

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

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Outline

Introduction to fluorescence nanoscopy

Method 1: Enhanced nanoscopy with single photon avalanche diode (SPAD) cameras

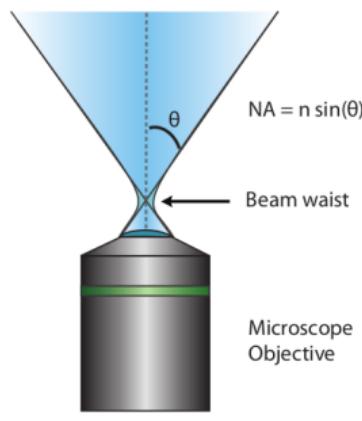
Method 2: Enhanced nanoscopy with deep generative models

Super-resolution of nucleosome nanodomains *in-vivo*

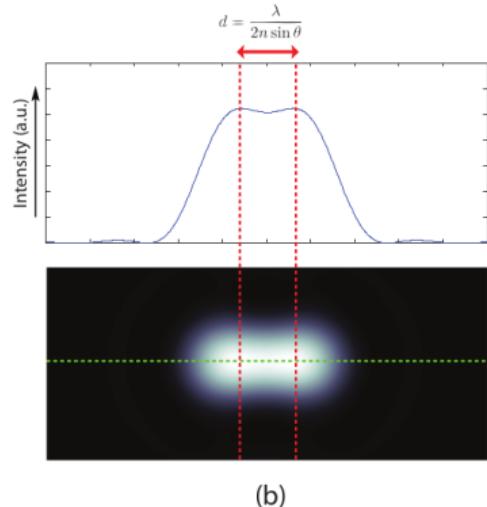
Introduction to fluorescence nanoscopy

Fluorescence microscopy and the diffraction limit

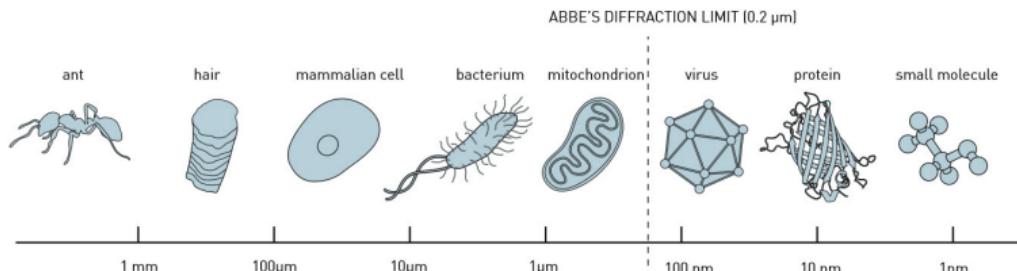
Minimal resolvable distance $d \sim \lambda$



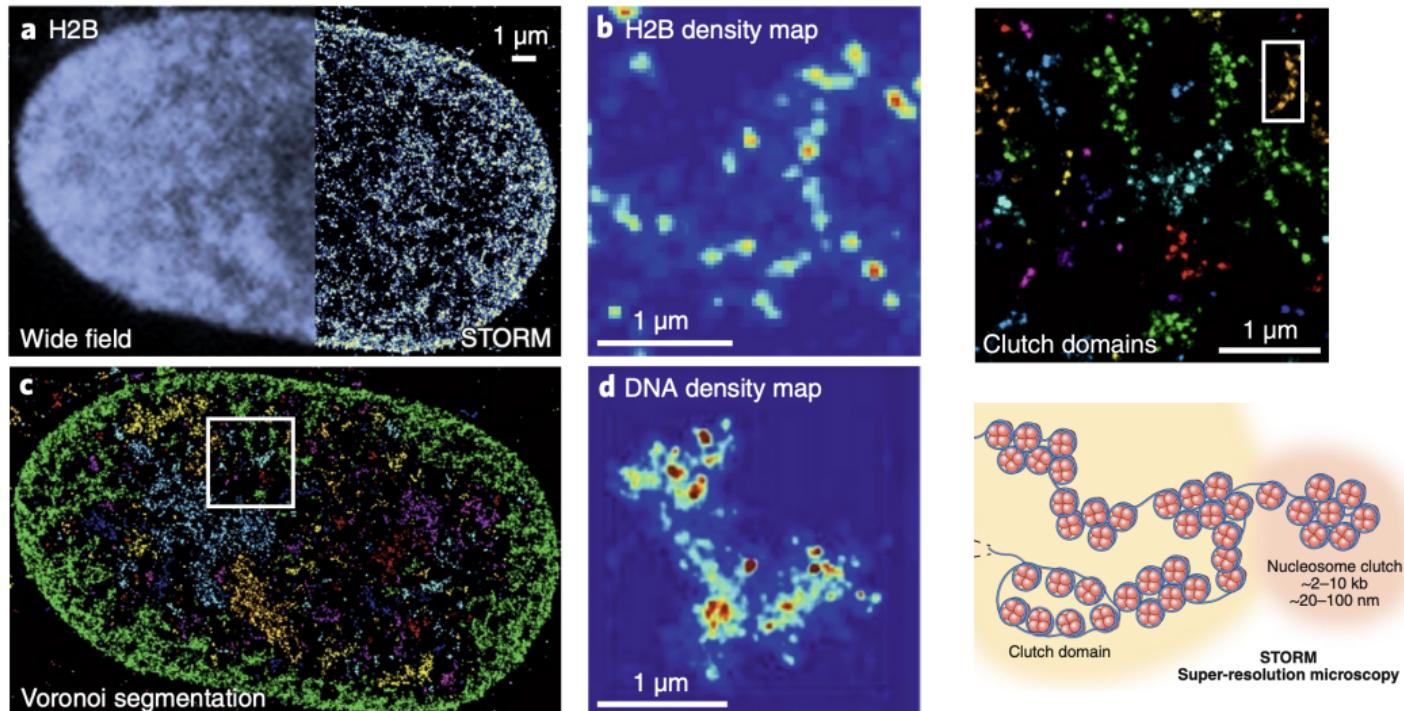
(a)



(b)

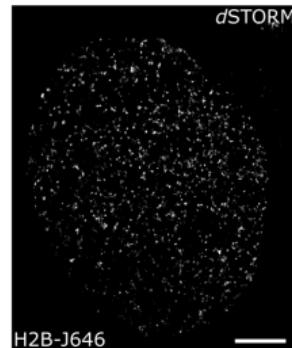
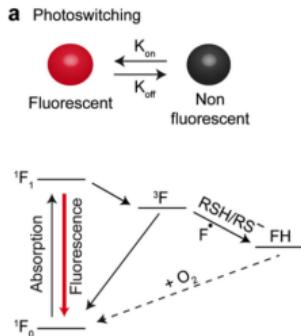


Stochastic optical reconstruction microscopy (STORM)



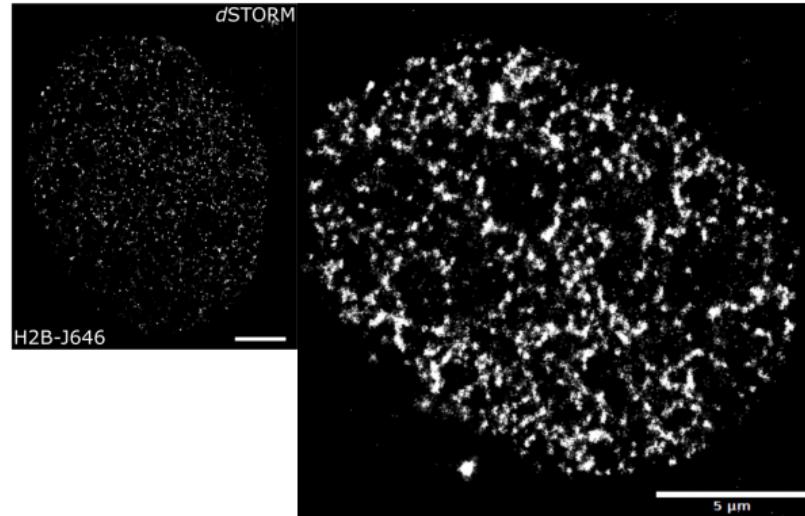
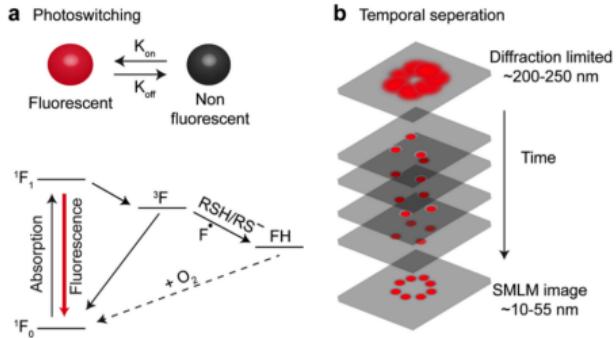
Lakadamyali, M. et al. Nature Methods 17, (2020).

Stochastic optical reconstruction microscopy (STORM)



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

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Vanilla SMLM by localizing isolated fluorescent emitters

Modeling the point spread function permits sub-pixel localization

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

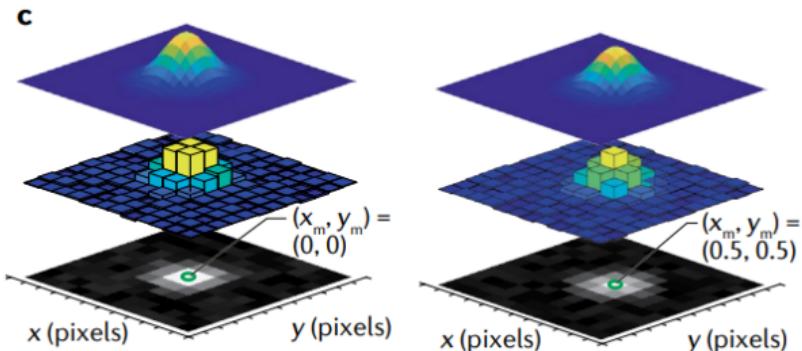
$$i_0 = g_k \eta N_0 \Delta$$

g_k – pixel gain

η – quantum efficiency

N_0 – photon emission rate

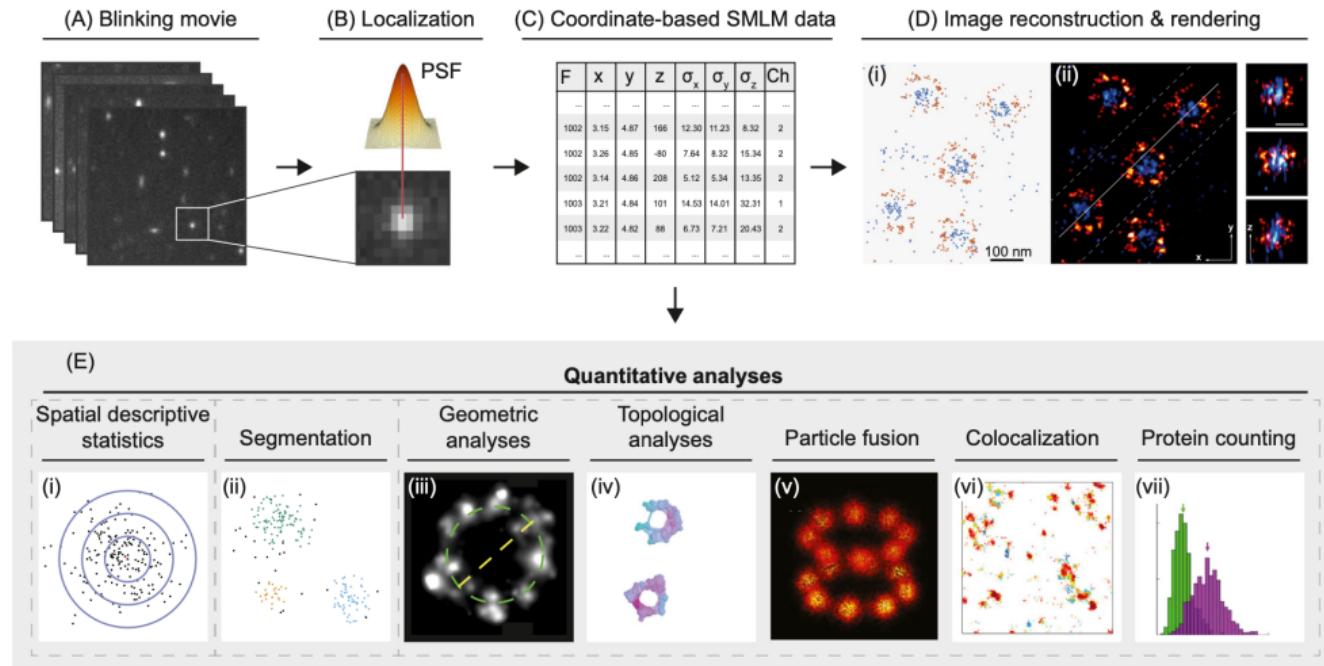
Δ – exposure time



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



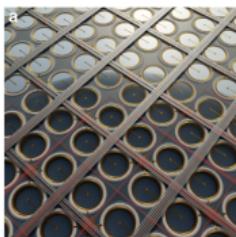
Wu et al. Trends in Cell Biology. 30 (2020)

Method 1: Enhanced nanoscopy with single photon avalanche diode (SPAD) cameras

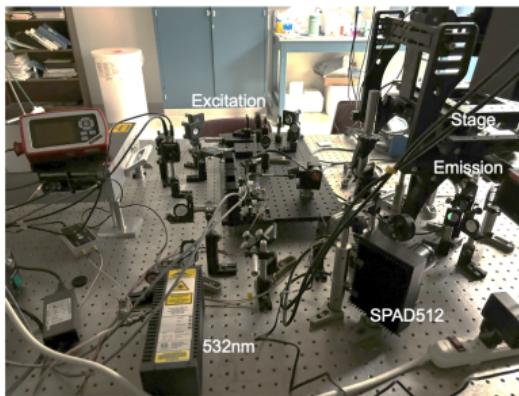
Enhanced nanoscopy with single photon avalanche diode (SPAD) cameras



SPAD512



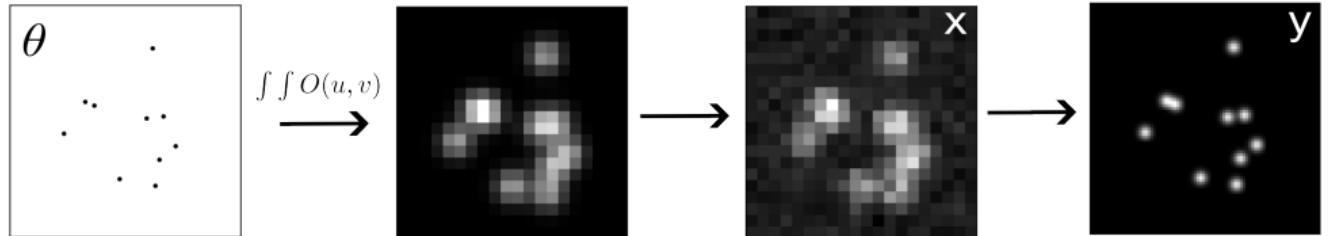
a



- ▶ Exposures as low as 1 microsecond
- ▶ Fast gated imaging for widefield fluorescence lifetime imaging
- ▶ Zero readout noise (imaging weak fluorescent signals)

Method 2: Enhanced nanoscopy with deep generative models

How to pack more localizations in a single frame?



$$p(\mathbf{x}_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\omega_k} \omega_k^q \frac{1}{\sqrt{2\pi w_k}} e^{-\frac{(\mathbf{x}_k - g_k q - o_k)^2}{2w_k^2}} \approx \text{Poisson}(\omega'_k)$$

- ▶ Would like to estimate a high-resolution \mathbf{y} from low-resolution \mathbf{x} , but it is many to one
- ▶ Must then model a *distribution* over \mathbf{y} i.e., $p_\theta(\mathbf{y}|\mathbf{x})$

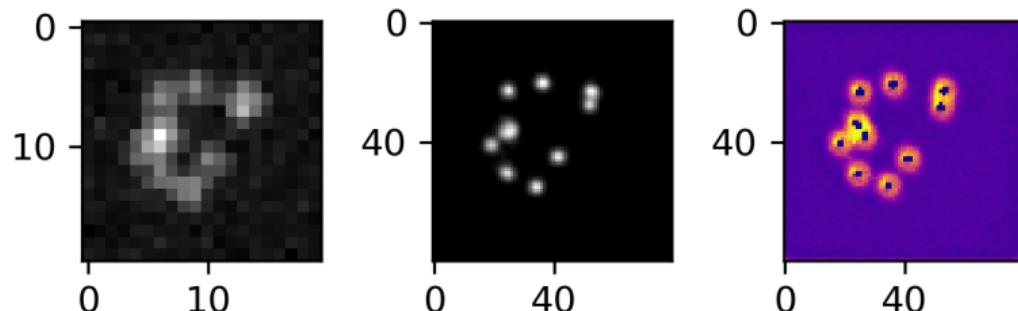
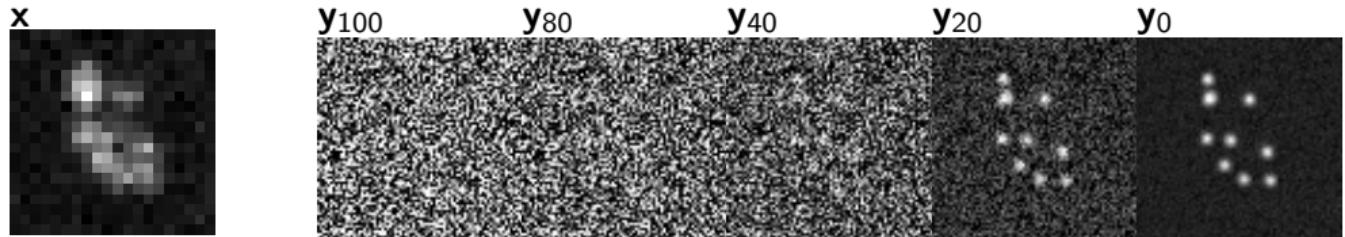
Trivial example of sampling from a mixture of Gaussians

Consider a two-component Gaussian mixture $p(\mathbf{x}) = \sum_{k=1}^2 \pi_k \mathcal{N}(\mu_k, \sigma_k^2)$

If we know $\nabla \log p(\mathbf{x})$ we can sample from $p(\mathbf{x})$ with Langevin dynamics:

$$\mathbf{x}_i = \mathbf{x}_{i-1} + \epsilon \nabla \log p(\mathbf{x}) + \sqrt{2\epsilon} \eta \quad \eta \sim \mathcal{N}(0, I)$$

Bayesian image restoration with diffusion models

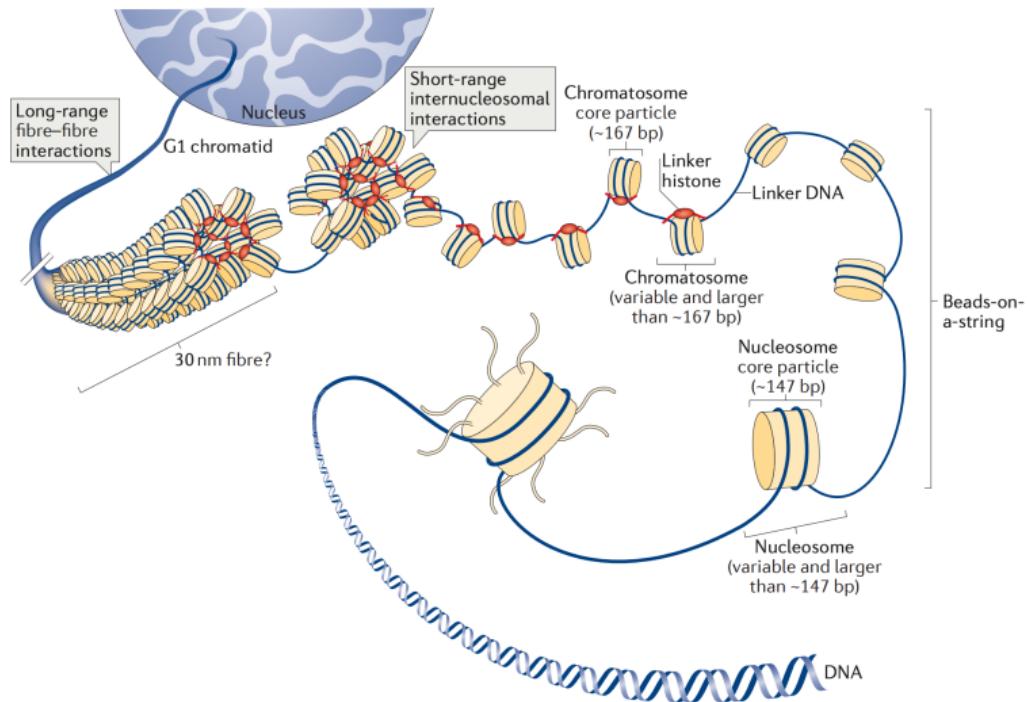


Need to approximate the gradient $s_\theta(\mathbf{y}_t) \approx \nabla \log p(\mathbf{x})$ and sample:

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

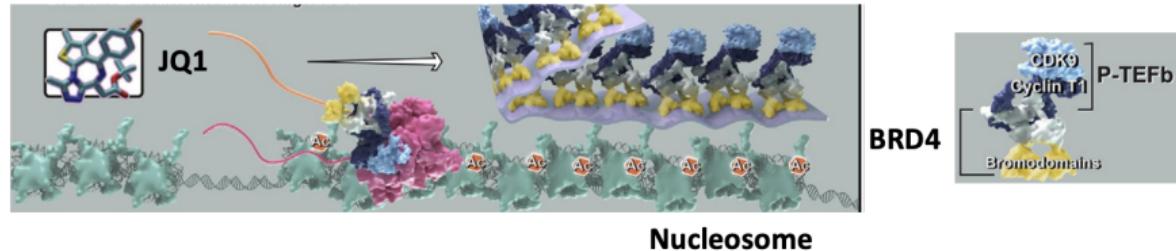
Super-resolution of nucleosome nanodomains *in-vivo*

Hierarchical structure of chromatin

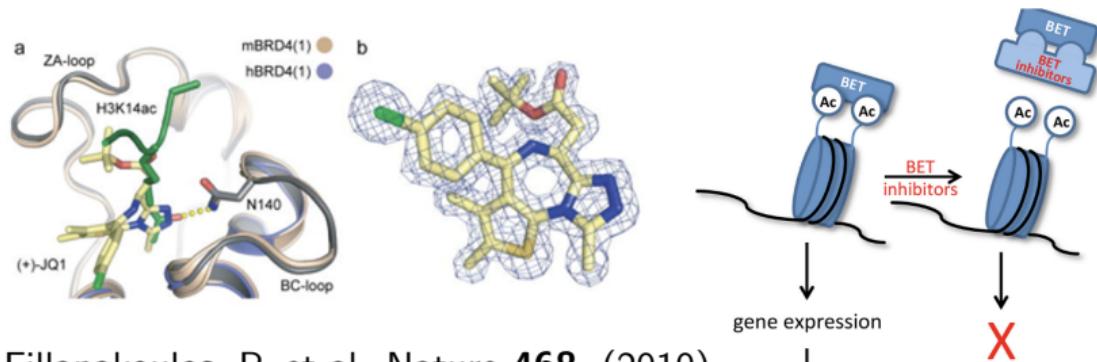


Fyodorov, D. et al. Nat Rev Mol Cell Biol **19**, (2018).

Bromodomain protein 4 (BRD4) binds acetylated chromatin

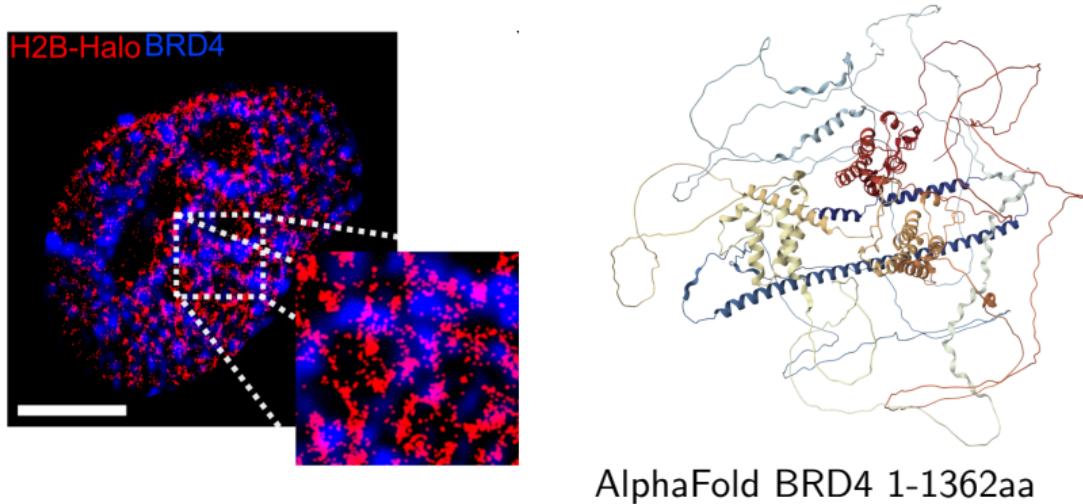


Zheng, B. et al. Molecular Cell **16**, (2023).

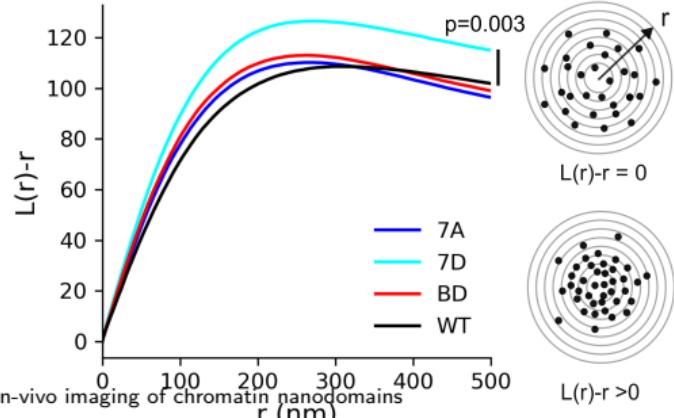
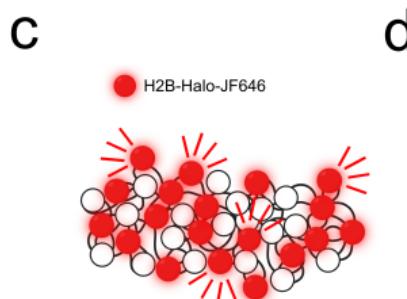
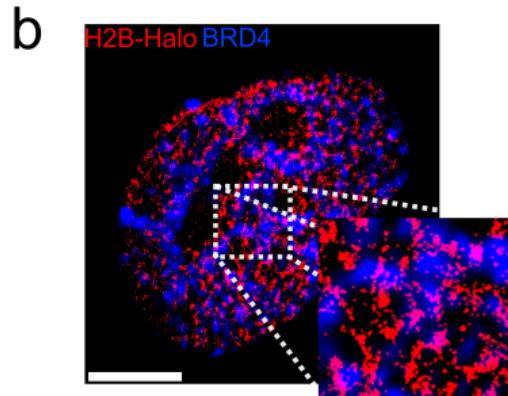
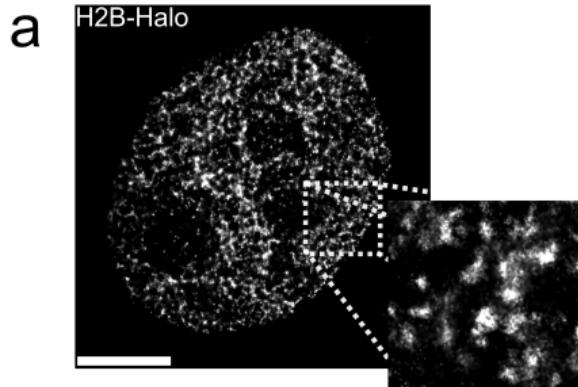


Fillapakoulos, P. et al. Nature **468**, (2010).

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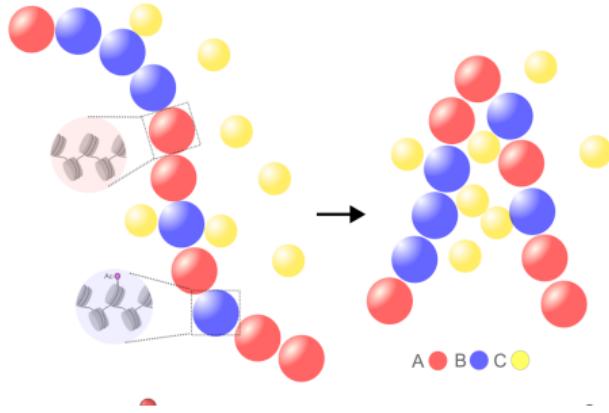


BRD4 phosphorylation state is necessary for maintenance of chromatin structure

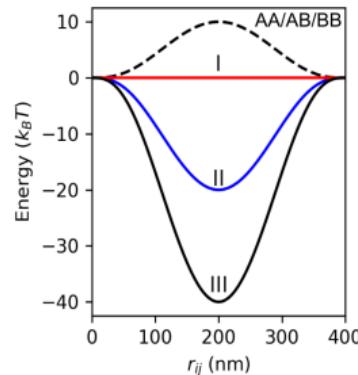


Coarse grained molecular dynamics of chromatin binders at 310K

a



b

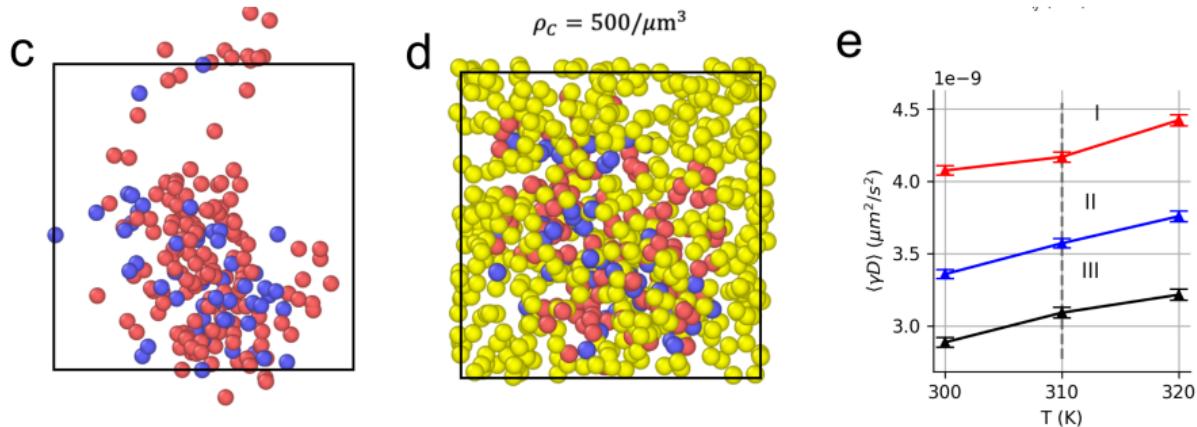


100kb chromatin chains interact with binders via the potential

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

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

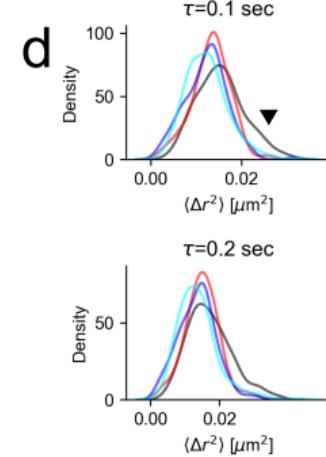
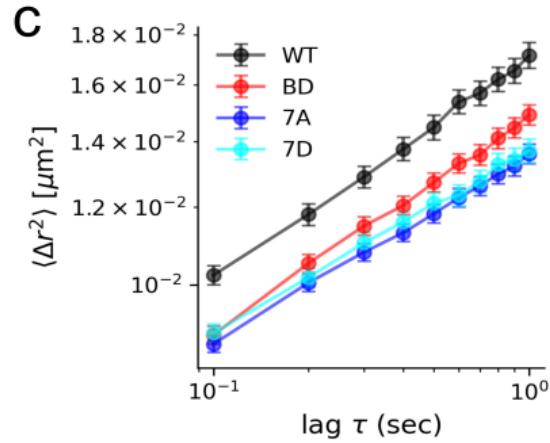
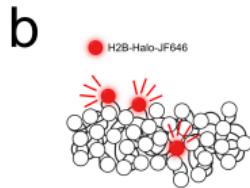
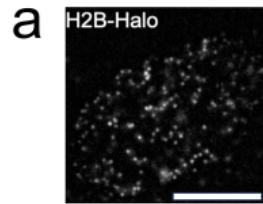
Multivalent chromatin binding reduces chromatin mobility



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