

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

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June 9, 2024

Outline

Introduction to fluorescence nanoscopy

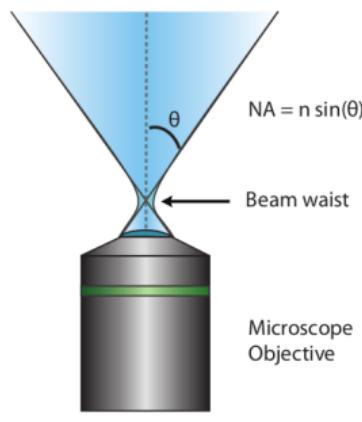
Enhanced nanoscopy with deep generative models

Super-resolution of nucleosome nanodomains *in-vivo*

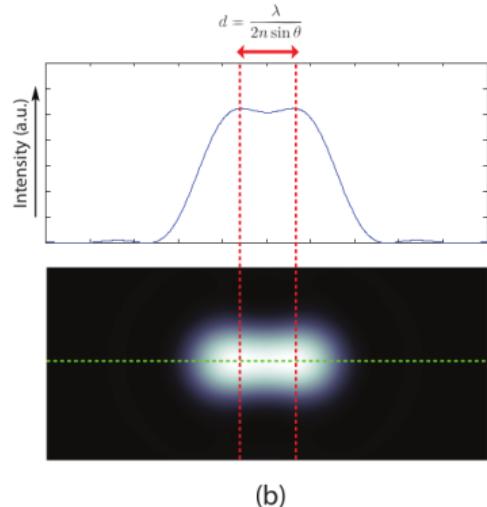
Introduction to fluorescence nanoscopy

Fluorescence microscopy and the diffraction limit

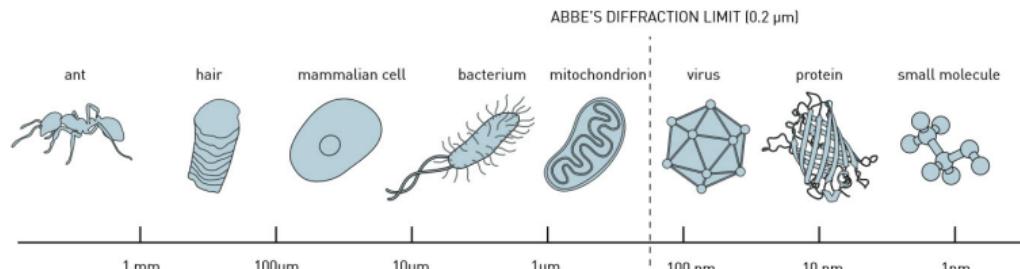
Minimal resolvable distance $d \sim \lambda$



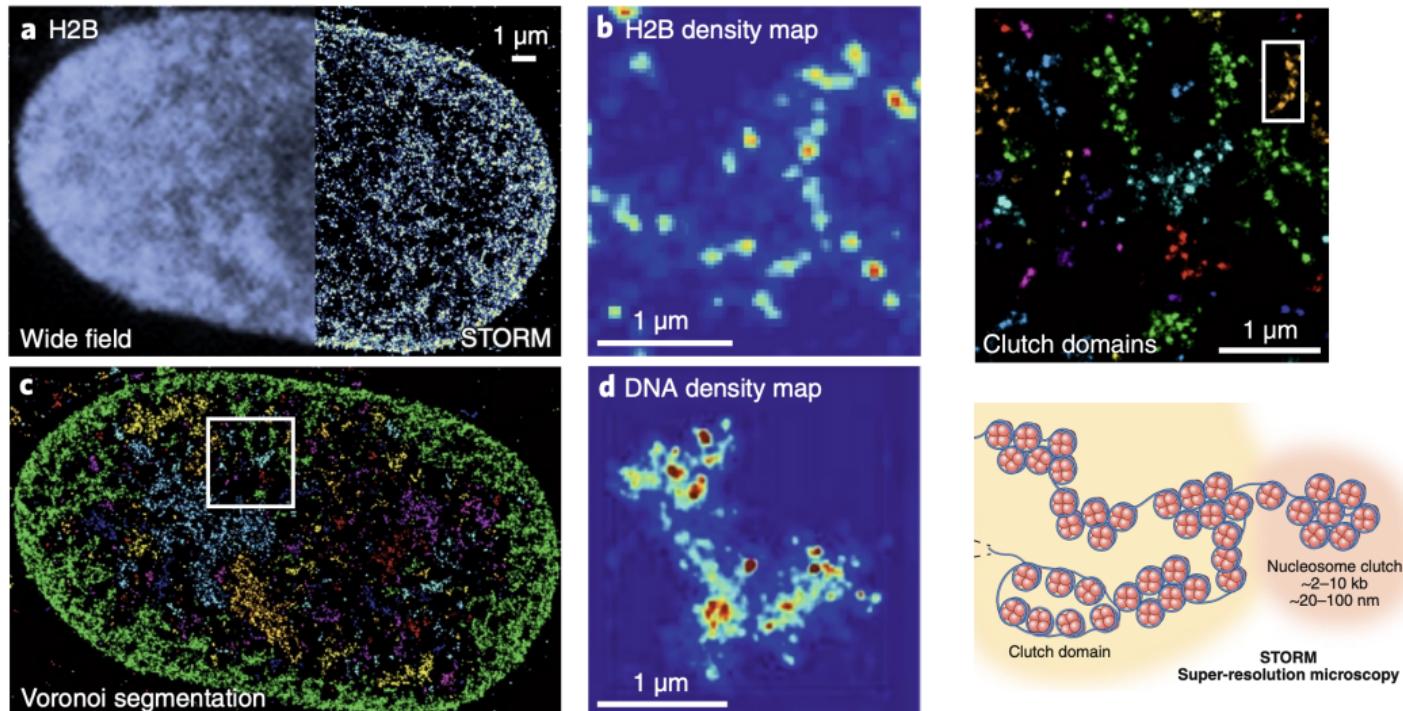
(a)



(b)



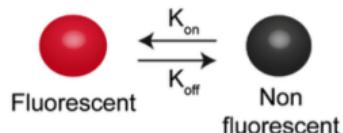
Stochastic optical reconstruction microscopy (STORM)



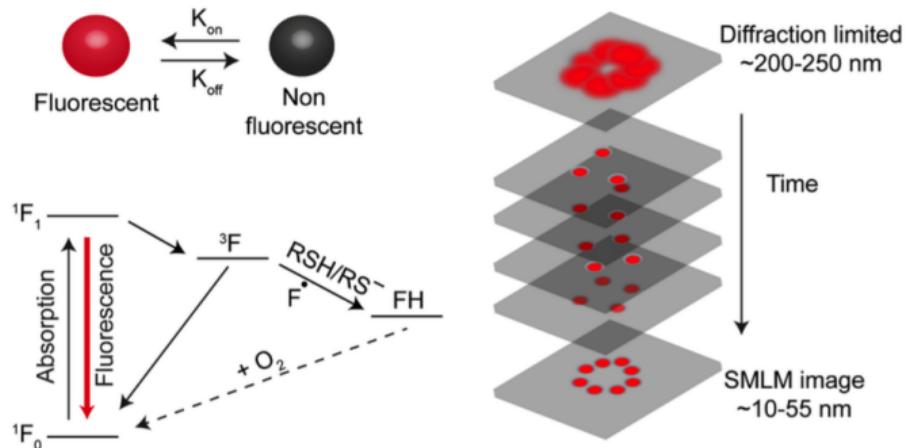
Lakadamyali, M. et al. Nature Methods 17, (2020).

Stochastic optical reconstruction microscopy (STORM)

a Photoswitching

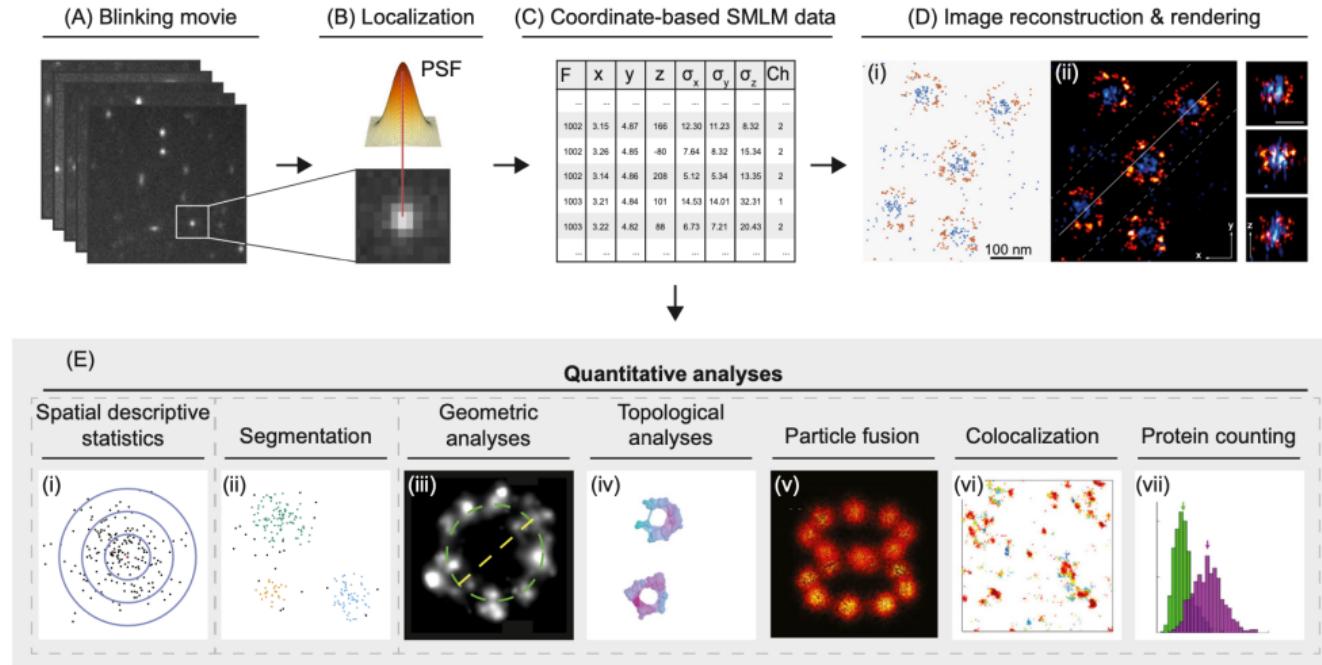


b Temporal separation



- ▶ Single molecule localization microscopy (SMLM) techniques are diffraction-unlimited
- ▶ Photoswitching enables resolution of emitters below the diffraction limit

Applications of single molecule localization microscopy



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

Enhanced nanoscopy with deep generative models

Localization of isolated fluorescent emitters

Modeling the point spread function permits sub-pixel localization

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

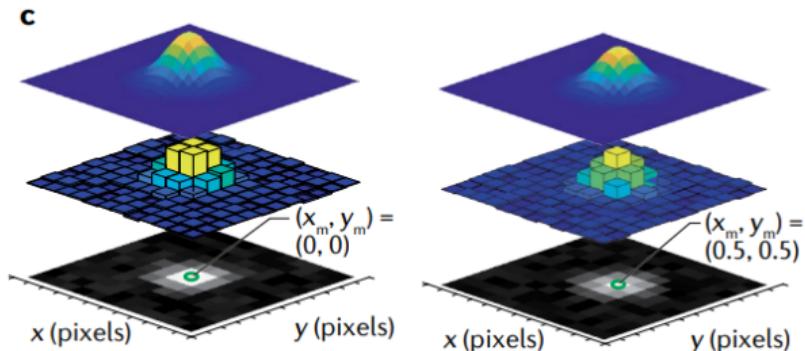
$$i_0 = g_k \eta N_0 \Delta$$

g_k – pixel gain

η – quantum efficiency

N_0 – photon emission rate

Δ – exposure time

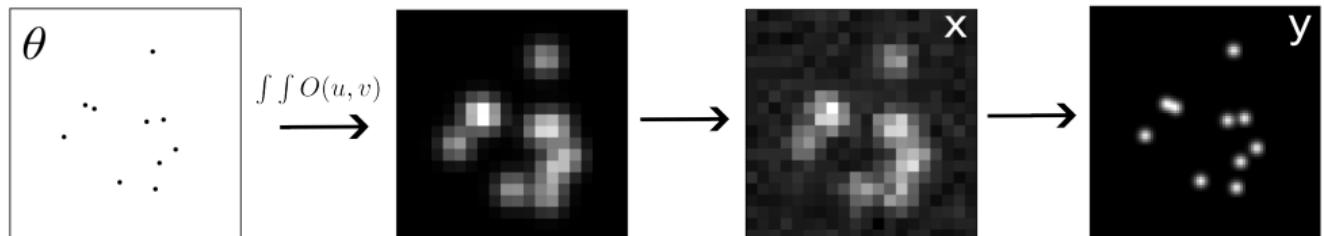


Assume N_0 is constant over Δ (homogeneous Poisson)

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

How to pack more localizations in a single frame?

Cast localization as *image restoration*



Seitz et al. Under Review. (2024)

- ▶ 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., $p(\mathbf{y}|\mathbf{x})$
- ▶ How to model a distribution over images?

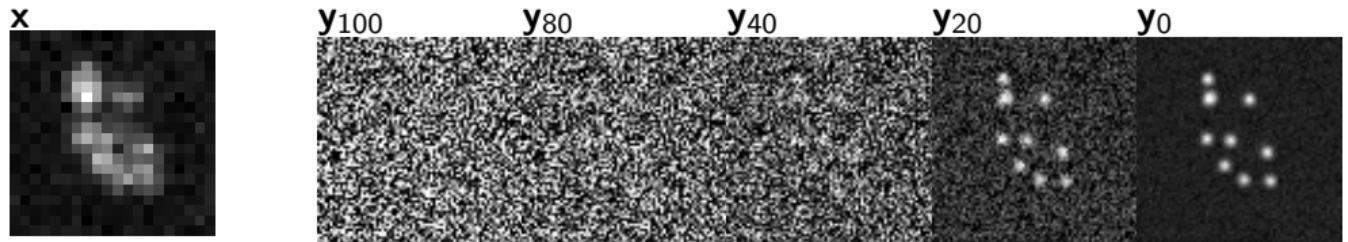
Trivial example of sampling from a mixture of Gaussians

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:

$$\mathbf{x}_i = \mathbf{x}_{i-1} + \epsilon \nabla \log p(\mathbf{x}) + \sqrt{2\epsilon} \eta \quad \eta \sim \mathcal{N}(0, I)$$

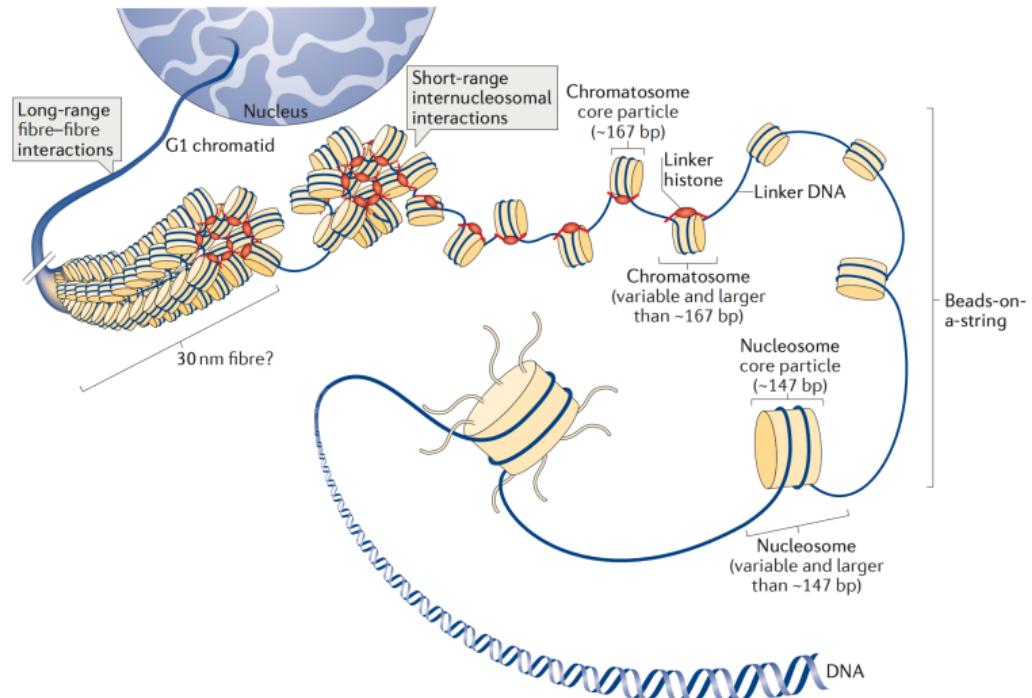
Bayesian image restoration with diffusion models



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

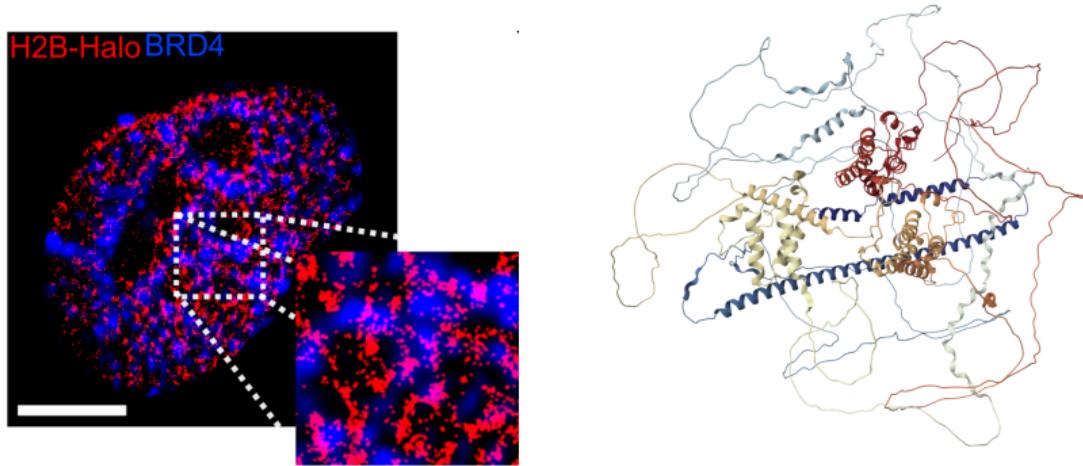
Super-resolution of nucleosome nanodomains *in-vivo*

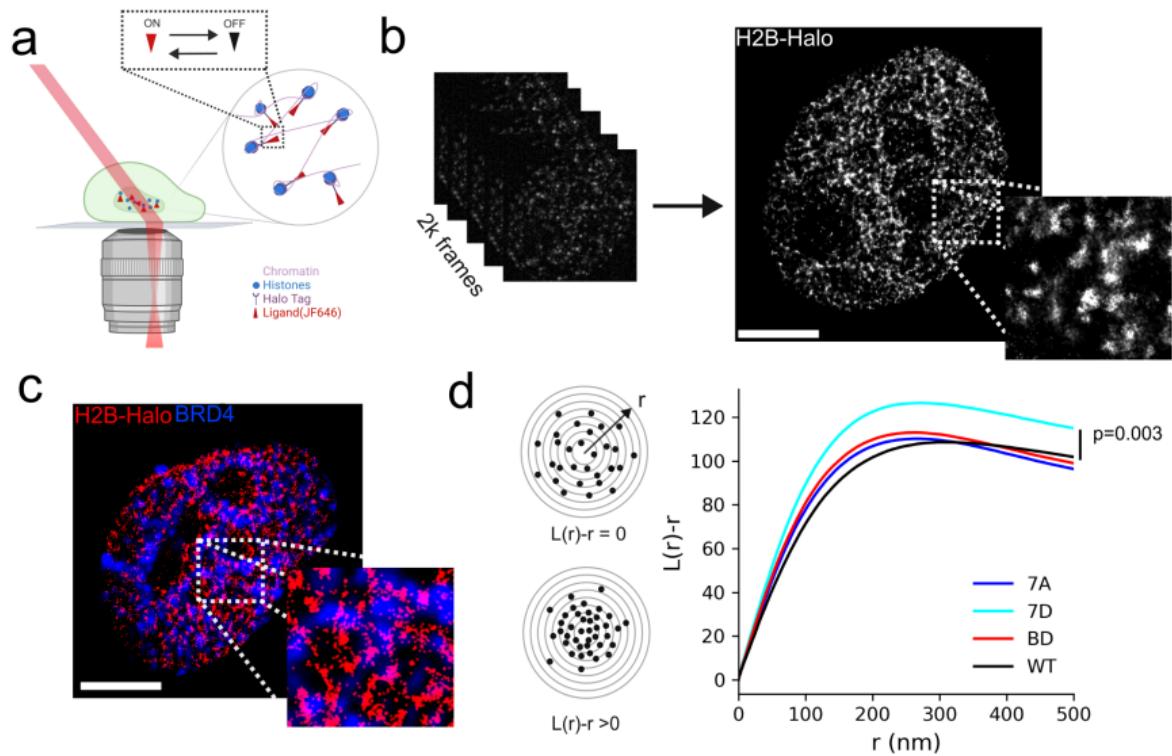
Hierarchical structure of chromatin



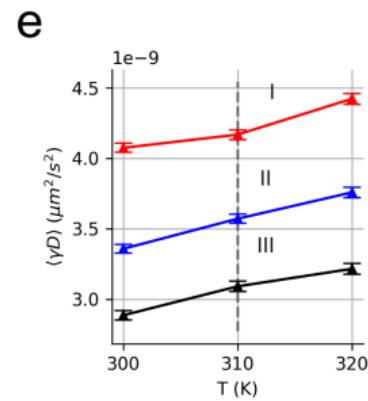
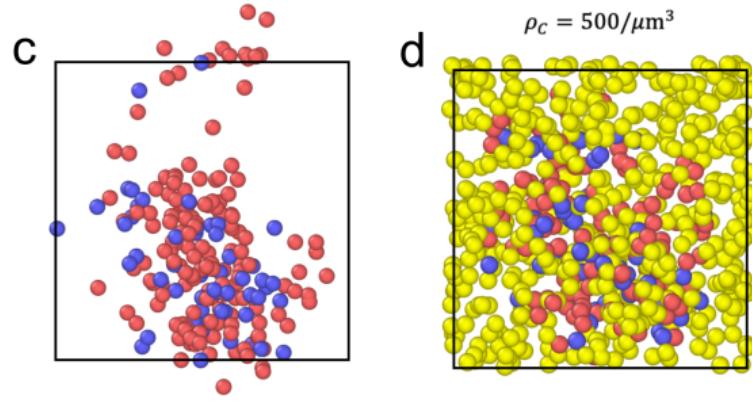
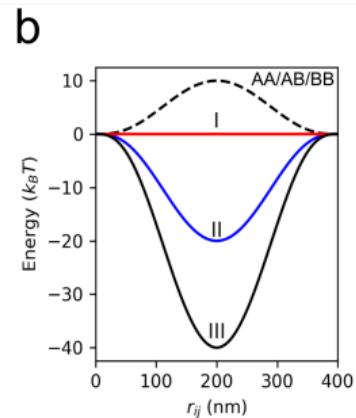
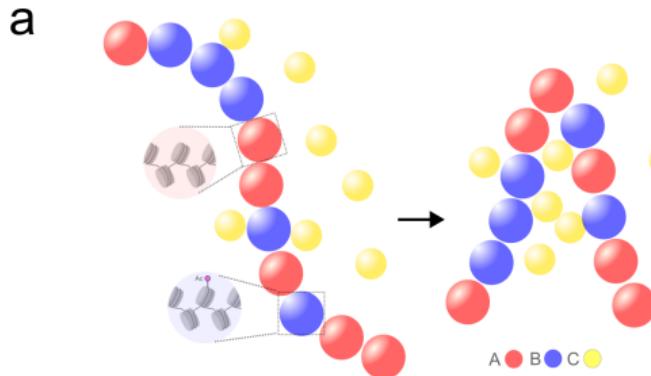
Fyodorov, D. et al. Nat Rev Mol Cell Biol 19, (2018).

Bromodomain protein 4 (BRD4) binds acetylated chromatin

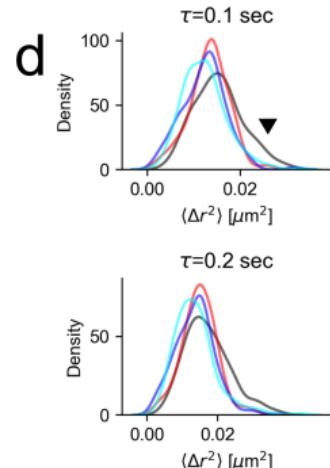
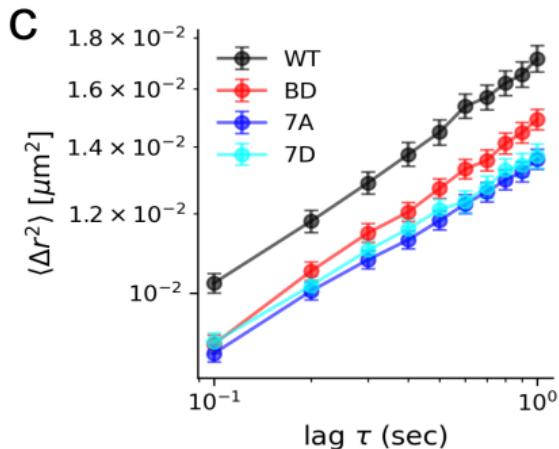
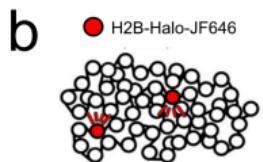
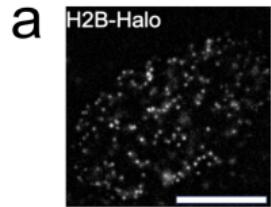




Seitz et al. Under Review. (2020)



Seitz et al. Under Review. (2020)



Seitz et al. Under Review. (2024)

Recent Publications

- ▶ Maelle Locatelli[†], Josh Lawrimore[†], Hua Lin[†], Sarvath Sanaullah, **Clayton Seitz**, ..., Pierre-Alexandre Vidi. *DNA damage reduces heterogeneity and coherence of chromatin motions.* PNAS. July 2022
- ▶ 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. January 2022.
- ▶ Wenting Wu, Farooq Syed, Edward Simpson, Chih-Chun Lee, Jing Liu, Garrick Chang, Chuanpeng Dong, **Clayton Seitz**, ..., Carmella Evans-Molina; *Impact of Proinflammatory Cytokines on Alternative Splicing Patterns in Human Islets.* Diabetes. January 2022

Acknowledgements



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



Pancho



Donghong Fu



Norbert Scherer

Thank you!