

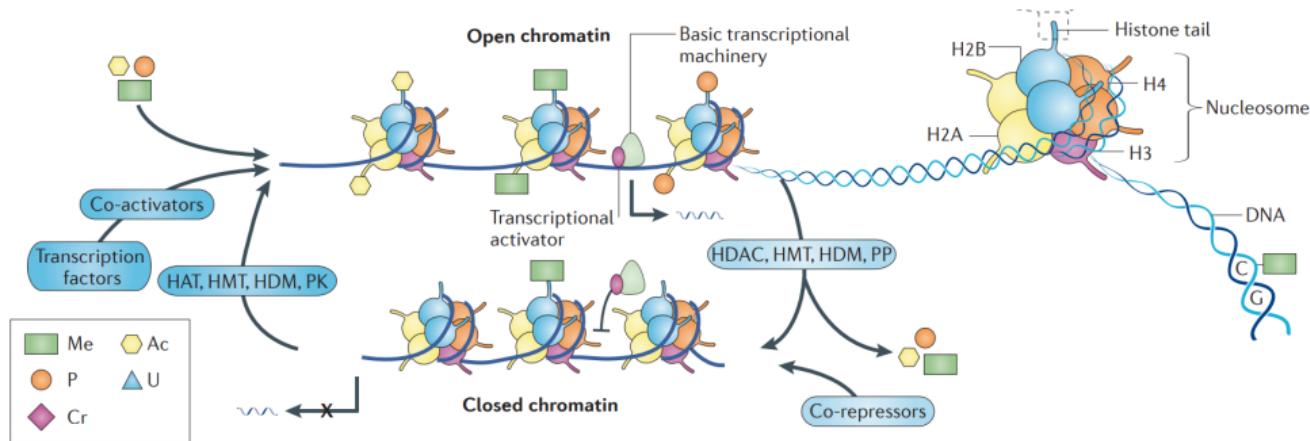
# Visualizing nucleosome cluster dynamics with dense single molecule localization microscopy

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# Introduction

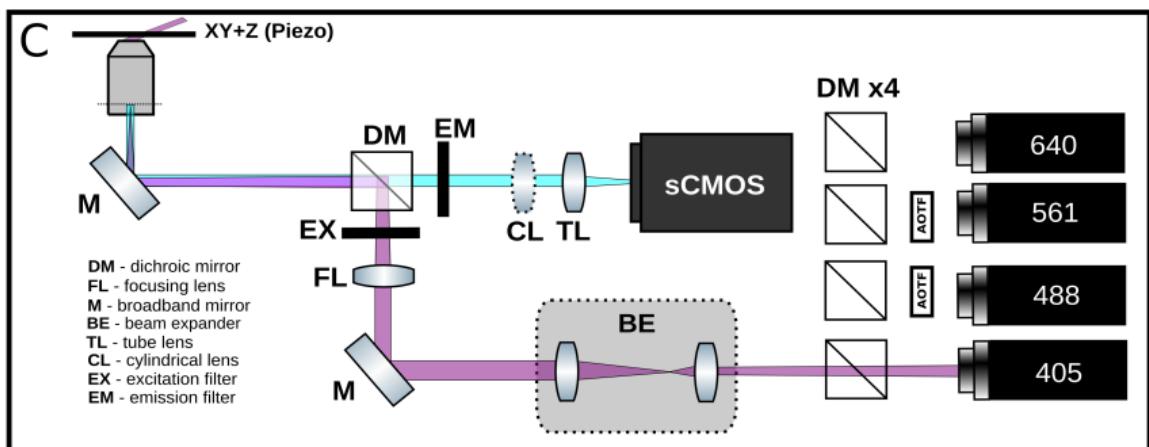
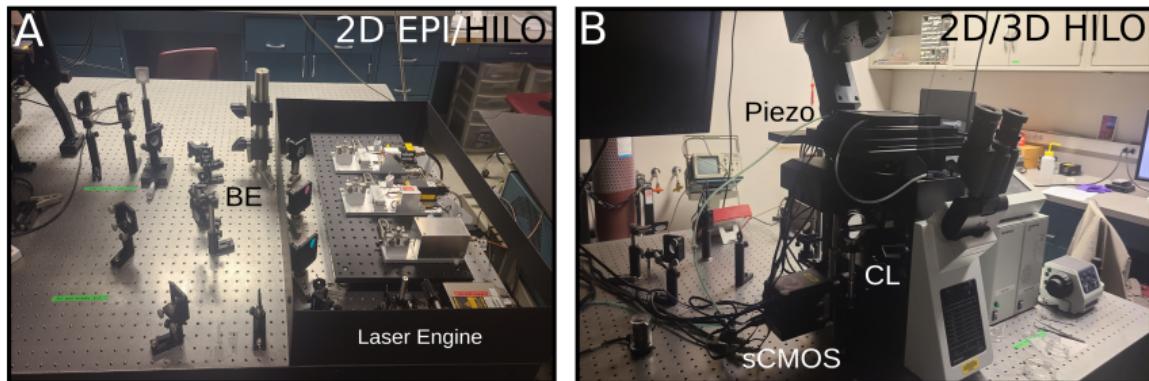
# The textbook view of histone acetylation



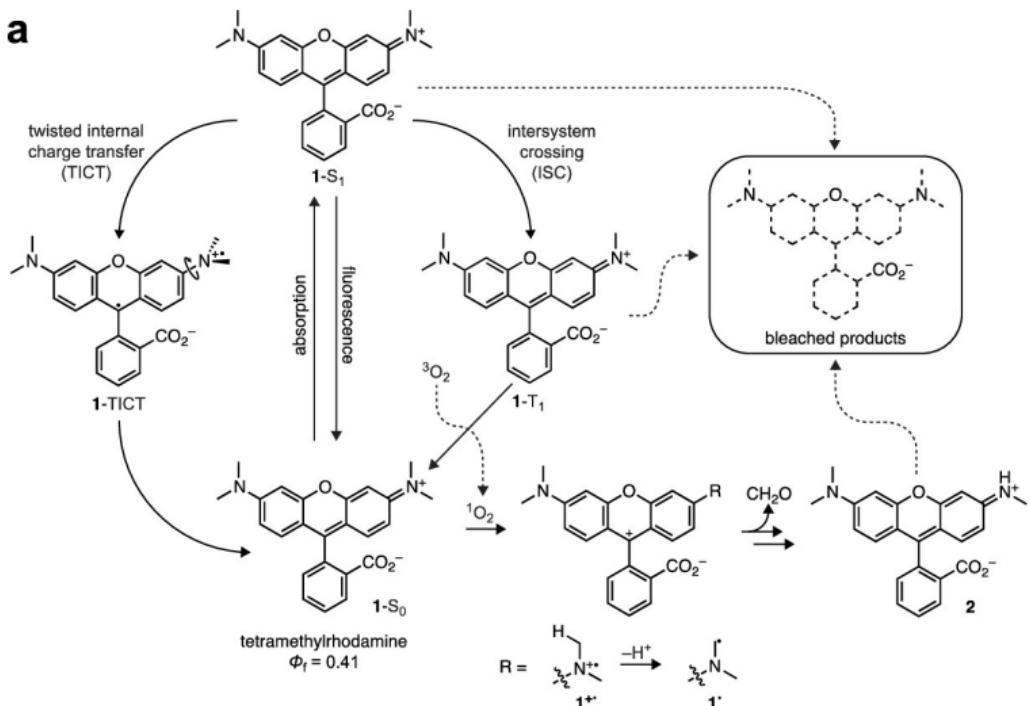
Graff et al. Histone acetylation: molecular mnemonics on the chromatin

- ▶ I am interested in the impact of BRD4 protein on chromatin structure
- ▶ Previous work has shown BRD4 is associated with histone acetylation
- ▶ Live cell super-resolution imaging is a useful tool

# Instrumentation for super-resolution and high throughput microscopy

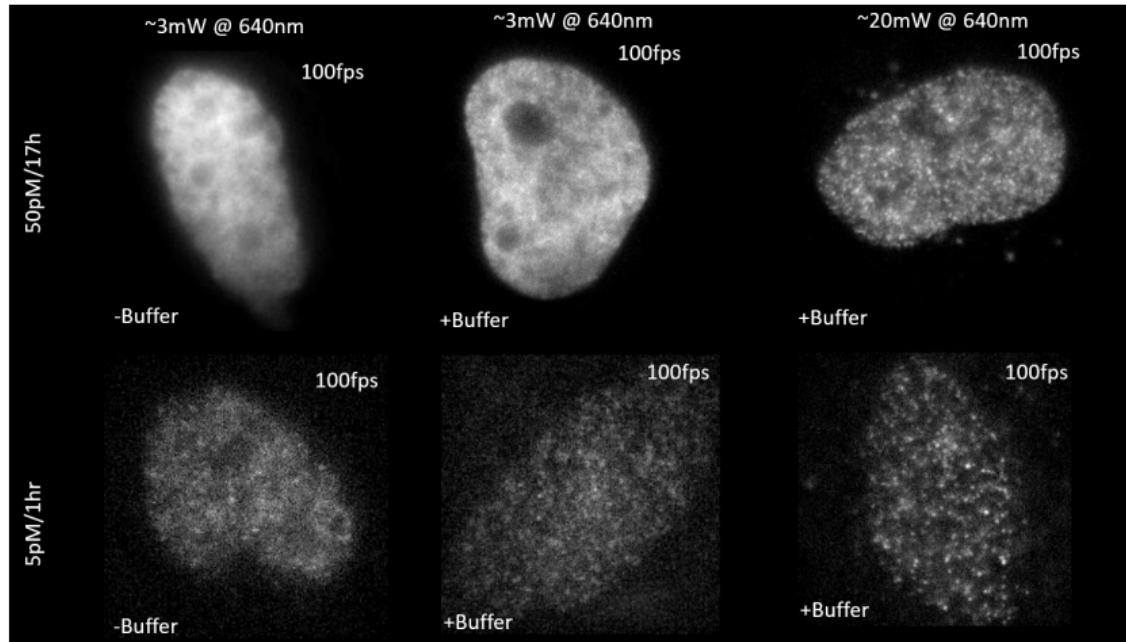


# The photophysics of rhodamines



- ▶ Reduction of the T<sub>1</sub> state yields a dark, long-lived, and stable radical state
- ▶ The reducing agent is usually a primary thiol like cysteamine (MEA)

# The OFF state of JF646 can be maintained with high laser power



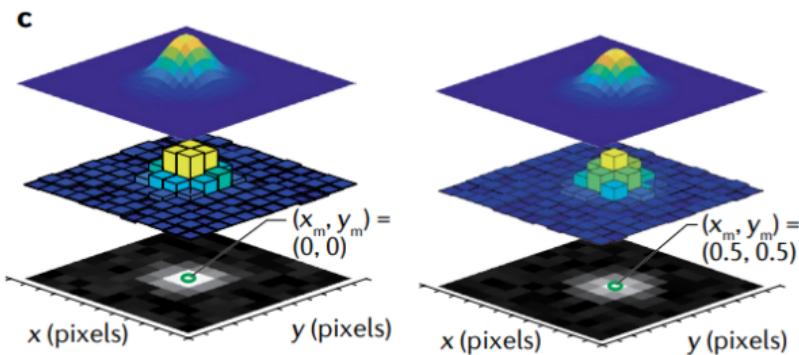
- ▶ High power maintains the OFF state, potentially by promoting triplet state formation

# Maximum likelihood localization of an isolated fluorescent emitter

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

$$\mu_k = g_k \eta N_0 \Delta \int_{\text{pixel}} G(x, y) dA$$

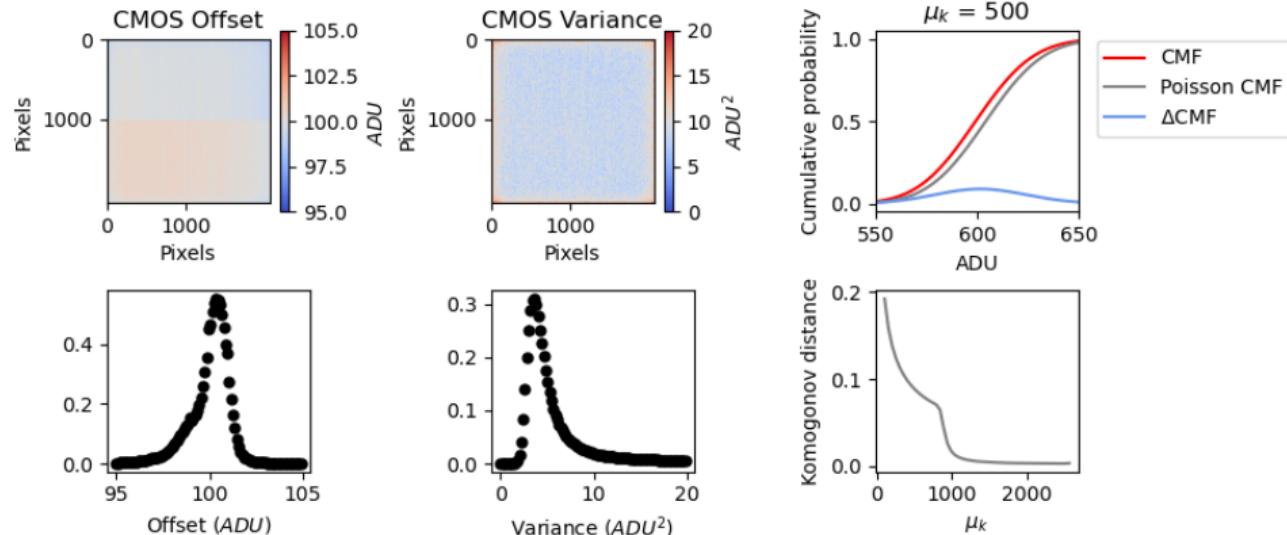
- $\eta$  – quantum efficiency
- $N_0$  – photon count
- $\Delta$  – exposure time



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

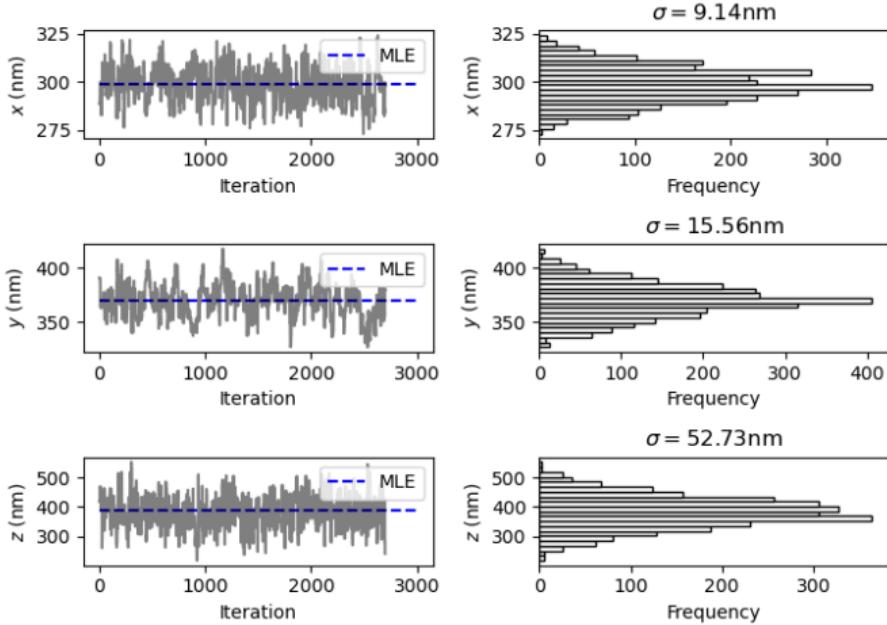
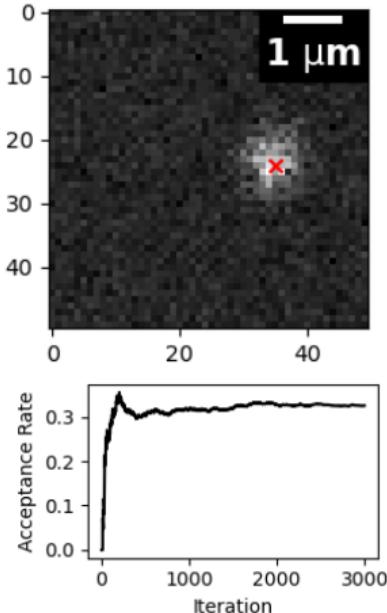
$P(H_k|\theta)$  can be approximated as Poisson at high signal-to-noise (SNR)

# A Poisson approximation at moderate SNR simplifies SMLM



- ▶  $P(H_k - o_k | \theta) = \text{Poisson}(\mu_k + \sigma_k^2 | \theta)$  for pixel offset  $o_k$  noise variance  $\sigma_k^2$
- ▶ Fisher information and Cramer-Rao lower bound (CRLB) can be computed analytically for Poisson log-likelihood  $\ell$  (Smith 2010, Huang 2013)

# Estimator precision sets the resolution limit in localization microscopy



- ▶ Variance of the posterior  $P(\theta|\vec{H})$  is a useful particle filter
- ▶ We assume uniform priors on coordinates

## Computing the CRLB for static errors in two dimensions

Fisher information (separable case):

$$I_{ij}(\theta) = \mathbb{E}_{\theta} \left( \frac{\partial \ell}{\partial \theta_i} \frac{\partial \ell}{\partial \theta_j} \right) \quad (1)$$

Let  $\mu'_k = \mu_k + \sigma_k^2$ . For an arbitrary parameter,

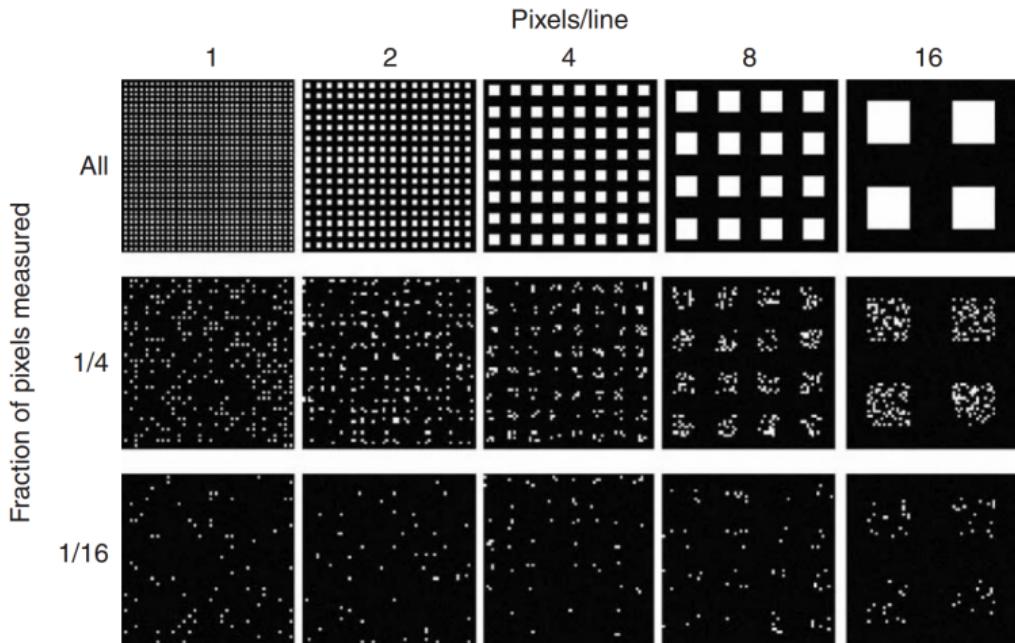
$$\frac{\partial \ell}{\partial \theta_i} = \frac{\partial}{\partial \theta_i} \sum_k x_k \log x_k + \mu'_k - x_k \log (\mu'_k)$$

$$= \sum_k \frac{\partial \mu'_k}{\partial \theta_i} \left( \frac{\mu'_k - x_k}{\mu'_k} \right)$$

$$I_{ij}(\theta) = \mathbb{E}_{\theta} \left( \sum_k \frac{\partial \mu'_k}{\partial \theta_i} \frac{\partial \mu'_k}{\partial \theta_j} \left( \frac{\mu'_k - x_k}{\mu'_k} \right)^2 \right) = \sum_k \frac{1}{\mu'_k} \frac{\partial \mu'_k}{\partial \theta_i} \frac{\partial \mu'_k}{\partial \theta_j}$$

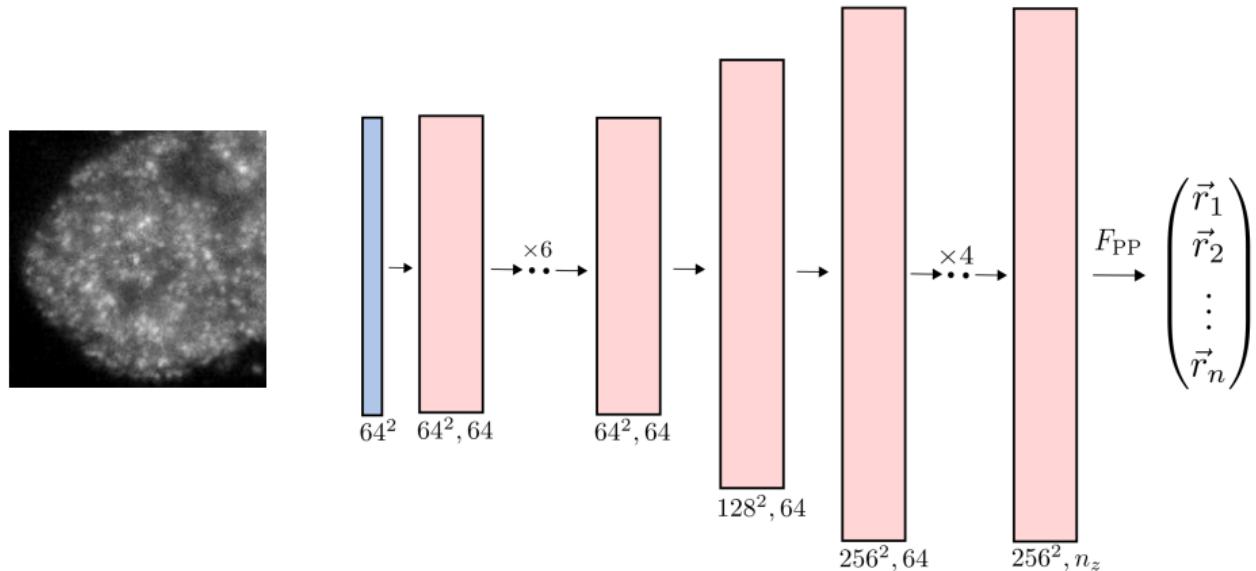
The CRLB is a frequentist

# The tradeoff between spatial and temporal resolution in SMLM



- ▶ SMLM is desirable for SR due to very high res and no scanning (e.g., STED)
- ▶ Less control over photophysical state, but high throughput

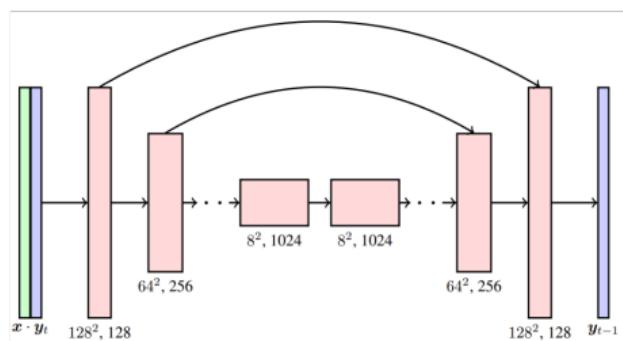
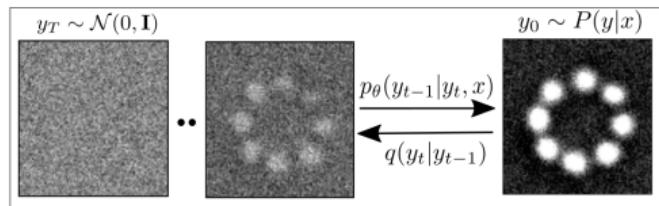
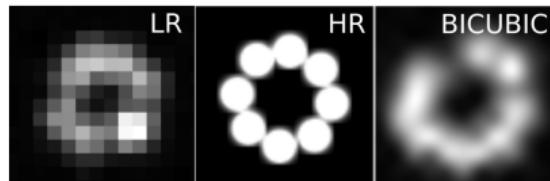
# Deep learning enables dense localization in two-dimensions



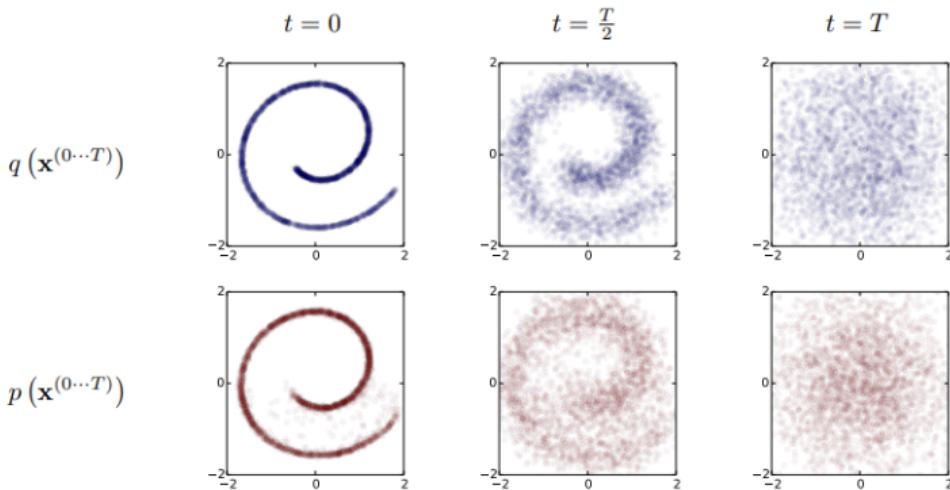
Localization is cast as semantic segmentation of the high resolution tensor:

$$\mathcal{L} = \sum_{i,j} \log p_{ij}(\tilde{x}) = \sum_{i,j} \log \frac{\exp(-s_{ij}(\tilde{x}))}{\sum_{x \in \chi} \exp(-s_{ij}(\tilde{x}))}$$

# Deep generative modeling with nonequilibrium thermodynamics

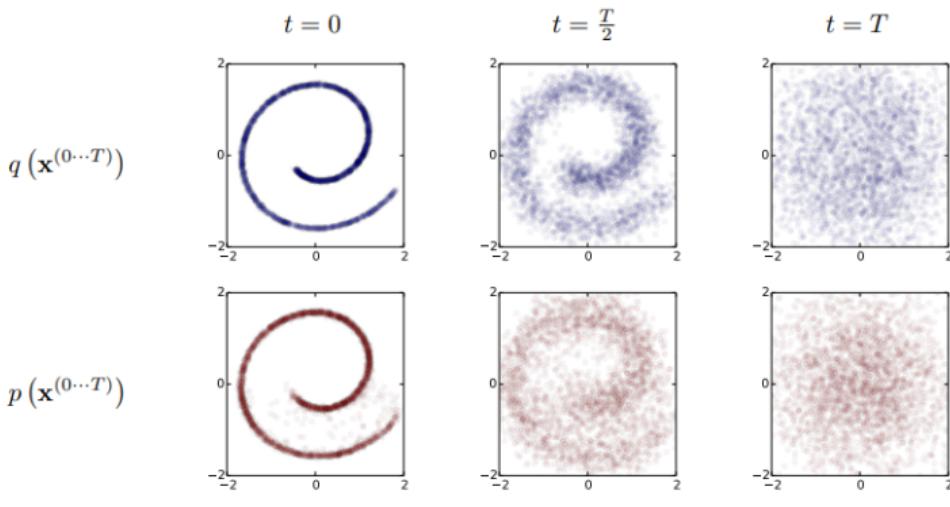


# Deep generative modeling with nonequilibrium thermodynamics



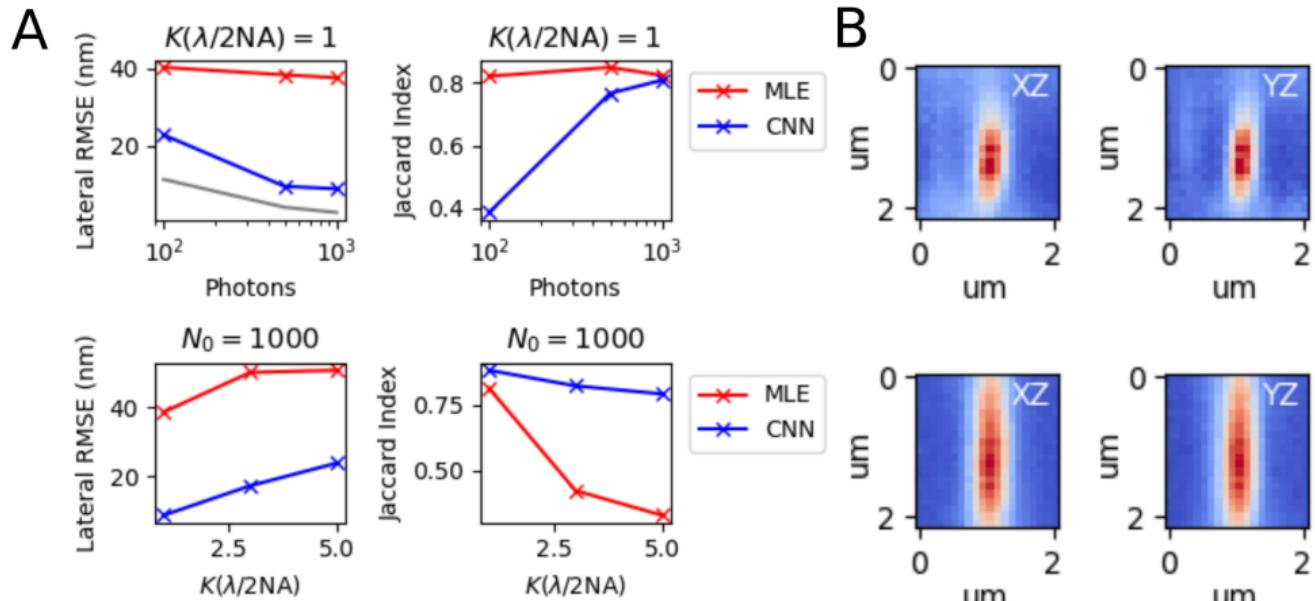
- ▶ The data distribution is gradually converted into a analytically tractable distributon e.g., Gaussian
- ▶ Repeated application of a Markov transition kernel destroys data structure
- ▶ Use deep neural networks to learn to "reverse time"

# Deep generative modeling with nonequilibrium thermodynamics



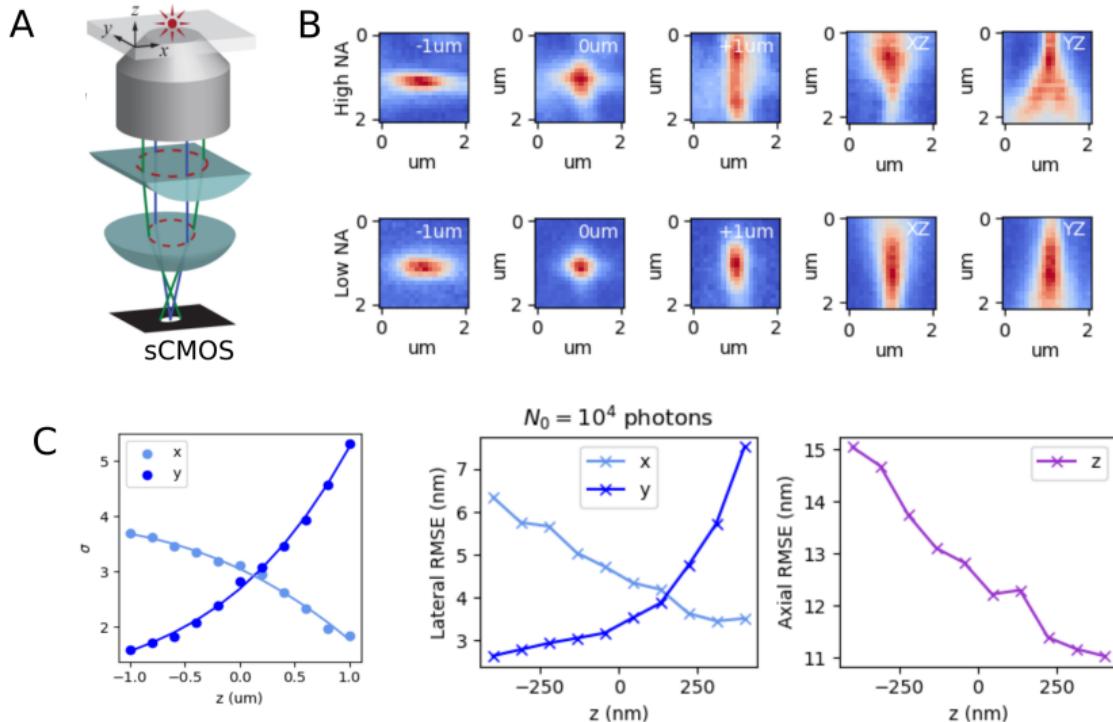
$$\pi(\mathbf{y}) = \int d\mathbf{y}' T_\pi(\mathbf{y}|\mathbf{y}'; \beta) \pi(\mathbf{y}')$$

# Estimator precision sets the resolution limit in localization microscopy



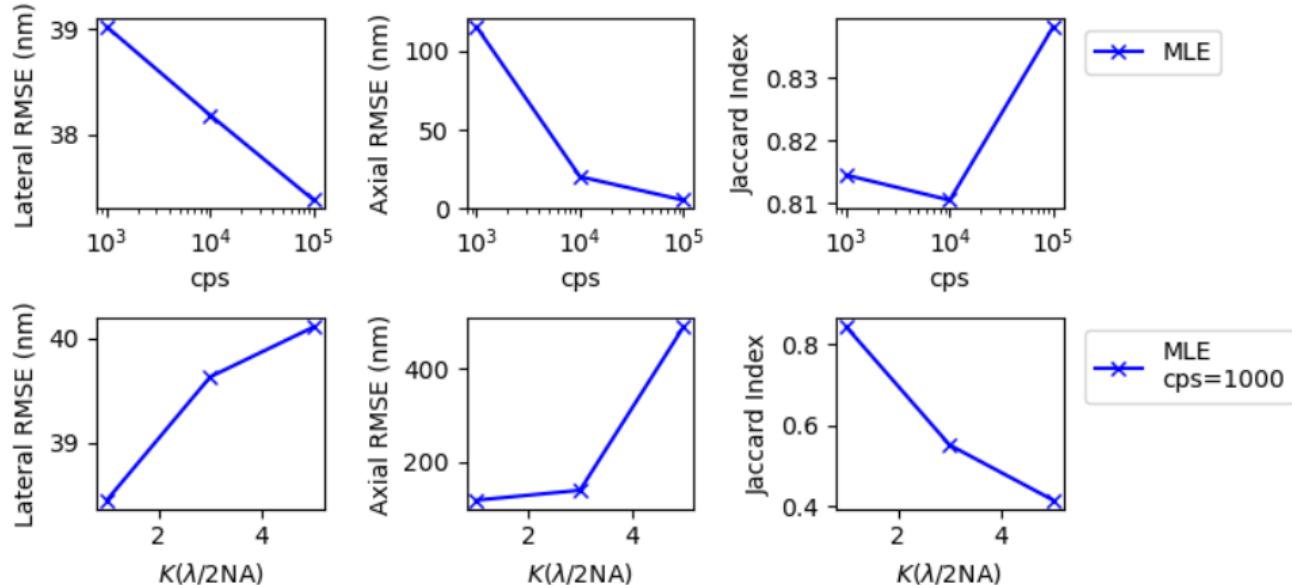
- ▶  $K(\lambda/2NA)$  is Ripley's K function at the diffraction limit ( $\lambda = 640\text{nm}$ )
- ▶ Convolutional neural networks (CNNs) approach the CRLB (gray) at high photon counts and generalize to the dense regime

# Astigmatism based three dimensional imaging



- A weak ( $f = 10\text{m}$ ) cylindrical lens breaks the axial symmetry of the PSF

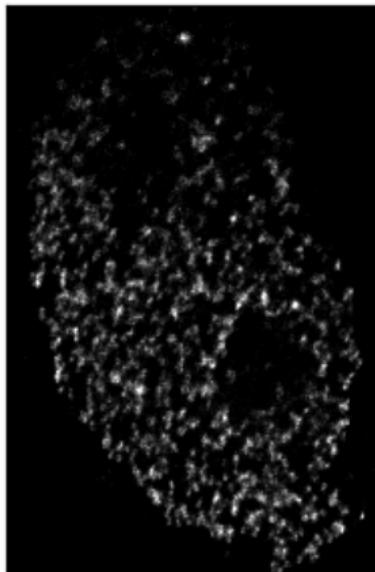
# Astigmatism based three dimensional imaging



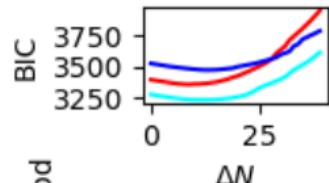
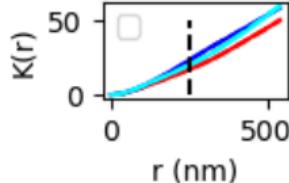
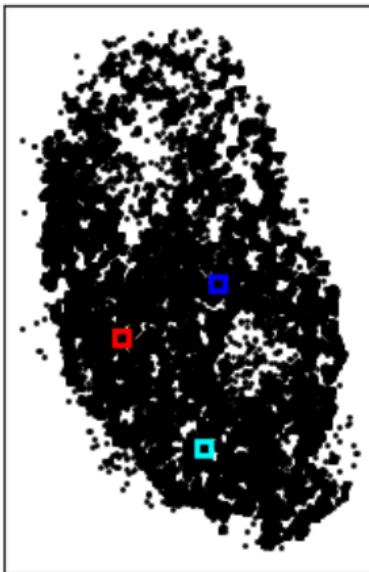
- ▶  $z_0 \sim U([-0.4, 0.4])$  um
- ▶ 3D imaging requires long exposure and sparse emitters for MLE
- ▶ Deep methods may be a suitable choice in future work

# Chromatin nanodomains in a living Hela cell nucleus at 37C

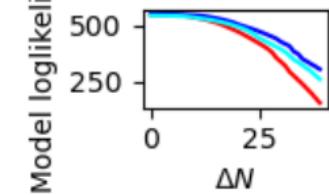
Density



Pointillist



Model loglikelihood



- ▶ Density estimation using 30x30nm bins
- ▶ Closest pairs are merged one at a time, until we minimized the BIC
- ▶ Data likelihood is computed under a Gaussian Mixture Model (GMM)

## Diffusion increases localization uncertainty in live-cell SMLM

Nucleosome diffusion has been modeled in various potentials:

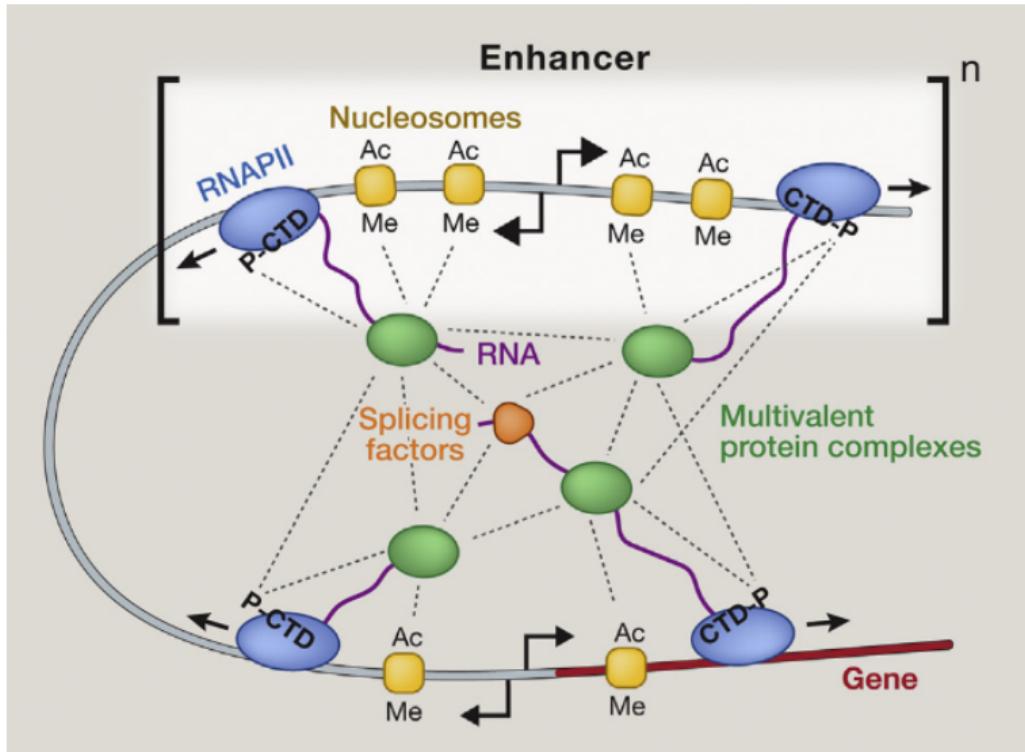
- ▶ Bead model:  $V(r_{ij}) = \epsilon_0(r_0/r_{ij})^{12} - \epsilon_{ij}(r_0/r_{ij})^6$  (Ashwin 2019)
- ▶ Harmonic:  $V(\vec{\Delta r}) = \frac{1}{2}k|\vec{\Delta r}|^2$  (XXX)

The latter is attractive because the stationary distribution of Brownian motion in a harmonic potential is known:

$$\partial_t P(r) = \hat{\mathcal{L}}_{FP} P(r); \hat{\mathcal{L}}_{FP} = \hat{\mathcal{L}}_{FP} = \left( -\frac{\partial}{\partial x} M^{(1)}(t) + \frac{1}{2} \frac{\partial^2}{\partial x^2} M^{(2)}(t) \right)$$

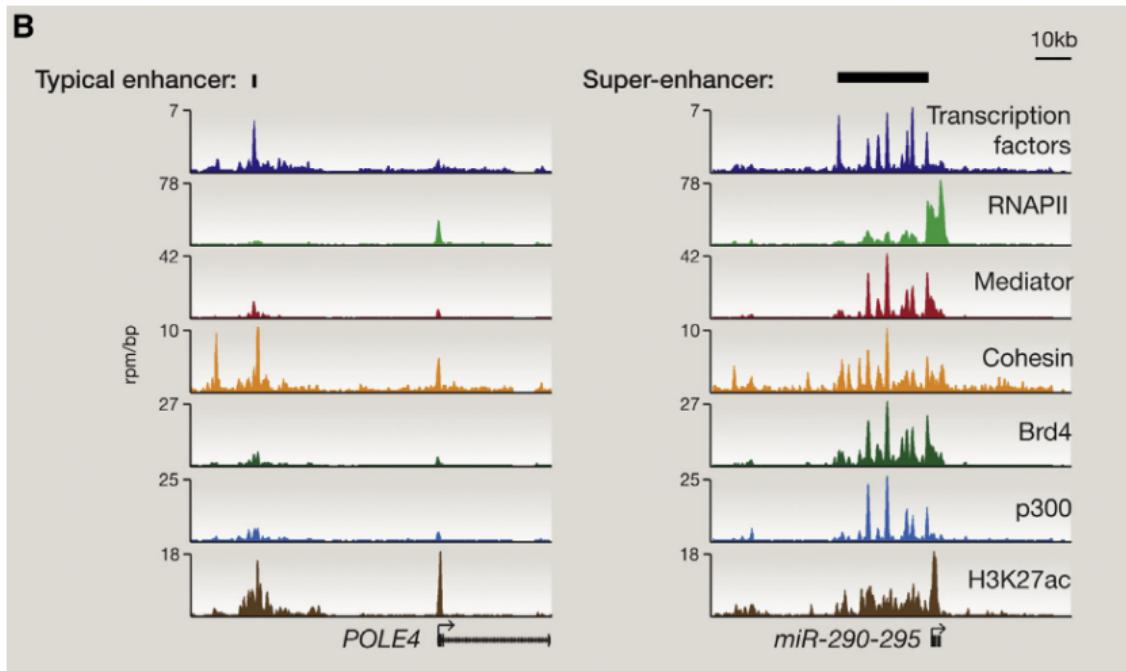
$$x', t' | x, t = \mathcal{N}(\mu, \Sigma)$$

# A phase separation model for transcriptional control



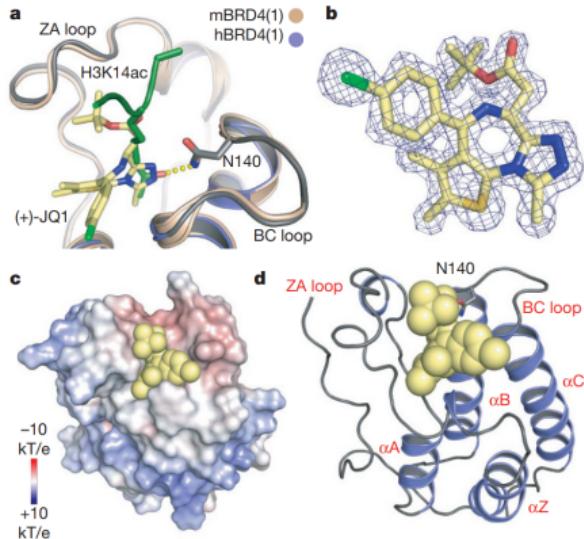
Hnisz et al. A phase separation model of transcriptional control. Cell 2017

# A phase separation model for transcriptional control



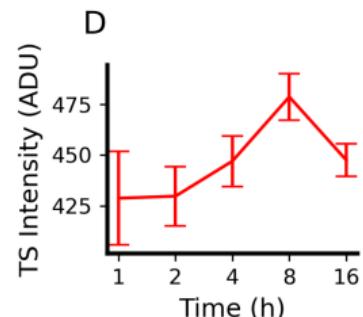
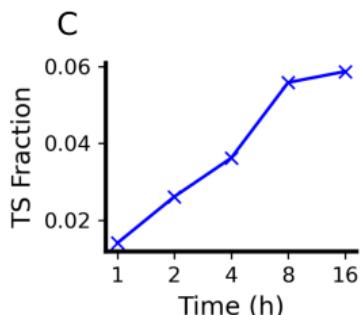
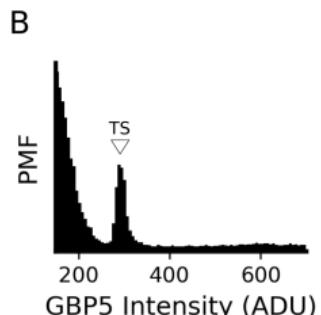
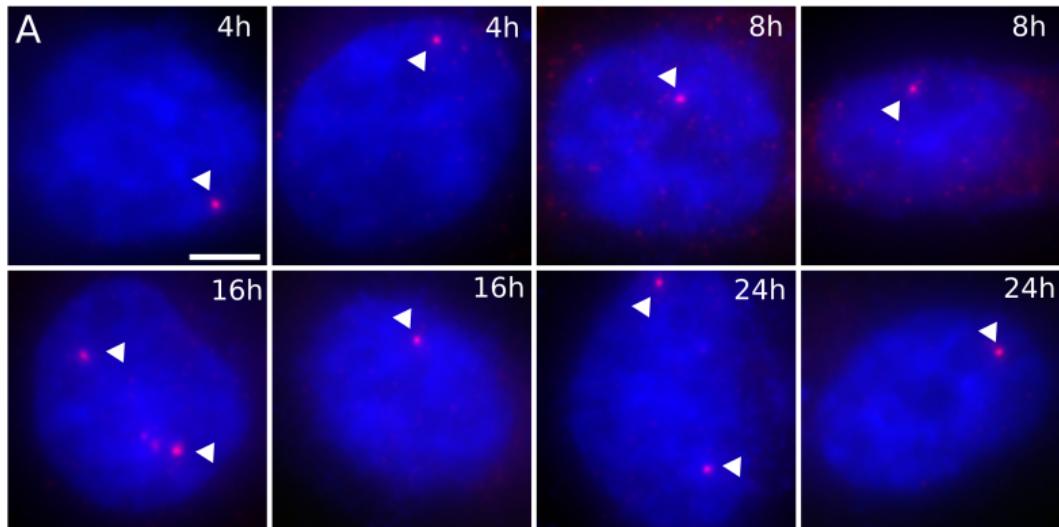
*Hnisz et al. A phase separation model of transcriptional control. Cell 2017*

# (+)-JQ1 in complex with BRD4 protein

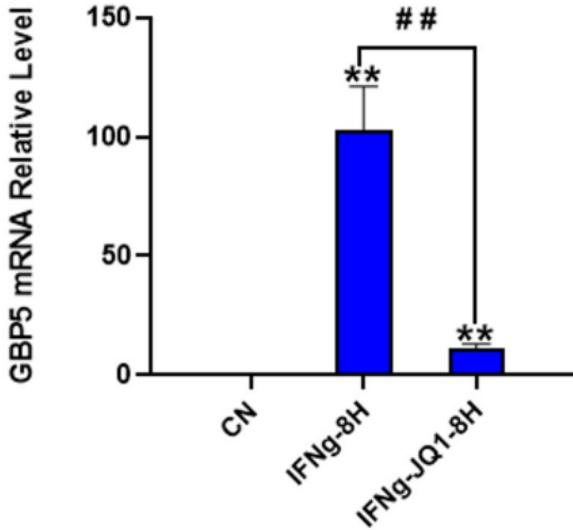
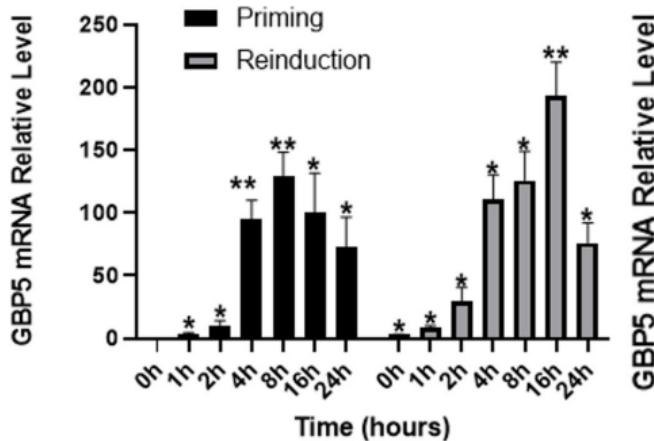


*Filippakopoulos. Selective inhibition of BET bromodomains. Nature Communications* A bromodomain is an approximately 110 amino acid protein domain that recognizes acetylated lysine residues BRD4 recognizes H3K14ac

# Induction of a BRD4-controlled gene with cytokine treatment



## Validation of JQ1 efficacy for BRD4 inhibition in HeLa cells



- RT-qPCR quantified using  $2^{-\Delta\Delta C_t}$  method using GAPDH as a reference gene
- \*: $P \leq 0.1$ , \*\*: $P \leq 0.01$

# Dissolving BRD4 condensates with 1,6 Hexanediol

Fixed cell results

Proposed two and three color imaging experiments