

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

Clayton W. Seitz

August 6, 2024

# Outline

Introduction to fluorescence nanoscopy

Enhanced nanoscopy with single photon avalanche diode (SPAD) cameras

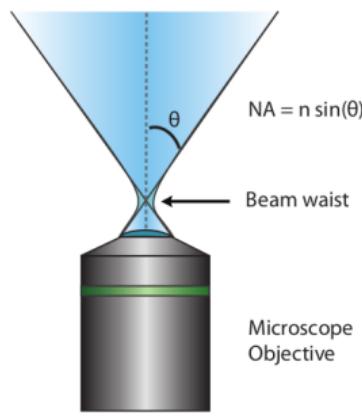
Enhanced nanoscopy with deep generative models

Super-resolution of nucleosome nanodomains *in-vivo*

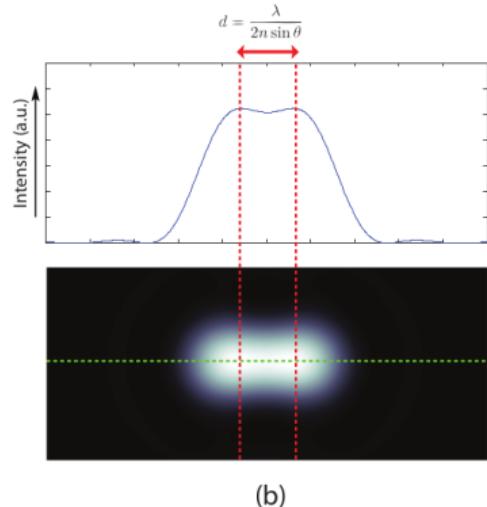
## Introduction to fluorescence nanoscopy

# Fluorescence microscopy and the diffraction limit

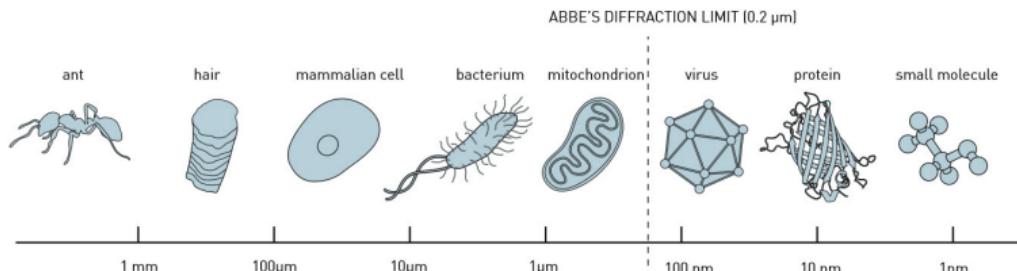
Minimal resolvable distance  $d \sim \lambda$



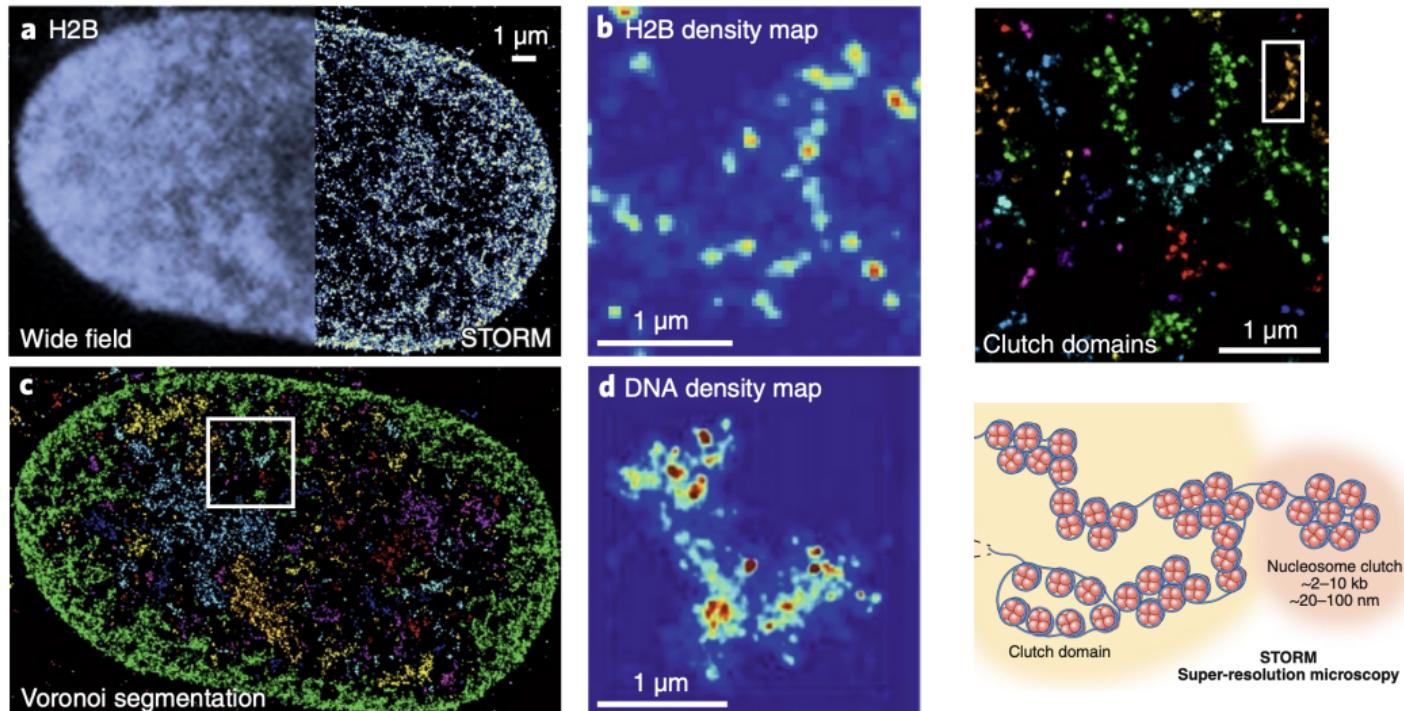
(a)



(b)

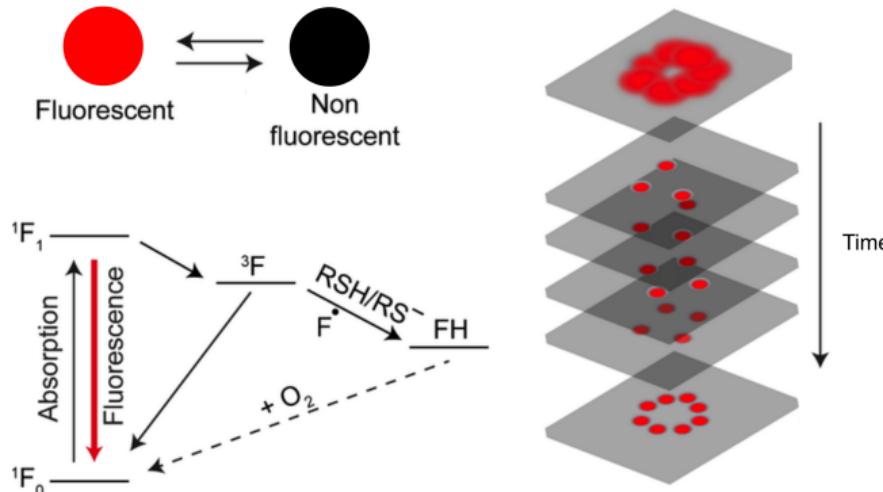


# Stochastic optical reconstruction microscopy (STORM)



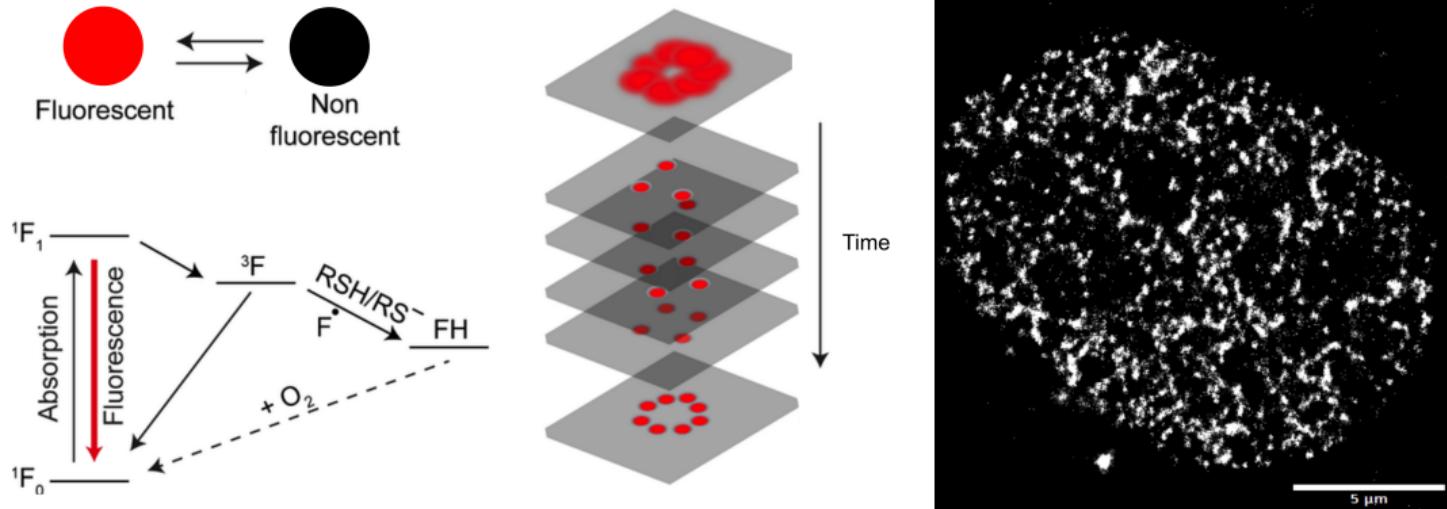
Lakadamyali, M. et al. Nature Methods 17, (2020).

# 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

# Vanilla SMLM by localizing isolated fluorescent emitters

Modeling the point spread function permits sub-pixel localization

$$\mu_k = i_0 \int \int O(u, v) du dv$$

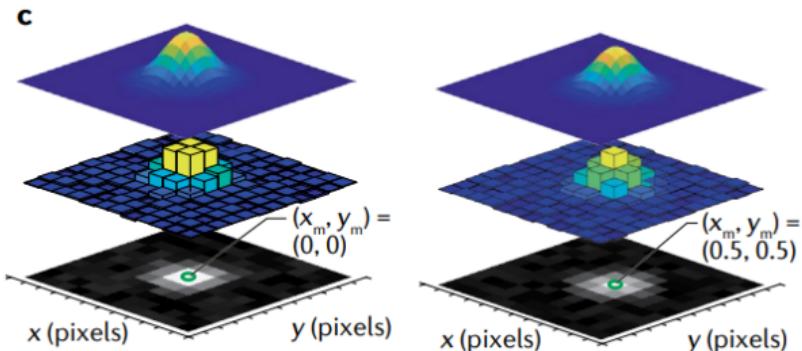
$$i_0 = g_k \eta N_0 \Delta$$

$g_k$  – pixel gain

$\eta$  – quantum efficiency

$N_0$  – photon emission rate

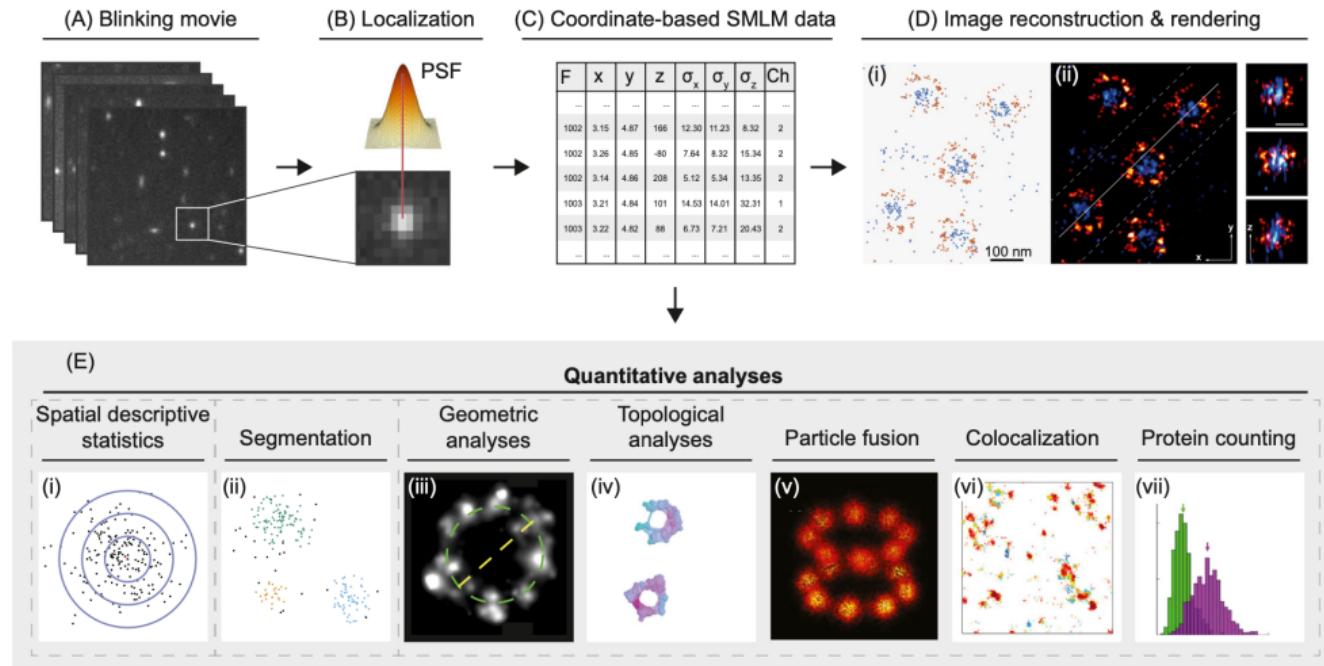
$\Delta$  – exposure time



Assume  $N_0$  is constant over  $\Delta$  (homogeneous Poisson)

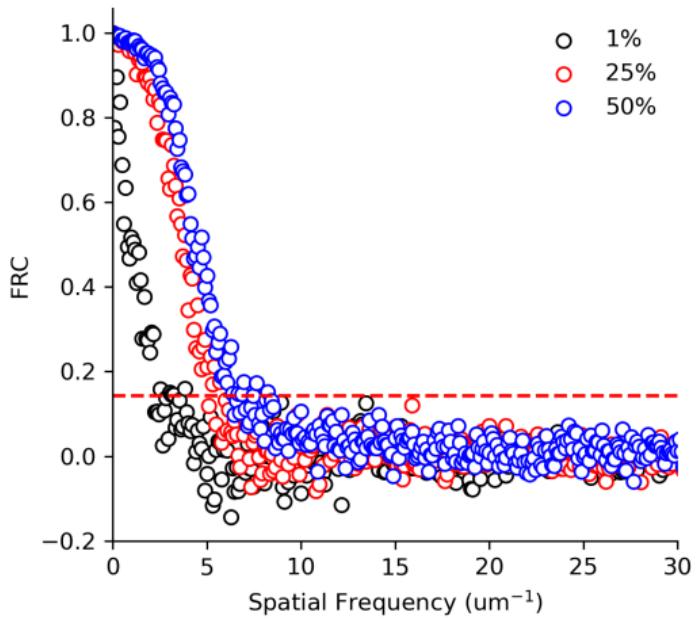
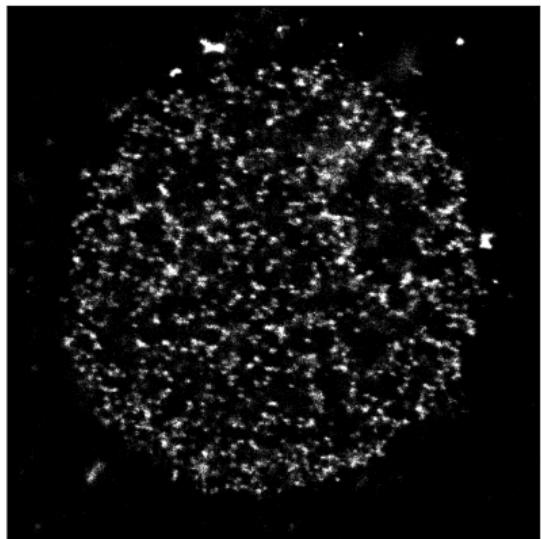
$$\theta^* = \operatorname{argmax}_{\theta} \prod_k p(\mathbf{x}_k | \theta) = \operatorname{argmin}_{\theta} - \sum_k \log p(\mathbf{x}_k | \theta)$$

# Applications of single molecule localization microscopy

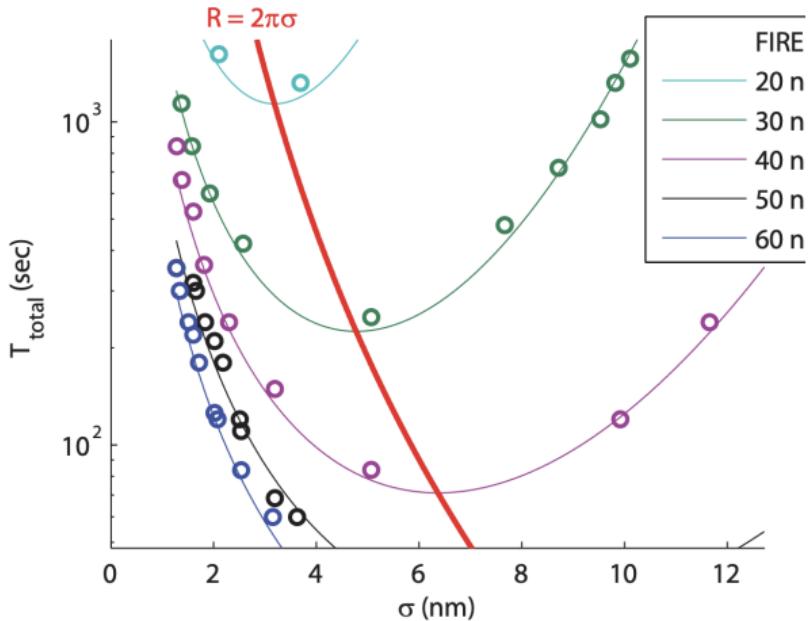


Wu et al. Trends in Cell Biology. 30 (2020)

# How do we define resolution in localization microscopy?



# Fourier Image Resolution (FIRE)

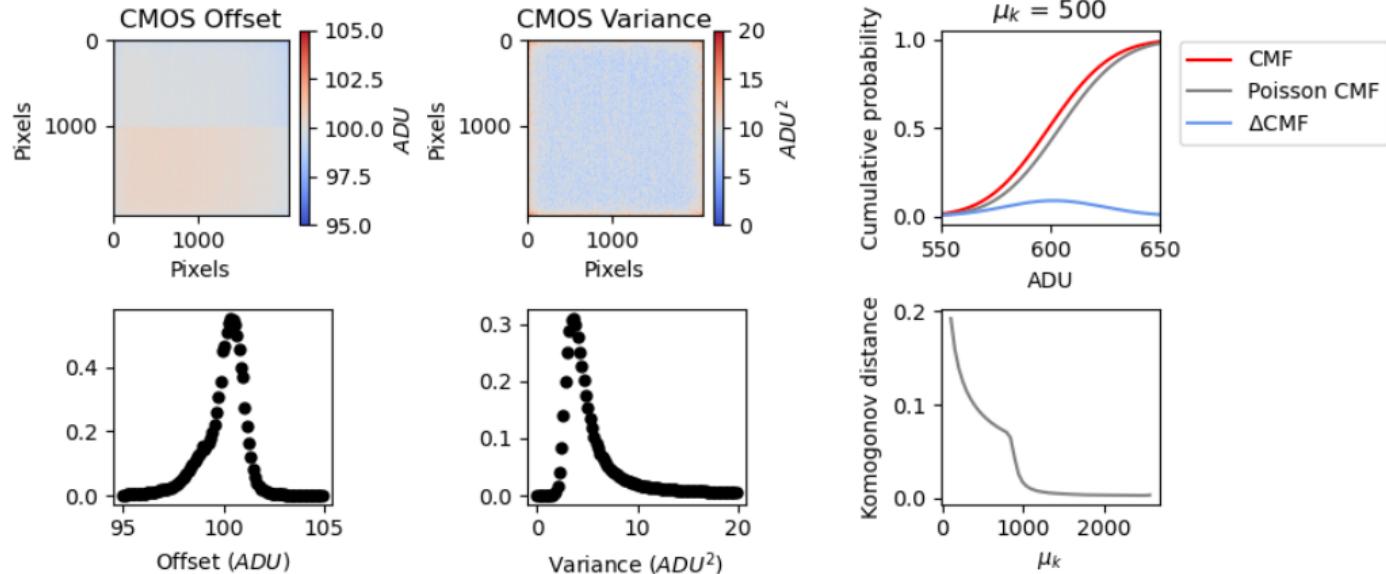


Nieuwenhuizen et al. Nature Methods. **10** (2013)

Nutshell: *How to relax the density limit in localization microscopy?*

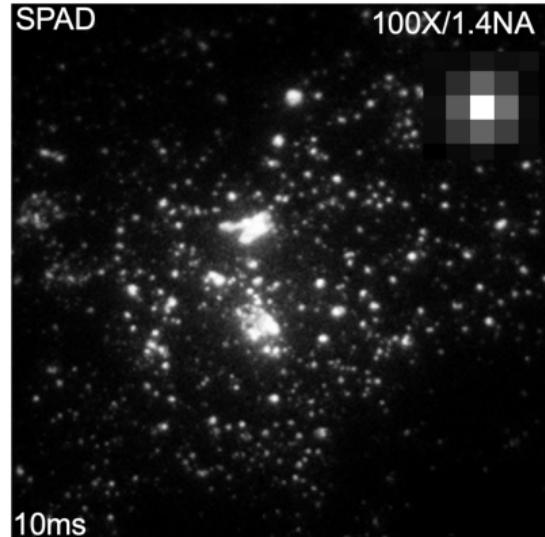
## Enhanced nanoscopy with single photon avalanche diode (SPAD) cameras

# Conventional CMOS cameras are noisy

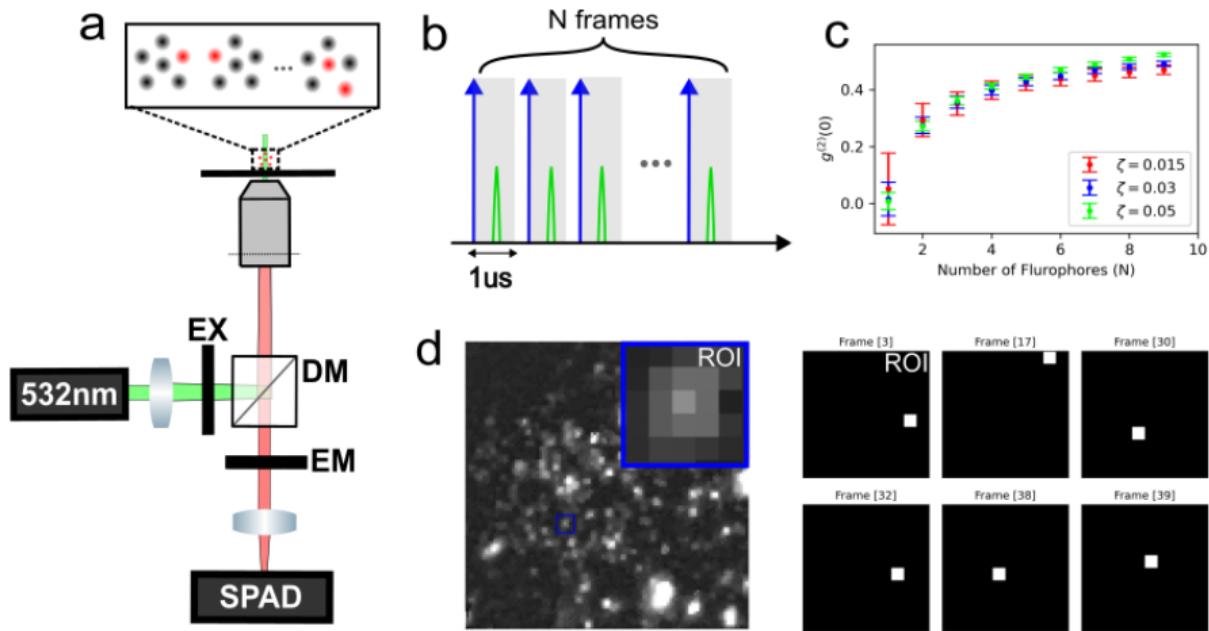


Noise characteristics for air-cooled Hamamatsu ORCA-Flash4.0

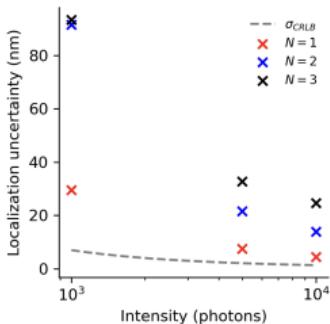
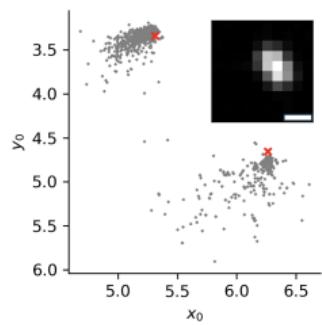
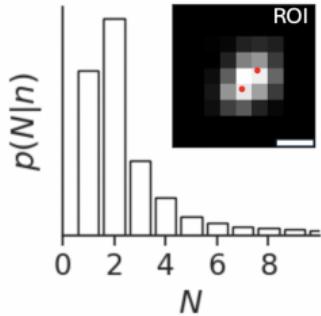
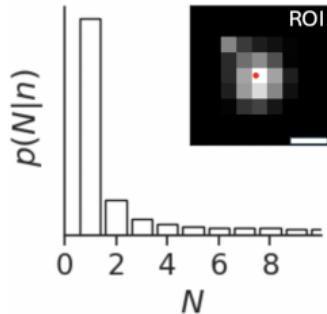
# Widefield photon counting



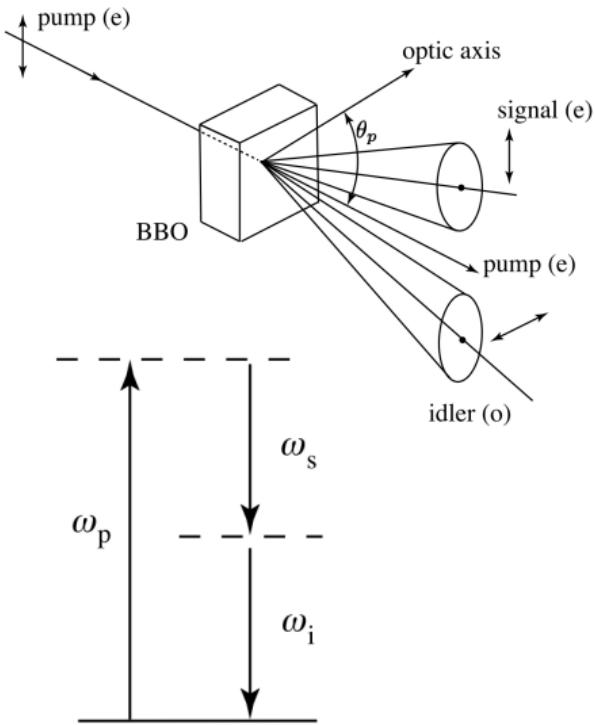
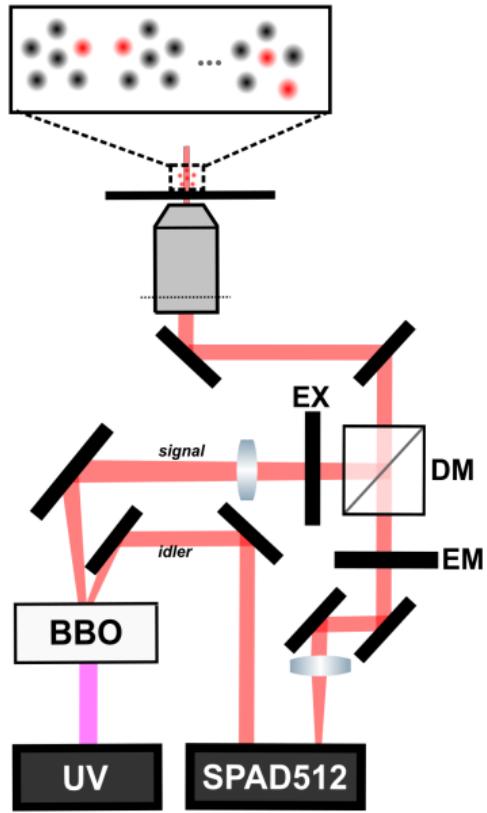
# Imaging Qdot655 photon by photon



# Constrained multi-emitter localization with photon counting



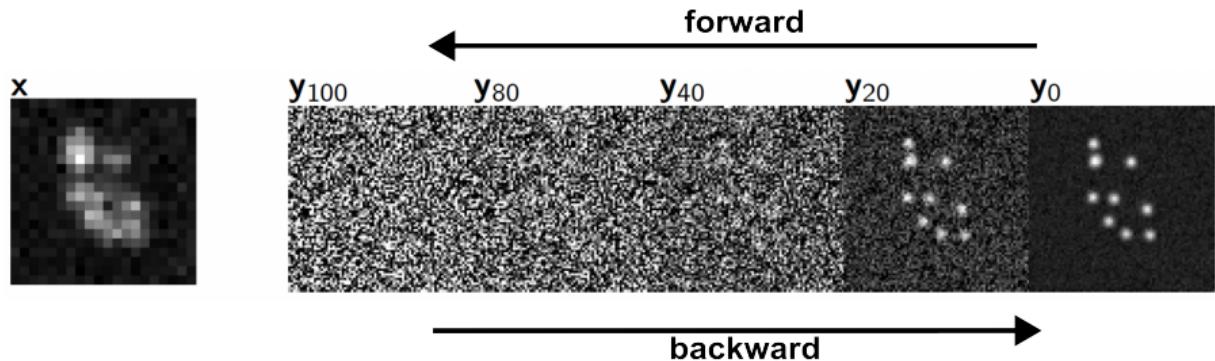
# Quantum illumination with spontaneous parametric downconversion



## Enhanced nanoscopy with deep generative models

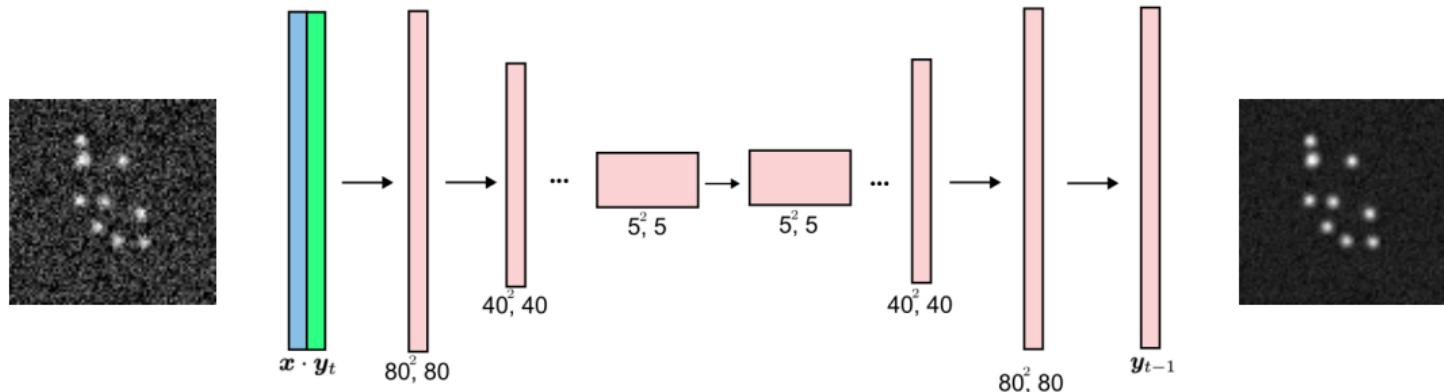
## Bayesian image restoration with diffusion models

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



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

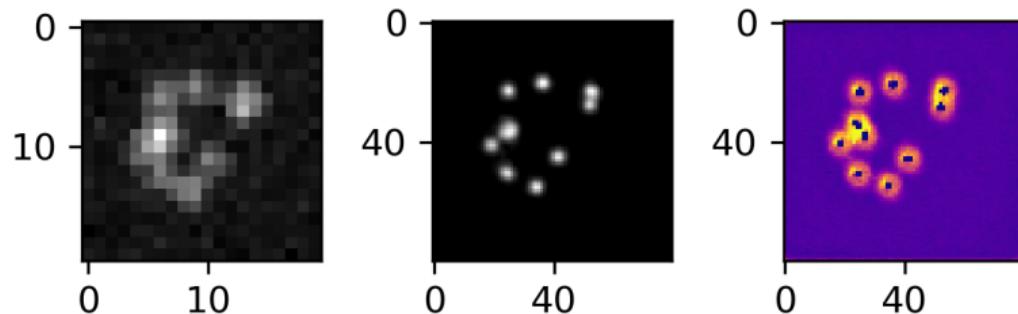
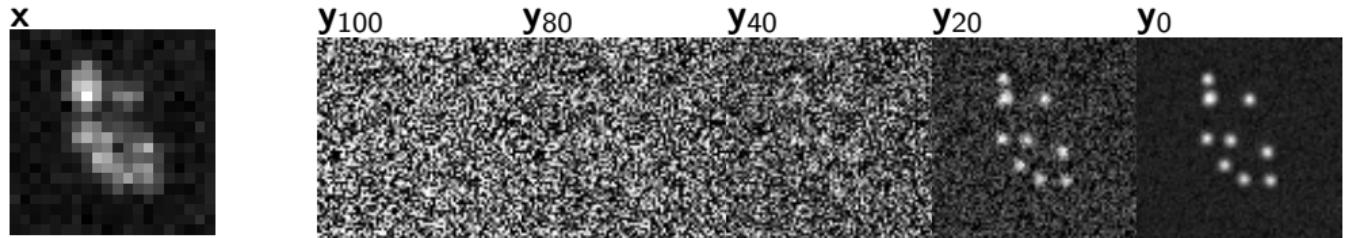
# Bayesian image restoration with diffusion models



A deep neural network estimates the gradient of the reverse process

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

## Bayesian image restoration with diffusion models

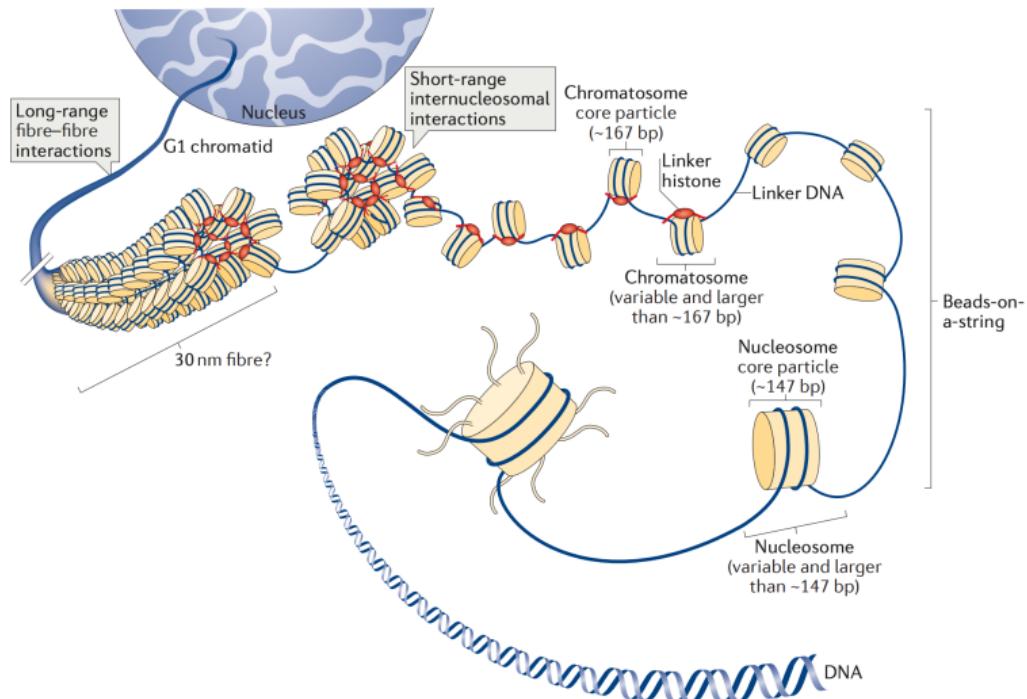


Need to approximate the gradient  $s_\theta(\mathbf{y}_t) \approx \nabla \log p(\mathbf{x})$  and sample:

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

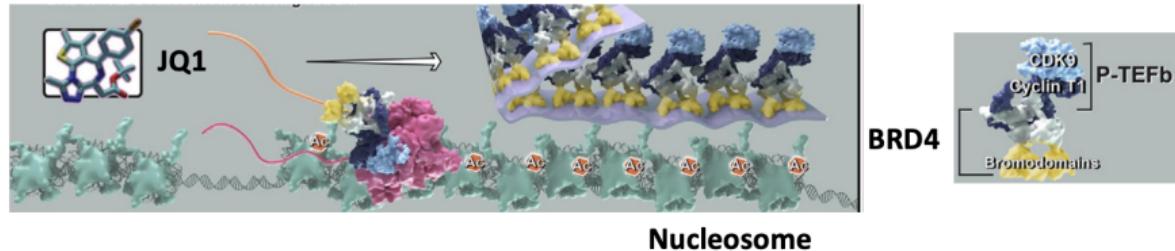
## Super-resolution of nucleosome nanodomains *in-vivo*

# Hierarchical structure of chromatin

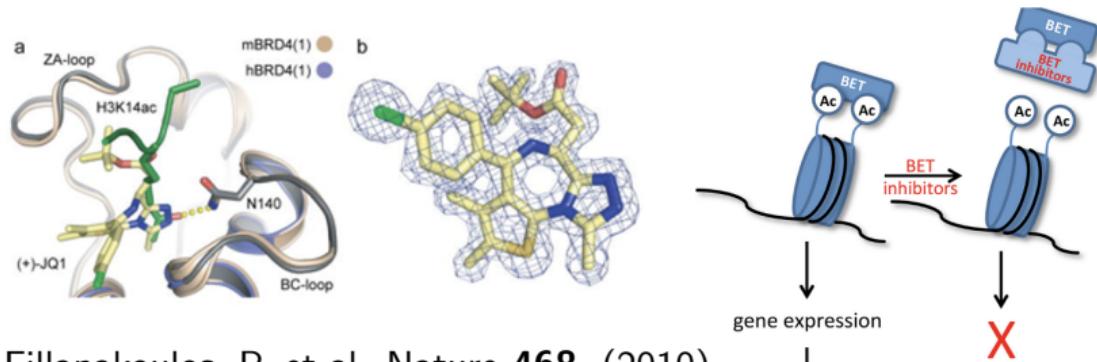


Fyodorov, D. et al. Nat Rev Mol Cell Biol **19**, (2018).

# Bromodomain protein 4 (BRD4) binds acetylated chromatin

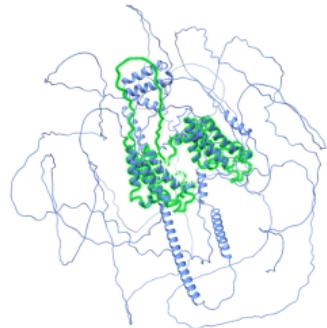
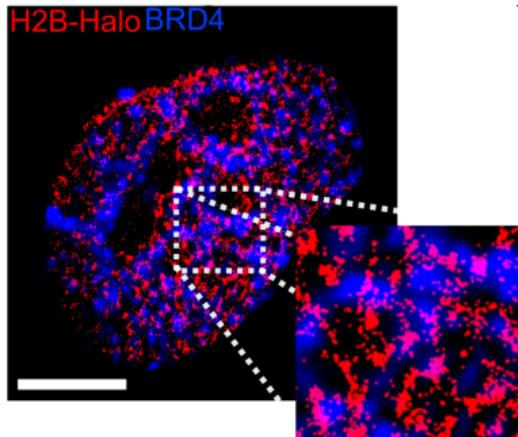


Zheng, B. et al. Molecular Cell **16**, (2023).



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

# Bromodomain protein 4 (BRD4) binds acetylated chromatin



# BRD4 phosphorylation state is necessary for maintenance of chromatin structure

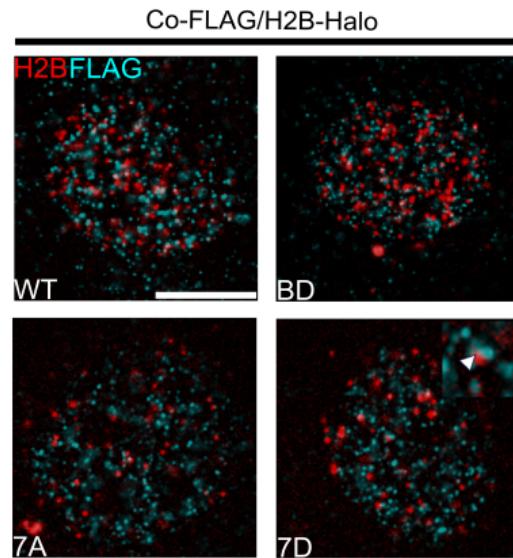
a



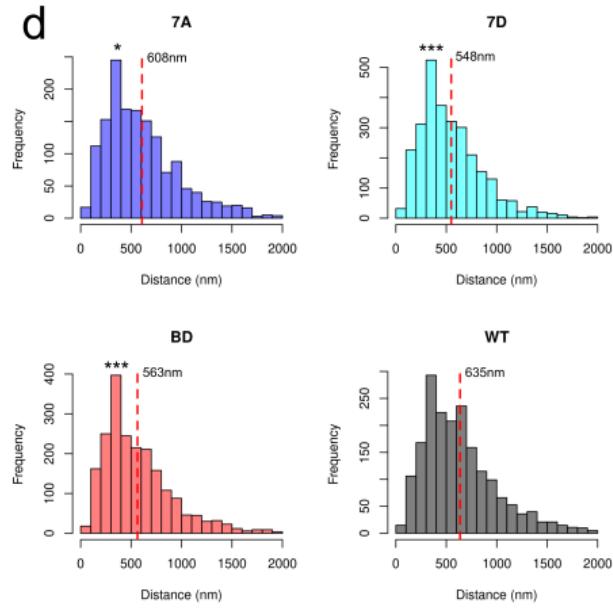
b



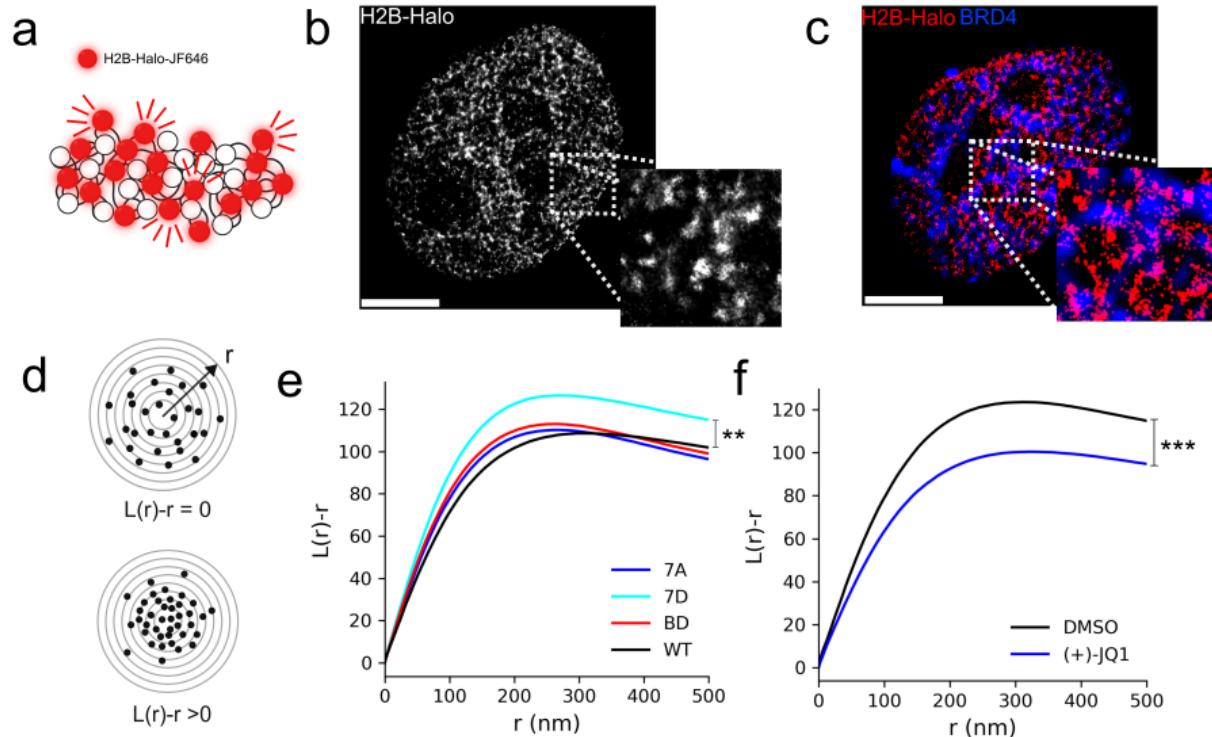
c



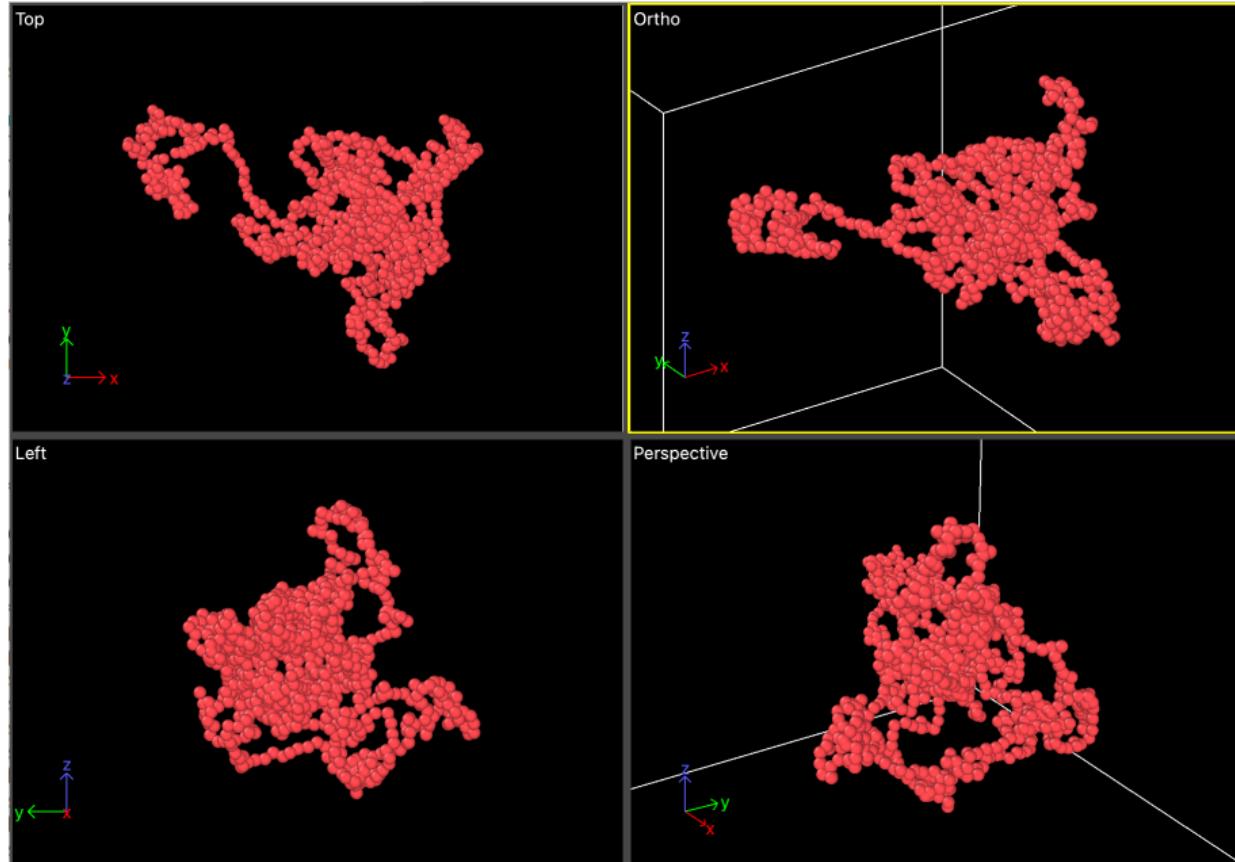
d



# BRD4 phosphorylation state is necessary for maintenance of chromatin structure

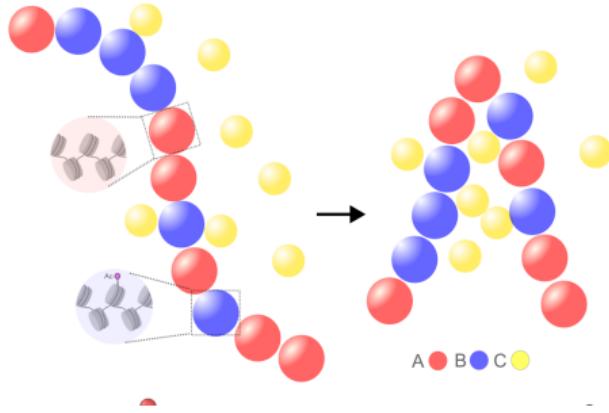


# Coarse grained molecular dynamics of chromatin at 310K

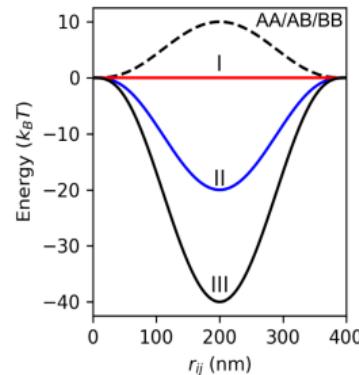


# Coarse grained molecular dynamics of chromatin binders at 310K

a



b

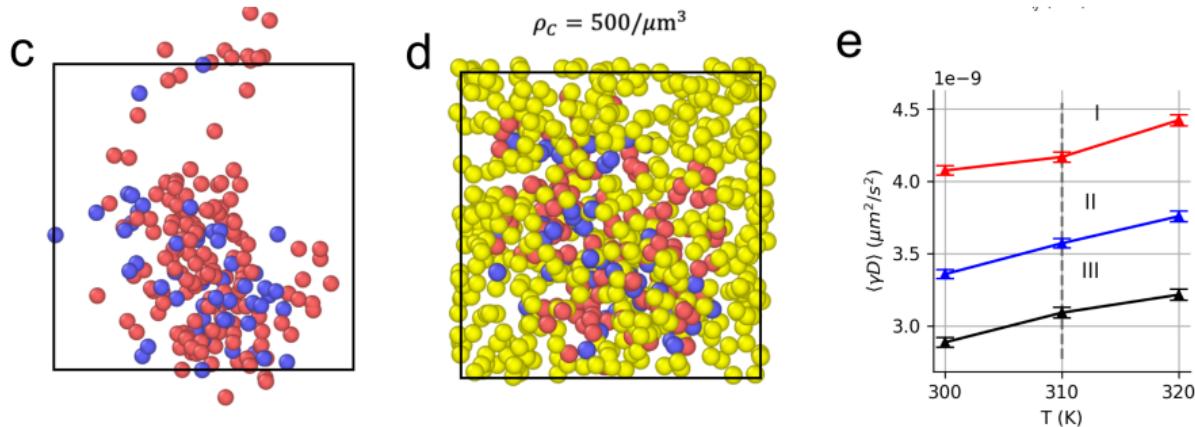


100kb chromatin chains interact with binders via the potential

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

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

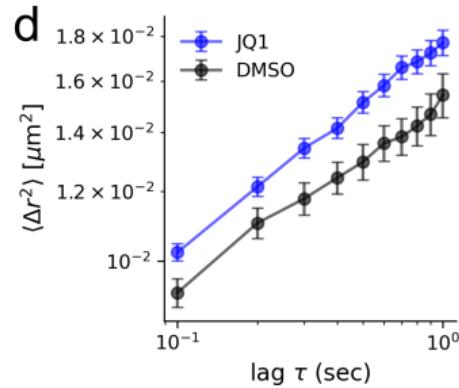
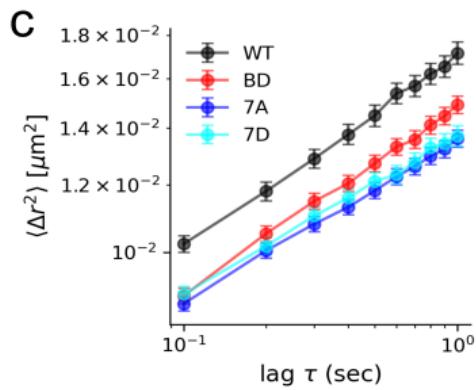
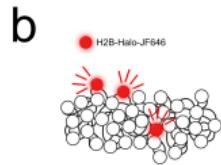
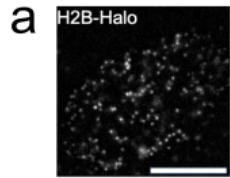
# Multivalent chromatin binding reduces chromatin mobility



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  
 $\xi \sim \mathcal{N}(0, 1)$ ,  $\langle \xi(t) \xi(t + \tau) \rangle = \delta(\tau)$

# Multivalent chromatin binding reduces chromatin mobility



Experiment:  $D_{W\tau} - D_{7D} \approx 10^{-3} \mu\text{m}^2/\text{s}, \gamma = 10^{-6}$