

Biochemistry—Proteins

A. CBS proteins, 3D representation

The protein we will consider today and periodically throughout the term is the yeast cystathionine beta synthase (CBS) protein. Today we will see gels visualizing wild-type and mutants versions of the yeast CBS protein. Yeast that lack CBS protein can not synthesize the amino acid cysteine. Mutant versions of the human CBS protein can lead to a very serious disease called homocystinuria. Some symptoms include mental retardation, early strokes and heart disease.

First consider a crystallographic model of a form of CBS protein. Open the CBS structure html file in Internet Explorer. Rotate the molecule and get an idea of the three dimensional structure of CBS.

The true 3-dimensional structure of CBS can be observed using the “**Show Spacefill**” button, and a trace of the peptide backbone can be observed using the “**Show Ribbon**” button.

1. What is the primary structure of a protein? What information does the modeling program provide us about the primary structure of CBS?
2. What is the secondary structure of a protein? What information does the modeling program provide us about the secondary structure of CBS?
3. What is the tertiary structure of a protein? What information does the modeling program provide us about the tertiary structure of CBS?
4. What is the quaternary structure of a protein? What information does the modeling program provide us about the quaternary structure of CBS? (Hint: Use the “Color Ribbon by Protein Subunit” button)

B. Protein gels

1. Yeast cells produce enough CBS to fulfill their needs depending on the conditions in which they are growing. In order to procure large quantities of the enzyme, we created special bacterial cells that mostly produce CBS. After we grew up a large number of these bacterial cells, what did we have to do to end up with pure CBS for loading on the gel?

Some technical issues:

2. What is a gel?
3. How does gel electrophoresis work?
4. If you run two proteins on the same gel, and one travels farther than the other, what can you say about the relative sizes of these proteins?
5. With gel electrophoresis, two different conditions are often used.
 - a. Denaturing gel: detergent is added to the gel and a reducing agent is added to the protein sample. Reducing agent converts the S-S disulfide bonds to SH and SH.
 - b. Native gel: Physiological pH conditions where no detergent or reducing agent is added.

Compare the 1°, 2°, 3°, and 4° structure of CBS for each of these conditions:

	Denaturing gel	Native gel
1°		
2°		
3°		
4°		

6. Information about what level(s) of protein structure do you expect to gain from a denaturing gel?

7. Information about what level(s) of protein structure do you expect to gain from a native gel?
8. What could the native gel tell you that the denaturing could not?

Let's now talk about the proteins themselves.

In the 3D representation of the CBS protein, consider a single subunit of CBS. Now consider the whole CBS protein again.

9. Which of these forms would we be visualizing with each kind of gel?

Imagine we are the researchers who first isolated the wild type and mutant versions of the protein. What kinds of things might we want to know about these proteins?

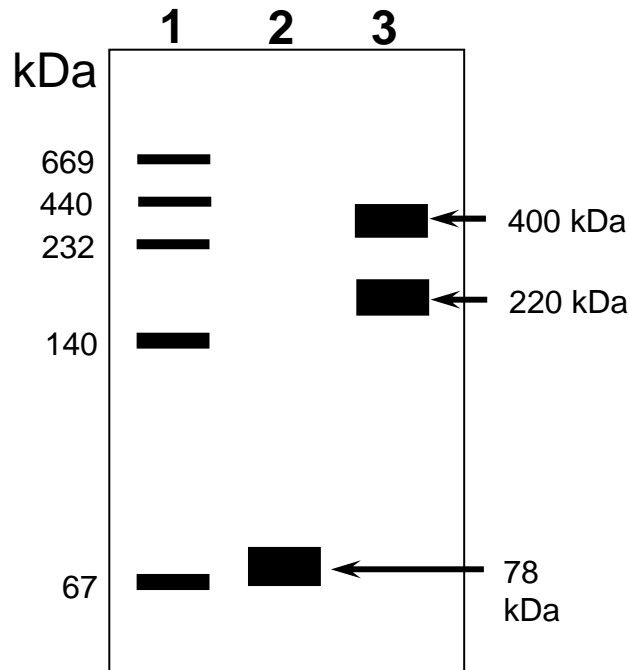
The five questions from journalism school are very much applicable here: Who? (What is this protein?) What? (What does it do in the cell?) When? (When does it act?) Where? (Where does it act?) How? (How does it perform its function? What does it interact with?). Note that these are all basic questions that can be asked from first principles.

10. Which of these things can we figure out by running the purified proteins on the gels and which ones can't we? Why?
11. What other techniques might you want to employ in your research of the purified protein? What would you hope to find out from these experiments?

C. Analyzing the data and interpreting the results

Take a look at the denaturing gels. One of our proteins migrates a shorter distance than the other.

1. What does this mean?
2. As we said earlier, we ran both the wild-type and mutant protein on the denaturing gel. Which do you expect to be longer—wild-type or mutant protein? Why?



Take a look at the photocopy of a native gel.

3. What can you say about the difference between the wild type and the mutant enzymes from the native gels?
4. Would you expect the same section of the monomer subunit of CBS to be used in forming dimers as in forming tetramers? Why or why not?
5. Do you think the 3D representation we are working with today is of the wild-type or mutant protein? Why?

Let's now look at the dimer interface that forms between the two chains. There are several important interactions that hold the two subunits together. We will look at some residues involved in keeping the interface together.

I. Choose **“Central Interaction”**

6. What are the residues involved in this interaction?
Hint: It may help to change the representation of the residues to ball and stick by clicking in the box labeled “Ball and Stick on/off” below the 3D window
7. What types of amino acids are these residues (acidic, basic, polar, or hydrophobic)?
8. What types of interactions occur between these residues?

9. Would the CBS dimer interface be more, less or equally as stable if we changed one of the phenylalanines to a glutamic acid? An alanine? A tryptophan?

II. Choose “**Interaction I**”

10. We have highlighted two identical clusters at opposite ends of the CBS structure. Each individual cluster contains an alanine and a leucine from chain A and an alanine and a leucine from chain B. What type of amino acids are alanine and leucine?

11. What type(s) of interaction(s) are the amino acids in these clusters involved in?

12. Would you expect the side chains in this cluster to be exposed to the cytoplasm? Why or why not?

III. Choose “**Interaction II**”

13. We have highlighted two identical clusters at far ends of the CBS structure. Each individual cluster contains a glutamine and an aspartic acid on one chain, and a lysine on the other. What types of amino acids are these residues?

14. What type(s) of interaction(s) are found in this cluster?

15. Select “**Interaction II with all atoms shown.**” This view shows all the atoms of CBS (not including hydrogens) as transparent dots. Are the amino acids involved in Interaction III found on the exterior or interior of the protein? Does this make sense if CBS is a soluble protein?

16. Choose “**Show Surface Hydrophobics.**” This colors all the hydrophobic residues of CBS pink. Are there a lot or a few surface exposed hydrophobic amino acids? Does this make sense? For what purpose could the protein use surface exposed hydrophobic residues?

Back to the gel data.

17. Do you expect the mutant enzyme to be active? Why or why not?

18. The in-gel activity assay actually indicates that the mutant protein is active. What do you think is the function of the missing domain?

19. Suppose a colleague in the lab next door has figured out that the mutant protein is active in the cell all the time, while the wild type protein is active only some of the time. What conclusion might you draw from this information in combination with your data?