Choose correct statements

2) What are enzymes?

A) Unsaturated fats that catalyze chemical reactions
B) Saccharides that catalyze chemical reactions
C) Proteins that catalyze chemical reactions
D) Nucleic acids that catalyze chemical reactions
E) DNA molecules that catalyze chemical reactions

6) Carboxypeptidase is an enzyme used in the determination of the primary structure of a protein.

Specifically, which of the following does it identify?
A) The disulfide bridges in the protein
B) N-terminal amino acid of the protein
C) The number of peptide bonds in the protein
D) C-terminal amino acid of the protein
E) The number and kind of amino acids in the protein

3.1. Which of the following statements is *false* about protein purification?

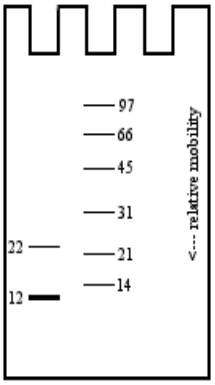
a) Gel filtration separates proteins by their Stokes-radius.
True, the Stokes-radius depends on both size and shape of proteins and determines whether or not they fit into the pores of the gel

b) Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) is a useful method to separate proteins by isoelectric point (*p*I).
-False, SDS-PAGE separates by molecular weight. Binding of SDS

c) Enzymes may be stabilized during purification by the presence of their substrate.
True, substrate binding reduces conformational freedom and thus Stabilizes the protein against denaturation.

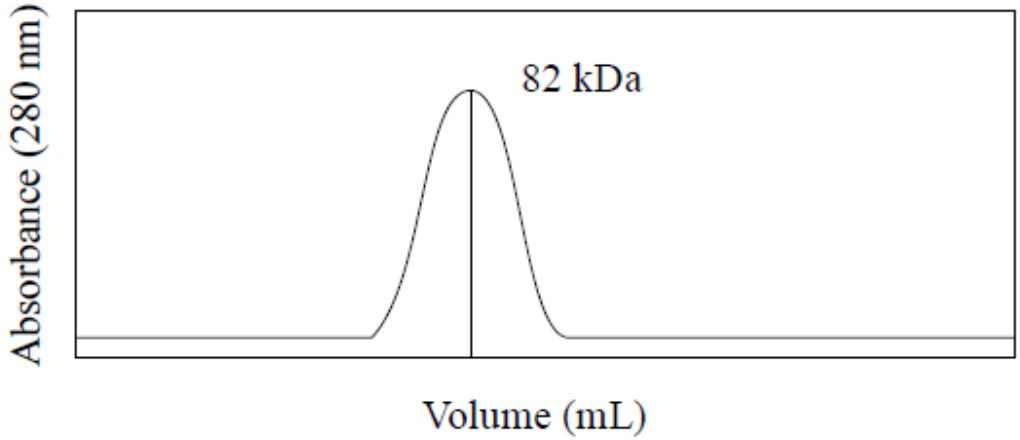
(24) What is the role of 2-mercaptoethanol (b-mercaptoethanol) in SDS-PAGE?
- 2-mercaptoethanol is a reducing agent that breaks disulfide bonds (cystines) into free thiols (cysteines). This breaks covalent attachments between subunits in multisubunit proteins.

(25) Vibrio cholera (causitive agent of "cholera") secretes a protein toxin with an AB5 quaternary structure.
The single "A" subunit has a molecular mass near 22 kDa and each "B" subunit has a mass of near 12 kDa.
(a) Sketch and label a figure of the result of an SDS-PAGE experiment.
-)The size information and relative intensity are required while the



molecular weight markers in lane 2 are not.
SDS-PAGE denatures the protein structure and 2-mercaptoethanol breaks any disulfide bonds. As a result, each polypeptide chain migrates according to its size.

(b) Sketch and label a figure of the result of size exclusion chromatography experiment.
(25b) The label and determined size of the peak are all required.
SEC does not disrupt the quaternary structure of the protein.



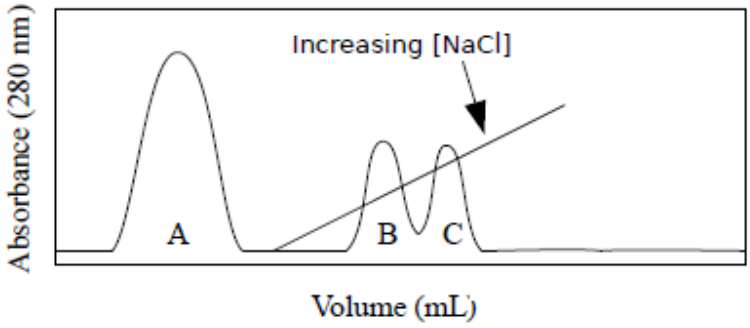
(26) What is the retarding force in size exclusion chromatography?
- Each bead of the stationary phase contains pores with a defined minimum and maximum size. Solute that diffuses into a bead flows more slowly through the column as the pores restrict flow down the column.

(27) You have isolated a novel, globular protein. Describe an experiment or set of experiments to prove (or disprove) the protein is composed of more than one subunit.
- The unknown protein must be exposed to conditions that
(i) disrupt quaternary structure and
(ii) maintain quaternary structure.
(i) SDS-PAGE in the presence of reducing agent (2-mercaptoethanol) will denature the protein and break any disulfide bonds. There are two possible results:
case 1: two or more bands are present and indicate the protein has multiple subunits
case 2: one band is present. This is an inconclusive result as the sample may have one subunit or be composed of multiple identical subunits

(ii) To resolve case 2, use SEC in the absence of denaturant:
If the mass determined by SEC is significantly larger (an ~ integer multiple) than that determined by SDS-PAGE the sample has multiple subunits. Otherwise the sample contains a single polypeptide chain.

(28) Consider the following cation exchange chromatogram run at pH 6.0. A mixture of proteins were applied to the column in a low [NaCl] buffer. Proteins retained by the cation

exchange column were eluted by gradually increasing the NaCl concentration within the buffer. What can be concluded regarding the pI and relative charge of the protein(s) in each chromatogram peak?



--Peak A - The protein(s) do not bind to the cation exchange column indicating their pI is likely 6.0 or less and their net charge is neutral or negative at pH 6.0.
(eg. A protein has a net charge of 0 at pH 5.0 (pI = 5.0); as the pH is raised (less H+) its charge will be negative)
Peak B - The protein(s) bind to the cation exchange column indicating the pI is likely greater than 6.0 and has a net positive charge at pH 6.0.
(eg. A protein has a net charge of 0 at pH 7.0 (pI = 7.0); as the pH is lowered (more H+) its charge will be positive)
Peak C - The protein binds to the cation exchange column indicating the pI is likely greater than 6.0 and has a net postive charge at pH 6.0. The net positive charge of Peak C is likely greater than that of Peak B as it requires higher salt concentration to elute.
Note - It does not follow that Peak C has a higher pI than Peak B.

3.7. Protein Purification.
An enzyme (MW 24 kDa, pI 5.5) is contaminated with two other proteins, one with a similar molecular mass and a pI of 7.0 while the other has a molecular mass of 100 kDa and a pI of 5.4. Suggest a procedure to purify the contaminated enzyme
A fast and simple way to separate these proteins is to first perform an ion exchange chromatography: it will then be possible to get rid to the protein whose pI is different that our favorite enzyme. As a second step, we can perform a molecular sieve (or size exclusion, that's the same thing) chromatography to separate our enzyme (Mr 24 kDa) from the other contaminating protein (Mr 100 kDa).
Note: Similar results would be obtained if one were to first perform the size exclusion chromatography and then the ion exchange chromatography.
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3.8. Protein Purification.
A procedure used to purify 6-gluconate dehydrogenase from E. coli is presented below.
a) Calculate (1) the specific activity, (2) the percent yield based on the *initial* quantity of the enzyme and (3) the degree of purification for each step (i.e. *fold* increase in purification).
b) Indicate which step purifies the protein the most.

c) Assuming the protein is pure after gel permeation chromatography (on Bio-Gel A), what percent of the initial extract contained 6-gluconate dehydrogenase?

Purification step	*Volume (mL)*	*Total protein (mg)*	*Enzymatic activity (µg/min)*
1- Cellular extract	2 800	70 000	2 700
2- Ammonium sulfate	3 000	25 400	2 300
3- Heat denaturation	3 000	16 500	1 980
4- DEAE chromatography	80.00	390.00	1 680
5- CM-cellulose chromatography	50.00	47.00	1 350
6- Bio-Gel A chromatography	7.00	35.00	1 120

a) ***Specific activity*** is defined as the enzymatic activity per mg protein and is an indication of the relative concentration of the enzyme in the solution (the greater the amount of enzyme in the solution *relative to all other proteins*, the greater the specific activity will be). All we have to do is to divide the enzymatic activity obtained at each step by the corresponding protein concentration. For example, for the heat treatment step, we get:
specific activity = enzymatic activity / mg protein
specific activity = 1 980 U/16 500 mg = **0,12 U/mg**
The ***percent yield*** is definec as the amount of enzyme (or protein) recovered at each step with reference to the amount present at the start of the purification procedure. This value is obtained by dividing the enzymatic activity at step X by the initial enzymatic activity. Again, for the heat treatment step, we get
Percent yield = Enzyme activity step X / Initial enzyme activity
Percent yield = (1 980 U/2 700U) x 100 = **73,3 %**
The ***degree of purification*** is obtained by dividing the specific activity after step X by the initial specific activity. We therefore obtain the fold increase in enzyme purification after the purification procedure. For the heat treatment step, we get:
Degree of purification = Specific activity step X/ Initial specific activity
Degree of purification = 0,12/0,039 = **3,07 fold.**

The results for each step of the purification procedure are shown in the table below:

Purification step	*Specific activity (U/mg)*	*Percent yield*	*Degree of purification (fold increase)*
Cell extract	0,039	100%	---
Ammonium sulphate	0,09	85,2%	2,30
Heat treatment	0,12	73,3%	3,07
DEAE chromato.	4,31	62,2%	110,50
CM-cellulose chromato.	28,72	50%	736,40
Bio-Gel A	32,00	41,5%	820,50

b) To determine which purification step was the most effective, all one has to do is divide the value for the degree of purification of step X by the value for the preceding step. Thus, the DEAE chromatography step was the most efficient, with a 36 fold increase in enzyme purity.
c) Considering that the protein is pure after molecular sieve chromatography, 35 mg of 6-gluconate dehydrogenase were obtained. This corresponds to 41,5 % of the amount of enzyme initially present in the extract (refer to percent yield). Thus, in the initial extract we had:
35 mg / 41,5% = 75,9 mg
And this amount of enzyme was initially present in an extract containinfg a total of 70 000 mg proteins. Thus, in the initial extract we had:
(75,9 mg / 70 000 mg) x 100 = **0,108 % of 6-gluconate dehydrogenase**

3.9 Protein Purification.

Why is SDS omitted when proteins need to undergo isoelectric focusing?

The aim of isoelectric focusing is to separate proteins according to their pI, thus according to their charge at different pH values. Adding SDS to the protein sample would give all proteins the same charge density and would prevent their separation by this type of electrophoresis.

3.10. Protein Purification.

A series of proteins with known molecular mass and an enzyme of unknown molecular mass are separated by chromatography on a Sephadex G-200 column. The elution volume (Ve) for each protein is indicated in the table below. Estimate the molecular mass of the unknown protein.

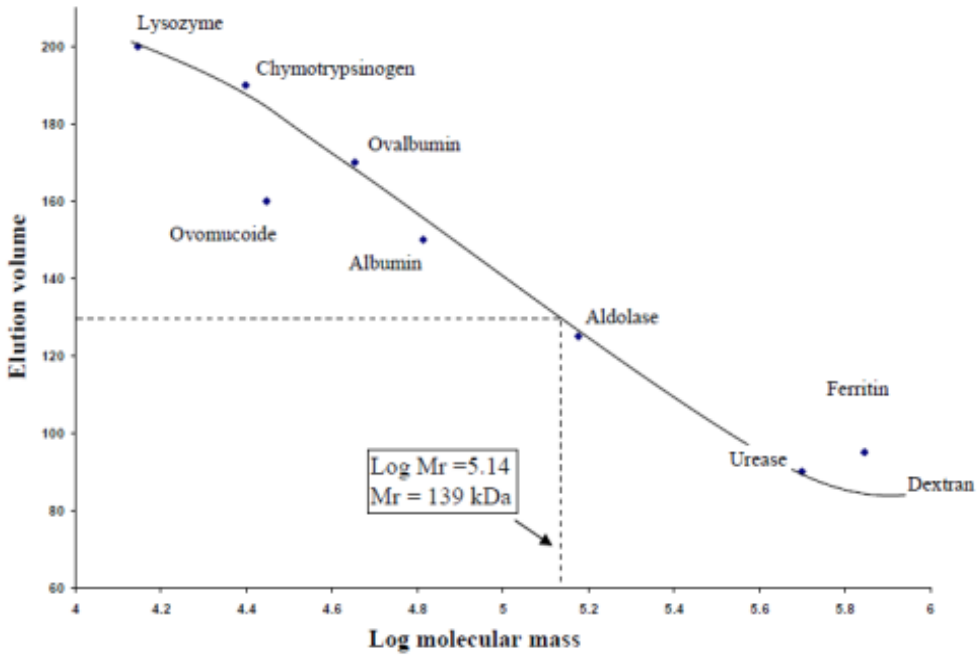
To determine the molecular mass of our unknown protein, we must first draw the graph of the elution profile of our sandards as a function of the log of their molecular mass (see graph on following page).

Protein	Log Mr	V_{el} (ml)
dextran blue	6.000	85.00
Lysozyme	4.146	200.00
Chymotrypsinogen	4.398	190.00
Ovalbumin	4.653	170.00
serum albumin	4.813	150.00
aldolase	5.176	125.00

urease	5.699	90.00
Ferritin	5.845	92.00
ovomucoide	4.447	160.00

From this graph, we obtain a molecular mass of **139 kDa** for our unknown protein.
Note: ferritin and ovomucoid were excluded of the standard curve because they obviously behaved differently to the other proteins when subjected to molecular sieve chromatography.

Graph, problem 3.10



***3.11. Protein Purification.**

Referring to the previous problem, give a plausible explanation for the bizarre behavior ferritin's elution from the sephadex column.

Ferritin has an iron core, giving it a greater density than other proteins of similar size and influencing its behaviour when subjected to molecular sieve chromatography.

3.12. Protein Purification.

A student isolates a protein from anaerobic bacteria and analyses the protein by polyacrylamide gel electrophoresis containing SDS (PAGE-SDS). Following protein staining, a single band bappears, which excites the student's supervisor. To be certain, the supervisor suggests that the student run a second electrophoresis under *native* conditions (i.e. non-denaturing, or without SDS). This gel shows two bands after staining. Assuming no errors were committed during these experiments, explain the observations.

In the presence of SDS, all the proteins have an identical negative charge density: this is what allows us to use PAGE-SDS to determine the molecular mass of proteins. Therefore, two proteins of different pI but identical molecular mass will co-migrate as a single band on SDS -PAGE.

However, in the absence of SDS (thus under *native* or *non-denaturing* conditions), protein migration towards the positive or negative electrodes will be driven by its net charge, in other words by the protein's pI. In this case, two proteins of identical molecular mass but different pI will give two distinct bands upon gel staining.

3.13. Protein Purification.

A student from CHMI 2227 analyses bovine serum albumin (BSA) with a polyacrylamide gel electrophoresis (PAGE-SDS). During the experiment, the student forgets to add β- mercaptoethanol to the sample. When comparing his sample to those of his classmates he realizes that the molecular mass of his BSA sample determined by PAGE-SDS is 57 kDa, while all the other students (those that added β-mercaptoethanol) found a molecular mass of 68 kDa.

Explain this difference.

In the absence of β-mercaptoethanol, the protein's disulfide bonds remain intact. Thus, the protein will have a more compact shape and will migrate more rapidly during PAGE-SDS than the same protein whose disulfide bonds have been reduced.

--Draw the complete chemical structures of the products of the enzyme catalyzed reaction below, catalyzed by chymotrypsin. Include appropriate charges on the products, assume that the pH is 6

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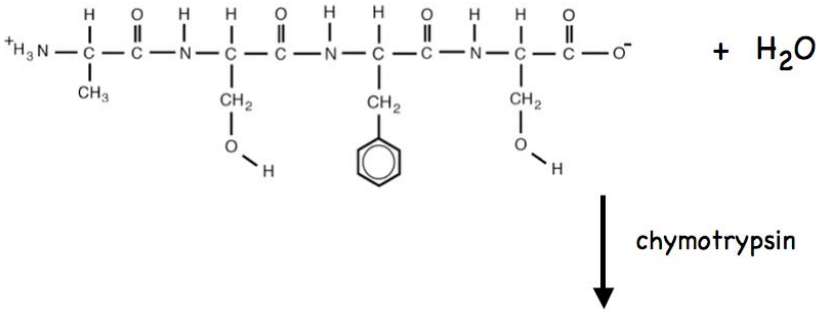

chymotrypsin cleaves peptide bonds that follow Phe or Tyr or Trp. So the product is a tripeptide of Ala-Ser-Phe and a serine amino acid.

-- If the previous problem used trypsin as an enzyme instead of chymotrypsin, how would your answer be different? Explain.

trypsin cleaves peptide bonds that follow Lys or Arg. So trypsin would not cleave this peptide.