



Loop Extrusion Dynamics at Chromatin Fountains

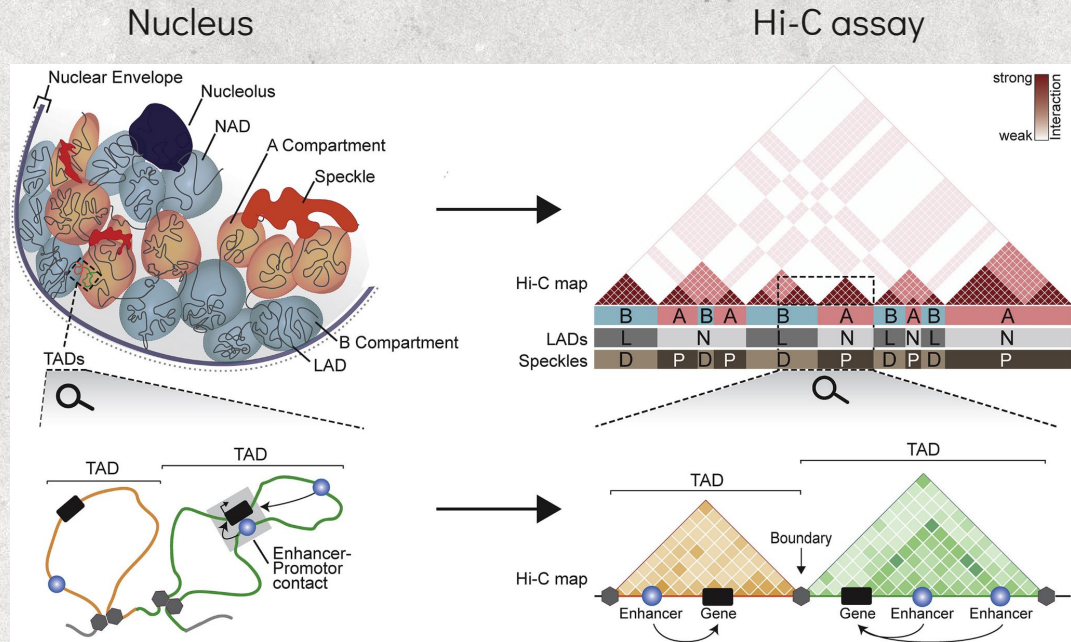
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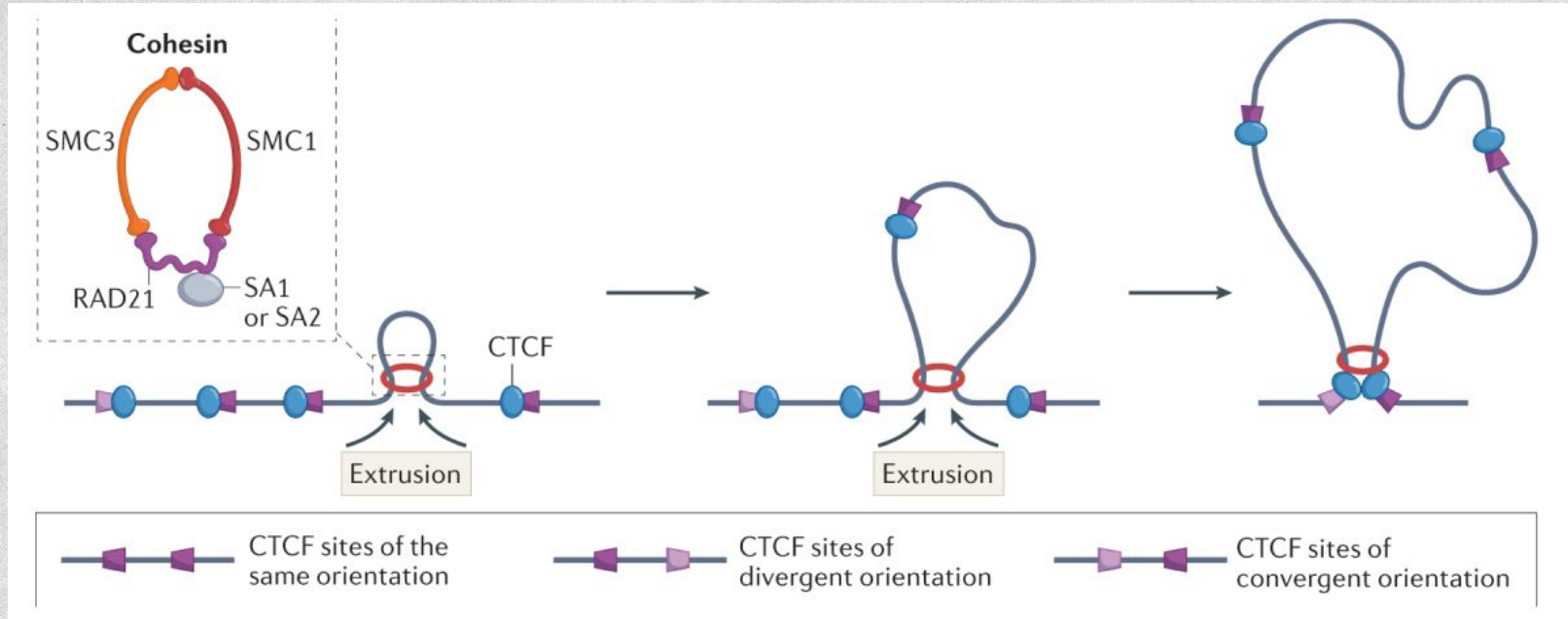
Promoter-enhancer interactions in nucleus

How do they find each other?

Promoter-enhancer interactions are essential for gene expression, but the biological mechanisms behind them are unknown. We theorise that they are brought together for interaction via loop extrusion.



Loop Extrusion Mechanism



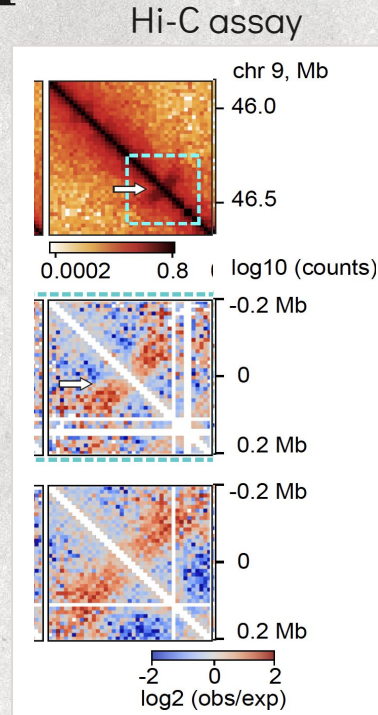
Fountain Formation

Necessary components:

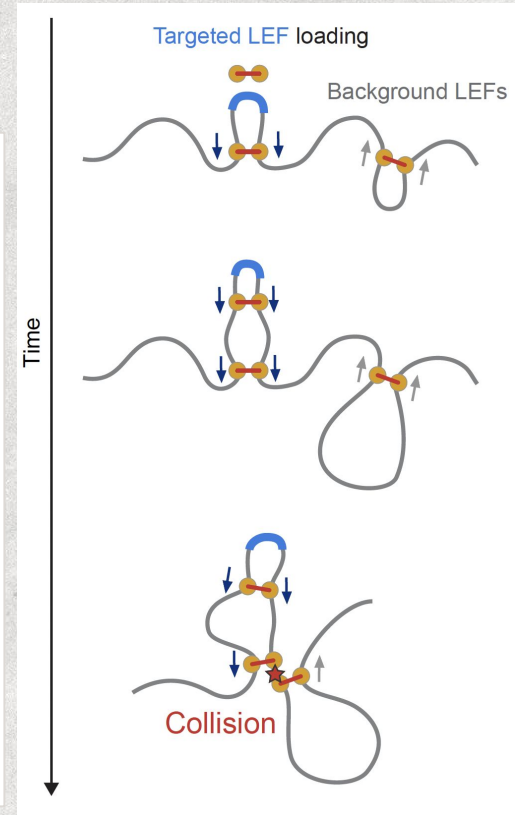
- Cohesin (the motor protein)
- Polymer
- Target site for cohesin to land on

Cohesin parameters:

- Lifetime – for how long does cohesin stay on the polymer
- Separation – linear distance between two cohesins



Mechanism



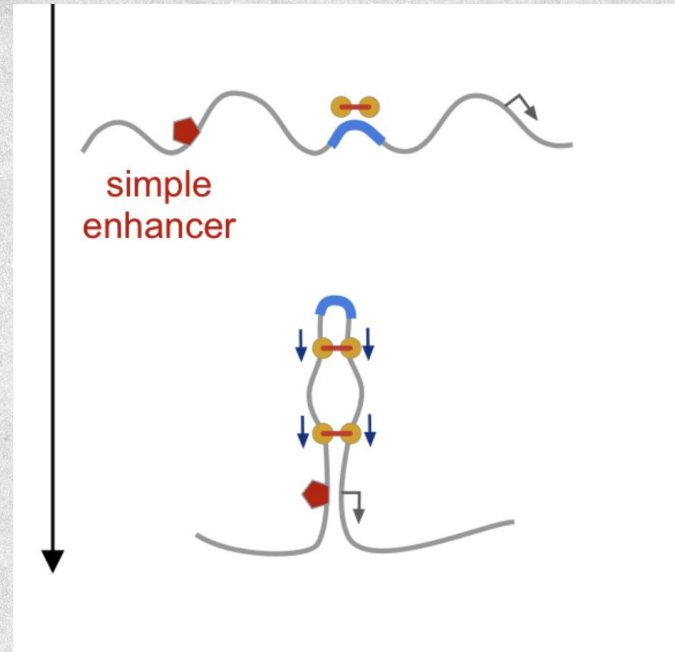
Research question: Does extrusion help promoter-enhancer interactions?

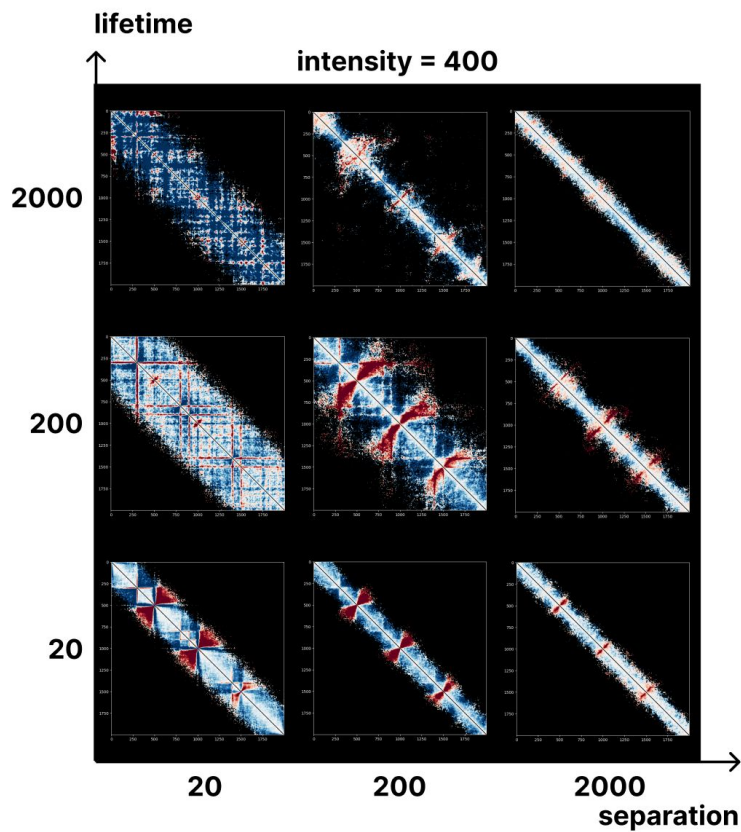
Fountains may help promoter enhancer interactions which regulate the gene expression in the DNA.

Can cohesin speed up promoter-enhancer interactions by forming a fountain and how much?

The time needed for promoter-enhancer interaction to occur is yet to be determined, but we can get the preliminary assessment through simulations.

One cohesin step in simulations is 1 second in reality.





Hi-C-like visualisation of fountains in simulations

We varied the cohesin parameters (lifetime and separation) to get the most realistic simulated fountain.



Neural Network for Hi-C fountain calling

We trained a neural network to recognise fountains on our Hi-C maps to then recognise the parameters we used, but only we achieved accuracy of 0.68.

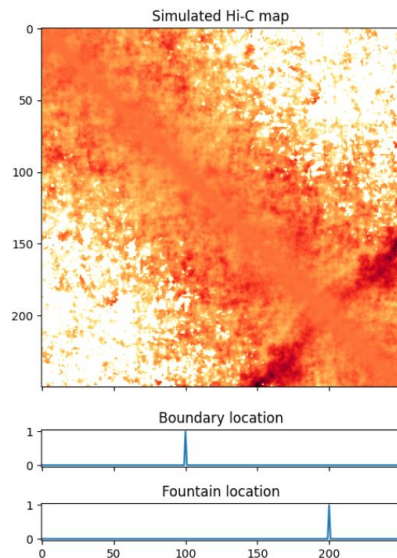


Figure 5A. Input Hi-C map and original data for comparison with the model's predictions.

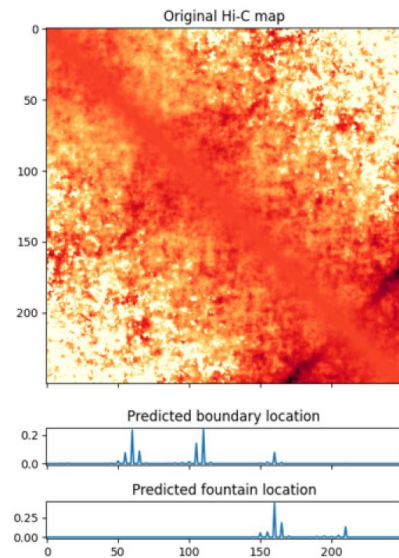


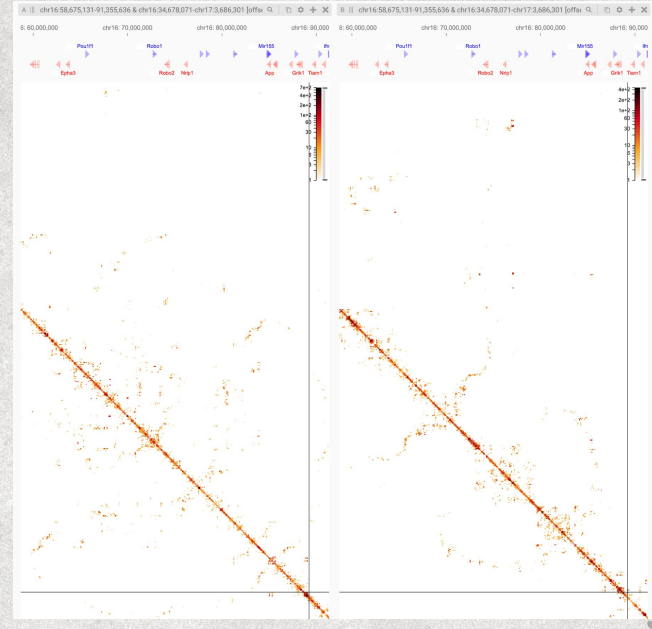
Figure 5B. Neural network predictions based on the original Hi-C map input.

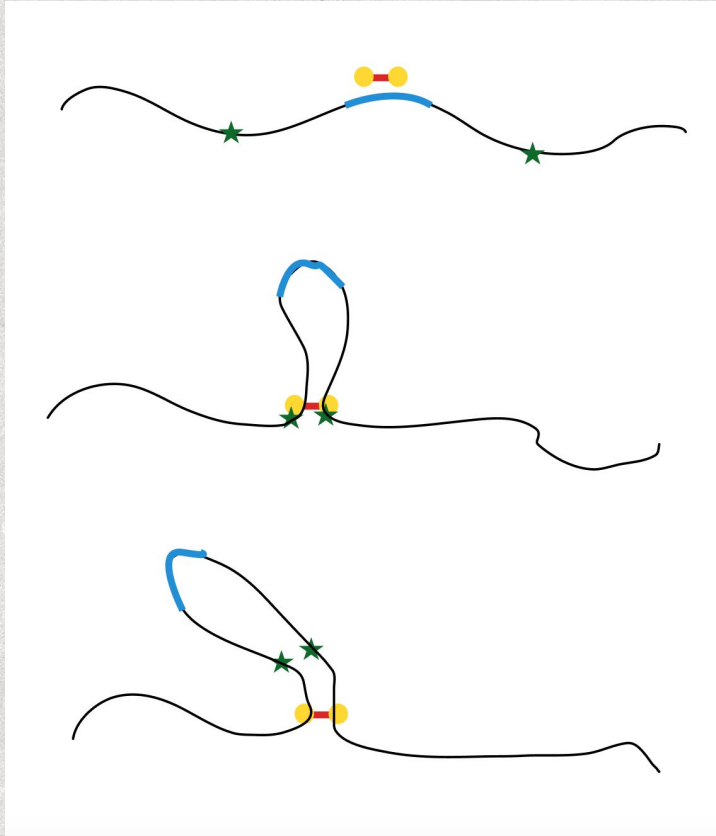


Single-cell Hi-C

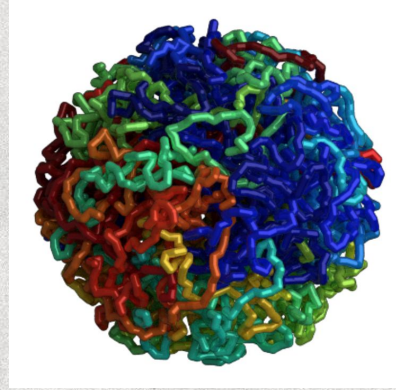
Do we see cohesin traces in single cell Hi-C maps?

Answer requires simulation of loop extrusion dynamics with the focus on the polymer/simulation parameters rather than cohesin dynamics. We twiddle the parameters in order to generate a detectable cohesin trace which should manifest in the single-cell Hi-C maps.





Cohesin trace



A detectable evidence of cohesion activity on the part of a genome. The lasting impact of cohesin activity visible in Hi-C. Cohesin brings the beads together and the longer they take to go back to the original 3D distance between them the more noticeable is the trace.



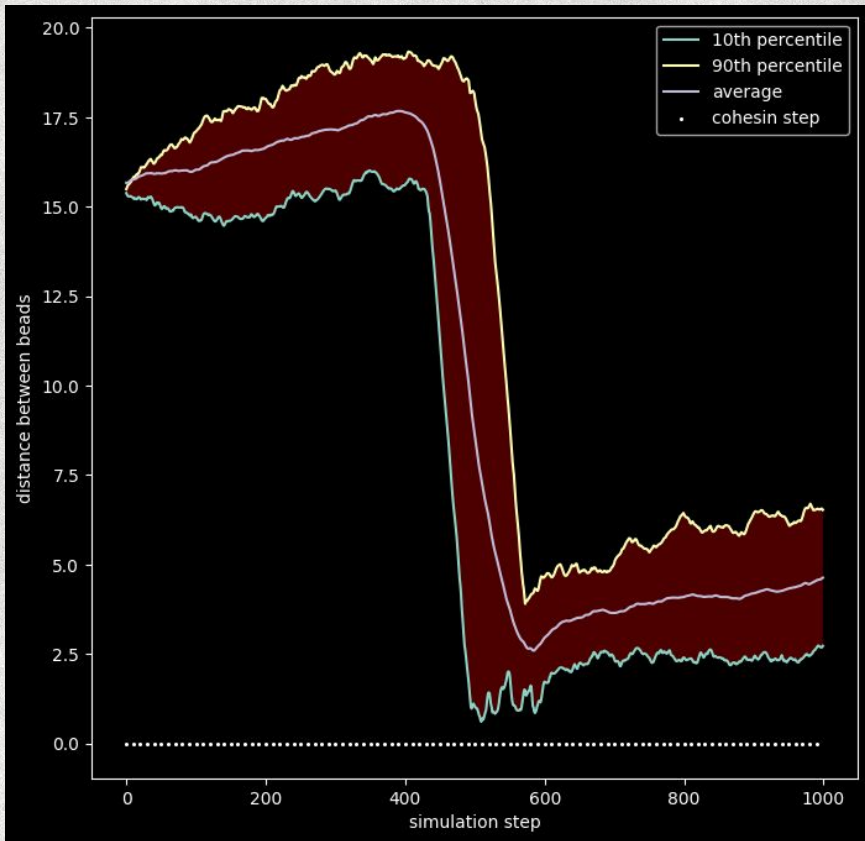
Structure of the simulation

Polymer & System structure

- We look at the polymer consisting of 10 systems each containing 1000 beads
- Each system has exactly one target site for cohesins, and due to specified cohesin parameters only one cohesin can land in one system
- We track the pair of beads in one system placed exactly in the middle of cohesin way (the beads are joined by cohesin and then let go so we can track them before and after their interaction with cohesin)

Variables & Readouts

- We are varying the polymer dynamics parameters: **density** of the simulation (i.e. how much space does the polymer have to expand), **collision rate** (the frequency of bead-solution collisions), and the cohesin-made **bond distance** (how close does cohesin bring the beads)
- To track the trace we save the positions of all of the beads in the system each simulation step (we set the number of simulation steps per cohesin step)
- Using the positions we make a graph of distance between chosen beads for each simulation step



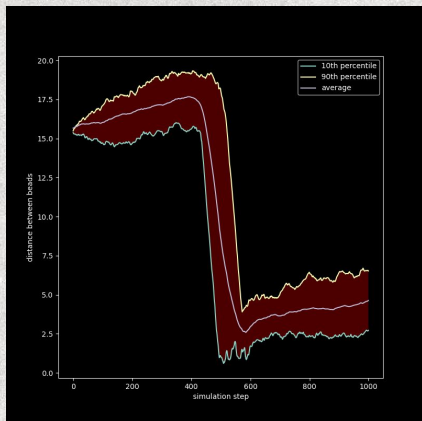
Cohesin trace analysis

The gap in the distance drop is dependent on the time cohesin landed on the target site.

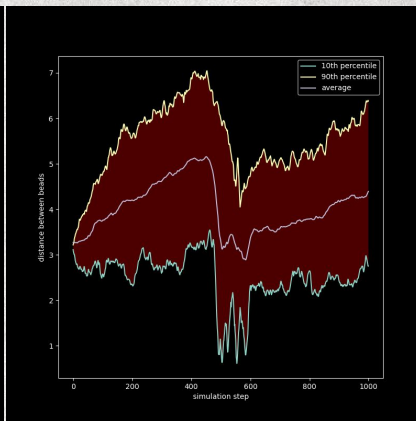
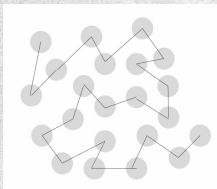
Since the beads in question are placed in the middle of cohesin path the distance drop happens roughly in the middle of the graph (so that we can see the original distance and the post-cohesin distance for comparison)



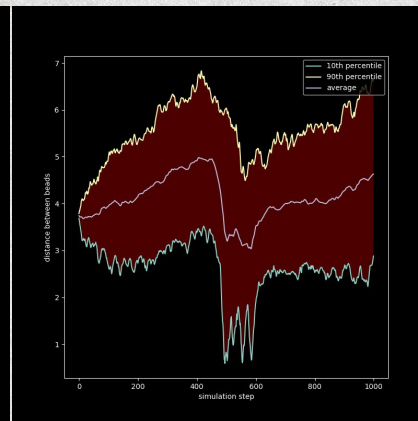
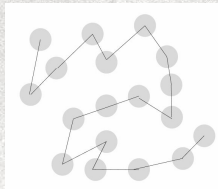
Changing density



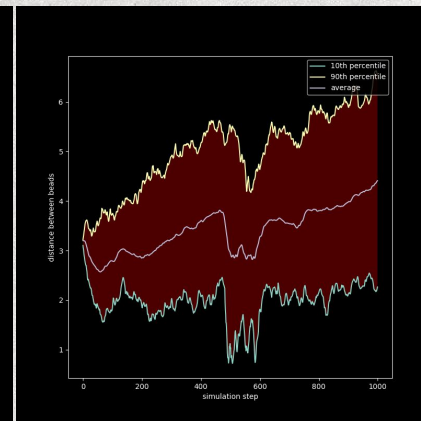
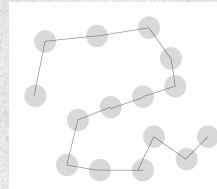
Density = 0.1



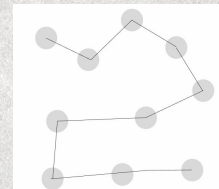
Density = 0.01



Density = 0.001

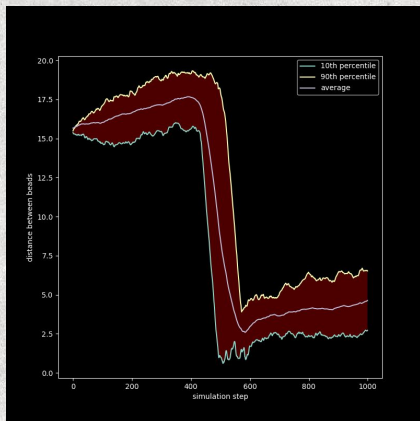


Density = 0.0001

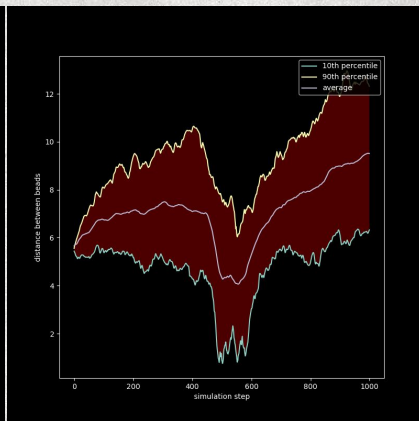
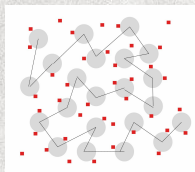




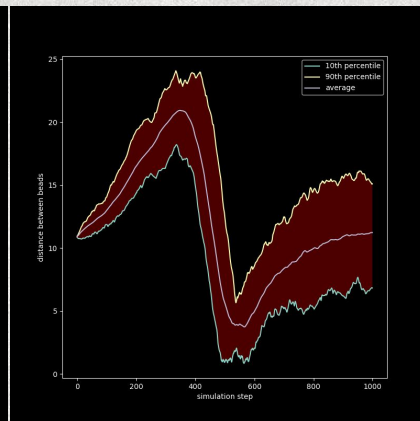
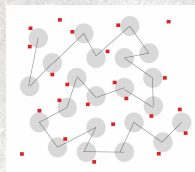
Changing collision rate



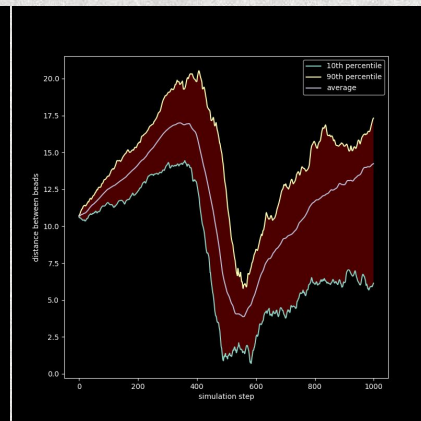
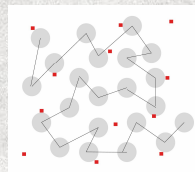
Collision rate = 0.3



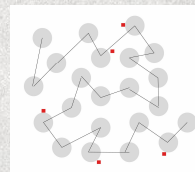
Collision rate = 0.03



Collision rate = 0.003

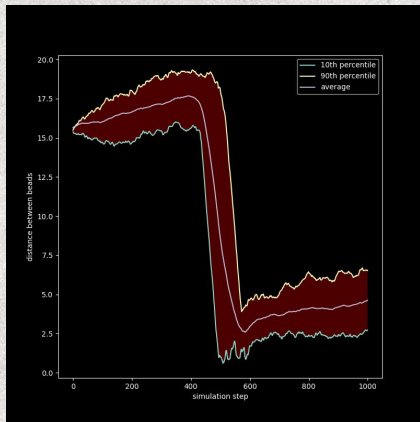


Collision rate = 0.0003

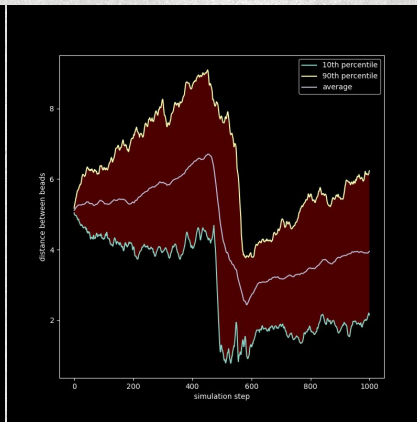
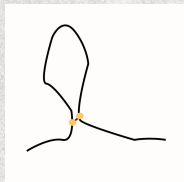




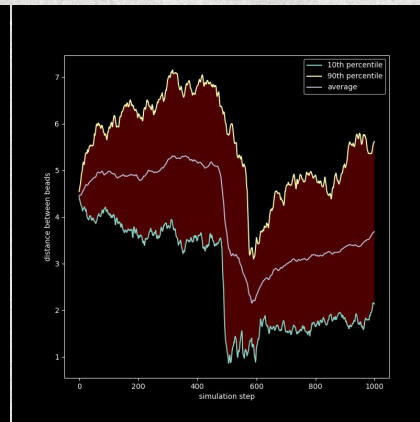
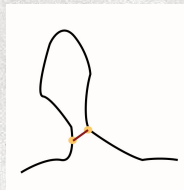
Changing bond distance



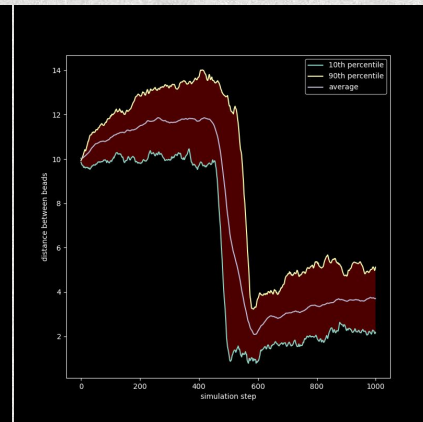
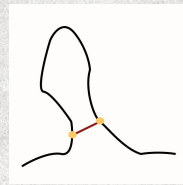
Bond distance = 0.5



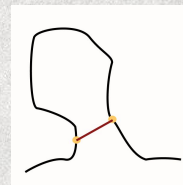
Bond distance = 1



Bond distance = 1.5



Bond distance = 2





Conclusions

Density

Density, perhaps, affects the cohesin trace the most, as the more dense polymer has less space and thus requires more time to return to the original level of separation between two beads

Collision rate

Does not change the traces as much with lower values, but the lower the rate the faster the trace dissipates.

Bond distance

Surprisingly, this parameter does not significantly affect the big picture of the trace, but it does change the “initial repulsion” of beads, even though other parameters affect the subsequent trace more.



Thanks!

Do you have any questions?

