

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

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# Outline of the talk

Introduction to fluorescence nanoscopy

Probabilistic modeling approaches to fluorescence nanoscopy

Specific Aim 1: Enhance nanoscopy with deep generative models

Specific Aim 2: Integrate single photon counting and widefield single molecule imaging

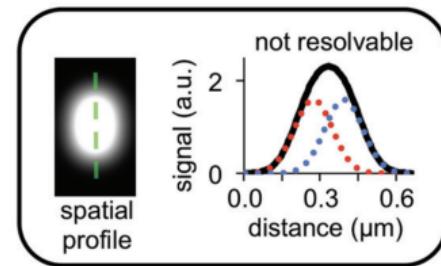
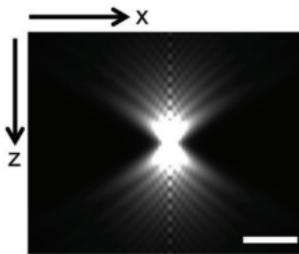
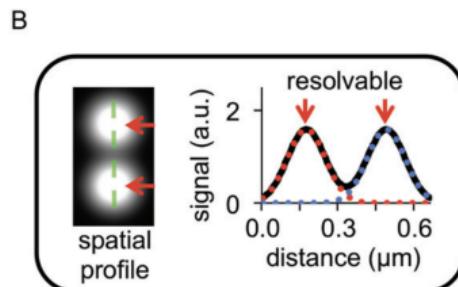
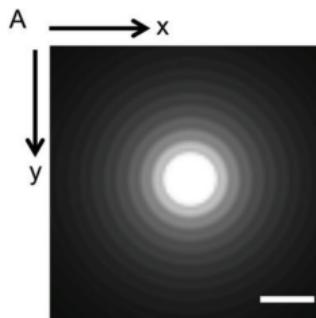
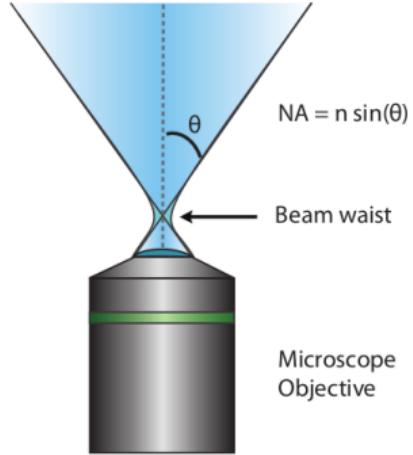
Super-resolution of nucleosome nanodomains *in-vivo*

Specific Aim 3: Interaction of transcriptional condensates with nucleosome nanodomains

## Introduction to fluorescence nanoscopy

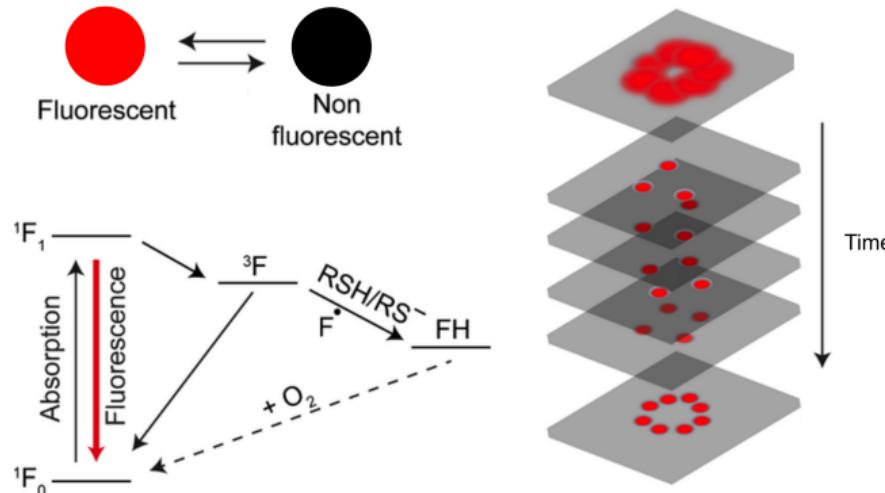
# Fluorescence microscopy and the diffraction limit

Minimal resolvable distance  $d \sim \lambda$



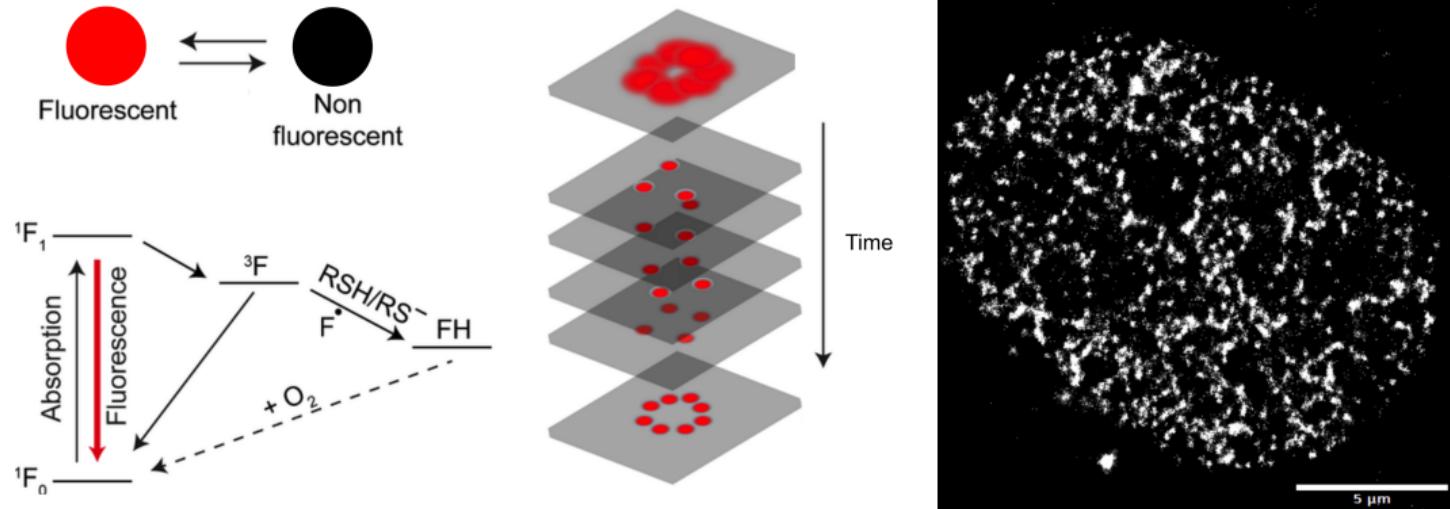
Herbert et al. Microscopy and Microanalysis.  
(2012)

# 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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# Nanoscopy by localizing isolated fluorescent emitters

Modeling the point spread function permits sub-pixel localization

$$\mu_k = i_0 \int \int O(u, v) dudv + \lambda$$

$$i_0 = g_k \eta \zeta \Delta$$

$g_k$  – pixel gain

$\eta$  – quantum efficiency

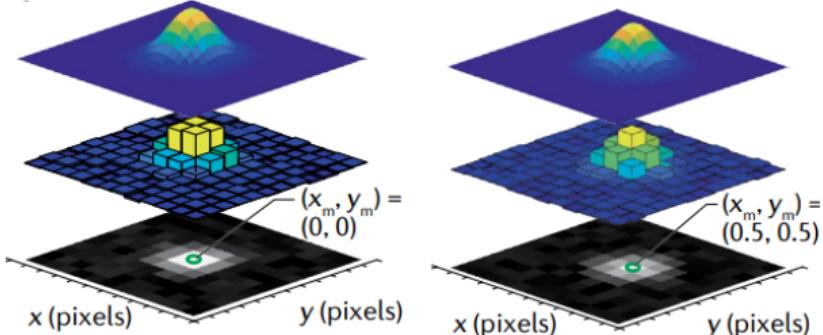
$\zeta$  – photon emission rate

$\Delta$  – exposure time

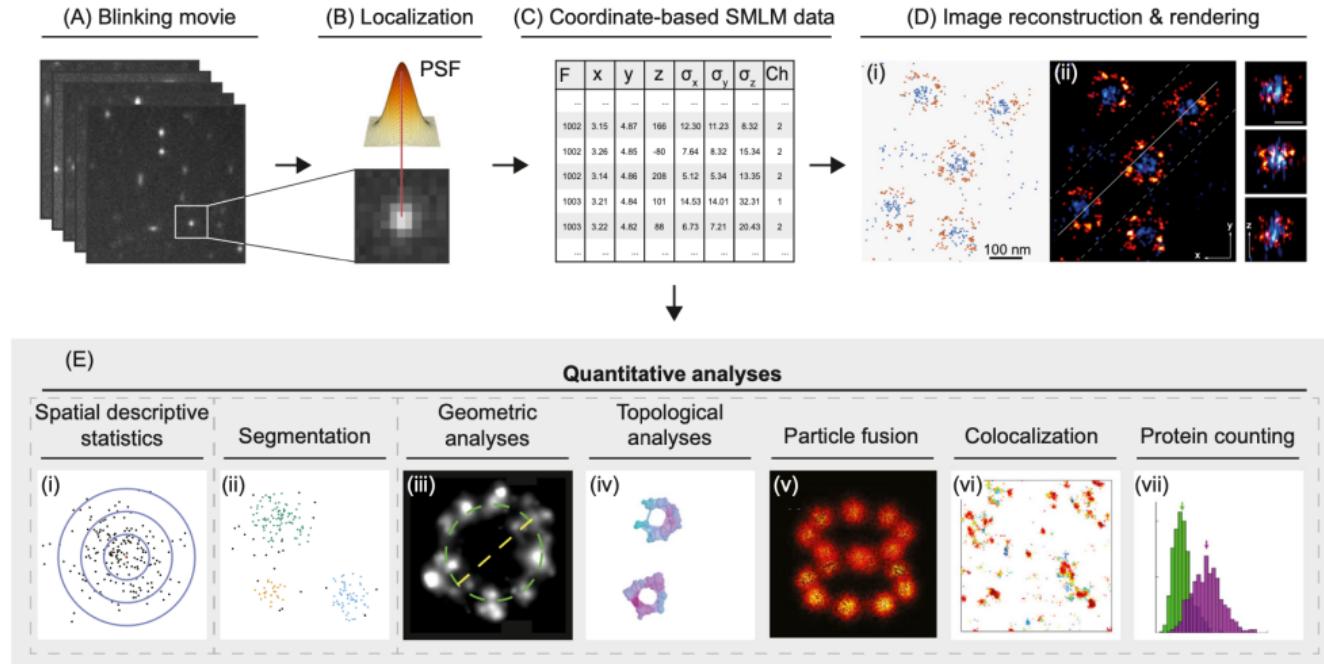
$\lambda$  – background rate

Maximum likelihood localization:

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

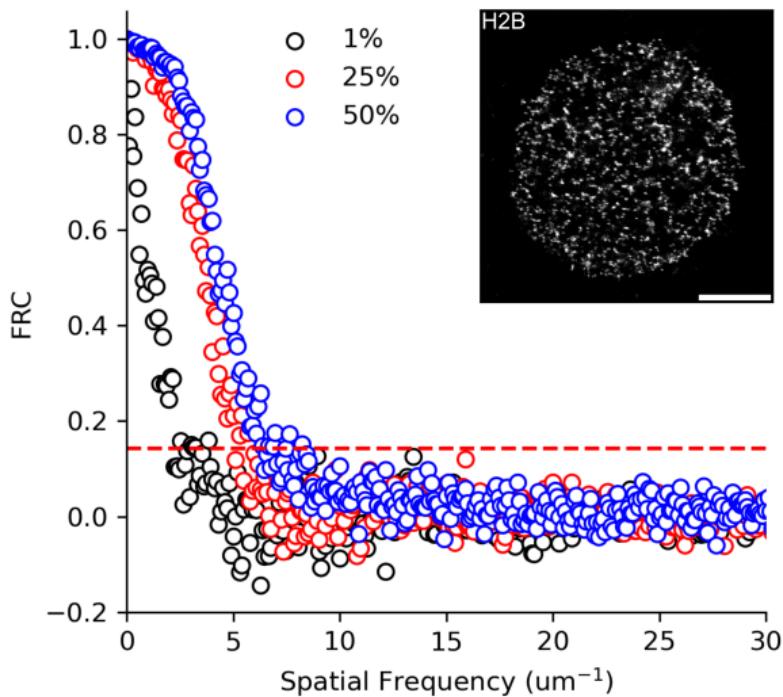


# Applications of single molecule localization microscopy



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

## Maximization of density, minimization of error



- ▶ **Maximize** density and in turn spatial/temporal resolution
- ▶ **Minimize** localization errors

## Maximization of density, minimization of error

Approach these objectives using two probabilistic modeling approaches:

- ▶ **Specific Aim 1:** Utilize deep generative modeling for *dense* widefield nanoscopy
- ▶ **Specific Aim 2:** Introduce single photon counting in widefield nanoscopy

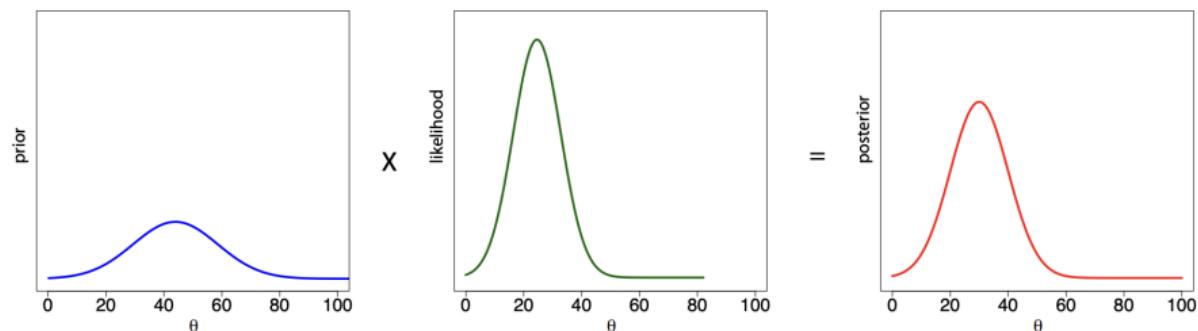
## Probabilistic modeling approaches to fluorescence nanoscopy

# The Bayesian calculation and variational inference

Let  $\theta$  be a latent variable (e.g. high resolution image) to be inferred from a measurement  $x$  (e.g. low resolution image)

## Bayes Rule

$$p(\theta|x) = \frac{p(x|\theta)p(\theta)}{\int p(x,\theta)d\theta} \propto p(x|\theta)p(\theta)$$

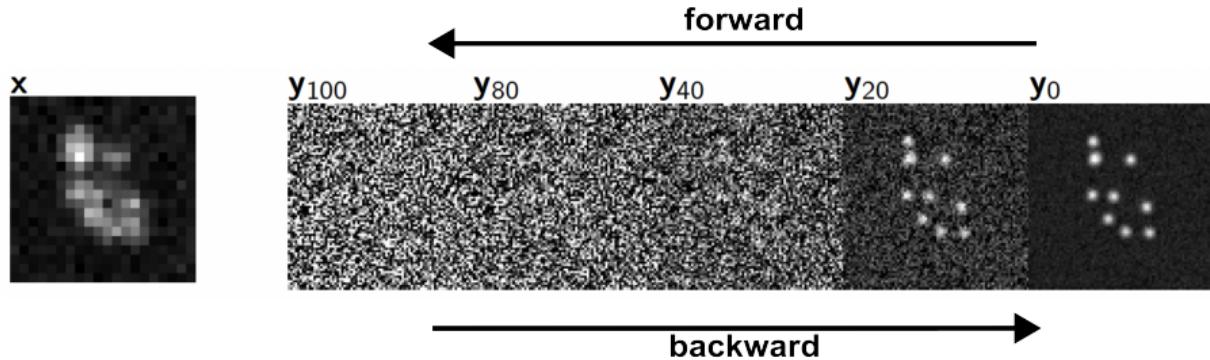


$p(\theta|x)$  is can be hard to obtain. One solution is to fit a model distribution  $p_\psi(\theta|x)$  that has parameters  $\psi$

## Bayesian image restoration with diffusion models

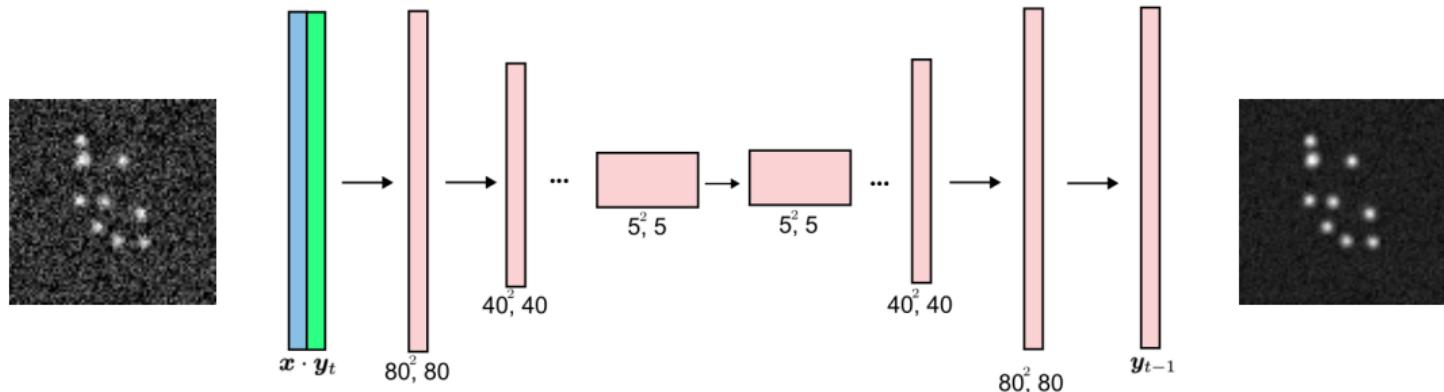
Inference of a high resolution image  $\mathbf{y}_0$  from low resolution  $\mathbf{x}$  is approached by modeling a distribution  $p_\psi(\mathbf{y}_0|\mathbf{x})$  with a diffusion model

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



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

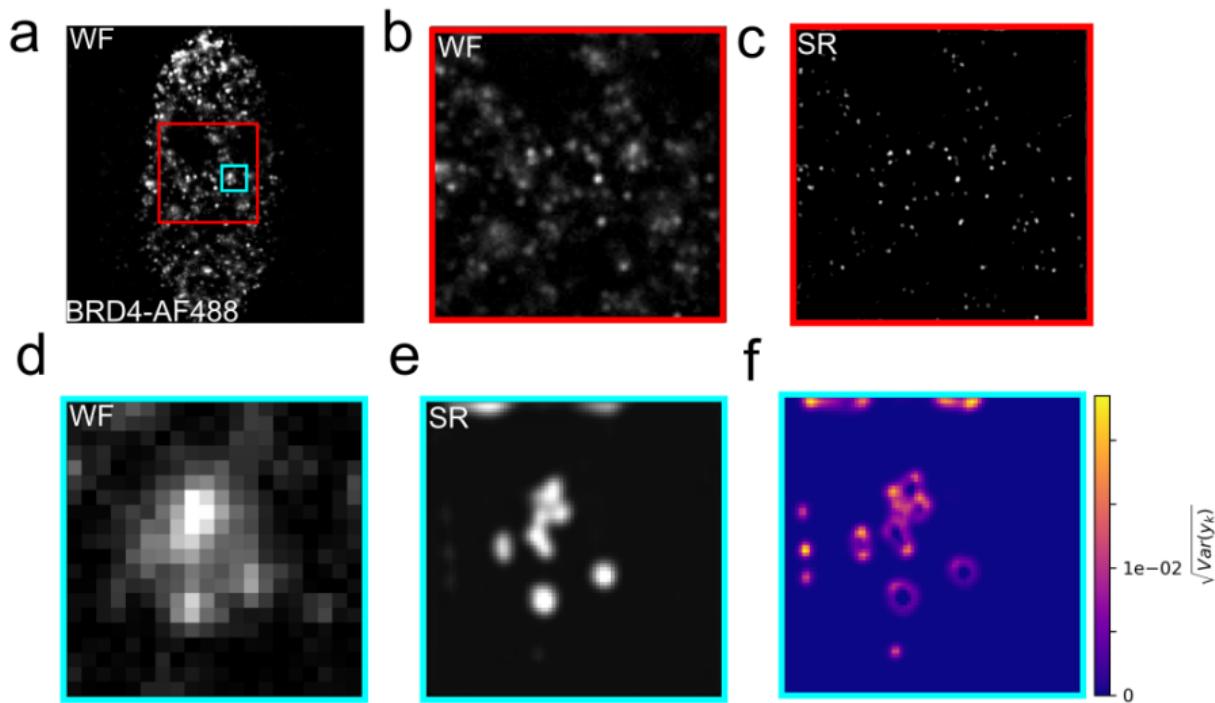
# Bayesian image restoration with diffusion models



A deep neural network estimates the gradient of the reverse process

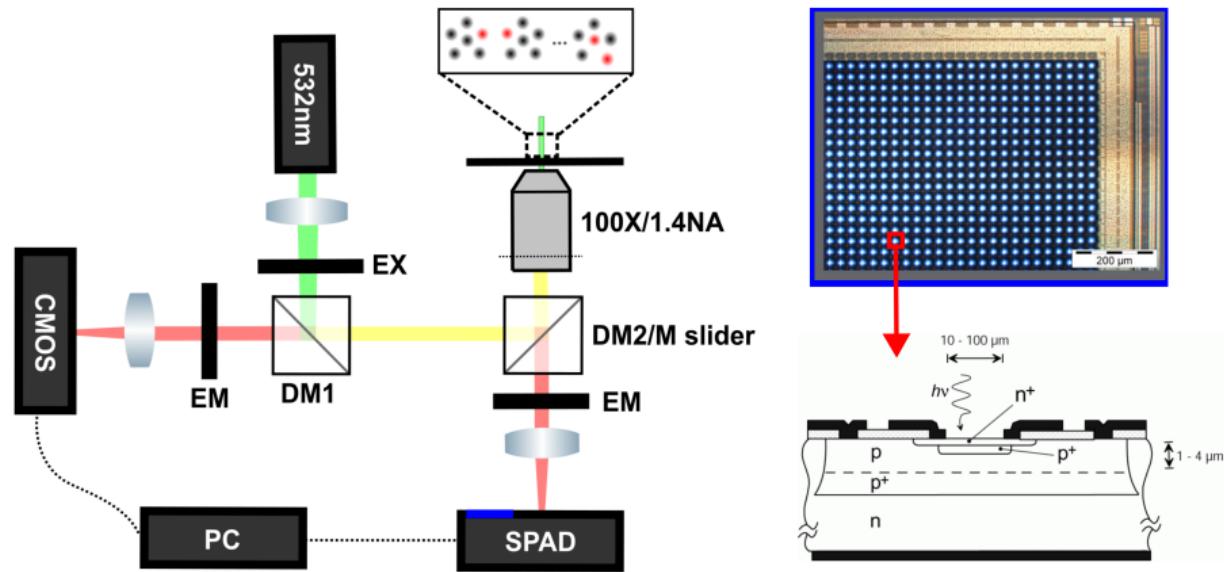
$$\mathbf{y}_{t-1} = \frac{1}{\sqrt{1 - \beta_t}} (\mathbf{y}_t + \beta_t s_\psi(\mathbf{y}_t)) + \sqrt{\beta_t} \xi \quad \xi \sim \mathcal{N}(0, I)$$

# Super resolution of BRD4 protein in a HeLa cell

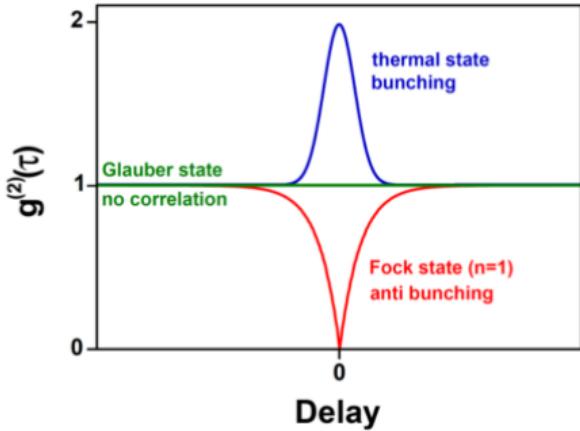
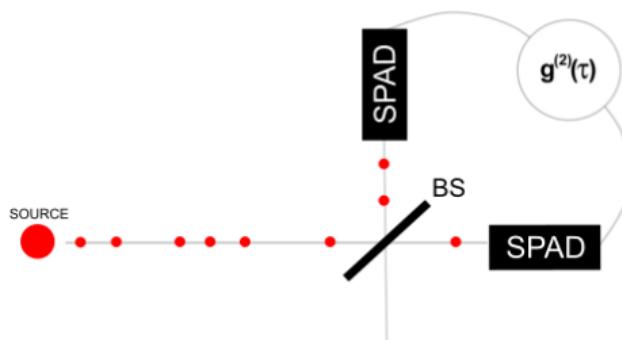


# An integrated single photon avalanche diode (SPAD) array

SPAD arrays open several doors in microscopy, including new modeling approaches



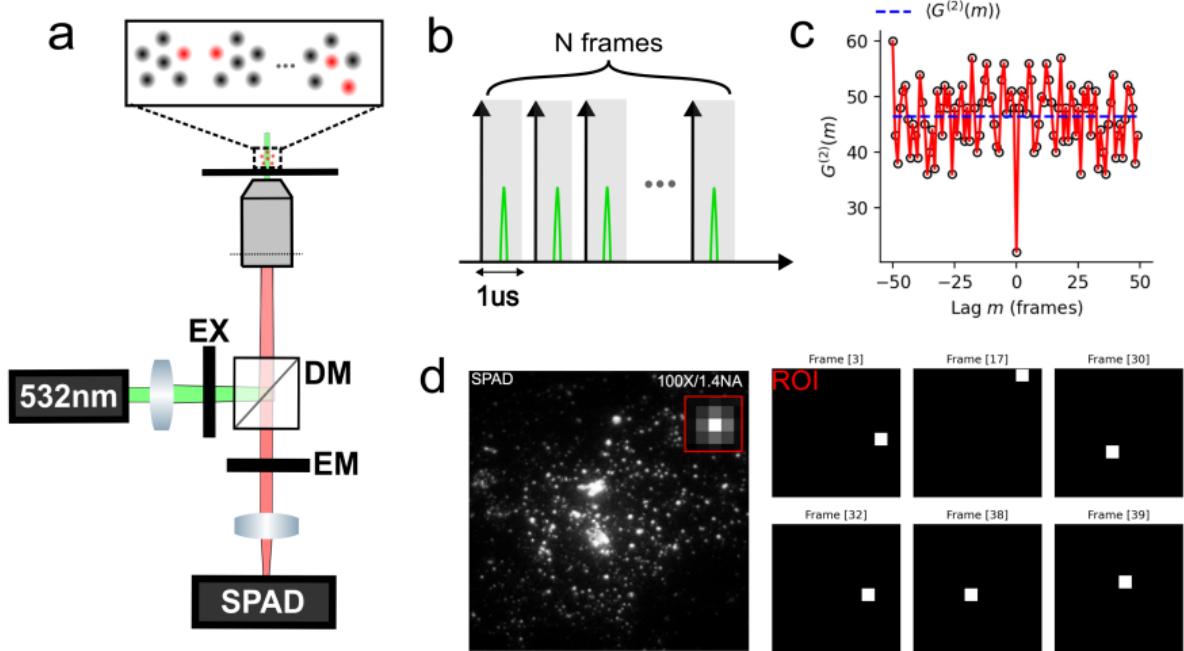
# The Hanbury Brown and Twiss Effect



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

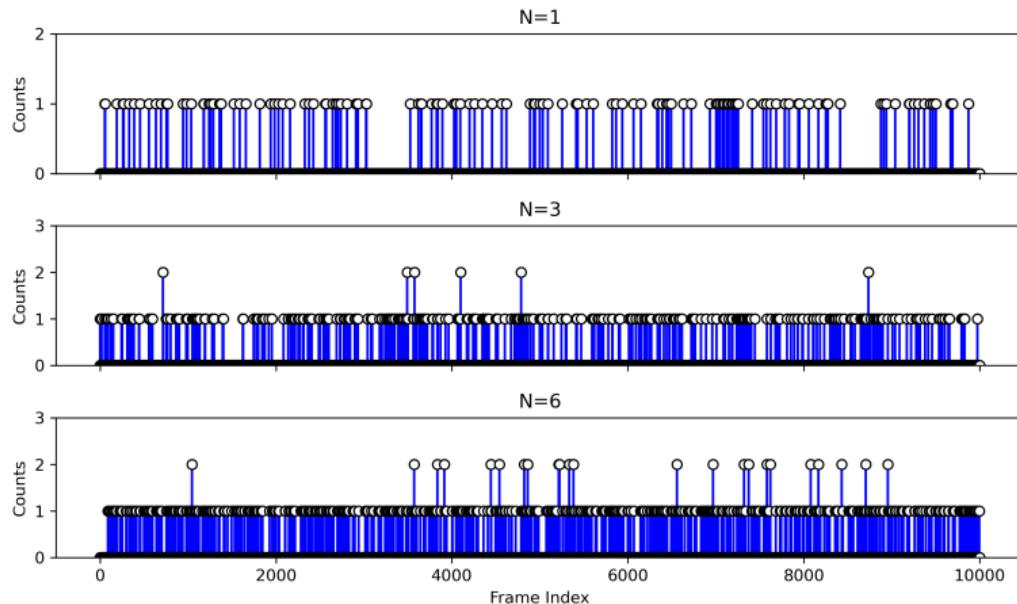
- ▶ Single photon sources (such as a fluorescent dye) exhibit antibunching
- ▶ Magnitude of  $g^{(2)}(0)$  "dip" depends on the number of fluorescent emitters

# Imaging Qdot655 photon by photon



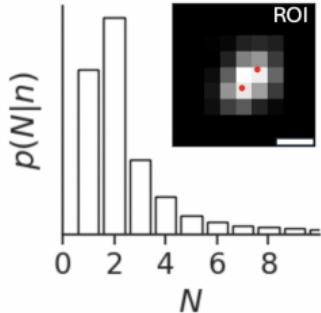
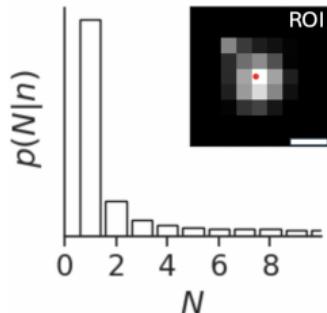
- ▶ 532nm picosecond pulsed laser pulsed at 500kHz to excite fluorescent quantum dots

## Poisson-Binomial photon count data at a single pixel (simulation)

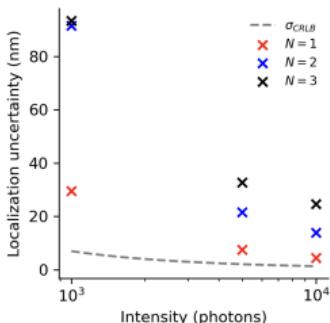
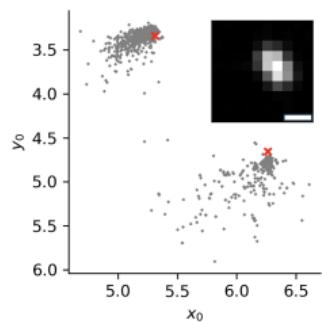


- ▶ Likelihood is a mixture of counts from  $N$  single photon sources, and background noise
- ▶ Can obtain posterior  $p(N, \zeta|n) \propto p(n|N, \zeta)p(\zeta)$

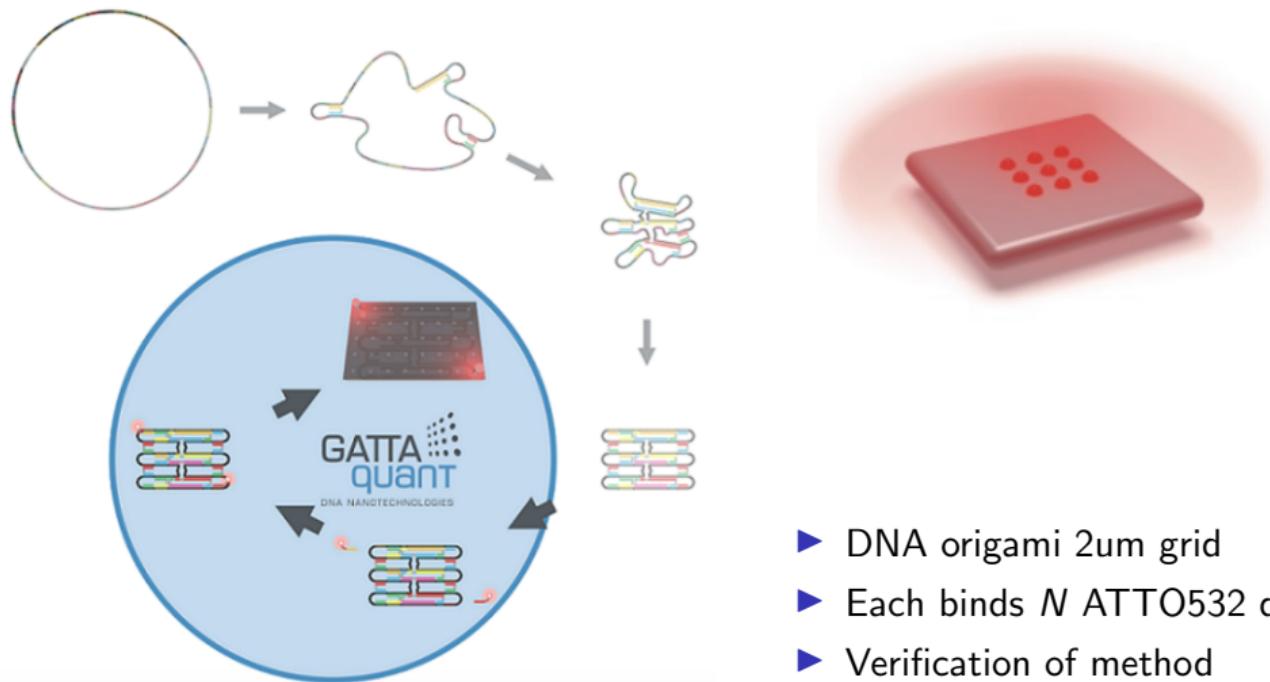
# Constrained multi-emitter localization with photon counting



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



## Counting ATTO532 dye bound to DNA origamis

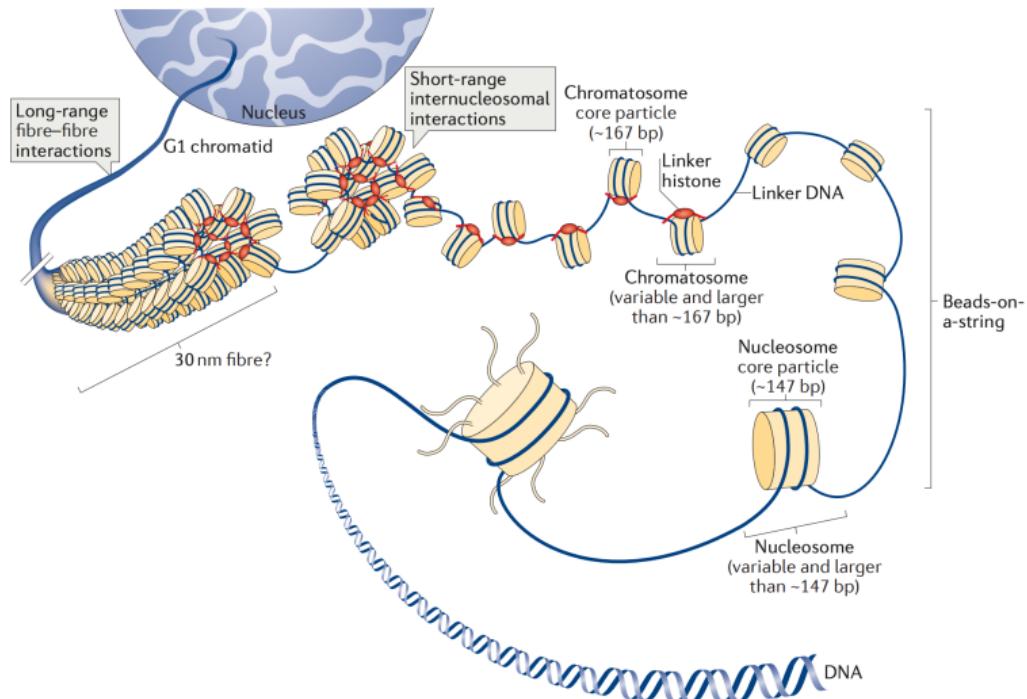


- ▶ DNA origami 2um grid
- ▶ Each binds  $N$  ATTO532 dyes
- ▶ Verification of method

Courtesy of GATTAquant DNA Nanotech

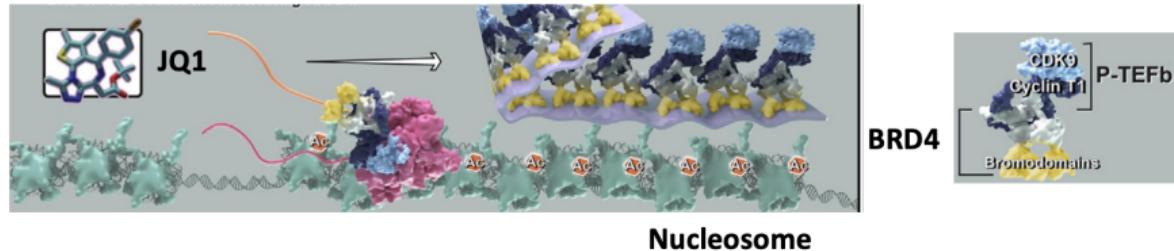
## Super-resolution of nucleosome nanodomains *in-vivo*

# Hierarchical structure of chromatin

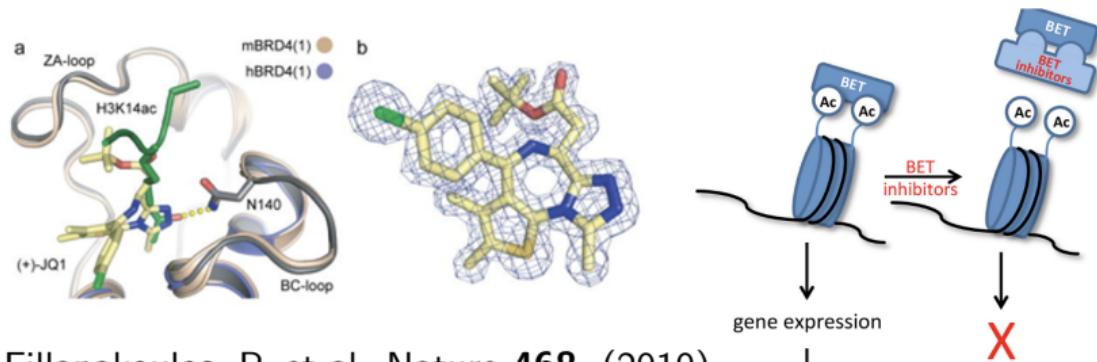


Fyodorov, D. et al. Nat Rev Mol Cell Biol **19**, (2018).

# Bromodomain protein 4 (BRD4) binds acetylated chromatin

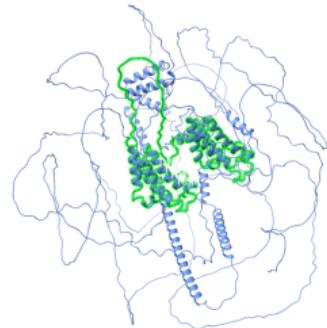
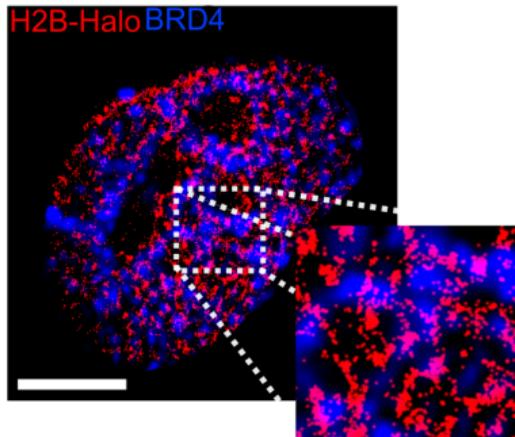


Zheng, B. et al. Molecular Cell **16**, (2023).



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

# BRD4 mutations to probe effects on chromatin structure

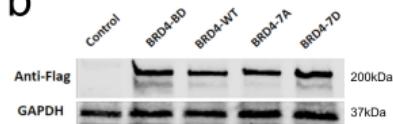


# BRD4 binding efficacy controls colocalization with nucleosome nanodomains

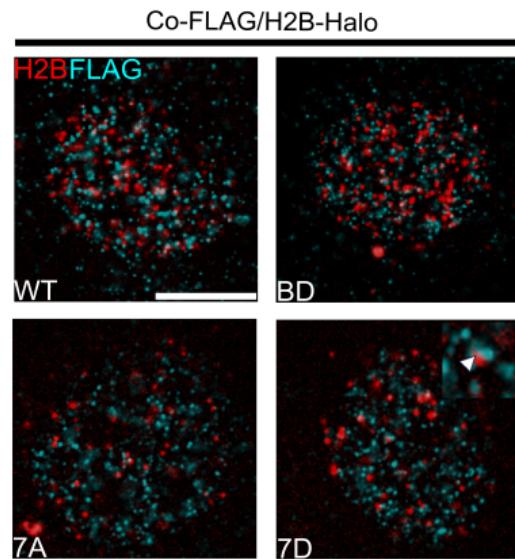
a



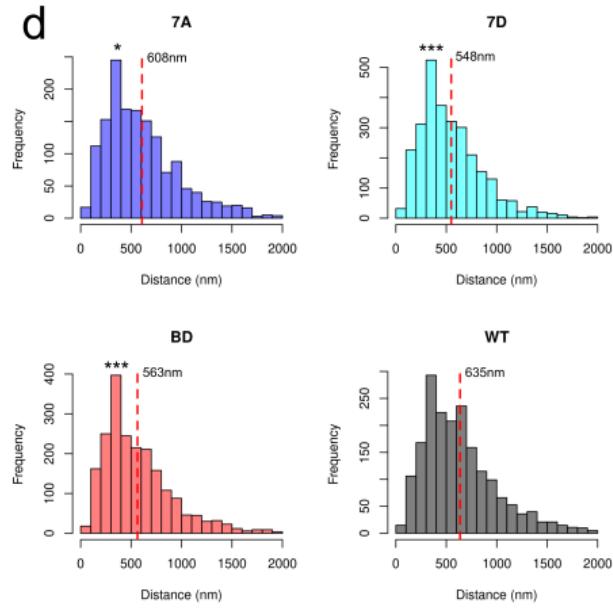
b



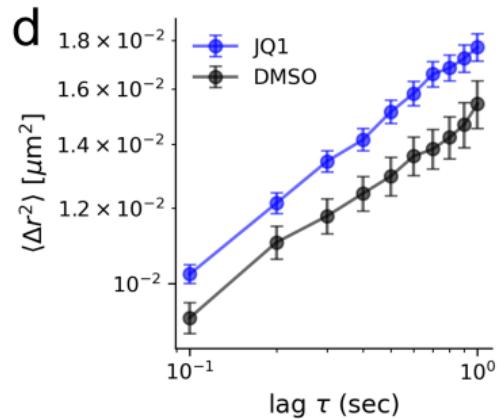
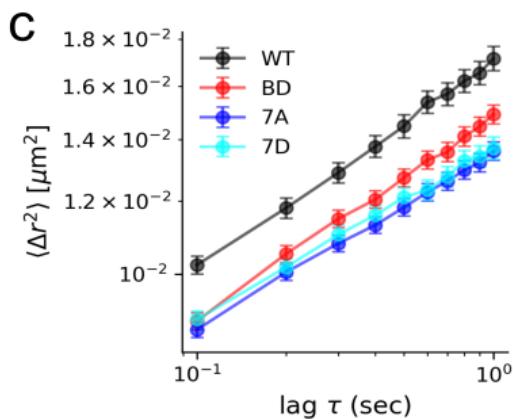
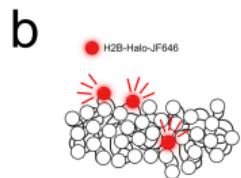
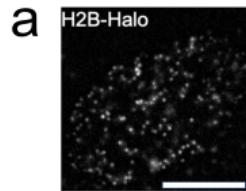
c



d

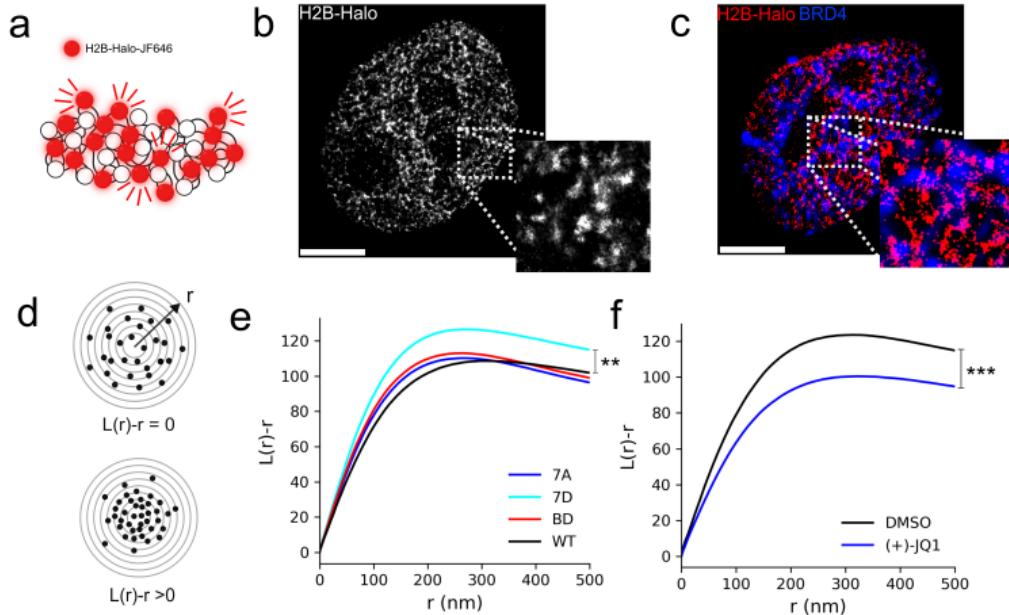


# BRD4 chromatin binding controls chromatin mobility



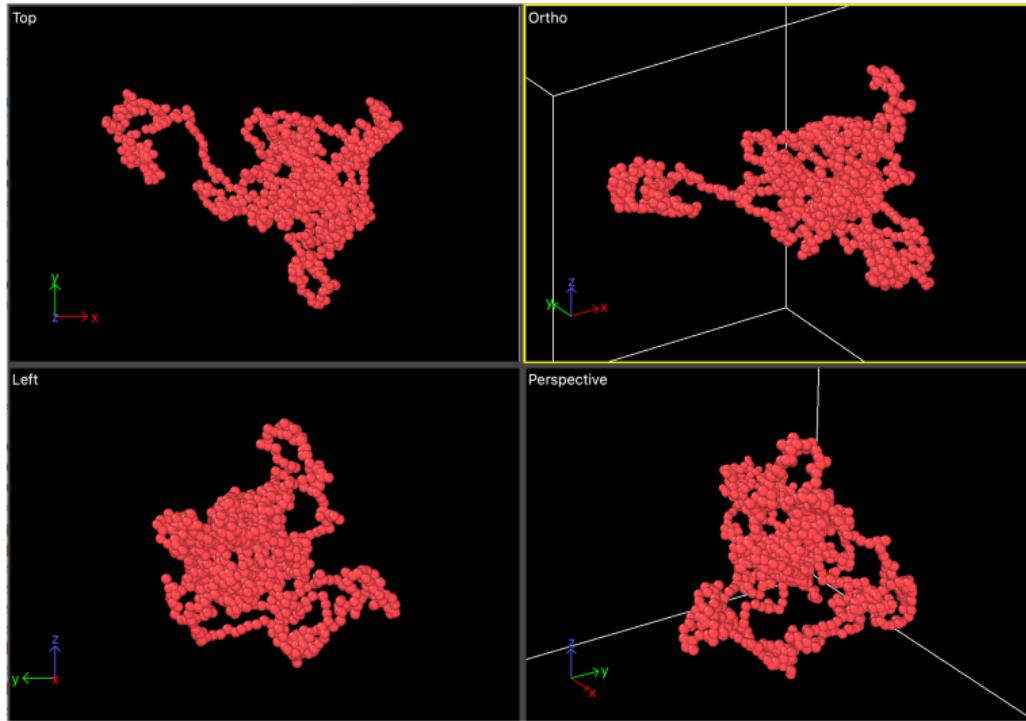
- ▶ H2B is sparsely labeled for particle tracking
- ▶ Reduced diffusion coefficient  $D$  in BRD4 mutants
- ▶ Increased  $D$  in cells exposed to (+)-JQ1

# BRD4 binding is necessary for maintenance of nucleosome nanodomains



- ▶ H2B is densely labeled for super-resolution imaging
- ▶ BRD4 chromatin binding activity controls nanodomain density

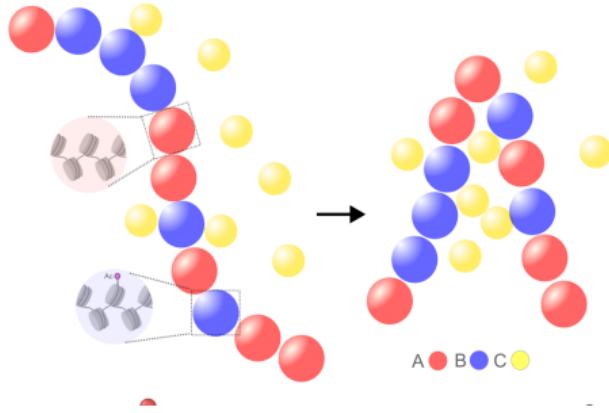
# Coarse grained molecular dynamics of chromatin at 310K



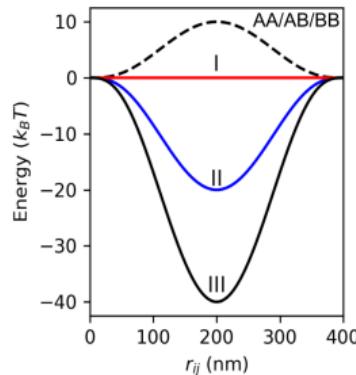
- ▶ 100kb chromatin chains connected by harmonic bonds (Rouse model)

# Coarse grained molecular dynamics of chromatin binders at 310K

a



b

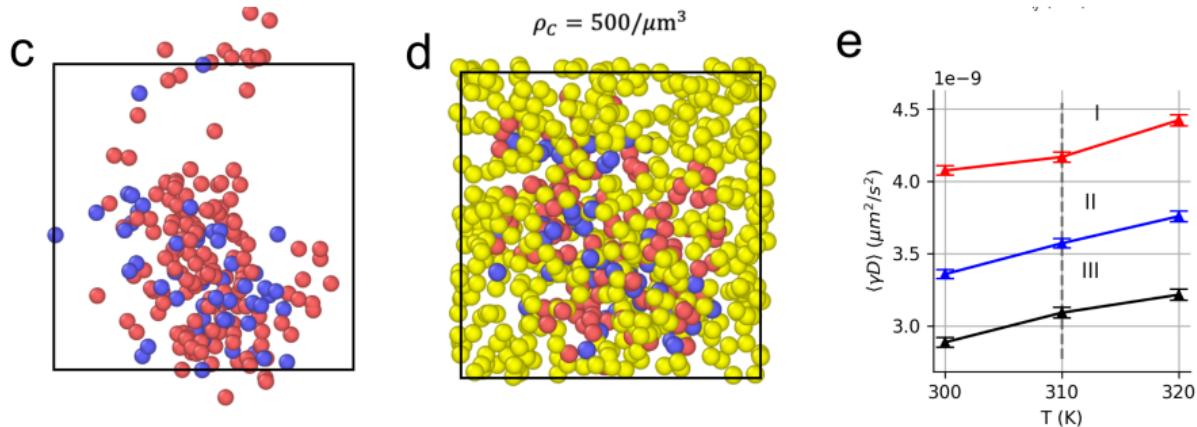


100kb chromatin chains interact with binders via the potential

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

- ▶ 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  
 $\xi \sim \mathcal{N}(0, 1)$ ,  $\langle \xi(t) \xi(t + \tau) \rangle = \delta(\tau)$

## Summary of contributions

### **First generative model of super-resolution microscopy images from low-resolution inputs**

- ▶ Modeled a distribution rather than a point estimate
- ▶ Quantified uncertainty

### **Applied SPAD array for counting fluorescent molecules in widefield microscopy**

- ▶ Novel Bayesian inference paradigm from single photon counts

### **First data supporting control of chromatin architecture by transcriptional condensates**

# Acknowledgements



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Norbert Scherer



Donghong Fu

Thank you!