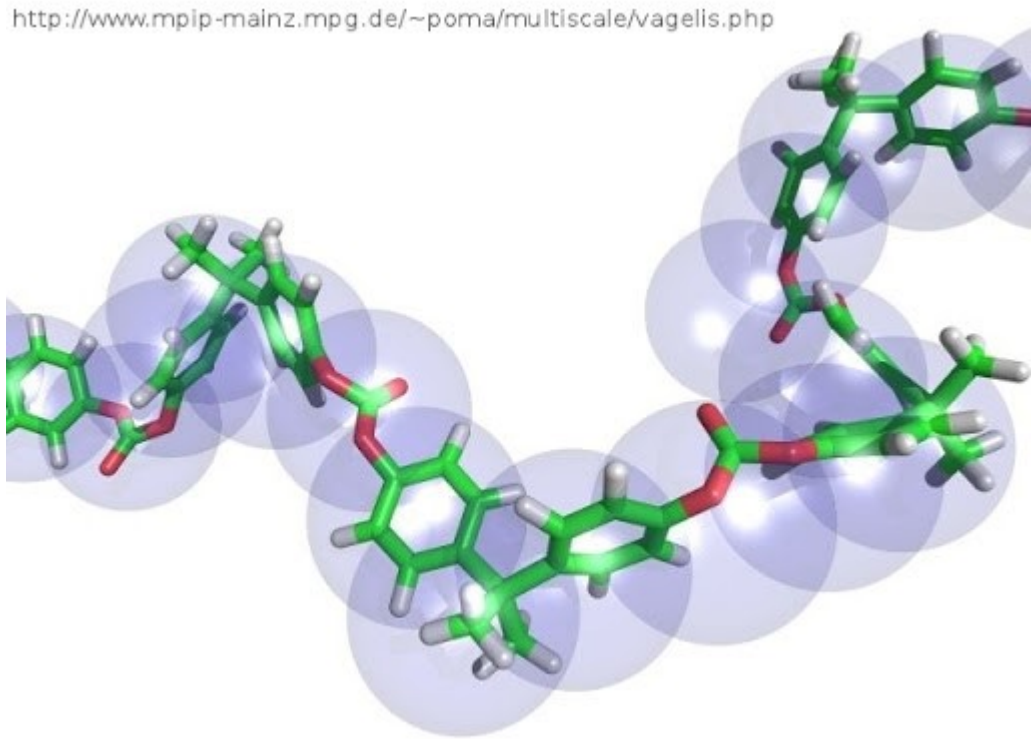
Coarse graining in science

Yet nature still resists
for it is not made of the details
but of all that manifest between …
from the answer, not to “Why”
but to “Why not?”

If we just distance the objective
from the subject
that is subjective by default
and take a glance from far enough
the universe unfolds
a whole much larger than its parts



Ohad Cohen, “Coarse graining”, 2020

Coarse grained approaches to cell physics

- In vivo mechanobiology – versus in-vitro, reduced systems where “exact” theory possible
  - Calcium oscillations in cardiomyocytes – why are they spontaneous?
    coarse-grain over activity of ion channels as per experiments (Cohen… PRL 2019)
  - Cell and nuclear volume regulation is modified by cell spreading on surfaces
    (expts: Guo/Weitz; Jiang)
    coarse-grain over Donnan equilibrium + active pumps (Adar… PNAS 2020)
  - Lamina hole formation, DNA repair factor loss and chromatin herniation
    in cells under mechanical stress (expts: Discher; Piel: Lammerding)
    coarse-grain over lamin layer and over nuclear pore complexes (Deviri… Nat. Phys. 2019)
  - **Chromatin concentration – coarse grained experiments and theory**

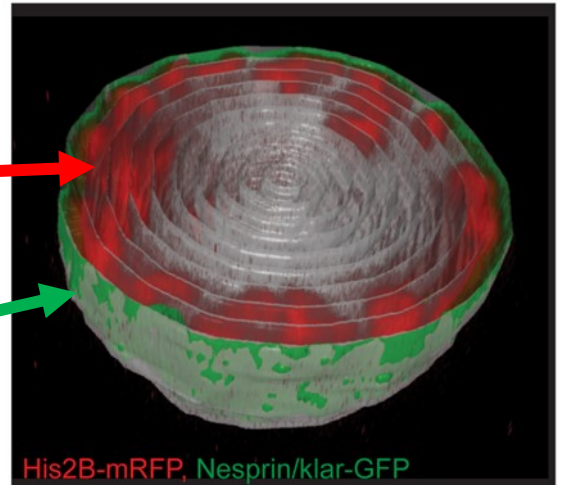
Nuclear-scale chromatin concentration profile: hydration and lamina interactions

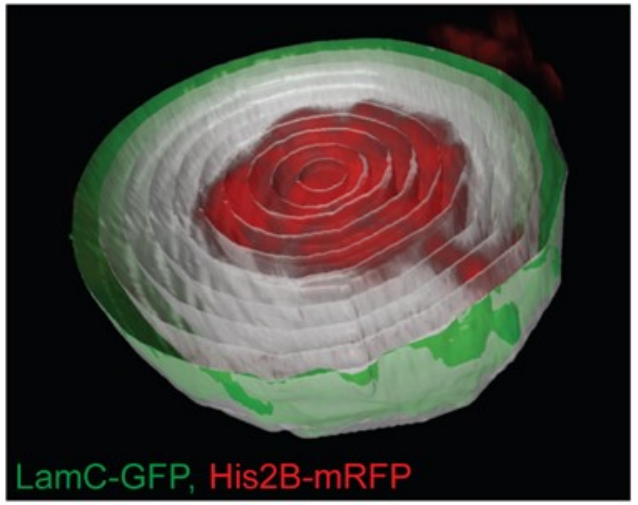
Expts. Volk group

Chromatin

Nuclear envelope: **lamina**

Lamina: network of lamin B, lamin A/C

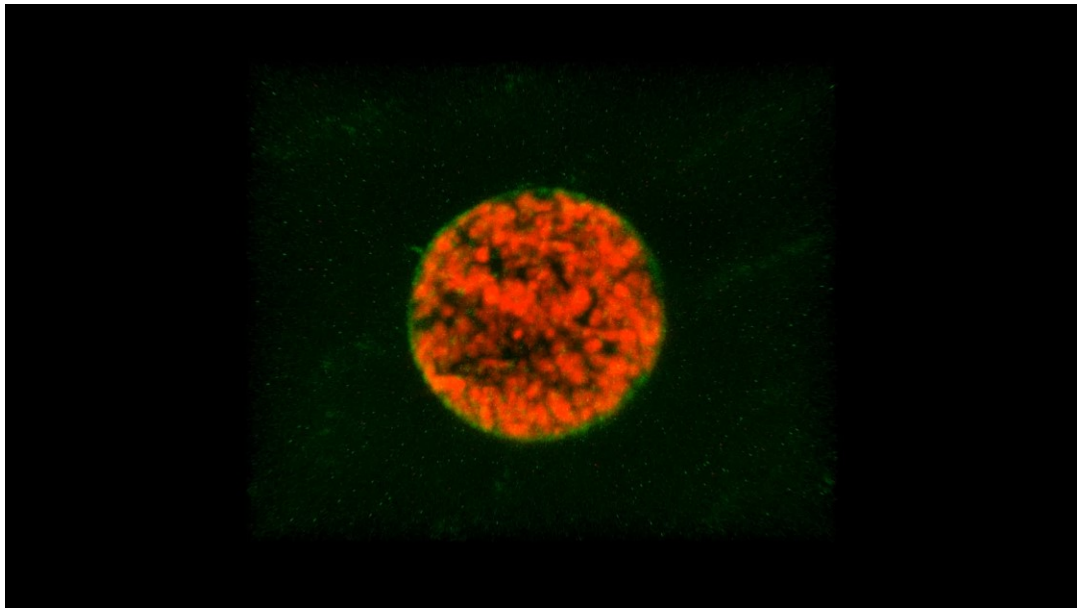


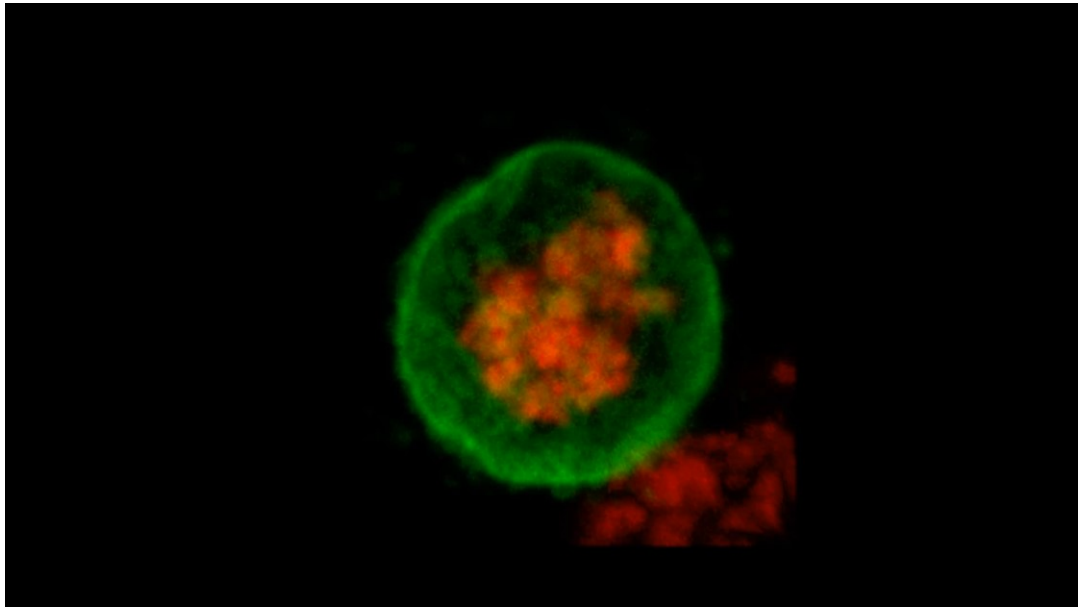


Theory: Gaurav Bajpai, Omar Adame-Arana, SAS (Dept. Chemical and Biological Physics)

Experiments: Dana Lorber, Daria Amiad-Pavlov, Talila Volk (Dept. Molecular Genetics)

Nuclear scale chromatin concentration profile: hydration and lamina interactions





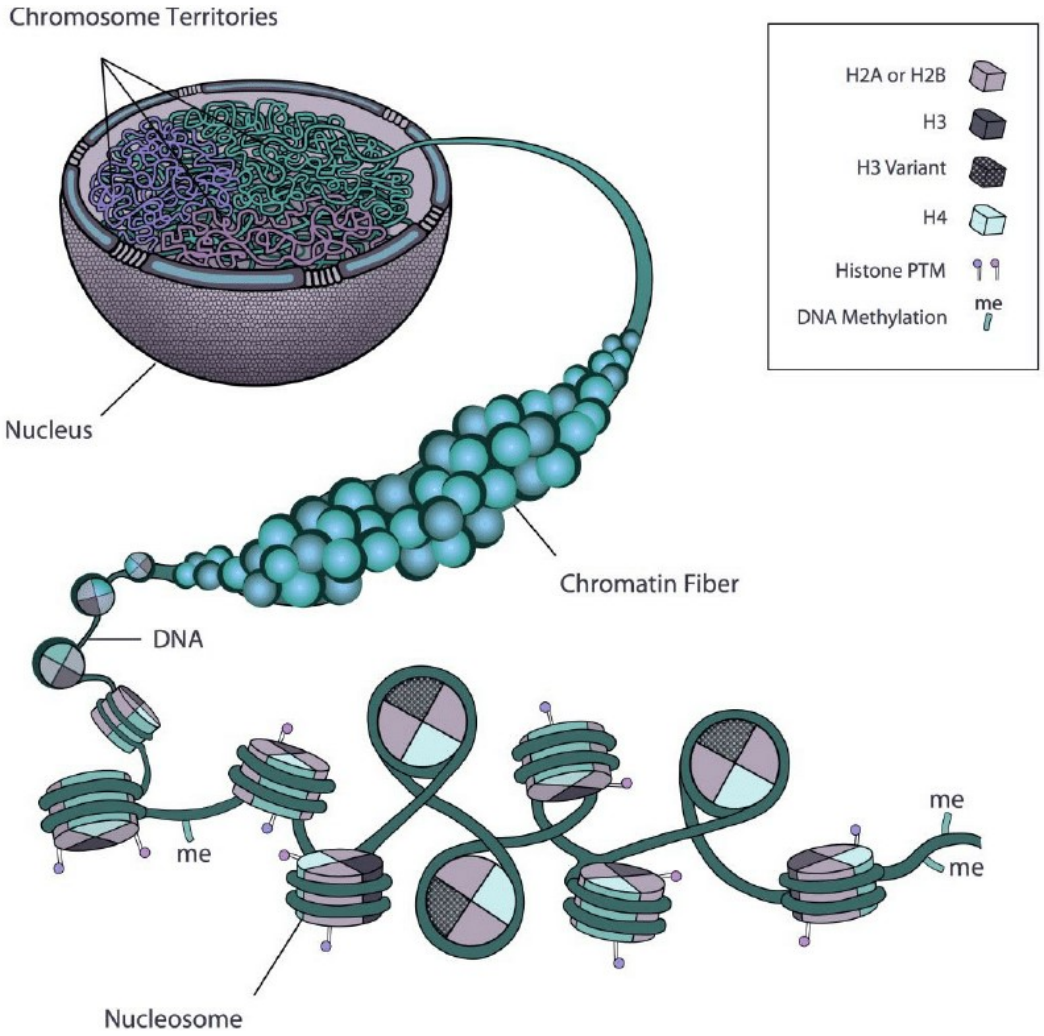
Peripheral

Central

Expts. Volk group

Conventional chromatin organization in nucleus:

chromatin fills the nucleus, uniform phase w/aqueous solvent

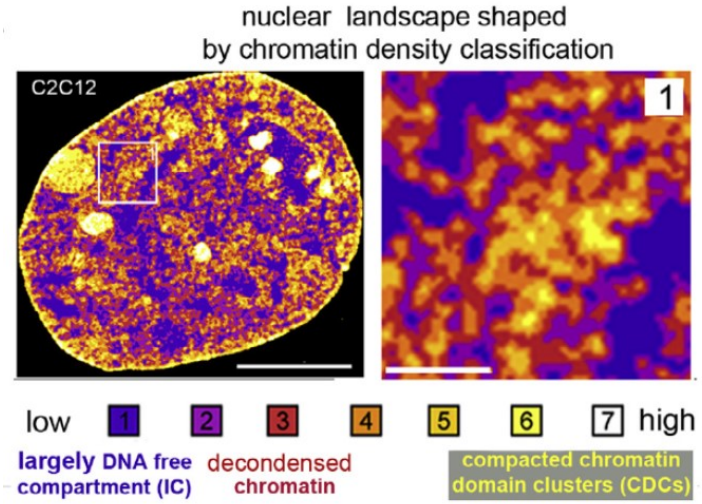


Rosa, Shaw – Biology 2013

Phase separation of chromatin

- Phase separation of AB blocks (e.g., eu- EC and hetero- chromatin HC) chromatin
  - Chromatin uniformly solubilized in aqueous phase: resolve HC and EC
  - Mirny, Thirumalai, …. theory
  - Karpen, Rosen … – experiments

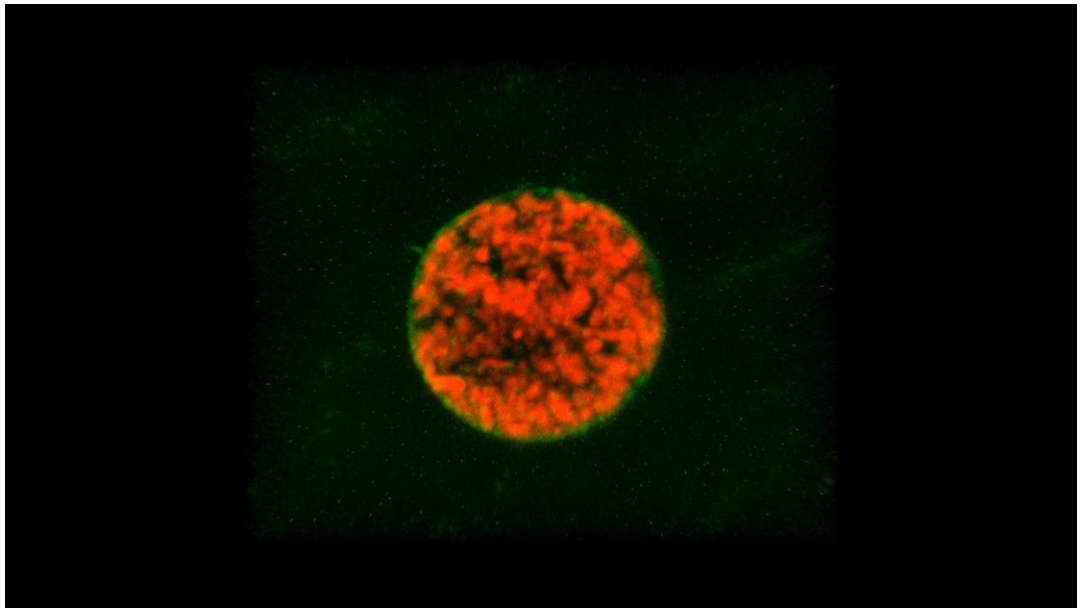
- Sub micron phase separation of chromatin from “aqueous phase”
  - Cremer and Cremer (FEBS Letters 2015) – “marshland”
    - compartment structure, water percolation
    - “crosslinks”: motors, HP1, proteins
    - Is chromatin a gel (mechanics – Discher, Marko..)?



Phase separation of chromatin and aqueous solvent: in vivo

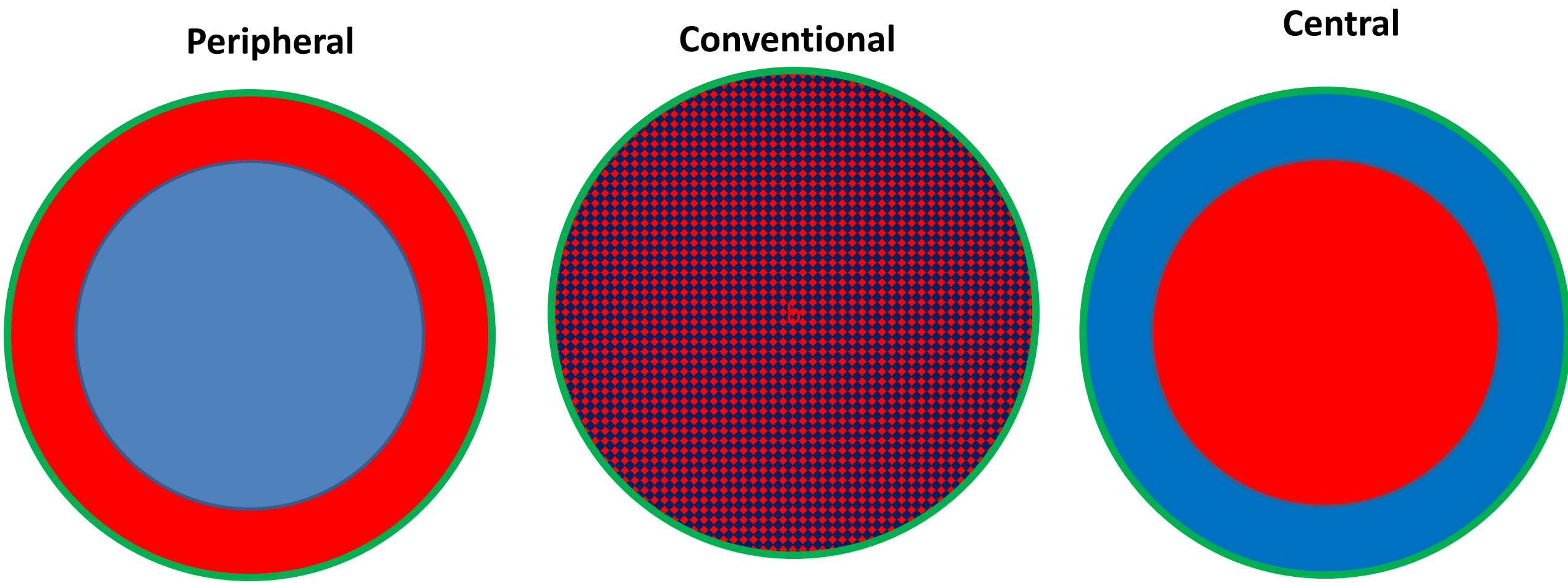
- **Experiments:** Live cell vs. fixed cell: Image histone marker His2B-mRFP (all chromatin) – coarse grained, resolves chromatin and aqueous phase (below, resolve EC and HC)

- **Theory:** coarse grained –strong lamina-HC (LAD) and chromatin-chromatin interactions relative to aqueous phase (EC-EC, HC-HC, HC-EC approximately equal, for now).



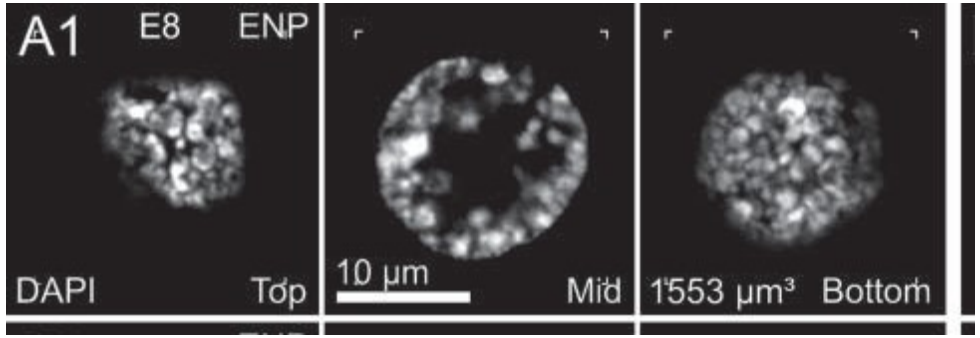
Peripheral, conventional, central: nuclear-scale chromatin vs. aqueous

Aqueous Lamina Chromatin

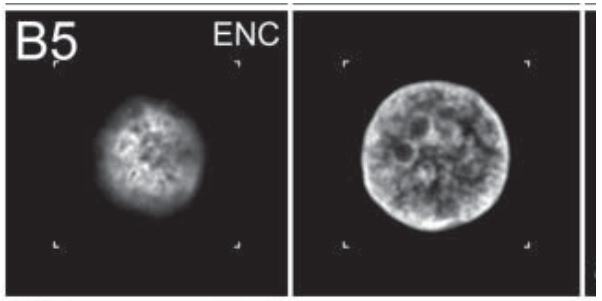


Peripheral, conventional, central: experiments

- Popken..Cremer (2014): multi-cell bovine IVF embryos
  - When major gene activation occurs – observe peripheral chromain organization (ENP)



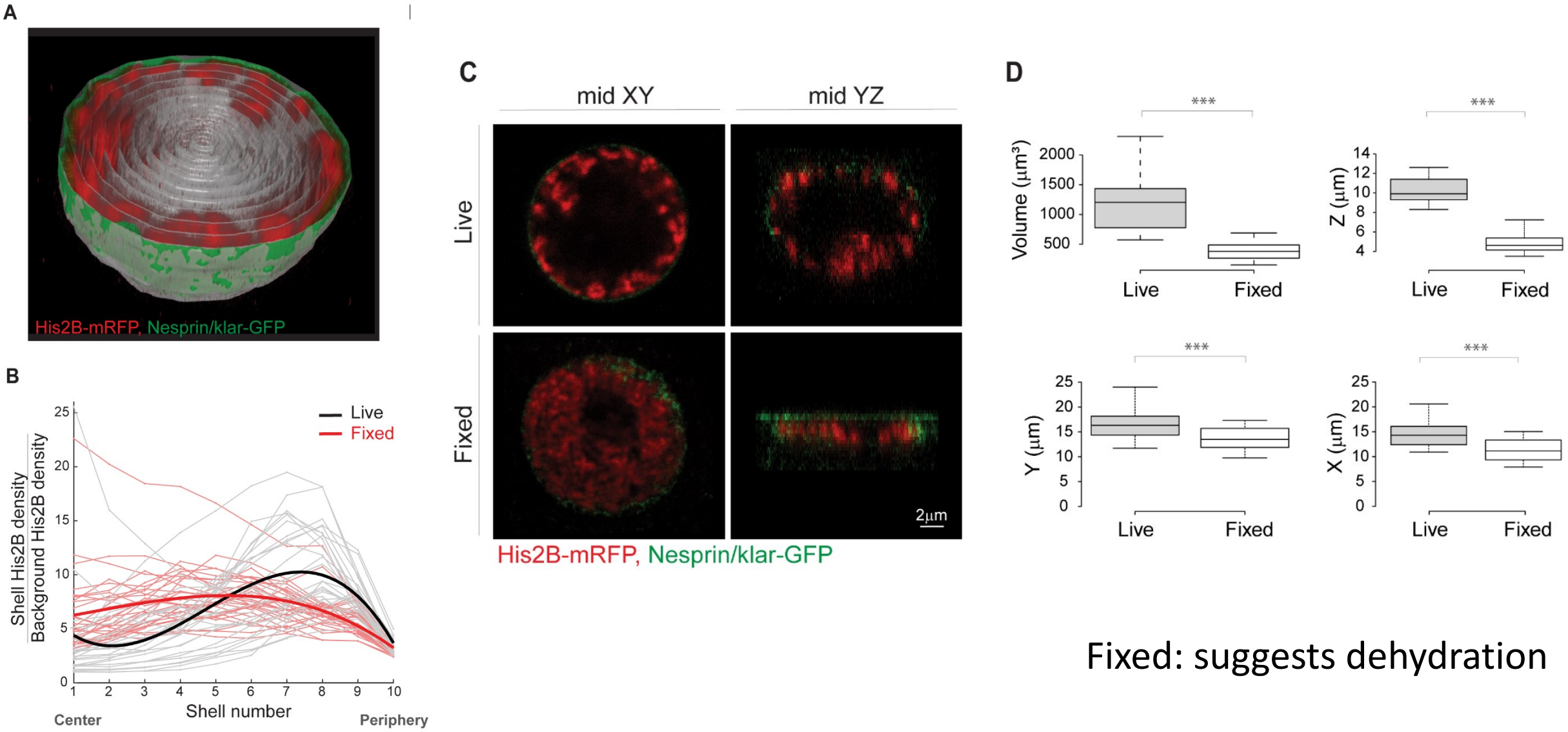
  - Later on: conventional chromatin concentration profile (ENC)



- **Experiments on *Drosophila* larvae, live imaging: peripheral, conventional, central**
  - **Fixed or dehydrated nuclei – conventional; lamin overexpression – central**

- **Simulations and theory: transitions of chromatin profiles: hydration, lamin interaction**
  - Ajoy et al. – Biophysical J. 2020 –good solvent; Chiang et al., Lamina, Cell Reports 2019.

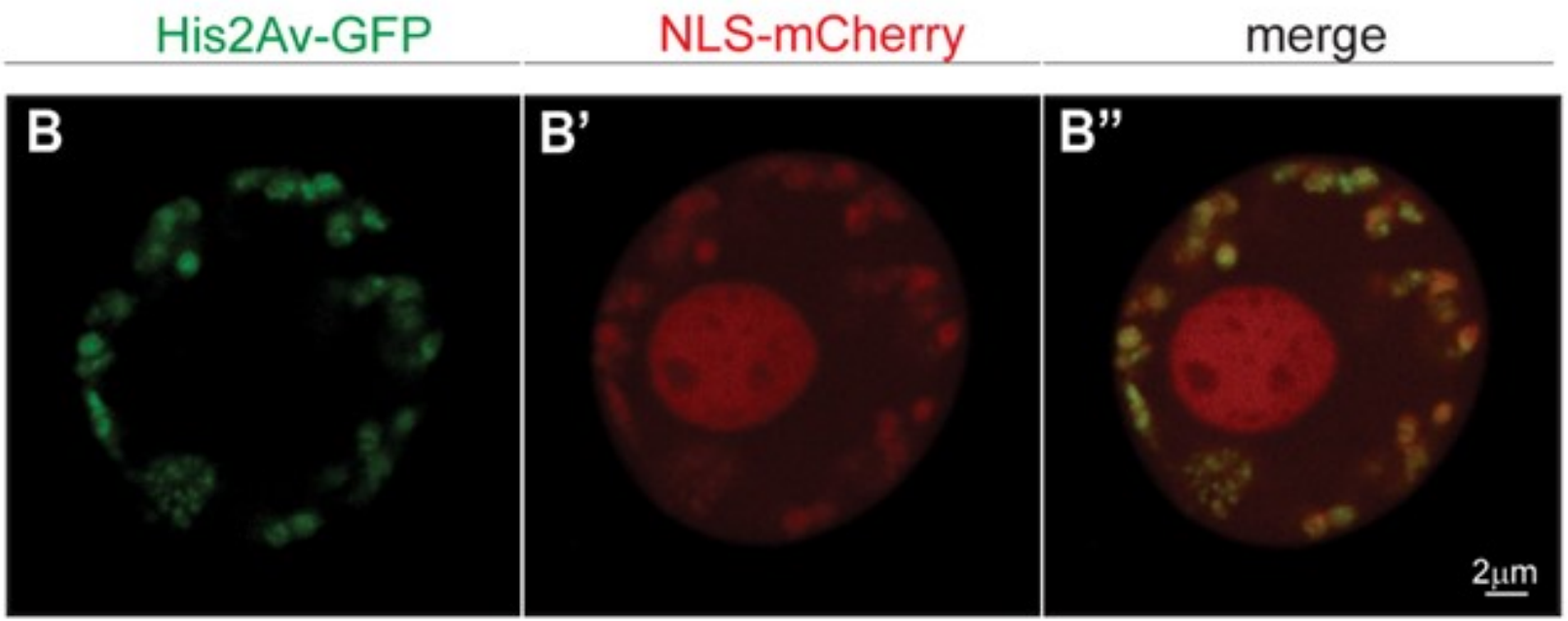
Live cell – peripheral, Fixed cell – “conventional”



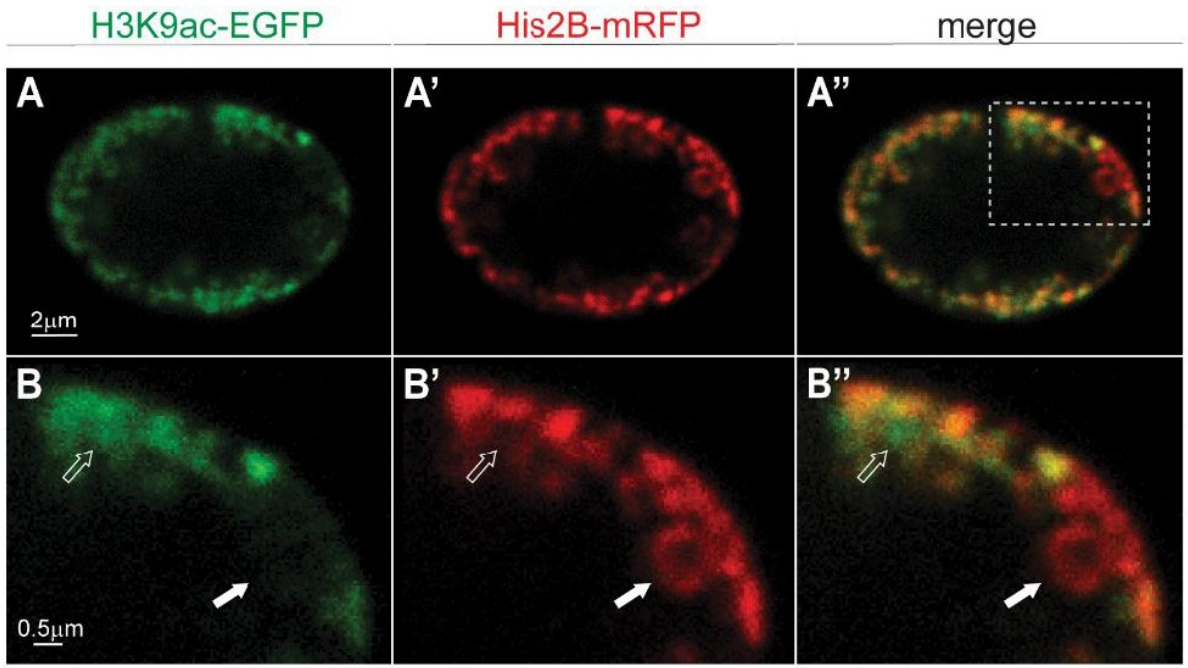
Fixed: suggests dehydration

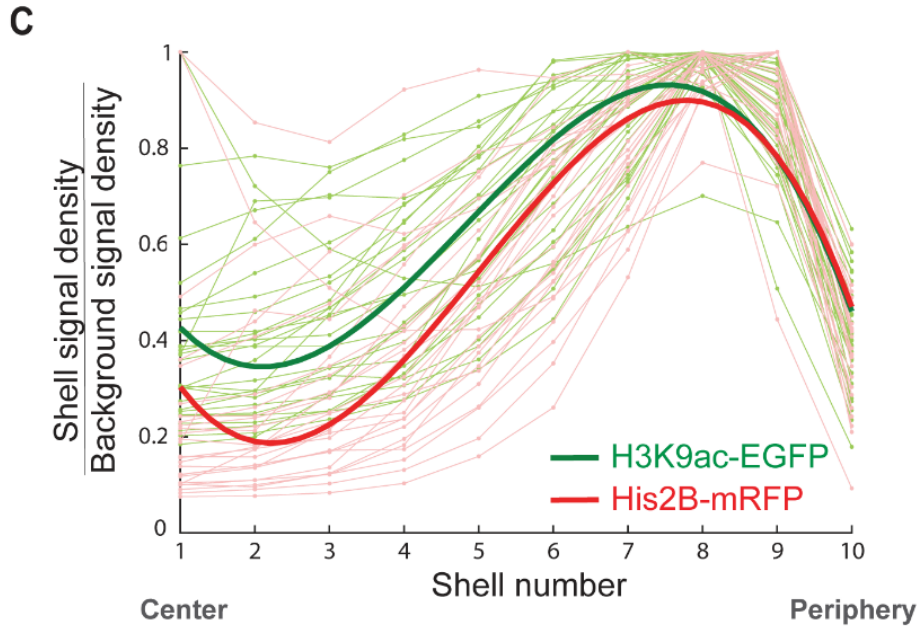
Is peripheral chromatin due to exclusion by nucleolus?

Probably not. Also expect chromatin more rigid than nucleolus (LLPS).



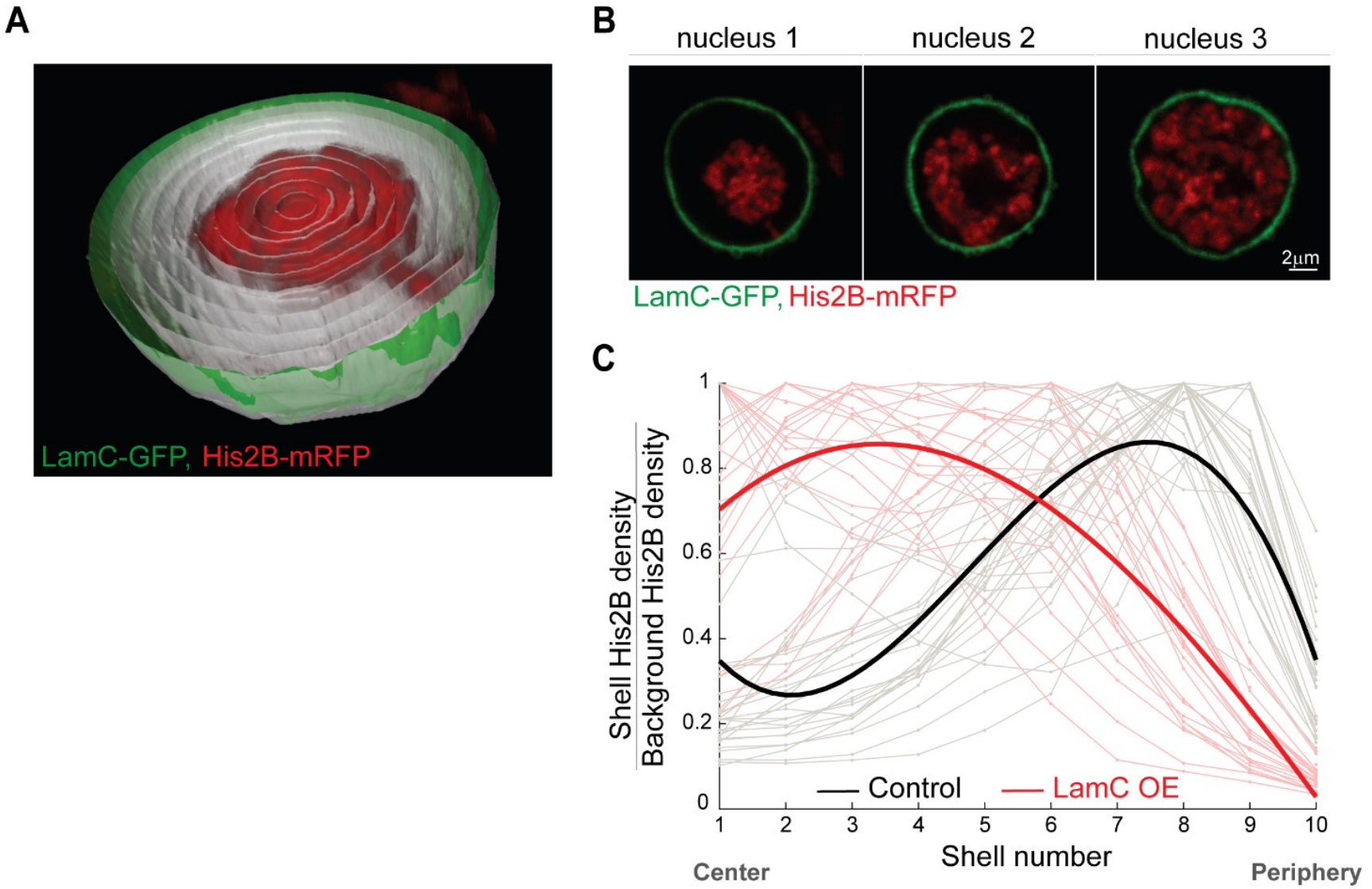
Peripheral: hetero and eu chromatin – angular distribution





Euchromatin mark All chromatin mark Merge

Lamin A/C overexpression: peripheral to central transition

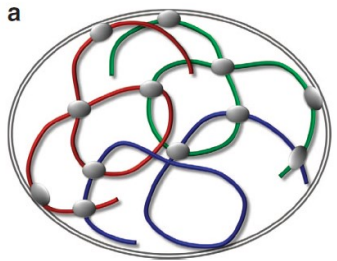


What controls transitions between peripheral, conventional and central chromatin profiles?

- Chromatin – chromatin interactions: repulsive, attractive
- Chromatin – aqueous phase (water+small proteins) interfacial tension

- Chromatin – lamina layer interactions: Lamin associated domains (LAD) of chromatin
  - Fly: 48% of X chromosome is LAD
  - LAD and heterochromatin: identical but not always
  - LAD domains: in fly about 90Kbp “chunks”
  - Not all LAD can be at periphery due to overpacking. Theory – consider various scenarios

- Chromatin – lamin A protein interactions in nucleoplasm: LAD – soluble lamin A protein
  - Garini (2015): lamin underexpression qualitatively modifies chromatin diffusion

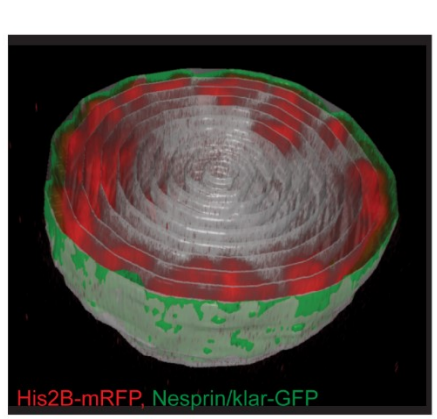


Chromatin in-vivo: self-attractions or good solvent

- Is chromatin a polymer in good solvent or poor solvent (self-attractive)

  - Chromatin-chromatin repulsion due to excluded volume
  - Positively charged histone tail can attract negative DNA linkers (distant along the chain but close in 3d space; entropic – counterion release) - Rosen
  - HP1 for heterochromatin, effective chromatin-chromain attraction - Karpen
  - Other proteins (soluble lamin A) can act as “crosslinkers” in vivo
  - Is chromatin a type of gel or just a self-attractive polymer (“collapsed”)?

- If there is no self-attraction, why is peripheral profile observed?
  In good solvent, a long polymer would fill the entire volume
  if radius of gyration is equal or larger than the nuclear size.

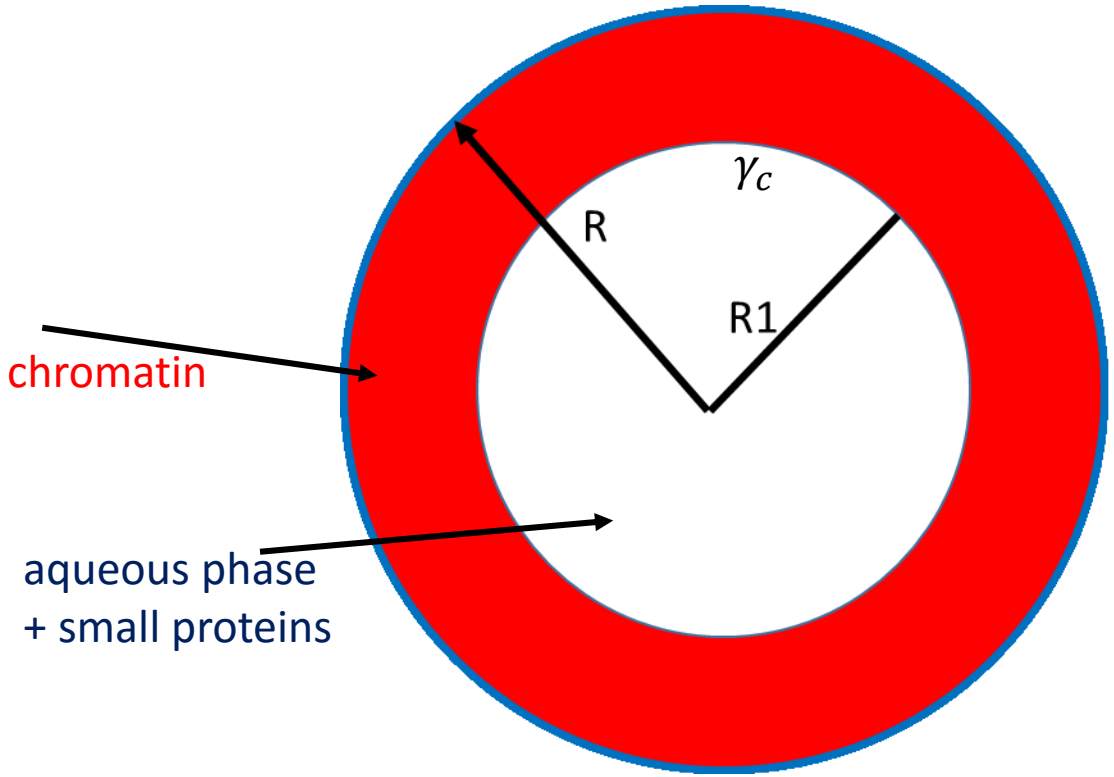


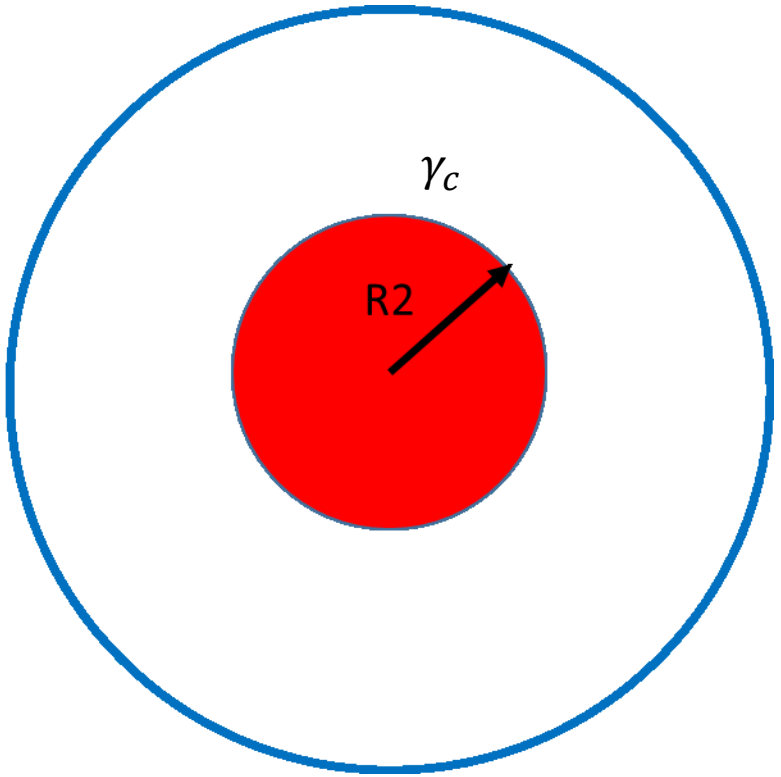
Naïve model for perhipheral to central transition

Lamin attraction $-\gamma_\ell$

Confinement in nucleus: R

surface tension w/aqueous γ_c





$$f_c = \gamma_c R_2^2$$

Relate R1 and R2 - Conserve chromatin: $\mathrm{R}^3 - R_1^3 = R_2^3$

$$f_p = -\gamma_l R^2 + \gamma_c R_1^2$$

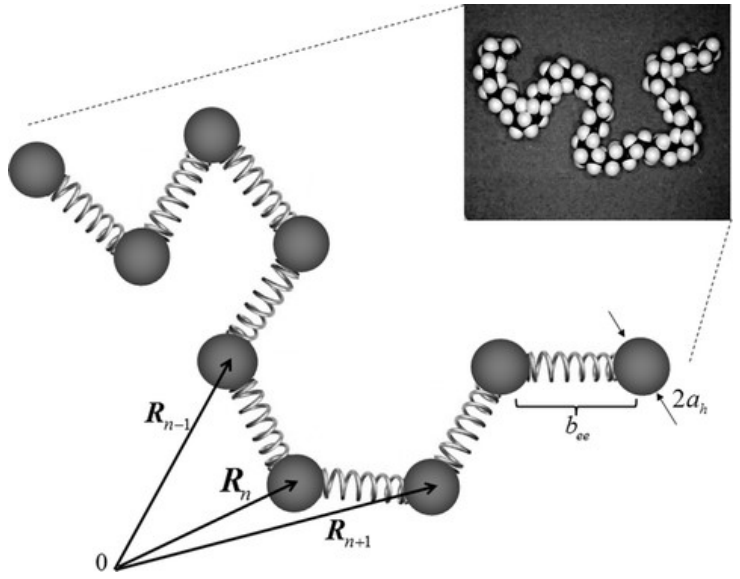
Transition from peripheral to central as function of volume fraction, surface energies.

But must yet include: polymeric topology of chromatin and non-uniform attraction of LAD

Simulations of transitions: hydration and lamina interactions

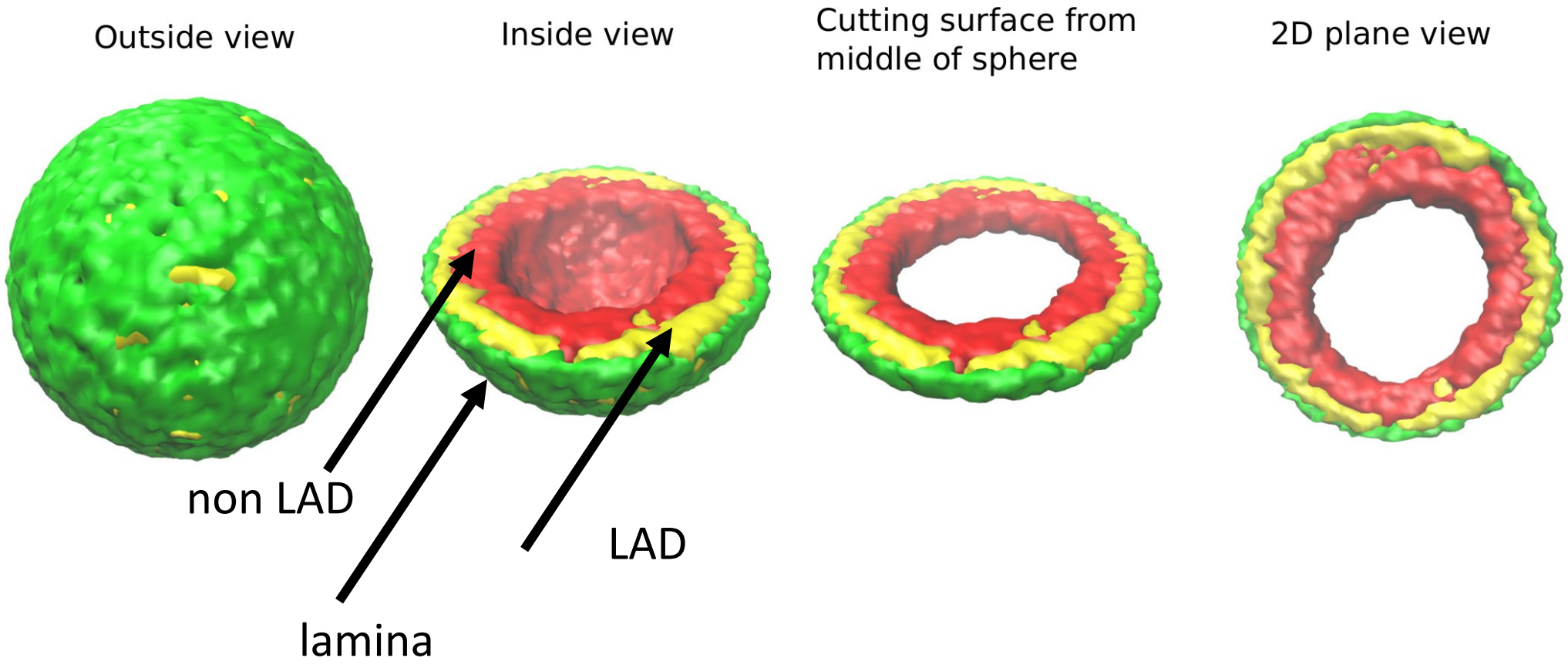
- Simulation model: Langevin equation includes polymer connectivity, interactions with itself, solvent, and lamina. LAMMPS molecular dynamics simulations
- Equilibration and data collection: ~10ms (step-size ~ ns)

- Bead-spring model of polymer:
  - Bead ~ 3 nucleosomes (10nm diameter).
  - Persistence length: 2 beads, 1.2Kbp of DNA
  - Fly X chromosome ~ 37,000 beads
  - Two types of chromatin beads (i) LAD (ii) non-LAD
  - Maximal LAD which bind to lamina: 48%
  - Two cases for LAD binding to lamina: (a) single beads, (b) distributed in clusters (~ 150 beads).



Mol. Dynamics Polymers - Springer

Strong lamina attraction, hydrated: peripheral

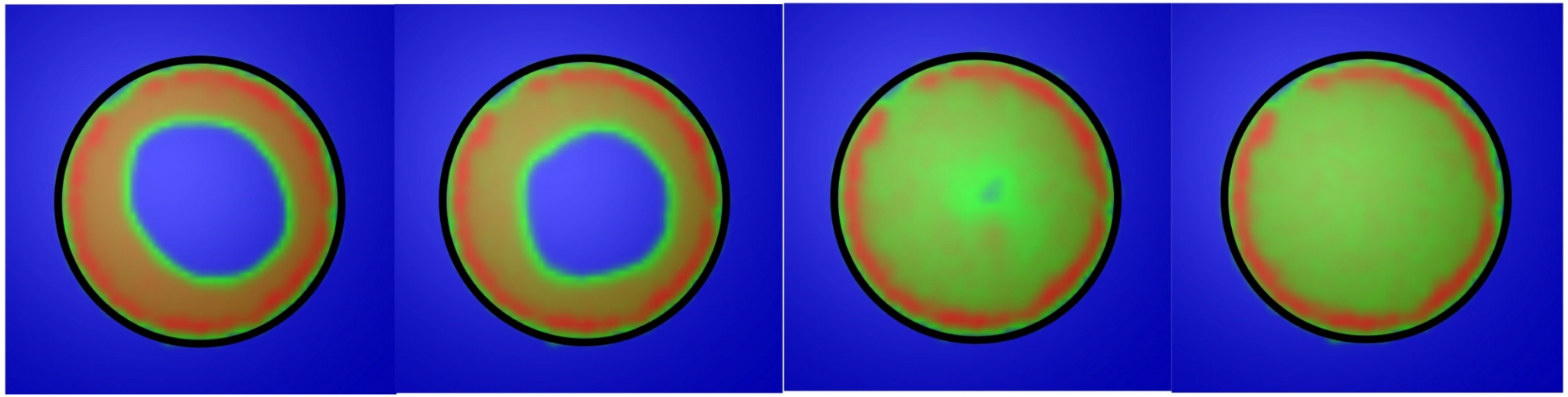


Simulations: snapshots

Decrease chromatin self-attraction:transition peripheral to conventional

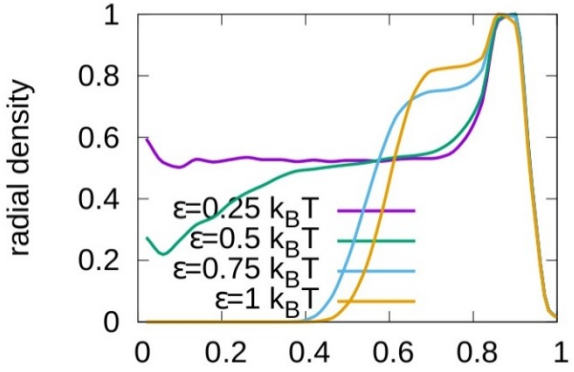
Chromatin volume fraction $\phi = 0.3$ maximal LAD-lamin bonds

self-attraction ε=1 ε=0.75 ε=0.5 ε=0.25



Chromatin concentration: red – high, green - low

Peripheral



Conventional

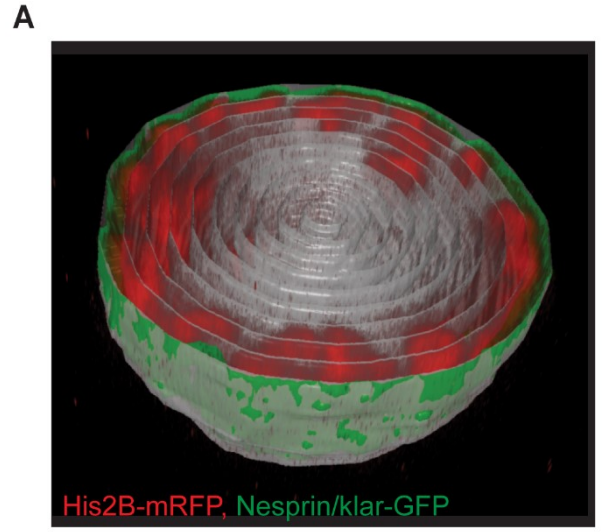
Simulations: average over time steps

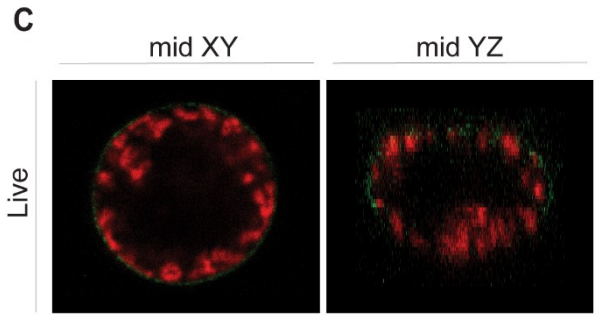
Decrease chromatin self-attraction:transition peripheral to conventional

Does observation of peripheral organization in hydrated nucleus indicate chromatin self-attraction?
If chromatin were long and in good solvent conditions, it would fill nucleus (entropy of excluded volume polymer).

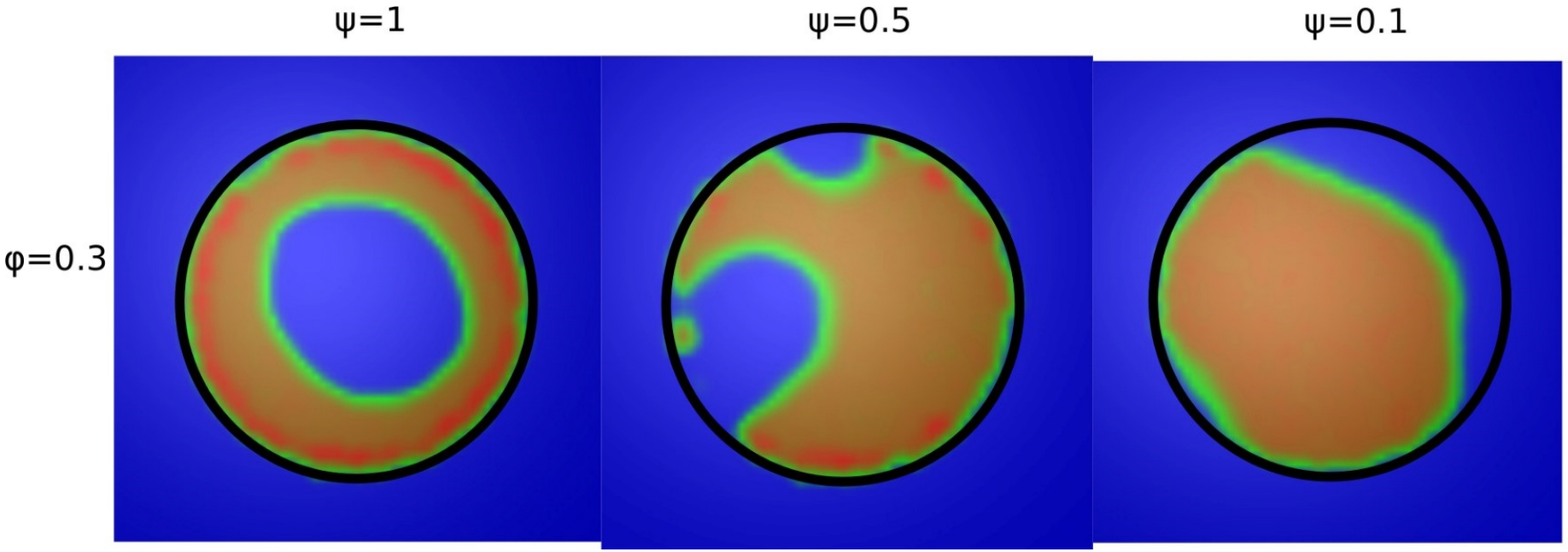
Depends on chromosome size vis a vis nuclear size.

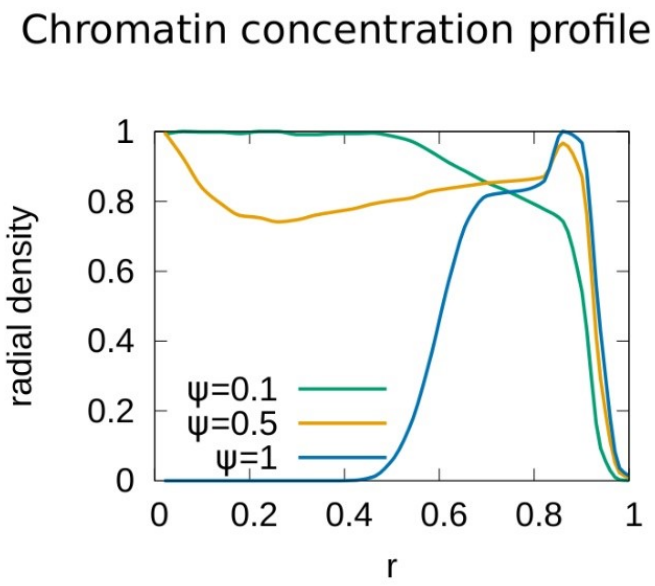
Fly X chromosome: 22Mbp DNA (shortest one);
Interphase persistence length, a, ~ 6 nucleosomes ~ 20nm ~ 1.2kbp
N=6,000 chromatin persistence lengths, $R_g = a\,N^{\nu} \sim 3.7\mu m$
8 chromosomes expected to overfill nucleus of diameter $8\mu m$





Decrease LAD fraction ψ, moderate hydration $\phi = 0.3$: Transition peripheral to conventional

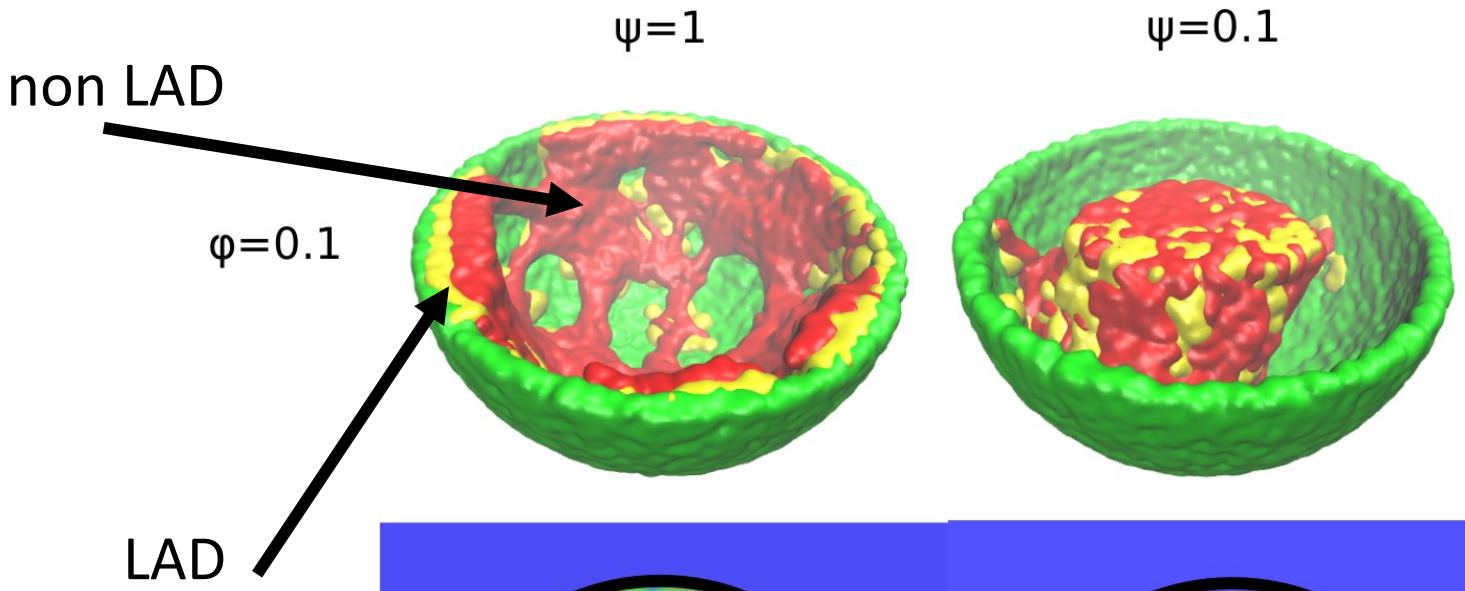




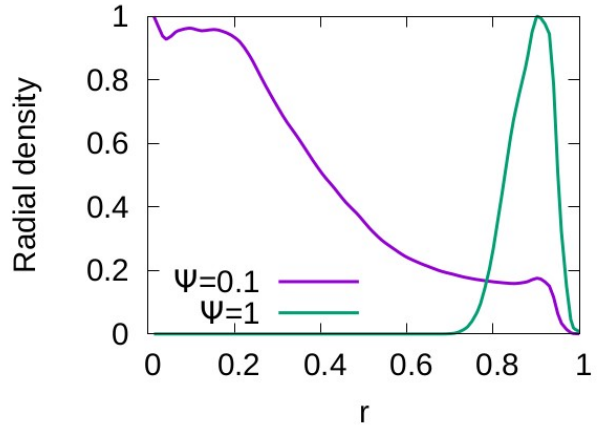
Chromatin volume fraction ϕ =0.3

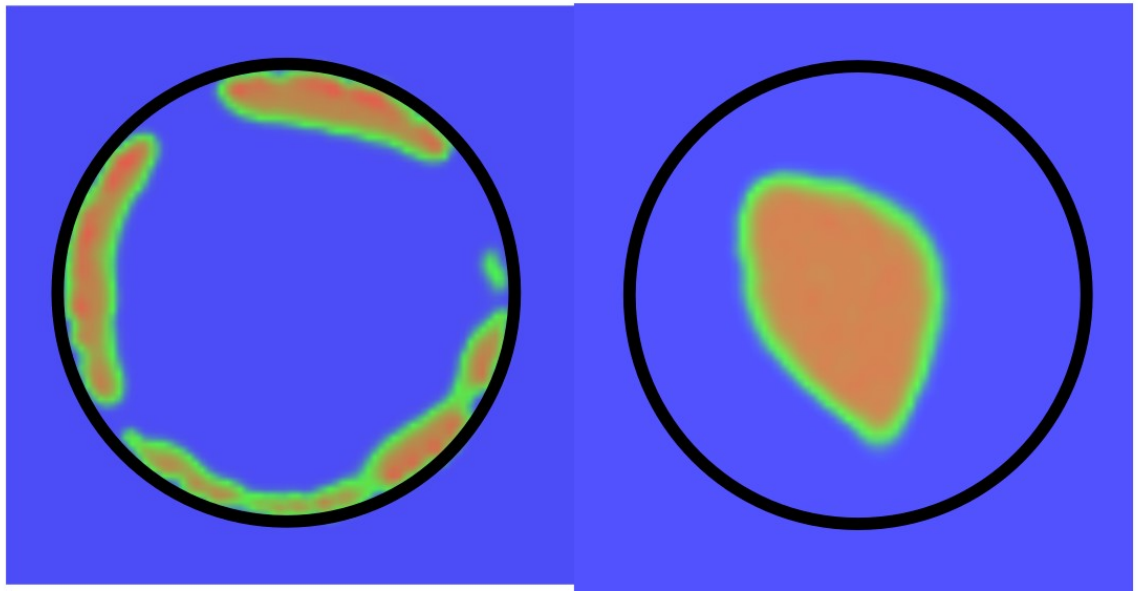
Chromatin concentration: red – high, green - low

Decrease LAD fraction ψ, high hydration $\phi = 0.1$: Transition peripheral to central



Chromatin concentration profile

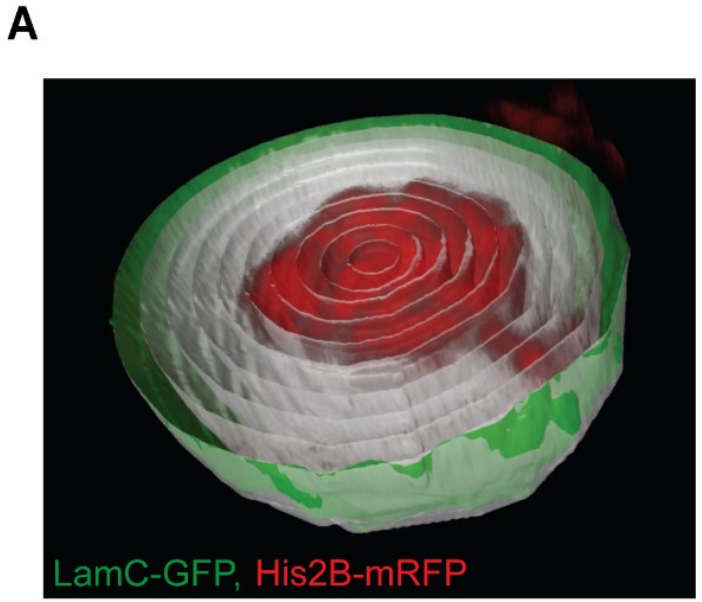


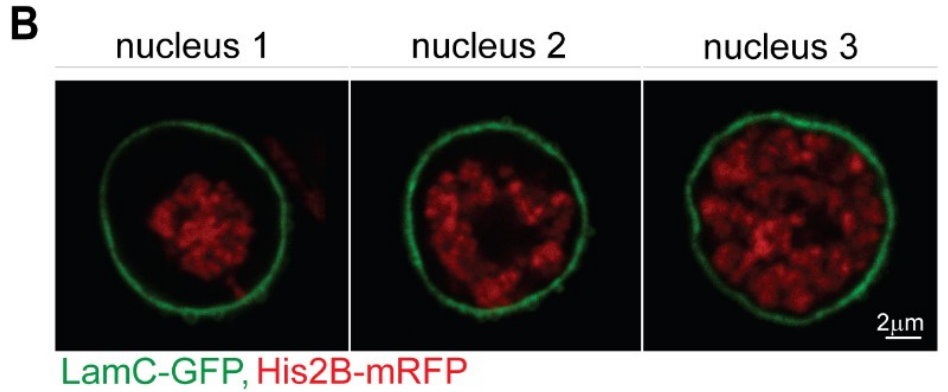


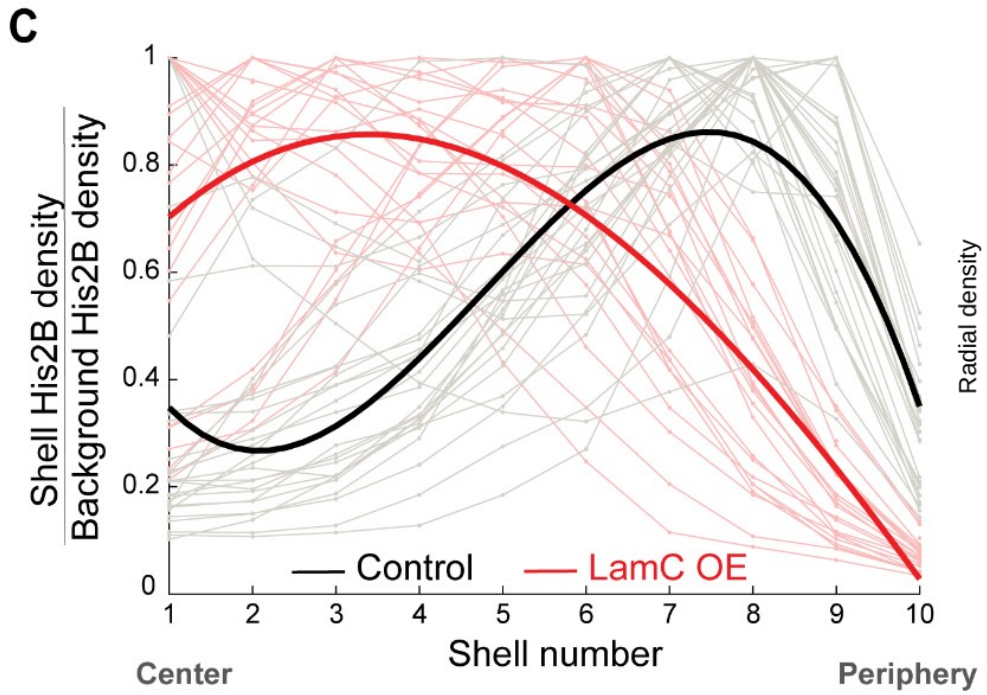
Chromatin volume fraction ϕ =0.3

Chromatin concentration:
red – high, green - low

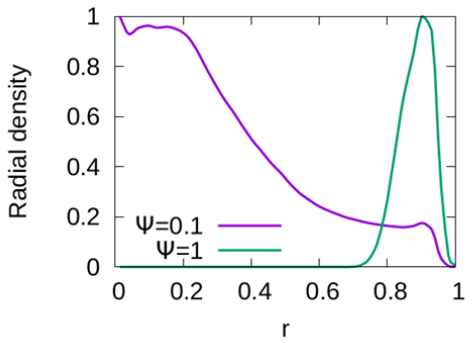
Lamin A/C overexpression: peripheral to central transition







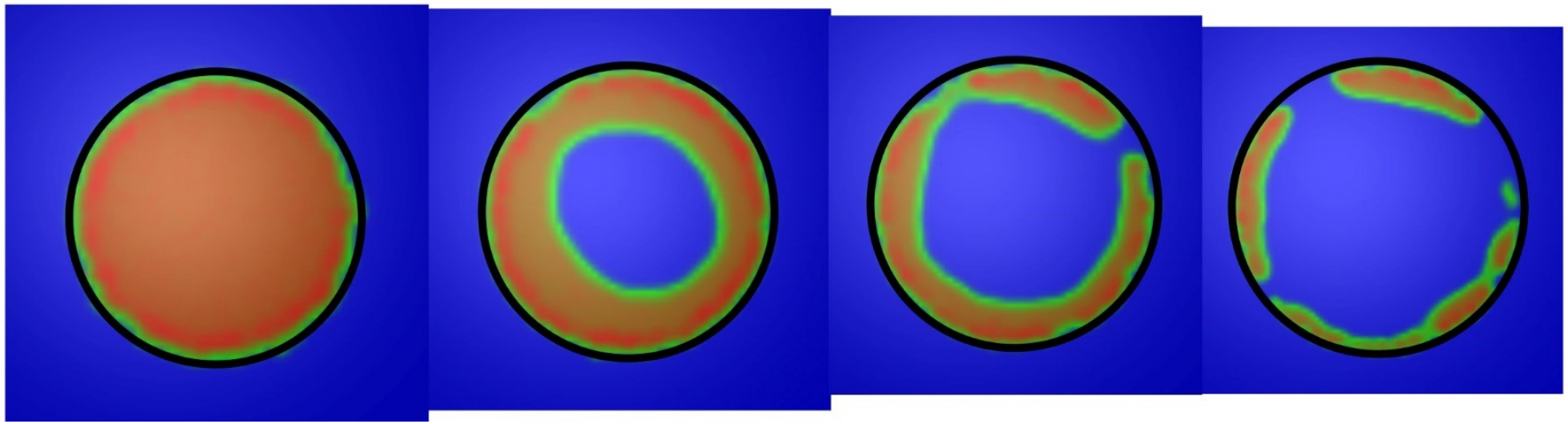
simulations



Overexpression: Hide LAD binding sites?
Perhaps, lamin in nucleoplasm binds LAD.

Increase hydration: Transition- conventional to peripheral

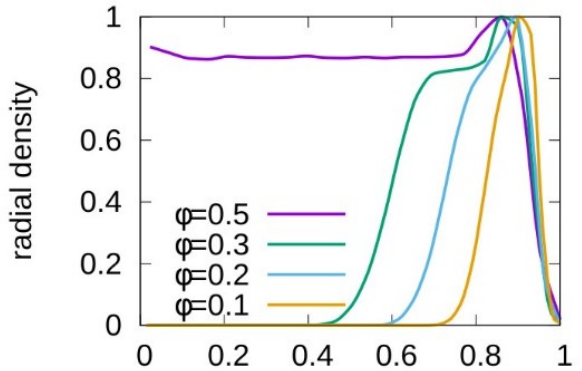
Chromatin volume fraction ϕ



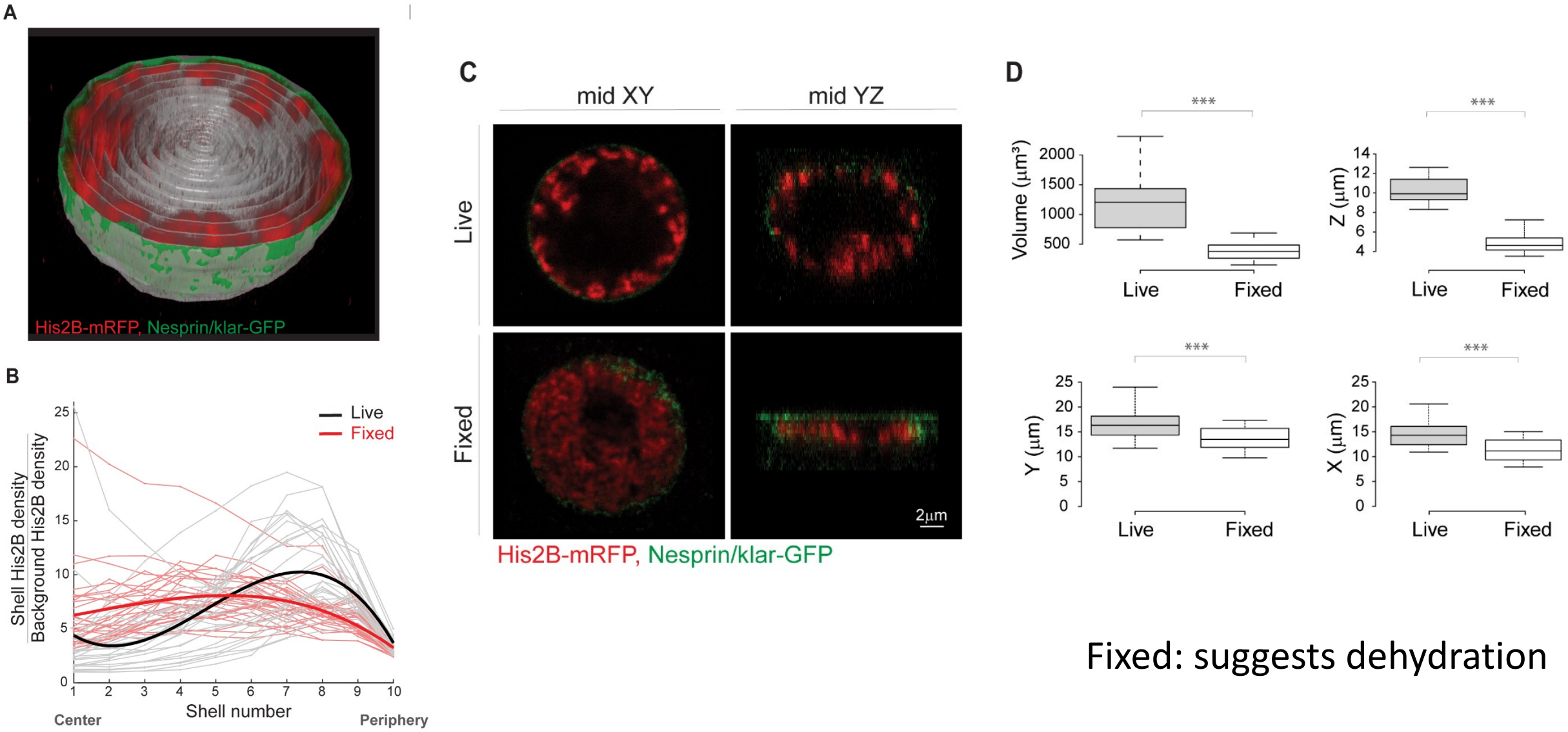
Self-attraction
$\epsilon = 1$

Chromatin concentration: red – high, green - low

Chromatin concentration profile

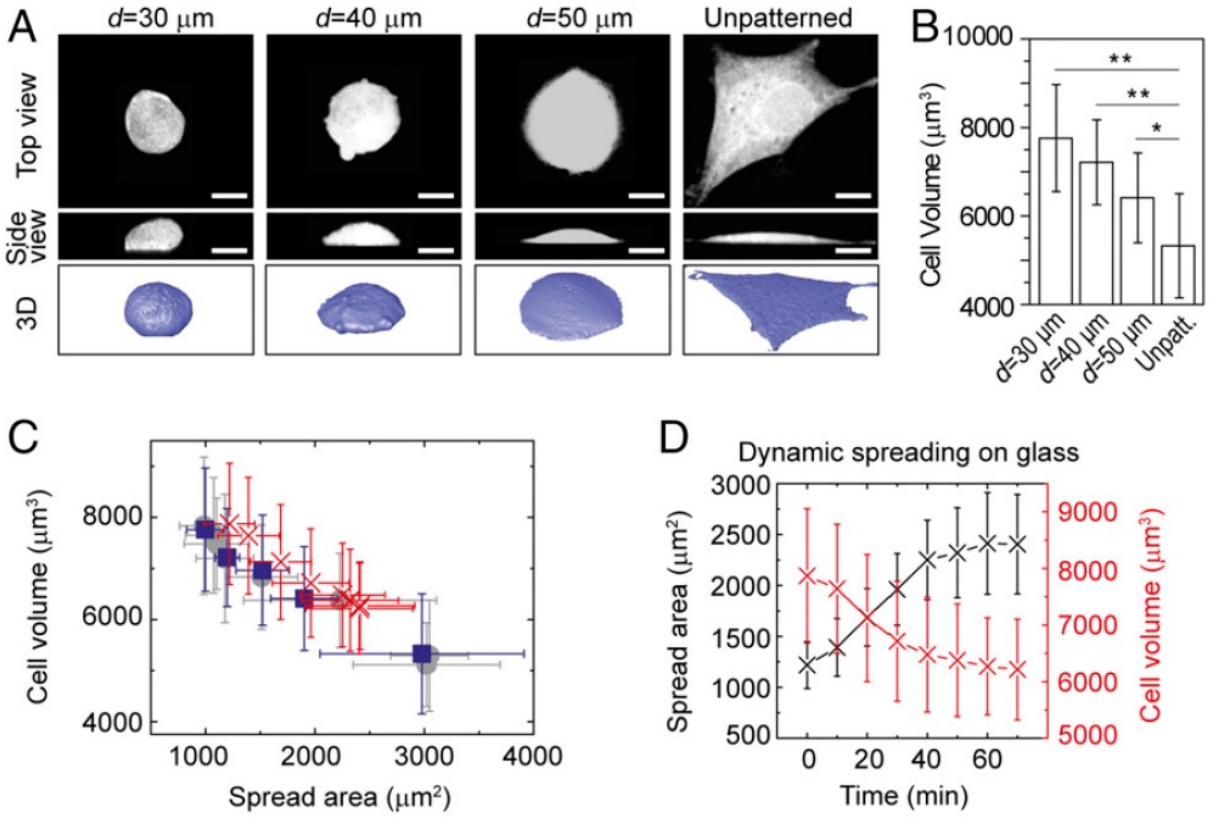


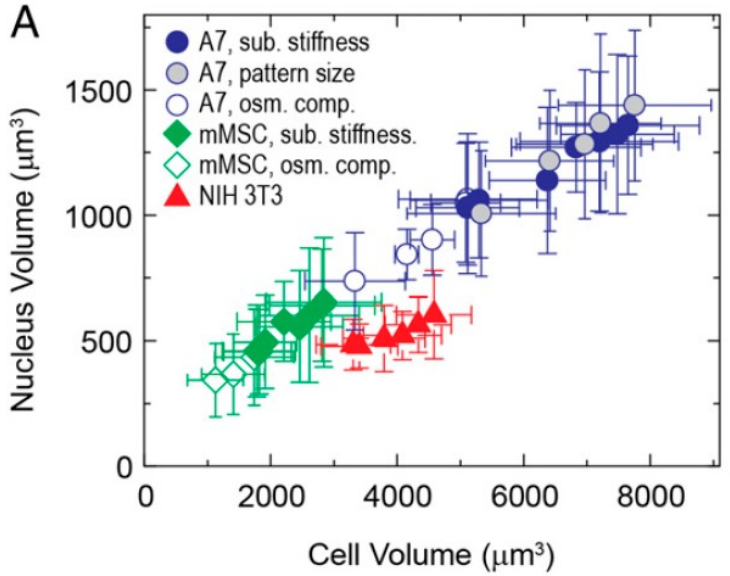
Live cell – peripheral, Fixed cell – “conventional”



Fixed: suggests dehydration

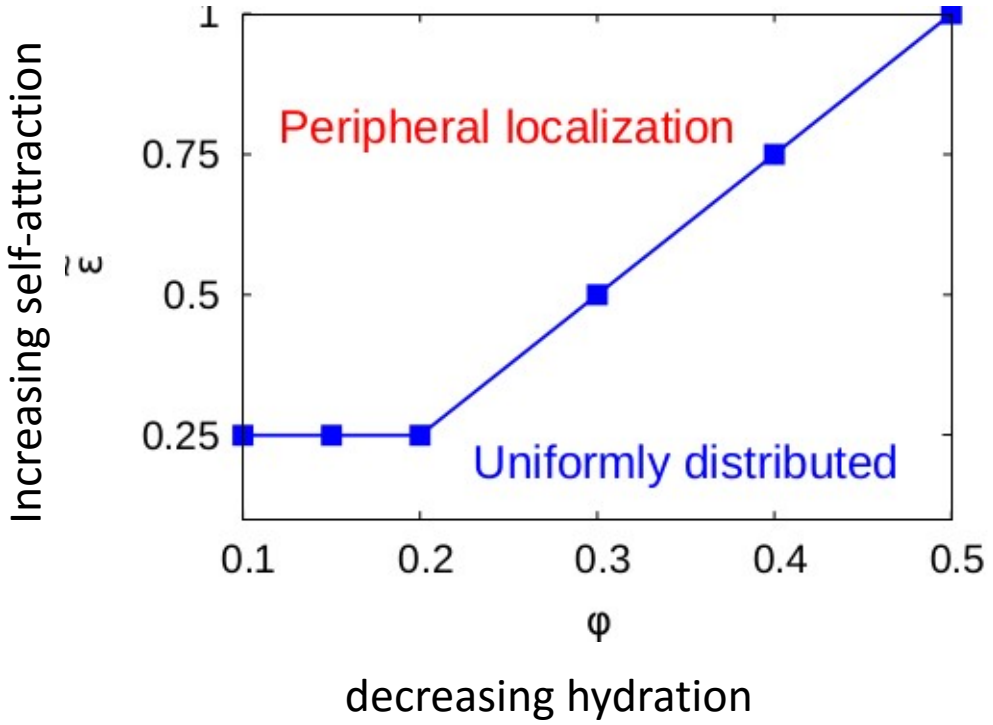
Dehydration of spread cells and nuclei



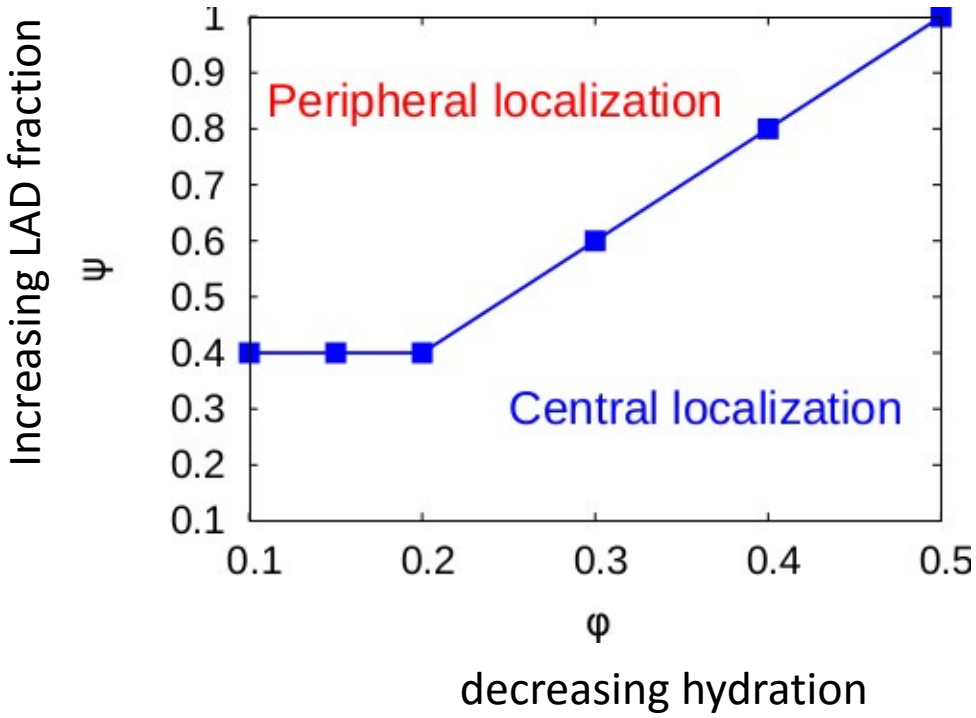


Expts.: Guo…Weitz PNAS 2017; Xie et al. Biophys. J. 2018
Theory: Adar… PNAS 2020

State diagram: peripheral, conventional (uniform), central



Self-attraction
$\epsilon = 1$



Current research: Further simulations

- Here: chromatin-aqueous-lamina profile with average chromatin self-attraction

- Next level of resolution: distinguish interactions within chromatin
  HC-HC, EC-EC, HC-EC, LAD-LAD, LAD-HC, LAD-EC

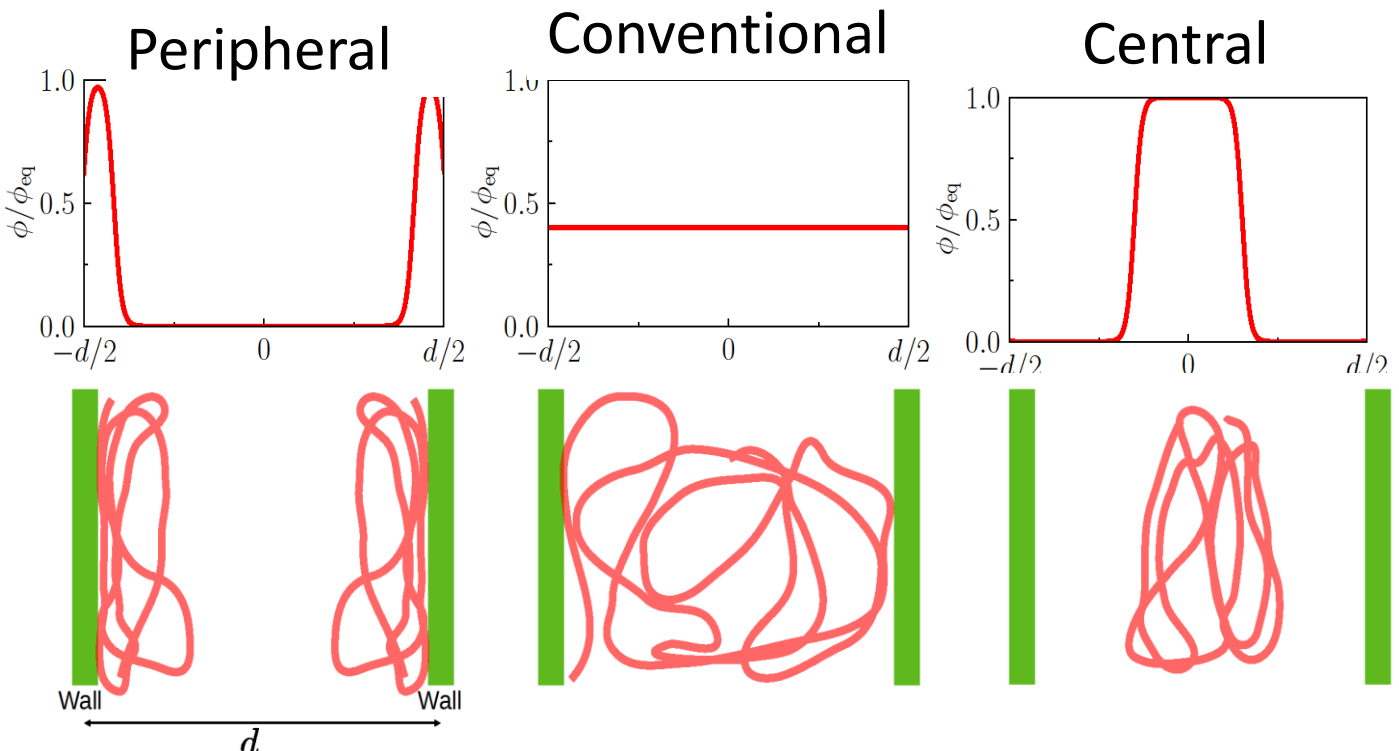
- Can we obtain new intuitive insight as we did with naïve model or does it just complicate matters?

- Add soluble lamin proteins in aqueous phase so have competitive adsorption of LAD on lamina on nuclear envelope and with soluble lamin A in nucleoplasm. (Garini, Nat. Comm., 2015 – lamin proteins “crosslink” DNA in bulk)

Current research: Analytical insights

Effective free energy (de Gennes - ground state approximation) with attractions and wall energy in linear geometry exact periodic solutions (Klein and Pincus; PGG – single wall)

How do transitions vary with changes in hydration, LAD interactions?



Needed: more coarse-grained experimental insight

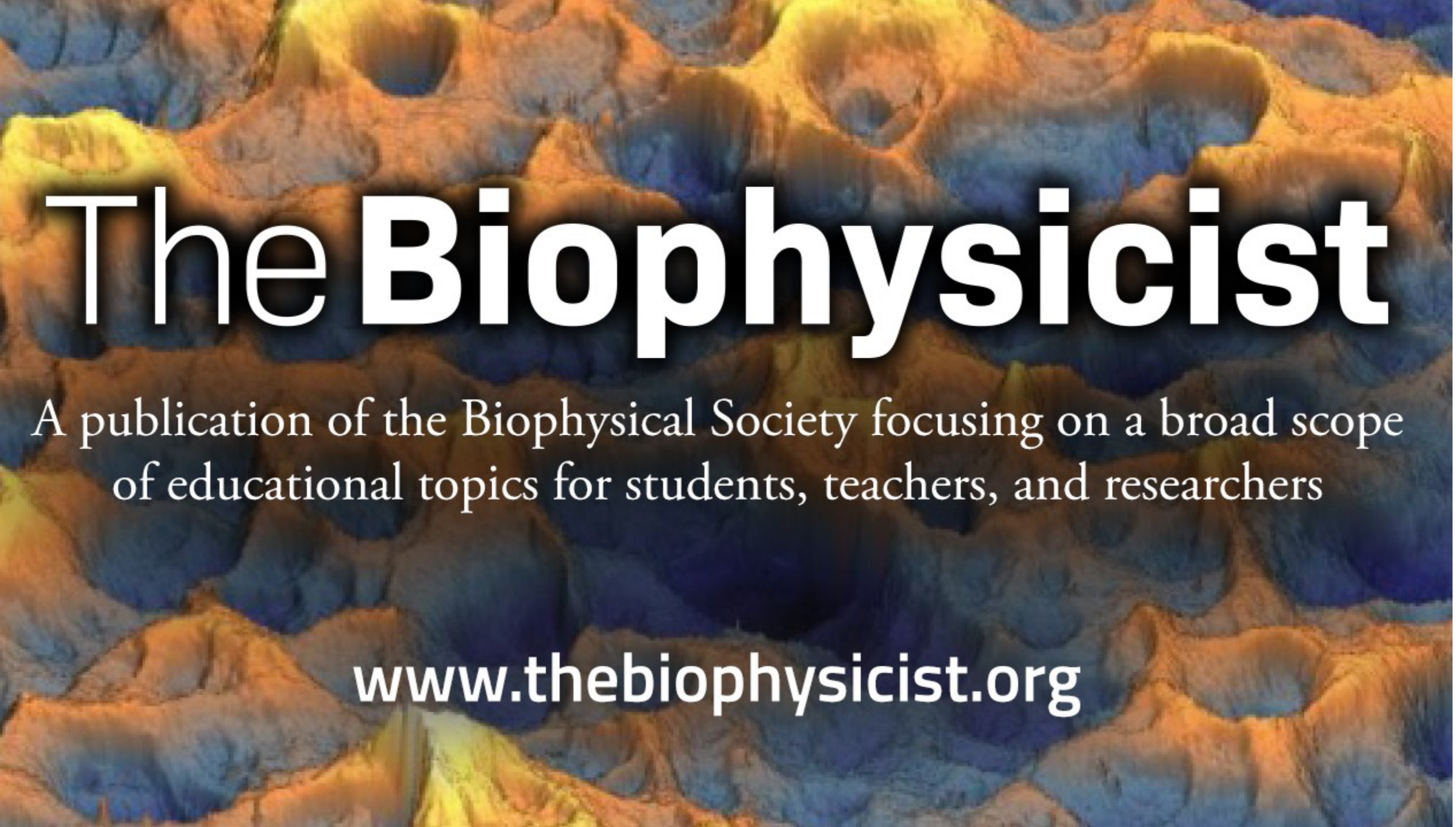
- Are chromosomes strongly condensed in the nucleus due to self-attractions (mediated by molecules in nucleoplasm and/or attractions of histone tails and distant DNA) or are they in "good solvent" but confined by the lamina and nuclear envelope?

  - Lyse nuclear envelope and observe chromatin spill-out as seen from bacteriophage (Physics World, 2013)

    Cell free, isolated nuclei (Elbaum, PNAS 2007).

- LAD domains – characterization, binding to lamin (strength, binding domain size)

- Cell and nuclear hydration in development, aging… : effects on chromatin profile in nucleus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