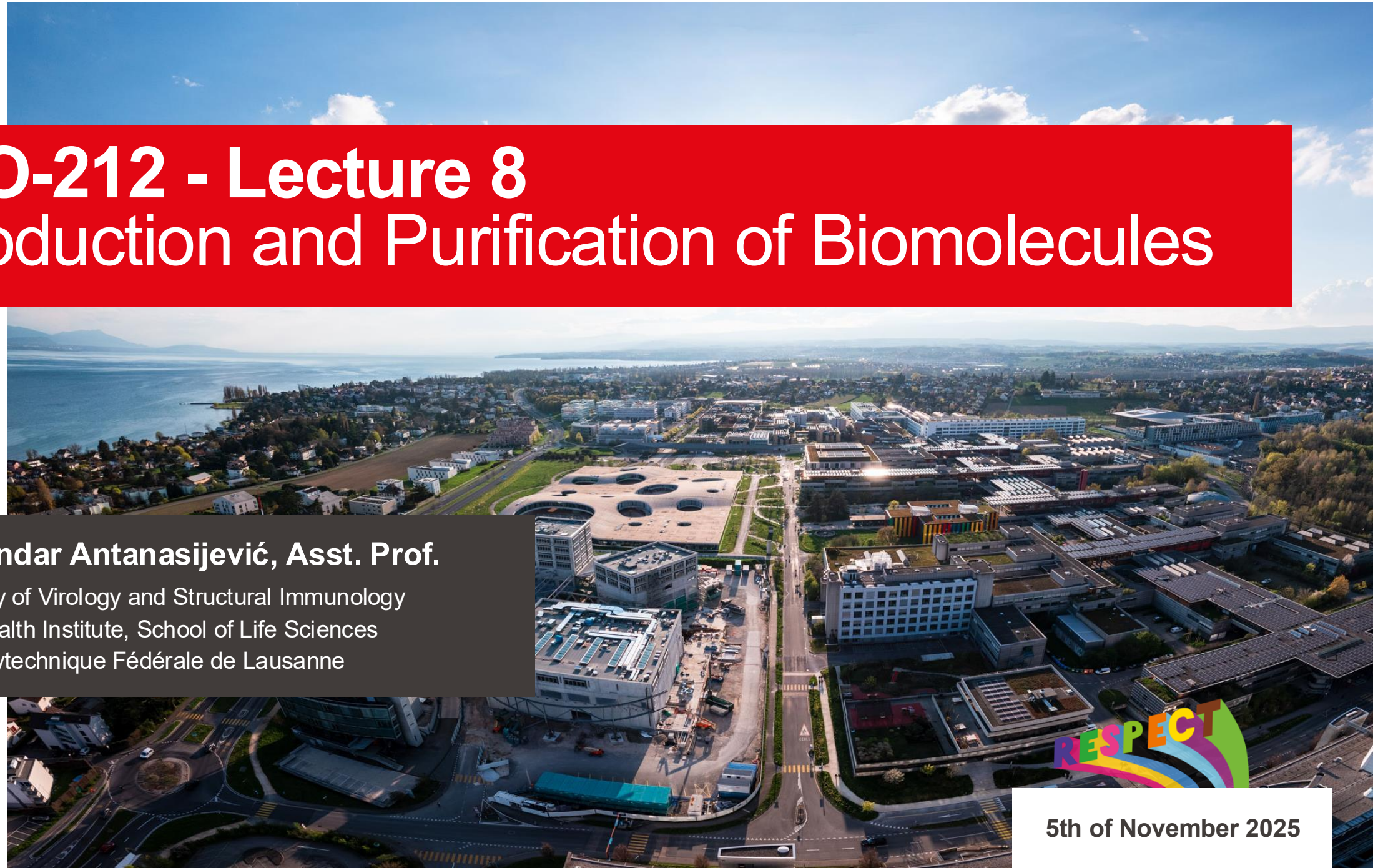
# BIO-212 - Lecture 8
# Production and Purification of Biomolecules



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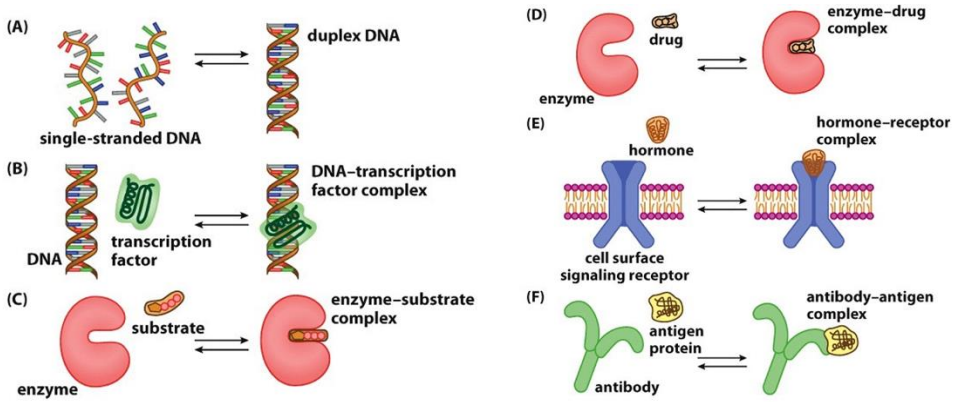
5th of November 2025

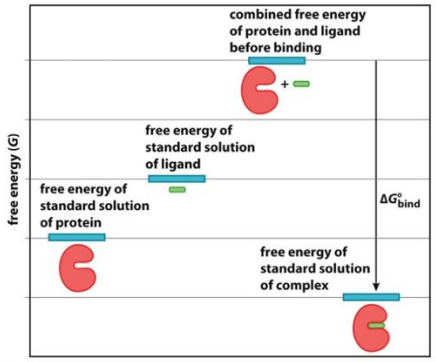
# Lecture 7 - Quick Summary

## • Biomolecular interactions (binding)

- Binding in different contexts




- Gibbs free energy and equilibrium constants (K)




$$P + L \rightleftharpoons P \bullet L$$

$$K_D = \frac{[P][L]}{[P \bullet L]} = \frac{1}{K_A}$$

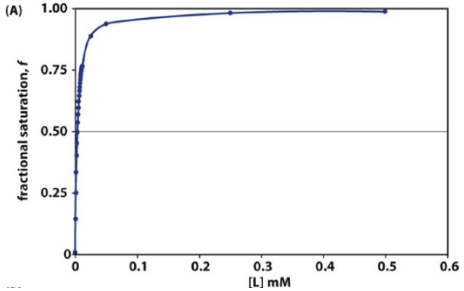
$$\Delta G^{o}_{bind} = -RT \ln K_A$$

$$\Delta G^{o}_{bind} = +RT \ln K_D$$

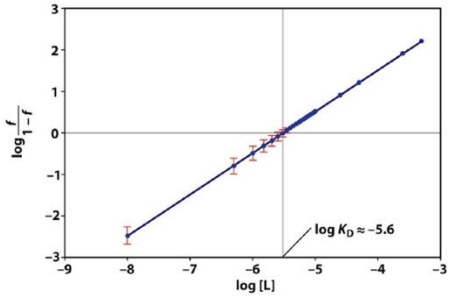
## • Different ways of presenting binding curves

- Fractional saturation (f):

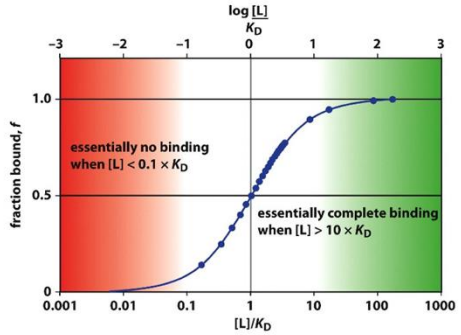
$$f = \frac{\text{concentration of protein with ligand bound}}{\text{total protein concentration}}$$




**f ~ [L]**




**$\log \frac{f}{1-f} \sim \log [L]$**




**f ~ [L]/Kd**

# Lecture 8 - Outline

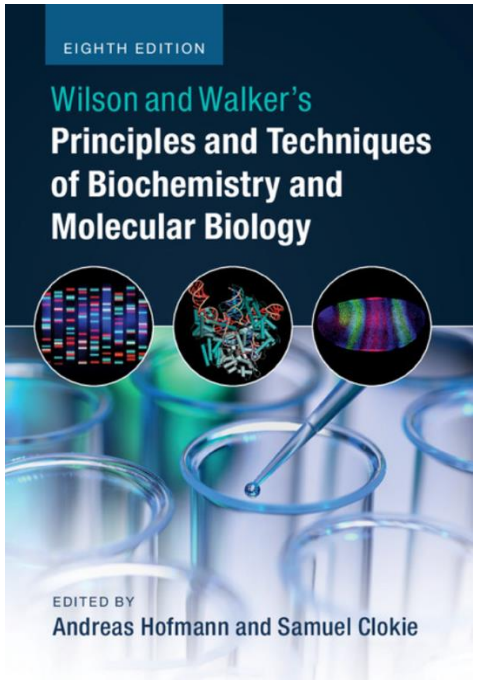
## Today:

- Different methods for biomolecule production
- Purification of biomolecules using liquid chromatography
- Liquid chromatography columns

## Reading suggestions:

- Principles and Techniques of B&MB

  Chapters 6, 8 and 11



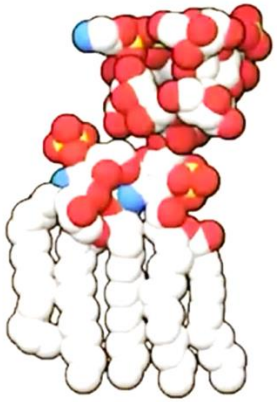



Link: https://bgc.ac.in/pdf/study-material/Keith-Wilson-John-Walker-Principles-and-Techniques-of-Biochemistry-and-Molecular-Biology-Cambridge-University-Press-2010.pdf

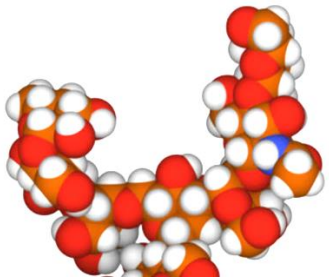
# Production and purification of biomolecules

- Why is it important to generate purified biomolecule samples?

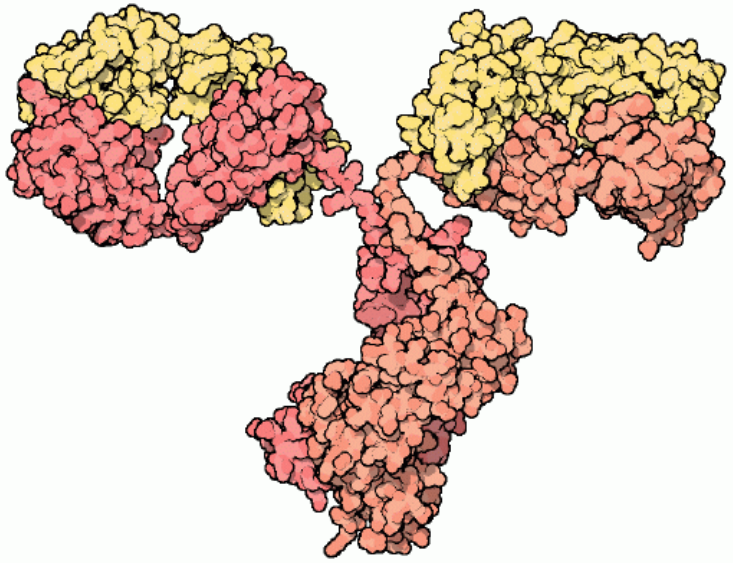
- To study their function
- To study how they fold (kinetics)
- To identify which other biomolecules they interact with
- To determine their sequence/composition
- To determine their structure (e.g., by X-ray crystallography)
- To apply for drug screening
- For therapeutic applications



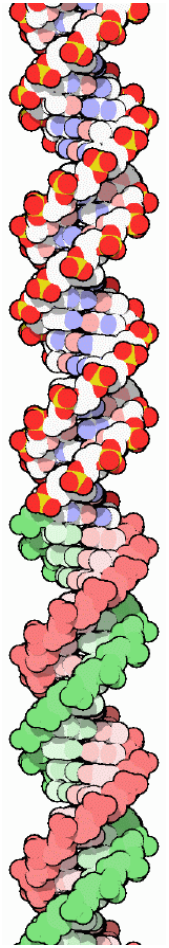
Lipid



Carbohydrate



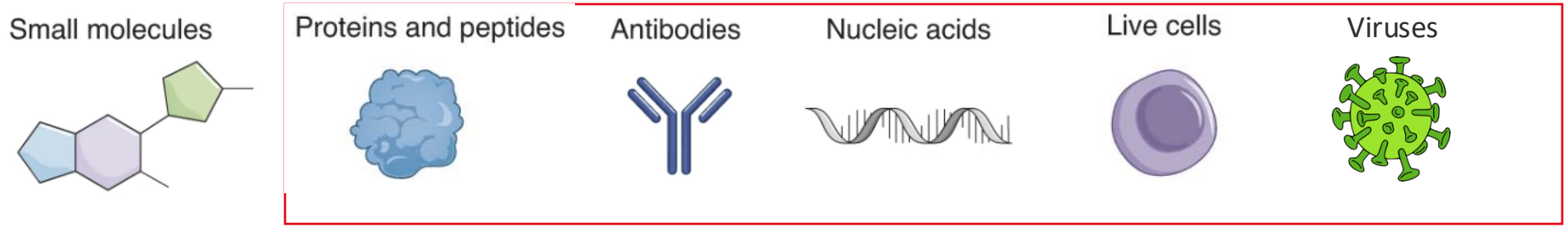
Protein



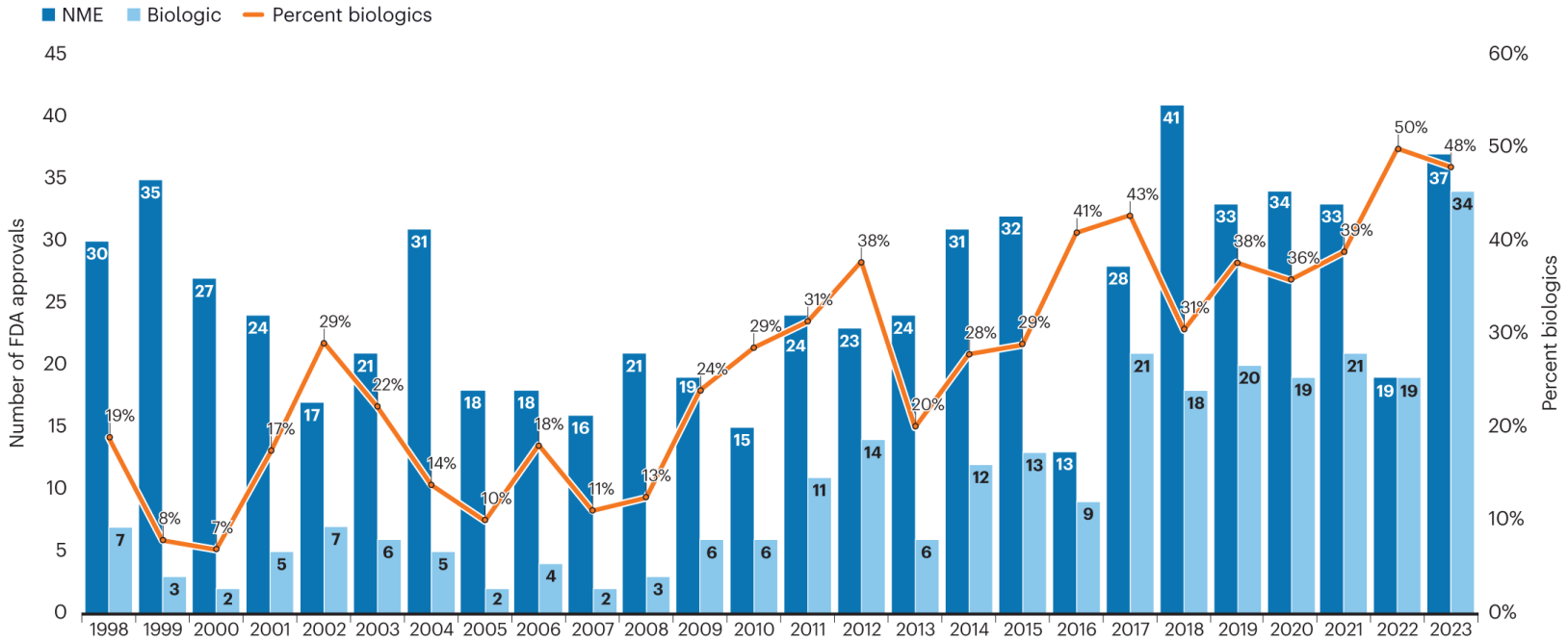
Nucleic Acids

# Biomolecules as therapeutics and vaccines

- Newly approved drugs by the US Food and Drug Administration (FDA)




Biologics



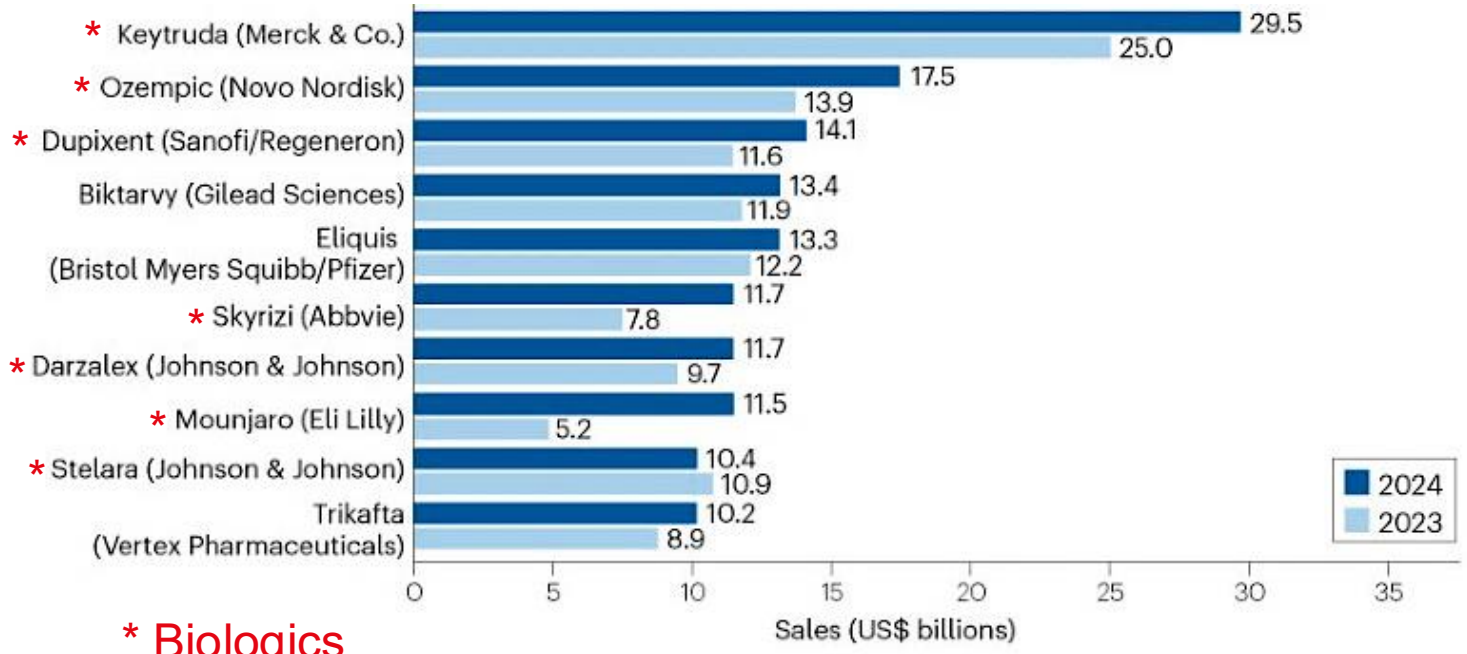

Source: Senior et al., Nature Biotechnology, 2024

- 2023 was a record year in terms of the # of new approved drugs
- 48% of newly approved drugs in 2023 are biologics
- All vaccines are essentially of biomolecular origin

# Biologics are important drugs

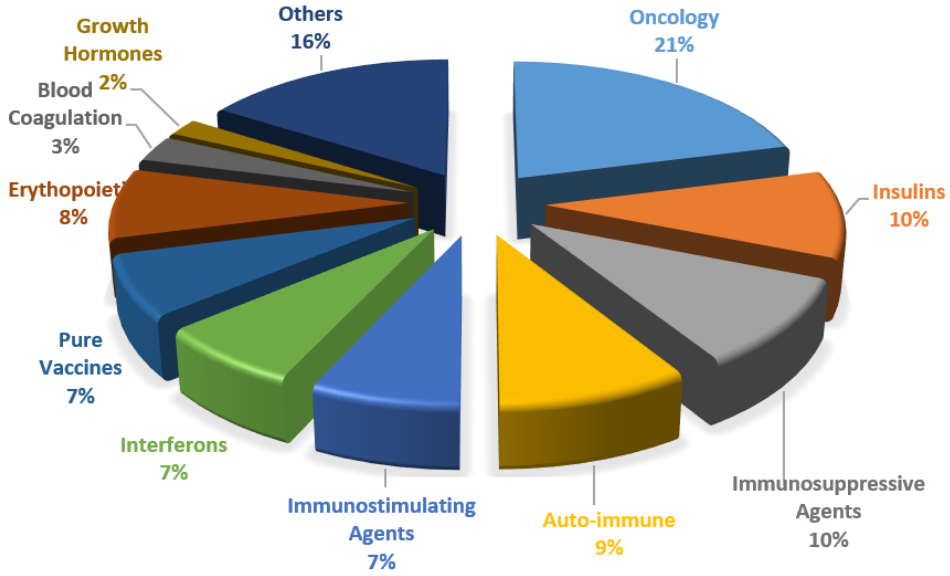
- Proteins and mRNA are a major class of biologics

Highest-selling drugs in 2023/2024




* Biologics

Applications of protein therapeutics




- Most protein therapeutics are based on monoclonal antibodies (more info during BIO-213)
- Advances in protein structure prediction and design are expected to lead to more biologics

EPFL

**Biomolecule Production**

↓

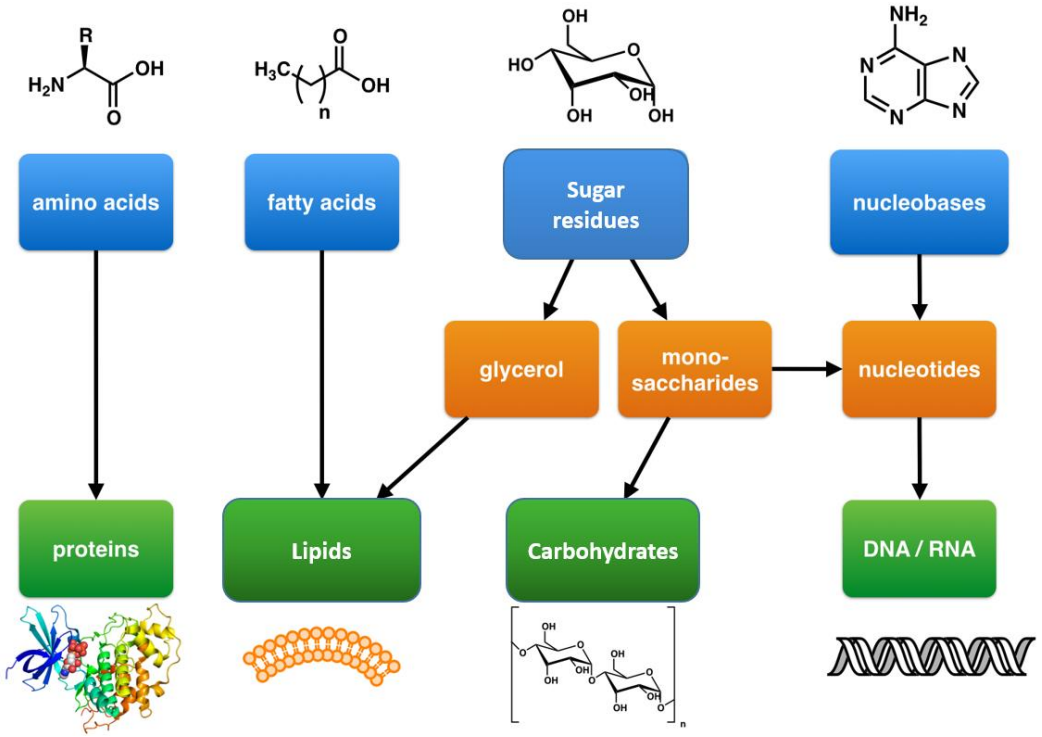
**Biomolecule Purification**

↓

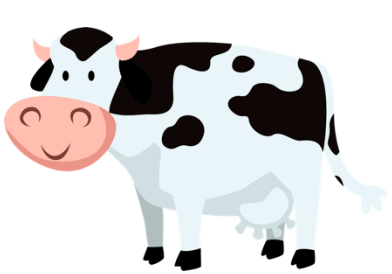
**Biomolecule characterization**

# Biomolecules are produced from building blocks

• **Biological macromolecules are produced from the corresponding building blocks** such as amino-acids, nucleotides, monosaccharides, fatty acids, which assemble into long linear or branched chains.




• First approaches for obtaining high purity biomolecules were based on **purification from biological samples** (e.g., animal tissues) enriched in the protein of interest.
• To date there are many biologic therapeutics that are still generated using these approaches (e.g., estrogen, heparin, antivenoms).



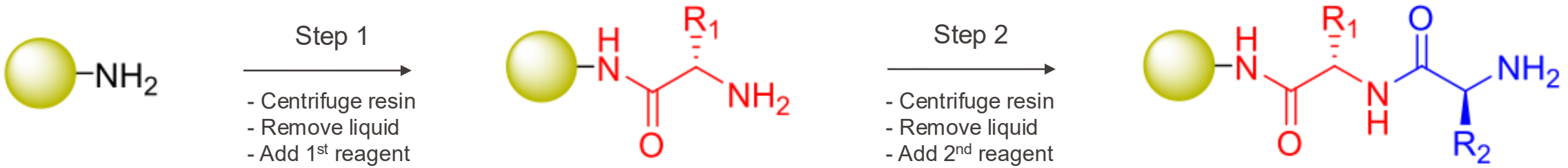
• Today's technology allows to generate target molecules without the need for natural source. There are 3 primary approaches to produce macromolecules:

- **Chemical synthesis**
- **Enzymatic synthesis**
- **Cell-based synthesis**

# Chemical synthesis approaches

• These approaches rely on chemical reactions to fuse building blocks in the correct order (e.g., amino-acid sequence) and using the correct chemical linkage (e.g., peptide bond)

• Most current approaches utilize **solid-phase synthesis** where one terminus of the biomolecule (e.g., N-terminal amino acid) is conjugated to solid support (e.g., resin)




• Resin beads are typically in the **~µm-mm range** which makes them much larger than the biomolecules. They can also be made to be magnetic (e.g., iron-oxide)

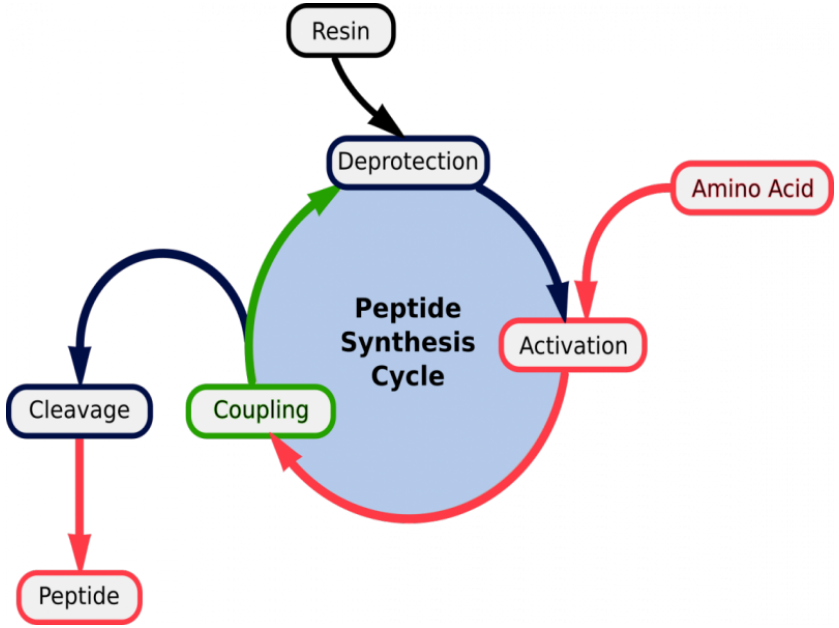
• This allows to use centrifugation, filtration or magnetic field to perform sequential removal and replacement of chemical groups in solution, allowing to **incorporate building blocks in a desired order (sequence)**

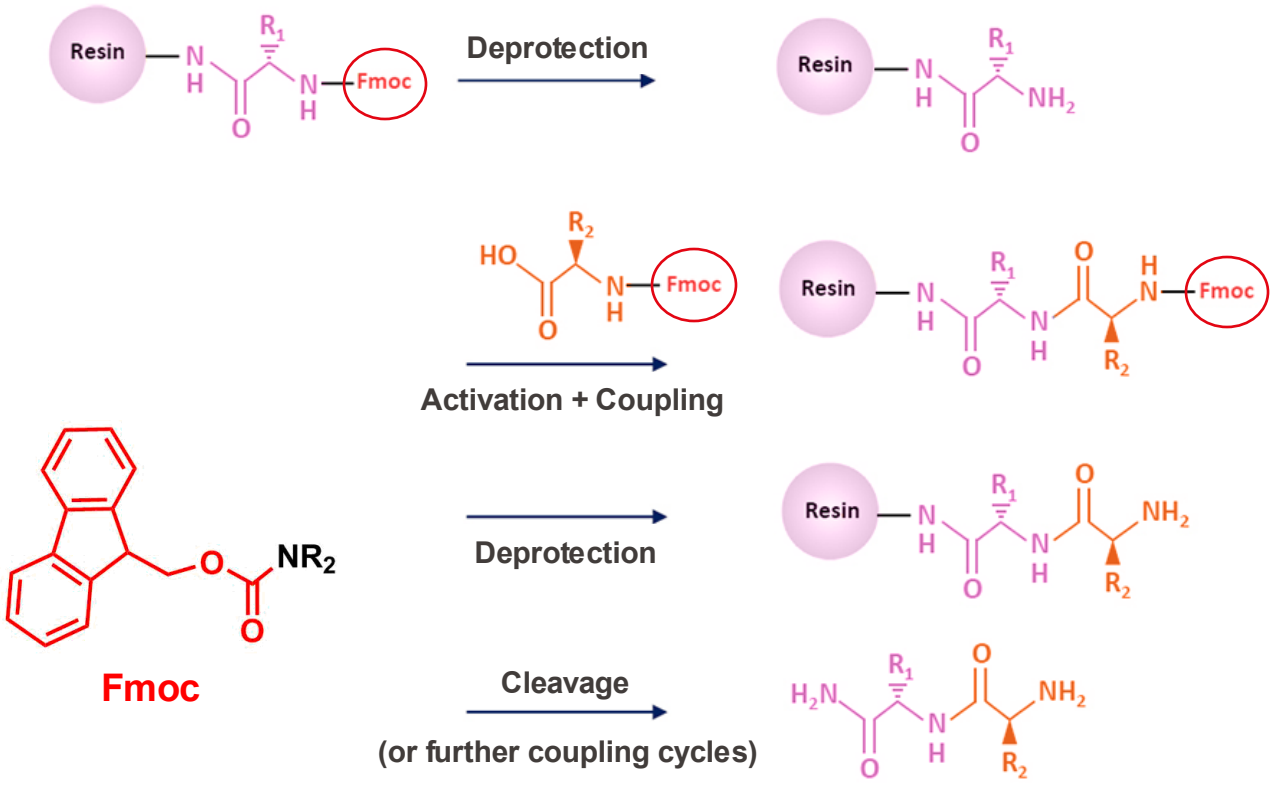
# Chemical synthesis approaches

• Protective groups (e.g., Fmoc) assure that **only a single amino-acid is coupled to the chain in one step**. The group is removed (**deprotection**) when the next building block is ready to be added.

• After adding the last building block of the desired sequence, the product is **cleaved** from the resin and ready for use.

Example: Peptide synthesis from amino-acids



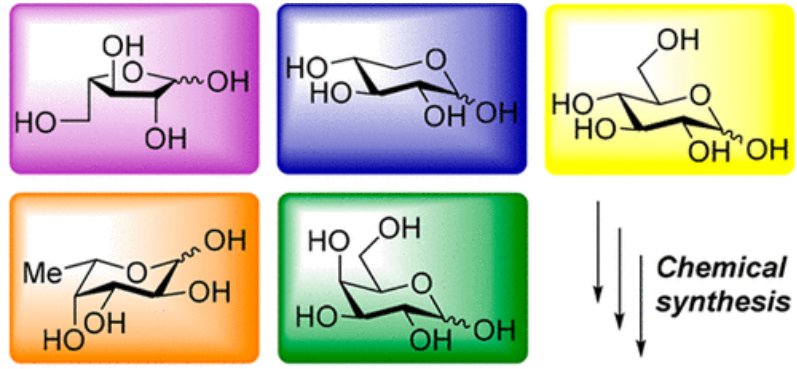
# Chemical synthesis approaches

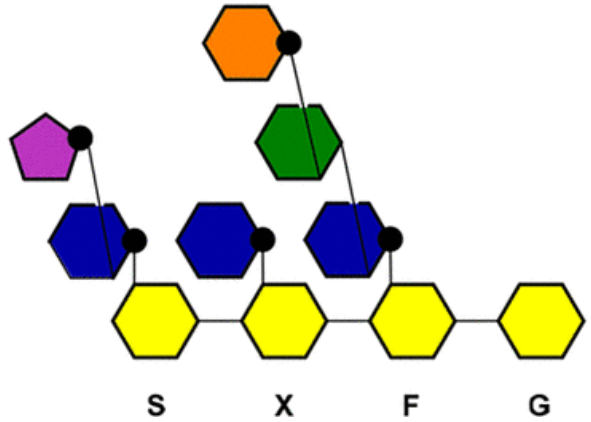
- Each biomolecule family has a corresponding set of chemical procedures and reagents that allow to perform synthesis of desired products.
- There are commercial kits that can be purchased, and many companies provide this service

- Advantages:
  - Simple and well-controlled (good in industry setting)
  - High-yield chemical reactions
  - Allows to produce molecules that are toxic to cells
  - Great for oligonucleotides and short peptides

- Disadvantages:
  - Requires specialized reagents (e.g., protected amino-acids)
  - Chemistry is not always simple (e.g., carbohydrates)
  - **Limited to shorter sequences (e.g., 10-200 residues)**
  - More reaction byproducts with more steps
  - Difficult to produce properly folded protein domains

Carbohydrates have many equivalent groups




Hard to assure linkage via a single group (e.g., just 1-1, or 1-4)

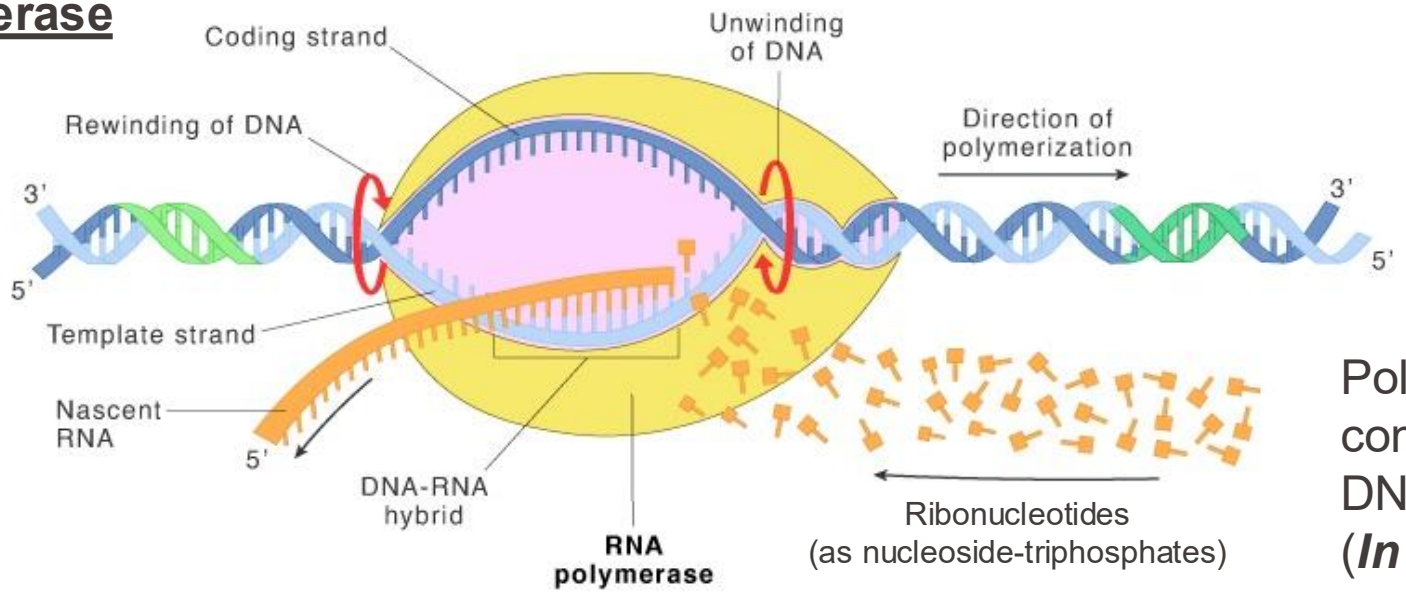
# Enzymatic approaches for biomolecule synthesis

• These approaches are based on ***in vitro* application of enzymes** that naturally catalyze the formation of the corresponding chemical linkages, such as:

- **DNA polymerase** -> Phosphodiester bonds between deoxyribonucleotides
- **RNA polymerase** -> Phosphodiester bonds between ribonucleotides
- **Ribosome** -> Peptide bonds
- **Carbohydrate synthases** -> Glycosidic bonds

• The reactions require the enzyme, corresponding building blocks, buffering components, and in some cases (e.g., protein and nucleic acids) a template nucleic acid sequence to be copied or translated.

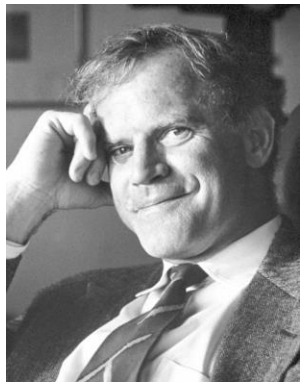
**RNA polymerase**



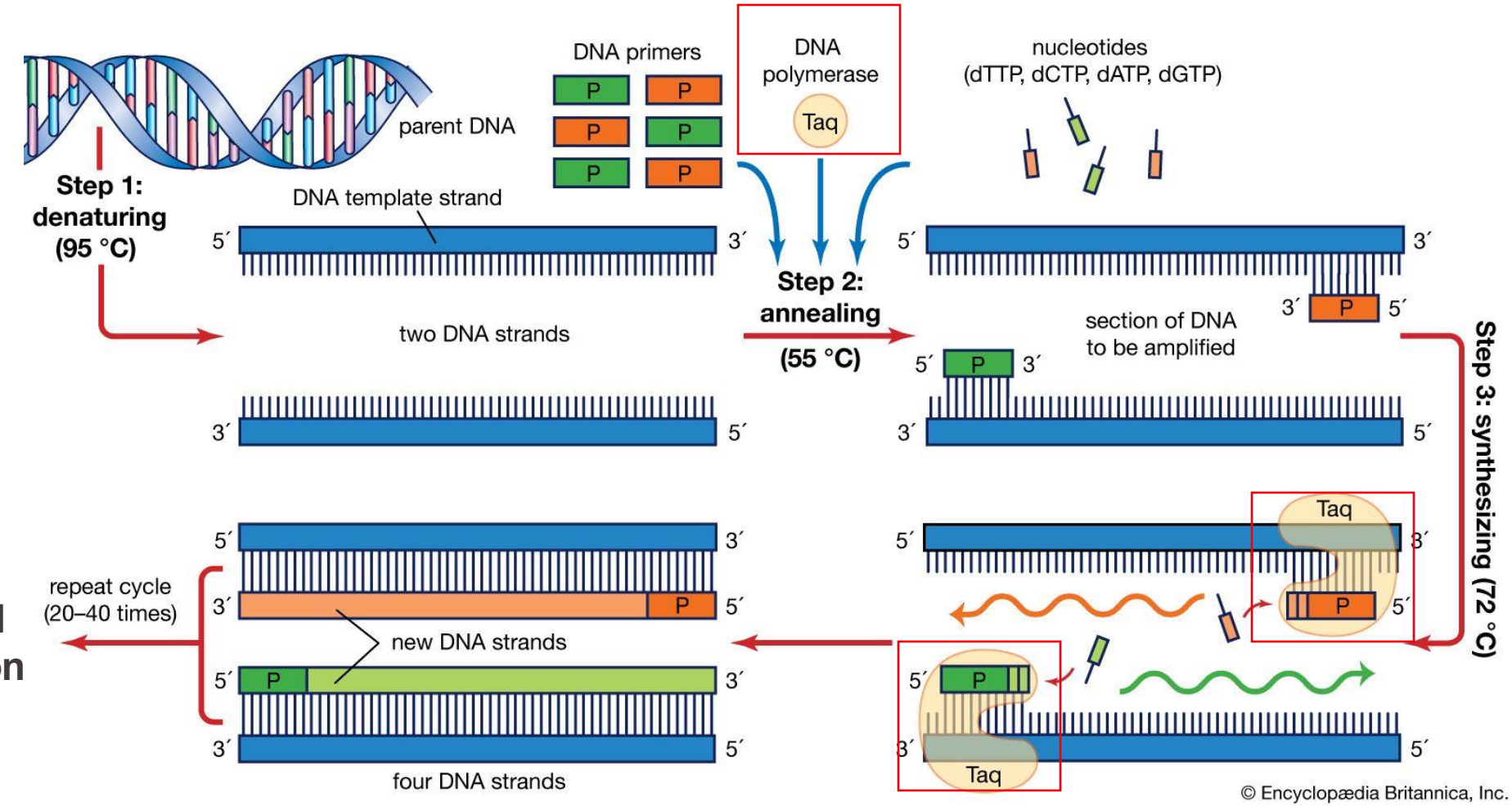

Polymerase builds RNA strand complementary to the tempate DNA strand using nucleotides (***In vitro* transcription**)

# Polymerase chain reaction (PCR)

- Enzymatic system for DNA production based on template (parent) DNA
- Watson-Crick pairing is used to generate copies of complementary DNA strands



Kary Banks Mullis
(1944-2019)

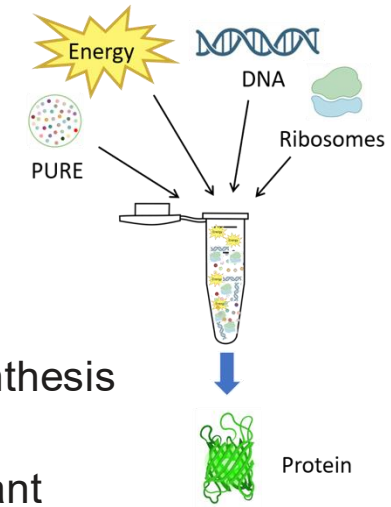




**Exponential amplification**

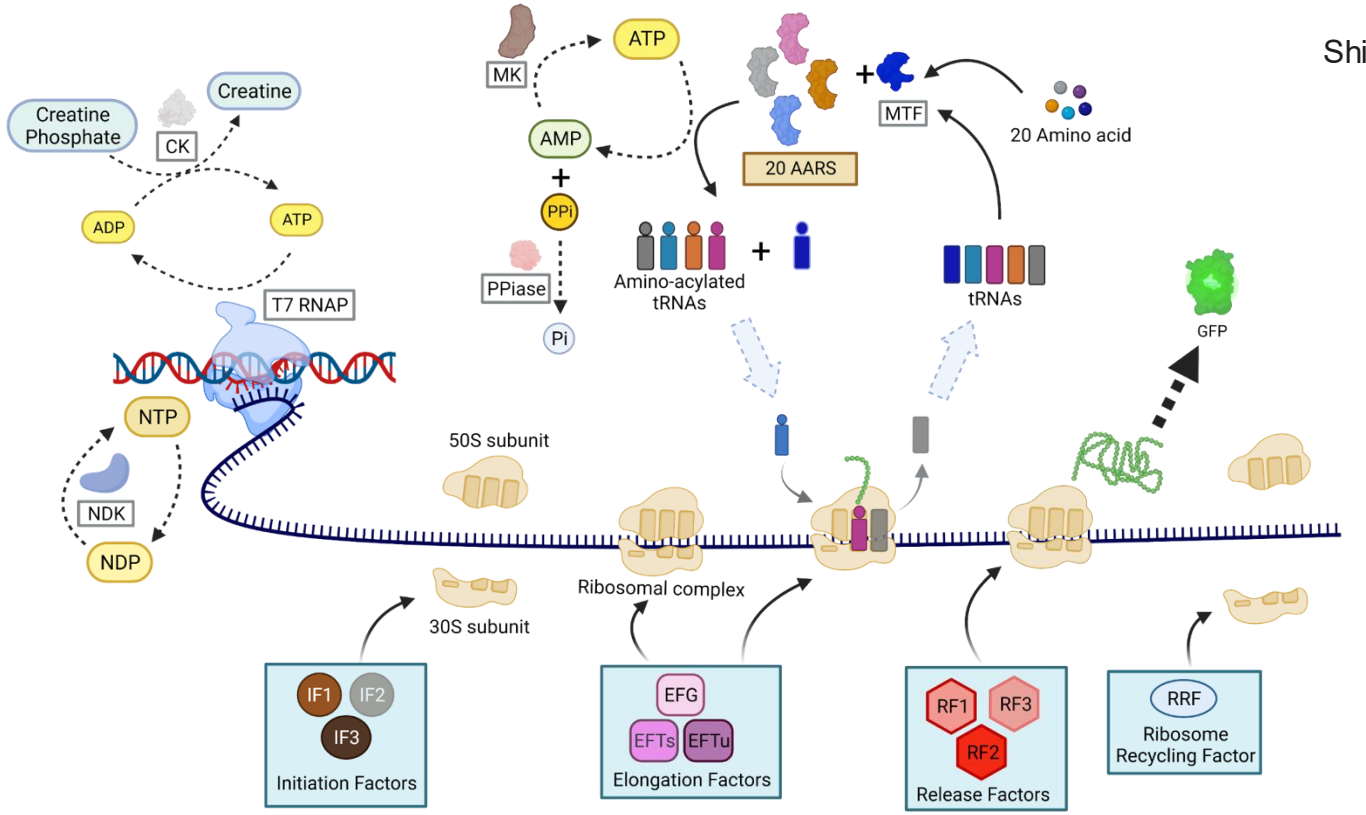
~$10^9$ copies

# PURE Technology for Protein Synthesis

- Protein synthesis is challenged by the need for template mRNA as well as loaded tRNA molecules
- The mix comprises all the building blocks and enzymes needed for **transcription** and **translation**




PURE =
Protein synthesis
Using
Recombinant
Elements




Shimizu Y et al. 2001

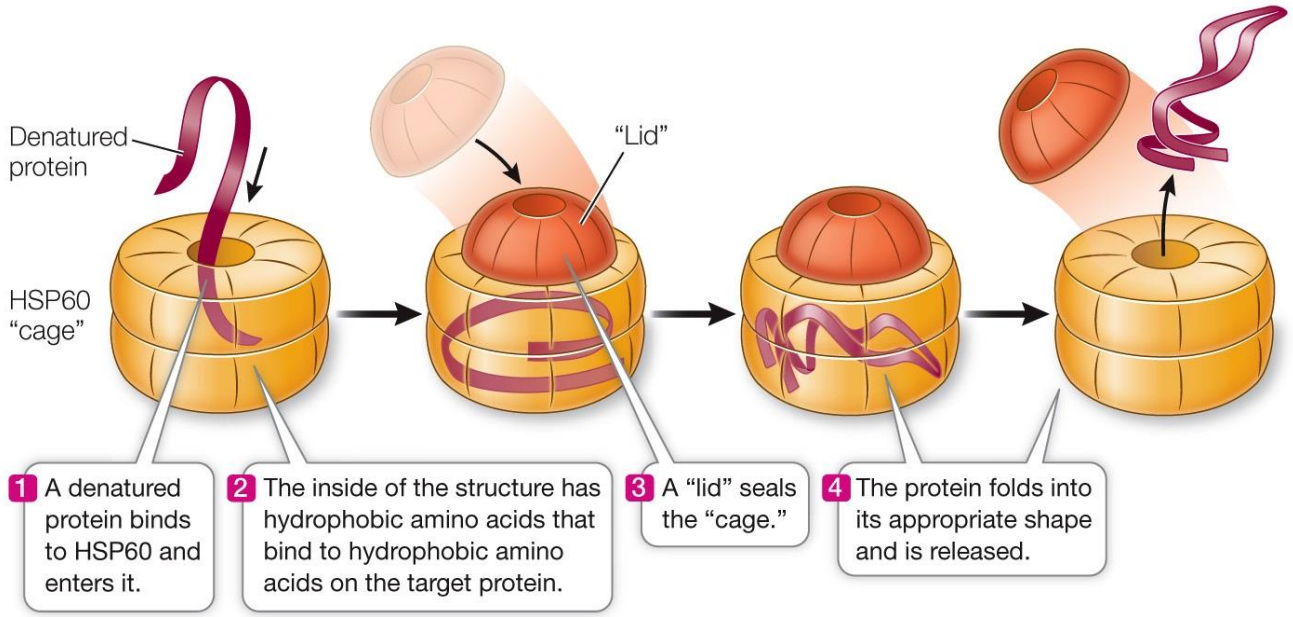
DNA -> RNA -> Protein

- Very useful for production of **toxic proteins and $^{13}C$ and $^{15}N$ isotope labeling for NMR**

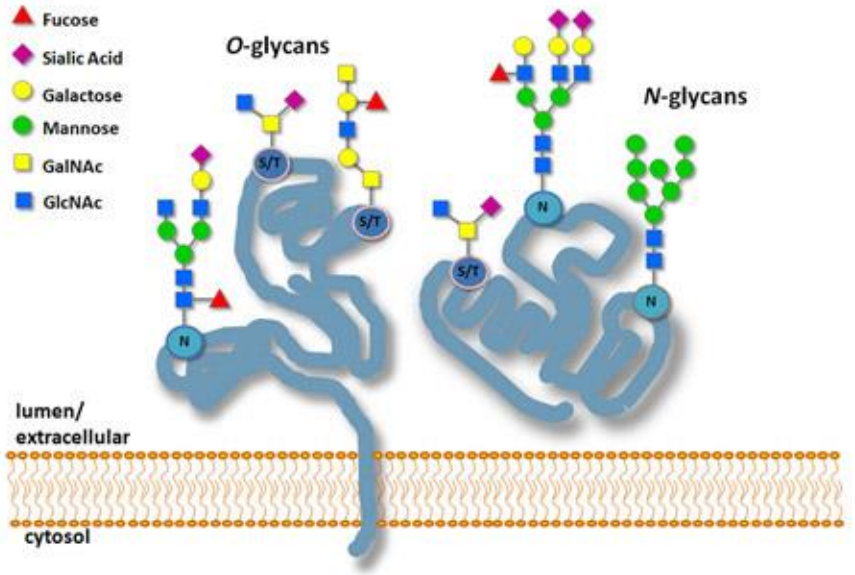
# Pros and Cons of enzymatic biosynthesis

• Enzymatic biosynthesis approaches are versatile (i.e., many enzymes exist and can be applied towards different purpose) and significantly faster compared to chemical methods (e.g., DNA polymerase copies up to 700 base pairs per second)

• However, application to protein synthesis is relatively limited due to the lack of cellular proteins that assist with protein folding (**chaperones**) and post-translational modifications (**e.g., glycosylation**)




**Chaperones** help newly synthesized proteins to fold into their functional states



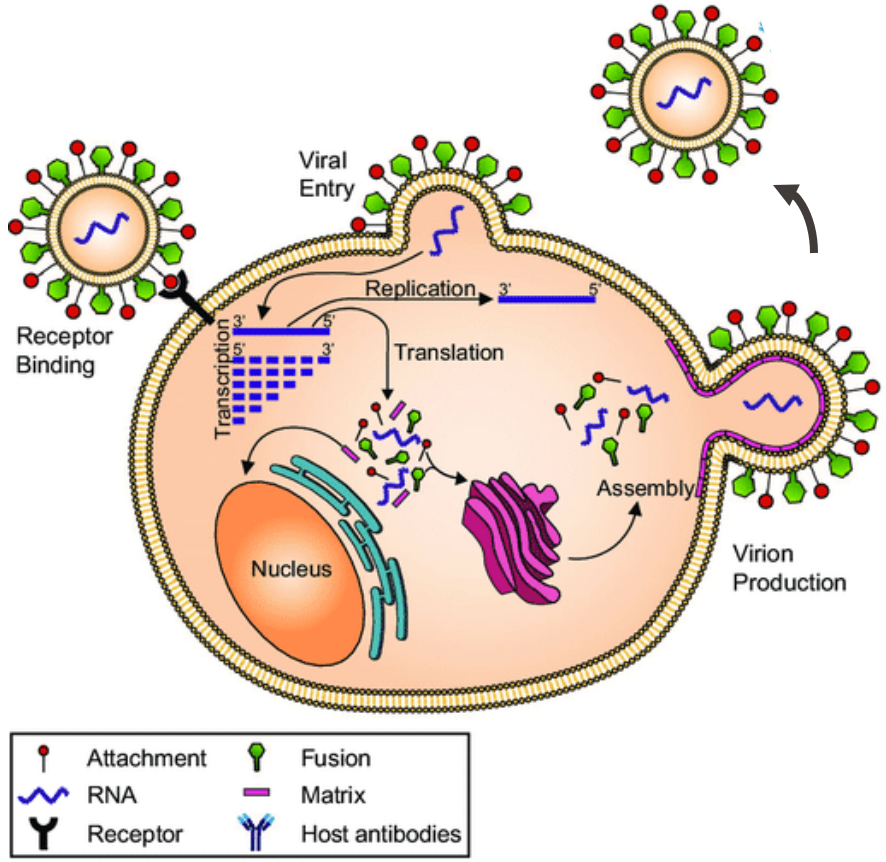

**Glycosylation** is attachment of oligosaccharides onto protein

# Cell-based approaches for biomolecule synthesis

• Biomolecule synthesis is performed using living cells, typically transformed/engineered to produce **high quantity** of the biomolecule of interest.

• The biggest benefit comes from using (hijacking) existing cell machinery for synthesis, folding, PTMs and other modifications necessary to produce functional biomolecule.

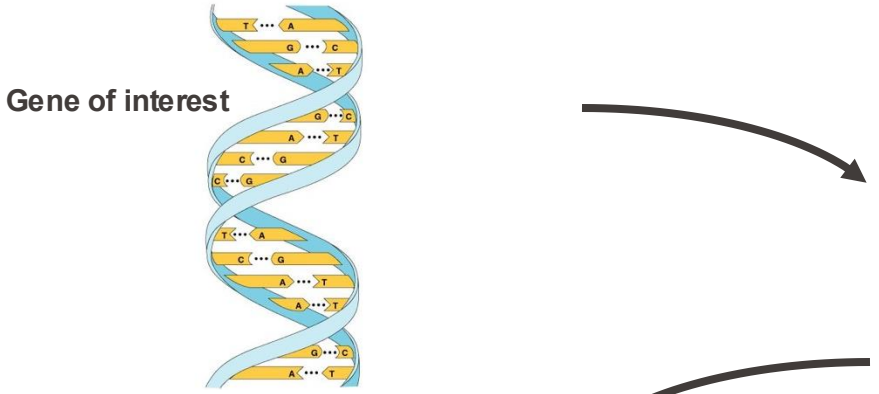
• A few examples:
  - DNA production at high quantities in bacterial cells
  - Synthesis of complex carbohydrates with specific branching points in mammalian cells
  - Production of proteins based on DNA sequence
  - Synthesis of modified, non-native lipids
  - Virus and vaccine manufacturing



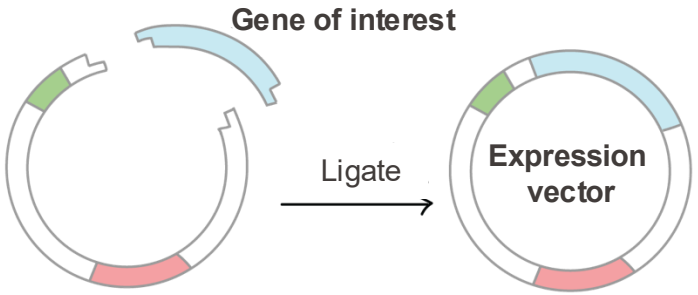

**Virus** = Nucleic Acids + (Glyco)proteins + Lipids

# Recombinant production of proteins

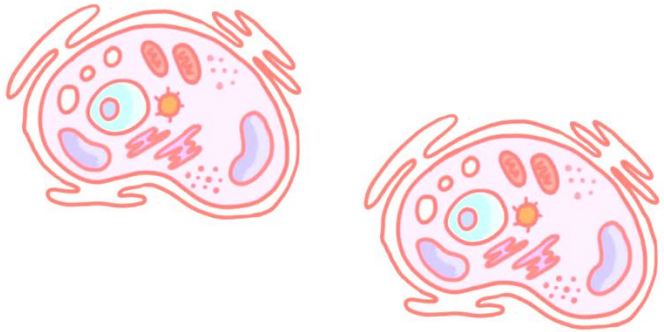
## 1) DNA encoding the protein of interest




## 2) Incorporate into an expression vector




## 3) Transduce the vector into host cells



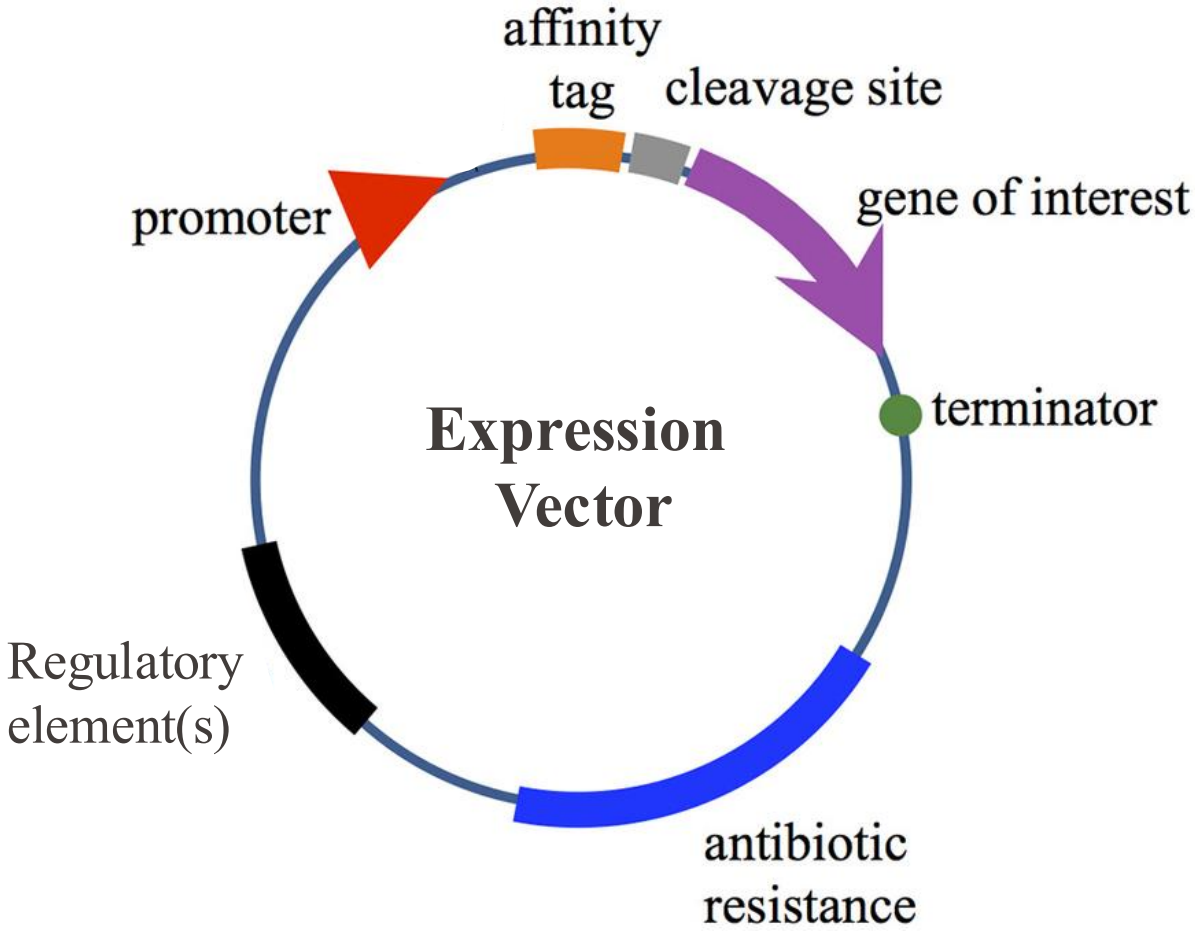
## 4) Cell growth in a suitable environment

# Expression vector components

• Expression vector is based on circular DNA that has the necessary genetic elements (**promoters**) to assure robust transcription of the gene of interest, thereby creating lots of mRNA for translation.

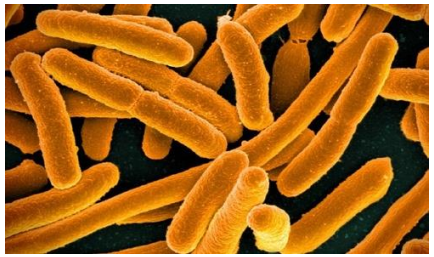
• Additional elements include **antibiotic resistance genes** (e.g., AmpR) for selection of cells that have taken up the vector and **regulatory elements** to (e.g., Lac) to switch on/off the transcription.

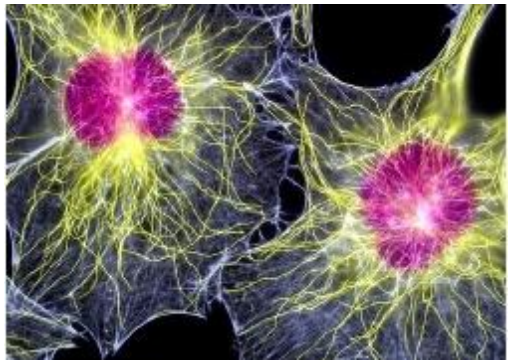
• Additional sequence can be added to the gene of interest to encode a short peptide extension on the protein that can be used for purification (i.e., **affinity tags**)



# Recombinant Protein Production Hosts

- Microorganisms
  - ***E. coli***
  - ***Pichia pastoris* (Yeast)**
  - ***Saccharomyces cerevisiae* (Yeast)**
- Cultivated animal cells
  - **Mammalian cells (e.g., HeLa, CHO)**
  - **Insect cells (e.g., SF9)**
- Less common
  - **Transgenic animals**
  - **Cultivated plant cells**



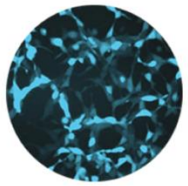



• Cell lines have typically been engineered to produce high quantity of nucleic acids, proteins, carbohydrates or any other biomolecule of interest.

# When to use which cell line?

- ***E. coli*:** Small to medium, single domain, no post-translational modifications (PTMs)
- ***Yeast*:** Secreted (extracellular) proteins, antibodies, simpler multi-domain proteins
- **Insect cells:** Larger, more complex multi-domain proteins, may contain some PTMs
- **Mammalian cells:** Larger, more complex multi-domain and membrane proteins with PTMs



| | Bacterial Expression System | Yeast Expression System | Insect Expression System | Mammalian Expression System |
|---|---|---|---|---|
| Speed | ★★★★ | ★★★ | ★★ | ★ |
| Yield | ★★★ | ★★★★ | ★★ | ★ |
| PTM (relative to human) | ✘ | ★★ | ★★★ | ★★★★ |
| Cost | ★★★★ | ★★★ | ★★ | ★ |
| Application | Small to medium, Simple single domain proteins | Secreted Protein, Disulfide-bonded protein, Glycosylated protein | Larger, more complex multi-domain proteins | Complex multi-domain and membrane proteins |

- Always consider *E. coli* first since it is the cheapest, fastest and most resilient method.
- However, proteins often need to be produced in cells that resemble their origin tissue
- Viruses are almost exclusively produced in cells where they naturally grow

# Cells use the expression vector to produce protein

• Following the transduction with the expression vector each cell line is placed in a suitable **medium rich with nutrients** to assure optimal growth and translation of protein of interest

• The environment is also adjusted to **maintain temperature, humidity and atmospheric composition**


Usually ~mL - L volumes

At the start

After ~18 hours

For any population with doubling time d, the exponential growth is approximated as:

$$N_{(t)} = N_0 \bullet 2^{t/d}$$

- $N_{(t)}$ is the cell count at time t
- $N_0$ is the initial cell count
- **d** is the doubling time
- **t** is the elapsed time

• *E. coli* divide (double) every 20-30mins and reach max confluency in less than 24 hours

• Mammalian cells double every ~18-32 hours depending on the cell line (need longer time)

# Cell bioreactors in pharmaceutical industry

- Industrial production of biomolecules requires scales that are in the ~ $10^2$ - $10^5$ L range

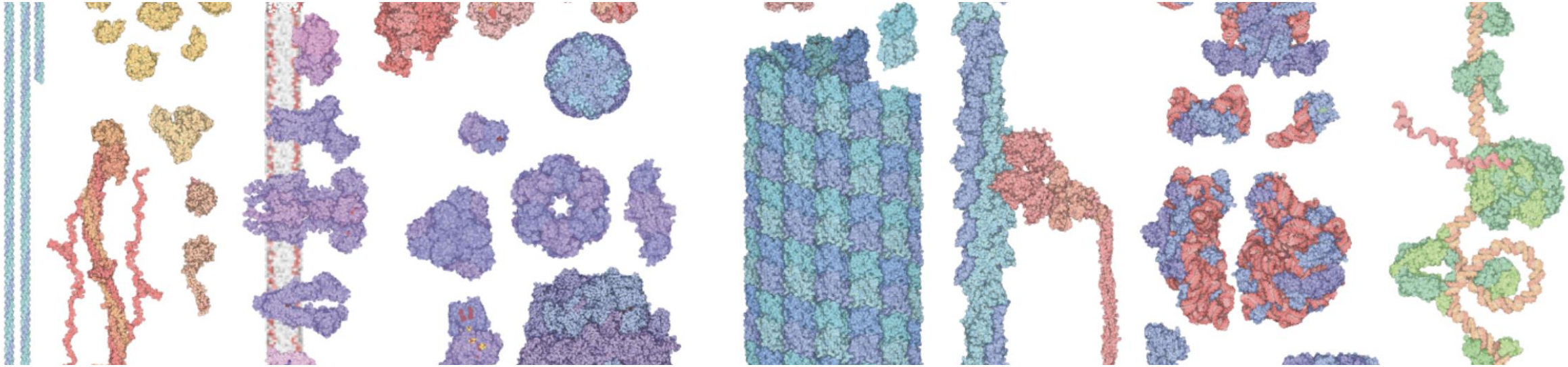

Roche

Sanofi

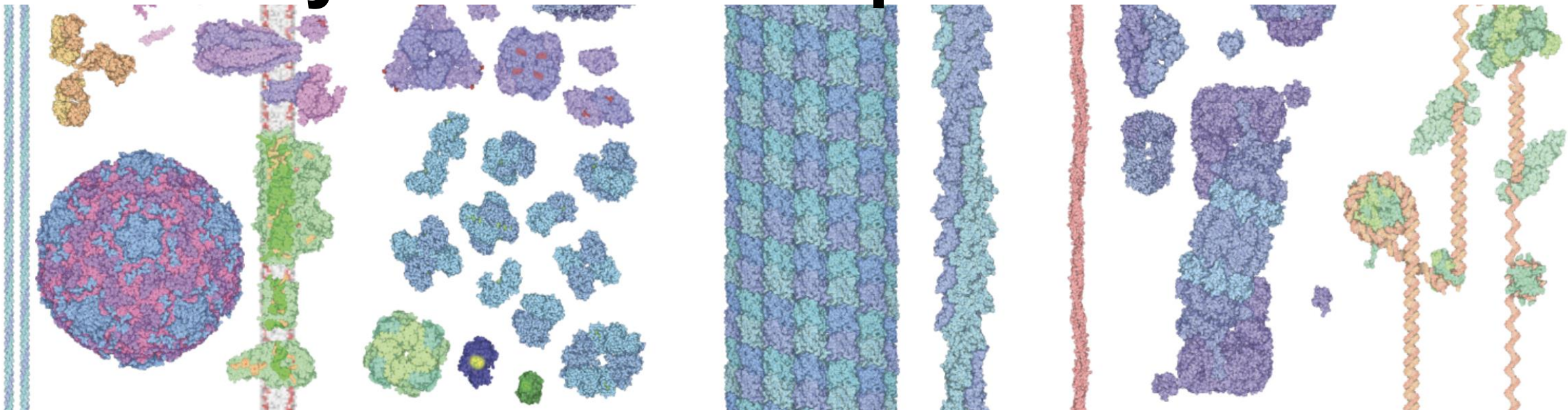
Lonza

- Additional safety measures need to be implemented if making vaccines using live pathogens

# Problem: Cells have many biomolecular components



# How do you isolate the protein of interest?



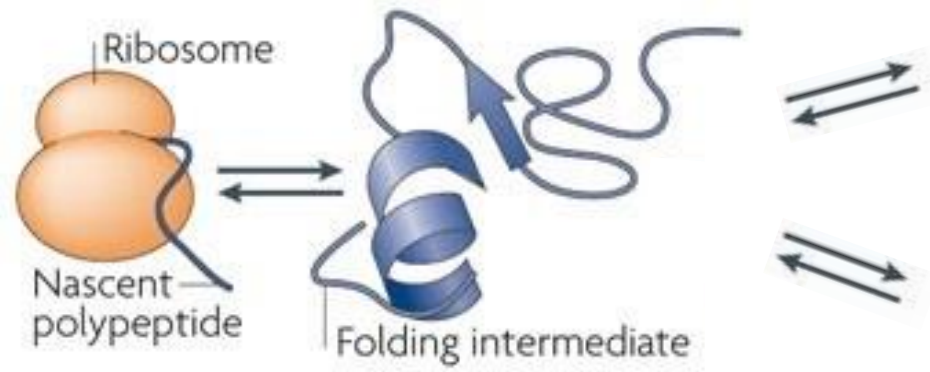
Biomolecule Production

**Biomolecule Purification**

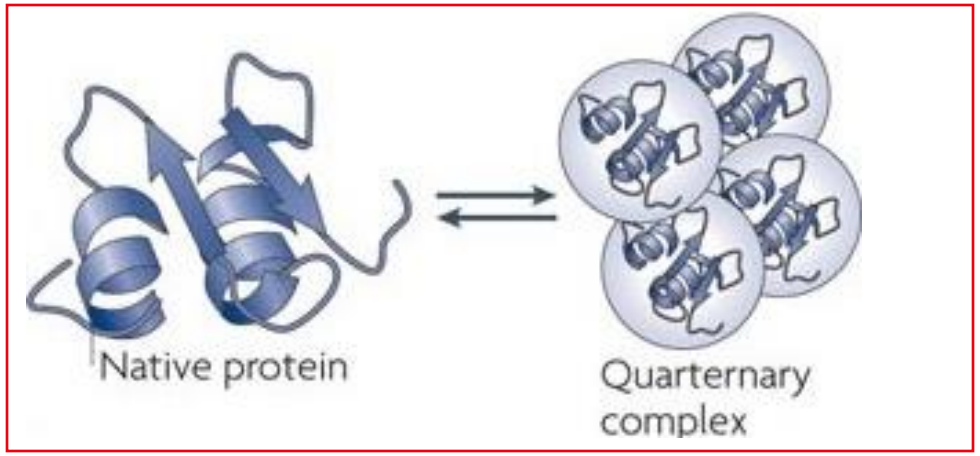
Biomolecule characterization

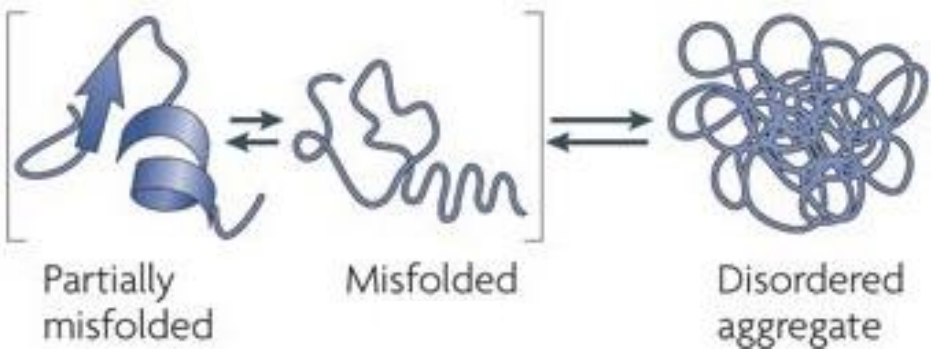
# What are the goals of biomolecule purification?

- Specific applications and needs can vary, but generally speaking the goals are:
  - Obtaining **~mg quantities** of the specific molecule
  - It needs to be **pure of the remaining cell material**
  - **Natively-folded** (=in active state)
  - Native **oligomeric state** (monomer, dimer, or other)
  - **Stable** at high concentrations (no aggregation)
  - Stable over time (days or even weeks)




Preferred outcome




- Protein expression can lead to a mix of folded and missfolded molecular species

# Typical steps in protein production and purification

Design expression vector

↓

Express protein in cell culture

↓

Collect cells by centrifugation (or medium if protein is secreted)

↓

Cell lysis to release proteins (not needed if secreted)

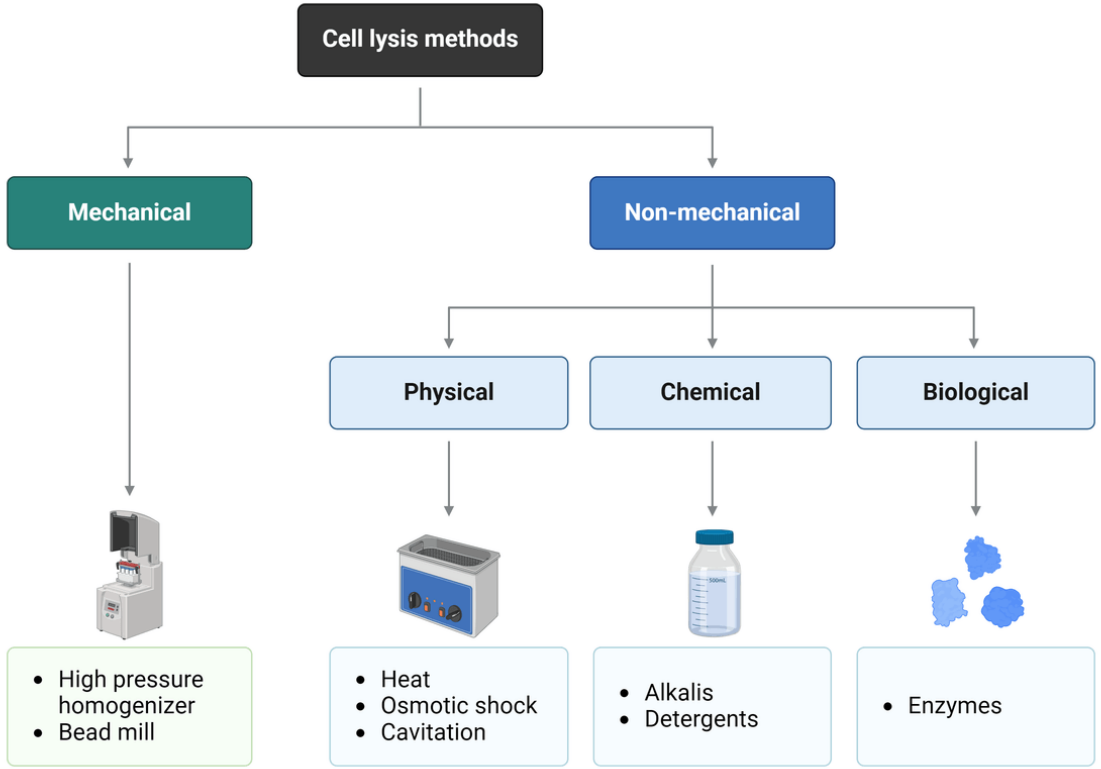
↓

Purify protein using liquid chromatography

↓

Assess quantity and purity (Biophysical methods)

- If the protein is expressed intracellularly (cytoplasm or specific organelle) the cells need to be lysed to release the proteins
- Cell lysis can be performed using several methods:




- If the protein is expressed extracellularly (into the medium) then the cells can be discarded, and medium should be collected

# Buffers - Liquid medium for biomolecule handling

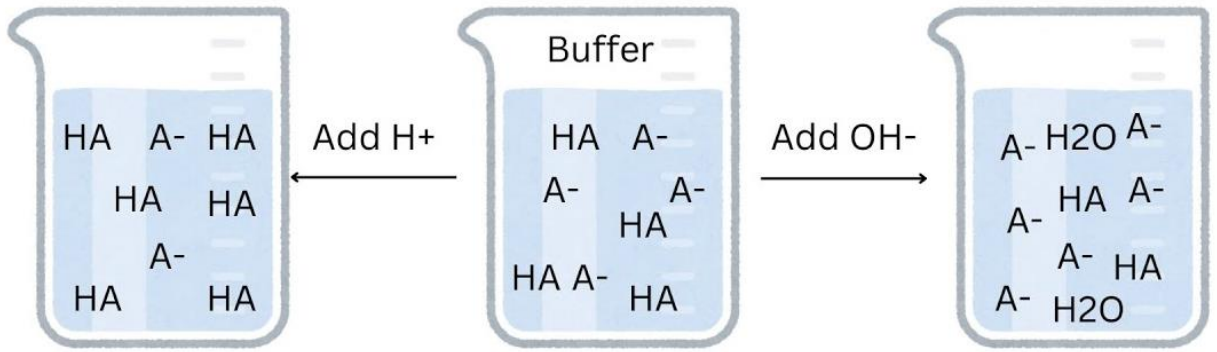
• A buffer is a solution that can resist pH change upon the addition of an acidic or basic components. It is able to neutralize small amounts of added acid or base, thus **maintaining the pH of the solution**.

Acid Base

$$HA \rightleftarrows A^- + H^+$$

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

Buffer components bind $H^+$ and $OH^-$ and maintain the pH




• Typically based on weak acids and bases with pKa value in the 3-10 range (e.g., phosphate, tris-HCl, MES, carbonate, acetate, citrate)

• **The buffering capacity is highest in pH ranges that are within 1 unit from the pKa**

# Buffers - Typical components

• In addition to the pH-regulating buffer components, the solutions can contain other reagents tailored to the molecule of interest

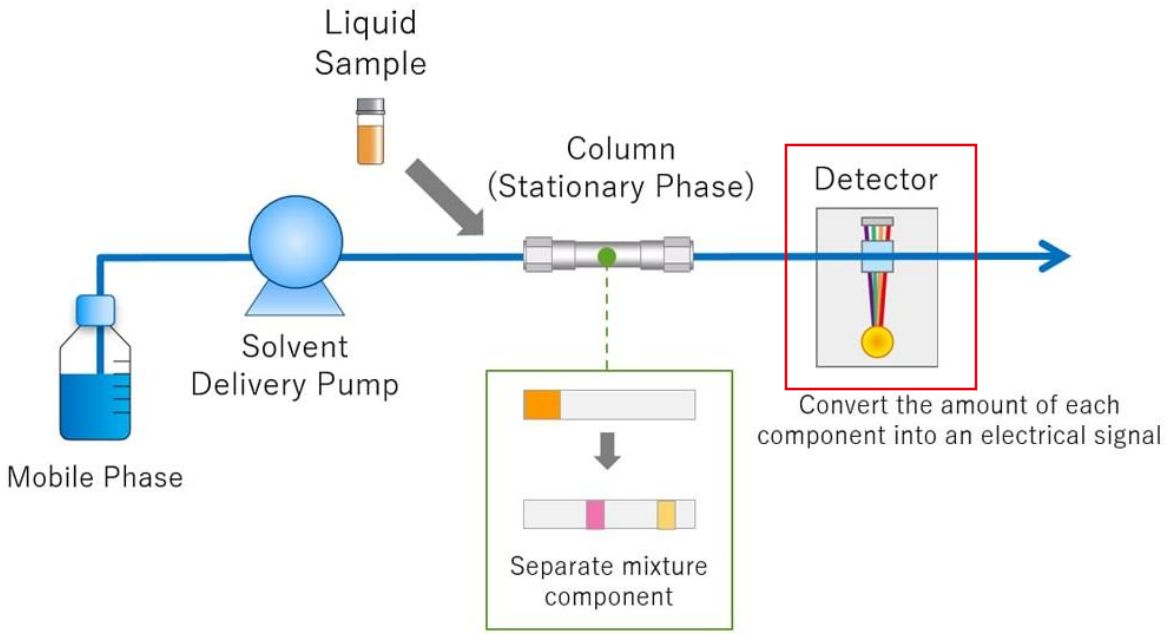
| Reagent Group | Typical Examples | Main Purpose / Function | Commonly Used For |
|---|---|---|---|
| **Buffering agents** | Phosphate, Tris, HEPES, MOPS, citrate | Maintain a **stable pH** environment; prevent conformational or chemical changes in biomolecules. | Proteins, nucleic acids, carbohydrates, lipids |
| **Salts** | NaCl, KCl, NaOAc, $(NH_4)_2SO_4$, $MgCl_2$ | Adjust **ionic strength**; support electrostatic stability, nucleic acid hybridization, and macromolecular solubility. | Proteins, nucleic acids, polysaccharides |
| **Reducing agents** | DTT, β-mercaptoethanol, TCEP, cysteine | Maintain **reducing conditions**, prevent disulfide crosslinks or oxidation of thiols and redox-sensitive groups. | Proteins, some redox-active enzymes, coenzymes |
| **Detergents / surfactants** | Triton X-100, Tween-20, CHAPS, SDS, NP-40 | Disrupt or solubilize **membranes**, emulsify lipids, prevent aggregation, reduce surface tension. | Membrane proteins, lipids, lipoproteins |
| **Cofactors / metal ions** | $Mg^{2+}$, $Mn^{2+}$, $Zn^{2+}$, $Ca^{2+}$, $Fe^{2+}$ | Essential for **enzyme catalysis, nucleic acid folding, or complex assembly**. | Enzymes, DNA/RNA, ribozymes |
| **Organic co-solvents** | Ethanol, methanol, DMSO, acetonitrile | Solubilize hydrophobic molecules (lipids, steroids, pigments); adjust dielectric constant. | Lipids, small molecules, metabolites |
| **Chelating agents** | EDTA, EGTA, citrate, DTPA | Bind **metal ions** (e.g., $Mg^{2+}$, $Ca^{2+}$, $Fe^{2+}$) to inhibit metal-dependent enzymes or control metal availability for nucleic acids or cofactors. | Proteins, nucleic acids |
| **Stabilizers / osmolytes** | Glycerol, sucrose, trehalose, sorbitol, PEG | Stabilize macromolecular structure; protect during storage, drying, or temperature changes. | Proteins, enzymes, nucleic acids, liposomes |
| **Protease / nuclease inhibitors** | PMSF, leupeptin, EDTA, RNaseOUT, DEPC | Prevent **enzymatic degradation** of biological macromolecules. | Proteins, RNA, DNA |
| **Chaotropic agents / denaturants** | Urea, guanidinium chloride, formamide | Disrupt noncovalent interactions; used for unfolding, solubilization, or nucleic acid denaturation. | Proteins, nucleic acids |
| **Antioxidants** | Ascorbate, glutathione, Trolox | Protect from **oxidative degradation** (especially lipids, cofactors, pigments). | Lipids, redox enzymes, small metabolites |
| **Antimicrobials / preservatives** | Sodium azide, thimerosal, chloroform | Prevent microbial contamination during storage. | General biochemical reagents |

Phosphate-buffered saline (PBS): **10mM Phosphate (pH 7.4) + 137mM NaCl + 2.7mM KCl**

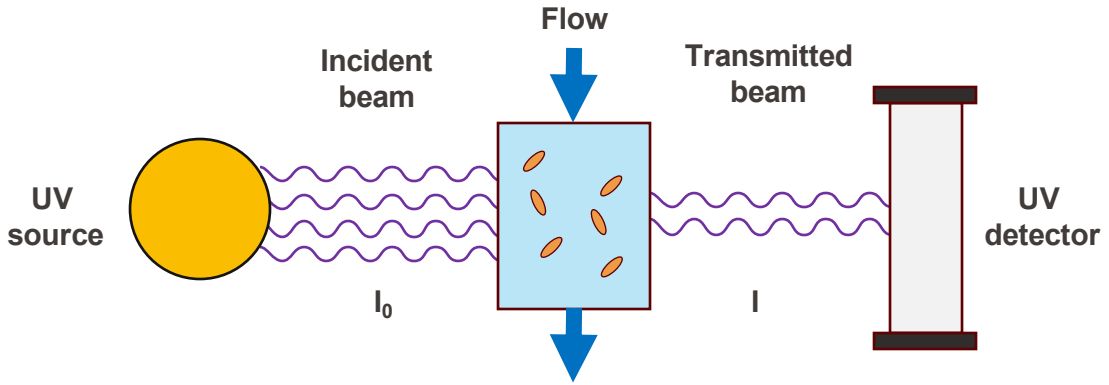
(very common buffer)

# Liquid chromatography

- Liquid chromatography is a technique used to separate a mixture of (biological) samples into its individual parts.
- This separation occurs based on the differential interactions of each sample with the **mobile** and **stationary** phases (“Columns”).



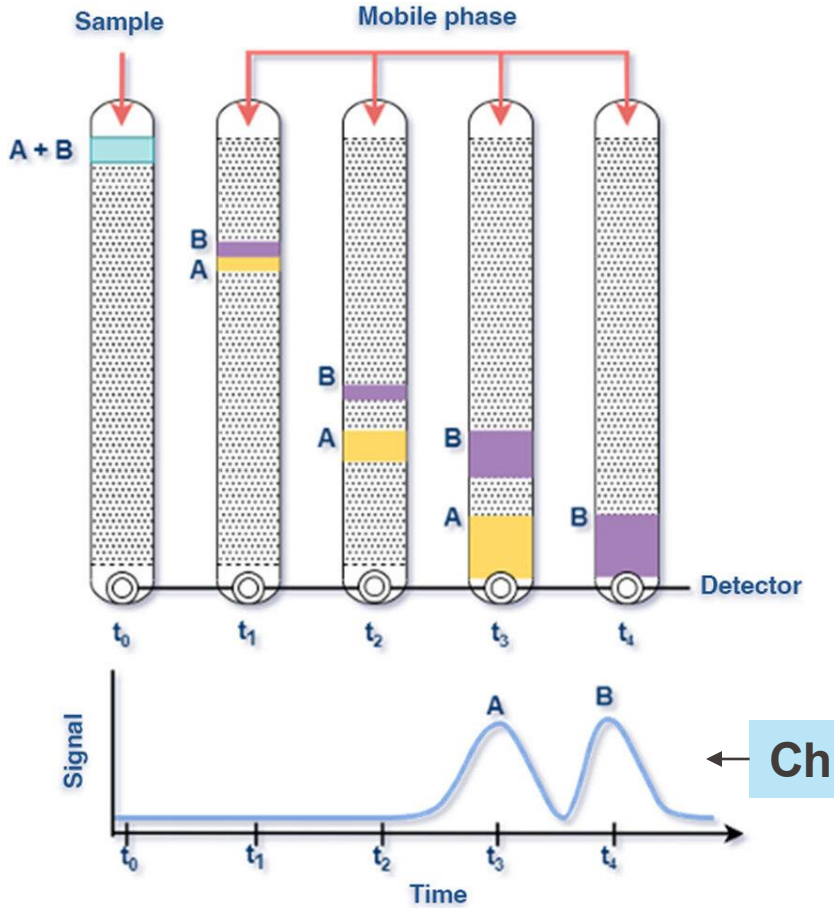

- Biomolecule passage through the column is monitored using detectors that measure:
  - **Absorbance**: Proteins and nucleic acids absorb UV
  - **Conductivity**: Useful for charged biomolecules
  - **Fluorescence**: Requires labeling with fluorescent dyes
  - **Refraction**: Useful for most biomolecules
- Detectors are typically downstream from the column and are reading post-separation content



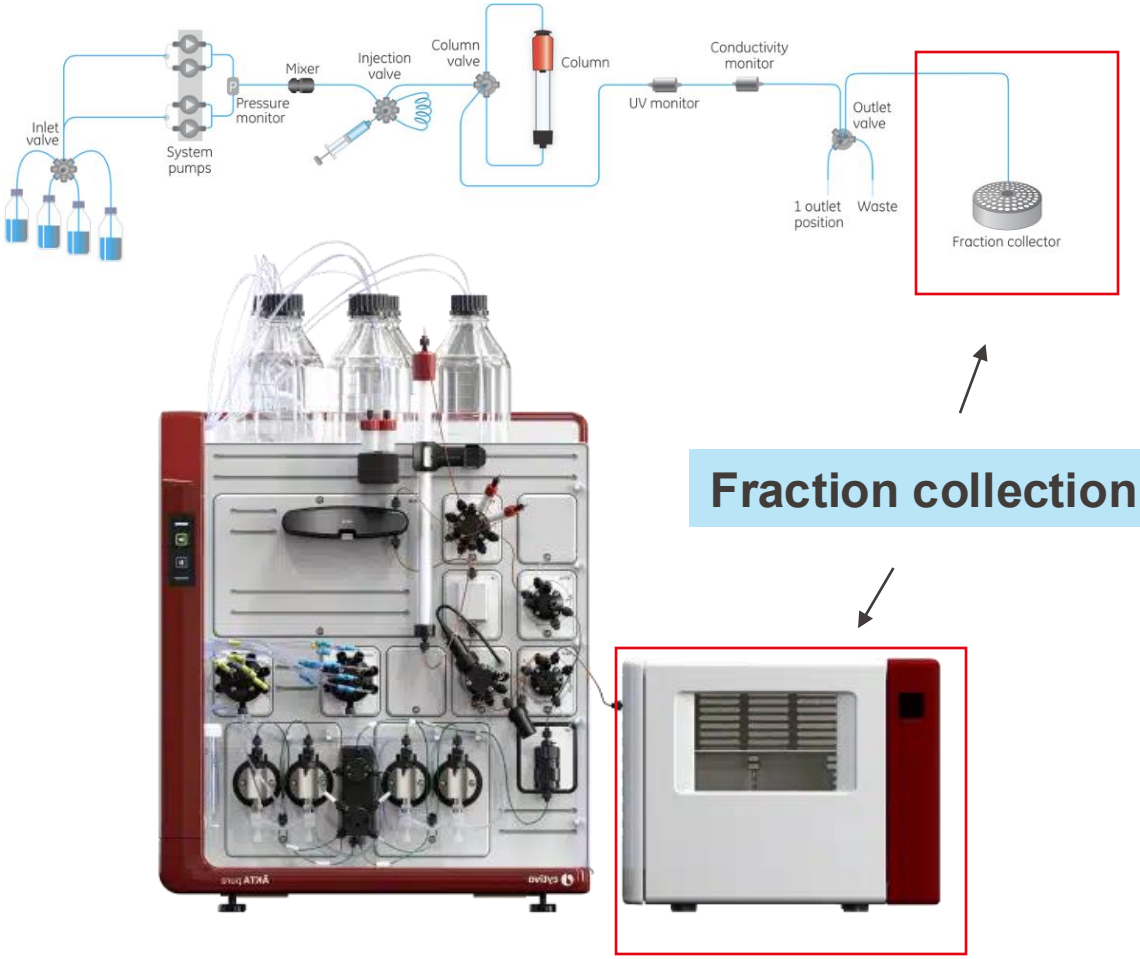

UV detection system **continuously** measures the reduction in signal intensity (**absorbance**) as the sample flows through

# Liquid chromatography

- Different biomolecules interact differently with the stationary phase leading to longer or shorter retention in the system (= different elution times [t]). This is the **separation principle**.




Chromatogram

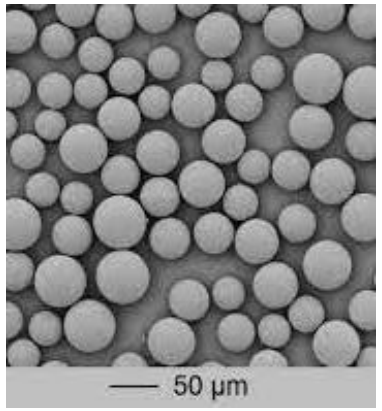



Fraction collection

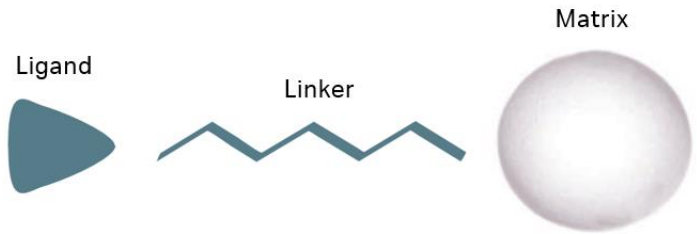
- Manual or automated system collects **temporal volume fractions** into different tubes or wells

# Stationary phases in liquid chromatography

- The stationary phase in liquid chromatography is the **immobile solid or liquid material** inside the column that interacts with analytes.
- This material is referred to as **matrix** or **resin** and it typically has the following properties:
  - Composed of **highly polymerized small gel beads** (typically ~10-100 µm in diameter)
  - Common materials are **polysaccharides** or **synthetic polymers** (need to be inert and mechanically stable)
  - Matrix is chemically derivatized with different groups (charged, polar, hydrophobic, antibody)
  - Total resin volume and column geometry also play an important role in separation




Matrix beads

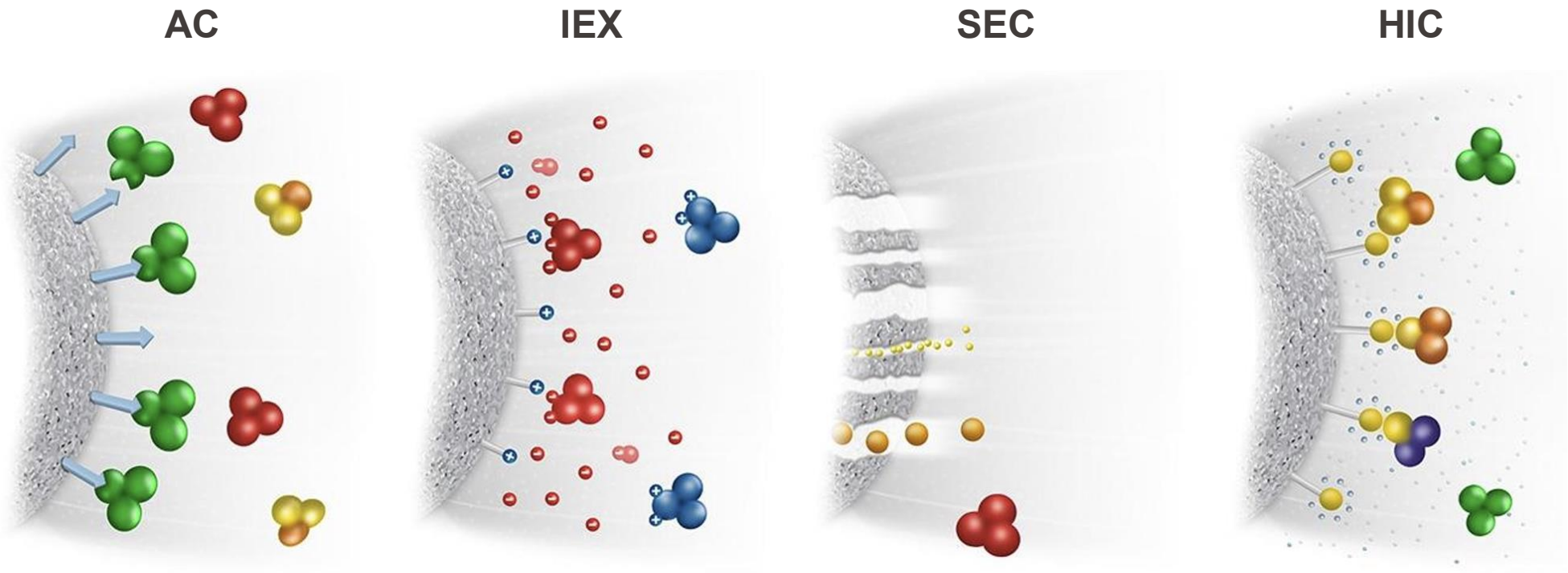



Functionalization with different ligand groups determines the application

Functionalized matrix packed into columns of different sizes

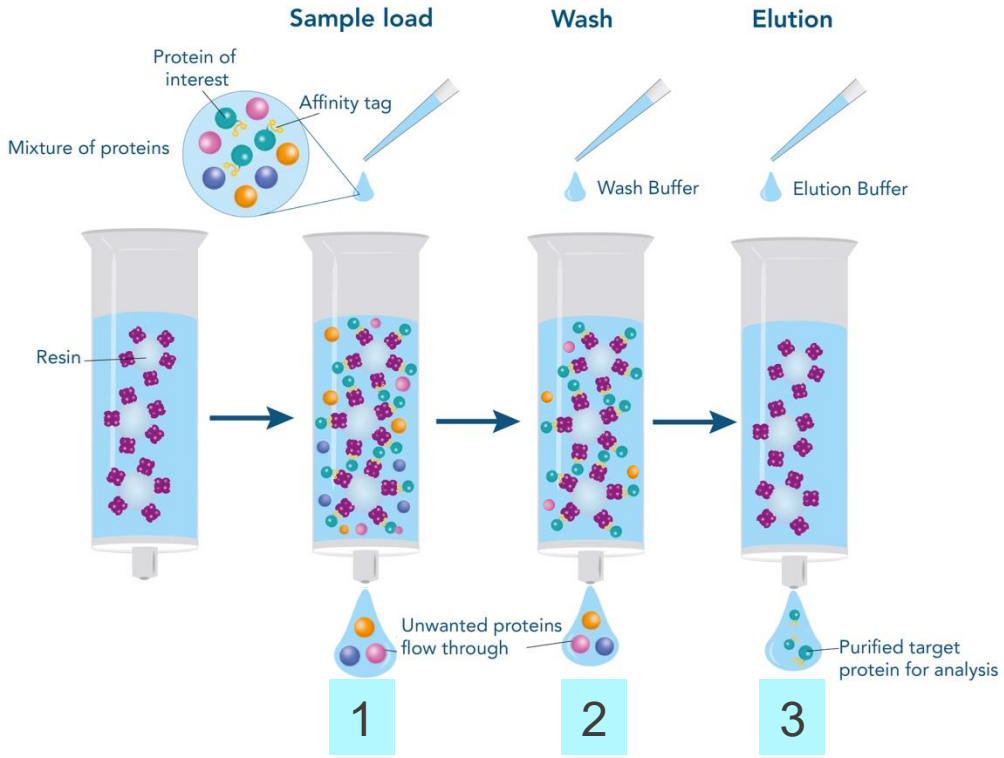
# The main types of chromatographic methods

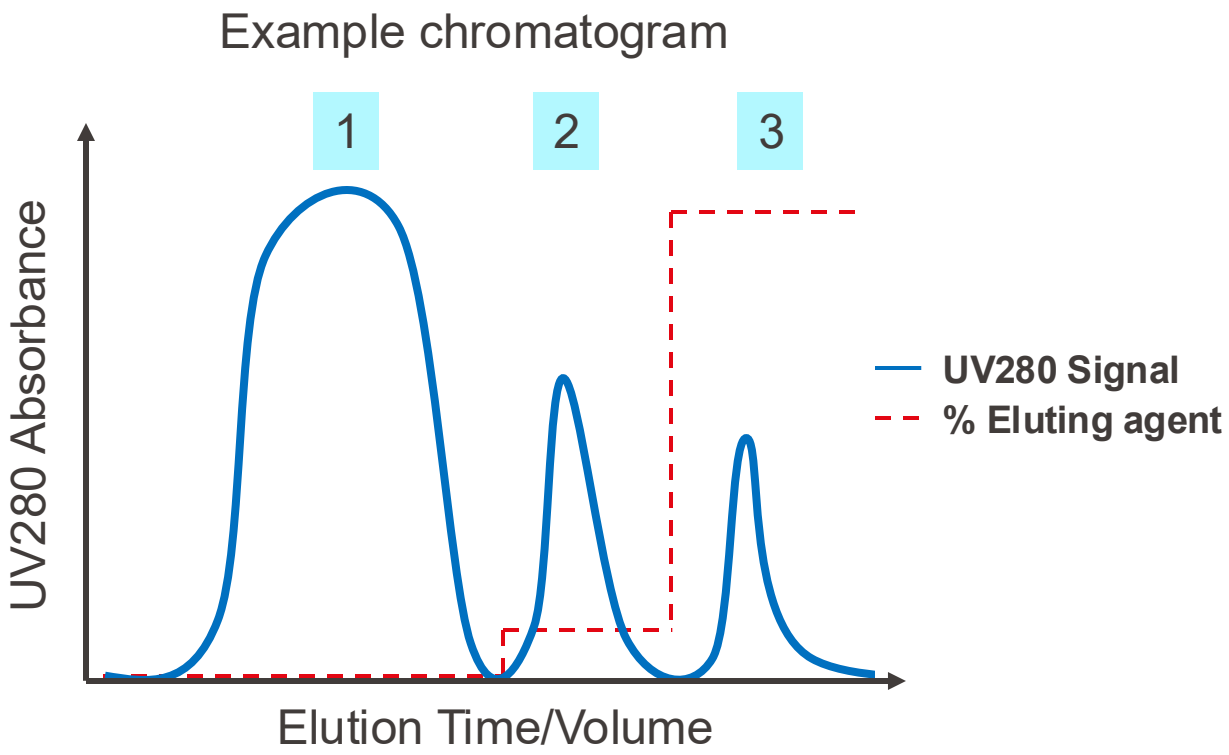
| Method | Biochemical principle | Applications |
|---|---|---|
| Ion-exchange chromatography (IEX) | Different charges of biomolecules | Protein, DNA, charged lipids |
| Size-exclusion chromatography (SEC) | Different sizes of biomolecules | Protein, DNA, lipids, sugars |
| Affinity chromatography (AC) | Binding to a chemical group | Mainly proteins |
| Hydrophobic interaction chromatography (HIC) | Differing hydrophobic properties | Lipids, sugars, proteins |



# Affinity chromatography

- Most commonly it is the first method for purification of target biomolecule from cell lysate.
- The stationary phase (matrix) is based on immobilized chemical or protein groups that selectively bind the protein of interest (via a tag), while they display minimal affinity to any other protein



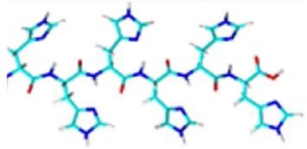



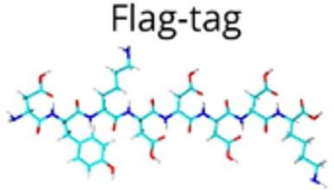

- A buffer containing low percentage of eluting agent (insufficient to detach the molecule of interest) is commonly used as a **wash** (= Step 2). This step is essential to remove weakly bound biomolecules

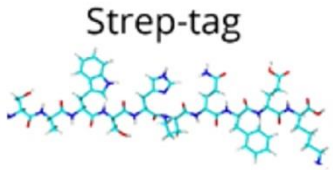
# Affinity chromatography - Some common tags

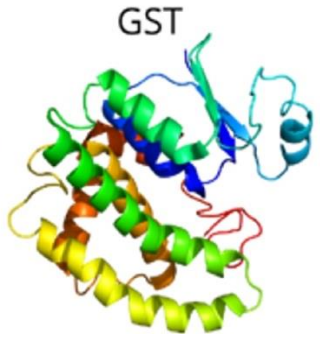
- Protein tags are added during the expression vector design and are fused in frame with the protein gene so that they can be coded into the protein sequence
- The location of the tag is typically at the N- or C-terminus of the protein
- Some common tag choices and their properties:

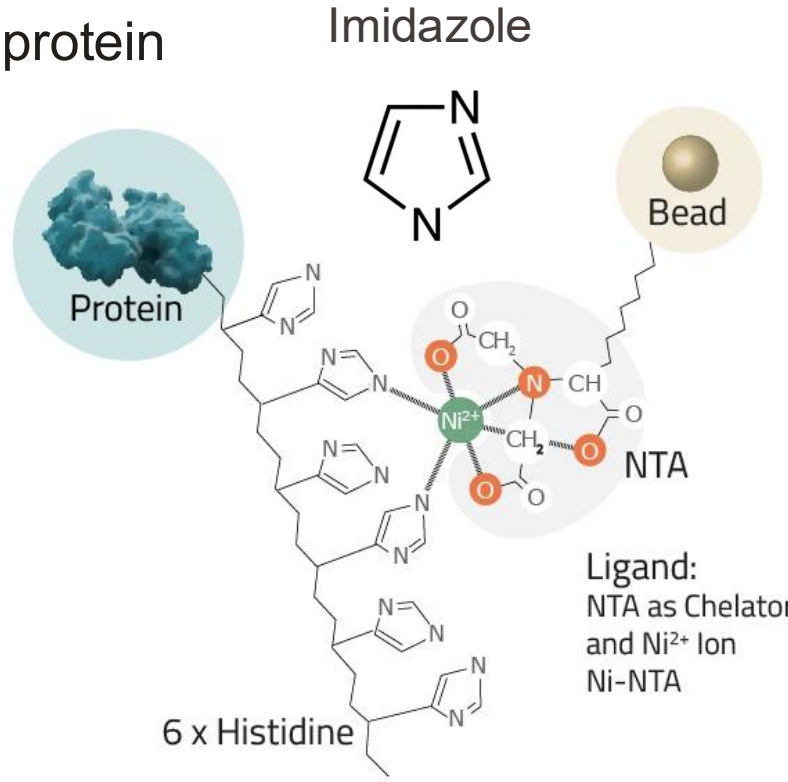
| Tag | Sequence | Resin ligand | Elution agent |
|---|---|---|---|
| His-tag | HHHHHH | Ni-NTA | 500mM Imidazole |
| Strep-tag | WSHPQFEK | Streptavidin | 10mM Biotin |
| Flag-tag | DYKDDDDKG | Anti-Flag Ab | 0.1mM Flag peptide |
| GST | Whole domain | Glutathione | 20mM Glutathione |



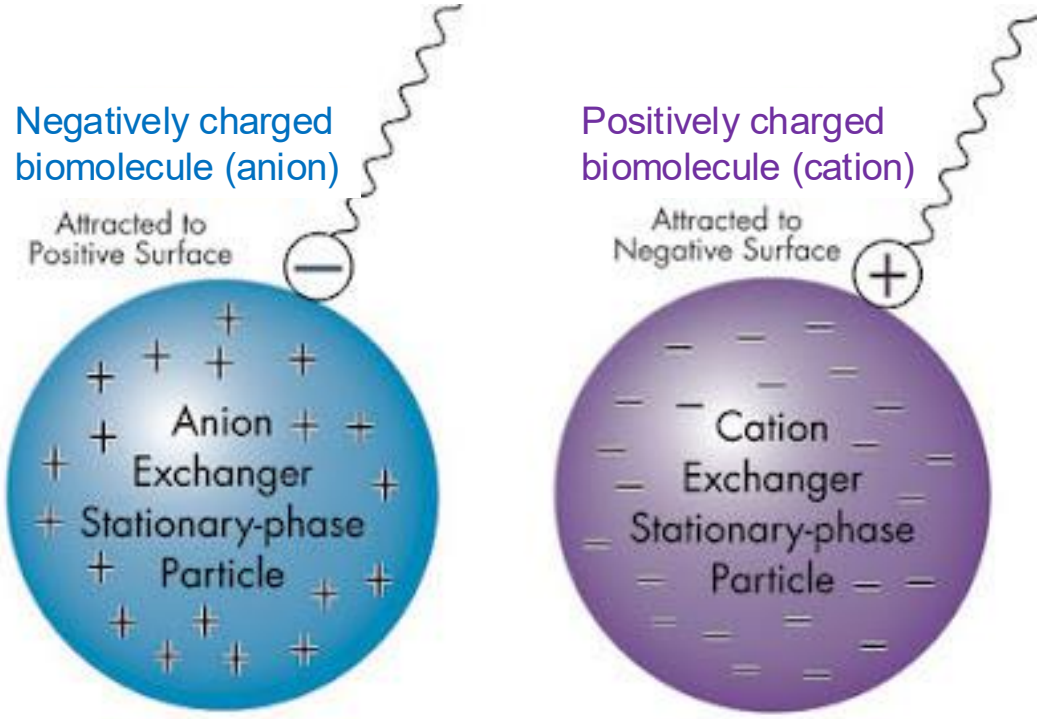




Typical Elution Buffer composition:
- 10-100mM Buffering component (e.g., Tris-HCl)
- 10-1000mM Salt (e.g., NaCl)
- Elution agent (from the table)
- Other components if/when needed

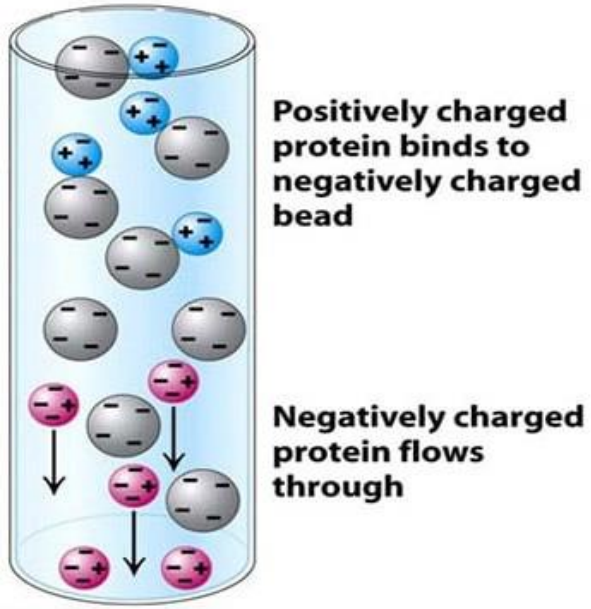
# Ion Exchange Chromatography

- Ion exchange chromatography is a **separation method based on different properties of charges and different amounts of charge** in target biomolecules

- The stationary phase (resins) comprise chemical groups that are **positively or negatively charged**




Principle of work

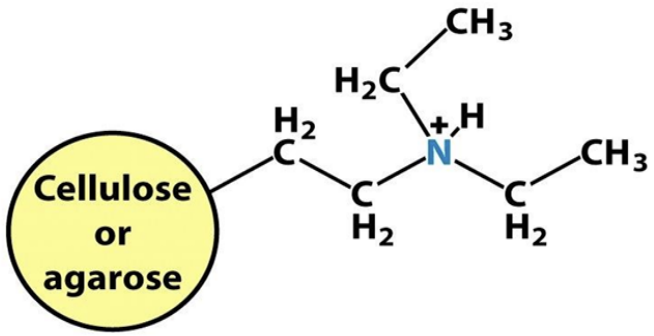


# Ion Exchange Chromatography

- Ion exchange chromatography is a **separation method based on different properties of charges and different amounts of charge** in target biomolecules

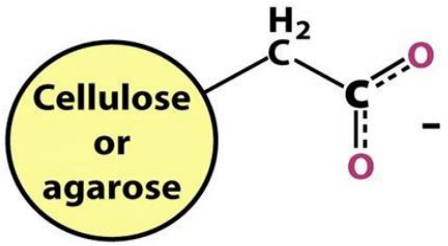
- The stationary phase (resins) comprise chemical groups that are **positively or negatively charged**

**Anion exchanger**



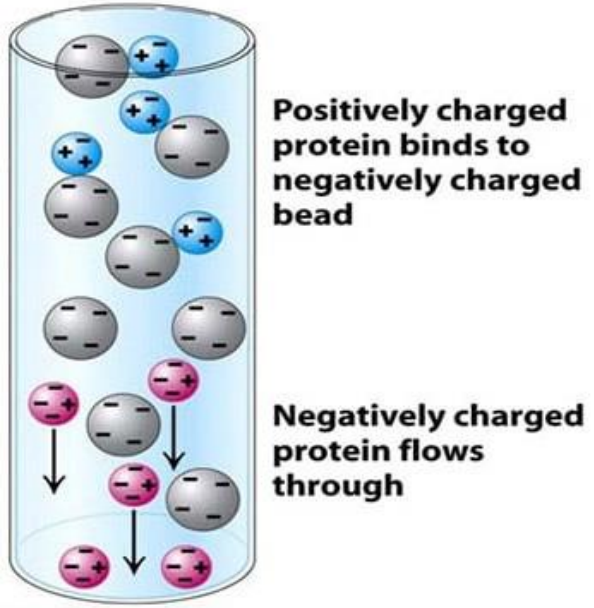

**Diethylaminoethyl (DEAE) group (protonated form)**

**Cation exchanger**




**Carboxymethyl (CM) group (ionized form)**

Principle of work




Which ion exchange resin would you use to purify DNA?

# Isoelectric point and its implications to column selection

- The separation of protein species is achieved by differing net charge at a given pH, which is based on the protonation state of the charged groups (e.g., **Lys, Arg, Asp, Glu, His, N- and C-termini**)

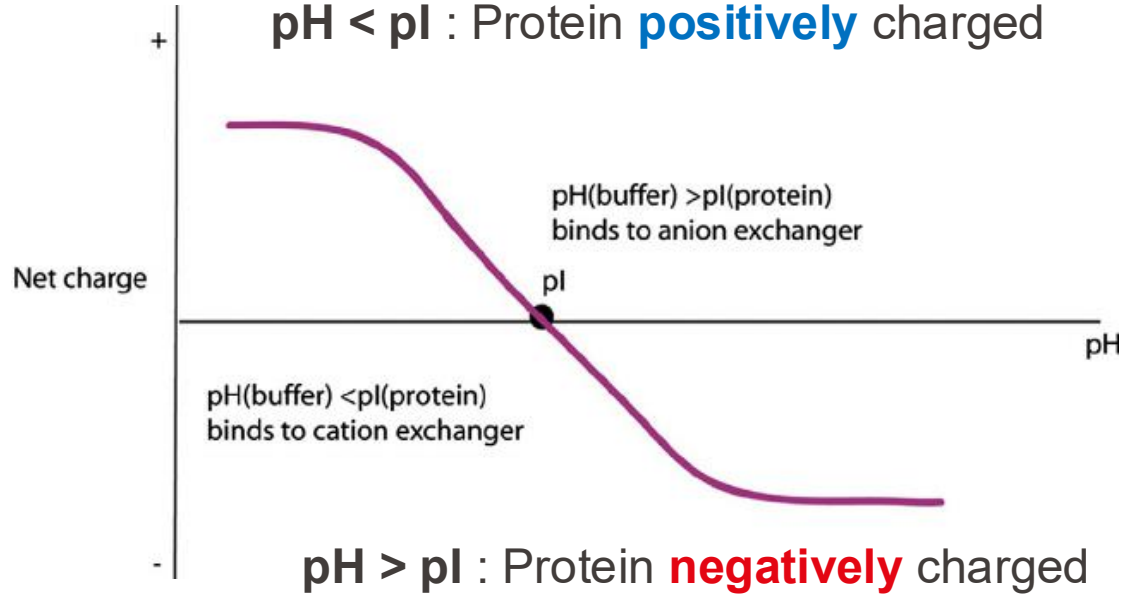
- **Isoelectric point (pI)** of a protein corresponds to the pH at which the protein has no net charge. It is the average of the two pKa values that bracket the point where the protein's net charge is zero.

$$pI = \frac{pKa_{(i)} + pKa_{(i+1)}}{2}$$

If the molecule has multiple pKa values:
1) Order them from lowest to highest and assign the pH range (bracket pKa values) between which the molecule is at neutral charge.
2) Take the two flanking pKa values (i and i+1) and average them

Selecting the optimal column for your protein

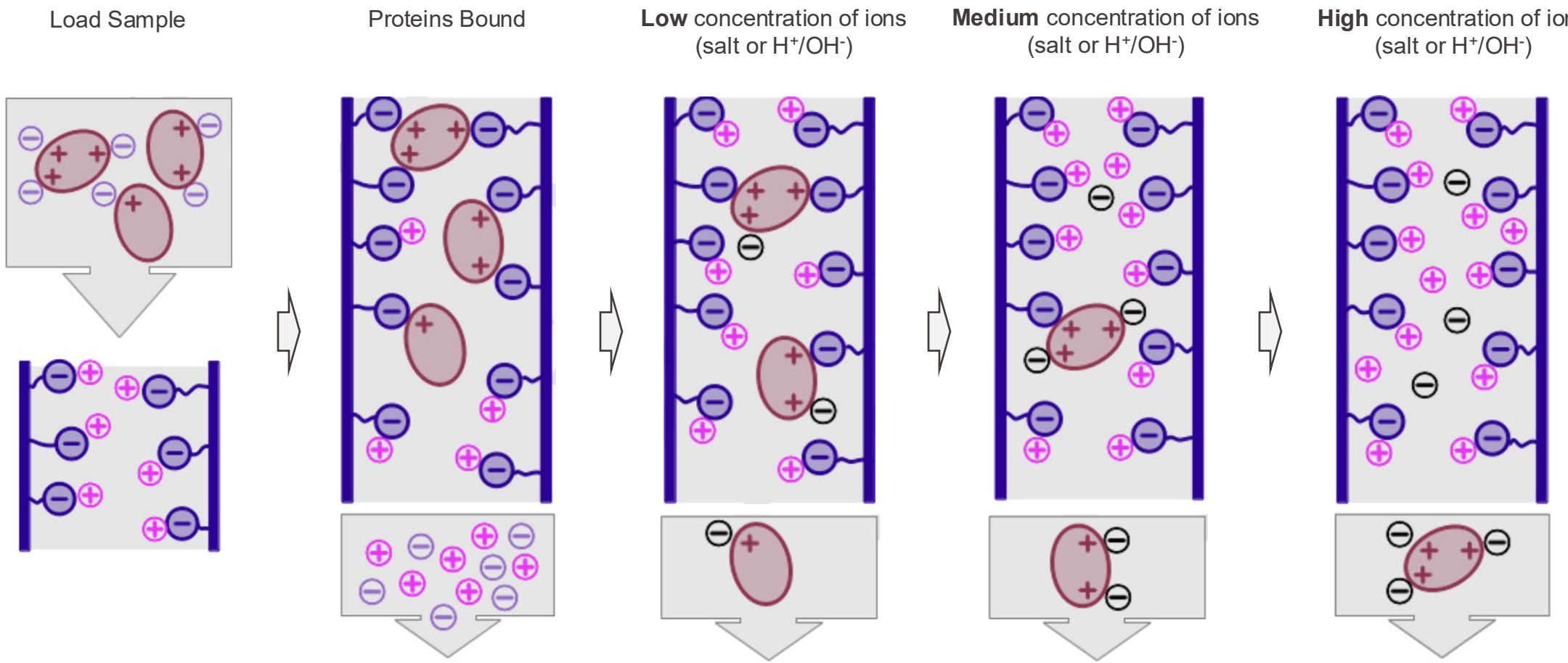
**pH < pI** : Protein **positively** charged




**pH > pI** : Protein **negatively** charged

# Ion Exchange Chromatography

- Each protein has a different amino-acid composition and **different net charge at neutral pH** which means that different proteins will interact weaker or stronger with the same ion exchange resin

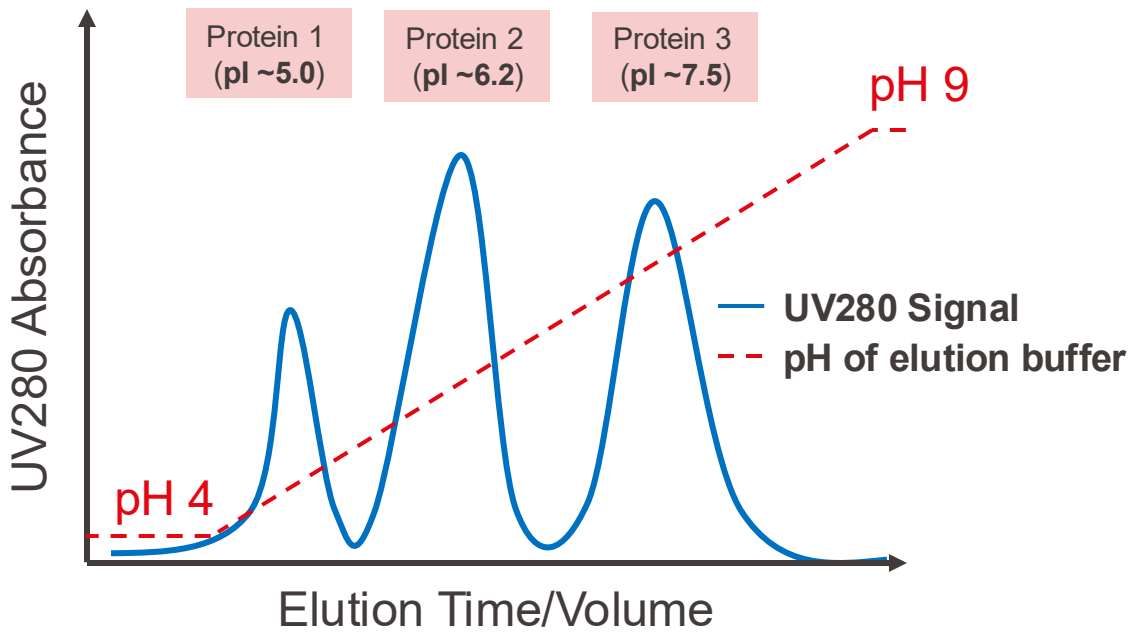



- Molecules are eluted when ion concentration is sufficient to dissociate them from the resin

# Ion Exchange Chromatography : Elution

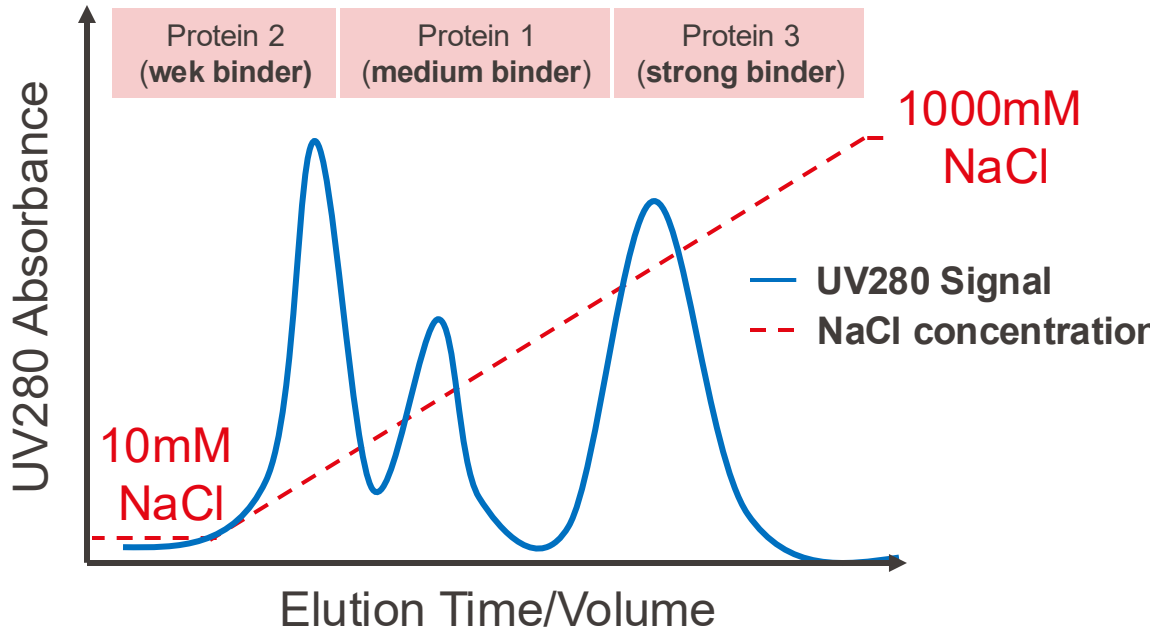
- After the protein is bound to the appropriate cation- or anion-exchange resin, the elution can be performed either by **(1) gradually changing the pH** or **(2) by changing the salt concentration**

pH gradient elution




• Each protein elutes when pH reaches their pI value due to charge reversal

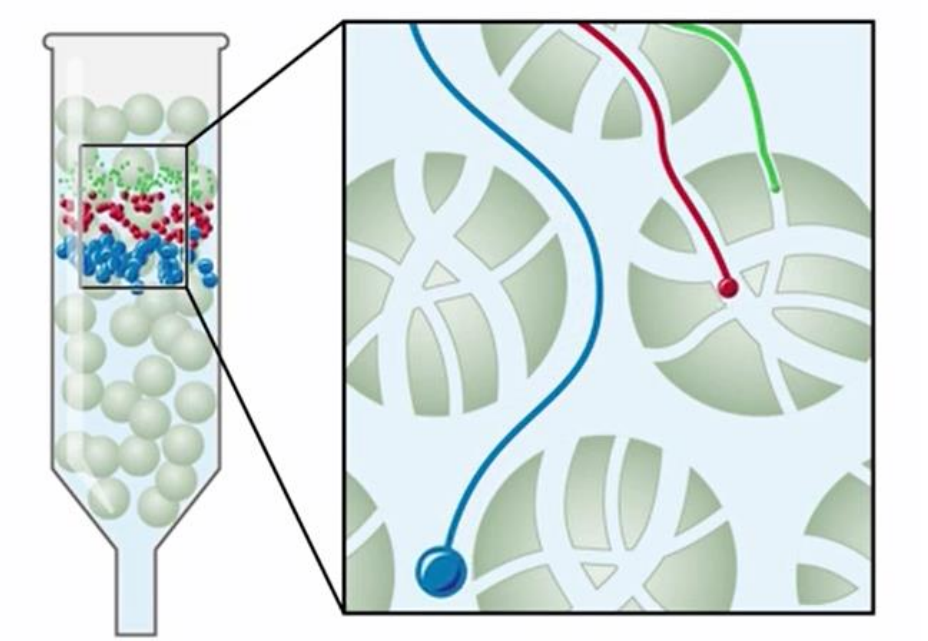
Salt gradient elution

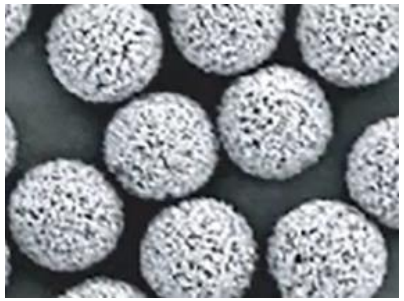


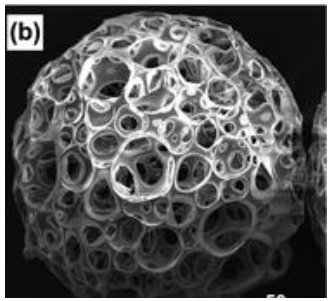

• Each protein elutes when the salt reaches sufficient concentration to outcompete it on the resin

# Size-exclusion (gel filtration) chromatography

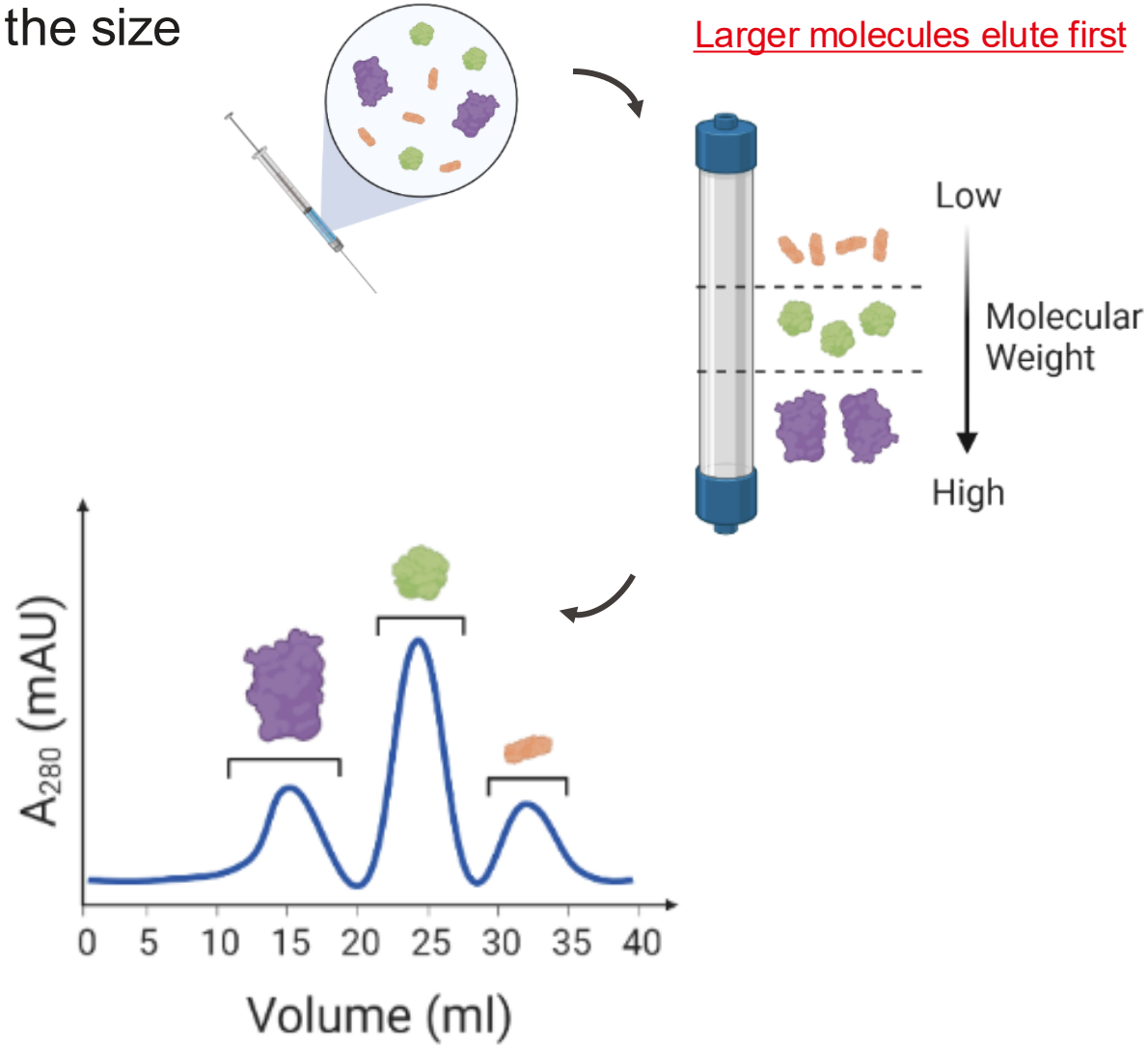
- Protein purification based on **different sizes (molecular weights)**
- Columns are based on porous resins through which the molecules migrate and get retained differently depending on the size





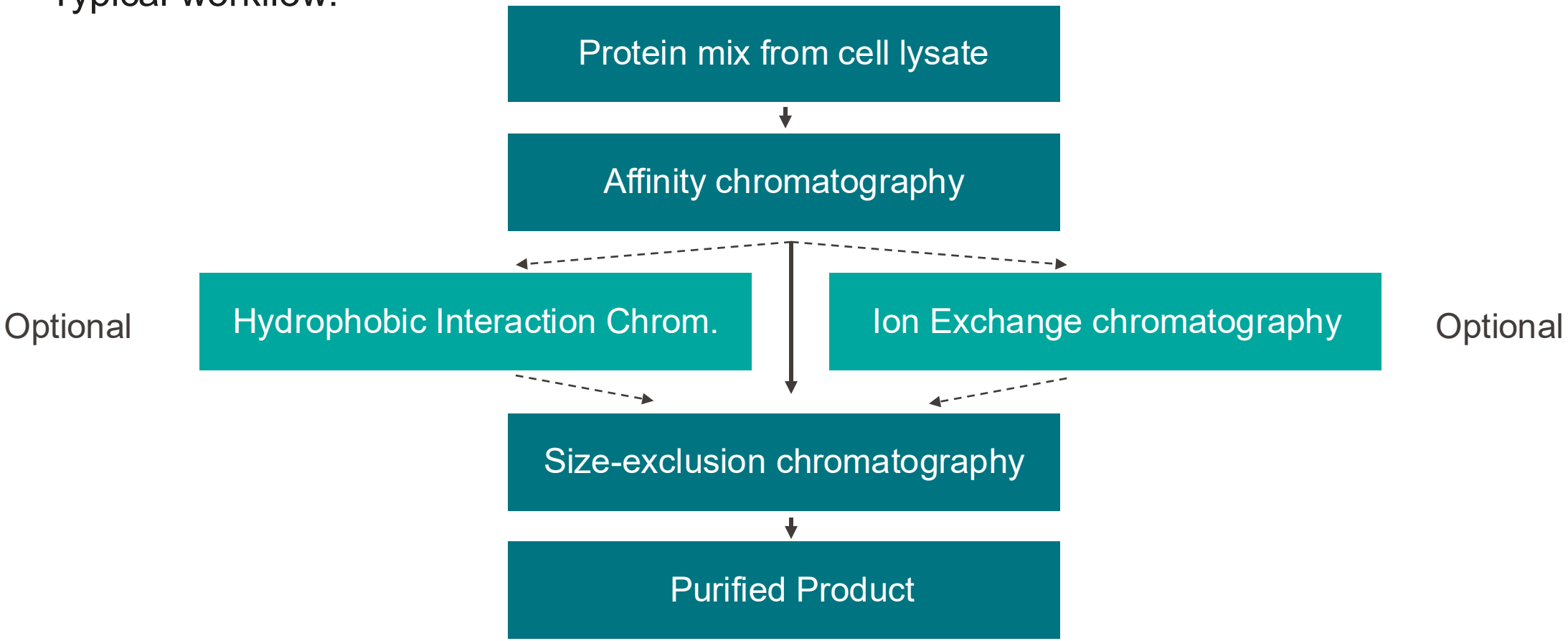



Larger molecules elute first



# Size-exclusion (gel filtration) chromatography

- Size exclusion chromatography is typically applied at the end of the purification experiment for sample polishing (removal of last impurities) as well as for separation of different oligomeric forms (e.g., monomer, dimer, aggregate) of the same protein.
- Typical workflow:



# Summary

- There are conceptually 3 different ways to synthetize biomolecules: chemical, enzymatic and cell-based
- Cell-based methods are the most versatile, particularly for protein production, but there are several steps to follow starting with the generation of expression vectors
- Selection of cell system for protein expression is done by taking into consideration the origin, size and complexity of the target protein, as well as the costs and time of using different systems
- Biomolecule purification is performed using liquid chromatography methods such as ion-exchange, size-exclusion and affinity chromatography
- A combination of approaches is usually applied to recover the protein of interest

Biomolecule Production

↓

Biomolecule Purification

↓

**Biomolecule characterization**

Lecture 9