Discovering molecular pathways from protein interaction and gene expression data

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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.
Motivation — Why search for pathways

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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.
Motivation — Why use two kinds of data

2 properties of (many) pathways

(A) Genes in the same pathway are activated together ⇒ exhibit similar expression profiles.

(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.
Motivation — Why use two kinds of data

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Intuitive Idea

- Detect group of genes that are co-expressed, and whose products interact in the protein data.
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- Create a model for **gene expression data**.
- Create a model for **protein interaction data**.
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- Create a model for **gene expression data**.
- Create a model for **protein interaction data**.
- Join them.
Basics

Gene

- Set of genes $G = \{1, \ldots, n\}$. 
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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 $\Rightarrow g.E = \{g.E_1, \ldots, g.E_m\}$. 

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Discovering molecular pathways from protein interaction and gene expression data
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

- \( g.E_i | g.C = k \sim N(\mu_{ki}, \sigma^2_{ki}) \)
- 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?
Model for expression profiles — Naive Bayes

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- What is the most likely expression matrix?

▶ (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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Expressing molecular pathways from protein interaction and gene expression data
Model for protein interaction — Markov random field

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.
Each $g_i.C$ is associated with a potential $\phi_i(g_i.C)$. 

$Z$ is a normalization constant.
Markov random field — Formalism

- Each $g_i.C$ is associated with a potential $\phi_i(g_i.C)$.
- Each edge $g_i.C - g_j.C$ is associated with a compatibility potential $\phi_{i,j}(g_i.C, g_j.C)$. 
Markov random field — Formalism

- Each \( g_i.C \) is associated with a potential \( \phi_i(g_i.C) \).
- Each edge \( g_i.C - g_j.C \) is associated with a compatibility potential \( \phi_{i,j}(g_i.C, g_j.C) \).

Joint distribution is

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

(1)

\( Z \) is a normalization constant.
Markov random field — Formalism

\[ \phi_{i,j}(g_i.C = p, g_j.C = q) = \begin{cases} \alpha & p = q \\ 1 & \text{otherwise} \end{cases} \]
Markov random field — Formalism

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

(\alpha \geq 1).
Unified Model

What we already have

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

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

What we want

Unified Model

What we already have

- Model for expression data (Naive Bayes)
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What we want

What we are missing

- Naive Bayes provides that prob. distribution, but does not use protein data.
Unified Model

What we already have

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

What we want

Probability distribution \( P(G.C, G.E) \), 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 $P(G.C)$. 
Unified Model

Solution

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

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Unified Model

\[ P(G.C, G.E) = \]

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

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

\[ P(G.C) \rightarrow \text{Markov random field.} \]
\[ P(G.E) \rightarrow \text{Gaussian distributions.} \]
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 → 3589 genes.
Running the algorithm

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

How to set the $\alpha$ parameter?
Setting $\alpha$

- Recall: $\alpha$ is the compatibility potential when two proteins interact and belong to the same pathway.
Recall: $\alpha$ is the *compatibility potential* when two proteins interact and belong to the same pathway.
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.
Prediction of held-out interactions

- Cross-validation — divide protein data into 5 disjoint sets (4 for training, 1 for testing)
Prediction of held-out interactions

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- Get average number of held-out interactions between genes in the same pathway
Prediction of held-out interactions

- 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: $222.4 \pm 13.2$
- (MCL) $383.2 \pm 29.1$
Biological coherence of the inferred pathways

General result
More functionally coherent than when using standard clustering or MCL
Biological coherence of the inferred pathways

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More functionally coherent than when using standard clustering or MCL

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
More functionally coherent than when using standard clustering or MCL

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 → 124 complexes in which more than 50% of members appear in the same pathway.
- Clustering → only 46 complexes that verify that condition.
Assigning New Roles to Unknown Proteins

Largest connected component of pathway 1 (cytoplasmic exosome):

- YHR081W is uncharacterized
Assigning New Roles to Unknown Proteins

Largest connected component of pathway 1 (cytoplasmic exosome):

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- Clustering — Only 4 genes in pathway
Assigning New Roles to Unknown Proteins

Largest connected component of pathway 1 (cytoplasmic exosome):

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

Summary

▶ Probabilistic model for integrating gene expression and protein interaction data
Conclusion

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- Method aims at finding co-expressed and connected genes (pathways)
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Comparison with single-source methods
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Summary

- Probabilistic model for integrating gene expression and protein interaction data
- Method aims at finding co-expressed and connected genes (pathways)

Comparison with single-source methods
Some pathways are only obtainable by combining both types of data
Conclusion

Limitations

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▶ Model for co-expression is too restrictive
Conclusion

Limitations

- Model for co-expression is too restrictive
- Assignment of each gene to a single pathway
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- 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 $\alpha$ parameter in the Markov random field be greater than one?

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