

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

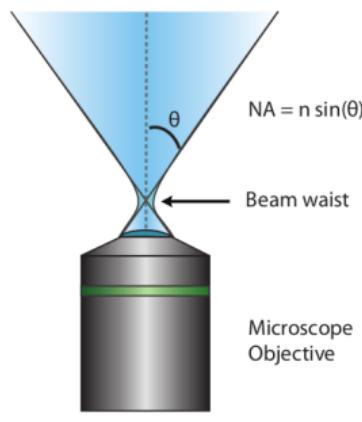
Method 2: Enhanced nanoscopy with deep generative models

Super-resolution of nucleosome nanodomains *in-vivo*

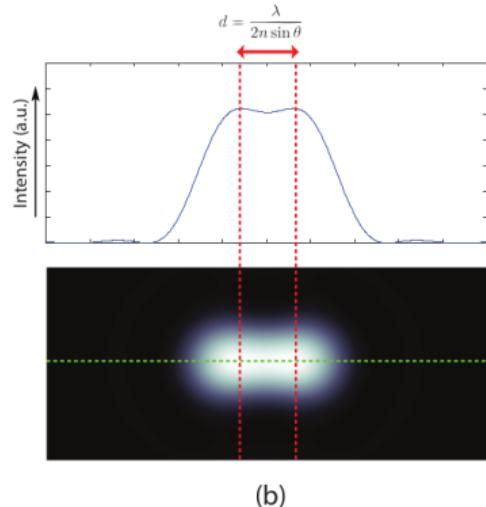
Introduction to fluorescence nanoscopy

Fluorescence microscopy and the diffraction limit

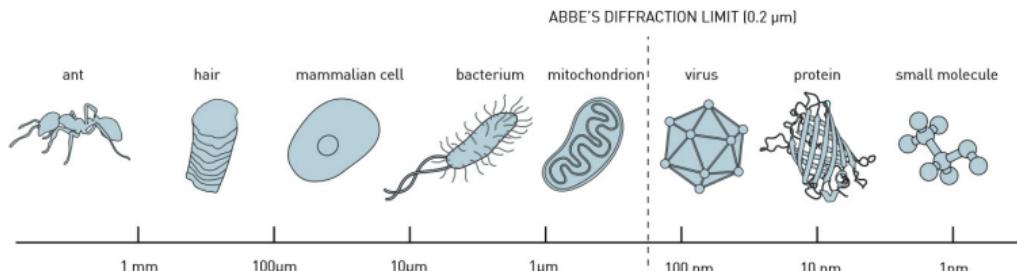
Minimal resolvable distance $d \sim \lambda$



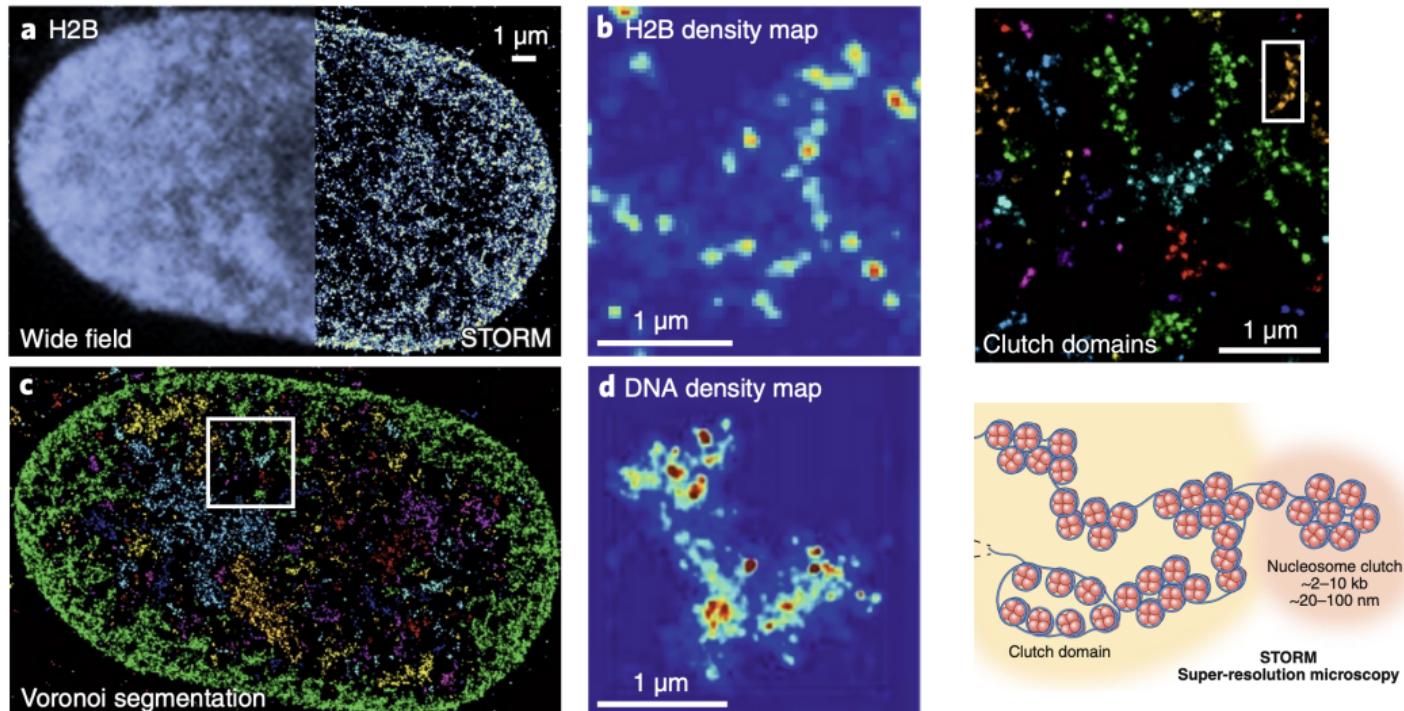
(a)



(b)



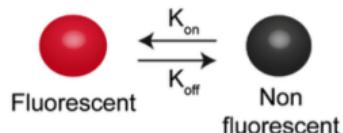
Stochastic optical reconstruction microscopy (STORM)



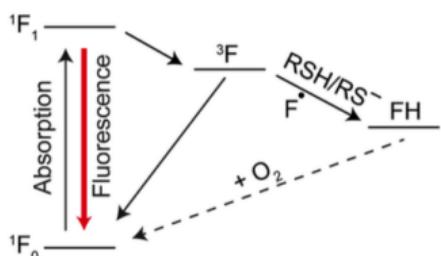
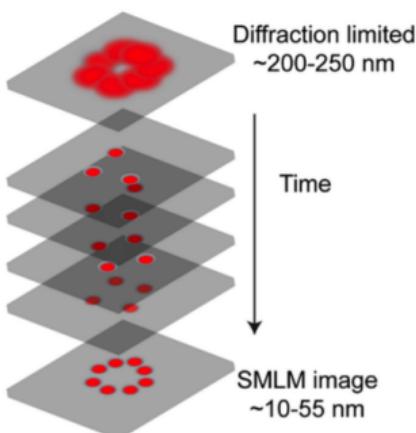
Lakadamyali, M. et al. Nature Methods 17, (2020).

Stochastic optical reconstruction microscopy (STORM)

a Photoswitching



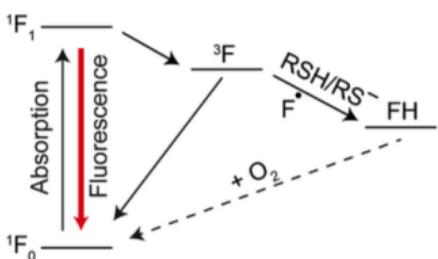
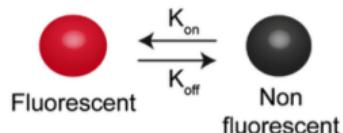
b Temporal separation



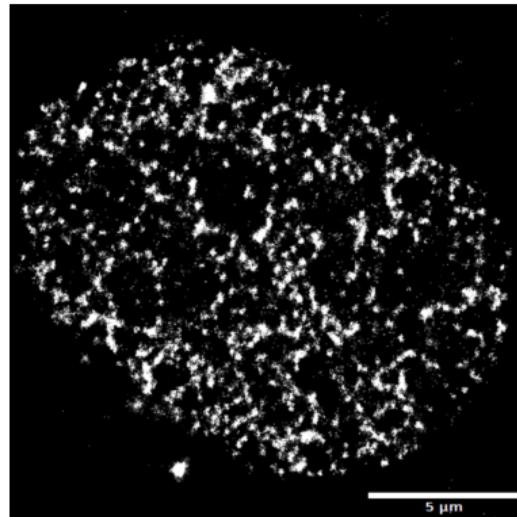
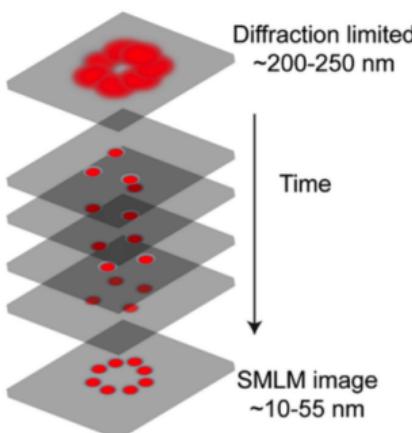
- ▶ STORM and similar nanoscopy techniques are diffraction-unlimited
- ▶ Photoswitching enables resolution of emitters below the diffraction limit

Stochastic optical reconstruction microscopy (STORM)

a Photoswitching



b Temporal separation



- ▶ 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

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

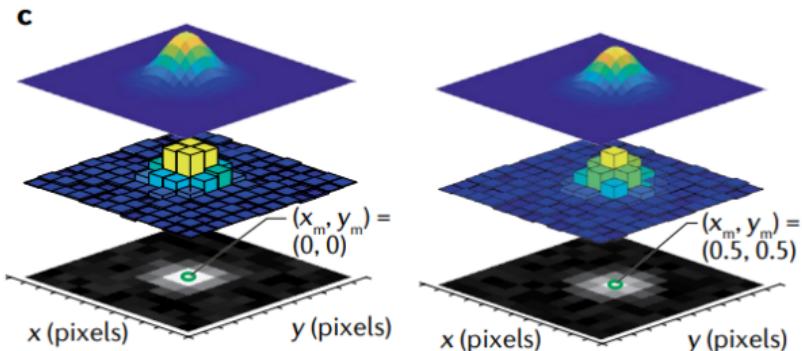
$$i_0 = g_k \eta N_0 \Delta$$

g_k – pixel gain

η – quantum efficiency

N_0 – photon emission rate

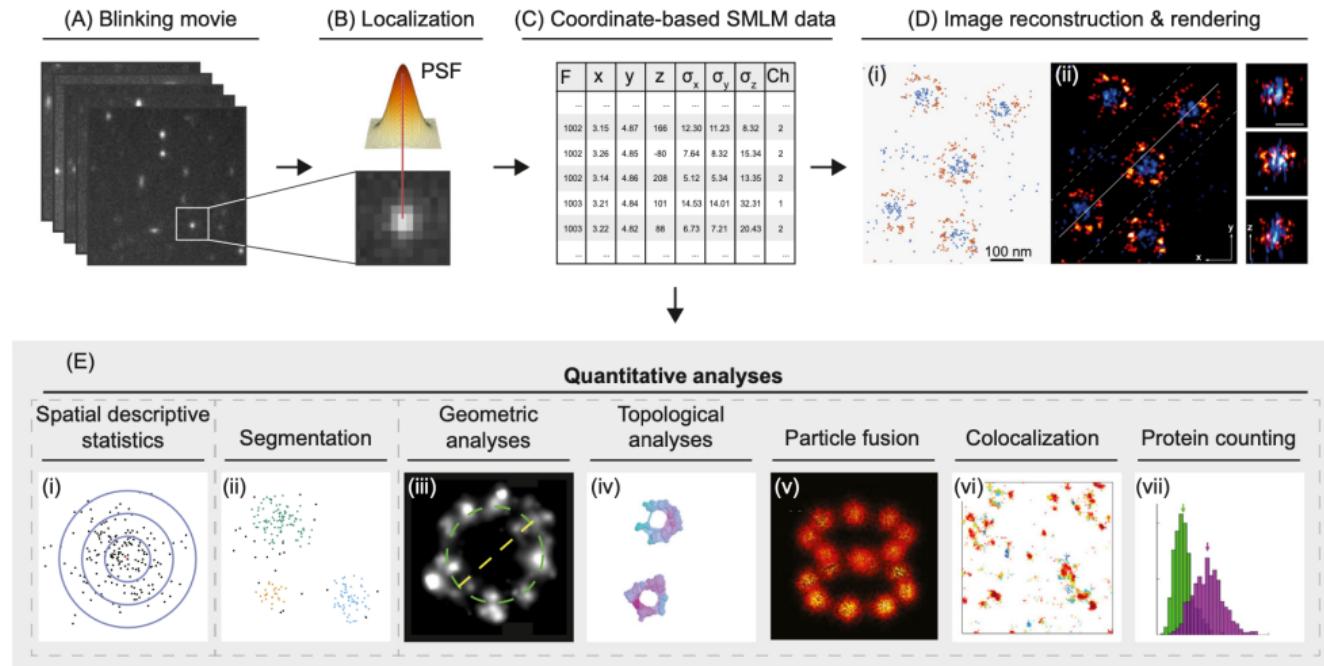
Δ – exposure time



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



Trends in Cell Biology

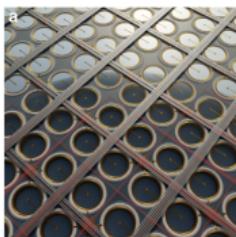
Wu et al. Trends in Cell Biology. 30 (2020)

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

Enhanced nanoscopy with single photon avalanche diode (SPAD) cameras



SPAD512

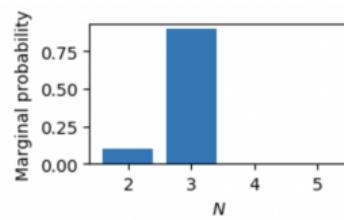
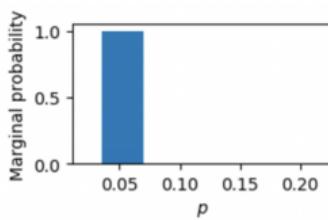
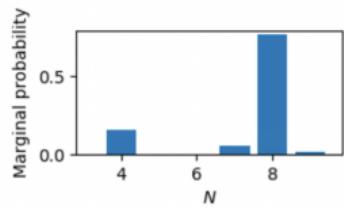
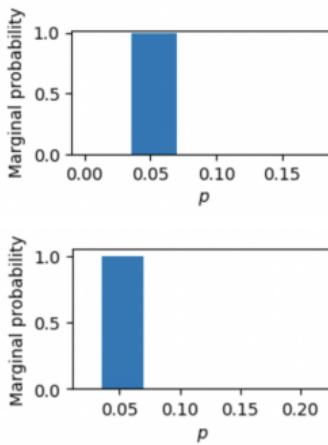
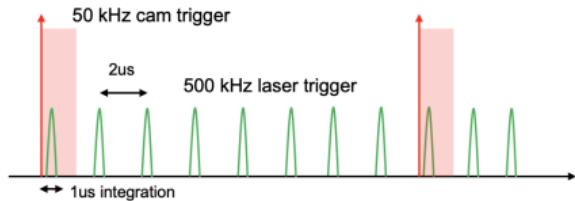
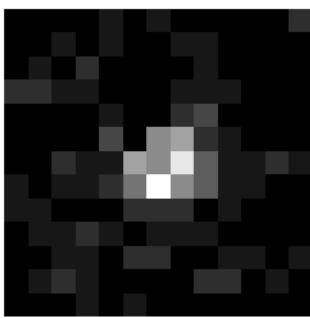
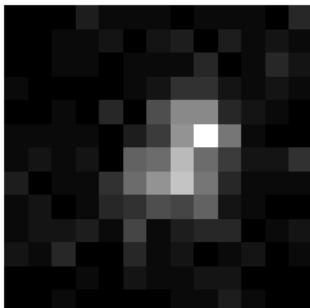


a



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

Enhanced nanoscopy with single photon counting



$$\mathbf{x} = \mathbf{x}_{\text{signal}} + \mathbf{x}_{\text{background}} \quad p(N, \zeta | \mathbf{x}) = p(\mathbf{x} | N, b) p(N) p(\zeta)$$

Inference of the number of fluorescent emitters N

Let $x = x_{\text{signal}} + x_{\text{background}}$ be observed photon counts after a single pulse

$$x_{\text{signal}} \sim \text{Binom}(N, \zeta) \quad x_{\text{background}} \sim \text{Poisson}(\lambda)$$

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

- ▶ $p(x|N, \zeta)$ is Poisson-Binomial which is hard to compute analytically
- ▶ We can estimate the likelihood by sampling := $\tilde{p}(x|N, \zeta)$

$$p(N = n|x) \propto \int_0^1 p(N = n, \zeta|x)d\zeta$$

Need to estimate:

$$p(N = n|x) \propto \int_0^1 e^{\frac{(\zeta - \zeta_0)^2}{2\sigma^2}} \prod_{i=1}^M \tilde{p}(x|N = n, \zeta)$$

Computing the coincidence ratio $g^{(2)}(0)$

Define $g^{(2)}(0) = \frac{G^{(2)}(0)-B}{G^{(2)}(m)-B}$ where $B = \langle x_{\text{background}} \rangle = N_{\text{frames}} \lambda \zeta$ is the expected number of background-signal coincidences in the acquisition

$$G^{(2)}(0) \sim \text{Binomial}(N_{\text{frames}}, P(b_i \geq 2))$$

$$G^{(2)}(m) \sim \text{Binomial}(N_{\text{frames}}, P(b_i \geq 1 \text{ and } b_{i+m} \geq 1))$$

where

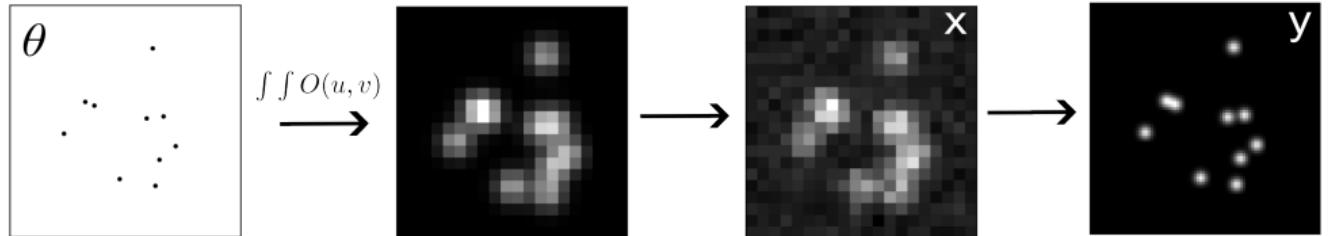
$$P(b_i \geq 2) = 1 - \binom{n}{0} \zeta^0 (1 - \zeta)^n - \binom{n}{1} \zeta^1 (1 - \zeta)^{n-1}$$

The probability that both elements in the sequence are one or greater:

$$P(b_i \geq 1 \text{ and } b_{i+m} \geq 1) = (1 - (1 - \zeta)^n)^2$$

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., $p_\theta(\mathbf{y}|\mathbf{x})$

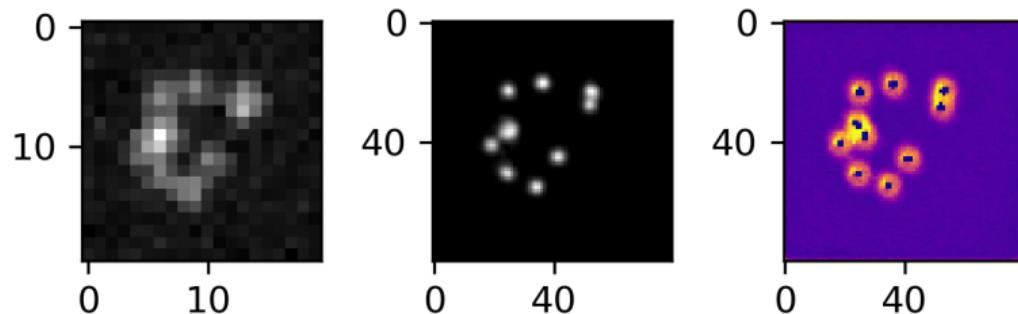
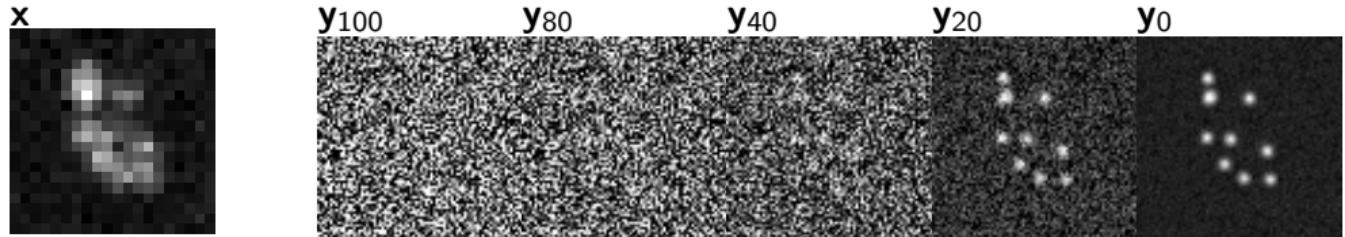
Trivial example of sampling from a mixture of Gaussians

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

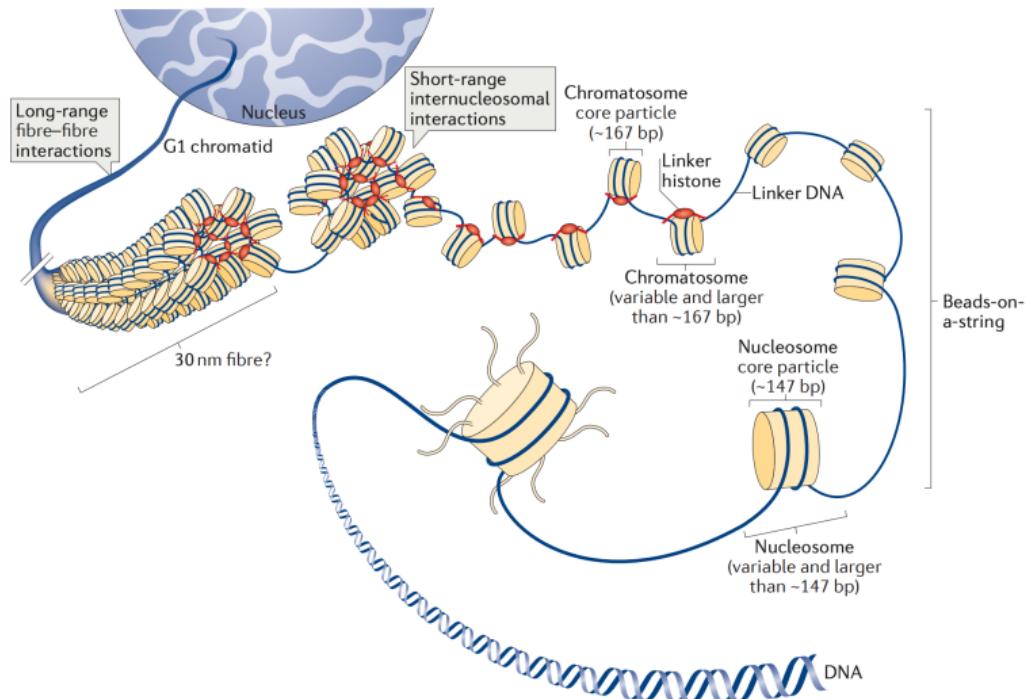


Need to approximate the gradient $s_\theta(\mathbf{y}_t) \approx \nabla \log p(\mathbf{x})$ and sample:

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

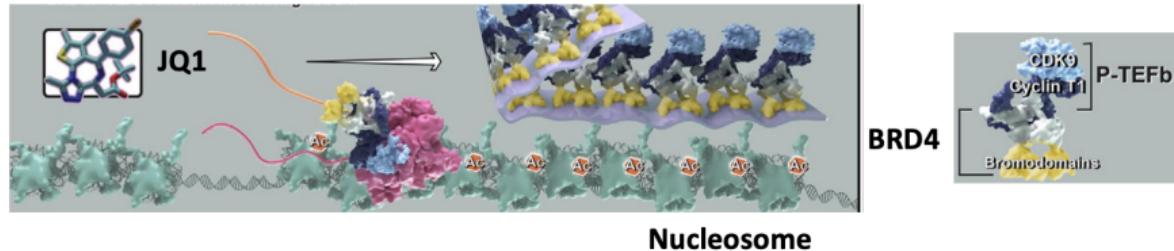
Super-resolution of nucleosome nanodomains *in-vivo*

Hierarchical structure of chromatin

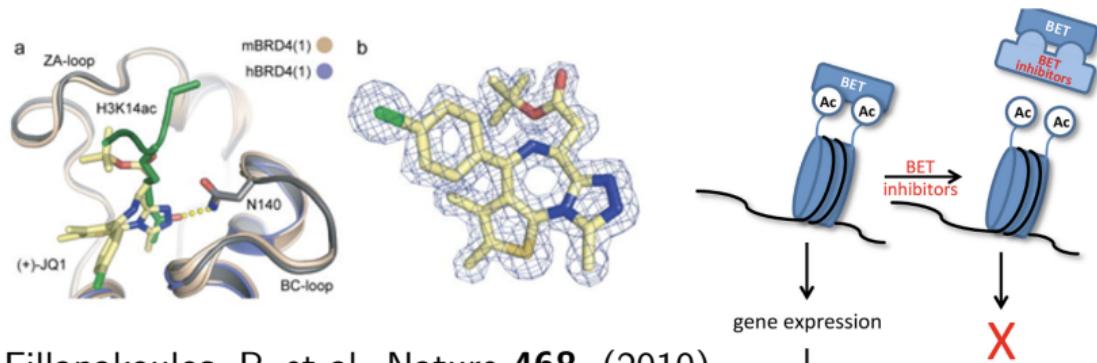


Fyodorov, D. et al. Nat Rev Mol Cell Biol **19**, (2018).

Bromodomain protein 4 (BRD4) binds acetylated chromatin

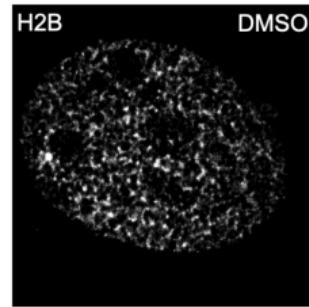
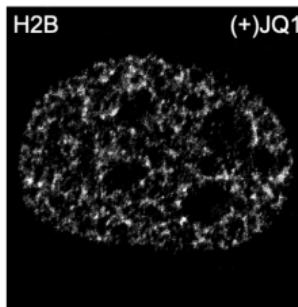
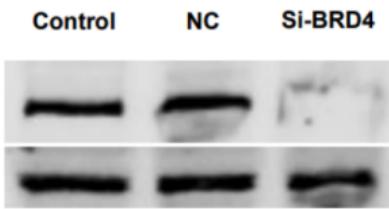
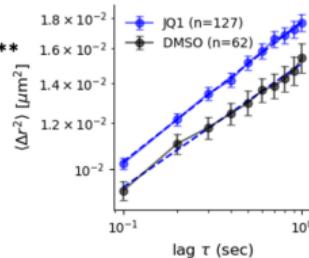
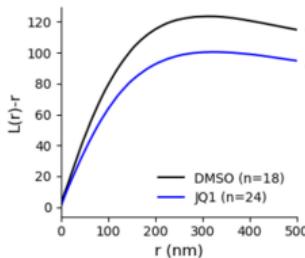
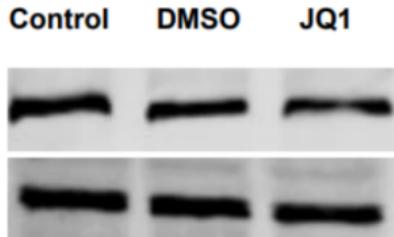


Zheng, B. et al. Molecular Cell **16**, (2023).



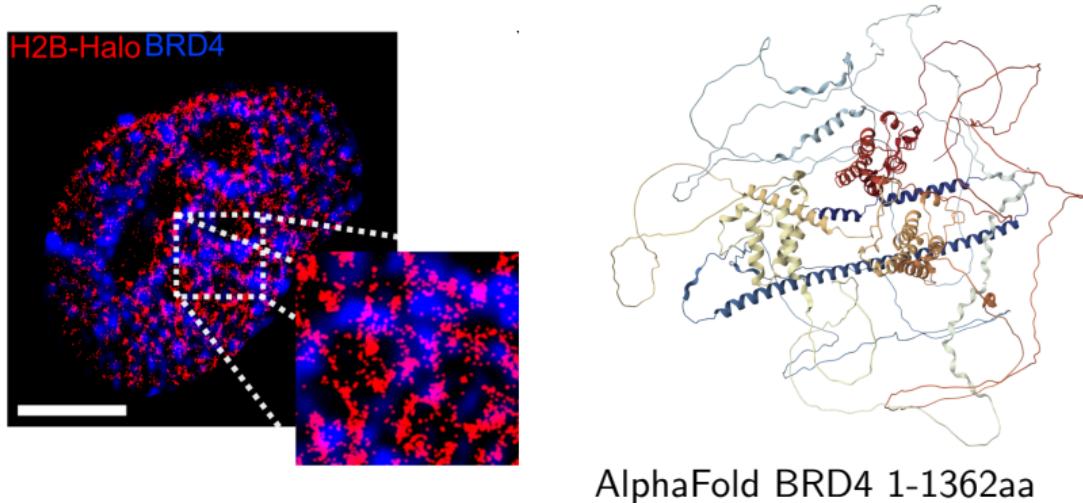
Fillapakoulos, P. et al. Nature **468**, (2010).

BET inhibitor (+)-JQ1 decondenses chromatin and increases mobility

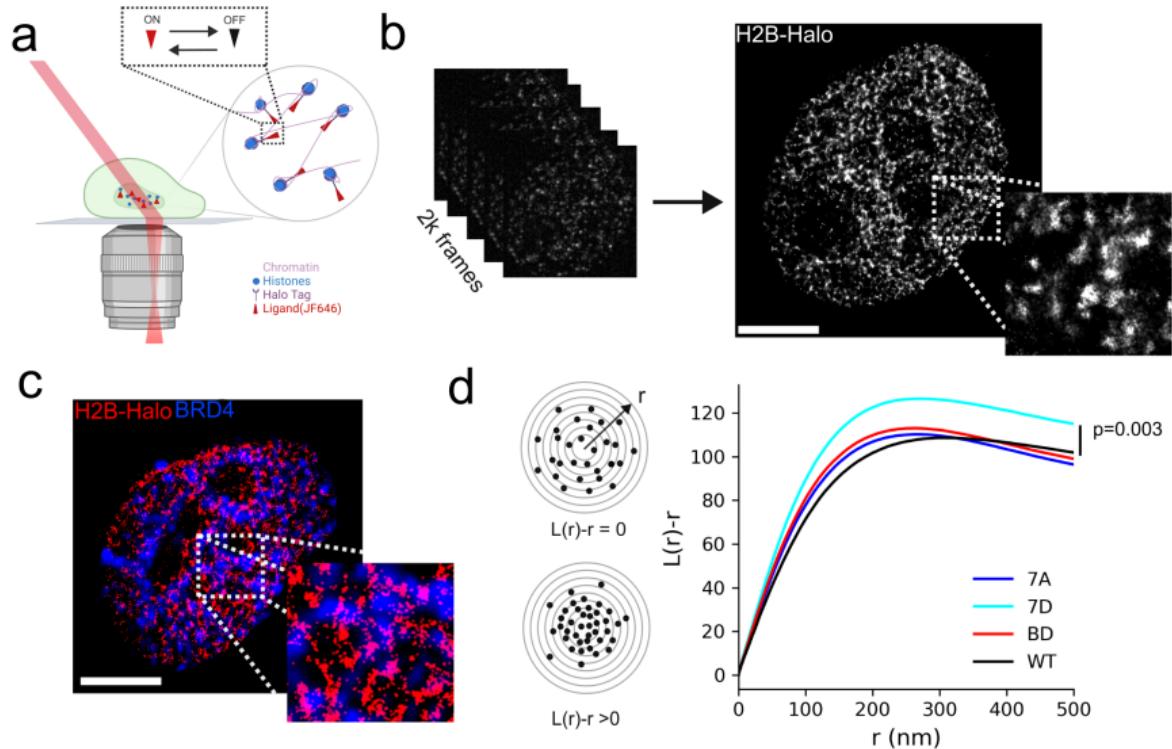


Blots: top - BRD4, bottom - GAPDH

Bromodomain protein 4 (BRD4) binds acetylated chromatin

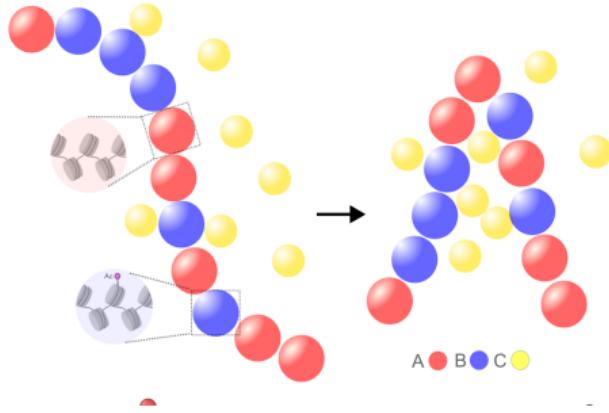


BRD4 phosphorylation state is necessary for maintenance of chromatin structure

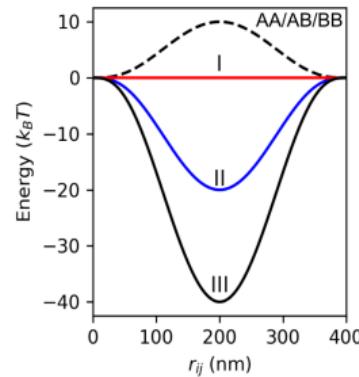


Coarse grained molecular dynamics of chromatin binders at 310K

a



b

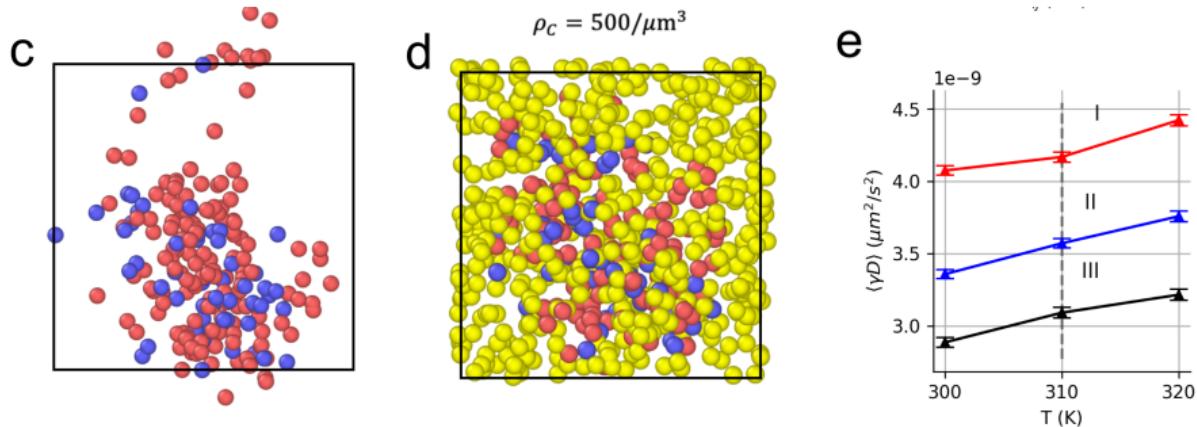


100kb chromatin chains interact with binders via the potential

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

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

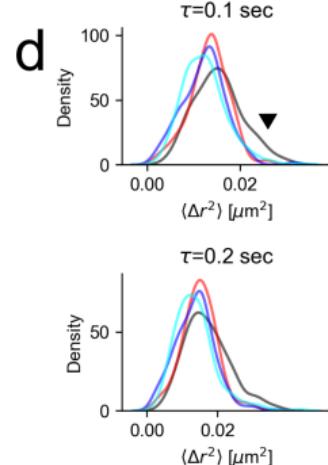
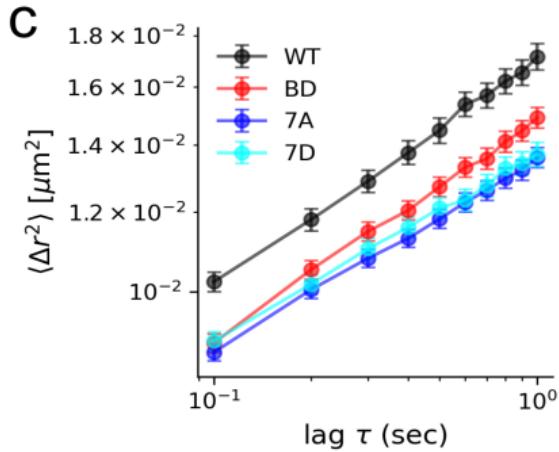
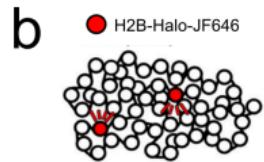
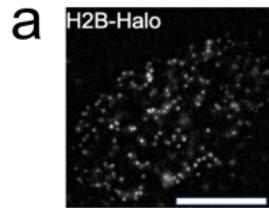
Multivalent chromatin binding reduces chromatin mobility



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