The social network of a cell:

Recent advances in protein-protein interaction

‘ ‘. . . any phenomenon, any event, or for that matter, any ‘knowledge’, any transfer of information implies *an interaction*’ ’

Jacques Monod

Nobel lecture (1968)

- Faced with a constantly increasing number of sequenced genomes, life science research is now focusing on the properties and functions of the encoded proteins

- One basic property of proteins is their ability to specifically target and form non-covalent complexes with other proteins

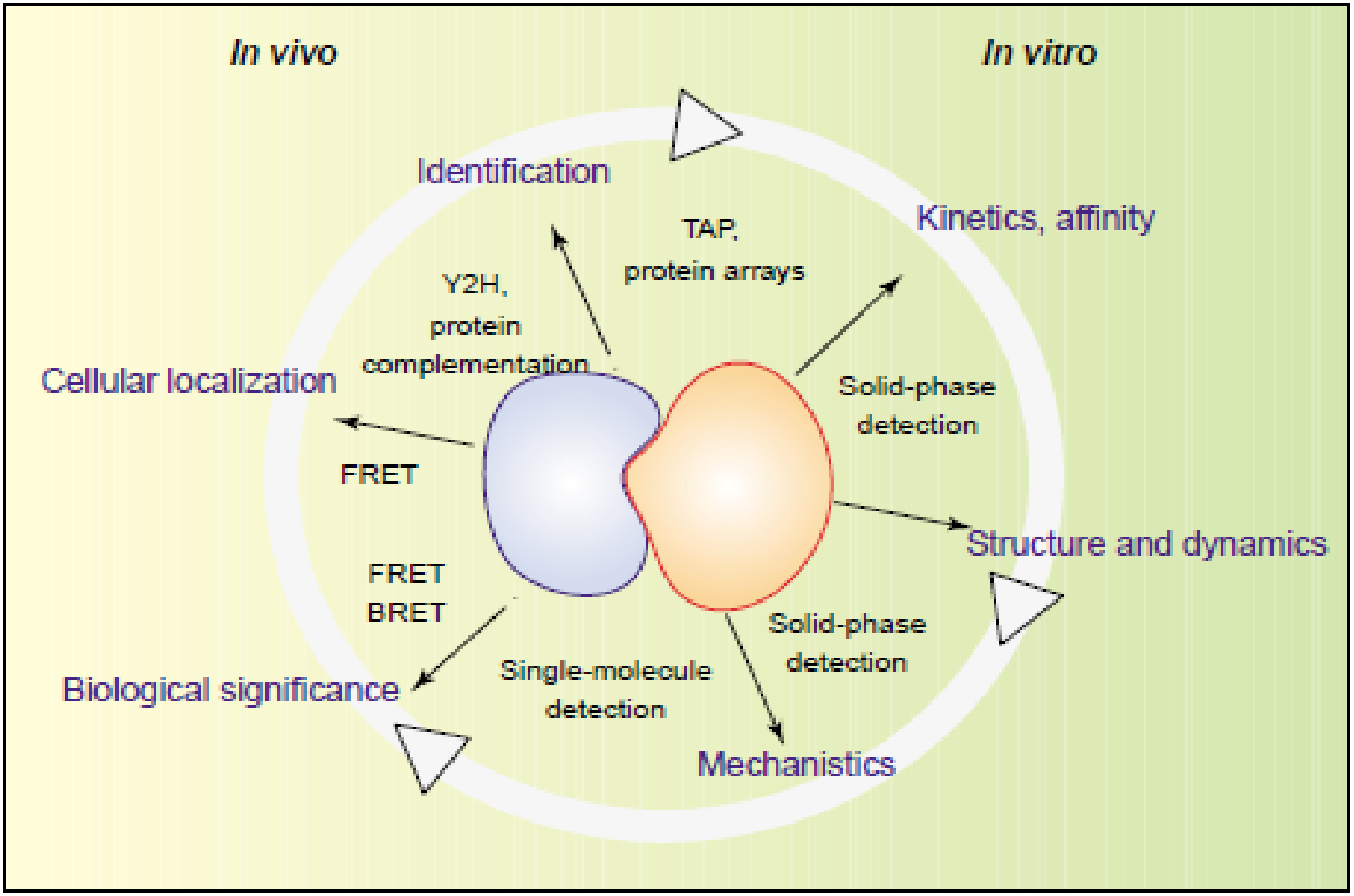
- Protein–protein interactions play key roles in all cellular processes and functions

- An average of five interaction partners per protein has been estimated, predicting a problem that far exceeds the complexity of the genome.

- For example, plant proteomes of 30 000 - 40 000 proteins are expected to have an estimated 75 000 -150 000 interaction pairs, extrapolating from estimates of the size of the yeast interactome

- Proteins interactions can be analyzed from many perspectives

Different levels of characterization of protein interactions *in vivo* and *in vitro*



- To *identify* protein interaction partners, techniques are required for the screening of large numbers of proteins (and that should preferably operate *in vivo* to maintain the cellular context).

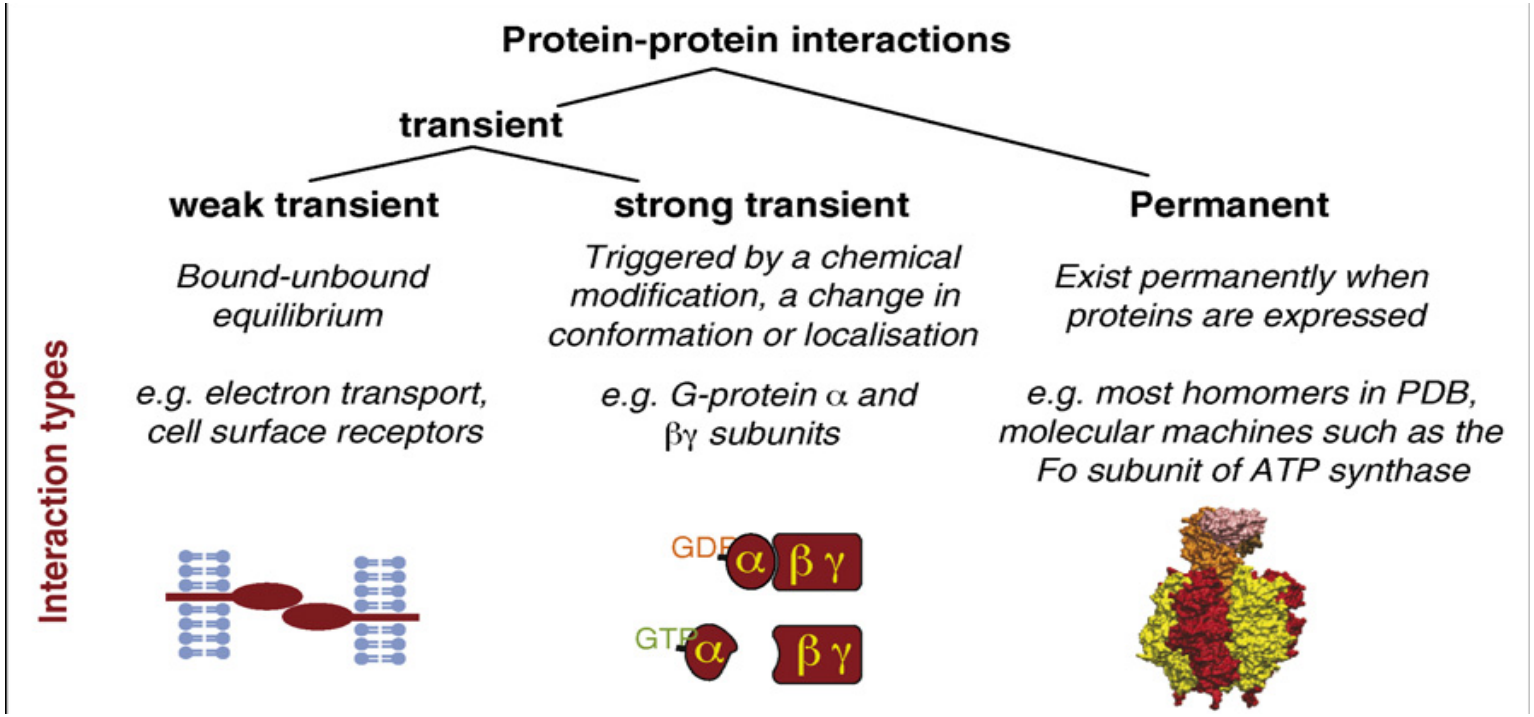
- Once specific and relevant interactions have been identified, the *molecular and biophysical properties* of the complexes have to be characterized in more detail

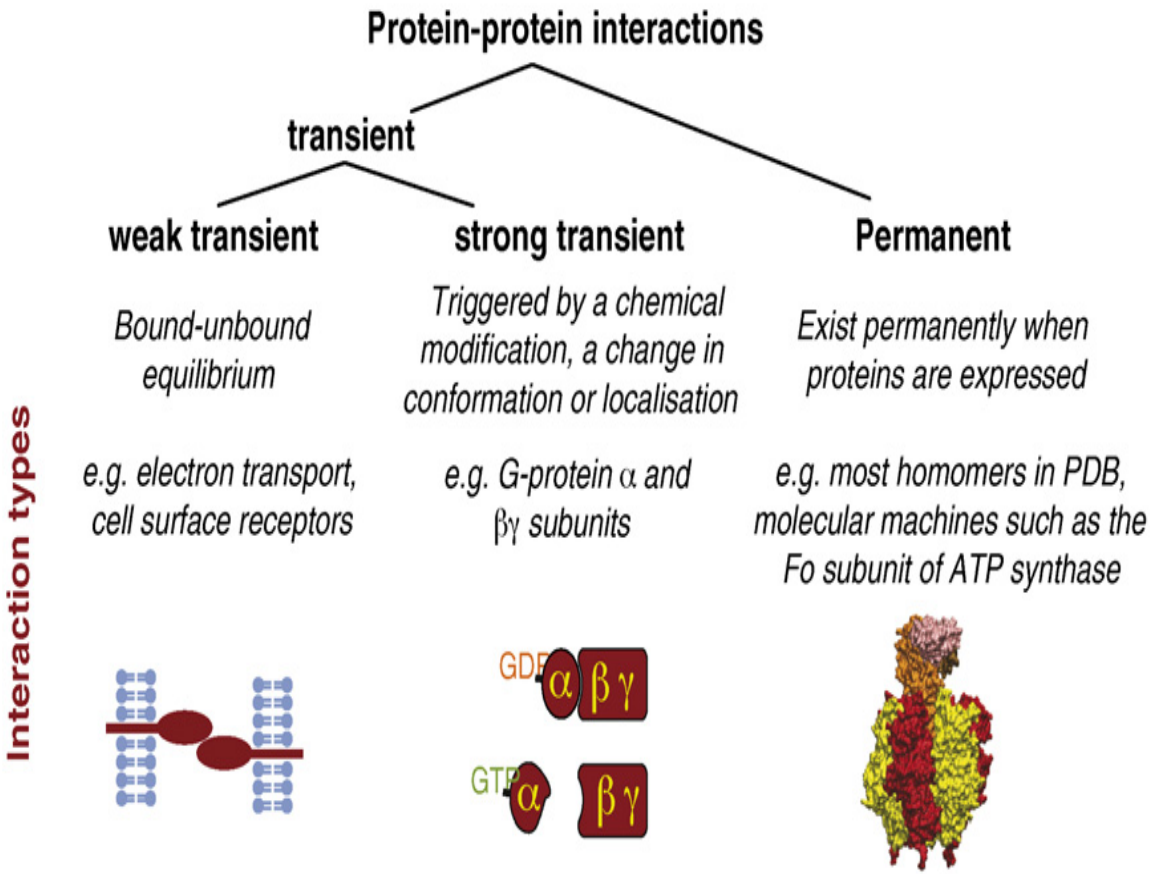
- Key parameters are: the oligomeric state of the interaction partner and the stoichiometric ratio in the complex, the affinity of the interaction partners for each other, the kinetic rate constants and the nature of the interaction sites. For this part of protein interaction analysis, *purified and well-characterized proteins* are required.

- However, a detailed understanding of protein interactions on the molecular level also has to take into account the cellular environment, requiring techniques for *studying interactions in vivo*

Dynamic of binary interactions

- Not all interactions occur at the same time and place, or have the same strength. Typical ***affinity constants*** of protein interactions span over six orders of magnitude, ***from the micromolar to the picomolar***.
- In spite of this great diversity, interactions can be conceptually divided into two groups: those that are ***permanent*** and those that are ***transient***





Current Opinion in Structural Biology 2008, 18:349–357

Different cellular functions require different interaction strengths.

Cell motility, for example, requires weak **transient** interactions between the extracellular receptors and the surface matrix.

By contrast, molecular machines such as ATP synthases or proteasomes have their subunits interacting more strongly and **permanently** with each other.

In other processes, such as in the association of the α and βγ subunits of heterotrimeric G proteins, the interaction is controlled by a **conformational change** that requires GTP hydrolysis

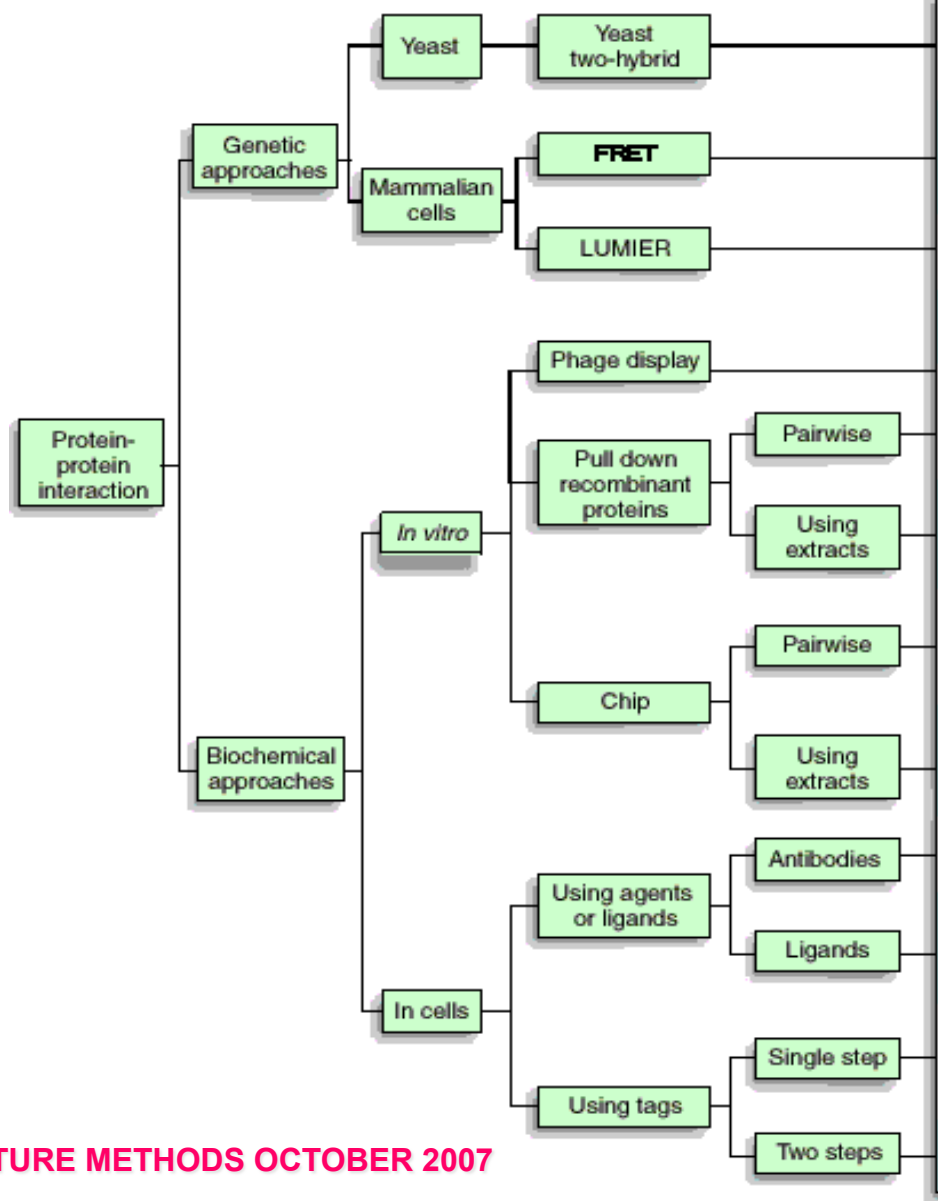
- **Goals of protein-protein interaction studies- Functional proteomics**

- Modern genetics and functional genomics experiments often lead to the identification of gene products with a putative biological function but a **poorly characterized** biochemical mode of action.

- Functional proteomics experiments allow researchers to **identify the interacting proteins** facilitating mapping of a protein to a particular biological pathway.

- The term ‘functional proteomics’ derives from the hypothesis that the **association** of proteins would suggest their common involvement in a **biological function**, analogous to the ‘guilt by association’ concept in criminal investigation.

Decision tree of options for the most common different protein-protein (or protein-ligand) interaction experimental strategies



Variants	Ideal to obtain	Resource intensity	References
Three-hybrid; reverse 2H; membrane 2H (mammalian 2H)	Potential interactions, map domains	Low	30,40
Technologies based on fluorescence interference	*In vivo* evidence, temporal spatial resolution	Medium	31
Other enzymatic readout	Very sensitive detection by enymatic amplification	Low	32
Ribosomal display	Quantitative binary data, direct binding	Medium	43
Co-immuno-precipitation, *in vitro* translation	Fast evidence for *in vitro* interaction	Low	53
Co-immuno-precipitation	Various detection possibilities (usually dirty with MS)	Low	53
Several variants	Biochemical interaction evidence, biochemical reactions	High	44–50
Several variants	Biochemical interaction evidence, biochemical reactions, medical samples	High	44–50
Affibodies, minibodies	Endogenous complexes	Low to Medium	54
Aptamers, drugs, lectins metabolites, nucleic acids	Specific information on type of affinity	Medium	55
In vitro and *in vivo* tagging, BirA	Fast purification of protein complexes, can be scaled up	High	57,58
TAP, GS-TAP	Sturdy interactors, can be scaled up	High	60–62, 64,65

NATURE METHODS OCTOBER 2007

in vivo identification of protein–protein interactions

1) **Two-hybrid techniques:** A breakthrough in the screening of protein interactions *in vivo* was made 15 years ago by implementing the yeast two-hybrid (Y2H) system.

- The concept of YTH exploits the modularity of eukaryotic transcription factors and the powerful genetics of the yeast, *Saccharomyces cerevisiae*, to monitor prot-prot interaction

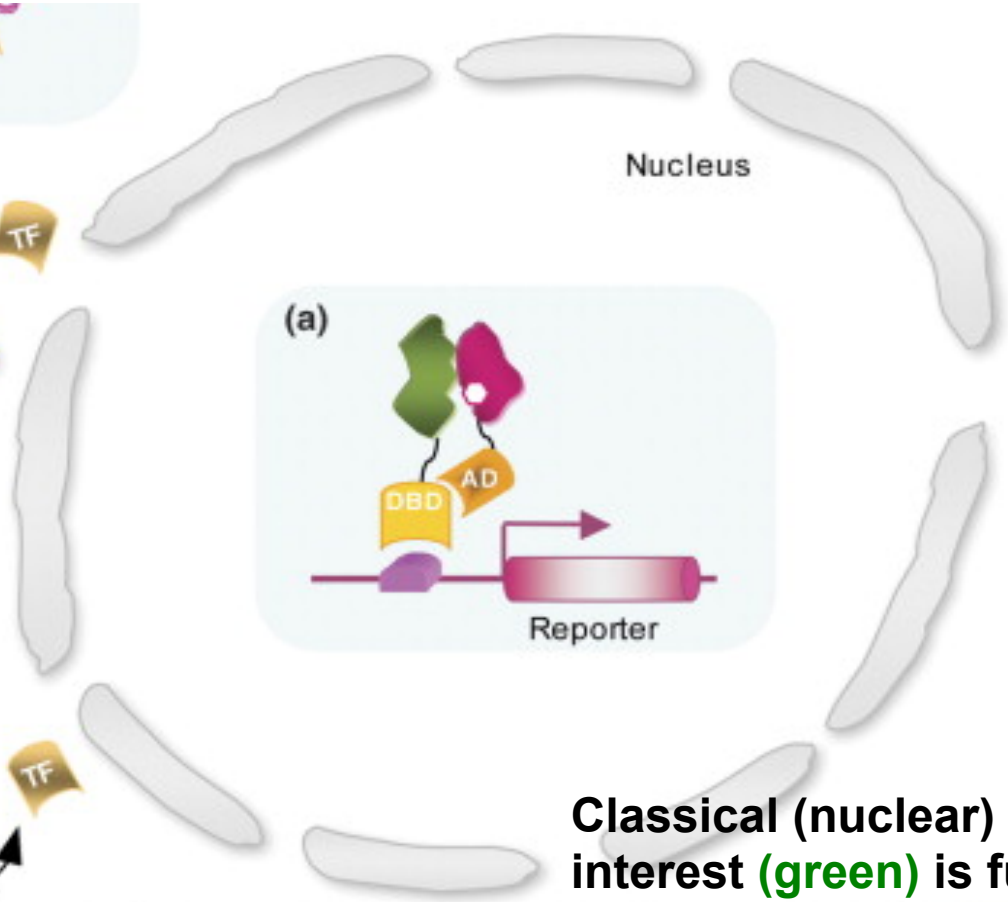
Principle

- The Y2H approach exploits the **modular** nature of transcription factors containing DNA-binding and activation domains.

- When these domains are split, the factor is functionally disabled. However, if each domain is fused to two interacting proteins as hybrid proteins (thus the name ‘two-hybrid’), the function of the factor can be restored to transcribe one or more selectable marker or reporter genes that then select for the interaction event

- A bait protein is fused to the DNA binding domain (DBD) and a prey protein is fused to the activation domain (AD) of a transcriptional activator.

- The functional transcription factor (TF) is reconstituted upon the physical interaction between bait and prey proteins. This results in the activation of a reporter gene and in a measurable output, which is either growth under selective conditions or a color signal (β-galactosidase assay)



Classical (nuclear) yeast two-hybrid.The bait protein of interest (green) is fused to the DNA-binding domain (DBD) of a transcriptional activator, and the prey protein (red) is fused to the activation domain (AD).
The interaction between bait and prey reconstitutes the functional transcription factor and results in the expression of the reporter gene for positive selection.

Transcription factors in Y2H are typically the Gal4 or LexA activator proteins

- The Y2H system today is a mature and very broadly applied method. Many improvements and variations of this technique have been reported.

- A very serious challenge to large-scale YTH is imposed by the frequent occurrence of false positives and false negatives.

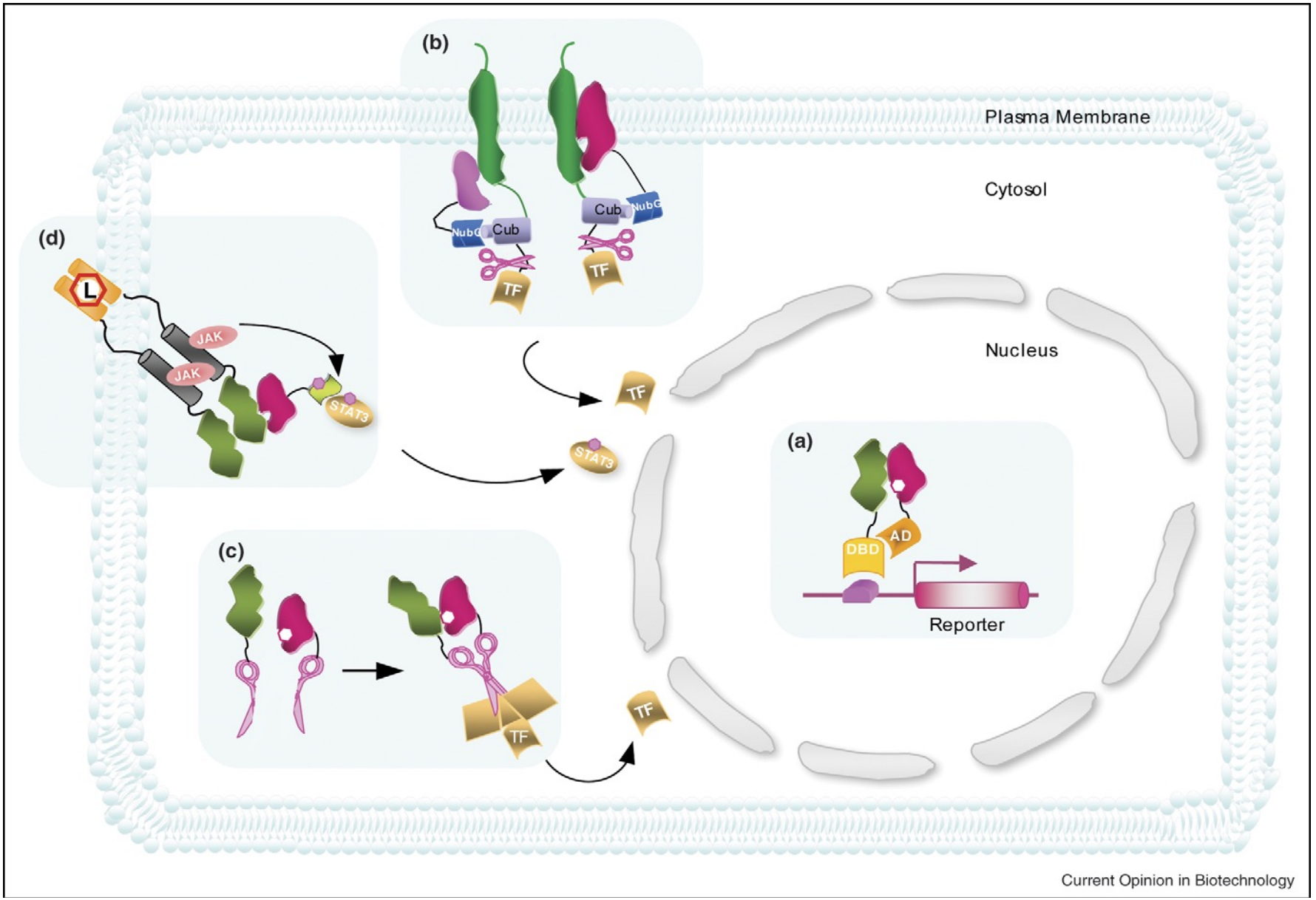
- False negatives are real interactions that go unnoticed in the screening procedure. They can arise by failures in the nuclear localization or improper folding of the fusion proteins.

- False positives are spurious interactions that occur only in the context of the YTH and are physiologically meaningless. Major sources for false positives are non-relevant PPIs forced by the overexpression of heterologous proteins and self-activation of the reporter gene by the bait protein. Estimations for false positives in large-scale YTH approaches range from 25 to 50%

Variant yeast two-hybrid technologies

- A variety of variant YTH methodologies have been developed to overcome the limitations of the original YTH regarding compartment- or modification-dependent PPIs

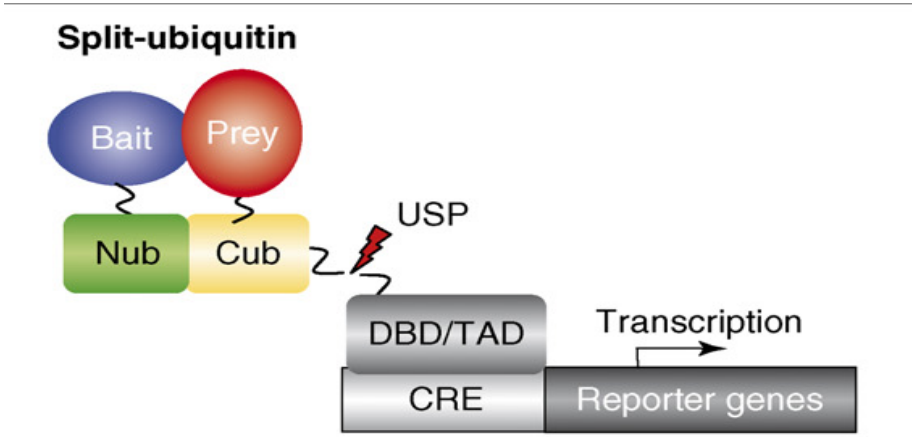
- However, only a few of these methods have been adapted for screens at the large-scale levels so far.

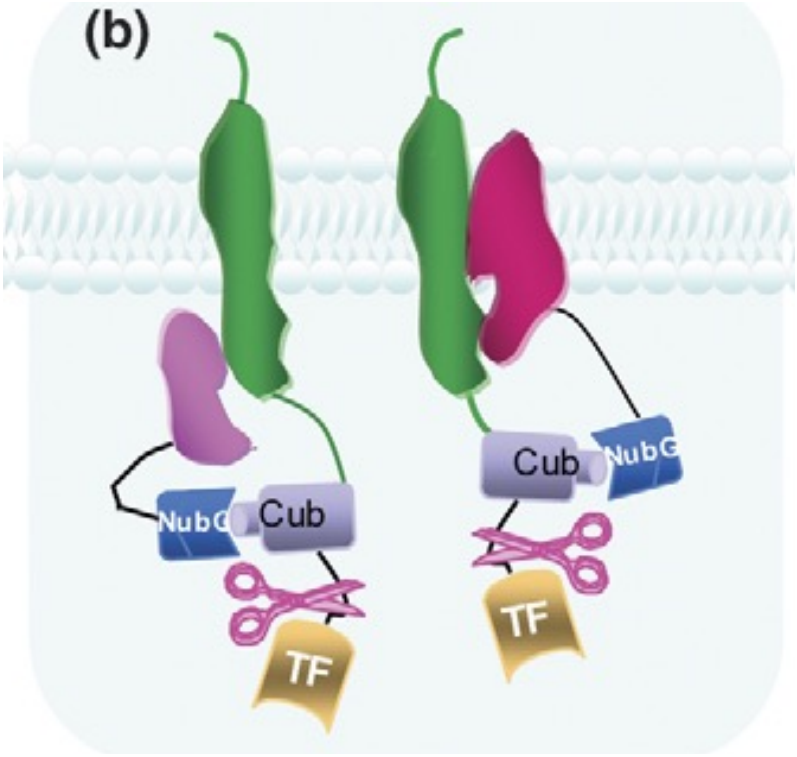


Yeast two-hybrid analysis and important variants in yeast and mammalian cells

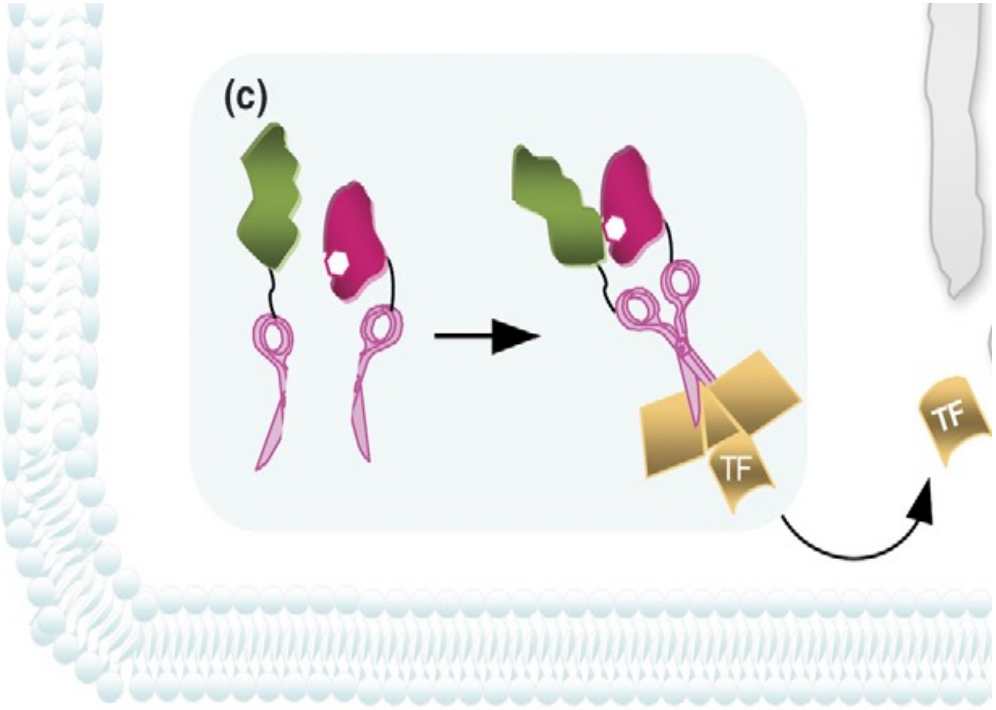
The variants are based on Protein fragment complementation

- Classic Y2H systems are limited to protein interactions in the nucleus, so interactions involving proteins integrated into or anchored to the plasma membrane are barely accessible. This was resolved by extending the two-hybrid approach to protein fragment complementation assays which were first implemented in the split ubiquitin technique.

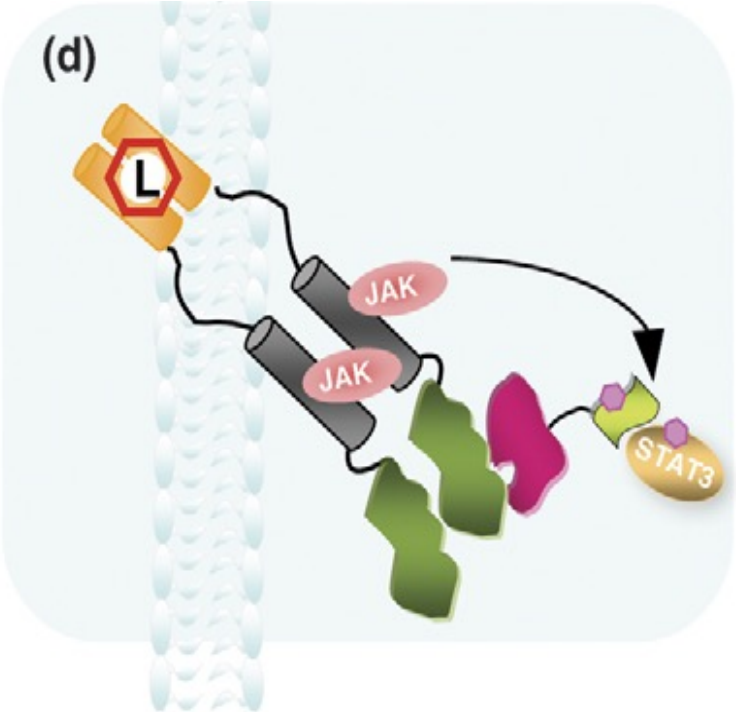




(b) **Membrane yeast two-hybrid system**. In MYTH, integral or peripheral membrane proteins **(baits)** are fused to the *C-terminal* half of ubiquitin **(Cub),** followed by a transcription factor **(TF).**
Preys (membrane or cytosolic proteins) are expressed as fusions with the *N-terminal* half of ubiquitin (Nub).
Bait–prey interaction reconstitutes native ubiquitin, which is then cleaved by endogenous **ubiquitin-specific proteases** (UBPs).
The transcription factor enters the nucleus and activates reporter gene expression.



(c) **Split-TEV system**. The tobacco etch virus (TEV) protease **(scissors)** is functionally reconstituted by a bait–prey interaction. TEV cleavage of the recognition sequence releases a transcription factor (TF). The activator enters the nucleus and drives reporter gene expression (transcription-coupled split-TEV).



(d) **Mammalian protein–protein interaction trap** (MAPPIT). Recruitment and activation of STAT3 transcription factor **(purple)** by Janus kinases **(JAK)** normally occurs when the receptor is activated and clustered by ligand **(L)** binding to the extracellular domain of the receptor. For MAPPIT, the bait protein is fused to a cytokine receptor variant **(gray)** that cannot recruit the STAT3 transcription factor.

Prey proteins are fused to a functional receptor that contains docking sites for STAT3 **(light green).** Bait–prey interaction results in the phosphorylation **(pink)** of the STAT transcription factor. Phosphorylated STAT3 transcription factor translocates to the nucleus and activates a reporter gene.

Directly monitoring protein interactions *in vivo*: resonance energy transfer

- Y2H and Protein fragment complementation are potent techniques for identifying interactions

BUT

real-time monitoring and localization of protein interactions in living cells requires an instant spectroscopic read-out.

- The principal challenge for direct spectroscopic investigation of protein interactions *in vivo* is the requirement for specifically attaching spectroscopic probes to the protein of interest in the cellular environment.

- Currently the most powerful techniques for the direct spectroscopic detection and monitoring of protein interactions in living cells is fluorescence resonance energy transfer (FRET) and bimolecular fluorescence complementation (BiFC).

Getting started: general considerations for FRET and BiFC studies

- First: all fluorophore-based methods require tagged variants of the protein of interest, modifications that may alter their physiological parameters. Thus, wherever possible, fluorophore-tagged protein should be tested for subcellular localization, stability, and biological activity.

- Second: the expression level of the tagged prot. In plants, frequently expression is driven by strong constitutive promoters (e.g., the cauliflower mosaic virus [CaMV] 35S promoter) that may result in ectopic expression and/or overexpression. This might result in artifacts that may possibly either promote or inhibit particular protein-protein interactions.
- Thus the *native gene promoters* should be used for driving the expression of fluorophore-tagged protein.

- Third: both FRET and BiFC represent methods that determine "only" the close physical proximity of two fluorophore-tagged fusion proteins *in vivo*.

- Is such a tight contact the proof of a true protein-protein interaction or, alternatively, it represents merely an indicator of close vicinity, as for example, the co-presence of two proteins in a large multi-protein complex???

- Evidence for a direct interaction requires *in vitro* assays using purified recombinant proteins, e.g. far Western blots or surface plasmon resonance (SPR) spectroscopy

The basic principle of FRET

- Förster (or Fluorescence) Resonance Energy Transfer (FRET) biophysical phenomenon. 50 yrs ago

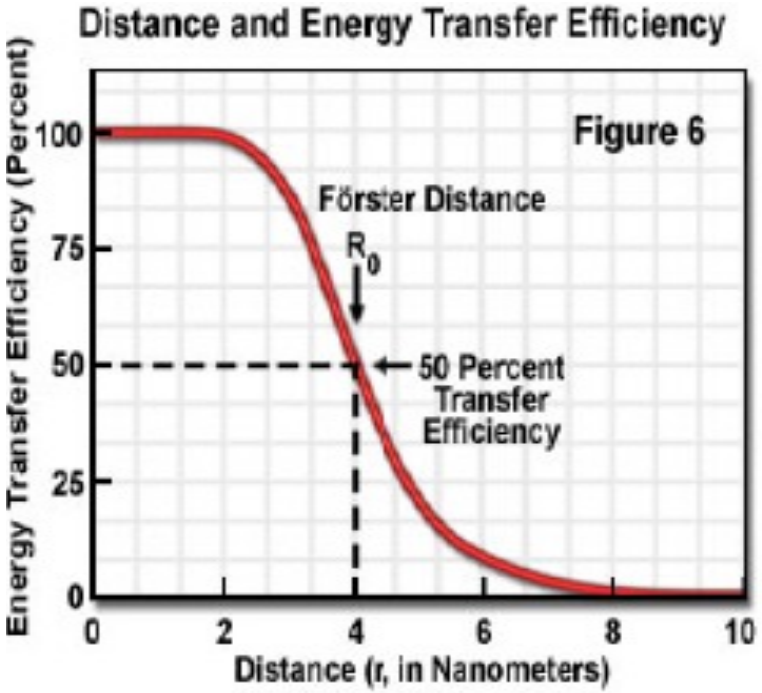
- Long-range dipole-dipole resonance interaction in which non-radiative energy is transferred from a chromophore in an electronic excited state (donor) to another molecule (fluorescent or otherwise) serving as the acceptor.

- This energy transfer leads to a reduction in the donor's fluorescence intensity and a decreased lifetime in the excited state. If the acceptor molecule is a fluorophore, then FRET additionally gets manifested in the form of an increase in the acceptor's emission intensity

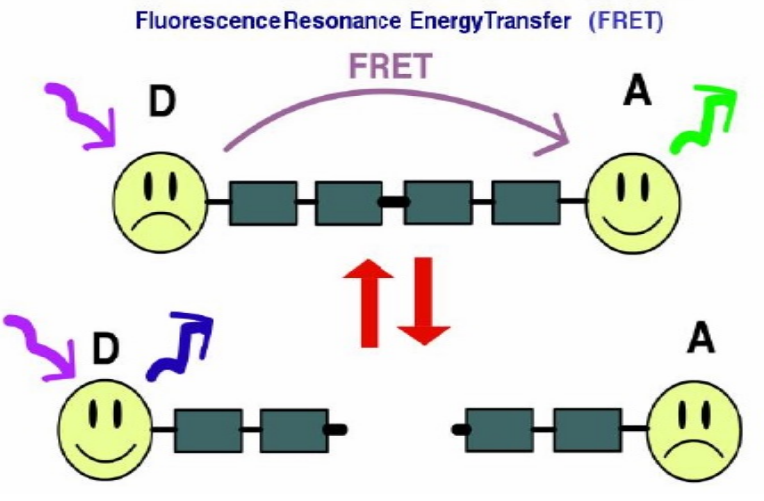
- The efficiency of energy transfer (E) is inversely proportional to the sixth power of the distance between the donor and the acceptor:

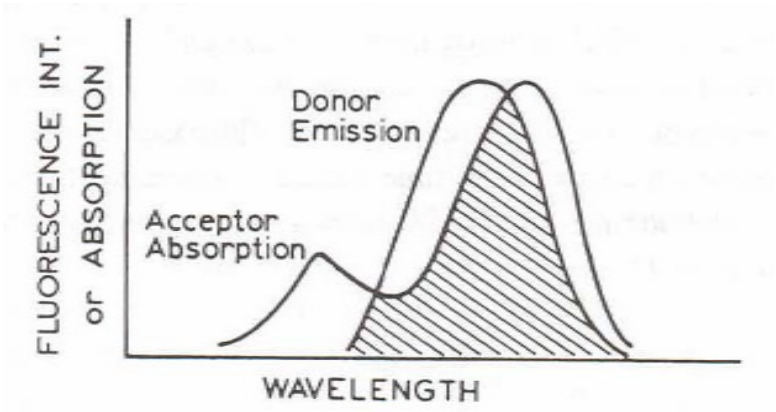
$$E = 1/\{1 + (R/Ro)6\}$$

Ro is the distance at which half of the energy is transferred from the donor to the acceptor. R0 is typically between 20–60 A° (2–6 nm) and thus in the range of conventional protein dimensions.



•The exact value of R0 is a function of the spectral overlap between donor emission and acceptor excitation spectra, the quantum yield of the donor in the absence of the acceptor, and the relative orientation of donor and acceptor chromophore





Emission spectrum of donor and excitation (absorption) spectrum of acceptor must significantly overlap (J, overlap integral)

•Effective between 10-100 Å only (Ro)

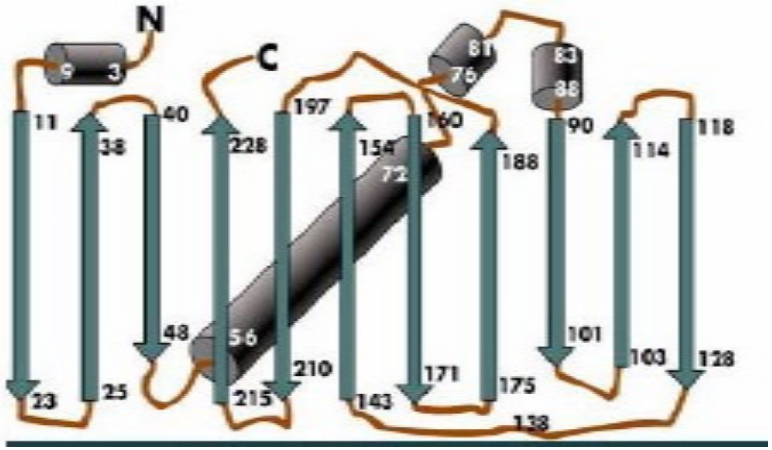
•Dipole-dipole interaction (Kappa square)

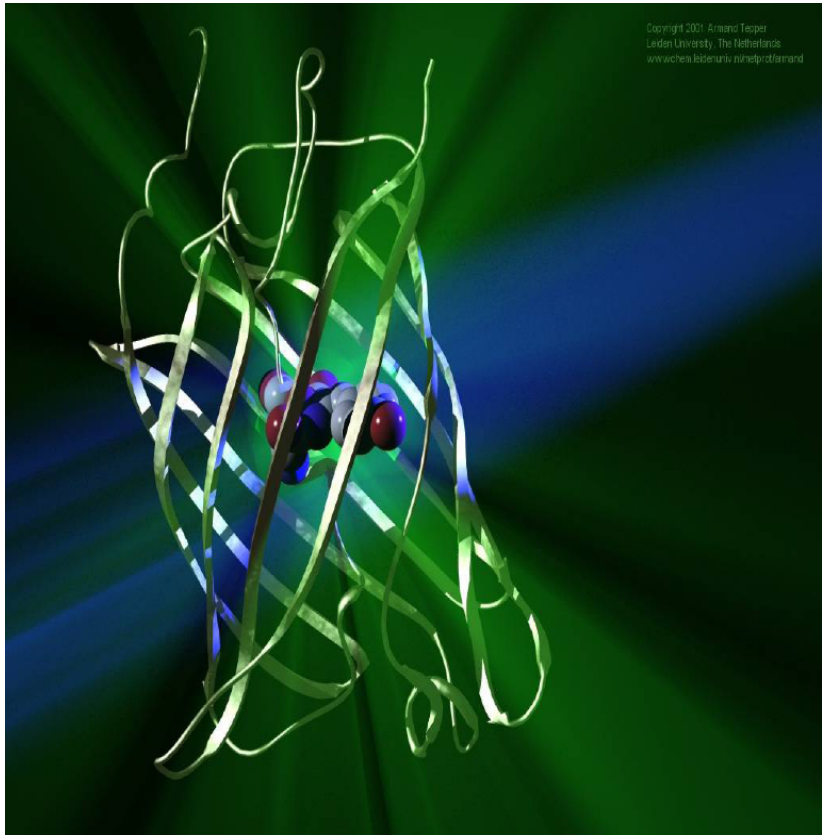
•Donor has high quantum yield (Qd)

- Since the discovery of autofluorescent proteins, their application to characterizing protein– protein interactions in living cells has led to stunning developments

A quick recall....

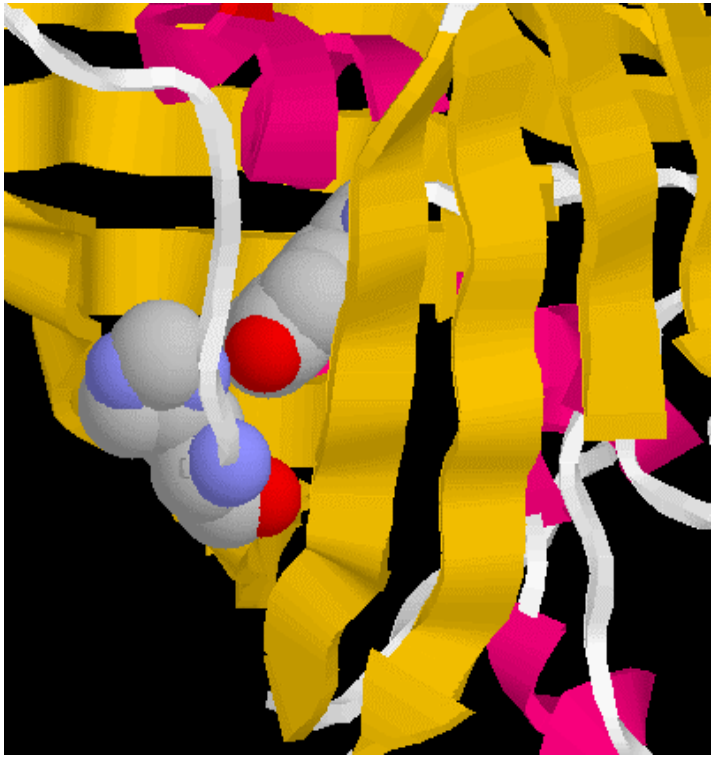
Green Fluorescent protein (GFP)

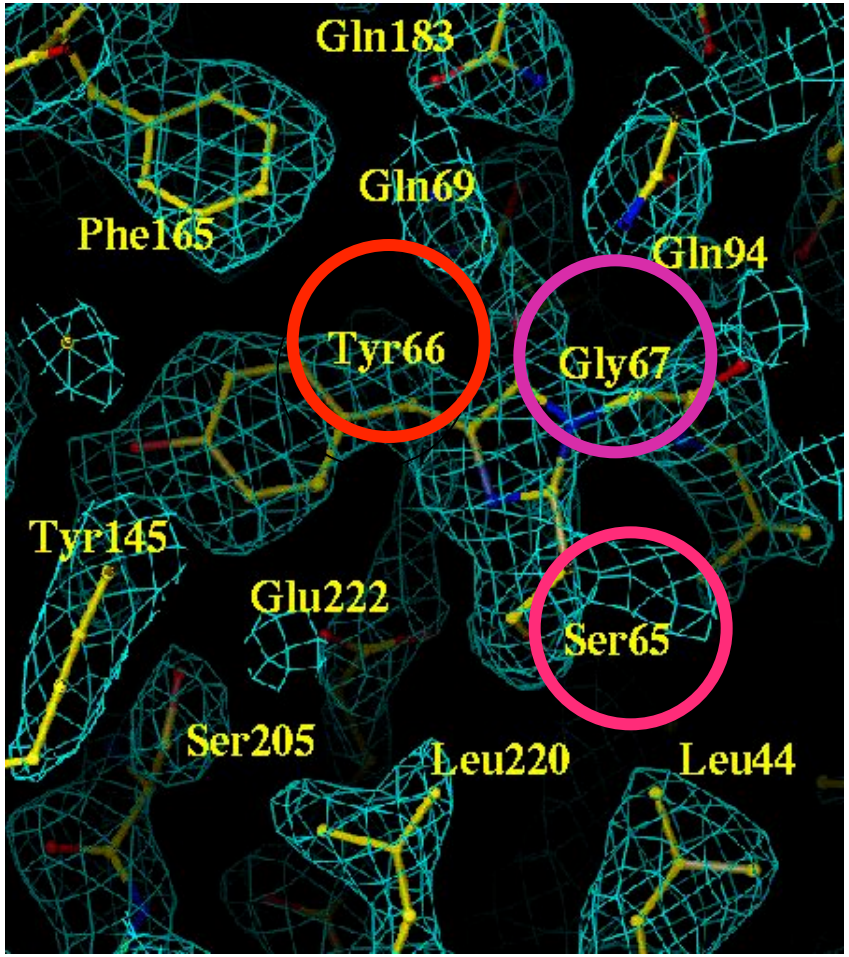




- 238 amino acids
- "Paint in a can"
- Each monomer composed of a central alpha-helix surrounded by an eleven stranded cylinder of anti-parallel beta-sheets
- Fluorophore located on central helix

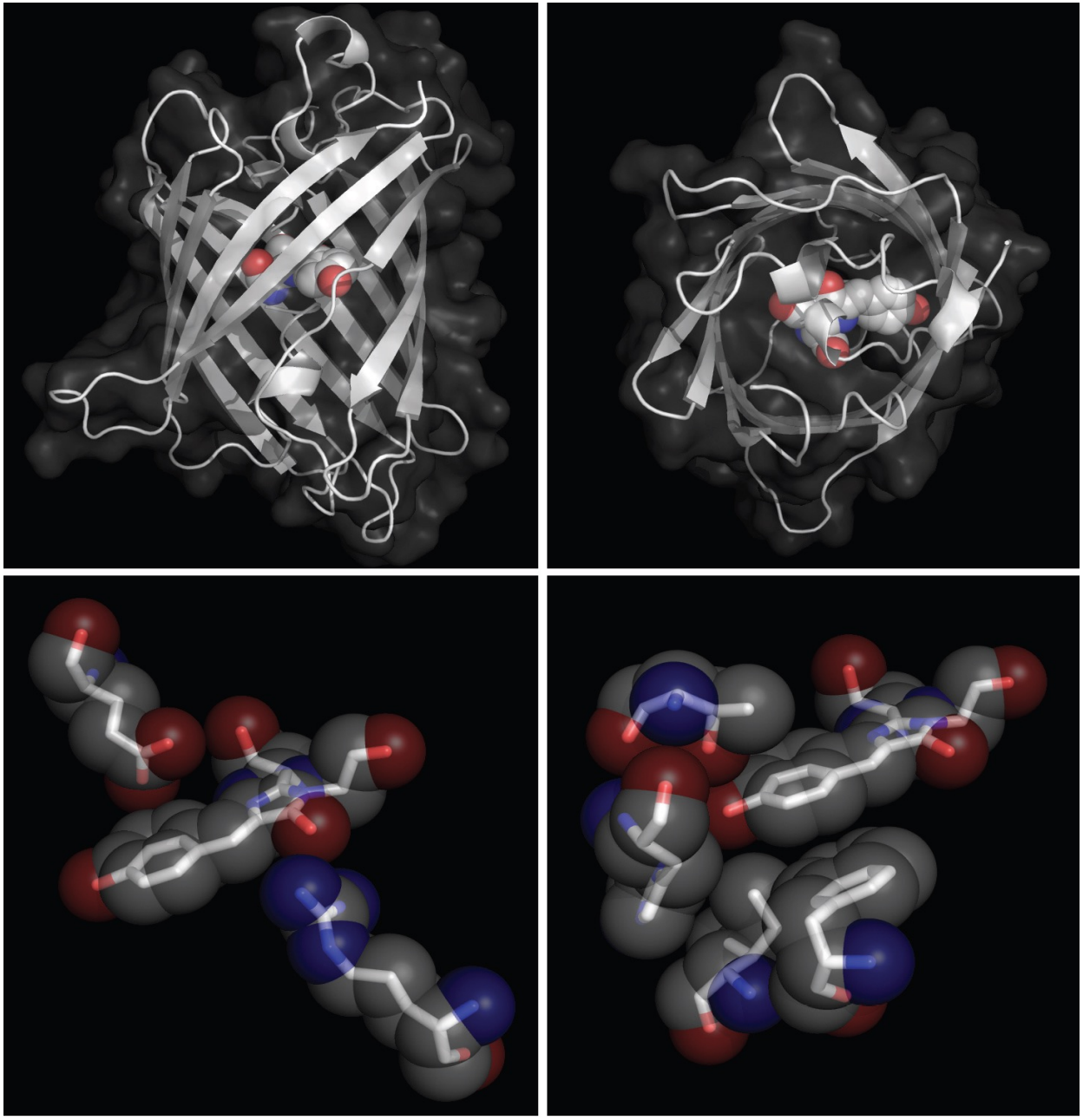
The fluorophore active site



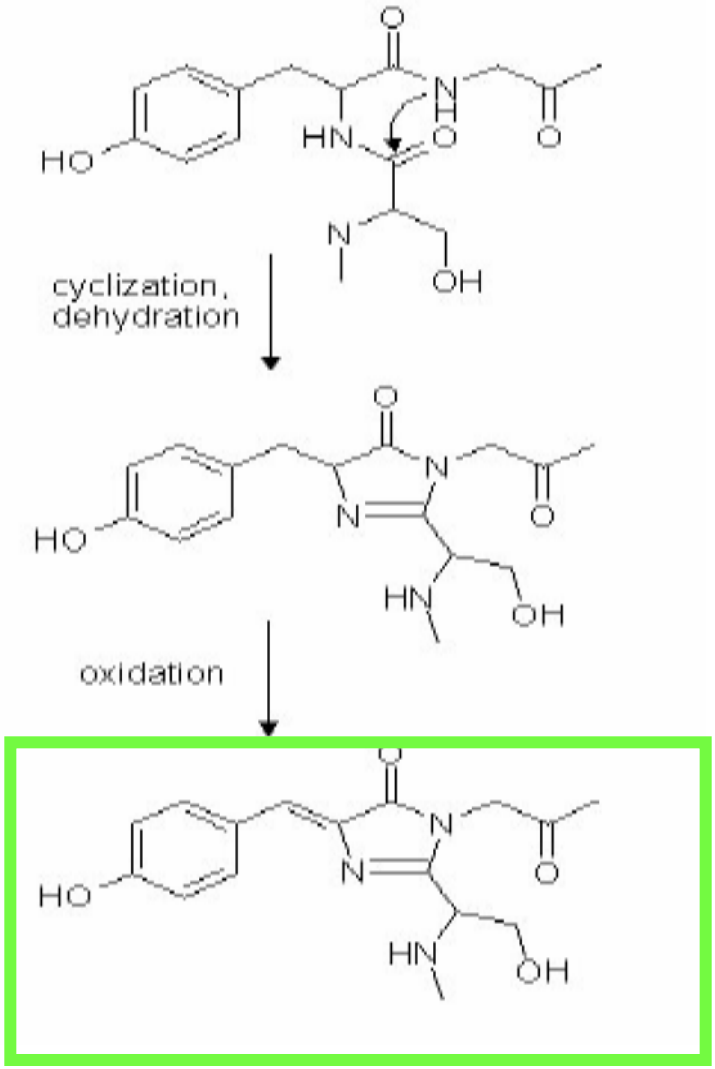


Ser65-Tyr66-Gly67

Deprotonated phenolate of Tyr66 is cause of fluorescence



GFP fluorophore formation



3AA: Serine65, Tyrosine66, Glycine67

2Step maturation:

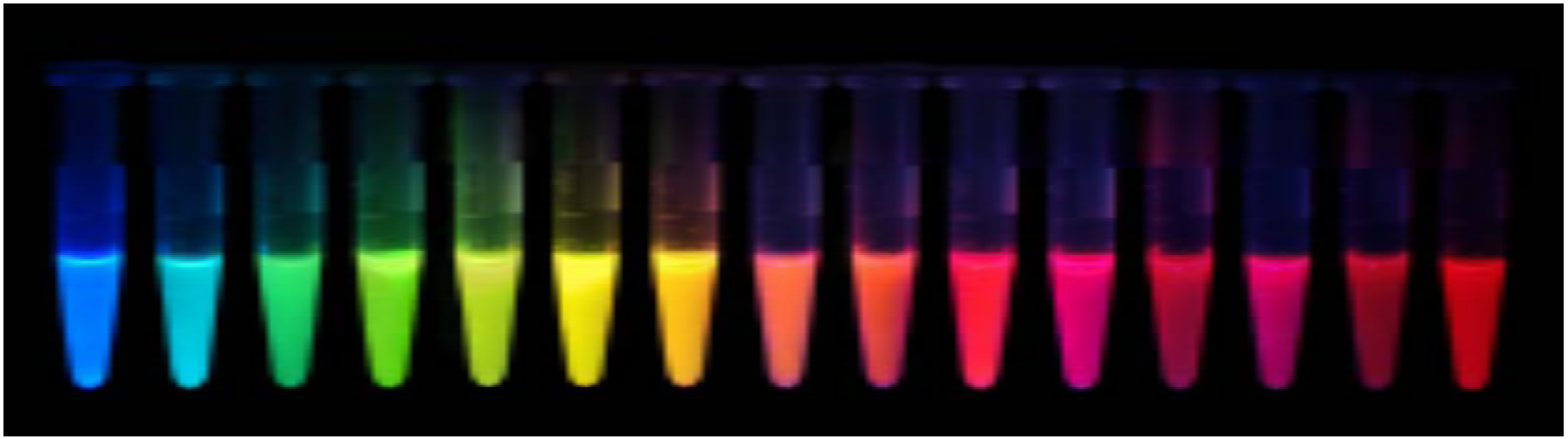
1)Cyclization, dehydration: formation of imidazol-ring

2)Oxidation: Extension of conjugated π- electron system (reversible with reducing agents)
→Environment matters:
- Oxidizing Environment
- Thermostable but temperature sensitive:
Lower temperature → better protein folding.
- Long maturation time (up to 6h)

RFP1 derived

GFP derived

Exc. 380 433/452 488 516 487/504 540 548 554 568 574 587 595 596 605 590 nm
Em. 440 475/505 509 529 537/562 553 562 581 585 596 610 620 625 636 648 nm



→ The spectroscopic and physicochemical properties of autofluorescent proteins are constantly being improved (mutants) and novel species are being discovered....

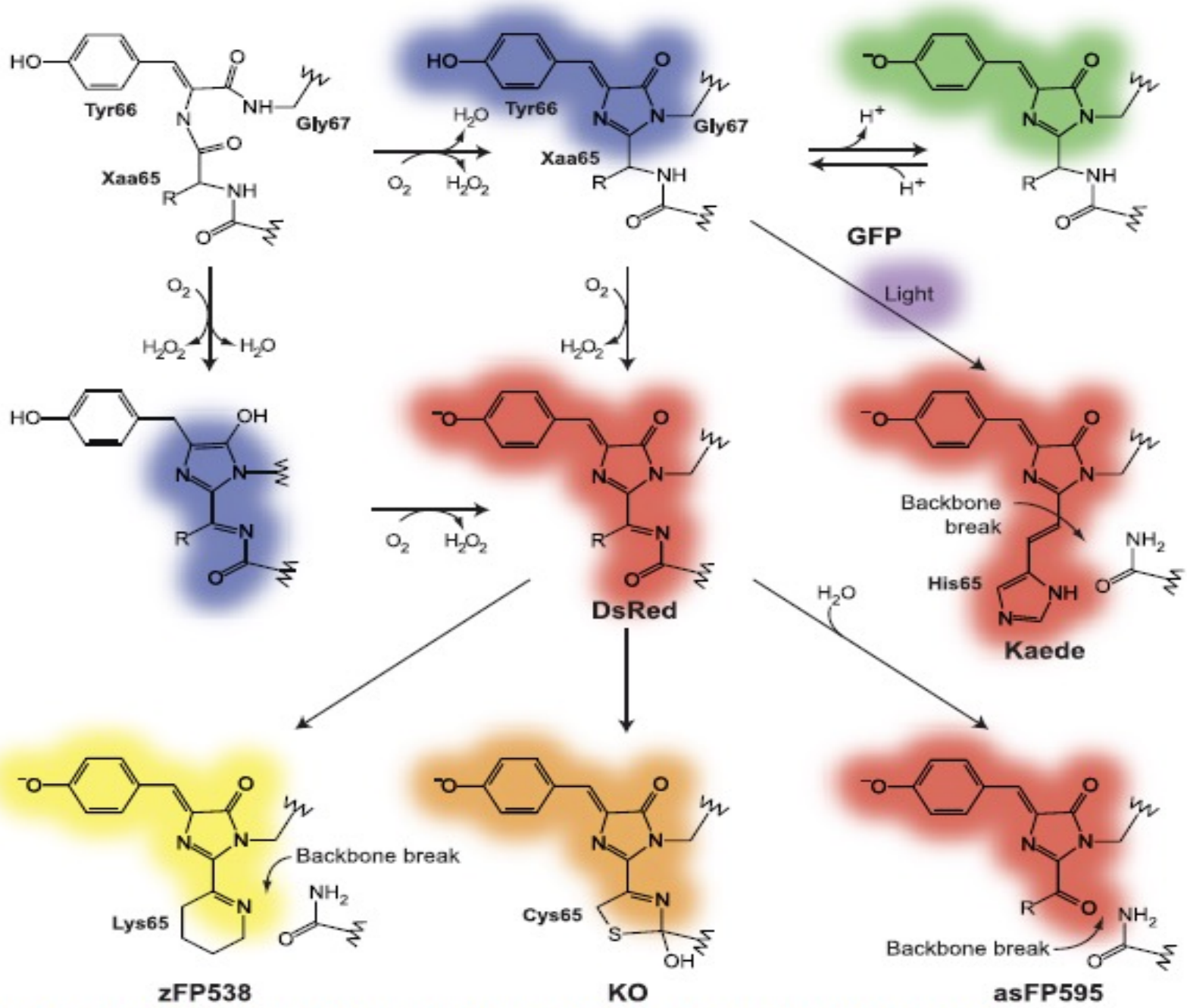
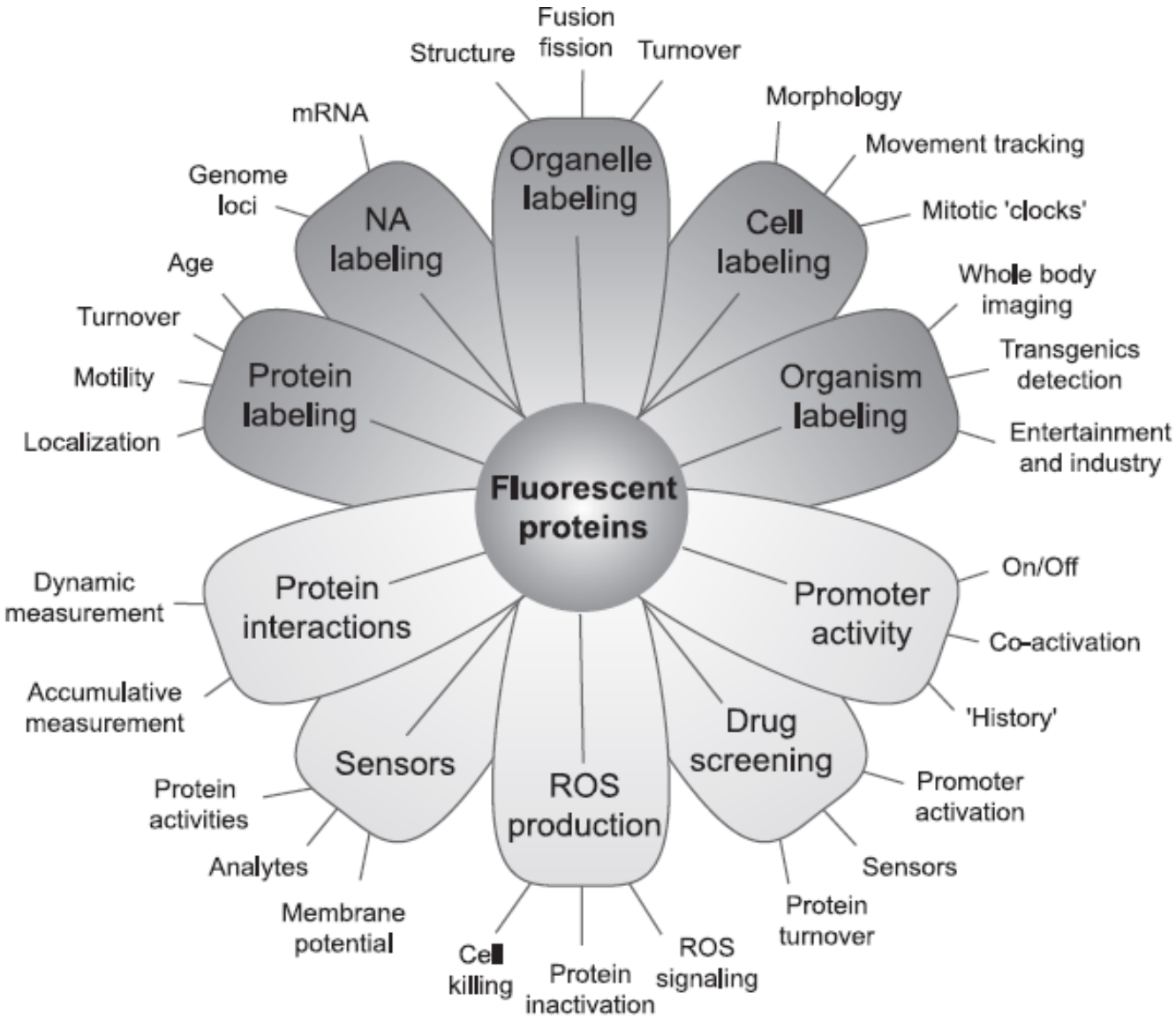
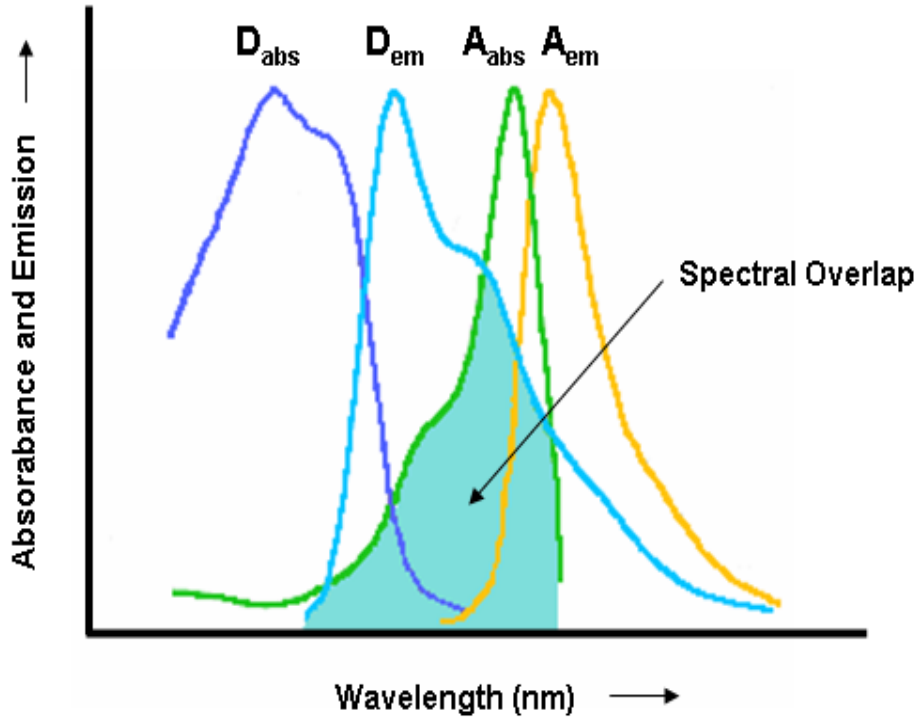


FIG. 6. Chemical structures and pathways of maturation of naturally occurring chromophores within GFP-like proteins.



Main areas of applications of fluorescent proteins. Dark gray and light gray **petals show structural and** functional **studies, respectively**



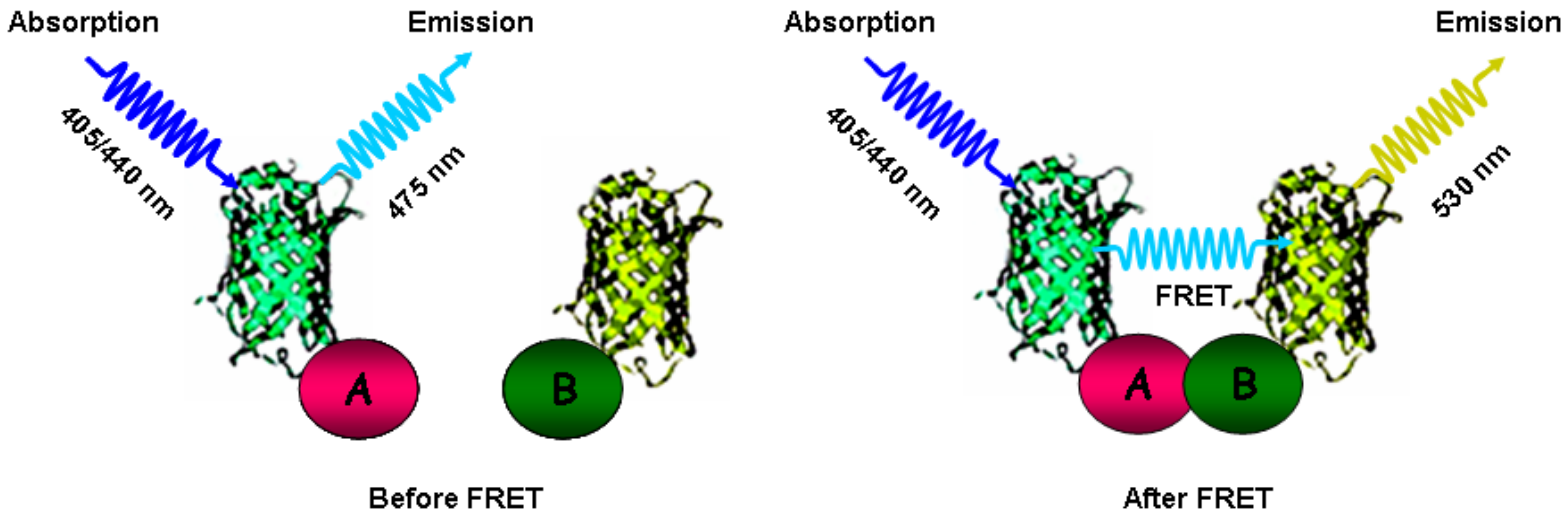
Dabs – Donor absorbance;
Dem – Donor emission;
Aabs – Acceptor absorbance;
Aem – Acceptor emission.

Excitation and emission spectra of a commonly used FRET pair.
Simplified absorbance and emission spectra of CFP (cyan fluorescent protein; donor; D) and YFP (yellow fluorescent protein; acceptor, A).
Overlap between CFP emission and YFP absorption (shaded region) is a prerequisite for FRET.

Thus, FRET is possible between different variants of GFP

FRET as a sensor of protein-protein interactions in living cells

- In living cells, FRET occurs when protein (domains) fused to suitable donor and acceptor fluorescent dyes physically interact, i.e. the fluorophores come in close spatial proximity



FRET between cyan fluorescent protein (CFP) as a donor fused to protein A and yellow fluorescent protein (YFP) fused as an acceptor to protein

- During the past few years, FRET has been extensively used to study protein-protein interactions in a diverse range of organisms and cell types, including yeast, animal and plant cells.

- However, despite the widespread interest in detecting protein-protein interactions using FRET microscopy, in the plant sciences reports of successful FRET are still limited in number.

- A major problem is *achieving* FRET(!!) because a successful FRET requires that the donor and acceptor fluorophores come into close proximity. This can be a limiting factor, especially in the case of large interaction partners.

- *Sterical orientation* of the fluorophores in the fusion proteins is another critical factor.

- Both fluorophore *distance* and *orientation* represent parameters that are difficult to control.

- FRET analysis generally requires high levels of protein expression to detect energy transfer.

- The fraction of proteins that form complexes must also be high enough to produce a sufficient change in the donor and acceptor fluorescence intensities.

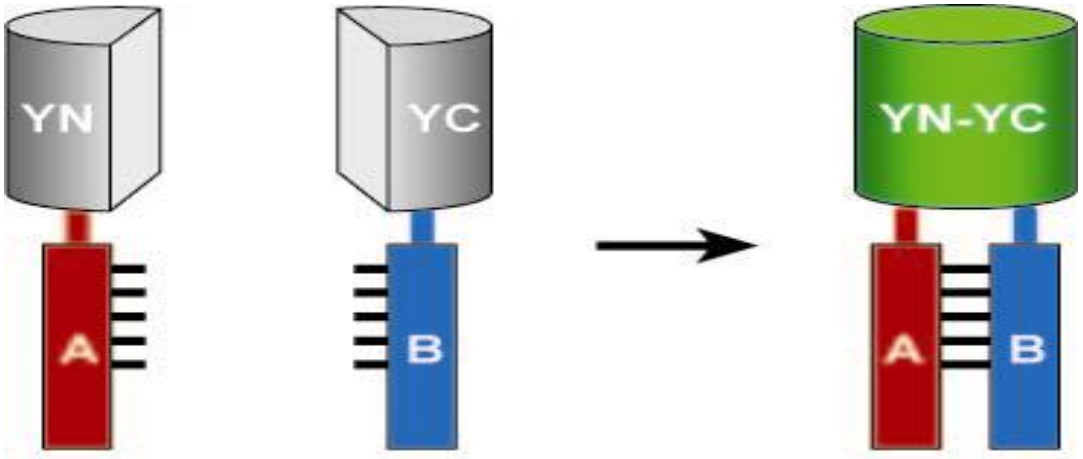
- To exclude alternative interpretations of the results, numerous controls must be performed and the fluorescence intensities must be measured with high quantitative accuracy

- The BiFC assay tries to overcome these problems

BiFC principle

- BiFC (Bimolecular fluorescence complementation) is based on the association between two nonfluorescent fragments of a fluorescent protein when they are brought in proximity to each other by an interaction between proteins fused to the fragments.

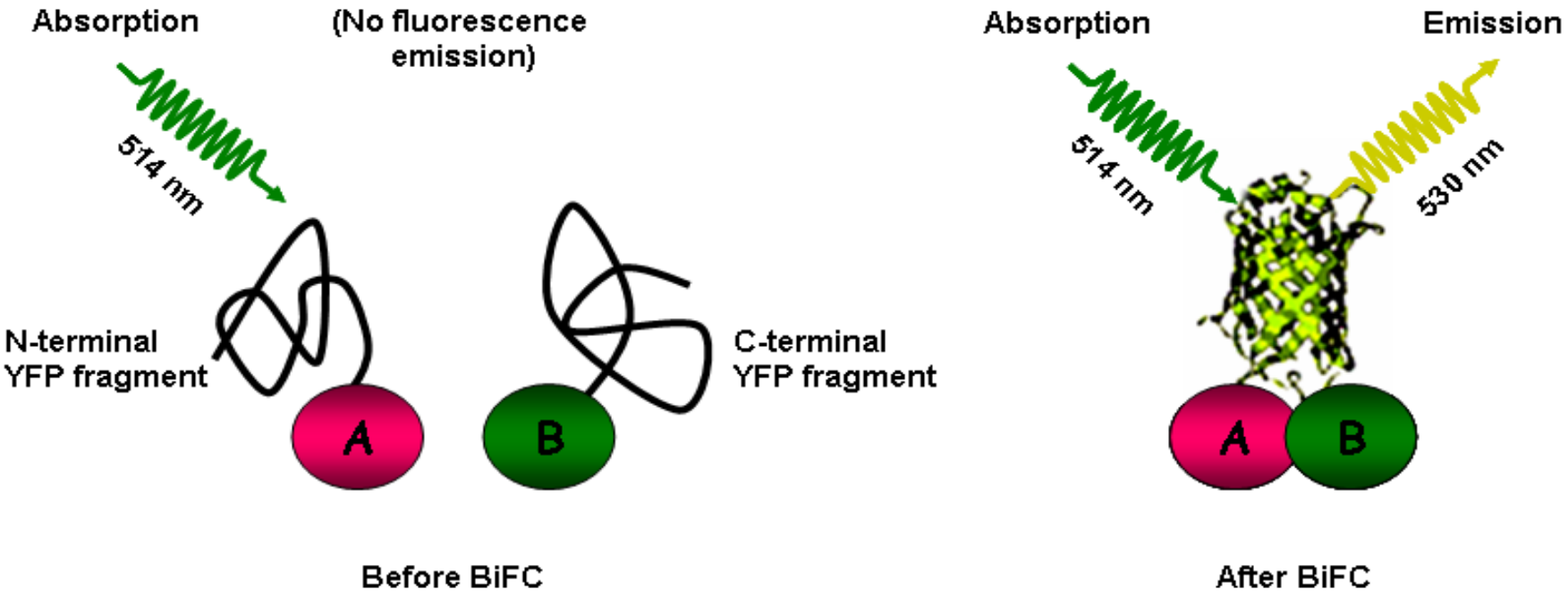
Schematic representation of the principle of the BiFC assay



Kerppola TK. 2008.
Annu. Rev. Biophys. 37:465–87.

Two nonfluorescent fragments (YN and YC) of the yellow fluorescent protein (YFP) are fused to putative interaction partners (A and B). The association of the interaction partners allows formation of a bimolecular fluorescent complex.

Bimolecular fluorescence complementation (BiFC) – also known split YFP



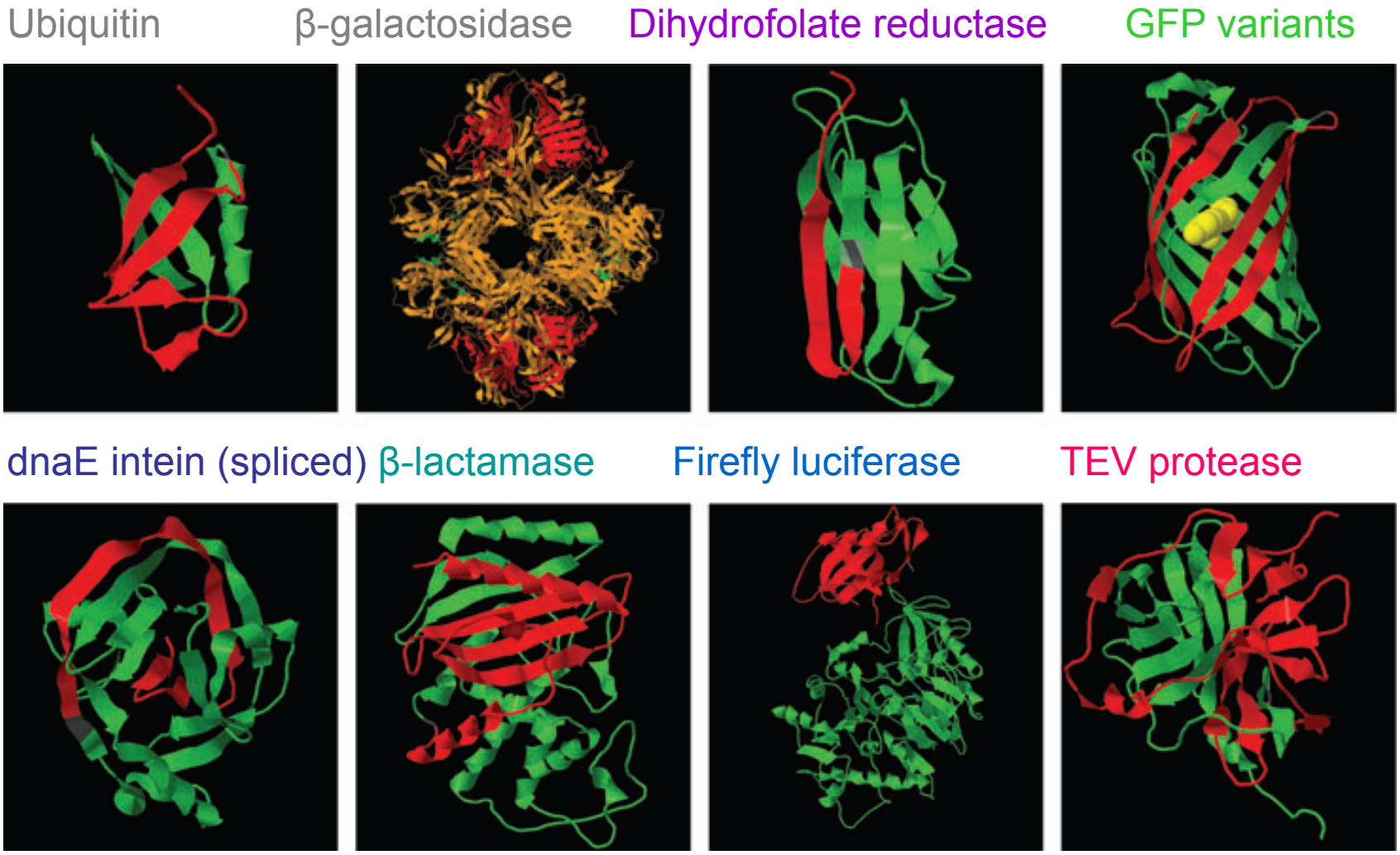
Principle of the BiFC assay, exemplified by a split YFP fluorophore.
Proteins A and B are fused to N- and C-terminal fragments of YFP, respectively.
In the absence of an interaction between A and B, the fluorophore halves remain non-functional.
Following interaction between A and B, a functional fluorophore is reconstituted which exhibits emission of fluorescence upon excitation with an appropriate wavelength

- Proteins with a variety of structures can be reconstituted from fragments.

- However, only a few of the peptide bonds in any particular protein can be broken to produce fragments that can associate to form a functional complex. This limitation may reflect the folding pathways of the respective proteins.

- Greater insight into the folding pathways of complexes formed by the protein fragments would be valuable for understanding the factors that determine which protein fragments can associate to produce a functional complex.

Structures of proteins that have been used to study protein interactions using complementation approaches. The two fragments that have been used are shown in **red** and **green** based on the X-ray crystal structures of the intact proteins.



Pitfalls and limitations of this technique

- irreversibility of complex formation: no information on the physiological time course of interactions,but also traps and accumulates transient and weak associations, thus increasing the sensitivity of detection; this attribute may be the cause of false-positive results and prevents the analysis of dynamic interactions.

- does the affinity of two interaction partners corresponds to the degree of cellular fluorescence? No. However, when studying several interactors for a given protein, BiFC is useful for discriminating strongly bound ligands from weakly bound ones;

- Maturation time of the fluorophore tag: Intact (full-size) GFP, for example, requires several hours to mature in the cell, so the intermolecular reconstitution of a split fluorophore may take even longer. Thus, proteins with *high turnover rates* is not amenable to BiFC studies.

Comparison between FRET and BiFC

- **BiFC is based on a gain of fluorescence, while FRET causes a quantitative change in fluorescence**
- FRET-based studies rely on specific detection of spectrally similar fluorophores → they require sophisticated, expensive instrumentation,
- **BIFC can be measured by standard epifluorescence microscopy equipment;**
- FRET assays need comprehensive post-imaging data analysis, **while this additional step is generally not required for BiFC studies;**
- **BiFC sensor peptides fluoresce only upon interaction of their fusion partners, it is impossible to visually confirm that both fusion proteins are being made in the absence of an interaction →in BiFC studies, immunoblot analysis is required to validate expression of the fusion proteins in the absence of interaction**. FRET sensor peptides are intrinsically fluorescent, which permits detection and quantification of fusion protein levels independently of their interaction status.

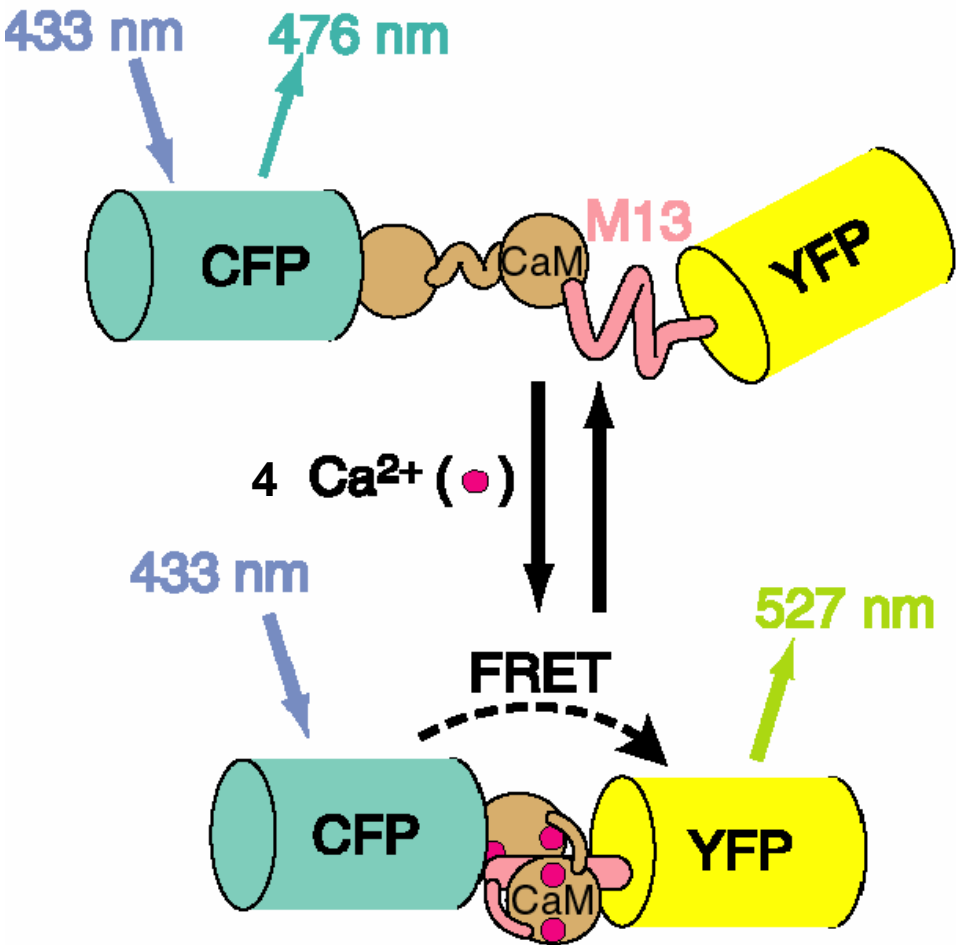
- Intracellular sensors based on *intramolecular FRET* gained increasing attention and are now routinely used as nanosensors to report various intracellular changes of metabolites, e.g. alterations in calcium levels

Fluorescent CaMeleons (CaM + chameleon)

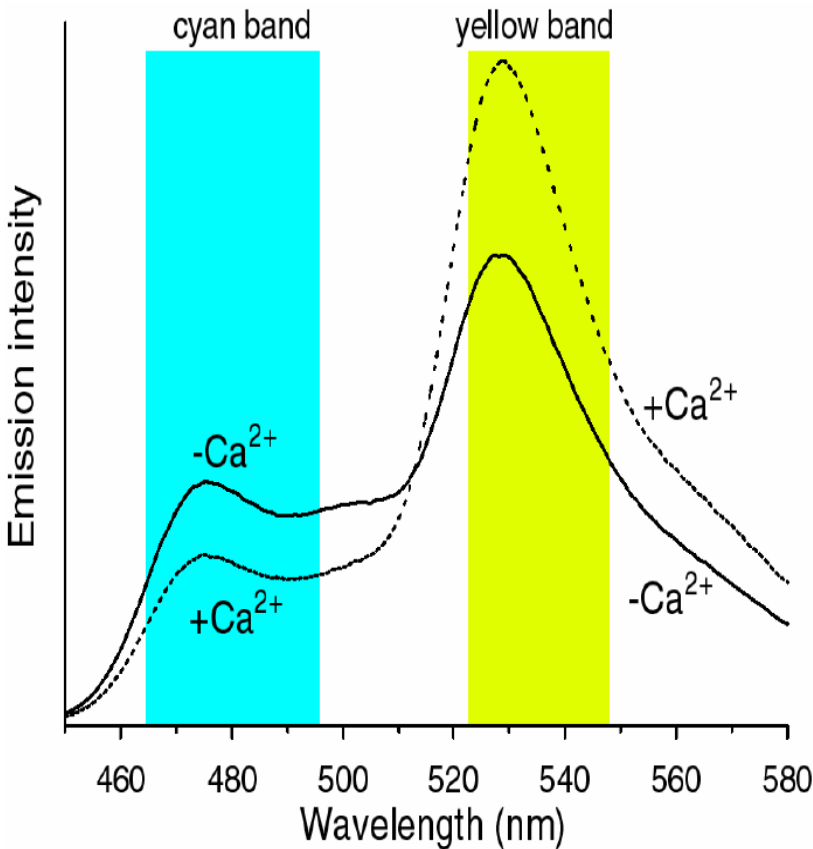
- They consist of *tandem fusions* of a blue- or cyan-emitting mutant of the green fluorescent protein (GFP), calmodulin, the CaM-binding peptide M13, and an enhanced green- or yellow-emitting GFP.

- Binding of Ca2+ makes calmodulin wrap around the M13 domain, increasing the fluorescence resonance energy transfer (FRET) between the flanking GFPs

Cameleons: Ca2+ indicators based on CaM + GFP mutants



Emission spectra of yellow cameleons ± Ca2+



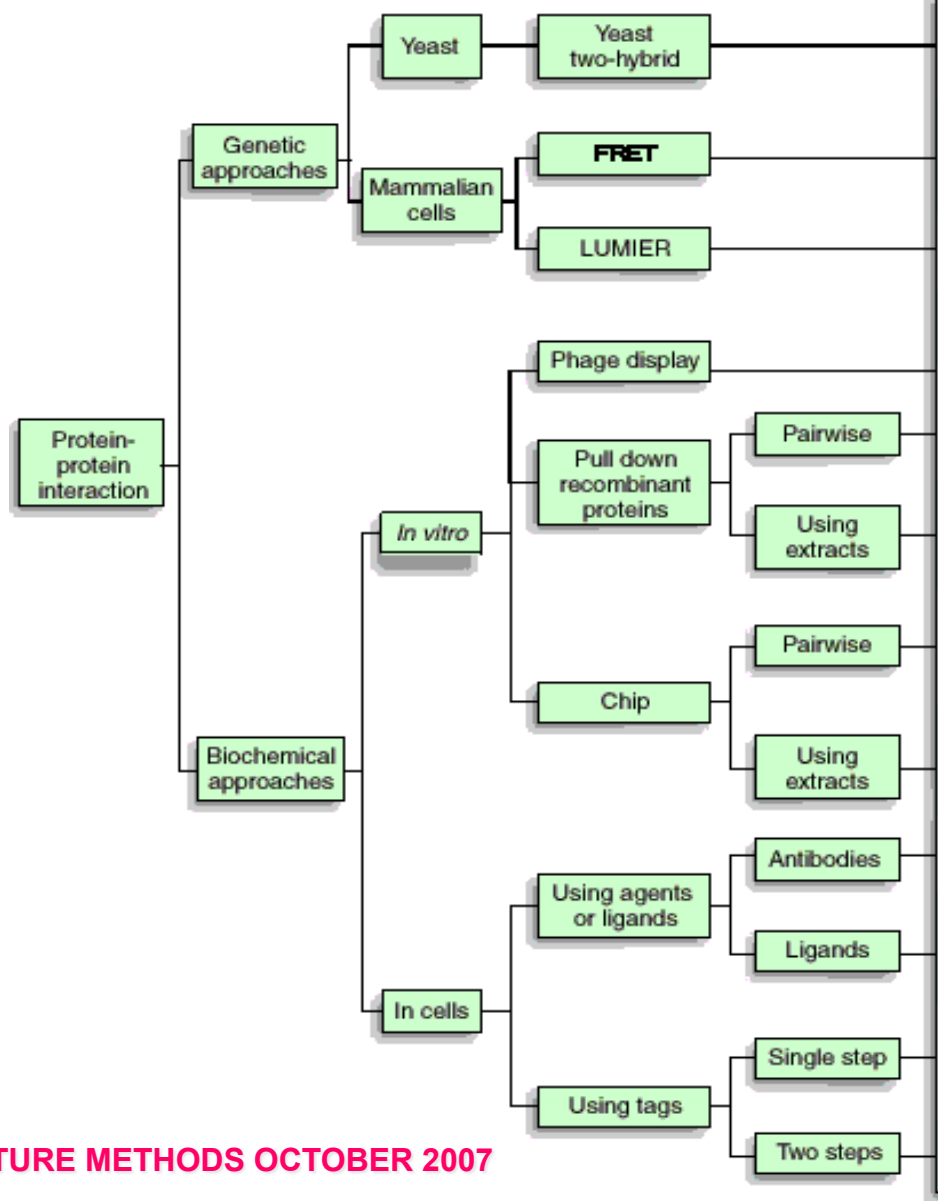
Identification of protein interactions in vitro

REMEMBER!!!

- The identification of protein interactions in vivo requires validation in vitro, as a high number of false positive results are generated.

- Furthermore, interacting partners need to be characterized in more detail with respect to post-translational modification, cofactor or other proteins in the complex.

Decision tree of options for the most common different protein-protein (or protein-ligand) interaction experimental strategies



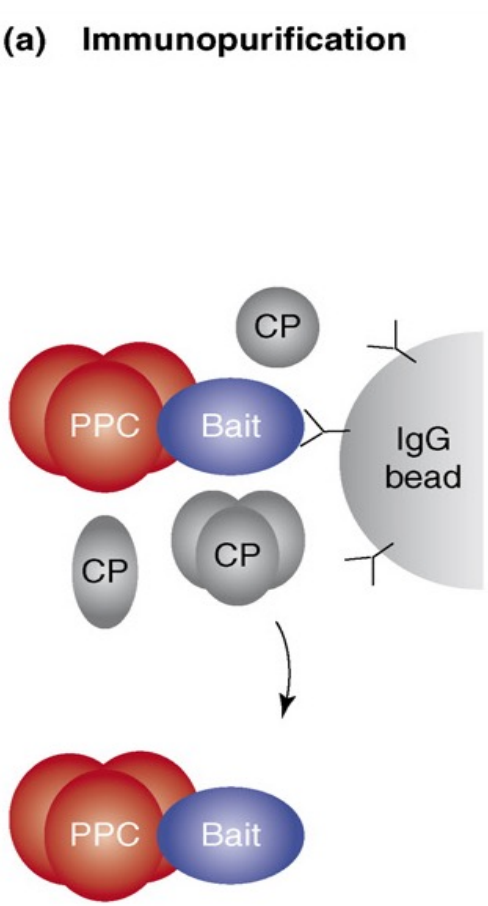
Variants	Ideal to obtain	Resource intensity	References
Three-hybrid; reverse 2H; membrane 2H (mammalian 2H)	Potential interactions, map domains	Low	30,40
Technologies based on fluorescence interference	*In vivo* evidence, temporal spatial resolution	Medium	31
Other enzymatic readout	Very sensitive detection by enymatic amplification	Low	32
Ribosomal display	Quantitative binary data, direct binding	Medium	43
Co-immuno-precipitation, *in vitro* translation	Fast evidence for *in vitro* interaction	Low	53
Co-immuno-precipitation	Various detection possibilities (usually dirty with MS)	Low	53
Several variants	Biochemical interaction evidence, biochemical reactions	High	44–50
Several variants	Biochemical interaction evidence, biochemical reactions, medical samples	High	44–50
Affibodies, minibodies	Endogenous complexes	Low to Medium	54
Aptamers, drugs, lectins metabolites, nucleic acids	Specific information on type of affinity	Medium	55
In vitro and *in vivo* tagging, BirA	Fast purification of protein complexes, can be scaled up	High	57,58
TAP, GS-TAP	Sturdy interactors, can be scaled up	High	60–62, 64,65

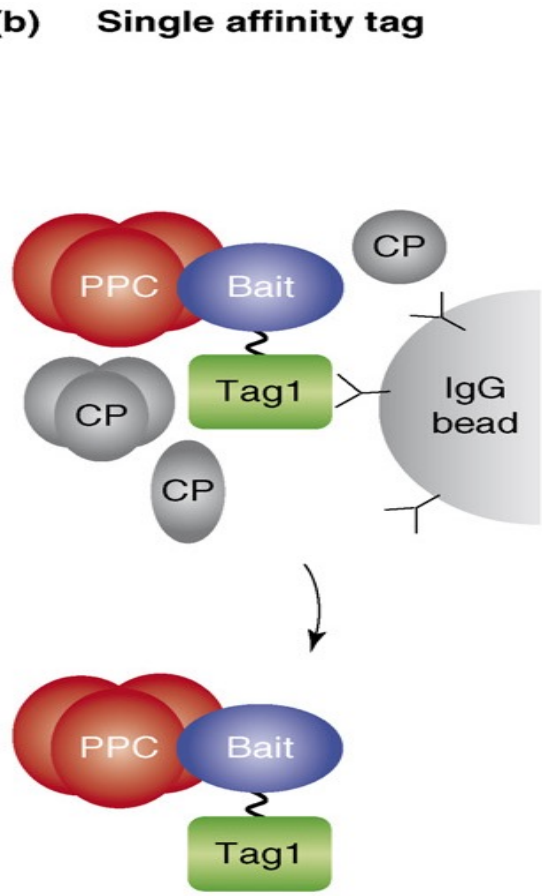
NATURE METHODS OCTOBER 2007

Affinity purification strategies

- The classic biochemical techniques for detecting protein interactions in vitro are immunoprecipitation and pull-down assays, both of which are based on *affinity purification* of a bait protein.

- Affinity-based purification (AP) methods typically result in protein preparations of suitable purity and reduced complexity that they can be interrogated efficiently by MS based protein identification approaches

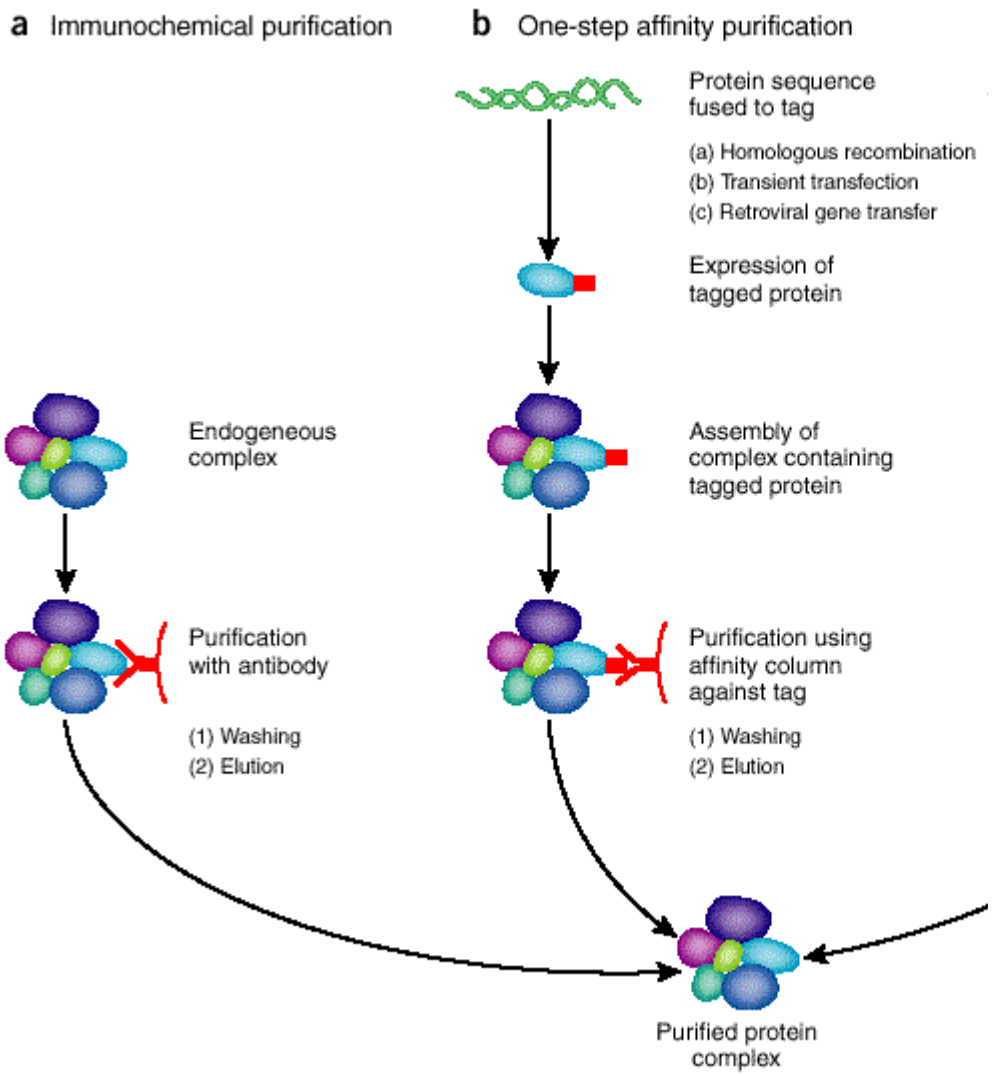




PPC: prey prot complex
CP: contaminating prot

Immunoprecipitation with bead-immobilized antibody directed against a bait protein of interest

Single-affinity tag with bead-immobilized antibody directed against a universal protein tag (Tag1);



(**a**)In immunochemical purification, the endogenous protein complex is precipitated using an antibody to the target protein, allowing protein-complex characterization without expression of a tagged protein.

(**b**) In one-step affinity purification, the purified protein complex is obtained by expression of the tagged construct in the cell, followed by specific binding and elution from an affinity column.

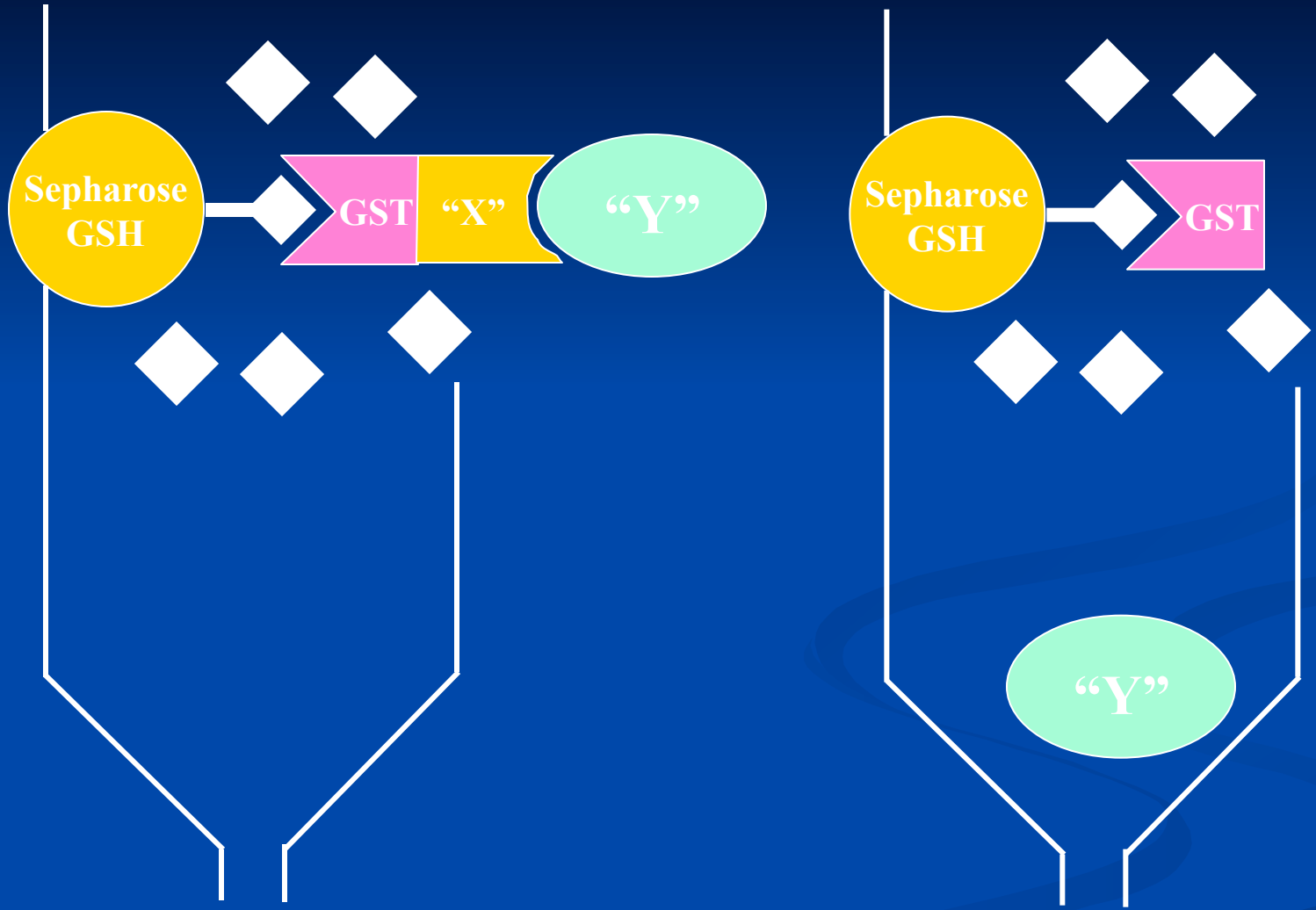
Pull-down assay: principle

- Is probably one of the widest spread techniques to identify biomolecular interactions;
- The assay monitors the ability of a ligand (bait), for example, a recombinant protein, a domain, a peptide bound to a matrix, to specifically capture proteins from a complex cell extract;
- The binding of the bait to the matrix can be achieved by chemical cross-linking, His-tag, GST-tag.

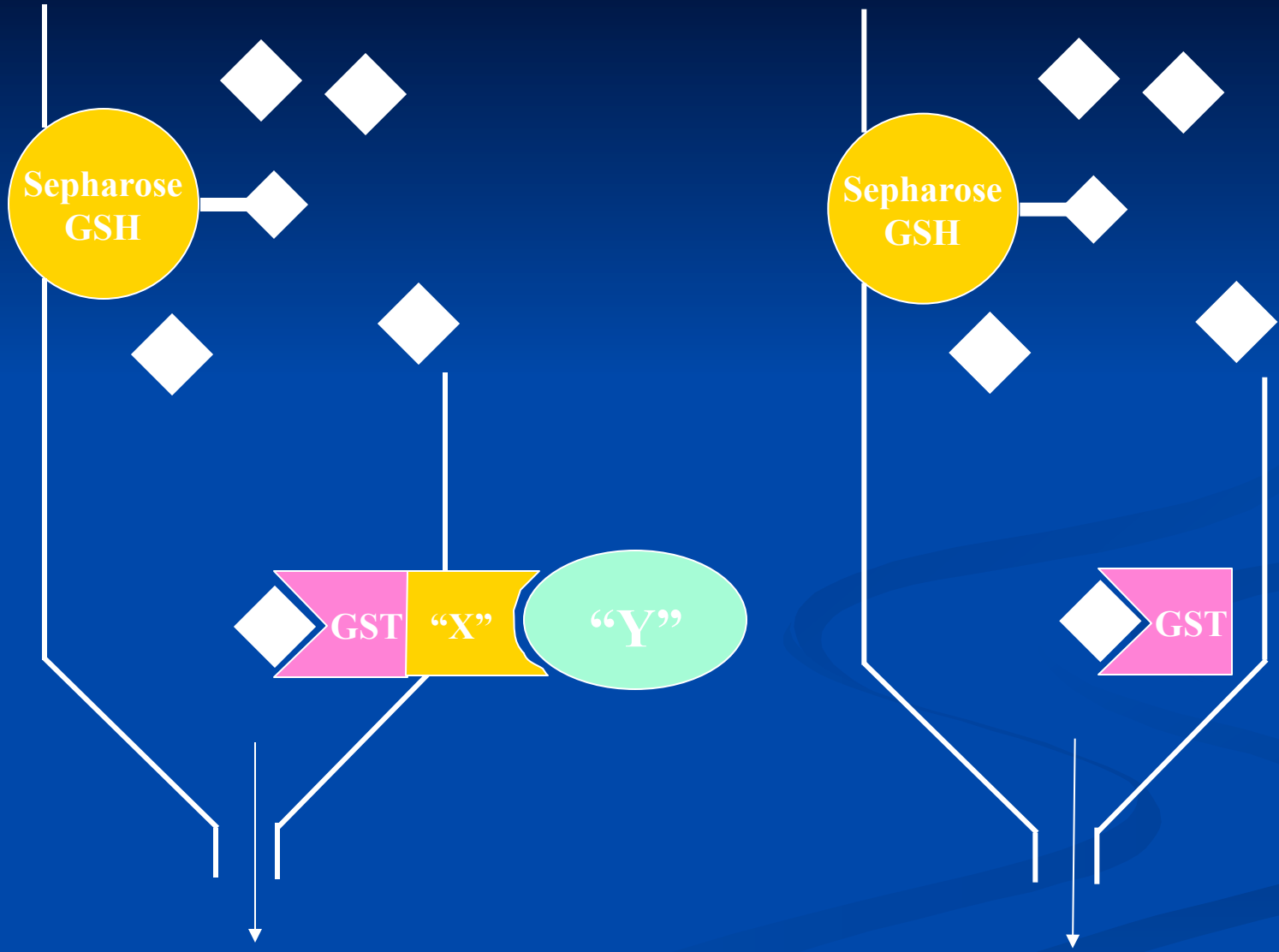
GST-Tag and His-Tag Pull-Down Assay

- . "Pull-down" is an affinity purification technique similar to immunoprecipitation, except that the antibody function of is replaced by some *other affinity system*.
- The affinity system can be either a GST-tagged protein that can be captured by glutathione agarose beads or a His-tagged protein that can be captured by metal chelate agarose beadst.
- The fusion-tagged protein acts as the "bait" to capture a putative binding partner (i.e., the "prey").
- In a typical pull-down assay, the immobilized bait protein is incubated with a cell lysate. After the prescribed washing steps, the 'interactors" are selectively eluted for analysis in-gel or by Western blot.

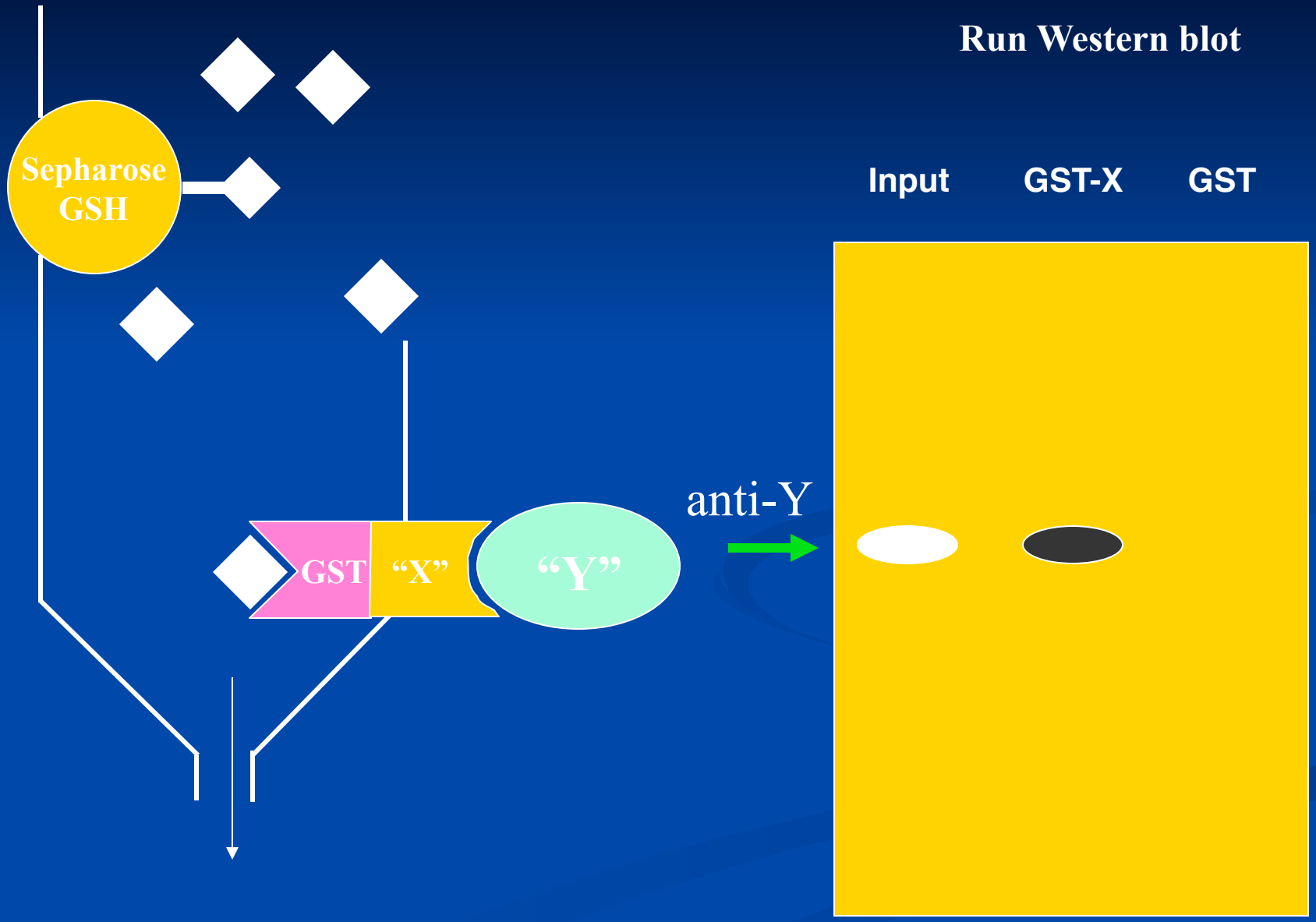
GST pull-down assay



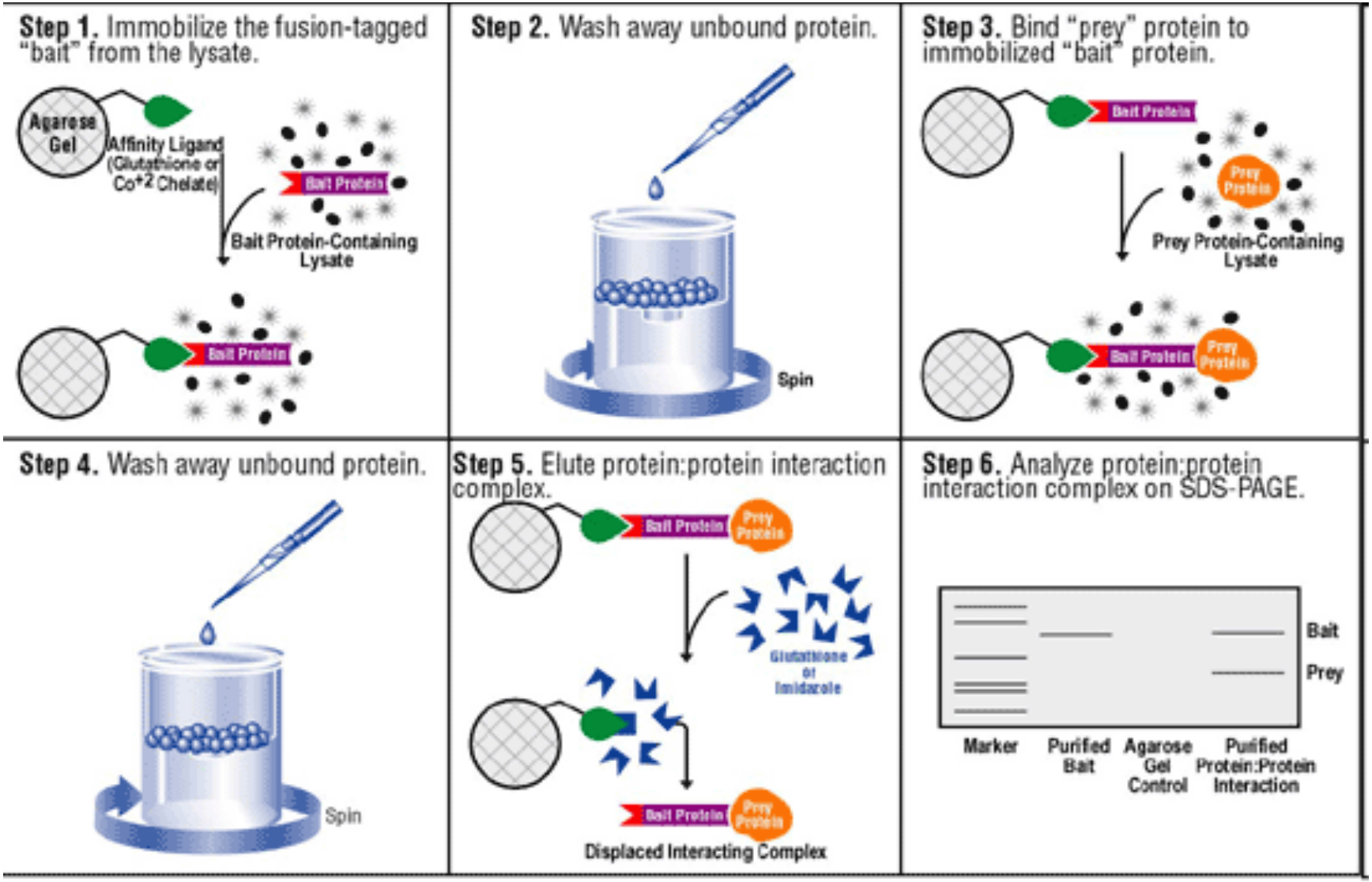
GST pull-down assay



GST pull-down assay



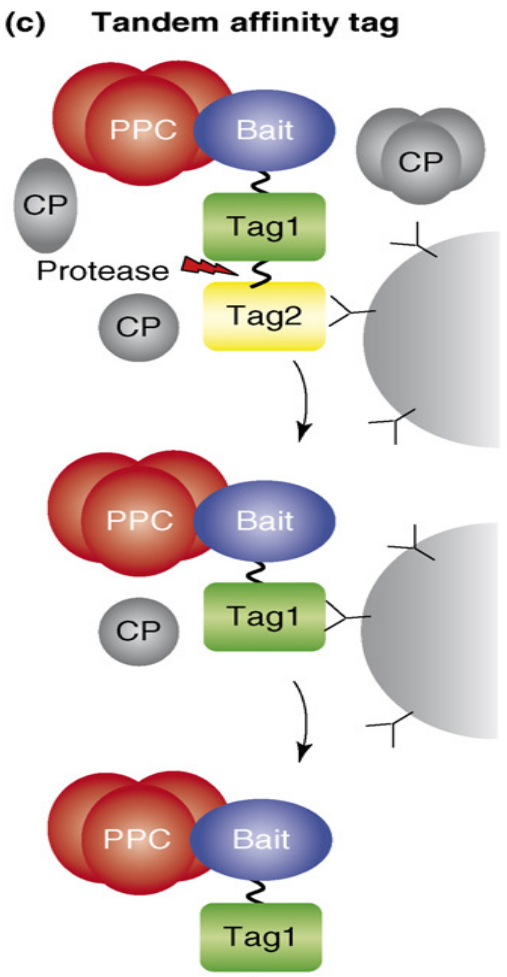
GST pull down assay

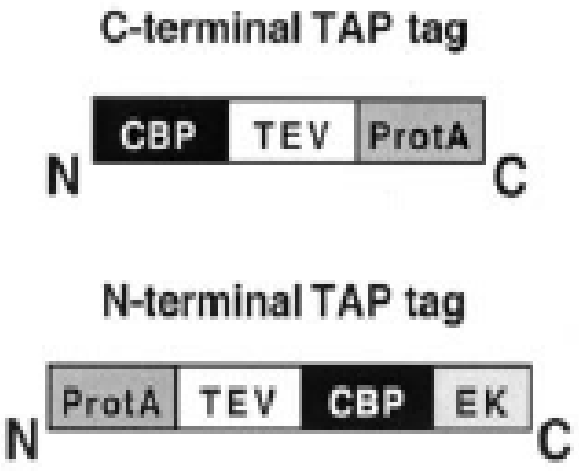


Tandem affinity purification TAP

- The use of *multiple* affinity tags permits two consecutive or ‘tandem’ affinity purification steps to be performed, usually under *very mild and selective* elution conditions.

- TAP is based on an affinity tag that is used for *two consecutive* steps of affinity purification under very mild and selective elution conditions



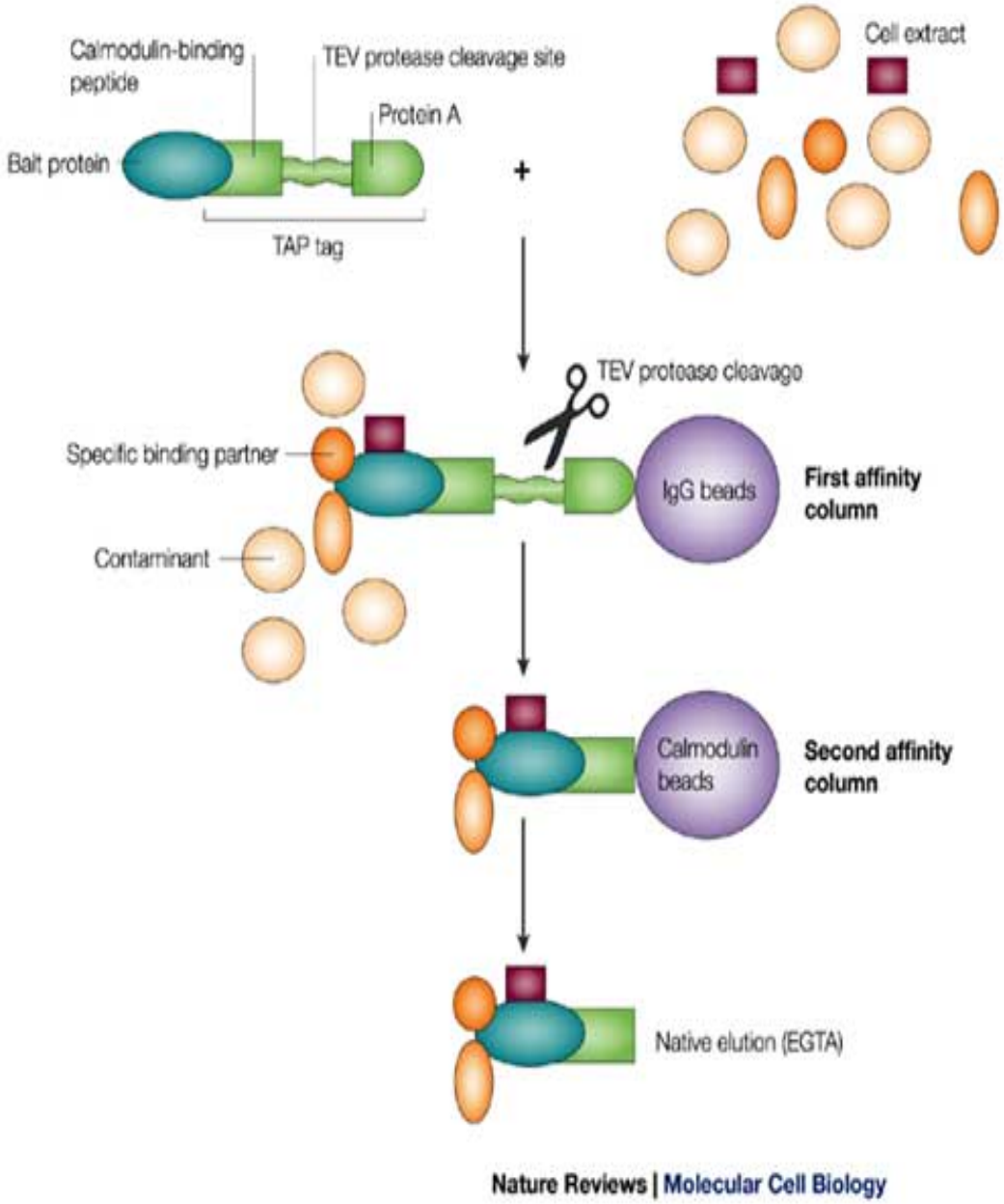


CBP: calmodulin binding protein

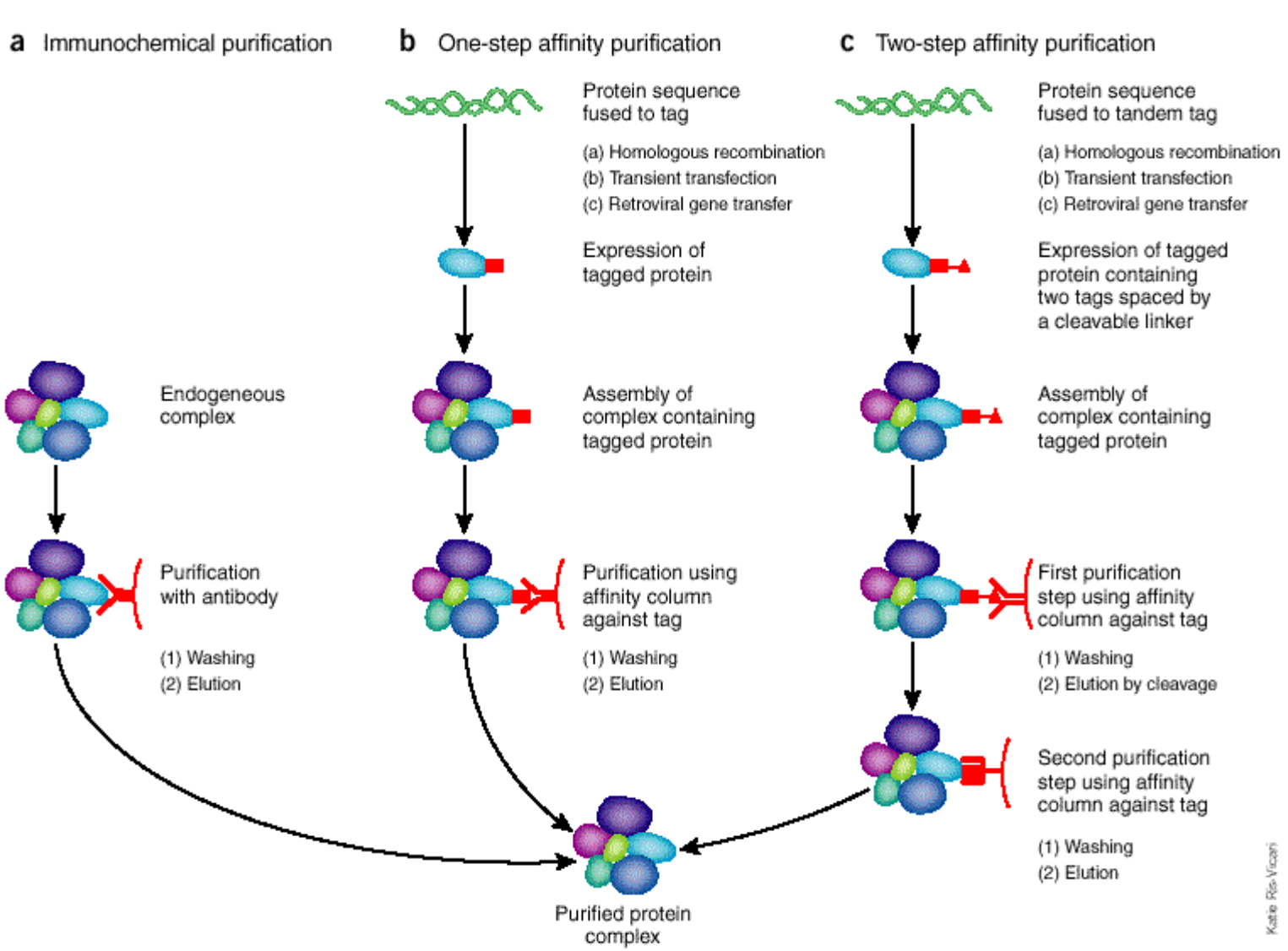
TEV: tobacco etch virus protease

ProtA: protein A

The TAP tag consists of three components: a CaM-binding peptide, a tobacco etch virus (TEV) protease cleavage site and Protein A as an immunoglobulin G (IgG)-binding domain



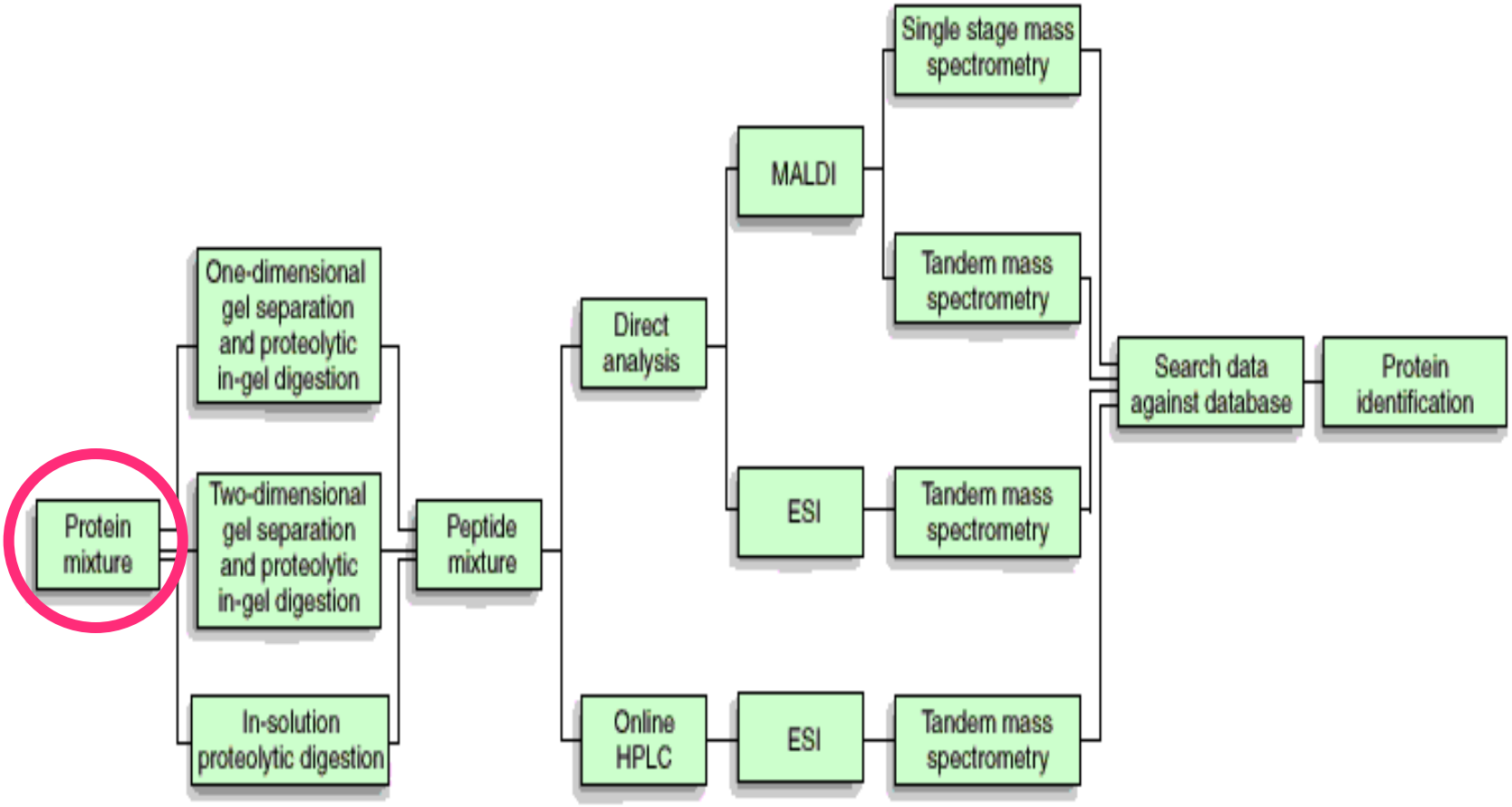
•TAP tag consists of three components: a CaM-binding peptide, a tobacco etch virus (TEV) protease cleavage site and Protein A as an (IgG)-binding domain.
•Cells are generated that contain TAP-tagged protein(s).
•Extracts are then prepared under mild conditions.
•The **first column** consists of IgG beads. TEV protease cleaves the immobilized multiprotein complexes.
•Another round of binding is carried out on a **second column** that consists of calmodulin beads. The native complex is then eluted by chelating calcium using EGTA.



In two-step affinity purification, two rounds of specific binding and specific elution assure a highly purified protein complex with little contaminating proteins at the cost of losing transient interactions.

Mass-spectrometric protein identification

- After chromatography experiments the purified proteins are separated by one-dimensional SDS-PAGE. Individual protein bands of interest are excised or the entire lane is cut into slices, followed by in-gel digestion with a specific protease such as trypsin to produce peptides for MS analysis


Protein mixture
One-dimensional gel separation and proteolytic in-gel digestion
Two-dimensional gel separation and proteolytic in-gel digestion
In-solution proteolytic digestion
Peptide mixture
Direct analysis
Online HPLC
MALDI
ESI
ESI
Single stage mass spectrometry
Tandem mass spectrometry
Tandem mass spectrometry
Tandem mass spectrometry
Search data against database
Protein identification
Karin Ris-Vicari

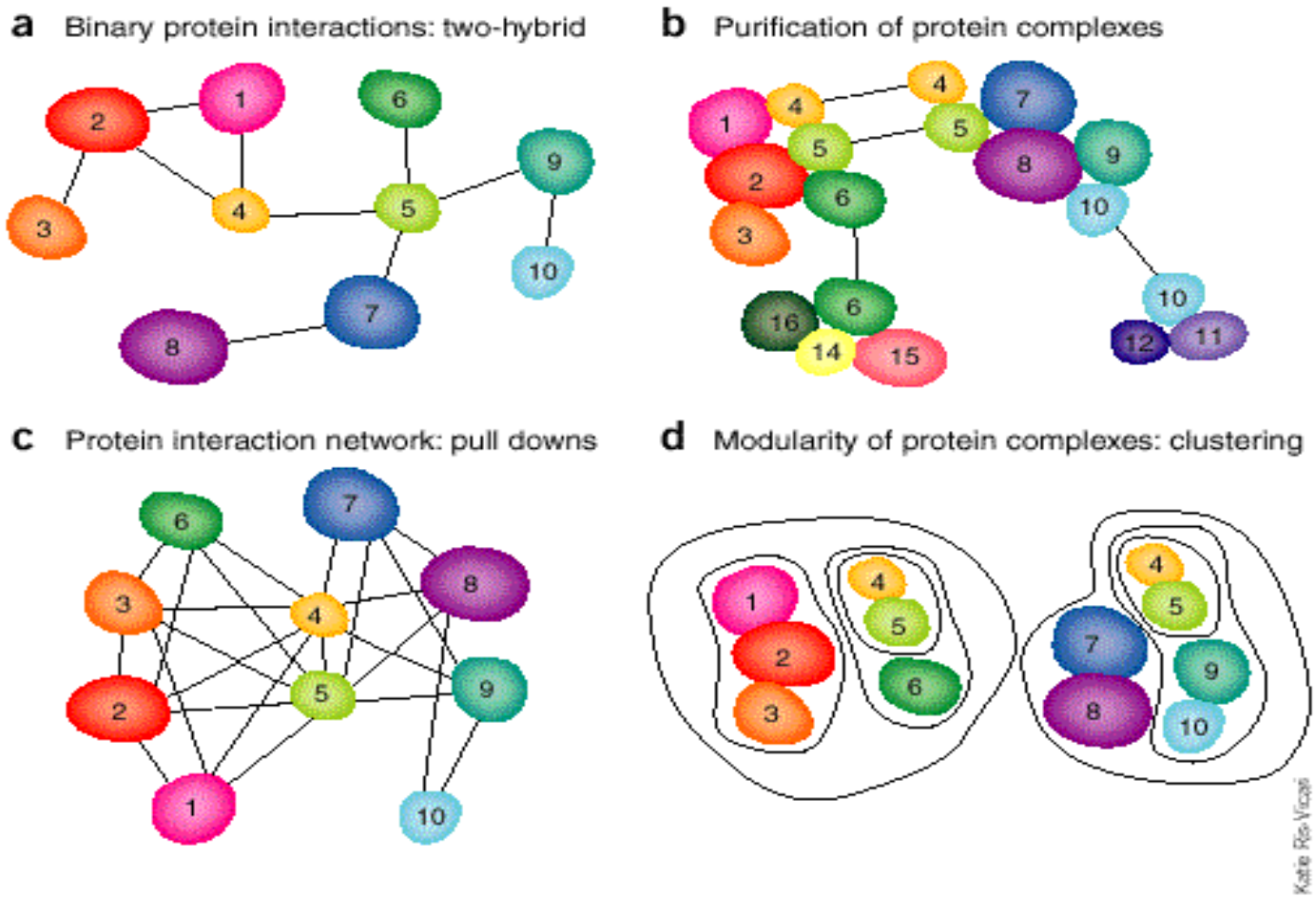


Illustration of the types of protein networks that can be elucidated with different experimental approaches. (**a**) Binary protein interactions are typically obtained from two-hybrid assays. (**b**) The purification of protein complexes leads to a corresponding protein network where two protein complexes are connected by sharing one or more proteins, indicated by lines. (**c**) Protein interaction networks can be generated from protein pulldown assays. The matrix model represents purified protein assemblies as if interacting all with each other. (**d**) Statistical analysis and clustering demonstrates the modularity of protein complexes.