Exercise 2
Discussion

Q1

Biophysical properties of proteins:

a) What biophysical properties influence the movement of a protein through solution, and how?

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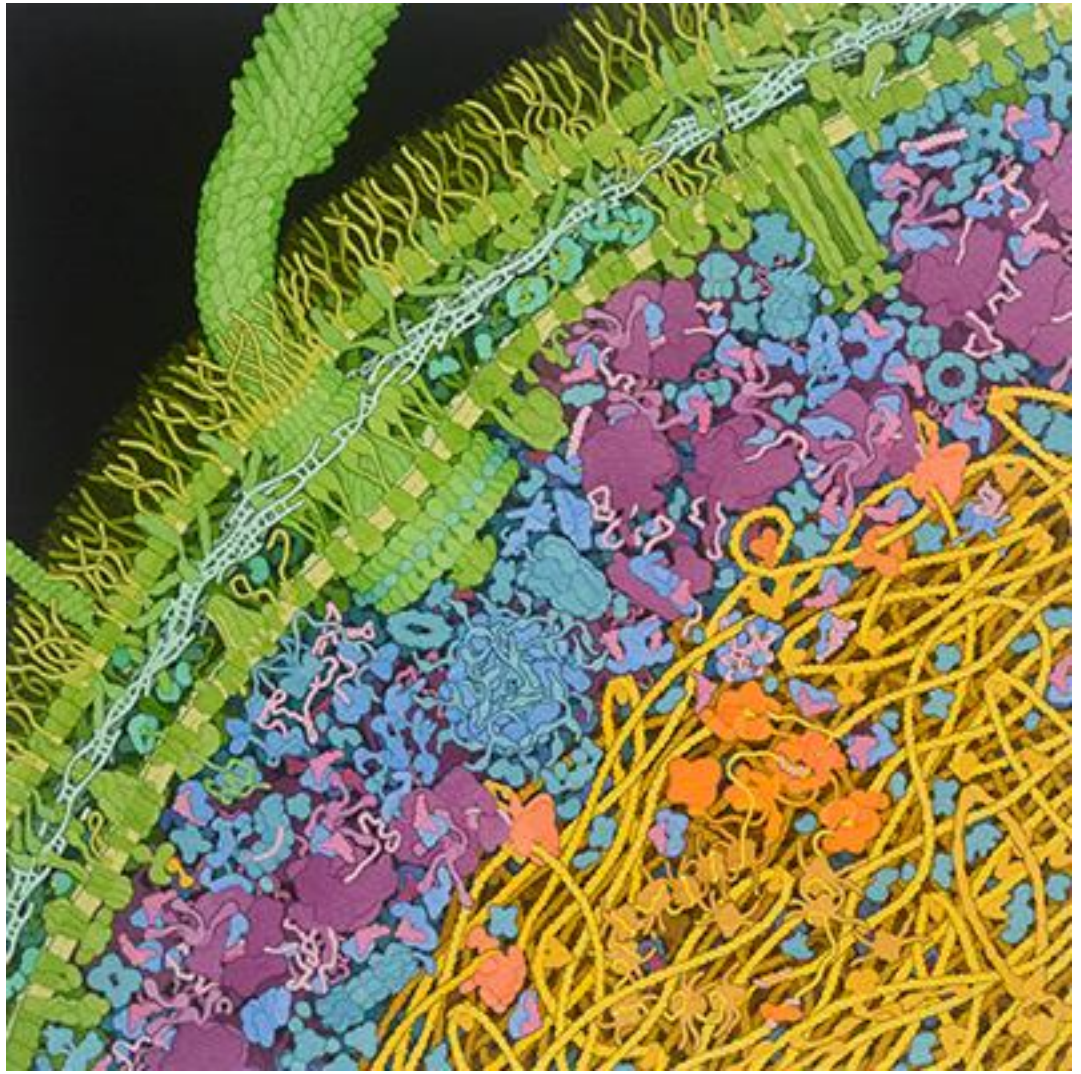


Q1

Biophysical properties of proteins:
a) What biophysical properties influence the movement of a protein through solution, and how?

- **Size & Molecular Weight:**
Larger proteins move more slowly due to greater resistance.

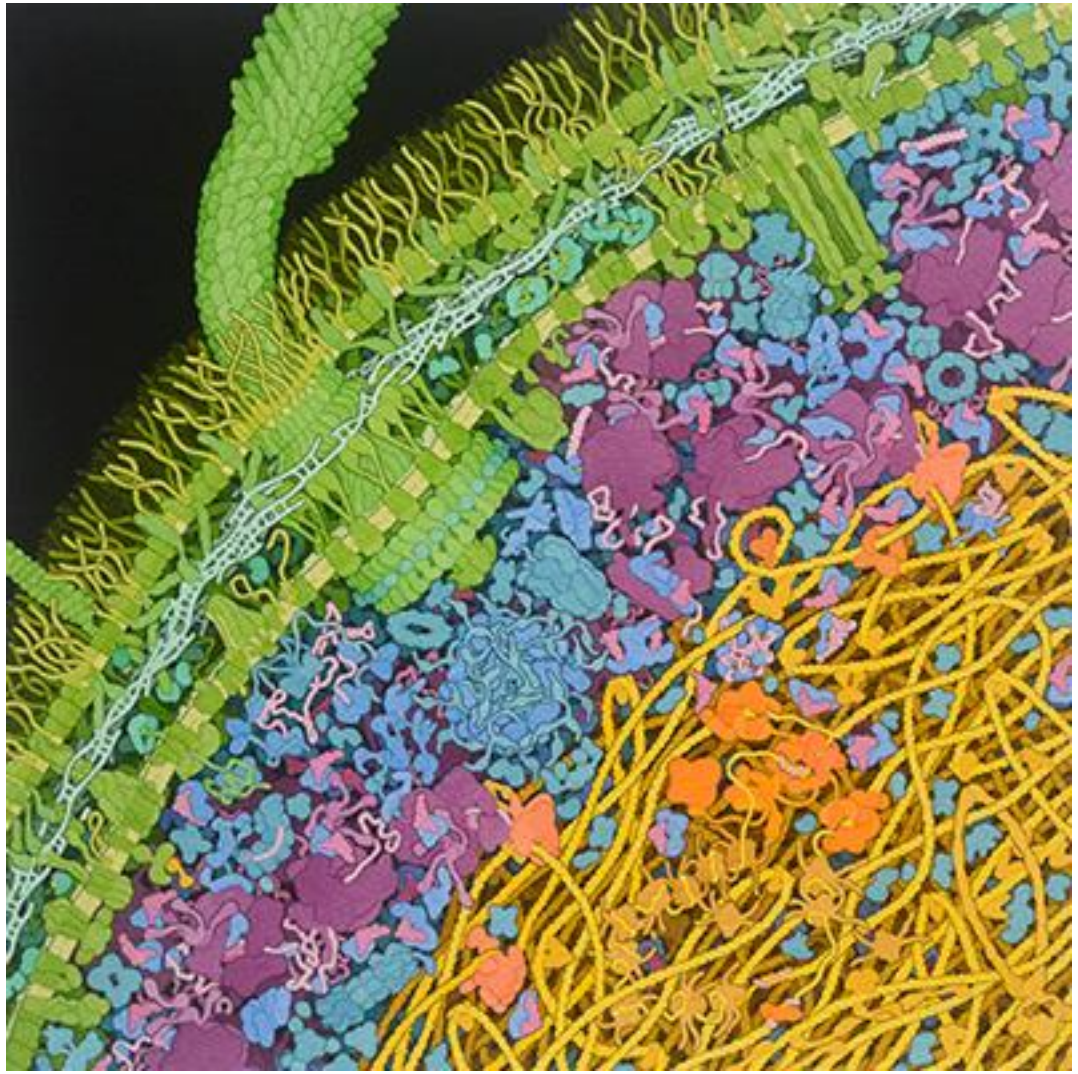
- **Shape & Hydrodynamic radius:**
Globular (compact) proteins diffuse faster than elongated ones.

- **Charge:**
Determines migration in an electric field (electrophoresis).

- **Hydrophobicity:**
Affects interactions with the solvent and potential aggregation.

- **Density & Buoyancy:**
Important for sedimentation in ultracentrifugation.

- **Solvent Viscosity:**
Higher viscosity slows diffusion and migration.



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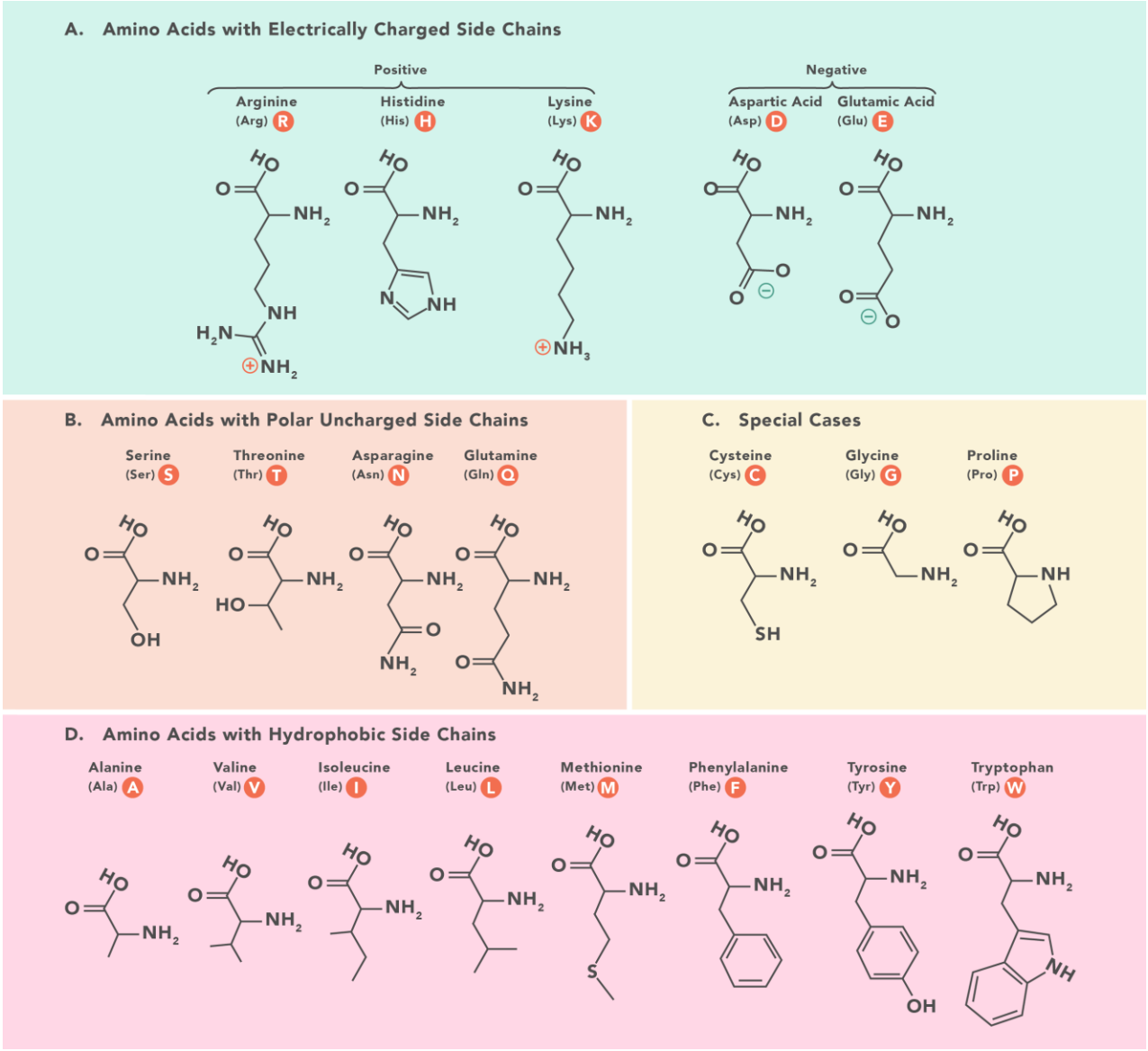
Biophysical properties of proteins:

b) How do amino acids determine the different biophysical properties of a protein?

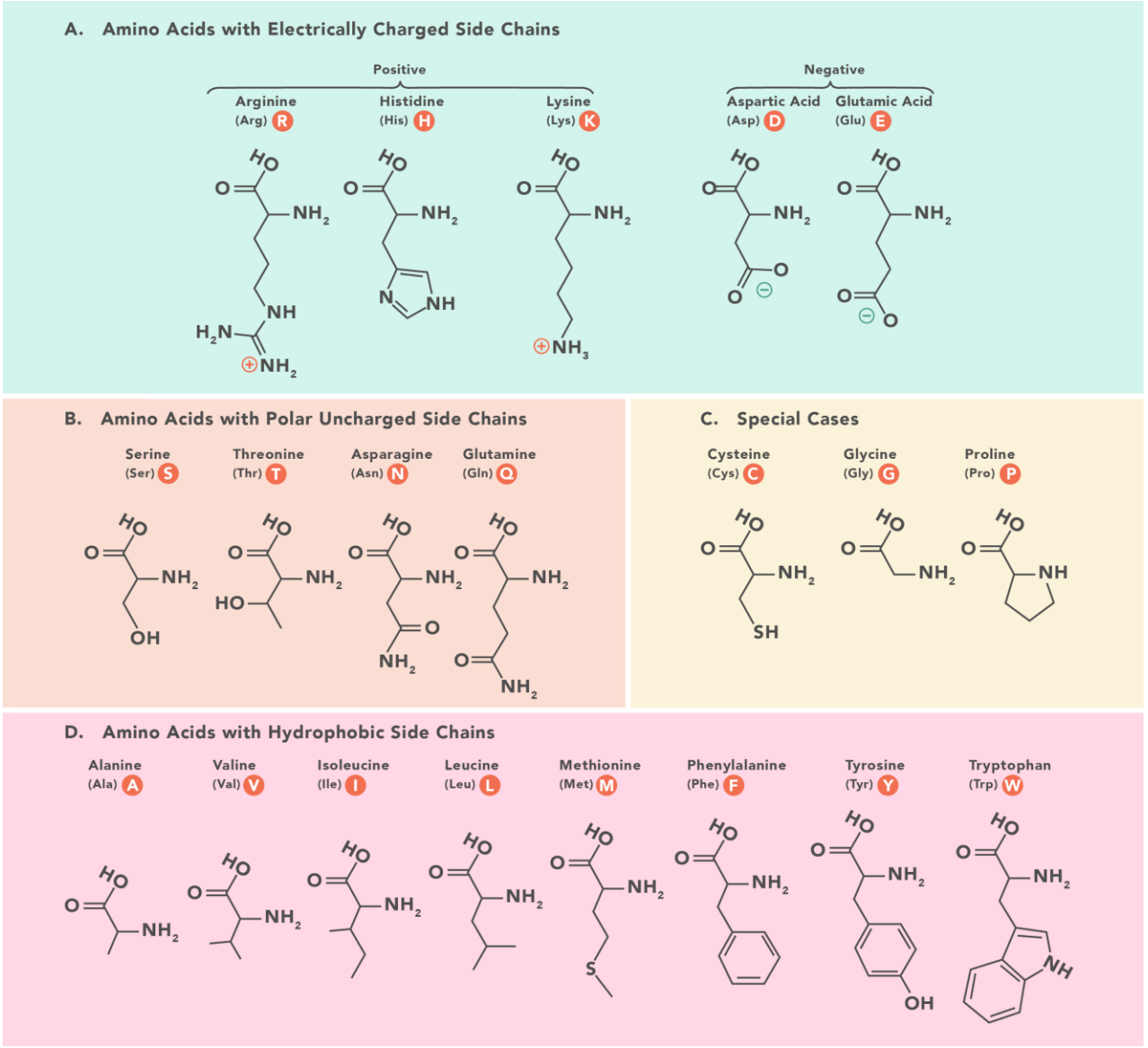


Q1

Biophysical properties of proteins:
b) How do amino acids determine the different biophysical properties of a protein?

Amino acids have different side chains that contribute to a protein's overall properties. Since proteins are composed of many amino acids, their **combined effects** determine the protein's biophysical characteristics. For example:

- **Size & Molecular Weight:** The total number of amino acids determines the protein's size and mass.
- **Charge & Isoelectric Point (pI):** The number and type of **charged amino acids** influence the protein's net charge at different pH levels.
- **Hydrophobicity & Solubility:** The proportion of **polar vs. nonpolar amino acids** affects solubility and interactions with water or lipids.
- **Shape & Folding:** The way amino acids **interact with each other and their environment** determines the protein's overall structure (globular vs. fibrous).



Q2

Chromatography columns used in biochemistry:

a) According to which property does a gel filtration column separate proteins?

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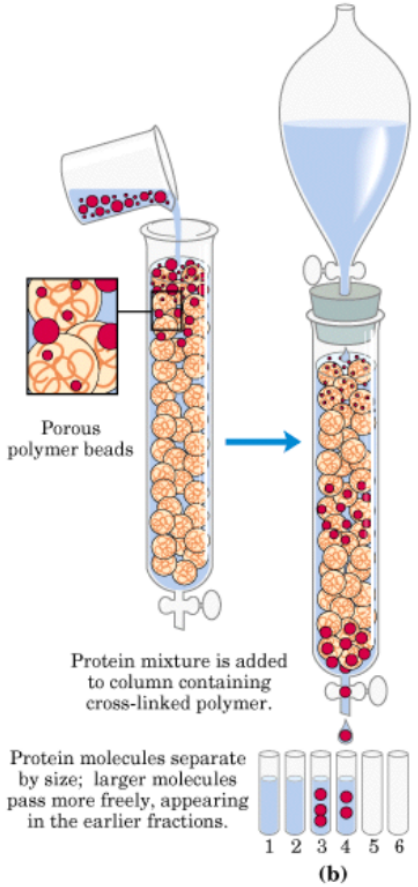

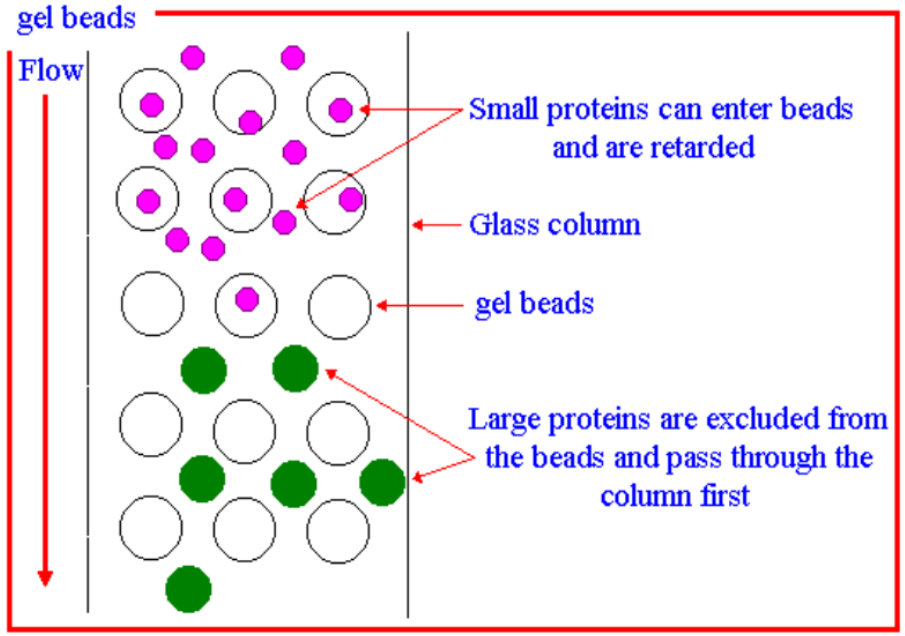

Size: Larger proteins elute **first** because they pass around the gel beads, while smaller proteins get **trapped in the pores** and elute **later**.

Q2

Chromatography columns used in biochemistry:

b) According to which property does an ion exchange column separate proteins?

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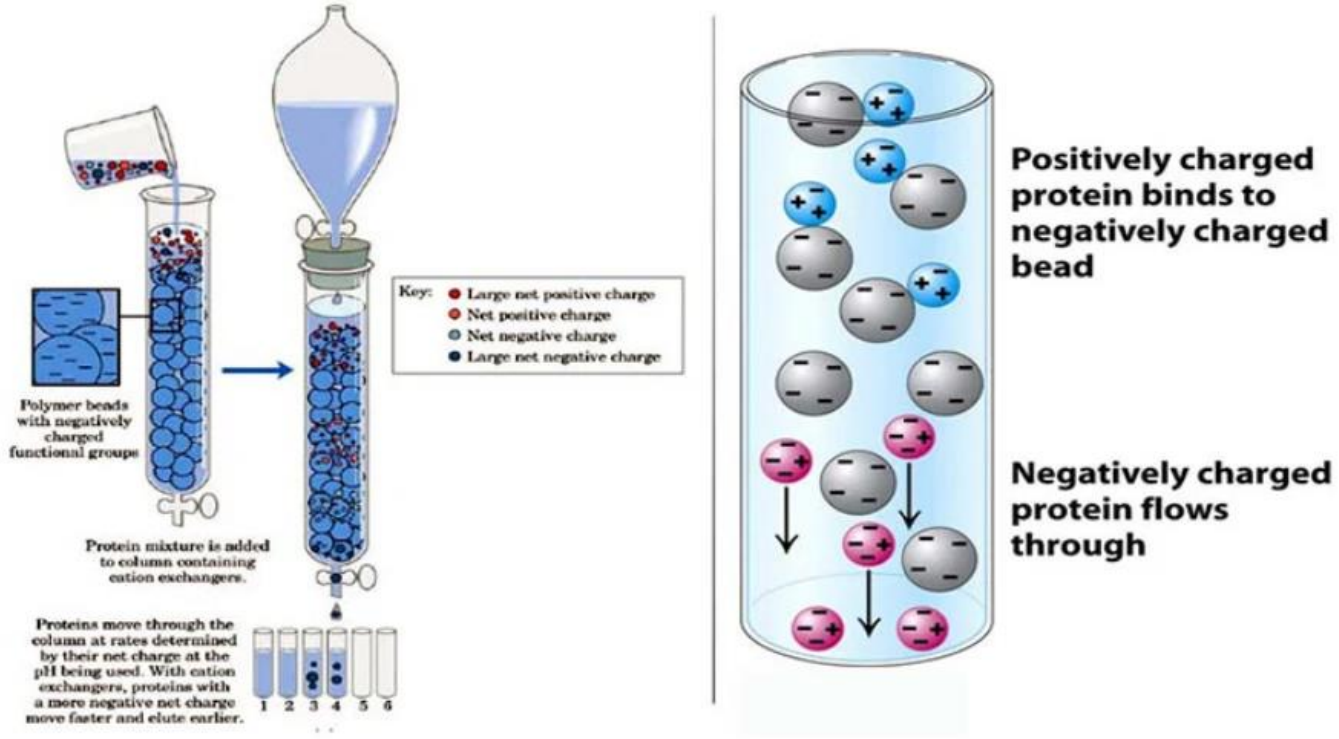

- **Charge**: Proteins bind to the charged beads in a column based on their **net charge** at a given pH.
- Proteins are eluted by changing the **salt concentration or pH**.
- Bound proteins will **elute as their pH approaches the pI** of the protein, weakening their interaction with the column.

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Chromatography columns used in biochemistry:

c) How can an IMAC column be used to purify one protein out of many?

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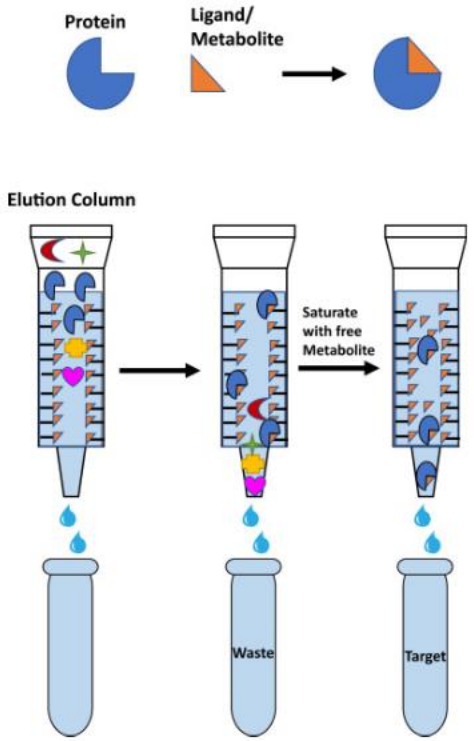

Affinity for metal ions:

- IMAC (**Immobilized Metal Affinity Chromatography**) binds proteins with **histidine (His-tag)** or other metal-binding sequences.
- The target protein binds to the **metal ions (e.g., Ni2+ or Co2+)** on the column.
- It is eluted using **imidazole or other competing agents**.

Q3

Gel electrophoresis

a) Explain/Draw + label the mechanism of SDS-PAGE

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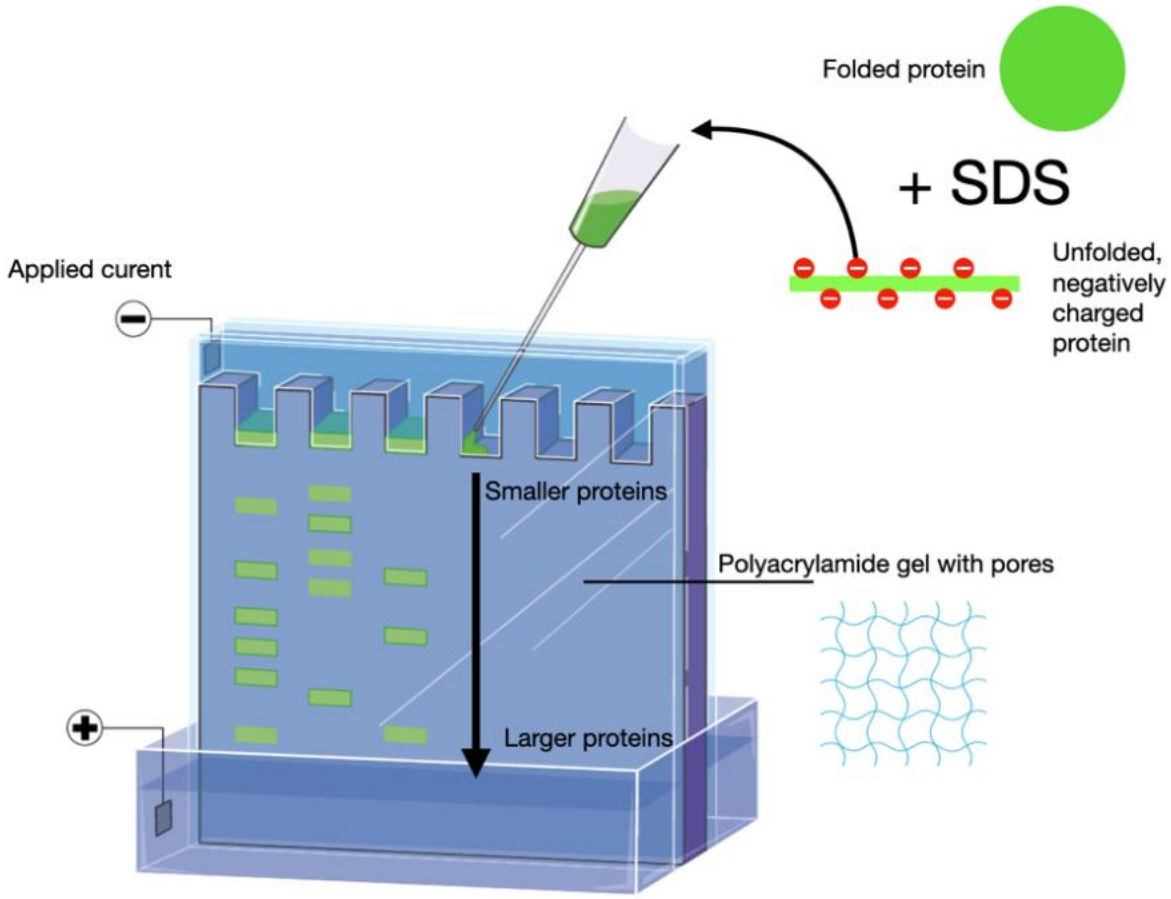
Mechanism:

- The detergent **SDS (sodium dodecyl sulfate)** binds to proteins, **denaturing them** and giving them a **uniform negative charge**.
- Proteins are loaded into a **polyacrylamide gel**, which forms a **porous network** that restricts movement based on size.
- An **electric current** is applied from **negative (cathode) to positive (anode)**.
- **Negatively charged proteins migrate** through the gel toward the positive terminus.
- **Smaller proteins migrate faster**, while **larger proteins move more slowly** due to greater resistance in the gel matrix.
- **Proteins are separated purely by size**, not by charge or shape.



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Gel electrophoresis

b) You run an SDS-PAGE gel and see multiple bands for your purified protein. What are possible explanations? How would you refine the purification?

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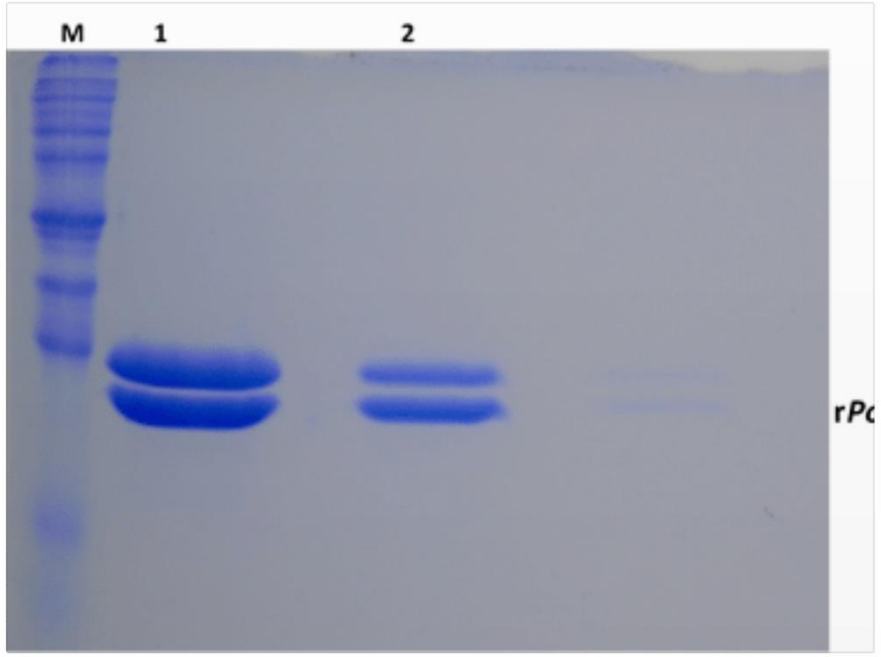
Question Asked 18 October 2018

Murilo Luiz Bazon
LIH Luxembourg Institute of Health

Recombinant Protein expression in Pichia pastoris appears in two bands in SDS-PAGE after purification in affinity chromatography. Why?

When expressed in *Pichia pastoris*, the recombinant protein Antigen 5 (~25 kDa) appears in two bands in SDS-PAGE after purification in affinity chromatography. The supperior band have a molecular weight of ~30 kDa. And the lower band have a molecular weight of ~25 kDa. Does someone have any idea of what the additional band might be? Maybe a glycosylation or peptide signal? We are waiting for the results of the sequencing of both bands.

The image bellow contain the purified protein with two bands.



Q3

Gel electrophoresis

b) You run an SDS-PAGE gel and see multiple bands for your purified protein. What are possible explanations? How would you refine the purification?

- Multiple bands may indicate **contaminants could be present.**
- **Additional chromatography steps, or** other purification techniques could be used to increase the purity of the protein.

Q4

1.Spectroscopy Calculation

A protein solution has an absorbance (A280) of 1.5 in a 1 cm cuvette.

The extinction coefficient for this protein is 50,000 $M^{-1}cm^{-1}$. What is the protein concentration?

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The extinction coefficient for this protein is 50,000 $M^{-1}cm^{-1}$. What is the protein concentration?

Using , the concentration is: or **30 µM**.

Using $c = \frac{A}{\varepsilon l}$, the concentration is: $c = \frac{1.5}{50{,}000 \times 1} = 3.0 \times 10^{-5} M$ or **30 µM**.

Q5

Membrane proteins

Explain/Draw + label how detergents can be used to aid in the purification of membrane proteins?

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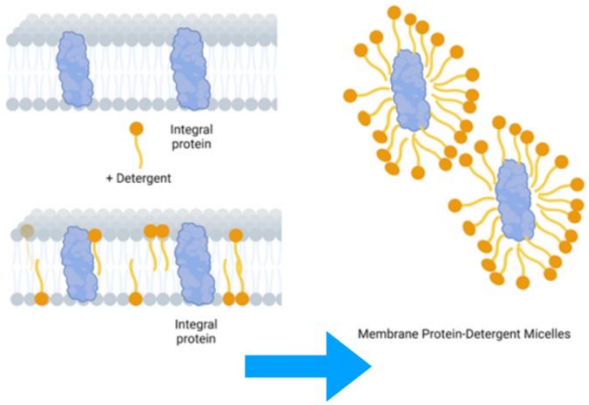
Membrane proteins

Explain/Draw + label how detergents can be used to aid in the purification of membrane proteins?

Detergents are essential in the purification of membrane proteins because these proteins are typically hydrophobic and embedded in the lipid bilayer of membranes. Here's how detergents aid in their isolation:

- **Hydrophobicity of Membrane Proteins**: Membrane proteins have hydrophobic regions that interact with the lipid bilayer, making them difficult to extract and purify without disrupting the membrane.
- **Detergent Structure**: Detergents are amphipathic molecules, meaning they have both hydrophilic (polar head) and hydrophobic (fatty acid tail) parts, which is similar to the structure of lipids in the membrane.
- **Insertion into the Membrane**: When added to the membrane, detergent molecules insert into the lipid bilayer. The hydrophobic tails of the detergent interact with the hydrophobic regions of the membrane protein, while the hydrophilic heads face the surrounding aqueous solution. This disrupts the membrane packing and destabilizes the bilayer structure.
- **Critical Micellar Concentration (CMC)**: As the detergent concentration increases, a point is reached where detergent molecules begin to form micelles—aggregates of detergent molecules in solution. At the critical micellar concentration (CMC), these micelles surround the hydrophobic regions of membrane proteins, preventing aggregation and stabilizing the proteins in their solubilized form.
- **Solubilization and Stabilization**: The detergent micelles keep the membrane proteins soluble by stabilizing their hydrophobic regions, allowing them to remain in solution without re-aggregating or losing activity.



= critical micellar concentration (CMC)