Comparative Analysis of Networks

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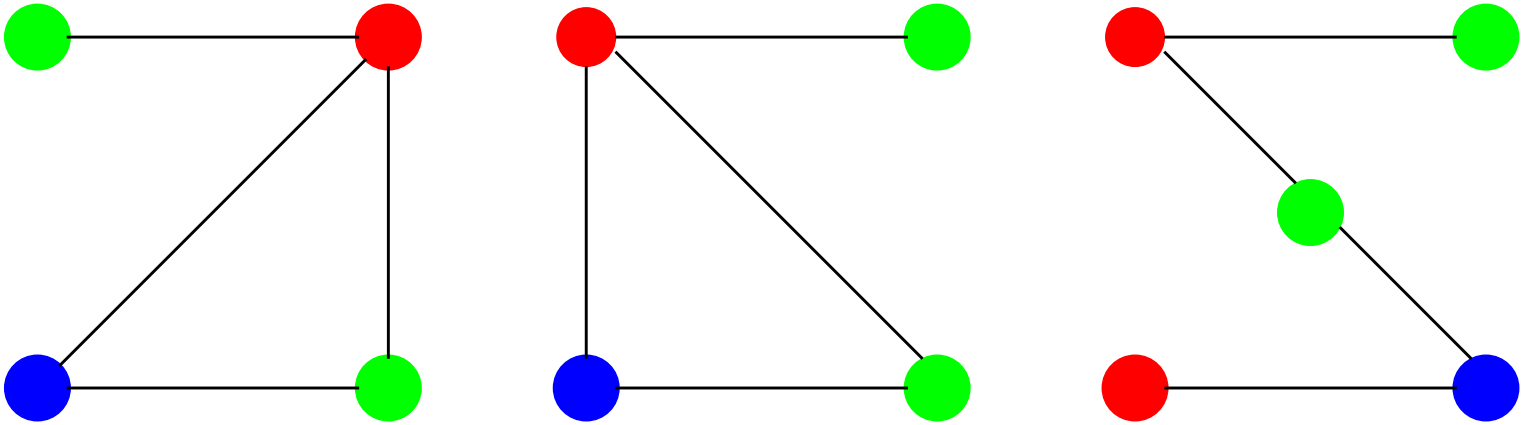
Outline

- Interaction Networks
  - Modeling, evolution, problems, practical implications
- Algorithms for Analyzing Interaction Networks
  - *Analyzing* biological networks for *conserved interaction patterns*
  - *Pairwise* Alignment *of networks*
  - Probabilistic *models/analyses for assessing* statistical significance
- Computational Synthesis of Interaction Networks
  - Inferring function from domain co-evolution

Conserved Interaction Patterns

- Given a collection of interaction networks, find sub-networks that are common to an interesting subset of these networks (Koyutürk, Grama, & Szpankowski, *ISMB*, 2004)
  - A sub-network is a group of interactions that are tied to each other (connected)
  - Frequency: The number of networks that contain a sub-network, is a coarse measure of statistical significance
- Computational challenges
  - How to relate nodes (proteins) in different networks (organisms)?
  - Requires solution of the intractable subgraph isomorphism problem
  - Must be scalable to potentially large number of networks
  - Networks are large (in the range of $10K$ edges)

Graph Analysis



Network database

Interaction patterns that are common to all networks

Problem Statement

- Given a set of proteins V, a set of interactions E, and a many-to-many mapping from V to a set of ortholog groups $\mathcal{L} = \{l_1, l_2, ..., l_n\}$, the corresponding interaction network is a labeled graph $G = (V, E, \mathcal{L})$.
  - $v \in V(G)$ is associated with a set of ortholog groups $L(v) \subseteq \mathcal{L}$.
  - $uv \in E(G)$ represents an interaction between u and v.
- S is a sub-network of G, *i.e.*, $S \sqsubseteq G$ if there is an injective mapping $\phi : V(S) \rightarrow V(G)$ such that for all $v \in V(S)$, $L(v) \subseteq L(\phi(v))$ and for all $uv \in E(S)$, $\phi(u)\phi(v) \in E(G)$.

Computational Problem

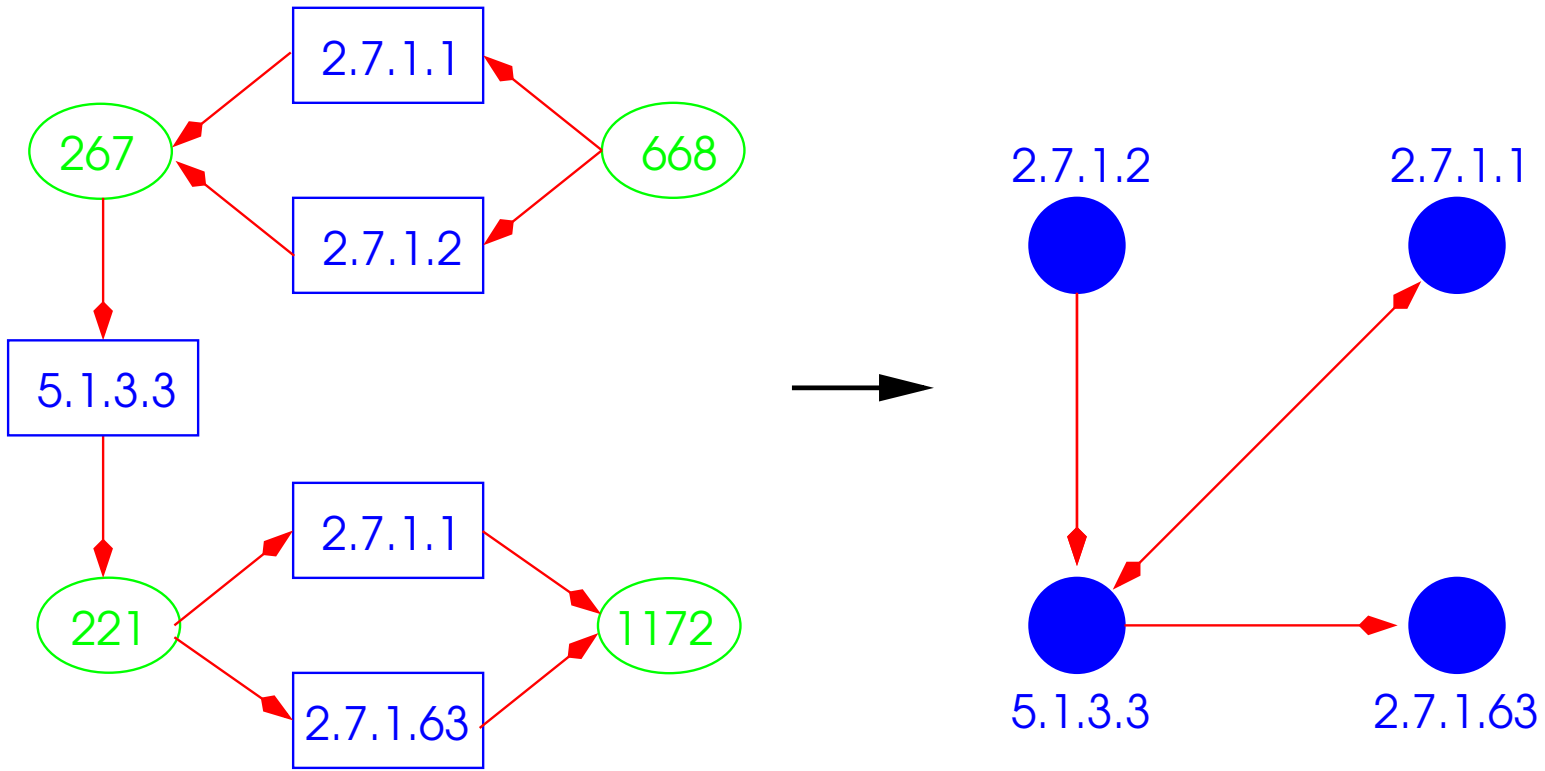
- Conserved sub-network discovery
  - Instance: A set of interaction networks $\mathcal{G} = \{G_1 = (V_1, E_1, \mathcal{L}), G_2 = (V_2, E_2, \mathcal{L}), ..., G_m = (V_m, E_m, \mathcal{L})\}$, each belonging to a different organism, and a frequency threshold σ^*.
  - Problem: Let $H(S) = \{G_i : S \sqsubseteq G_i\}$ be the occurrence set of graph S. Find all connected subgraphs S such that $|H(S)| \geq \sigma^*$, *i.e.*, S is a frequent subgraph in $\mathcal{G}$ and for all $S' \sqsupset S$, $H(S) \neq H(S')$, *i.e.*, S is maximal.

Algorithmic Insight: Ortholog Contraction

- Contract orthologous nodes into a single node
- No subgraph isomorphism
  - Graphs are uniquely identified by their edge sets
- Key observation: Frequent sub-networks are preserved $\Rightarrow$ No information loss
  - Sub-networks that are frequent in general graphs are also frequent in their ortholog-contracted representation
  - Ortholog contraction is a powerful pruning heuristic

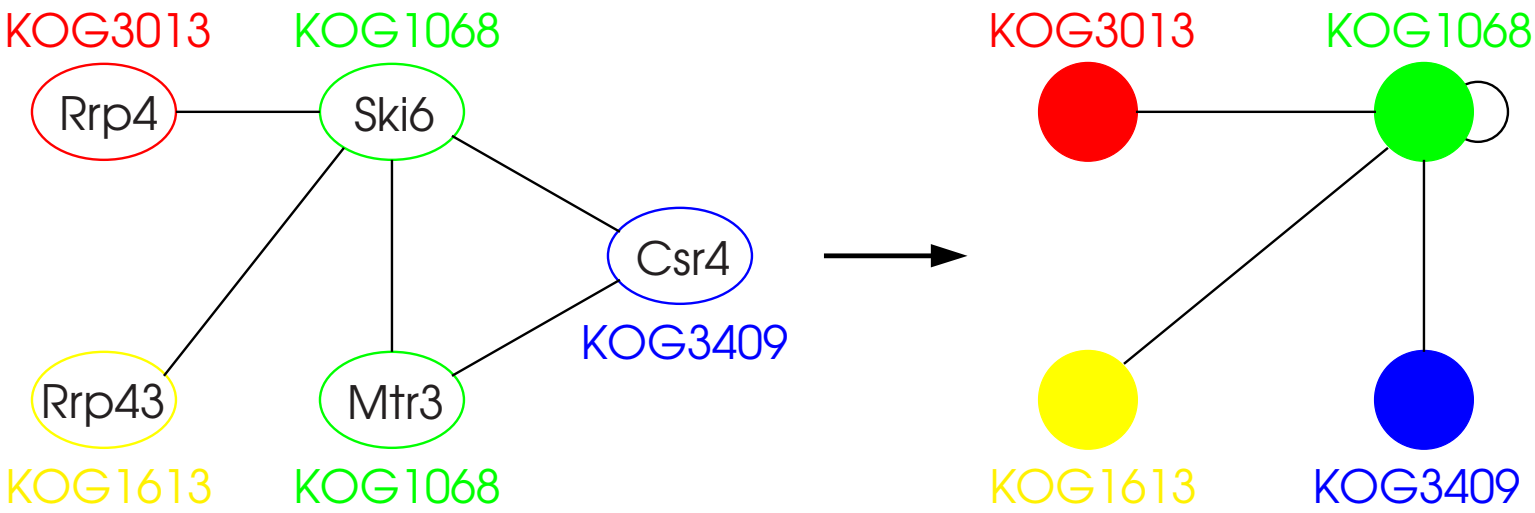
Example: Ortholog Contraction in Metabolic Pathways

- Directed hypergraph → uniquely-labeled directed graph
  - Nodes represent enzymes
  - Global labeling by enzyme nomenclature (EC numbers)
  - A directed edge from one enzyme to the other implies that the second consumes a product of the first



Example: Ortholog Contraction in Protein Interaction Networks

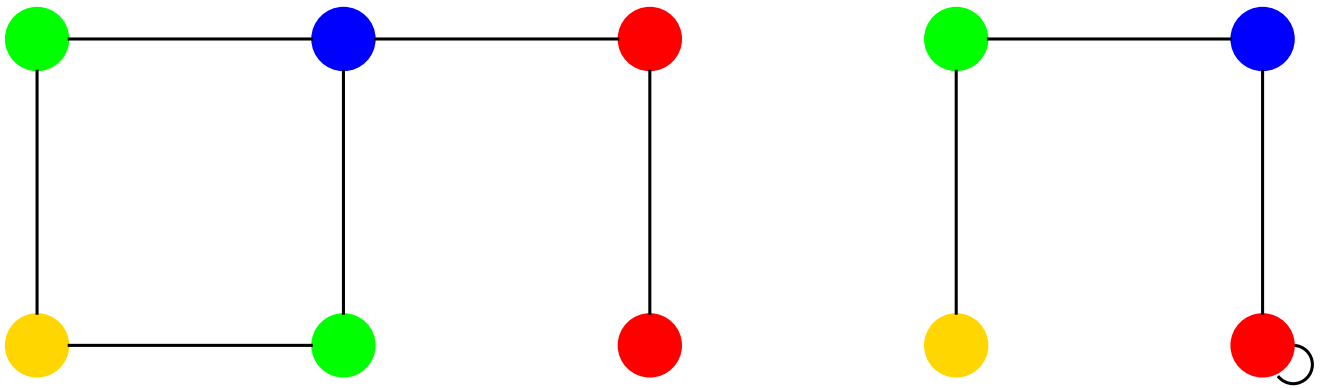
- Interaction between proteins → Interaction between ortholog groups or protein families



Preservation of Sub-networks

Theorem: Let $\tilde{G}$ be the ortholog-contracted graph obtained by contracting the orthologous nodes of network G. Then, if S is a subgraph of G, $\tilde{S}$ is a subgraph of $\tilde{G}$.

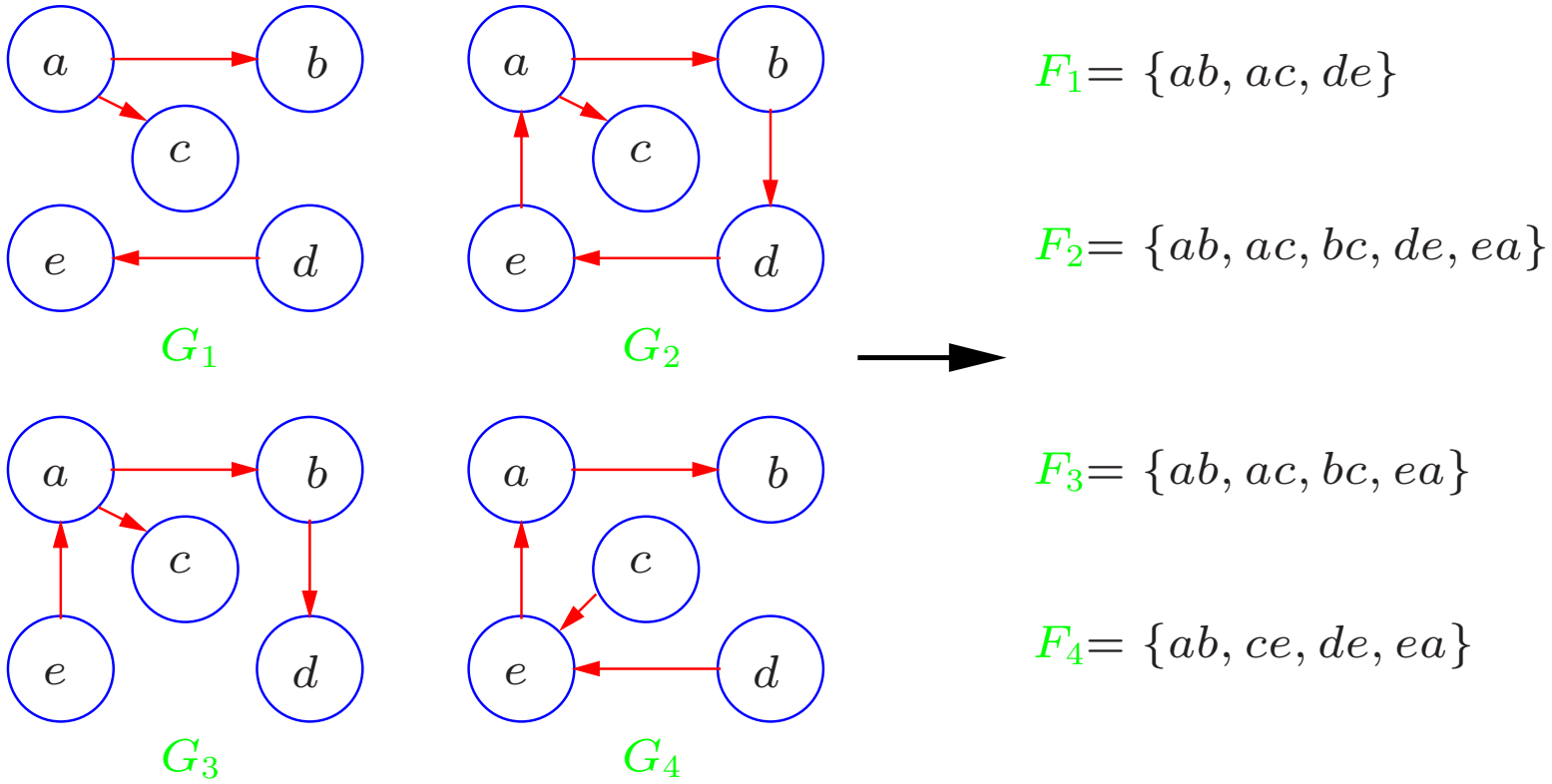
Corollary: The ortholog-contracted representation of any frequent sub-network is also frequent in the set of ortholog-contracted graphs.



G $\tilde{G}$

Simplifying the Graph Analysis Problem

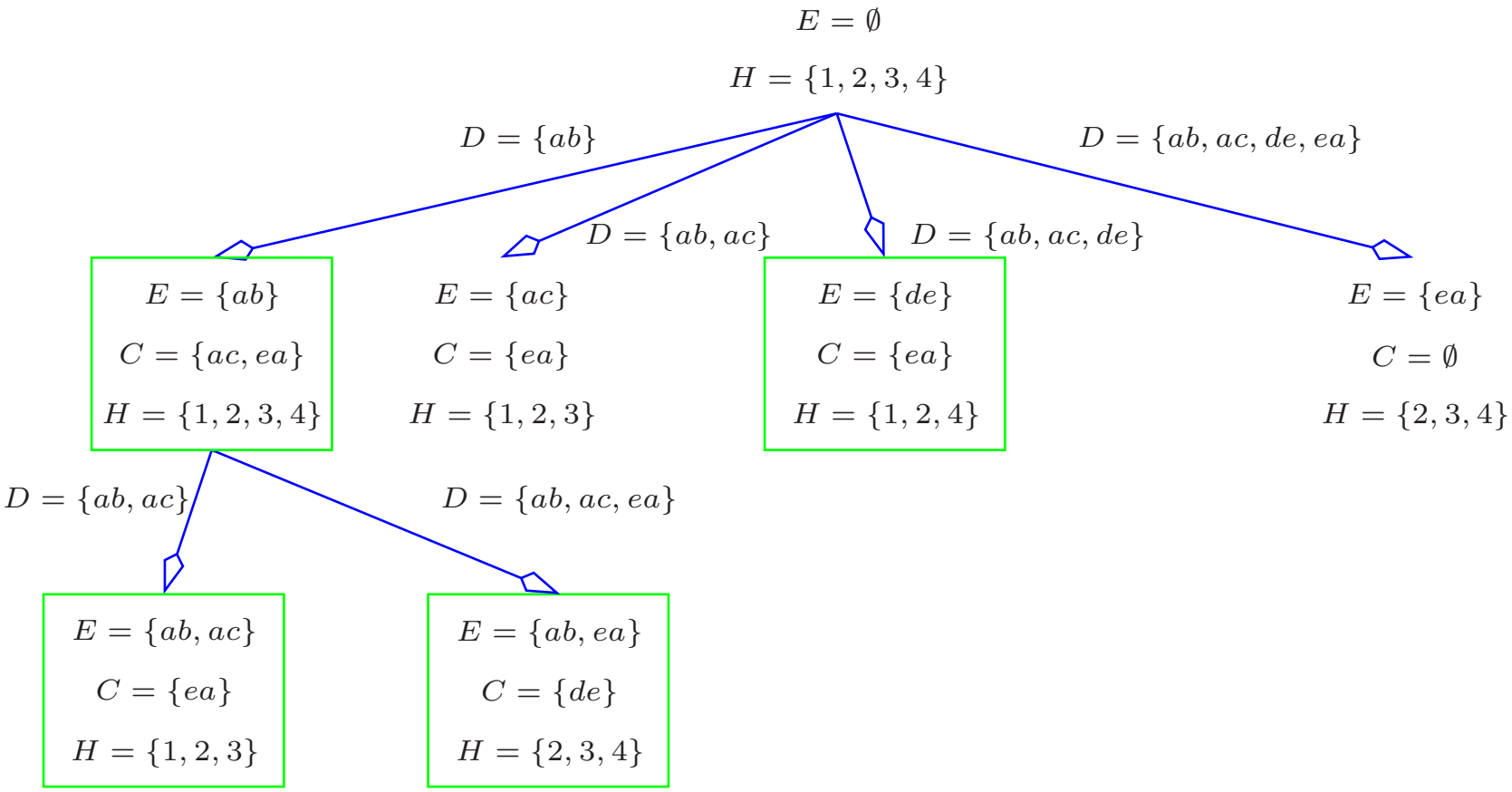
- Observation: An ortholog-contracted graph is uniquely determined by the set of its edges.
  - Conserved Sub-network Discovery Problem → Frequent Edge set Discovery Problem



Extending Frequent Itemset Mining to Graph Analysis

- Given a set of transactions, find sets of items that are frequent in these transactions
  - Extensively studied in data mining literature
- Algorithms exploit downward closure property
  - An edge set is frequent only if all of its subsets are frequent
  - Generate edge sets (sub-networks) from small to large, pruning supersets of infrequent sets
- No redundancy
- No subgraph enumeration

MULE: Analyzing Ortholog-Contracted Networks

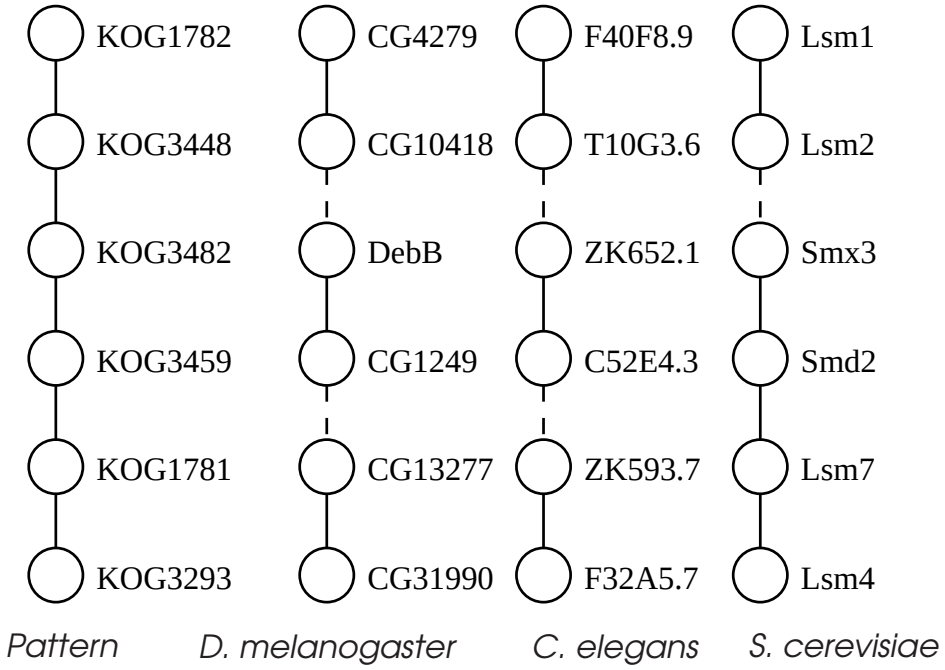


Sample run of MULE for identifying maximal sub-networks that are common to at least 3 organisms

Case Study: Analyzing Protein Interaction Networks

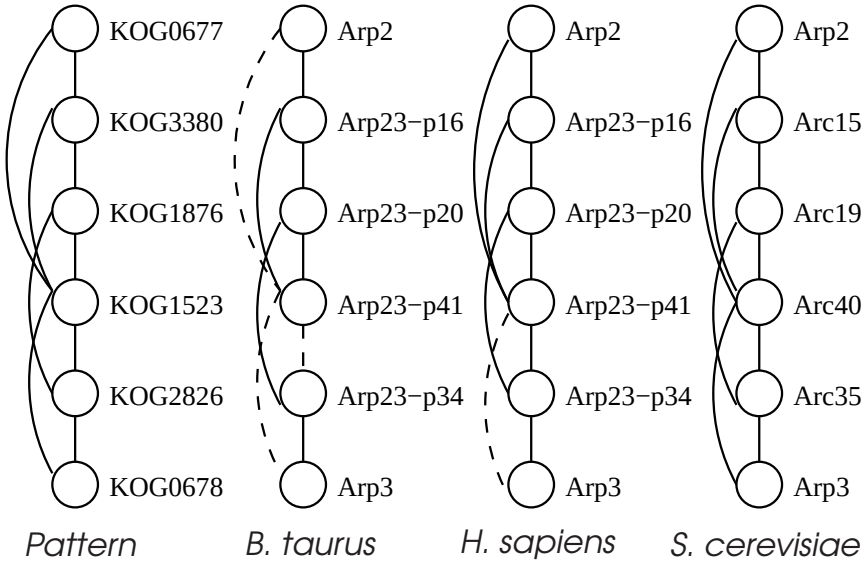
- PPI networks for 9 eukaryotic organisms derived from BIND and DIP
  - *A. thaliania, O. sativa, S. cerevisiae, C. elegans, D. melanogaster, H. sapiens, B. taurus, M. musculus, R. norvegicus*
  - # of proteins ranges from 288 (*Arabidopsis*) to 8577 (*fruit fly*)
  - # of interactions ranges from 340 (*rice*) to 28829 (*fruit fly*)
- Ortholog contraction
  - Group proteins according to existing COG ortholog clusters
  - Merge Homologene groups into COG clusters
  - Cluster remaining proteins via BLASTCLUST
  - Ortholog-contracted *fruit fly* network contains 11088 interactions between 2849 ortholog groups
- MULE is available at `http://www.cs.purdue.edu/pdsl/`

Conserved Protein Interaction Patterns



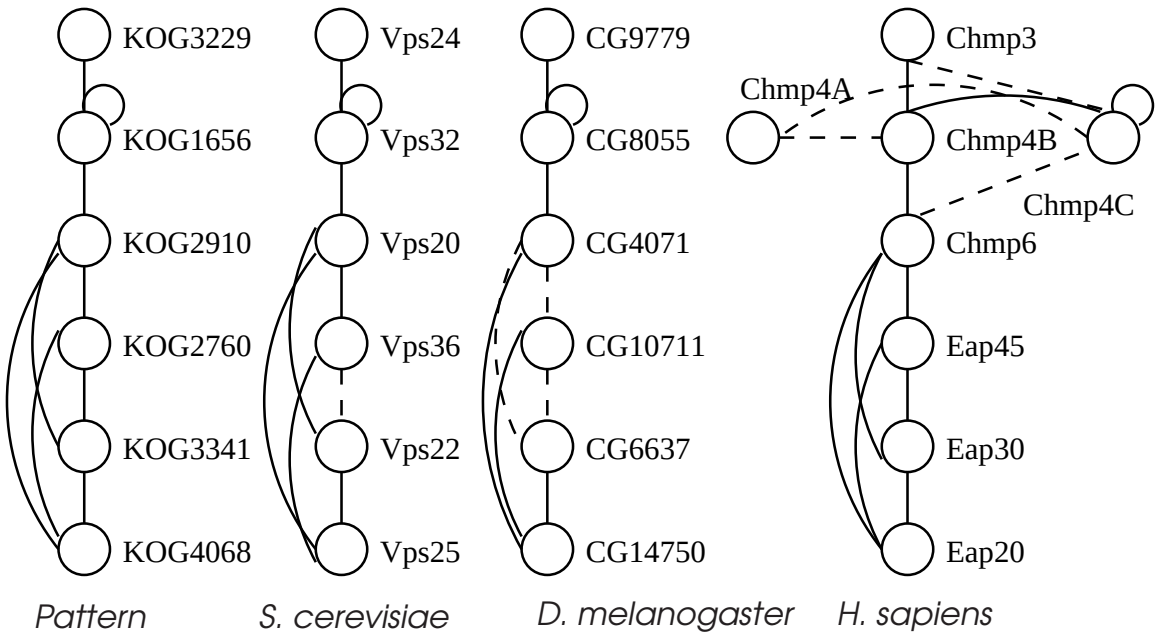
Small nuclear ribonucleoprotein complex ($p < 2e - 43$)

Conserved Protein Interaction Patterns



Actin-related protein Arp2/3 complex ($p < 9e - 11$)

Conserved Protein Interaction Patterns



Endosomal sorting ($p < 1e - 78$)

Runtime Characteristics

Comparison with isomorphism-based algorithms

FSG (Kuramochi & Karypis, *IEEE TKDE*, 2004), gSpan (Yan & Han, *KDD*, 2003)

		FSG			MULE		
Dataset	Minimum Support (%)	Runtime (secs.)	Largest pattern	Number of patterns	Runtime (secs.)	Largest pattern	Number of patterns
Glutamate	20	0.2	9	12	0.01	9	12
	16	0.7	10	14	0.01	10	14
	12	5.1	13	39	0.10	13	39
	10	22.7	16	34	0.29	15	34
	8	138.9	16	56	0.99	15	56
Alanine	24	0.1	8	11	0.01	8	11
	20	1.5	11	15	0.02	11	15
	16	4.0	12	21	0.06	12	21
	12	112.7	17	25	1.06	16	25
	10	215.1	17	34	1.72	16	34

Extraction of contracted patterns

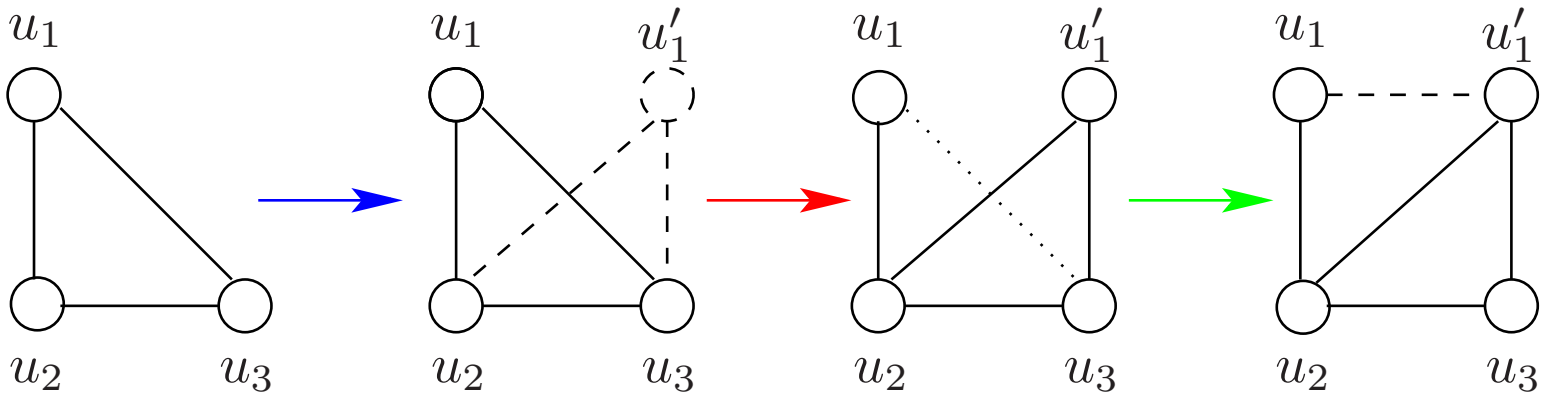
Glutamate metabolism, $\sigma = 8\%$				Alanine metabolism, $\sigma = 10\%$			
Size of contracted pattern	Extraction time (secs.) FSG	gSpan	Size of extracted pattern	Size of contracted pattern	Extraction time (secs.) FSG	gSpan	Size of extracted pattern
15	10.8	1.12	16	16	54.1	10.13	17
14	12.8	2.42	16	16	24.1	3.92	16
13	1.7	0.31	13	12	0.9	0.27	12
12	0.9	0.30	12	11	0.4	0.13	11
11	0.5	0.08	11	8	0.1	0.01	8
Total number of patterns: 56				Total number of patterns: 34			
Total runtime of FSG alone: 138.9 secs.				Total runtime of FSG alone :215.1 secs.			
Total runtime of MULE+FSG: 0.99+100.5 secs.				Total runtime of MULE+FSG: 1.72+160.6 secs.			
Total runtime of MULE+gSpan: 0.99+16.8 secs.				Total runtime of MULE+gSpan: 1.72+31.0 secs.			

Alignment of Networks

- Given two networks, identify sub-networks that are similar to each other
  - Biological implications
  - Mathematical modeling
- Existing algorithms
  - PathBLAST aligns pathways (linear chains) to simplify the problem while maintaining biological meaning (Kelley et al., *PNAS*, 2004)
  - NetworkBLAST compares conserved complex model with null model to identify significantly conserved subnets (Sharan et al., *J. Comp. Biol.*, 2005)
- Our approach (Koyutürk et al., *RECOMB*, 2005) (Koyutürk et al., *J. Comp. Biol.*, 2006)
  - Guided by models of evolution
  - Scores evolutionary events
  - Identifies sets of proteins that induce high-scoring sub-network pairs

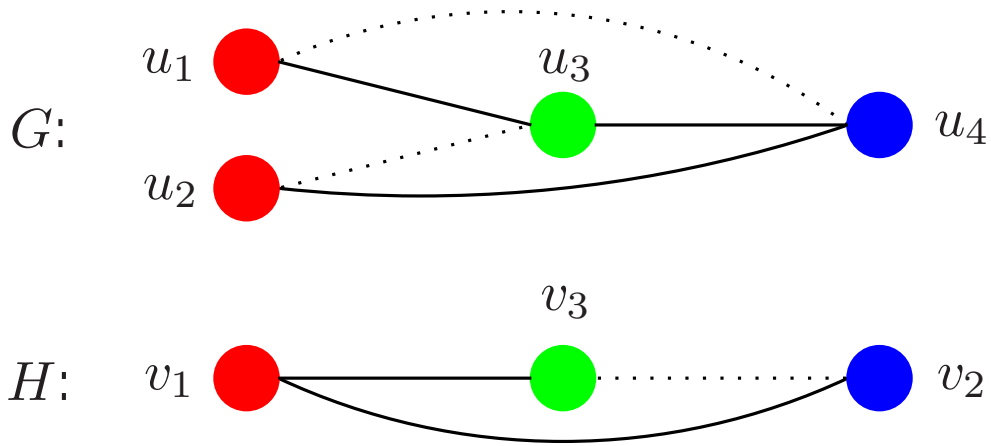
Evolution of Networks

- Many networks evolve through the process of Duplication/ and Divergence.
  - Interactions of duplicated nodes are also duplicated
  - Duplicated nodes rapidly lose interactions through mutations
- Allows defining and scoring evolutionary events as graph-theoretical concepts



Match, Mismatch, and Duplication

- Evolutionary events as graph-theoretic concepts
  - A match $\in \mathcal{M}$ corresponds to two pairs of homolog nodes from each network such that both pairs interact in both networks. A match is associated with score μ.
  - A mismatch $\in \mathcal{N}$ corresponds to two pairs of homolog nodes from each graph such that only one pair is interacting. A mismatch is associated with penalty ν.
  - A duplication $\in D$ corresponds to a pair of homolog nodes that are in the same network. A duplication is associated with score δ.



Scoring Matches, Mismatches and Duplications

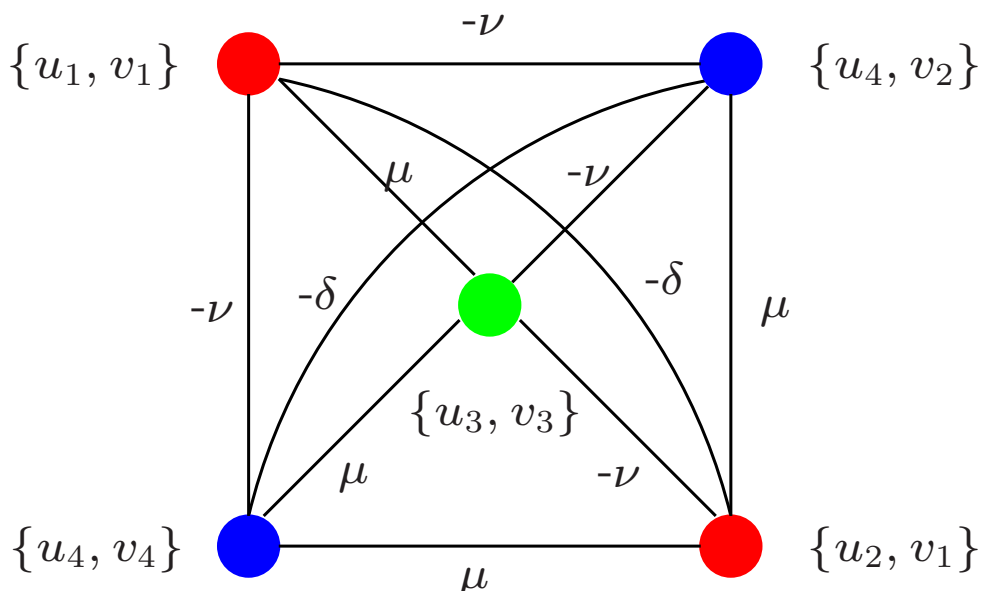
- Quantizing similarity between two nodes
  - Confidence in two nodes being orthologous
  - E-value: $S(u,v) = log_{10}\frac{p(u,v)}{p_{random}}$
  - Ortholog clustering: $S(u,v) = c(u)c(v)$
- Match score
  - $\mu(uu', vv') = \bar{\mu}\min\{S(u,v), S(u',v')\}$
- Mismatch penalty
  - $\nu(uu', vv') = \bar{\nu}\min\{S(u,v), S(u',v')\}$
- Duplication score
  - $\delta(u,u') = \bar{\delta}(\hat{\delta} - S(u,u'))$
  - $\hat{\delta}$ specifies threshold for sequence similarity to be considered functionally conserved

Pairwise Alignment of Networks as an Optimization Problem

- Alignment score:
  $\sigma(\mathcal{A}(P)) = \sum_{M \in \mathcal{M}} \mu(M) - \sum_{N \in \mathcal{N}} \nu(N) + \sum_{D \in \mathcal{D}} \delta(D)$
  - Matches are rewarded for conservation of interactions
  - Duplications are rewarded/penalized for functional conservation/differentiation after split
  - Mismatches are penalized for functional divergence (what about experimental error?)
- Scores are functions of similarity between associated proteins
- Problem: Find all protein subset pairs with significant alignment score
  - High scoring protein subsets are likely to correspond to conserved modules
- A graph equivalent to BLAST

Weighted Alignment Graph

- $\mathbf{G(V, E)}$: $\mathbf{V}$ consists of all pairs of homolog proteins $\mathbf{v} = \{u \in U, v \in V\}$
- An edge $\mathbf{vv'} = \{uv\}\{u'v'\}$ in $\mathbf{E}$ is a
  - match edge if $uu' \in E$ and $vv' \in V$, with weight $w(\mathbf{vv'}) = \mu(uv, u'v')$
  - mismatch edge if $uu' \in E$ and $vv' \notin V$ or vice versa, with weight $w(\mathbf{vv'}) = -\nu(uv, u'v')$
  - duplication edge if $S(u, u') > 0$ or $S(v, v') > 0$, with weight $w(\mathbf{vv'}) = \delta(u, u')$ or $w(\mathbf{vv'}) = \delta(v, v')$



Maximum Weight Induced Subgraph Problem

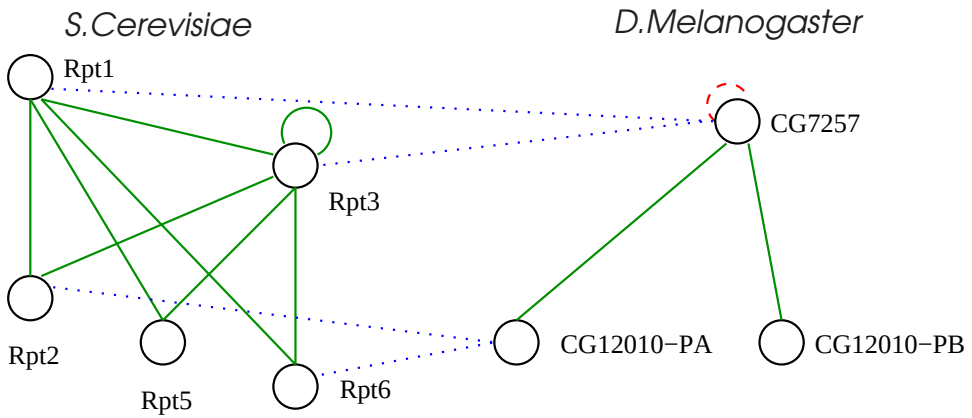
- Definition: (MAWISH)
  - Given graph $\mathcal{G}(\mathcal{V}, \mathcal{E})$ and a constant ϵ, find $\tilde{\mathcal{V}} \in \mathcal{V}$ such that $\sum_{\mathbf{v},\mathbf{u}\in\tilde{\mathcal{V}}} w(\mathbf{vu}) \geq \epsilon$.
  - NP-complete by reduction from Maximum-Clique
- Theorem: (MAWISH $\equiv$ Pairwise alignment)
  - If $\tilde{\mathcal{V}}$ is a solution for the MAWISH problem on $\mathcal{G}(\mathcal{V}, \mathcal{E})$, then $P = \{\tilde{U}, \tilde{V}\}$ induces an alignment $\mathcal{A}(P)$ with $\sigma(\mathcal{A}) \geq \epsilon$, where $\tilde{\mathcal{V}} = \tilde{U} \times \tilde{V}$.
- Solution: Local graph expansion
  - Greedy graph growing + iterative refinement
  - Linear-time heuristic
- Source code available at `http://www.cs.purdue.edu/pdsl/`

Case Study: Alignment of Yeast and Fruit Fly Networks

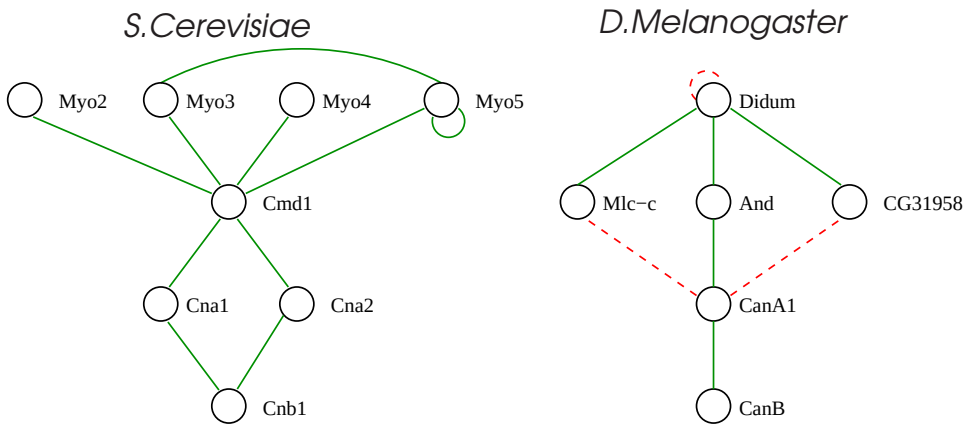
Rank	Score	z-score	# Proteins	# Matches	# Mismatches	# Dups.
1	15.97	6.6	18 (16, 5)	28	6	(4, 0)
	protein amino acid phosphorylation (69%)					
	JAK-STAT cascade (40%)					
2	13.93	3.7	13 (8, 7)	25	7	(3, 1)
	endocytosis (50%) / calcium-mediated signaling (50%)					
5	8.22	13.5	9 (5, 3)	19	11	(1, 0)
	invasive growth (sensu Saccharomyces) (100%)					
	oxygen and reactive oxygen species metabolism (33%)					
6	8.05	7.6	8 (5, 3)	12	2	(0, 1)
	ubiquitin-dependent protein catabolism (100%)					
	mitosis (67%)					
21	4.36	6.2	9 (5, 4)	18	13	(0, 5)
	cytokinesis (100%, 50%)					
30	3.76	39.6	6 (3, 5)	5	1	(0, 6)
	DNA replication initiation (100%, 80%)					

Subnets Conserved in Yeast and Fruit Fly

Proteosome regulatory particle subnet



Calcium-dependent stress-activated signaling pathway



Analytical Assessment of Statistical Significance

- What is the significance of a dense component in a network?
- What is the significance of a conserved component in multiple networks?
- Existing techniques
  - Mostly computational (*e.g.*, Monte-Carlo simulations)
  - Compute probability that the pattern exists rather than a pattern with the property (*e.g.*, size, density) exists
  - Overestimation of significance

Random Graph Models

- Interaction networks generally exhibit power-law property (or exponential, geometric, etc.)
- Analysis simplified through independence assumption (Itzkovitz et al., *Physical Review*, 2003)
- Independence assumption may cause problems for networks with arbitrary degree distribution
- $P(uv \in E) = d_u d_v / |E|$, where d_u is expected degree of u, but generally $d_{\max}^2 > |E|$ for PPI networks
- Analytical techniques based on simplified models (Koyutürk, Grama, Szpankowski, *RECOMB*, 2006)
  - Rigorous analysis on $G(n, p)$ model
  - Extension to piecewise $G(n, p)$ to capture network characteristics more accurately

Significance of Dense Subgraphs

- A subnet of r proteins is said to be ρ-dense if $F(r) \geq \rho r^2$, where $F(r)$ is the number of interactions between these r proteins
- What is the expected size of the largest ρ-dense subgraph in a random graph?
  - Any ρ-dense subgraph with larger size is statistically significant!
- $G(n, p)$ model
  - n proteins, each interaction occurs with probability p
  - Simple enough to facilitate rigorous analysis
  - If we let $p = d_{\max}/n$, largest ρ-dense subgraph in $G(n, p)$ stochastically dominates that in a graph with arbitrary degree distribution
- Piecewise $G(n, p)$ model
  - Few proteins with many interacting partners, many proteins with few interacting partners
  - Captures the basic characteristics of PPI networks
  - Analysis of $G(n, p)$ model immediately generalized to this model

Largest Dense Subgraph

- Theorem: If G is a random graph with n nodes, where every edge exists with probability p, then

$$\lim_{n\to\infty} \frac{R_\rho}{\log n} = \frac{1}{\kappa(p,\rho)} \qquad (pr.), \tag{1}$$

where

$$\kappa(p,\rho) = \rho \log \frac{\rho}{p} + (1-\rho)\log \frac{1-\rho}{1-p}. \tag{2}$$

More precisely,

$$P(R_\rho \geq r_0) \leq O\left(\frac{\log n}{n^{1/\kappa(p,\rho)}}\right), \tag{3}$$

where

$$r_0 = \frac{\log n - \log\log n + \log \kappa(p,\rho)}{\kappa(p,\rho)} \tag{4}$$

for large n.

Piecewise $G(n,p)$ model

- The size of largest dense subgraph is still proportional to $\log n/\kappa$ with a constant factor depending on number of hubs

- Model:

$$P(uv \in E(G)) = \begin{cases} p_h & \text{if } u,v \in V_h \\ p_l & \text{if } u,v \in V_l \\ p_b & \text{if } u \in V_h, v \in V_l \text{ or } u \in V_l, v \in V_h \end{cases}$$

- Result:
  Let $n_h = |V_h|$. If $n_h = O(1)$, then $P(R_n(\rho) \geq r_1) \leq O\left(\frac{\log n}{n^{1/\kappa(p_l,\rho)}}\right)$, where

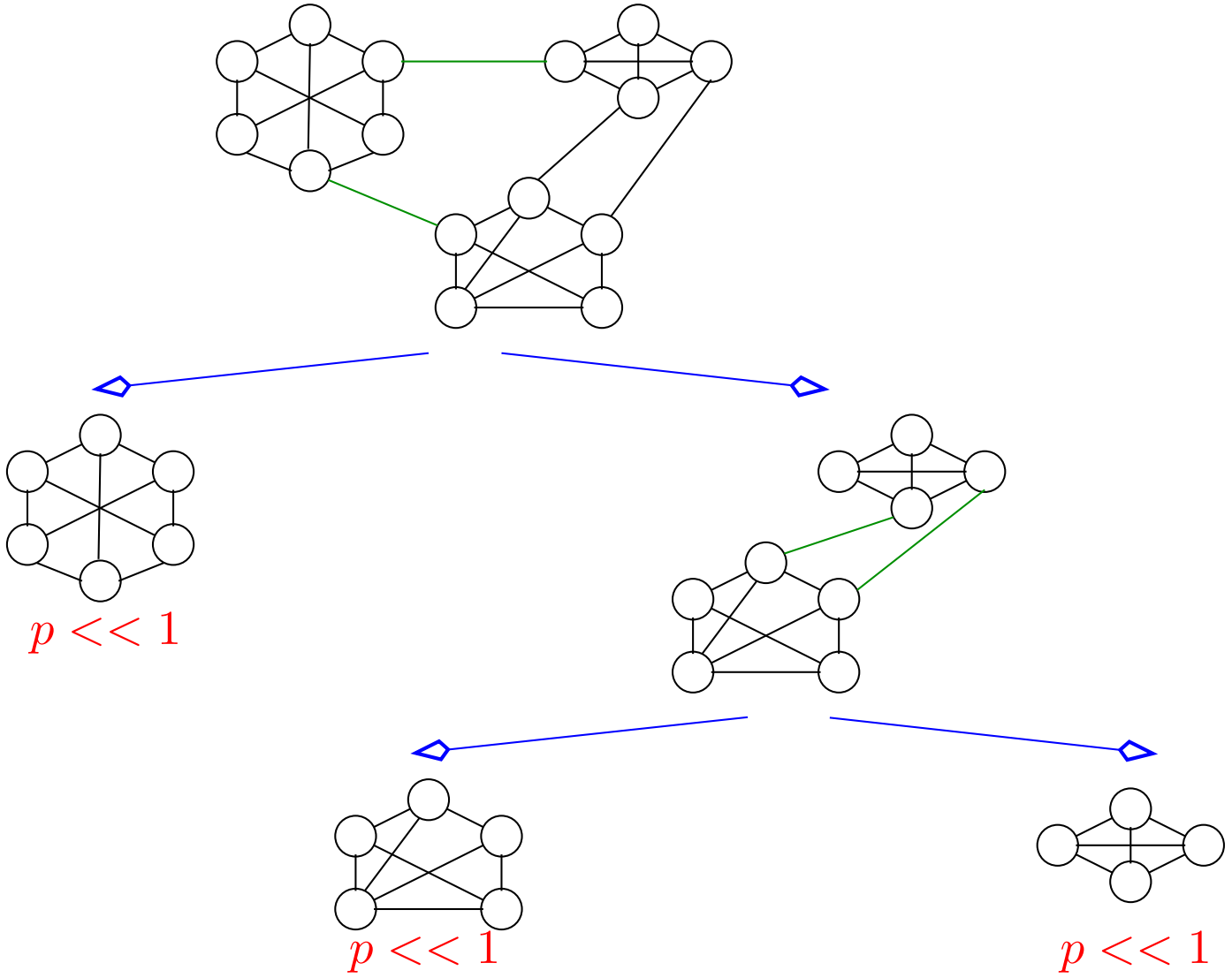
$$r_1 = \frac{\log n - \log\log n + 2n_h \log B + \log \kappa(p_l,\rho) - \log e + 1}{\kappa(p_l,\rho)}$$

  and $B = \frac{p_b q_l}{p_l} + q_b$, where $q_b = 1 - p_b$ and $q_l = 1 - p_l$.

Algorithms Based on Statistical Significance

- Identification of topological modules
- Use statistical significance as a stopping criterion for graph clustering heuristics
- HCS Algorithm (Hartuv & Shamir, *Inf. Proc. Let.*, 2000)
  - Find a minimum-cut bipartitioning of the network
  - If any of the parts is dense enough, record it as a dense cluster of proteins
  - Else, further partition them recursively
- SIDES: Use statistical significance to determine whether a subgraph is sufficiently dense
  - For given number of proteins and interactions between them, we can determine whether those proteins induce a significantly dense subnet

SIDES **Algorithm**



SIDES is available at `http://www.cs.purdue.edu/pdsl`

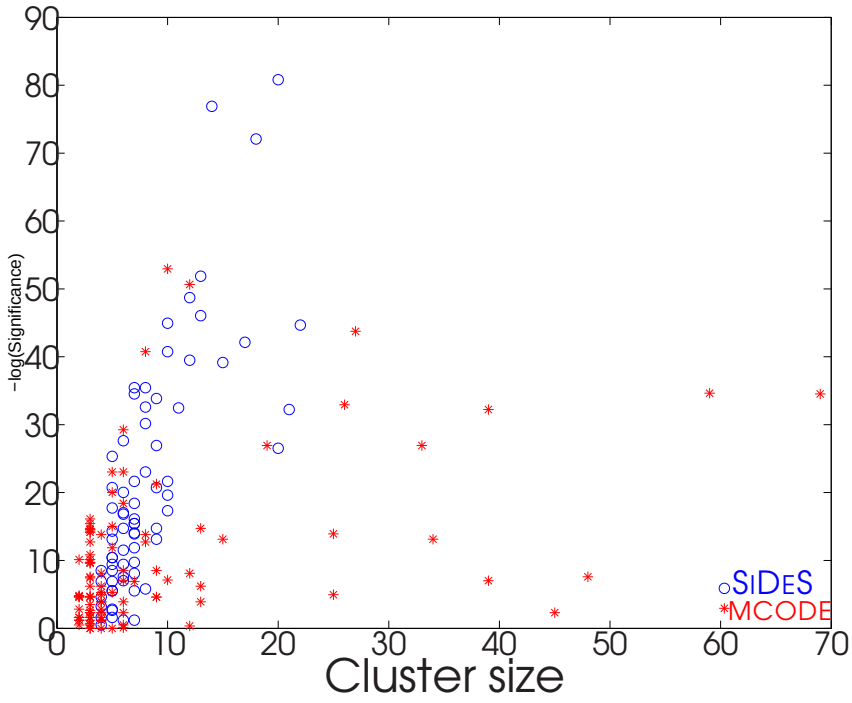
Performance of SIDES

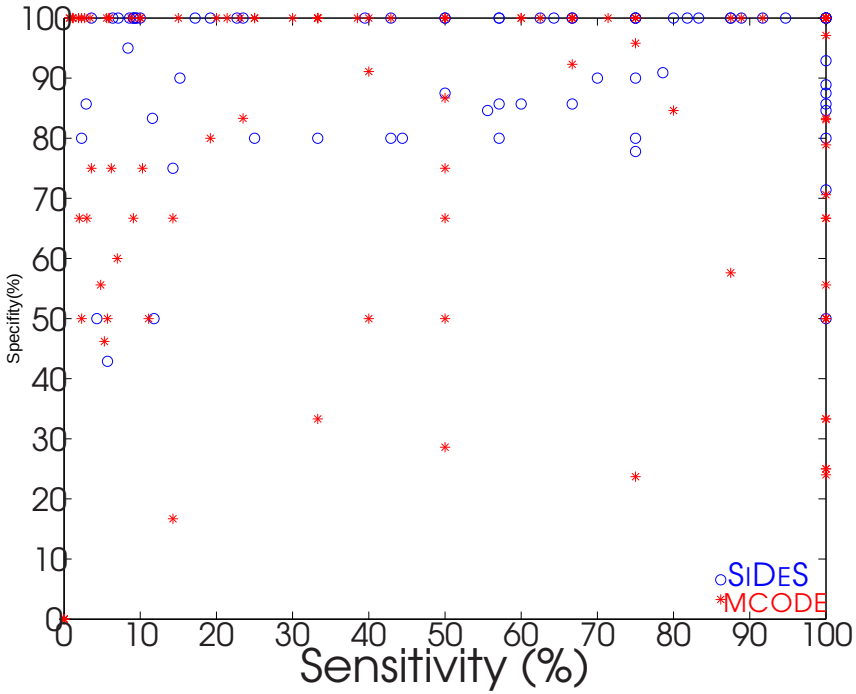
- Biological relevance of identified clusters is assessed with respect to Gene Ontology (GO)
  - Estimate the statistical significance of the enrichment of each GO term in the cluster
- Quality of the clusters with respect to GO annotations
  - Assume cluster C containing n_C genes is associated with term T that is attached to n_T genes and n_{CT} of genes in C are attached to T
  - specificity = $100 \times n_{CT}/n_C$
  - sensitivity = $100 \times n_{CT}/n_T$

	SIDES			MCODE		
	Min.	Max.	Avg.	Min.	Max.	Avg.
Specificity (%)	43.0	100.0	91.2	0.0	100.0	77.8
Sensitivity (%)	2.0	100.0	55.8	0.0	100.0	47.6

Comparison of SIDES with MCODE (Bader & Hogue, *BMC Bioinformatics*, 2003)

Performance of SIDES





Size vs Significance

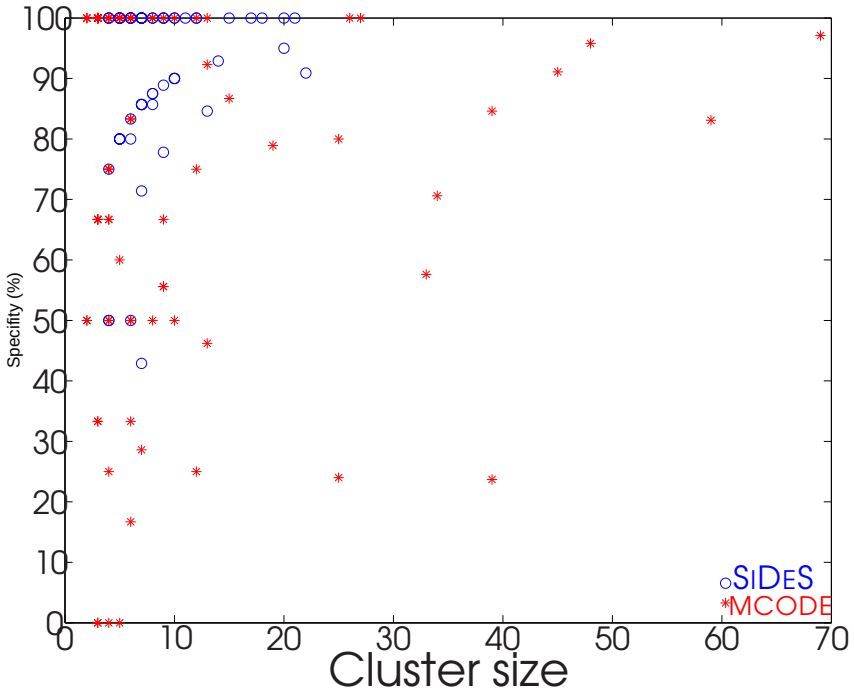
Sensitivity vs Specifity

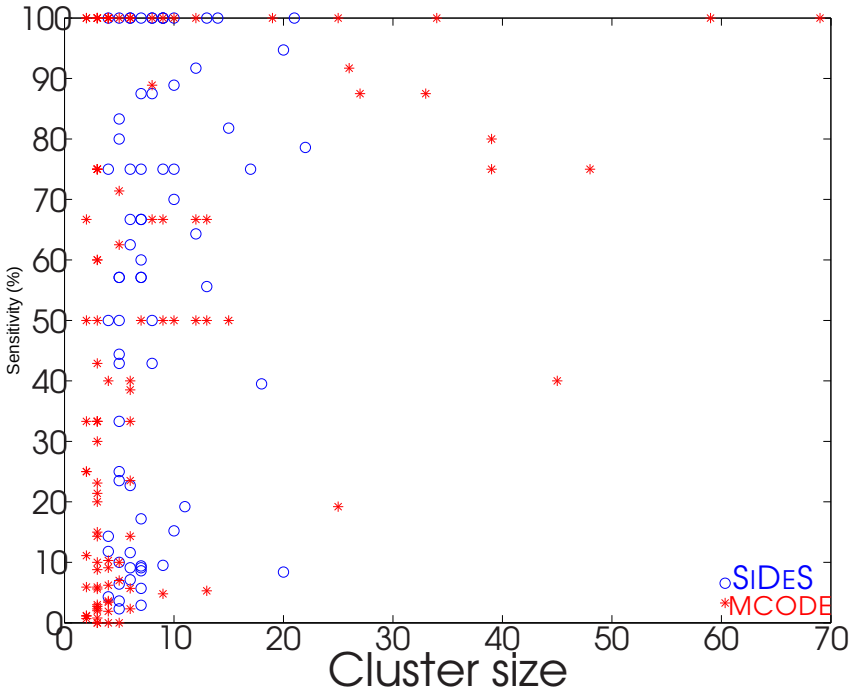
Correlation

SIDES: 0.76

MCODE: 0.43

Performance of SIDES





Size vs Specificity

Size vs Sensitivity

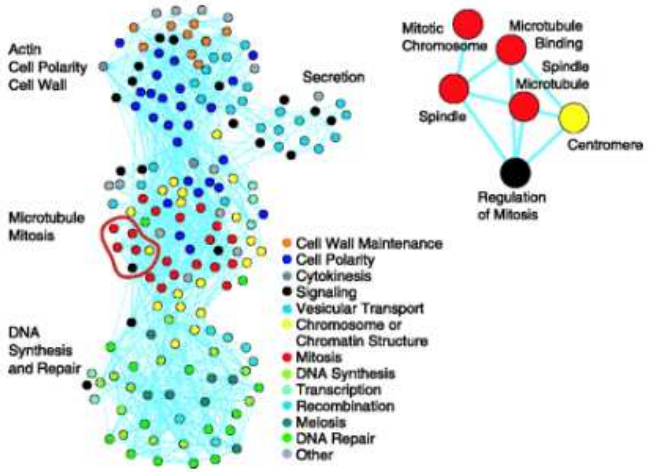
Correlation

SIDES: 0.22
MCODE: -0.02

SIDES: 0.27
MCODE: 0.36

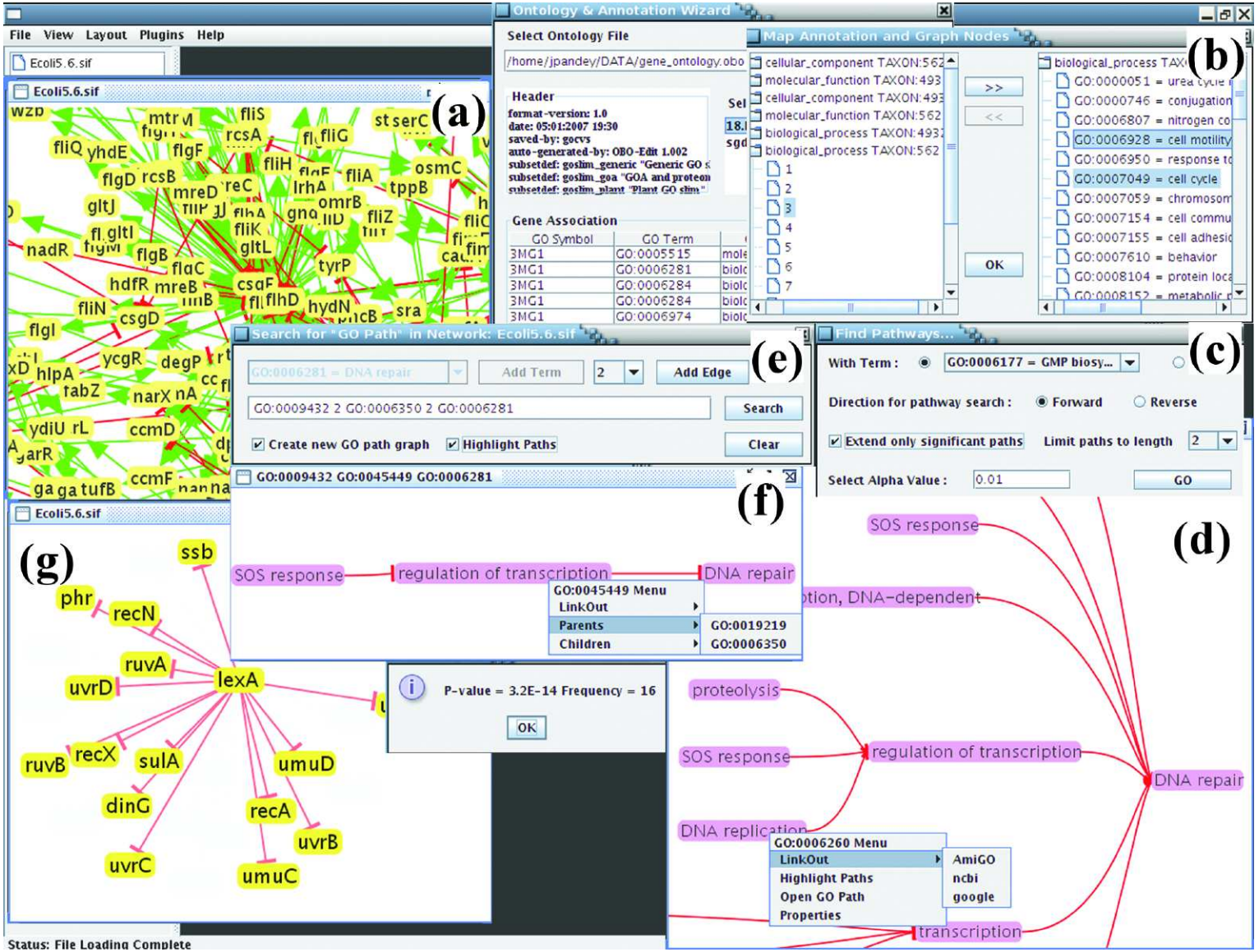
Functional Annotation of Pathways: From Molecules to Systems

- Annotation is at the node level
- Map networks to function space (can generate a library of annotated modular (sub-) networks)



Network of Gene Ontology terms based on significance of pairwise interactions in yeast synthetic gene array (SGA) (Tong et al., Science, 2004)

Narada Functionality



Narada Network Annotation