Name Key

This exam is schedule for 75 minutes and I anticipate it to take the full time allotted. You are free to leave if you finish.

1. Which is not a class of enzymes?

Transferase Fumarase Lyase Oxidoreductase Isomerase

2. Which of these is not commonly involved in redox reaction in biological reactions?

PLP NADH NADPH metal ions $FADH_2$

3. Which of these residues is never involved in general acid-base catalysis?

Histidine Aspartic Acid Lysine Cysteine Arginine None of the above

4. Formation of a Schiff Base is always an example of what type of catalysis?

General Acid-Base Metal ion Covalent Proximity

5. Which of the following is not an example of a secondary messenger?

PKC IP3 cAMP Ca^{2+} ← not really a correct answer, but I'll accept it.

6. Based on the model we discussed for enzyme kinetics, which constant refers to the rate limiting step?

K_M k_1 k_{-1} k_2 k_{-2}

7. The lysozyme mechanism proceeds through a covalent intermediate.

True False

8. What amino acid forms a covalent bond with the substrate in trypsin, chymotrypsin, and elastase?

Histidine Aspartic Acid Lysine Asparagine Arginine None of the above

9. Uncompetetive inhibitors interact with the active site of an enzyme

True False

10. Which corresponds to conformation of hemoglobin that binds O_2 with high affinity?

R-State S-State T-State U-State Allstate

11. Which of the following increases the affinity of hemoglobin for O_2? Choose all that apply.

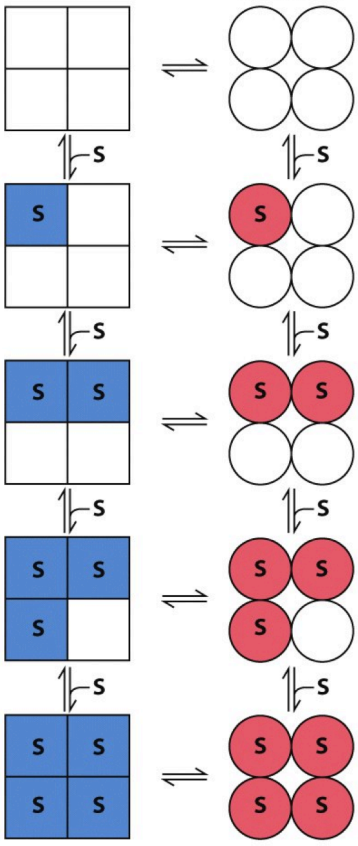
CO_2 Basic pH 2,3-BPG Acidic pH CO

12. Which of the following is not a type of gated ion channel?

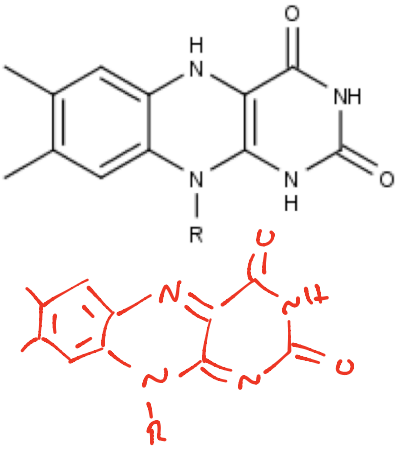
Ligand-gated redox-gated voltage-gated mechanosensitive

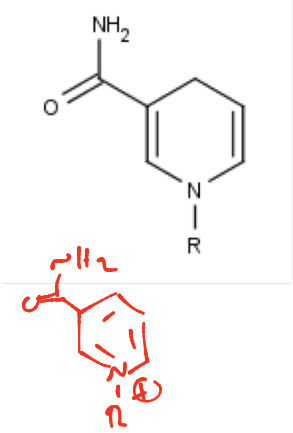
13. The image to the right is consistent with which model of allostery?

Symmetry – or MWC molel



14. Draw the oxidized form of each cofactor:





15. Given the following data, please calculate k_{cat} (1^{st} order) and catalytic efficiency (2^{nd} order). **You must include units**.

K_M = 0.1 μM V_{max} = 10 μM s^{-1} $[E]_{total}$ = 0.1 μM

$k_{cat} = \frac{V_{max}}{[E]_{tot}} = 100\ s^{-1}$

k_{cat} 100 s^{-1}

Catalytic Efficiency 1000 $\mu M^{-1} s^{-1}$

$\frac{k_{cat}}{K_M}$

16. What would a graph of J_A vs. [A] look like for **mediated** transport?

17. What assumptions are needed to derive the following equation: $v^0 = \frac{v_{max}[S]}{K_M+[S]}$

[ES] is constant ($\frac{\Delta[ES]}{\Delta t} = 0$)

The enzyme only exists in the E and ES form ($[E]_{tot}$ = [ES] + [E].

Other assumptions are made about the model, but none are really necessary to derive the equation. Credit is awarded for other correct assumptions (e.g. $k_2 << k_{-1}$)

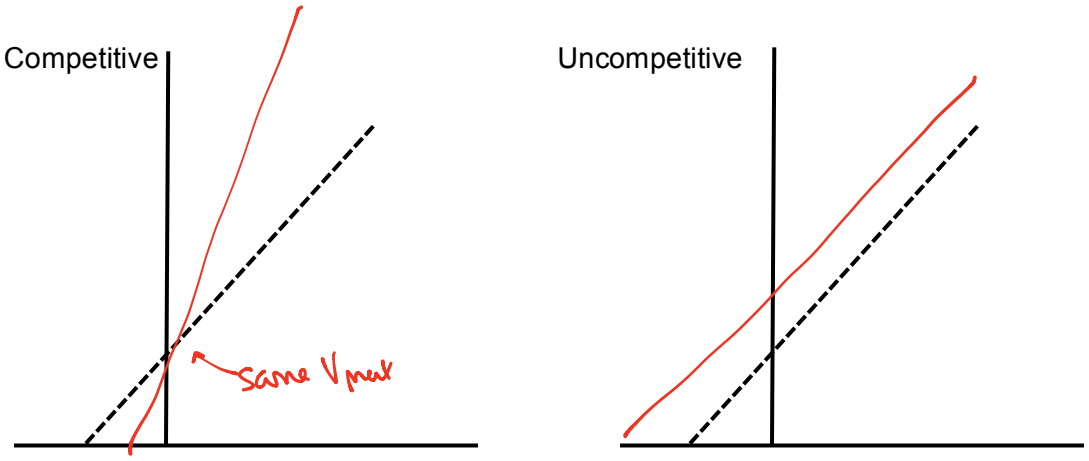
18. How does O_2 binding to hemoglobin induce a change from the T state to the R state?

O_2 binding to Fe^{2+}/heme casues the proximal histidine to move slightly toward the heme group. This small change causes the F-Helix to move and causes the entire $\alpha\beta$ dimer to rotate relative the other $\alpha\beta$ dimer.

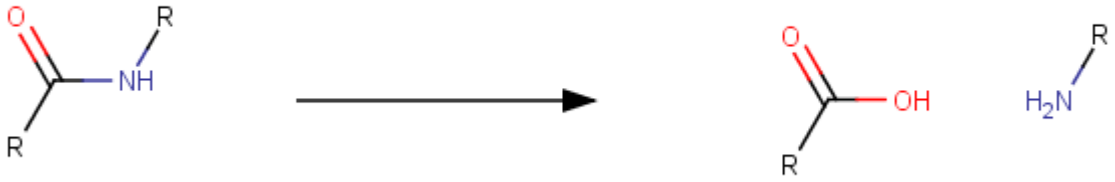
19. The activity of nearly all enzymes is pH-sensitive. Please clearly justify this observation.

Nearly all enzymes rely on general acid/base catalysis. Therefore, there must be an optimal pH to ensure that the general acids are protonated and the general bases are deprotonated. If the pH shifts below the pKa of a general base, that base gets protonated and will not be nearly as effective at deprotonating a substrate. The same arguement can be made for a general acid. Basic pH will make them deprotonate and won't be able to donate a proton when they need to.

20. Sketch a Lineweaver-Burk plot for competitive and uncompetitive inhibition. The line that is drawn is from data without a competitor present. Please label the axis on at least one plot.

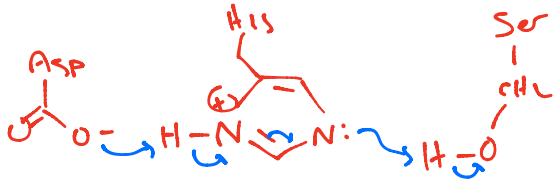


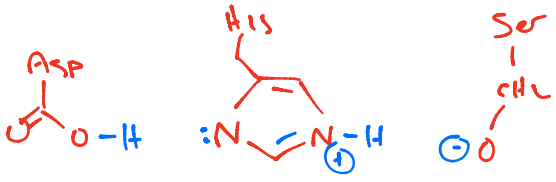
21. The reaction below is catalyzed by a very common class of enzymes called serine proteases. These enzymes use serine as a nucleophile to initiate the reaction. For the next several questions, please refer to this reaction. You may use the blank sheet of paper at the end of the exam if you need more space.



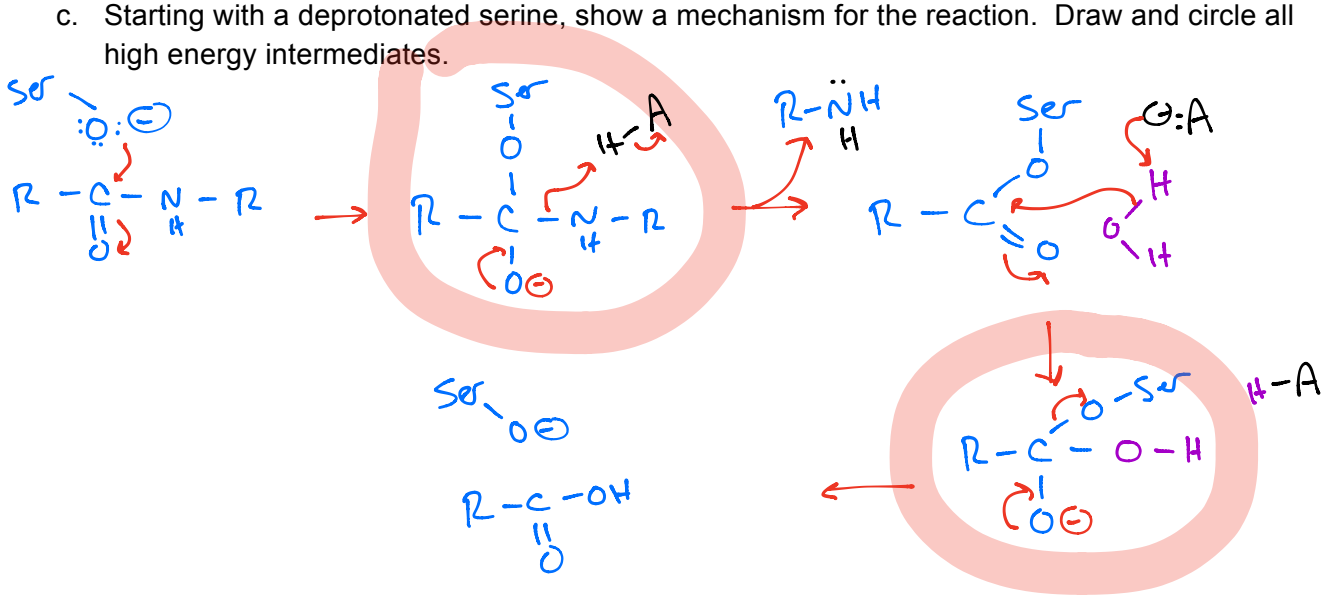
a. What enzyme class catalyzes this reaction? hydrolase

b. Serine is typically not a good nucleophile. How do serine proteases activate serine for nucleophilic attack? A sketch is required for full credit. Catalytic Triad (Asp/His/Ser)





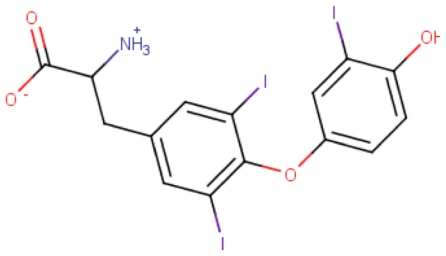
c. Starting with a deprotonated serine, show a mechanism for the reaction. Draw and circle all high energy intermediates.



d. Based on your mechanism, sketch a diagram that shows the energy of the reaction as a function of the reaction progress. You need to show both transition states for full credit

e. In addition to providing a way to activate Ser as a nucleophile, how else do these enzymes increase the rate of the reaction? Give a specific example. They stabilize the transition state. Both of the high energy intermediates identified in this reaction are tetrahedral intermediate that contain an oxyanion. Enzymes have an oxyanion hole (a partially or fully positive cluster of amino acids or metal ions) positioned to accept the anion.

22. The most active pituitary hormone, T_3 (triiodothyronine), is shown below. This hormone is decarboxylated by a PLP-dependent enzyme and then further deiodonated by selenium containing enzymes to produce a different hormone, T_1a. T_1a is an activator of TAR1, a GPCR in neurons that triggers increased synthesis of cAMP.



a. Discuss how T_1a can trigger cAMP synthesis.

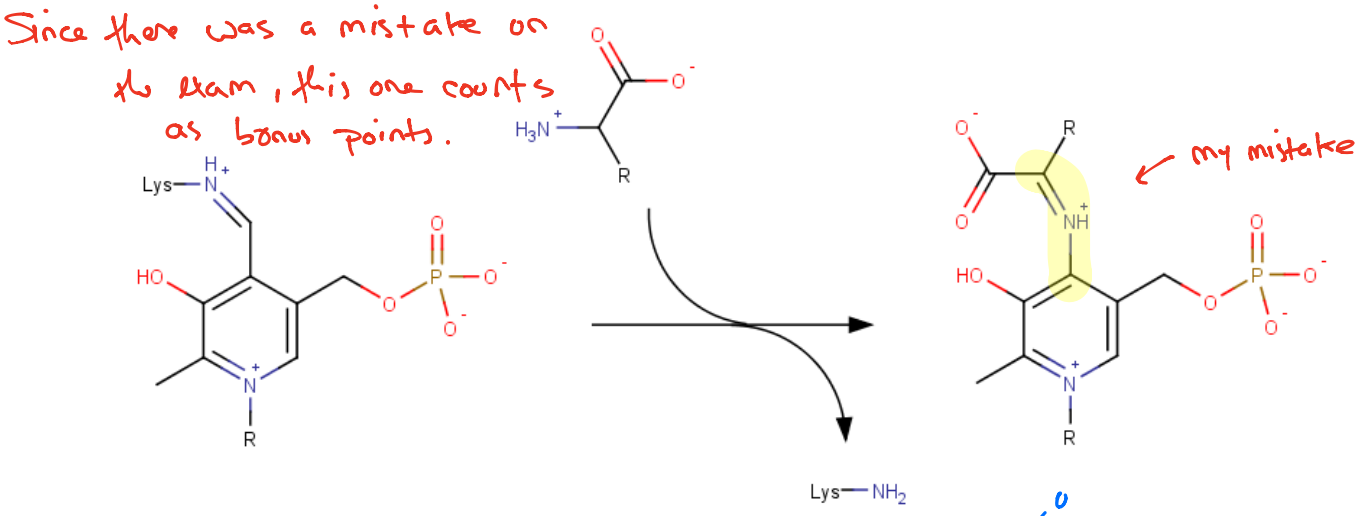
This molecules interacts with a GPCR. Once bound to the receptor, the heterotrimeric ($\alpha_{GDP}\beta\gamma$) subunits dissociate from the receptor (this is inside the cell). GTP can now displace GDP from the active site causing the $\beta\gamma$ subunit to dissociate from the α_{GTP}. The α_{GTP} interacts with adenylate cyclase, activating it, and triggering production of cAMP. The cAMP production is terminated when GTP is hydrolyzed to GDP.

b. What class of enzyme is this decarboxylase? lyase

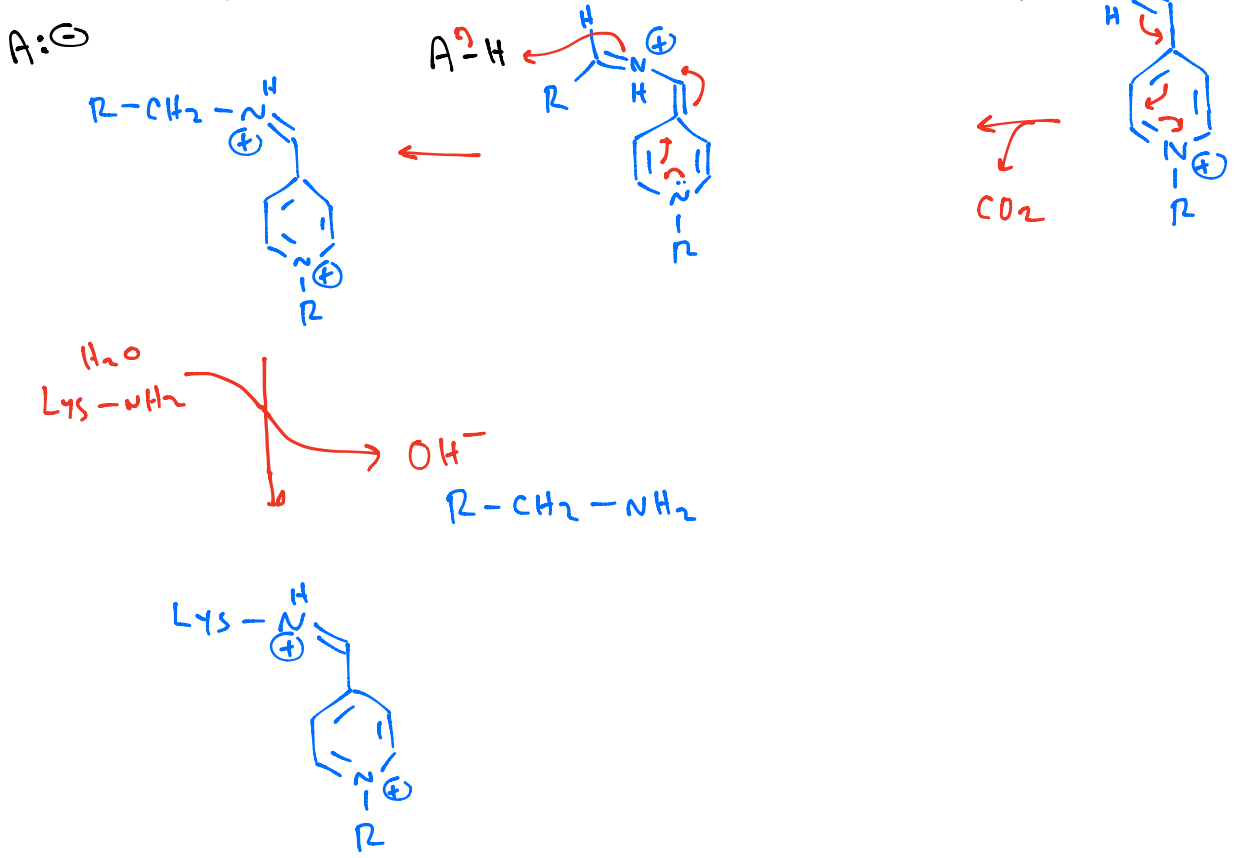
c. How do PLP-dependent enzymes ensure that the proper bond is broken? Only the bond in the plane of the pi system is reactive. Typically, these enzymes present a (+) pocket to orient the CO_2- ensuring that the Cα is oriented properly.

d. Please propose a mechanism of this reaction. The first step is shown. You may abbreviate the side chain as 'R' and show the last step in a similar way that I have drawn the 1st step.

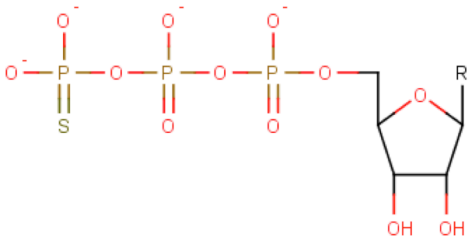
Since there was a mistake on the exam, this one counts as bonus points.



Starting with the corrected structure



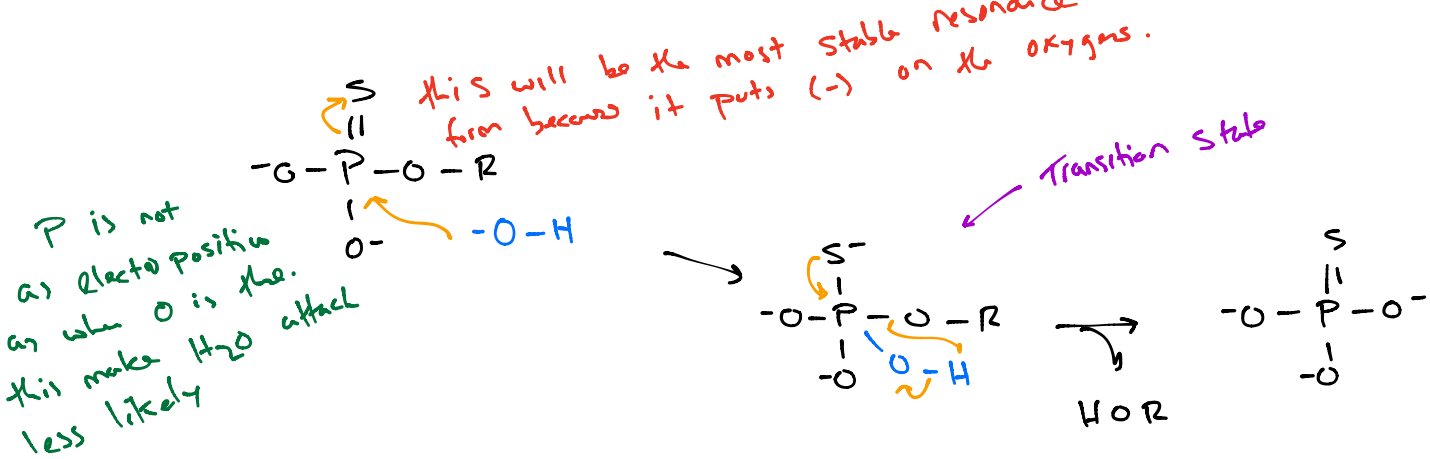
23. The molecule shown here is a sufficient substrate analogue to determine the structure of GTPases; in fact, hydrolysis of the gamma phosphate has never been observed. However, it is hydrolyzed by ATPases quickly enough that it cannot be used for structural studies.



a. What is meant by a substrate analogue and why are they needed for structural studies? They have all the structural properties of the actual substrate but they are not reactive.

b. Noting that the turnover number for GTPase is ~0.02 min^{-1} and ATPase ~400 min^{-1}, propose a reason for this observation. Reaction rates are most strongly associated with the activation energy. Since the GTPase has a significantly faster k_2, the Ea must be much higher. This happens for two reasons that both relate to the electronegativity difference between oxygen and sulfur. First, the gamma phosphorus is not as electropositive, so its potential for nucleophilic attack is much lower. Second, the transition state ($P\text{-}S^-$) contains a sulfur anion; since sulfur is not as electronegative as oxygen, this transition state will be at a higher energy.

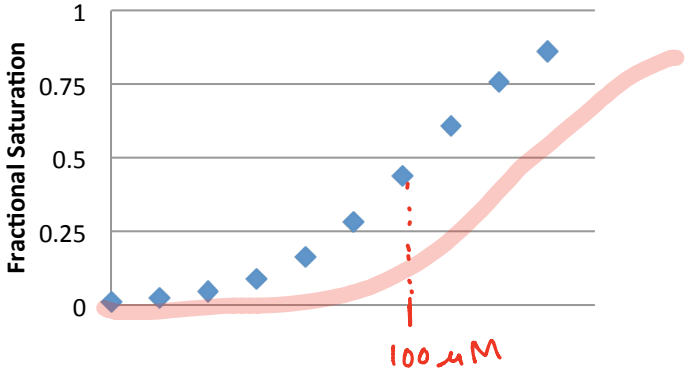
Now, these observations are also true for ATPase, but the activation energy is already so small that catalysis will still happen – at a reduced rate for sure, but still quickly enough that these molecules can't be used as substrate analogues.



24. Based on the data to the right:

a. Approximate K_D for the substrate binding to this enzyme. 100 μM (pdf cut off the x-axis, but its on your exam)

b. Show how the curve would change if a competitive inhibitor were present.



Amino Acid	α-carboxylic acid	α-amino	Side chain
Alanine	2.35	9.87	
Arginine	2.01	9.04	12.48
Asparagine	2.02	8.80	
Aspartic Acid	2.10	9.82	3.86
Cysteine	2.05	10.25	8.00
Glutamic Acid	2.10	9.47	4.07
Glutamine	2.17	9.13	
Glycine	2.35	9.78	
Histidine	1.77	9.18	6.10
Isoleucine	2.32	9.76	
Leucine	2.33	9.74	
Lysine	2.18	8.95	10.53
Methionine	2.28	9.21	
Phenylalanine	2.58	9.24	
Proline	2.00	10.60	
Serine	2.21	9.15	
Threonine	2.09	9.10	
Tryptophan	2.38	9.39	
Tyrosine	2.20	9.11	10.07
Valine	2.29	9.72	