

# Building and manipulating complex molecular systems

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ESPCI  PARIS



# Cell's unique properties

- Grow, divide and replicate
- Dissipative, active, responsive





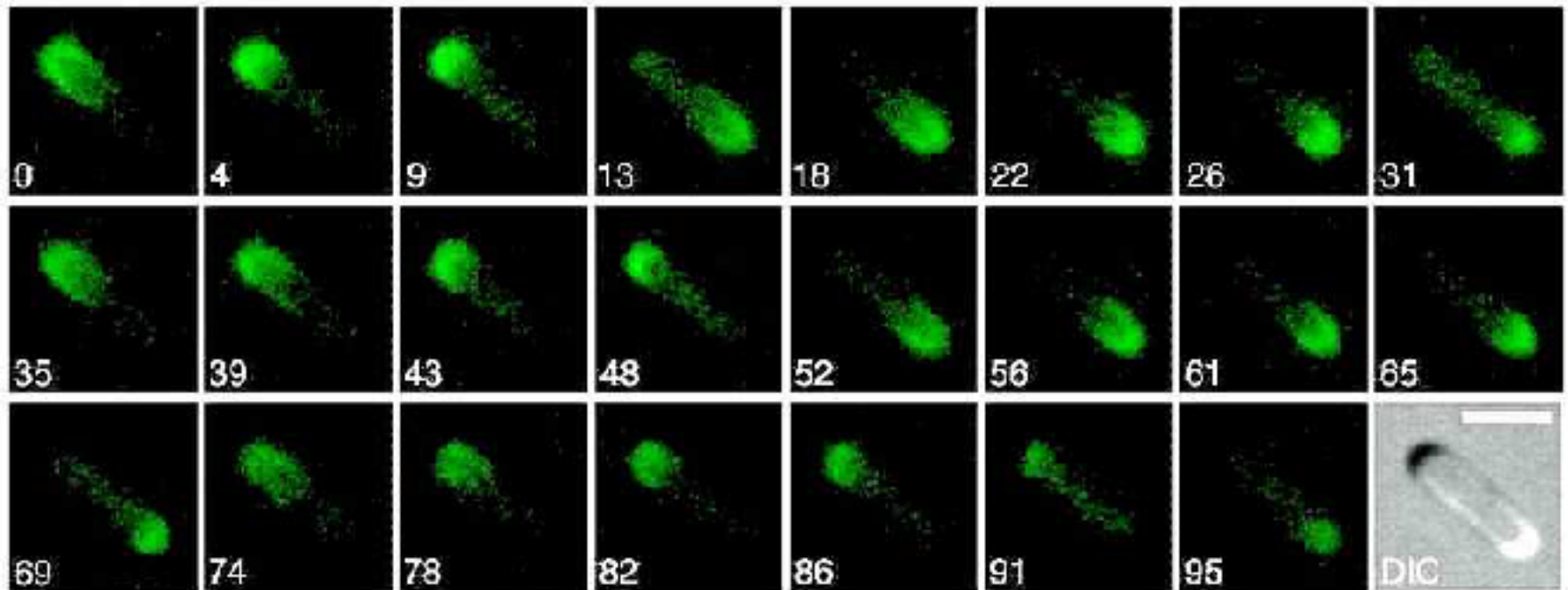
Neutrophil chasing a bacteria (D. Roger 1950)



# How does a bacteria finds its middle?

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

The min system (minC,D,E)

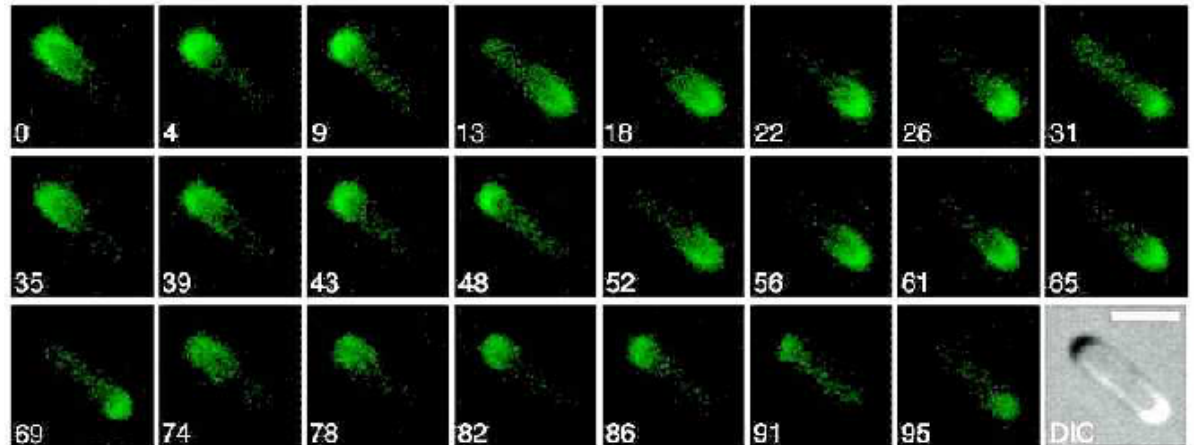
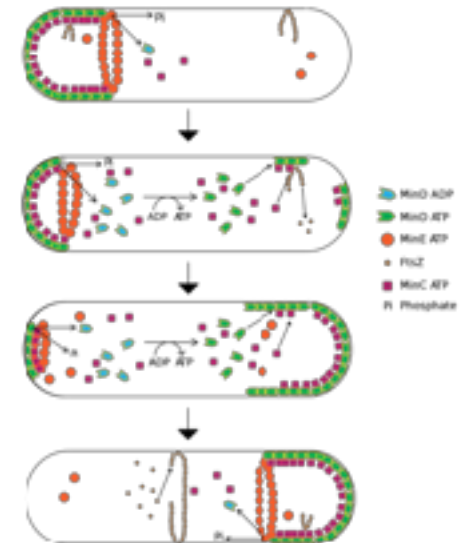
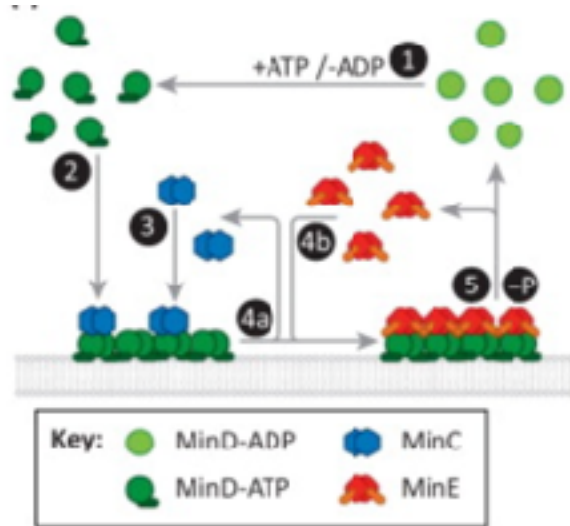
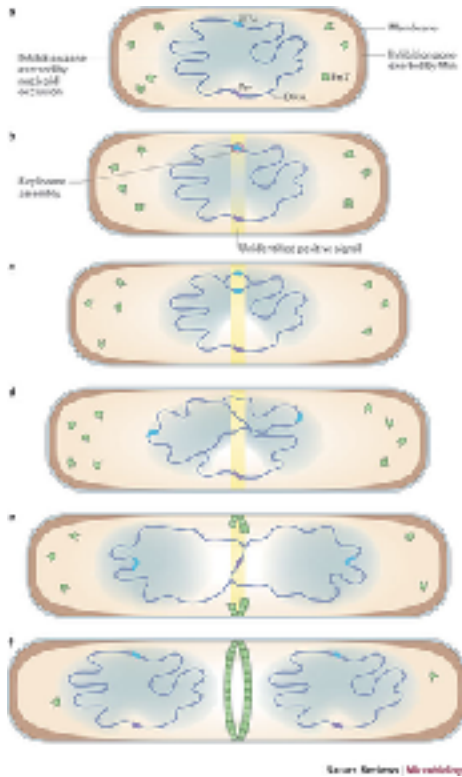


Raskin, D. M., and de Boer, P. A. J. (1999b). Rapid pole-to-pole oscillation of a protein required for directing division to the middle of *Escherichia coli*. PNAS **96**, 4971-4976



# A bacteria compute its midpoint with a few % accuracy

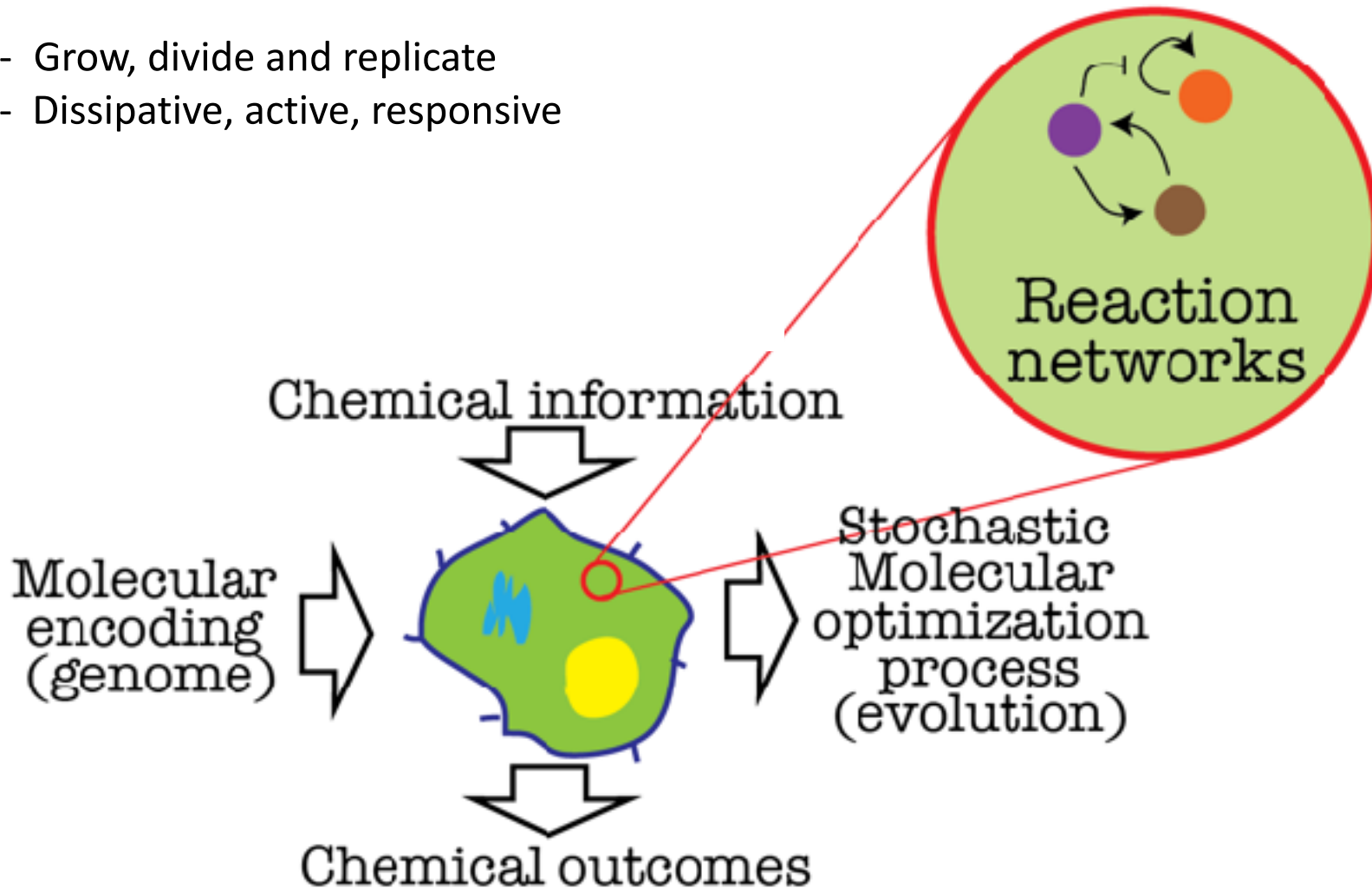
Three proteins called the min system (minC,D,E)



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

# Cell's unique properties

- Grow, divide and replicate
- Dissipative, active, responsive



# Mimicking cells as **information-processing** units

## **Ingredients:**

- Compartments
- Components
- Networks

## **Computation method:**

- Rational
- Optimization



# Mimicking cells as **information-processing** units

## The **rational** approach

Structure<>behavior at the level of networks  
Programmable thermodynamics (DNA)

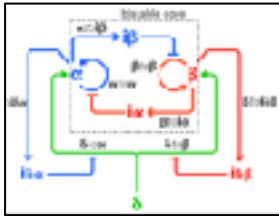


## The **stochastic optimization** approach

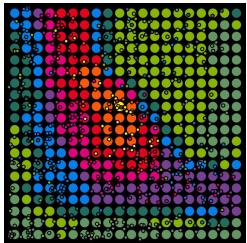
Structure<>function at the level of components  
(macromolecules)



# Outline



Molecular programming with the DNA toolbox



High-throughput landscape exploration for molecular program

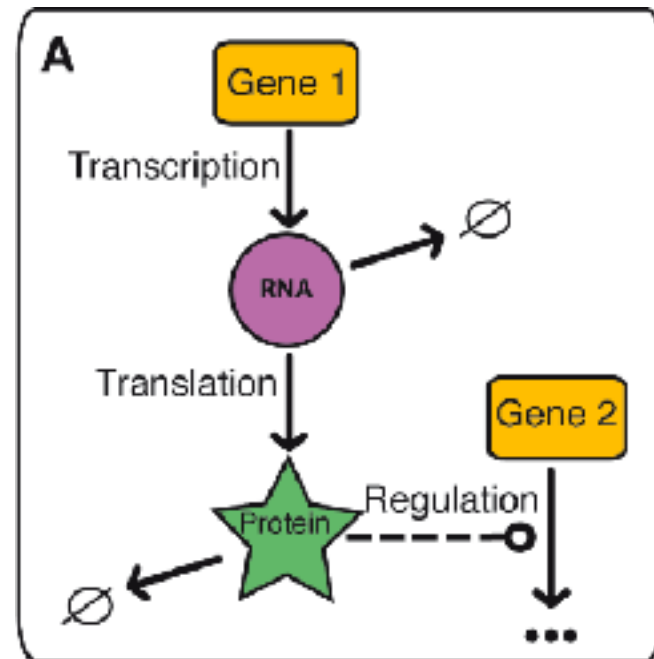
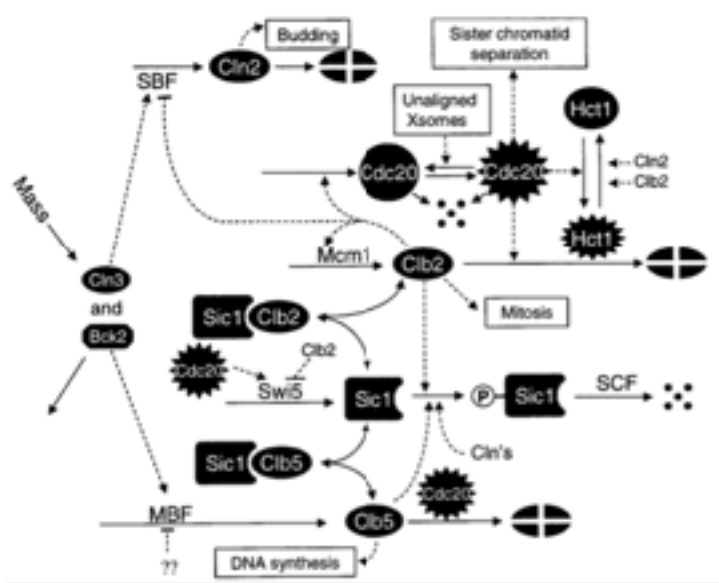


Programmable evolution

(Adèle Dramé Maigné PhD thesis)



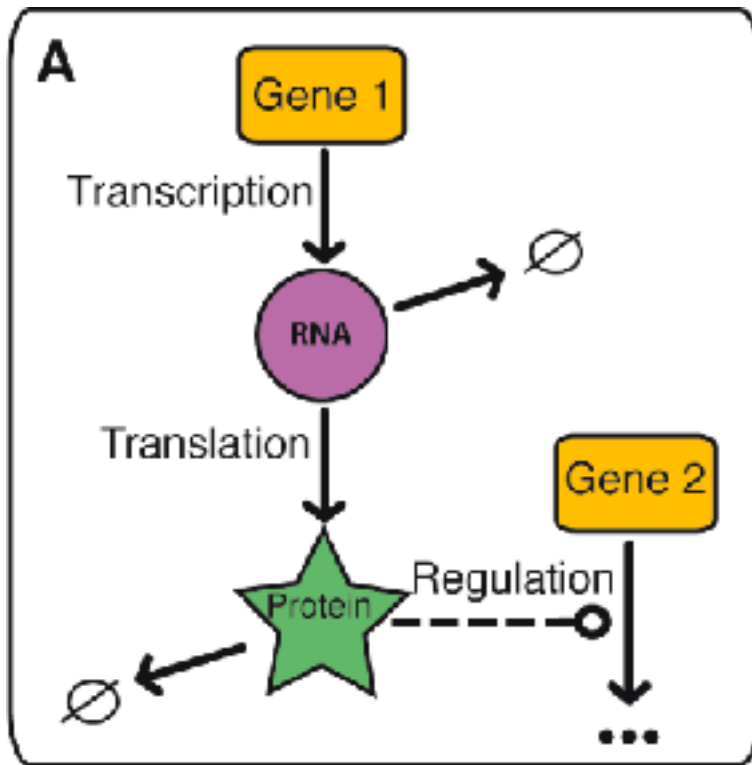
# The **rational** approach for information processing in chemistry



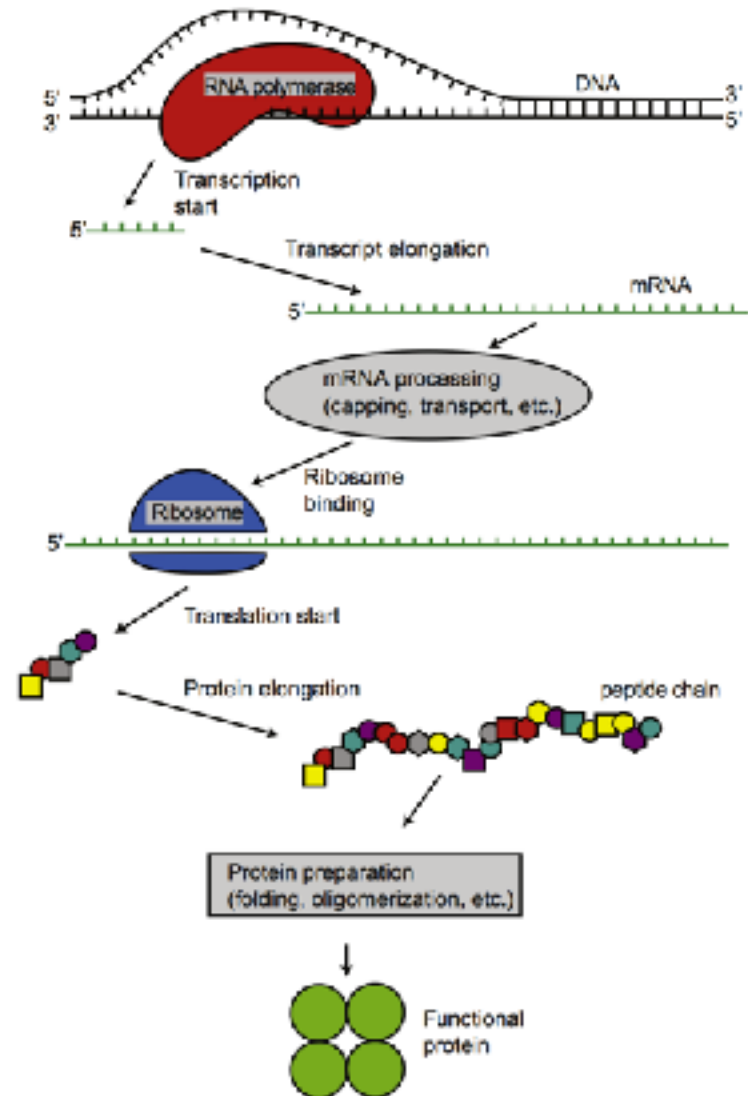
Regulatory network of the budding yeast cell cycle (Tyson 2006)



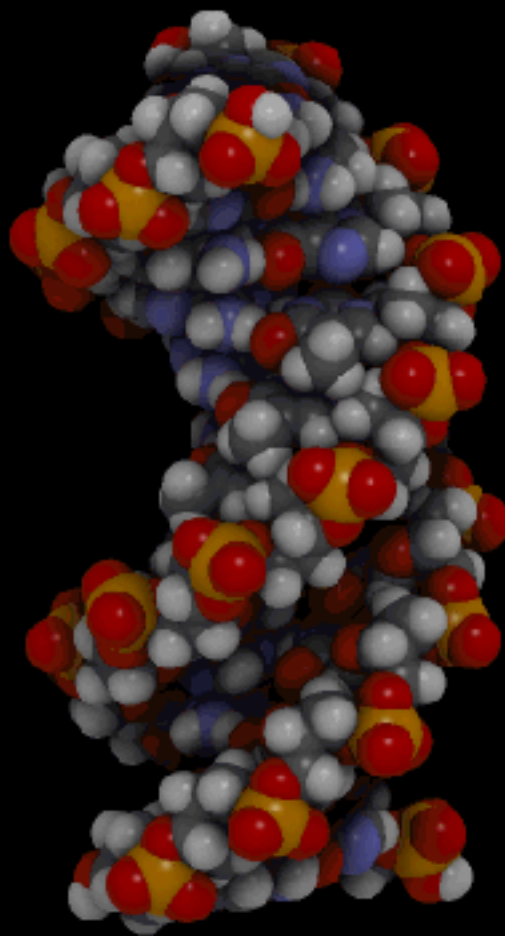
# A modular motif, based on complex, DNA encoded biochemistry



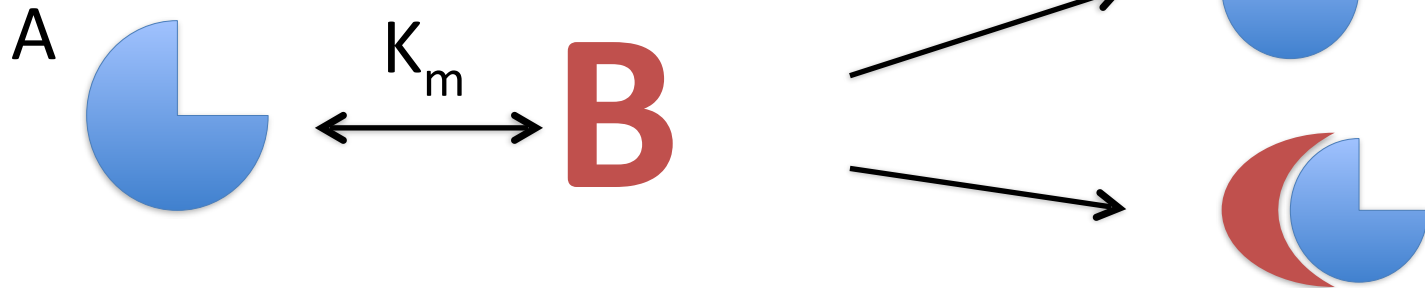
- ✓ A gene can activate another gene
- ✓ A gene can repress another gene



# The **rational** approach for information processing in chemistry



# Programmability of DNA



"DNA as a universal substrate for chemical kinetics" PNAS 2010



# Programmability of DNA



"DNA as a universal substrate for chemical kinetics" PNAS 2010

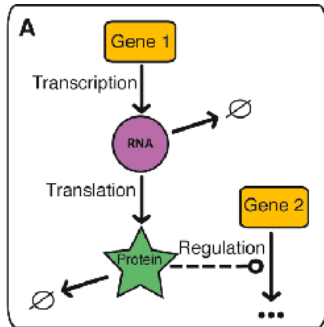
## Enzymes:

Copy: Polymerases

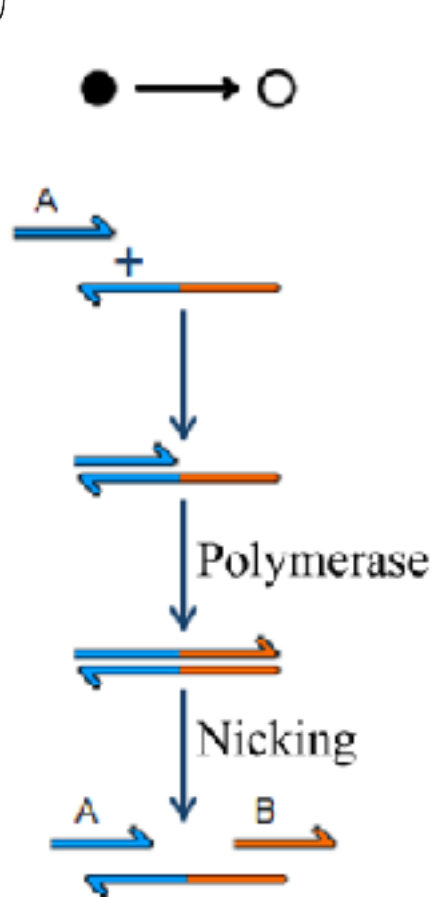
Cut-paste: Restriction enzymes and Ligases

Delete: Nucleases

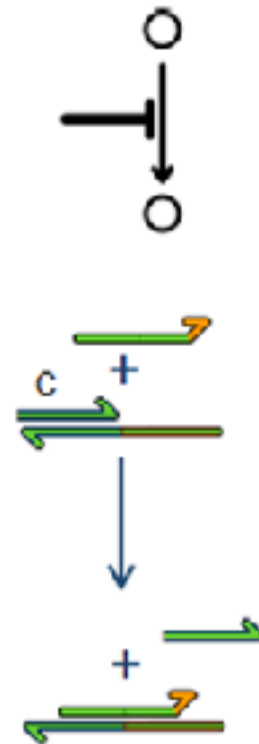
# Programmable networking strategy using DNA oligos



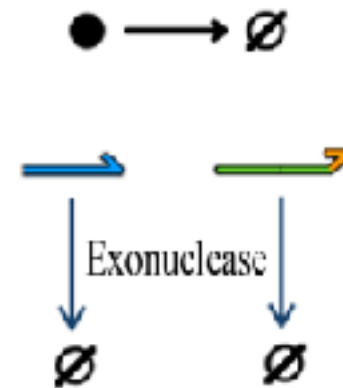
(a) Activation



(b) Inhibition



(c) Degradation



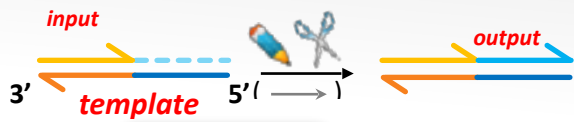
# The PEN DNA toolbox

## A molecular programming language

### PEN toolbox

Polymerase Exonuclease Nickase

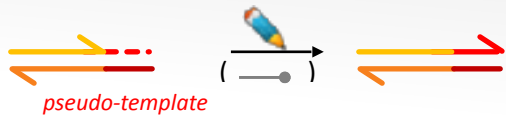
#### ACTIVATION



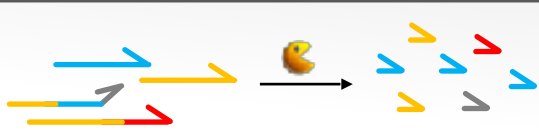
#### INHIBITION



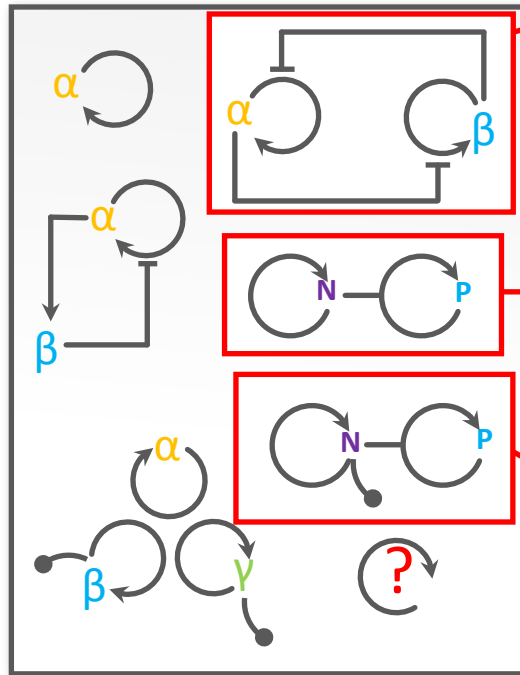
#### DEACTIVATION



#### DEGRADATION

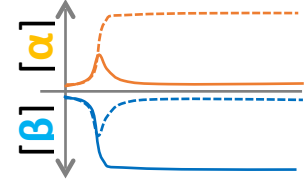


### Circuits



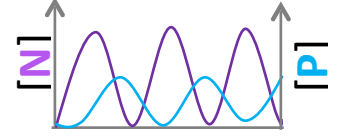
#### Bistable switch

Padirac, A. et al. PNAS 2012



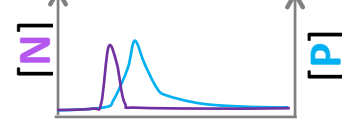
#### Predator-Prey

Fujii, Rondelez, ACS Nano 2013

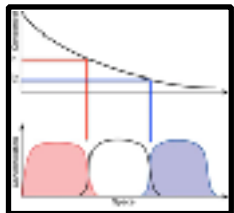


#### Excitable

Montagne et al. Nat. Comm. 2016

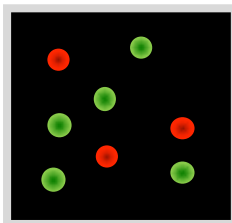


Synthesis and materialization of a reaction-diffusion French flag pattern.



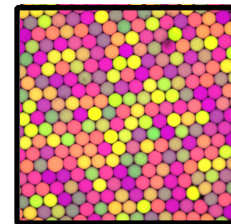
Zadorin, et al. Nat Chem. 2017

Microscopic Agents programmed by DNA circuits.



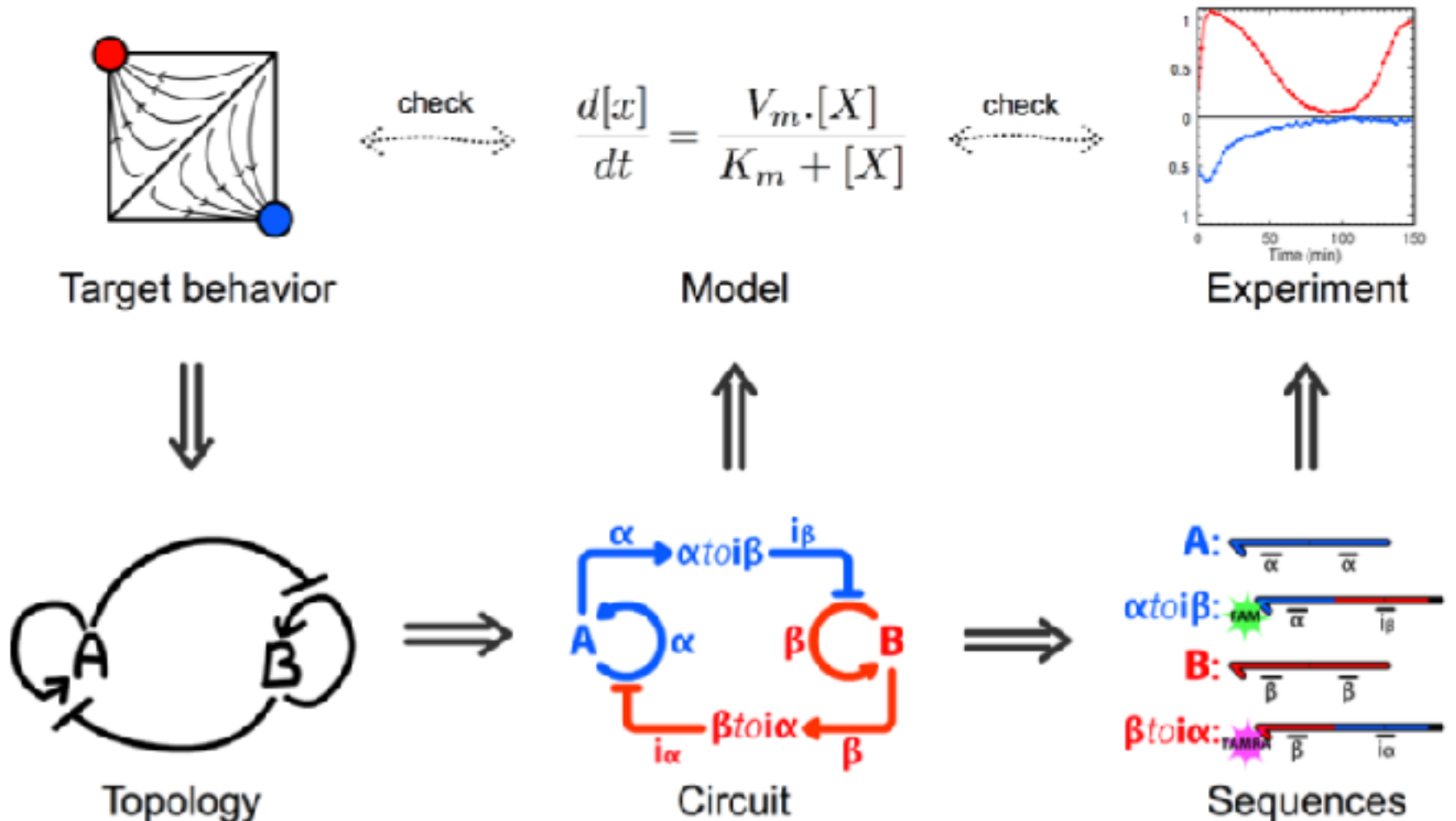
Gines, et al. Nat. Nanotech. 2017

High-resolution mapping of bifurcations in nonlinear DNA circuits

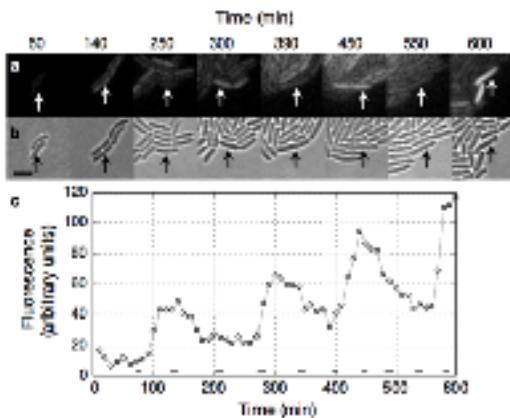
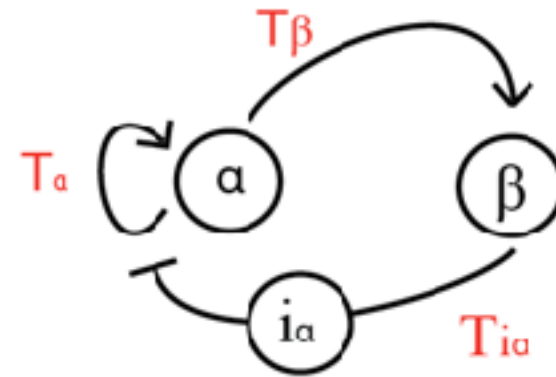
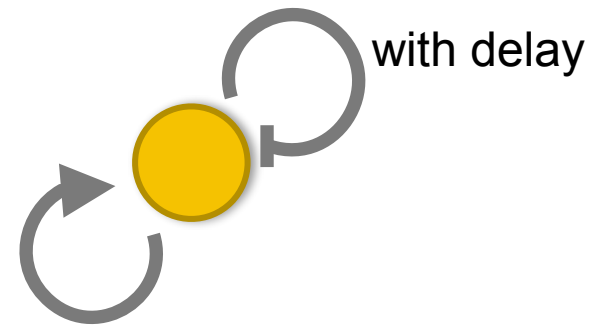
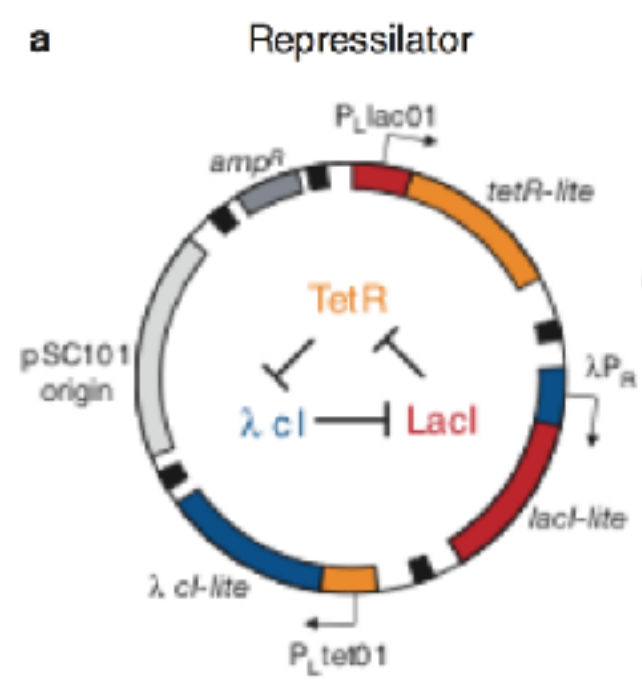


Genot, Baccouche et al. Nat. Chem. 2016

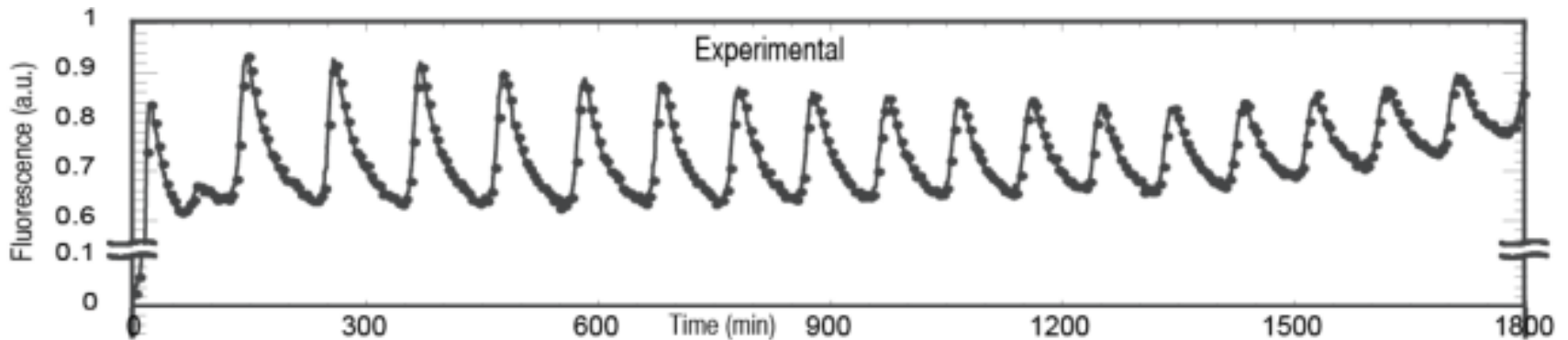
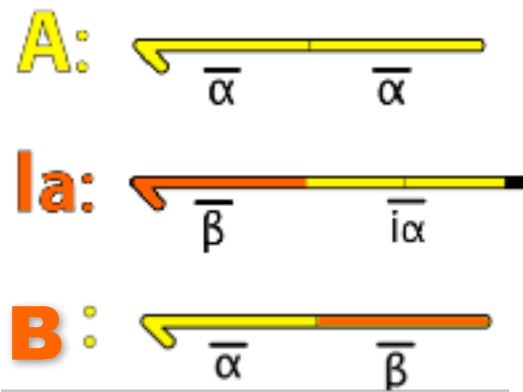
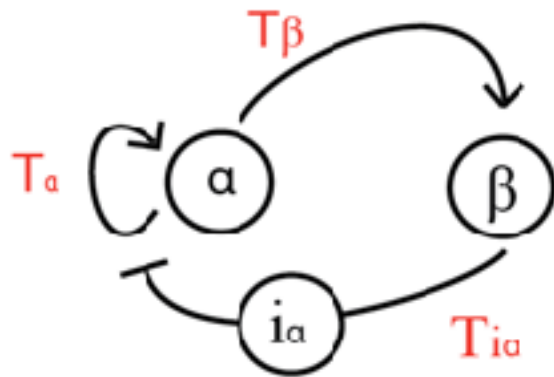
# Synthesizing chemical reaction networks with dynamical behaviours



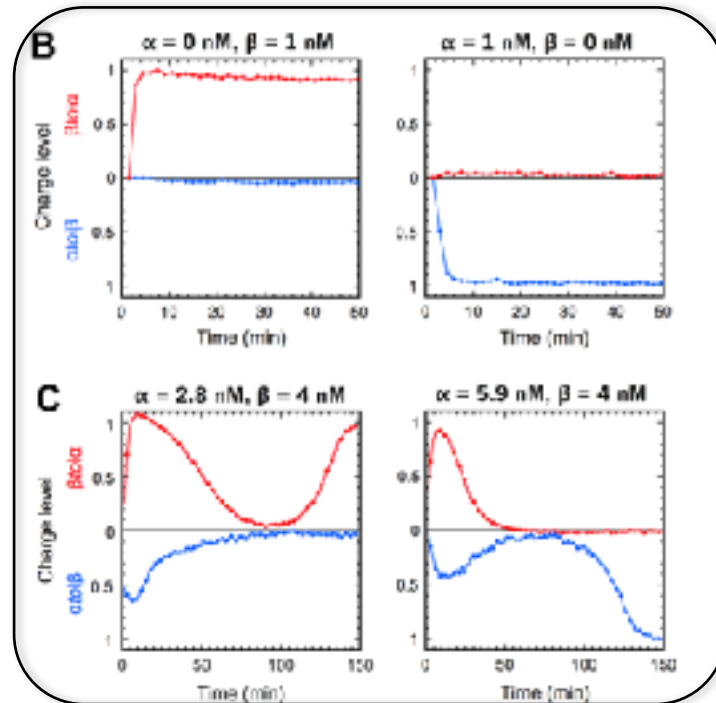
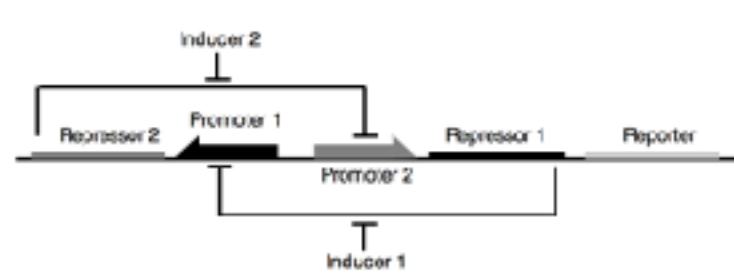
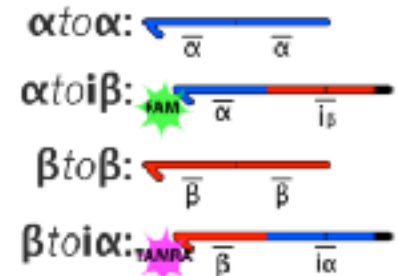
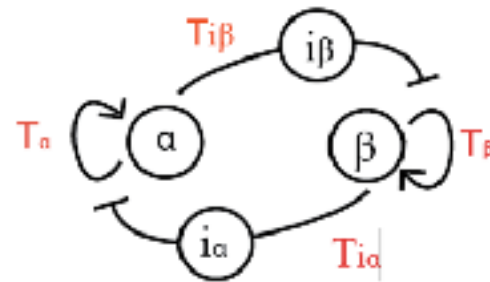
# An in vitro clock (oscillator)



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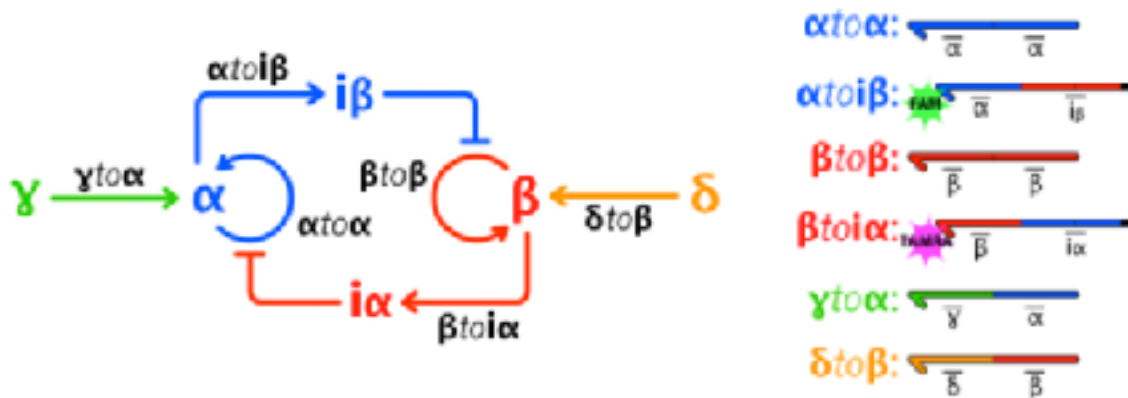
# A Toggle switch



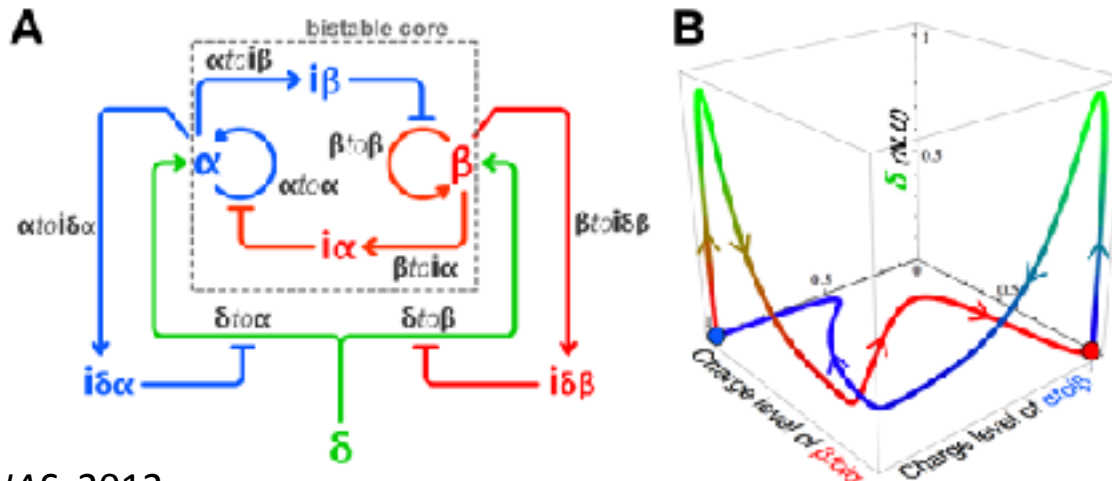


# Toggle switch => Push-push button

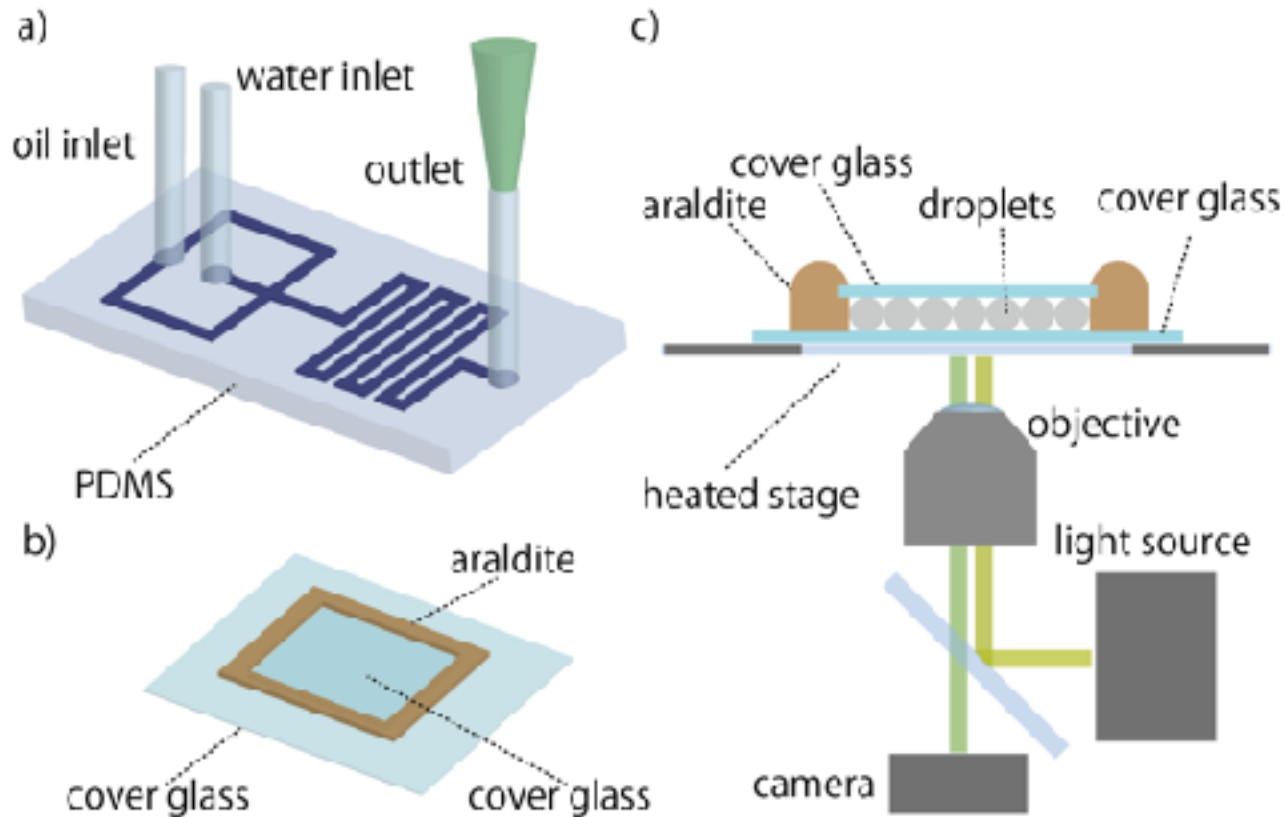
First, add input modules to force-switch the bistable back and forth



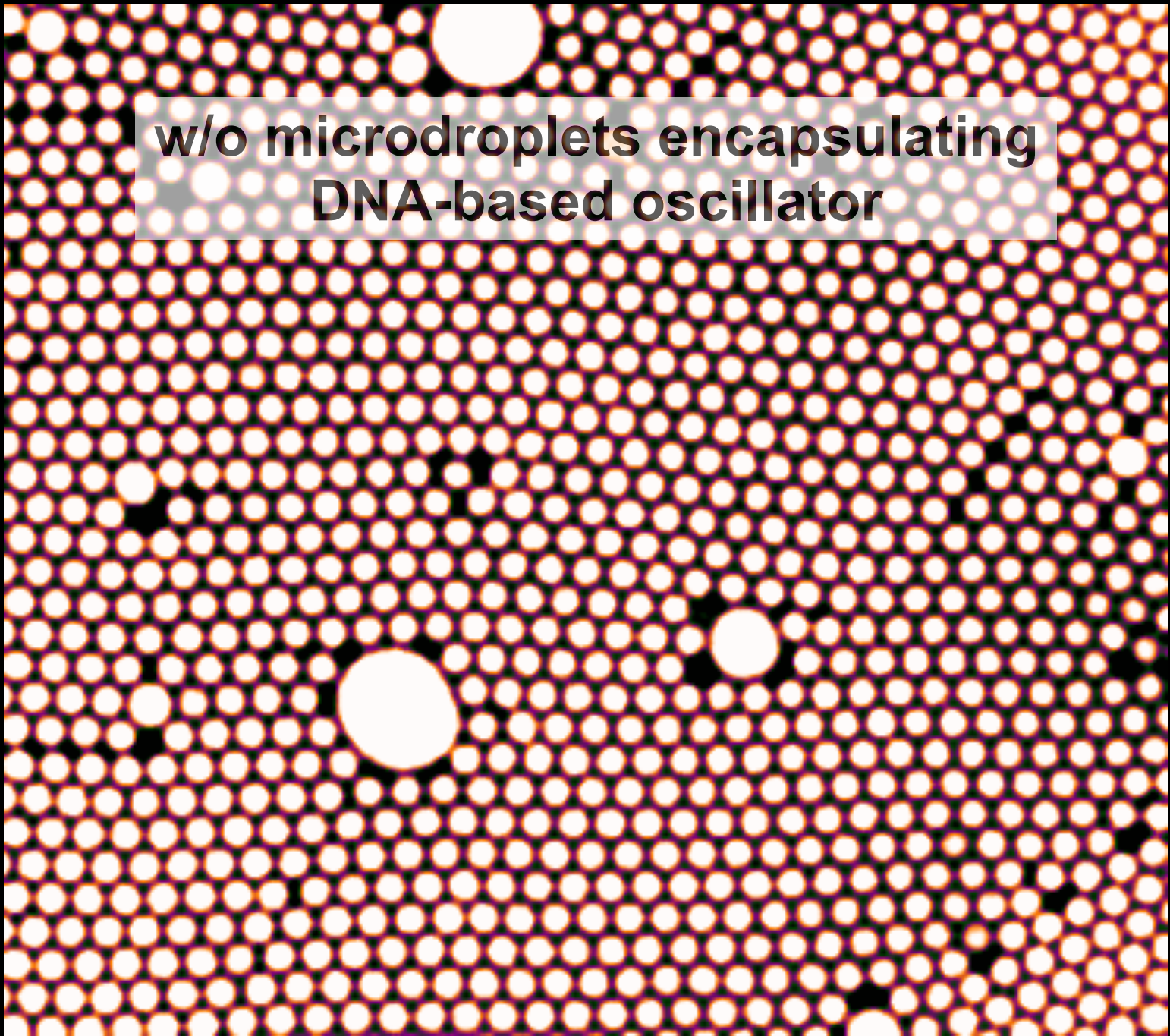
Second, fold the topology to accept a single input and combine with current state



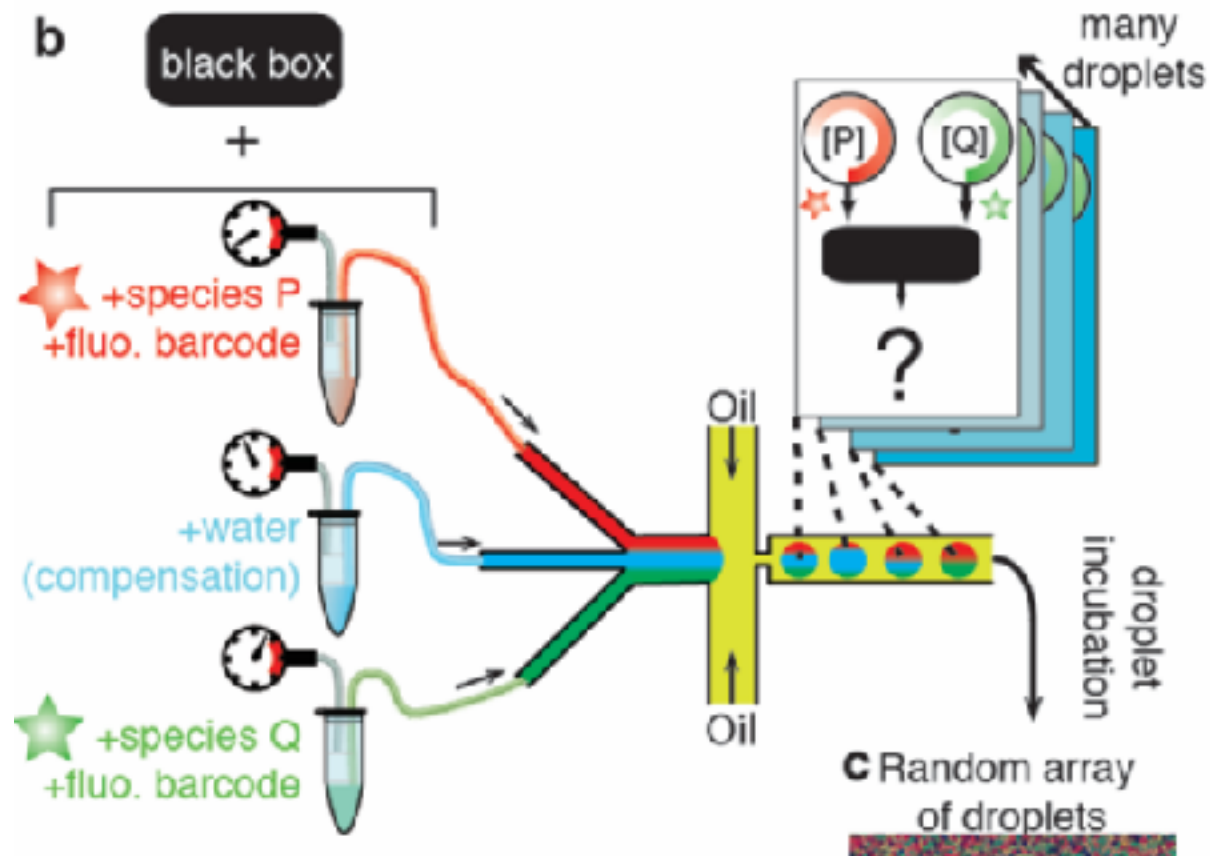
# Micro-compartments



**w/o microdroplets encapsulating  
DNA-based oscillator**

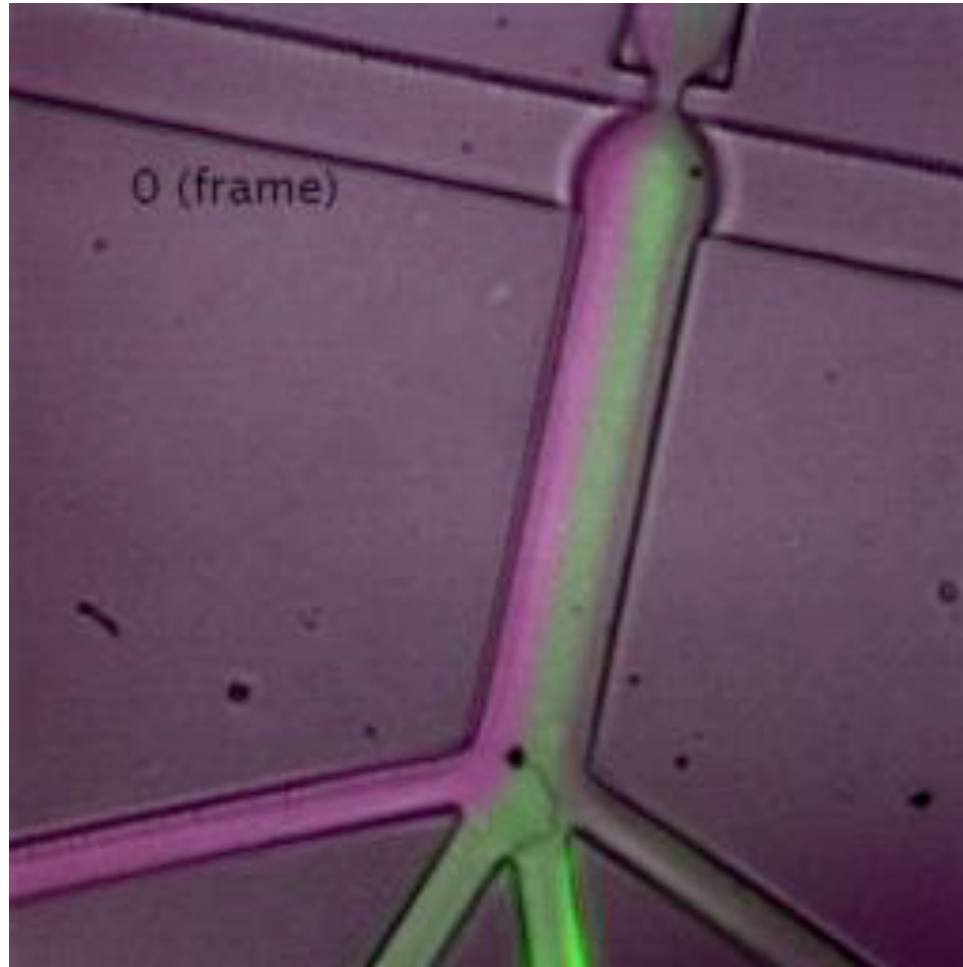


# The droplet scanning technique





The pressure protocol converts into a  $(n-1)$ D concentration map



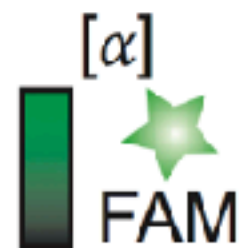
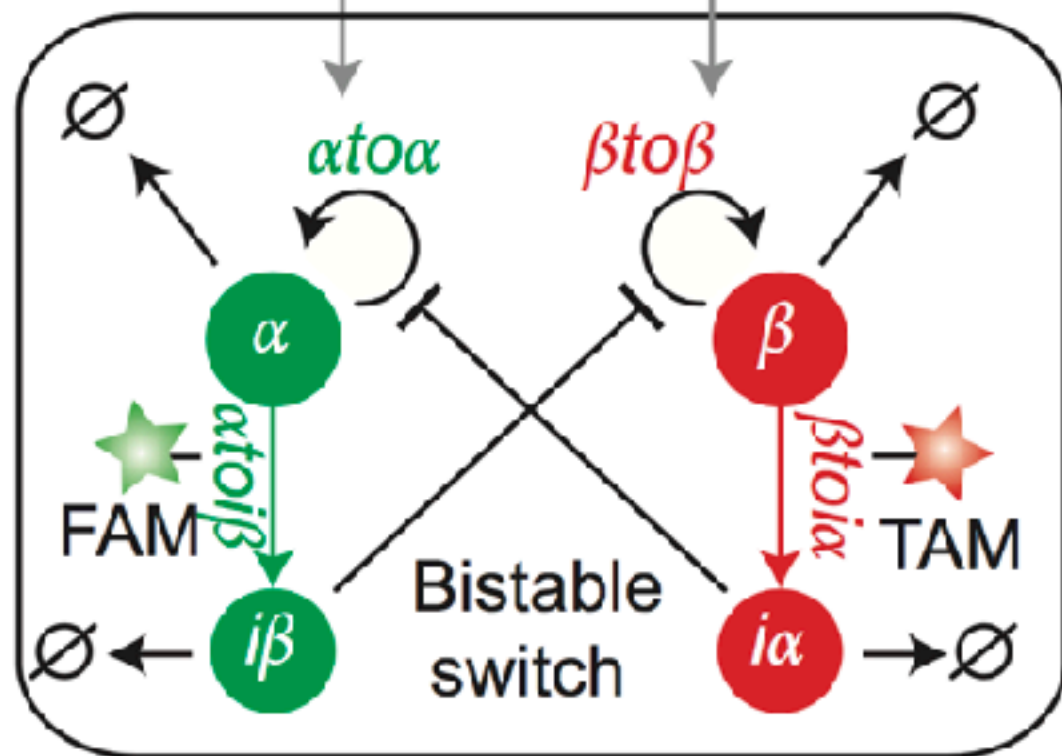
## Two parameters

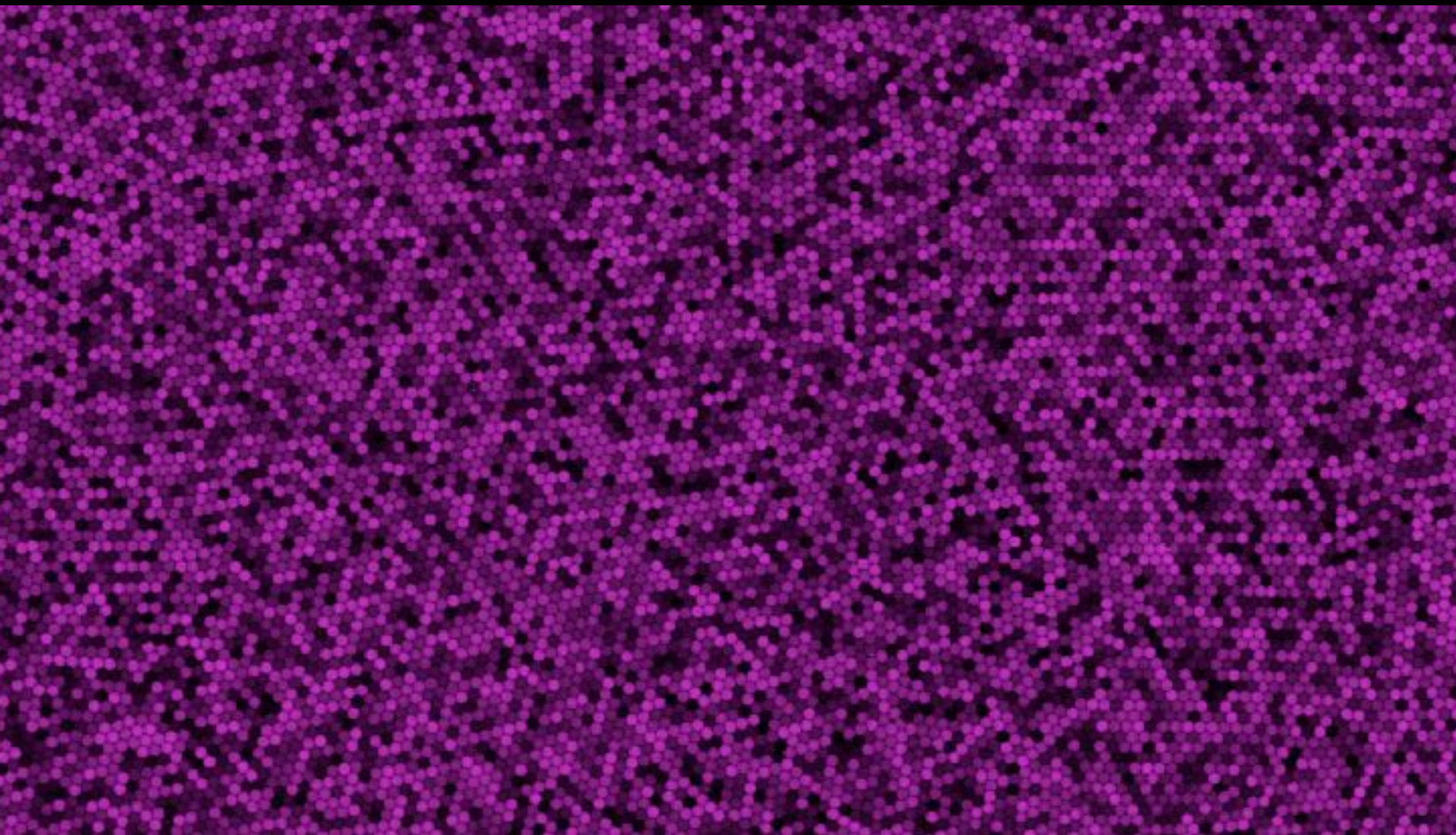
$[\alpha\text{to}\alpha]$  (replication rate of  $\alpha$ )

$[\beta\text{to}\beta]$  (replication rate of  $\beta$ )

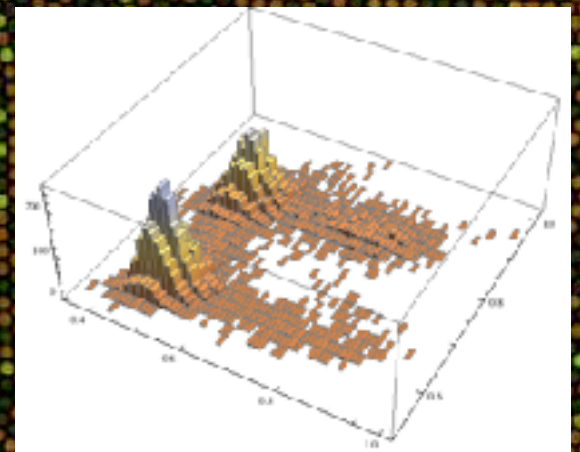
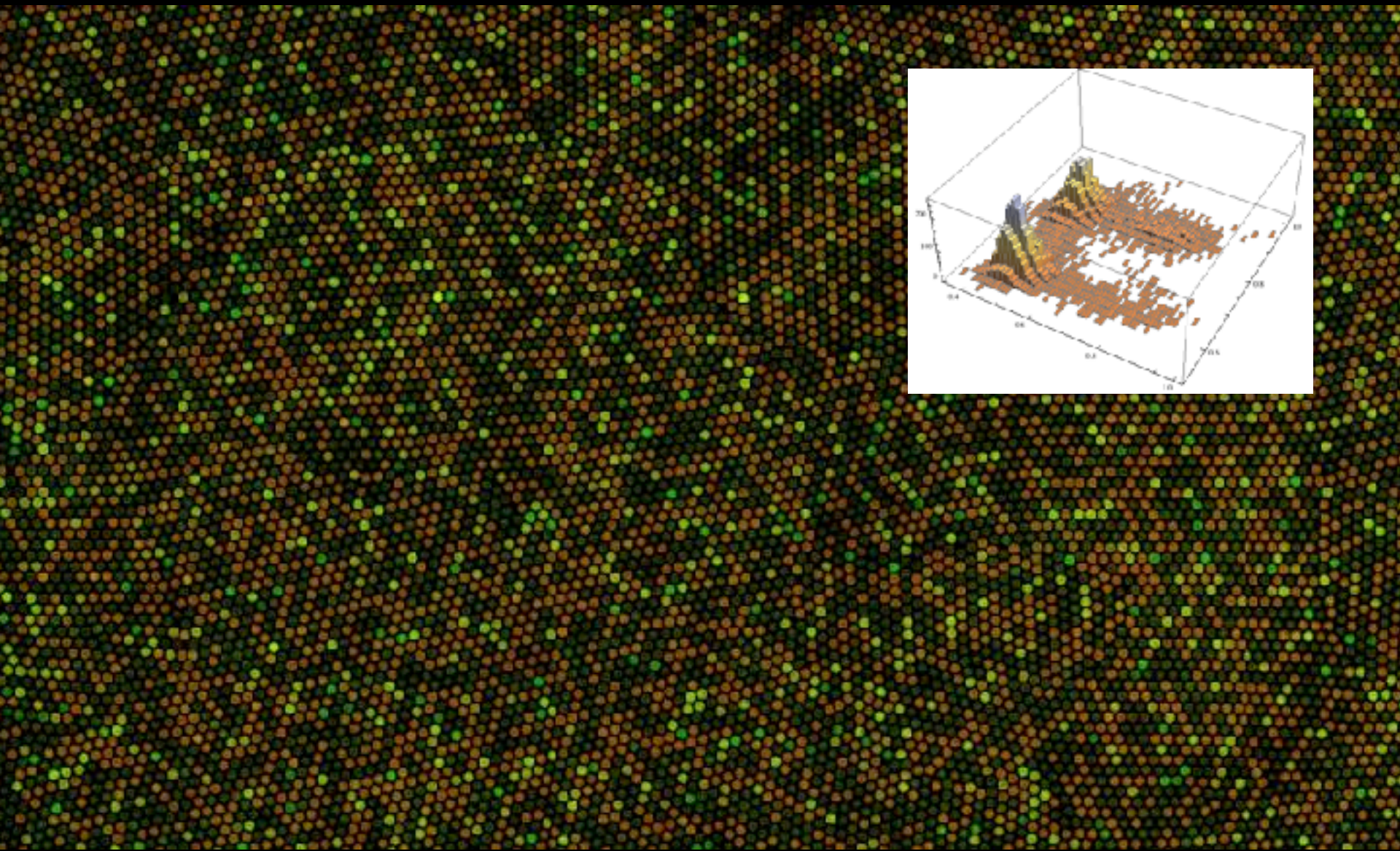


Two dynamical observables  
 $\alpha, \beta$  after 11 h

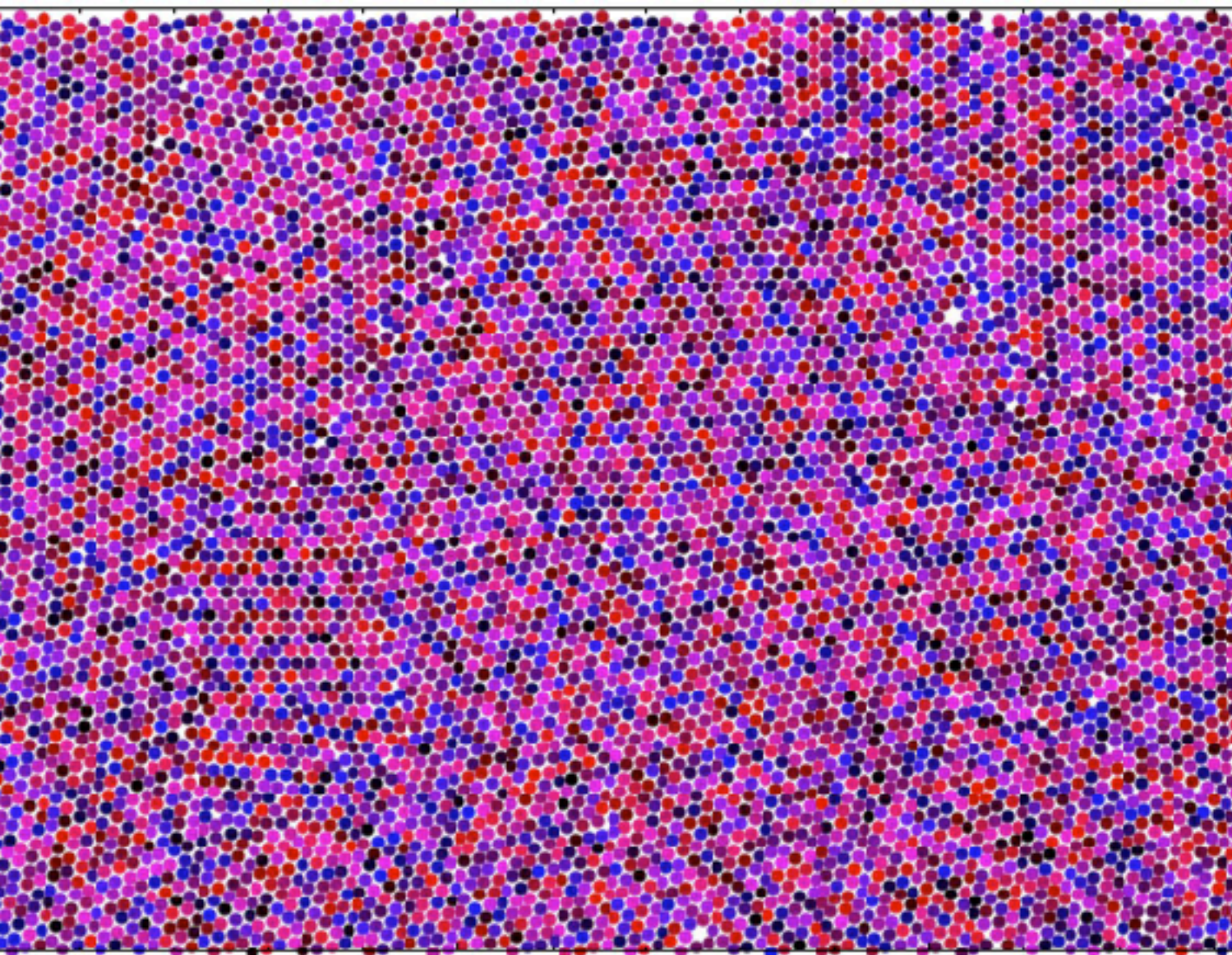








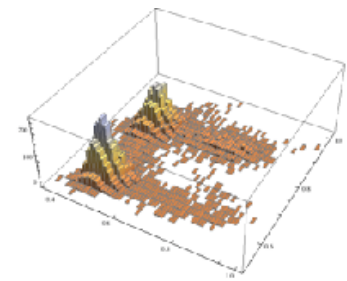
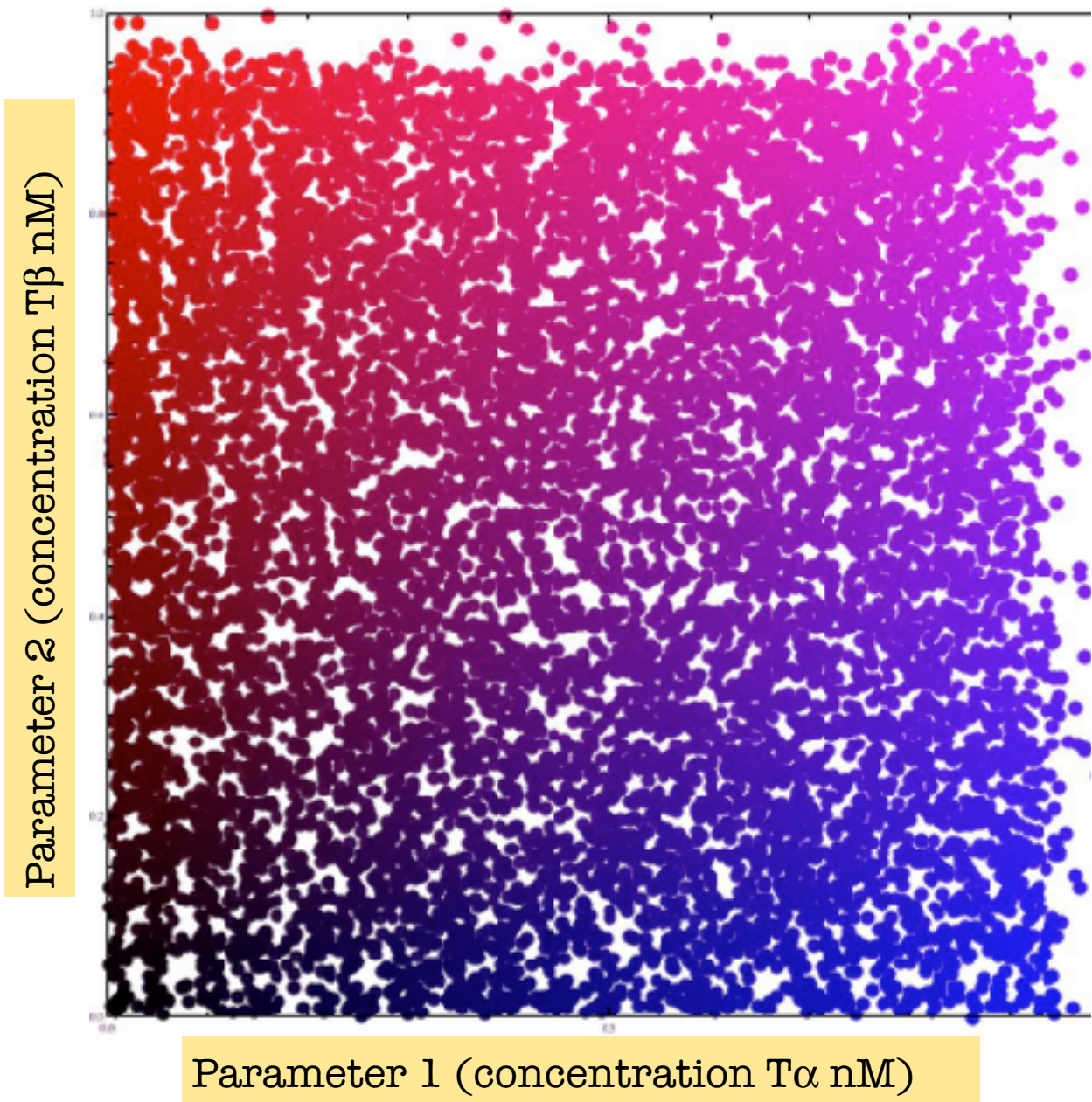




0.5

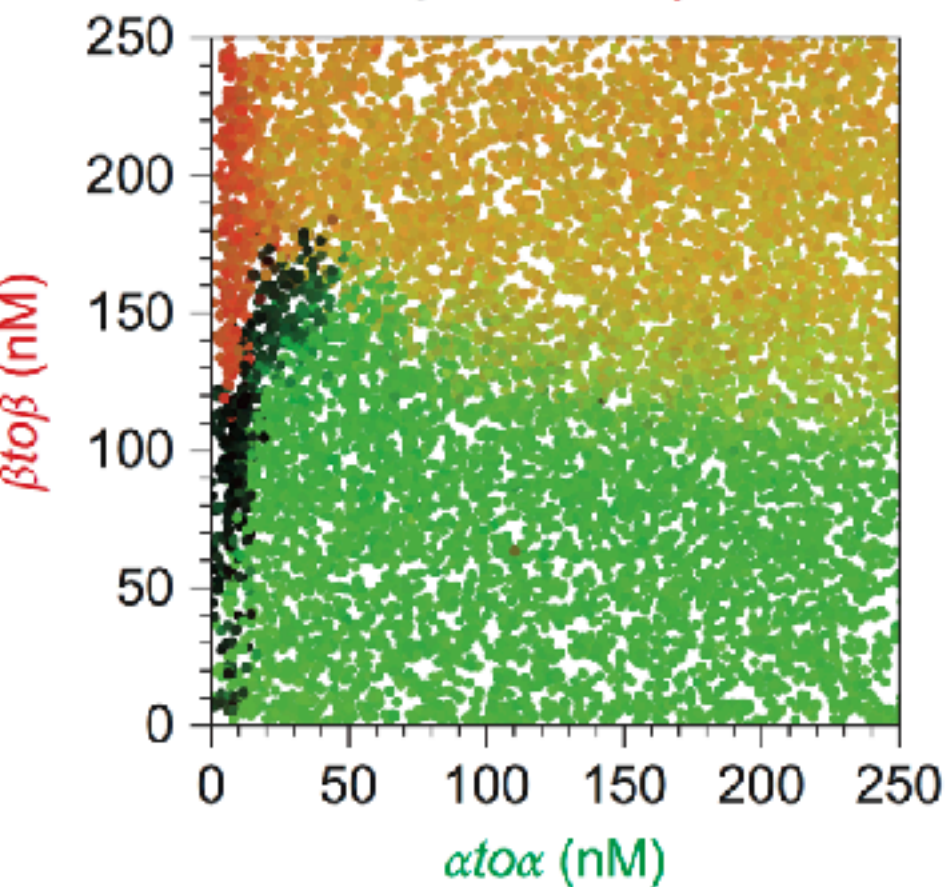
1.0



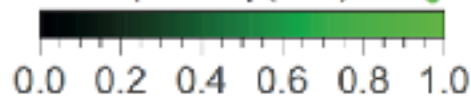


- $\alpha$  ↑  $\beta$  ↑
- $\alpha$  ↓  $\beta$  ↑
- $\alpha$  ↑  $\beta$  ↓
- $\alpha$  ↓  $\beta$  ↓

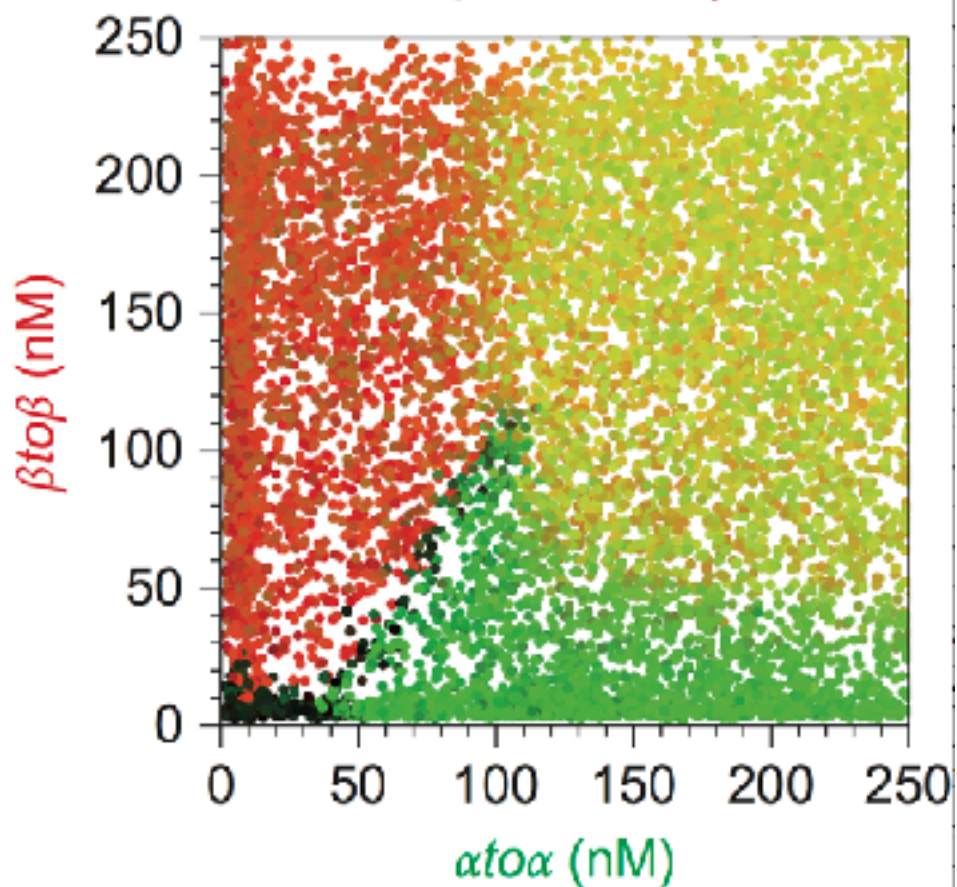
Starting close to  $\alpha\beta = 10$



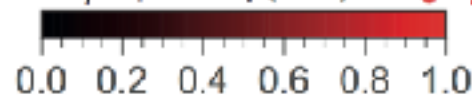
Low  $\alpha$   $|\Delta\text{FAM}|$  (a.u.) High  $\alpha$



Starting close to  $\alpha\beta = 01$

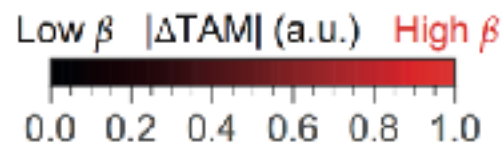
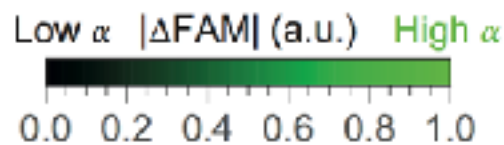
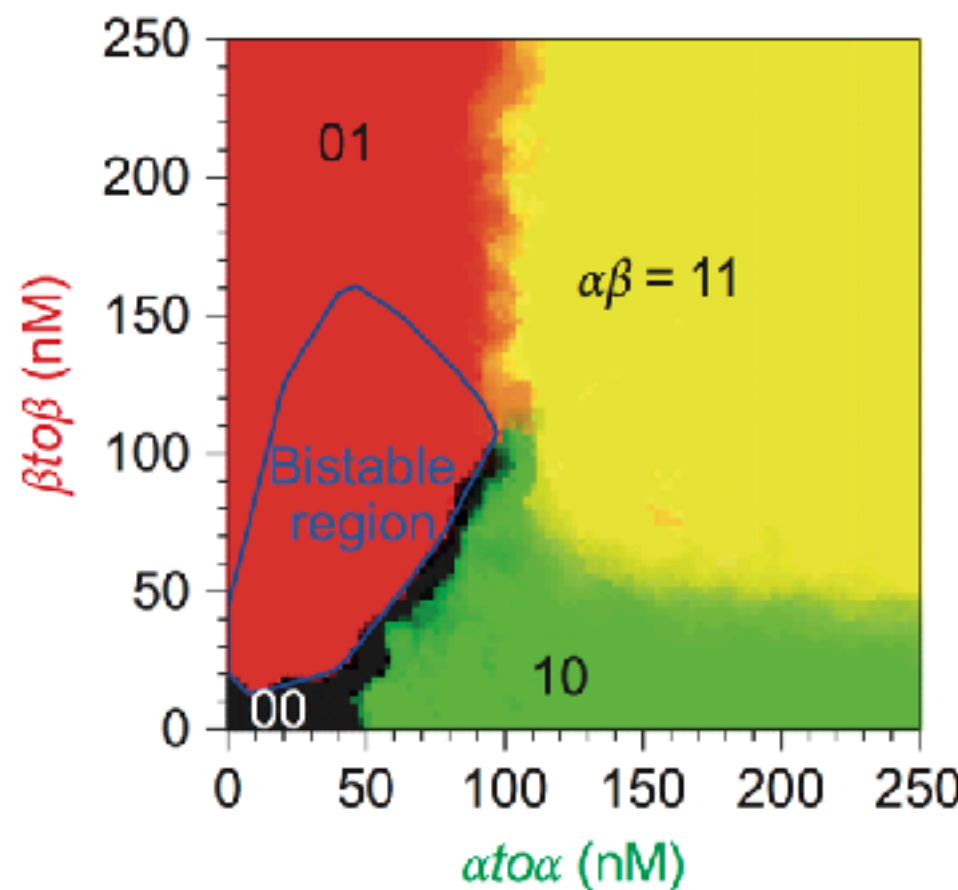
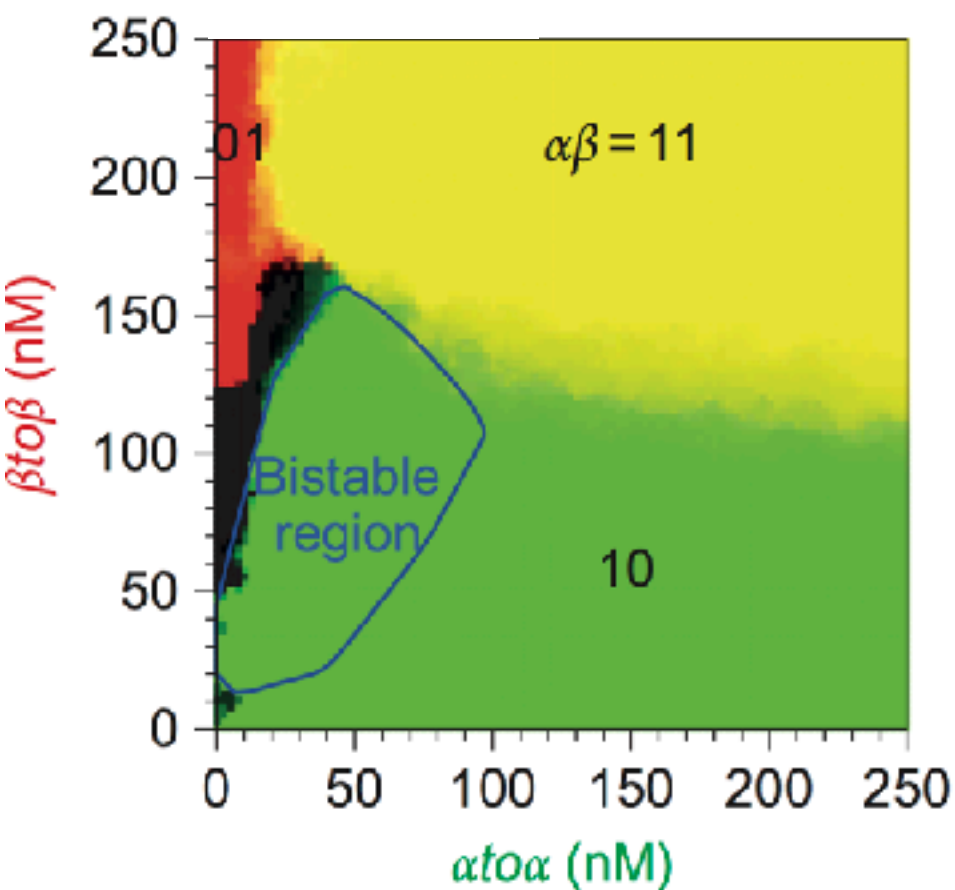


Low  $\beta$   $|\Delta\text{TAM}|$  (a.u.) High  $\beta$

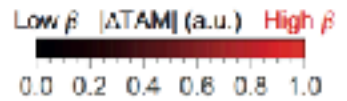
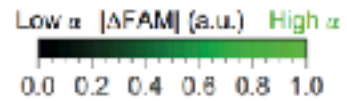
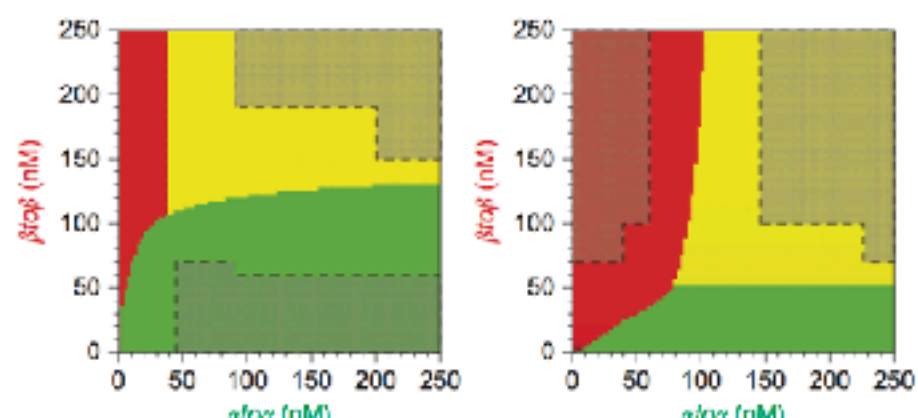
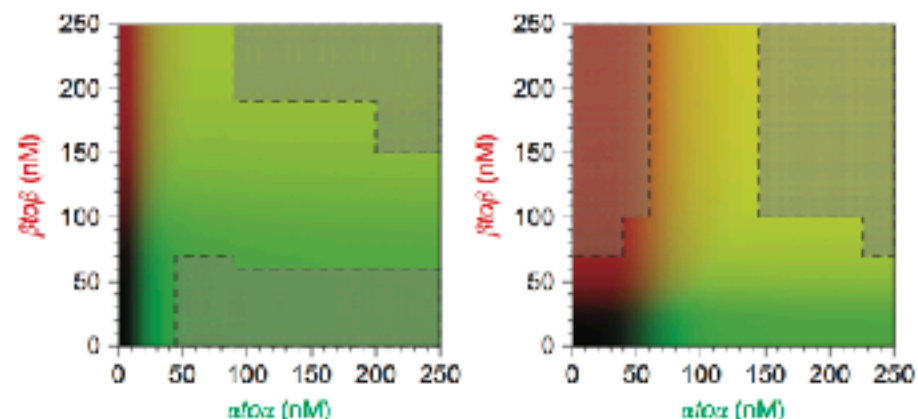
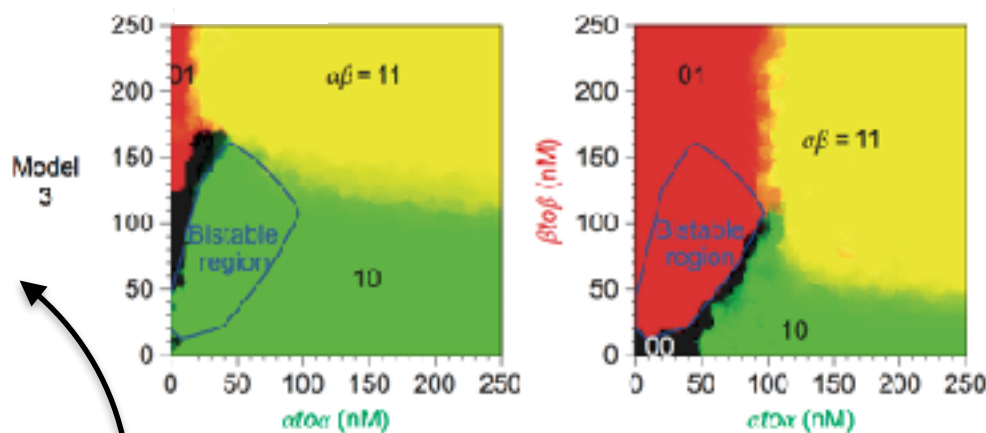
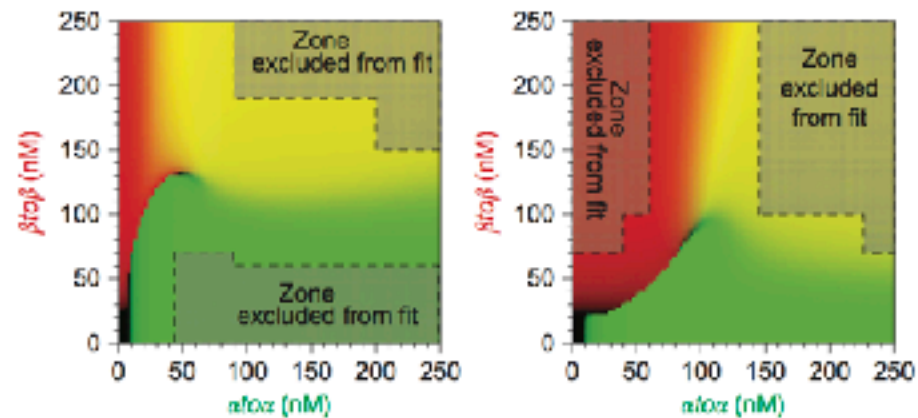


Fluorescence shift  
at 11 h (a.u.)





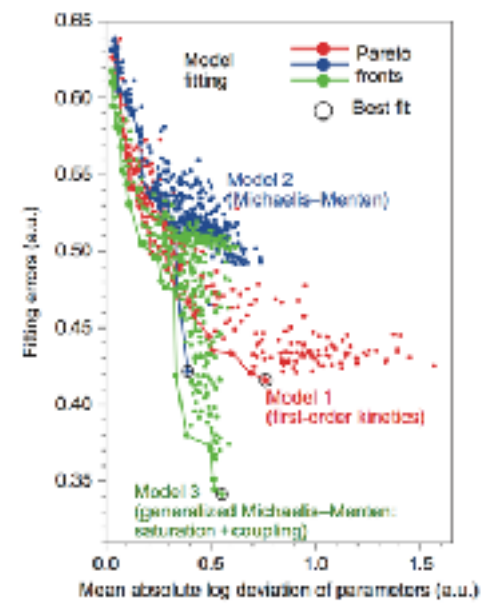
Fluorescence shift  
at 11 h (a.u.)



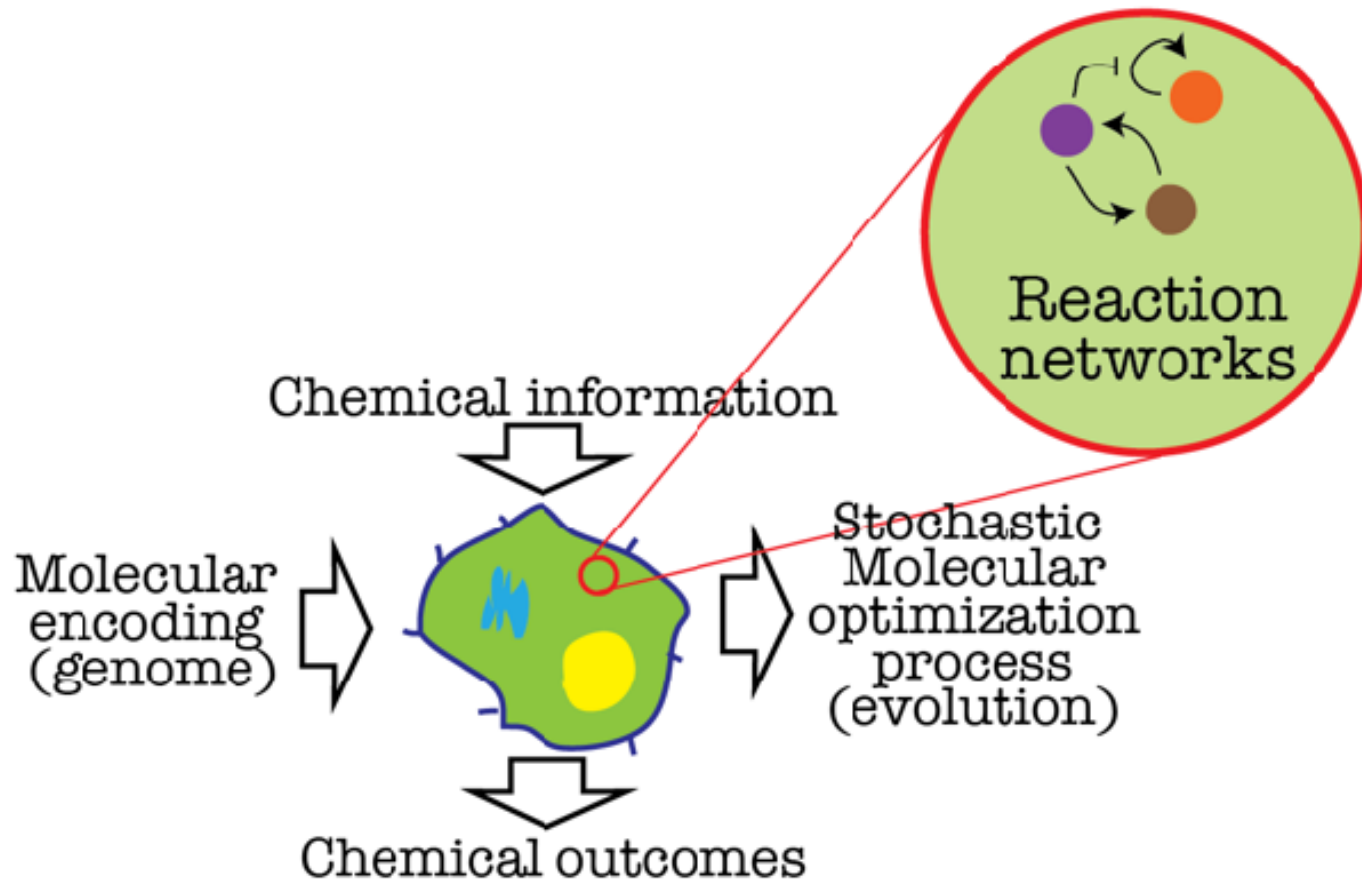
Fluorescence shift at 11 h (a.u.)

Experimental

Model 3 includes competition effects associated with enzymatic catalysis

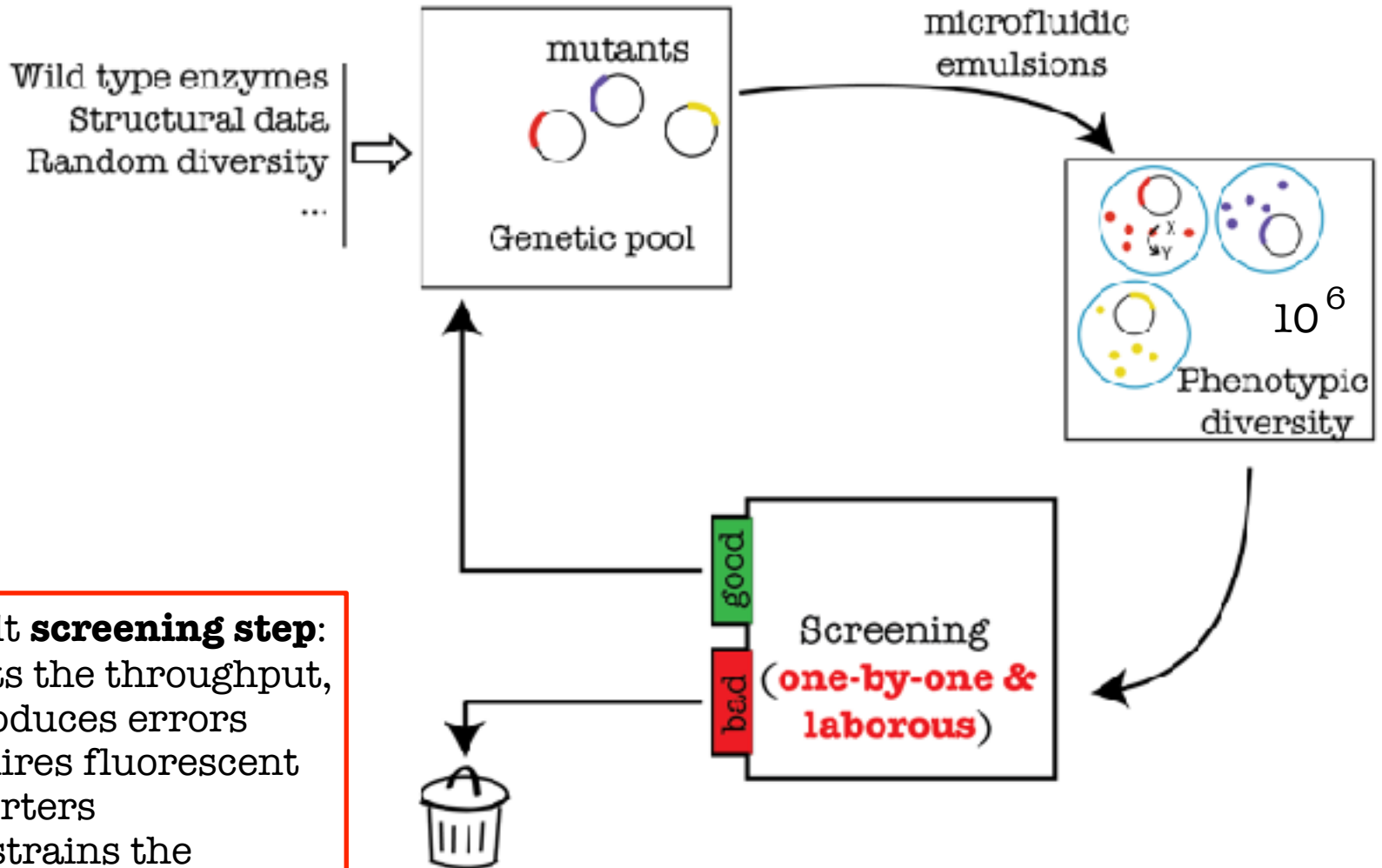


# Programmable molecular evolution





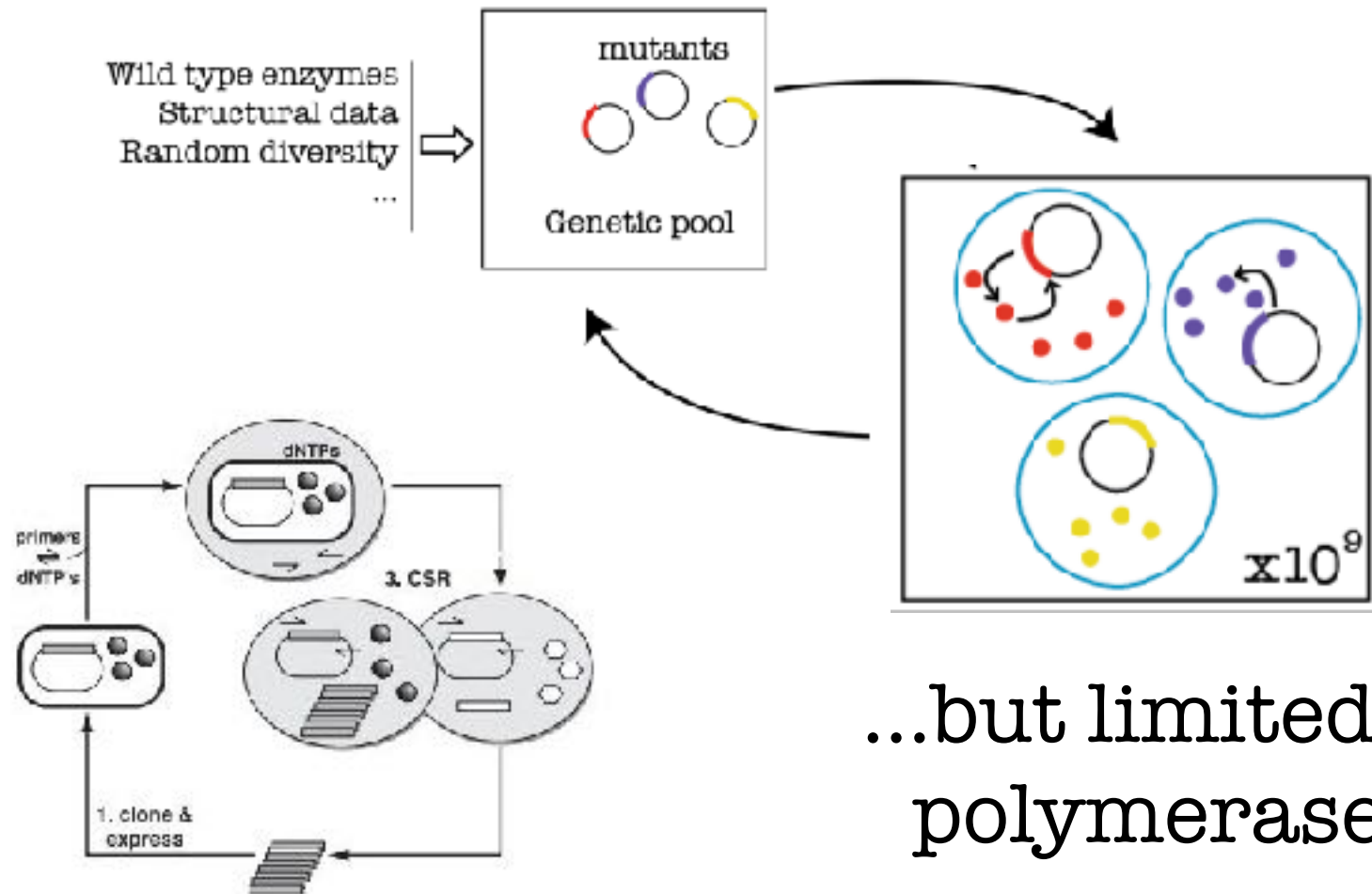
# High throughput artificial evolution of enzymes



## The difficult **screening step**:

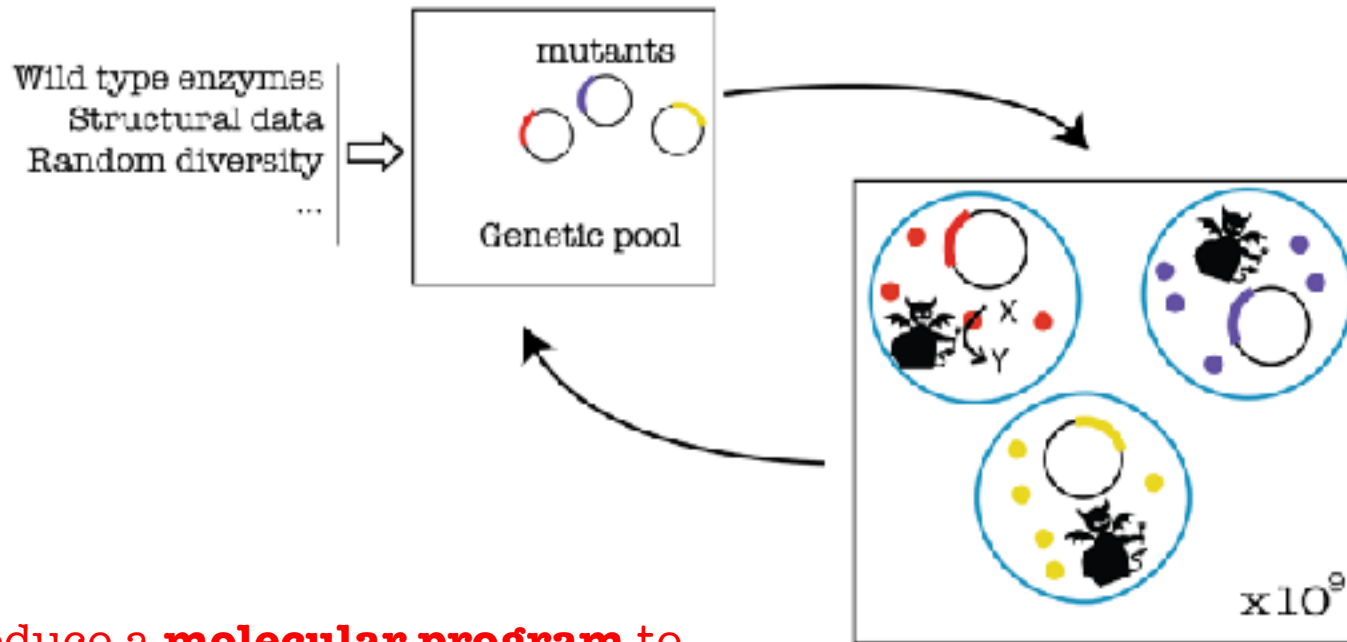
- ➔ limits the throughput,
- ➔ introduces errors
- ➔ requires fluorescent reporters
- ➔ Constrains the evolutionary walk

# Self-replication: a special case providing super high throughput



...but limited to polymerases

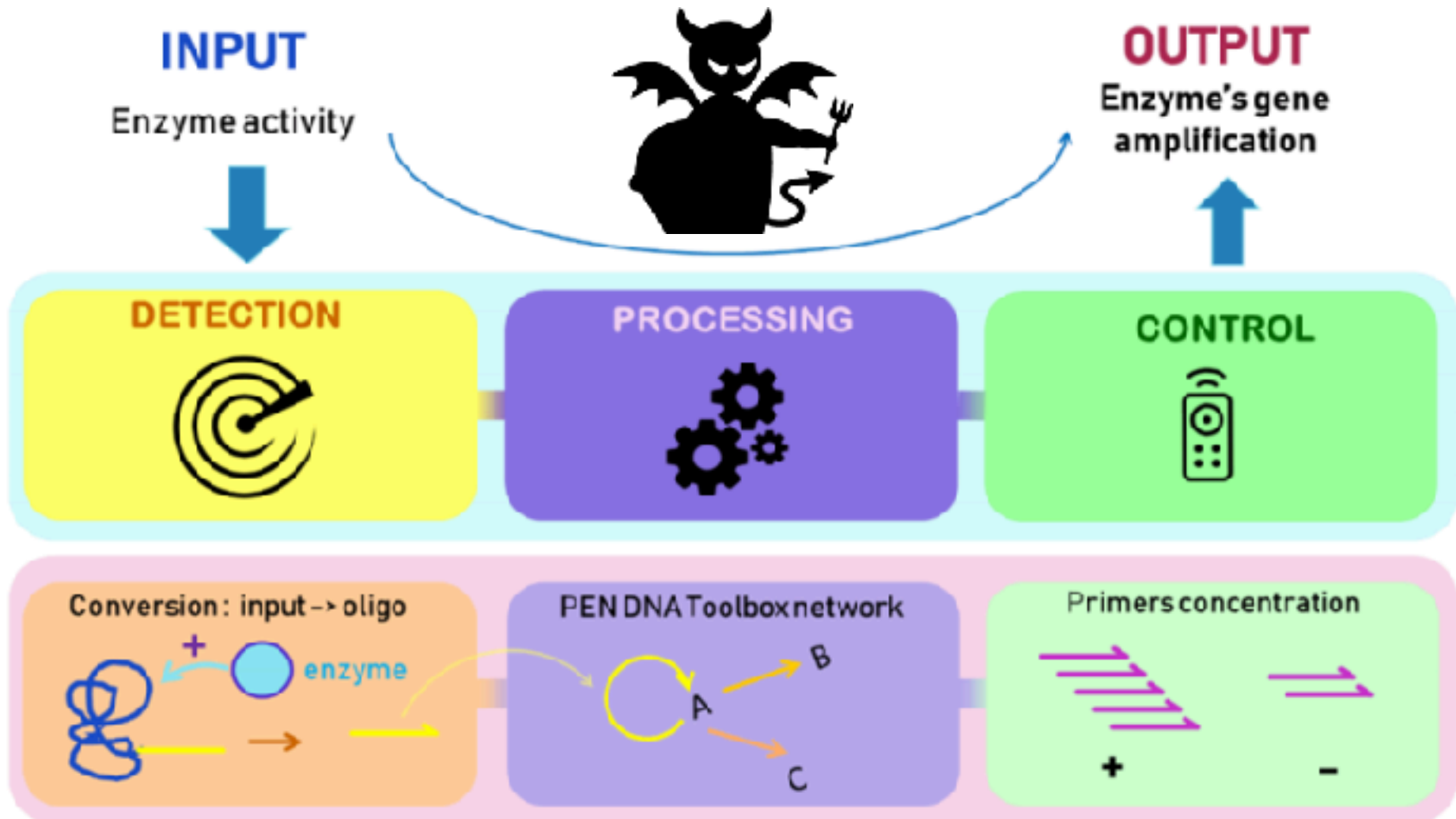
# Generalizing self-replication with programmed artificial cells



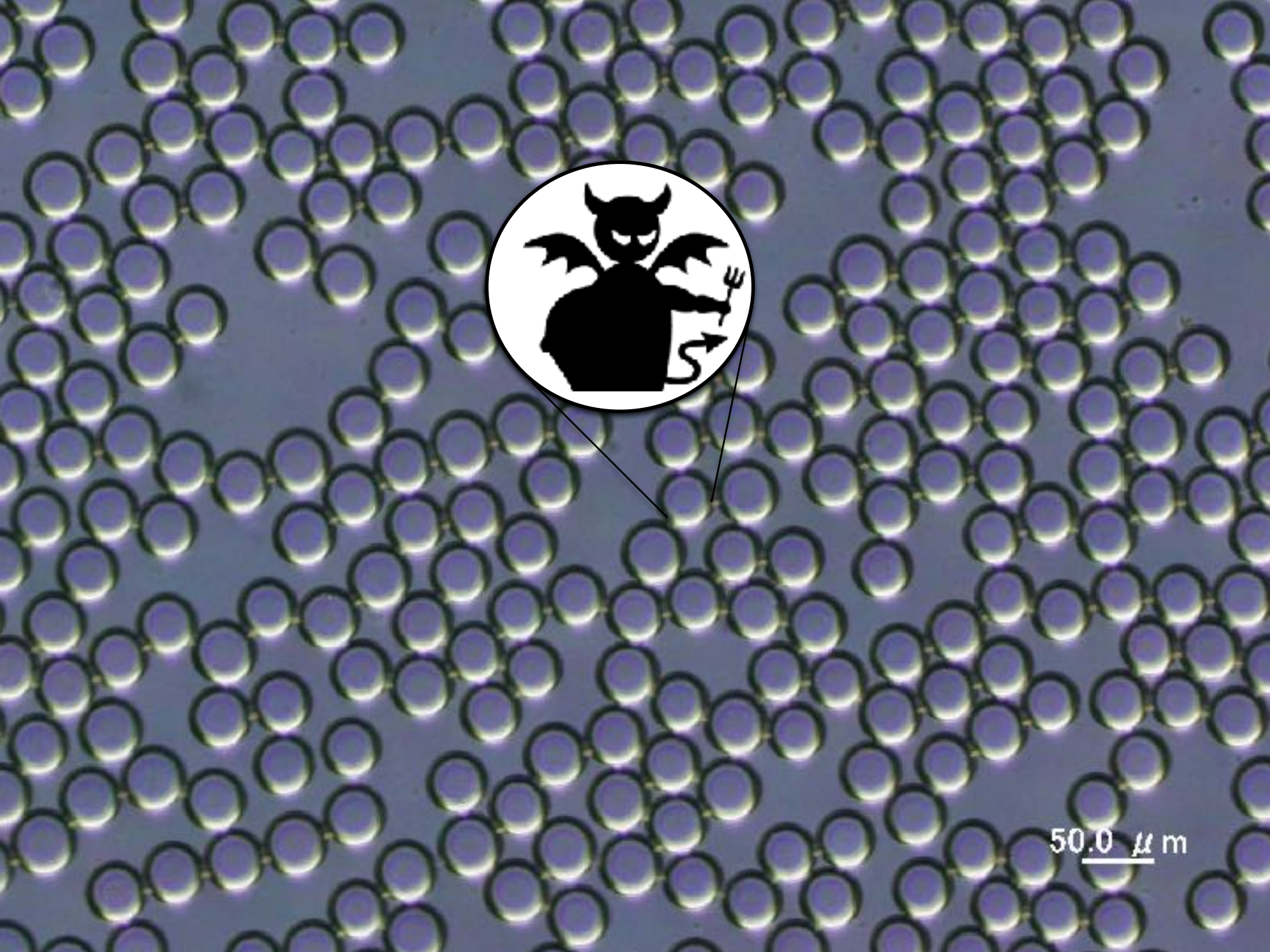
We introduce a **molecular program** to apply a controlled evolutionary pressure in each compartment.

- ➔ No more screening
- ➔ General approach
- ➔ Precise control (target & dynamics)
- ➔ Ultra-high throughput

# A molecular system programmed to amplify genes conditionally to the activity of the gene product





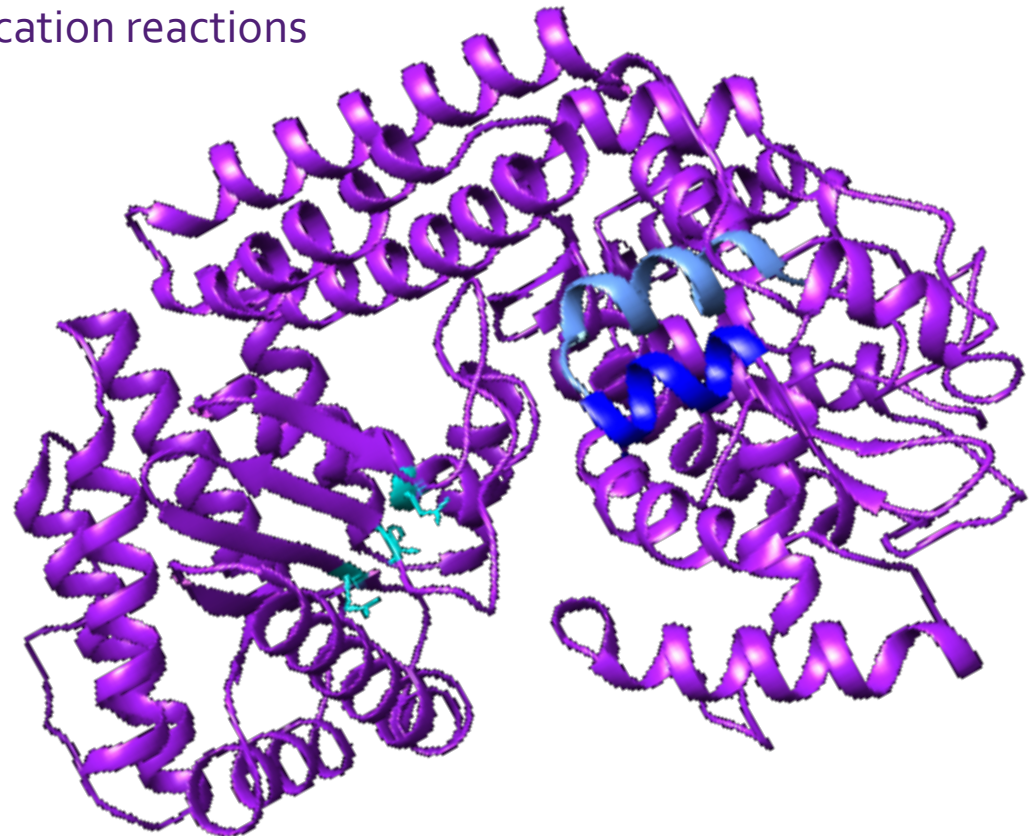
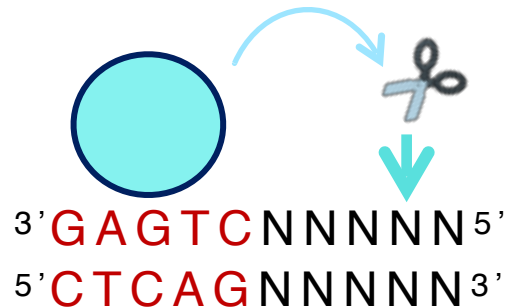


50.0  $\mu$  m

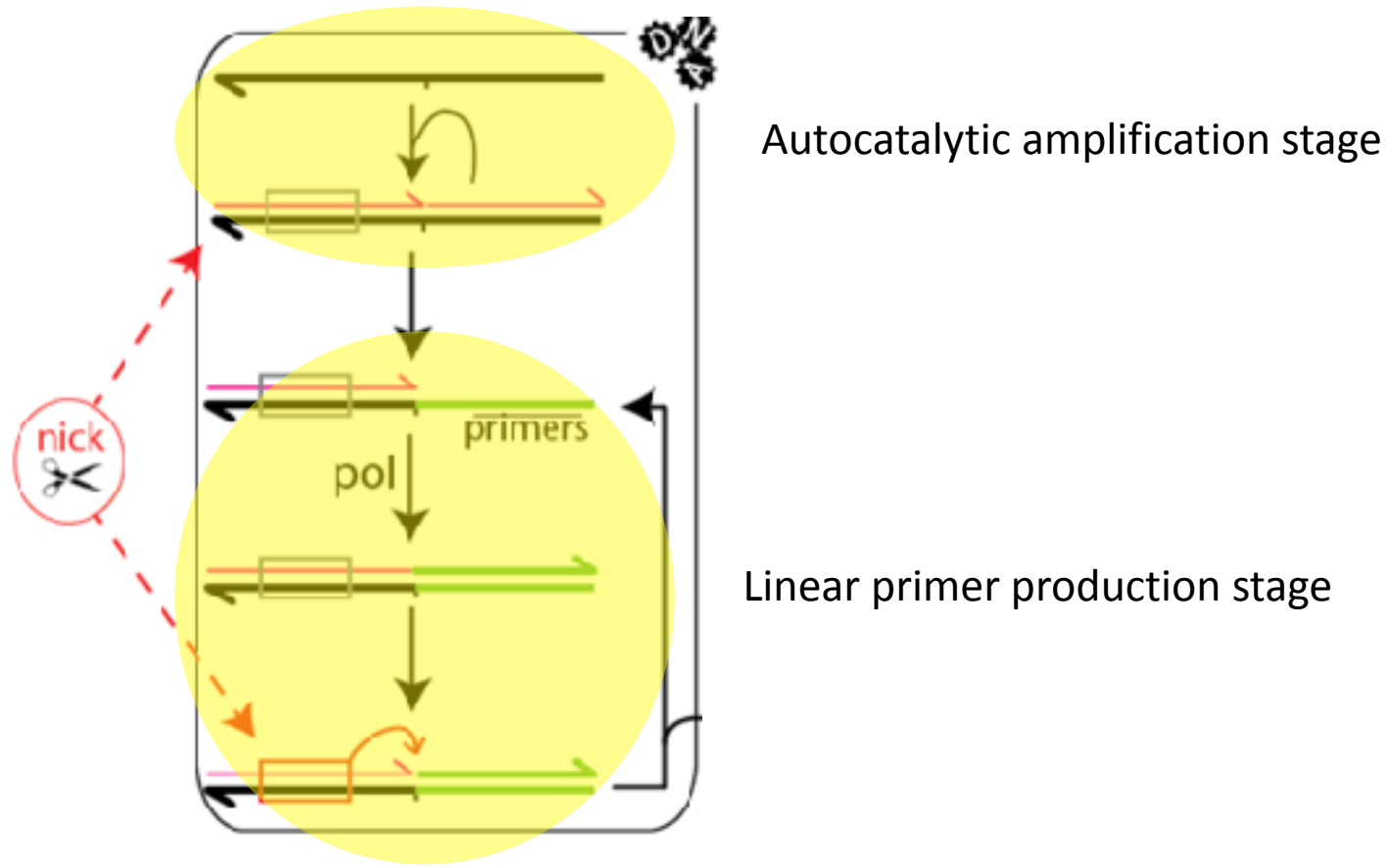
# Proof of concept target is a nickase

## Nt.bstNBI

- Nickase
- Recognizes 5'-GAGTC-3'
- Cut the top strand 4bp further
- Mostly used in isothermal amplification reactions
- 604 amino acids

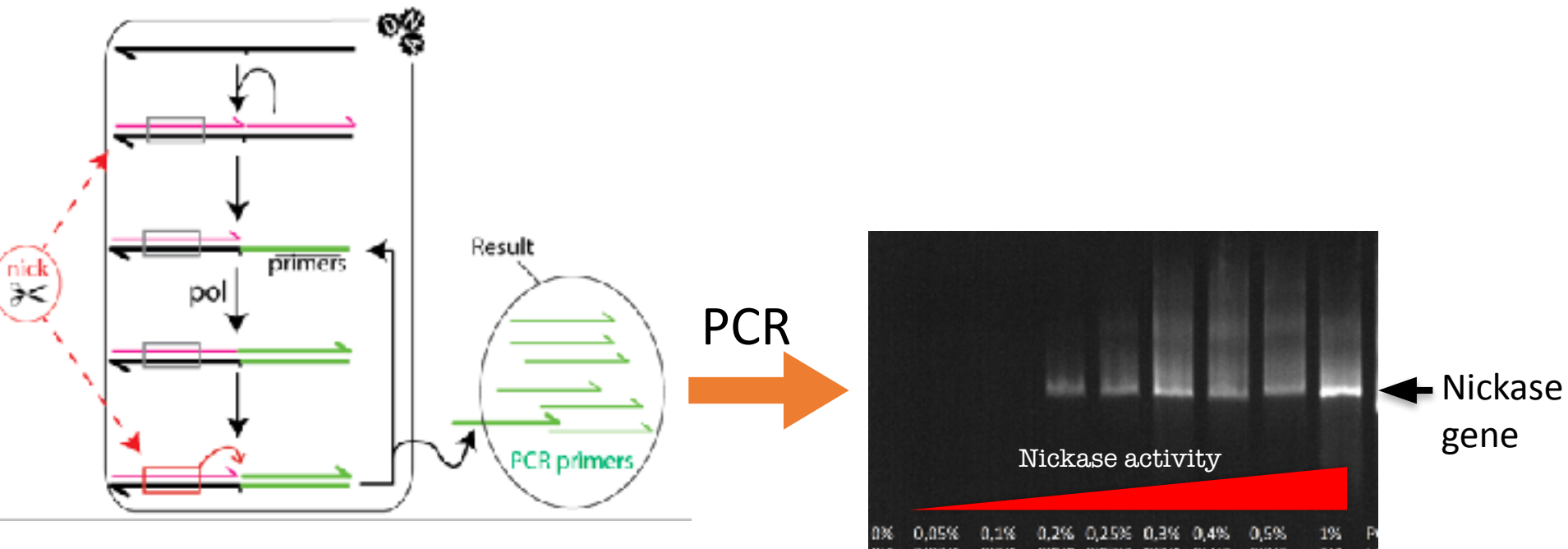


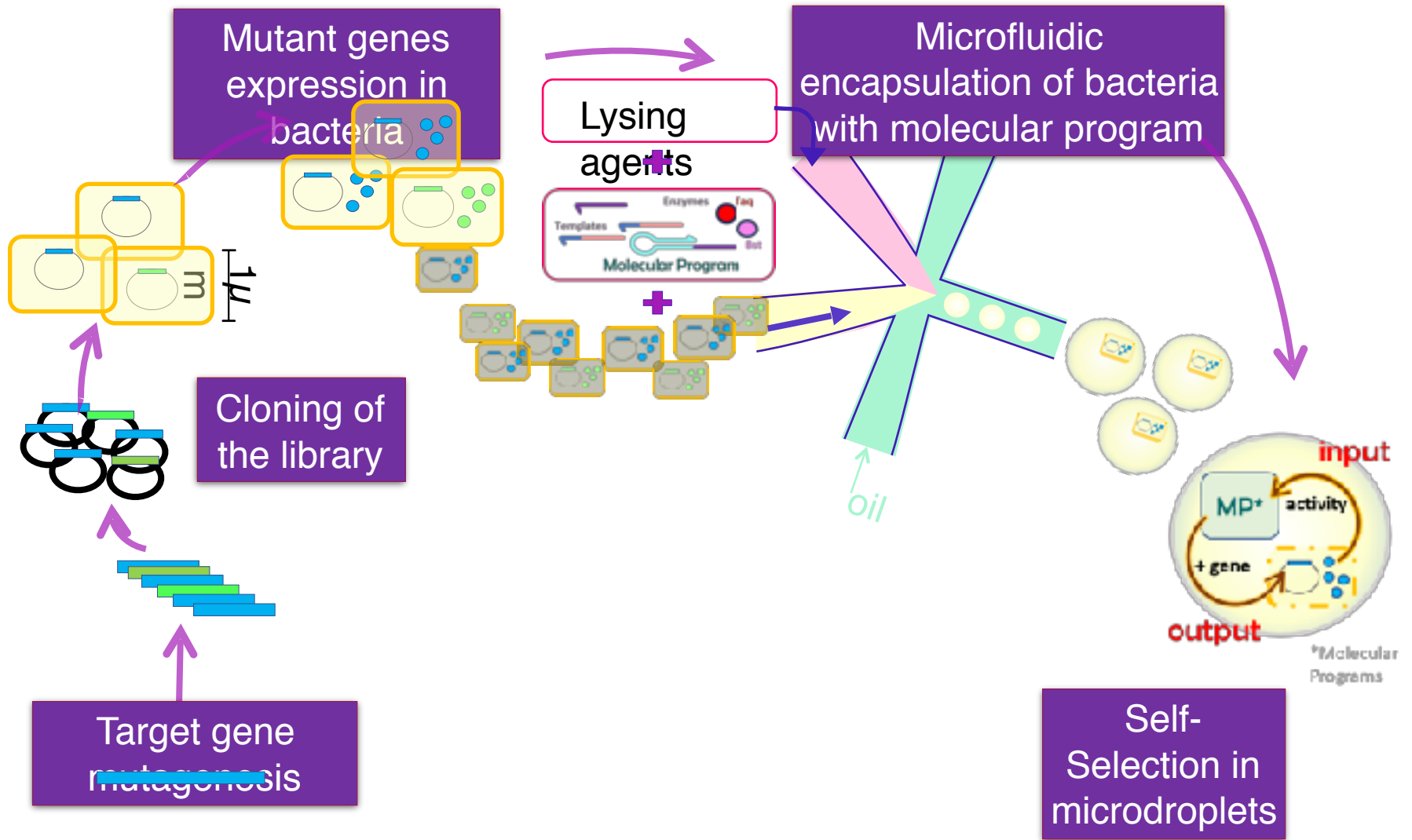
# Linking **nickase** activity to PCR

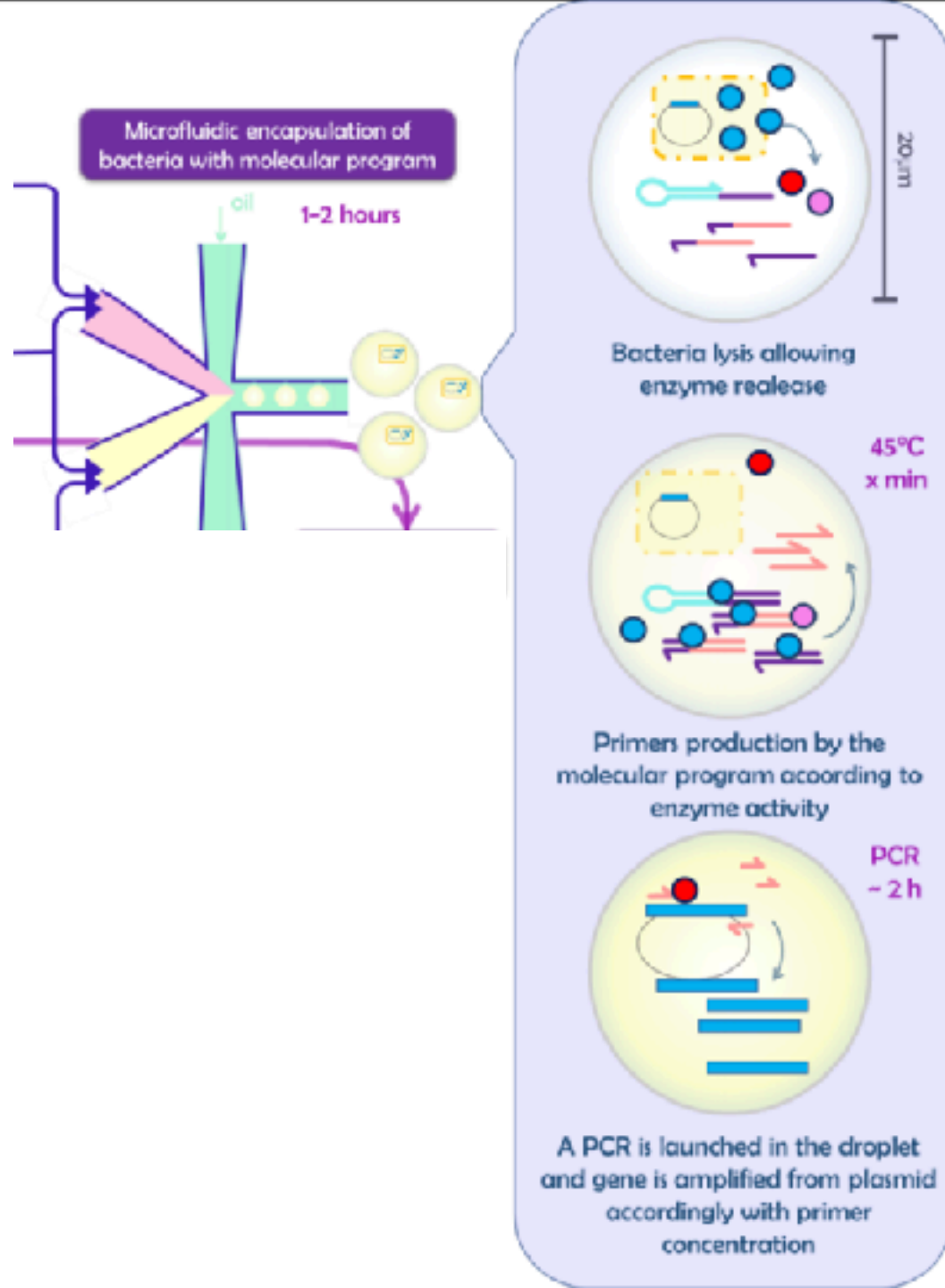




# Linking nickase enzyme activity to nickase gene PCR





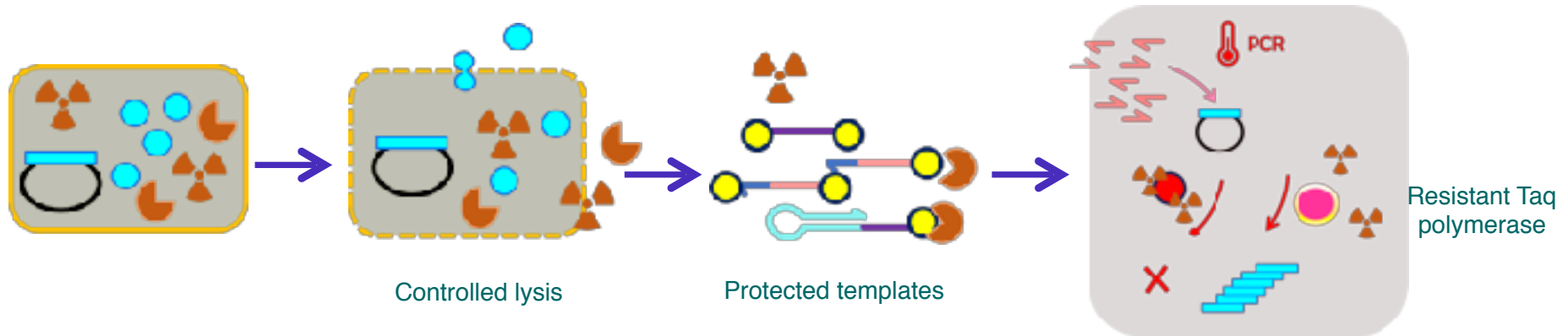
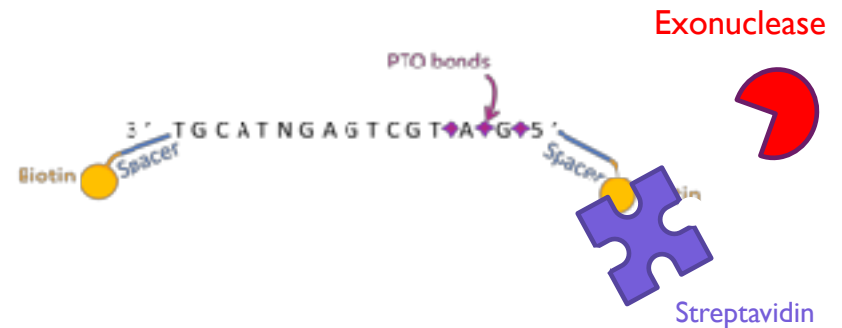


# Dealing with toxicity of bacterial lysate

(beside buffer optimization to perform PCR and MP reaction in the same droplet)

- MP templates protection with biotin / streptavidin
- Controlled lysis of the bacteria
- Resistant Taq polymerase

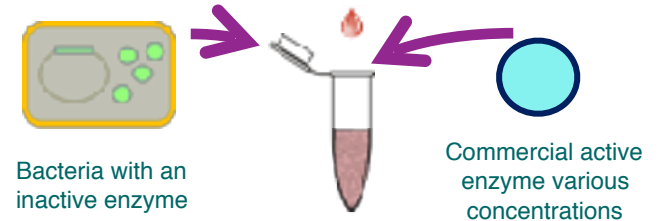
Finally, an optimal bacteria concentration of  
~100 bact/nL  
-> ~1 bact per droplet of 27µm diameter



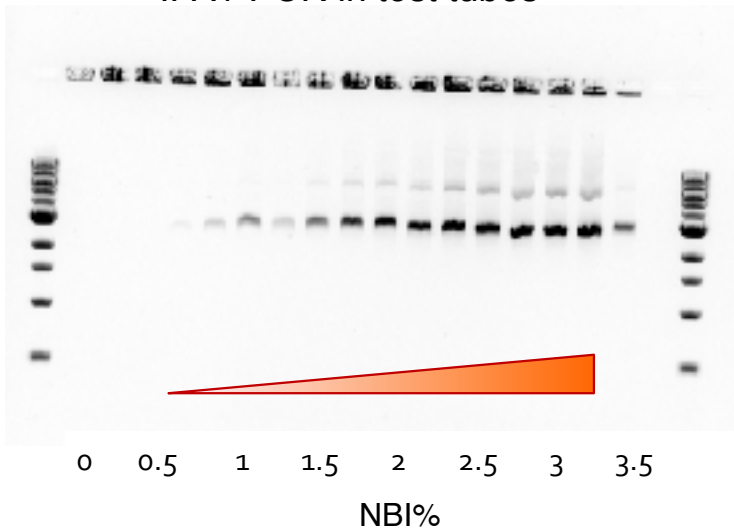


# Selection function with bacteria

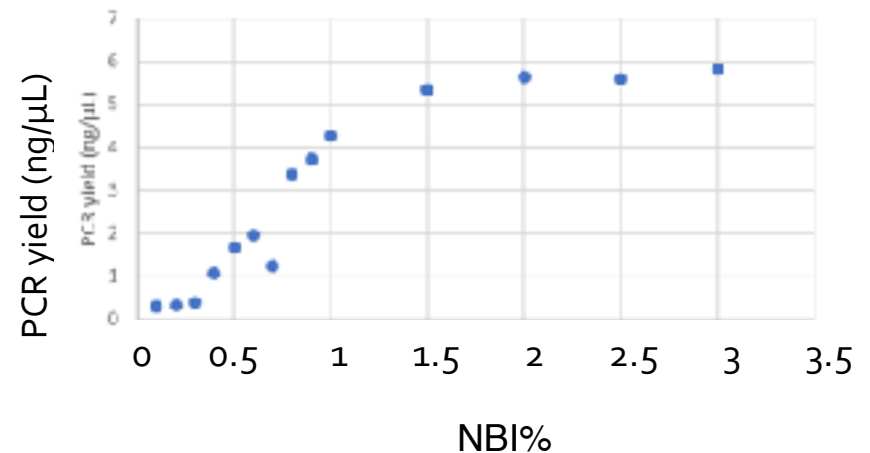
Bacteria expressing the inactive version of the enzyme were supplemented with various quantity of commercial NBI to mimic a variation in activity.



IPA+ PCR in test tubes

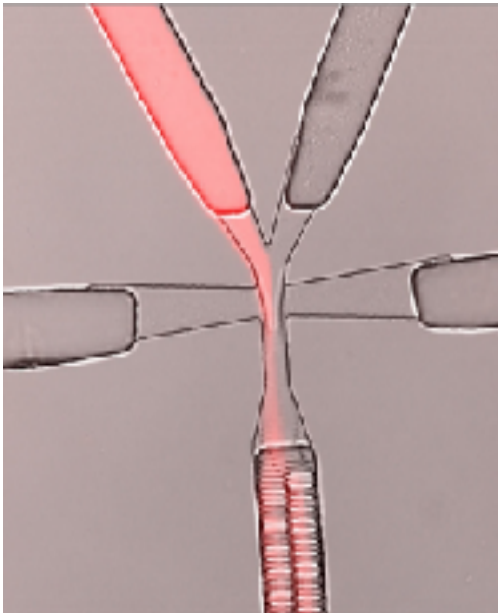


Quantification of the DNA

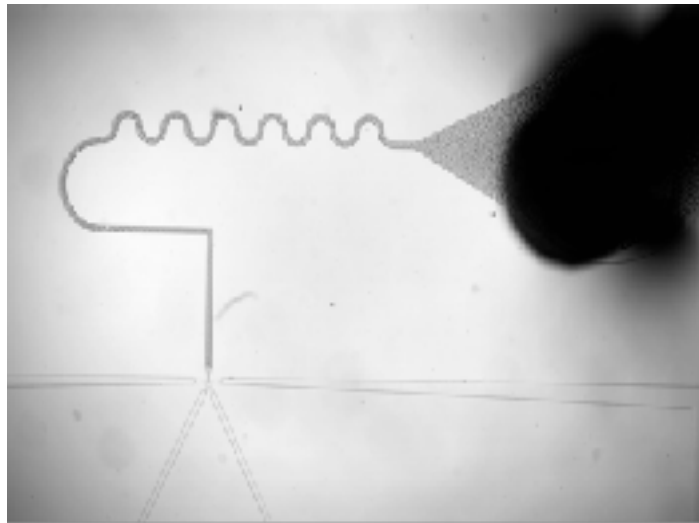


# High-Throughput compartmentalization

Bacteria encapsulation 👍

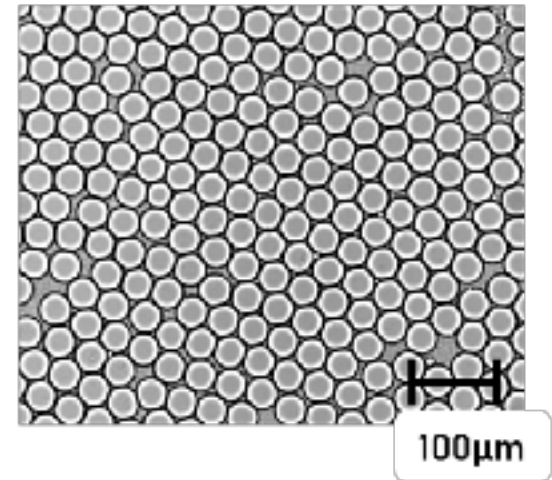


High throughput 👍



$\sim 10^7$  droplets/hour

Monodispersity 👍



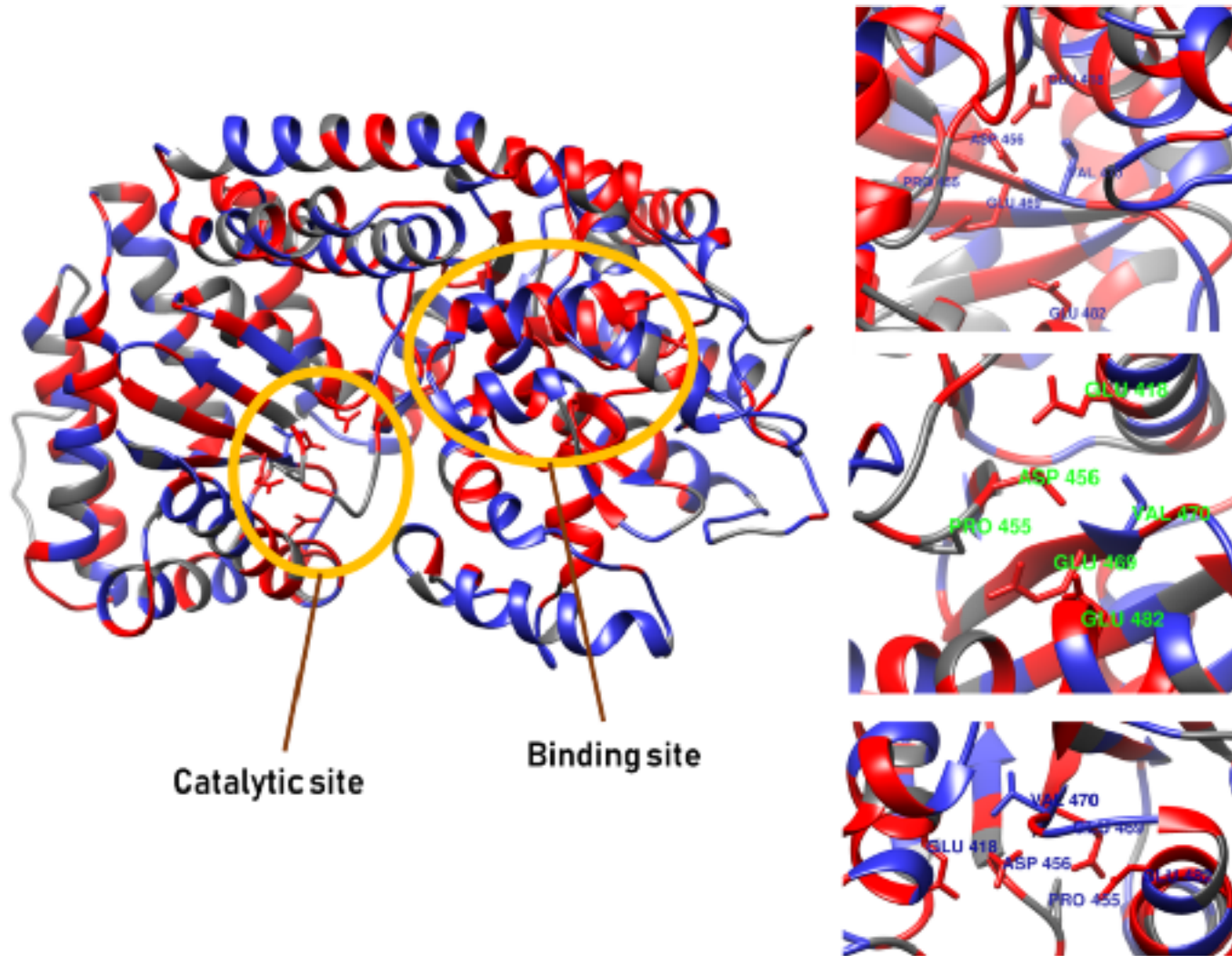
Diameter =  $27.6 \mu\text{m} \pm 3\%$

# Purifying a mock library at high throughput ( $10^8$ )





One round of purifying selection on a randomised nickase library provides mutational sensitivity mapping





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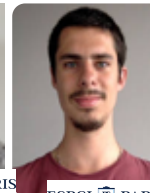
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Eindhoven University (de Greef)

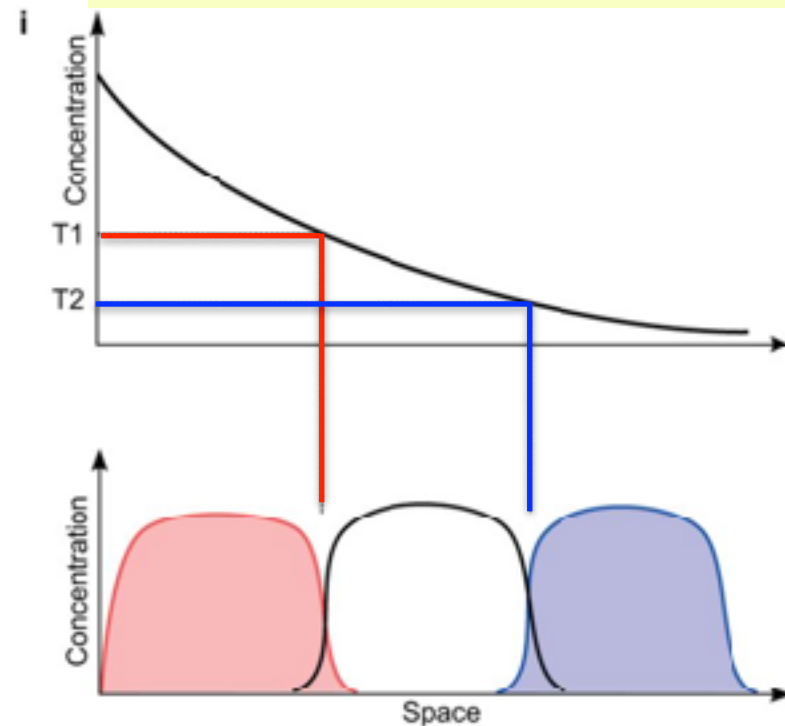
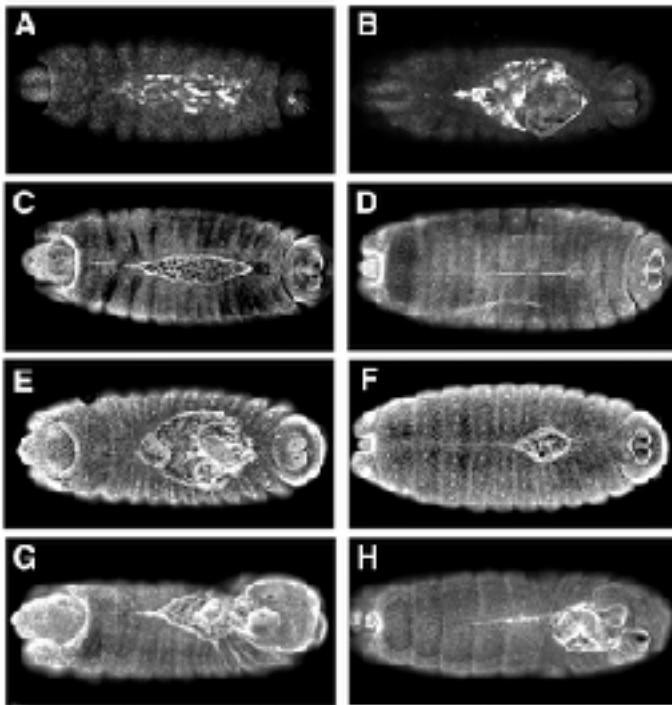


# Artificial morphogenesis

(With A. Estevez-Torres et JC Galas, UPMC)

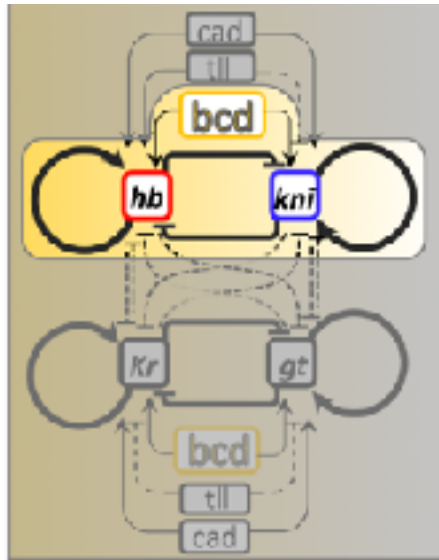
**creating materials that contain their own developmental program**

The French-Flag patterning, a fundamental building block of morphogenesis

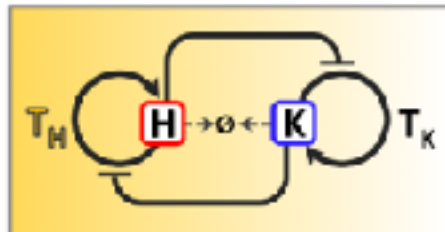


# Artificial morphogenesis

The biological network ... in an embryo

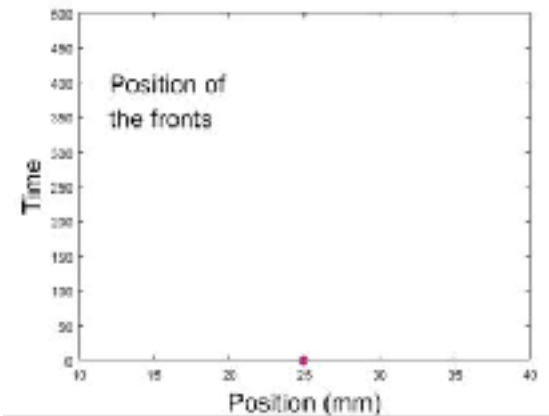
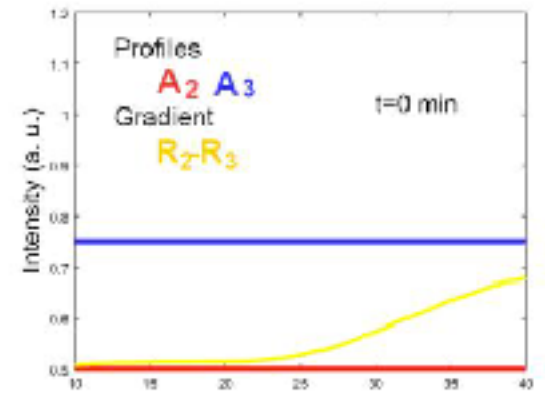
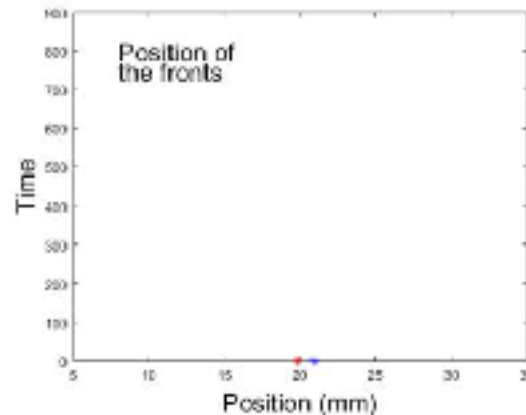
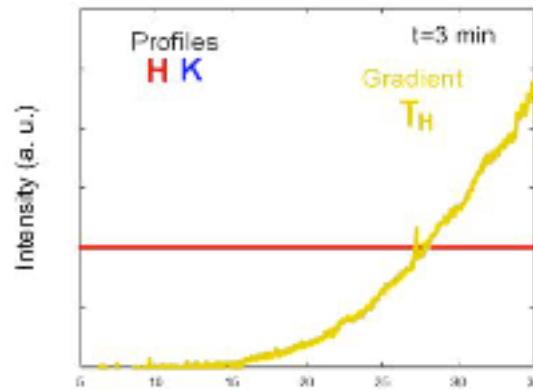
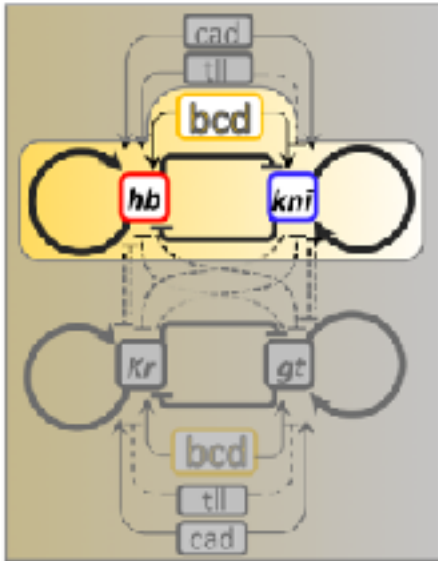


Our synthetic analog ... in a capillary tube

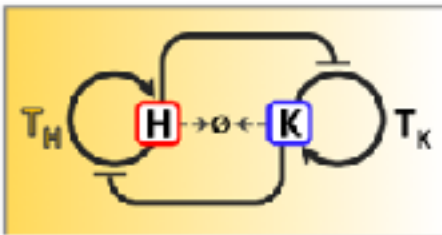


# Artificial morphogenesis

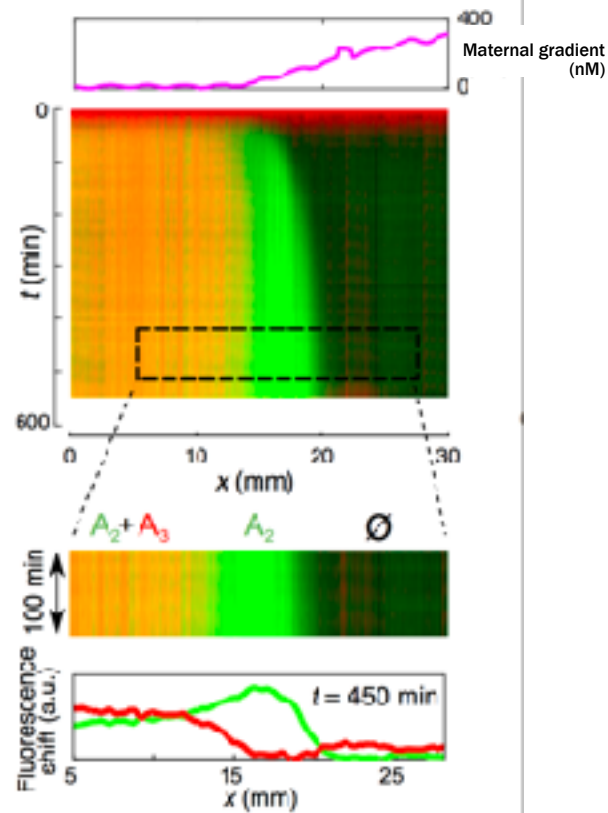
A



B



# Artificial morphogenesis





# Artificial morphogenesis

## Materialization

