

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

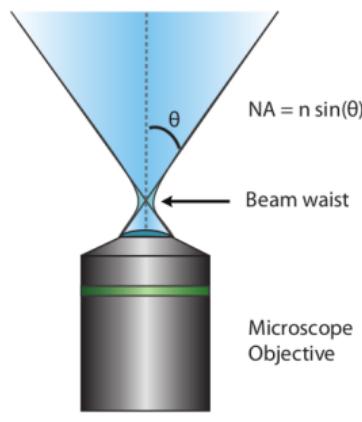
Super-resolution of nucleosome nanodomains *in-vivo*

Specific Aim 3: Interaction of transcriptional condensates with nucleosome nanodomains

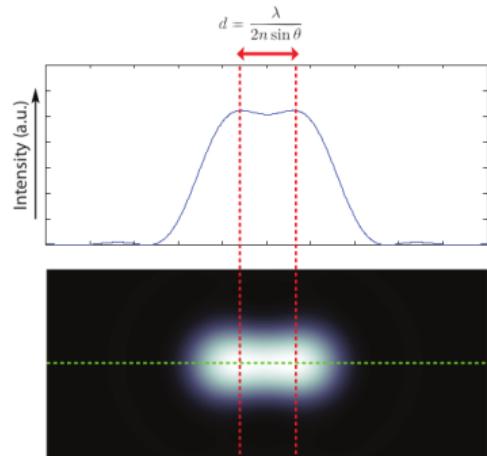
Introduction to fluorescence nanoscopy

Fluorescence microscopy and the diffraction limit

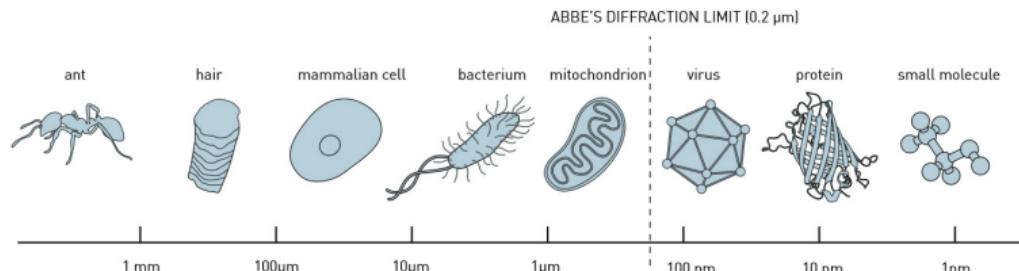
Minimal resolvable distance $d \sim \lambda$



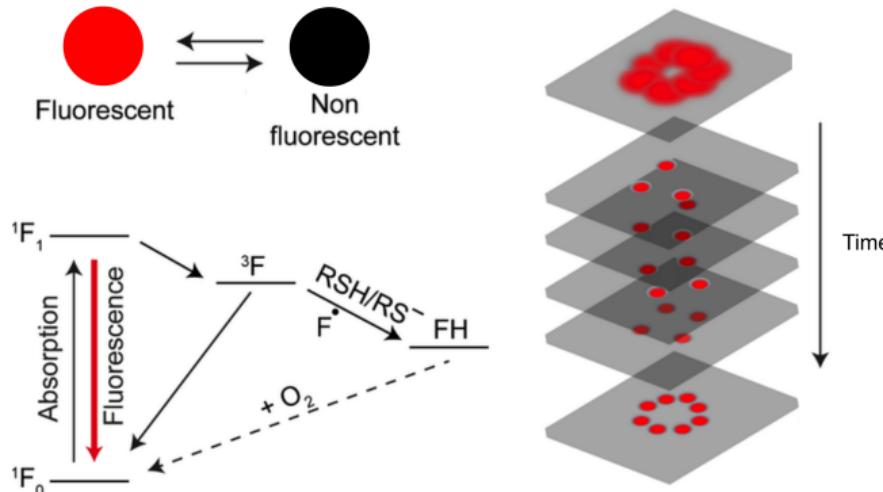
(a)



(b)

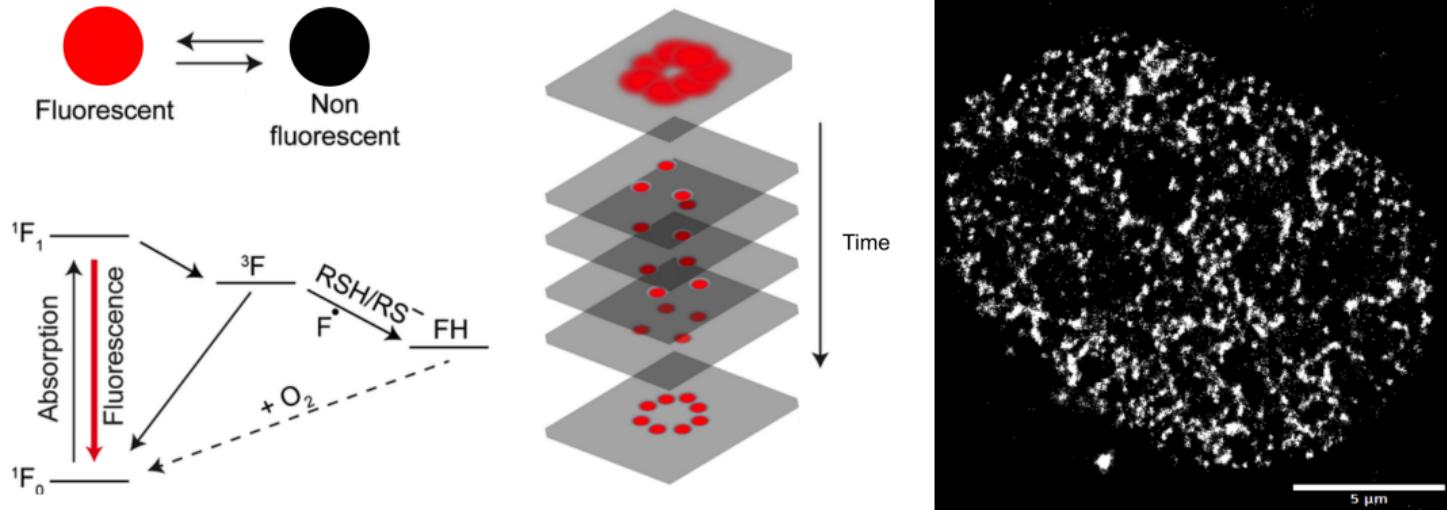


Stochastic optical reconstruction microscopy (STORM)



- ▶ 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

η – quantum efficiency

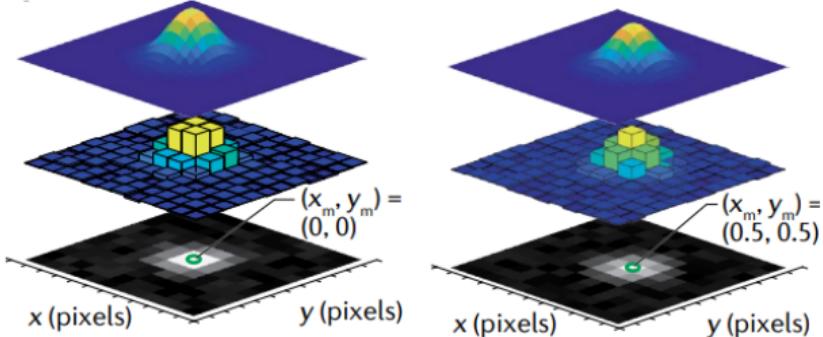
ζ – photon emission rate

Δ – exposure time

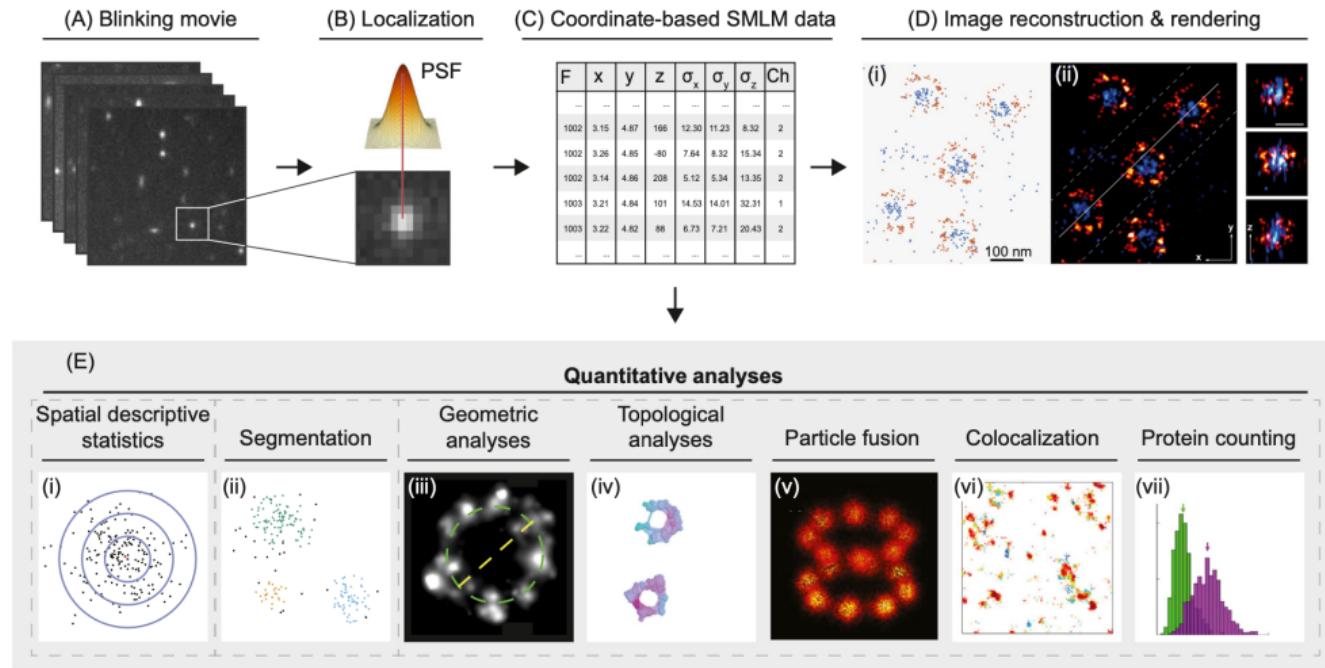
λ – background rate

Maximum likelihood localization:

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

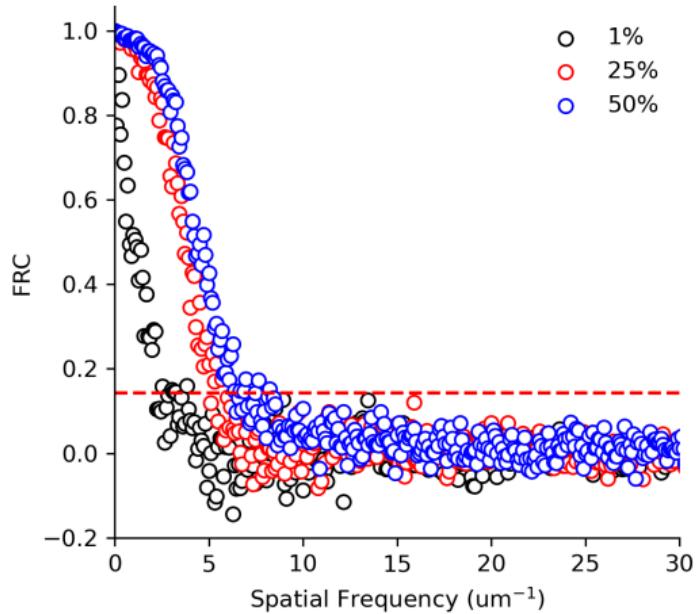
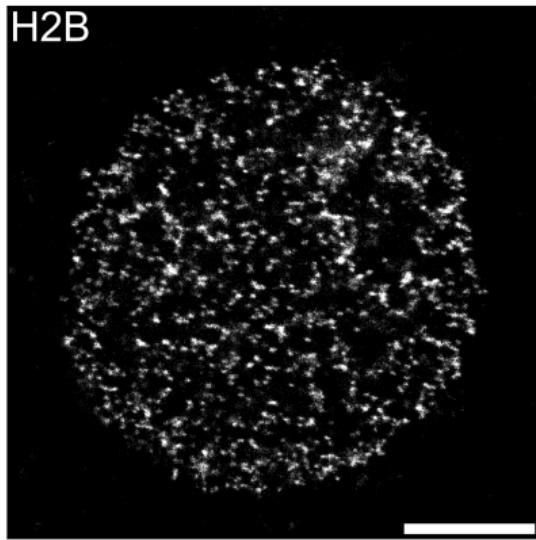


Applications of single molecule localization microscopy



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

How do we define resolution in localization microscopy?



More samples → higher spatial/temporal resolution

Can we extract more information to enhance localization microscopy?

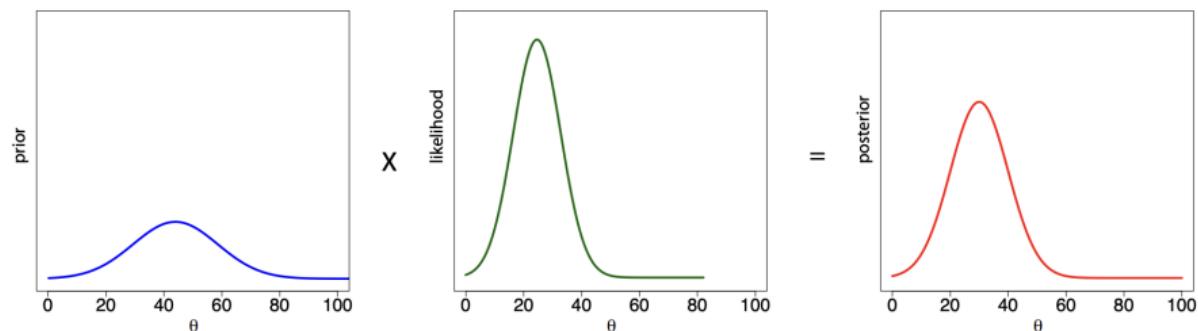
Probabilistic modeling approaches to fluorescence nanoscopy

The Bayesian calculation and variational inference

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

Bayes Rule

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

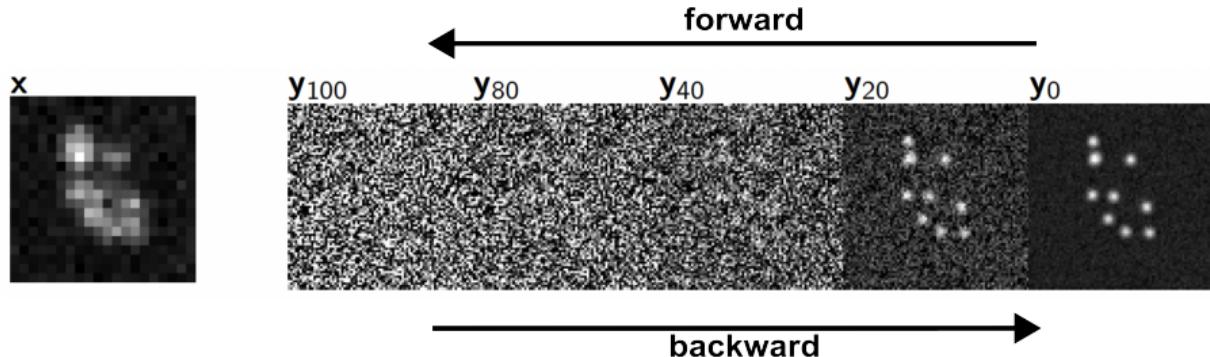


$p(\theta|x)$ is can be hard to obtain. One solution is to fit a model distribution $p_\psi(\theta|x)$ that has parameters ψ

Bayesian image restoration with diffusion models

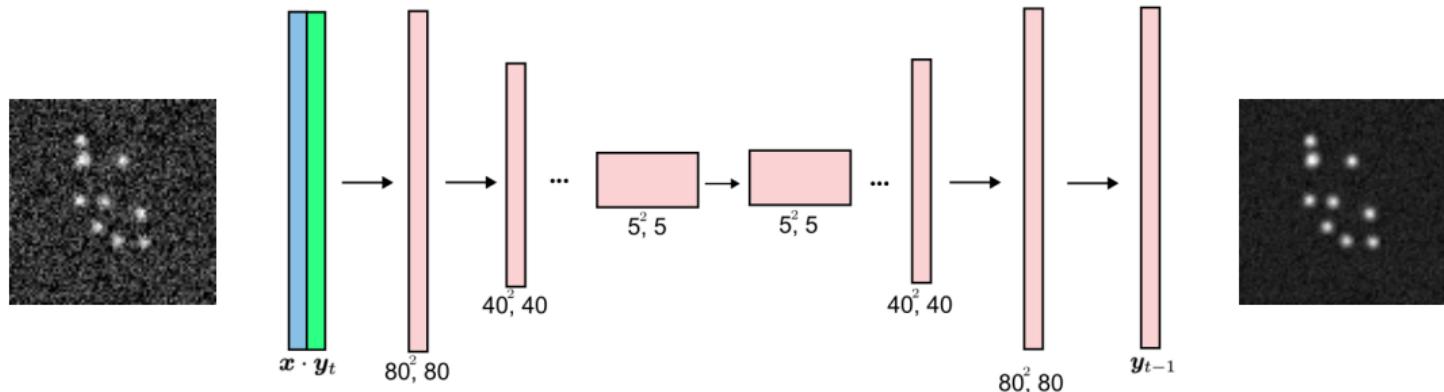
Inference of a high resolution image \mathbf{y}_0 from low resolution \mathbf{x} is approached by modeling a distribution $p_\psi(\mathbf{y}_0|\mathbf{x})$ with a diffusion model

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



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

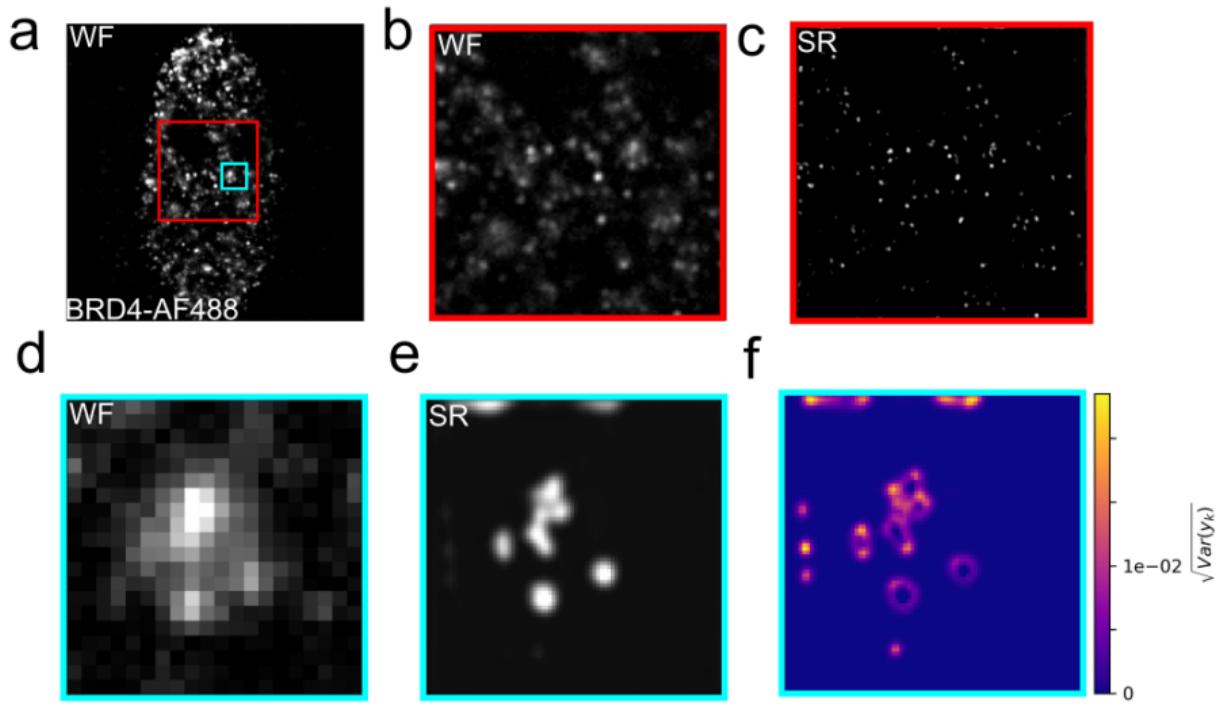
Bayesian image restoration with diffusion models



A deep neural network estimates the gradient of the reverse process

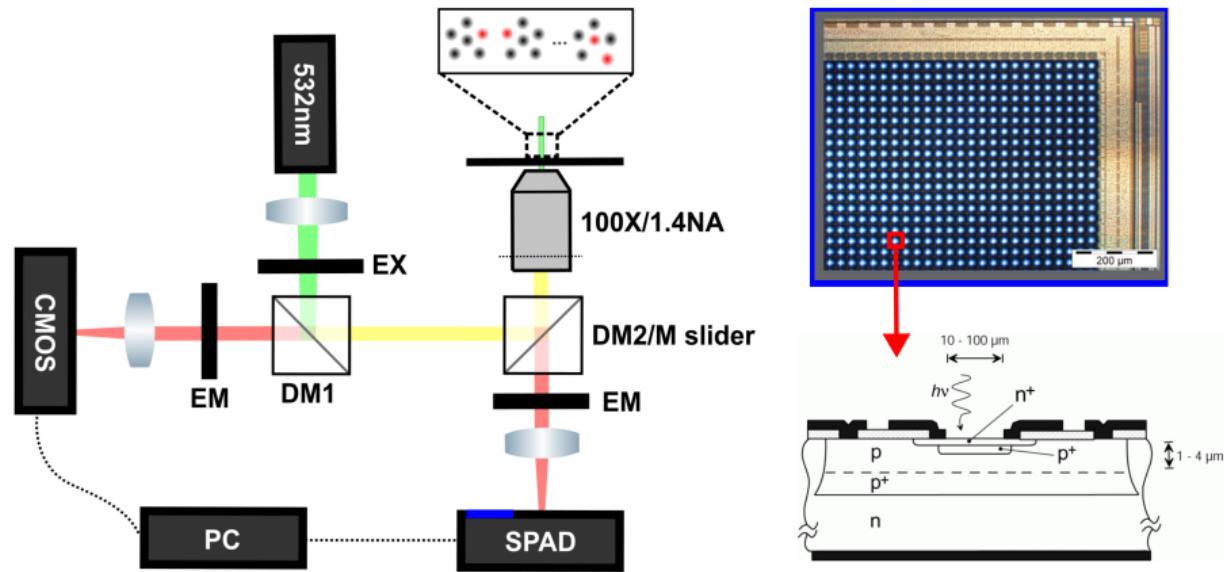
$$\mathbf{y}_{t-1} = \frac{1}{\sqrt{1 - \beta_t}} (\mathbf{y}_t + \beta_t s_\psi(\mathbf{y}_t)) + \sqrt{\beta_t} \xi \quad \xi \sim \mathcal{N}(0, I)$$

Super resolution of BRD4 in a HeLa cell

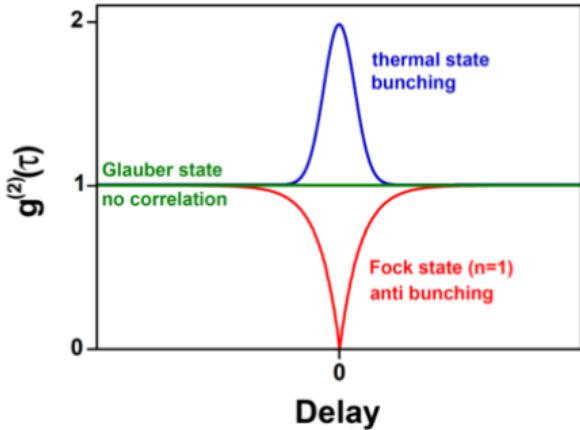
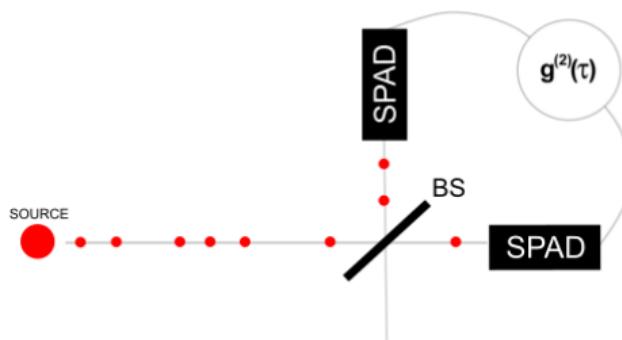


An integrated single photon avalanche diode (SPAD) array

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



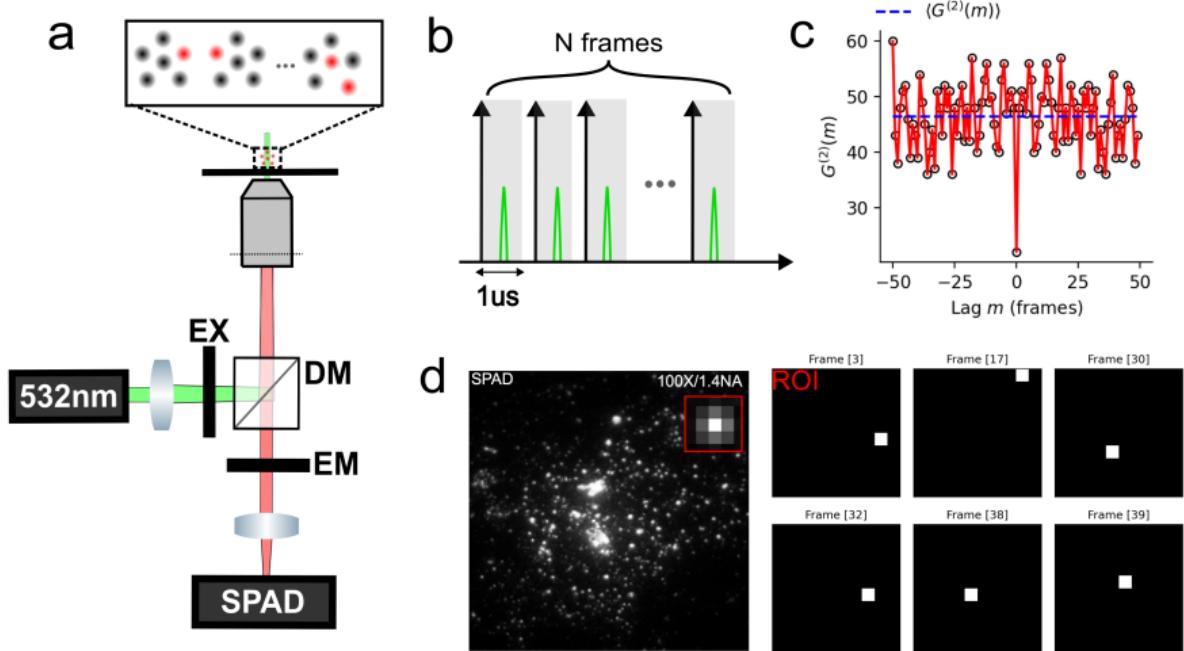
The Hanbury Brown and Twiss Effect



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

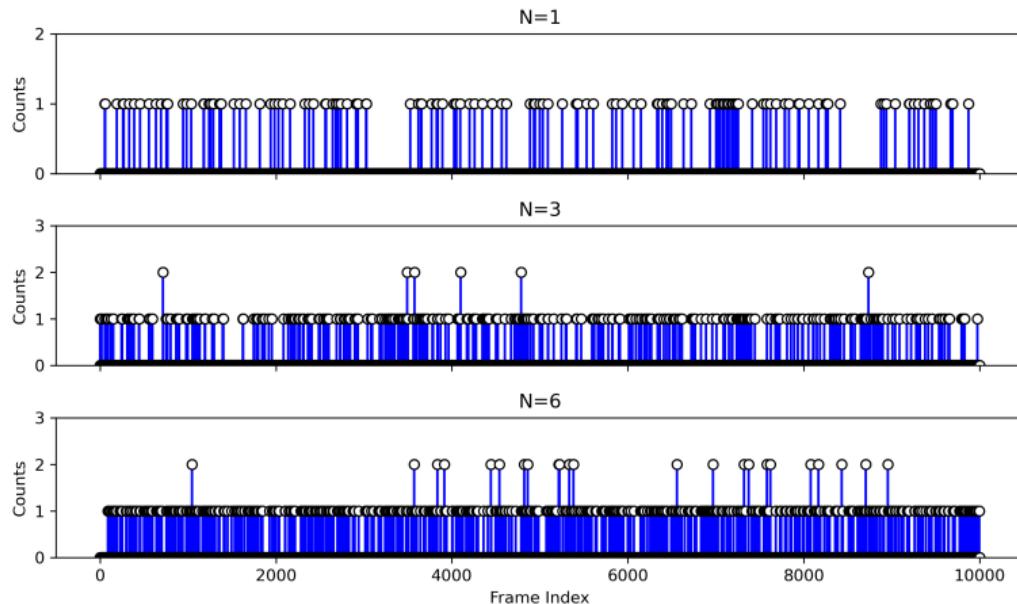
- ▶ Single photon sources (such as a fluorescent dye) exhibit antibunching
- ▶ Magnitude of $g^{(2)}(0)$ "dip" depends on the number of fluorescent emitters

Imaging Qdot655 photon by photon



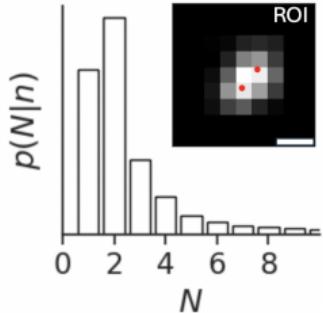
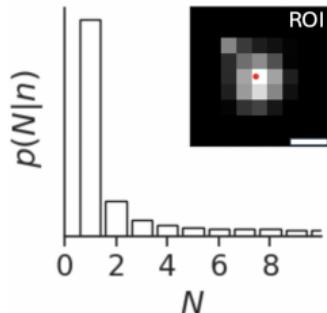
- ▶ 532nm picosecond pulsed laser pulsed at 500kHz to excite fluorescent quantum dots

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

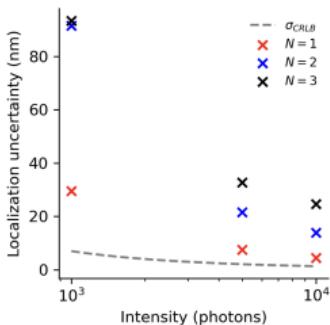
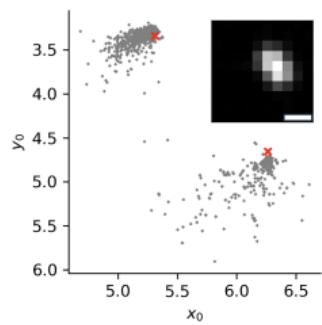


- ▶ Likelihood is a mixture of counts from N single photon sources, and background noise
- ▶ Can obtain posterior $p(N, \zeta|n) \propto p(n|N, \zeta)p(\zeta)$

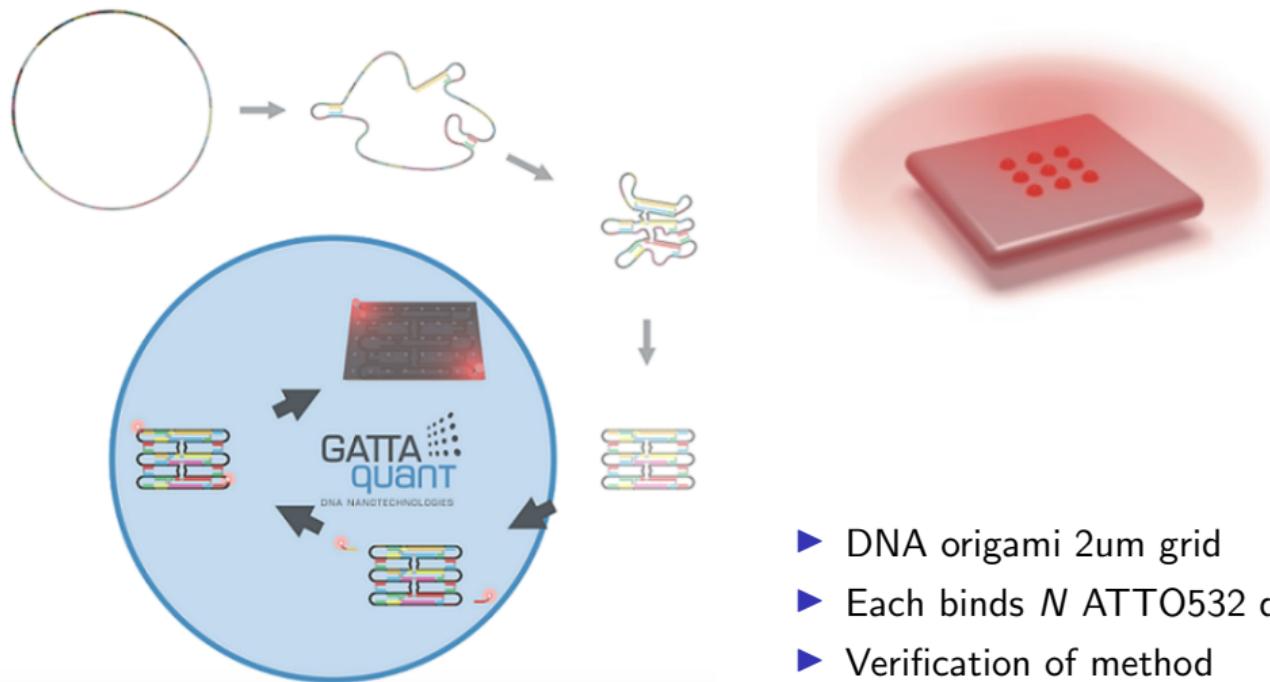
Constrained multi-emitter localization with photon counting



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



Counting ATTO532 dye bound to DNA origamis

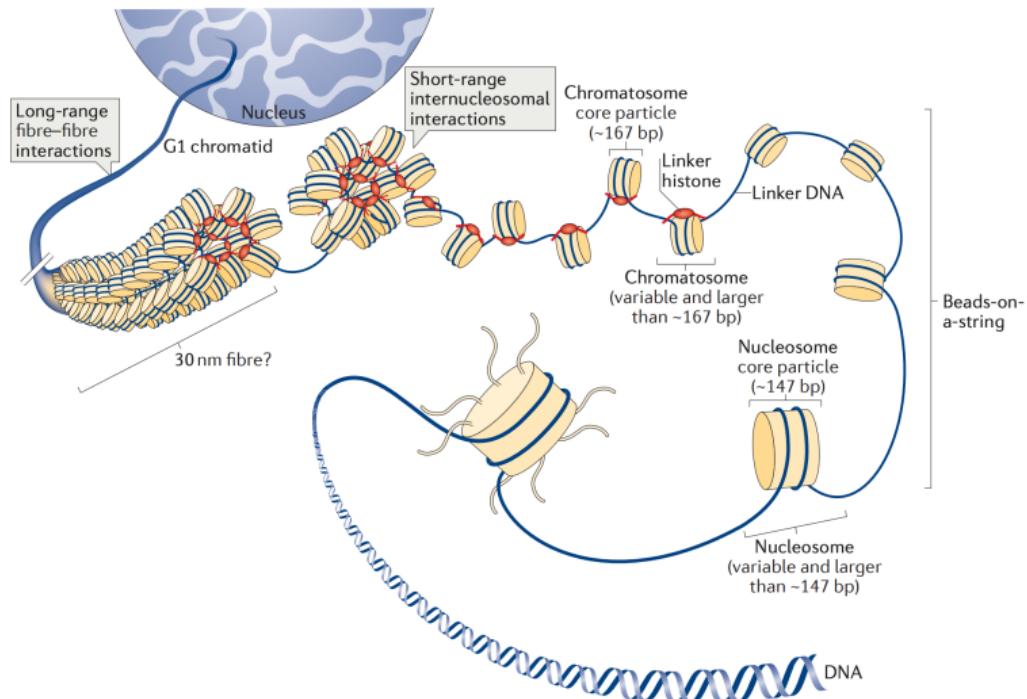


- ▶ DNA origami 2um grid
- ▶ Each binds N ATTO532 dyes
- ▶ Verification of method

Courtesy of GATTAquant DNA Nanotech

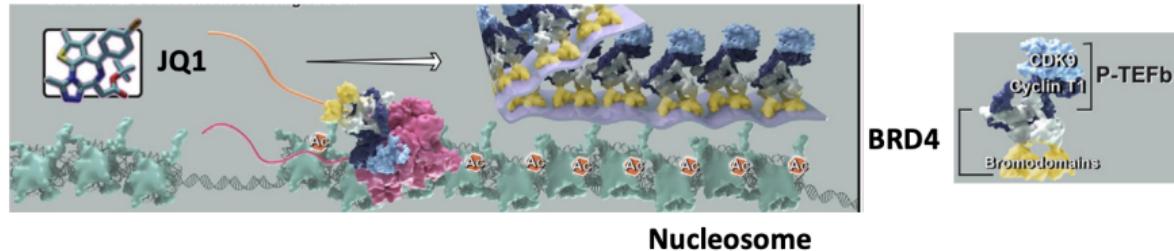
Super-resolution of nucleosome nanodomains *in-vivo*

Hierarchical structure of chromatin

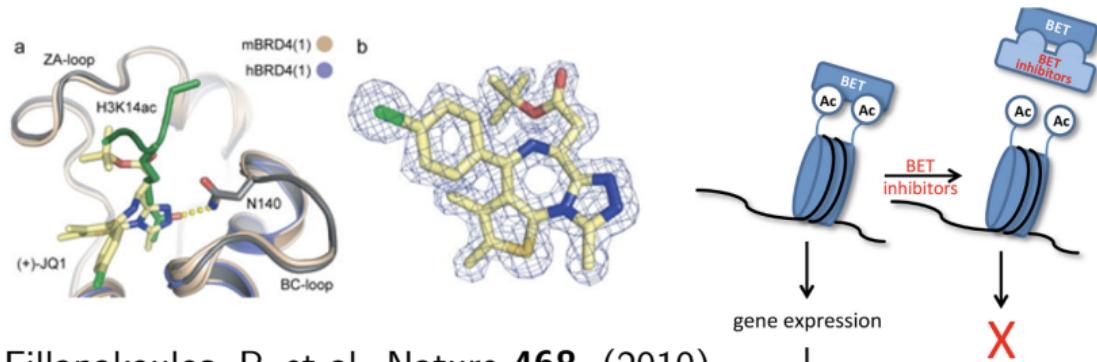


Fyodorov, D. et al. Nat Rev Mol Cell Biol **19**, (2018).

Bromodomain protein 4 (BRD4) binds acetylated chromatin

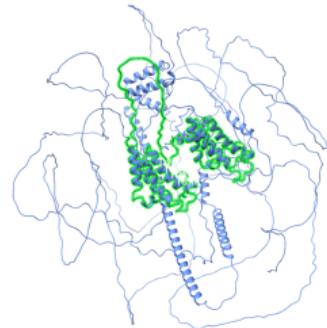
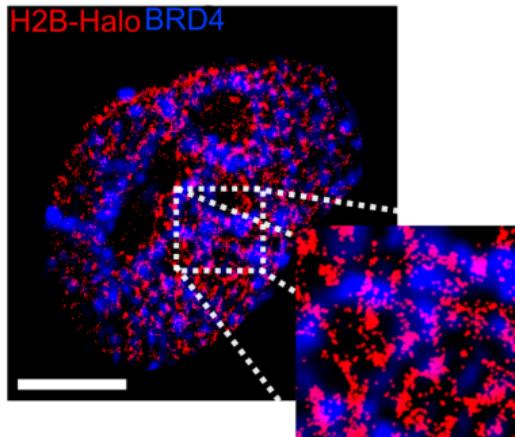


Zheng, B. et al. Molecular Cell **16**, (2023).



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

BRD4 mutations to probe effects on chromatin structure

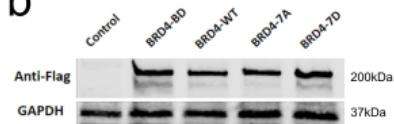


BRD4 binding efficacy controls colocalization with nucleosome nanodomains

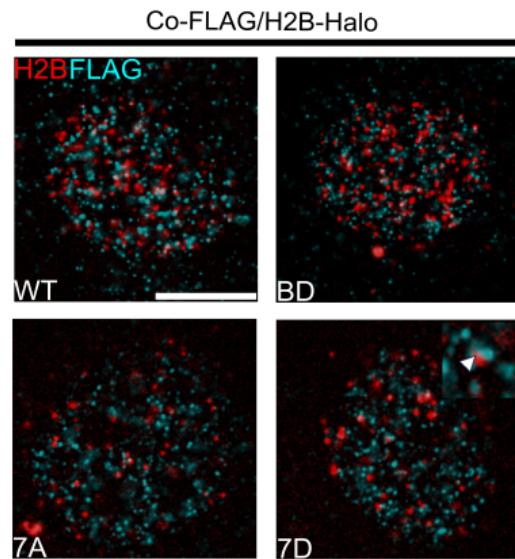
a



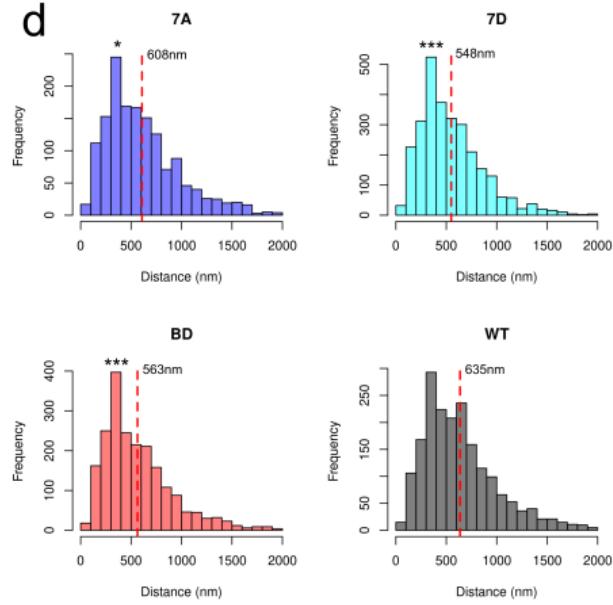
b



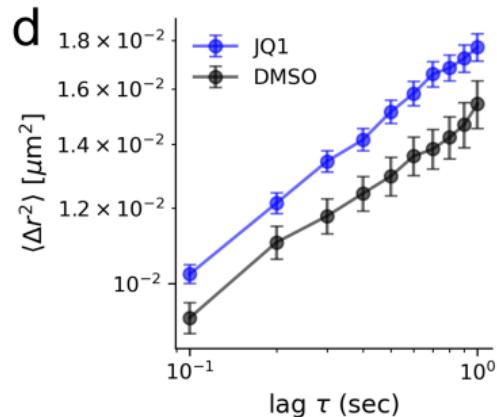
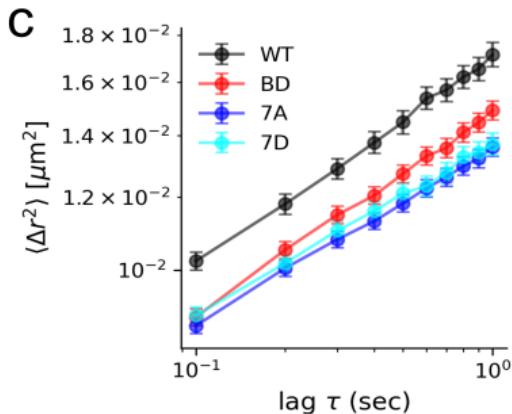
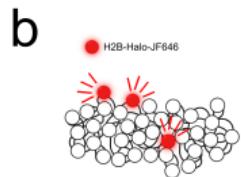
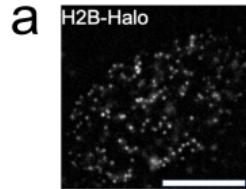
c



d

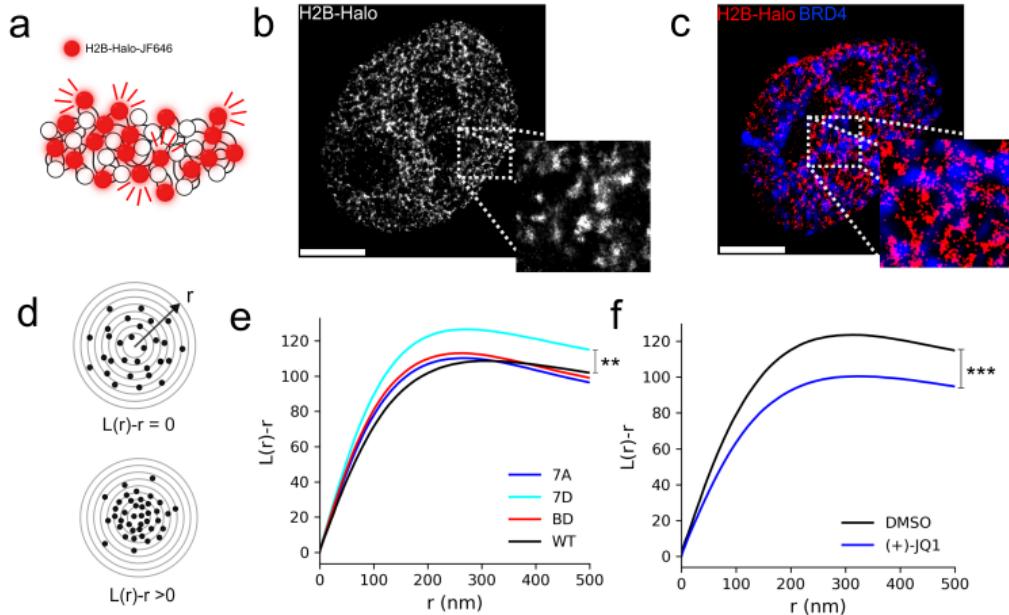


BRD4 chromatin binding controls chromatin mobility



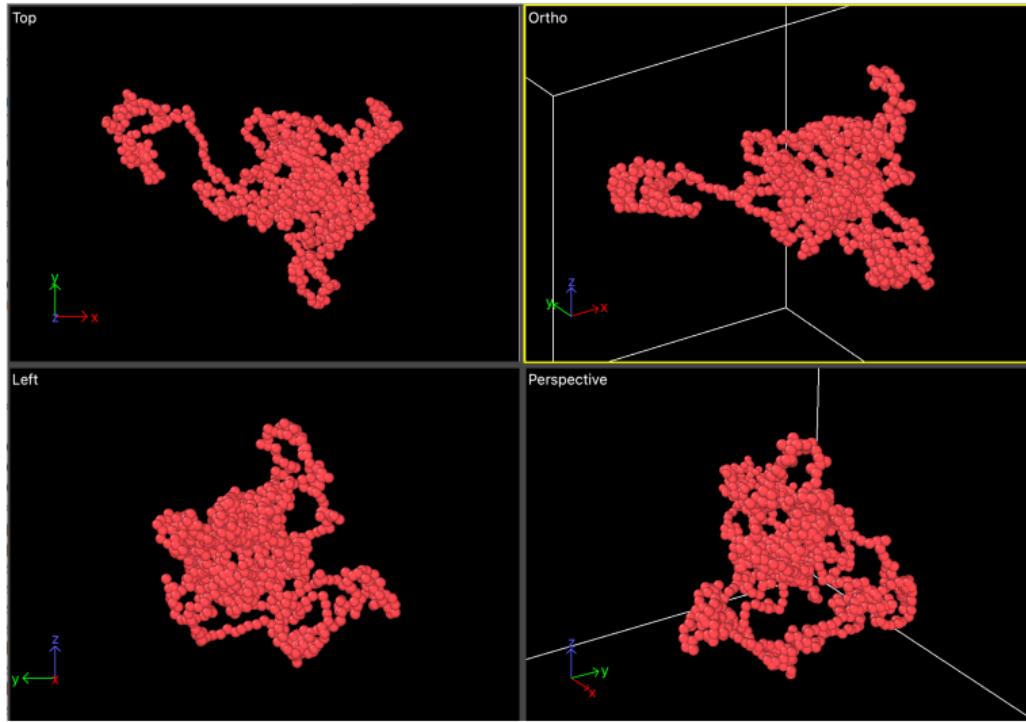
- ▶ H2B is sparsely labeled for particle tracking
- ▶ Reduced diffusion coefficient D in BRD4 mutants
- ▶ Increased D in cells exposed to (+)-JQ1

BRD4 binding is necessary for maintenance of nucleosome nanodomains



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

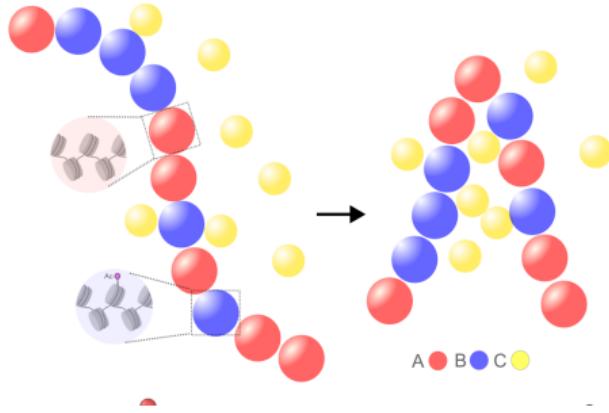
Coarse grained molecular dynamics of chromatin at 310K



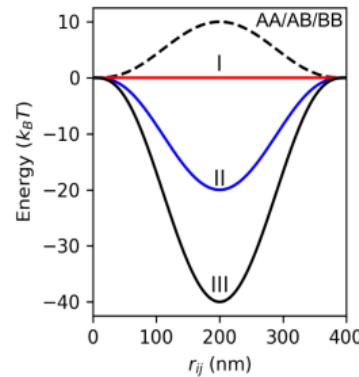
- ▶ 100kb chromatin chains connected by harmonic bonds (Rouse model)

Coarse grained molecular dynamics of chromatin binders at 310K

a



b

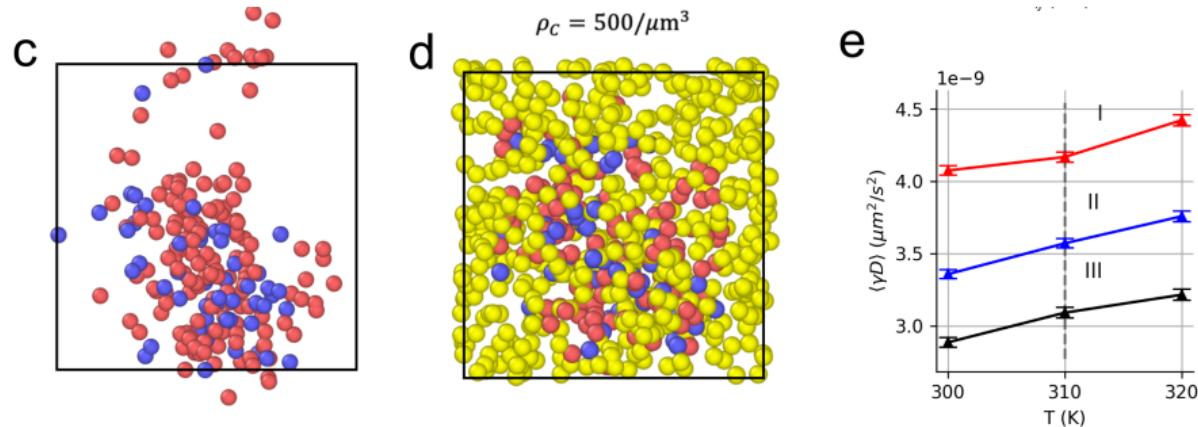


100kb chromatin chains interact with binders via the potential

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

- ▶ 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



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



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Donghong Fu

Thank you!