Master Module
Proteinbiochemistry and Bioinformatics
December 2023

Protein interaction networks

Katja Luck, PhD

Institute of Molecular Biology

Emmy Noether-Programm
DFG Deutsche Forschungsgemeinschaft

JOHANNES GUTENBERG UNIVERSITÄT MAINZ

JG|U

Some organizational information

- Questions throughout the lecture are welcome
- I will ask questions, too!
- Happy to receive feedback on the course

Outline

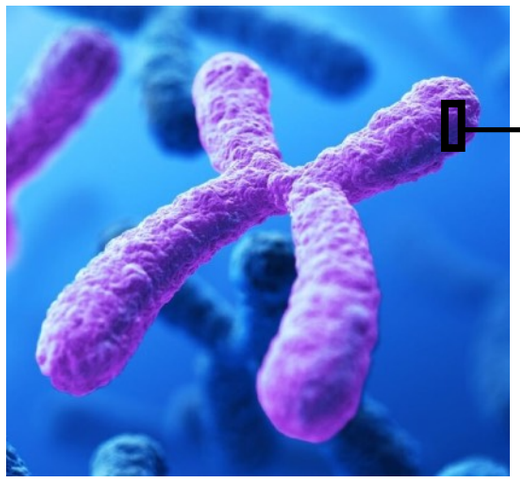
1. What are protein interactions?
2. Methods to detect protein interactions
3. Bioinformatic resources for protein interactions
4. Graph theoretical aspects of protein interaction networks
5. Visualizing and analyzing networks using Cytoscape

Master Module
Proteinbiochemistry and Bioinformatics
December 2023

Session: Protein interaction networks

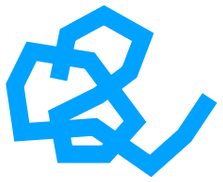
1. What are protein interactions?

Why do protein interactions matter?

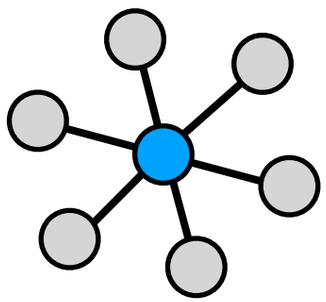


humanoriginproject.com

Gene X functions in Y - How?



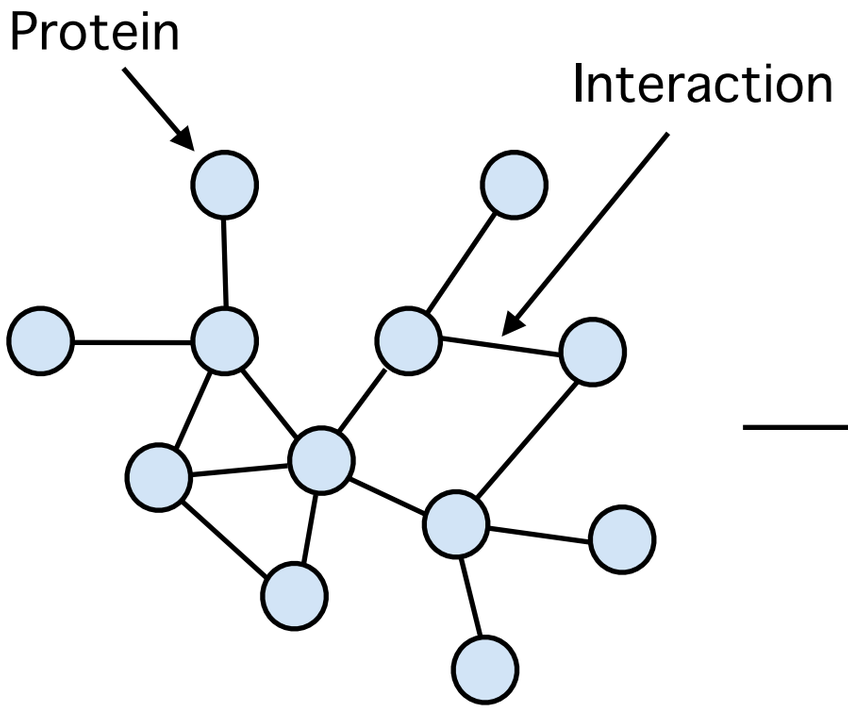
Protein X functions in Y - How?



- Interactions mediate a gene's function
- Interactions inform on a gene's function

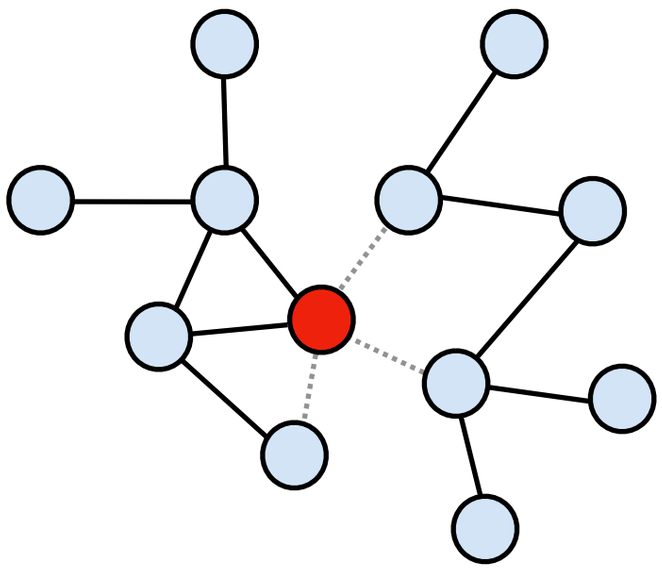
Protein interactions mediate cellular function

Genome,
Environment



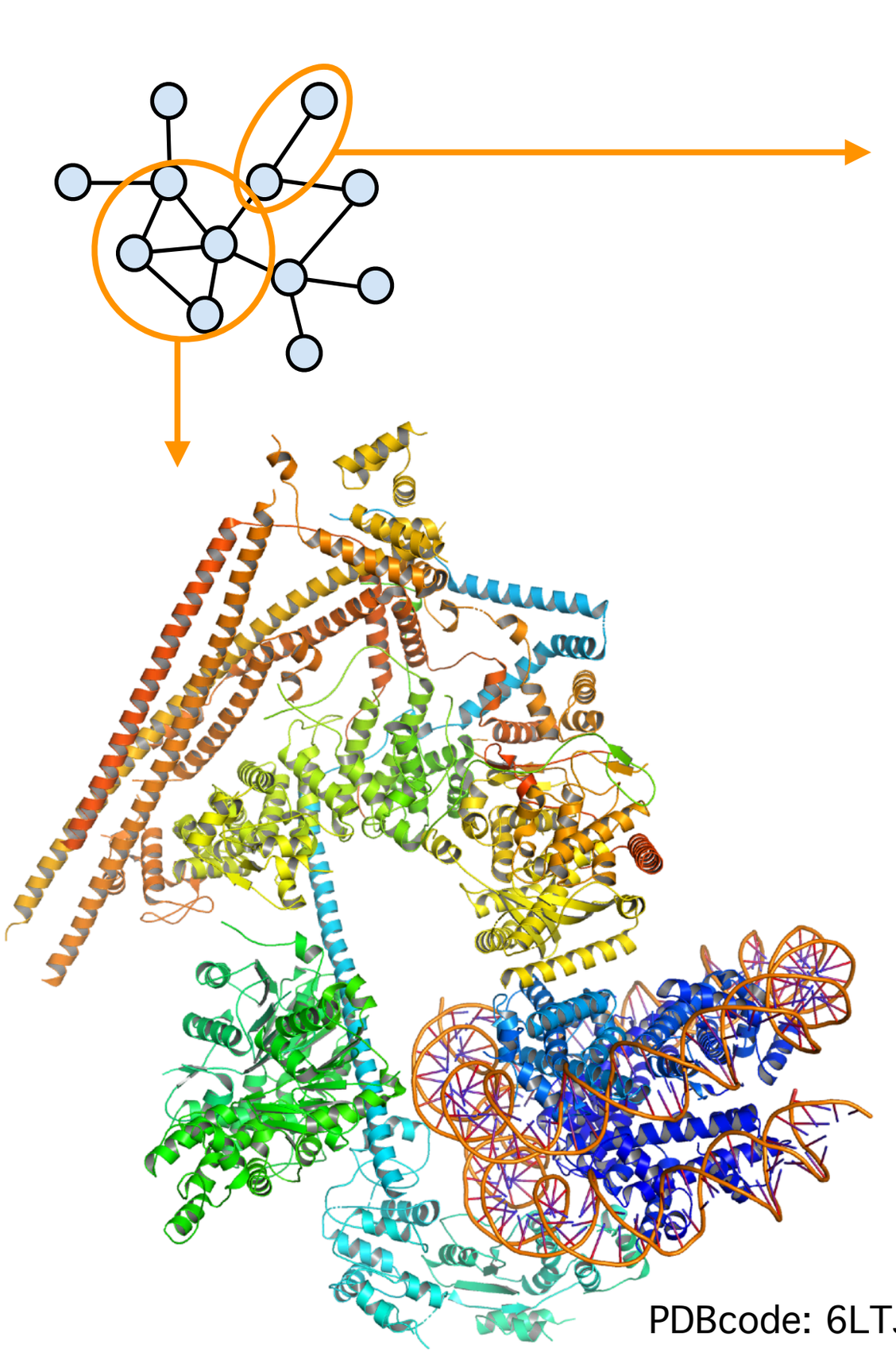
Cellular function
and organization

Mutation,
pathogen,
toxin

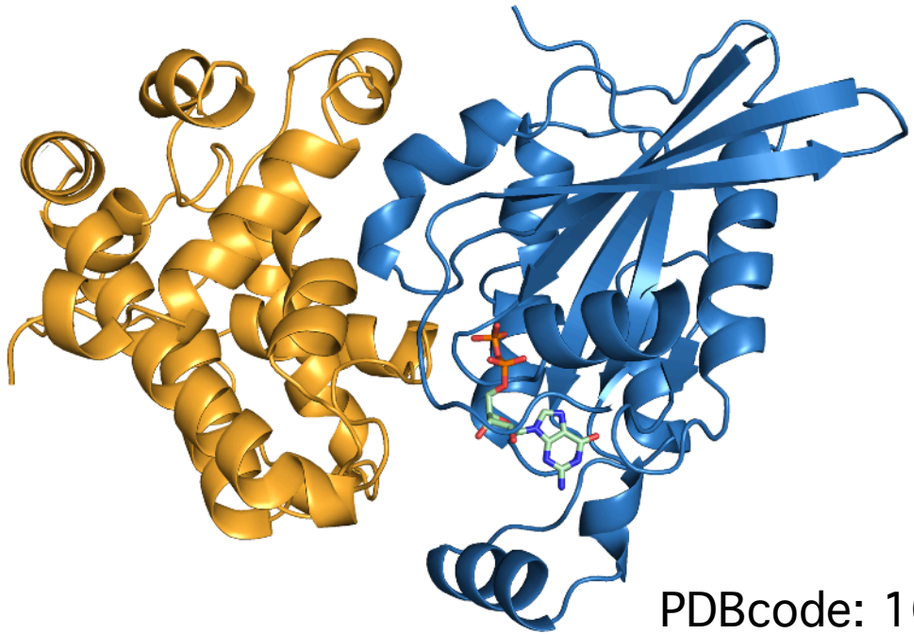


Disease

Protein interactions are complex

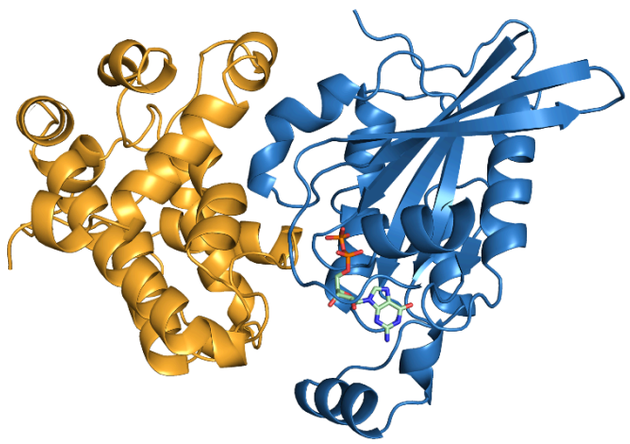


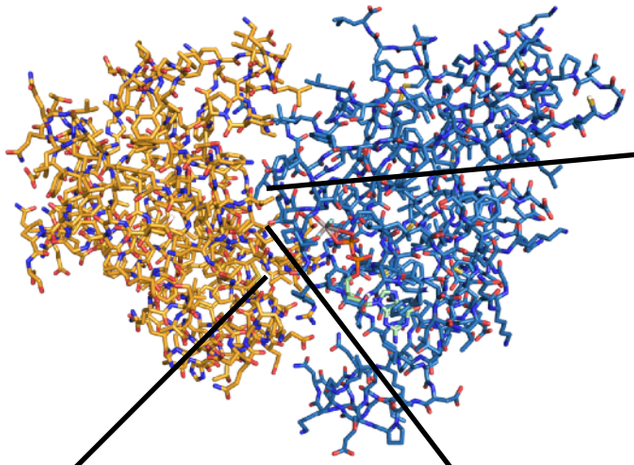
PDBcode: 6LTJ



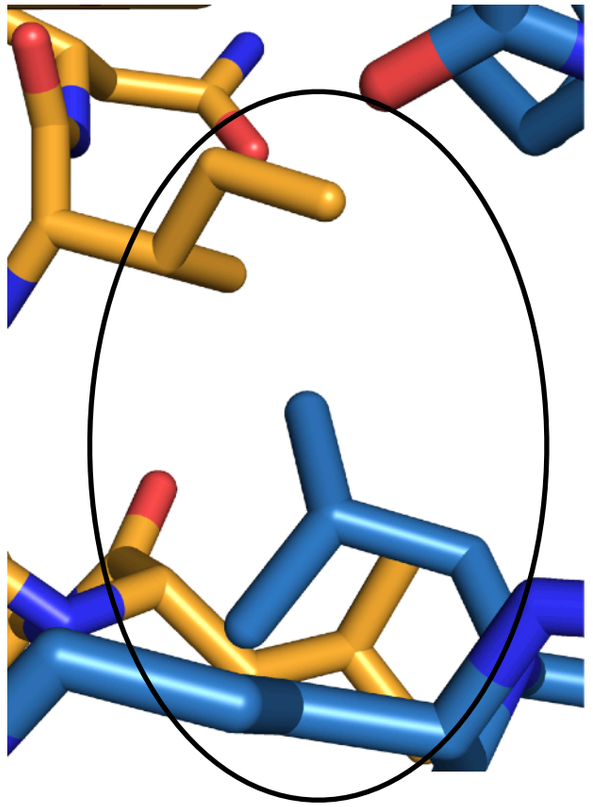
PDBcode: 1GRN

Non-covalent contacts between amino acids mediate protein interactions

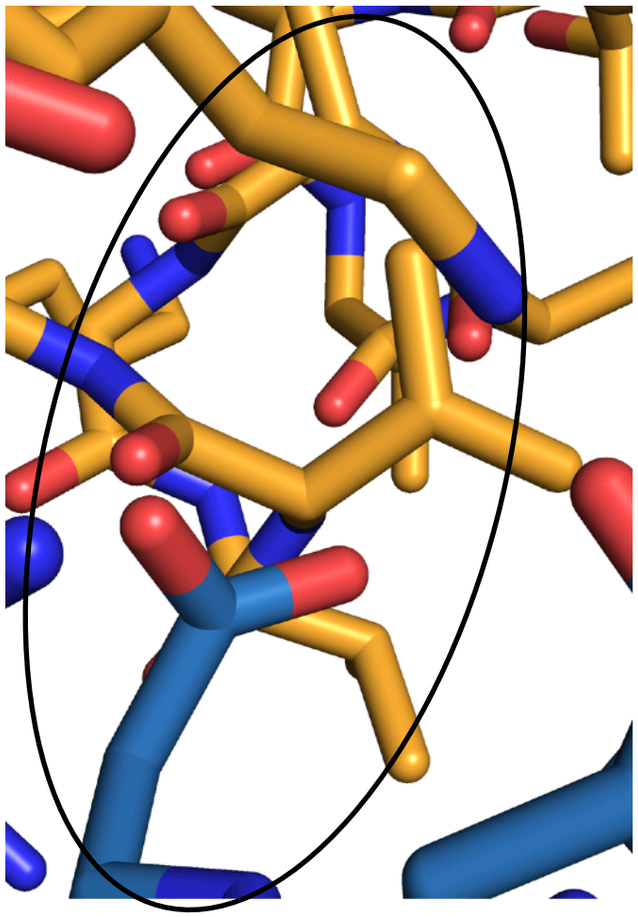




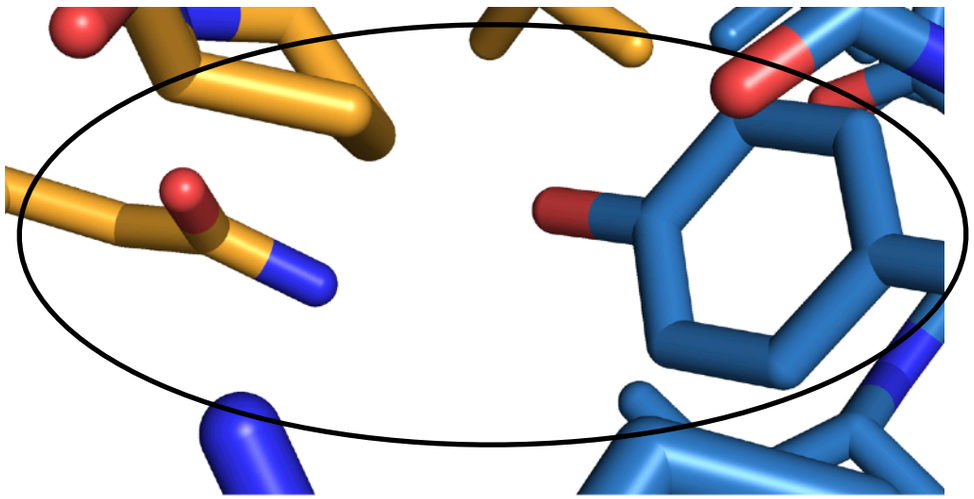
Hydrophobic contact (van-der-Waals)



Charge contact (salt bridge)



Polar contact (H-bond)



Protein interaction strength is expressed as dissociation constant K_D

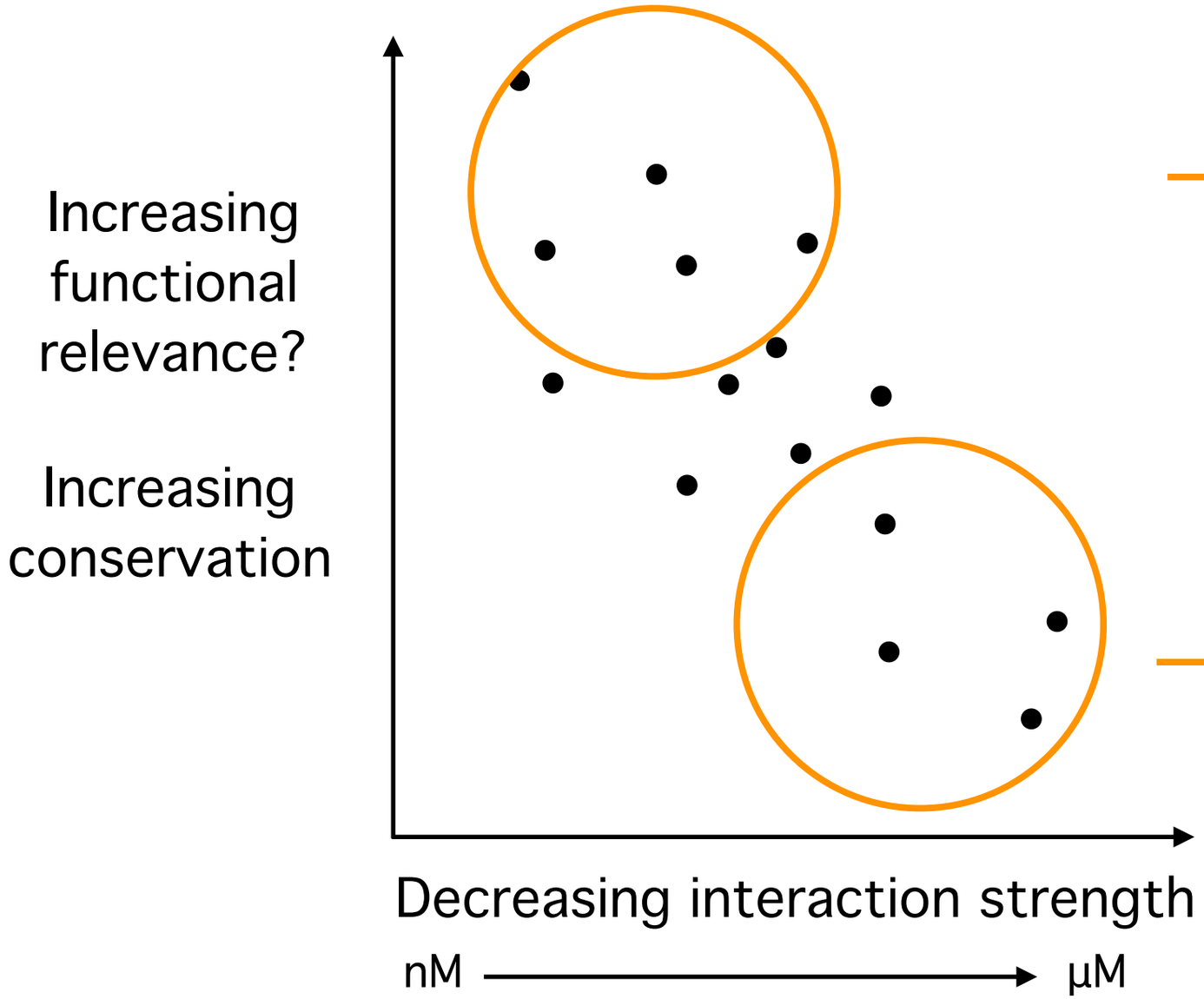
$$[A] + [B] \rightleftharpoons [AB]$$

$$K_D = \frac{[A][B]}{[AB]}$$

- the smaller the K_D, the stronger the interaction
- nM -> very strong, µM -> rather weak
- it is a continuum!

When can we say that two proteins interact with each other?

- interaction strength (K_D) is a continuum
- there is no universal cutoff on the K_D
- discrimination into binding/no binding is assay-dependent

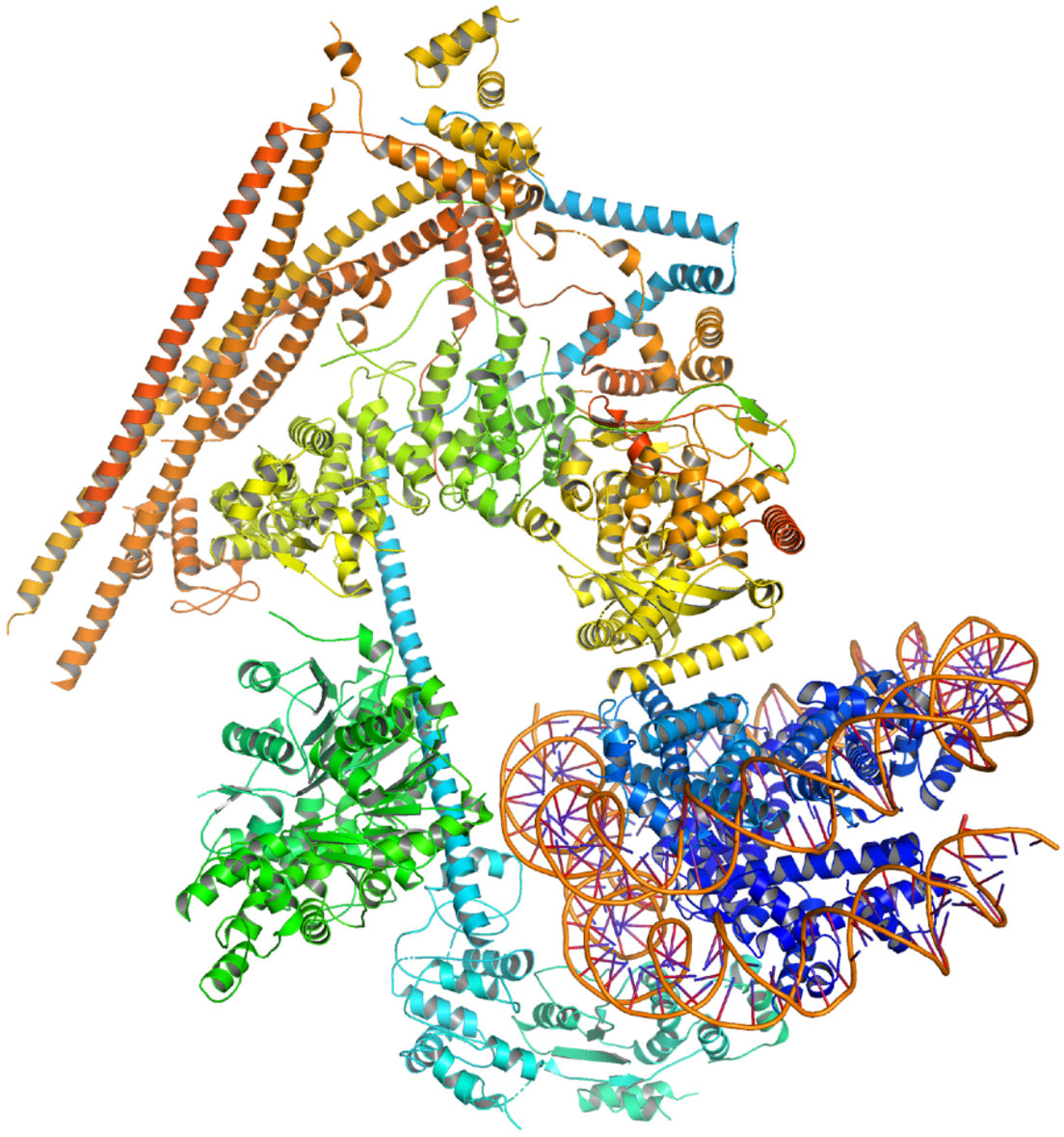


- core biological processes
- proteasome, transcription complex, ...

- multicellularity
- regulation, signaling

Wan et al *Nature* 2015

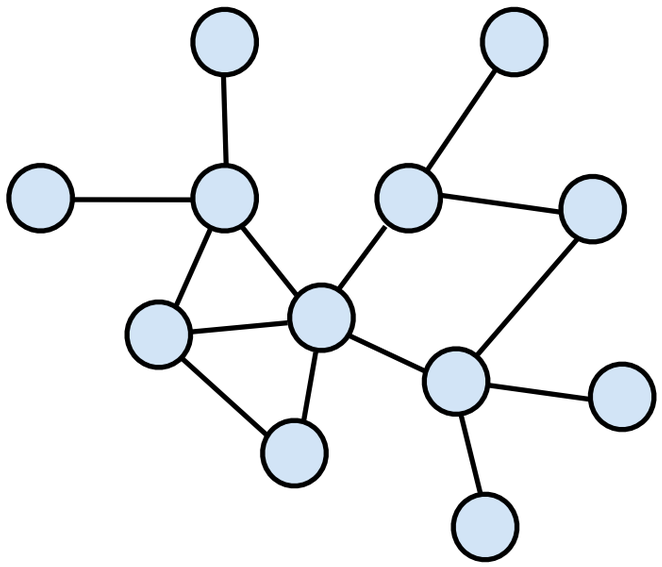
All life depends on the proper formation and dissociation of protein interactions



Mechanisms of protein interaction specificity?

If we knew all (human) protein interactions...

Complete functional annotation of all genes



Complete understanding of human biology

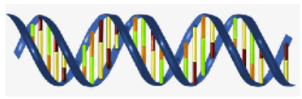
Master Module
Proteinbiochemistry and Bioinformatics
December 2023

Session: Protein interaction networks

2. Methods to detect protein interactions

Why is it so hard to detect protein interactions?

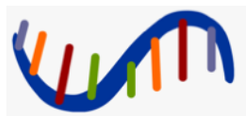
Genome



Next generation sequencing

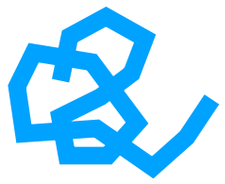
Great sensitivity
-> Amplification via PCR

Transcriptome



Next generation sequencing

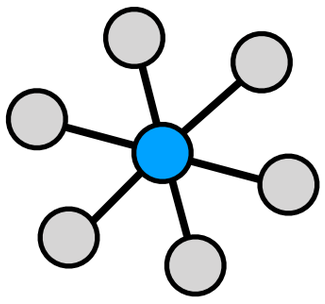
Proteome



Mass spectrometry

Moderate sensitivity
-> no PCR for proteins

Interactome



?

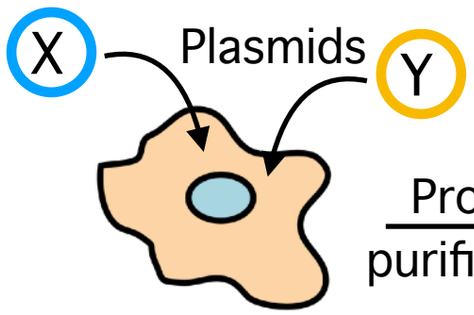
- No PCR for interactions
- Interactions are a state, not a molecule

Approaches to detect protein interactions

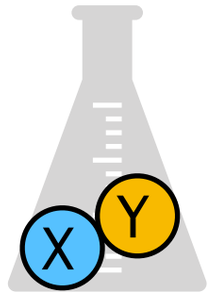
- interaction detection method
  - experimental interaction detection
    - biochemical
      - affinity technology
      - aggregation assay
      - chromatography technology
      - cosedimentation
      - cross-linking study
      - electrophoretic mobility-based method
      - enzymatic study
      - footprinting
      - nucleotide exchange assay
      - polymerization
      - probe interaction assay
      - virotrap
    - biophysical
      - biosensor
      - circular dichroism
      - detection by mass spectrometry
      - differential scanning calorimetry
      - electron diffraction
      - electron resonance
      - enzyme-mediated activation of radical sources
      - equilibrium dialysis
      - filter trap assay
      - fluorescence technology
      - force measurement
      - force spectroscopy
      - infrared spectroscopy
      - isothermal titration calorimetry
      - light scattering
      - luminiscence technology
      - microscale thermophoresis
      - molecular sieving
      - neutron diffraction
      - neutron fiber diffraction
      - nuclear magnetic resonance
      - rheology measurement
      - scintillation proximity assay
      - small angle neutron scattering
      - thermal shift binding
      - ultraviolet-visible spectroscopy
      - x-ray crystallography
    - genetic interference
      - chemical rna modification plus base pairing prediction
      - random spore analysis
      - synthetic genetic analysis
    - imaging technique
      - atomic force microscopy
      - confocal microscopy
      - electron microscopy
      - fluorescence microscopy
      - fluorescent protein-protein interaction-visualization
      - light microscopy
      - super-resolution microscopy
      - x-ray tomography
    - phenotype-based detection assay
      - nuclear translocation assay
    - post transcriptional interference
      - antisense oligonucleotides
      - antisense rna
      - miRNA interference luciferase reporter assay
      - rna interference
    - protein complementation assay
      - Split Intein-Mediated Protein Ligation
      - adenylate cyclase complementation
      - beta galactosidase complementation
      - beta lactamase complementation
      - bimolecular fluorescence complementation
      - dihydrofolate reductase reconstruction
      - kiss
      - mammalian protein protein interaction trap
      - protein kinase A complementation
      - reverse ras recruitment system
      - split luciferase complementation
      - tox-r dimerization assay
      - transcriptional complementation assay

https://www.ebi.ac.uk/ols/ontologies/mi

Detection of direct interactions

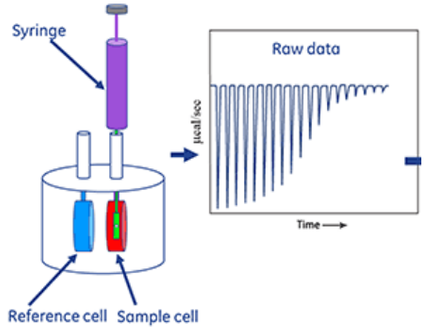


Protein purification



Measure-ment

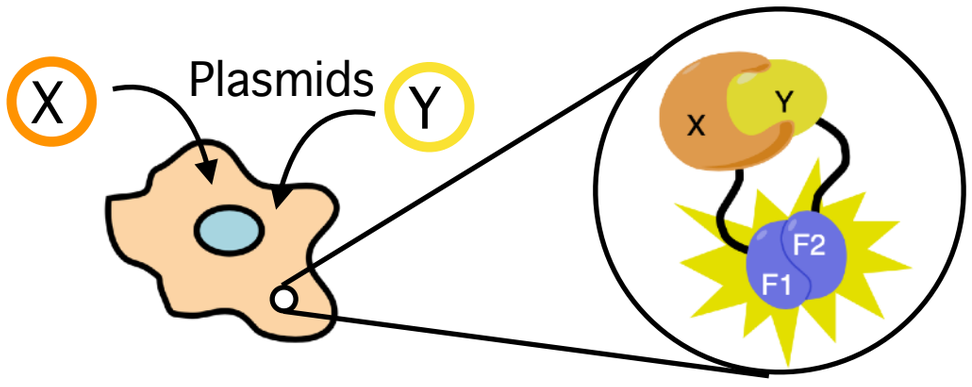
Isothermal titration calorimetry



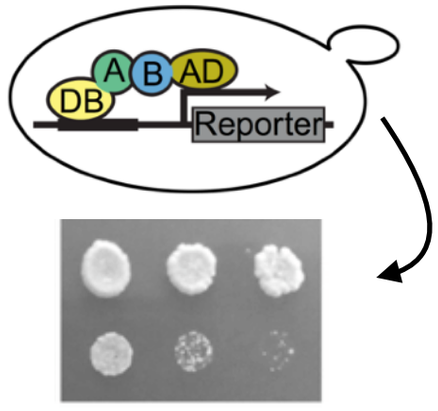
Direct interaction

Detection of binary interactions

Protein complementation assay

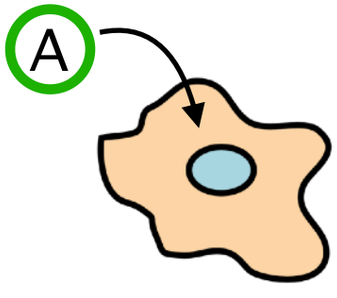


Yeast two-hybrid

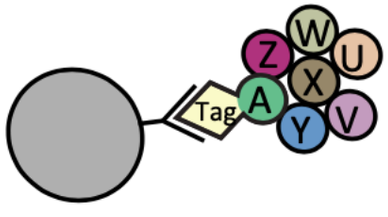


Binary interaction

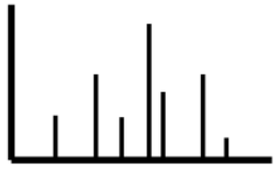
Detection of co-complex associations



Pull down



Mass spectrometry



Co-complex interaction

Different assays produce different types of protein interaction data

Direct assays

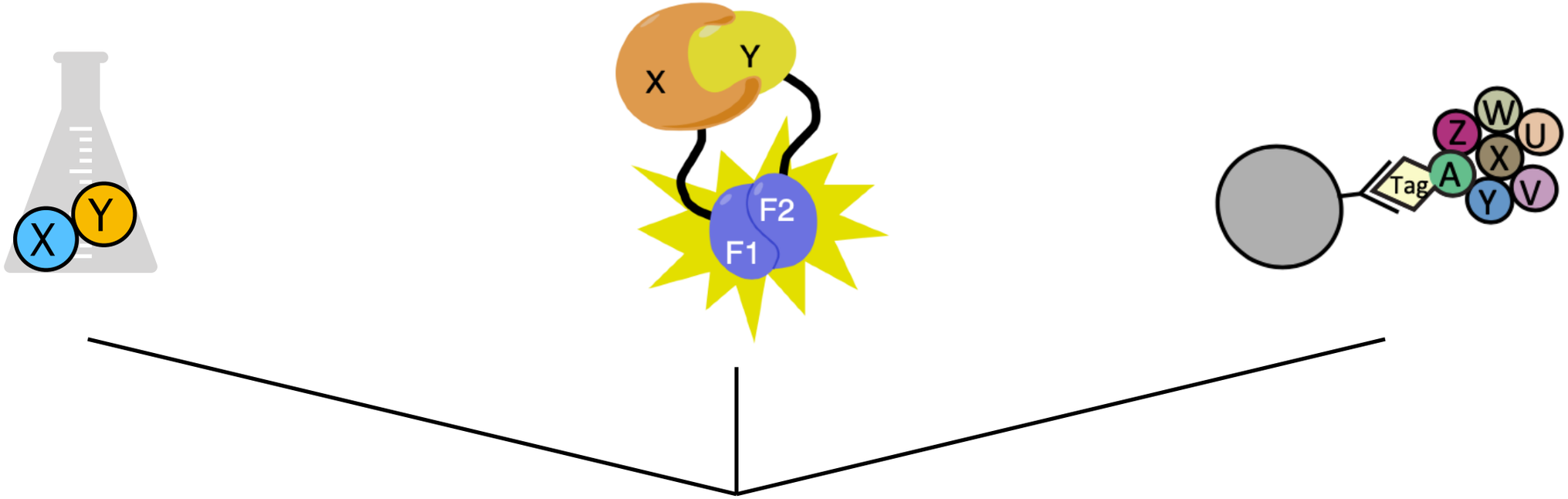
- Direct interactions
- Protein fragments
- With K_D
- Low-throughput

Binary assays

- Binary interactions
- Full length proteins
- No K_D
- Over-expression

Co-complex assays

- Co-complex associations
- Full length proteins
- No K_D
- Over-expression and endogenous



- All are called protein interactions
- Assays differ in which interactions they can detect

Accuracy of protein interaction assays

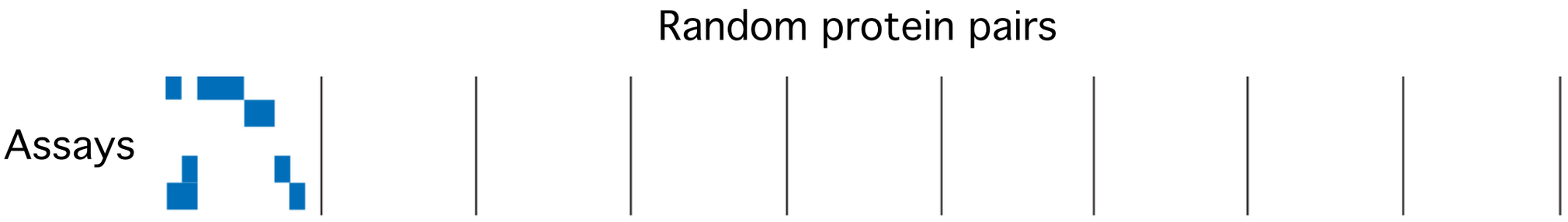
Sensitivity of protein interaction assays

Why are some interactions detected by some assays and not by others?

Braun et al *Nature Methods* 2009

Accuracy of protein interaction assays

Specificity of protein interaction assays

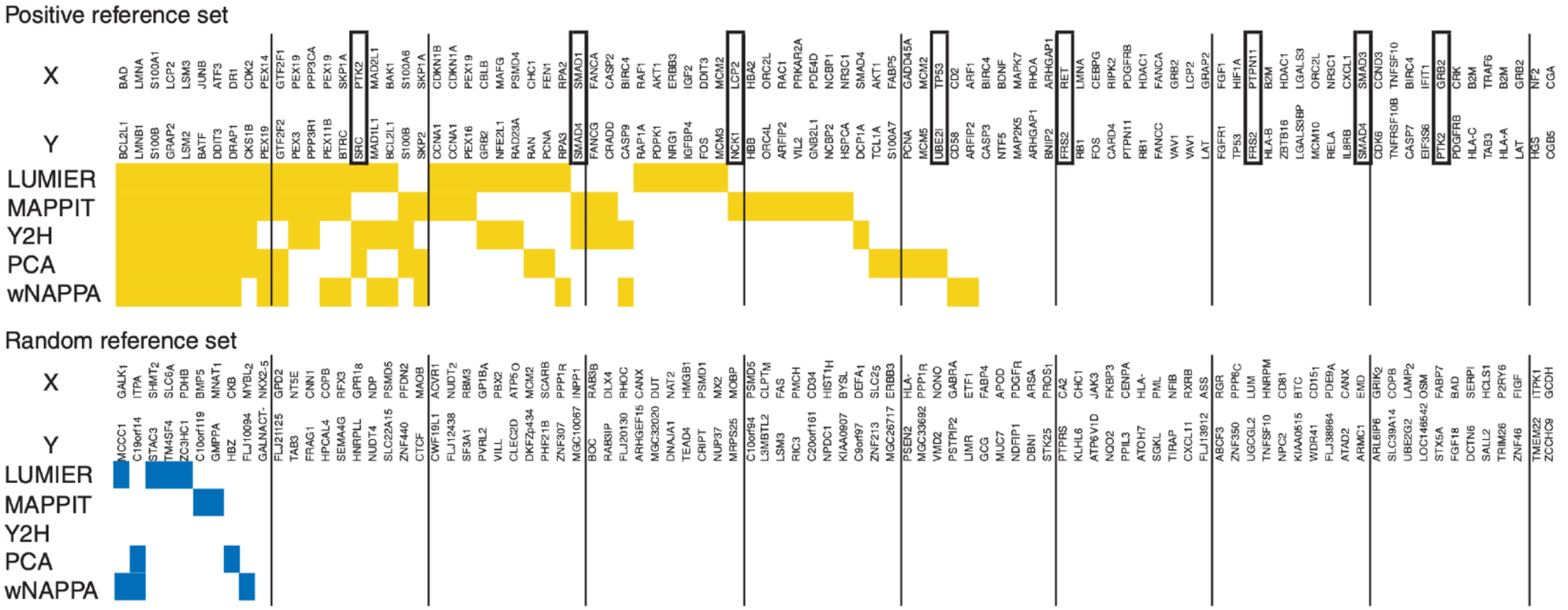


Why would an assay erroneously report a protein interaction?

Braun et al *Nature Methods* 2009

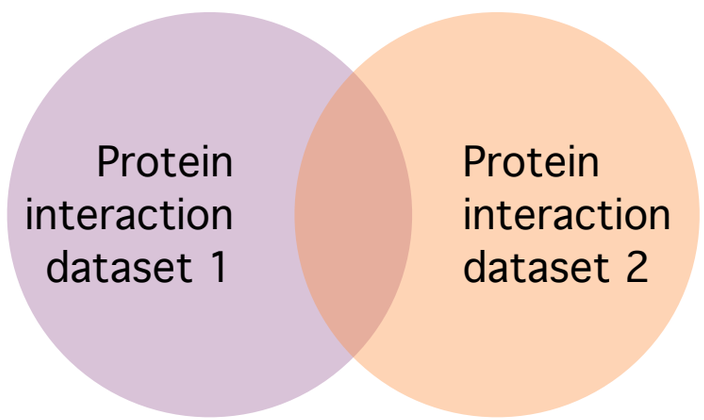
Accuracy of protein interaction assays

Correct benchmarking of assays



Correct interpretation of protein interaction data

Low overlap



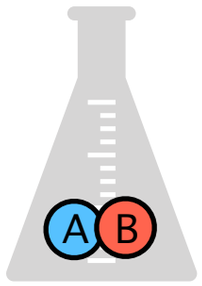
→ Low sensitivity
High specificity

Braun et al *Nature Methods* 2009, Luck et al *TiBS* 2017

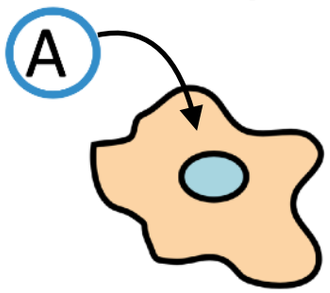
Functional relevance of detected protein interactions

Often artificial context when protein interaction is detected

In vitro

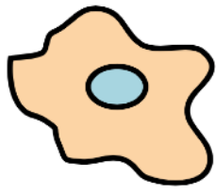


Exogenous expression



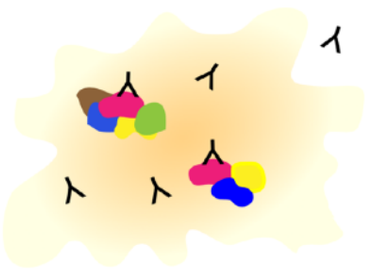
Cellular context

yeast



cell line

Cell lysis



At which cellular context is a detected protein interaction functional?

Should we delete ‘non-functional’ interactions?

Methods to detected protein interactions

Summary

- Interaction strength is a continuum
- Most common methods are direct, binary, and co-complex assays
- Different methods detect different types of protein interactions
- Many interactions remain undetected
- If properly controlled interaction data can be of high quality
- It is difficult to distinguish between functional and non-functional protein interactions