Surf 64 - XP practice

Biological Network Inference with Sparse Graphical Models

Julien Chiquet

Anglet, 27 June 2018

https://github.com/jchiquet/JC2BIM18

Outline

Statistical analysis of Networks

Different questions

Understanding the network topology

- Data = observed network
- Questions: central nodes? cluster structure? small-world property?

Inferring/Reconstructing the network

- Data = repeated signal observed at each node
- Questions: which nodes are connected?

Using the network

- Data = a given network + signal on nodes
- Questions: how the epidemic spreads along the network?

Each to be combined with

covariates, time, heterogeneous data set, missing data, ...

Automatic reconstruction of biological networks

E. coli regulatory network

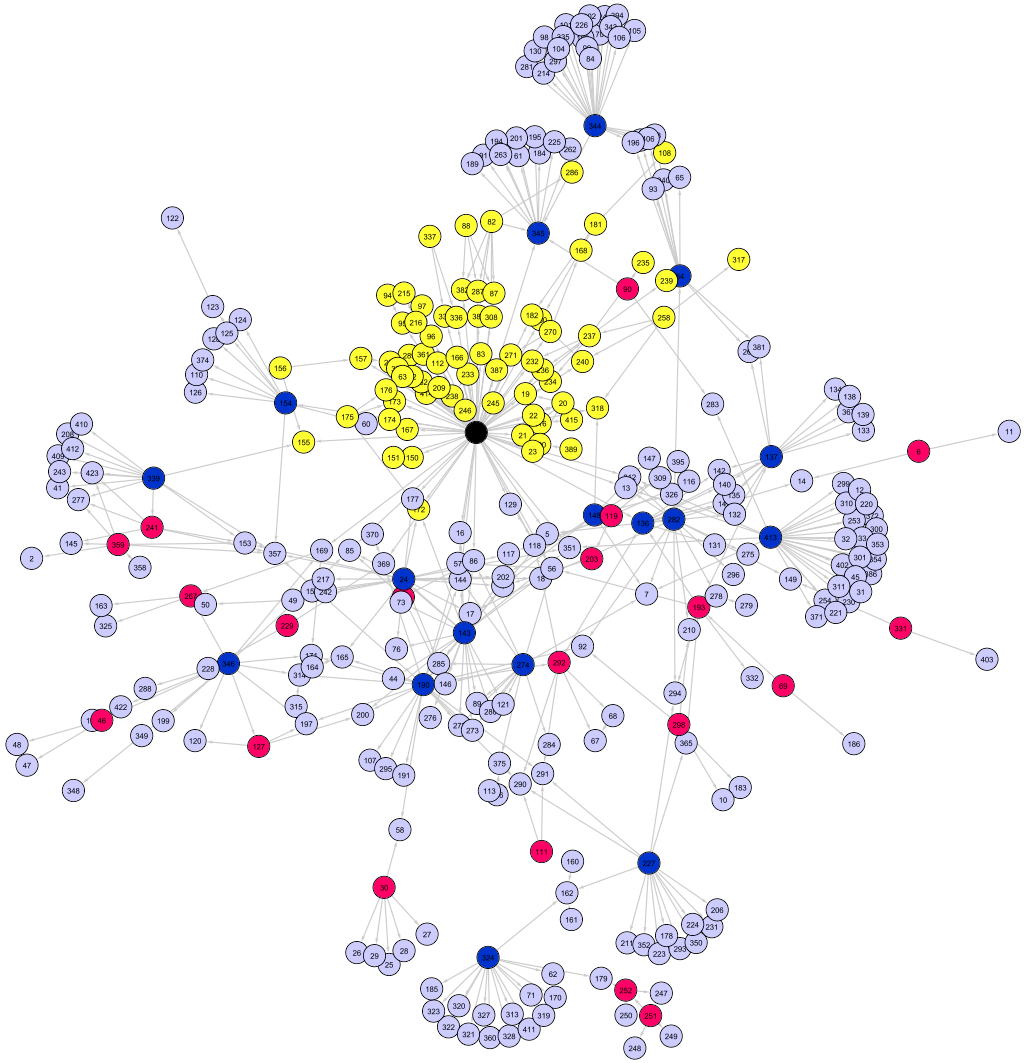
Target network

Relations between genes and their products

- highly structured
- always incomplete

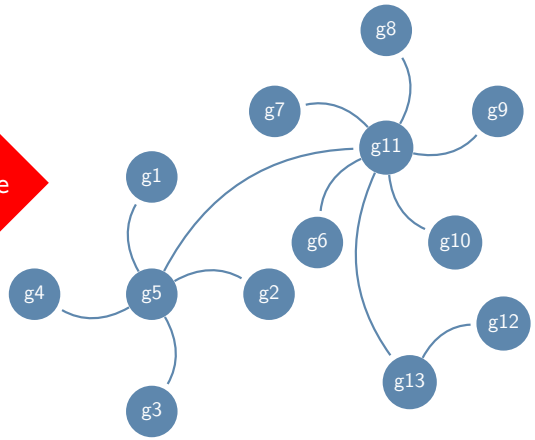
Data and method

- transcriptomic data
- Gaussian graphical model with sparse methods



A challenging problem

Inference



$\approx$ 10s/1,000s assays

$\approx$ 1,000s/1,000,000s features

Model point of view

1. Nodes (genes, OTUS, ...)
   - fixed variables
2. Edges (biological interactions)
   - use (partial) correlations or others fancy statistical concepts
3. Data (intensities, counts)
   - a tidy $n \times p$ dat matrix

$\rightsquigarrow$ Quantities and goals well defined

Data point of view: non classical statistics

- (Ultra) High dimensionality ($n < p$, $n \ll p$)
- Heterogeneous data

Biological point of view: not well defined goals and questions

- What interaction? Direct? Indirect? Causal?
- Whole network? Subnetwork? Groups of key actors?

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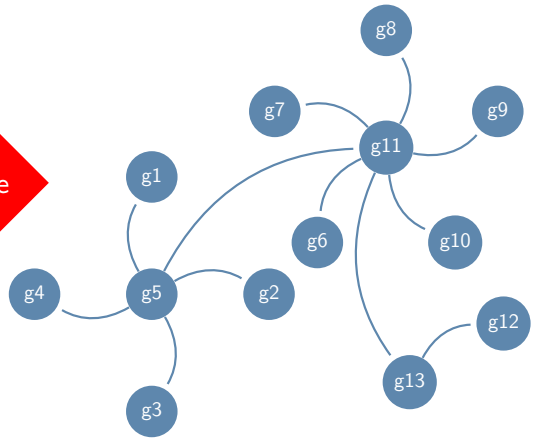

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Canonical model settings

Biological microarrays in comparable conditions

Notations

1. a set $\mathcal{P} = \{1, \ldots, p\}$ of p variables:
   these are typically the genes (could be proteins);
2. a sample $\mathcal{N} = \{1, \ldots, n\}$ of individuals associated to the variables:
   these are typically the microarray (could be sequence counts).

Basic statistical model

This can be view as

- a *random vector* X in $\mathbb{R}^p$, whose jth entry is the jth variable,
- a n-size sample $(X^1, \ldots, X^n)$, such as X^i is the ith microarrays,
  - could be independent identically distributed copies (steady-state)
  - could be dependent in a certain way (time-course data)
- assume a parametric probability distribution for X (Gaussian).

Canonical model settings

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Canonical model settings

Biological microarrays in comparable conditions

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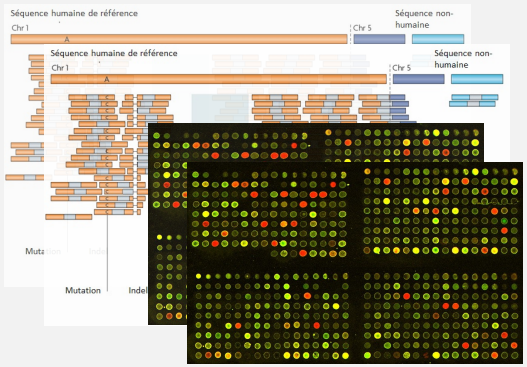
The data

Stacking $(X^1, \ldots, X^n)$, we met the usual individual/variable table $\mathbf{X}$



stacked in

$$\mathbf{X} = \begin{pmatrix} x_1^1 & x_1^2 & x_1^3 & \ldots & x_1^p \\ \vdots & & & & \\ x_n^1 & x_n^2 & x_1^2 & \ldots & x_n^p \end{pmatrix}$$

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sparse Gaussian Graphical Models

Modeling relationship between variables (1)

Independence

Definition (Independence of events)

Two events A and B are independent if and only if

$$\mathbb{P}(A, B) = \mathbb{P}(A)\mathbb{P}(B),$$

which is usually denoted by $A \perp\!\!\!\perp B$. Equivalently,

- $A \perp\!\!\!\perp B \Leftrightarrow \mathbb{P}(A|B) = \mathbb{P}(A)$,
- $A \perp\!\!\!\perp B \Leftrightarrow \mathbb{P}(A|B) = \mathbb{P}(A|B^c)$

Example (class vs party)

	party	
class	Labour	Tory
working	0.42	0.28
bourgeoisie	0.06	0.24

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Table: Joint probability (left) vs. conditional probability (right)

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Conditional independence

Generalizing to more than two events requires strong assumptions (mutual independence). Better handle with

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Consider the events $A =$ "having low QI", $B =$ "having low weight".

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Example (Does QI depends on weight?)

Consider the events $A =$ "having low QI", $B =$ "having low weight". Estimating[1] $\mathbb{P}(A, B)$, $\mathbb{P}(A)$ and $\mathbb{P}(B)$ in a sample would lead to

$$\mathbb{P}(A, B) \neq \mathbb{P}(A)\mathbb{P}(B)$$

[1] stupidly

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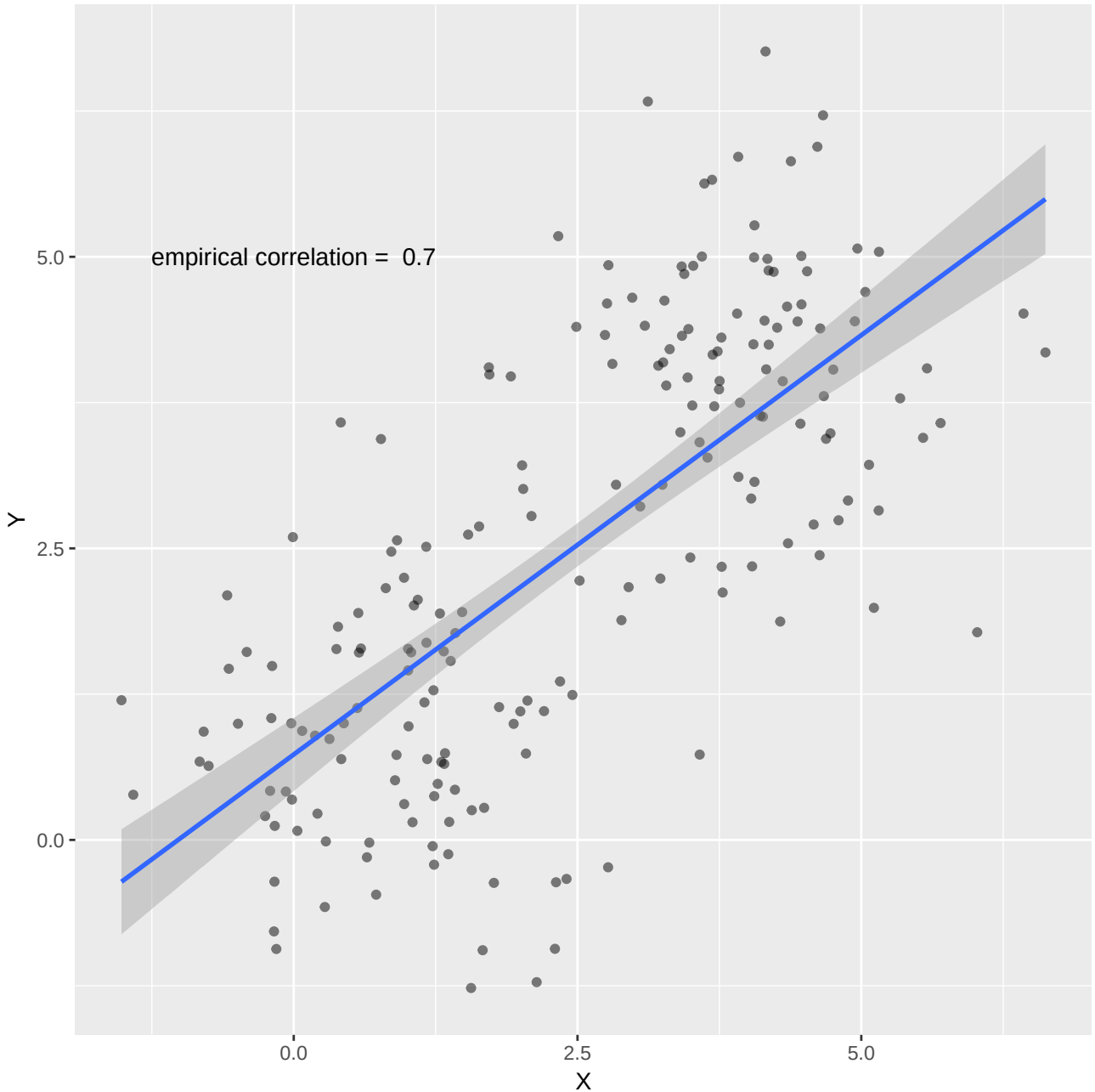
Example (Does QI depends on weight?)

Consider the events $A =$ "having low QI", $B =$ "having low weight". But in fact, introducing $C =$ "having a given age",

$$\mathbb{P}(A, B|C) = \mathbb{P}(A|C)\mathbb{P}(B|C)$$

Limits of correlation for network reconstruction



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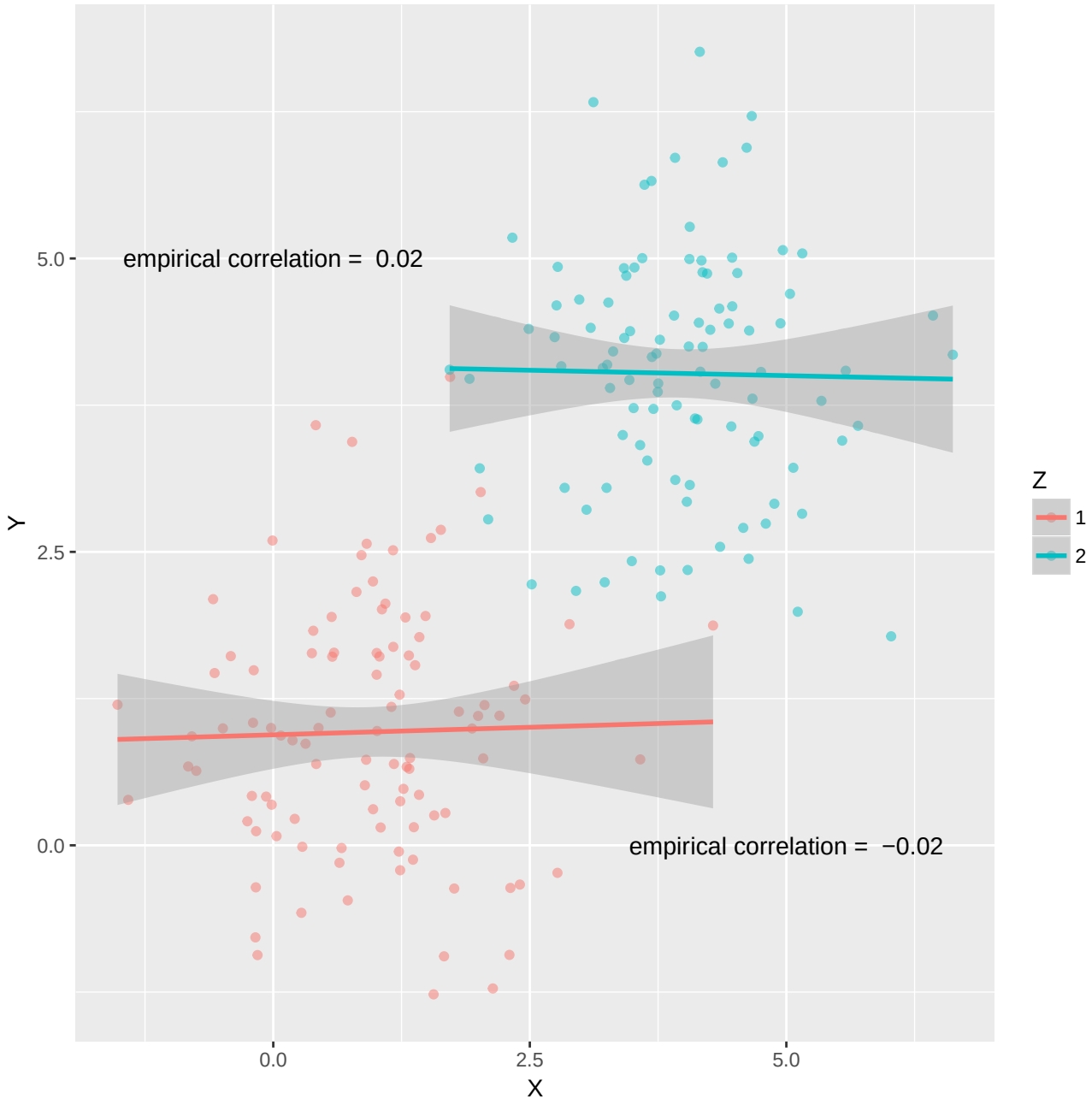

Outline

sparse Gaussian Graphical Models

Correlation networks

Correlation (association network)

Similar expression profile $\rightsquigarrow$ high-correlation

1. Compute the correlation matrix (Pearson, Spearman, ...)
2. Predict an edge between two actors if their absolute correlation is above a given threshold

Questions

- How to set up the threshold?
- If we target actors with similar profiles, why not clustering?
- Information is drowned (all actors are correlated ...)

Graphical models

Definition

A graphical model gives a graphical (intuitive) representation of the dependence structure of a probability distribution, by linking

1. a random vector (or a set of random variables.) $X = \{X_1, \ldots, X_p\}$ with distribution $\mathbb{P}$,

2. a graph $\mathcal{G} = (\mathcal{P}, \mathcal{E})$ where
   - $\mathcal{P} = \{1, \ldots, p\}$ is the set of nodes associated to each variable,
   - $\mathcal{E}$ is a set of edges describing the dependence relationship of $X \sim \mathbb{P}$.

Conditional independence graph

It is the undirected graph $\mathcal{G} = \{\mathcal{P}, \mathcal{E}\}$ where

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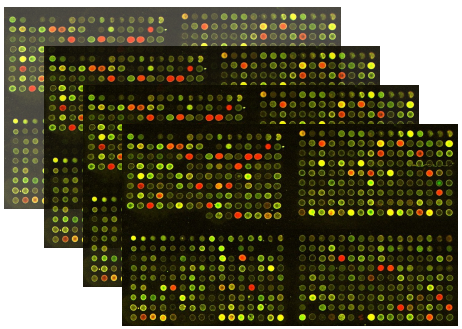
The Gaussian case

The data



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Assuming $f_X(\mathbf{X})$ multivariate Gaussian

Greatly simplifies the inference:

- $\rightsquigarrow$ naturally links independence and conditional independence to the covariance and partial covariance,
- $\rightsquigarrow$ gives a straightforward interpretation to the graphical modeling previously considered.

Why Gaussianity helps?

Case of 2 variables or size-2 random vector

Let X, Y be two real random variables.

Definitions

$$\operatorname{cov}(X,Y) = \mathbb{E}\Big[\big(X - \mathbb{E}(X)\big)\big(Y - \mathbb{E}(Y)\big)\Big] = \mathbb{E}(XY) - \mathbb{E}(X)\mathbb{E}(Y).$$

$$\rho_{XY} = \operatorname{cor}(X,Y) = \frac{\operatorname{cov}(X,Y)}{\sqrt{\mathbb{V}(X) \cdot \mathbb{V}(Y)}}.$$

Proposition

- $\operatorname{cov}(X,X) = \mathbb{V}(X) = \mathbb{E}[(X - \mathbb{E}X)(Y - \mathbb{E}Y)]$,
- $\operatorname{cov}(X+Y,Z) = \operatorname{cov}(X,Z) + \operatorname{cov}(X,Z)$,
- $\mathbb{V}(X+Y) = \mathbb{V}(X) + \mathbb{V}(Y) + \operatorname{cov}(X,Y)$.
- $X \perp\!\!\!\perp Y \Rightarrow \operatorname{cov}(X,Y) = 0$.
- $X \perp\!\!\!\perp Y \Leftrightarrow \operatorname{cov}(X,Y) = 0$ *when* X, Y *are Gaussian.*

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The bivariate Gaussian distribution

The Covariance Matrix

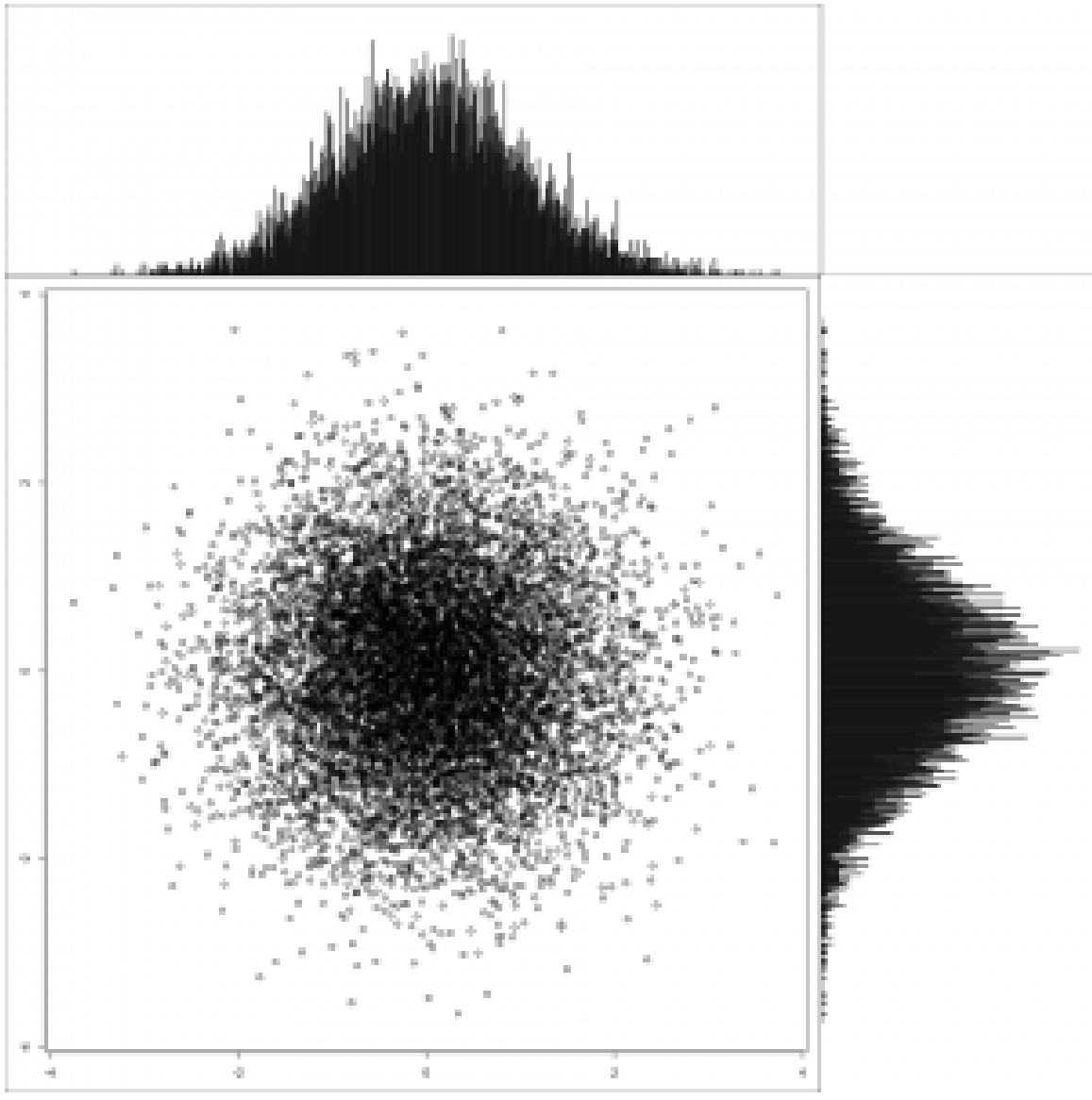
Let

$$X \sim \mathcal{N}(\mathbf{0}, \mathbf{\Sigma}),$$

with unit variance and $\rho_{XY} = 0$

$$\mathbf{\Sigma} = \begin{pmatrix} 1 & 0 \\ 0 & 1 \end{pmatrix}.$$

The shape of the 2-D distribution evolves accordingly.



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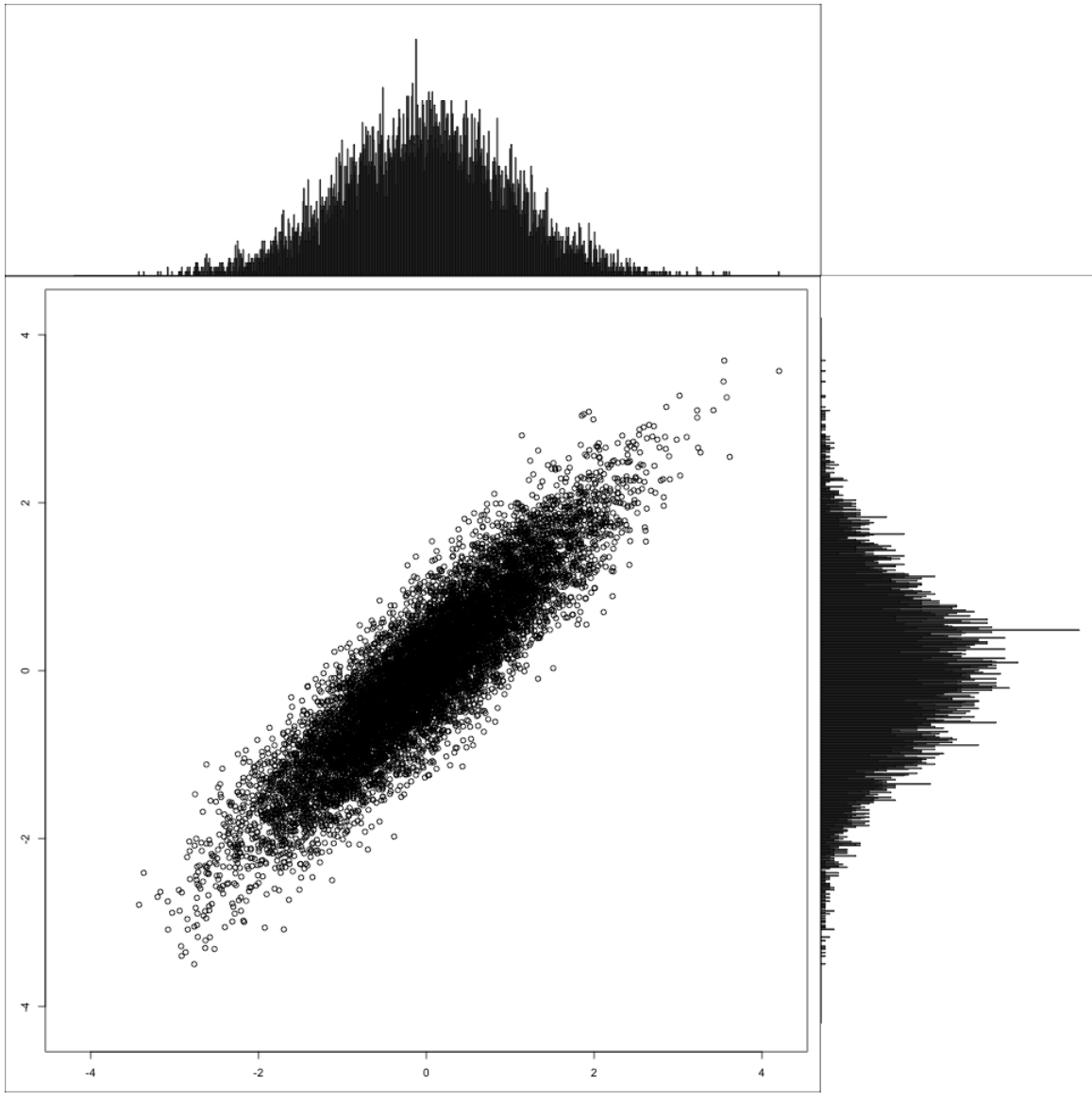

Generalization: multivariate Gaussian vector

Now need partial covariance and partial correlation

Let X, Y, Z be real random variables.

Definitions

$$\mathrm{cov}(X, Y|Z) = \mathrm{cov}(X, Y) - \mathrm{cov}(X, Z)\mathrm{cov}(Y, Z)/\mathbb{V}(Z).$$

$$\rho_{XY|Z} = \frac{\rho_{XY} - \rho_{XZ}\rho_{YZ}}{\sqrt{1-\rho_{XZ}^2}\sqrt{1-\rho_{YZ}^2}}.$$

$\rightsquigarrow$ Give the interaction between X and Y once removed the effect of Z.

Proposition

When X, Y, Z are jointly Gaussian, then

$$\mathrm{cov}(X, Y|Z) = 0 \Leftrightarrow \mathrm{cor}(X, Y|Z) = 0 \Leftrightarrow X \perp\!\!\!\perp Y|Z.$$

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Important properties of Gaussian vectors

Proposition (Gaussian vector and conditioning)

Consider a Gaussian vector with the following decomposition

$$Z = \begin{pmatrix} Z_1 \\ Z_2 \end{pmatrix} \sim \mathcal{N}(\mathbf{0}, \boldsymbol{\Sigma}), \quad \boldsymbol{\Sigma} = \begin{pmatrix} \boldsymbol{\Sigma}_{11} & \boldsymbol{\Sigma}_{12} \\ \boldsymbol{\Sigma}_{21} & \boldsymbol{\Sigma}_{22} \end{pmatrix}, \quad \boldsymbol{\Omega} = \boldsymbol{\Sigma}^{-1} = \begin{pmatrix} \boldsymbol{\Omega}_{11} & \boldsymbol{\Omega}_{12} \\ \boldsymbol{\Omega}_{21} & \boldsymbol{\Omega}_{22} \end{pmatrix}.$$

Then,

$$Z_2 | Z_1 = \mathbf{z} \sim \mathcal{N}\left(-\boldsymbol{\Omega}_{22}^{-1}\boldsymbol{\Omega}_{21}\mathbf{z}, \boldsymbol{\Omega}_{22}^{-1}\right)$$

and

$$\boldsymbol{\Omega}_{22}^{-1} = \boldsymbol{\Sigma}_{22} - \boldsymbol{\Sigma}_{21}\boldsymbol{\Sigma}_{11}^{-1}\boldsymbol{\Sigma}_{12}.$$

Corollary

Partial correlations are related to the inverse of the covariance matrix:

$$\operatorname{cor}(Z_i, Z_j | Z_k, k \neq i, j) = -\frac{\Omega_{ij}}{\sqrt{\Omega_{ii}\Omega_{jj}}}$$

Gaussian Graphical Model: canonical settings

Biological experiments in comparable Gaussian conditions

Profiles of a set $\mathcal{P} = \{1, \ldots, p\}$ of genes is described by $X \in \mathbb{R}^p$ such as

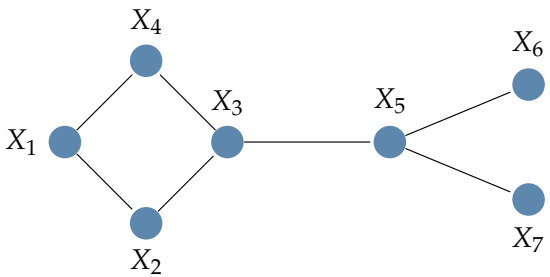
1. $X \sim \mathcal{N}(\boldsymbol{\mu}, \boldsymbol{\Sigma})$, with $\boldsymbol{\Theta} = \boldsymbol{\Sigma}^{-1}$ the precision matrix.
2. a sample $(X^1, \ldots, X^n)$ of exp. stacked in an $n \times p$ data matrix $\mathbf{X}$.

Conditional independence structure

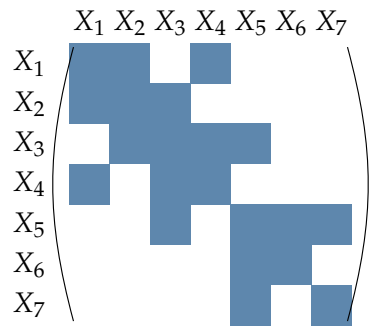
$$(i,j) \notin \mathcal{E} \Leftrightarrow X_i \perp\!\!\!\perp X_j | X_{\setminus\{i,j\}} \Leftrightarrow \Theta_{ij} = 0.$$

Graphical interpretation

$$\mathcal{G} = (\mathcal{P}, \mathcal{E})$$



$\boldsymbol{\Theta}$



$\rightsquigarrow$ "Covariance" selection

Gaussian Graphical Model and Linear Regression

Linear regression viewpoint

Gene expression X_i is linearly explained by the other genes':

$$X_i | X_{\setminus i} = -\sum_{j \neq i} \frac{\Theta_{ij}}{\Theta_{ii}} X_j + \varepsilon_i, \quad \varepsilon_i \sim \mathcal{N}(0, \Omega_{ii}^{-1}), \quad \varepsilon_i \perp X$$

Conditional on its neighborhood, other profiles do not give additional insights

$$X_i | X_{\setminus i} = \sum_{j \in \text{neighbors}(i)} \beta_j X_j + \varepsilon_i \quad \text{with } \beta_j = -\frac{\Theta_{ij}}{\Theta_{ii}}.$$

$\rightsquigarrow$ "Neighborhood" selection

Gaussian Graphical Model and AR process (1)

Time course data

Time course- data experiment can be represented as a multivariate vector $X = (X_1, \ldots, X_p) \in \mathbb{R}^p$, generated through a first order vector autoregressive process $VAR(1)$:

$$X^t = \mathbf{\Theta} X^{t-1} + \mathbf{b} + \boldsymbol{\varepsilon}^t, \quad t \in [1, n]$$

where $\boldsymbol{\varepsilon}^t$ is a white noise to ensure the Markov property and $X^0 \sim \mathcal{N}(0, \mathbf{\Sigma}^0)$.

Consequence: a Gaussian Graphical Model

- Each $X^t | X^{t-1} \sim \mathcal{N}(\theta X^{t-1}, \mathbf{\Sigma})$,
- or, equivalently, $X_j^t | X^{t-1} \sim \mathcal{N}(\mathbf{\Theta}_j X^{t-1}, \mathbf{\Sigma})$

where $\mathbf{\Sigma}$ is known and $\mathbf{\Theta}_j$ is the jth row of $\mathbf{\Theta}$.

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Gaussian Graphical Model and AR process (2)

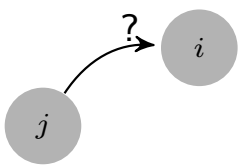
Interpretation as a GGM

The VAR(1) as a covariance selection model

$$\theta_{ij} = \frac{\text{cov}\left(X_i^t, X_j^{t-1} | X_{\mathcal{P}\setminus j}^{t-1}\right)}{\text{var}\left(X_j^{t-1} | X_{\mathcal{P}\setminus j}^{t-1}\right)},$$

Graphical Interpretation

$\rightsquigarrow$ The matrix $\mathbf{\Theta} = (\theta_{ij})_{i,j\in\mathcal{P}}$ encodes the network $\mathcal{G}$ we are looking for.

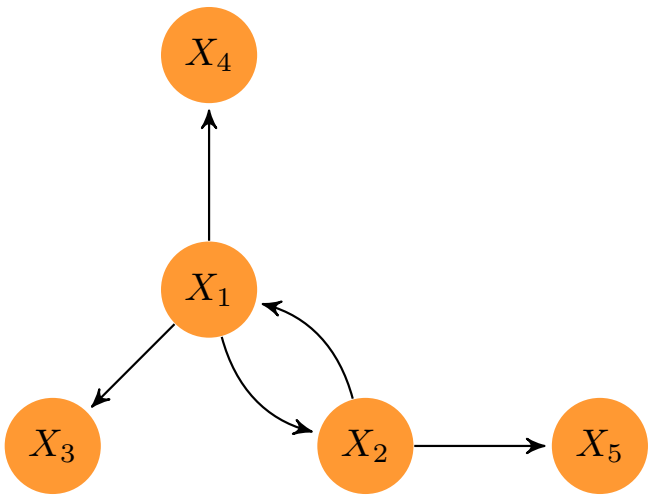


conditional dependency between X_j^{t-1} and X_i^t
or
non-null partial correlation between X_j^{t-1} and X_i^t
$\Updownarrow$
$\theta_{ij} \neq 0$

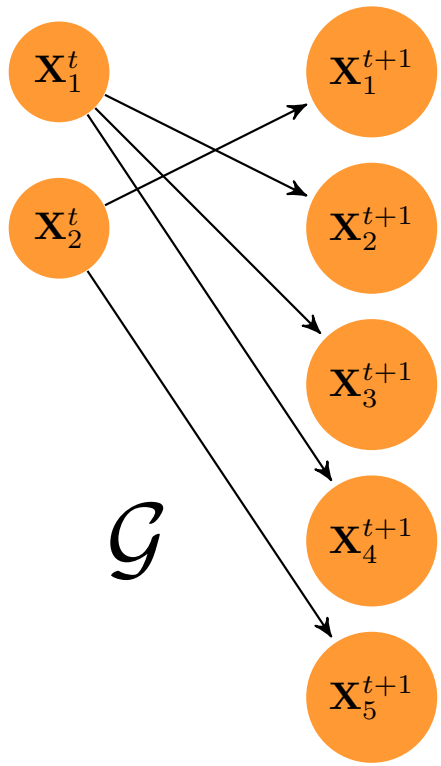
Gaussian Graphical Model and AR process (3)

Graphical interpretation

1. Follow-up of one single experiment/individual;
2. Close enough time-points to ensure
   - dependency between consecutive measurements;
   - homogeneity of the Markov process.



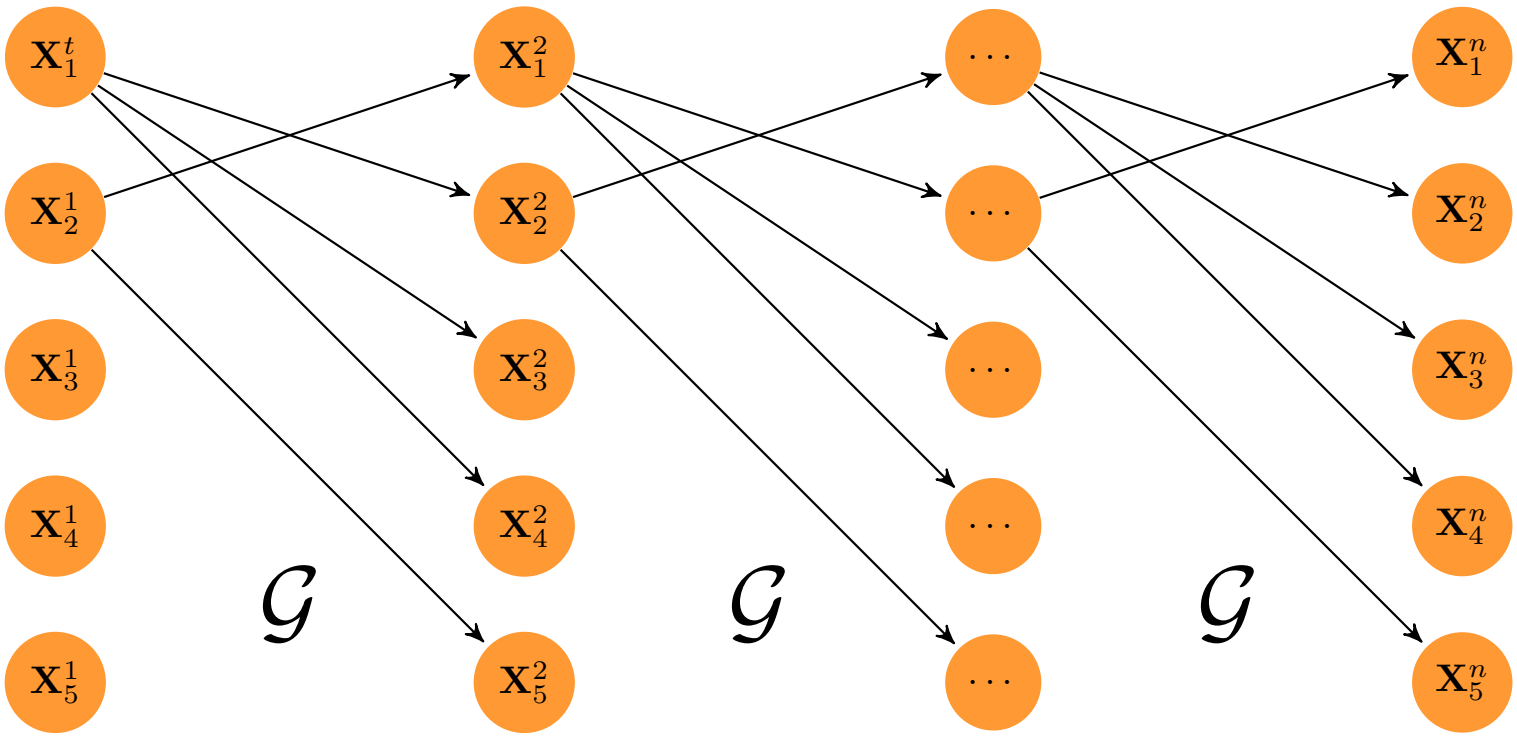
stands for



Gaussian Graphical Model and AR process (3)

Graphical interpretation

1. Follow-up of one single experiment/individual;
2. Close enough time-points to ensure
   - dependency between consecutive measurements;
   - homogeneity of the Markov process.



Outline

Some families of methods for network reconstruction

Test-based methods

- Tests the nullity of each entries
- Combinatorial problem when $p > 30$ ...

Sparsity-inducing regularization methods

- induce sparsity with the ℓ_1-norm penalization
- Use results from convex optimization
- Versatile and computationally efficient

Bayesian methods

- Compute the posterior probability of each edge
- Usually more computationally demanding
- For special graphs, computation gets easier

Inference: maximum likelihood estimator

The natural approach for parametric statistics

Let X be a random vector with distribution defined by $f_X(x; \boldsymbol{\Theta})$, where $\boldsymbol{\Theta}$ are the model parameters.

Maximum likelihood estimator

$$\hat{\boldsymbol{\Theta}} = \arg\max_{\boldsymbol{\Theta}} \ell(\boldsymbol{\Theta}; \mathbf{X})$$

where ℓ is the log likelihood, a function of the parameters:

$$\ell(\boldsymbol{\Theta}; \mathbf{X}) = \log \prod_{i=1}^{n} f_X(\mathbf{x}_i; \boldsymbol{\Theta}),$$

where $\mathbf{x}_i$ is the ith row of $\mathbf{X}$.

Remarks

- This a convex optimization problem,
- We just need to detect non zero coefficients in $\boldsymbol{\Theta}$

The multivariate Gaussian log-likelihood

Let $\mathbf{S} = n^{-1}\mathbf{X}^\intercal\mathbf{X}$ be the empirical variance-covariance matrix: $\mathbf{S}$ is a sufficient statistic of $\mathbf{\Theta}$.

The log-likelihood

$$\ell(\mathbf{\Theta}; \mathbf{S}) = \frac{n}{2}\log\det(\mathbf{\Theta}) - \frac{n}{2}\mathrm{Trace}(\mathbf{S}\mathbf{\Theta}) + \frac{n}{2}\log(2\pi).$$

- $\rightsquigarrow$ The MLE $= \mathbf{S}^{-1}$ of $\mathbf{\Theta}$ is not defined for $n < p$ and never sparse.
- $\rightsquigarrow$ The need for regularization is huge.

Application to GGM: the "Graphical-Lasso"

A penalized likelihood approach

$$\hat{\mathbf{\Theta}}_\lambda = \arg\max_{\mathbf{\Theta} \in \mathbb{S}_+} \ell(\mathbf{\Theta}; \mathbf{X}) - \lambda \|\mathbf{\Theta}\|_{\ell_1}$$

where

- ℓ is the model log-likelihood,
- $\|\cdot\|_{\ell_1}$ is a penalty function tuned by $\lambda > 0$.
  1. *regularization* (needed when $n \ll p$),
  2. *selection* (sparsity induced by the ℓ_1-norm),
- solved in `R`-packages **glasso**, **quic**, **huge** ($\mathcal{O}(p^3)$)

Application to GGM: "Neighborhood selection"

A close cousin, thank to the relationship between Gaussian vector and linear regression

Remember that

$$X_i | X_{\setminus i} = \sum_{j \in \text{neighbors}(i)} \beta_j X_j + \varepsilon_i \quad \text{with } \beta_j = -\frac{\Theta_{ij}}{\Theta_{ii}}.$$

A penalized least-square approach

Let $\mathbf{X}_i$ be the ith column of the data matrix (i.e data associated to variable (gene) i), and $\mathbf{X}_{\setminus i}$ deprived of colmun i. We select the neighbors of variable i by solving

$$\widehat{\boldsymbol{\beta}}^{(i)} = \arg\min_{\boldsymbol{\beta} \in \mathbb{R}^{p-1}} \frac{1}{n} \left\| \mathbf{X}_i - \mathbf{X}_{\setminus i} \boldsymbol{\beta} \right\|_2^2 + \lambda \left\| \boldsymbol{\beta} \right\|_1$$

- not symmetric, not positive-definite
- \+ p Lasso solved with Lars-like algorithms ($\mathcal{O}(npd)$ for d neighbors).

Model selection

Cross-validation

Optimal in terms of prediction, not in terms of selection

Information based criteria

- GGMSelect (Girault *et al*, '12) selects among a family of candidates.
- Adapt IC to sparse high dimensional problems, e.g.

$$\text{EBIC}_\gamma(\widehat{\mathbf{\Theta}}_\lambda) = -2\text{loglik}(\widehat{\mathbf{\Theta}}_\lambda; \mathbf{X}) + |\mathcal{E}_\lambda|(\log(n) + 4\gamma \log(p)),$$

Resampling/subsampling

Keep edges frequently selected on an range of λ after sub-samplings

- Stability Selection (Meinshausen and Bühlman, 2010, Bach 2008)
- Stability approach to Regularization Selection (StaRS) (Liu, 2010).

Concluding remark about GGM

Sparse GGM

+ very solid statistical and computational framework
+ competitive to other inference methods (DREAM 5 benchmark, 2012)
− performances remain questionable on real data, as for other methods

$\rightsquigarrow$ Network inference is a very difficult problem
$\rightsquigarrow$ Some biological questions can be answered without network inference

Outline

Assess the standard GGMs approaches

Full analysis can be found at
`http://julien.cremeriefamily.info/exposome.html`

```
suppressMessages(library(huge, quietly = TRUE))
```

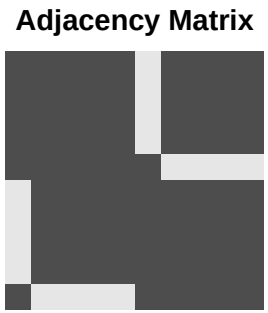
1. Simulated data
   - Test that an approach is working under some simple conditions
   - Especially usefull when the approach has no underlying model
   - Essential sanity check
2. Breast cancer data (pinpoint interesting genes/pathways)
   - Several hundred breast cancers (estrogen receptor + and -)
   - Several thousand genes
   - Goal: How can GGMs approaches help ?

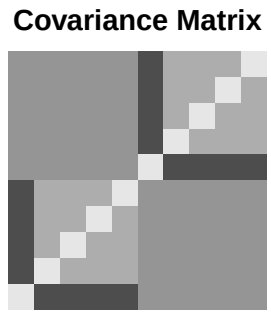
Simple simulations (network with hubs)

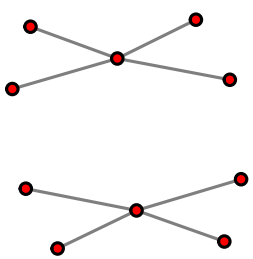
```
set.seed(11)
n <- 80; d <- 10;
rd.net  <- huge.generator(
  n, ## number of samples
  d, ## number of genes
  graph="hub", ## type of net
  g = 2, ## number of group)
  verbose=FALSE)
```

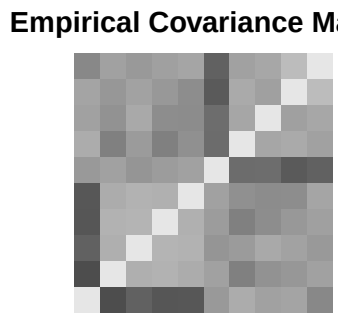
Simple simulations (network with hubs)

```
plot(rd.net)
```









Inference using GGMs and correlation

Inference

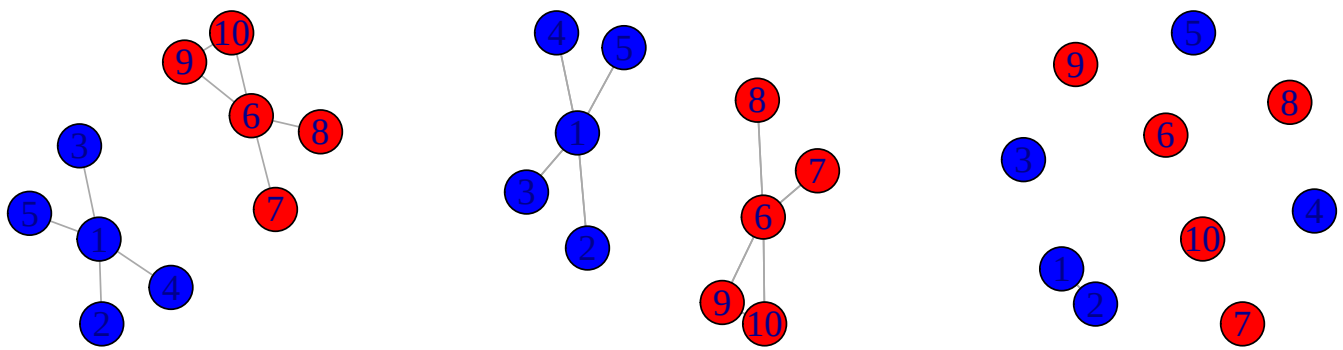
```
## glasso, mb and ct
glasso <- huge(rd.net$data, method="glasso",
               nlambda=50,  verbose=F)
mb <- huge(rd.net$data, method="mb",
           nlambda=50, verbose=F)
corthr <- huge(rd.net$data, method="ct",
              nlambda = 50, verbose=F)
```

Selection

```
## glasso, mb and ct
glasso.sel <- huge.select(glasso, "stars", verbose=F)
mb.sel <- huge.select(mb, "stars", verbose=F)
corthr.sel <- huge.select(corthr, "stars", verbose=F)
```

Inference using GGMs and correlation (results)

```
gr.glasso <- graph.adjacency(glasso.sel$refit)
V(gr.glasso)$label.cex <- 2
V(gr.glasso)$color <- rep(c("blue", "red"), each=5)
par(mfrow=c(1, 3))
plot(gr.glasso, vertex.size=30, edge.arrow.mode = "-")
plot(gr.mb, vertex.size=30, edge.arrow.mode = "-")
plot(gr.cor, vertex.size=30, edge.arrow.mode = "-")
```



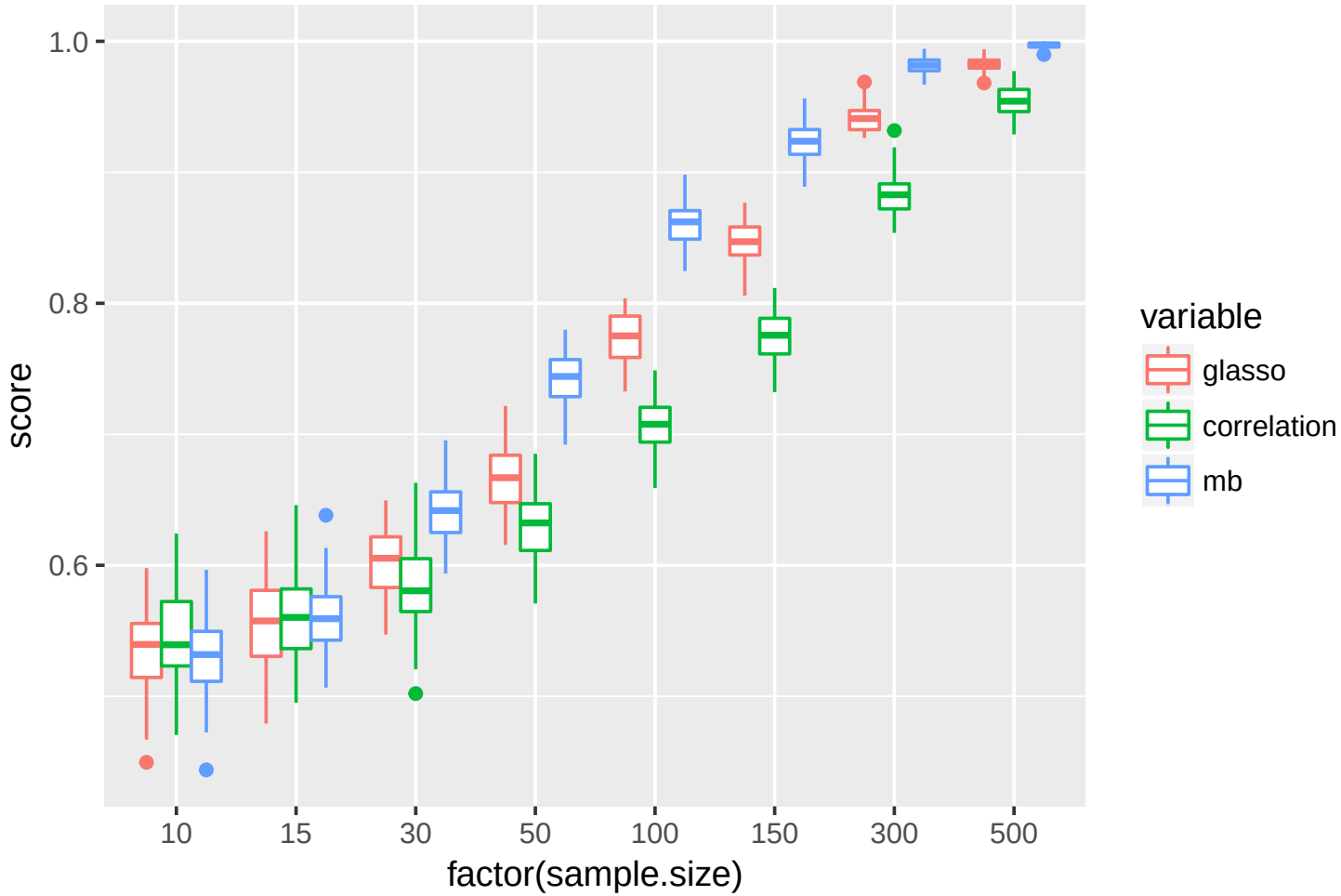
A bit of code to run a simulation

```
suppressMessages(require(reshape2))
one.simu <- function(i) {
  lbd.c <- seq(1, 0, -10^-2);
  d <- 25; seq.n <- c(10, 15, 30, 50, 100, 150, 300, 500)
  out <- data.frame(t(sapply(seq.n, function(n) {
   exp <- huge.generator(n, d, graph="cluster",
                         g=3, prob=1, verbose=F)
   gl <- huge(exp$data, method="glasso", nlambda=50, verbose=F)
   mb <- huge(exp$data, method="mb", nlambda=50, verbose=F)
   cthr <- huge(exp$data, method="ct", lambda=lbd.c, verbose=F)
   res.cthr <- perf.auc(perf.roc(cthr$path, exp$theta))
   res.gl <- perf.auc(perf.roc(gl$path, exp$theta))
   res.mb <- perf.auc(perf.roc(mb$path, exp$theta))
   return(setNames(c(res.gl,res.cthr,res.mb,n,i),
   c("glasso","correlation","mb", "sample size", "simu")))
  })))
return(melt(out, measure.vars = 1:3, value.name = "score"))}
```

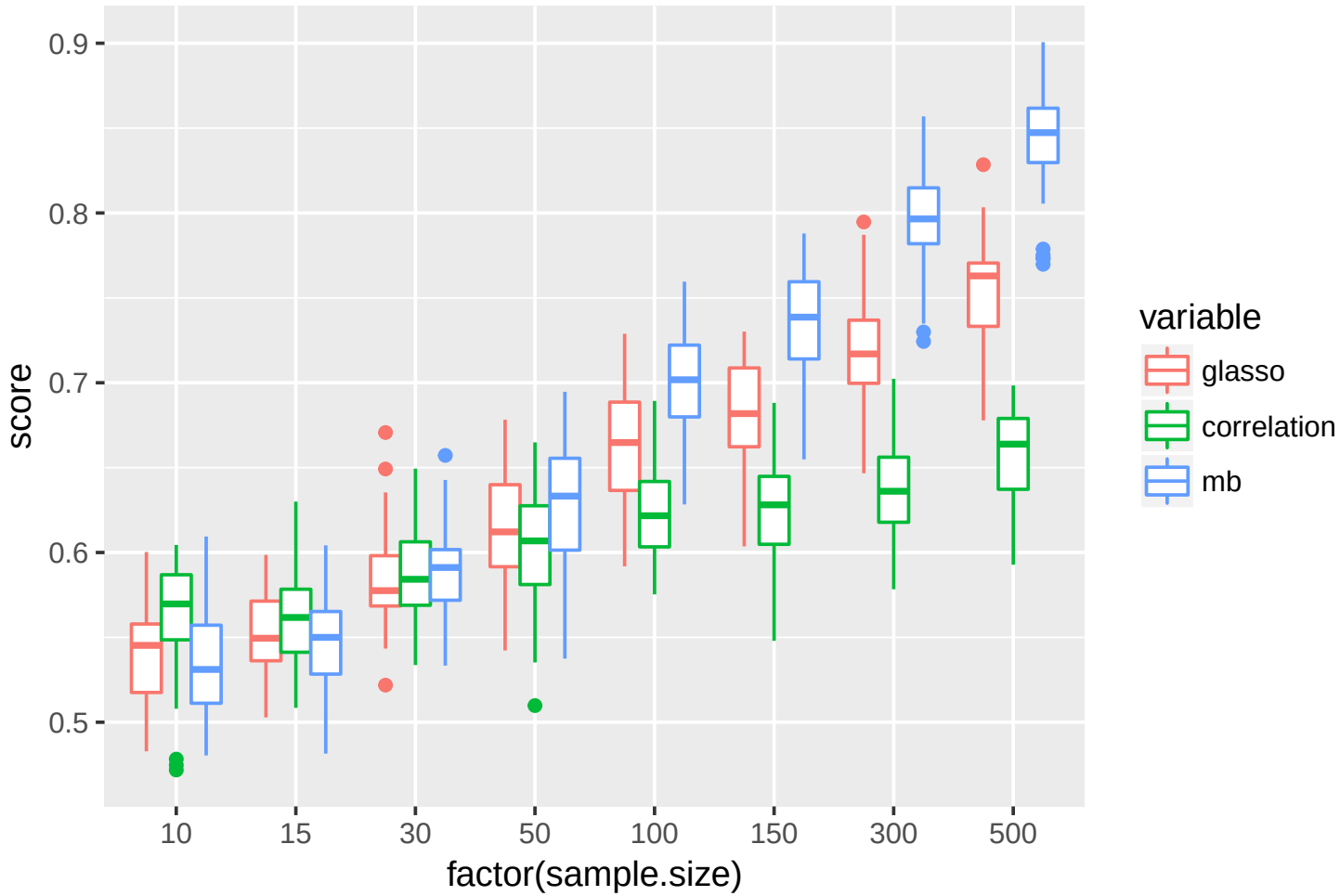
Run

```
suppressMessages(library(parallel))
res <- do.call(rbind, mclapply(1:40, one.simu, mc.cores=4))
```

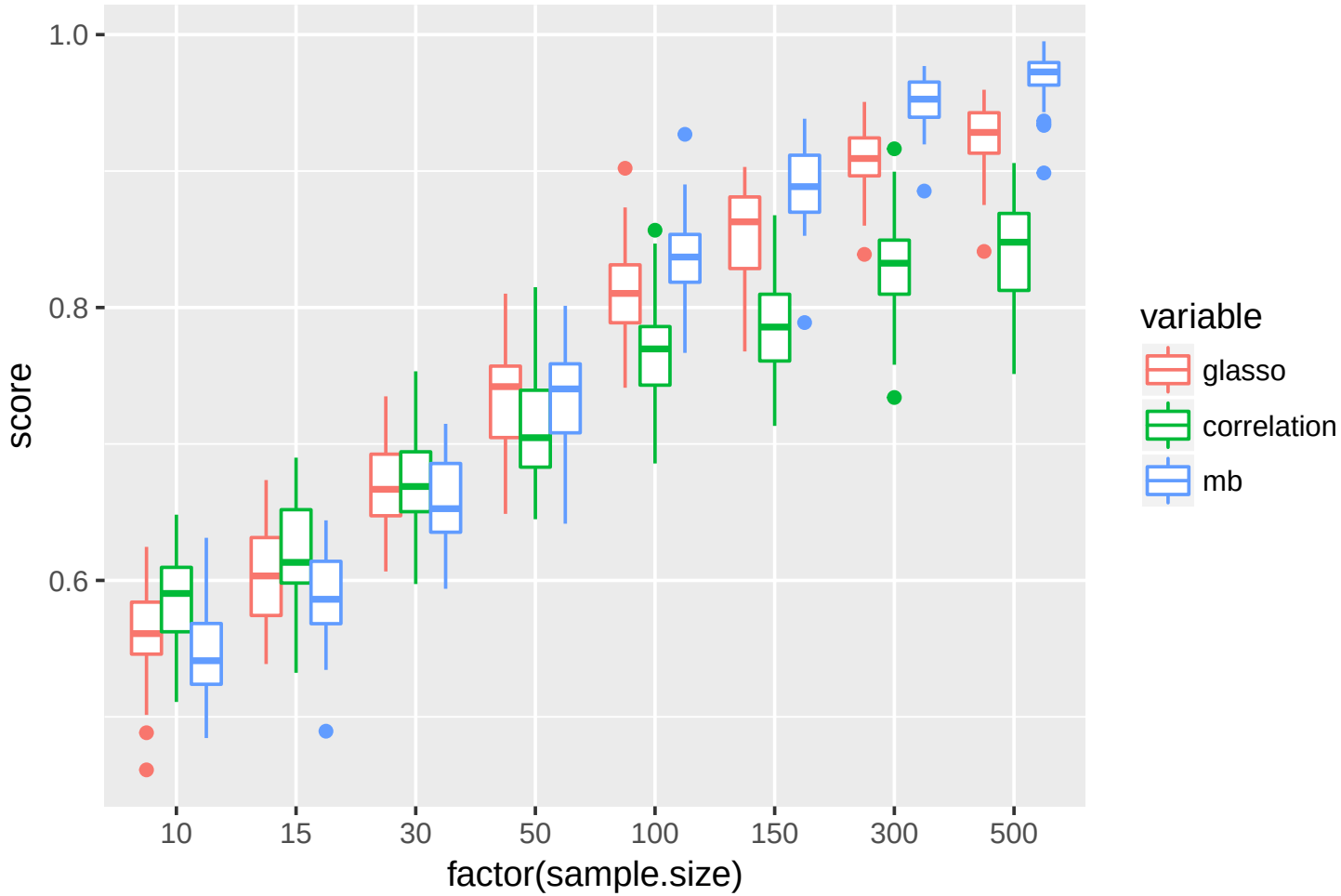
Simulation results (cluster - clique)



Simulation results (cluster, connection probability of 0.5)



Simulation results (random, connection probability of 0.3)



Breast cancer: transcriptomics for ER+ and ER- tumors

We look at a large public datasets from Guedj et al. 2011 with two main subgroups

- Estrogen receptor positive
- Estrogen receptor negative

```
load ("huge/breast_cancer_guedj11.RData")
load ("huge/gen_name.RData")
gene.name <- unlist(gene.name)
data.raw <- expr
table(class.ER)

## class.ER
## ERm ERp
## 162 375
```

Filtering Unknown genes

```
toDiscard <- which(gene.name == "Not.Known")
gene.name <- gene.name[-toDiscard]
data.raw <- data.raw[-toDiscard, ]
```

We get

```
dim(data.raw)

## [1] 41248   537
```

Differential analysis

Do we detect some gene expression differences ?

```
load ("huge/breast_cancer_guedj11.RData")
suppressMessages(library(limma))
design <- cbind(Moy=1, Erp=(class.ER == "ERp")+0)
fit <- lmFit(data.raw, design=design)
fit <- eBayes(fit)
res <- topTable(fit, coef="Erp", number=10^5,
                genelist=fit$genes, adjust.method="BH",
              sort.by="none", resort.by=NULL,
              p.value=1, lfc=0, confint=FALSE)
```

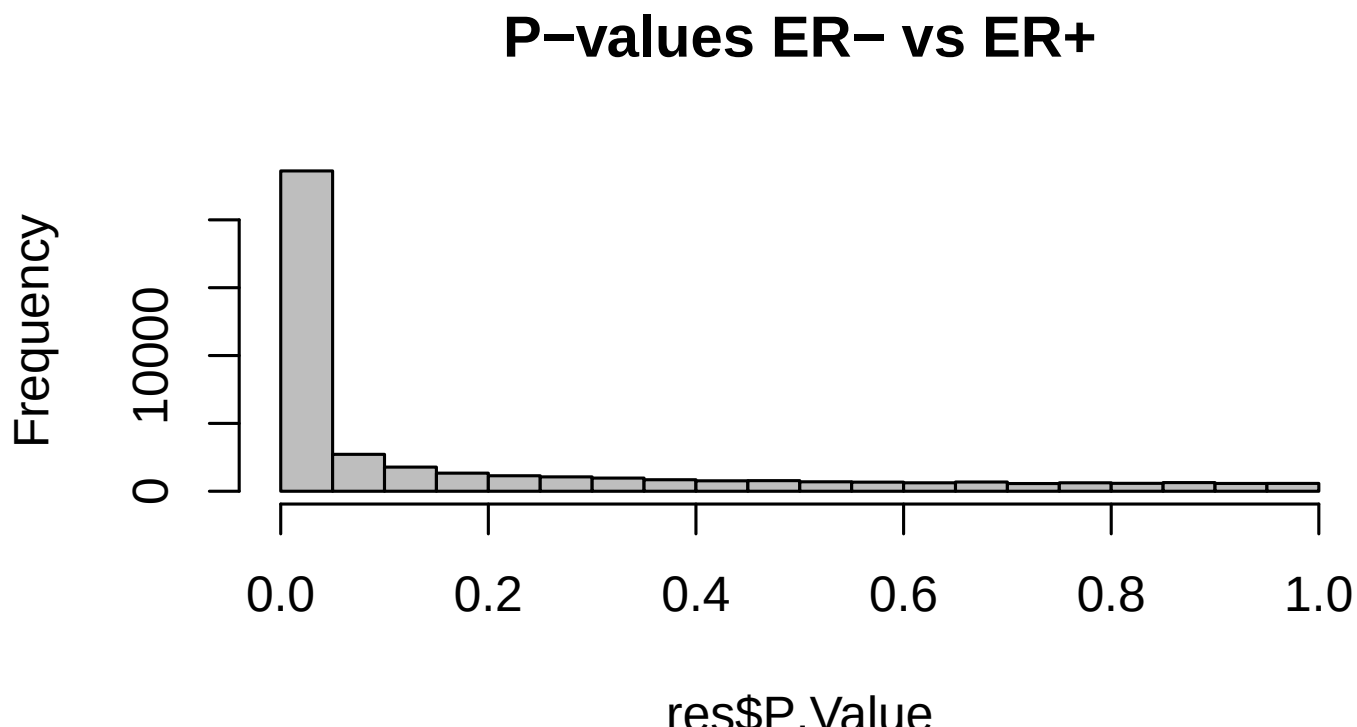
Many genes are differentially expressed

- The histogram of p-values looks good
- This is a well known fact (ER+ and ER- are very different)

```
sum(res$adj.P.Val < 10^-5)

## [1] 5907

hist(res$P.Value, breaks=30, col="grey",
     main="P-values ER- vs ER+")
```



What to do with this list of genes?

ESR1 has the most significant p-values

```
gene.name[order(res$adj.P.Val)[1]]

## 205225_at
##    "ESR1"
```

Network analysis

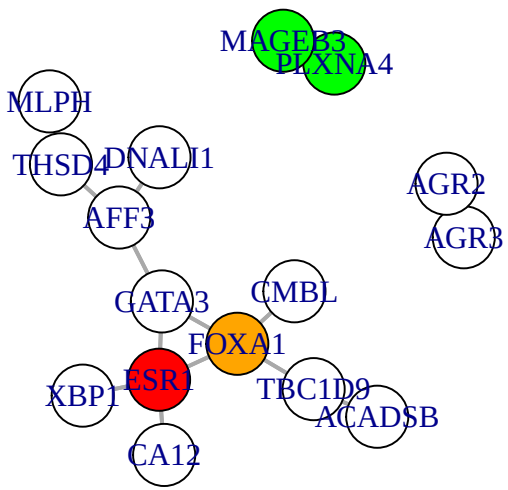
- Could we find partners of ESR1 that are specific to ER+?
- We cannot infer a network on 41000 genes (Verzelen 2011)
  - ⇝ Most differentially expressed genes
  - ⇝ Most varying genes
  - ⇝ Look at a specific pathway ...

Selecting some probes

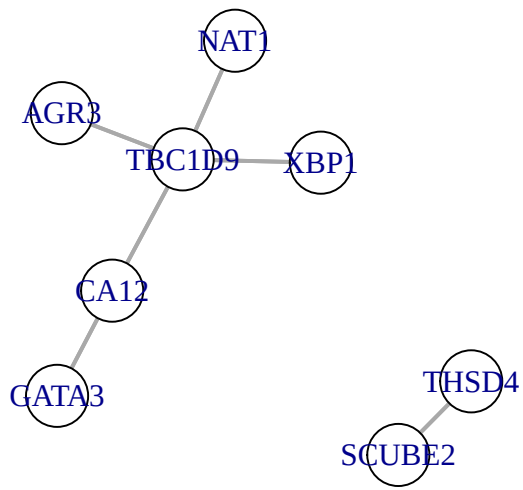
Take the 20 most differentially expressed plus some random

```
## Error in graph_from_adjacency_matrix(net_Mspec_):  could not find function
"graph_from_adjacency_matrix"
## Error in graph_from_adjacency_matrix(net_Pspec_):  could not find function
"graph_from_adjacency_matrix"
```

ER+ specific



ER− specific



FOXA1, ESR1, GATA3 a well known interaction

1. FOXA1 is a key determinant of estrogen receptor function and endocrine response. Antoni Hurtado et al. 2011 (Nat. Genet.):
   $\rightsquigarrow$ "FOXA1 is a key determinant that can influence differential interactions between ER and chromatin"
2. GATA3 acts upstream of FOXA1 in mediating ESR1 binding by shaping enhancer accessibility. Theodorou et al. 2013 (Genome Res.)
3. Estrogen receptor regulation of carbonic anhydrase XII through a distal enhancer in breast cancer. Barnett DH et al 2008 (Cancer Res.)
   $\rightsquigarrow$ "we show that CA12 is robustly regulated by estrogen via ER alpha in breast cancer cells"

Outline

Tutorial

Let us have a look together at

`http://julien.cremeriefamily.info/doc/teachings/exposome/transcriptomics_networks_inference.html`