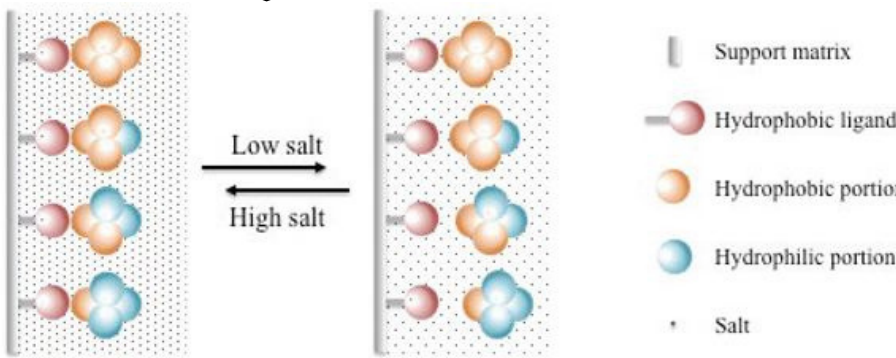


In this lab you will lyse (burst open) the bacteria you transformed in Lab 5A, then use a column that separates proteins based on their solubility in water to obtain purified **RFP** made by the cloned rfp gene. This is how human therapeutic proteins like insulin are isolated via hydrophobic interaction chromatography or HIC.



How do different salt concentrations help purify a protein?

Binding Buffer: has a high salt concentration that causes proteins to unfold and expose hydrophobic regions. These regions attach to the resin beads, the hydrophilic cell waste parts pass through the column while **rfp protein is retained**.

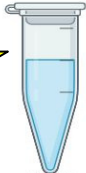
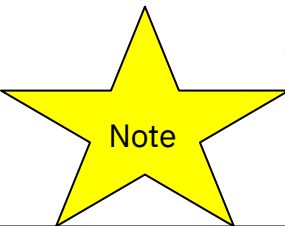
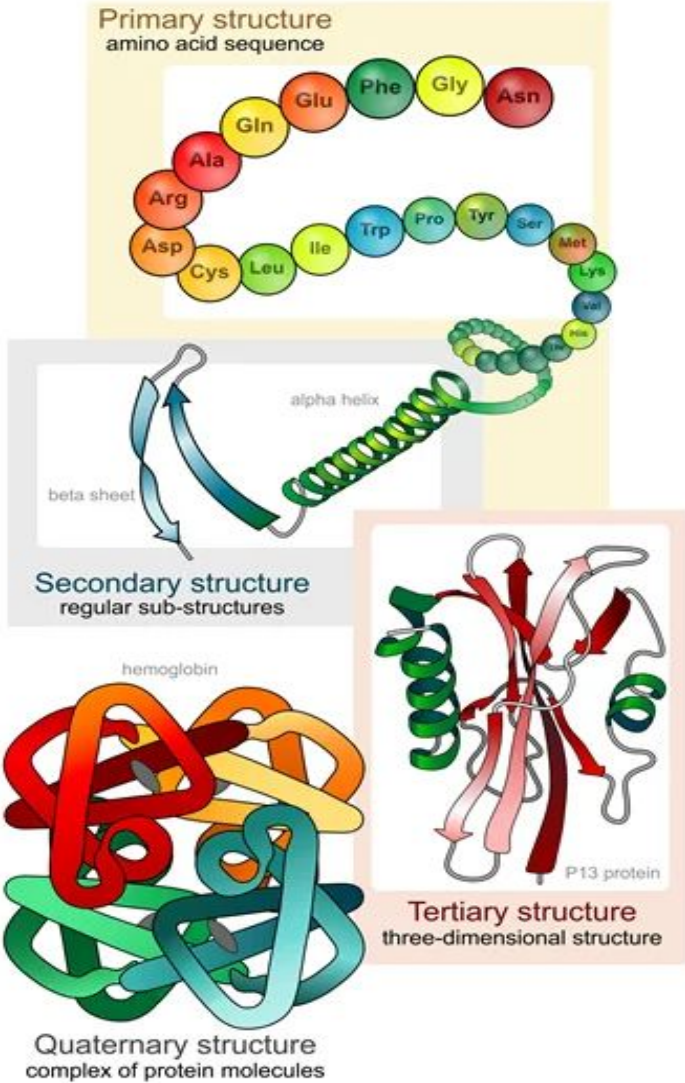
Wash buffer: has a medium salt concentration solution that releases moderately hydrophobic regions from the resin further purifying the protein.

Elution buffer: Addition of EB (no salt) causes the hydrophobic regions of the protein to bury deep inside the protein again releasing it from the column resin.

Summary: The salt concentration gradient allows the protein to refold gradually leading to the eventual release from the resin.

More on proteins:

Proteins are made of chains of amino acids, a large number of amino acid chains is called a polypeptide (long linear molecule forming the total or part of a protein). When a polypeptide is made, it immediately folds into a 3D conformation (shape) this is what we call a protein. Protein folding is a result of weak noncovalent (no e- sharing) bonds between amino acids. Hydrophobic amino acids usually become buried inside of the protein when in an aqueous environment. Interactive protein link: <https://lab.concord.org/embeddable.html#interactiv es/samples/5-amino-acids.json>



The contents of your tube can vary depending on teacher prep and lab start point!

When growing a bacterial culture, when do we add arabinose?

Remember that arabinose sugar interacts with the arabinose operon to “turn on” rfp production.

Between the middle and end of the log phase, at this point there are a large number of healthy cells. Earlier there are too few cells and later there are too many cells and death soon follows.

