Biochemical principles

1. **Compare and contrast the major non-covalent forces: H-bonds, hydrophobic effect, ionic bonds, Van der Waals interactions**
    a. H-bonds: O, N and OH, NH. 20kJ/mol Gibb's free energy
    b. Hydrophobic effect: occurs between large, nonpolar molecules
    c. Ionic: between charged molecules
    d. Van der Waals: between large molecules
2. **Define enthalpy and entropy and explain how the changes in both contribute to Gibbs energy**
    a. Gibb's energy is enthalpy minus entropy.
    b. In the hydrophobic effect, the separated state has more enthalpy, since there are more hydrogen bonds and they have a cage-like structure. The joined state has more entropy, since the water molecules are able to move more freely.
3. **Compare and contrast delta G knot and delta G, and how they relate to Keq**
    a. Delta G = Delta G knot + RTln[B]/[A]. That is, Delta G tells you how far you are from Delta G which is equilibrium
    b. Delta G = 0 means you are at equilibrium, Delta G knot = 0 means 50/50 at equilbrium
4. **Describe the properties of water that determine the conformation of a biological molecule and facilitate interactions between biological molecules**
    a. Hydrogen bonds because of polarity. Hydrogen interactions are also directional.
5. **Define pH**
    a. Measures proton concentration
6. **Estimate the proportion of protonated/deprotonated molecules at various pHs**
    a. At pKa, 1:1. For each pH down 10x more protonated, and for each pH up, 10x more deprotonated. At a lower pH, excess H+ in solution, so will get protonated. At a higher pH, donate proton to solution.
7. **Estimate the ratio of acid/base forms of a molecule when given sufficient information, and explain how this ratio is related to pKa and pH**
    a. How many magnitudes away from pKa? For each magnitude, 10-fold difference.
    b. If below pKa, more protonated because more willing to take protons from environment. If above pKa, more deprotonated since more willing to share protons.
8. **Draw and interpret panels of populations of molecules in reference to the equilibrium**
    a. A ←→ B, K_eq = [B]/[A]
    b. Favor products if Keq > 1, favor reactants if Keq < 1
    c. 10 fold difference in concentration corresponds with 5kJ/mol Gibb's
    d. If more products, Gibb's is negative. If more reactants, Gibb's is positive.
9. **Predict the behavior of polar, apolar, and amphipathic molecules in aqueous environments**
    a. Amphipathic molecules, nonpolar will have hydrophobic effect

Protein Structure

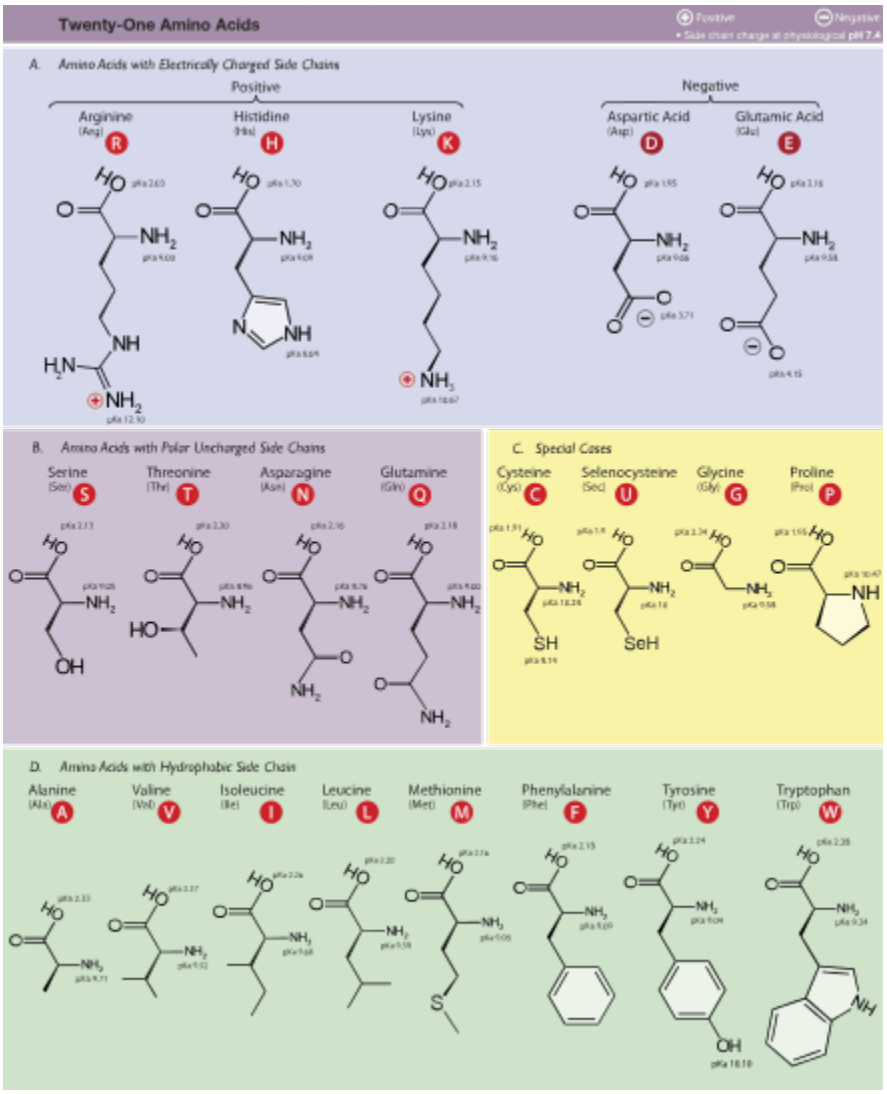
1. **Given the full name, three letter code, one letter code, or structure of an amino acid, list or draw the other three**

Table 2.2 Abbreviations for amino acids

Amino acid	Three-letter abbreviation	One-letter abbreviation	Amino acid	Three-letter abbreviation	One-letter abbreviation
Alanine	Ala	A	Methionine	Met	M
Arginine	Arg	R	Phenylalanine	Phe	F
Asparagine	Asn	N	Proline	Pro	P
Aspartic acid	Asp	D	Serine	Ser	S
Cysteine	Cys	C	Threonine	Thr	T
Glutamine	Gln	Q	Tryptophan	Trp	W
Glutamic acid	Glu	E	Tyrosine	Tyr	Y
Glycine	Gly	G	Valine	Val	V
Histidine	His	H	Asparagine or aspartic acid	Asx	B
Isoleucine	Ile	I	Glutamine or glutamic acid	Glx	Z
Leucine	Leu	L			
Lysine	Lys	K			

a.

b.

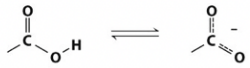
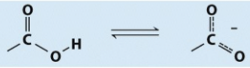
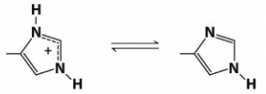
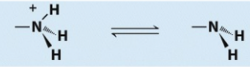
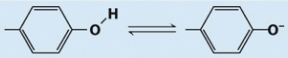
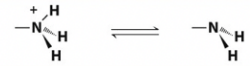
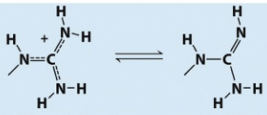


2. **Identify amino acids (structure, pKa) and apply knowledge of their characteristics (hydrophobicity, size, shape)**

Know these

Our Values

Table 2.1 Typical pK_a values of ionizable groups in proteins

Group	Acid ⇌ Base	Typical pK_a*	Our Values
Terminal α-carboxyl group		3.1	2.0
Aspartic acid Glutamic acid		4.1	4.0
Histidine		6.0	6.0
Terminal α-amino group		8.0	9.0
Cysteine		8.3	8.5
Tyrosine		10.9	11.0
Lysine		10.8	11.0
Arginine		12.5	12.5

*pK_a values depend on temperature, ionic strength, and the microenvironment of the ionizable group.

a.

3. **Describe the properties of a peptide bond**
    a. Formation removes a water
    b. Double bond resonates, preventing rotation
    c. 9B identify
4. **.**
5. **Draw the molecular structure of a peptide. Identify the correct ionization states and calculate the charge at a given pH**
    a. At pKa, +/-0.5 depending on whether the molecule goes from (neutral to negative) or (positive to neutral)
6. **Compare and contrast protein purification techniques discussed in class, and propose, interpret or predict results of protein purification techniques**
    a. Isoelectric focusing: negatively charged molecules need many protons to be neutral, so will go to low pH. Positively charged molecules few protons to be neutral, so high pH.
    b. Ion chromatography: cation has negative beads that attract positive, anion has positive beads that attract negative. Choose a pH between pIs.
    c. SDS-Page: small molecules move faster, smaller elute first. Denatures protein, gives it uniform negative charge.
    d. Size exclusion/gel filtration: small molecules get stuck in matrix/beads, bigger elute first. Does not prevent molecules sticking together.
7. **Explain how identical protein molecules reliably adopt the same structure**
    a. Anfinsen experiment
    b. Hydrophobic effect, determined by primary structure, helps determine native state
    c. Allowing cysteine to form bridges first ends up with scrambled state
8. **Identify covalent and non-covalent interactions between amino acids that affect the conformation of a protein**
    a. Primary structure - amino acid chain
    b. Secondary structure - alpha helices and beta sheets
        i. Beta: R groups alternate face up and down. NPNPNP

    ii. Alpha: R groups face away from center of helix NPPNPPPN
    iii. Turns - small molecules in turns
    iv. Loops
    v. In beta and alpha, R groups hydrogen bond together, but beta more likely to aggregate because they are non-specific, they do not a particular primary structure to aggregate
  c. Tertiary structure - protein folding
    i. Cysteines can form disulfide bonds
    ii. Hydrophobic can get pushed to the inside
  d. Quaternary structure - protein subunit interactions
9. **Predict hydrogen bonding interactions that occur in alpha helices and beta sheets**
  a. Alpha: Hydrogen bonds between the backbone (between *i* and *i+4*) bind the helix together, with CO's pointing towards the C terminus and NH's pointing towards the N terminus.
  b. Beta: Hydrogen bonds between the backbone bind multiple sheets together. Antiparallel is more stable than parallel.
10. **Represent protein folding as an equilibrium and describe the relative contributions of different forces on the overall equilibrium constant**
  a.
11. **List factors that favor protein folding or unfolding (enthalpy, entropy, temperature, pH). Predict the effect of changing those factors on protein folding, interactions, and binding**
  a.
12. **Describe possible outcomes of protein misfolding**
  a. No active binding site, lose its function
13. **Describe the mechanism of how specific molecules (detergents, reducing agents, or proteins) affect folding**
  a. Detergents: cover hydrophobic regions
  b. Reducing agents: denatures, e.g. mercaptoethanol and disulfide

Protein function

1. **Explain how changes in protein sequence might affect protein structure and function**
  a. Proline can break secondary structure
  b. Nonpolar versus polar can affect hydrophobic effect
2. **Define binding affinity and Kd**
  a. Binding affinity: strength of two molecules binding together. High binding affinity means that a molecule will stick to it, e.g. R state binds O2 well
  b. Kd: dissociation constant. high dissociation means that molecules do not stick easily, e.g. T state's high Kd means that O2 will not stick easily to it.
3. **Draw and interpret a binding plot and label the Kd**
  a. Kd is 50% fractional saturation
  b. Shift to the right means higher dissociation and lower binding affinity, while shift to the left means lower dissociation and higher binding affinity
4. **Apply principles of equilibrium to protein function**
  a. Kd = [P][L]/[PL]. This can be useful, since you can move [L] to the other side, and this tells you the relative concentration of P and PL.
5. **Compare and contrast myoglobin and hemoglobin**
  a. Myoglobin is a monomer with no allosterics, hemoglobin a tetramer with cooperative binding
  b. Myoglobin curve is rectangular hyperbola, while hemoglobin is more S shape.
6. Explain the role of allostery, cooperativity, and conformational change in hemoglobin function
7. List ways proteins can be regulated and describe specific examples

8. Predict the impact of a ligand on a given equilibrium and on protein function
9. Predict the effects of molecules (BPG, proton, CO_2) on hemoglobin cooperativity and describe the physiological importance of each