

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

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# Outline of the talk

## Introduction to fluorescence nanoscopy

Novel methods: Probabilistic modeling approaches to fluorescence nanoscopy

Approach I: Enhance nanoscopy with deep generative models

Approach II: Integrated single photon counting and widefield single molecule imaging

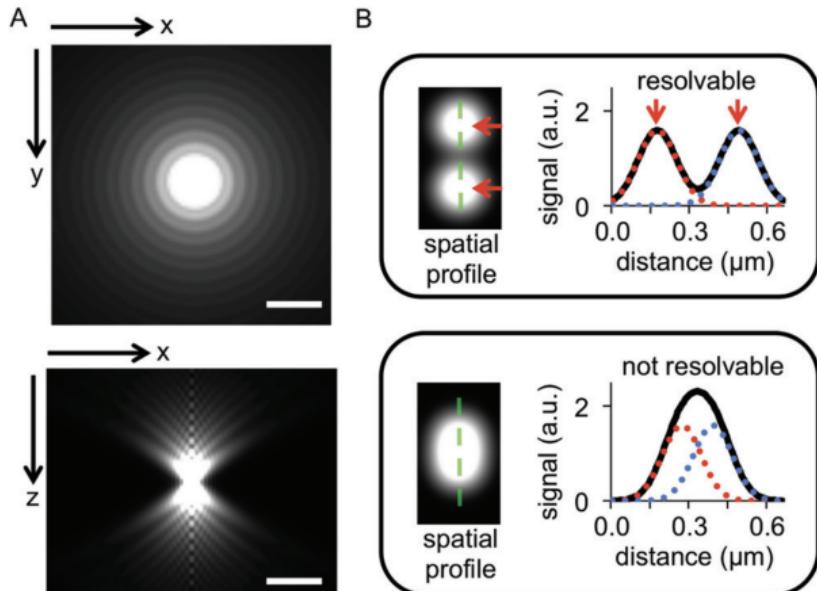
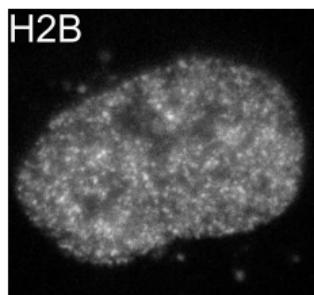
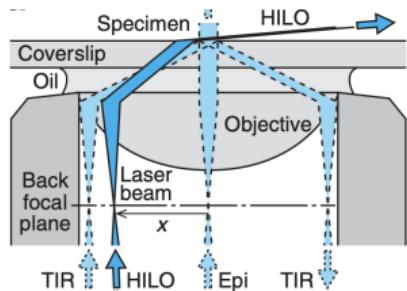
## Super-resolution of nucleosome nanodomains *in-vivo*

Interaction of transcriptional condensates with nucleosome nanodomains

## Introduction to fluorescence nanoscopy

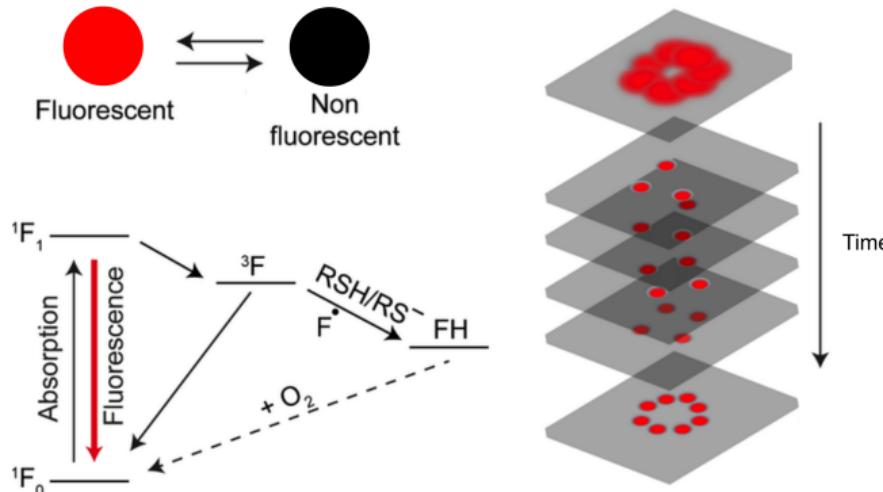
# Fluorescence microscopy and the diffraction limit

- Minimal resolvable distance  $d \sim \lambda/2$



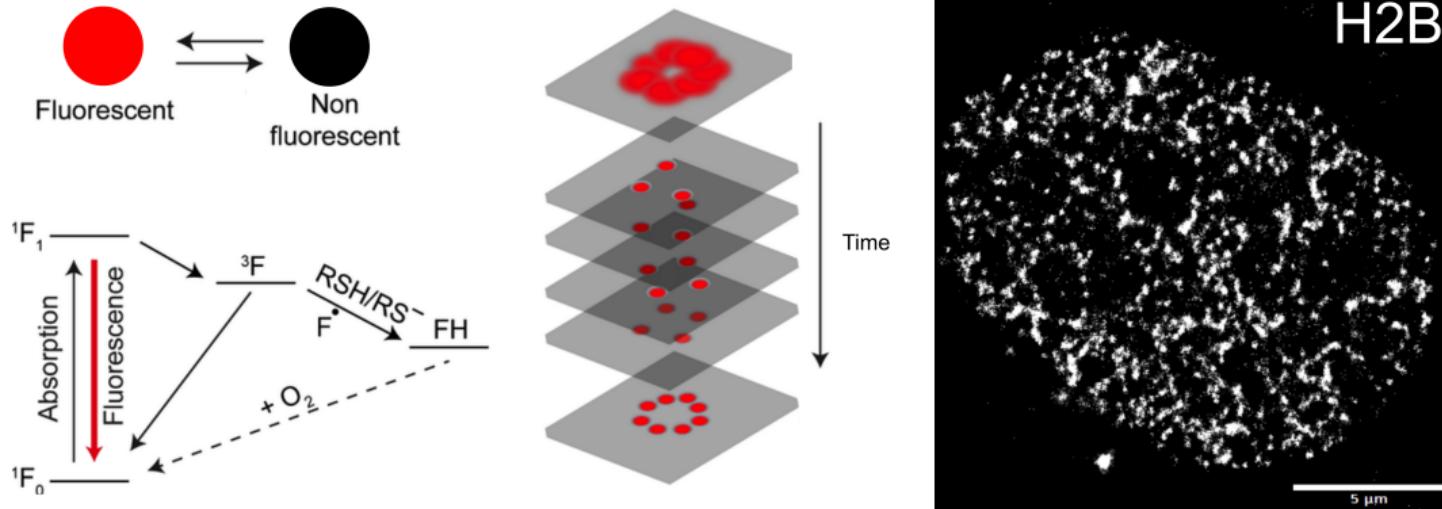
Herbert et al. Microscopy and Microanalysis.

# Stochastic optical reconstruction microscopy (STORM)



- ▶ STORM and similar nanoscopy techniques are limited by localization precision
- ▶ Higher lateral/axial resolution than other methods (e.g., SIM, STED, Confocal)
- ▶ Poor time resolution

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# Nanoscopy by localizing isolated fluorescent emitters

- Modeling the point spread function permits sub-pixel localization

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

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

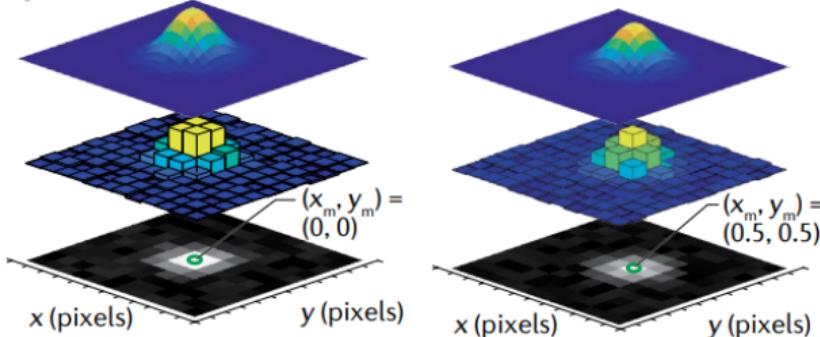
$g_k$  – pixel gain

$\eta$  – quantum efficiency

$\zeta$  – photon emission rate

$\Delta$  – exposure time

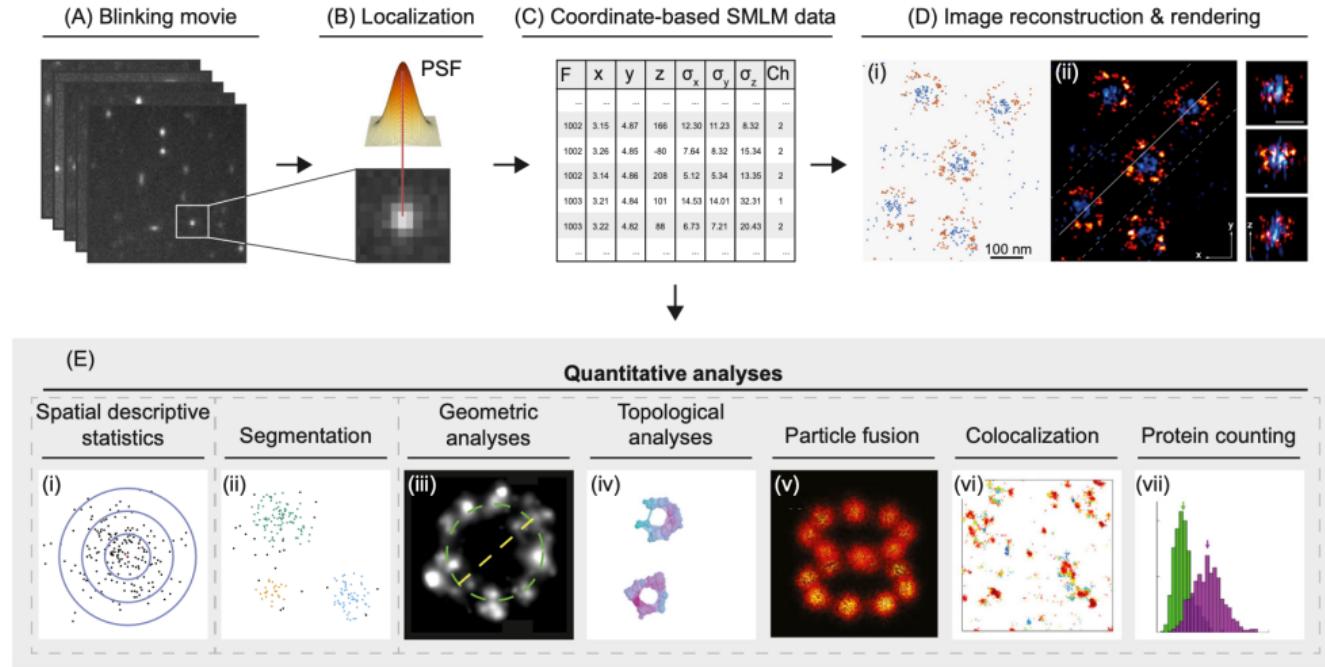
$\lambda$  – background rate



Maximum likelihood localization:

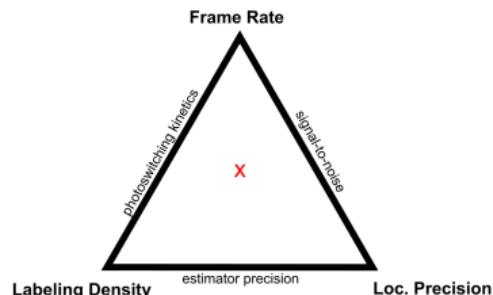
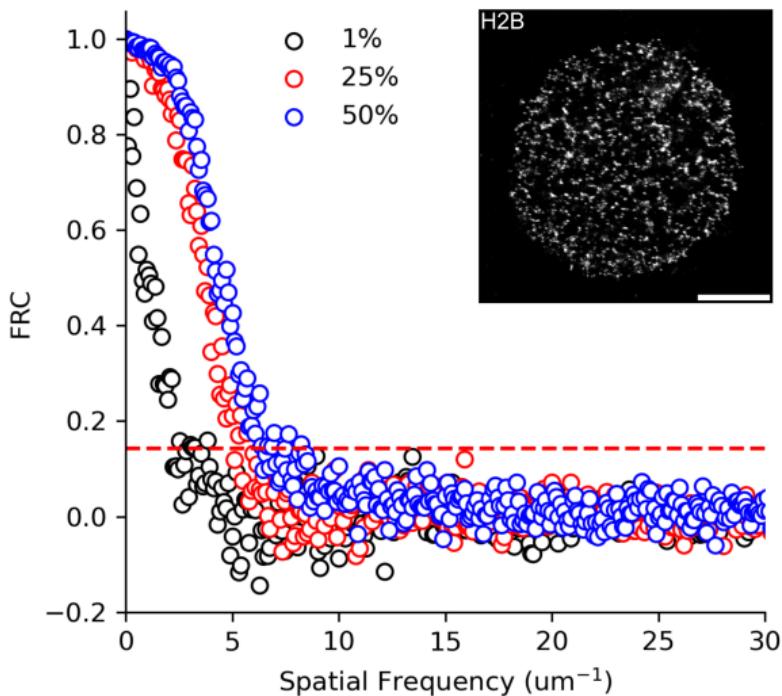
$$\theta^* = \operatorname{argmax}_{\theta} \prod_k p(\mathbf{x}_k | \theta) = \operatorname{argmin}_{\theta} - \sum_k \log p(\mathbf{x}_k | \theta)$$

# Applications of single molecule localization microscopy



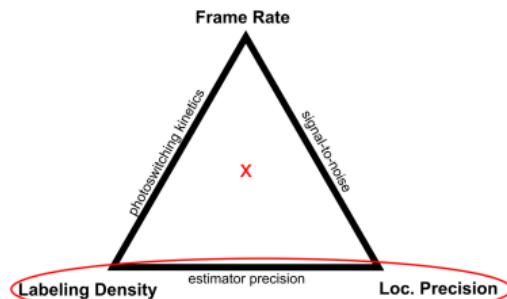
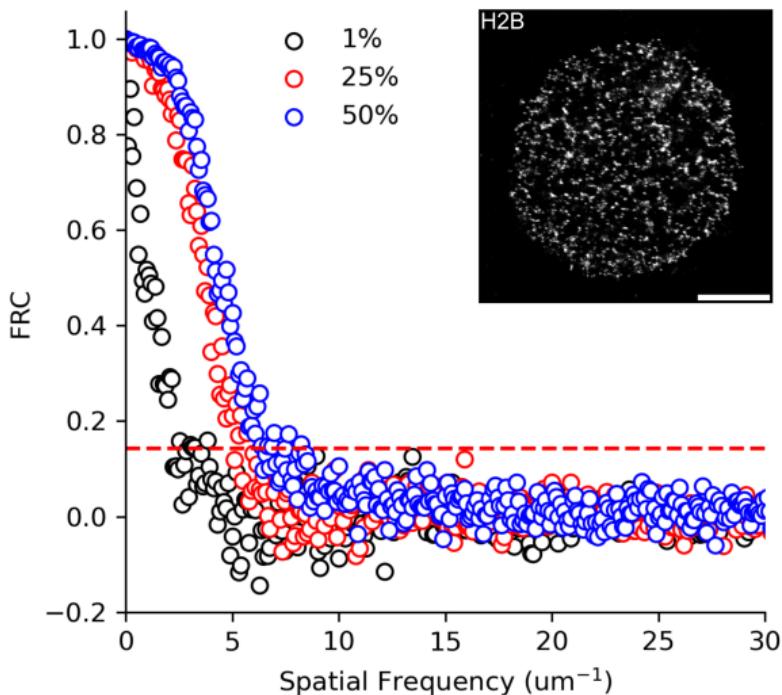
Wu et al. Trends in Cell Biology. 30 (2020)

# Maximization of density, minimization of error



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

## Maximization of density, minimization of error

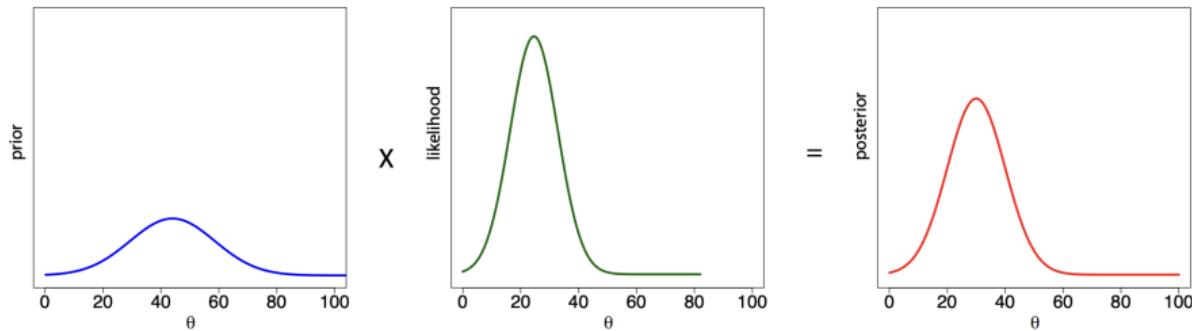


- ▶ Maximize density and in turn spatial/temporal resolution
- ▶ Minimize localization errors

## Novel methods: Probabilistic modeling approaches to fluorescence nanoscopy

# The Bayesian calculation and variational inference

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



- ▶ The posterior  $p(\theta|x)$  is can be hard to obtain
- ▶ Variational inference: fit a model distribution  $p_\psi(\theta|x)$  with parameters  $\psi$
- ▶ Can naturally represent uncertainty during inference

## Approach I: Resolution enhancement with a diffusion model

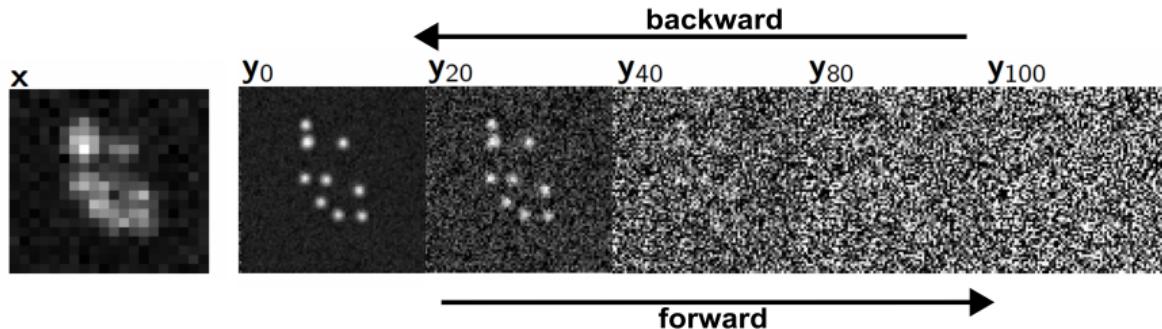
- ▶ Can sample from  $p(\theta|x)$  using a stochastic process called Langevin dynamics

Drift and diffusion:  $\theta_t = \overbrace{\theta_{t-1} - \frac{\beta}{2} \nabla f(\theta)}^{\mu} + \sqrt{\beta} \xi \quad \xi \sim \mathcal{N}(0, I)$

## Approach I: Resolution enhancement with a diffusion model

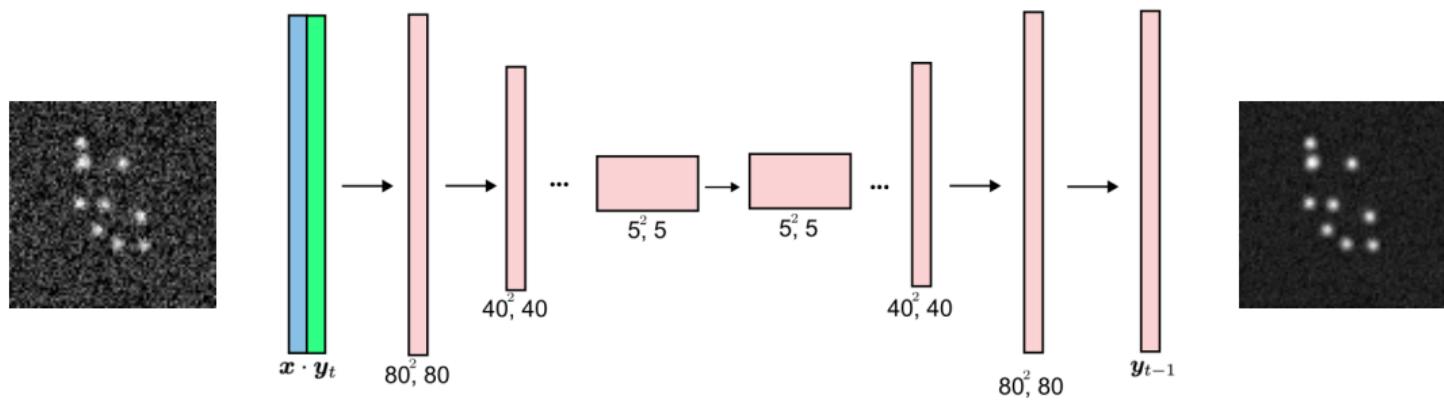
- ▶ Task: infer a high resolution image  $\mathbf{y}_0$  from low resolution  $\mathbf{x}$
- ▶ Drift is not available for image data, but can be learned from pairs  $(\mathbf{x}, \mathbf{y}_0)$

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



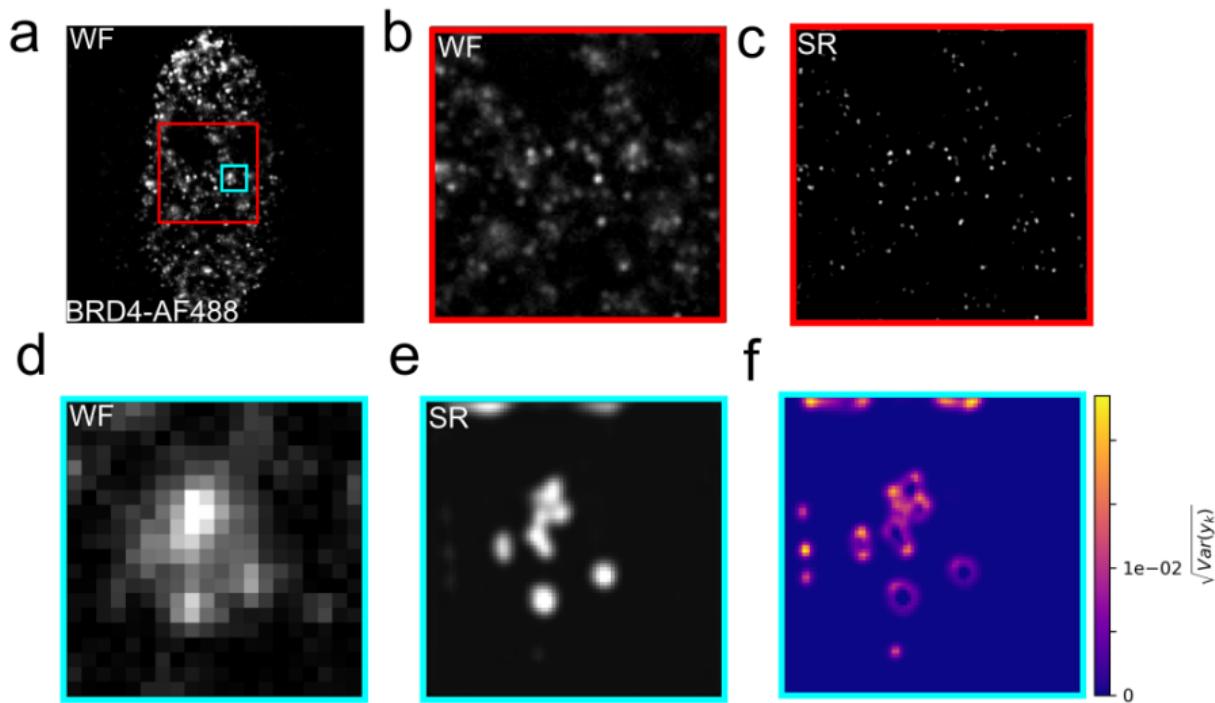
$$q(\mathbf{y}_t | \mathbf{y}_{t-1}) = \mathcal{N}\left(\sqrt{1 - \beta_t} \mathbf{y}_{t-1}, \beta_t I\right)$$

## Approach I: Resolution enhancement with a diffusion model



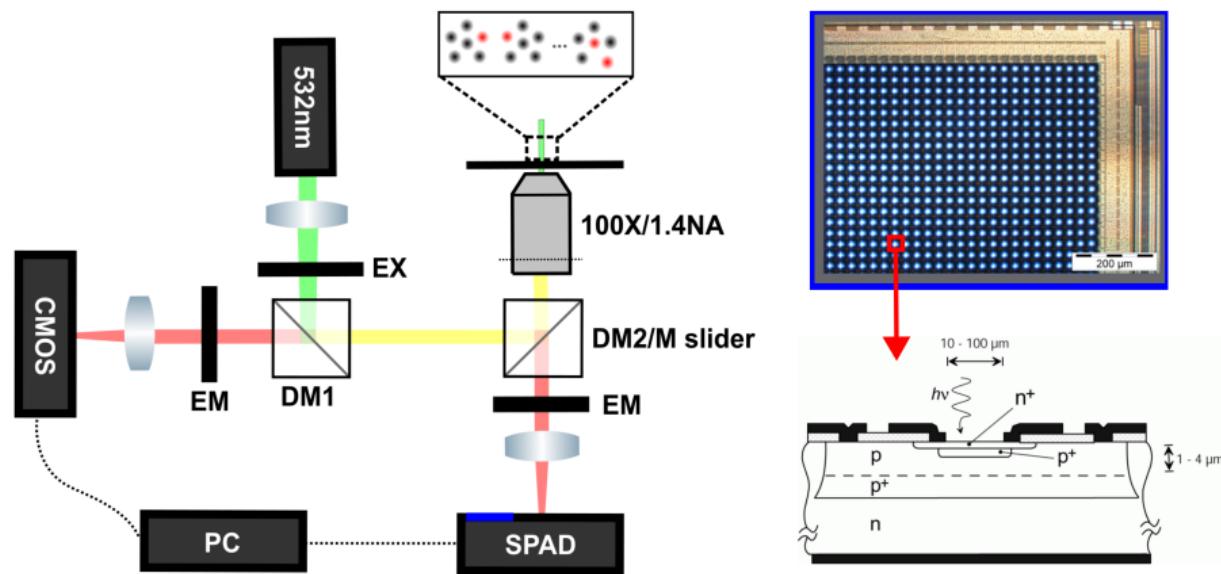
- ▶ A convolutional neural network  $\psi$  estimates the drift  $\mu_\psi$
- ▶ Denoising step:  $\mathbf{y}_{t-1} \sim p_\psi(\mathbf{y}_{t-1} | \mathbf{y}_t, \mathbf{x}) = \mathcal{N}(\mu_\psi, \beta_t I)$

# Super resolution of BRD4 protein in a HeLa cell

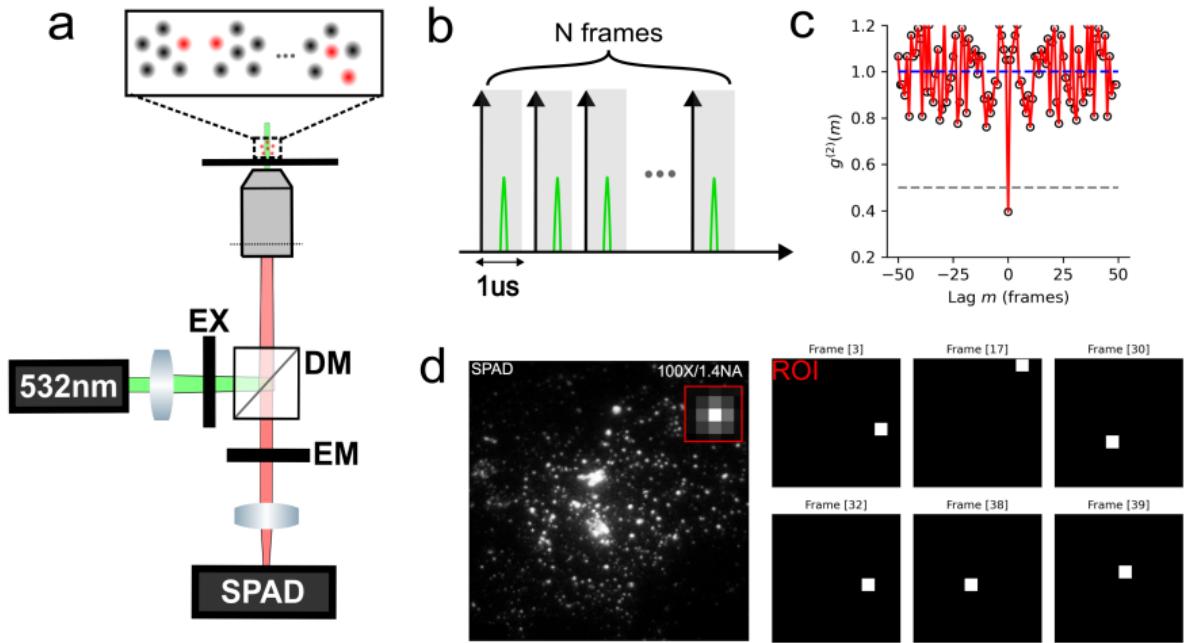


## Approach II: Integrated single photon counting and widefield single molecule imaging

- ▶ Single photon avalanche diode (SPAD) arrays give us new modeling approaches

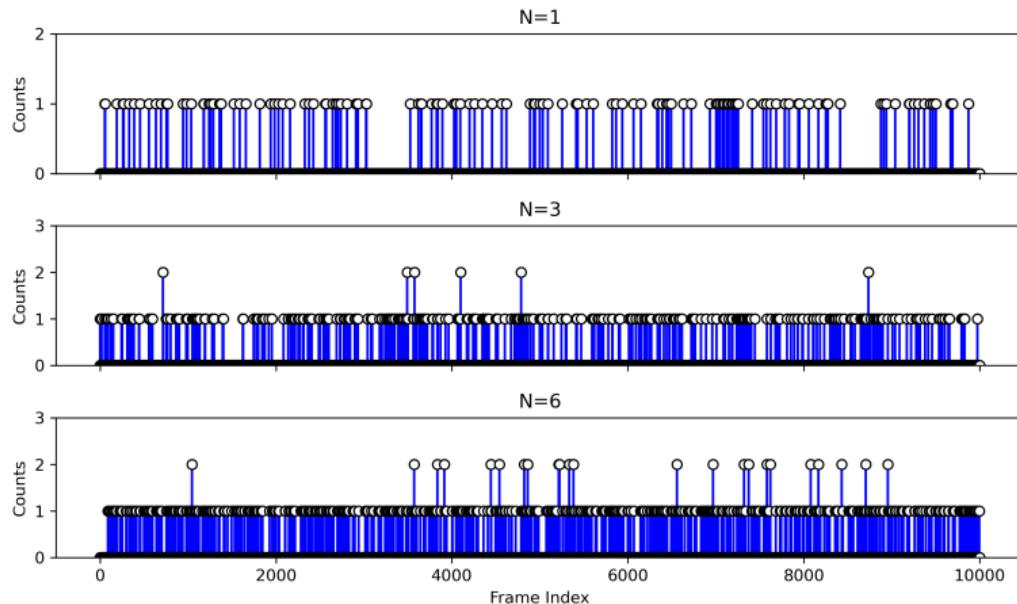


# Imaging Qdot655 photon by photon



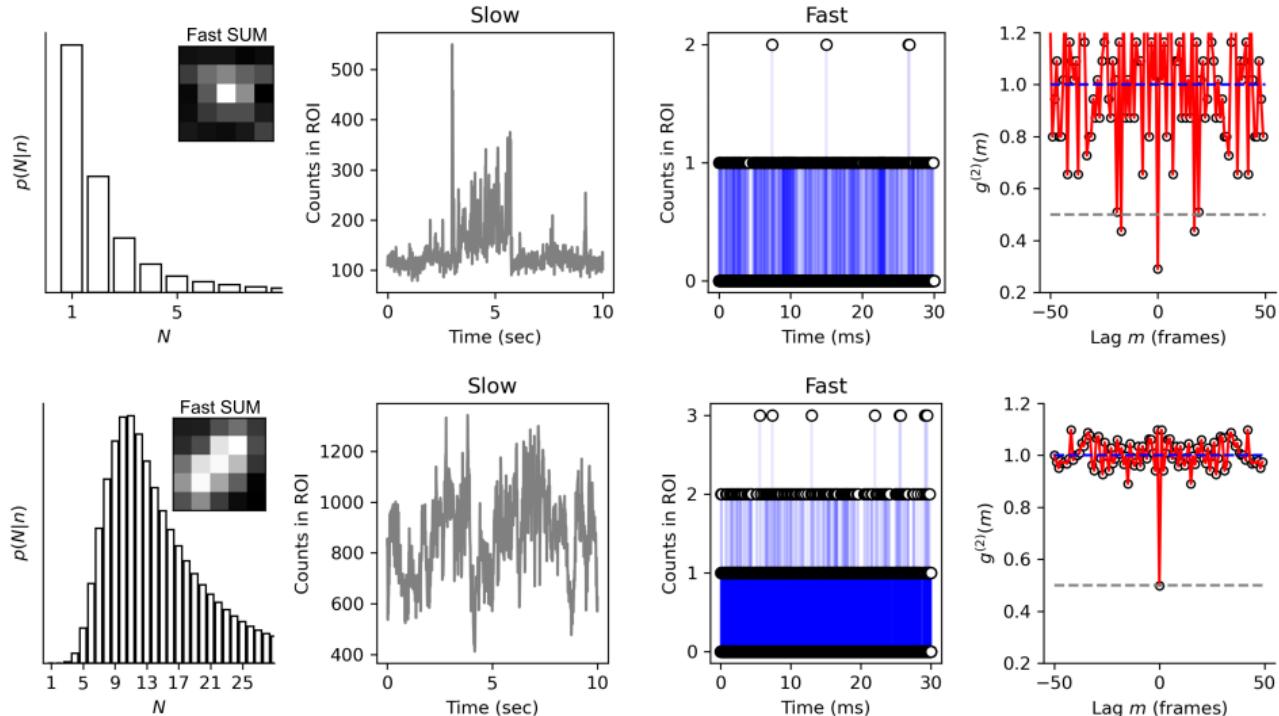
►  $g^{(2)}(m) = G^{(2)}(0)/\langle G^{(2)}(m) \rangle$

## Poisson-Binomial photon count data (simulation)



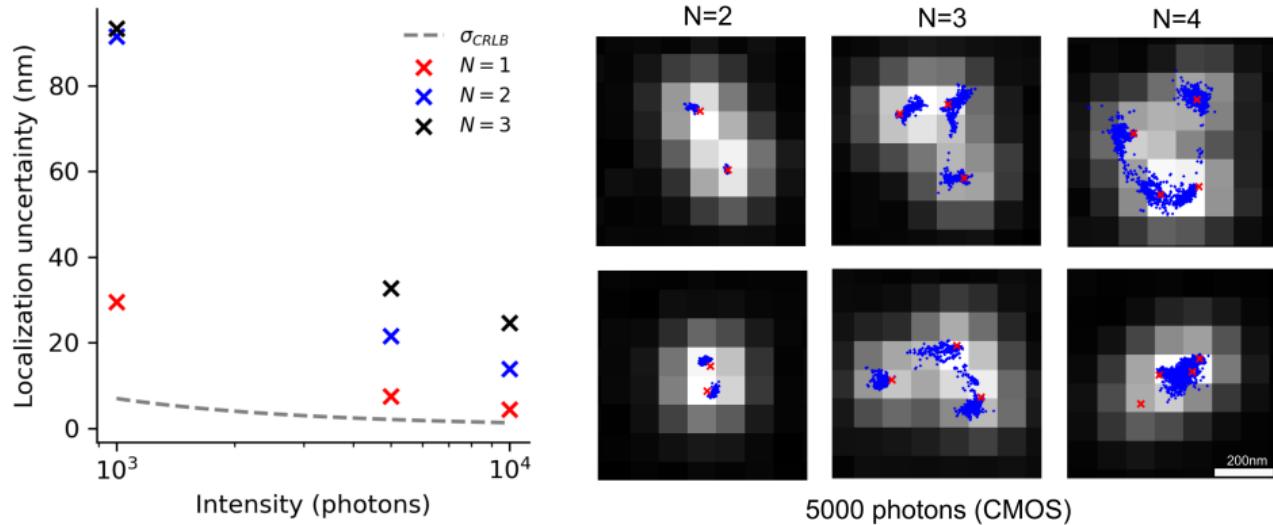
- ▶ SPAD arrays permit modeling of time-series of photon counts  $n_t$
- ▶ Likelihood on counts:  $\mathcal{L}(n_t|N, \zeta, \lambda) = \text{Poisson}(\lambda) * \text{Binomial}(N, \zeta)$

# Discrimination of single and multiple quantum dots



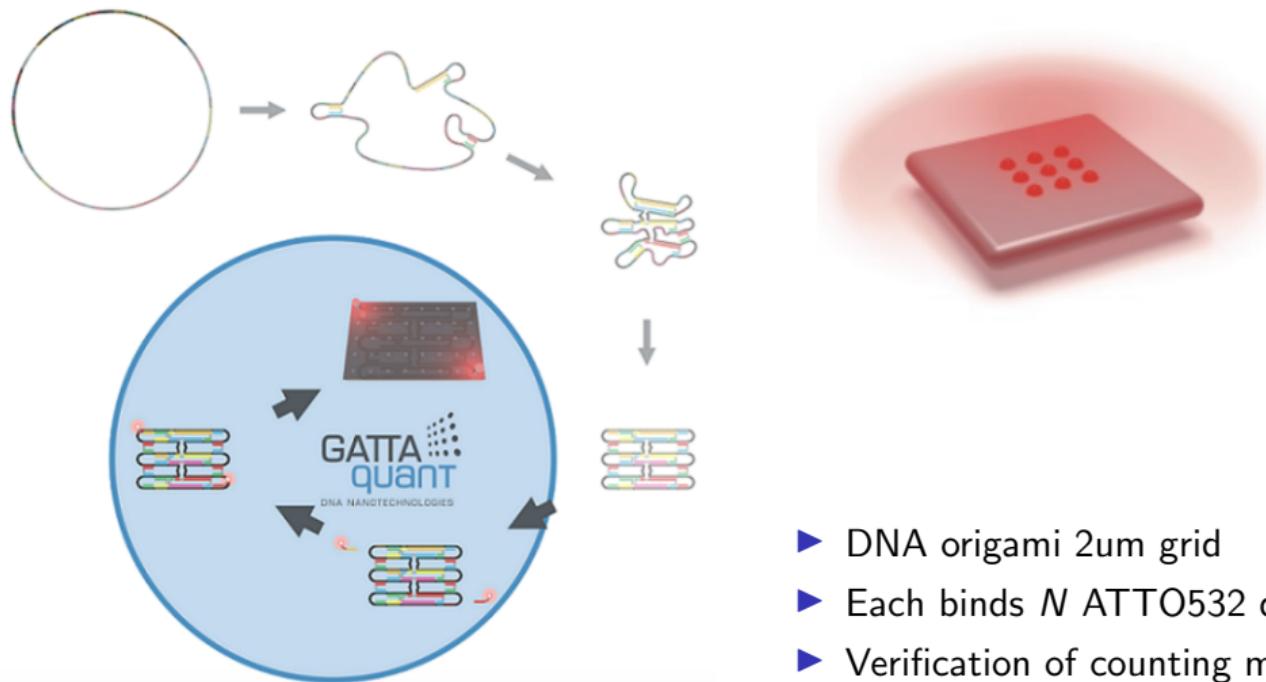
Blinking in quantum dots occurs due to non-radiative relaxation pathways

## Constrained multi-emitter localization (simulation)



- ▶ Multiple emitters can be fit simultaneously using MCMC sampling
- ▶ Constrained multi-emitter localization when  $N$  is known

## Upcoming work: counting ATTO532 dye bound to DNA origamis

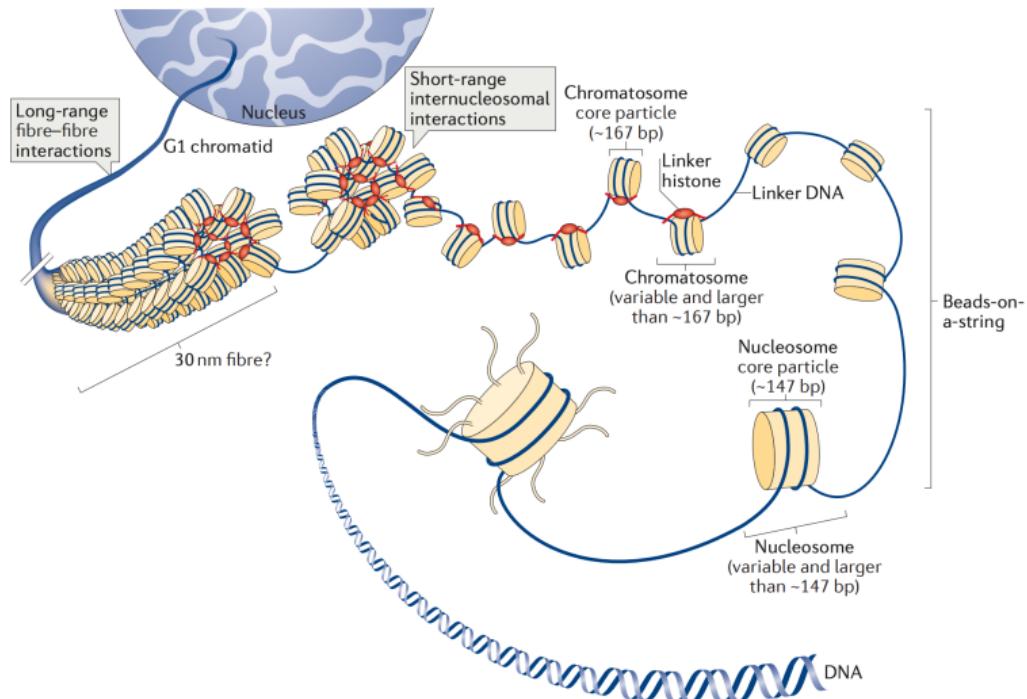


- ▶ DNA origami 2um grid
- ▶ Each binds  $N$  ATTO532 dyes
- ▶ Verification of counting method

Courtesy of GATTAquant DNA Nanotech

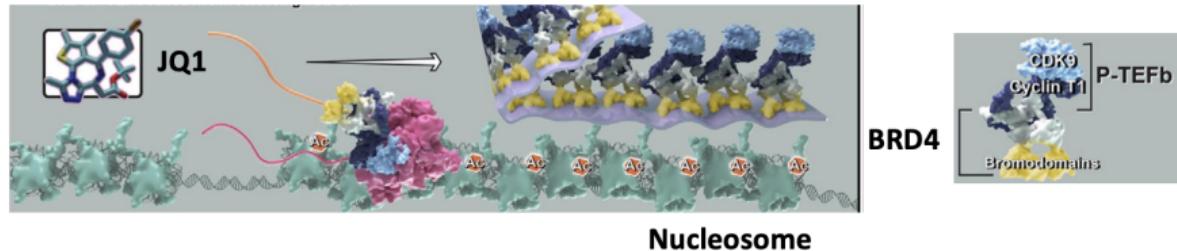
## Super-resolution of nucleosome nanodomains *in-vivo*

# Hierarchical structure of chromatin

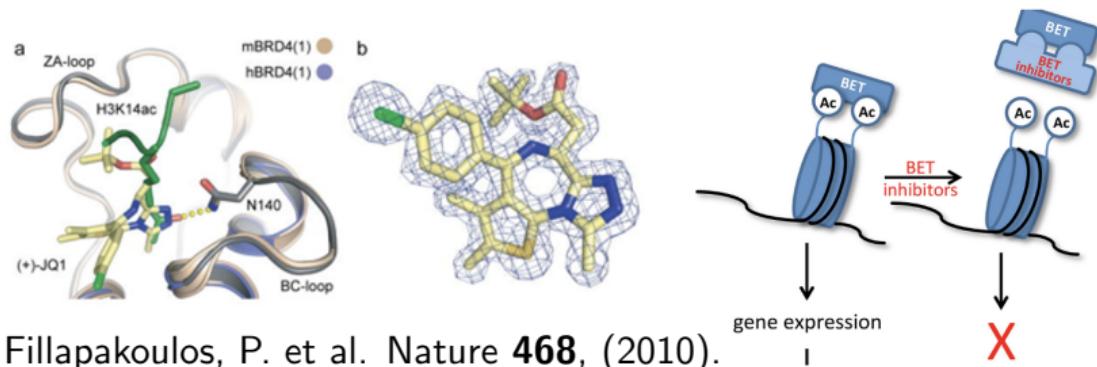


Fyodorov, D. et al. Nat Rev Mol Cell Biol **19**, (2018).

# Bromodomain protein 4 (BRD4) binds acetylated chromatin

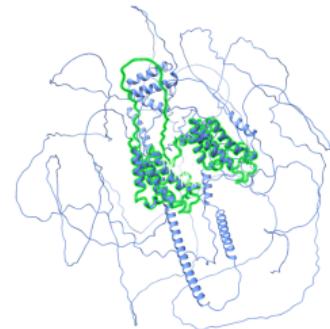
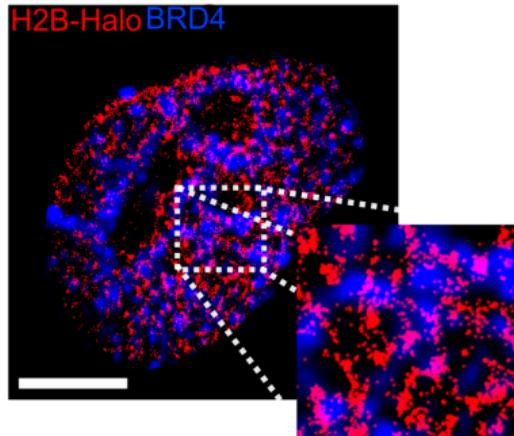


Zheng, B. et al. Molecular Cell **16**, (2023).



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

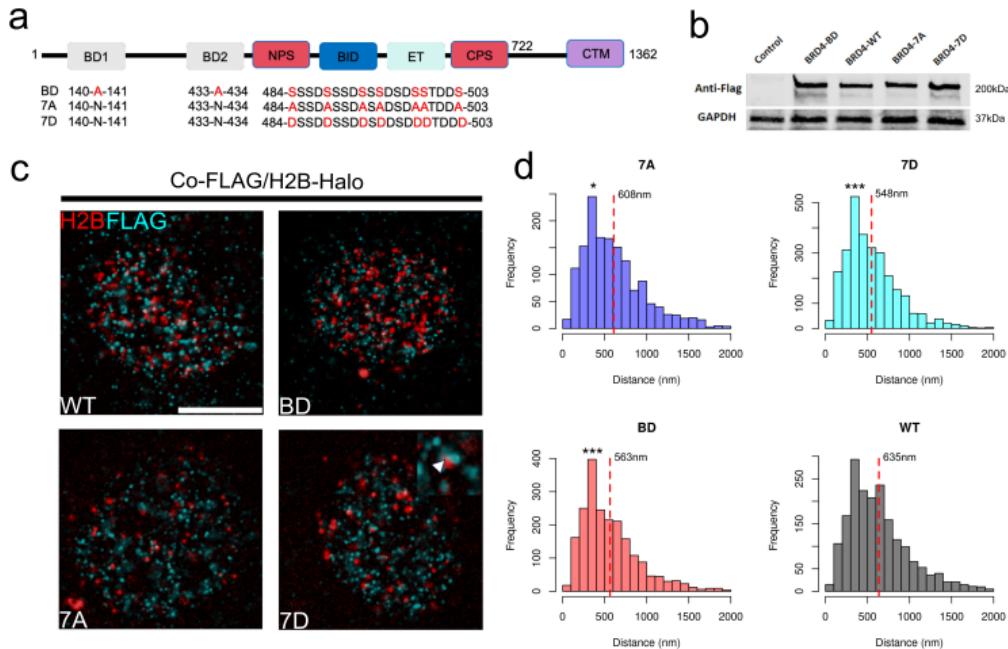
## BRD4 mutations to probe effects on chromatin structure



AlphaFold BRD4 1-1362aa

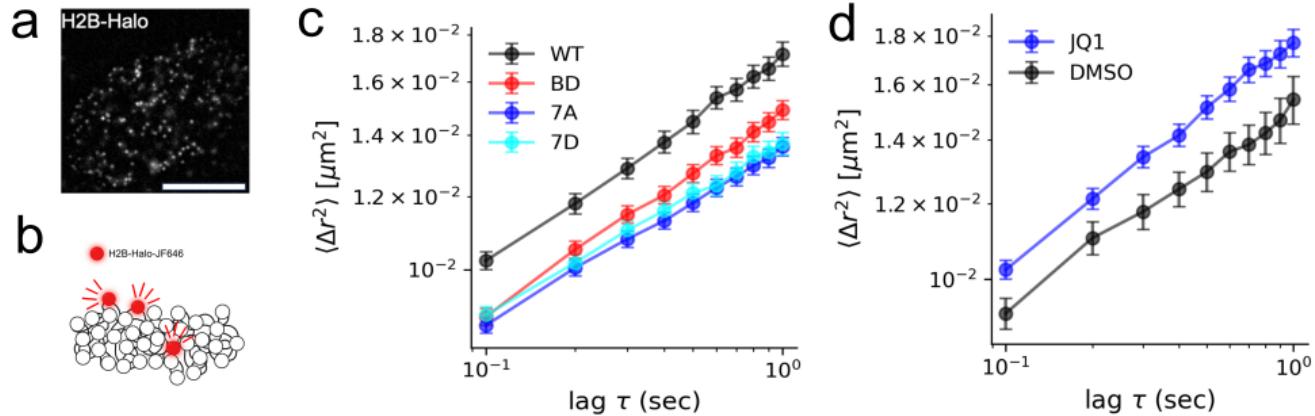
- ▶ 7A mutant - Constitutively unphosphorylated
- ▶ 7D mutant - Constitutively phosphorylated
- ▶ BD mutant - Bromodomain deactivated

# BRD4 recruitment to nucleosome nanodomains



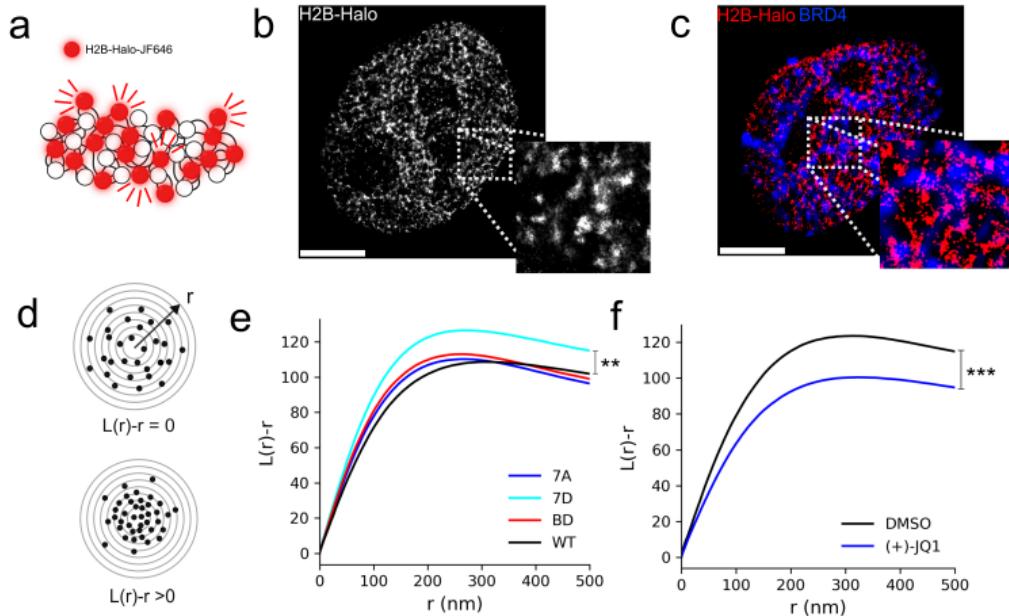
- ▶ Phosphorylation enhances BRD4 binding to acetylated chromatin
- ▶ Hyperphosphorylation - resistance mechanism to BD loss (Shu 2016)

# BRD4 chromatin binding controls chromatin mobility



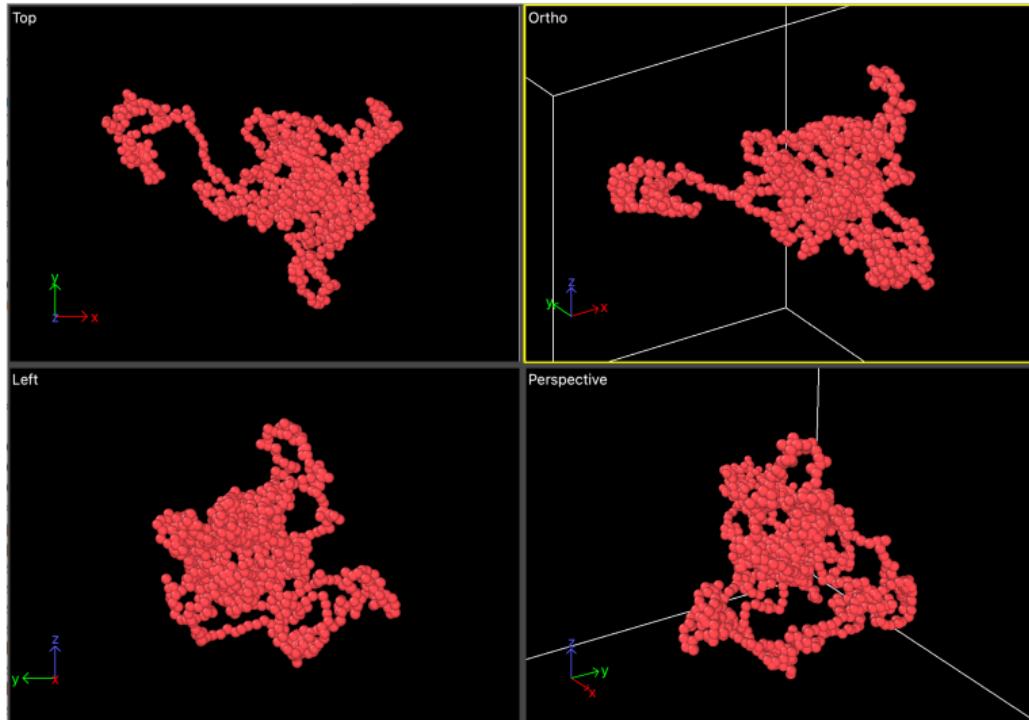
- ▶ H2B is sparsely labeled for particle tracking
- ▶ Reduced diffusion coefficient  $D$  in BRD4 mutants
- ▶ Increased  $D$  in cells exposed to (+)-JQ1

# BRD4 binding is necessary for maintenance of nucleosome nanodomains



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

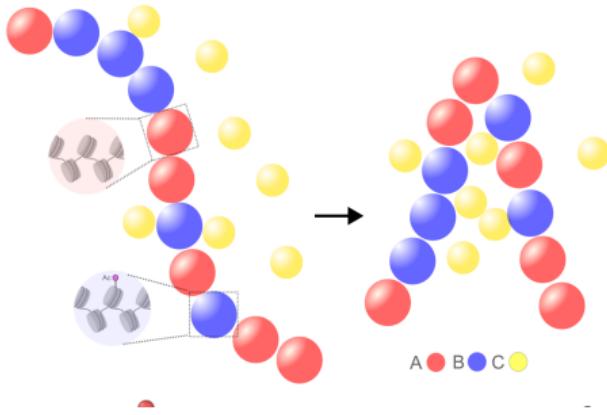
# Coarse grained molecular dynamics of chromatin at 310K



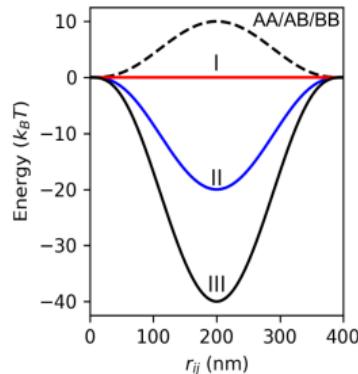
- ▶ 100kb chromatin beads connected by harmonic bonds (Rouse model)

# Coarse grained molecular dynamics of chromatin binders at 310K

a



b

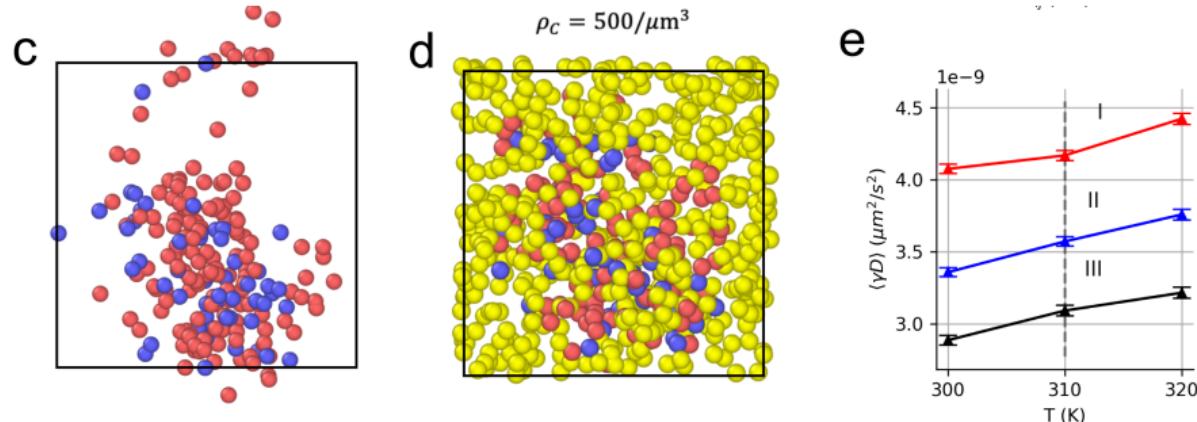


Chromatin chains interact with binders via the potential

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

- ▶ 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 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
- ▶ Applied SPAD array for counting fluorescent molecules in widefield microscopy
- ▶ First data supporting control of chromatin architecture by transcriptional condensates

## Selected Publications

- ▶ **C. Seitz**, D. Fu, M. Liu, H. Ma, and J. Liu. *BRD4 phosphorylation regulates the structure of chromatin nanodomains*. In Review. Phys Rev Lett. 2024
- ▶ **C. Seitz** and J. Liu. *Uncertainty-aware localization microscopy by variational diffusion*. In Progress. 2024
- ▶ **C. Seitz** and J. Liu. *Quantum enhanced localization microscopy with a single photon avalanche diode array*. In Progress. 2024
- ▶ M. Locatelli<sup>†</sup>, J. Lawrimore<sup>†</sup>, H. Lin<sup>†</sup>, S. Sanaullah, **C. Seitz**, D. Segall, P. Kefer, S. Moreno Naike, B. Lietz, R. Anderson, J. Holmes, C. Yuan, G. Holzwarth, B. Kerry, J. Liu, K. Bonin, P. Vidi. *DNA damage reduces heterogeneity and coherence of chromatin motions*. PNAS 12 July 2022; 119 (29): 1-11
- ▶ M. Zhang, **C. Seitz**, G. Chang, F. Iqbal, H. Lin, and J. Liu *A guide for single-particle chromatin tracking in live cell nuclei*. Cell Biology International 15 January 2022; 46 (5): 683-700
- ▶ S. Roy, **C. Seitz**, M. Samaddar, J. Liu *Bayesian Inference of Transcriptional Kinetics of GBP5 by Single-Molecule Imaging*. In Progress. 2024

# Acknowledgements



(left to right) Charles Park, Garrick Chang, Jing Liu, David Buchanan, Mengyuan Liu, Hailan Ma



Norbert Scherer



Donghong Fu

Thank you!