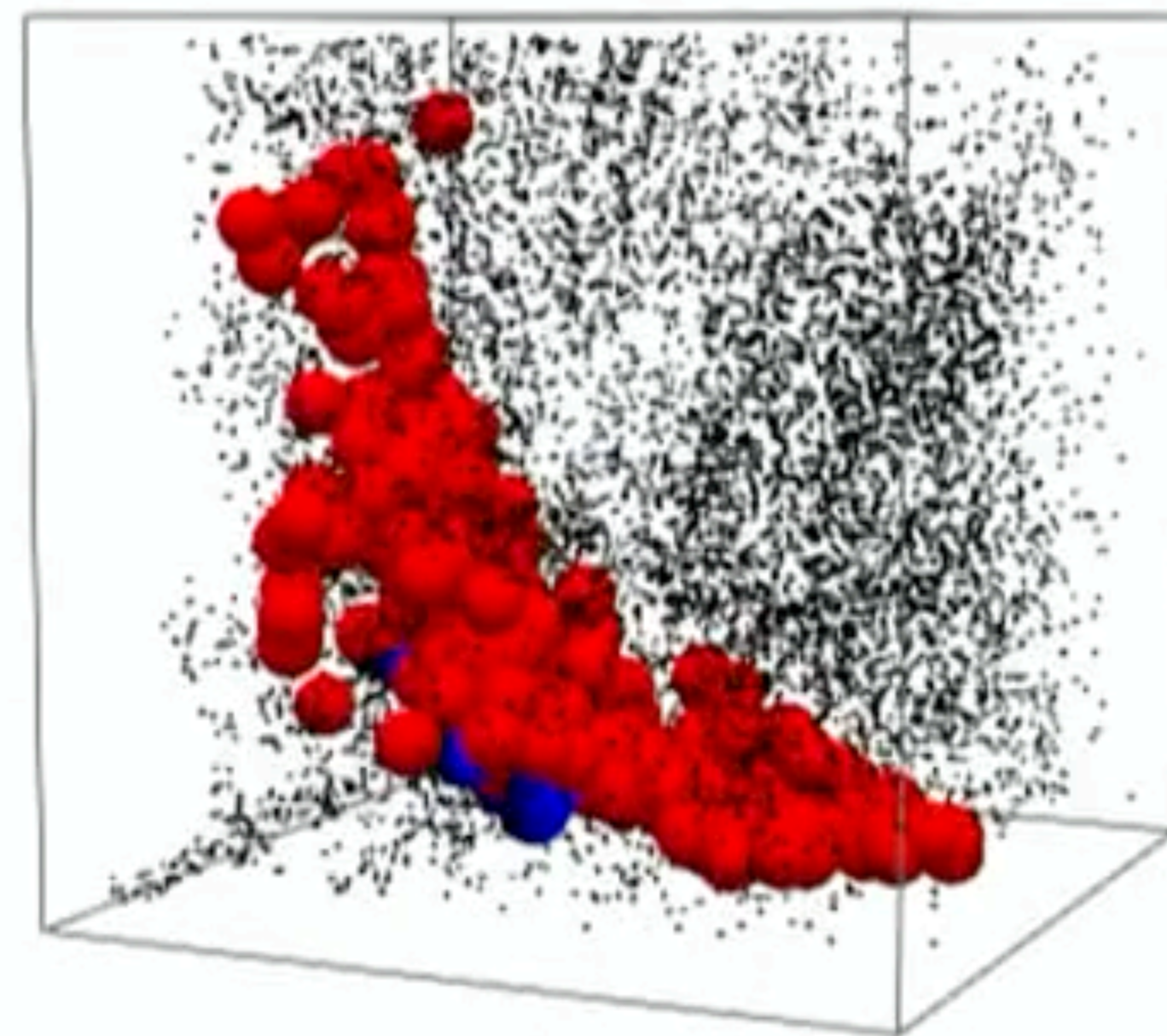
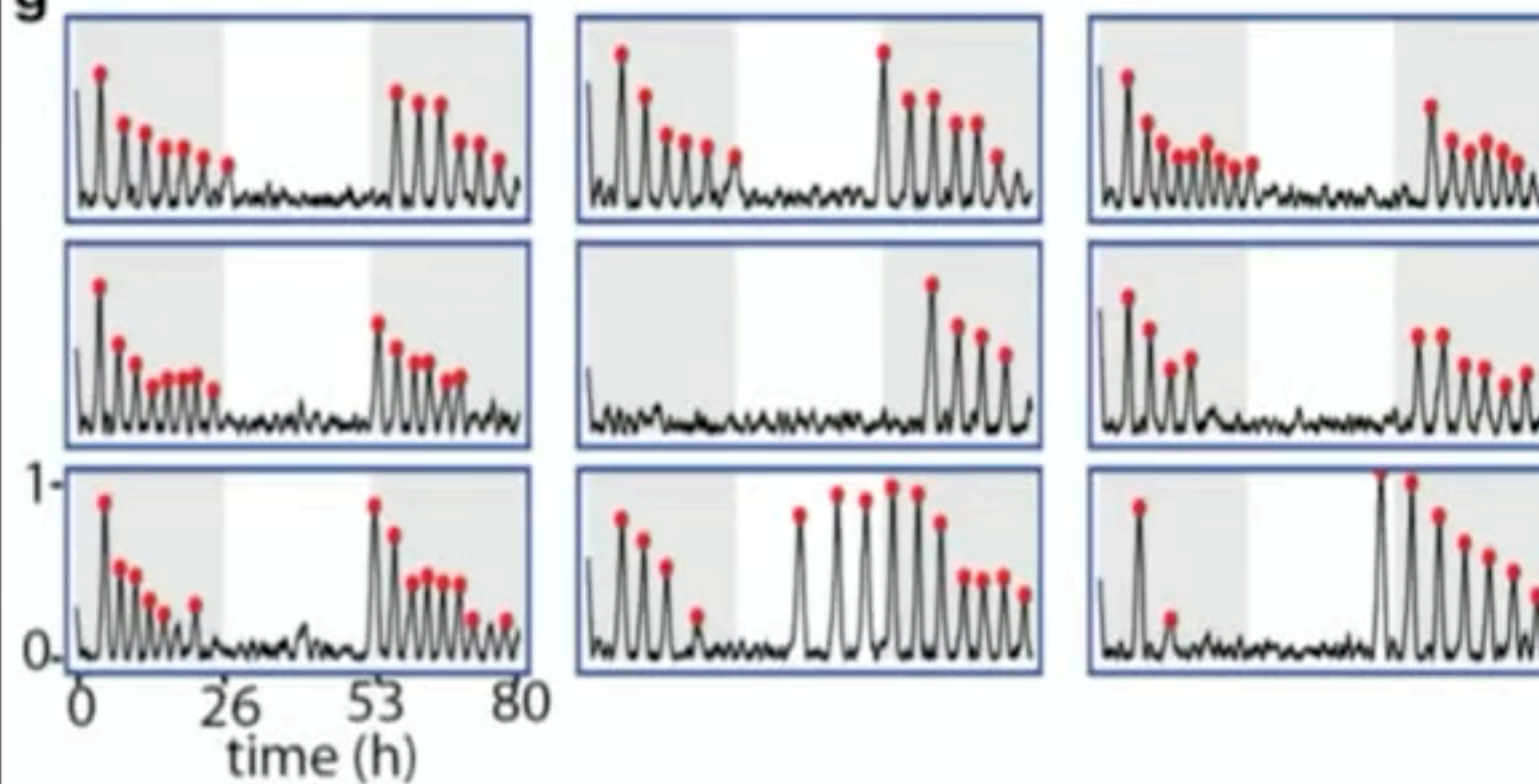


3-dimensional bifurcation diagram of an oscillator

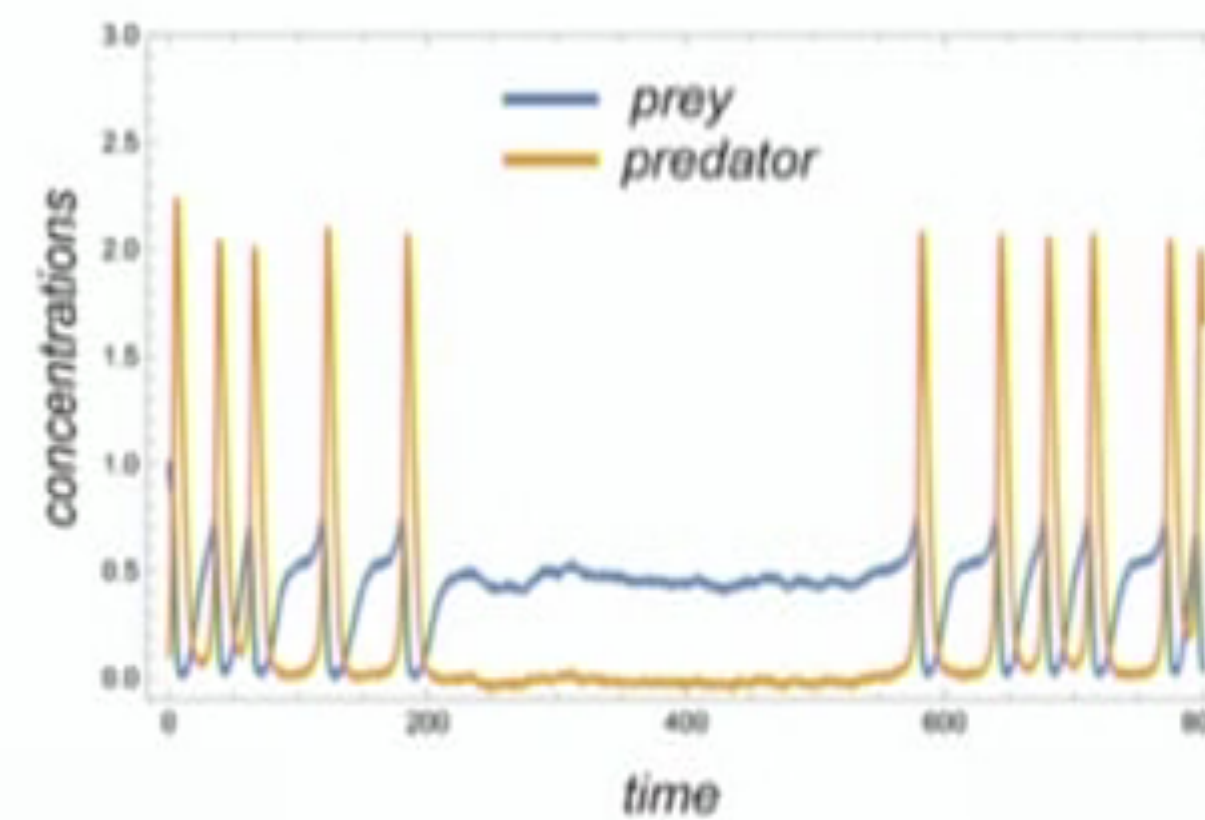
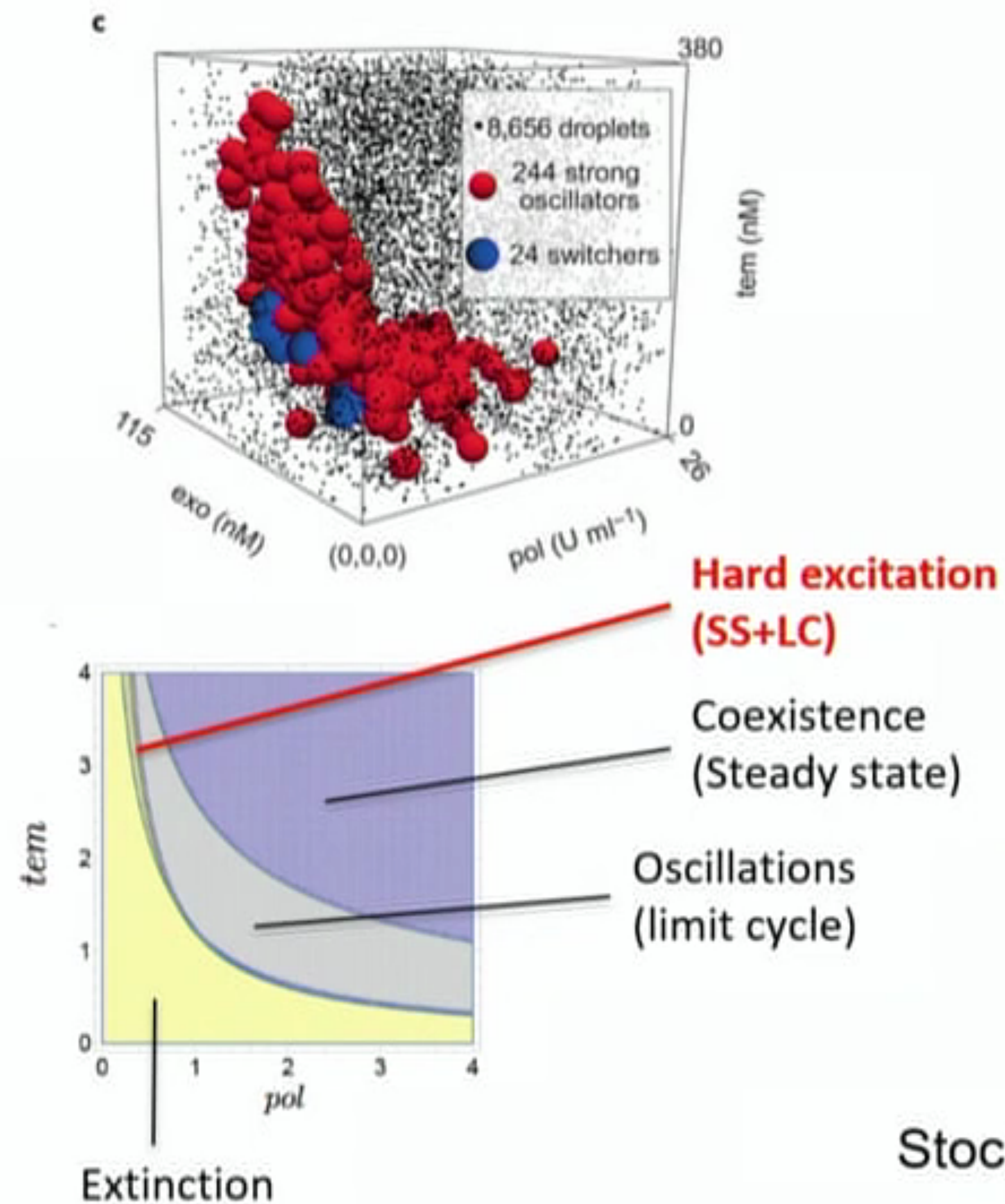


Stochastic bursters located at the Hopf bifurcation

g



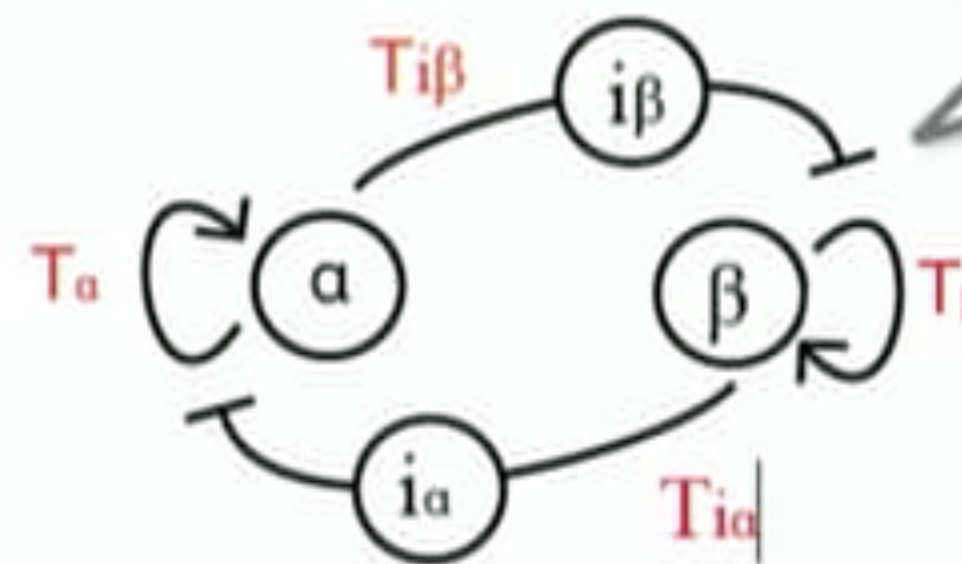
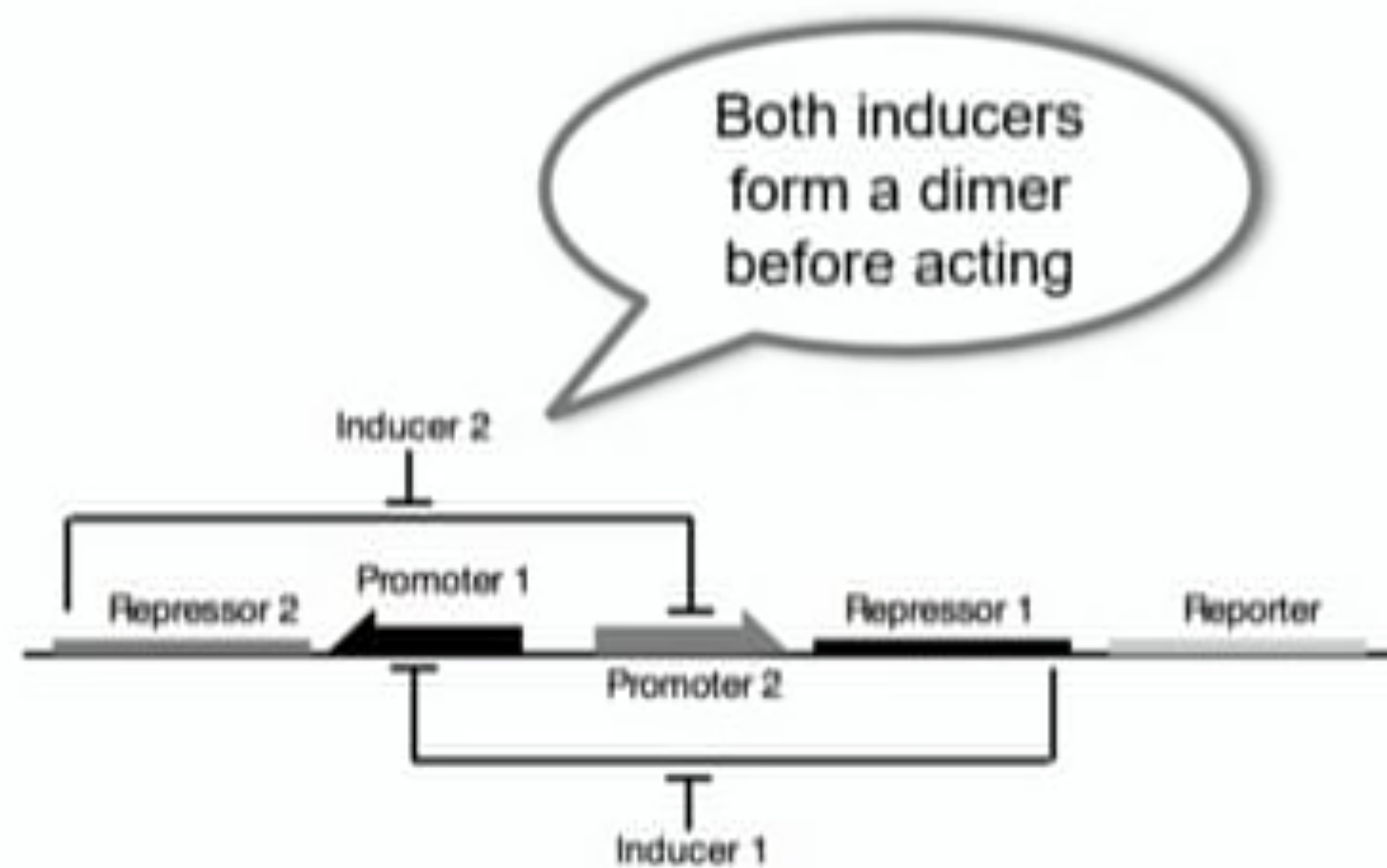
Bursting droplets



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



Positive Feed back loops are necessary for function

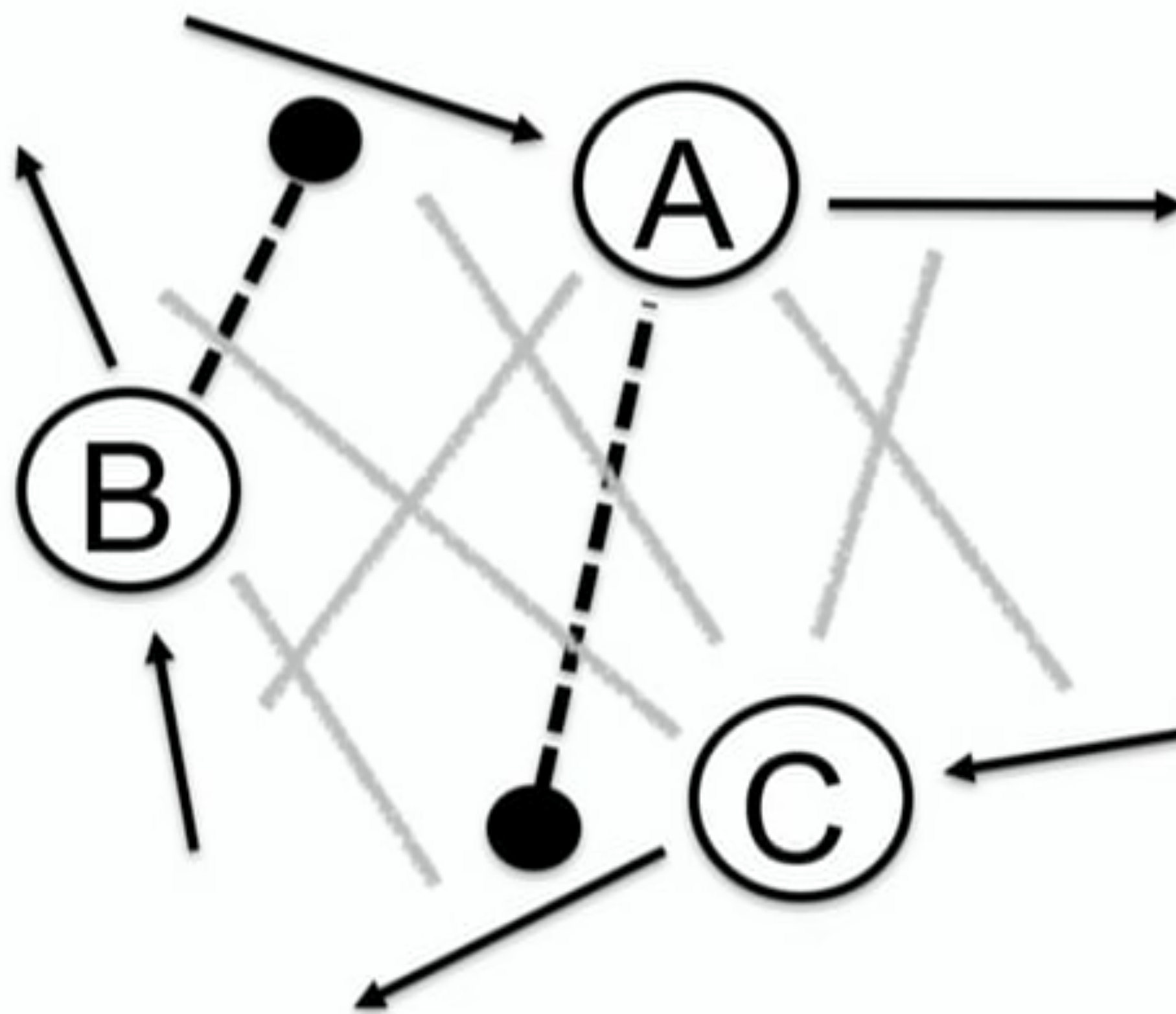
Learning by building

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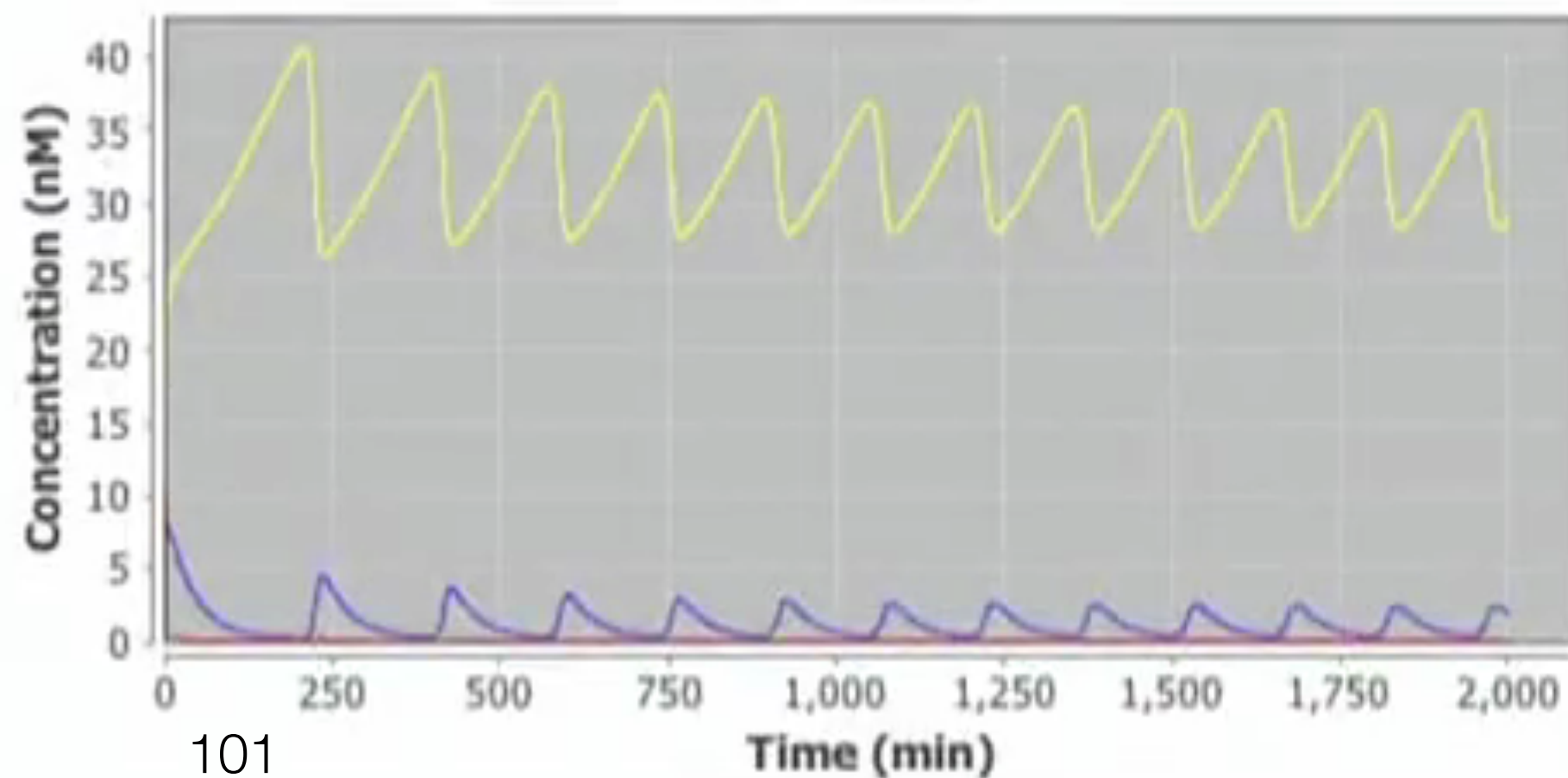
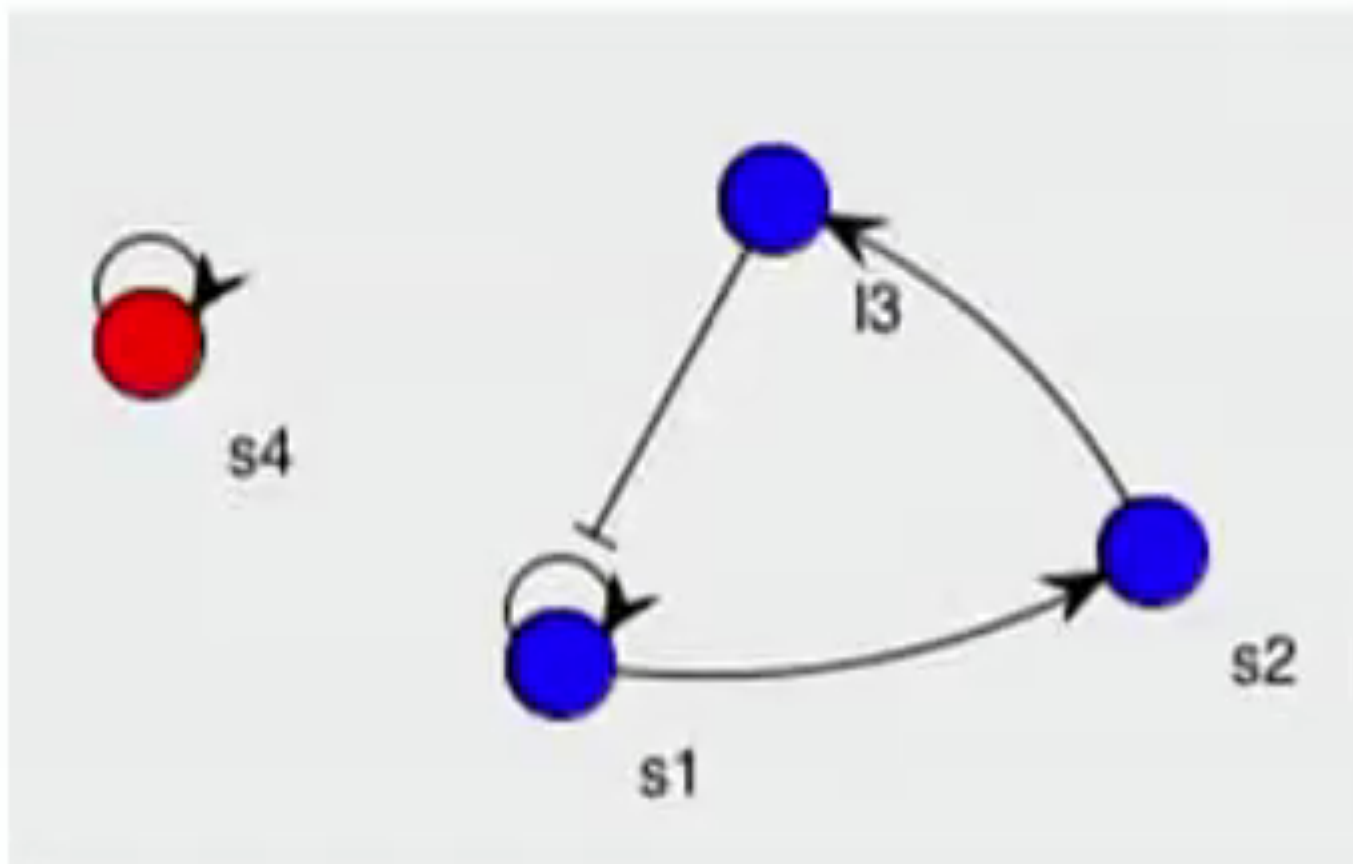
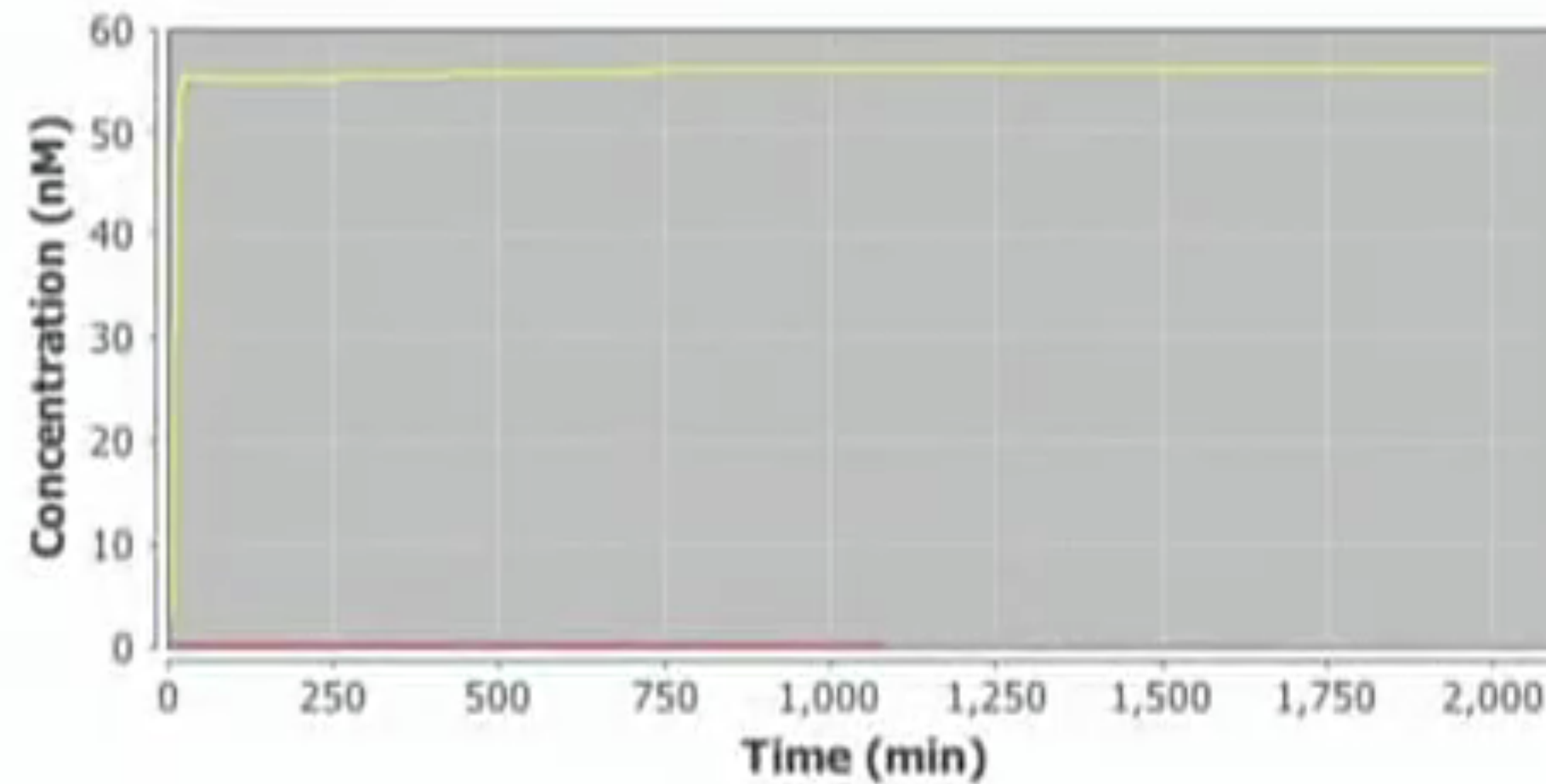
Parasites (chemical virus) are ubiquitous free riders when a powerful molecular machine is provided

The role of global couplings: Ideal versus real molecular networks

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

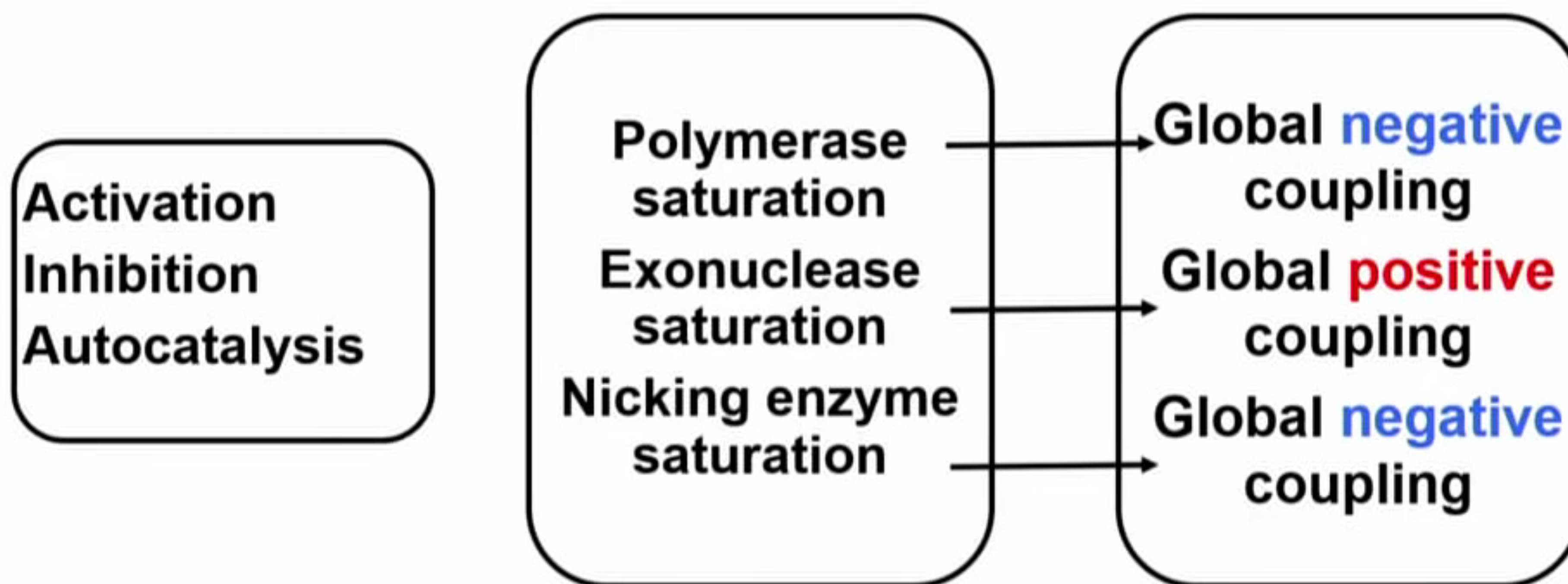


In (bio)-chemistry, networks with a “discrete” structure are an idealisation

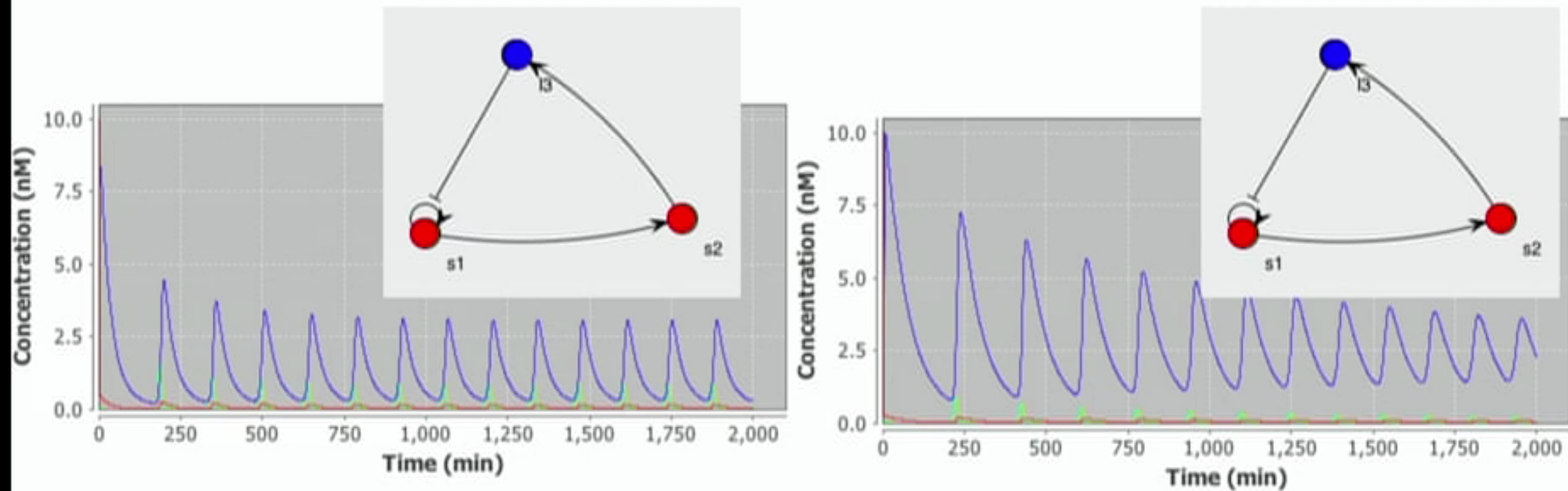


In chemistry, networks with a “discrete” structure are an idealisation

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



Global loads can negatively affect function



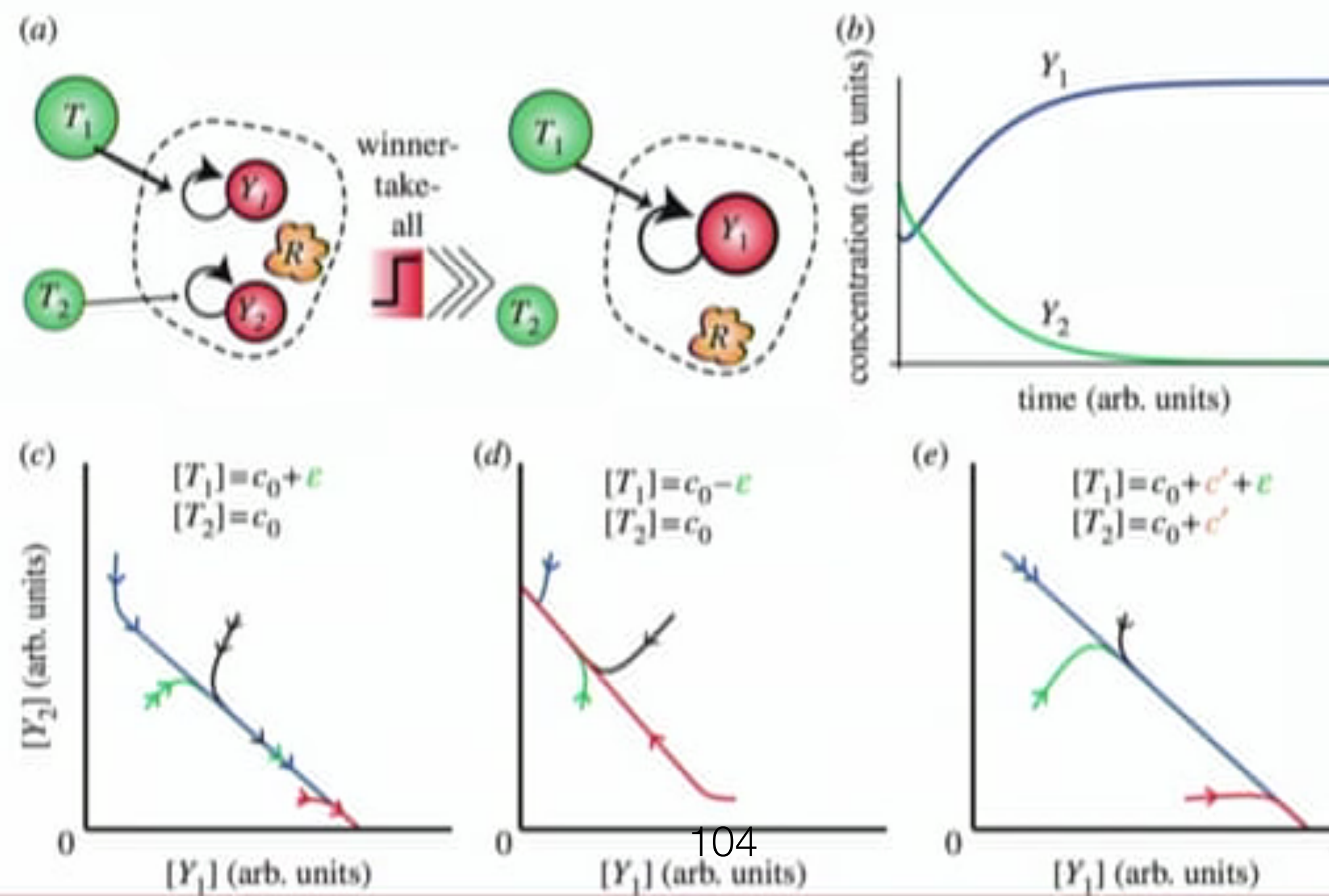
Global competition can also provide **new** functions

- 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.