

# Probing phase transitions of DNA-protein condensates using single molecule localization microscopy

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# Outline

- ▶ Introduction: biological motivation and broad overview of microscopy approaches, IDR<sub>s</sub> of BRD4/MED1
- ▶ Methods: High-throughput and super-resolution imaging, PSF engineering, dSTORM chemistry, photoswitching dynamics
- ▶ Methods: Motivation for deep learning methods for 3D imaging at high density
- ▶ Methods: Bayesian clustering algorithms on high density data
- ▶ Results: Induction of gene expression, colocalization with phase separation markers
- ▶ Results: Statistics of nucleosome organization in phase separated clusters

# A phase separation model for transcriptional control

IDRs of BRD4/MED1

# The role of nucleosome organization in phase separation of DNA-protein condensates

HaloTag labeling method

# Single molecule localization microscopy for super-resolution

Direct stochastic optical reconstruction microscopy Fourier shell correlation

# Statistics of sCMOS cameras

# Point spread function engineering for three-dimensional imaging

# Localization microscopy as statistical inference

# Localization microscopy as statistical inference

Photoswitching dynamics are a major determinant of localization precision

# A deep learning framework for localization microscopy

Deep learning performs better than maximum likelihood estimators

# A deep learning framework for localization microscopy

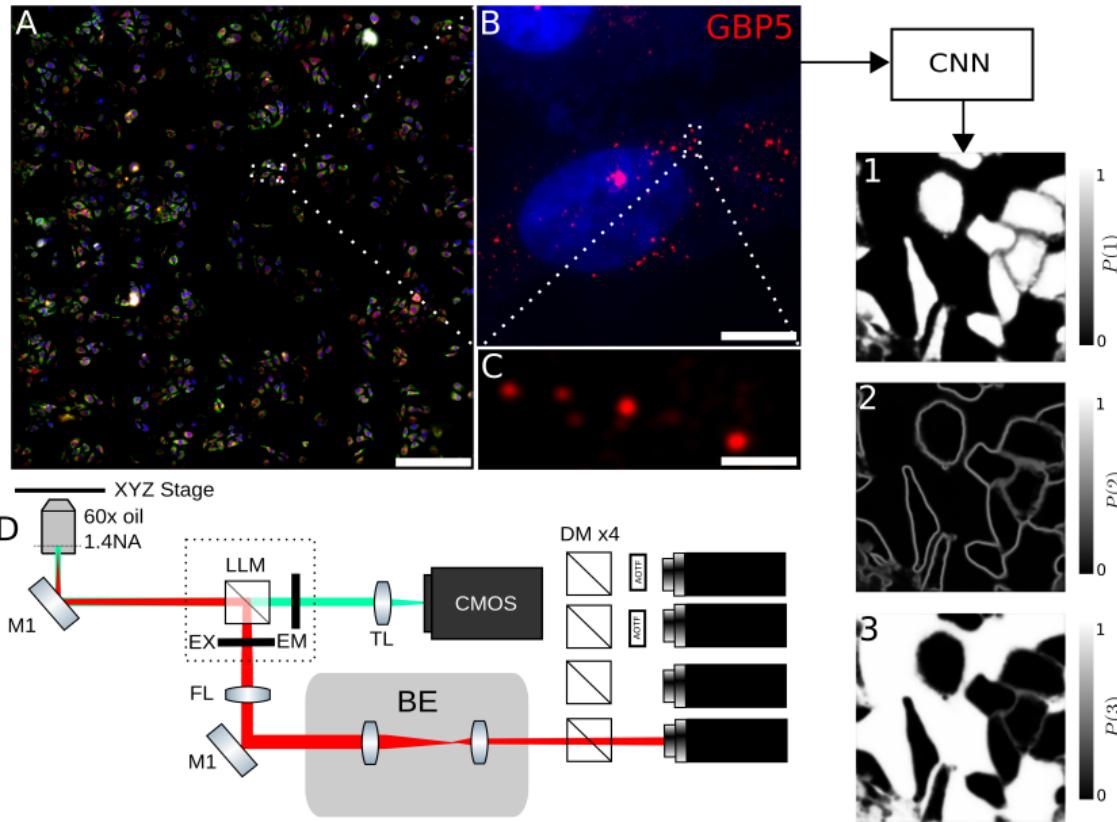
# Bayesian clustering algorithms on high density data

Bayesian nonparametrics in general

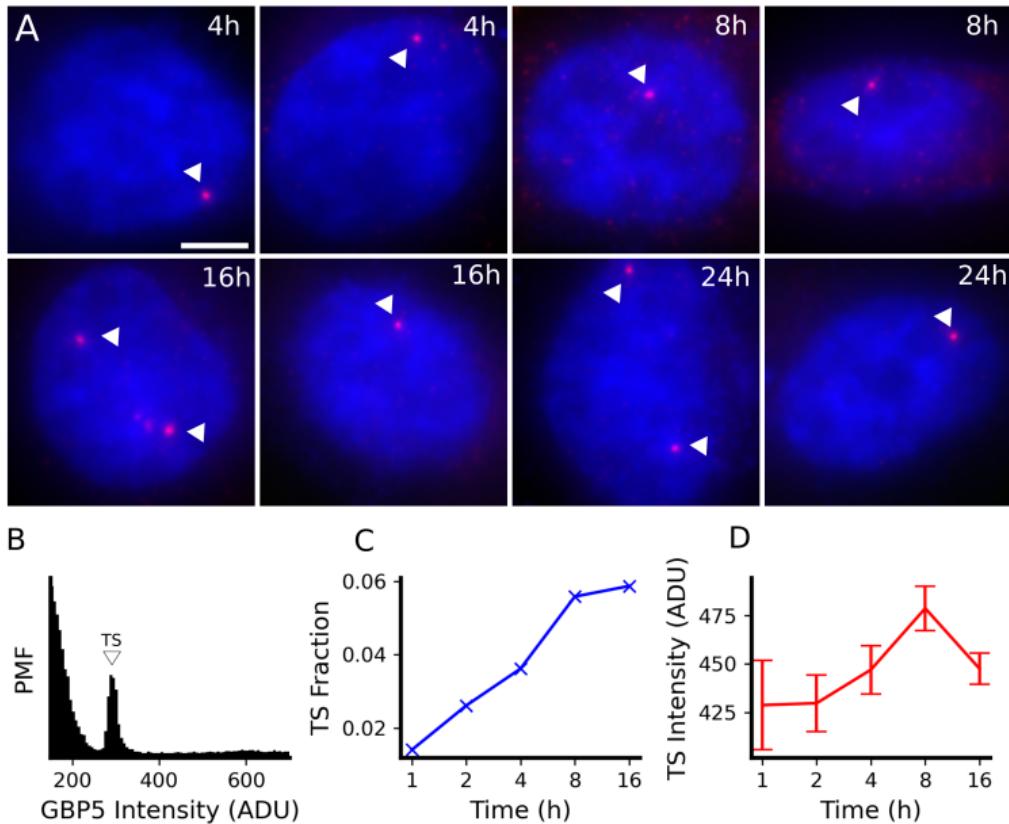
# Bayesian clustering algorithms on high density data

Bayesian nonparametrics

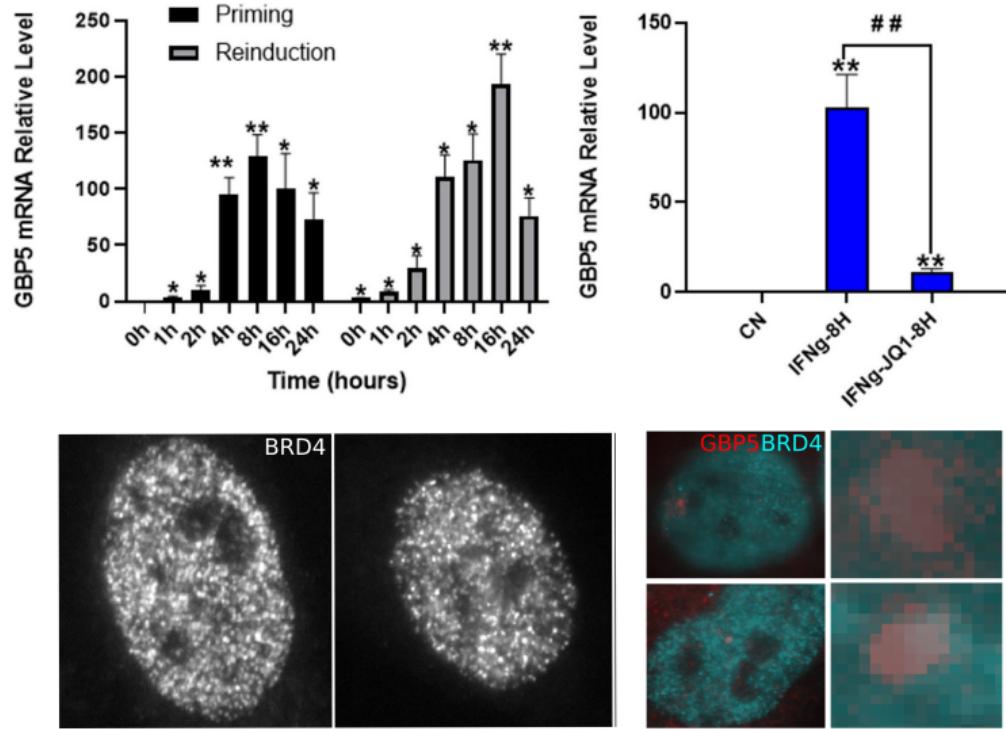
# Inducing GBP5 gene expression with Interferon- $\gamma$



# Colocalization of nascent GBP5 mRNA with phase separation markers



# Colocalization of nascent GBP5 mRNA with phase separation markers



## Costaining of H2B/BRD4/MED1 in interphase HeLa cells

## Cluster analysis of H2B at putative transcriptional condensates

# Physical cluster analysis of H2B at putative transcriptional condensates