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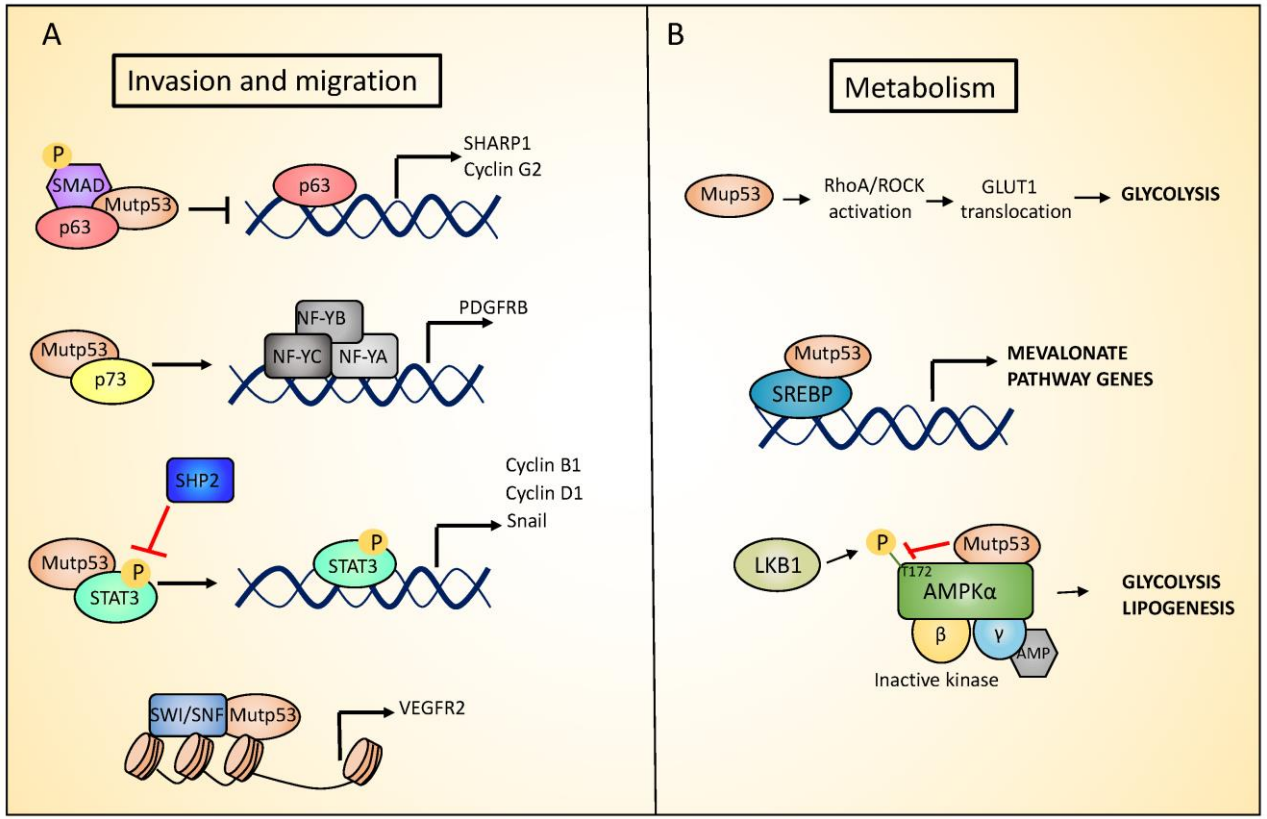
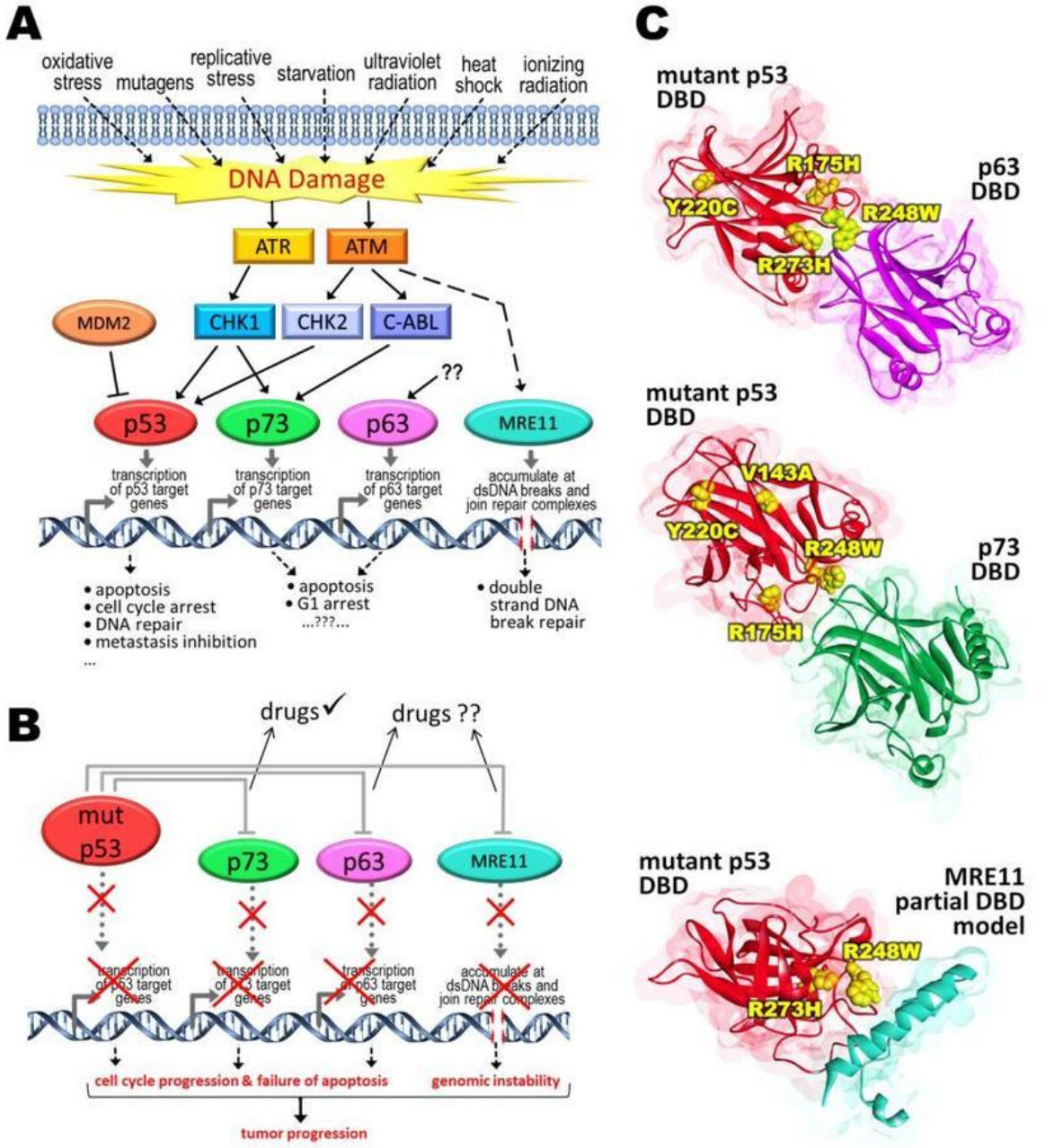
10. Methods for studying: a) protein-protein, b) protein-ligand



https://www.mdpi.com/1422-0067/20/24/6241/htm

a) protein-protein,
b) protein-ligand
c) protein-DNA



https://www.researchgate.net/figure/Mutant-p53-proteins-carry-out-novel-oncogenic-interactions-A-Signaling-in-the-p53_fig2_277087873

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Methods for studying protein-protein and other interactions

- methods for detecting interactions

characterization of protein-protein interactions (e.g. Kd, quantification, stoichiometry, structure...)

immunoprecipitation, co-immunoprecipitation, pull-down,

fluorescence anisotropy-polarization,

SPR, ITC...

affinity purification, co-purification, gel filtration, ultracentrifugation

TAP-tag (and other tags) purification and MS analysis

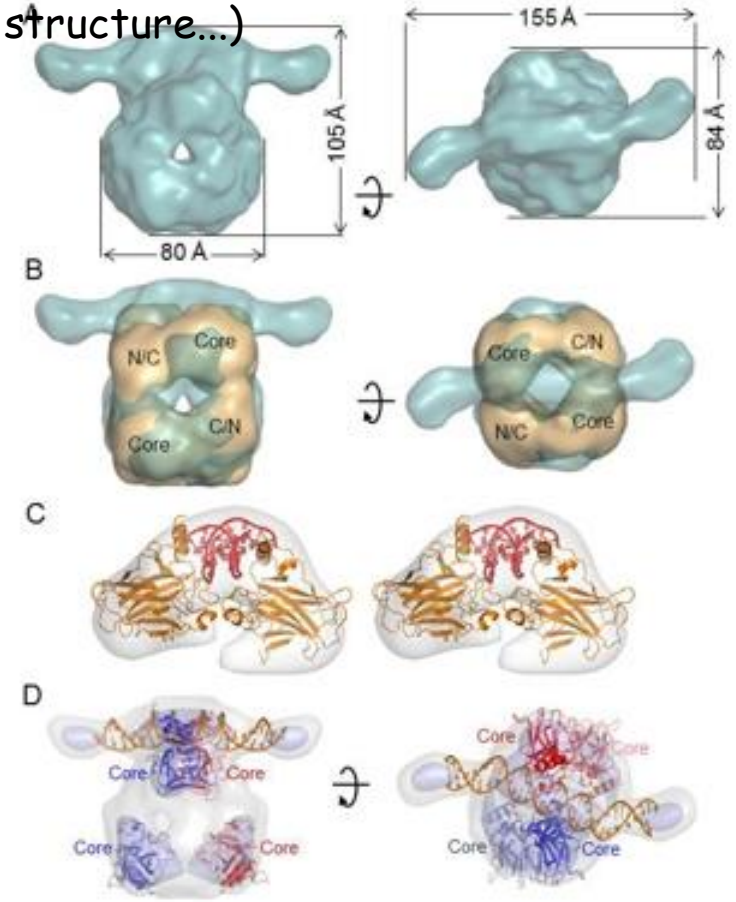
yeast two-hybrid system

FRET, co-localization, co-expression

co-crystallization, cryoEM...

database (interactome and complexes...)

genetic methods (synthetic lethality, suppression)



D reconstruction of p53-DNA complex. (A) Side and top views of the p53-DNA complex. The length of the stem is limited by the size of the image frames. (B) Superimposition of the DNA-free p53 map on the p53-DNA complex map. The two maps were compared in order to determine locations of core domains and dimers within the complex. N/C ◆ areas of N and C termini interaction. (C) Stereo view of the crystal structure of two core domains bound to DNA (PDB entry: 2ata) fitted into the high density areas of the map (threshold used: 2s (light grey). (D) Fitting of the core domains and DNA in the EM map. Core domains are displayed in blue and red, DNA in orange. Docking was performed with both Chimera and Veda/UROX. The best fits from each software produced similar results.

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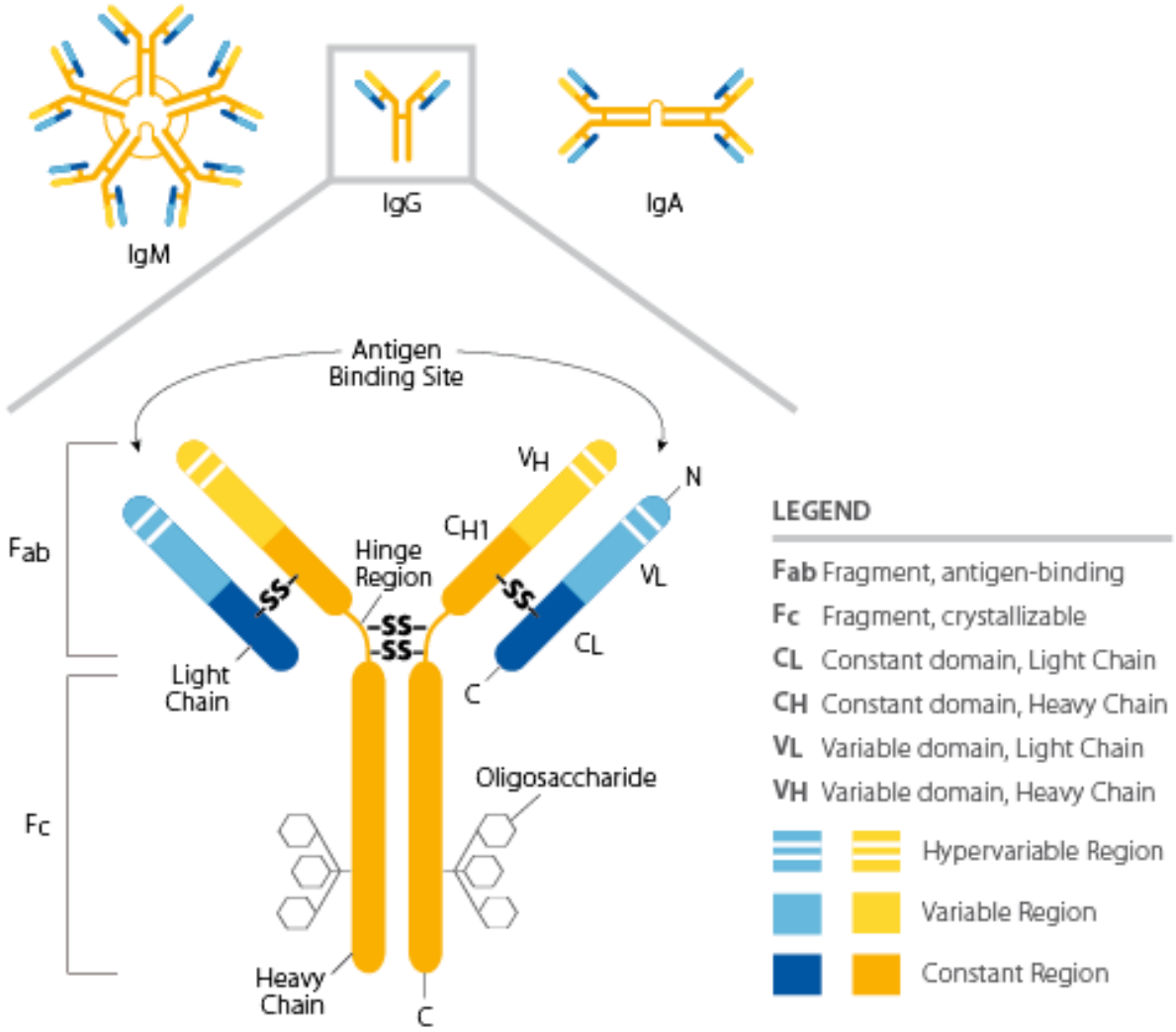
Immunochemical methods

Immunochemical methods are based on antigen-antibody interaction. Using these methods, we determine the presence of pathogens or demonstrate whether or not the sample contains specific antibodies against the given antigen. An antigen is a macromolecular substance of natural or artificial origin that the organism recognizes as foreign. An antibody is a molecule that is able to bind to an antigen and thereby trigger the body's defense reaction. We distinguish between polyclonal antibodies (directed against several epitopes of a certain antigen), monoclonal antibodies (directed against one epitope of the antigen) and recombinant antibodies (combination of the two previous ones).

Selected methods: - Immunoprecipitation, Pull-down assay, WB-immunodetection, ELISA, SPR, ITC, FP,



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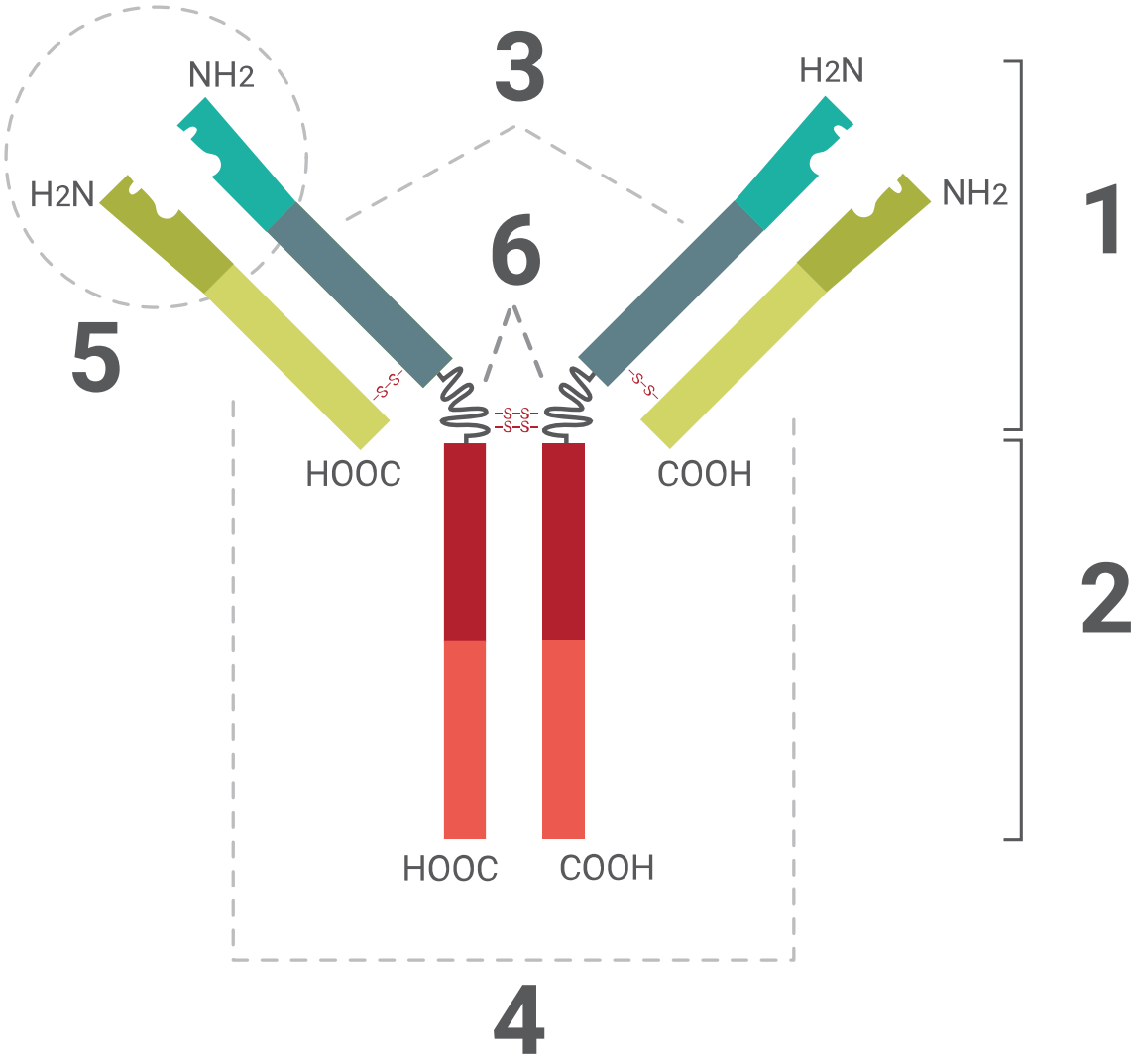
Imunoprecipitation

- method of isolating specific proteins from protein mixtures (lysates, purified…) using antibodies
- antibodies in a complex with their antigens are separated from other molecules using proteins A or G (source of bacteria), which bind immunoglobulins and at the same time are immobilized on a solid substrate (BEADS)
- proteins A and G bind to the Fc region of heavy chains
- the Fab region is still available for antigen binding

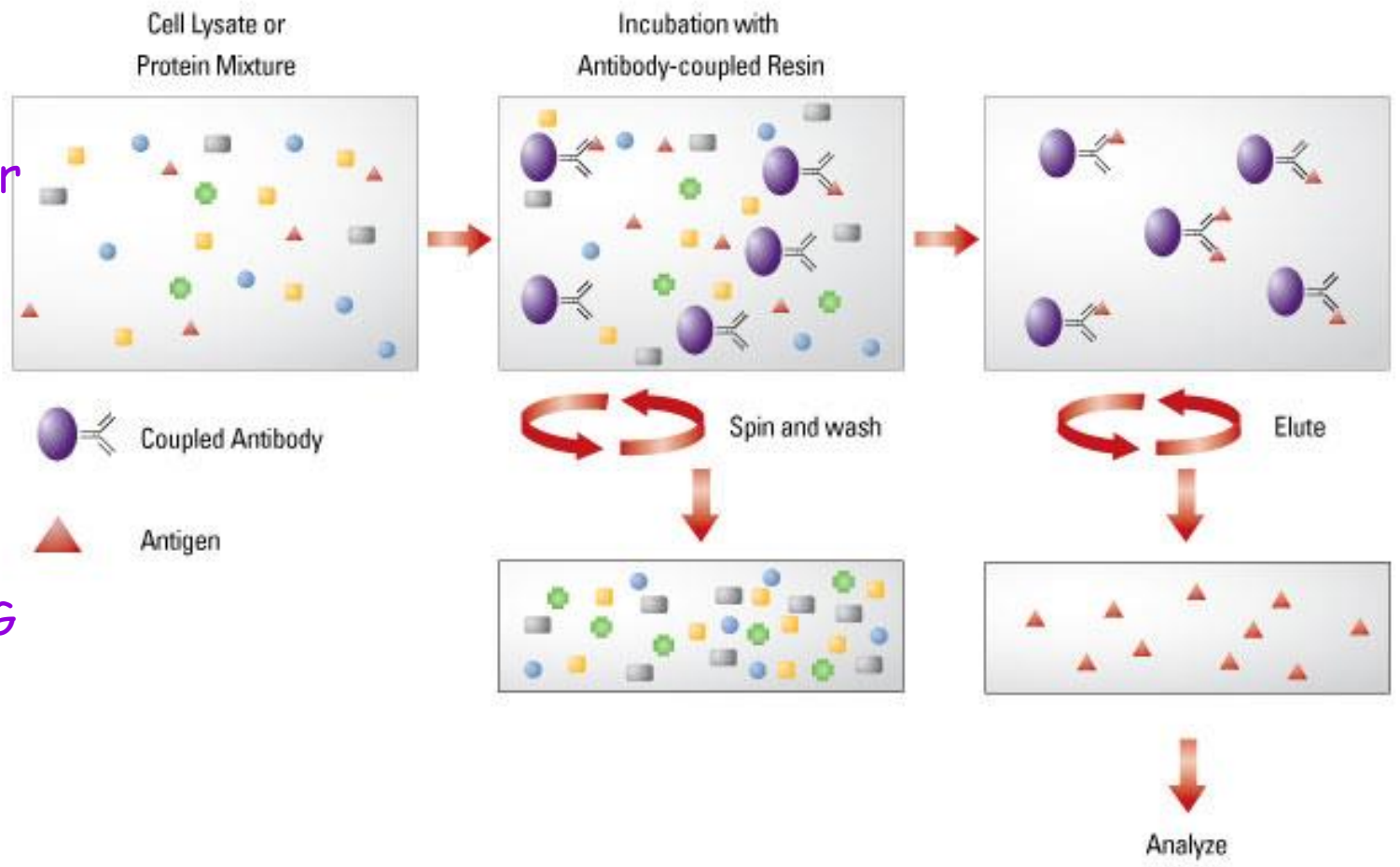


1.Fab region
2.Fc region
3.Heavy chain with one variable (V_H) domain followed by a constant domain (C_H1), a hinge region, and two more constant (C_H2 and C_H3) domains.
4.Light chain with one variable (V_L) and one constant (C_L) domain
5.Antigen binding site (paratope)
6.Hinge regions

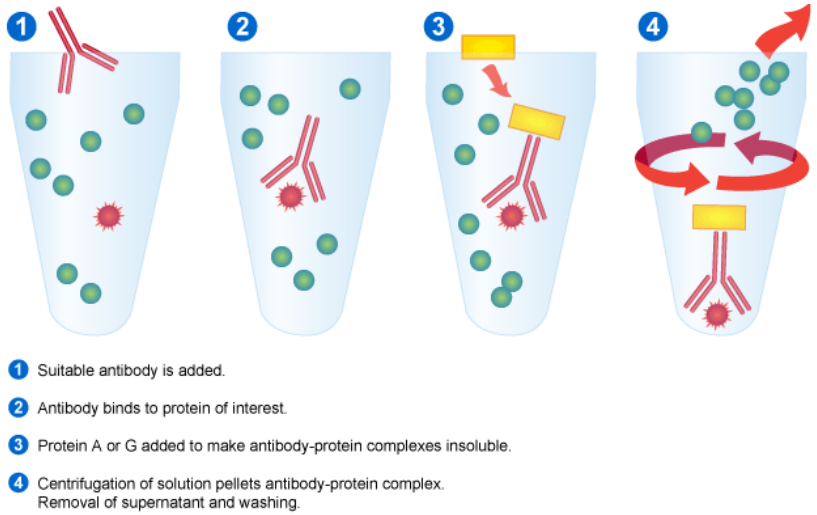
Imunoprecipitation

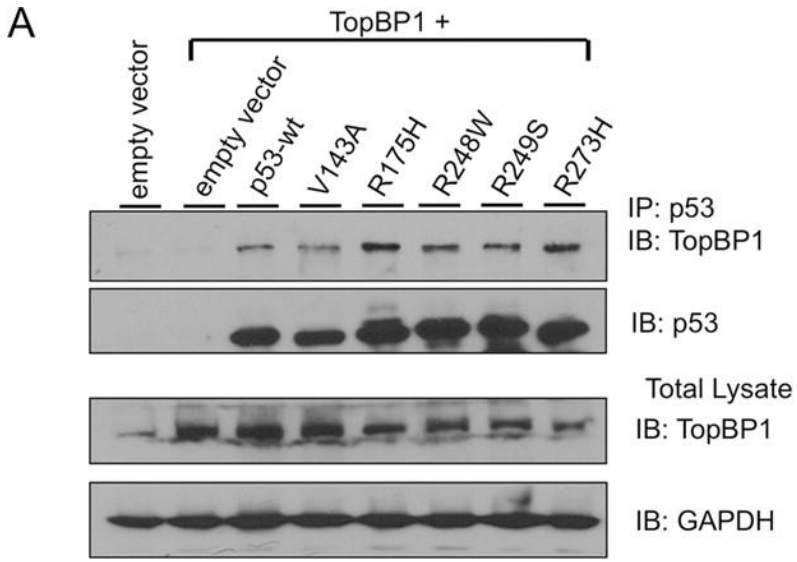
the antibody is immobilized on a solid support (e.g. paramagnetic or agarose/non-magnetic beads), binds the antigen-protein and removes it from the mixture (we obtain a native or denatured protein)

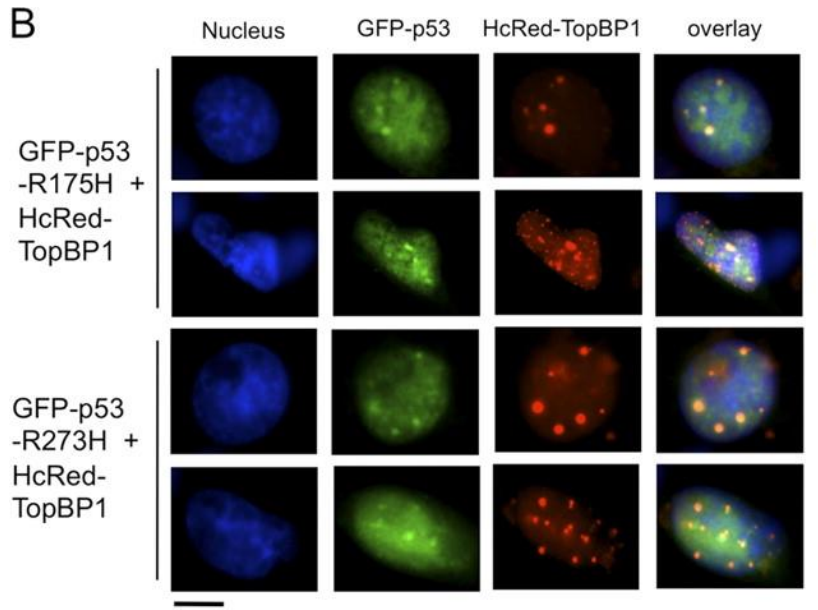
Procedure:
- cell lysis, in vitro translation, protein mixture
- incubation of the sample (cell extracts...) with the antibody
-precipitation with beads with proteins A/G
- washing
- protein separation from beads and detection (WB, further use)
- protein detection (proteins are most often separated from immunoglobulins and beads by denaturing electrophoresis, WB)

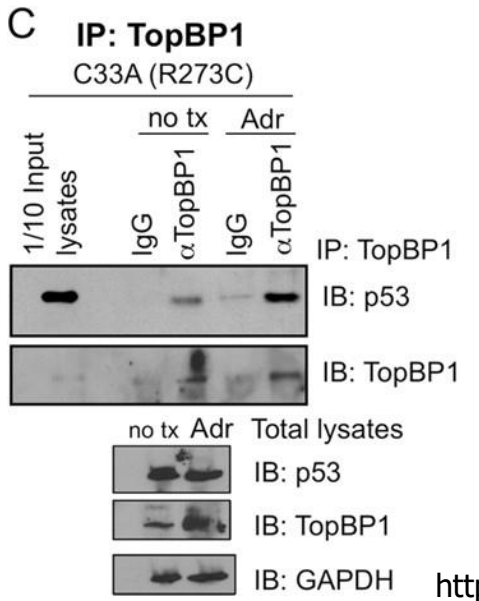


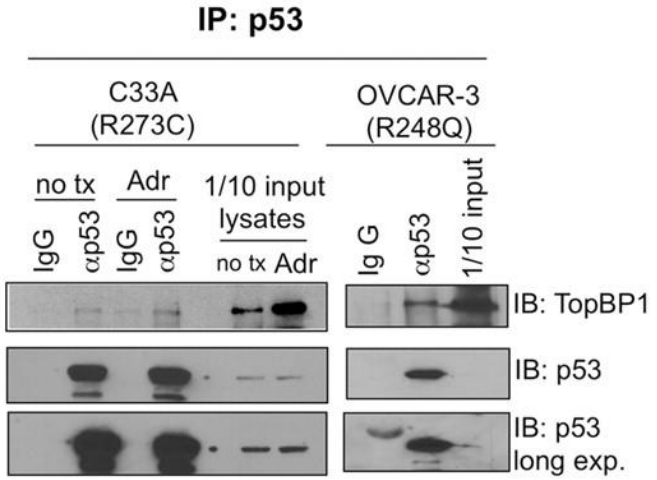
https://www.youtube.com/watch?v=QRgTpCEY2nU

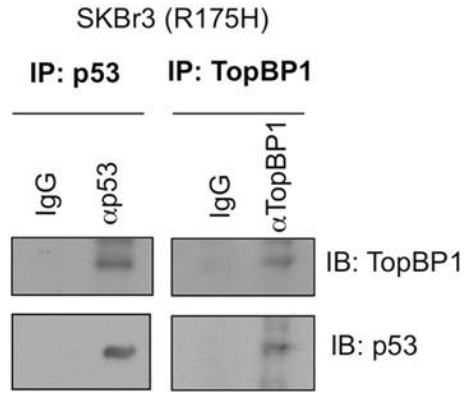












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10. Interaction protein-protein IP

https://www.youtube.com/watch?v=QRgTpCEY2nU

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Various systems of affinity tags and their interaction partners used for protein purification or the study of protein-protein interactions

Tab. 1. Různé systémy afinitních značek a jejich interakčních partnerů využívané pro purifikaci proteinů nebo studium protein--proteinových interakcí (afinitní koprecipitaci).

	TAG afinitní značka	sekvence afinitní značky	imobilizovaný interakční partner
Peptidové značky	FLAG	DYKDDDDK	protilátka anti-FLAG
	HA	YPYDVPDYA	protilátka anti-HA
	oligoHis (6-10mer)	HHHHHH(HHHH)	chelát niklu nebo kobaltu
	Myc	EQKLISEEDL	protilátka anti-Myc
	SBP	MDEKTTGWRGGHVVEGLAGELEQLR ARLEHHPQGQREP	streptavidin
	Avi	GLNDIFEAQKIEWHE	streptavidin
	Strep	WSHPQFEK	streptavidin
	V5	GKPIPNPLLGLDST	protilátka anti-V5
Proteinové značky	GST (glutathione S-transferase)		glutathion
	MBP (manose-binding protein)		amylóza

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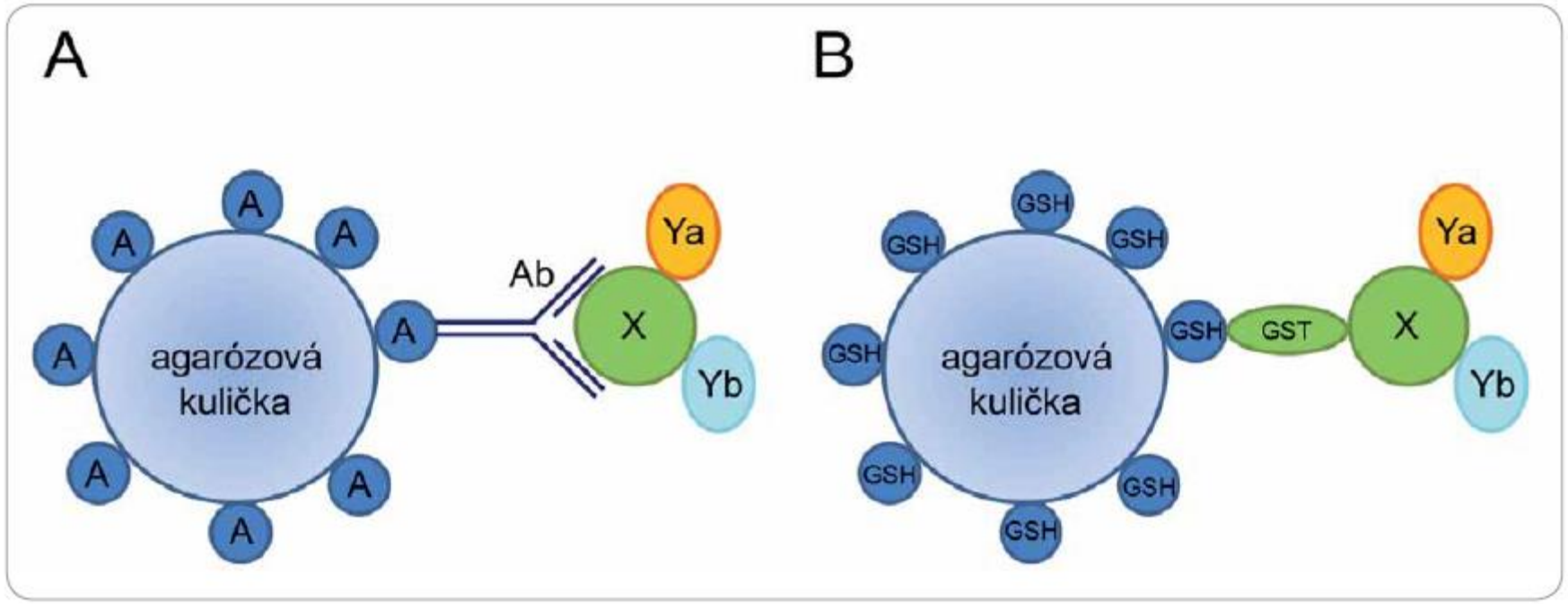
Coimunoprecipitation (Co- IP) and affinity coprecipitation, (pull- down analýzy)



Obr. 1. Princip koimunoprecipitace (A) a afinitní koprecipitace (B).

A. Protein X spolu s jeho interakčními partnery (proteiny Ya a Yb) je navázán na specifickou protilátku (Ab). Vzniklý imunokomplex je ze směsi vychytán pomocí agarózových kuliček s imobilizovaným proteinem A, který rozeznává Fc fragment protilátek. B. Komplex tří proteinů (X, Ya, Yb) je vychytán ze směsi pomocí silné interakce proteinu GST (fúzovaného s proteinem X) a glutationu (GSH) imobilizovaného na agarózových kuličkách.

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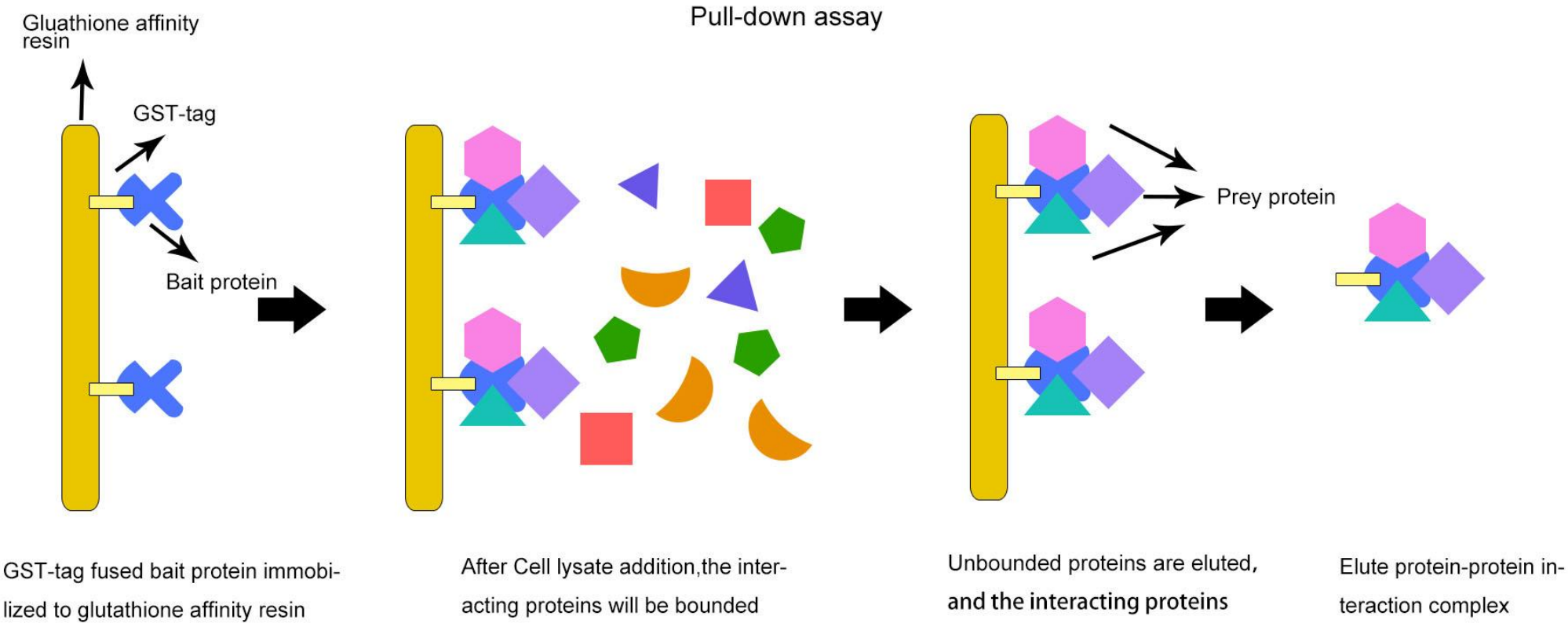
Izolation of specific proteins (tzv. pull-down assay)



https://www.youtube.com/watch?v=euHjpUs20YE

Objectives:

Qualitative parameters - proteins, interaction partners, understanding of interactions

WB, IP

Quantitative definition

- affinity (Kd...)

ELISA

SPR

FP, fluorescence anisotropy

- affinity, kinetic and thermodynamic parameters (DG, DH, DS) - ITC

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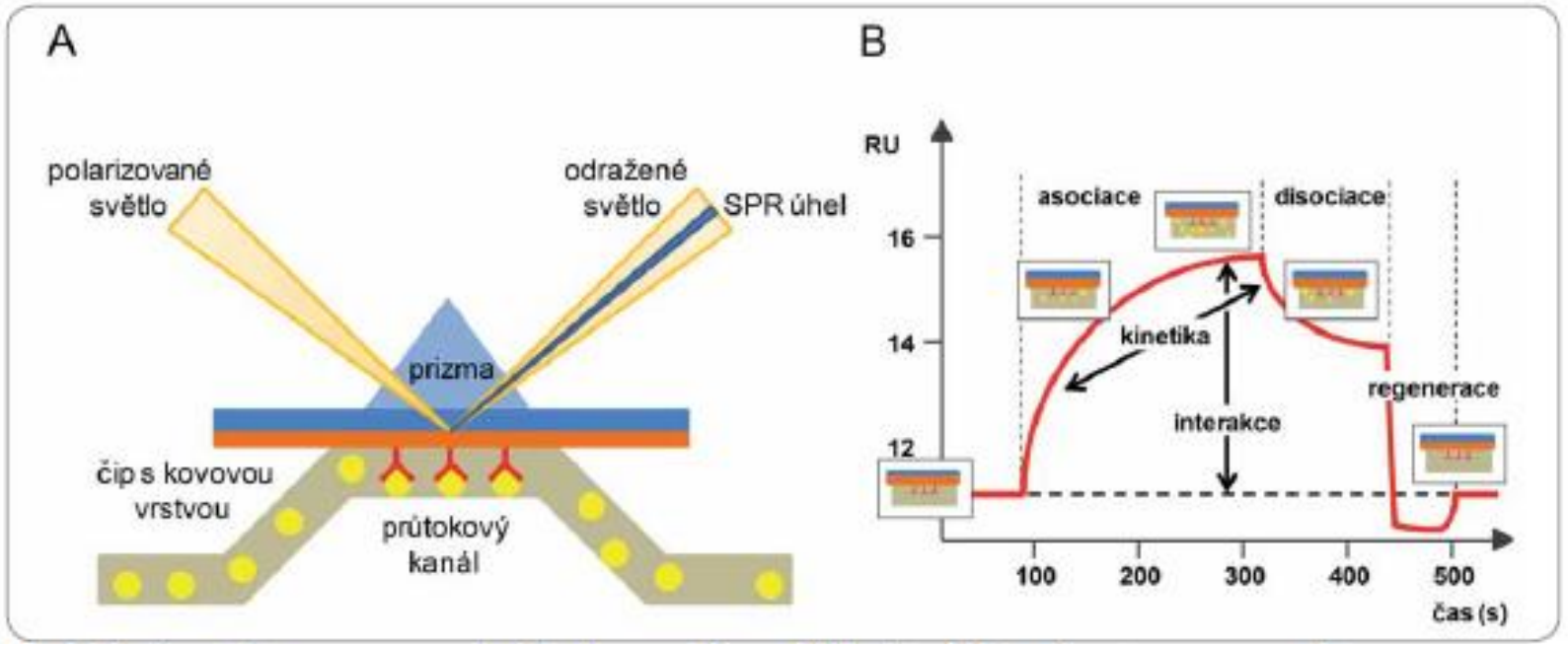
Surface plasmon resonance (SPR)



A highly sensitive and temperature-stabilized SPR biosensor system for real-time monitoring of biomolecular interactions without the need for labeling biomolecules. Using the T200 SPR system, information can be obtained on the kinetics, affinity, concentration, specificity, selectivity and thermodynamics of biomolecular interactions, and this method can thus be used in various areas from basic research to biotherapeutics and drug research. In principle, one of the binding partners is immobilized on the surface of the biosensor and the other is present freely in the loading buffer.

10. Interaction protein-protein IP

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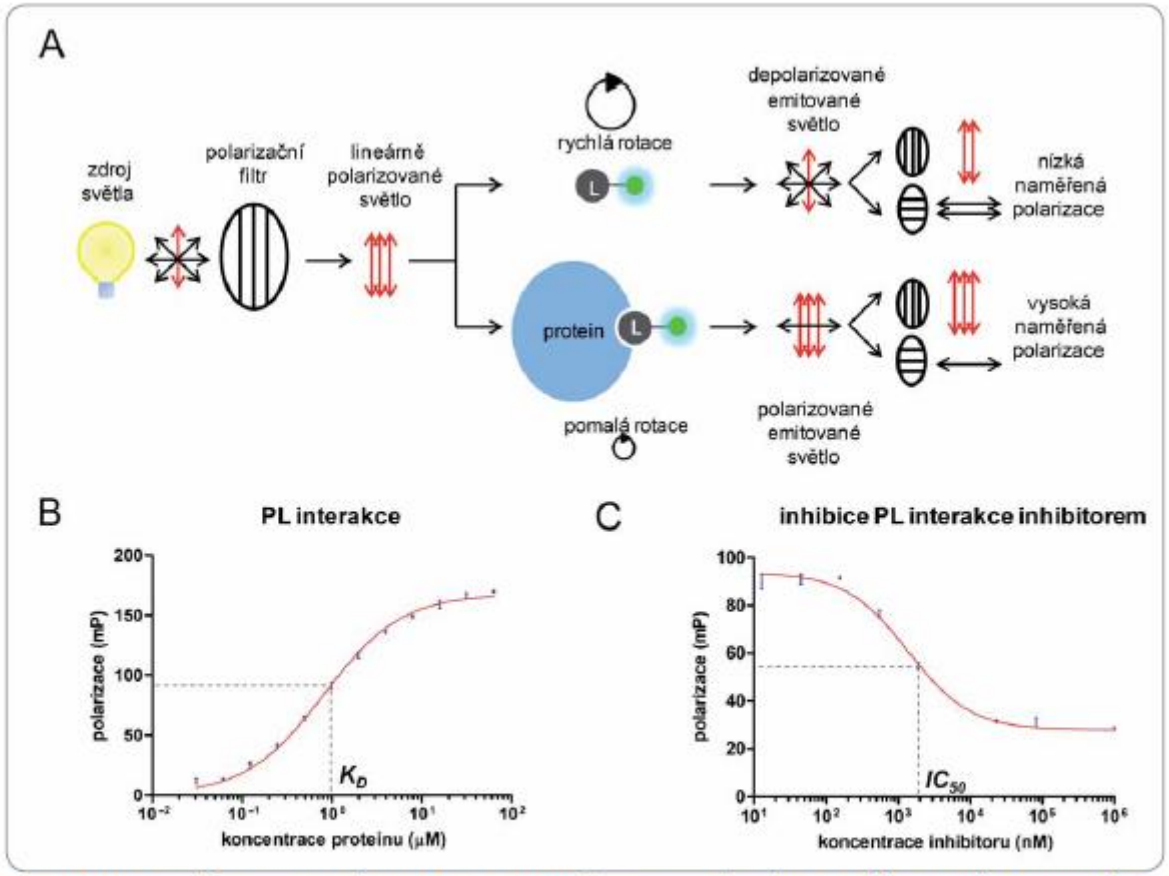
he principle of the fluorescence polarization method (A), measurement of protein-ligand interaction (B) and inhibition of protein-ligand interaction (C) by the FP method.



Obr. 3. Princip metody fluorescenční polarizace (A), měření interakce proteinu s ligandem (B) a inhibice interakce proteinu s ligandem (C) metodou FP.

A. Fluoroforem značený ligand (L) je ozářen lineárně polarizovaným světlem. V důsledku jeho vysoké rotace dochází k emisi depolarizovaného světla a k naměření nízké hodnoty fluorescenční polarizace. Interakce ligandu s větší molekulou (proteinem) způsobí zpomalení jeho rotace a emisi polarizovaného světla. Převzato z [13]. B. Titrace fluoroforem značeného ligandu o konstantní koncentraci vzrůstající koncentrací proteinu. C. Měření IC_{50} inhibitoru protein-ligandové interakce. Směs proteinu a fluoroforem značeného ligandu byla titrována vzrůstající koncentrací inhibitoru.

Fluorescence polarization (FP) is a fluorescence-based detection method that is widely used to monitor molecular interactions in solution.
Unlike fluorescence intensity which focuses on the quantification of emission intensity at a specific wavelength and neglects its polarization, fluorescence polarization specifically analyses as output the emission intensity of different polarization planes.
FP is typically used to assess biomolecular interactions such as protein-protein and protein DNA binding, as well as enzyme activity. It has been adopted in basic research as well as high-throughput screening.
Initially, fluorescence polarization had been mainly used in the diagnostic field. A FP immunoassay was first described in the 1960s. In life science research, applications were at first limited due to the lack of sensitive instruments. Currently, FP is experiencing strong popularity in biological research. This is mainly due to the increased performance and sensitivity of modern microplate readers that can deliver robust results with minimal variability and larger assay windows.

https://www.bmglabtech.com/en/fluorescence-polarization/

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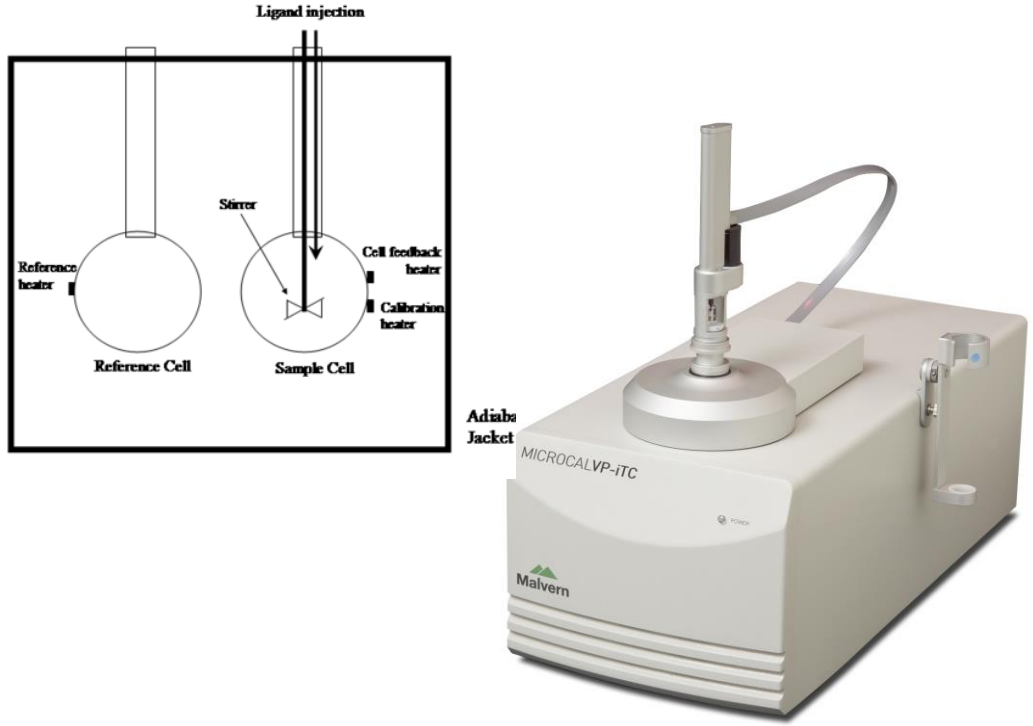
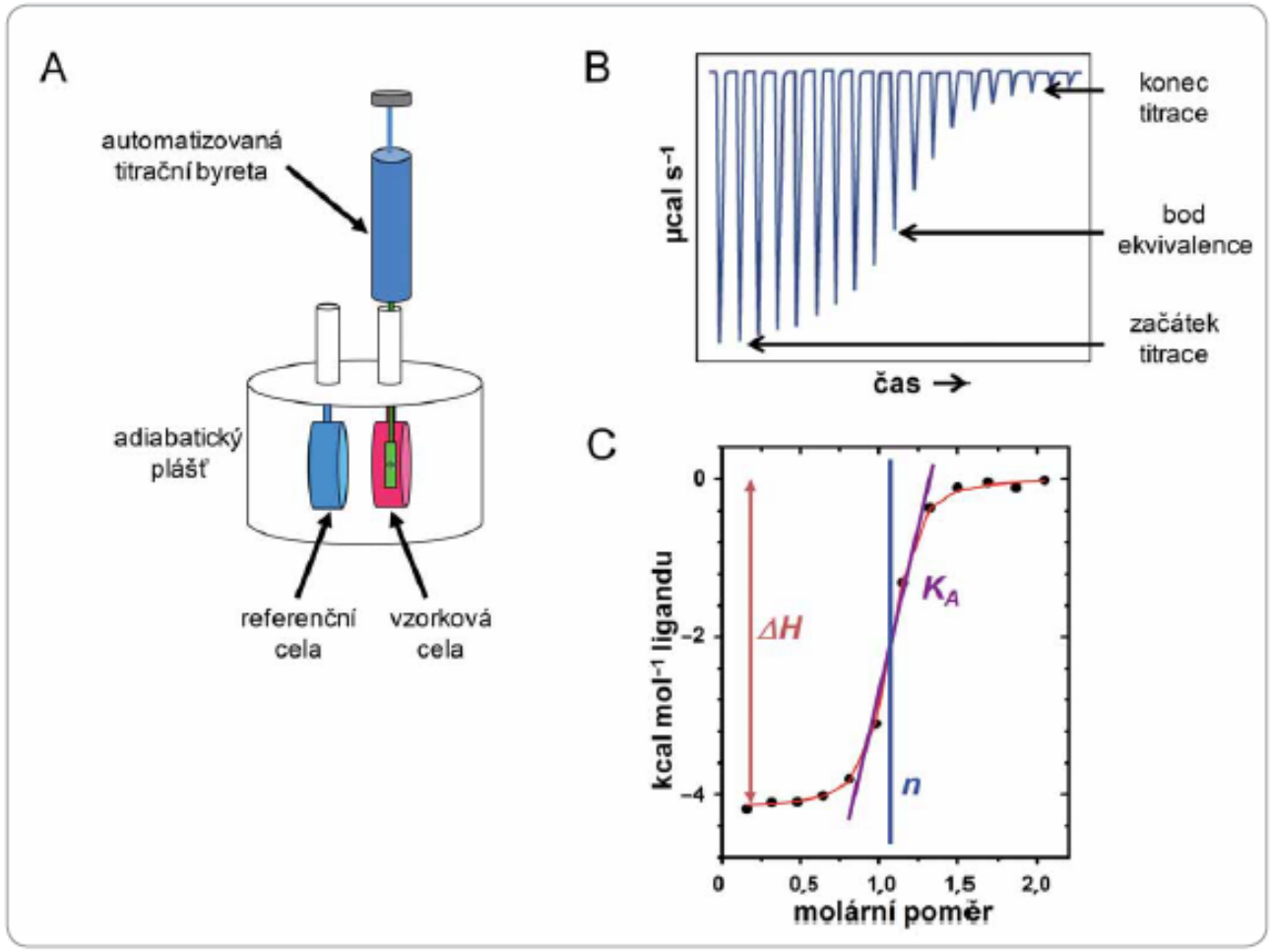
Isothermal titration calorimetry (ITC)

sothermal titration calorimetry is used to characterize biomolecular interactions of small molecules, proteins, antibodies, nucleic acids, lipids, and others. A complete thermodynamic profile (stoichiometry, Ka, ΔH and ΔS) can be obtained during one experiment.





Obr. 4. Schematické znázornění přístroje MicroCal® (A).

Teplo uvolněné při interakci dvou interakčních partnerů je zaznamenáno jako funkce času (B) a po integraci jako závislost entalpie na molárním poměru interakčních partnerů (C). Z výsledného grafu je možné přímo definovat hodnoty změny entalpie, asociační konstantu i stechiometrii interakce. Převzato z manuálu GE Healthcare [23].

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