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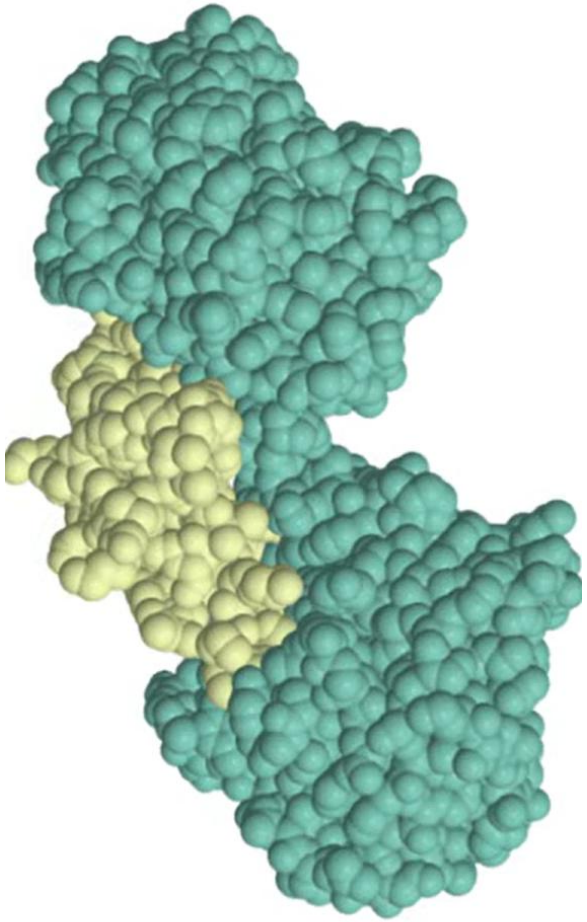
THE BIOLOGICAL KNOWLEDGE DISCOVERY (BKD)
CONFERENCE
2026



Protein protein interactions - steering force of biochemical reactions

Dr. Mohana Priya A,
School of Biociences and Technology,
VIT, Vellore

Why Protein Interactions?

- Complete genomes are now available
- Knowing the **genes** is not enough to understand how biology **functions**

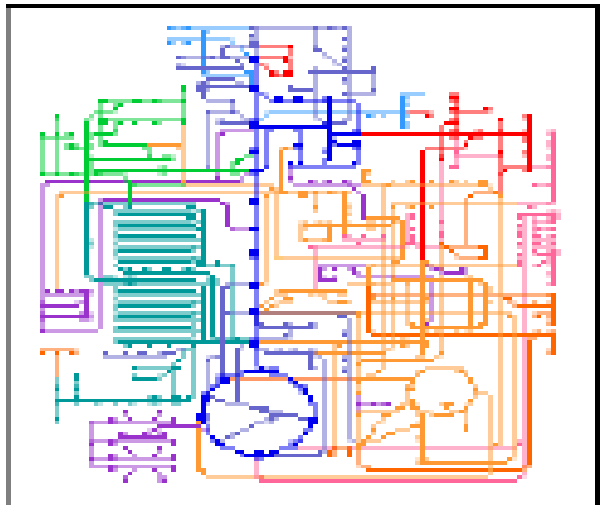
- **Proteins**, not genes, are responsible for many cellular activities

- Proteins function by **interacting** w/ other proteins and biomolecules

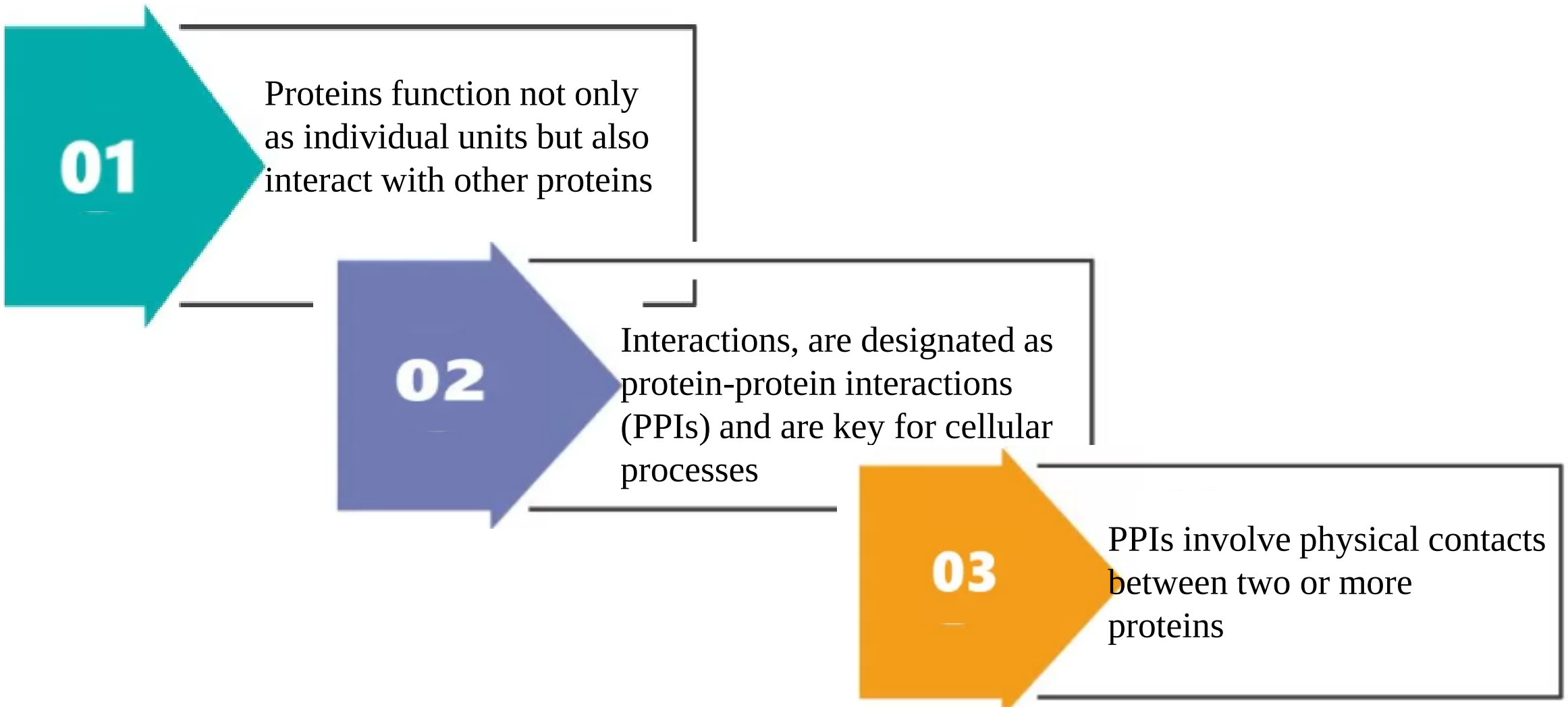
GENOME

PROTEOME

"INTERACTOME"



Protein Protein Interactions (PPI) – Steers biological functions!



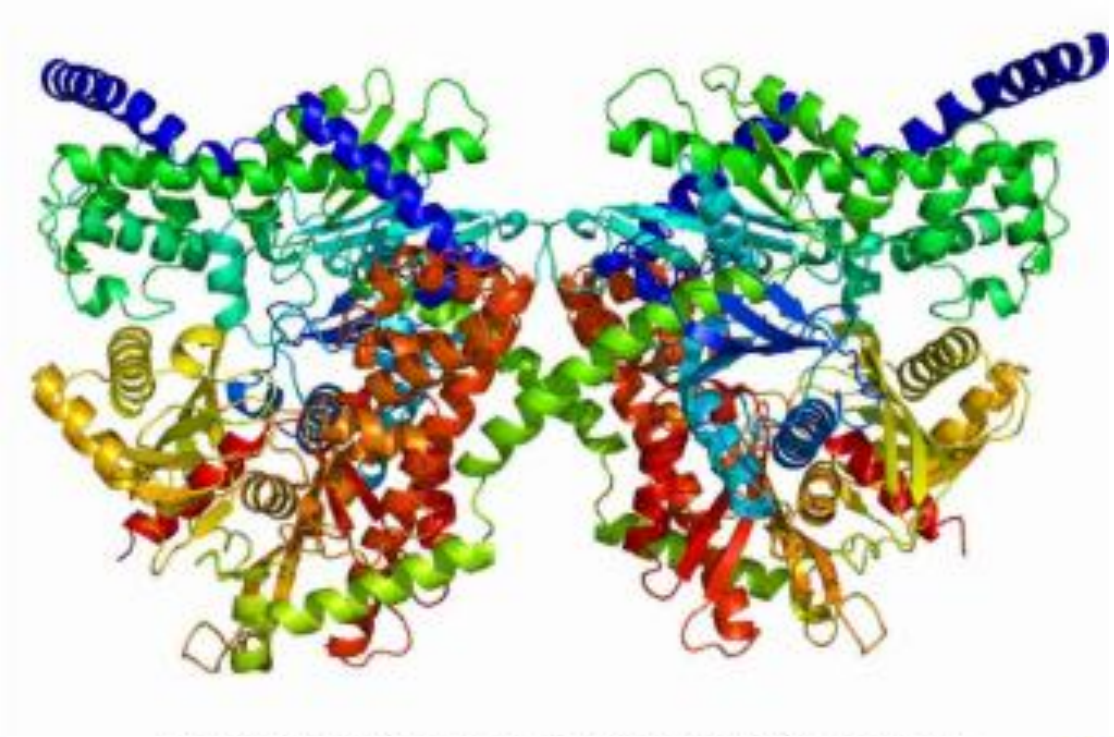
PPIs are central to numerous cellular processes

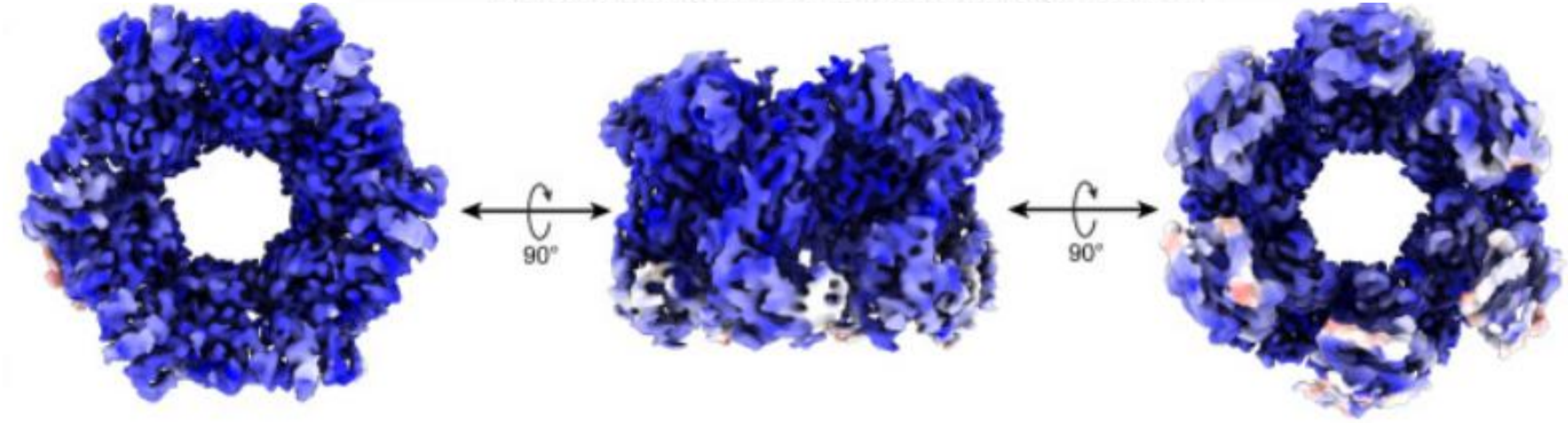
1. Signal transduction
2. Structural Support
3. Cell Transport
4. Regulation of cellular activities

Types of Protein-Protein Interactions

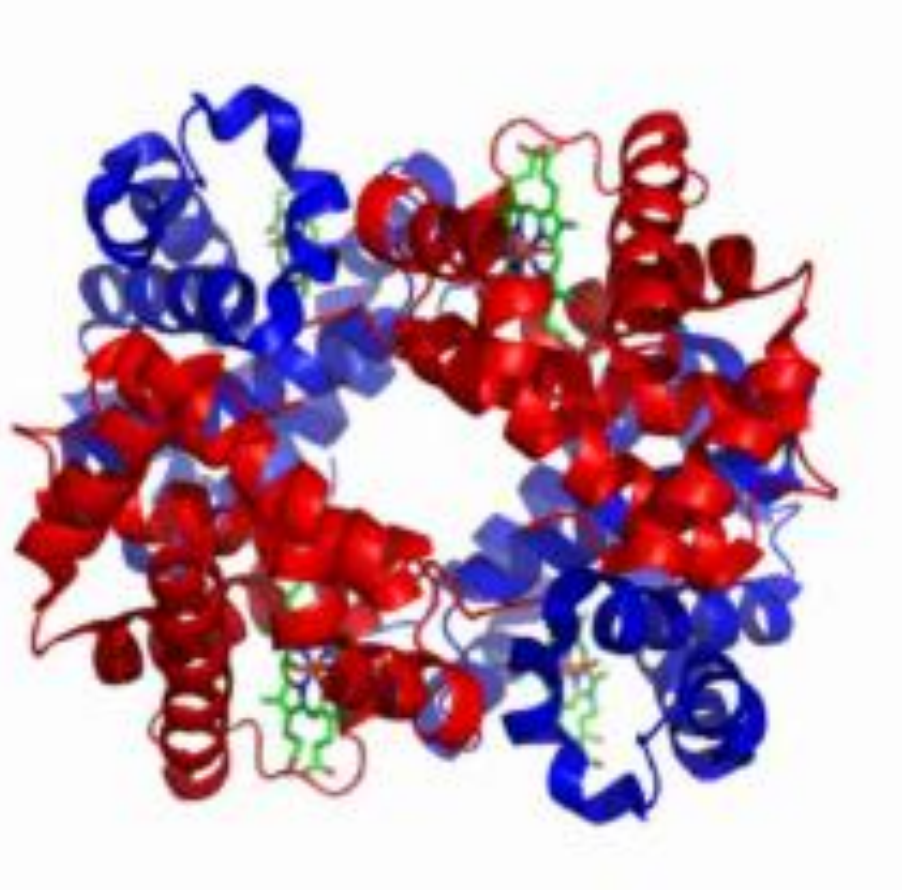
- Homo-Oligomers vs. Hetero-Oligomers
- Obligate Vs. Non-Obligate interactions
- Stable Interactions vs. Transient Interactions

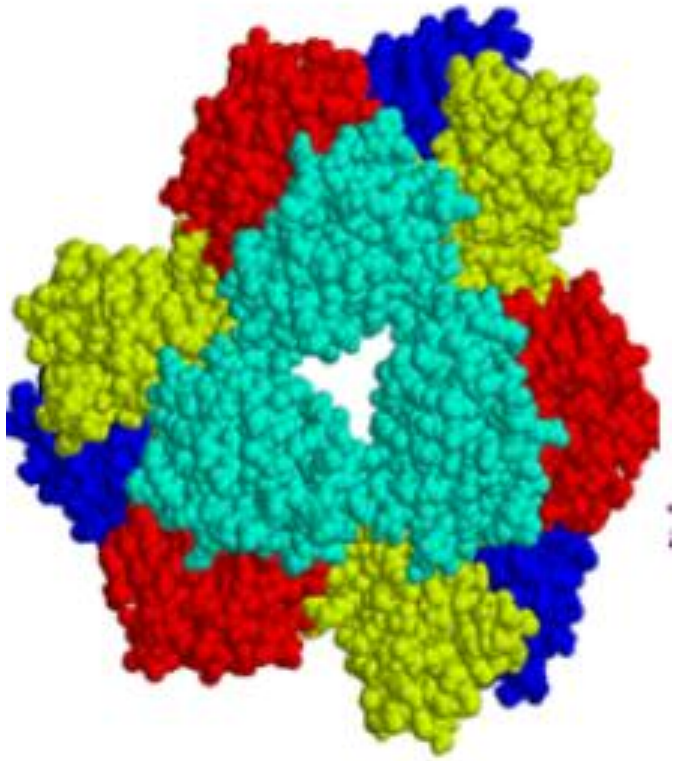
Homo-Oligomers: Homodimer/homohexamer





Hetero-Oligomers





Homodimer

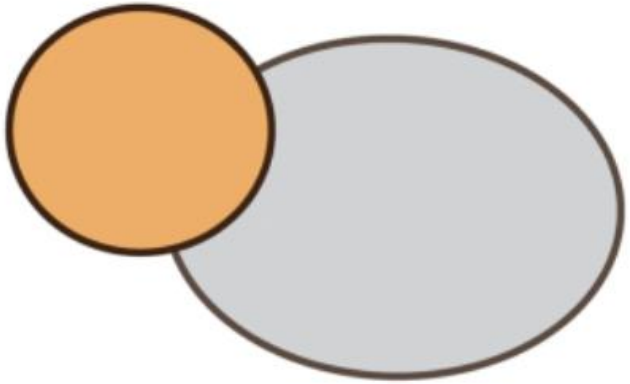
Vs

Heterodimer

- Combination of identical protein subunits.
- Frequently display symmetrical and highly ordered arrangements (dimers, trimers, tetramers)
- Increases the stability of the individual protein subunits.
- Increases enzymatic activity or facilitates regulatory functions.
- Notable Eg : hexokinase 1; collagen.

- Formed by different proteins or different isoforms of a single protein.
- No display of symmetry
- The structural and functional diversity allows for the implementation of intricate regulatory mechanisms.
- Confer specificity to the complex, thereby enabling precise cellular functions. .
- Notable Eg: ribosome; DNA polymerase holoenzyme.

Obligate Vs. Non-Obligate interactions

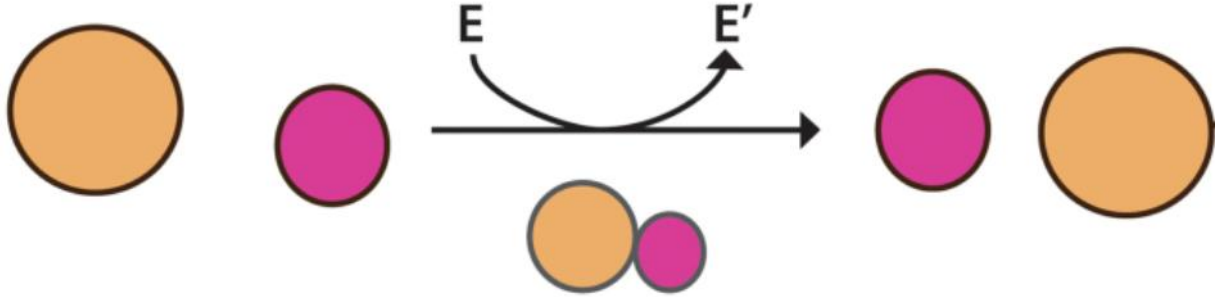


- Protein partners are unable to function independently
- Only stable when they form a complex.
- Example: subunits of the hemoglobin molecule.

Obligate

- Proteins are capable of existing independently
- Interact under specific conditions.
- Example: Enzyme-inhibitor interactions.

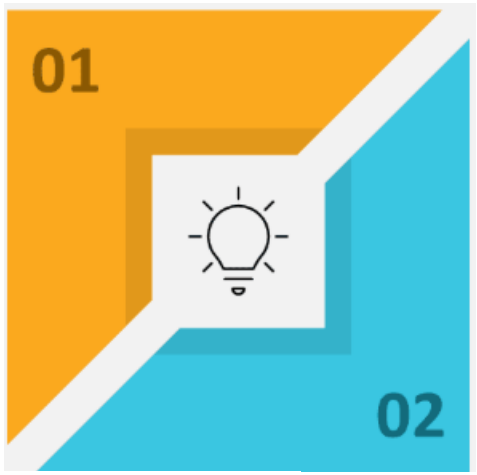
Non-Obligate

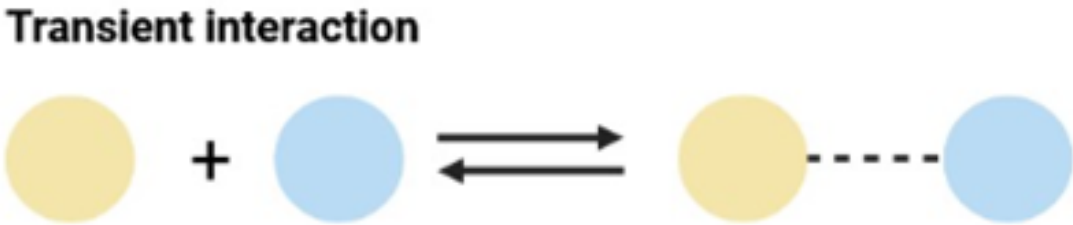


Stable Interactions vs. Transient Interactions

Stable Interactions

- Forms long-lived complexes
- Serve as structural and functional components of cellular machinery
- Examples: ribosomes and proteasomes.

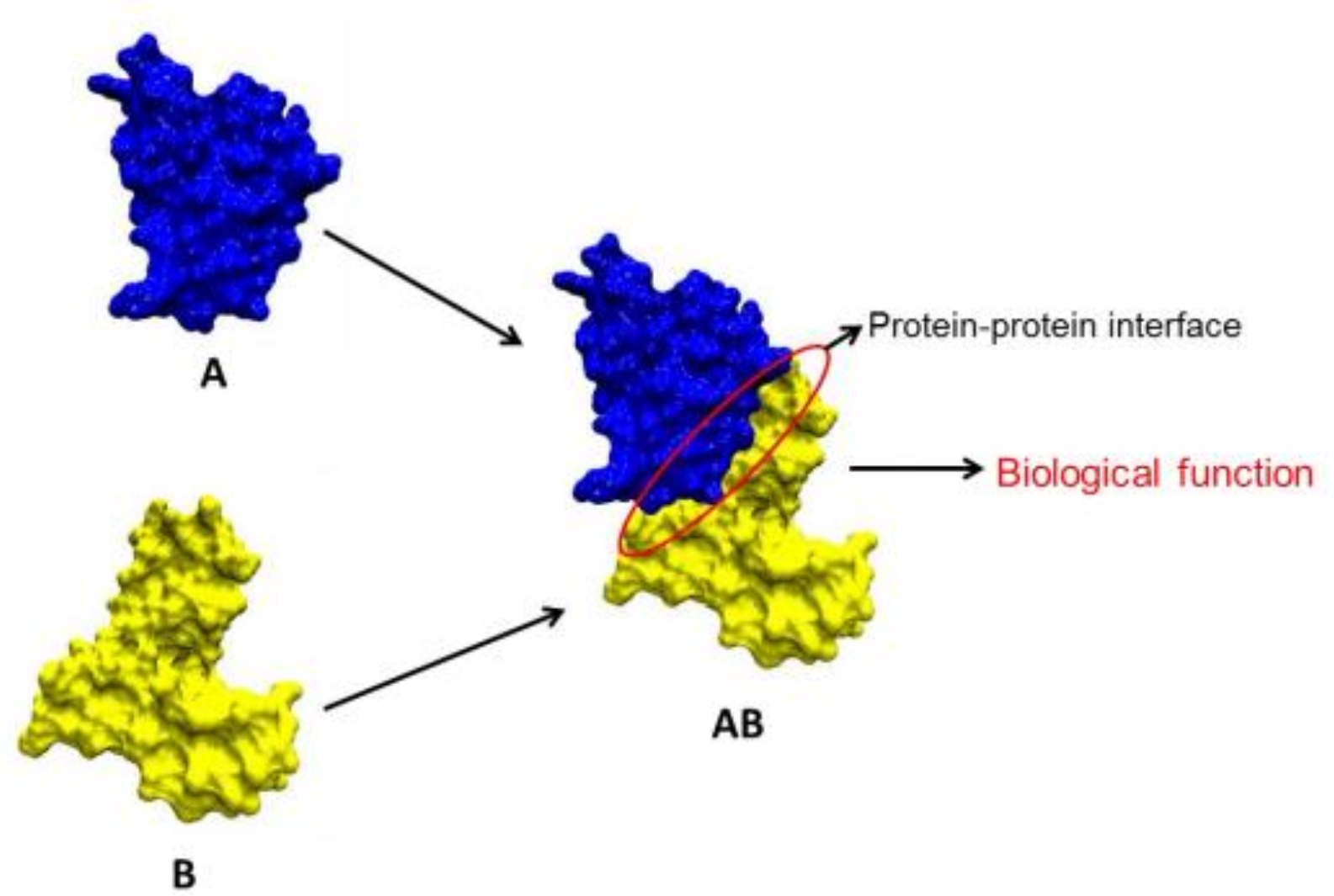




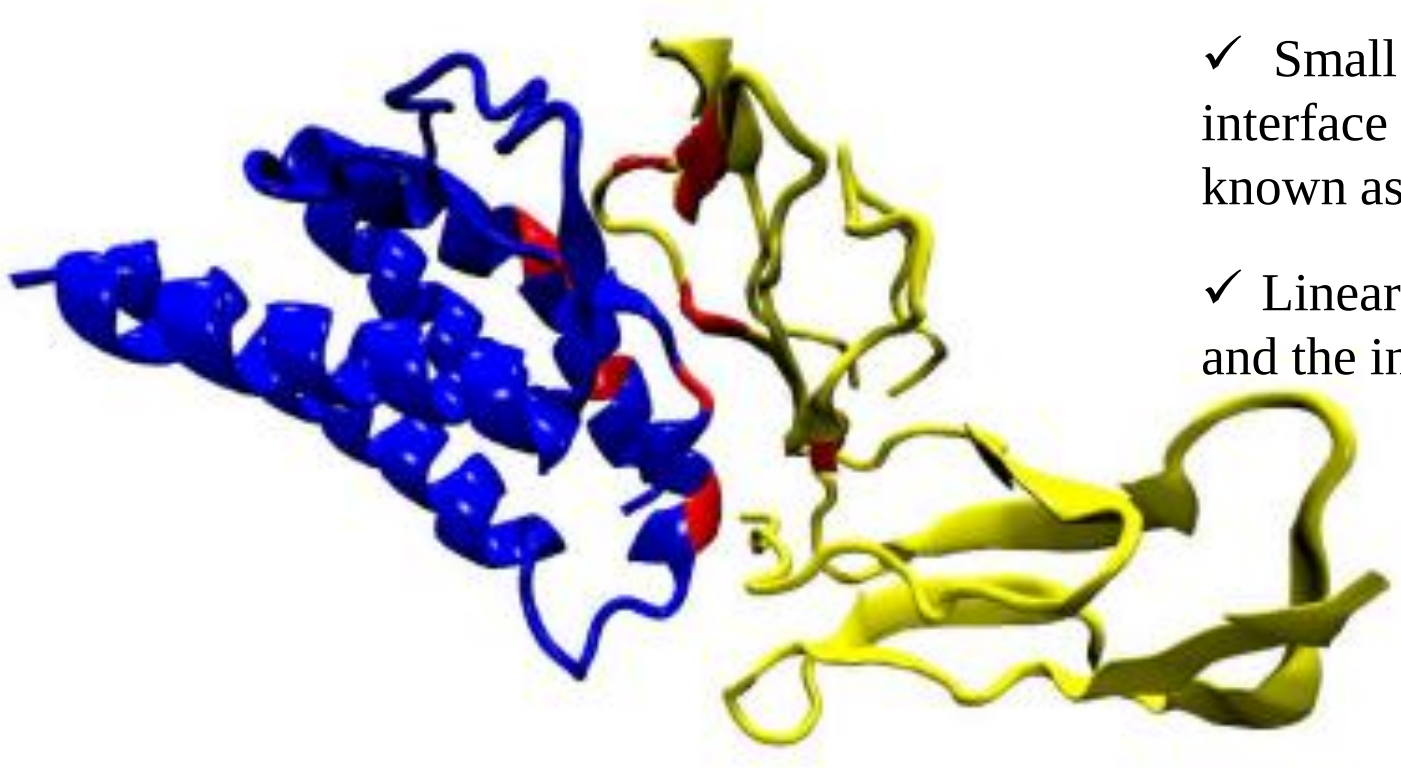
Transient Interactions

- Characterized by a relatively short lifespan, occurring and dissociating rapidly.
- Implicated in signal transduction and regulatory processes.
- Examples:kinase-substrate interactions and antigen-antibody interactions.

Complex of interleukin-2 and alpha receptor with interface



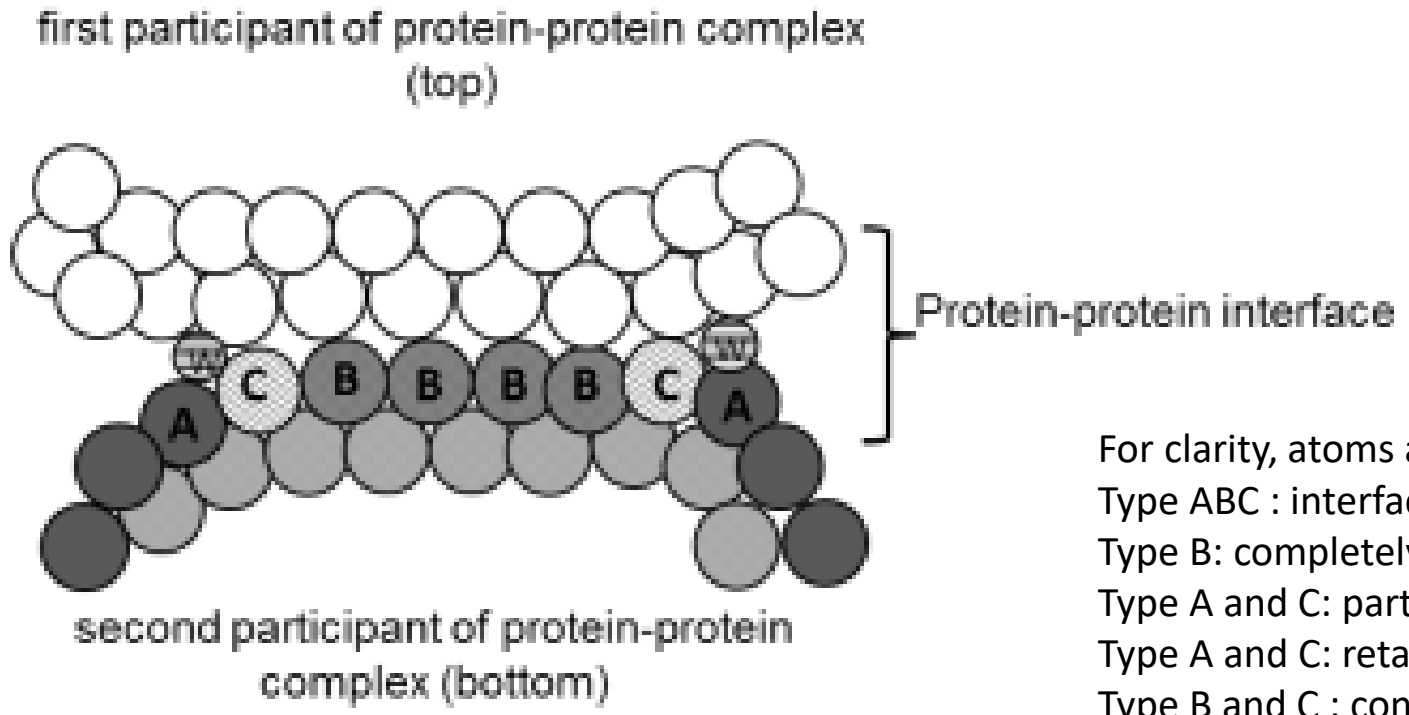
Hot spots: The regions on the Protein-Protein interface responsible for binding



Structure of interleukin-2 (blue) with its alpha receptor (yellow).
The hot spots are represented in red.

- ✓ Small subsets of residues at the protein-protein interface that contribute to the binding energy are known as ‘hot spots’
- ✓ Linear correlation between the numbers of hotspots and the interface size

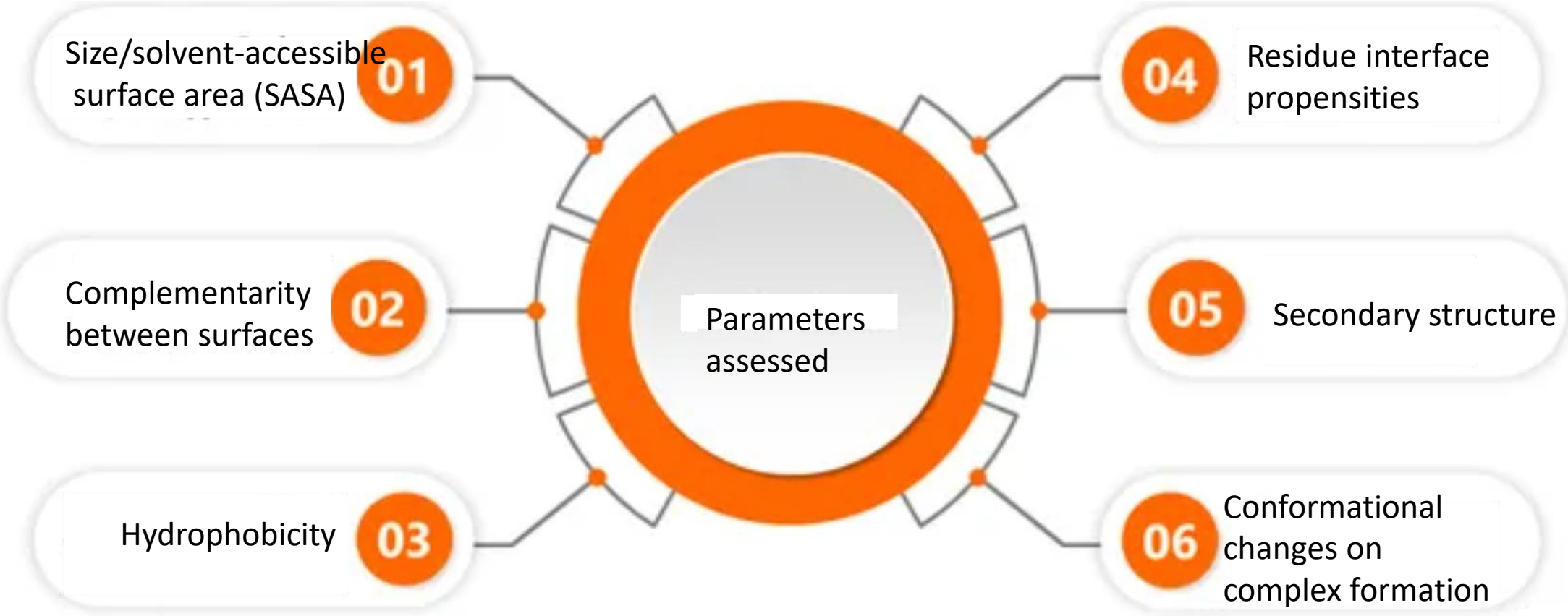
Types of atoms at the protein-protein interface



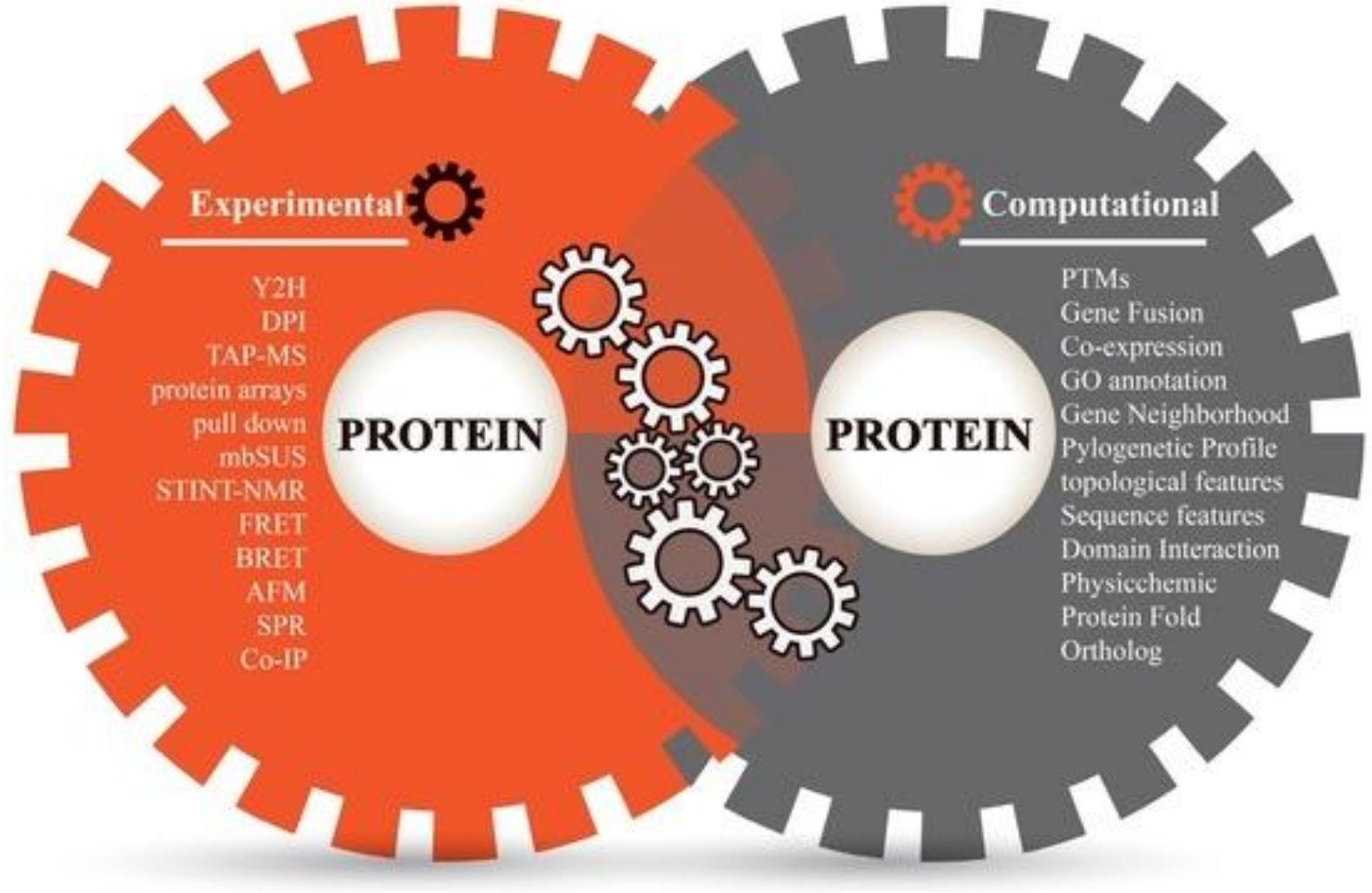
For clarity, atoms are specified for the bottom protein only.
Type ABC : interface atoms
Type B: completely buried atoms
Type A and C: partially buried atoms
Type A and C: retain partial solvent accessibility
Type B and C : contact atoms that make vdw contacts with the top protein partner.
The sphere with W represents water molecule

The figure is taken from "The atomic structure of protein-protein recognition sites," L. Jo Conte et al, 1999, J. Mol. Biol.,285, p. 2182

Properties of the interface



Methods for Detection of Protein Protein Interactions



Yeast two hybrid system

- Molecular genetic tool which facilitates the study of protein-protein interactions
- Pioneered by Stanley Fields and Song in 1989
- Based on the properties of the yeast GAL4 protein
- Consists of separable domains responsible for DNA-binding and transcriptional activation

The key to the two-hybrid screen

- Most euk. transcription factors(TF), are comprised of the activating and binding domains
- Functions in close proximity to each other without direct binding
- Even though the TF is split into 2 fragments, it can still activate transcription when the two fragments are indirectly connected

Gal4 transcription factor of Yeast

- *Gal4* transcription factor gene produces a two domain protein (DNA binding domain: D*BD* &

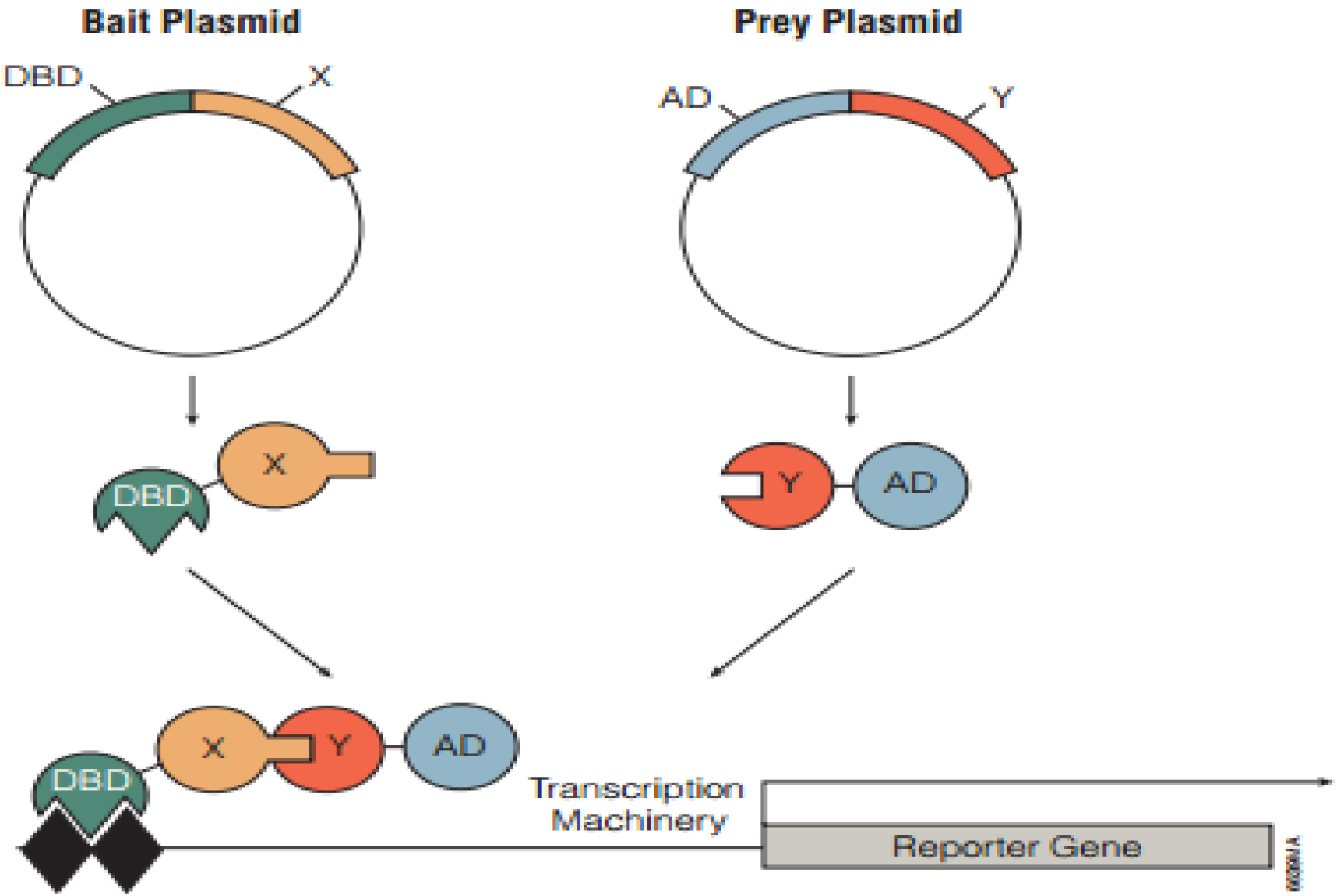
  activation domain: *AD*)

- The DBD recognizes a specific DNA sequence

- The AD coordinates the assembly of the elements required for transcription

- Both domains - essential for transcription of the reporter gene (*LacZ*)

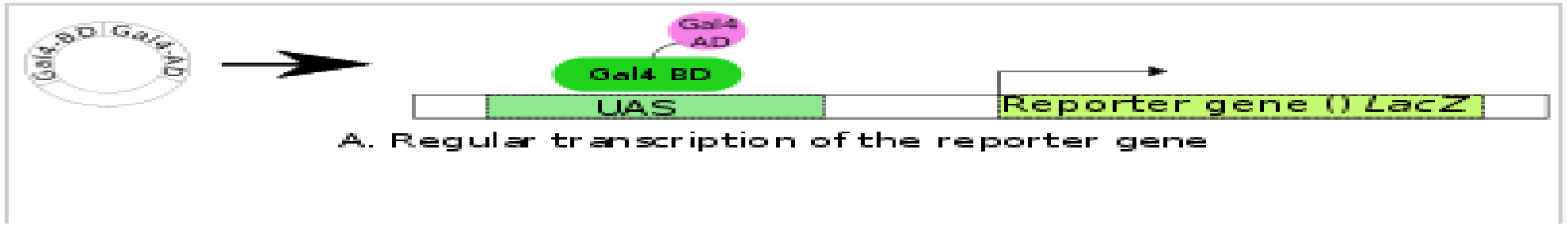
Two fusion proteins from plasmids



Bait – Prey interaction

- If the bait and prey proteins interact (i.e., bind), then the AD and BD of the transcription factor are indirectly connected
- Hence transcription of reporter gene(s) occurs
- If the two proteins do not interact, there is no transcription of the reporter gene.

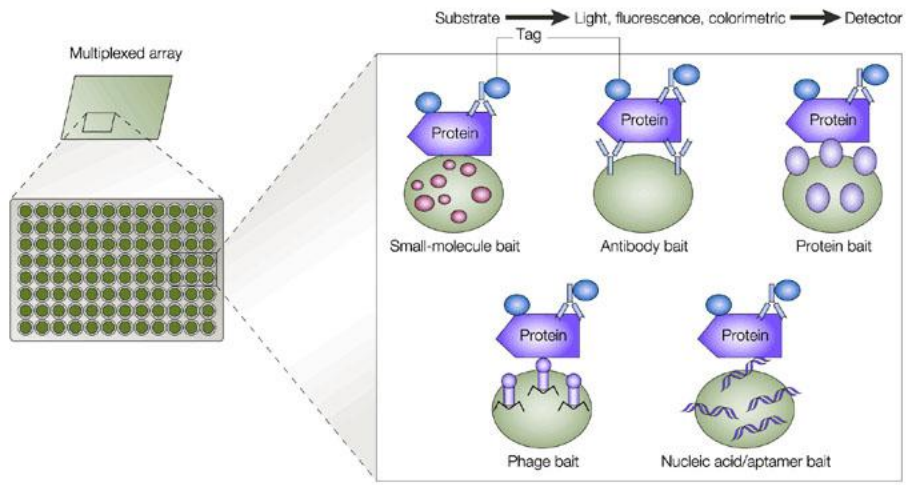
Overview of two-hybrid assay



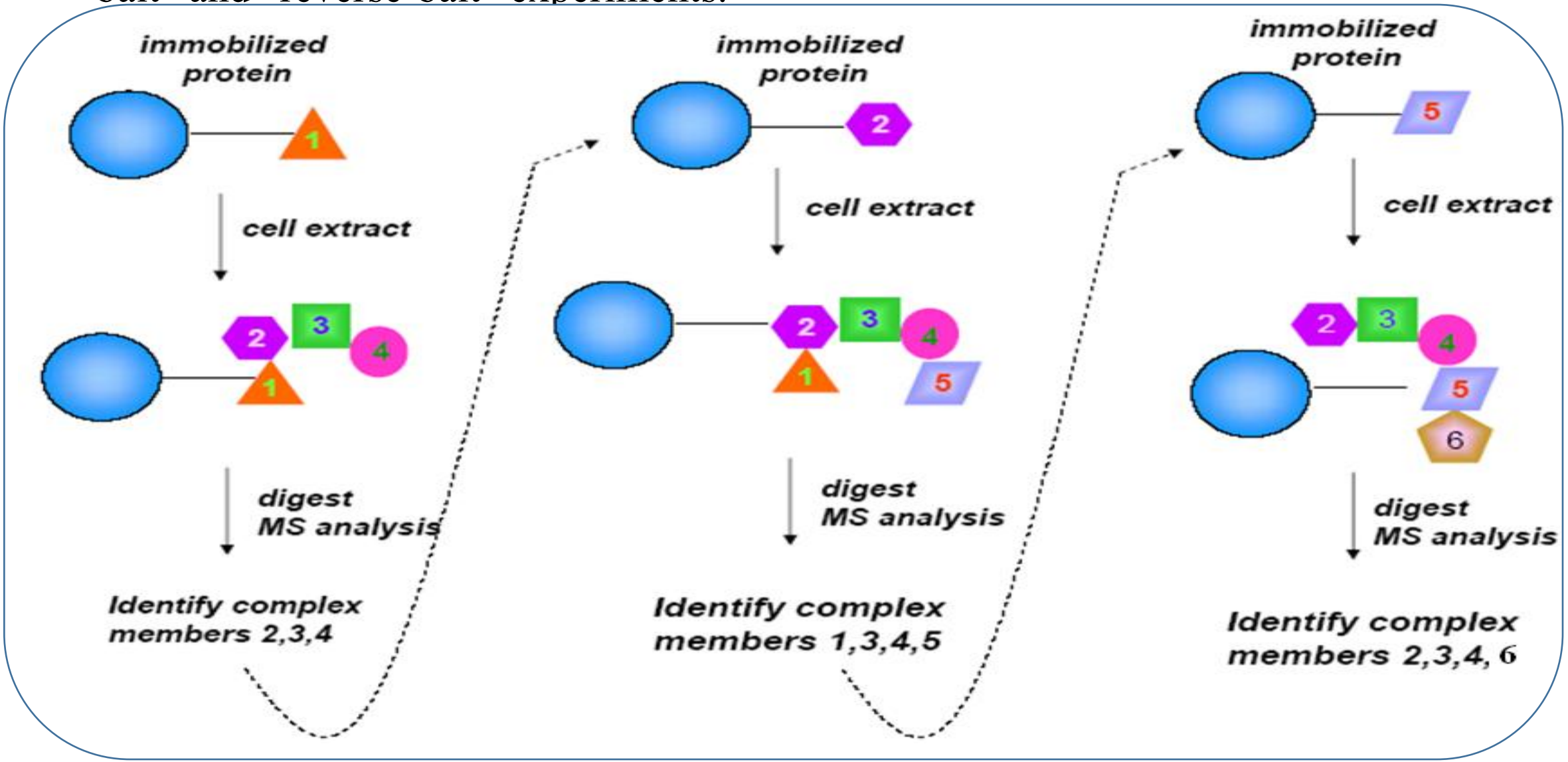
Protein microarrays

- Protein microarrays are rapidly becoming established as a powerful means to detect proteins, monitor their expression levels, and probe protein interactions and functions.
- A protein microarray is a piece of glass on which various molecules of protein have been affixed at separate locations in an ordered manner.
- The objective behind protein microarray development is to achieve efficient and sensitive high-throughput protein analysis, carrying out large numbers of determinations in parallel by automated process.

Protein microarrays



Mapping a protein-interaction network with a series of linked "bait" and "reverse-bait" experiments.



Bait and reverse bait

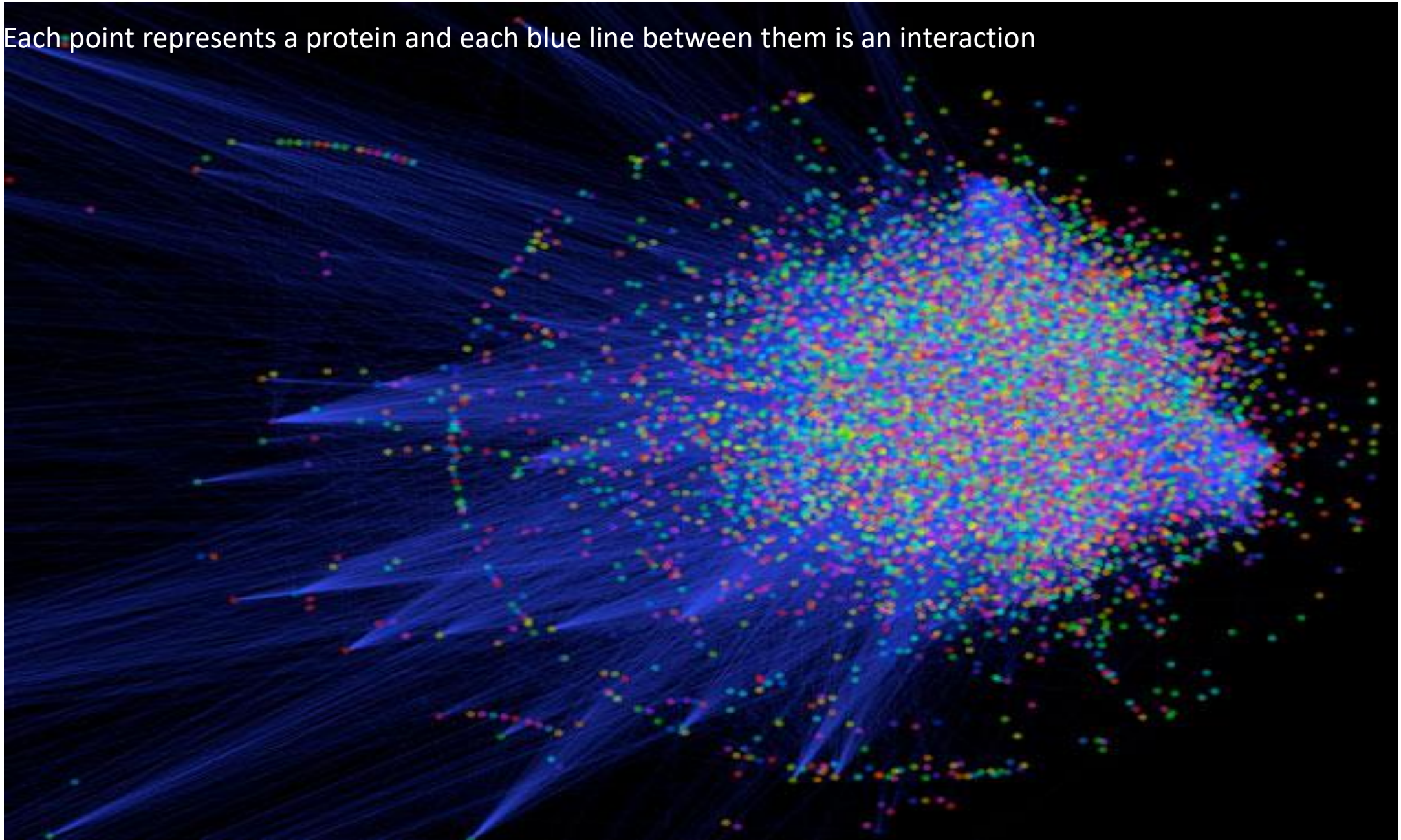
- Net result of this experiment is to *map a network of protein –protein association*

- This also gives clues to function of a protein

Databases

- Interactions are collected together in specialized biological databases
- Allows the interactions to be assembled and studied further
- The first of these databases was DIP, the Database of Interacting Protein

Network visualization of the human interactome



Protein – protein interaction map construction software

- HIPPE - Human Integrated Protein-Protein Interaction rEference

http://cbdm.mdc-berlin.de/tools/hippie/index.php

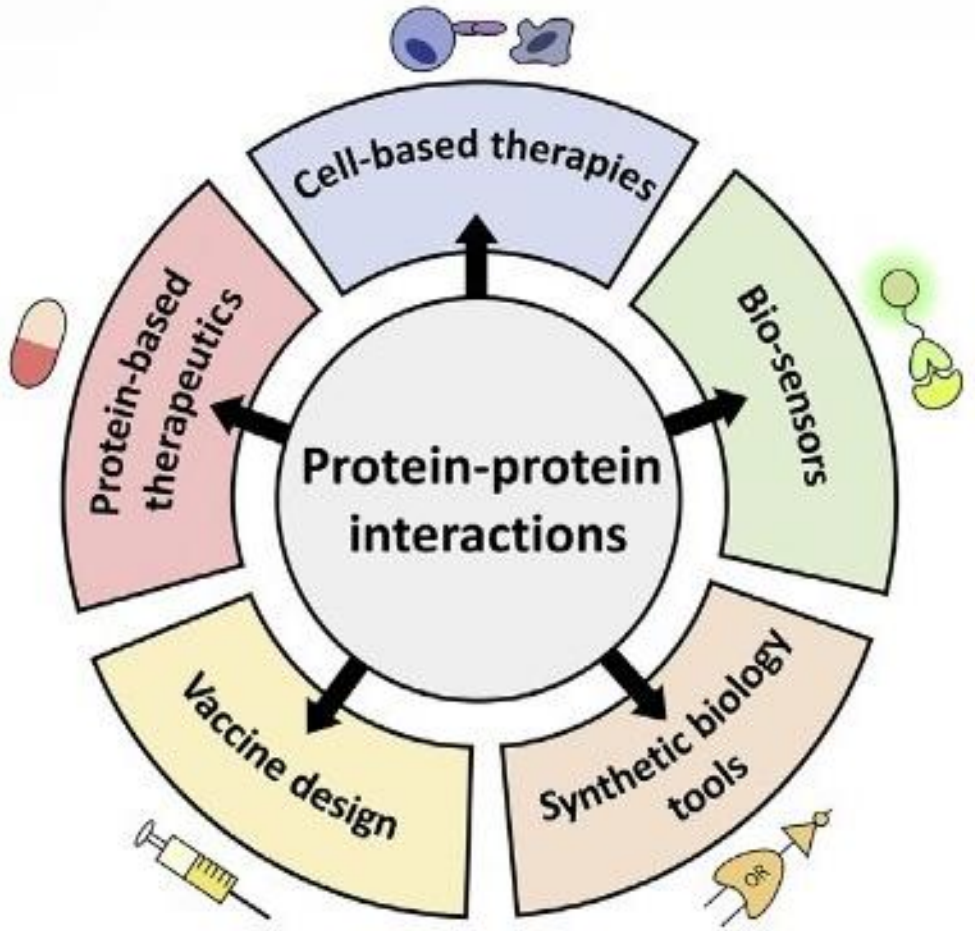
- PINA: Protein Interaction Network Analysis platform

http://cbg.garvan.unsw.edu.au/pina/

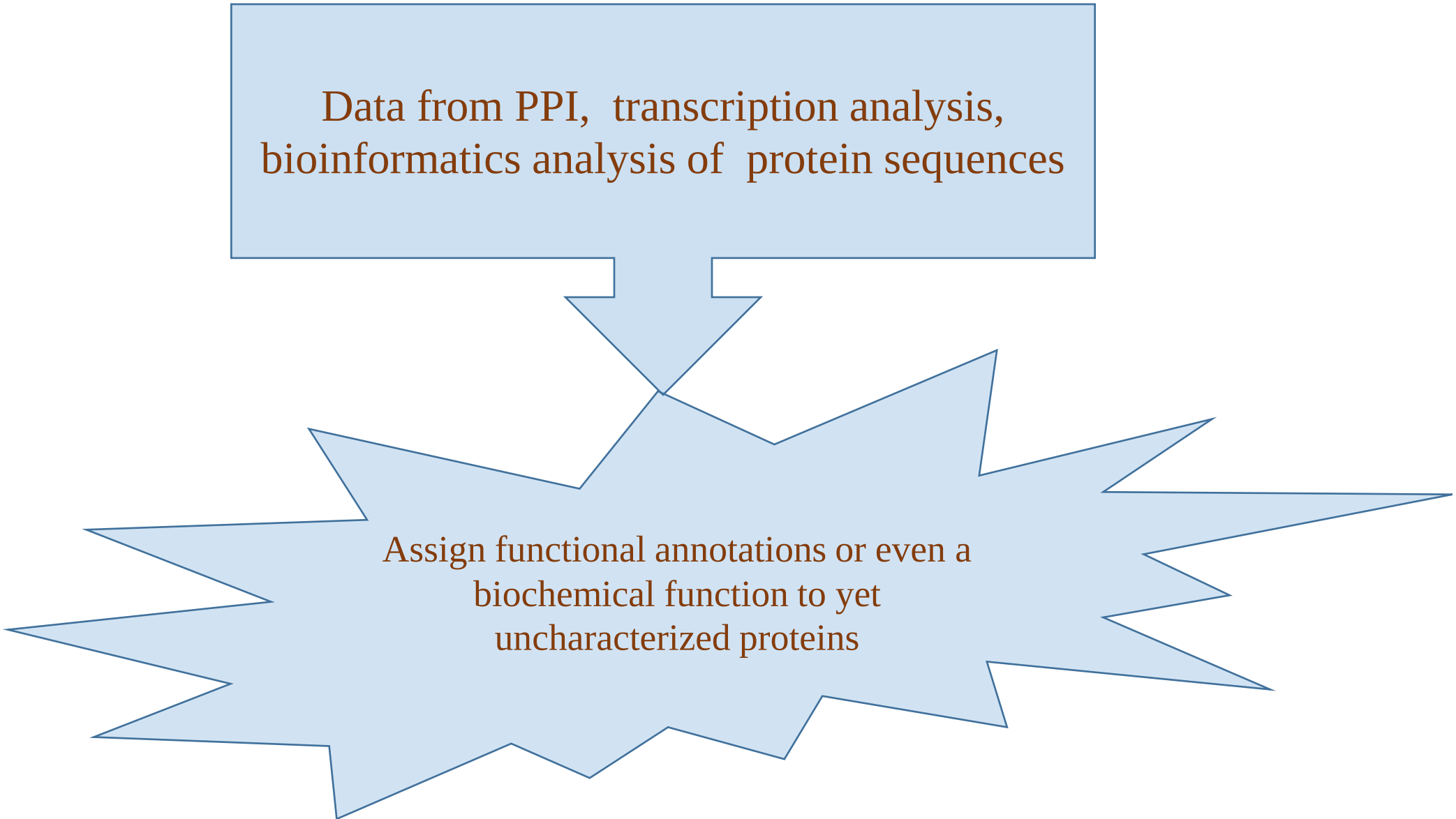
- Cytoscape (for visualization of networks)

http://www.cytoscape.org/

Overview of potential applications for novel PPIs (Marchand et al., 2022).



Ultimate aim!

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Thank you!