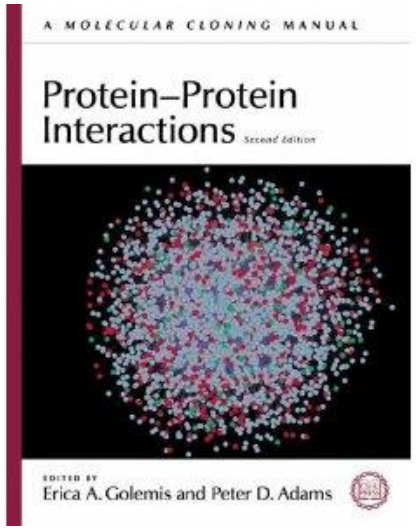
Protein-protein interaction analysis



Protein-protein interactions
Golemis & Adams
CSHL Press, 2005

Review and research papers
(referenced on slides)

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

Protein-protein interaction analysis

- Matrix/beads-based:
  - **pull-down assay**
  - **co-purification ï gel filtration**
  - **co-immunoprecipitation**
    - **Analysis of protein domains**
    - **Analysis of interaction surfaces**
    - **Peptide libraries**
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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

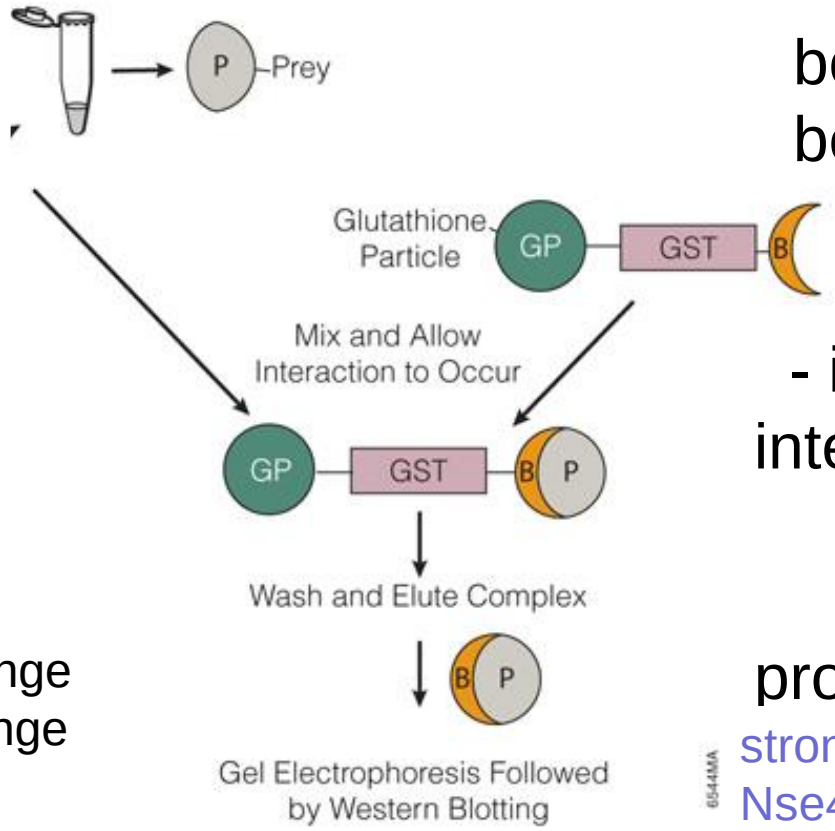
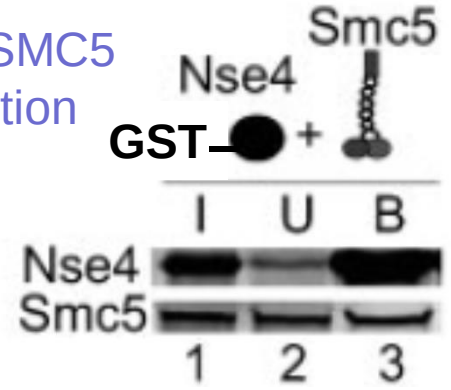


Figure 1. Schematic of pull-down

strong = nM-pM range
weak = mM-μM range

Palecek et al, JBC, 2006

strong
Nse4-SMC5
interaction



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

Pull-down

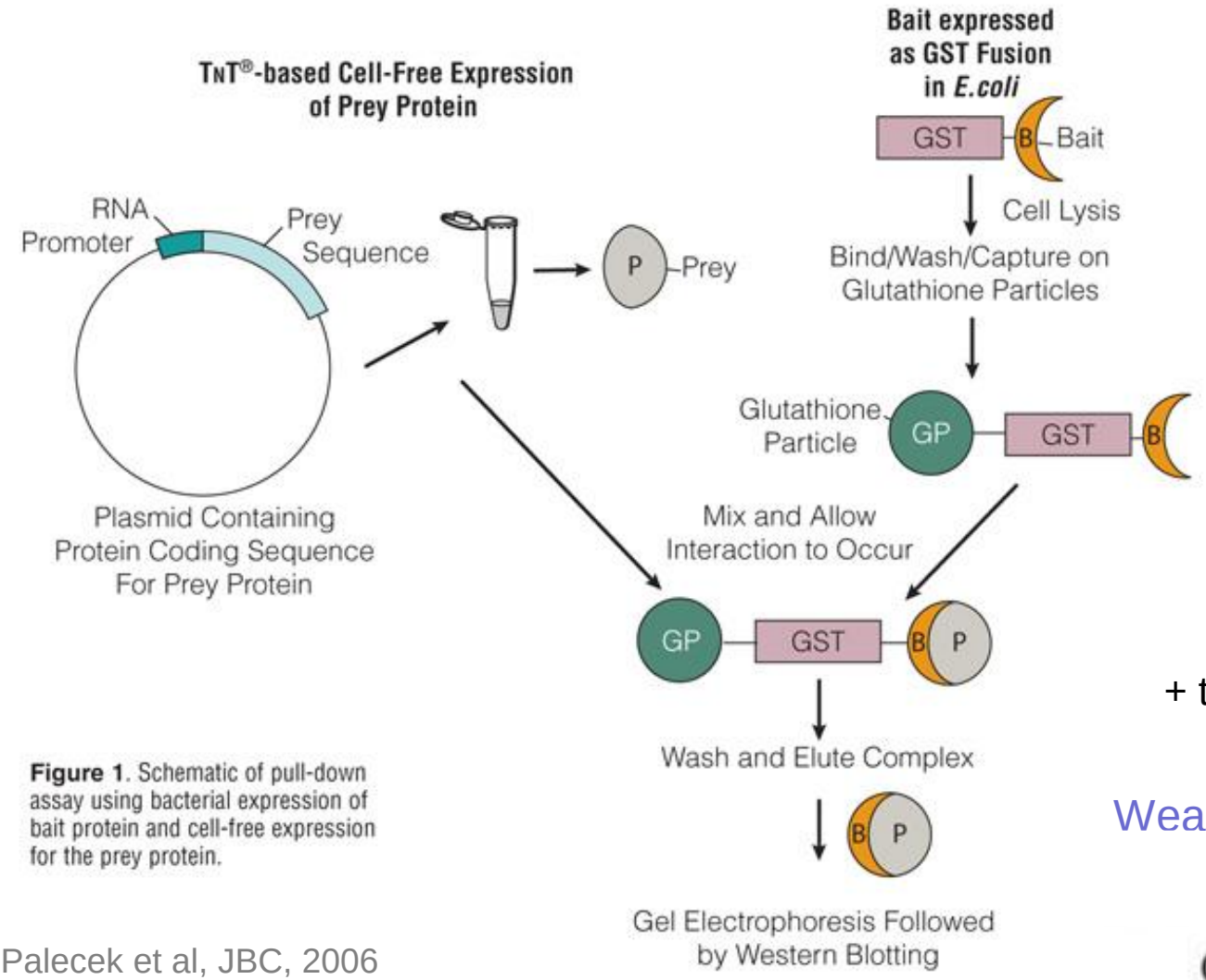


Figure 1. Schematic of pull-down assay using bacterial expression of bait protein and cell-free expression for the prey protein.

Palecek et al, JBC, 2006

Common tags for pull-down assay:

GST (glutathione)

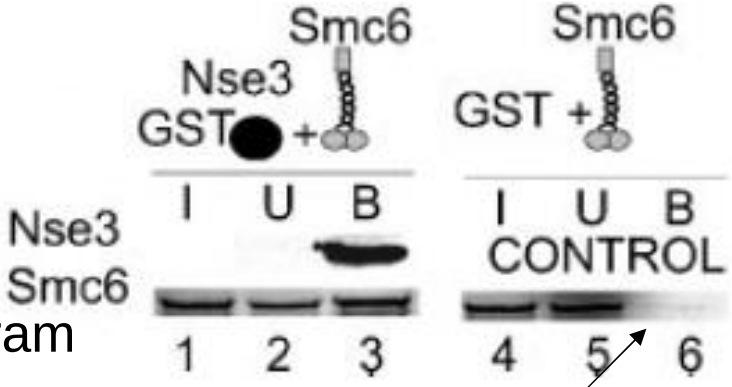
Streptactin (biotin-streptavidin)

MBP (maltose)

S-tag (protein S)

+ tags recognized by antibodies (see co-immunoprecipitation)

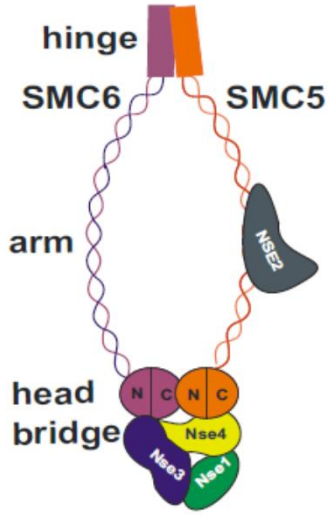
Weak Nse3-SMC6 interaction

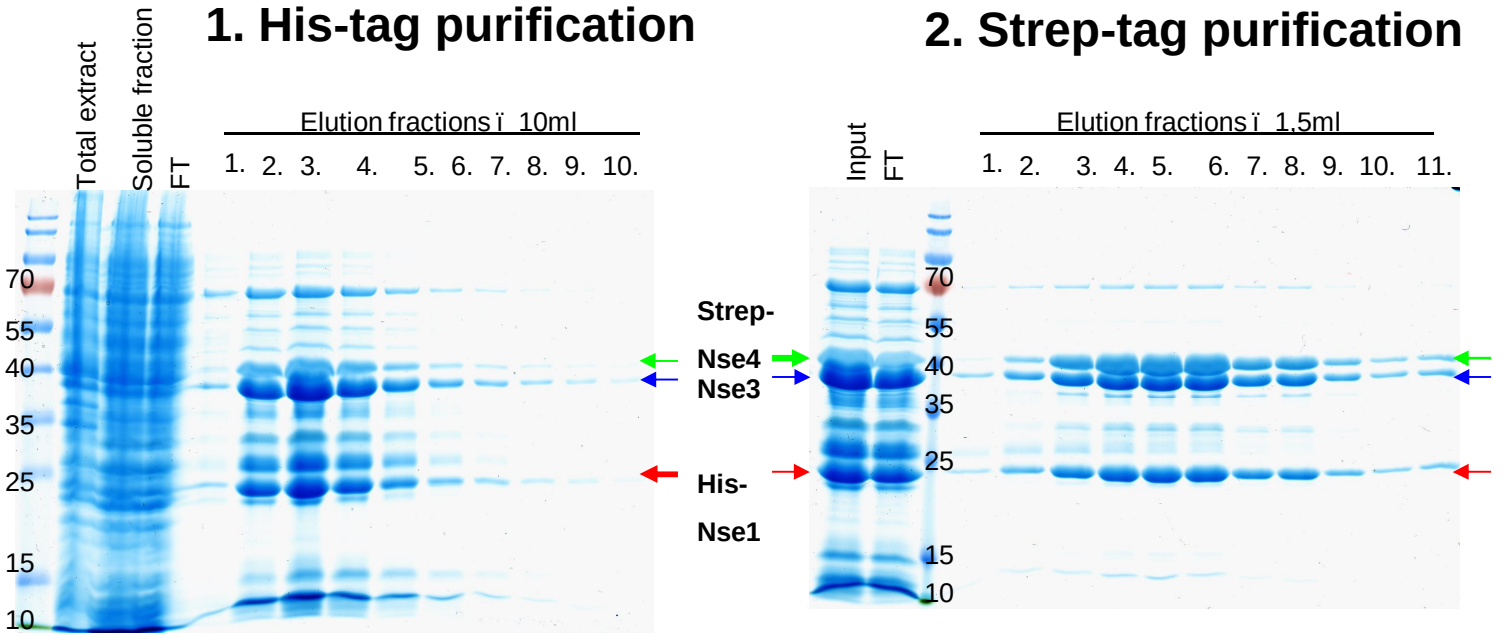


- GST-Nse3 expressed in bacteria ï (pre)purified on glutathione particles ï detected with anti-GST
- Smc6 expressed in TnT (radiolabeled) ï radiogram detection (neither tag nor antibody needed)
- control nonspecific binding of prey (Smc6 does not bind to GST-bound beads)

co-purification

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





Zabrady et al, NAR, 2016

Nse1-Nse3-Nse4 co-purify (interact strongly)

co-purification

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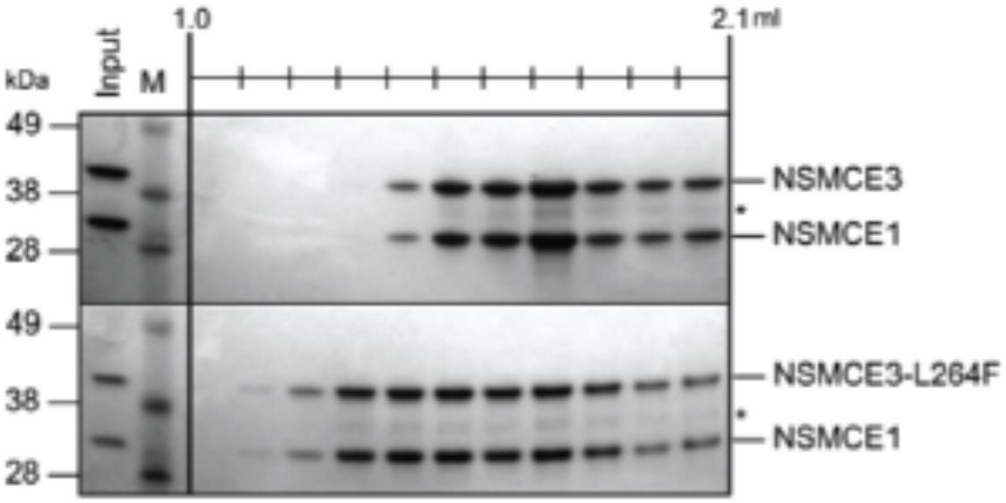
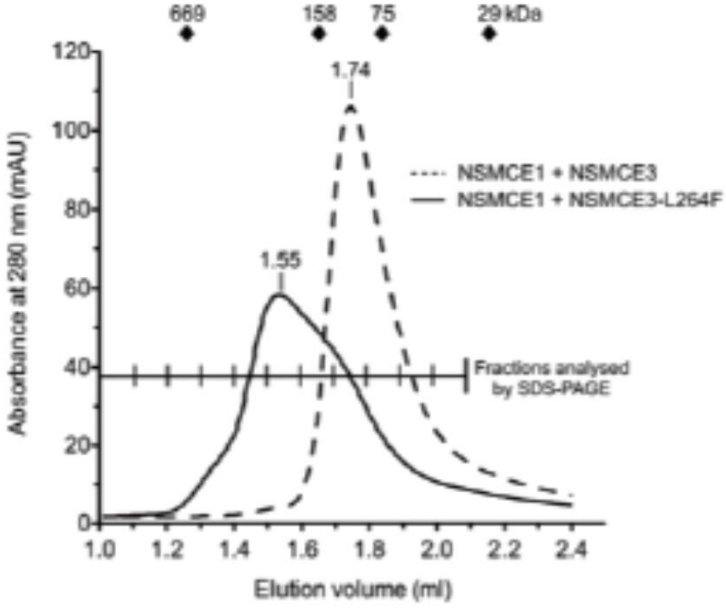


Gel filtration



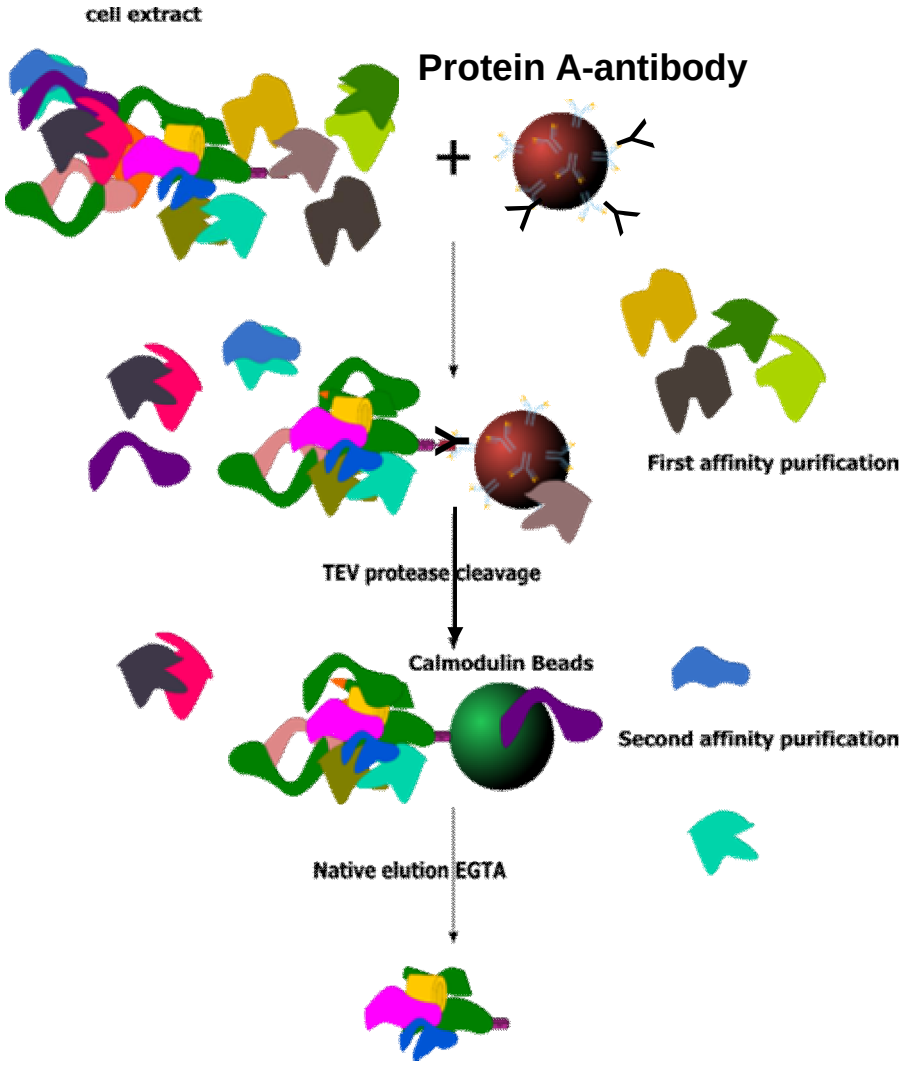
Nse1-Nse3 co-purify (interact strongly)

Interaction strength/stability can be compared by gel filtration (NSE3-L264F mutation affects the structure and interaction of NSE3 with NSE1 ï resulting in broader elution peak in gel filtration)

Van Crabben et al, JCI, 2016

Co-immunoprecipitation

Similar to pull-down assay, beads/matrix/particles are used to precipitate bait protein with its bound partners



Common tags for co-immunoprecipitation assay:
GFP, FLAG, myc, HA é

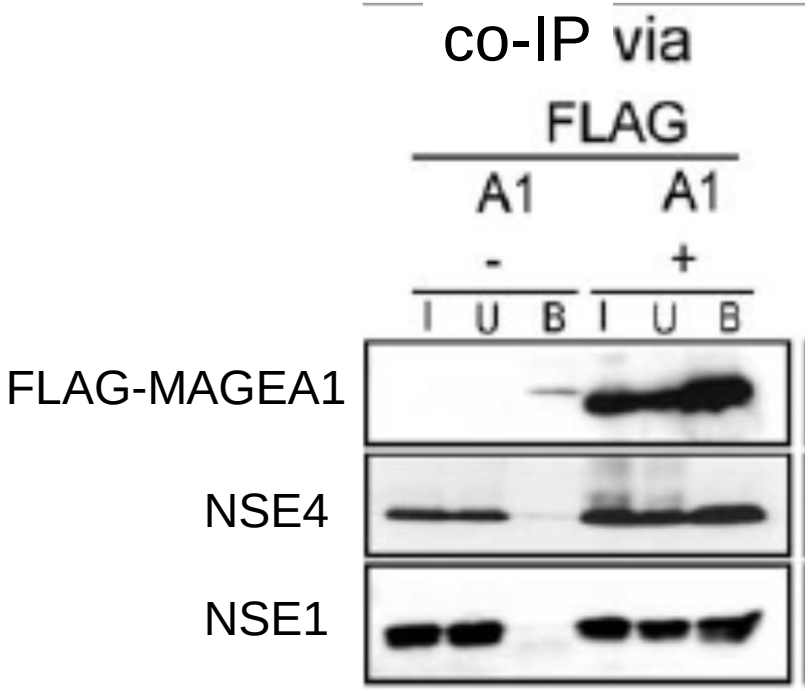
These tags are recognized by specific antibodies (commercially available)

TAP-tag (can be used as well):
ĂTandem-affinity purificationñ
= immunoglobulin tag + calmodulin tag
(usually used to purify complexes)

Co-immunoprecipitation

Similar to pull-down assay, beads/matrix/particles are used to precipitate bait protein with its partners bound

However, whole cell extracts are used (instead of purified proteins)



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

These tags are recognized by specific antibodies (commercially available)

Hudson et al, PLoS One, 2011

precipitated proteins may be associated indirectly (NSE1 is bound via NSE4 linker protein to MAGEA1) with the bait fusion protein (pull-down with pre-purified proteins is more reliable)

Protein-protein interaction analysis

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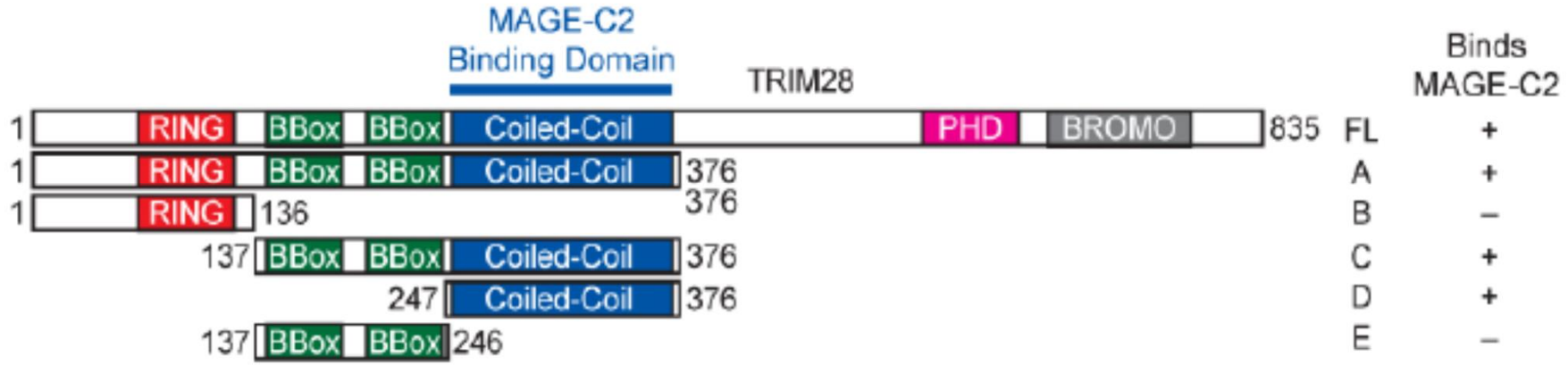
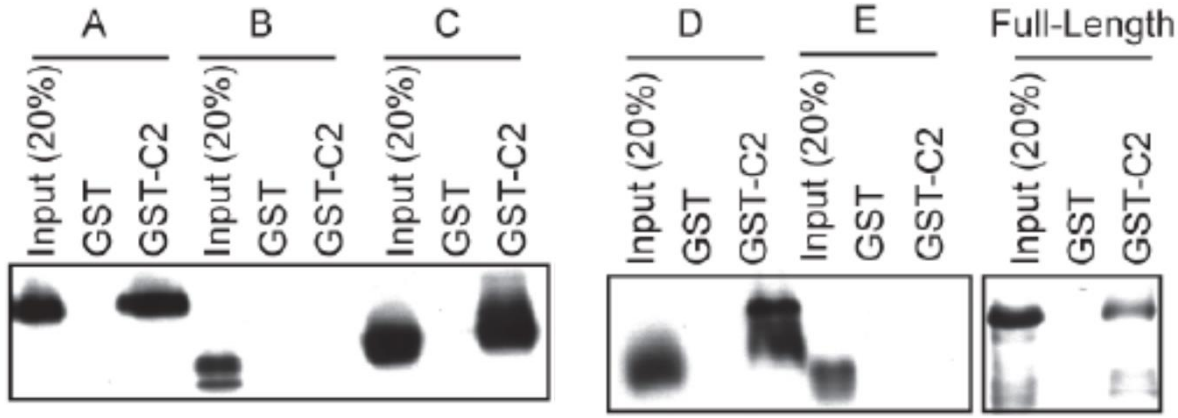
Characterization of binding domain

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

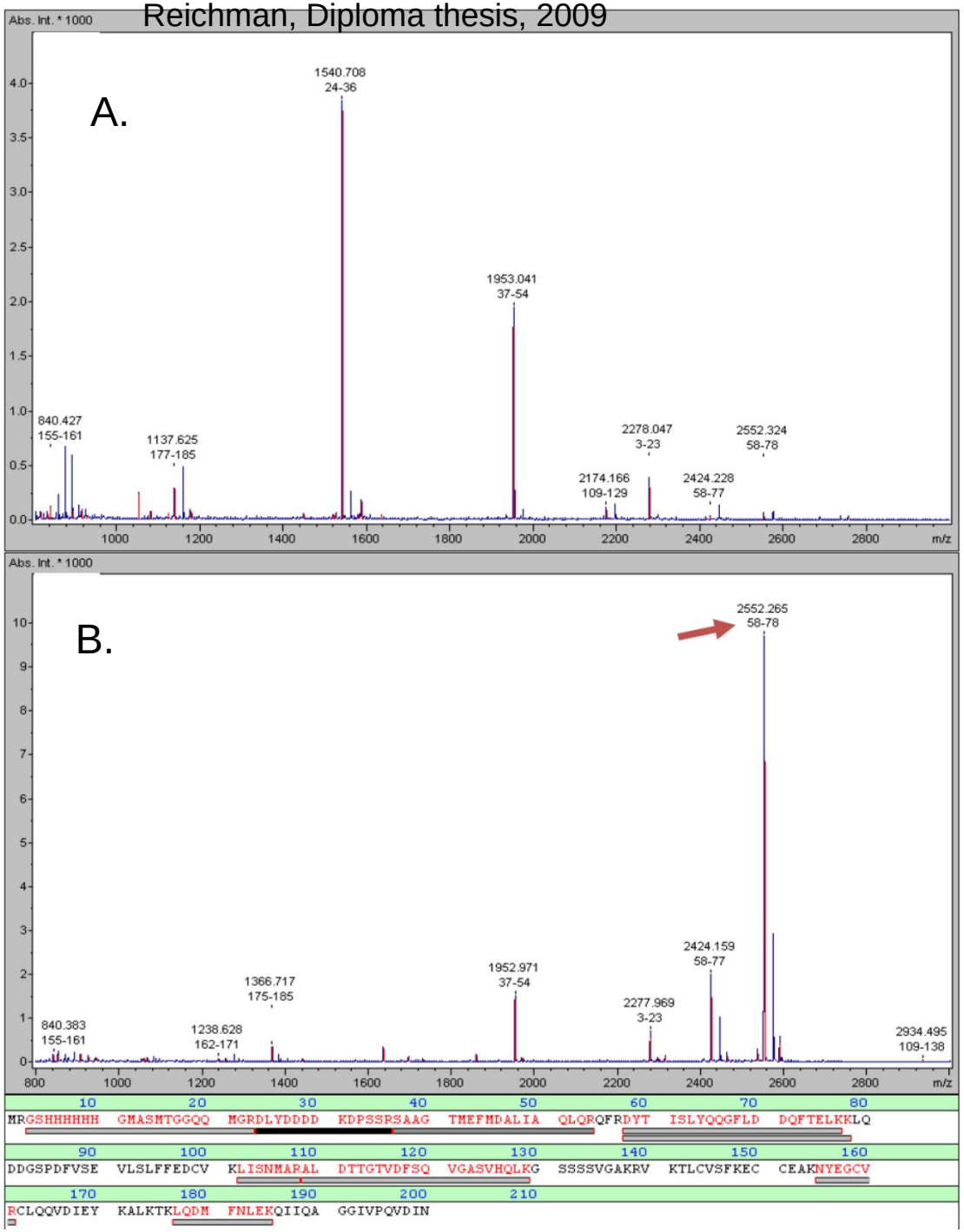


Doyle et al, Mol Cell, 2010



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

Reichman, Diploma thesis, 2009



Characterization of binding regions

Proteins interact via their domains (motifs) ï (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)

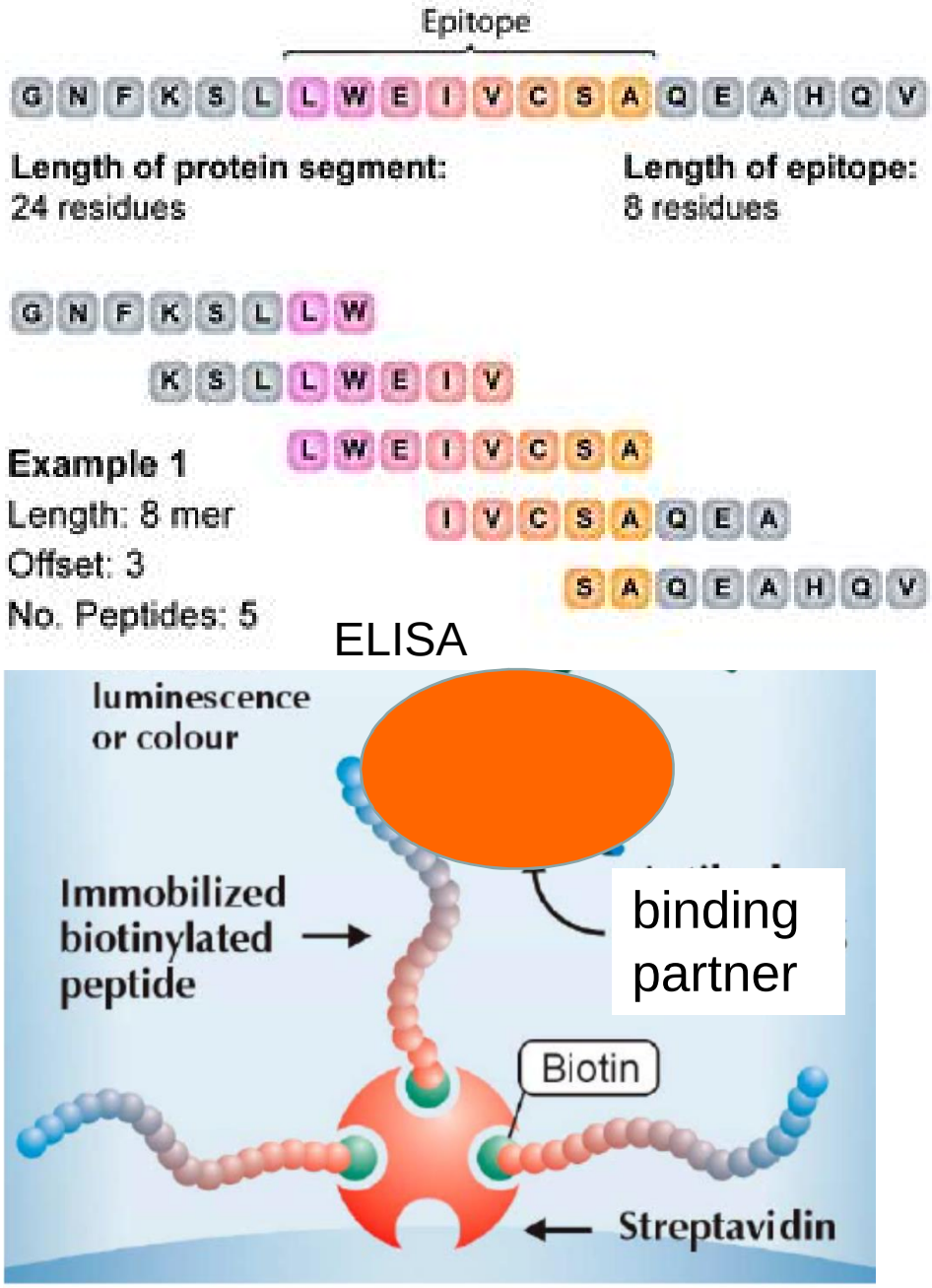
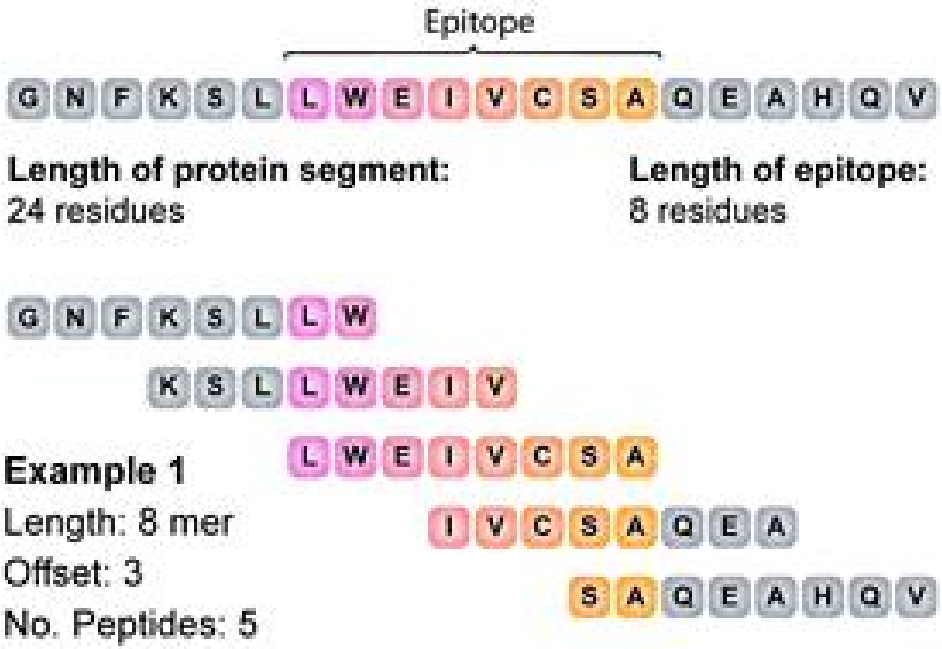


Figure 1: An ELISA using biotinylated peptides and coated plates

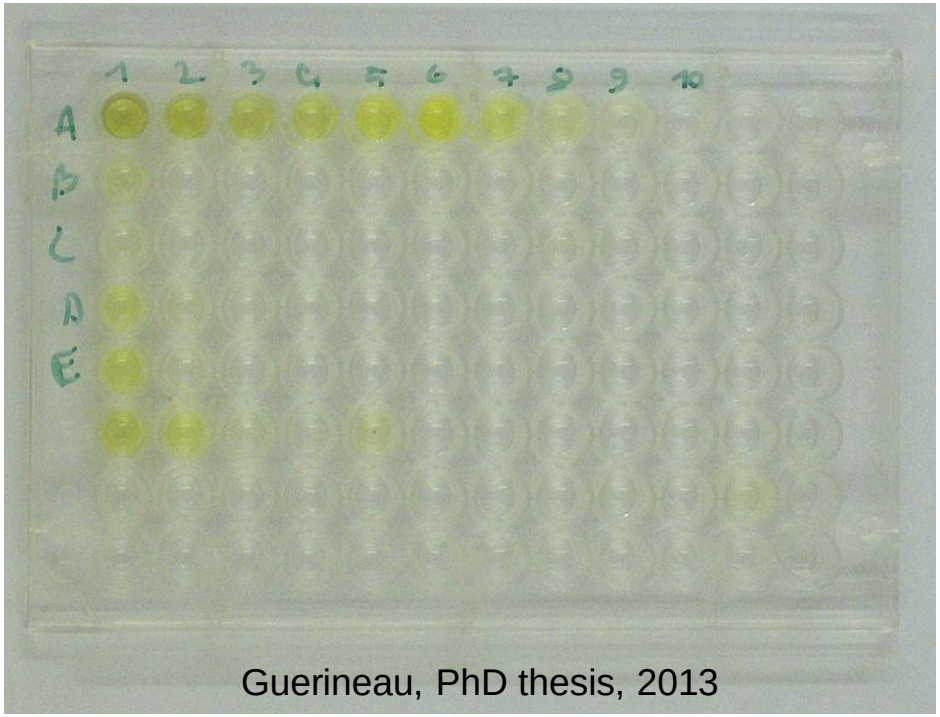
Peptide libraries ï region definition

Proteins interact via their domains (motifs) ï (sometimes) only fragments of the domain can interact (can be precipitated) - peptide library can be synthetized (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 ï binding partner interacts with peptide ï antibody against the partner with conjugated enzyme (or 2nd antibody-enzyme) is applied - luminescence or colour detection



ELISA



Guerineau, PhD thesis, 2013

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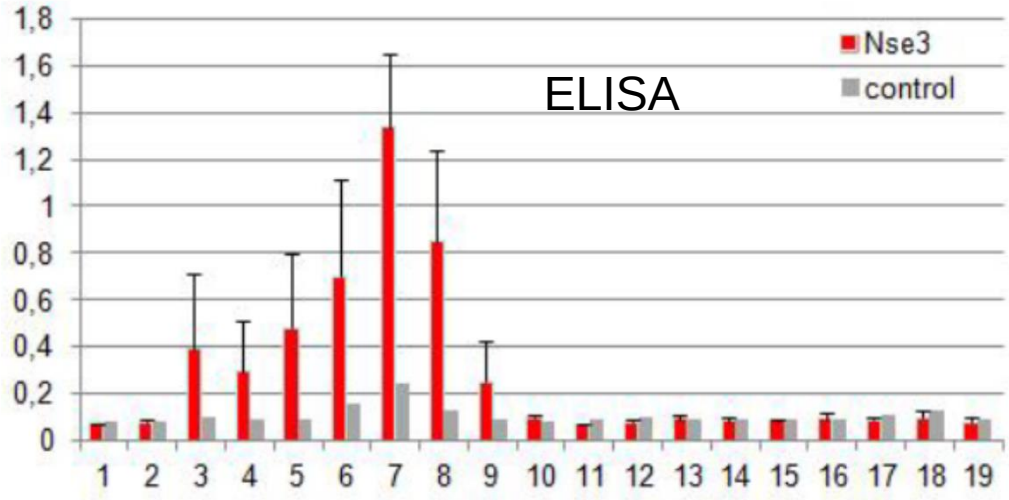
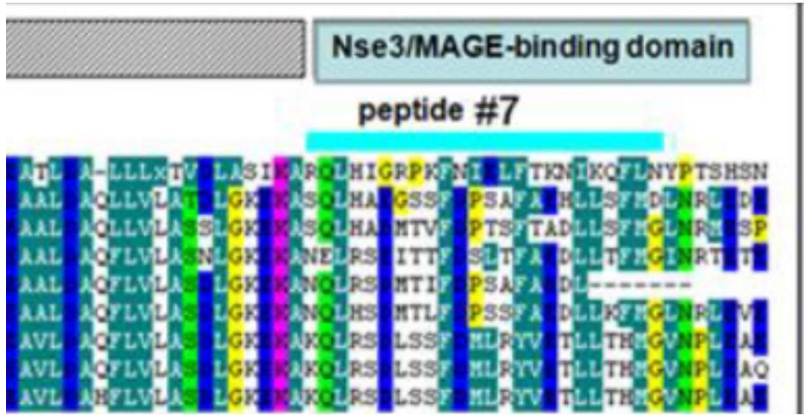
Peptide libraries





	peptide sequence
peptide #1	DAPTEATLDALLLTKTVDLASIKAR
peptide #2	--------EATLDALLLTKTVDLASIKARQLHI
peptide #3	----------DALLLTKTVDLASIKARQLHIGRPK
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	----------QFLNYPTSHSNVTRIQEIDTAWSRL
peptide #13	---------- YPTSHSNVTRIQEIDTAWSRLGKLA
peptide #14	---------- HSNVTRIQEIDTAWSRLGKLASNCE
peptide #15	---------- TRIQEIDTAWSRLGKLASNCEKQPA
peptide #16	---------- EIDTAWSRLGKLASNCEKQPASLNL
peptide #17	---------- AWSRLGKLASNCEKQPASLNLMVGP
peptide #18	---------- LGKLASNCEKQPASLNLMVGPLSFR

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

WT peptide	QRNPHRVDLDILTFTIALTAS
peptide #1	**A**RNPHRVDLDILTFTIALTAS
peptide #2	Q**A**NPHRVDLDILTFTIALTAS
peptide #3	QR**A**PHRVDLDILTFTIALTAS
peptide #4	QRN**A**HRVDLDILTFTIALTAS
peptide #5	QRNP**A**RVDLDILTFTIALTAS
peptide #6	QRNPH**A**VDLDILTFTIALTAS
peptide #7	QRNPHR**A**DLDILTFTIALTAS
peptide #8	QRNPHRV**A**LDILTFTIALTAS
peptide #9	QRNPHRVD**A**DILTFTIALTAS
peptide #10	QRNPHRVDL**A**ILTFTIALTAS
peptide #11	QRNPHRVDLD**A**LTFTIALTAS
peptide #12	QRNPHRVDLDI**A**TFTIALTAS
peptide #13	QRNPHRVDLDIL**A**FTIALTAS
peptide #14	QRNPHRVDLDILT**A**TIALTAS
peptide #15	QRNPHRVDLDILTF**A**IALTAS
peptide #16	QRNPHRVDLDILTFTA**A**LTAS
peptide #17	QRNPHRVDLDILTFTIA**A**TAS
peptide #18	QRNPHRVDLDILTFTIAL**A**AS
peptide #19	QRNPHRVDLDILTFTIALTA**A**

Guerineau, PLoS One, 2012

Peptide libraries ï surface mapping

Proteins interact via their domains (motifs) ï amino acids essential for the interaction can be identified (via mutational analysis ï e.g. alanine substitutions = ñalanine scanò)

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

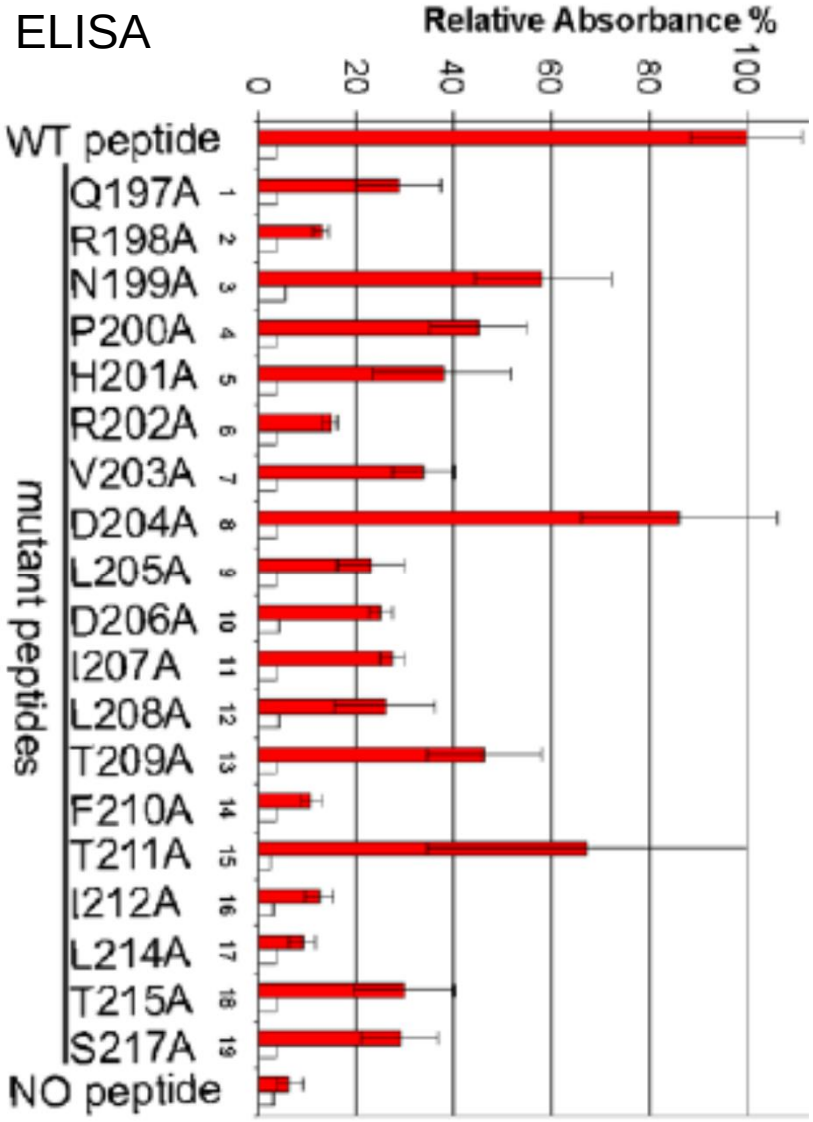
21 amino acids long (20) peptides library with single amino acid alanine substitution (covering every non-Ala amino acid)

Peptide libraries ï alanine scan

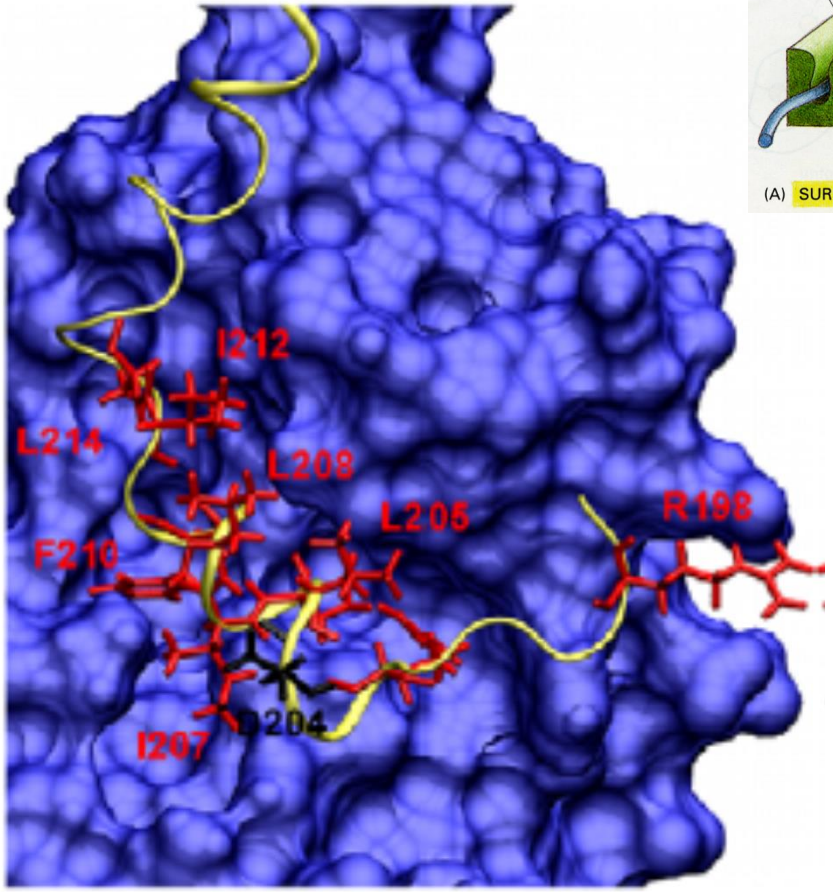
	197	217
WT peptide	QRNPHRVDLDILTFTIALTAS	
peptide #1	**A**RNPHRVDLDILTFTIALTAS	
peptide #2	Q**A**NPHRVDLDILTFTIALTAS	
peptide #3	QR**A**PHRVDLDILTFTIALTAS	
peptide #4	QRN**A**HRVDLDILTFTIALTAS	
peptide #5	QRNP**A**RVDLDILTFTIALTAS	
peptide #6	QRNPH**A**VDLDILTFTIALTAS	
peptide #7	QRNPHR**A**DLDILTFTIALTAS	
peptide #8	QRNPHRV**A**LDILTFTIALTAS	
peptide #9	QRNPHRVD**A**DILTFTIALTAS	
peptide #10	QRNPHRVDL**A**ILTFTIALTAS	
peptide #11	QRNPHRVDLD**A**LTFTIALTAS	
peptide #12	QRNPHRVDLDI**A**TFTIALTAS	
peptide #13	QRNPHRVDLDIL**A**FTIALTAS	
peptide #14	QRNPHRVDLDILT**A**TIALTAS	
peptide #15	QRNPHRVDLDILTF**A**IALTAS	
peptide #16	QRNPHRVDLDILTFTA**A**LTAS	
peptide #17	QRNPHRVDLDILTFTIA**A**TAS	
peptide #18	QRNPHRVDLDILTFTIAL**A**AS	
peptide #19	QRNPHRVDLDILTFTIALTA**A**	

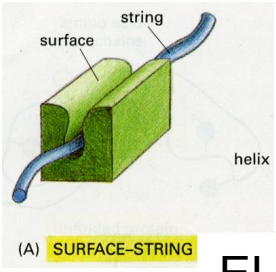
Guerineau, PLoS One, 2012

ELISA



Peptide libraries ï surface mapping





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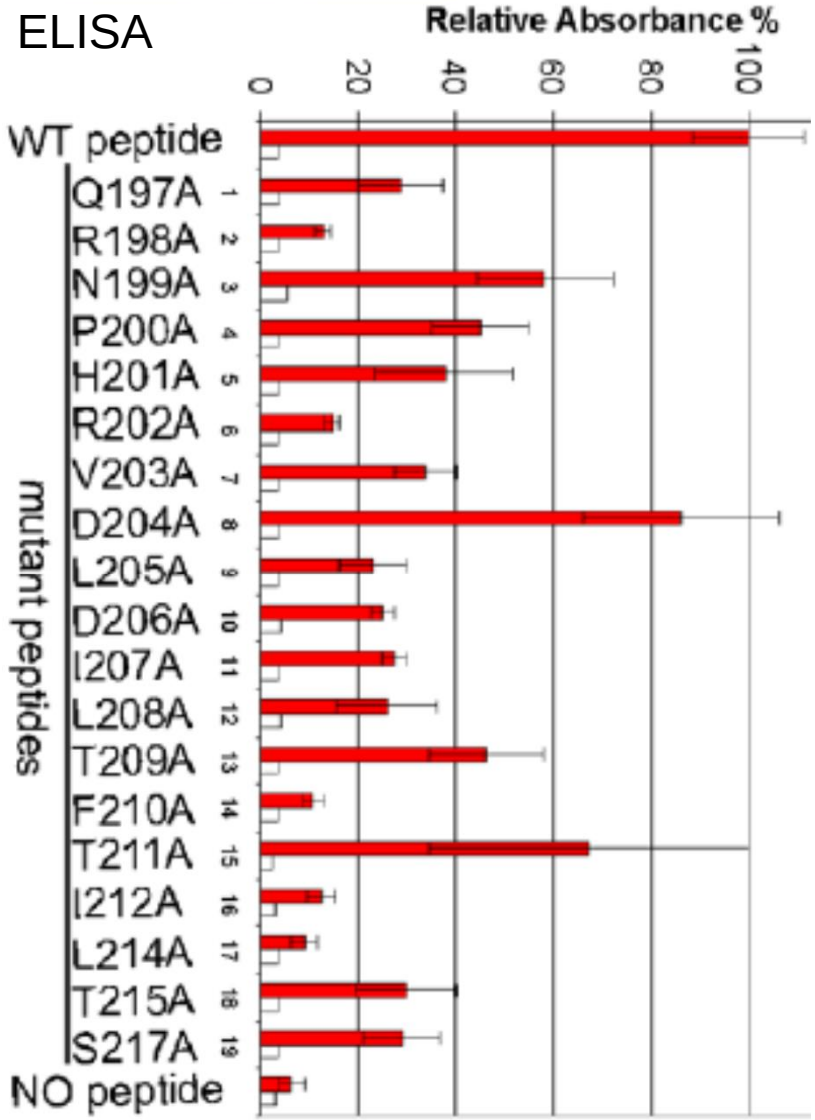

Helical peptide is sitting in the pocket of the partner protein ï most peptide residues are in contact (red labeled) with the pocket (so, their mutations reduced the mutant peptide affinity), while the D204 (black labeled) residue is exposed to solvent

Guerineau, PLoS One, 2012

Protein-protein interaction analysis

- matrix/beads-based: pull-down (*in vitro*), coIP é
- **hybrid-based:**
  - **classical systems-** *domain*
    - transcription 2-hybrid systems
      - reverse systems
    - multi-hybrid systems
    - alternative (membrane) systems
  - **complementation systems ï** *fold*
    - BiFC, DHFR
  - proximity/transfer system - FRET
- proximity-based: PLA, BioID é
- MS-based: crosslink, D/H-exchange é
- Quantitative methods: SPR, ITC é
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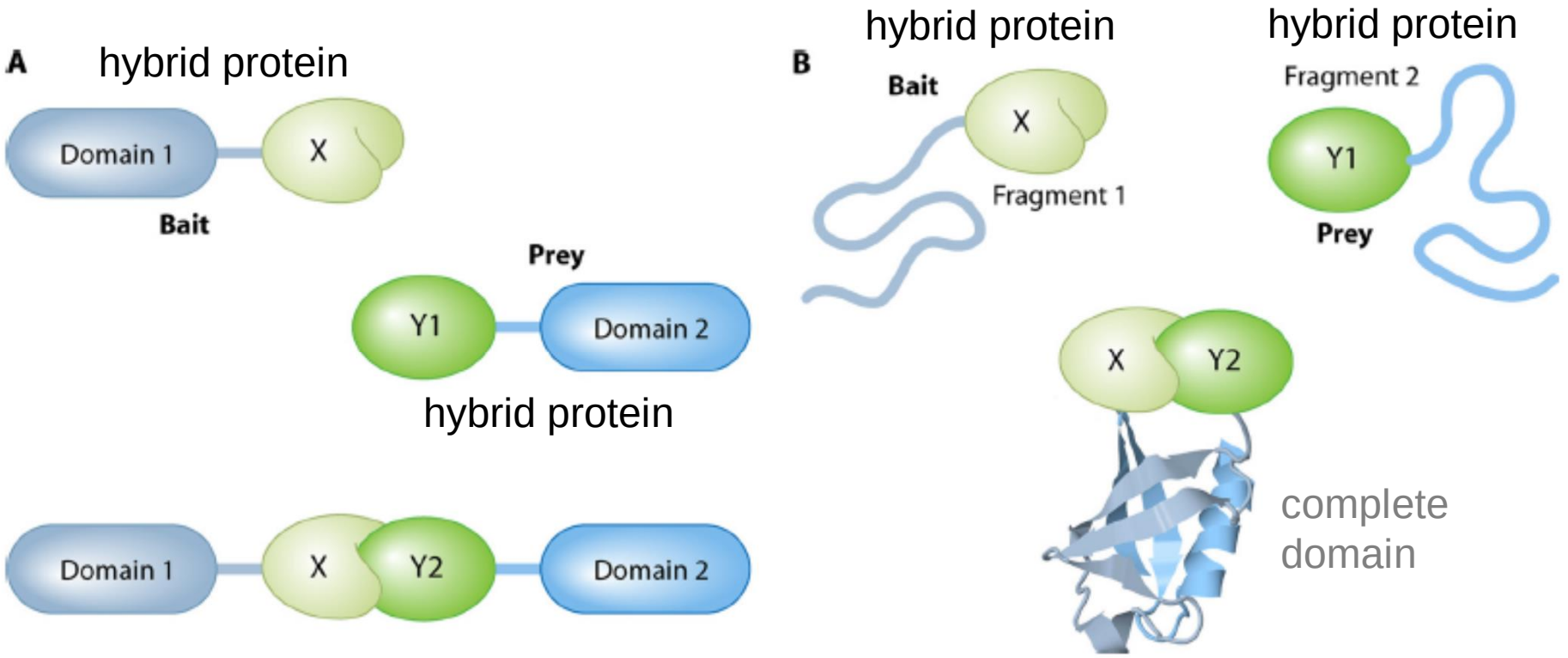
Principal differences in hybrid systems



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

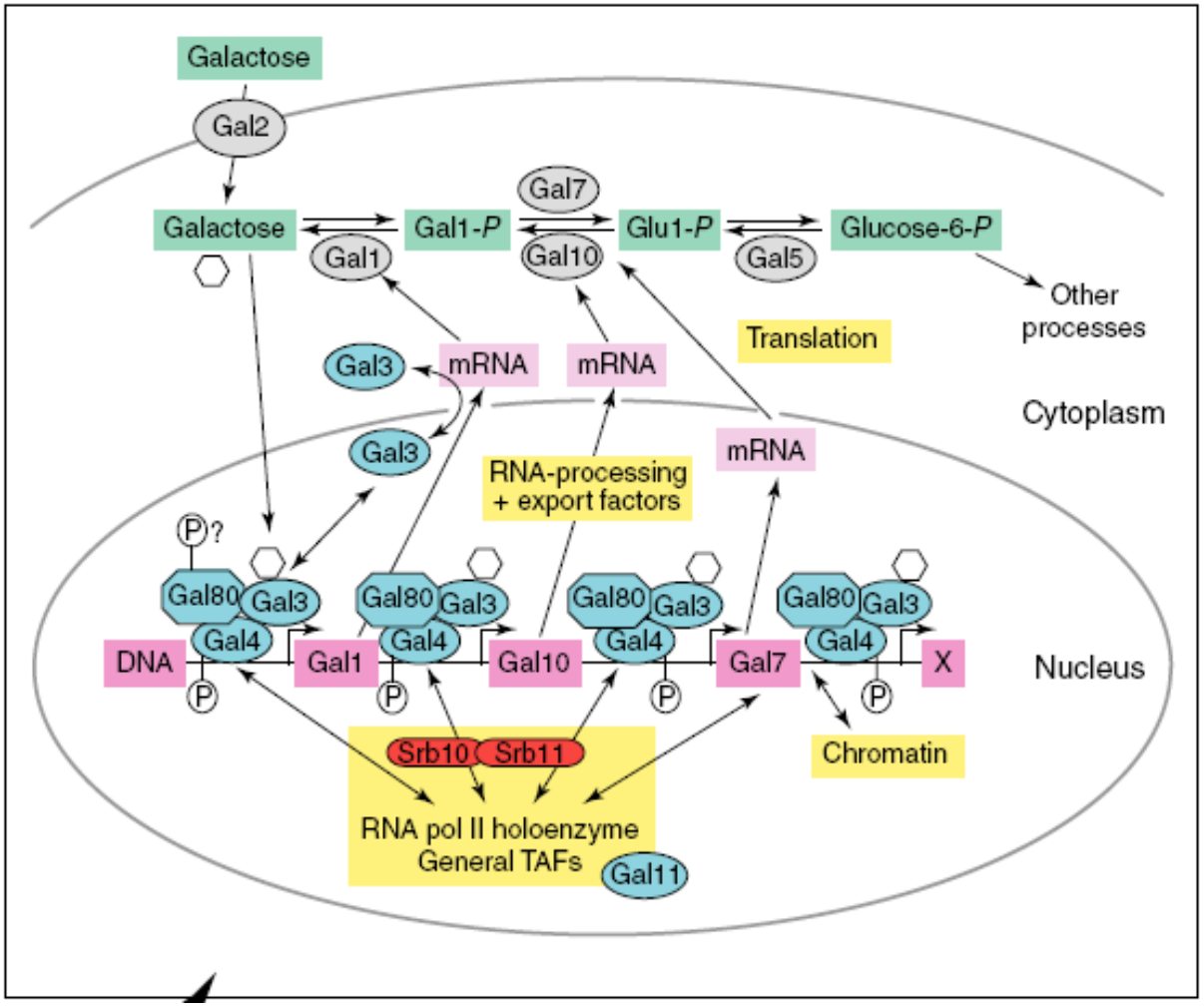
B. In complementation systems, PPI reconnects fragments of one domain and reconstitutes its fold

In FRET system, PPI enables energy transfer (see below)

Stynen et al, MMBR, 2012

Classical yeast two-hybrid system

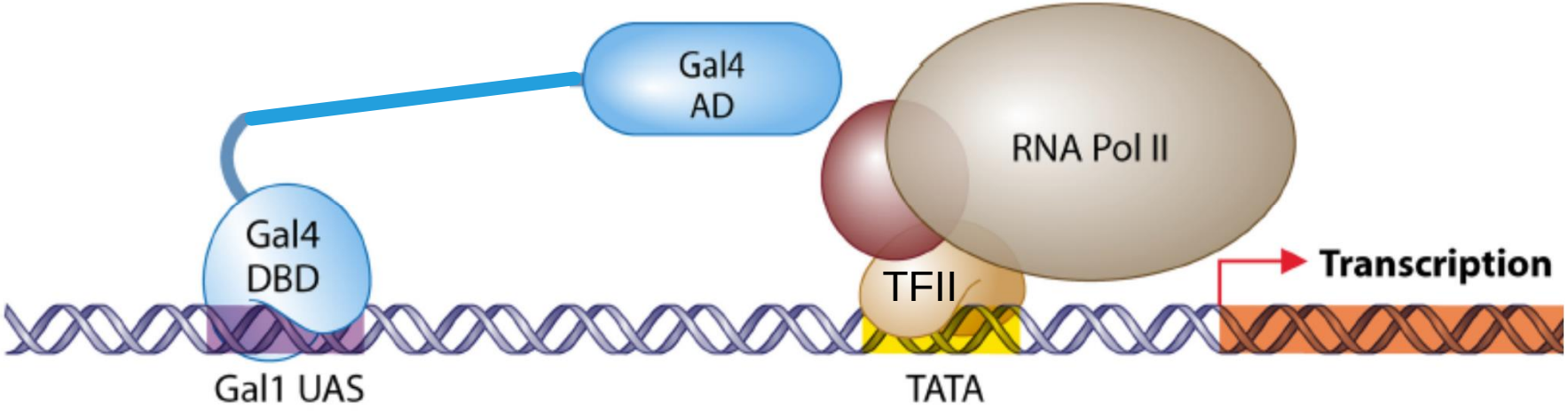
Classical (first) yeast two-hybrid system is based on transcription factor Gal4 function ï Gal4 binds promotor regions (sequences) of *GAL* genes and activates their transcription



Uetz and Finley, FEBS lett., 2005

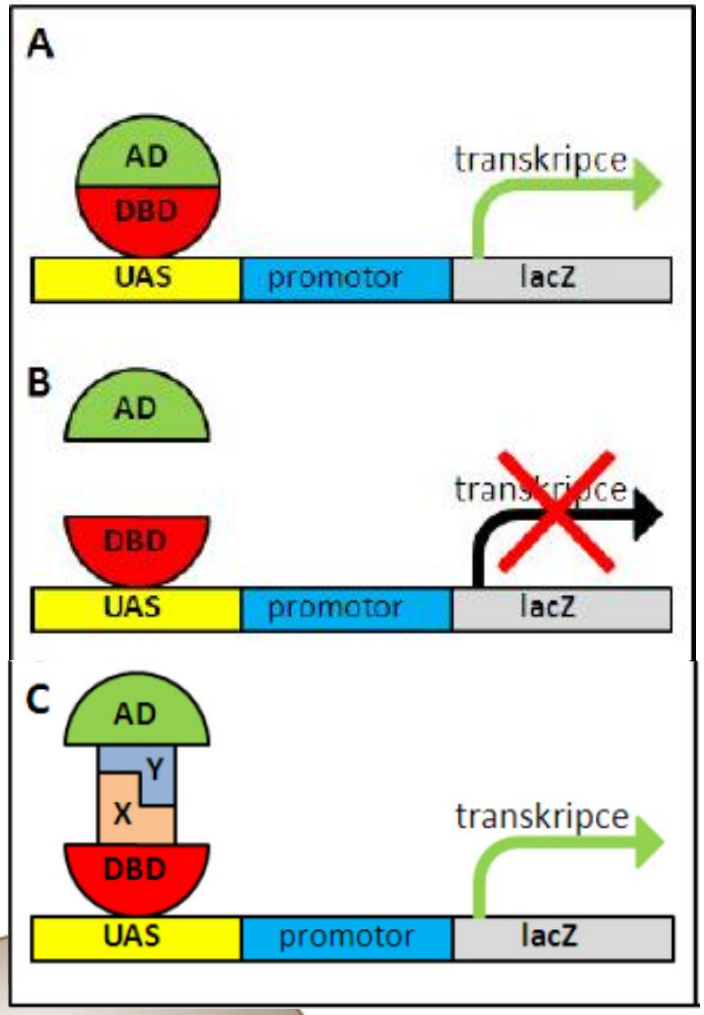
Gal4-based two-hybrid system

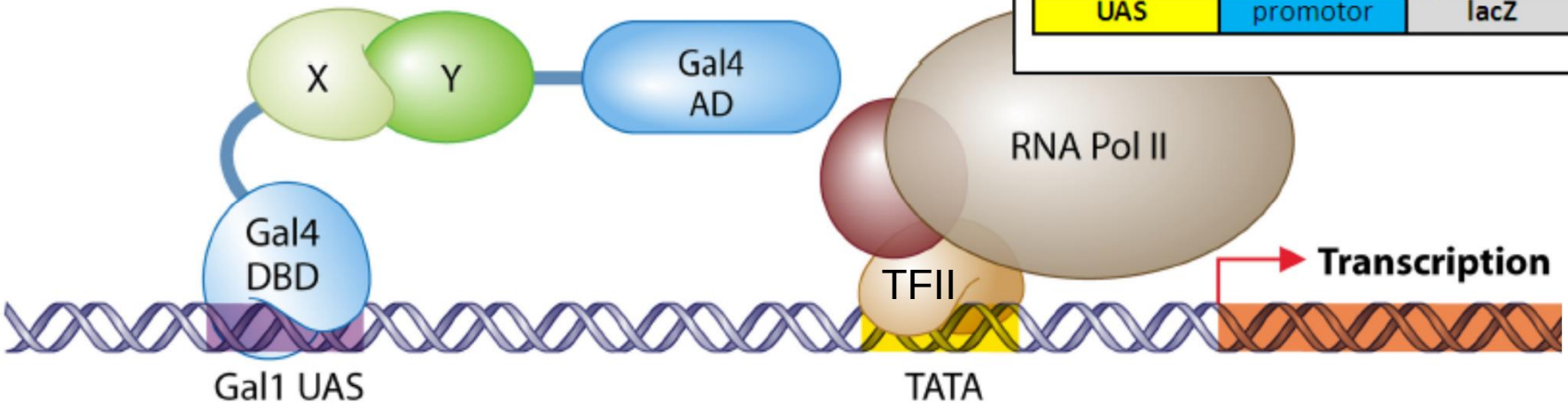
Gal4 transcription factor binds specific DNA sequence through its DNA-binding domain (DBD) - Gal4 transcription activation domain (AD) binds to general TFII factors/RNA polymerase II and activates transcription machinery



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

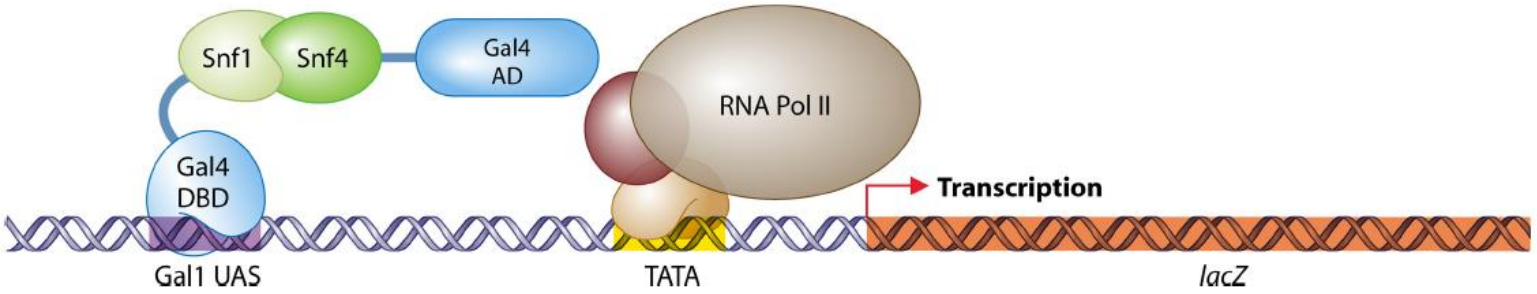




Other transcription factors have been employed in two-hybrid variants:

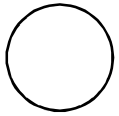
Prey activation domains	
S. cerevisiae Gal4 AD	Gal4 activating region II (aa 768 to 881), moderate strength (178)
Herpes simplex virus VP16 AD	VP16 activating region (aa 413 to 490), high strength (673)
E. coli B42 AD	Bacterial polypeptide, weak strength (234)
Bait DNA-binding domains	
S. cerevisiae Gal4 DBD*	Binds *GAL1*, *GAL2*, and *GAL7* upstream activating sequences (178)
E. coli repressor LexA DBD*	Binds LexA operator sequences (234)
H. sapiens estrogen receptor DBD	Binds estrogen receptor elements (374)
Bacteriophage λ repressor cI	Binds cI operator sequences (580)
Tet repressor	Binds Tet operator sequences (716)

Stynen et al, MMBR, 2012



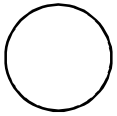
To detect/score transcription activation (i.e. ñseeò interaction of partner proteins), different reporter genes are used

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) ï lacZ enzymatic activity can be measured (thus, the strength of the PPI can be quantified)

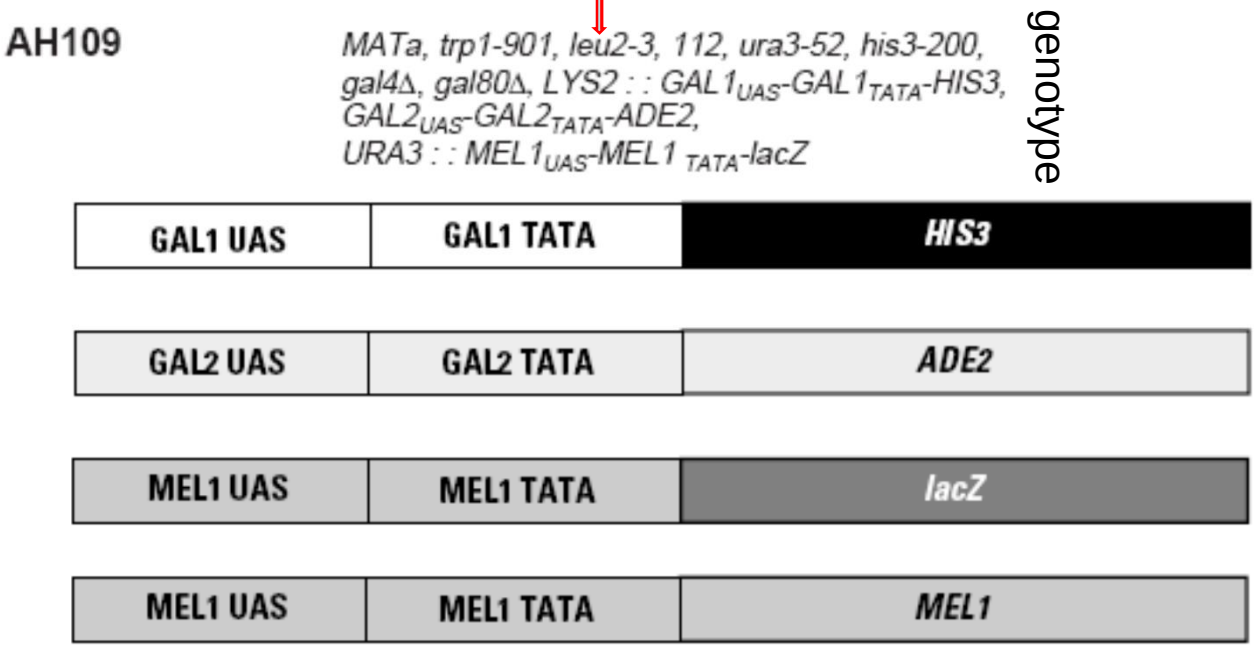
Reporter genes

Reporter genes		
*E. coli lacZ**	β-Galactosidase chromogenic reporter (178)	quantitative
S. cerevisiae MEL1	Secretory α-galactosidase chromogenic reporter (5)	
E. coli gusA	β-Glucuronidase chromogenic reporter (580)	
Aspergillus oryzae lacA3	Engineered secretory β-galactosidase chromogenic reporter (318)	
*S. cerevisiae HIS3**	Prototrophic reporter for histidine biosynthesis (673)	⟵ His3 enzyme activity can be titrated by its 3-aminotriazol inhibitor
*S. cerevisiae LEU2**	Prototrophic reporter for leucine biosynthesis (234)	
S. cerevisiae URA3	Prototrophic reporter for uracil biosynthesis (374)	
*S. cerevisiae ADE2**	Prototrophic reporter for adenine biosynthesis (299)	auxotrophy (selective)
S. cerevisiae LYS2	Prototrophic reporter for lysine biosynthesis (580)	
Aequorea victoria GFPuv	Fluorescent reporter (107)	FACSorting
EGFP	Fluorescent reporter (613)	
Yeast *EGFP*	Fluorescent reporter for flow cytometry screens (88)	
Aureobasidium pullulans AUR1-C	Aureobasidin A resistance reporter (167)	antibiotic resistance

Stynen et al, Microbiol Mol Biol Rev, 2012

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 promotors (GAL1 and MEL1, respectively)

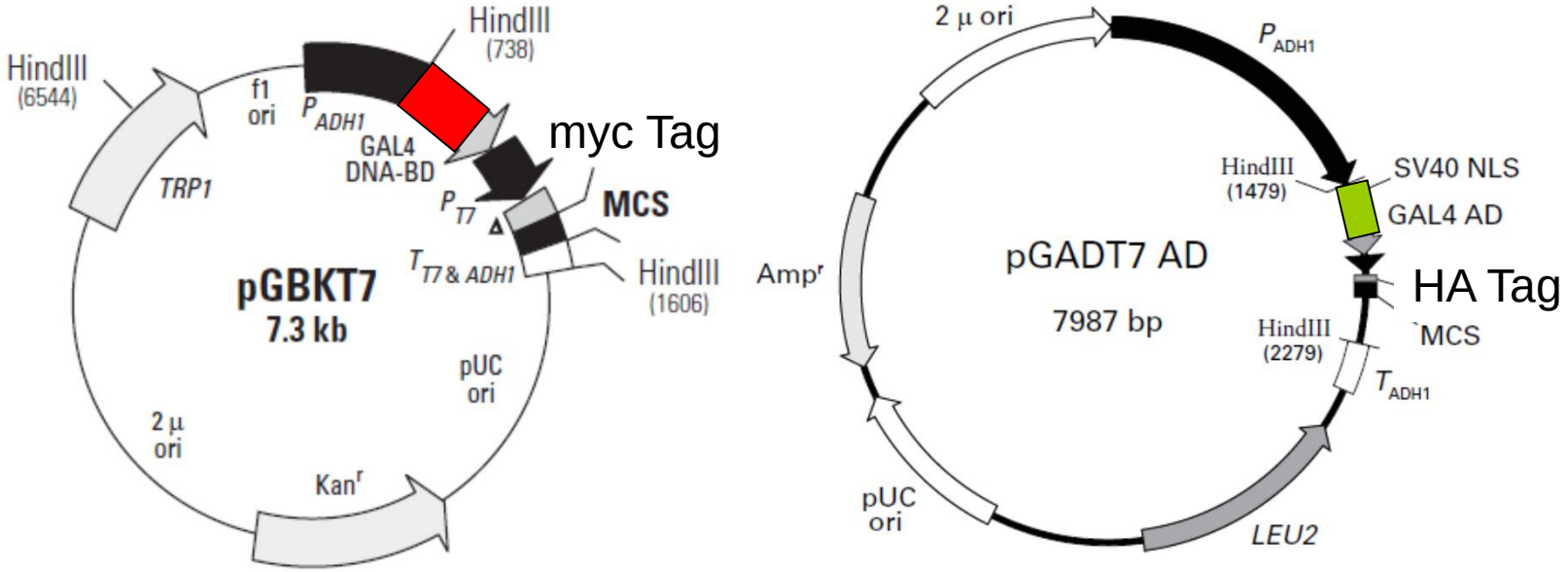


Trp1 and *Leu2* genes must be mutated to enable (auxotrophy) selection of plasmids (bearing hybrid genes)

- many yeast strains exist; systems adopted to bacterial and mammalian cells exist as well

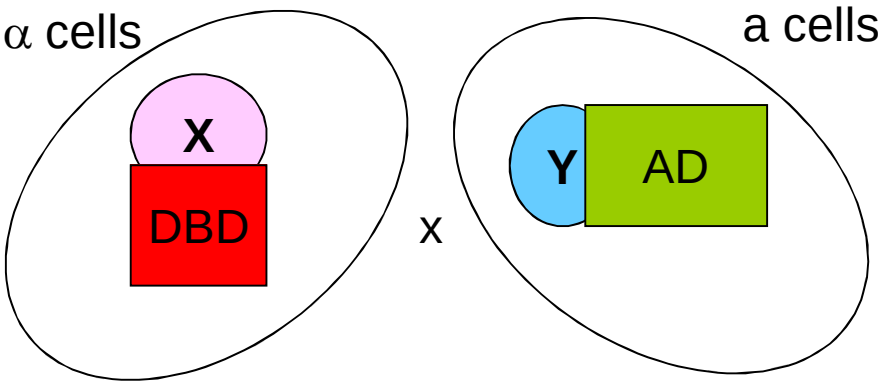
Yeast 2-hybrid plasmid example

pGBKT7 and pGADT7 plasmids contain **G**al4 **B**D and **A**D elements (to make hybrid proteins) as well as selective markers (Trp1 and Leu2 for yeast selection)

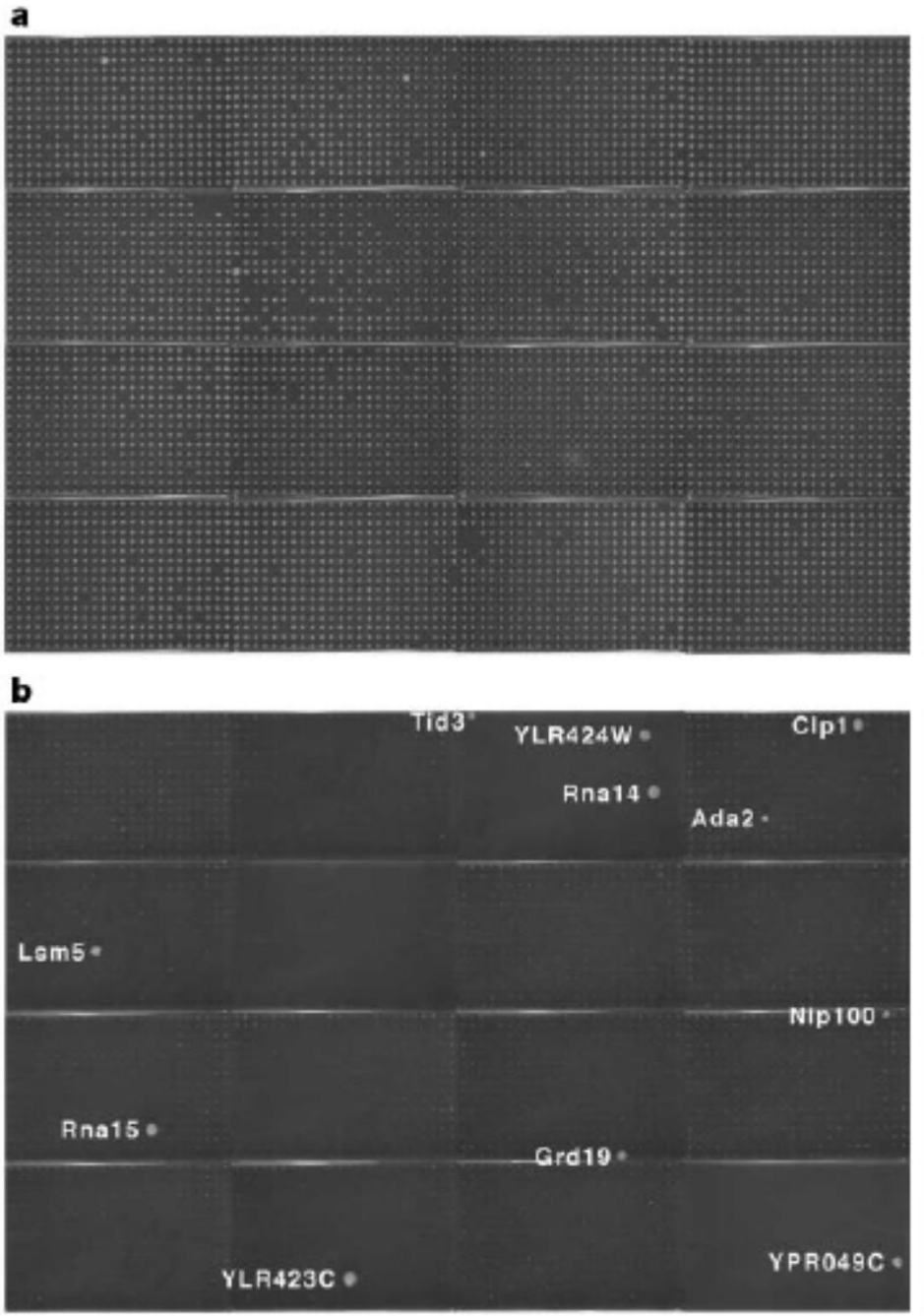


T7 promoters in front of myc and HA tag, respectively, are suitable for additional pull-down experiments (see previous slides)

Yeast 2-hybrid screens

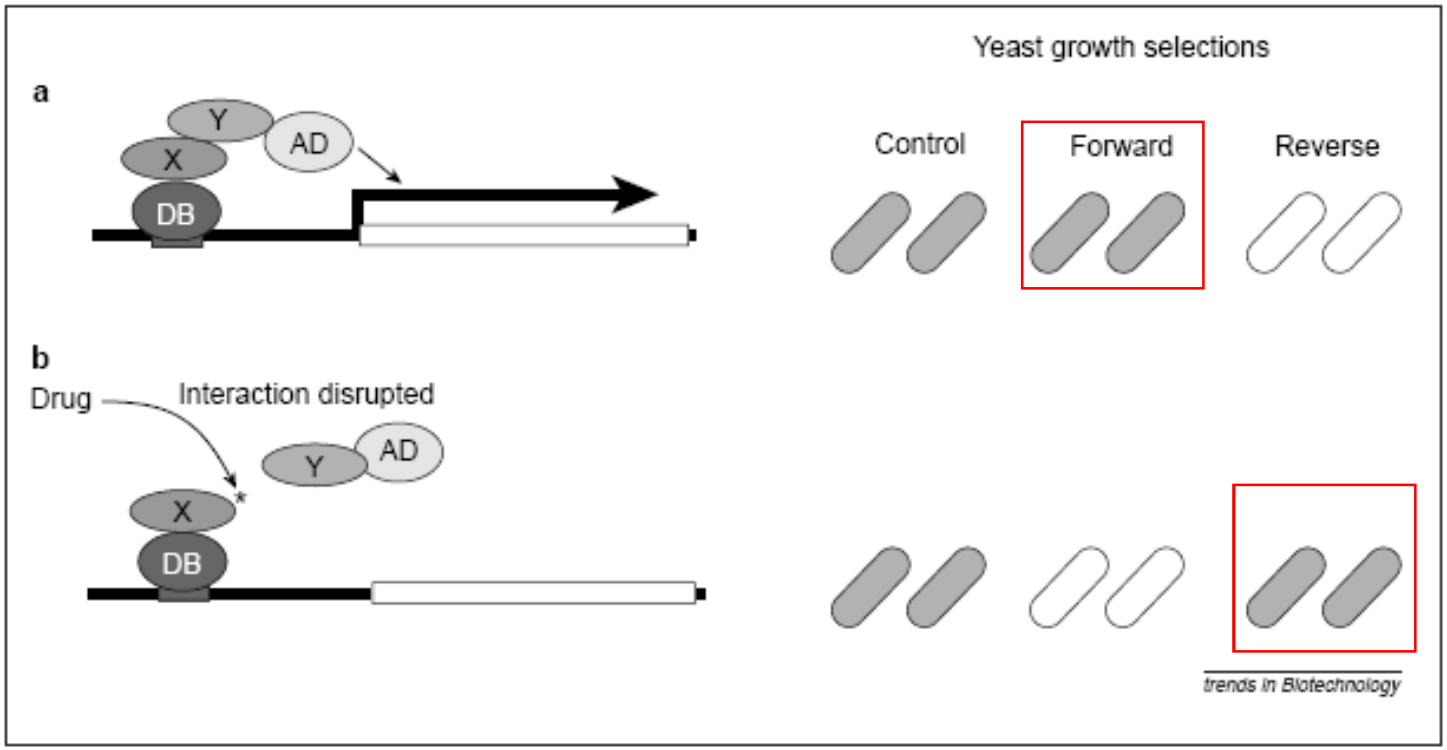


High-throughput screens can be done as ï 1. simple study: one bait is screened against AD-library (e.g. of all human hybrid proteins) - or ï 2. interactom study: collection of all BD-proteins is screened against AD-library (e.g. 6000x6000 yeast proteins = yeast interactom)



Uetz et al, Nature, 2000

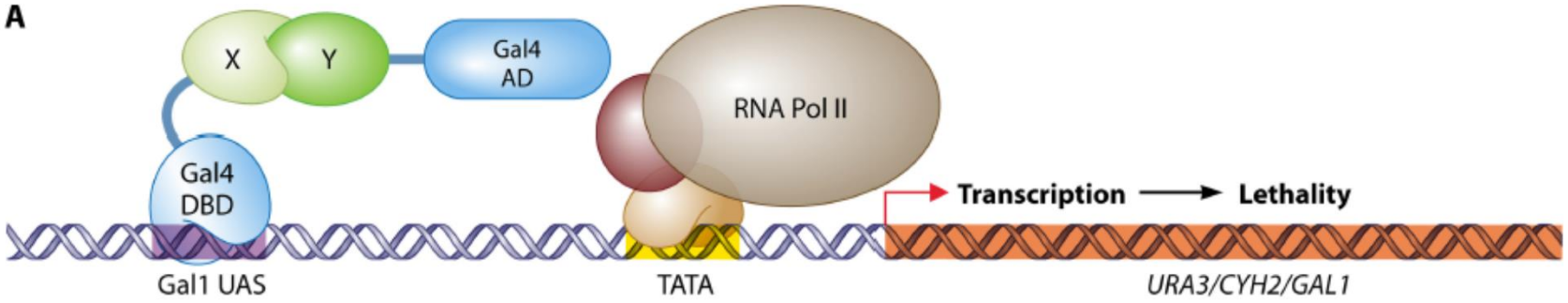
Reverse systems



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) ï reverse systems were developed to ñvisualizeòloss of interaction é

Reverse systems

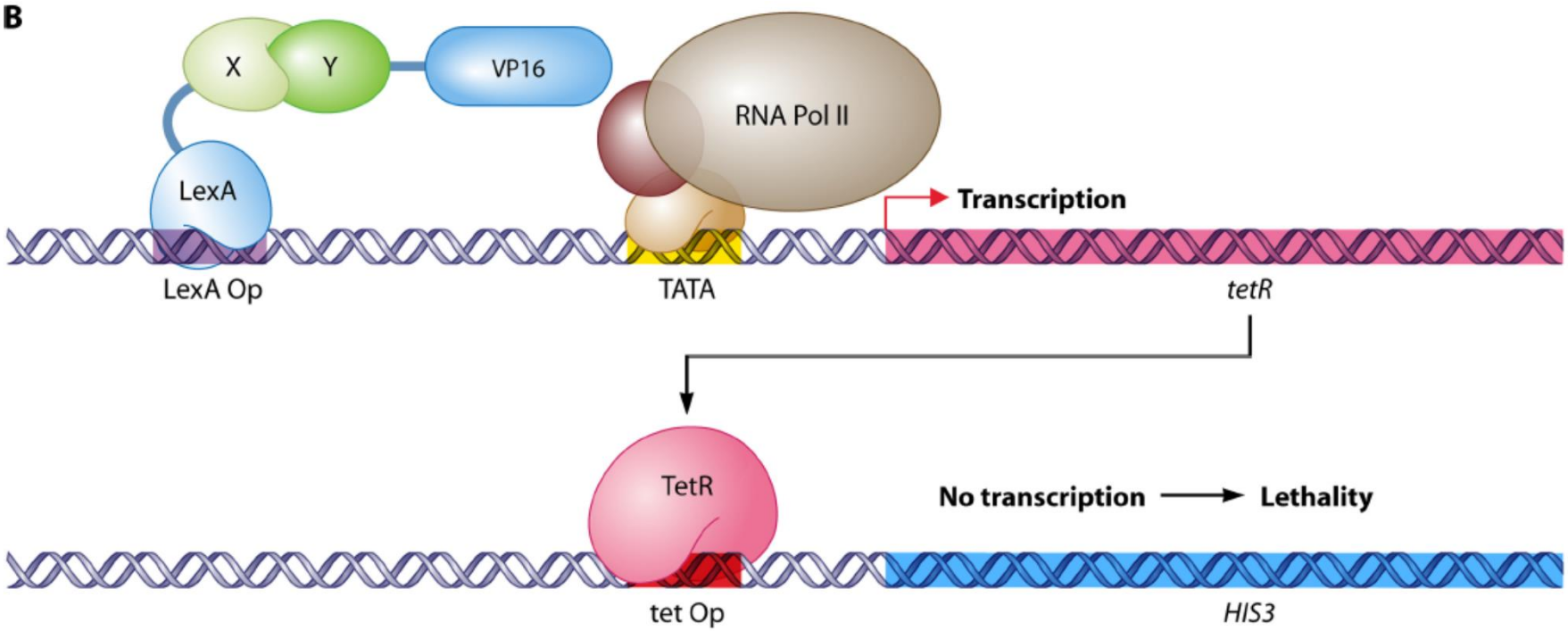


é in reverse systems, PPI results in lethal phenotype ï yeast cells will not grow until PPI is disturbed (by mutation or drug) ï for example, cells expressing URA3 reporter gene will grow on plates without uracil, but these cells will be killed by 5-flouro-orothic acid (Ura3 enzyme converts FOA to toxic 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

Stynen et al, MMBR, 2012

Reverse systems

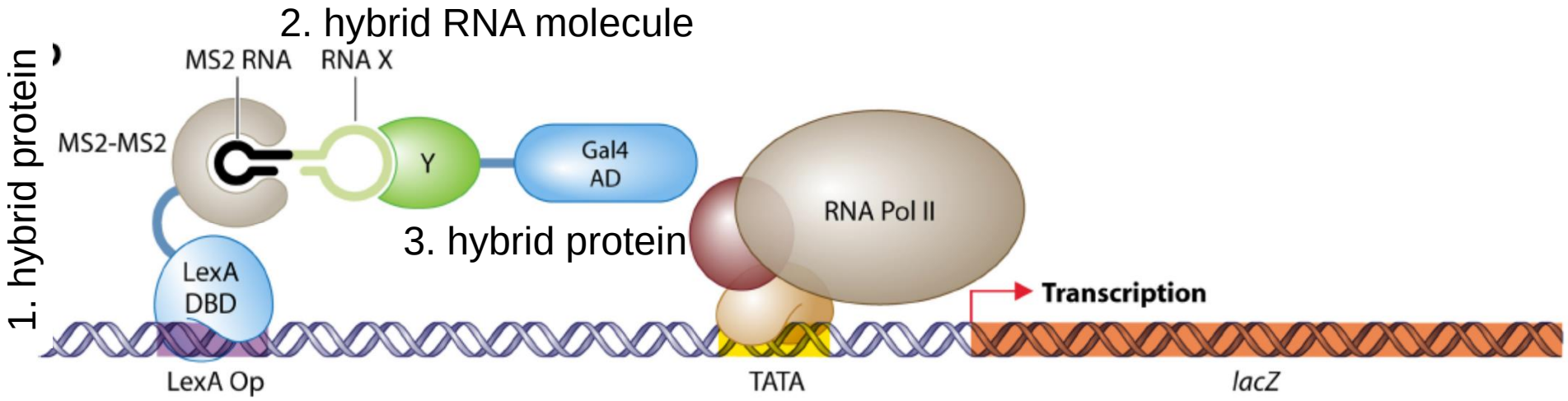
é new reverse system (also called split system) is based on two transcription regulation steps: PPI activates transcription of repressor which blocks transcription of reporter gene (only when PPI is disturbed, the His3 reporter gene is transcribed)



Stynen et al, MMBR, 2012

(multi) three-hybrid systems

First three-hybrid system was developed to study RNA-binding proteins ï 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) ï this RNA-protein complex will switch on lacZ reporter gene transcription ï in this way, you can screen AD-hybrid library for RNA-X binding proteins

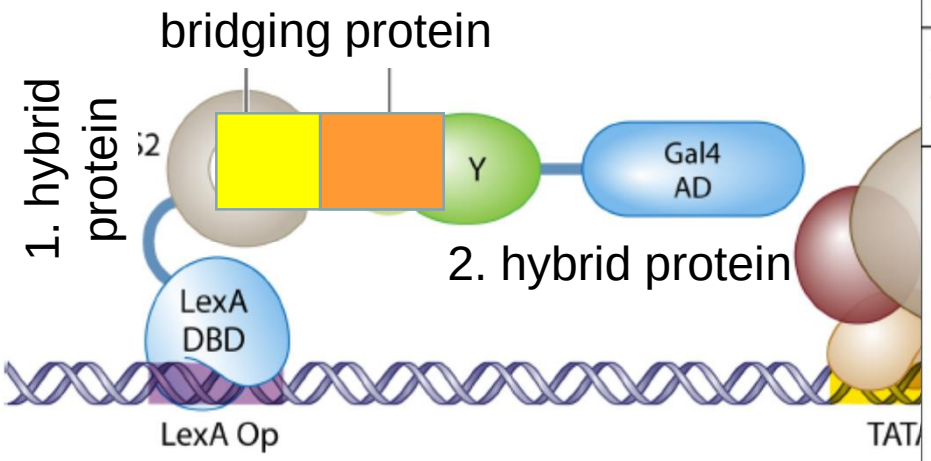


SenGupta et al, PNAS, 1996

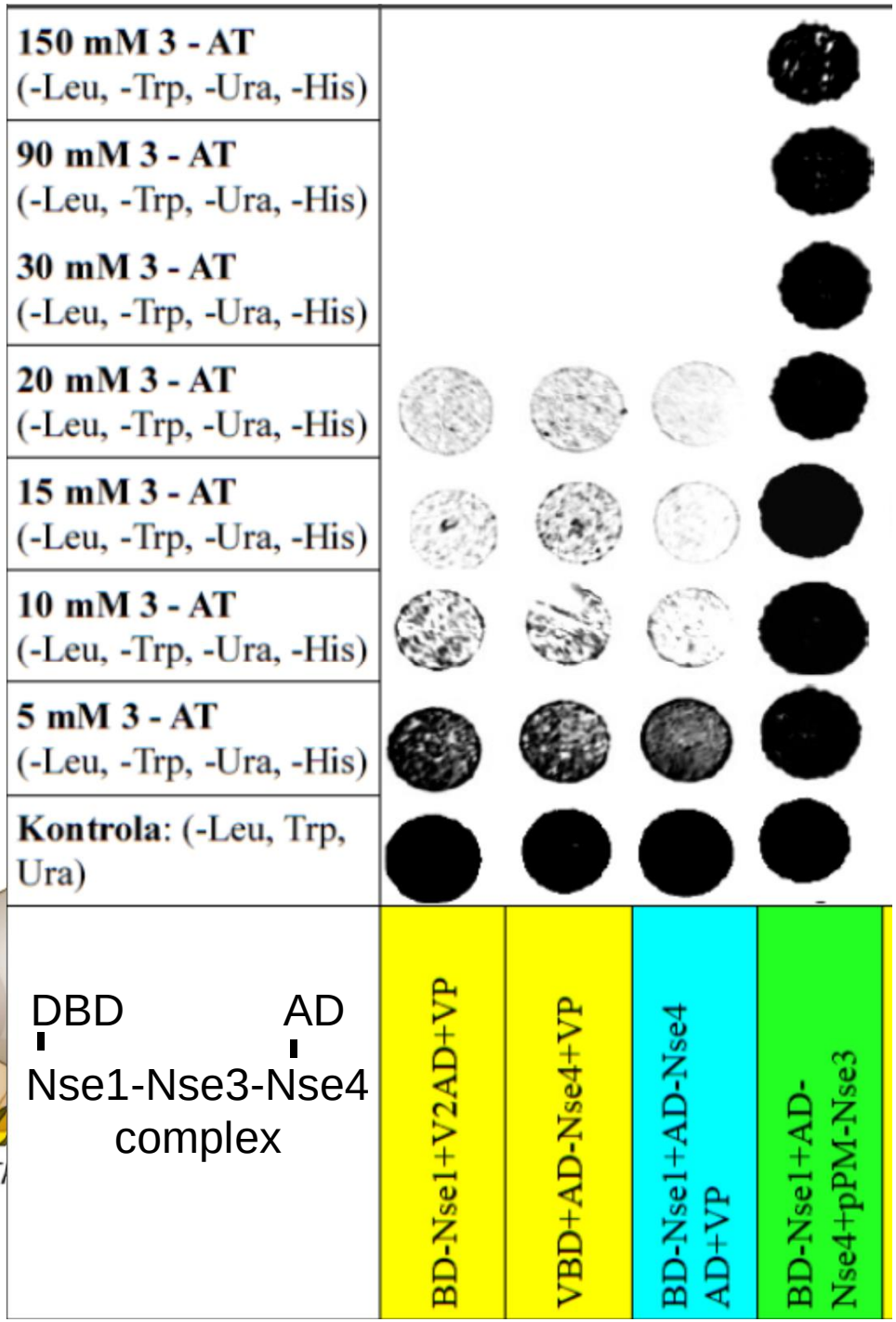
Stynen et al, MMBR, 2012

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)

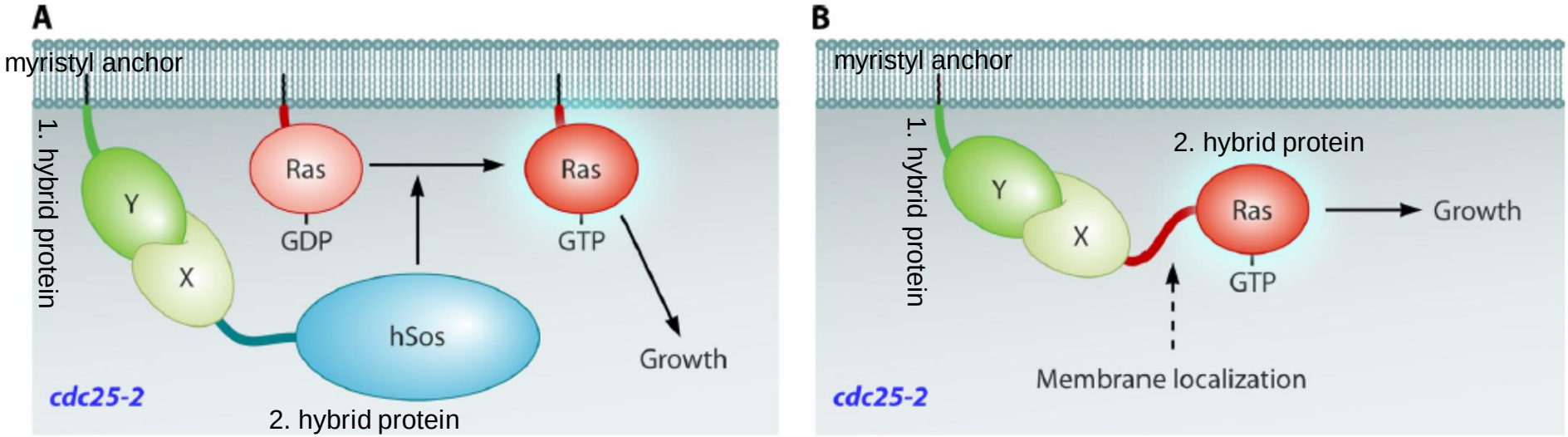


Bednarova, Diploma thesis, 2009



Alternative membrane systems - Ras

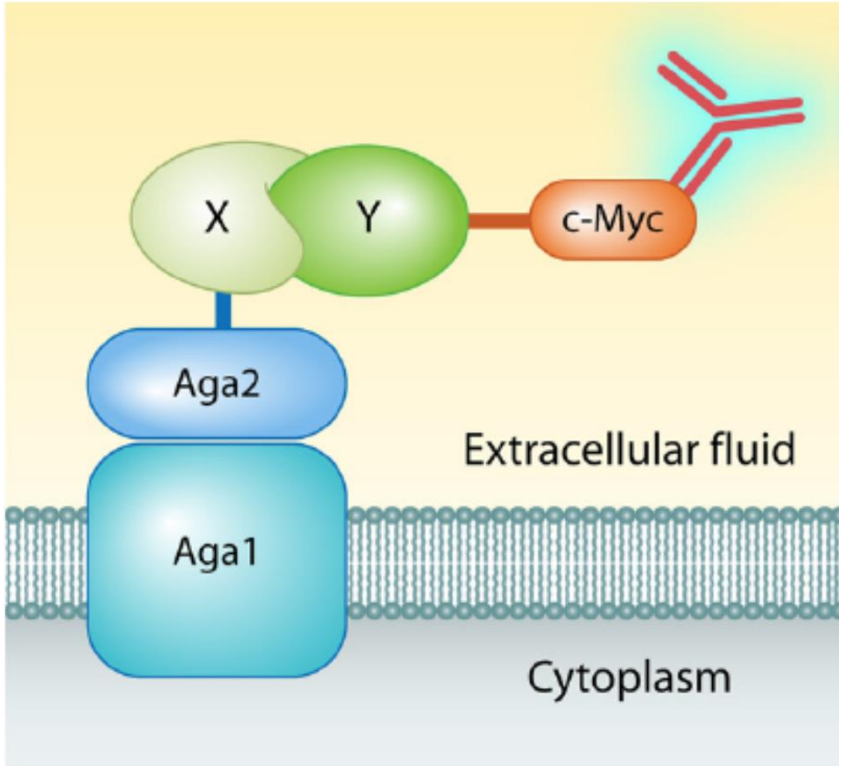
Number of proteins cannot be used in transcription-based hybrid systems (e.g protein cannot be localized to the yeast cell nucleus) ï CytoTrap (Ras recruitment) system is based on membrane-anchored Ras pathway reactivation ï **A.** RAS protein is activated only when human hSOS-hybrid, otholog of yeast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



Stynen et al, Microbiol Mol Biol Rev, 2012

Alternative membrane systems - Aga

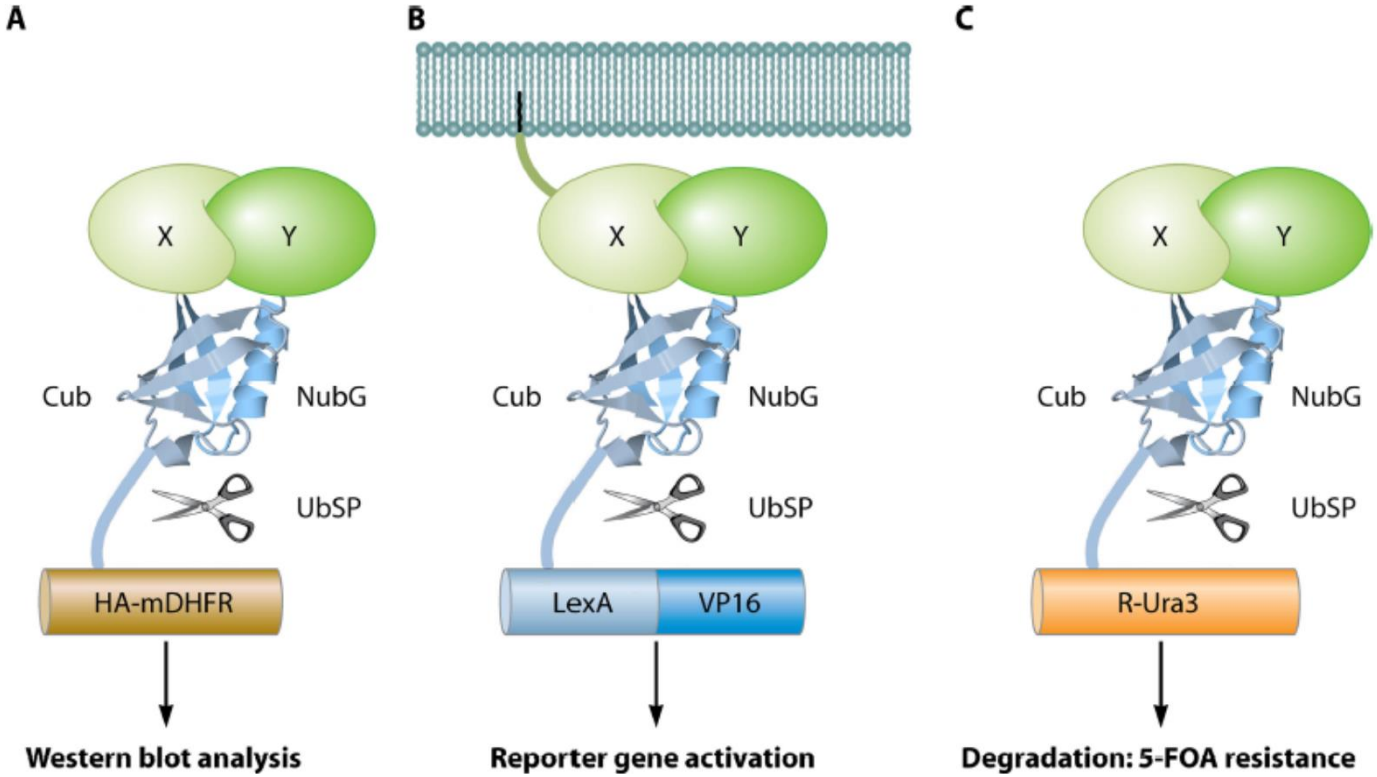
Yeast surface display system ï Aga2-hybrid protein is localized at the yeast surface ï tagged-partner interaction anchors it at the yeast surface ï anti-tag antibody recognizes the tagged protein ï fluorescence of the antibody (primary or secondary antibody) is detected and can be used for yeast strain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



Stynen et al, MMBR, 2012

Complementation systems

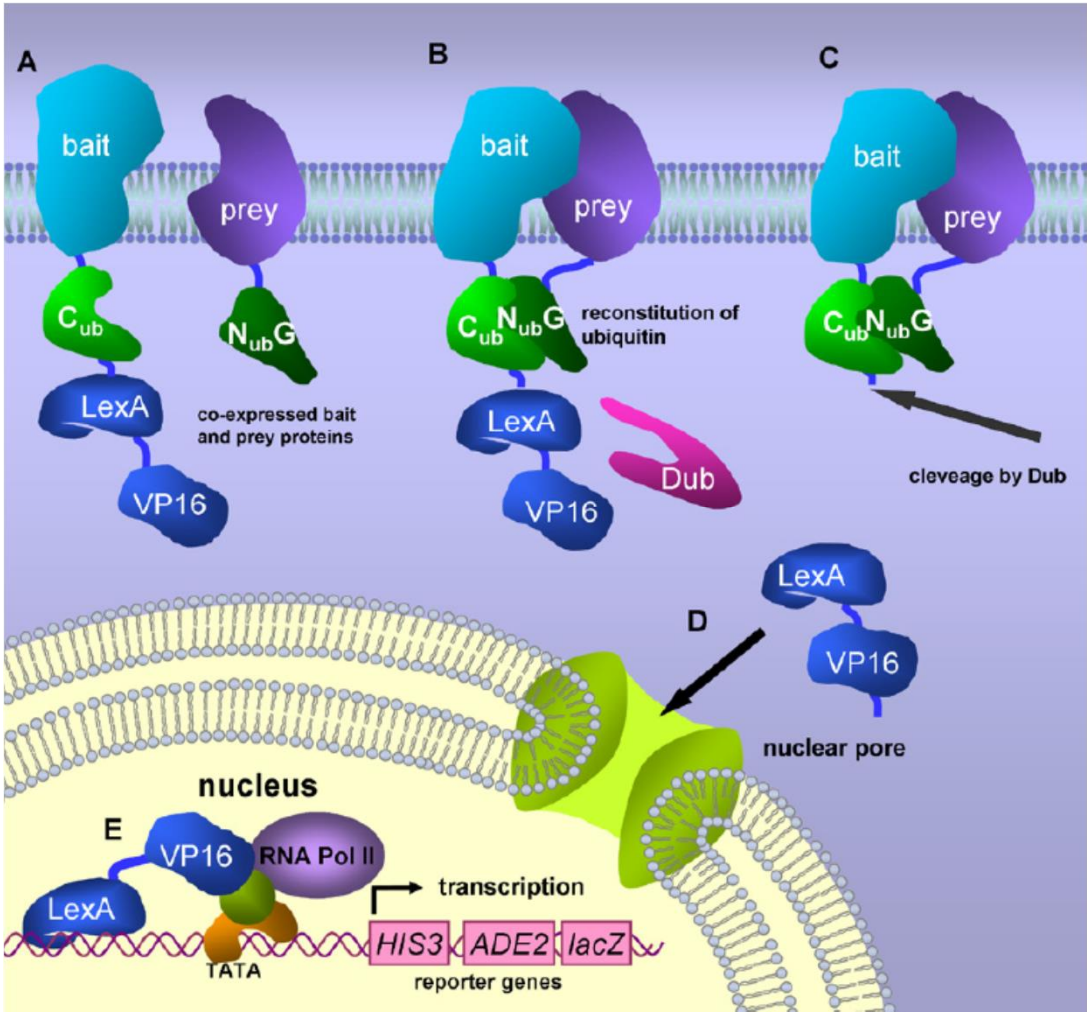
PPI reconnects fragments of one domain and reconstitutes its fold ï original (A) assay based on reconstitution of ubiquitin (western blot analysis of protein degradation) ï new alternative versions use different detection approaches ï for example (B), in transcription-based approach, reporter gene is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



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

Complementation systems

PPI reconnects fragments of ubiquitin molecule ï ubiquitin attracts Dub (de-ubiquitination) enzyme, which releases LexA-VP16 transcription factor from membrane - LexA-VP16 transcription factor goes to the cell nucleus and activates transcription of reporter genes

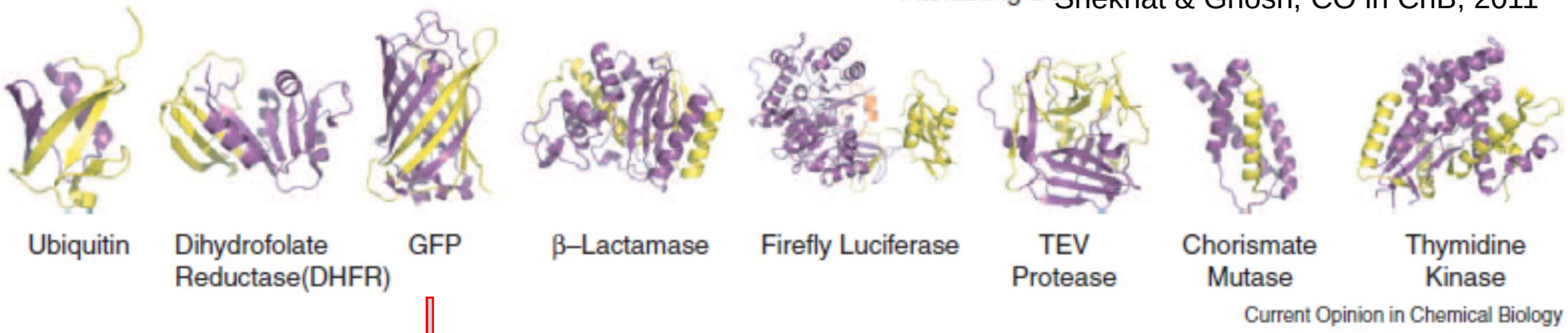


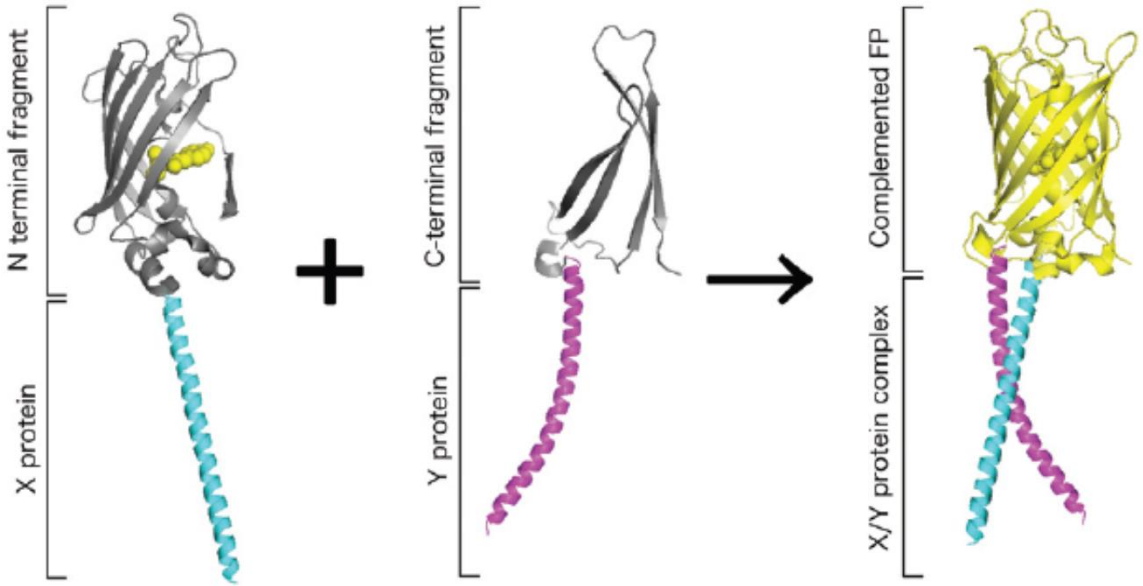
Ivanusic et al, BioTech, 2015

Complementation systems

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

Shekhat & Ghosh, CO in ChB, 2011

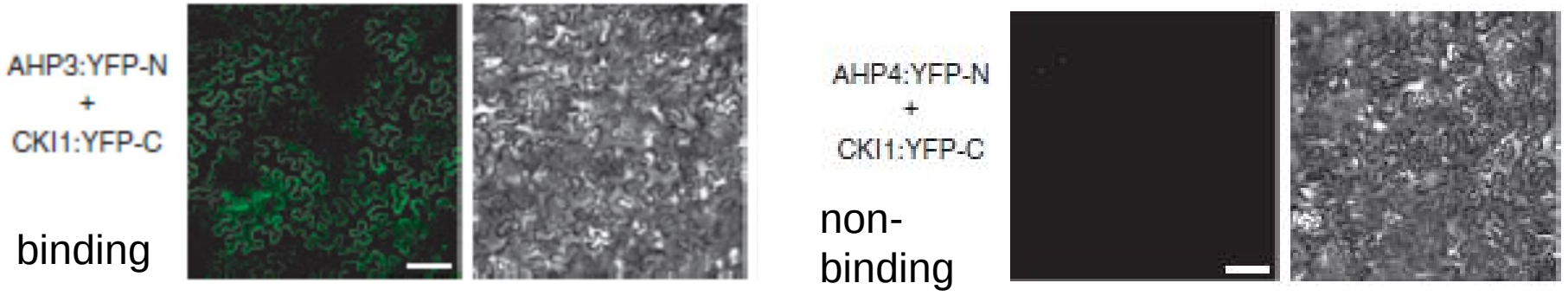




Bimolecular fluorescence complementation (BiFC) ï PPI reconnects GFP

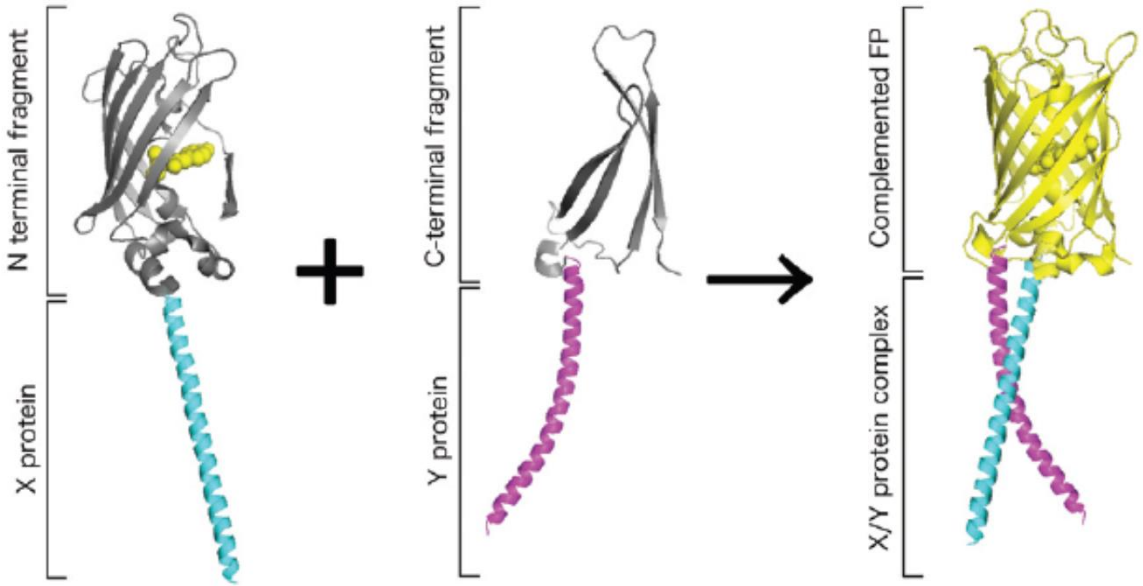
Kodama & Hu, Biotechniques, 2012

Bimolecular fluorescence complementation (BiFC)



Pekarova et al, Plant J., 2011

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



Kodama & Hu, Biotechniques, 2012

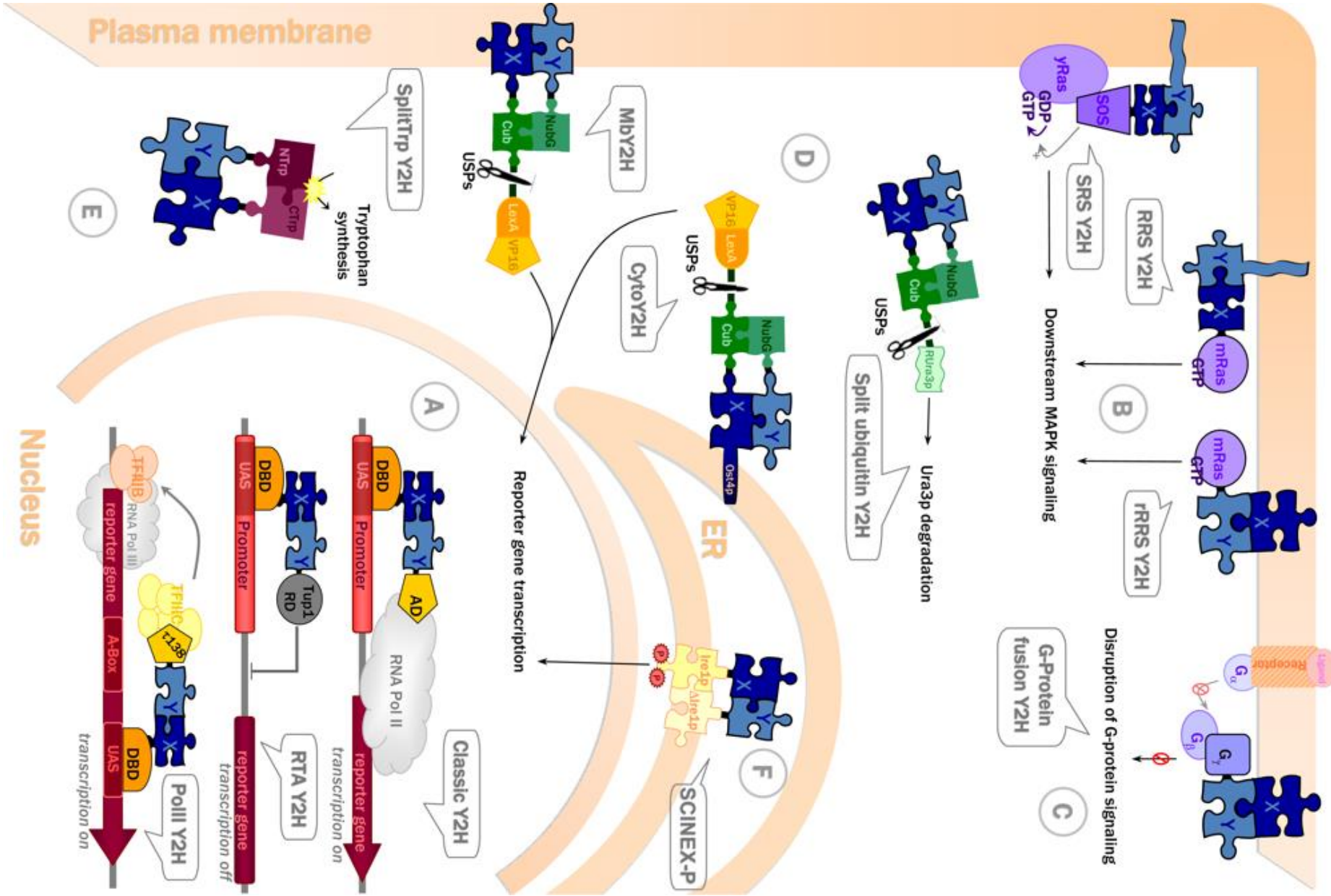
Table 1. List of fluorescent proteins used in BiFC assays.

Fluorescent protein	Excitation Peak (nm)[1]	Emission Peak (nm)[1]	Cell type or organism in the first use	Additional mutation	References
EBFP	382*	448*	Mammalian (COS-1)	None	8, 11
Cerulean	439	479	Mammalian (COS-1)	None	21
ECFP	452	478	Mammalian (COS-1)	None	8
EGFP	488	512	Bacteria (*E. coli*)	None	7, 8
GFP-S65T	489*	510	Plant (Onion epidermis)	V163A	6, 24
frGFP	485*	510*	Bacteria (*E. coli*)	None	22
sfGFP	503*	518*	Mammalian (HeLa)	None	23
Dronpa	503*	518*	Mammalian (HEK293)	None	34, 35
EYFP	514/515	527	Mammalian (COS-1)	None	8, 21
Venus	515	528	Mammalian (COS-1)	None	21
Citrine	516	529	Mammalian (COS-1)	None	21
mRFP	549*	570*	Plant (Tobacco BY2 and Onion epidermis)	Q66T	31
DsRed monomer	556*	556*	Plant (Onion epidermis)	None	20, Clontech[2]
mCherry	587*	610*	Mammalian (Vero)	None	12, 32
mKate	587*	621*	Mammalian (COS-7)	S158A	33

[1]The excitation and the emission spectra of BiFC systems are shown. Asterisks (*) indicate that the spectra of the full-length fluorescent proteins was used when no measurement for the spectra of the reconstituted fluorescent protein was available. [2]Excitation and the emission spectra as shown at the Clontech website (http://www.clontech.com/)

Kodama & Hu, Biotechniques, 2012

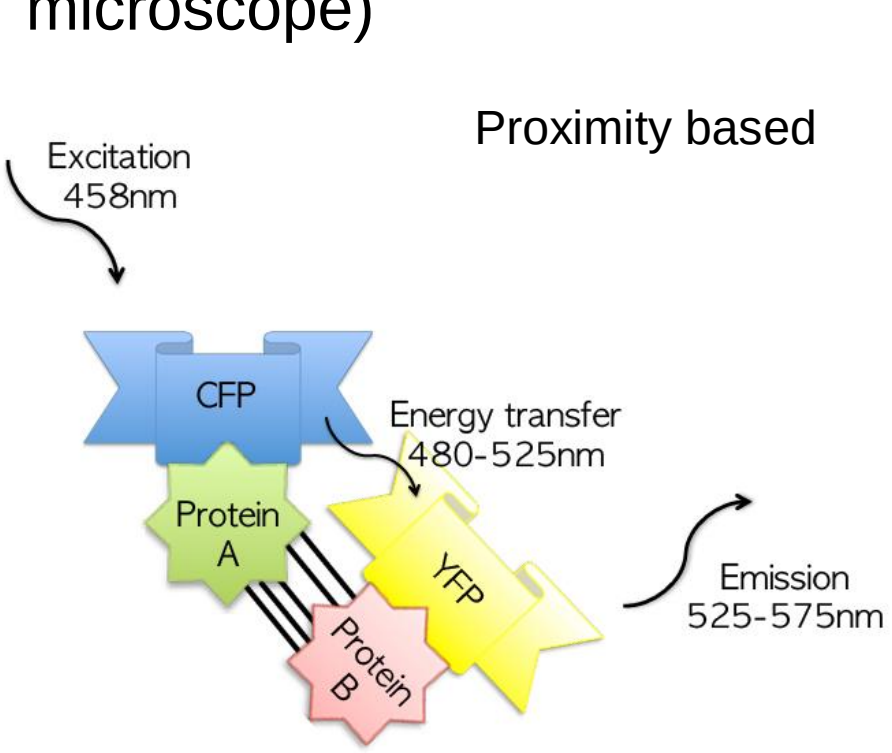
Overview of yeast 2-hybrid systems

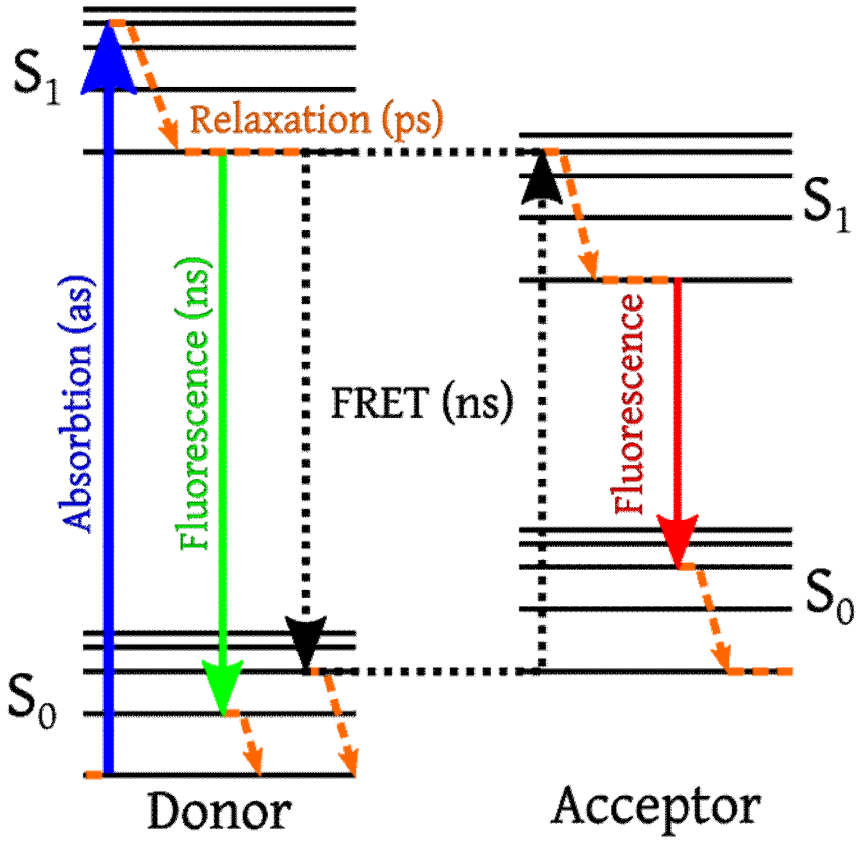


Bruckner et al, IJMS, 2009

FRET (Forster/fluorescence resonance energy transfer)

- CFP-hybrid protein emits 480-525nm light when excited (by 458nm light) ï 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)

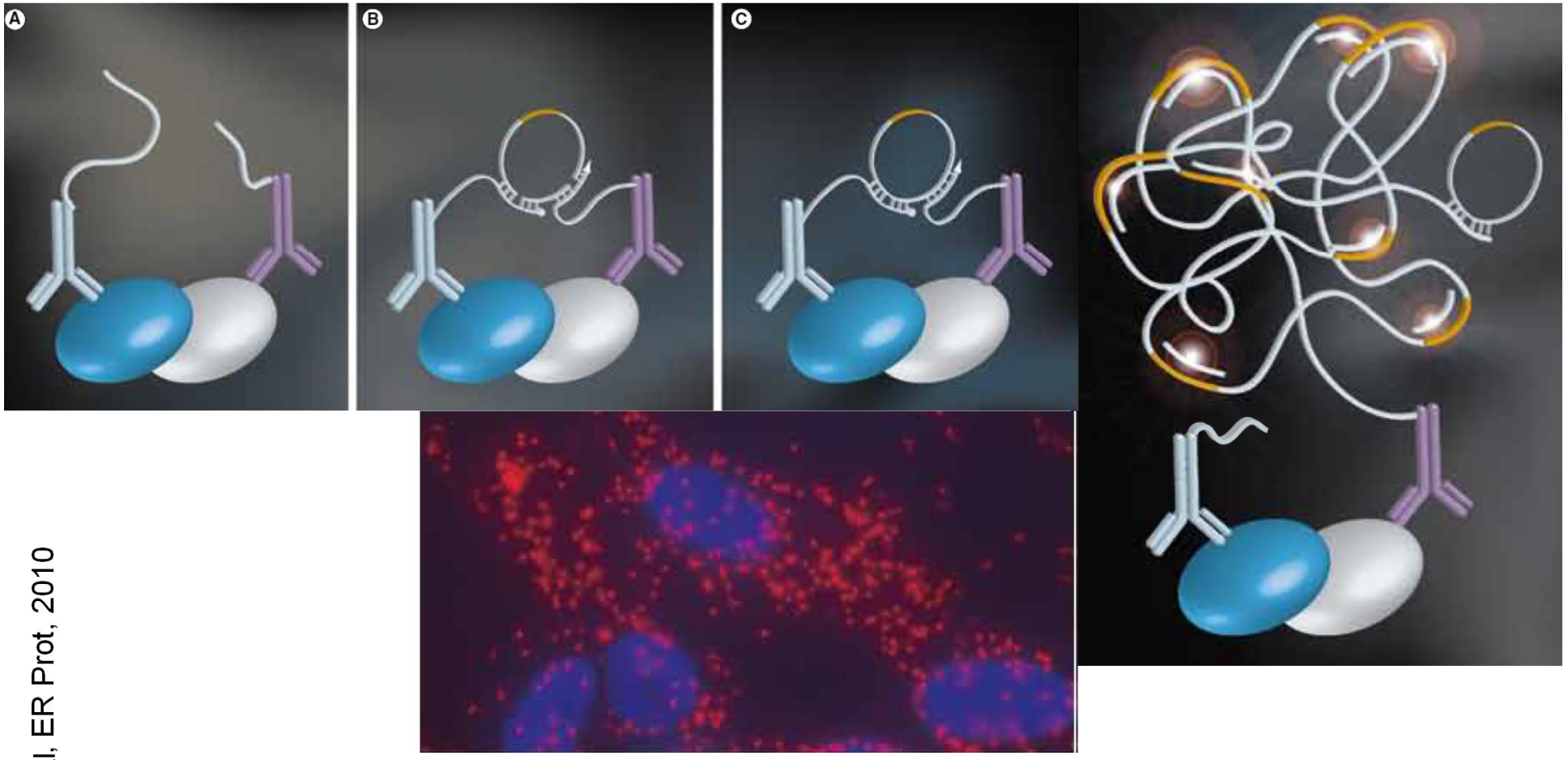




Protein-protein interaction analysis

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

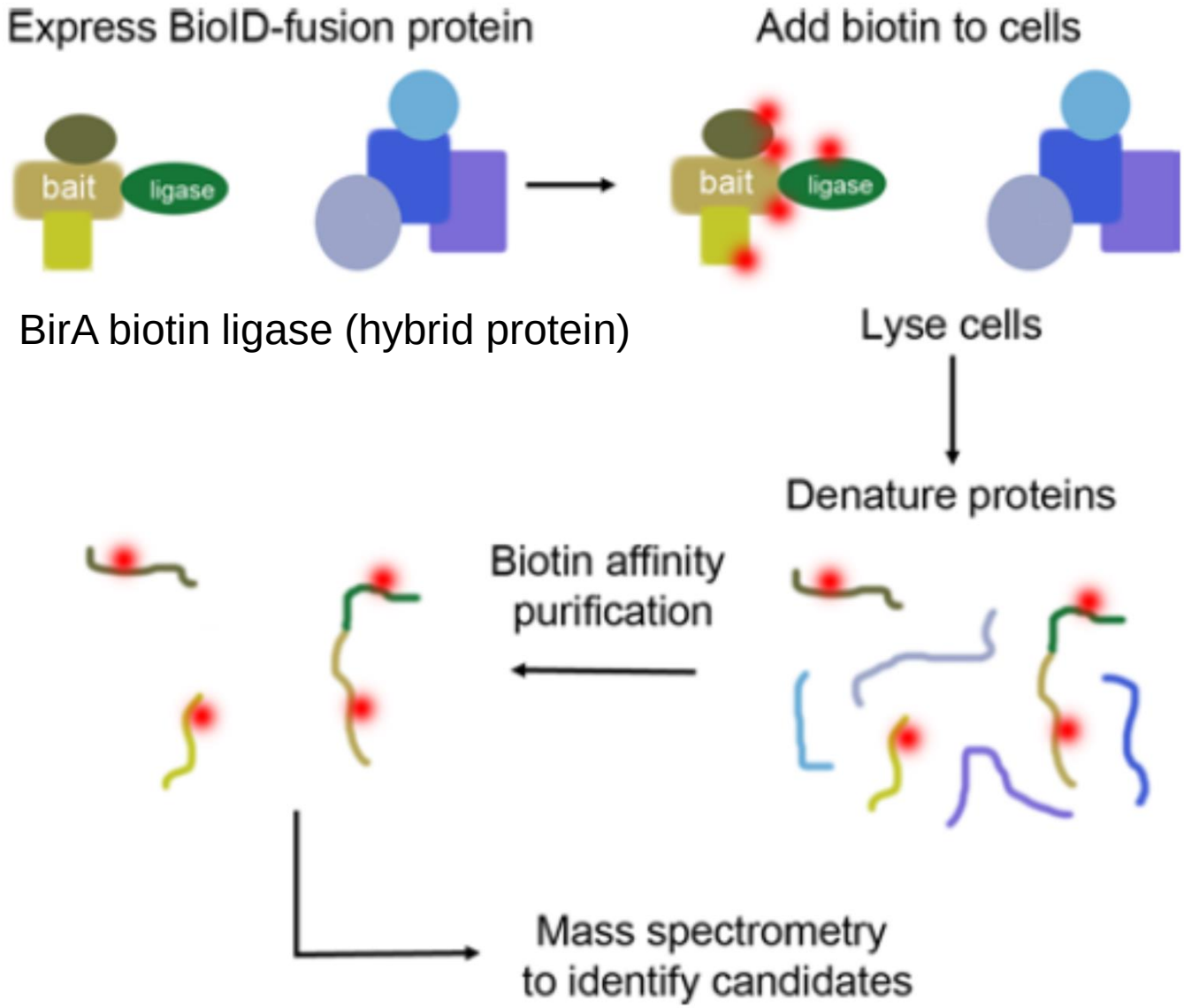
Proximity ligation assay - PLA



Weibrecht et al, ER Prot, 2010

- Specific antibodies conjugated with oligonucleotides, which are complementary to circular DNA ï if the antibodies come close (<16nm) via PPI of they target proteins then ï polymerase synthesis reaction can run

BioID assay



Roux, CMLS, 2013

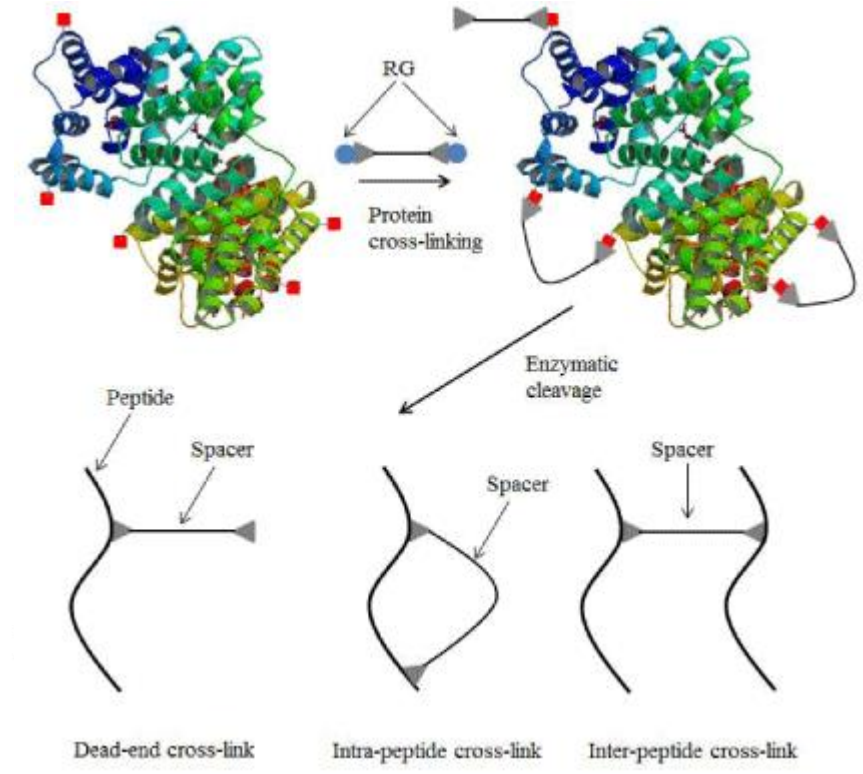
biotin ligase domain biotinylates interacting (or close proximity <20nm) partner

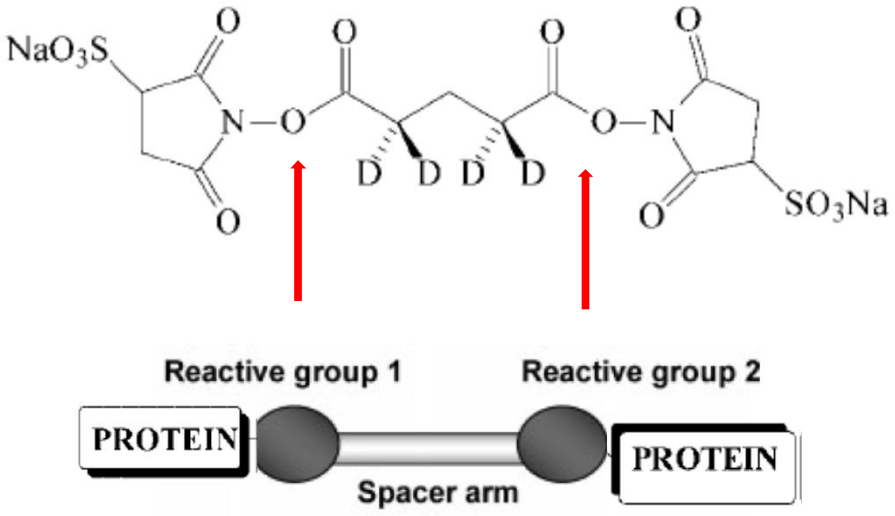
(highly sensitive method ï covalently bound biotin persists even after transient interaction dissociates)

Protein-protein interaction analysis

- beads-based: pull-down (*in vitro*), coIP é
- hybridní: Y2H (kvasinkový 2-hybridní), BiFC é
- proximity-based: FRET, PLA é
- **MS-based:**
  - **crosslink**
  - **D/H-exchange é**
- Quantitative methods: SPR, ITC é
- Structural methods: co-crystalization, NMR é
- Genetic methods: synthetic lethality é
- Bioinformatics methods: databases, docking é

Protein cross-linking

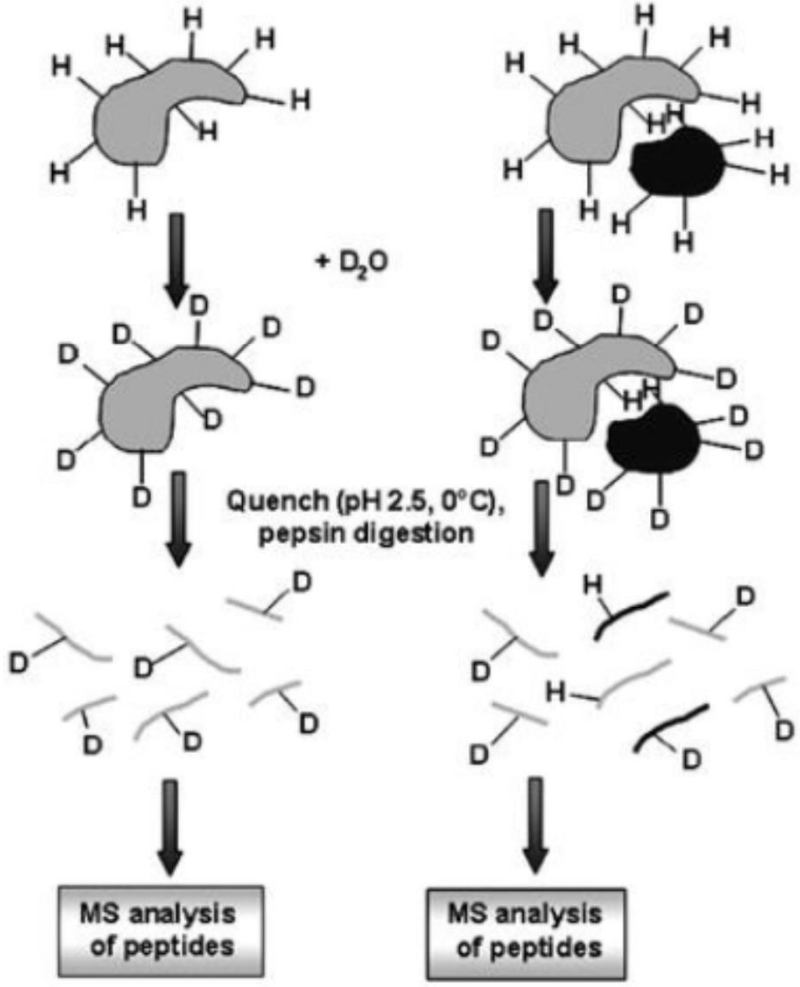




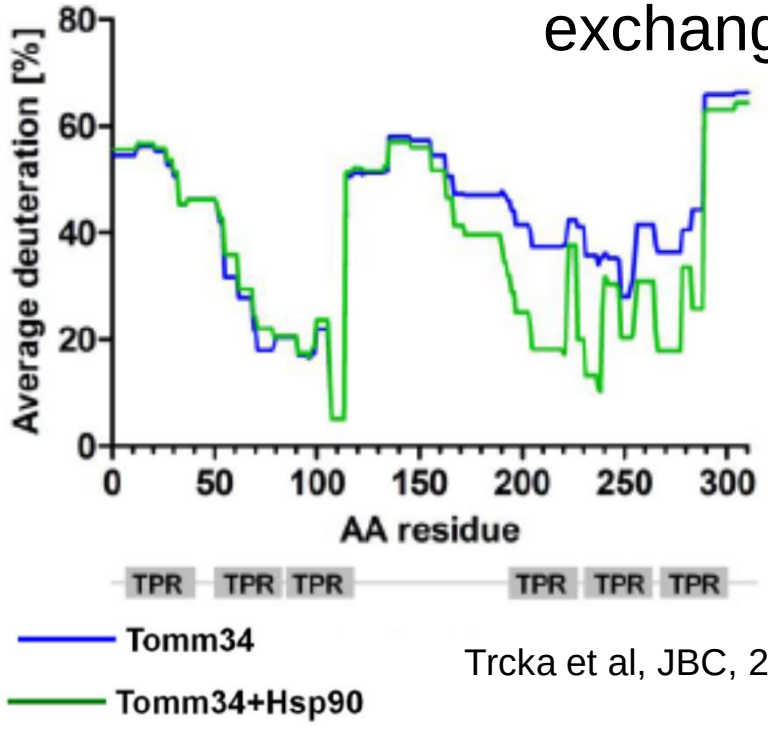
- ε-amine lysine groups react with cross linking reagent and form covalent ester bonds
- MS analysis of dipeptides can show partner peptides in close proximity

Sinz, MS Reviews, 2006
Bian, AJBE, 2014

Hydrogen/deuterium exchange

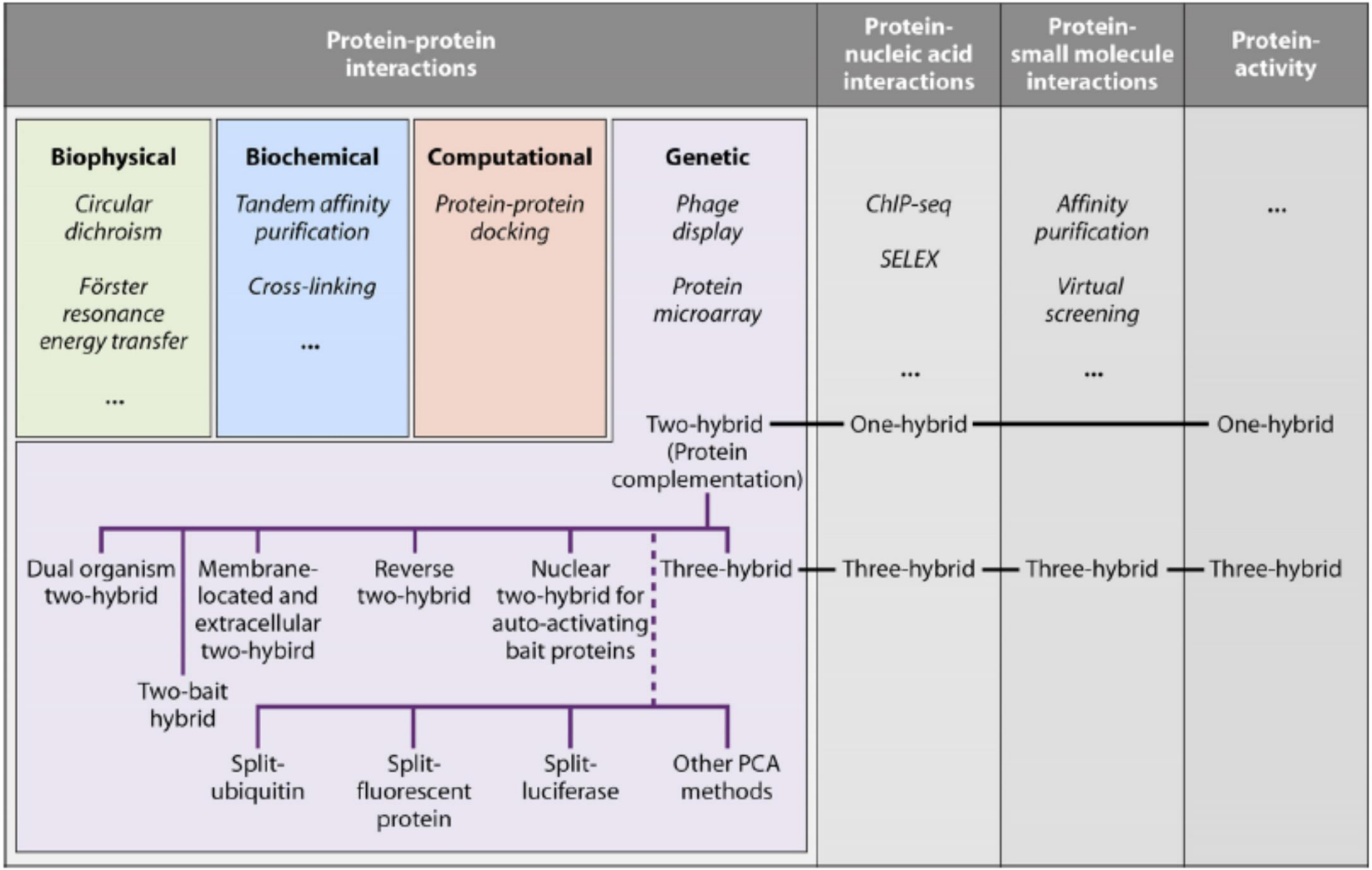


single protein deuteriated peptide profile is compared to profile of the partner-bound protein (deuteriated after partnerŝ interaction) - peptides buried inside the contact zones are not available for H/D exchange)



Trcka et al, JBC, 2014

Protein-protein interaction analysis - overview



Stynen et al, MMBR, 2012