

Master Module
Proteinbiochemistry and Bioinformatics
December 2024

Protein interaction networks

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Some organizational information

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

Outline

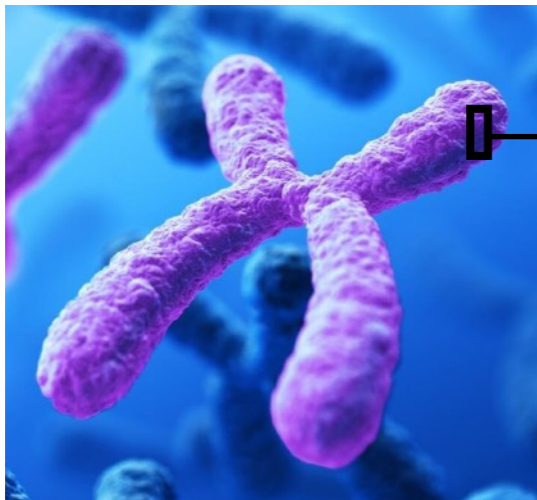
1. What are protein interactions?
2. Methods to detect protein interactions
3. Bioinformatic resources for protein interactions
4. Graph theoretical aspects of protein interaction networks
5. Visualizing and analyzing networks using Cytoscape

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Session: Protein interaction networks

1. What are protein interactions?

Why do protein interactions matter?

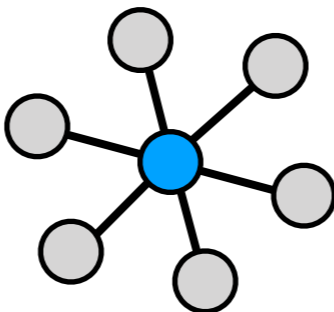


humanoriginproject.com

Gene X functions in Y - How?

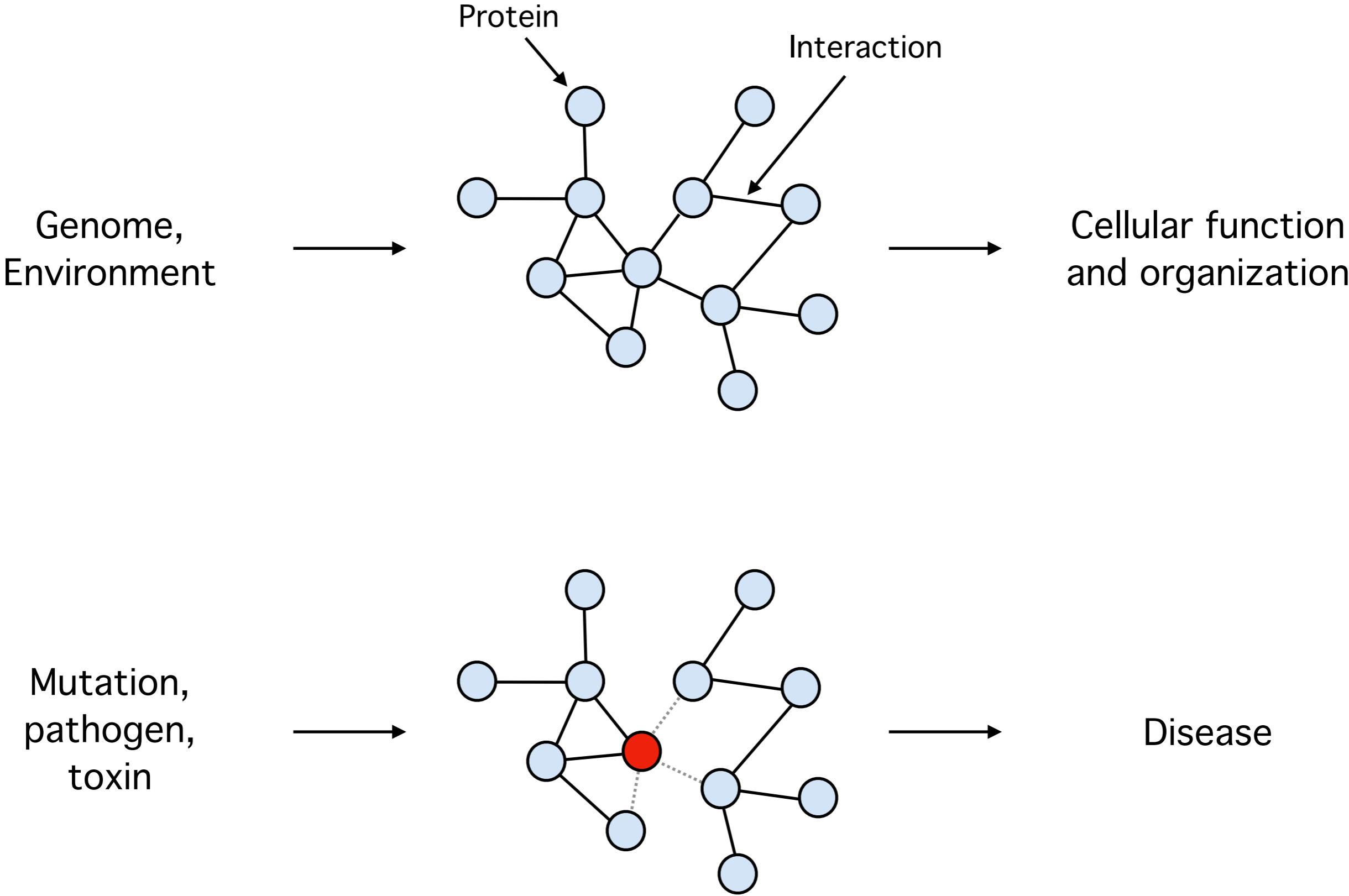


Protein X functions in Y - How?

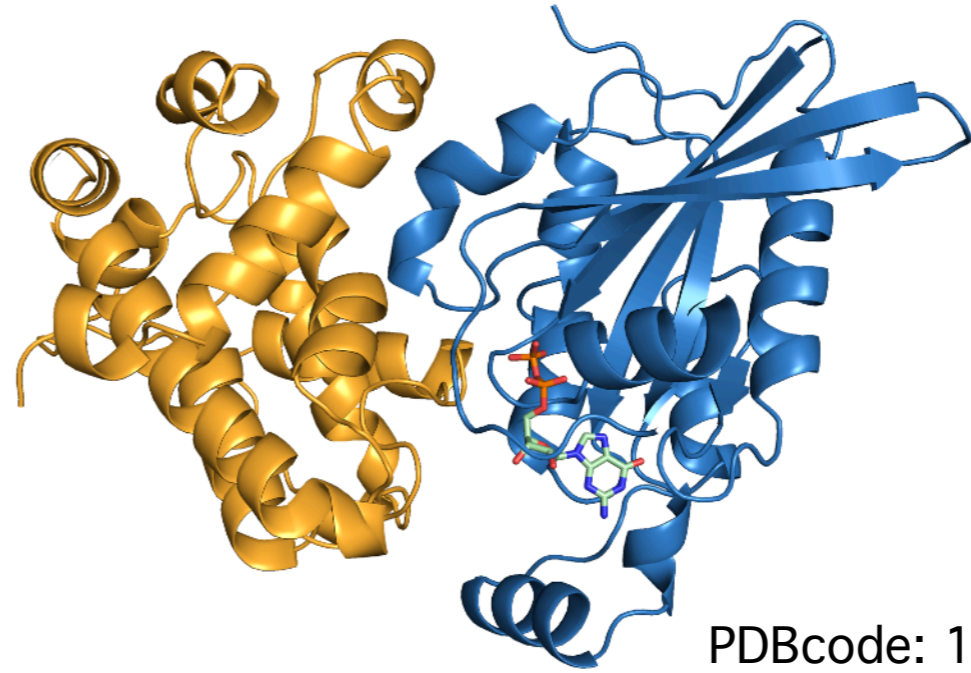
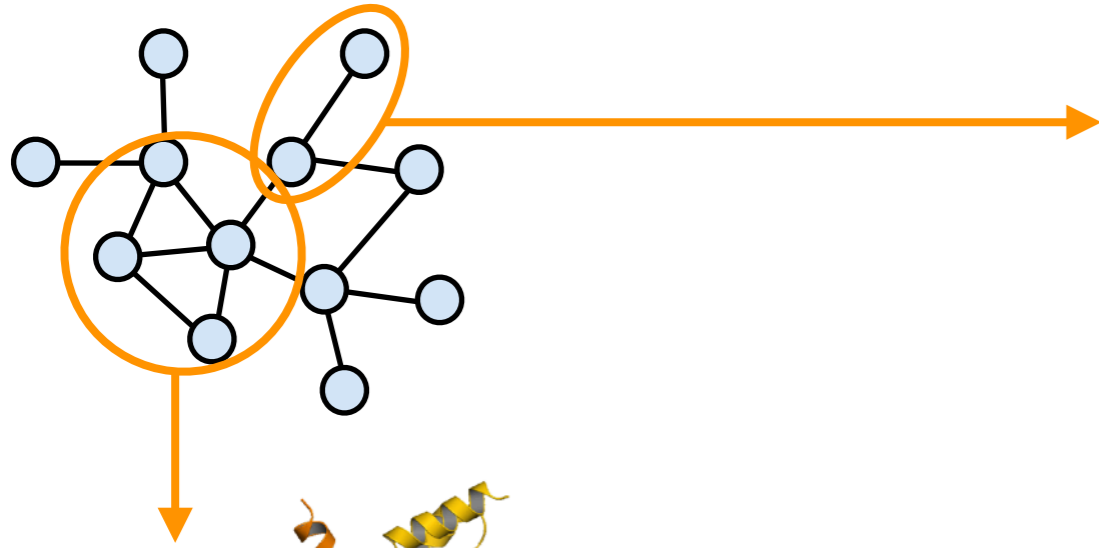


- Interactions mediate a gene's function
- Interactions inform on a gene's function

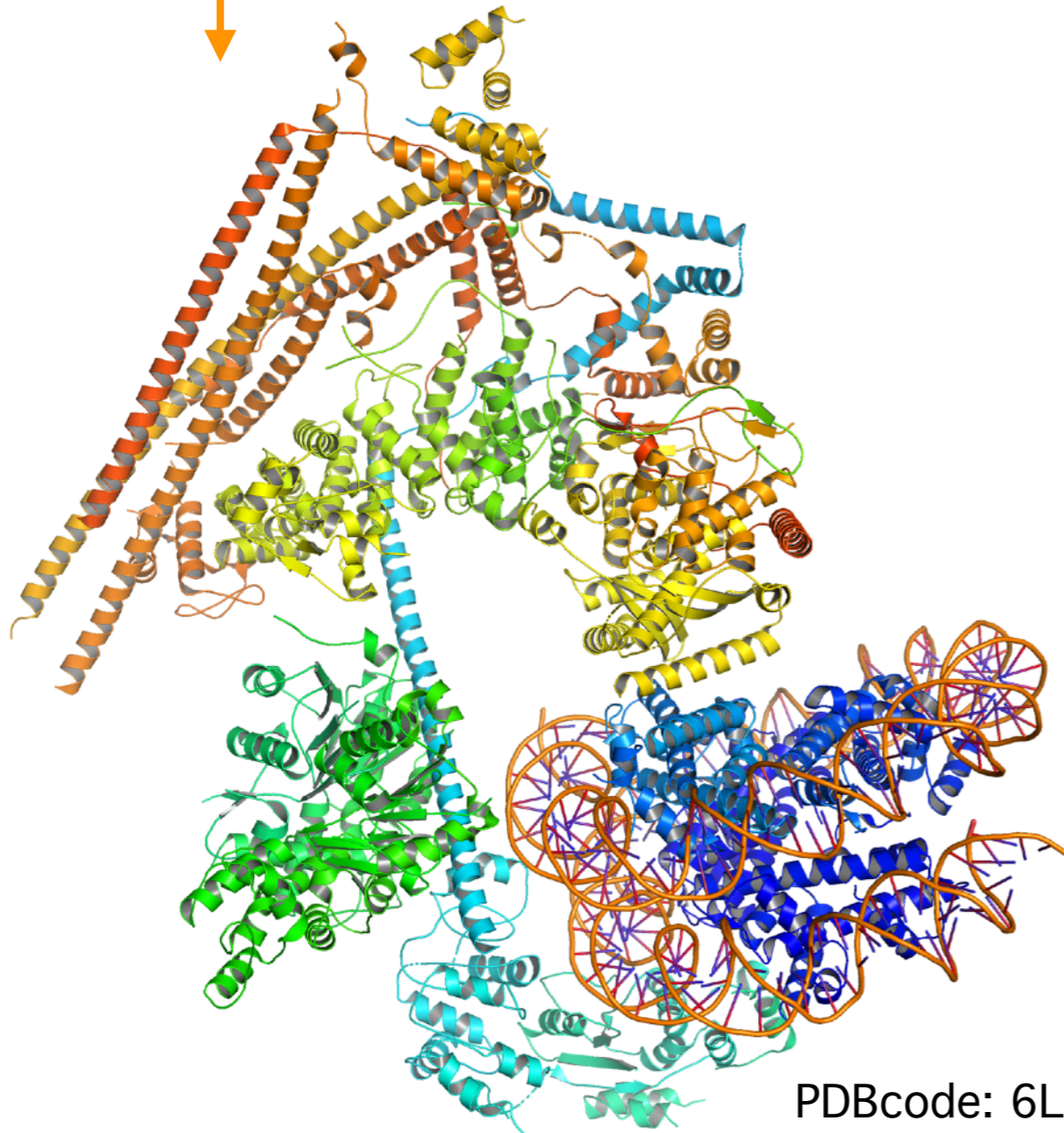
Protein interactions mediate cellular function



Protein interactions are complex

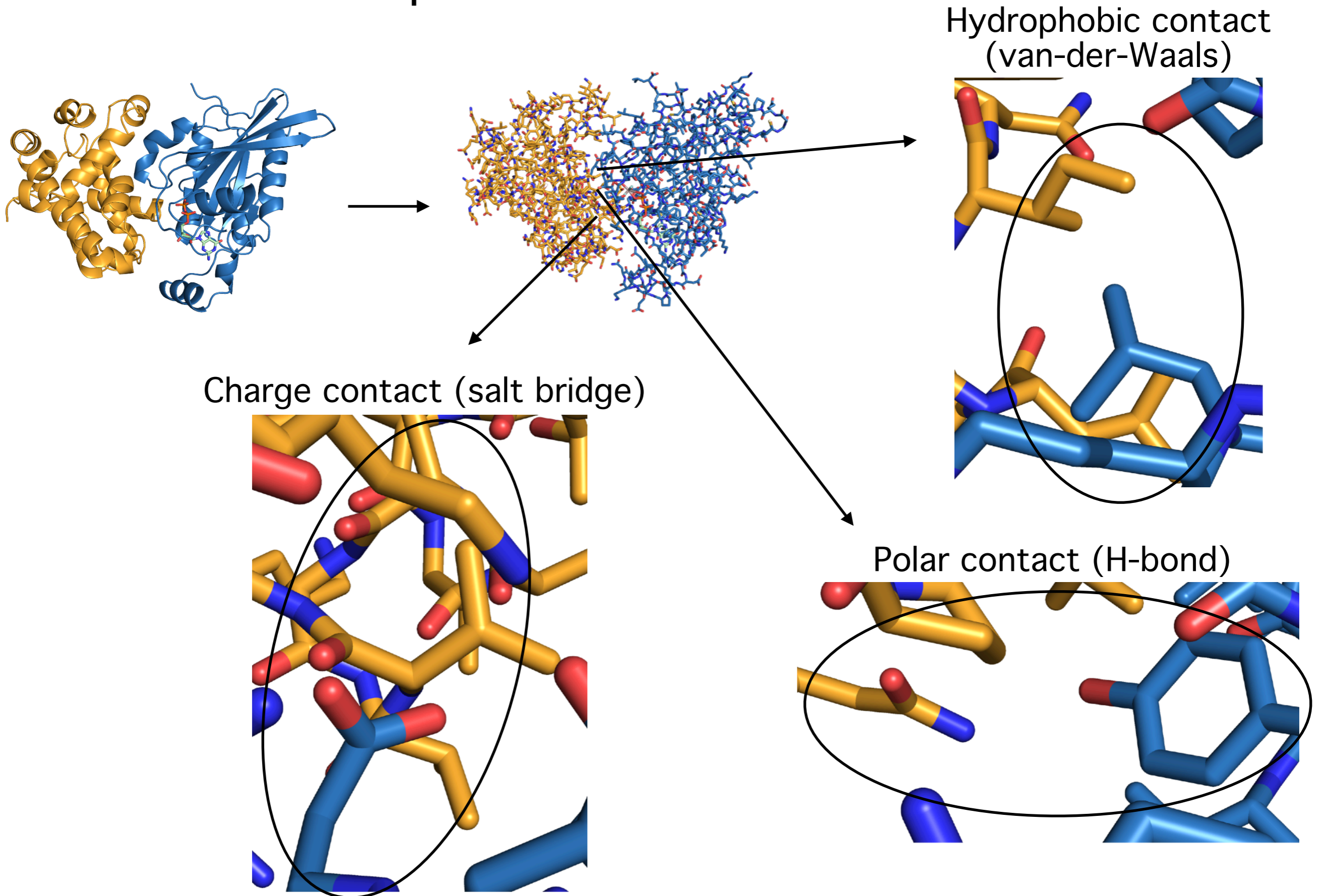


PDBcode: 1GRN



PDBcode: 6LTJ

Non-covalent contacts between amino acids mediate protein interactions



Protein interaction strength is expressed as dissociation constant K_D

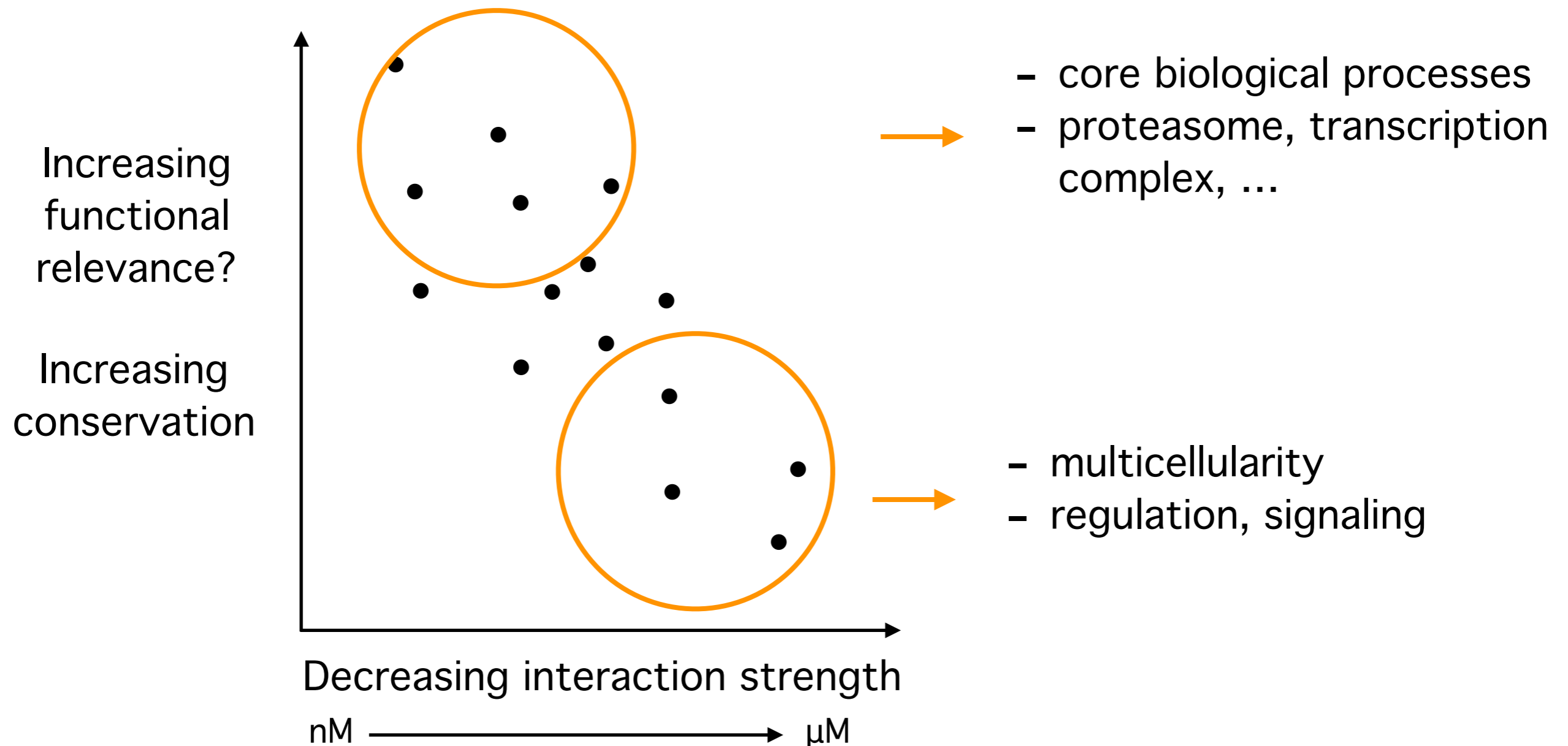


$$K_D = \frac{[A][B]}{[AB]}$$

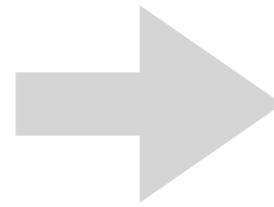
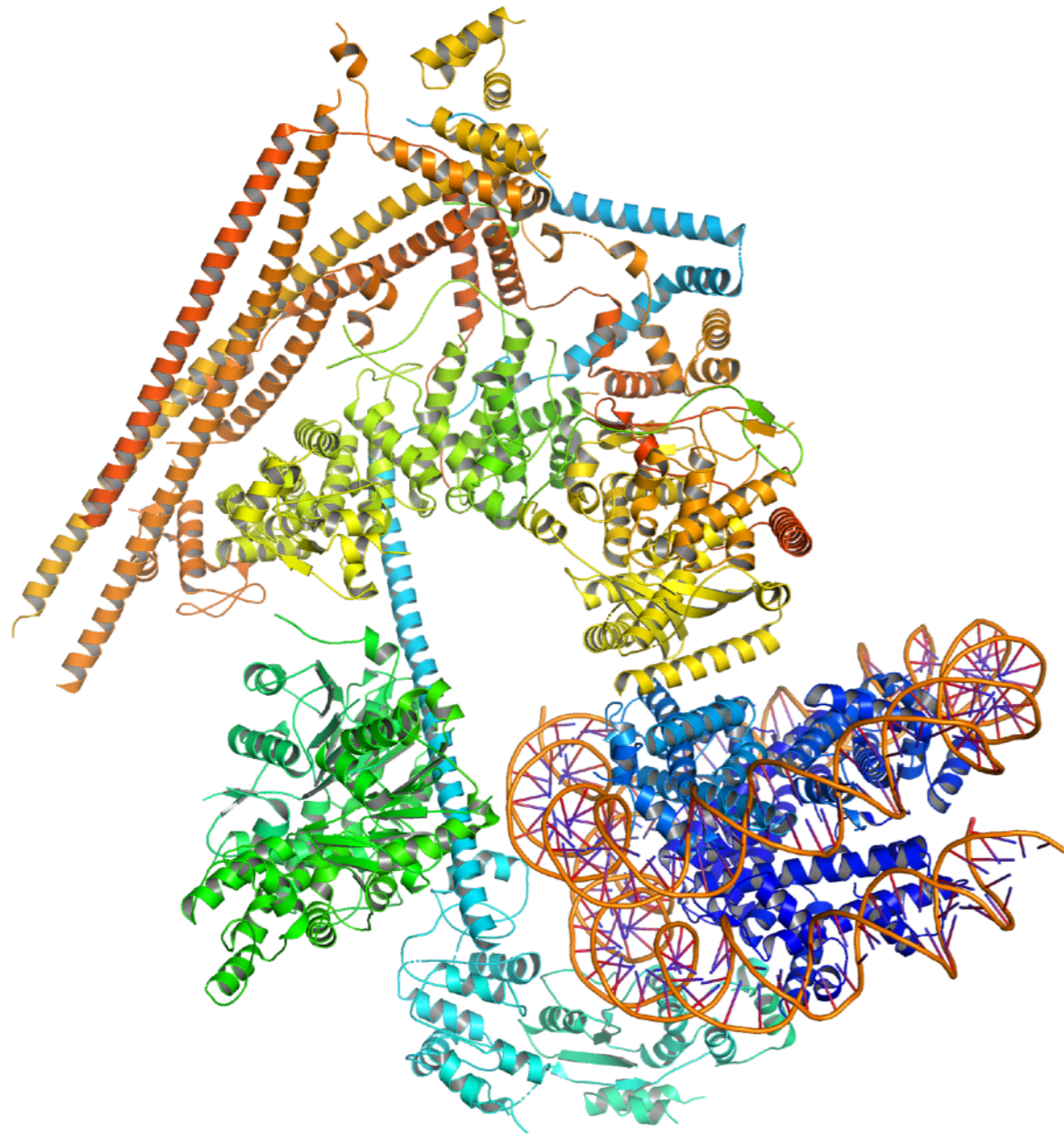
- the smaller the K_D , the stronger the interaction
- nM \rightarrow very strong, μ M \rightarrow rather weak
- it is a continuum!

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



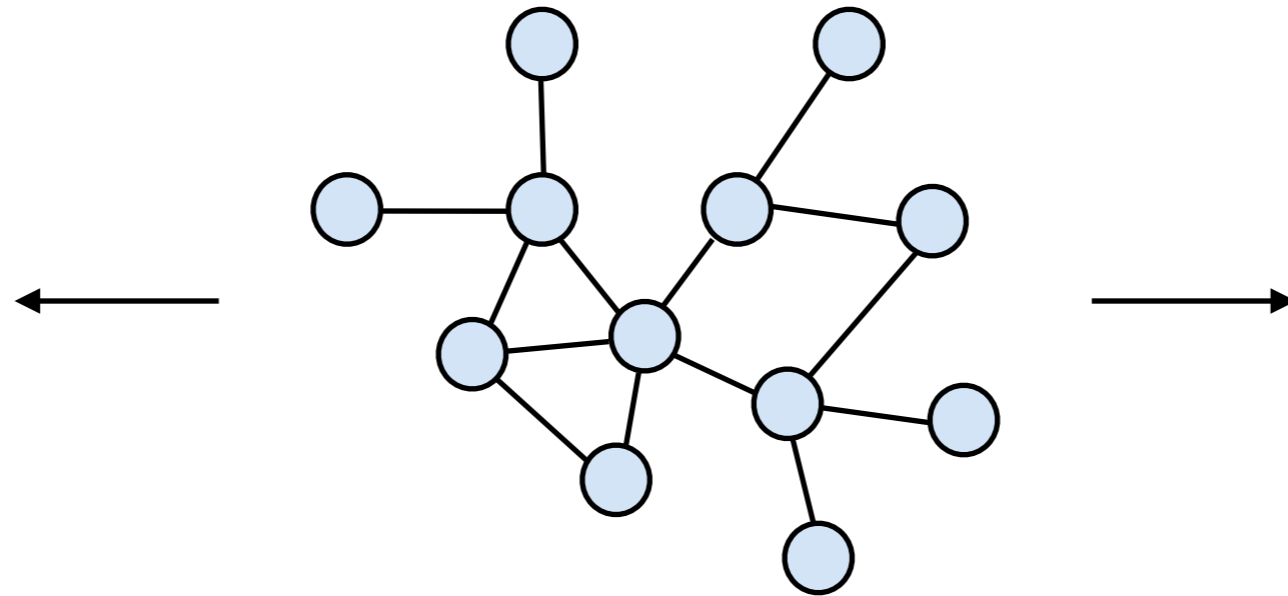
All life depends on the proper formation and dissociation of protein interactions



Mechanisms of protein interaction specificity?

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

Complete
functional
annotation
of all genes



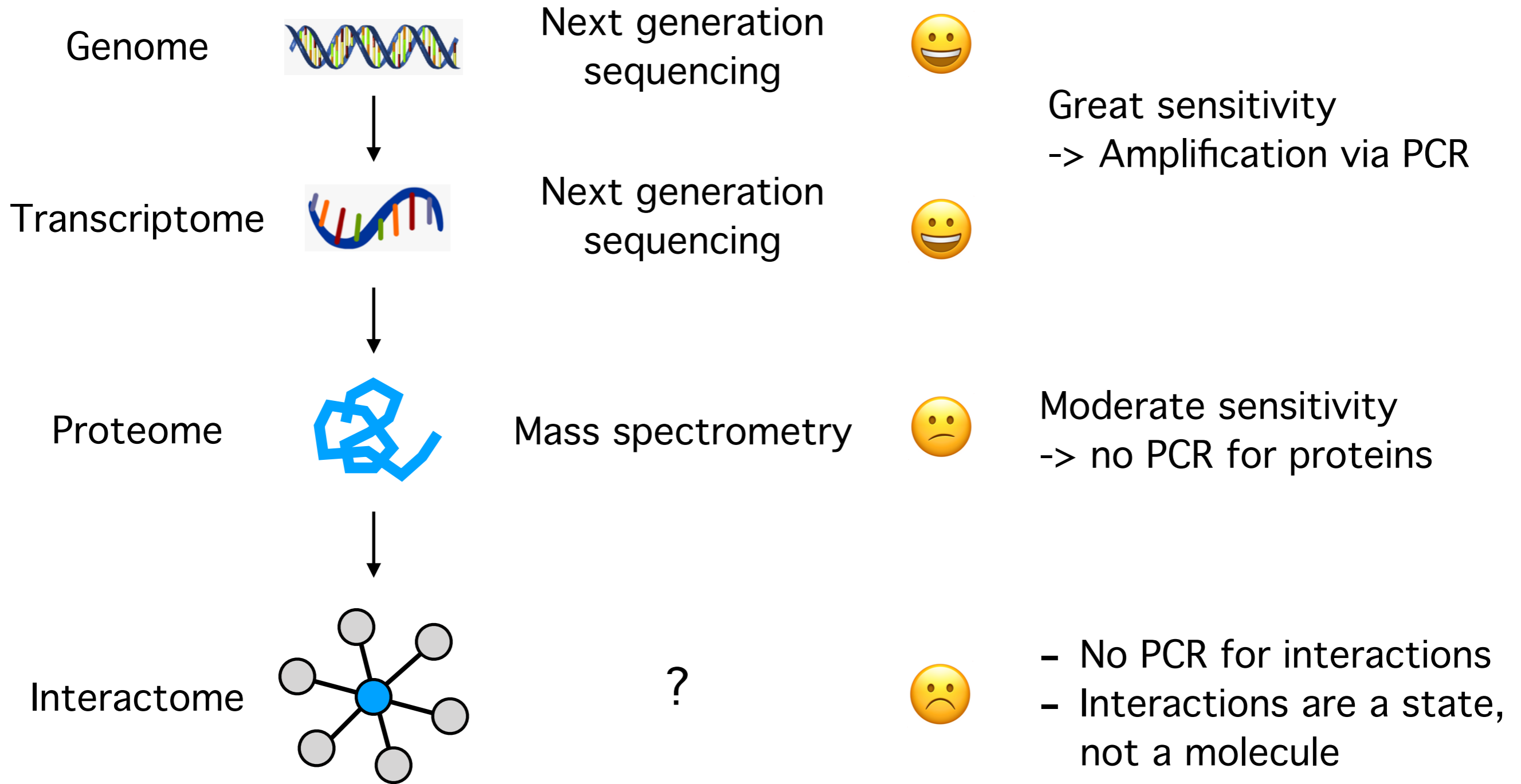
Better
understanding of
human biology

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Session: Protein interaction networks

2. Methods to detect protein interactions

Why is it so hard to detect protein interactions?



Approaches to detect protein interactions

- [-] 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

- [-] 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

- [-] 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>

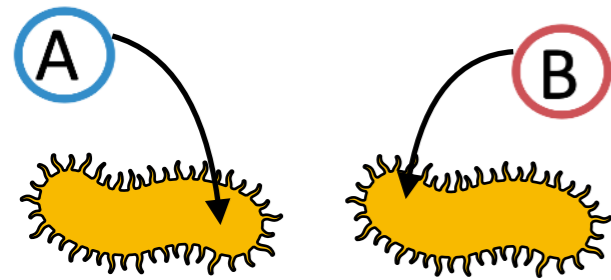
Direct assays

Binary assays

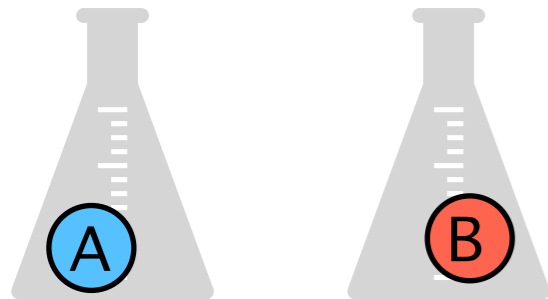
Co-complex assays

Assays to detect **direct** protein interactions

Principle



Production of interaction partners in bacteria



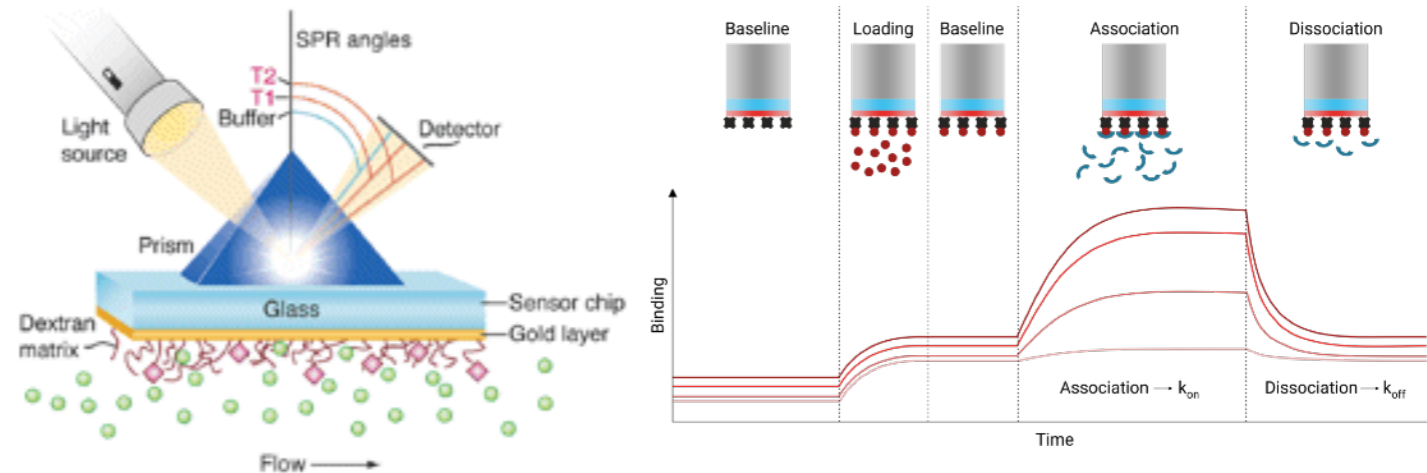
Purification of interaction partners



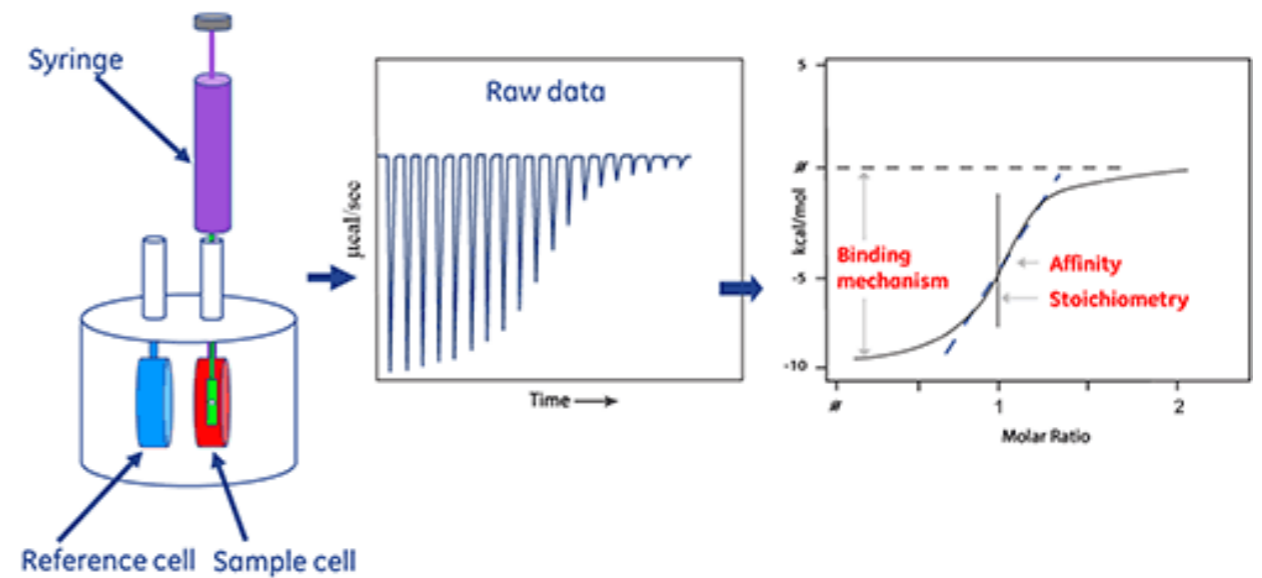
Measure

Examples of assays

Surface Plasmon Resonance (SPR)

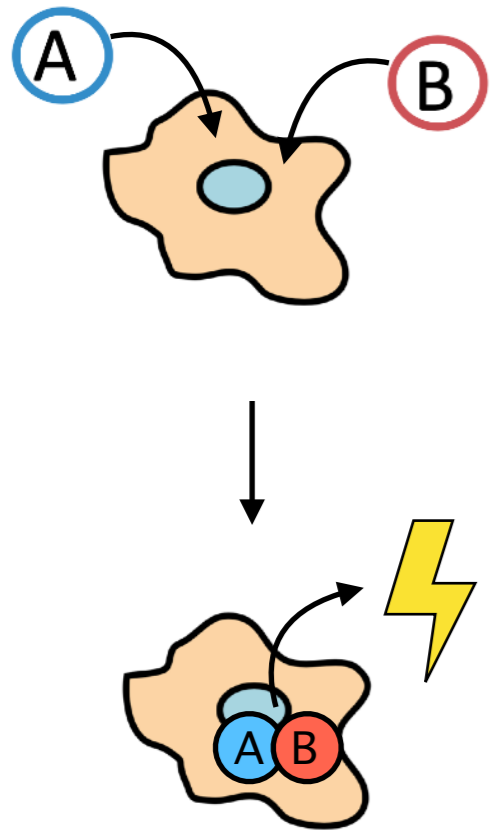


Isothermal titration calorimetry (ITC)



Assays to detect **binary** protein interactions

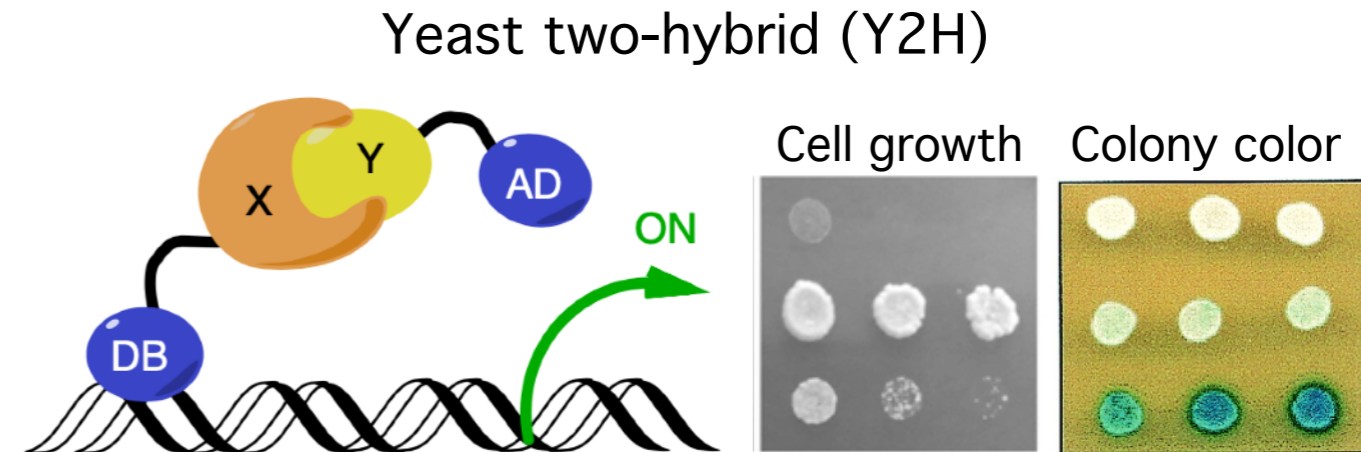
Principle



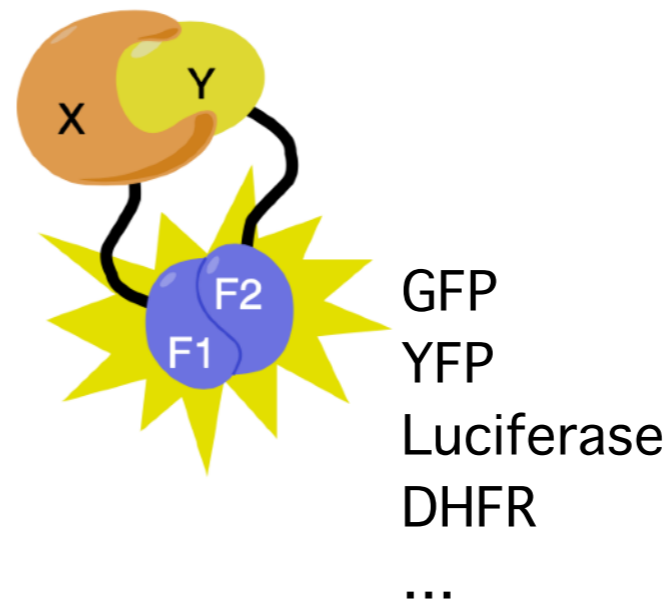
Exogenous expression of interaction partners as fusion constructs in cellular system

Interaction creates a signal in form of fluorescence, light, cellular growth, or others

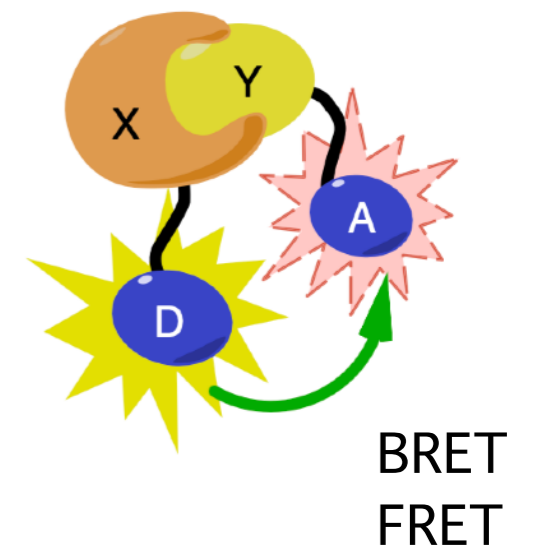
Examples



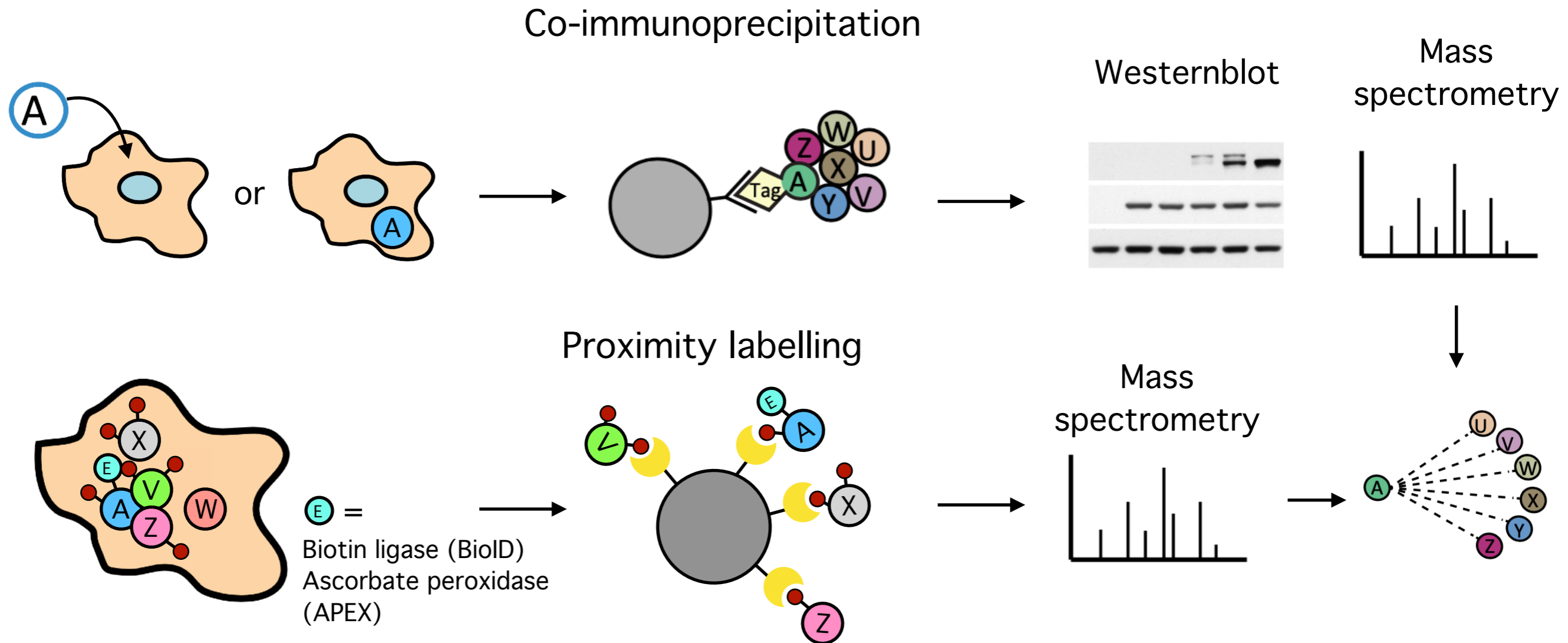
Protein complementation assays (PCA)



Energy transfer assays



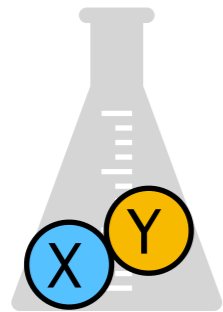
Assays to detect **co-complex** protein interactions



Different assays produce different types of protein interaction data

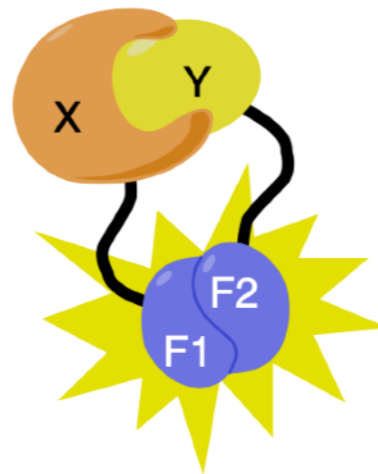
Direct assays

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



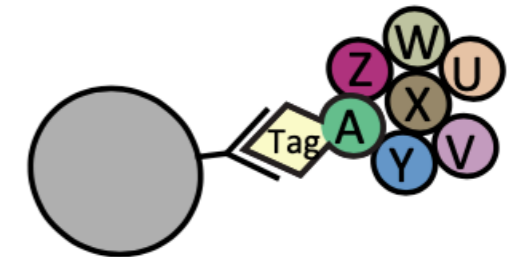
Binary assays

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



Co-complex assays

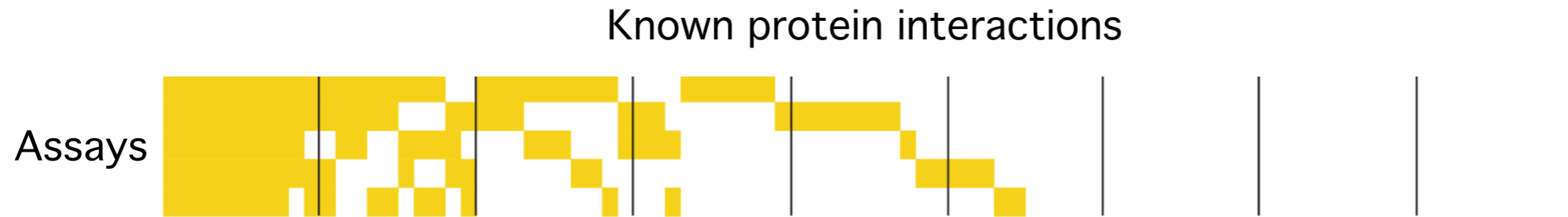
- Co-complex associations
- Full length proteins
- No K_D
- Over-expression and endogenous



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

Accuracy of protein interaction assays

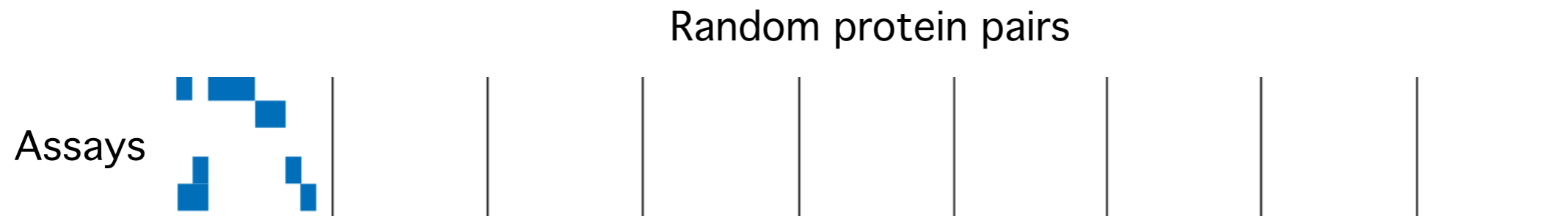
Sensitivity of protein interaction assays



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

Accuracy of protein interaction assays

Specificity of protein interaction assays

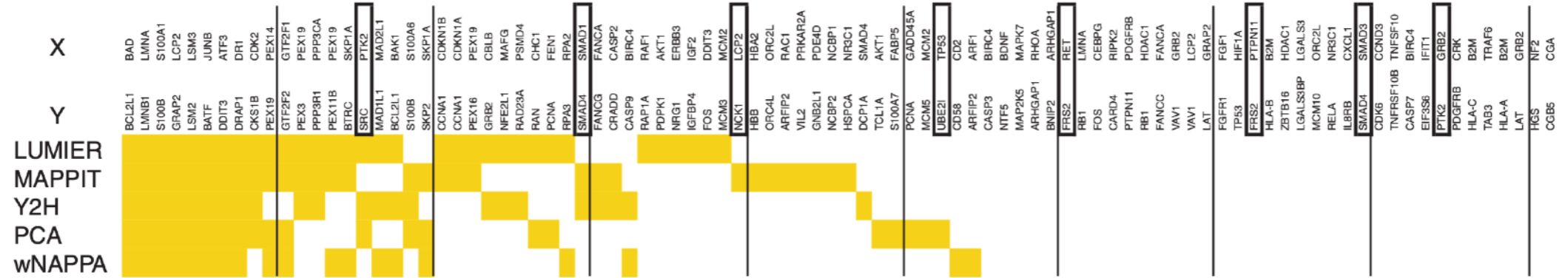


Why would an assay erroneously report a protein interaction?

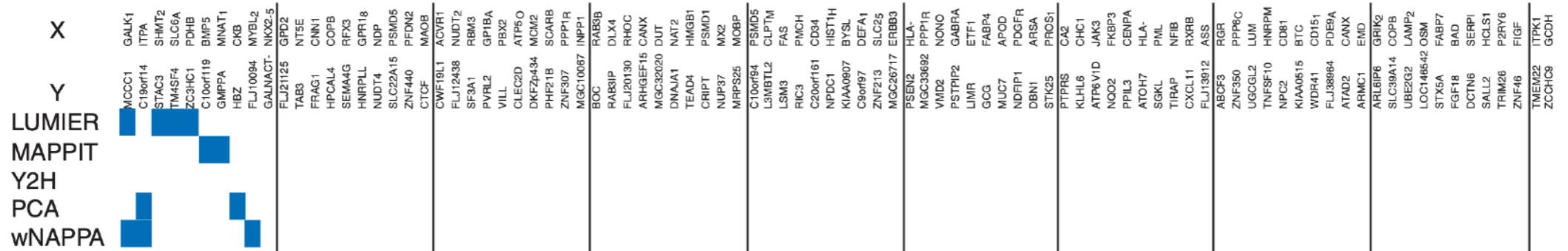
Accuracy of protein interaction assays

Correct benchmarking of assays

Positive reference set

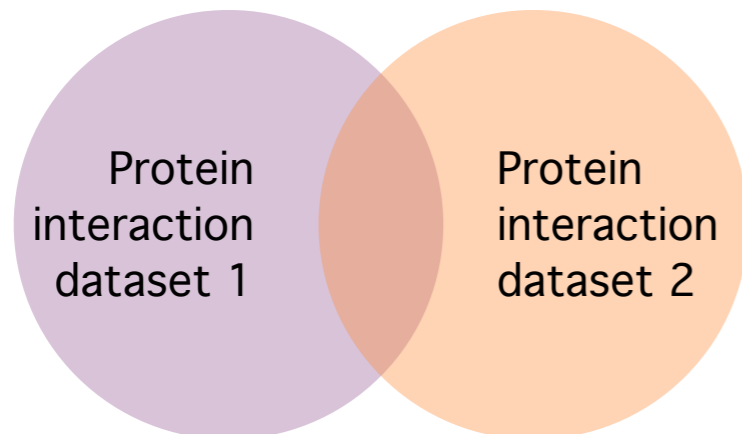


Random reference set



Correct interpretation of protein interaction data

Low overlap



Low sensitivity
High specificity

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