

CG990 – Methods in proteomics

# Protein-protein interaction analysis

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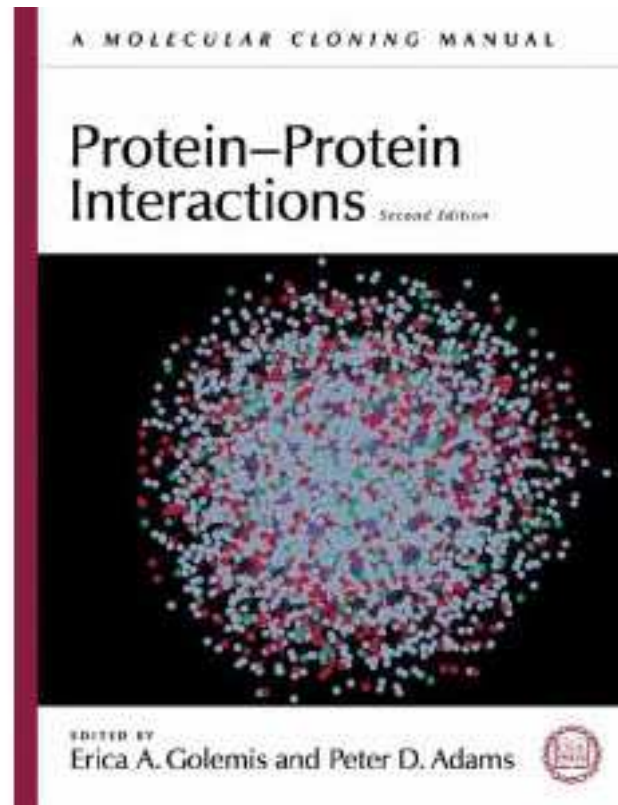
[jpalecek@sci.muni.cz](mailto:jpalecek@sci.muni.cz)



# Information sources

Golemis a Adams: Protein-protein interactions ...

**... the newest methods from current literature ...**



Databases of protein-protein interactions: <http://string-db.org/newstring.cgi> ...  
<http://www.ebi.ac.uk/intact/?conversationContext=1>



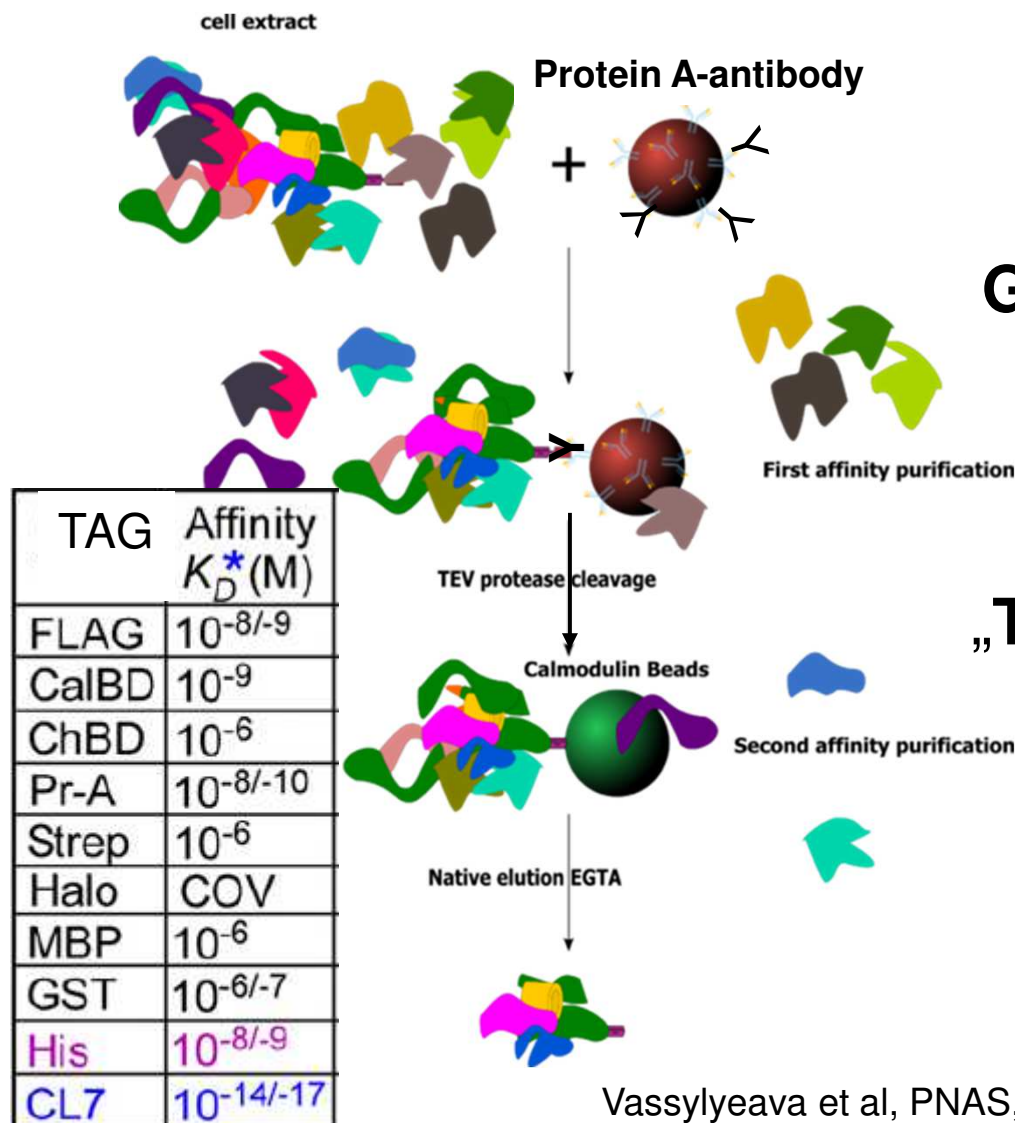
# Protein-protein interaction analysis

- Matrix/beads-based:
  - **pull-down assay**
  - **co-purification – gel filtration**
  - **co-immunoprecipitation**
    - **Analysis of protein domains**
    - **Analysis of interaction surfaces**
    - **Peptide libraries**
- Hybrid-based: Y2H (yeast 2-hybrid), BiFC ...
- Proximity-based: PLA, BioID ...
- MS-based: crosslink, D/H-exchange ...
- Quantitative methods: SPR, ITC ...
- Structural methods: co-crystalization, NMR ...
- Genetic methods: synthetic lethality ...
- Bioinformatics methods: databases, docking ...



# Co-immunoprecipitation

beads/matrix/particles are used to precipitate bait protein with its bound partners



Common tags for co-immunoprecipitation assay:  
**GFP, FLAG, myc, HA, T7, PK**

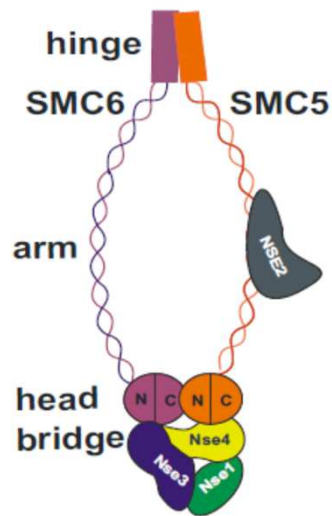
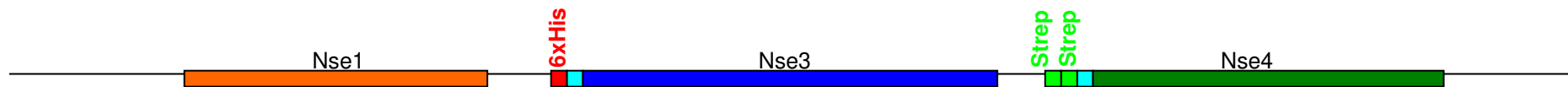
These tags are recognized by specific antibodies (commercially available)

TAP-tag (can be used as well):  
 „Tandem-affinity purification“ = immunoglobulin tag + calmodulin tag  
 (usually used to purify complexes)

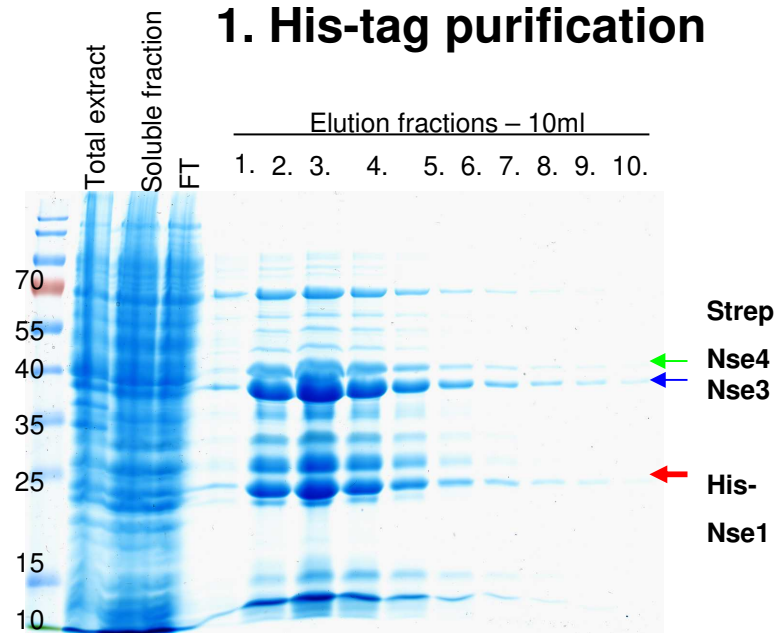
Risk of indirect interactions  
 (in same organism)

# co-purification

Strong interactions (protein complexes) can be recognized during the purification of the proteins (similar approach to pull-down assay) – proteins can be co-purified through different tags and using gel filtration



## 1. His-tag purification

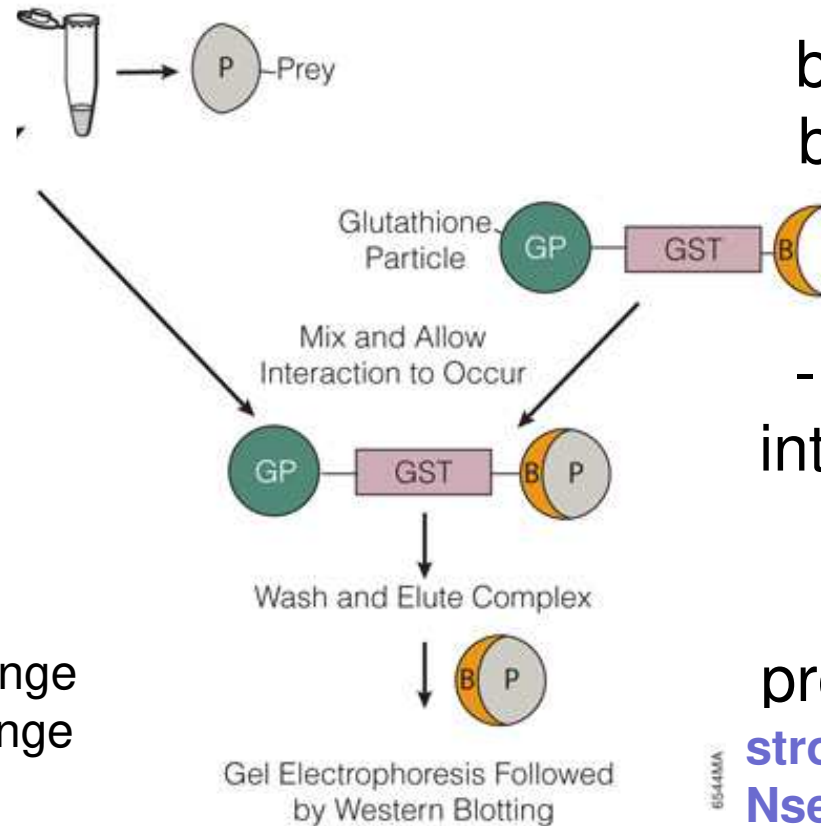


Common tags:  
**GST** (glutathione)  
**Streptactin** (biotin-streptavidin)  
**MBP** (maltose)  
**S-tag** (protein S)  
...

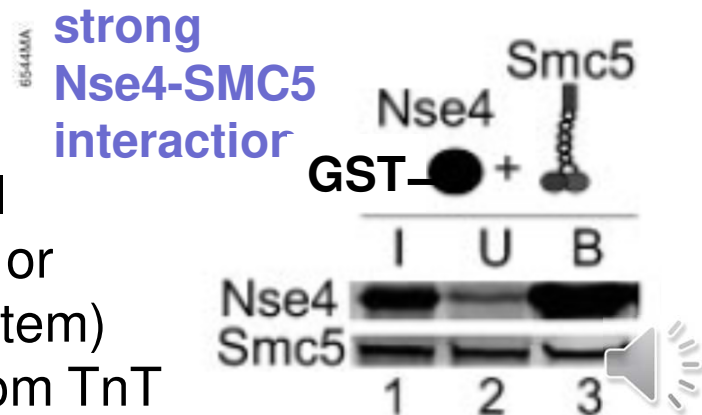


# Pull-down

1. tagged (e.g. GST) protein (*bait*) is bound to (glutathione) beads/particles (GP)
2. Partner protein (*prey*) is added - if the bait and prey interact then prey will be pulled down (together with bait protein) on the beads



strong interaction – both proteins can be at equal concentrations (expressed/purified from bacteria or expressed/labelled in TnT in vitro expression system)  
weak interaction – bait overexpressed vs prey from TnT



# Pull-down

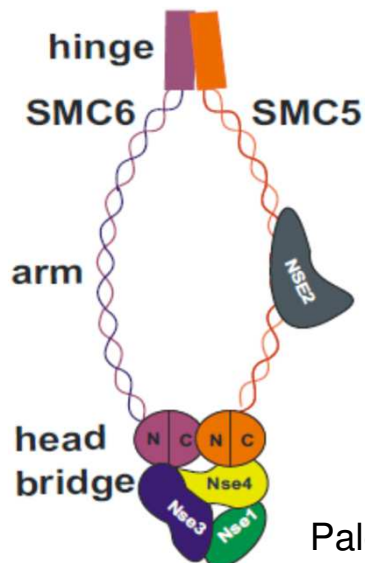
Similar to co-purification/precipitation

***in vitro*** TNT (Transcription and Translation) system

methionin S<sup>35</sup>

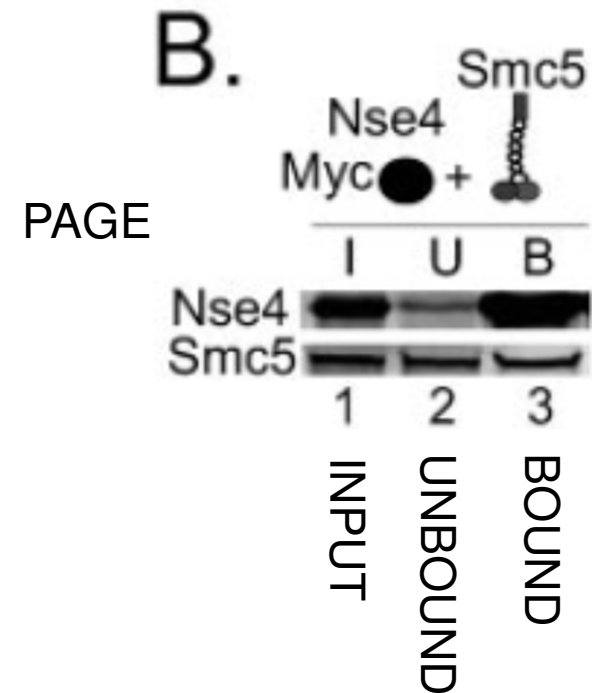
advantages:

- No need to purify proteins
- No need for antibodies
- No toxicity in cell
- Higher solubility ...



Palecek et al, JBC, 2006

**Strong interaction** – both proteins  
v TNT (nM-pM)



Nse4 protein well expressed in TNT  
(insoluble in cells)



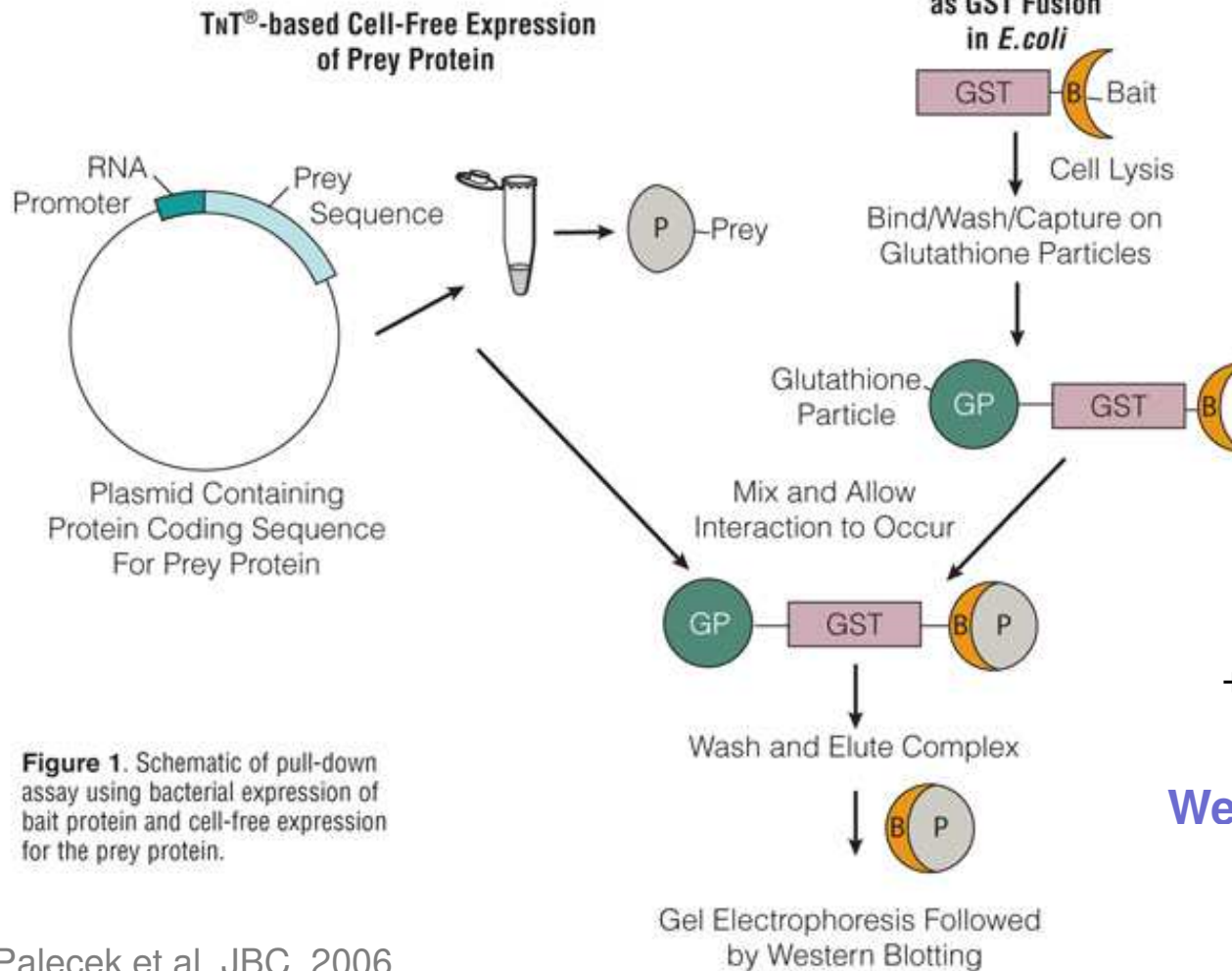
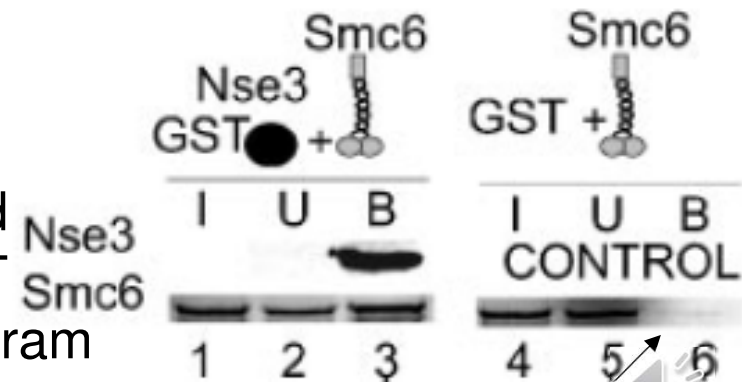


# Pull-down

Common tags for pull-down assay:  
**GST** (glutathione)  
**Streptactin** (biotin-streptavidin)  
**MBP** (maltose)  
**S-tag** (protein S)  
 ...

+ tags recognized by antibodies  
 (see co-immunoprecipitation)

## Weak Nse3-Smc6 interaction



Palecek et al, JBC, 2006

- GST-Nse3 expressed in bacteria – (pre)purified on glutathione particles – detected with anti-GST
- Smc6 expressed in TnT (radiolabeled) – radiogram detection (neither tag nor antibody needed)
- control nonspecific binding of prey (Smc6 does not bind to GST-bound beads)



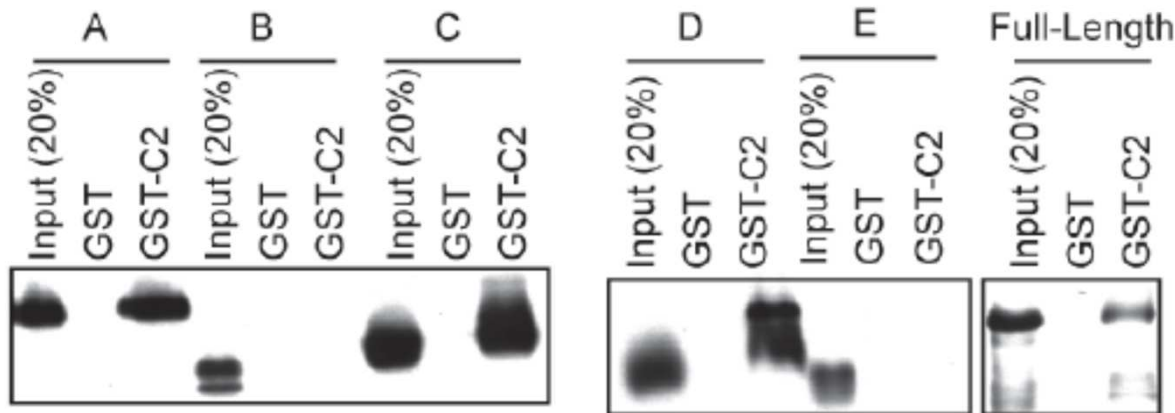
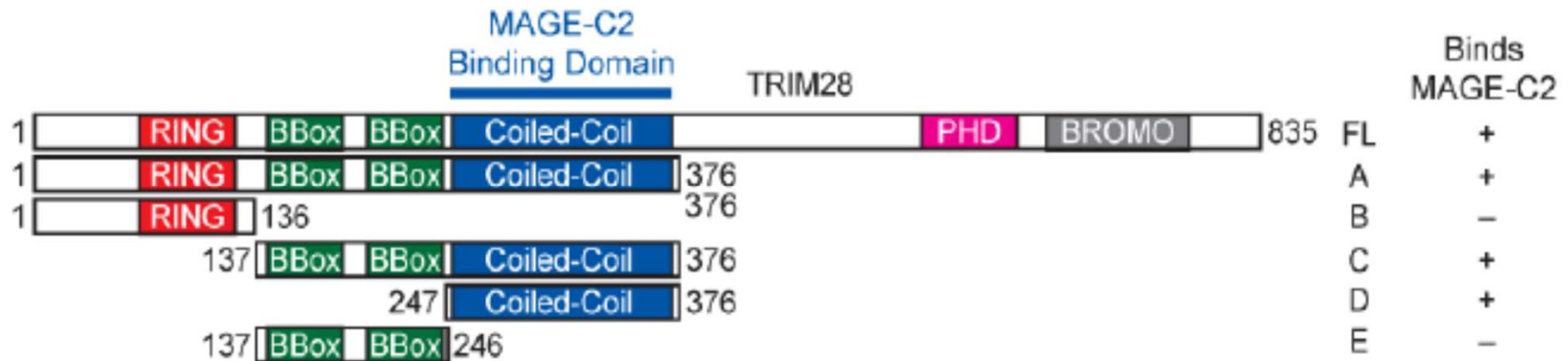
# Protein-protein interaction analysis

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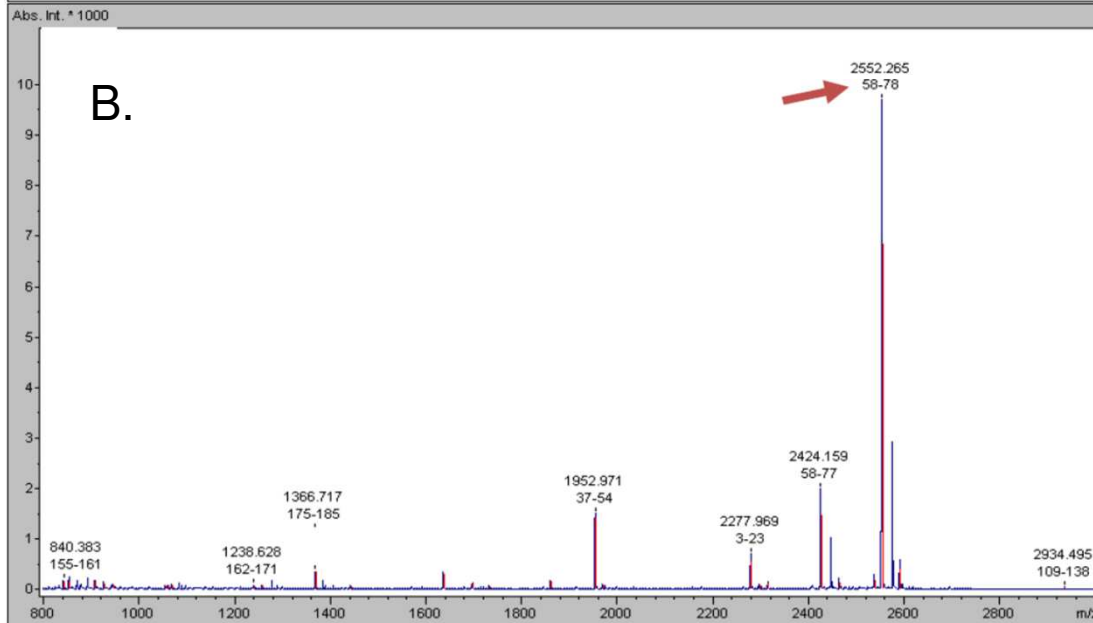
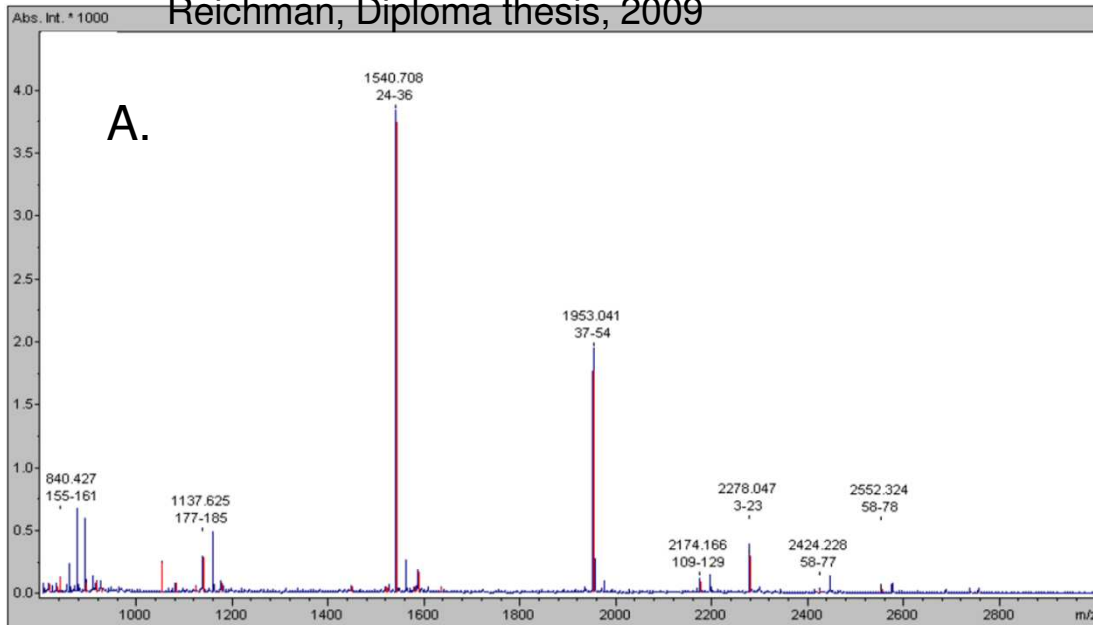
# Characterization of binding domain

Proteins interact via their domains (motifs) – analyze domain composition of your protein – prepare fragments of your protein defined by domain boundaries – test them in pull-down, co- ...



Truncated versions of TRIM28 were used to determine MAGEC2-binding domain (only fragments A, C, D overlapping in coiled-coil domain do interact)





10	20	30	40	50	60	70	80
MRGSHHHHH	GMASNTGGQQ	MGRDLVDDDD	KDPSSRSAAG	TNEFMALIA	QLQRQFRDYT	ISLYQQGFLD	DQFTELKQLQ
90	100	110	120	130	140	150	160
DDGSPDFVSE	VLSLFFEDCV	KLISNARAL	DTTGTVDFSQ	VGASVHQLKG	SSSSVGARRV	KTLCVSPFKEC	CEAKNYEGCV
170	180	190	200	210			
RCLQQVDIEY	KALKTKLQDM	FNLEKQIIQA	GGIVPQVDIN				

# Characterization of binding regions

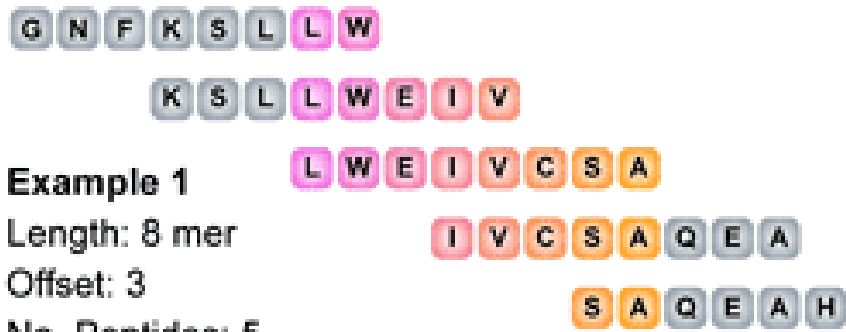
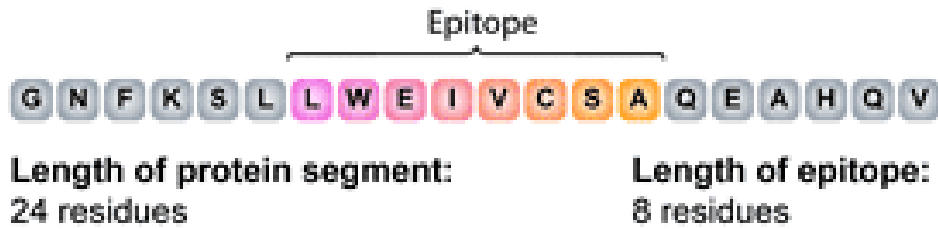
Proteins interact via their domains (motifs) – (sometimes) only fragments of the domain can interact (can be precipitated)

A. Peptide coverage of the protein

B. Peptide enrichment after co-immunoprecipitation with the bait protein

(red arrow points to enriched/bound peptide in MS spectra)

# Peptide libraries – region definition



**Example 1**  
 Length: 8 mer  
 Offset: 3  
 No. Peptides: 5

ELISA

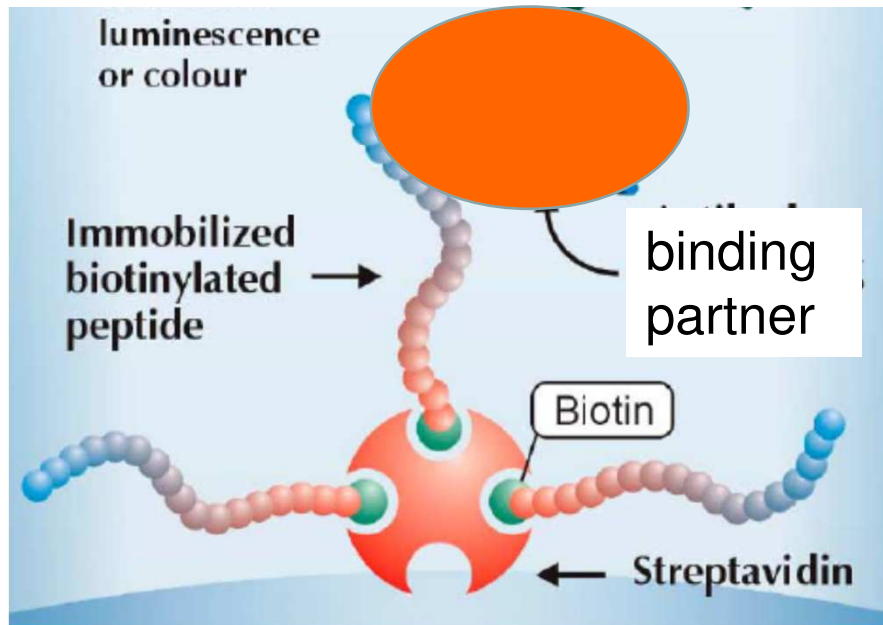
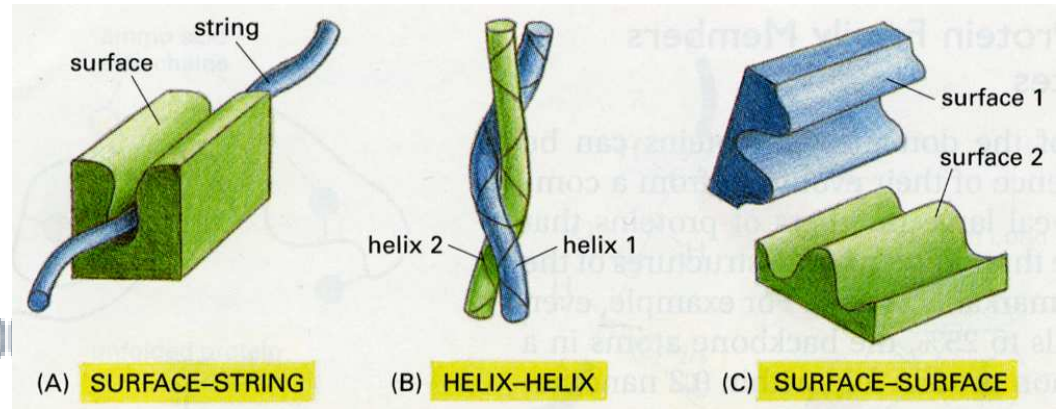
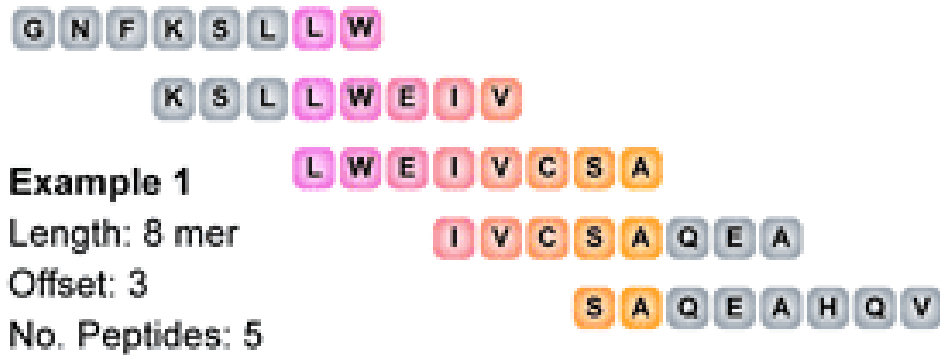
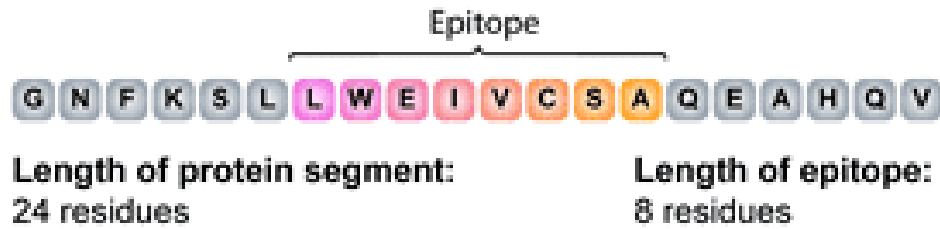


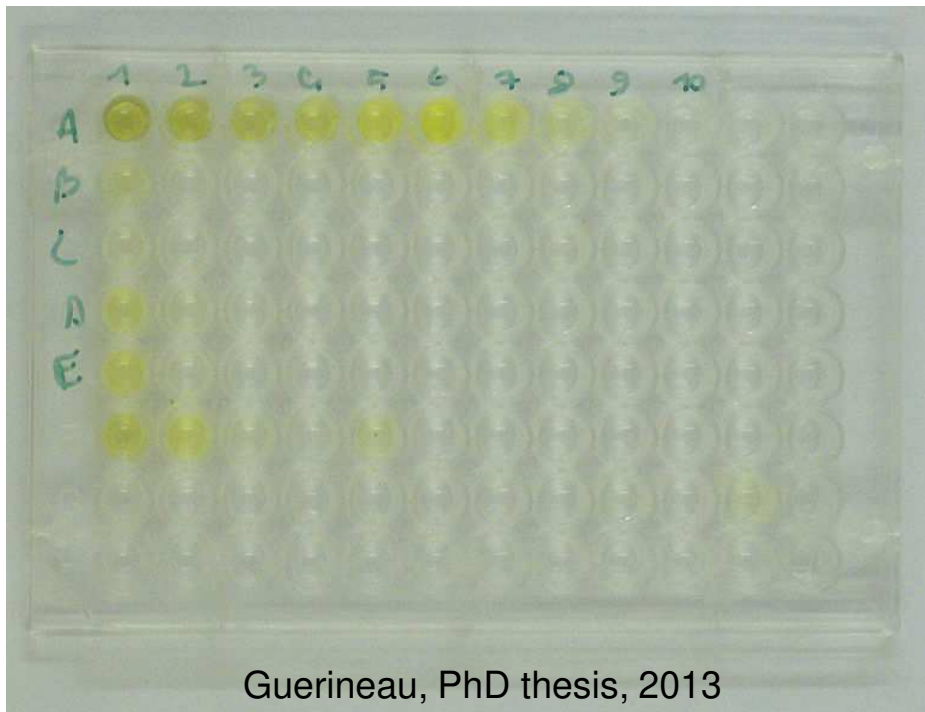
Figure 1: An ELISA using biotinylated peptides and coated plates

peptide library can be synthesized (with conjugated biotin tag) and used in pull-down or ELISA assays (similar to antigen-epitope mapping)

wells are coated with streptavidin which anchors biotinylated peptides – binding partner interacts with peptide – antibody against the partner with conjugated enzyme (or 2<sup>nd</sup> antibody-enzyme) is applied - luminescence or colour detection



ELISA



Guerineau, PhD thesis, 2013

## Peptide libraries – region definition

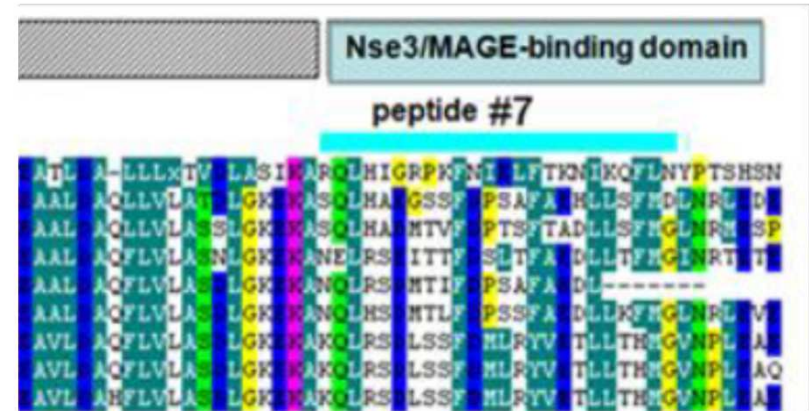
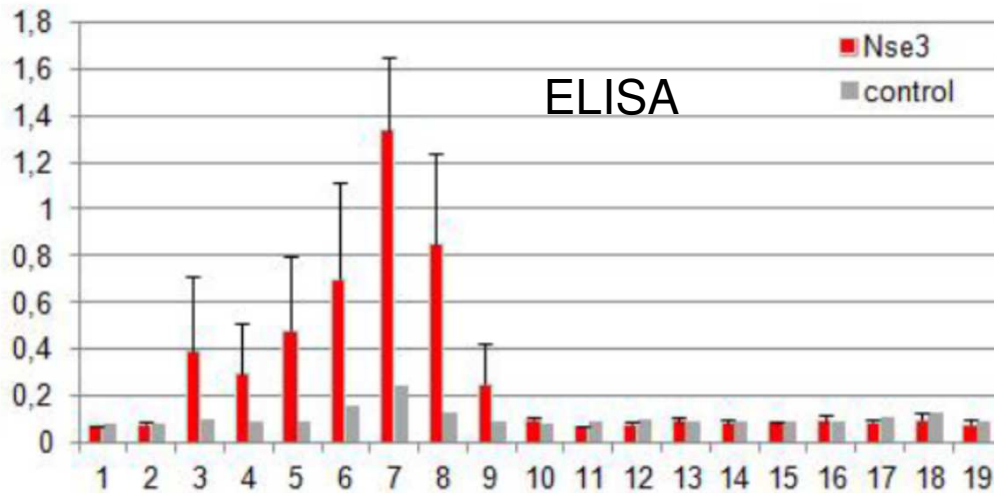
Proteins interact via their domains (motifs) – (sometimes) only fragments of the domain can interact (can be precipitated) - peptide library can be synthesized (with conjugated biotin tag) and used in pull-down or ELISA assays (similar to antigen-epitope mapping)

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# Peptide libraries



## peptide sequence

peptide #1	DAPTEATLDALLTKTVDLASIKAR
peptide #2	-----EATLDALLTKTVDLASIKARQLHI
peptide #3	-----DALLTKTVDLASIKARQLHIGRPK
peptide #4	-----LTKTVDLASIKARQLHIGRPKFNIE
peptide #5	-----VDLASIKARQLHIGRPKFNIELFTK
peptide #6	-----SIKARQLHIGRPKFNIELFTKNIKQ
peptide #7	-----RQLHIGRPKFNIELFTKNIKQFLNY
peptide #8	-----IGRPKFNIELFTKNIKQFLNYPTSH
peptide #9	-----KFNIELFTKNIKQFLNYPTSHSNVT
peptide #10	-----ELFTKNIKQFLNYPTSHSNVTRIQE
peptide #11	-----KNIKQFLNYPTSHSNVTRIQEIDTA
peptide #12	-----QFLNYPTSHSNVTRIQEIDTAW SRL
peptide #13	-----YPTSHSNVTRIQEIDTAW SRLGKLA
peptide #14	-----HSNVTRIQEIDTAW SRLGKLASNCE
peptide #15	-----TRIQEIDTAW SRLGKLASNCEKQPA
peptide #16	-----EIDTAW SRLGKLASNCEKQPASLNL
peptide #17	-----AWSRLGKLASNCEKQPASLNL MVGP
peptide #18	-----LGKLASNCEKQPASLNL MVGPLSFR

25 amino acids long (18) peptides library with 4 amino acids overlap (covering 90 amino acids region of Nse4 protein) – peptides #6-8 bind with highest affinity, suggesting the core of the binding region



WT peptide	QRNPHRVLDLILTFTIALTAS
peptide #1	<b>A</b> RNPHRVLDLILTFTIALTAS
peptide #2	Q <b>A</b> NPHRVLDLILTFTIALTAS
peptide #3	QR <b>A</b> PHRVLDLILTFTIALTAS
peptide #4	QRN <b>A</b> H RVLDLILTFTIALTAS
peptide #5	QRNP <b>A</b> RVLDLILTFTIALTAS
peptide #6	QRNPH <b>A</b> VLDLILTFTIALTAS
peptide #7	QRNPHR <b>A</b> DLILTFTIALTAS
peptide #8	QRNPHRV <b>A</b> LILTFTIALTAS
peptide #9	QRNPHRV <b>D</b> ADILTFTIALTAS
peptide #10	QRNPHRV <b>L</b> AILTFTIALTAS
peptide #11	QRNPHRV <b>L</b> DAL TFTIALTAS
peptide #12	QRNPHRV <b>L</b> DIATFTIALTAS
peptide #13	QRNPHRV <b>L</b> DILAF TIALTAS
peptide #14	QRNPHRV <b>L</b> DILTAI TIALTAS
peptide #15	QRNPHRV <b>L</b> DILTFAI TIALTAS
peptide #16	QRNPHRV <b>L</b> DILTF TAAI TIALTAS
peptide #17	QRNPHRV <b>L</b> DILTF TIAA TIALTAS
peptide #18	QRNPHRV <b>L</b> DILTF TIALA A S
peptide #19	QRNPHRV <b>L</b> DILTF TIALTA A

## Peptide libraries – surface mapping

Proteins interact via their domains (motifs) – amino acids essential for the interaction can be identified (via mutational analysis – e.g. alanine substitutions = “alanine scan”)

- peptide library or yeast two-hybrid system (see below) can be used

21 amino acids long (20) peptides library with single amino acid alanine substitution (covering every non-Ala amino acid)



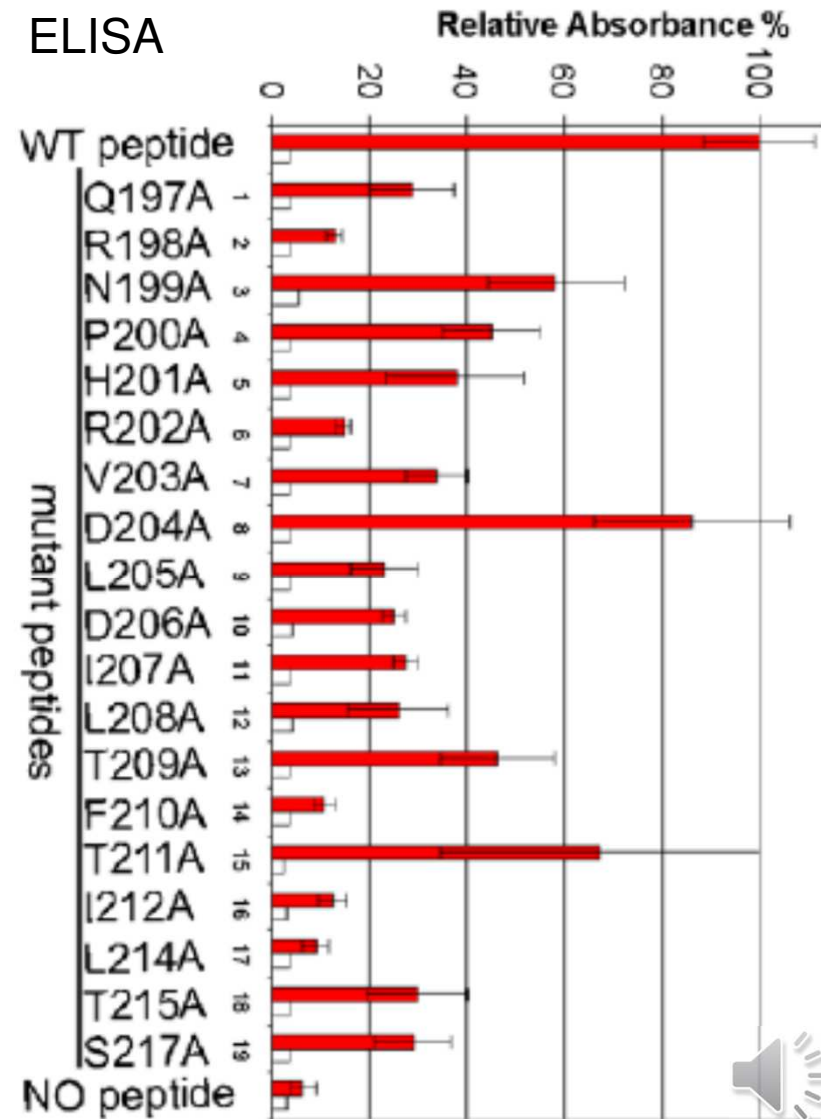


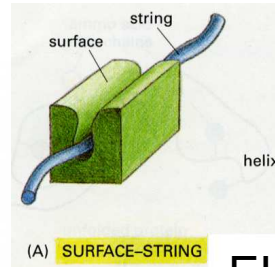
	197	217
WT peptide	QRNPHRV	LDILTFTIALTAS
peptide #1	<b>A</b> RNPHRV	LDILTFTIALTAS
peptide #2	Q <b>A</b> NPHRV	LDILTFTIALTAS
peptide #3	QR <b>A</b> PHRV	LDILTFTIALTAS
peptide #4	QRN <b>A</b> HRV	LDILTFTIALTAS
peptide #5	QRNP <b>A</b> RV	LDILTFTIALTAS
peptide #6	QRNP <b>H</b> AV	LDILTFTIALTAS
peptide #7	QRNP <b>H</b> RA	LDILTFTIALTAS
peptide #8	QRNP <b>H</b> RV	<b>A</b> LDILTFTIALTAS
peptide #9	QRNP <b>H</b> RV	<b>D</b> ILFTFTIALTAS
peptide #10	QRNP <b>H</b> RV	<b>L</b> AILTFTIALTAS
peptide #11	QRNP <b>H</b> RV	<b>D</b> ALFTFTIALTAS
peptide #12	QRNP <b>H</b> RV	<b>D</b> IL <b>A</b> TFTIALTAS
peptide #13	QRNP <b>H</b> RV	<b>D</b> IL <b>L</b> AFTIALTAS
peptide #14	QRNP <b>H</b> RV	<b>D</b> IL <b>T</b> AFTIALTAS
peptide #15	QRNP <b>H</b> RV	<b>D</b> IL <b>T</b> F <b>A</b> IALTAS
peptide #16	QRNP <b>H</b> RV	<b>D</b> IL <b>T</b> F <b>T</b> A <b>A</b> LTAAS
peptide #17	QRNP <b>H</b> RV	<b>D</b> IL <b>T</b> F <b>T</b> I <b>A</b> A <b>T</b> AS
peptide #18	QRNP <b>H</b> RV	<b>D</b> IL <b>T</b> F <b>T</b> I <b>A</b> L <b>A</b> AS
peptide #19	QRNP <b>H</b> RV	<b>D</b> IL <b>T</b> F <b>T</b> I <b>A</b> L <b>T</b> A <b>A</b>

Guerineau, PLoS One, 2012

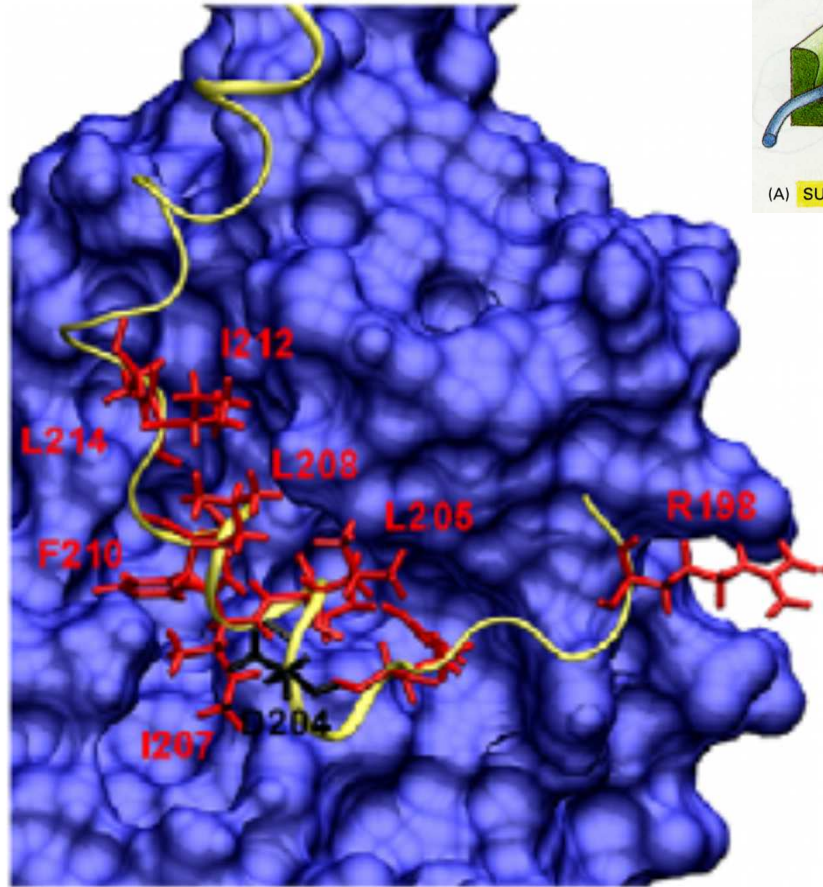
## Peptide libraries – alanine scan

ELISA



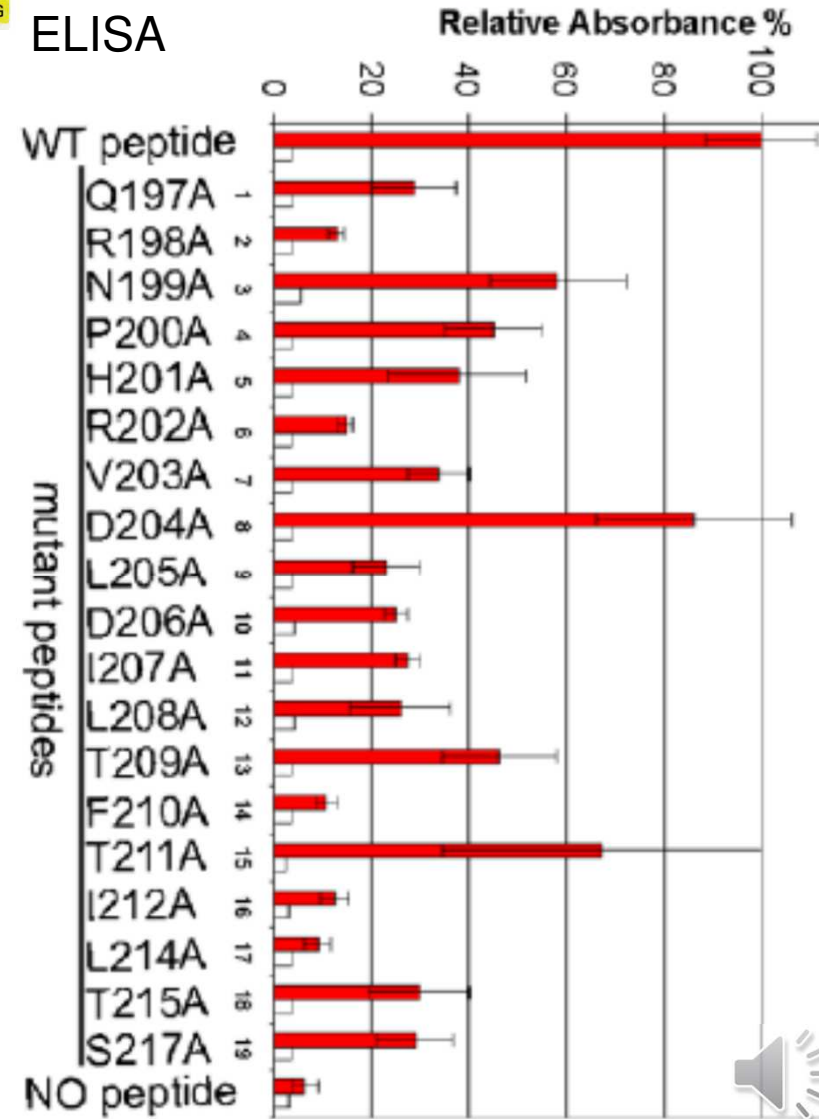


# Peptide libraries – surface mapping



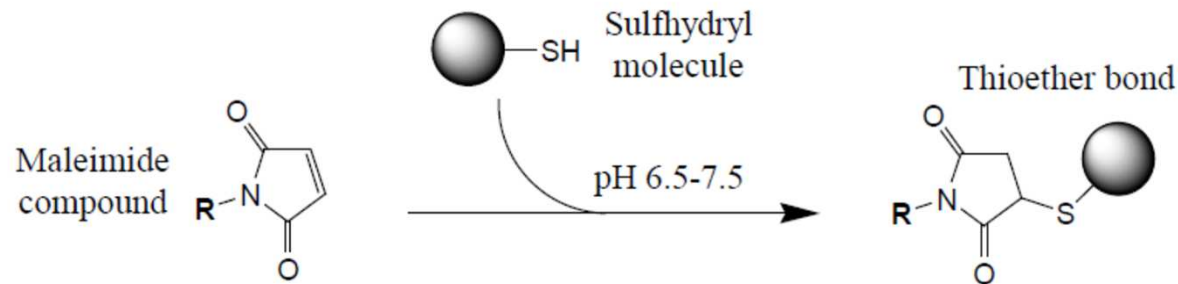
Helical peptide is sitting in the pocket of the partner protein – most peptide residues are in contact (red labeled) with the pocket (so, their mutations reduced the mutant peptide affinity), while the D204 (black labeled) residue is exposed to solvent

## ELISA



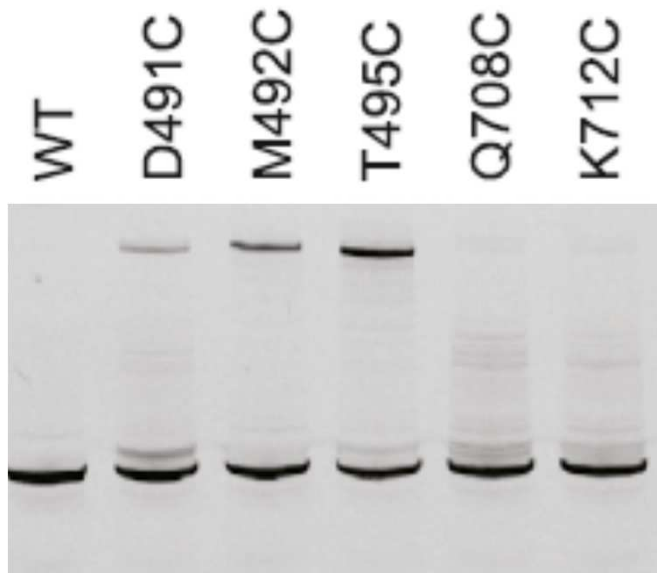
# Mapování interakcí - crosslinking

- maleimid reacts with sulph-hydryl group of Cys (covalent bond)
- directed crosslink (low abundant Cys)

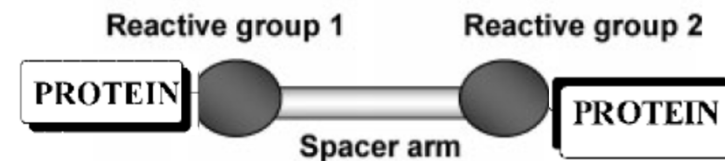
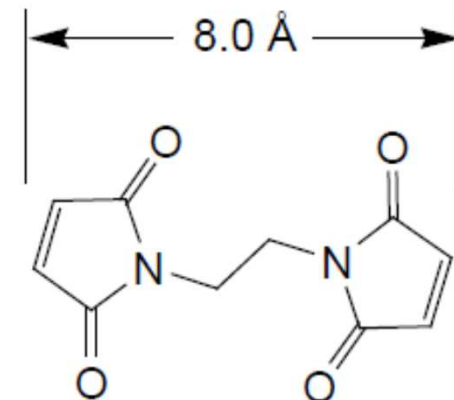


BMOE, bis(maleimido)ethane.

- detection on SDS-PAGE



intensity correlates  
With distance



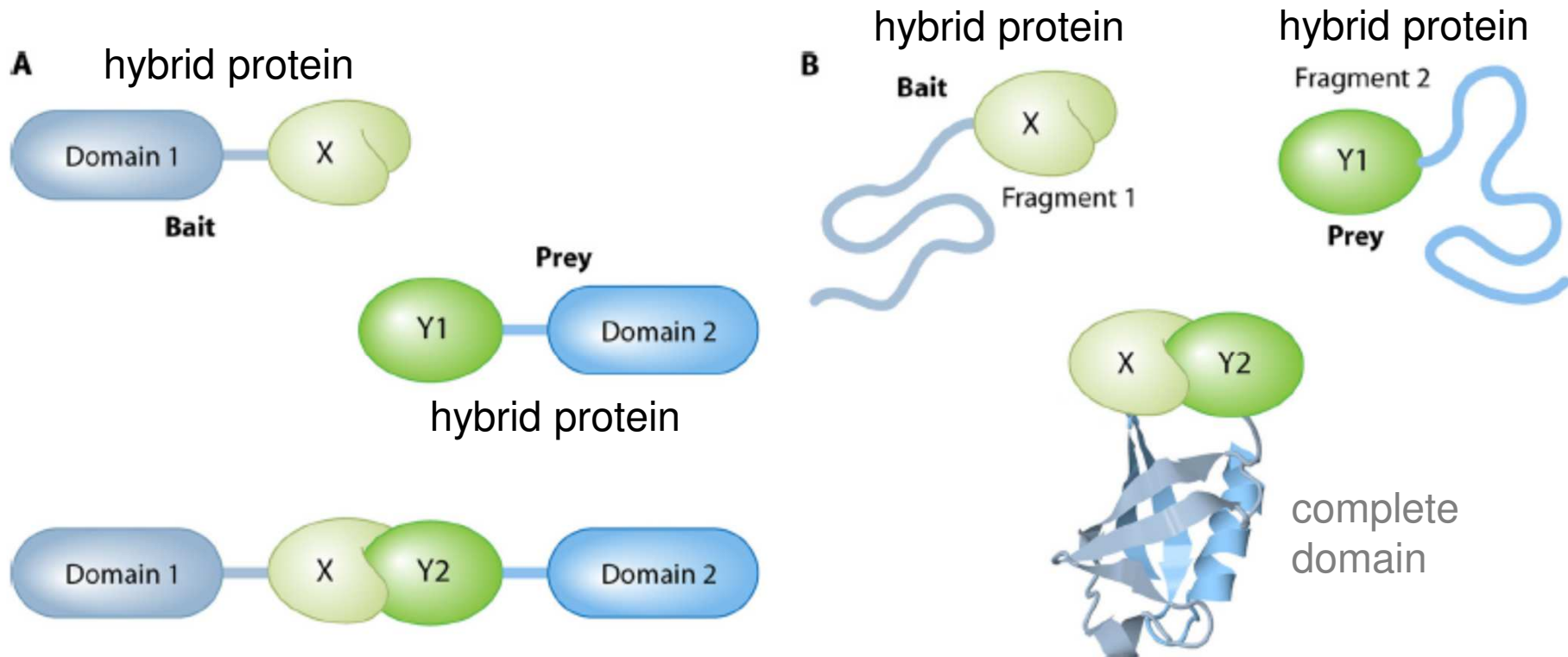
# Protein-protein interaction analysis

- matrix/beads-based: pull-down (*in vitro*), coIP ...
- **hybrid-based:**
  - **classical systems- *domain***
    - transcription 2-hybrid systems
      - reverse systems
    - multi-hybrid systems
    - alternative (membrane) systems - *pathway*
  - **complementation systems – *fold***
    - BiFC, DHFR
- proximity-based: FRET, PLA, BioID ...
- MS-based: crosslink, D/H-exchange ...
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# Principal differences in hybrid systems



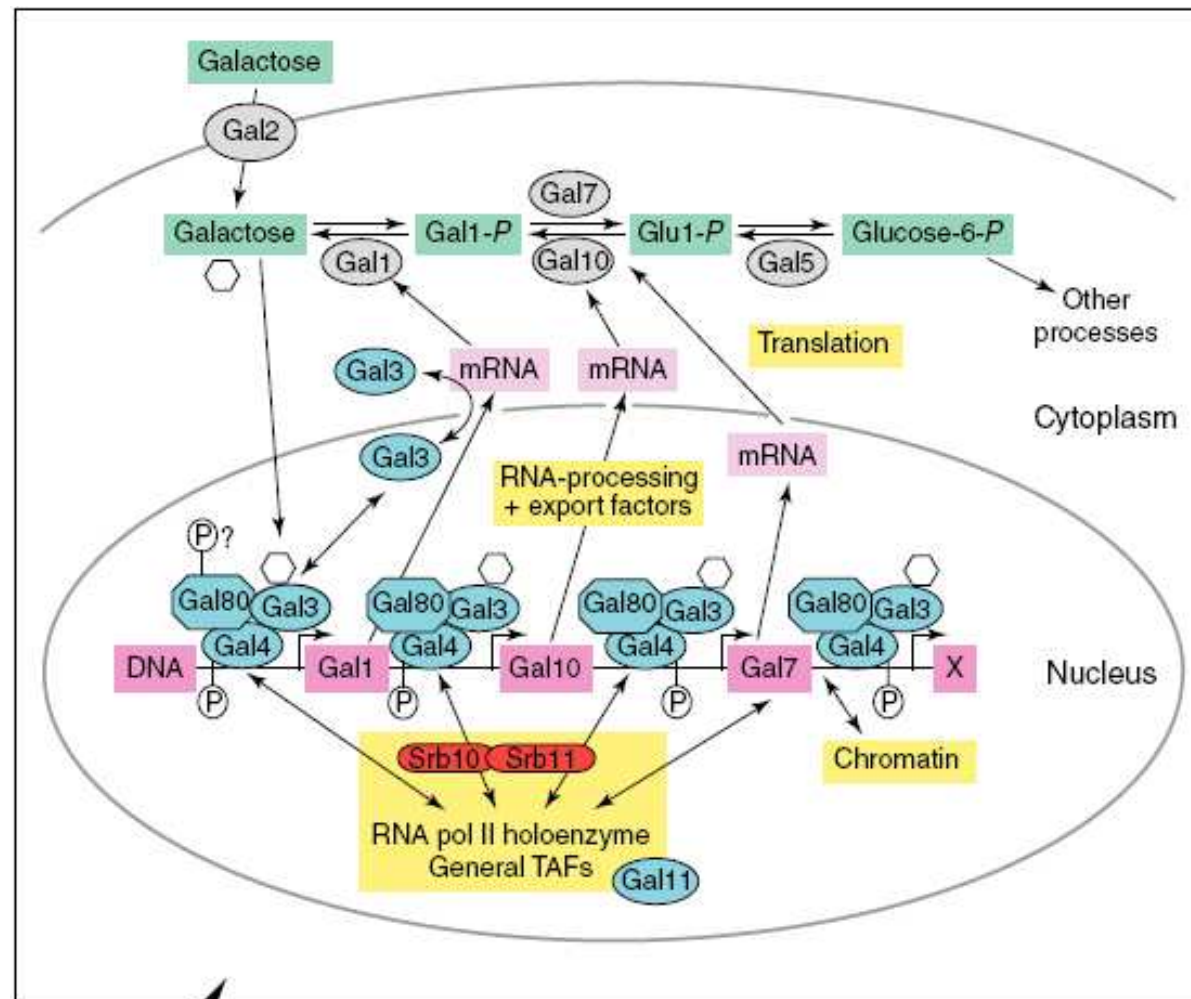
A. In classical systems, PPI reconnects two separated domains (normally present in one protein) back to one tight complex

B. In complementation systems, PPI reconnects fragments of one domain and reconstitutes its fold



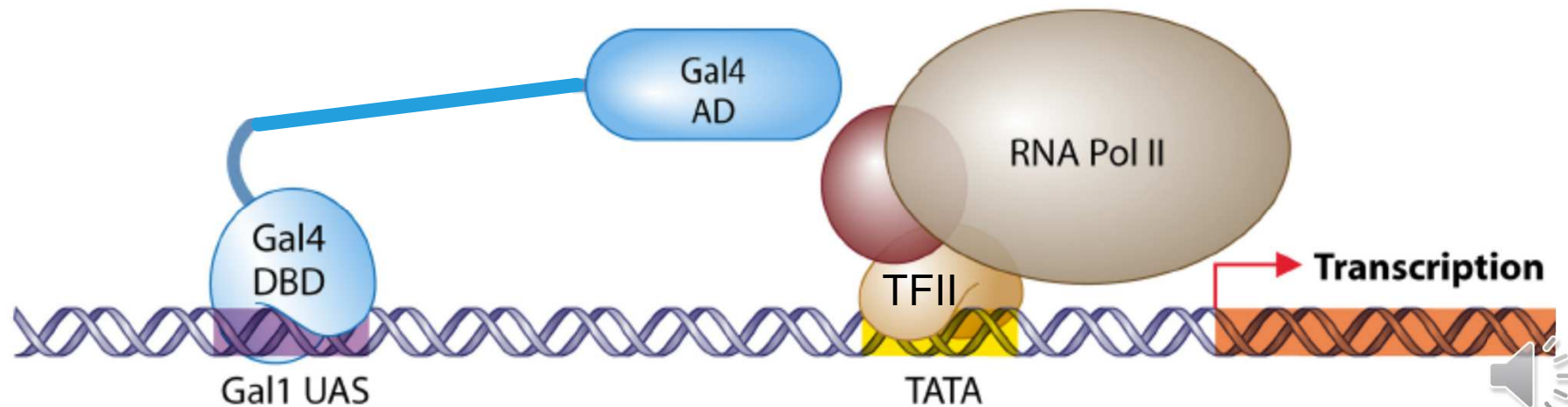
# Classical yeast two-hybrid system

Classical (first) yeast two-hybrid system is based on transcription factor Gal4 function – Gal4 binds promoter regions (sequences) of *GAL* genes and activates their transcription



# Gal4-based two-hybrid system

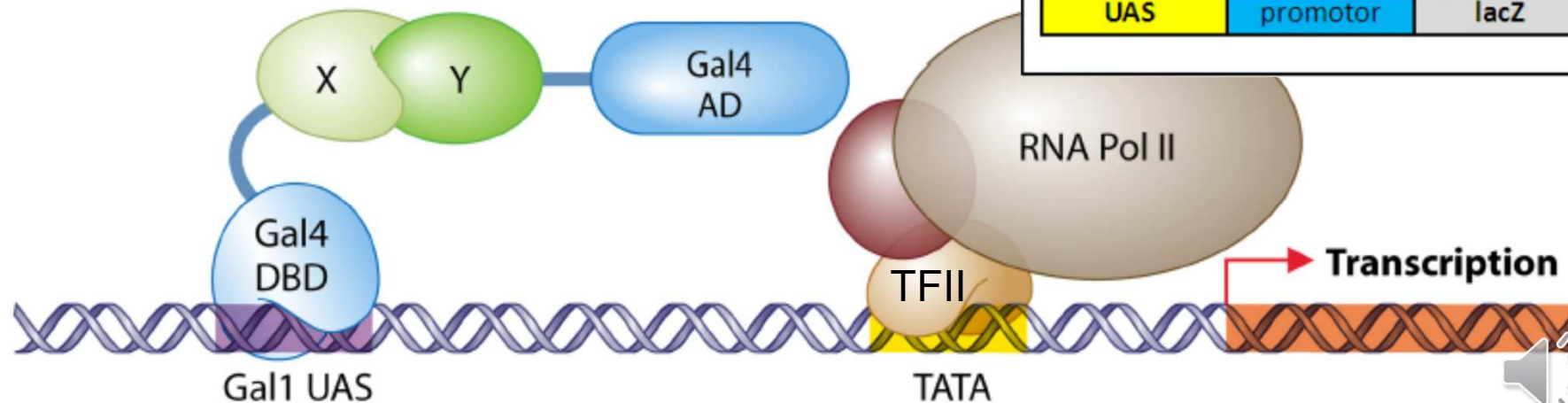
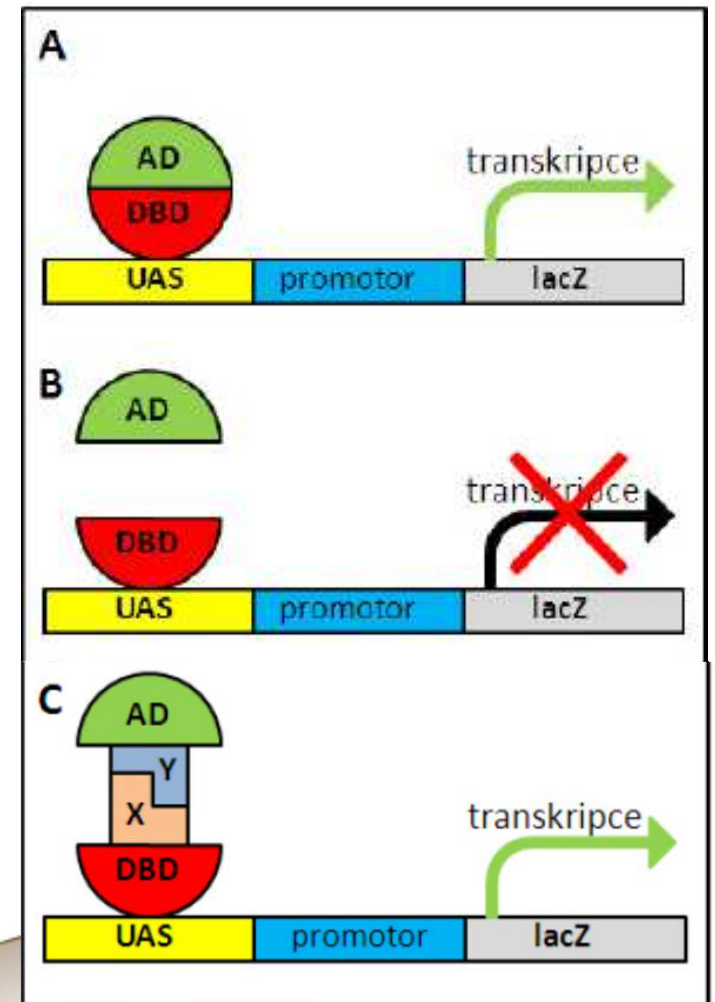
Gal4 transcription factor binds specific DNA sequence through its DNA-binding domain (DBD) - Gal4 transcription activation domain (AD) binds to general TFII factors/RNA polymerase II and activates transcription machinery





# Gal4-based 2-hybrid system

- A. Gal4 (DBD-AD) protein activates reporter gene (*lacZ*)
- B. When DNA-binding domain (DBD) and activation domain (AD) are separated, they are not able to activate transcription machinery
- C. When DBD and AD are fused in frame to interacting proteins (X and Y), then PPI reconnects DBD-AD and enables transcription



Other transcription factors have been employed in two-hybrid variants:

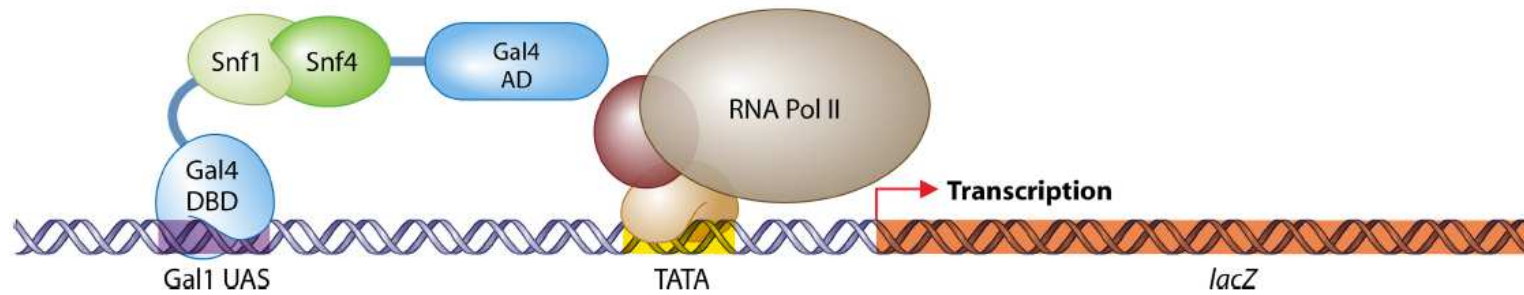
Prey activation domains

<i>S. cerevisiae</i> Gal4 AD	Gal4 activating region II (aa 768 to 881), moderate strength (178)
Herpes simplex virus VP16 AD	VP16 activating region (aa 413 to 490), high strength (673)
<i>E. coli</i> B42 AD	Bacterial polypeptide, weak strength (234)

Bait DNA-binding domains

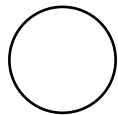
<i>S. cerevisiae</i> Gal4 DBD*	Binds <i>GAL1</i> , <i>GAL2</i> , and <i>GAL7</i> upstream activating sequences (178)
<i>E. coli</i> repressor LexA DBD*	Binds LexA operator sequences (234)
<i>H. sapiens</i> estrogen receptor DBD	Binds estrogen receptor elements (374)
Bacteriophage $\lambda$ repressor cI	Binds cI operator sequences (580)
Tet repressor	Binds Tet operator sequences (716)





To detect/score transcription activation (i.e. “see” interaction of partner proteins), different reporter genes are used

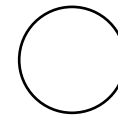
BD-Snf1/-



BD-Snf1/AD-Snf4



-/AD-Snf4



Only yeast cells expressing binding partners will turn blue (as the *lacZ* reporter will be transcribed/expressed and will convert transparent X-gal substrate to blue product) – *lacZ* enzymatic activity can be measured (thus, the strength of the PPI can be quantified)



# Reporter genes

## Reporter genes

<i>E. coli lacZ*</i>	$\beta$ -Galactosidase chromogenic reporter (178)
<i>S. cerevisiae MEL1</i>	Secretory $\alpha$ -galactosidase chromogenic reporter (5)
<i>E. coli gusA</i>	$\beta$ -Glucuronidase chromogenic reporter (580)
<i>Aspergillus oryzae lacA3</i>	Engineered secretory $\beta$ -galactosidase chromogenic reporter (318)
<i>S. cerevisiae HIS3*</i>	Prototrophic reporter for histidine biosynthesis (673)
<i>S. cerevisiae LEU2*</i>	Prototrophic reporter for leucine biosynthesis (234)
<i>S. cerevisiae URA3</i>	Prototrophic reporter for uracil biosynthesis (374)
<i>S. cerevisiae ADE2*</i>	Prototrophic reporter for adenine biosynthesis (299)
<i>S. cerevisiae LYS2</i>	Prototrophic reporter for lysine biosynthesis (580)
<i>Aequorea victoria GFPuv</i>	Fluorescent reporter (107)
<i>EGFP</i>	Fluorescent reporter (613)
Yeast <i>EGFP</i>	Fluorescent reporter for flow cytometry screens (88)
<i>Aureobasidium pullulans AUR1-C</i>	Aureobasidin A resistance reporter (167)

quantitative

← His3 enzyme activity can be titrated by its 3-aminotriazol inhibitor

auxotrophy (selective)

FACS sorting

antibiotic resistance



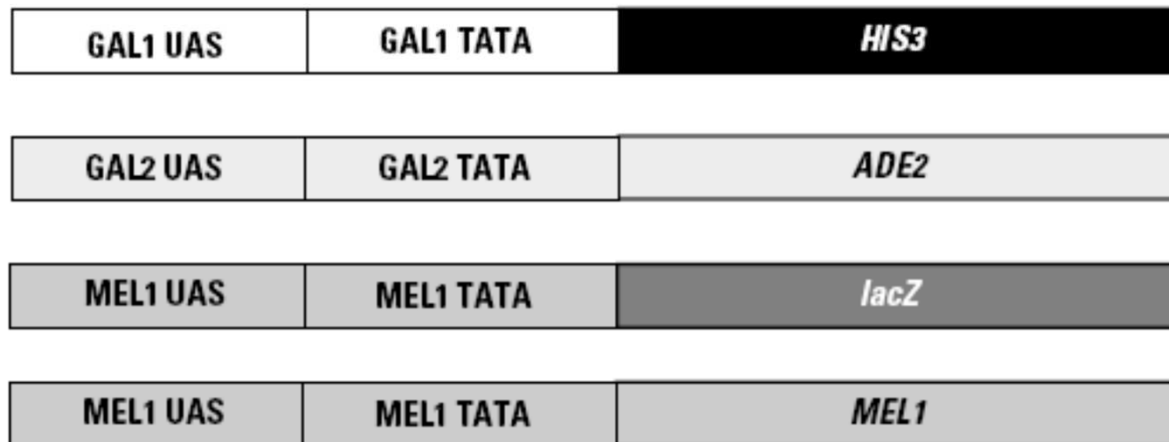
# Yeast 2-hybrid strain example

AH109 (and other strains) contains *His3* and *lacZ* reporter genes (integrated in *LYS2* and *URA3* genes, respectively) under different Gal4-binding promoters (GAL1 and MEL1, respectively)

AH109

*MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2 :: GAL1<sub>UAS</sub>-GAL1<sub>TATA</sub>-HIS3, GAL2<sub>UAS</sub>-GAL2<sub>TATA</sub>-ADE2, URA3 :: MEL1<sub>UAS</sub>-MEL1<sub>TATA</sub>-lacZ*

genotype



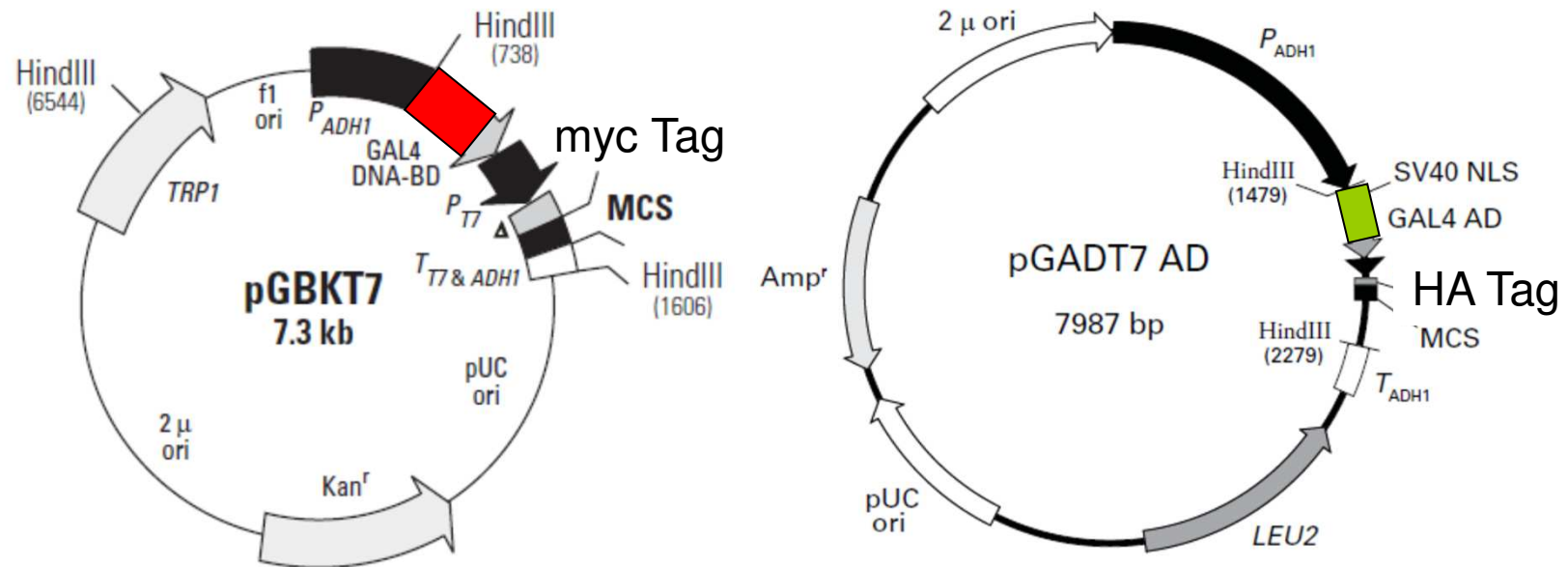
*Trp1* and *Leu2* genes must be mutated to enable (auxotrophy) selection of plasmids (bearing hybrid genes)  
 - many yeast strains exist; systems adopted to bacterial and mammalian cells exist as well





# Yeast 2-hybrid plasmid example

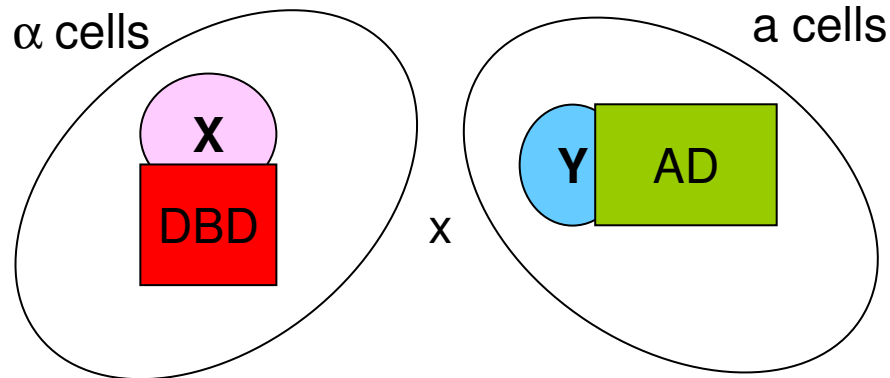
pGBKT7 and pGADT7 plasmids contain **Gal4 BD** and **AD** elements (to make hybrid proteins) as well as selective markers (Trp1 and Leu2 for yeast selection)



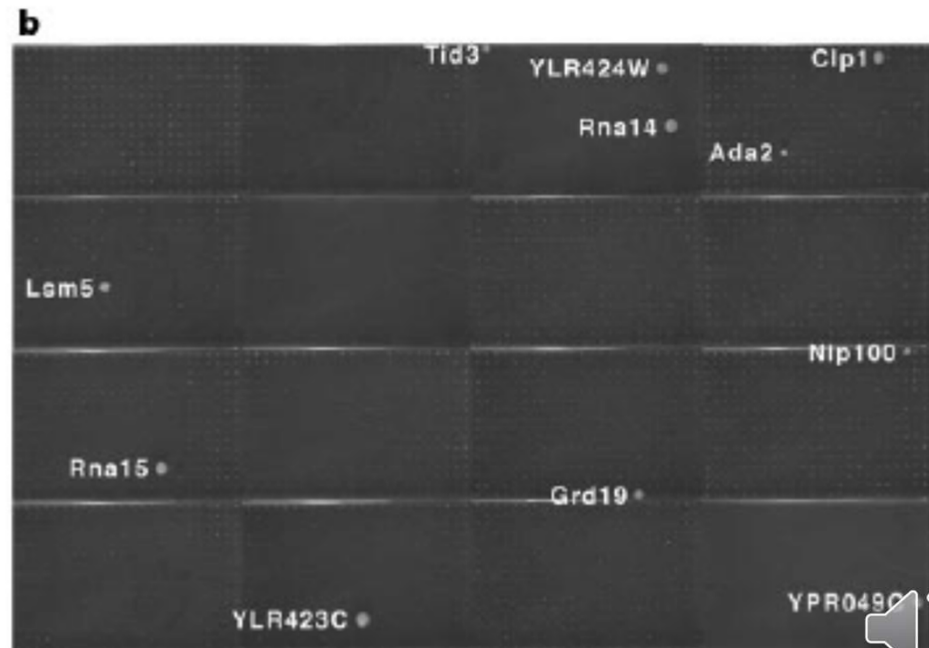
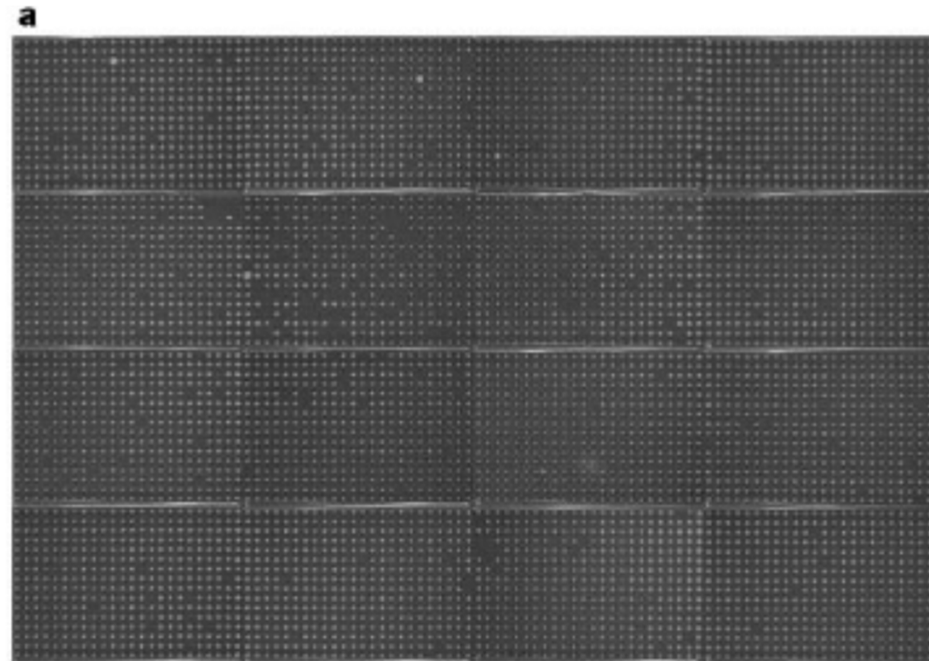
**T7** promoters in front of myc and HA tag, respectively, are suitable for additional pull-down experiments (see previous slides)



# Yeast 2-hybrid screens

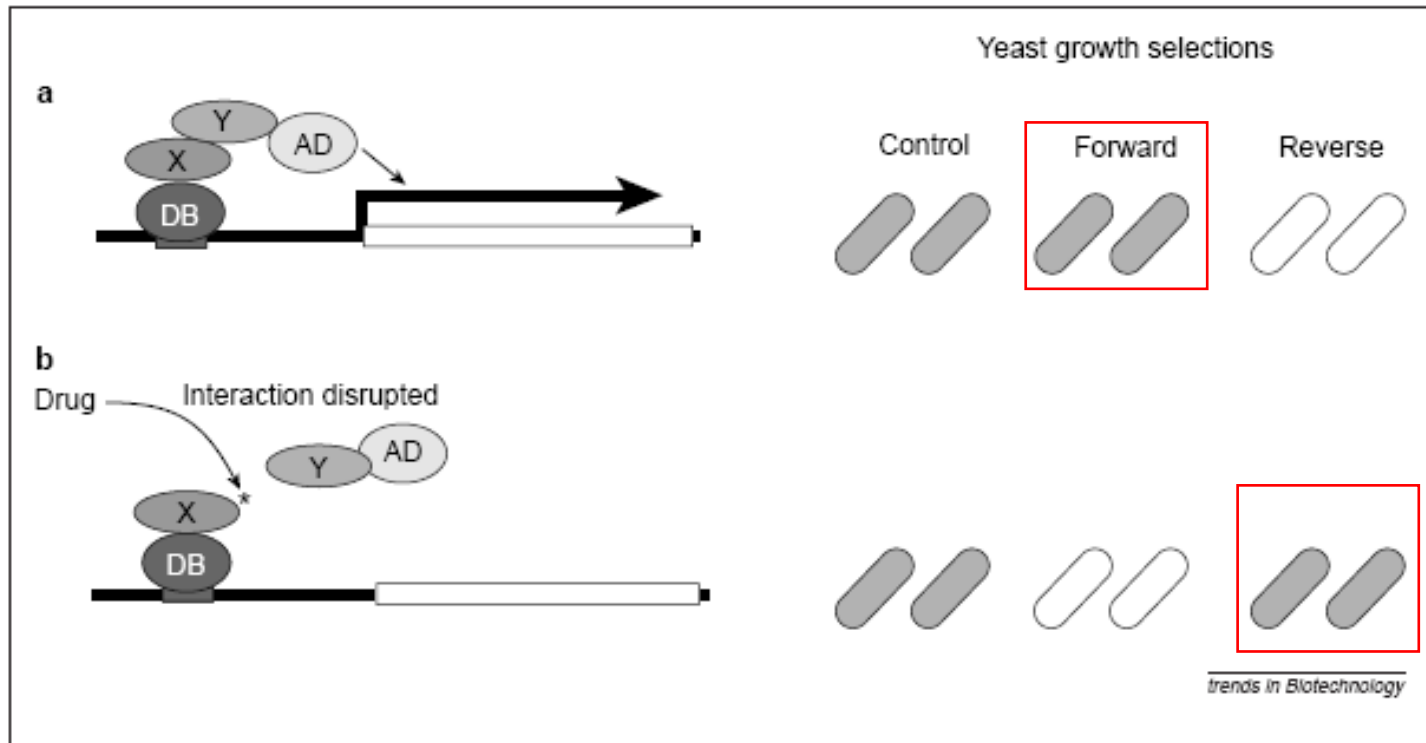


High-throughput screens can be done as – 1. simple study: one bait is screened against AD-library (e.g. of all human hybrid proteins) - or – 2. interactom study: collection of all BD-proteins is screened against AD-library (e.g. 6000x6000 yeast proteins = yeast interactom)





# Reverse systems

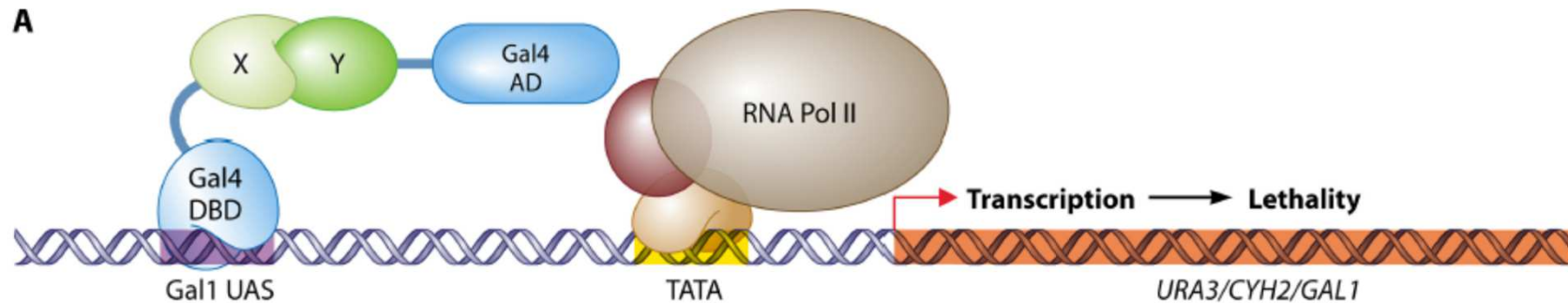


Vidal & Endoh, T in Biotech, 1999

For detail PPI analysis (e.g. binding surface mapping), mutation (drug) will disturb interaction - it (loss of interaction) is detected by the loss of growth of the yeast cells on selective plate (or inability to turn on the blue colour) – reverse systems were developed to “visualize” loss of interaction ...



# Reverse systems

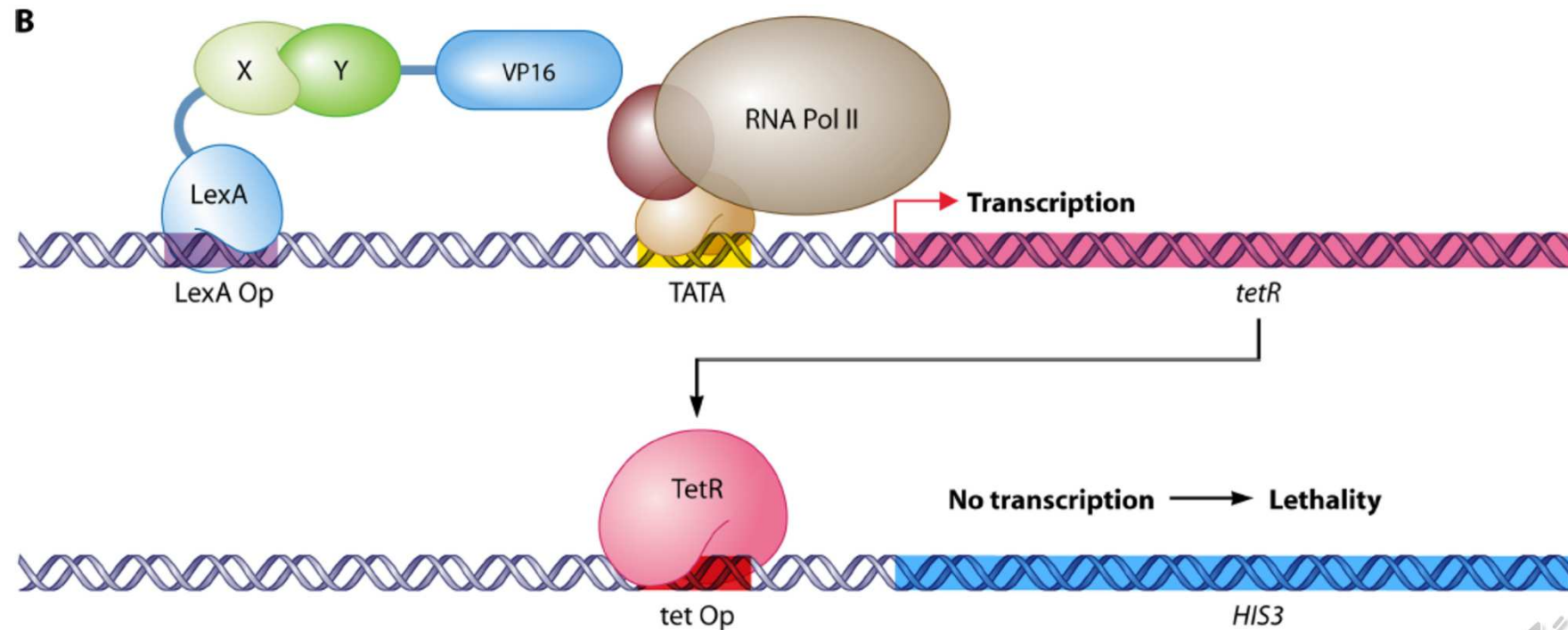


... in reverse systems, PPI results in lethal phenotype – yeast cells will not grow until PPI is disturbed (by mutation or drug) – for example, cells expressing URA3 reporter gene will grow on plates without uracil, but these cells will be killed by 5-flouro-orothic acid (Ura3 enzyme converts FOA to toxic compound); in contrast, when PPI is disturbed, yeast cells will not express URA3 reporter gene (will not grow on plates without uracil), but these cells will not convert 5-flouro-orothic acid and therefore they will be able to grow on plates with FOA



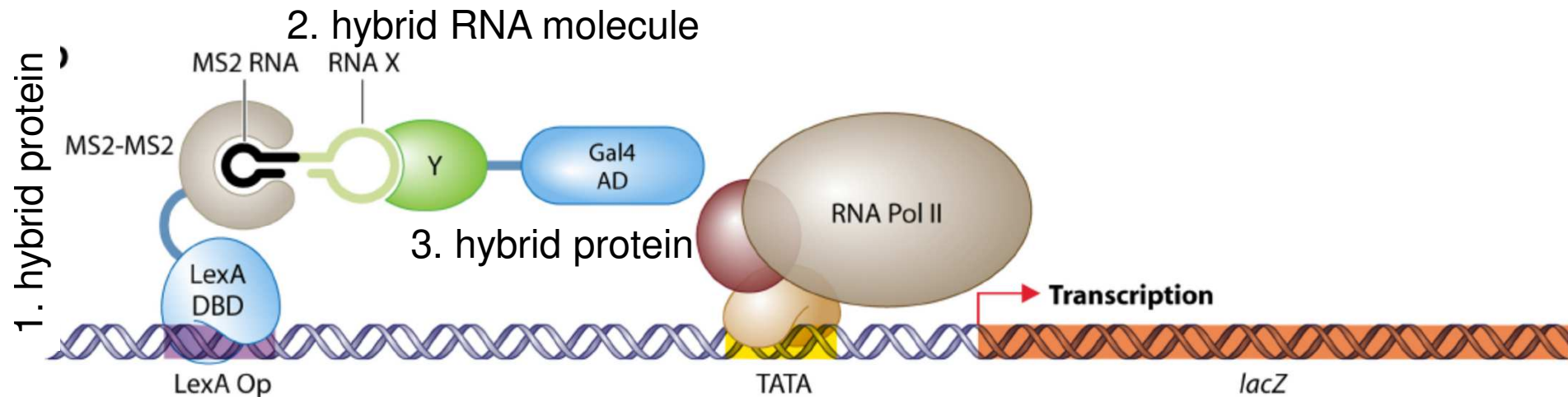
# Reverse systems

... new reverse system (also called split system) is based on two transcription regulation steps: PPI activates transcription of repressor which blocks transcription of reporter gene (only when PPI is disturbed, the His3 reporter gene is transcribed)



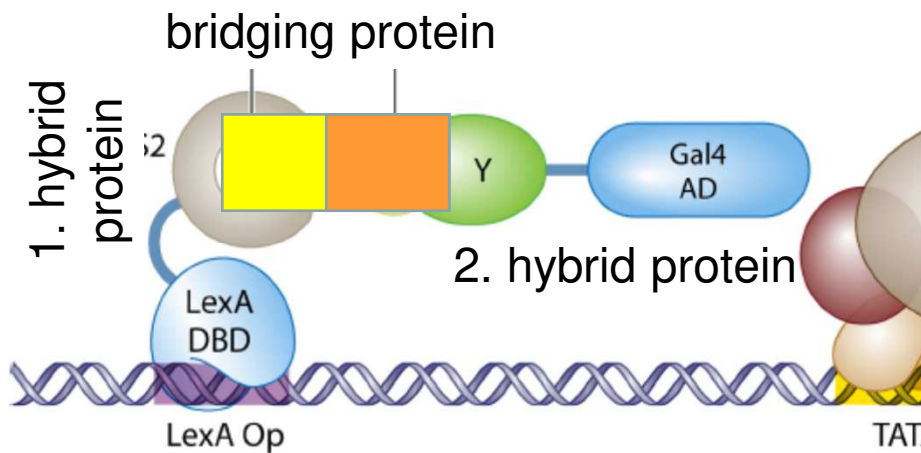
# (multi) three-hybrid systems

First three-hybrid system was developed to study RNA-binding proteins – DBD-hybrid protein (1) binds one RNA motif (MS2) within the RNA-hybrid molecule (2), while the other part of the RNA-hybrid molecule (X) is recognized by AD-hybrid protein (3) – this RNA-protein complex will switch on *lacZ* reporter gene transcription – in this way, you can screen AD-hybrid library for RNA-X binding proteins



# Three-component 2-hybrid system

DBD-hybrid protein binds one part of bridging protein, while the other part of the bridging (non-hybrid) protein is bound by AD-hybrid protein (several bridging proteins can be used)



Bednarova, Diploma thesis, 2009

150 mM 3 - AT (-Leu, -Trp, -Ura, -His)				
90 mM 3 - AT (-Leu, -Trp, -Ura, -His)				
30 mM 3 - AT (-Leu, -Trp, -Ura, -His)				
20 mM 3 - AT (-Leu, -Trp, -Ura, -His)				
15 mM 3 - AT (-Leu, -Trp, -Ura, -His)				
10 mM 3 - AT (-Leu, -Trp, -Ura, -His)				
5 mM 3 - AT (-Leu, -Trp, -Ura, -His)				
Kontrola: (-Leu, Trp, Ura)				
DBD Nse1-Nse3-Nse4 complex	AD			
	BD-Nse1+V2AD+VP	VBD+AD-Nse4+VP	BD-Nse1+AD-Nse4 AD+VP	BD-Nse1+AD- Nse3+PPM-Nse3

# Protein-protein interaction analysis

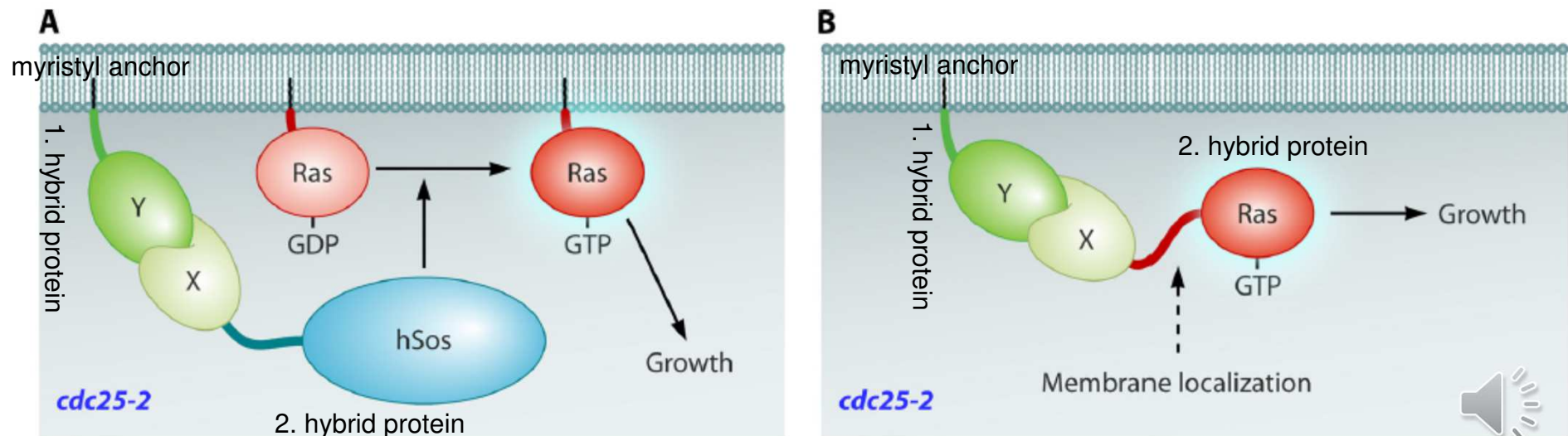
- matrix/beads-based: pull-down (*in vitro*), coIP ...
- **hybrid-based:**
  - classical systems- *domain*
    - transcription 2-hybrid systems
      - reverse systems
    - multi-hybrid systems
    - **alternative (membrane) systems** - *pathway*
  - complementation systems – *fold*
    - BiFC, DHFR
- proximity-based: FRET, PLA, BioID ...
- MS-based: crosslink, D/H-exchange ...
- Quantitative methods: SPR, ITC ...
- Structural methods: co-crystalization, NMR ...
- Genetic methods: synthetic lethality ...)
- Bioinformatics methods: databases, docking ...





# Alternative membrane systems - Ras

Number of proteins can't be used in transcription-based hybrid systems (e.g protein can't be localized to the yeast cell nucleus) – CytoTrap (Ras recruitment) system is based on membrane-anchored Ras pathway reactivation – **A.** RAS protein is activated only when human hSOS-hybrid, ortholog of yeast *cdc25* (guanine exchange factor; *cdc25-2* mutant cells are used), is anchored at the cytoplasmic membrane via interaction of myristylated hybrid-protein partner – **B.** RAS-hybrid protein works when it binds to myristylated hybrid-protein partner

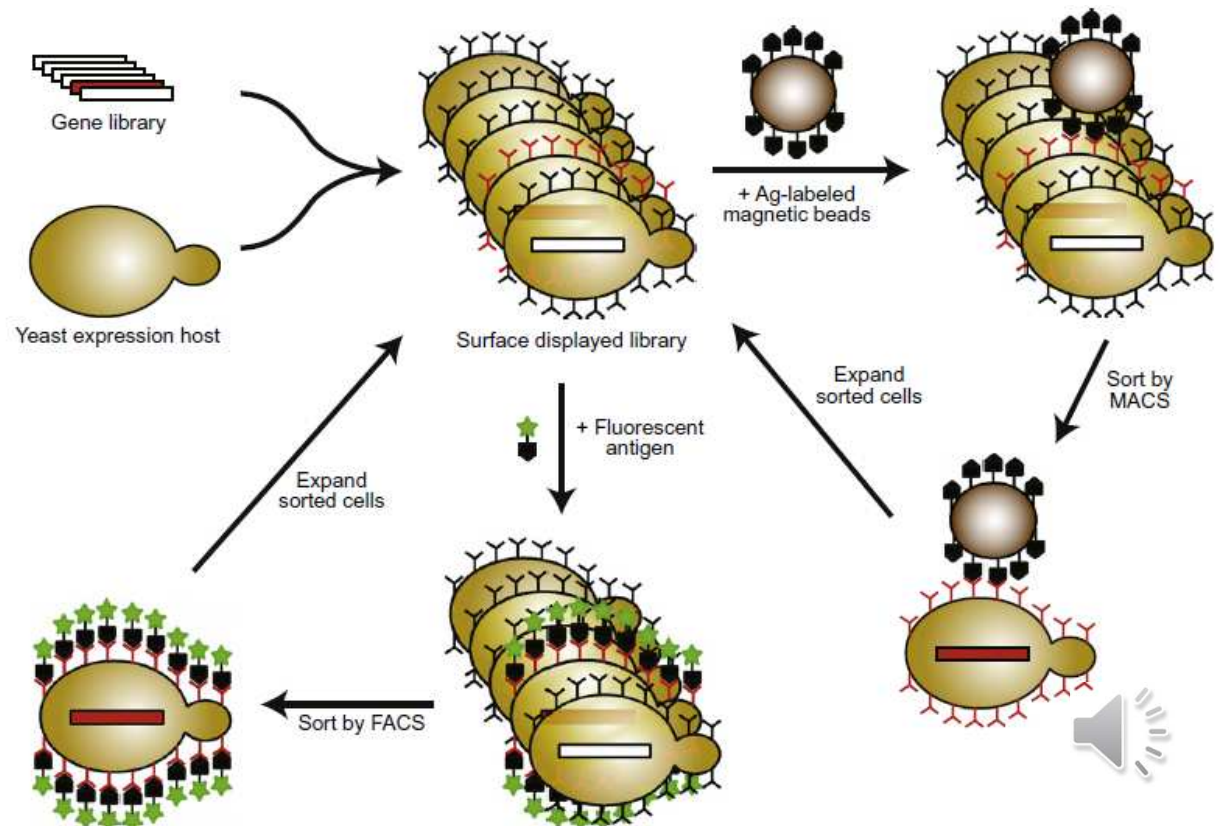
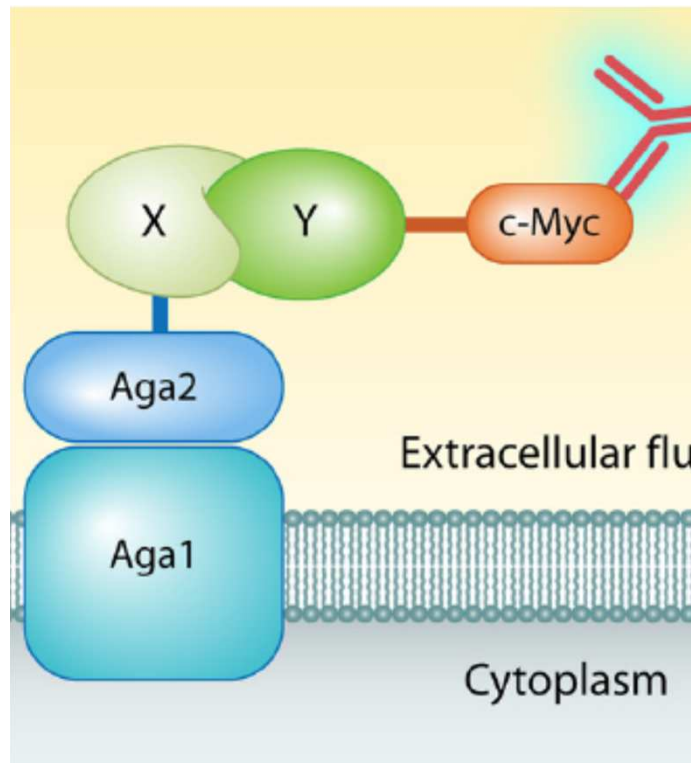




# Yeast surface display system

Aga2-hybrid protein is localized at the yeast surface – tagged-partner interaction anchors it at the yeast surface – anti-tag antibody recognizes the tagged protein – fluorescence of the antibody (primary or secondary antibody) is detected and can be used for yeast strain selection (by FACS)

Stynen et al, MMBR, 2012



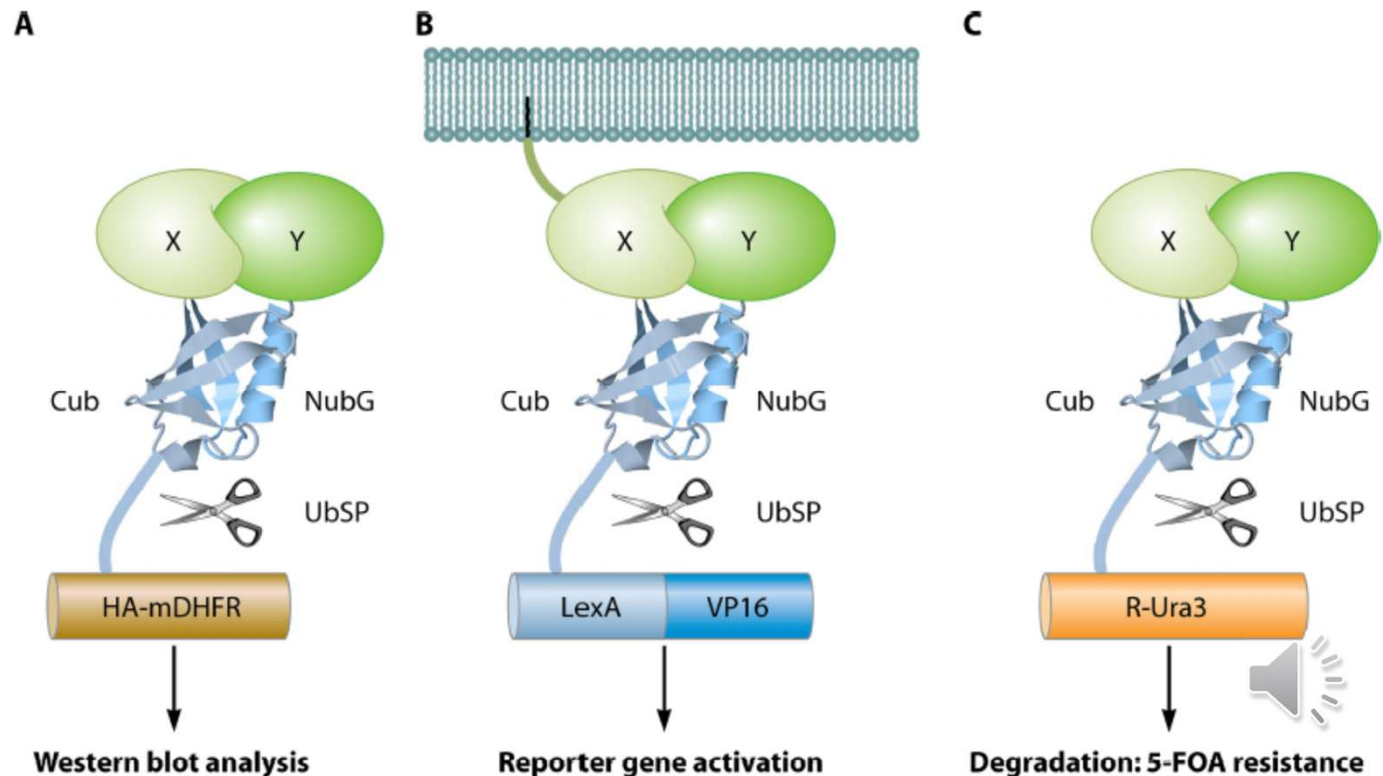
# Protein-protein interaction analysis

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- Genetic methods: synthetic lethality ...)
- Bioinformatics methods: databases, docking ...



# Complementation systems

PPI reconnects fragments of one domain and reconstitutes its fold – original (A) assay based on reconstitution of ubiquitin (western blot analysis of protein degradation) – new alternative versions use different detection approaches – for example (B), in transcription-based approach, reporter gene is transcribed only when LexA-VP16 transcription factor is released from membrane localization

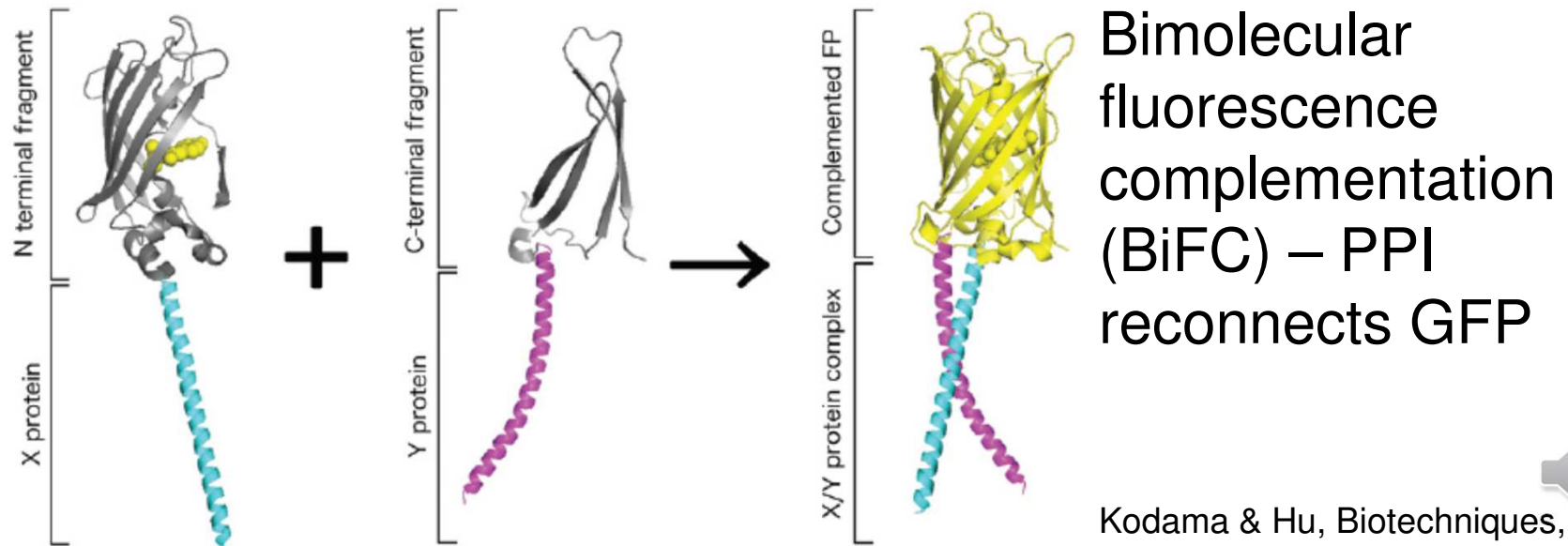
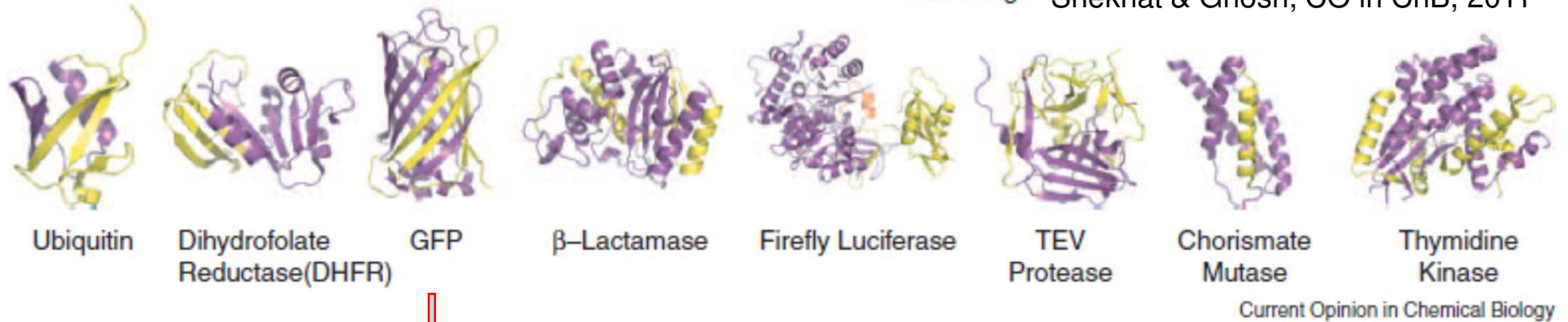


Johnsson et al, PNAS, 1994  
Stynen et al, MMBR, 2012

# Complementation systems

Several systems based on complementation of different protein folds have been developed

Shekhat & Ghosh, CO in ChB, 2011



Kodama & Hu, Biotechniques, 2012

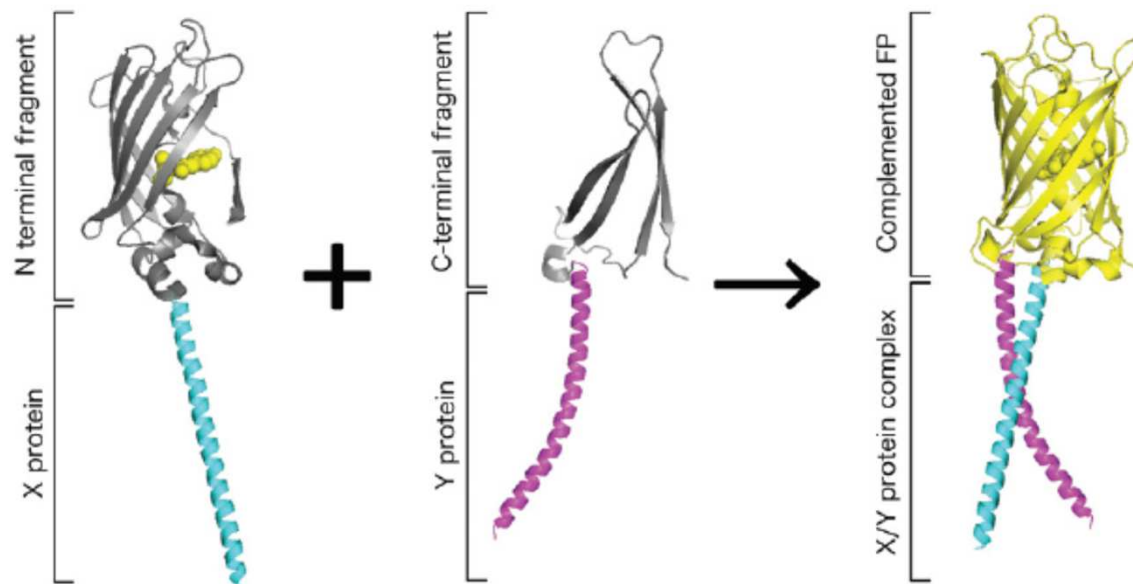


# Bimolecular fluorescence complementation (BiFC)



Pekarova et al, Plant J., 2011

Bimolecular fluorescence complementation (BiFC) – PPI  
reconnects GFP and its fluorescence is detected

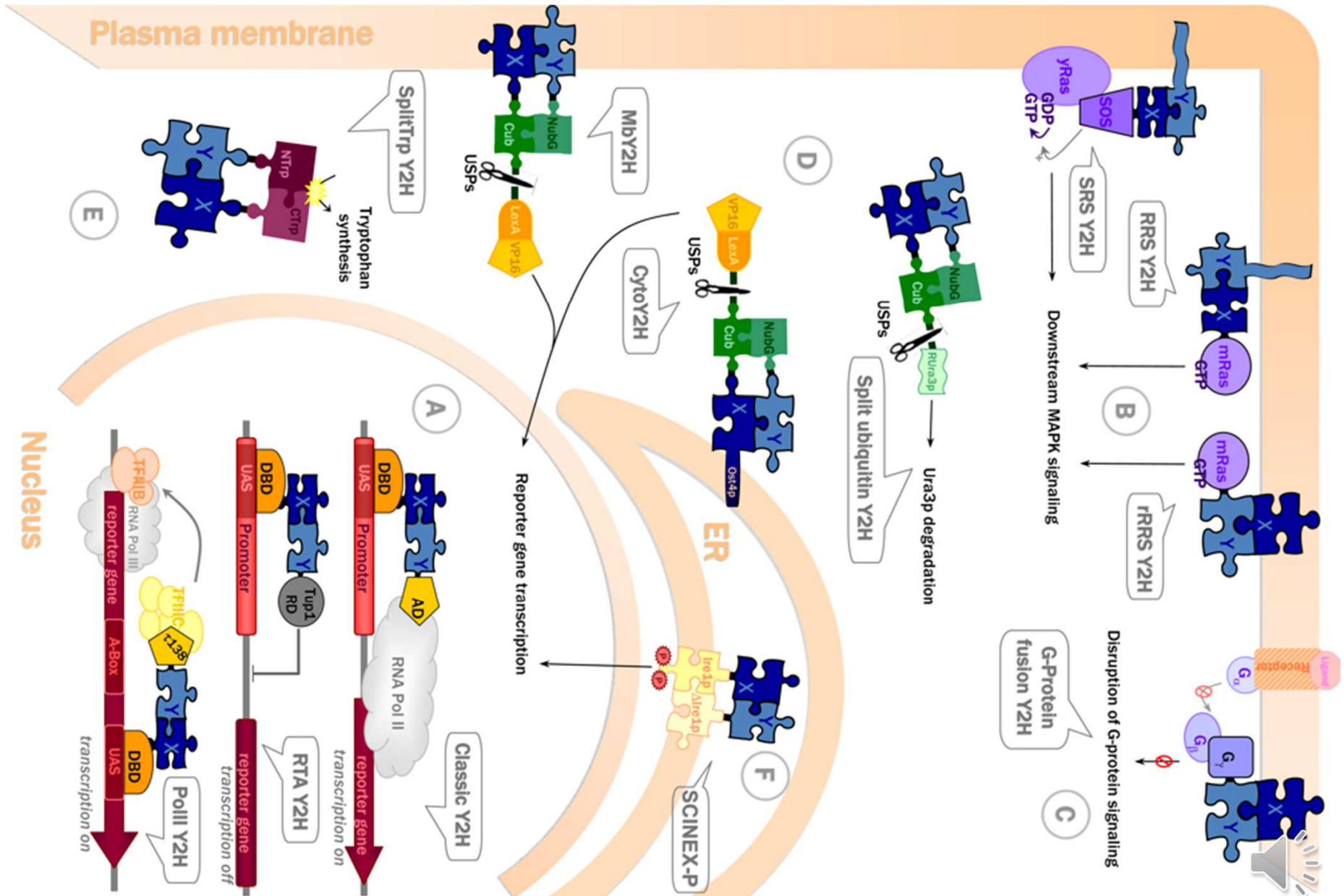


Kodama & Hu, Biotechniques, 2012





# Overview of yeast 2-hybrid systems



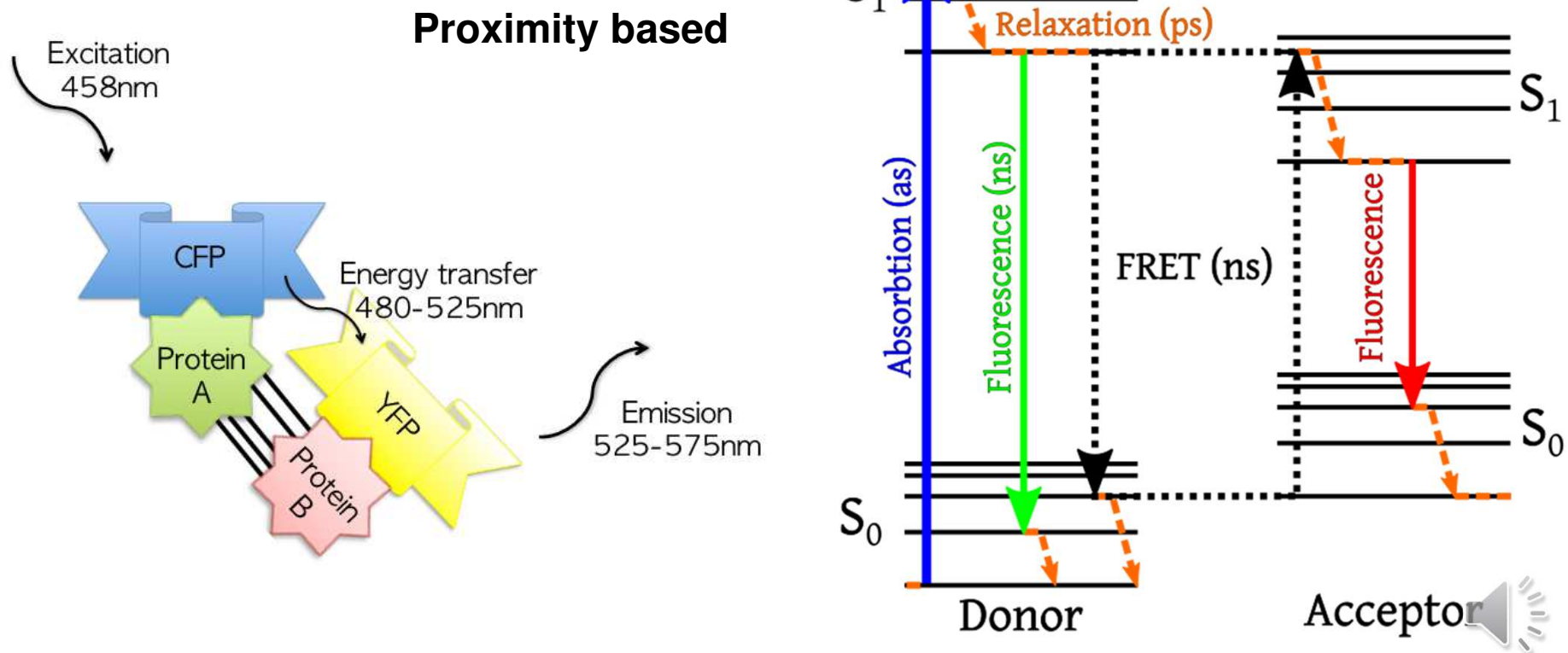
# Protein-protein interaction analysis

- matrix/beads-based: pull-down (*in vitro*), coIP ...
- Hybrid-based: Y2H (yeast 2-hybrid), BiFC ...
- **Proximity-based:**
  - **FRET**
  - **PLA**
- MS-based: crosslink, D/H-exchange ...
- Quantitative methods: SPR, ITC ...
- Structural methods: co-crystalization, NMR ...
- Genetic methods: synthetic lethality ...
- Bioinformatics methods: databases, docking ...

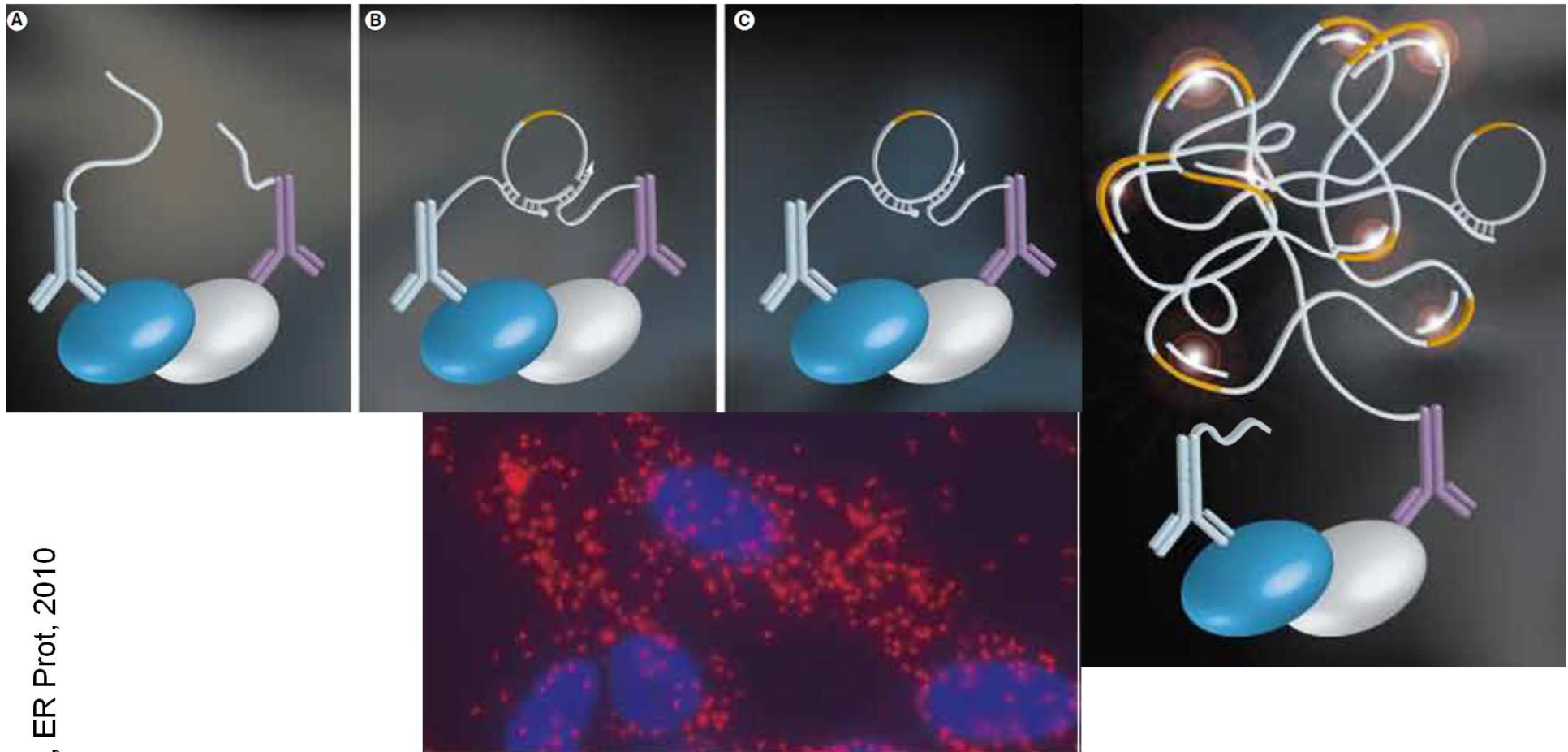


# FRET (Forster/fluorescence resonance energy transfer)

- CFP-hybrid protein emits 480-525nm light when excited (by 458nm light) – when CFP-hybrid protein binds partner YFP-hybrid protein, the 480-525nm emitted light excites YFP which then emits 525-575nm light (detected in the fluorescence microscope)



# Proximity ligation assay - PLA



Weibrecht et al, ER Prot, 2010

- Specific antibodies conjugated with oligonucleotides, which are complementary to circular DNA – if the antibodies come close ( $<16\text{nm}$ ) via PPI of their target proteins then – DNA is ligated and polymerase synthesis reaction can run

