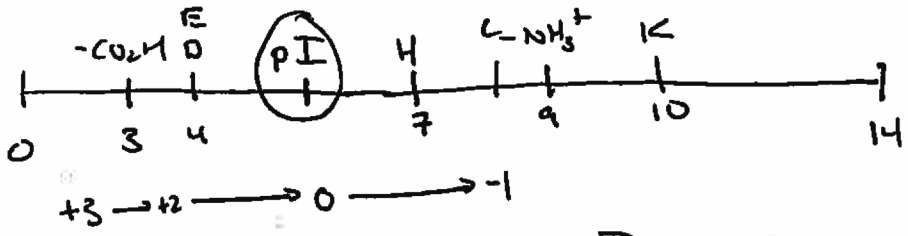
1. Consider a short peptide, CDEHKLF. What would be the total charge on the peptide at pH 0, and what is the approximate pI of the peptide?

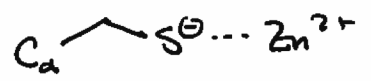
$H_3\overset{\oplus}{N}$-CDE$\overset{\oplus}{H}\overset{\oplus}{K}$LF-$CO_2H$ total charge = +3



pI ≈ 5.5

2. Predict the effects of the following environments on side chain pK_a. Provide a brief rationale for each of your answers.

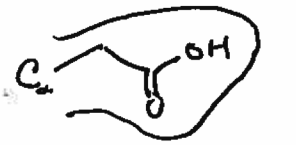
a. A cysteine side-chain interacting with a Zn^{2+} ion.



Zn^2 stabilizes conjugate base (anion)

pK_a Cys ↓ (stronger acid)

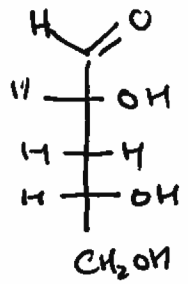
b. An aspartic acid side chain buried in the hydrophobic core.

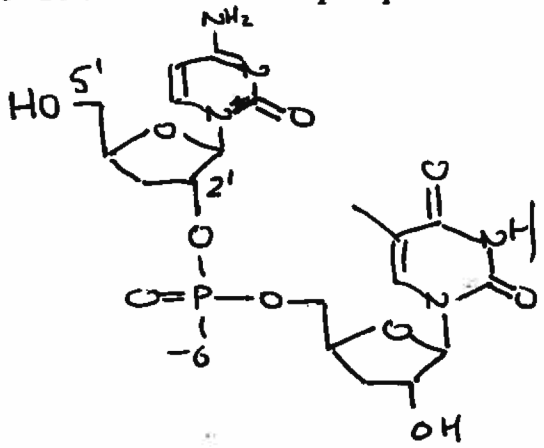


core would destabilize conj. base

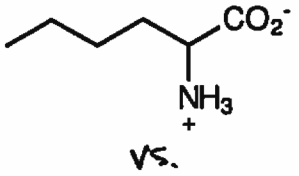
pK_a Asp ↑ (weaker acid)

3. Consider an artificial polynucleic acid system derived from **3'-deoxyribose**. Draw a dinucleotide with the sequence 5'-CT-2'. Assume a **2'-5' phosphodiester** linkage.

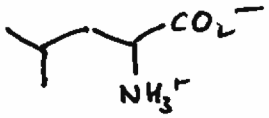




4. Alpha helix dimers are stabilized by the heptad repeat of leucine residues at every 1st and 4th position of a seven residue repeat. Let's say we wanted to try to improve on nature by using an unnatural residue such as norleucine (see right) in place of leucine.



vs.



a. Briefly identify any enthalpic effects you suspect such a substitution would have on the stability of a helix dimer.

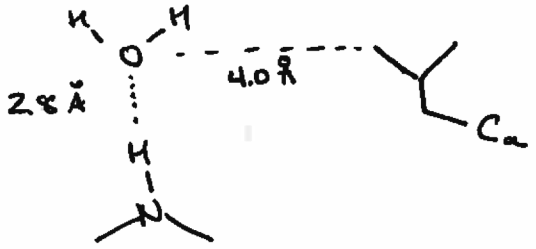
vdW interactions key. Unbranched alkane have more surface area/pack better, so mildly stronger vdW's?

b. Briefly identify any entropic effects you expect such a substitution would have on the stability of a helix dimer.

→ solvent entropy will decrease if helices unfold. norleucine has modestly more S.A than Leu, so unfolding worse.

→ side chain entropy - Norleucine would give up more C-C bond rotation than Leu. in helix.

5. You see a ball of green in your electron density map. You wonder, could it be a water. It is near a backbone amide nitrogen and the side chain of leucine residue. How far should it be from each if it is really a water interacting with your protein?



6. You obtain a mass spectrum of a peptide obtained via trypsin digestion of a protein. The four smallest peaks in the spectrum have masses of 58.0, 147.1, 157.1 and 234.1. What are the masses of the first two (N-terminal) residues in the peptide? Briefly give your logic.

147.1 is y_1 (Lys)

58.0 is definitely b_1

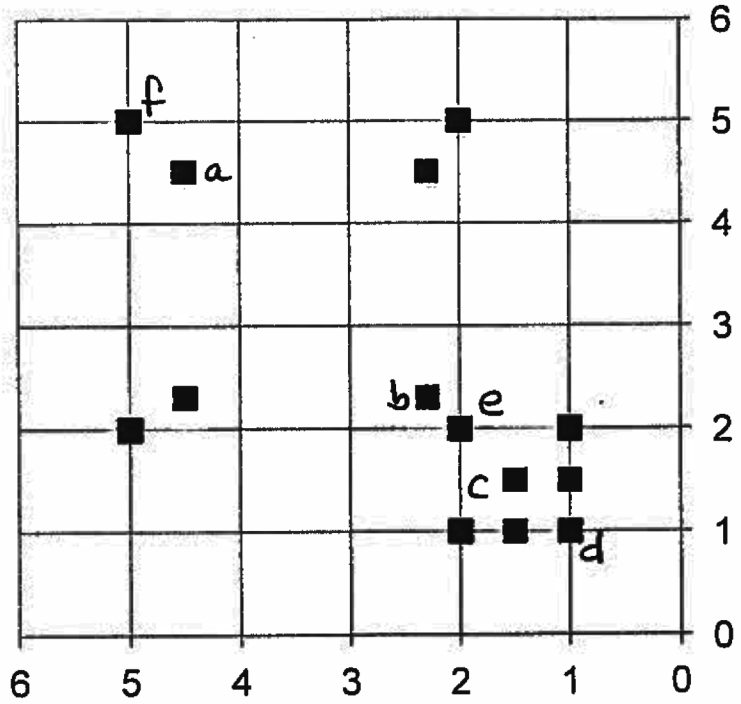
157.1 is b_2, since 157.1 - 58.0 = 99.1

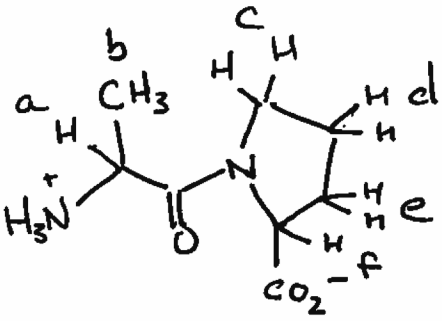
but 157.1 - 147.1 = 10

too small for an a.a.

7. I have prepared a simulated 2D COSY spectrum of the dipeptide AlaPro, showing only the region from 0-6 ppm (below).

a. Draw the structure of AlaPro and use it to identify the chemical shifts of all proton resonances. It may help to label the set of equivalent hydrogens on your structure as "a", "b", "c"…





b. AlaPro can adopt two conformations separated by 180° rotation about the peptide bond, *cis-* or *trans-*. How can 2D NMR be used to identify the presence of either one or the other of the two conformations? Specify which conformation you want to identify, the technique and what result you would hope to see from the technique.

NOESY

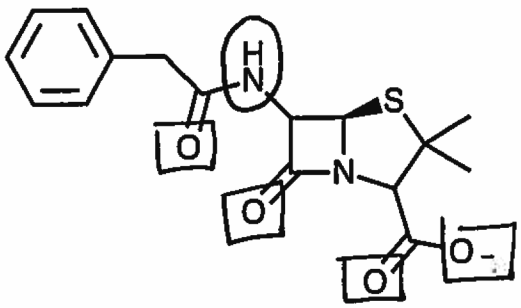
In trans- conformer, I'd expect to see x-peak between ⓒ (proton on α carbon) & ⓐ/ⓑ

8. For the following molecule on the right:

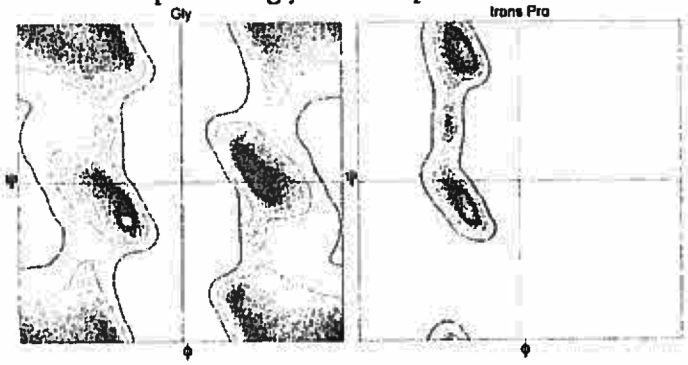
Draw a circle all H-bond donors

Draw a square around all acceptors

Put an asterisk* next to groups that can do both



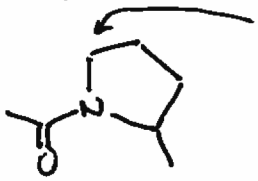
8. The Ramachandran plots for glycine and proline are shown below.



a. Based upon these plots alone, which residue – Gly or Pro – is more likely to support secondary structure in proteins? Explain briefly.

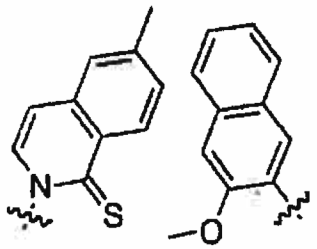
Proline has less intrinsic flexibility so loses less entropy in adopting 2° struct.

b. Neither residue is commonly associated with secondary structure formation. For the residue you ID'd as more likely to support it in "a", why would it too be a poor choice for stabilizing an α helix or β sheet?



no capacity to support internal H-bonding.

9. The "base" pair has been successfully placed in a bacterial chromosome and can be replicated through several generations of growth.



d5SICS dNaM

a. Briefly comment on the enthalpic contributions (positive or negative) that you think this base pair will make to duplex stability.

Polarizable π clouds could improve vdW interactions in stacking

a. Briefly comment on the entropic contributions (positive or negative) that you think this base pair will make to duplex stability.

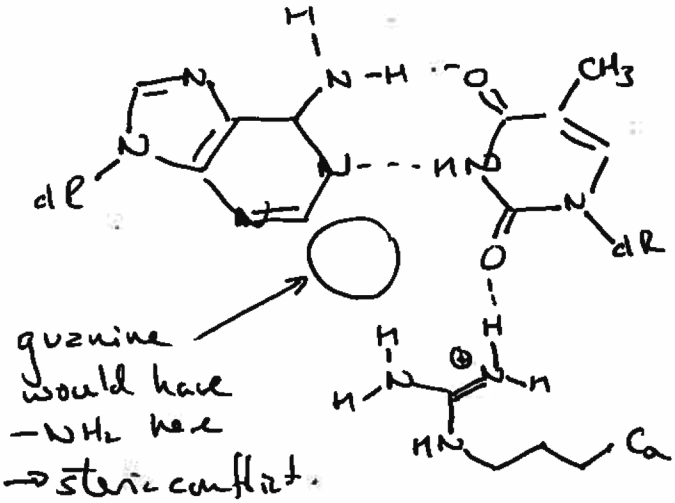
Solvent entropy benefits from burial of these bases in the interior of duplex.

c. Briefly comment on why these bases do not pair successfully with other, natural, DNA bases (and can therefore be sustained independently in a bacterial chromosome).

Inability to H-bond leads to enthalpic penalty for a natural base in a mismatch.

10. A mentioned in class, the 434 repressor protein, an active dimer, is selective for AT base pairs at the center of the palindromic DNA sequence it binds. At one point, it was thought that Arg43 might be responsible for direct readout of the AT base pair through contacts in the minor groove.

a. **Draw** a potential example of direct readout that could take place in the minor groove between Arg43 and an AT base pair. **Briefly describe** how your proposed contact would exclude a GC base pair from that position.



b. The Arg43→Ala mutation raises the dissociation constant for the 434 repressor-DNA complex from 0.02 μM to 2 μM. Could that difference in K_d be related to the kind of contact you show in "a"? Calculate the difference in $\Delta G_{dissociation}$ ($\Delta\Delta G$) between the wild-type and Ala43 mutant in formulating your answer.

$$\Delta\Delta G_{dis} = -RT\ln\left(\frac{2\mu M}{.02\mu M}\right) = 2.8\ \frac{kcal}{mol}$$

mutant dissociate more readily, due to loss of H-bond.

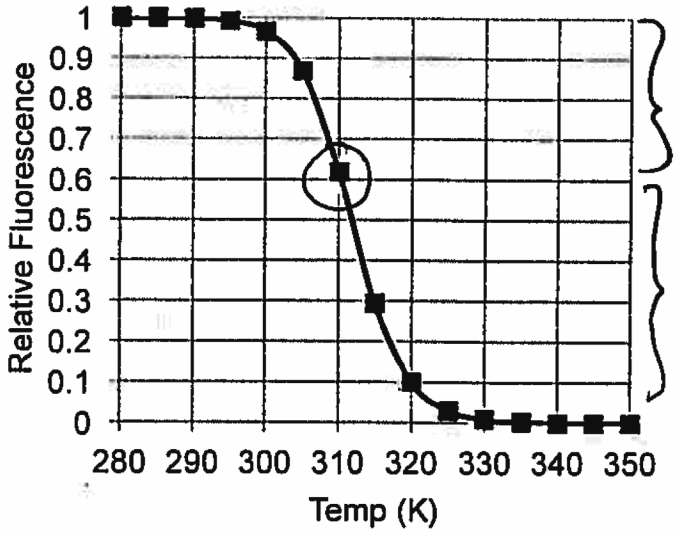
c. When the same experiment, comparing K_d between the wild type (Arg43) and mutant (Ala43) variants of 434 repressor is performed with a GC base pair, the K_d increases from 1.0 μM to 200 μM. Is that result consistent with direct readout of the minor groove as described above? Explain briefly.

$$\Delta\Delta G_{dis} = -RT\ln\left(\frac{200\mu M}{1\mu M}\right) = -3.2\ kcal/mol$$

About the same as above. Suggests that Arg → Ala mutation has the same effect in both cases, not an H-bond specific for A·T.

11. An examination of protein stability is performed.

a. From the following plot, determine the equilibrium constant for protein unfolding (K_{unfold}) at 310 K.



$K_u = \frac{[U]}{[F]} = \frac{0.4}{0.6} = 0.67$

b. A plot of $\ln(K_{unfold})$ vs. $1/T$ is prepared. The y-intercept is 86 and the slope is -2.7×10^4 K. What is ΔH° and ΔS° for the unfolding process.

Intercept $= \Delta S^\circ/R$; $\Delta S^\circ = 86\left(1.987 \frac{cal}{mol\,K}\right) = 171 \frac{cal}{mol\,K}$

Slope $= -\Delta H^\circ/R$; $\Delta H^\circ = +(27000\,K)\left(1.987 \frac{cal}{mol\,K}\right)$

$= 54000 \frac{cal}{mol}$

$= 54 \frac{kcal}{mol}$

12. NMR and crystallographic experiments have greater or lesser success in producing high quality models of molecular structure. In the space below, briefly specify how you would determine, from a table of experimental statistics, whether the final structure was reliable in…

a. An x-ray structure.

$R_{factor}/R_{free} \leq 25\%/30\%$

RMSD bonds $< .02$ Å or agreement w/ Ramachandran plot.

angles $< 2^\circ$

b. An NMR structure.

RMSD between models in ensemble

Few violations/model

Good geometry