Protein Structure & Purification

1. Disruption of forces that stabilize protein structure cause folded proteins to unfold. Unfolded proteins are not functional. We describe them as denatured. Denaturing agents include heat, detergent, acid or base.

2. RNase is an unusual enzyme. It is very stable. After being heated to boiling temperatures, it will renature and regain its activity. Most proteins don't do this.

3. Folding of a protein is how it gets its 3D shape. Folding is a process that is determined by the sequence of amino acids in a proteins.

4. Misfolded proteins cause enormous problems sometimes. A misfolded protein is the cause of mad cow disease. The misfolded protein is called a prion and it works by inducing other copies of the same protein to misfold.

5. Chaperonins are complexes that facilitate the proper folding of proteins.

6. To study a protein, one must purify it away from all the other proteins in a cell. Steps in the purification typically include 1) busting cells open; 2) centrifuging cellular components apart from the cytoplasm; and 3) using techniques that separate molecules by several different processes.

7. Most proteins require more than one method to be purified.

8. Techniques for protein purification often rely on the structural components of proteins. Isolation of proteins from cells requires breaking open the cells, centrifugation to remove insoluble debris, and the application of protein isolation techniques to purify desired proteins from the soluble fraction of the cells.

9. Gel exclusion chromatography is a technique for isolating proteins on the basis of their different sizes. The method uses 'beads' with uniform holes in them. The holes are openings to tunnels through the bead. Small molecules that fit into the holes travel throught the tunnels and take longer to pass through the column than large molecules that do not fit into the holes.
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10. Affinity chromatography uses the structure of a molecule that a protein binds (such as ATP) as a means of purifying the protein. For example, proteins that bind ATP would be retained by a column full of beads with ATP on their surface. The non-ATP-binding proteins will pass through first. ATP-binding-proteins can be removed from the column by adding ATP.

11. Ion exchange chromatography uses ionic interactions as a means of separating proteins. Cation exchange chromatography uses negatively charged beads. These attract the positively charged proteins in a mixture, whereas the negatively charged proteins pass through quickly. Anion exchange chromatography has an opposite strategy.

12. Gel electrophoresis involves gelatinous support materials (agarose - for DNA or polyacrylamide - usually for proteins) and an electric current that drags molecules through the gel. Electrodes are arranged such that the "top" or beginning of the gel is where the negative electrode is placed and the positive electrode is placed at the bottom or end of the gel. DNA is negatively charged, so it is repelled away from the top and towards the bottom of the gel. Separation is on the basis of size. Large molecules

travel slowest in the gel, whereas the small molecules travel fastest. DNA fragments appear as bands on a gel and bands can be excised separately from the other bands for further manipulation.

13. Proteins can have negative, positive, or no charge. To work in electrophoresis, they must be converted from folded entities into rods with uniform negative charge. This is done using the detergent called SDS. It unfolds the protein and coats it with negative charges. This coated protein is loaded onto a gel of polyacrylamide and the complexes are sorted on the basis of size, just like the DNA fragments were - smallest moves fastest and largest moves slowest.