# Landscape of Differential Network Analysis Methods & Software

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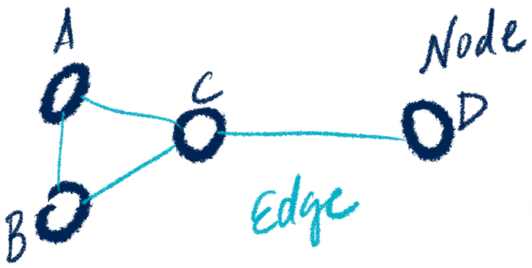
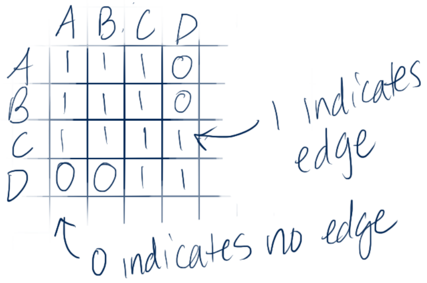
# Presentation Overview

- Background on graphical models and differential networks
- Overview of statistical landscape for differential network analysis
- Overview of available software
- Links to practical application using a few software options
- Discussion & feedback!

# Background: Graphical Model

- Graphical models express connections between variables. When undirected, the connection doesn't imply any directionality.




- Connected edges can be seen in an **Adjacency Matrix**, where anything with a zero is considered "conditionally independent", and anything with a 1 is considered "conditionally dependent"

- In this example, A and B are **conditionally independent** of D

# Gaussian Graphical Model

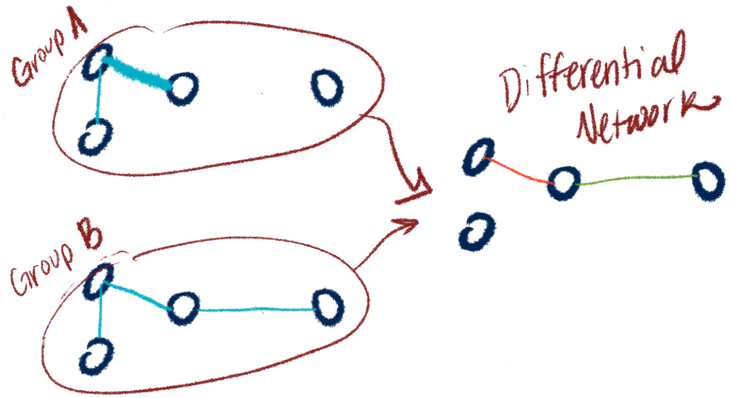
- Gaussian Graphical Models (GGMs) are the most widely used probabilistic graphical models (See Kate Shutta's recently published tutorial on GGMs for full details! [Shu+22])
- Assume data $\mathbf{y}$ is distributed as multivariate Gaussian $N(\mu, \Sigma)$ with mean vector $\mu$ and precision matrix $\Sigma^{-1} = \Omega$ whose entries correspond to partial correlation between variables
- So any two entries are conditionally independent if entry in $\Omega$ is zero.
- In low dimensional setting, the Likelihood function:

$$l(\Omega; S) = ln|\Omega| - tr(S\Omega)$$

- Where $\Omega = \Sigma^{-1}$ is the "precision matrix" and $S$ is sample covariance matrix.
- We want an estimate for this which we will call $P = S^{-1}$. This will give us the graph structure.

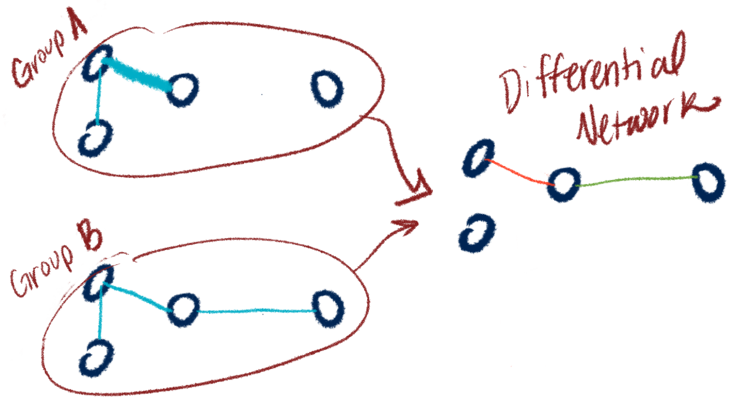
# More than one graphical model

- Say you have data from two groups, like disease and healthy control.
- Say you estimate a graphical model for each group, then want to compare the resulting networks.



# But are the networks different??

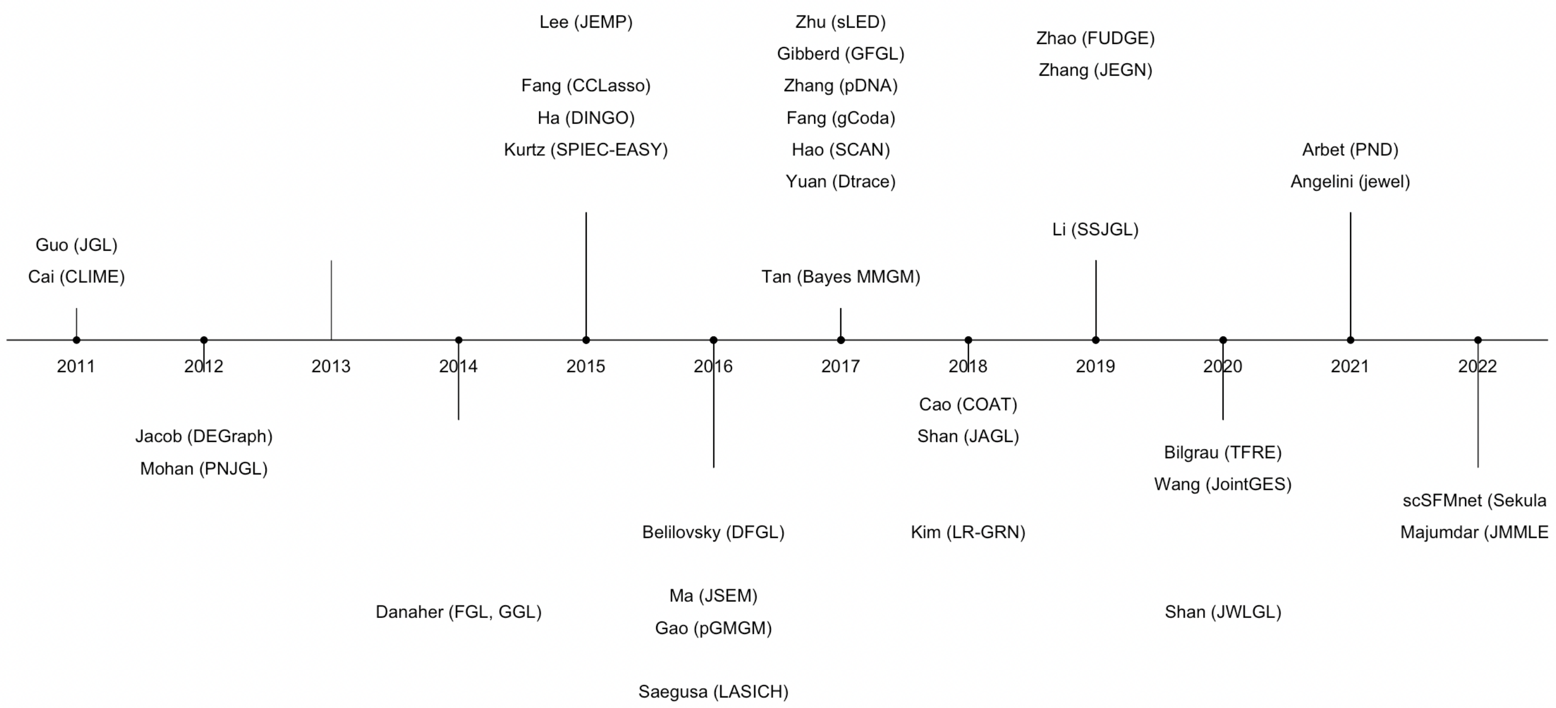
- How do you estimate them?
- How do you test the difference?
- How do you even *characterize* the difference? (edges? nodes? hubs? general structure?)
- This all falls under DIFFERENTIAL NETWORK ANALYSIS! (DiNA)



# Statistical Landscape of DiNA methods

# Timeline

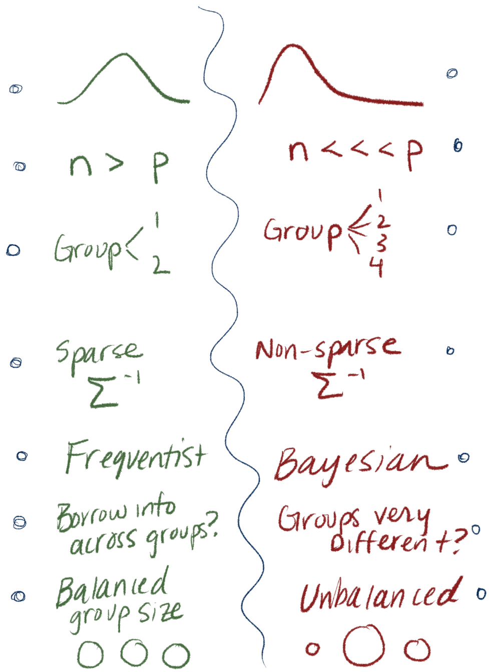
- I found 40+ methods papers on DiNA methods published in the last 10 years
- The wide variety is due to addressing many subtly different problems



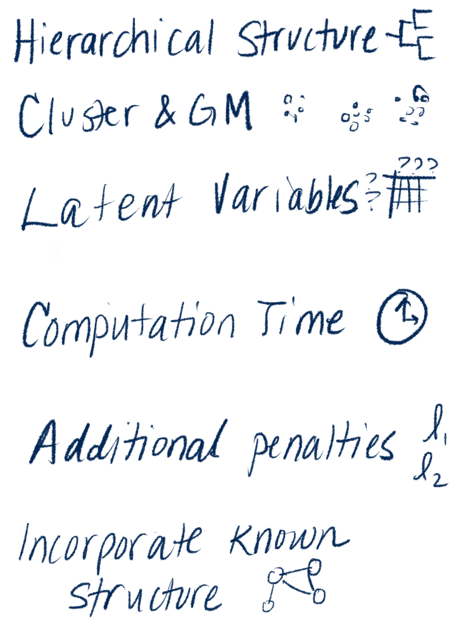
# Why so many methods?

To address various data and modeling situations!

What's your data like?




Other model considerations...



# DiNA Methods Summary

- Gaussian:
  - Graphical Lasso: JGL (Guo 2011)
    - Additional penalties for encouraging similar sparsity across groups: FGL & GGL (Danaher 2014)
      - Incorporating structural information: JSEM (Ma 2016)
        - Extension which doesn't require post-processing: jewel (Angelini 2021)
      - Doesn't require sparse inputs: DTrace (Yuan 2017)
        - Extension for multi-modal data: pDNA (Zhang 2017)
    - Node-based learning framework: PNJGL (Mohan 2014)
    - Unbalanced groups: JAGL (Shan 2018)
    - Hierarchical structure: JWLGL (Shan 2020)
  - Graphical Ridge: TFRE (Bilgrau 2020)
  - Adjust for global conditional dependencies to identify "driver" group-specific components: Dingo (Ha 2015)
  - Group-wise heterogeneous structure: LASICH (Saegusa 2016)
  - Direct estimation of difference: Zhao 2014
  - Uses latent nodes: Na 2019
  - Simultaneous clustering & GM estimation: SCAN (Hao 2017), Price 2021

# DiNA Methods Summay (cont'd)

- Non-Gaussian: SPIEC-EASY (Kurtz 2015), pDNA (Zhang 2017)
- Semi-parametric: Xu 2016
- Comparison across 3+ groups: BioNetStat (Jardim 2019)
- Group-wise structure: JMMLE (Majumdar 2022)
- High dimensional: JointGES (Wang 2020), FUDGE (Zhao 2022)
- Bayesian: Peterson 2015, Mitra 2016, Tan 2017, Li 2019, Sekula 2022

# Graphical Lasso (gLasso)

- Because majority of available methods are some variation on gLasso, I'm going to go into the details of the optimization problem and penalty terms here.

- Convex optimization problem for graphical lasso, where $\lambda$ is a tuning parameter and $||\Theta||_1$ is the sum of absolute values of the elements of $\Theta$. The solution gives an estimate for $\Sigma^{-1}$, the precision matrix:

$$maximize_{\Theta}\{logdet\Theta - tr(S\Theta) - \lambda||\Theta||_1\}$$

- *Graphical lasso* can be used even when $p >> n$, and when $\lambda$ is large then it forces the estimated precision matrix to be sparse (so few edges!).

- Joint graphical lasso builds upon this by estimating *multiple, related GGMs* from data with observations belonging to distinct classes (for example, cancer vs normal tissue).

- The idea is to leverage information across the classes while still letting there be class-specific edges. Sparsity and similarity between graphs modified by penalty functions.

# Notation

- $K$ number of classes 2+. Index classes using $k$ = 1, ... $K$.
- $\Sigma_k^{-1}$: True precision matrix for the kth class
- $Y^{(k)}$: $n_k$ x $p$ matrix consisting of $n_k$ observations from the $k$th class on a set of $p$ features which are common to all $K$ data sets
- $S^{(k)}$: Empirical covariance matrix for $Y^{(k)}$
- $\Theta^{(k)}$: argument to convex optimization problem used for estimating $\Sigma_k^{-1}$
- Index matrix arguments by using $i$ = 1, ..., $p$ and $j$ = 1, ..., $p$
- $\lambda_1$ and $\lambda_2$: non-negative tuning parameters used in penalty function

# Major assumptions

- We assume the observations **within** each class are iid.
- Also assume $\mu_k$, the mean for each class, is 0. i.e:

$$Y_1^{(k)}, \ldots, Y_{nk}^{(k)} \sim N(0, \Sigma_k)$$

# Optimization problem for Joint Graphical Lasso

- Our goal is to estimate $\Sigma_1^{-1}$, ..., $\Sigma_K^{-1}$ by using penalized log-likelihood approach.
- Again, we want each class to have it's own precision matrix, but to be able to use information across the classes to make them.
- Seek $\hat{\Theta}$ by solving:

$$maximize_{\{\Theta\}} \left( \sum_{k=1}^{K} n_k [log\{det(\Theta^{(k)})\} - tr(S^{(k)}\Theta^{(k)}) - P(\{\Theta\}) \right)$$

- A **major innovation of the Danaher 2014 paper**, is the generalization of the optimization problem to multiple classes, in addition to using the penalty function $P(\{\Theta\})$, for which the authors provide two different versions.

# Penalty functions

- The general form for the penalty function is:

$$P(\{\Theta\}) = \lambda_1 \sum_{k=1}^{K} \sum_{i \neq j} |\theta_{ij}^{(k)}| + \widetilde{P}\{\Theta\}$$

- Notice that the $P(\{\Theta\})$ is **not class specific**. It takes information from all the classes!
- The form of this penalty function will encourage the solutions to share certain characteristics such as locations of sparsity or value.
- Depending on the form we choose and the value of the tuning parameters, we could essentially force joint graphical lasso to just perform unrelated graphical lasso on each of the $K$ classes (i.e. if $\widetilde{P}\{\Theta\}$ is zero.)
- Let's look at the possible forms for $\widetilde{P}\{\Theta\}$!

# Fused Graphical Lasso

- Fused Graphical Lasso (FGL) uses the following penalty function:

$$P(\{\Theta\}) = \lambda_1 \sum_{k=1}^{K} \sum_{i \neq j} |\theta_{ij}^{(k)}| + \lambda_2 \sum_{k<k'} \sum_{i,j} |\theta_{ij}^{(k)} - \theta_{ij}^{(k')}|$$

- When $\lambda_1$ is **large**, FGL makes sparse estimates of $\hat{\Theta}^{(1)}, \ldots, \hat{\Theta}^{(K)}$
- When $\lambda_2$ is **large**, many elements of $\hat{\Theta}^{(1)}, \ldots, \hat{\Theta}^{(K)}$ will be the same across classes
- So, FGL "borrows information aggressively across classes, encouraging similar network structure and similar edge values"

# Group Graphical Lasso

- Group Graphical Lasso (GGL) uses the following penalty function:

$$P(\{\Theta\}) = \lambda_1 \sum_{k=1}^{K} \sum_{i \neq j} |\theta_{ij}^{(k)}| + \lambda_2 \sum_{i \neq j} \left( \sum_{i,j} {\theta_{ij}^{(k)}}^2 \right)^{1/2}$$

- Lasso penalty applied to elements of the precision matrices
- Group lasso penalty is applied to the (i, j) element across all K precision matrices
- When $\lambda_1$ is **large**, GGL makes sparse estimates of $\hat{\Theta}^{(1)}, \ldots, \hat{\Theta}^{(K)}$
- So, GGL just encourages a shared pattern of *sparsity*, not shared *edge values* (unlike FGL which encourage sharing across both)

# Other penalty functions

- Here is a selection of penalty functions for comparison. For full treatment see Tsai or Shojaie review papers.

The OG Joint Graphical Lasso

JGL $\lambda_1 \sum_{i \neq j} \theta_{i,j} + \lambda_2 \sum_{k=1}^{K} \sum_{i \neq j} |\gamma_{i,j}^{(k)}|$

Important ☆ Danaher 2014

FGL $\lambda_1 \sum_{k=1}^{K} \sum_{i \neq j} |\theta_{i,j}^{k}| + \lambda_2 \sum_{k<k'} \sum_{i,j} |\theta_{i,j}^{(k)} - \theta_{i,j}^{(k')}|$

GGL $\lambda_1 \sum_{k=1}^{K} \sum_{i \neq j} |\theta_{i,j}^{(k)}| + \lambda_2 \sum_{i \neq j} \left( \sum_{i,j} \theta_{i,j}^{(k)2} \right)^{1/2}$

LASICH $\lambda_1 \sum_{k=1}^{K} \sum_{i \neq j} |\theta_{i,j}^{(k)}| + \lambda_1 \lambda_2 \sum_{i \neq j} \left\{ \sum_{k,k'}^{K} W_{k,k'} (\theta_{i,j}^{(k)} + \theta_{i,j}^{(k')})^2 \right\}^{1/2}$

Group-wise heterogeneous structure

JSEM $\sum_{j \neq i} \sum_{g \in G_{i,j}} \lambda_{i,j}^{(g)} \|\theta_{i,j}^{(g)}\|_2$

Neighborhood selection

JAGL $\sum_{k=1}^{K} \frac{1}{n_k} \sum i \neq j \; \underbrace{\frac{1}{|(1-\pi)\hat{t}_{i,j} + \pi \hat{s}_{i,j}|^{r}}}_{\text{weight avg of pooled and individual precision matrices}} |\theta_{i,j}^{(k)}|$

For K unbalanced groups

# Bayesian Inference of Multiple GGMs

- Peterson, Stingo, and Vannucci (2015) offer an alternative Bayesian approach.
- They use Markov random field (MRF) prior which encourages common structures across groups, but does not assume that all subgroups are related
- Place a spike-and-slab prior on parameters that measure network relatedness in an effort to learn which groups have a shared graph structure
- Posterior probabilities of inclusion for those parameters summarize the network similarity.

# Notation

- $K$ number of classes 2+. Index classes using $k$ = 1, ... $K$.
- $\Sigma_k^{-1}$: True precision matrix for the kth class
- $Y^{(k)}$: $n_k$ x $p$ matrix consisting of $n_k$ observations from the $k$th class on a set of $p$ features which are common to all $K$ data sets. $n_k$ need not be identical size.
- $S^{(k)}$: Empirical covariance matrix for $Y^{(k)}$
- $\Omega^{(k)}$: symmetric positive definite matrix constrained by a graph $G_k$
- $\mathbf{g}_{ij}$: Binary vector representing inclusion of edge (i, j) in graphs 1, ..., K.
- Index matrix arguments by using $i$ = 1, ..., $p$ and $j$ = 1, ..., $p$

# Markov Random Field Prior

Probability of the binary vector of edge inclusion indicators $\mathbf{g}_{ij}$ given by:

$$p(\mathbf{g}_{ij}|v_{ij},\Theta) = C(v_{ij},\Theta)^{-1} exp(v_{ij}\mathbf{1}^T g_{ij} + \mathbf{g}_{ij}^T \Theta \mathbf{g}_{ij})$$

- $\mathbf{1}$: unit vector of dimension K
- $v_{ij}$ is a parameter specific to each set of edges $\mathbf{g}_{ij}$
- $\Theta$ is a KxK symmetric matrix representing pairwise relatedness of the graphs for each sample group.
  - Diagonals are zero, off-diagonals which are non-zero represent connections between related networks.
- $C(v_{ij},\Theta)$: Normalizing constant

# MRF continued

So, prior probability that edge (i, j) is absent from all K graphs is:

$$p(\mathbf{g}_{ij} = 0 | v_{ij}, \Theta) = 1/C(v_{ij}, \Theta)$$

- Joint prior on graphs is product of densities for each edge:

$$p(G_1, \ldots G_K | v, \Theta) = \prod_{i<j} p(\mathbf{g}_{ij} = 0 | v_{ij}, \Theta)$$

Conditional probability of the inclusion of edge in $G_k$, given inclusion of edge in remaining graphs, is:

$$p(g_{k,ij} | g_{m,ij_{m \neq k}}, v_{ij}, \Theta) = \frac{exp(g_{k,ij}(v_{ij} + 2\sum_{m \neq k} \theta_{km} g_{m,ij}))}{1 + exp(v_{ij} + 2\sum_{m \neq k} \theta_{km} g_{m,ij})}$$

# Selection prior on network similarity

Impose priors on $v$ and $\Theta$ to reduce false selection of edges.

- Use spike-and-slab prior on the off-diagonal entries of $\Theta$: $\theta_{km}$
    - "Slab" portion defined on positive domain
    - Appropriate choice is $Gamma(x|\alpha, \beta)$ with $\alpha > 1$
    - So mixture prior on $\theta_{km}$ is $p(\theta_{km}|\gamma_{km})$
    - Joint prior on off-diagonal entries is product of marginal densities:
    - $p(\Theta|\gamma) = \prod_{k<m} p(\theta_{km}|\gamma_{km})$
    - Place Bernoulli prior on latent indicators

# Edge-specific informative prior on v

- Given a known reference network $G_0$, define a prior that encourages higher selection probabilities for edges included in $G_0$.
- Impose a prior on probability of inclusion in edge in $G_k$ which reflects belief that it is similar to the reference network. Use Beta(a,b) prior.
- In cases where no prior knowledge on graph structure, use a prior that favors lower values such as Beta(1, 4) to encourage overall sparsity.

# Joint Posterior & MCMC Sampling

- Joint posterior given $\Psi$ is set of all parameters and $X$ is observed data for all groups:

$$p(\Psi|X) \propto \prod_{k=1}^{K}[p(X_k|\mu_k, \Omega_k)p(\mu_k|\Omega_k)p(\Omega_k|G_k)] \prod_{i<j}[p(g_{ij}|v_{ij}, \Theta)p(v_{ij})]p(\Theta|\gamma)p(\gamma$$

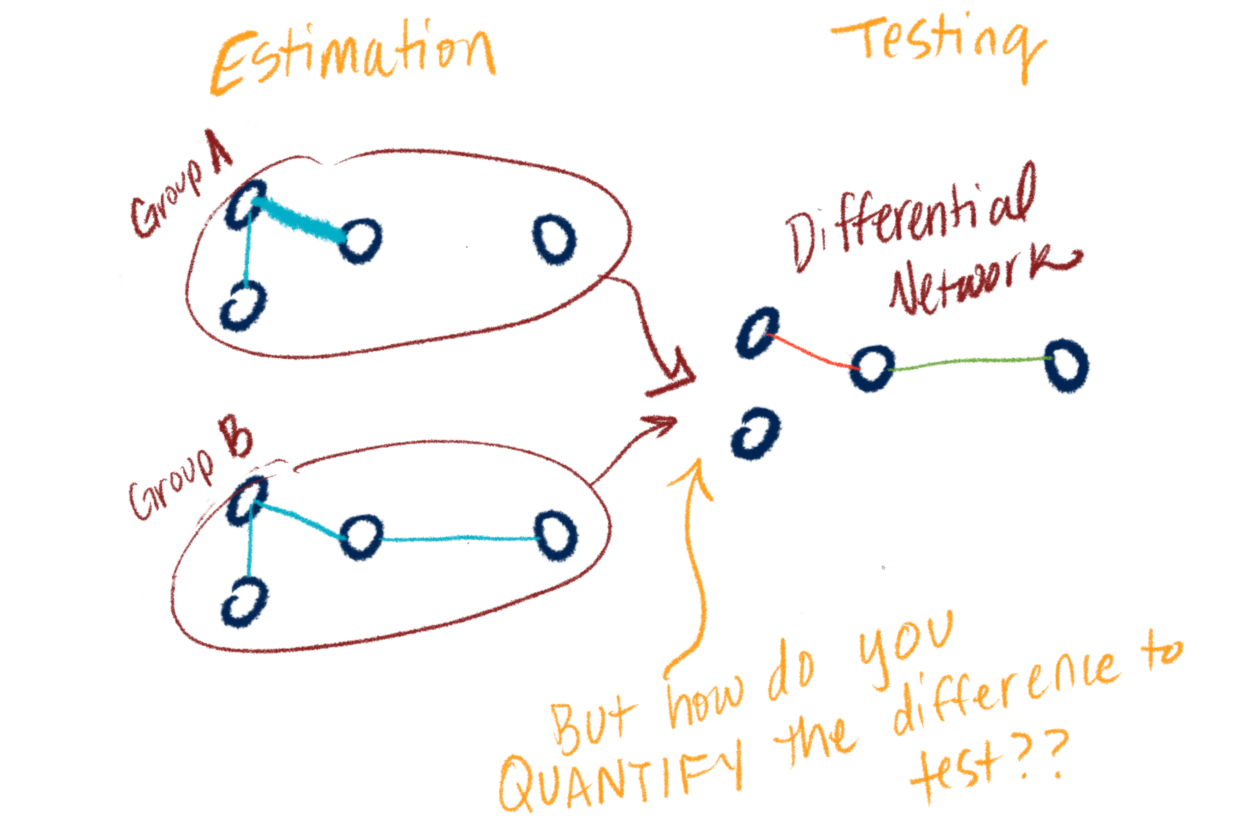
- Use a MCMC sampler to obtain a posterior sample of the parameters of interest.
  - At iteration t:
  - Update graph $G_k^{(t)}$ and precision matrix $\Omega_k^{(t)}$ for each group k = 1, ..., K
  - Update parameters for network relatedness $\theta_{km}^{t}$ and $\gamma_{km}^{t}$ for $1 \leq k < m \leq K$
  - Update edge-specific parameters $v_{ij}^{(t)}$ for $1 \leq i < j \leq p$

# Other Bayesian methods for inferring multiple GGMs

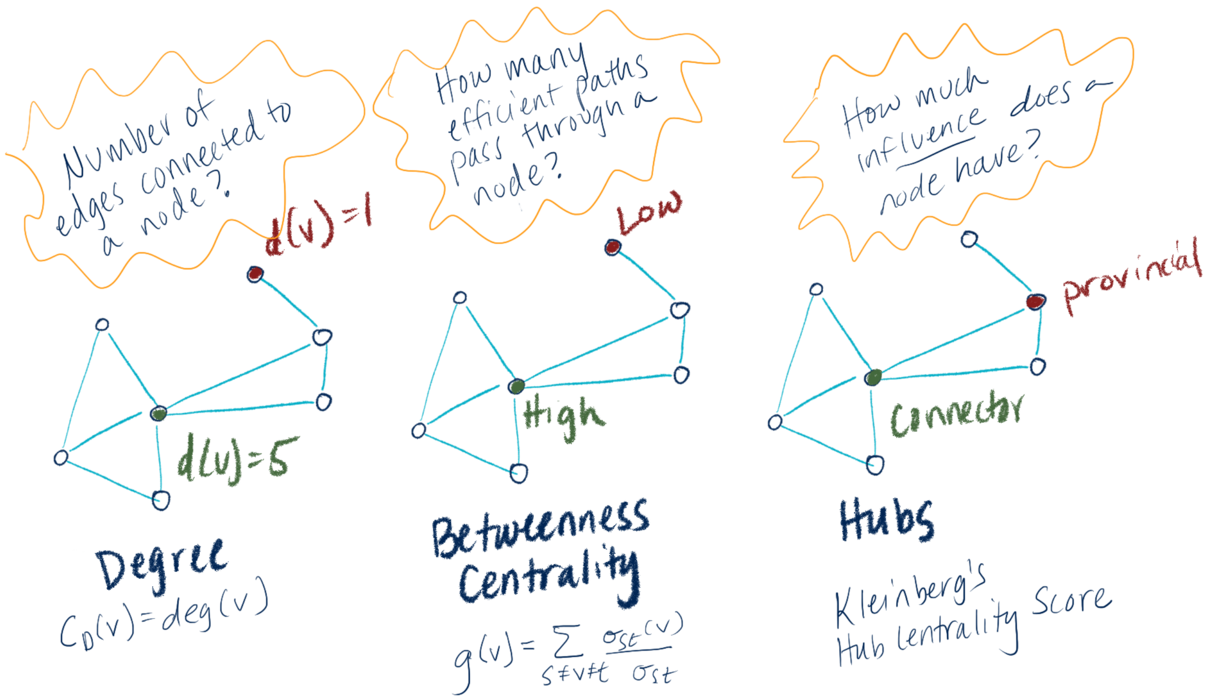
- Method for non-normal and mixed discrete continuous 'omic data (Bhadra 2018)
- Bayesian counterpart of JGL with simultaneous shrinkage and model selection (Li 2019)
- Multi-layered genomic networks - good for when you have multiple data types/hierarchical structure (Ha 2020)
- Hierarchical Bayesian factor model for count data (good for single-cell differential network analysis) (Sekula 2021)

# Takeaway from methods landscape

- There are many available methods to jointly estimate multiple graphical models
- Choice of method depends on your data type, goal of analysis, and ease of implementation (more on that in software section)


Estimation
Testing
Group A
Group B
Differential
Network
But how do you
QUANTIFY the difference to
test??

# Some Node Importance Measures



# And finally… test the difference

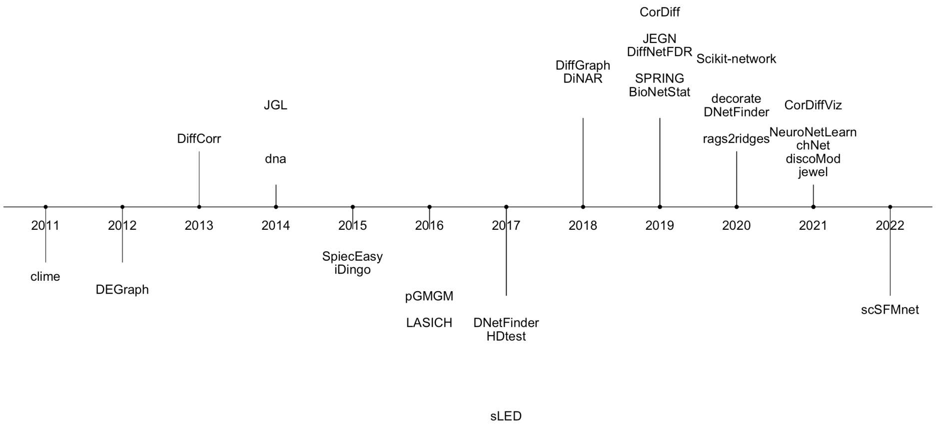
# Methods & Software for testing

- Lichtblau 2017 Compares 10 methods for quantifying node-specific differences between groups
- Identify pairs of nodes with difference (Ha DINGO 2015, McKenzie DGCA 2016)
- Identify subsets (3+) nodes that whose connections are different between groups (Jardim BioNetStat 2019, Arbet PND 2021)
- Various p-value options, e.g. permutation
- Adjust for multiple testing!! Bonferoni for conservative estimate, FDR for less stringent.
- For Bayesian methods: posterior mean and 100(1-$\alpha$)% Credible Interval for each gene-gene pair correlation difference are obtained from the posterior. (Sekula 2022)

# Software Landscape of DiNA methods

# Overview DiNA software landscape

- I found 26 different R packages and 2 Python packages that implement a variety of subtly different DiNA algorithms/pipelines



# Notes on software

- `JGL`, `iDingo`, `rags2ridges`, and `SpiecEasy` seem to be most popular and cited.
- I have a full tutorial for `JGL` posted on my GitHub, and Kate has one available for `iDingo`.
- Currently working making tutorials for for `rags2ridges`, `Spiec-Easy` and will work through the other available methods
- For implementation in Python, see my repository: https://github.com/mljaniczek/diff_net_python which contains scripts on running sparse inverse covariance estimation methods in Python (Graphical Lasso and Ledoit-Wolf shrinkage methods).
- Bayesian methods: I am working on the `spikeyglass` package which implements Li's 2019 method for Bayesian Joint Spike-and-Slab Graphical Lasso. The `scSFMnet` package is also available for Sekula 2022 method for hierarchical Bayesian factor model, which can be used on zero-inflated count data.

# JGL

- JGL package runs Fused Graphical Lasso (FGL) and Group Graphical Lasso from Danaher et al 2014
- Estimates sparse covariance matrices that are *similar* across classes
- Has a lot of useful functions to analyze the networks after estimating them, for example extracting hubs, edges, degree etc.
- Graphical lasso uses L1 penalty, which encourages sparsity and as a result selects edges in the graph in the process of estimating precision matrix
- (If time allows we can open up my JGL tutorial for a practical metabolomics example)

# Takeaways

- DiNA has potential to be a useful tool in biomedical research
- There are many ways to customize the estimation and testing process to fit research question and data types
- However the broad landscape of methods and software and the current lack of practical applied tutorials comparing software methods seems like a barrier to widespread use
- I'm working on trying to bridge the gap between statistical methodology and applied researchers! Full tutorial forthcoming!

# Questions & Comments?

# Thank you!

- Dr. Raji Balasubramanian
- Members of Balasubramanian Lab
- Dr. Kate Hoff Shutta
- Dr. Zehang Richard Li

Github: @mljaniczek
Website: mljaniczek.github.io/
Slides created via the R package **xaringan**.

# References

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