

BCH222 - The Ribosome

Reading

If you want a refresh on basic nucleic acid structure, read chapter 7 in Branden & Tooze, and refer to c7DNA.kin to see base atom types, and to see base pairing and stacking in 3D. In the exercise, you'll see the extra 2'OH on the ribose ring of RNA and some of its interactions.



(3.4MB) [Ban et al \(2000\)](#) "The Complete Structure of the Large Ribosomal Subunit at 2.4 Å Resolution", *Science* **289**: 905-920 - especially color figures 3 and 4.



(896KB) [Dunkle et al \(2000\)](#) "Structures of the Bacterial Ribosome in Classical and Hybrid States of tRNA Binding", *Science* **332**: 981-984.

Graphics assignment

The *Haloarcula marismortui* 50S ribosomal subunit -- RNA tertiary interactions

Look at kinemage [1ffk.bb.kin](#) (704KB) in Mage. The colors and the startup view are as in Fig. 4A of the Ban et al. paper. Turn on individual RNA domains with their appropriate views, and compare with Fig. 4E (remember to rotate a bit, slowly, for 3D; or use stereo). In domain I, find a place at one edge where two parts distant in sequence touch like curled hands back-to-back; this is near residues _____ and _____.

Turn off the 23S and 5S RNA's and turn on the proteins. Note that both their names (e.g., "L10e") and chainID's (e.g., "f") are given in the button labels. Move them around in View1, and confirm that they really avoid the center of the particle except for their long "tails".

Turn on the 5S RNA and choose its view. Five proteins interact with the 5S; identify them by picking a point to get the chainID, then looking up the protein name on that button and confirming by turning the button off and on again. The 5 protein names are _____, _____, _____, _____, and _____.

Which one seems to interact most extensively? _____

Look up the interaction list for 5S in Table 2 to check if you got them right.

Turn off the proteins and try each of the 23S domains one at a time to identify the two that are close to 5S; these are _____ and _____.

One of these has especially tight interactions: find a place where two double helices, one on 5S and one on domain _____, have their backbones very close (over at least half a dozen base pairs), this is near residues _____ and _____.

To look at the above interaction in detail, open kinemage [1ffk_5Sto38.kin](#) (436KB). The 5S backbone is in pink tint and helix 38 of 23S domain II is in blue tint, while the bases are color-coded by type: G green, C yellow, A pink, and U blue. Find helix 38 in Fig. 4C. Rotate the overview to look in sidewise

at the interaction, as indicated by the all-atom contact dots. Which base type predominates near the contact? _____

This is indeed an "A minor motif", a common and important type of RNA tertiary-structure interaction. Views 2 and 3 are closeups of the two halves of the contact, one half with A bases on the 5S and the other with A's on helix 38. How many A BASES actually contact the other RNA, in View2?

_____ in View3? _____

For each view, are the A's all on the same strand of their double helix? _____

There are two different types of A-base-to-backbone interactions; describe each briefly: most A bases _____, while some A bases _____.

In addition to interaction of atoms on the A base, what other part of the two RNA's interact closely and frequently? _____ & _____

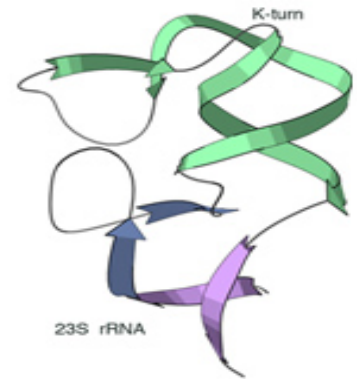
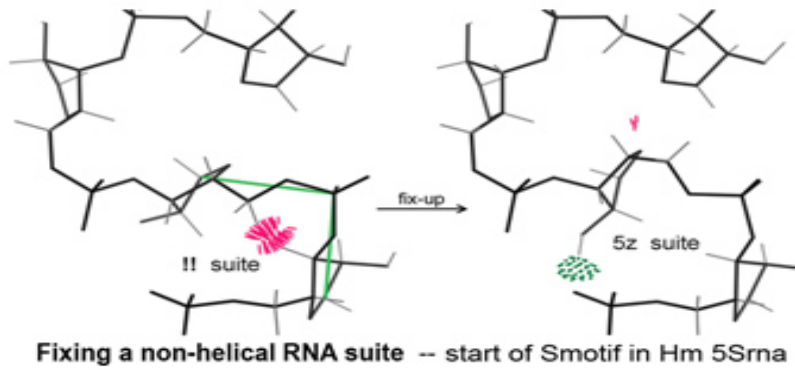
What does that imply about RNA vs DNA structure?

_____.

Where are the phosphates relative to these backbone contacts?

Open file [1ffk5SHstack.kin](#) (1.9MB) to see base stacking in the 5S RNA, as shown by contact dots between the bases. Rotate (slowly!), to see where the stacking is continuous and where it has large gaps. Go thru the views, especially to see typical patterns of single-base stacking where loops cap the double-helical stems. Notice that essentially all the dots between bases are the blues, greens, and yellows of well-fit contacts.

Open file [1ffk5S_mcdots.kin](#) (1.1MB), which shows just the bad overlaps (in hotpink) and the H-bonds (in greentint) between the RNA backbone atoms. Turn off the bases ("sidechains" button). There are three really big clashes. For one of those, pickcenter and zoom in close, and identify the two heavy atoms to which the clashing hydrogens are attached: _____ and _____. Turn off "bb dots" and measure the distance between the two clashing H atoms: _____ Å. Ideal van der Waals contact between two non-polar H's is considered to be 2.34 Å, so these two H's are overlapping by _____ Å? That is a physical impossibility, so the local backbone conformation must be wrong in these places. Bases can be placed very accurately in nucleic-acid crystal structures because they are large and easy to see in the electron density map, and they are rigid with no internally variable angles. Backbone is more difficult to get correct, however, because there are too many variable angles per observable atom, with only the P clear and unambiguous. Therefore, we are encouraging crystallographers to look at the H atom contacts, in order to provide additional information that can increase accuracy. The figure at left below shows correction of the largest 5S clash.



The six 23S "domains" identified from RNA secondary-structure plots turned out not all to be compact in 3D. However, some smaller pieces are compact and domain-like, one of which is shown in file [1ffk_hlx7H.kin](#) (433KB). The startup view shows a backbone ribbon, color-coded bases, and greentint contact dots for the base-base H-bonds. This complex small piece includes helix 7 of domain I. By comparing the 3D structure (turning on "pseudo-mc") with the secondary structure shown in Ban et al Fig. 4C and then Fig. 3 (left half), figure out how much of the structure (i.e., which helices) are included here: _____

Look for the most obvious tertiary (long-range) base pairs, and identify them on Ban et al Fig. 3. What is one of those base pairs? _____ with _____

Choose View2 to look at the "kinked turn" at the top of this structure. Does it connect two stretches with double-helical base pairs? _____

The kink-turn motif is involved in many of the ribosomal-protein binding sites, and this one is no exception. Turn off the ribbon, and turn on the backbone. On the file menu, use "Append" to bring in contacts for [protein L29](#) (240KB) from the revised 50S ribosome structure (PDB 1S72) with full coordinates for the proteins. There seem to be a great variety of interactions between proteins and kink-turns, but they do share the general nature of most protein/nucleic acid interactions in having both sequence-specific H-bonds between polar sidechains and RNA bases, and also contacts between charged or polar sidechains and the phosphate backbone which are non-specific in DNA, but are often specific to recognizing unusual backbone conformations in RNA (such as a kink-turn).

Go to the K-turn contact view and selectively turn on the contact dots just for H-bonds, or just for vdW. Identify one protein sidechain that H-bonds with part of the kink-turn backbone: _____

Does it also have vdW contacts? _____

Identify the protein sidechain that makes a specific H-bond to the flipped-out base at the kink: _____ to _____ atom of _____ base number _____

Which sidechain stacks with that base? _____

File [1ffk_piece.pdb](#) (37KB) is an edited file containing short sections of two interacting double helices in the 23S RNA. Start up MolProbity, and browse to upload this file. Add hydrogens (you don't need flips, since it's all RNA). Back on the main page, choose to visualize interactions, and ask for contacts between the two different pieces ("chains" 1 vs 2). View the resulting kin in KiNG.

From the analysis in the 2000 Science paper, what kind of interaction do you think this is? _____

Turn off all contact types except H-bonds. Which type of base is most strongly involved? _____

H-bonding to what other type of atom? _____ What atom type forms several backbone-backbone H-bonds? _____

Turn off the H-bond dots and turn on all the other contacts. Describe the most characteristic type of van der Waals contact in this interface:

When you're done, log out of MolProbity and delete your files.

Note: If you want later to study more of the protein-RNA interactions in the Hm 50S ribosome, use either file 1S72 with full coordinates for the proteins, or file 3CC2 with even more accurate coordinates for the rRNA.

The E coli 70S ribosome -- tRNA and ratchet dynamics

File [Ecoli70S_with_tRNAs.kin](#) (2.3MB) shows the A, P, and E site tRNAs positioned along the mRNA in the unrotated E coli 70S ribosome. From the startup side view you can see that they are inside an open groove between the large and small subunits. Try the front view, then the A-P-E close view, then turn off virtual backbone and Calphas to see the tRNAs clearly. The A-site tRNA is _____ (color), the P-site is _____, and the E-site is _____. Find the close-neighbor CCA ends of the A and P tRNAs, at residue _____. Center on one, and turn to look down the narrow opening of the peptide exit tunnel; turn on "exit tunnel" to check that you identified it right, then choose the back exit tunnel view to see where it comes out.

File [Ecoli70S_ratchet_demo.kin](#) (1.3MB) animates the ratcheting motion of the ribosome during a step of peptide elongation and tRNA translocation along the message. The startup view shows a "top" view from the direction of the 50S (large) subunit, in the classic unratcheted state. Animate to see the large rotational motion of the 50S (in blue). Try the different views, with animation or with both states turned, with and without the different parts. The two ribosomes in the asymmetric unit of the crystal structure from the Dunkle paper were superimposed, here, on the tRNA anticodon loop, which also keeps the mRNA and the 30S fairly still. Where in the tRNA is the major hinge point for its bending between the PP and the hybrid PE-site states? _____

Choose the L1 stalk view, and animate to see the large motion of the rRNA stalk next to L1 protein. Click on the worm ribbon at the base of the long helix where the stalk bends, to get the residue numbers that bracket the stalk at its bend point: _____, _____. Turn on both animation states, and identify an equivalent pair of points (same or close residue numbers) in the structure at the end of the stalk; about how far does it move? _____ Å What piece of structure does the end of the stalk move to interact closely with in the ratcheted state? _____