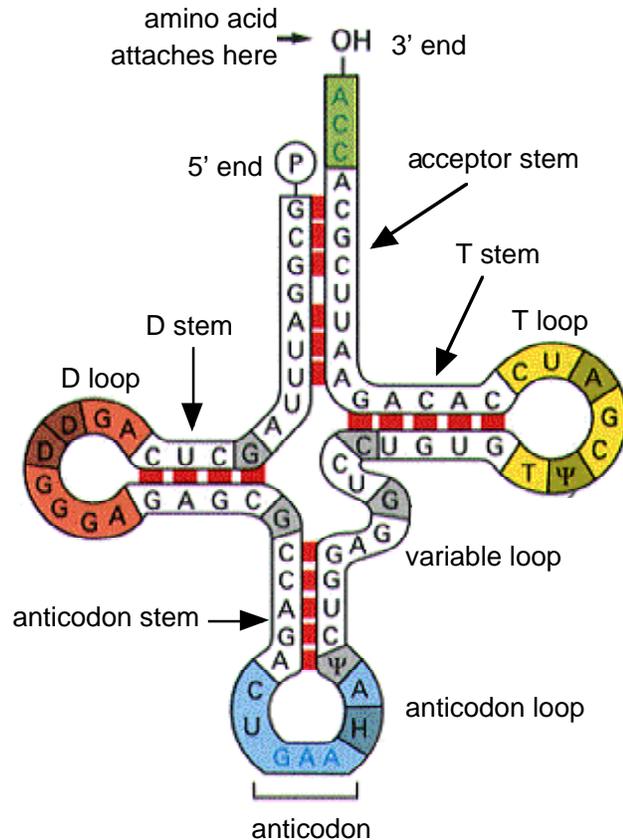


A Short Rasmol Tutorial: tRNA

Note that this tutorial is due at the beginning of class on Wednesday, October 3.

tRNA secondary structure. The sequence of yeast tRNA^{Phe} is shown on the standard cloverleaf. You may find it useful to number the residues (1-76) to assist in the identification of the various structural elements in the exercises that follows. Figure taken from B.A. Alberts et al., *Molecular Biology of the Cell* (Third Edition, 1994). © Garland Publishing, New York.



SETUP:

Go to the HarveyLab website:

<http://uracil.cmc.uab.edu/~harvey/Tutorials/cmbi/>

If you don't have a copy of RasMol, follow the link to the [RasMol Home Page](#) and pick up the program. (Both Mac and PC versions are available. Instructions given here are for the Macintosh version, but you should be able to figure out the PC modifications.)

Download the PDB file for tRNA^{Phe} by clicking on the appropriate link. Open the PDB file in RasMol and bring the tRNA structure onto the screen. If you put the cursor into the RasMol window, hold down the mouse button and move it around, the molecule should rotate. Rotate it into the familiar upside-down "L" configuration.

There should be two windows open, one showing the molecule and one showing text communication between you and the program. Set the sizes and positions of the two windows so you can see both of them.

DISPLAY:

Press the shift key. Hold down the mouse button and move the cursor back and forth **vertically**. What happens? What happens if you move the cursor back and forth **horizontally** while the shift key is depressed? (Be sure you're moving as horizontally as possible, with no vertical component.) Release everything. Hold the option key down and move the mouse around. Hold the mouse button down and move the mouse around, repeatedly holding then releasing the option key.

Adjust the size of the molecule so that you can easily see individual atoms and bonds. Put the cursor over any atom. Click. Look in the text window.

From the menu bar, pull down the **Display** menu. Check out the various displays one at a time, i.e., *wireframe*, *backbone*, *sticks*, . . . , *strands*, *cartoons*. Resize the molecular image as necessary to examine each of these.

One of the displays allows you to see the magnesium ions clearly. (They're green in my display.) Using that display, rotate the molecule around and search visually to answer the question, how many Mg⁺⁺ ions are there in this structure? (There are six or less.)

How many magnesium ions are there? _____

DEFAULT COLORING SCHEME:

Pull down the **Display** menu again and set the display to *sticks*.

Scale the molecular size and position so that you can clearly see the individual atoms of a single residue. Write down the colors of the atoms:

carbon _____
nitrogen _____
oxygen _____
phosphorus _____

FINDING YOUR WAY AROUND THE MOLECULE:

For the next exercise, you'll want the display set to either *wireframe* or *sticks*. Note that the atomic coloring scheme is the same in these two display modes.

Successive atoms in the 5' to 3' direction of the chain are P-O5'-C5'-C4'-C3'-O3'-P. The backbone thus has a fundamental asymmetry, because of the C5' carbon on one side of the ribose ring. Let's pick a phosphorus atom and see if you can determine the 5' to 3' direction:

Find a phosphate group. Click on the phosphorus atom.

What residue did you click on? _____

Without clicking on any atoms, examine the backbone and figure out which direction is 5' to 3'; you may rotate the molecule as necessary, and scale it up and down as needed. Be very careful and take your time. Figure out which phosphorus atom corresponds to the next residue along the backbone. When you're absolutely sure, click on it and verify that you got it right.

USER-CONTROLLED COLORING SCHEMES:

Search visually through the molecule, rescaling, moving and rotating as necessary, and find the single-stranded 3' terminus. The last four residues of this tRNA are A73, C74, C75 and A76.

In the text window, type: select 73-76

then: color yellow

Note what happens when you pull down the **Display** menu and change the display to some other option (such as *spacefill*) now. (Be sure you can see the whole molecule.) It is important to understand that any change in display affects only those atoms that are currently selected (residues 73-76 in the present case). If you want to get the display of all atoms to be similar, go to the text window and type:

select all

Now pull down the **Display** menu and the display to *sticks*.

Pull down the **Colours** menus and go through the various coloring options. These apply to the whole molecule, because of the “select all” command above. The most interesting color option is *group*, because it uses the rainbow colors, going from deep blue at the 5' end of the chain to red at the 3' end.

Alternatively, you can change the colors in the text window with the commands:

```
color red
color group
color cpk
```

To illustrate how you use a coloring scheme to examine the structure, reset the display to *wireframe*, and set the coloring to CPK, using either the `color cpk` command or *cpk* in the **Colours** menu.

DEFINING GROUPS:

One very useful feature is the ability to define groups of residues by short names. Let's illustrate this by defining and displaying the acceptor stem (“accstem”), which, for tRNA^{Phe}, consists of basepairs 1-72, 2-71, ..., 7-66:

```
define accstem 1-7,66-72
```

Note that you have *defined* the acceptor stem, but you haven't *selected* it. The whole molecule is still selected. This is illustrated by the following set of commands:

```
color green
color cpk
select accstem
color green
```

As you can see, it is often very useful to regularly *select all* before changing the display of any subset of the atoms in the molecule. This will also be seen in the example below.

The following set of commands will define the other stems of the cloverleaf structure, i.e., the D, anticodon, and T stems:

```
define Dstem 10-13,22-25
define acnstem 26-31,39-44
define Tstem 49-53,61-65
```

Let's use these group definitions to investigate the difference in the stem stacking in the two arms of tRNA. One arm consists of the stacked acceptor and T stems, while the other consists of the stacked D and anticodon stems. Which has better stacking? The following sequence of commands will help you figure this out:

```
select accstem,Tstem
color yellow
select Dstem,acnstem
color red
```

In order to examine these separately, we need to make all atoms except those in a selected region disappear. RasMol uses the restrict command to display only a subset of the atoms. The only problem is figuring out how to display the whole molecule again when you're done looking at only the restricted region. The following set of commands will walk you through this, first restricting your attention to only the acceptor/T arm, then returning to display of the whole molecule, then looking at only the D/anticodon stem.

```
restrict accstem,Tstem
```

Take a good look at the geometry of the stacking in that region. When you're done and want to look at the stacking in the other arm, you must first get the whole molecule back:

```
select all
```

and then pull down the **Display** menu and display *wireframe*.

Then you must select the other arm only:

```
restrict Dstem,acnstem
```

Which arm has the best stacking and looks most like an ideal A-RNA double helix? You may want to go back and forth between those two arms and look at various views before deciding. [You may also want to take a look at one of the A-DNA structures on the webpage cited above. This will require that you *quit* RasMol (**File** menu), load the A-DNA pdb file from the webpage, examine it in Rasmol, then reload tRNA.]

The arm with the better stacking contains the _____ and _____ stems.

Now let's define the D, anticodon, variable and T loops:

```
define Dloop 14-21
define acnloop 32-38
define Vloop 45-48
```

define Tloop 54-60

Reset everything by selecting all, displaying wireframe and coloring CPK.

INVESTIGATING STRUCTURAL QUESTIONS:

First let's look at the structure of the anticodon loop. You'll find it useful to use the restrict command to show only the anticodon stem and loop.

There are seven nucleotides in the anticodon loop. Look closely at the anticodon (the stacked residues 34-35-36 and at nucleotide 37, which is stacked on top of the anticodon. The base on residue 37 is a hypermodified purine. Can you tell whether it is derived from an adenine or a guanine?

(Circle one): adenine guanine

One residue in tRNA^{Phe} is intercalated between two others. The outer two residues are 45 and 46. Select these residues (select 45,46), color them some distinguishing color, and display them as sticks. Examine them visually. Are these residues A, C, G, U, or what? Can you figure out what each of them is by looking at them? Double-check your answer by clicking on each one and write the identities of these residues here:

Residue 45 is _____
Residue 46 is _____

Color residues 45-46 yellow. Search the rest of the tRNA (displayed as *wireframe*) to find the intercalated residue. (Use *stereo* in the **Options** menu if that's helpful.) You can get its identity by clicking on it. Specify both residue number and type:

The residue intercalated between residues 45 and 46 is _____

The central region of the cloverleaf structure, where all four stems come together, has a very interesting three-dimensional structure. Similarly, the interactions where the two arms join in the "elbow region" has a number of interesting features. These interactions, revealed crystallographically in the early 1970's, showed that RNAs can form globular and rather complex structures. RNAs do this even though they have

Name or SSN _____

only four different kinds of residues (in contrast with the twenty amino acids that make up proteins), and even though those four residues have much less physical and chemical variability than do amino acids.

You should change the display as necessary to answer the following questions. (Turn on and off the various parts of the molecule, color in different ways, display as wireframe, sticks, or spacefill, etc.)

There are four loops (D, anticodon, variable and T), and you've already defined them to facilitate examination of their interactions with one another and with other parts of the tRNA molecule.

Which two loops have extensive interactions with one another? _____ and _____.

Those two loops are held together by a set of interactions involving seven bases. Basepairing, stacking, and intercalation all contribute to stabilizing the interaction. Identify three basepairs (two interloop and one intraloop) in that interaction.

The three basepairs are _____, _____, and _____.

Identify the two sets of intercalation interactions, with three bases in each intercalated set. (Intercalation is like a sandwich, with #1 and #3 being the bread, and #2 the meat in the sandwich.) One set of three bases has somewhat less than perfect intercalation geometry. One base is the bread in one triple and the meat in the other triple.

One intercalated triple involves bases _____

The other intercalated triple involves bases _____

One intercalated triple involves bases _____, _____, and _____.

One intercalated triple involves bases _____, _____, and _____.

Of the four loops mentioned above, three really do look like loops, while one is extended.

Which loop has the most extended conformation? _____

Name or SSN _____

Which stem does this loop interact with? _____

Does the loop lie in the major or minor groove of that stem? _____

Two bases from a different part of the molecule are intercalated between the bases of this extended loop. The result is a stack of five bases, sort of like a double cheeseburger, with the three pieces of bread corresponding to the bases of the loop, and the two pieces of meat corresponding to the two bases from the other part of the molecule. What five bases are in that stack? (Your answer should read from one end of the stack to the other, i.e., bread, meat, bread, meat, bread.)

Answer: _____

There are several nonstandard basepairing patterns in this part of the molecule, along with some base triples. Find as many of those as you can, identifying the bases clearly and figuring out where you think hydrogen bonding might be holding things together. You'll find it easiest if you start at the top of the D stem and work your way upward toward the elbow from there. (Each base pair or base triple is approximately coplanar.)

For each interesting basepair or base triple, you can print out a figure directly from RasMol. For clarity, change the background color from black to white:
background white

If you're drawing things from any perspective other than one in which all the bases lie in the plane of the paper, it will often be helpful to turn on *stereo* in the **Options** menu so I can see your answers clearly. (To turn stereo off, just click on *stereo* again.)

You should print out and hand in at least four figures showing unusual basepairs and base triples. Attach the figures securely to this tutorial.

OPTIONAL EXERCISES ON DNA STRUCTURE:

Now that you know how to use RasMol, you might want to look at the differences between A-DNA and B-DNA and some other DNA structures. The following files are all located on the webpage given at the very beginning of this tutorial:

A-DNA-GC-GC.pdb	A-DNA: self-complementary, alternating GC
A-DNA-Gn-Cn.pdb	A-DNA: G's on one strand and C's on the other
B-DNA-GC-GC.pdb	B-DNA: self-complementary, alternating GC
B-DNA-Gn-Cn.pdb	B-DNA: G's on one strand and C's on the other
B-DNA-dickerson-bdl001.pdb	The <i>E. coli</i> restriction site
DNA-tetraplex-udl018.pdb	A four-stranded structure containing G-quartets

HELP FACILITY WITHIN RasMol:

RasMol has a help facility which you can activate in the text window. The general command is

help

while you can get information on any specific topic by typing a command such as

help color

or

help define

To get a list of all RasMol commands, type

help commands