

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

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Outline

Introduction to fluorescence nanoscopy

Toward noiseless fluorescence nanoscopy

Enhanced nanoscopy with deep generative models

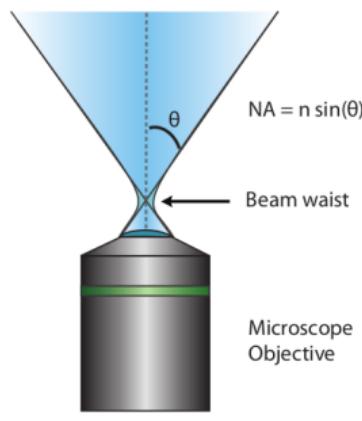
Enhanced nanoscopy with a single photon avalanche diode array

Super-resolution of nucleosome nanodomains *in-vivo*

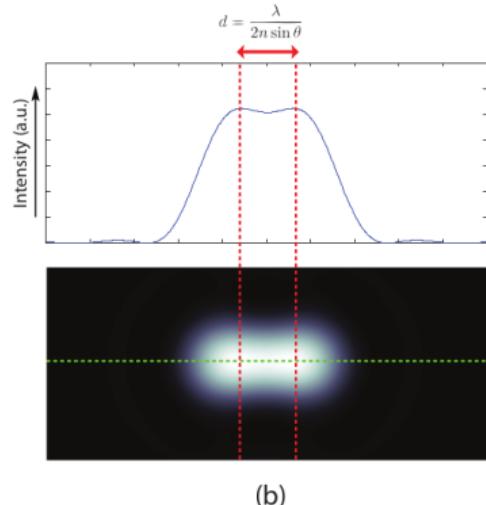
Introduction to fluorescence nanoscopy

Fluorescence microscopy and the diffraction limit

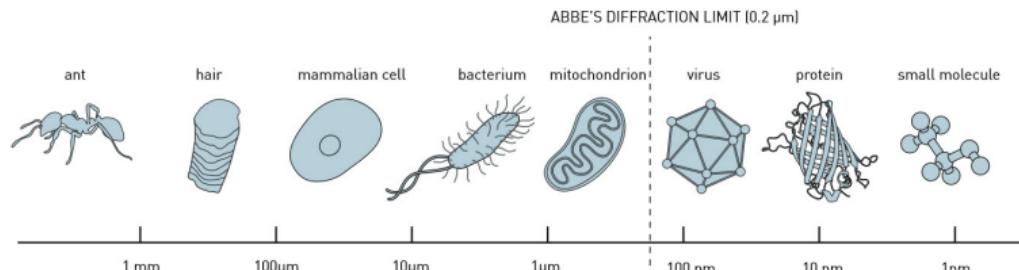
Minimal resolvable distance $d \sim \lambda$



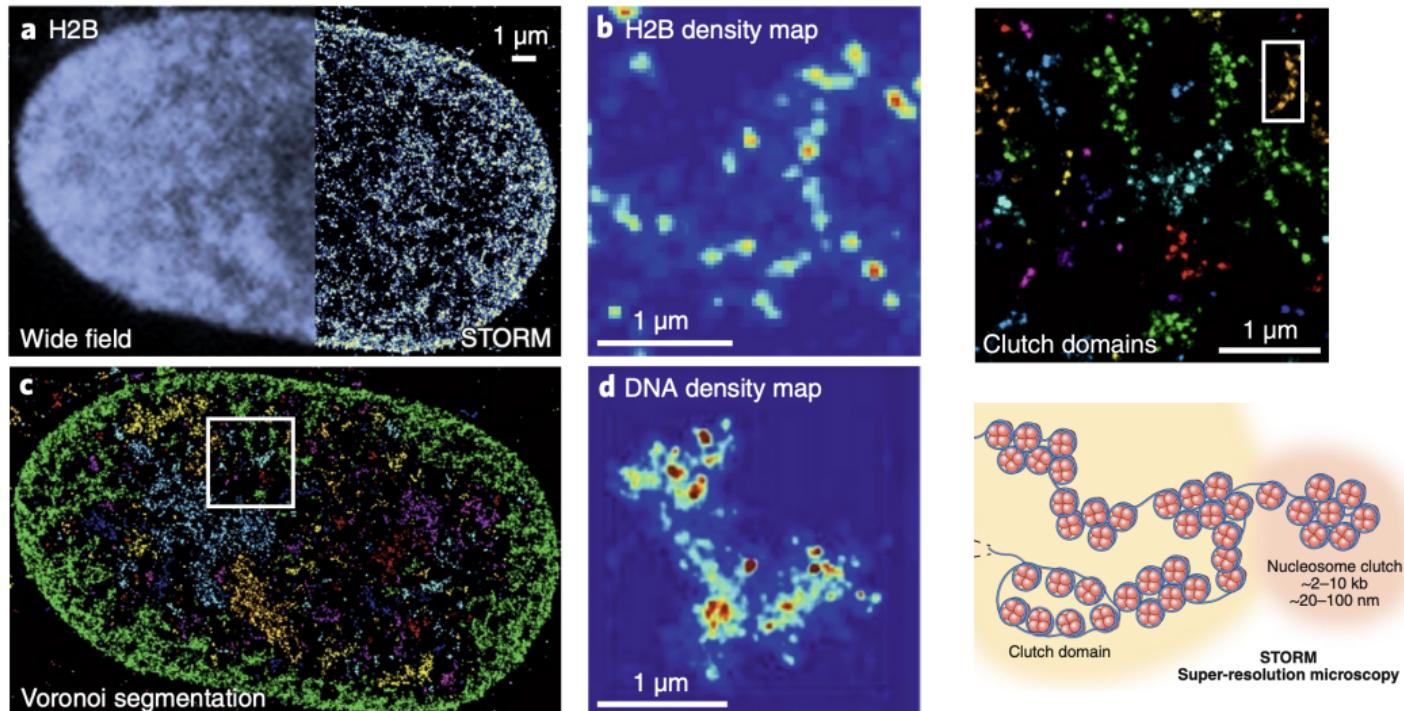
(a)



(b)

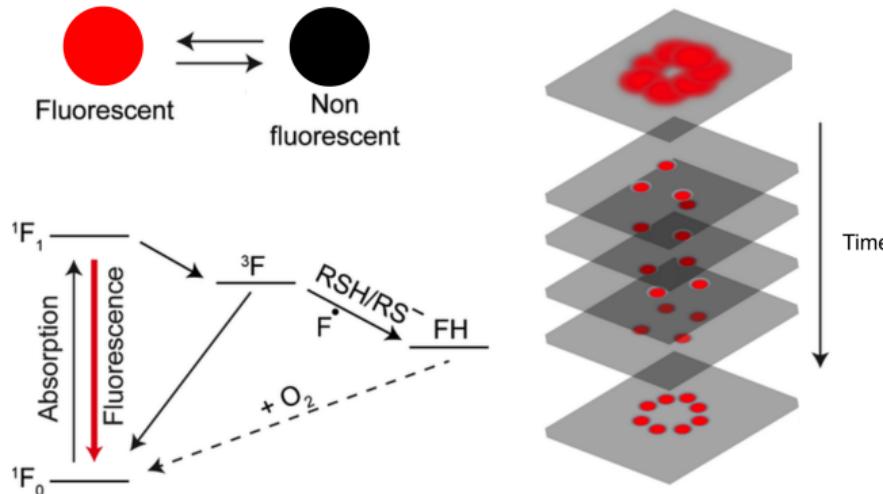


Stochastic optical reconstruction microscopy (STORM)



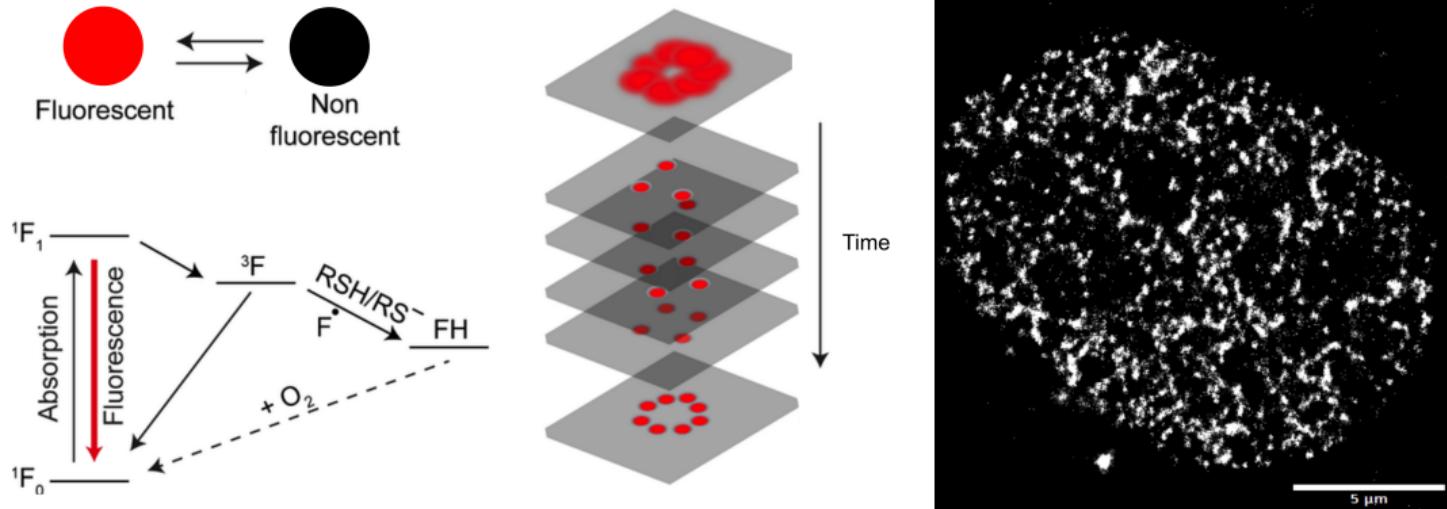
Lakadamyali, M. et al. Nature Methods 17, (2020).

Stochastic optical reconstruction microscopy (STORM)



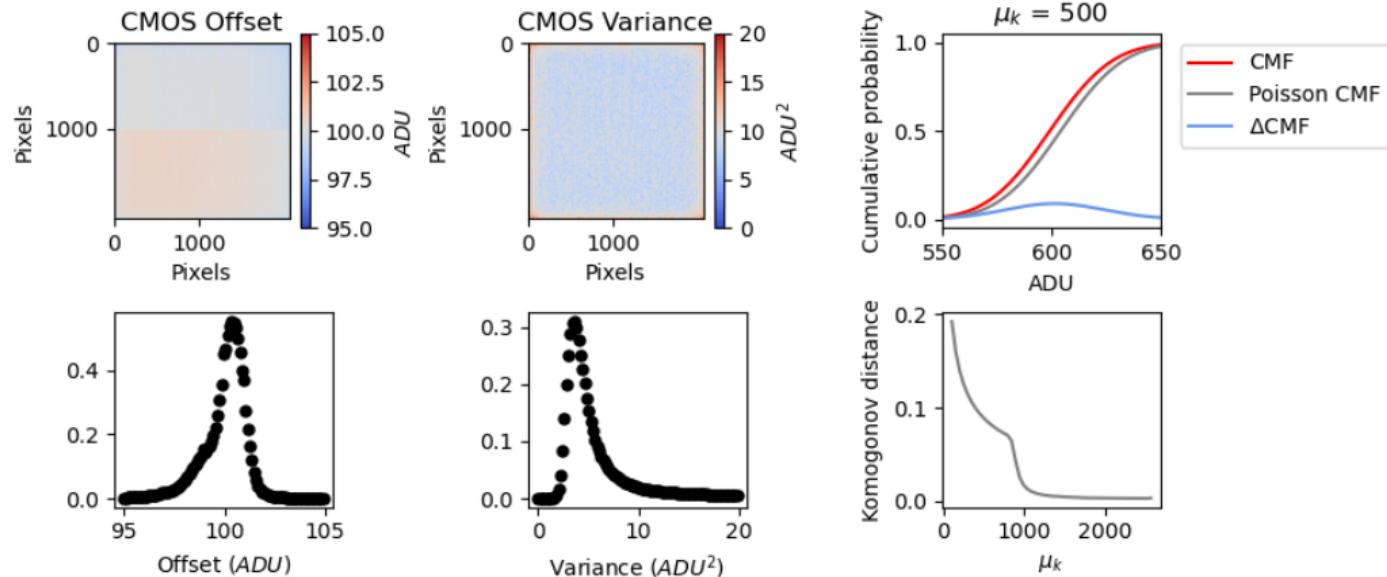
- ▶ STORM and similar nanoscopy techniques are diffraction-unlimited
- ▶ Photoswitching enables resolution of emitters below the diffraction limit

Stochastic optical reconstruction microscopy (STORM)



- ▶ STORM and similar nanoscopy techniques are diffraction-unlimited
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Experimental facts of life



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

Nanoscopy by localizing isolated fluorescent emitters

Modeling the point spread function permits sub-pixel localization

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

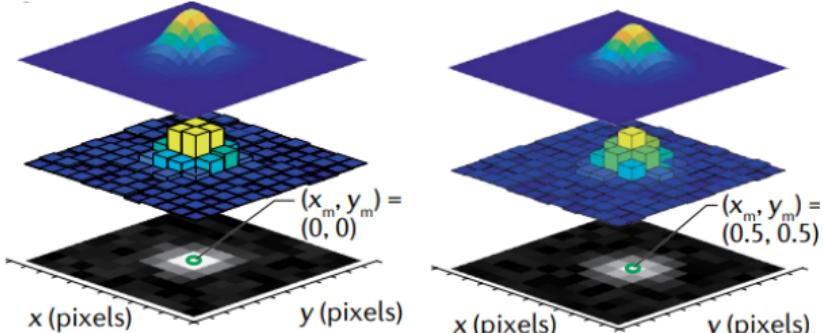
$$i_0 = g_k \eta N_0 \Delta$$

g_k – pixel gain

η – quantum efficiency

N_0 – photon emission rate

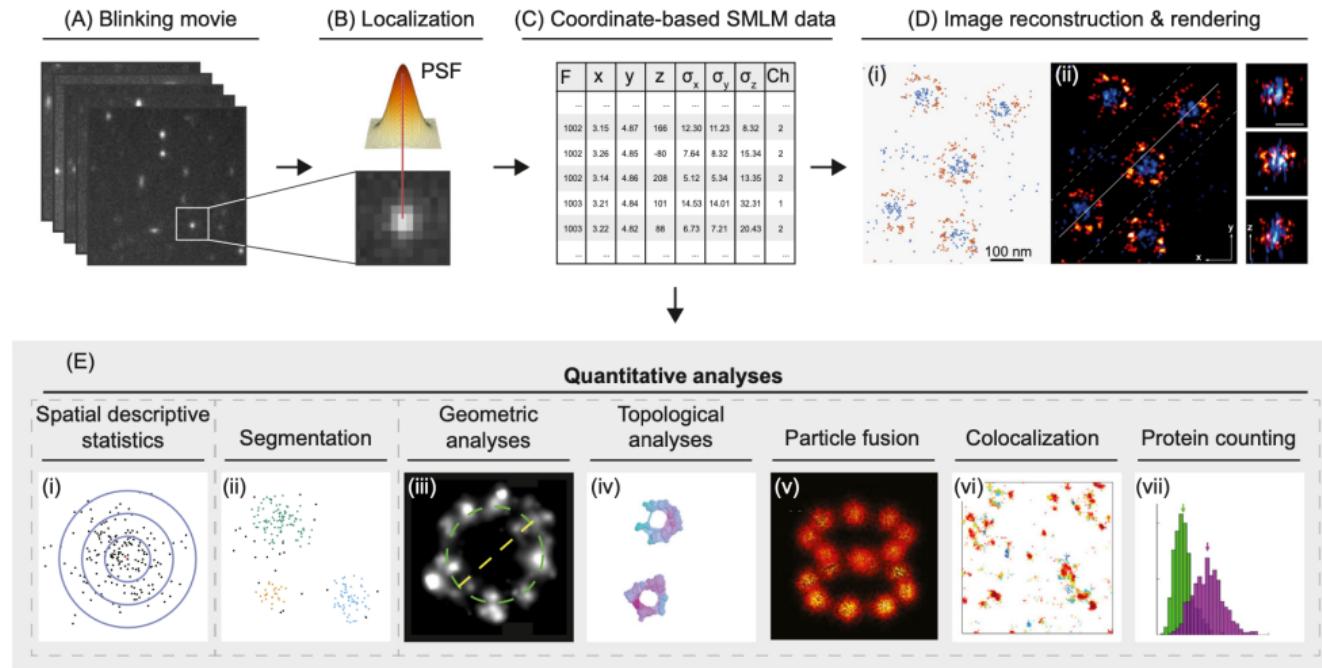
Δ – exposure time



Assume N_0 is constant over Δ (homogeneous Poisson)

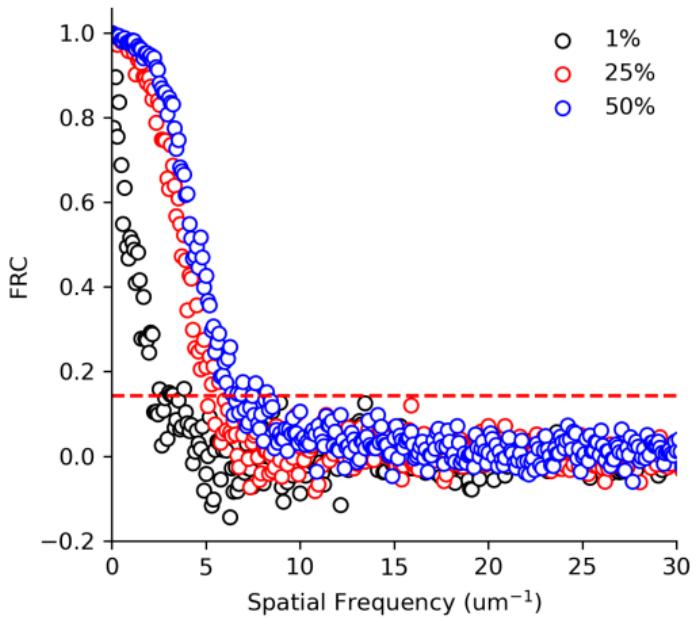
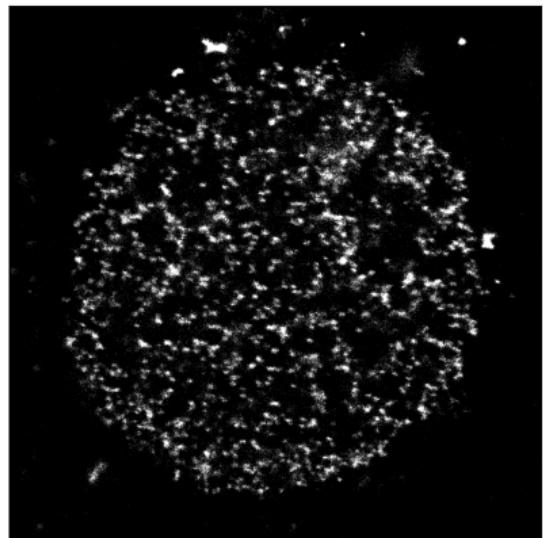
$$\theta^* = \operatorname{argmax}_{\theta} \prod_k p(\mathbf{x}_k | \theta) = \operatorname{argmin}_{\theta} - \sum_k \log p(\mathbf{x}_k | \theta)$$

Applications of single molecule localization microscopy

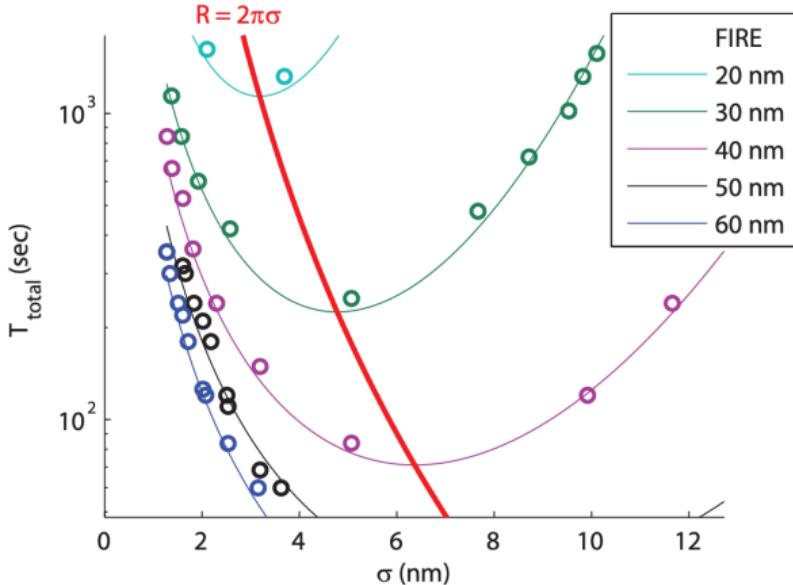


Wu et al. Trends in Cell Biology. 30 (2020)

How do we define resolution in localization microscopy?



Fourier Image Resolution (FIRE)



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

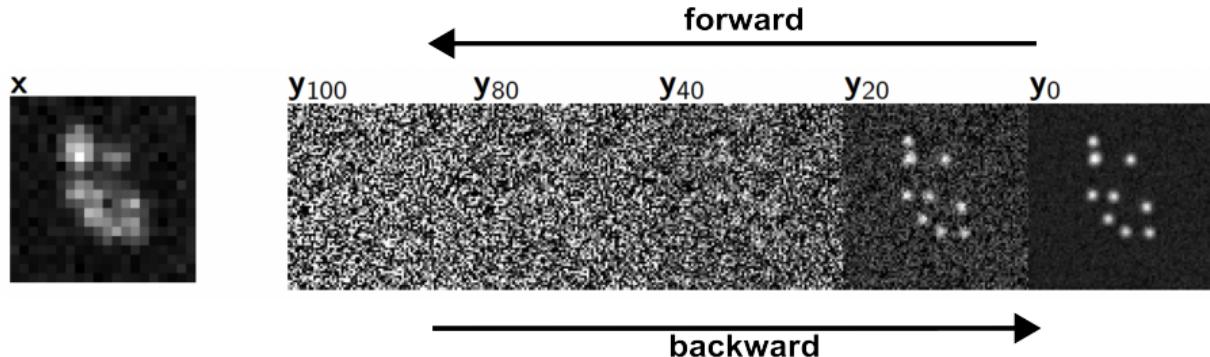
Nutshell: *How to relax the density limit in localization microscopy?*

Toward noiseless fluorescence nanoscopy

Bayesian image restoration with diffusion models

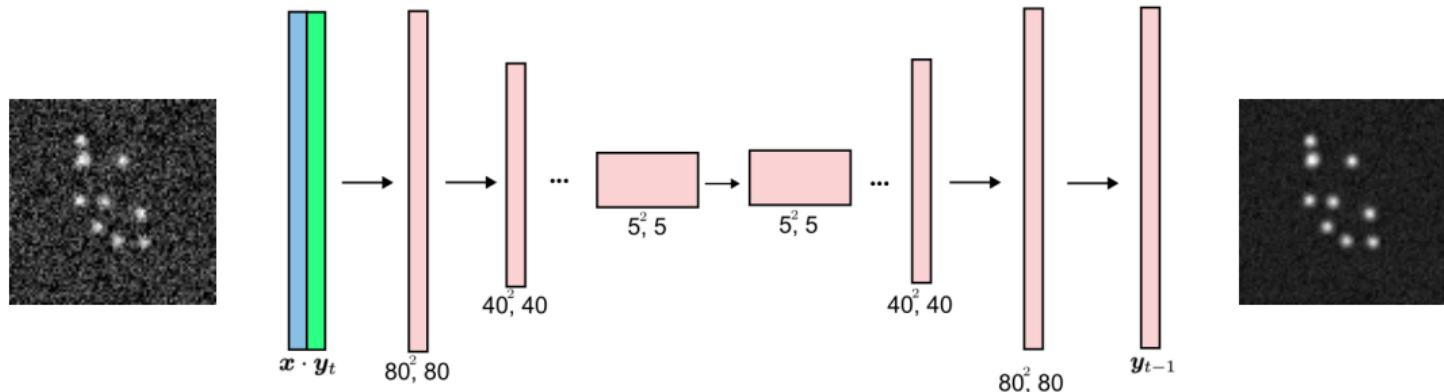
Inference of a high resolution image \mathbf{y} from low resolution \mathbf{x} is approached by modeling a distribution $p_\psi(\mathbf{y}|\mathbf{x})$ with a diffusion model

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



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

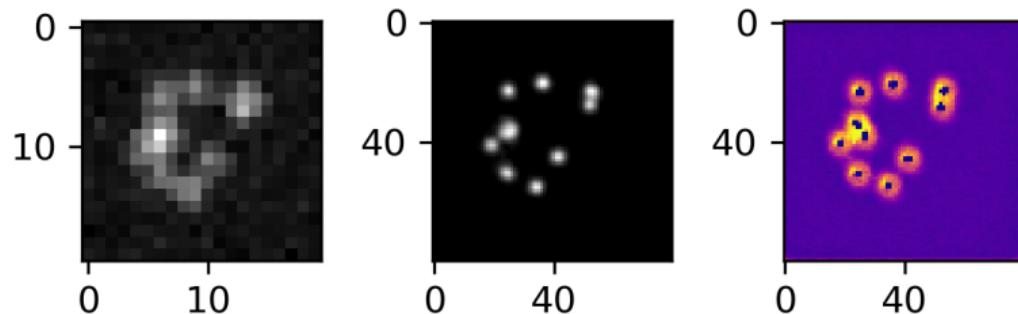
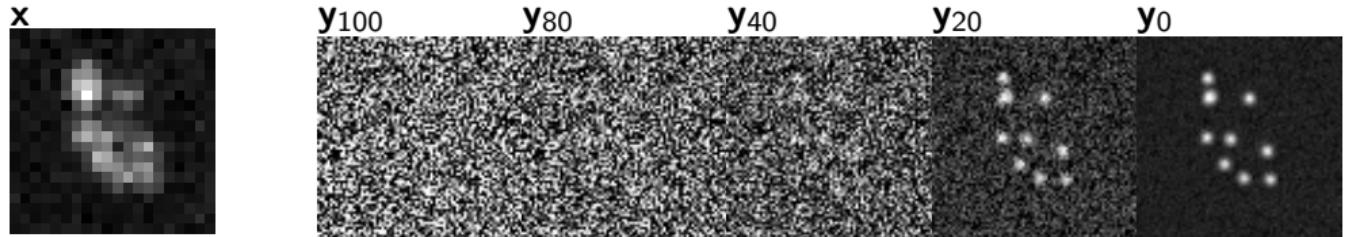
Bayesian image restoration with diffusion models



A deep neural network estimates the gradient of the reverse process

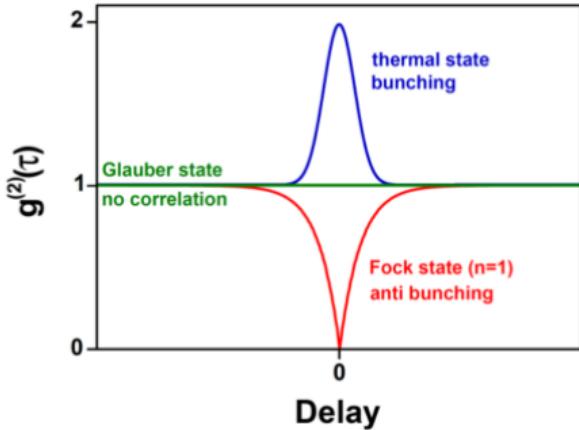
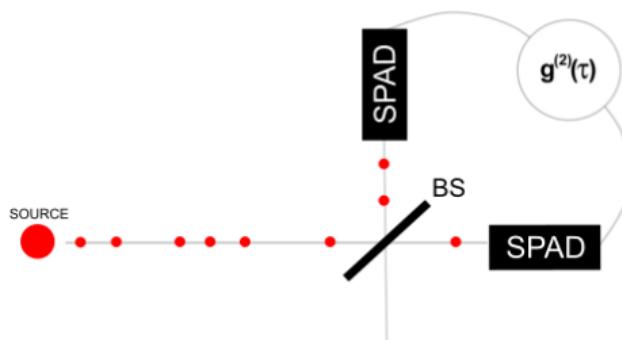
$$\mathbf{y}_{t-1} = \frac{1}{\sqrt{1 - \beta_t}} (\mathbf{y}_t + \beta_t s_\psi(\mathbf{y}_t)) + \sqrt{\beta_t} \xi \quad \xi \sim \mathcal{N}(0, I)$$

Bayesian image restoration with diffusion models



A powerful class of methods but generally lacks a physically constrained number of fluorescent emitters...

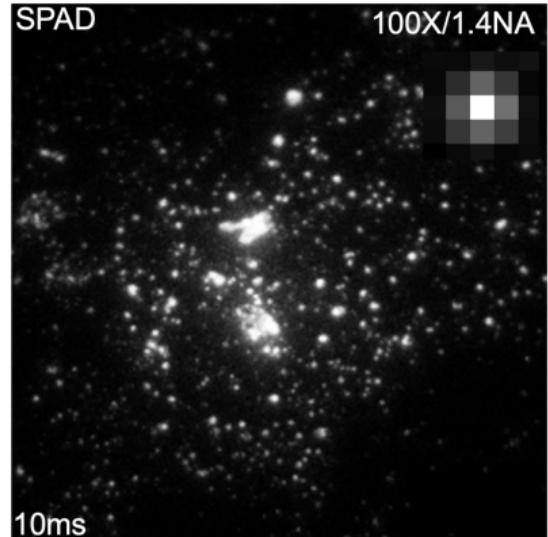
The Hanbury Brown and Twiss Effect



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

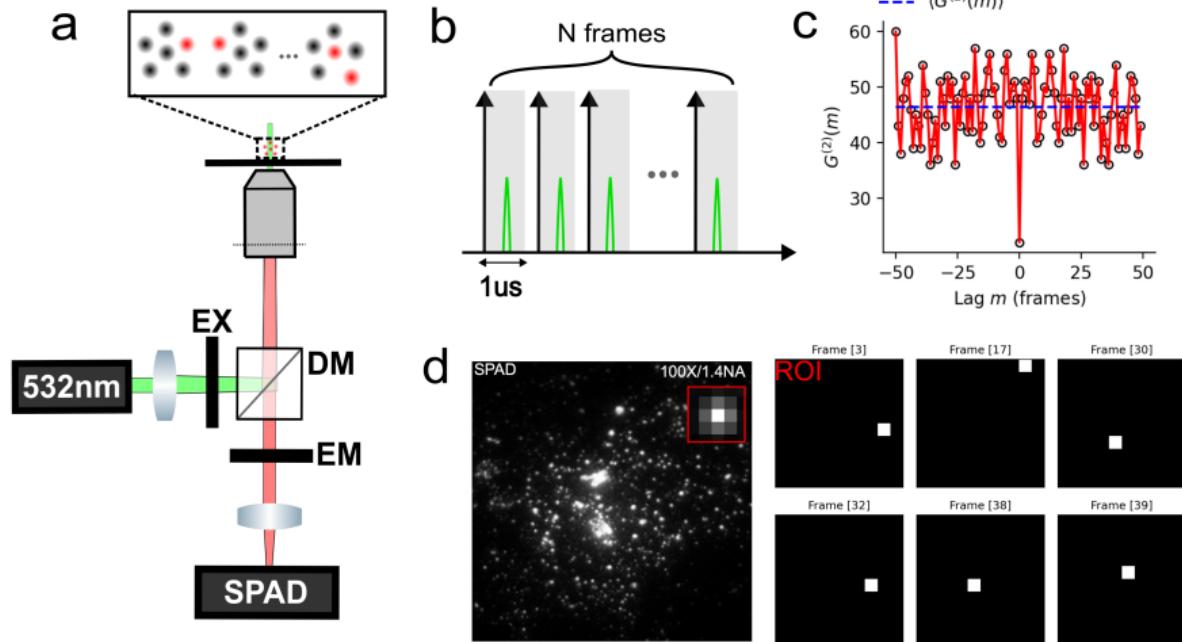
- ▶ Single photon sources (such as a fluorescent dye) exhibit antibunching
- ▶ Magnitude of $g^{(2)}(0)$ "dip" depends on the number of fluorescent emitters
- ▶ Provides a means of counting fluorescent emitters

Widefield photon counting with a SPAD array

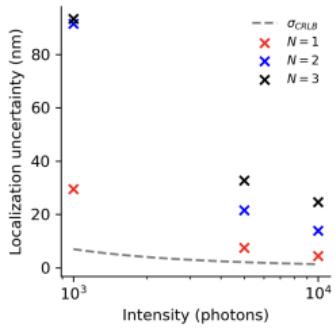
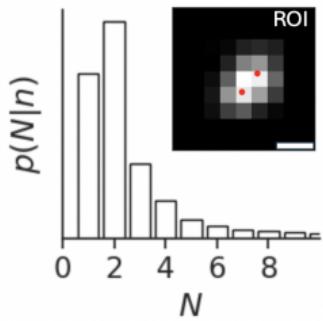
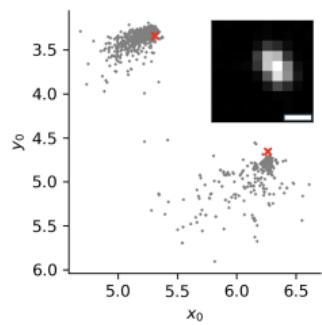
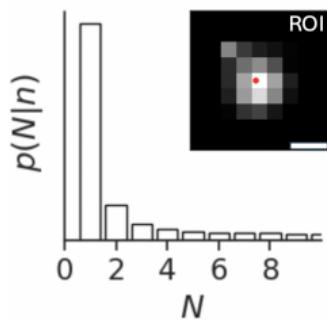


- ▶ Spatial resolution of photon counts
- ▶ Fast exposures as low as 20 nanoseconds

Imaging Qdot655 photon by photon



Constrained multi-emitter localization with photon counting

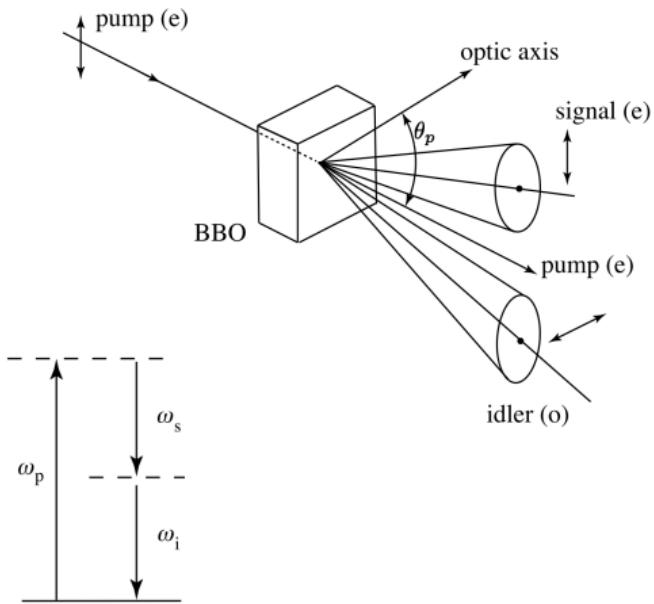
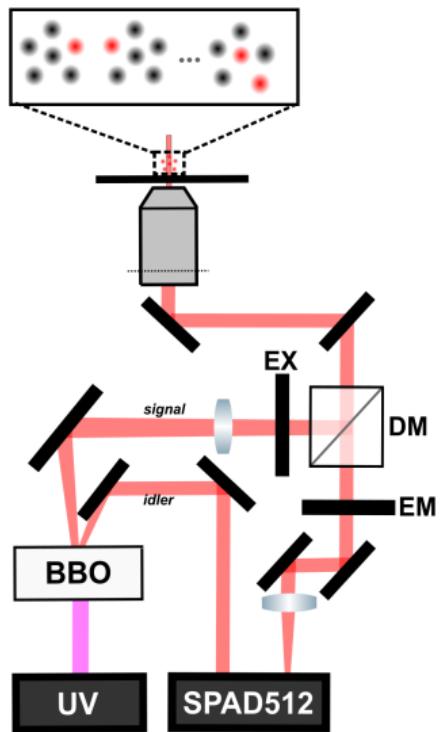


Posterior distribution on (N, ζ) :

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

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

Heralding single photons with parametric downconversion



Heralding single photons with parametric downconversion



ROI 1

ROI 2

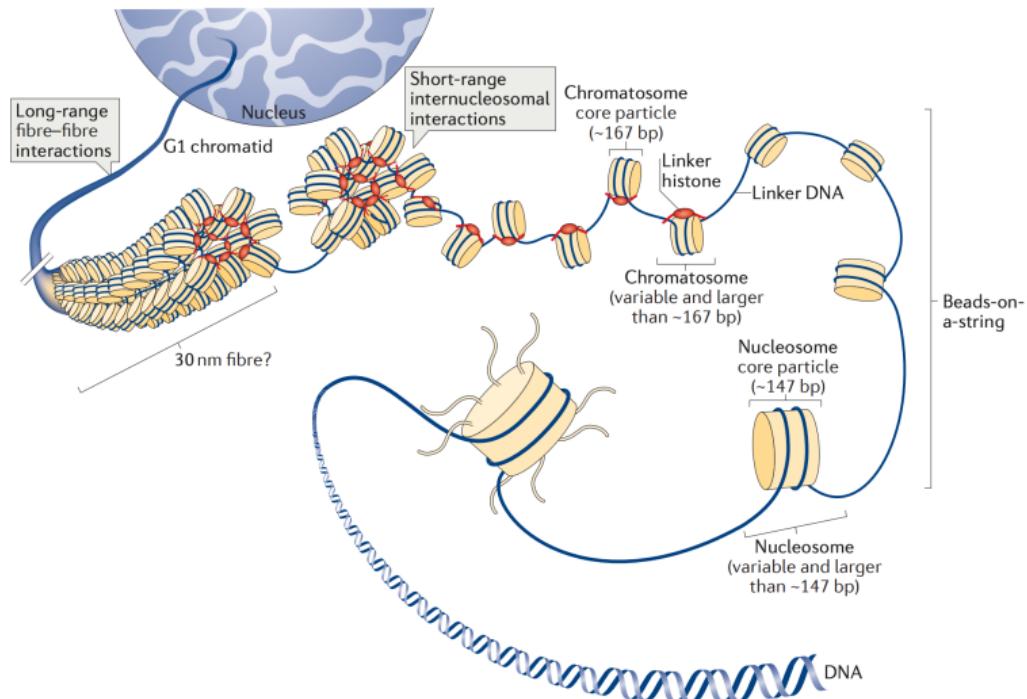
AND-image

$\left(\begin{array}{l} \text{rotated by} \\ \text{angle of } \pi \end{array} \right)$

- ▶ Detection of dark counts or background counts
- ▶ Noise free nanoscopy

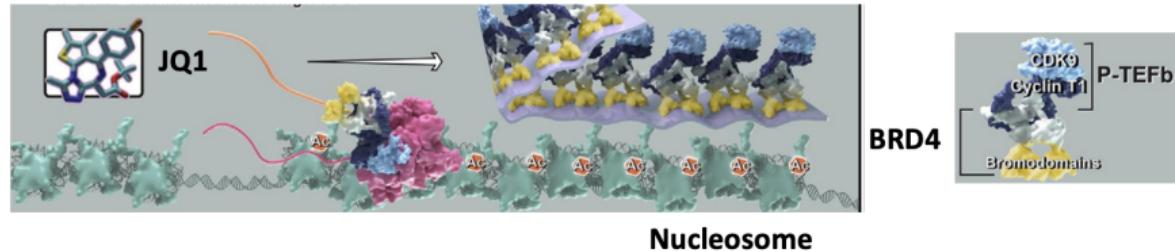
Super-resolution of nucleosome nanodomains *in-vivo*

Hierarchical structure of chromatin

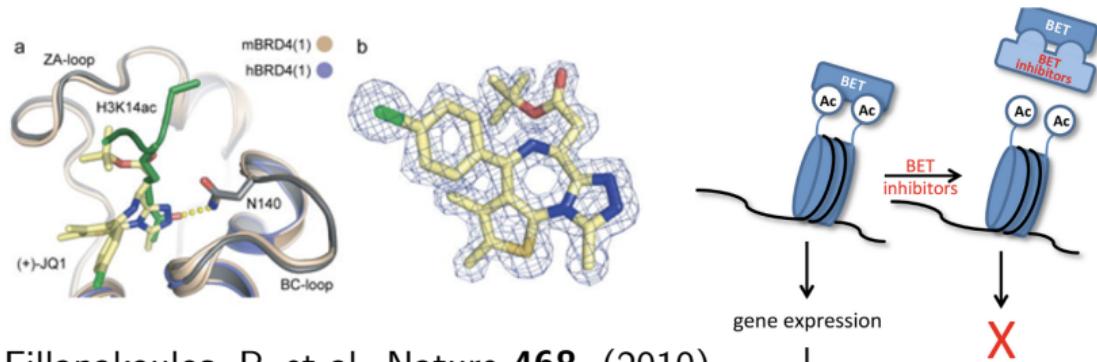


Fyodorov, D. et al. Nat Rev Mol Cell Biol **19**, (2018).

Bromodomain protein 4 (BRD4) binds acetylated chromatin

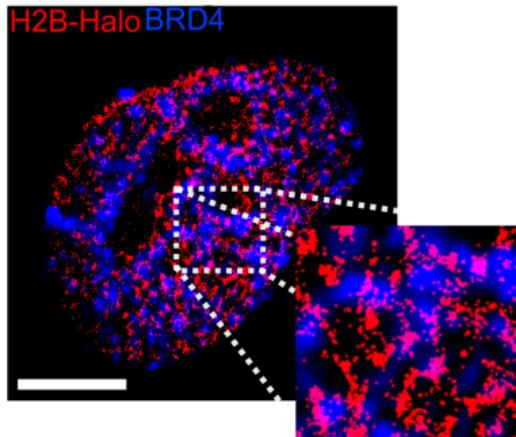


Zheng, B. et al. Molecular Cell **16**, (2023).



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

Bromodomain protein 4 (BRD4) binds acetylated chromatin

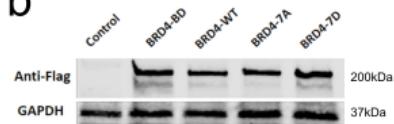


BRD4 phosphorylation state is necessary for maintenance of chromatin structure

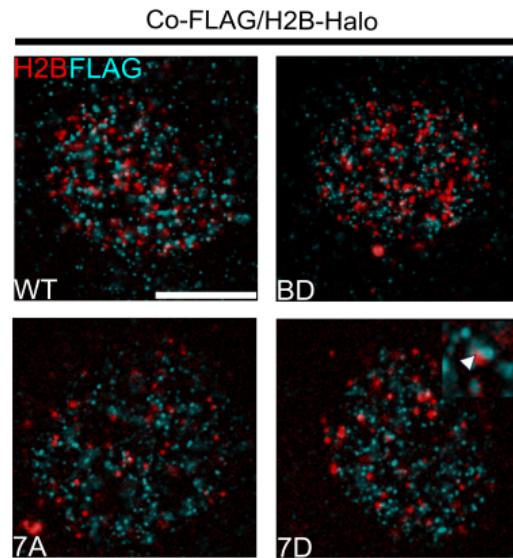
a



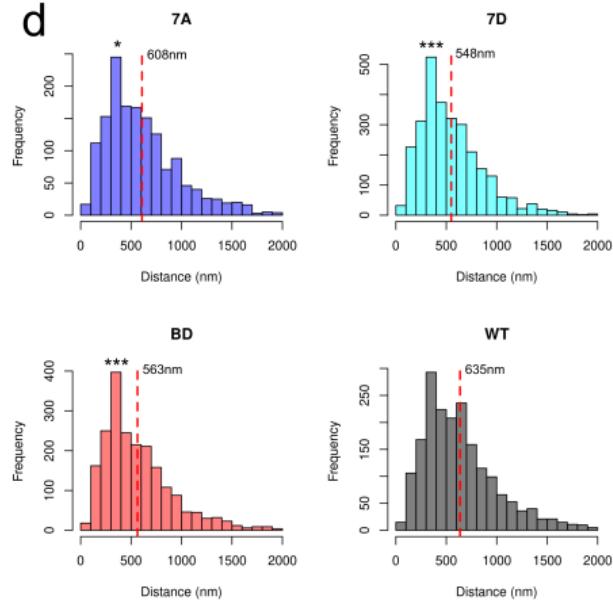
b



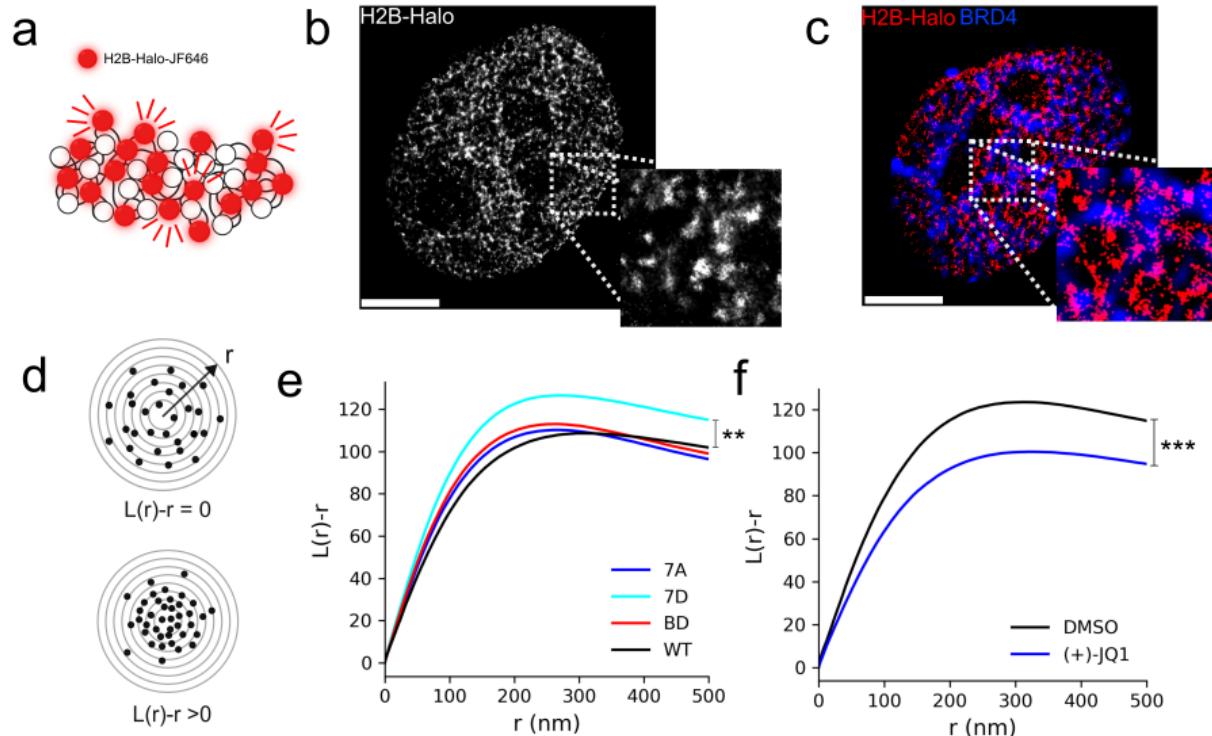
c



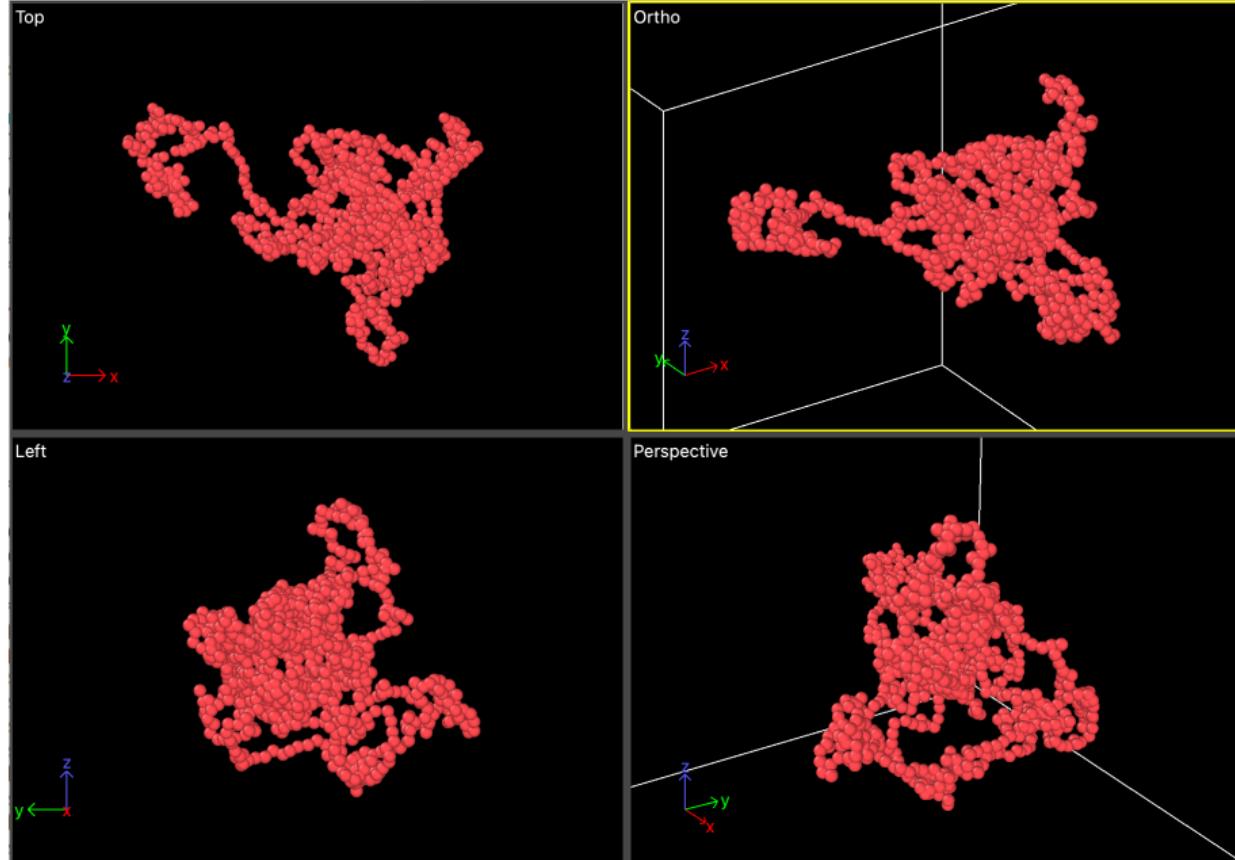
d



BRD4 phosphorylation state is necessary for maintenance of chromatin structure

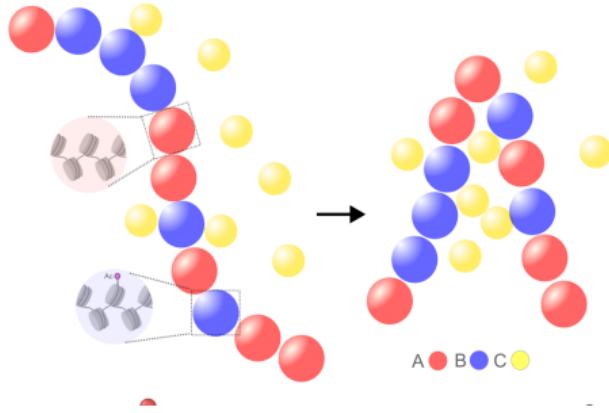


Coarse grained molecular dynamics of chromatin at 310K

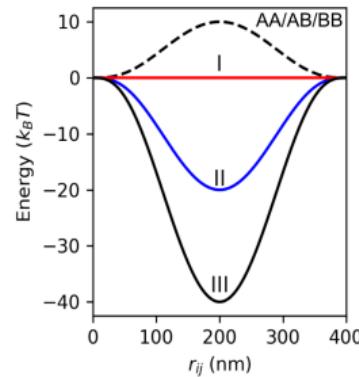


Coarse grained molecular dynamics of chromatin binders at 310K

a



b

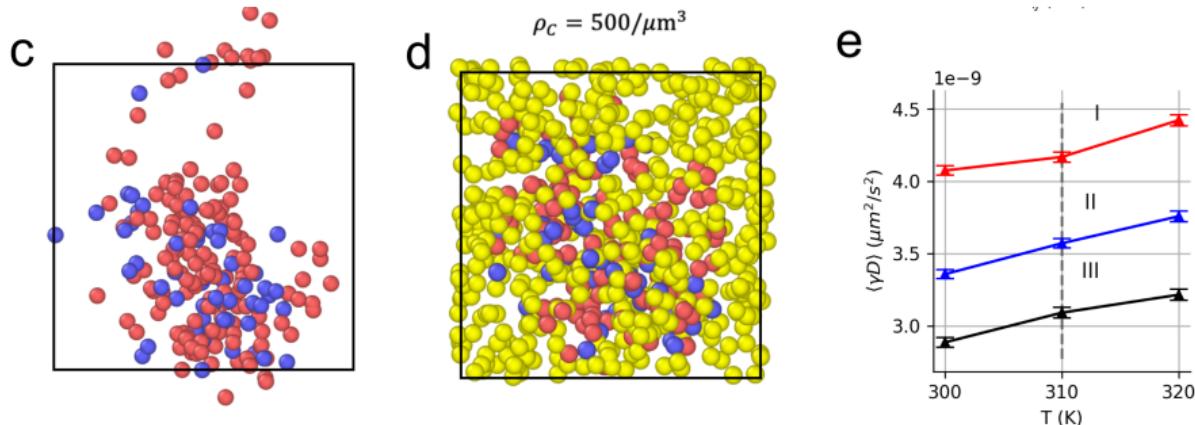


100kb chromatin chains interact with binders via the potential

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

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

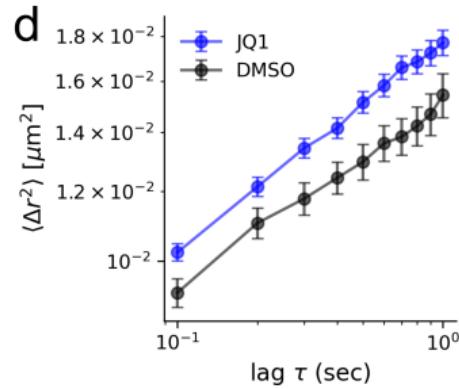
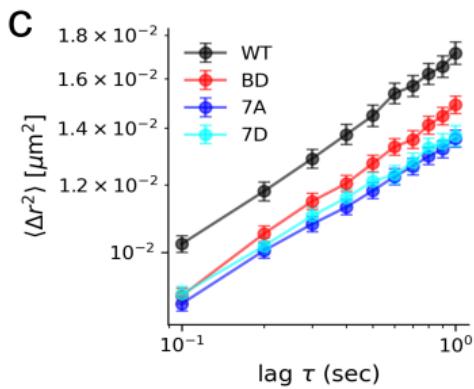
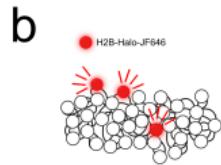
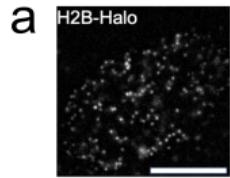
Multivalent chromatin binding reduces chromatin mobility



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