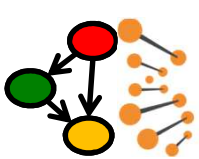
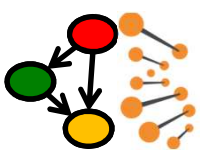
Lecture 16: Correlation vs. Conditional Dependence

Learning the human chromatin network from all ENCODE ChIP-seq data



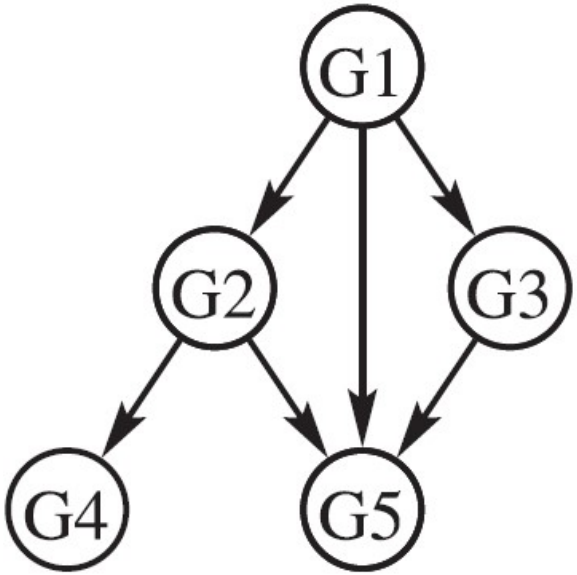
GENOME 560

Su-In Le, CSE & GS (suinlee@uw.edu)

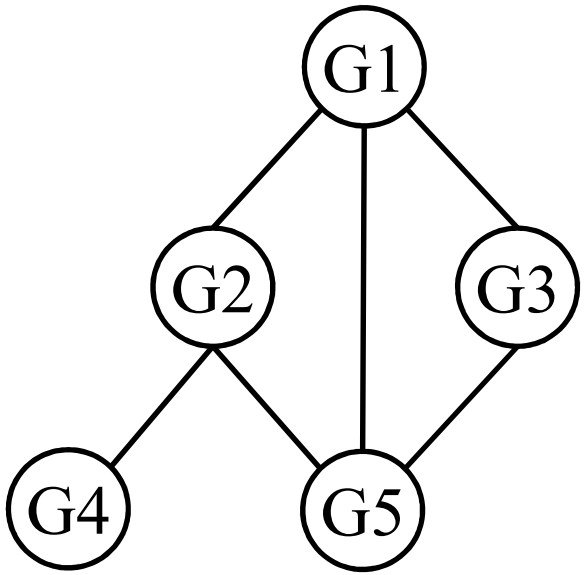


Directed vs. undirected graphical models

Directed graphical model (Bayesian network)



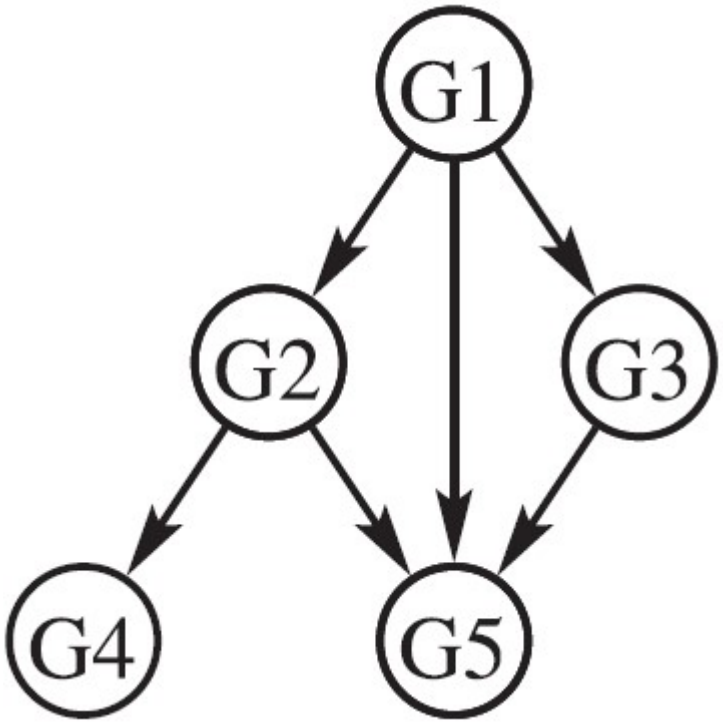
Undirected graphical model (Gaussian graphical model)



- Different conditional independence assumptions

Directed graphical models

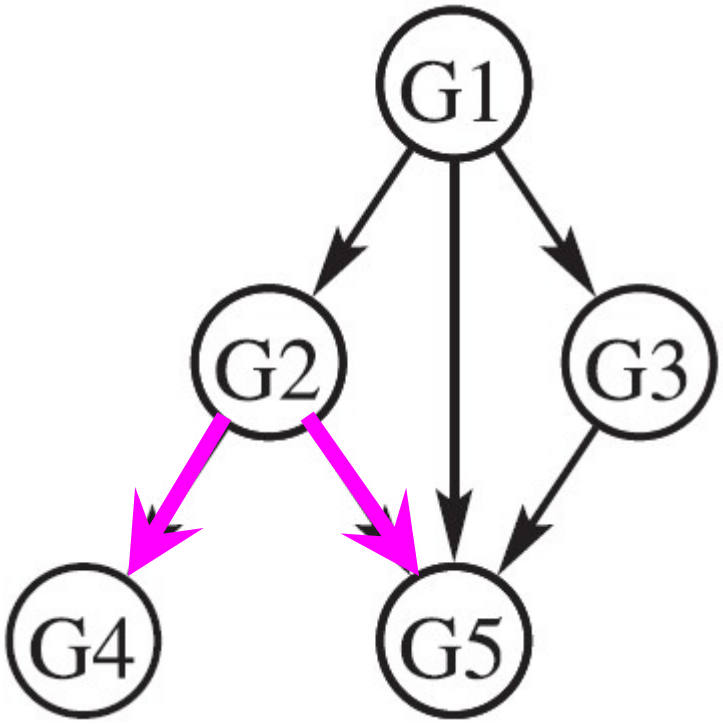
- Probability distribution for a gene depends only on its regulators (parents) in the network.



Review: Conditional Dependency Represented in Bayesian Networks

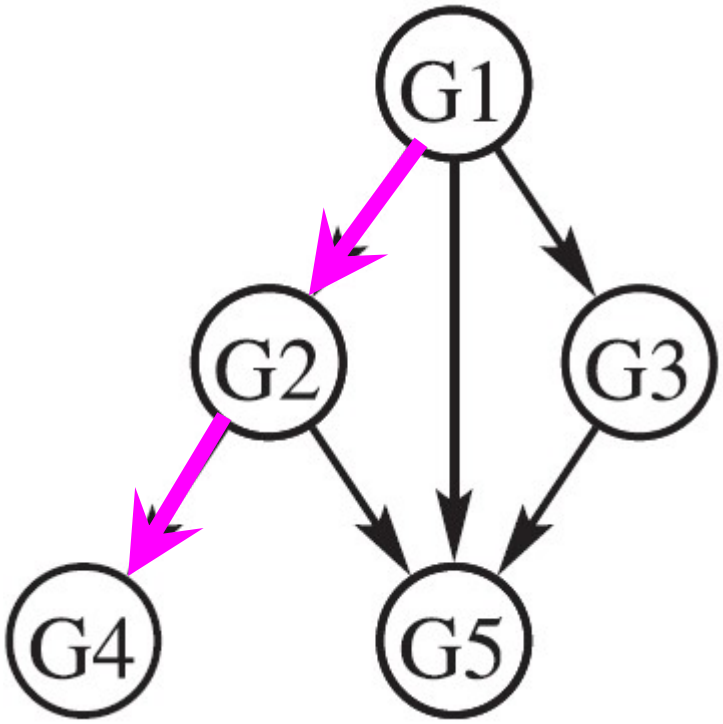
- The expression levels of G4 and G5 are related only because they share a common regulator G2.
- In mathematical term, G4 and G5 are conditionally independent given G2.

$G4 \perp G5 \mid G2$



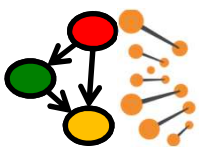
Review: Conditional Dependency Represented in Bayesian Networks

- The expression levels of G4 and G1 are related only because of gene G2.



$G4 \perp G5 \mid G2$

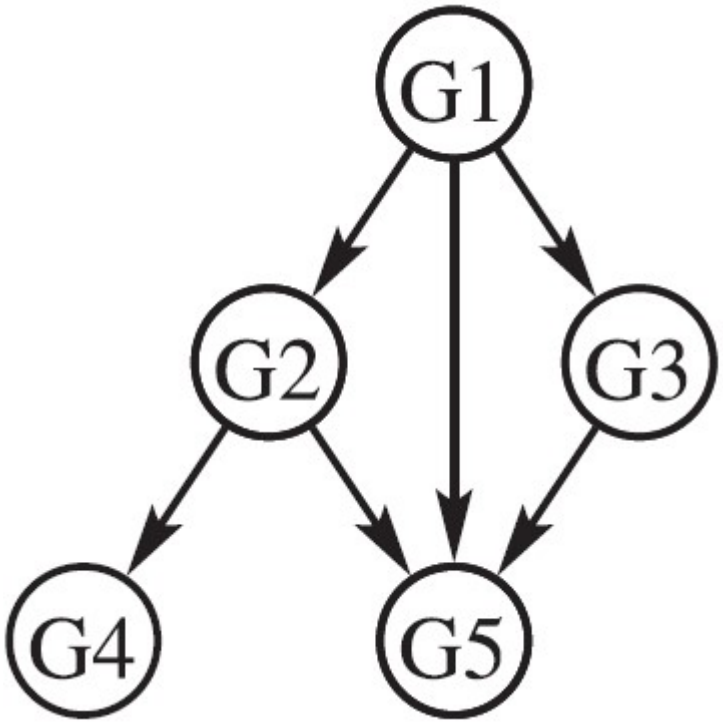
$G1 \perp G4 \mid G2$



Review: Conditional Dependency Represented in Bayesian Networks

- P(G1,G2,G3,G4,G5)

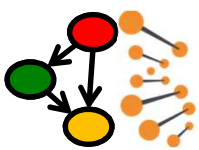
  = P(G1) P(G2|G1) P(G3|G1) P(G4|G2) P(G5|G1,G2,G3)



$G4 \perp G5 \mid G2$

$G1 \perp G4 \mid G2$

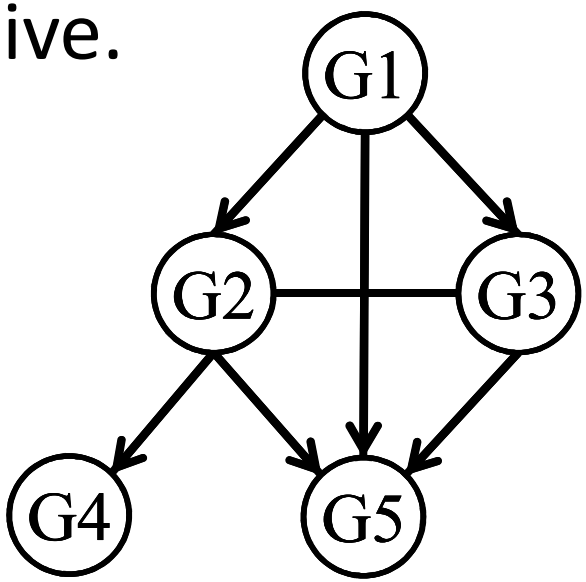
:

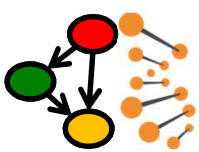


Convert to undirected model

- Step 1: “Moralize” the graph (marry parents)
- Step 2: Remove arrow heads.
- This is the best approximation that an undirected model can give.

Pro: No concerns about cycles!
Pro: More stable inference.
Con: No “direction” of influence.

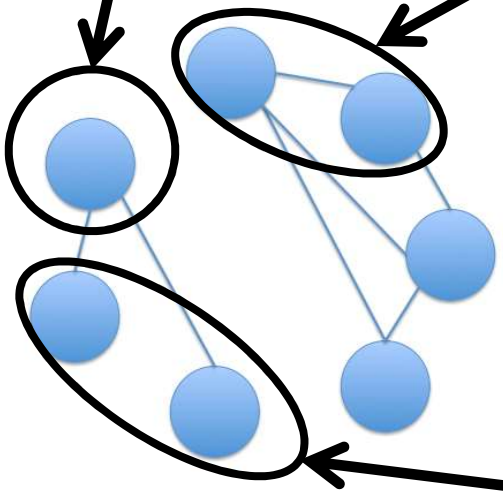




In undirected graphical models…

Each node represents a gene

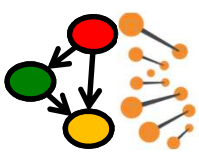
Edge indicates 2 genes are conditionally dependent



2 genes that are conditionally independent

Starbucks Example

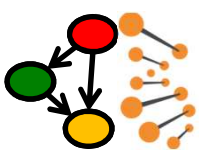
Credit: Daniela Witten



Coffeeshop data

	Like Coffeeshops?	Like Coffee?	Like Tea?
Person 1	Y	Y	N
Person 2	N	N	N
.			
.			
.			
.			
.			
.			
.			
.			
Person 100	Y	N	Y

Credit: Daniela Witten



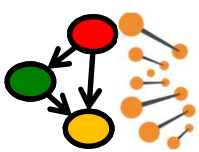
Correlation matrix

$$\Sigma = \begin{bmatrix} 1 & 0.58 & 0.58 \\ 0.58 & 1 & 0.33 \\ 0.58 & 0.33 & 1 \end{bmatrix}$$



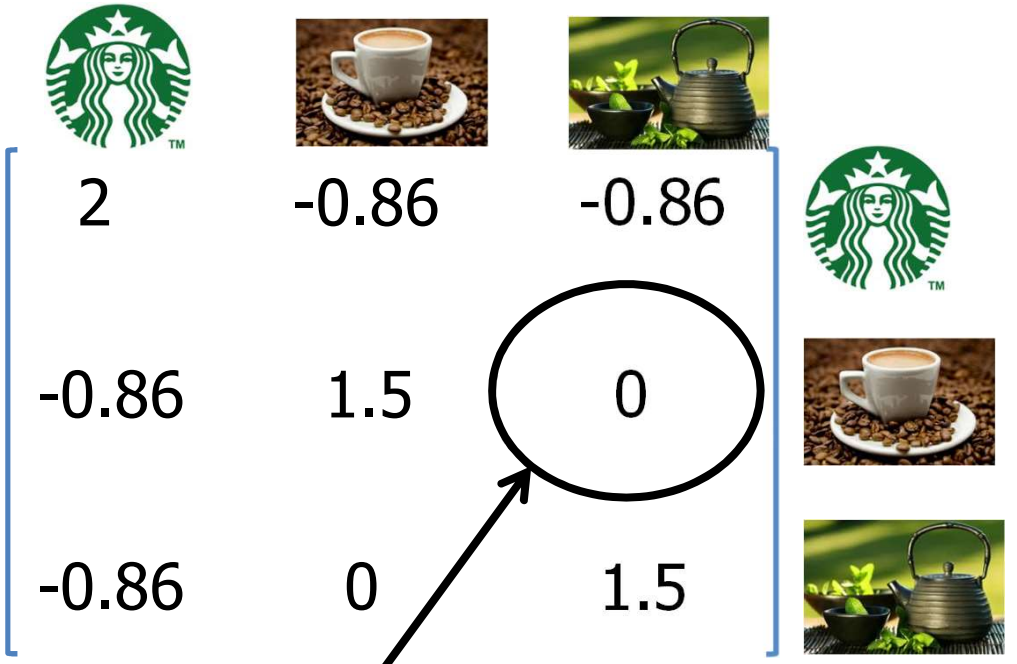



Credit: Daniela Witten



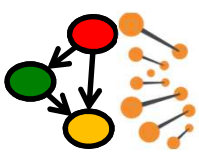
Inverse correlation matrix

$$\Sigma^{-1} =$$



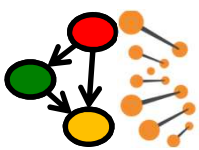
Zero in the inverse correlation matrix indicates that coffee and tea are conditionally independent!

Credit: Daniela Witten



The network

Credit: Daniela Witten

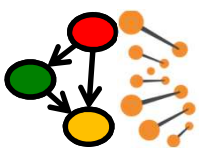


Correlation versus inverse correlation

- Every population correlation matrix $\sum$ has an associated inverse correlation matrix $\sum^{-1}$.
- We might be tempted to estimate $\sum$ in order to learn the network structure…

But this includes all indirect interactions!

- Instead, we can estimate $\sum^{-1}$.
- What we really need: a good way to estimate $\sum^{-1}$!



Outline

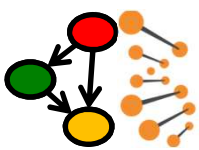
- Motivation
  - Learning a network of a large number of ChIP-seq datasets
- Key features of ChromNet
- Visualization tool
- Future directions

Regulatory factors co-localize in the genome to interact with each other.

- Our goal is to learn this network of interactions, we call the *chromatin network*.*

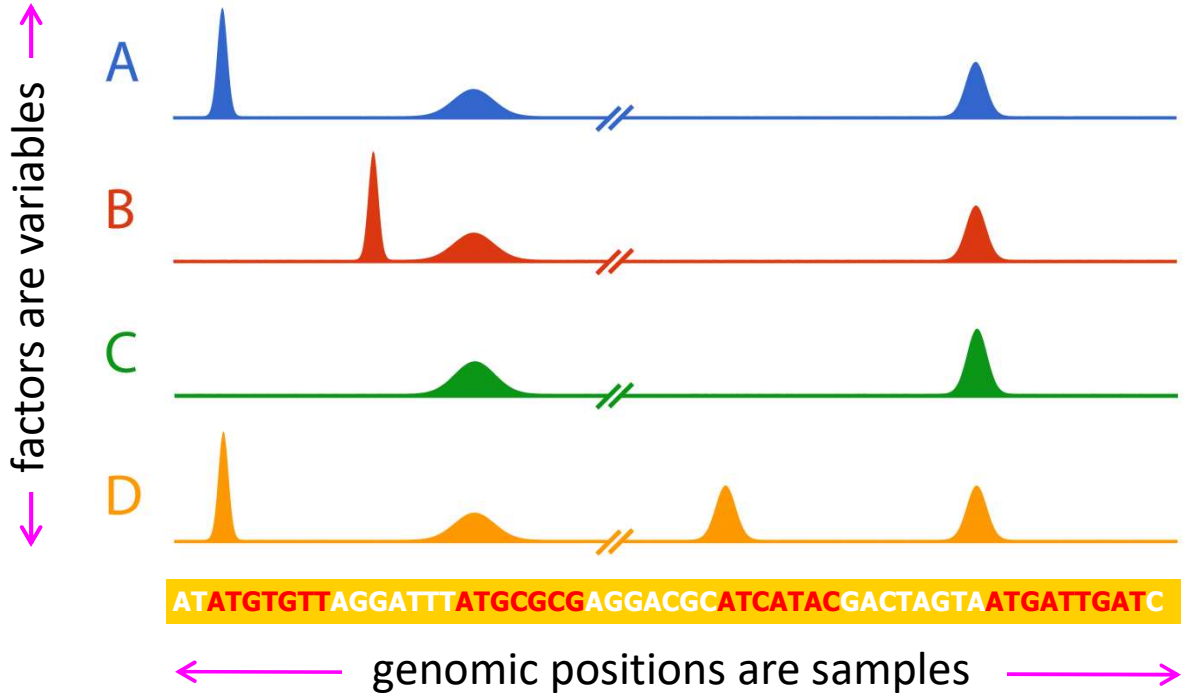
Artwork based on http://commons.wikimedia.org/wiki/File:Chromatin_immunoprecipitation_sequencing.svg

* Lundberg et al. Accepted to *Genome Biology*

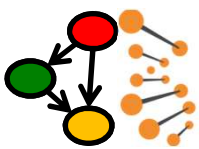


ChIP-seq data measure genome-wide localization of regulatory factors.

Consider the following simulated ChIP-seq data:



How would you infer which regulatory factors worked together?



One idea is to see if the regulatory factors tend to show up together.

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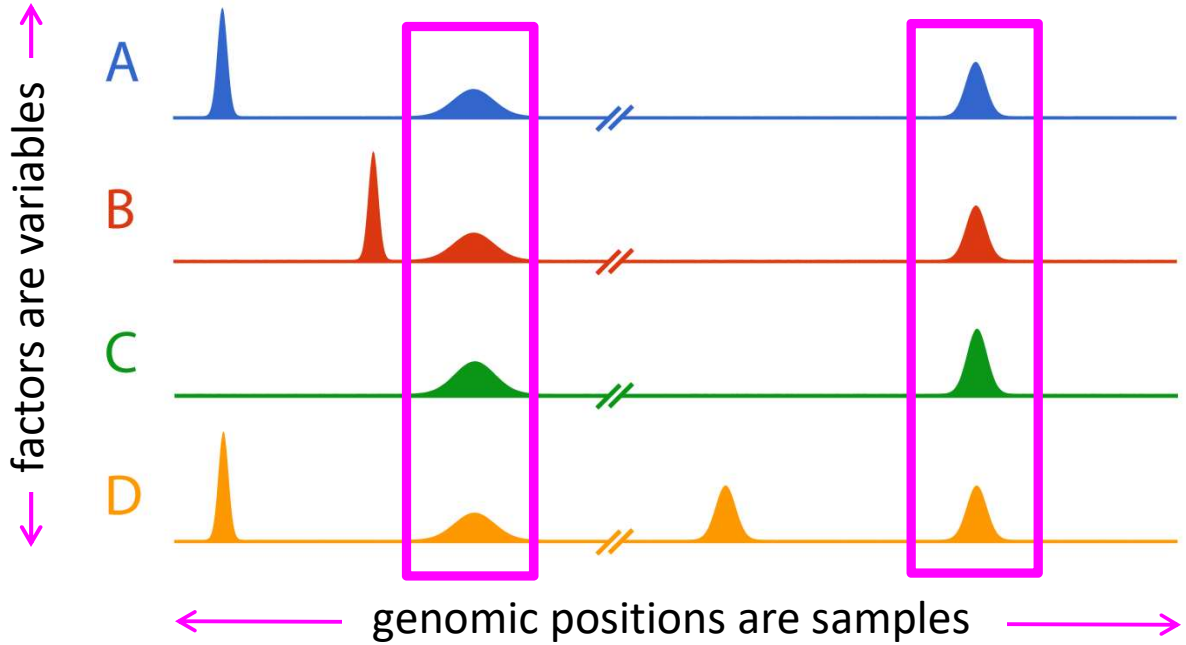
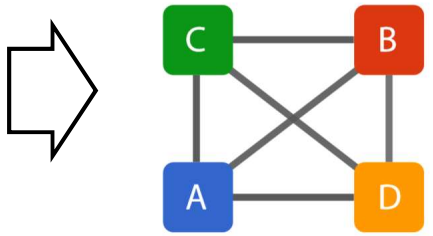
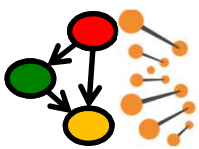


Co-occurrences



The problem is they *all* co-occur in a statistically significant manner!



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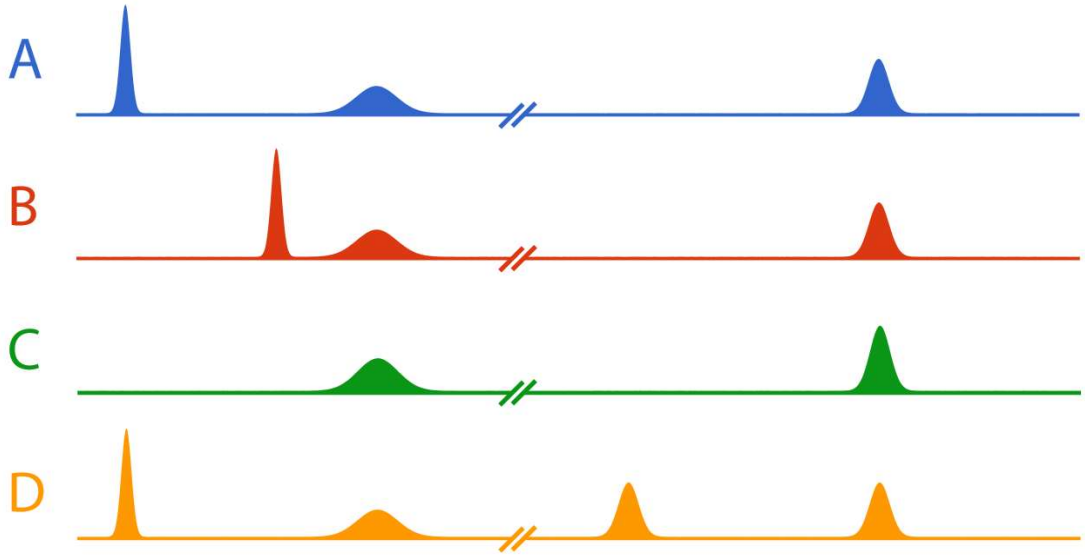

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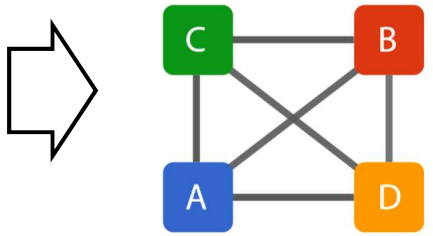


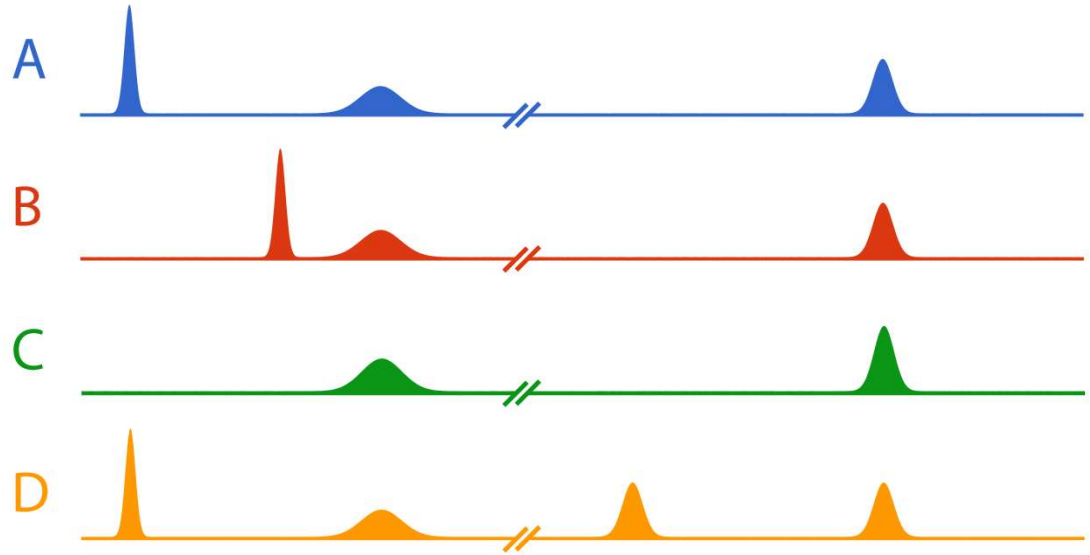
The problem is they *all* co-occur in a statistically significant manner!

- **Direct interaction:** *physical contact or functional coupling* that requires spatial proximity

- **Indirect interaction:** *Transitive effect* of direct interactions

Here is a possible scenario of interactions among these 4 factors

C recruits A and B, and A in turn recruits D



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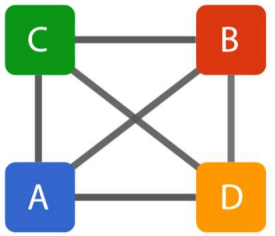
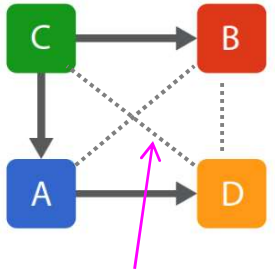
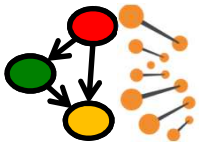


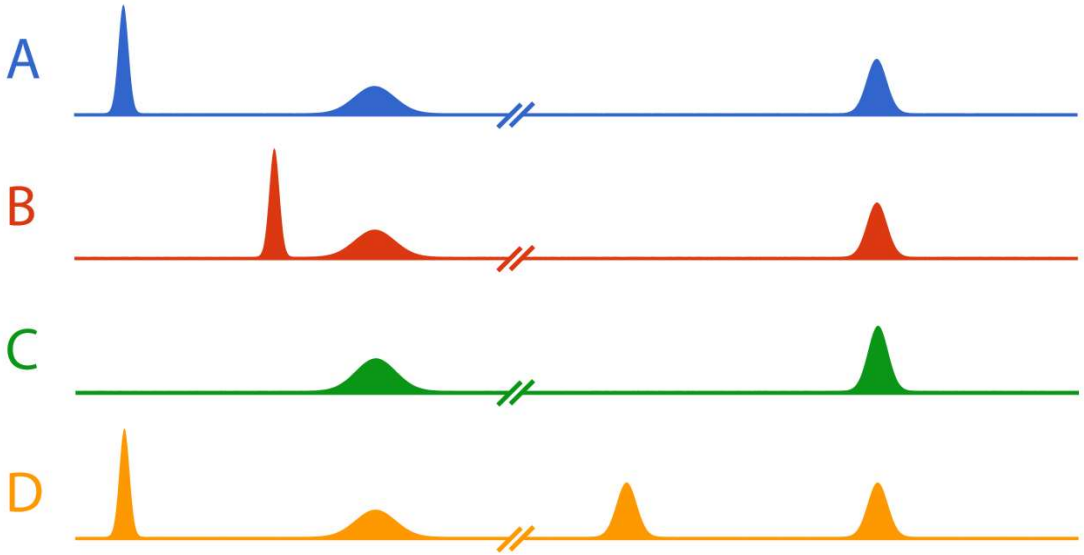
True interactions

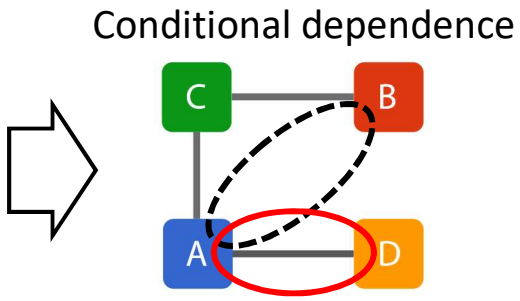


indirect interactions



A better idea is to use conditional dependence to estimate direct interaction.





Edge indicates 2 factors are *conditionally dependent*.

2 factors that are not connected are conditionally independent.

Conditional dependence represents a "direct" interaction.

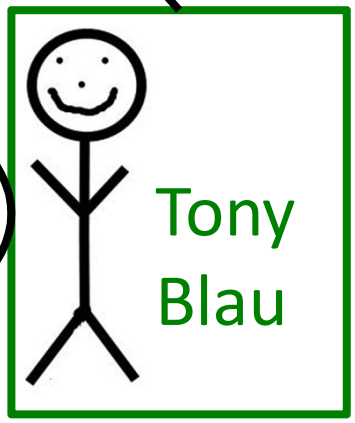
Hypothetical example: What is conditional dependence relationship?

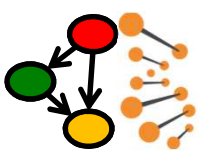
GS collaboration network

Su-In and Judit has a shared interest in proteomics

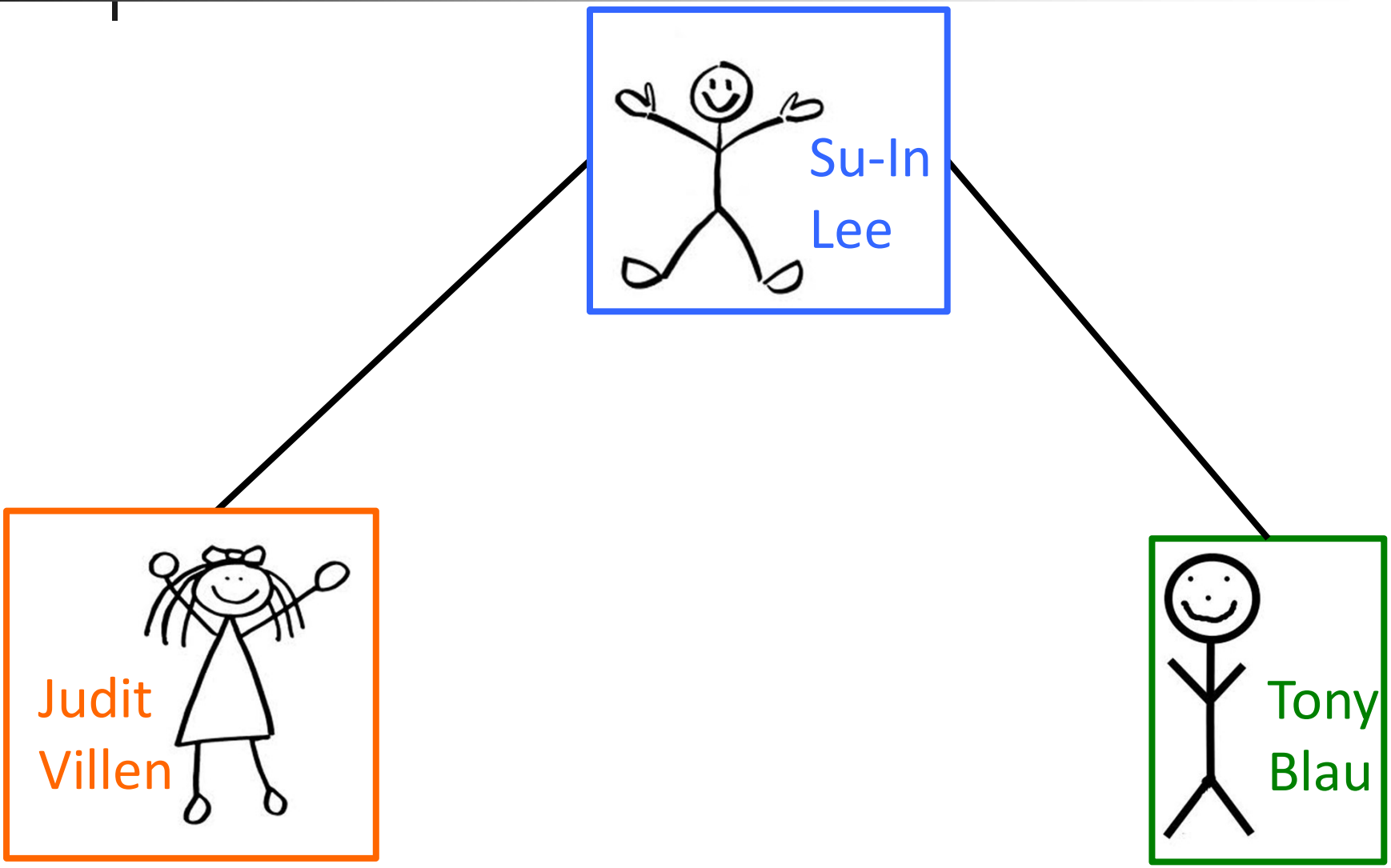
Su-In and Tony are very interested in personalized oncology

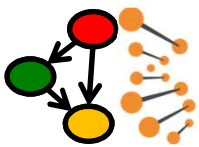
SHARED PREFERENCE CAN BE FULLY EXPLAINED BY **Su-In**



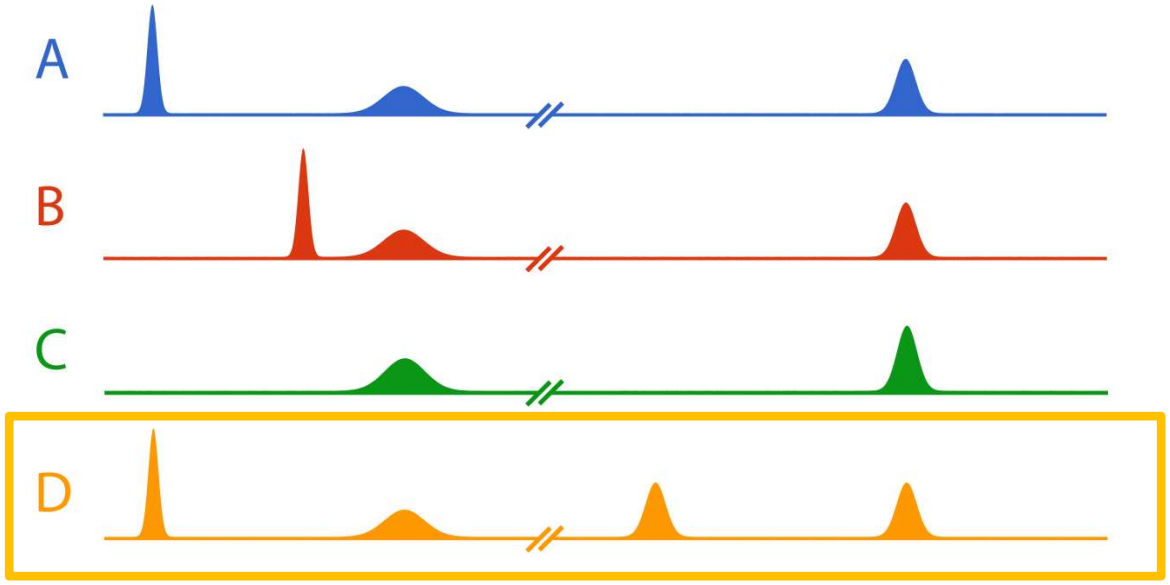


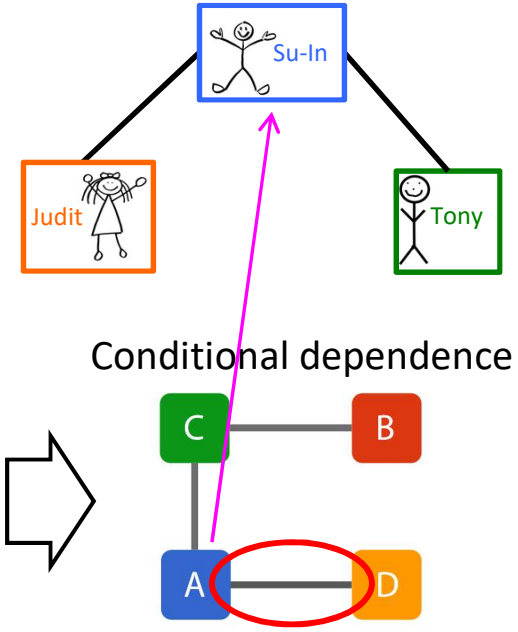
Conditional dependencies capture direct relationships





How can we capture conditional dependence relationships from data?

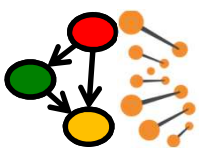




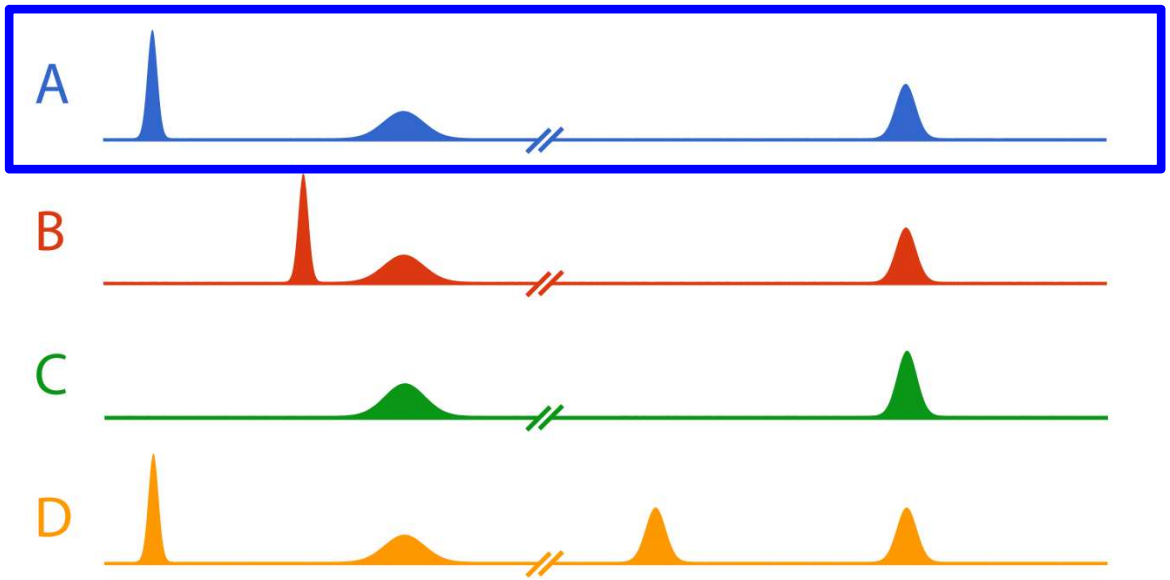
If we run a linear regression of D against all the other variables which coefficients will be non-zero?

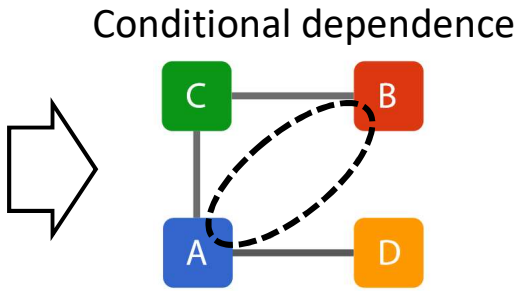
$$D = w_A A + w_B B + w_C C$$

B and C give no more information about D after accounting for A.



A better idea is to use conditional dependence to estimate direct interaction.



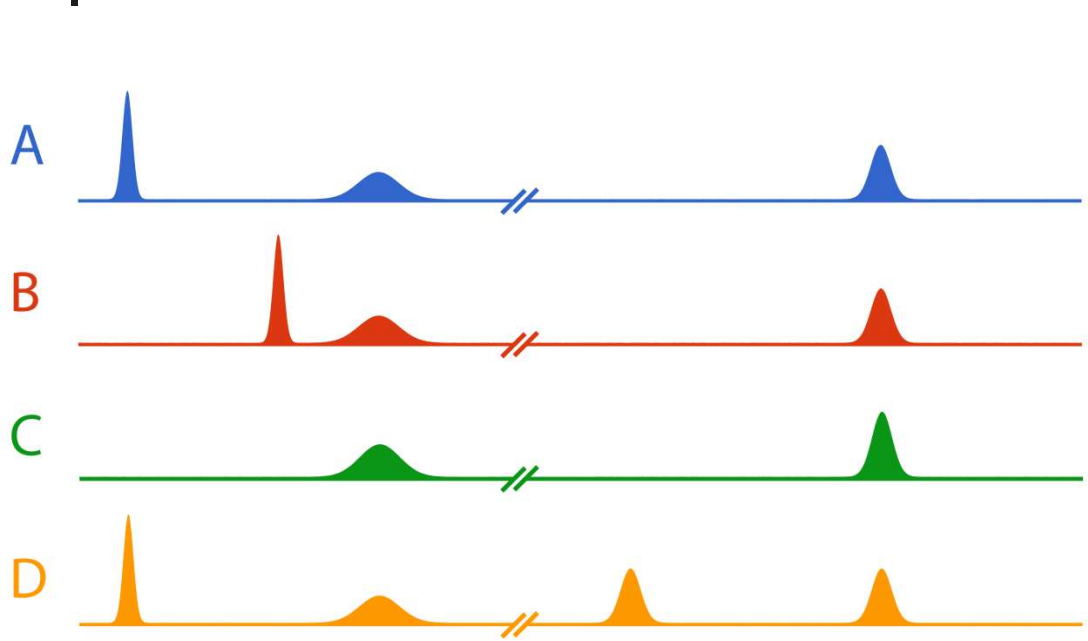


If we run a linear regression of A against all the other variables which coefficients will be non-zero?

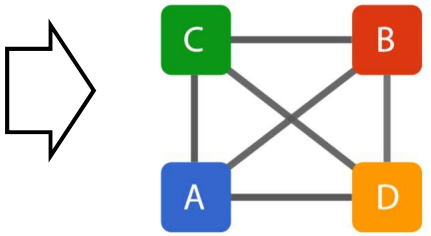
$$A = v_B B + v_C C + v_D D$$

B gives no more information about A after accounting for C and D.

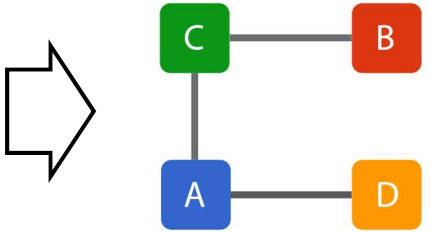
Conditional dependencies can better capture direct interactions



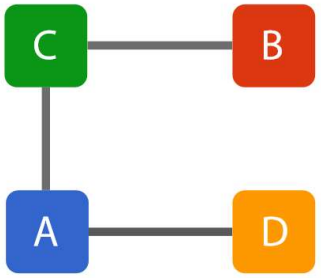
Co-occurrences



Conditional dependence



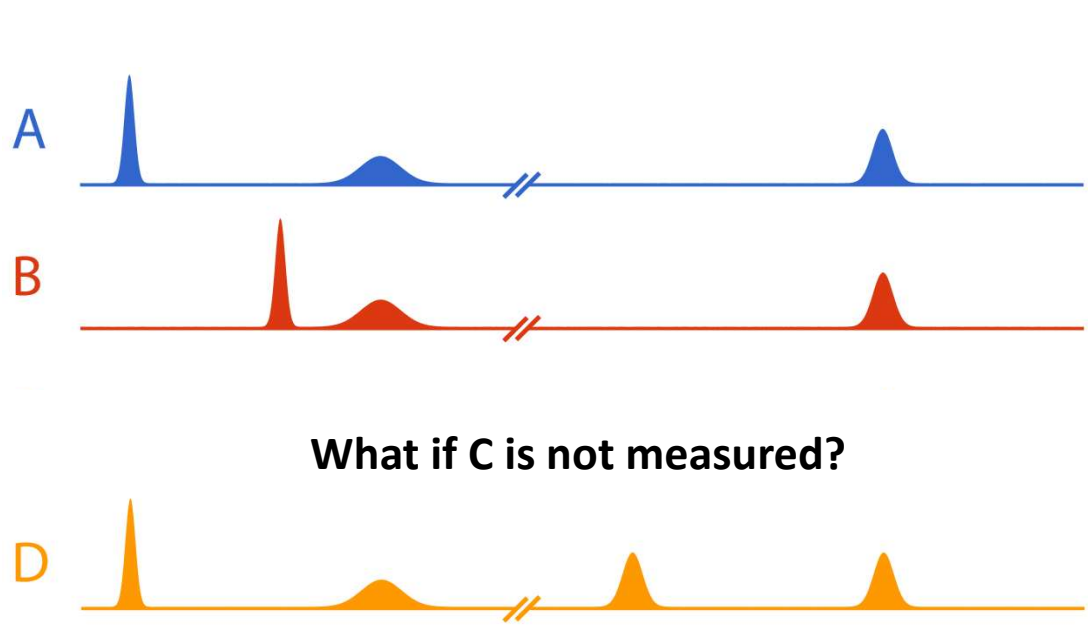
True interactions

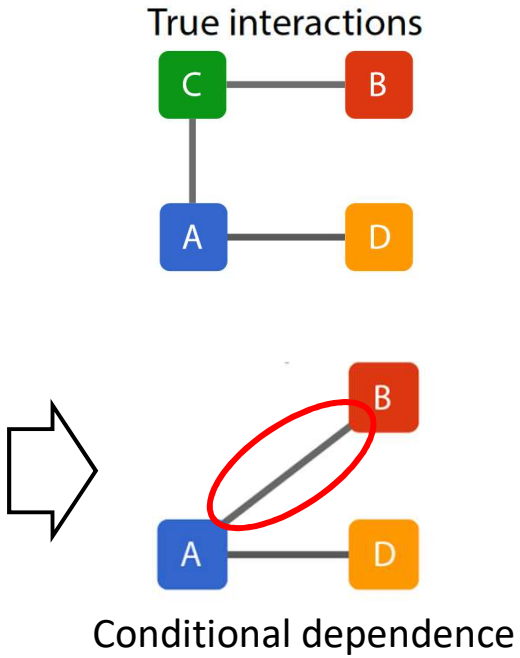


Direct interaction: physical contact or functional coupling that requires spatial proximity

Indirect interaction: Transitive effect of direct interactions

It is important to observe many factors.



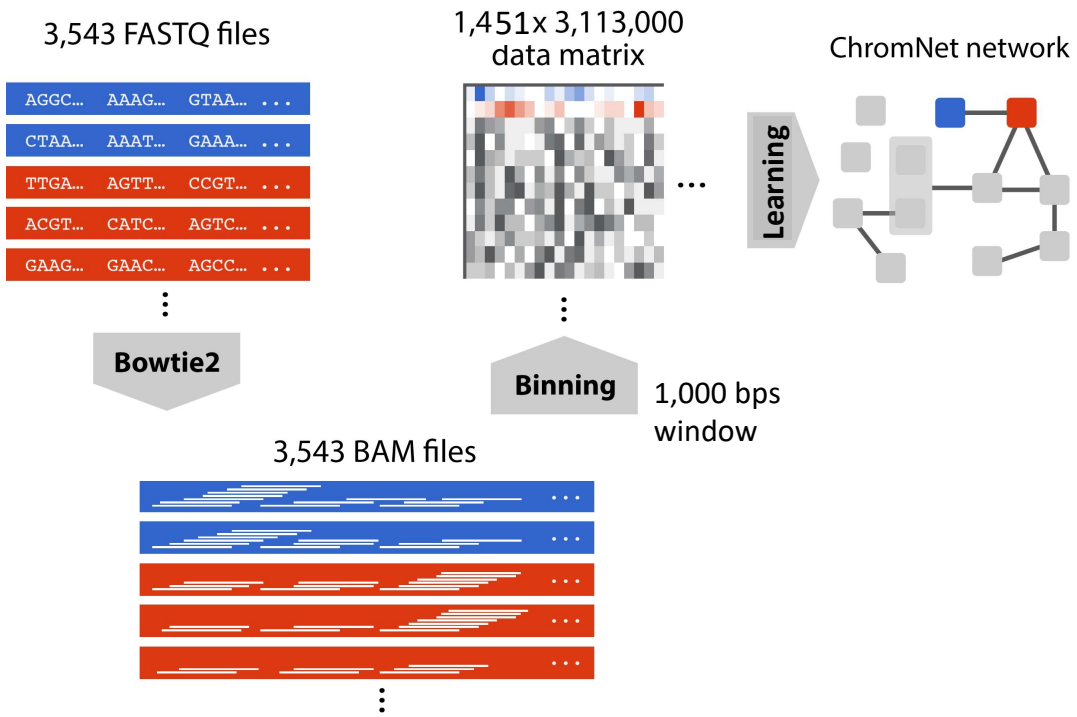


Then the connection between A and B can no longer be explained by other variables.

Incorporating more ChIP-seq data sets would improve the network.

ChromNet – Learning a conditional dependence network from 1,451 ENCODE ChIP-seq datasets

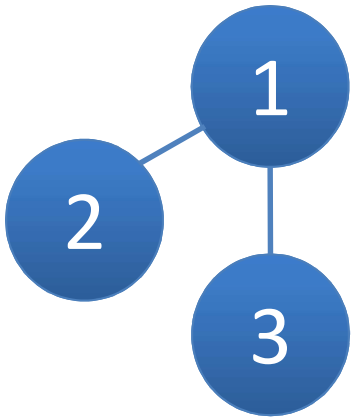
1,451 ChIP-seq datasets – 223 transcription factors and 14 histone marks from 105 cell types.



- Key motivations
  - The more variables are included, the more chance we have to remove indirect interactions.
  - The human genome has billions of positions, giving statistical power to infer conditional dependencies.
  - Integrating ChIP-seq datasets from many cell types into a single network provides unique advantages.

Key Challenge I – Large data

- Learning a network among thousands of ChIP-seq datasets based on millions of samples (1,451 x 3,000,000) is highly computationally intensive.
- Our approach: Compute the inverse covariance matrix Σ^{-1}.
  - A zero element $(\Sigma^{-1})_{ij} = 0$ means that the *i*-th and *j*-th variables are conditionally independent to each other (Gaussian*, binary **)

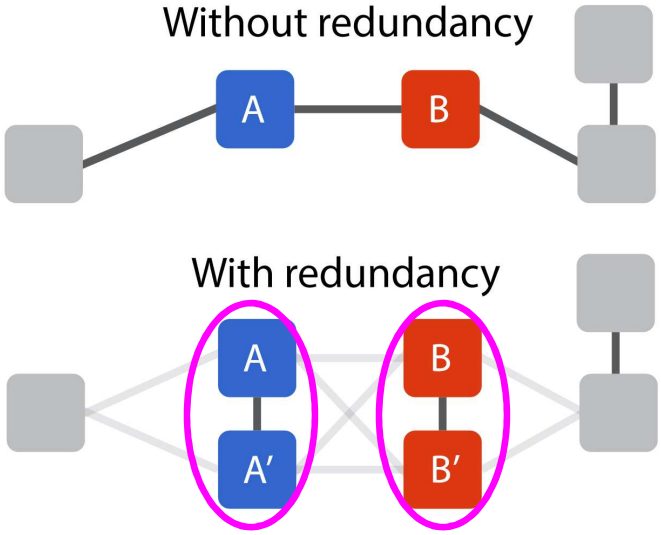


$$\Sigma^{-1} = \begin{bmatrix} 1 & 0.2 & 0.2 \\ 0.2 & 1 & 0 \\ 0.2 & 0 & 1 \end{bmatrix}$$

* Lauritzen, *Graphical Models*, 1996 ** Loh, Wainwright et al. *The Annals of Statistics*, 2013

Key Challenge II – High redundancy in data

- Conditional dependence has been applied before to smaller datasets (e.g. 23 histone marks*).
- Large networks contain much more redundancy.
  - Same factors measured in different labs, conditions or cell types.
  - Some factors are functionally very much related.
- When variables are highly correlated each other, they explain each other and disconnect from other nodes.
- Solution: We developed the group graphical model**.

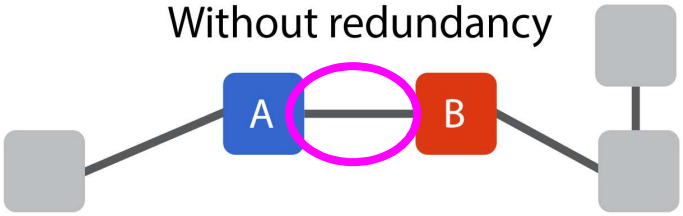


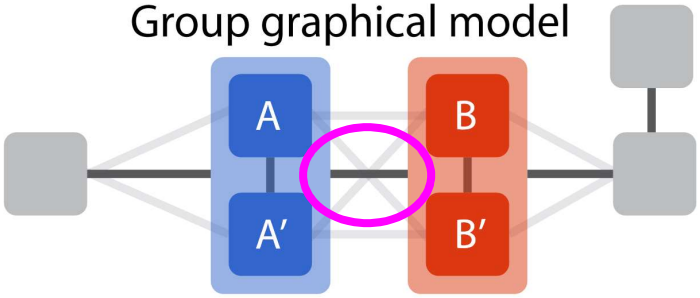
** Lundberg et al. Accepted to *Genome Biology*

* Lasserre et al., *PLoS Computational Biology*, 2013

To address this problem, we developed the group graphical model*

- In a group graphical model, we want to preserve the magnitude of the edge between (A, A') and (B, B') in the model without redundancy.

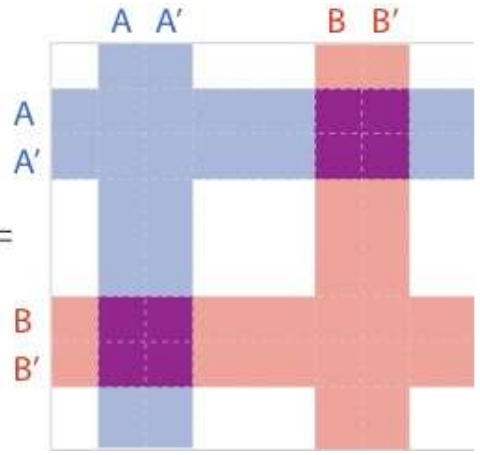




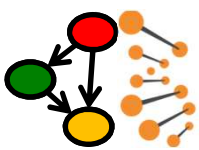
- We prove that this can be estimated by summing the entries in the Σ^{-1} *:

$$G_{[A,A'][B,B']} = \Sigma^{-1}_{AB} + \Sigma^{-1}_{AB'} + \Sigma^{-1}_{A'B} + \Sigma^{-1}_{A'B'}$$

$\Sigma^{-1} =$



* Lundberg et al. Accepted to *Genome Biology*

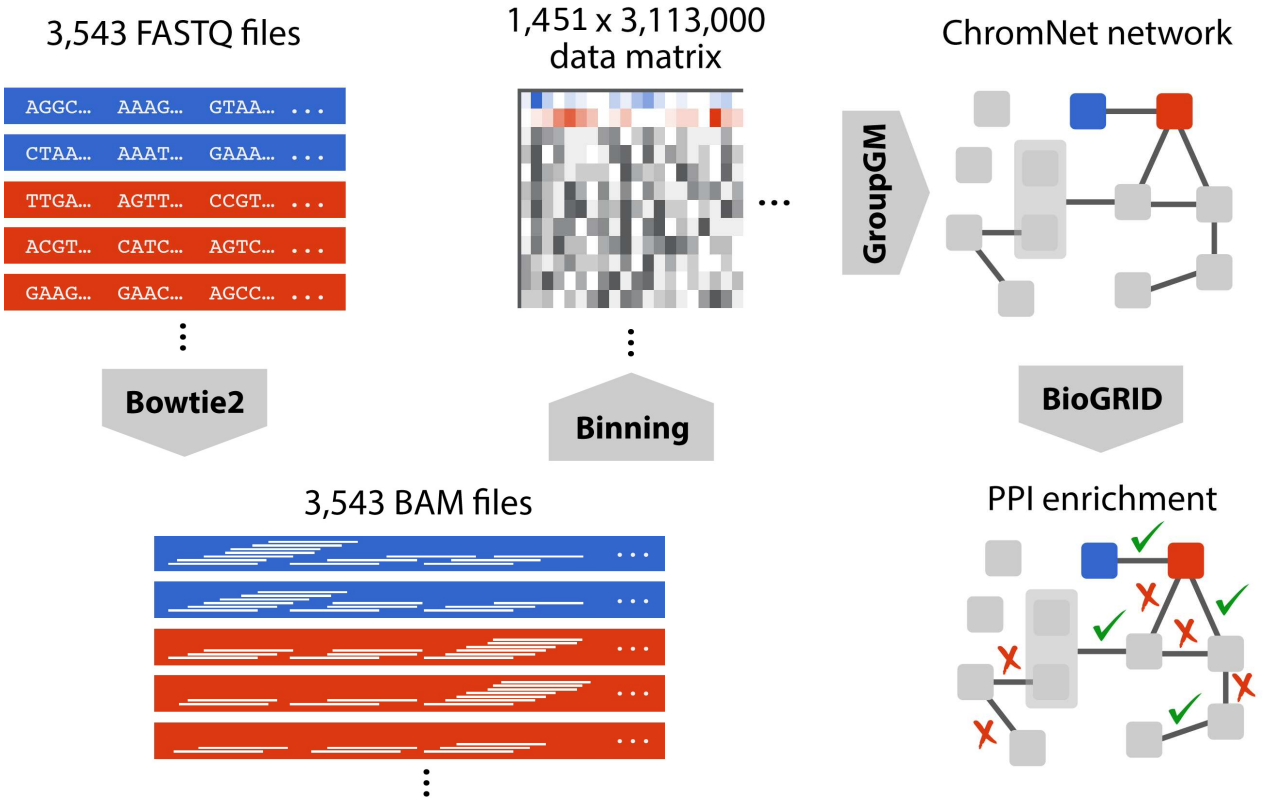


Outline

- Motivation
- Key features of ChromNet
  - Identifying conditional dependence
  - Group-based modeling
  - Learning a joint model of all cell types
  - Identifying context-specificity
- Visualization tool
- Discussion

Group graphical model (GroupGM)

Integrating 1,451 ChIP-seq datasets into a group graphical model



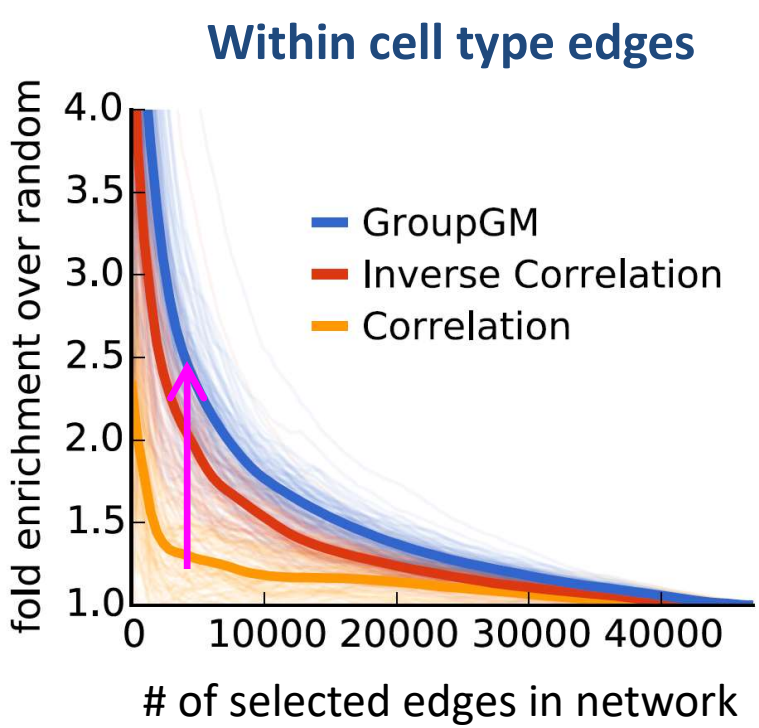
Evaluation

Comparison with

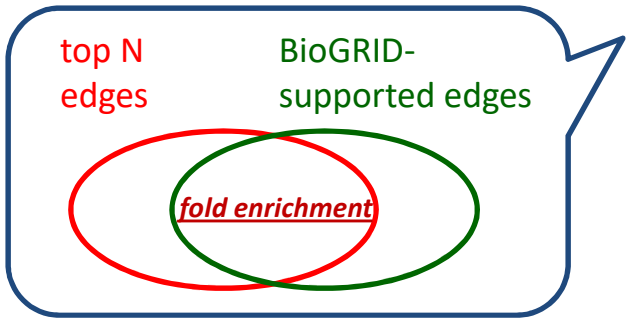
- Correlation network (co-occurrences)
- Inverse correlation Σ^{-1} (conditional dependence without group modeling)

Evaluating how well the network estimate reveals known interactions

- The human chromatin network is not fully understood.
- As a "silver standard", we use previously known physical interactions between proteins (PPIs) annotated in BioGRID.



- Consider *N* strongly weighted edges (x-axis).
- Compute the fold enrichment of BioGRID-supported edges over random *N* edges (y-axis).



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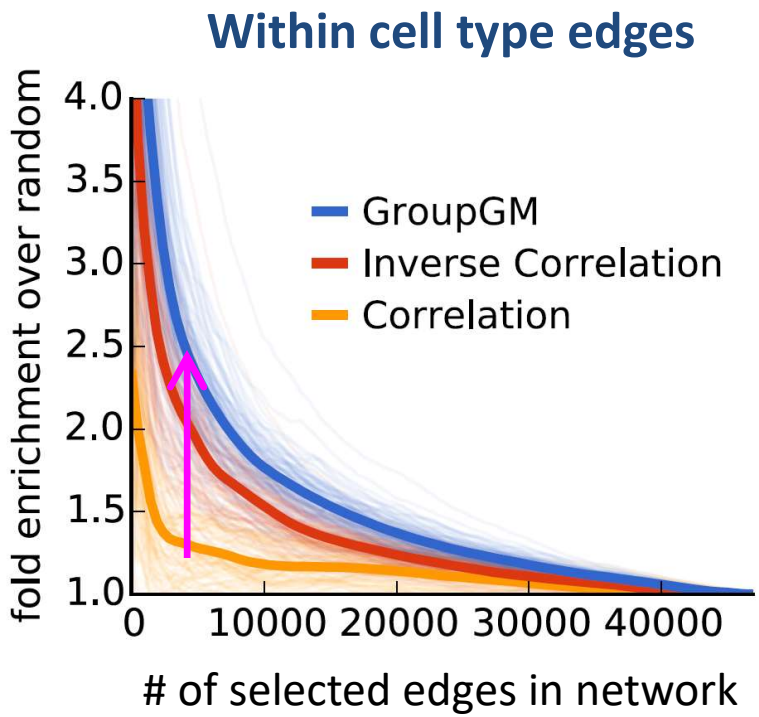
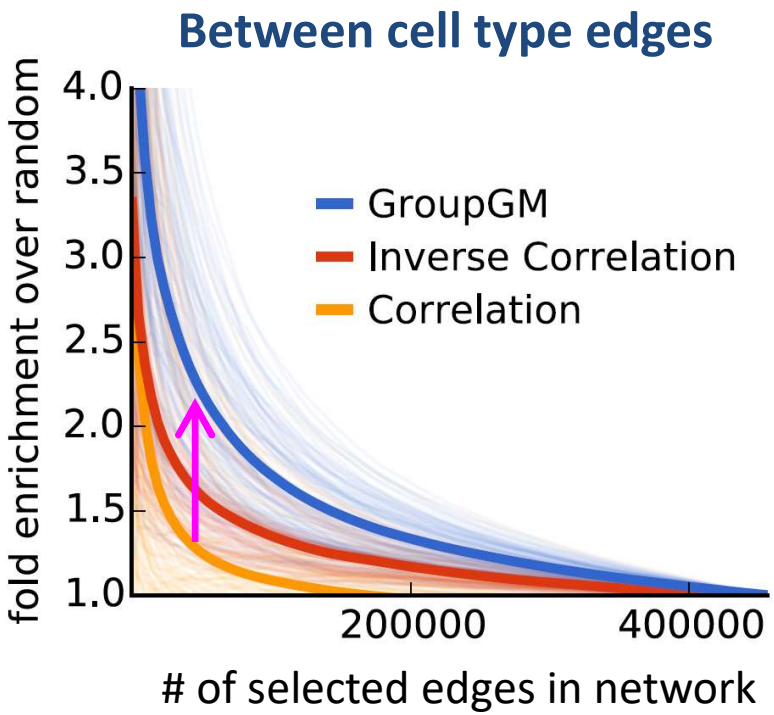
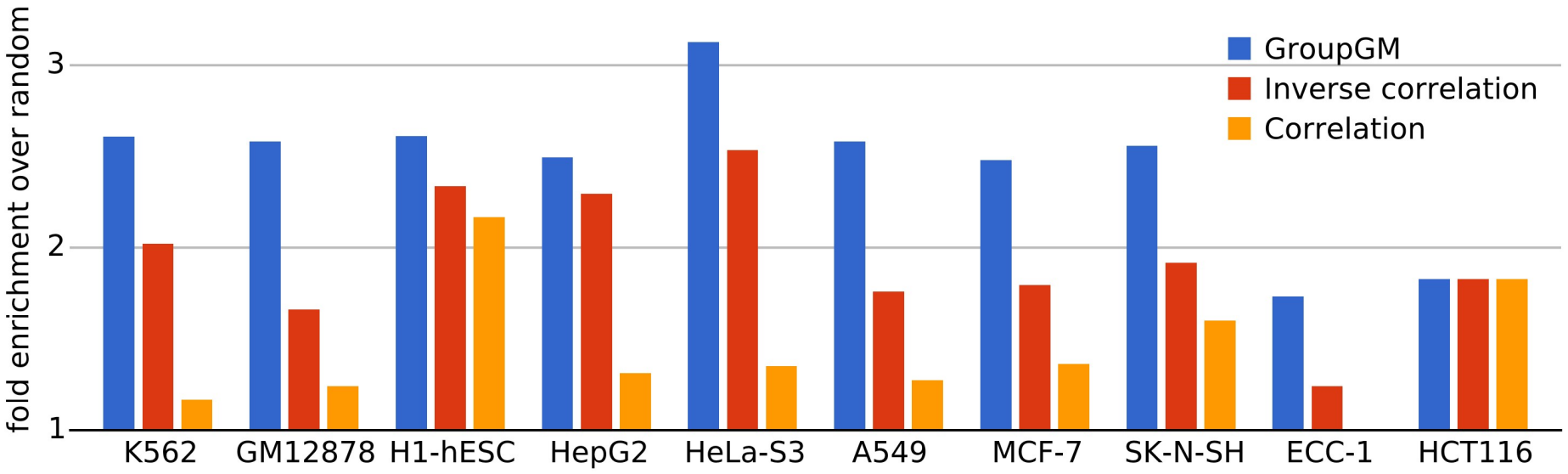




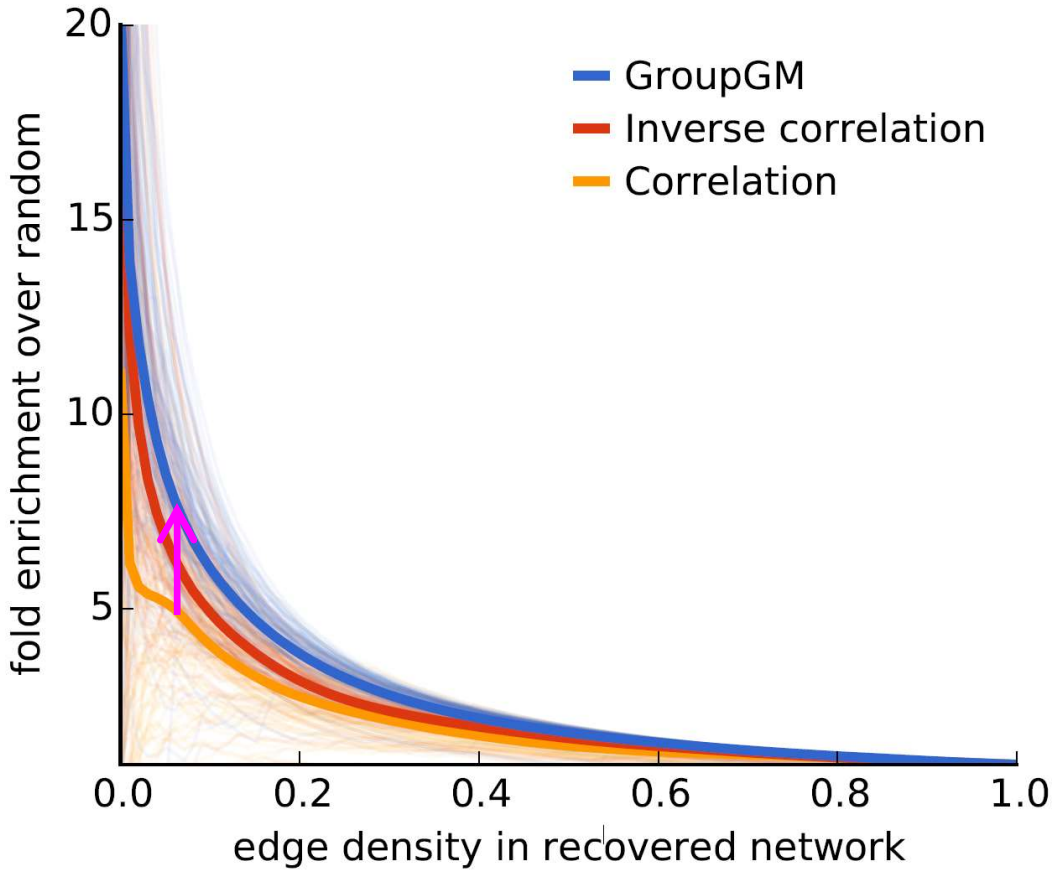
GroupGM is consistently better than alternative methods across cell types

- We measure the fold enrichment across different cell types, for a fixed *N* value

  (= total number of BioGRID-supported edges).



Chromnet reveals known TF-histone marks interactions



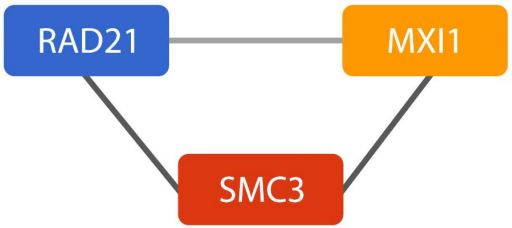
- We used known histone mark/writer combinations* to validate TF/histone mark edges
- GroupGM is better than the alternative methods

* HIstome database: Khare et al. *Nucleic Acids Research* (2012)

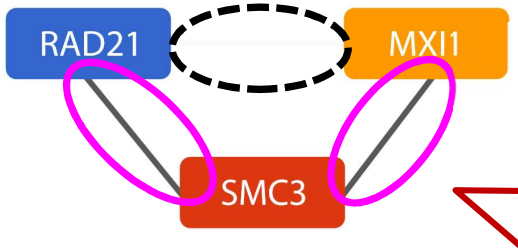
Importance of conditional dependence: SMC3 separates RAD21 and MXI1

A previous study identified 200 potential interactors of RAD21. MXI1 was not one of them*.

Correlation



Conditional Dependence

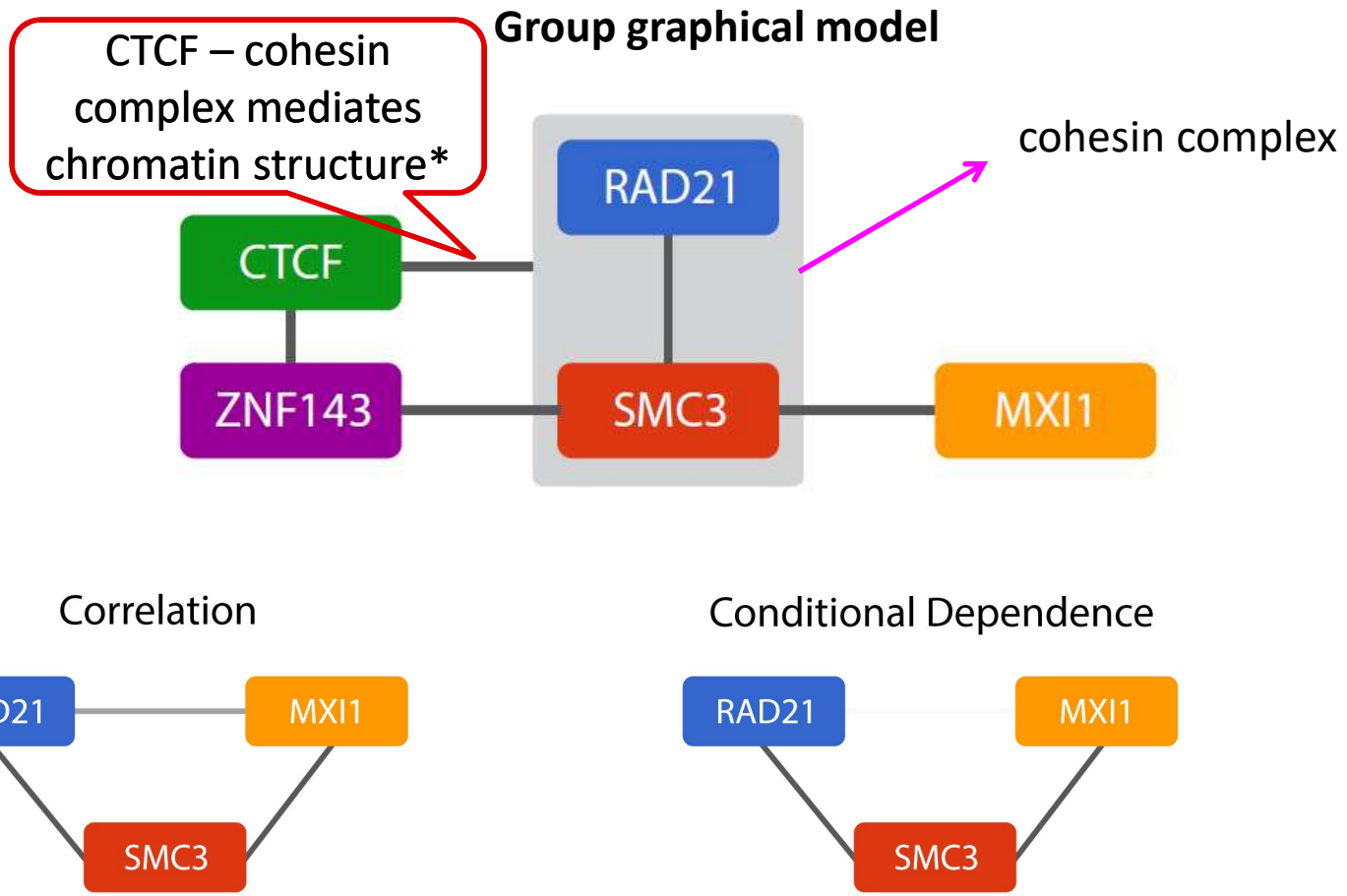


RAS21-SMC3, and SMC3-MXI1 are known PPI pairs (BioGRID)

HeLa-S3 cervical carcinoma cells

* Panigrahi et al., “A cohesin-RAD21 interactome”. *Biochemical Journal* 442.3 (2012)

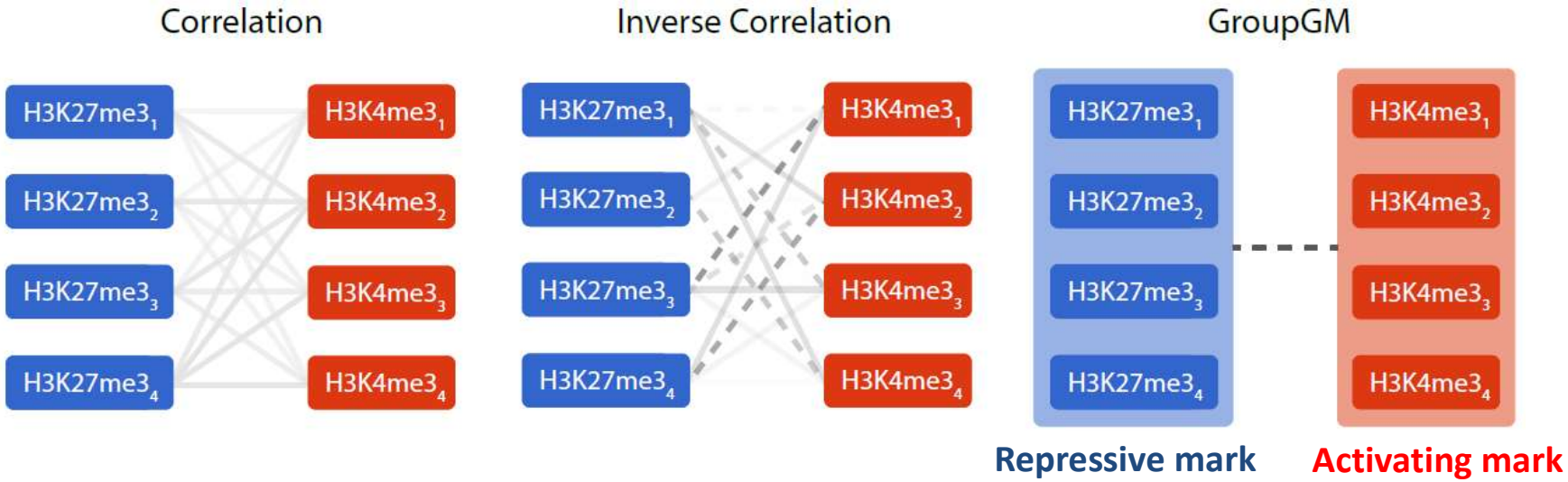
Here is what the group graphical model captured



* Parelho et al. “Cohesins functionally associate with CTCF on mammalian chromosome arms”. *Cell* (2008)

Recovering a connection between H3K27me3 and H3K4me3

- Typically the problem of redundancy is avoided by either averaging or removing redundant variables.
  - However, such choices require knowledge of both the data and the scale of the interactions being considered.



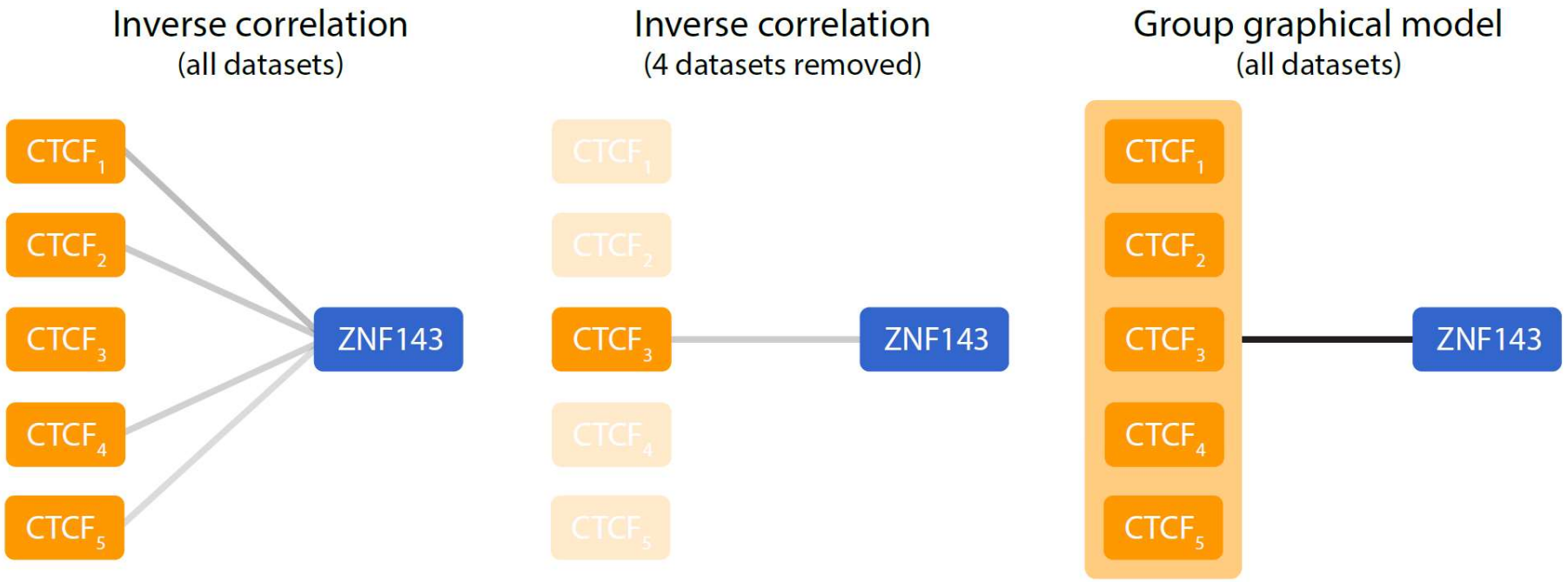
H7-hESC embryonic stem cells

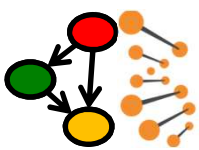
H3K28me3 and H3K4me3 were measured at UW at different time points of differentiation.

Another example to show how GroupGM mitigates problems with highly correlated variables

- ZNF143 – CTCF connections in K562

standard conditional dependence model





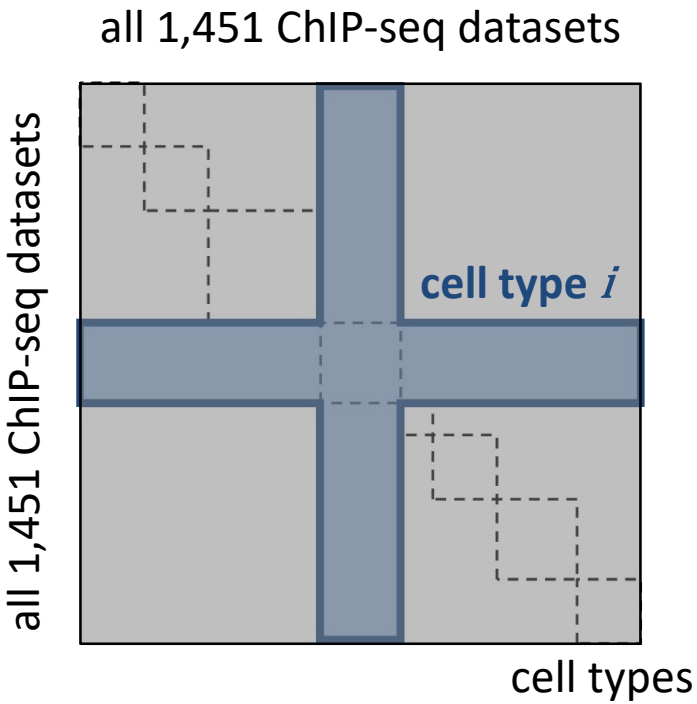
Outline of Talk

- Motivation
-
  - Increased number of possible interactions
  - Direct comparison across cell types when conditioned on the same global set of datasets
  - Extraction of global pattern of the network
  - Learning a joint model of all cell types
  - Identifying context-specificity
- Visualization tool
- Discussion

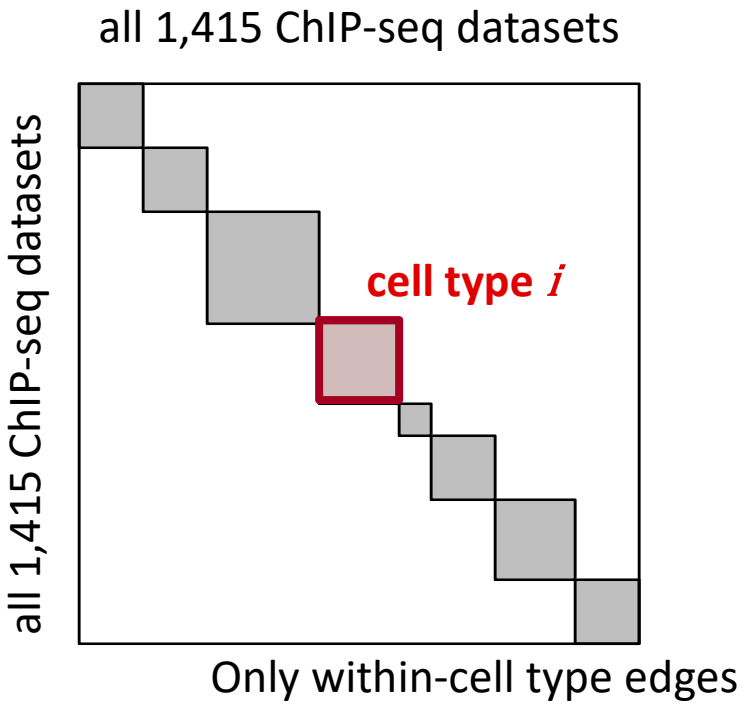
The value of learning a joint network of ChIP-seq datasets from multiple cell types

network estimates (Σ^{-1})

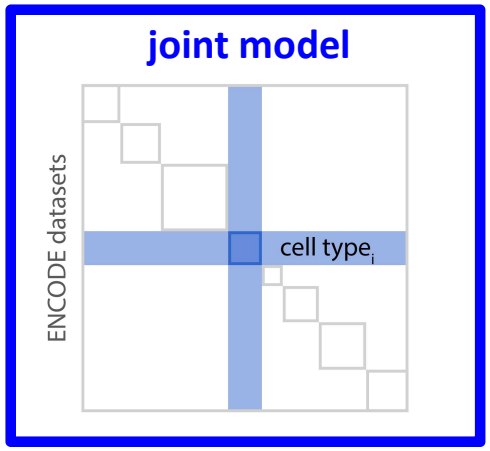
joint model

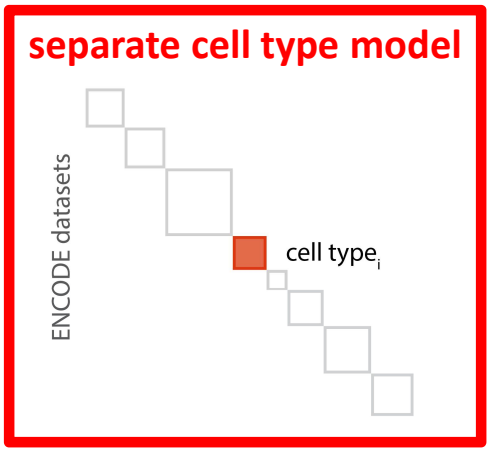


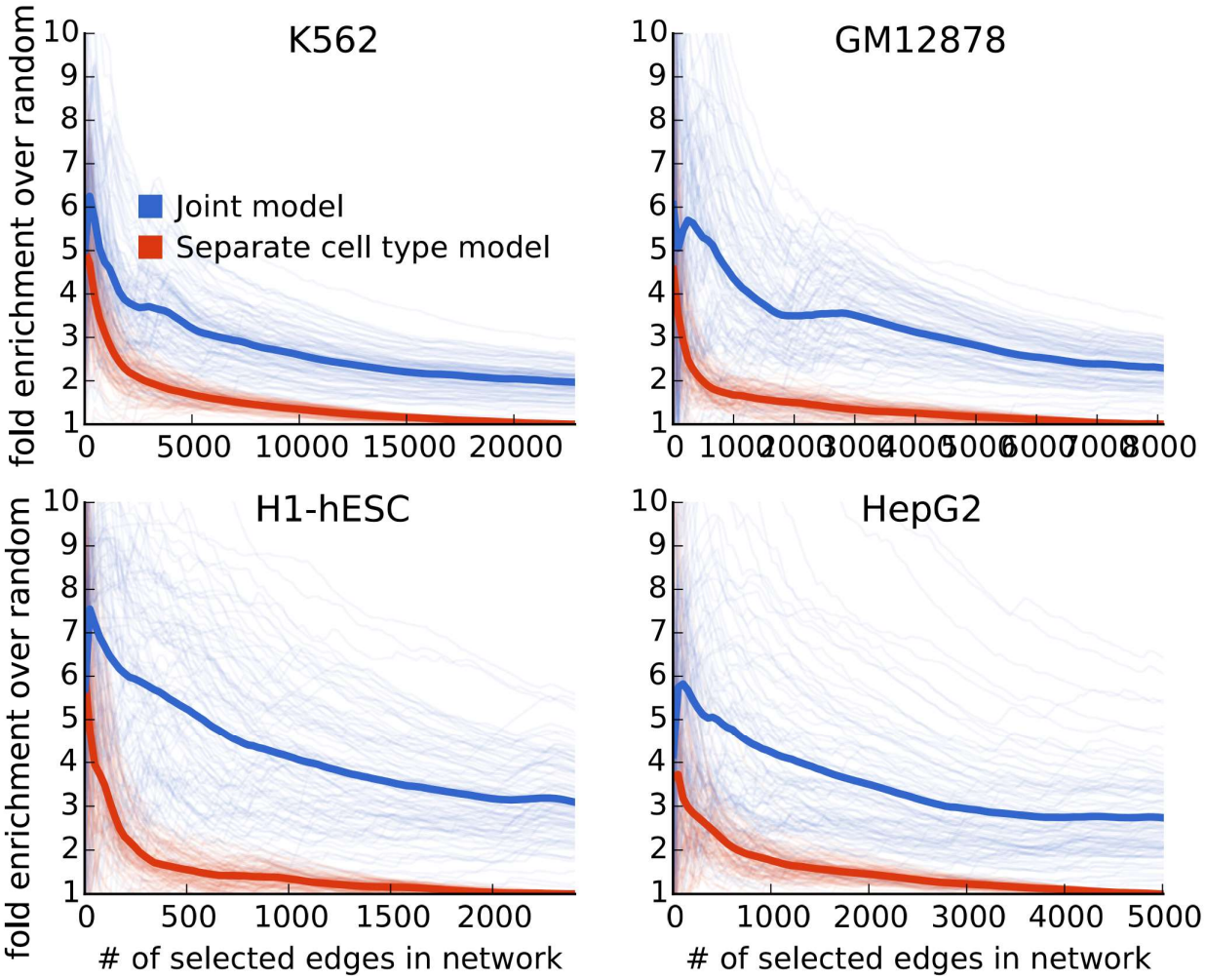
separate cell type model



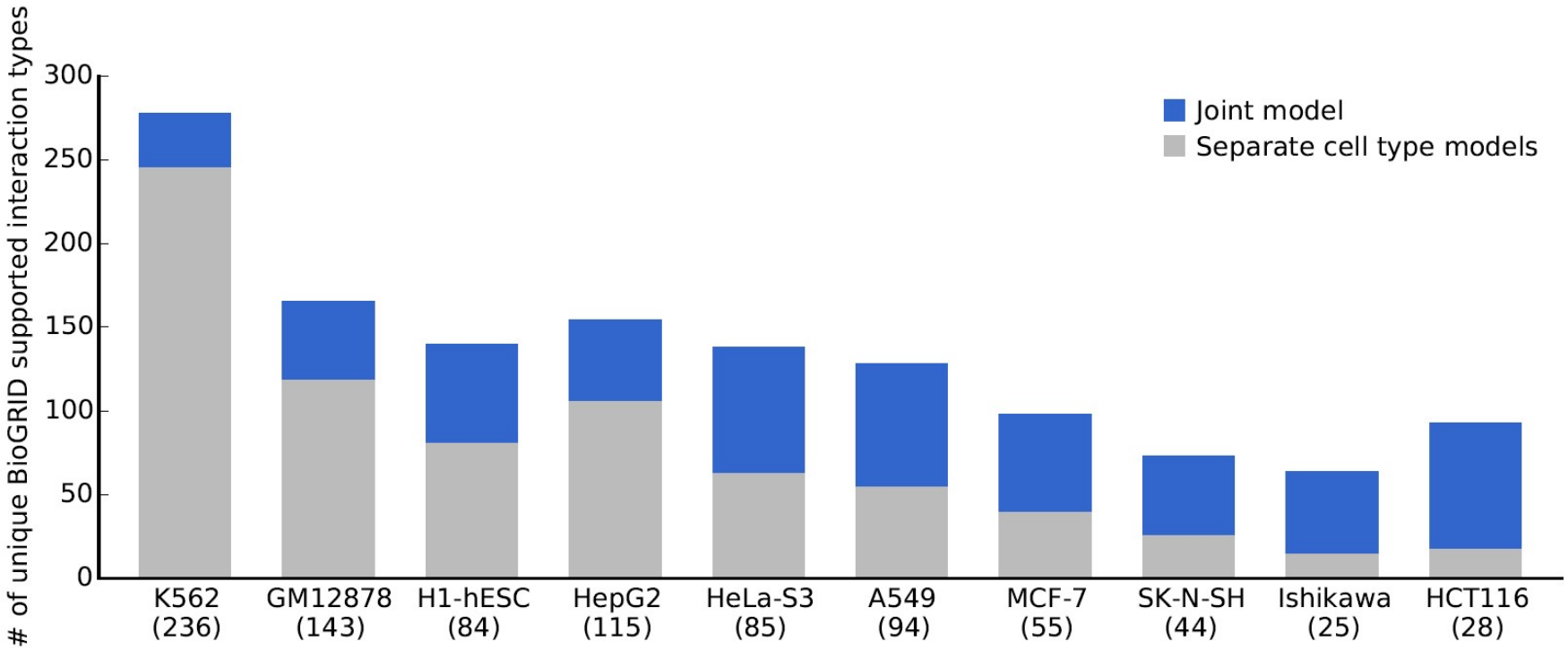
Comparison between joint model and separate cell type-model



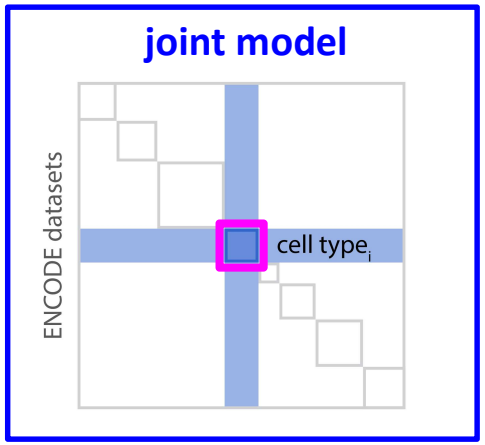


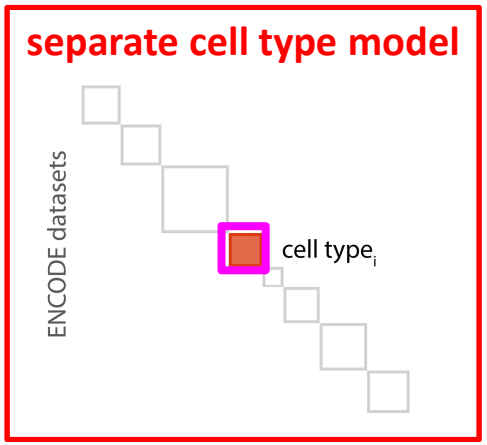


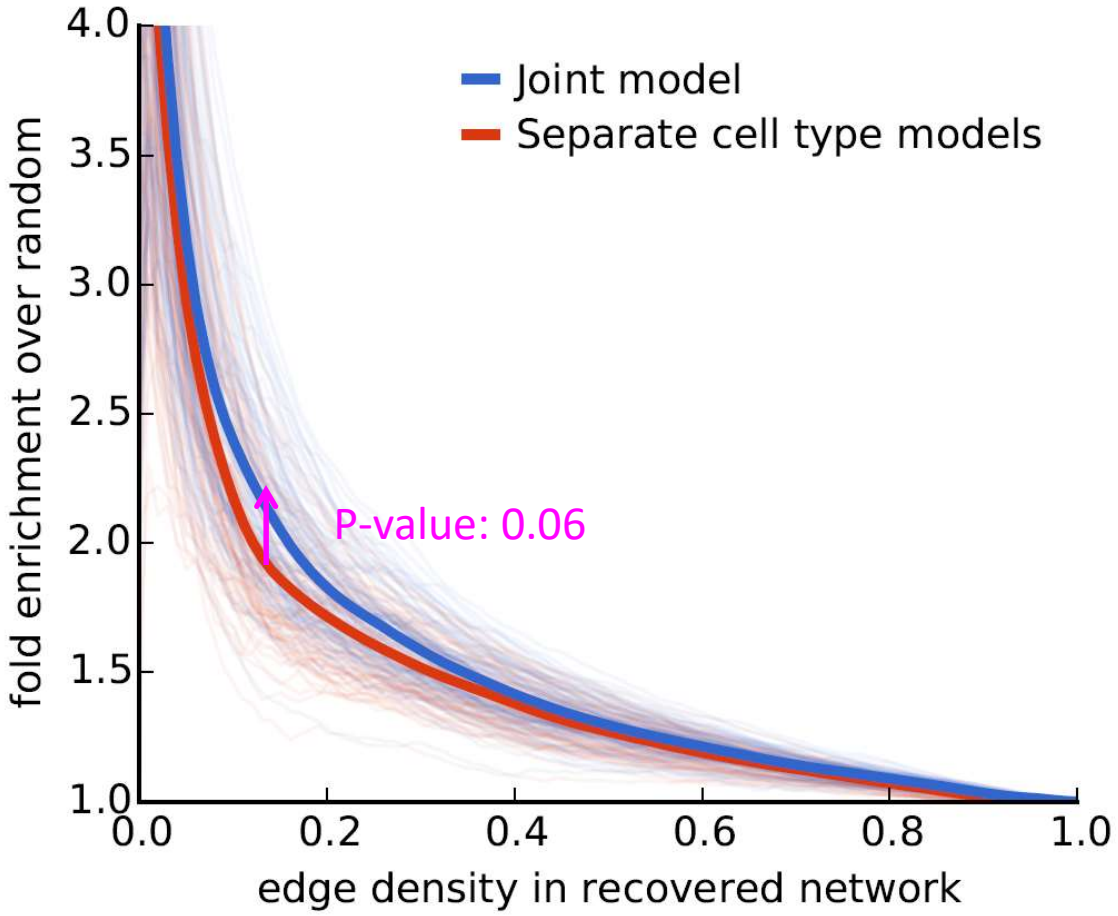
Increased number of unique factor-factor interaction types



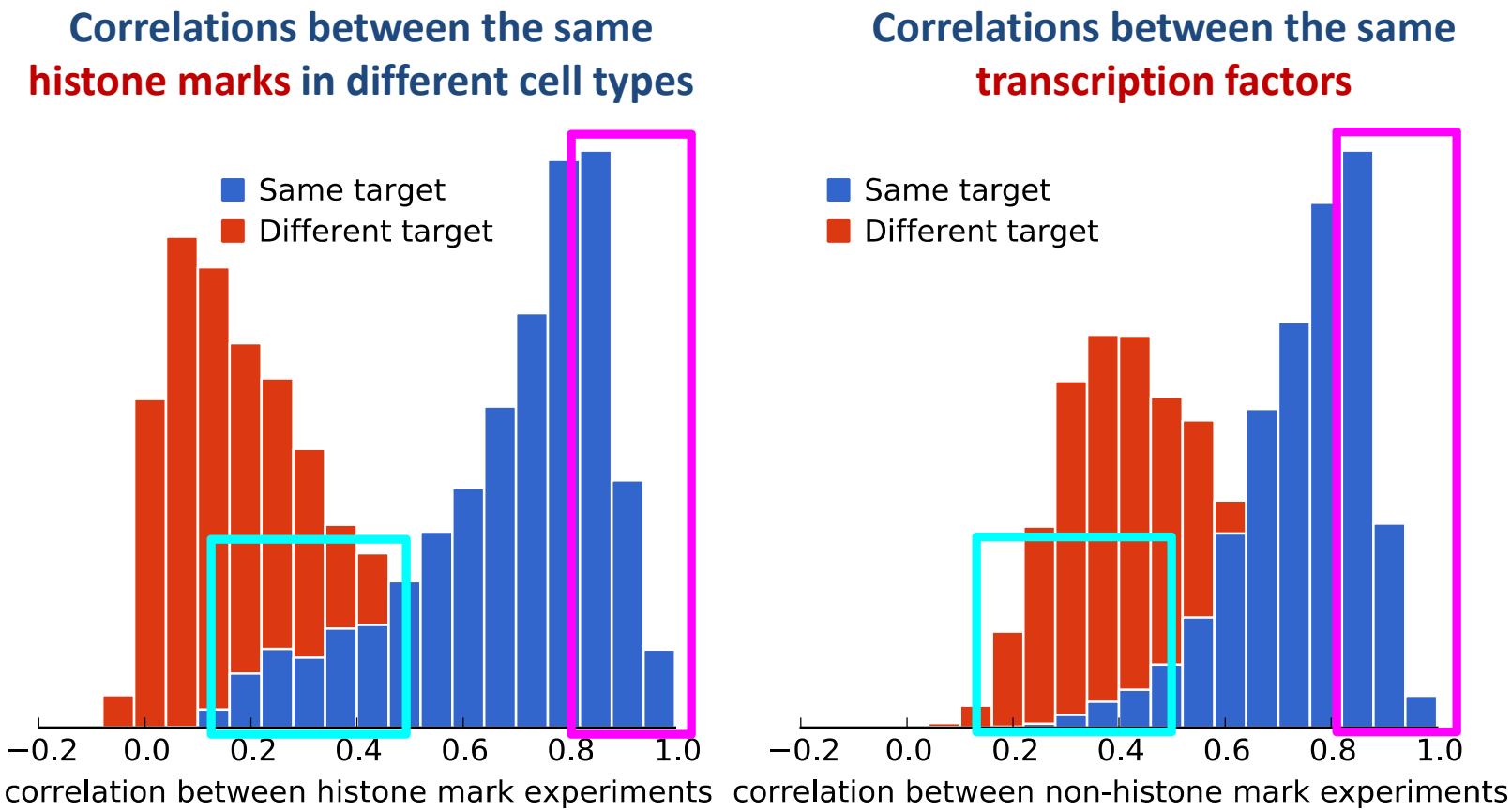
Focusing on only within cell type edges…



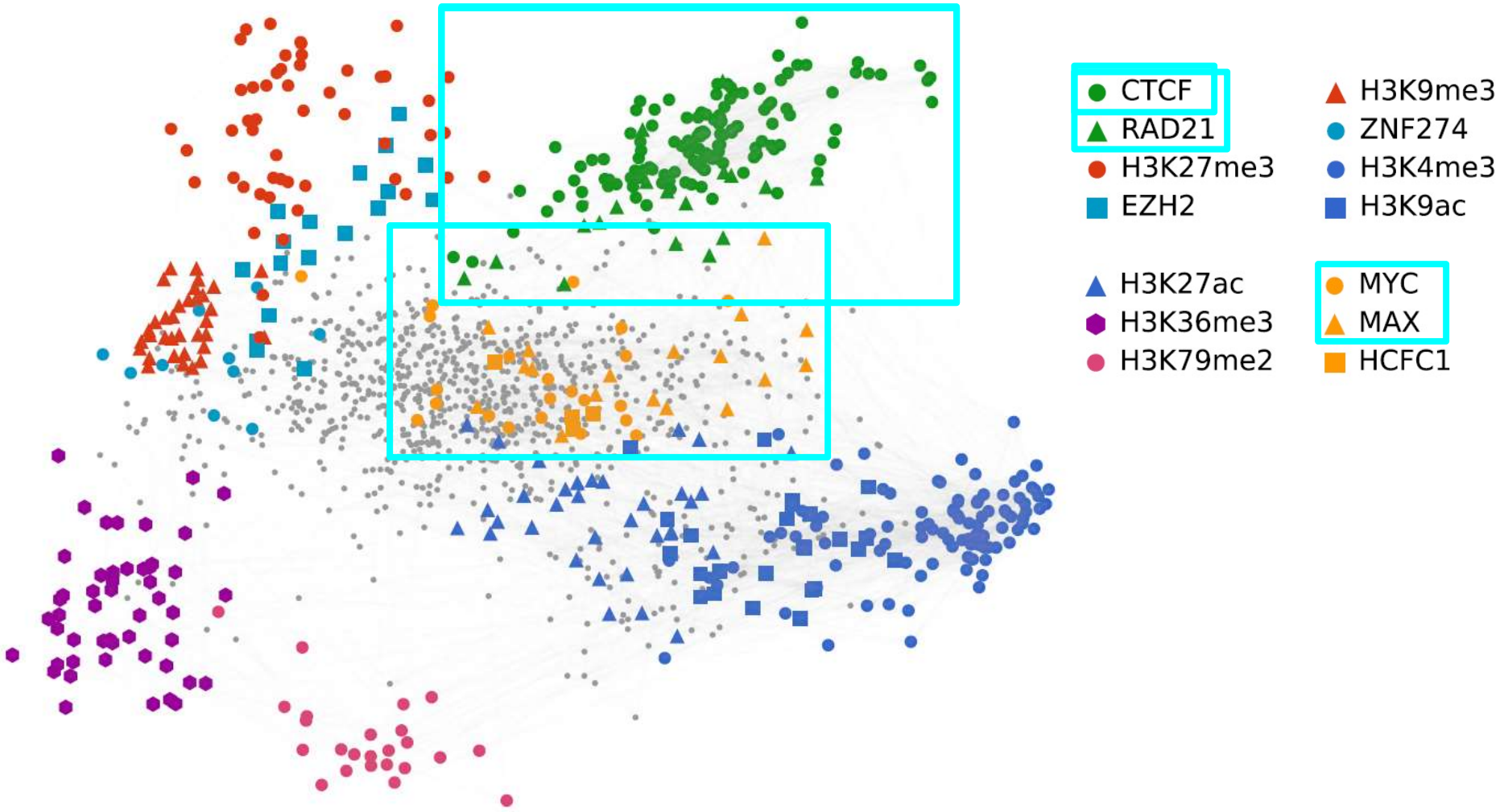




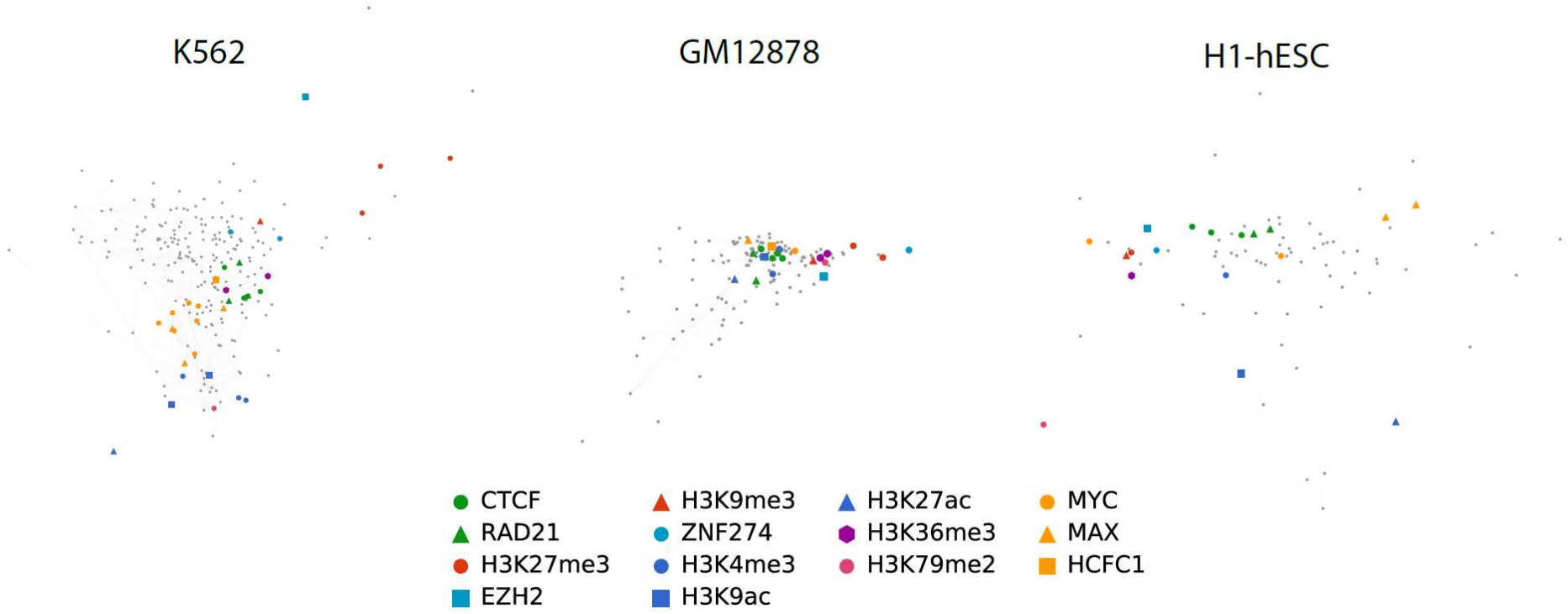
Correlation of the same factor between different cell types



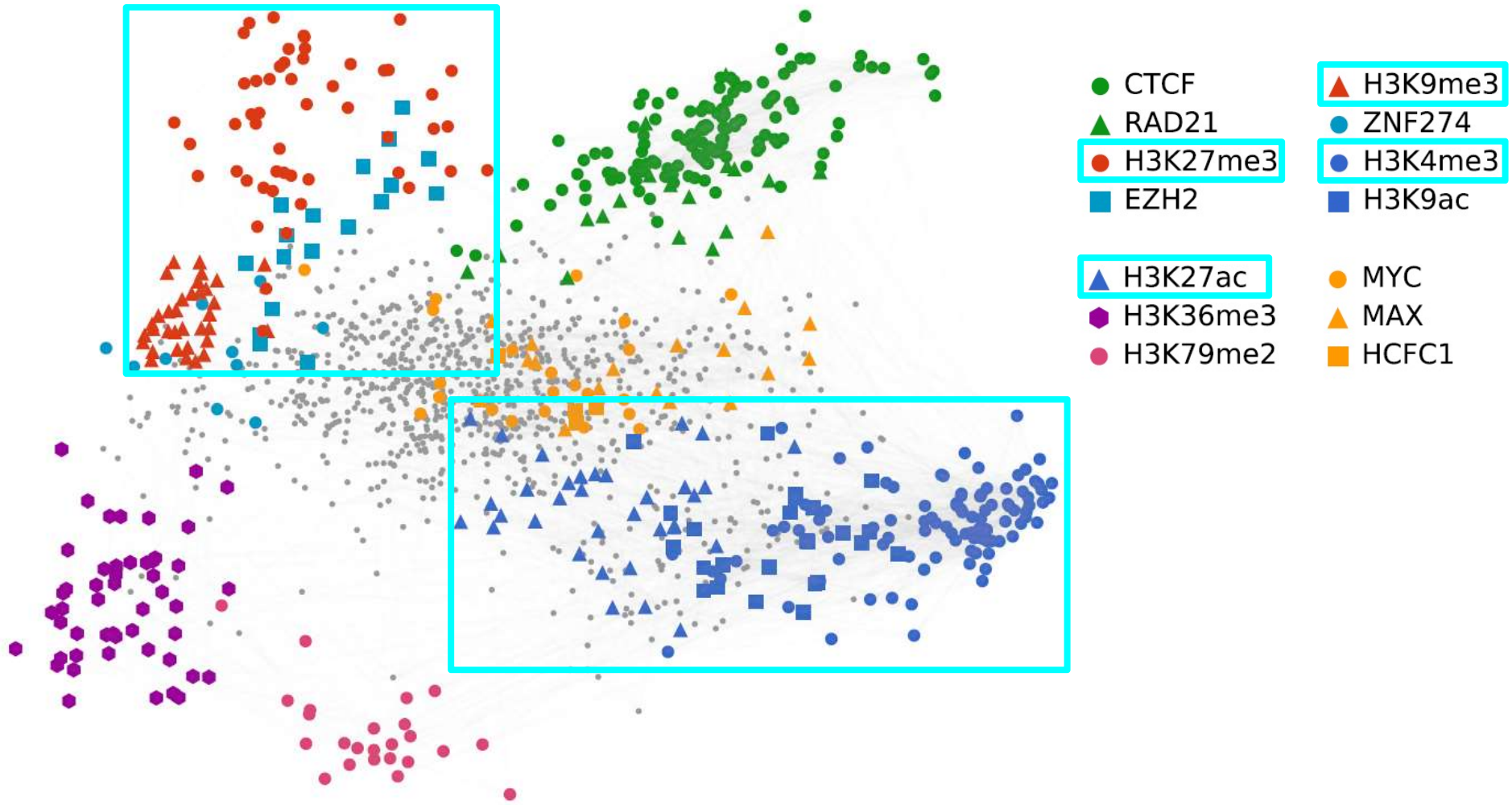
Global pattern of the entire network

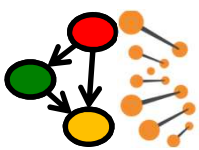


Global pattern in individual cell type networks



Global pattern of the entire network





Outline

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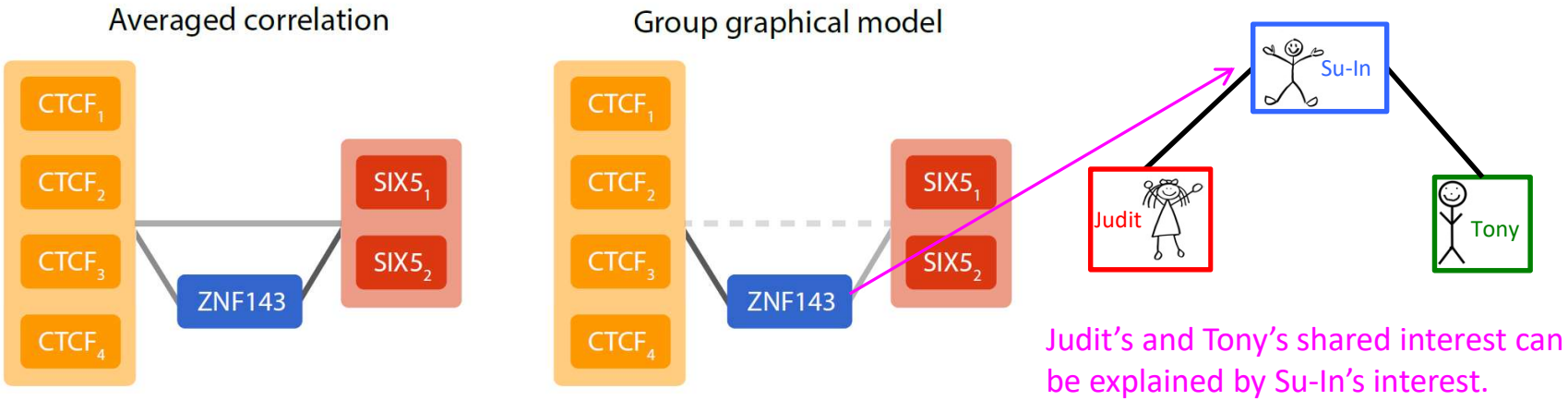
- Key features of ChromNet
  - Identifying conditional dependence
  - Group-based modeling
  - Learning a joint model of all cell types
  - Identifying context-specificity
- Visualization tool
- Discussion

How context-specificity is encoded in a conditional dependence network?

- Transcription factors co-associate in a combinatorial and context-specific fashion.
  - For example, CTCF and SIX5 closely co-associate specifically when ZNF143 is also present.*
- This means that CTCF and SIX5 are *conditionally independent (not connected with each other)* given ZNF143
- ***CTCF – SIX5 relationship is context specific and ZNF is the context.***

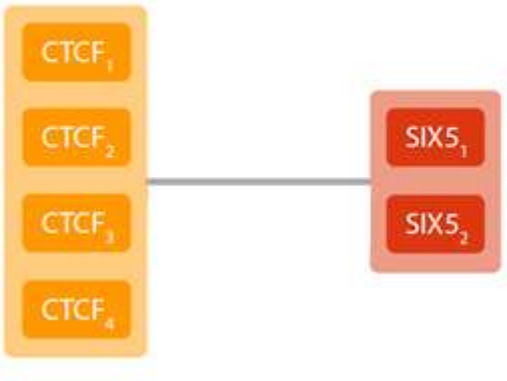


* Gerstein et al. Nature (2012)

What if the mediator is not captured?

- It is important to understand the genomic context in which any given edge occurs.
- We developed a new efficient method to estimate the contribution of the subset of data for each position to each network edge.
  - Say that ZNF143 is not present.

Group graphical model without ZNF143



Measuring the difference in the $\Sigma^{-1}(\Sigma^{-1})_{ij}$

$$
\begin{aligned}
\bar{\Sigma} &= (D^{-1}(\Sigma - uu^T)D^{-1})^{-1} \\
&= D(\Sigma - uu^T)^{-1}D \\
&= D(\Sigma + uBu^T)^{-1}D \\
&= D(\Sigma^{-1} - \Sigma^{-1}u(B^{-1} + u^T\Sigma^{-1}u)^{-1}u^T\Sigma^{-1})D \\
&= D(\Sigma^{-1} - \Sigma^{-1}u(-1 + u^T\Sigma^{-1}u)^{-1}u^T\Sigma^{-1})D \\
&= D(\Sigma^{-1} - v(-1 + u^Tv)^{-1}v^T)D \\
&= D(\Sigma^{-1} - \frac{vv^T}{u^Tv - 1})D
\end{aligned}
$$

estimated contribution of each position to CTCF – SIX5 edge

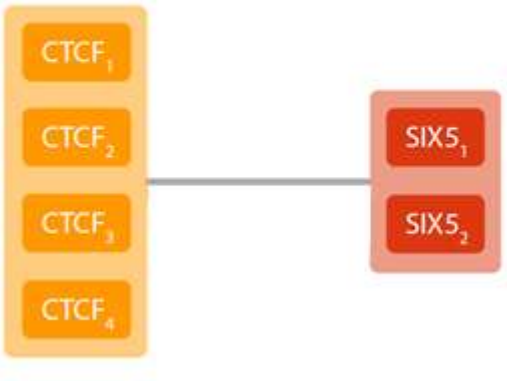
ATATGTGTTAGGATTTATGCGCGAGGACGCATCATACGACTAGTAATGATTGATC

genomic positions are samples

What if the mediator is not captured?

- It is important to understand the genomic context in which any given edge occurs.
- We developed a new efficient method to estimate the contribution of the subset of data for each position to each network edge.
  - Say that ZNF143 is not present.

Group graphical model without ZNF143



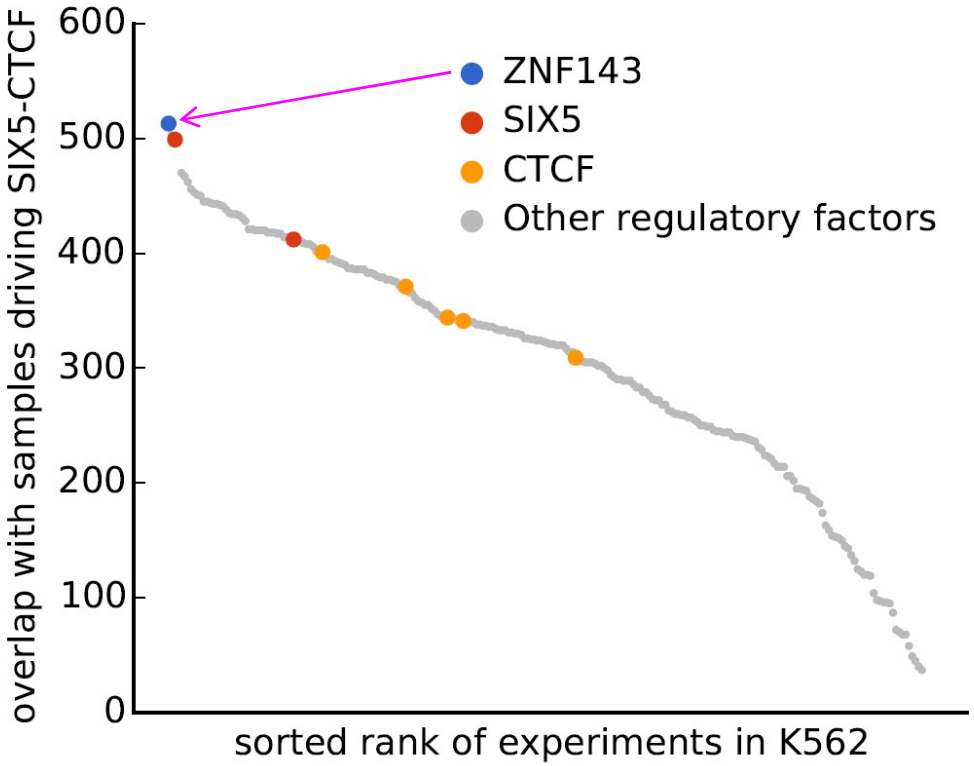
Does the estimated amount of contribution overlap with localization of another factor ?

estimated contribution of each position to CTCF – SIX5 edge

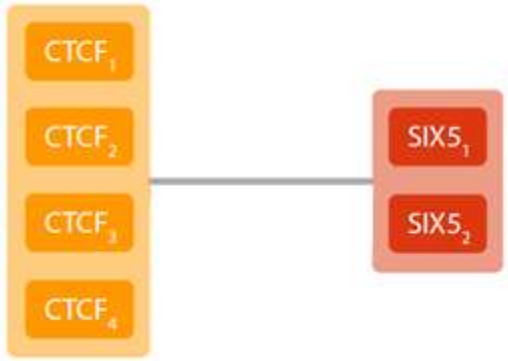
ATATGTGTTAGGATTTATGCGCGAGGACGCATCATACGACTAGTAATGATTGATC

genomic positions are samples

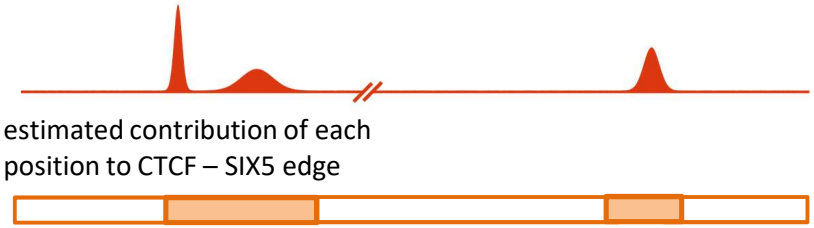
ZNF143 is captured as the context



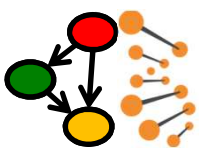
Group graphical model without ZNF143



Does the estimated amount of contribution overlap with localization of another factor ?



estimated contribution of each position to CTCF – SIX5 edge

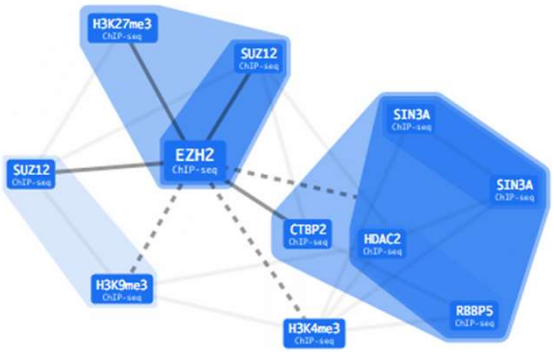


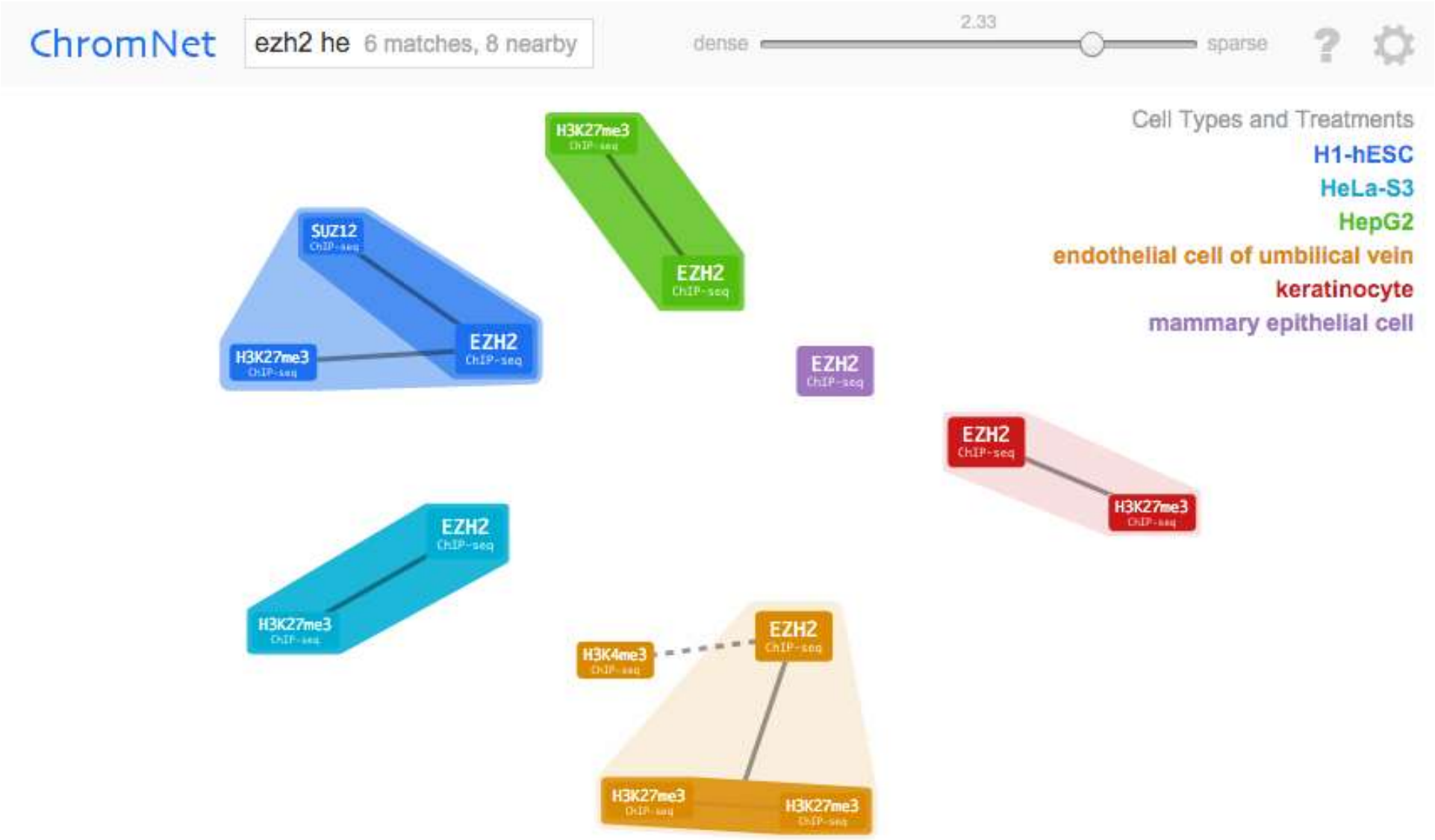
Outline

- Motivation
- Key features of ChromNet
  - Identifying conditional dependence
  - Group-based modeling
  - Learning a joint model of all cell types
  - Identifying context-specificity
- Visualization tool
- Discussion

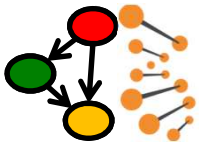
Simultaneously visualizing groups and network edges

- Easy access to the network information is important.
  - Interactive tool to navigate the human chromatn network
  - http://chromnet.cs.washington.edu/#/
- The group graphical model perspective requires a unique visual encoding.
- This encoding must simultaneously display both clusters and networks.
- Ideally it should be both dynamic and interactive in order to allow exploration.





ChromNet browser: http://chromnet.cs.washington.edu/

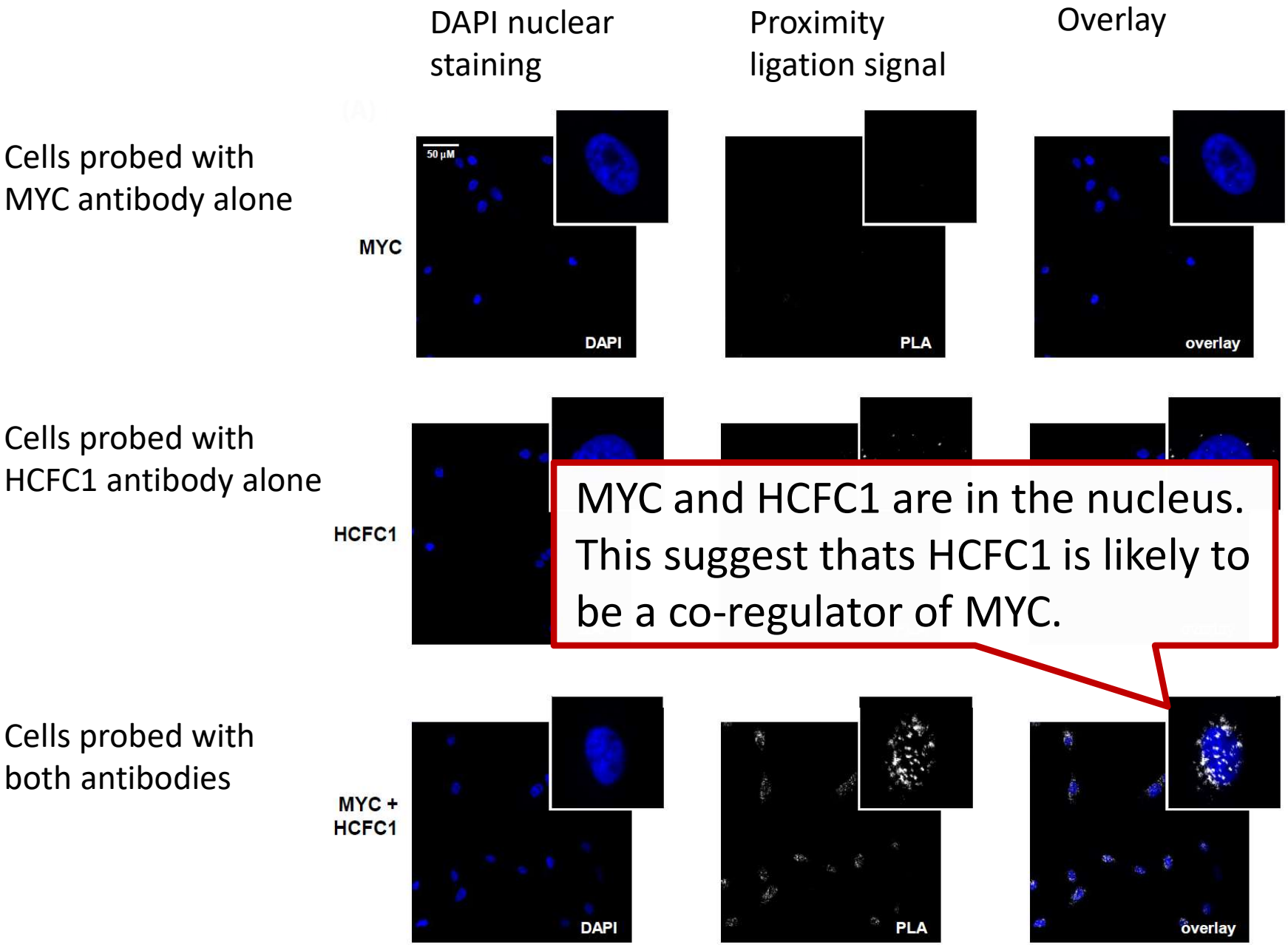


An example of a novel interaction MYC-HCFC1 and wet-lab validation

- One of the strongest edges that connect proteins not known to interact with each other is MYC-HCFC1 (Host Cell Factor C1)
  - **MYC:** cell growth, cell cycle progression, oncogenesis
  - **HCFC1:** cell cycle progression, oncogenesis

- To validate the potential novel MYC-HCFC1 interaction, we performed a proximity ligation assay (PLA) in MCF10A mammary epithelial cells.

- When two proteins that are probed with specific antibodies are within close proximity of each other, fluorescence signals are produced that are measured and quantified using fluorescence microscopy.


DAPI nuclear staining
Proximity ligation signal
Overlay
Cells probed with MYC antibody alone
MYC
50 µM
DAPI
PLA
overlay
Cells probed with HCFC1 antibody alone
HCFC1
MYC and HCFC1 are in the nucleus. This suggest thats HCFC1 is likely to be a co-regulator of MYC.
Cells probed with both antibodies
MYC + HCFC1
DAPI
PLA
overlay

More information is available

- Preprint is available on bioRxiv
- The ChromNet brower
  - http://chromnet.cs.washington.edu/

Genome Biology

Learning the human chromatin network from all ENCODE ChIP-seq data

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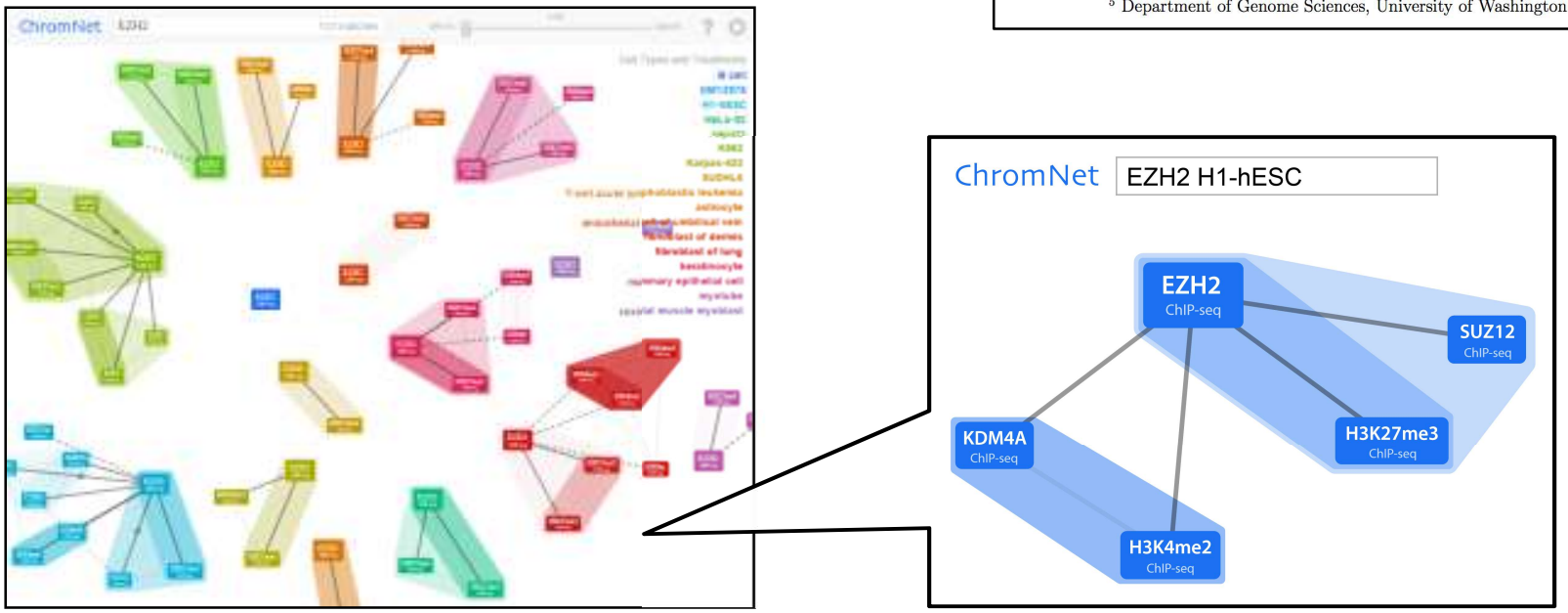
[3] Princess Margaret Cancer Centre
[4] Department of Computer Science, University of Toronto
[5] Department of Genome Sciences, University of Washington



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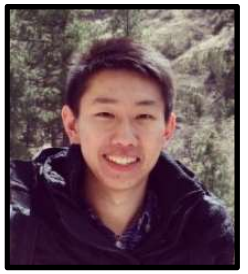
William Tu


Brian Raught

computational biologist

experimental biologists

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