Cancer Biology I :

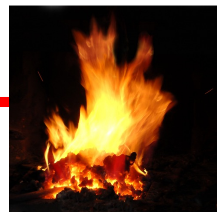
Topics covered

Week 5:
Lecture 5/Exercises-paper: Telomeres and cellular senescence
(Chapters 10 (Weinberg))

Weeks 6:
Lecture 6/Exercises-Q&A: Telomeres: length and cancer, aging, mouse models;
CDKs and G1/S control
Wednesday: CDKs as drug target, protein-protein interactions; Q&A session
(Chapter 8 (Weinberg book): pRb and control of the cell cycle clock)

1 week break

Week 7, Monday: Q & A session: discussion of your questions
(to be submitted via email to me **by October 24**!!!)
Wednesday October 29, 2025: exam (contrôle continu)



From Monday: **Pairing of Cyclins with Cyclin-Dependent Kinases**

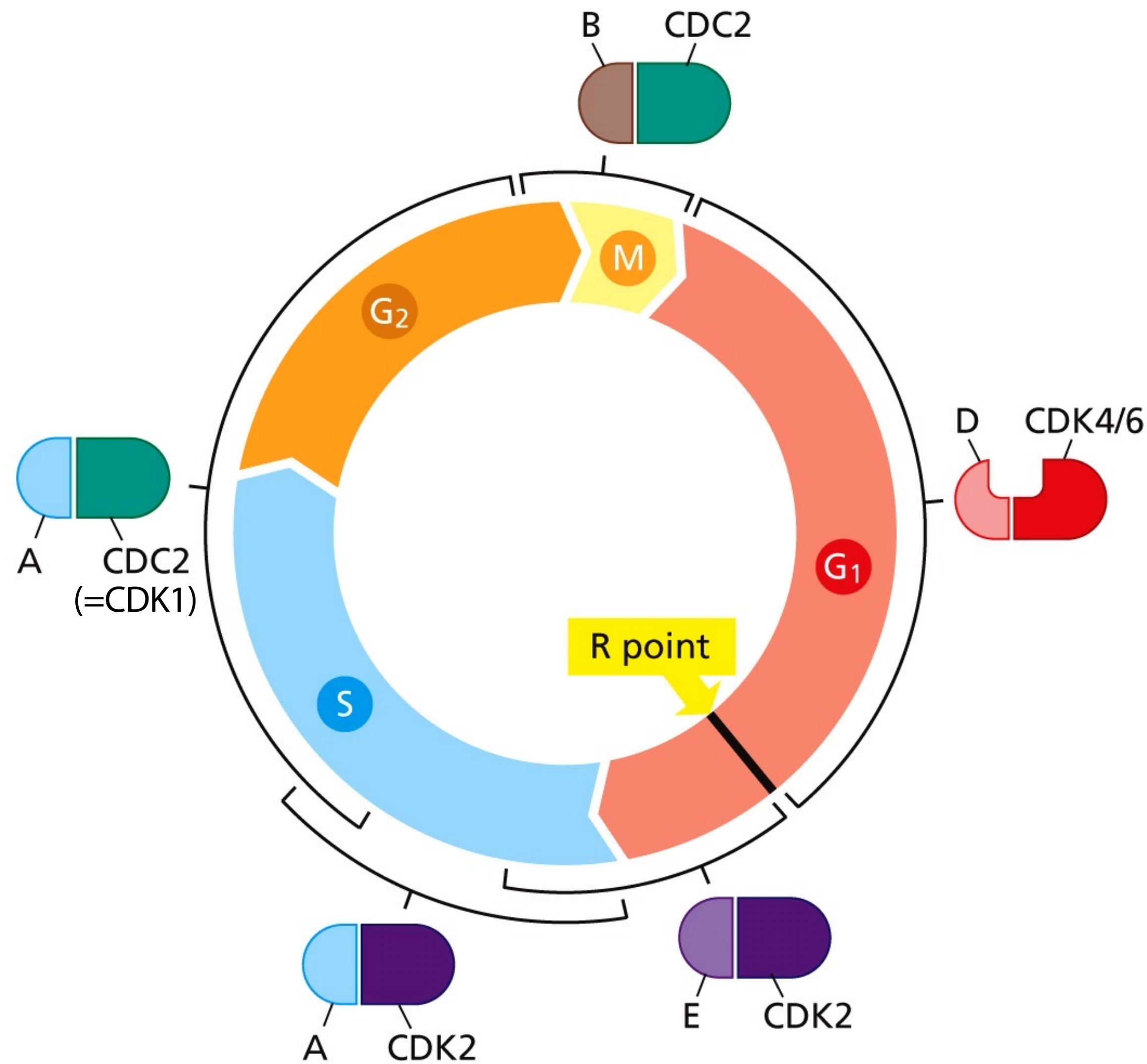


Figure 8.6. Weinberg, The Biology of Cancer

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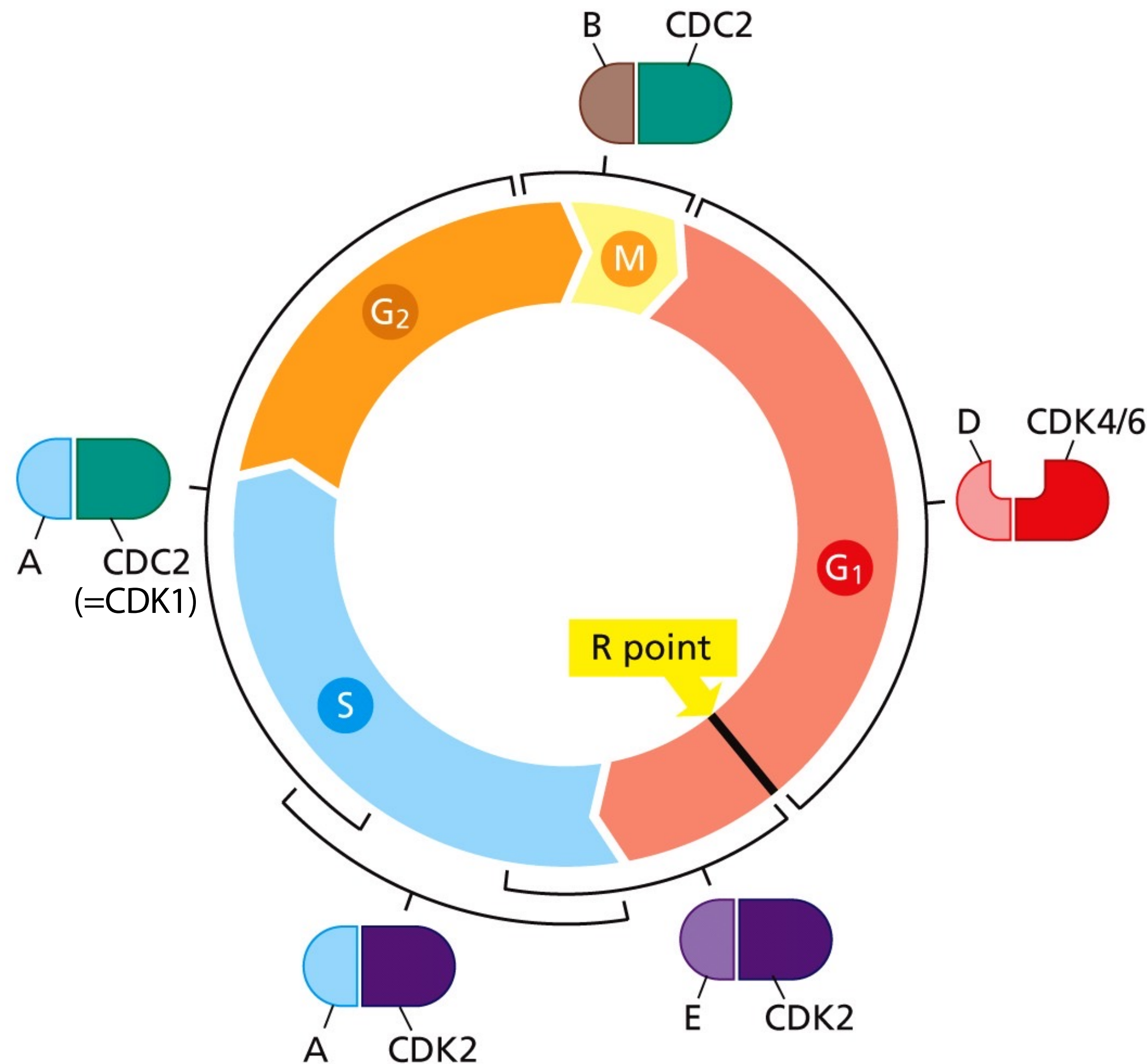
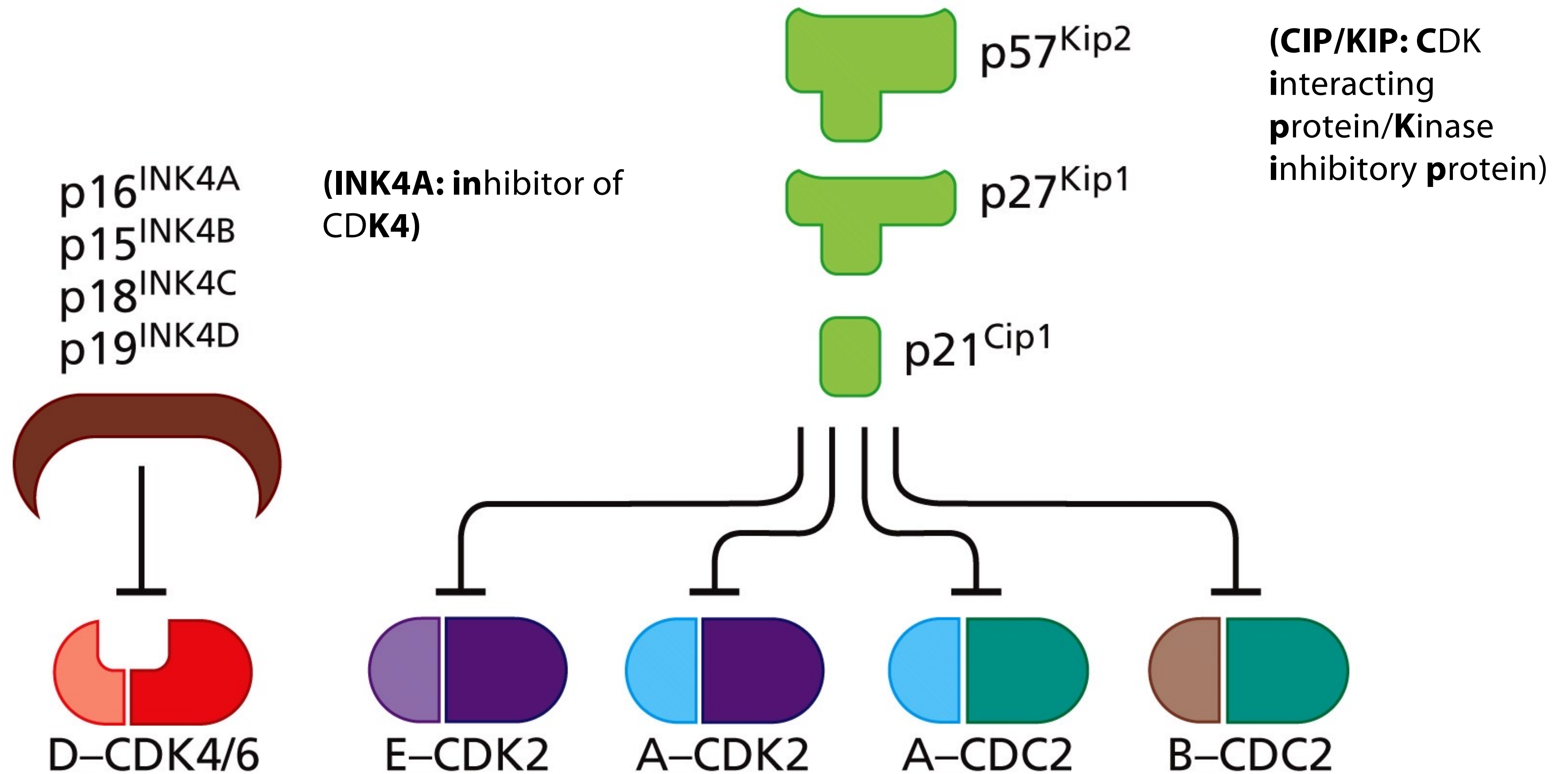


Figure 8.6. Weinberg, The Biology of Cancer

- D-type cyclins: convey signals from the extracellular environment.
- D-type cyclins assemble with CDK4 and CDK6 both of which have similar enzymatic activities.
- Cyclin D1, D2, D3: induced by different transcription factors.

From Monday:

Cyclin-CDK complexes: regulation by 7 CDK-inhibitors (CKIs)



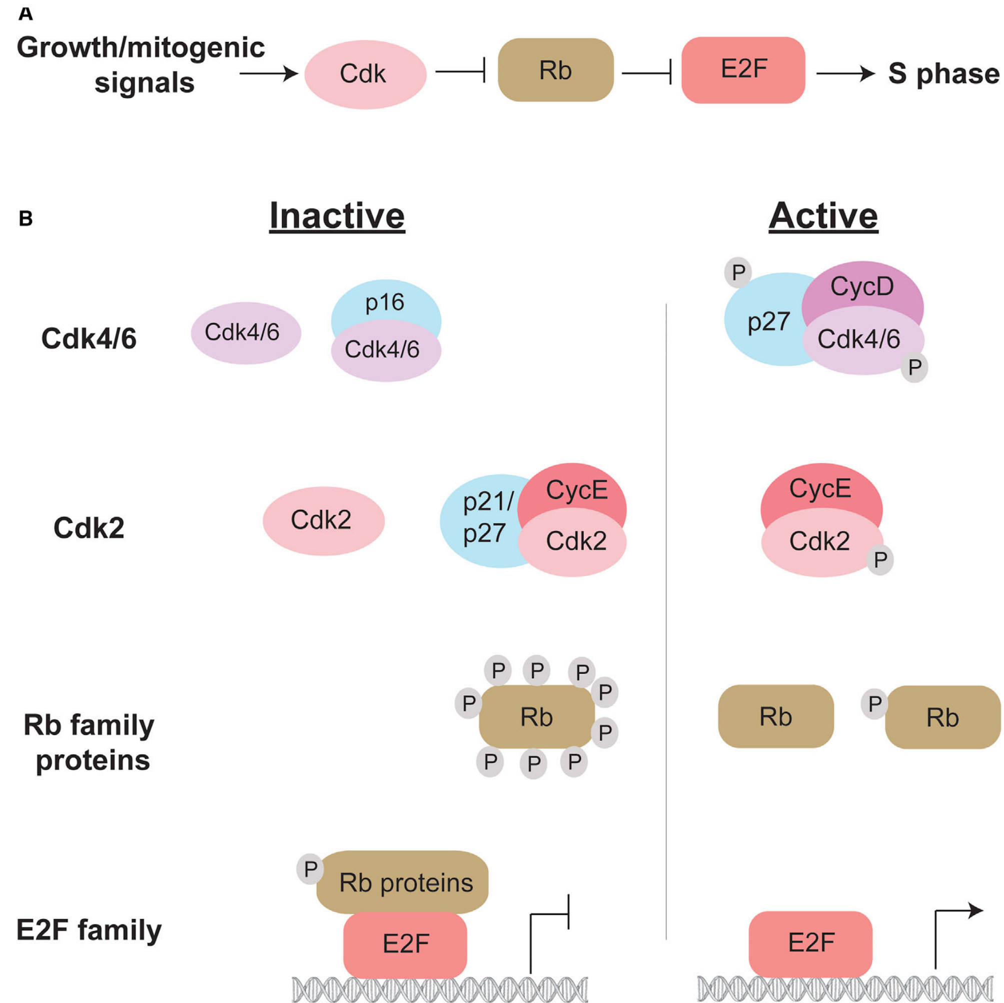


Figure 1. Components of the Cdk-Rb-E2F Pathway Controlling the G1/S Transition

(A) Simplified linear model for the pathway.

(B) Inactive and active states of the key players in the Rb pathway. Cdk4/6 have relatively high sequence homology among Cdks. They are inactive as monomers, bound to p16 family proteins, or bound by unphosphorylated p21/p27 proteins. Cdk4/6 are activated by association with CycD family proteins, but full activity also requires a phosphorylated form of p27 in the complex and phosphorylation on the kinase activation loop. Cdk2 is inactive as a monomer or in complex with p21/p27 family proteins, and it is activated by CycE binding in G1 (or by CycA later in the cell cycle) and activation loop phosphorylation. Rb is considered active when hypophosphorylated or monophosphorylated; in this state, it binds and inhibits E2F. Hyperphosphorylation of Rb leads to its inactivation, dissociation from E2F, and subsequent E2F activation.

Why should CDKs be valuable drug targets?

- Canonical CDK genes were thought to be essential; however, viable mice develop from knockout of CDK2, CDK4, or CDK6 and only CDK1 knockout mice are embryonic lethal. (though tissue-specific defects are seen)
- Studies in mice describing compound knockout of CDKs 2, 4, and 6 illustrate the ability of CDK1 to compensate and support cellular proliferation by binding various cyclins.

…but some tumor subsets depend on CDK4/6 or CDK2 for their proliferation.

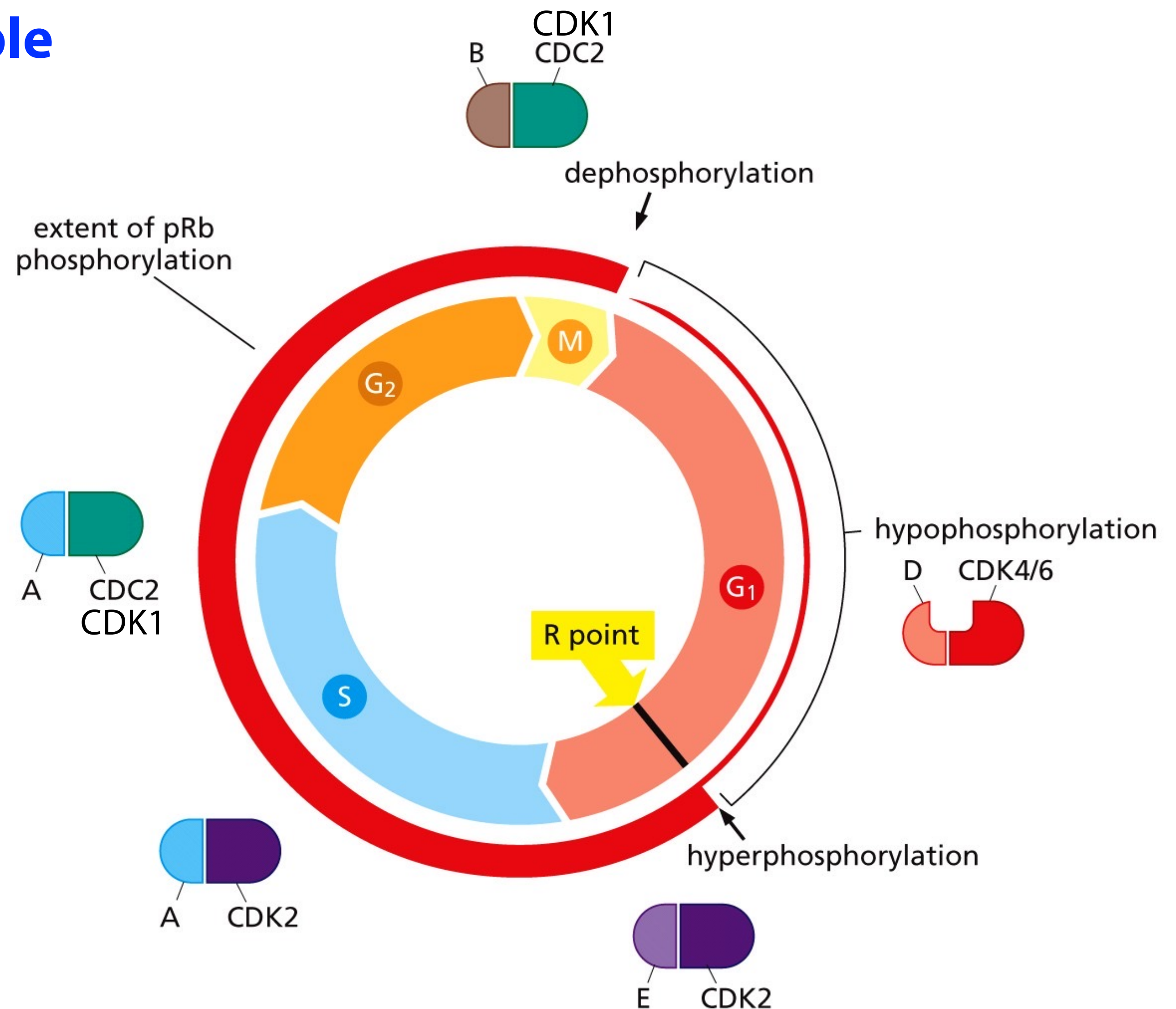
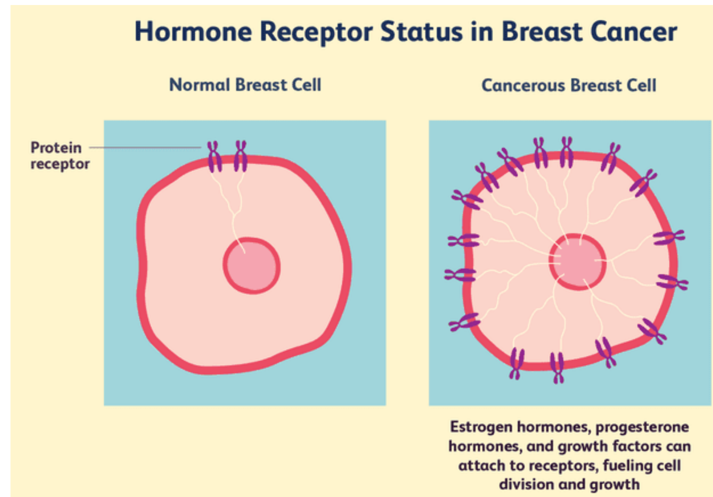
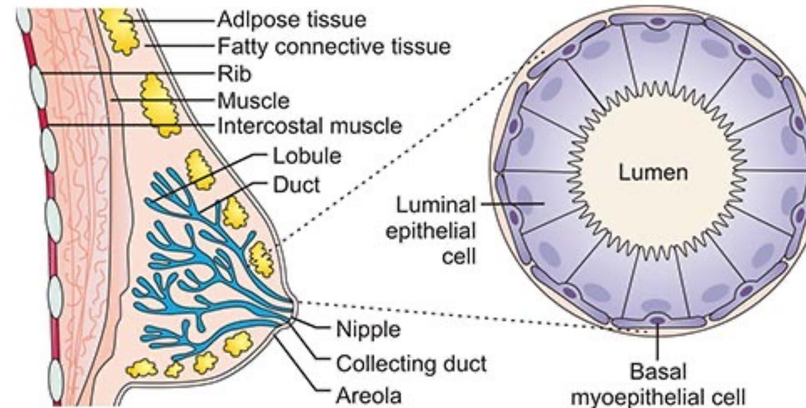


Figure 8.19 The Biology of Cancer (© Garland Science 2014)

The CDK4/6 inhibitor palbociclib (PAL) significantly improves progression-free survival in hormone receptor positive breast cancer (combined with anti-hormonals (tamoxifen)).





In **luminal estrogen receptor (ER) positive breast cancer**, representing approximately 75% of breast cancer, **ER signaling activates the cyclin D1 promoter**.
ER-positive, luminal breast cancer presents the archetypal model for **CDK4/6 inhibitors**, reflecting the particular dependence of luminal breast cancer on cyclin D1 to initiate G1-S phase transition.

In contrast to luminal breast cancer, **basal-like triple negative breast cancer** is characterized by **loss of RB1** and by **high expression of cyclin E**. Consequently basal-like breast cancer cell lines are resistant to CDK4/6 inhibition (triple negative: breast cancers that are not fueled by estrogens, progesterone, or growth factors binding to HER2 receptors)

Classification:
Breast cancers that are estrogen receptor-positive (ER+) and/or progesterone receptor-positive (PR+) are "fueled" by hormones. They are different from breast cancers that are HER2-positive (human epidermal growth factor receptor 2), in which tumor growth is driven by growth factors that bind to HER2 receptors on the cancer cells (see week 1: Herceptin monoclonal antibodies for the treatment of HER+ breast cancer).

Breast cancers that don't have any of these receptors are called triple-negative.

Some breast cancers are both hormone receptor-positive and HER2-positive, meaning that estrogen, progesterone, ***and*** growth factors can stimulate cell growth. These cancers are often referred to as triple-positive breast cancers.

Types of Protein Interactions

- Stable or transient
- Strong or weak

Stable: purified as multi-subunit complexes (e.g. hemoglobin, core RNA polymerase)
-->studied by gel filtration, sedimentation, co-immunoprecipitation.
Transient: require a set of conditions to become detectable
-->captured e.g. by cross-linking, label transfer methods but also 2-hybrid.

Coimmunoprecipitation

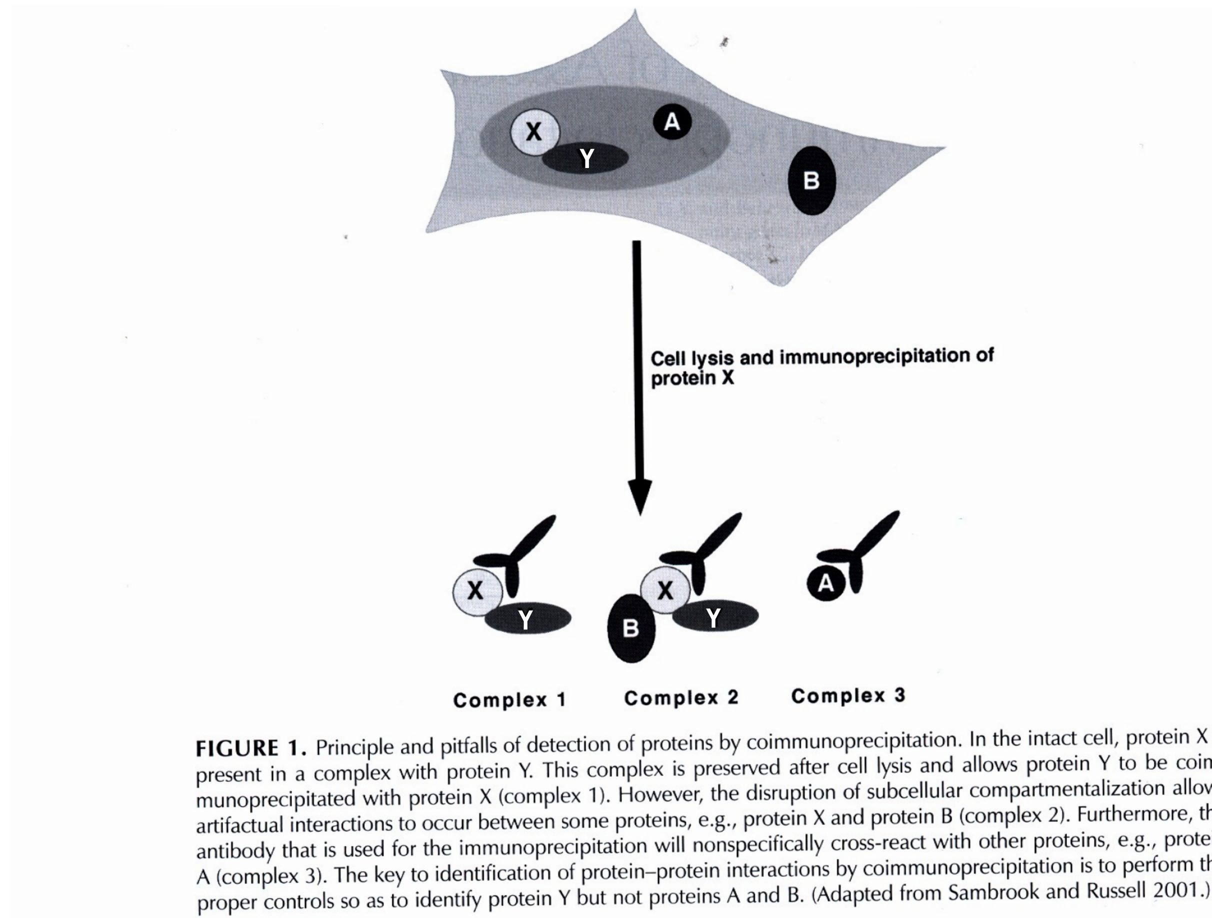


FIGURE 1. Principle and pitfalls of detection of proteins by coimmunoprecipitation. In the intact cell, protein X is present in a complex with protein Y. This complex is preserved after cell lysis and allows protein Y to be coimmunoprecipitated with protein X (complex 1). However, the disruption of subcellular compartmentalization allows artifactual interactions to occur between some proteins, e.g., protein X and protein B (complex 2). Furthermore, the antibody that is used for the immunoprecipitation will nonspecifically cross-react with other proteins, e.g., protein A (complex 3). The key to identification of protein–protein interactions by coimmunoprecipitation is to perform the proper controls so as to identify protein Y but not proteins A and B. (Adapted from Sambrook and Russell 2001.)

Already discussed: **Epitope tags**

Table 1. Common Epitope Tags

Name	Sequence	Detection	Purification	Reference
FLAG	DYKDDDDK	M1,M2, M5	Immunoaffinity	1
6 × His	HHHHHH	Anti-His	Metal affinity	2
HA	YPYDVPDYA	12CA5	Immunoaffinity	4
c-myc	EQKLISEEDL	9E10	Immunoaffinity	5
GST	220 aa GST	Anti-GST	Glutathione	3
Protein A	IgG-binding domain	IgG	IgG	10
CD	18 aa exon	12CA5	Immunoaffinity	19
Strep-tag	WSAPQFEK	Strep-Tactin	Strep-Tactin	11
MBP	Maltose-binding protein	Anti-MBP	Maltose	13
CBD	Chitin-binding domain	Anti-CBD	Chitin	14
S-tag	S-peptide	Anti-S peptide	S-peptide	16
Avitag	GLNDIFEAQKIEWHE	Avidin	Avidin	12
CBP	CBP peptide	Anti-CBP	Calmodulin	15
TAP	Calmodulin- and IgG-binding domains	Anti-CBP	Calmodulin and IgG	15
SF-TAP	Strep Tag II and FLAG	Anti-FLAG	Strep-Tactin	28

GST, glutathione-S-transferase; CBP, calmodulin-binding peptide.

694 | BioTechniques 25th Anniversary | www.biotechniques.com Vol. 44 | No. 5 | 2008

For **Halo-tag**: see next slide.

GFP and derivatives: https://en.wikipedia.org/wiki/Green_fluorescent_protein

Epitope tagging is a technique in which a known epitope is fused to a recombinant protein by means of genetic engineering. By choosing an epitope for which an antibody is available, the technique makes it possible to detect proteins for which no antibody is available.

HaloTag is a self-labeling protein tag. It is a 297 residue peptide (33 kDa) derived (and modified) from a bacterial enzyme, designed to covalently bind to a synthetic ligand. The bacterial enzyme can be fused to various proteins of interest.

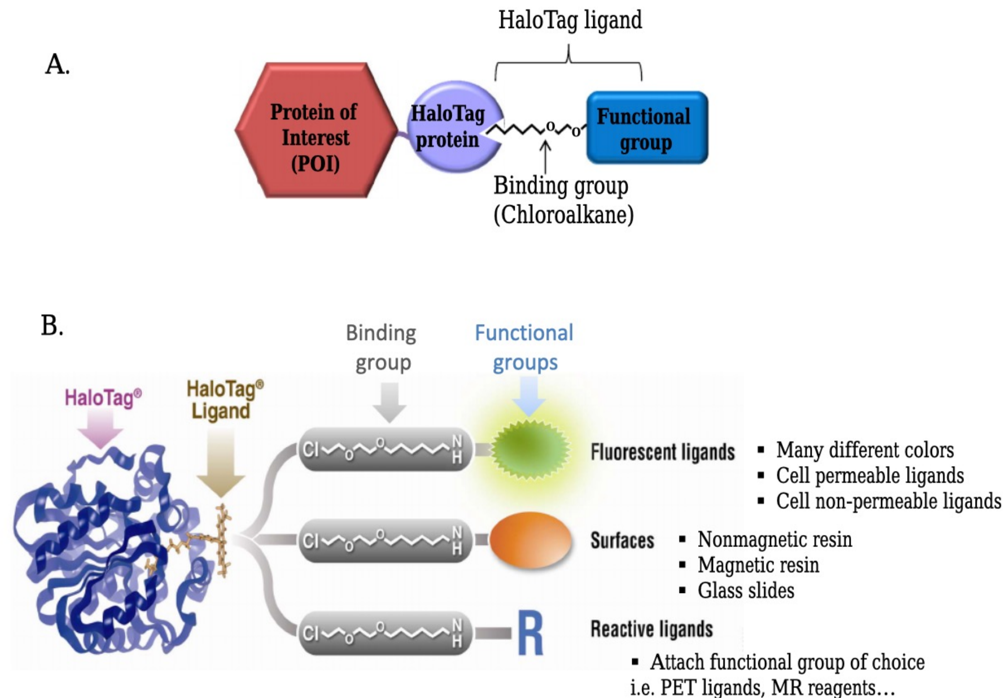


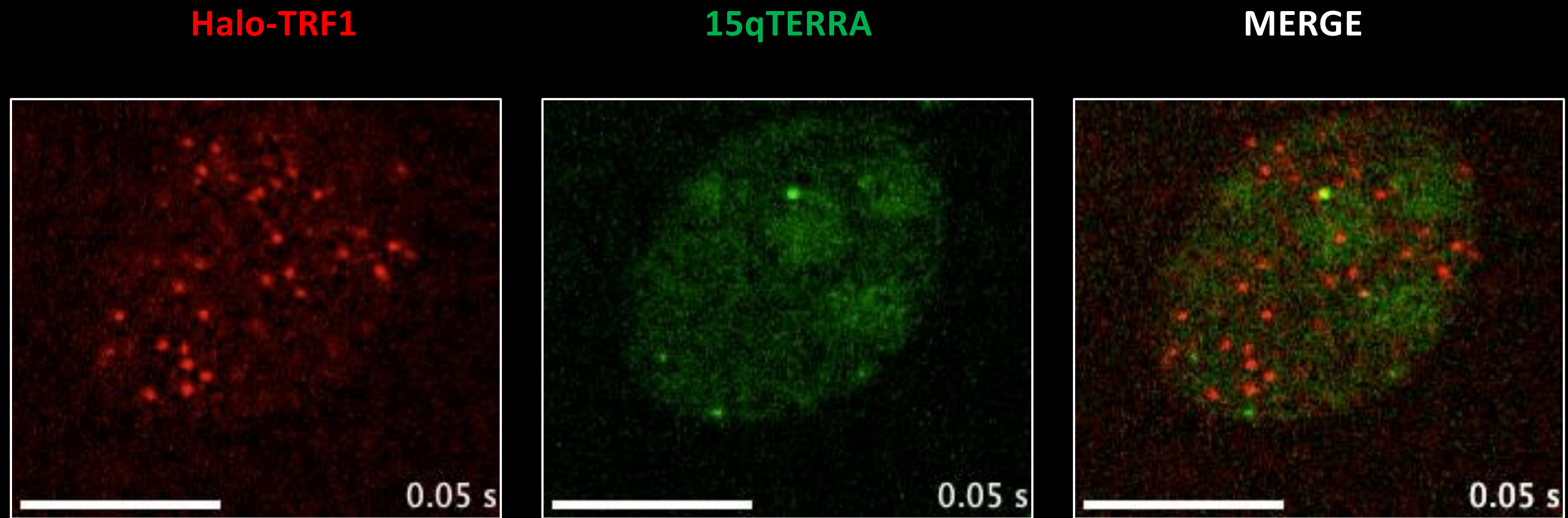
Fig. (1). Multiple functionalities of the HaloTag™ Technology.

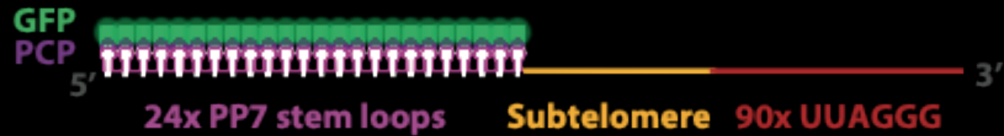
A. Schematic of the HaloTag technology consisting of the HaloTag protein and a selection of synthetic HaloTag ligands carrying different functional groups. HaloTag protein can be easily fused to any protein of interest (POI). HaloTag ligands specifically and covalently bind to the HaloTag fusions.

B. Schematic representation of HaloTag-TMR ligand binding to the active site in the binding tunnel of HaloTag protein. HaloTag ligands with different functional groups such as surfaces, fluorescent dyes or reactive groups are shown; the constant binding group (chloroalkane) is highlighted by the grey shaded rectangle.The ligands, depending on functional group impart multiple functions to a HaloTag fusion protein including imaging, immobilization and others. Thus one genetic construct can be used in various in vitro and in vivo assays.

https://www.youtube.com/watch?v=dDtY2iO41cU

Telomeres and TERRA Live Imaging

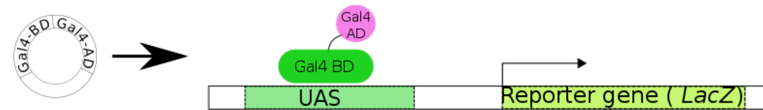




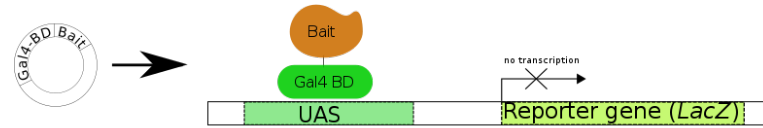
20fps, 200 frames, 10s
scale bars: 10µm

Eftychia Kyriacou

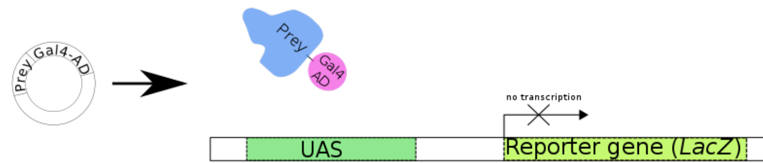
Yeast 2-hybrid System



A. Regular transcription of the reporter gene

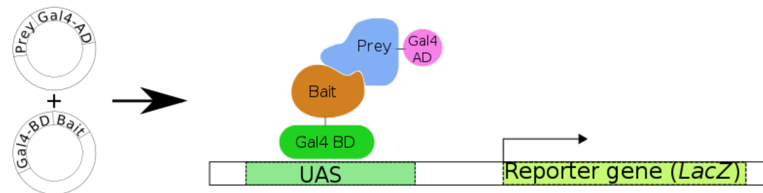


B. One fusion protein only (Gal4-BD + Bait) - no transcription



C. One fusion protein only (Gal4-AD + Prey) - no transcription

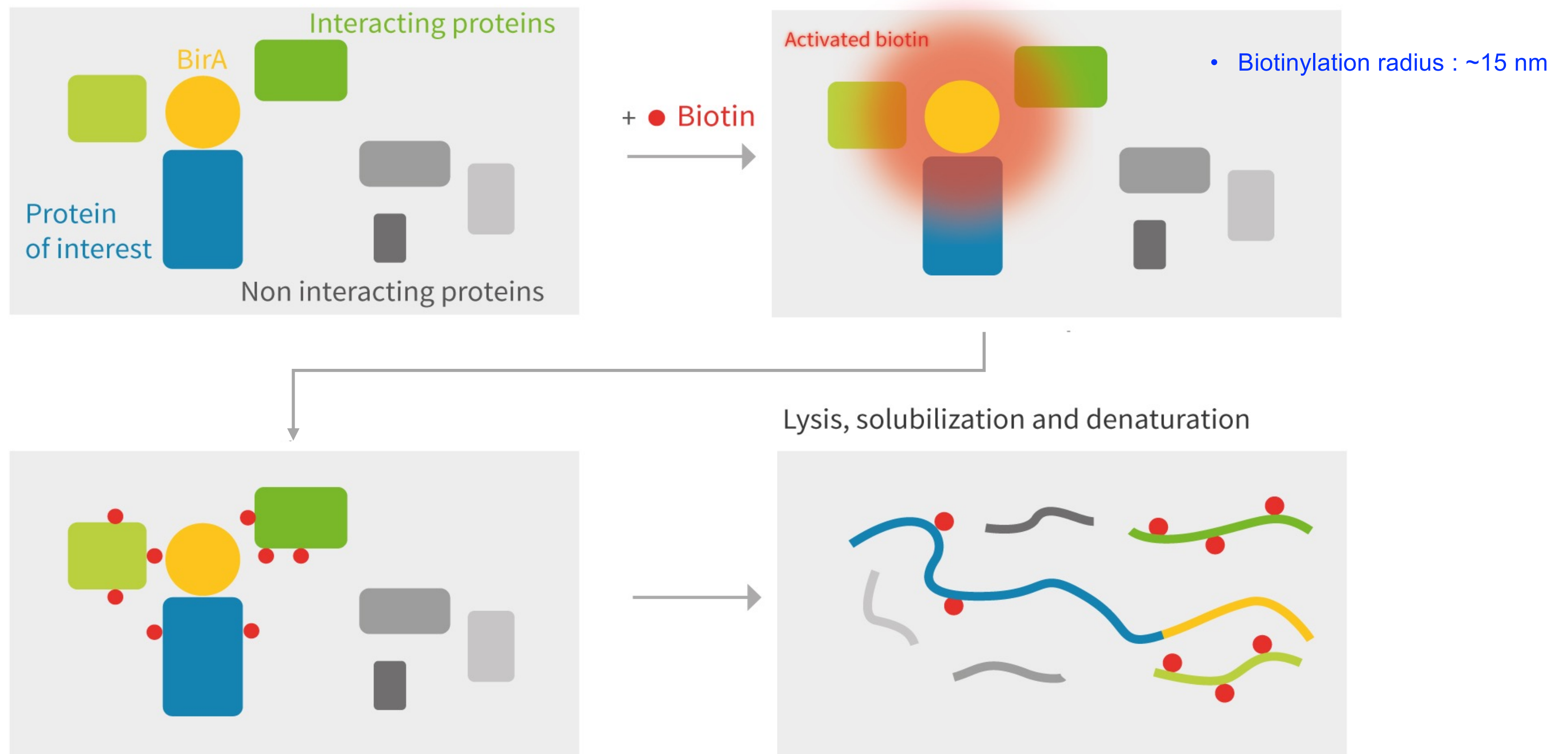
First developed: Fields S. Song O.
Nature. 1989 Jul 20;340(6230):245-6



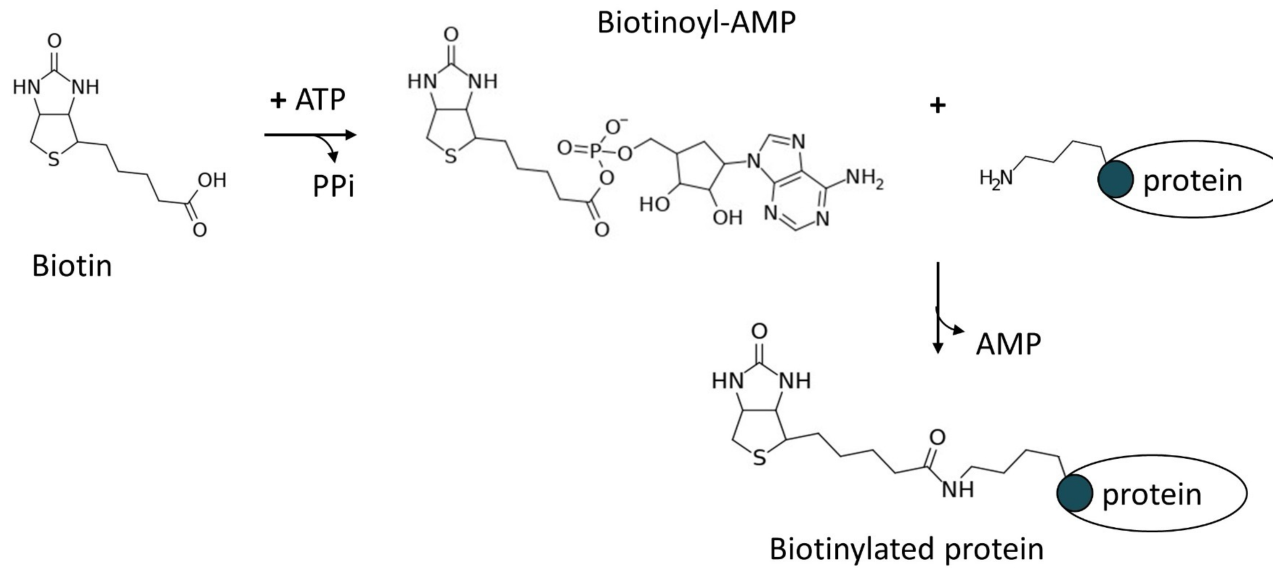
D. Two fusion proteins with interacting Bait and Prey

Reporter gene can also be a drug-selectable marker etc.

BioID Method: express protein of interest as fusion protein with a biotin ligase (BirA)



- Biotinylation radius : ~15 nm



BirA catalyzes an ATP-dependent two-step reaction in which **biotin** is used to form biotinyl-5'-adenylate and is transferred to a specific **lysine residue of the accepting protein** via an amide linker.

BioID- Advantages

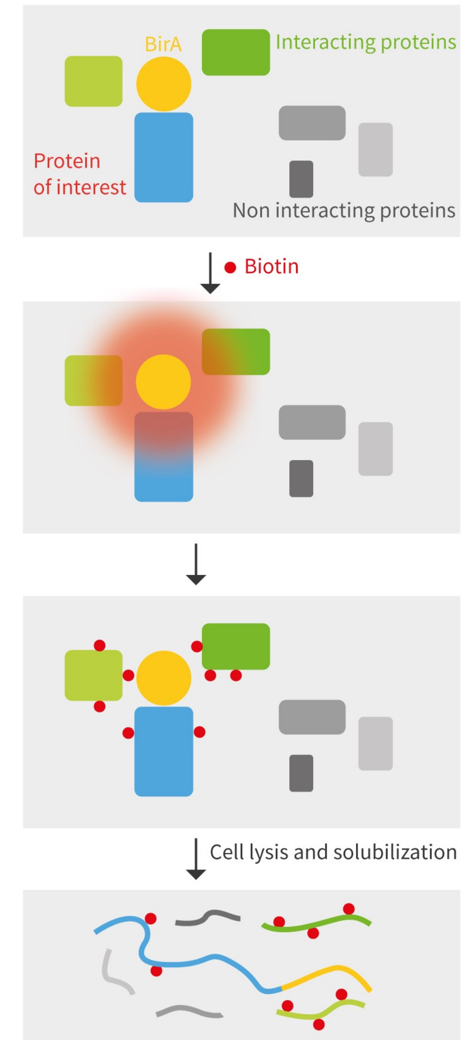
Interacting proteins are covalently labeled → no need to maintain the native interaction through the purification process

→ Application of harsh lysis and solubilization conditions

→allows identification of week and transient interactions

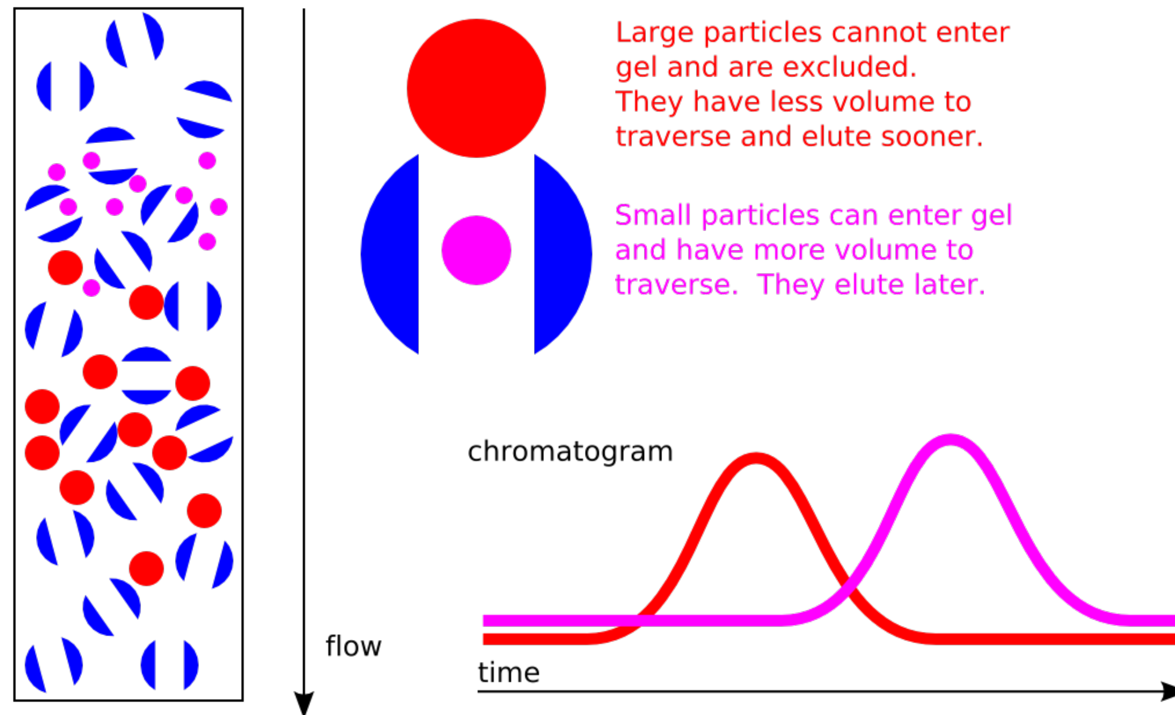
→ Detects not only direct interactions but also the environment of protein

Purification of biotinylated proteins using streptavidin beads



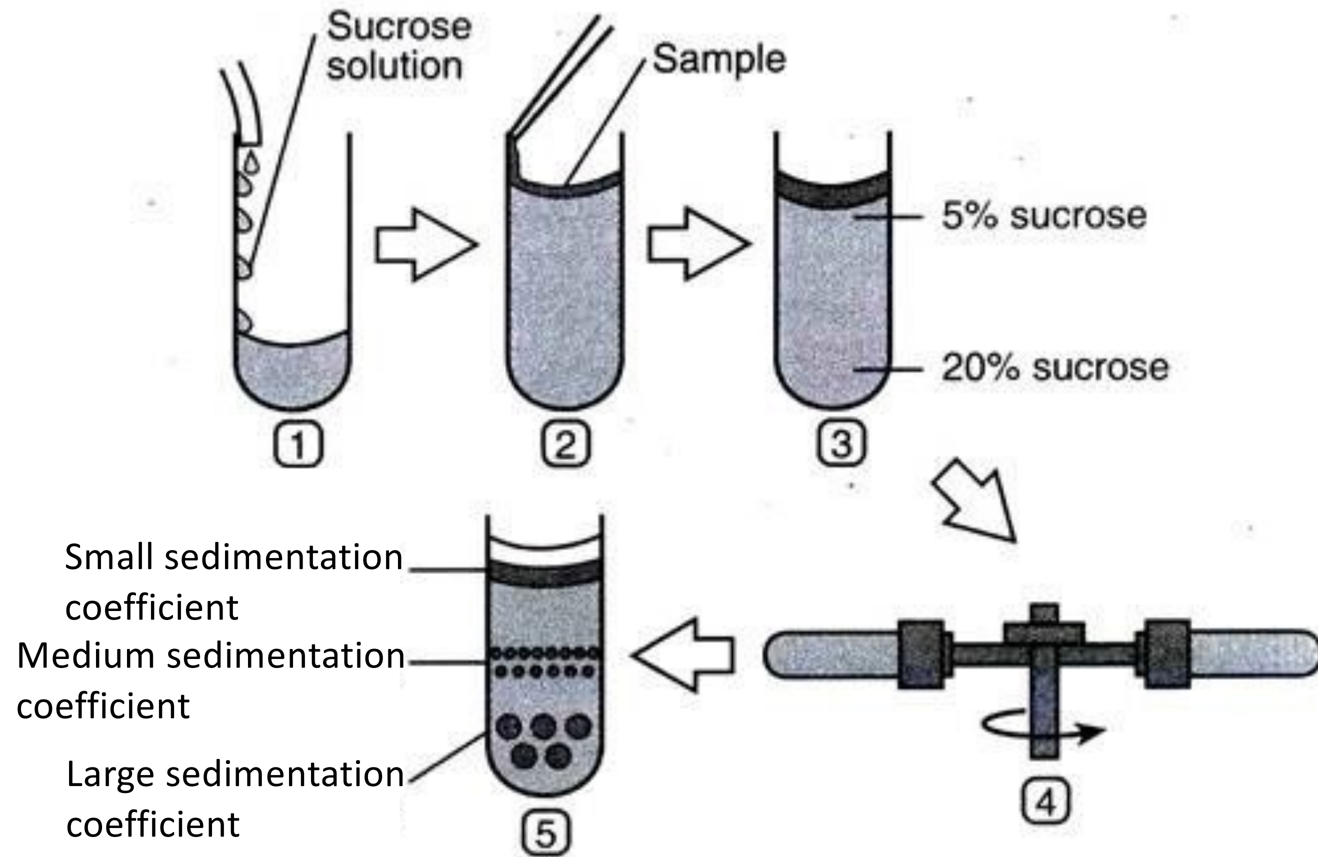
Native size of proteins and protein complexes

Size exclusion chromatography / Gel filtration



…Fractionation according to Stokes' radius

Sucrose gradient ultracentrifugation



…Fractionation according to sedimentation coefficient