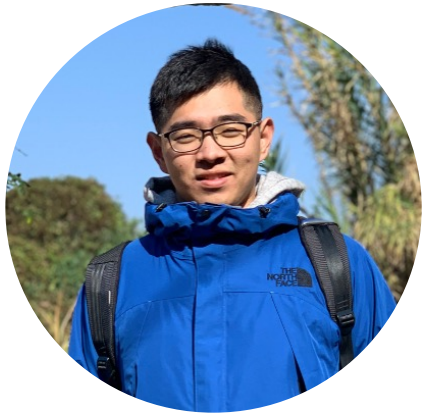
Lecture 4
Multi-Omics Causal Mediation Analysis and Single-Cell Multi-Omics Analysis

April 20, 2023



Instructor: **Rick Chang**

Outline

Multi-Omics Causal Mediation Analysis

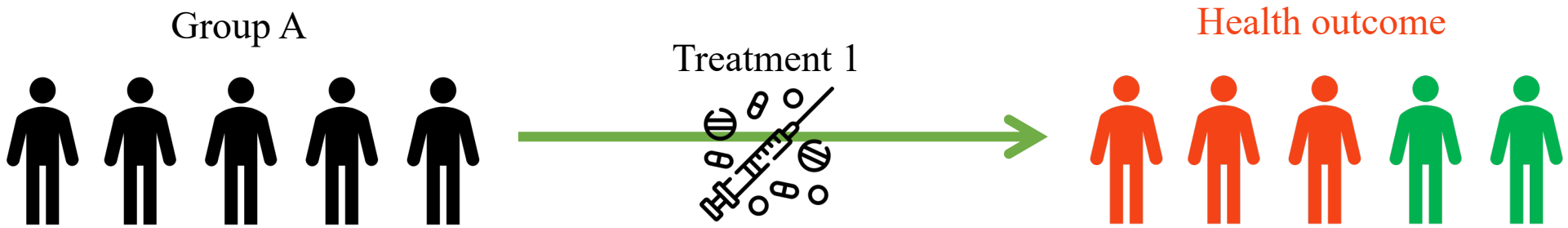
1. Causal mediation analysis
2. The difficulty of mediation analysis in omics data
3. Overview of high-dimensional mediation analysis
4. Penalization-based method: HIMA (lab session)

Single-Cell Multi-Omics Analysis

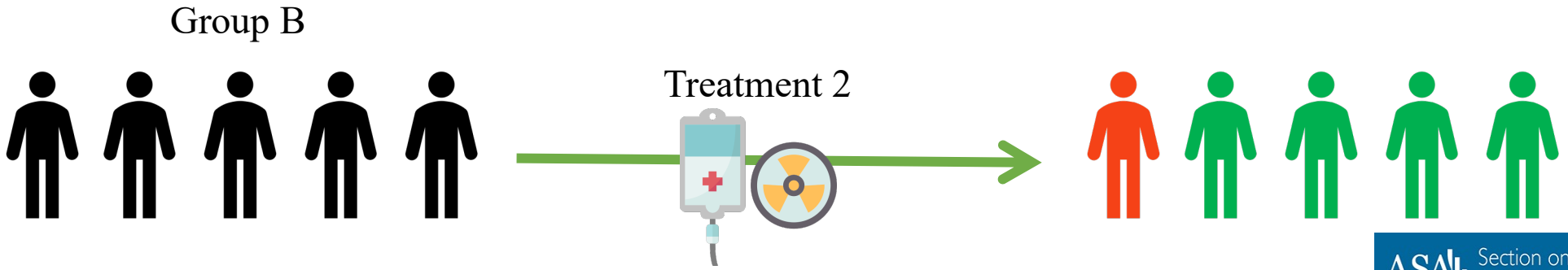
1. Bulk vs. Single-Cell
2. Single-Cell multi-omics data and integration methods
3. Integrated analysis: Seurat 4.0 (lab session)

Causation vs. Association

- Causal inference is an essential component for the discovery of disease mechanism.

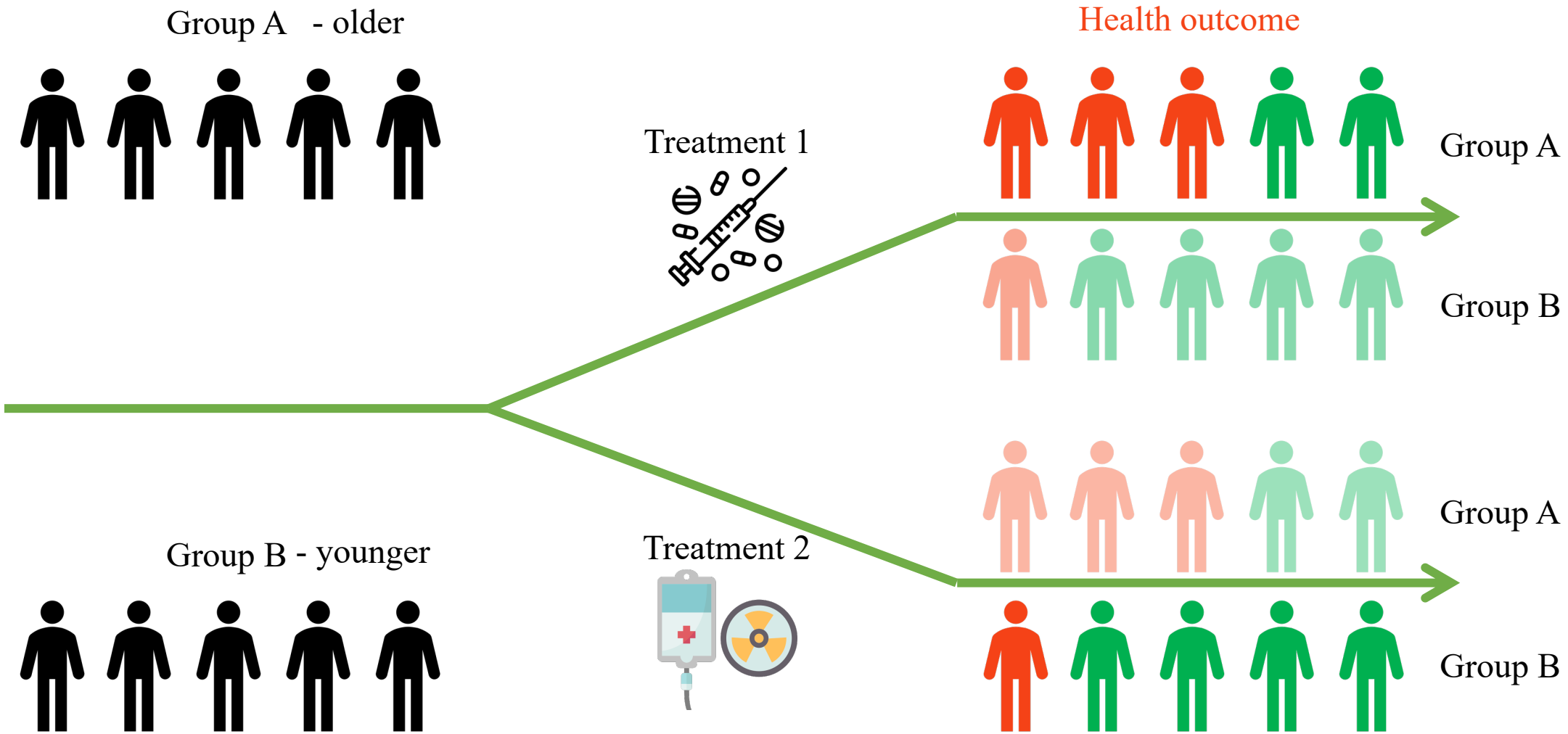


Exposure X ····?····> Outcome Y



Causation vs. Association

- To claim causation, we could do randomized experiment or control all confounding variables.
- If we control for age, each group would have the same outcome, regardless of treatment.



Mediation Effect

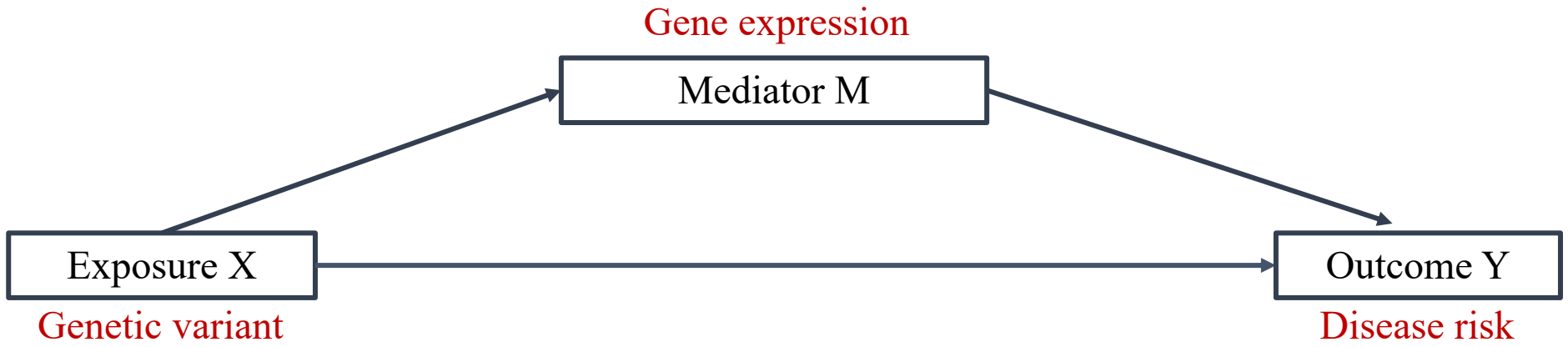
Mediation analysis: to further explore the mechanism behind the causation

Causal mediation effect:

- Exposure has a causal effect on the mediator
- Mediator has a causal effect on the outcome conditional on the exposure

Example

- Genetic variant leads different expression level which may increase the risk of disease.



Causal Mediation Analysis In Omics Studies

- Causal mediation analysis seeks to investigate the intermediate mechanism through an exposure on the outcome of interest.

- Rising interest in omics studies to identify the mechanism of molecular-level traits
  - E.g. DNA → RNA → Protein → outcome

- Mediation analysis in omics studies is challenging:
  - High-dimensional mediators → identifiability problem
  - Composite null hypothesis → weak power

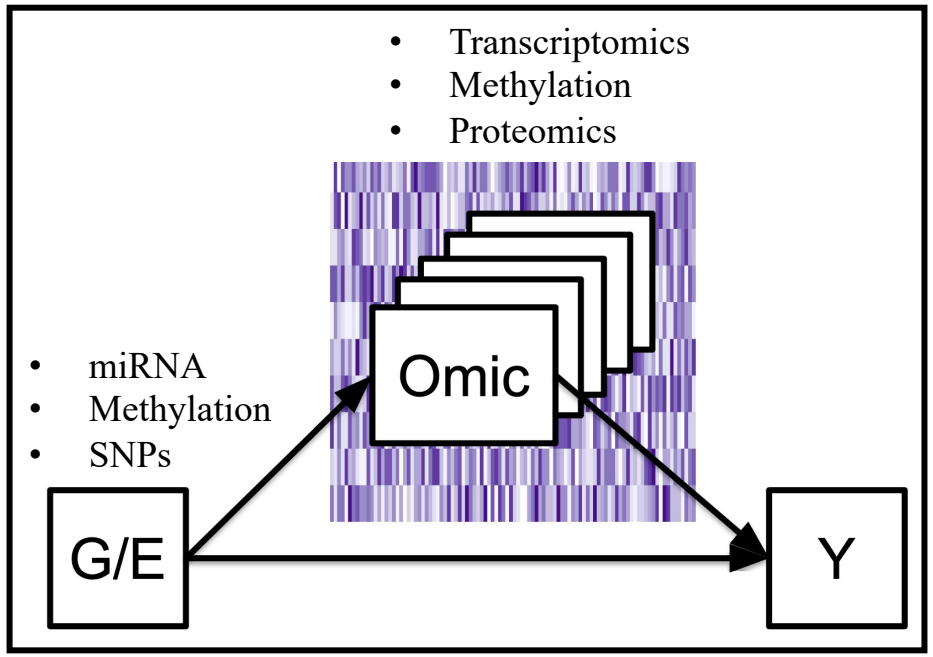
Some Applications

General setting: One exposure → multiple mediators → one outcome

- Environment → DNA Methylation → Outcome
  - E.g. Normative Aging Study (Bind et al., 2014 Epigenetics; Zhang et al., 2016 Bioinformatics; Liu et al., 2022 JASA)
    Prostate Cancer (Dai et al., 2022 JASA)
    Atherosclerosis (Song et al., 2020 Biometrics; Clark-Boucher et al., 2023 medRxiv)
  - Also known as epigenome-wide mediation analysis

- microRNAs → Gene expression → Outcome
  - E.g. Glioblastoma (Huang et al., 2014 AOAS; Huang and Pan, 2016 Biometrics)
    Brain cancer (Loh et al., 2020 Biometrics)

- Others
  - E.g. Air Pollution (Inoue et al., 2020 JASA)
    Neuroimaging (Chén et al., 2018 Curr. Environ. Health Rep.; Zhao et al., 2021 CSDA)



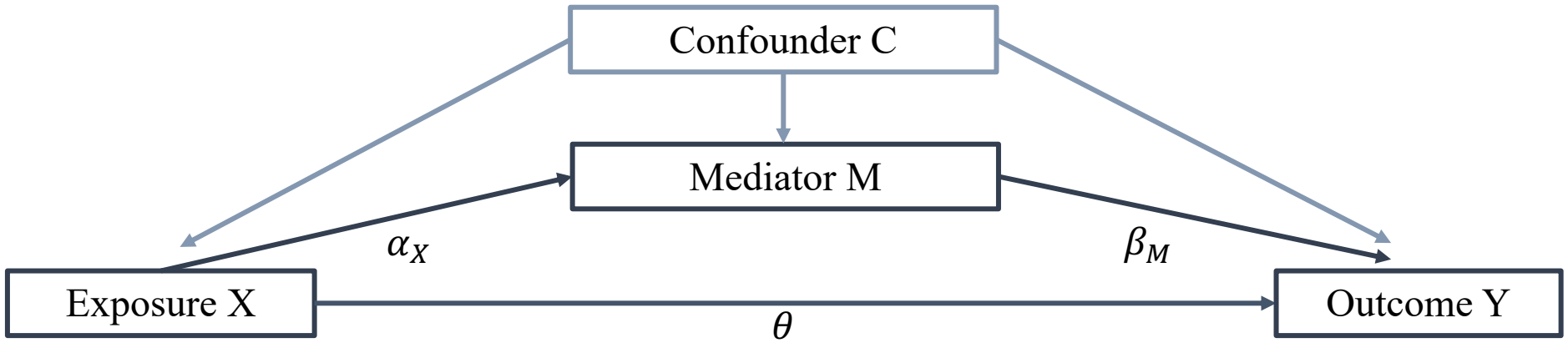
Causal Mediation Model

Two linear regressions method proposed by Baron and Kenny, 1986 J Pers Soc Psychol

- (Model X → M) $M = C\alpha_C + X\alpha_X + \epsilon_M$
- (Model M → Y) $Y = C\beta_C + X\theta + M\beta_M + \epsilon_Y$, where $\epsilon_Y \sim N(0, \sigma_Y^2)$ and $\epsilon_M \sim N(0, \sigma_M^2)$
- Since $Y = C\beta_C + X\theta + M\beta_M + \epsilon_Y$

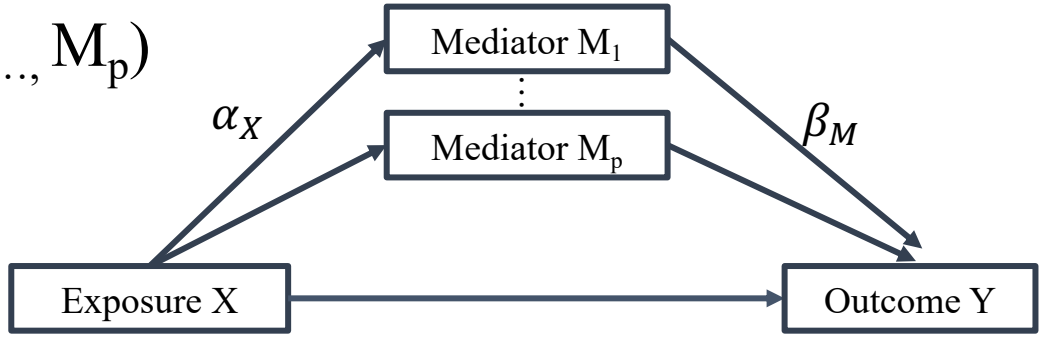
  $= C\beta_C + X\theta + (C\alpha_C + X\alpha_X + \epsilon_M)\beta_M + \epsilon_Y$

  $= C\beta_C + X\theta + C\alpha_C\beta_M + X\alpha_X\beta_M + \epsilon_Y^*$
- Direct effect is θ, and indirect effect (mediation effect) can be expressed as $\alpha_X\beta_M$
- Total effect $\gamma = \theta + \alpha_X\beta_M$



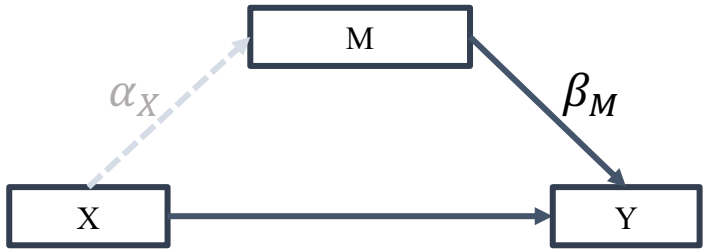
High-Dimensional Mediation Analysis

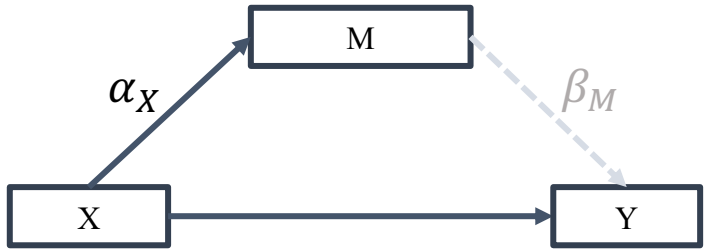
- Challenge 1: High-dimensional mediators ($M_{1,\ldots,} M_p$)
  - (Model M → Y) $Y = C\beta_C + X\theta + \sum_j M_j\beta_{M,j} + \epsilon_Y$
  - When the number of mediators (p) is much greater than the sample size (N), $\beta_{M,j}$ are not estimable.

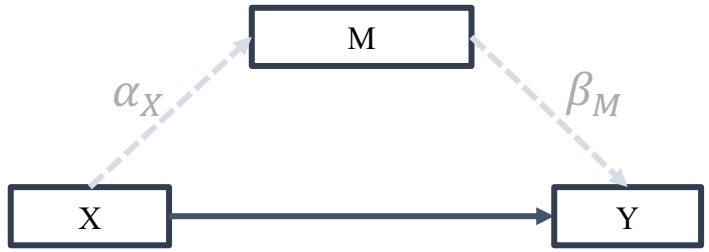


  - Identification assumptions could be easily violated after dimension reduction (Huang and Pan, 2016 Biometrics)

- Challenge 2: Composite null hypothesis (H_0: $\alpha_X\beta_M = 0$)
  - Traditional hypothesis tests are underpowered for testing composite null hypothesis. (Liu et al., 2022 JASA)
    E.g. Sobel's test and joint significant test (MaxP)

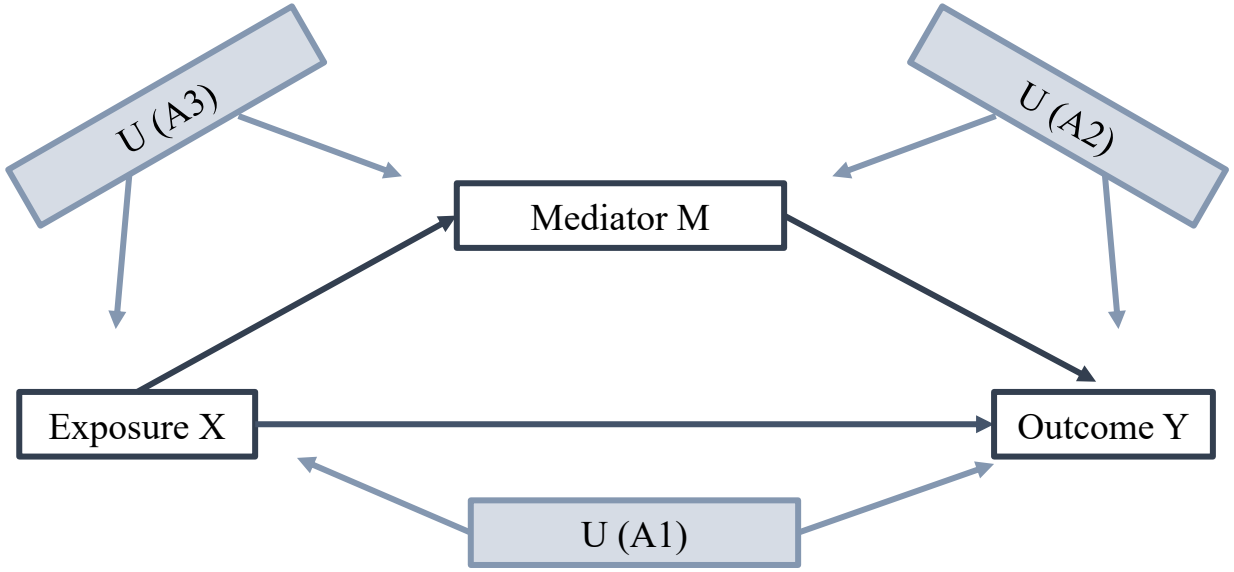


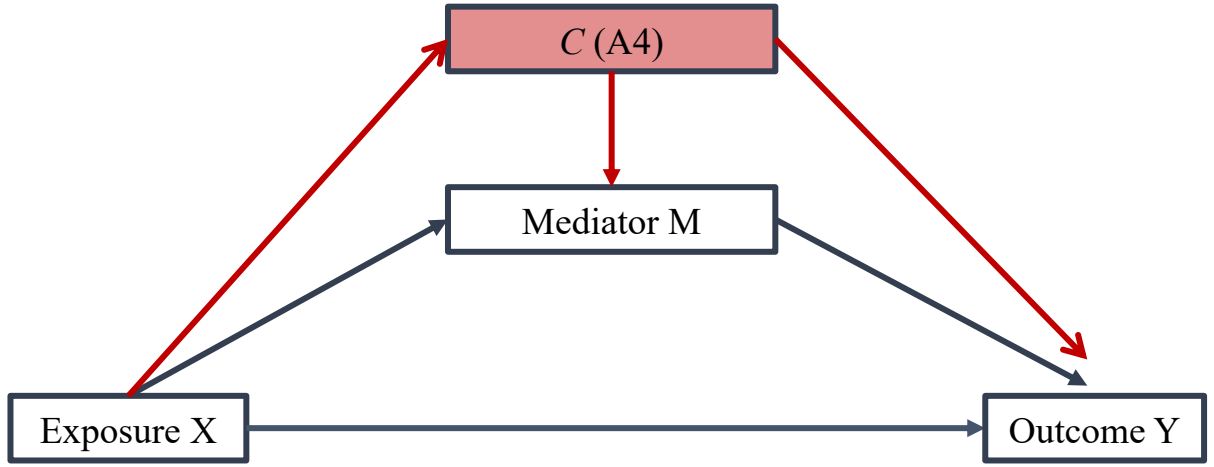




Four Identification Assumptions

- [A1] $Y(x) \coprod X|C$: no unmeasured confounding for the association of Y and X
- [A2] $Y(x, \boldsymbol{m}) \coprod \boldsymbol{M}|(X, C)$: no unmeasured confounding for the association of Y and **M** given X
- [A3] $\boldsymbol{M}(x) \coprod X|C$: no unmeasured confounding for the association of **M** and X
- [A4] $Y(x, \boldsymbol{m}) \coprod \boldsymbol{M}(x^*)|C$: no X-induced confounder for the **M**-Y association (cross-world assumption)





Since the causal ordering of mediators is unknown, it is hard to identify causal effect through the single mediator. (Clark-Boucher et al., 2023 medRxiv)

VanderWeele and Vansteelandt, 2014 Epidemiologic methods

Overview Of High-Dimensional Mediation Analysis

First category: Mediation methods based on dimension reduction or mediator screening

Methods	Test Statistics	Null Distribution
correlation-based method	P_{max}	permutation
Huang-Pan method	marginal and component-wise ME based on PCA	Monte Carlo (normal-based or bootstrapping)
causal inference test (CIT)	P_{max}	permutation
direction of mediation	PCA-based	bootstrapping
MCP-subset	P_{max}	screening followed by multiple comparison procedure
MCP-subset based on Westfall-Young	P_{max}	screening followed by multiple comparison procedure
MCP-subset based on multivariate	P_{max}	screening followed by multiple comparison procedure
HDMA	P_{max}	screening followed by debiased estimation
gHMA#	ACAT combining gHMA-L and gHMA-NL	screening followed by multiple comparison procedure
global test + ScreenMin#	P_{min} followed by P_{max}	screening followed by multiple comparison procedure

Second category: Mediation methods accounting for the composite nature of the null

Methods	Test Statistics	Null Distribution
JTV-comp#	mixture of multiple-mediator based *P* value without estimating the proportions	composite null
JT-comp	mixture of single-mediator based *P* value without estimating the proportions	composite null
DACT	mixture of single-mediator based *P* value with estimated proportion	composite null
JS-mixture	mixture of single-mediator based *P* value with estimated proportion	composite null

Third category: Penalization-based mediation regression methods and Bayesian mediation methods

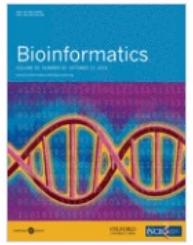
Methods	Prior Effects Assumptions	Optimization Procedure
pathway Lasso	penalization based method	ADMM
HIMA	P_{max}	screening followed by minimax concave penalty estimation
BAMA	spike-and-slab prior	MCMC
BAMA with joint priors	Gaussian mixture prior and, product threshold Gaussian prior	MCMC
BAMA with joint priors considering correlation among mediators	the Potts prior and logistic normal prior	MCMC

Popular methods

*Lab session

Zeng et al., 2021 Computational and structural biotechnology journal

High-Dimensional Mediation Analysis (HIMA)



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Original Paper

Genetics and population analysis

Estimating and testing high-dimensional mediation effects in epigenetic studies

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- The most user-friendly tools for high-dimensional mediation analysis

Supported mediator types:
- Epigenetics
- Transcriptomics
- Proteomics
- Metabolomics
- Microbiome

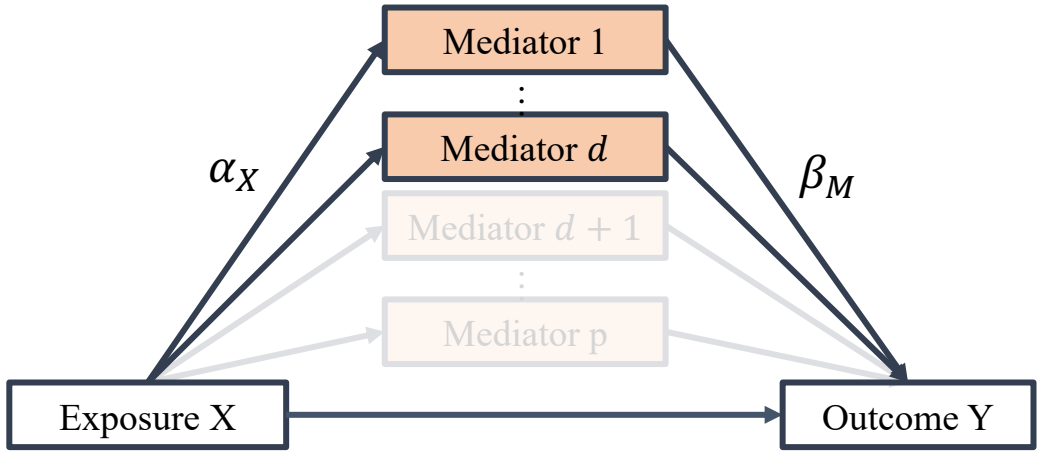
Supported outcomes:
- Continuous
- Binary
- Count
- Survival

Zhang et al., 2016 Bioinformatics

High-Dimensional Mediation Analysis (HIMA)

- HIMA assumes that the true mediators are sparse and applied Sure independence screening and penalty regression to reduce the dimensionality.

- Workflow
  1. Sure independence screening to identify those mediators with large absolute β_k
  2. Penalty regression: Minimax concave penalty for variable selection
  3. Joint significance test (MaxP) of mediator effect (p-value of α_X and β_M)
     $T_{MaxP} = \max(p_\alpha, p_\beta)$
  4. Control the family wise error rate (Bonferroni)



(Model X → M)

- $M_1 = C\alpha_{C,1} + X\alpha_{X,1} + \epsilon_{M1}$

  ⋮

- $M_d = C\alpha_{C,d} + X\alpha_{X,d} + \epsilon_{Md}$

(Model M → Y)

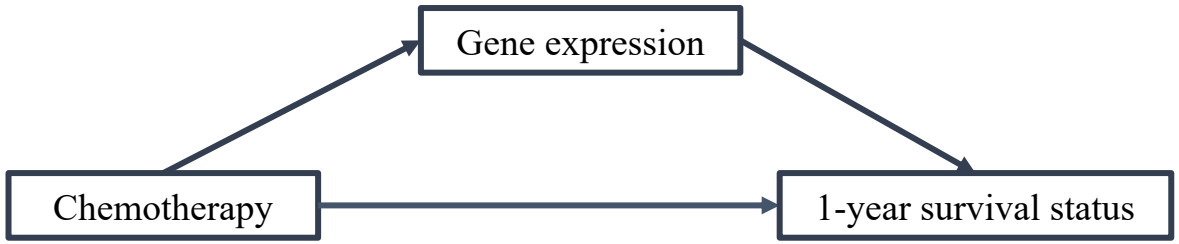
- $Y = C\beta_C + X\theta + M_1\beta_{M,1} + \cdots + M_d\beta_{M,d} + \epsilon_Y$

SIS: Fan and Lv, 2008 JRSS B; **MCP**: Zhang, 2010 Ann. Stat

TCGA Glioblastoma Multiforme

- 469 patients of glioblastoma multiforme have complete genomic data on gene expression (UNC AgilentG4502A-07) archived in The Cancer Genome Atlas (TCGA).
- **Chemotherapy** have been reported to be associated with **survival** of cancer patients.
- Hypothesis: chemotherapy affects survival outcome mainly through its influence on gene expression levels

Exposure (X): chemotherapy (Yes/No)
Mediator (M): gene expression (17450 genes)
Outcome (Y): dichotomous 1-year survival status
Confounders (C): Age, Gender



	alpha	beta	gamma (Total effect)	alpha*beta (Mediation effect)	% total effect	Bonferroni.p	BH.FDR
DHRS12	0.2270880	-0.3282940	1.517597	-0.0745516	-4.9124789	0.0202180	0.0067547
NDUFA7	0.1575399	1.0372184	1.517597	0.1634033	10.7672359	0.0086816	0.0024884
OR52R1	-0.1427211	0.0156191	1.517597	-0.0022292	-0.1468885	0.3949076	0.0987269
PIGS	-0.1480009	0.5642783	1.517597	-0.0835137	-5.5030204	0.0202642	0.0067547

To understand the biological mechanism across multi-omics, you can also try different exposure.
E.g. methylation and miRNA

Outline

Multi-Omics Causal Mediation Analysis

1. Causal mediation analysis
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3. Overview of high-dimensional mediation analysis
4. Penalization-based method: HIMA (lab session)

Single-cell Multi-Omics Analysis

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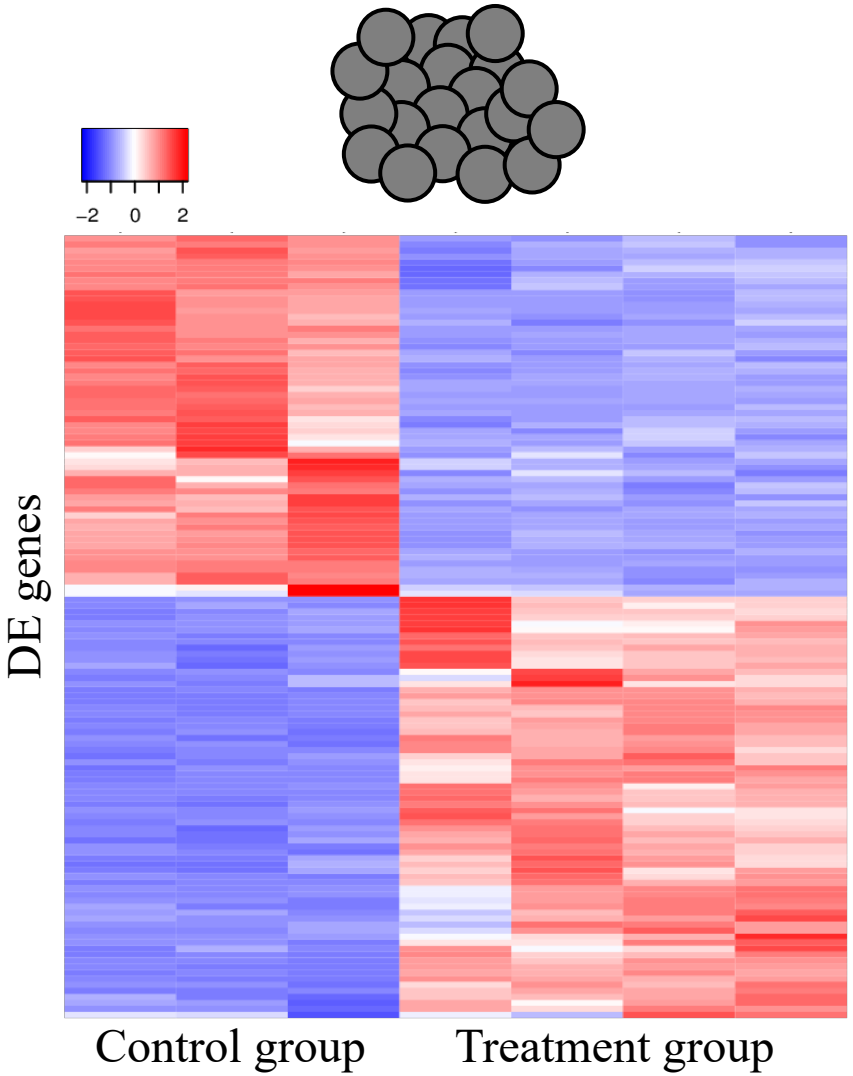
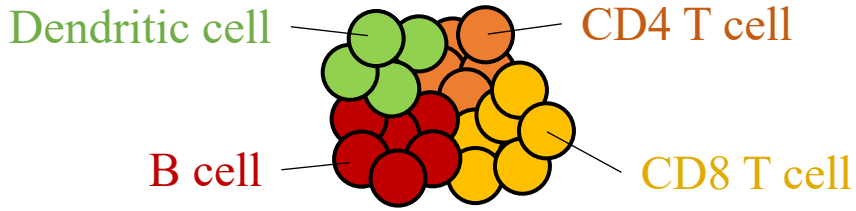
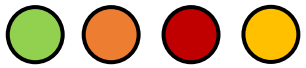
From Bulk To Single-Cell

Bulk RNA-Seq



Single-Cell level



Bulk Expression change	Single-Cell possibility 1	Single-Cell possibility 2
Increase	Increase in: 	Increase in: 
Decrease	Decrease in: 	Decrease in: 
No change	No change in: 	Increase in:  Decrease in: 

https://www.youtube.com/watch?v=y1mFdkDVc-c&t=651s&ab_channel=ActiveMotif

Heterogeneity And Rarity

- Understanding disease machenism is challenging because of heterogeneity and rarity of target cells.
- E.g. HIV cells < 0.1% (Collora et al., 2022 Immunity)

Bulk data

Single-Cell data

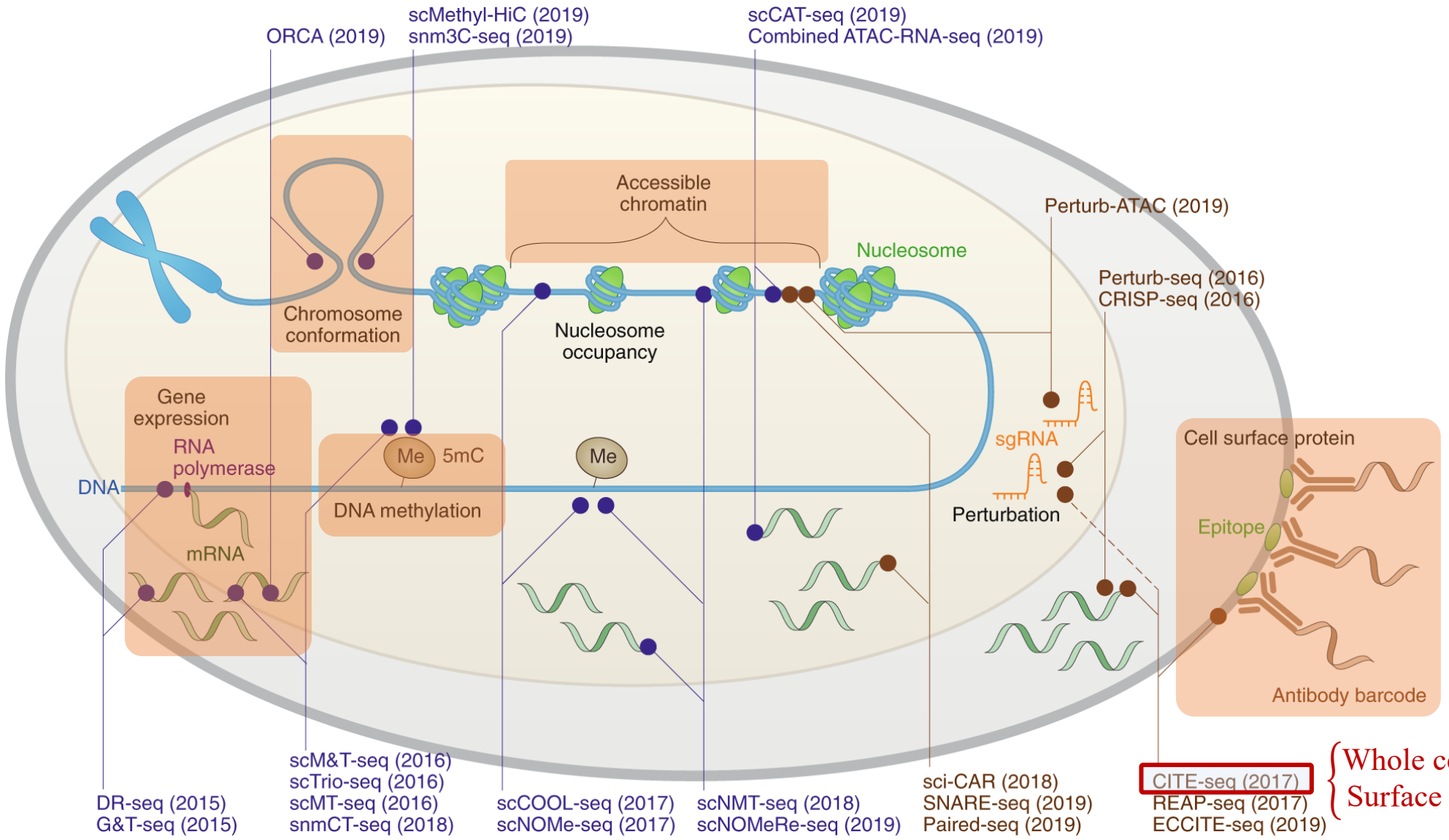
Heterogeneity

Rarity

Analogy from Ya-Chi Ho's talk

Pan and Jia, 2021 Frontiers in Molecular Biosciences; Liu et al., 2019 Nature Communications; Collora et al., 2022 Immunity

Single-Cell Multi-Omics Data



Zhu et al., 2020 Nature methods

Single-Cell (Multi-)Omics Methods

- CITE-Seq and 10X Multiome are the two frequently used methods in single-cell multi-omics.

	Epigenome	Transcriptome		Proteome
	Chromatin accessibility	Nuclear RNA	Whole cell RNA	Protein abundance
scRNA-Seq			✓	
snRNA-Seq		✓		
scATAC-Seq	✓			
CITE-Seq			✓	✓ (surface)
ASAP-Seq	✓			✓ (surface + intracellular)
10X Multiome	✓	✓		
inCITE-Seq		✓		✓ (intranuclear)
DOGMA-Seq	✓		✓	✓(surface + intracellular)

- DOGMA = CITE-Seq + 10X Multiome

From Wei Chen's lab

CITE-Seq

Example: CITE-Seq = scRNA-Seq + ADT

- scRNA: gene expression (Transcriptome)
- ADT: surface protein (Proteome)

Zero expression because of "technical" dropouts

scRNA-Seq

Genes (36,601)

SLC35E2A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
NADK	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
GNB1	1	.	.	.	6	.	2	1	.	.	2	1	1	.	1	2	.	2		
AL109917.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CALML6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
TMEM52	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
CFAP74	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AL391845.2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
GABRD	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AL391845.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PRKCZ	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
AL590822.2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
PRKCZ-AS1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
FAAP20	.	.	.	.	1	3	.	.	.	.	1	.	1	1	1	2	1	.		
AL590822.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Noisy but broad

✓ Large number of genes

✗ High rate of false negative

Antibody-Derived Tags (ADTs)

Antibodies (163)

CD86-A0006	2	.	1	1	.	.	2	1	1	2	1
CD274-A0007	23	20	10	29	24	43	18	69	25	13	58
CD270-A0020	39	45	39	40	41	31	30	27	33	29	29
CD155-A0023	7	6	4	13	4	4	1	4	4	8	6
CD112-A0024	9	21	4	33	13	5	16	10	6	12	16
CD47-A0026	166	140	118	66	82	102	113	107	146	57	125
CD48-A0029	73	66	54	33	136	127	101	55	111	65	84
CD40-A0031	2	1	4	8	3	5	3	2	3	5	5
CD154-A0032	23	40	19	92	20	54	27	28	24	29	35
CD52-A0033	55	33	18	31	18	40	33	34	33	18	37
CD3-A0034	7	1	2	3	2	5	4	3	6	2	2
CD8-A0046	2	.	2	142	.	14	64	.	309	140	1
CD56-A0047	6	5	2	8	9	2	5	5	5	10	6
CD19-A0050	1	.	1	.	.	.	.	.	1	.	.
CD33-A0052	4	4	1	1	.	1	5	2	1	5	2

Targeted but narrow

✓ Doesn't have dropout problem

✗ Limited number of antibodies

Data from Stuart et al., 2019 Cell

Data Integration Strategy for Matched and Unmatched Data

Table 1 | **Methods for matched data analysis**

Tool	Data type	Model	Additional notes	Documentation	Ref.
BREM-SC	T + P	Early integration, probabilistic modelling	This method models the observed data by multinomial distributions and assumes data from both modalities to be generated in a cluster-specific manner	https://github.com/tarot0410/BREMSC	35
scAI	T + C	Early integration, latent space modelling	scAI iteratively updates a regularized matrix factorization model to obtain an optimal common cell-loading matrix across two modalities	https://github.com/sqjin/scAI	36
MOFA+	T + C	Early integration, latent space modelling	MOFA and MOFA+ were built on the framework of group factor analysis but extend the model to enable the integration of different data types (count versus binary)	https://github.com/bioFAM/MOFA2	38
TotalVI	T + P	Early integration, latent space modelling	This method uses a variational autoencoder framework built on scVI. In this method, the protein measurements are modelled with a negative binomial mixture distribution to account for background reads	https://github.com/YosefLab/scvi-tools	39
CiteFuse	T + P	Late integration, latent space modelling	The similarity measurement for protein data is based on a proportionality coefficient and the similarity measurement for RNA data is constructed with the Pearson correlation	https://github.com/SydneyBioX/CiteFuse	42
Seurat 4.0	T + P	Late integration, latent space modelling	Computes a weighted average cell affinity matrix from modality-specific affinity matrices. The weights are computed to reflect the predictive information within a cell's local neighbourhood defined within each modality	https://github.com/satijalab/seurat	40

BREM-SC, Bayesian random effects mixture model-single cell; C, chromatin accessibility; MOFA, multi-omics factor analysis; P, proteome; scAI, single-cell aggregation and integration; scVI, single-cell variational inference; T, transcriptome.

Table 2 | **Methods for unmatched data analysis**

Strategy	Tool	Data type	Feature matching	Algorithm	Additional notes	Documentation	Ref.
Group matching	Stereoscope	T + ST	R	Deconvolution	This method assumes negative binomial distributions of genes and tolerates differential gene capture efficiencies between two technologies	https://github.com/almaan/stereoscope	53
	MAESTRO	T + C	R	CCA + MNN	This method implements ChIP–seq data-based TF enrichment score calculators to define core TFs in each cell-type cluster	https://github.com/liulab-dfci/MAESTRO	49
Comon features	STvEA	MI + ET	R	MNN	This method also provides a framework to transfer cell-type annotations from one modality to another	https://github.com/CamaraLab/STvEA	54
	Clonealign	T + D	R	Variational Bayes	This method assumes correlation between DNA copy number and gene expression within the same region	https://github.com/kieranrcampbell/clonealign	56
	Seurat 3.0	T + C	R	CCA + SNN	This method identifies anchor cells between datasets based on SNN across modalities; these anchor cells serve as a bridge for matching	https://github.com/satijalab/seurat	57
	LIGER	T + M, T + C	R	iNMF	The relative contribution of dataset-specific factors and shared factors is determined by a hyperparameter λ, which can be used to fine-tune the integration results	https://github.com/welch-lab/liger	58
Aligning spaces	MAGAN	MI + T	R	GAN	This method identifies cell-to-cell correspondence by adding a loss function defined by similarity of cell matching; such loss function requires at least some shared features between two datasets	https://github.com/KrishnaswamyLab/MAGAN	60
	MATCHER	T + C	NR	Manifold alignment	This method assumes 1D structure (pseudotime) with a pre-specified direction	https://github.com/jw156605/MATCHER	61
	MMD-MA	T + M	NR	MMD	In addition to the MMD loss, the loss function also has a distortion loss and a penalty to ensure the dimensionality and orthogonality of each projection	https://bitbucket.org/noblelab/2019_mmd_wabi/src/master/	62
	UnionCom	T + M	NR	GUMA	The algorithm generalizes the GUMA method to achieve soft matching between datasets, enabling matching with different numbers of cells	https://github.com/caokai1073/UnionCom	63
	SCOT	T + C	NR	GWOT	A late integration method in which a similarity matrix is constructed by each modality separately, after which probabilistic transportation between datasets is achieved by GWOT	https://github.com/rsinghlab/SCOT	64

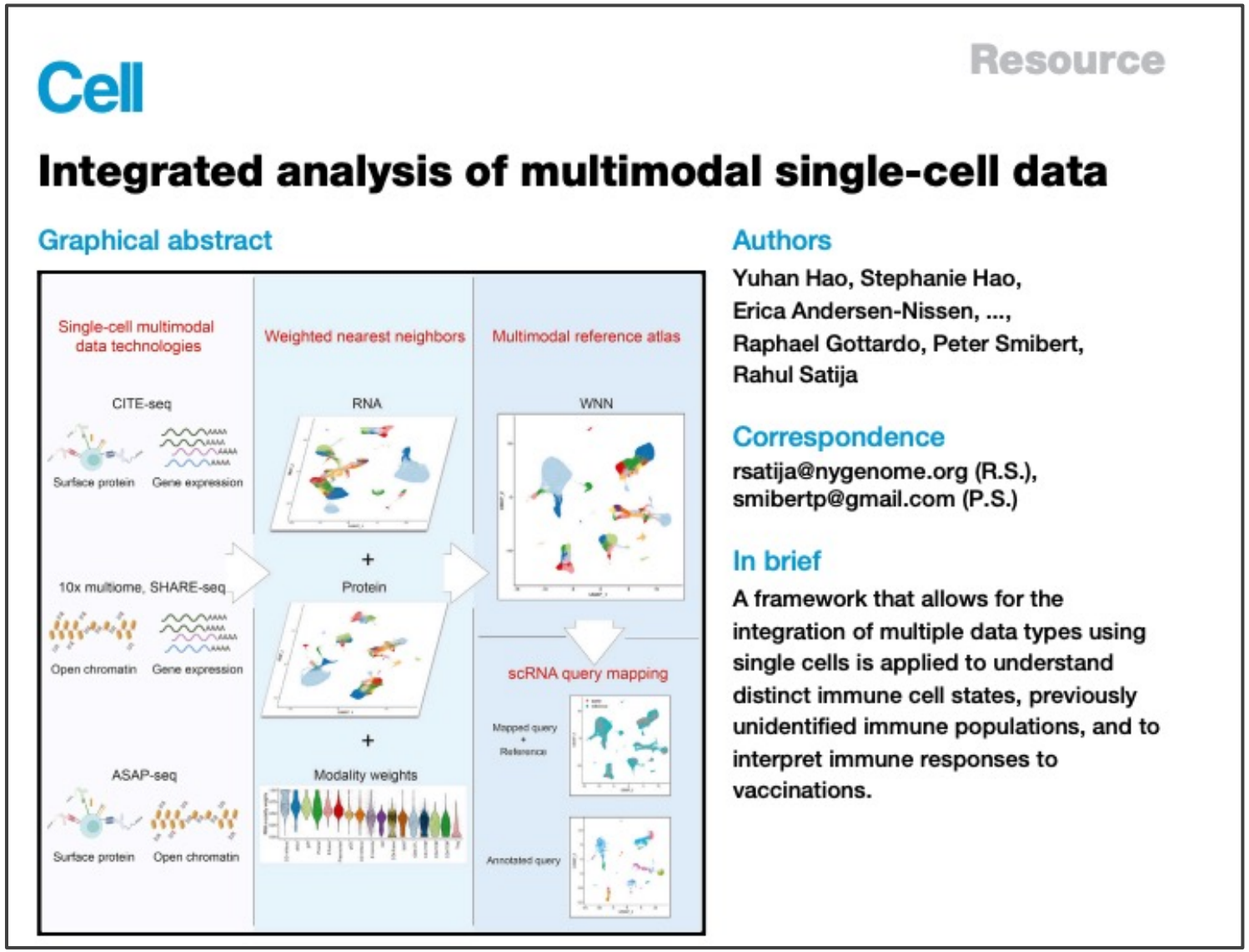
C, chromatin accessibility; CCA, canonical correlation analysis; ChIP–seq, chromatin immunoprecipitation followed by sequencing; D, DNA; ET, simultaneous epitope and transcriptome; GAN, generative adversarial networks; GUMA, generalized unsupervised manifold alignment; GWOT, Gromov–Wasserstein optimal transport; iNMF, integrative non-negative matrix factorization; M, methylome; MI, multiplexed immunohistochemistry; MMD, maximum mean discrepancy; MNN, mutual nearest neighbours; NR, not required; R, required; SNN, shared nearest neighbours; ST, spatial transcriptome; T, transcriptome; TF, transcription factor.

Matched data: different modalities were profiled from the same cell

Unmatched data: different modalities were profiled from different cells

Miao et al., 2021 Nephrology

Seurat 4.0



Cell

Resource

Integrated analysis of multimodal single-cell data

Graphical abstract

Authors

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In brief

A framework that allows for the integration of multiple data types using single cells is applied to understand distinct immune cell states, previously unidentified immune populations, and to interpret immune responses to vaccinations.

- Introduce to WNN
- Hands-on CITE-Seq analysis
- Omics vs. Multi-Omics analysis

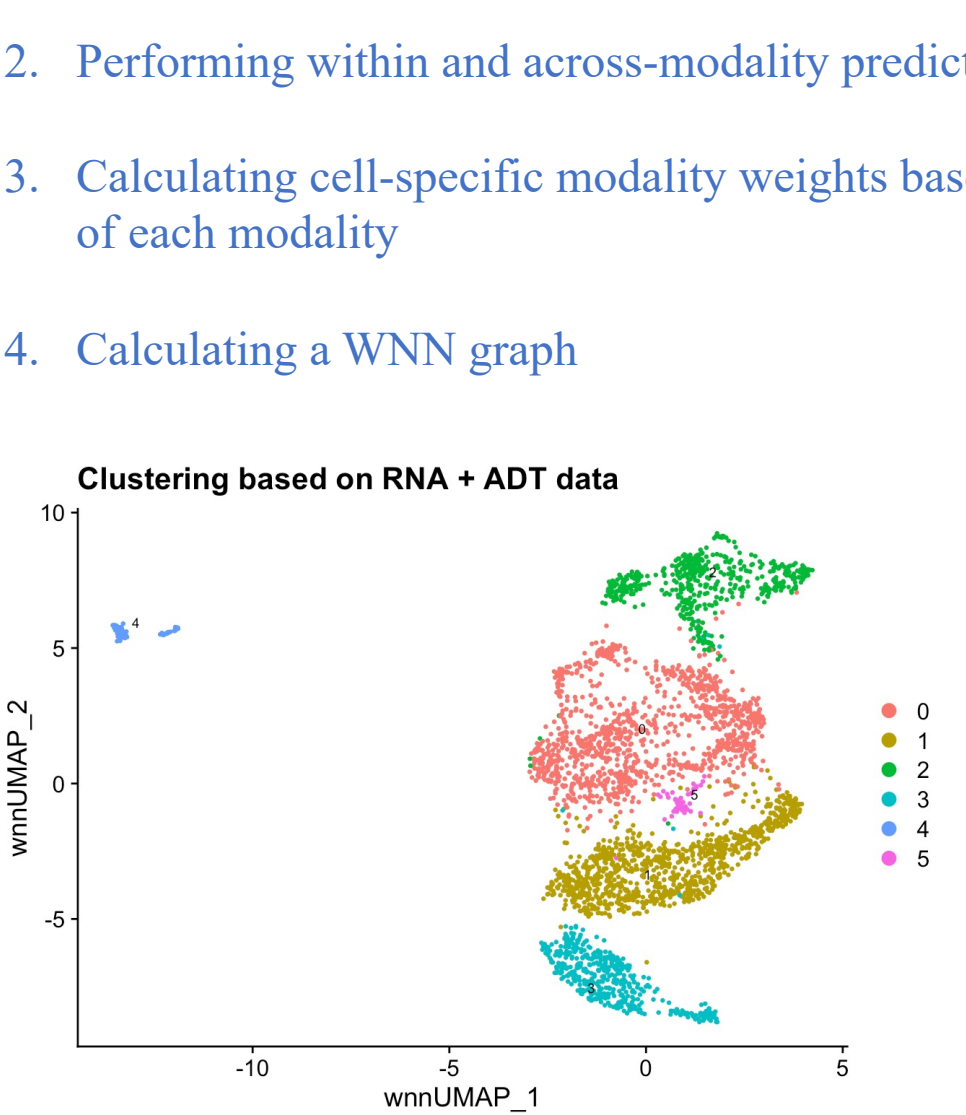
Weighted Nearest Neighbor Analysis

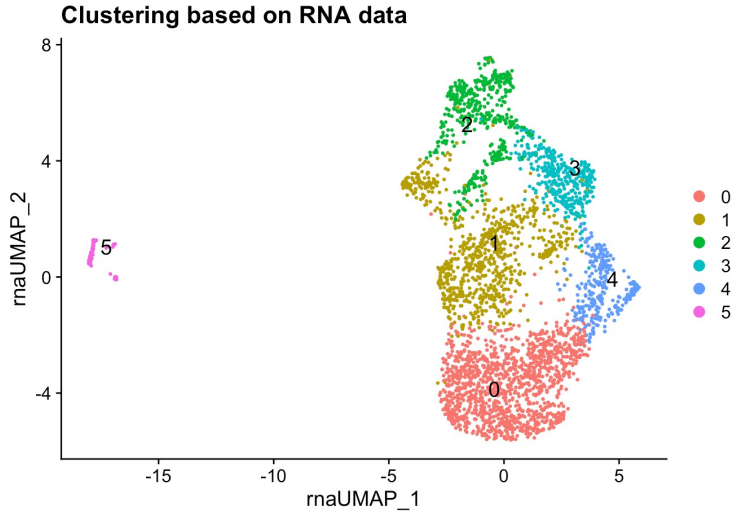
- Parallele intergration analysis
- An unsupervised framework to learn the relative utility of each data type in each cell, enabling an integrative analysis of multiple modalities.

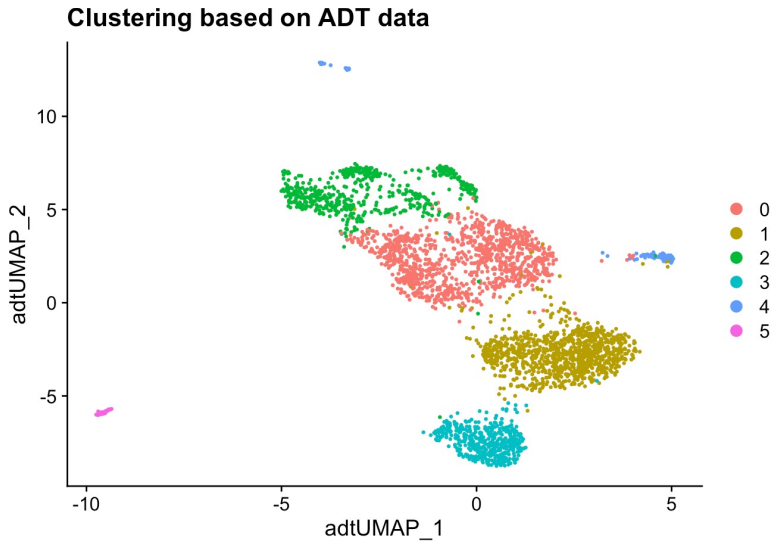
Hao et al., 2021 Cell

Weighted Nearest Neighbor Analysis (WNN)

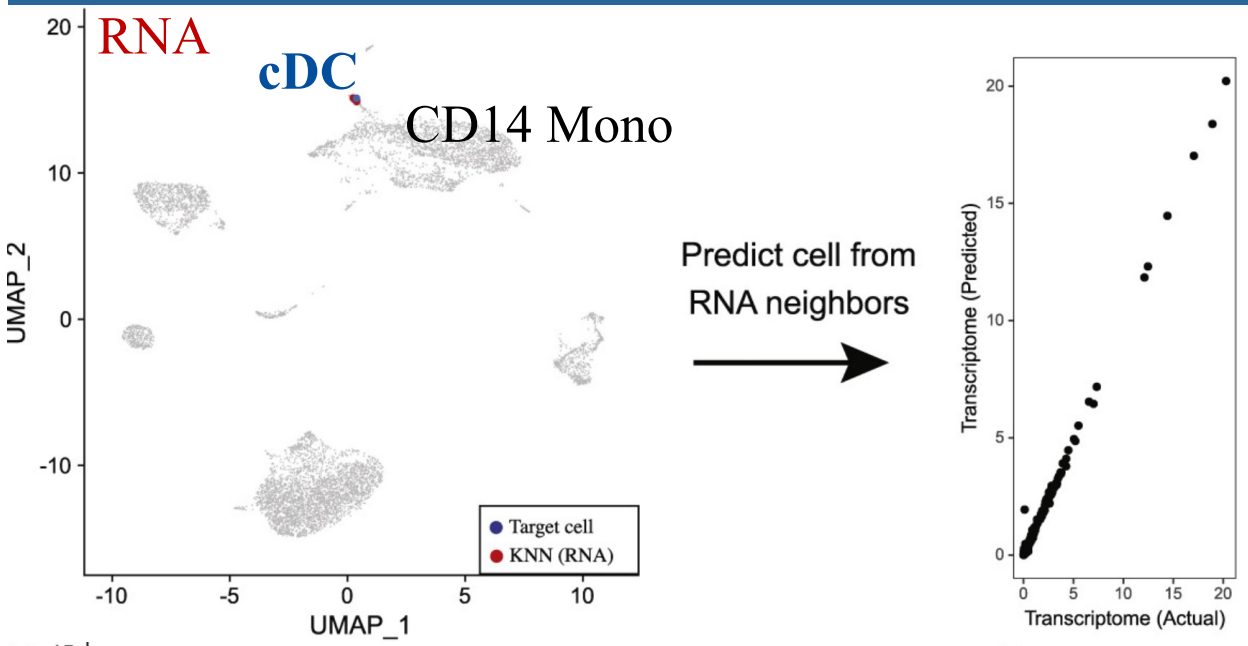
1. Constructing independent k-nearest neighbor (KNN) graphs for each modality
2. Performing within and across-modality prediction
3. Calculating cell-specific modality weights based on the relative accuracy of each modality
4. Calculating a WNN graph



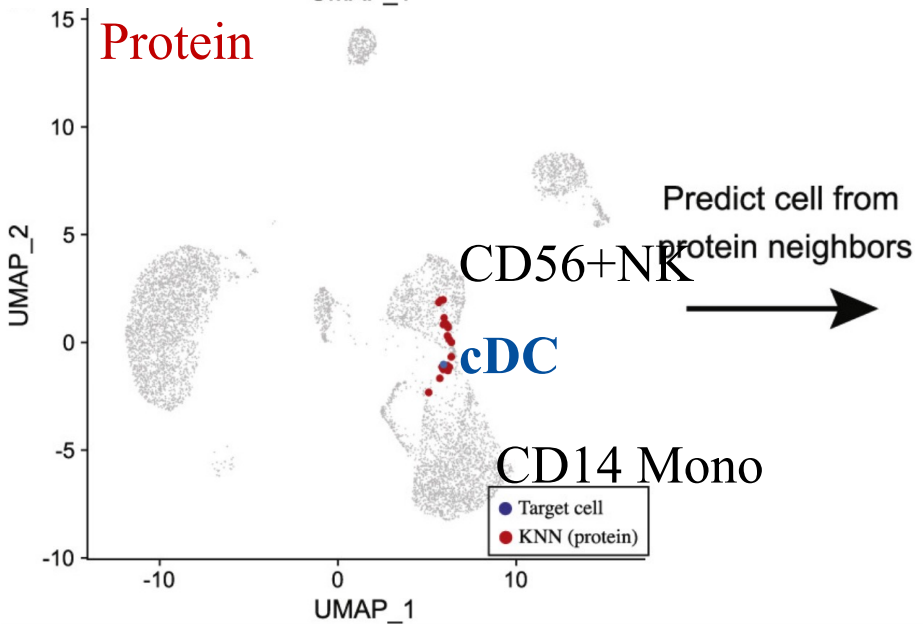




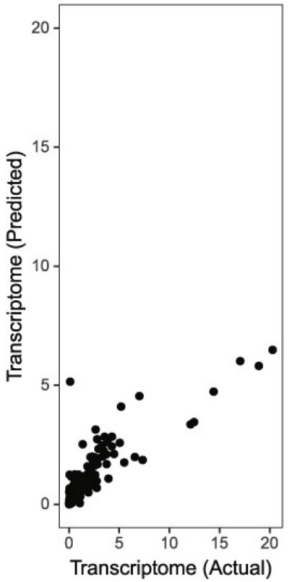
Case I: Protein Is Worse Than RNA



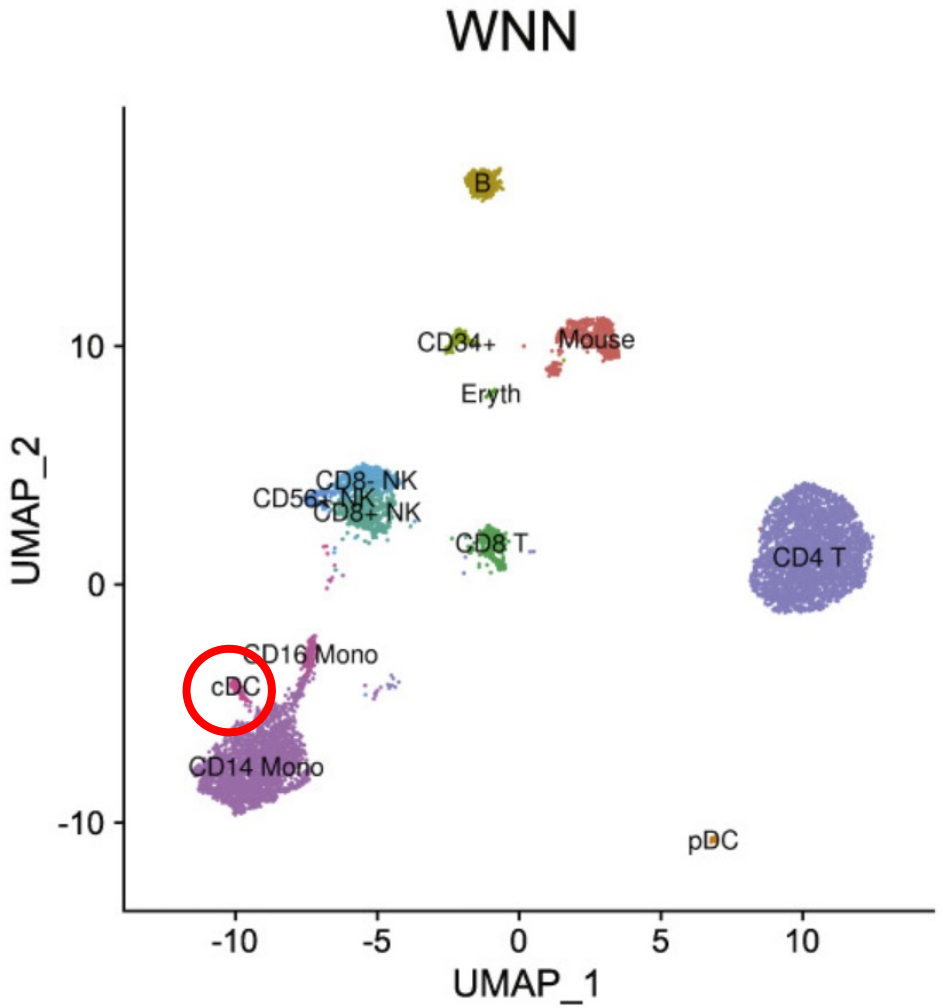
Predict cell from RNA neighbors



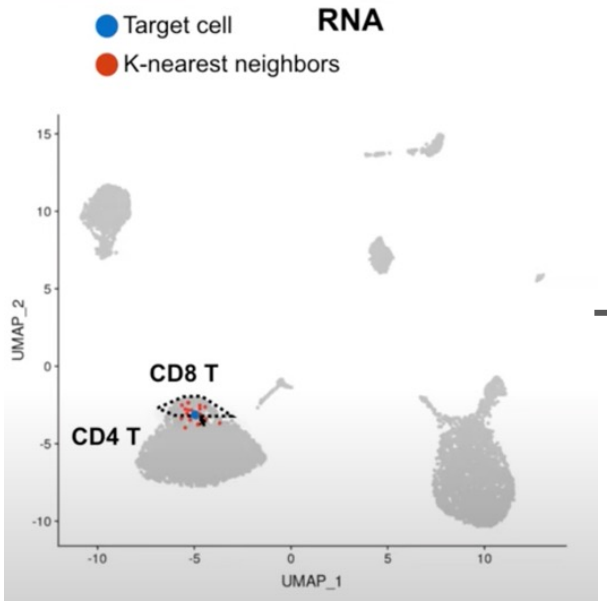
Predict cell from protein neighbors

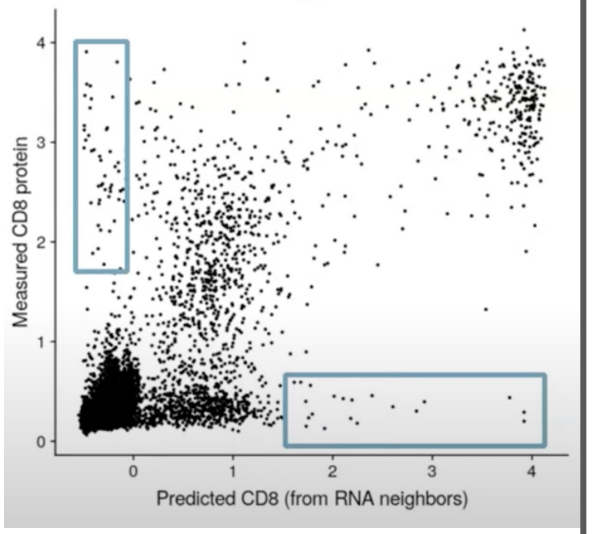


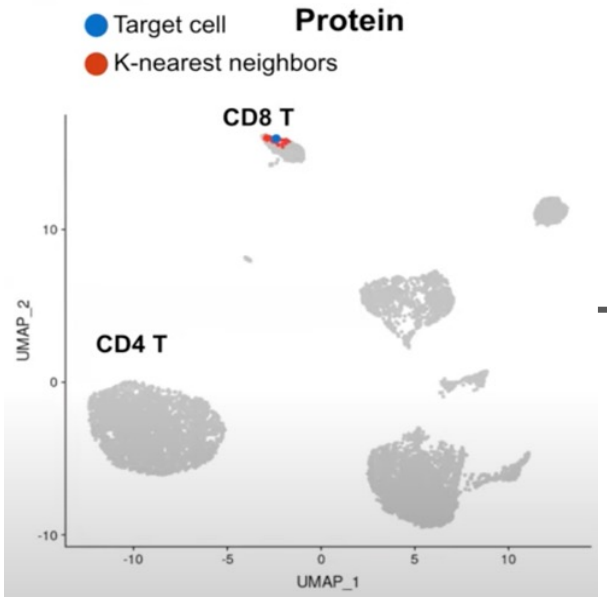
Target cell: **cDC**

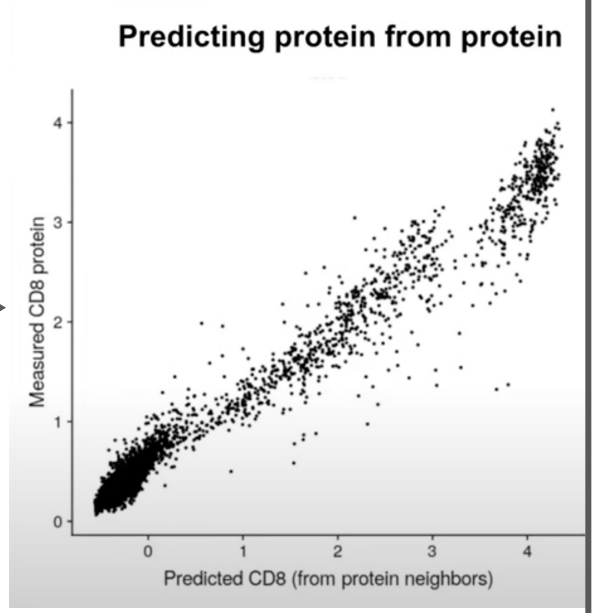


Case II: Protein Is better Than RNA

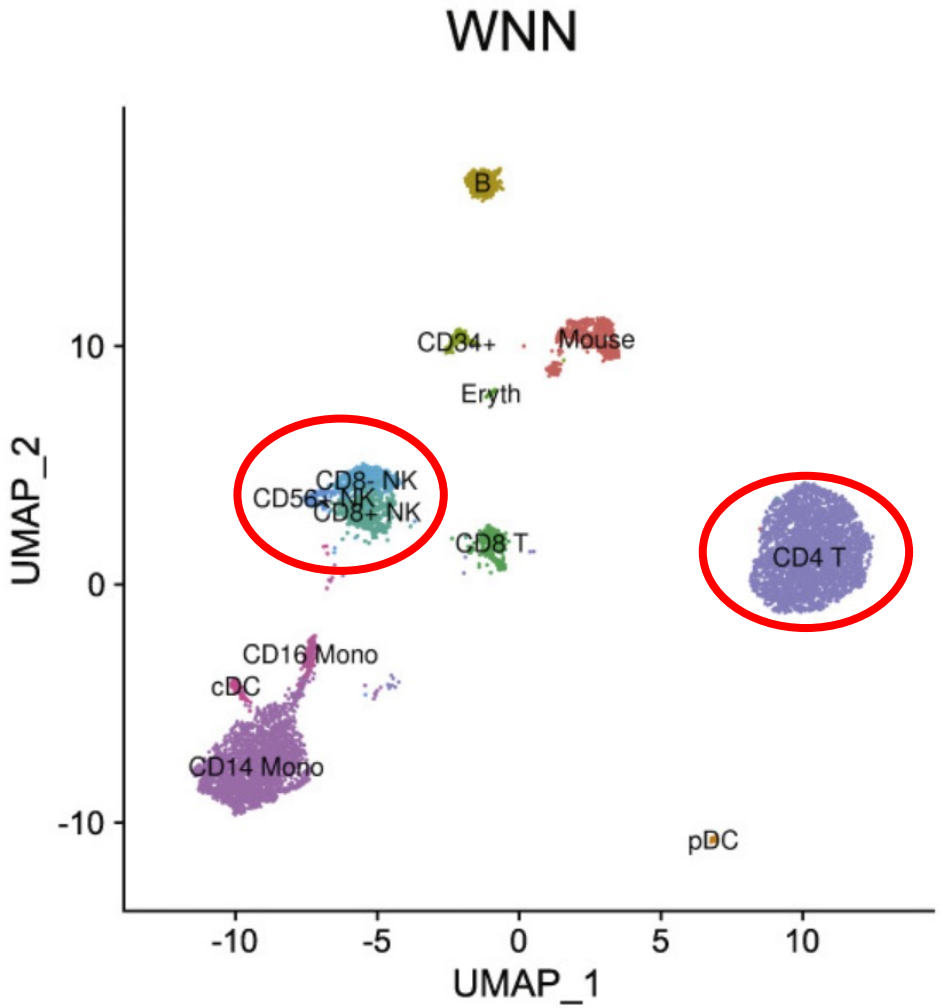




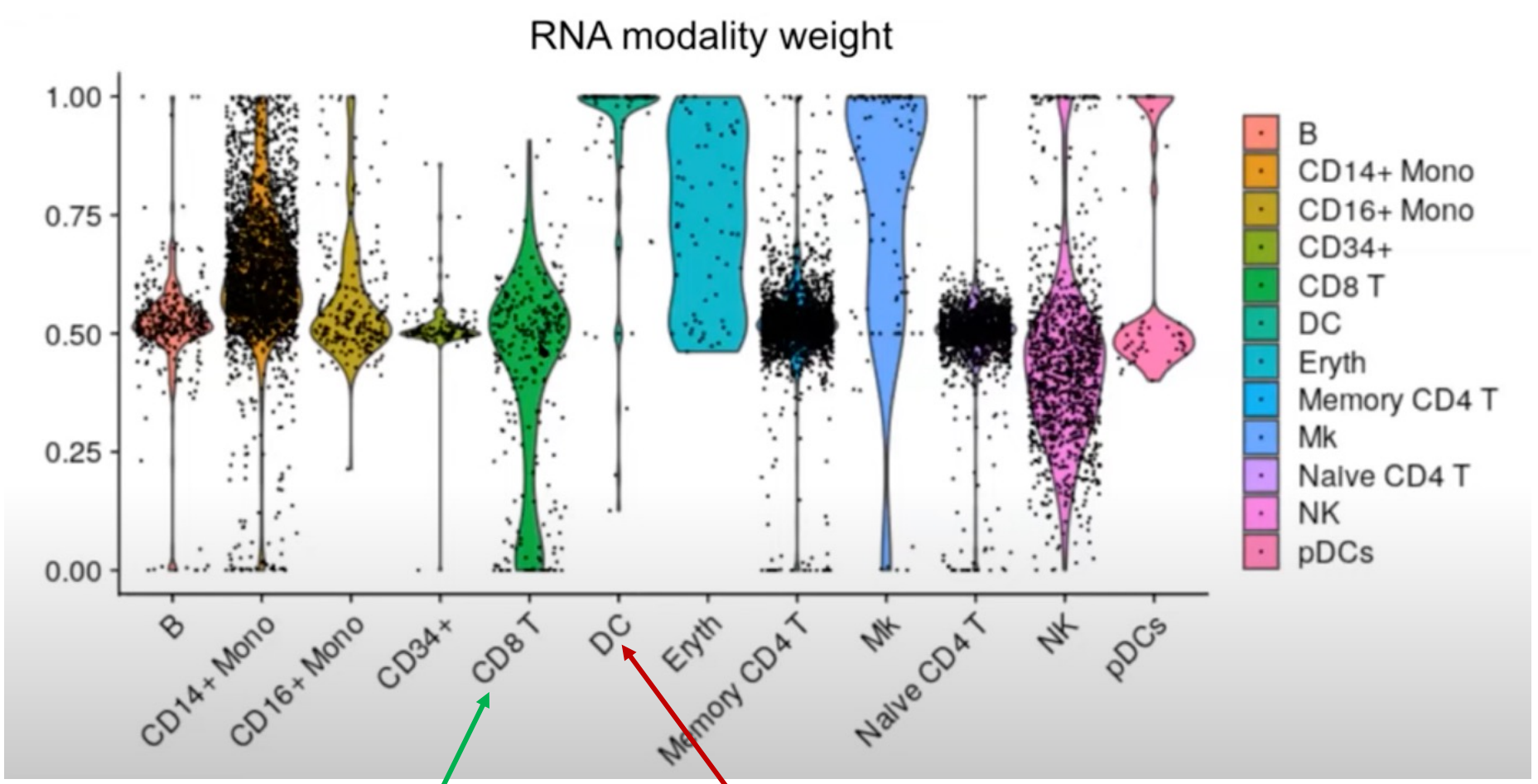


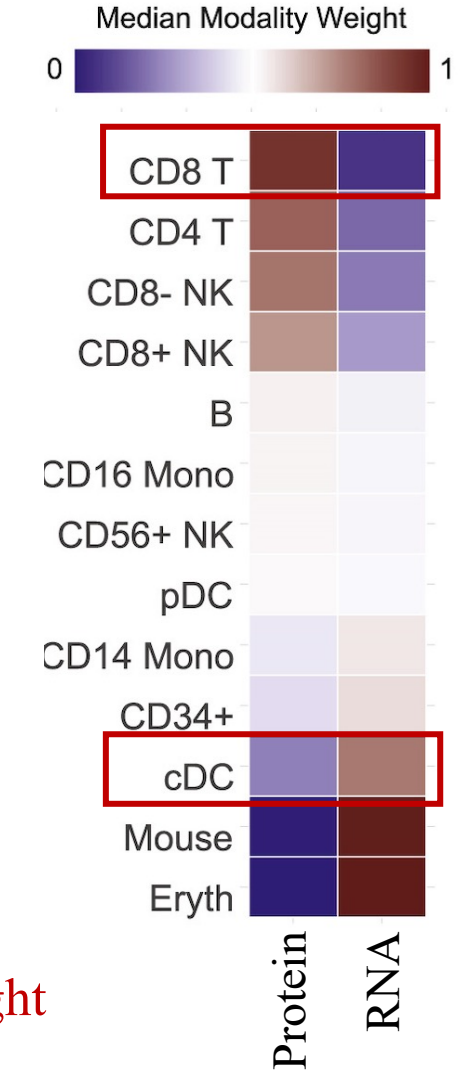


Target cell: **CD8 T**



Modality Weight From WNN





CASE I: High RNA weight & low protein weight

Case II: Low RNA weight & high protein weight

https://www.youtube.com/watch?v=9MP2y3ZgD4M&ab_channel=AllenDiscoveryCenterforCellLineageTracing

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