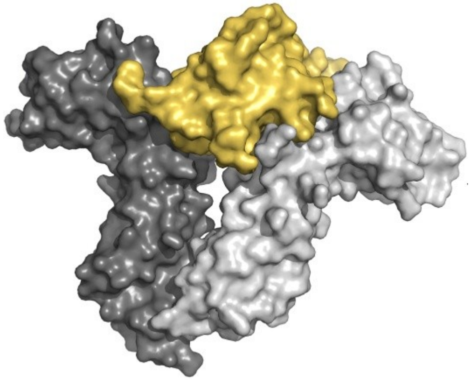
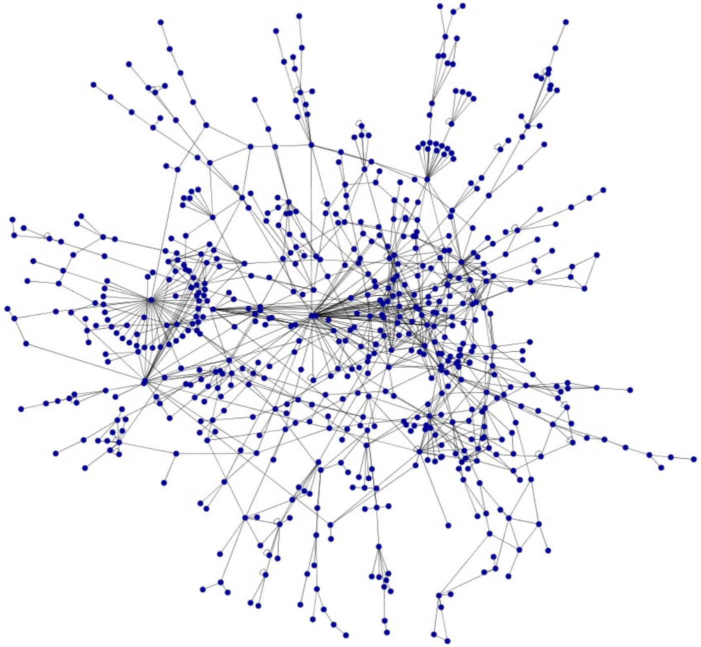
Protein-Protein-Interaction Networks

Ulf Leser, Samira Jaeger

This Lecture





- 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, metabolism, …
  - Transient or permanent
  - Directed effect (regulates), undirected (binds), specific (activates)
- 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 in a cell (type, species, …)
- Complex: Permanent binding of two or more proteins

Context-dependency

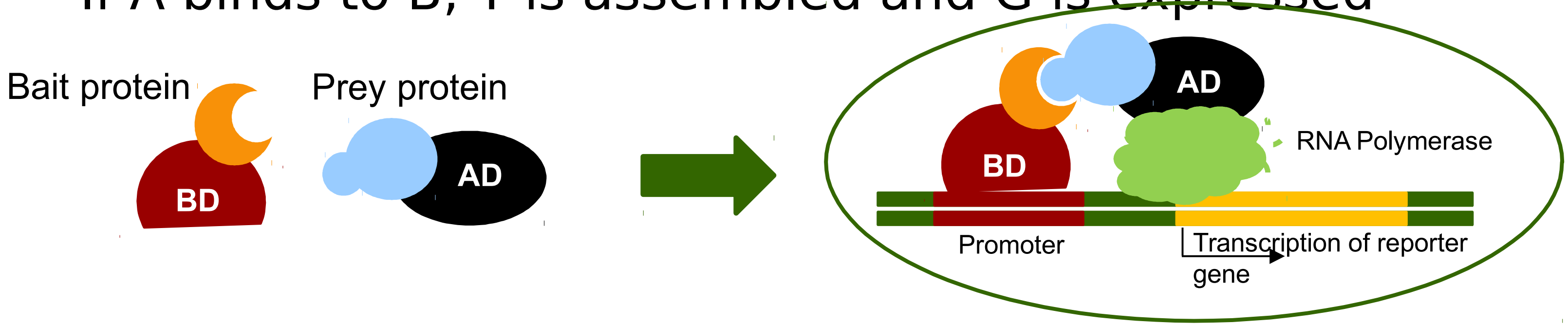
- PPI often is context-dependent
  - Cell type, cell cycle phase and state
  - Environmental conditions
  - Developmental stage
  - Protein modification
  - Presence of cofactors and other binding partners
  - ...
- Disregarded by many PPI detection methods
- Low quality of typical data sets

Experimental detection methods

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

Yeast two-hybrid screens

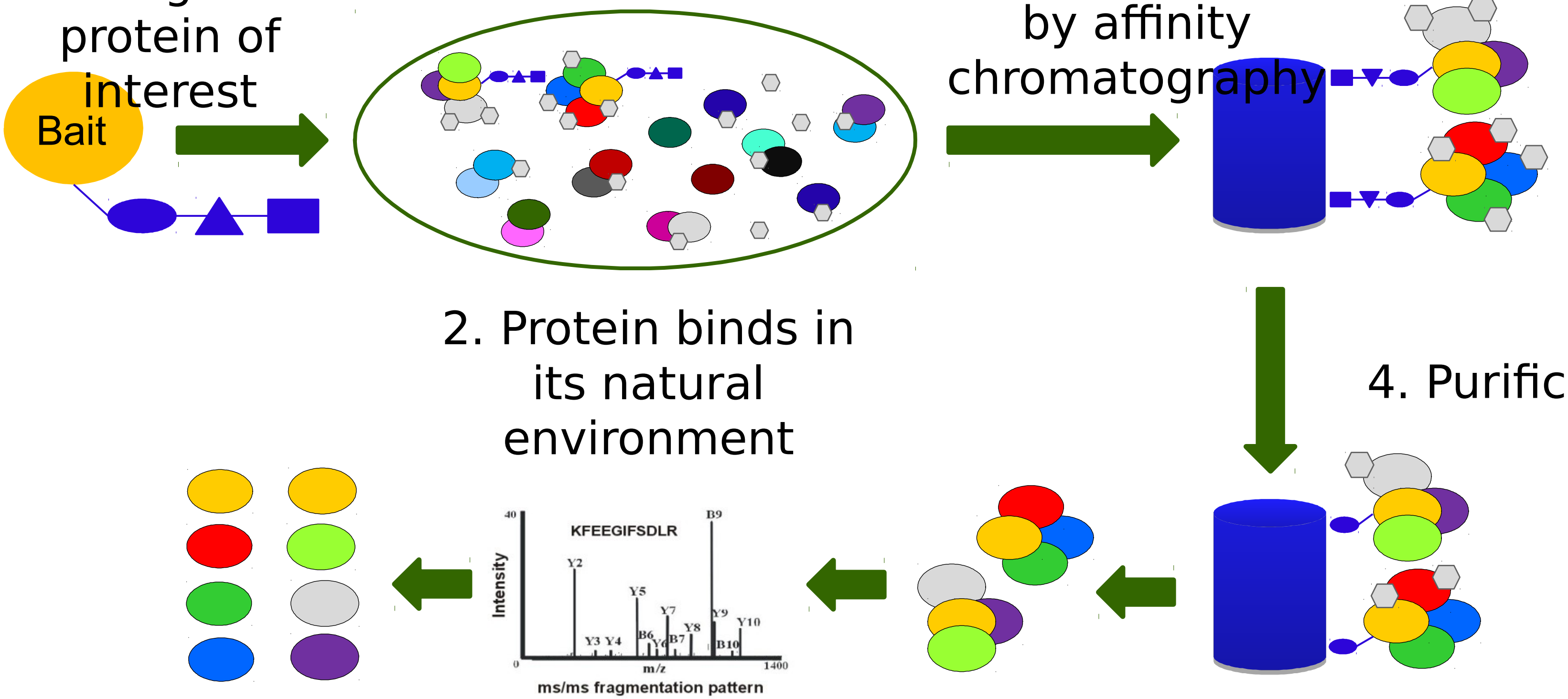
- Test if protein A (bait) is interacting with B (prey)
  - Choose a transcription factor T and reporter gene G such that
    - If activated T binds to promoter of G, G is expressed
    - Expression of G can be measured
    - T can be split in two domains: DNA binding and activation
  - Bait is fused 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 can be tested with same bait (and vice versa)
  - Can be automized – high coverage of interactome
  - Readout can be very sensitive
- Problems
  - High rate of false positives (up to 50%)
    - Artificial environment: Yeast cells
    - No post-translational modifications
    - No protein transport
    - Unclear if proteins in vivo are ever expressed at the same time
    - ...
  - Fusion influences binding behavior – false negatives

Tandem affinity purification and mass spectrometry



1. Tag the protein of interest

2. Protein binds in its natural environment

3. Complexes are fished by affinity chromatography

4. Purification

5. Purified protein complexes

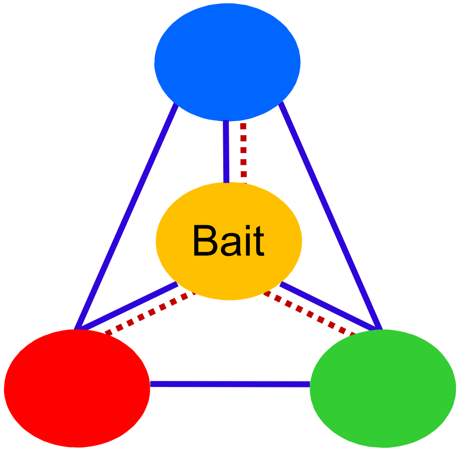
6. Identification of associated proteins by mass spectrometry

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
  - Difficult MS since the input is a mixture of different proteins
  - Individual complexes are not identified
  - Internal structure of complex is not resolved

Matrix / Spokes Model

- Direct interactions can not be distinguished from interactions mediated by other proteins in a complex
- Matrix model: infers interactions between all proteins of a purified complex → (N*(N-1))/2
- Spokes model: infers only interactions between the bait and the co-purified proteins → N–1

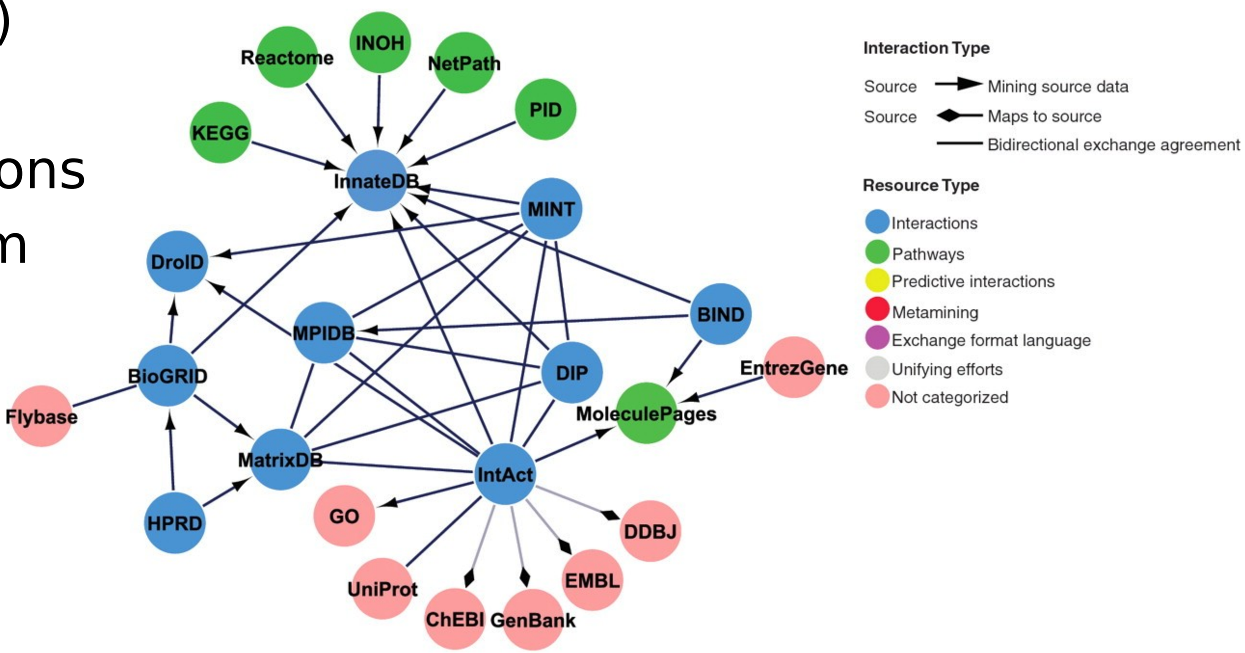


# Proteins	Matrix	Spokes
4	6	3
10	45	9
80	3540	79

PPI Databases [KP10]

- There are >300 DBs related to PPI and pathways
  - See http://www.pathguide.org

- Manually curated "source" DBs (blue)
  - Experimentally proven interactions
  - Gather data from low-throughput methods



PPI Databases

- There are >300 DBs related to PPI and pathways
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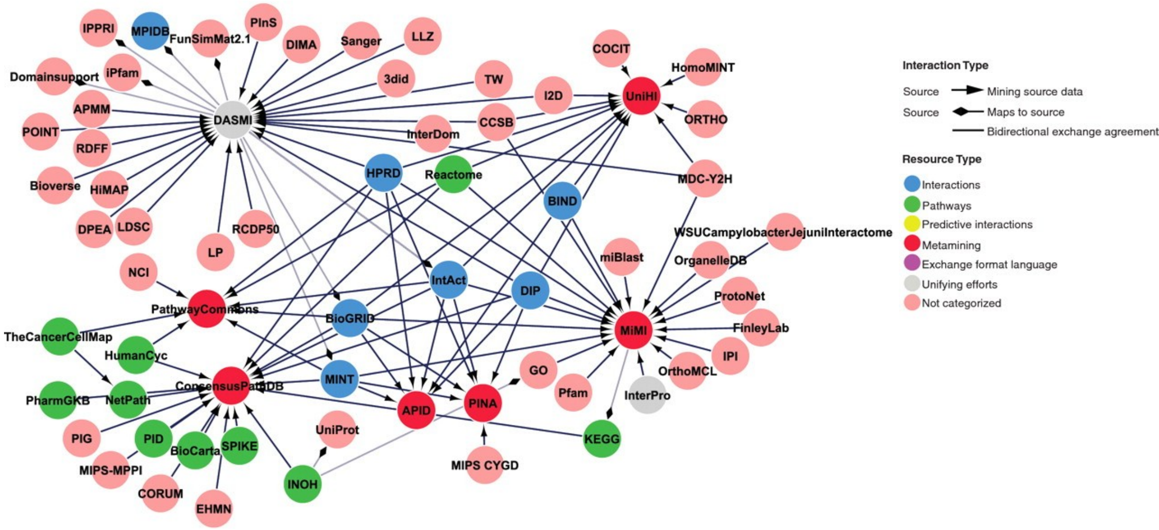
- Manually curated "source" DBs
- DBs integrating other DBs and HT data sets (red)



PPI Databases

- There are >300 DBs related to PPI and pathways
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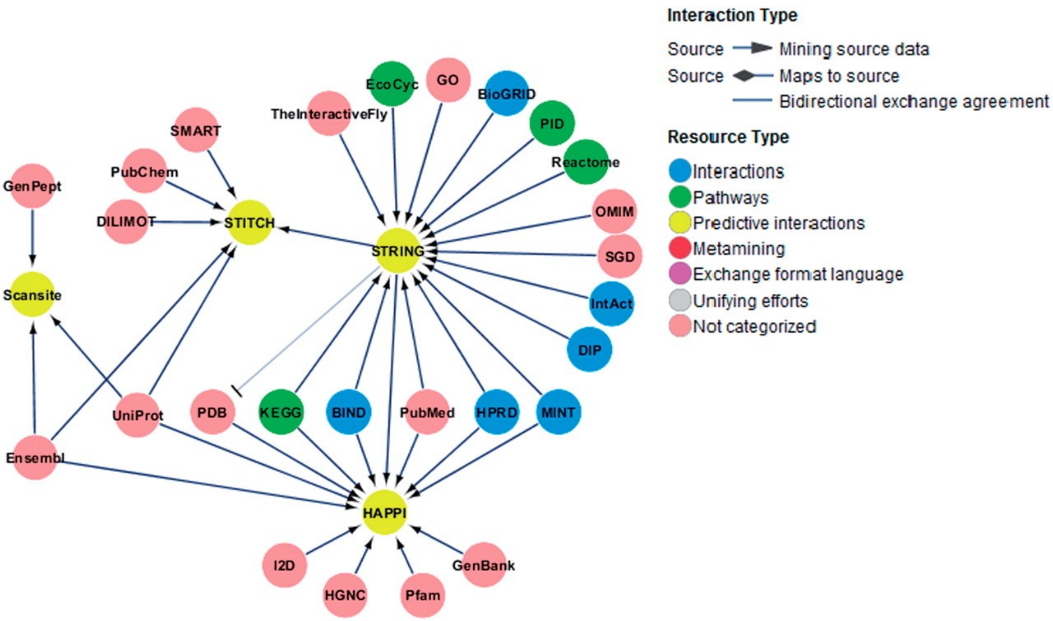
- Manually curated “source” DBs
- DBs integrating others and HT data sets
- Predicted interactions (yellow)



PPI Databases

- There are >300 DBs related to PPI and pathways
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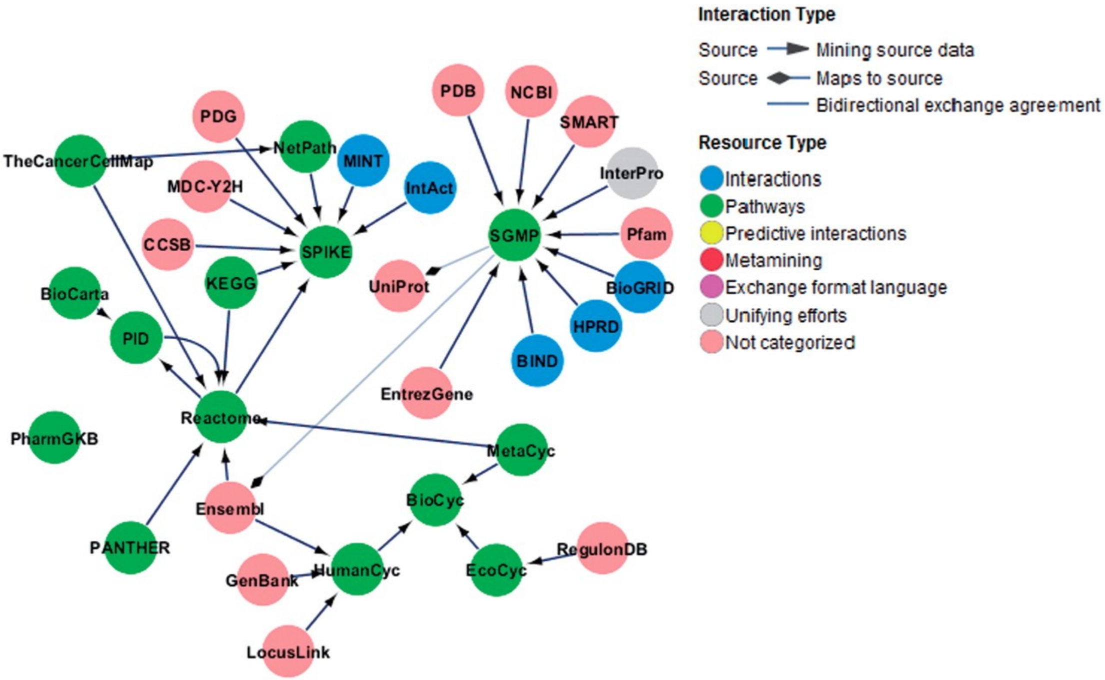
- Manually curated “source” DBs
- DBs integrating ot and HT data sets
- Predicted interact
- Pathway DBs (green)



A Mess [KP10]

- Different definitions of a PPI
  - Binary, physical interaction
  - Complexes, pairs, pathways
  - Transient, functional association
- 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, …

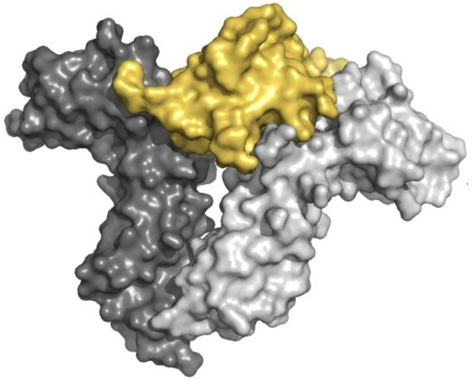
Concrete Examples

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		

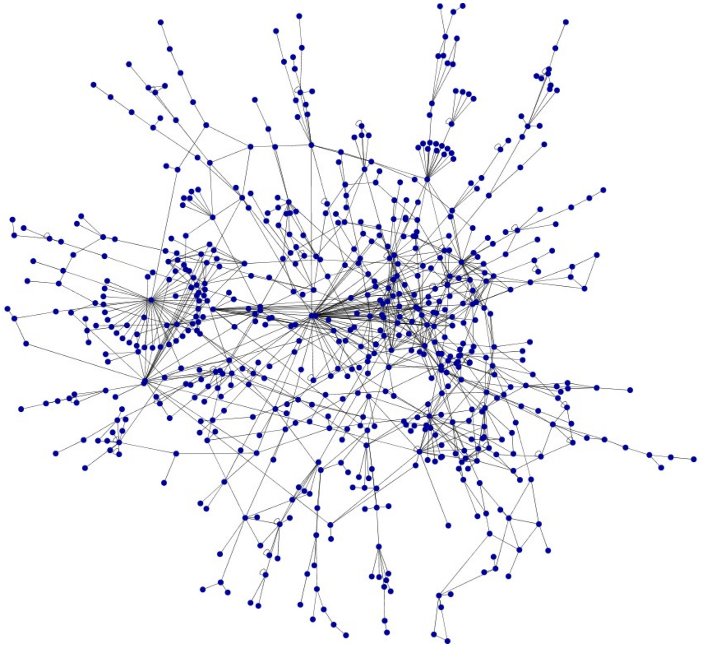
Experimentally verified

Experimentally verified and / or predicted

This Lecture

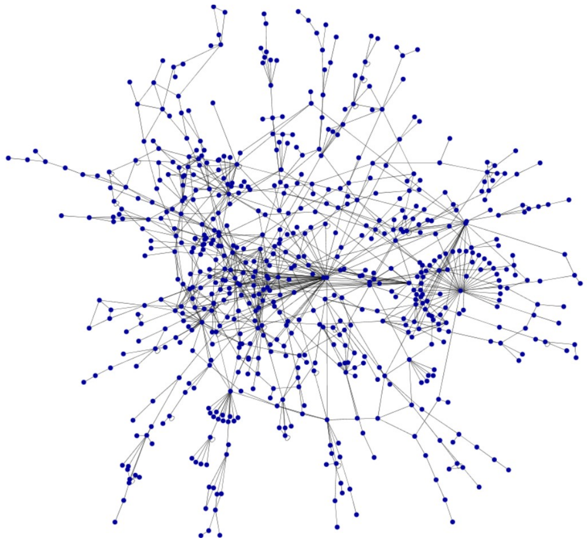


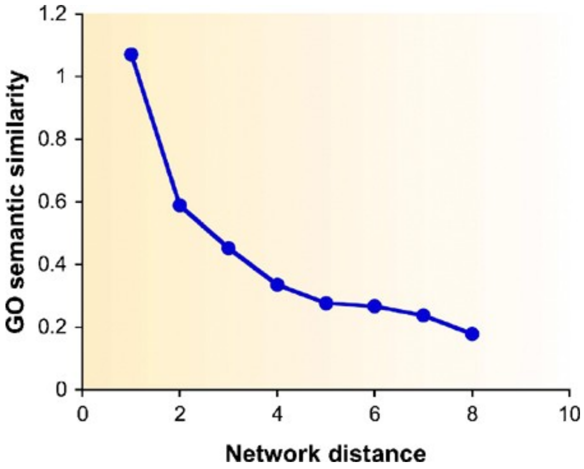
- Protein-protein interactions
- Biological networks
  - Scale-free graphs
  - Cliques and dense subgraphs
  - Centrality and diseases



Some Fundamental Observations

- Proteins that are close in the network share function more frequently
- 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?)



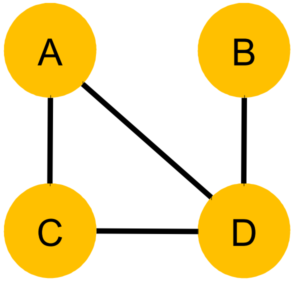


Protein-protein interaction networks

- Networks are represented as undirected graphs
- Definition of a graph: *G = (V,E)*
  - *V* is the set of nodes (proteins)
  - *E* is the set of binary, undirected edges (interactions)
- Computational representation

Adjacency lists

{ (A,C), (A,D), (B,D), (C,A), (C,D) (D,B) , (D,C), (D,A) }

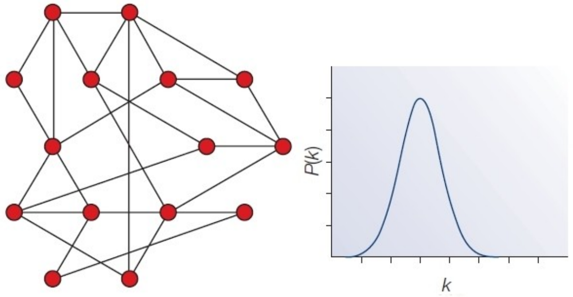


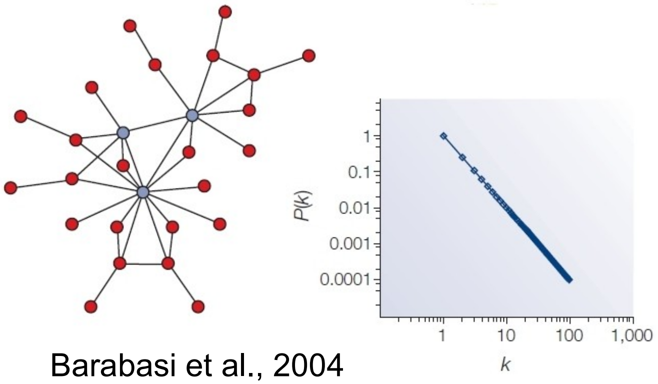
Adjacency matrix

	A	**B**	**C**	**D**
A	0	0	1	1
B	0	0	0	1
C	1	0	0	1
D	1	1	1	0

Degree distribution

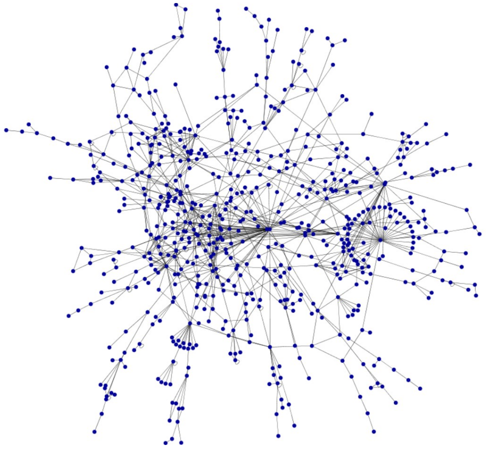
- Degree distribution P(k): relative frequency of nodes with degree *k*
- Used to define different classes of networks
- Common distributions
  - Poisson $P(k) \sim \frac{\lambda^k}{k!} e^{-\lambda}$
    - Random networks
  - Power-law $P(k) \sim k^{-\gamma}$
    - Scale-free networks



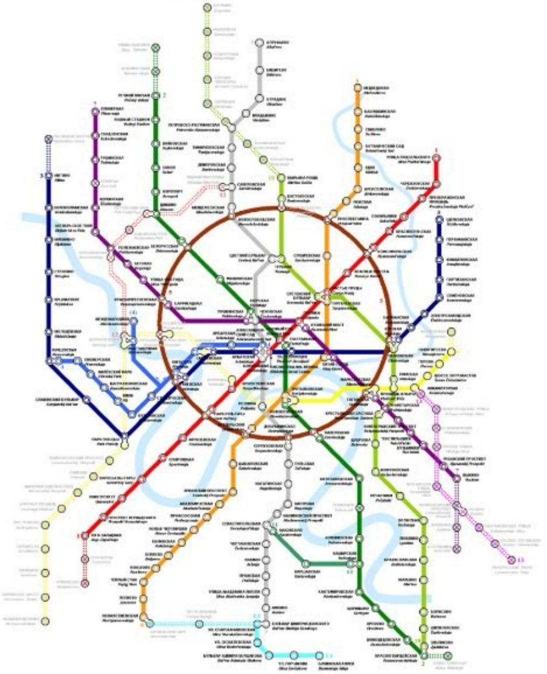


Barabasi et al., 2004

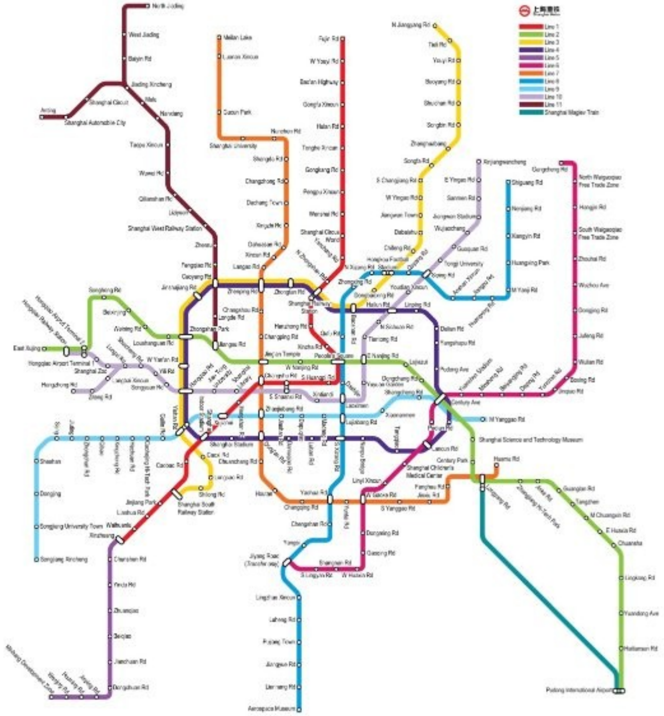
Scale-free Networks



- Biological networks are (presumabl 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 chances to connect to nodes
  - Hub-structure emerges naturally



MetroNorte
MetroEste
TFM
MetroSur

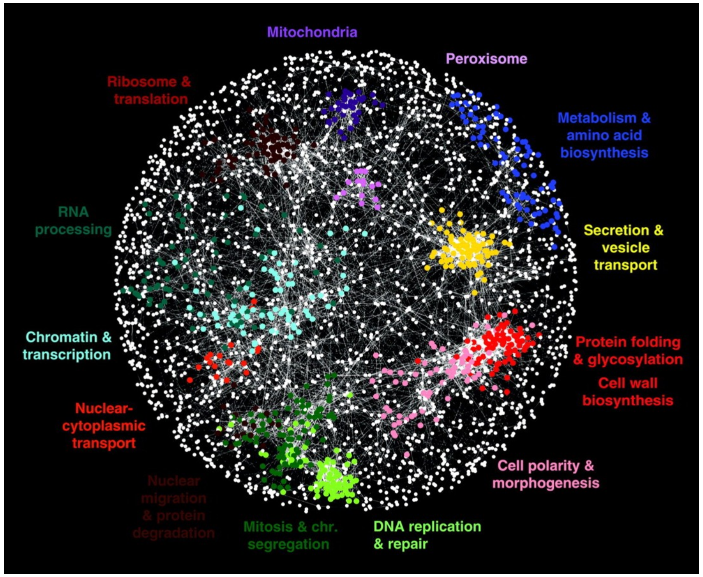


Other Biological Networks

- 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
  - Important, for instance, in cell differentiation
- Signal networks: Molecular reaction to external stimulus
  - Transient interactions including small molecules
  - Temporal dimension important (fast)
  - Important, for instance, in oncology
- Metabolic networks
- Protein-protein interaction networks

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

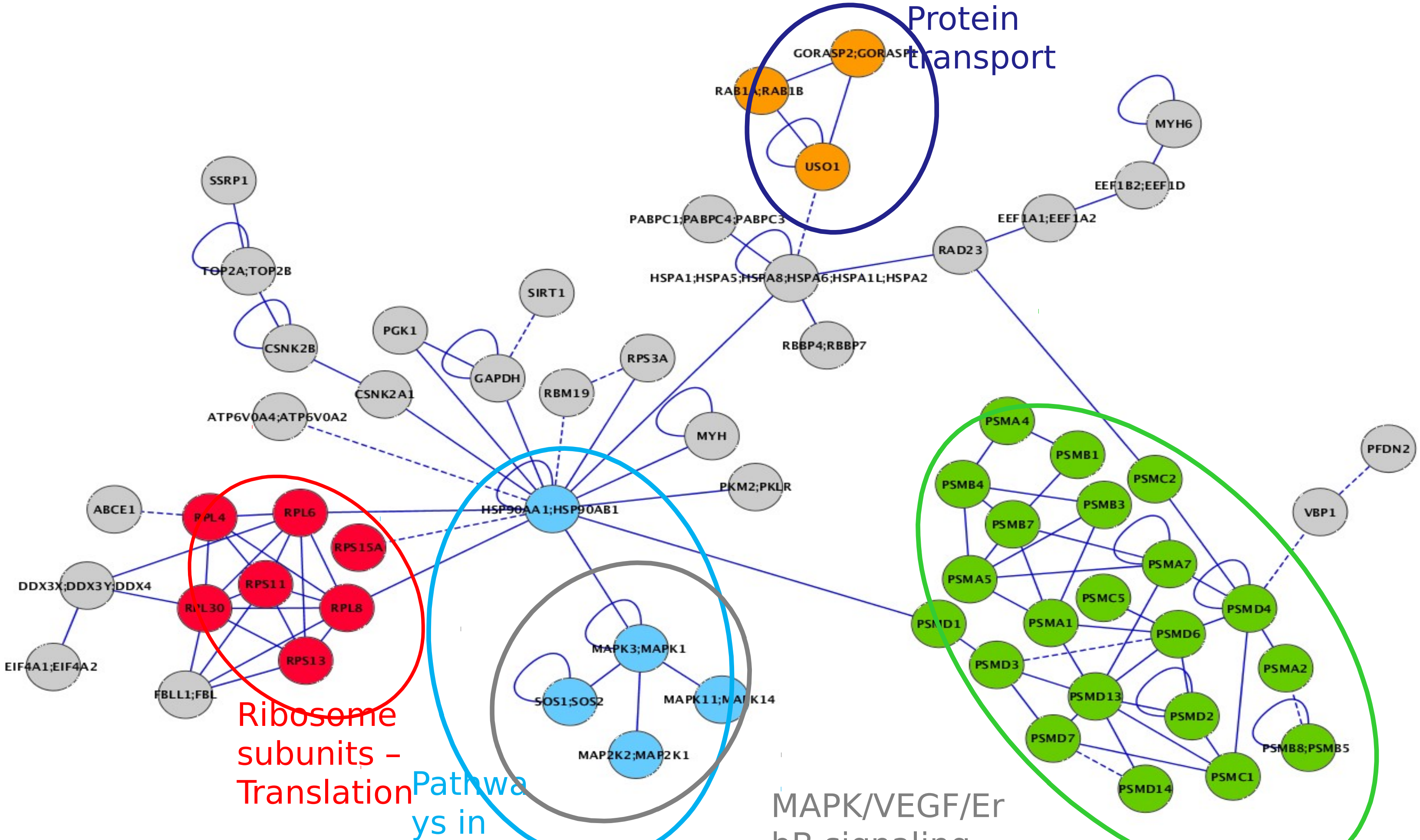

Mitochondria
Peroxisome
Ribosome & translation
Metabolism & amino acid biosynthesis
RNA processing
Secretion & vesicle transport
Chromatin & transcription
Protein folding & glycosylation
Cell wall biosynthesis
Nuclear-cytoplasmic transport
Cell polarity & morphogenesis
Nuclear migration & protein degradation
Mitosis & chr. segregation
DNA replication & repair

Don‘t be fooled by layout

Modules must be dense, not close

Costanzo et al., Nature, 2010

Functional Modules



Protein transport

Ribosome subunits – Translation

Pathways in cancer

MAPK/VEGF/ErbB signaling pathway

Proteasome subunits – Protein degradation

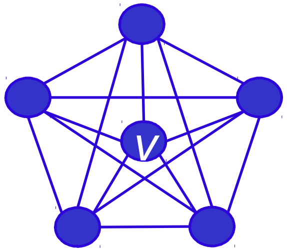
Clustering Coefficient

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

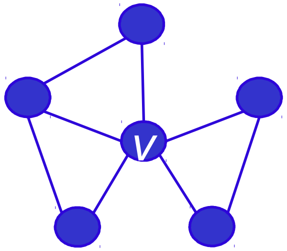
$$C_v = \frac{2E_v}{d_v(d_v - 1)} \longrightarrow C = \frac{1}{|V|} \sum_{v \in V} C_v$$

  - 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

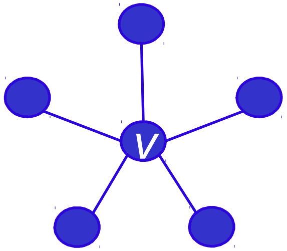
Example



$C_v = 10/10 = 1$



$C_v = 3/10 = 0.3$



$C_v = 0/10 = 0$

- 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

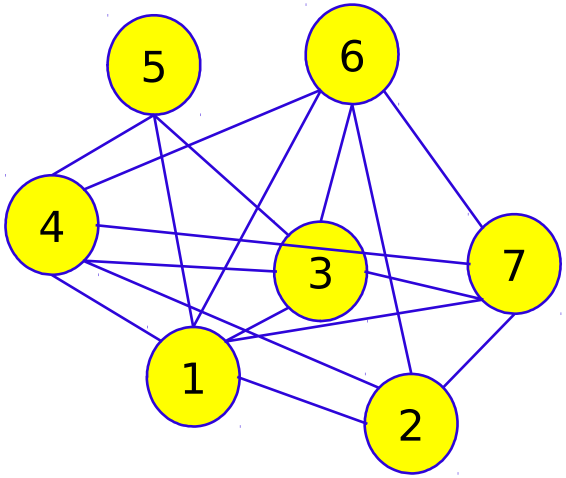
Finding Modules / Cliques

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

```
build set $S_2$ of all cliques of size 2
i:= 2;
repeat
  i := i+1;
  $S_i$ := ∅;
  for j := 1 to $|S_{i-1}|$
    for k := j+1 to $|S_{i-1}|$
      T := $S_{i-1}$[j] ∩ $S_{i-1}$[k];
      if |T|=i-1 then
        N := $S_{i-1}$[j] ∪ $S_{i-1}$[k];
        if N is a clique then
          $S_i$ := $S_i$ ∪ N;
        end if;
      end if;
    end for;
  end for;
until $|S_i|$ = 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 clique

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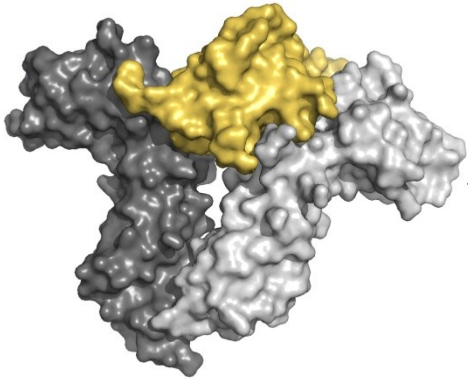
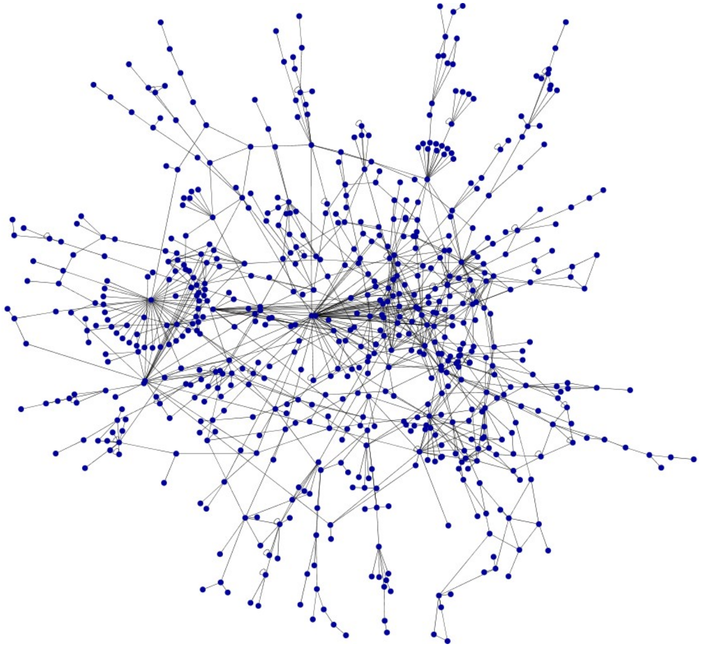
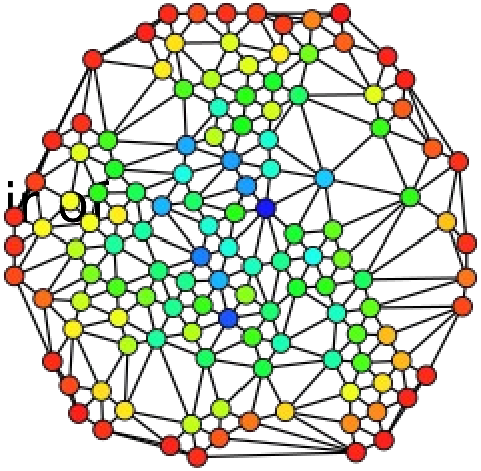


- Protein-protein interactions
- Biological networks
  - Scale-free graphs
  - Cliques and dense subgraphs
  - Centrality and diseases

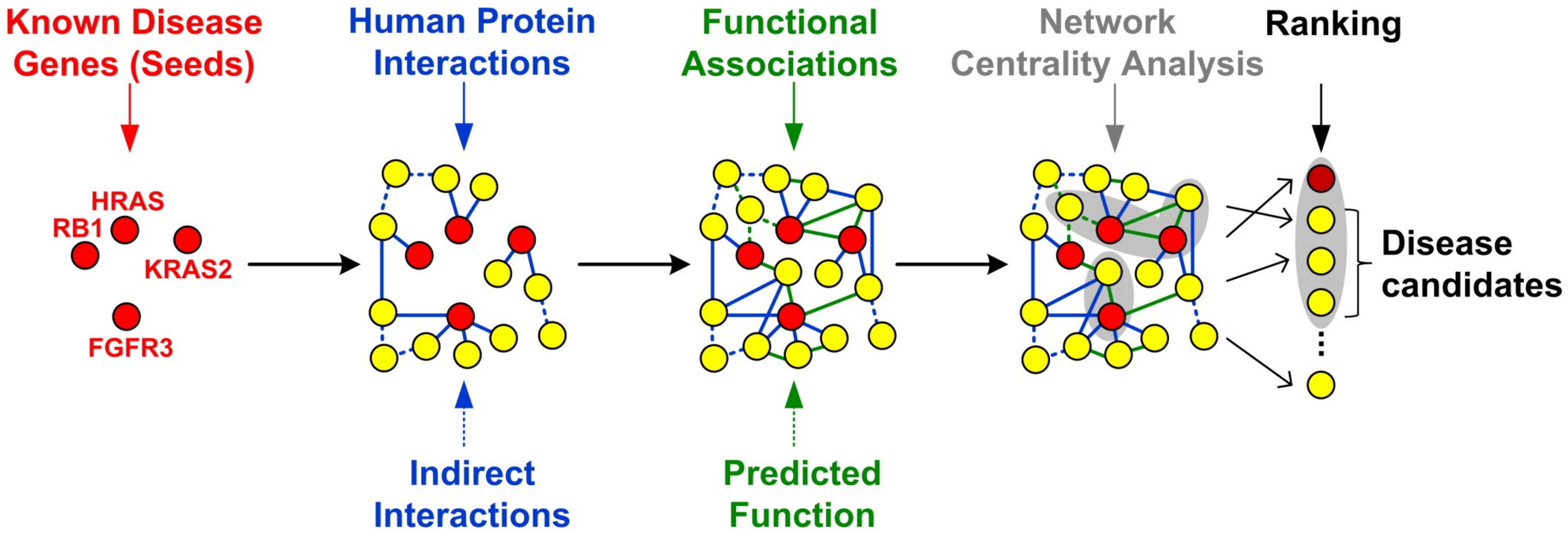


Network centrality

- 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
  - …

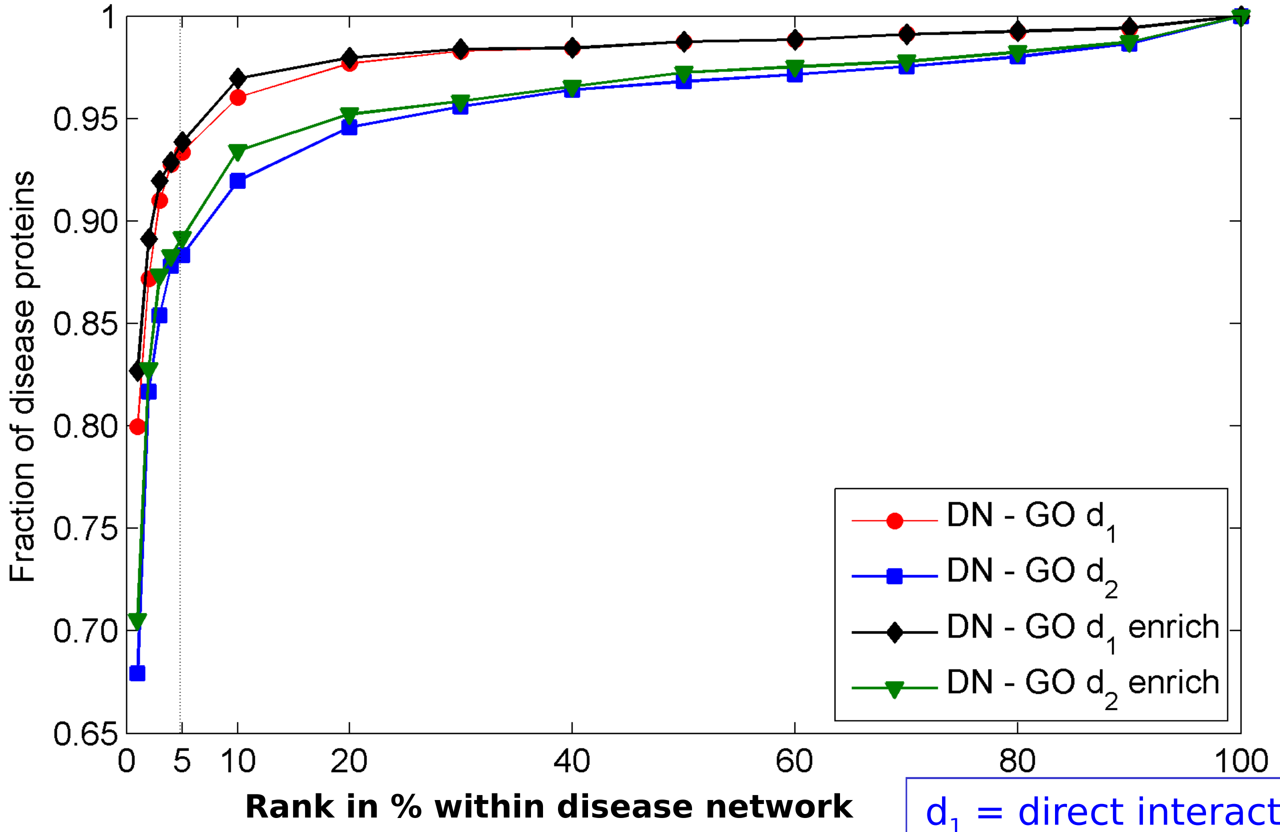


Network-based Disease Gene Ranking



Centrality of Seeds in (OMIM) Disease Networks

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

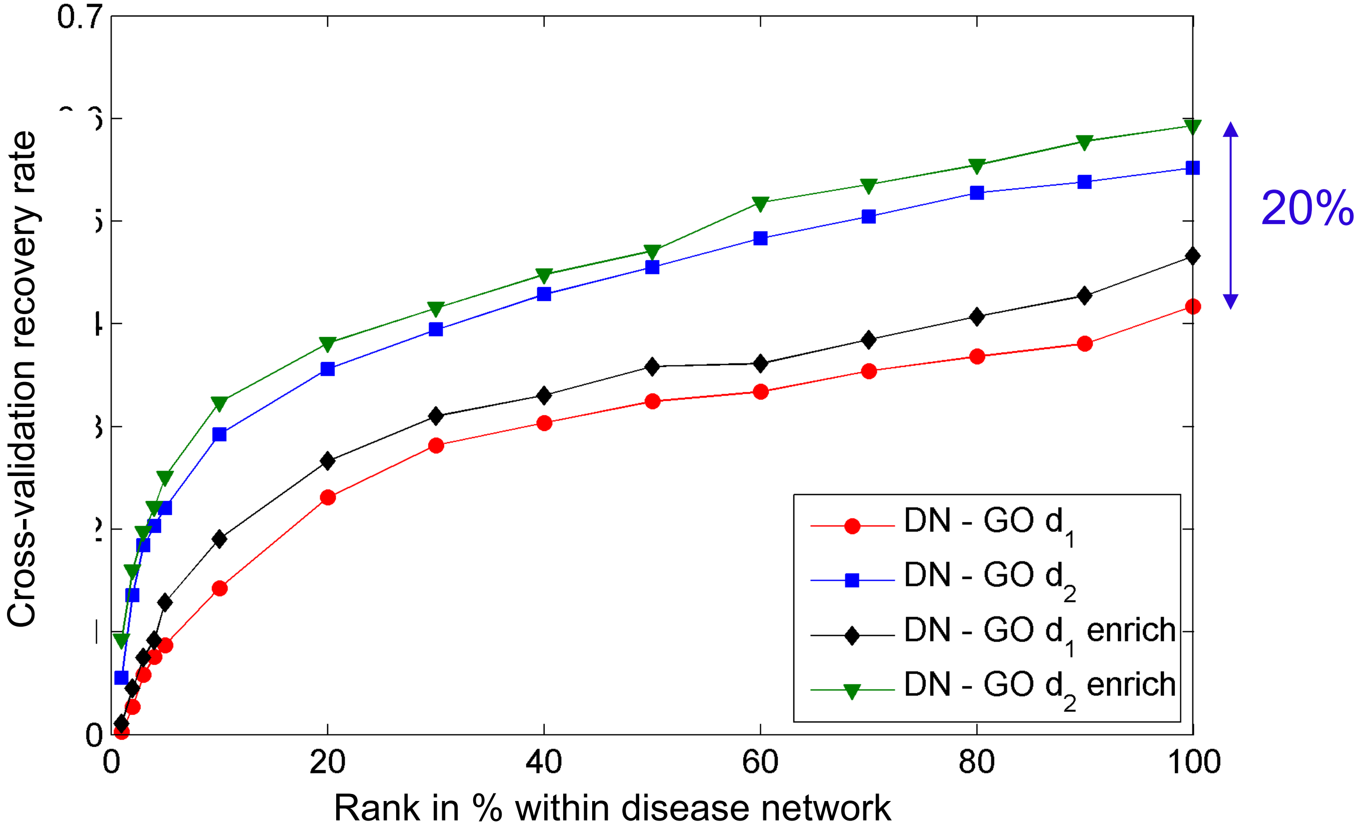


d_1 = direct interactions

d_2 = direct and indirect interactions

Cross-Validation

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



Further Reading

- Jaeger, S. (2012). "Network-based Inference of Protein Function and Disease-Gene Associations". Dissertation, Humboldt-Universität zu Berlin.
- 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.
- Ideker, T. and Sharan, R. (2008). "Protein networks in disease." Genome Res 18(4): 644-52.
- Barabasi, A. L. and Oltvai, Z. N. (2004). "Network biology: understanding the cell's functional organization." Nat Rev Genet 5(2): 101-13.

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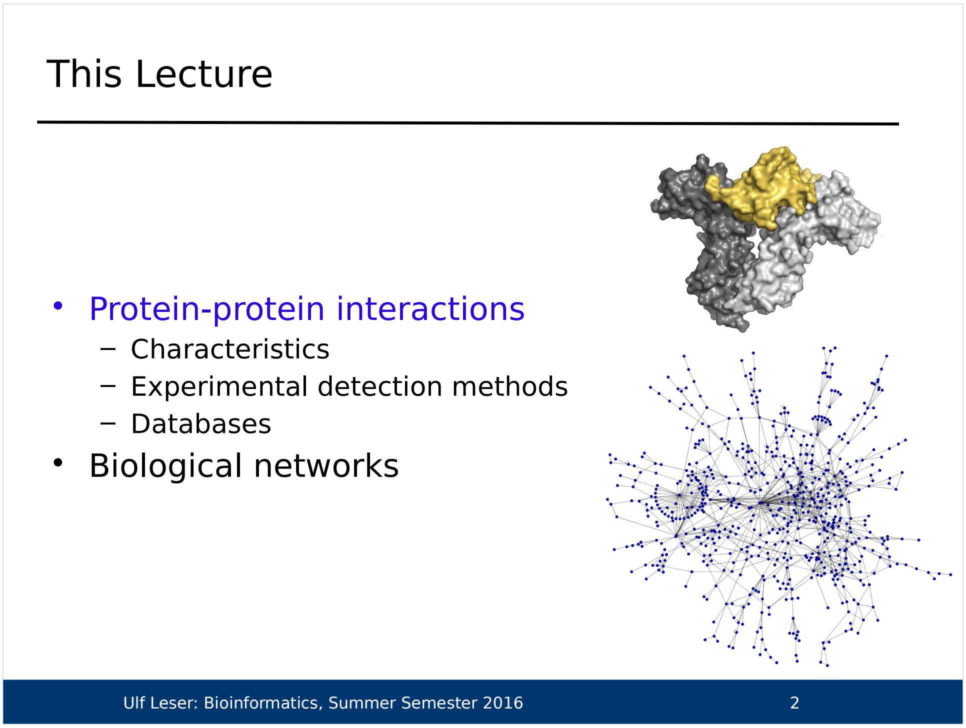

This Lecture

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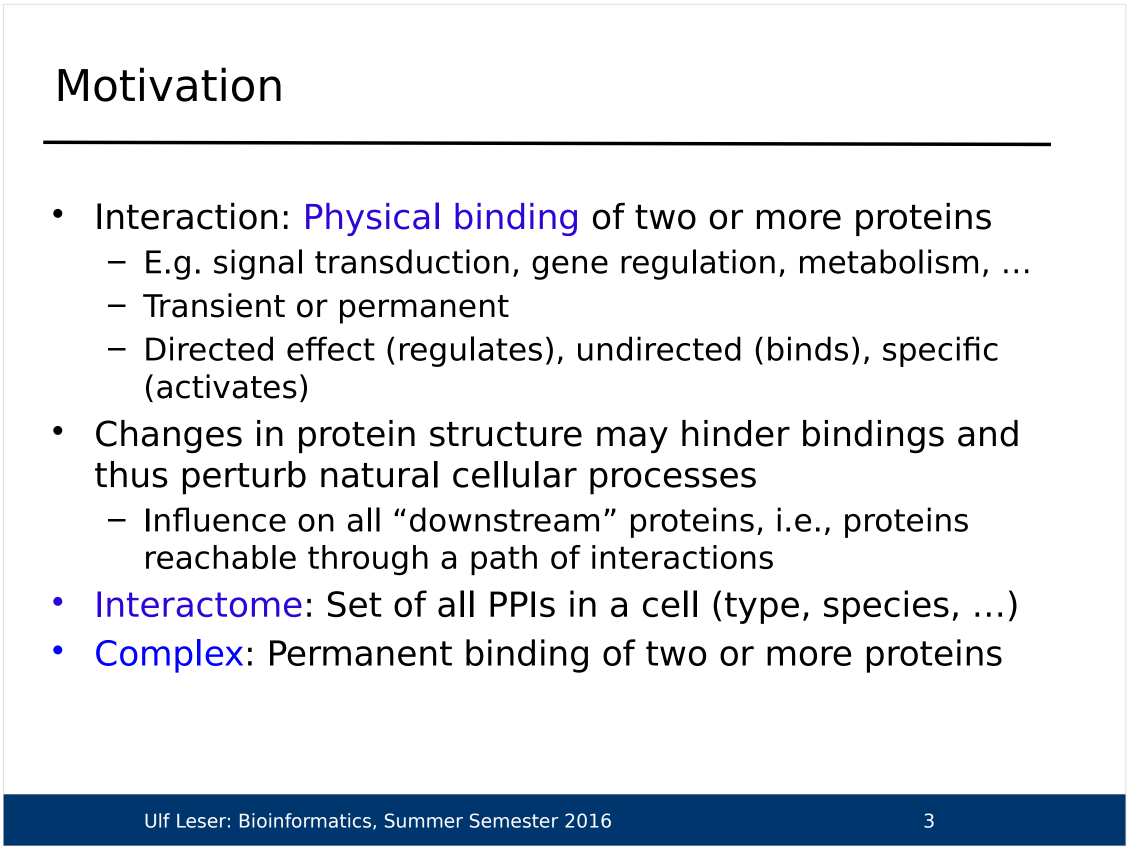
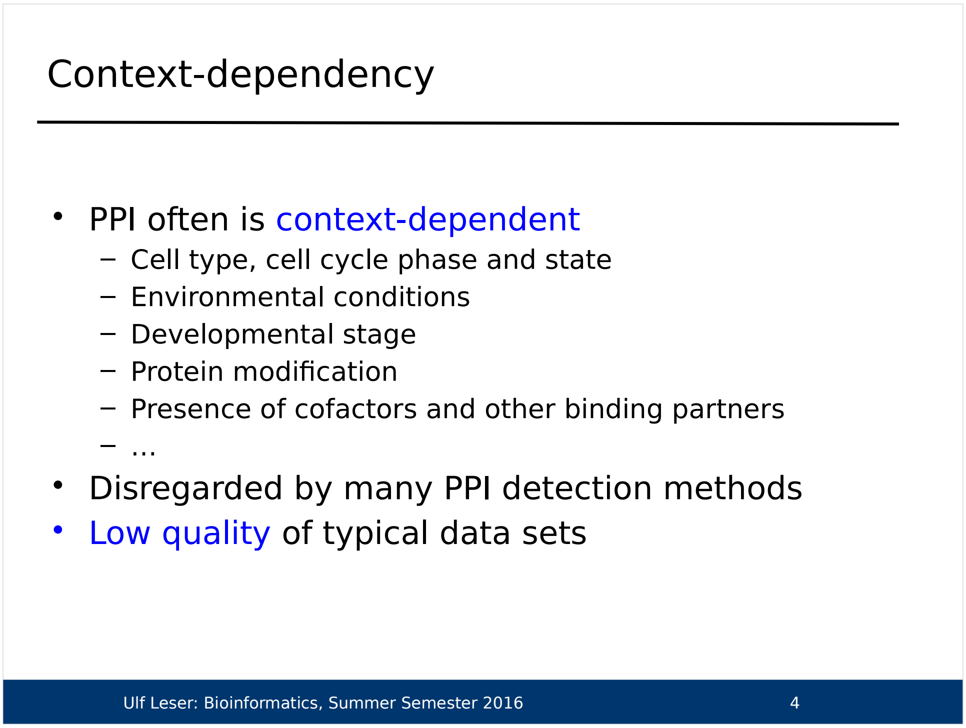
  - Characteristics
  - Experimental detection methods
  - Databases
- Biological networks



Motivation

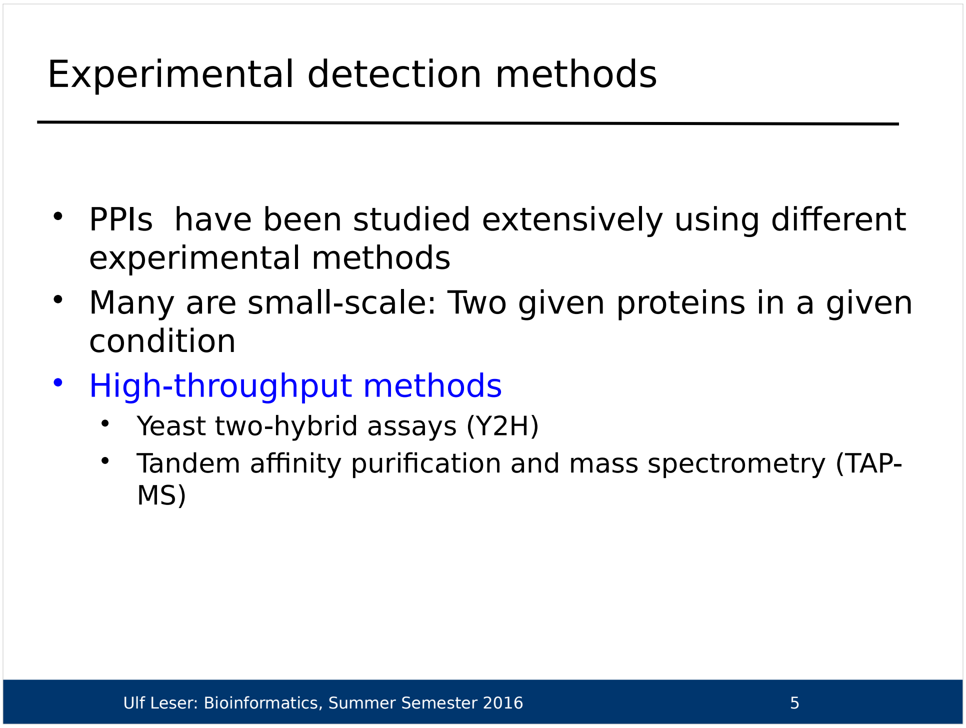
- Interaction: Physical binding of two or more proteins
  - E.g. signal transduction, gene regulation, metabolism, …
  - Transient or permanent
  - Directed effect (regulates), undirected (binds), specific (activates)
- Changes in protein structure may hinder bindings and thus perturb natural cellular processes
  - Influence on all “downstream” proteins, i.e., proteins reachable through a path of interactions
- Interactome: Set of all PPIs in a cell (type, species, …)
- Complex: Permanent binding of two or more proteins

* Interaktion = Wechselwirkung, physikalische Bindung zwischen zwei Proteinen, beruhen meist auf nicht-kovalenten Bindungen wie van-der-Waals Kräfte, Wasserstoffbrücken usw.
* PPI spielen wichtige Rolle bei allen biologischen Prozessen, z.B. Signaltransduktionsprozesse, Metabolismus (Enzyme)
* PPI können permanent (stable) oder transient sein
* transient: nur für eine kurzen Effekt kurzzeitig
* permanent: Bildung von Multiprotein Komplexen (zb Cytochrome c)
* Arten von Bindungen: gerichtet (Regulation), ungerichtet (Bindung), spezifisch (Aktivierung)



Context-dependency

- PPI often is context-dependent
  - Cell type, cell cycle phase and state
  - Environmental conditions
  - Developmental stage
  - Protein modification
  - Presence of cofactors and other binding partners
  - …
- Disregarded by many PPI detection methods
- Low quality of typical data sets



Experimental detection methods

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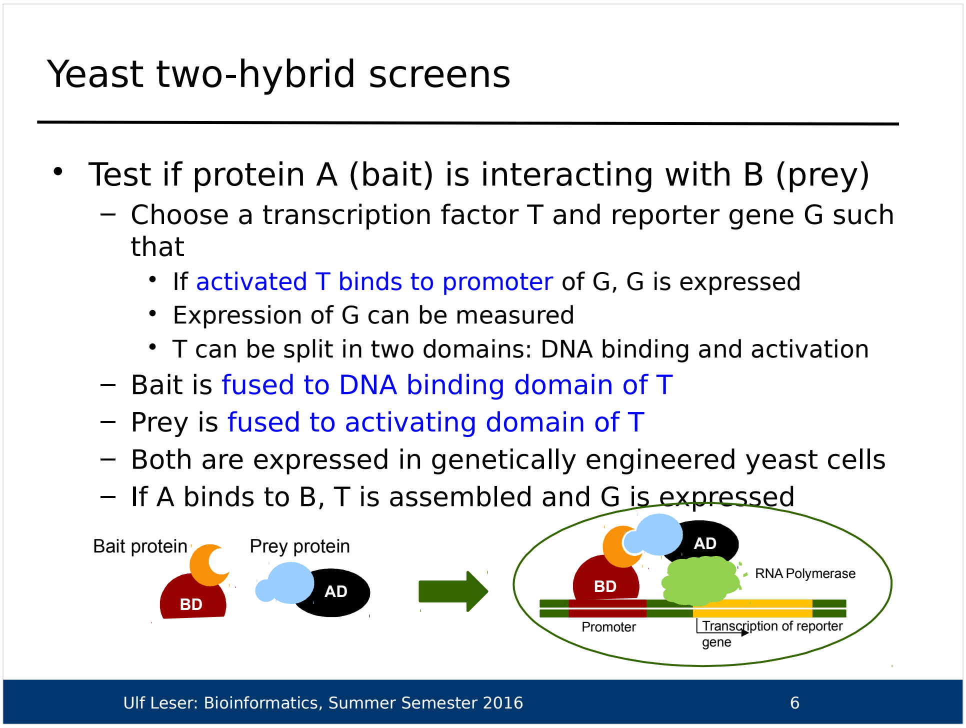
- Many are small-scale: Two given proteins in a given condition
- High-throughput methods
  - Yeast two-hybrid assays (Y2H)
  - Tandem affinity purification and mass spectrometry (TAP-MS)

Experimentelle Methoden: Protein Microarrays, MS usw

Small scale: less than 100 PPI are considered, typischeerweise mittels X-Ray Kristallographie, Studien beschäftigen sich zumiest mit einem bestimmten Protein

Binary methods: direct physical interactions between proteins (Y2H)
Co-compex: direct and indirect interactions (TAP-MS)

Beide sind large-scale



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  - Both are expressed in genetically engineered yeast cells
  - If A binds to B, T is assembled and G is expressed

Hefe-Zwei-Hybrid-System

* testen ob Protein A (bait = Köder) und Protein B (prey = Beute) miteinander interagieren
* dabei wird Prinzip der transkriptionellen Aktivierung genutzt

BD: Binding domain
AD: Activation domain

* die Voraussetzung für den Test ist das Binden eines TFs an einen Promoter und die darauffolgende Transkription eines downstream liegenden Gens
* Schlüssel: AD und BD funktionieren auch wenn sie nur nahe beieinander sind, sie müssen nicht direkt binden

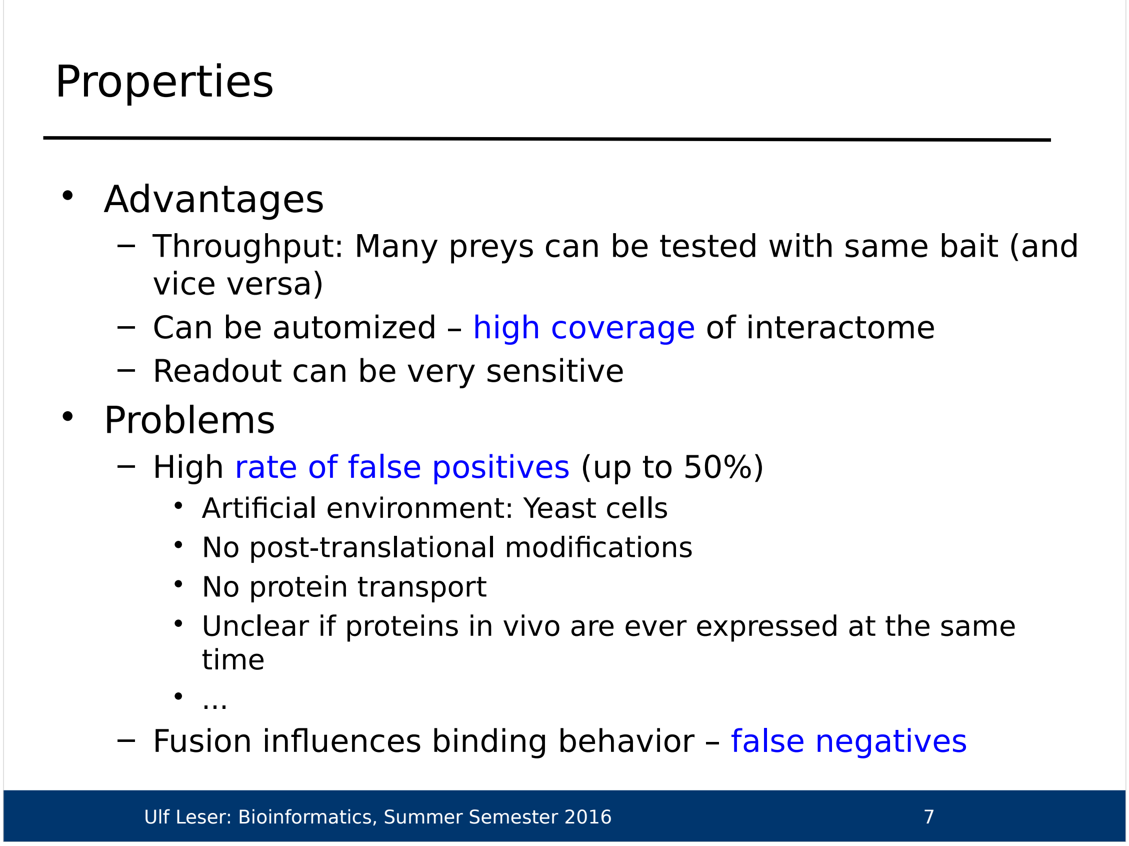
Samira:

- Two fusion protein reconstitute a transcription factor – Bait and prey

- utilizes the fact that Transcription Factors commonly require two domains - a DNA binding domain and an activation domain promoting transcription. In order to find out which proteins interact with a certain protein of interest (termed bait), the bait is fused to a DNA binding domain which binds to the promoter of a reporter gene, while the other proteins (termed prey) are fused with an activation domain. When a physical interaction between the bait and some
prey occurs, an expression of the reporter gene can be detected.
An interaction between a protein containing a DNA binding domain (bait) and an activation domain (prey) can be detected by measuring the expression level of the reporter gene.

Which proteins interact with protein of interest ?
Fuse protein of interest, bait, to DNA binding domain
Fuse potential binding partners to activation domain

Physical interaction between bait and prey forms an intact functional transcriptional activator → Initiation of the expression of reporter gene



Properties

- Advantages
  - Throughput: Many preys can be tested with same bait (and vice versa)
  - Can be automized – high coverage of interactome
  - Readout can be very sensitive
- Problems
  - High rate of false positives (up to 50%)
    - Artificial environment: Yeast cells
    - No post-translational modifications
    - No protein transport
    - Unclear if proteins in vivo are ever expressed at the same time
    - ...
  - Fusion influences binding behavior – false negatives

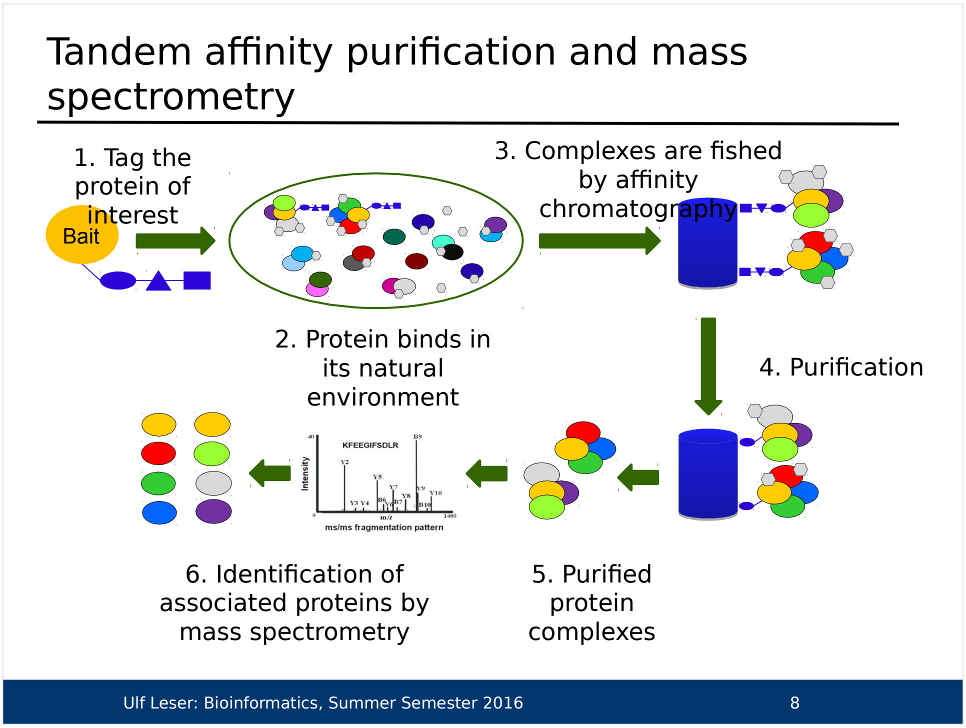
Vorteile

* ermöglicht Untersuchung von PPI in in vivo-ähnlichen Verhältnissen (d.h. im Milieu einer Zelle und mit in Eukaryoten vorkommenden posttranslationalen Modifikationen, z.B. Glykosylierung, Palmitoylierung)
* Hefe ist relativ billig und leicht zu handhaben

Probleme

* Interaktion der untersuchten Proteine muss im Zellkern stattfinden, da dort Trankription stattfindet. Aber: Proteine falten sich in diesem Milieu ggf.anders als in dem wo sie üblicherweise auftreten
* Modifikationen der Hefe sind teilweise anders als in anderen Eukaryoten (führt zu veränderter Faltung) → insgesamt also falsch-positive Interaktionen möglich: Proteine interagieren nur wegen falscher Faltung miteinander
* auch falsch-negative resultieren daraus: keine Interaktion aufgrund von veränderter Faltung im Kern
* evt. Interaktion im Y2H Versuch, aber in Realität kein gemeinsamen Auftreten im Zellzyklus, Zellorganell oder Zelltyp

nur ca. 50% der mittels Y2H ermittelten Interaktionen sind biologisch bedeutend
Große Anzahl PPI kann mit Y2H nicht detektiert werden



Tandem affinity purification and mass spectrometry

1. Tag the protein of interest

3. Complexes are fished by affinity chromatography

2. Protein binds in its natural environment

4. Purification

6. Identification of associated proteins by mass spectrometry

5. Purified protein complexes

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- In a single TAP-MS experiment, a protein of interest, “bait”, is modified by integrating a polypeptide tag into the protein via standard recombinant DNA techniques
- The bait protein is then expressed inside a cell where it may carry out its function as part of one or more protein complexes.
- To retrieve other proteins in a complex, the complex is purified from a cell lysate via affinity chromatography

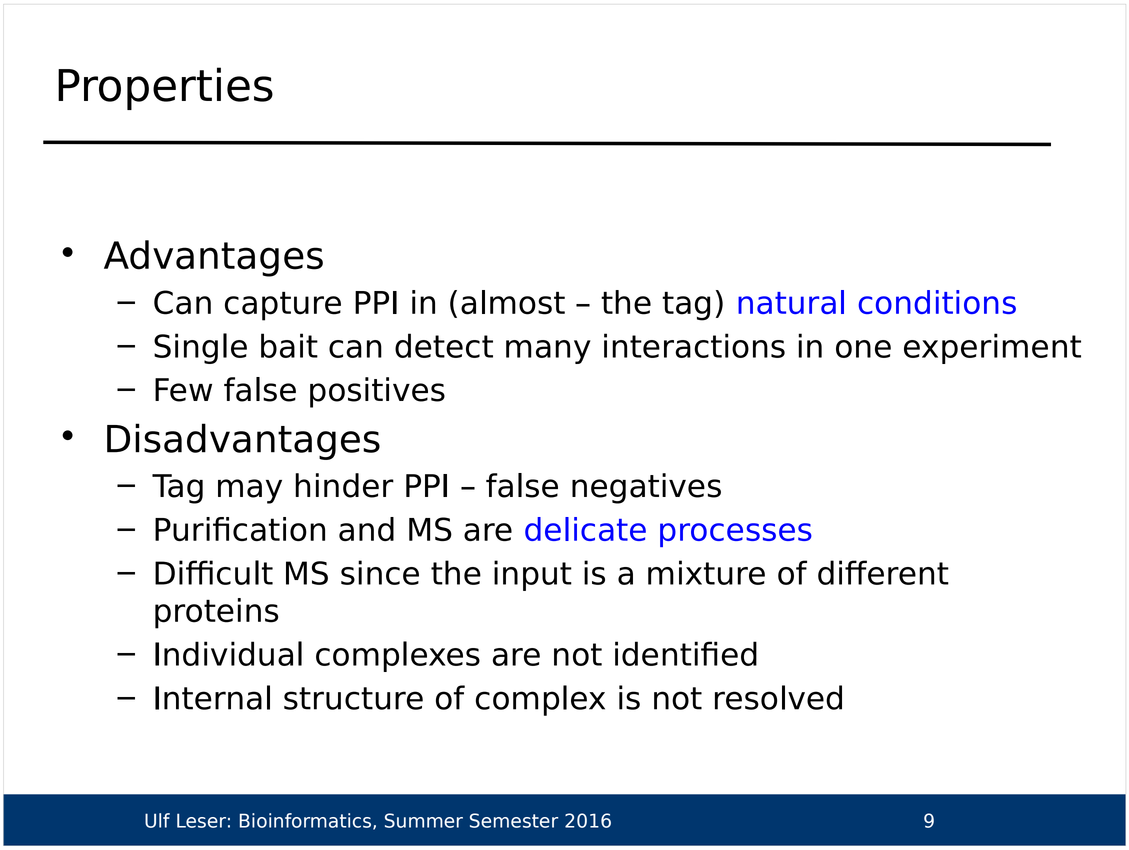
using the tag of the bait protein. A single experiment is referred to as a *purification.*

- Protein complexes are bound to an IgG column, and washed to remove majority of contaminants
- *A* single purification is supposed to identify “prey” proteins forming protein complexes with the bait

protein.

- TEV protease is then used to elute the semi-purified protein complexes which are subsequently absorbed onto a calmodulin column. After further washing, purified protein complexes containing the protein of interest and its associated proteins
- The purified proteins are identified by standard mass spectrometry identification.
- Ideally, these purified proteins would constitute the entire complex encompassing all proteins interacting

with the bait protein.

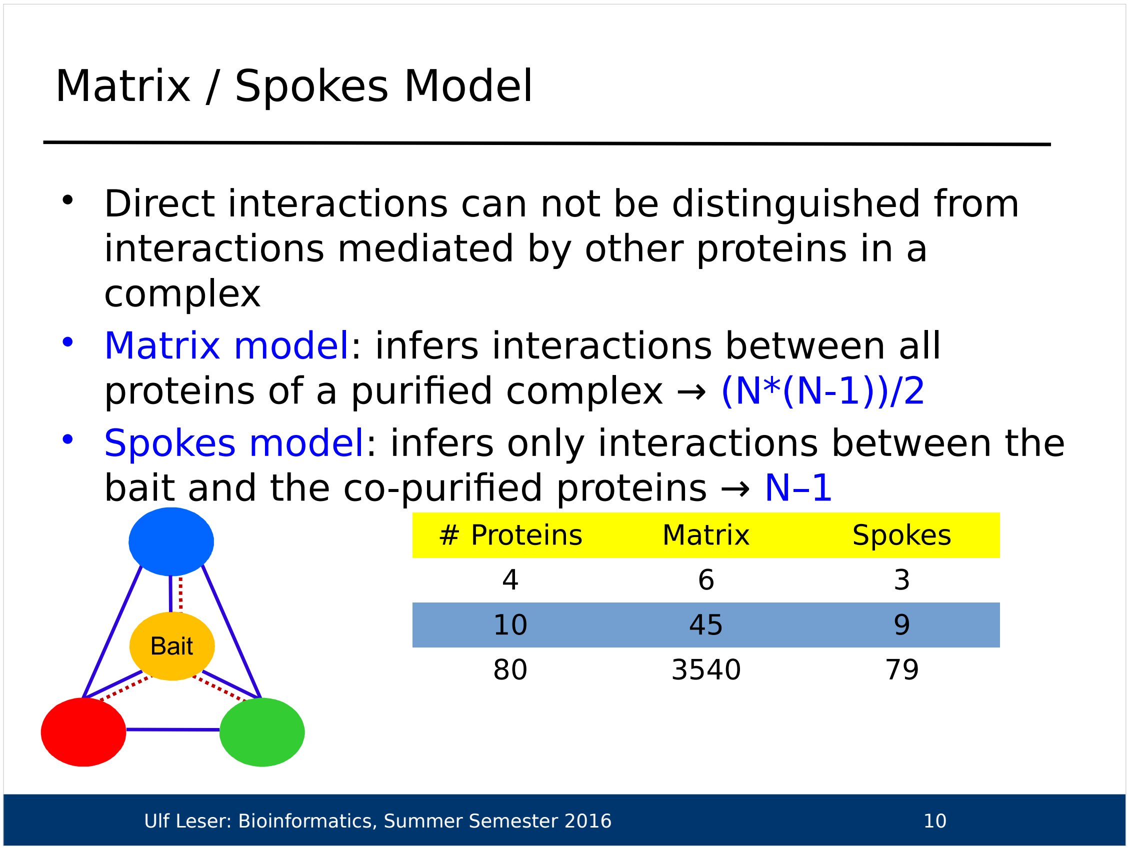


Properties

- 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
  - Difficult MS since the input is a mixture of different proteins
  - Individual complexes are not identified
  - Internal structure of complex is not resolved

Vorteile:

Nachteile:
* MS/Purification sind anfällige,schwierige Experimente

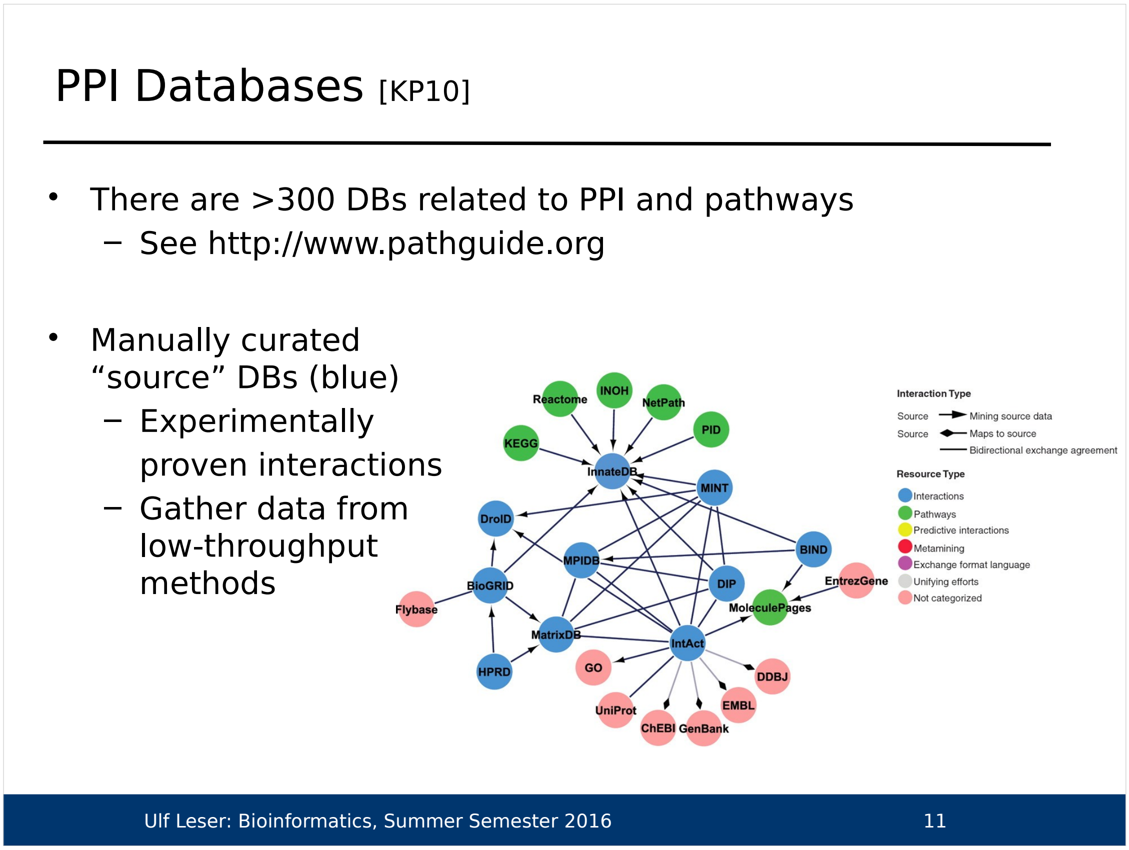


Matrix / Spokes Model

- Direct interactions can not be distinguished from interactions mediated by other proteins in a complex
- Matrix model: infers interactions between all proteins of a purified complex → (N*(N-1))/2
- Spokes model: infers only interactions between the bait and the co-purified proteins → N–1

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

Matrix model: direct and indirect interactions
Spokes model: only direct interactions, less false-positives



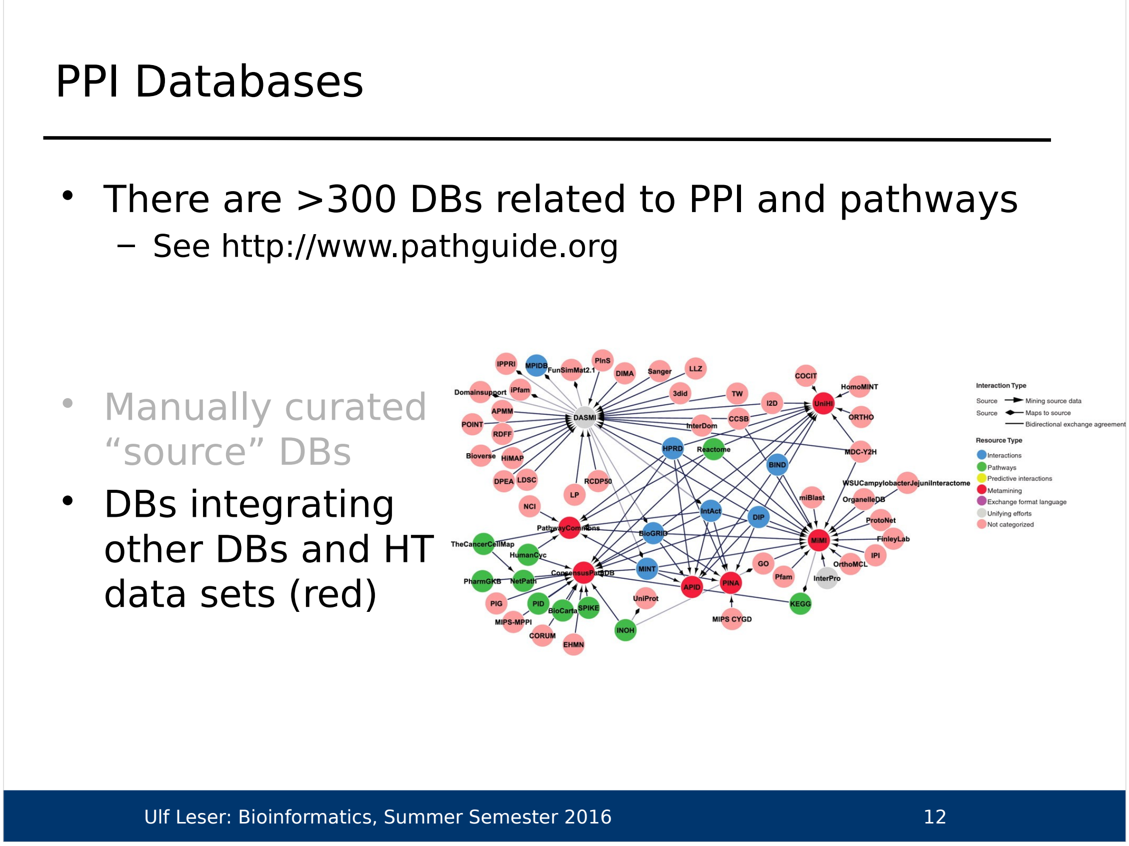
PPI Databases [KP10]

- There are >300 DBs related to PPI and pathways
  - See http://www.pathguide.org

- Manually curated "source" DBs (blue)
  - Experimentally proven interactions
  - Gather data from low-throughput methods

Drei verschiedene Typen von Datenbanken:

* primary databases: containing only experiemnetally proven interactions
    * enthalten PPI aus small scale experiments
    * berühmte Beispiele auf Folien:
        * BioGRID: Biological General Repository for Interaction datasets
        * DIP: Database of interacting Proteins
        * IntAct: molecular interaction database
        * MNT: molecular interaction database
        * HPRD: Human Protein Reference Database
* DBs list Spezies, Experiment, Publikation, …
* kaum Überlappung zwischen den Datenbanken: in sechs Resourcen gibt es drei PPI, die in allen Quellen vorkommen, im vergleich sind die Anzahl der Interaktionen, die nur in einer DB vorkommen sehr hoch, zb ~20k in HPRD



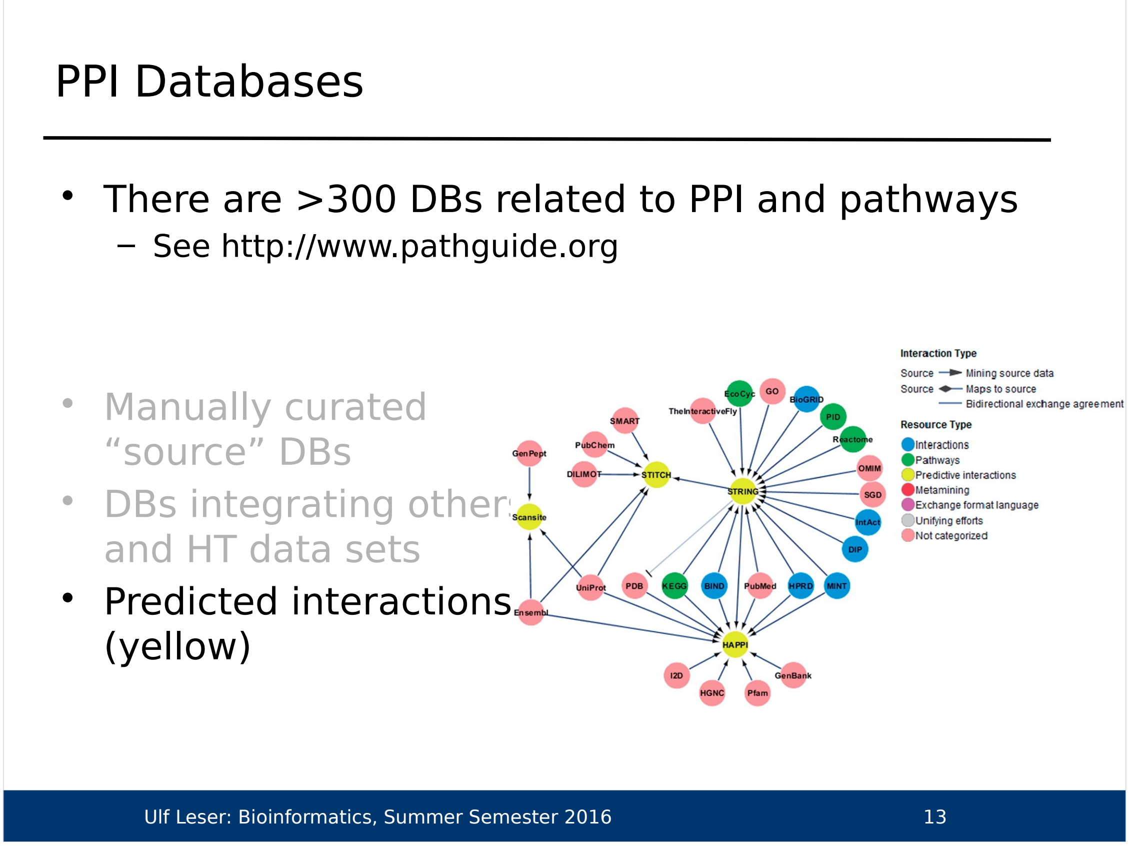
PPI Databases

- There are >300 DBs related to PPI and pathways
  - See http://www.pathguide.org

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

* Wegen der Heterogenität der Daten in den DBs gibt es inzwischen Meta-Dbs, Daten werden kombiniert um zuverlässigere Daten zu erhalten

* nicht einfach: unterschiedliche Ids für Proteine (selbst innerhalb einer DB)



PPI Databases

- There are >300 DBs related to PPI and pathways
  - See http://www.pathguide.org

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

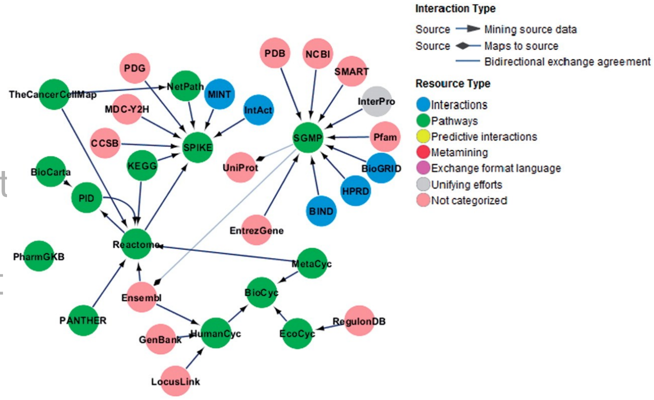
* kombinieren Daten aus den anderen Datenbanken mit in silico methoden (am computer bestimmt)
* bekannteste: STRING DB
* STRING integriert aus versch.Dbs und gewichtet die PPI
* methoden um PPI zu predicten:

* aus sequenze Struktur ableiten, ähnliche Domänen die interagieren, interagieren vllt auch zwischen zwei Proteinen mit ähnlichen Domänen
* ableitung von homologen Proteinen
* co-expression usw.

PPI Databases

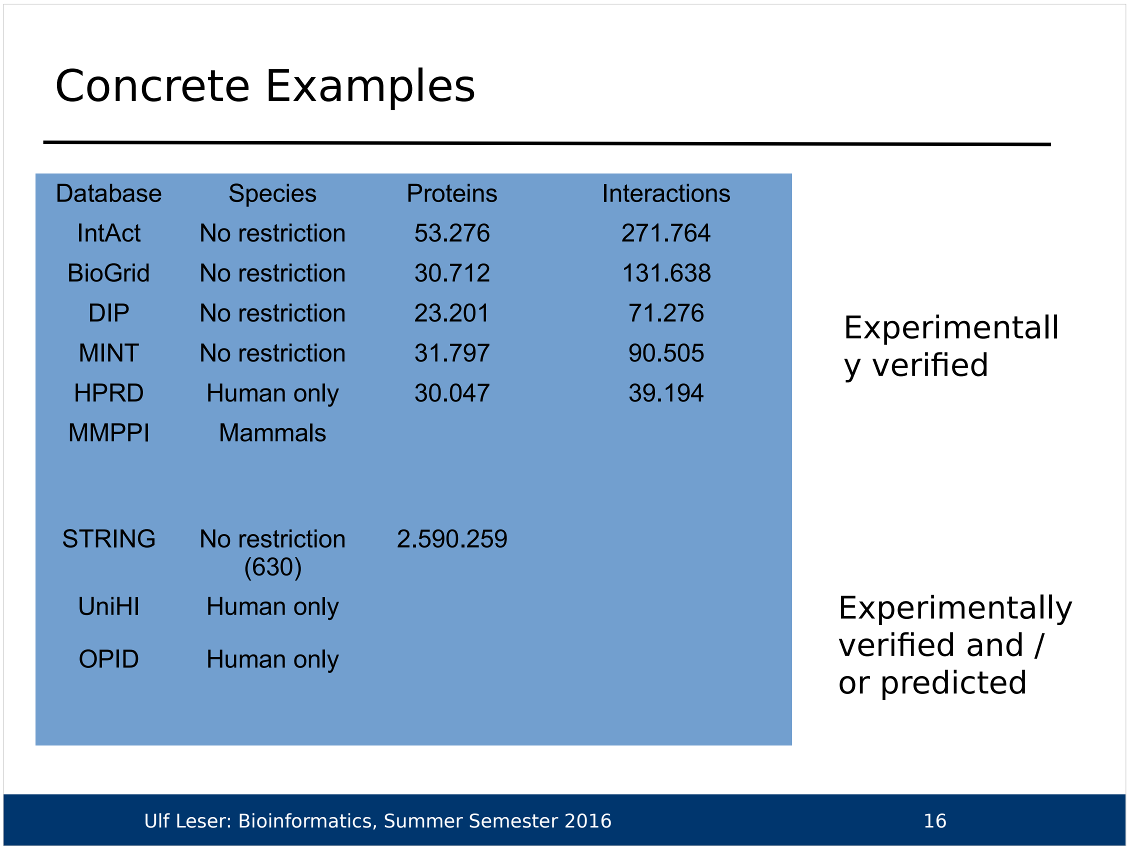
- There are >300 DBs related to PPI and pathways
  - See http://www.pathguide.org

- Manually curated “source” DBs
- DBs integrating ot and HT data sets
- Predicted interact
- Pathway DBs (green)



A Mess [KP10]

- Different definitions of a PPI
  - Binary, physical interaction
  - Complexes, pairs, pathways
  - Transient, functional association
- 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

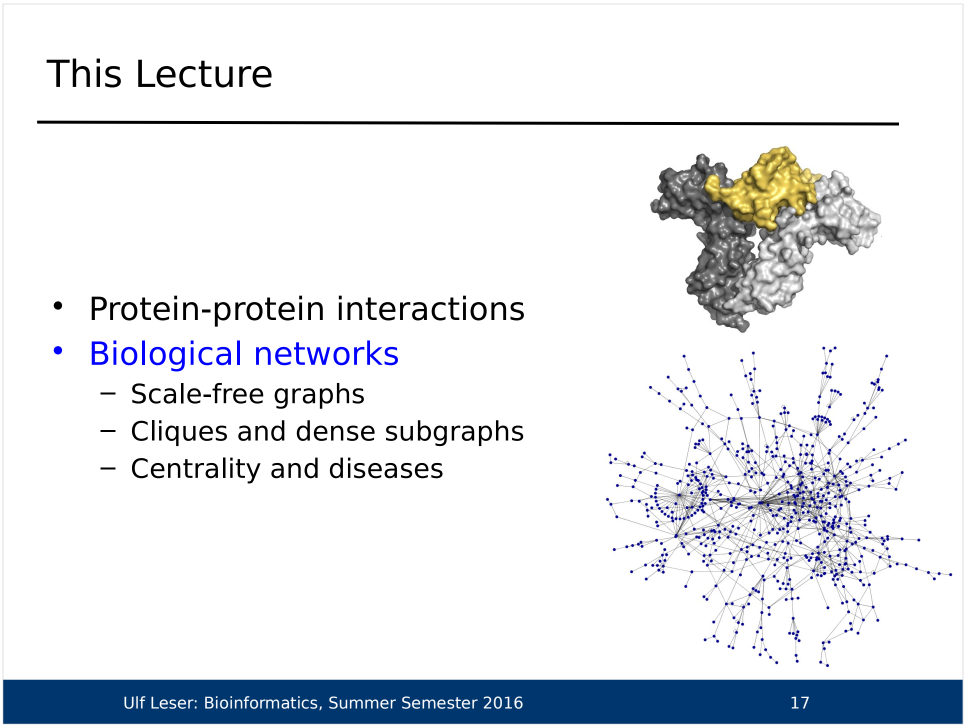
Database	Species	Proteins	Interactions
IntAct	No restriction	53.276	271.764
BioGrid	No restriction	30.712	131.638
DIP	No restriction	23.201	71.276
MINT	No restriction	31.797	90.505
HPRD	Human only	30.047	39.194
MMPPI	Mammals		
STRING	No restriction (630)	2.590.259	
UniHI	Human only		
OPID	Human only		

Experimentall y verified

Experimentally verified and / or predicted

Variierende Datenqualität, deshalb verschiedene Methoden um Verläasslichkeit zu erhöhen:

* filtern nach Interaktionen, die in mehreren DBs vorkommen, berechnen eines Scores für eine PPI (unterscheidliche Gewichtung der Quellen, nach Detektionsmethode)
* weitere Konfidenz über einbeziehung der Funktion – interagierende Proteine haben wahrscheinlich ähliche Funktionen/üben Funktion im gleichen Zellkompartiment aus usw



This Lecture

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

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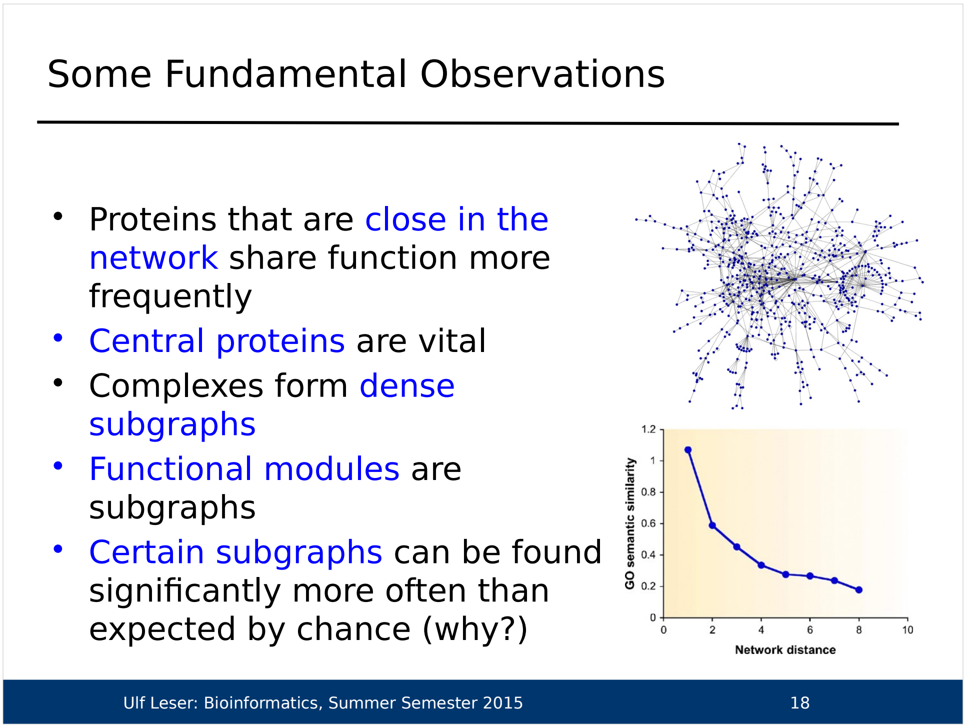
- Vom Gen zum Protein
- mRNA, andere Arten RNA
- Kernidee von Microarrays
- Affy versus two-color
- Populäre chip Typen, kurzer Exkurs neue Sachen (Exon, tiling)
- Probleme mit den Daten
- Normalisierung (1-2 Methoden) und Qualitätssicherung (*wenige* Plotarten mit Kochrezept-artigen Hinweisen)
- Technische / biologische Replikate
- Wenn möglich was "echtes„

- http://plmimagegallery.bmbolstad.com/



Some Fundamental Observations

- Proteins that are close in the network share function more frequently
- 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?)

1.2
1
0.8
0.6
0.4
0.2
0
GO semantic similarity
0
2
4
6
8
10
Network distance

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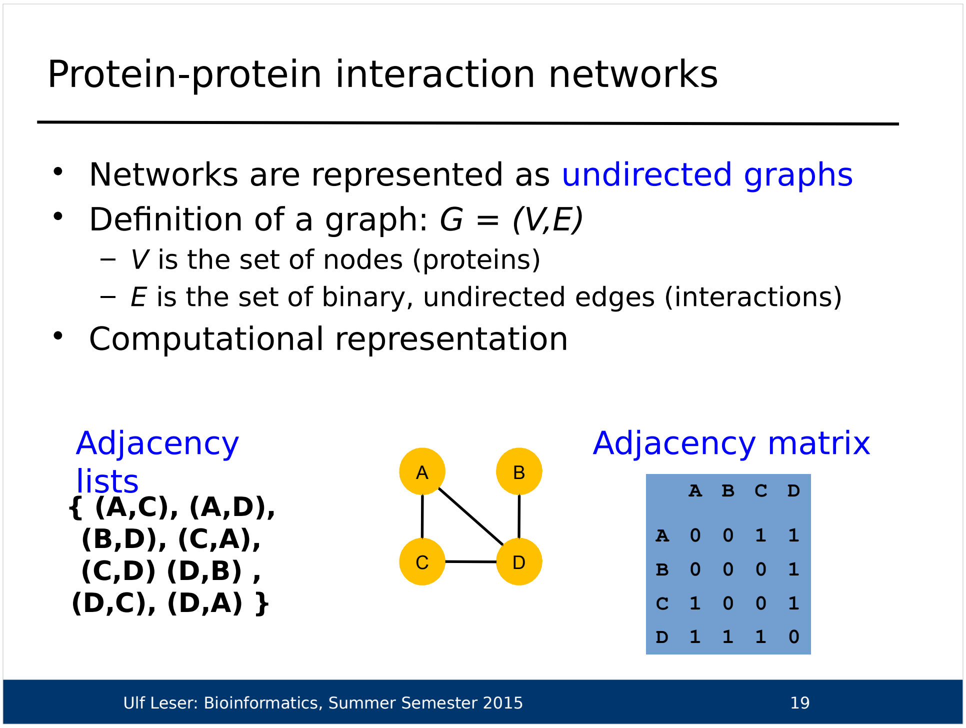
For computational analysis, protein interaction networks and their structure

Nodes V represent proteins

Edges E between two nodes *u* and *v* represent evidence for the presence of a physical interaction between the two proteins



Protein-protein interaction networks

- Networks are represented as undirected graphs
- Definition of a graph: *G = (V,E)*
  - *V* is the set of nodes (proteins)
  - *E* is the set of binary, undirected edges (interactions)
- Computational representation

Adjacency lists

{ (A,C), (A,D), (B,D), (C,A), (C,D) (D,B) , (D,C), (D,A) }

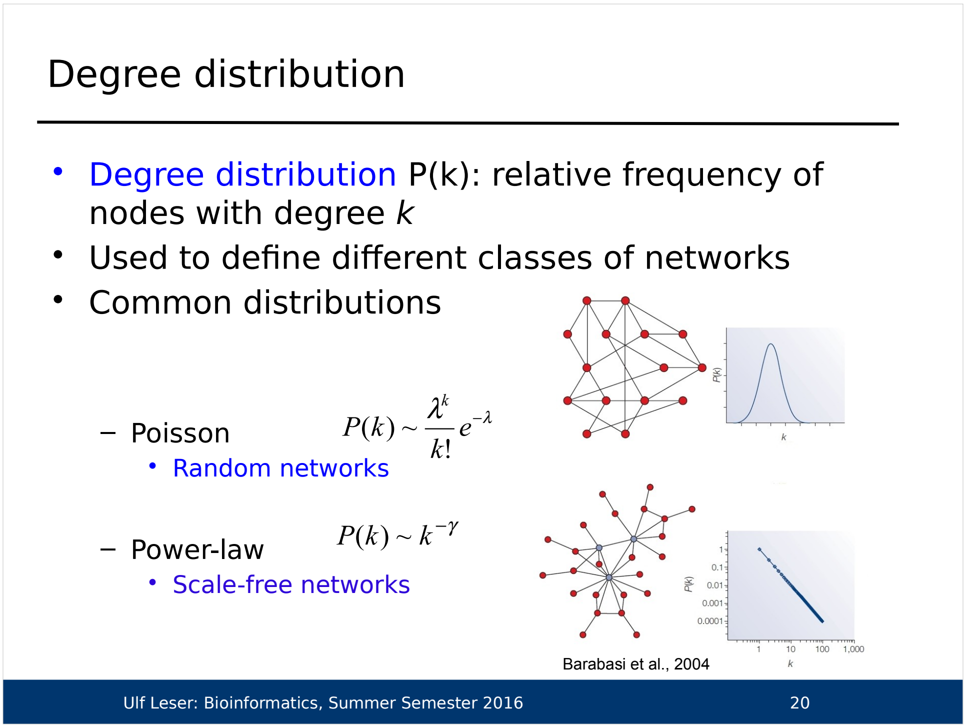
Adjacency matrix

	A	B	C	D
A	0	0	1	1
B	0	0	0	1
C	1	0	0	1
D	1	1	1	0

For computational analysis biological networks are modeled as graphs, protein interaction networks and their structure

Nodes V represent proteins

Edges E between two nodes *u* and *v* represent evidence for the presence of a physical interaction between the two proteins



Degree distribution

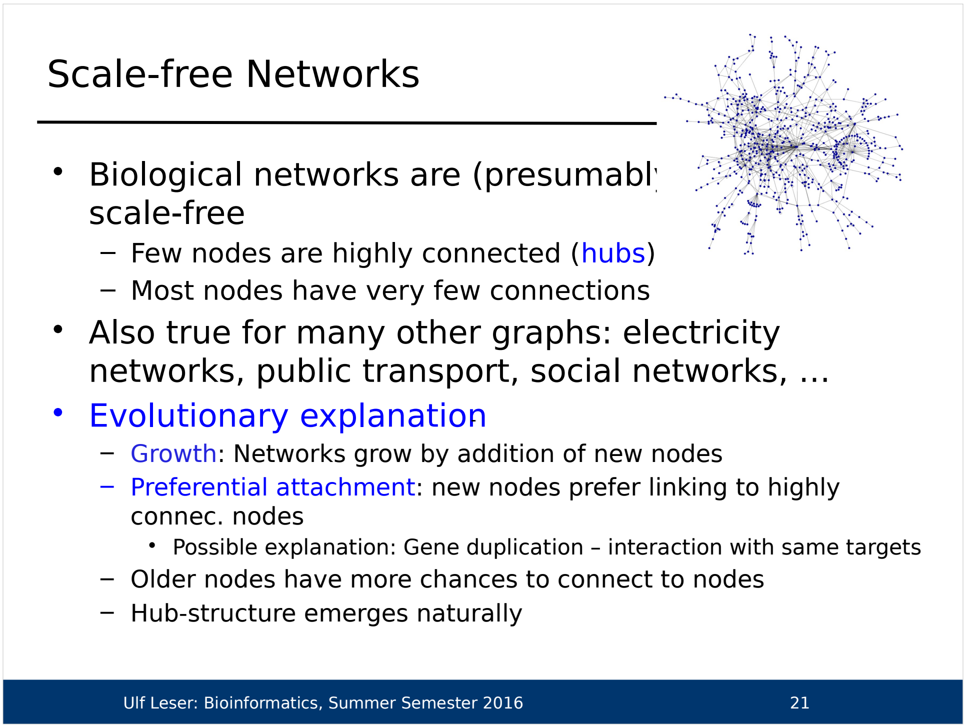
- Degree distribution P(k): relative frequency of nodes with degree *k*
- Used to define different classes of networks
- Common distributions
  - Poisson $P(k) \sim \frac{\lambda^k}{k!} e^{-\lambda}$
    - Random networks
  - Power-law $P(k) \sim k^{-\gamma}$
    - Scale-free networks

Barabasi et al., 2004

* P(k) Wahrscheinlichkeit, dass ein beliebiger Knoten Grad k hat

* number of nodes with degree k N(k) divided by the total number of nodes in the network

* allows to distinguish between different network classes, eg random networks, scale free networks
* in a random network the nodes follow a poisson distribution, which indicates that most nodes have approximately the same number of links
* random networks: jeder Knoten hat in etwa den gleichen Knotengrad
* scale free networks: wenige Knoten haben hohen Knotengrad, viele einen geringen



Scale-free Networks

- Biological networks are (presumabl 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 chances to connect to nodes
  - Hub-structure emerges naturally

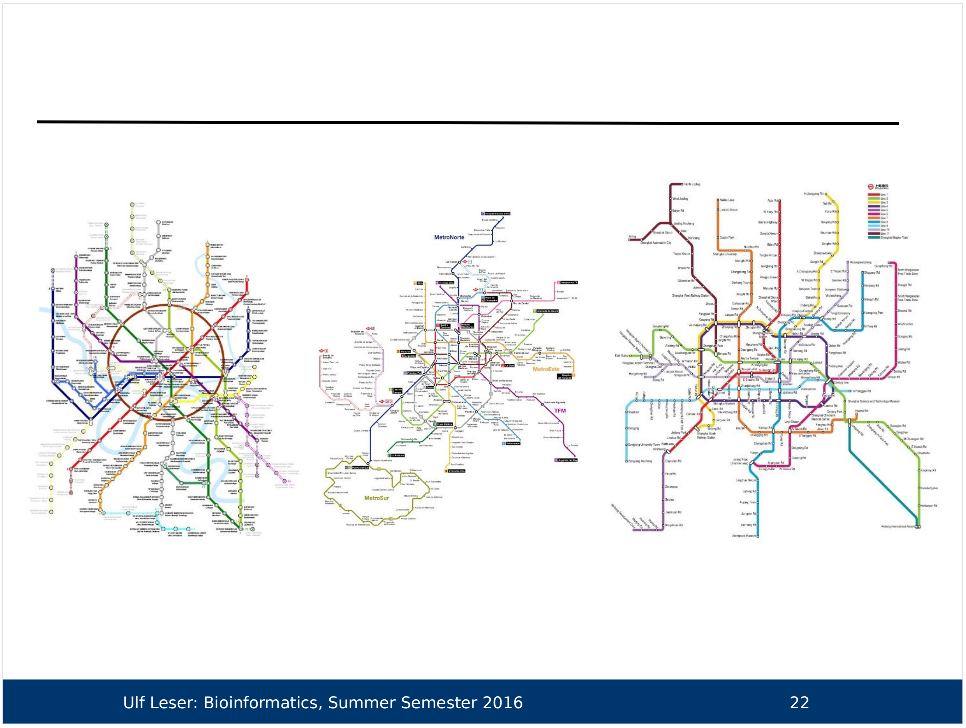
* in biological networks gamma is between 2 and 3
* highly connected nodes are called hubs
* typisch für scale free networks ist, dass es keinen typischen Knotengrad gibt
* The smaller gamma, the more important the rate of the hubs in the network
* zwei Mechanismen verantwortlich für scale-free Topologien:

* Growth (networks wachsen durch Hinzufügen neuer Knoten)

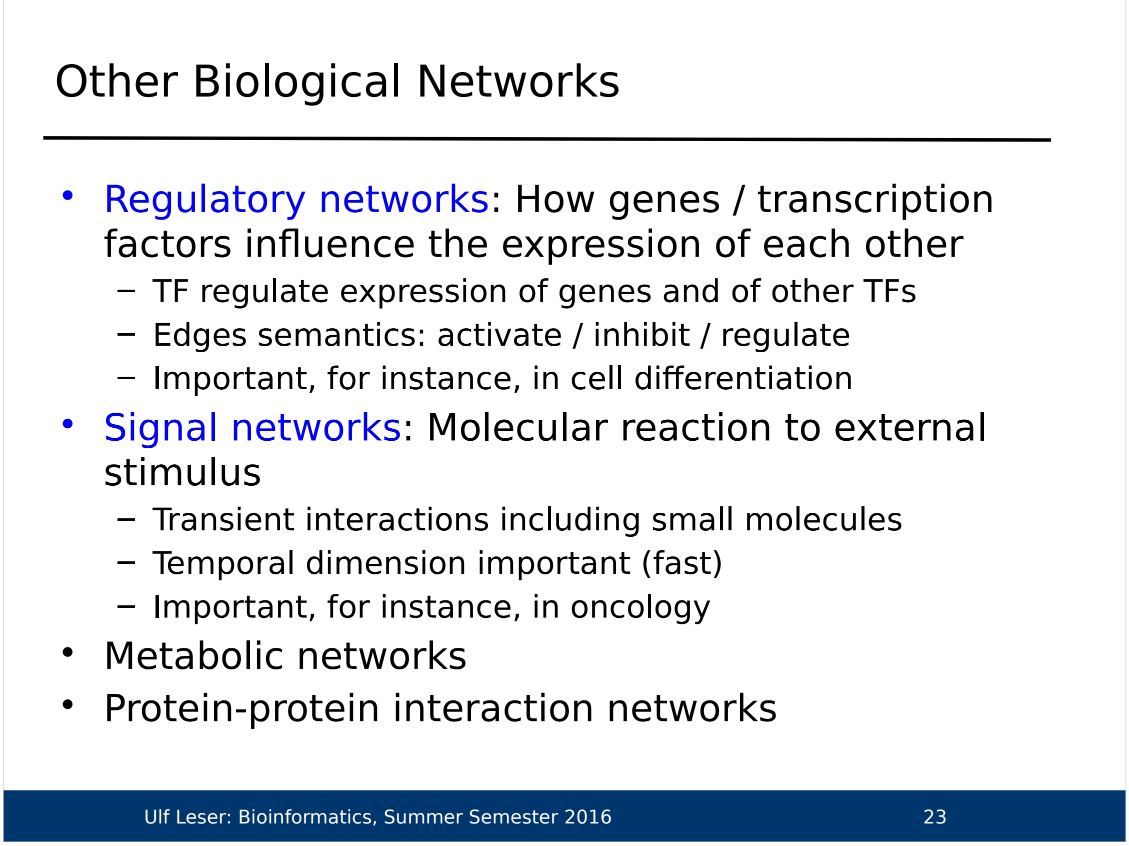
* preferential attachment: nodesae mainly attached to nodes that are already highly connected

* gemeinsamer Ursprung: duplikation

* duplikation: zwei dientische Gen Produkte entstehen (Growth), außerdem werden duplizierte Gene mit den gleichen Gen Produkten interagieren wie ihr Vorfahr-Gen, da strukturelle Ähnlichkeit



Einige Metro Netzwerke

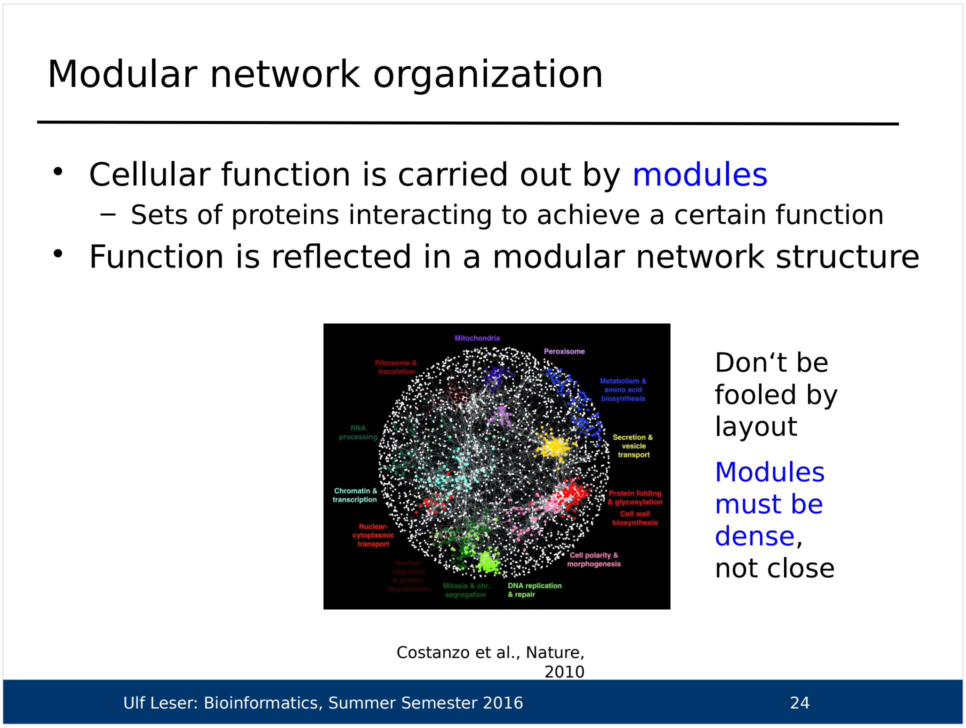


Other Biological Networks

- 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
  - Important, for instance, in cell differentiation
- Signal networks: Molecular reaction to external stimulus
  - Transient interactions including small molecules
  - Temporal dimension important (fast)
  - Important, for instance, in oncology
- Metabolic networks
- Protein-protein interaction networks

Regulatory networks: The activity of genes is regulated by transcription factors, proteins that typically bind to DNA. Most transcription factors bind to multiple binding sites in a genome. As a result, all cells have complex gene regulatory networks. For instance, the human genome encodes on the order of 1,400 DNA-binding transcription factors that regulate the expression of more than 20,000 human genes.[9] Technologies to study gene regulatory networks include ChIP-chip, ChIP-seq, CliP-seq, and others.

Signal networks: Signals are transduced within cells or in between cells and thus form complex signaling networks. For instance, in the MAPK/ERK pathway is transduced from the cell surface to the cell nucleus by a series of protein–protein interactions, phosphorylation reactions, and other events. Signaling networks typically integrate protein–protein interaction networks, gene regulatory networks, and metabolic networks.



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

Mitochondria
Peroxisome
Ribosome & translation
Metabolism & amino acid biosynthesis
RNA processing
Secretion & vesicle transport
Chromatin & transcription
Protein folding & glycosylation
Cell wall biosynthesis
Nuclear-cytoplasmic transport
Cell polarity & morphogenesis
Nuclear migration & protein degradation
Mitosis & chr. segregation
DNA replication & repair

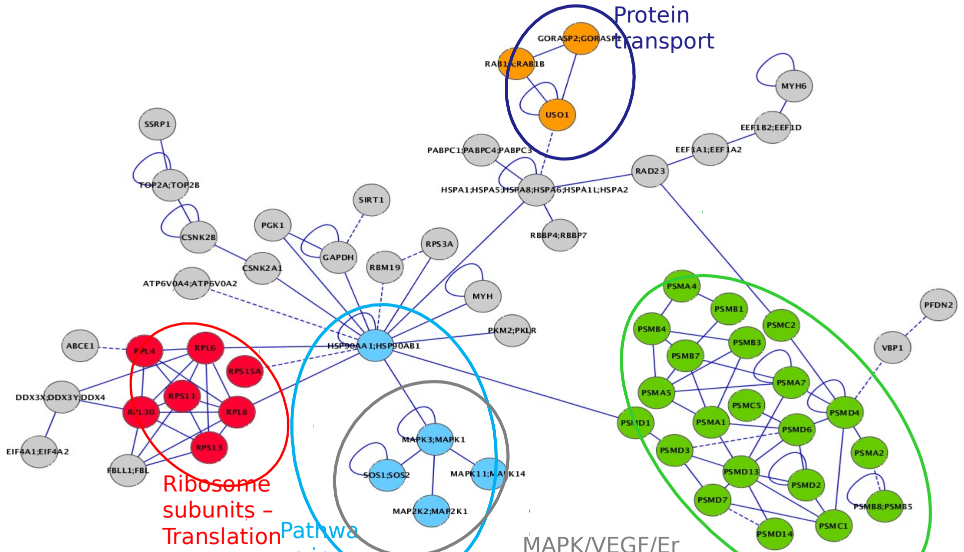
Don‘t be fooled by layout

Modules must be dense, not close

Costanzo et al., Nature, 2010

This modularity refers to a group of physically or functionally linked molecules/proteins that work together to achieve a (relatively) distinct function

Functional Modules



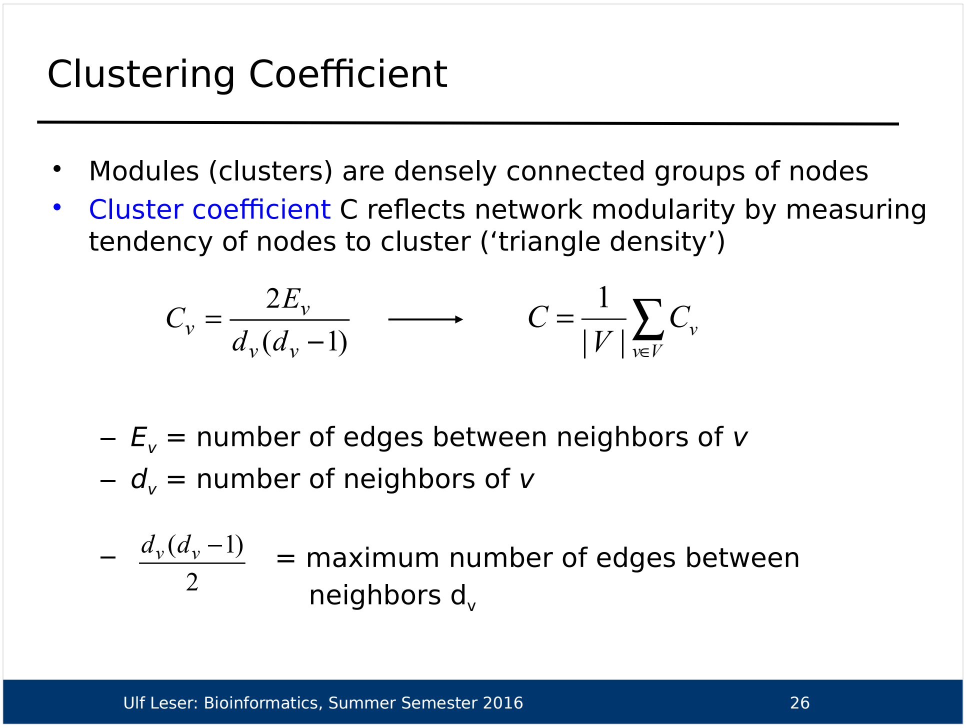
Protein transport

Ribosome subunits – Translation

Pathways in cancer

MAPK/VEGF/ErbB signaling pathway

Proteasome subunits – Protein degradation



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

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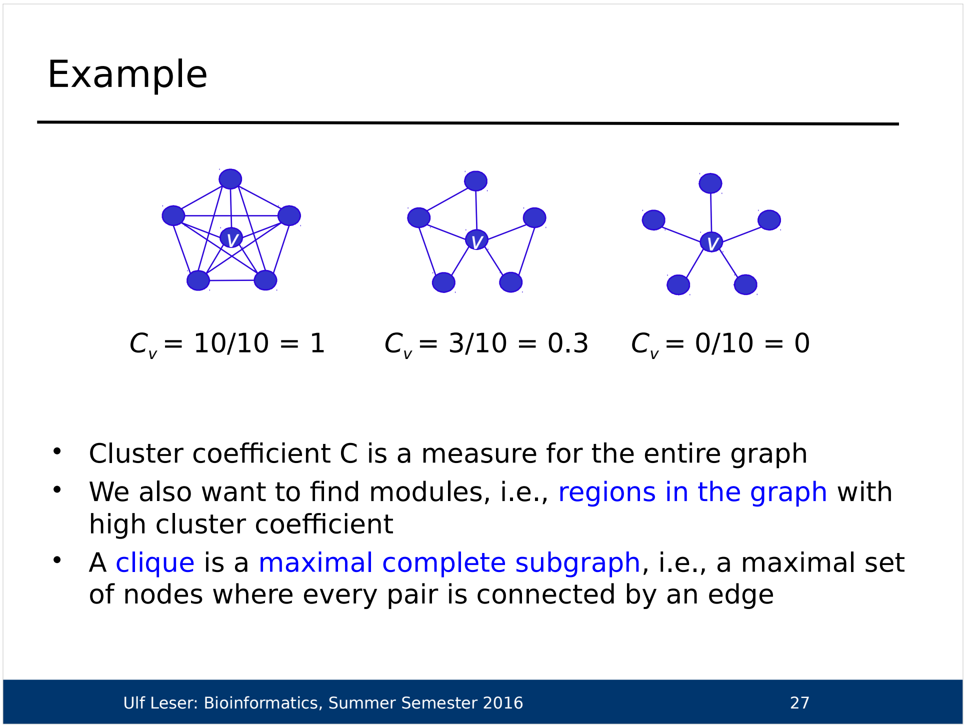
Der Clusterkoeffizient (engl. clustering coefficient) ist in der Graphentheorie ein Maß für die Cliquenbildung bzw. Transitivität in einem Netzwerk. Sind alle Nachbarn eines Knotens paarweise verbunden, also jeder mit jedem, dann bilden sie eine Clique. Dies ist gleichbedeutend mit dem Begriff der Transitivität, denn innerhalb einer Clique gilt: Ist A mit B verbunden und A mit C, so sind auch B und C verbunden.[1] Man unterscheidet zwischen dem globalen Clusterkoeffizienten, der das gesamte Netzwerk charakterisiert und dem lokalen Clusterkoeffizienten, der einen einzelnen Knoten charakterisiert.

Lokaler CC: Quotient aus der Anzahl der Kanten, die zwischen seinen k_i Nachbarn tatsächlich verlaufen, und der Anzahl Kanten, die zwischen diesen Nachbarn maximal verlaufen könnten (ungerichteter Graph)

Each module can be reduced to a set of triangles – high density of triangles is reflected by a high clustering coefficient

For cliques C = 1

For non-triangle graphs = 0



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

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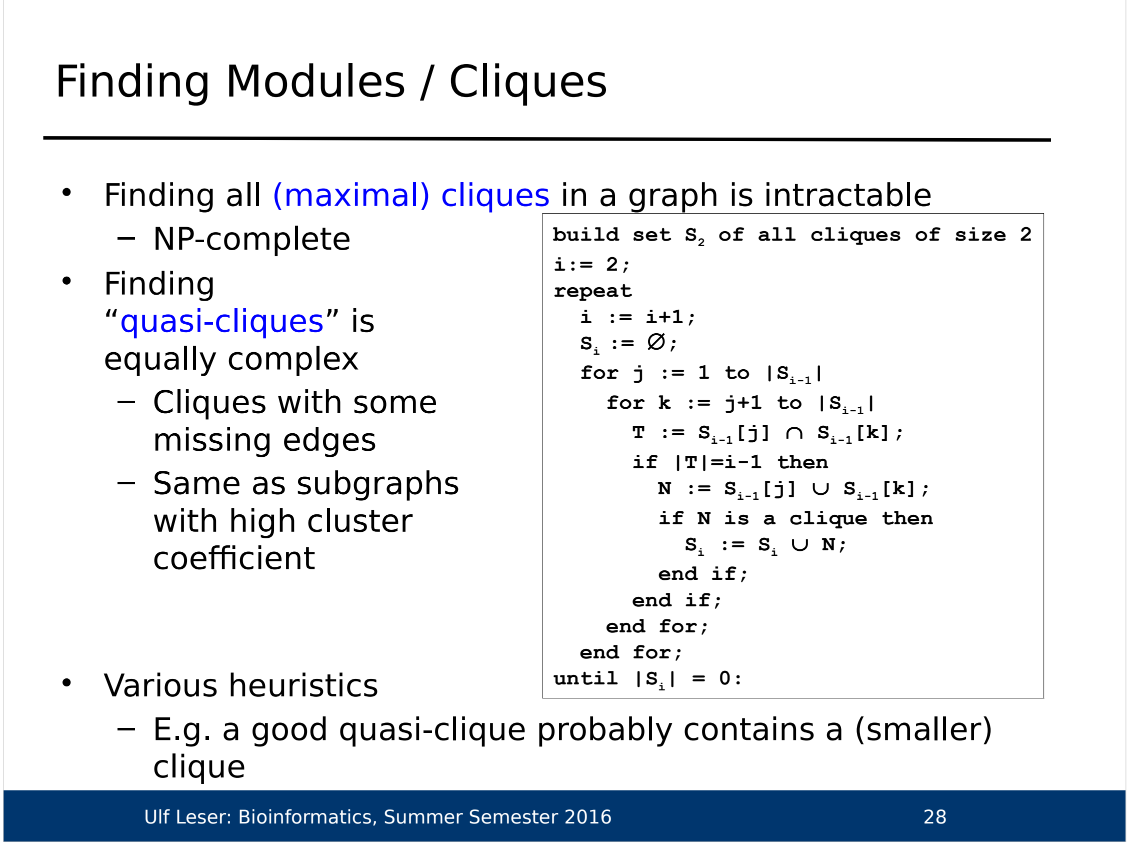
Each module can be reduced to a set of triangles – high density of triangles is reflected by a high clustering coefficient

For cliques C = 1

For non-triangle graphs = 0



Finding Modules / Cliques

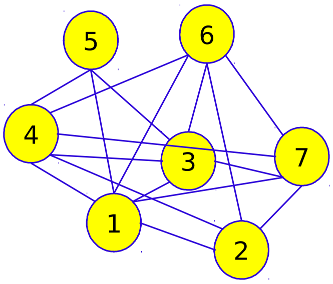
- Finding all (maximal) cliques in a graph is intractable
  - NP-complete
- Finding "quasi-cliques" is equally complex
  - Cliques with some missing edges
  - Same as subgraphs with high cluster coefficient

```
build set $S_2$ of all cliques of size 2
i:= 2;
repeat
  i := i+1;
  $S_i$ := $\emptyset$;
  for j := 1 to $|S_{i-1}|$
    for k := j+1 to $|S_{i-1}|$
      T := $S_{i-1}$[j] $\cap$ $S_{i-1}$[k];
      if |T|=i-1 then
        N := $S_{i-1}$[j] $\cup$ $S_{i-1}$[k];
        if N is a clique then
          $S_i$ := $S_i$ $\cup$ N;
        end if;
      end if;
    end for;
  end for;
until $|S_i|$ = 0:
```

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

Anderes Beispiel: social network, finden des größten Subset an Leuten, die sich alle kennen

Example



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

|(1,3,4,6)∩(1,3,4,7)|=3

(1,3,4,6)∪(1,3,4,7)=(1,3,4,6,7)

Edge (6,7) exists

5-clique

|(1,3,4,5)∩(1,3,4,6)|=3

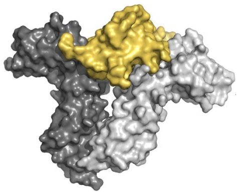
(1,3,4,5)∪(1,3,4,6)=(1,3,4,5,6)

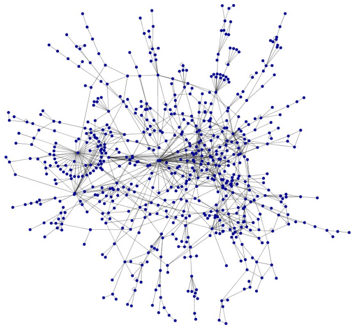
Edge (5,6) does not exists

No clique

This Lecture

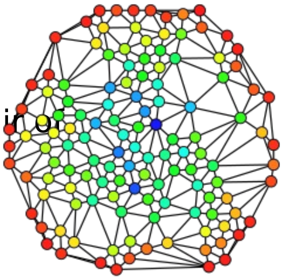
- Protein-protein interactions
- Biological networks
  - Scale-free graphs
  - Cliques and dense subgraphs
  - Centrality and diseases

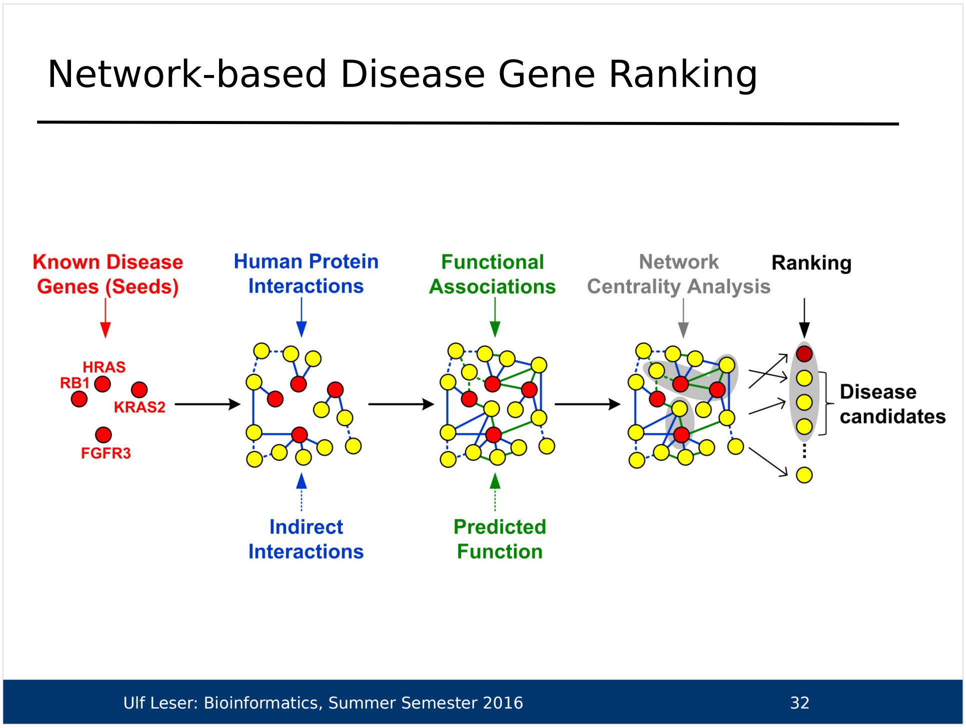




Network centrality

- 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

Known Disease Genes (Seeds)

HRAS
RB1
KRAS2
FGFR3

Human Protein Interactions

Indirect Interactions

Functional Associations

Predicted Function

Network Centrality Analysis

Ranking

Disease candidates

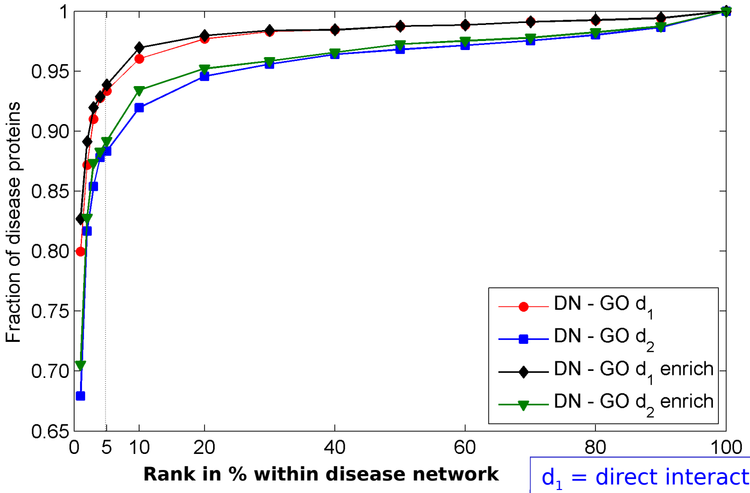
-For associating proteins with disease we follow a strategy that is based on finding disease-related proteins according to their similarity to known disease-related genes.
-Similarity with disease genes is measured in functional similarity and shared interactions

-For a given disease we first extract all genes that are known to be involved in the disease as seeds
-From these seeds we compile a disease specific network by integrating direct and indirctly interacting proteins as well as proteins with functional similarity

- Problem: many (especially novel) genes have little information attached
- Under-annotated genes are under-represented in the network

Centrality of Seeds in (OMIM) Disease Networks

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

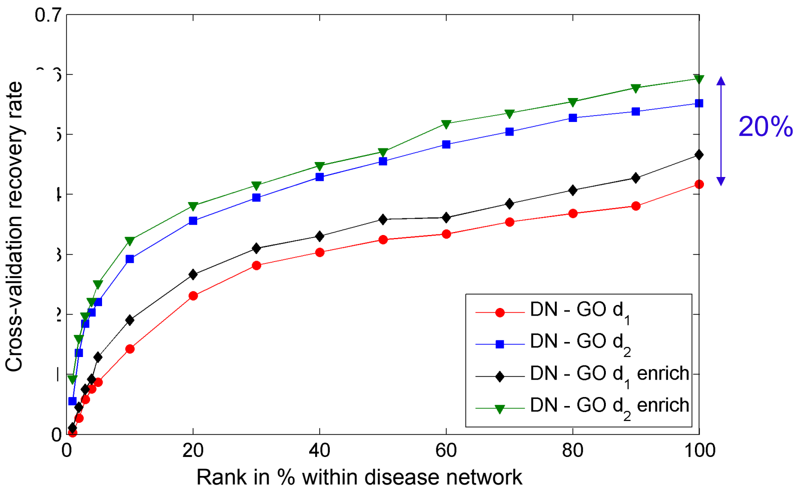


d_1 = direct interactions

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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