# Protein Interactions, Pathways and Networks

- The original one-gene-one-function theory is not always true.

- As usual biology is more complicated and this idea has changed

- Many proteins interact with each other and other molecules in metabolic systems

- This results in metabolic networks, regulatory systems and what some call Interactomes

What are Interacting Proteins?

- Interacting proteins span a range of structures and functions

- Simple self interactions:
  dimers, trimers, oligomers...

- Large fibrous proteins can form polymers, such as keratins

- Protein complexes where multiple proteins form a functional unit i.e. ribosomes, transcription complexes, chromatin.

- Cell signaling pathways
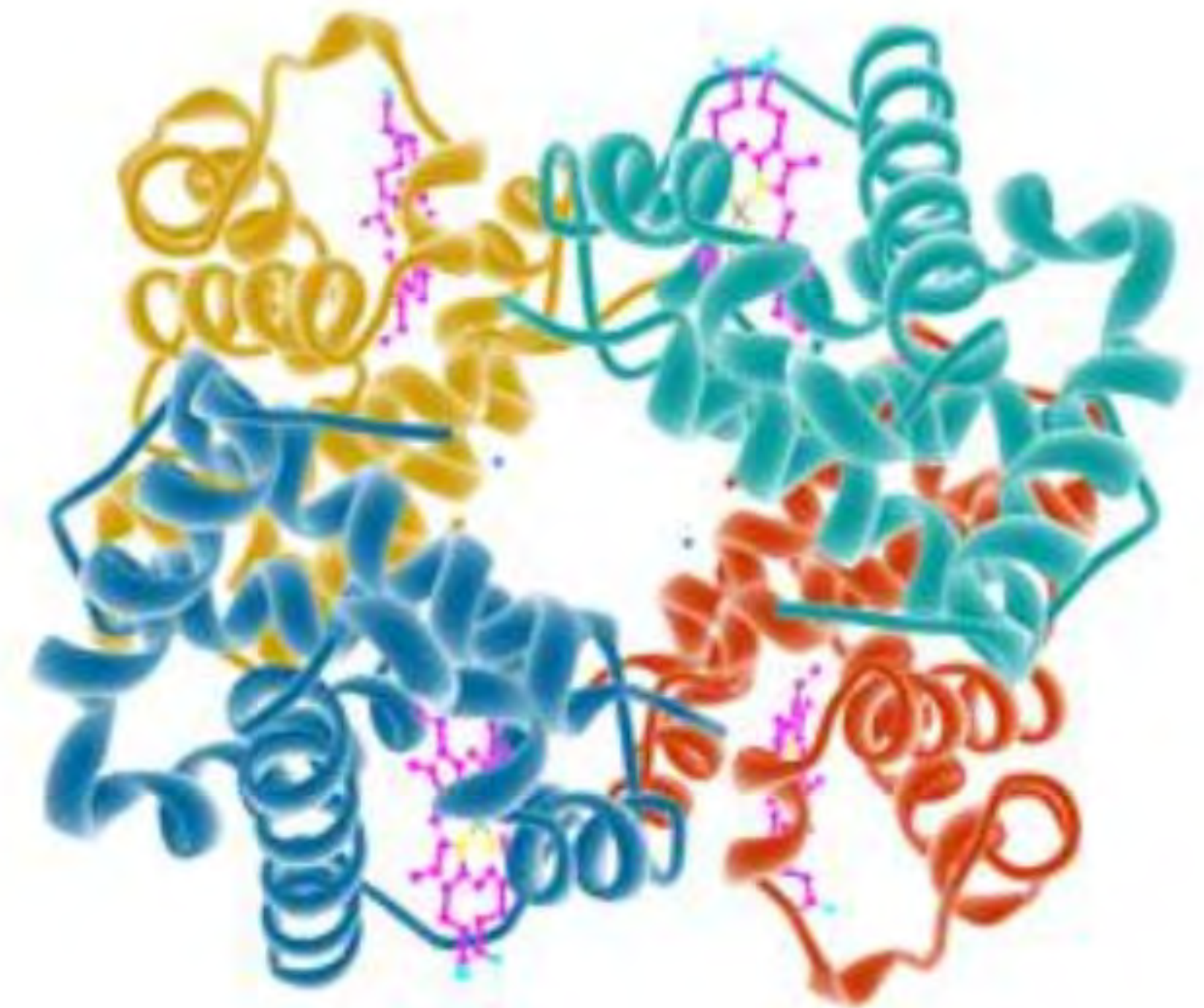
Experimental Methods to look for Protein-Protein interactions

Yeast two-hybrid assay (in vivo)

Co-precipitation (in vitro)

Co-Immunoprecipitation
Pull-down ligand assay
Tandem affinity purification (TAP)

Protein Microarrays

3D-Structure of complexes

Yeast two-hybrid assay *(in vivo)*

- Developed by Fields and Song in 1989

- Determines the interaction of two proteins by expression of a reporter gene

- A Binding Domain (BD) and Acceptor Domain (AD) in close proximity are required for transcription of a reporter gene.

- One protein called the Bait is cloned to the BD and another protein called Prey is cloned to the AD. If they bind each other the reporter gene is triggered.

Yeast two-hybrid assay
(High-troughput in vivo assay)

A. Regular transcription of the reporter gene
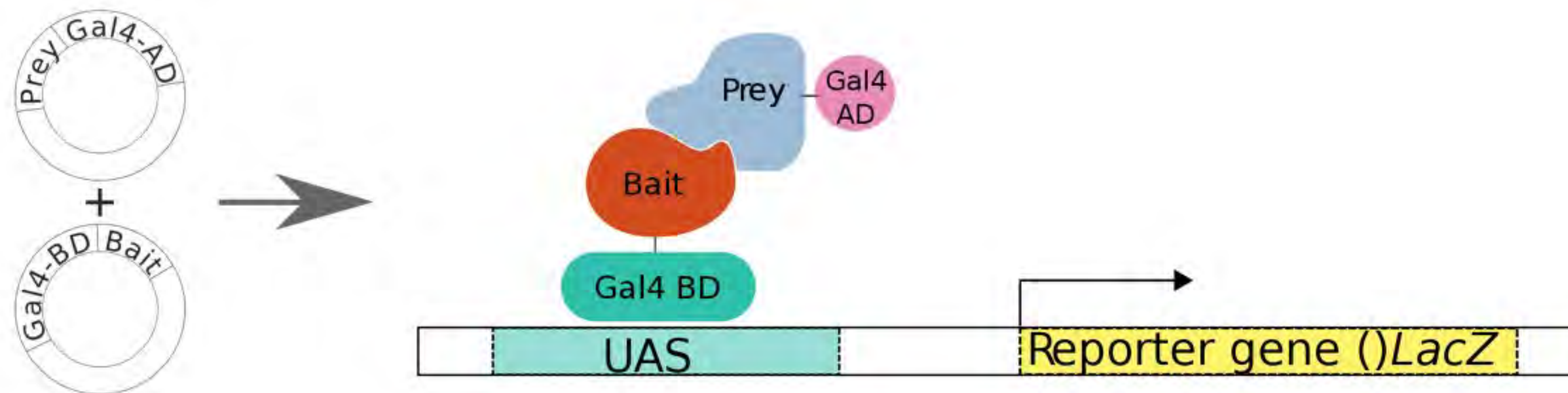
D. Two fusion proteins with interacting bait and prey

Animal cell

Antibody

Protein A-agarose bead

1 • Cell lysis

2 • Antibody binding to protein A-agarose bead

3 • Antigen isolation on antibody bead bead

Co-Immunoprecipitation (IP)

Washing and analysis (1D-2D gel, western)

Tandem affinity purification

- Considered more sensitive than Y2H

Mass spectrometry
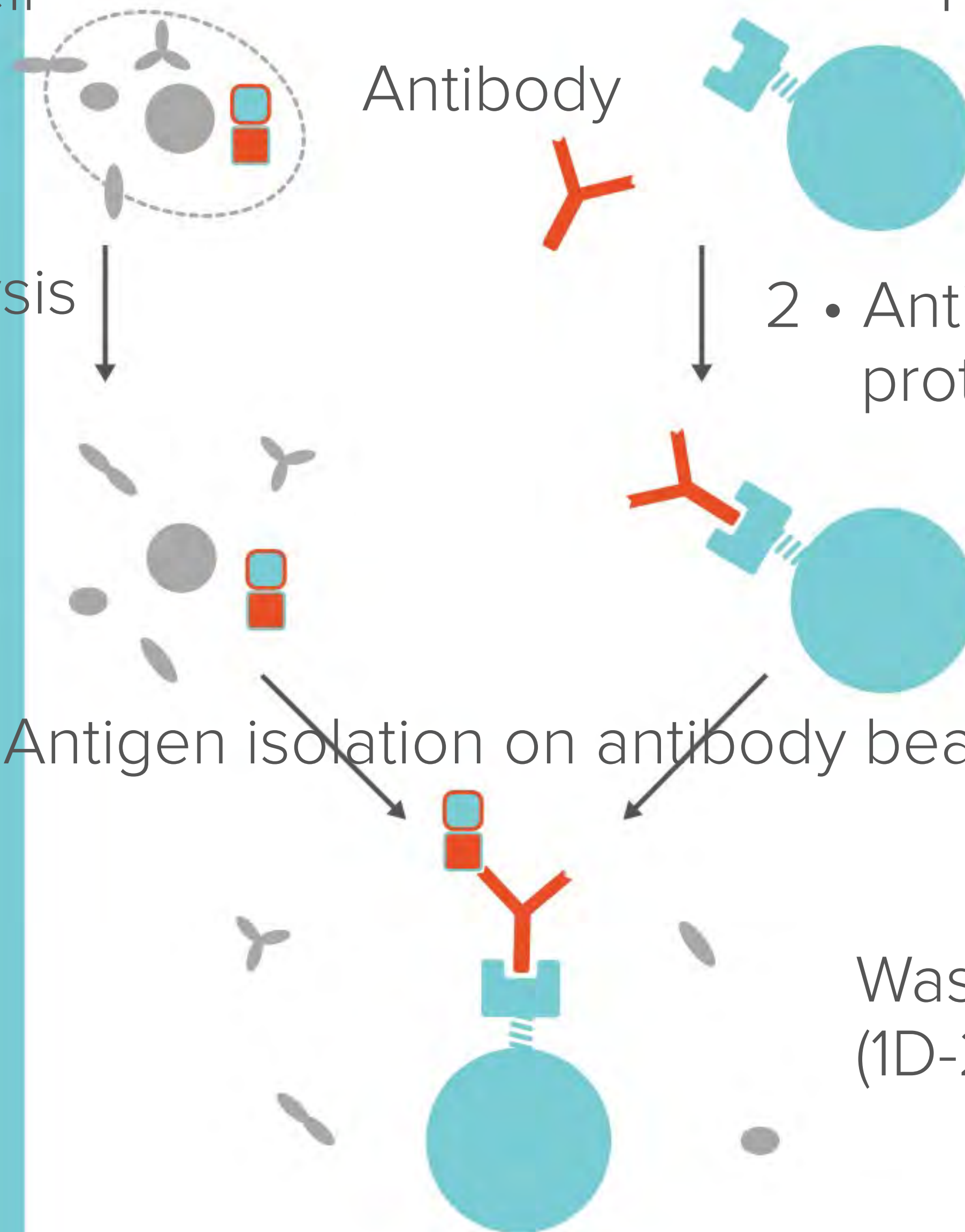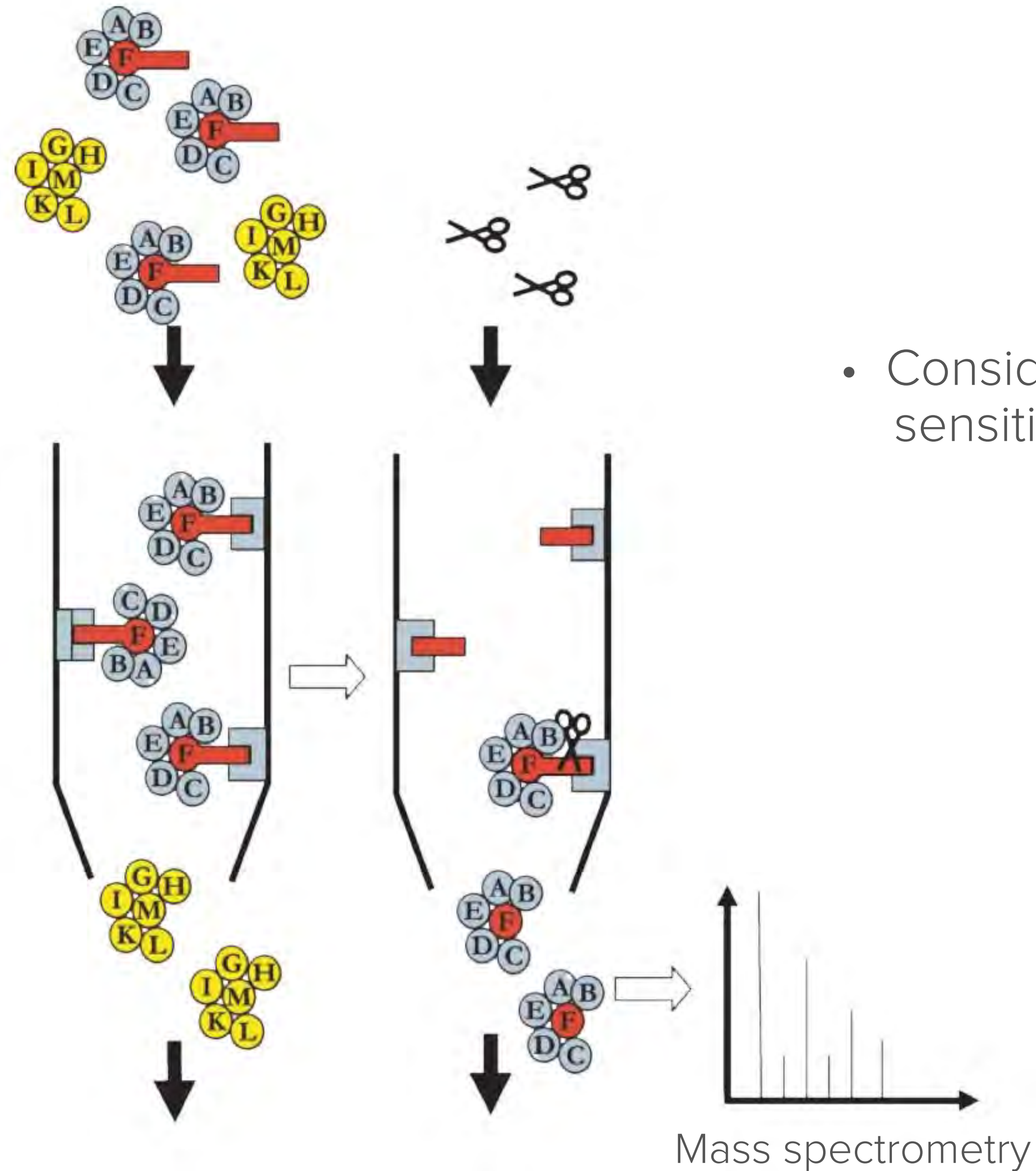
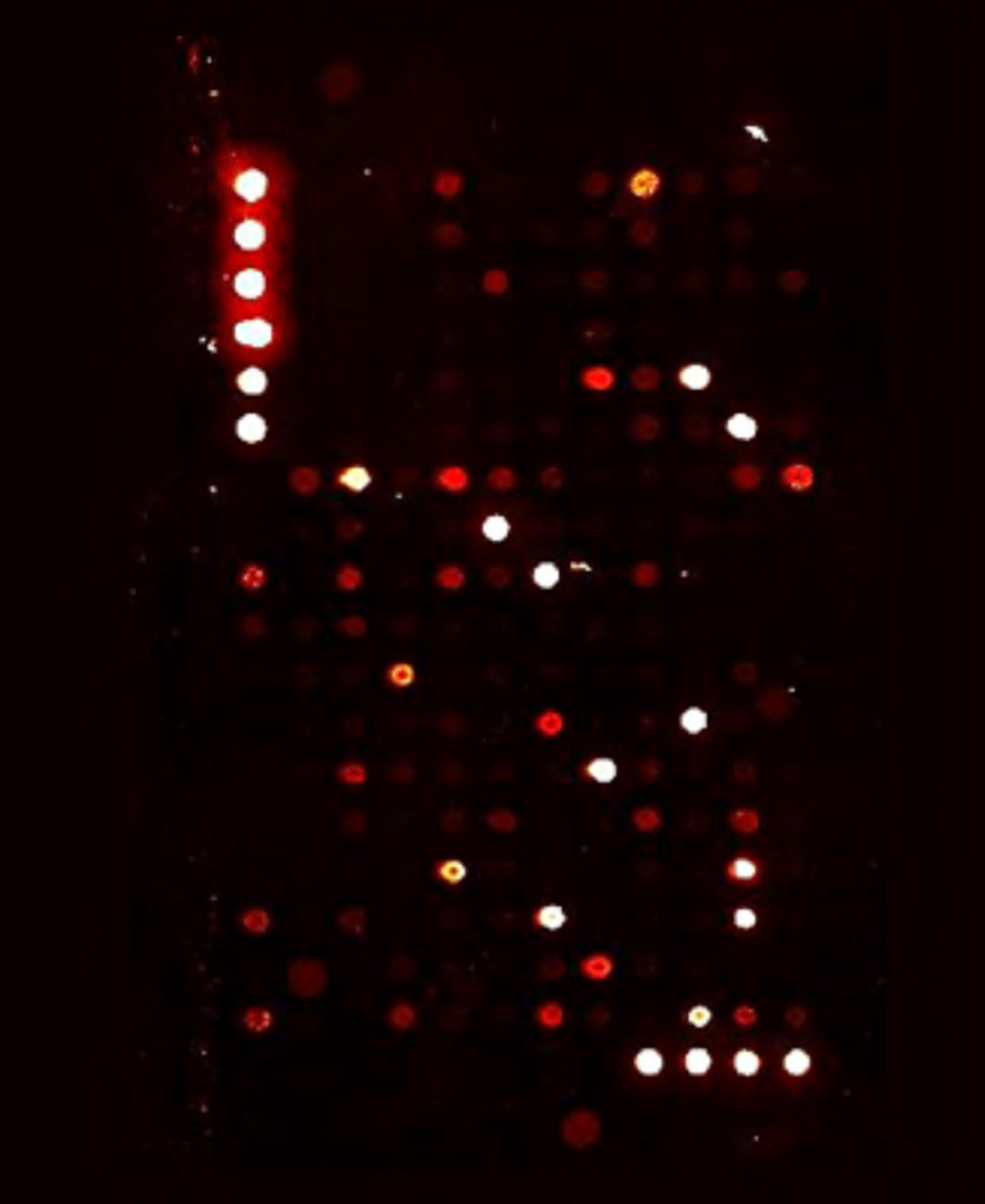# Protein Microarrays

Reverse phase protein array (RPPA)

Antibody arrays consisting of a glass slide on which antibodies to known proteins are bound > exposed to proteins > exposed to a detection system.

Protein Microarrays (PMA)

Full-length cloned proteins printed on glass slides > exposed to proteins, DNA, RNA > exposed to a detection system.

- Entire 5800 proteome of yeast and
- Over 9,000 full length human have been cloned, over expressed and placed on slides.

Various detection techniques exist that produce fluorescent light

- Statistical validation of data extremely important

- So is knowledge of Biology
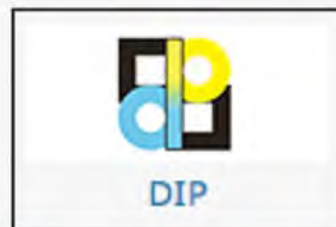
What to do with interaction data?

How to find data?

How to represent, visualize and analyze?

Sources of Protein Data 2
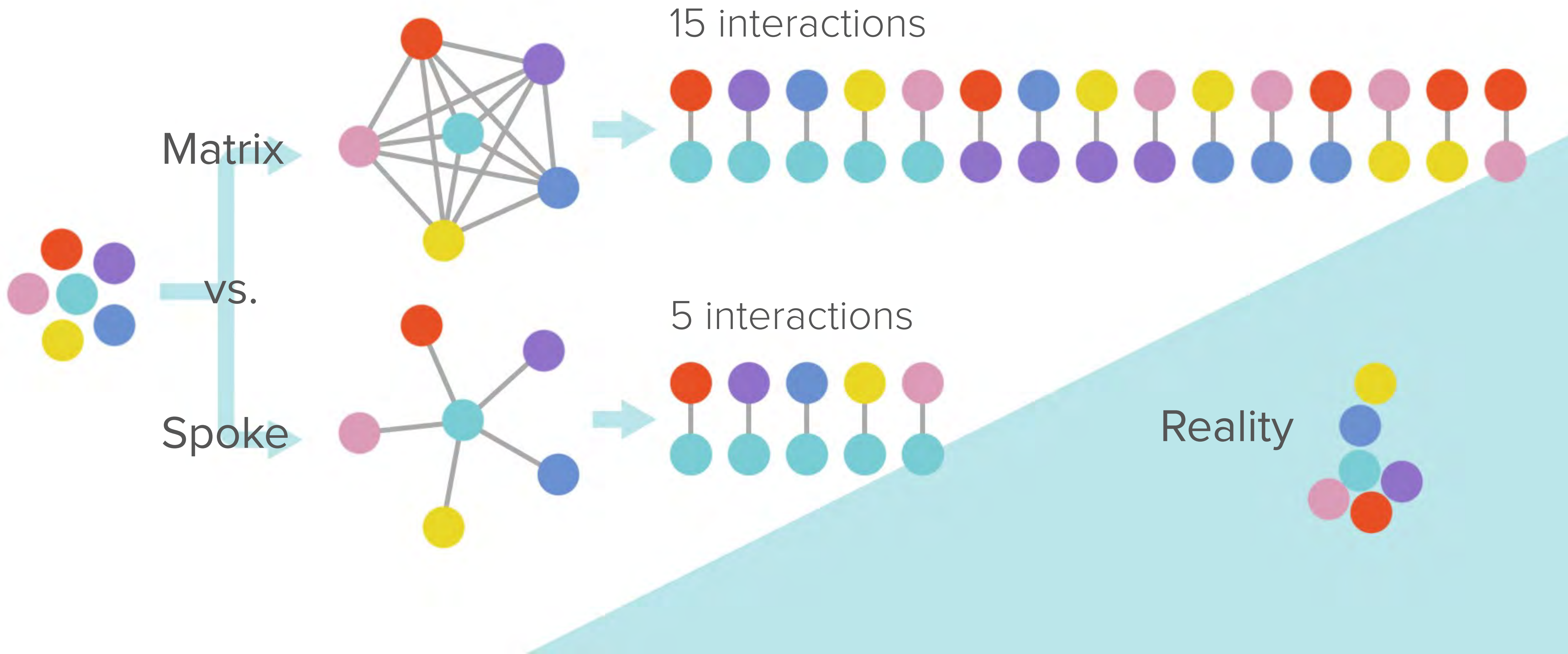
Protein Interaction Data

IMEx Consortium Databases   (www.imexconsortium.org)
IntACT < annotated   (www.ebi.ac.uk/intact/)
BioGrid < annotated   (thebiogrid.org)
String   < computational annotation   (string-db.org)

How to represent interactions?

Matrix vs. Spoke — 15 interactions, 5 interactions, Reality

IntAct Search for PTEN human

Customize view   MI-TAB 2.7   Download

| Dts		Molecule 'A'	Molecule 'B'	Species 'A'	Interaction Detection Method	Publication Identifier	Interaction AC	Source Database
●	🔍	PTEN	SLC9A3R1	Homo sapiens	pull down	**imex :** IM-21392 24012959	EBI-8697411 **imex :** IM-21392-2	MINT
●	🔍				pull down	**imex :** IM-21392 24012959	EBI-8697424 **imex :** IM-21392-3	MINT
●	🔍				two hybrid	16456542 **imex :** IM-11354 MINT-5218301	EBI-8056751 MINT-2832448 **imex :** IM-11354-1	MINT
●	🔍				pull down	16456542 **imex :** IM-11354 MINT-5218301	EBI-8056782 MINT-2832476 **imex :** IM-11354-4	MINT
●	🔍				filter binding	16456542 **imex :** IM-11354 MINT-5218301	EBI-8057097 MINT-2832508 **imex :** IM-11354-12	MINT
●	🔍	PTEN	PDGFRB	Homo sapiens	proximity ligation assay	25241761 **imex :** IM-18707	EBI-6594292 **imex :** IM-18707-418	IntAct
●	🔍				filter binding	16456542 **imex :** IM-11354 MINT-5218301	EBI-8057283 MINT-2832984 **imex :** IM-11354-17	MINT
●	🔍	PTEN	FRK	Homo sapiens	pull down	**imex :** IM-13590 19345329	EBI-2567618 **imex :** IM-13590-11	IntAct
●	🔍				anti bait coimmunoprecipitation	**imex :** IM-13590 19345329	EBI-2567666 **imex :** IM-13590-2	IntAct

SLC9A3R1

PTEN

PIK3CD PIK3CA

PDGFRB AKT1 UBC

PIK3CB

PTK2 TP53

JUN
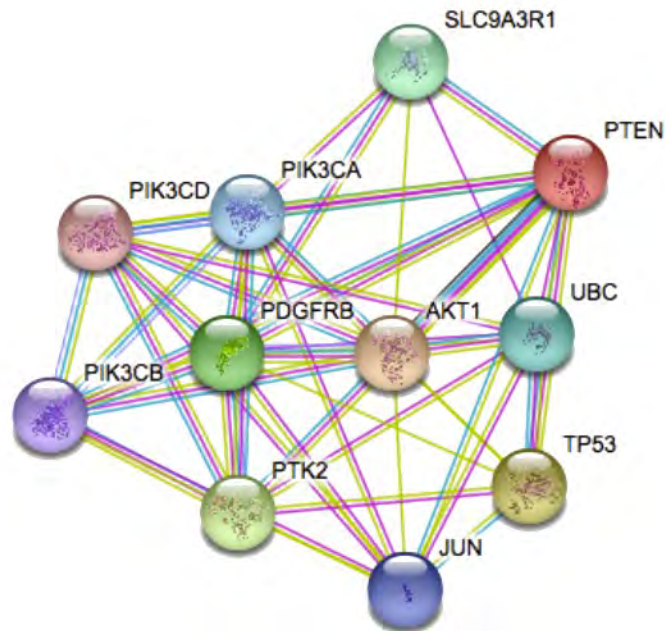
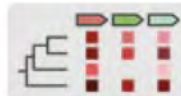ⓘ Legend ›   ⚙ Data Settings ›   ⚙ View Settings ›   ▦ Tables / Exports ›   👁 Evidence ⌄   Σ Analysis ›

Experiments
Co-purification, co-crystallization, Yeast2Hybrid, Genetic Interactions, etc ... as imported from primary sources.

Databases
Known metabolic pathways, protein complexes, signal transduction pathways, etc ... from curated databases.

Textmining
Automated, unsupervised textmining - searching for proteins that are frequently mentioned together.

Coexpression
Proteins whose genes are observed to be correlated in expression, across a large number of experiments.

Cooccurrence
Gene families whose occurrence patterns across genomes show similarities.

Neighborhood
Groups of genes that are frequently observed in each other's genomic neighborhood.

Fusion
Genes that are sometimes fused into single open reading frames.