Simulations of LLPS

Fernando Muzzopappa

06/09/24

Simulation setup

- The idea of these simulations is to use a minimal system to see if chromocenter binders can undergo LLPS when binding to different chromocenters and if the system can evolve to a situation where only one (or a few) chromocenters are the result of phase separation (and show a dip in mochafrap).
- For the simulations and its analysis I used a mix of python and LAMMPS. LAMMPS is a software that is used to simulate large systems with many components (like this).

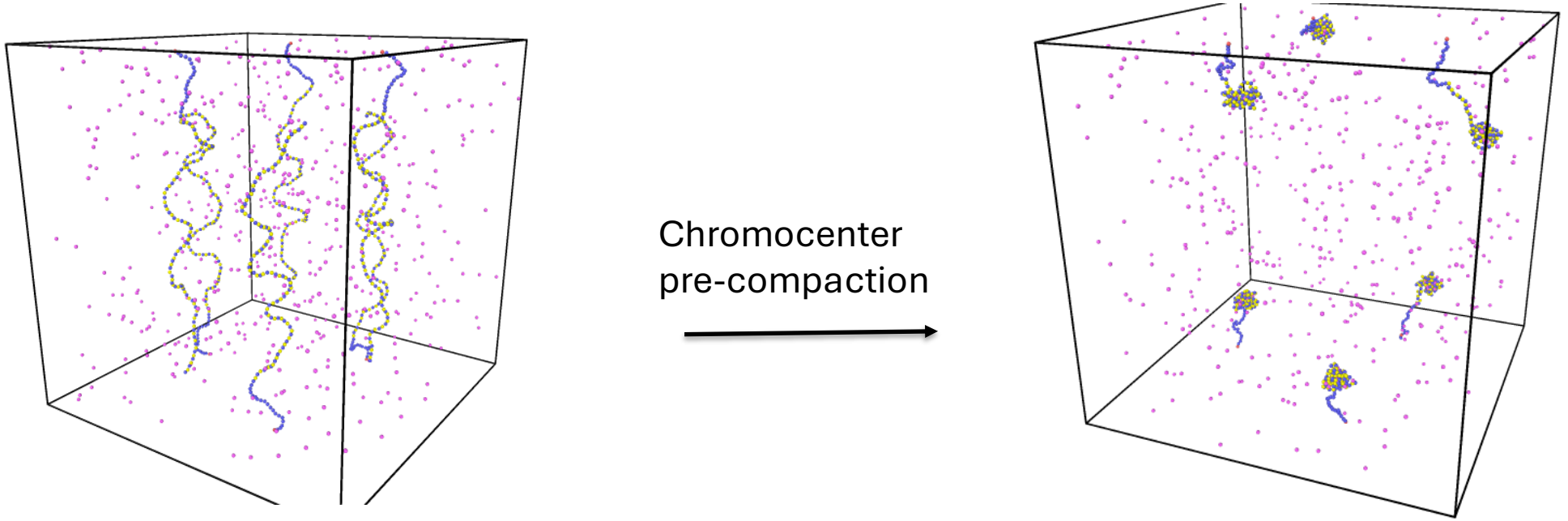
Simulation setup

- Disclaimer 1: in order to simulate large amounts of proteins, is necessary to coarse-grain, meaning that in the simulation the proteins are represented by a single particle. So, the simulation doesn't aim to model MeCP2, or another specific protein. This is more of a "toy simulation" to test one specific hypothesis.

Simulation setup

- These particles can move and interact with other protein particles, and with another type of particles that form a polymer that represent the chromocenters, which are tethered to the simulation box to avoid chromocenter fusion. As a first simulation step, the particles forming the chromocenter can self-interact, so the chromocenters form structures from the start of the simulation:

protein Chromocenter binding site Chromocenter "neutral" particle

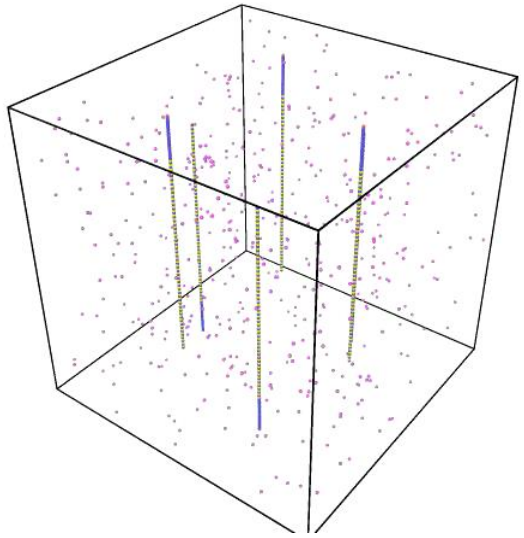


Simulation setup

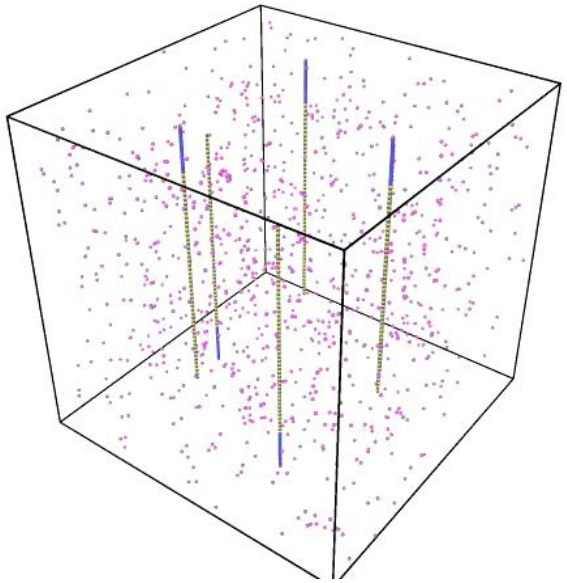
- The energy of interaction between proteins (Epp) and proteins – binding sites (Epc) is the same. This energy does not lead to protein LLPS in the absence of chromocenters, we did it this way because in the cells all these protein form clusters at the chromocenters and "only-protein" foci are extremly rare.

Binding to chromocenters increases the local concentration and this induce clustering (or LLPS). I run 3 different simulations, in which I gradually increased the protein concentration.

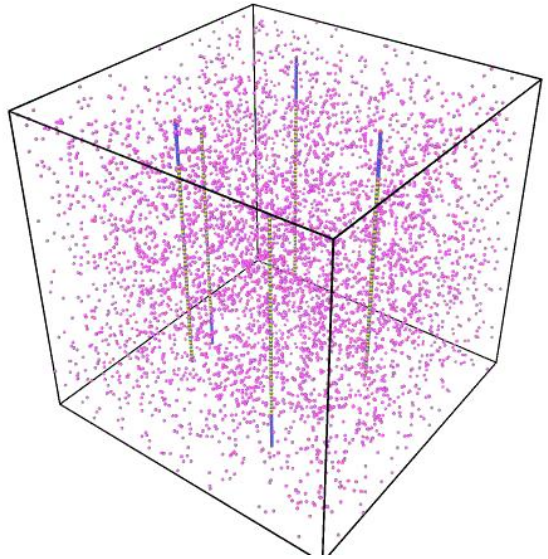
Low.mp4



Mid.mp4

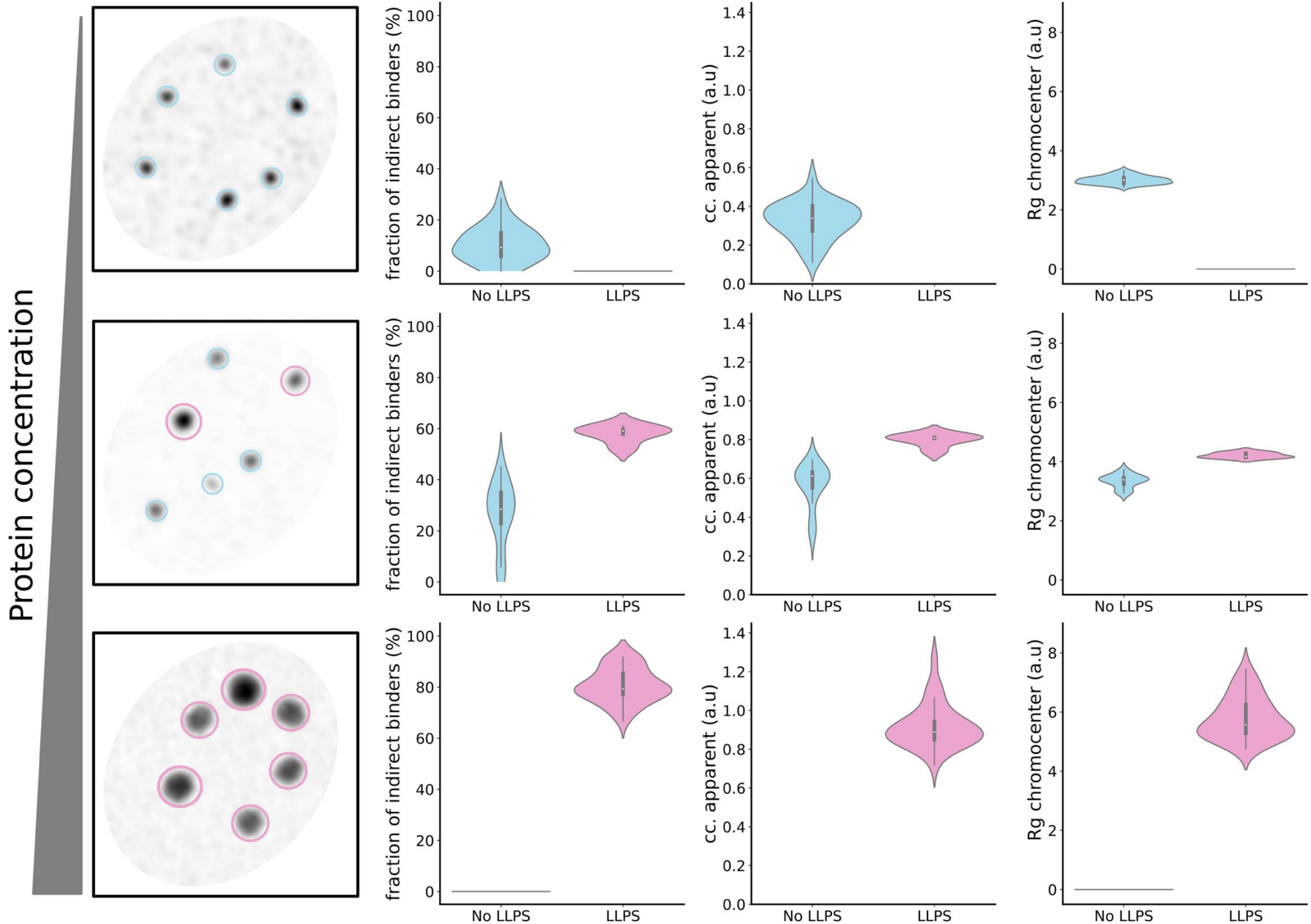


High.mp4



Simulation setup

- At low protein levels, the proteins only bind to the chromocenters. At high concentration, they form large clusters (that will give a dip in a mochafrap exp) in all chromocenters. The most interesting case is at medium levels, where proteins initially bind to chromocenters, then in some of them form a dense phase (LLPS) but the system evolves to a situation where only one or two chromocenters retain the dense phase and the others contain only bound proteins.
- In each simulation, I quantified the number of proteins directly bound and the number of proteins indirectly bound (dense phase) and I used the latter to distinguish LLPS vs no-LLPS. I also quantified the total protein concentration of each chromocenter (that could be compared to the enrichment of microscopy images, in different units/scale) and the radius of gyration of the chromocenter (that could be compared to the chromocenter size, in different units/scale). I also generated simulated microscopy images from the simulations


Protein concentration
fraction of indirect binders (%)
cc. apparent (a.u)
Rg chromocenter (a.u)
No LLPS
LLPS

Conclusion:

- In a scenario where proteins bind to chromatin: low number of proteins will lead to binding, a protein concentration slighly above the critical concentration (like in the 'medium' case) leads to formation of a dense phase in 1-2 chromocenters, that will show a dip in mocha and are a bit more protein-enriched and large, while the rest of the chromocenter will contain proteins from the dilute phase that bind to the binding sites. Both type of chromocenters are visible and the microscope, as the simulated-microscopy image shows.