Protein-Protein-Interaction Networks

Ulf Leser, Samira Jaeger

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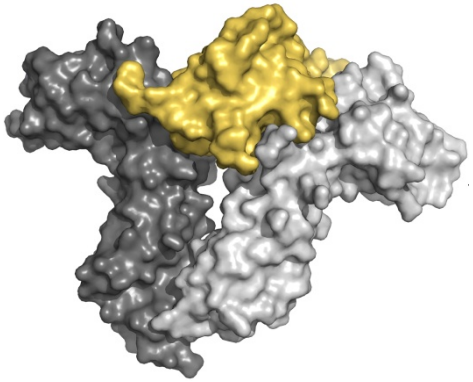
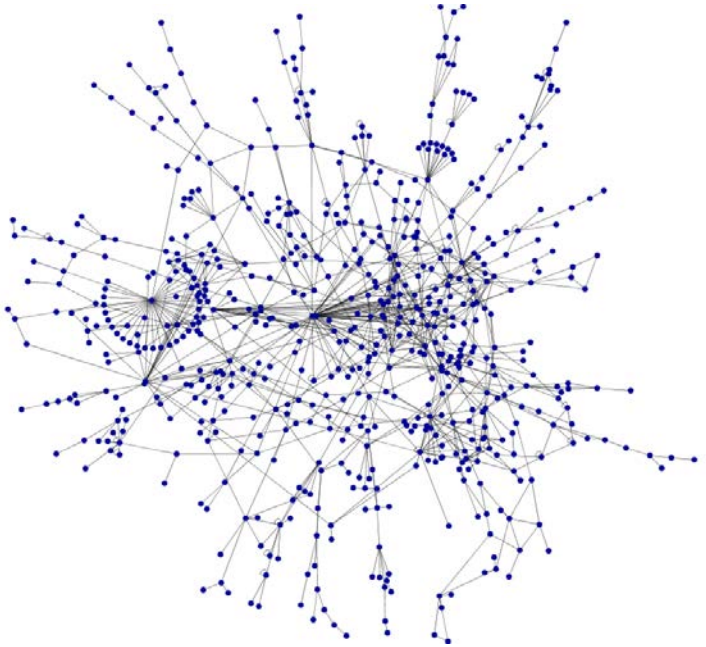


- Protein-protein interactions
  - Characteristics
  - Experimental detection methods
  - Databases
- Biological networks



Motivation

- Interaction: Physical binding of two or more proteins
  - E.g. signal transduction, gene regulation, protein transport, ...
  - Transient (signal) or permanent (complex formation)
  - Directed effect (regulates) or undirected (binds)
  - Specific (activates) or unspecific (binds, interacts)
- Changes in protein structure may hinder bindings and thus perturb natural cellular processes
  - Influence on all “downstream” proteins, i.e., proteins reachable through a path of interactions
- Interactome: Set of all PPIs happening in a cell
  - Typically includes complex formation

Context-dependency

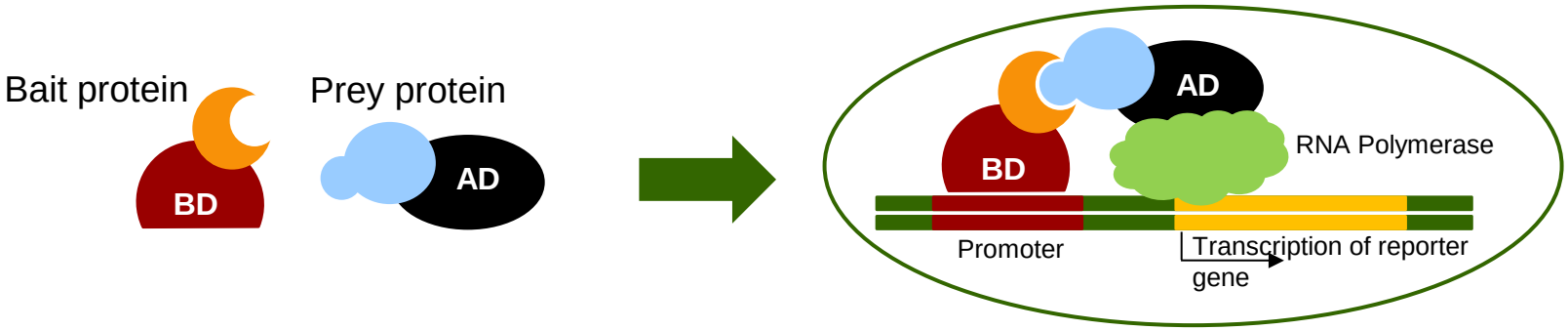
- Most PPIs are context-dependent
  - Cell type, cell cycle phase and state
  - Environmental conditions
  - Developmental stage
  - Protein modification
  - Presence of cofactors and other binding partners
  - Species
  - ...
- Disregarded by many PPI detection methods
- Low quality of typical data sets
  - Predicted / measured PPI do not happen in (most) real cells

Experimental detection methods

- PPIs have been studied extensively using different experimental methods
- Many are small-scale: Two given proteins in a given condition
  - Classical biochemistry
- High-throughput methods
  - Yeast two-hybrid assays (Y2H)
  - Tandem affinity purification and mass spectrometry (TAP-MS)

Yeast two-hybrid screens

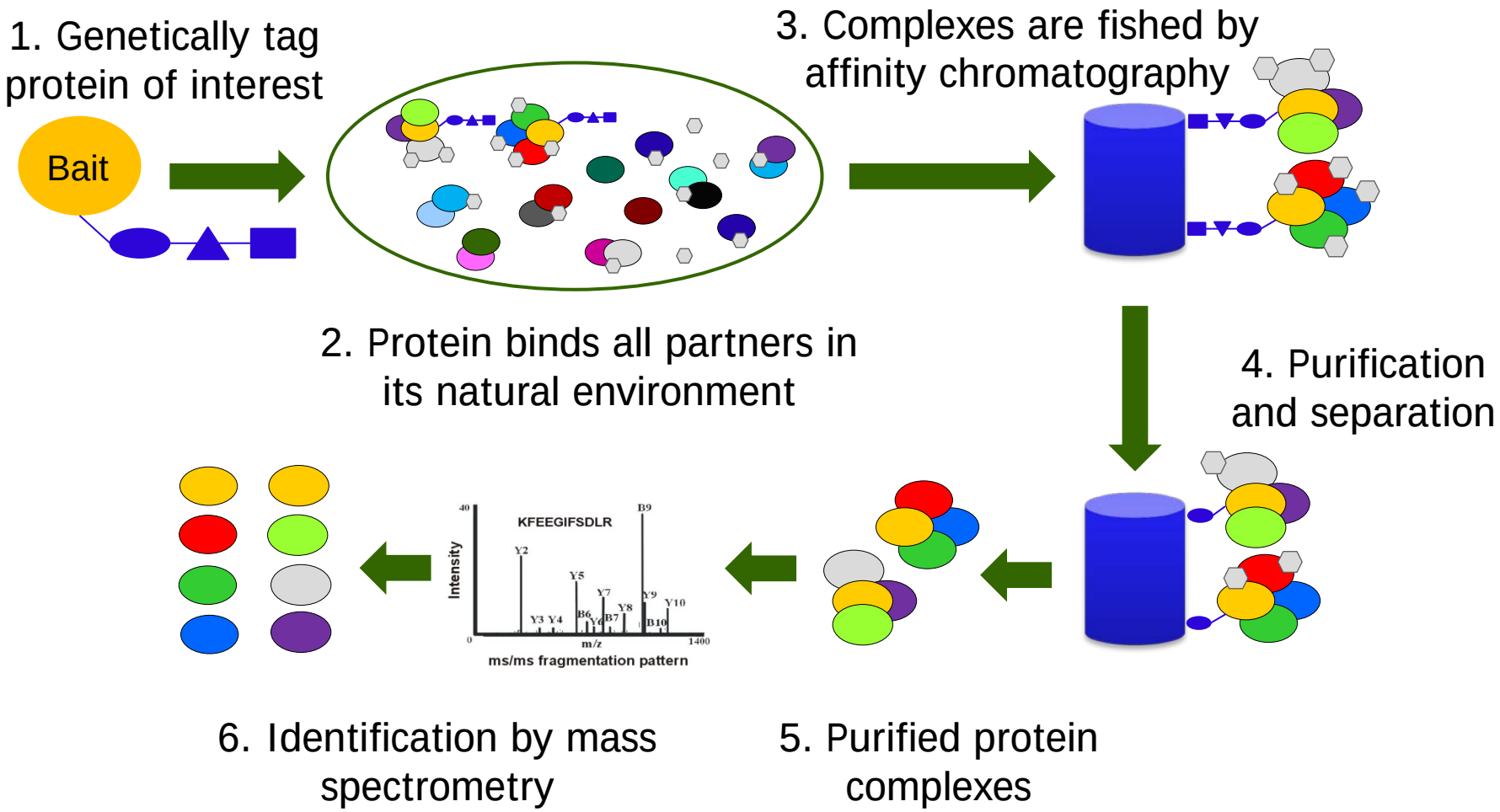
- Test if protein A (bait) is interacting with B (prey)
  - Choose a transcription factor T and reporter gene G such that
    - If activated T binds to promoter of G, G is expressed
    - Expression of G can be measured
    - T has two domains: Binding domain (BD) and Activation Domain (AD)
  - Bait is fused to DNA binding domain of T
  - Prey is fused to activating domain of T
  - Both are expressed in genetically engineered yeast cells
  - If A binds to B, T is assembled and G is expressed



Properties

- Advantages
  - Throughput: Many preys tested with same bait (and vice versa)
  - Can be automized – high coverage of interactome
    - Insertion of specifically designed gene complexes into yeast
  - Readout very sensitive (PCR)
- Problems
  - High rate of false positives (up to 50%)
    - Artificial environment and regulation: Yeast cells
    - No post-translational modifications, no spatial context
    - Unclear under which conditions the two proteins in vivo are expressed at the same time
    - ...
  - Fusion influences binding behavior – false negatives

Tandem affinity purification and mass spectrometry

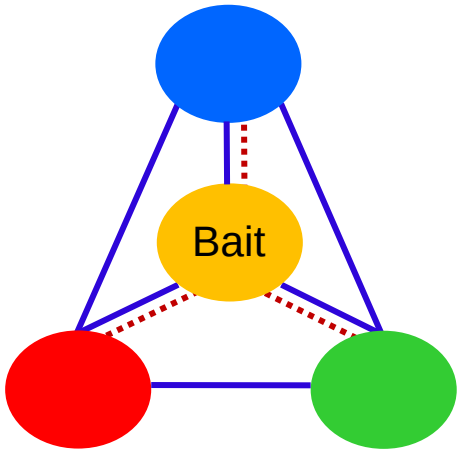


Properties

- Advantages
  - Can capture PPI in (almost – the tag) natural conditions
  - Single bait can detect many interactions in one experiment
  - Few false positives
- Disadvantages
  - Tag may hinder PPI – false negatives
  - Purification and MS are delicate processes
  - MS needs to measure a mixture of different proteins (complex)
  - Internal structure of complex is not resolved
    - Who binds whom?

Matrix / Spokes Model

- General: Methods cannot discern direct interactions from interactions mediated by other proteins (complexes)
- Matrix model: Assume interactions between all proteins of a purified complex → (N*(N-1))/2
- Spokes model: Assume only interactions between the bait and the co-purified proteins → N–1

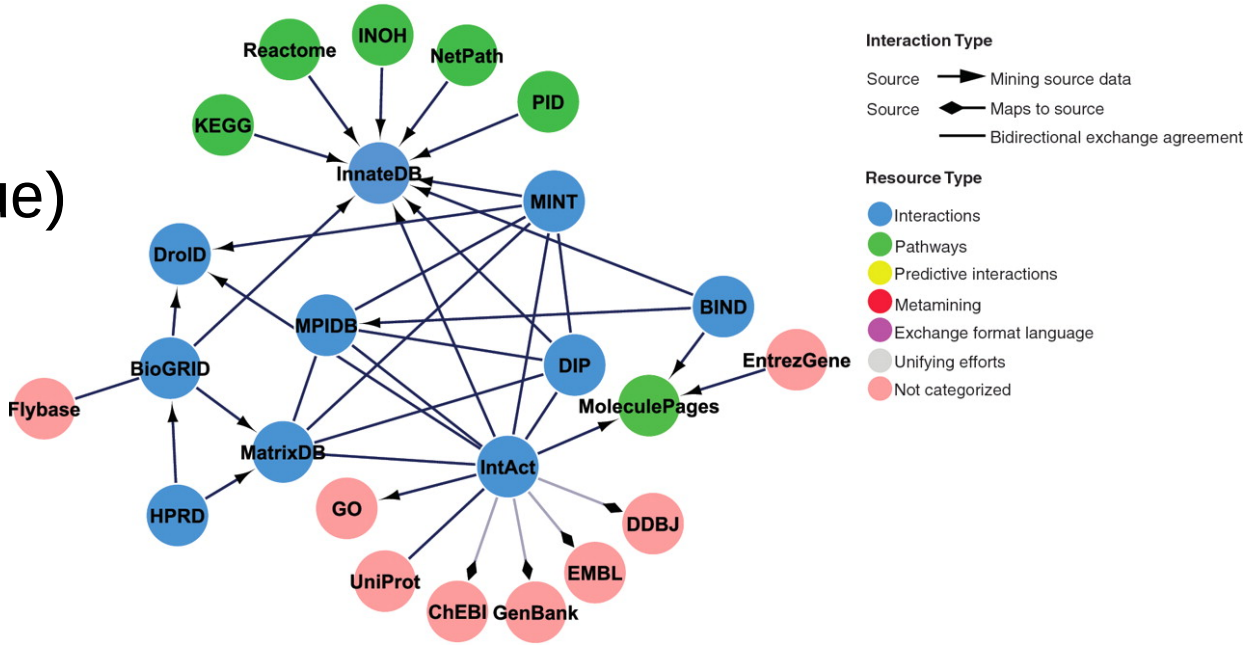


# Proteins	Matrix	Spokes
4	6	3
10	45	9
80	3540	79

PPI Databases [KP10]

- There are >700 DBs related to PPI and pathways
  - See http://www.pathguide.org

- Manually curated "source" DBs (blue)
  - Gather data from published low-throughput methods (text mining, curation)



PPI Databases

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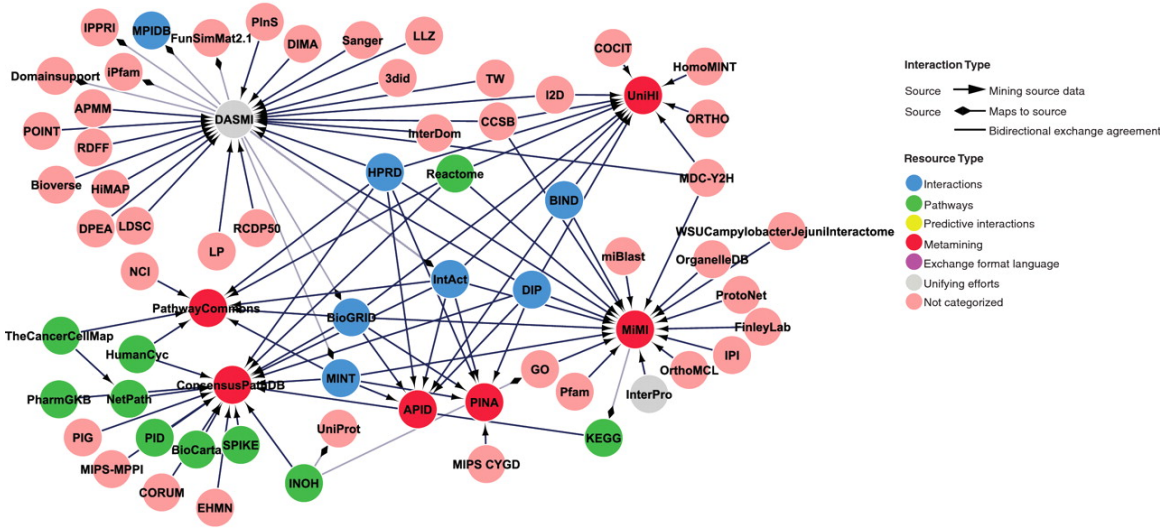
- Manually curated "source" DBs
- DBs integrating other DBs and HT data sets (red)



PPI Databases

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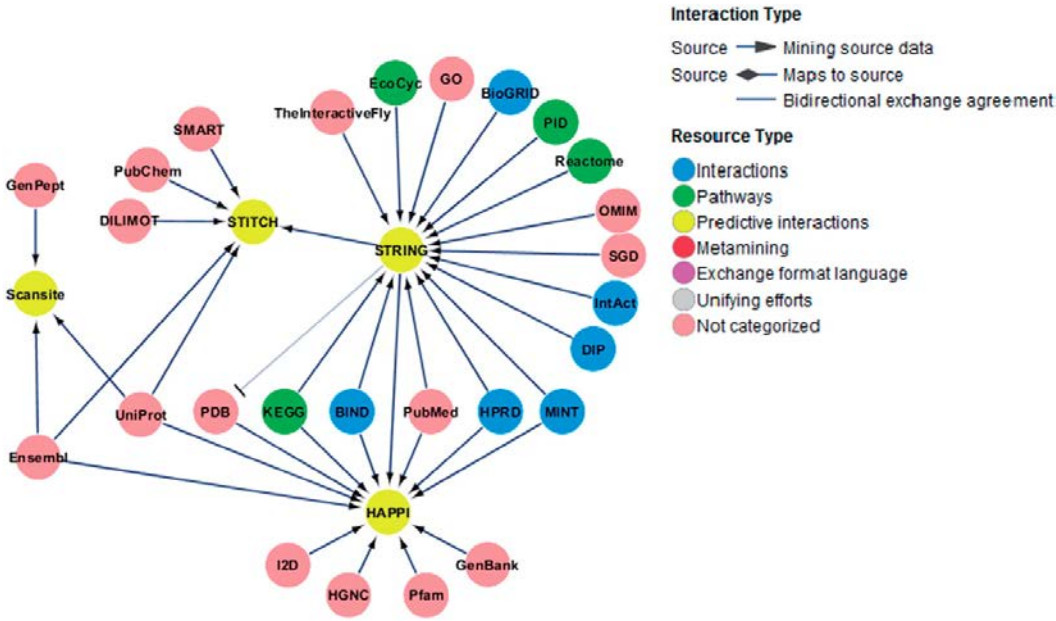
- Manually curated "source" DBs
- DBs integrating others and HT data sets
- Predicted interactions (yellow)



PPI Databases

- There are >700 BDBs related to PPI and pathways
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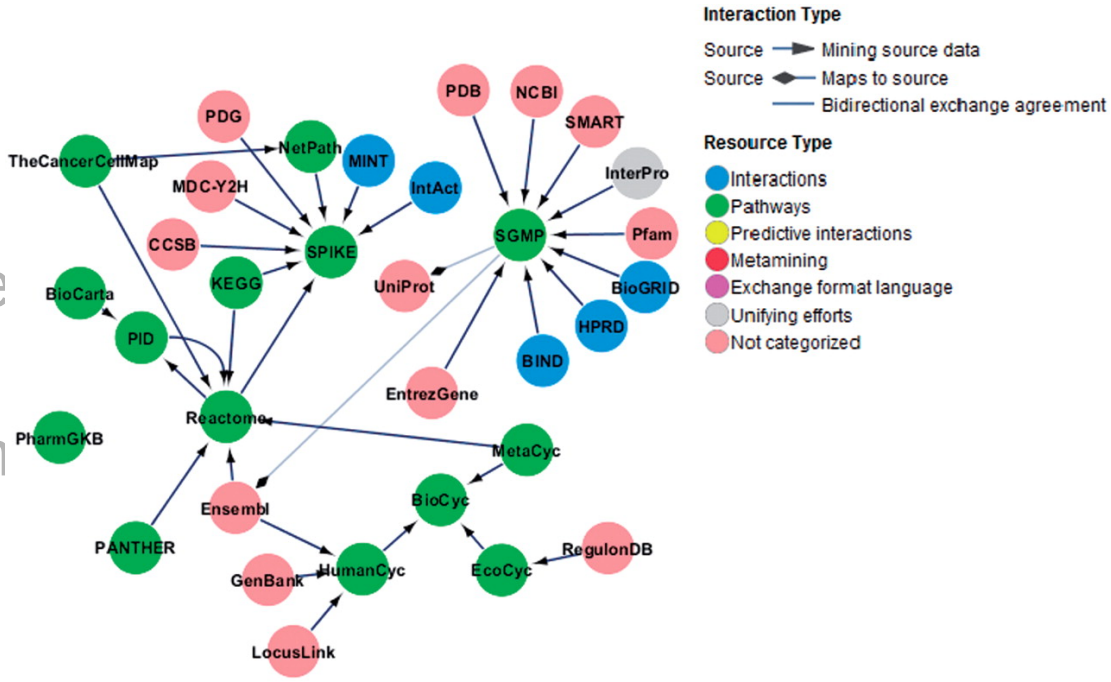
- Manually curated "source" DBs
- DBs integrating othe and HT data sets
- Predicted interaction
- Pathway DBs (green)



A Mess [KP10]

- Different definitions of a PPI
  - Only binary, physical interactions?
  - Inclusion of complexes or pathways?
  - Transient interactions? Functional associations?
- Consistency: Some integrated DBs have “imported” more data than there is in the sources
- Databases overlap to varying degrees
- Different reliability of content
- Literature-curated DBs do not guarantee higher quality than high-throughout experiments [CYS08]
  - Re-annotation reveals inconsistencies, subjective judgments, errors in gene name assignment, ...

Concrete Examples [KP10]

Database	Species	Proteins	Interactions
IntAct	No restriction	53.276	271.764
BioGrid	No restriction	30.712	131.638
DIP	No restriction	23.201	71.276
MINT	No restriction	31.797	90.505
HPRD	Human only	30.047	39.194
MMPPI	Mammals		
.			
STRING	No restriction (630)	2.590.259	
UniHI	Human only		
OPID	Human only		
:			

Experimentally verified

Experimentally verified and / or predicted

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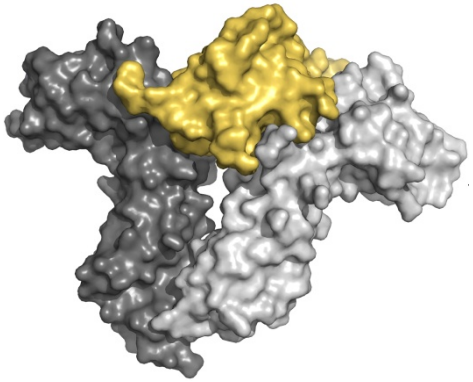
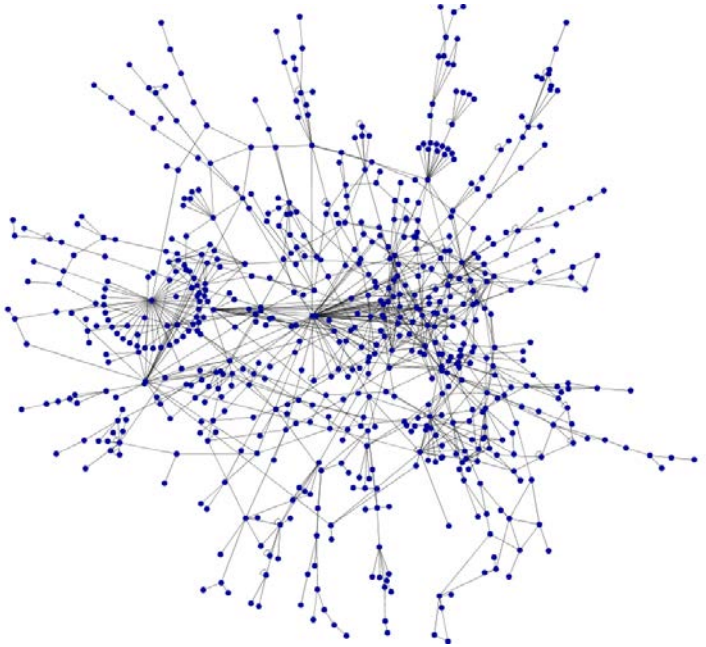
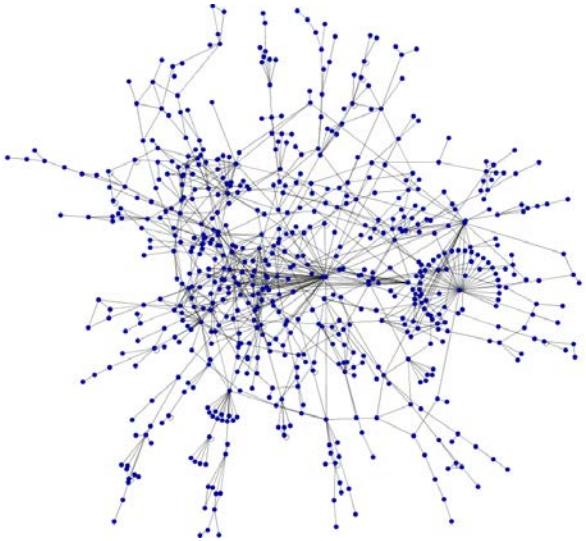


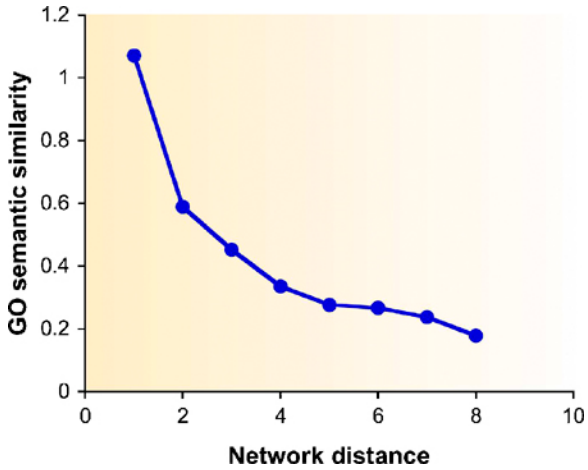
- Protein-protein interactions
- Biological networks
  - Scale-free graphs
  - Cliques and dense subgraphs
  - Centrality and diseases



Some Fundamental Observations

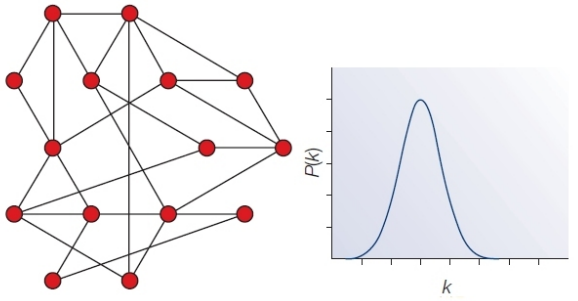
- Proteins that are close in the PPI-network of a cell share function more frequently than distant proteins
- Central proteins are vital
- Complexes form dense subgraphs
- Functional modules are subgraphs
- Certain subgraphs can be found significantly more often than expected by chance (why?)

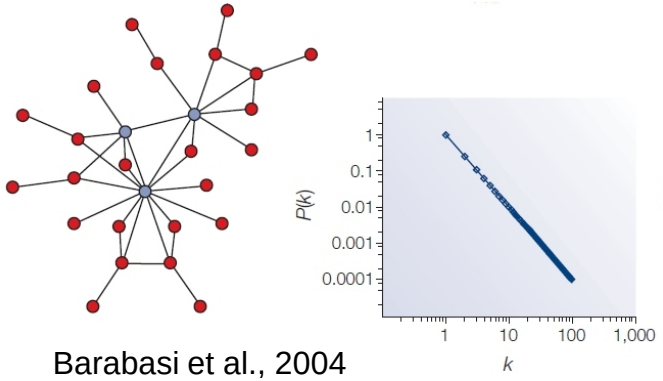




Degree distribution

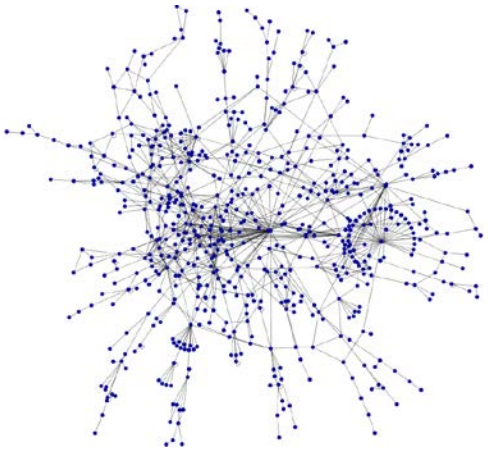
- Degree distribution P(k): relative frequency of nodes with degree *k*
- Used to define different classes of networks
- Common distributions
  - Poisson $P(k) \sim \frac{\lambda^k}{k!} e^{-\lambda}$
    - Random networks
  - Power-law $P(k) \sim k^{-\gamma}$
    - Scale-free networks



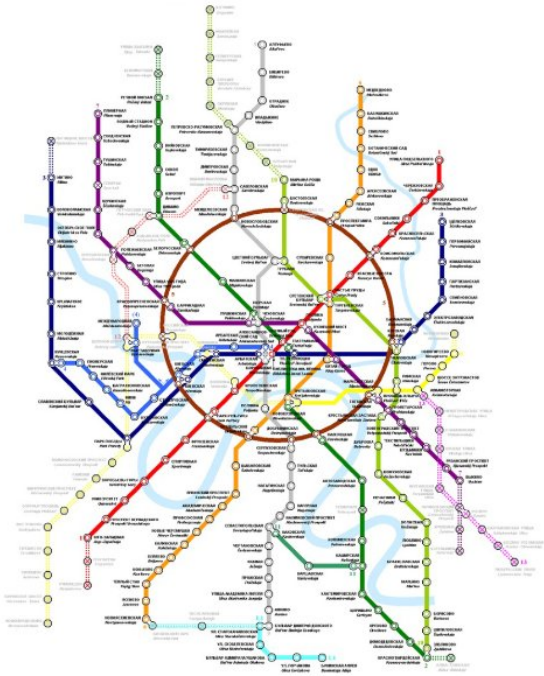


Barabasi et al., 2004

Scale-free Networks



- Biological networks are (presumably) scale-free
  - Few nodes are highly connected (hubs)
  - Most nodes have very few connections
- Also true for many other graphs: electricity networks, public transport, social networks, ...
- Evolutionary explanation -
  - Growth: Networks grow by addition of new nodes
  - Preferential attachment: new nodes prefer linking to highly connec. nodes
    - Possible explanation: Gene duplication – interaction with same targets
  - Older nodes have more changes to connect to nodes
  - Hub-structure emerges naturally



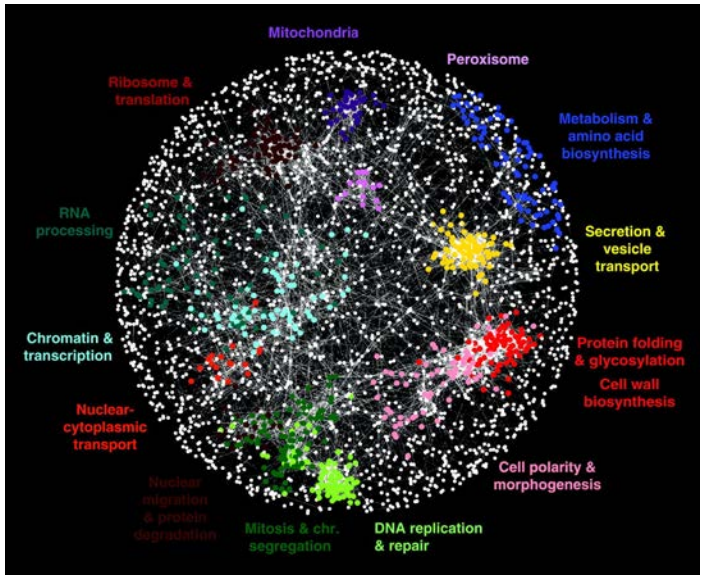

MetroNorte
MetroEste
TFM
MetroSur

Other Biological Networks

- Regulatory networks: How genes / transcription factors influence the expression of each other
  - TF regulate expression of genes and of other TFs
  - Edges semantics: activate / inhibit / regulate
- Signal networks: Molecular reaction to external stimulus
  - Transient interactions including small molecules
  - Temporal dimension important (fast)
- Metabolic networks
- Protein-protein interaction networks
- Stoecheometric networks: Flow of atoms in chemical reactions
  - Kinetic networks: Include energy consumption

Modular network organization

- Cellular function is carried out by modules
  - Sets of proteins interacting to achieve a certain function
- Function is reflected in a modular network structure

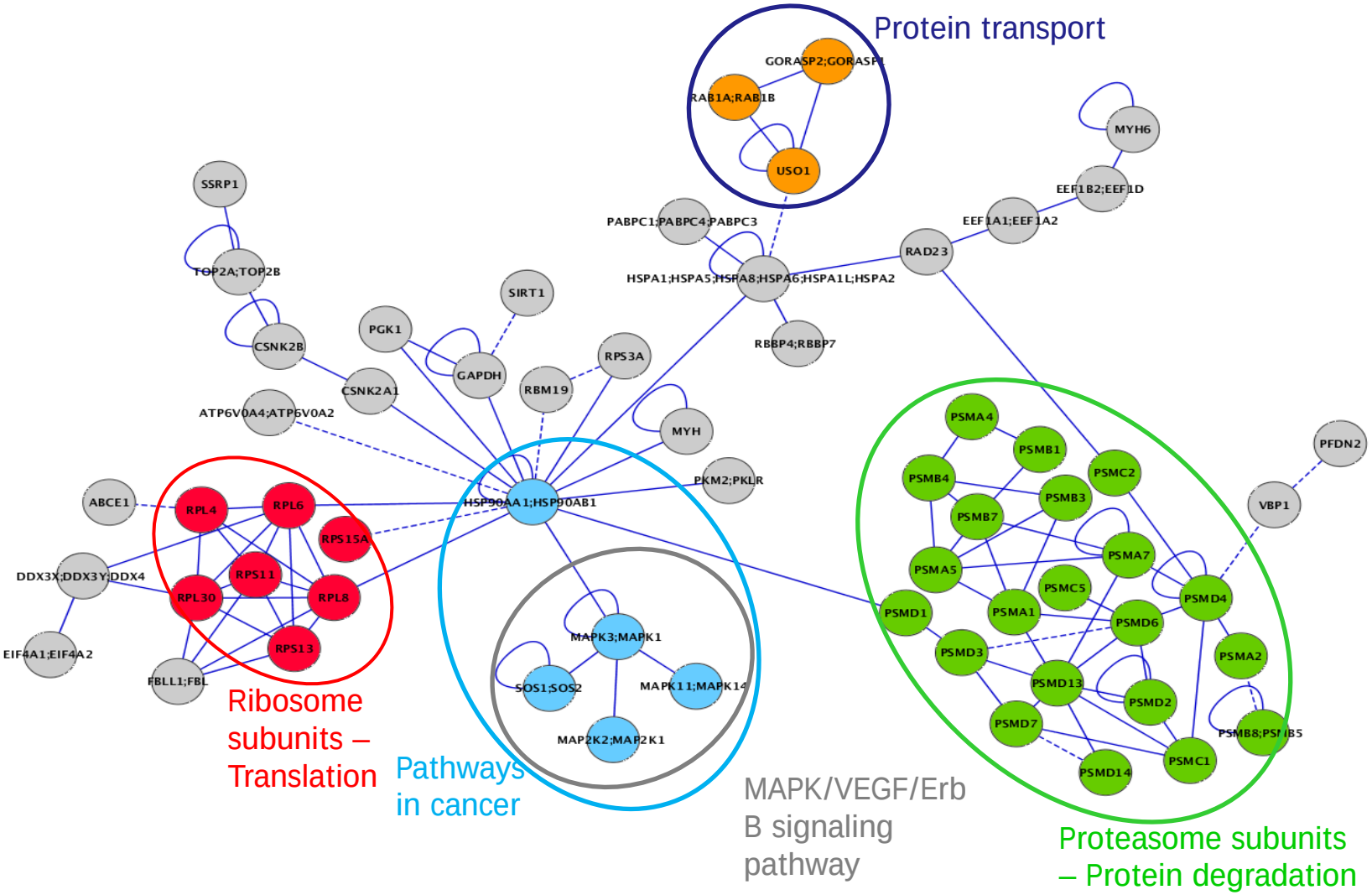


Don‘t be fooled by layout

Modules must be dense, not close

Costanzo et al., Nature, 2010

Functional Modules



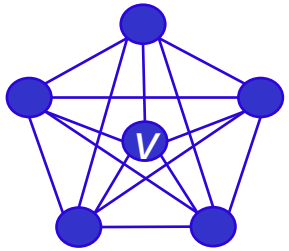
Clustering Coefficient

- Modules (clusters) are densely connected groups of nodes
- Cluster coefficient C reflects network modularity by measuring tendency of nodes to cluster ('triangle density')

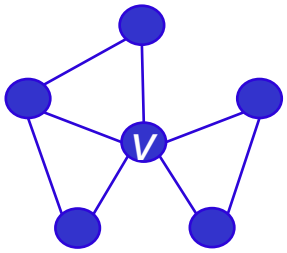
$$C_v = \frac{2E_v}{d_v(d_v - 1)} \longrightarrow C = \frac{1}{|V|}\sum_{v \in V} C_v$$

  - E_v = number of edges between neighbors of v
  - d_v = number of neighbors of v
  - $\frac{d_v(d_v - 1)}{2}$ = maximum number of edges between neighbors d_v

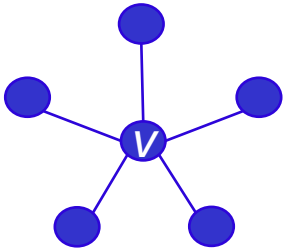
Example



$C_v = 10/10 = 1$



$C_v = 3/10 = 0.3$



$C_v = 0/10 = 0$

- Cluster coefficient C is a measure for the entire graph
- We also want to find modules, i.e., regions in the graph with high cluster coefficient
- A clique is a maximal complete subgraph, i.e., a maximal set of nodes where every pair is connected by an edge

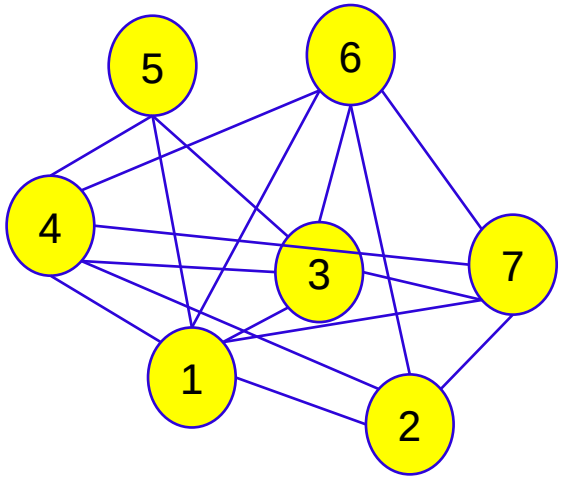
Finding Modules / Cliques

- Finding all (maximal) cliques in a graph is intractable
  - NP-complete
- Finding quasi- cliques is equally complex (yet much faster)
  - Cliques with some missing edges
  - Same as subgraphs with high cluster coefficient

```
build set S2 of all cliques of size 2
i:= 2;
repeat
  i := i+1;
  Si := ∅;
  for j := 1 to |Si-1|
    for k := j+1 to |Si-1|
      T := Si-1[j] ∩ Si-1[k];
      if |T|=i-2 then
        N := Si-1[j] ∪ Si-1[k];
        if N is a clique then
          Si := Si ∪ N;
        end if;
      end if;
    end for;
  end for;
until |Si| = 0:
```

- Various heuristics
  - E.g. a good quasi-clique probably contains a (smaller) clique

Example



- 4-cliques: (1,3,4,5) – (1,3,4,6) – (1,3,4,7) - ...
- Merge-Phase

$|(1,3,4,6) \cap (1,3,4,7)| = 3$

$(1,3,4,6) \cup (1,3,4,7) = (1,3,4,6,7)$

Edge (6,7) exists

5-clique

$|(1,3,4,5) \cap (1,3,4,6)| = 3$

$(1,3,4,5) \cup (1,3,4,6) = (1,3,4,5,6)$

Edge (5,6) does not exists

No 5-clique

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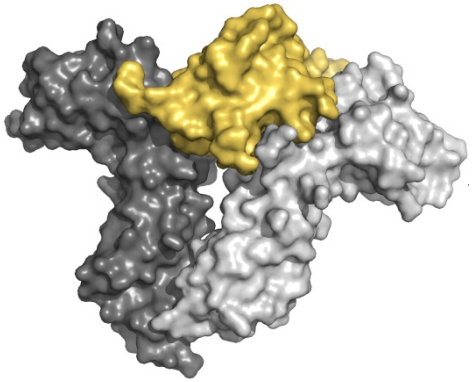

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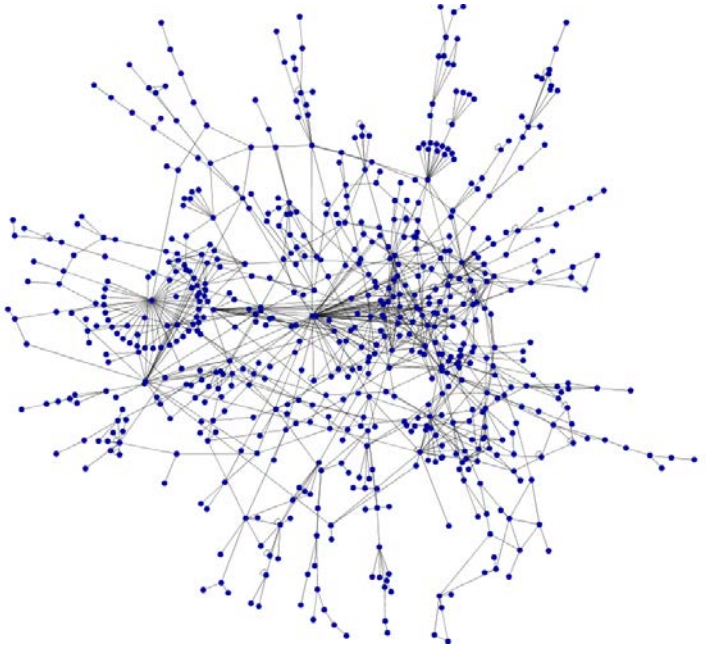
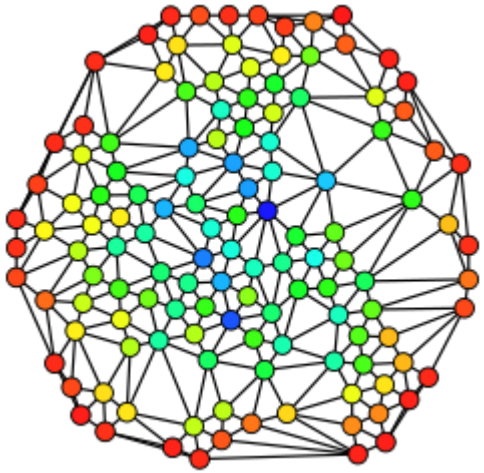
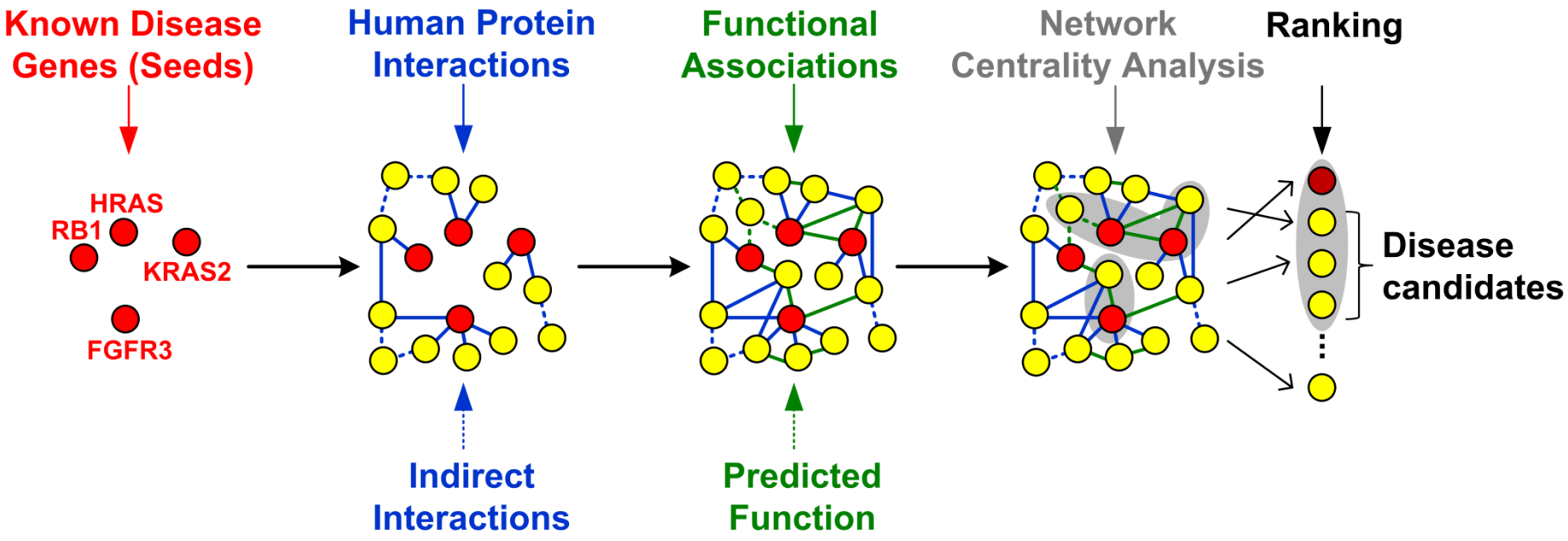


Network centrality

- Central proteins exhibit interesting properties
  - Essentiality – knock-out is lethal
  - Much higher evolutionary conservation
  - Often associated to (certain types of) human diseases
- Various measures exist
  - Degree centrality: Rank nodes by degree
  - Betweenness-centrality: Rank nodes by number of shortest paths between any pair of nodes on which it lies
  - Closeness-centrality: Rank nodes by their average distance to all other nodes
  - PageRank
  - ...

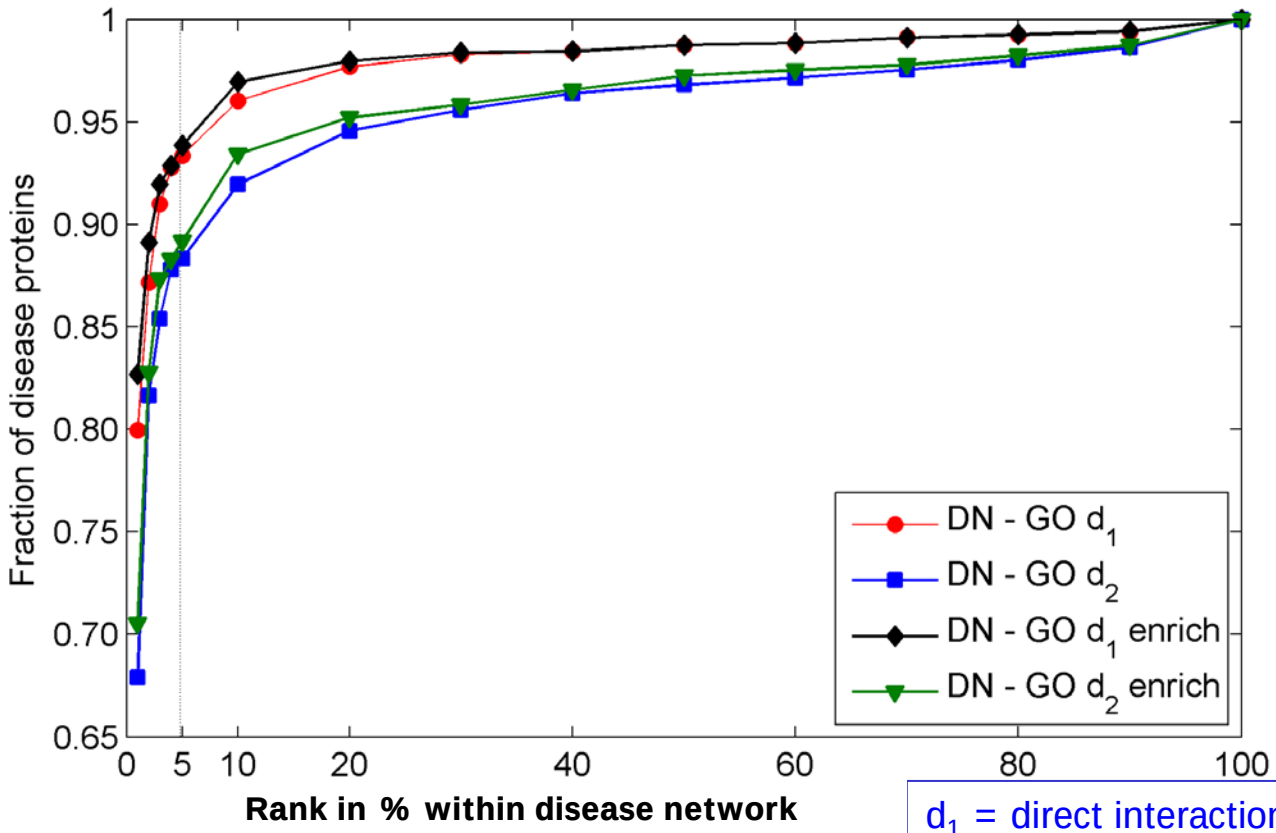


Network-based Disease Gene Ranking



Centrality of Seeds in (OMIM) Disease Networks

Fraction of seeds among top k% proteins; ~600 diseases from OMIM

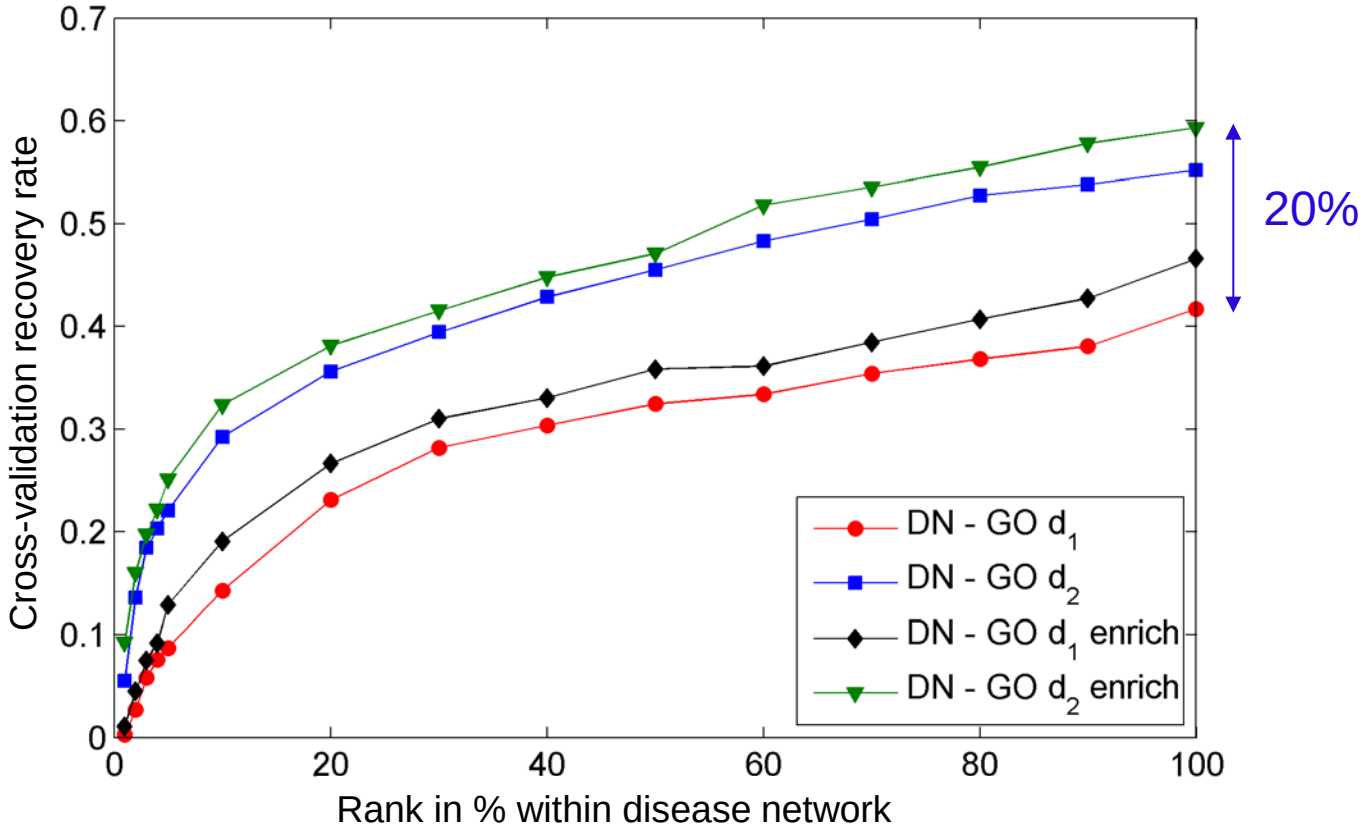


d_1 = direct interactions

d_2 = direct and indirect interactions

Cross-Validation

- If a disease gene is not yet known – can we find it?



Further Reading

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- Goh, K. I., Cusick, M. E., Valle, D., Childs, B., Vidal, M. and Barabasi, A. L. (2007). "The human disease network." Proc Natl Acad Sci U S A 104(21): 8685-90.
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