

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

Approach I: Enhance nanoscopy with deep generative models

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

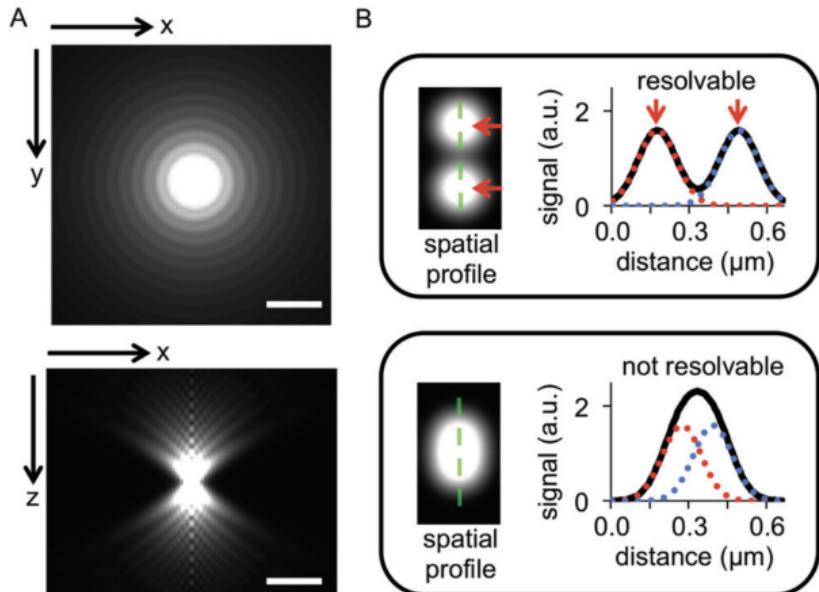
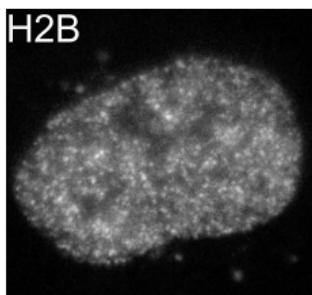
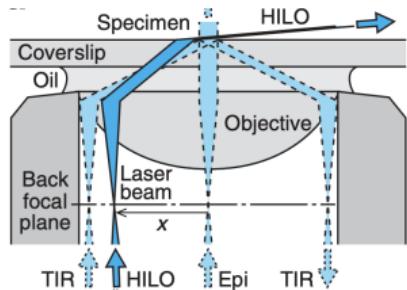
Super-resolution of nucleosome nanodomains *in-vivo*

Interaction of transcriptional condensates with nucleosome nanodomains

Introduction to fluorescence nanoscopy

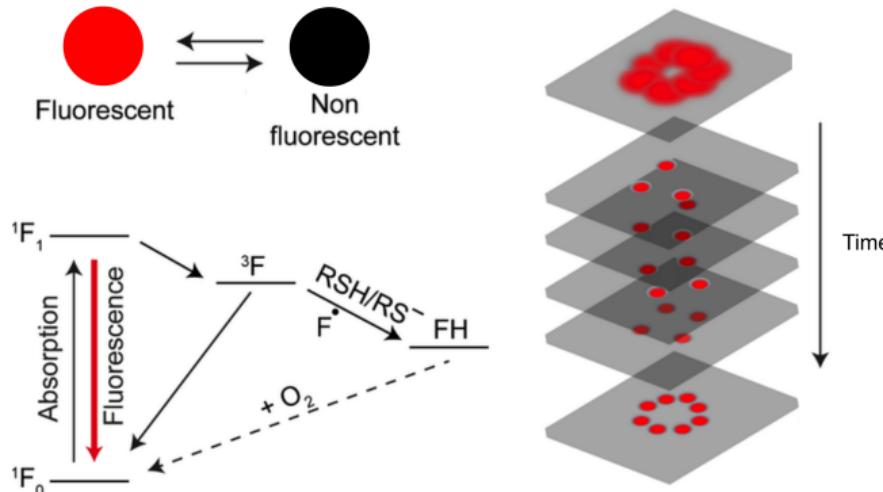
Fluorescence microscopy and the diffraction limit

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



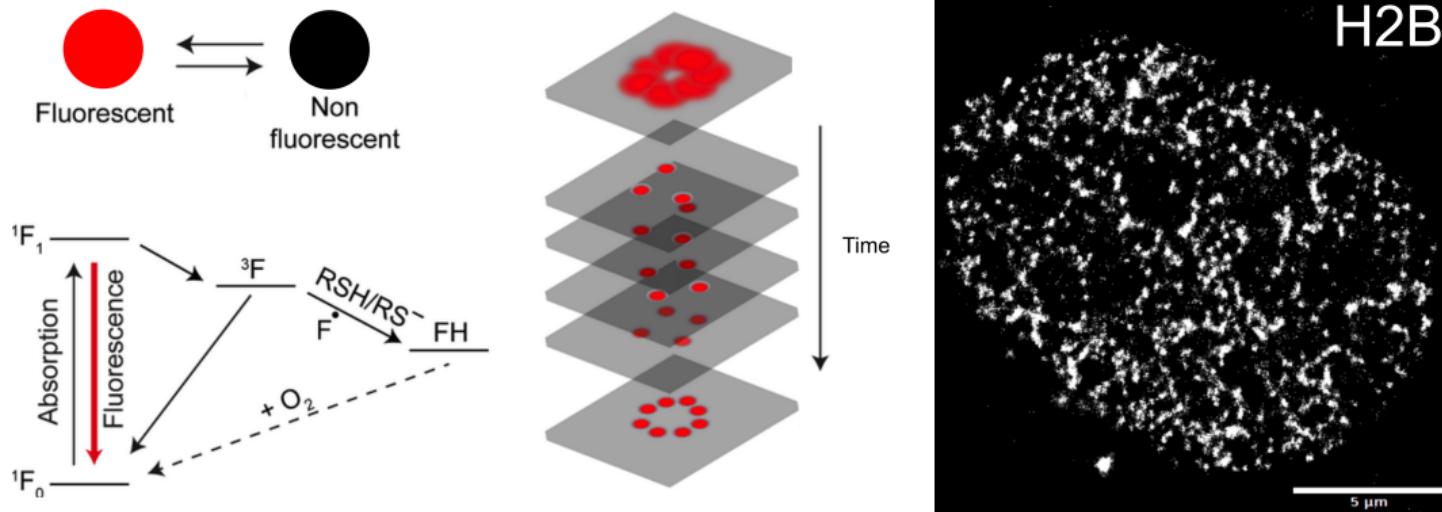
Herbert et al. Microscopy and Microanalysis.

Stochastic optical reconstruction microscopy (STORM)



- ▶ STORM and similar nanoscopy techniques are 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

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

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

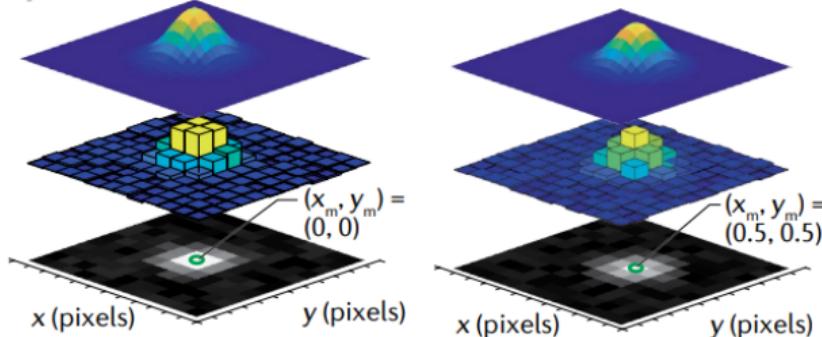
g_k – pixel gain

η – quantum efficiency

ζ – photon emission rate

Δ – exposure time

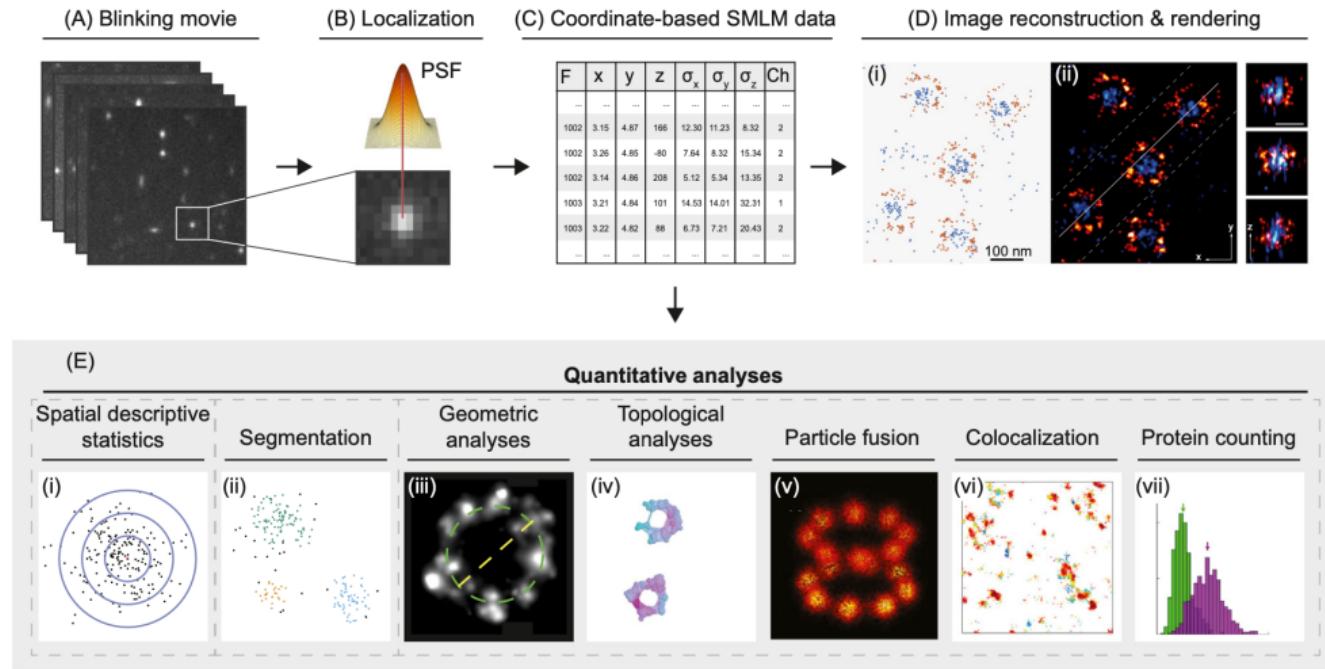
λ – background rate



Maximum likelihood localization:

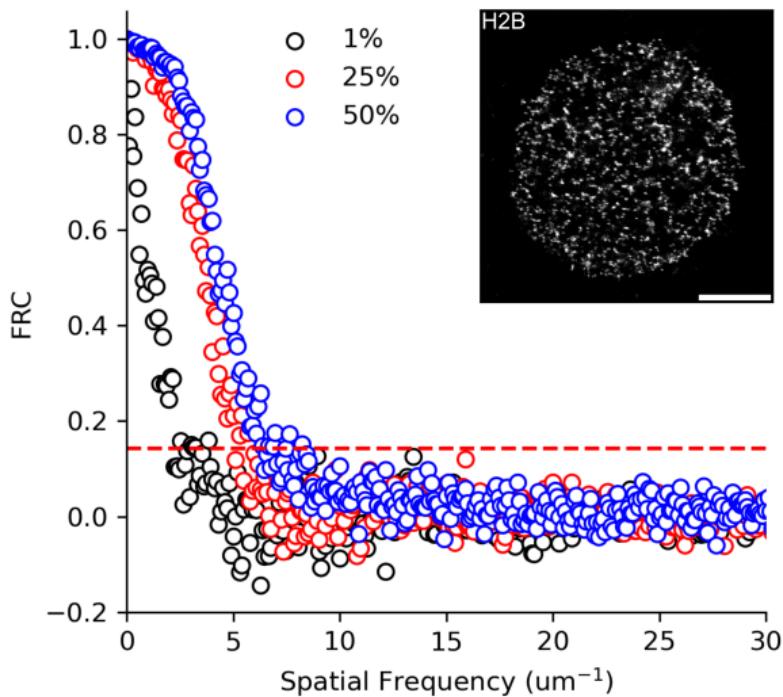
$$\theta^* = \operatorname{argmax}_{\theta} \prod_k p(\mathbf{x}_k | \theta) = \operatorname{argmin}_{\theta} - \sum_k \log p(\mathbf{x}_k | \theta)$$

Applications of single molecule localization microscopy



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

Maximization of density, minimization of error

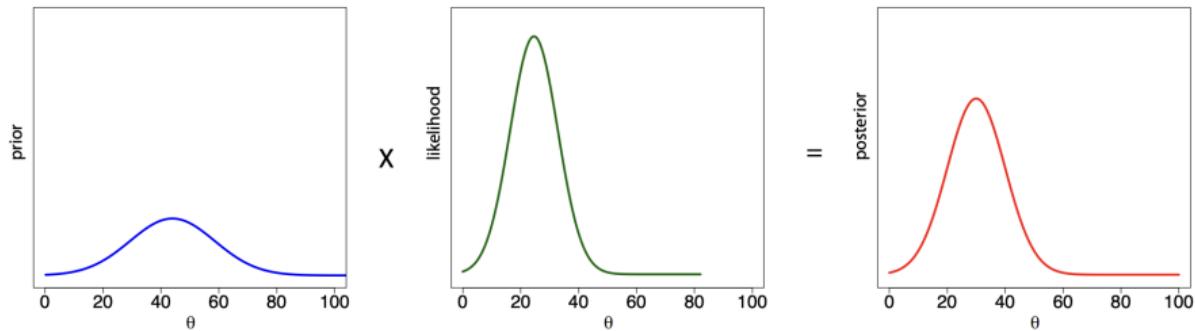


- ▶ **Maximize** density and in turn spatial/temporal resolution
- ▶ **Minimize** localization errors

Probabilistic modeling approaches to fluorescence nanoscopy

The Bayesian calculation and variational inference

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



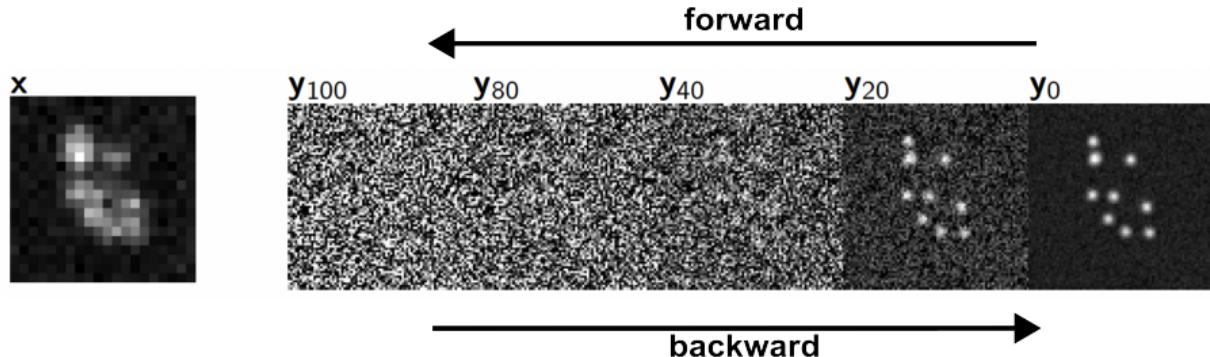
- ▶ $p(\theta|x)$ is can be hard to obtain
- ▶ Variational inference: fit a model distribution $p_\psi(\theta|x)$
- ▶ ψ can be a multivariate Gaussian, neural network, etc.

Approach I: Resolution enhancement with a diffusion model

Objective: Infer of a high resolution image \mathbf{y}_0 from low resolution \mathbf{x}

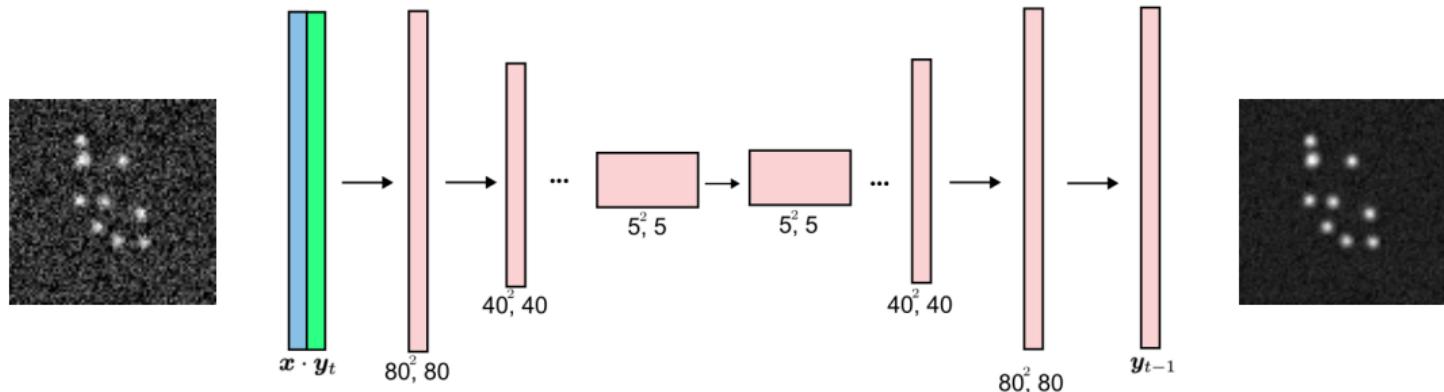
- ▶ A diffusion model can approximate the posterior distribution $p_\psi(\mathbf{y}_0|\mathbf{x})$

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



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

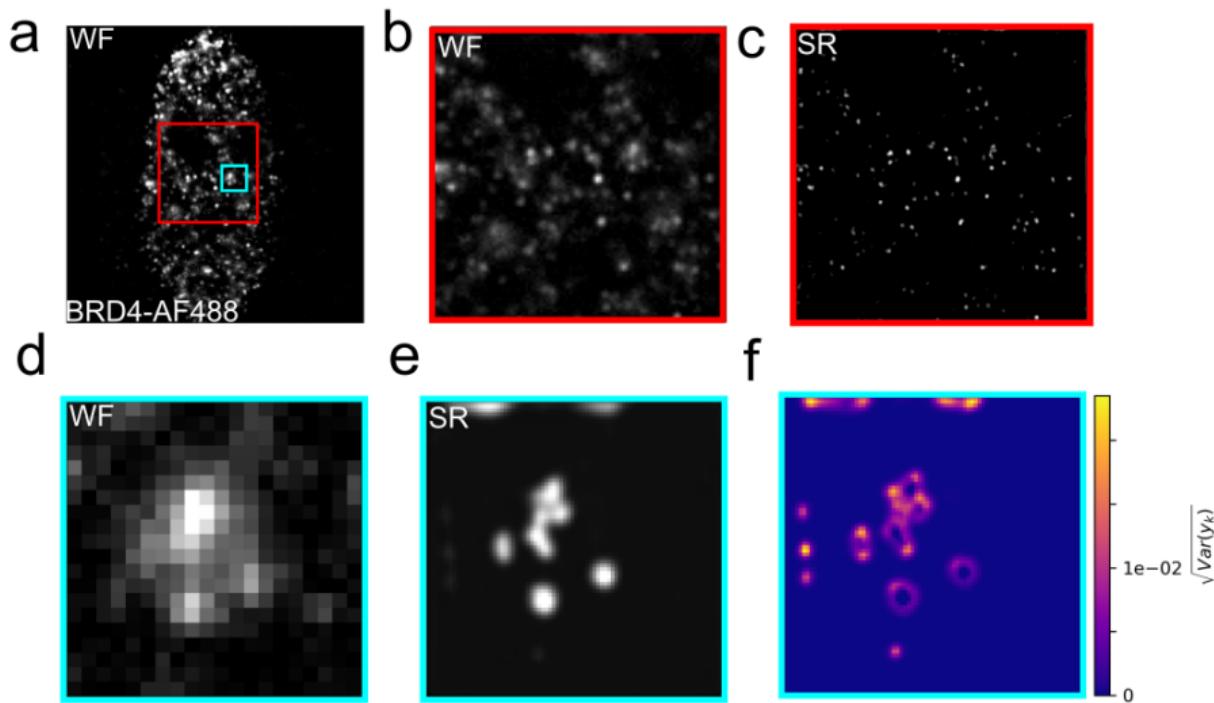
Approach I: Resolution enhancement with a diffusion model



A deep neural network estimates the gradient of the reverse process

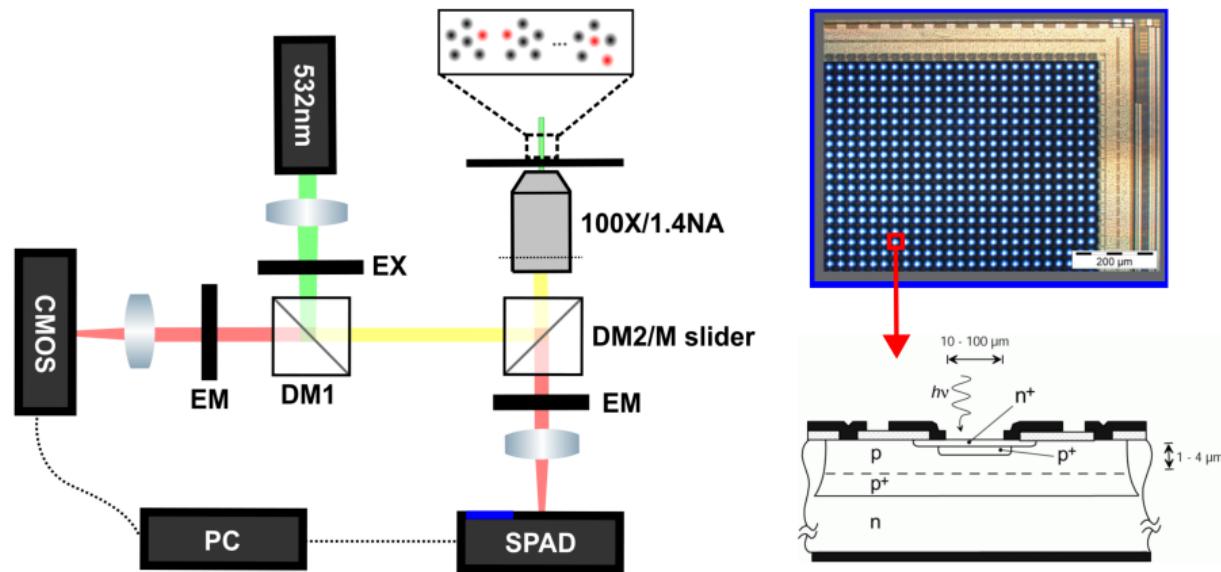
$$\mathbf{y}_{t-1} = \frac{1}{\sqrt{1 - \beta_t}} (\mathbf{y}_t + \beta_t s_\psi(\mathbf{y}_t)) + \sqrt{\beta_t} \xi \quad \xi \sim \mathcal{N}(0, I)$$

Super resolution of BRD4 protein in a HeLa cell

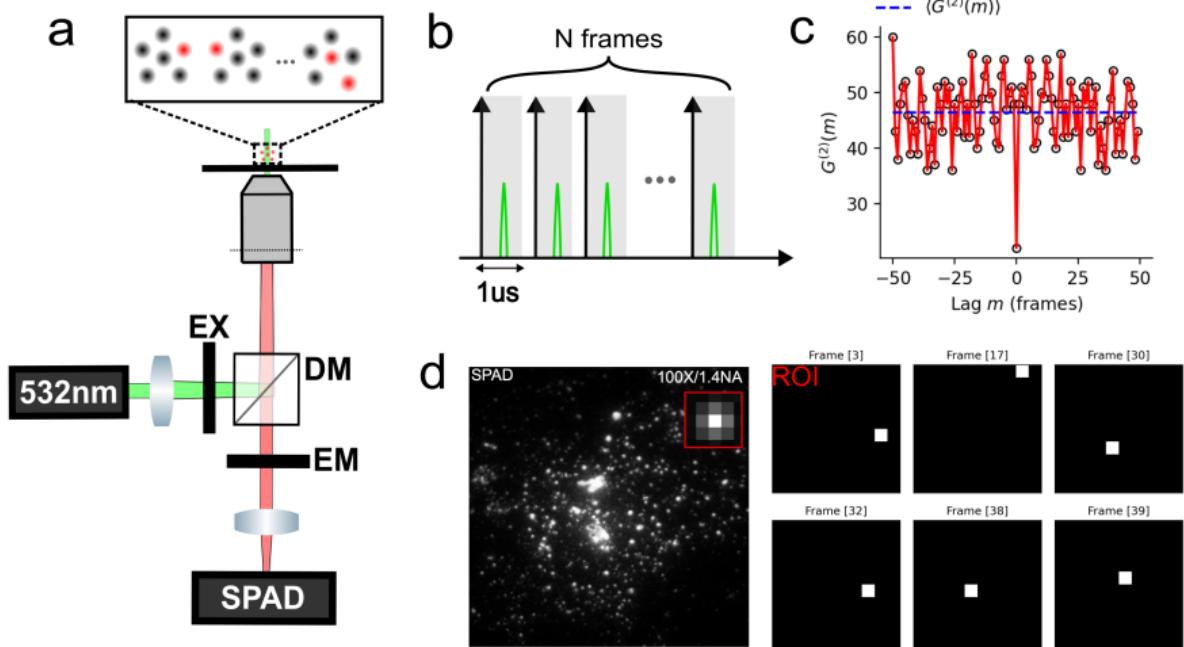


Approach II: Integrated single photon counting and widefield single molecule imaging

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

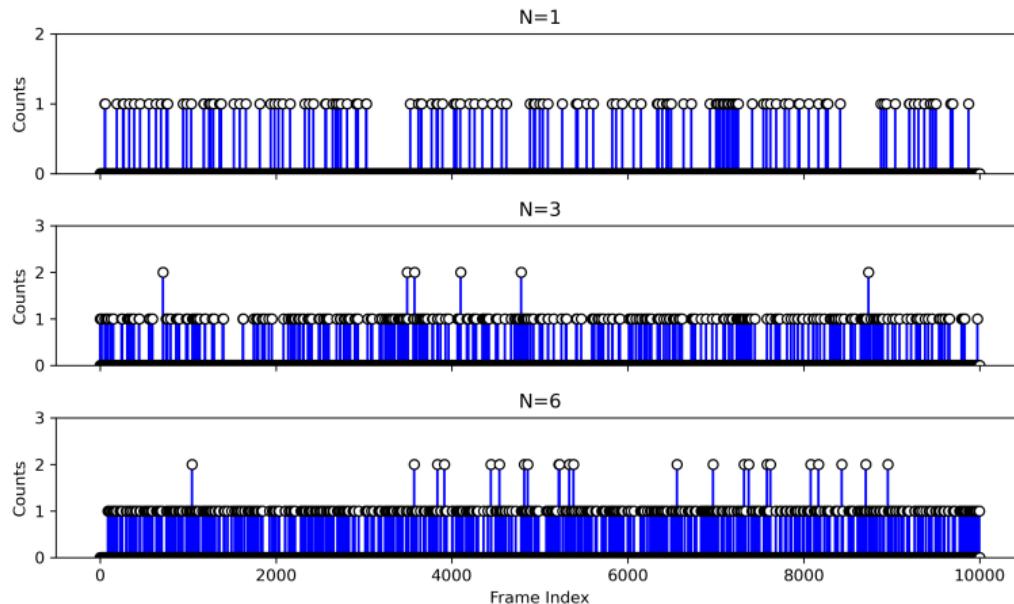


Imaging Qdot655 photon by photon



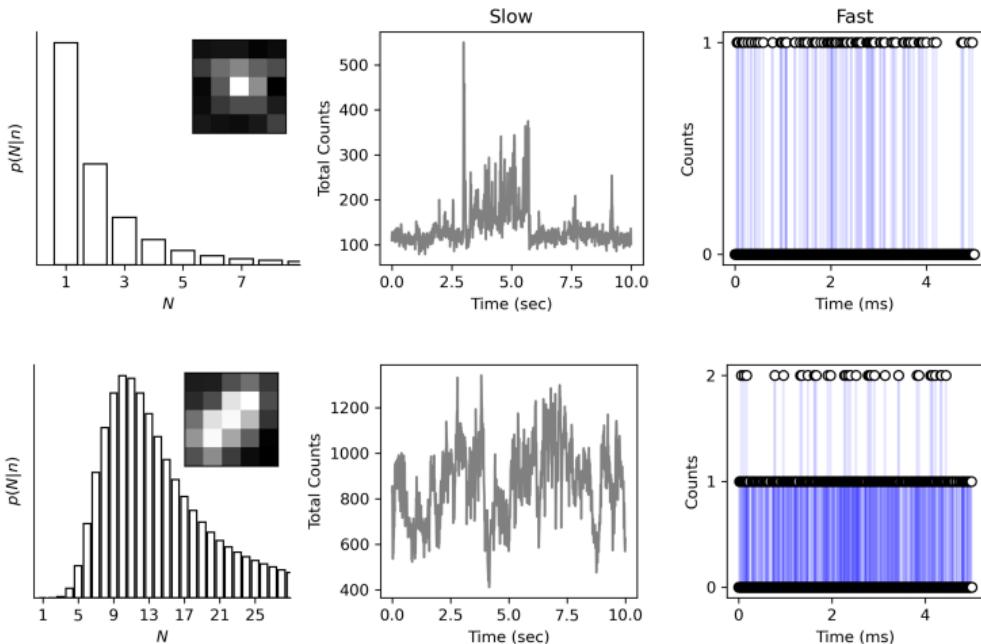
- ▶ 532nm picosecond pulsed laser pulsed at 500kHz to excite fluorescent quantum dots

Poisson-Binomial photon count data (simulation)



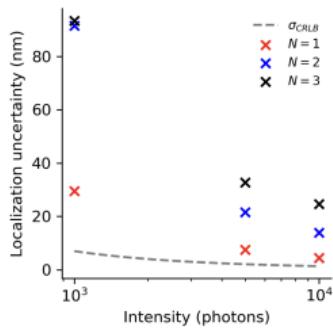
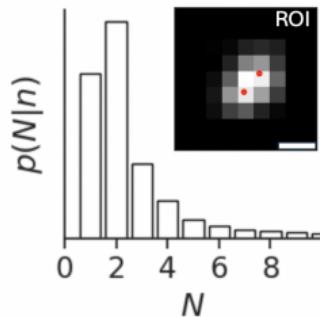
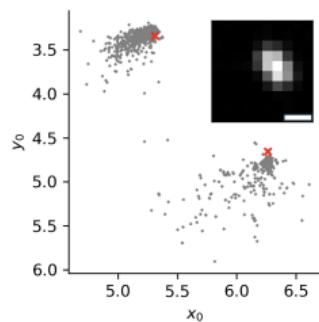
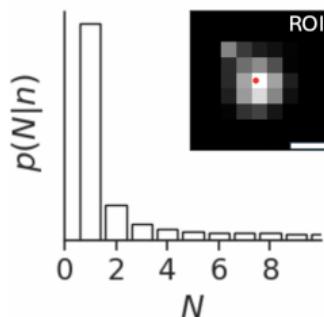
- ▶ SPAD arrays permit modeling of single photon counts, not just total intensity
- ▶ A simple form of a Hidden Markov Model (HMM)

Discrimination of single and multiple quantum dots



- ▶ Likelihood \mathcal{L} is a mixture of counts from N single photon sources, and background noise

Constrained multi-emitter localization with photon counting



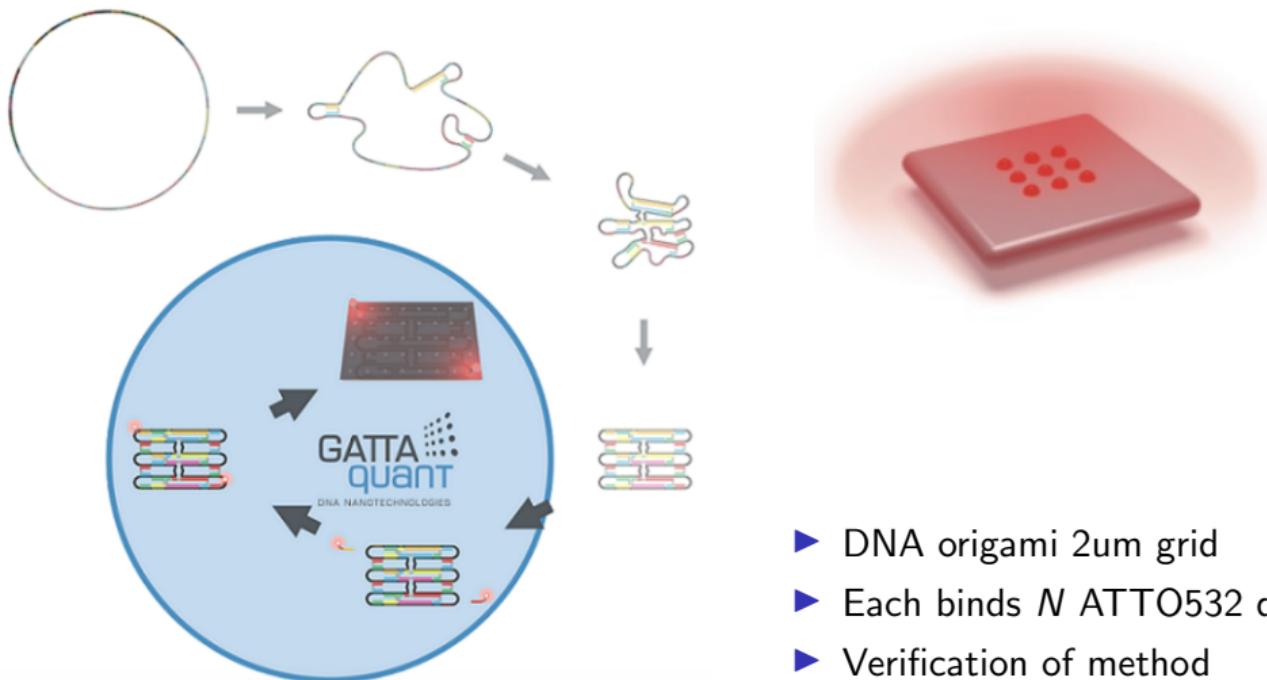
Can obtain a posterior on N :

$$\mathcal{L} = \text{Poisson}(\lambda) * \text{Binomial}(N, \zeta)$$

$$p(N|n) \propto \int_0^1 \mathcal{L}(n|N, \zeta) p(\zeta) d\zeta$$

- ▶ Posterior \rightarrow parameterization of multi-emitter fitting
- ▶ Approaches lower bound on localization error at high counts

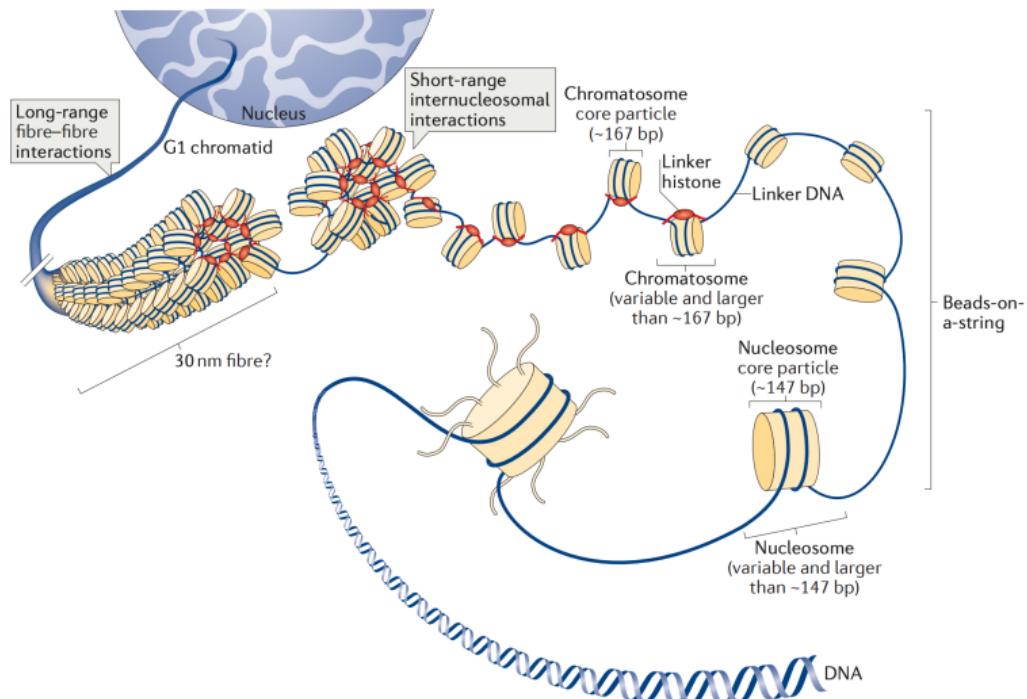
Counting ATTO532 dye bound to DNA origamis



Courtesy of GATTAquant DNA Nanotech

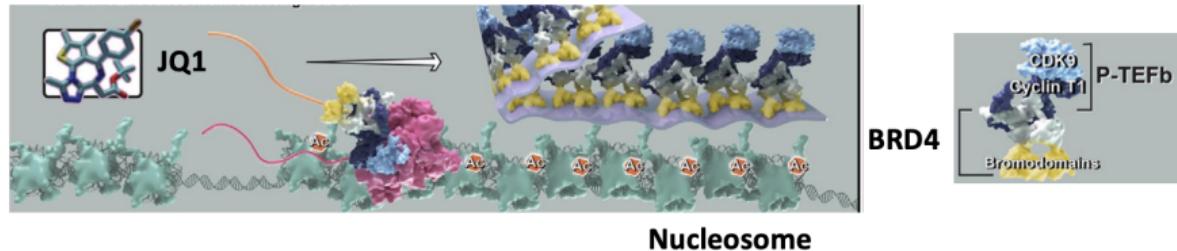
Super-resolution of nucleosome nanodomains *in-vivo*

Hierarchical structure of chromatin

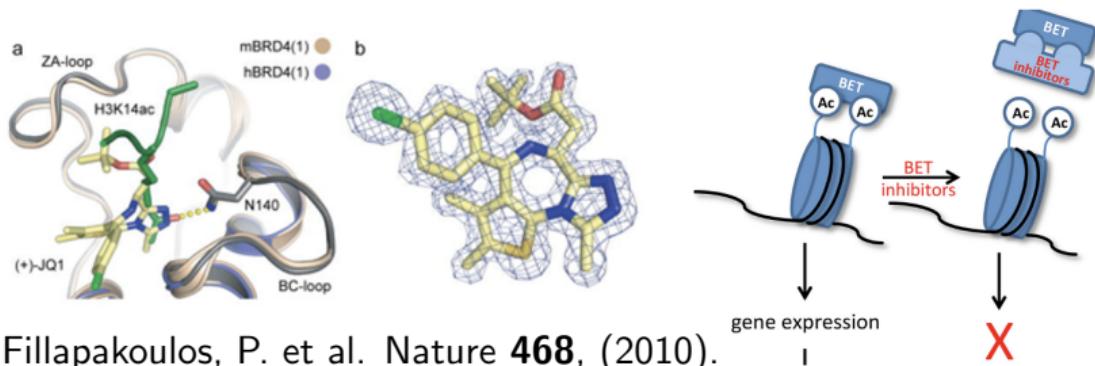


Fyodorov, D. et al. Nat Rev Mol Cell Biol **19**, (2018).

Bromodomain protein 4 (BRD4) binds acetylated chromatin

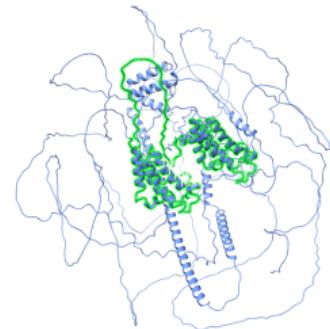
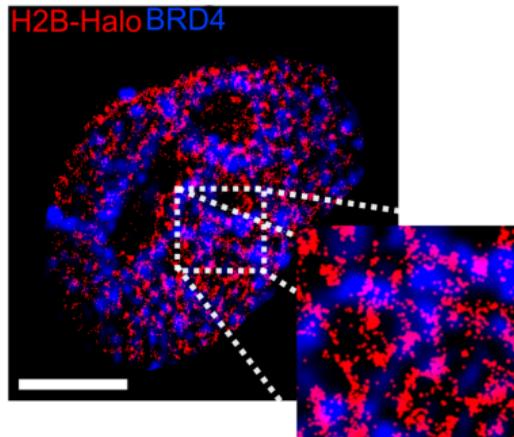


Zheng, B. et al. Molecular Cell **16**, (2023).



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

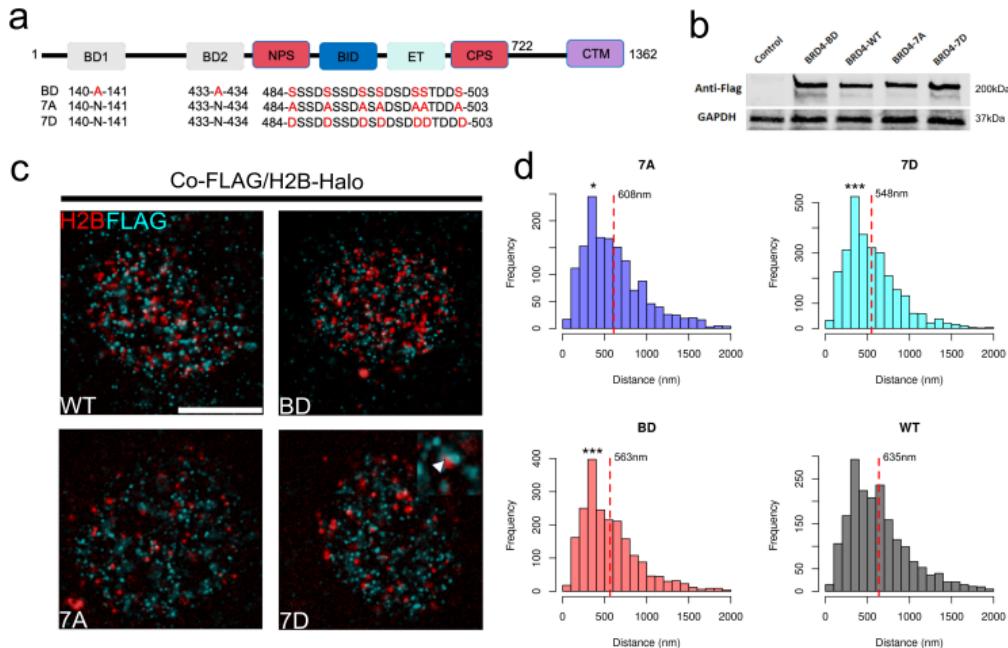
BRD4 mutations to probe effects on chromatin structure



AlphaFold BRD4 1-1362aa

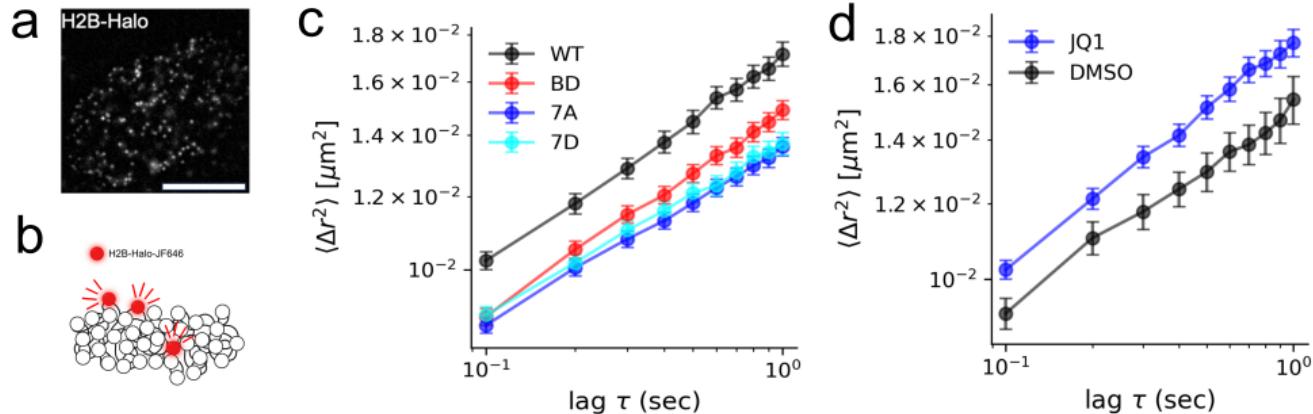
- ▶ 7A mutant - Constitutively unphosphorylated
- ▶ 7D mutant - Constitutively phosphorylated
- ▶ BD mutant - Bromodomain deactivated

BRD4 recruitment to nucleosome nanodomains



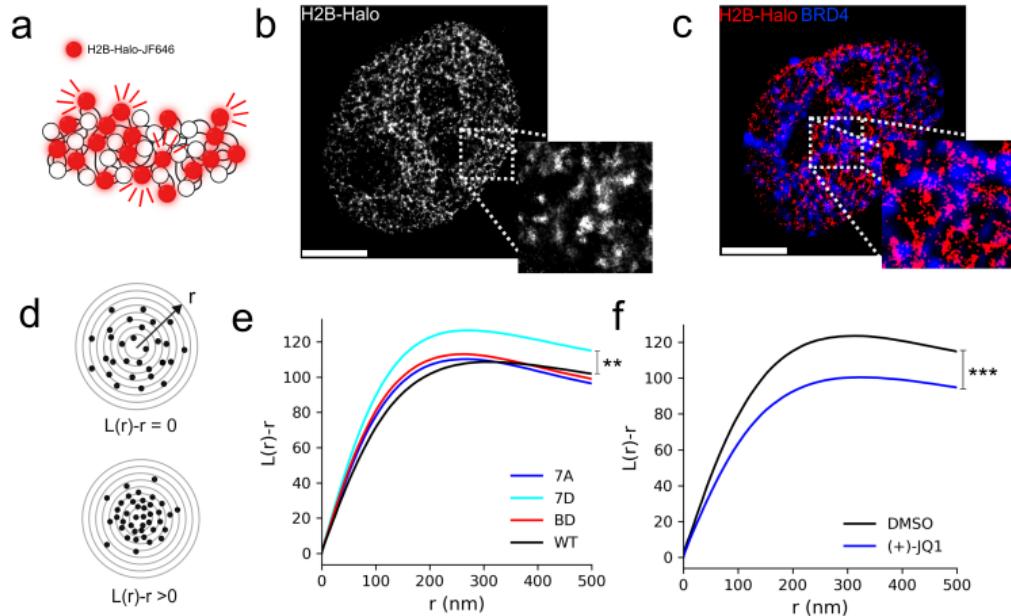
- ▶ Phosphorylation enhances BRD4 binding to acetylated chromatin
- ▶ Hyperphosphorylation - resistance mechanism to BD loss (Shu 2016)

BRD4 chromatin binding controls chromatin mobility



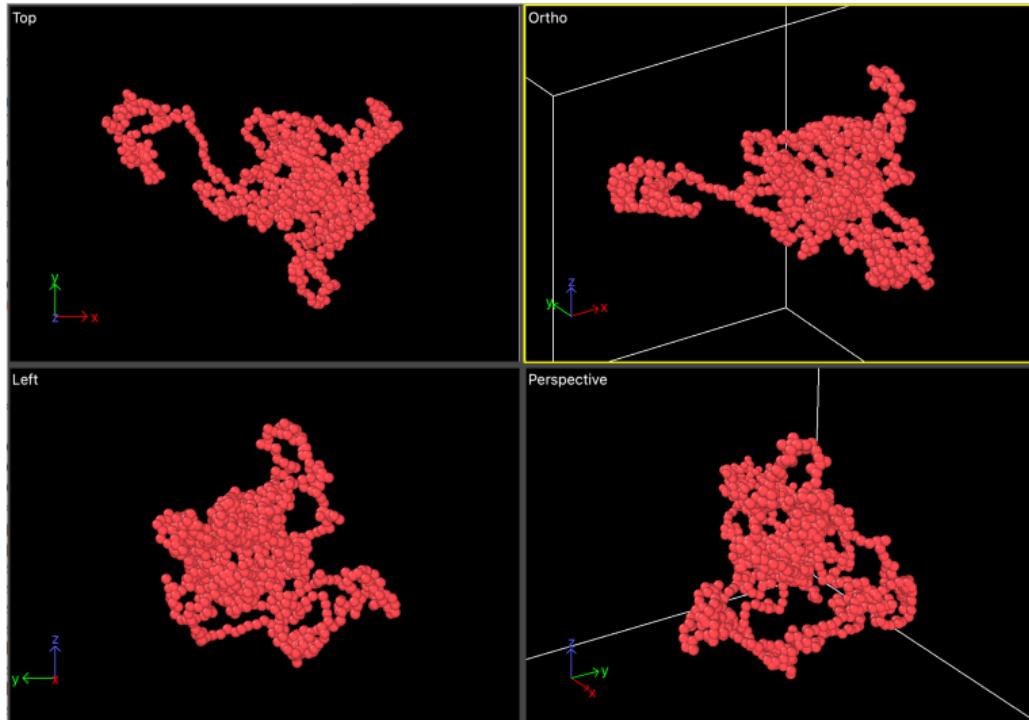
- ▶ H2B is sparsely labeled for particle tracking
- ▶ Reduced diffusion coefficient D in BRD4 mutants
- ▶ Increased D in cells exposed to (+)-JQ1

BRD4 binding is necessary for maintenance of nucleosome nanodomains



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

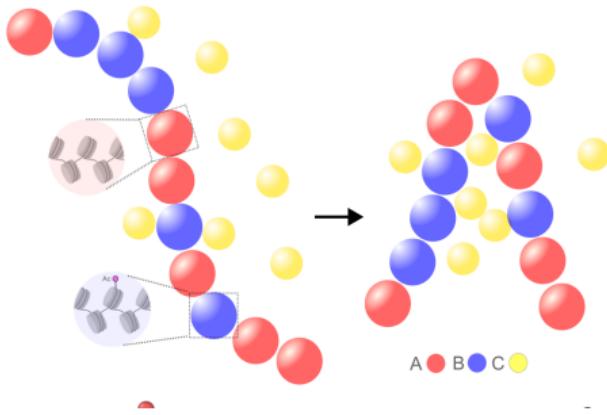
Coarse grained molecular dynamics of chromatin at 310K



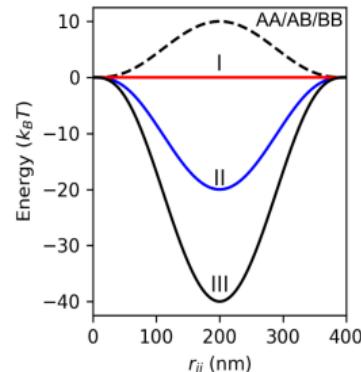
- ▶ 100kb chromatin beads connected by harmonic bonds (Rouse model)

Coarse grained molecular dynamics of chromatin binders at 310K

a



b

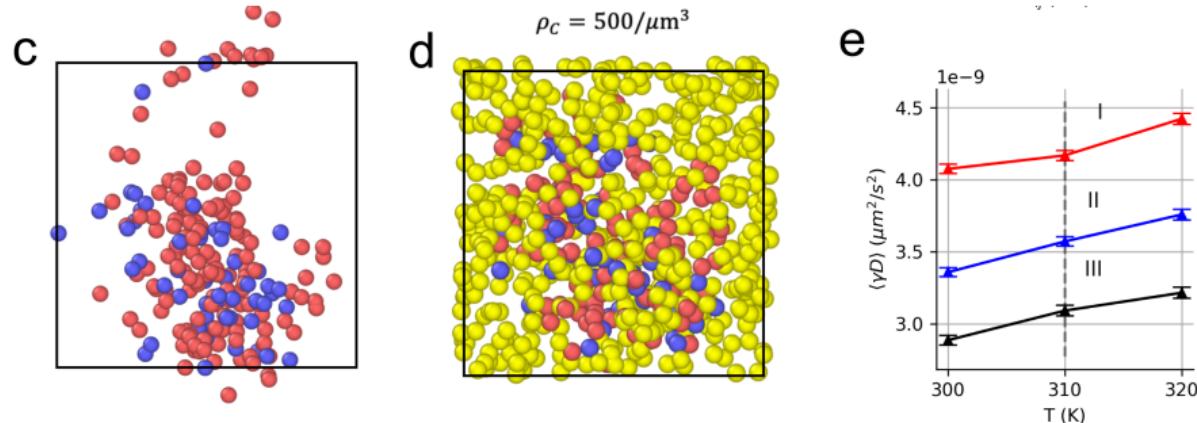


Chromatin chains interact with binders via the potential

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

- ▶ 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

- ▶ **Clayton Seitz**, Donghong Fu, Mengyuan Liu, Hailan Ma, and Jing Liu. *BRD4 phosphorylation regulates the structure of chromatin nanodomains.* <https://doi.org/10.1101/2024.09.03.611057>. 2024
- ▶ **Clayton Seitz** and Jing Liu. *Uncertainty-aware localization microscopy by variational diffusion.* In Review. 2024
- ▶ **Clayton Seitz** and Jing Liu. *Quantum enhanced localization microscopy with a single photon avalanche diode array.* In Progress. 2024
- ▶ Maelle Locatelli[†], Josh Lawrimore[†], Hua Lin[†], Sarvath Sanaullah, **Clayton Seitz**, Dave Segall, Paul Kefer, Salvador Moreno Naike, Benton Lietz, Rebecca Anderson, Julia Holmes, Chongli Yuan, George Holzwarth, Bloom Kerry, Jing Liu, Keith D Bonin, Pierre-Alexandre Vidi. *DNA damage reduces heterogeneity and coherence of chromatin motions.* PNAS 12 July 2022; 119 (29): 1-11
- ▶ Mengdi Zhang, **Clayton Seitz**, Garrick Chang, Fadil Iqbal, Hua Lin, and Jing Liu *A guide for single-particle chromatin tracking in live cell nuclei.* Cell Biology International 15 January 2022; 46 (5): 683-700

Acknowledgements



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



Norbert Scherer



Donghong Fu

Thank you!