STAT 35510
Lecture 11

Spring, 2024
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Outline

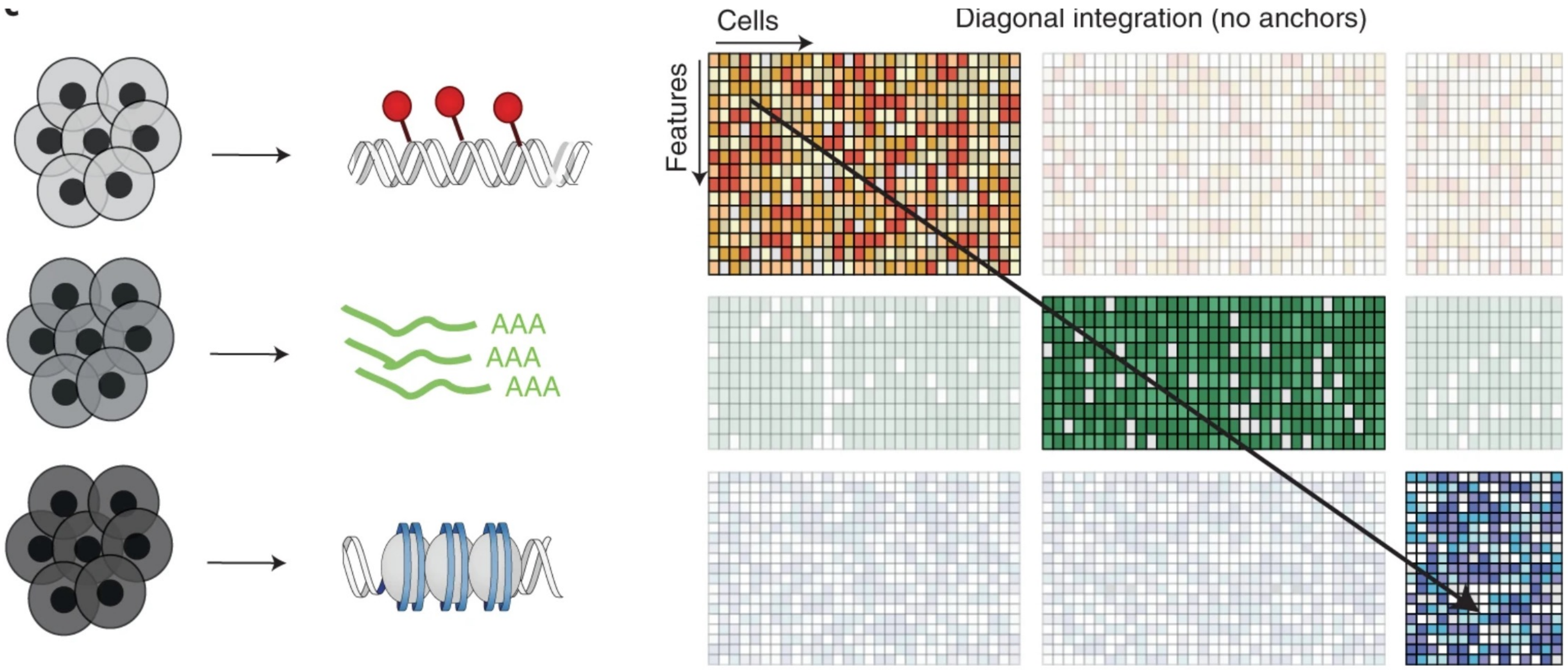
- Multi-omics data integration
  - Integrate unpaired multi-omics data
    - Integration of scATAC-seq and scRNA-seq
  - Integrate paired multi-omics data
  - Integrate unpaired multi-omics data using paired data as bridges

Integration between scRNA-seq and scATAC-seq

Why do we integrate?

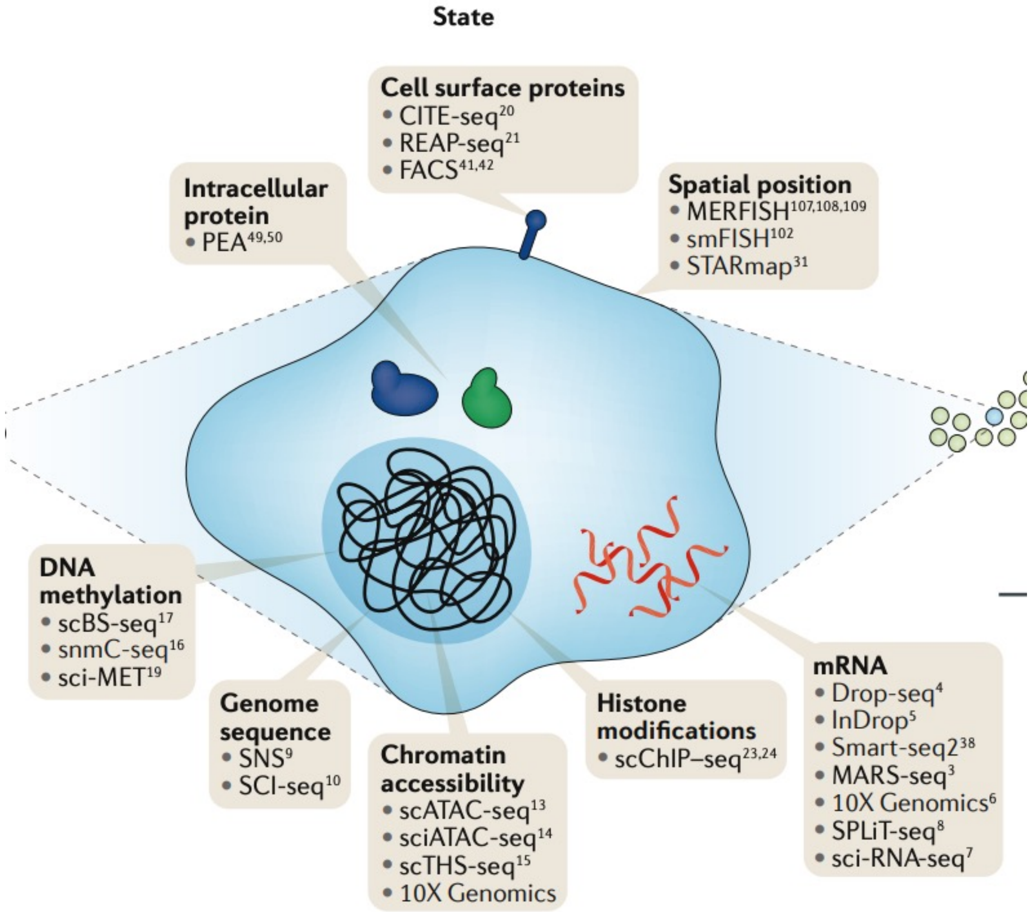
- Identify cell-specific regulatory network
- scATAC-seq data is extremely sparse → borrow information from scRNA-seq for better cell type annotation

Challenge: require extra information about feature connections



Integrative single-cell analyses

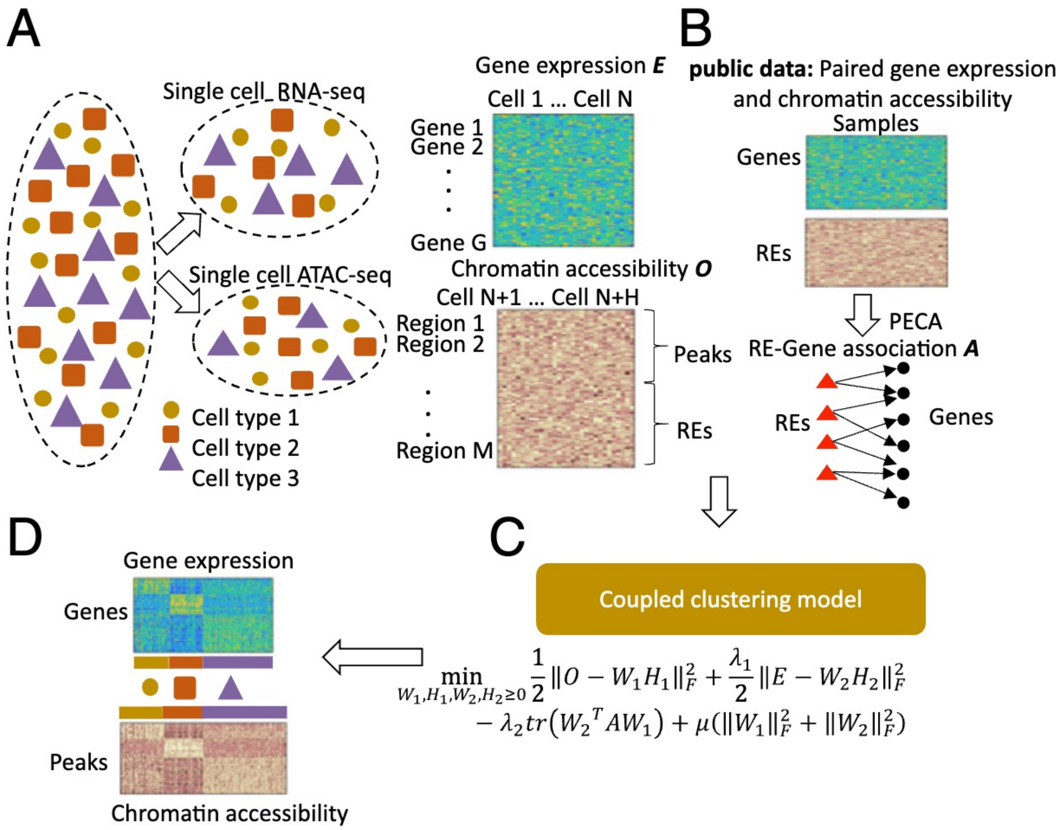
- Many technology only measure one modality of the single cells → unpaired multi-omics data
- Experimental methods have been developed to measure multiple modalities but can be more expensive



Integration of scRNA-seq and scATAC-seq

- Seurat v3 (Stuart et. al. Cell, 2019) :
  - Obtain gene activity matrix using Signac for scATAC-seq, treat as scRNA-seq data and integrate
  - Similar ideas used in scJoint (Lin et. al., Nature Biotech, 2022) and LIGER (Liu et. al., Nature Protocols, 2020)

- Coupled NMF (Daren et. al., PNAS, 2018)



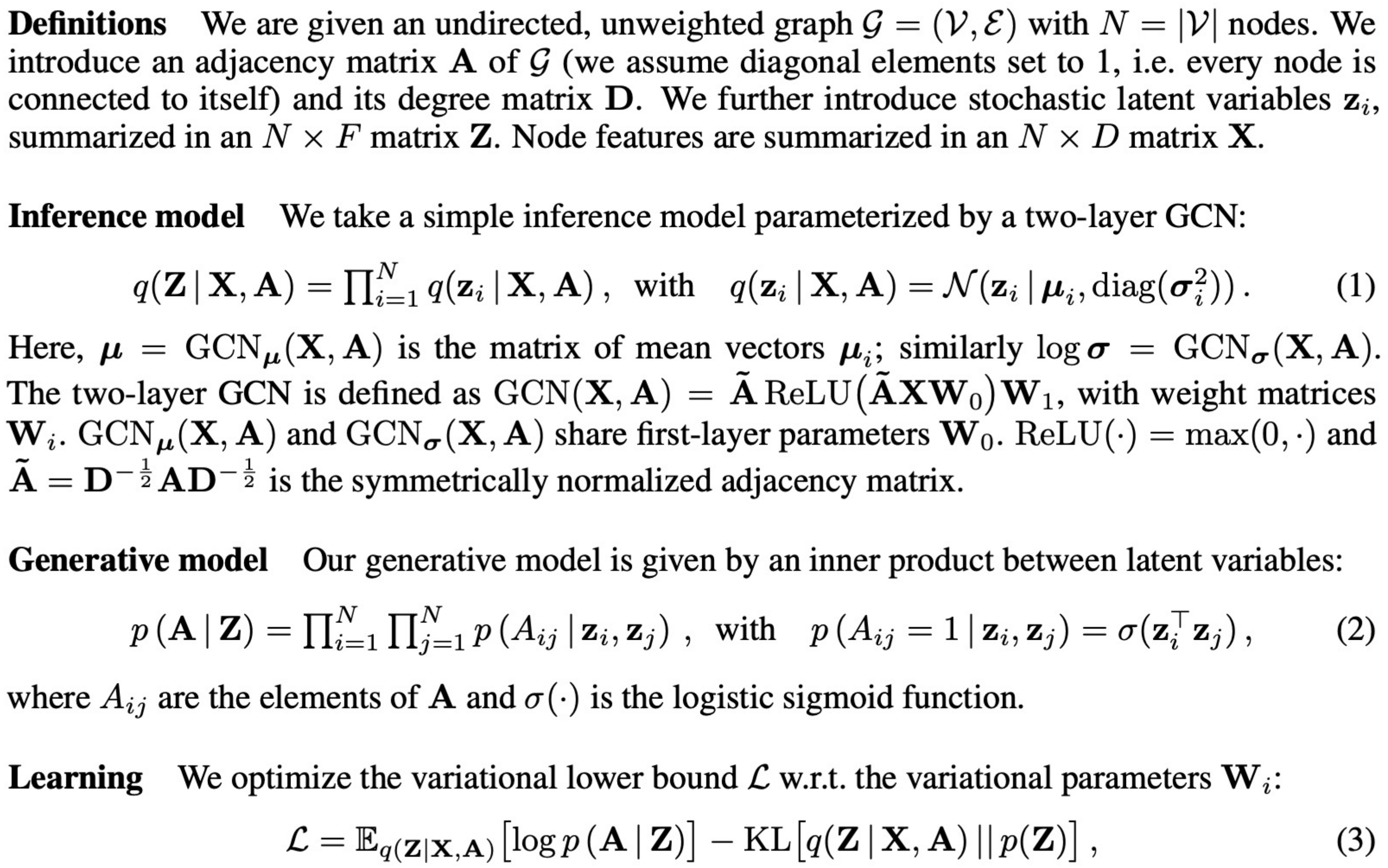
$$\min_{W_1,H_1,W_2,H_2\geq 0} \frac{1}{2}\|O-W_1H_1\|_F^2+\frac{\lambda_1}{2}\|E-W_2H_2\|_F^2 - \lambda_2 tr\left({W_2}^T A W_1\right)+\mu(\|W_1\|_F^2+\|W_2\|_F^2)$$

- Core idea: perform coupled clustering, making sure that feature loadings are similar after transformations
- A: coupling matrix, gene-peak prediction matrix where each peak is predicted by sets of genes learnt from paired mRNA-ATACseq bulk data

- Challenges:
  - Single-cell and bulk level data can have platform specific biases
  - Can not guarantee that H_1 and H_2 can be properly merged

GLUE (Cao and Gao, Nature Biotech, 2022)

- General integration of unpaired multi-omics data
- Make use of variational graph auto-encoders (VGAE, Kipf and Welling, Arxiv, 2016)



Definitions We are given an undirected, unweighted graph $\mathcal{G} = (\mathcal{V}, \mathcal{E})$ with $N = |\mathcal{V}|$ nodes. We introduce an adjacency matrix $\mathbf{A}$ of $\mathcal{G}$ (we assume diagonal elements set to 1, i.e. every node is connected to itself) and its degree matrix $\mathbf{D}$. We further introduce stochastic latent variables $\mathbf{z}_i$, summarized in an $N \times F$ matrix $\mathbf{Z}$. Node features are summarized in an $N \times D$ matrix $\mathbf{X}$.

Inference model We take a simple inference model parameterized by a two-layer GCN:

$$q(\mathbf{Z} \,|\, \mathbf{X}, \mathbf{A}) = \textstyle\prod_{i=1}^{N} q(\mathbf{z}_i \,|\, \mathbf{X}, \mathbf{A}) \,, \quad \text{with} \quad q(\mathbf{z}_i \,|\, \mathbf{X}, \mathbf{A}) = \mathcal{N}(\mathbf{z}_i \,|\, \boldsymbol{\mu}_i, \operatorname{diag}(\boldsymbol{\sigma}_i^2)) \,. \tag{1}$$

Here, $\boldsymbol{\mu} = \mathrm{GCN}_{\boldsymbol{\mu}}(\mathbf{X}, \mathbf{A})$ is the matrix of mean vectors $\boldsymbol{\mu}_i$; similarly $\log \boldsymbol{\sigma} = \mathrm{GCN}_{\boldsymbol{\sigma}}(\mathbf{X}, \mathbf{A})$. The two-layer GCN is defined as $\mathrm{GCN}(\mathbf{X}, \mathbf{A}) = \tilde{\mathbf{A}} \operatorname{ReLU}\big(\tilde{\mathbf{A}} \mathbf{X} \mathbf{W}_0\big) \mathbf{W}_1$, with weight matrices $\mathbf{W}_i$. $\mathrm{GCN}_{\boldsymbol{\mu}}(\mathbf{X}, \mathbf{A})$ and $\mathrm{GCN}_{\boldsymbol{\sigma}}(\mathbf{X}, \mathbf{A})$ share first-layer parameters $\mathbf{W}_0$. $\operatorname{ReLU}(\cdot) = \max(0, \cdot)$ and $\tilde{\mathbf{A}} = \mathbf{D}^{-\frac{1}{2}} \mathbf{A} \mathbf{D}^{-\frac{1}{2}}$ is the symmetrically normalized adjacency matrix.

Generative model Our generative model is given by an inner product between latent variables:

$$p\left(\mathbf{A} \,|\, \mathbf{Z}\right) = \textstyle\prod_{i=1}^{N} \prod_{j=1}^{N} p\left(A_{ij} \,|\, \mathbf{z}_i, \mathbf{z}_j\right) \,, \quad \text{with} \quad p\left(A_{ij} = 1 \,|\, \mathbf{z}_i, \mathbf{z}_j\right) = \sigma(\mathbf{z}_i^{\top} \mathbf{z}_j) \,, \tag{2}$$

where A_{ij} are the elements of $\mathbf{A}$ and $\sigma(\cdot)$ is the logistic sigmoid function.

Learning We optimize the variational lower bound $\mathcal{L}$ w.r.t. the variational parameters $\mathbf{W}_i$:

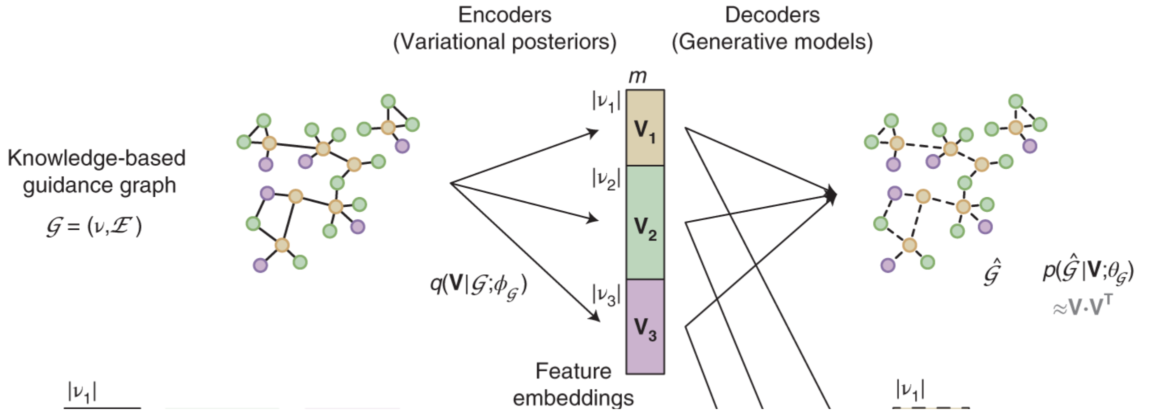
$$\mathcal{L} = \mathbb{E}_{q(\mathbf{Z}|\mathbf{X},\mathbf{A})}\big[\log p\left(\mathbf{A} \,|\, \mathbf{Z}\right)\big] - \mathrm{KL}\big[q(\mathbf{Z} \,|\, \mathbf{X}, \mathbf{A}) \,||\, p(\mathbf{Z})\big] \,, \tag{3}$$

GLUE (Cao and Gao, Nature Biotech, 2022)

Core steps:

- Build a separate VAE for each modality data
- Build a guidance graph (signed and weighted, possibly multi edges between two nodes) based on prior knowledge on regulatory interactions across features from different modalities
  - Peak and gene are linked if they overlap with the gene body or proximal promoter regions
  - GLUE is robust to corruption of the graph even 90% of the edges are random

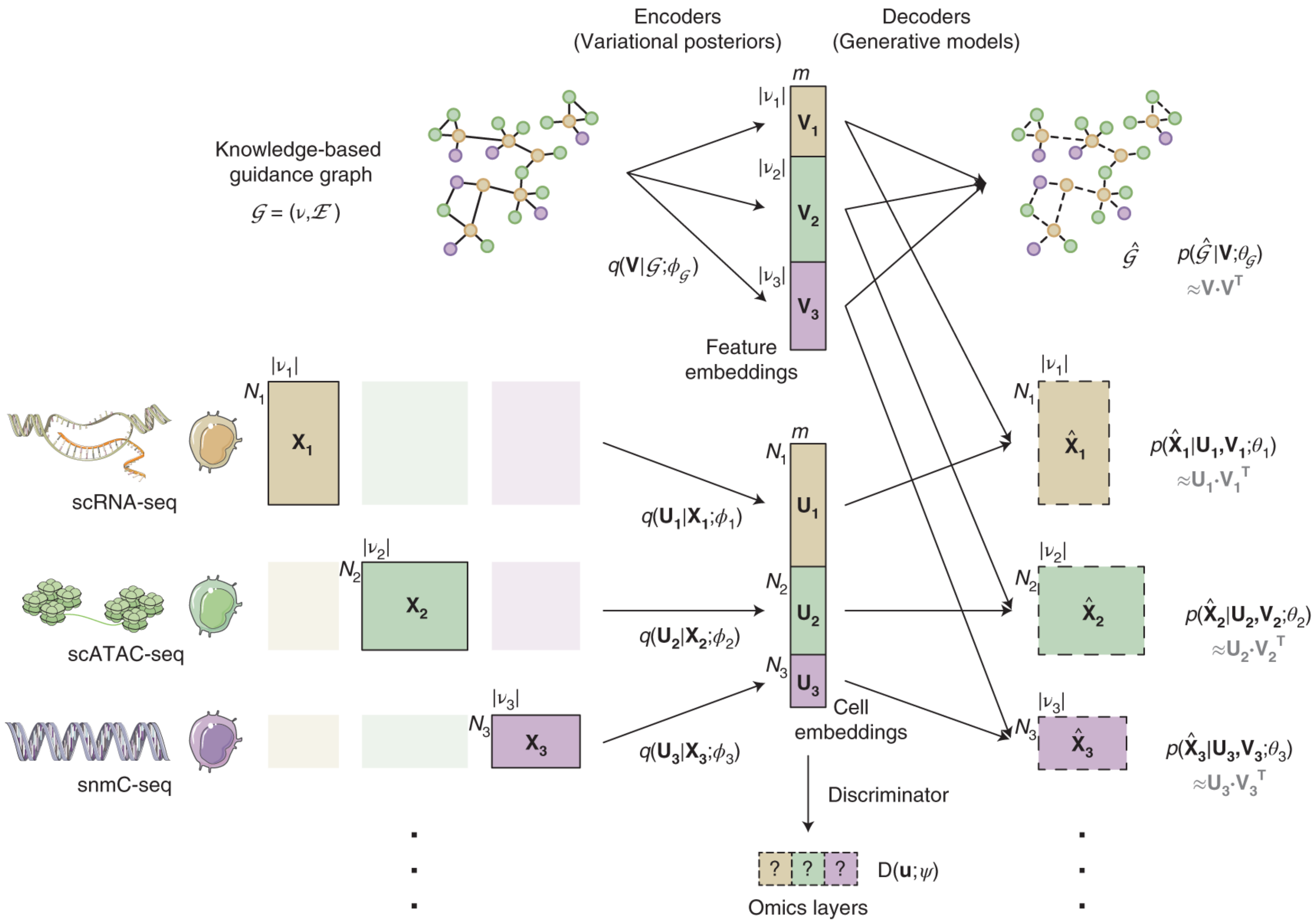
- Build feature embeddings using the same idea as VGAE encoder



- Cell embeddings are transformed based on feature embeddings
  - Linear decoder like SVD: for a cell i in dataset k, the predicted data has the form

$$\hat{\mu}_i^{(k)} = U_i\left(V^{(k)}\right)^T$$

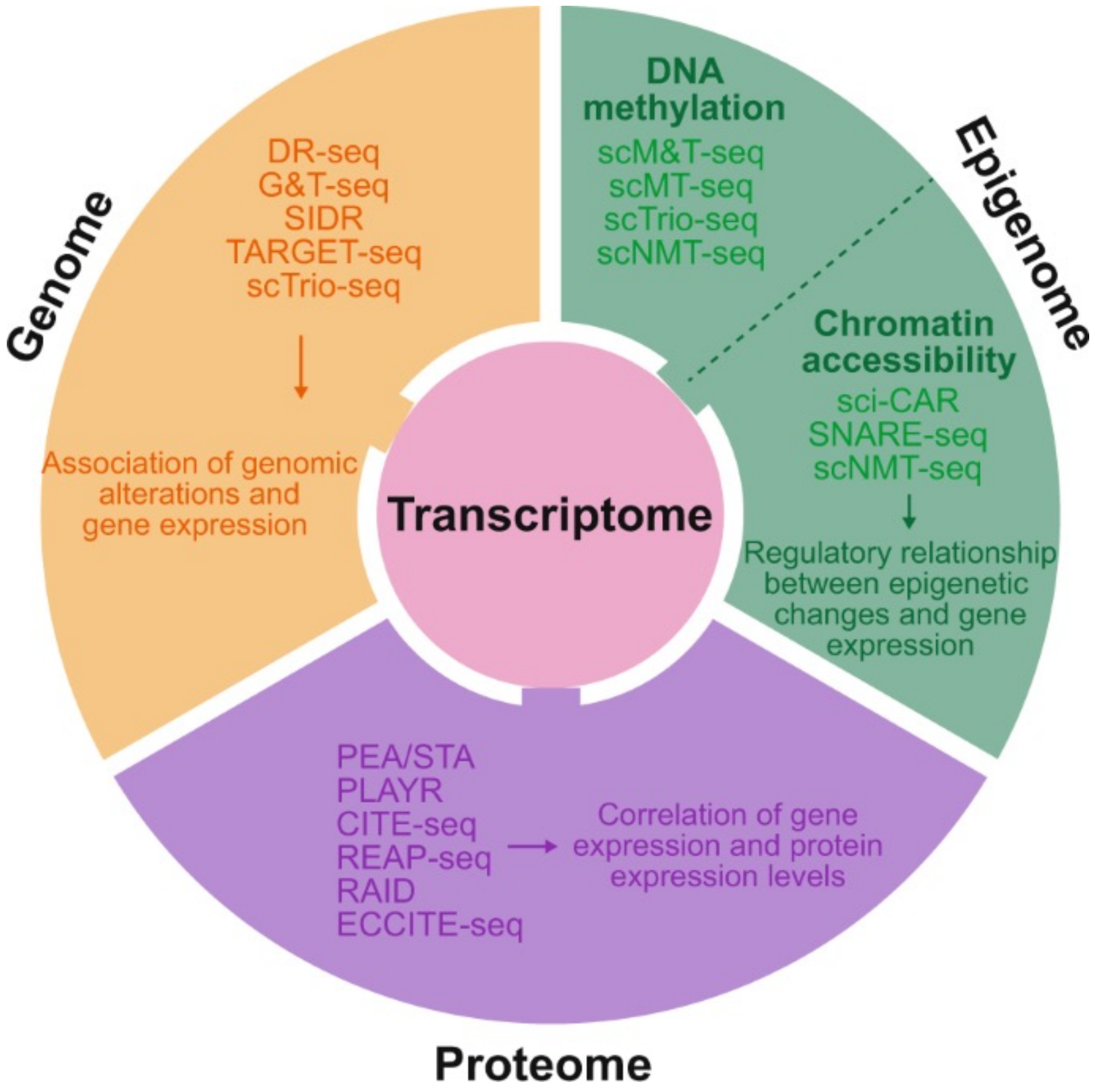
GLUE (Cao and Gao, Nature Biotech, 2022)



Encoders
(Variational posteriors)
Decoders
(Generative models)
Knowledge-based
guidance graph
$\mathcal{G} = (\mathcal{V}, \mathcal{E})$
$q(\mathbf{V}|\mathcal{G};\phi_{\mathcal{G}})$
m
$|\mathcal{V}_1|$
$|\mathcal{V}_2|$
$|\mathcal{V}_3|$
$\mathbf{V}_1$
$\mathbf{V}_2$
$\mathbf{V}_3$
Feature
embeddings
$\hat{\mathcal{G}}$
$p(\hat{\mathcal{G}}|\mathbf{V};\theta_{\mathcal{G}})$
$\approx \mathbf{V} \cdot \mathbf{V}^T$
scRNA-seq
scATAC-seq
snmC-seq
N_1
N_2
N_3
$\mathbf{X}_1$
$\mathbf{X}_2$
$\mathbf{X}_3$
$q(\mathbf{U}_1|\mathbf{X}_1;\phi_1)$
$q(\mathbf{U}_2|\mathbf{X}_2;\phi_2)$
$q(\mathbf{U}_3|\mathbf{X}_3;\phi_3)$
$\mathbf{U}_1$
$\mathbf{U}_2$
$\mathbf{U}_3$
Cell
embeddings
Discriminator
? ? ?
$D(\mathbf{u};\psi)$
Omics layers
$\hat{\mathbf{X}}_1$
$\hat{\mathbf{X}}_2$
$\hat{\mathbf{X}}_3$
$p(\hat{\mathbf{X}}_1|\mathbf{U}_1,\mathbf{V}_1;\theta_1)$
$\approx \mathbf{U}_1 \cdot \mathbf{V}_1^T$
$p(\hat{\mathbf{X}}_2|\mathbf{U}_2,\mathbf{V}_2;\theta_2)$
$\approx \mathbf{U}_2 \cdot \mathbf{V}_2^T$
$p(\hat{\mathbf{X}}_3|\mathbf{U}_3,\mathbf{V}_3;\theta_3)$
$\approx \mathbf{U}_3 \cdot \mathbf{V}_3^T$

- Need extra penalty to assure that cell embeddings are aligned across modalities
  - Train a classifier (discriminator) to separate different datasets based on the cell embeddings
  - Penalize the loss if the discriminator has small classification error

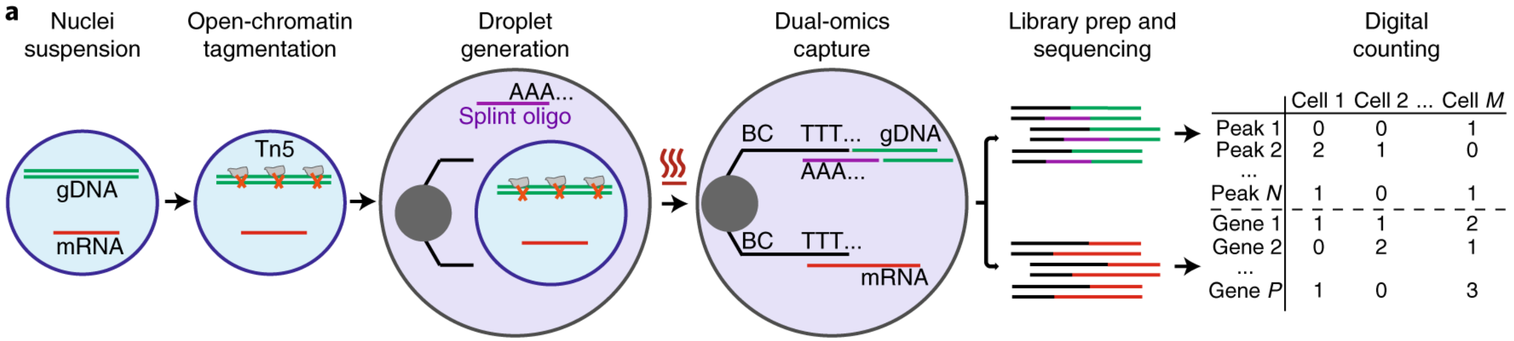
Single-cell multi-omics



- Paired single-cell multi-omics can be used as bridges to learn feature relationships across modalities

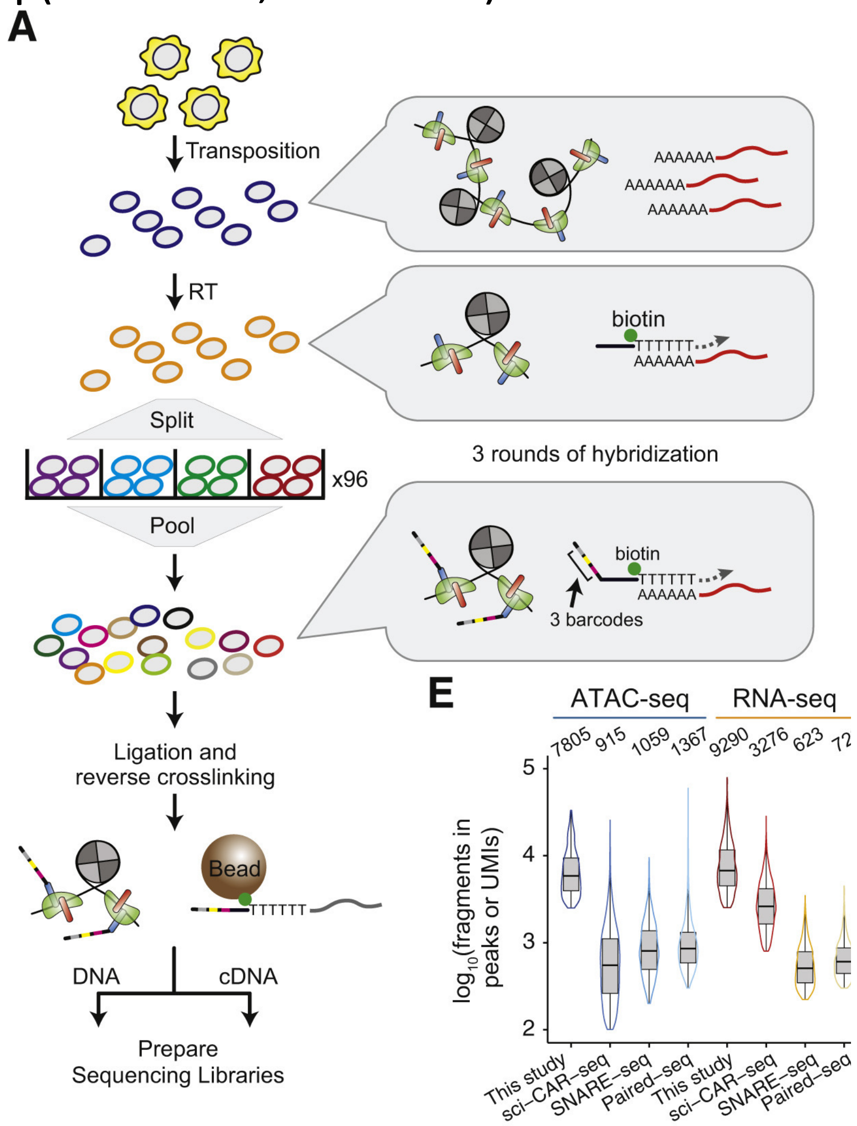
Simultaneous measure of mRNA and chromatin accessibility

SNARE-seq (Chen et. al., Nature Biotech 2019)

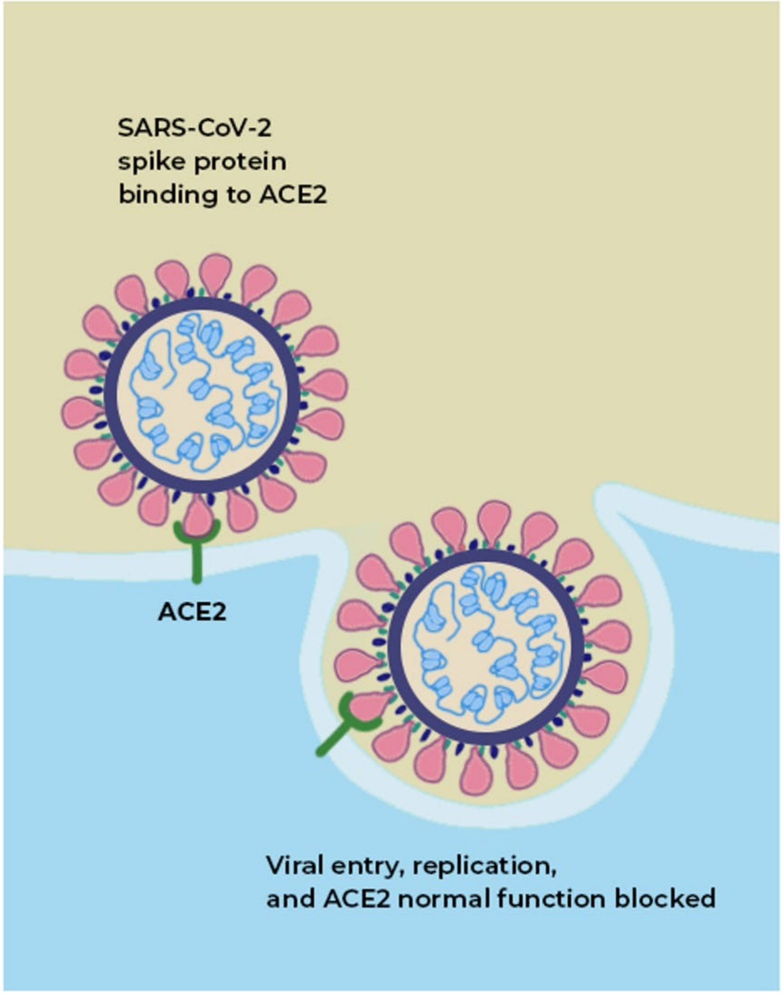


	Cell 1	Cell 2	...	Cell M
Peak 1	0	0		1
Peak 2	2	1		0
...				
Peak N	1	0		1
Gene 1	1	1		2
Gene 2	0	2		1
...				
Gene P	1	0		3

Share-seq (Ma et. al., Cell 2020)

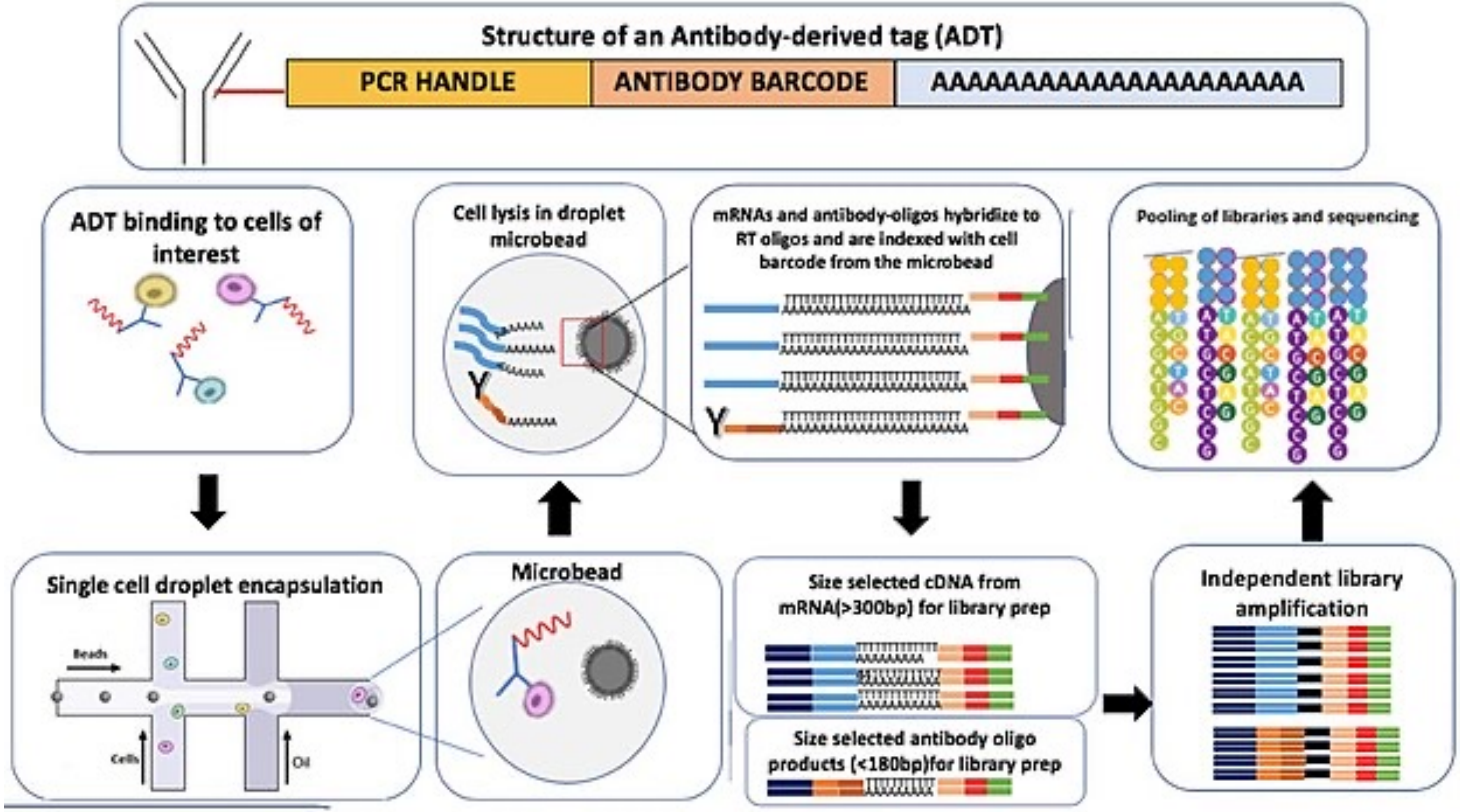


Simultaneous measure of mRNA and surface protein



- Proteins can more reliably indicate cellular activity and function
- Cell surface proteins: play crucial role in effective communication between the cell and its environment
- About 25% to 30% of human genes encode for membrane proteins
- Common technologies: REAP-seq (Peterson et. al., Nature Biotech 2017), CITE-seq (Stoeckius et. al., Nature Methods 2017)

CITE-seq workflow



Structure of an Antibody-derived tag (ADT)

PCR HANDLE | ANTIBODY BARCODE | AAAAAAAAAAAAAAAAAAAAA

ADT binding to cells of interest

Single cell droplet encapsulation

Microbead

Cell lysis in droplet microbead

mRNAs and antibody-oligos hybridize to RT oligos and are indexed with cell barcode from the microbead

Size selected cDNA from mRNA(>300bp) for library prep

Size selected antibody oligo products (<180bp)for library prep

Independent library amplification

Pooling of libraries and sequencing

Integrate paired single cell multi-omics data

- Seurat v4 (Hao et. al. Cell, 2021)

- Core challenge: need to consider multiple sets of features when calculating cell-cell similarity

- Core idea: calculate a weighted NN graph with cell-specific weights
  - Generate KNN graph within each modality
  - Within-modality and cross-modality prediction based on KNN (4 prediction values)
    - Calculate similarity between predicted values and observed values
      - For example:

$$\theta_{rna}\left(r_i, \hat{r}_{i,knn_r}\right) = \exp\left(\frac{-\max\left(d\left(r_i, \hat{r}_{i,knn_r}\right) - d\left(r_i, r_{knn_{r,i,1}}\right), 0\right)}{\sigma_{r,i} - d\left(r_i, r_{knn_{r,i,1}}\right)}\right)$$

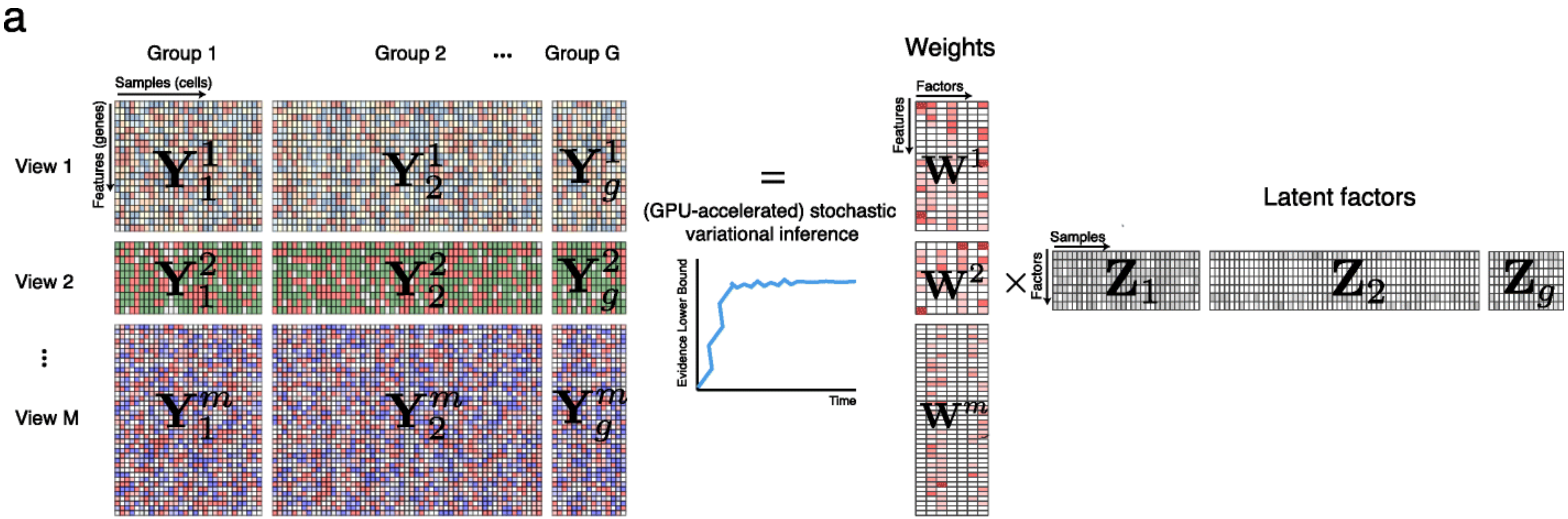
  - Calculated cell-specific modality weights: higher weights on protein if protein neighbors predict better than mRNA neighbors → the neighbors better reflect the molecular state of the cell

$$s_{rna}(i) = \frac{\theta_{rna}\left(r_i, \hat{r}_{i,knn_r}\right)}{\theta_{rna}\left(r_i, \hat{r}_{i,knn_p}\right) + \varepsilon}, \quad s_{protein}(i) = \frac{\theta_{protein}\left(p_i, \hat{p}_{i,knn_p}\right)}{\theta_{protein}\left(p_i, \hat{p}_{i,knn_r}\right) + \varepsilon}$$

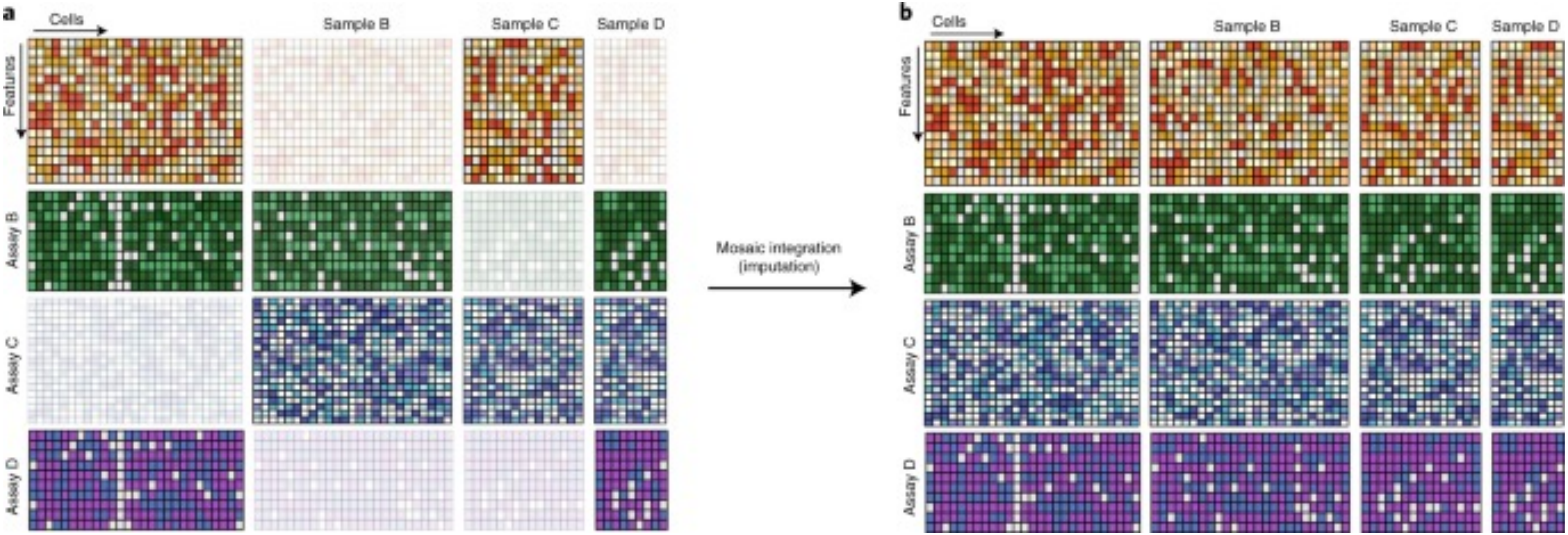
$$w_{rna}(i) = \frac{e^{s_{rna}(i)}}{e^{s_{rna}(i)} + e^{s_{protein}(i)}}, \quad w_{protein}(i) = \frac{e^{s_{protein}(i)}}{e^{s_{rna}(i)} + e^{s_{protein}(i)}}$$

MOFA+ (Argelaguet et. al., Genome Biology 2020)

- Apply Linear factor model on the data
- Apply spike-and-slab prior on both the feature factors and cell factors
  - Very challenging to solve, the authors used stochastic variational inference
  - Can deal with non-Gaussian likelihood, but very slow
- Should be (easy) to allow missing blocks (mosaic data) when performing the factor analysis (not implemented in the paper)



Multi-omics cells as bridges to integrate unpaired data



StabMap (Ghazanfar et. al., Nature Biotech, 2024)

- Essential idea: imputing the missing entries using linear factor analyses
  - Simpler example integrating three datasets, scRNA-seq, scATAC-seq, SNARE-seq

- Core steps:
  - For each reference data r (a reference data can have only one modality), obtain a linear embedding of the cells (for example, use PCA)

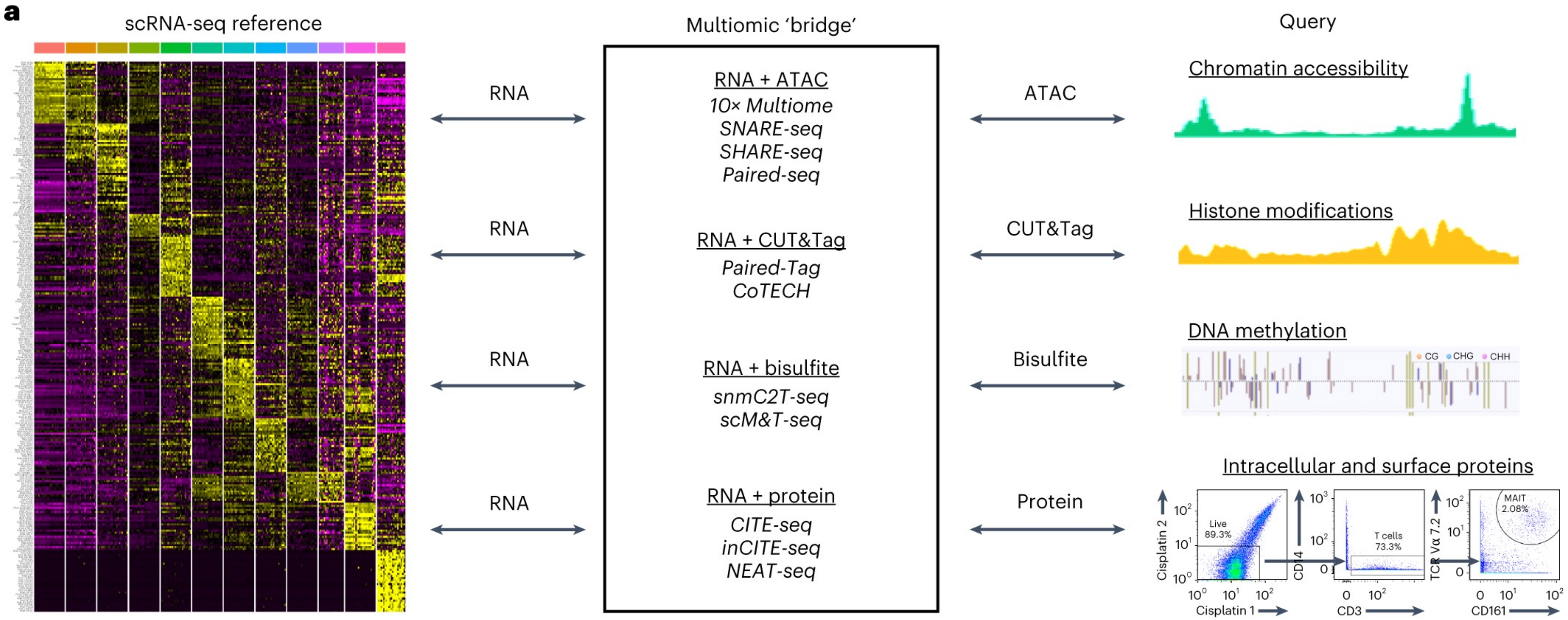
$$S_r = D_r^T \times A_r$$

  - For dataset i that only overlap part of the features with r, treat the cell embedding as outcome of each cell and train a linear prediction model of the embeddings using only the shared features using reference data r
    - Then predict the cell embeddings S_i^r using the prediction model
  - If dataset i does not have overlapping features with r, estimate S_i^r iteratively through a sequence of datasets that have overlapping features with each other
  - For each dataset, concatenate all embeddings as the final embedding

- Still need to perform batch correction on the final embedding

Seurat v5 (Hao et. al., Nature Biotech, 2024)

- Build reference using scRNA-seq and map cells of any modality onto a shared latent space



RNA + ATAC
10× Multiome
SNARE-seq
SHARE-seq
Paired-seq

RNA + CUT&Tag
Paired-Tag
CoTECH

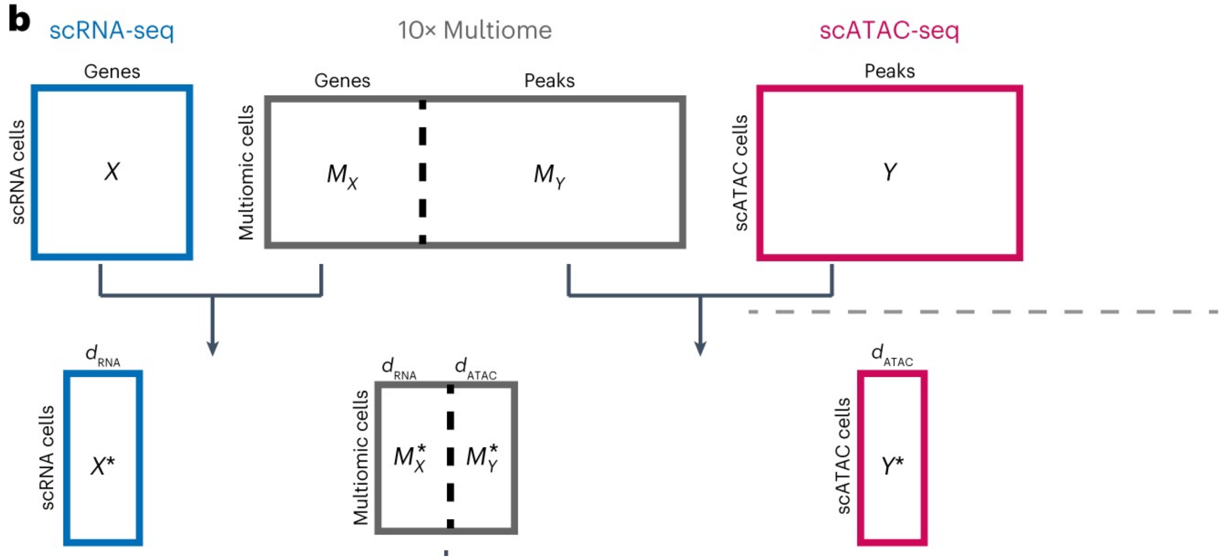
RNA + bisulfite
snmC2T-seq
scM&T-seq

RNA + protein
CITE-seq
inCITE-seq
NEAT-seq

Seurat v5 (Hao et. al., Nature Biotech, 2024)

Core steps:

- Data integration within modality across all datasets



Harmonize (within-modality) unimodal and bridge datasets

- Only need to integrate low-dimensional space.
- When merging between multiome and query data, can use other modality as supervision in dimension reduction
  - Supervised PCA: Construct a cell-cell similarity matrix L using both modalities
    - Find U that maximized the Hilbert-Schmidt Independence Criterion (HSIC):

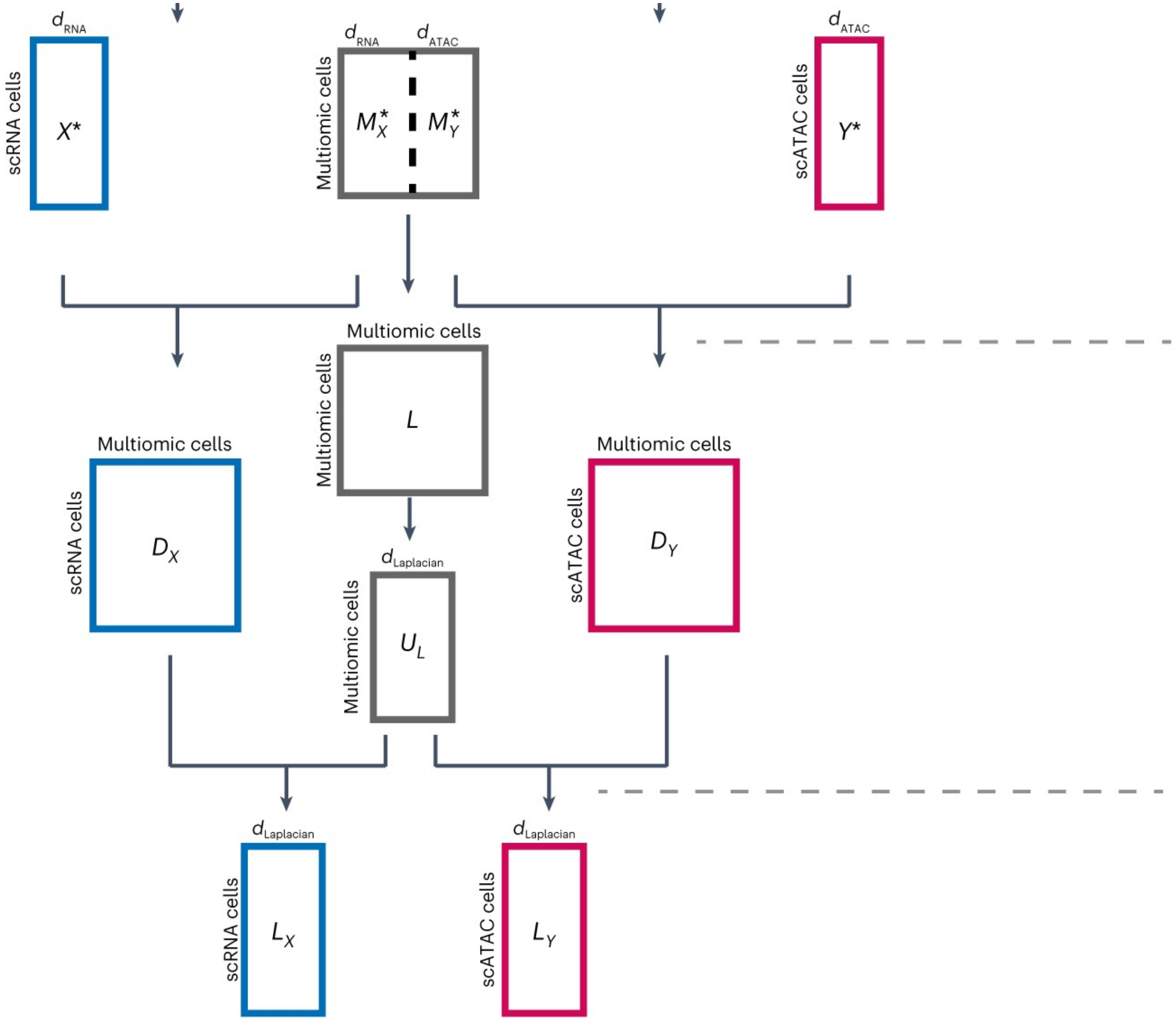
$$HSIC\left(\left(U^T X\right)^T U^T X, L\right)$$

$$= \frac{1}{(n-1)^2} tr\left(X^T U U^T X H L H\right)$$

Seurat v5 (Hao et. al., Nature Biotech, 2024)

Core steps:

- Construct dictionaries for each unimodal dataset



$$\arg\min_{D_X}(||D_X(M_X^*) - X^*||_F^2 + ||D_X||_F^2)$$

$$D_X = X^*(M_X^*)^\dagger$$

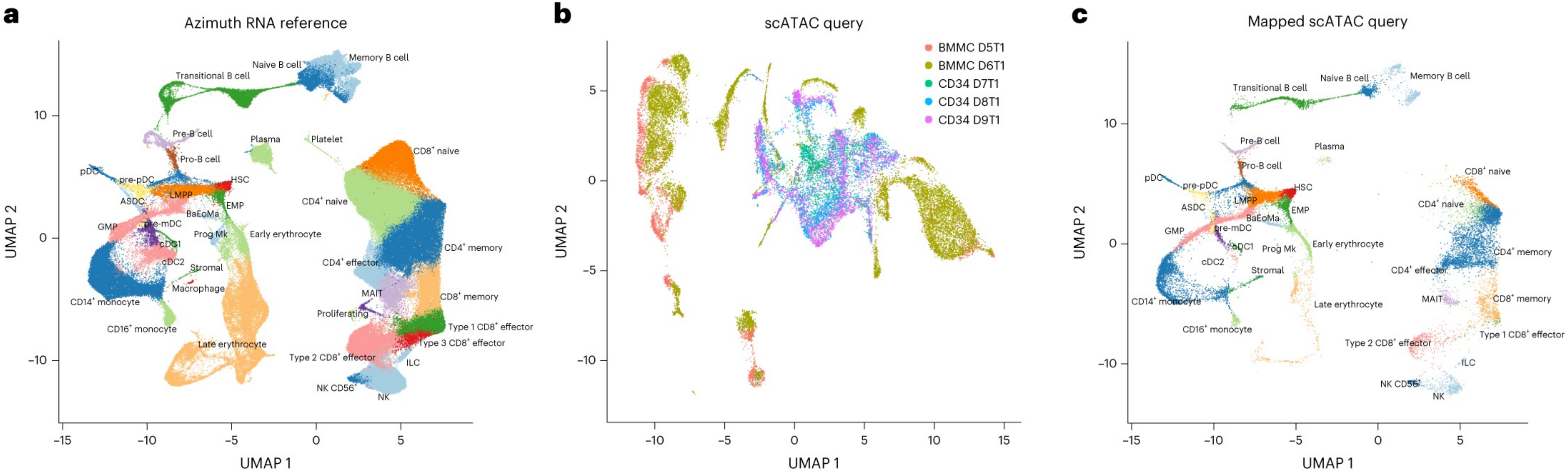
- Dimension reduction based on the multiomics data: $L = I - D^{-\frac{1}{2}}GD^{-\frac{1}{2}}$
  - Find U_L as the eigenvectors of the k smallest eigenvalues (except 0) of L
  - Map the unimodal data as the weighted average of the multi-omics cells

$$L_X = D_X U_L = X^*\left((M_X^*)^\dagger U_L\right)$$

$$L_Y = D_Y U_L = Y^*\left((M_Y^*)^\dagger U_L\right)$$

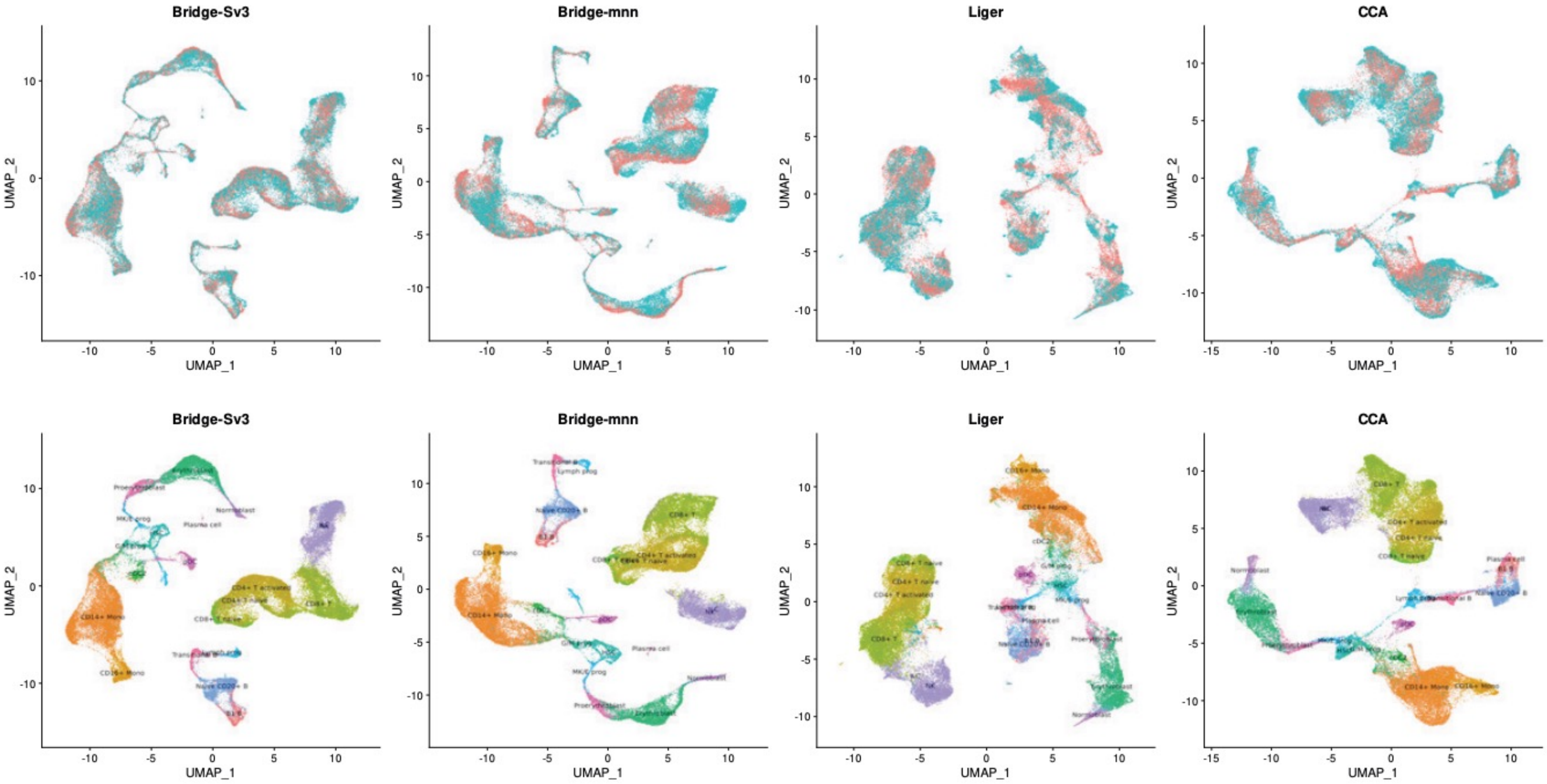
- Align the two datasets

Seurat v5 (Hao et. al., Nature Biotech, 2024)

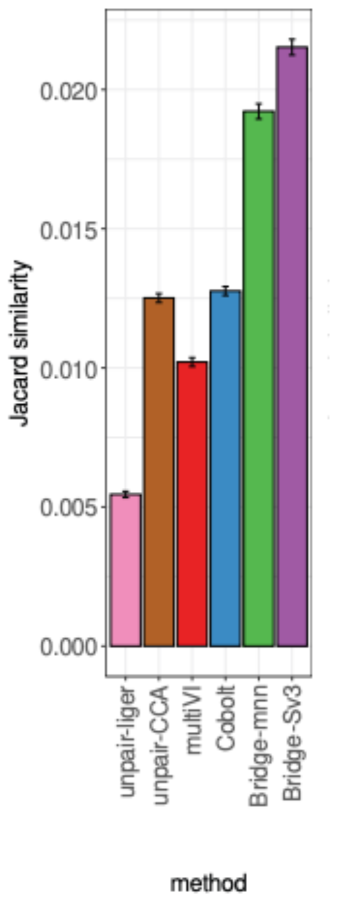


- Comparison with Seurat v3?

Seurat v5 (Hao et. al., Nature Biotech, 2024)



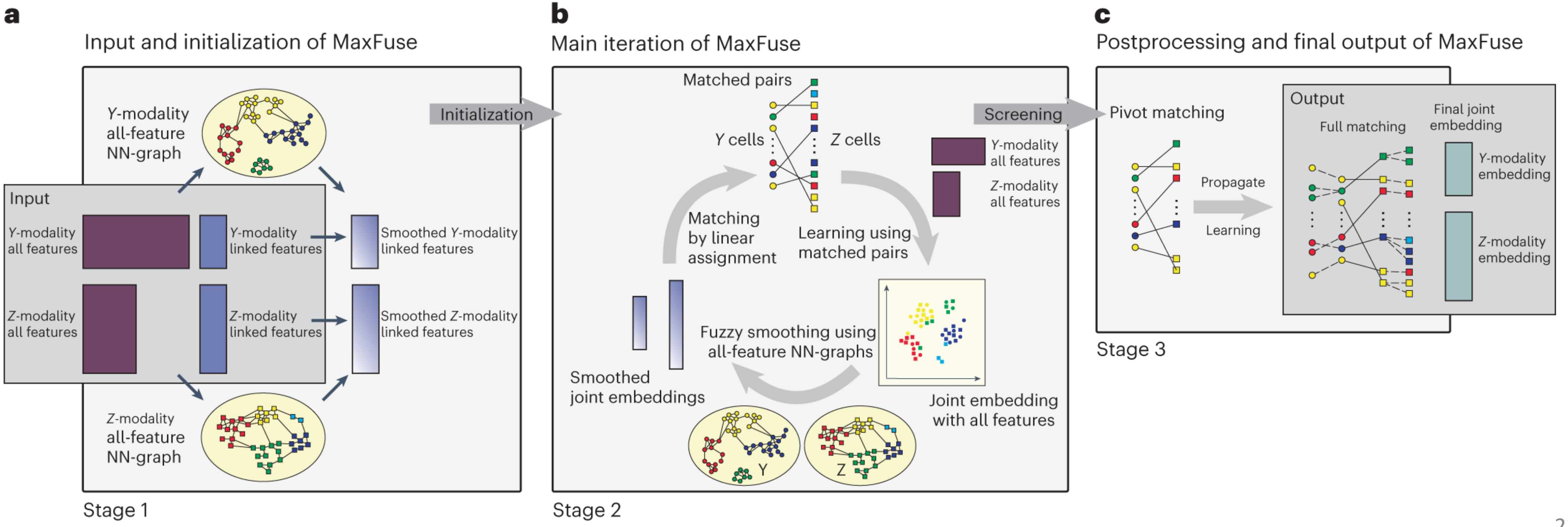
Matched cell label similarity



MaxFuse (Chen et. al., Nature Biotech, 2023)

- Core idea: smooth over similar cells and features to help find cell-cell pairs across modalities

- Inputs:
    - two unpaired single modality datasets
    - A pre-trained feature prediction model projecting both datasets on the same space
    - Noisy projection because the pre-trained model may not be reliable


a
Input and initialization of MaxFuse
Y-modality all-feature NN-graph
Initialization
Input
Y-modality all features
Y-modality linked features
Smoothed Y-modality linked features
Z-modality all features
Z-modality linked features
Smoothed Z-modality linked features
Z-modality all-feature NN-graph
Stage 1
b
Main iteration of MaxFuse
Matched pairs
Y cells
Z cells
Screening
Y-modality all features
Z-modality all features
Matching by linear assignment
Learning using matched pairs
Fuzzy smoothing using all-feature NN-graphs
Smoothed joint embeddings
Joint embedding with all features
Y
Z
Stage 2
c
Postprocessing and final output of MaxFuse
Pivot matching
Propagate
Learning
Output
Full matching
Final joint embedding
Y-modality embedding
Z-modality embedding
Stage 3

MaxFuse (Chen et. al., Nature Biotech, 2023)

- Initial smoothing of the projected data
  - Create meta cells within modality by Louvain clustering if data is too sparse
  - (fuzzy) smooth the projected data by similar cells within each modality

- Find initial matched pairs by optimal matching
  - D^0: Euclidean distance between two cells cross modalities based on projected data

$$\begin{aligned} \text{minimize} \quad & \langle \Pi, D^\circ \rangle \\ \text{subject to} \quad & \Pi \in \{0,1\}^{n_y \times n_z} \\ & \sum_i \Pi_{ij} \le 1, \forall j, \quad \sum_j \Pi_{ij} \le 1, \forall i, \\ & \sum_{i,\,j} \Pi_{ij} = n_{\min}\,. \end{aligned}$$

- Joint embedding of two datasets in the original space using CCA (CCA for the features instead of cells in Seurat) and the matched pairs
- Iterative refinement
  - Compute joint mapping via CCA using matched pairs of cells
  - (fuzzy) smoothing over similar cells
  - Apply optimal matching to find matched pairs of cells

- Similar to Seurat, only using a subset of pairs of cells as the anchor (pivot) pairs

Related papers

- Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck, W. M., ... & Satija, R. (2019). Comprehensive integration of single-cell data. *cell*, *177*(7), 1888-1902.
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- Chen, S., Zhu, B., Huang, S., Hickey, J. W., Lin, K. Z., Snyder, M., ... & Ma, Z. (2023). Integration of spatial and single-cell data across modalities with weakly linked features. *Nature Biotechnology*, 1-11.