

# Advancing super resolution microscopy for quantitative in-vivo imaging of chromatin nanodomains

Clayton W. Seitz  
PhD Candidate (IUI), MS (UChicago)

August 31, 2024

# Outline

Introduction to fluorescence nanoscopy

Contemporary approaches to fluorescence nanoscopy

Enhanced nanoscopy with deep generative models

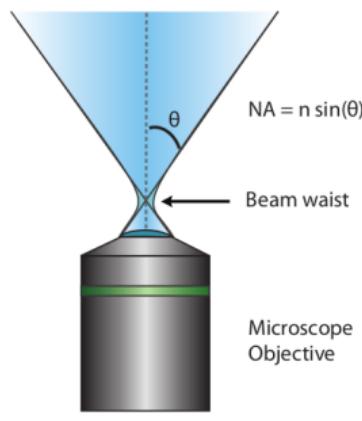
Enhanced nanoscopy with a single photon avalanche diode array

Super-resolution of nucleosome nanodomains *in-vivo*

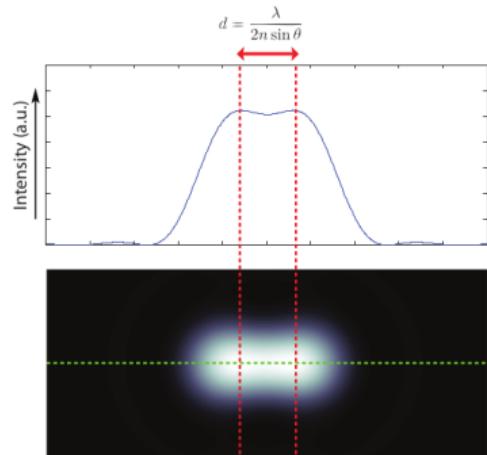
## Introduction to fluorescence nanoscopy

# Fluorescence microscopy and the diffraction limit

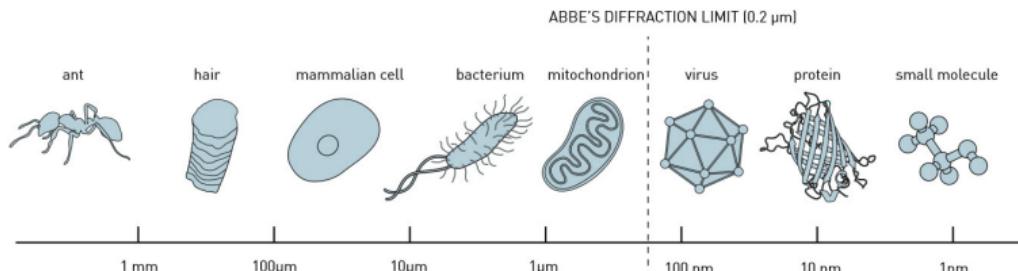
Minimal resolvable distance  $d \sim \lambda$



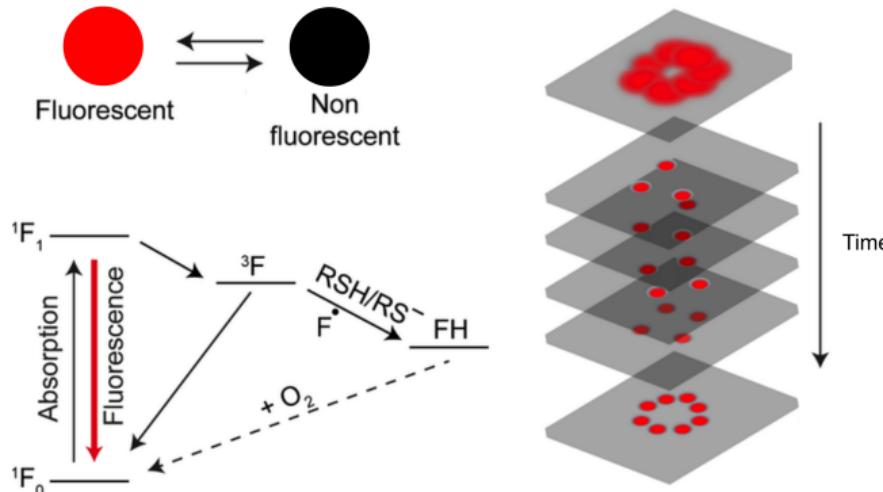
(a)



(b)

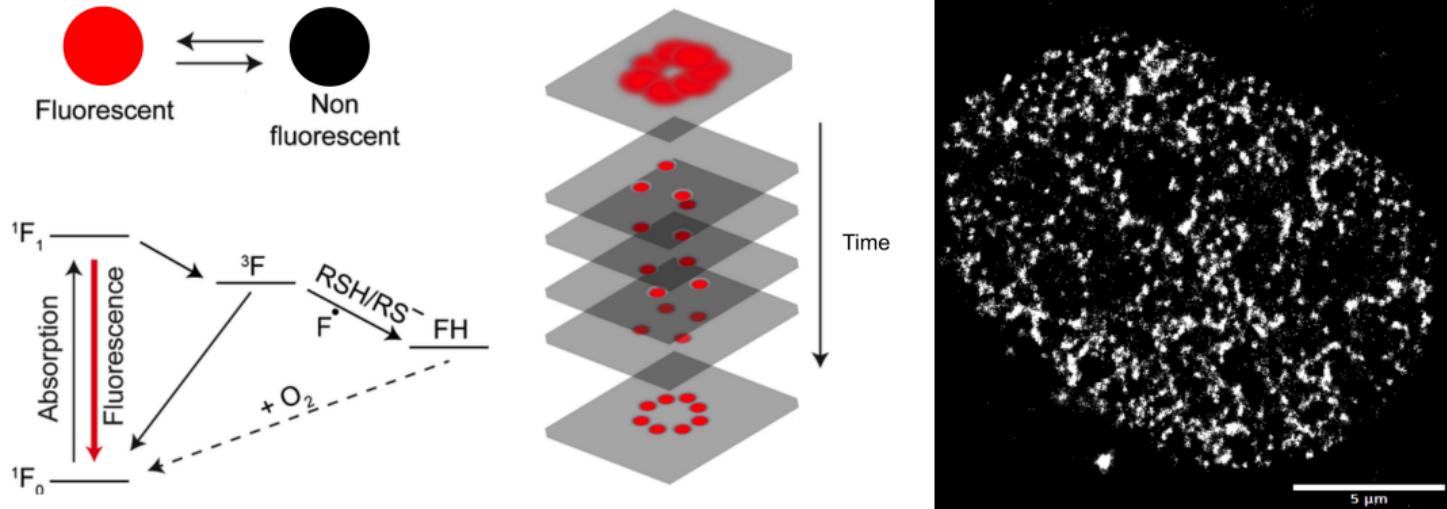


# Stochastic optical reconstruction microscopy (STORM)



- ▶ STORM and similar nanoscopy techniques are diffraction-unlimited
- ▶ Photoswitching enables resolution of emitters below the diffraction limit

# Stochastic optical reconstruction microscopy (STORM)



- ▶ STORM and similar nanoscopy techniques are diffraction-unlimited
- ▶ Photoswitching enables resolution of emitters below the diffraction limit

# Nanoscopy by localizing isolated fluorescent emitters

Modeling the point spread function permits sub-pixel localization

$$\mu_k = i_0 \int \int O(u, v) dudv + \lambda$$

$$i_0 = g_k \eta \zeta \Delta$$

$g_k$  – pixel gain

$\eta$  – quantum efficiency

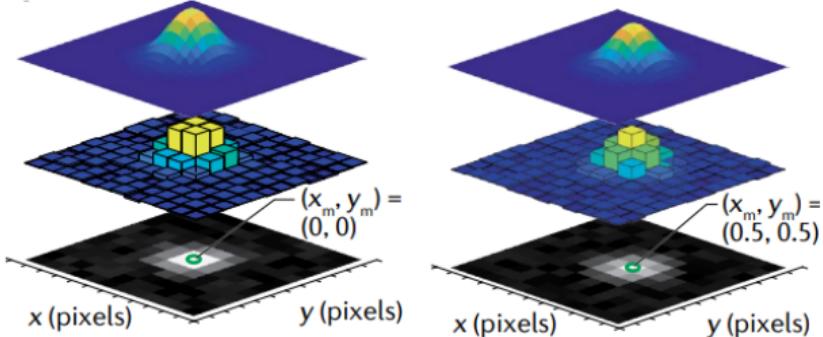
$\zeta$  – photon emission rate

$\Delta$  – exposure time

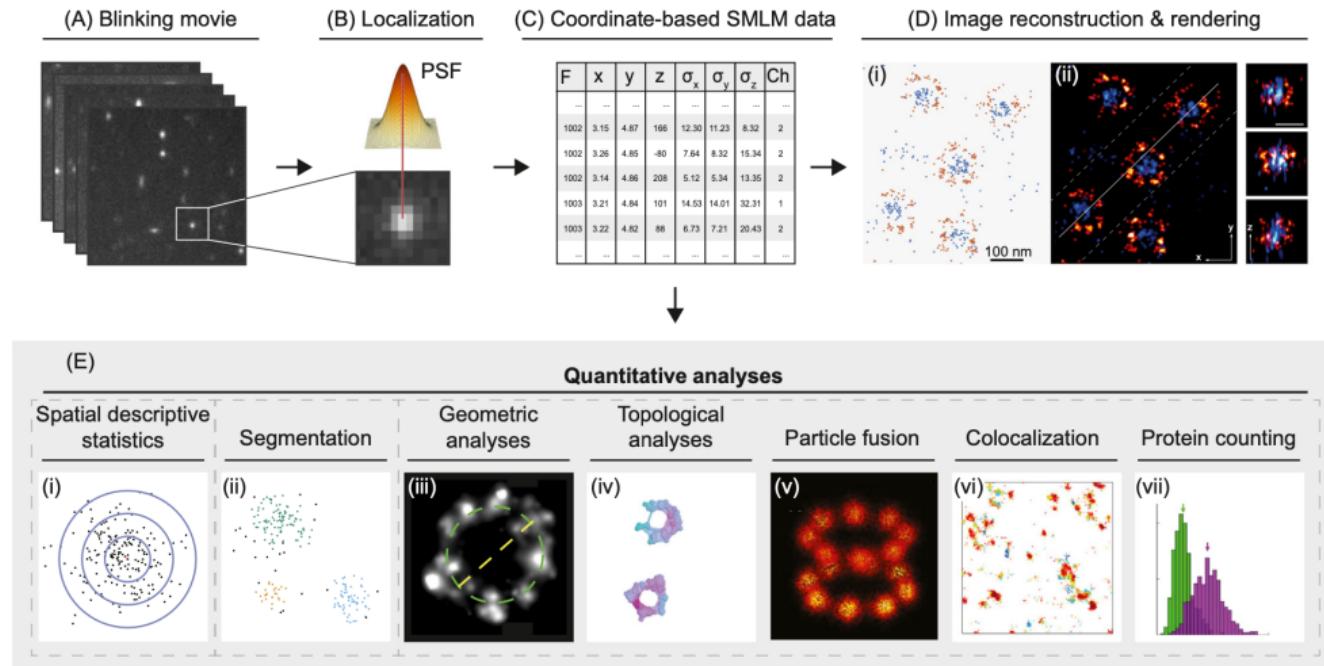
$\lambda$  – background rate

Maximum likelihood localization:

$$\theta^* = \operatorname{argmax}_{\theta} \prod_k p(\mathbf{x}_k | \theta) = \operatorname{argmin}_{\theta} - \sum_k \log p(\mathbf{x}_k | \theta)$$

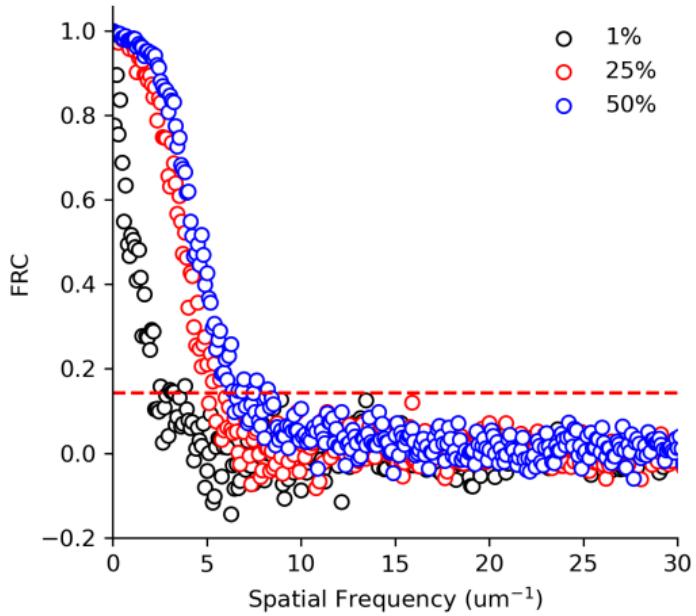
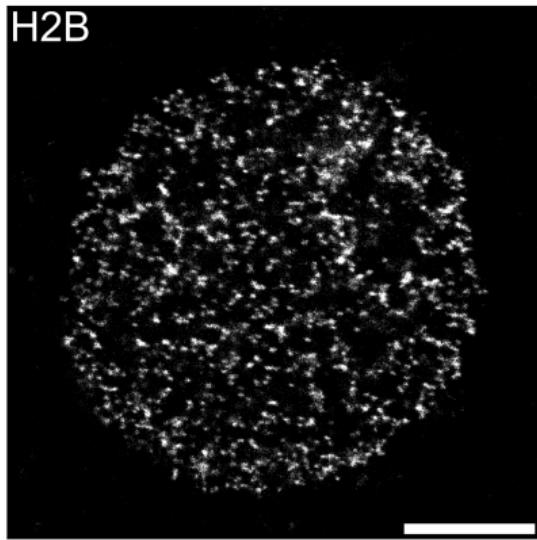


# Applications of single molecule localization microscopy



Wu et al. Trends in Cell Biology. 30 (2020)

# How do we define resolution in localization microscopy?



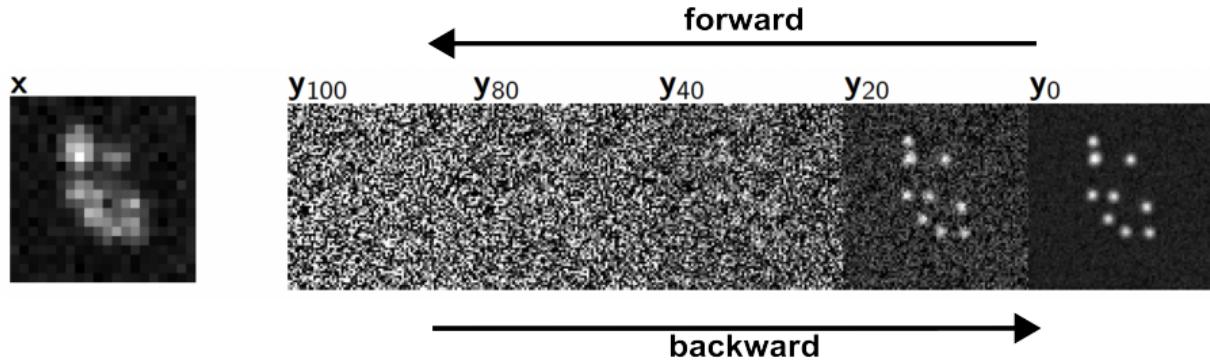
More samples → higher spatial/temporal resolution  
How to relax the density limit in localization microscopy?

## Contemporary approaches to fluorescence nanoscopy

# Bayesian image restoration with diffusion models

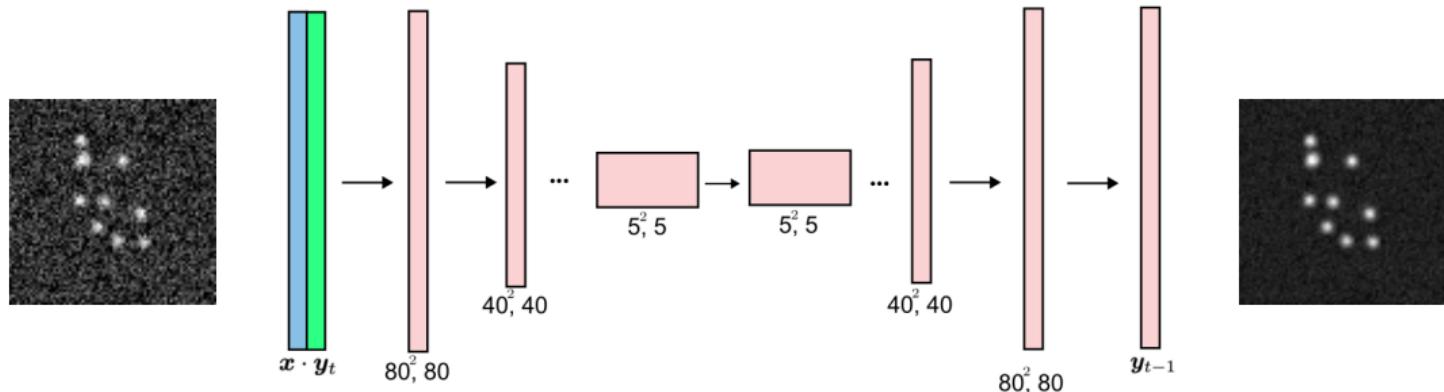
Inference of a high resolution image  $\mathbf{y}$  from low resolution  $\mathbf{x}$  is approached by modeling a distribution  $p_\psi(\mathbf{y}|\mathbf{x})$  with a diffusion model

$$q(\mathbf{y}_t | \mathbf{y}_{t-1}) = \mathcal{N}\left(\sqrt{1 - \beta_t} \mathbf{y}_{t-1}, \beta_t I\right)$$



$$p_\psi(\mathbf{y}_{t-1} | \mathbf{y}_t, \mathbf{x}) = \mathcal{N}(\mu_\psi(\mathbf{y}_t, \gamma_t), \beta_t I)$$

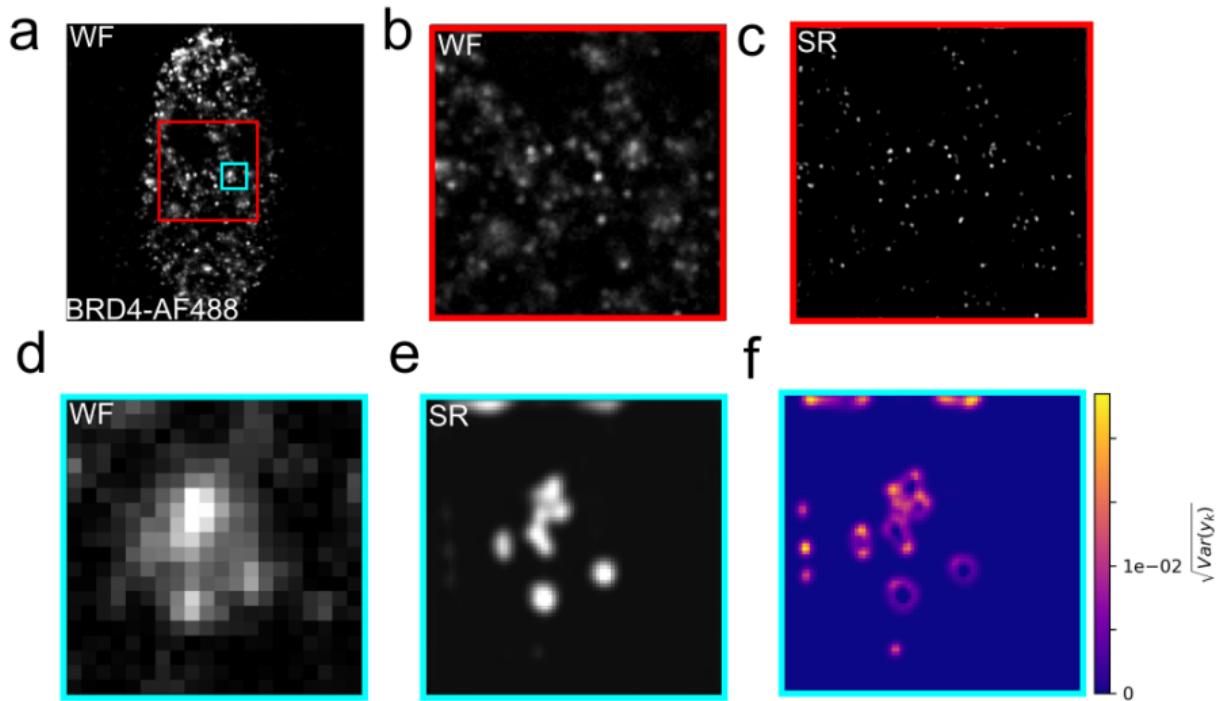
# Bayesian image restoration with diffusion models



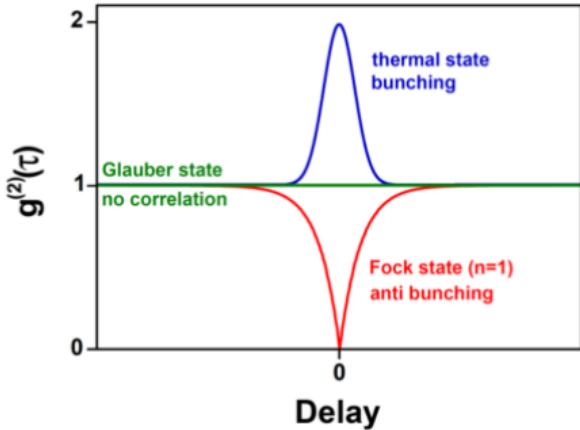
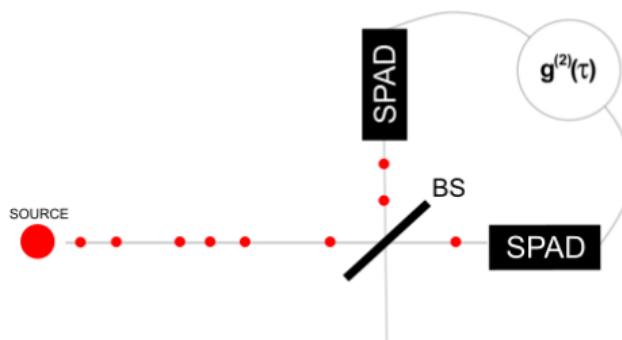
A deep neural network estimates the gradient of the reverse process

$$\mathbf{y}_{t-1} = \frac{1}{\sqrt{1 - \beta_t}} (\mathbf{y}_t + \beta_t s_\psi(\mathbf{y}_t)) + \sqrt{\beta_t} \xi \quad \xi \sim \mathcal{N}(0, I)$$

# Super resolution of BRD4 in a HeLa cell



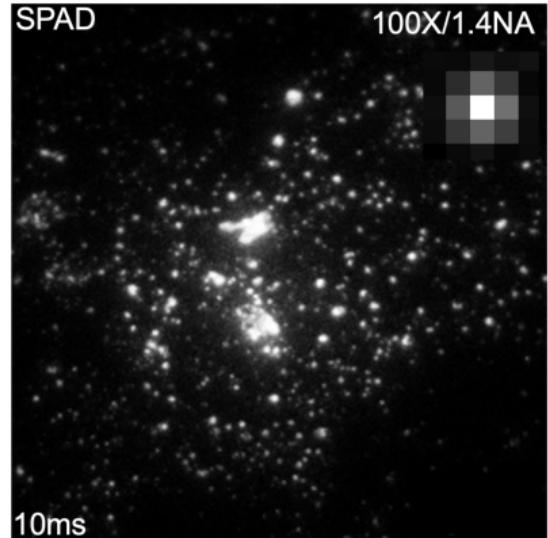
# The Hanbury Brown and Twiss Effect



$$g^{(2)}(\tau) = \frac{\langle n(t)n(t + \tau) \rangle}{\langle n(t) \rangle^2}$$

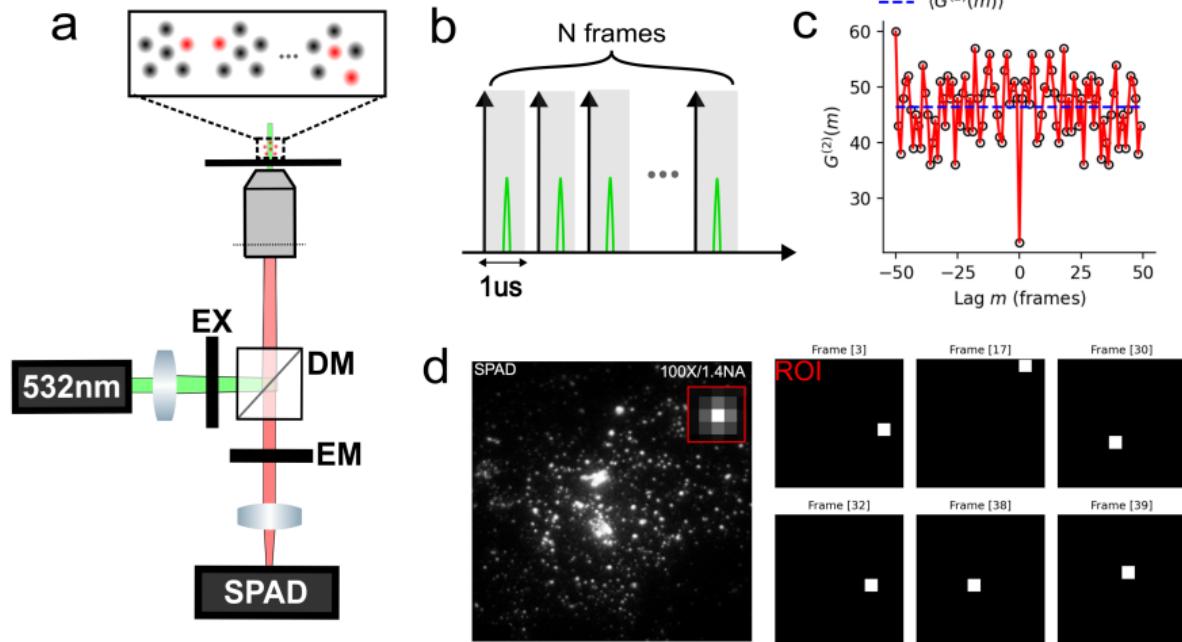
- ▶ Single photon sources (such as a fluorescent dye) exhibit antibunching
- ▶ Magnitude of  $g^{(2)}(0)$  "dip" depends on the number of fluorescent emitters
- ▶ Provides a means of counting fluorescent emitters

# Widefield photon counting with a SPAD array

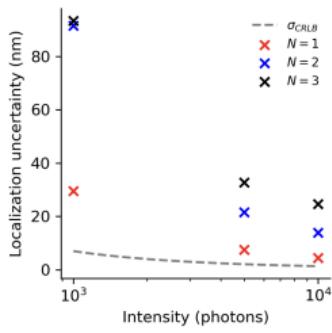
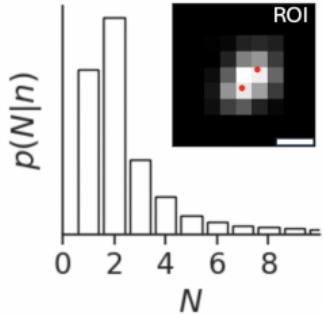
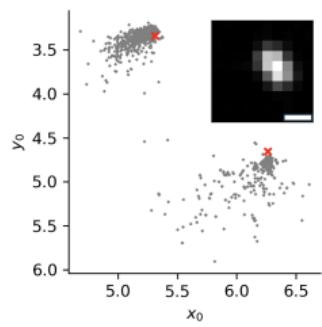
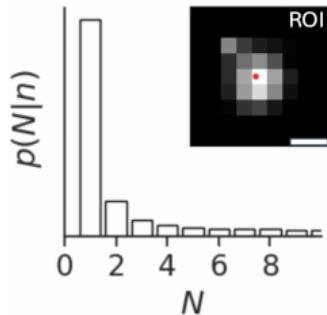


- ▶ Spatial resolution of photon counts
- ▶ Fast exposures as low as 20 nanoseconds

# Imaging Qdot655 photon by photon



# Constrained multi-emitter localization with photon counting

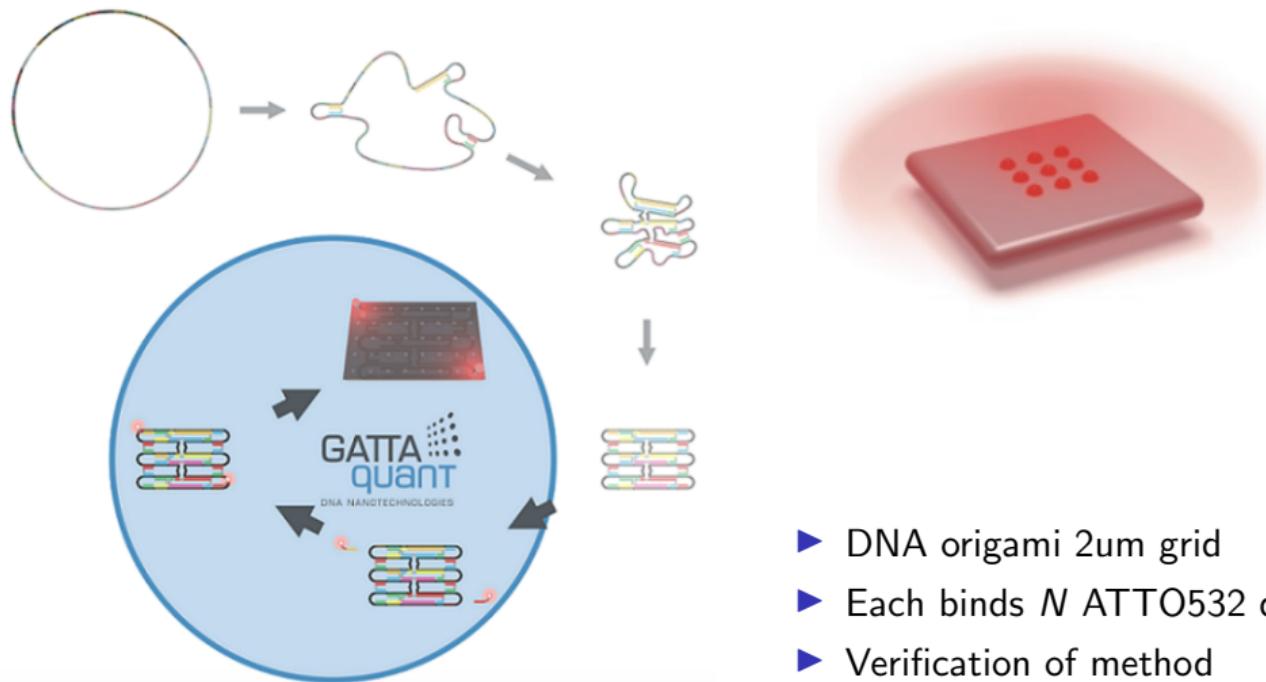


Posterior distribution on  $(N, \zeta)$ :

$$p(N, \zeta|n) \propto p(n|N, \zeta)p(\zeta)$$

- ▶ MAP estimation on  $N$
- ▶ Parameterization of multi-emitter fitting
- ▶ Approaches  $\sigma_{\text{CRLB}}$  for  $N = 1$

## Counting ATTO532 dye bound to DNA origamis

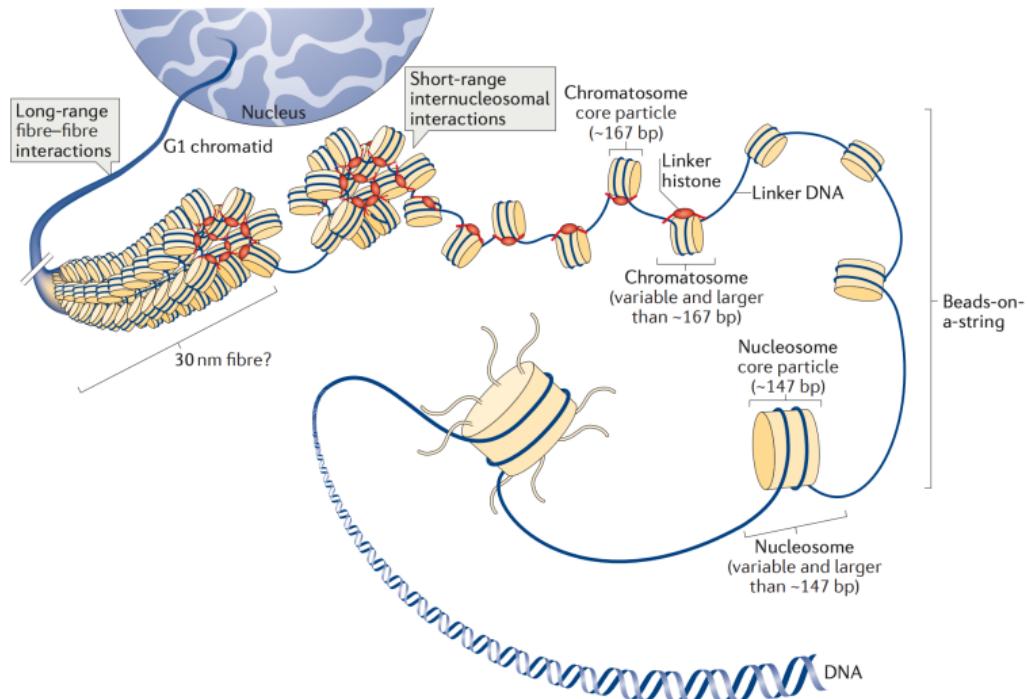


- ▶ DNA origami 2um grid
- ▶ Each binds  $N$  ATTO532 dyes
- ▶ Verification of method

Courtesy of GATTAquant DNA Nanotech

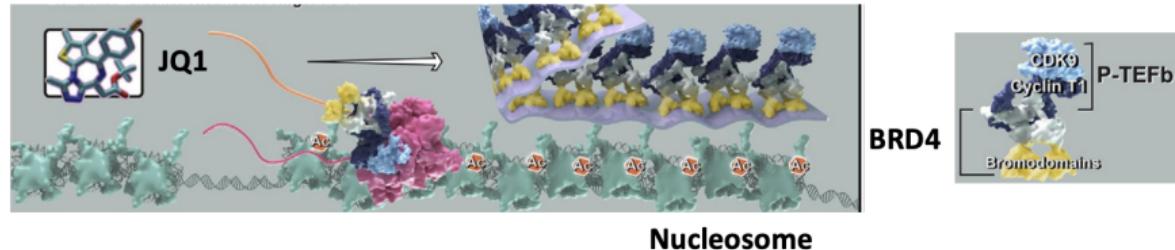
## Super-resolution of nucleosome nanodomains *in-vivo*

# Hierarchical structure of chromatin

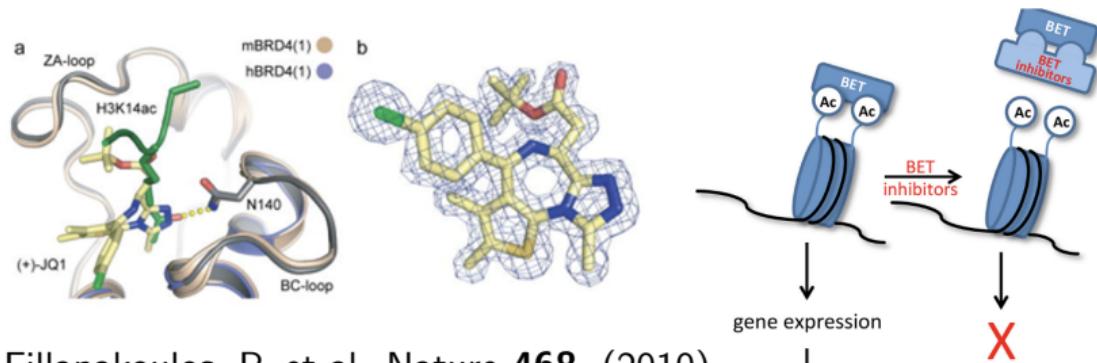


Fyodorov, D. et al. Nat Rev Mol Cell Biol **19**, (2018).

# Bromodomain protein 4 (BRD4) binds acetylated chromatin

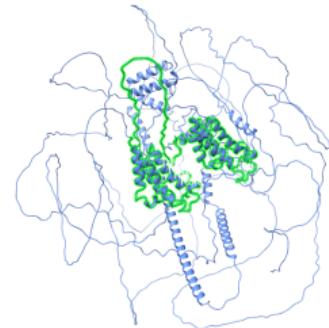
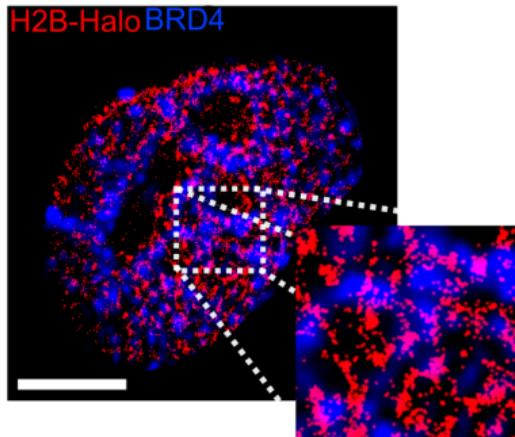


Zheng, B. et al. Molecular Cell **16**, (2023).



Fillapakoulos, P. et al. Nature **468**, (2010).

# BRD4 mutations to probe effects on chromatin structure



AlphaFold BRD4 1-1362aa

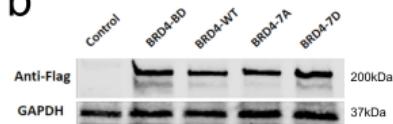


# BRD4 binding efficacy controls colocalization with nucleosome nanodomains

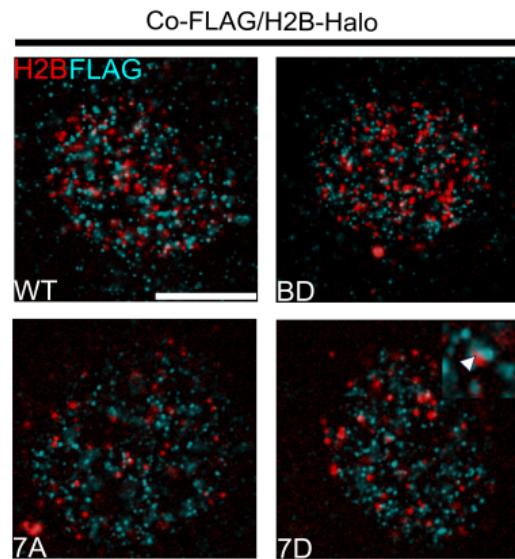
a



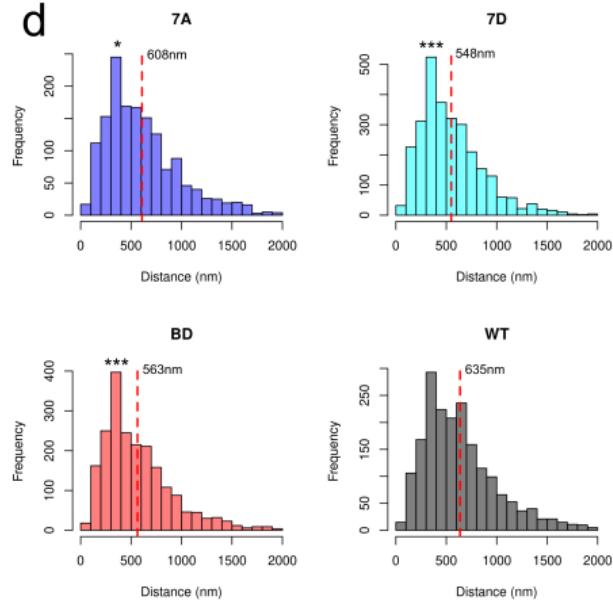
b



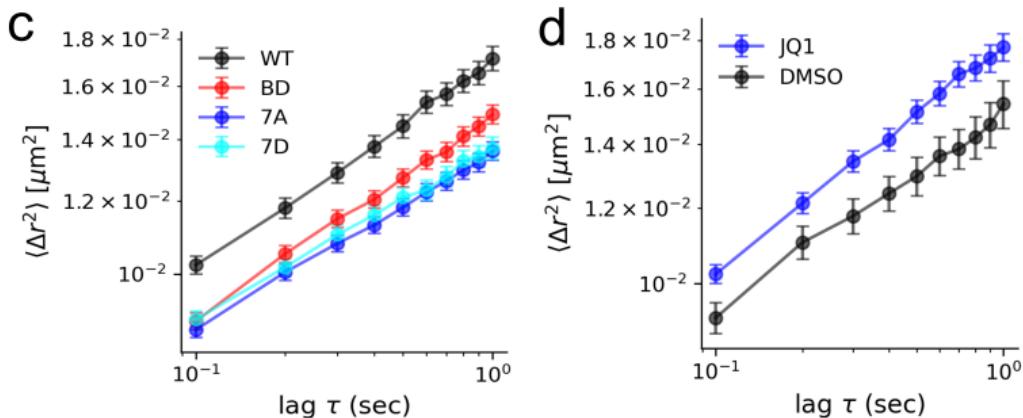
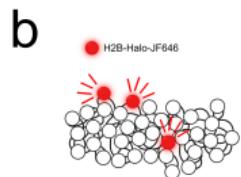
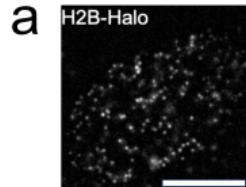
c



d

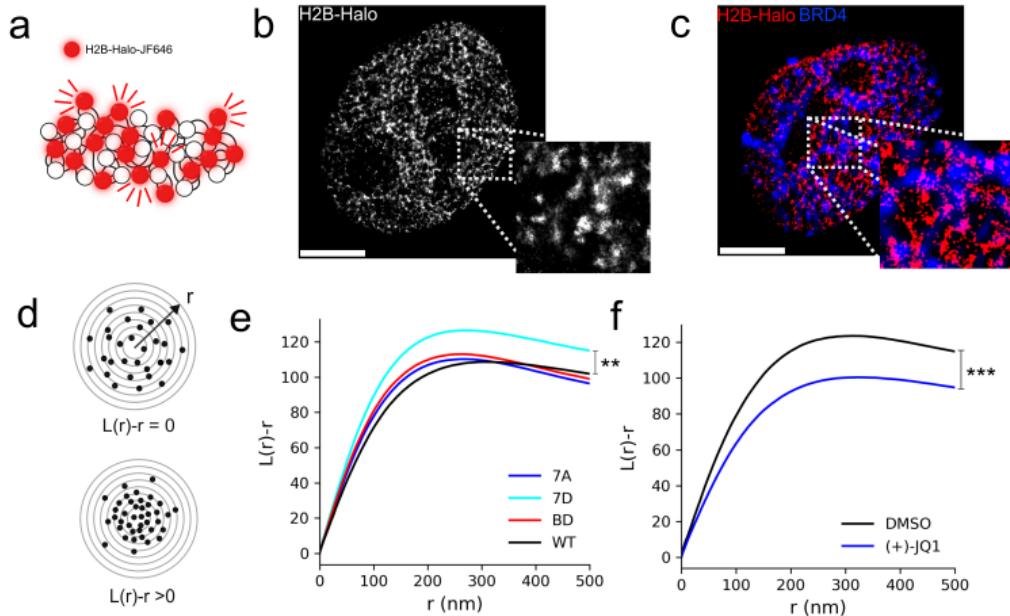


# BRD4 chromatin binding controls chromatin mobility



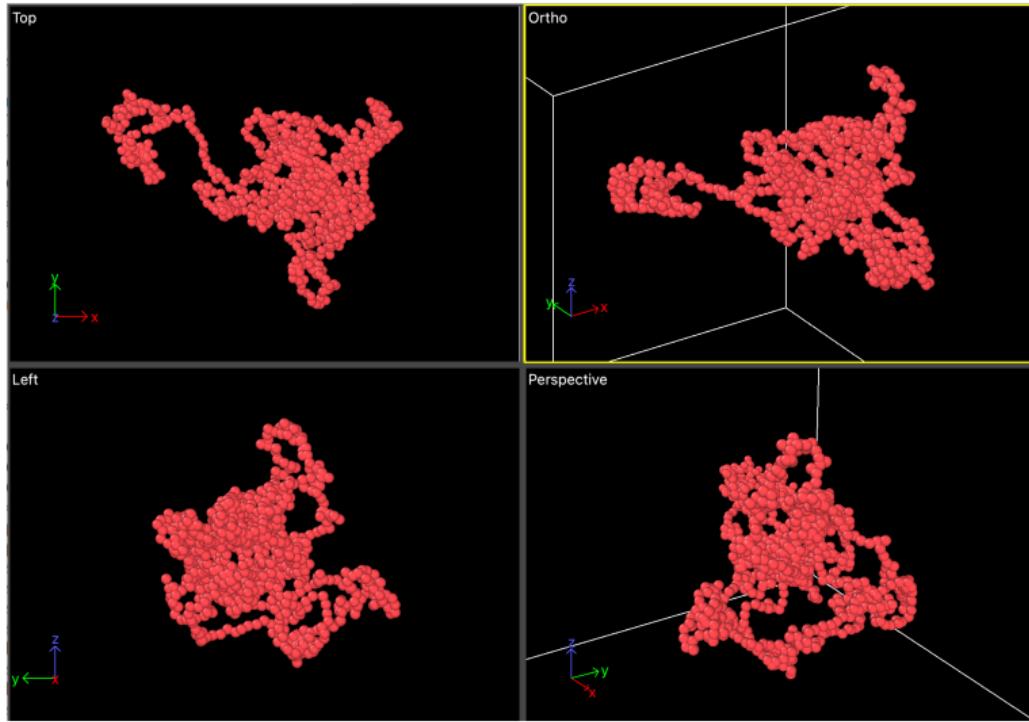
- ▶ H2B is sparsely labeled for particle tracking
- ▶ Reduced diffusion coefficient  $D$  in BRD4 mutants
- ▶ Increased  $D$  in cells exposed to (+)-JQ1

# BRD4 binding is necessary for maintenance of nucleosome nanodomains



- ▶ H2B is densely labeled for super-resolution imaging
- ▶ BRD4 chromatin binding activity controls nanodomain density

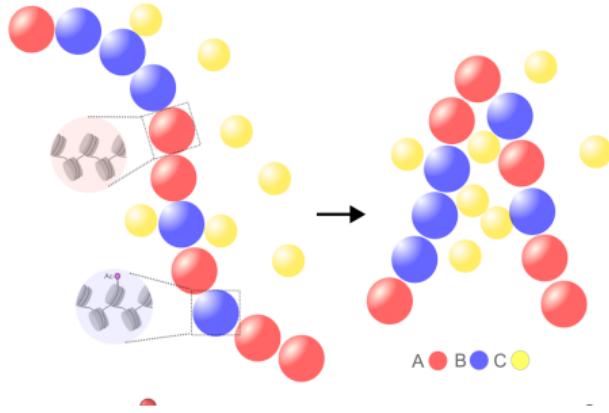
# Coarse grained molecular dynamics of chromatin at 310K



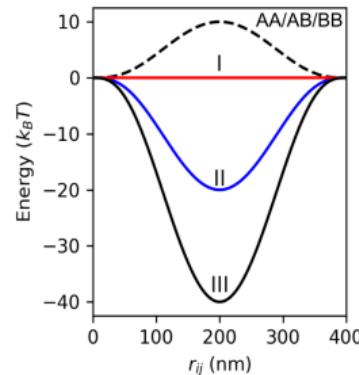
- ▶ 100kb chromatin chains connected by harmonic bonds (Rouse model)

# Coarse grained molecular dynamics of chromatin binders at 310K

a



b

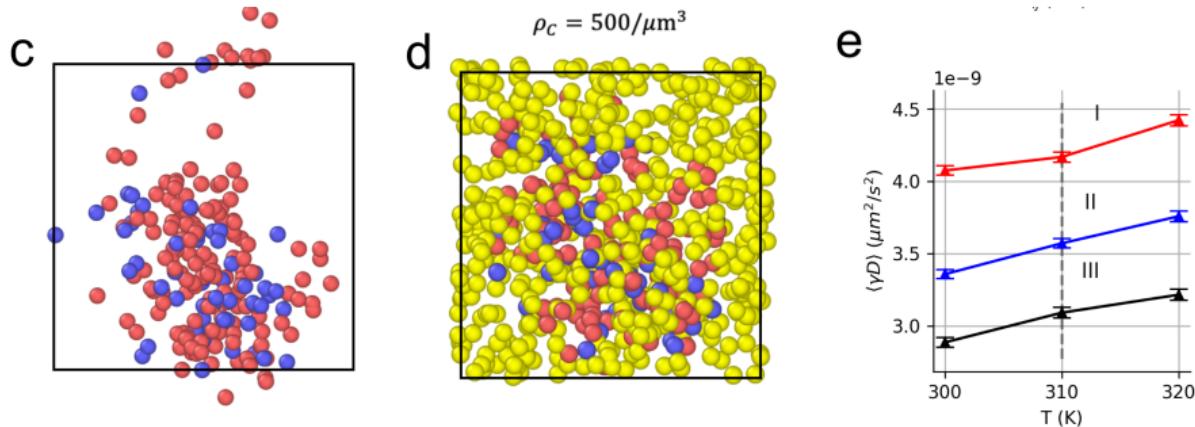


100kb chromatin chains interact with binders via the potential

$$U_{ij} = \epsilon \left( 1 - \left( \frac{|r_{ij}|}{R_0} \right)^2 \right)^3$$

- ▶  $A$  ( $B$ ) type particles represent unacetylated (acetylated) chromatin beads
- ▶ BRD4-like  $C$  particles bind  $B$  type particles with variable energies

# Multivalent chromatin binding reduces chromatin mobility



Integrate Brownian dynamics:  $\dot{r} = \gamma^{-1} \nabla U + \sqrt{2k_B T} \gamma^{-1/2} \xi$   $\gamma = 10^{-6}$

Stochastic forcing is a delta-correlated white-noise  
 $\xi \sim \mathcal{N}(0, 1)$ ,  $\langle \xi(t) \xi(t + \tau) \rangle = \delta(\tau)$

## Summary

- ▶ Developed novel algorithms for computational super-resolution microscopy
- ▶ Developed single photon counting methods for fluorophore quantification
- ▶ Provided evidence for BRD4 regulation of nucleosome nanodomain architecture

# Acknowledgements



(left to right) Charles Park, Garrick Chang, Jing Liu, David Buchanan, Mengyuan Liu, Hailan Ma



Norbert Scherer



Donghong Fu

Thank you!