

# The impact of bound protein on the sub-diffusion of a DNA locus

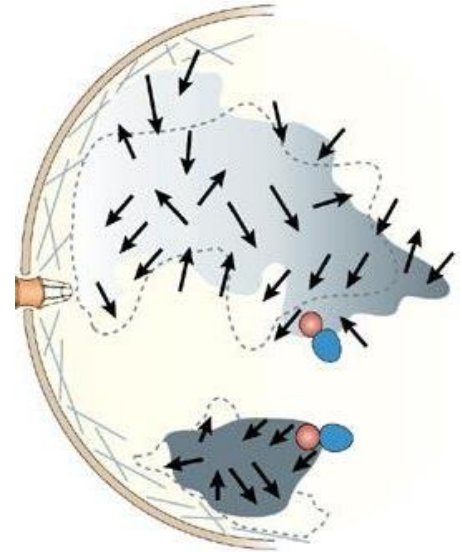
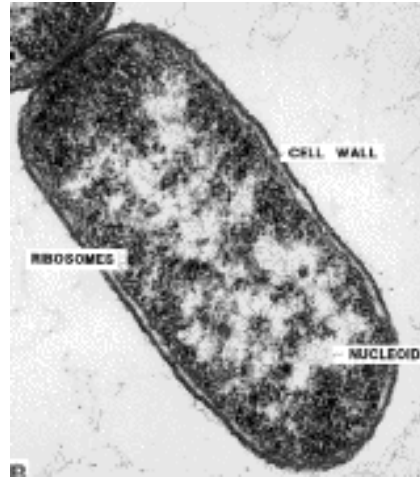
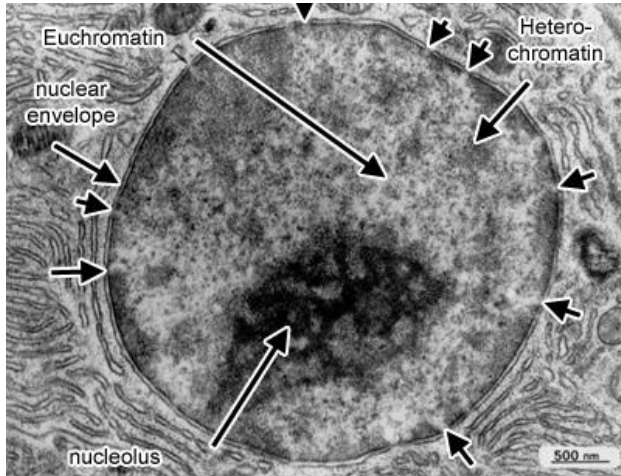
Andrew Luo

Mentors: Geoff Fudenberg & Maxim Imakaev



# Chromosomes function in a complex & dynamic environment

- A gene's expression dynamically depends on position in nucleus

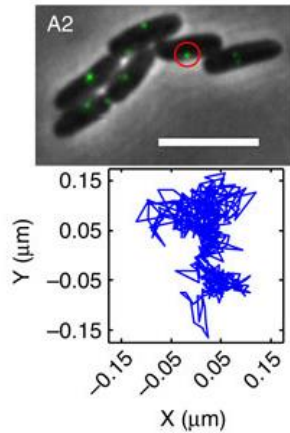


Eukaryotic nucleus, electron micrograph, Univ Leeds (Online Primer)

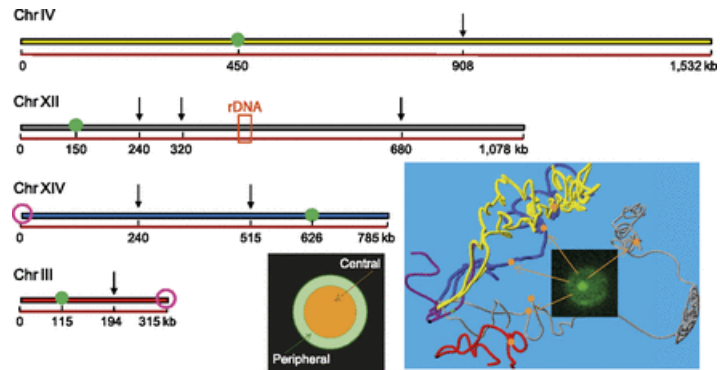
Bacterial cell, UCMP Berkeley

Lanctôt et al.  
*Nat Rev Gen* 2007

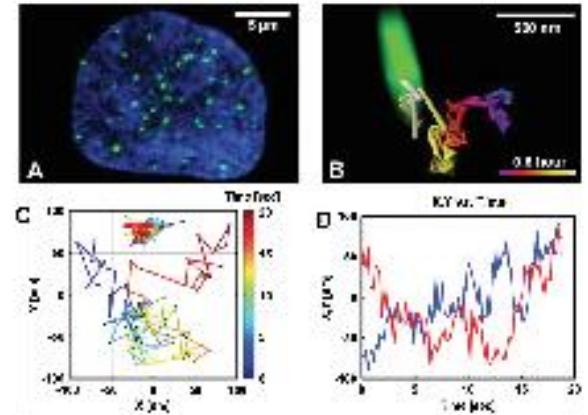
# Tracking DNA loci in living cells is a widely used method for investigating chromatin dynamics/organization



**E. coli:** Javer *et al*,  
*Nat. Comm.* 2014



**Yeast:** Hajjoul *et al*,  
*Genome Res.* 2013

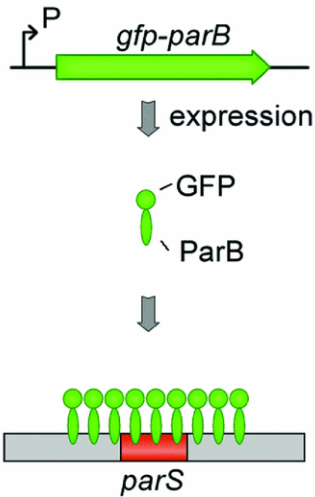


**Human:** Bronstein *et al*,  
*Phys Rev Lett* 2009

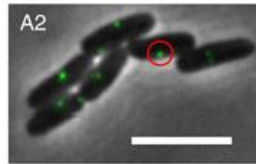
and many others !

# Tracking DNA loci allows the measurement of diffusive behavior (MSD vs. time)

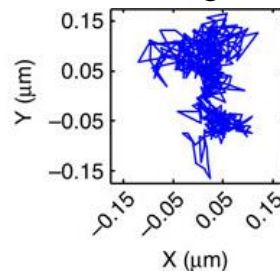
genetic engineering



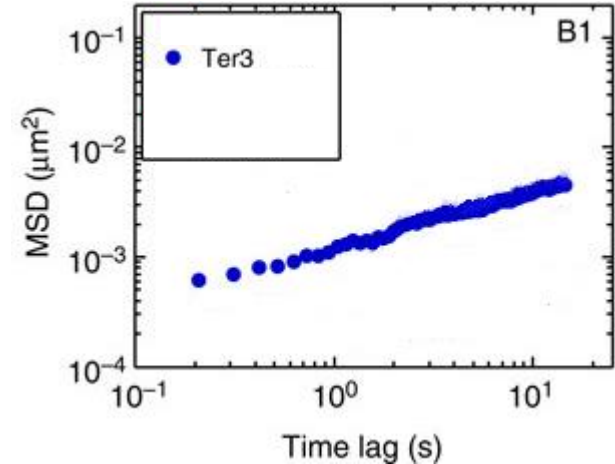
microscopy



Tracking over time



analysis



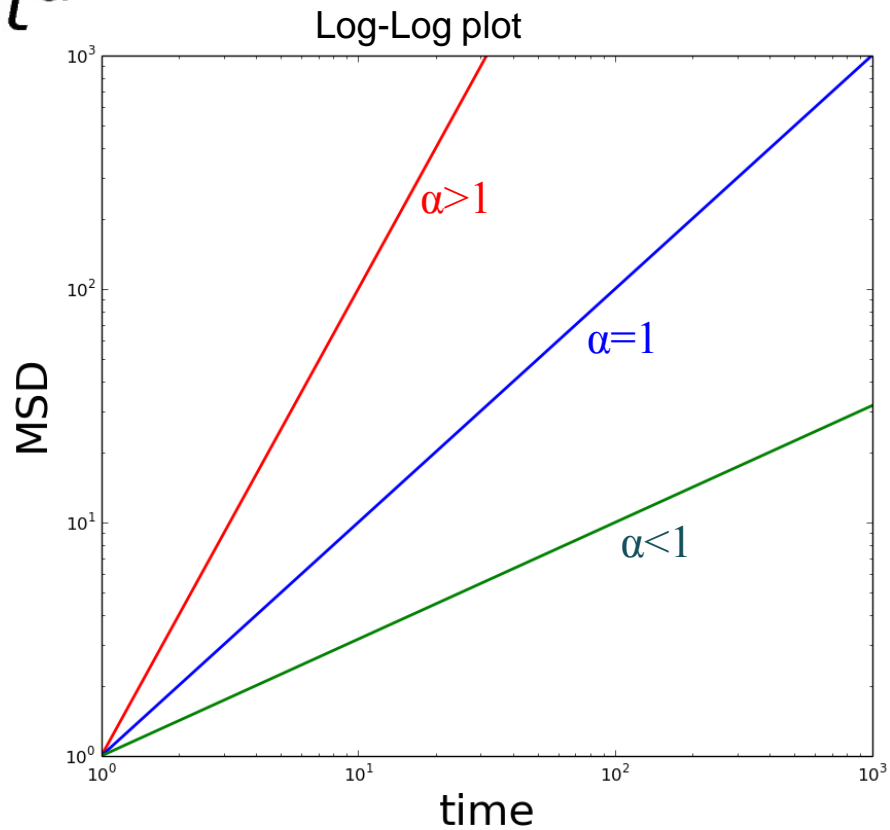
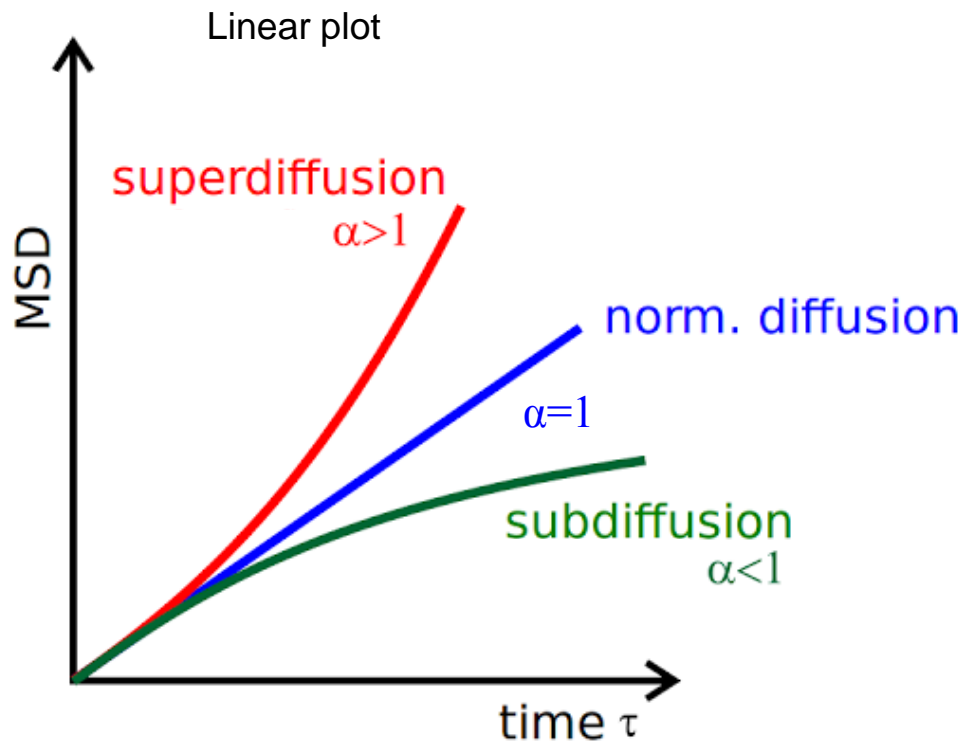
**ParB-parS tracking system:**

Wang *et al*, *Biochem. Soc. Trans.* (2008)

**Ecoli:** Javer *et al*, *Nat. Comm.* 2014

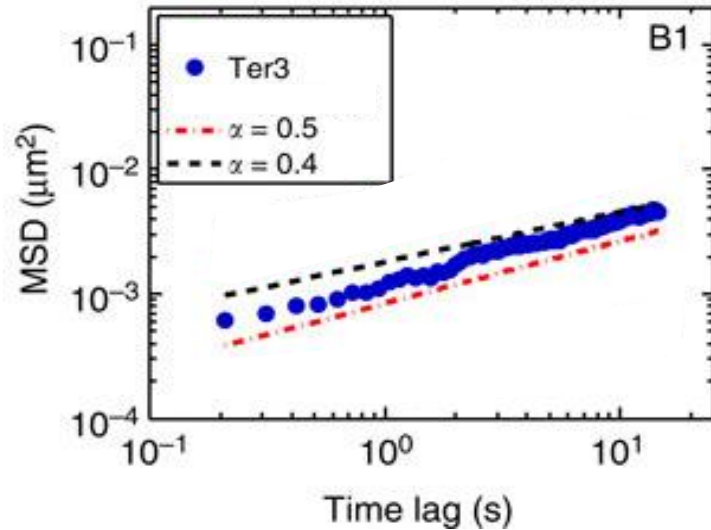
# MSD plots characterize diffusive behavior: regular vs. subdiffusion

$$MSD = Dt^\alpha$$



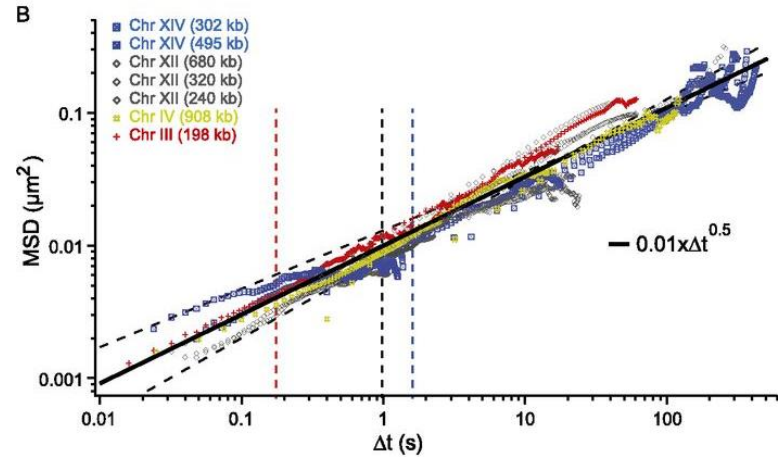
# Live-cell DNA tracking experiments observe subdiffusive behavior:

$$MSD = Dt^\alpha, \text{ alpha} < 1$$



**E. coli:  $\alpha \sim .4-.5$ :**

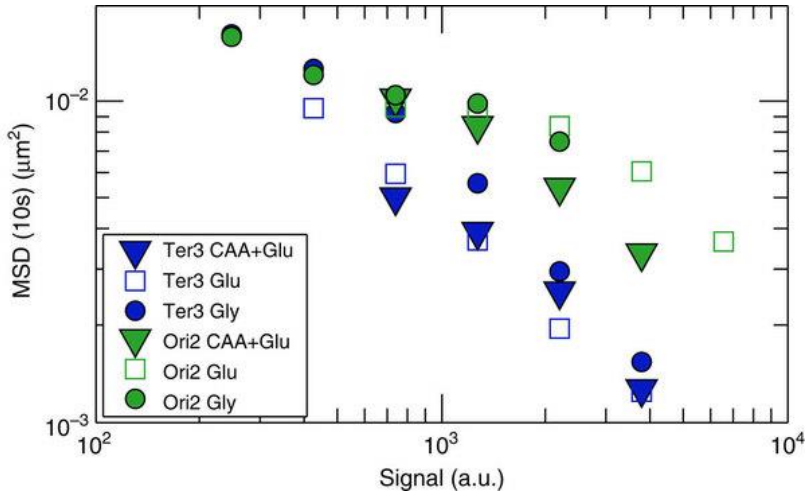
Javer *et al*, *Nat. Comm.* 2014



**Yeast,  $\alpha \sim .5$ :**

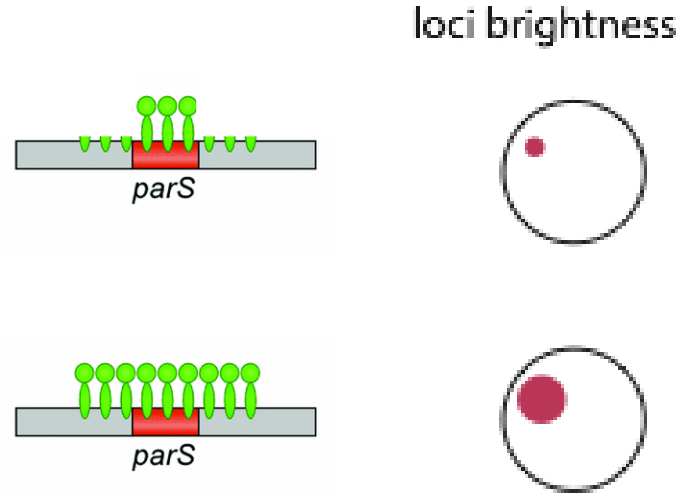
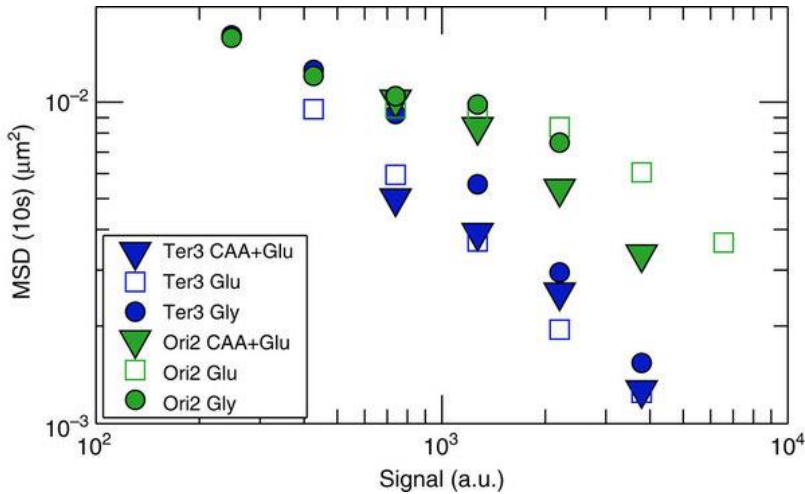
Hajjoul *et al*, *Genome Res.* 2013

# A surprising observation: MSD depends on brightness of tracked loci



- Certain experiments observed lower MSD for brighter tracked loci (i.e. those with more bound protein)

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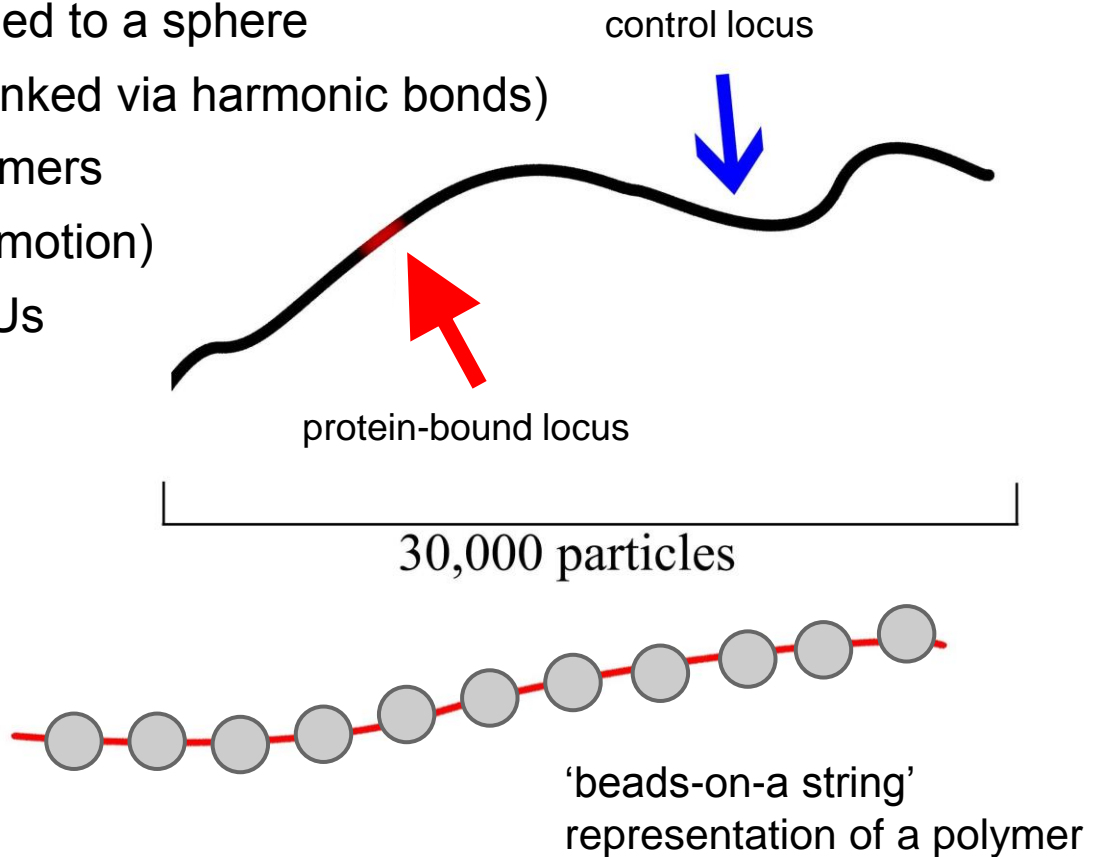


# How does bound protein affect observed subdiffusion??

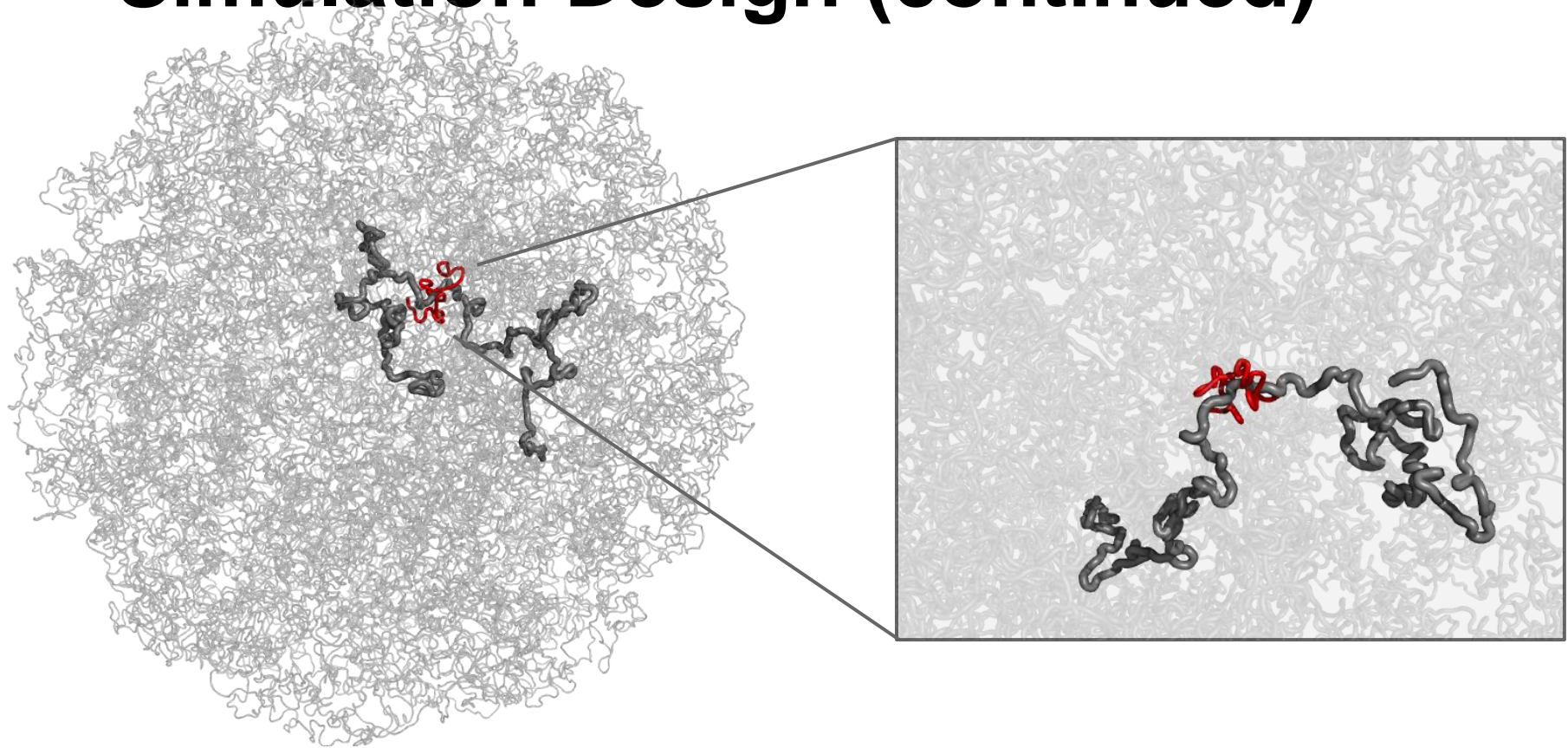
- Bound protein is commonly assumed not to affect DNA diffusion of a locus
- However, certain experiments observed lower MSD for loci with more bound protein
- Amount of bound protein (and RNA) varies dramatically genome-wide (e.g. RNA polymerase complex, condensin, etc.)
- **Approach**: test how, and to what extent, binding of protein affects diffusion in **simulations**

# Simulation Design: Locus Tracking *In Silico*

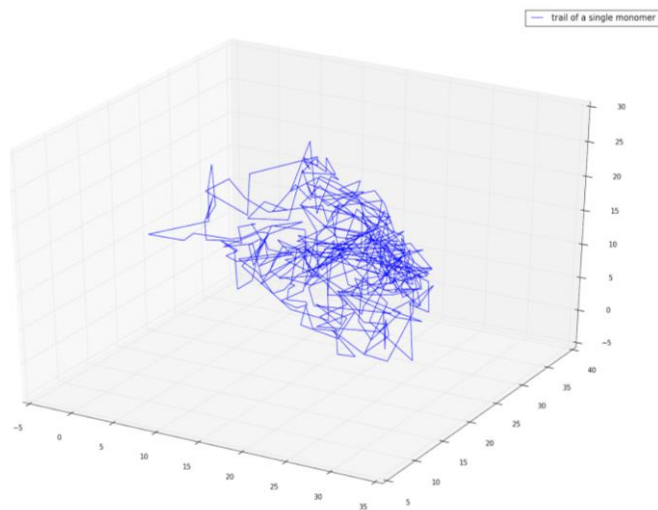
- 30,000 monomer polymer confined to a sphere
- ‘beads-on-a string’ (monomers linked via harmonic bonds)
- Repulsive forces between monomers
- Stochastic dynamics (Brownian motion)
- Simulated with OpenMM on GPUs
- Proteins are attached at  $\frac{1}{3}$  position
- Other  $\frac{2}{3}$  position is used as control



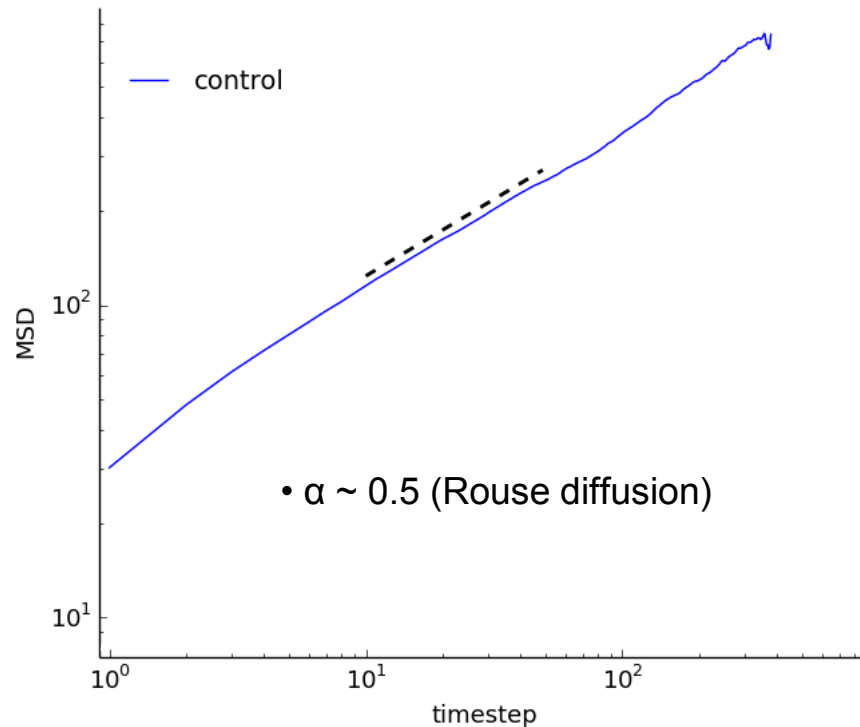
# Simulation Design (continued)



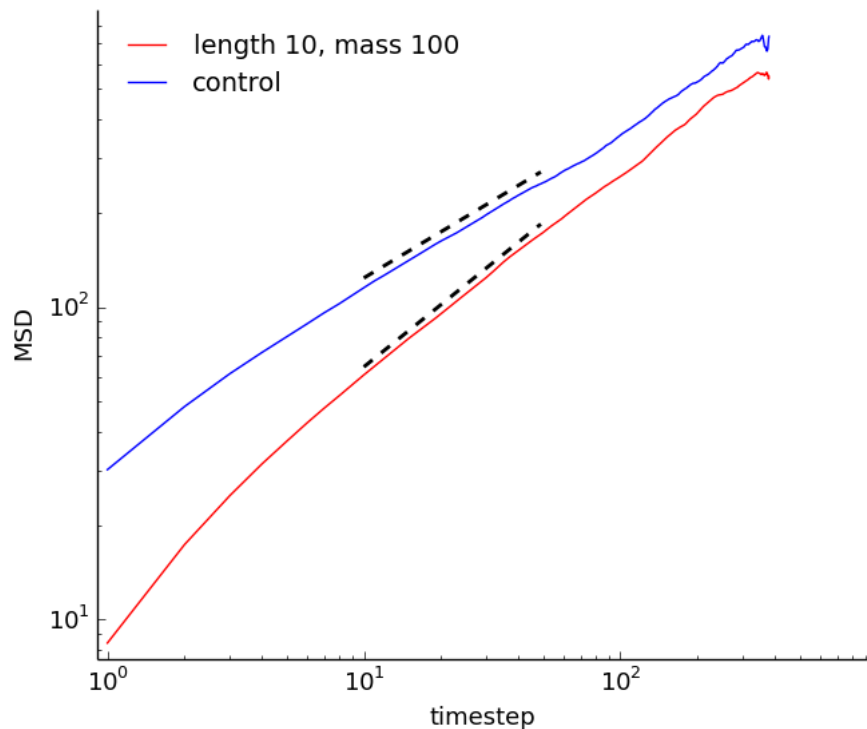
# Expected polymer subdiffusion observed at the control locus



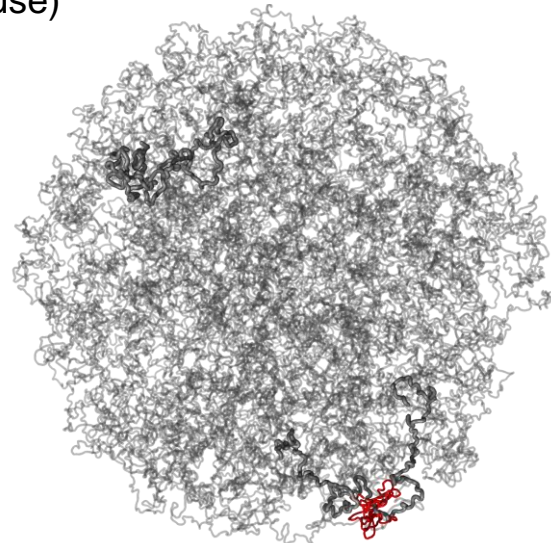
*in silico* locus tracking



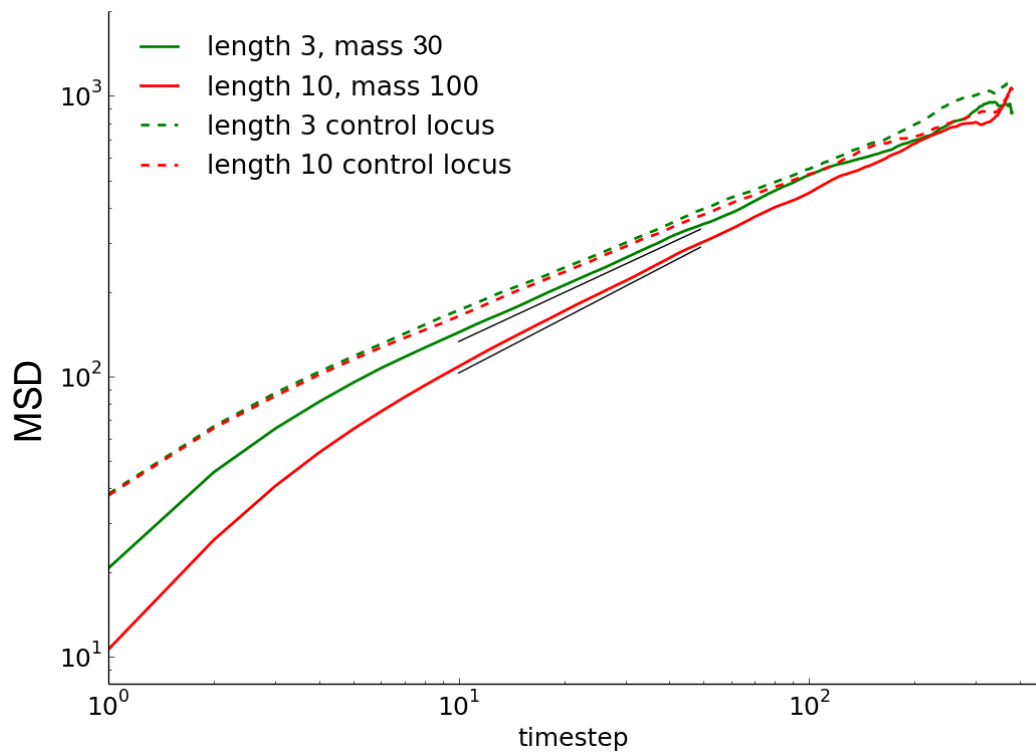
# Subdiffusion is drastically altered at the protein-bound locus



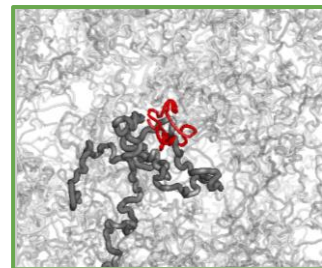
protein-bound locus:  $\alpha \sim 0.66$  (instead of Rouse)



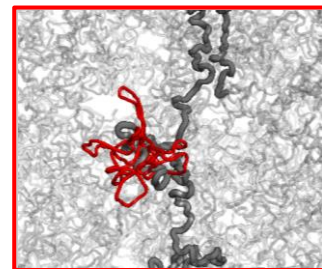
# Larger Probes Further Alter subdiffusion



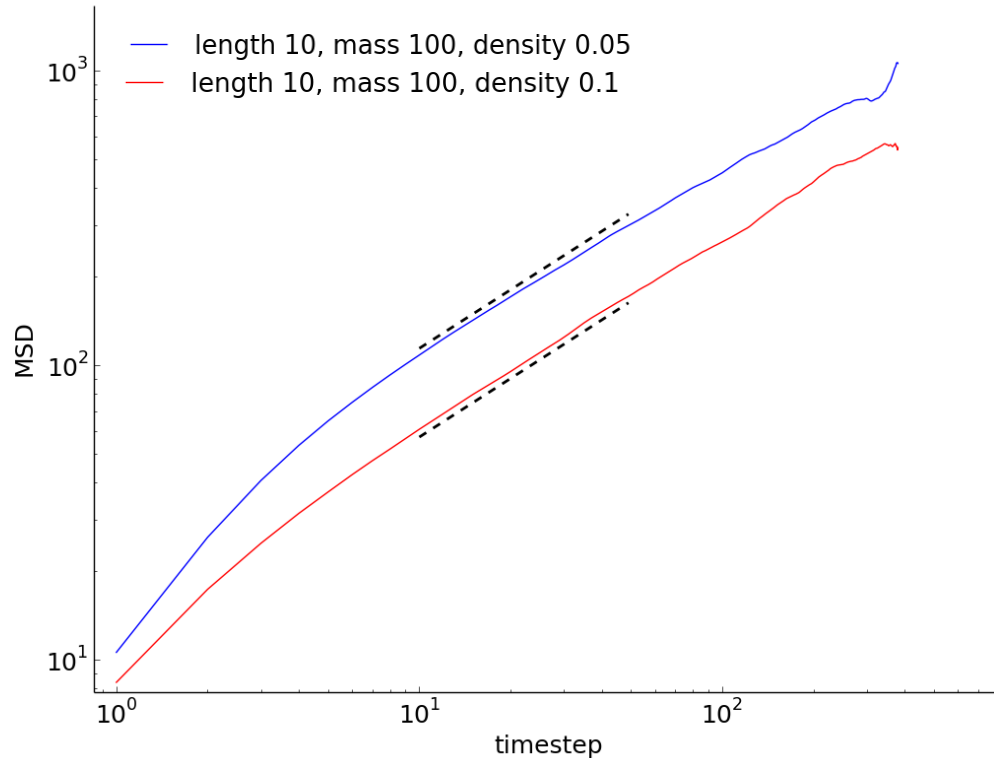
$\alpha \sim 0.53$



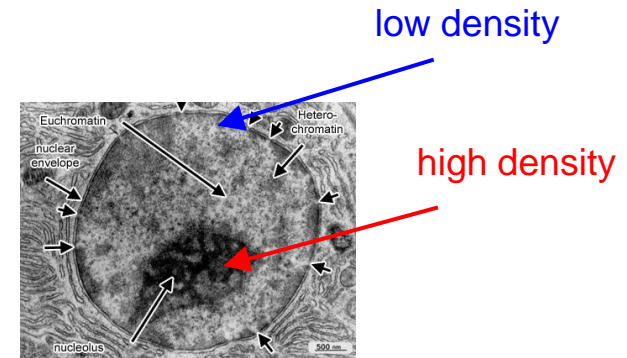
$\alpha \sim 0.66$



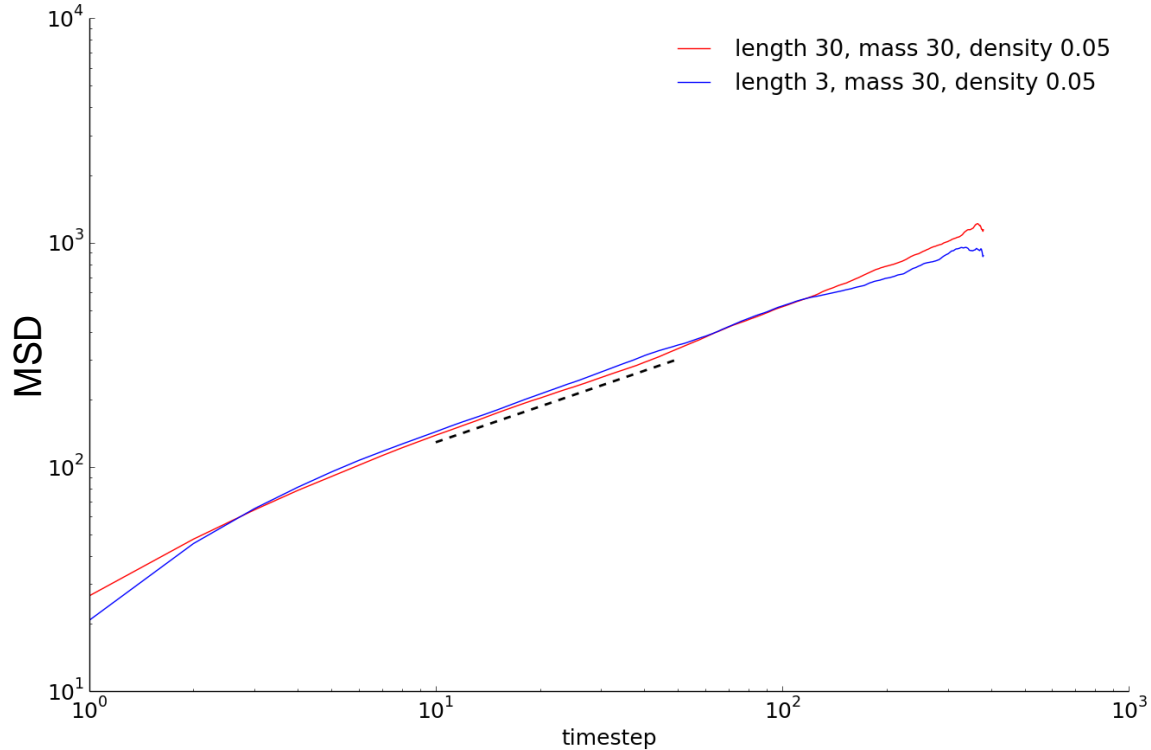
# Different density (i.e. different size of confining volume) has a minimal impact on $\alpha$



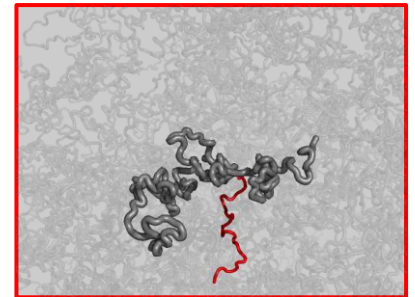
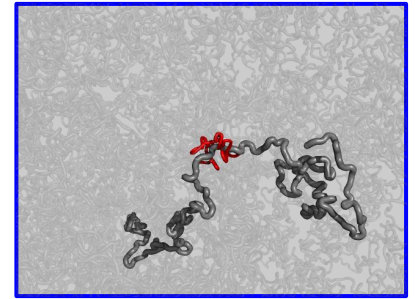
- Identical  $\alpha$
- Different subdiffusion coefficients



# Mass distribution has no effect on subdiffusion

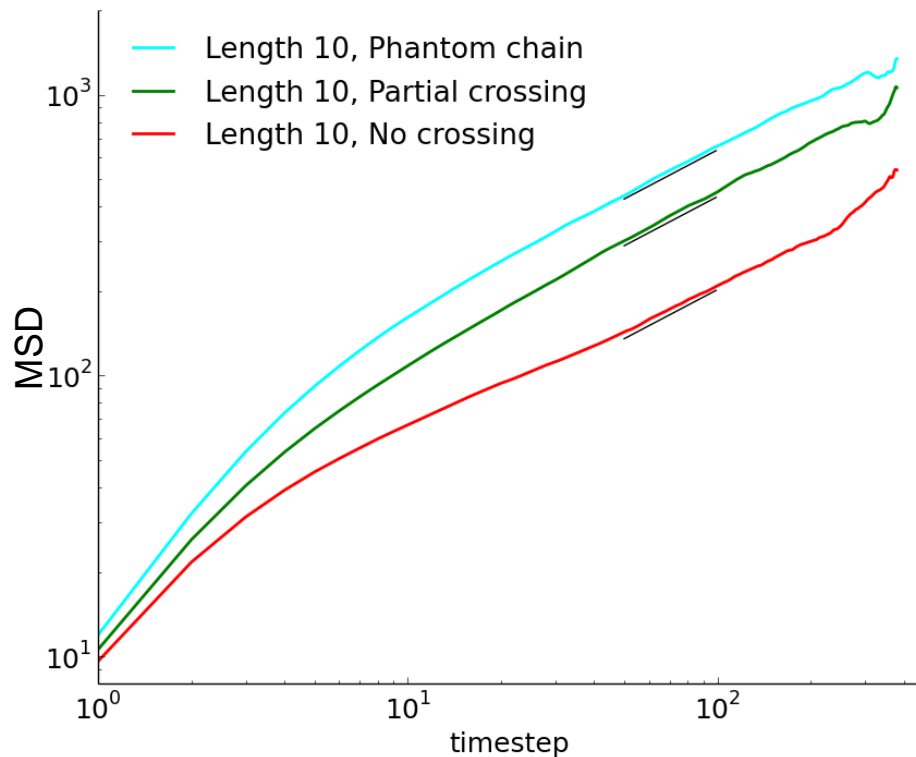


- Identical  $\alpha \sim 0.53$

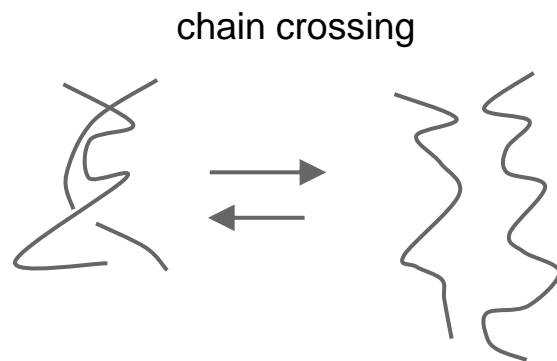




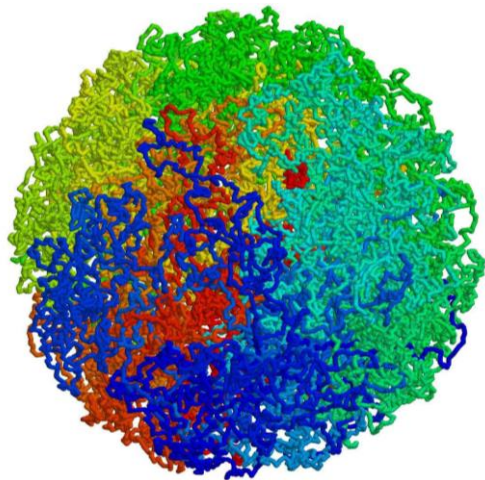
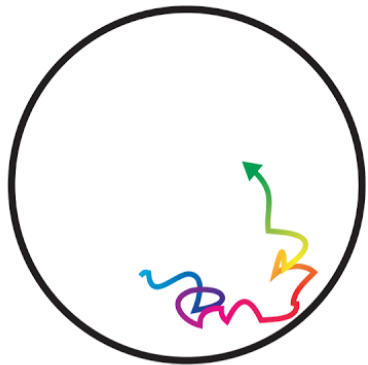
# Lower crossing frequency slows diffusion



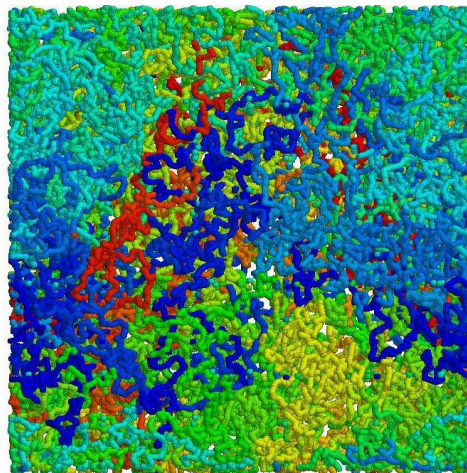
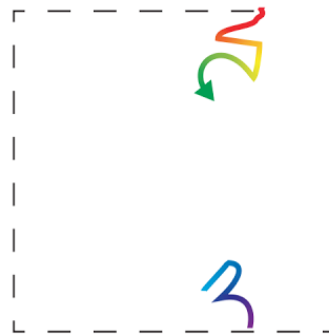
- Primarily affects the diffusion coefficient
- Very little effect on  $\alpha$



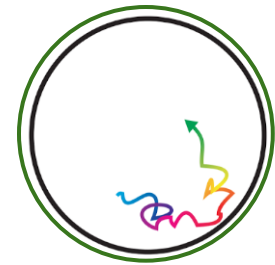
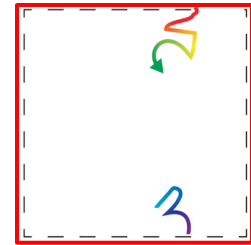
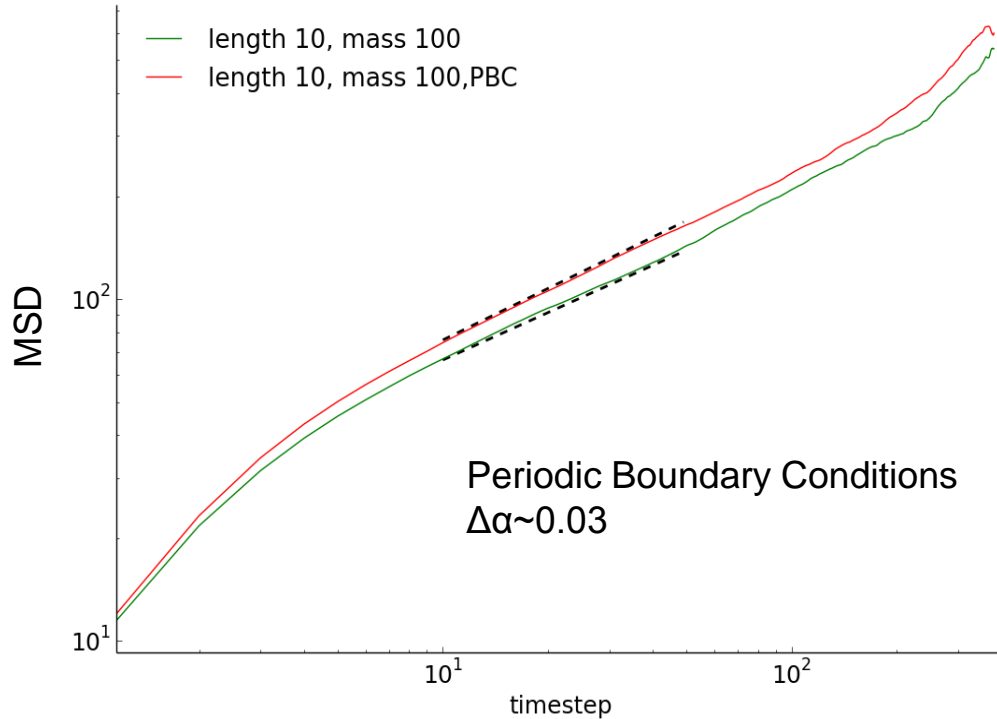
## Spherical Confinement



## Periodic Boundary Conditions ( $V_{\text{PBC}}=V_{\text{sphere}}$ )



# PBC has a small effect on observed subdiffusion



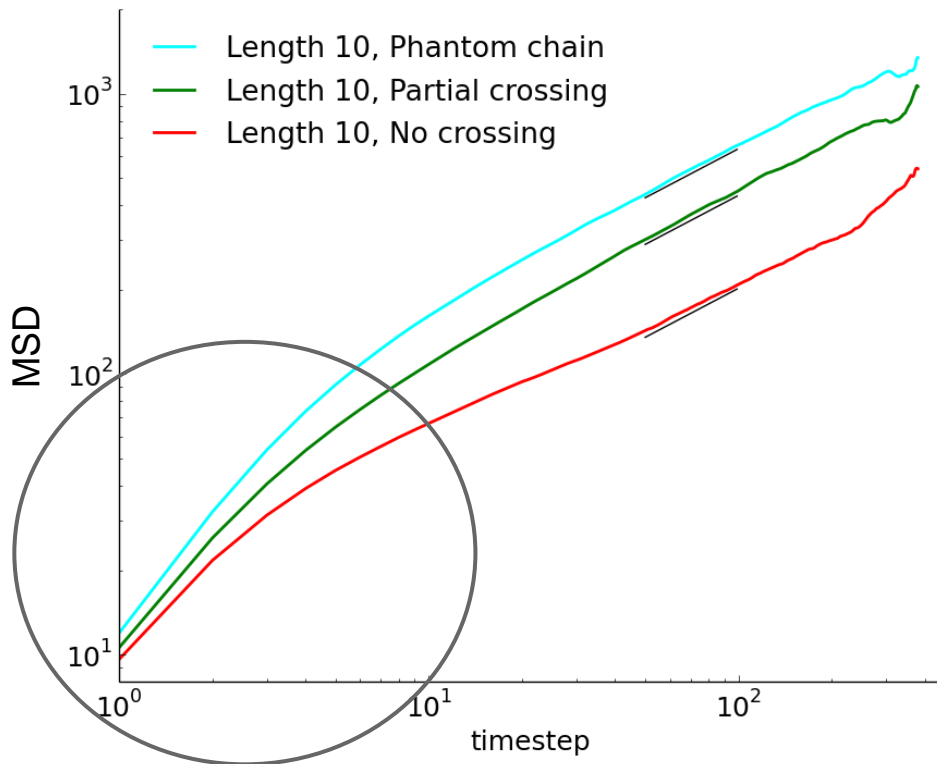
# Summary

- Loci with bound proteins had lower MSD at given times, but larger slopes for MSD vs. time
- More bound protein = stronger effect
- The distribution of the proteins and the density of the system have minimal effects
- More chain crossing accelerates the rate of diffusion, slower MSD not solely due to excluded volume
- On the timescales we tested, spherical confinement vs. PBC had minimal effects on our results

# Conclusions

- In simulations, bound protein slows diffusion at the bound locus
- This might explain observation of slower diffusion for brighter loci in experiments
- Future experiments can be designed to control for this
- **Caution is required:** when fitting homogeneous polymer models to diffusion of DNA loci, since chromosomes are non-uniform and may have vastly different amounts of protein bound at different loci

# Future directions



- Interesting behavior at start of diffusion
- This can be examined by collecting simulated data more frequently

# Acknowledgements

Geoff and Max  
Professor Mirny  
MIT PRIMES

