

Bio390: Introduction to Bioinformatics

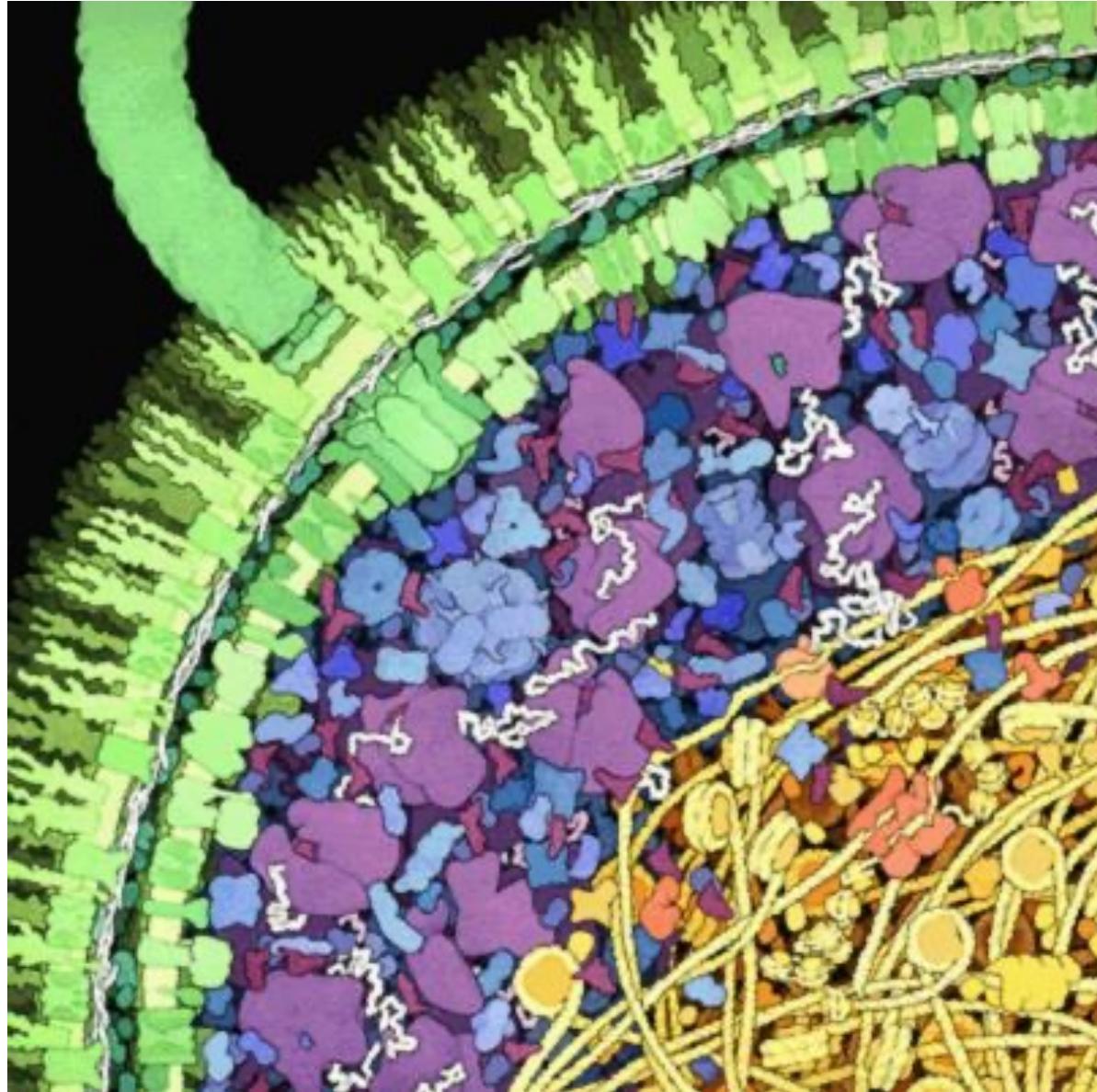
Protein – Protein Interactions

12.11.2018

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Cells are crowded places!



Protein interactions

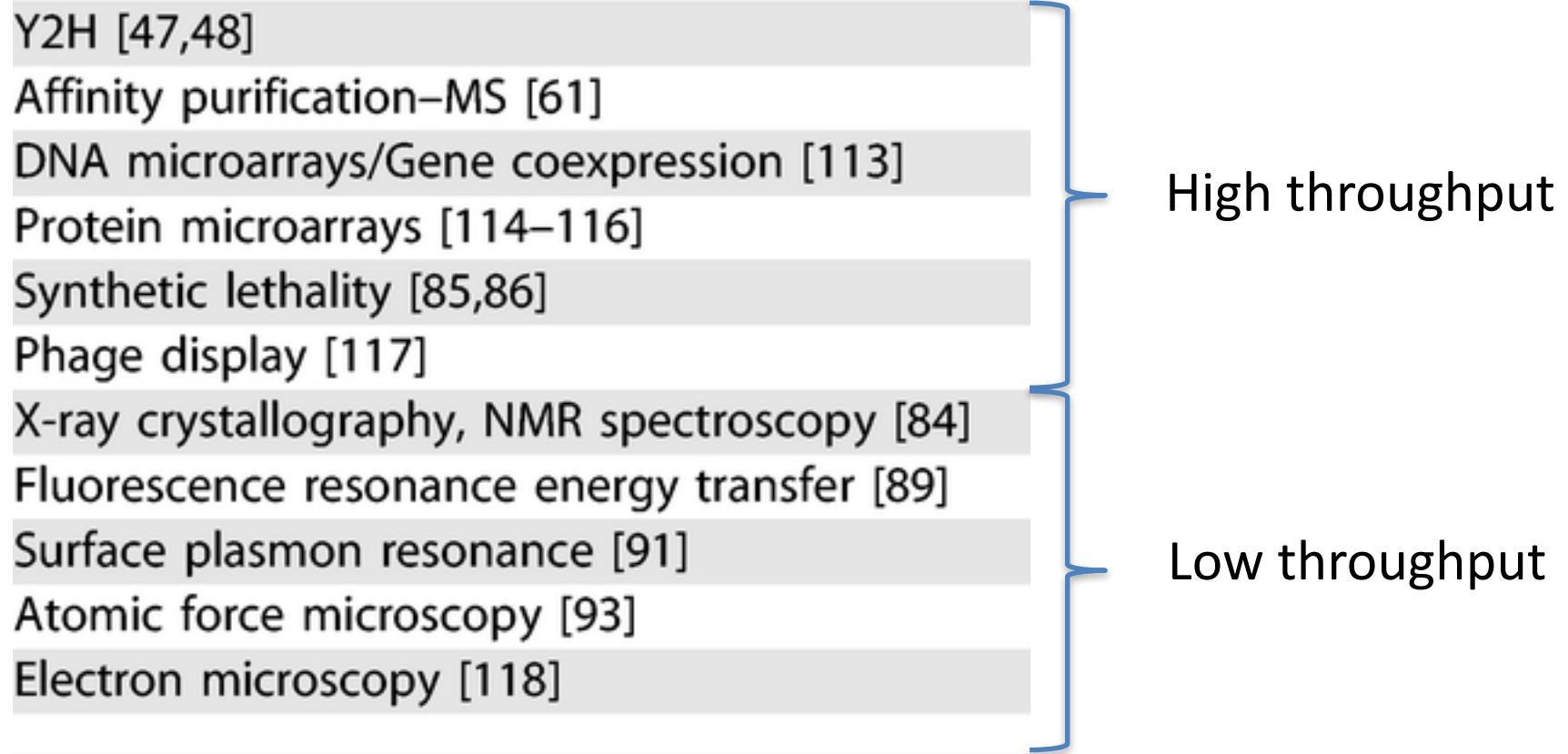
- Proteins interact with
 - Other proteins (protein complexes)
 - Metabolites (enzymatic activity)
 - DNA (regulatory activity)
 - Signaling molecules (signaling activity)

All carefully regulated

Overview

- Collect PPI data
 - Experimental methods to identify PPI
 - Text mining
 - Computational tools to predict PPI
- Curate PPI data - databases
- Analyze PPI data
 - Molecular level
 - Network level
- Predict novel PPIs

Protein-protein Interactions - Methods



Protein-protein Interactions - Methods

Low throughput (molecular level)

- Small scale
- One or a few proteins at a time
- Small number of data points
- Expensive and slow

High throughput

- Hundreds/thousands of proteins
- Noisy and incomplete data
- Little overlap across data sets

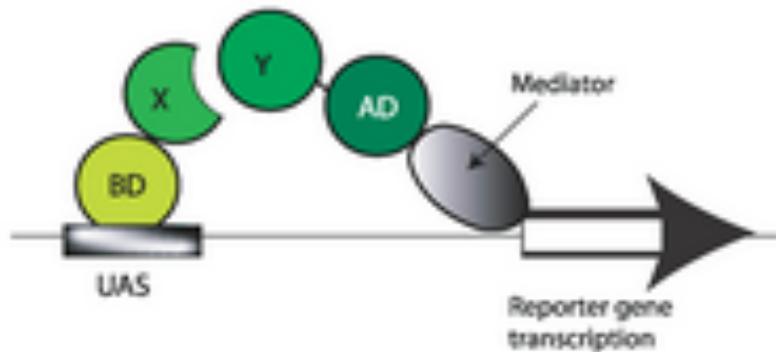
Protein-protein Interactions - Methods

- Various techniques are used to investigate protein-protein interactions, including:
 - Biochemical/biophysical methods (*in vitro*)
 - Isothermal calorimetry
 - Surface plasmon resonance (e.g. BIACore)
 - Mass spectrometry e.g. from protein complexes
 - “Pull-down” assays – one protein can be bound by an antibody (immunoprecipitation) or via a “tag”
 - Molecular/cellular biological methods (*in vivo*)
 - Two-hybrid experiments
 - Fluorescent proteins

Experimental Techniques for High-Throughput PPI analysis

A

Y2H



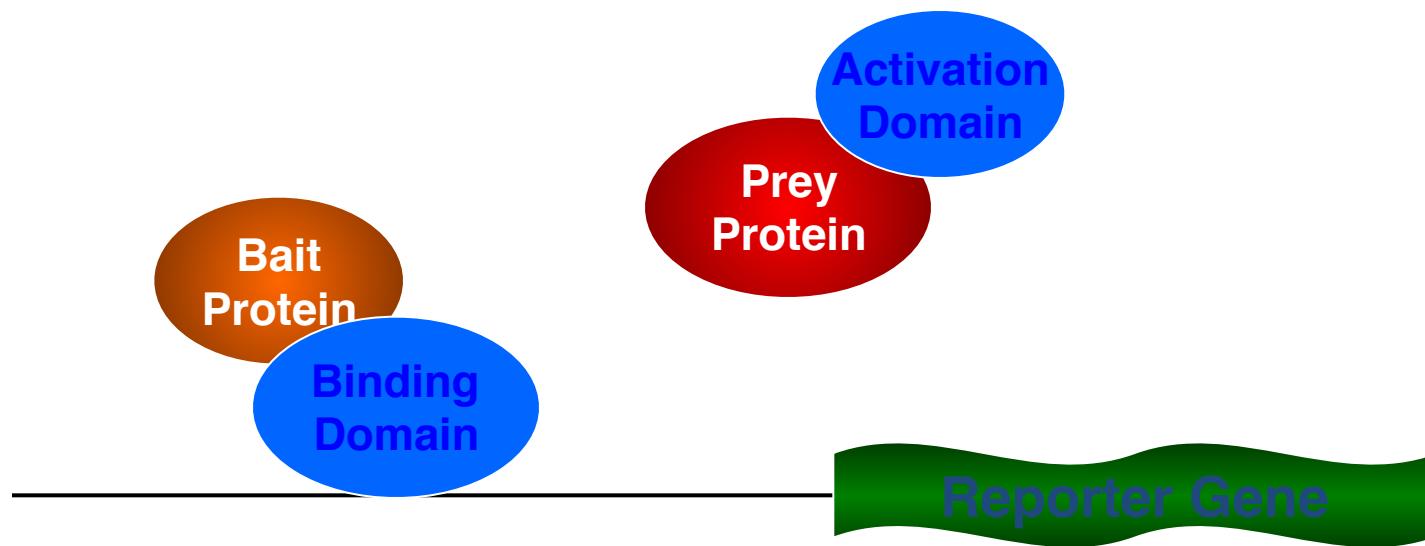
Most transcription activators have at least two distinct domains, one that directs binding to a promoter DNA sequence (BD) and another that activates transcription (AD)

In Y2H, X is fused to BD (bait). Y is fused to AD (prey).

If X and Y interact, AD and BD are brought together and the gene is activated.

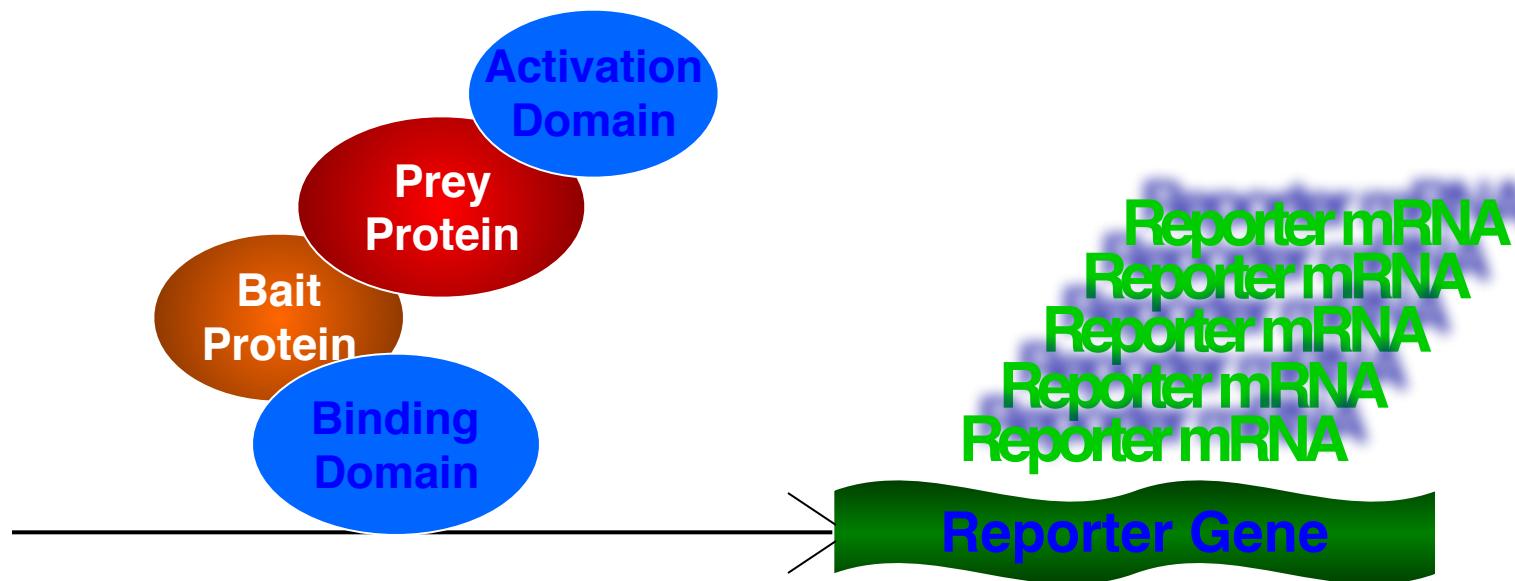
The Two-Hybrid System

- Two hybrid proteins are generated with transcription factor domains
- Both fusions are expressed in a yeast cell that carries a reporter gene whose expression is under the control of binding sites for the DNA-binding domain



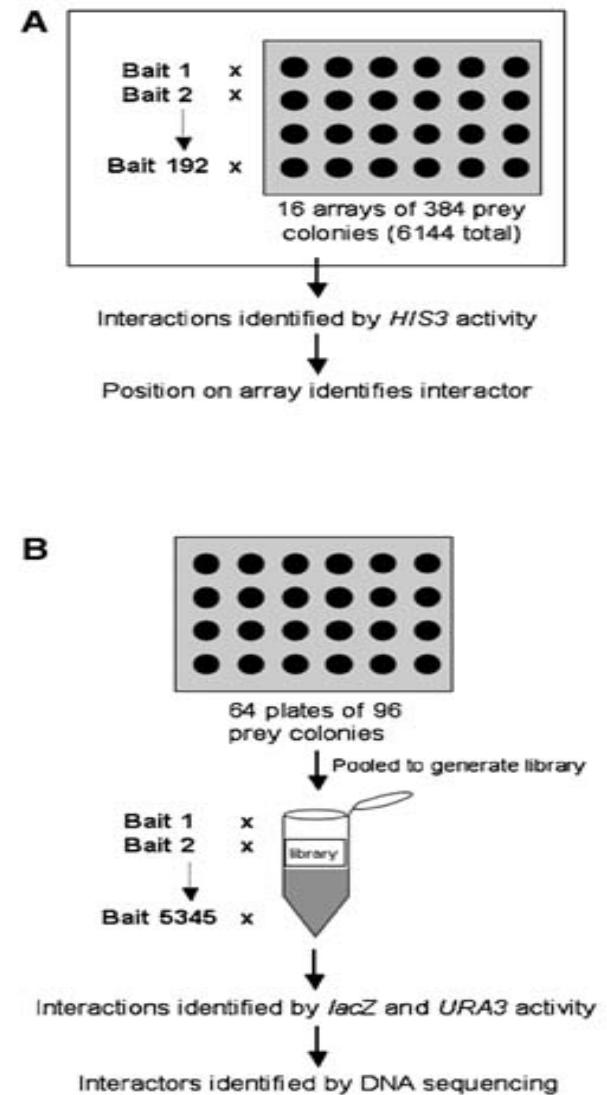
The Two-Hybrid System

- Interaction of bait and prey proteins localizes the activation domain to the reporter gene, thus activating transcription.
- Since the reporter gene typically codes for a survival factor, yeast colonies will grow only when an interaction occurs.



Genome-wide analysis by Y2H

- **Matrix approach:** a matrix of prey clones is added to the matrix of bait clones. Diploids where X and Y interact are selected based on the expression of a reporter gene.
- **Library approach:** one bait X is screened against an entire library. Positives are selected based on their ability to grow on specific substrates.



Limitations of Y2H

- Fusion of a protein into chimeras can change the structure of a target
- Protein interactions can be different in yeast and the organisms where the genes came from
- It is difficult to target extracellular proteins
- It is hard to detect interactions between proteins active only in a *complex*
- Proteins which can interact in two-hybrid experiments, may never interact *in vivo*

Identifying Protein–Protein Interactions

Protein complex isolation

- Epitope tag one protein in the complex
- Isolate tagged protein to isolate stably interacting proteins
- Separate and identify all isolated proteins

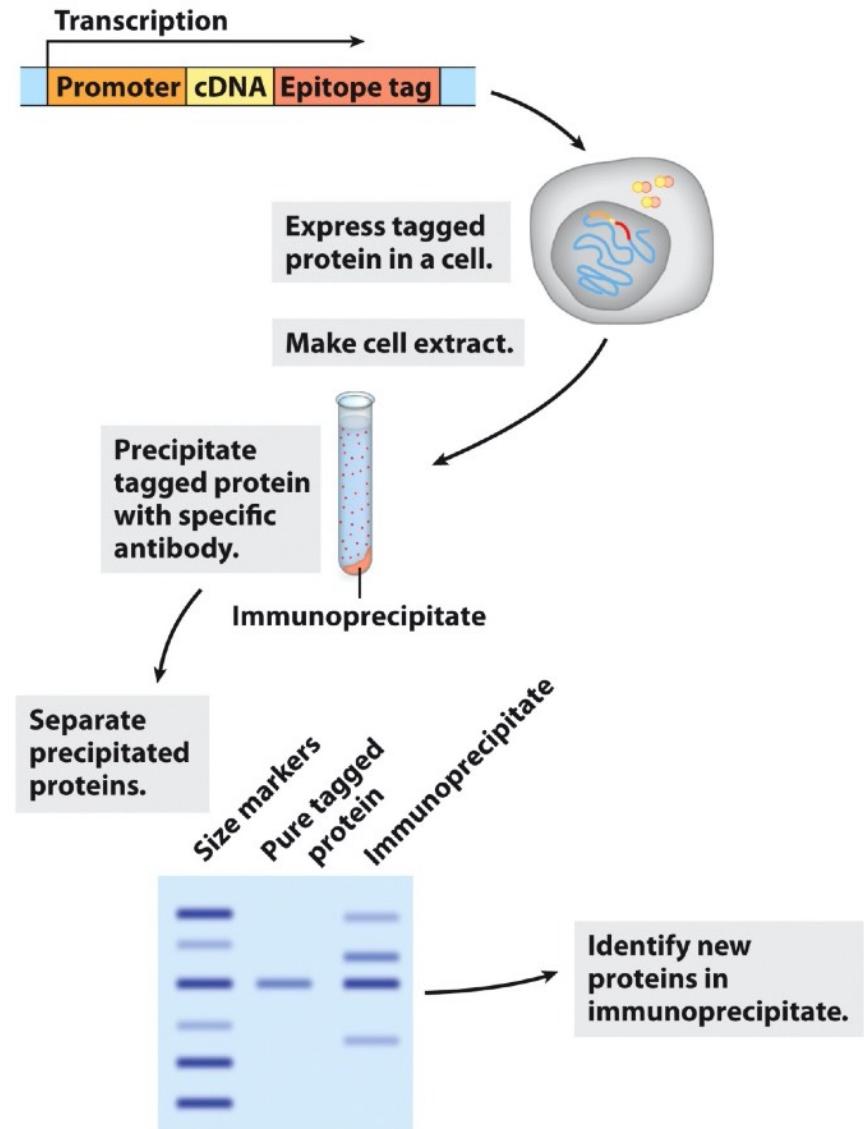


Figure 9-19
Lehninger Principles of Biochemistry, Sixth Edition
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Tandem Affinity Purification

A target gene is fused with the TAP tag (protein A + calmodulin binding peptide) and is expressed in yeast.

Protein forms native complexes with other proteins.

1st step of purification: protein A interacts with IgG matrix.

Eluate incubated with calmodulin coated beads.

After washing: protein complexes harvested and identified by MS.

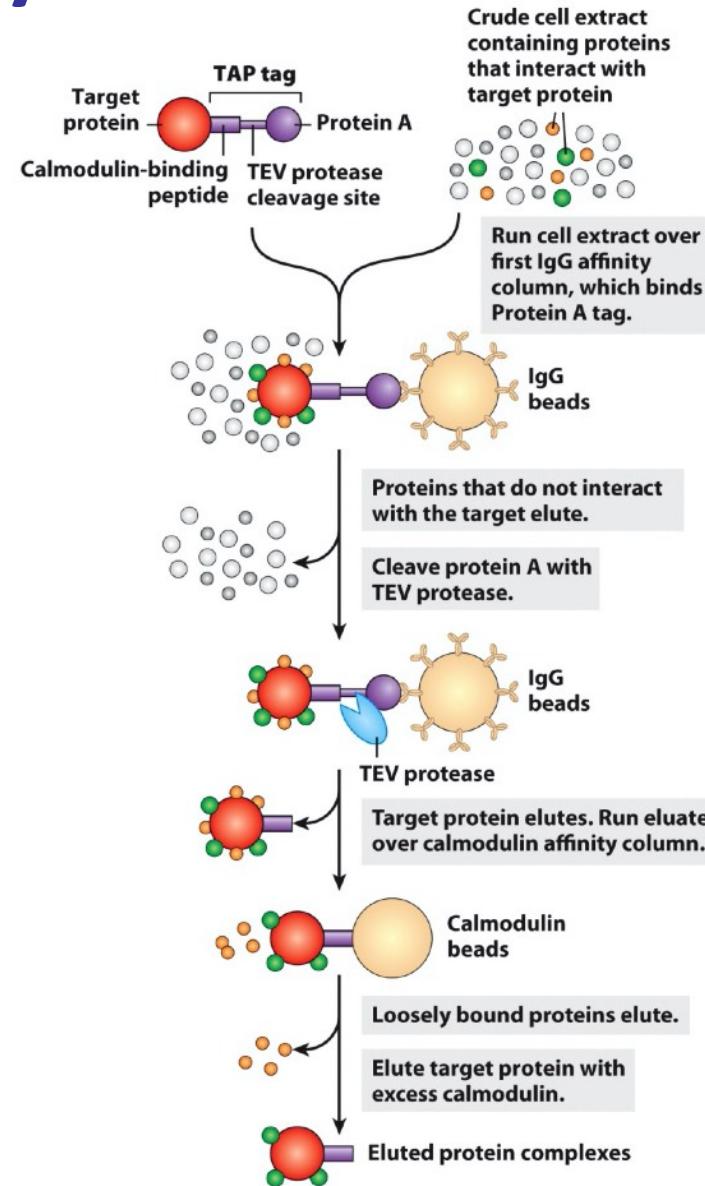


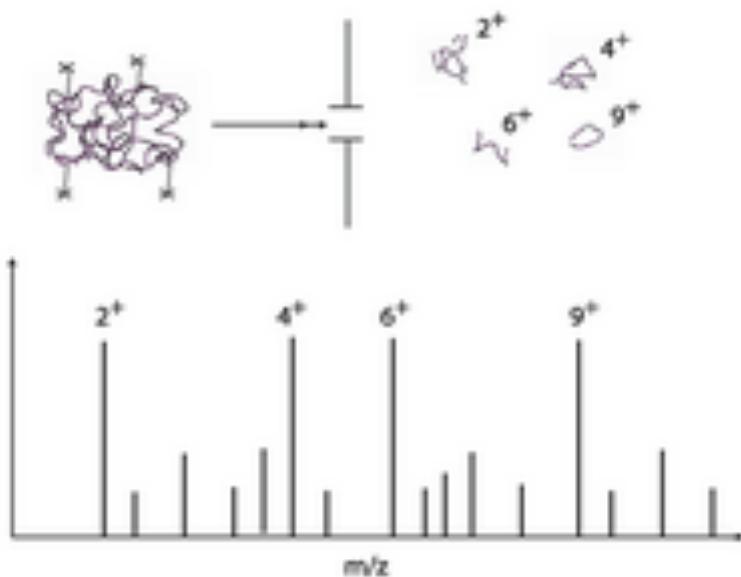
Figure 9-20

Lehninger Principles of Biochemistry, Sixth Edition
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Experimental Techniques for High-Throughput PPI analysis

B

MS



Mass spectroscopy

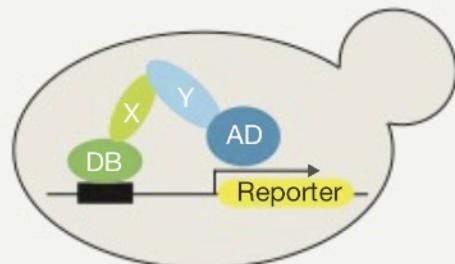
Protein complexes are purified.

MS identifies polypeptide sequence.

(a)

Binary mapping

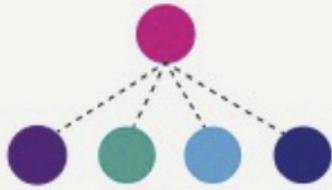
Yeast two-hybrid (Y2H)



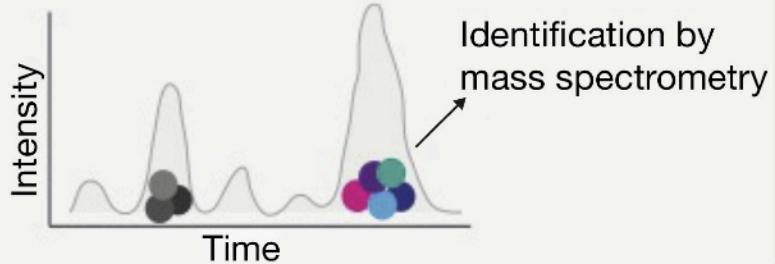
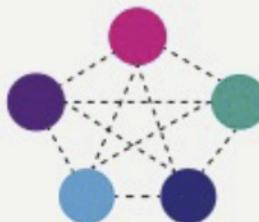
(b)

Co-complex mapping

Affinity purification
followed by mass
spectrometry
(AP-MS)



Co-fractionation
followed by mass
spectrometry



● Protein — Direct physical interaction ----- Protein association

----- Protein association

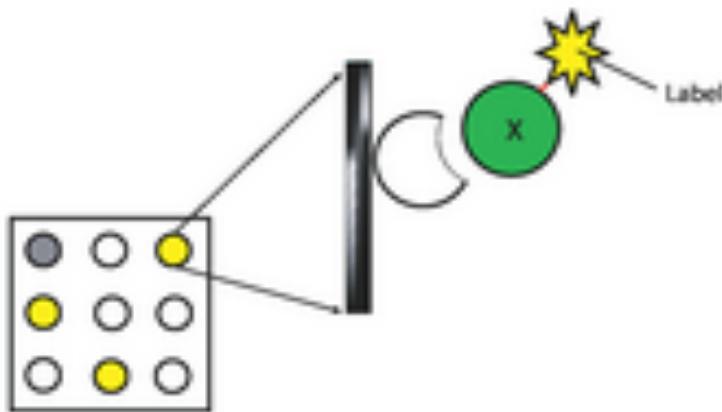
Differences and similarities between Y2H and MS-TAP

- TAP permits protein *complexes* to be isolated, but cannot detect weak/transient PPIs
- Both methods generate a lot of false positives, only ~50% interactions are biologically significant
- Y2H is an *in vivo* technique
- MS can detect large stable complexes and networks of interactions

Experimental Techniques for High-Throughput PPI analysis

E

Protein Microarray



Protein microarrays (protein chips) can detect interactions between actual proteins rather than genes: target proteins immobilized on the solid support are probed with a fluorescently labeled protein.

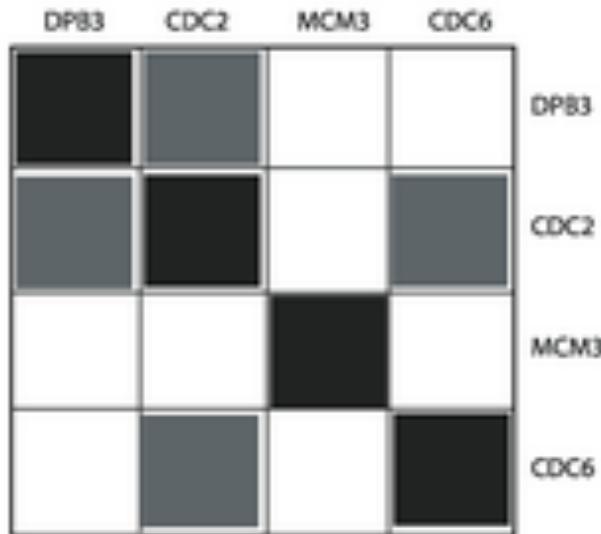
Protein-protein Interactions

- Direct methods
 - Yeast 2 hybrid
 - TAP tagging
 - Mass spectrometry
- Indirect
 - Correlated mRNA expression profiles
 - Genetic interaction data
 - Interolog analysis
 - Co-localization, co-expression
 - Correlated mutations

Experimental Techniques for High-Throughput PPI analysis

D

Gene co-expression



Hypothesis: Functional subunits of a protein complex should be present in stoichiometric amounts in cell.

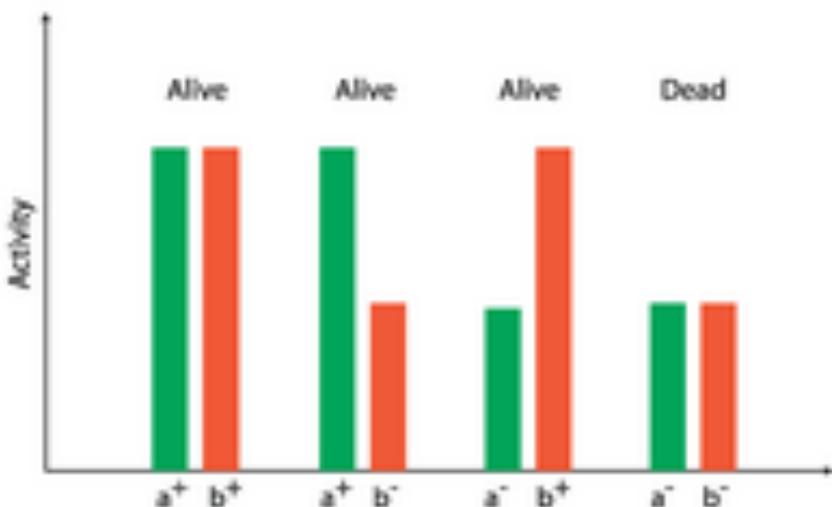
Gene coexpression analysis produces a correlation matrix where the dark areas show high correlation between expression levels of corresponding genes.

Especially high correlation for permanent complexes (ribosome, proteasome).

Experimental Techniques for High-Throughput PPI analysis

F

Synthetic lethality



Synthetic lethality method describes the genetic interaction when two individual, nonlethal mutations result in lethality when combined together ($a^- b^-$).

Study of Protein-protein Interactions *In Vivo*

- Various fluorescent techniques are also in use:
 - FRET – fluorescence resonance energy transfer; reports on distance between 2 fluorophores
 - Fluorescent reporters – expressed proteins emit fluorescence at specific wavelength
 - FRAP (FLIP) – fluorescence recovery after photobleaching (fluorescence loss in photobleaching); allows movement of reporters to be monitored

Identifying Regions Involved in Protein-protein Interactions

- Once protein-protein interactions have been identified, it is important to establish how the interactions occur i.e. what regions or specific amino acids are important for the interaction?
- Determine effect of mutations on the protein-protein interaction to identify critical residues for binding
- Results usually confirmed by more than one experimental technique

Structural information on protein complexes

- Protein Data Bank: maintained by the Research Collaboratory of Structural Bioinformatics(RCSB)
 - <http://www.rcsb.org/pdb/>
 - As of Sunday Sep 30, 2018 there are 144,682 Structures
 - including structures of Protein/Nucleic Acid Complexes, Nucleic Acids, Carbohydrates
 - The number of protein complexes is around 26,000.
- Each structure has a **PDB ID**: a 4 character unique identifier
- Most structures are determined by X-ray crystallography (about 130000 entries). Some other methods are NMR (about 12000 entries) and electron microscopy (EM) (about 2500 entries).

Structural information

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PDB-101 Worldwide PDB EMDataBank Nucleic Acid Database Worldwide Protein Data Bank Foundation

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A Structural View of Biology

This resource is powered by the Protein Data Bank archive-information about the 3D shapes of proteins, nucleic acids, and complex assemblies that helps students and researchers understand all aspects of biomedicine and agriculture, from protein synthesis to health and disease.

As a member of the wwPDB, the RCSB PDB curates and annotates PDB data. The RCSB PDB builds upon the data by creating tools and resources for research and education in molecular biology, structural biology, computational biology, and beyond.

Openings with RCSB PDB at UCSD

JOIN OUR TEAM

September Molecule of the Month

Bioinformatics - tasks

- Collect PPI information
- Analyze PPIs
 - Molecular level

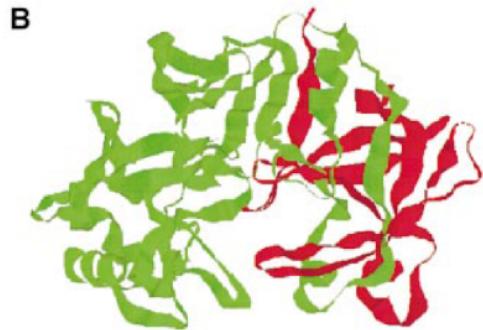
Types of protein-protein interactions (PPI)

Obligate PPI

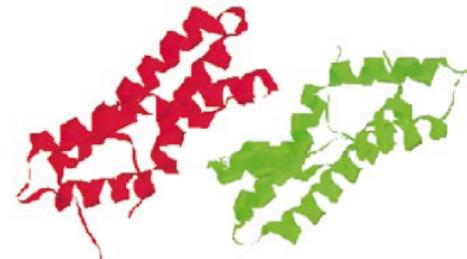
the protomers are not
found as stable
structures on their own
in vivo



Obligate
homodimer
P22 Arc
repressor DNA-
binding



Obligate heterodimer
Human cathepsin D
[1LYB](#)



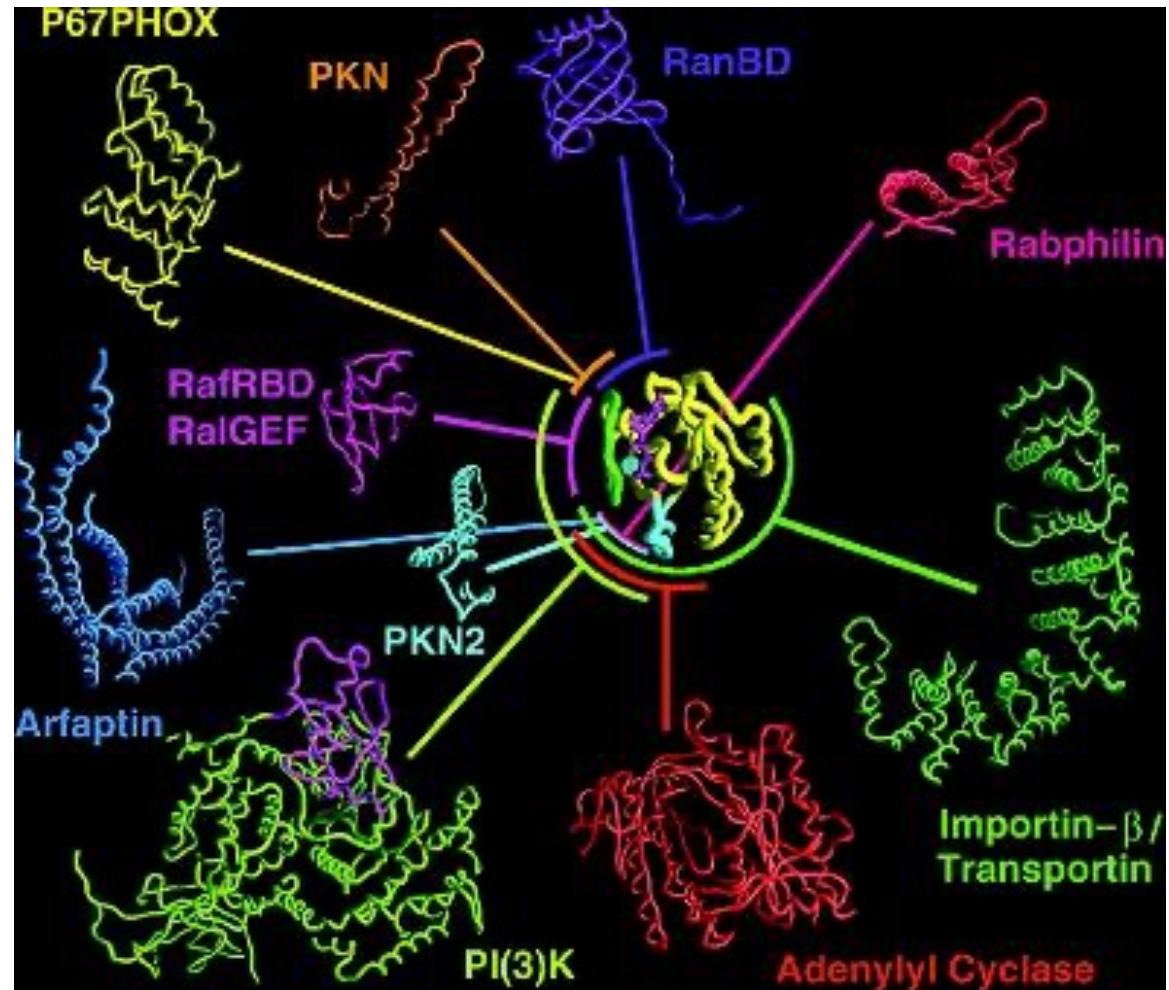
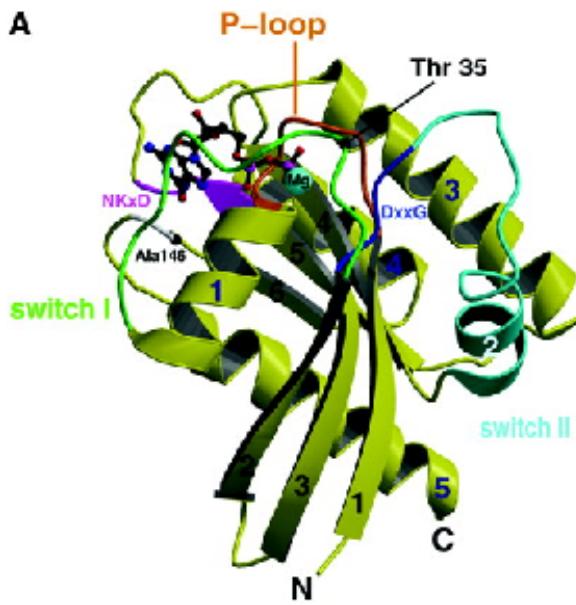
Non-obligate homodimer
Sperm lysin



Non-obligate heterodimer
RhoA and RhoGAP signaling complex

Multiple interactions: Guanine-nucleotide binding protein

A



Adapted from Vetter & Wittinghofer, *Science* 2001

Protein protein complex formation driven by encounter, concentration of components and free energy of complex relative to unbound form

PPIs can be controlled by

- Altering local concentration of the protein components
- Influencing the binding affinity, determined by the physicochemical and geometrical interface properties

General Properties of Protein-Protein Binding Sites

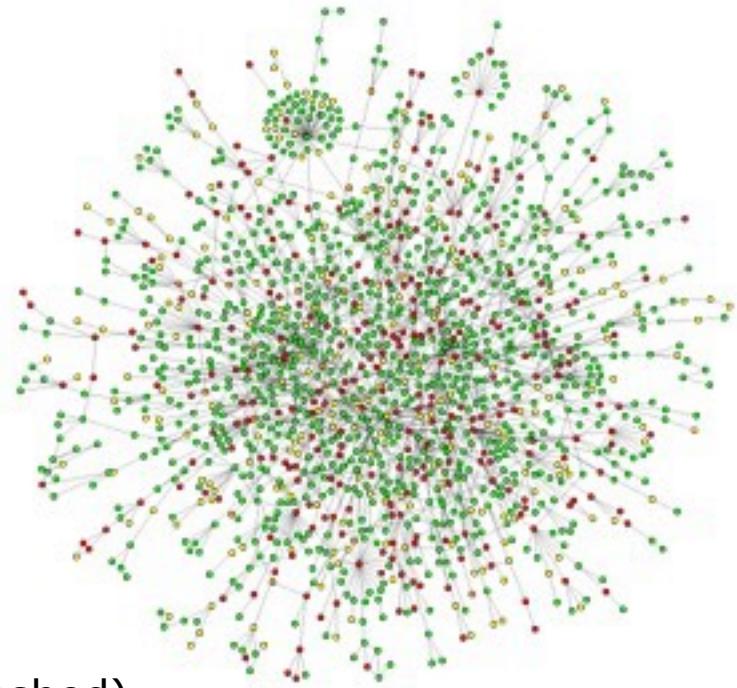
- More **hydrophobic** than the rest of the protein surface.
- Relatively **flat** (except for enzyme-substrate binding sites) with a core (buried) and rim (solvent accessible)
- The binding free energy is not distributed equally across the protein interfaces: a small subset of residues at the interfaces forms energy **hot spots**, enriched in tyrosine, tryptophan, and arginine.
- **Structurally conserved residues**, especially polar residues, correspond to the energy hot spots.
- Most sites are **specific** but some have **promiscuous** binding characteristics

Bioinformatics - tasks

- Collect PPI information
- Analyze PPIs
 - Molecular level
- Curate PPIs in a database

PPI Databases

- DIP
 - <http://dip.doe-mbi.ucla.edu/>
- MIPS (small scale)
 - <http://mips.gsf.de/proj/ppi/>
- BIND (PPI, Prot-DNA, Prot-SM)
 - <http://www.bind.ca> (now owned by Unleashed)
- OPHID (predicted interactions)
 - <http://ophid.utoronto.ca/ophid/>
- MINT - Molecular Interactions Database
 - <http://mint.bio.uniroma2.it/mint/Welcome.do>
- IntAct (EBI)
 - <http://www.ebi.ac.uk/intact/site/>
- InterDom (domain interactions)
 - <http://interdom.lit.org.sg/>
- STRING (EMBL)
 - <http://string.embl.de/>



PPI databases

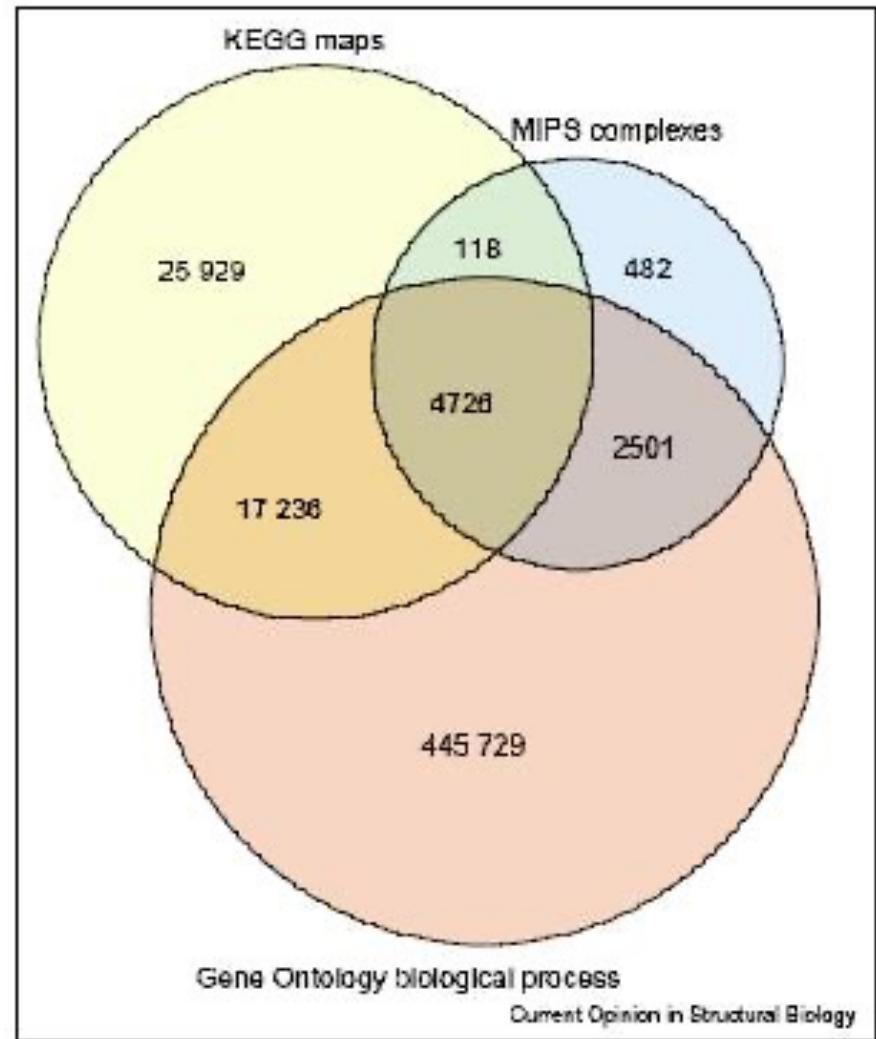
Types

- Experiment (E)
- Structure detail (S)
- Predicted
 - Physical (P)
 - Functional (F)
- Curated (C)
- Homology modeling (H)
- *International Molecular Exchange (IMEx) consortium

Database	Proteins/Domains	Type
DIP*, LiveDIP	P	E,S
BIND*	P	E,C,S
MPact/MIPS*	P	E,C,F
STRING	P	E,P,F
MINT*	P	E,C
IntAct*	P	E,C
BioGRID*	P	E,C
HPRD	P	E,C
3did, Interprets	D	S,H
Pibase, ModBase	D	S,H
CBM	D	S
iPfam	D	S
InterDom	D	P
DIMA	D	F,S
Prolinks	P	F

Comparing the DBs

- High FP rate in high-throughput experiments
- Disagreement between benchmark sets
- Experimental PPI data is sparse relative to all PPIs, so dataset overlap is small and hard to confirm with multiple sources



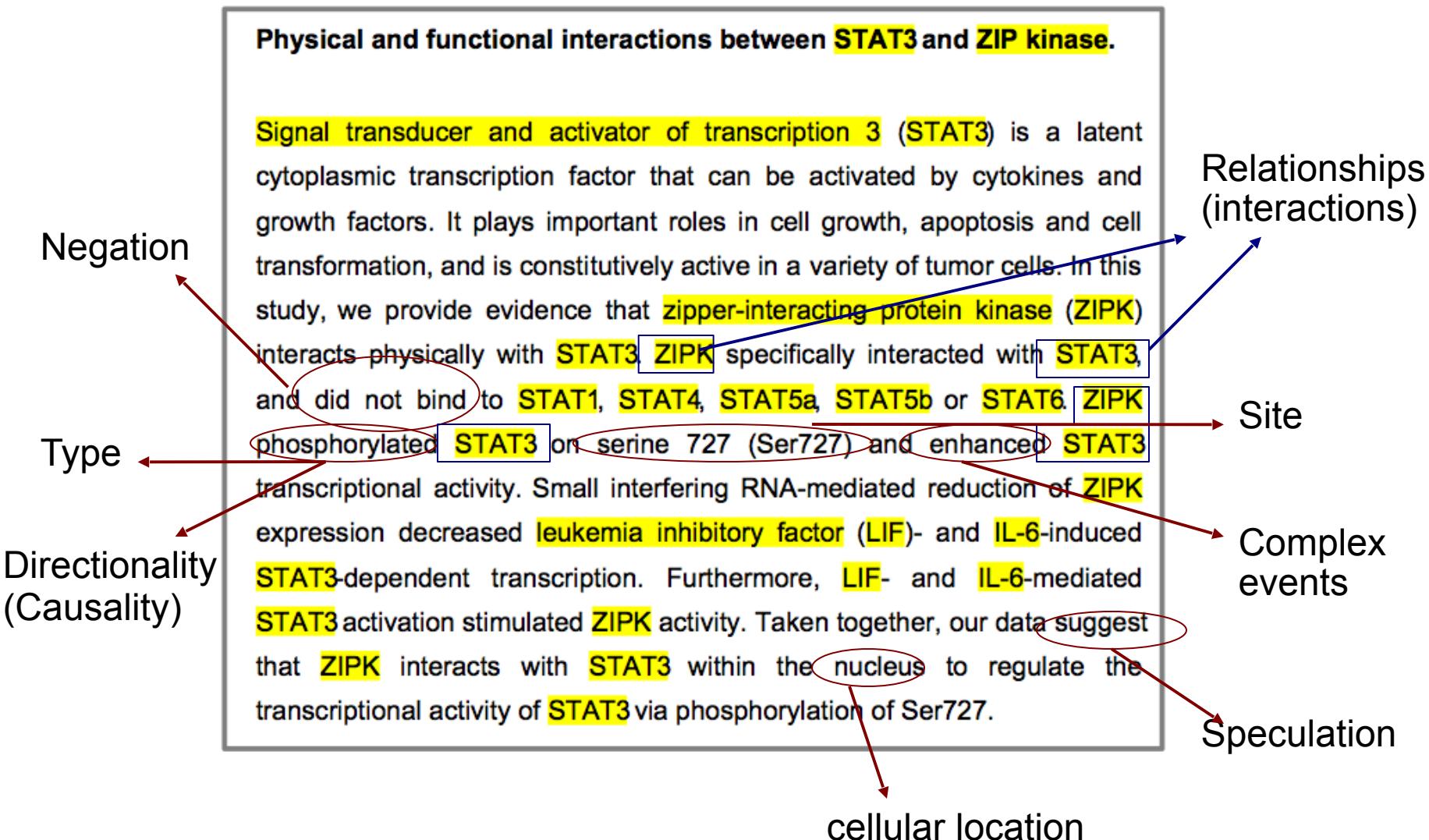
Bioinformatics - tasks

- Collect PPI information
- Analyze PPIs
 - Molecular level
- Curate PPIs in a database
- Extract information about PPI from texts

Text Mining

- Searching Medline or PubMed for words or word combinations
- Co-occurrence of terms is the simplest metric, yet lends to a higher FP rate
- Natural Language Processing (NLP) methods are more specific (e.g., “X binds to Y”; “X interacts with Y”; “X associates with Y” etc.) yet are difficult to detect so it has a higher FN rate
- Normally requires a list of known gene names or protein names for a given organism

What can be extracted



NLP pipeline

Tokenization

- What are the words?
- Break sentence into words

Morphology

- Named entity recognition (NER)
- Part of speech tagging (classification)

Syntax

- Dependency parsing
- Using grammar rules

Semantics

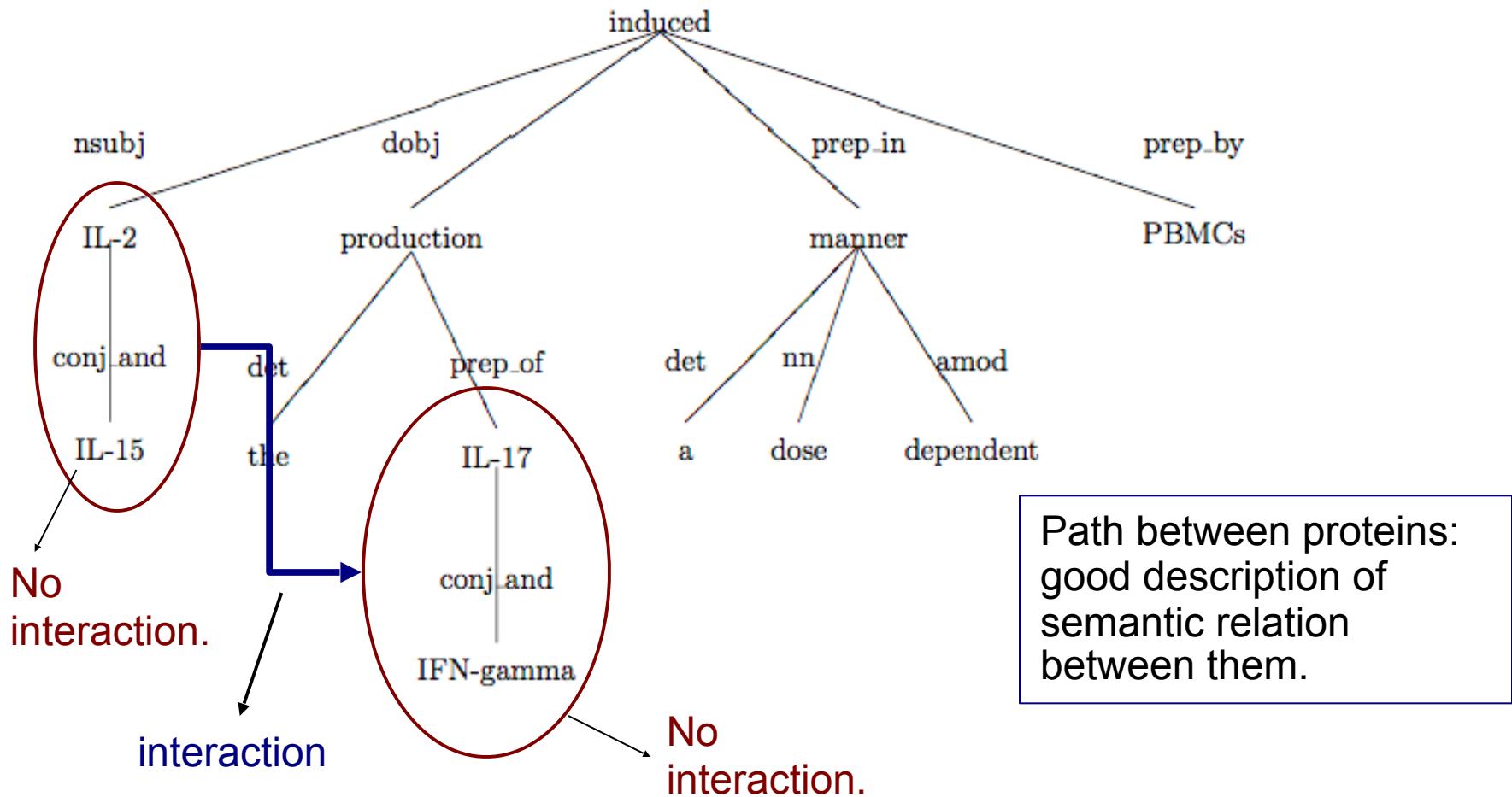
- Codependency resolution
- Use context info

Applications

- Machine translation
- Language generation + dialog

Interaction Extraction

IL-2 and IL-15 induced the production of IL-17 and IFN-gamma in a dose dependent manner by PBMCs.
(Genia Tagger: 71% F-measure)

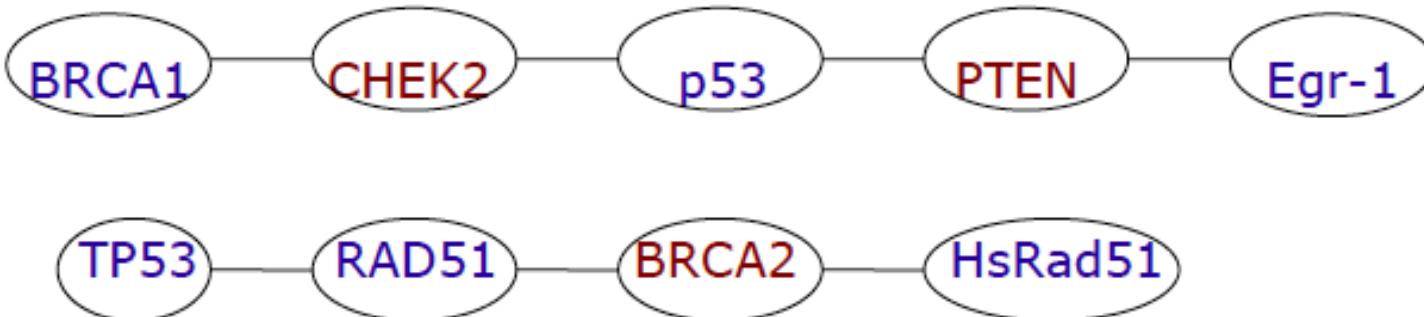


Stanford Parser is used to generate the dependency parse trees (de Marneffe et al., 2006).

Constructing the Interaction Network

Sample extracted interaction sentences:

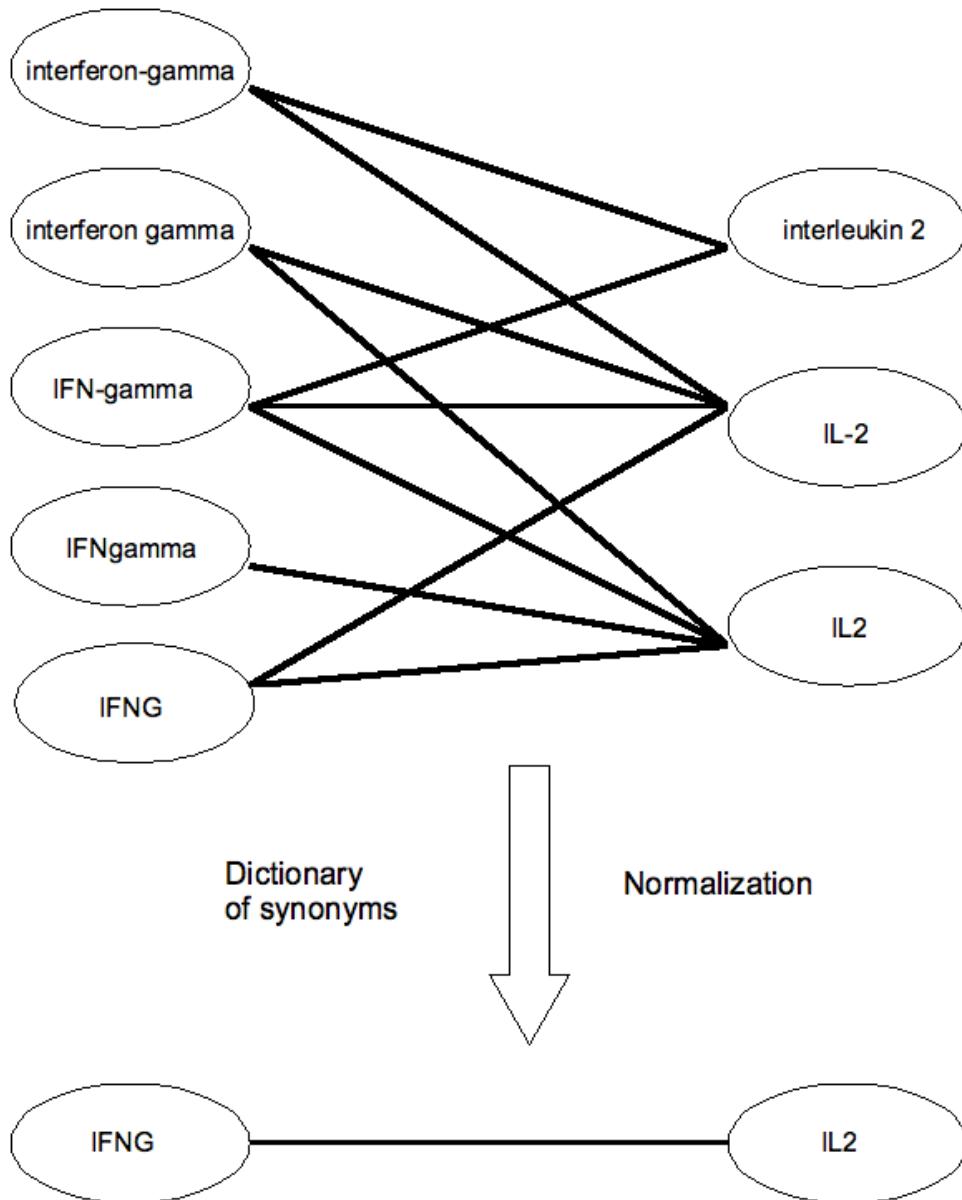
- PTEN is transcriptionally regulated by transcription factors such as p53 and Egr-1.
- In response to DNA damage, the cell-cycle checkpoint kinase CHEK2 can be activated by ATM kinase to phosphorylate p53 and BRCA1, which are involved in cell-cycle control and apoptosis.
- The interactions of RAD51 with TP53, RPA and the BRC repeats of BRCA2 are relatively well understood (see Discussion).
- The interaction of BRCA2 with HsRad51 is significantly more different to both RadA and RecA (Figure 2c).
- The constructed graph:



seed genes:

CHEK2
PTEN
BRCA2

Gene Name Normalization

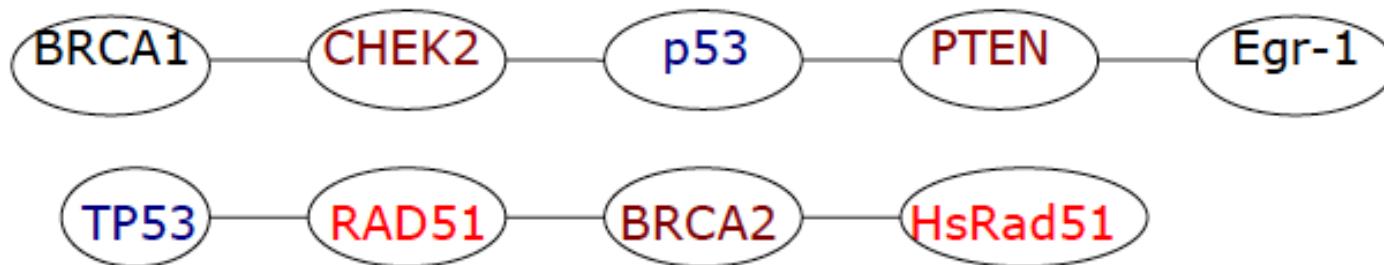


HUGO Gene Nomenclature Committee (HGNC) database used as the dictionary for gene names and their synonyms.
~28,000 gene records

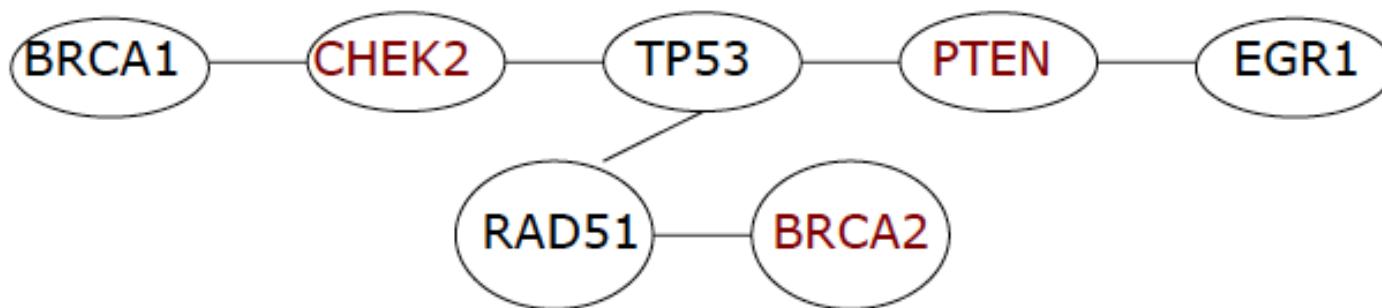
(<http://www.genenames.org/>)

Constructing the Interaction Network

- The graph before gene name normalization:



- The graph after gene name normalization:



Pre-BIND

- Used Support Vector Machine (SVM) to scan literature for PPIs
- Precision, accuracy and recall of 92% for correctly classifying PPI abstracts
- Estimated to capture 60% of all abstracted protein interactions for a given organism

Bioinformatics - tasks

- Collect PPI information
- Analyze PPIs
 - Molecular level
- Curate PPIs in a database
- Extract information about PPI from texts
- Analyze PPIs
 - Systems level

Assessment of Protein-protein Interaction Data

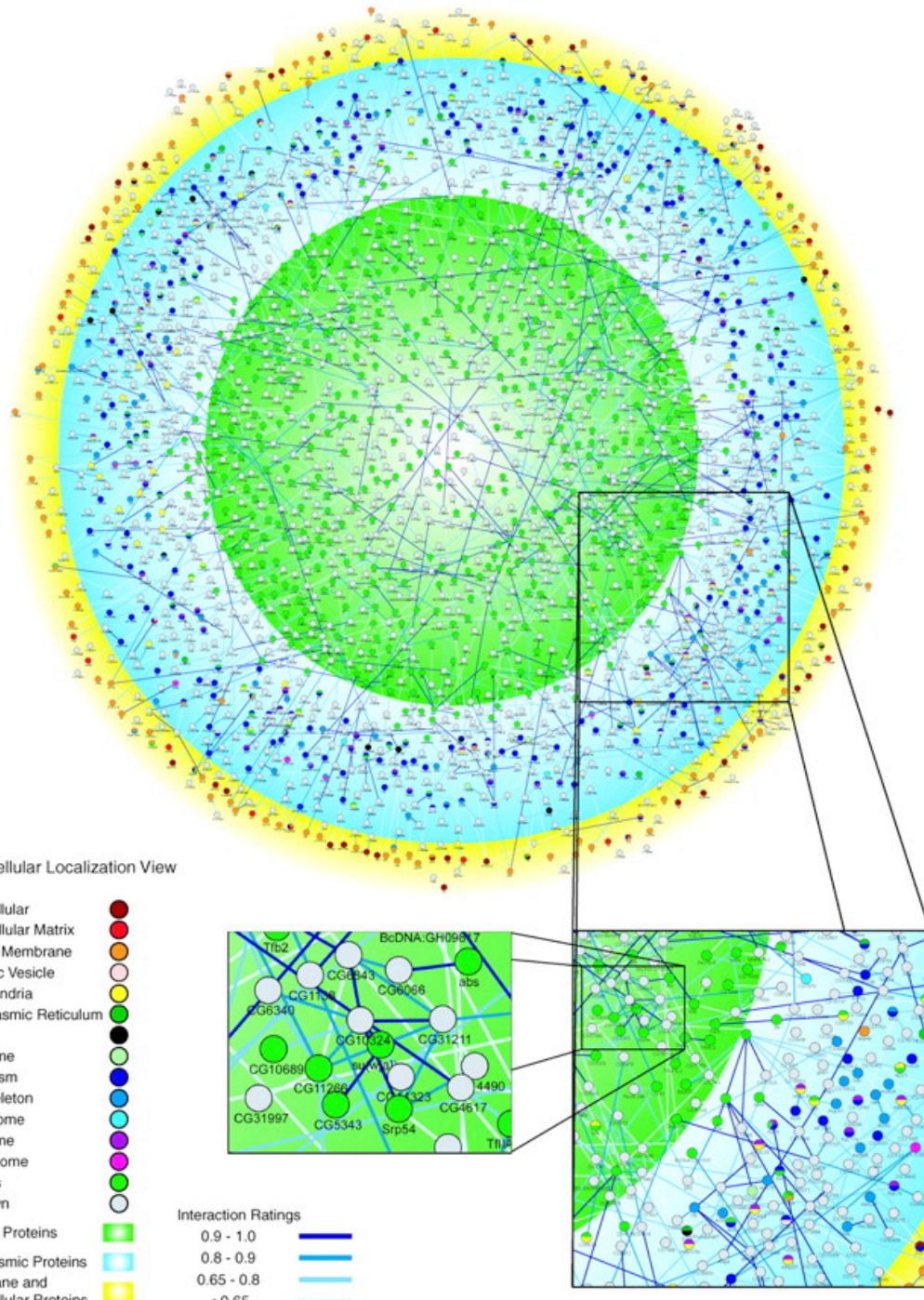
- Currently believed that yeast has >30,000 different interactions (for ~6,000 proteins)
- Overall conclusion is: different techniques identify different complexes!
- Results from protein-protein interaction studies should be confirmed by more than one experimental technique
- Especially important for considering if *in vitro* observations are relevant for *in vivo* situations

PPI network properties

Nodes & connections

B

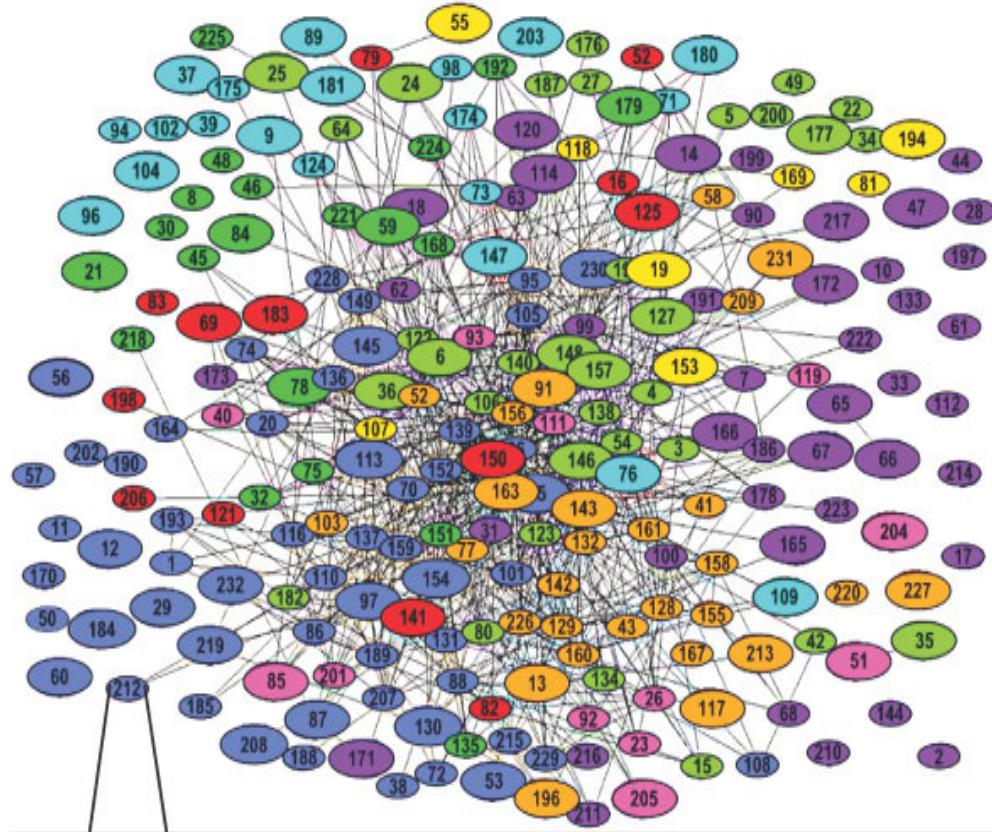
Drosophila interaction map

**From:****A Protein Interaction Map of Drosophila**

Giot et al. Science 302, 1727-1136 (2003)

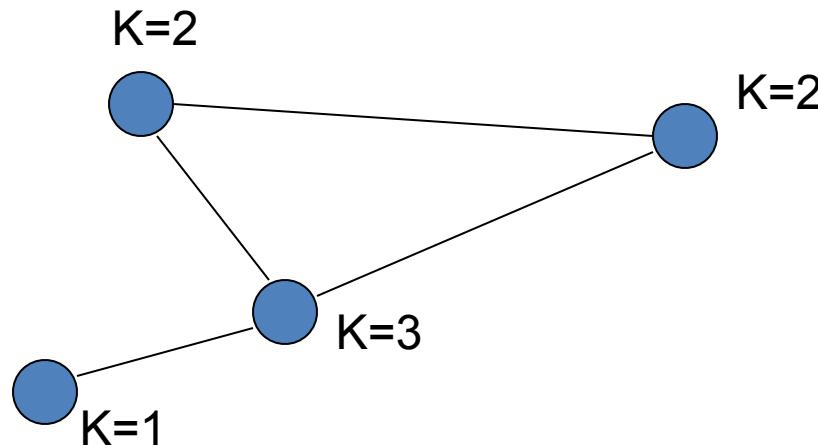
Functional organization of yeast proteome: network of protein complexes

- Essential gene products are more likely to interact with essential rather than nonessential proteins
- Orthologous proteins interact with complexes enriched with orthologs



Characteristics of networks

$n = \text{nodes}$, $k = \text{connections or "edges"}$



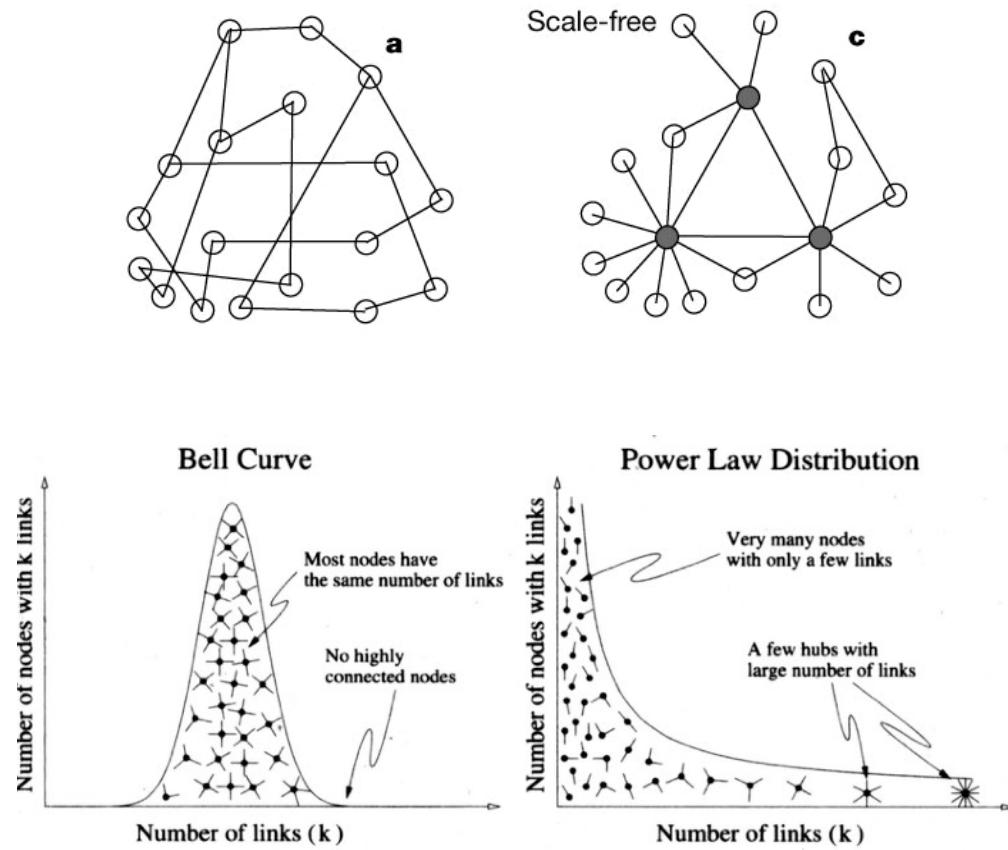
- In biology, n refers to genes/proteins (and/or metabolites) while k refers to interactions

Difference between scale-free and random graph models.

Random networks are homogeneous, most nodes have the same number of links.

Scale-free networks have a few highly connected hubs and many nodes with few connections.

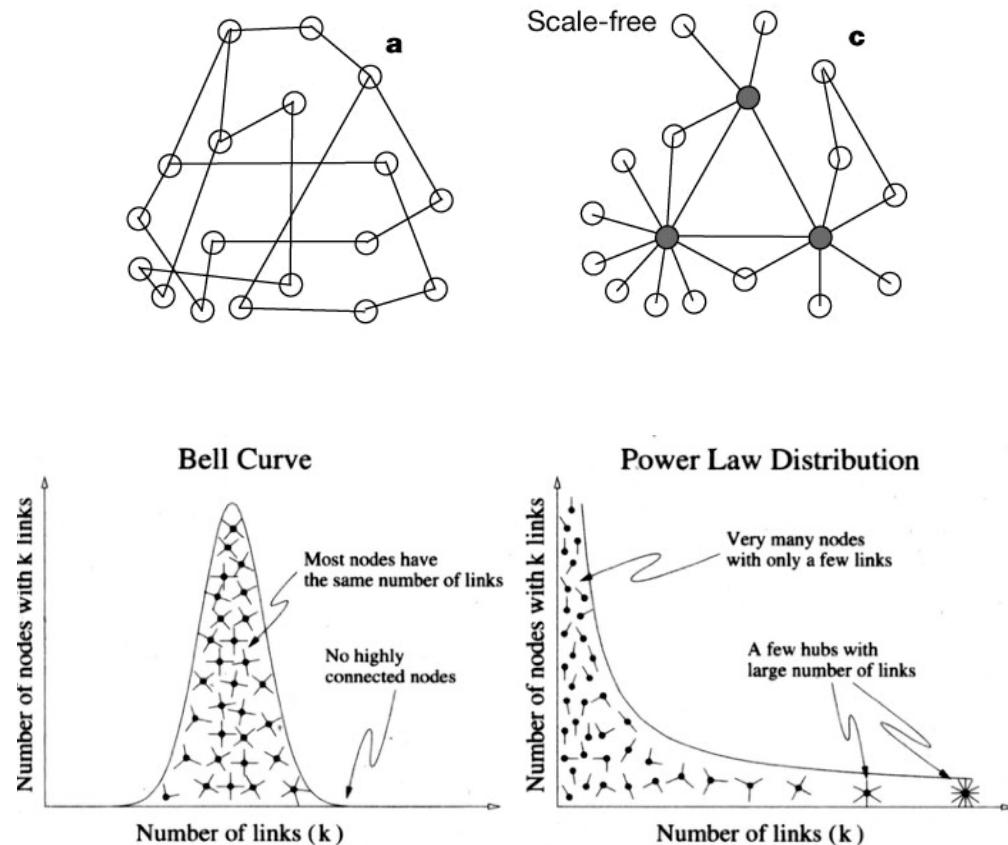
The presence of the hubs ensures that the nodes with few connections (low degree) are still connected to the network. Thus ensuring efficiency of communication.



Difference between scale-free and random graph models.

As a result, scale free networks are attack tolerant. A **random** attack does not break network down.

Attacks targeting hubs (highly connected nodes) are more dangerous.

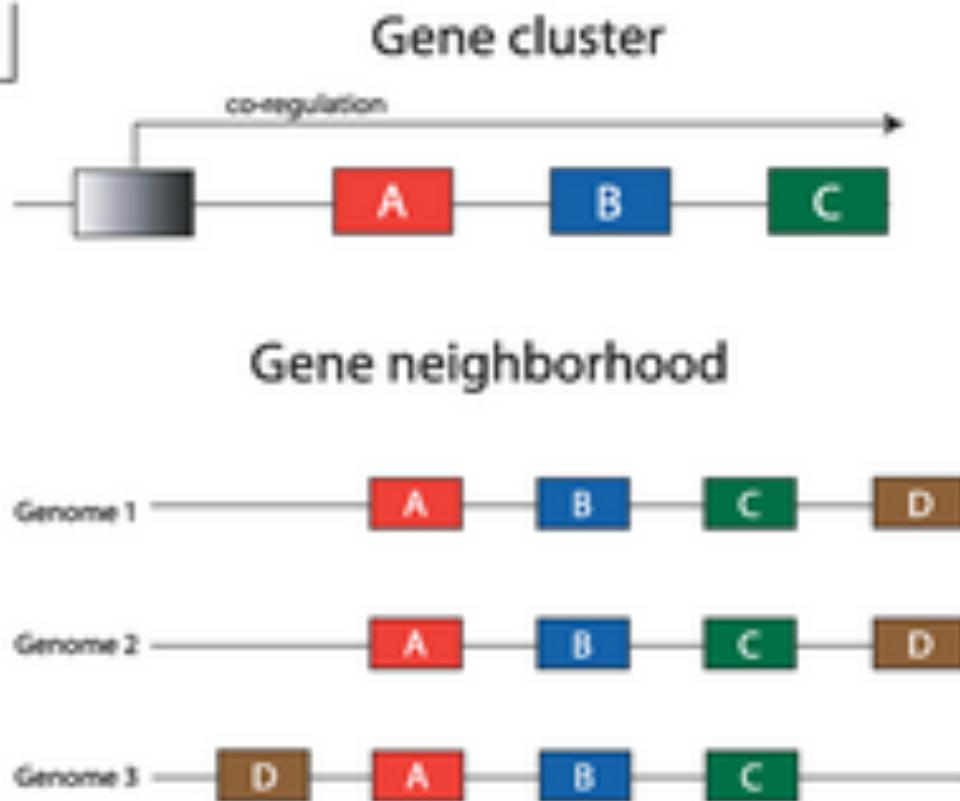


Bioinformatics - tasks

- Collect PPI information
- Analyze PPIs
 - Molecular level
- Curate PPIs in a database
- Extract information about PPI from texts
- Analyze PPIs
 - Systems level
- Prediction
 - Identify PPIs (systems level)

Computational Methods for Protein Interaction Prediction

A



Gene cluster and gene neighborhood methods

Genes with closely related functions encoding potentially interacting proteins are often co-regulated in eukaryotes.

Gene clusters or operons encoding for co-regulated genes are usually conserved

Computational Methods for Protein Interaction Prediction

B

Phylogenetic profile

Proteins	Genomes		
	EC	HI	BS
P1	0	1	1
P2	0	0	1
P3	1	0	0
P4	0	1	1

P1 and P4 are functionally linked

Phylogenetic profile method, showing the presence/absence of four proteins in three genomes.

Hypothesis: functionally linked and potentially interacting nonhomologous proteins co-evolve

A phylogenetic profile is constructed for each protein, as a vector of N (number of genomes). Proteins that form clusters are functionally related.

Computational Methods for Protein Interaction Prediction

C

Rosetta Stone



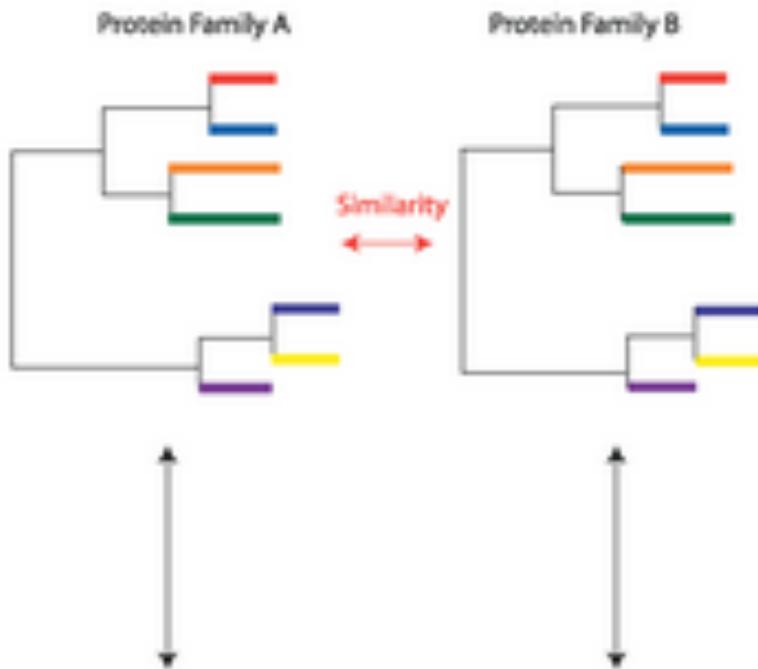
Rosetta Stone method

Based on the observation that some interacting proteins/domains have homologs in other genomes that are fused into one protein chain, a so-called Rosetta Stone protein

Computational Methods for Protein Interaction Prediction

D

Sequence co-evolution



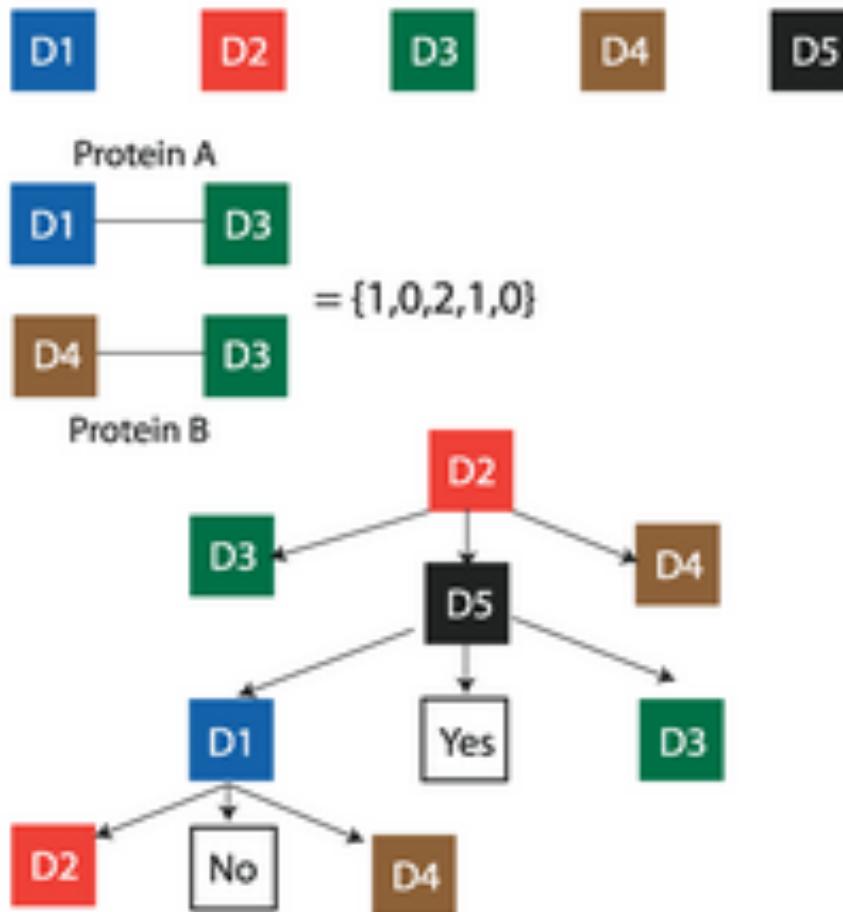
Sequence co-evolution method looking for the similarity between two phylogenetic trees/distance matrices

Proteins co-evolve so that changes in one protein leading to the loss of function or interaction should be compensated by the correlated changes in another protein.

Calculated based on similarity between phylogenetic trees.

Computational Methods for Protein Interaction Prediction

E Classification/ Random Decision forests



Classification methods shown with the example of Random Decision Forest (RDF) method. Here, 5 different features/domains are used and each pair is encoded as a string of 0, 1, and 2.

The decision trees are constructed based on the training set of interacting protein pairs and decisions are made if proteins under the question interact or not.

Bioinformatics - tasks

- Collect PPI information
- Analyze PPIs
 - Molecular level
- Curate PPIs in a database
- Extract information about PPI from texts
- Analyze PPIs
 - Systems level
- Prediction
 - Identify PPIs (systems level)
 - Identify PPI interfaces (molecular level)

Computational Methods to Identify Protein-Protein Binding Sites

Use evolutionary information from sequence alignments, and/or residue properties, and/or the geometric information from structures.

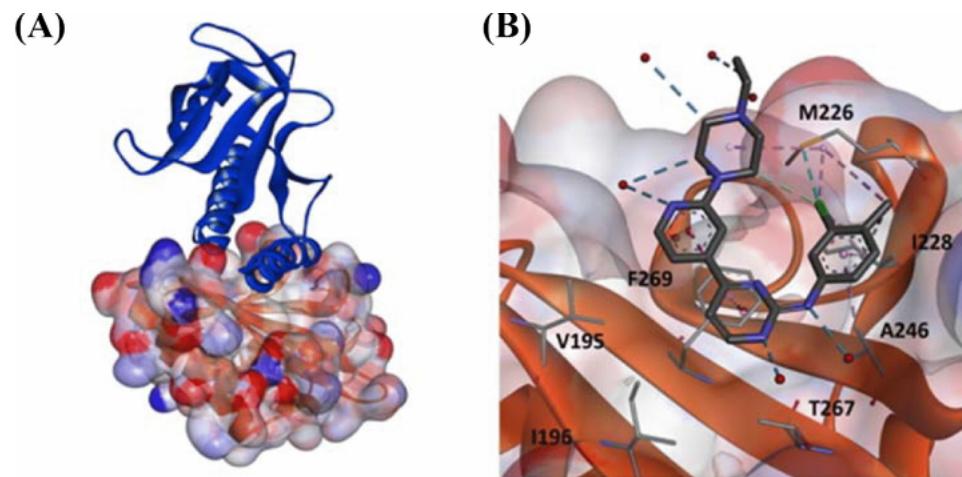
- Docking
- Threading and homology modeling
- Evolutionary tracing
- Correlated mutations
- Properties of patches
- Hydrophobicity
- Neural networks and support vector machines

Perspective – PPI sites as drug binding sites

PPIs can be modulated to **target** cancer, viruses, autoimmune disorders.

Direct binding occurs when small molecule modulators bind to the protein-protein interface, resulting in disruption or stabilization of the PPI.

Or allosteric binding occurs when a small molecule binds to a site away from the active site, resulting in a conformational change to the PPI binding site which then inhibits or enhances the PPI.



References

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