

Visualizing chromatin organization with single molecule localization microscopy

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Outline

Single molecule localization microscopy

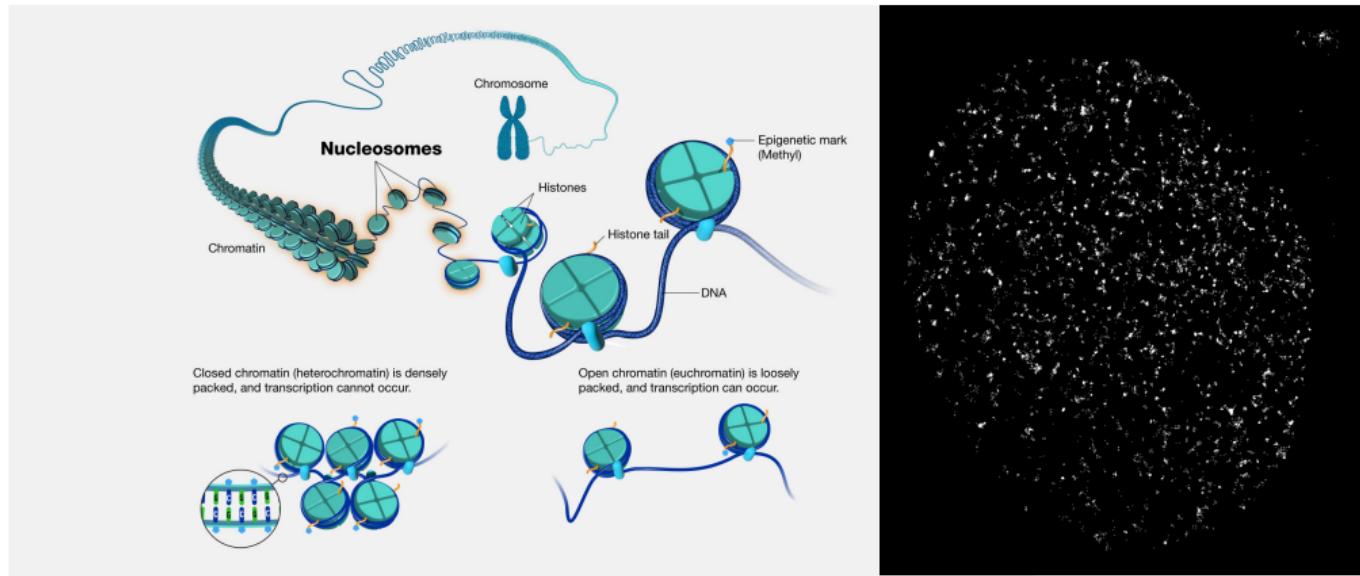
The time resolution of *d*STORM

Dense localization with deep learning

Dense localization by fluorescence antibunching

Phase separation of chromatin

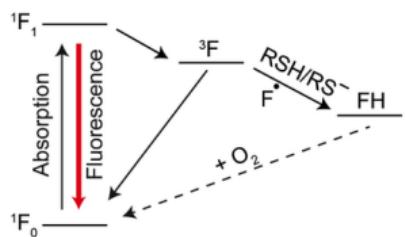
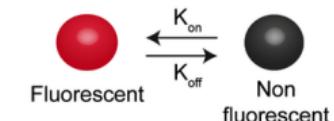
Genome organization and single molecule localization microscopy



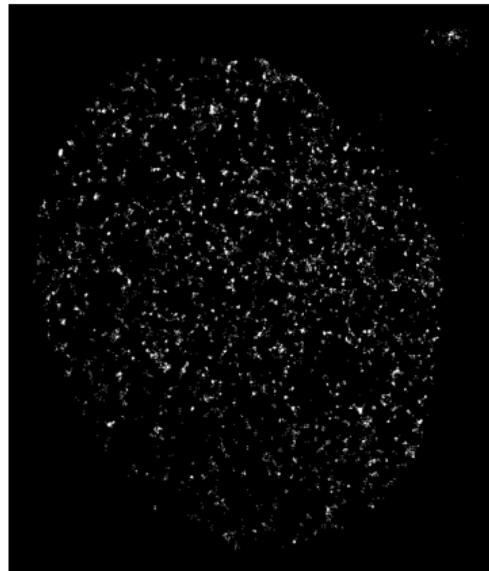
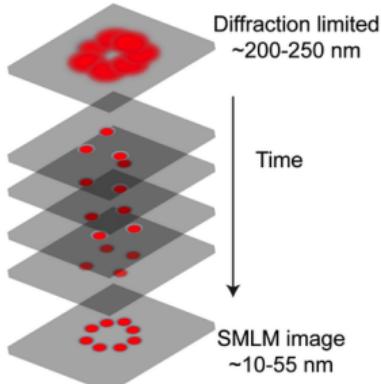
- ▶ Genome has a hierarchical structure, fundamental unit is the nucleosome
- ▶ We study chromatin organization by localizing fluorescently tagged nucleosomes

Single molecule localization microscopy

a Photoswitching



b Temporal separation



- ▶ SMLM techniques are diffraction-unlimited
- ▶ Photoswitching enables resolution of emitters in time rather than space

Single molecule localization microscopy

Modeling the point spread function permits sub-pixel localization

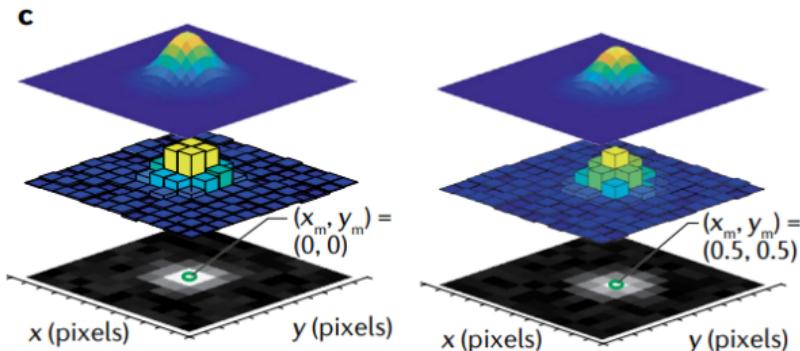
$$\mu_k = i_0 \int_{\mathbb{K}} h_\theta(x_0, y_0) dx dy$$

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

η – quantum efficiency

N_0 – photon count

Δ – exposure time



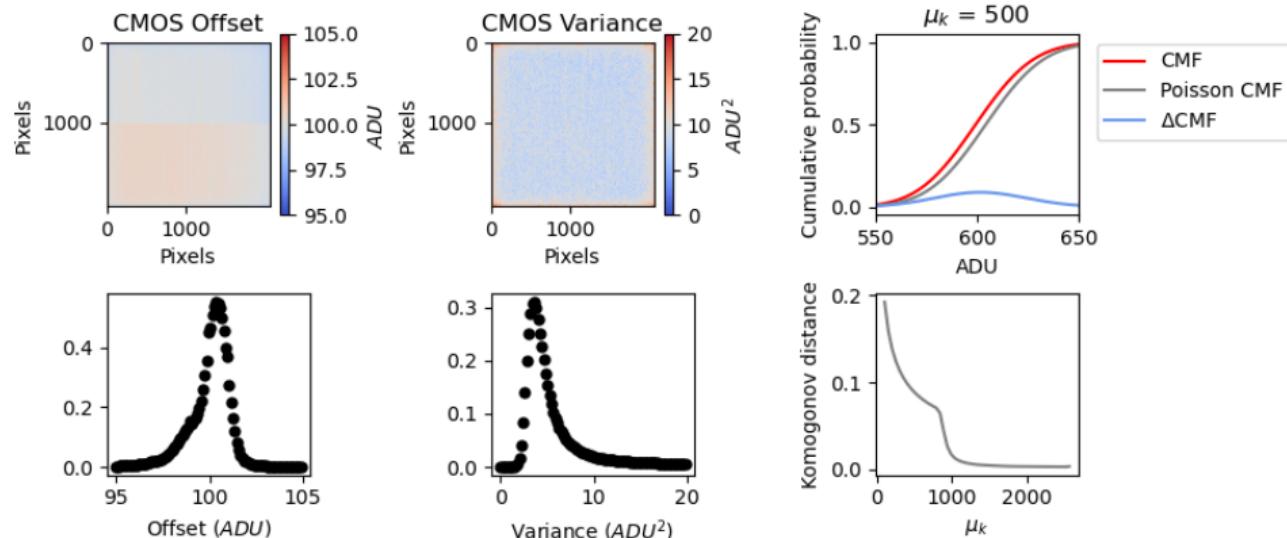
Long $\Delta \rightarrow$ pixels are iid:

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

What is $P(H_k | \theta)$?

Classical emission statistics of fluorescent markers

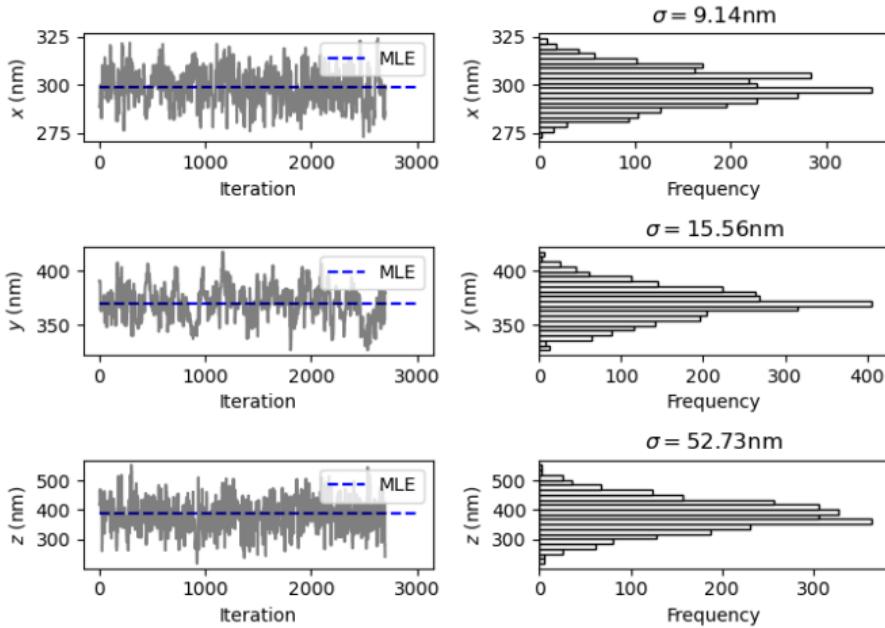
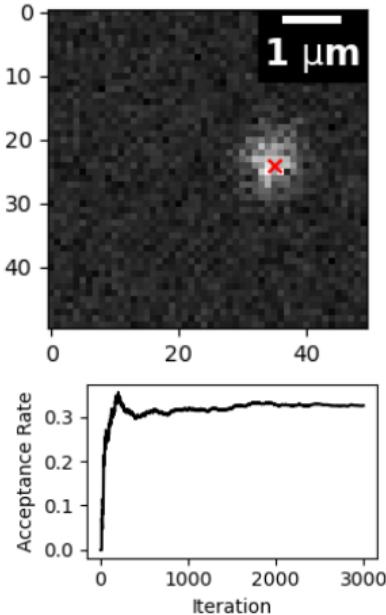
Long integration times $\Delta \rightarrow$ intensity fluctuations are Poisson



$$P(H_k|\theta) = A \sum_{q=0}^{\infty} \frac{1}{q!} e^{-\mu_k} \mu_k^q \frac{1}{\sqrt{2\pi}\sigma_k} e^{-\frac{(H_k - g_k q - \sigma_k)^2}{2\sigma_k^2}}$$

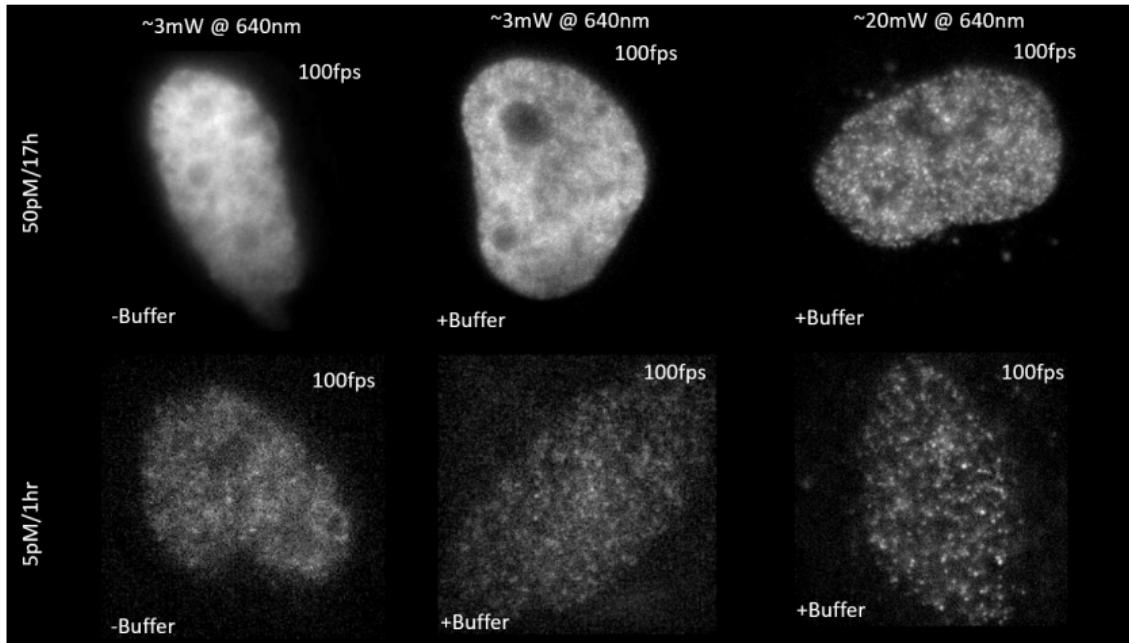
$P(H_k|\theta)$ can be approximated as Poisson at high signal-to-noise (SNR)

Estimator precision determines resolution in localization microscopy



- One can derive a lower bound on the variance of a statistical estimator of the coordinates θ

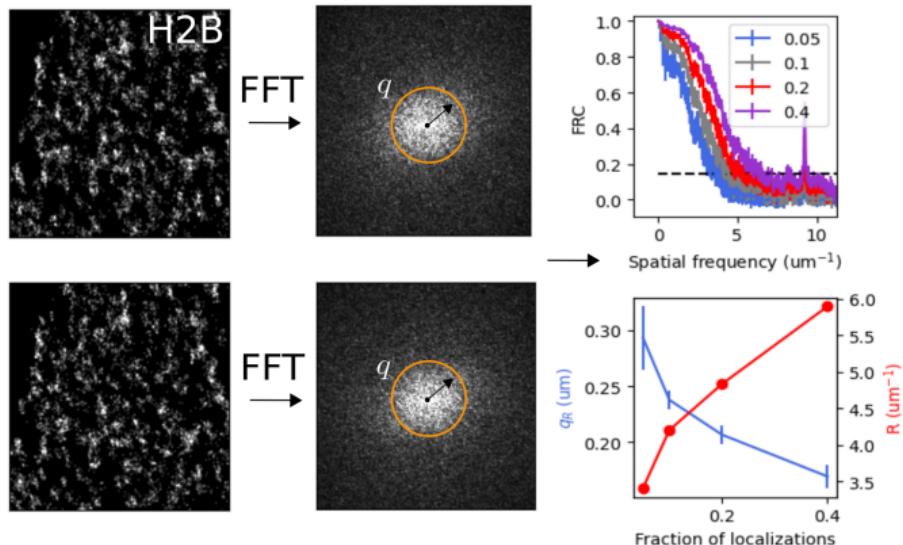
Dense labeling of histone H2B in fixed cells at RT



- ▶ Dense labeling of H2B-Halotag w/ fluorescent ligand JF646
- ▶ Reducing buffer is usually a primary thiol like cysteamine (MEA)
- ▶ Photoswitching of JF646 allows us to beat the diffraction limit

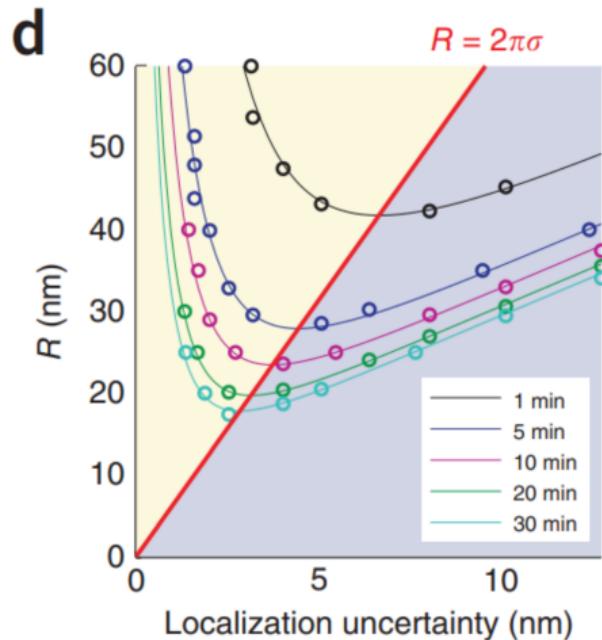
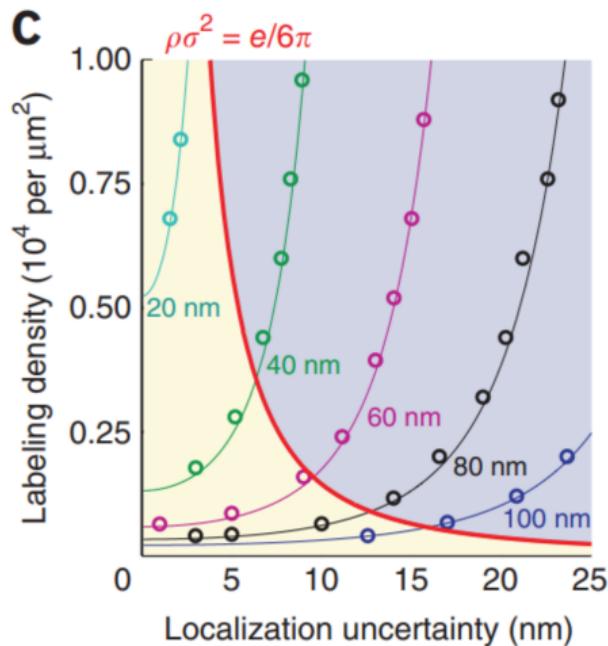
Dense localization increases time resolution

- We can view dSTORM as sampling from a density



$$\text{FRC}(q) = \frac{\sum_{\vec{q} \in \text{circle}} \tilde{f}_1(\vec{q}) \tilde{f}_2(\vec{q})^*}{\sqrt{\sum_{\vec{q} \in \text{circle}} |f_1(\vec{q})|^2} \sqrt{\sum_{\vec{q} \in \text{circle}} |f_2(\vec{q})|^2}}$$

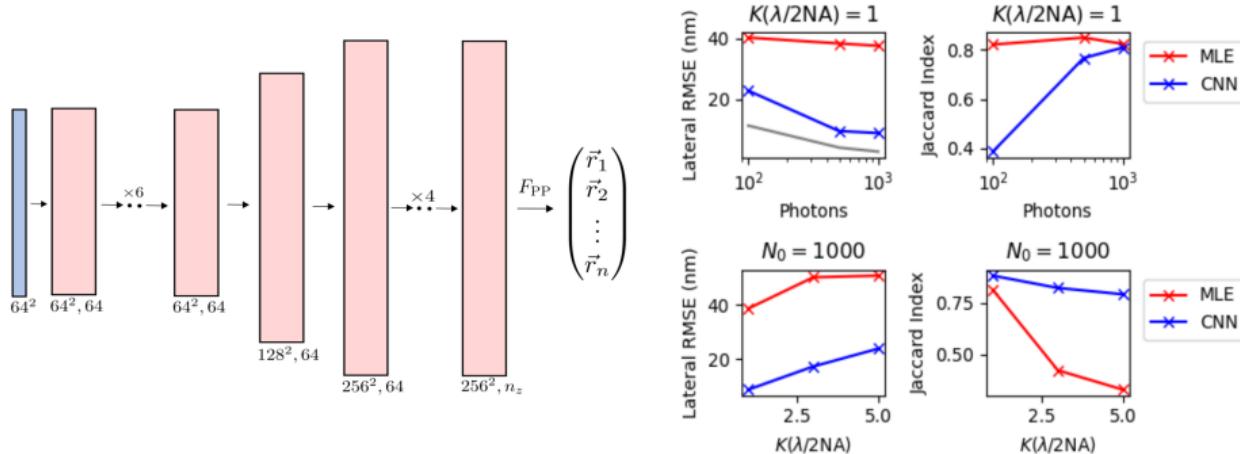
Dense localization increases time resolution



Nieuwenhuizen et al. Measuring image resolution in optical nanoscopy.

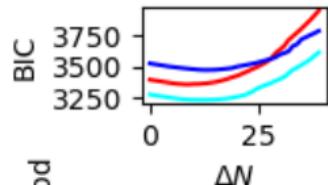
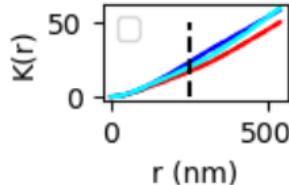
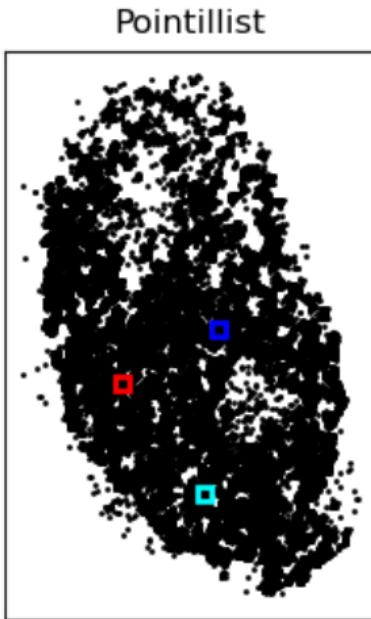
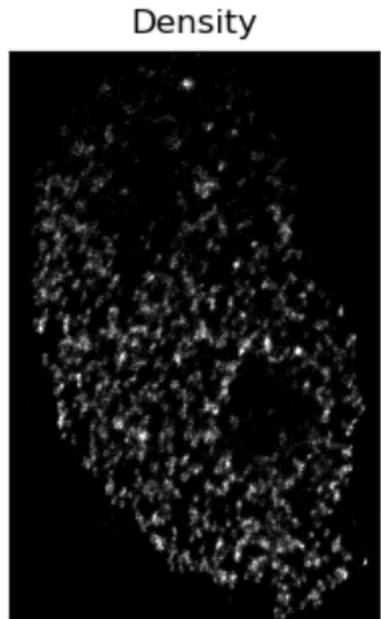
- ▶ Increased localization uncertainty requires higher density for same resolution
- ▶ Longer acquisitions have higher resolution

Estimator precision sets the resolution limit in localization microscopy

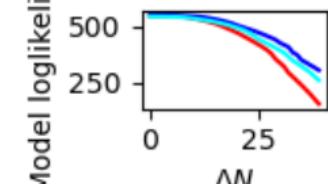


- ▶ $K(\lambda/2NA)$ is Ripley's K function at the diffraction limit ($\lambda = 640\text{nm}$)
- ▶ Convolutional neural networks (CNNs) approach the Cramer-Rao lower bound (gray)

Chromatin nanodomains in a living Hela cell nucleus



Model loglikelihood



- ▶ Histone DE using 30x30nm bins
- ▶ Likelihood is computed under a Gaussian Mixture Model (GMM)

Future work: dense localization with fluorescence antibunching

We need to compute the joint distribution $P(X_i, X_j)$. We compute $P(X_i = N_i, X_j = N_j)$ by considering now microstates α_i, α_j , which are binary vectors, s.t. $\sum \alpha_i = N_i$ and $\sum \alpha_j = N_j$ and have $\alpha_i \text{ AND } \alpha_j = 0$

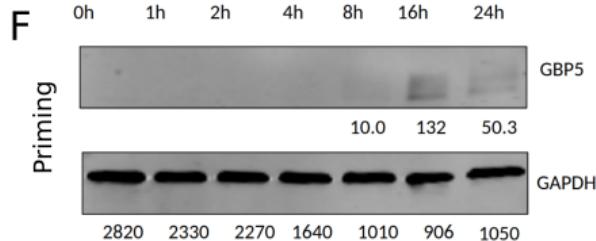
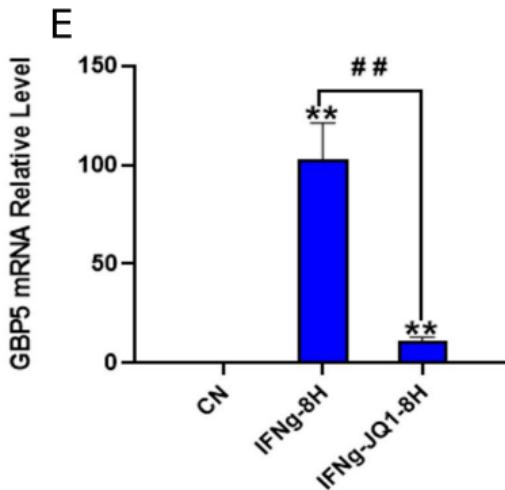
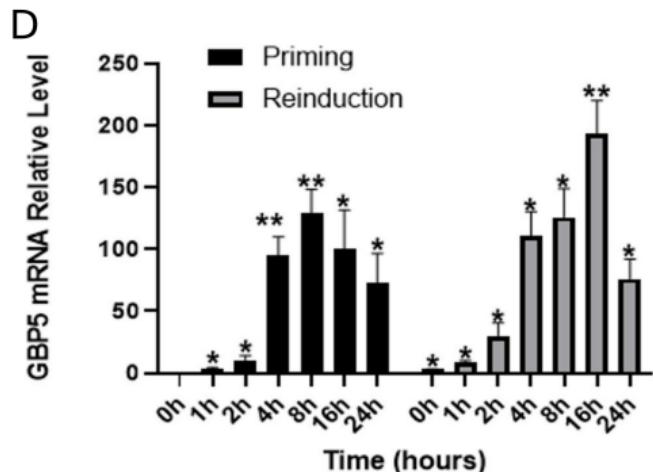
$$P(X_i = N_i, X_j = N_j) \propto \sum_{\alpha, \beta \in \mathcal{A} \otimes \mathcal{B}} \prod_n \mathbf{p}_i^\alpha \mathbf{p}_j^\beta$$

But now consider

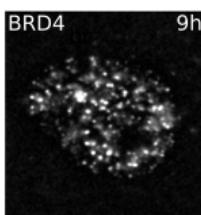
$$\langle X_i X_j \rangle = \sum_{(N_i, N_j)} N_i N_j P(X_i = N_i, X_j = N_j)$$

Antibunching now becomes apparent. If only a single emitter exists (and we have designed α 's correctly) then this expectation must be zero for all (i, j) .

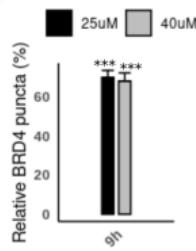
Inhibition of a super-enhanced gene with JQ1



G

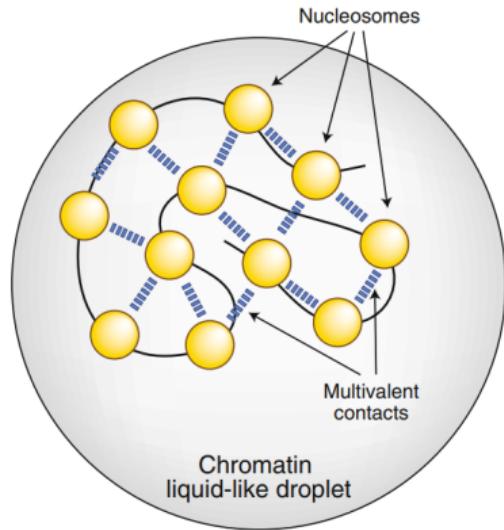


H



► *: $P \leq 0.1$, **: $P \leq 0.01$

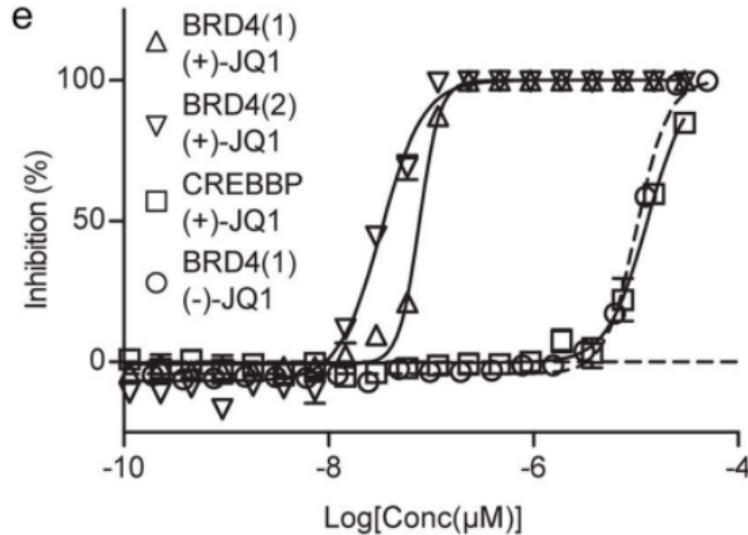
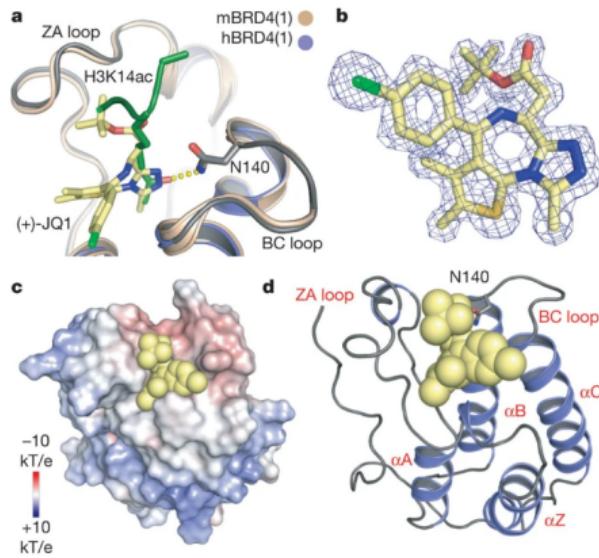
Chromatin has an intrinsic ability to undergo phase separation



Regulatory factors of chromatin LLPS
Histone H1
DNA length between nucleosomes
Histone post-translational modifications
Nucleosome dynamics
Multivalent binding of proteins

- ▶ Super-enhanced genes are regulated by large molecular assemblies
- ▶ We study nucleosome clustering dynamics using super-resolution microscopy

(+)-JQ1 in complex with BRD4 protein



Filippakopoulos. Selective inhibition of BET bromodomains. *Nature*

- ▶ BRD4 is an interesting target since specific and non-specific inhibitors exist
- ▶ BET mimics including +JQ1 prevent binding of BRD4 to acetylated histones

BET inhibitors reduce nucleosome-BRD4 interactions in BRD4 condensates

BET inhibitors promote disordered BRD4 condensates