Princeton High School

Protein Modeling 19-20 Captains' Tryouts

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Score: __ / 359

Names:

Part 1: Amino Acids

In which direction are proteins synthesized? (4 pts)

N-terminus to C-terminus

What term describes the flexibility of the third base pair in tRNA anticodon binding? (4 pts)

Wobble

What charge is arginine at a pH of 12.3 according to the pKa table below? (18 pts)

-1

	Amino Acid	pKa Value		
	Name	Alpha Carboxy	+Alpha Amino	Side Chain
Non-Polar Amino Acids	Glycine	2.34	9.60	
	Alanine	2.34	9.69	
	Valine	2.32	9.62	
	Leucine	2.36	9.60	
	Isoleucine	2.36	9.68	
	Methionine	2.28	9.21	
	Phenylalanine	1.83	9.13	
	Tryptophan	2.38	9.39	
	Proline	1.99	10.60	
Polar Amino Acids	Serine	2.21	9.15	
	Threonine	2.63	9.10	
	Cysteine	1.71	10.78	8.33
	Tyrosine	2.2	9.11	10.07
	Asparagine	2.02	8.84	
	Glutamine	2.17	9.13	
Acidic Amino Acids	Aspartic Acid	2.09	9.82	3.86
	Glutamic Acid	2.19	9.67	4.25
Basic Amino acids	Lysine	2.18	8.95	10.79
	Arginine	2.17	9.04	12.48
	Histidine	1.82	9.17	6.04

Amino Acid Tutorials + Cheat Sheet Leah4sci.com/AminoAcids

What is the term for the pH at which the alpha amino group and the alpha carbon group is charged? What chemical term describes an amino acid at this pH? (12 pts)

Isoelectric point, zwitterion (6 each)

What stereoisomeric form are amino acids commonly in? (8 pts)
L

What type of acid is glycine? (think titration) (6 pts)

Diprotic acid

What are proteins that have permanently attached chemical groups other than amino acids called? What is this attached chemical group called? (8 pts)

Conjugated amino acids, prosthetic groups (4 each)

What amino acids are helix-breakers? (4 pts)

Glycine and Proline (2 each)

What special amino acid is present in collagen? What vitamin is important for collagen synthesis? (6 pts)

4-hydroxyproline, vitamin C (3 each)

Part 2: Protein Chemistry

If I wanted to separate two proteins different in size but both hydrophobic, which type of chromatography would I use and which protein would elute first, the smaller or the larger? (18 pts)

Size-exclusion chromatography. The larger protein would elute first because the smaller protein would be impeded by entering the small pores in the beads. (6 for correct chromatography, 12 for correct reasoning)

During protein purification, low salt concentrations increase the solubility of proteins but high salt concentrations cause elution. Give the term for these effects and describe why it occurs. (30 pts)

Salting-in and salting-out (10 pts)
Increasing the ionic strength of the solution causes proteins to be better dissolved in solution because there is greater activity of the solvent (typically water) so the free energy of the system is reduced by having greater electrostatic interactions. However, at high salt concentrations, the salt outcompetes the protein for interactions with the solvent and causes the protein-protein interactions to be more favored than the solvent-protein interactions, thus concentrating the protein. (20 pts, 4 per underlined)

What does sodium dodecyl sulfate (SDS) do and how does it work? (5 pts)

SDS acts as a detergent, binding to both hydrophobic and hydrophilic molecules. SDS binds to the hydrophobic amino acids of the protein and thereby unfolds it. (2 pts for detergent, 3 pts for binding to hydrophobic domains)

What two properties does 2D-electrophoresis use to separate proteins? (8 pts)

Isoelectric point and size (4 each)

Describe the purpose and procedure of Edman degradation. (18 pts)

Edman degradation is used to sequence an amino acid sequence. A series of chemicals are used to degrade the peptide bond of the amino acid on the amino

terminus, removing only the amino-terminal amino acid. This amino acid is then isolated through certain organic solvents and the cycle repeats again.

(6 pts for purpose, 12 pts for correct procedure)

What three amino acids are in the catalytic triad? (6 pts)

Histidine, Serine, Aspartate (2 each)

What character does the peptide bond have and why? (10 pts)

Partial double bond character because of resonance (4 pts for identification of partial double bond character, 6 pts for resonance)

Part 3: Protein Folding

What is the primary thermodynamic force driving protein folding? (3 pts)

Entropy

What are two simple rules exhibited in protein folding patterns? (8 pts)

Hydrophobic amino acids are on the inside of the protein. Maximize hydrogen bonds on the inside. (4 pts for each

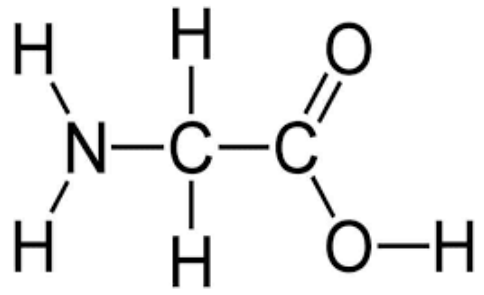
What is the common handedness of the alpha helix? (6 pts)

Right

Give an example of another type of alpha helix, denoting its *i* + x hydrogen bonding. (4 pts)

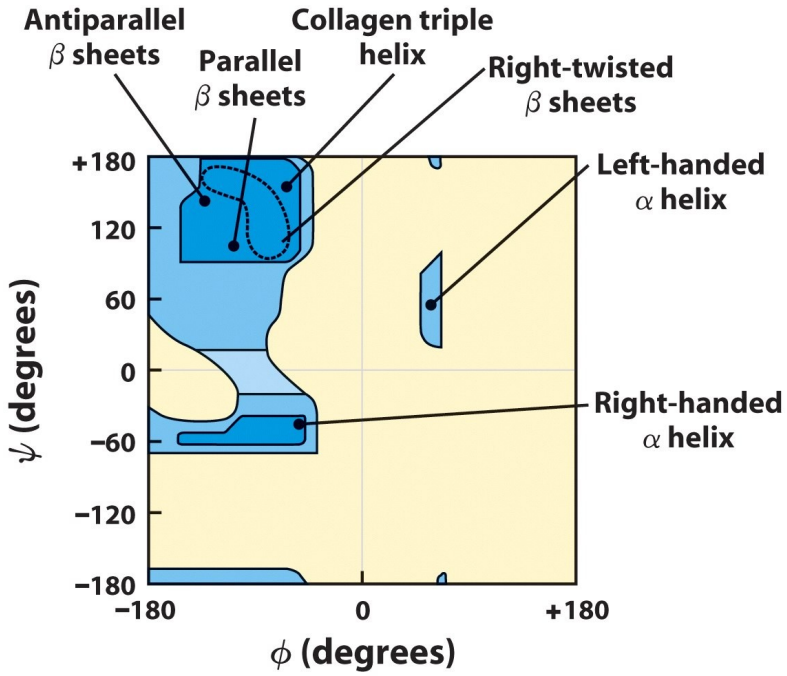
3_{10} helix (i + 3), pi (the Greek letter) helix (i + 5) (4 pts for one)

Identify phi and psi on the amino acid below: (6 pts)



phi is the angle between the central carbon and the amino group, psi is the angle between the central carbon and the carboxyl carbon. (3 pts each)

What is the name of the below graph? What is its purpose? (8 pts)



Ramachandran plot. Used to determine what protein secondary structures are possible and would form based on phi and psi angles.
(3 pts for name of graph, 5 pts for purpose)

How many amino acids apart are positively and negatively charged residues or two aromatic residues? (6 pts)

3

Name the five different kinds of constraints on the stability of an *a*-helix. (15 pts)

1) Electrostatic repulsion or attraction between successive residues
2) Bulkiness of adjacent R groups
3) Interactions between residues 3 to 4 residues apart
4) Occurrence of Pro or Gly residues
5) The inherent electric dipole at the end of the alpha helix

3 pts each

What type of protein is keratin? (4 pts)

Fibrous

What was the first protein to have its structure determined by X-ray crystallography? (5 pts)

Myoglobin

Describe how NMR spectroscopy works and how it can be used to determine protein structure: (12 pts)

NMR takes advantage of nuclear spin angular momentum which is a property of atomic nuclei that only certain atoms possess such as ^{1}H or ^{13}C. Giving the atom a pulse of electromagnetic radiation, typically in the radio range, some of that energy is absorbed as the nuclei resonate and the absorption spectrum that results contains information about the spectrum and their immediate chemical environment, letting scientists to determine what amino acids are involved and how the protein folds. (4 pts for mentioning each underlined portion)

Describe what a molten globule is. (4 pts)

It is an intermediate structure between the unfolded protein and the native conformation, having secondary structure due to hydrophobic interactions but side chains that are not entirely fixed.

What enzyme catalyzes the formation of disulfide bonds in the ER? (4 pts)

Protein disulfide isomerase

Part 4: CRISPR-Cas9

What does CRISPR stand for? (8 pts)

Clustered Regularly Interspaced Short Palindromic Repeats

What is the original purpose of CRISPR in bacteria? (4 pts)

It acts as a prokaryotic immune system, recognizing and destroying foreign DNA that it has encountered before.

What does CRISPR induce in the genomic DNA? (6 pts)

Double-stranded breaks

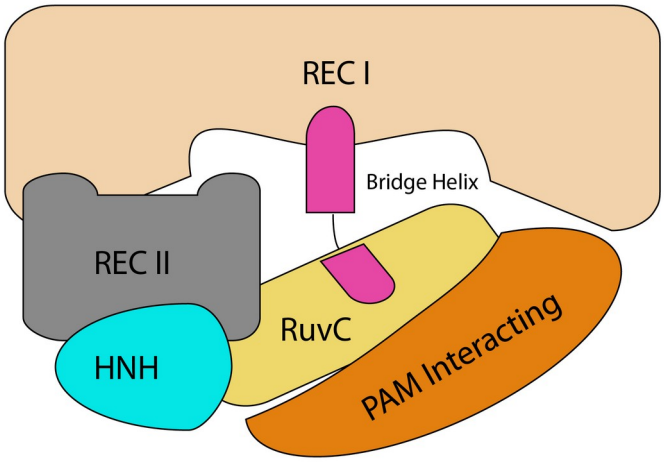
What two types of repair can CRISPR perform and what are they important for? (6 pts)

Error-prone non homologous end-joining (NHEJ) used for loss of function screens or homology directed repair important for gene editing. (3 pts each)

What does PAM stand for? (2 pts)

Protospacer adjacent motif

Identify the functions of each domain: (20 pts)



REC I and II: has three alpha-helices that help recognize the guide RNA.
HNH: nuclease utilizing one-metal ion *b-a-b* motif
RuvC: nuclease utilizing two-metal ion system.
Bridge helix: arginine rich and responsible for recognizing sgRNA and conforming apo-Cas9 into holo-Cas9, also helps initiate cleavage.
PAM Interacting: recognizes PAM site on the target strand, a site which helps differentiate between self and non-self DNA.

(4 pts each)

What type of DNA sequence are the repeats? (hint it's in the name) What structure does the spacer form that is important for recognition by Cas1 and Cas2? (6 pts)

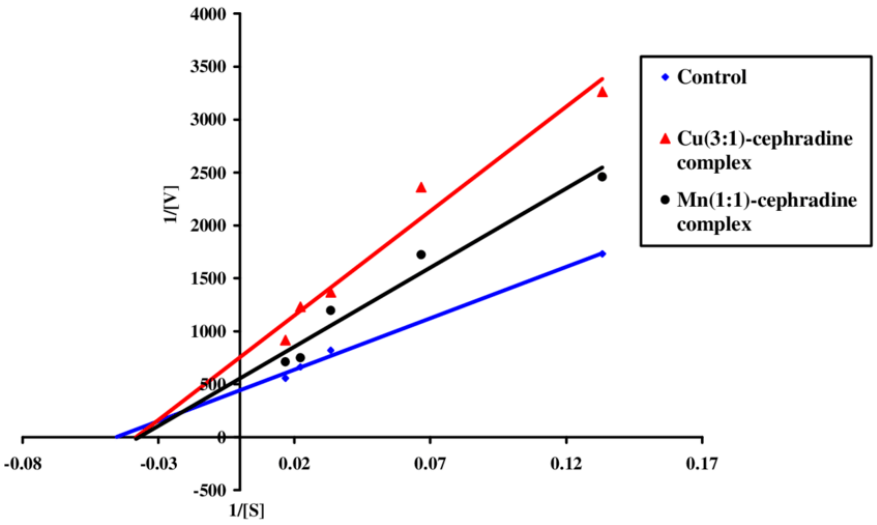
Palindromic. The spacer forms a cruciform structure that recruits Cas1 and Cas2 (2 pts for palindromic, 4 pts for cruciform)

Part 5: Biochemistry

What (infamous) equation describes the relationship between Vmax, Km, and substrate concentration? (5 pts)

Michaelis-Menten equation

According to the Lineweaver-Burk Plot below, what is the the K_m and V_{max} of "Control"? (12 pts)



Km is -(1/-0.04) and Vmax is 1/750 (6 pts each)

Derive the Michaelis-Menten Equation starting from $v_0 = k_{cat}[ES]$ (36 pts):

Enzyme Kinetics

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P \qquad (1)$$

- Michaelis-Menten

$v_0 = k_{cat} [ES]$

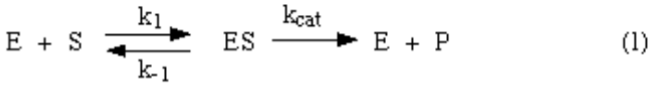
- Assumptions
  - Product is negligible
  - Substrate is in excess of enzyme
  - Steady state approximation: [ES] is constant

$k_{-1} [ES] + k_{cat} [ES] = k_1 [E] [S]$

$[ES] (k_{-1} + k_{cat}) = k_1 [E] [S]$

$(k_{-1} + k_{cat}) / k_1 = [E] [S] / [ES]$

Enzyme Kinetics



(1)

$(k_{-1} + k_{cat}) / k_1 = [E] [S] / [ES]$

Let $K_M = (k_{-1} + k_{cat}) / k_1$

$[E] [S] / [ES] = K_M$

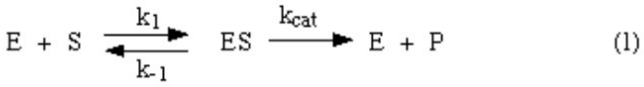
$[E]_0 = [E] + [ES]$, $[E] = [E]_0 - [ES]$

$K_M = ([E]_0 - [ES]) [S] / [ES]$

$K_M [ES] + [ES] [S] = [E]_0 [S]$

$[ES] = [E]_0 [S] / (K_M + [S])$

Enzyme Kinetics



(1)

$[ES] = [E]_0 [S] / (K_M + [S])$

$v_0 = k_{cat} [E]_0 [S] / (K_M + [S])$

$V_{max} = k_{cat} [E]_0$

$v_0 = V_{max} [S] / (K_M + [S])$

4 pts for assumptions, 4 pts for first slide, 8 pts for letting $K_m = (k_{-1} + k_{cat})/k_1$, 4 pts for second slide, 4 pts for third slide, 10 pts for just writing out Michaelis-Menten equation.