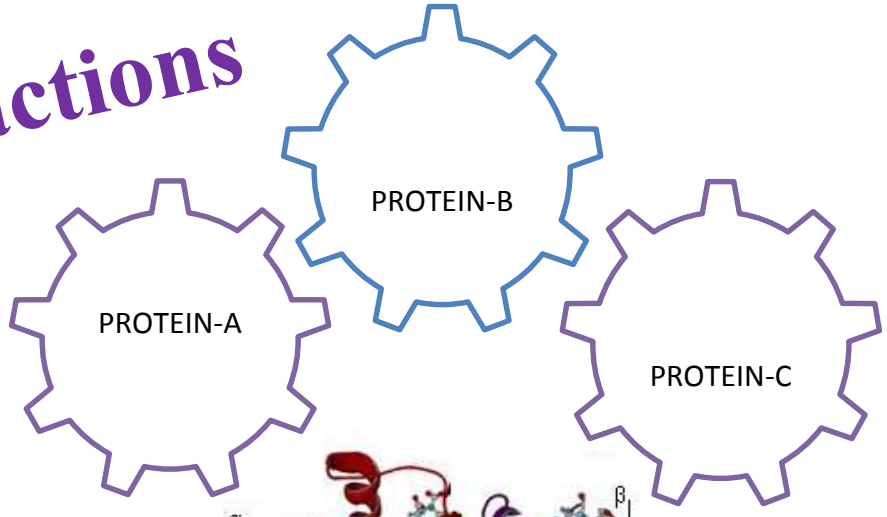
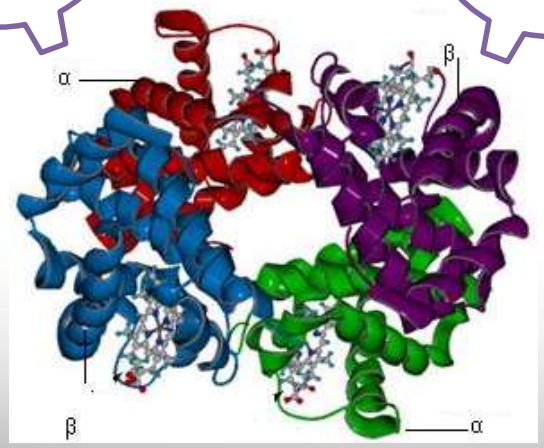
Protein-Protein Interactions
PPIs





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IABT, UAS DHARWAD

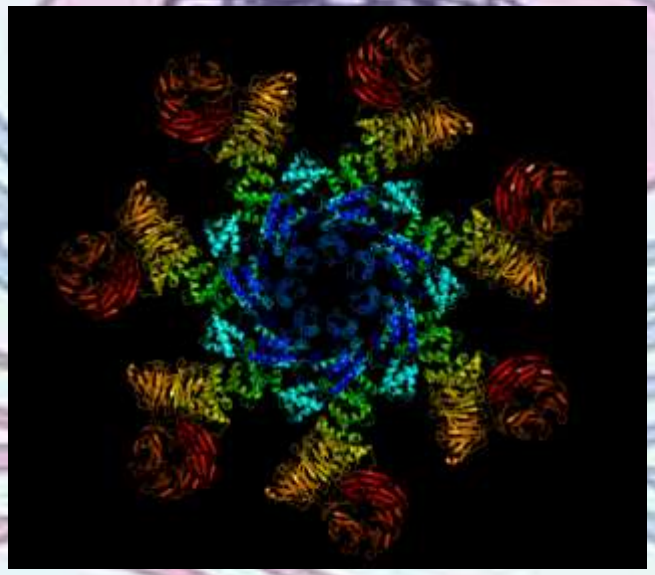
Contents....

INTRODUCTION

motility

apoptosis

nutrient uptake,



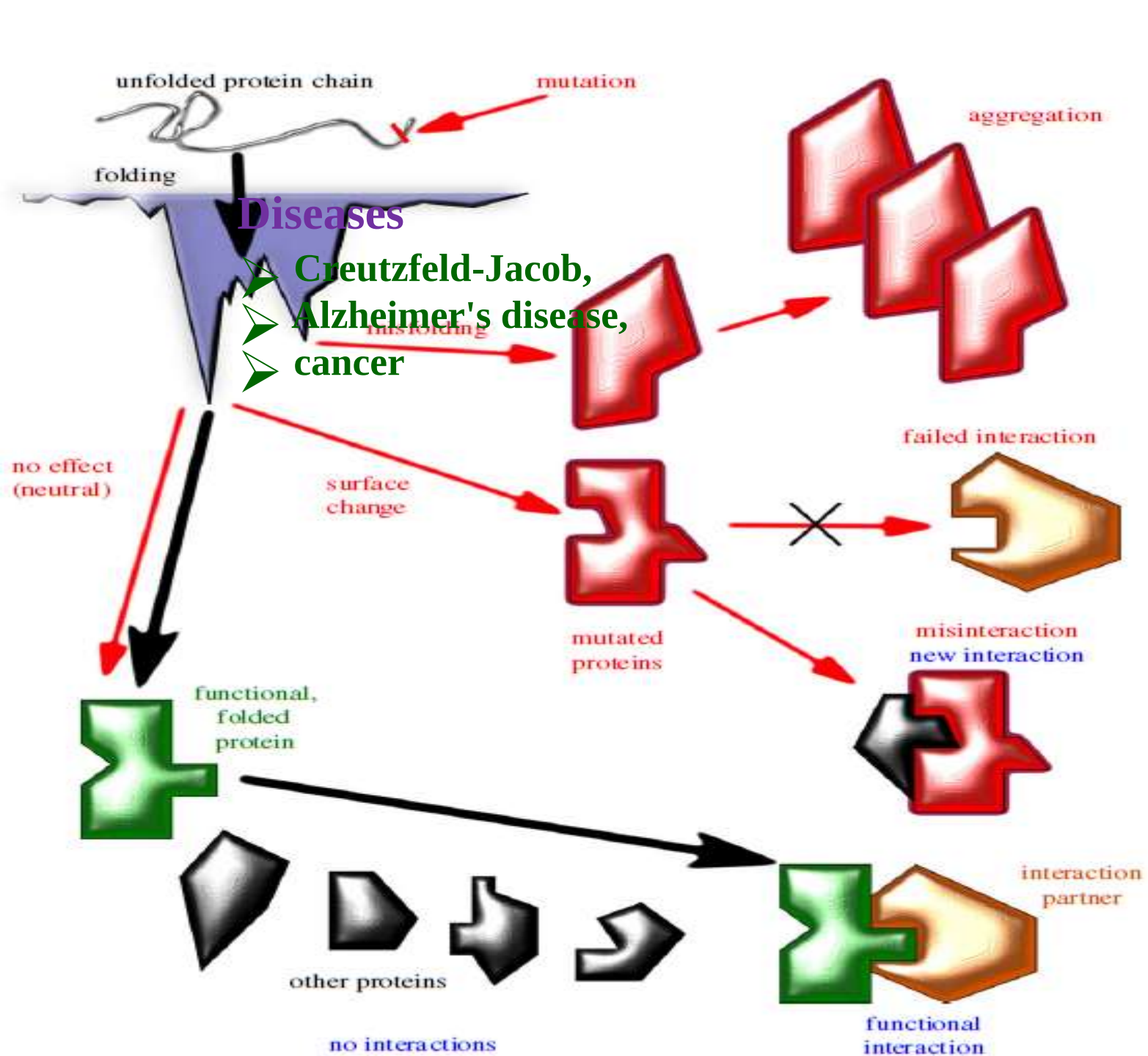
gene expression

intercellular communication

proliferation

cell growth

morphology

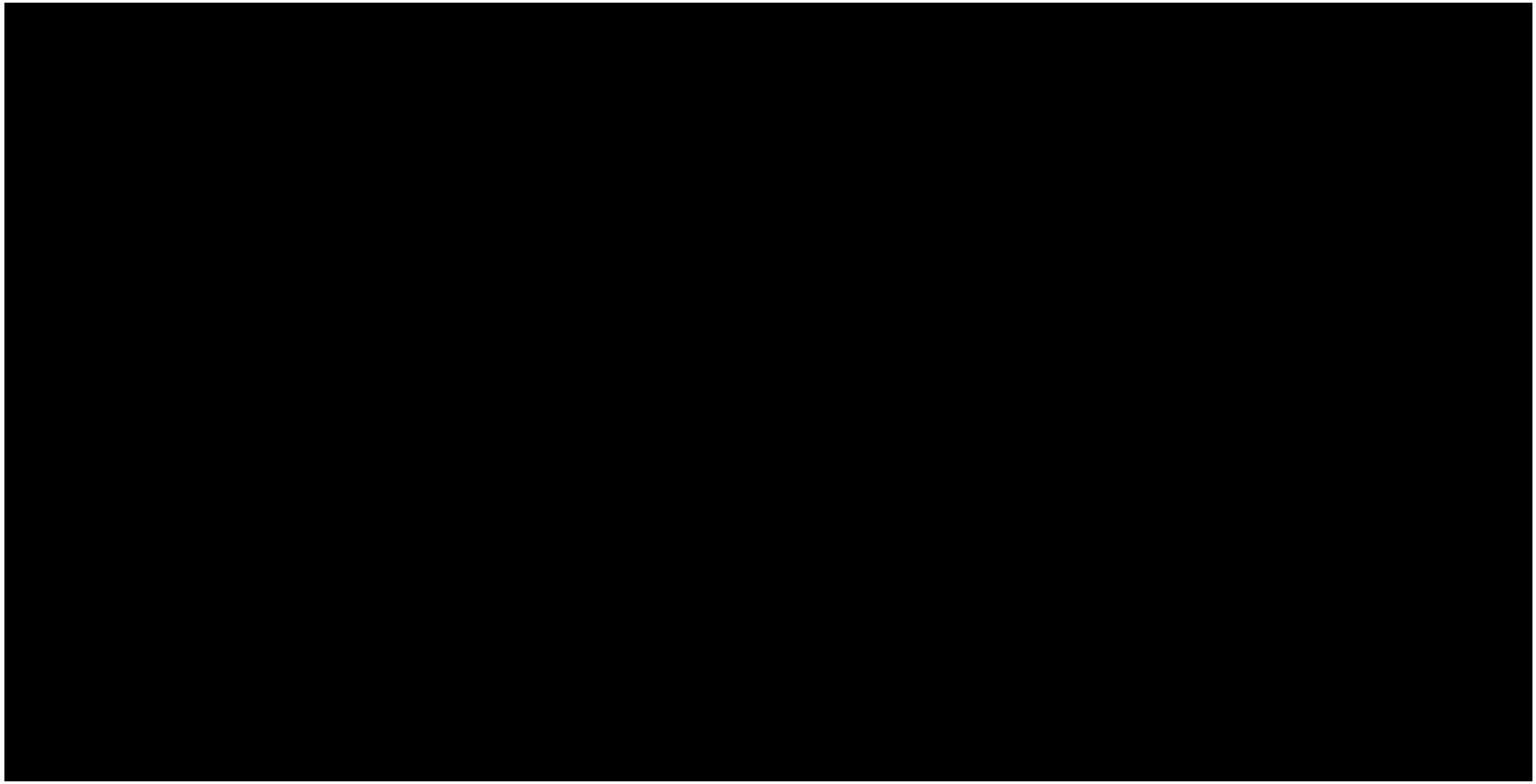


Diseases

- Creutzfeld-Jacob,
- Alzheimer's disease,
- cancer

DEFINITION

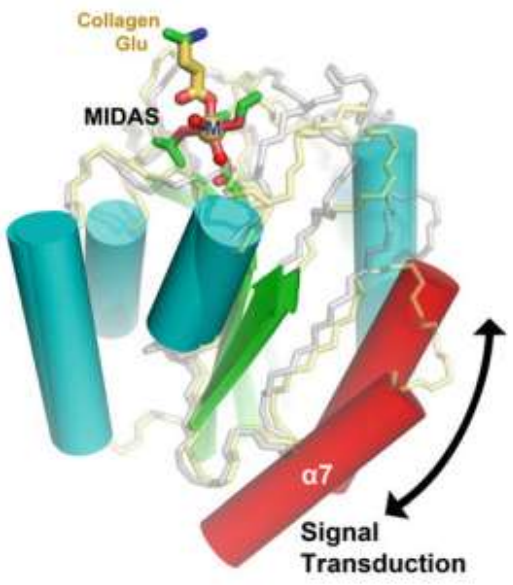
PPIs refer to intentional physical contacts established between two or more proteins as a result of biochemical events and/or electrostatic forces



Examples of protein–protein interactions

Signal transduction

The activity of the cell is regulated by extracellular signals



Transport across membranes

A protein may be carrying another protein.

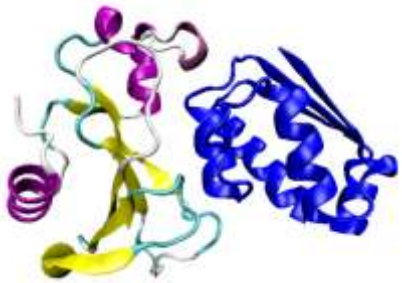
Cell metabolism

In many biosynthetic processes enzymes interact with each other to produce small compounds or other macromolecules.

Muscle contraction

Myosin filaments act as molecular motors and by binding to actin enables filament sliding.

TYPES OF PROTEIN–PROTEIN INTERACTIONS



Homo-oligomers

Hetero-oligomers

Transient

Stable

Covalent :

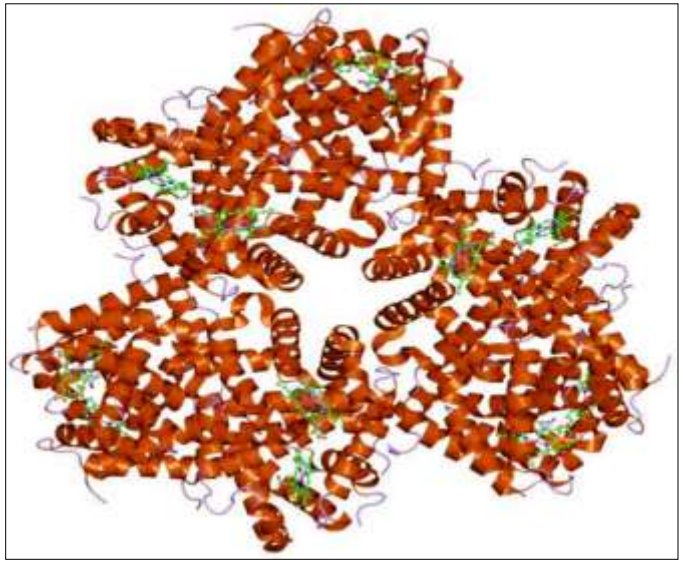
Non-covalent :

ON THE BASIS OF THEIR COMPOSITION

Homo-oligomers

Homo-oligomers are macromolecular complexes constituted by only one type of protein subunit

Homo-oligomers complex



Protein subunits assembly is guided by the establishment of non-covalent Interactions in the quaternary structure of the protein

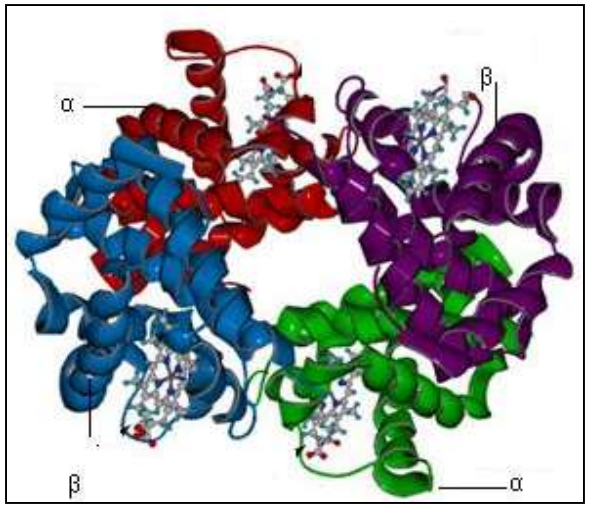
E.g. : PPIs in Muscle Contraction

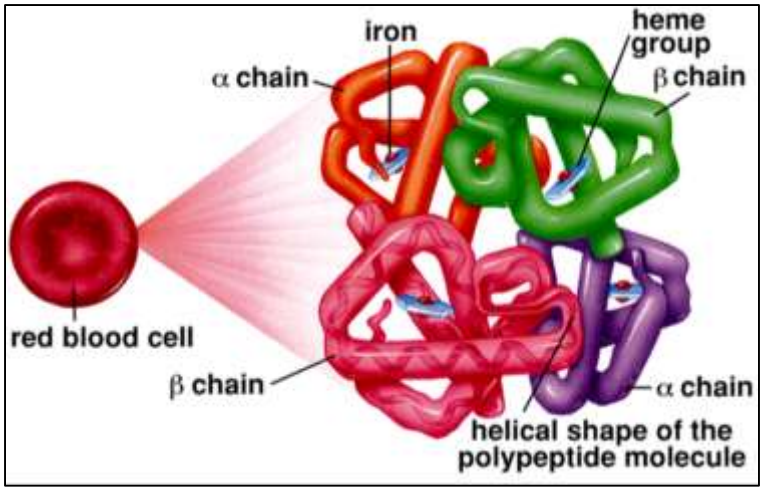
Several enzymes, carrier proteins and transcriptional regulatory factors carry out their functions as homo-oligomers.

Joel Edt and Shoshana Wodak, 2002

Hetero-oligomers

Distinct protein subunits interact in hetero-oligomers, which are essential to control several cellular functions





Hetero-oligomers complex Eg: Hemoglobin Hb or Hgb

Heterologous proteins - cell signaling events

E.g. : PPI between Cytochrome Oxidase and TRPC3 (Transient receptor potential cat ion channels)

2. ON THE BASIS OF THEIR BONDING

Covalent :

Strongest association - disulphide bonds or electron sharing

- Post translational modifications

E.g.: ubiquitination and SUMOylation

Non-covalent :

Established during transient interactions by the combination of weaker bonds

- Hydrogen bonds,
- Ionic interactions,
- Van der waals forces, or
- Hydrophobic bonds

Ubiquitination

- Plays a role in the degradation of defective and superfluous proteins , single-chain polypeptid
- Ubiquitination (or ubiquitylation) is an enzymatic post-translational modification in which a ubiquitin protein is attached to a substrate protein

- **Steps**: activation, conjugation, and ligation,
- By: ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin ligases (E3s)

ON THE BASIS OF THEIR DURATION OF INTERACTION

Transient Interactions :

Interactions that last a short period of time

reversible manner

E.g.: G protein-coupled receptors only transiently bind to $G_{i/o}$ proteins when they are activated by extracellular ligands

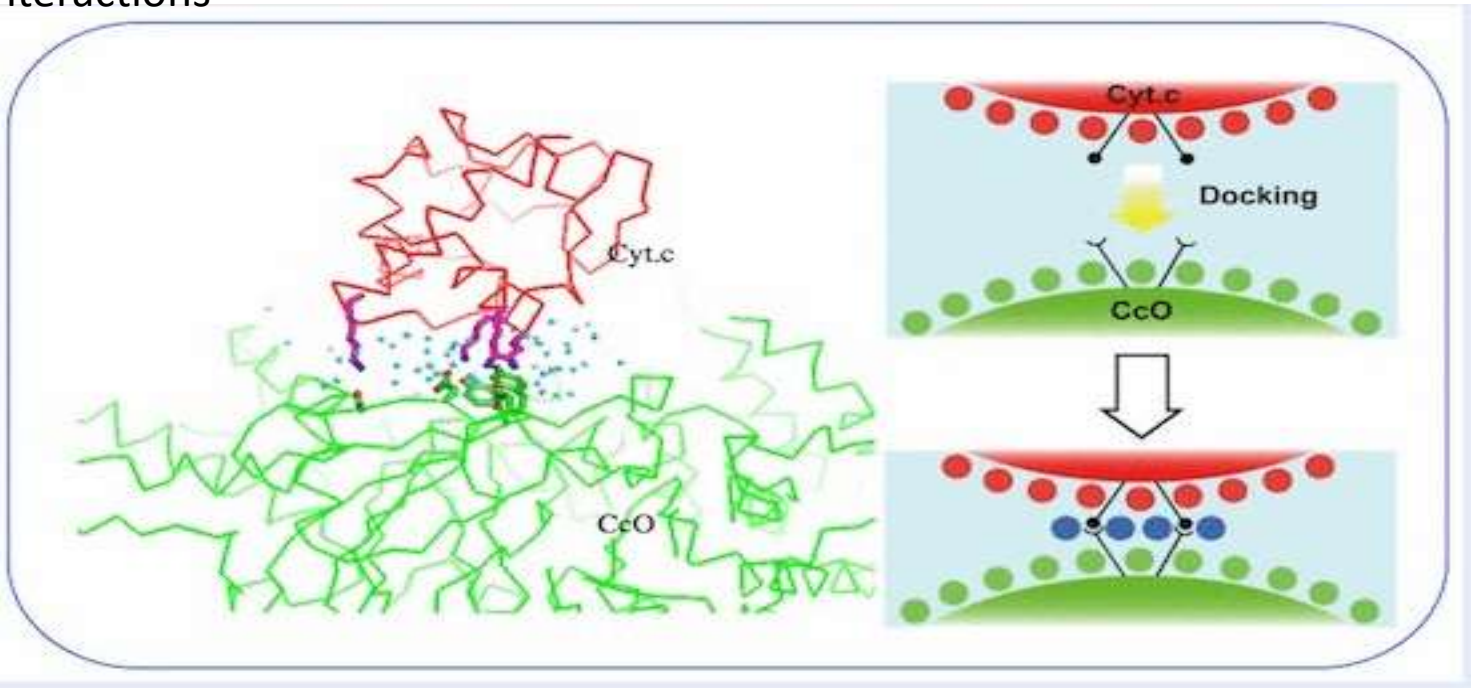
Stable Interactions:

Proteins - interact for a long time, taking part of permanent complexes as subunits

-carry out Functional or Structural roles

e.g. Cytochrome c

Eg: Stable Interactions



cytochrome *c* – **C*c*O complex

stabilized by a few electrostatic interactions between long side chains within a small contact surface.

In contrast to other Cyt.*c* complexes, numerous water molecules are found in the long inter-molecular span between Cyt.*c* and C*c*O..

**Cytochrome *c* oxidase

Table 1. Data sets of protein–protein complexes

PDB code	Protein	Resolution Å
	*Nonhomologous homodimers**	
1cdt	Cardiotoxin	2.5
1fc1	Fc fragment (immunoglobulin)	2.9
1il8	Interleukin	NMR
1msb	Mannose binding protein	2.3
1phh	*p*-Hydroxybenzoate hydrolase	2.3
1pp2	Phospholipase	2.5
1pyp	Inorganic pyrophosphatase	3.0
1sdh	Hemoglobin (clam)	2.4
1utg	Uteroglobin	1.35
1vsg	Variant surface glycoprotein	2.9
1ypi	Triose phosphate isomerase	1.9
2ccy	Cytochrome *c*3	1.67
2cts	Citrate synthase *c*	2.0
2gn5	Gene 5 DNA-binding protein	2.3
2or1	434 repressor	2.5
2rhe	Bence–Jones protein	1.6
2rus	Rubisco	2.3
2rve	*Eco*RV endonuclease	3.0
2sod	Superoxide dismutase	2.0
2ssi	Subtilisin inhibitor	2.6
2ts1	Tyrosyl transferase RNA synthase	2.3
2tsc	Thymidylate synthase	1.97
2wrp	Trp repressor	1.65
3aat	Aspartate aminotransferase	2.8
3enl	Enolase	2.25
3gap	Catabolite gene activator protein	2.5
3grs	Glutathione reductase	1.54
3ied	Isocitrate dehydrogenase	2.5
3sdp	Iron superoxidase	2.1
4mdh	Cytoplasmic malate dehydrogenase	2.5
5adh	Alcohol dehydrogenase	2.9
5hvp	HIV protease	2.0
	Enzyme–inhibitor complexes†	
1ach	α-Chymotrypsin–eglin C	2.0
1cho	α-Chymotrypsin–ovomucoid third domain	1.8
1cse	Subtilisin Carlsberg–eglin C	1.2
1mct	Trypsin–inhibitor from bitter gourd	1.6
1mcc	Peptidyl peptide hydrolase–Eglin C	2.0
1stf	Papain–inhibitor stefin B mutant	2.37
1tab	Trypsin–Bowman–Birk inhibitor	2.3
1tgs	Trypsinogen–Pancreatic secretory trypsin inhibitor	1.8
2ptc	β-Trypsin–pancreatic trypsin inhibitor	1.9
2sic	Subtilisin–streptomyces subtilisin inhibitor	1.8
	Antibody–antigen complexes‡	
1fdl	D1.3 Fab–hen egg white lysozyme	2.5
1jel	Fab JE142–histidine containing protein	2.8
1jhl	D11.15 Fv–pheasant egg lysozyme	2.4
1nca	NC41 Fab/influenza virus N9 neuraminidase	2.5
2hfl	HYHEL-5 Fab–chicken-lysozyme	2.54
3hfm	HYHEL-10 Fab–chicken lysozyme	3.0
	Other heterodimeric complexes§	
1atn	Deoyribonuclease I–actin	2.8
1gln	Glycerol kinase–glucose-specific factor III	2.6
1hrp¶	Human chorionic gonadotropin	3.0
1lpa	Lipase–colipase	3.04
1lya¶	Cathepsin D	2.5
2btf	β-Actin–profilin	2.55
2pch	Yeast cytochrome *c* peroxidase–horse cytochrome *c*	2.8
3hhr‖	Human growth hormone–human growth hormone receptor	2.8
3hvt¶	Reverse transcriptase	2.9
6rlx¶,**	Relaxin	1.5

Protein Domains

- Interactions only possible due to structural domains within the proteins
- A **protein domain** is a conserved part of a given protein sequence and (tertiary) structure that can evolve, function, and exist independently of the rest of the **protein** chain
- Proteins hold structural domains that allow their interaction with and bind to specific sequences on other proteins

1. phosphotyrosine-containing motifs,

- Examples for protein who carry this motif: activated receptors for growth factors, cytokines and antigens.

- Recognizing protein protein interaction domain:

  a. SH2 domains

  b. PTB domains, also binds unphosphorylated peptides

2. phosphoserine/threonine motifs,

- Recognizing protein protein interaction domain:

  a. 14-3-3 proteins

  b. FHA domains

  c. WW domains, also binds unphosphorylated peptides,

     Proline-rich

  d. WD40-repeat domains

3. acetylation of lysine residues

- Proteins who carry the motif: histones
- Recognizing proteins: creates binding sites for the Bromo domain

4. methylation of lysine residues

- Proteins who carry the motif: histones
- Recognizing proteins: creates binding sites for the Chromo domains,

Other protein-protein interaction domains

Apoptosis

- DD - death domain
- DED - Death Effector Domain
- CARD- caspase recruitment domain
- BH1-4 - Bcl-2 Homology

Chromatin

- CSD - Cold-shock domain

Proteolysis

- F-box
- Hect - homologous to the E6AP carboxyl terminus
- RING - really interesting new gene

Dimerization

- SAM - Sterile α Motif

Vessicle Traffic

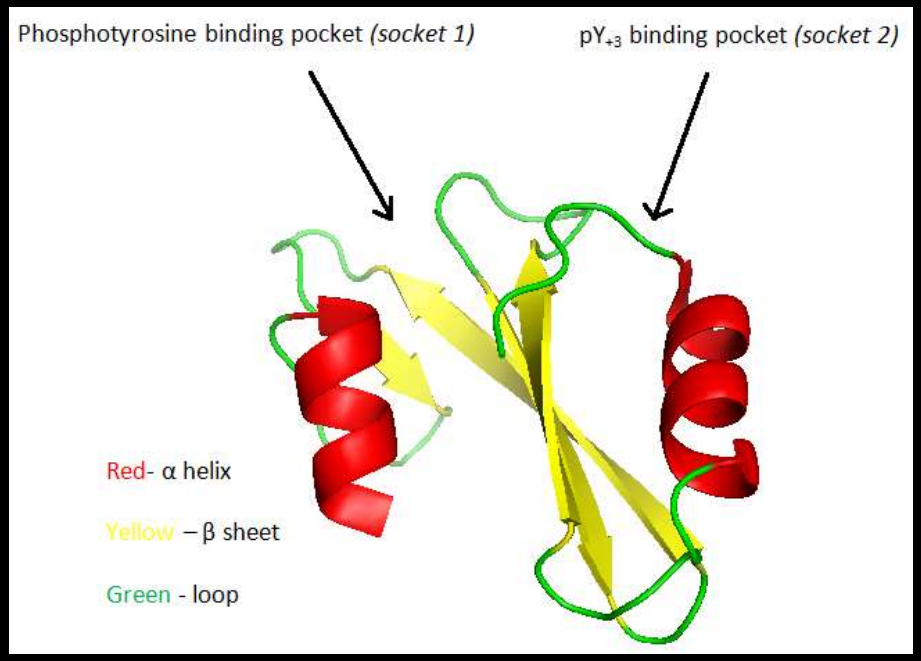
- GYF
- Snare
- VHS

Undefined

- ANK
- ARM
- WD40
- LIM

Src homology 2 (SH2) domain

- Role - cellular communication
- Structure - contains 2 alpha helices and 7 beta strands
- It has a high affinity to phosphorylated tyrosine residues
- It is known to identify a sequence of 3-6 amino acids within a peptide motif
- Represent the largest class of known pTyr-recognition domains.



PPIs Identification Methods

Experimental *(In vivo)*	• **Yeast two-hybrid system** • split ubiquitin system • split lactamase / split galactosidase system • split yellow fluorescent protein (YFP) system
Experimental *(In vitro)*	• **Co-immunoprecipitation** • Tagged Fusion Proteins • X-ray Diffraction • Biacore • Phage display
Computational *(In silico)*	• BIND • DIP • MINT • IntAct

Methods to Investigate PPIs

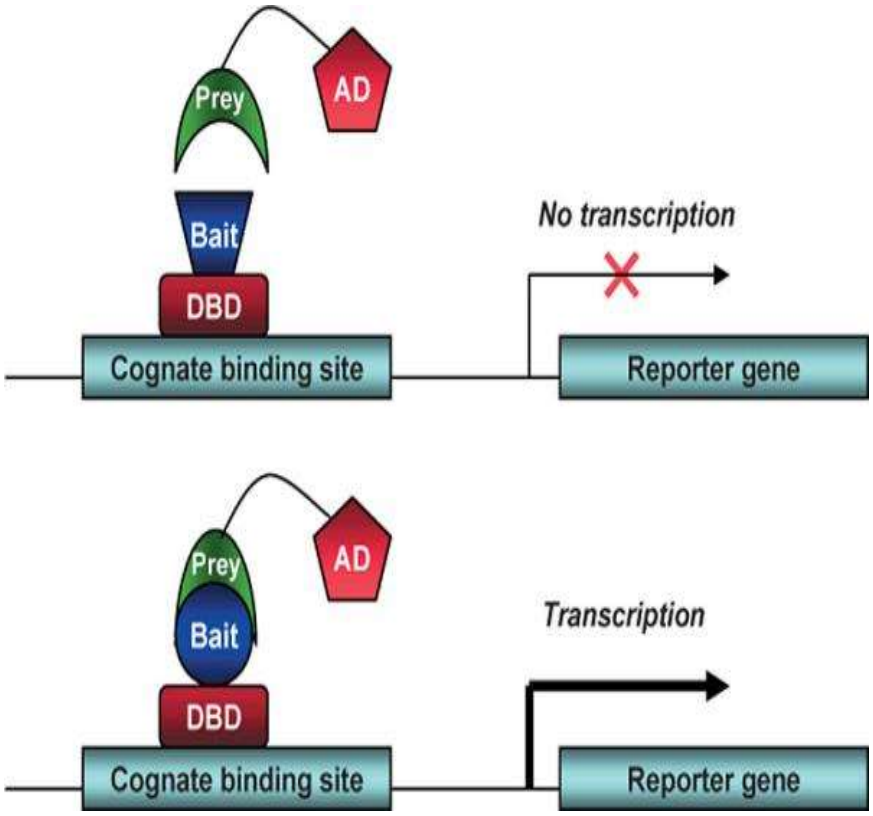
- Immuno-precipitation,
- Protein microarrays,
- Analytical ultracentrifugation,
- Light scattering,
- Fluorescence spectroscopy,
- Resonance-energy transfer systems,
- Surface Plasmon resonance, protein-fragment complementation assay, and Calorimetry etc…
- The two most prominent methods used for investigating PPIs are: Yeast two-hybrid screening and Affinity purification coupled to mass spectrometry

Xue-Wen Chen andMei Liu

Yeast two-hybrid

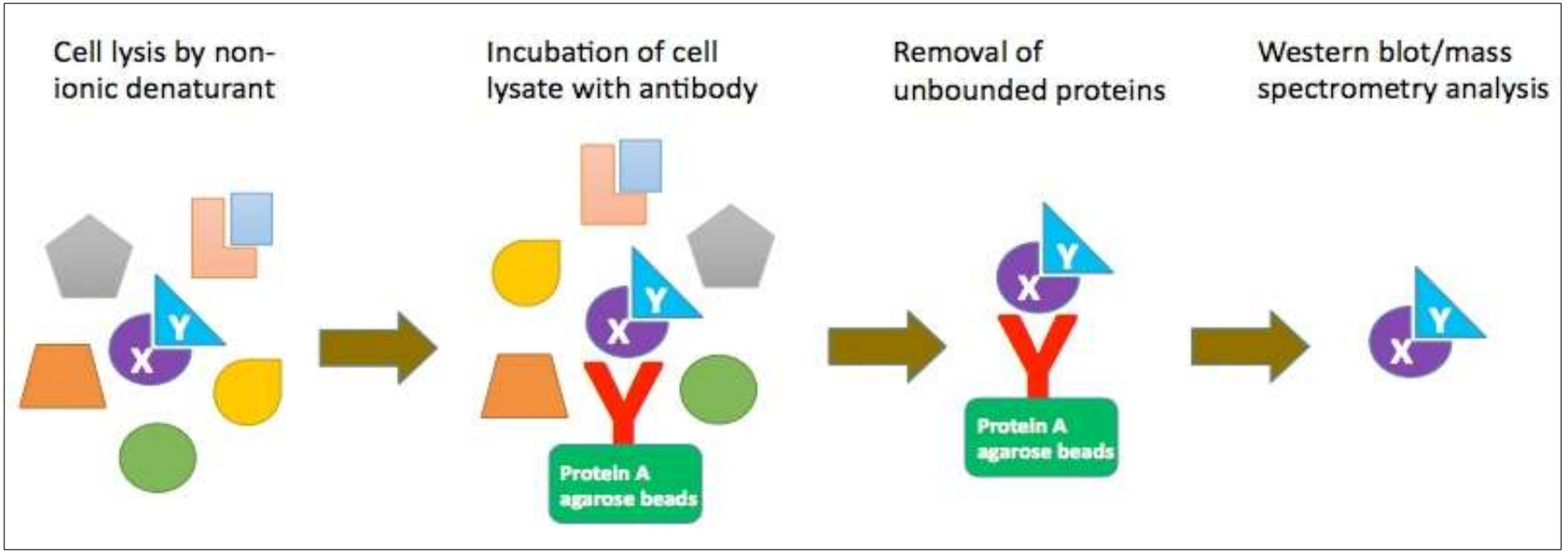
- Testing for physical interactions between two proteins
- first proven using ***Saccharomyces cerevisiae*** as biological model by Fields and Song

- Bait – The protein fused to the DBD is referred to as the 'bait' (yeast transcription factor, like Gal4)
- Prey- The protein fused to the AD
- Reporter gene: LacZ reporter - Blue/White Screening



Co-immunopercipitation

- Co-IP is a classic technology widely used for protein-protein interaction identification and validation
- New binding partners, binding affinities, the kinetics of binding and the function of the target protein



Principle of co-Immunoprecipitation

The advantage of this technology includes:

- Both the bait and prey proteins are in their **native conformation in the co-IP assay**
- The interaction between the bait and prey proteins happens in vivo with little to no external influence

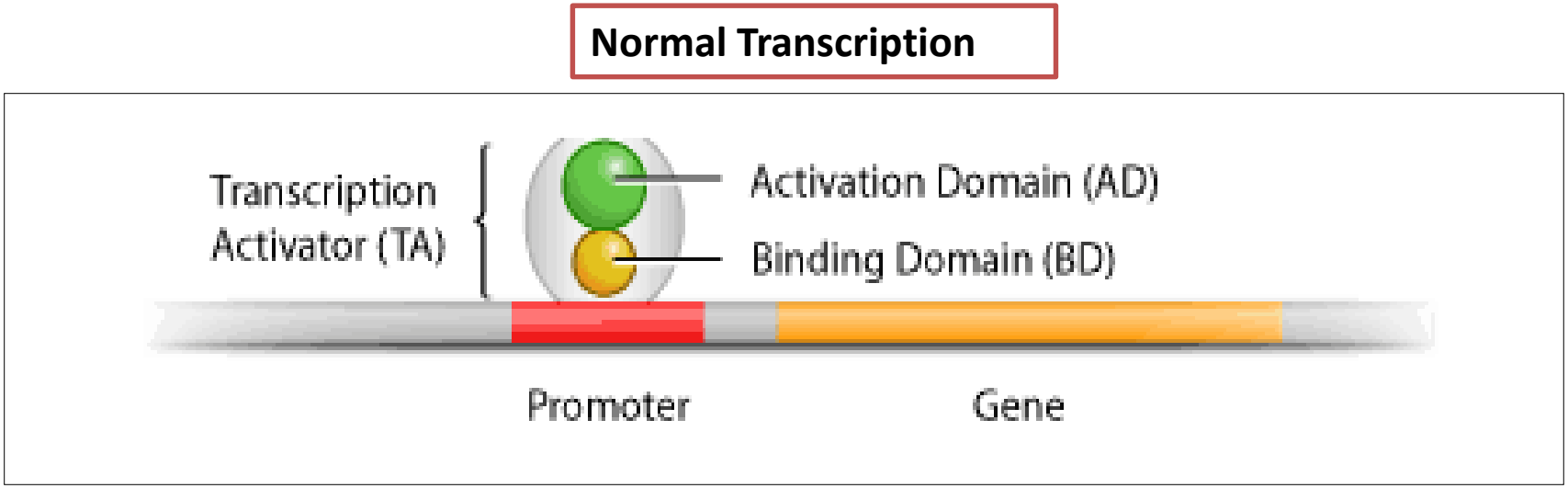
The limitation of this technology lies in

- Low affinity or transient interaction between proteins may not be detected.

Yeast two-hybrid

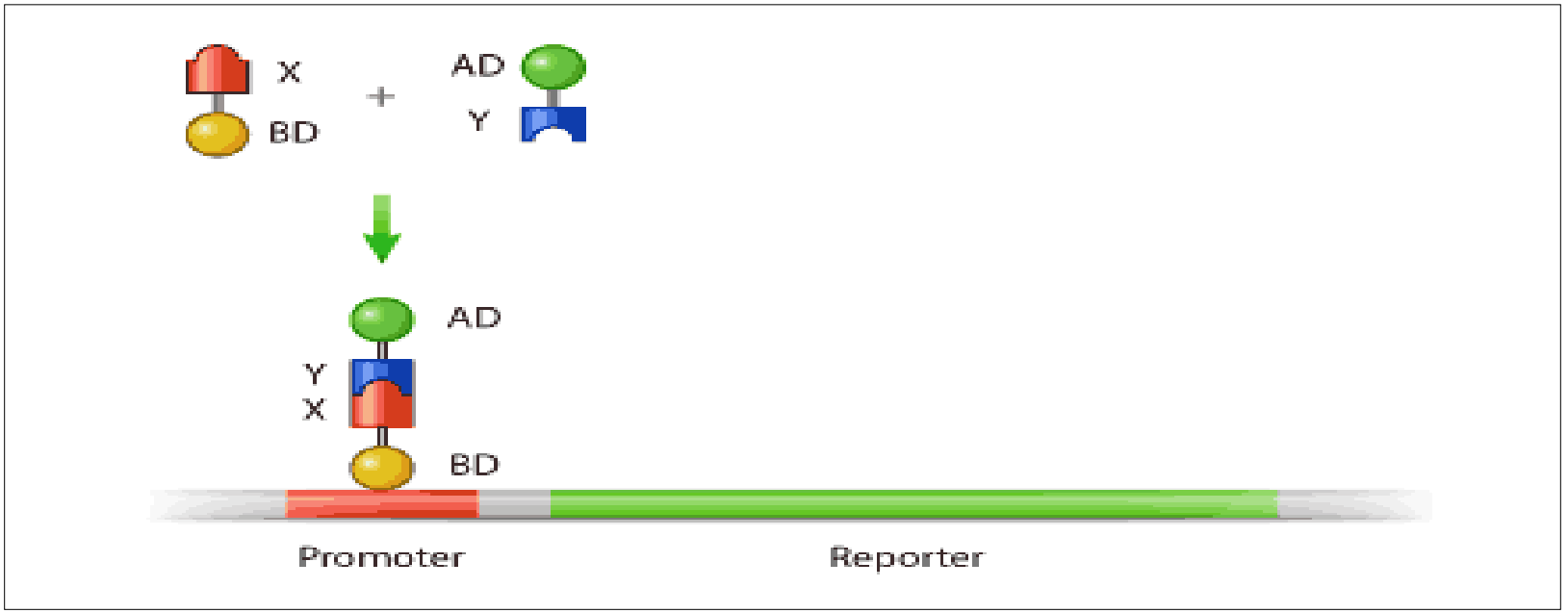
➢ ***Saccharomyces cerevisiae*** as biological model by Fields and Song

➢ One technique that can be used to study protein-protein interactions is the "yeast two hybrid" system



transcription requires both the DNA-binding domain (BD) and the activation domain (AD) of a transcriptional activator (TA)

Basic principle

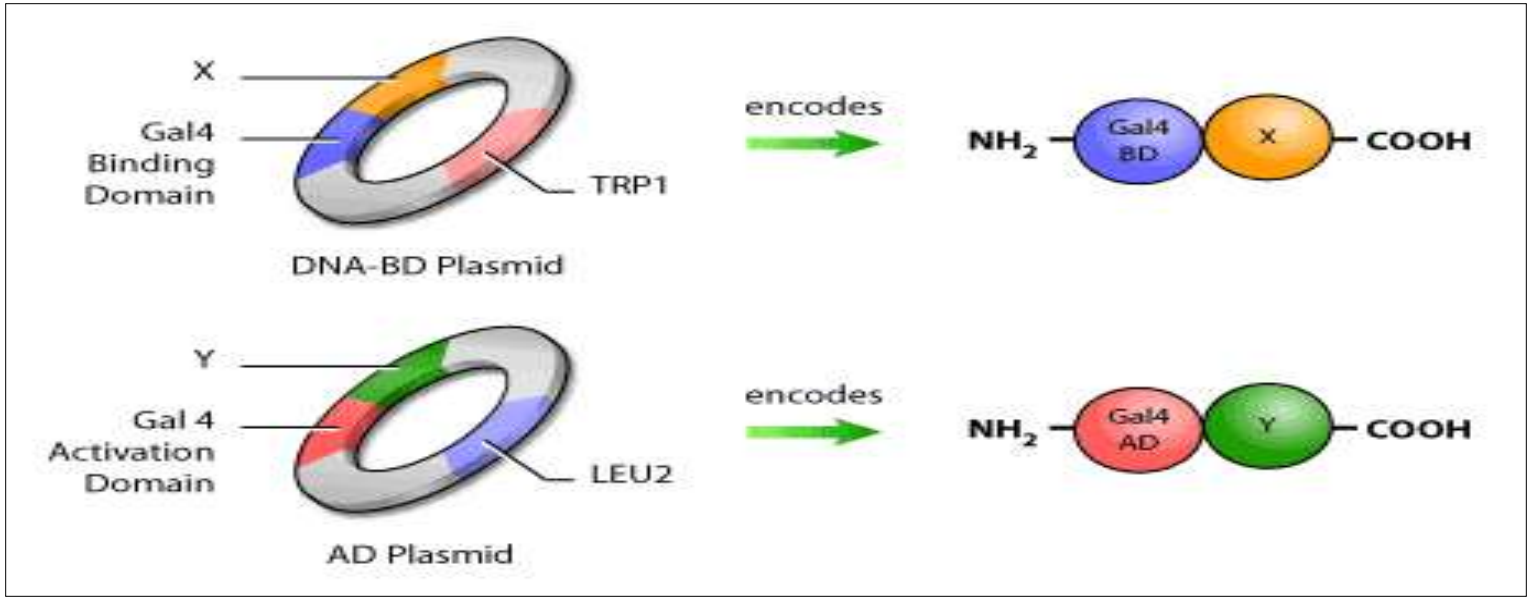


If protein X and protein Y interact, then their DNA-binding domain and activation domain will combine to form a functional transcriptional activator (TA). The TA will then proceed to transcribe the reporter gene that is paired with its promoter

The yeast two-hybrid assay uses two plasmid constructs

Bait plasmid,

Hunter plasmid

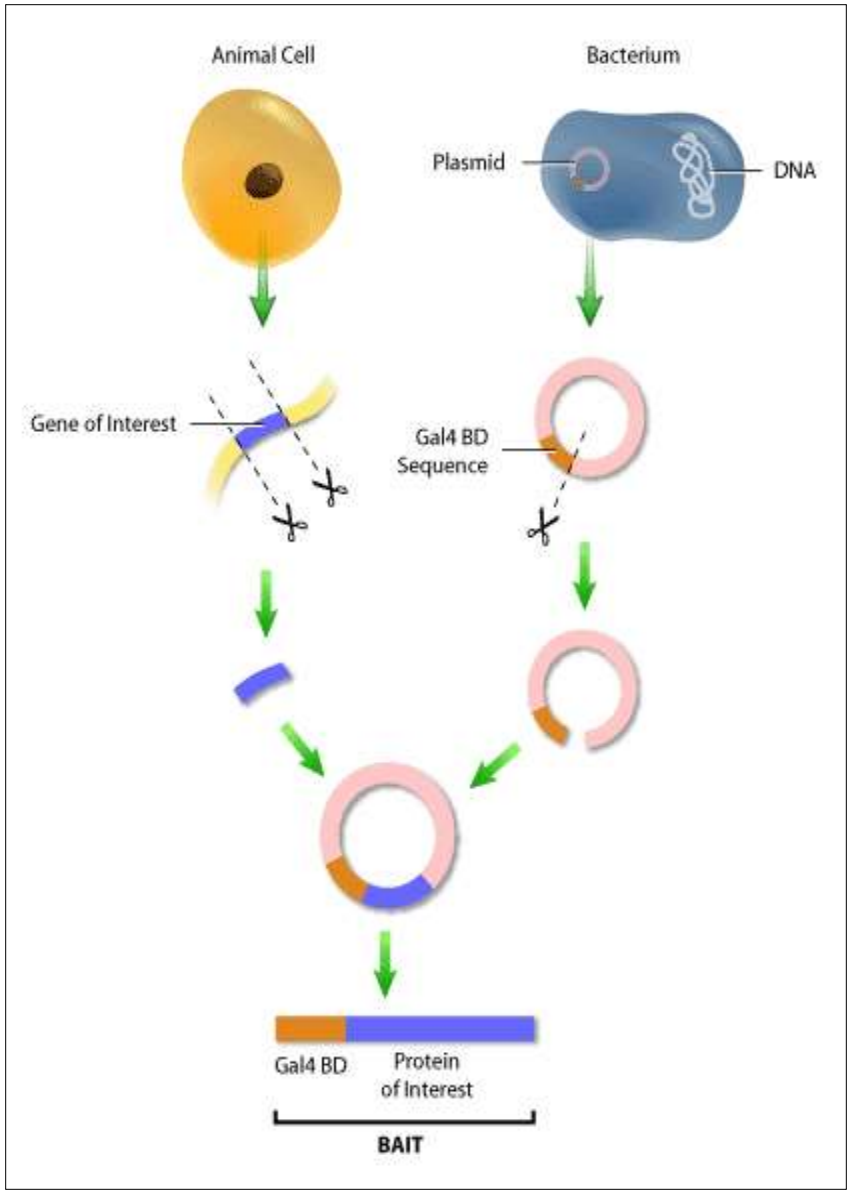


- The bait plasmid, which is **the protein of interest fused to a GAL4 binding** domain, and the hunter plasmid, which is the potential binding partner fused to a GAL4 activation domain

- Selection genes encoding for amino acids, such as histidine, leucine and tryptophan

Solmaz Sobhanifar (2005)

Plasmid construction



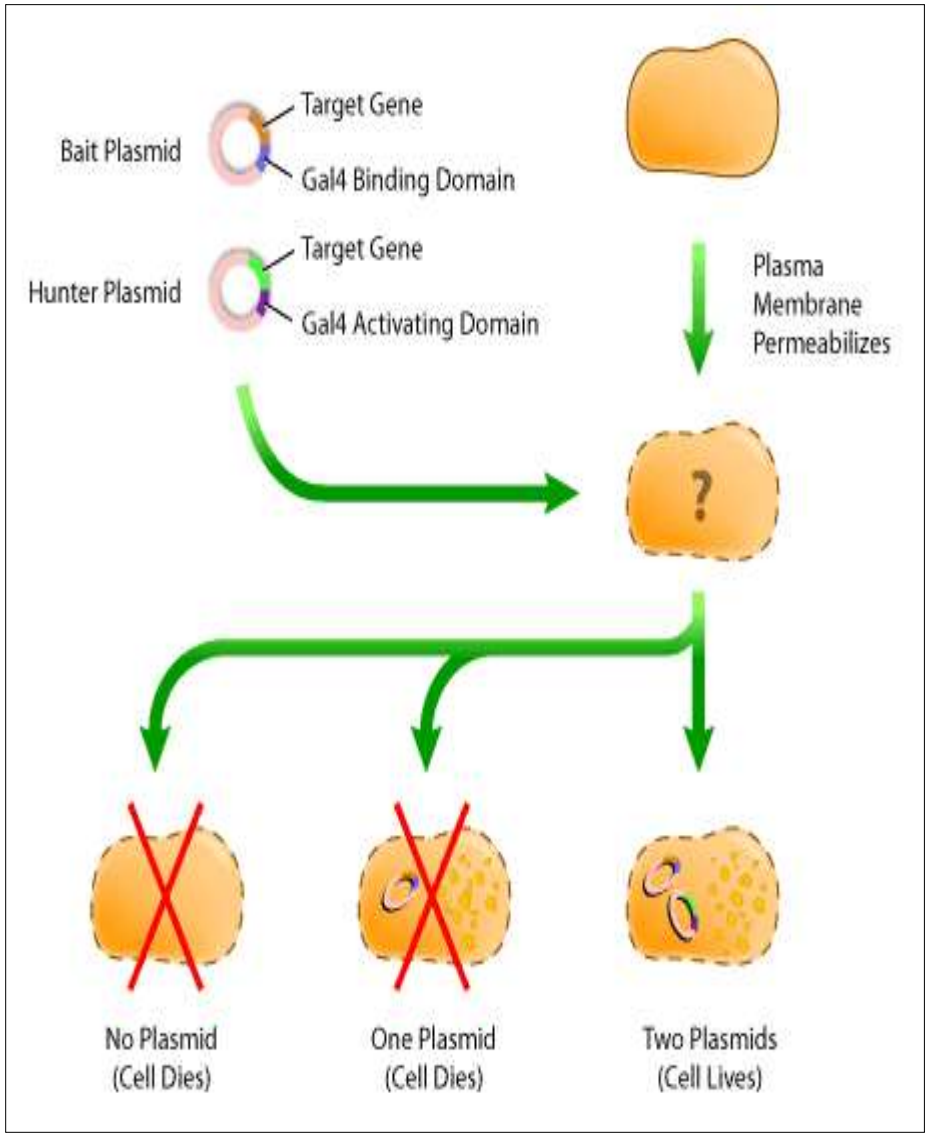
- The 'bait' DNA is isolated and inserted into a plasmid adjacent to the GAL4 BD DNA.

- When this DNA is transcribed, the 'bait' protein will now contain the GAL4 DNA-binding domain as well. The ‘Prey‘/ Hunter fusion protein contains the GAL4 AD

Solmaz Sobhanifar (2005)

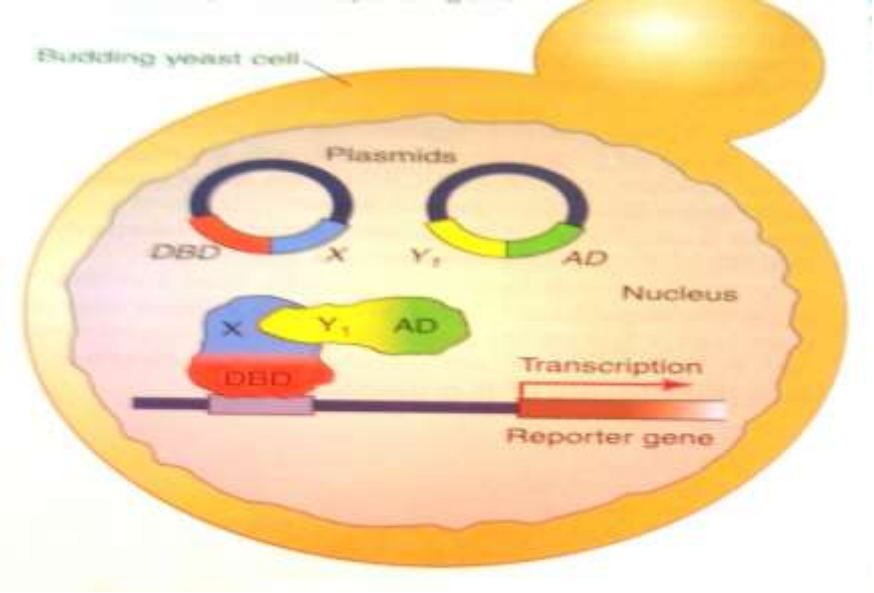
Transfection :

The 'bait' and 'hunter' plasmids are introduced into yeast cells by transfection.

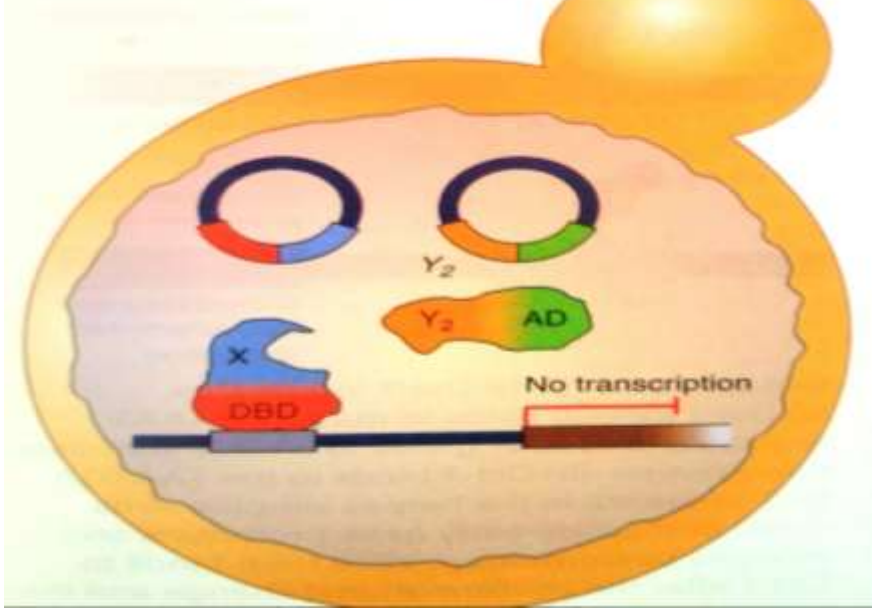


cells containing both plasmids are selected for by **growing cells on minimal media**. Only cells containing both plasmids have both genes encoding for missing nutrients, and consequently, are the only cells that will survive.

Solmaz Sobhanifar (2005)



Transcription of reporter gene



No transcription of reporter gene

- The reporter gene most commonly used in the Gal4 system is LacZ, an E. coli gene whose transcription causes cells to turn blue4

- LacZ gene is inserted in the yeast DNA immediately after the Gal4 promoter

Solmaz Sobhanifar (2005)

Applications

- Identify novel protein-protein interactions
- Characterize interactions already known to occur
  - protein domains
  - Conditions of interactions
- manipulating protein-protein interactions in an attempt to understand its biological relevance
- To know how mutation affects a protein's interaction with other proteins

Genome-wide protein-protein interaction networks in different organisms

Species	Genome wide methods	Interactions	Reference
Fly (*Drosophila melanogaster*)	Screening of 10,623 yeast-two hybrid (Y2H) baits	20,405 interactions (with 7,048 proteins)	Giot et al. 2003
Worm (*Caenorhabditis elegans*)	Screening of 1,873 Y2H baits	4,000 interactions	Li et al. 2004
Human (*Homo sapiens*)	Yeast mating of 8,100 ORF (7,200 unique genes)	2,800 interactions	Rual et al. 2005
Yeast (*Saccharomyces cerevisiae*)	Affinity purification of 4,562-tagged proteins	7,123 interactions (with 2,708 proteins)	Krogan et al. 2006
Plant (*Arabidopsis thaliana*)	Y2H screening of 8,000 ORF (9,000 predicted protein coding genes)	6,200 interactions (with 2,700 proteins)	*Arabidopsis* Interactome Mapping Consortium 2011

Bang et al 2011

List of rice genes used as baits for YTH screening

Gene class and predicted products[a]	Number of genes
Genes involved in plant defense responses/disease resistance Resistance genes (Pi-a, Pto, Mlo, NBS-LRR) Genes involved in defense signal transduction pathways (NPR1, NDR1, LSD1, LLS1, COI1-like, Pti1, MAP kinases, NOS, NOS inhibitors, Pti4/5/6) Genes involved in defense responses (PR proteins, oxidases, peroxidase, GSTs, glucanase, chitinases, lipoxygenases, PAL, proteinase inhibitors, 14-3-3 proteins)	58
Genes involved in other signal transduction pathways Auxin (Nitrilases, IAA-AA hydrolases, IAA) Ethylene (Ein3-like, ERF1) Brassinosteroids (BRI1) Light regulation (CRY1, COP9) General signal transduction (G proteins, calmodulins, casein kinases, phophatases, phospholipase, adenyl cyclase) DNA binding proteins [bZIP proteins (TGAs and GBFs), Myb proteins, HMG protein, MADS-box proteins, WD-40 repeat protein, homeodomain proteins (Knox class)	52

Fang et al., 2002

List of interacting proteins found for eight bait proteins

Baits and interacting proteins[a]	Number of hits
Pti1 (serine/threonine kinase)	
Protein kinase homolog	5
Receptor-like protein kinase homolog	2
Putative homeodomain transcription factor	3
Auxin-induced basic helix-loop-helix transcription factor	1
Abscisic acid and salt stress-responsive protein	1
Late embryogenesis Lea protein	1
Voltage-dependent anion channel protein 2	2
H(+)-transporting ATPase-like protein	1
Putative lipase homolog	1
Lipid transfer protein	3
Indole-3-acetate beta-glucosyltransferase homolog	1
Subtilisin-like proteinase	1
Methanobacterium thermoautotrophicum transcriptional regulator	4
Unknown proteins	29
Pti4/6 (rls6.pk0076.e6) EREBP proteins	
Jun activation domain binding or Jab1 protein	13
D. melanogaster sno homolog	1
Unknown proteins	19
Pti5 (rlr24.pk0042.d3) EREBP protein	
CONSTANS protein	2
Glutathione S-transferase (auxin-induced)	18
Inorganic phosphate transporter 1	3
Neoxanthin cleavage enzyme	3
Lipid transfer protein precursor	1
PCF1	1
Uroporphyrinogen decarboxylase	1
Unknown proteins	91
Calmodulin (rls24.pk0093.f4)	
Jab1 protein	7
10-kDa chaperonin	6
3-hydroxyisobutyryl-coenzyme A hydrolase-like protein	1
rNPR1-1 (rr1.pk0001.a11)	
bZIP DNA-binding protein	4
Acyl carrier protein precursor	7
Proteasome proteins	8
rNPR1-2 (rl0n.pk0063.d10)	
bZIP DNA-binding protein	4
Putative serine/threonine-specific receptor protein kinase	1
Pathogenesis-related protein 1	1
Dehydration-induced protein ERD15	1
Abscisic acid and salt stress-responsive protein (osr40g3)	1
Senescence-associated protein sen1	1

Fang et al., 2002

Protein interactions database

- Protein interactions are collected together in specialized biological databases
- Databases can be subdivided into **primary databases**, **meta-databases, and prediction databases**
- **Primary databases** - published PPIs proven to exist via small-scale large-scale experimental methods. Eg: DIP, Biomolecular Interaction Network, BIND, BioGRID), HPRD
- **Meta-database –** Primary and original data Eg: APID, The Microbial, MPIDB, and PINA , and GPS-Prot etc.
- **Prediction Databases –** predicted using several techniques Eg: Human Protein–Protein Interaction Prediction Database (PIPs), I2D, STRING, and Unified Human Interactive (UniHI).

BIND
(Biomolecular Interaction Network Database)

- http://bind.ca
- A free, open-source database for archiving and exchanging molecular assembly information.

- The database contains
  - Interactions
  - Molecular complexes
  - Pathways

Conclusions

- PPI methodologies have been developed in yeast-methods are sometimes not suitable for plant systems
- Proteomic approaches still challenging
- International Plant Proteomics Organization (www.inppo.com), global initiative to develop and improve connections between plant proteomics researchers and related fields

Thank you