

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

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Outline

Introduction to fluorescence nanoscopy

Enhanced nanoscopy with single photon avalanche diode (SPAD) cameras

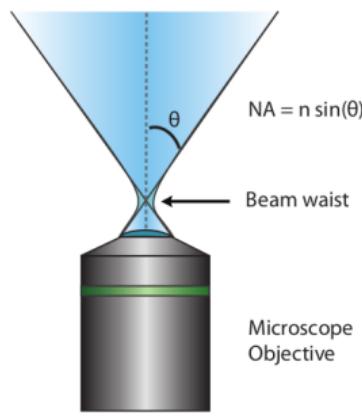
Enhanced nanoscopy with deep generative models

Super-resolution of nucleosome nanodomains *in-vivo*

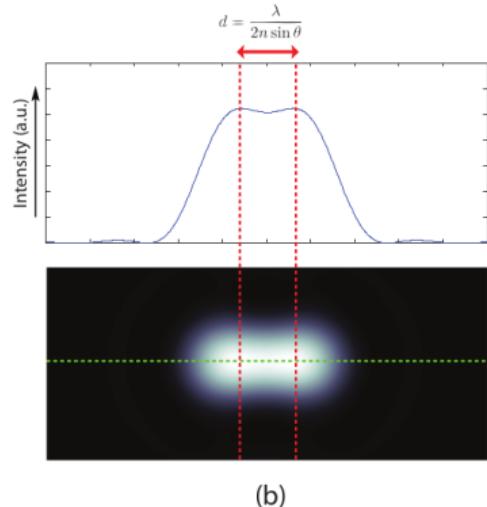
Introduction to fluorescence nanoscopy

Fluorescence microscopy and the diffraction limit

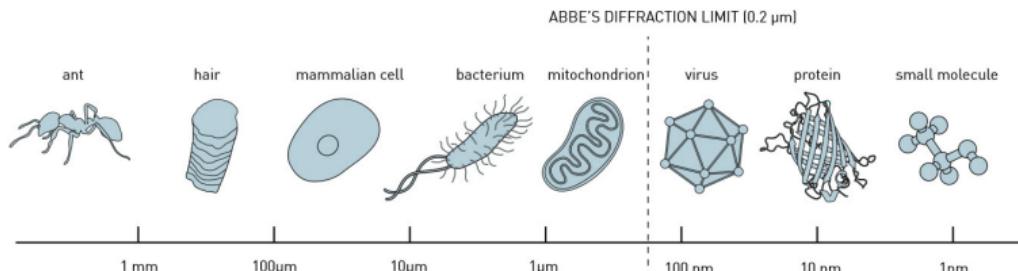
Minimal resolvable distance $d \sim \lambda$



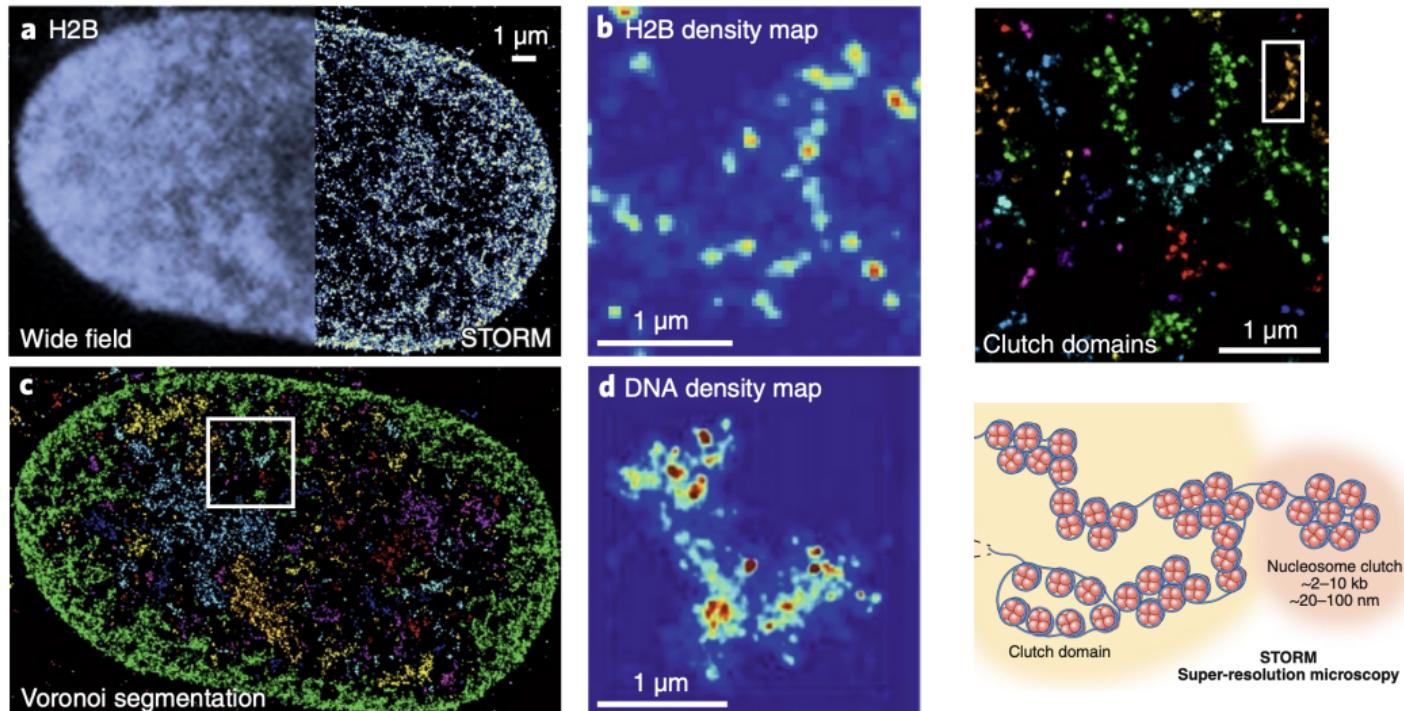
(a)



(b)

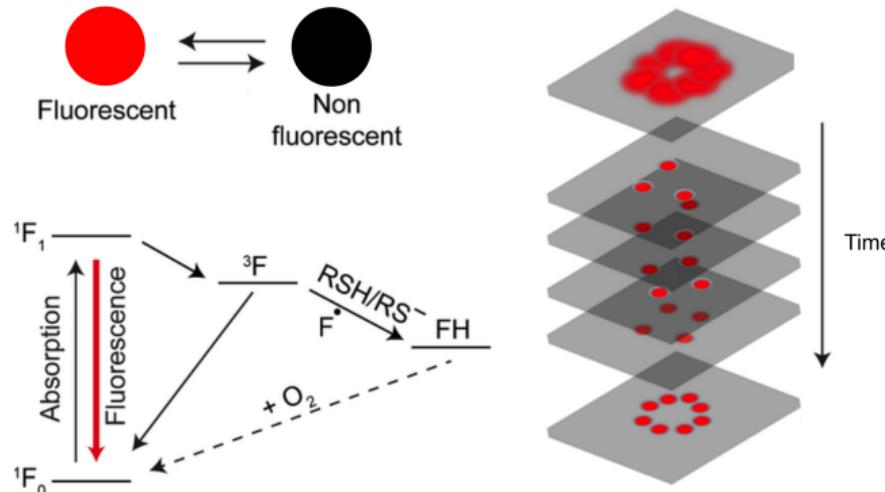


Stochastic optical reconstruction microscopy (STORM)



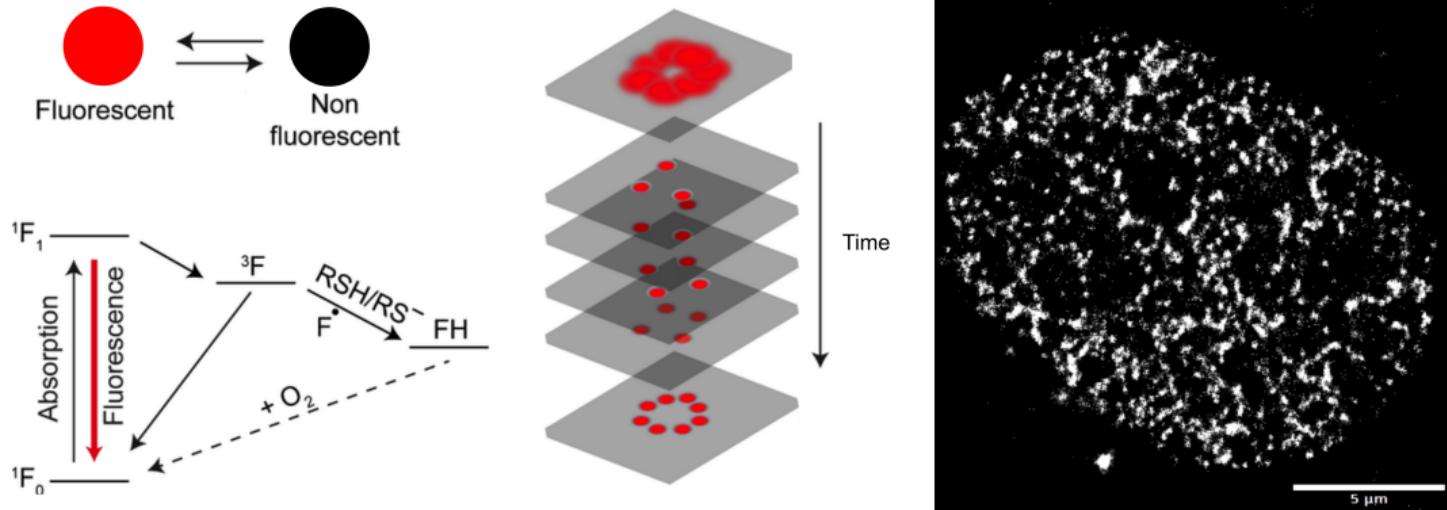
Lakadamyali, M. et al. Nature Methods 17, (2020).

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
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Vanilla SMLM by localizing isolated fluorescent emitters

Modeling the point spread function permits sub-pixel localization

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

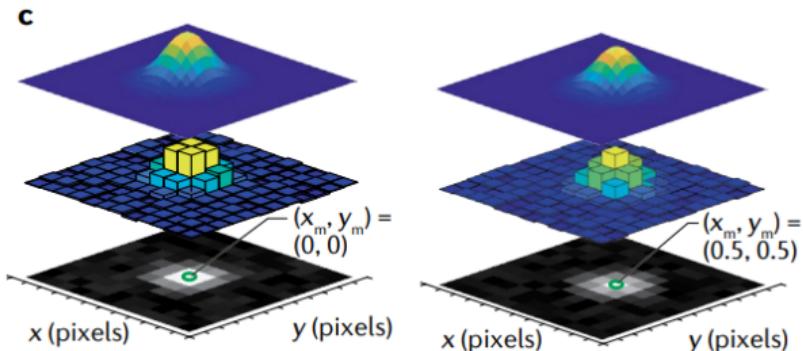
$$i_0 = g_k \eta N_0 \Delta$$

g_k – pixel gain

η – quantum efficiency

N_0 – photon emission rate

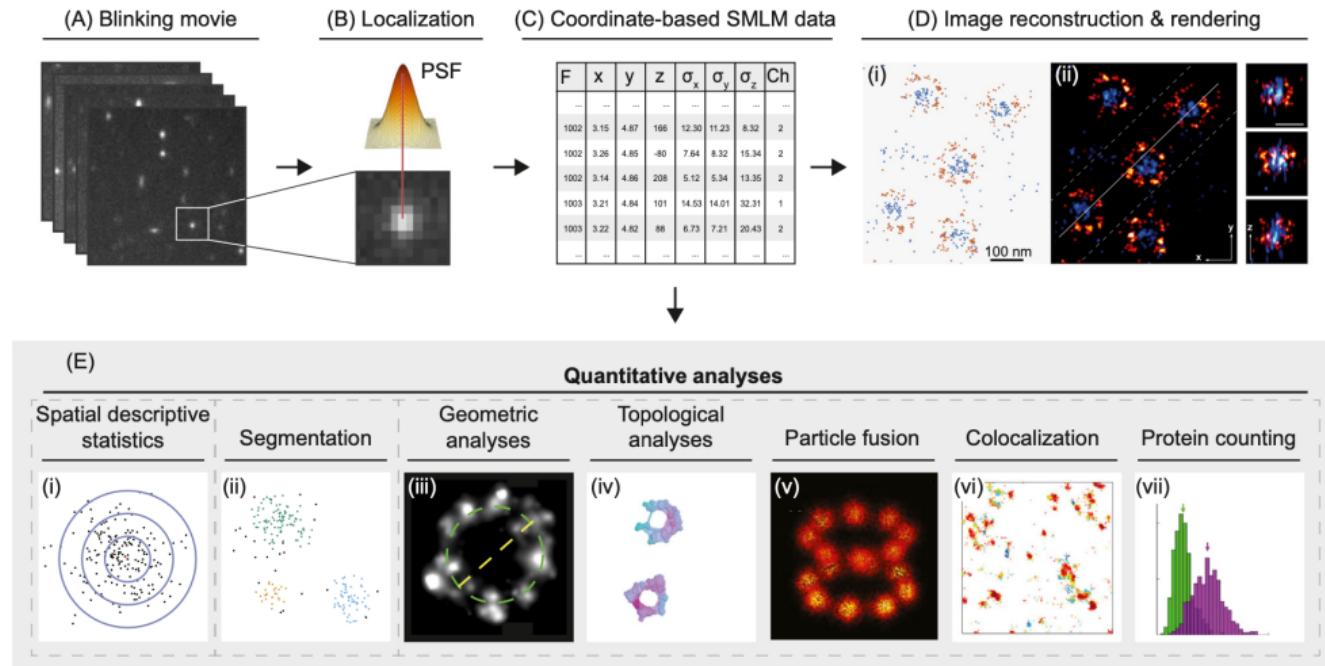
Δ – exposure time



Assume N_0 is constant over Δ (homogeneous Poisson)

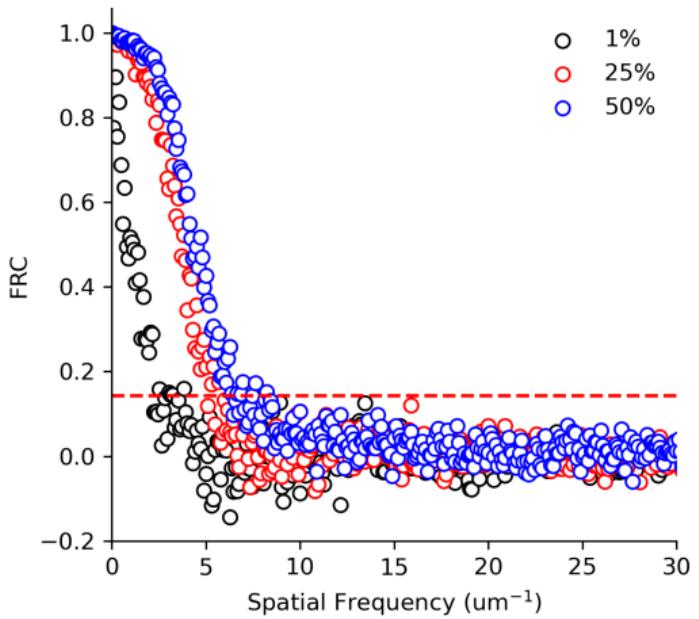
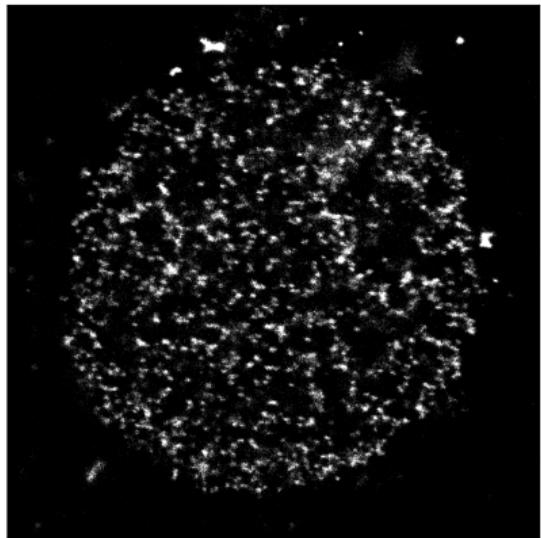
$$\theta^* = \operatorname{argmax}_{\theta} \prod_k p(\mathbf{x}_k | \theta) = \operatorname{argmin}_{\theta} - \sum_k \log p(\mathbf{x}_k | \theta)$$

Applications of single molecule localization microscopy

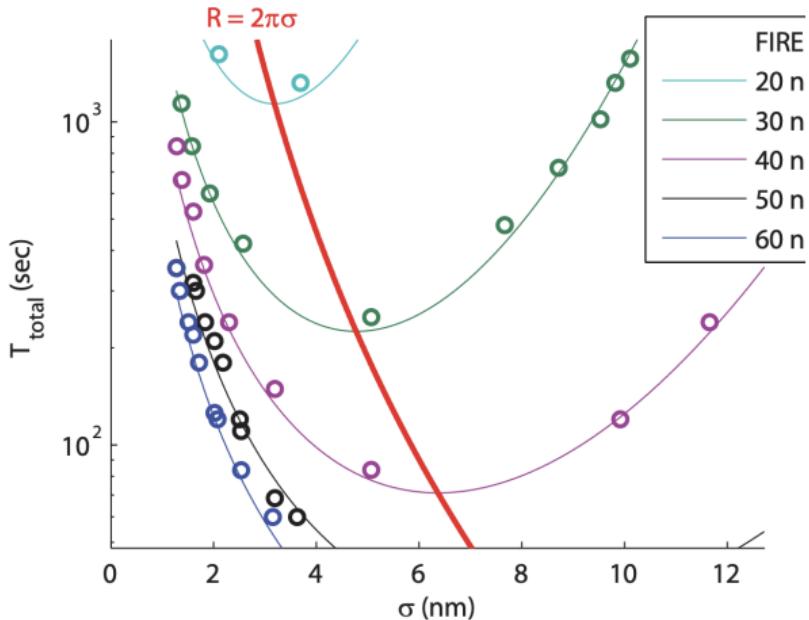


Wu et al. Trends in Cell Biology. 30 (2020)

How do we define resolution in localization microscopy?



Fourier Image Resolution (FIRE)

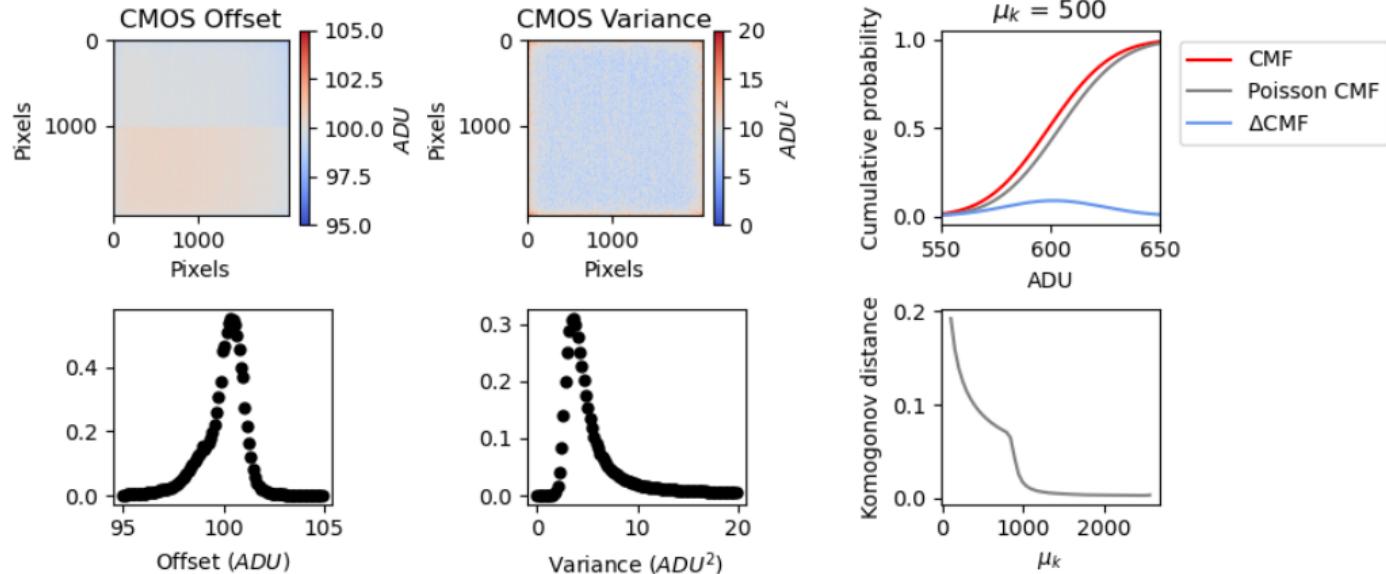


Nieuwenhuizen et al. Nature Methods. **10** (2013)

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

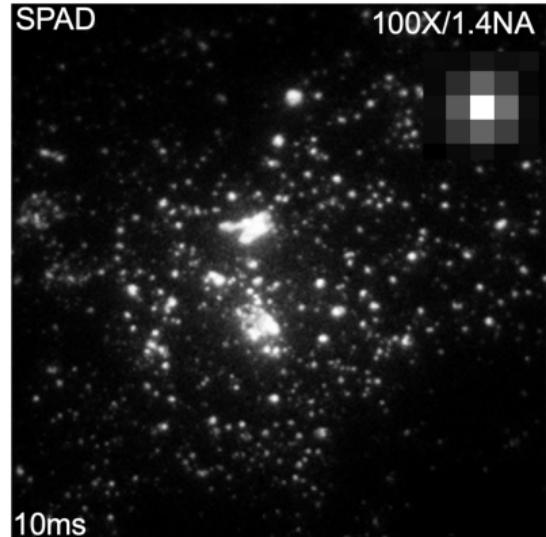
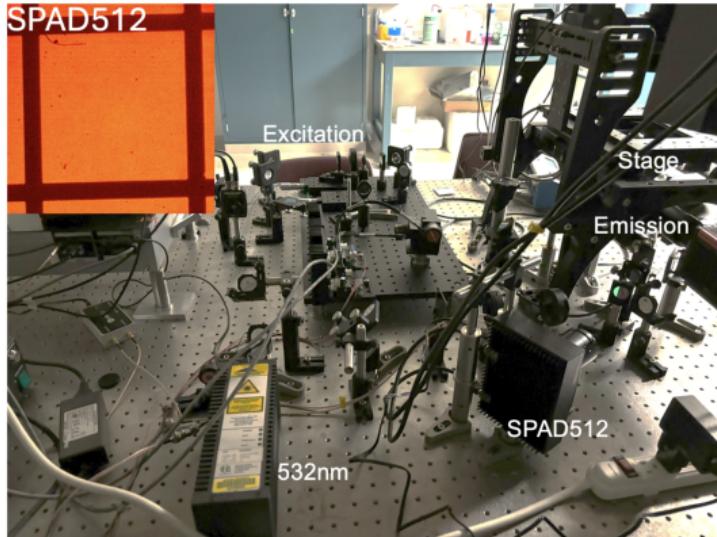
Enhanced nanoscopy with single photon avalanche diode (SPAD) cameras

Conventional CMOS cameras are noisy

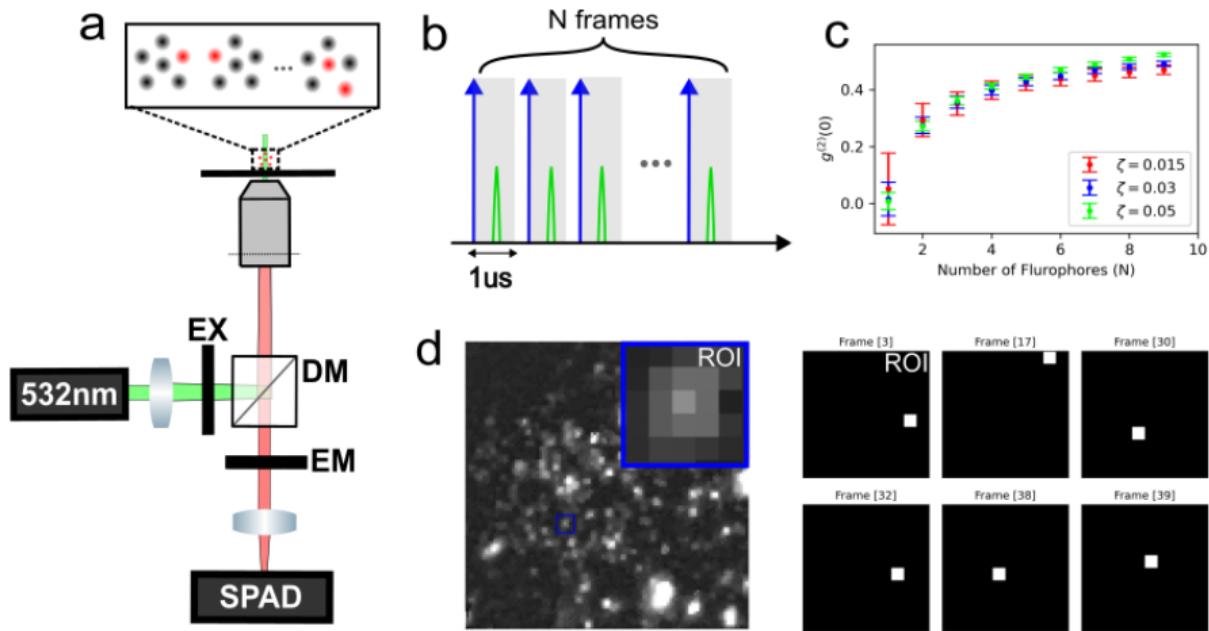


Noise characteristics for air-cooled Hamamatsu ORCA-Flash4.0

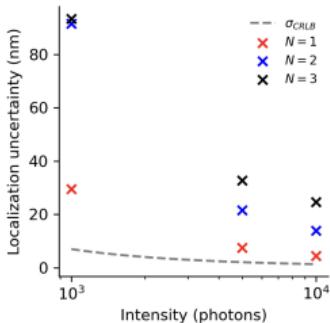
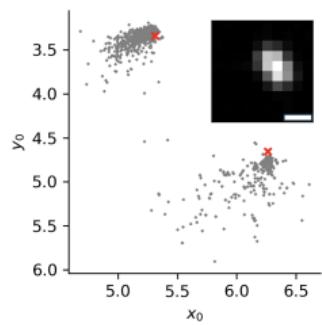
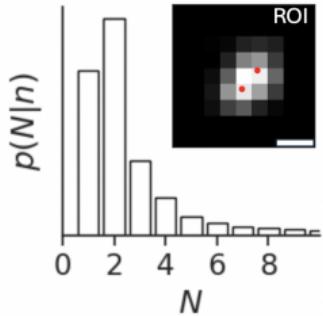
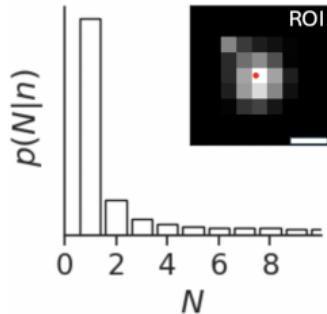
Widefield photon counting



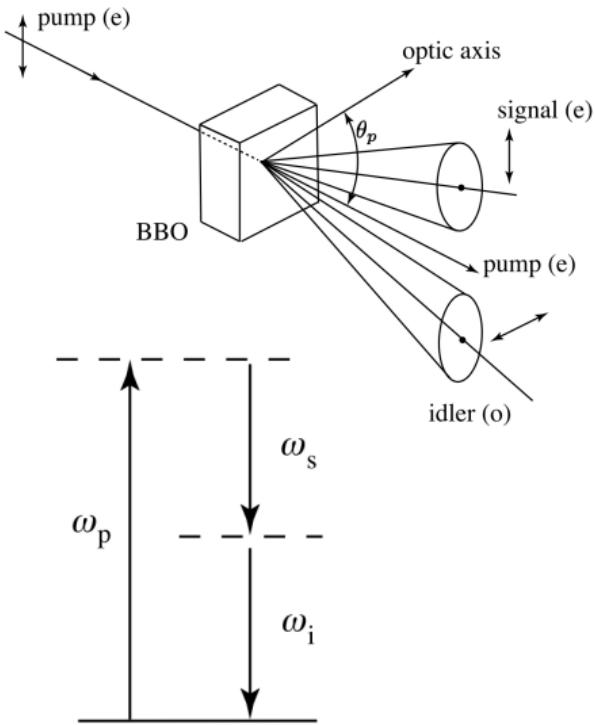
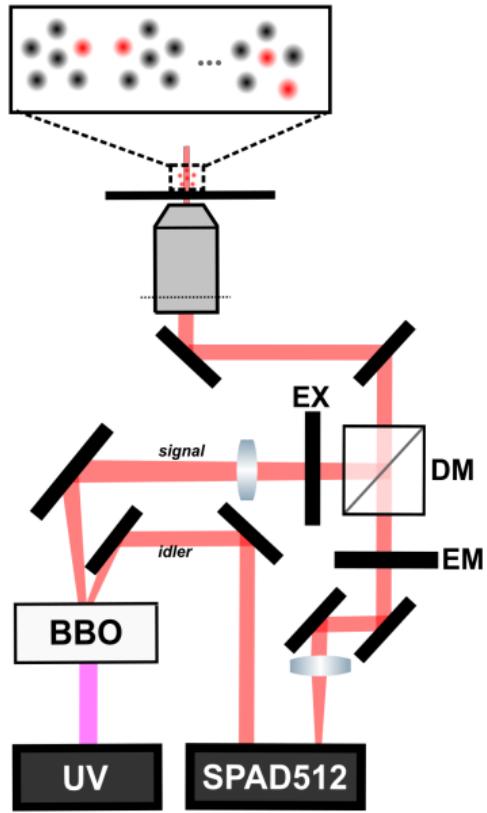
Imaging Qdot655 photon by photon



Constrained multi-emitter localization with photon counting



Quantum illumination with spontaneous parametric downconversion

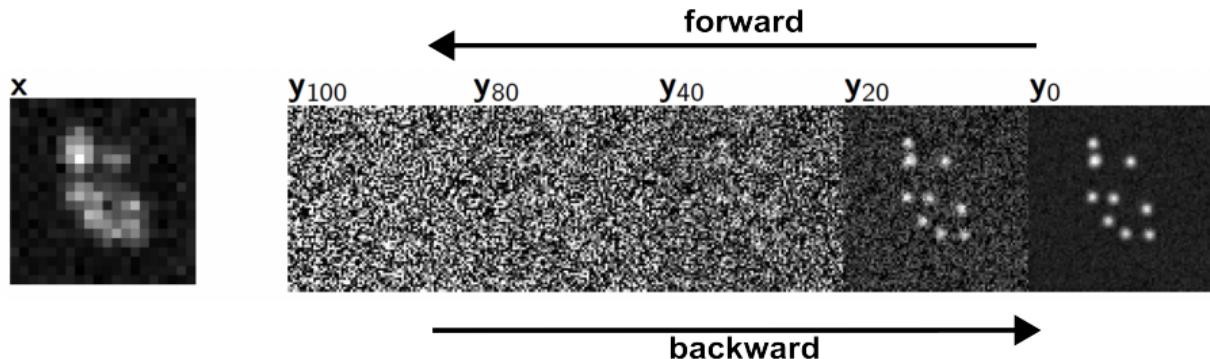


Enhanced nanoscopy with deep generative models

Bayesian image restoration with diffusion models

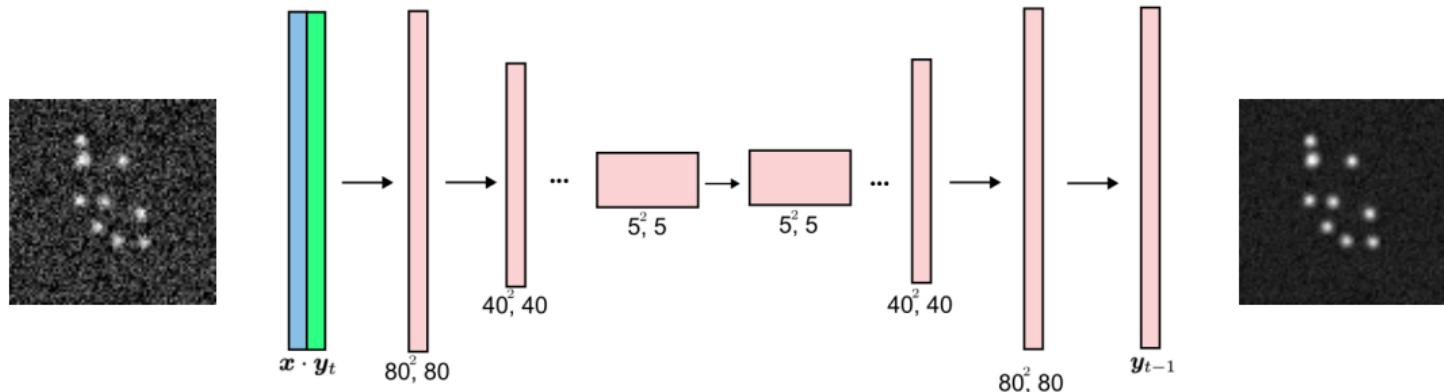
Inference of a high resolution image \mathbf{y} from low resolution \mathbf{x} is approached by modeling a distribution $p_\psi(\mathbf{y}|\mathbf{x})$ with a diffusion model

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



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

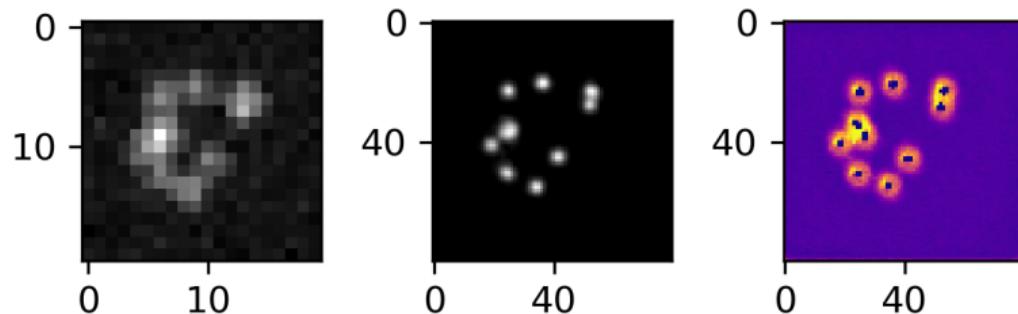
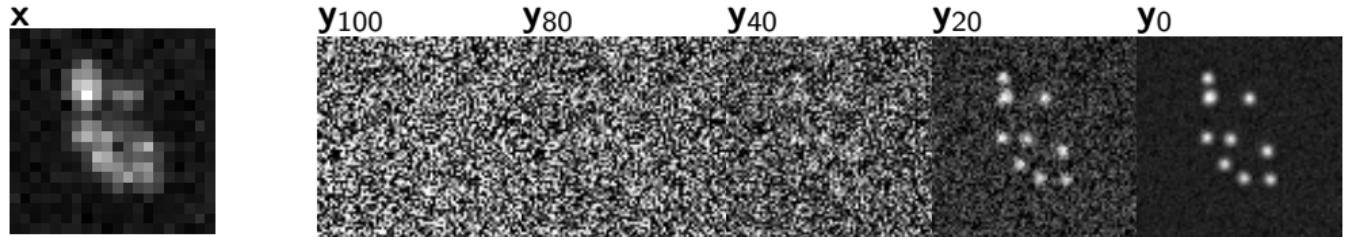
Bayesian image restoration with diffusion models



A deep neural network estimates the gradient of the reverse process

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

Bayesian image restoration with diffusion models

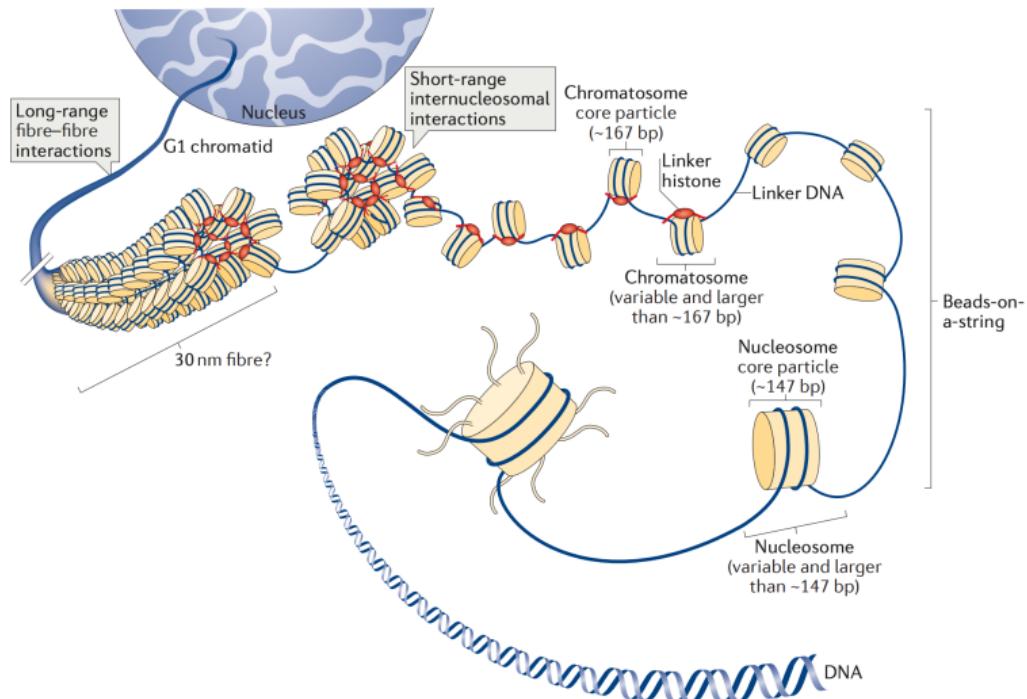


Need to approximate the gradient $s_\theta(\mathbf{y}_t) \approx \nabla \log p(\mathbf{x})$ and sample:

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

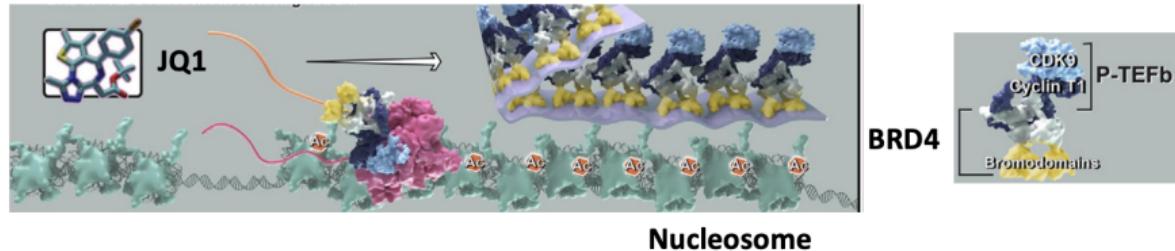
Super-resolution of nucleosome nanodomains *in-vivo*

Hierarchical structure of chromatin

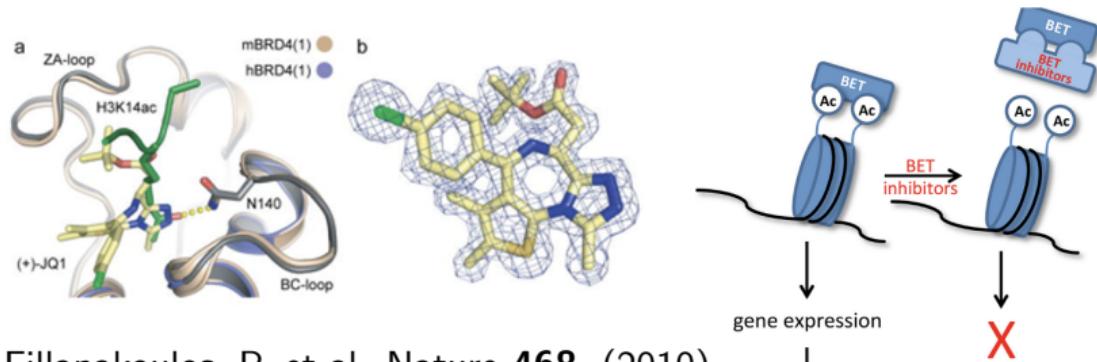


Fyodorov, D. et al. Nat Rev Mol Cell Biol 19, (2018).

Bromodomain protein 4 (BRD4) binds acetylated chromatin

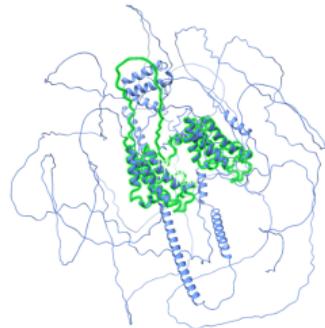
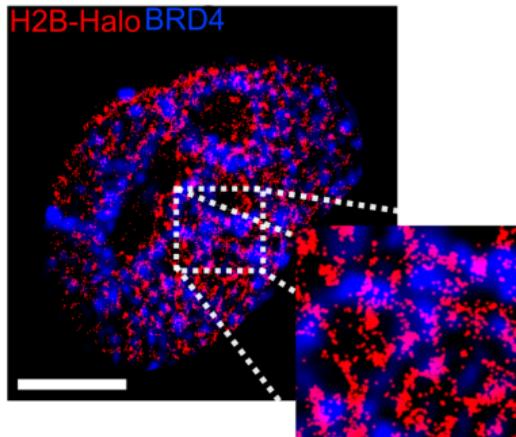


Zheng, B. et al. Molecular Cell **16**, (2023).



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

Bromodomain protein 4 (BRD4) binds acetylated chromatin



BRD4 phosphorylation state is necessary for maintenance of chromatin structure

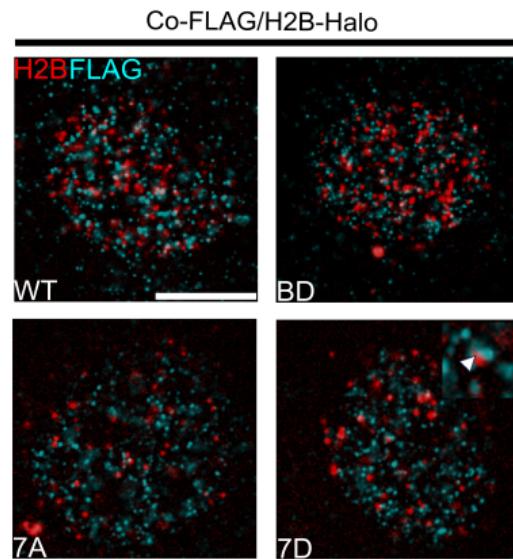
a



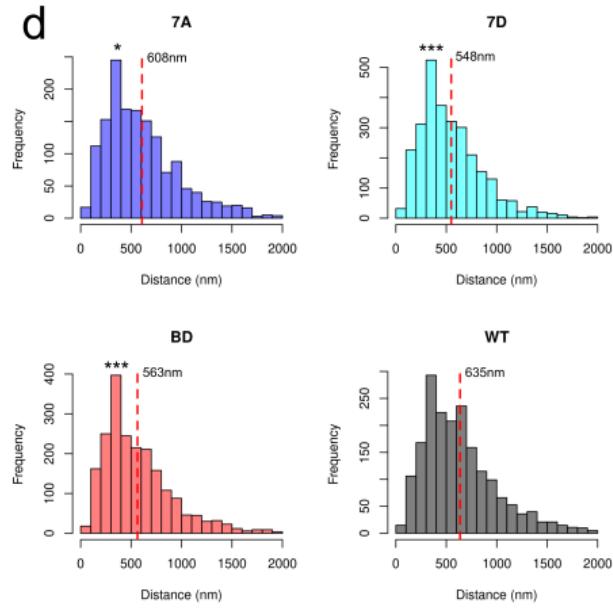
b



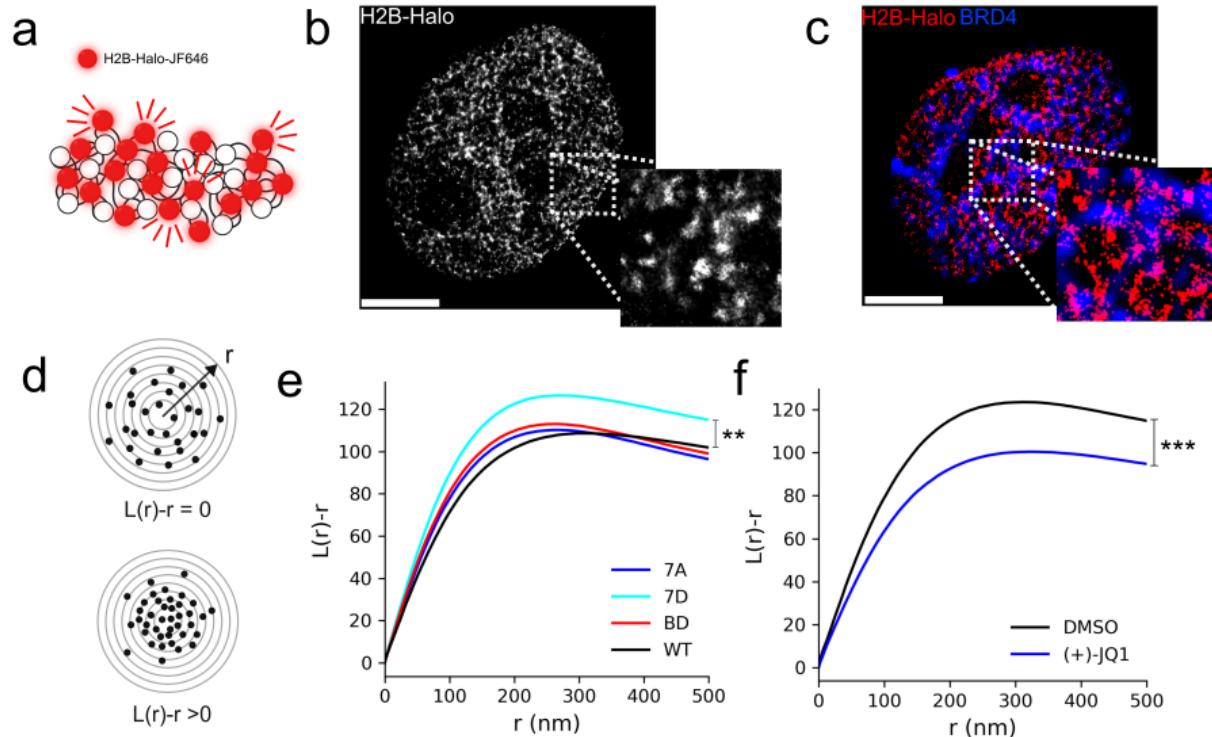
c



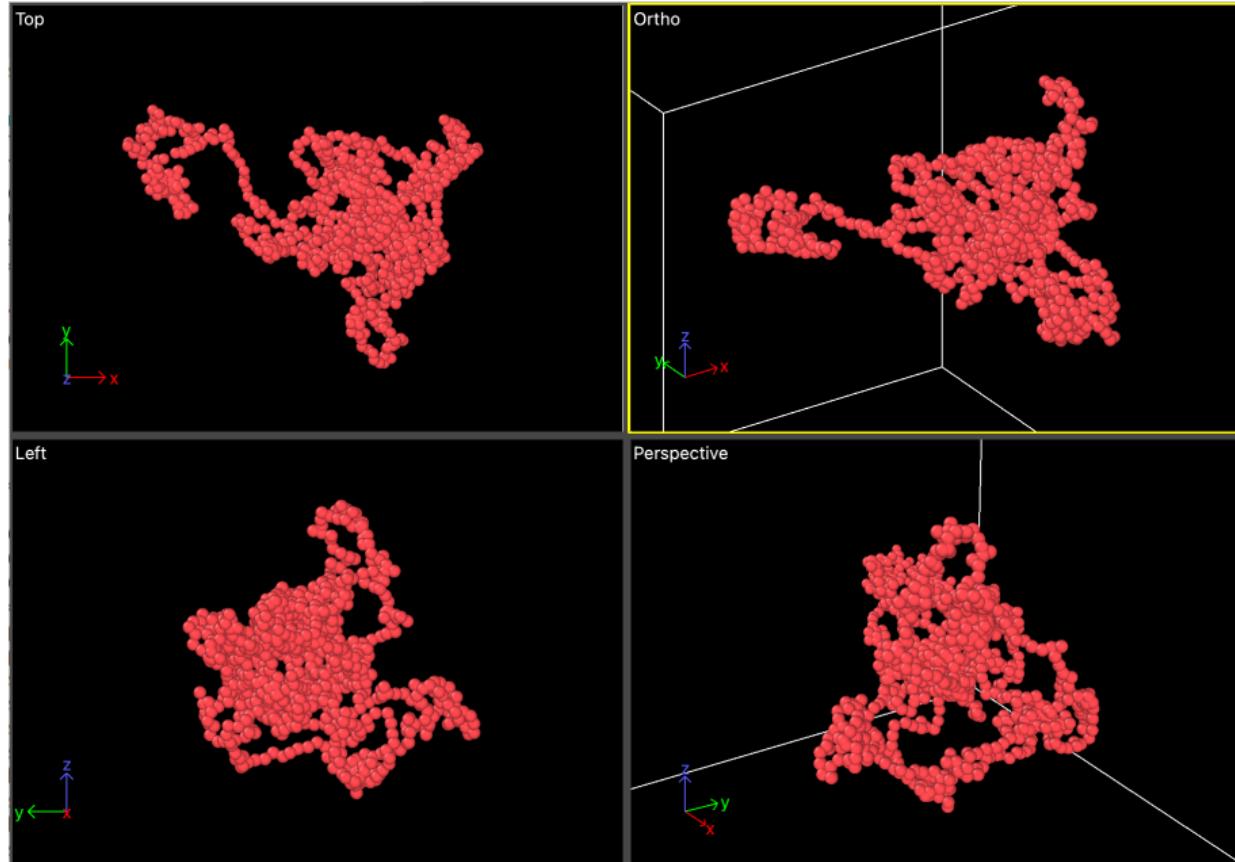
d



BRD4 phosphorylation state is necessary for maintenance of chromatin structure

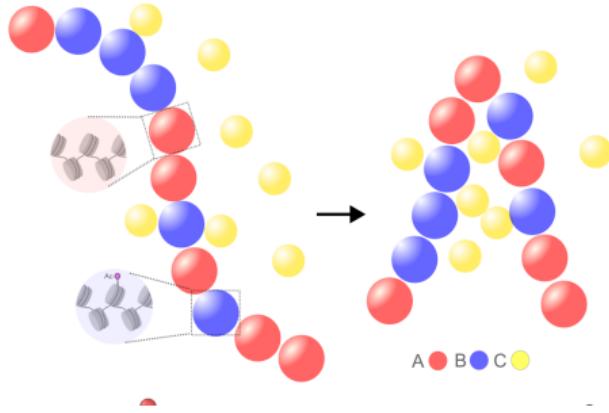


Coarse grained molecular dynamics of chromatin at 310K

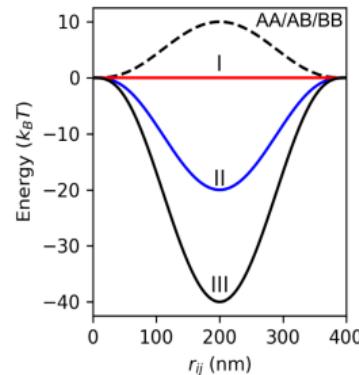


Coarse grained molecular dynamics of chromatin binders at 310K

a



b

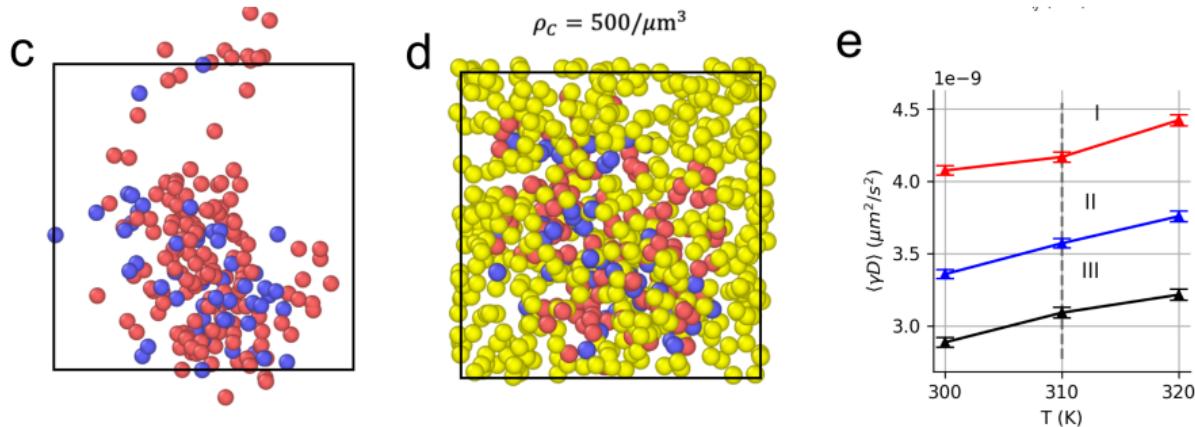


100kb chromatin chains interact with binders via the potential

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

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

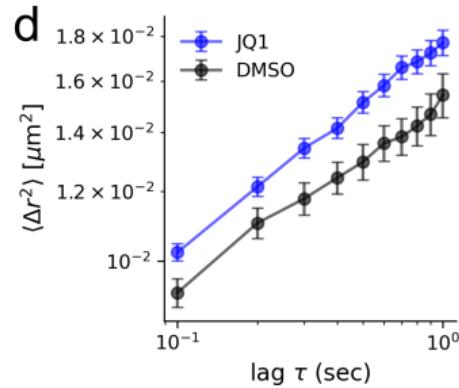
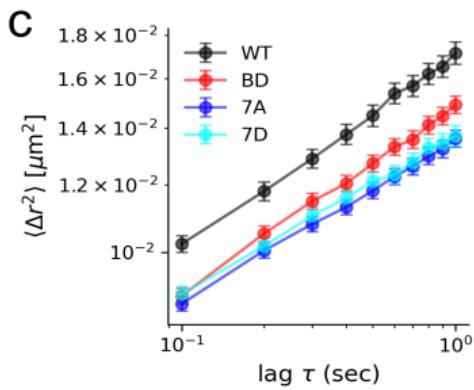
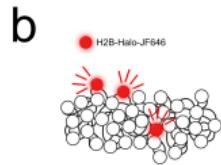
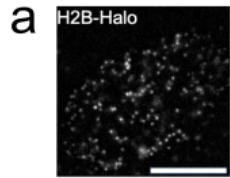
Multivalent chromatin binding reduces chromatin mobility



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