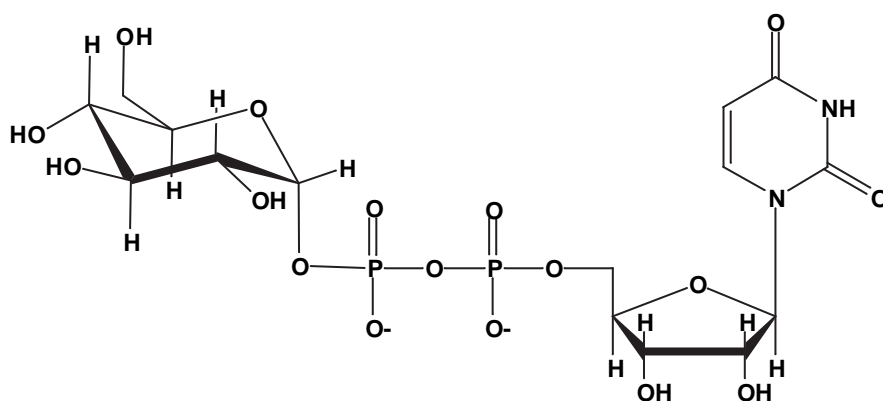


PLEASE WRITE YOUR ANSWERS ON A SEPARATE SHEET

Topics:

Bioinformatics of proteins
Amino acids
Peptides
Protein Purification
Protein Structure
Protein Folding
Unknown Assignment

1) Answer the following questions about UDP-Glucose.



- Identify all of the chiral centers by placing an asterisk next to them on the structure.
- Determine the absolute stereochemistry of C2'' (C2 of glucose) and C3' (C3 of ribose) (Hint: those should be on your list for 1a)
- Indicate all potential hydrogen bond donors by drawing a circle around the appropriate atom(s).
- Indicate all potential hydrogen bond acceptors by drawing a square around the appropriate atom(s).
- Indicate all of the acidic groups (pKa's between 1 – 7) by showing an equilibrium with their conjugate base (ex. $\text{ROH} \rightleftharpoons \text{RO}^-$).
- Indicate all of the basic groups (pKa's between 8 and 16) by showing an equilibrium with the conjugate acid.

2) The acid-base properties of the amino acids are critical for protein function. Answer the following questions about arginine and its titration behavior. You may want to look at

<http://cti.itc.Virginia.EDU/~cmg/Demo/front.html> while doing this problem. A link to this site is provided (Interactive Biochemistry) through the C484 web site.

- Draw the titration curve for the amino acid arginine and indicate pK_1 , pK_2 , pK_R and pI .
- Draw the forms of arginine that are dominant in each pH region.
- List the net charge of each species.

3) The following protein is human alpha chain hemoglobin.

```
>sp | P01922 | HBA_HUMAN HEMOGLOBIN ALPHA CHAIN - Homo sapiens  
(Human)  
VLSPADKTNV KAAWGKVG AH AGEYGAEALE RMFLSFPTTK TYFPHFDLSH  
GSAQVKGHGK KVADALTNAV AHVDDMPNAL SALSDLHAHK LRVDPVNFKL  
LSHCLLVTLA AHLPAEFTPA VHASLDKFLA SVSTVLTSKY R
```

- Identify any trypsin cleavage sites by indicating the resulting peptide fragments. (e.g. The first fragment is Val1-Lys7)
 - Identify any chymotrypsin cleavage sites (major cleavage sites only).
 - Identify any cyanogen bromide cleavage sites.
 - For the trypsin digests above, indicate the net charge of the largest peptide fragment at pH 5.0.
- 4) The peptide below is a fragment from a de novo designed protein called alpha-4. This molecule was made by Lynne Regan and William DeGrado and was one of the first designer proteins. Answer the following questions about this small polypeptide.

```
MGELEELLKK LKELLGPRR GELEELLKKL KELLGPRRG ELEELLKKLK  
HELIX I HELIX II HELIX III  
ELLGPRRGE LEELLKKLKE LLKG  
HELIX IV
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- Notice that each helical segment has the same sequence. Make a helical wheel representation of any one alpha helical segment.
- Draw the backbone of the helix. On your helix, add Glu₆ and Lys₁₀. Explain how this pair of amino acids helps to stabilize helix formation. Refer to your drawing to illustrate the point.
- Why do you think Regan and DeGrado chose the sequence PRR for the loops?

d) Use the helical wheel you made in part a and your knowledge of the forces that drive protein folding to predict what this protein might look like in solution. Draw a cartoon that represents this fold. Be sure to indicate the orientation of each helix.

5) Finding Information about Unknown Protein

Follow instructions on the handout that lead you through an exploration of the ExPASy and KEGG databases. You will use the information from those sites to answer questions about your unknown protein. Please submit copies of the NiceZyme and NiceProt pages for your unknown protein with your problem set.

a) What is the complete amino acid sequence of your protein? Provide it in Fasta format. Indicate on the protein sequence the peptides that you were provided as unknown fragments by highlighting those regions of the sequence.

b) What chemical reaction does your enzyme catalyze?

c) Does your enzyme require a cofactor for catalytic activity?

d) Interpret the E.C. number associated with your enzyme. Use the link to the Enzyme nomenclature page to find a hyperlink table that will allow you to explore the way proteins are classified.

e) In what metabolic pathway does your protein participate?

f) Is there a disease associated with a mutation of your protein? If so, what is it? If there are multiple, describe one. Include information on what that disease is, what the mutation is that causes it and whether the disease is related to altered activity, altered expression levels or improper regulation of the activity.

g) Tell me something you discovered about your unknown protein in step 13 of the instructions for exploring ExPASy.