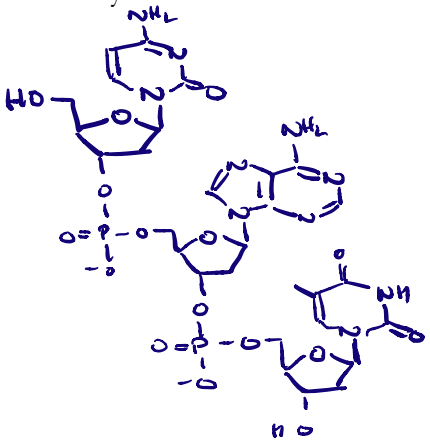
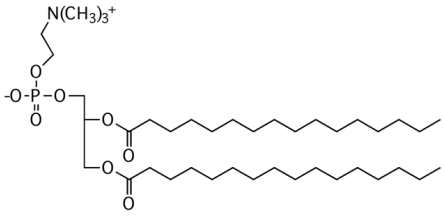
1. Draw the deoxyribotrinucleotide CAT.



2. At some critical concentration, lipids self-assemble into micelles and vesicles. Propose a **thermodynamic** argument, including enthalpic and entropic considerations, for why the following phospholipid would self-assemble at a higher or lower (your choice) concentration than its constituent fatty acids would alone. Assume a solution pH of 7.0.



Enthalpically, the positively charged quaternary ammonium ion and negatively charged phosphate will be attracted to each other. I would expect it to be more enthalpically favorable for the phospholipid to assemble.

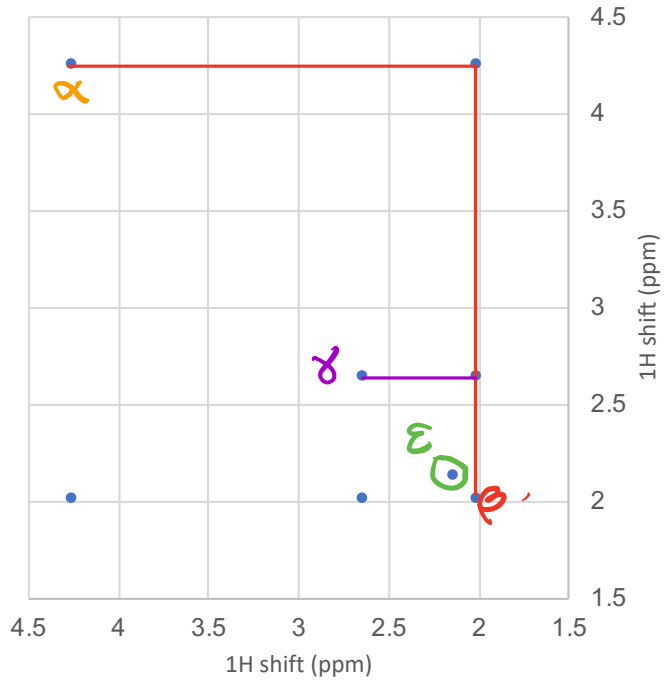
Entropically, two fatty acids are pre-associated, meaning that there is a smaller loss of entropy of aggregation when a phospholipid enters the assembled state vs. two separate fatty acids. Possibly the hydrophobic effect would be smaller though given that these two are already associated with each other.

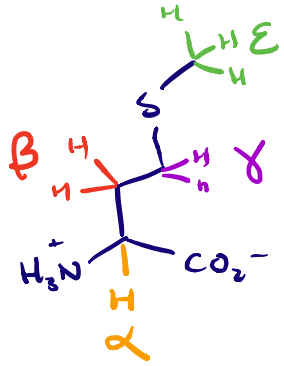
3. The following sequence appears at the C-terminus of a protein. Suggest whether this sequence is likely to adopt a short stretch of alpha-helix or beta-strand. Then suggest a minimal number of rearrangements necessary to convert it to the alternate conformation. In addressing this question make it clear whether you're changing a strand to helix or vice versa. Also, be clear regarding the logic of your choices (a sketch may be useful).

E L S D M K R T F N This is an amphiphilic helix with a heptad repeat of nonpolar residues (AbcDefgA'…)

E S L D M K L T F N I see at least two changes being required. The L/S are swapped early in the sequence and then the "R" in "KRT" is mutated to a non-polar leucine to achieve the alternating pattern needed for an amphiphilic strand.

4. I hereby present a 2-D COSY spectrum of one of the 20 naturally occurring amino acids at pH 7. Note that the amino protons are not visible in the spectrum due to exchange. Please identify the amino acid and make your logic clear, including chemical shift assignments for each set of protons in the amino acid.





The assignments are possible because the alpha H is furthest downfield. It only couples to the beta protons (red) but the beta protons couple to both alpha and gamma. The epsilon protons are not coupled to any others. That is perhaps the easiest way to identify the amino acid as **methionine**. That lone methyl grup is an unusual feature.

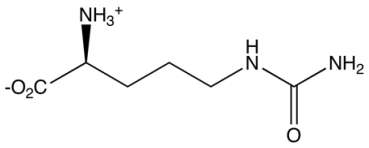
5. I wish to test the stability of myristoleic acid vesicles as compartments that can retain small molecules like amino acids and nucleotides over time. Suggest an experiment that would allow me to conduct such a study.

I would do something similar to the Chen et al. paper. Start with vesicles containing radioactive sucrose (or fluorescent label) and incubate over time, periodically running a sample over a size exclusion column to see if free small molecule has escaped and can be separated from the large vesicles that encapsulate the small molecules.

6. What is the importance of the R-factor in determining the quality of a crystallographic model?

The R-factor is a measure of the % (or fractional) disagreement between model and data.

7. A study was performed to isolate functional RNA molecules that act as receptors capable of distinguishing between L-arginine and L-citrulline (right).



The following dissociation constants were obtained from one of the isolated RNA receptor molecules.

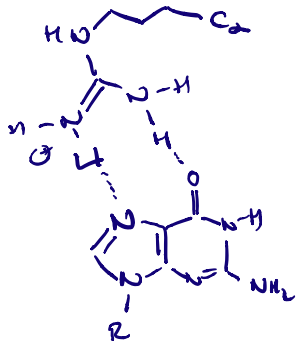
	K_d (mM)
L-arginine	0.060
L-citrulline	7.2

a. What is the difference free energies of dissociation of L-arginine and L-citrulline from the RNA receptor (kcal/mol)? Feel free to give an estimated value if you like, but be clear on how you reached your estimate.

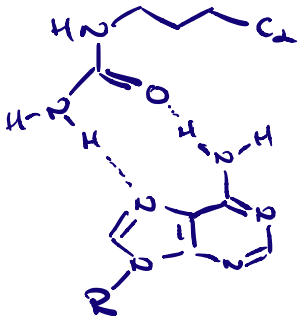
$\Delta\Delta G^\circ = -RT\ln(7.2/0.060) = -(0.001987\ \text{kcal/molK})(298\text{K})(4.8) = 2.8\ \text{kcal/mol}$

b. A guanine in the RNA receptor described above is makes two H-bonds to the side chain of the bound arginine ligand, but when that guanine replaced with an adenine, the aptamer shows selectivity for citrulline. Show what the original interaction between guanine and arginine might look like and then draw a prediction of the new interaction between citrulline and adenine to the side.

i. Arg + guanine

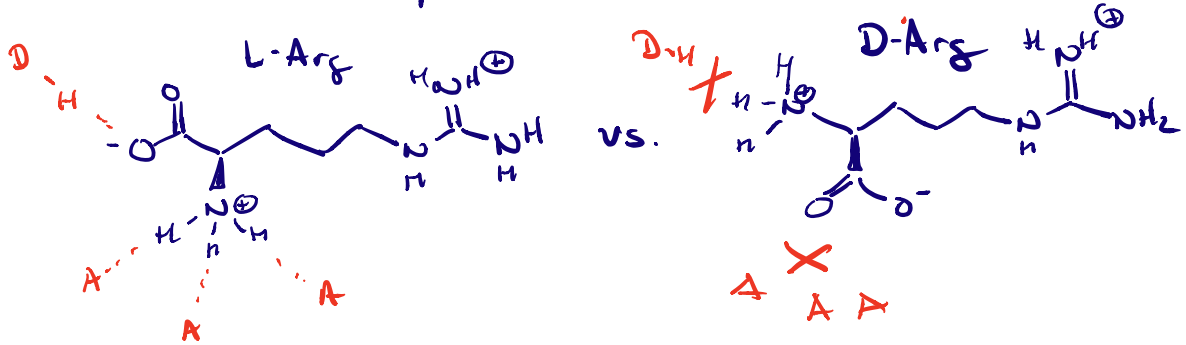


ii. Citrulline + adenine



c. The study also found that D-arginine does not bind to the L-Arg receptor. Explain how such selectivity might be achieved. I suspect that a drawing will be required.

A receptor will have H-bond donors and acceptors located carefully to interact w/ relevant atom groups.



8. In a recent study, the structure and function of an RNA-binding zinc finger protein was explored. It was found that the protein has no affinity for dsDNA but binds dsRNA without any sequence specificity.

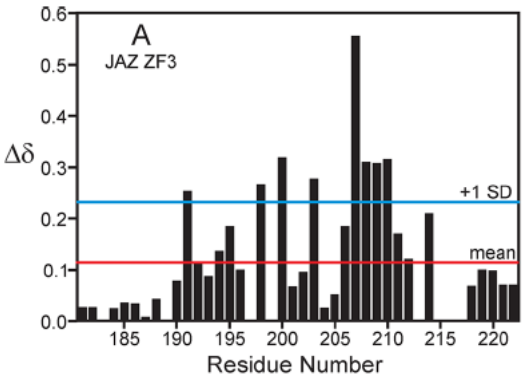
a. Suggest what you believe to be the most important distinguishing structural feature between duplex RNA and DNA and indicate how that might lead to the observed preference for RNA.

Aside from the 2' OH and uracil, the big difference is conformation. RNA is in the A-conformation and DNA is the B-conformer. A protein evolved to bind RNA would have no affinity for a duplex in the B-conformation (extreme indirect readout of the 2' OH group).

b. Given what you know of the origins of sequence selectivity of dsDNA-binding zinc finger proteins, why might it not be a surprise that a dsRNA-binding zinc finger does not possess sequence selectivity.

Zn fingers bind DNA through direct readout – through intermolecular interactions taking place in the major groove of DNA. Side chains from the α-helix of the finger interact with H-bonding groups from bases. Since the A conformation makes the major groove less accessible than the B conformation, it's harder for zinc fingers to contact base edges in RNA.

An NMR study was conducted on the structure of the zinc finger/RNA complex. Some information from the paper is reproduced below.



c. This figure shows differences in the resonances of backbone amides upon the finger binding RNA. How does this assist in identifying residues that interact with RNA?

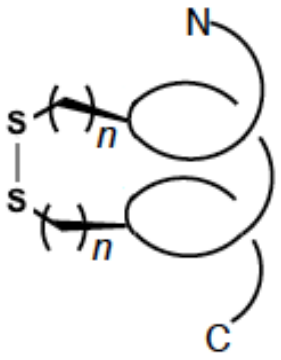
The shift reflects a change in chemical environment for the ^{1}H in question. Presumably binding alters the environment of those residues in direct contact with the RNA more than others.

NMR Constraints		
total unambiguous distance restraints	d	209
Violation Analysis		
maximum distance violation (angstroms)	o	0.19
maximum dihedral angle violation (degrees)	o	0
Deviation from Idealized Geometry		
bond lengths (angstroms)	g	0.01
bond angles (degrees)	g	2.5
rmsd from mean structure	m	0.43
rmsd for all heavy atoms	m	1.04
Ramachandran Plot		
most favorable regions (%)	g	89.6
additionally allowed regions (%)	g	10.4
disallowed regions (%)	g	0

d. The table at left has statistics for the NMR structure of the 40-residue protein. Indicate which of the following issues is addressed by a given datum by writing one of the following one-letter codes to the left of each numerical entry.

d – data quality
m – agreement between models
g – agreement with ideal geometry
o – other

9. I plan to explore a series of disulfide "staples" as possible stabilizers of alpha helical stability. They will be incorporated into short test peptides via chemical methods where the number of methylene groups (n) in the staple varies from 1-6 on each of the two substituted positions as shown at right.



a. Suggest an experimental procedure I can use to examine the stability of the helices that I'm creating. The procedure should allow me to extract enthalpic and entropic data. Please be specific and brief. Diagrams may be useful.

I would do a van't Hoff experiment, where a thermal "melt" of the helix is followed by far-UV CD spectroscopy to monitor the loss of negative ellipticity at 222 nm. That melting experiment can be used to obtain equilibrium constants at various temperatures, and a subsequent replot of the data as ln(K) vs. 1/T will give ΔH and ΔS.

b. Let's say that I find some of these staples are shorter than the "ideal" in stabilizing helices. Do you believe that their failing will be chiefly enthalpic or entropic? Explain briefly.

If the staple is too short, that suggests it would exhibit strain if the helix succeeds in forming. Strain is a classically unfavorable enthalpic concern.

c. On the other hand, some staples will be longer than the ideal. Predict whether the deficiency of these longer staples will be chiefly enthalpic or entropic? Again, please give a brief explanation.

Here I go entropy. A longer staple will do less to constrain the conformational entropy of the polypeptide, so the benefit of stapling (reducing the loss of entropy via backbone preorganization) would be lost.

10. Several neurological disorders (Alzheimer's, Parkinson's, ALS) are associated with the aggregation of proteins to form "amyloid fibrils" through the formation of extensive β-sheets, with each protein contributing one strand to the growing insoluble aggregate anchored by the β-sheet comprising these individual strands. One idea to block fibril growth is to place methyl groups on one-half the backbone amide nitrogens of a peptide taken from the protein of interest and use it as an inhibitor of β-sheet growth. Sketch a methylation scheme that these folks have in mind for a hexapeptide (you can use "R" groups in place of specific residues) and explain how it will work, in a mixture of diagrams and/or words.

Fibrils are aggregated by stand-stand interactions