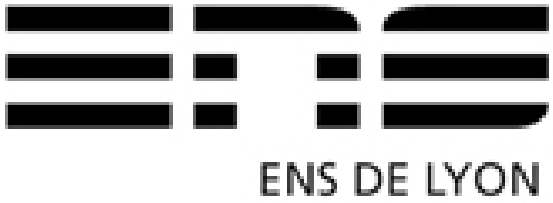
# BIOPHYSICS
# Lab. Phys. ENS Lyon

## Rencontres ENS-SISSA





Scuola Internazionale Superiore
di Studi Avanzati

## Main activities

1. Physics of virus :
   Cendrine Moskalenko, Martin Castelnovo
2. Physics of bacteria swimming near interfaces :
   C. Place, J.-F Palierne, C. Vaillant ; L. Lemelle (Geol. ENS)
3. Physics of nuclear pores :
   F. Montel
4. Physics cell motility :
   A. Pumir
5. Physics of chromosome organisation, gene transcription and DNA replication :
   R. Everaers, B. Audit, C. Vaillant, Angelo Rosa (SISSA).

# Physics of Virus. Atomic Force Microscopy (AFM) Experiments

## Capsid self-assembly and control of viral DNA/RNA packaging/ejection ?

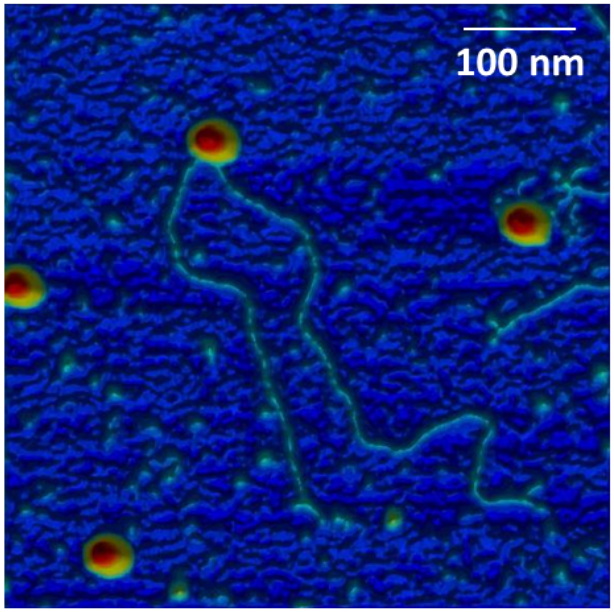
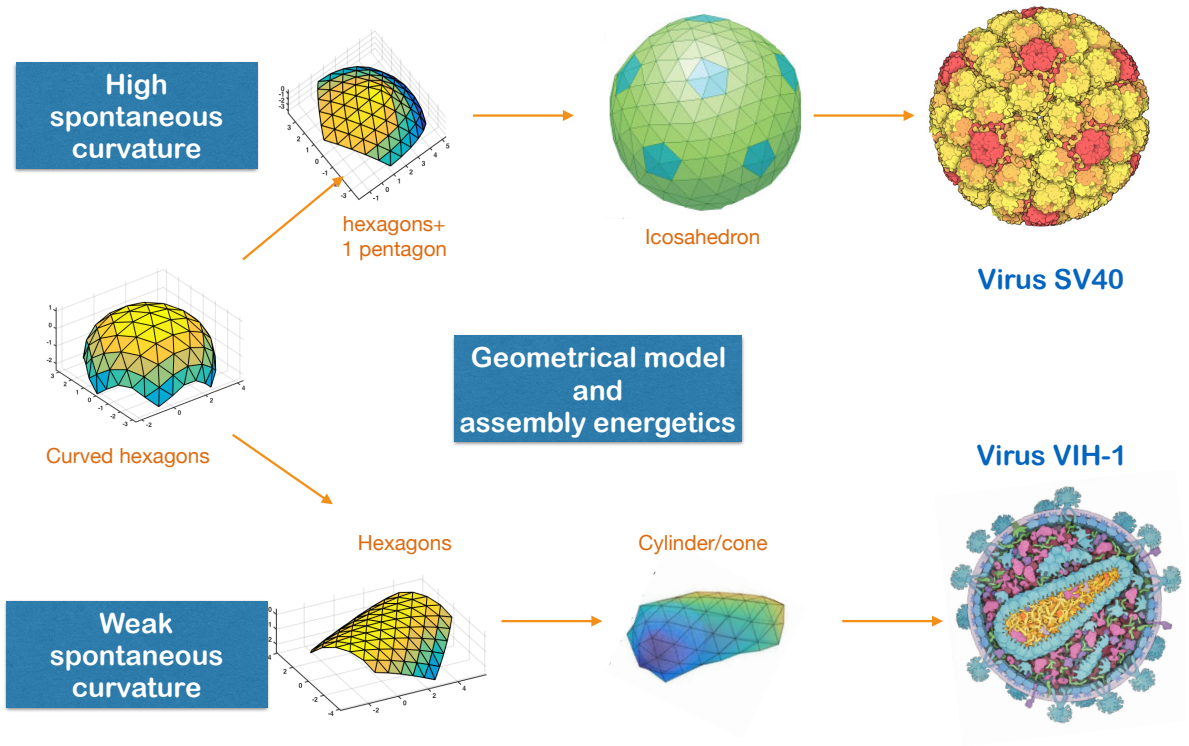



Figure: AFM image of AAV8 capsids incubated for 15 minutes @T=60°C, with partially ejected single-stranded DNA genomes. Using such high resolution images, the length of ejected DNA can be measured over viral capsid population as a function of temperature or micro-environment conditions.

- C. Moskalenko, Y. Carrasco Salas, M. Castelnovo,A. Salvetti (CRCL)

# Physics of Virus. Theory : From proteins structure to capsid self-assembly ?




- M. Castelnovo, L. Menou
- Ground state : Minimisation of elastic energy (bending + stretching).
- Excited states ?

# Physics of R-Loops : AFM experiments

## Explaining R-Loops toxicity by their conformation

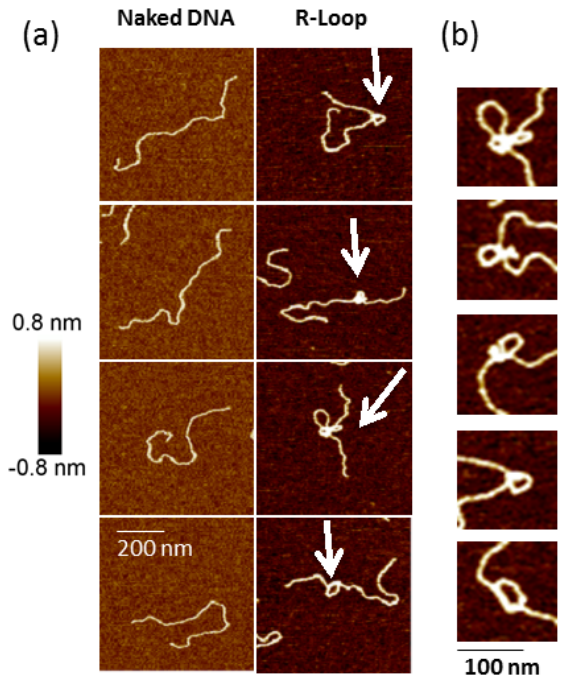
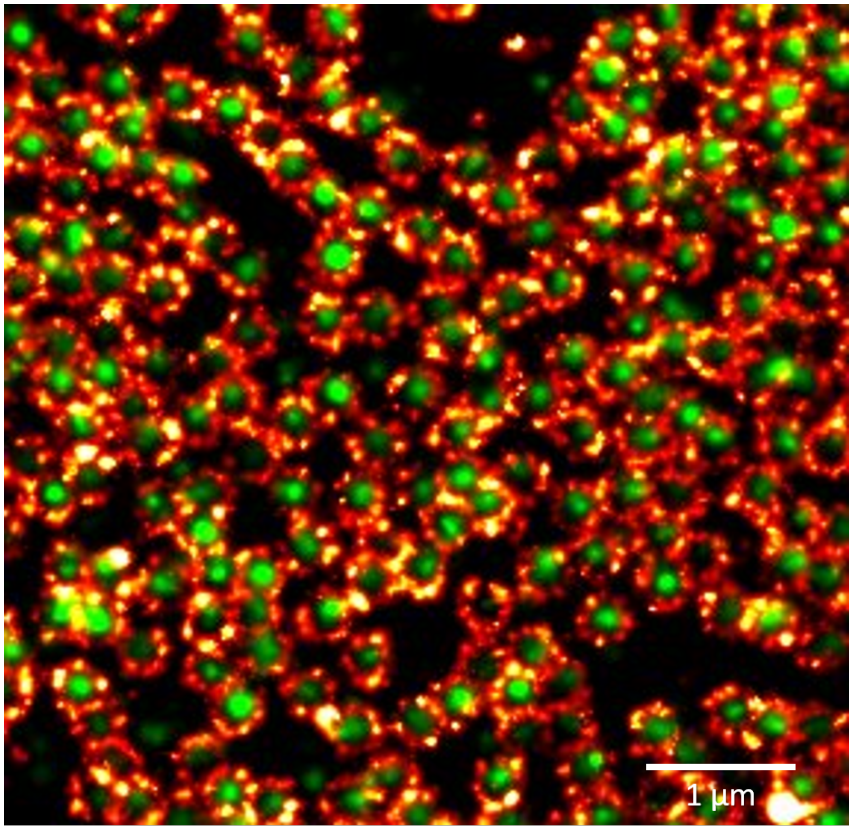



**Figure:** R-loops formed in the AIRN gene after *in vitro* transcription and visualized by AFM microscopy. (a) The left column represents the naked DNA before transcription. The DNA of the right column has been transcribed and accumulates complex structures indicated by arrows. (b) magnification of observed R-Loops structures.

- C. Moskalenko, V. Vanoosthuyse (LBMC)

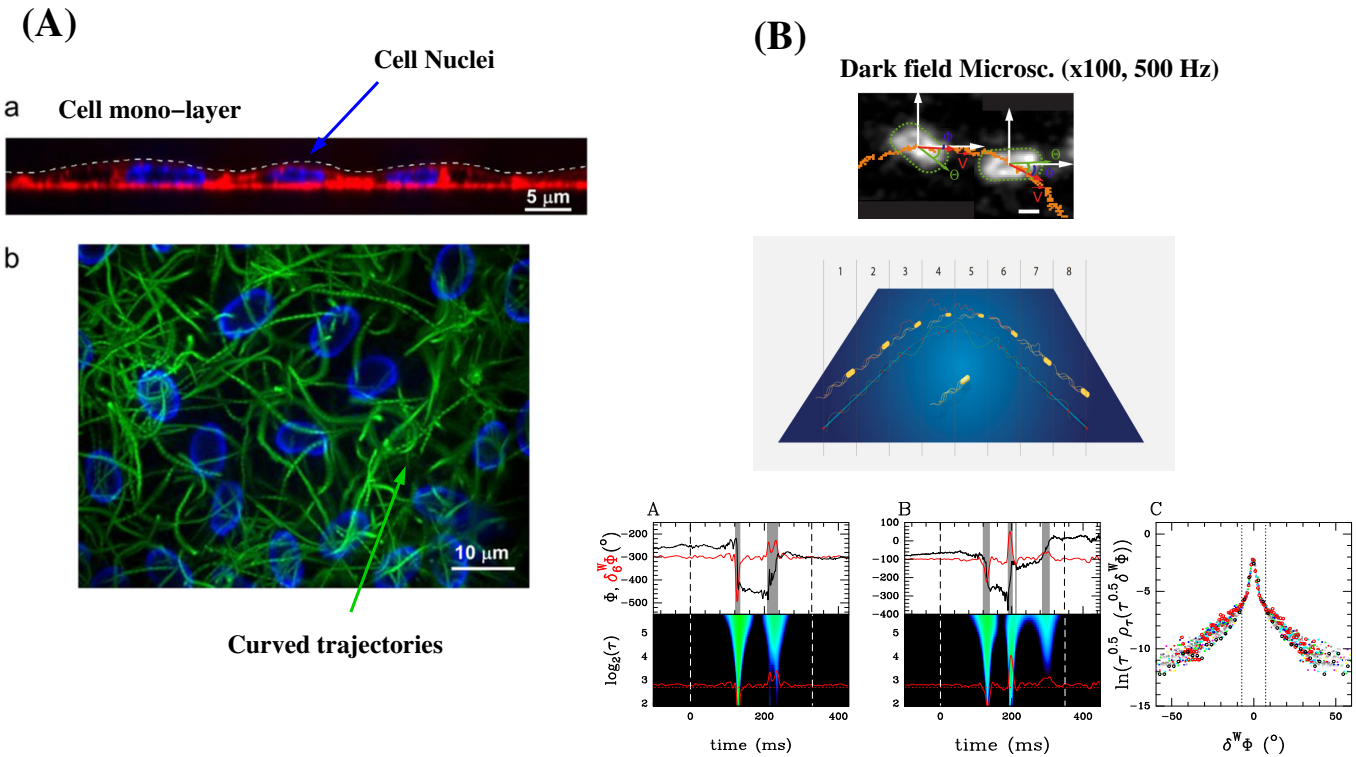
## Mecanism of high selectivity and directionality ?




- F. Montel
- In vitro reconstitution of nuclear nanopores.
- Super-resolution imaging of real nanopores.

# Physics of bacteria motility near interfaces

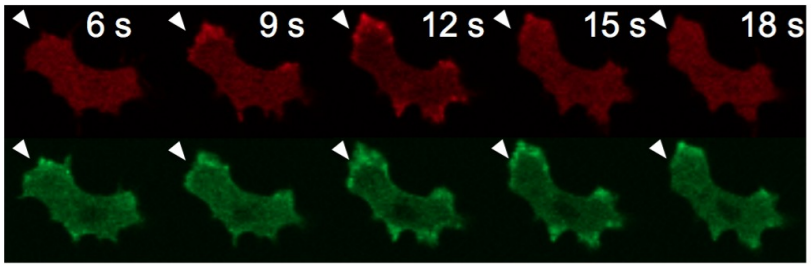
## How interfaces modify swimming and chemotaxis ?




- L. Lemelle, C. Place, JF Palierne, C. Vaillant
- At interfaces : circular motion (run) + tumbles.
- (A) Swimming of pathogen *P. aeruginosa* at cell mono-layer interface
- (B) Cell-body kinematics during tumbling of swimming *Escherichia coli* near a solid surface

# *Noise and oscillations in chemotactic cells.*

- Oscillations in the cytosol go with the formation of new pseudopods. No cAMP stimulation => no preferred motion direction.



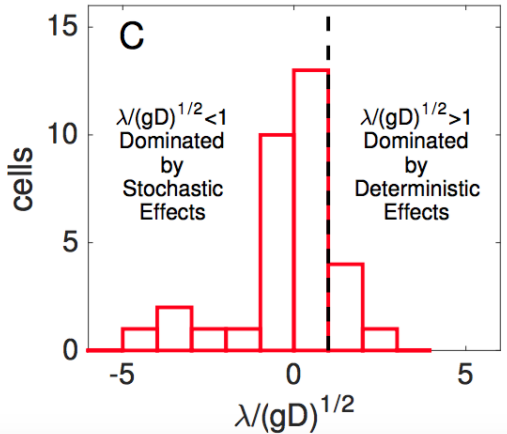

[Coronin-GFP; LimE-mRFP]

***Model:*** *Stuart-Landau oscillator with noise.*

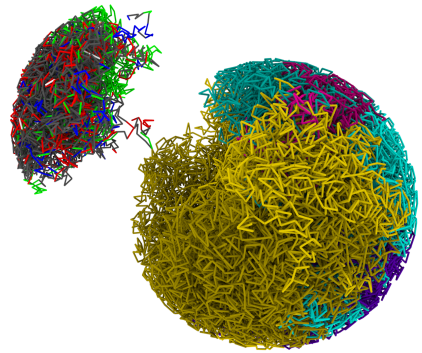
$$\partial_t z = (\lambda + i\omega_0)\, z - g\,|z|^2\, z + (2D)^{1/2}\,\eta$$

$D$ : noise amplitude; $\eta$ : Gaussian, white-noise term: $\langle \eta(t_1)\eta(t_2)\rangle = \delta(t_1\text{-}t_2)$

Explicitly measure the parameters of the model
=> the cells are close to the transition point; in a regime where noise dominates

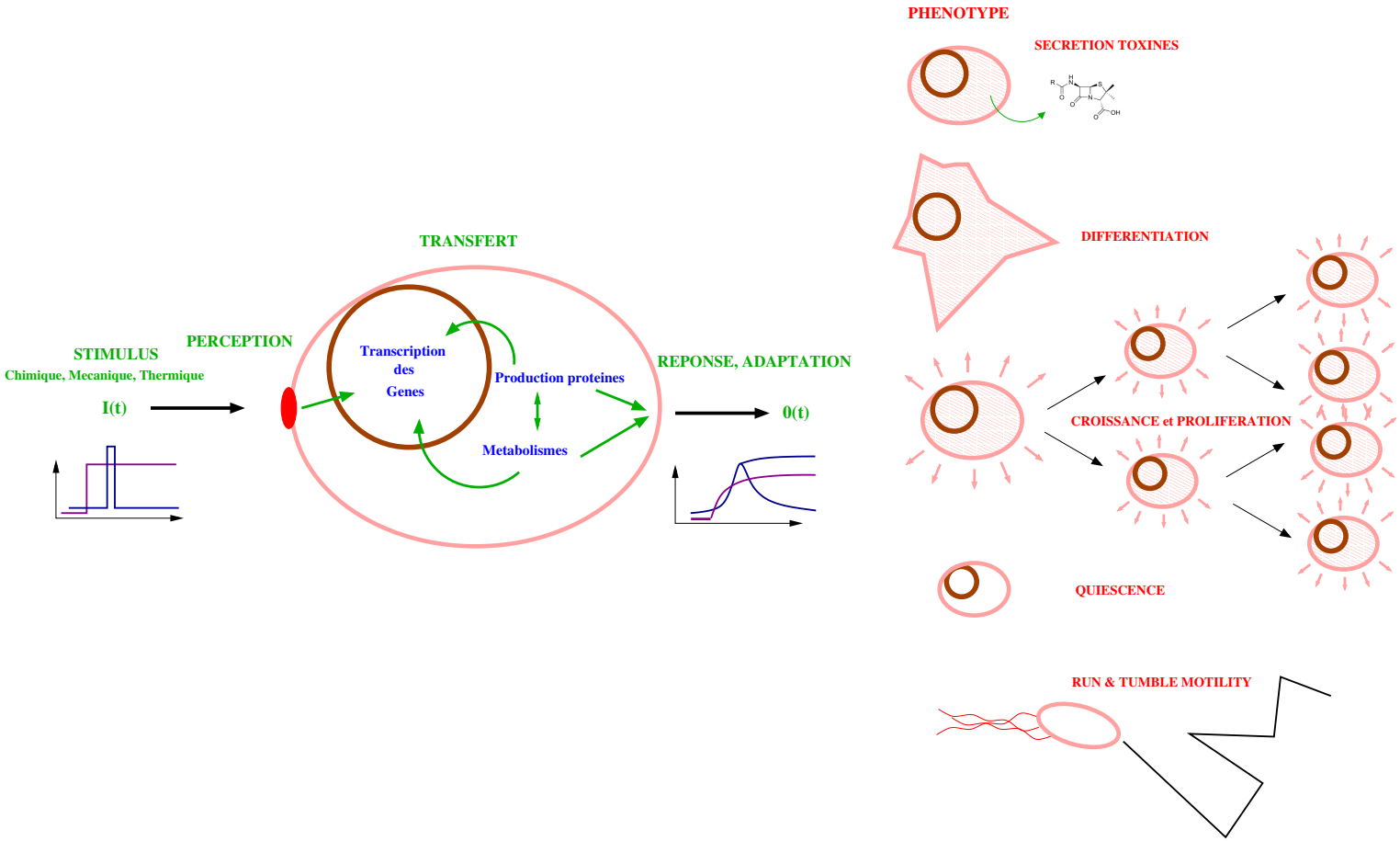


# The folding landscape of the epigenome



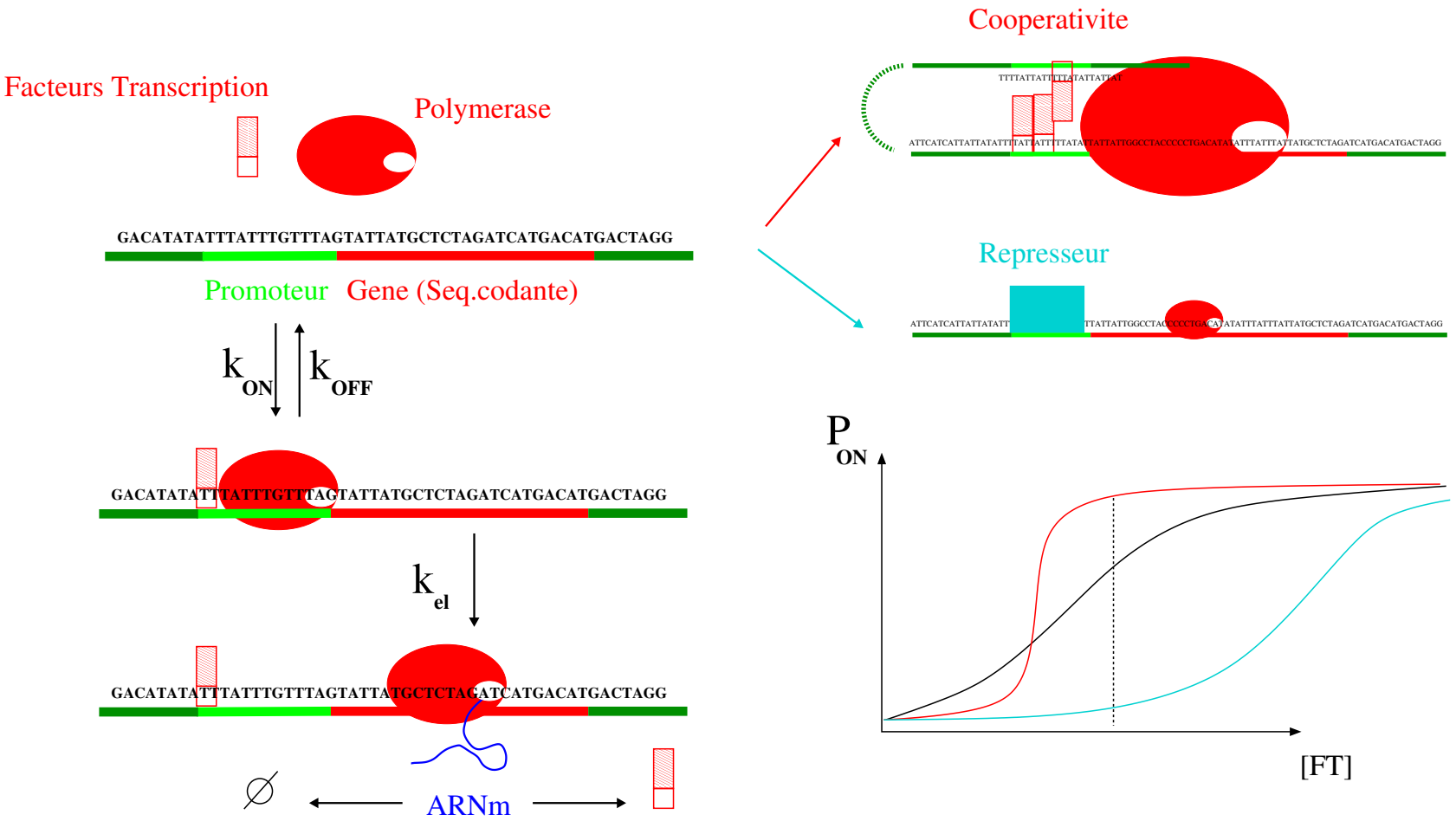
**C. Vaillant, R. Everaers, B. Audit, P. Carrivain, JM Arbona, R. Schram**
Laboratoire de Physique - ENS de Lyon
**D. Jost, S. Ghosh**, TIMC-Imag, UJF, Grenoble
**G. Cavalli, Y. Ogiyama** IGH, Montpellier.

# GENERAL QUESTION : Cellular Response




- Gene regulation in mecanisms of cellular adaptation ?
- Short term response : stress
- Long-term response : epigenetic memory during development.
- Dérégulation (environnement, mutations) : pathologies.

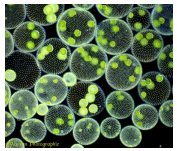
# Gene regulation : 1. Initiation 2. Elongation 3. Translation

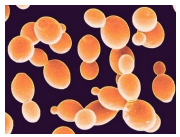


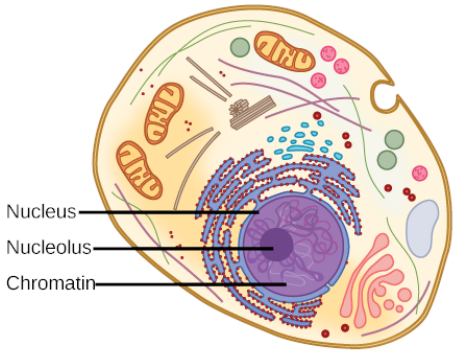

**Regulation of transcription by** Control of genomic regulatory sites (gene) accessibility.

# Context : Eucaryote → Chromatin

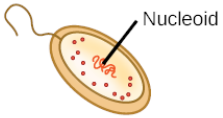
**Eukaryotes**

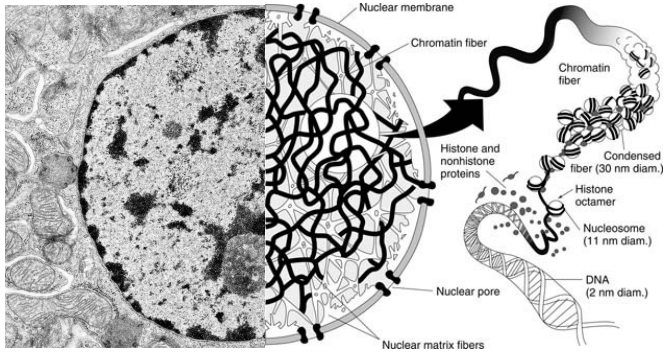

Eukaryote

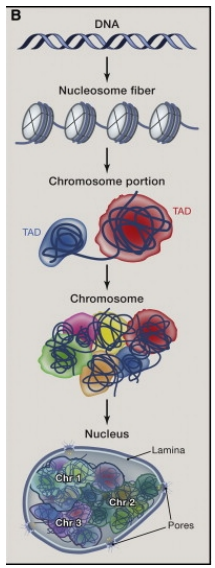



Prokaryote

**Nucleus**

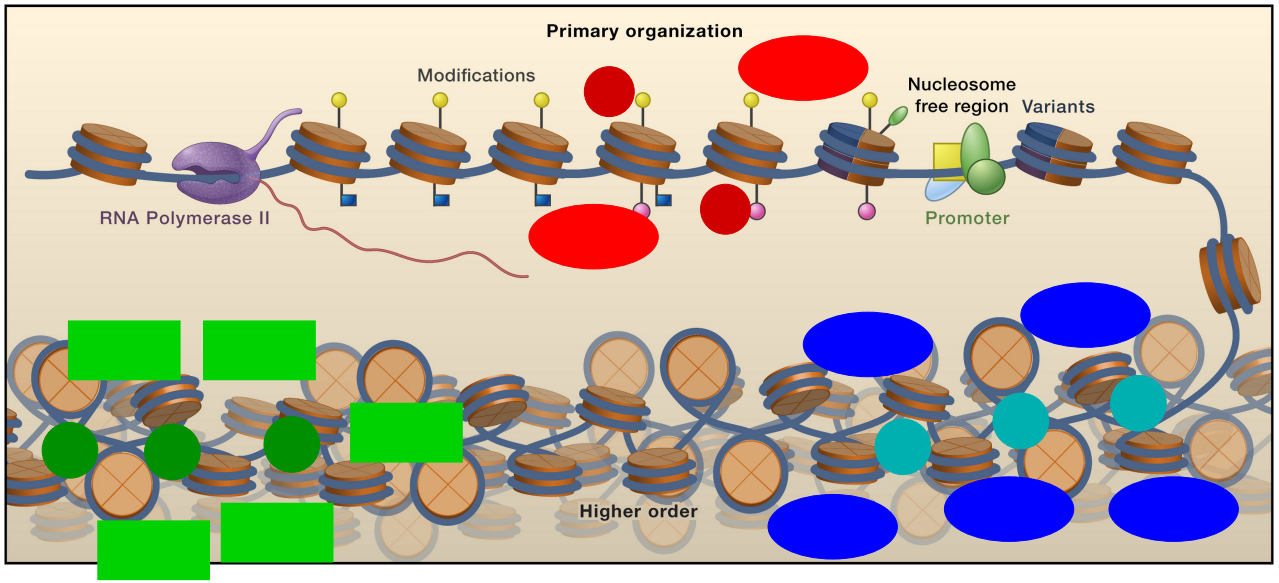
**cryo–EM**




Gene regulation and chromatin ?

**Euchromatine, H3/H4KAc**

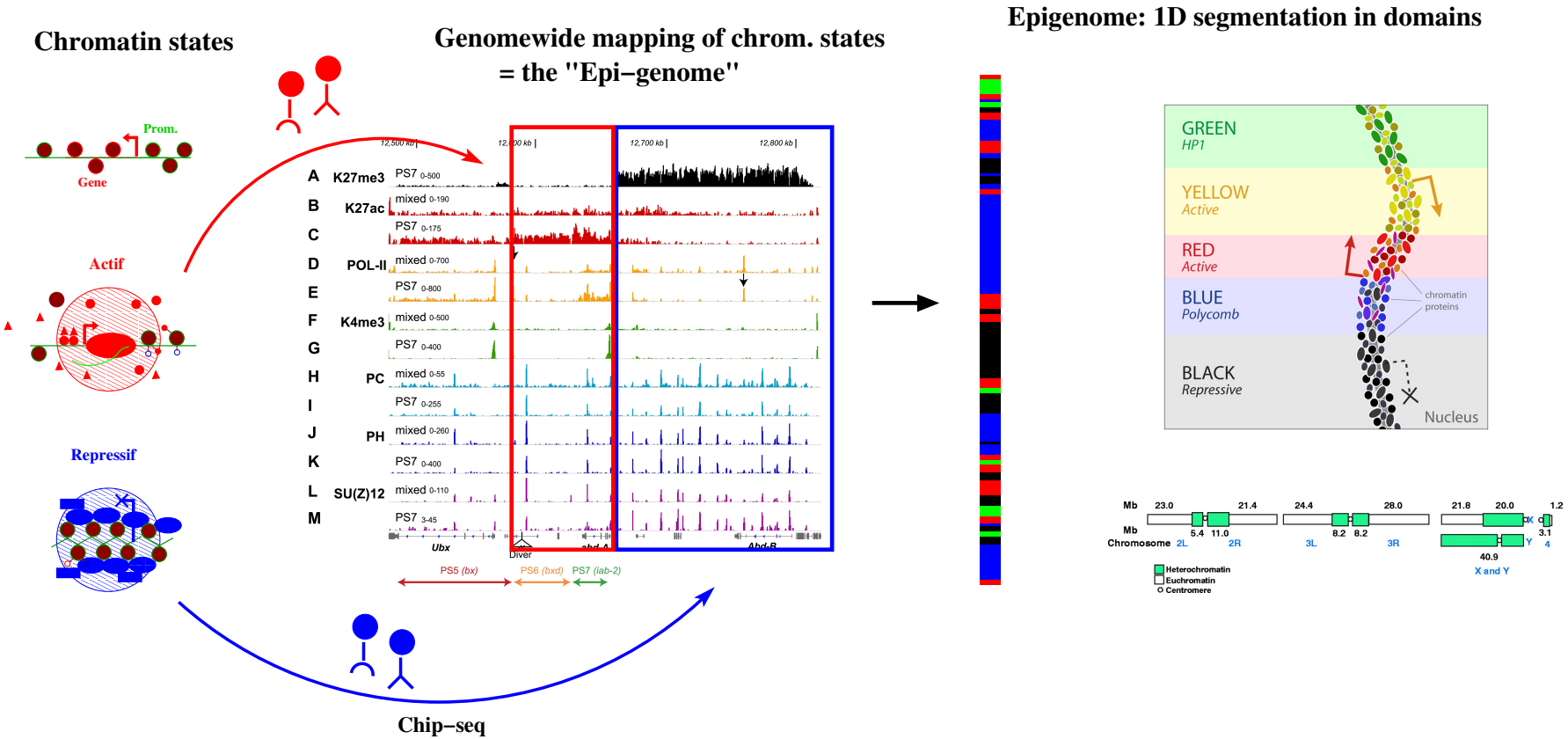



**Constitutive het. HP1,H3K9me3**

**Facultative het. PcG,H3K27me3**

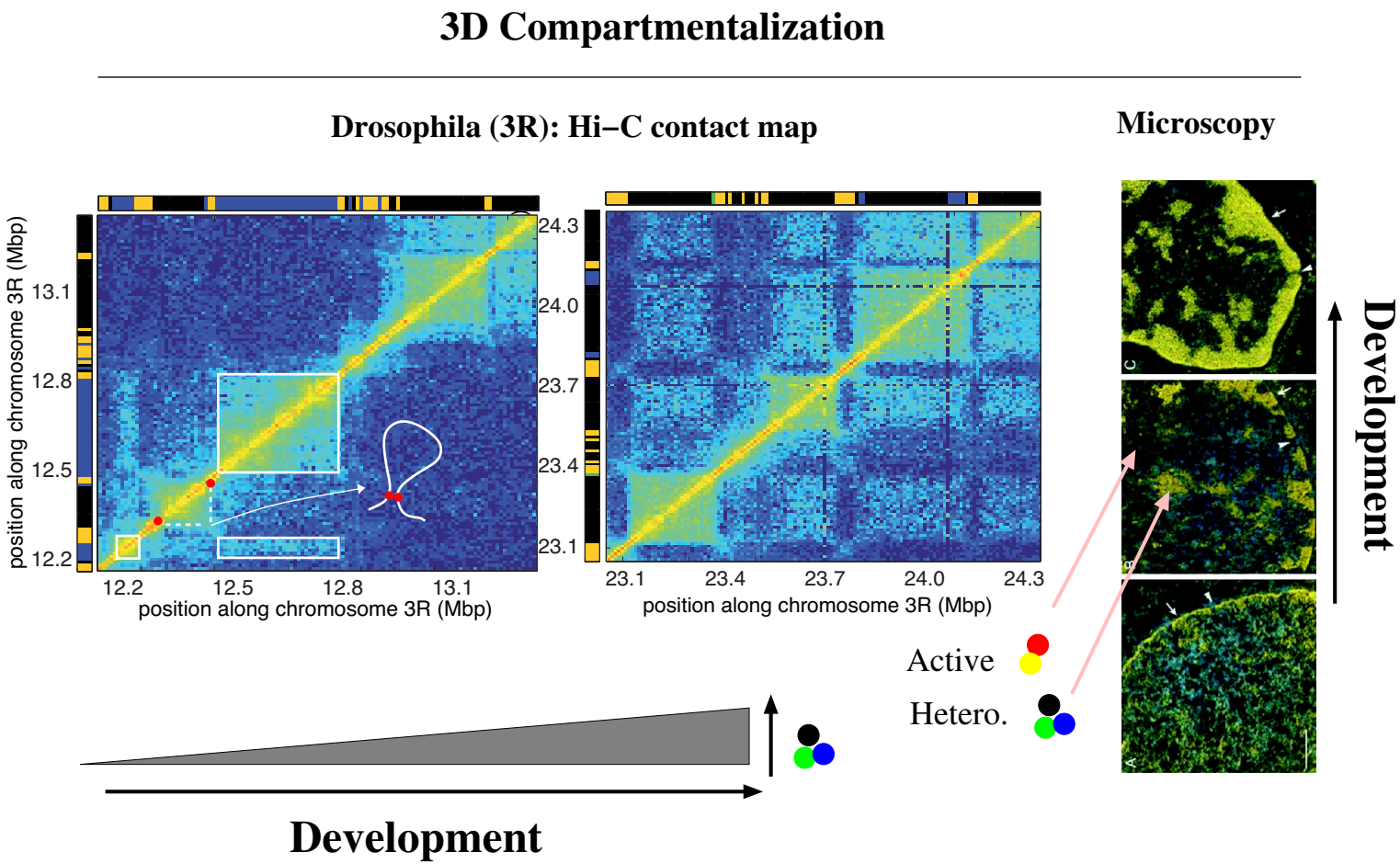
- Local chromatin states : eu- (Active) *vs* hetero- (inactive) chromatin

# Epigenome, 1D segmentation




- Epigenome = distribution of chromatin states
- 1D segmentation of the epigenome in large “mono”-chromatic domains
- Signature of cell identity. Control of Cell identity ?

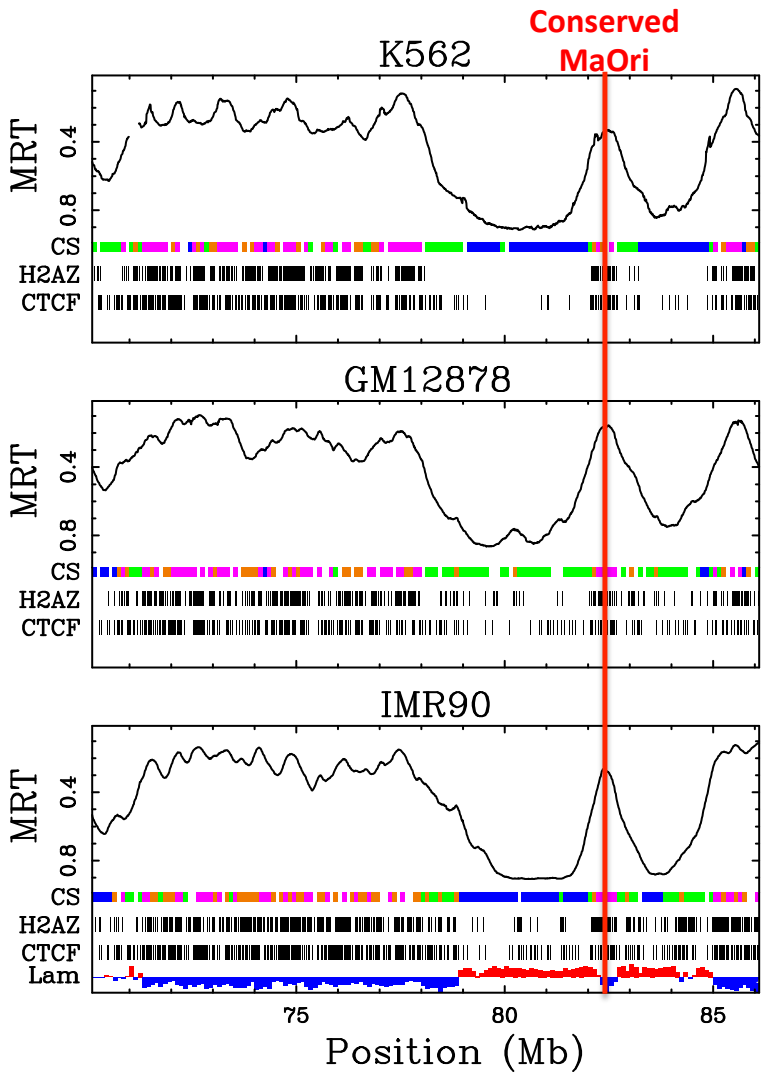
# 3D compartmentalization of the epigenome : sub-chromosome scale

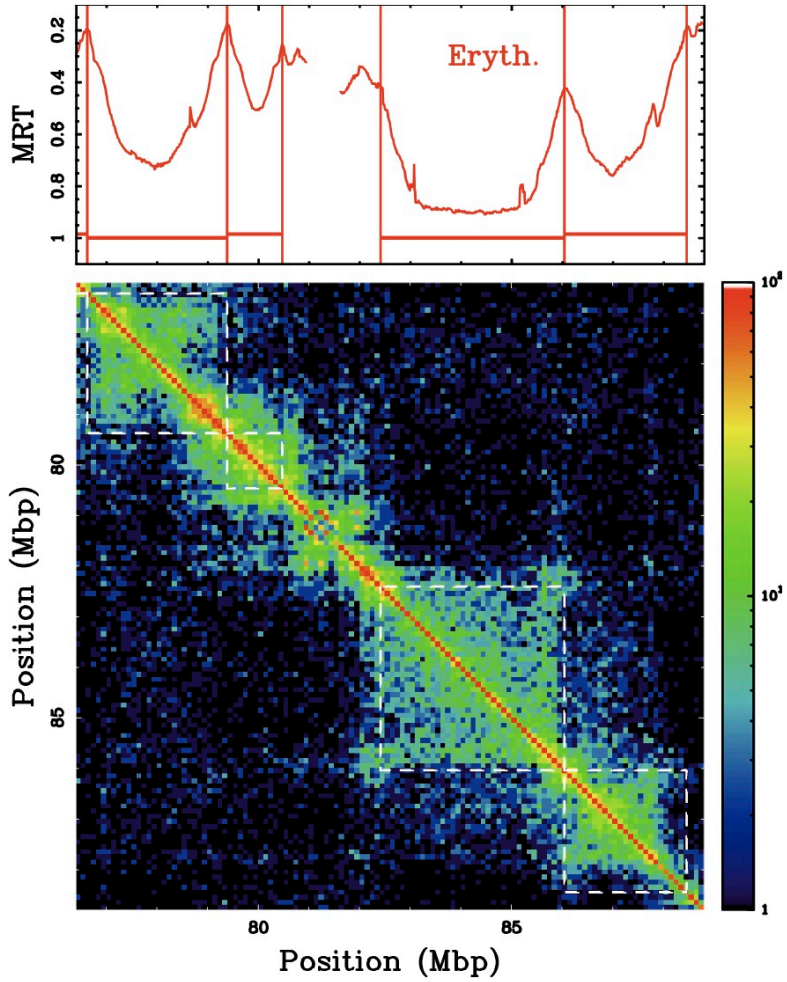



- "TADs" : internal folding of epigenomic domains & insulation with neighbours.
- "Like-like" interaction between "TADs" of the same chromatin state : Global spatial and functional compartmentalization (cf Microscopy)

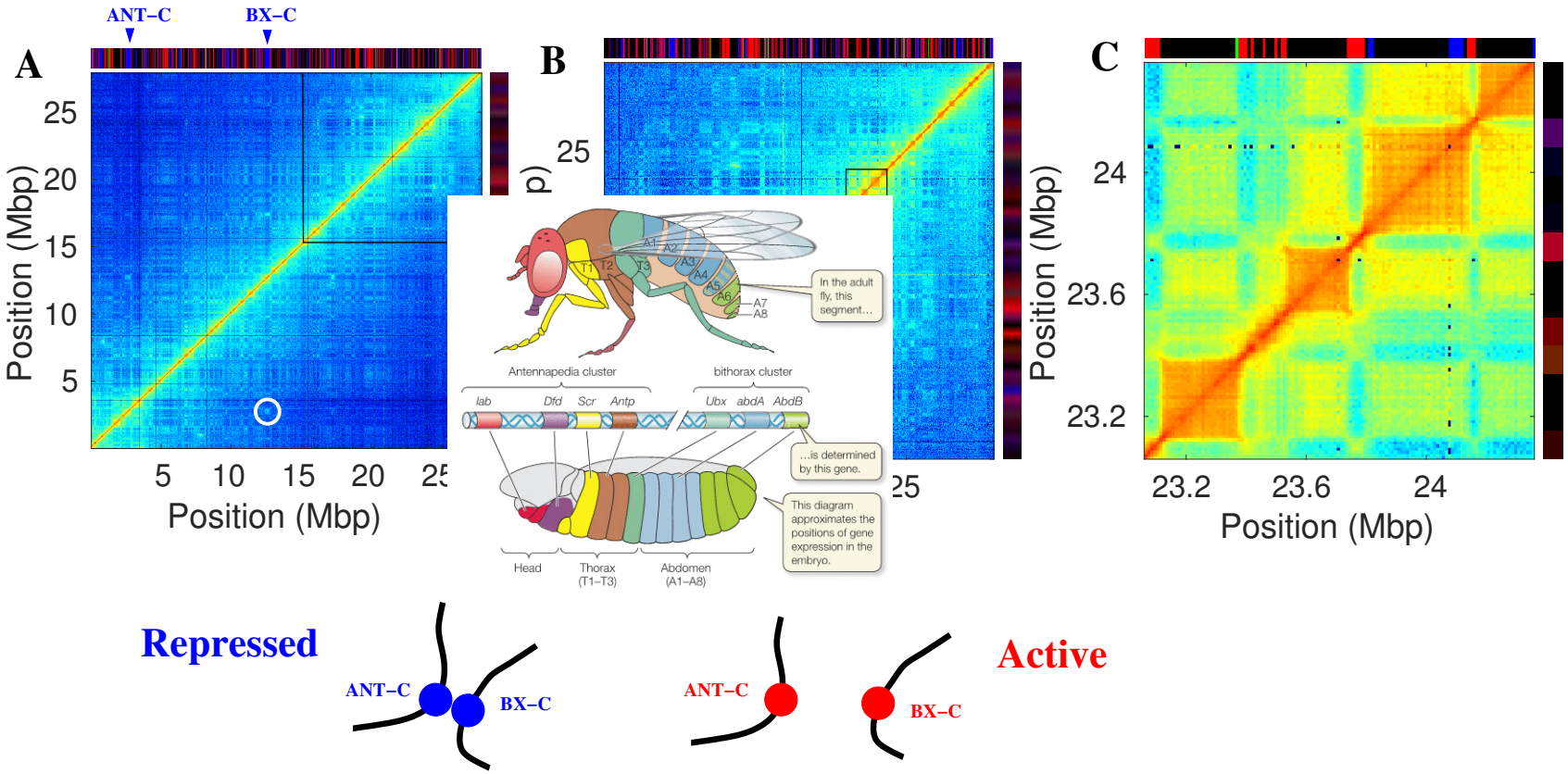
# Chromatin states and the DNA replication program

Baker, PLoS Computational Biology (2012)
Moindrot, Nucleic Acids Research (2012)
Julienne, PLoS Computational Biology (2013)



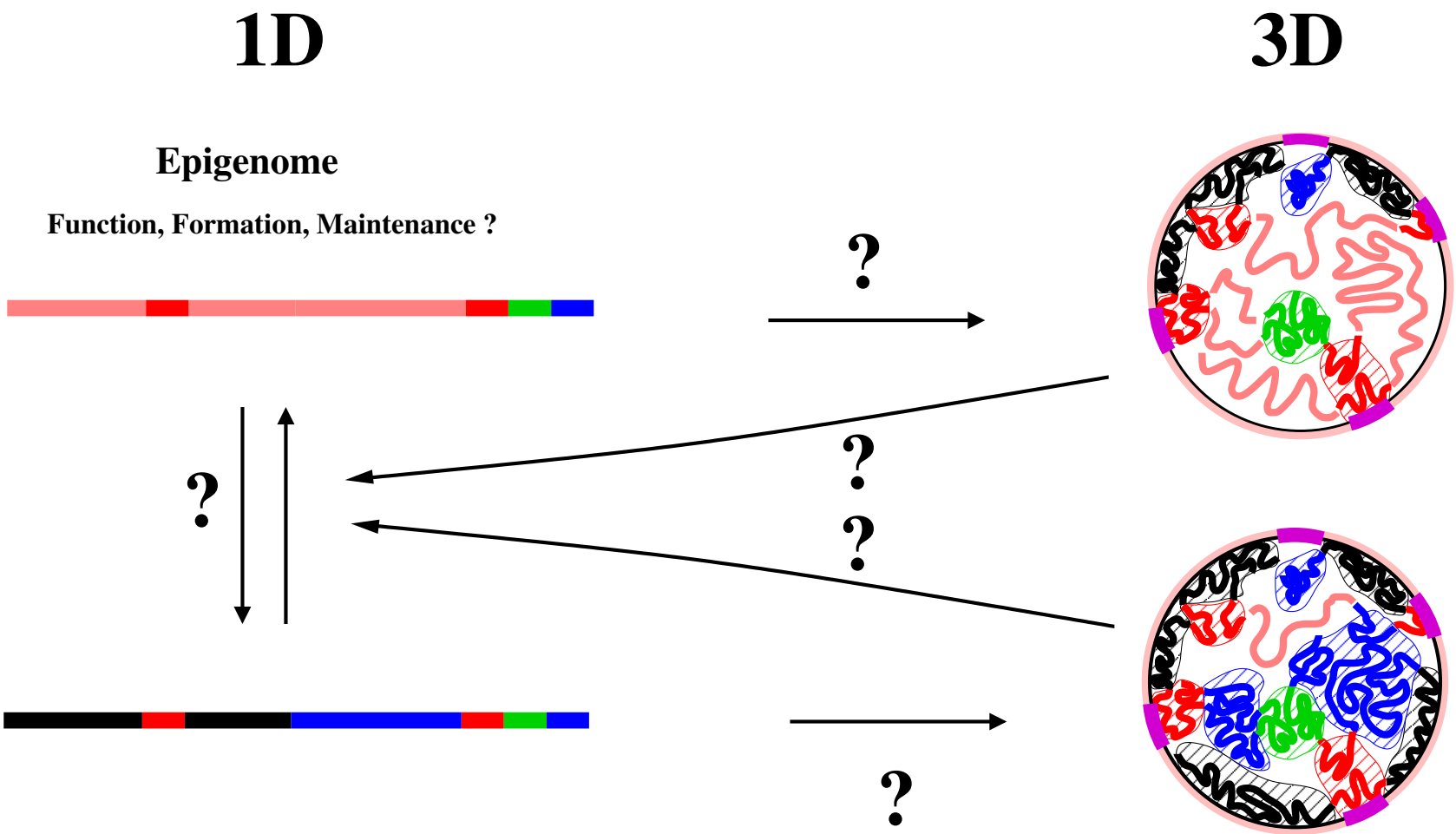



# 3D compartmentalization and epigenome regulation

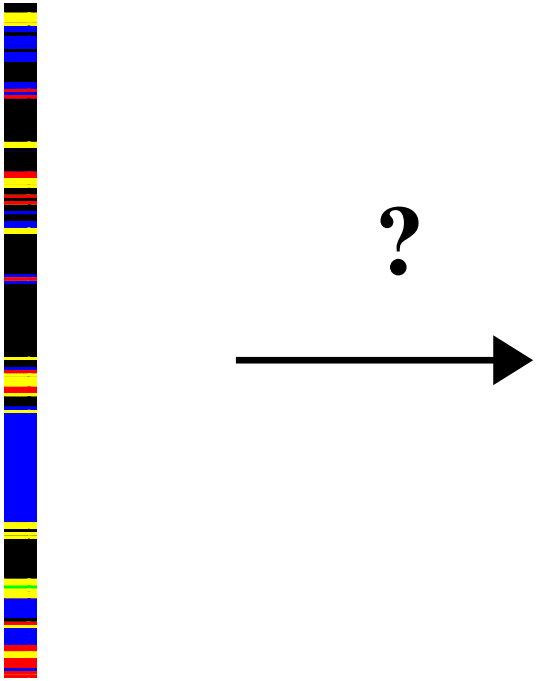


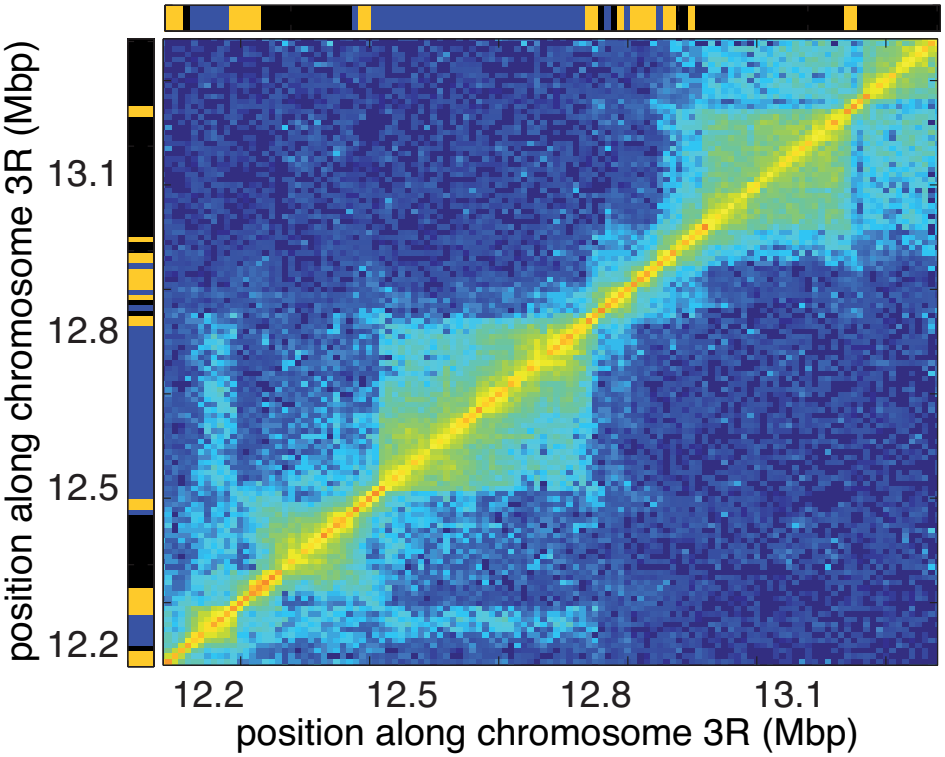

- Hox genes : developmentally regulated genes by the PcG/Trx system
- Specification of body axis/morphology (Spatial colinearity).
- Very long-range functional clustering of ANT-C and BX-C hox genes ($> 10Mbp$).
- The repression level at ANT-C and BX-C depend on their clustering.

# Questions : Coupling between epigenome (1D) and folding (3D) ?



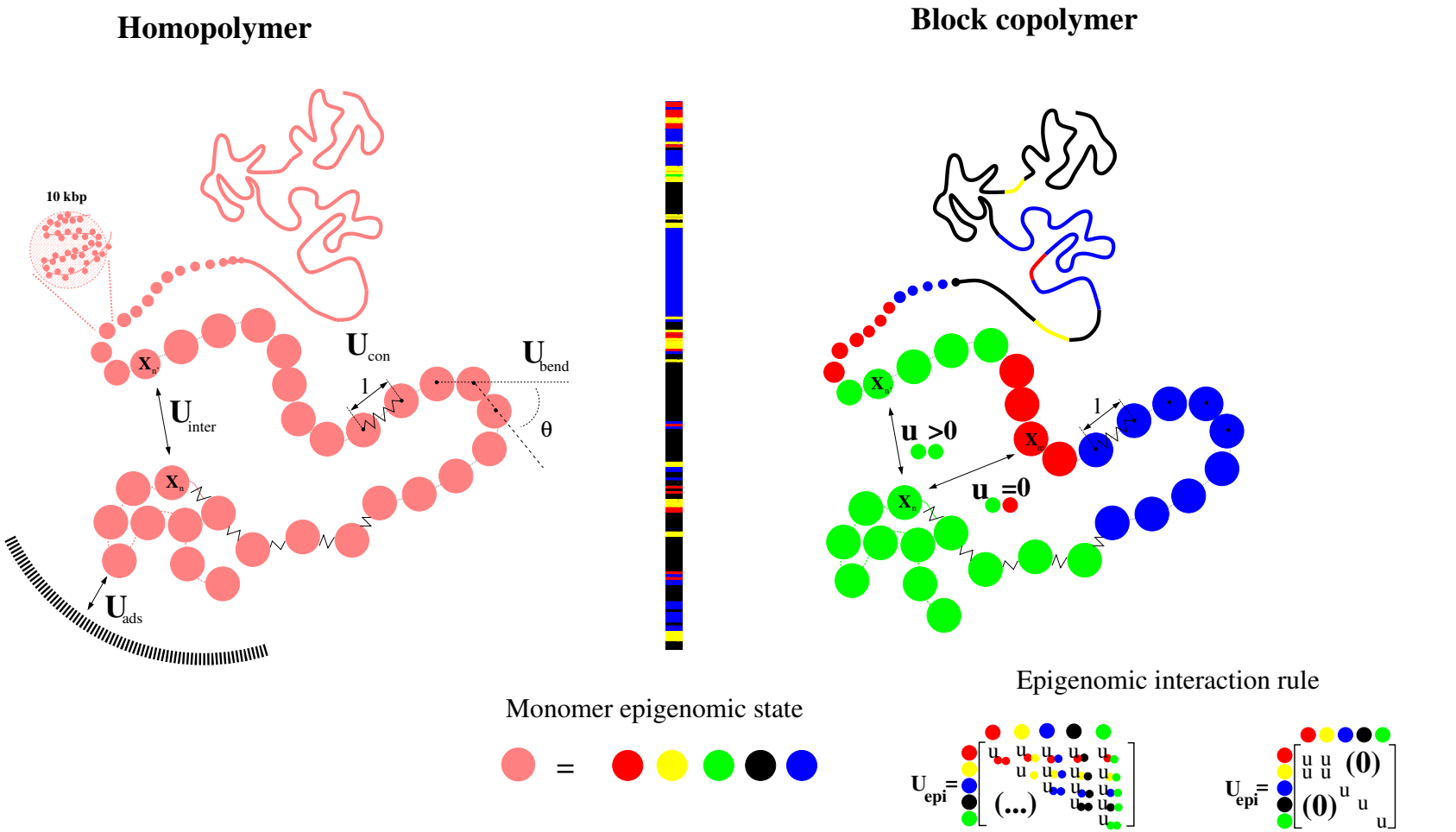
# Questions (I) Modeling the folding of the epigenome



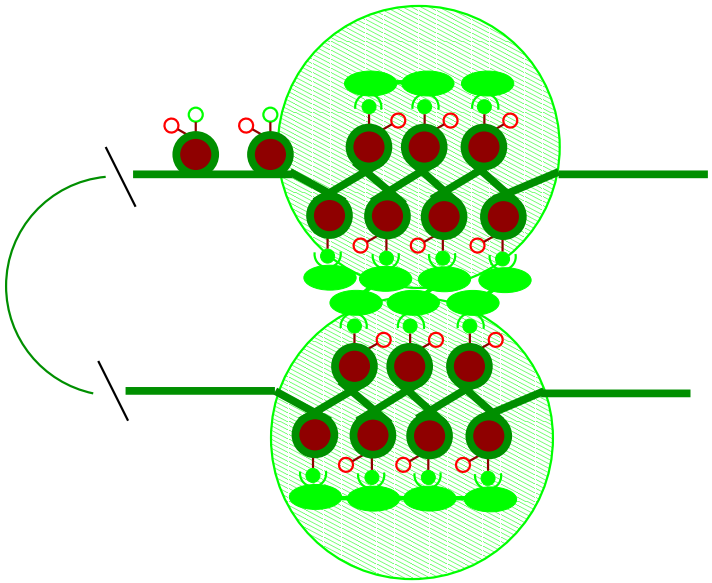



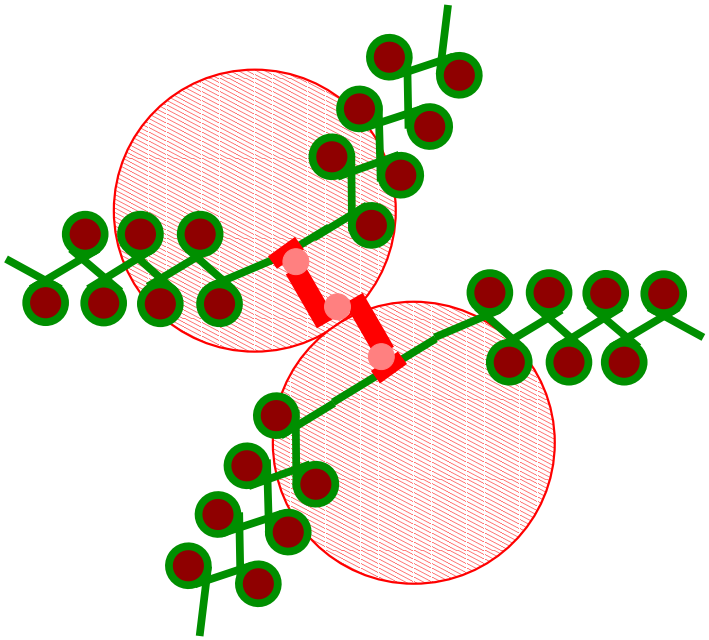
# "1D → 3D" : Block co-polymer framework

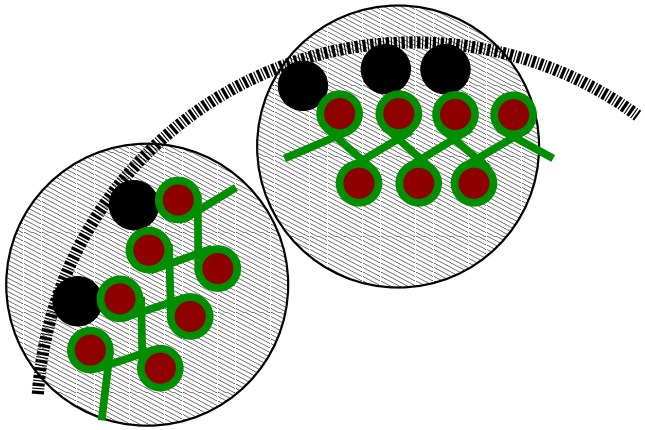
D. Jost, P. Carrivain, G. Cavalli, C. Vaillant. Nucleic Acids Res. 42, 9553-9561 (2014).



# Molecular origin of self-attraction







Hamiltonian (Energy) : $H = H_{chain} + H_{inter}$

- Self-avoiding "bead-and-spring" :

$$H_{chain} = \frac{k}{2}\sum_{n=2}^{N}(\mathbf{X}_n - \mathbf{X}_{n-1})^2 + \sum_{n<m} U_{hc}(r_{n,m})$$

- Interactions :

$$H_{inter} = \sum_{n<m}(u_{ns} + u_s\delta_{e_n,e_m})\exp[-r_{n,m}^2/(2r_0^2)]$$

Block co-polymer model with 2 parameters : $u_{ns}$ and $u_s$.

- Dynamic : Langevin equation

$$m\frac{d\dot{\mathbf{X}}_n}{dt} = -\frac{\partial H}{\partial \mathbf{X}_n} - \xi\frac{d\mathbf{X}_n}{dt} + \boldsymbol{\eta}_n(t) \quad n = 1, ..., N$$

- Molecular Dynamics : Conformation of a chain vs time (Parameters=interactions)
- Kinetic Monte-Carlo : Conformation of a chain vs time (Parameters=transition rates)
- "Gaussian Sef-Consistent" approach : Mean conformation of chains vs time (Parameters=interactions)

The idea : Approximate, at each time point, $H$ by a Gaussian hamiltonian $H_g = {}^t XVX$.
From the Fokker-Planck equation :

$$\partial_t P(\{\mathbf{X}_n\}, t) = \frac{1}{\xi} \sum_n \left[ \frac{\partial}{\partial \mathbf{X}_n} \left( P \frac{\partial H}{\partial \mathbf{X}_n} \right) + k_B T \frac{\partial^2 P}{\partial \mathbf{X}_n^2} \right]$$

Approximate $P$ with the multivariate Gaussian distribution :

$$P_g = \frac{1}{(2\pi)^{3N/2} |\det(\bar{C})|^{1/2}} \exp \left[ -\frac{1}{2} (\{\mathbf{X}_n\})^\dagger \bar{C}(t)^{-1} (\{\mathbf{X}_n\}) \right]$$

By Minimisation of the Kullback-Leibler divergence :

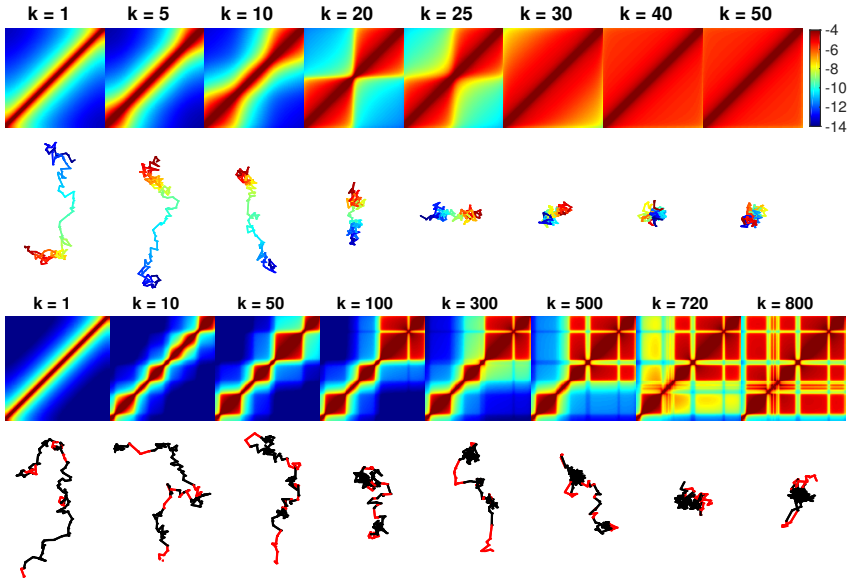
$$d_{KL}(P_g || P) = \int dY' P_g(Y') \log \left( \frac{P_g(Y')}{P(Y')} \right)$$

This gives a Self-consistent equation : for the covariance $C$ and distance matrices $D$, $D_{n,m} = \langle (\mathbf{X}_n - \mathbf{X}_m)^2 \rangle / 3$

$$\xi \frac{dD_{m,n}}{dt} = 4k_B T - \sum_k (\langle J_{m,k} \rangle - \langle J_{n,k} \rangle)(D_{m,k} - D_{n,k})$$

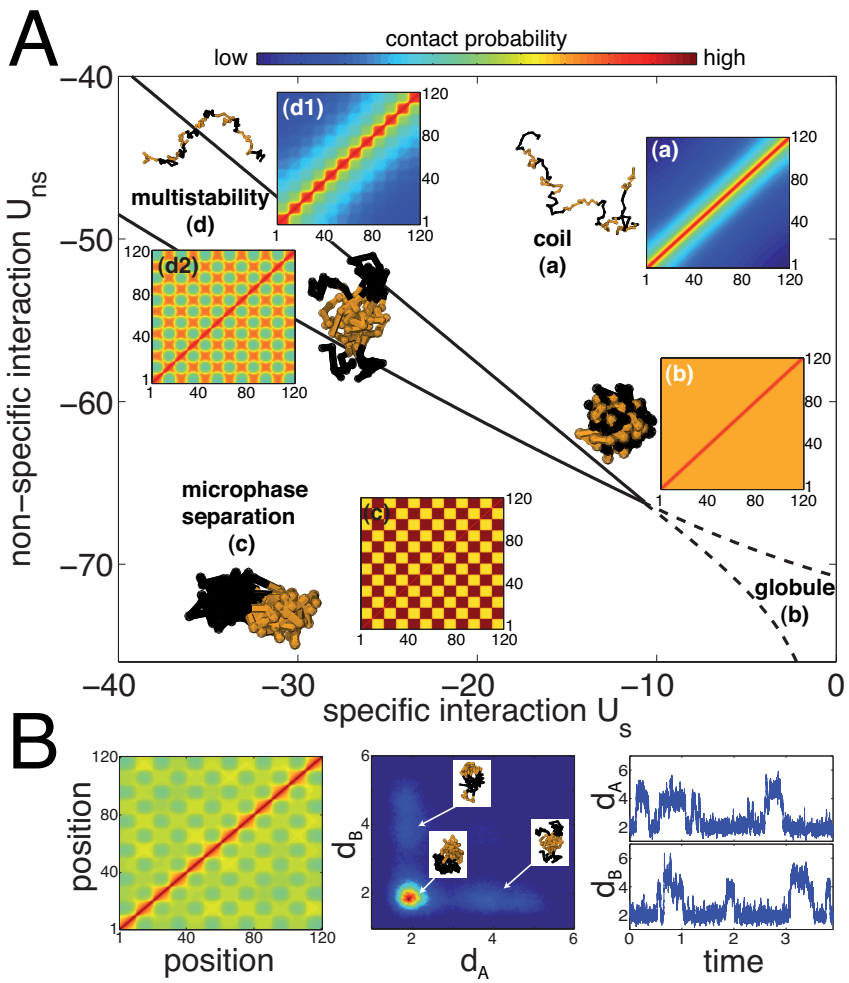
where $J_{n,m} = -\frac{\partial^2 H}{\partial X_n \partial X_m}$ and thus $< J >$ function of $u_s, u_{ns}$.

# “GSC” appoach




- “Ensemble”-averaged properties (contact map, $R_g$...) vs. time.
- From $D$, the probability of contact : $P_{mn} \sim AD_{mn}^{-3/2}$
- Convergence toward stationnary states = “fixed points” of the dynamics. (Solved by Runge-Kutta algorithm).
- Equilibrium = weighted average of this different states.

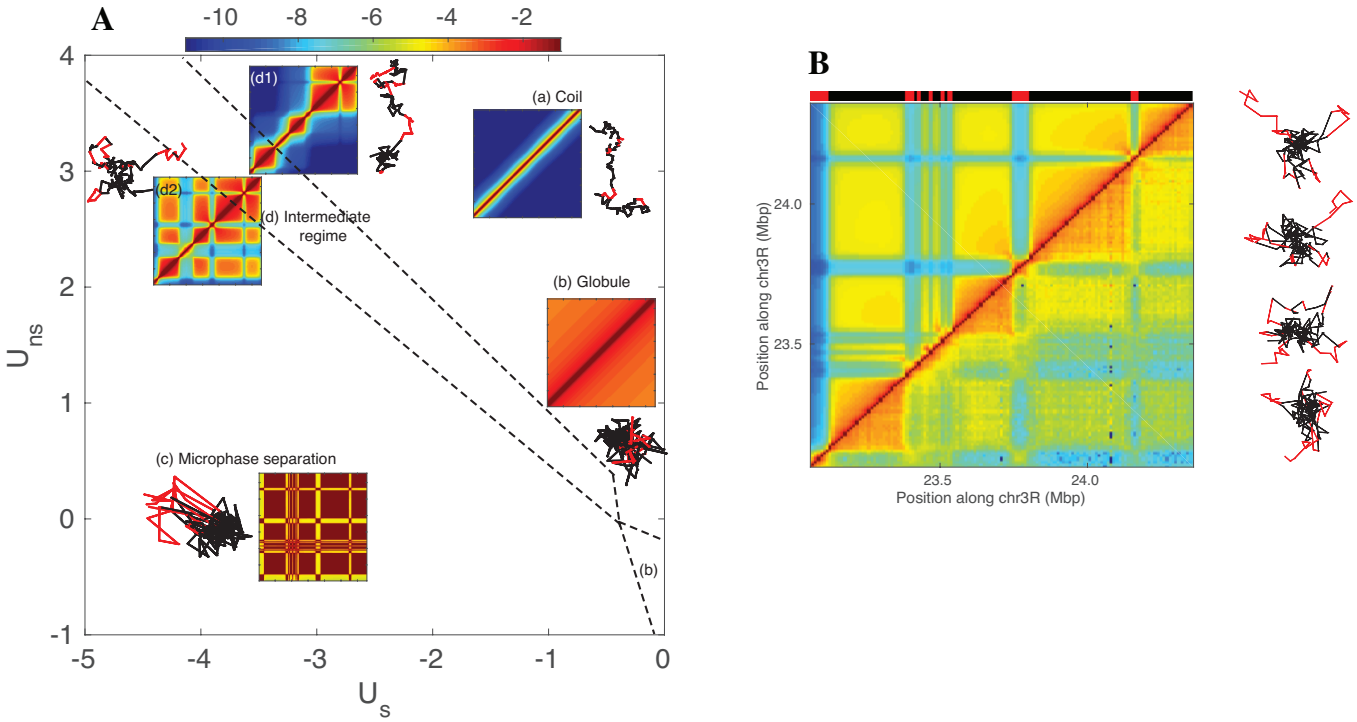
# A toy example : Block copolymer $(A_{10}B_{10})_6$ :




- Multistability region, between the Coil phase and MPS.
- Metastable states = pearl-necklace configurations of the collapsed domaines (TADs) with transient "like-like" associations between distal TADs.

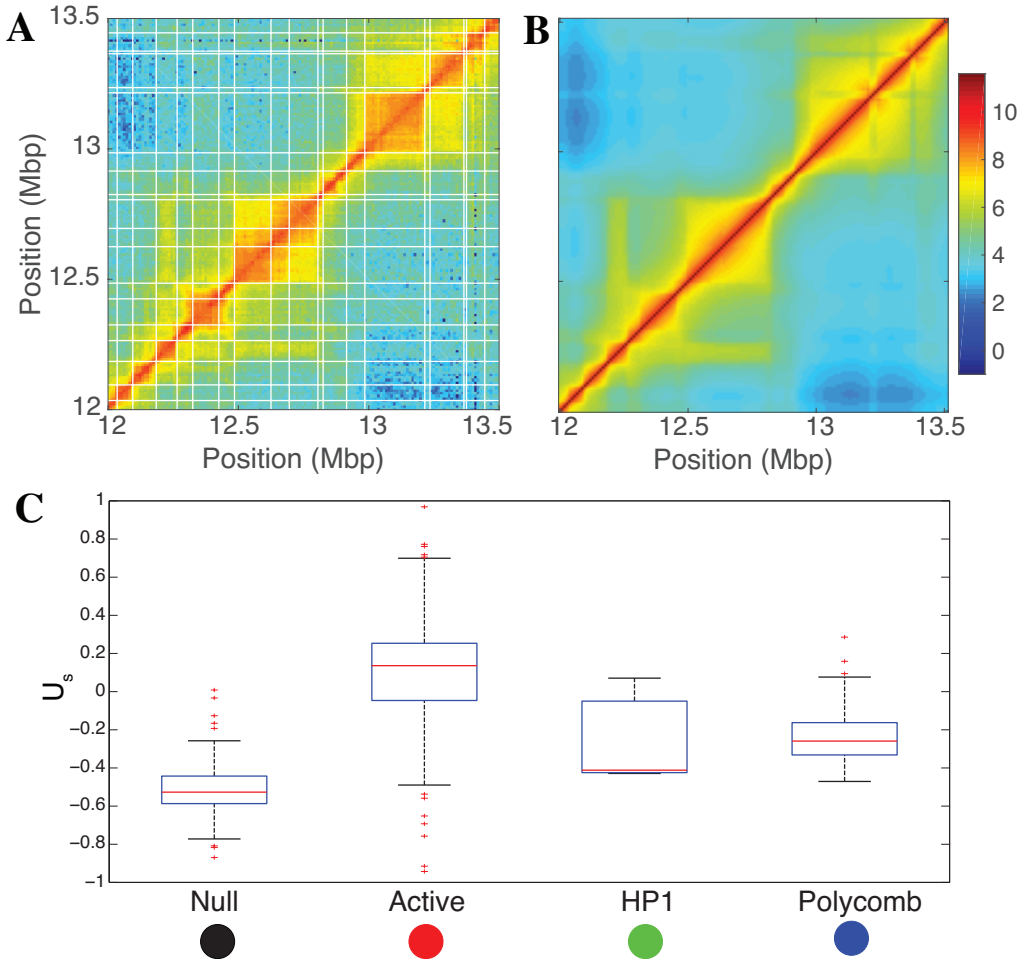
# Application to Drosophila : sub-chromosomal scale with GSC

**Simulation of short fragments of $\sim$ few Mbp.**
2 parameters : $u_s$, specific attraction between chrom states ; $u_{ns}$.




- Very good agreement
- *In vivo* folding consistent with multistability : intermediate microphase separation
- Multistability : Robust TADs and variability of inter-TAD contact

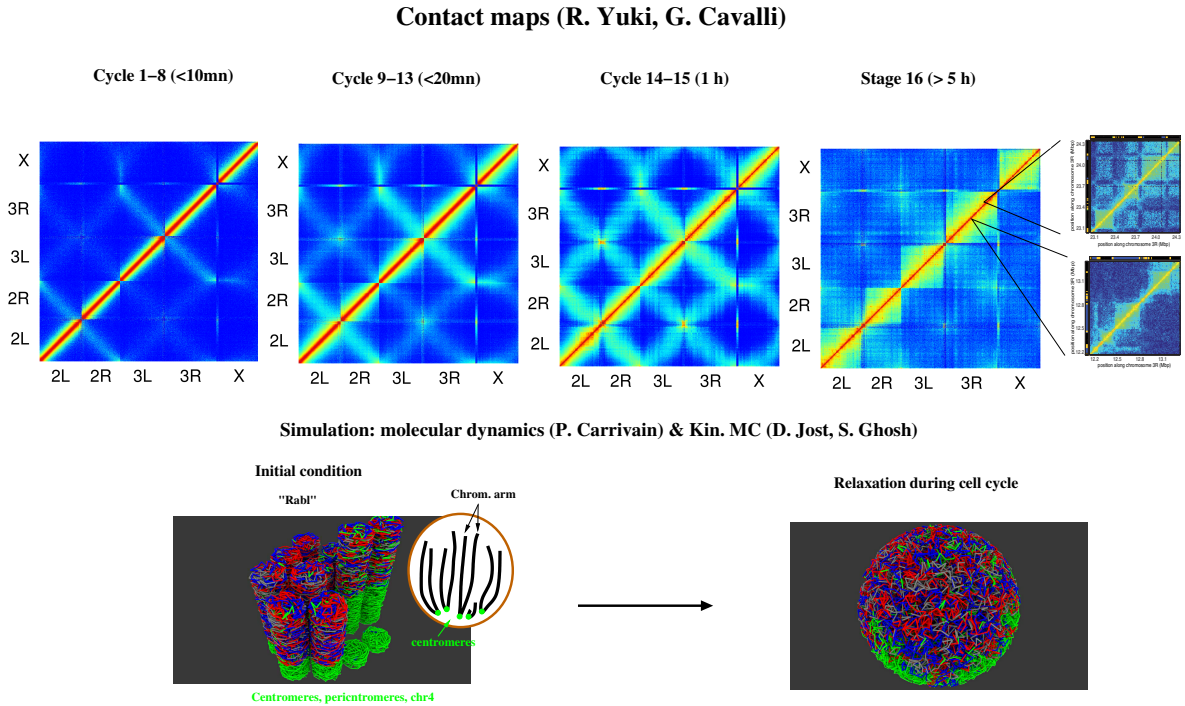
**Inference : Simulation of short fragments of $\sim$ few Mbp.**




Different modes of interaction in Active vs Inactive domains : $u_{A,A} \sim 0,\ u_{II} < 0$

# Drosophila : whole chromosomes relaxation dynamics

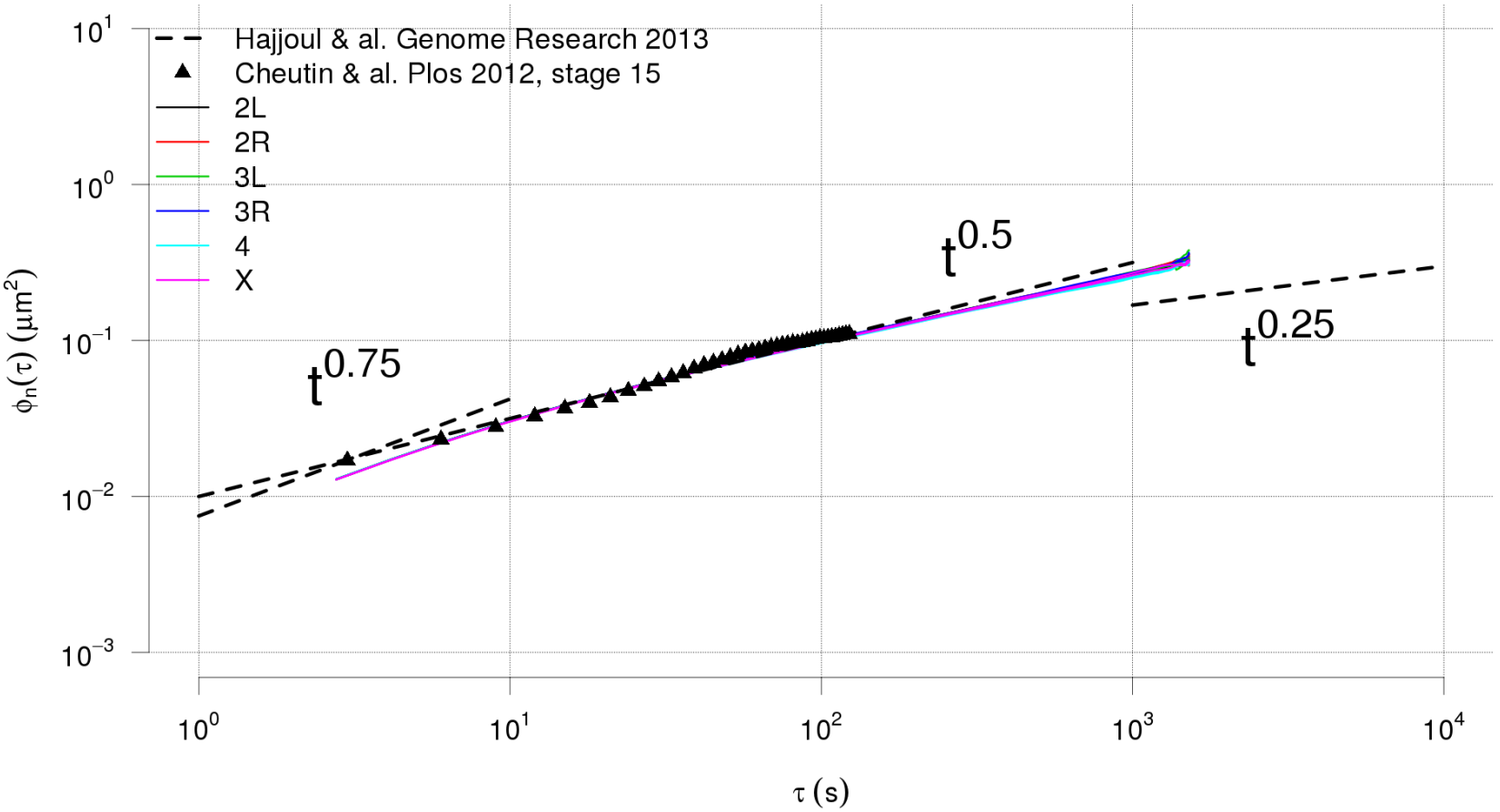
Molecular Dynamics of all chromosomes ($\sim 300 Mbp$, $\sim 30000$ monomers)




- Relaxation from Rabl-like diploid mitotic chromososomes.
- Proper mapping of the time, *in silico* CPU vs *in vivo* real time.
- Interaction parameter : like-like attraction (value $\sim$ inference by GSC).
- Comparison with Hi-C experiments at different stages (with $\neq$ cell cycle duration times).

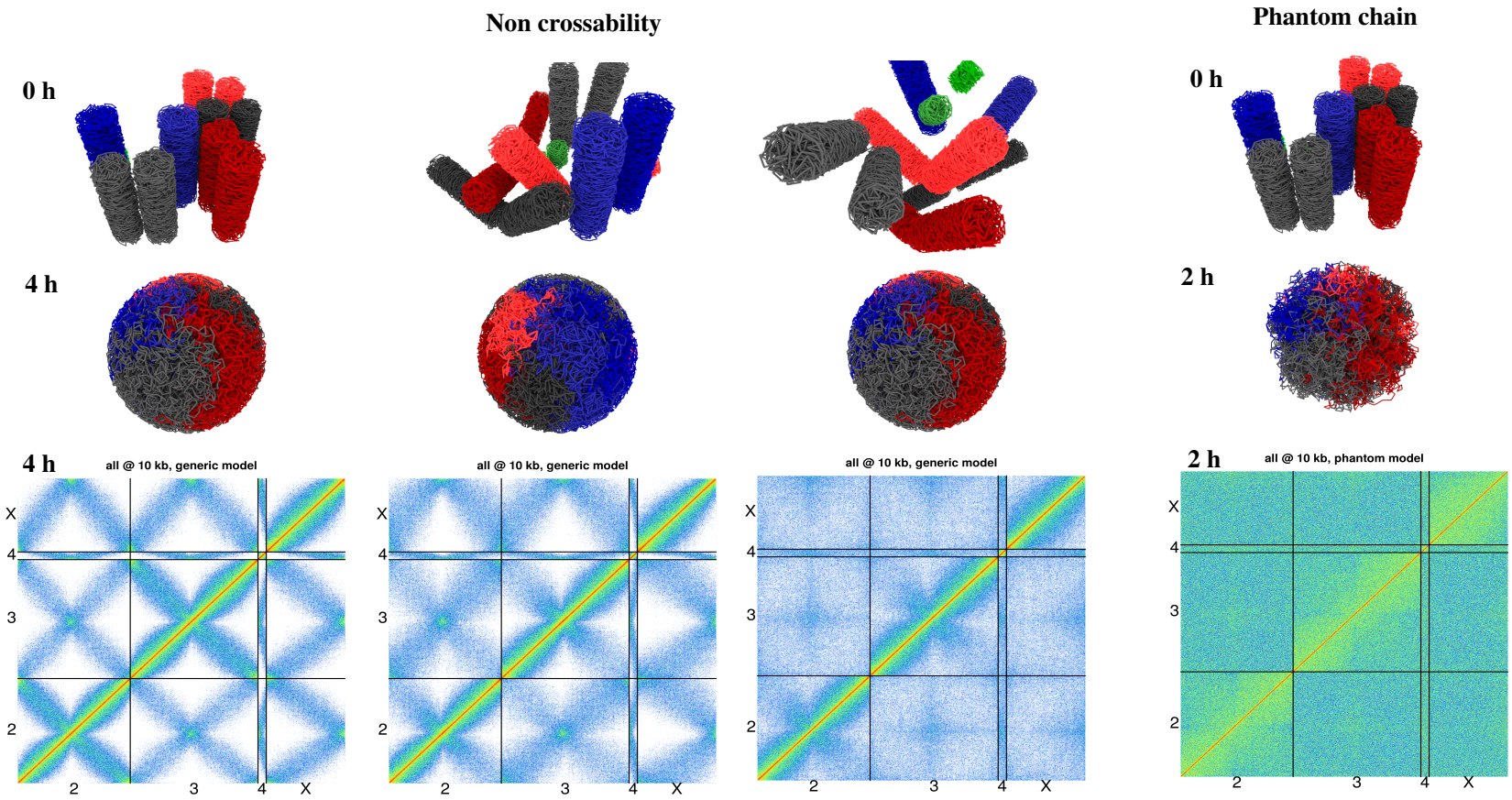
# Mapping of the CPU vs real time

Experiments : MSD vs time



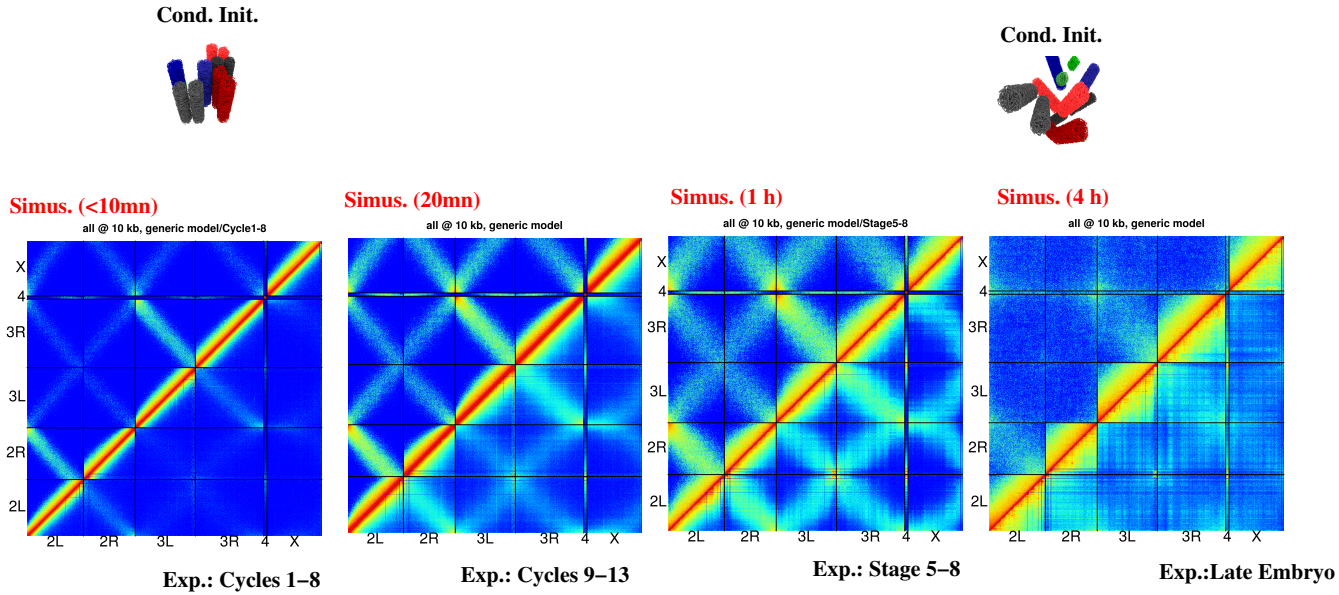

- 4 hours (real) $\sim$ a week (CPU)

# Very slow relaxation of large scale organisation



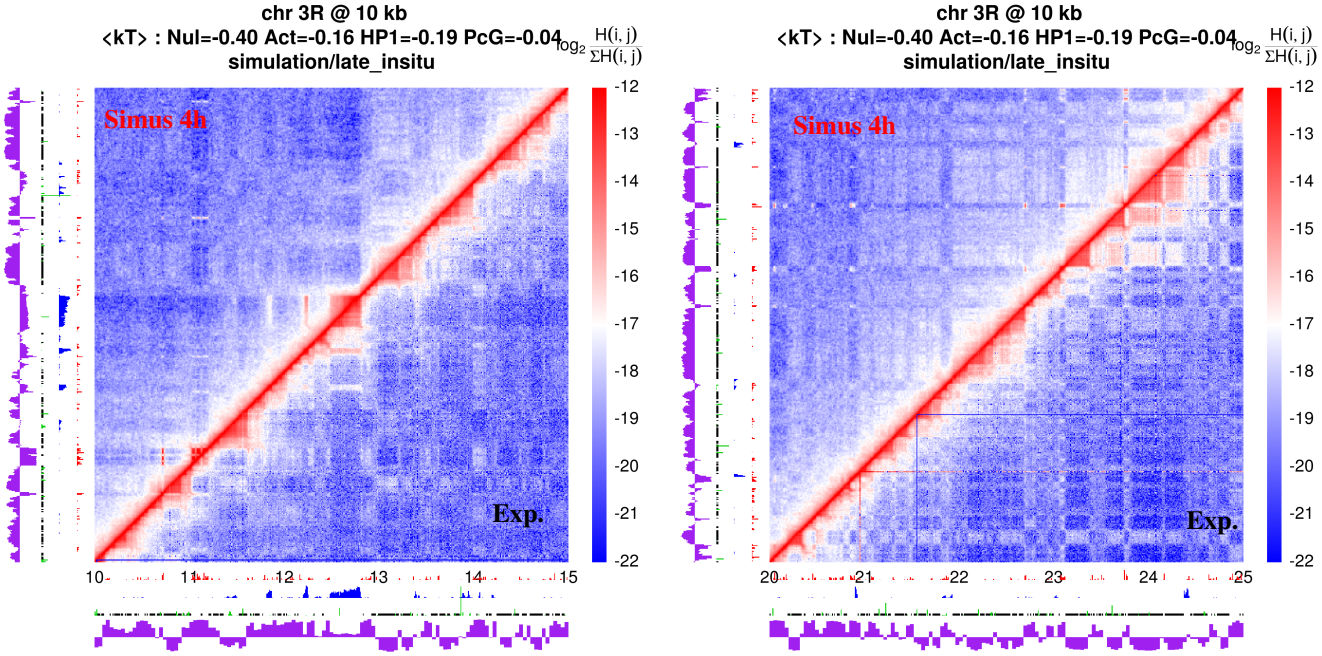

- Equilibration is very slow ($L^3$) due to topological constraint
- For cell-cycle relevant time, Large scale = memory of initial condition.
- Relaxation of topology may promote faster equilibration

# Large scale organization : Exp vs Simus.




- Generic model (no self-attraction).
- Good description of large scale organizations at the $\neq$ devpt stages
- Need a proper initial condition
- Very long range contact challenged by cell-cycle duration.

# Compartmentalization of the epigenome : microphase separation

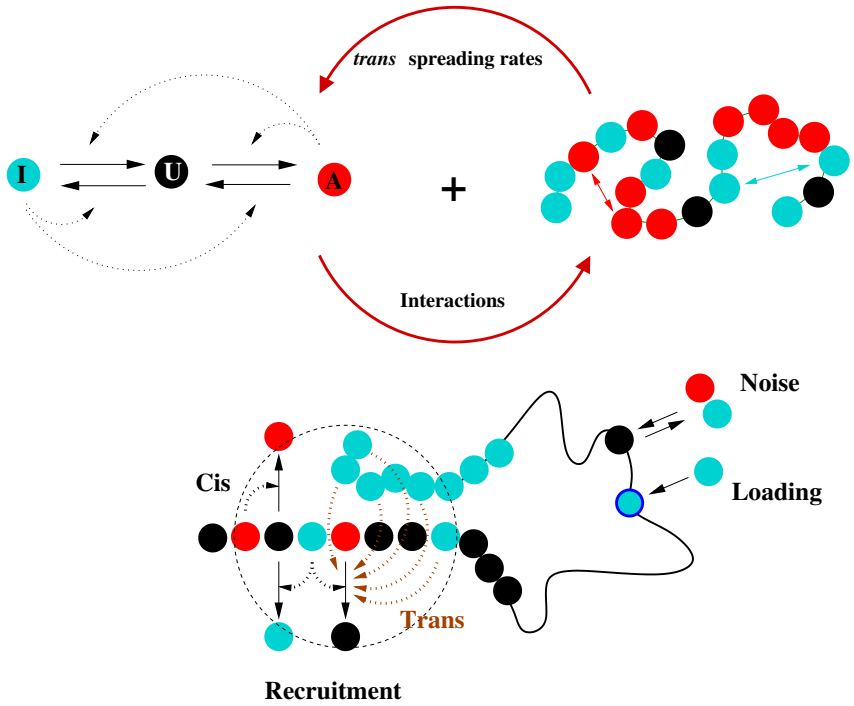



- Epigenome 1D segmentation in late embryo.
- Good agreement, with weak self-attractions
- “Intermediate” Microphase separation (No strong clustering)

# Conclusion

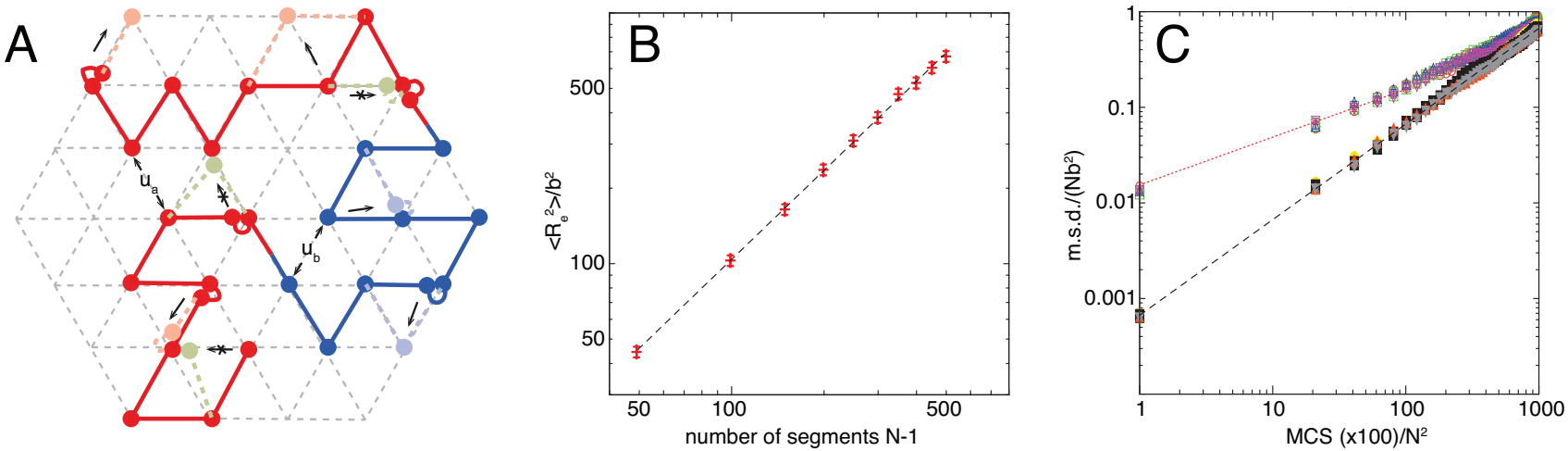
- **Block Co-polymer framework** : Nuclear organization as a phase separation.
- **Small scales** : multistability, weak self-attraction
- **Large scales** : memory of init. cond. ; weakly depend on self-attraction ; weak TAD-TAD, centro. clustering
- Confirmation by super-resolution imaging (M. Nollman).
- Improvement of the models : parameter inference, other interactions (Discrete bridging, Membranes...), active processes (loop extrusion)
- Predictions and tests : Droso. (G. Cavalli), Human Senescence (G. Cavalli)
- **Living-chromatin framework** : Epigenome is not fixed ; Regulation coupled with 3D.

# The model : The Living Chromatin framework



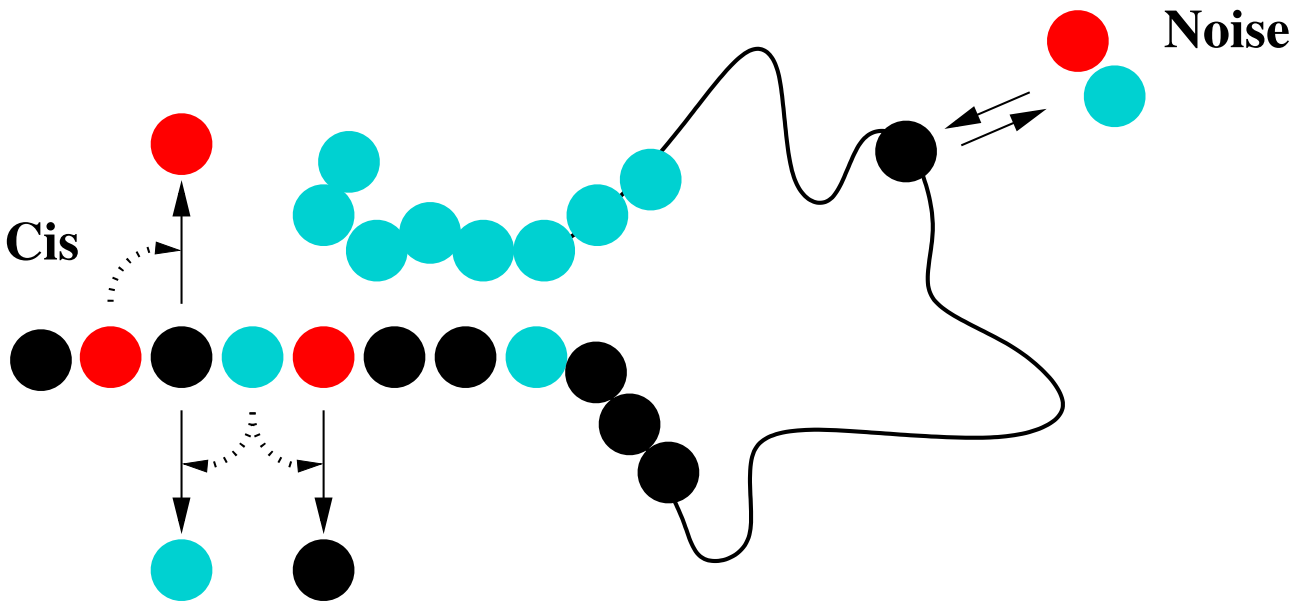

- Annealed copolymer with "like-like" self-attraction : $u$
- Noisy conversion : leaky activity of enzymes, turn-over... : rate $\epsilon_o$
- Recruited conversion : the "reader-writer" principle
  ($cis$, $trans$) spreading rate $\epsilon_{cis}, \epsilon_{trans}$
- "Loading sites" : silencers, promoter/enhancer : rate $h$

# The method




- Co-polymer : Kinetic Monte Carlo on a lattice
- Isolated chain of $N = 100$ monomers.
- Self-attraction : bonding rate $k_b$ and unbonding rate $k_u$
- Conversion of monomer $i$ with rate (*e.g.* if in Active state) : $k_{A\to U}(i) = h^I(i) + \epsilon_0 + \epsilon_{cis} n_{cI} + \epsilon_{trans} n_{tI}$
- *trans* "recruited" conversion : the nearest 3D neighborhoud.

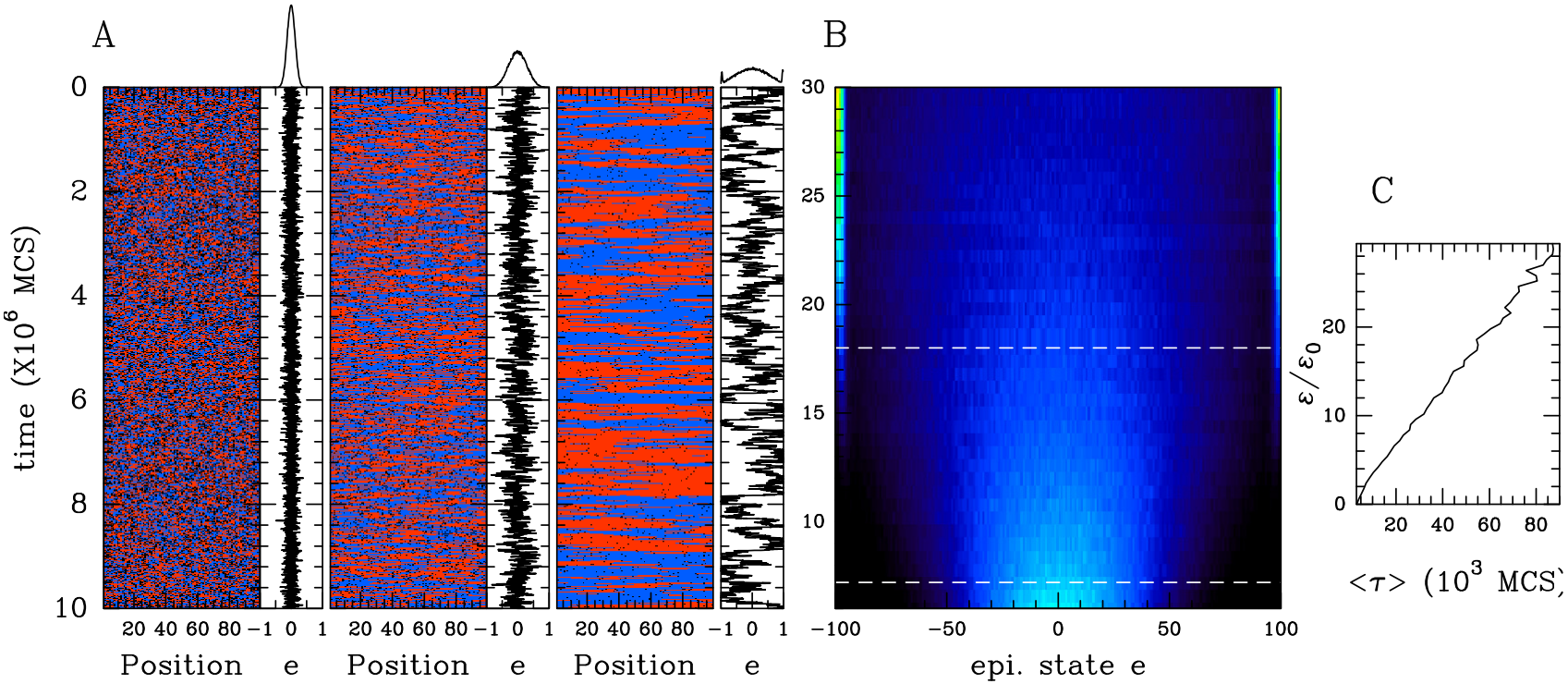
# Nearest neighbour (1D) Spreading




**Spreading in cis (1D)**

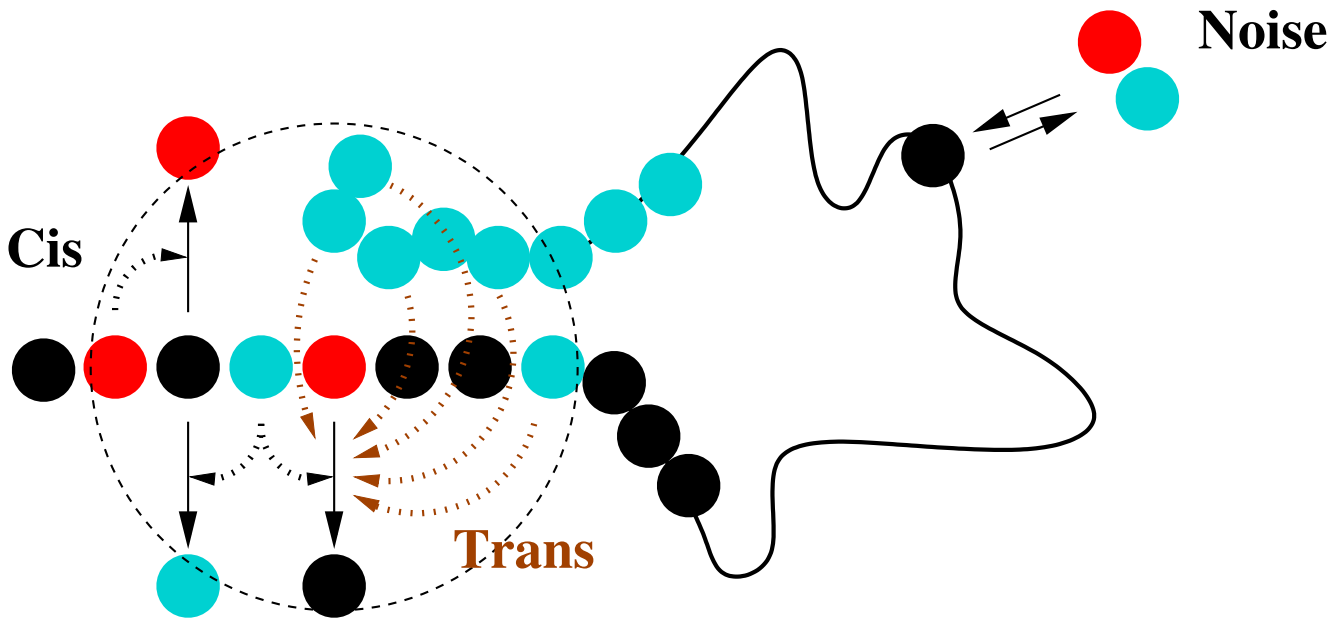
- No influence of 3D.
- Control parameter : 1D spreading rate $\epsilon_{cis}/\epsilon_o$.

# Epigenome dynamics vs $\epsilon_{cis}/\epsilon_o$




- Random distribution of short-lived active and inactive microdomains
- No stable "coherent" epigenomic state.
- The system remains monostable around the "zero-mean" state.

# 1D and 3D Spreading

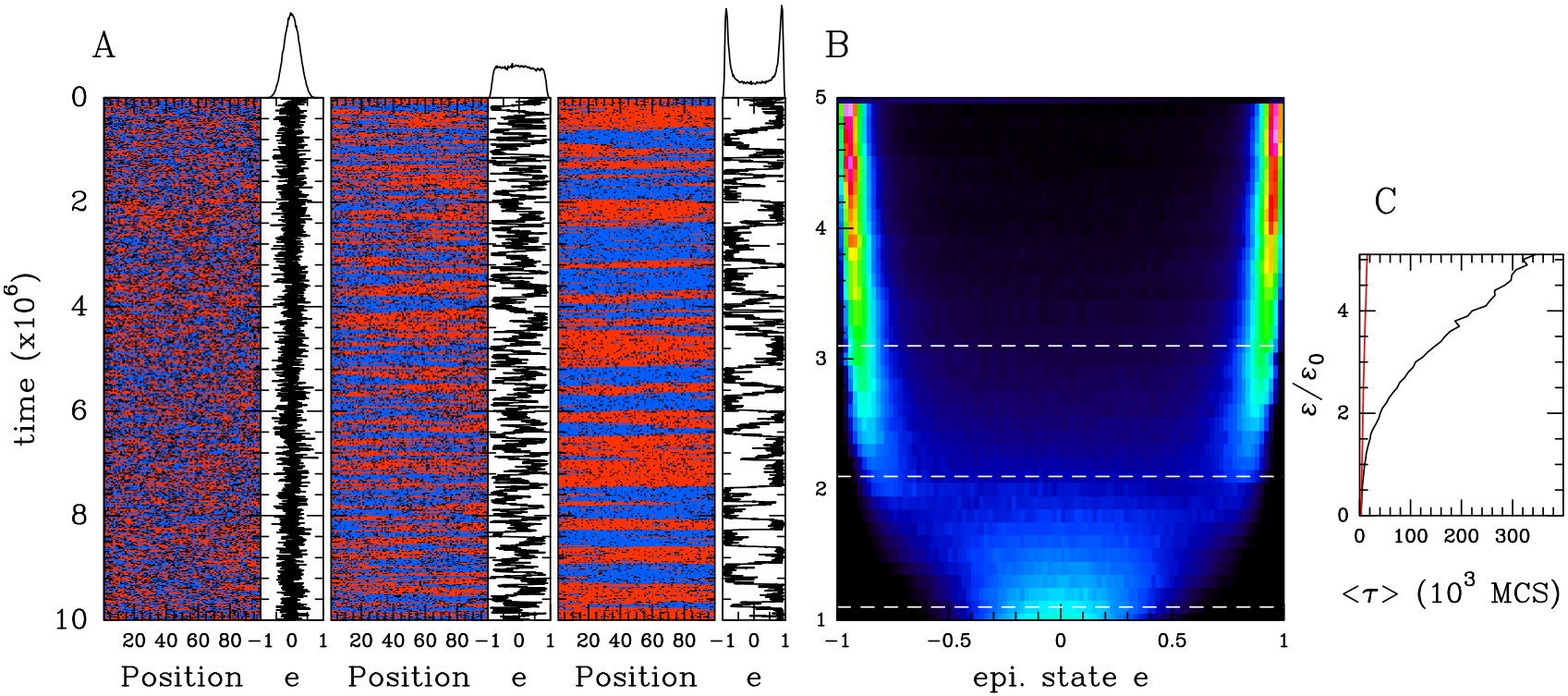



**Spreading in 1D and 3D**

- Influence of 3D.
- Control parameter : 1D and 3D spreading rate $\epsilon_{cis}/\epsilon_o$, $\epsilon_{trans}/\epsilon_o$ + Self-attraction $k_b/k_u$.

# (I) no self-attraction

$k_b = 0$, $\epsilon_{cis} = \epsilon_{trans} = \epsilon$.




Increasing the spreading rate leads to bistability for $\epsilon > \epsilon_{crit.} = 2.1\epsilon_o$

# (II) self-attraction : Phase diagram $(\epsilon/\epsilon_o, k_b/k_u)$