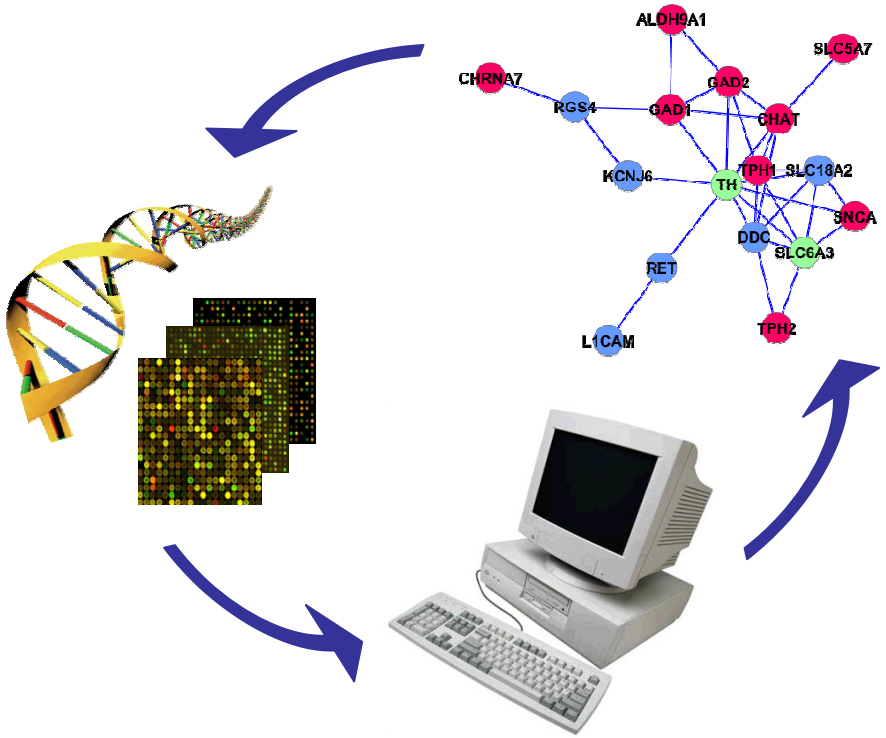


Integrating prior biological knowledge into omics data analysis

Enrico Glaab, Luxembourg Centre for Systems Biomedicine

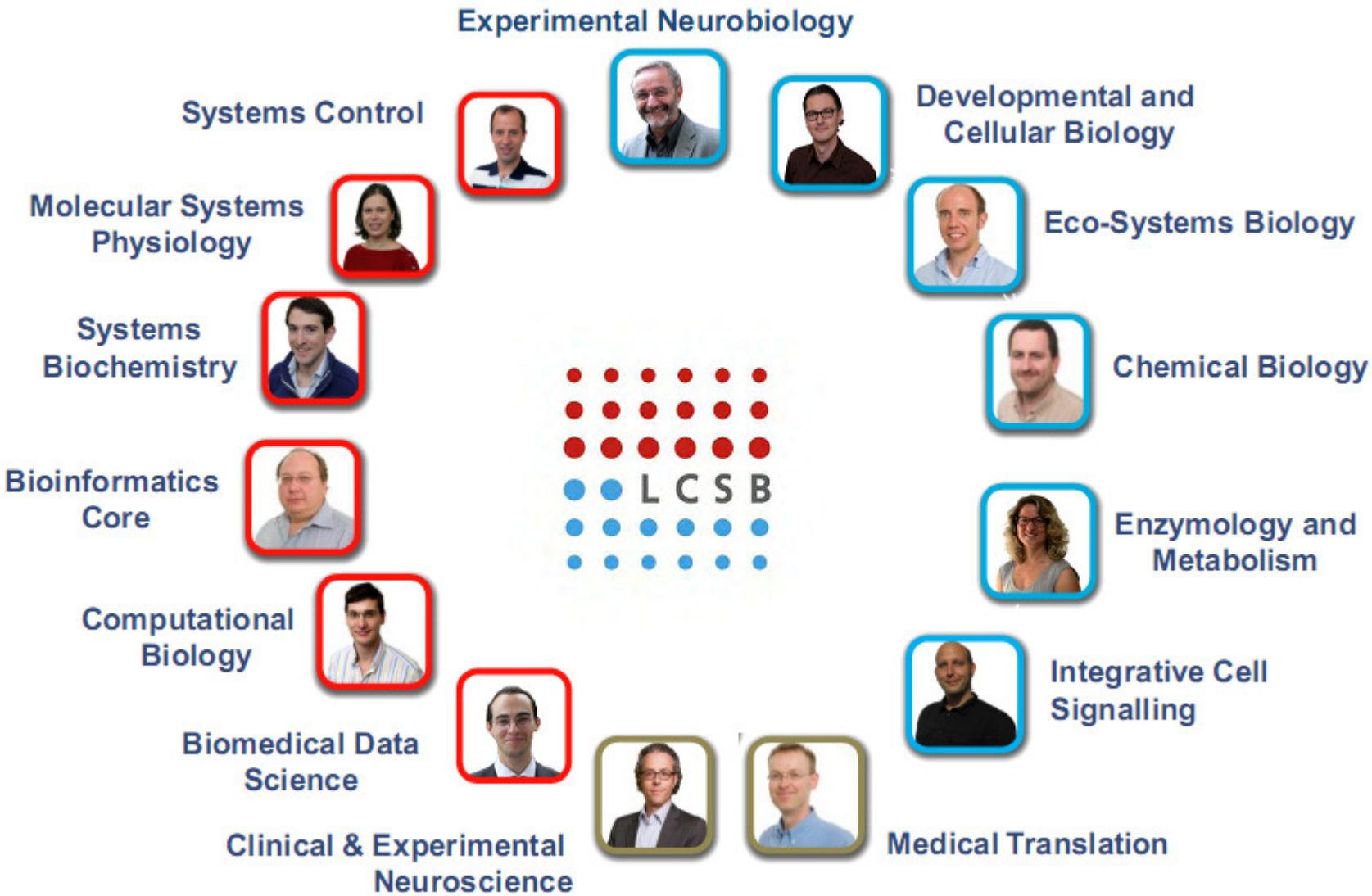
Outline

- Background and focus of research group
- Addressing common statistical challenges in omics data analysis
- Exploiting information from cellular pathways and molecular networks for machine learning analyses of omics data
- Summary & Discussion

Luxembourg Centre for Systems Biomedicine (LCSB)

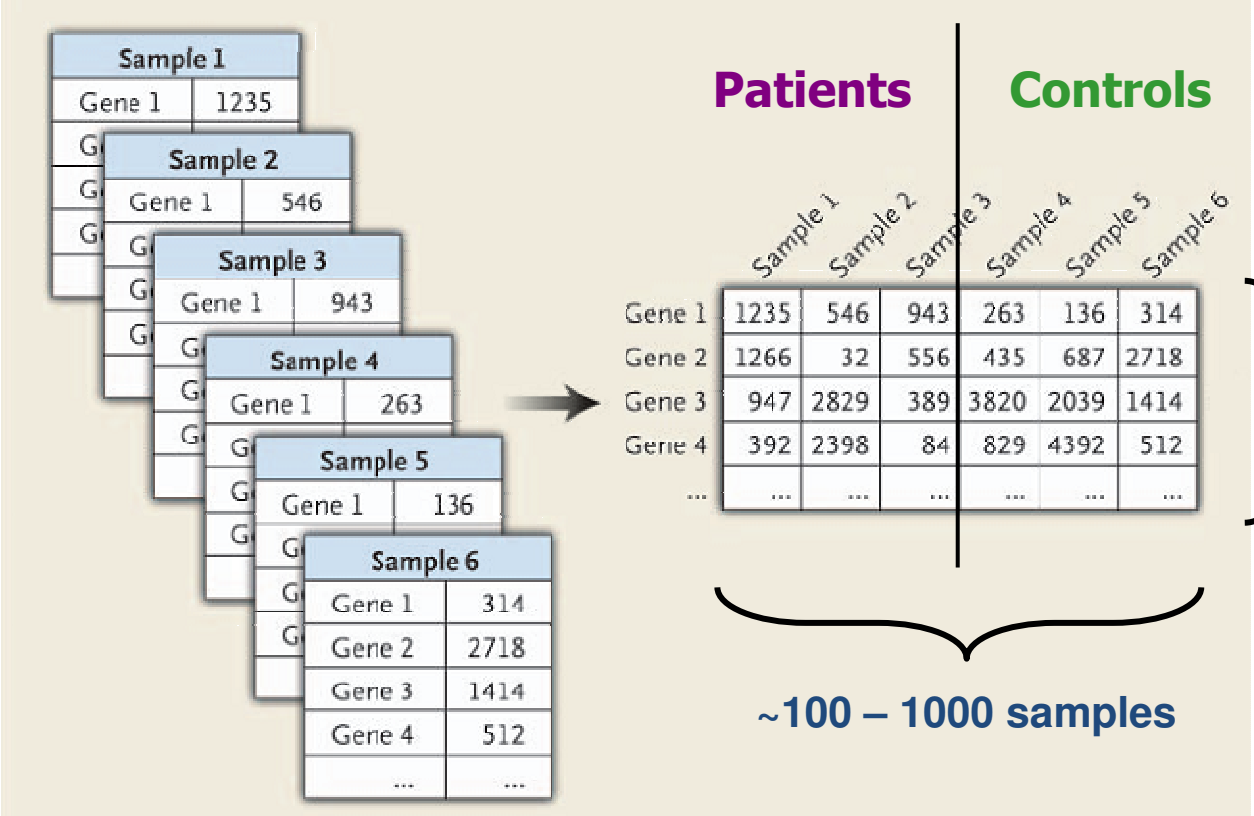
Luxembourg Centre
for Systems Biomedicine

LCSB – Research Groups & Interdisciplinarity



Research Focus & Main Goals

<u>Research focus</u>: Analysis of omics data from case/control studies



"Small N, Large P" problem

→ **<u>GOALS</u>: Interpret biological differences between patients and controls, identify candidate disease genes & biomarkers for validation**

Overview of machine learning analysis types for omics data

Unsupervised Analyses:
(no sample labels used)

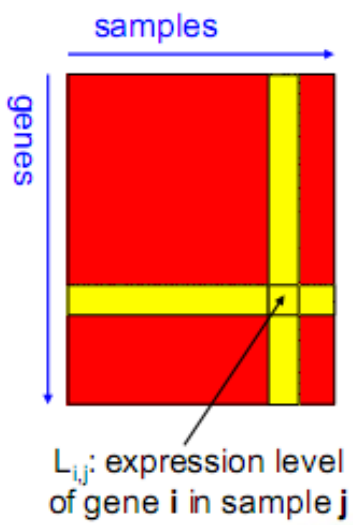
Complexity:

- Clustering samples (columns) *"hard"*
- Clustering biomolecules (rows) *"hard"*
- Bi-Clustering (rows & columns) *"hard"*

Supervised Analyses
(using labelled training data):

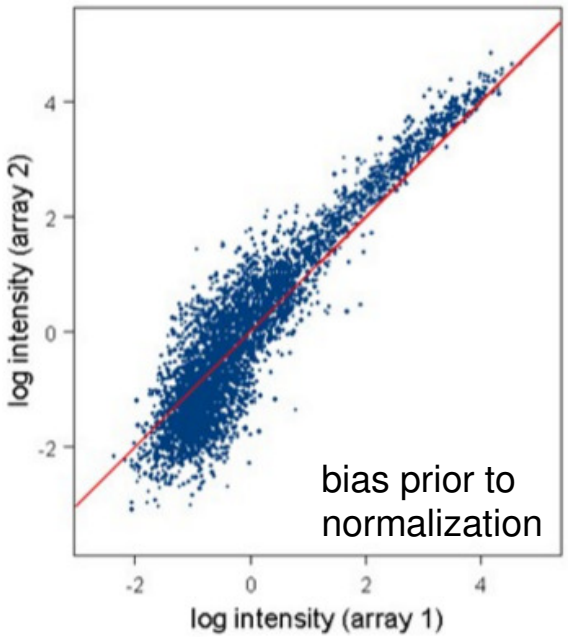
- Differential expression analysis *"easy"*
- Pathway enrichment analysis *"easy"*
- Network/causal reasoning canalysis *"hard"*
- Sample classification/regression *"hard"*
- Gene/protein function classification *"hard"*

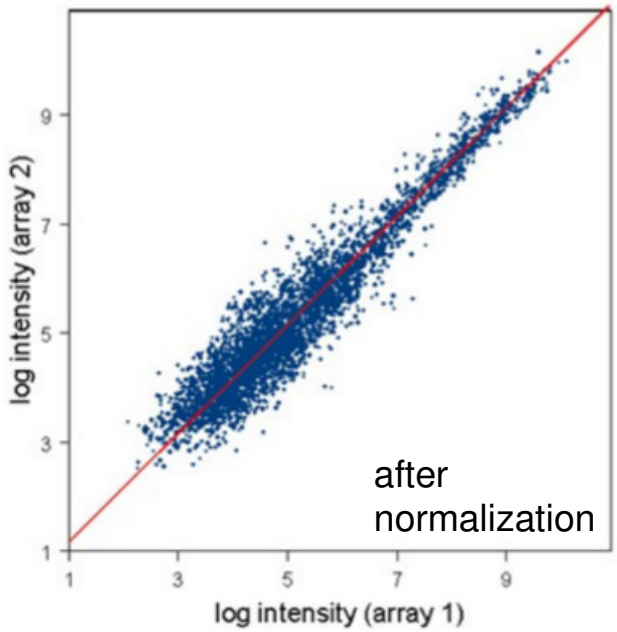
Example: High-throughput gene expression data



Common challenges for functional genomics data analysis (1)

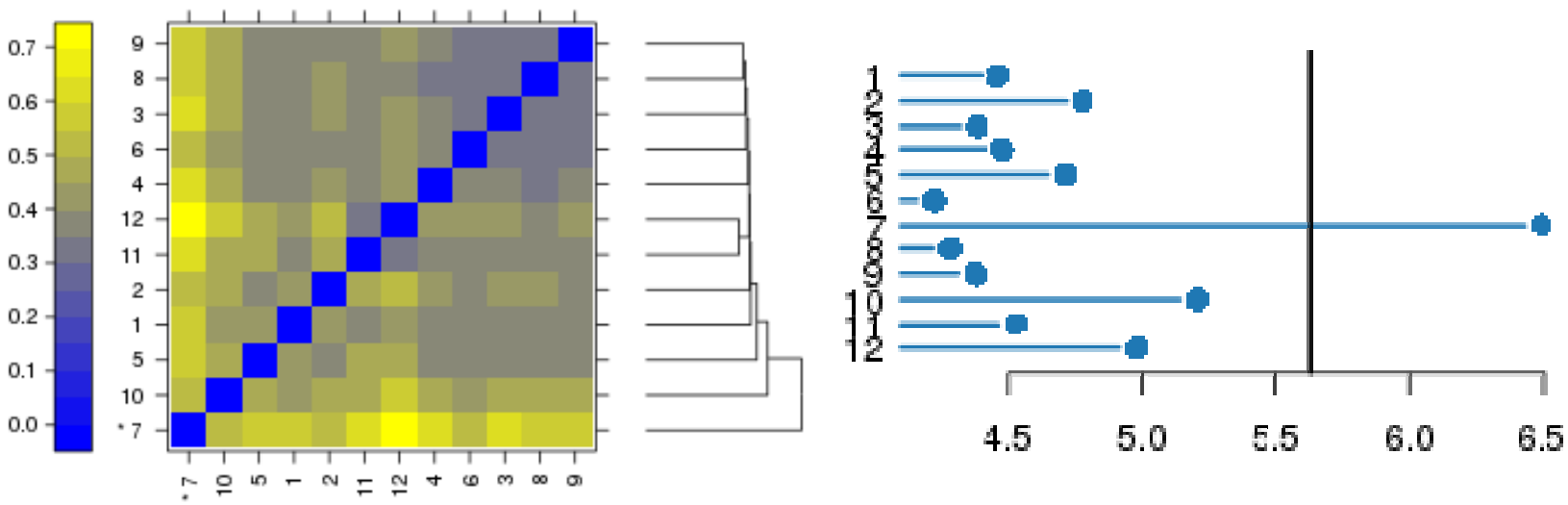
- Small number of samples in relation to large number of biomolecules ("Small N, Large P" problem) → "curse of dimensionality"
- Large number of uninformative and/or functionally redundant biomolecules (shared function & expression/activation pattern)
- Real signal shifted and scaled by additive and multiplicative noise





Common challenges for functional genomics data analysis (2)

- Outliers (among biomolecules or samples) and transcriptional amplification in sample subset
- Imbalances in no. of samples per condition (e.g. lack of control samples)
- Confounding factors and inadequate matching of patients & controls



False colour heat map (left) and bar chart (right) of distances between microarrays

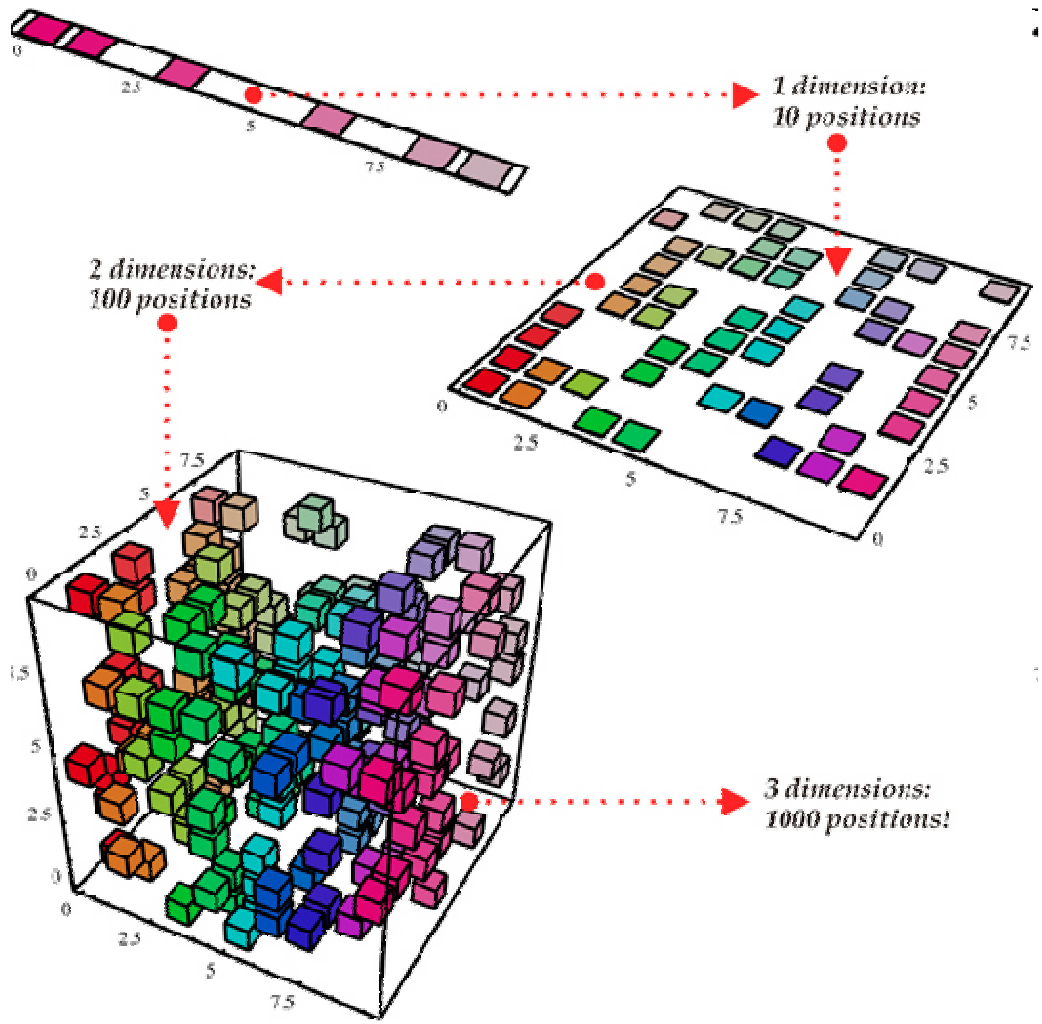
The “curse of dimensionality“

For increasing numbers of biomolecules/features:

- the space spanned by these features grows exponentially

  → the available data becomes sparse

  → more data points needed to train reliable diagnostic models



Strategies to address common issues in omics analysis

Statistical approaches:

- Use dimension reduction techniques, dedicated methods to exploit on-chip replicates and spike-in controls, model averaging methods for machine learning (e.g. ensemble classification, consensus clustering)

Data integration methods / exploiting prior biological knowledge:

- Apply meta-analyses across multiple studies, combining information across complementary omics & clinical data in supervised machine learning models
- Analyse and integrate data on the level of cellular pathways & molecular networks

Computer-assisted study design / power calculation:

- Design the study with a sufficient number of replicates per class/condition and balanced classes; reduce impact of confounding factors via algorithmic sample selection/matching

Using prior knowledge in omics data analysis - Overview

Data integration at the level of biomolecules:

- Exploit functional relationships between biomolecules:
  → cellular pathway membership
  → protein complex membership
  → interaction in gene regulatory or protein interaction networks

- Exploit biomolecular relationships across different omics:
  → genes encoding proteins
  → enzymes converting metabolites
  …

	Patient samples			Control samples		
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Gene 1	1235	546	943	263	136	314
Gene 2	1266	32	556	435	687	2718
Gene 3	947	2829	389	3820	2039	1414
Gene 4	392	2398	84	829	4392	512
…	…	…	…	…	…	…

Data integration at the level of samples:

- Exploit meta information for each sample (clinical data, sample quality, storage duration)
- Exploit correlation patterns across different omics data collected for the same samples

…

Pathway-based sample classification (PathVar software)

Motivation: Gene/protein expression alterations in diseases tend to be co-ordinated at the level of cellular pathways

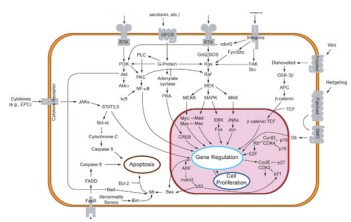
→ Use "**pathway fingerprints**" (weighted sums of gene expression levels from all pathway members) as candidate biomarkers with increased robustness

Pathway databases

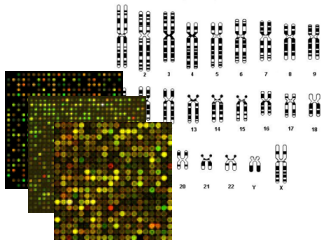
Cellular pathways

Omics data

Min. size: 10 genes

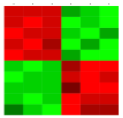


Map expression levels



Compute weighted sum of expression levels for each pathway (e.g. using PCA)

Pathway-level activity measures (fingerprints)



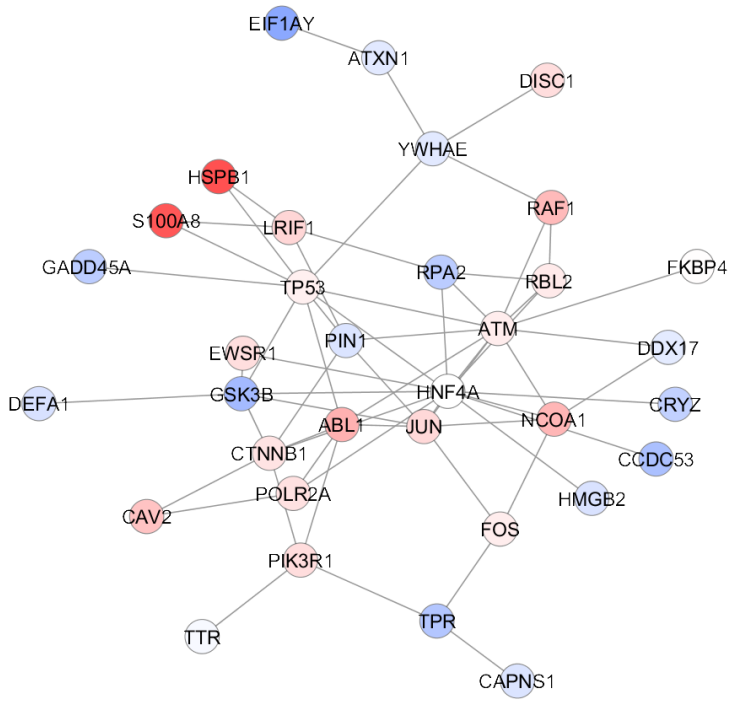
Pathway-level sample classification results

- Map omics data onto Gene Ontology (GO) biological processes (example: Parkinson's disease case/control post-mortem brain transcriptomics data)
- Use "pathway fingerprints" and a Support Vector Machine for classification (10-fold cross-validation; feature selection: empirical Bayes moderated t-statistic)

	Accuracy and stddev. for different numbers of selected attributes			
Attribute type	**10**	**30**	**50**	**100**
Gene-level model	89.2 ± 14.2	89.2 ± 14.2	92.5 ± 12.1	92.5 ± 12.1
GO - Mean	84.2 ± 13.9	90 ± 12.9	92.5 ± 12.1	89.2 ± 14.2
GO - Median	84.2 ± 13.9	91.7 ± 13.6	**95 ± 10.5**	91.7 ± 13.6
GO - Stddev.	76.7 ± 18.8	81.7 ± 17.5	79.2 ± 20.1	86.7 ± 14.3
GO - Min.	71.7 ± 21.9	68.3 ± 25.1	79.2 ± 23.3	71.7 ± 24.9
GO - Max.	81.7 ± 17.5	84.2 ± 13.9	90 ± 12.9	84.2 ± 18.2
GO - PCA	89.2 ± 14.2	**95 ± 10.5**	92.5 ± 12.1	**95 ± 10.5**
GO - MDS	**91.7 ± 13.6**	86.7 ± 18.5	84.2 ± 18.2	87.5 ± 17.7

Molecular networks as prior knowledge (GenePEN software)

Motivation: Disease-associated perturbations are often localized in biological networks. Finding these network clusters may help us to develop more robust biomarker models.



Example sub-network (Meta-analysis of 8 post-mortem microarray datasets for *substantia nigra* tissue) :

- Over-expressed in PD
- Under-expressed in PD

Question: How can we find clustered gene/protein groups efficiently, accounting for their predictivity and connectedness in the network?

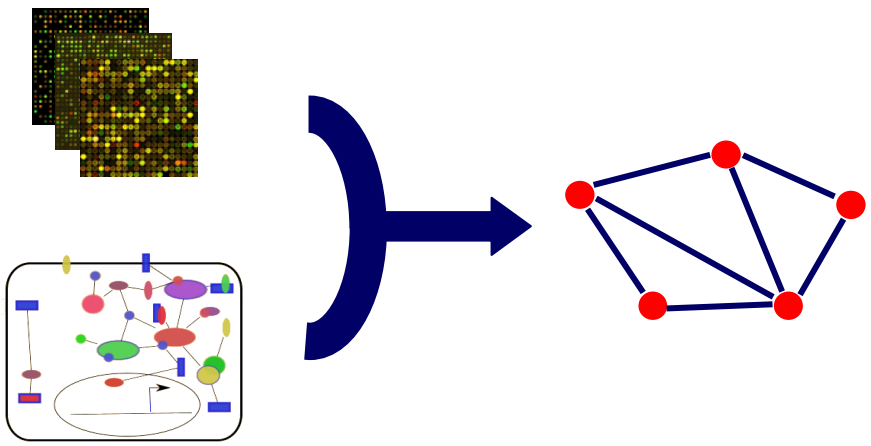
GenePEN - Workflow

Input:

- Omics dataset **X** (p rows = biomolecules, n columns = samples)
- Class labels **y** (e.g. “patient” or “control”)
- Table **A** of interactions/similarities between rows in X (e.g. protein-protein interactions)

Output:

- A subset of discriminative biomolecules (rows in X) representing a **connected** component in A (→ an altered sub-network) that provides a signature to classify new samples



GenePEN - Approach

Idea: Cast the feature selection as an optimization problem, maximizing two quantities:

- the estimated diagnostic prediction accuracy of the classifier
- the connectedness of selected features/biomolecules in the network

→ formulate an objective function (details not shown):

$$\min_{w} \underline{loss(w)} + \underline{\lambda} \cdot \underline{penalty(w)}$$

loss-function (minimize error) trade-off parameter penalty-function (network grouping)

→ Output after optimization procedure: A selection of features (***w***) that minimizes the objective function (features which minimize the prediction error and are well-grouped in the network)

GenePEN – Application to Parkinson‘s disease data

- **Parkinson’s disease test dataset**: Microarray gene expression data from *post mortem* brain samples (*substantia nigra*) of 43 PD patients and 50 controls (Zhang et al., 2005)

- **Network data**: Human genome-scale protein-protein interaction network constructed from 80,543 public, direct physical interactions between 10,042 proteins.

- **Comparison to other approaches**: GenePEN was compared against related methods with other penalty functions:

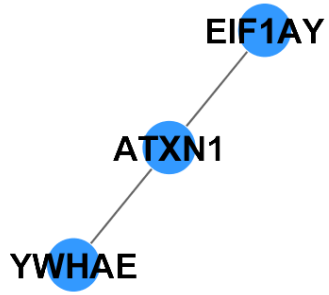
→ Lasso (Tibshirani, 1996) → sparse feature selection, but no feature grouping

→ Elastic Net (Zou & Hastie, 2005) → cannot account for external network data

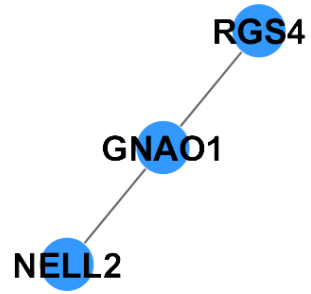
→ Pairwise Elastic Net (Lorbert, 2010 & 2013) → can take external network data into account to achieve a partial grouping of features

Comparison: Largest clusters found for 50 selected genes

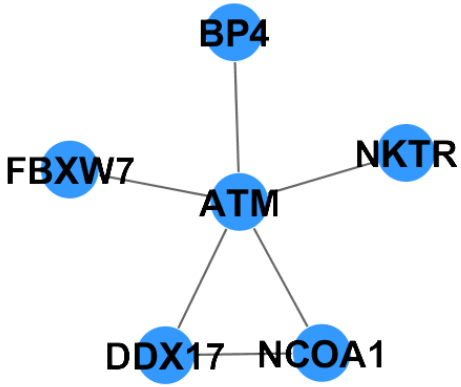
Lasso



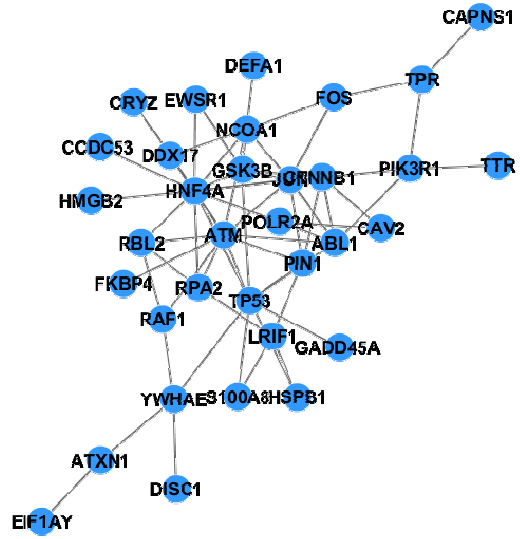
Elastic Net



PEN (2010)



PEN (2013)



GenePEN → cluster of 34 genes
(accuracy comparable to best alternative)

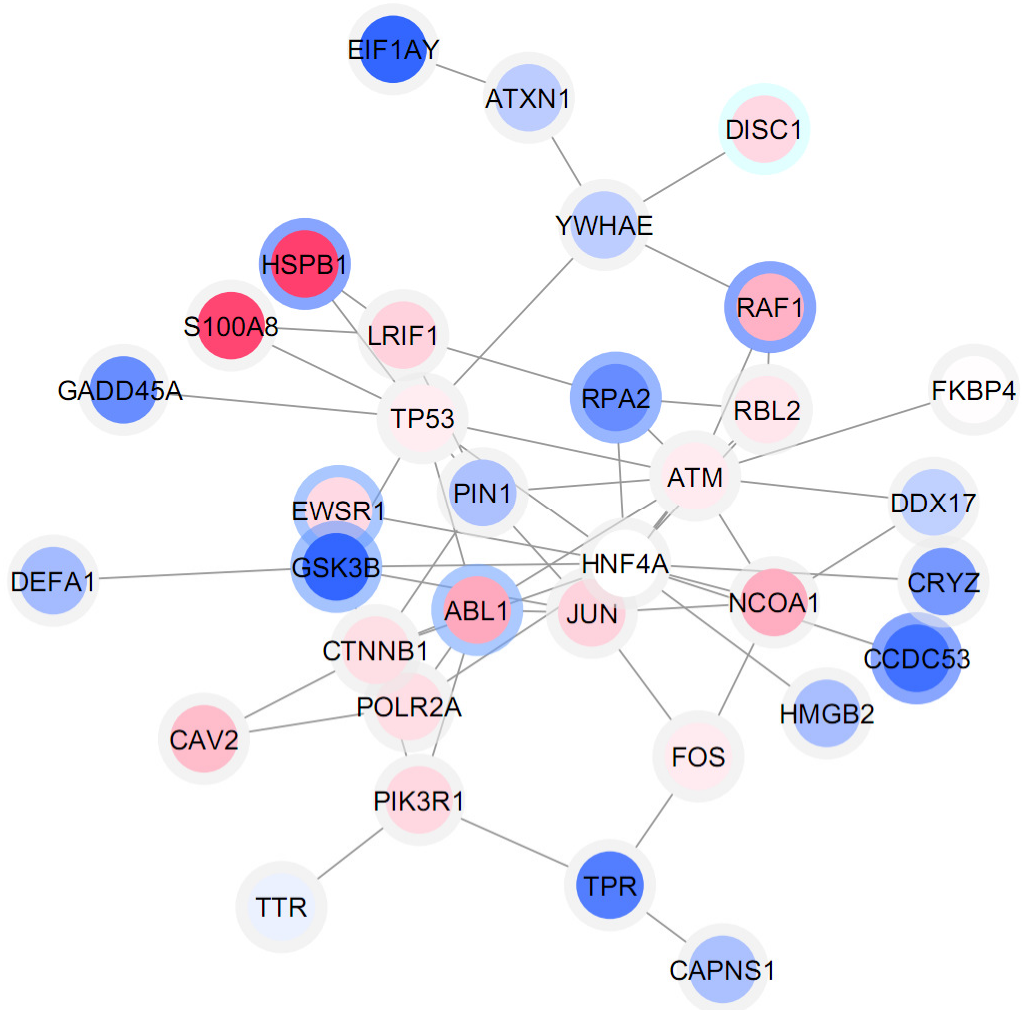
GenePEN: Biological analysis of predictive sub-networks

Largest connected graph component identified for Parkinson's disease:

- red = over-expressed in PD
  blue = under-expressed in PD
  node borders = individual statistical significance (from gray to blue with increasing significance)

- individually significant genes are significantly over-represented in the sub-network (p = 0.01)

- GSK3B contains polymorphisms associated with Parkinson's disease



Summary

- Many tools are available to address statistical challenges in omics data analysis

  → computer-guided **study design**, dedicated **normalization** methods,

  exploiting **prior knowledge** from molecular networks and pathways

- **PathVar** uses "pathway activity fingerprints" derived from omics data and known pathway definitions to build robust diagnostic machine learning models

- **GenePEN** discovers discriminative sub-networks for diagnostic sample classification and enables an interpretation of disease-associated molecular alterations at the network level

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