

# 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

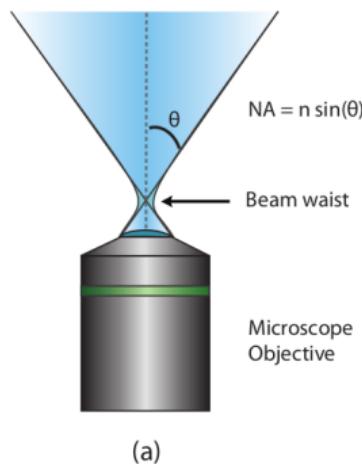
Method 2: Enhanced nanoscopy with deep generative models

Super-resolution of nucleosome nanodomains *in-vivo*

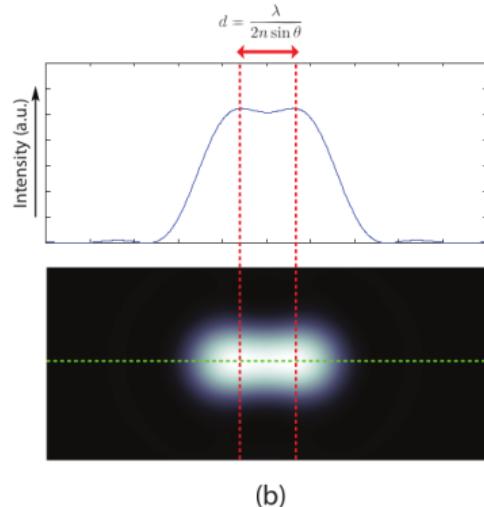
## Introduction to fluorescence nanoscopy

# Fluorescence microscopy and the diffraction limit

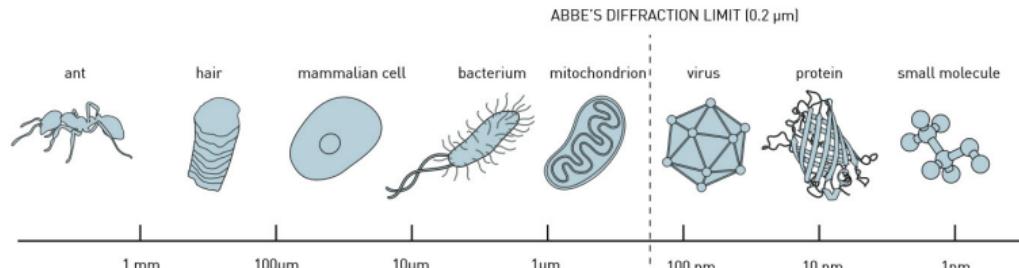
Minimal resolvable distance  $d \sim \lambda$



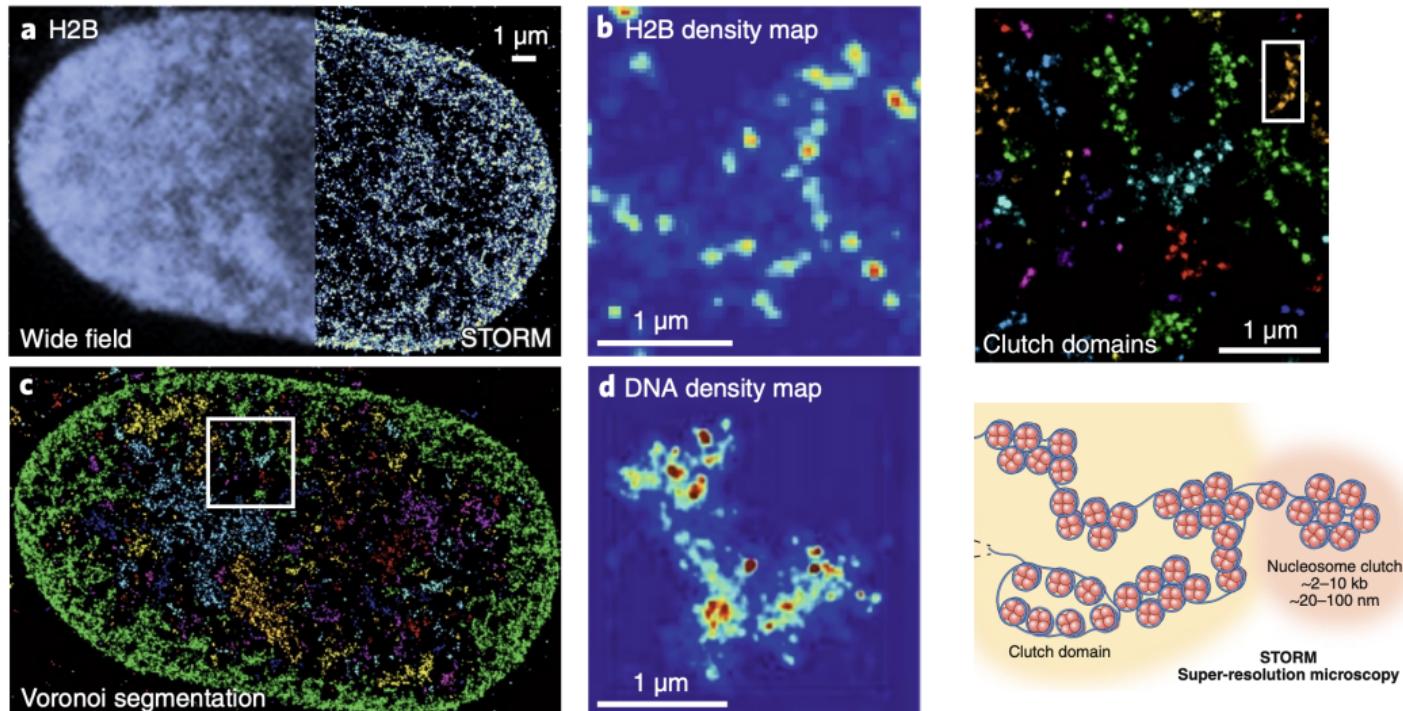
(a)



(b)



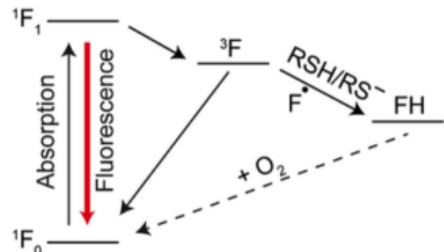
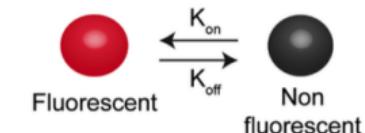
# Stochastic optical reconstruction microscopy (STORM)



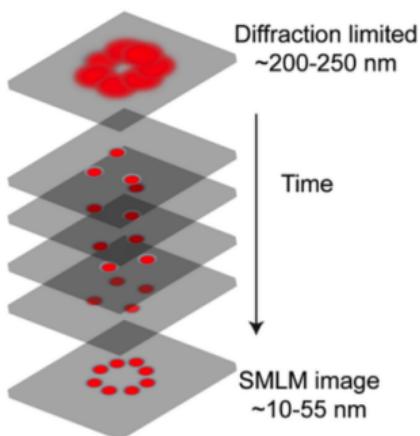
Lakadamyali, M. et al. Nature Methods **17**, (2020).

# Stochastic optical reconstruction microscopy (STORM)

## a Photoswitching



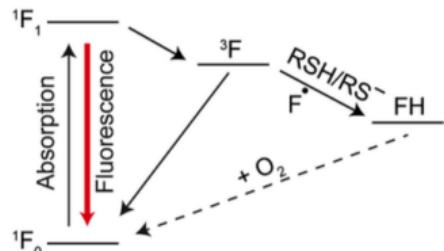
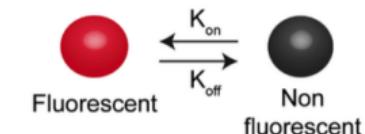
## b Temporal separation



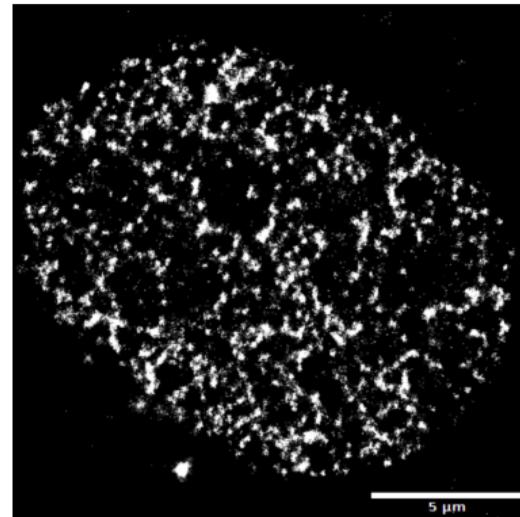
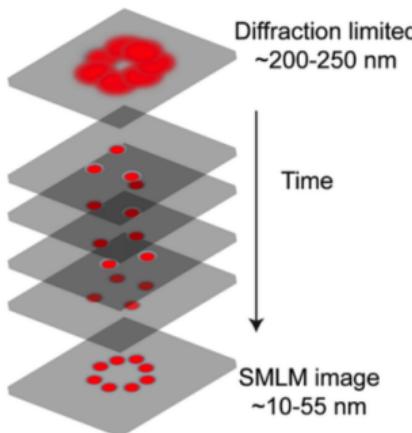
- ▶ STORM and similar nanoscopy techniques are diffraction-unlimited
- ▶ Photoswitching enables resolution of emitters below the diffraction limit

# 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

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

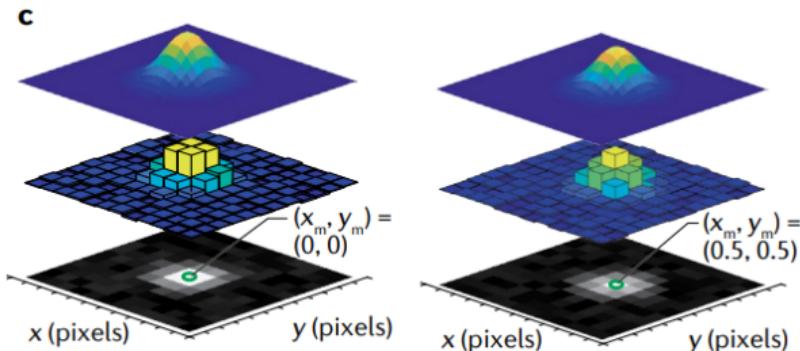
$$i_0 = g_k \eta N_0 \Delta$$

$g_k$  – pixel gain

$\eta$  – quantum efficiency

$N_0$  – photon emission rate

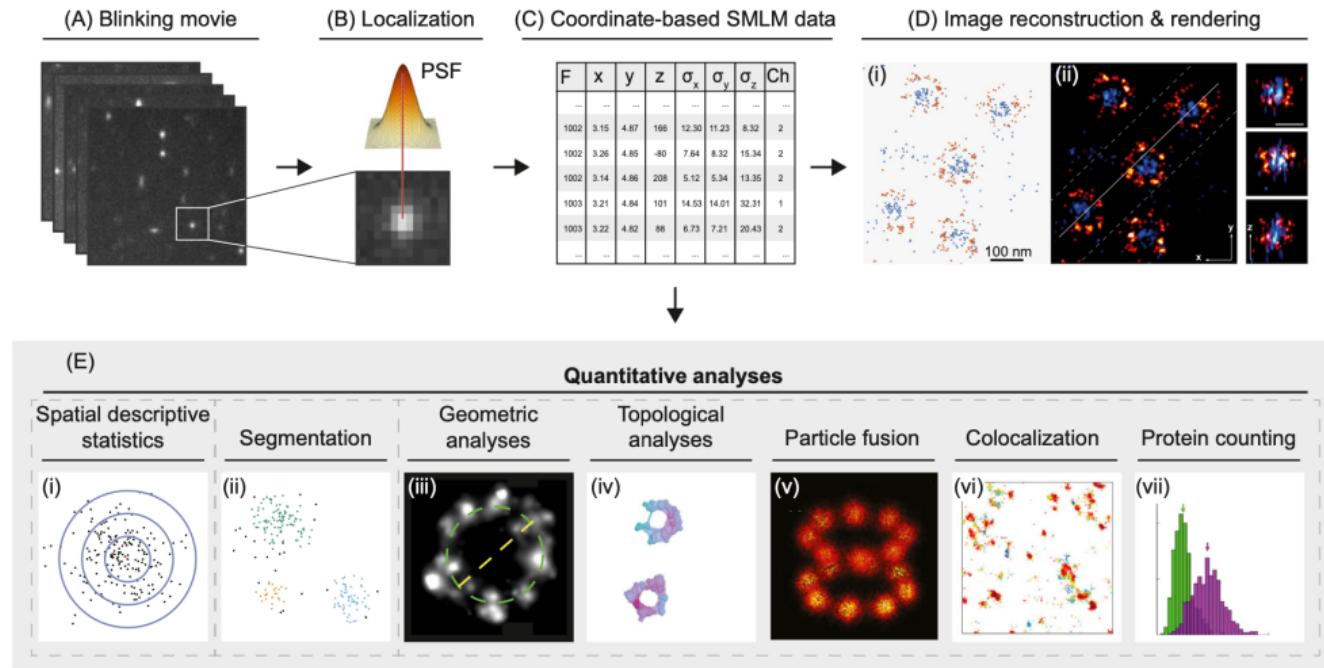
$\Delta$  – exposure time



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

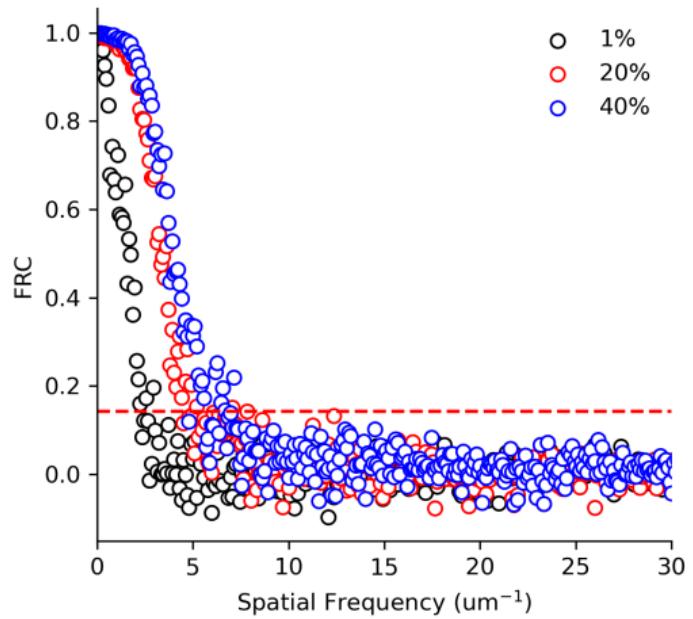
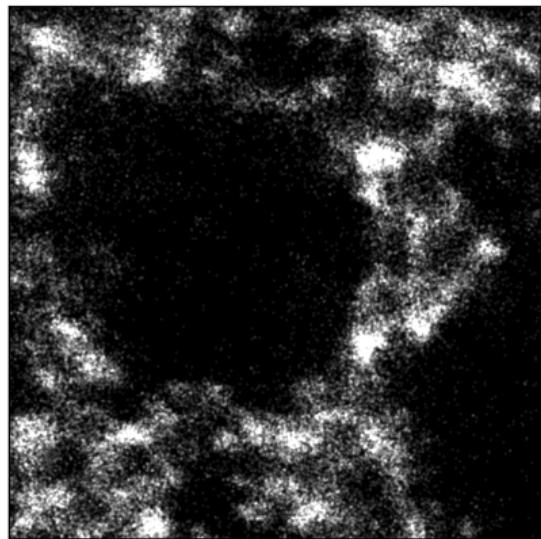
$$\theta^* = \operatorname{argmax}_{\theta} \prod_k p(\mathbf{x}_k | \theta) = \operatorname{argmin}_{\theta} - \sum_k \log p(\mathbf{x}_k | \theta)$$

# Applications of single molecule localization microscopy

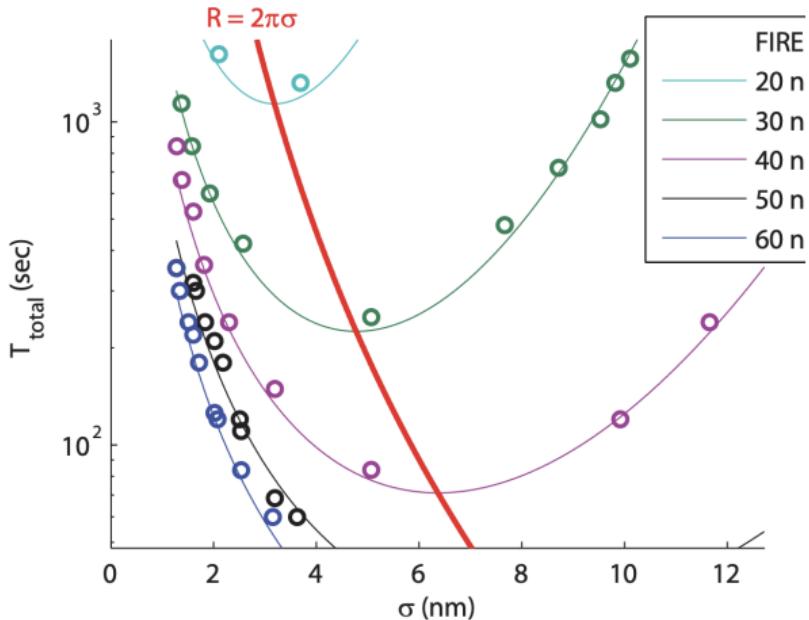


Wu et al. Trends in Cell Biology. 30 (2020)

# How do we define resolution in localization microscopy?



# Fourier Image Resolution (FIRE)



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

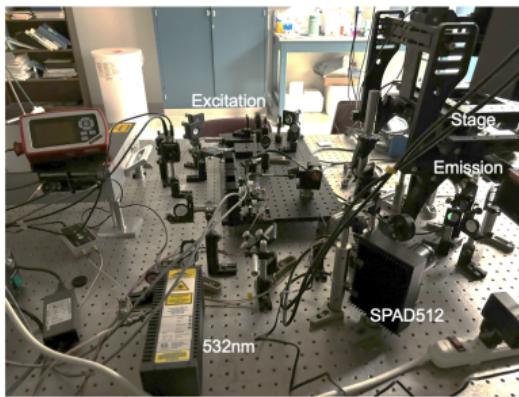
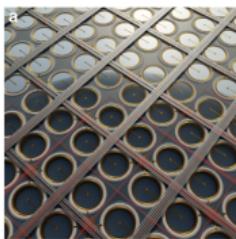
Nutshell: *How to relax the density limit in localization microscopy?*

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

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



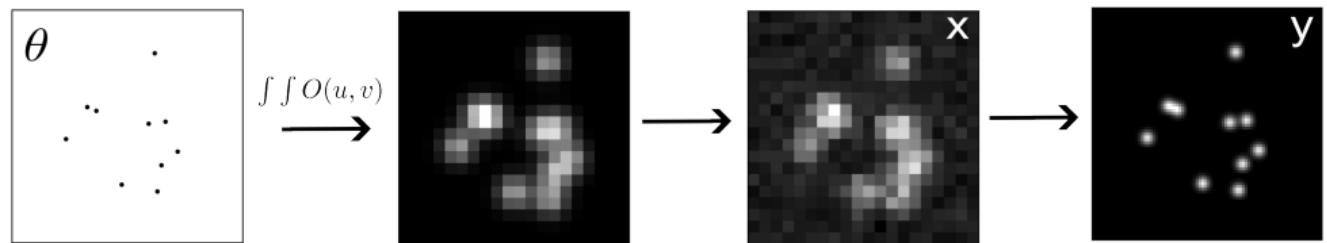
SPAD512



- ▶ 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

## Inference of a high-resolution image from a low-resolution one



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

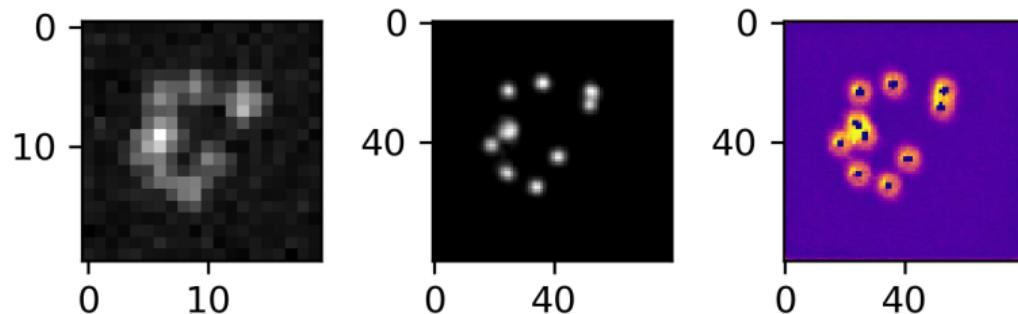
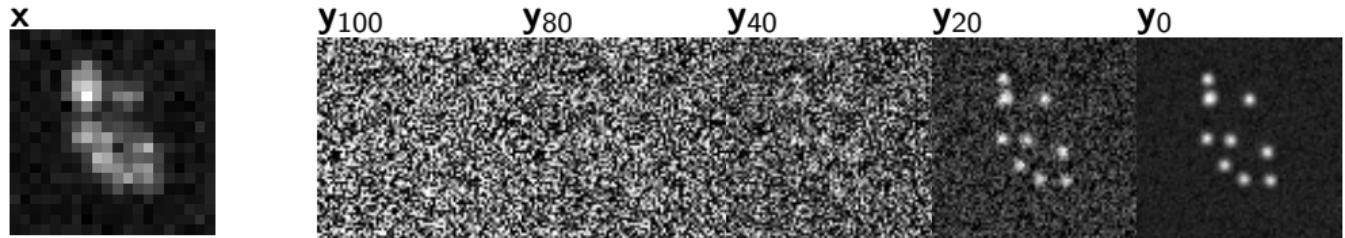
## Trivial example of sampling from a mixture of Gaussians

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

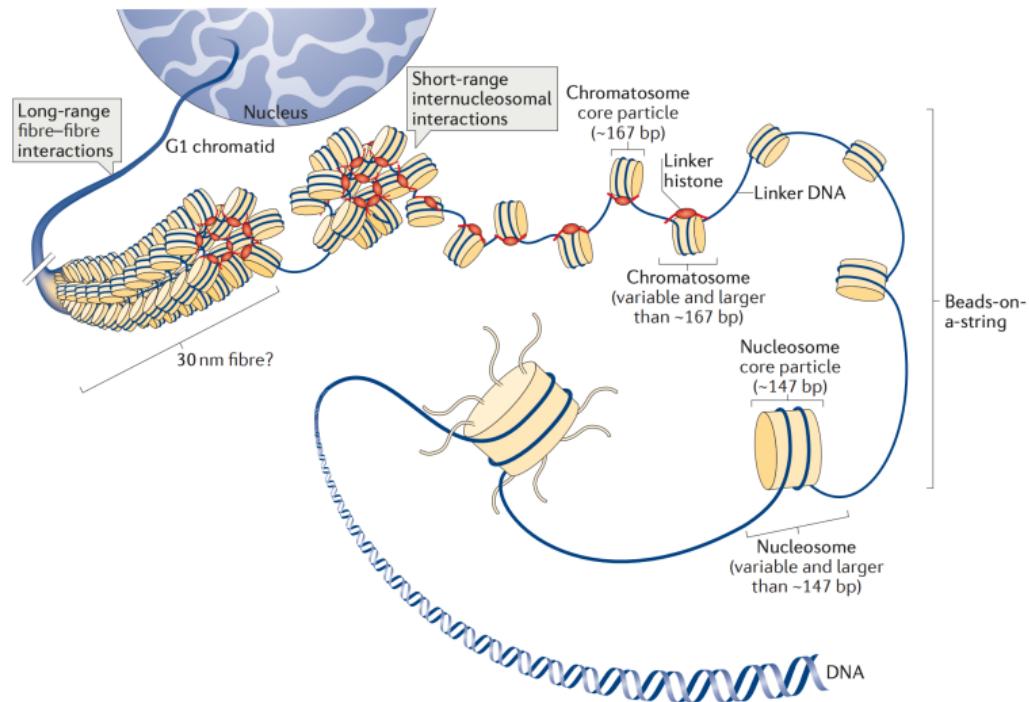


Need to approximate the gradient  $s_\theta(\mathbf{y}_t) \approx \nabla \log p(\mathbf{x})$  and sample:

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

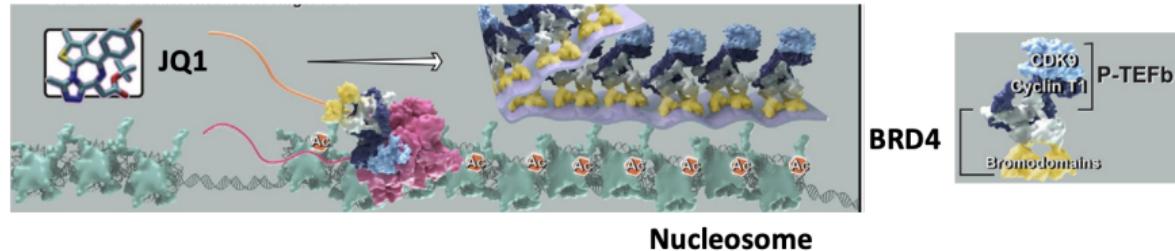
## Super-resolution of nucleosome nanodomains *in-vivo*

# Hierarchical structure of chromatin

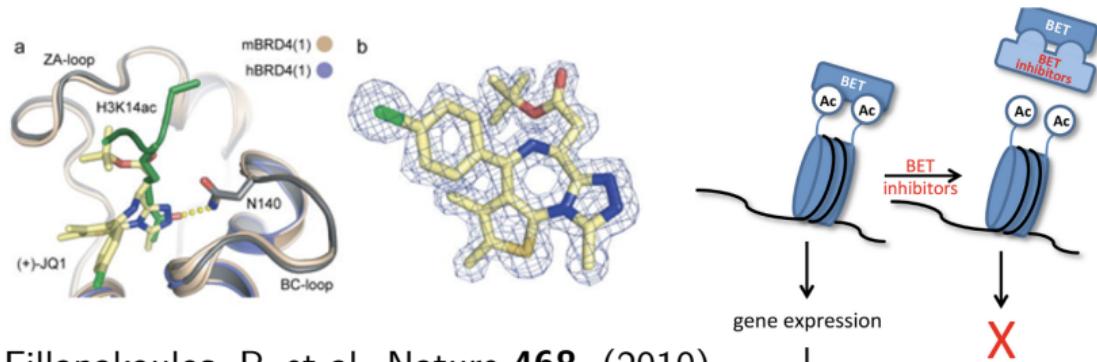


Fyodorov, D. et al. Nat Rev Mol Cell Biol 19, (2018).

# Bromodomain protein 4 (BRD4) binds acetylated chromatin

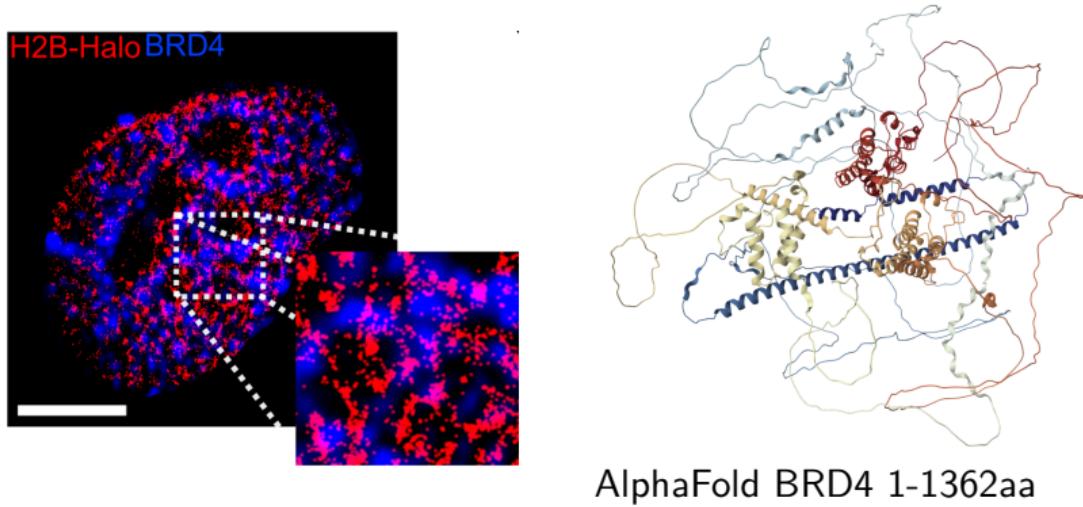


Zheng, B. et al. Molecular Cell **16**, (2023).

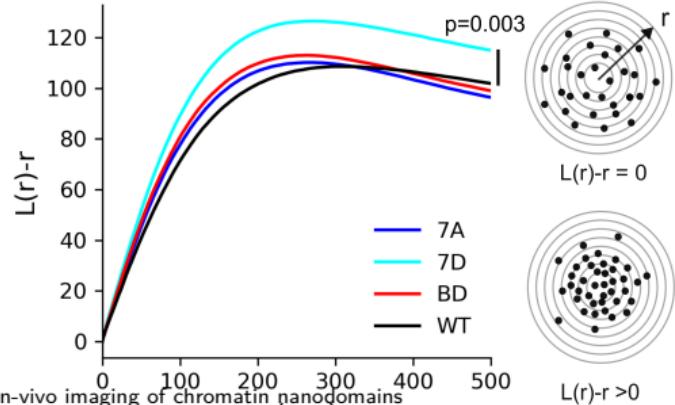
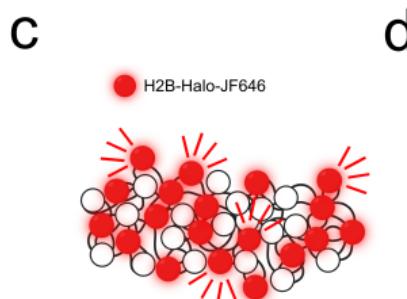
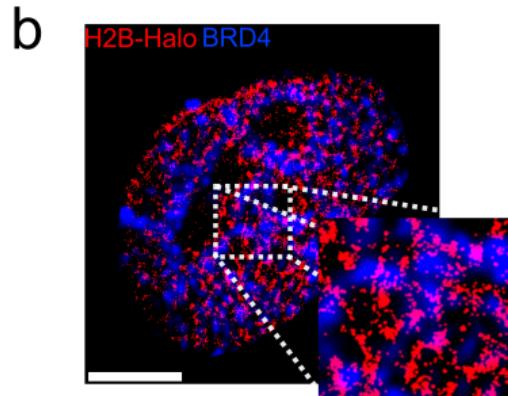
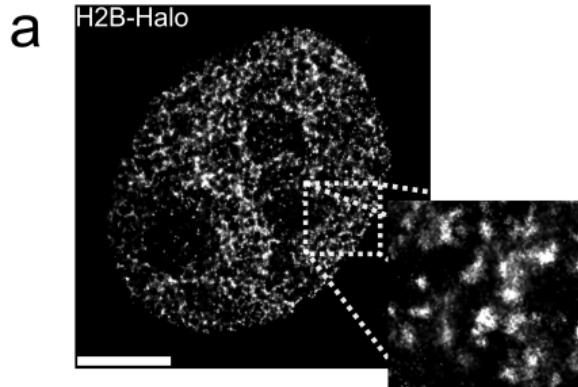


Fillapakoulos, P. et al. Nature **468**, (2010).

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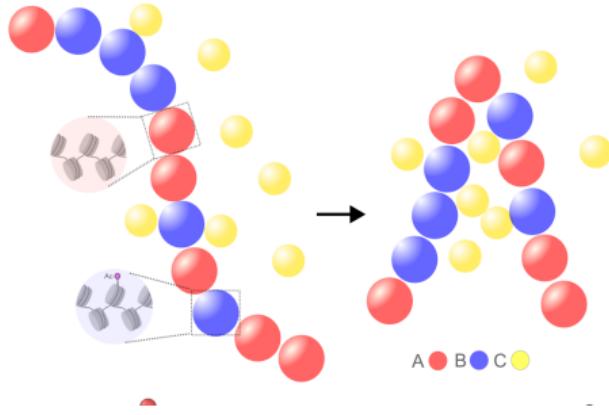


# BRD4 phosphorylation state is necessary for maintenance of chromatin structure

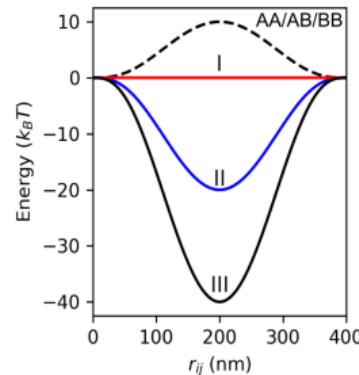


# Coarse grained molecular dynamics of chromatin binders at 310K

a



b

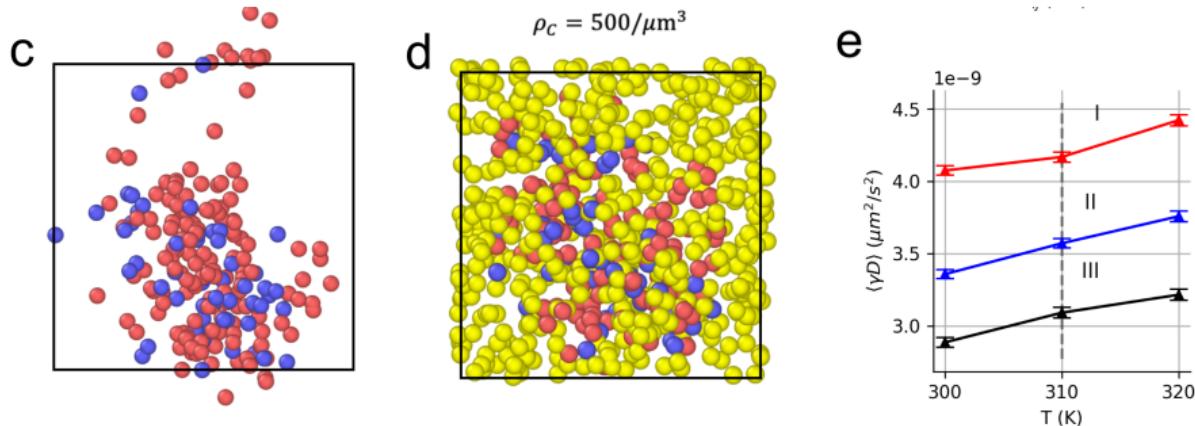


100kb chromatin chains interact with binders via the potential

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

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

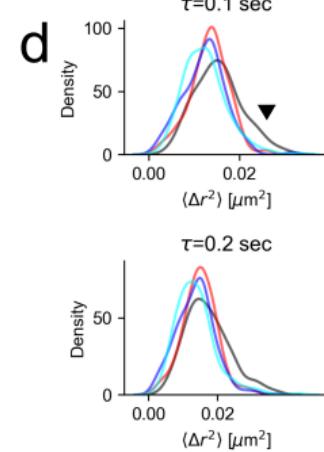
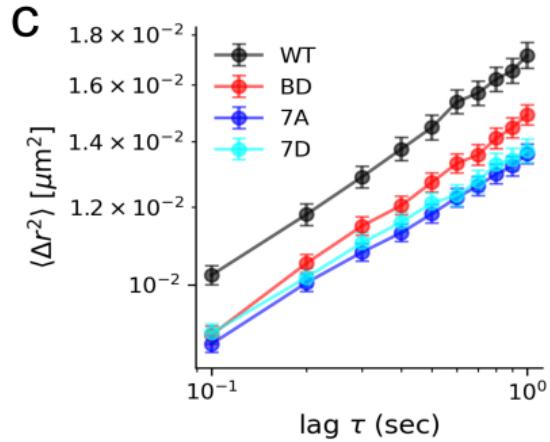
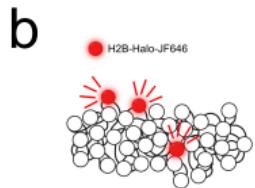
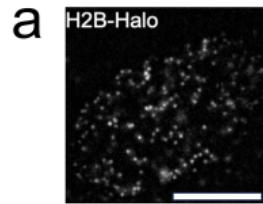
# Multivalent chromatin binding reduces chromatin mobility



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