Greater than the sum of the parts: Learning relationships between histone modifications in single cells

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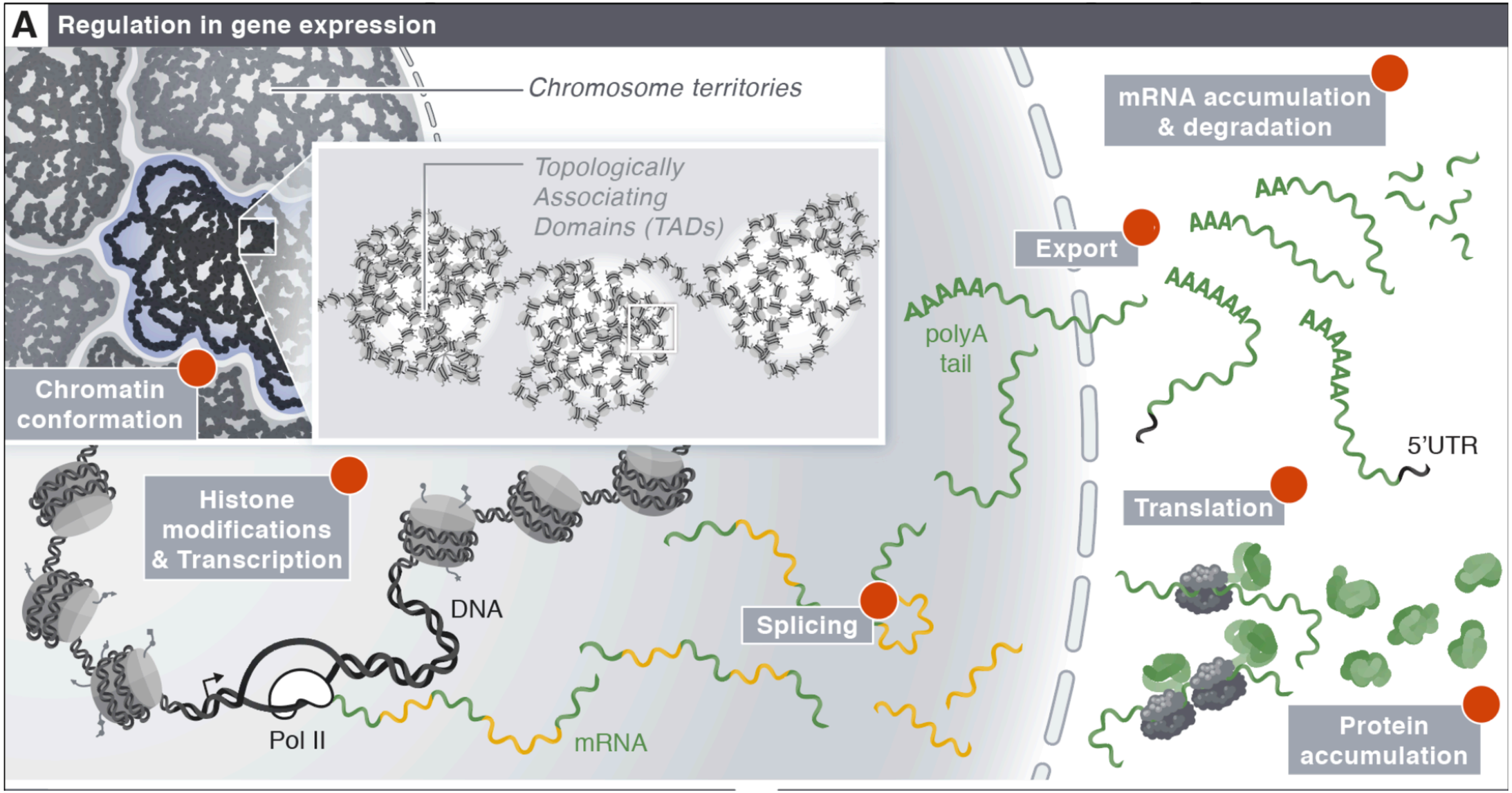
PhD 2019 EPFL
HFSP Fellow 2019-2022 at Hubrecht, Sanger, ISTA
Incoming Group Leader (2024): Genentech

Monday July 3, 2023
BIRS : Data Science Challenges in Single-Cell Research

Single-cell epigenomics is increasingly multimodal, can we infer relationships between modalities?



Adapted from Mermet, Yeung, Naef, 2017 *Cold Spring Harbor Perspectives*

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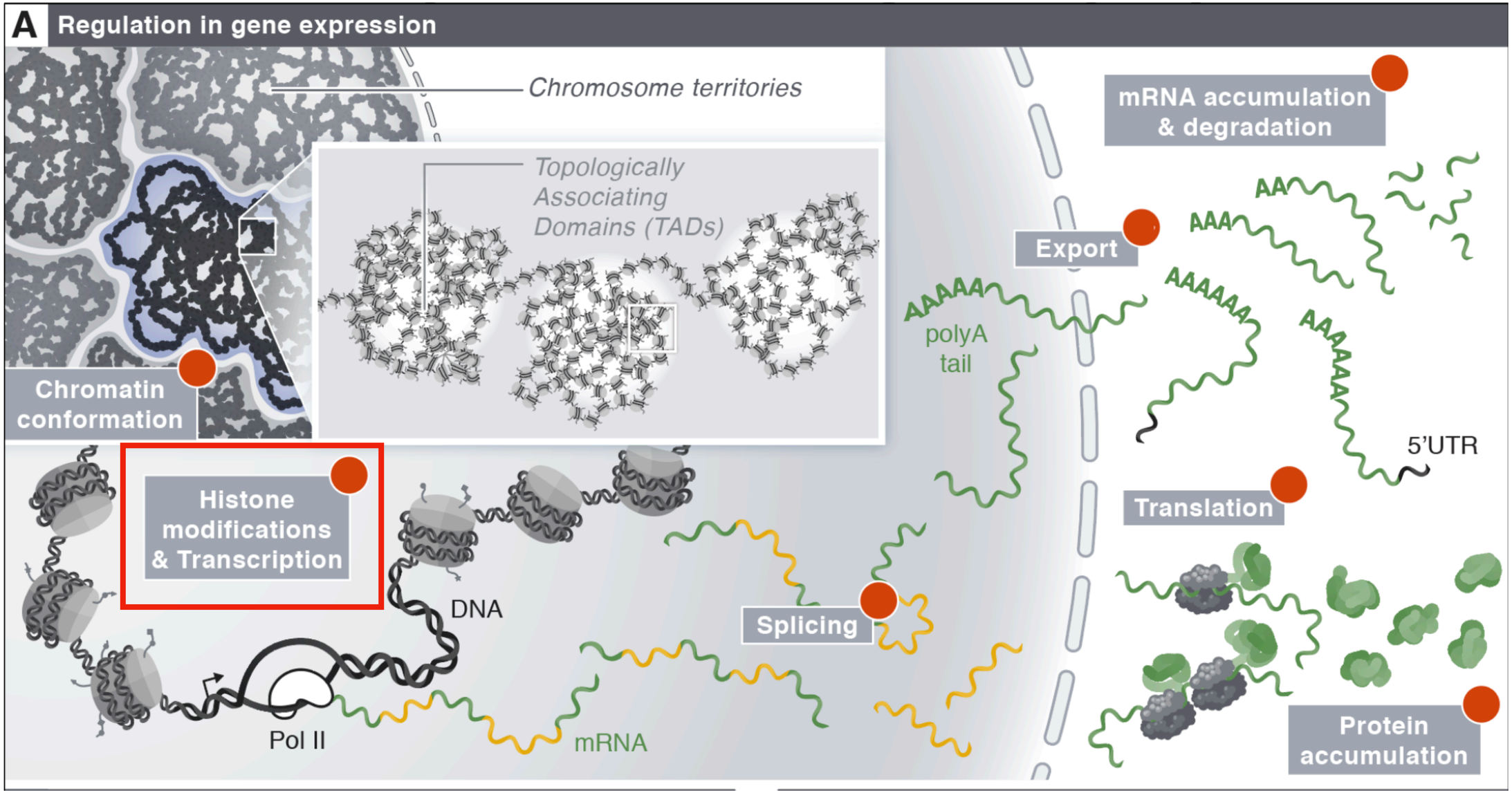

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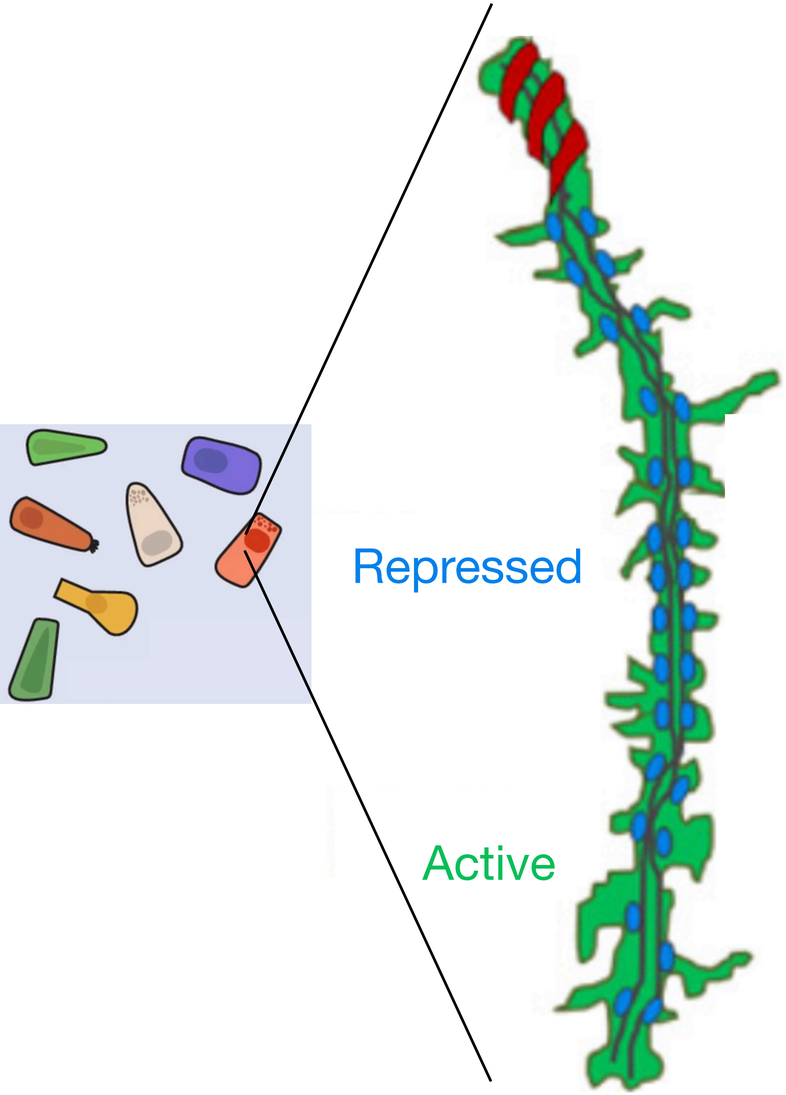
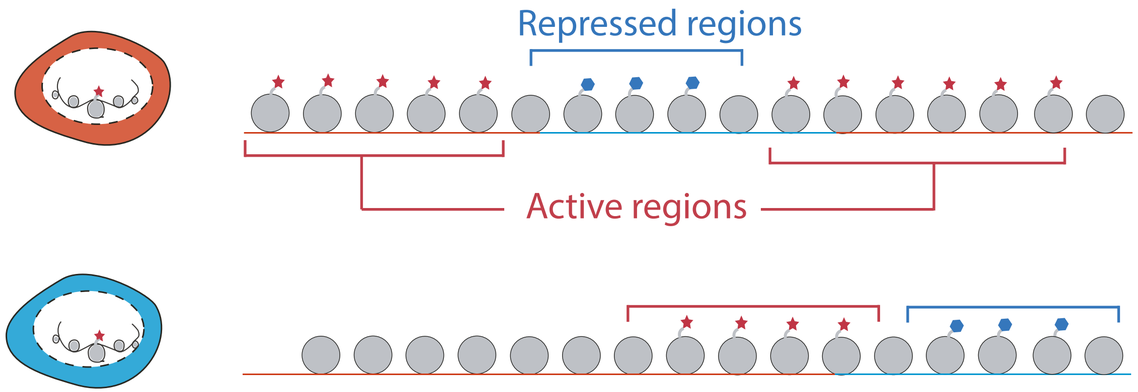
Most chromatin profiling techniques measure only one histone modification per cell





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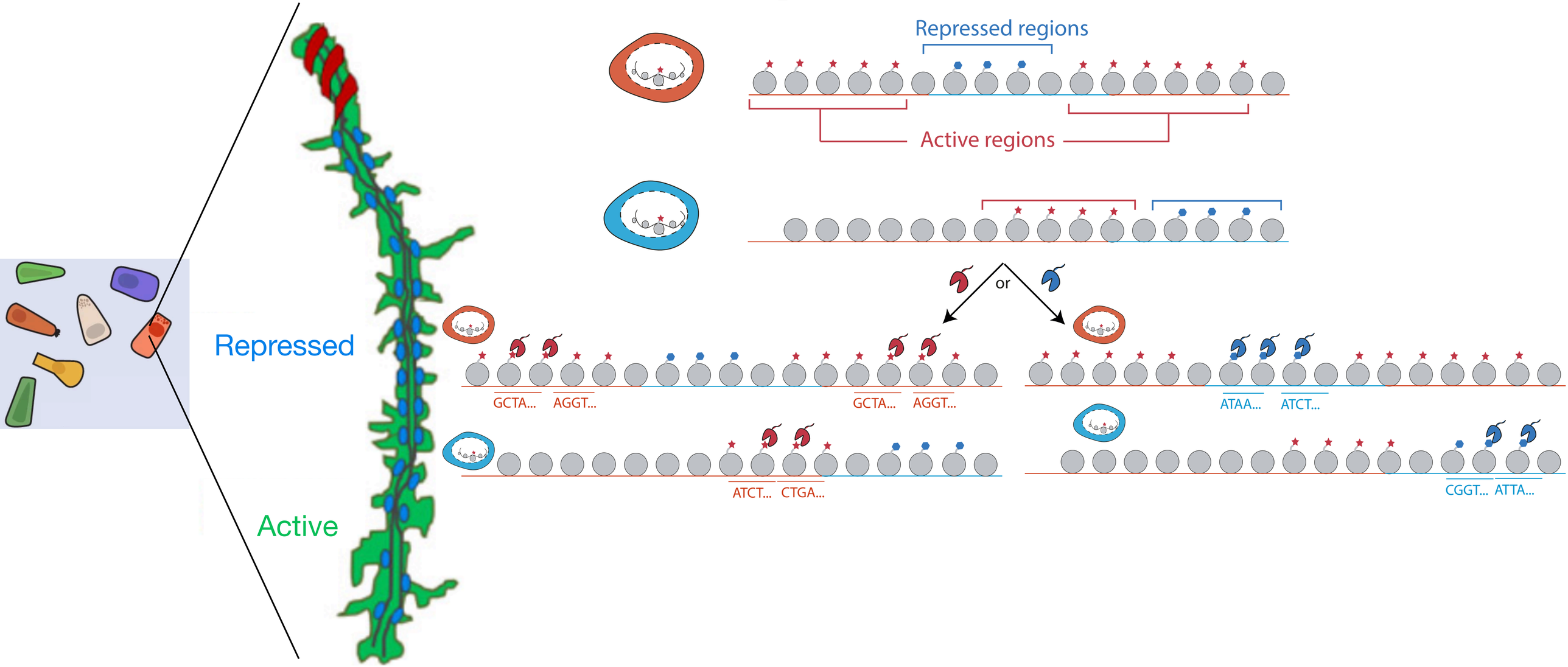

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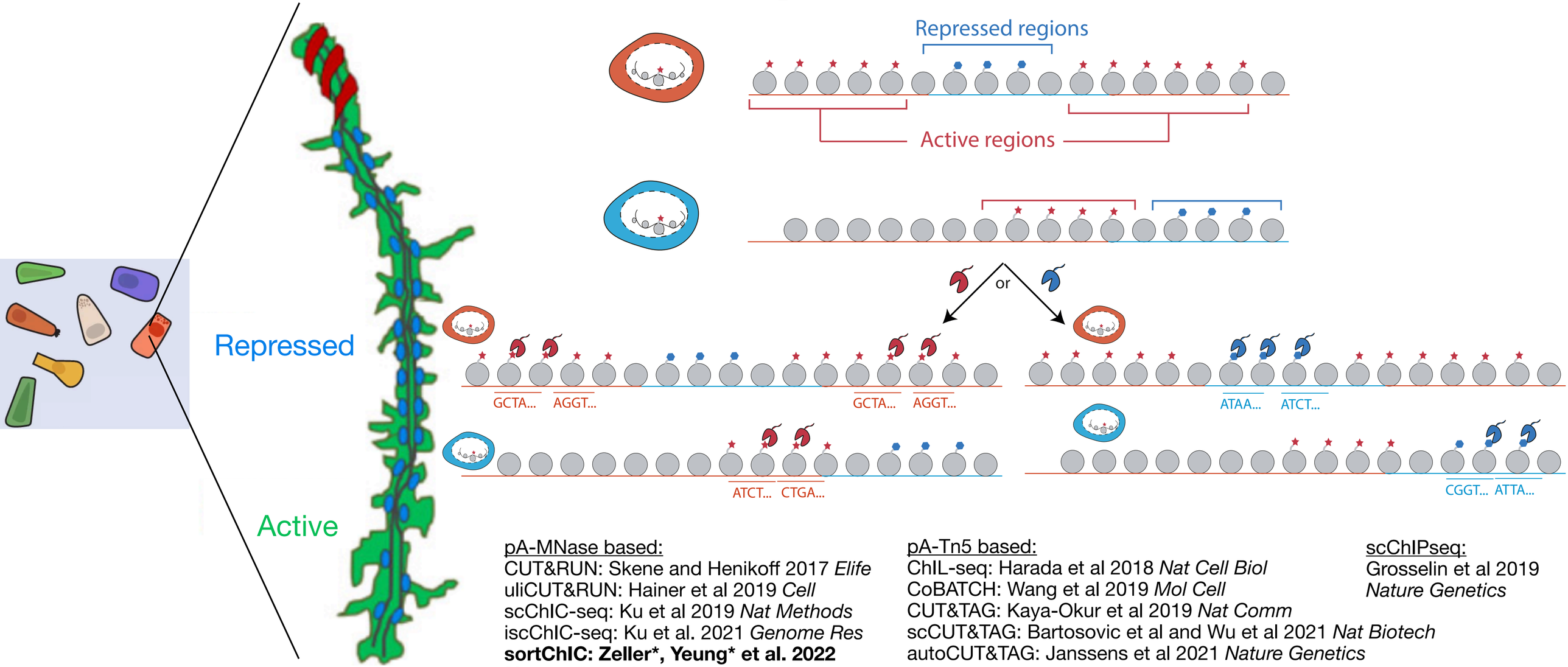


pA-MNase based:
CUT&RUN: Skene and Henikoff 2017 *Elife*
uliCUT&RUN: Hainer et al 2019 *Cell*
scChIC-seq: Ku et al 2019 *Nat Methods*
iscChIC-seq: Ku et al. 2021 *Genome Res*
sortChIC: Zeller*, Yeung* et al. 2022 *Nature Genetics*

pA-Tn5 based:
ChIL-seq: Harada et al 2018 *Nat Cell Biol*
CoBATCH: Wang et al 2019 *Mol Cell*
CUT&TAG: Kaya-Okur et al 2019 *Nat Comm*
scCUT&TAG: Bartosovic et al and Wu et al 2021 *Nat Biotech*
autoCUT&TAG: Janssens et al 2021 *Nature Genetics*

scChIPseq:
Grosselin et al 2019 *Nature Genetics*

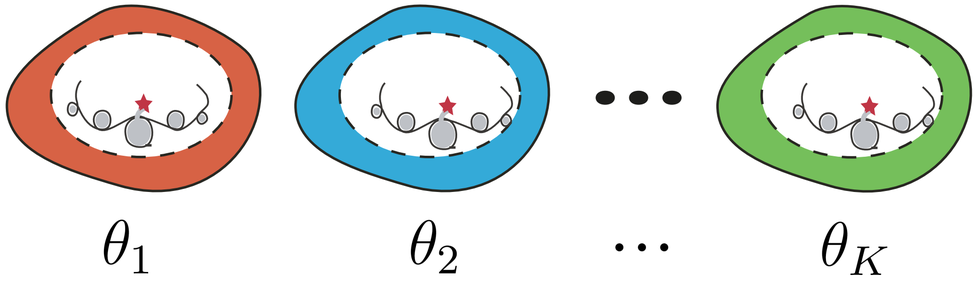
For single-modality analysis, latent Dirichlet allocation (LDA) is a natural way to model sparse counts

To generate a cut location $w_{d,n}$ in cell d for the nth read:

1) Choose a latent variable (topic)

$$z_{d,n} \sim \text{Multinomial}\left(1, \vec{\theta}_d\right)$$

Latent factors



See also:
Structure from Pritchard, Stephens, Donnelly 2000
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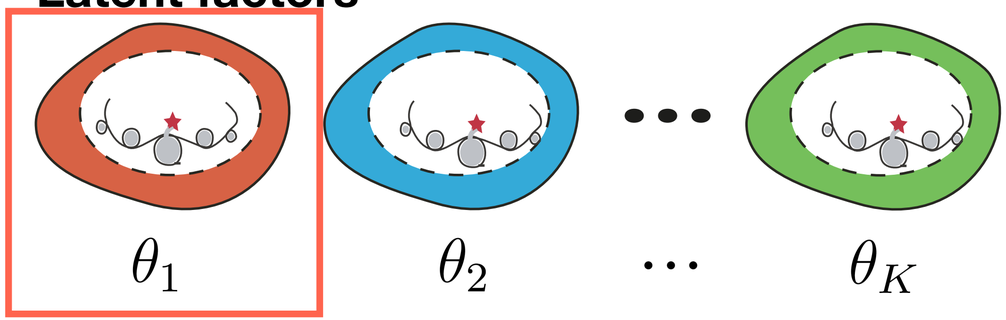

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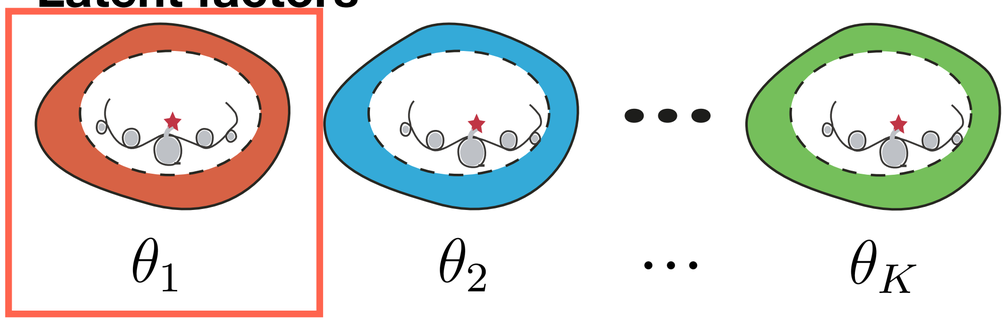

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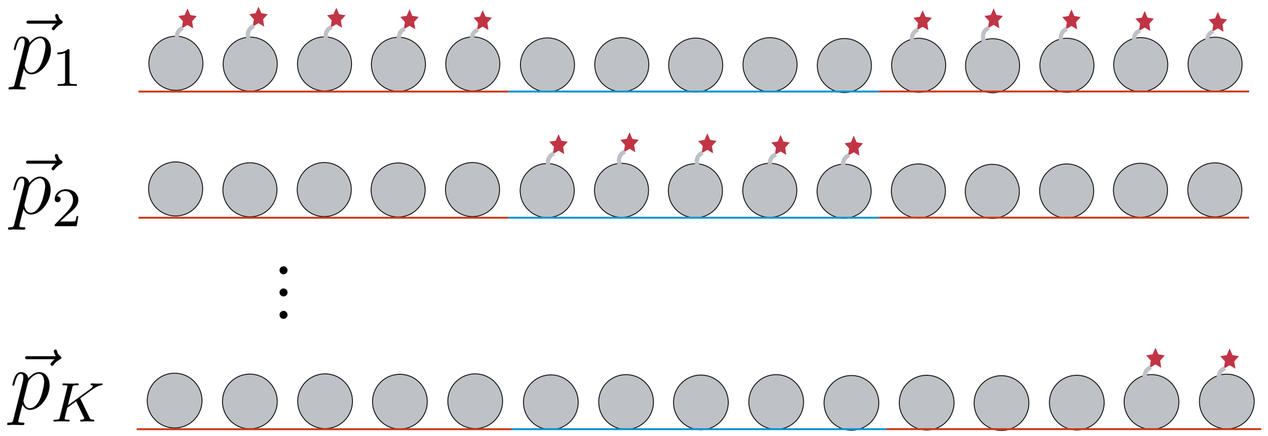

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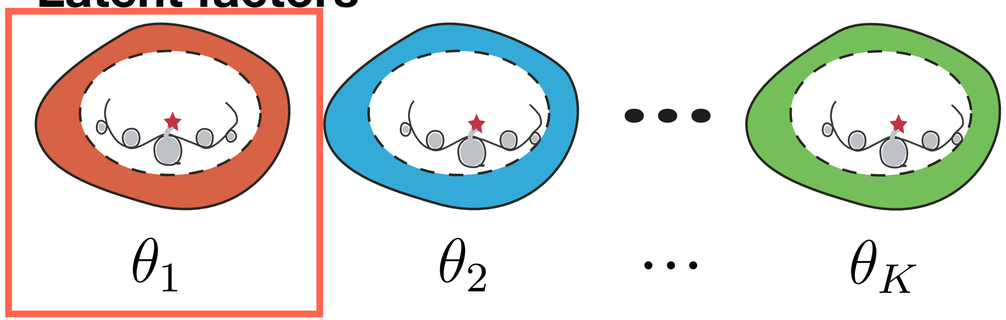

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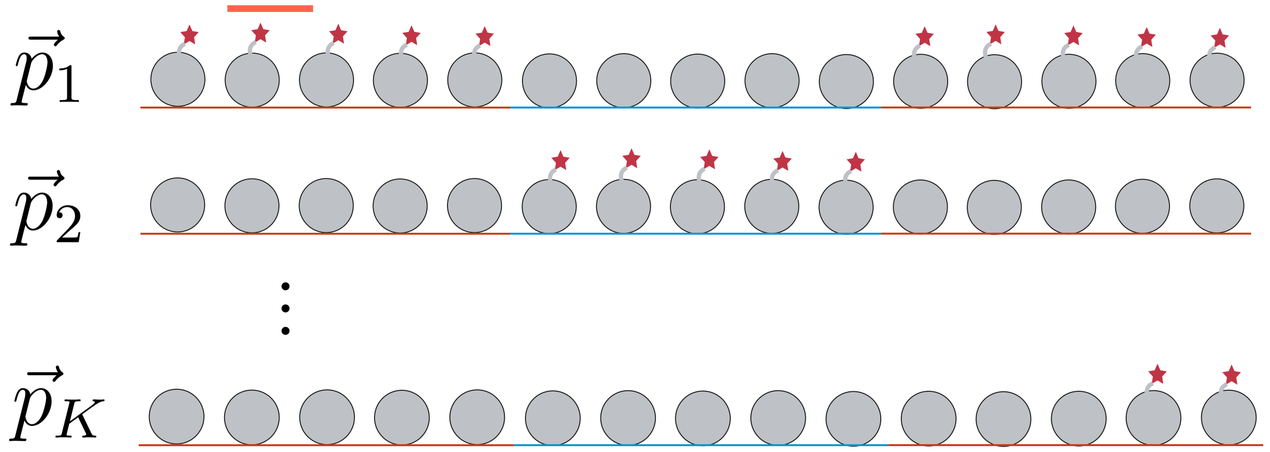

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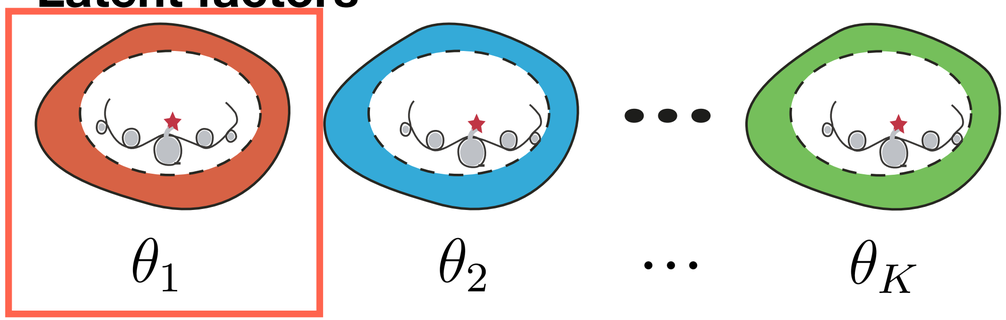

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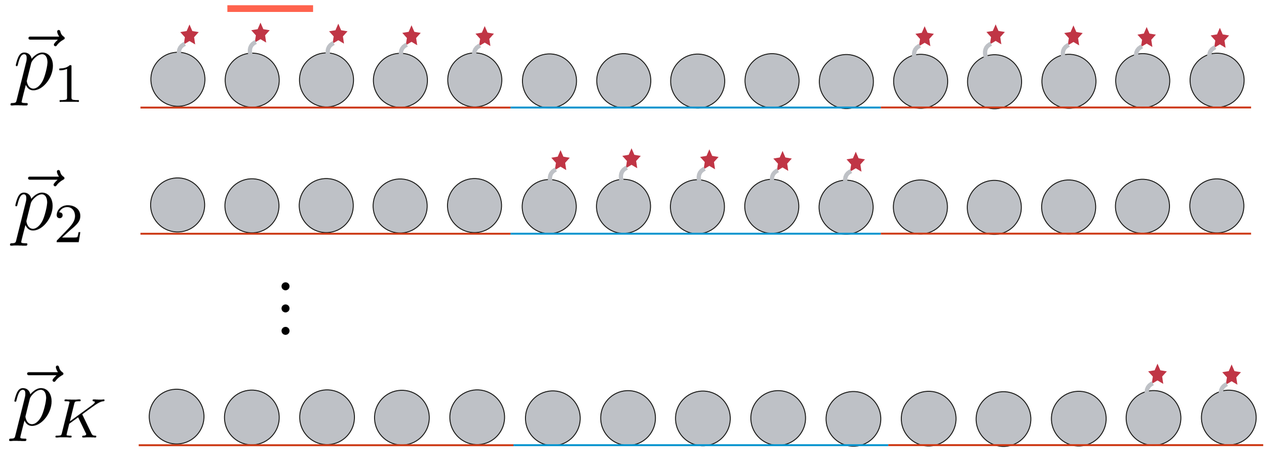

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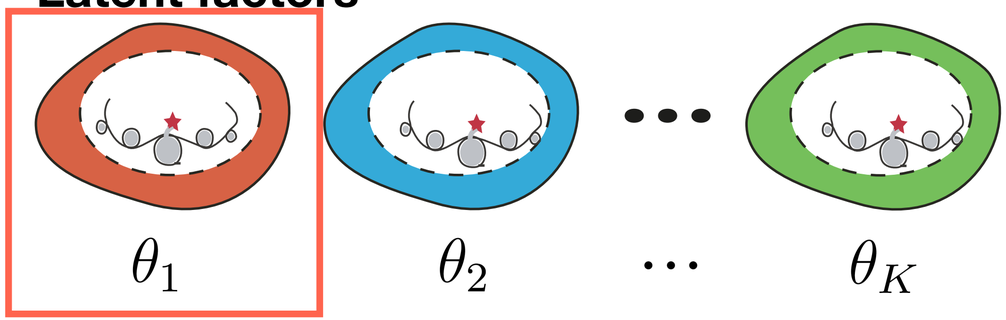

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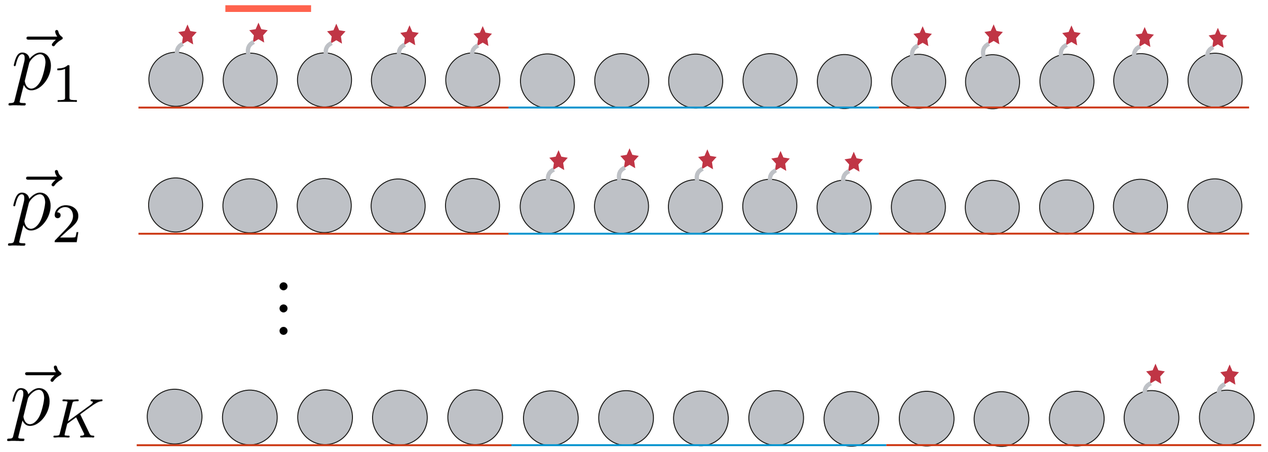

Parameters **θ** and **P** are inferred by collapsed Gibbs sampling

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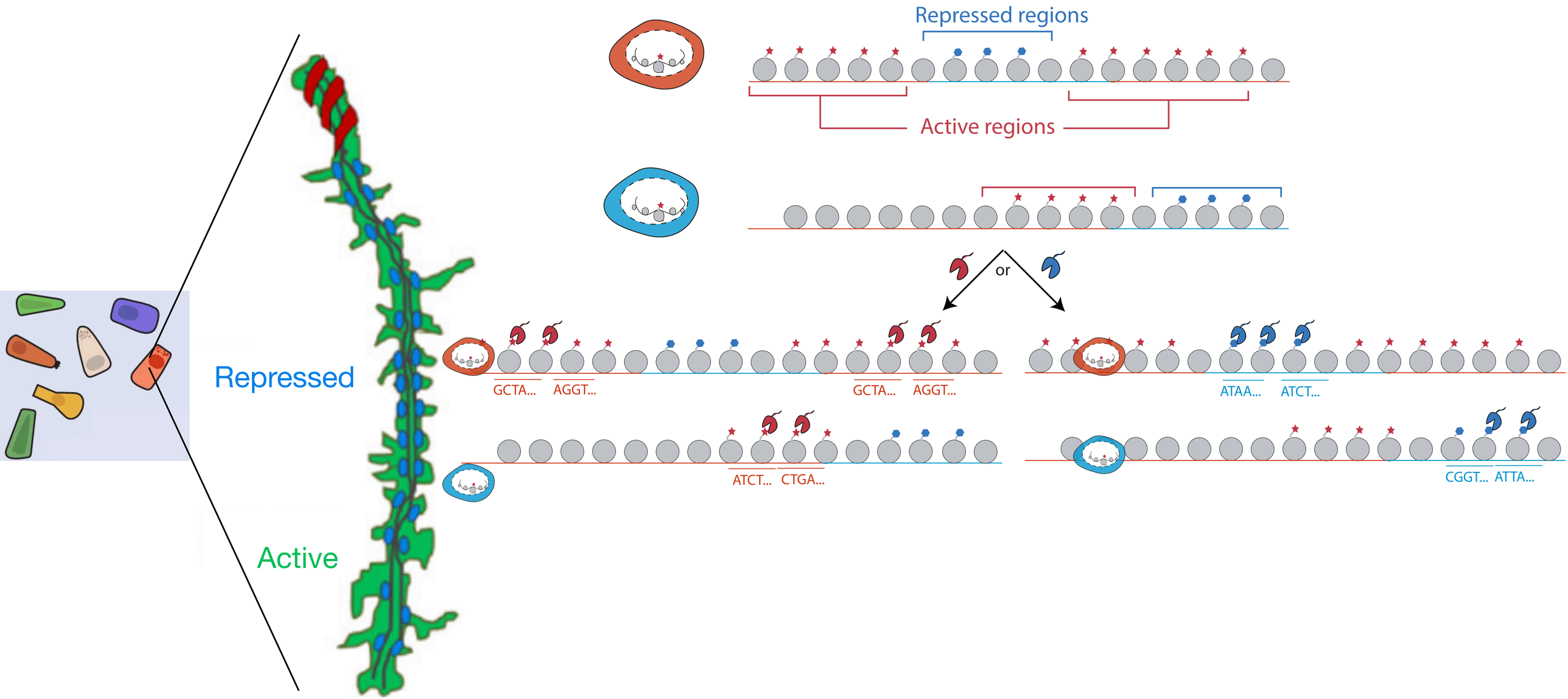
Can we go beyond one histone mark per cell?



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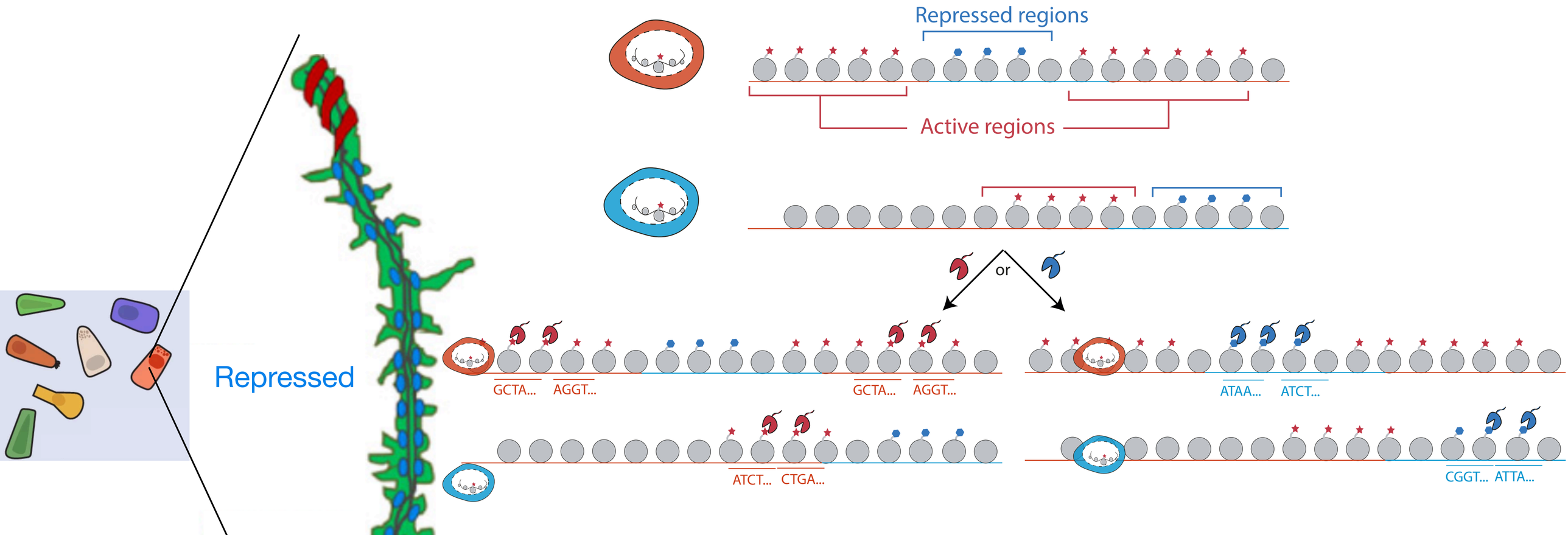

Experimental solutions (barcoding):
Gopalan et al 2021 *Mol Cell*
Meers et al 2022 *Nat Biotech*
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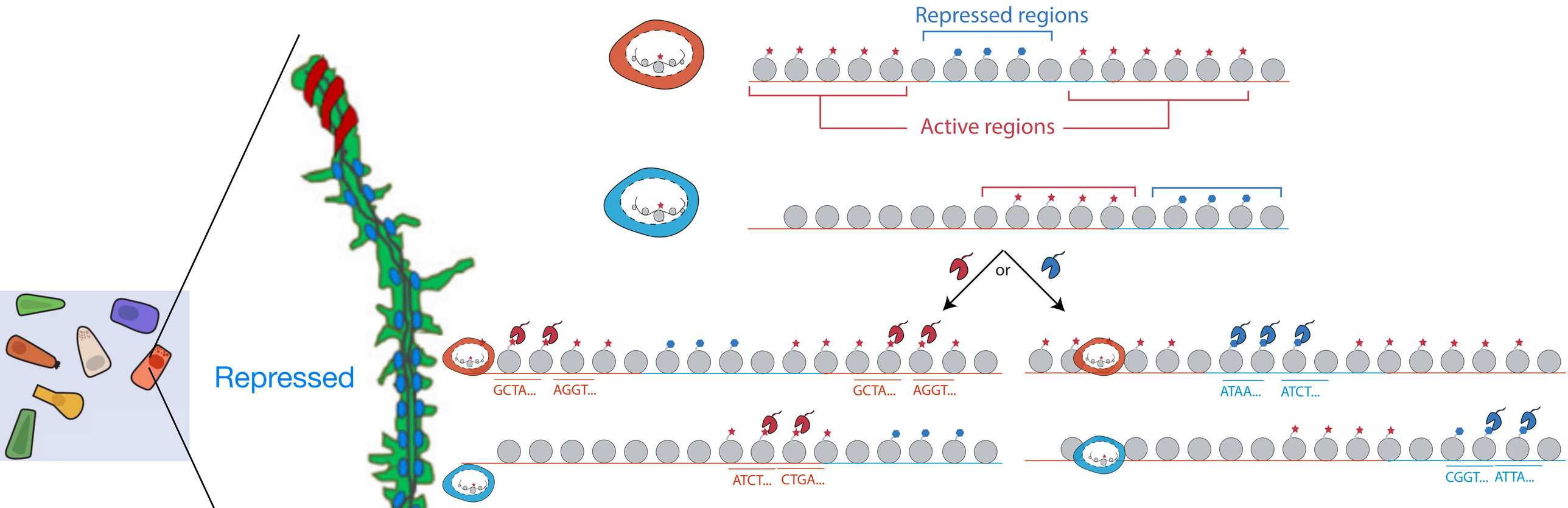

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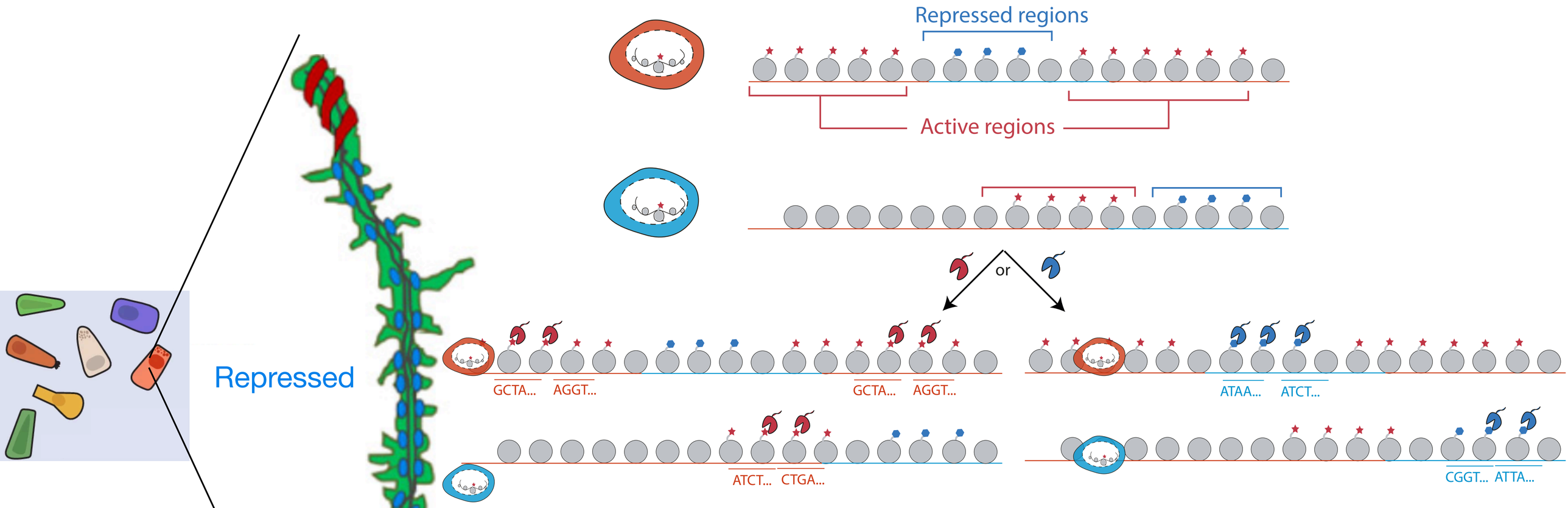

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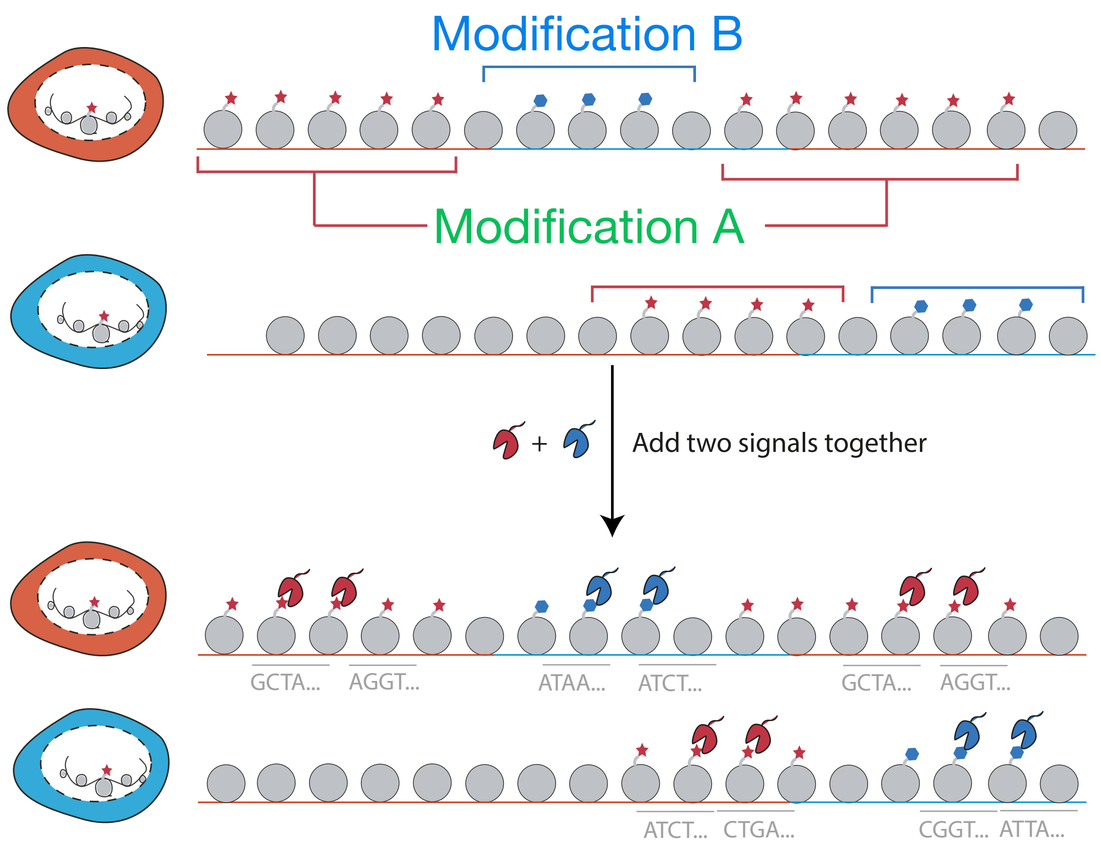
How can we extract unique insights from this multimodal data?

scChIX-seq multiplexes two histone modifications together, then deconvolves the mixed signal



scChIX-seq: chromatin immunocleavage and unmixing
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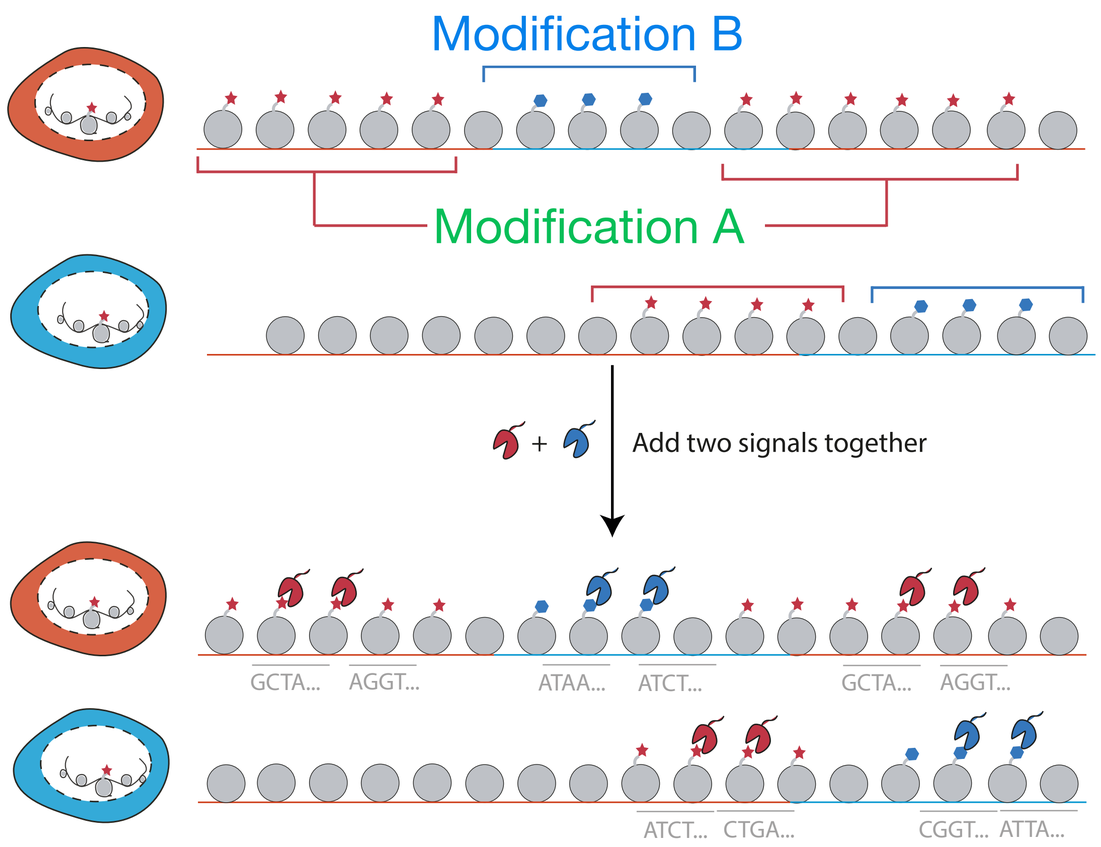

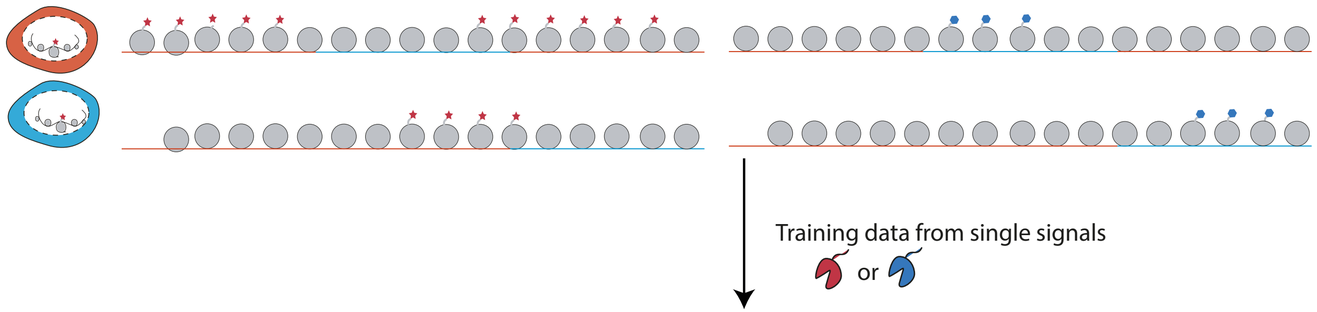

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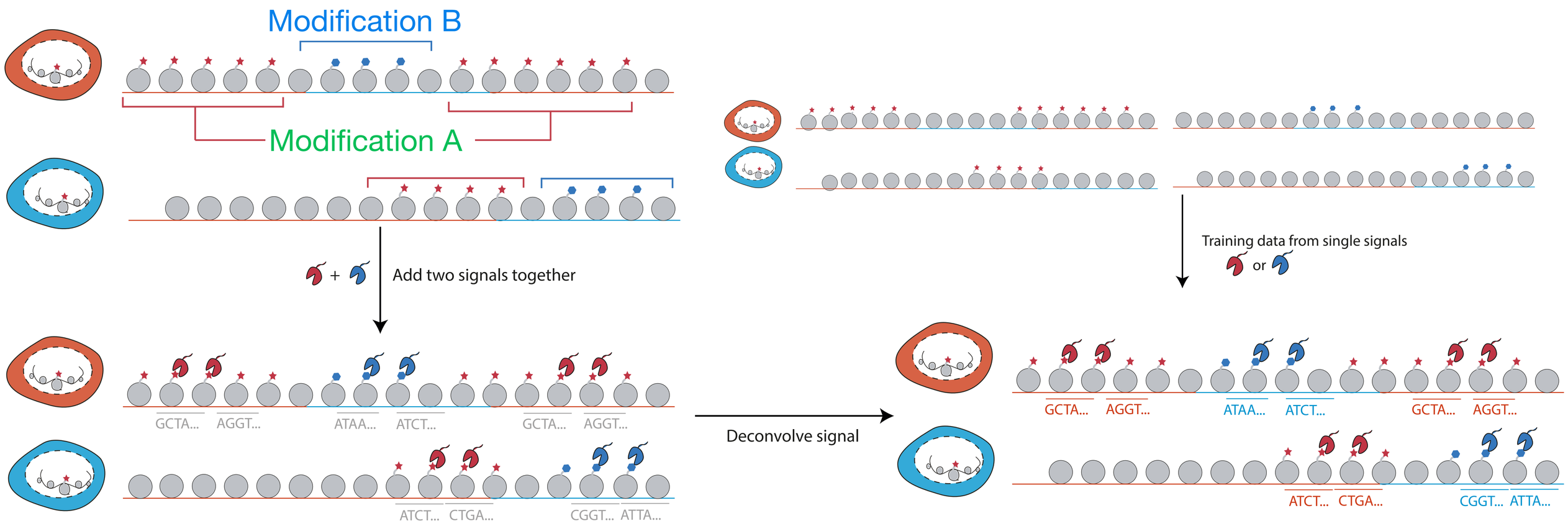

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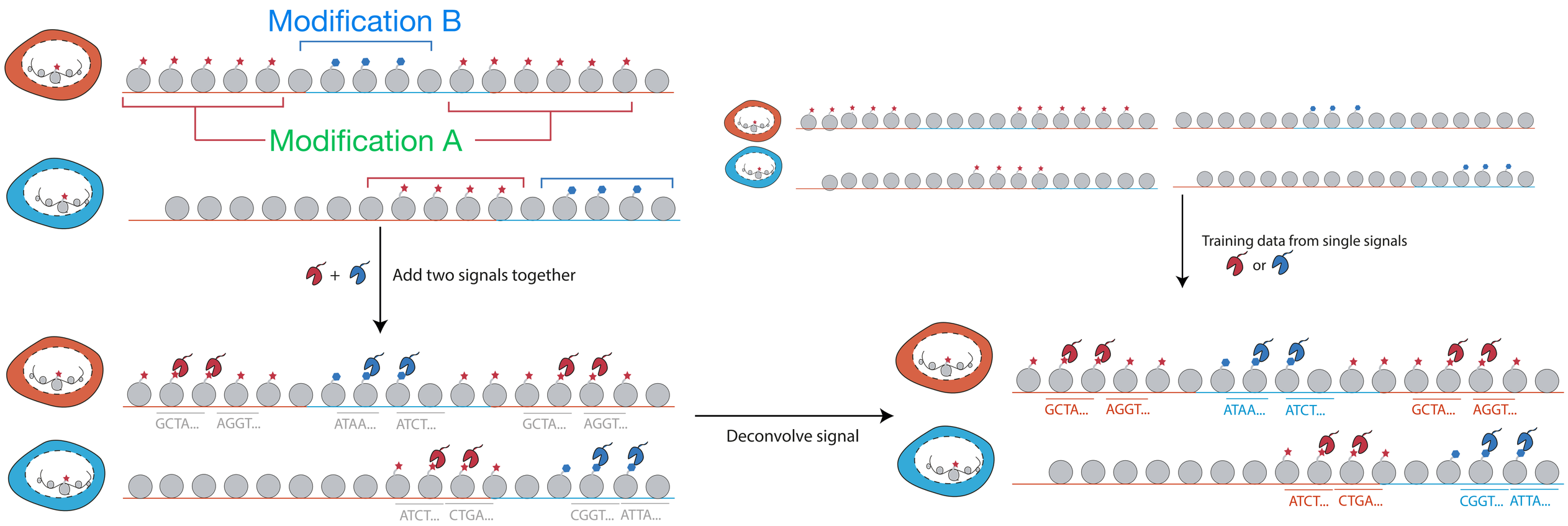

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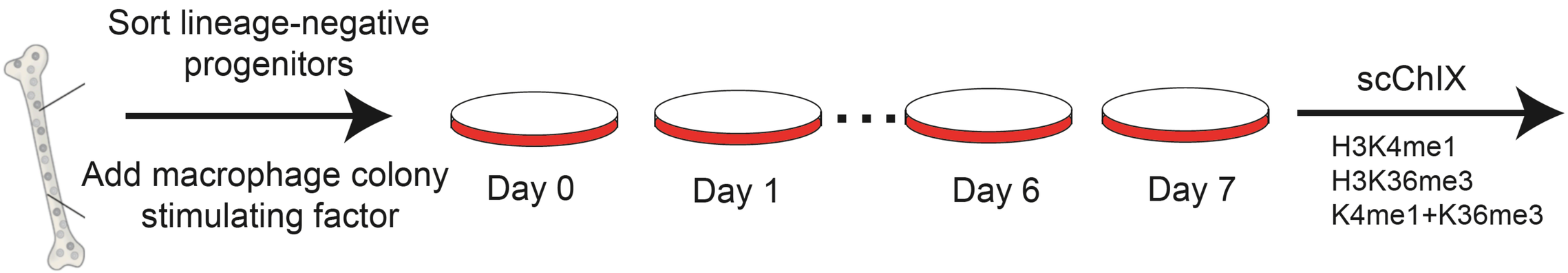
Extending multinomial models to allow linear combinations of profiles

Apply scChIX-seq to uncover dynamic relationships between two active histone marks



Experimentalists:

Maria Florescu

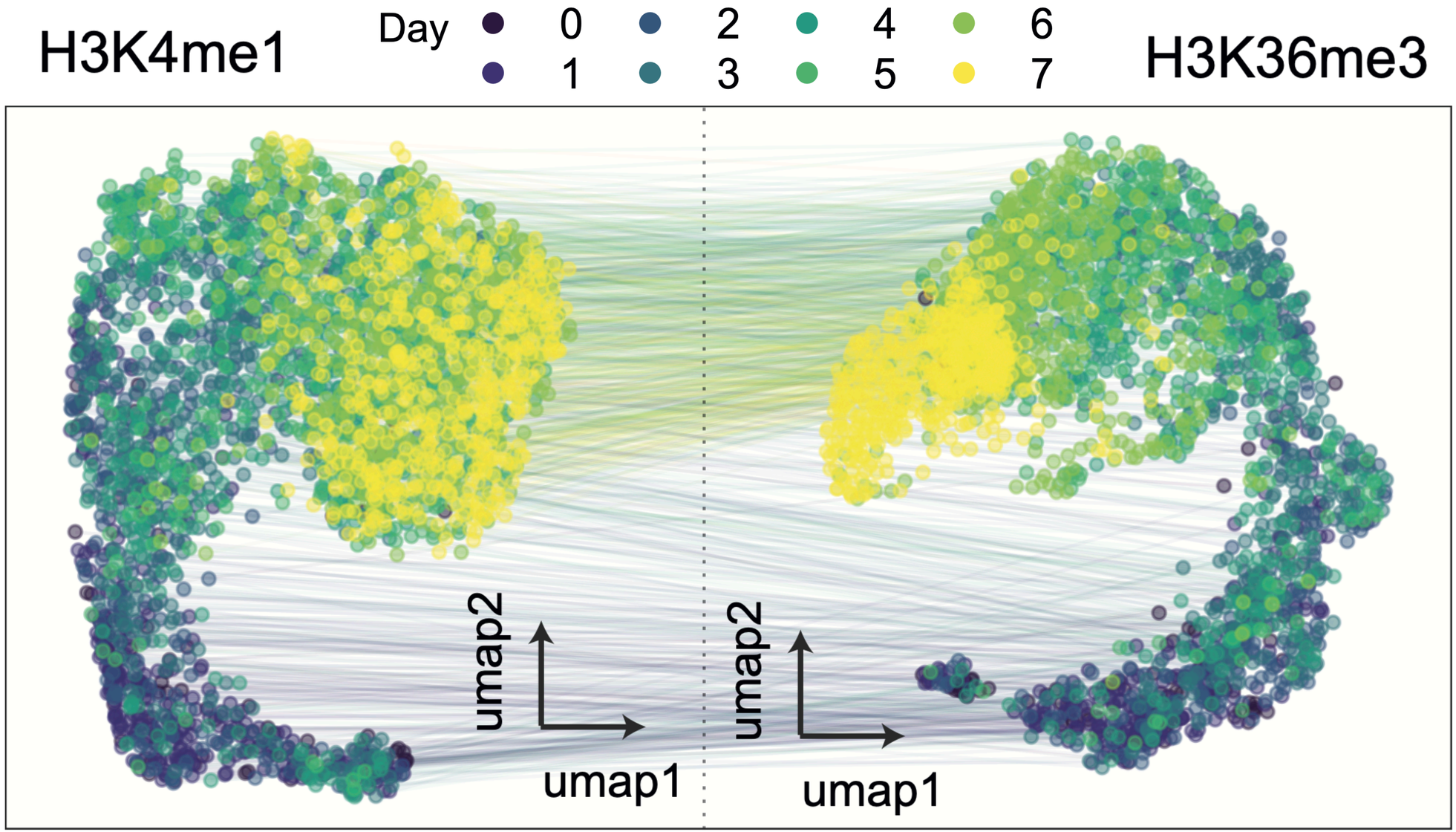
Max Wellenstein

Peter Zeller

Alexander van Oudenaarden group

H3K4me1: active and primed regions
H3K36me3: transcription

scChIX-seq connects H3K4me1 and H3K36me3 dynamics in single cells



UMAP of cell-cell relationship matrix

scChIX-seq can uncover relationships *between* histone modifications

Axis of variation:
histone modification B

Axis of variation:
histone modification A

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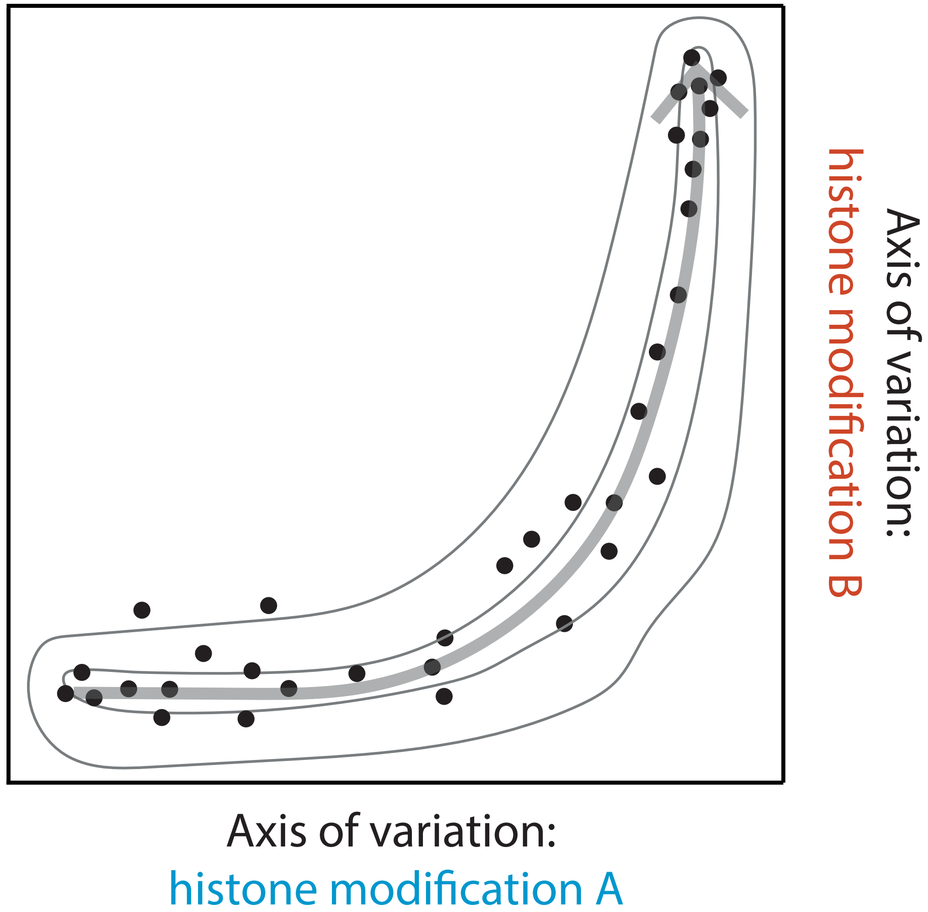

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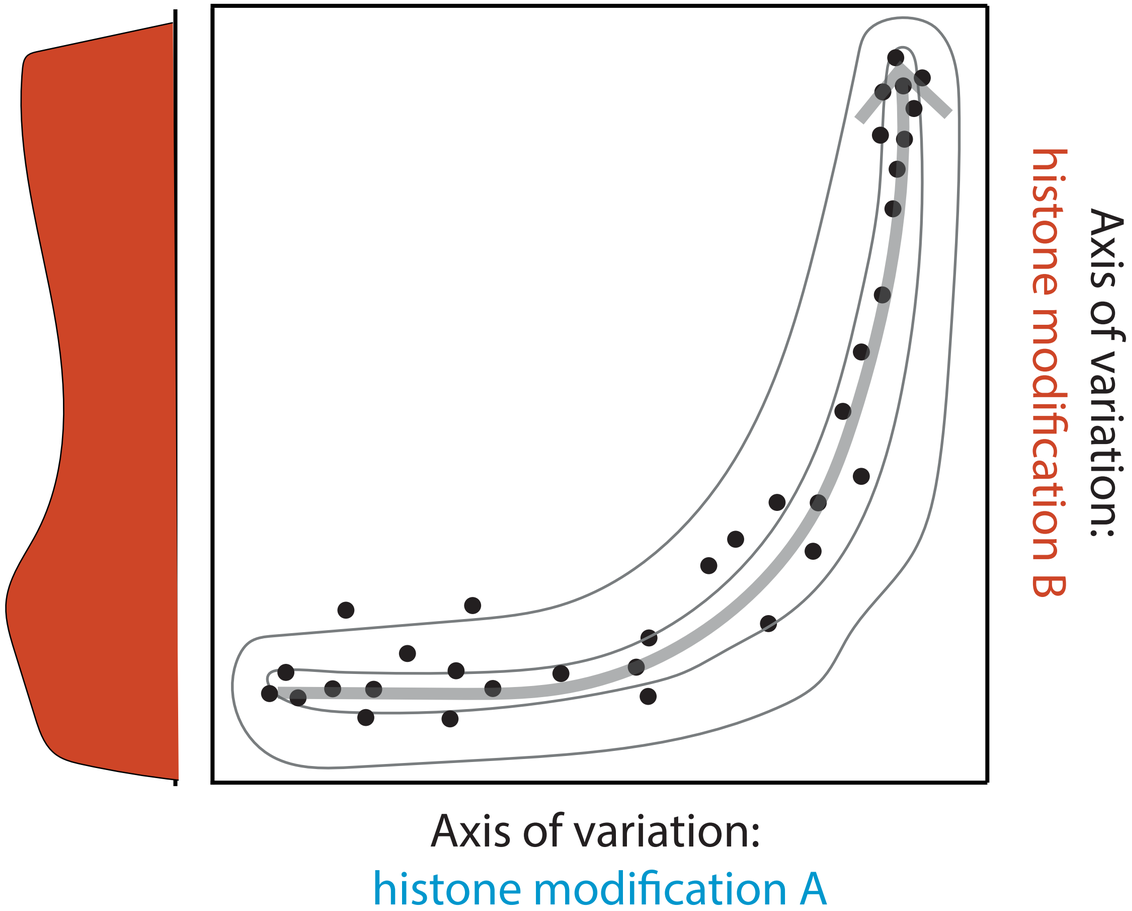

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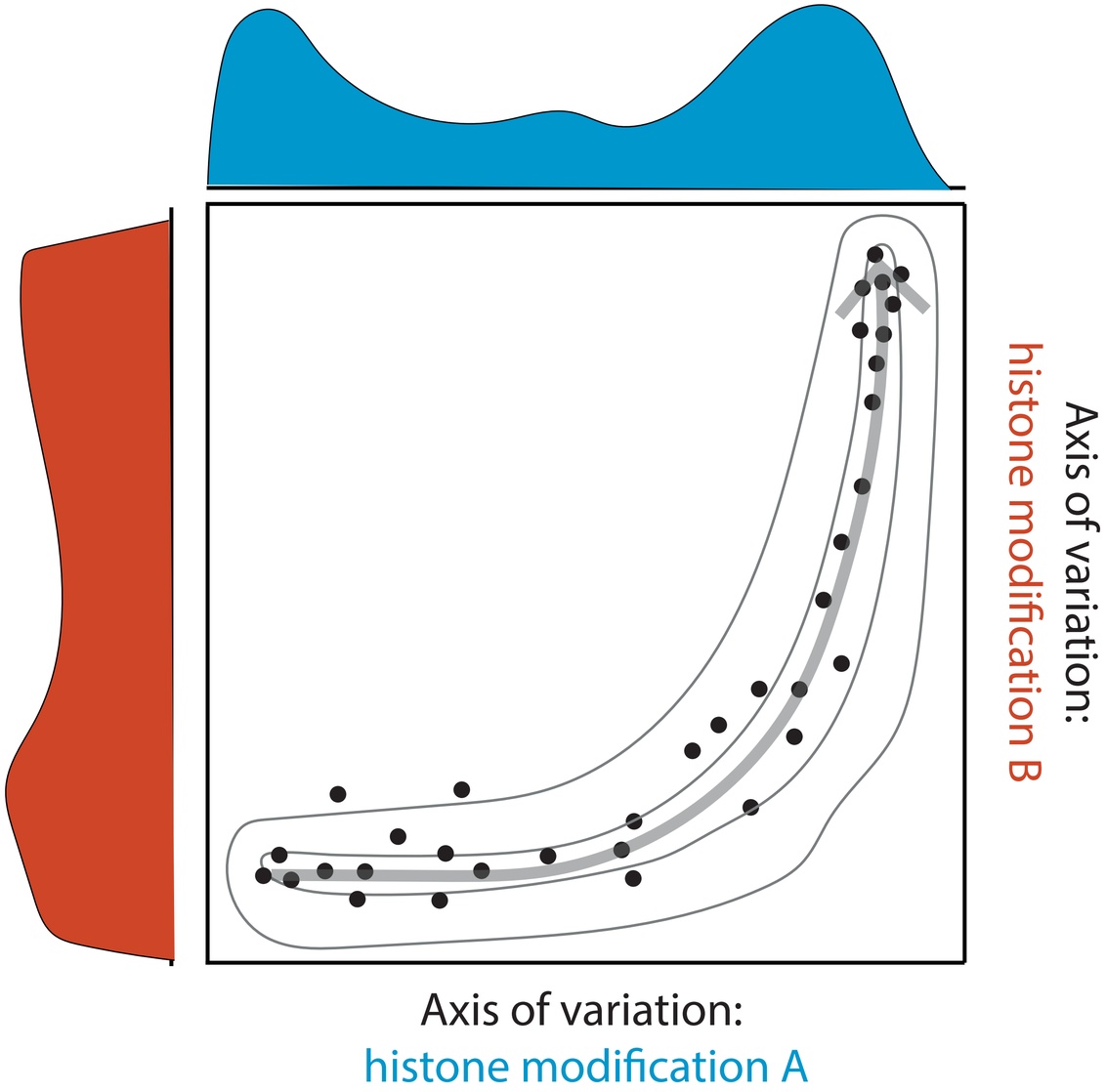

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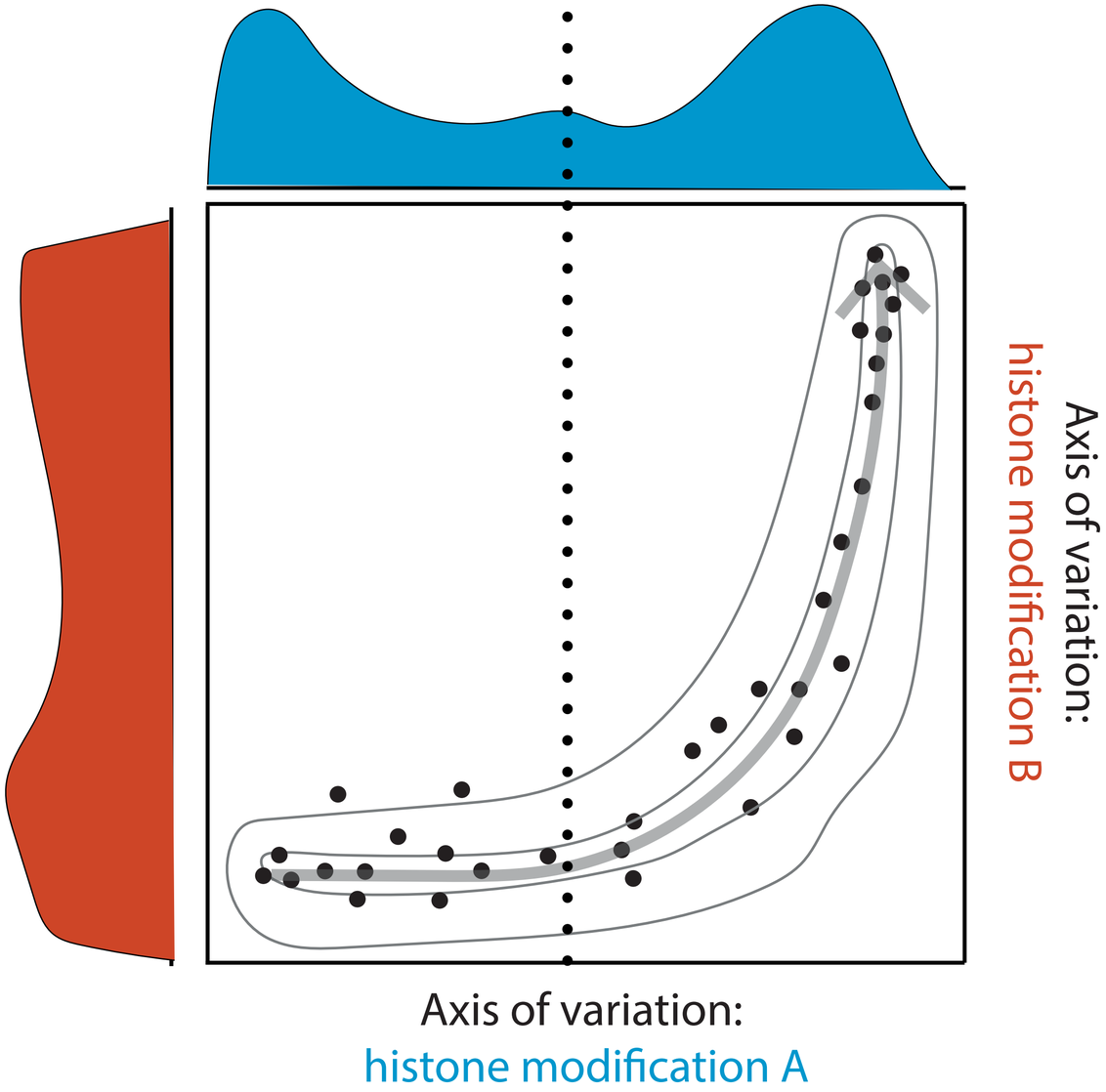

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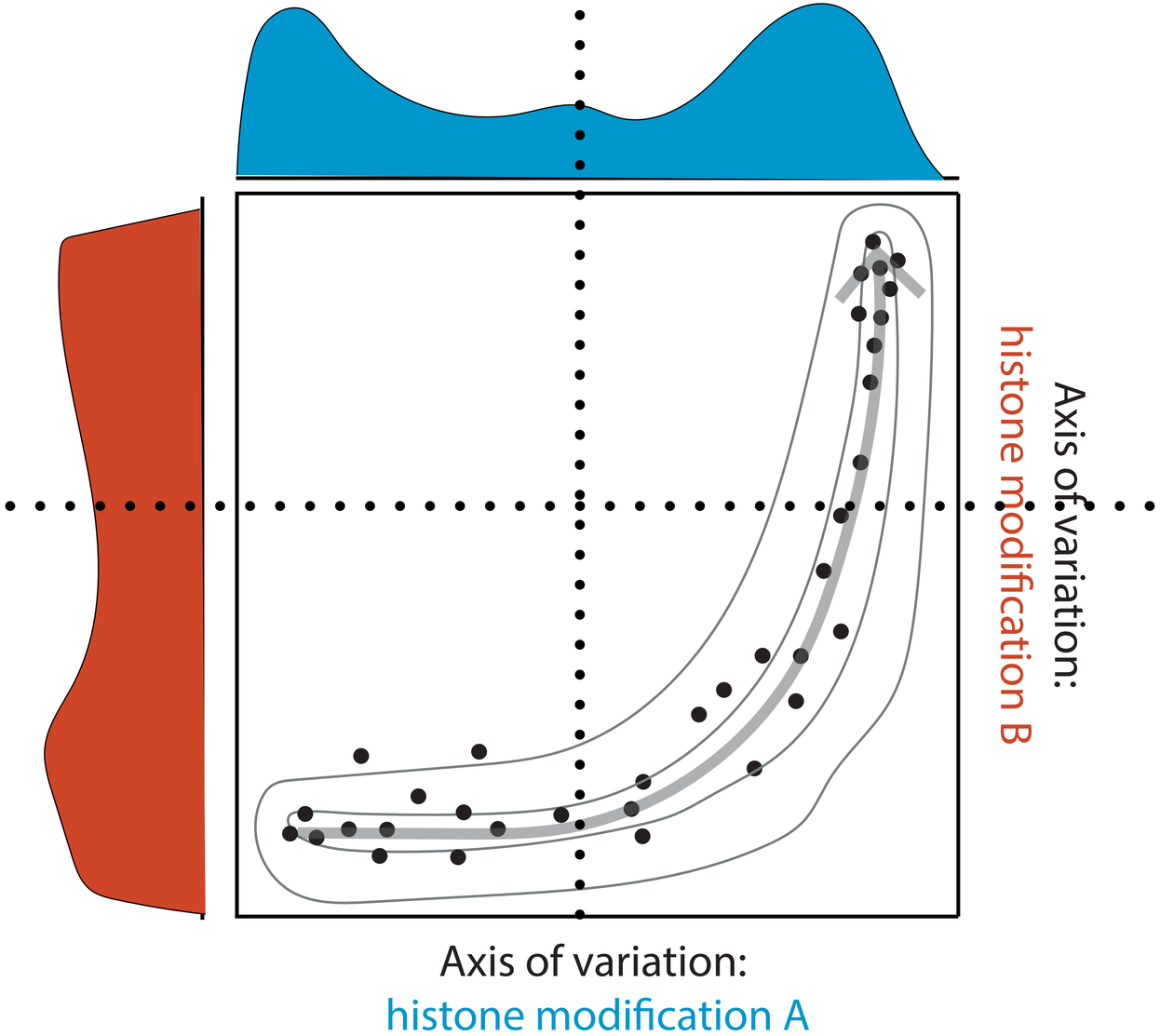

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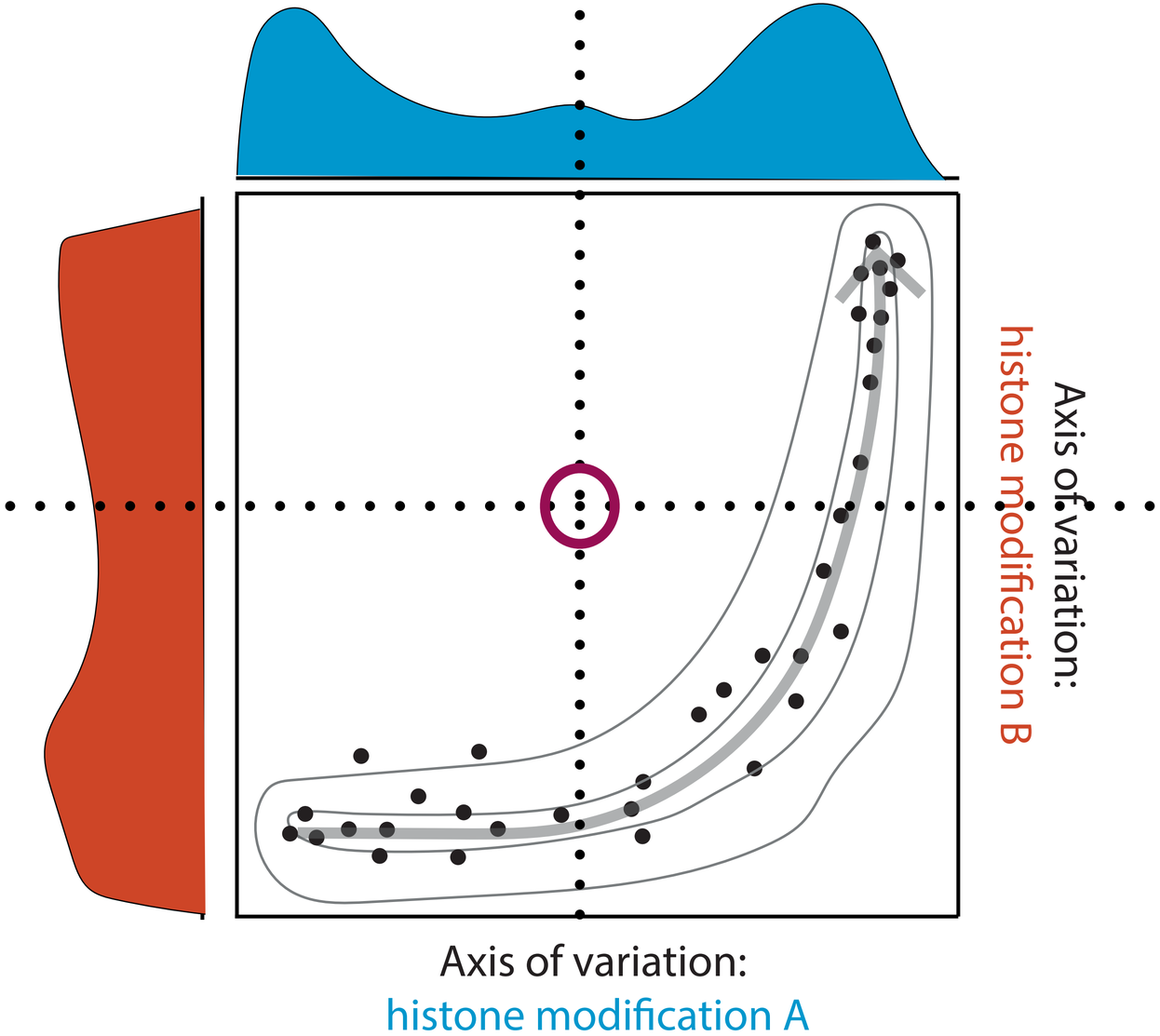
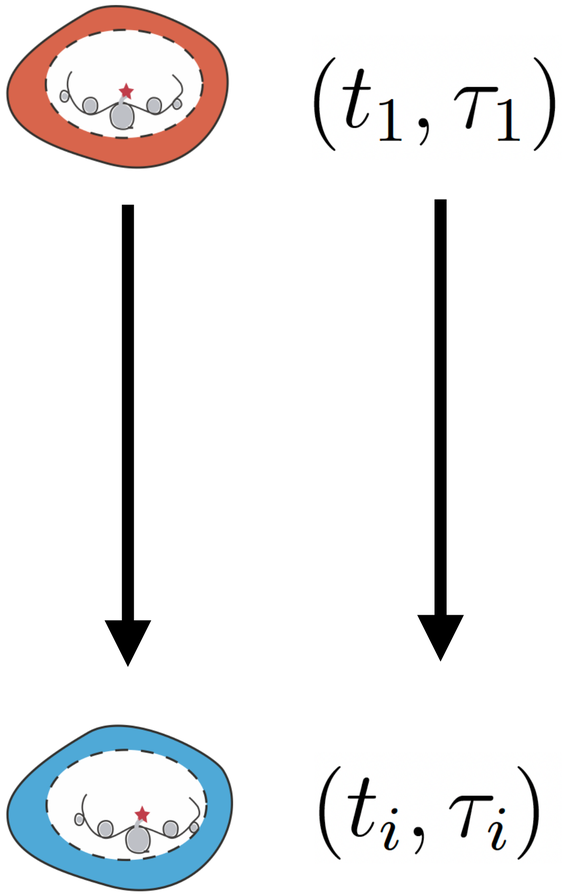


Inferring pseudotime along both H3K4me1 and H3K36me3 reveals distinct dynamics



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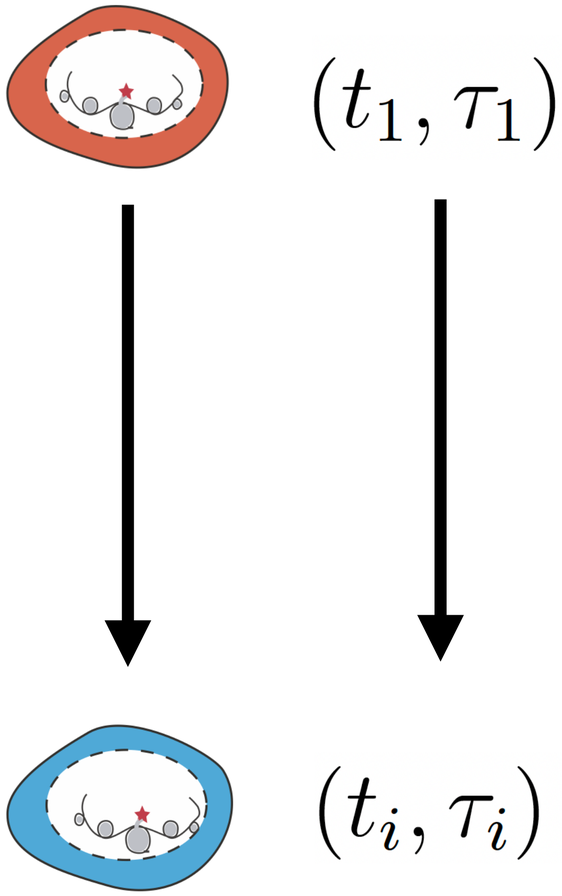

Find t and $\boldsymbol{\tau}$ that maximizes multinomial likelihood:

$$L(t, \tau) = \log\left(\Pr\left(\vec{y} | \vec{p}(t), \vec{q}(\tau), w\right)\right) \propto \sum_{g=1}^{G} y_g \log\left(w\vec{p}_g(t) + (1-w)\,\vec{q}_g(\tau)\right)$$

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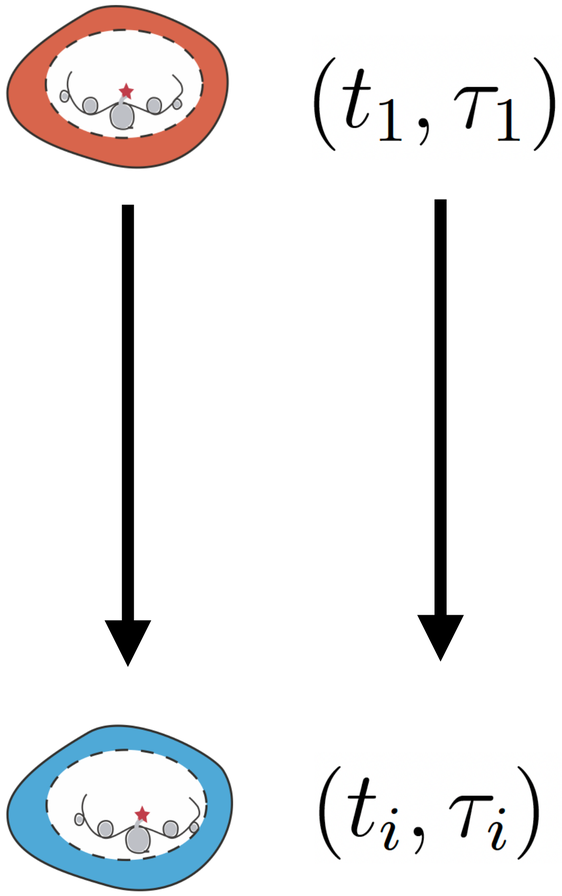

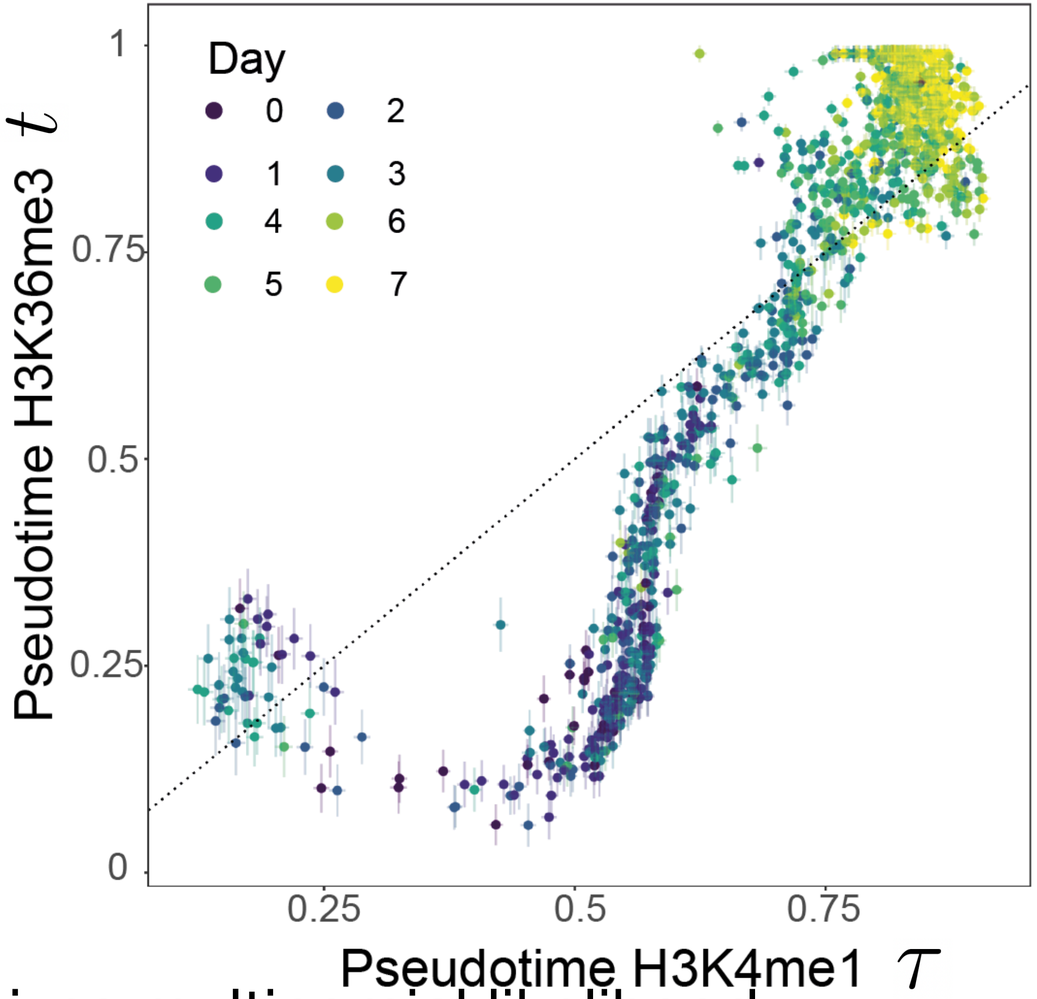

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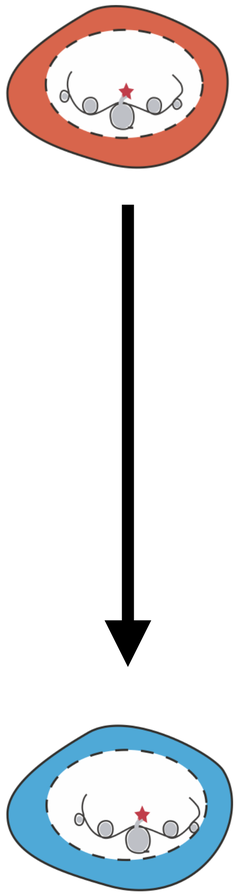
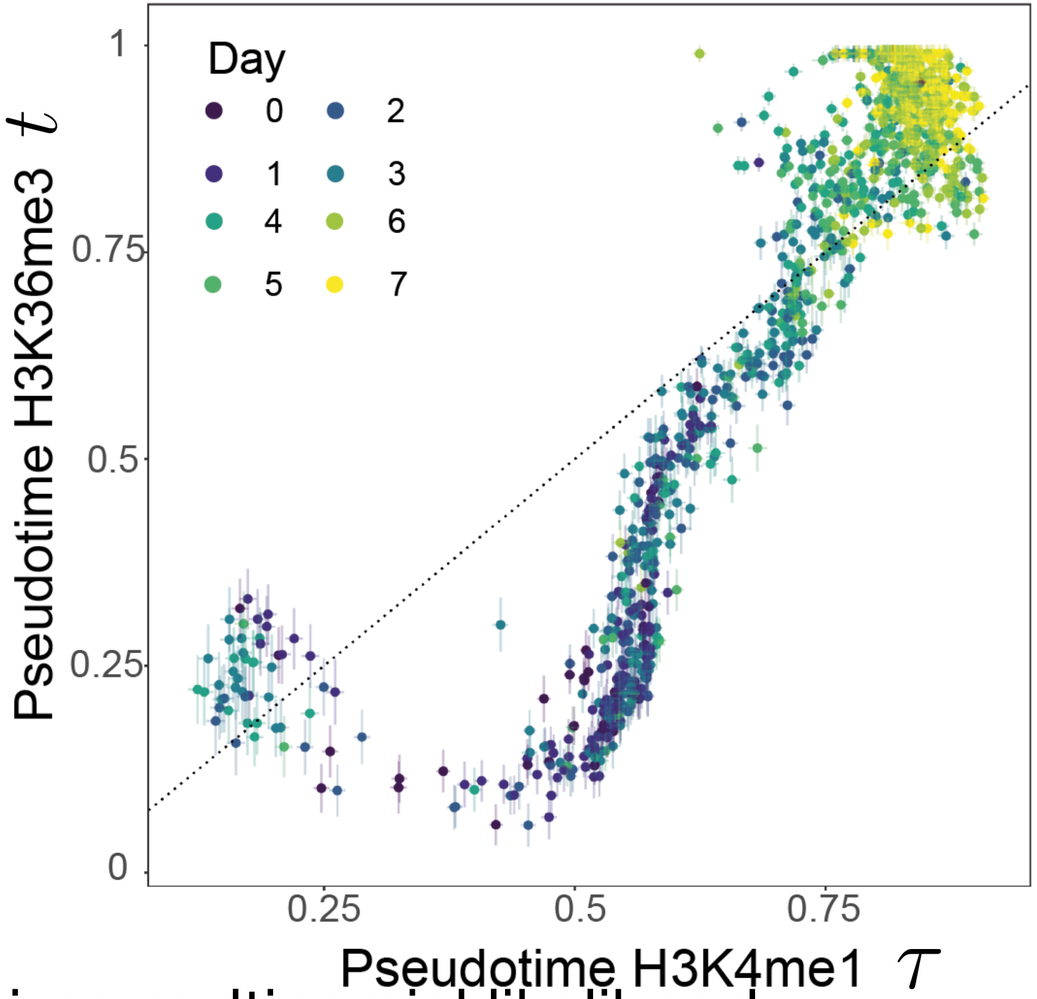


(t_1, τ_1)

(t_i, τ_i)



K4me1 primes genes for transcription (K36me3)

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Modeling the dynamics of both histone modifications reveals chromatin velocity

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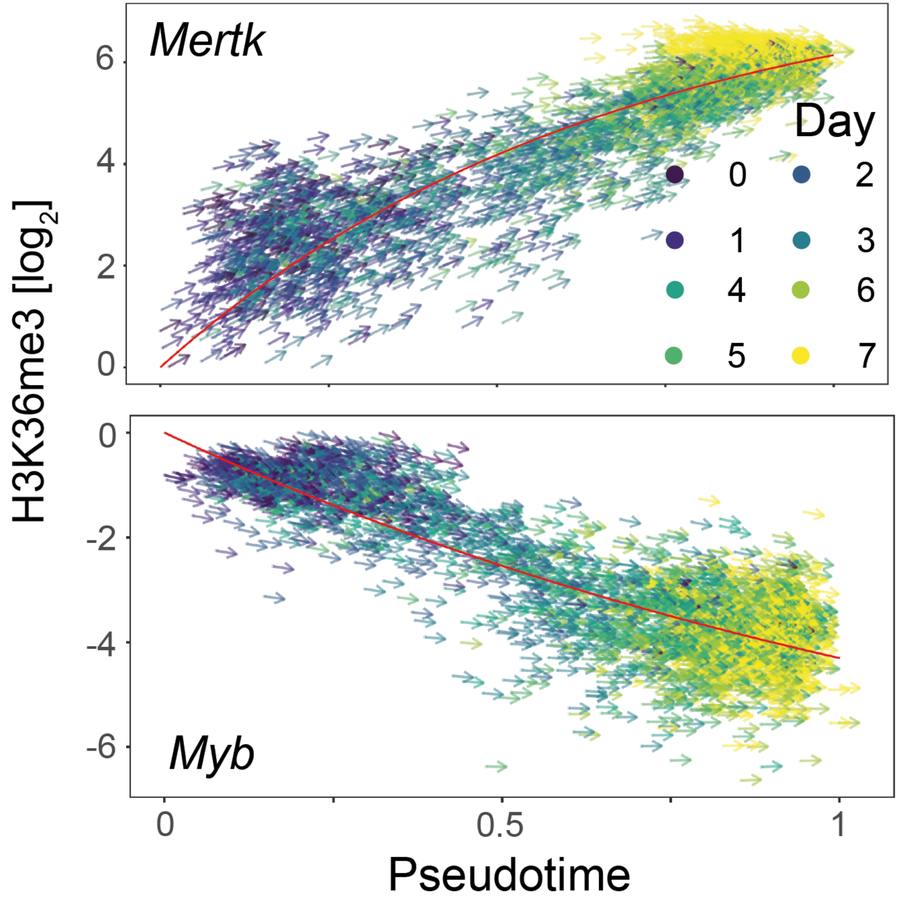

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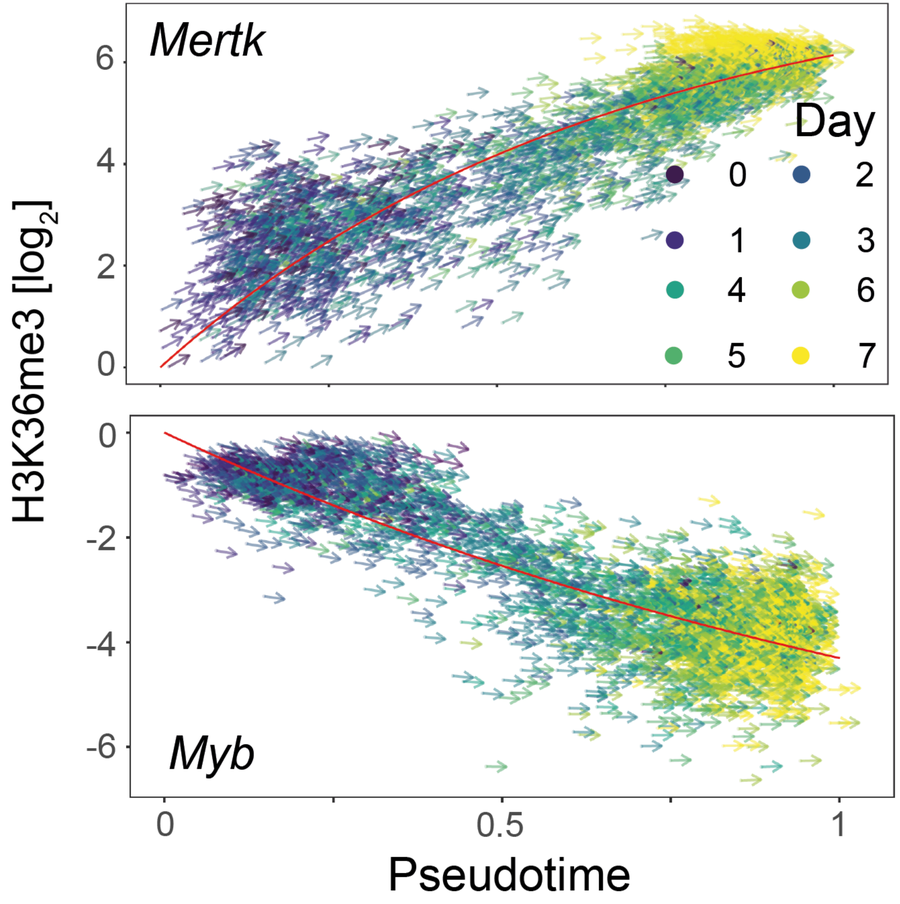
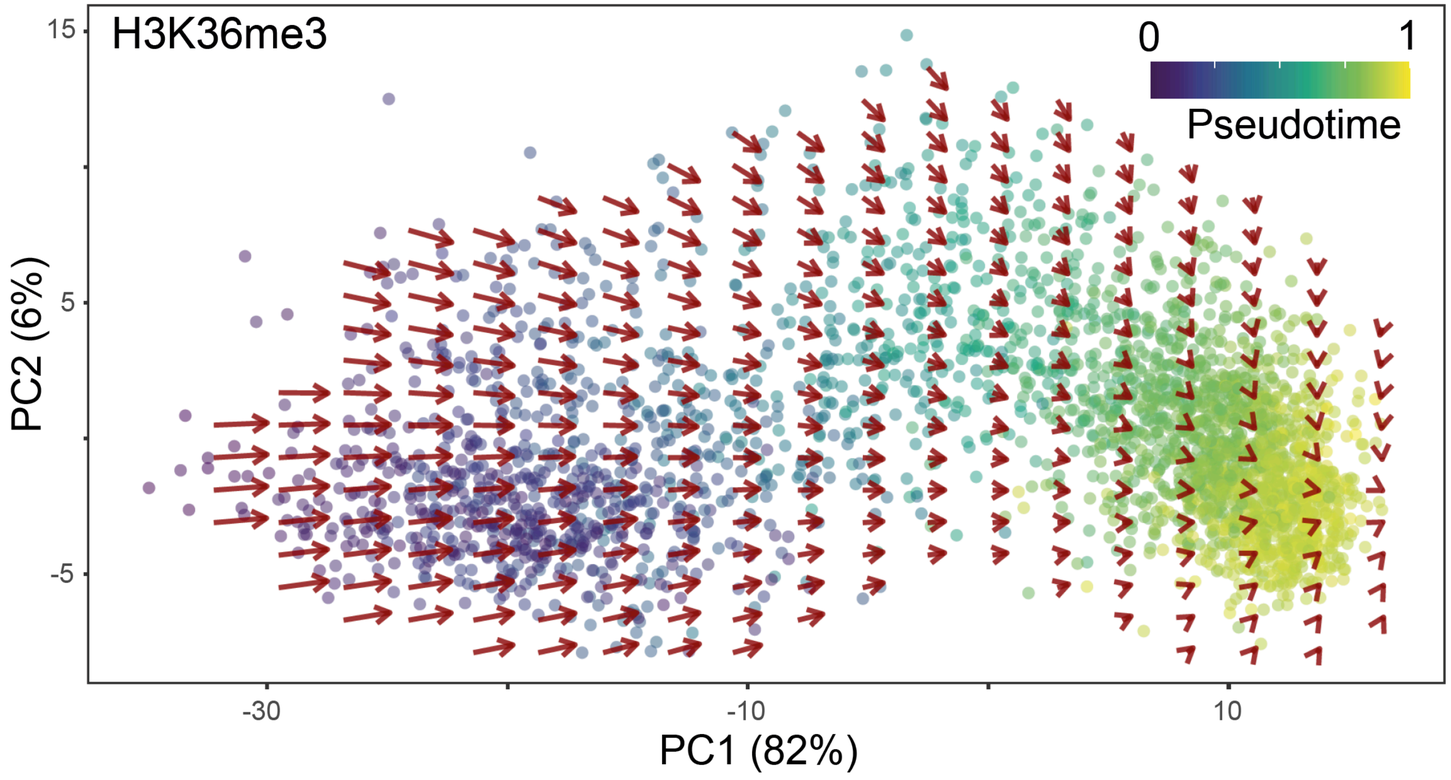
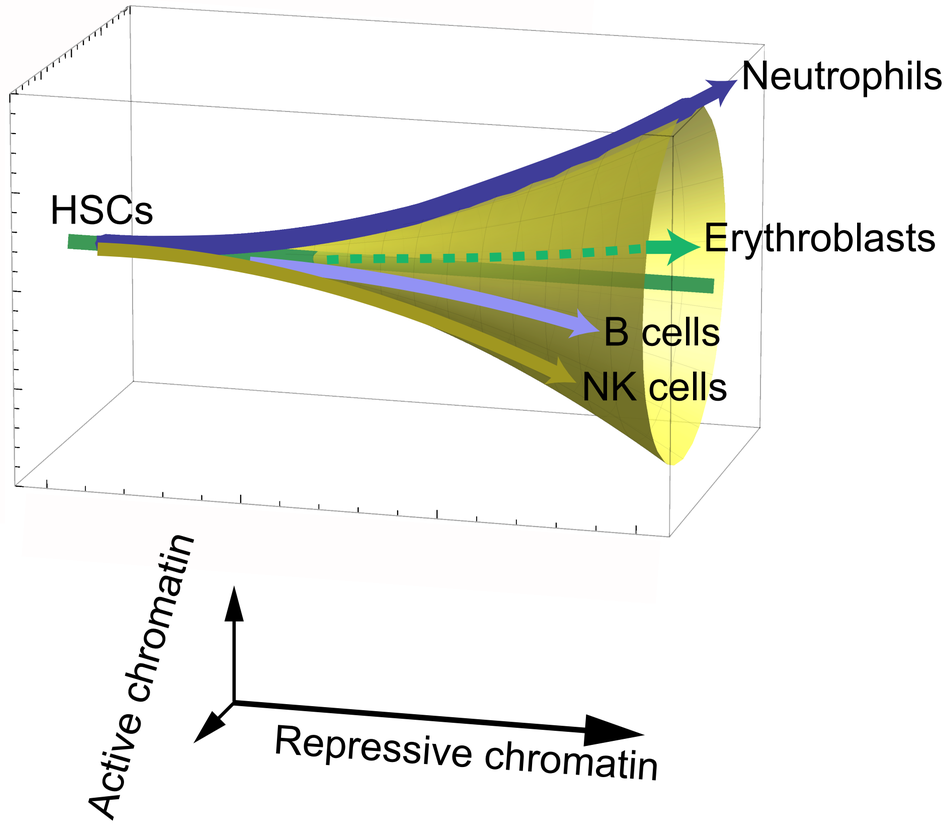
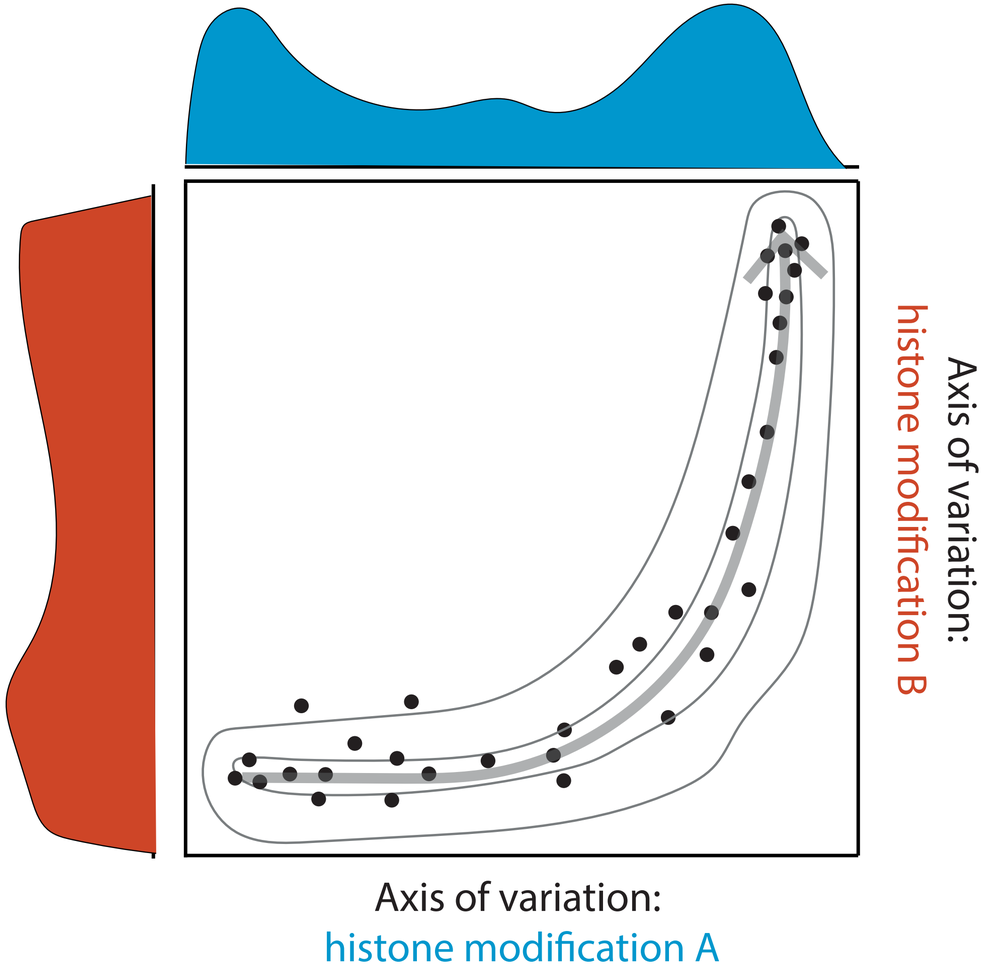


Summary of 206 genes



Integrative methods reveal interactions that are "greater than the sum of the parts"





sortChIC: Zeller*, Yeung* et al. *Nat Genetics* 2022

scChIX-seq: Yeung*, Florescu*, et al. *Nat Biotech* 2023

Challenges: towards data science-driven experimental methods and design

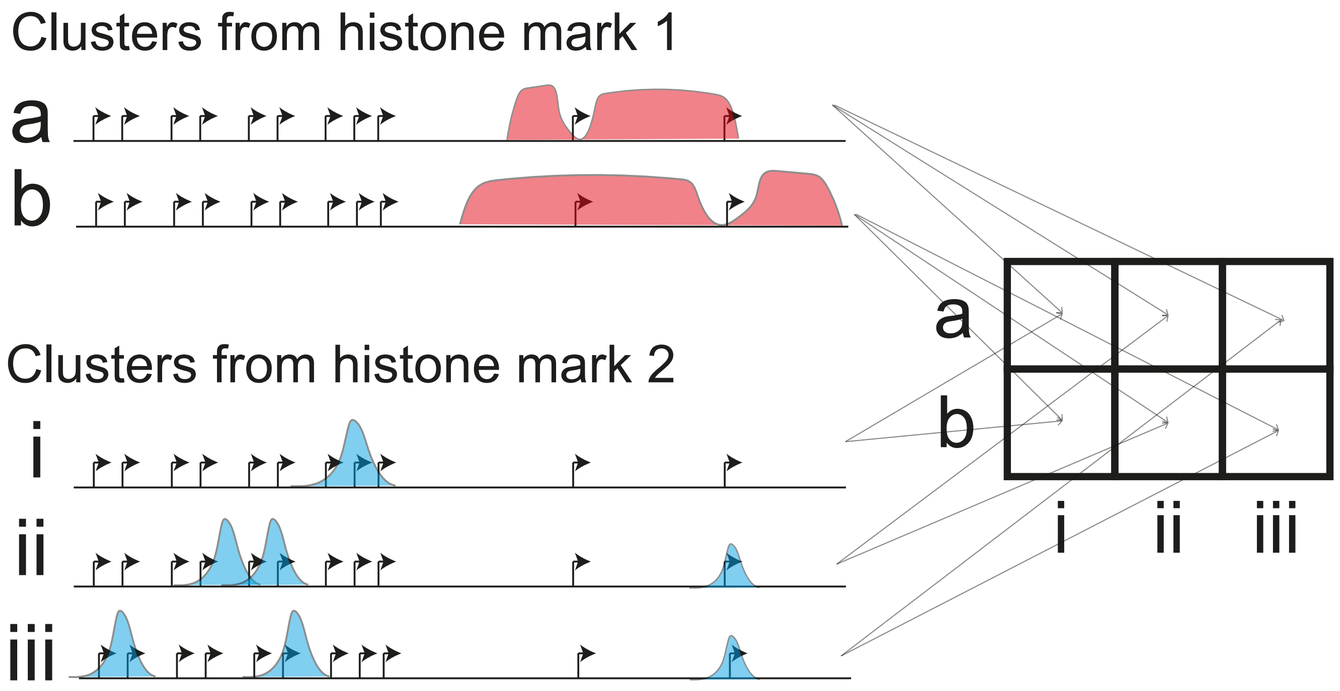
- Data science-driven solutions can reveal experimental insights that expand the gene regulatory picture captured by single-cell genomics.

- Dynamics of different chromatin states can be distinct: why and how much they differ influences experimental design and integrative analysis.

- What are the limits of analyzing noisy snapshot data to learn the real underlying stochastic trajectories? Can they be (partially) alleviated?

We use single-incubated data as training to infer cell type and heterochromatin identity in double-incubated cells

Single-incubated data (training)



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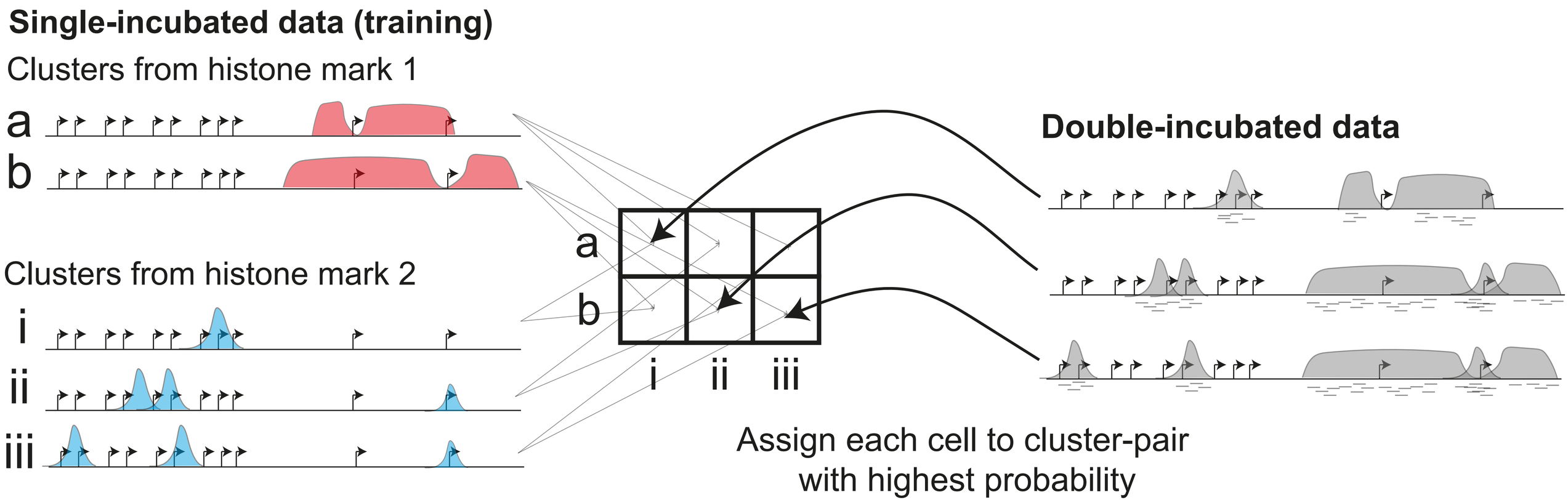
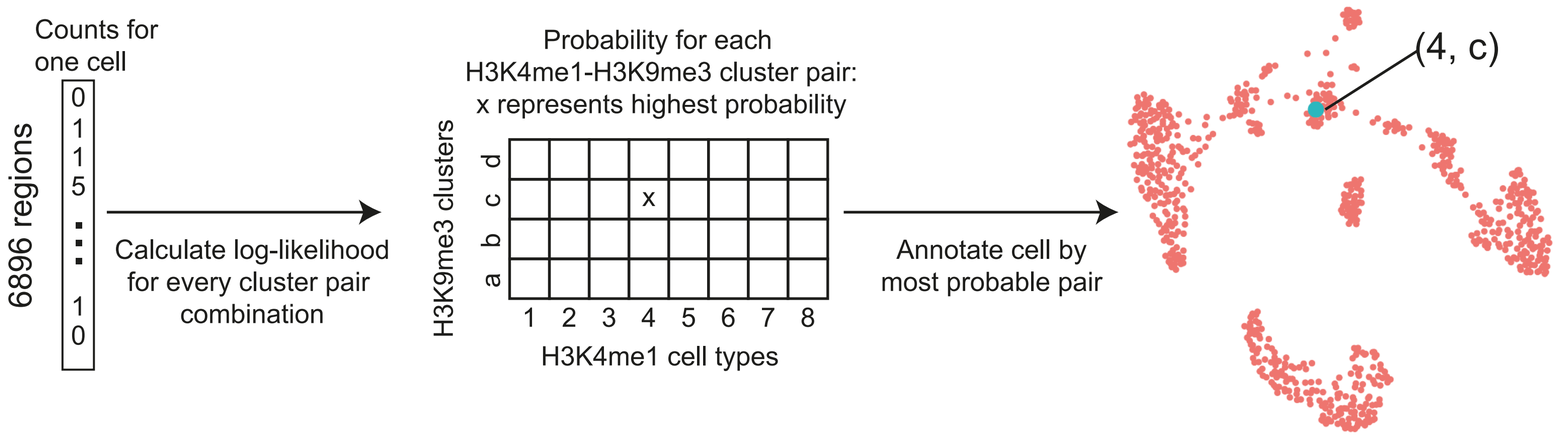


Each double-incubated cell generates a likelihood grid, which gives probabilities for each cluster-pair

Double-incubated single cells (observed)



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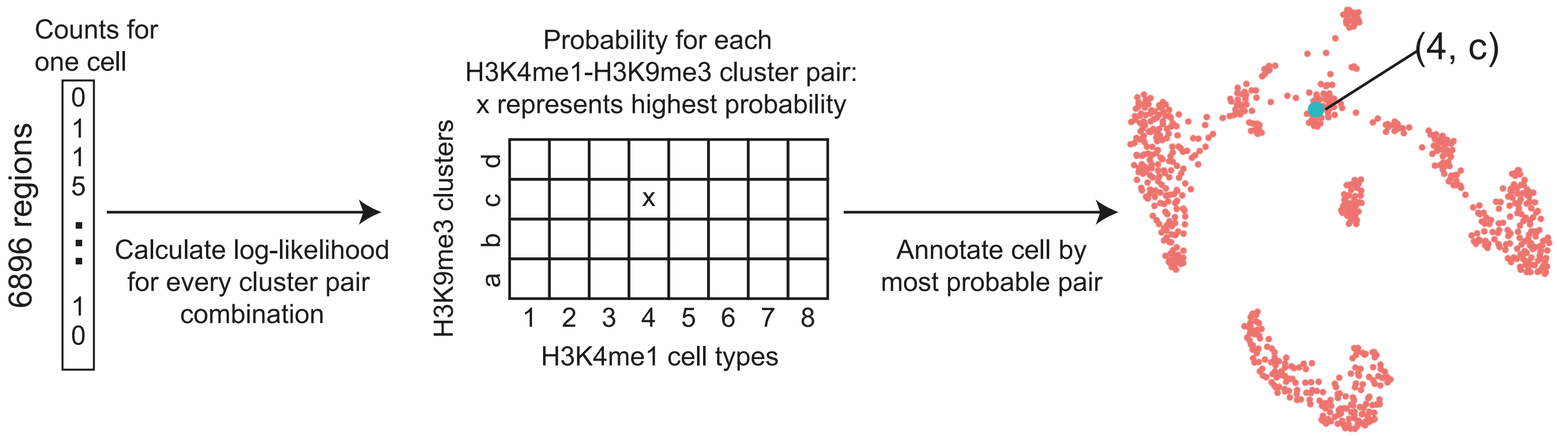
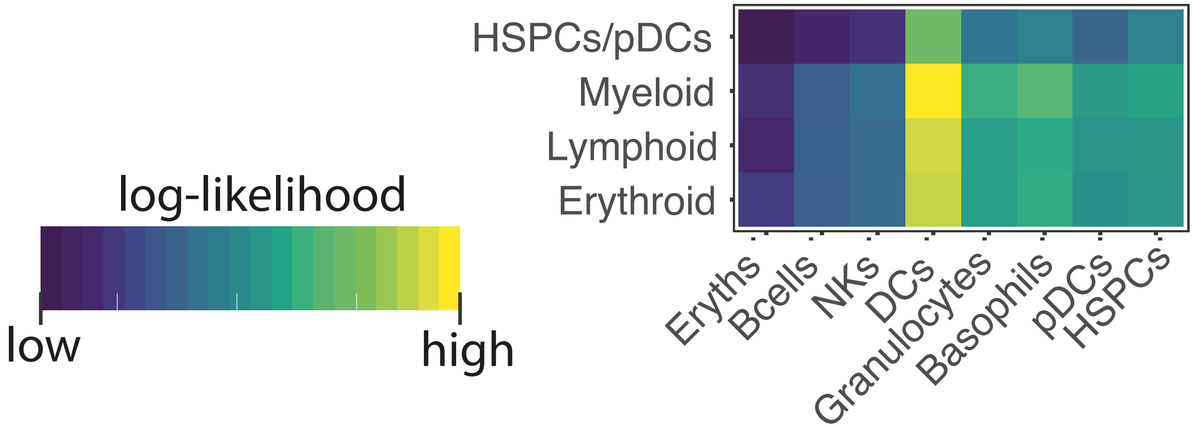


Likelihood map for one cell



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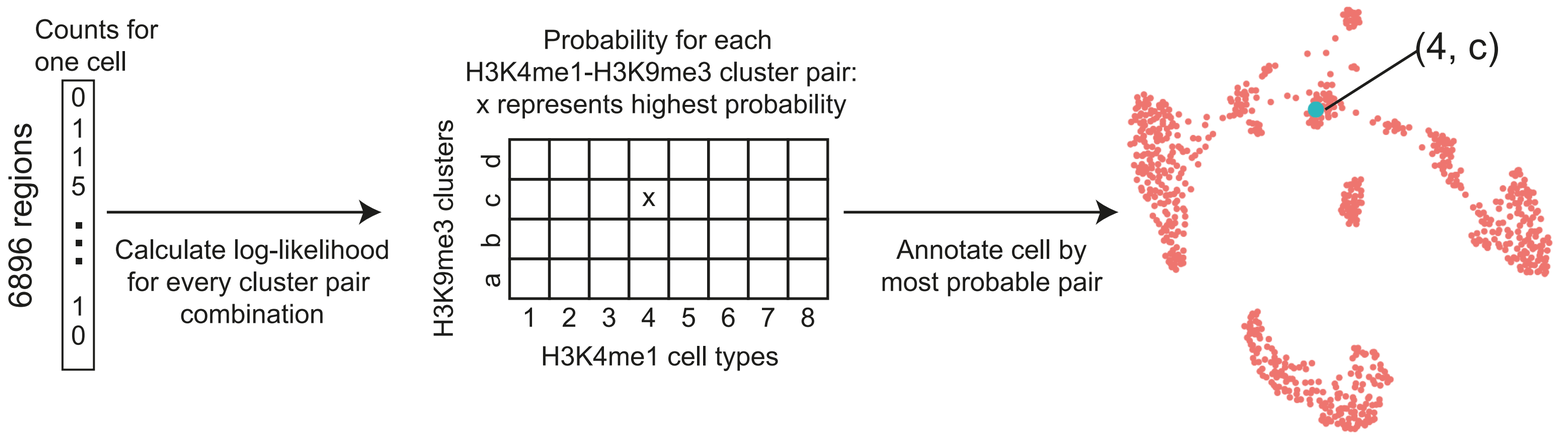

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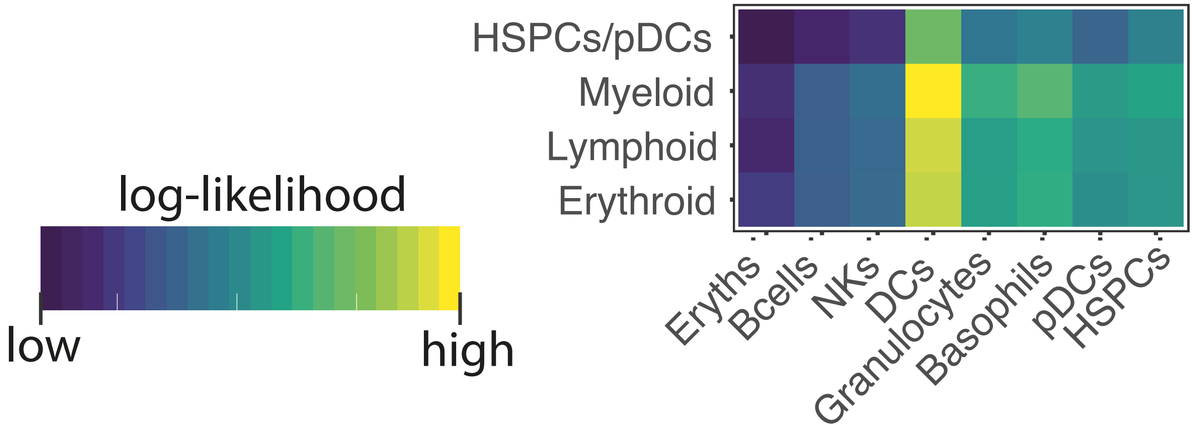


Example: likelihood at (4, c) is:

$$\text{logLikelihood}|\vec{p}_4, \vec{p}_c \propto \sum_{k=1}^{K} y_k \log\left(w p_{4,k} + (1-w)\, p_{c,k}\right)$$

K9 probability at region *k*

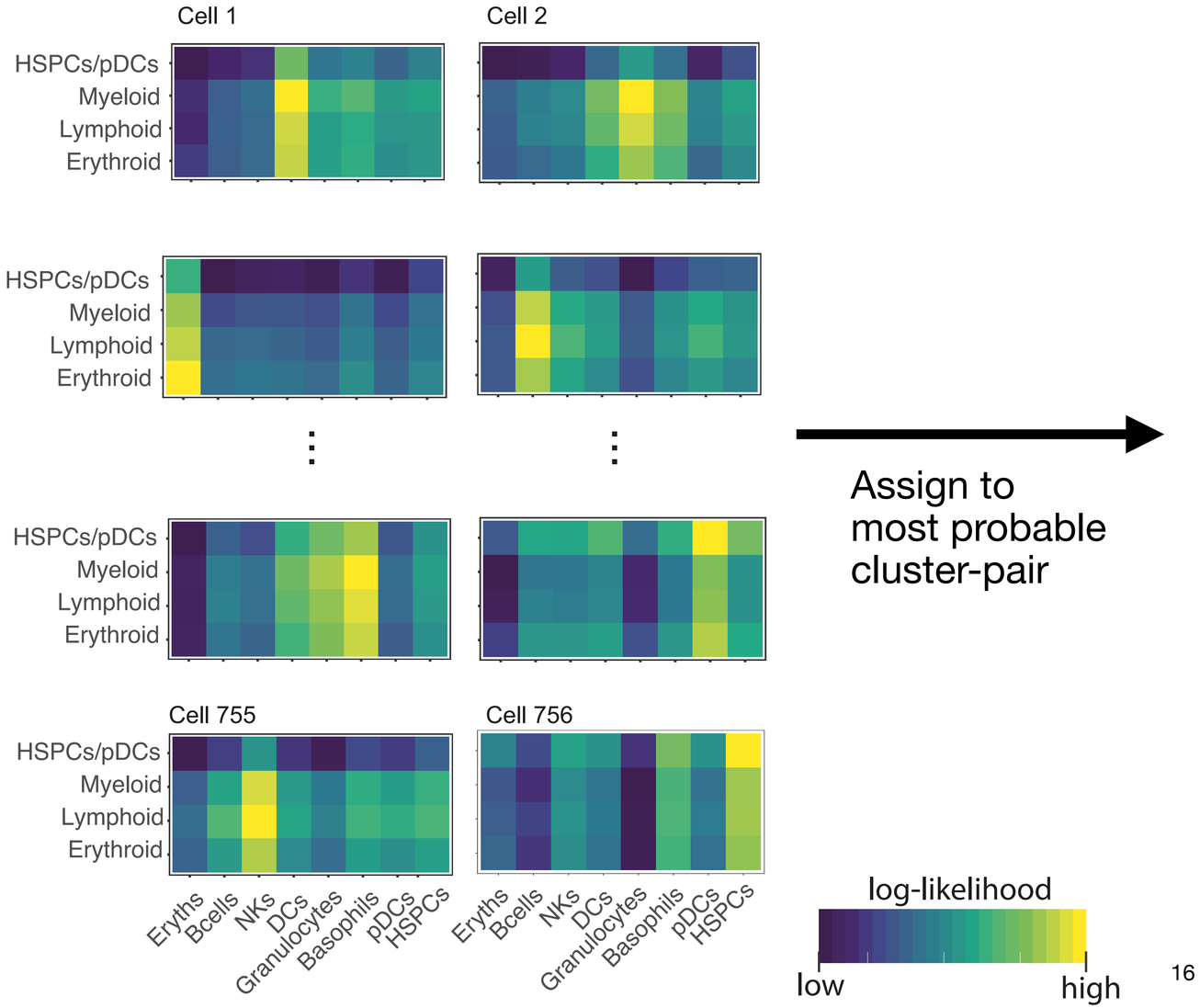
K4 probability at region *k*

Mixing weight (inferred)

Double cuts at region *k*

Double-incubated analysis reveals heterochromatin can be shared across related cell types

Calculate logLikelihood grid for each double-incubated cell:



Double-incubated analysis reveals heterochromatin can be shared across related cell types

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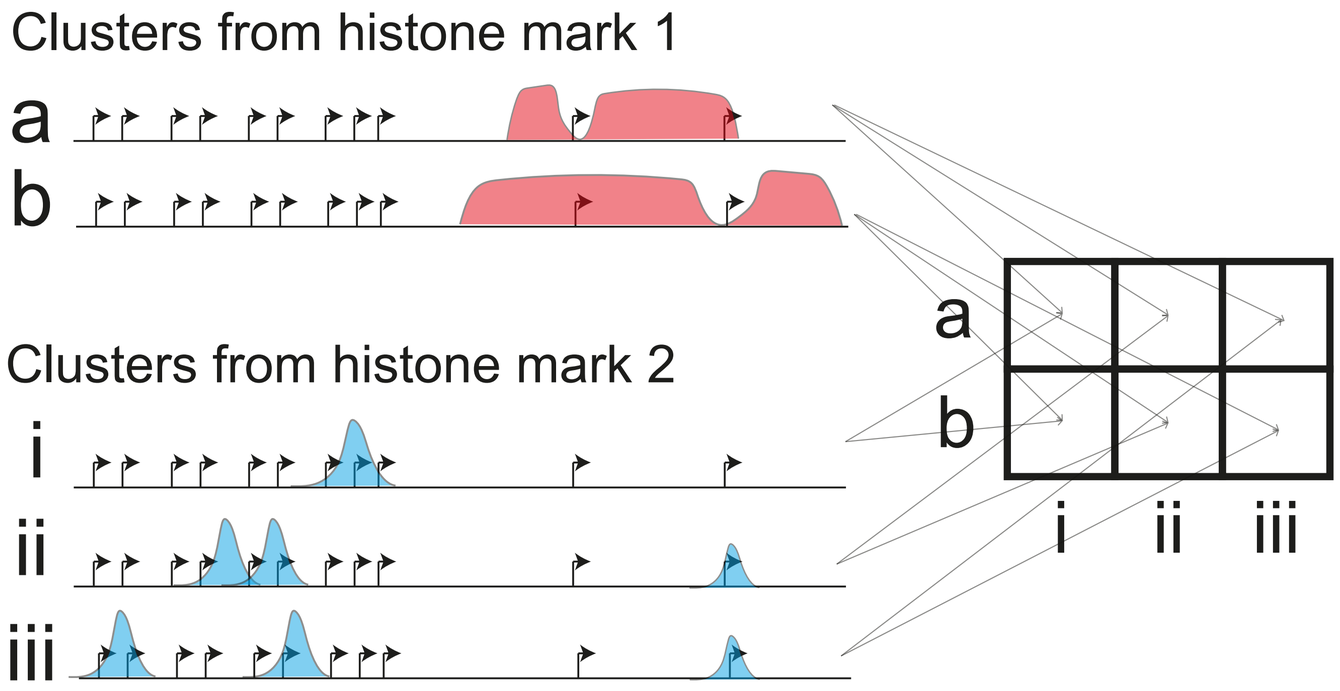
We use single-incubated data as training to infer cell type and heterochromatin in double-incubated cells

Single-incubated data (training)



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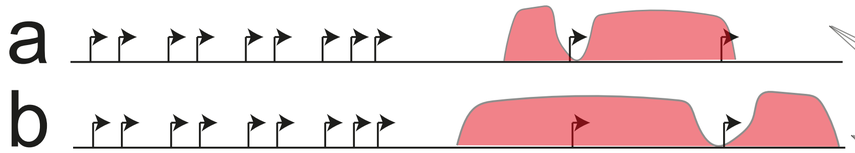
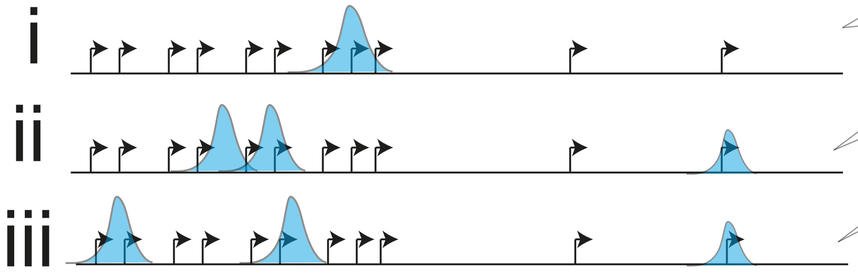
Single-incubated data (training)

Clusters from histone mark 1

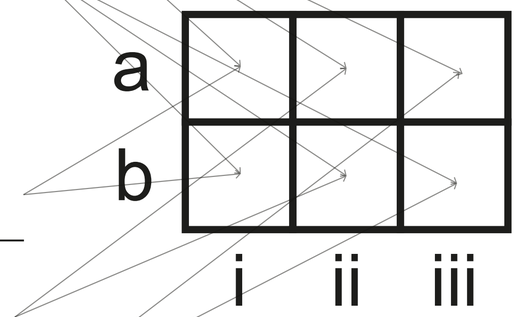


$\vec{p}_a = [0.01, 0.05, ..., 0.3]$

Clusters from histone mark 2



$\vec{p}_{ii} = [0.3, 0.05, ..., 0.01]$

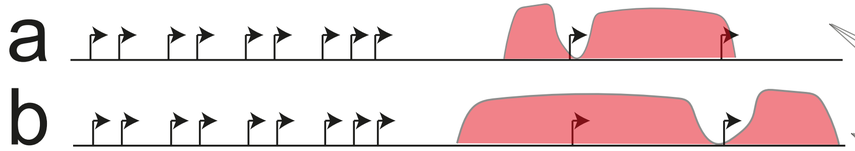


LDA gives these probabilities for free

We use single-incubated data as training to infer cell type and heterochromatin in double-incubated cells

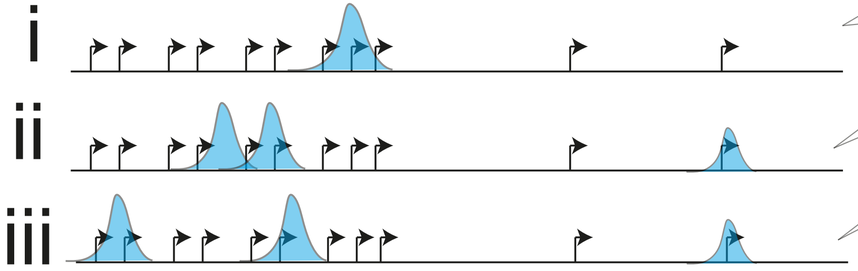
Single-incubated data (training)

Clusters from histone mark 1



$\vec{p}_a = [0.01, 0.05, ..., 0.3]$

Clusters from histone mark 2



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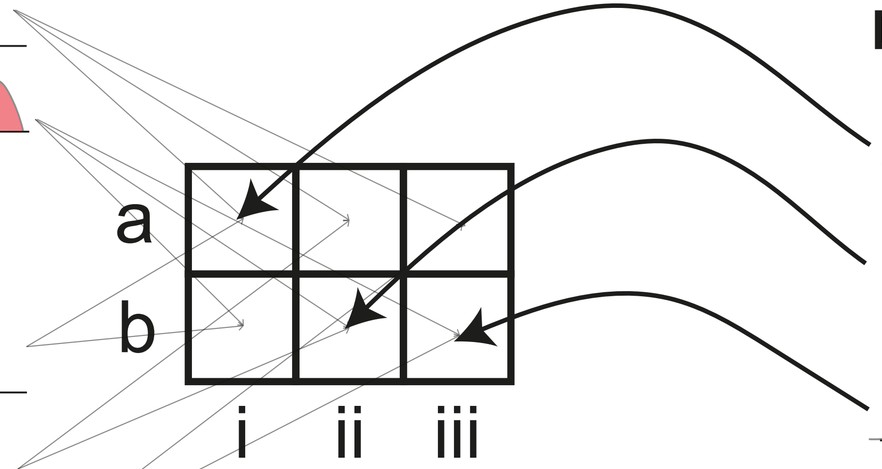
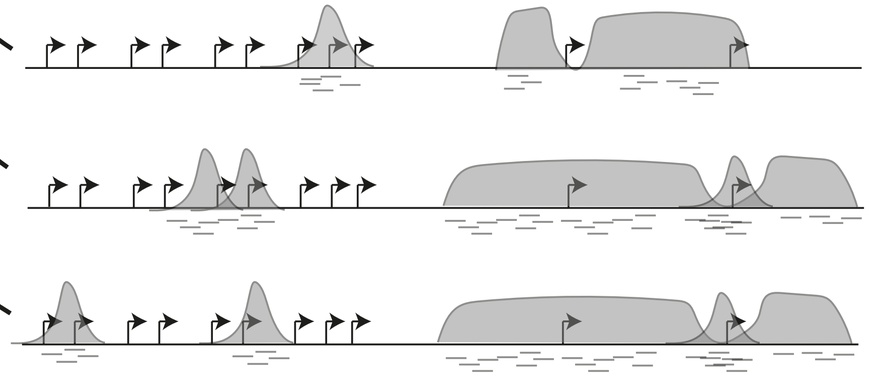


Double-incubated data



Assign each cell to cluster-pair with highest probability

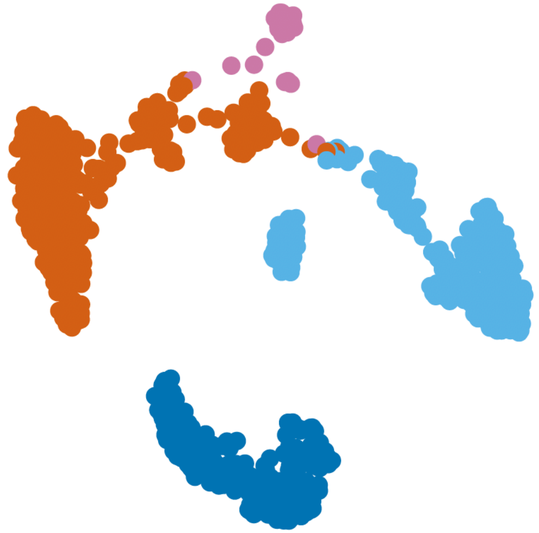
LDA gives these probabilities for free

Model for double-incubated counts coming from cluster b and ii:

$$\vec{y}|\vec{p}_b, \vec{p}_{ii} \sim \text{Multinomial}\left(w\vec{p}_b + (1-w)\vec{p}_{ii}, N\right)$$

Distinct cell types from related lineage share similar heterochromatin

H3K4me1+H3K9me3



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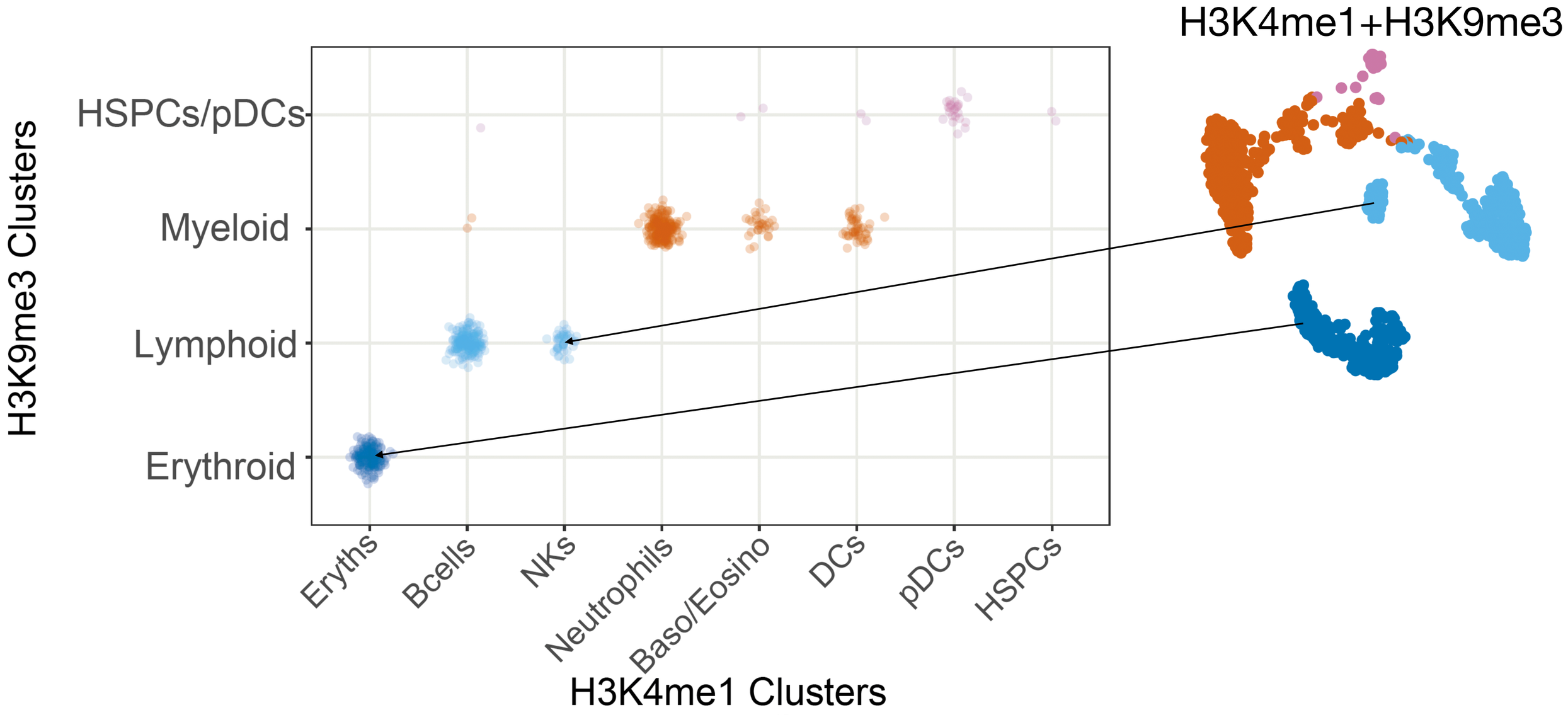
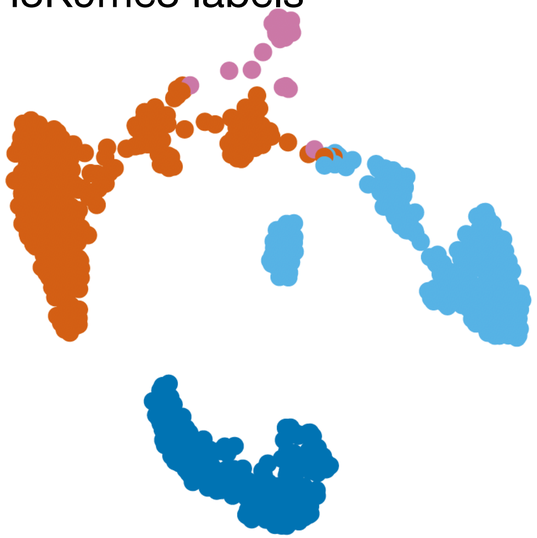


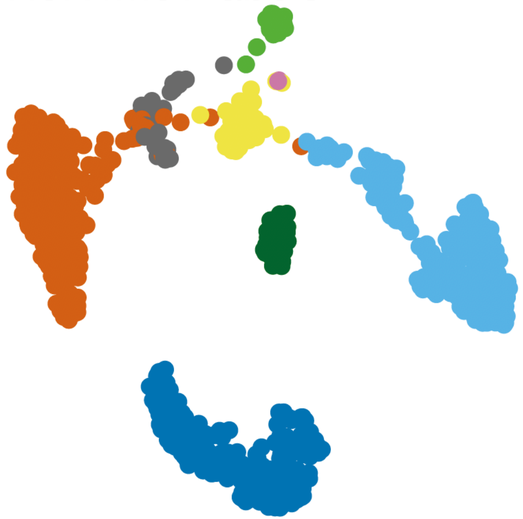
Chromatin regulation gives information of its cell type *and* its lineage

Each cell has two labels:

H3K9me3 labels



H3K4me1 labels



- NKs
- Baso/Eosino
- Eryths
- Bcells/Lymphoid
- pDCs
- DCs
- Neutrophils/Myeloid
- HSPCs

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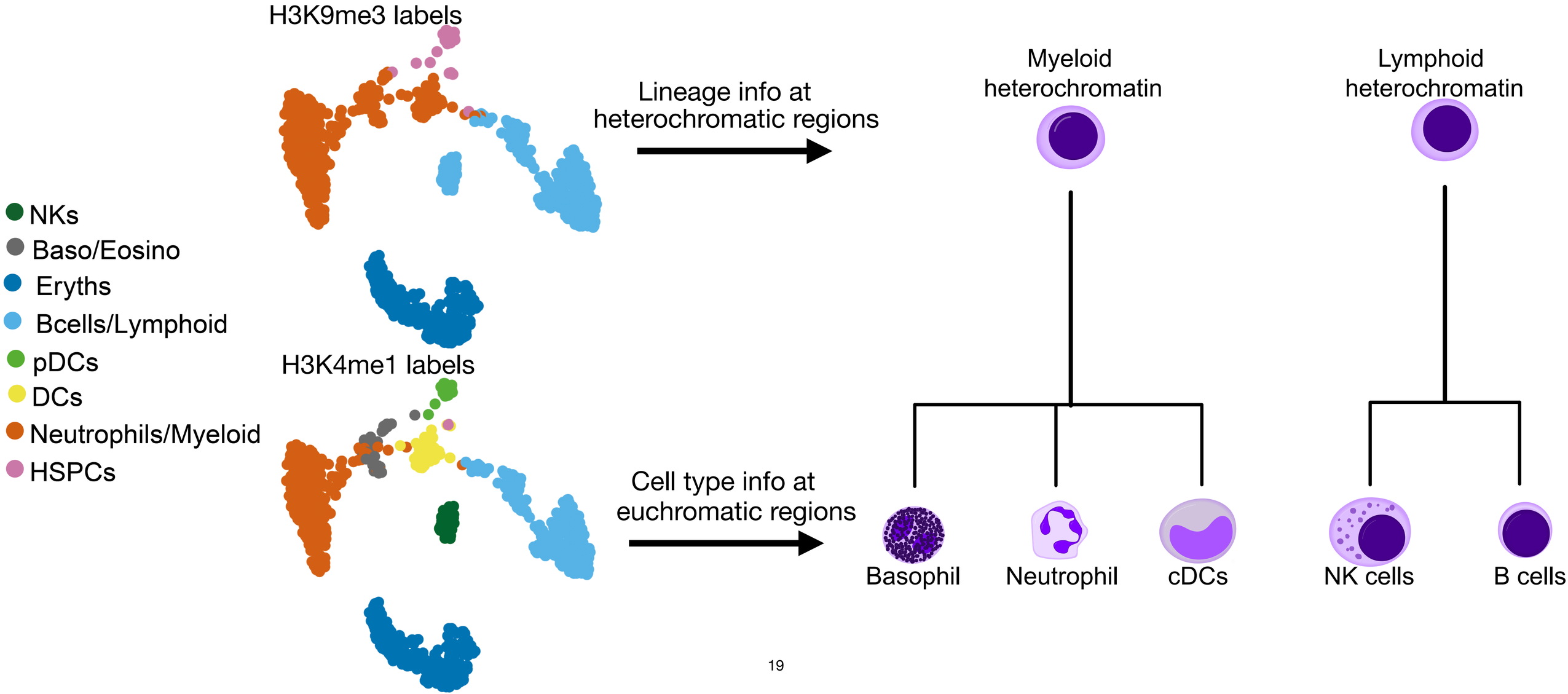
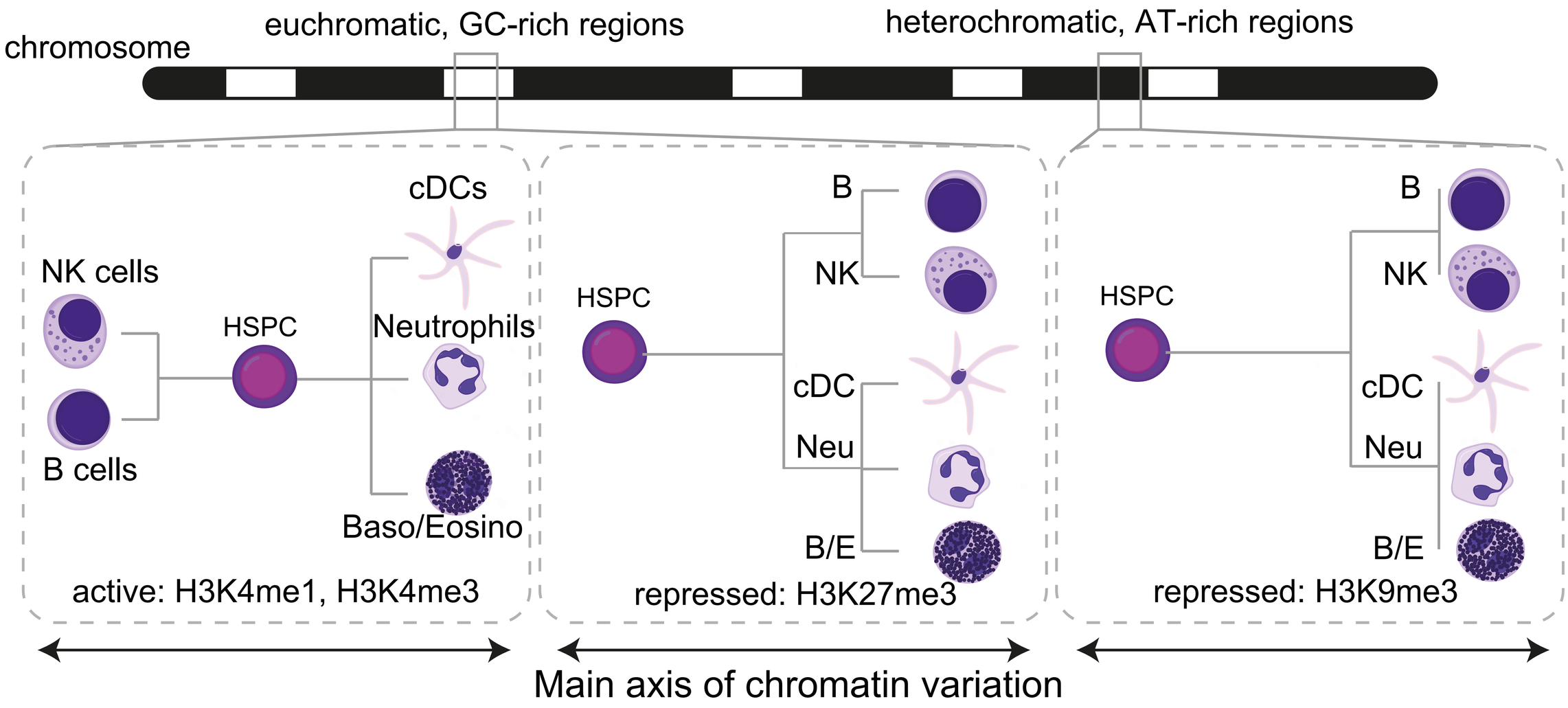


Repressive chromatin dynamics are distinct from active dynamics, and reveal hierarchical structure



Full model is complex, but integration simplifies the update equation for Gibbs sampling

$$\text{Prob}\left(\vec{z}, \vec{w}, \vec{\theta}, \vec{p} | \alpha, \lambda\right) = \text{Prob}\left(\text{topic}\right) \text{Prob}\left(\text{cell}\right) \text{Prob}\left(\text{genomic location} | \text{topic}\right)$$

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$$\text{Prob}\left(\vec{z}, \vec{w} | \alpha, \lambda\right) = \int_{\vec{p}} \prod_{k=1}^{K} \text{Prob}\left(p_k | \lambda\right) \text{Prob}\left(z_{d,n} | \theta\right) \text{Prob}\left(w_{d,n} | \lambda_{z_{d,n}}\right) \int_{\vec{\theta}} \prod_{d=1}^{D} \text{Prob}\left(\theta_d | \, \alpha\right)$$

Collapsed Gibbs sampling updates efficiently

Probability of assigning read *n* to topic *k:*

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$u_{d,k}$: number of times cell *d* uses topic *k*
$v_{k,w_{d,n}}$: number of times topic *k* uses locus $w_{d,n}$
α : Dirichlet prior for cell-to-topic distribution
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How much a cell likes a topic

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How much a cell likes a topic

How much a topic likes a genomic locus

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