Functional Characterization of Molecular Interaction Networks

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Outline

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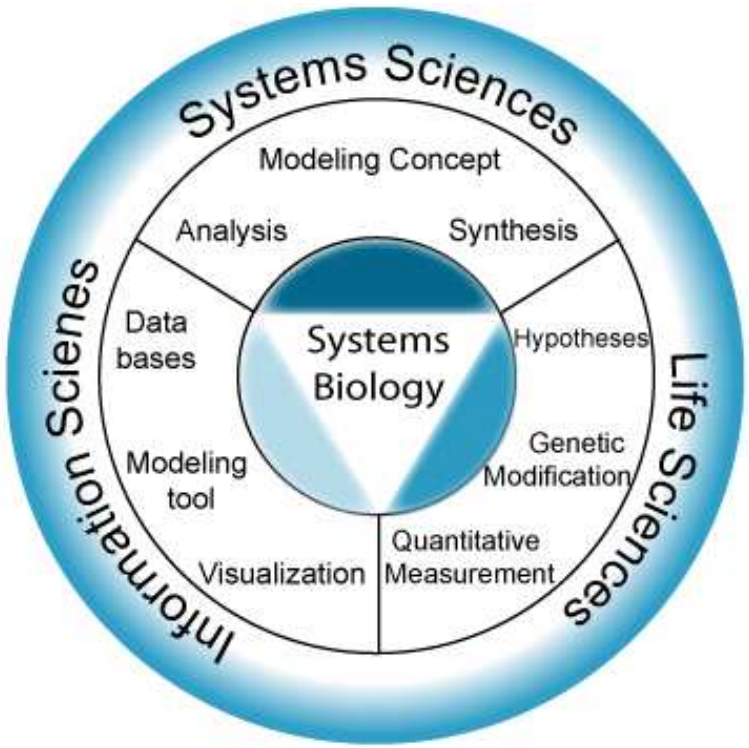
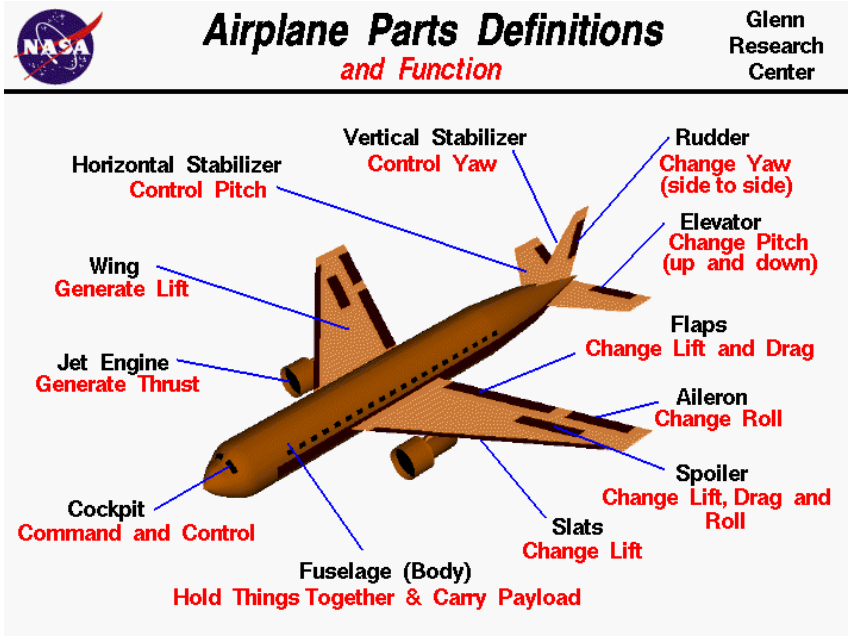
Systems Biology

- Life is an emergent property
  - "To understand biology at the system level, we must examine the structure and dynamics of cellular and organismal function, rather than the characteristics of isolated parts of a cell or organism." (Kitano, *Science*, 2002)
- Systems biology complements molecular biology



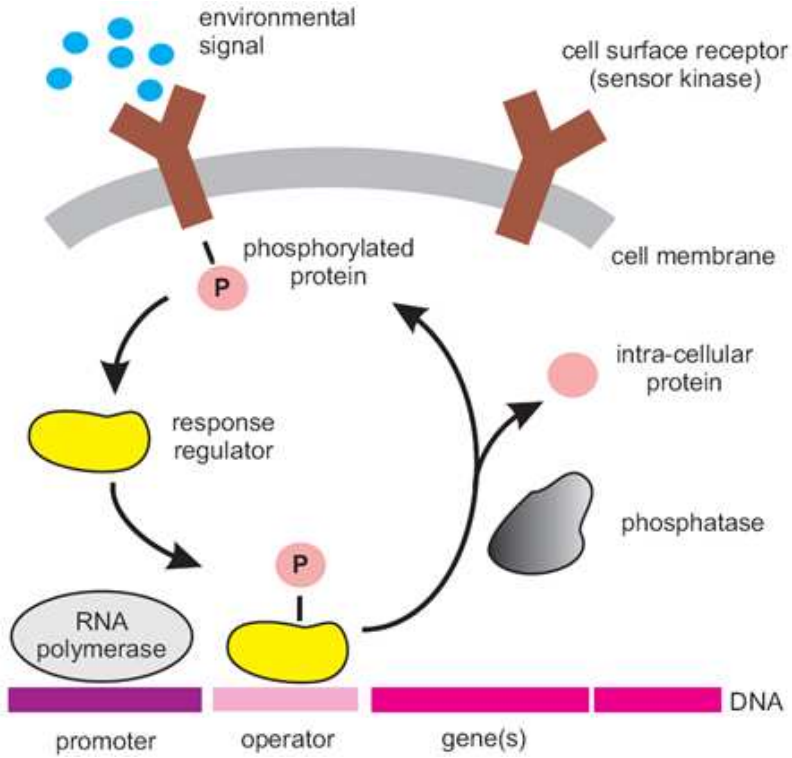
Organization & Dynamics of Systems



- Understanding how an airplane (cell) works
  - Listing parts (genes, proteins)
  - Understanding how parts are connected (interactions)
  - Characterizing the electrical and mechanical dynamics (cellular dynamics)

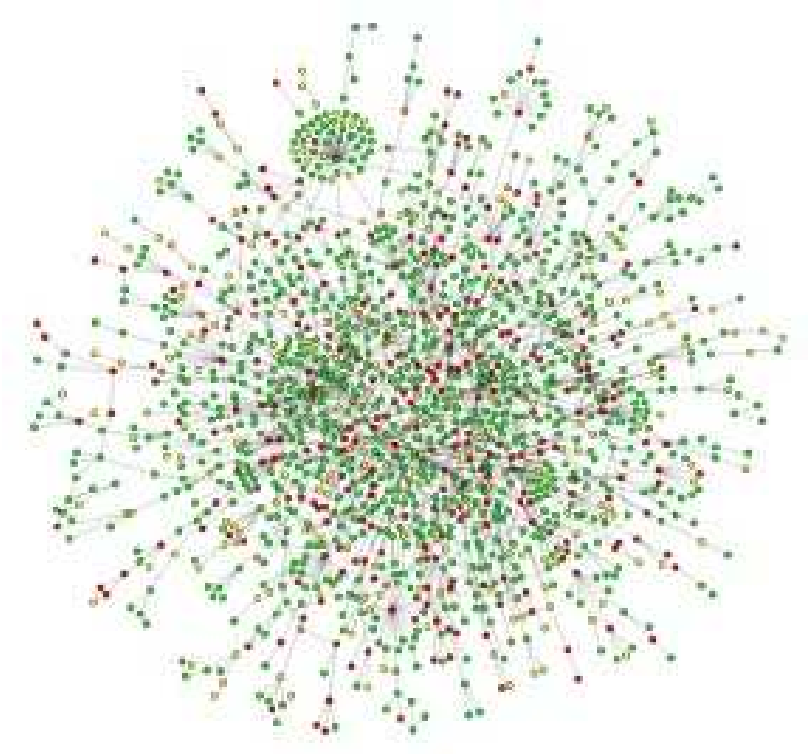
Molecular Interactions

- Regulation of molecular activity
  - Transcriptional regulation: Which genes will be expressed?
  - Post-transcriptional regulation & signaling: Phosphorylation, degradation, transport...
- Cooperation between molecules
  - Protein complexes: Macromolecular machines



Modeling Molecular Interactions: Networks

- High level description of cellular organization
- Nodes represent cellular components
  - Protein, gene, enzyme, metabolite
- Edges represent interactions
  - Binding, regulation, modification, complex membership, substrate-product relationship

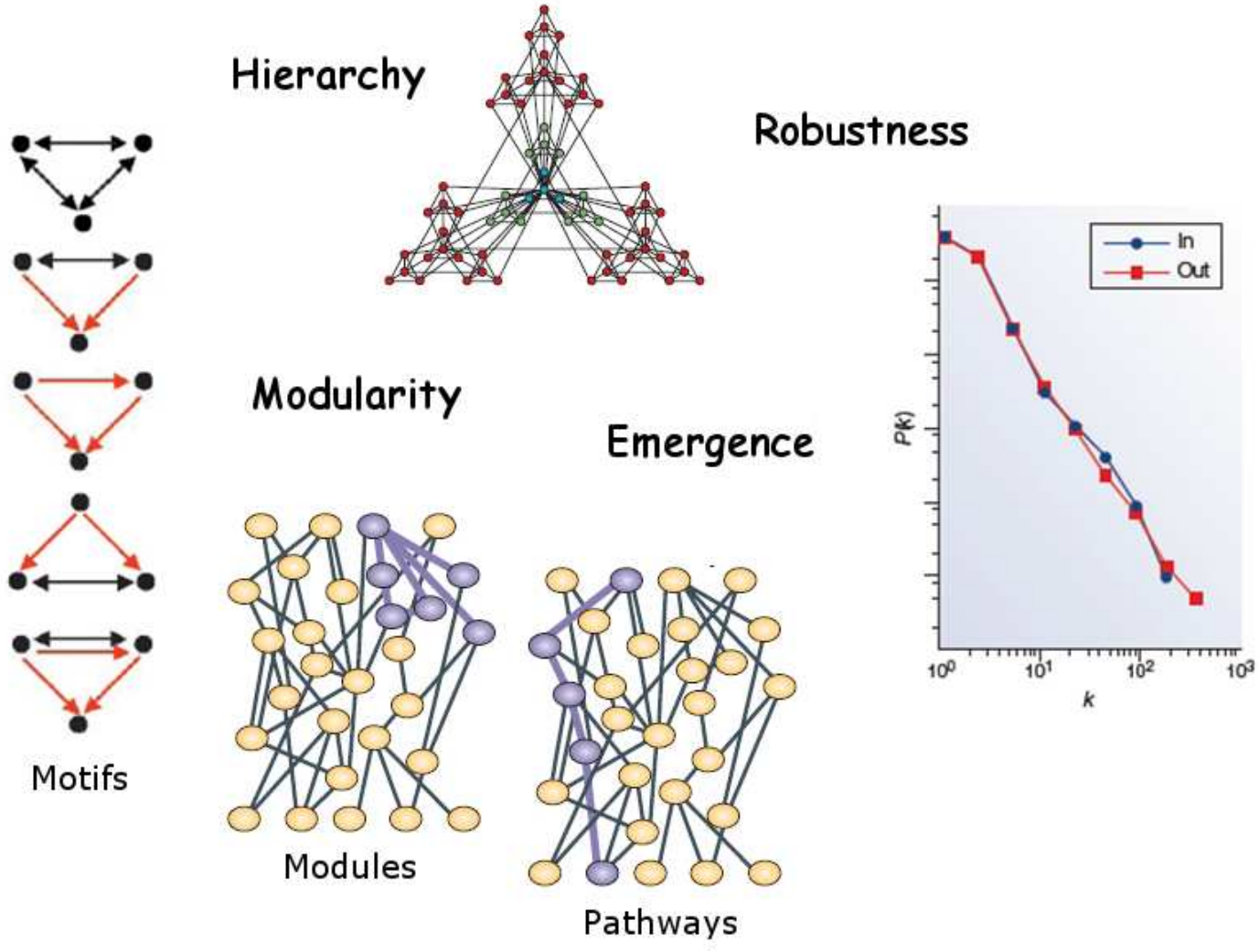


S.cerevisiae

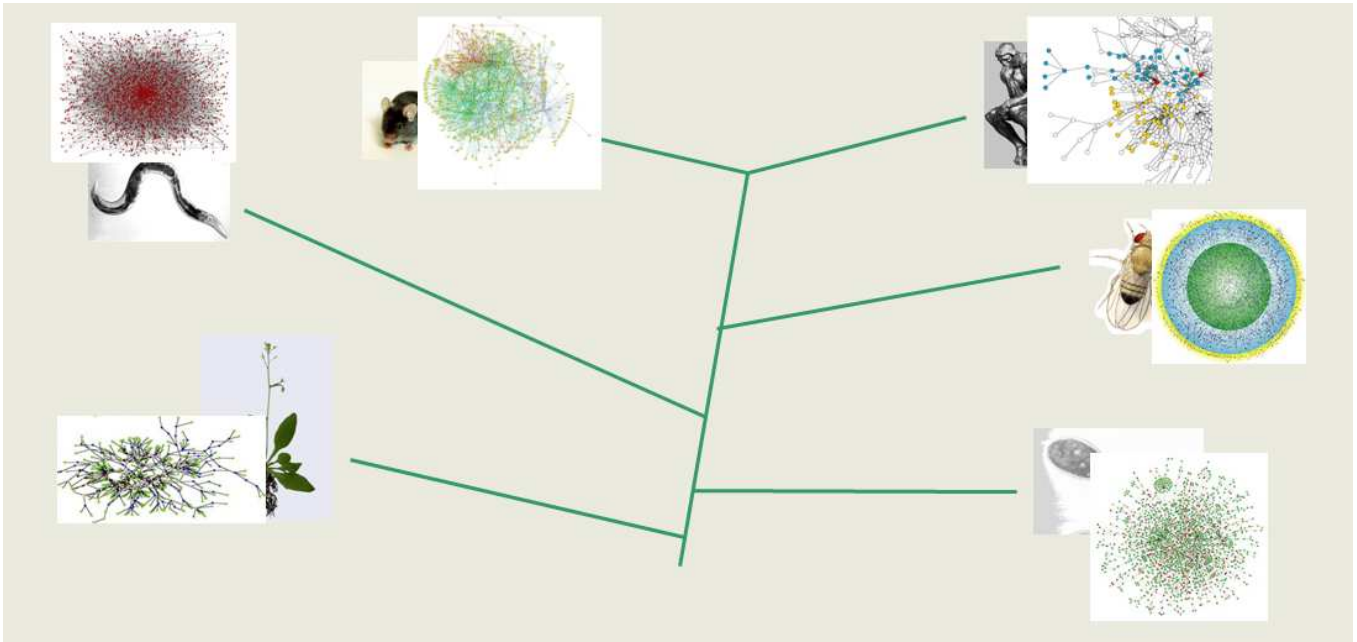
Protein-Protein Interaction (PPI) Network

Function & Topology in Molecular Networks

How does function relate to network topology?



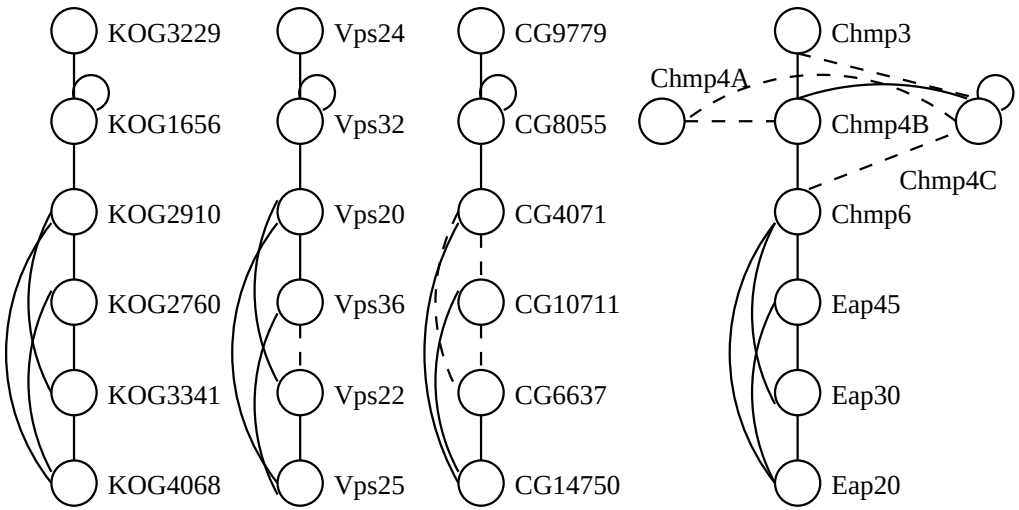
Comparative Network Analysis



- What is common to the networks that belong diverse species?
- Do conserved subgraphs correspond to functionally modular network components?

Frequent Protein Interaction Patterns

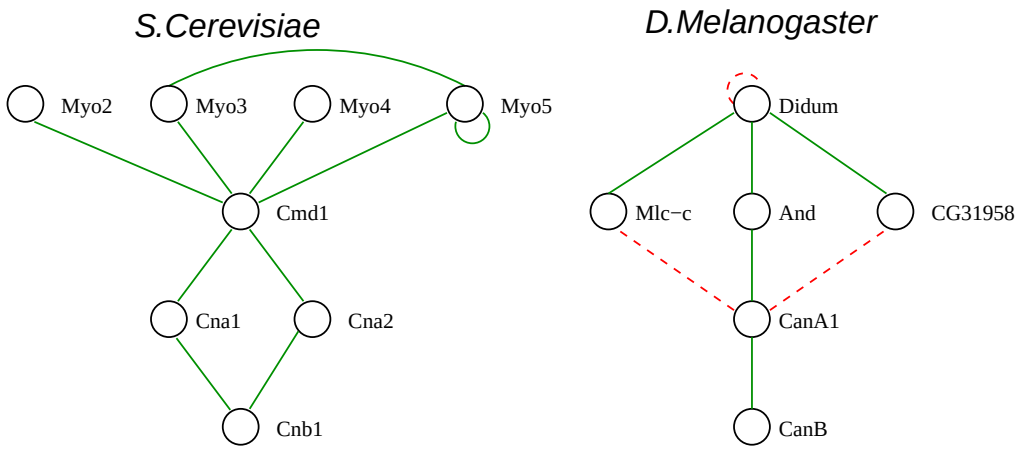
- Identification of frequent subgraphs in networks of multiple species
  - Graph mining: Networks with thousands of nodes
  - Our solution: Homolog contraction (Koyutürk et al., *ISMB*, 2004; Koyutürk et al., *JCB*, 2006)
  - Reduces graphs to sets, preserves frequent subgraphs



Conserved subgraph related to endosomal sorting

Pairwise Network Alignment

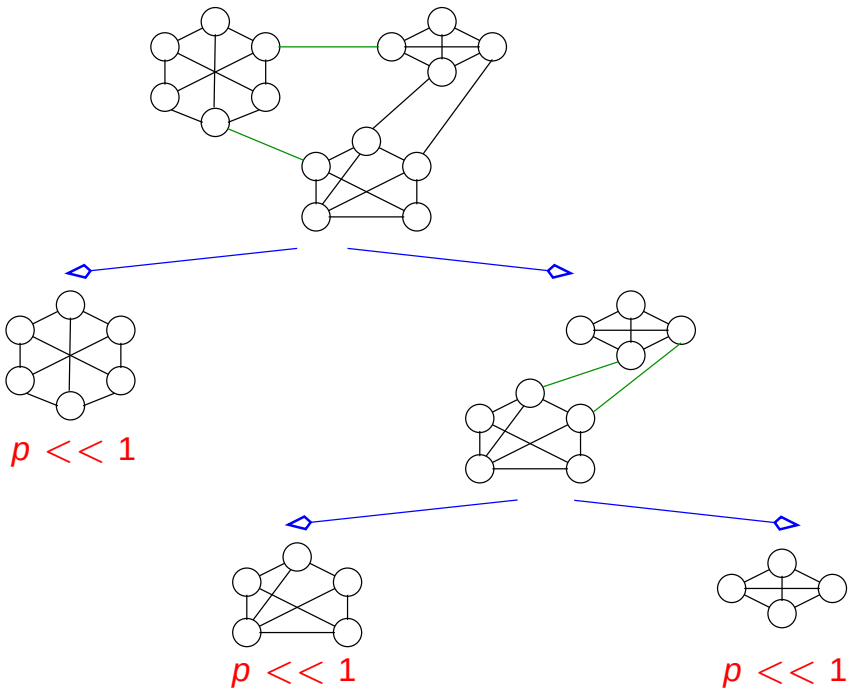
- Allowing approximate matches (evolution, noise in data)
  - Generalization of subgraph isomorphism, no well-defined matching between nodes
  - Our solution: Score evolutionary events, formulate problem as one of identifying heavy subgraphs (Koyutürk et al., *RECOMB*, 2005; Koyutürk et al., *JCB*, 2006)
  - Computationally very efficient



Calcium-dependent stress-activated signaling pathway

Assessing The Significance of Patterns

- Are the identified subgraphs statistically significant?
  - Most of the literature is based on Monte Carlo simulations
  - Our approach: Rigorously characterize the distribution of largest dense (conserved) subgraph based on random graph models (Koyutürk et al., *RECOMB*, 2006; Koyutürk et al., *JCB*, 2007)



SIDES

(Uses statistical significance as stopping criterion)

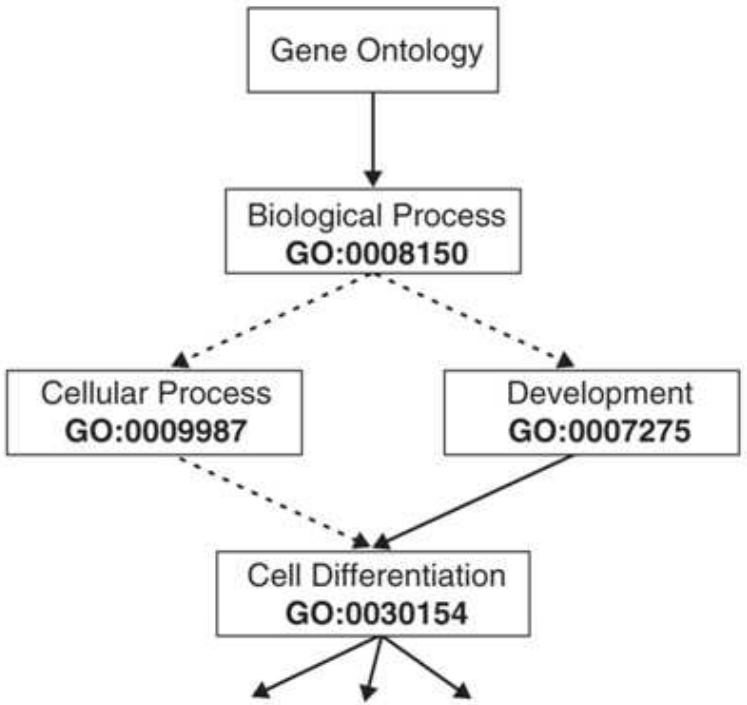
In This Talk

1. Recurrent functional interaction patterns
   - Crosstalk between different processes
   - "Periodic table of systems biology"
2. Functional coherence with respect to different types of interaction
   - What does proximity mean in domain-domain interaction networks?
   - Assessing functional similarity between two molecules
3. Where are we going with all these?
   - Using network proximity to identify implicated genes in human colorectal cancer

Outline

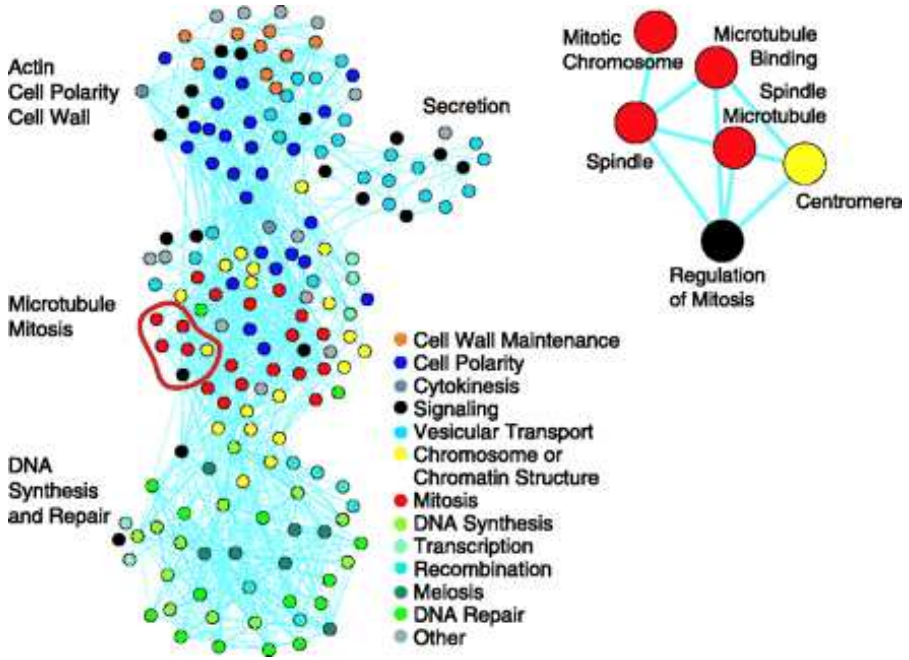
Characterizing Molecular Function: Ontologies

- Significant progress on standardizing knowledge on biological function at the molecular level
  - Protein/domain families (COG, PFAM, ADDA)
- Gene Ontology
  - A controlled vocabulary of molecular functions, biological processes, and cellular components



Functional Annotation: From Molecules to Systems

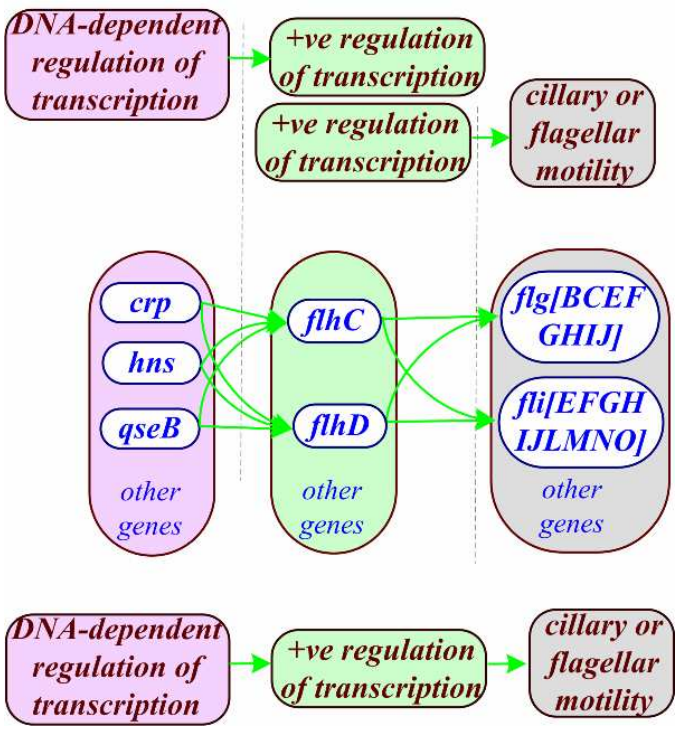
- Networks are species-specific
- Functional ontologies are described at the molecular level
- Can we map networks from gene space to an abstract (and unified) function space?

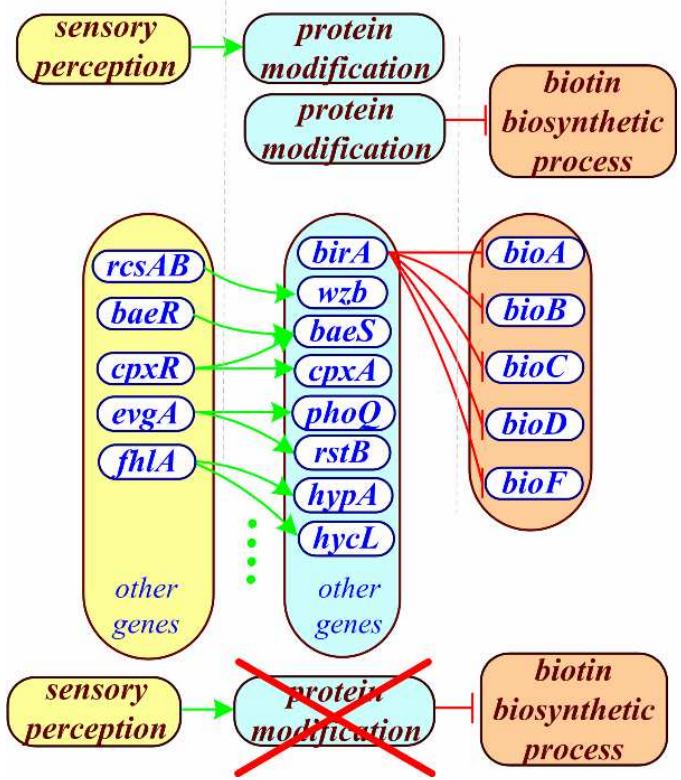


Network of GO terms based on significance of pairwise interactions in *S. cerevisiae* Synthetic Gene Array (SGA) network (Tong *et al.*, *Science*, 2004)

Gene Regulatory Networks: Indirect Regulation

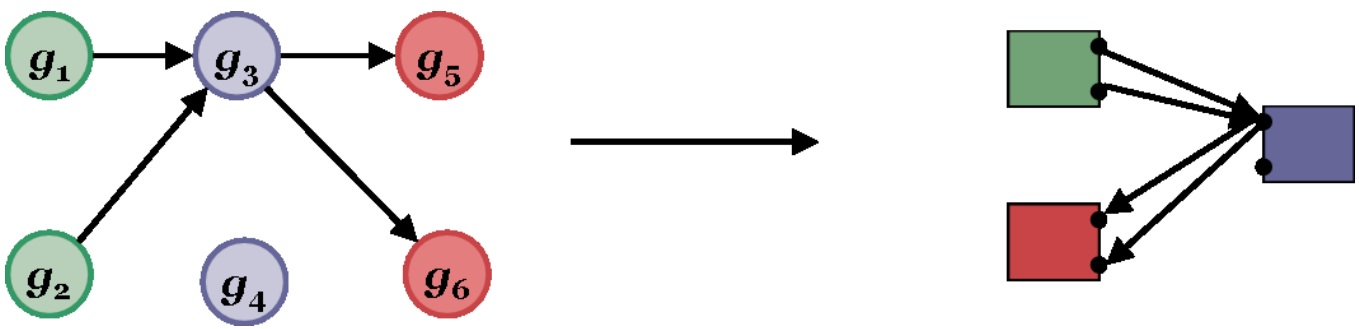
- Assessment of pairwise interactions is simple, but not adequate





Functional Attribute Networks

- Multigraph model
  - A gene is associated with multiple functional attributes
  - A functional attribute is associated with multiple genes
  - Functional attributes are represented by nodes
  - Genes are represented by ports, reflecting context

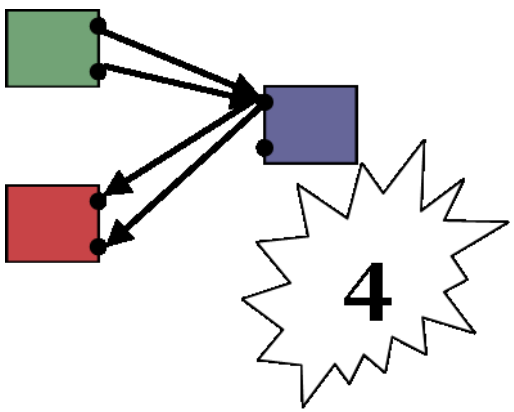


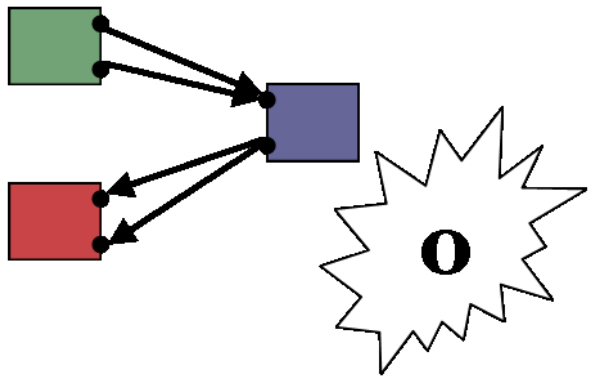
Gene Network

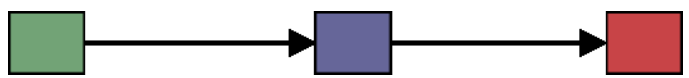
Functional Attribute Network

Frequency of a Multipath

- A pathway of functional attributes occurs in various contexts in the gene network
  - Multipath in the functional attribute network





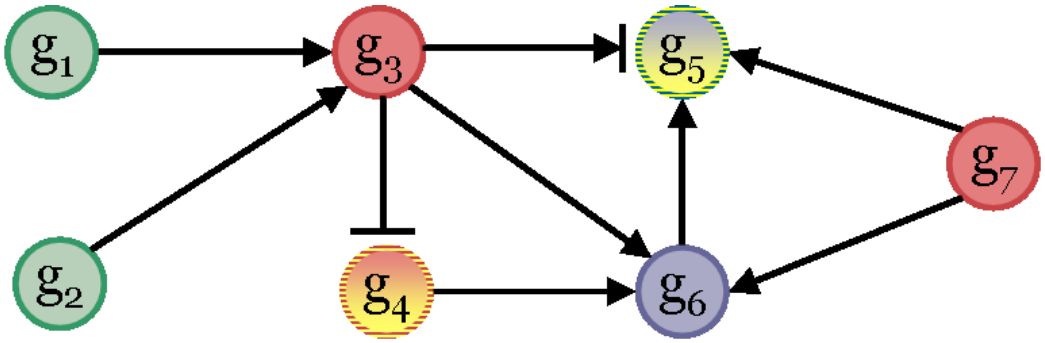
Frequency of Multipath  ?

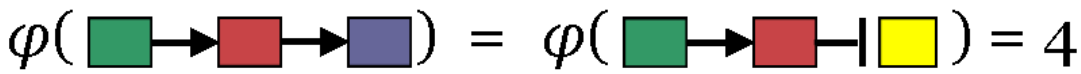
Frequency *vs.* Statistical Significance

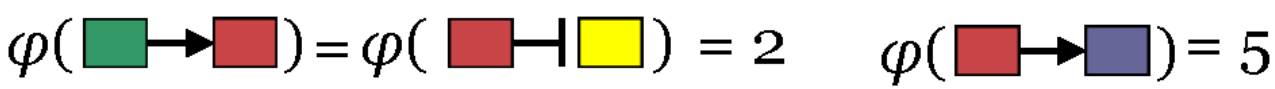
- We want to identify overrepresented pathways
  - These might correspond to modular pathways
- Frequency alone is not a good measure of statistical significance
  - The distribution of functional attributes among genes is not uniform
  - The degree distribution in the gene network is highly skewed
  - Pathways that contain common functional attributes have high frequency, but they are not necessarily interesting

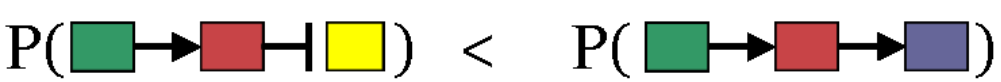
Statistical Significance of a Pathway

- Emphasize modularity of pathways (Pandey, Koyutürk *et al.*, *ISMB*, 2007)
  - Condition on frequency of building blocks
  - Evaluate the significance of the coupling of building blocks









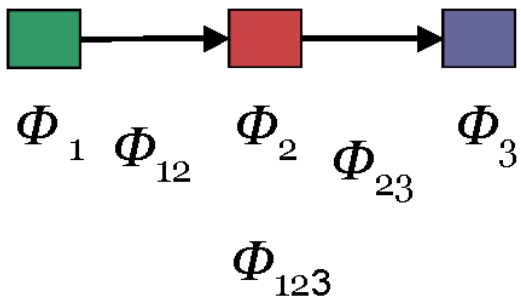
Significance of Pairwise Interactions

- A single regulatory interaction is the shortest pathway
  - Arbitrary degree distribution: The number of edges leaving and entering each functional attribute is specified
  - Edges are assumed to be independent
- The frequency of a regulatory interaction is a hypergeometric random variable
- $p_{ij} = P(\Phi_{ij} \geq \phi_{ij} | \mathcal{B}) = \sum_{\ell=\phi_{ij}}^{\min\{\beta_i \delta_j, n\}} \frac{\binom{\beta_i \delta_j}{\ell} \binom{m-\beta_i \delta_j}{n-\ell}}{\binom{m}{n}}.$
  - β_i = in-degree and δ_i = out-degree
  - m = pool of potential edges, n = number of edges in network

Significance of a Pathway

- We denote each frequency random variable by ϕ, their observed value by φ

π_{123}:



- Significance of pathway π_{123} (p_{123}) is defined as

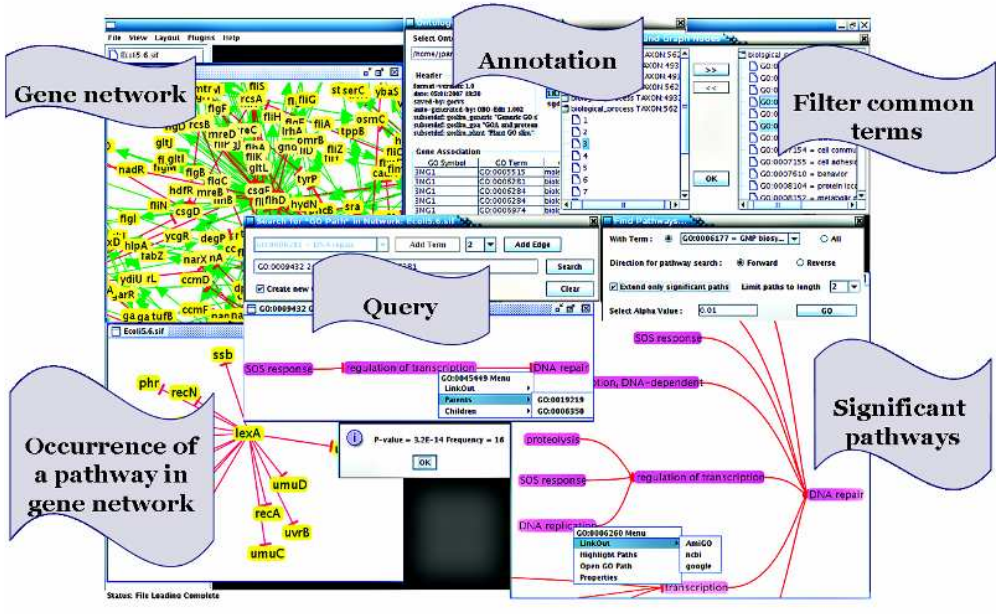
$$P(\phi_{123} \geq \varphi_{123} | \phi_{12} = \varphi_{12}, \phi_{23} = \varphi_{23}, \phi_1 = \varphi_1, \phi_2 = \varphi_2, \phi_3 = \varphi_3)$$

Computing Significance

- Assume that interactions are independent
  - There are $\varphi_{12}\varphi_{23}$ possible pairs of π_{12} and π_{23} edges
  - The probability that a pair of π_{12} and π_{23} edges go through the same gene (corresponds to an occurrence of π_{123}) is $1/\varphi_2$
- The probability that at least φ_{123} of these pairs go through the same gene can be bounded by
  - $p_{123} \leq exp(\varphi_{12}\varphi_{23}H_q(t))$ where $q = 1/\varphi_2$ and $t = \varphi_{123}/\varphi_{12}\varphi_{23}$
  - $H_q(t) = t\log(q/t) + (1-t)\log((1-q)/(1-t))$ is divergence
  - Bonferroni-corrected for multiple testing (adjusted by $\prod_{j=1}^{k} |\cup_{g_\ell \in T_{i_j}} \mathcal{F}(g_\ell)|$)

NARADA

- A software for identification of significant pathways (Pandey, Koyutürk *et al.*, *PSB*, 2008)
  - Given functional attribute T, find all significant pathways that originate (terminate) at T
  - User can explore back and forth between the gene network and the functional attribute network



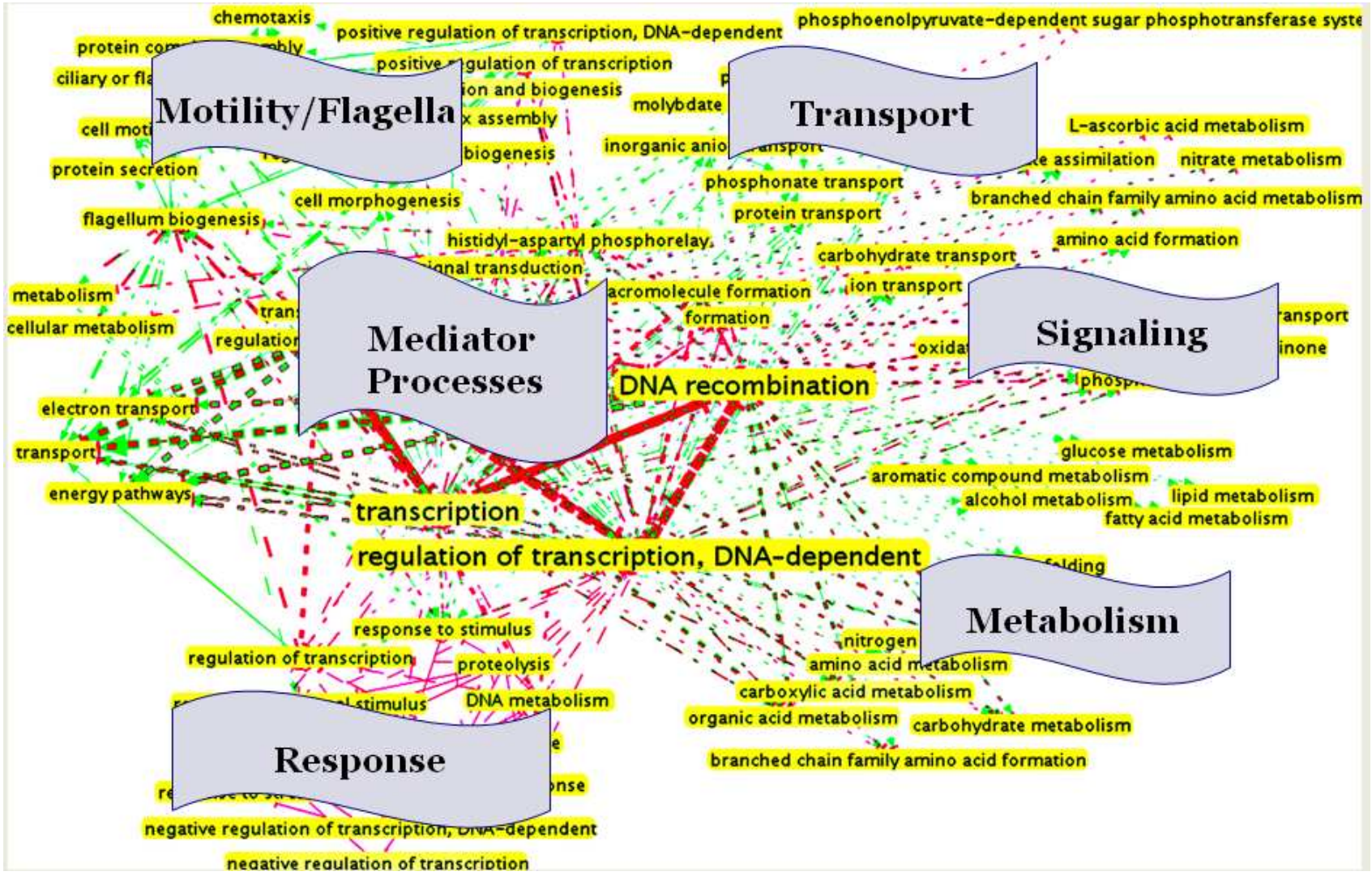
Significant Regulatory Pathways in Bacteria

- We use NARADA to identify significant pathways in the transcriptional networks of two bacterial species
  - *E. coli*: 1364 genes, 3159 regulatory interactions (RegulonDB)
  - *B. subtilis*: 562 genes, 604 regulatory interactions (DBTBS)

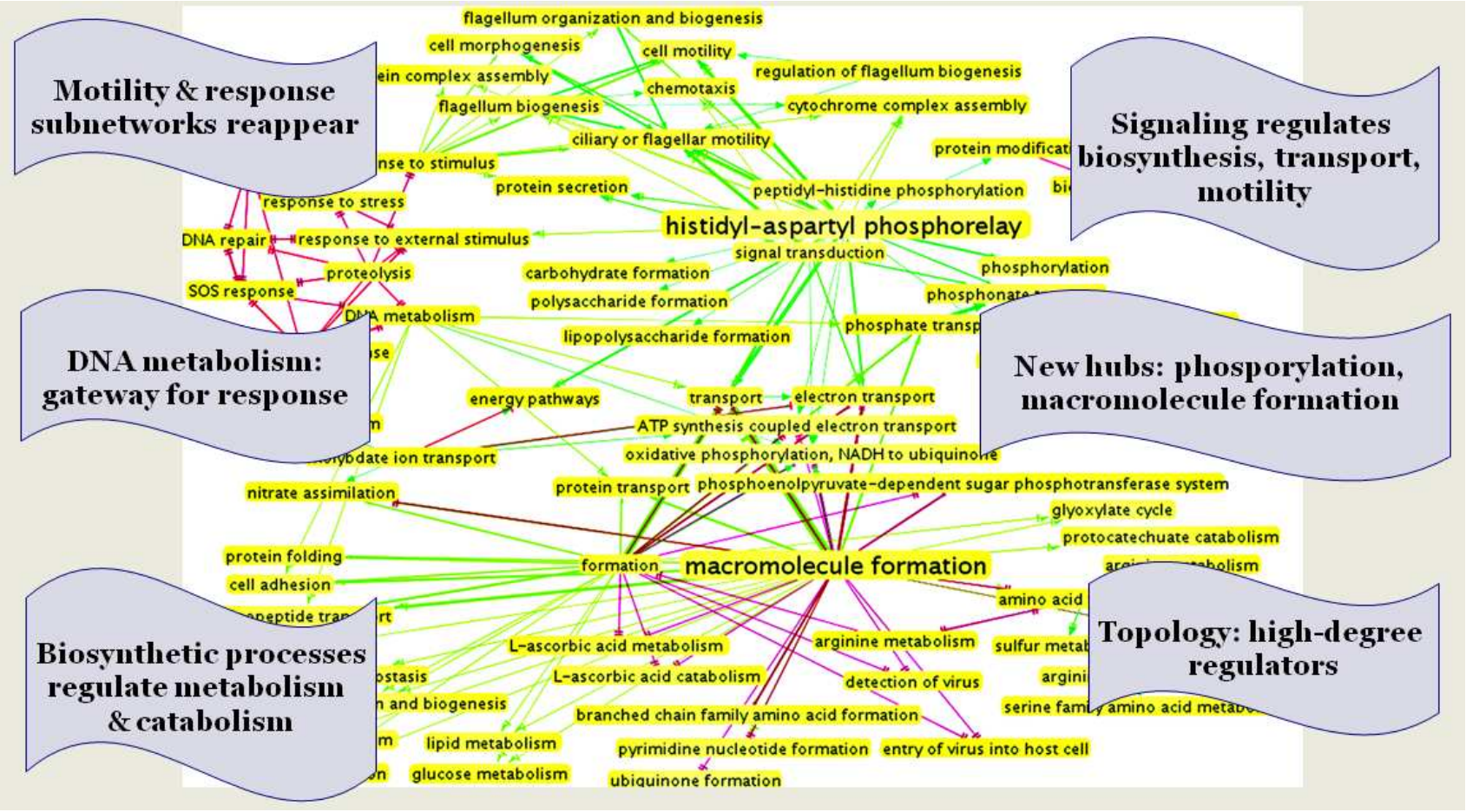
Strongly significant pathways ($p < 0.01$)

Pathway length	2	3	4
E. coli	106	1436	5250
B. subtilis	39	111	524
Common	22	67	365
Expected	5	8	26

Functional View of *E. coli* Regulatory Network



Short-Circuiting Mediator Processes



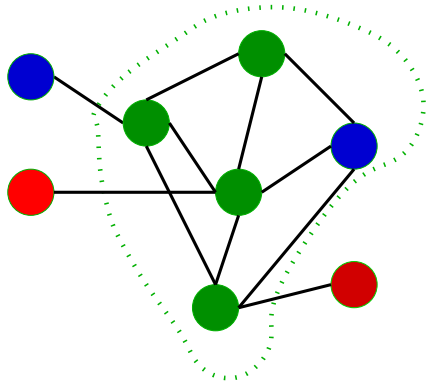
Applications

- Projecting from functional space back to molecular space
  - Pattern-based functional annotation (Kirac *et al.*, *RECOMB*, 2008)
  - Pathway identification through cross-species projection (Cakmak *et al.*, *Bioinformatics*, 2008)
- Ongoing work: Interaction prediction
  - Identify significant functional pathways in *E. coli* transcriptional network
  - Find (partial) occurrences of these pathways in the *B.subtilis* transcriptional network
  - "Interpolate" these pathways to predict novel interactions

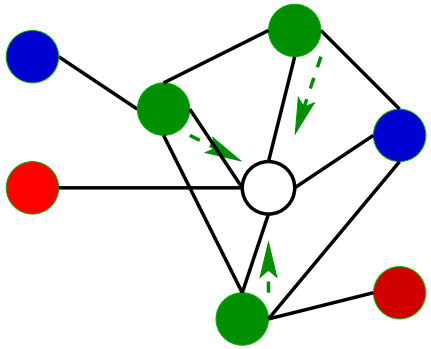
Outline

Functional Coherence in Networks

- Modularity manifests itself in terms of high connectivity in the network
  - Identification of modular subgraphs
  - Functional annotation of a group of molecules

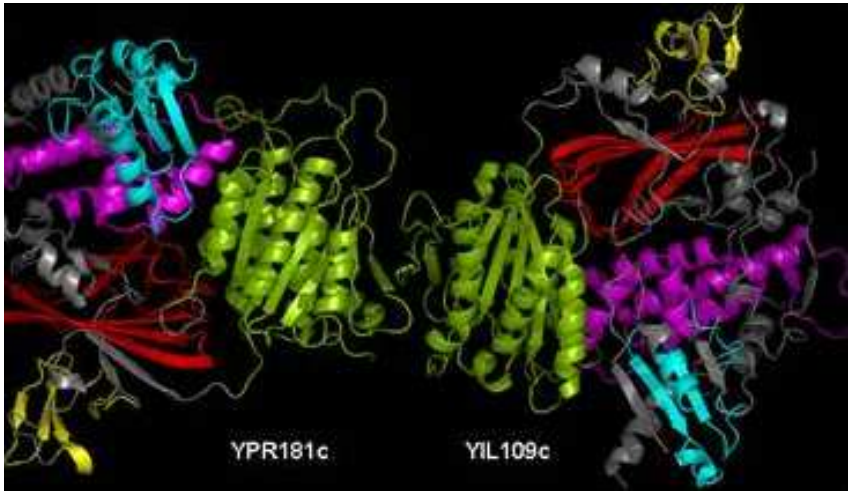


- Functional association (similarity) is correlated with network proximity
  - Network based functional annotation
  - Identification of multiple disease markers



Domain-Domain Interactions

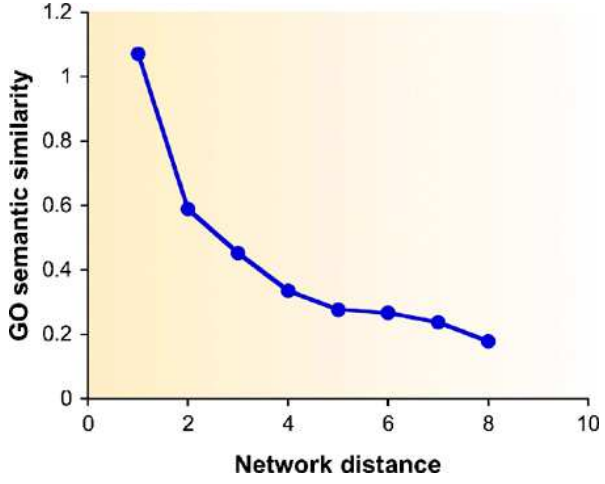
- Most proteins are composed of multiple domains
- Many domains are reused in several (evolutionarily/functionally related) proteins
- Interactions between domains underlie observed protein-protein interactions
- Many algorithms exist to infer domain-domain interactions



Jothi *et al.*, *JMB*, 2006

PPI Networks *vs.* DDI Networks

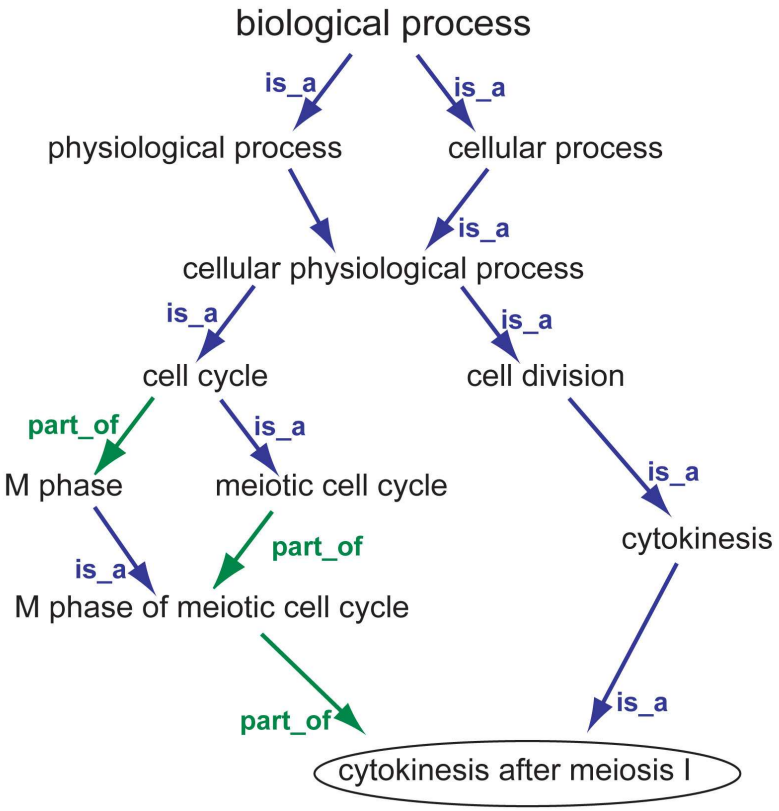
- Protein-protein interaction (PPI) networks are used extensively for functional inference
  - Network-based functional annotation
  - Identification of functional modules
- In PPI networks, functional coherence manifests itself in terms of network proximity
  - How about DDI "networks"?



Sharan *et al.*, *MSB*, 2007

Assessing Functional Similarity

- Gene Ontology (GO) provides a hierarchical taxonomy of biological function
- Assessment of semantic similarity between concepts in a hierarchical taxonomy is well studied (Resnik, *IJCAI*, 1995)



Semantic Similarity of GO Terms

- Resnik's measure based on information content:

$$I(c) = -\log_2(|G_c|/|G_r|)$$

$$\delta_I(c_i, c_j) = \max_{c \in A_i \cap A_j} I(c)$$

  - G_c: Set of molecules that are associated with term c
  - r: Root term
  - A_i: Ancestors of term C_i in the hierarchy
  - $\lambda(c_i, c_j) = \text{argmax}_{c \in A_i \cap A_j} I(c)$: Lowest common ancestor of c_i and c_j

Functional Similarity of Molecules

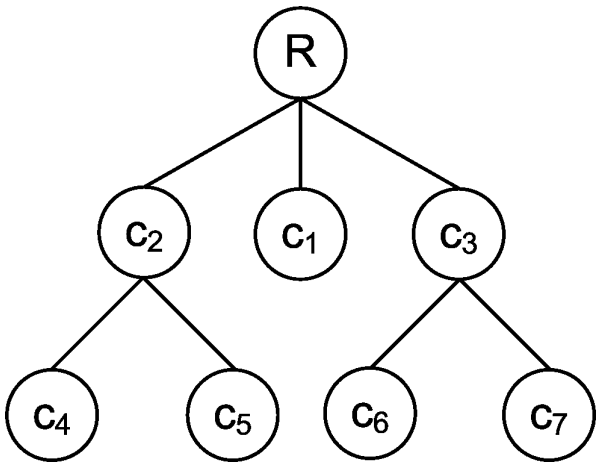
- Each molecule (protein or domain) is associated with multiple GO terms
- Available annotations are incomplete
- Domain annotations are often derived from protein annotations
  - A domain is associated with terms at the intersection of proteins that contain the domain
- Is it possible to compare functional similarity between domains and functional similarity between proteins at all?

Properties of Admissible Measures

What are the basic required properties of an admissible measure of similarity between two sets?

1. Symmetry: $\rho(S_i, S_j) = \rho(S_j, S_i)$ for all S_i, S_j
2. Consistency: $\rho(S_i, S_j) \leq \rho(S_j, S_j)$ for all S_i, S_j
3. Monotonicity: $\rho(S_i, S_j) \leq \rho(S_i \cup c_k, S_j \cup c_k)$
4. Generality: $\rho(S_i, S_j) \leq \rho(S_i, S_j \cup S_k)$ for all S_i, S_j, S_k
   - Incompleteness-aware measures: No conclusions based on negative evidence!

Illustration of Properties



$$S_1 = \{c_4\}$$

$$S_2 = \{c_7\}$$

$$S_3 = \{c_6\}$$

$$S_4 = \{c_4, c_6\}$$

$$S_5 = \{c_6, c_7\}$$

- Monotonicity:

  $\rho(S_1, S_2) \leq \rho(S_4, S_5)$

- Generality:

  $\rho(S_2, S_3) \leq \rho(S_2, S_4)$

Existing Measures are not Admissible

- Average (Lord *et al.*, *Bioinformatics*, 2003)

$$\rho_A(S_i, S_j) = \frac{1}{|S_i||S_j|} \sum_{c_k \in S_i} \sum_{c_l \in S_j} \delta(c_k, c_l)$$

  - Fails consistency, monotonicity, generality

- Maximum (Sevilla *et al.*, *IEEE TCBB*, 2005)

$$\rho_M(S_i, S_j) = \max_{c_k \in S_i, c_l \in S_j} \delta(c_k, c_l)$$

  - Principle: Similarity in a single pair of terms is sufficient
  - Fails monotonicity

Existing Measures are not Admissible

- Average of Maxima (Schlicker *et al.*, *Bioinformatics*, 2007)

$$\rho_H(S_i, S_j) = \max\left\{ \frac{1}{|S_i|} \sum_{c_k \in S_i} \max_{c_l \in S_j} \delta(c_k, c_l), \frac{1}{|S_j|} \sum_{c_l \in S_j} \max_{c_k \in S_i} \delta(c_k, c_l) \right\}$$

  - Principle: Similarity with a single term is sufficient for each term
  - Fails consistency, monotonicity, generality

Information Content Based Set Similarity

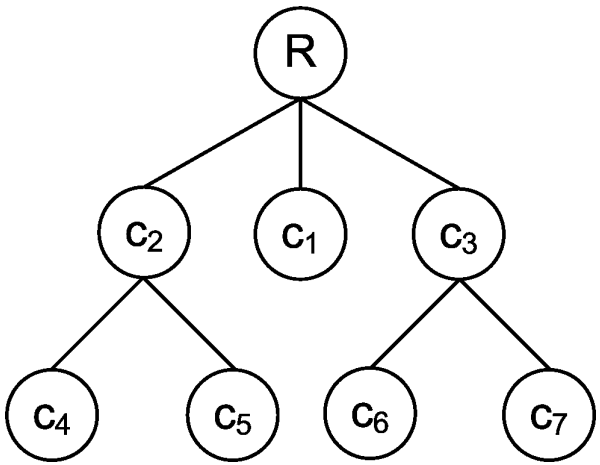
- Generalize the concept of lowest common ancestor to sets of terms (Pandey, Koyutürk *et al.*, *ECCB*, 2008)

$$\Lambda(S_i, S_j) = \bigsqcup_{c_k \in S_i, c_l \in S_j} \lambda(c_k, c_l)$$

$$\rho_I(S_i, S_j) = I(\Lambda(S_i, S_j)) = -\log_2 \left(\frac{|G_{\Lambda(S_i,S_j)}|}{|G_r|} \right)$$

  - $G_{\Lambda(S_i,S_j)} = \bigcap_{c_k \in \Lambda(S_i,S_j)} G_{c_k}$ is the set of molecules that are associated with all terms in the MCA set

Illustration of Information Content Based Measure



$$S_1 = \{c_4, c_6, c_7\}$$

$$S_2 = \{c_4\}$$

$$S_3 = \{c_4, c_6\}$$

$$S_4 = \{c_6, c_7\}$$

$$S_5 = \{c_4, c_3\}$$

- $\lambda(c_4, c_4) = c_4,$
  $\lambda(c_6, c_4) = \lambda(c_7, c_4) = R$
- $\Lambda(S_1, S_2) = \{c_4\} \Rightarrow$
  $\rho_I(S_1, S_2) =$
  $-\log_2(|G_{c_4}|/|G_R|) =$
  $\log_2(5/4)$
- $\Lambda(S_1, S_3) = \{c_4, c_6\} \Rightarrow$
  $\rho_I(S_1, S_3) = \log_2(5/2)$

Information Content Based Measure Is Admissible

1. Symmetry: Trivially, $\rho_I(S_i, S_j) = \rho_I(S_j, S_i)$ for all S_i, S_j.
2. Consistency: Clearly, $c_k \preceq \lambda(c_k, c_l)$ for any c_k, c_l. Now consider any $c_m \in \Lambda(S_i, S_j)$. Since $c_m = \lambda(c_k, c_l)$ for some $c_k \in S_i$ and $c_l \in S_j$, there always exists $c_n \in \Lambda(S_i, S_i)$ such that $c_n \preceq c_k \preceq c_m$. Consequently, we must have $G_{\Lambda(S_i,S_i)} \subseteq G_{\Lambda(S_i,S_j)}$, leading to $\rho_I(S_i, S_j) \leq \rho_I(S_i, S_i)$.
3. Monotonicity: Since $c_k \nsim c_n$ for all $c_n \in S_i \cup S_j$, we have

   $\Lambda(S_i \cup c_k, S_j \cup c_k) = \Lambda(S_i, S_j) \sqcup \Lambda(S_i \sqcup S_j, \{c_k\}) \sqcup \{c_k\} \supseteq \Lambda(S_i, S_j) \cup \{c_k\}$,

   leading to $G_{\Lambda(S_i \cup c_k, S_j \cup c_k)} \subseteq G_{\Lambda(S_i,S_j)}$ and $|G_{\Lambda(S_i \cup c_k, S_j \cup c_k)}| \leq |G_{\Lambda(S_i,S_j)}|$.

   Consequently, $\rho_I(S_i \cup c_k, S_j \cup c_k) \geq \rho_I(S_i, S_j)$.
4. Generality:

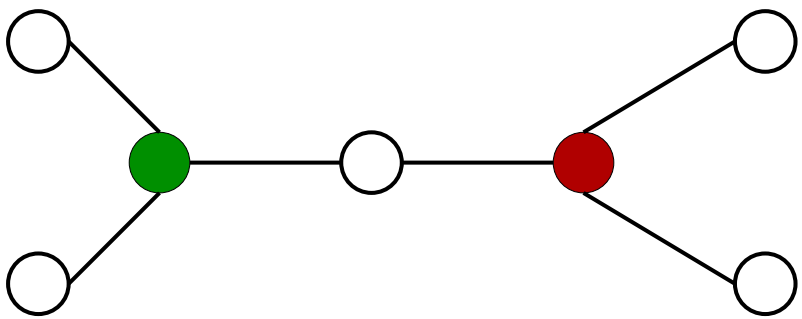
   $\Lambda(S_i, S_j \cup S_k) = \Lambda(S_i, S_j) \sqcup \Lambda(S_i, S_k) \sqsupseteq \Lambda(S_i, S_j)$.

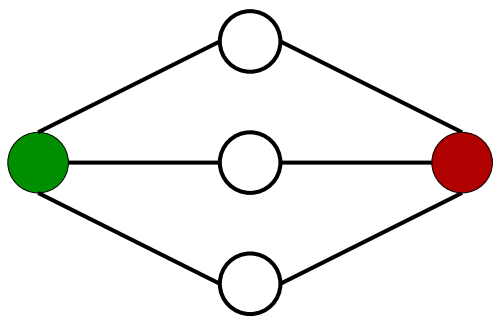
   Therefore, $G_{\Lambda(S_i, S_j \cup S_k)} \subseteq G_{\Lambda(S_i,S_j)}$, leading to

   $\rho_I(S_i, S_j \cup S_k) \geq \rho_I(S_i, S_j)$.

Accounting for Multiple Paths

- Is "shortest path" a good measure of network proximity?
  - Multiple alternate paths might indicate stronger functional association
  - In well-studied pathways, redundancy is shown to play an important role in robustness & adaptation (*e.g.*, genetic buffering)





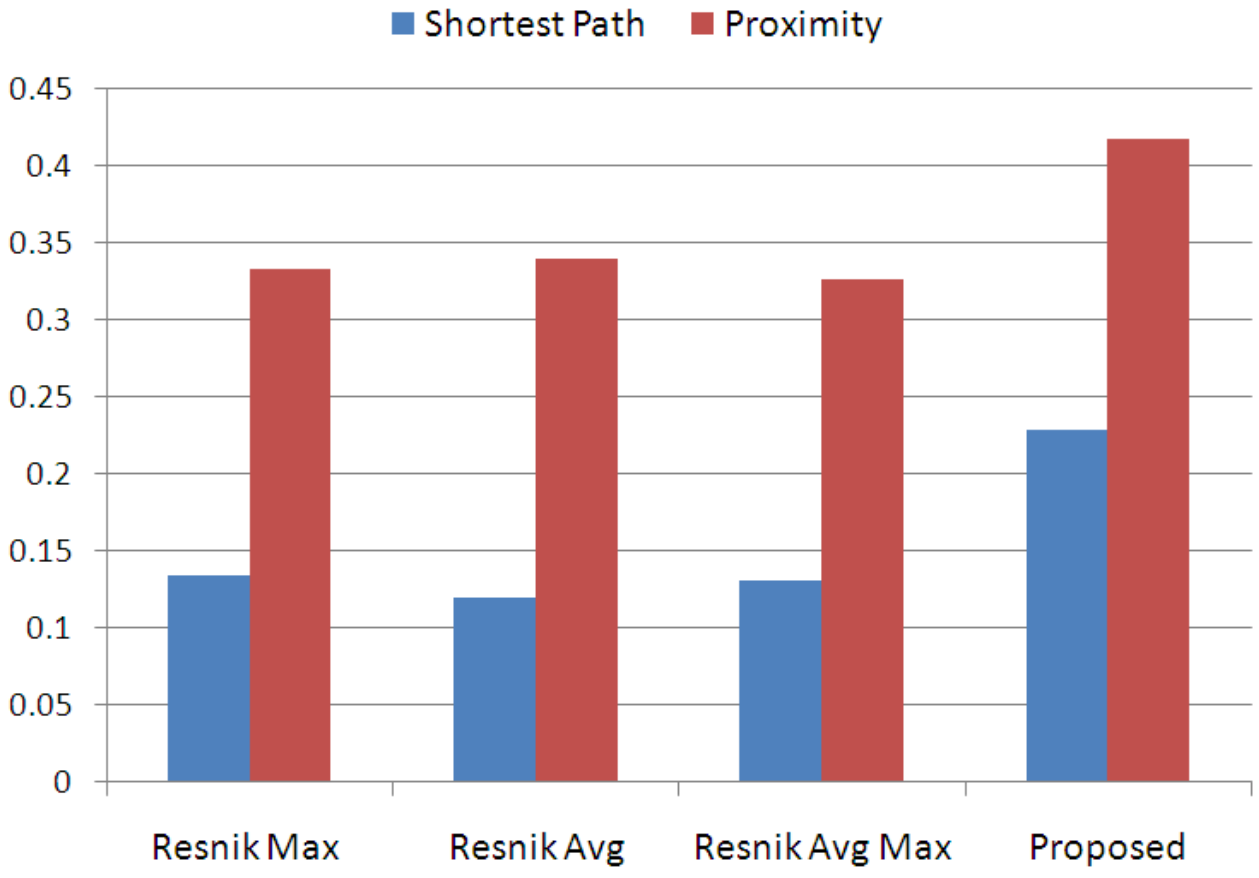
Proximity Based On Random Walks

- Simulate an infinite random walk with random restarts at protein i
- Proximity between proteins i and j is given by the relative amount of time spent at protein j

$$\Phi(0) = I,\ \Phi(t+1) = (1-c)A\Phi(t) + cI,\ \Phi = \lim_{t\to\infty} \Phi(t)$$

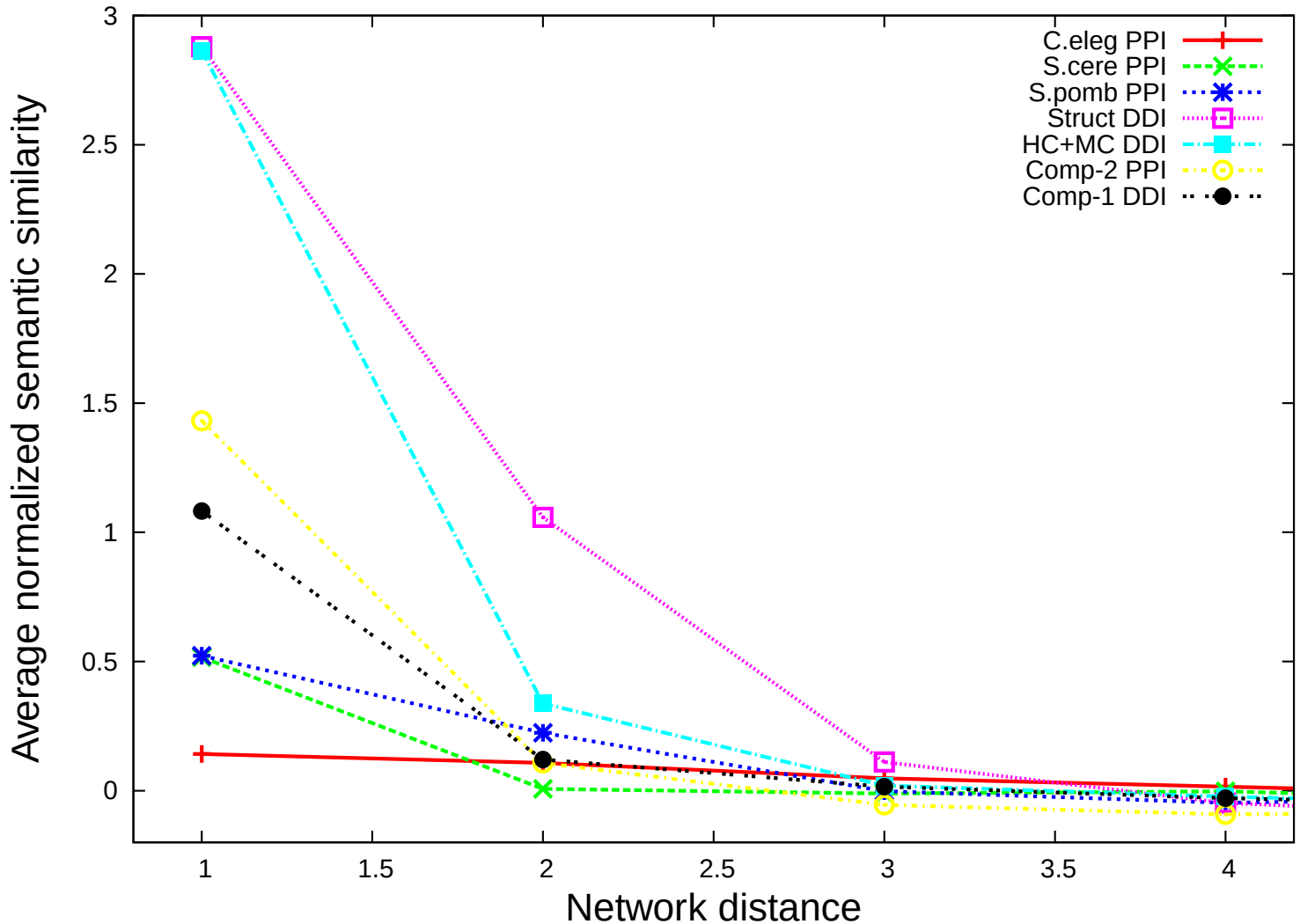
  - $\Phi(i,j)$: Network proximity between protein i and protein j
  - A: Stochastic matrix derived from the adjacency matrix of the network
  - I: Identity matrix
  - c: Restart probability

Network Proximity & Functional Similarity



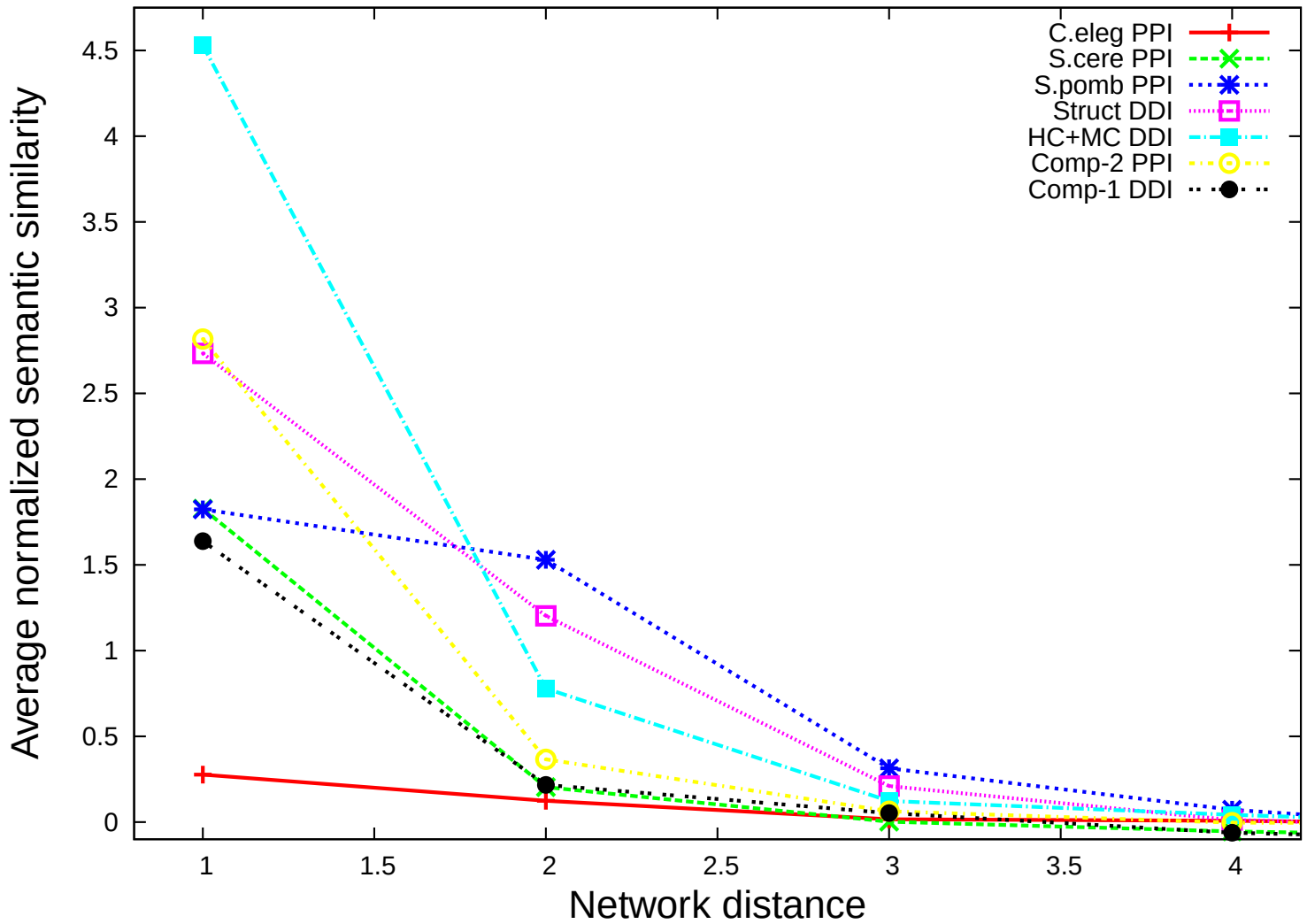
Correlation between functional similarity and network proximity on nine PPI and DDI networks

Comparison of PPI and DDI Networks



Network distance *vs.* functional similarity based on molecular functions

Comparison of PPI and DDI Networks

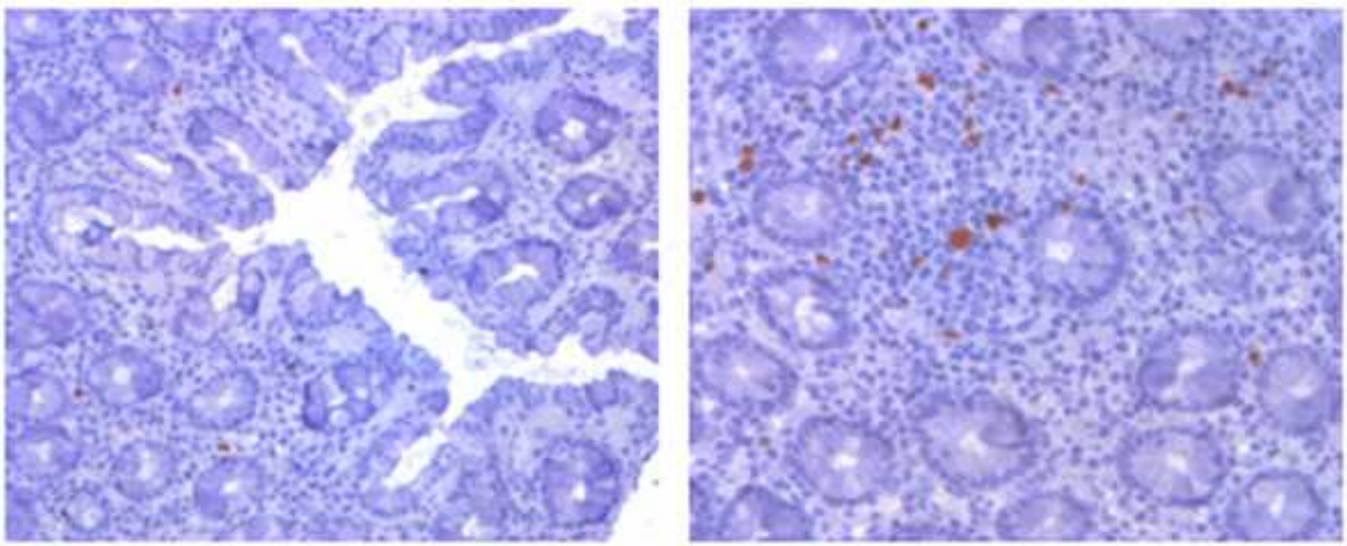


Network distance *vs.* functional similarity based on biological processes

Outline

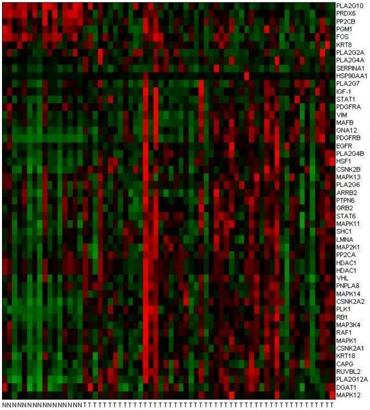
Proteomic Studies of Disease Markers

- Human colorectal cancer
  - One out of every 19 individuals will be diagnosed with this disease in their lifetime
  - We have to identify markers (for diagnosis), drug targets (for intervention), and mechanisms (for intelligent intervention)



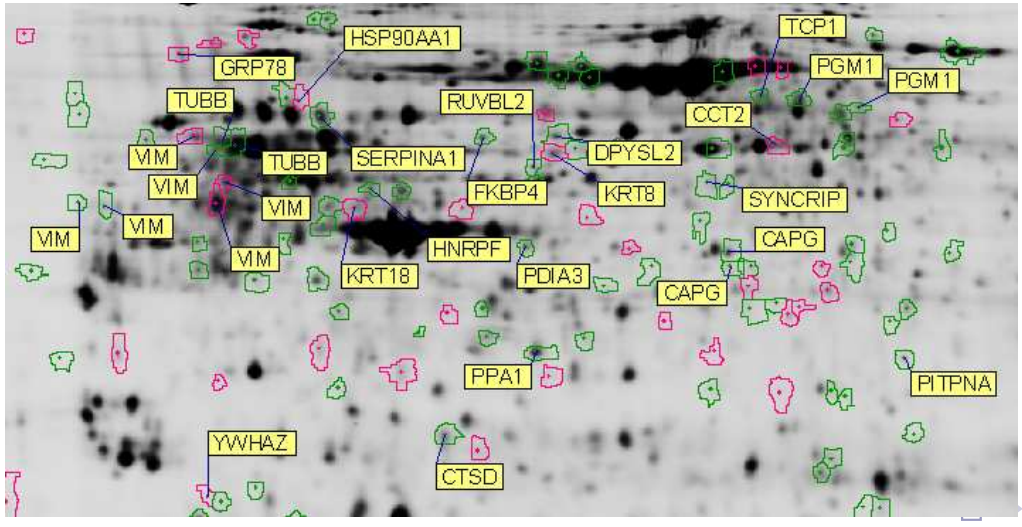
Traditional Approach

- Differential gene expression
  - Collect tissue samples from affected and control individuals
  - Measure mRNA expression for each gene, identify differentially expressed genes
- Problems
  - Many differentially expressed genes (driver *vs.* passenger genes)
  - mRNA expression captures activity to a limited extent
  - Weak signals may be lost



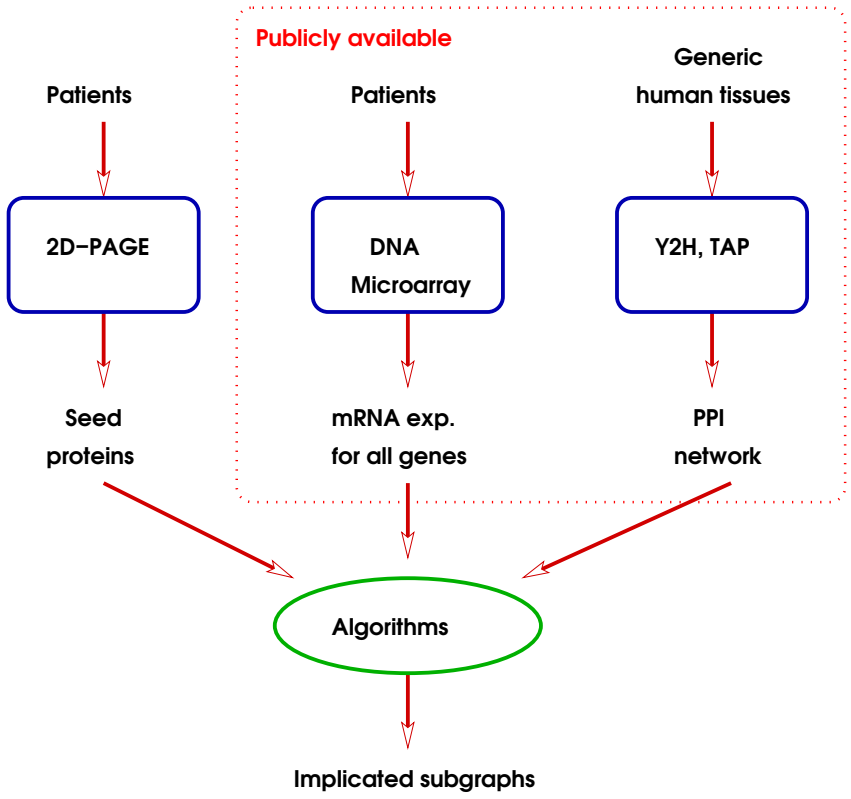
Incorporating Protein Expression

- Protein expression captures post-transcriptional activity better
- Can be measured using various screening techniques
  - 2D-PAGE, Mass Spectrometry
  - Significantly less coverage compared to mRNA expression
- Transcriptomic (mRNA expression) and proteomic (protein expression) data complement each other



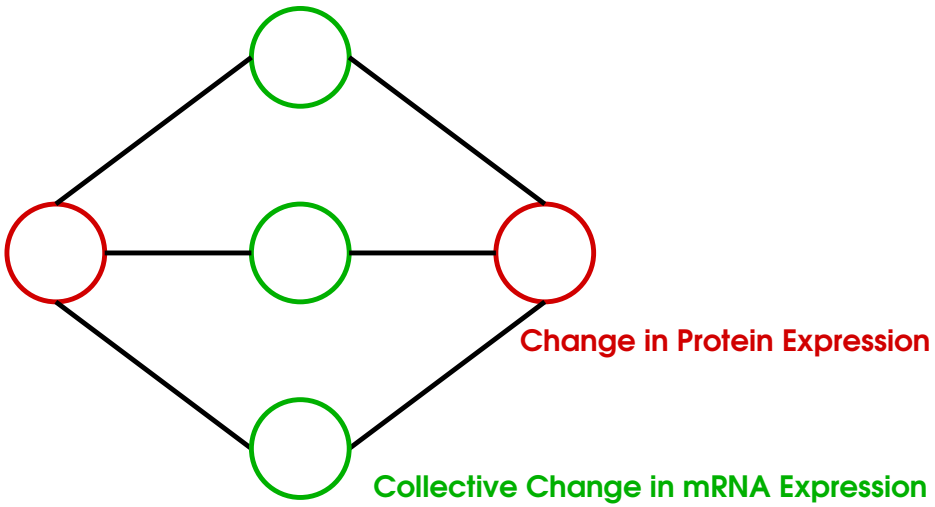
Proteomics-First Approach

- Premise: Small changes in mRNA expression may lead to significant changes in post-transcriptional activity
  - Find "seed proteins" using 2D-PAGE
  - Map seed proteins on the PPI network
  - Refine subgraphs based on "collective" change in mRNA expression



Finding Implicated Subgraphs

- Compute topological scores for all proteins in the network
  - Proteins with high proximity to seed proteins have high topological scores
  - Combine topological scores with differential expression to identify subgraphs with high topological score and significant differential expression when considered together



Computing Topological Scores

- Proximity to a set of seed proteins
  - Generalize random walk with restarts: Restart at any of the seed proteins!

$$\phi(0) = r,\ \phi(t+1) = (1-c)A\phi(t) + cr,\ \phi = \lim_{t\to\infty} \phi(t)$$

- $\phi(j)$: Proximity of protein j to seed proteins
- r : Restart vector, $||r||_1 = 1$
- $r(i) = |z_i|$ if fold change z_i of protein i is significant
- Prioritize all proteins in the network based on $\phi(j)$

Genes Implicated by Network Proximity

Rank	Gene	Score	E-value	p-value	Partners	Seed Partners
2	SUMO4	5.40E-03	8.70E-04	1.00E-03	75	11
7	GFAP	3.70E-03	4.60E-04	1.00E-03	40	7
8	NEFL	3.50E-03	3.60E-04	1.00E-03	31	7
21	UCHL1	3.00E-03	3.40E-04	1.00E-03	29	6
22	UNG	3.00E-03	2.40E-04	1.00E-03	21	6
16	STXBP1	3.10E-03	4.60E-04	1.00E-03	40	6
17	APBB1	3.10E-03	4.20E-04	1.00E-03	36	6
10	MAP3K5	3.40E-03	6.30E-04	2.00E-03	54	6
42	CCT4	2.20E-03	1.60E-04	2.00E-03	14	4
20	CRYAB	3.00E-03	3.40E-04	3.00E-03	29	6
43	CCT6A	2.20E-03	1.30E-04	3.00E-03	11	4
9	DNM1	3.40E-03	7.70E-04	4.00E-03	66	6
23	DCTN1	2.90E-03	3.90E-04	4.00E-03	34	6
37	CCT7	2.30E-03	2.00E-04	4.00E-03	17	4
12	MBP	3.30E-03	7.20E-04	5.00E-03	62	6
44	CCT8	2.10E-03	1.20E-04	5.00E-03	10	4
15	SPTAN1	3.20E-03	6.30E-04	6.00E-03	54	6
18	NSF	3.10E-03	5.20E-04	6.00E-03	45	6
29	TUBA1B	2.40E-03	3.00E-04	7.00E-03	26	4
257	LGALS13	8.40E-04	3.50E-05	7.10E-03	3	2
34	CCT5	2.30E-03	2.60E-04	8.00E-03	22	4
5	APP	4.00E-03	1.10E-03	8.10E-03	99	7
40	CCT3	2.20E-03	2.10E-04	8.10E-03	18	4

Outline

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