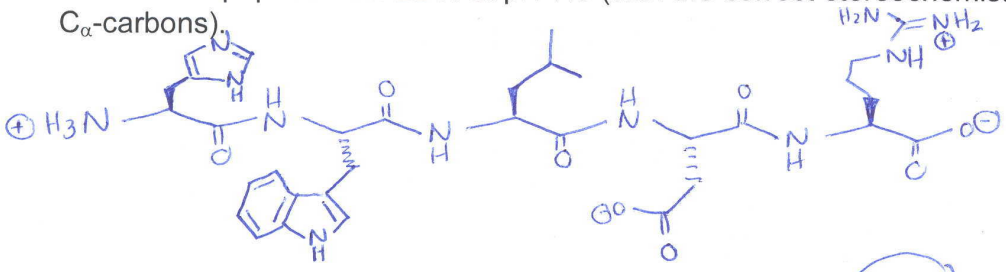
1. a. Draw the peptide: **HWLDR** at pH 7.0 (with the correct stereochemistry at ALL C_α-carbons). (15 pts)



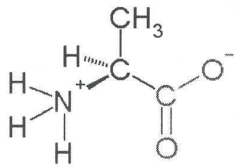
b. What is the total charge of the peptide at pH 1? (3 pts)

+3

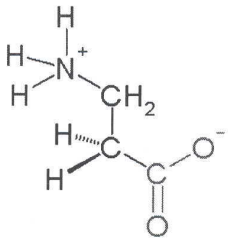
c. What is the total charge of the peptide at pH 14? (3 pts)

-2

2. β-Peptides are protein mimics that contain β-amino acids, which differ from α-amino acids in that the amino group is attached to the β- rather than the α-carbon as shown below. Naturally-occurring β-amino acids, like β-alanine, exist but they have not yet been found in ribosomally-synthesized peptides or proteins. However, β-amino acids have been incorporated into synthetic peptides and proteins and found to adopt structured folds and can also be used to design orally-active peptide-like drugs that are protected from degradation by proteases.

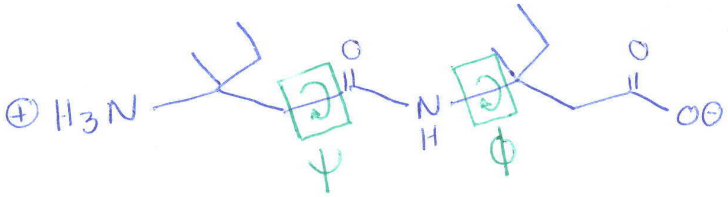


L-α-alanine



β-alanine

a. Like α-peptides, the β-peptide backbone is also formed by the linkage of amino acids by amide bonds. Draw a β-isoleucine dipeptide and mark the ϕ and ψ dihedral angles. (9 pts.)

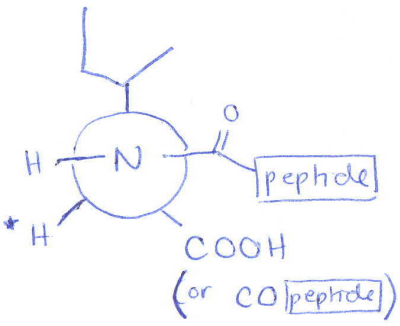


You must draw a dipeptide to get any credit. The angles ψ + ϕ must surround the peptide bond to get credit.

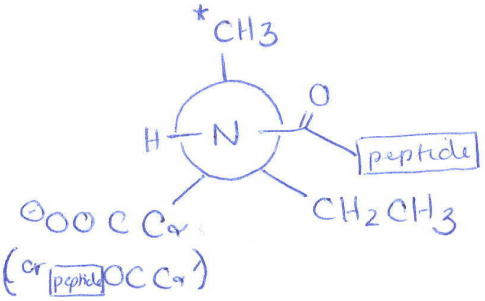
b. Draw the Newman projections for the ϕ dihedral angle for α-isoleucine and β-isoleucine. Do you expect the allowable conformational space in ϕ to be the same? Please briefly explain.

9 pts.

α-isoleucine



β-isoleucine



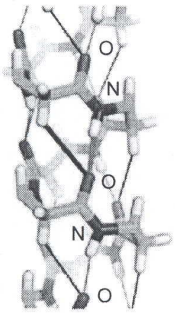
The allowable conformational space in ϕ will be different.

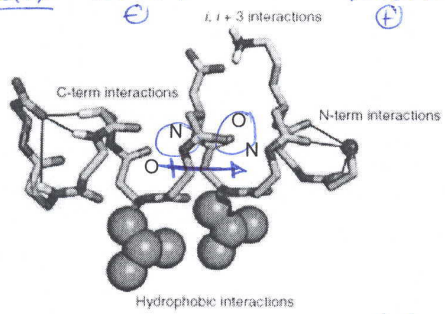
~~β pepti~~ β-isoleucine has more restricted conformational space due to the replacement of H with a bulky methyl group (* in picture)

c. β-Peptides can also form stable three-dimensional structures that have been structurally characterized by X-ray crystallography. One predominant structure is the right-handed 14-helix (shown below). Looking at the structure below, is the helix dipole pointing in the same direction as the α-helix?

3 pts.

α helix C term ⊖ ← → N term ⊕

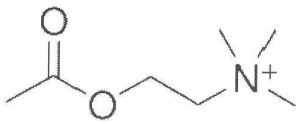




β-peptide helix C term ⊕ → N term ⊖

No. The helix dipole is pointing in the opposite direction

3. Acetylcholine (shown below) is an important neurotransmitter in the peripheral and central nervous system that is especially important in diverse functions from regulating muscle contractions to controlling REM sleep. Acetylcholine esterases carry out the hydrolysis of acetylcholine at the synapses formed between neural cells in order to clear the synaptic cleft for reactivation.



a. Draw the products of the reaction of acetylcholine with acetylcholine esterase.

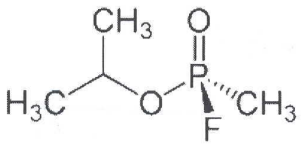
2 pts.

b. Canonical acetylcholine esterases (AchE1) contain a catalytic triad similar to those found in serine proteases such as chymotrypsin. In the course of your studies of Brazilian tree frogs, you find a new family of acetylcholine esterases (AchE2). After solving the crystal structure of AchE2, you find several polar residues in the active site you that could be involved in catalysis. You carry out mutagenesis studies and measure the k_{cat} and K_m values for the various AchE2 mutants. Looking at the table of mutations below, fill in the most important predicted function of the residue in terms of catalytic, substrate recognition, or none.

Sprouty	k_{cat} (s^{-1})	K_m (μM)	Predicted function of residue?
Wild-type	100	100	
S23A	85	120	none
E75A	102	1000	substrate recognition
D89A	<0.1	110	catalysis
D89N	<0.1	112	
H105A	<0.1	150	catalysis
H105K	35	145	
D178A	10	118	catalysis
D178N	66	95	
K210A	93	118	none
S257A	92	5000	substrate recognition
H292A	98	103	none

8 pts.

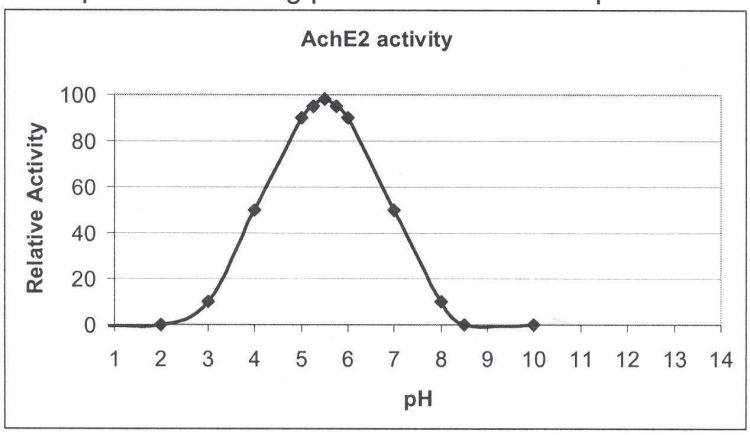
c. Nerve gases such as sarin (shown below) have been found to inactivate the canonical AchE1 family of enzymes by covalent modification of the catalytic serine with elimination of fluoride, leading to convulsions and eventual death by suffocation. When you test your new AchE2 with Sarin, you find that it binds with the same K_D but does not modify any of the serines in the active site. Please briefly explain based on the data from part 3b.



Serine is not participating in Covalent catalysis

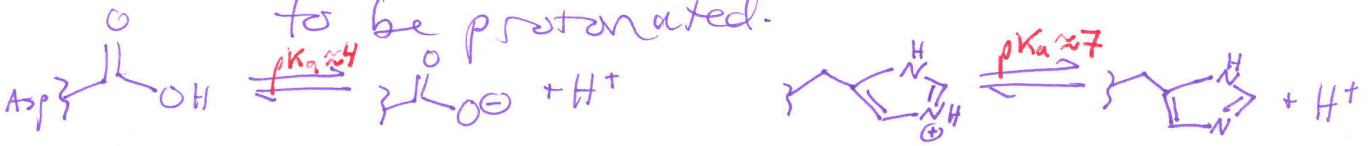
2 pts

d. In order to further explore the mechanism of AchE2s, you also measure a pH-rate profile for your new enzyme (shown below). Using the data from part 3b, please briefly explain the increase at pH 4 and the decrease at pH 7 using chemical equations showing protonation and/or deprotonation events.



4 pts

The catalytic mechanism requires Asp to be deprotonated and His to be protonated.



e. Are the pK_as of any of these residues shifted? If so, please give a possible reason for this shift given the data from part d.

2 pts

The pKa of His is shifted to 7.0. The shift may be due to the His residing near a negative charge.

3 covalent mech

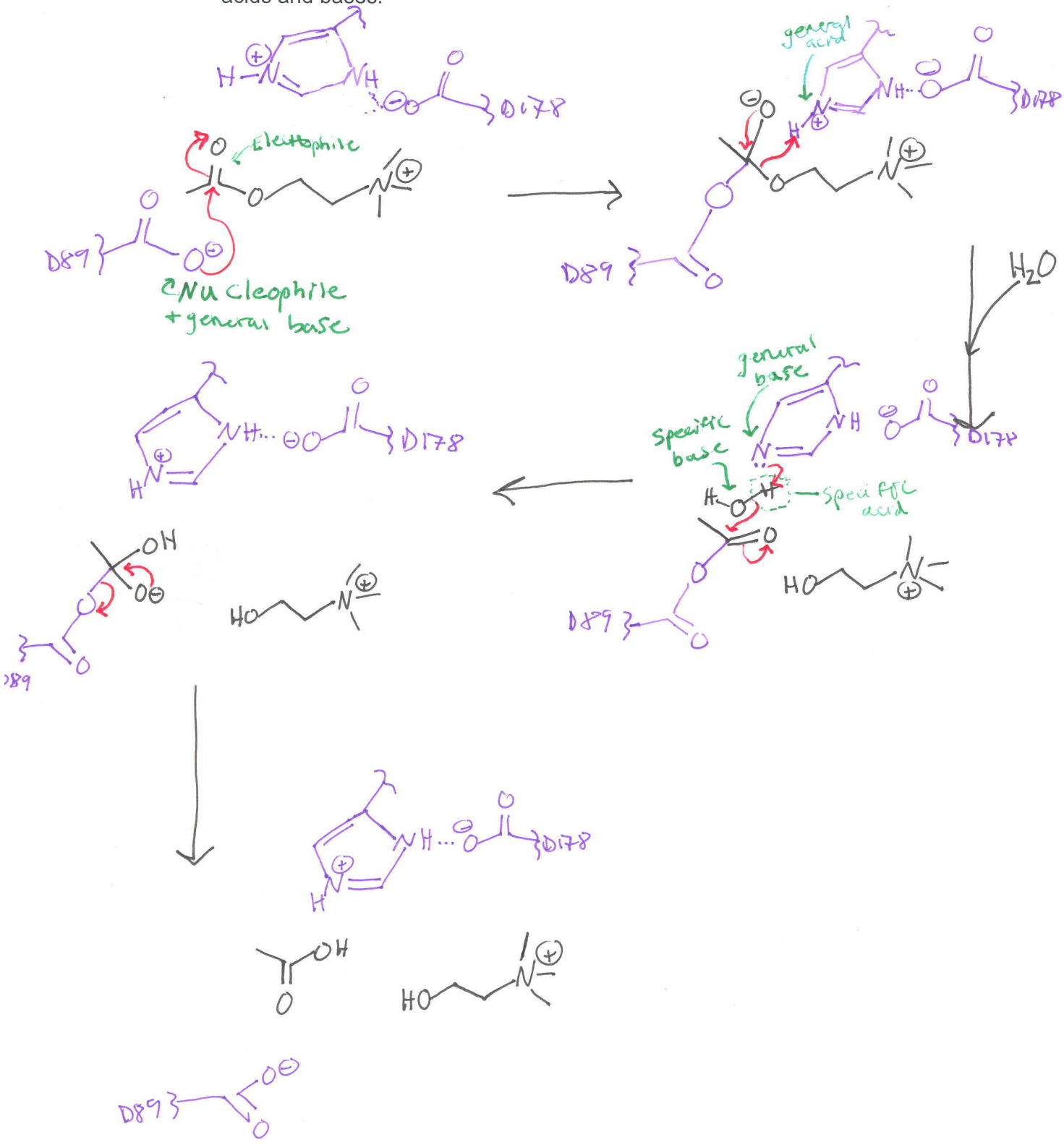
3 right residues

3 labeling

3 pts for reasonable

(12pt)

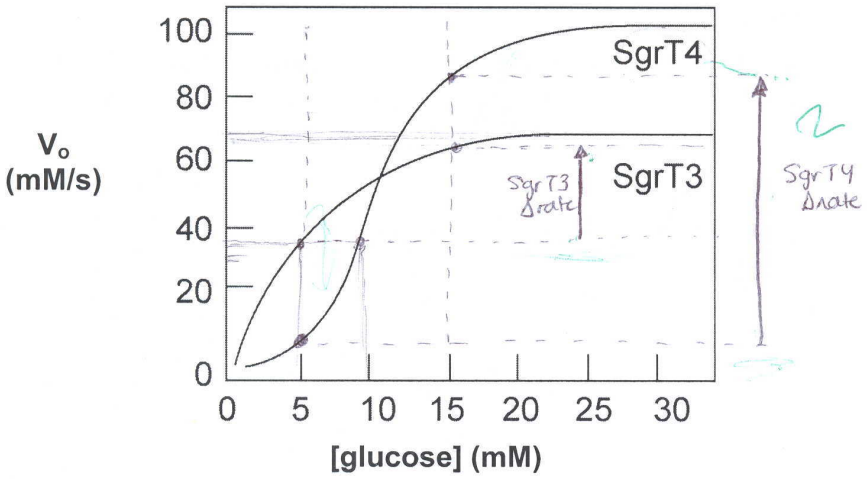
f. Draw a detailed arrow-pushing mechanism for AchE2 for the hydrolysis of acetylcholine based on the data from part 3b-3d. Please only show the catalytic residues and label the nucleophile, electrophile, and general/specific acids and bases.



4. Studies have shown that humans are highly colonized by bacterial communities that differ greatly from person to person. Different bacteria in the gut are thought be influence human caloric uptake. You have been studying four different sugar transporters, SgrT1-4, from a unique bacterium *E. saccharific* found only in gut of patients with high caloric uptake.

a. SgrT1 and SgrT2 belong to known families of transporters, so the first experiment that you do is to test the response of SgrT3 and SgrT4 to glucose. Do both enzymes follow Michaelis-Menten kinetics? Please briefly explain why or why not and estimate V_{max} and K_m for any enzymes that show Michaelis-Menten behavior.

10 pts



2 SgrT3 follows Michaelis-Menten kinetics.

2 SgrT4 does not.

2 Why? SgrT3 displays pseudo first order kinetics at low [S]

For, SgrT4 we cannot assume pseudo first order kinetics at low [S]

SgrT3 Vmax: 2 70 mM/s

Km: 2 [S] at ½ Vmax ~~10 mM~~ ~ 5 mM

b. The resting glucose concentration in the gut is typically 5 mM and shifts to 15 mM immediately after eating a large meal. Which enzyme has a greater increase in sugar uptake before and after eating? Please draw two arrows on the graph in part 4a representing the difference in rates for both SgrT3 and SgrT4 and briefly explain which enzyme you would expect to perform better as a sugar sensor and why.

6

SgrT4 has a larger difference in rate for sugar uptake before + after eating, so it would perform better as a sugar sensor

c. SgrT1 and SgrT2 are already known to follow Michaelis-Menten kinetics, so you measure their V_{max} and K_m using 1 μM enzyme. Fill in the box with the k_{cat} of each enzyme, including the units.

4

$V_{max} = [E]_{TOT} \cdot k_{cat}$ when $[S] \gg K_m$

$k_{cat} = \frac{V_{max}}{[E]_{TOT}}$

Enzyme	V_{max} (mM s^{-1})	K_m (mM)	k_{cat}
SgrT1	1000	1	$k_{cat} = \frac{1000\ mM/s}{0.001\ mM} = 10^6/s$
SgrT2	100	50	$k_{cat} = \frac{100\ mM/s}{0.001\ mM} = 10^5/s$

d. Calculate the rate of sugar transport of both SgrT1 and SgrT2 at [glucose] = 10 mM.

4

$V_o = \frac{V_{max}[S]}{[S] + K_m}$ OR if $[S] << K_m$, estimate $v_o = V_{max}[S]/K_m$

if $[S] >> K_m$, estimate $v_o = V_{max}$

SgrT1: $V_o = \frac{(1000\ \frac{mM}{s})(10\ mM)}{10\ mM + 1\ mM} = \frac{10{,}000\ mM^2/s}{11\ mM} = 909\ mM/s$

or $V_o = V_{max} = 1000\ mM/s$

SgrT2: $V_o = \frac{(100\ mM/s)(10\ mM)}{10\ mM + 50\ mM} = \frac{1000\ mM^2/s}{60\ mM} = 17\ mM/s$

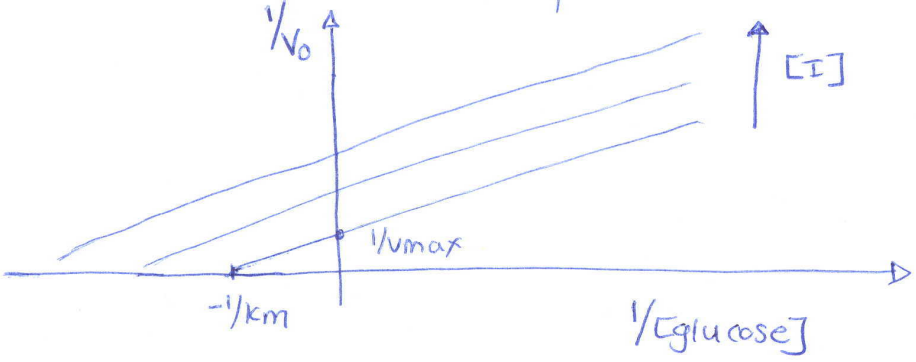
or $V_o = \frac{V_{max}[S]}{K_m} = \frac{(1000\ mM)(10\ mM)}{50\ mM} = 20\ mM/s$

(4)

e. When the glucose concentration reaches the high levels observed in diabetic patients that also harbor *E. saccharific*, the increased sugar metabolism in this bacterium can cause acidosis of the gut. Although this is a very rare condition, you decide that you know enough about the main sugar transporter, SgrT1, of *E. saccharific* to design an inhibitor to turn it off during high sugar episodes. Since you want to avoid targeting other glucose transporters with a glucose analog, you decide to target a regulatory site that is unique to SgrT1 and is not found in any other glucose transporter. Please briefly explain what type of inhibitor you would design and show using a graph how the glucose transport concentration dependence would change as a function of your inhibitor concentration.

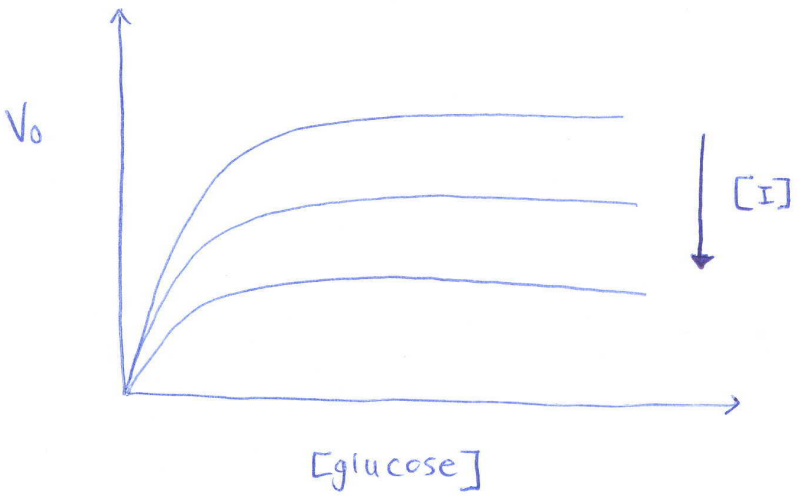
uncompetitive inhibition

The best way to show this is by a Lineweaver-Burk plot.



Vmax and Km are decreased

Another depiction:



Vmax and Km are decreased