

## 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.~~

## 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?

*We had to grow up a lot of cells, break them all open, and then go through a gradual process of removing all parts of the cell we didn't need. We removed membranes, nucleic acids, carbohydrates, and small molecules. We then removed the majority of other proteins that were in the cell at the time of harvest, leaving us with mostly pure CBS.*

Some technical issues:

2. What is a gel?  
*A gel is a jelly-like matrix. Bigger objects have more trouble fitting through the matrix, and, therefore, travel slower through the gel.*
3. How does gel electrophoresis work?  
*Our sample preparations and the buffer used in the gel set-up ensure that unfolded proteins have negative charge overall. When the electric current is applied, they move toward the anode.*
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?  
*The one that travels farther has lower molecular weight.*
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°	<i>Primary structure is not disrupted. Same as that for CBS proteins present in a cell and for those run on native gels.</i>	<i>Primary structure is not disrupted. Same as that for CBS proteins present in a cell and for those run on denaturing gels.</i>
2°	<i>Secondary structure lost due to presence of detergent and reducing agent.</i>	<i>Secondary structure is not disrupted. Same as that for CBS proteins present in a cell.</i>
3°	<i>Tertiary structure lost due to presence of detergent and reducing agent.</i>	<i>Tertiary structure is not disrupted. Same as that for CBS proteins present in a cell.</i>
4°	<i>Unable to form quaternary structures due to presence of detergent and reducing agent.</i>	<i>Quaternary structure is not disrupted. Same as that for CBS proteins present in a cell.</i>

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

*Adding detergent and a reducing agent ensures that the protein will lose its secondary and tertiary structure, so the information we get from running such a denaturing gel is the size of each subunit of the protein complex.*

7. Information about what level(s) of protein structure do you expect to gain from a native gel?  
*Not adding detergent and a reducing agent allows the protein to enter the gel as a 3D structure, so we would be able to gain information about its apparent 3D size.*
8. What could the native gel tell you that the denaturing could not?  
*The native gel would provide information on the number of subunits involved in the protein complex.*

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?  
*A native gel would give us an opportunity to visualize the whole CBS protein, while the denaturing gel would tell us the size of the individual subunit. These two pieces of information put together would tell us how many subunits are in each complete CBS protein.*

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?  
*We can only get a part of the answer to the last question from the gel. This is because we can not definitively answer questions about the protein's function and mode of operation in the cell without looking in the cell.*
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?  
*If we determined by other means that this is an enzyme, we could try to determine what reaction the protein catalyzes by seeing whether any of the known or suspected substrates will be acted upon. We could also determine whether the enzyme needs a cofactor, such as any of the known small molecule co-factors.*

### ~~C. Analyzing the data and interpreting the results~~

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