# Estimation of Metabolomic Networks with Gaussian Graphical Models

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## Abstract

- Network-based metabolomic analyses have high potential to capture signatures of complex biological processes [1].
- Gaussian graphical model (GGM) estimation is one approach to network estimation. Recently, several open-source R packages have been developed for this purpose [2, 3].
- GGM estimation involves several choices with regard to scoring criteria, precision matrix estimation algorithms, and data transformations.
- We present results from a simulation study designed to investigate these choices, with the goal of providing practical guidance to researchers applying GGM approaches to metabolomic data.

## GGM vs Correlation Network

A Gaussian graphical model begins with the assumption an $p$-dimensional random vector of metabolite measurements that follows the multivariate normal distribution [4].

$$\mathbf{X} = (X_1, \ldots, X_p) \sim MVN(\underline{\mu}, \mathbf{\Sigma}) \qquad (1)$$

In this setting, $\underline{\mu} = \underline{0}$ and $\mathbf{\Sigma}$ represents the between-metabolite covariance matrix. Under the MVN assumption, this framework allows us to estimate two different types of networks:

**Correlation network** (Edges from $\mathbf{\Sigma}$)

- $X_i \perp X_j \iff \mathbf{\Sigma}_{i,j} = 0$
- Edges correspond to pairwise dependence
- This marginal dependence may be able to be explained by other metabolites in the network

**GGM network** (Edges from $\mathbf{\Sigma}^{-1}$)

- $X_i \perp X_j | \{X_{k \neq i,j}\} \iff \mathbf{\Sigma^{-1}}_{i,j} = 0$
- Edges correspond to conditional dependence
- This dependence is conditioned on the state of the rest of the network metabolites
- The observed relationship between two metabolites cannot be explained through any of the other metabolites in the network

## Algorithms

- Meinshausen-Bühlmann (mb): uses penalized regression to model each individual metabolite on the others in the network [5]
- Correlation Thresholding (ct): applies a threshold to the correlation matrix
- Graphical LASSO (glasso): uses penalized regression to estimate a sparse inverse covariance matrix [2]

## Scoring Criteria

- Rotation Information Criterion (ric): estimates optimal tuning parameter by permutation-based approach [6]
- Stability Approach to Regularization Selection (StARS): estimates optimal tuning parameter by subsampling approach [7]

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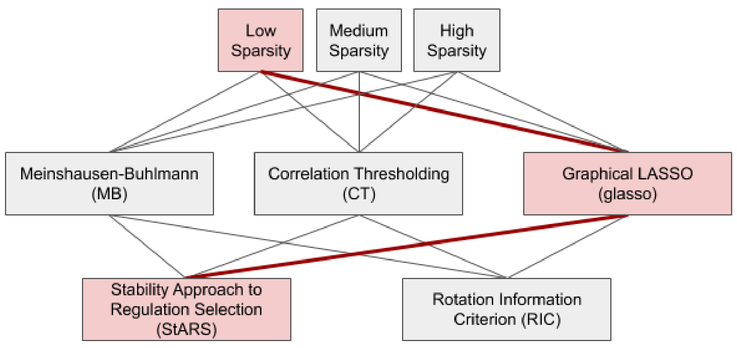
## Simulated Networks



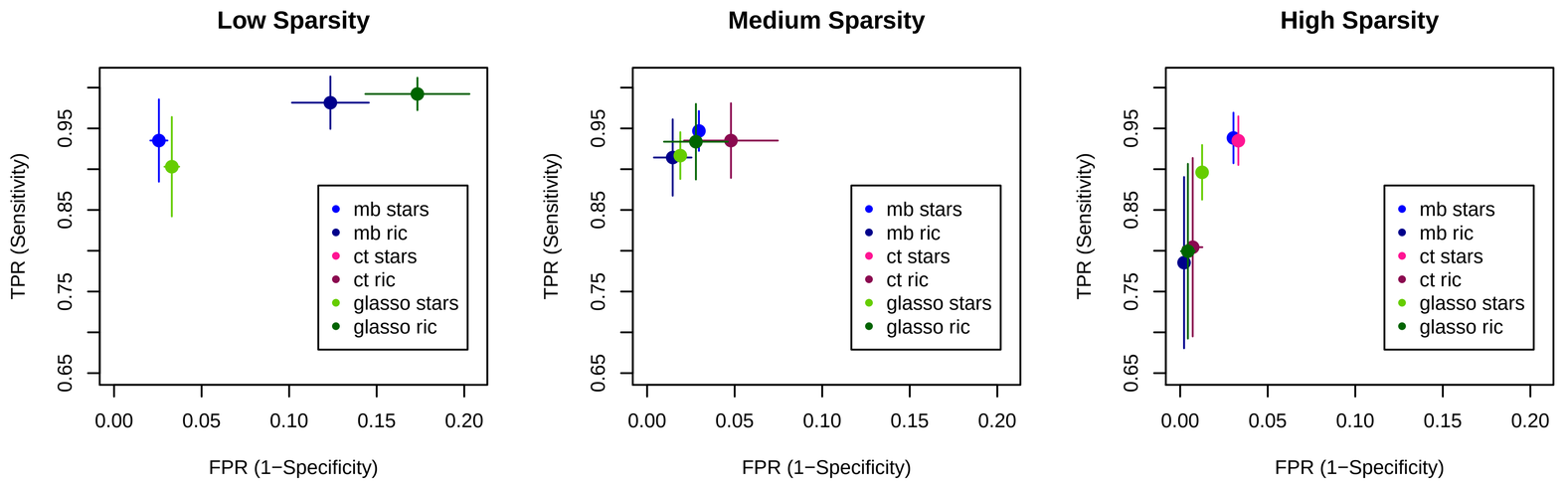

As a gold standard for reference, we generated 3 precision matrices corresponding to random graphs using the Erdos-Renyi random graph generation process in `igraph` [8]. The sparsities modeled were high (edge probability 0.01), medium(0.025), and low(0.1). For chosen simulation settings (e.g., in the highlighted example, a low sparsity matrix estimated with the `glasso` algorithm and scored with StARS), we repeated the following 100 times:

1. Draw 100 samples from the $MVN(\underline{0}, \mathbf{\Sigma})$ distribution
2. Obtain the 400 x 400 sample covariance matrix
3. Apply the chosen algorithm and scoring criterion to obtain an estimated adjacency matrix
4. Compare the estimated adjacency matrix to the gold-standard precision matrix $\mathbf{\Sigma}^{-1}$ from which the data were generated

## Edge Recovery Performance

With three estimation algorithms and two scoring criteria, we studied a total of six network estimation approaches for each sparsity level. To assess the sensitivity and specificity of each algorithm and criterion combination, the following definitions were used (where $\mathbf{\Sigma}^{-1}$ is the gold-standard precision matrix for the simulation):

**True Positive:** an edge in $\mathbf{\Sigma}^{-1}$ with magnitude of conditional correlation $> \rho^* \approx 0.2$ that was detected by the estimation. ($\rho^*$ is the threshold for significance testing of null hypothesis $\rho = 0$ at $\alpha = 0.05$ for a sample of size $n = 100$.)
**True Negative:** an edge in $\mathbf{\Sigma}^{-1}$ with magnitude of conditional correlation exactly 0 that was not detected by the estimation.
**False Positive:** an edge detected in the estimation which has weight exactly 0 in $\mathbf{\Sigma}^{-1}$.
**False Negative:** an edge not detected in the estimation that has absolute weight $> \rho^*$ in $\mathbf{\Sigma}^{-1}$.
Edges in the gold-standard precision matrix with absolute edge weight between 0 and $\rho^*$ were not considered in this analysis.




**Low sparsity network**

- Both CT approaches detected a very small number of edges and are not shown (TPR, FPR $\approx 0$)
- StARS criterion has lower sensitivity and higher specificity than the RIC criterion for both the MB and glasso algorithms

**Medium sparsity network**

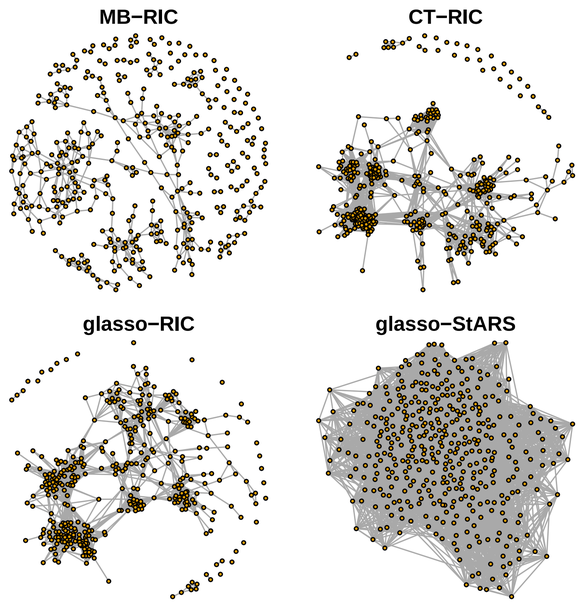
- Performance is comparable among all methods with the exception of CT-StARS
- CT-StARS detects a very small number of edges and is not shown

**High sparsity network**

- StARS criterion has higher sensitivity and lower specificity than RIC
- Difference in criterion has more impact than difference in algorithm

## Application: CATHGEN

We used the three algorithms and two criteria to fit six estimated networks for a dataset of targeted metabolomic data from the CATHGEN Biorepository [9]. The estimated topologies varied depending on choice of algorithm. Not shown are the MB-StARS and CT-StARS estimated networks; almost no edges were estimated for these approaches.




The table below shows the edge count for each approach.

| | MB | CT | glasso |
|---|---|---|---|
| RIC | 610 | 4530 | 2542 |
| StARS | 0 | 1 | 6045 |

## Conclusion

Estimated GGMs can vary broadly depending on method, and this variability may depend on network topology. Cross-validation and sensitivity analyses are recommended.

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