Chromatin organisation and (non-)equilibrium phase separation

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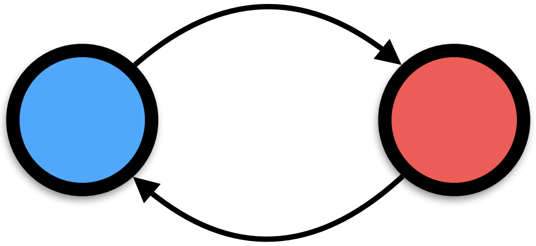
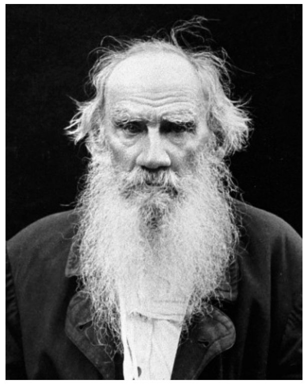
THEORY
GROUP

Fyl-osophy

"If you're in equilibrium you're dead."
Fyl Pincus (UCSB)

- Equilibrium - no net macroscopic flows of matter and energy
- Detailed balance - each elementary process is equilibrated by its reverse process





"All equilibrium systems are alike, each non-equilibrium system is out of equilibrium in its own way."
Tolstoy; Joanny, Grosberg, PRE (2015)

- An example of non-equilibrium phase separation in genome organisation - the longer the "active" polymer, the easier it is.

Outline

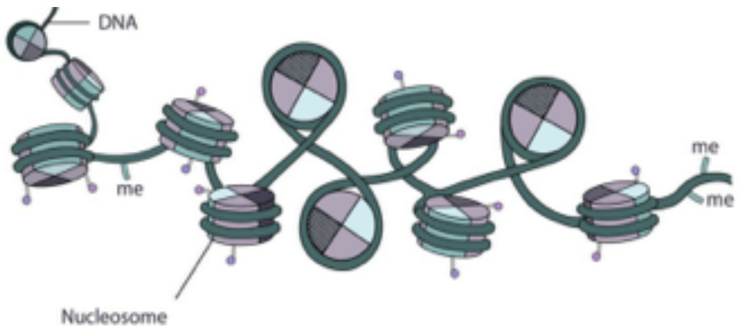
- Phase separation behind Chromatin domain formation
  - Equilibrium - what it can tell us and what not?
  - Equilibrium vs Out-of-equilibrium
  - Many ways to be non-equilibrium
  - Spatial and temporal scales matter

- Structure-Function vs Structure-Function-Structure (Deterministic vs Self-organizing models[1])
  - Out of equilibrium the function can affect the structure
  - Relevance of non-equilibrium phase separation in genome folding and organisation
  - New physics, new biophysical phenomena

[1] Misteli, Cell 2007

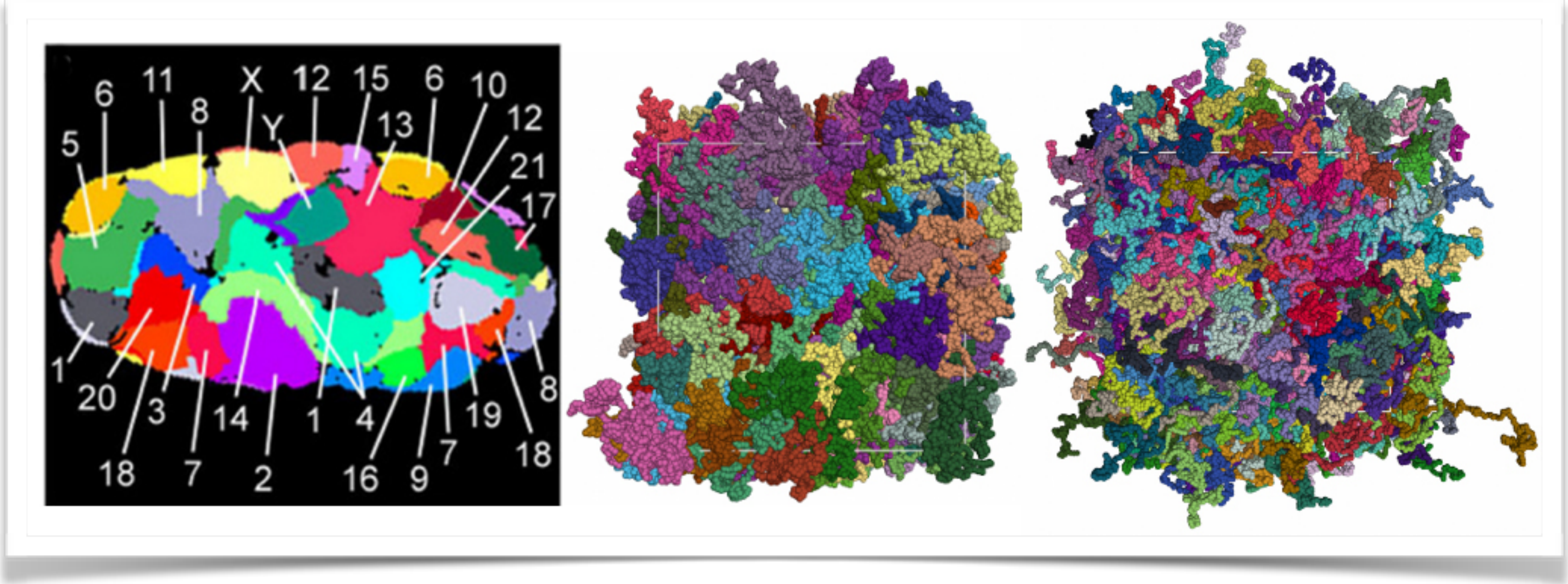
Genome folding and equilibrium

Human DNA (2m), double helix, nucleosomes 10nm fiber, in cell nucleus (10um), interphase.



Homopolymer (simplest view)

- High level of compaction
- (Mostly) uncrossable - relaxation time hundreds of years
- Generic features, e.g. territories[1] and genome/population averaged looping probability (Hi-C) $P(s) \sim s^{-\gamma} \quad \gamma \simeq 1$

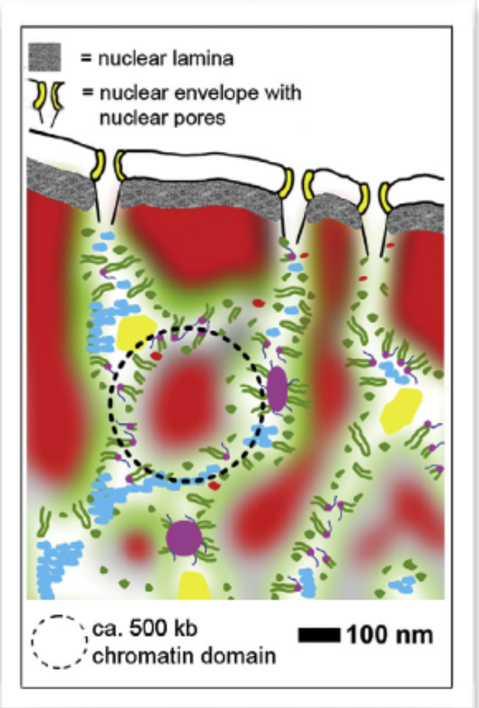


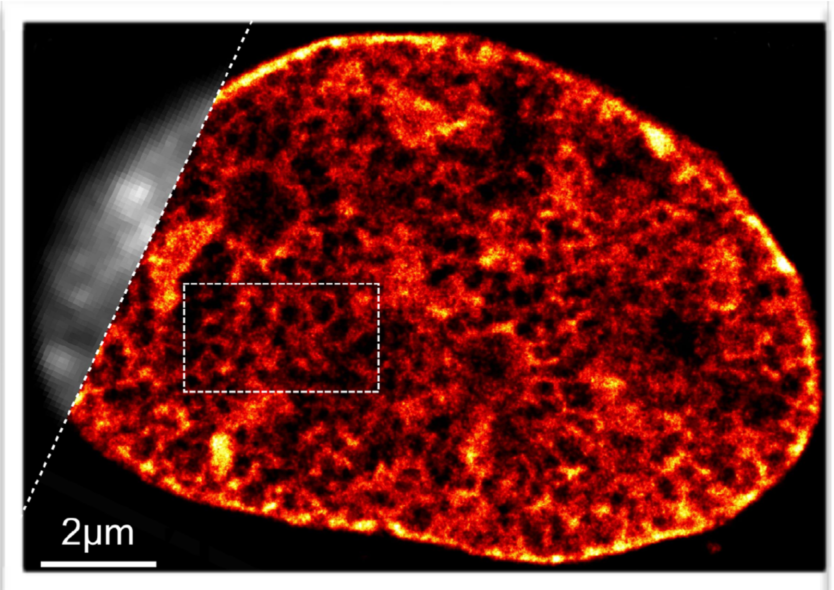
- Large scales > 1Mbp: Mappable to (equilibrium) solution of rings (uncrossable) and space filling-curves (fractal globules)[2].

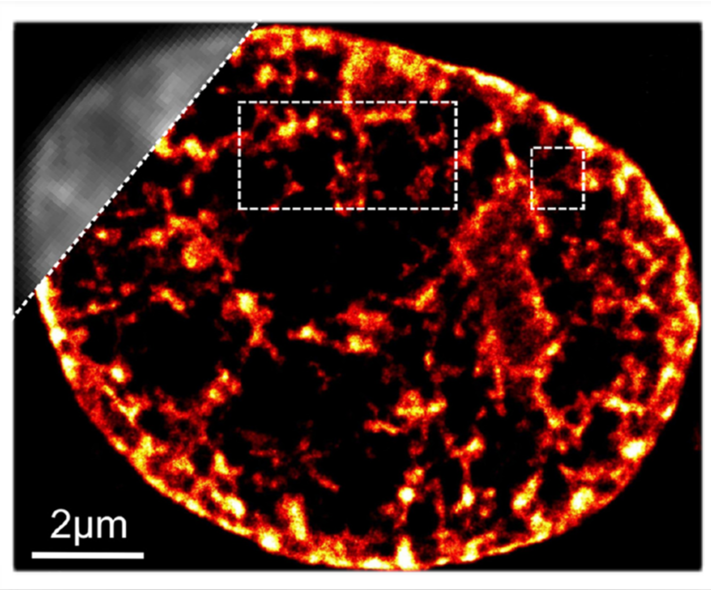
[1]Bolzer et al PLOS Biol.(2005) [2]Halverson, Smrek, Kremer, Grosberg, Rep. Prog. Phys. (2014)

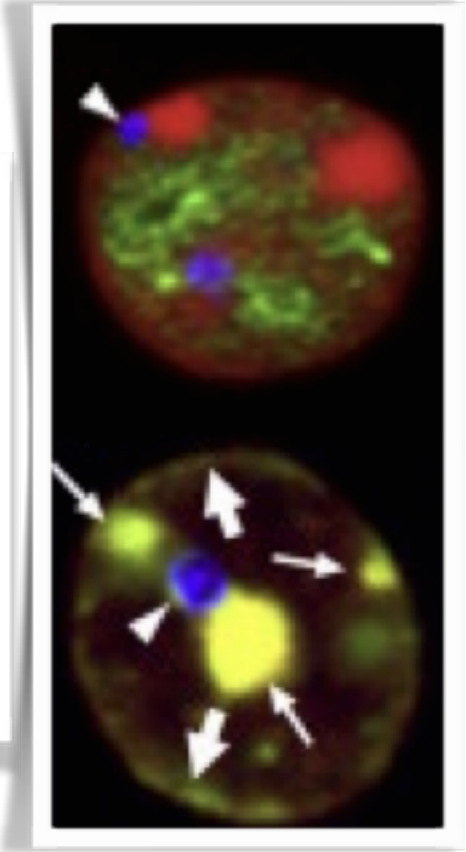
Beyond simplest view

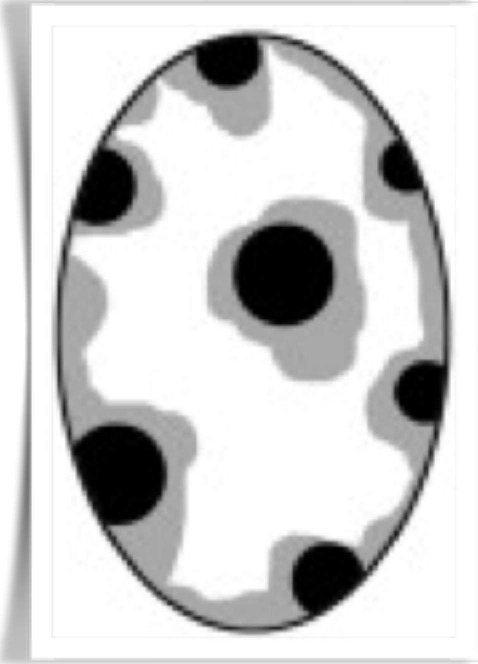
- Heteropolymer (epigenome)
- TADs (C-methods): higher self-contacts in (kbp-Mbp)
- Transcription/replication/repair foci (factories) likely self-organizing (e.g. replication factories formation kinetics correlate with rate of replication[1])
- Active/Inactive compartments: Eu-/Hetero-chromatin[2]
- ATP dependent conformation[3] and fluctuations[4]
- Eu-/Hetero-chromatin positioning







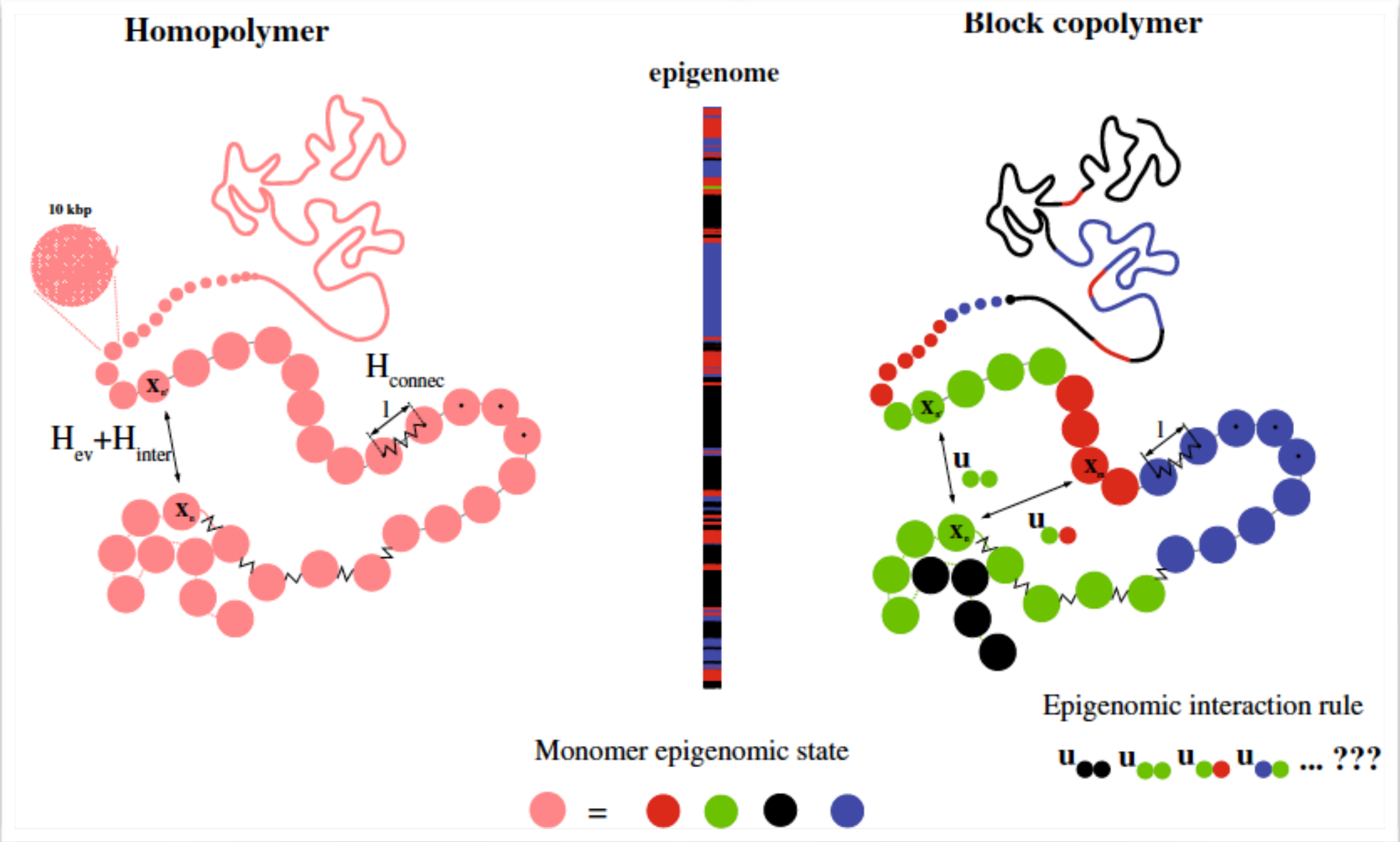




[1] Misteli J Cell Biol (2001), Kitamura et al Cell (2006) [2] Cremer et al, 2009, FEBS Lett. 2015;
[3] Szczurek et al NAR (2017), [4] Zidovska et al, PNAS (2013)

Finer Equilibrium (Deterministic) Models

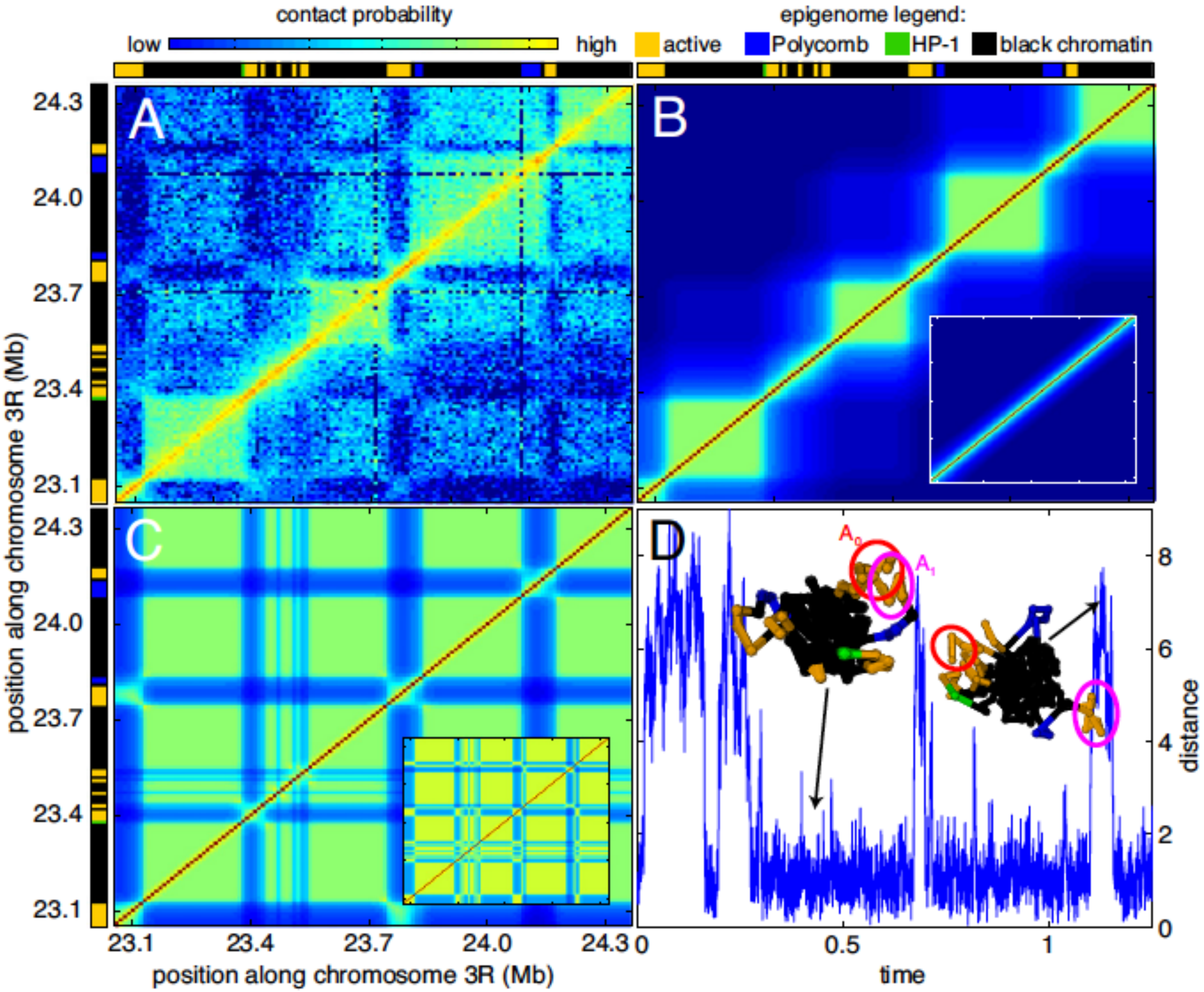
- Structure, Interactions (H)
- Competition of energy and entropy: Free energy: min(F)



[1] Haddad, Jost, Vaillant, Chrom. Res. (2017)

Finer Equilibrium (Deterministic) Models

- Small scales < 1Mb
- TADs
- Hi-C maps
- Multi(meta)stability - short lengths, dependence on initial state
- Equilibration from smaller scales - Local equilibrium
- Timescale of. metastable states - emergence of non-equilibrium
- Spatially spreading epigenomic marks



[1]Jost, Carrivain, Cavalli, Vaillant, NAR (2014)

Finer Equilibrium (Deterministic) Models

- Expression-dependent chromatin folding[1]
- Strings and binders switch model[2]

TADs specificity vs. checkerboard pattern vs. relaxation time

Equilibrium models are useful

- Modelling non-equilibrium conformational ensembles - large scale topological constraints (rings)
- Stable structures - e.g. nuclear bodies, unclear functional role (e.g. elimination of lamins - little effect on organization)
- Simple modelling small-scale features - TADs, BUT

Let's not forget:

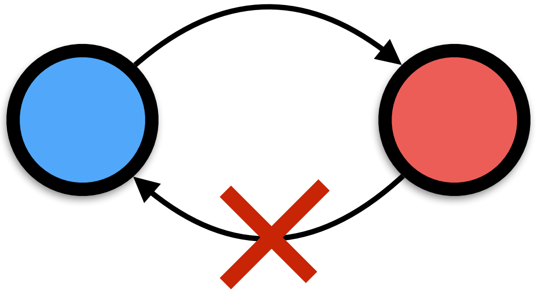
[1]Jerabek, Heermann, PloS ONE (2012) [2]Barbieri et al, PNAS (2012)

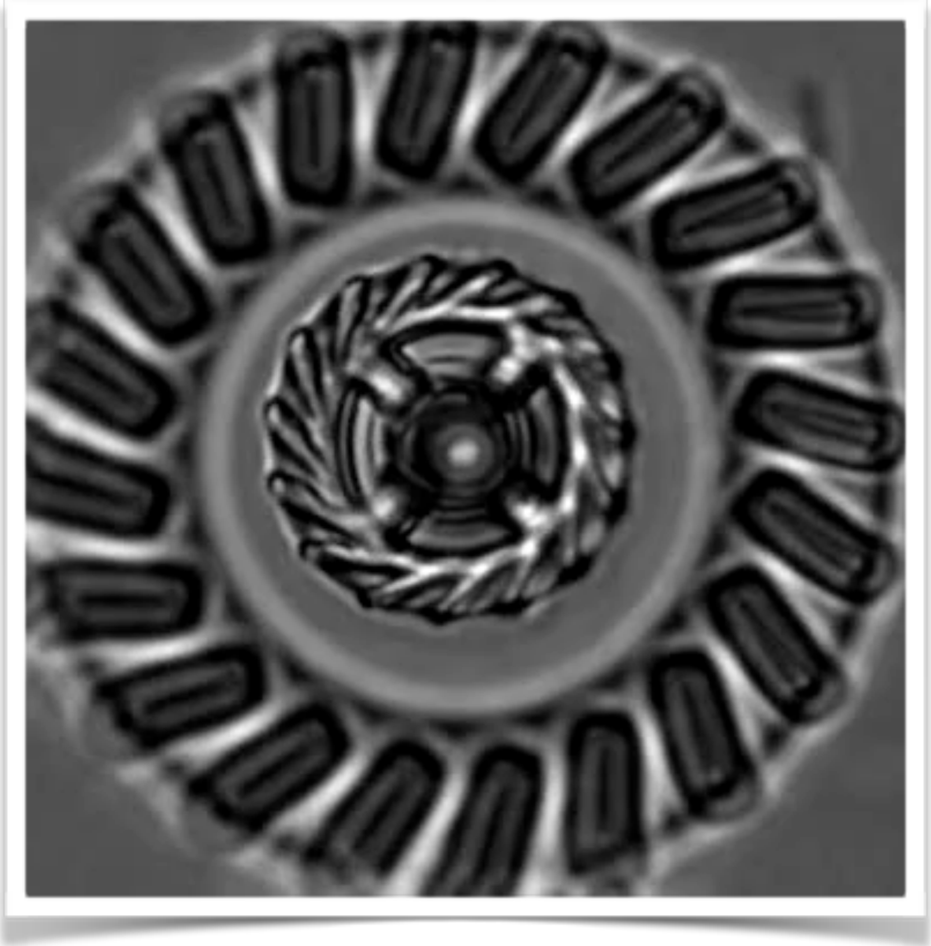
Out-of-equilibrium: Active Matter

- Previously: Unrelaxed topological constraints (global)
- Now: Local mechanical work at the expense of free energy.

Emergence of structures through dynamic phase transitions

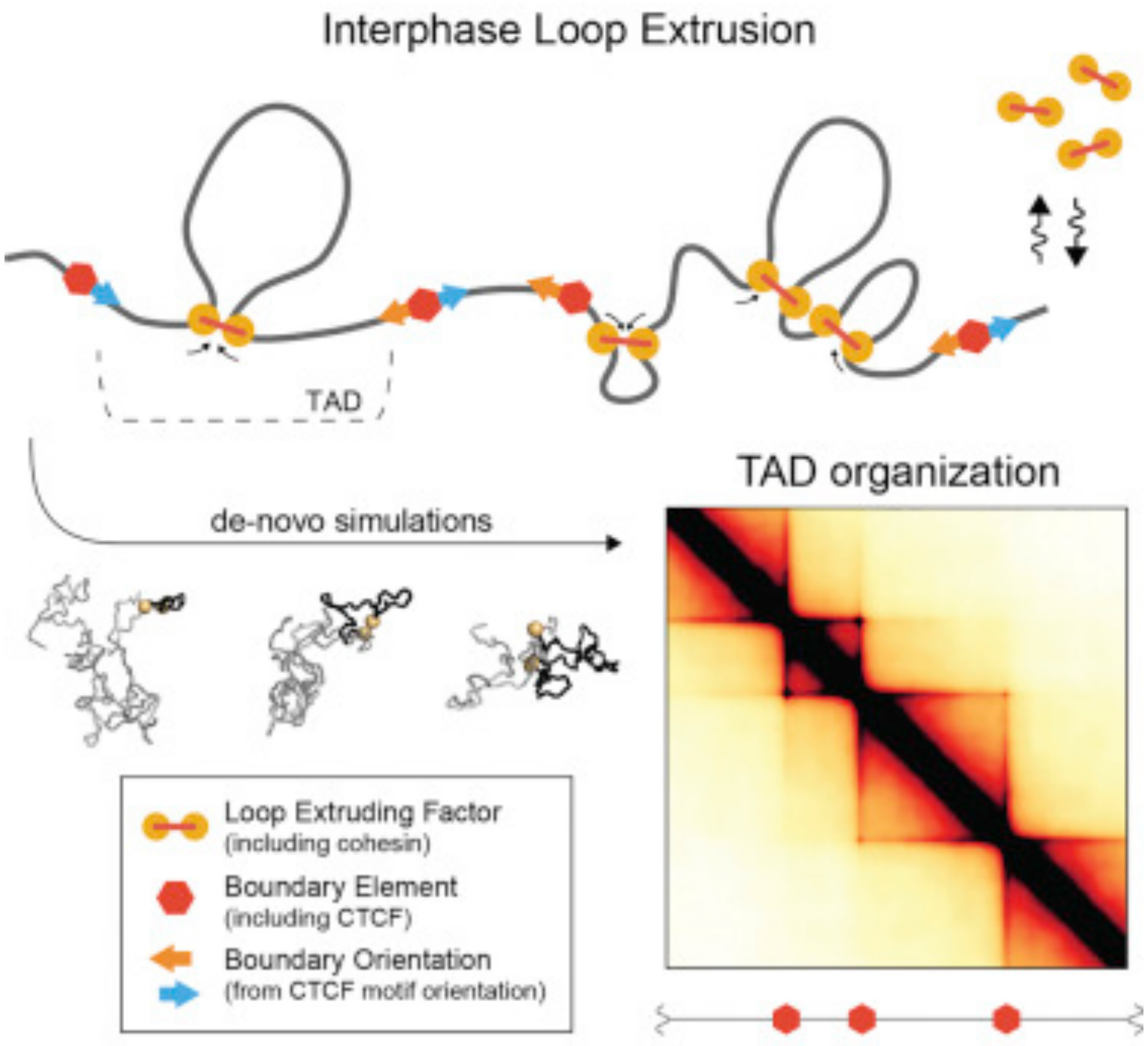
- Collective flows (cells, pigeons)[1] ``Flocking''[2]
- Directed rotational motion[3]
- Active-passive separation - DNA in cell nucleus?





[1]Szabó et al, 2006 [2]Palacci et al, 2013 [3]Di Leonardo et al, 2010, Vizsnyiczai et al, 2017.

Active Nucleus - Loop extrusion

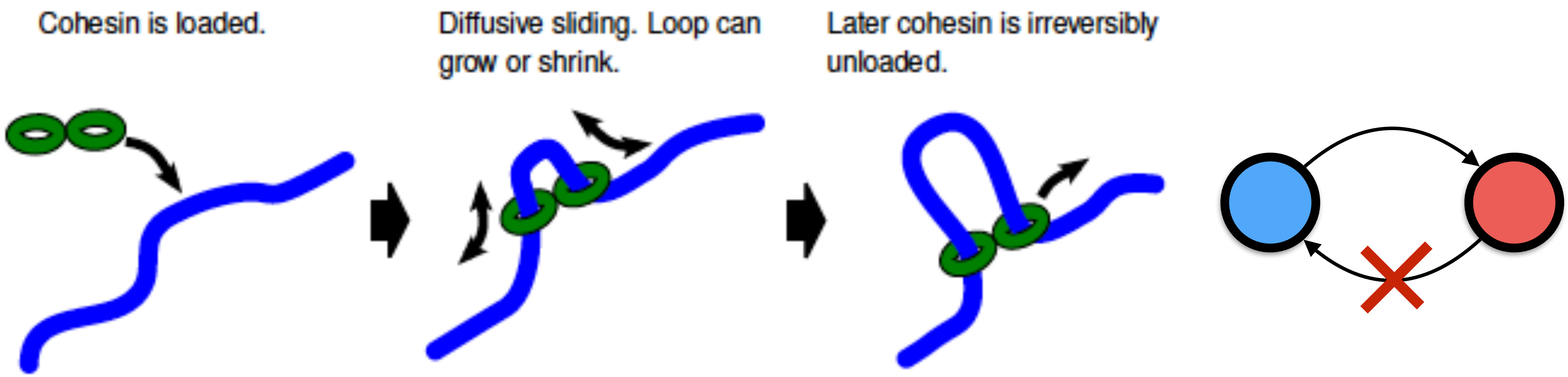


[1]Fudenberg et al (2016) [2]Sanborn et al (2015)

- LEF and BE (Cohesin? and CTCF)
- TADs and corner peaks
- Absent interaction peaks between distal BEs
- TADs merging in BE depletion experiments
- Directionality at TAD boundaries

- Non-uniform agreement along chromosomes - other effects? (active-inactive compartments)
- Requires motor activity

Loop extrusion without motor



Cohesin is loaded.

Diffusive sliding. Loop can grow or shrink.

Later cohesin is irreversibly unloaded.

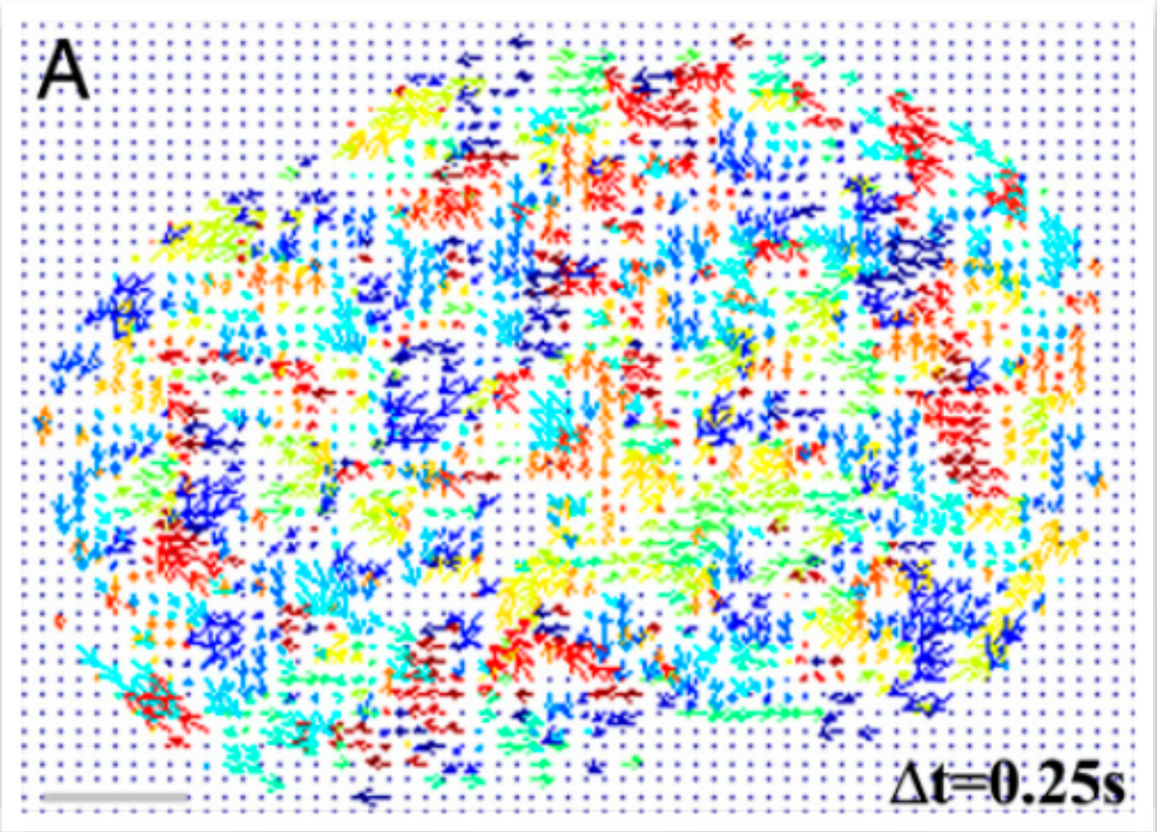
- Diffusional motion of “handcuffs”, ATP only for (un)loading
- 100-1000kbp loops in 20mins -> 2-20kbp/min (>> 1 RNApoly)
- D > 0.025um^2/s - in vitro could work, in vivo - obstacles, … ?
- Reproduces loop probability between CTCF sites, but not HiC

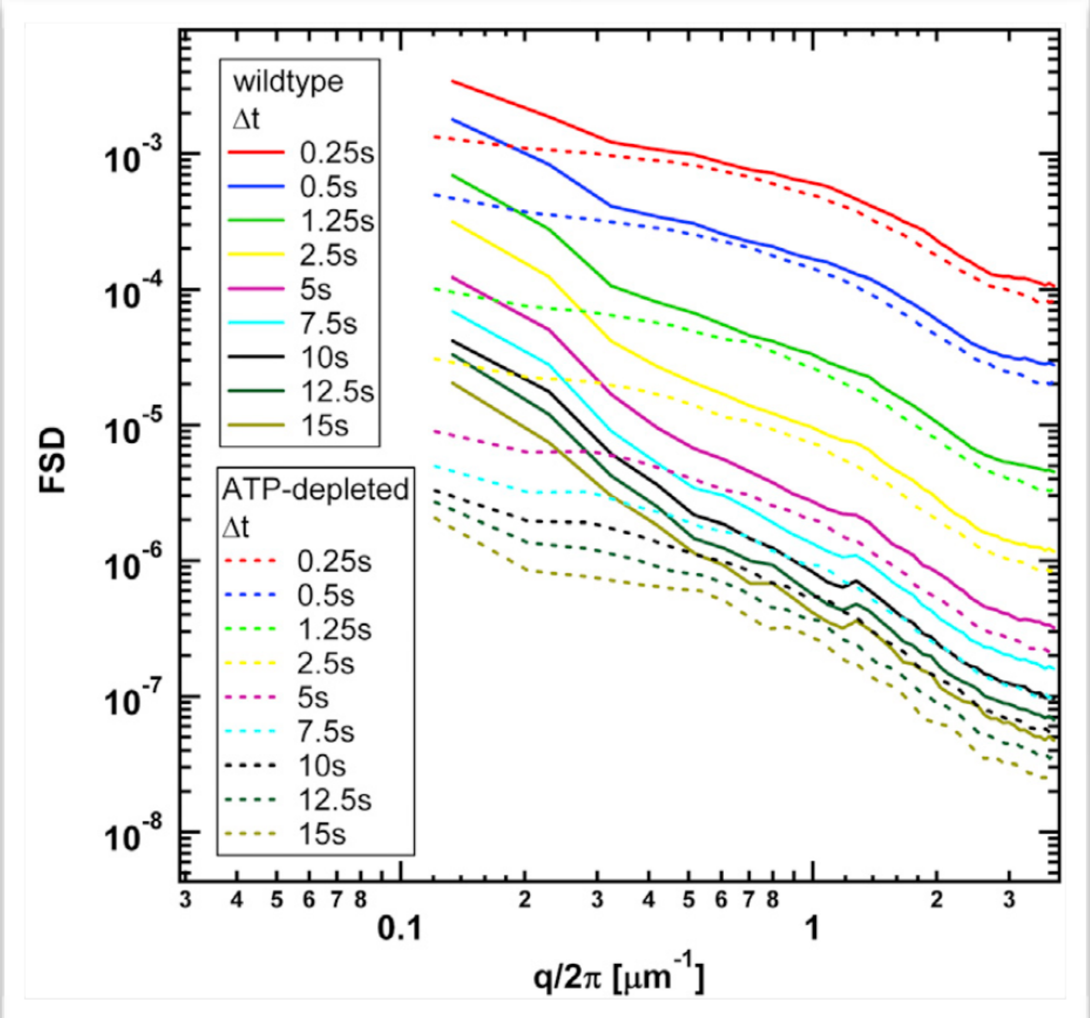
- Ratchet effect - two handcuffs loaded - increases mobility
- In vitro diffusivity ATP dependent
- Pore size vs Polymerase pushing cohesins vs active non-directed translocation
- Still Loop-less Active/Inactive compartments (even when CTCF and Cohesin are off)

[1]Brackley et al Nucleus (2018); Phys. Rev. Lett. (2017), [2]Stigler et al Cell Rep (2016)
[3]Davidson et al EMBO J (2016)

DCS and two fluid model

- Displacement correlation spectroscopy





$$\zeta(\vec{v}^p - \vec{v}^s) = \vec{\nabla} \cdot \overset{\leftrightarrow}{\sigma} - \vec{\nabla}\Pi - \phi_0 \vec{\nabla} P$$

$$\zeta(\vec{v}^s - \vec{v}^p) = -(1-\phi_0)\vec{\nabla} P$$

$$\Pi(\vec{r},t) = K\delta\phi(\vec{r},t) - \sum_k s_k \delta(\vec{r}-\vec{r}_k)\theta(t)\exp(-t/\tau)$$

- Relation of spatial and velocity correlations to complex viscoelastic modulus & active source correlations
- Local concentration fluctuations - elevated temperature $\simeq 2T$

Zidovska et al PNAS 2013; Bruinsma et al Biophys. J. 2014

Activity models, active-passive separation

- Vectorial - bacteria, colloids[1] $v(\rho)$
- Scalar - identical particles with different “temperature”[2] $T_h/T_c \gg 1$
- Analytic theory[3] - low density “virial” expansion predicts instability for $T_h/T_c \geq 42$

$$\zeta_i \dot{x}_i = -\partial_i U + (2T_i \zeta_i)^{1/2} \xi_i(t) \quad \rightarrow \quad \zeta_r \dot{r} = F + (2\bar{T}\zeta_r)^{1/2} \xi_r$$

$$\bar{T} = (\zeta_h T_c + \zeta_c T_h)/\zeta_h + \zeta_c$$

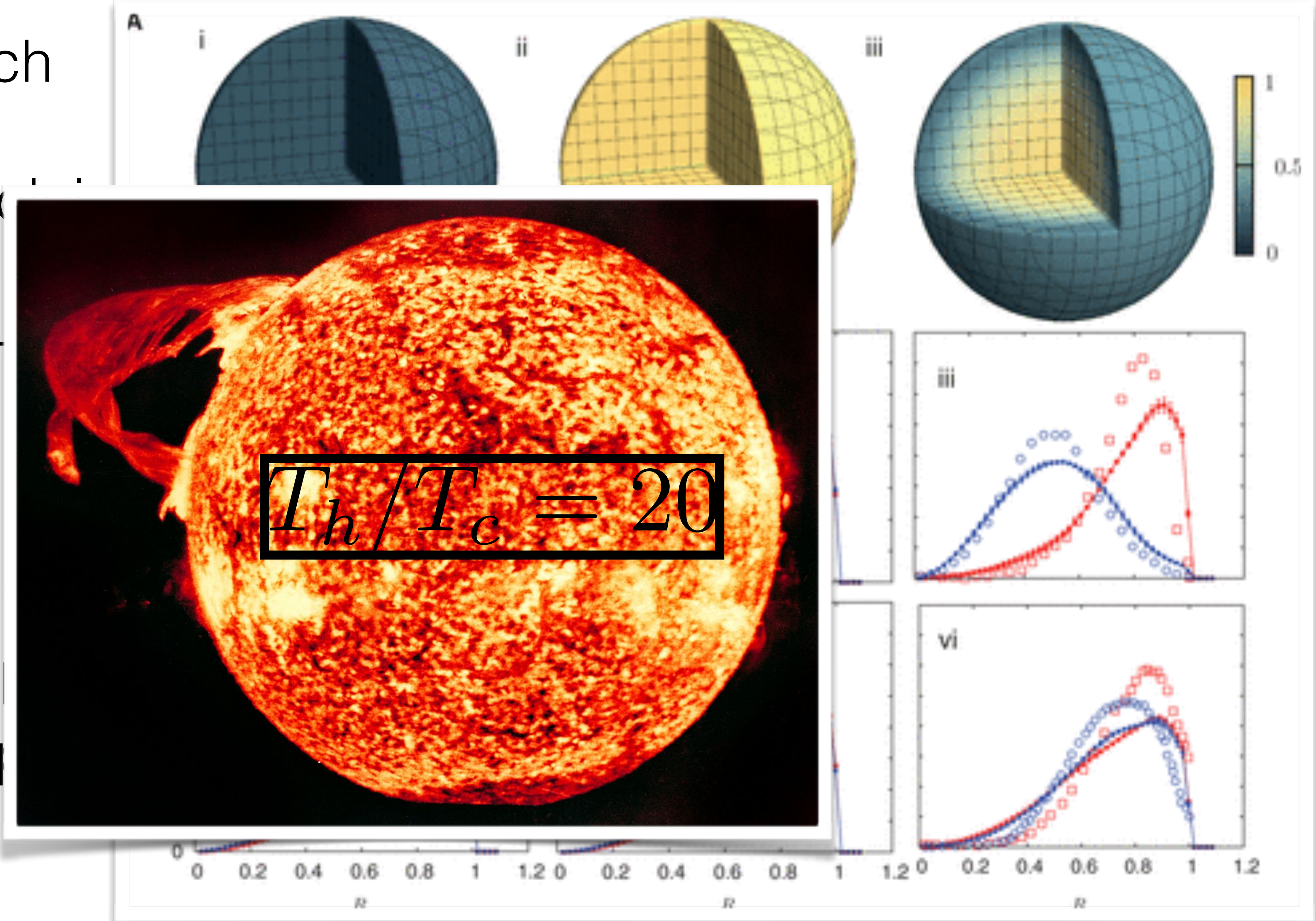
$$P(r) = Z^{-1} \exp(-U(r)/k\bar{T})$$

- Langevin - Fokker Planck - hierarchy - p^3=0, stability analysis
- DNA two-temperature model[4] of polymers with isolated monomers $T_h/T_c = 20$; Random-loop model; activity-based chromosome positioning and segregation

[1] Stenhammar et al, PRL (2015). [2] Weber^2, Frey, PRL (2016). [3] Joanny, Grosberg, PRE (2015)., [4] Ganai et al NAR (2014), Awazu PRE (2014), [2] Sewitz et al biorXiv (2017)

Activity-based positioning and separation

- Ch18&19 and ch 12&20; human lymphocyte nu
- Territories = compact conf activity segregation
- Positioning inversion (nocturnal), wit nuclear envelo association



$T_h/T_c = 20$

- Reports no territories if random-loop model is off
- How to compare eq. and noneq. “strength of interaction”?

[1] Ganai et al NAR 2014

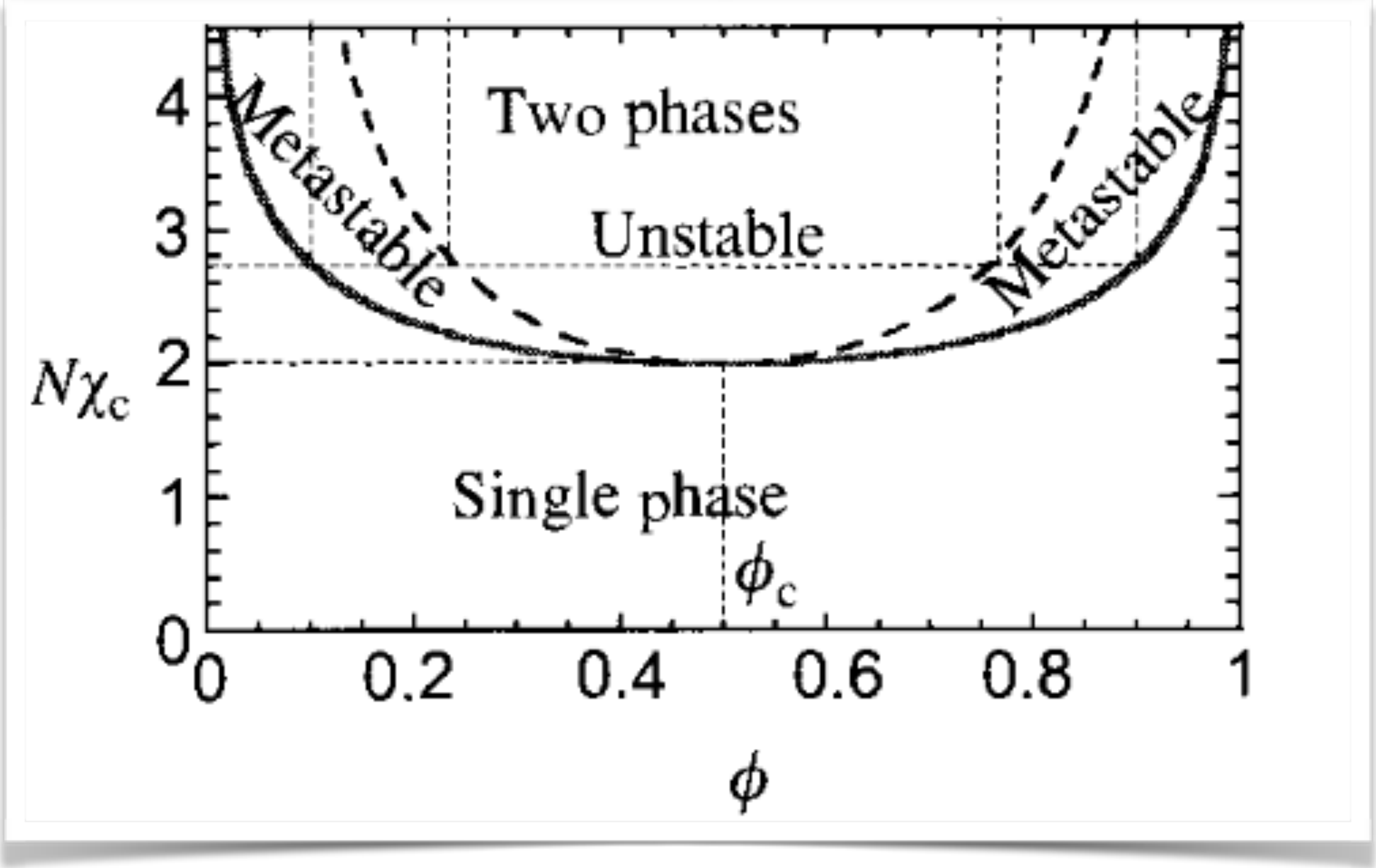
Once upon a time in equilibrium

- Phase separation in purely passive polymer blends
- Flory-Huggins (MF) theory:

$$S \sim M$$

$$U \sim \chi N M$$

$$\chi_{\mathrm{crit}} \sim 1/N$$



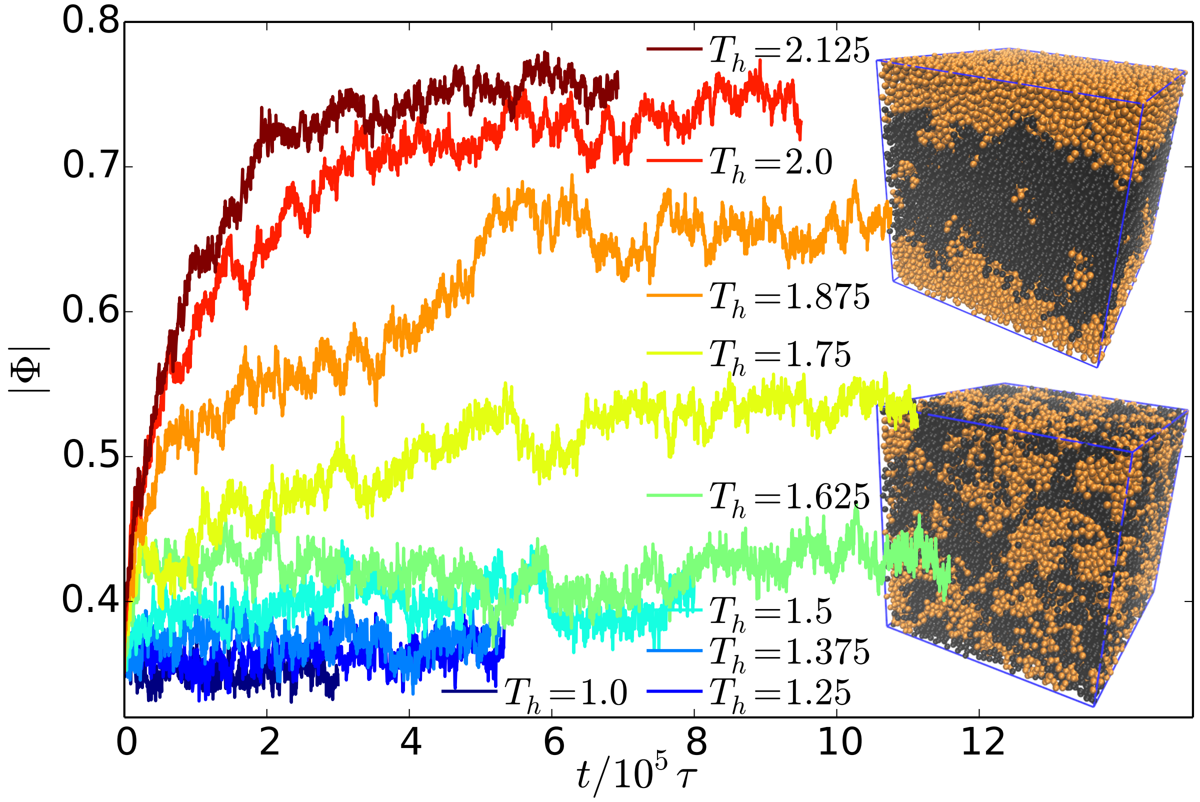
- How is this for active-passive polymer blends?

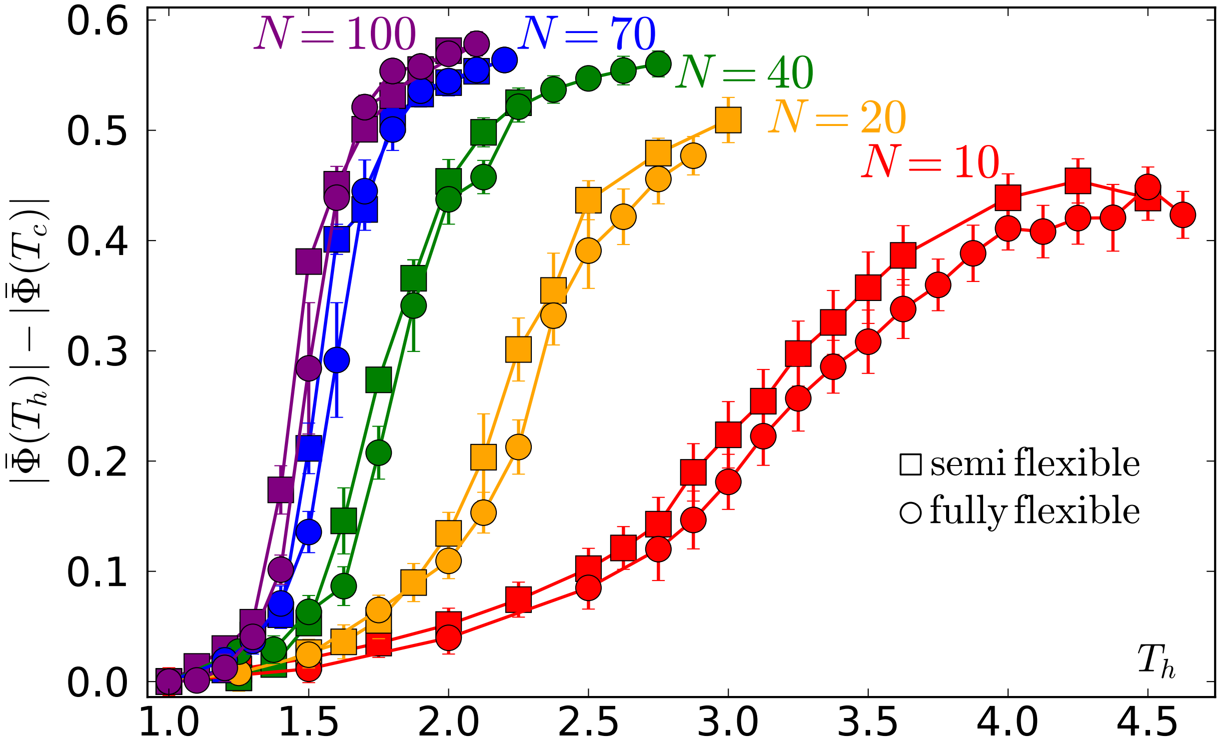
Rubinstein, Colby

MD

- Standard (KG) polymer blend 50:50, identical interactions type-specific Langevin thermostat , E++

$$(T_h; T_c = 1.0\varepsilon, \gamma = 10\tau^{-1})$$





- Higher effective excluded volume - higher pressure - concentration change
- Semi-flexible: less pronounced correlation hole - more unfavourable contacts

[1] Smrek, Kremer, PRL (2017),

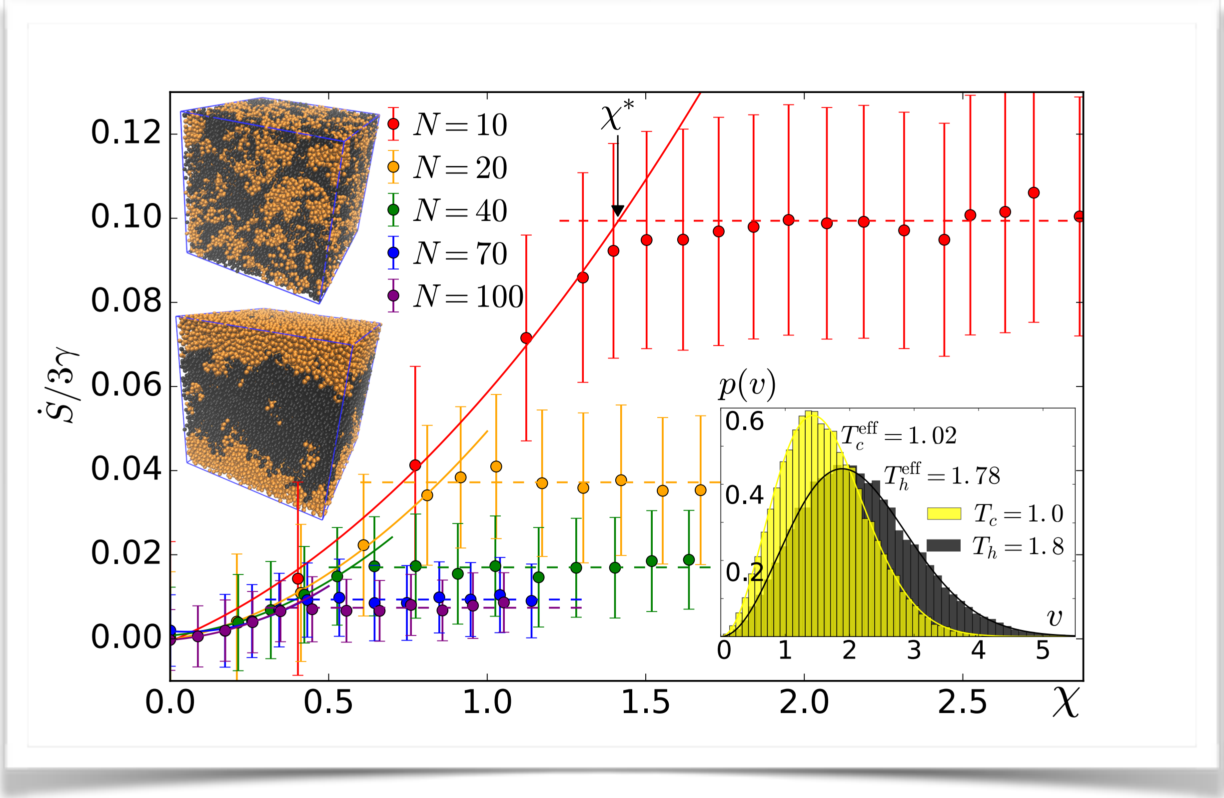
Entropy production per particle

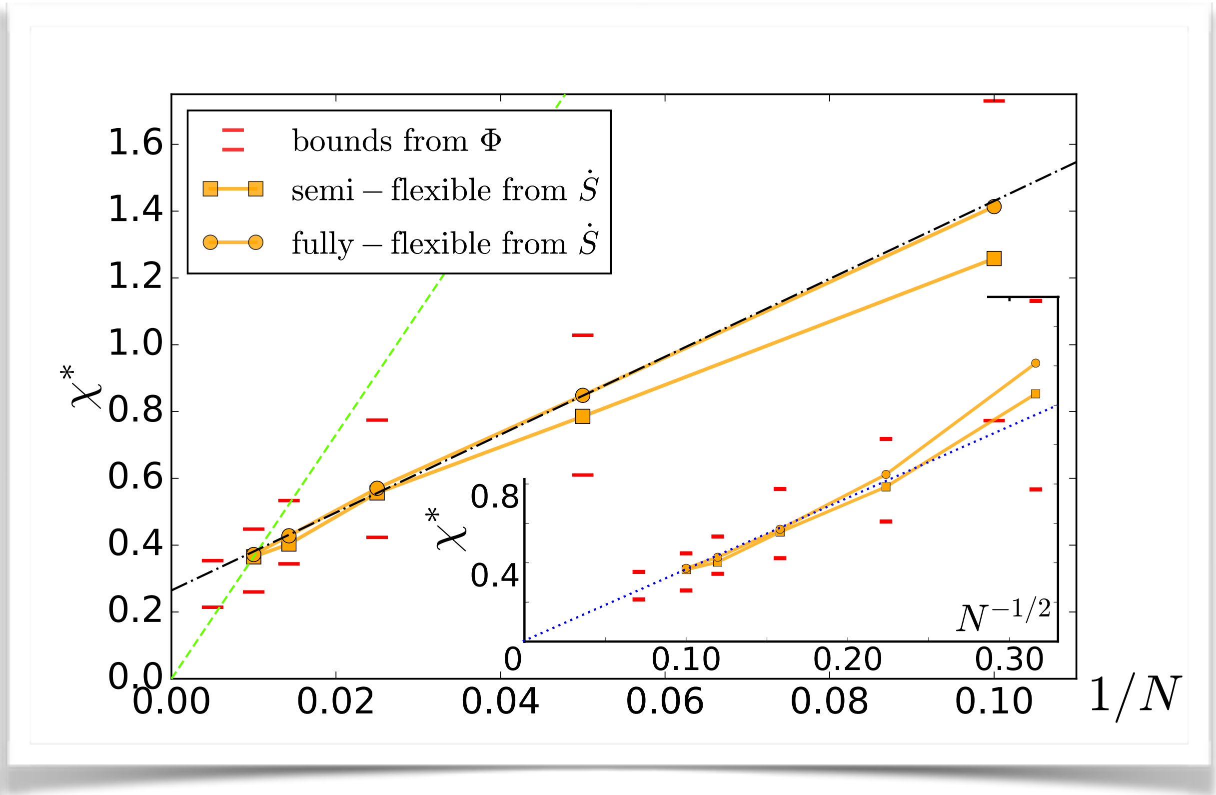
- Heat transfer between hot and cold species

$$T_h > T_h^{\mathrm{eff}} > T_c^{\mathrm{eff}} > T_c$$

- Analogue of Flory parameter $\chi = \dfrac{T_h^{\mathrm{eff}} - T_c^{\mathrm{eff}}}{T_c^{\mathrm{eff}}}$

$$\frac{\dot{S}}{3\gamma} = \frac{T_h^{\mathrm{eff}}}{T_h} + \frac{T_c^{\mathrm{eff}}}{T_c} - 2$$

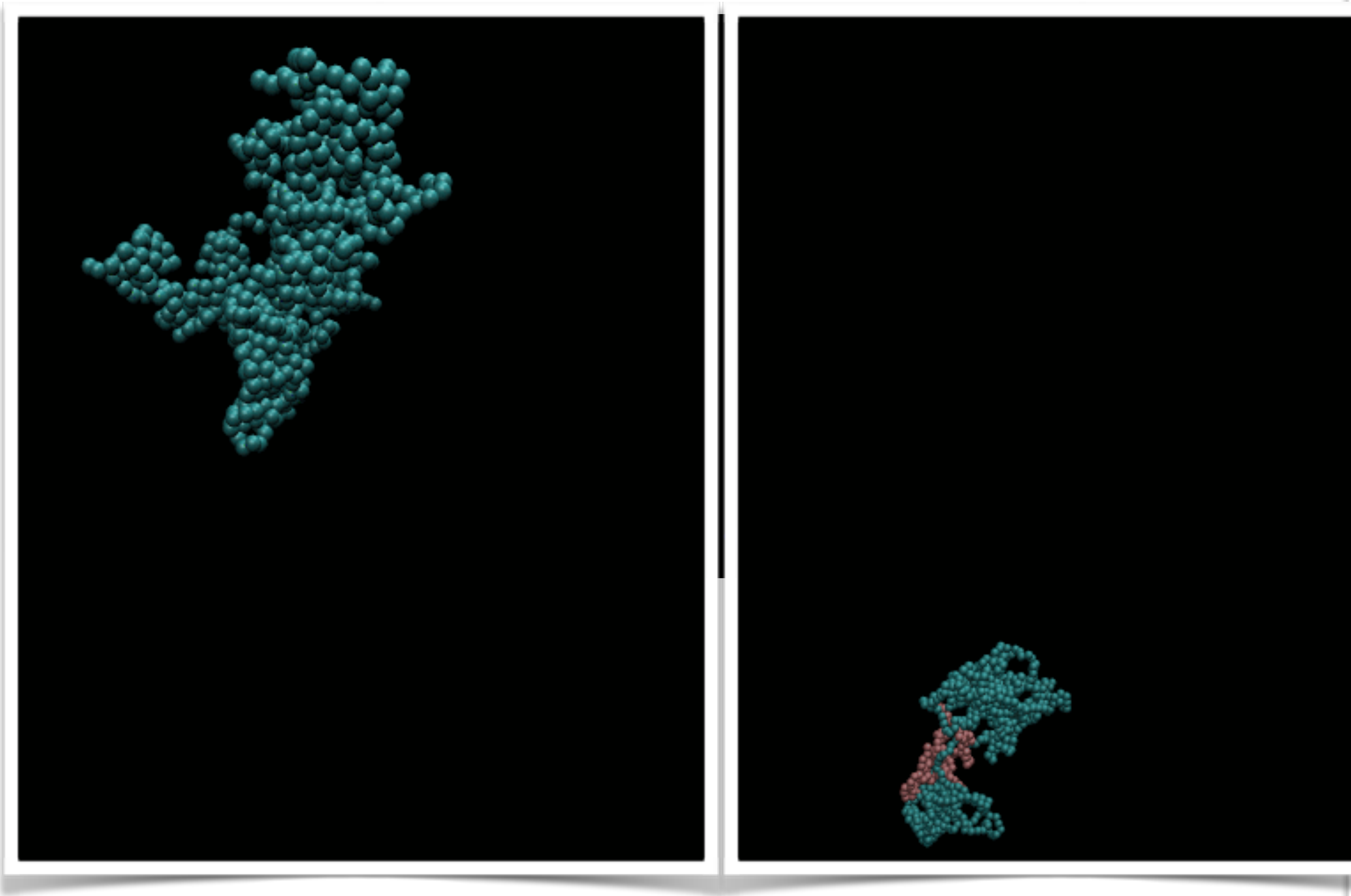


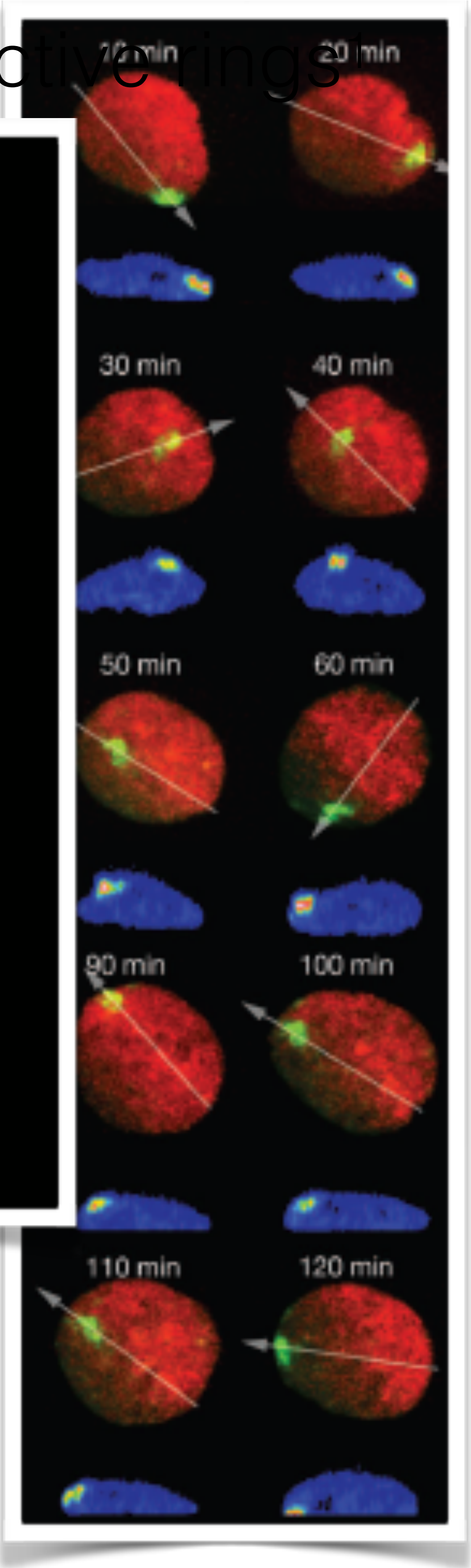


[1] Smrek, Kremer, PRL (2017)

Dynamic effects

- Active compartment/Transcription factories: partly active rings





- Hydrodynamics + directional[2] active matter = spontaneous circulation[3,1]

[1] Smrek, Kremer In prep (2018) [2] Chuang et al Curr Biol (2006), [3] Woodhouse, Goldstein, PRL (2012), Strickfaden et al Nucleus (2010)

Take-home messages

- Life is non-equilibrium and it is relevant
- Equilibrium physics is useful to
  - capture stable structures
  - small spatial scales or short times
  - understand non-equilibrium - analogies/dissimilarities
  - get characteristic time/space/energy scales to judge:
- Self-organizing (non-equilibrium) behaviour
  - plasticity, responsiveness to stimuli
  - new phases and effects
  - critical activity difference decreases with polymer length

Thanks

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