Discovering molecular pathways from protein interaction and gene expression data

José Caldas

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Aim

To have a mechanism for inferring pathways from **gene expression** and **protein interaction** data.

Motivation — Why search for pathways

Pathway

Set of genes that coordinate to achieve a specific task.

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What do we gain from understanding pathways

1. A coherent global picture of (condition-specific) cellular activity.
2. Application to disease mechanisms.

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(B) When genes coordinate to achieve a particular task, their protein products often interact.

Each data type alone is a **weaker indicator** of pathway activity.

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- Join them.

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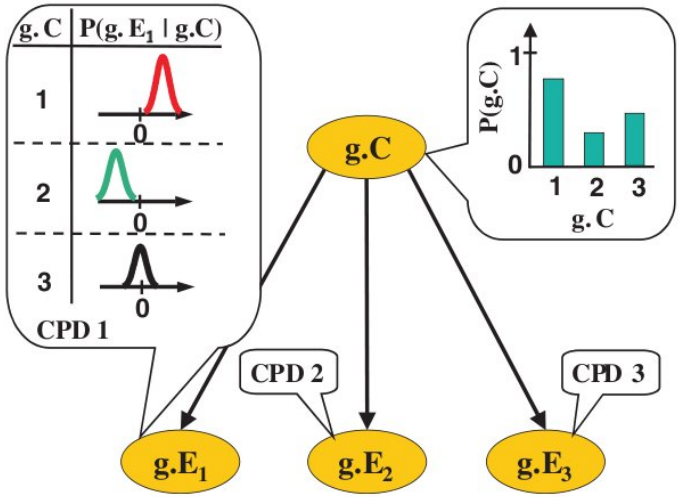
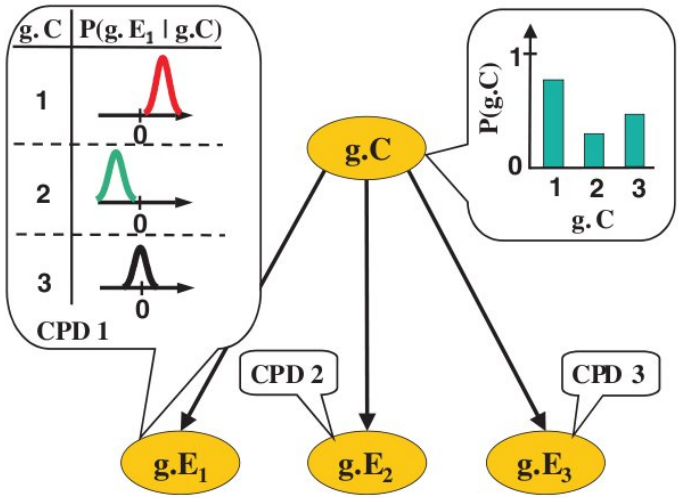
- Each gene g has two attributes:
  - Class (pathway), denoted by $g.C$ (discrete value).
  - Expression in microarray i, denoted by $g.E_i$.
  - If there are m microarrays $\Rightarrow g.\mathbf{E} = \{g.E_1, \ldots, g.Em\}$.

Model for expression profiles — Naive Bayes



Naive Bayes — given the class label $g.C$, $g.E_i$ and $G.E_j$ are independent.

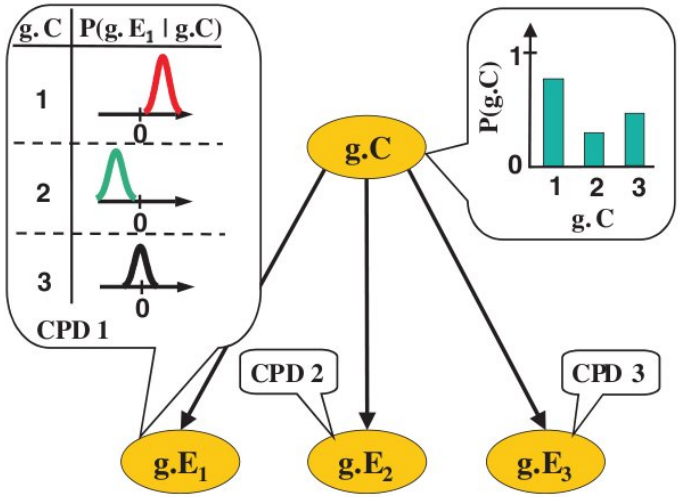
Model for expression profiles — Naive Bayes



Class probability

- $g.C$ follows a multinomial probability distribution
- $p(g.C = k) = \theta_k$
- $\sum_{i=1}^{K} \theta_i = 1$

Model for expression profiles — Naive Bayes



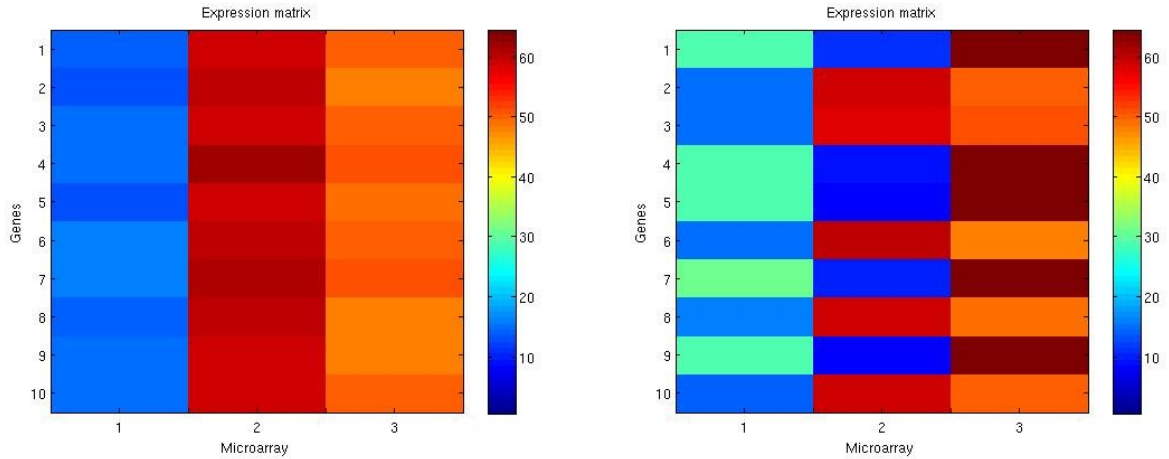
Expression profiles

- $g.E_i|g.C = k \sim N(\mu_{ki}, \sigma^2_{ki})$
- A pathway i specifies the **average** expression level for each microarray and also the variance.

Model for expression profiles — Naive Bayes

Example:

- 1 pathway, 10 genes, 3 microarrays
- Pathway specifies the averages $\mu = (15, 60, 50)$
- What is the most likely expression matrix?



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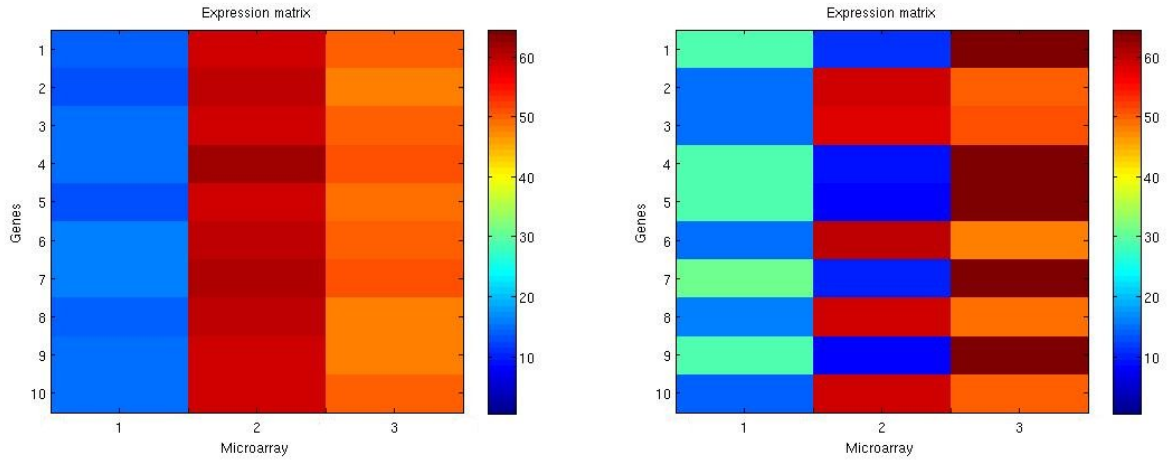
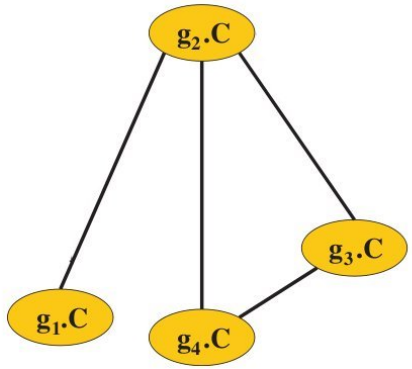


- (The matrix on the left)

Model for protein interaction — Markov random field



Undirected graph, $V = \{g_1.C, \ldots, g_n.C\}$, E =set of protein interactions.

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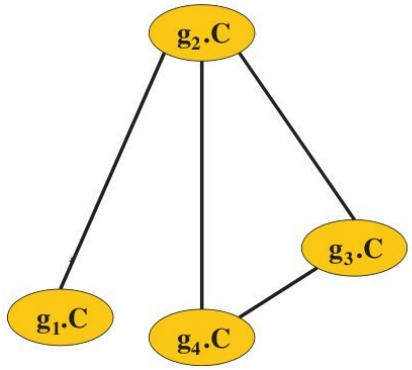

Undirected graph, $V = \{g_1.C, \ldots, g_n.C\}$, E =set of protein interactions.

Assumption

Interacting proteins are more likely to be in the same pathway.

Intuitive idea

If a pair of nodes share the same class $\Rightarrow$ likelihood is higher

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Joint distribution is

$$P(g_1.C, \ldots, g_n.C) = \frac{1}{Z} \prod_{i=1}^{n} \phi_i(g_i.C) \prod_{\{g_i.C - g_j.C\} \in \mathrm{E}} \phi_{i,j}(g_i.C, g_j.C) \tag{1}$$

Z is a normalization constant.

Markov random field — Formalism

$$\phi_{i,j}(g_i.C = p,\, g_j.C = q) = \begin{cases} \alpha & p = q \\ 1 & \text{otherwise} \end{cases}$$

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$(\alpha \geq 1)$.

Unified Model

What we already have

- Model for expression data (Naive Bayes)
- Model for class probability (Markov random field)

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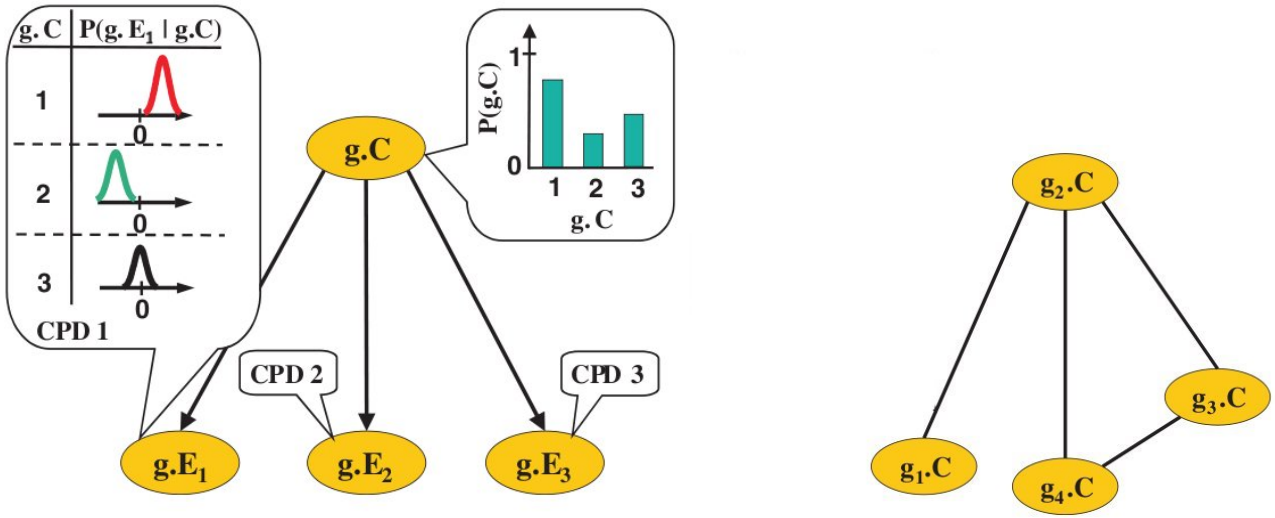
Probability distribution $P(\mathbf{G}.C, \mathbf{G}.E)$, using expression and protein data.

What we are missing

- Naive Bayes provides that prob. distribution, but does not use protein data.
- We haven't specified the potentials $\phi_i(g_i.C)$.

Unified Model



Solution

- Use Markov random field as $P(\mathbf{G}.C)$.

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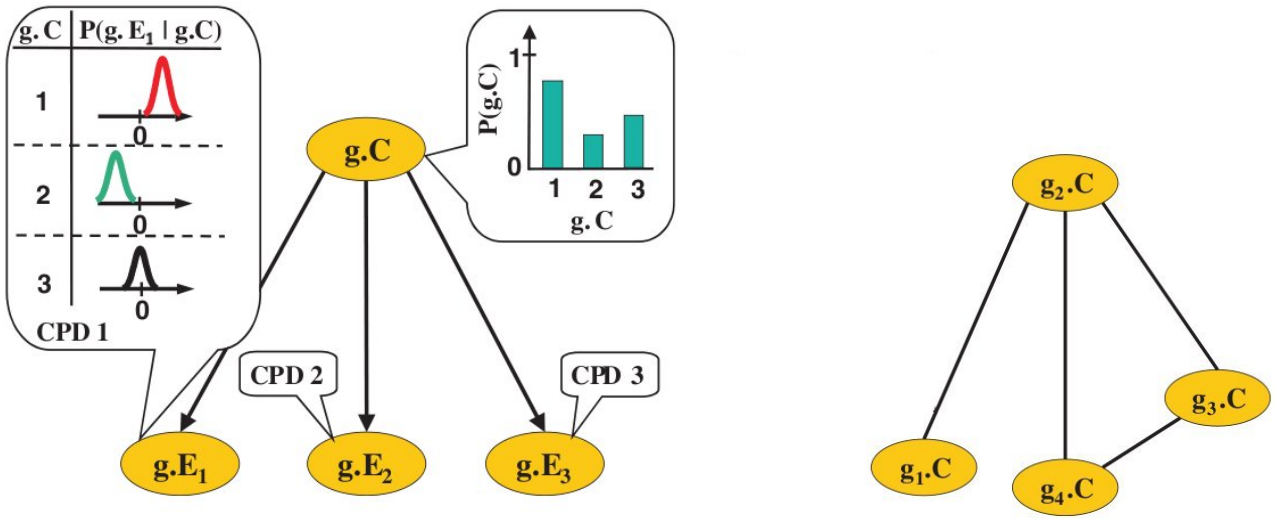

Solution

- Use Markov random field as $P(\mathbf{G}.C)$.
- Use multinomial dist. $P(g_i.C)$ from Naive Bayes as potential $\phi_i(g_i.C)$.
- Call it $P^*(g_i.C)$.

Unified Model

$$P(\mathbf{G}.C, \mathbf{G}.\mathbf{E}) =$$

$$\frac{1}{Z} \prod_{i=1}^{n} P^*(g_i.C) \prod_{\{g_i.C - g_j.C\} \in \mathrm{E}} \phi_{i,j}(g_i.C, g_j.C) \cdot$$

$$\prod_{i=1}^{n} \prod_{j=1}^{m} P(g_i.E_j | g_i.C)$$

$P(\mathbf{G}.C) \rightarrow$ Markov random field.
$P(\mathbf{G}.\mathbf{E}) \rightarrow$ Gaussian distributions.

Learning Algorithm

EM algorithm

Parameters to be estimated

- Multinomial distribution $\rightarrow (\theta_1, \ldots, \theta_K)$.
- Mean and variance for gaussian distributions

Datasets

Gene Expression

- 173 arrays (Gasch *et al.* 03)
- 77 arrays (Spellman *et al.* 98)

Protein Interaction

10705 interactions (Xenarios *et al.* 05)

After preprocessing $\longrightarrow$ 3589 genes.

Running the algorithm

- EM for optimizing parameters
- Number of pathways fixed as 60
- Starting point for parameters $\longrightarrow$ use hierarchical clustering

How to set the α parameter?

Setting α

- Recall: α is the *compatibility potential* when two proteins interact and belong to the same pathway.

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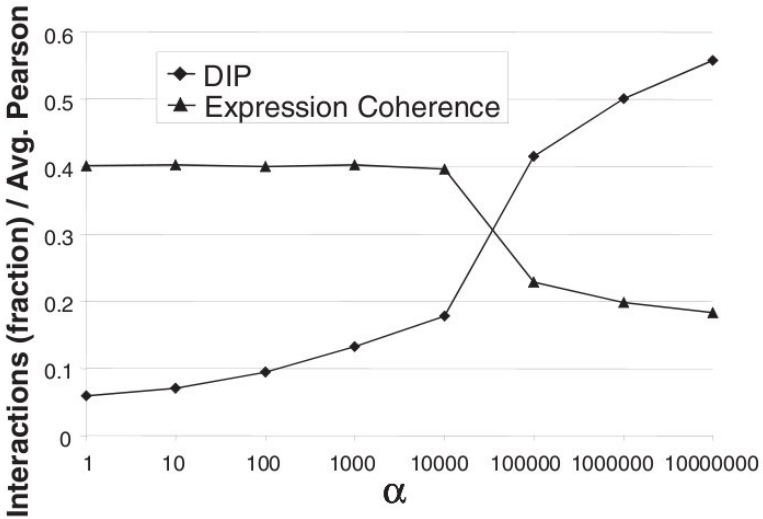

Comparisons with other methods

Methods that use only one type of data

- Markov Cluster (Enright *et al.* 02)
- Hierarchical clustering (Eisen *et al.* 98)

Tests

- Prediction of held-out interactions.
- Functional enrichment in Gene Ontology.
- Coverage of protein complexes.
- Assigning new roles to unknown proteins.

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- Cross-validation — divide protein data into 5 disjoint sets (4 for training, 1 for testing)
- Get average number of held-out interactions between genes in the same pathway
- Result: 222.4 ± 13.2
- (MCL) 383.2 ± 29.1

Biological coherence of the inferred pathways

General result

More functionally coherent than when using standard clustering or MCL

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Example — Pathways related to translation, protein degradation, transcription, and DNA replication

- Genes in these pathways interact with many genes from other categories.
- They are also co-expressed.

Biological coherence of the inferred pathways

General result

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Example — Pathways related to translation, protein degradation, transcription, and DNA replication

- Genes in these pathways interact with many genes from other categories.
- They are also co-expressed.
- MCL cannot isolate them.

Protein Complexes

Motivation

The components of many pathways are protein complexes. Thus, a good pathway model should assign the member genes of many of these complexes to the same pathway.

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Procedure

- Use experimental assays (Gavin *et al.* 02) and (Ho *et al.* 02)
- Associate each gene to the complexes to which it belongs.
- Measure enrichment in pathways.

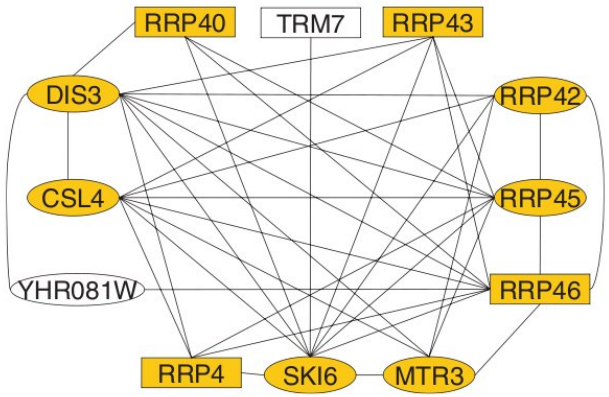
Protein Complexes — Results

In general, better than clustering:

- 374 complexes significantly enriched (higher than in clustering).
- Stress data $\rightarrow$ 124 complexes in which more than 50% of members appear in the same pathway.
- Clustering $\rightarrow$ only 46 complexes that verify that condition.

Assigning New Roles to Unknown Proteins

Largest connected component of pathway 1 (cytoplasmic exosome):



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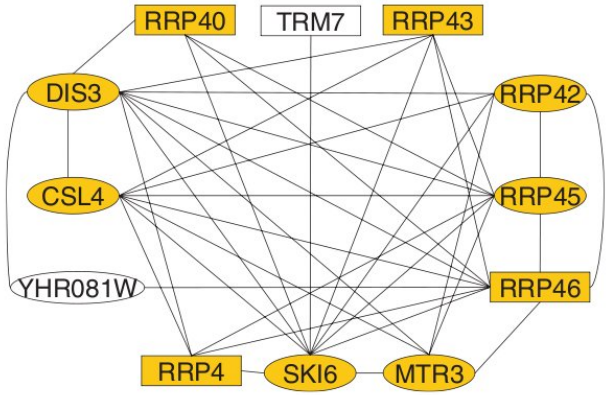

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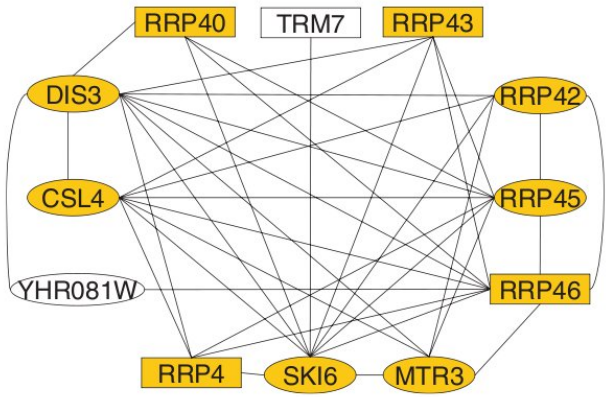

- YHR081W is uncharacterized
- Clustering — Only 4 genes in pathway
- MCL — Includes 114 additional genes in connected component

Conclusion

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Comparison with single-source methods

Some pathways are only obtainable by combining both types of data

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Limitations

- Model for co-expression is too restrictive
- Assignment of each gene to a *single* pathway
- Pathways should be condition-specific (same goes for protein interaction)

Questions

(1) On which two assumptions about pathways is the model based?

(2) Map each of the previous assumptions into a property of the model

(3) Why must the α parameter in the markov random field be greater than one?

(4) What happens when (a) $\alpha = 1$ or when (b) α is close to infinity?