

Computational Biochemistry

Your ID

Your Name

Created with: Release 2020-3

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In this tutorial, you will learn how to draw small molecules, make double stranded RNA, and examine enzyme in complex with RNA and an inhibitor in Maestro molecular modeling software.

Words found in the Glossary of Terms are shown like this: Workspace

File names are shown with the extension like this: 1fjs.pdb

Items that you click or type are shown like this: **File > Import Structures**

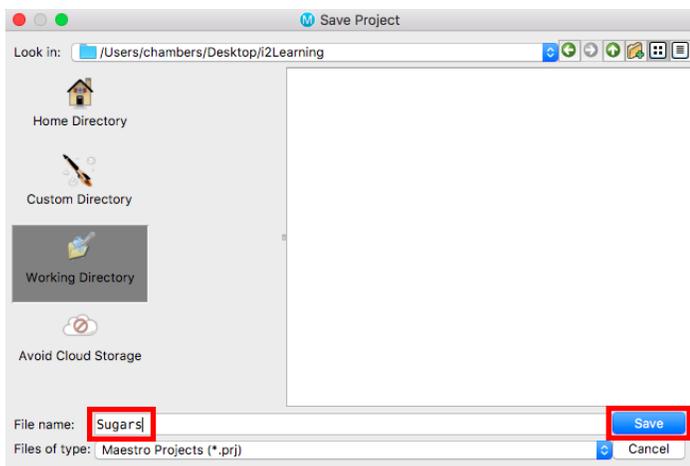
This tutorial is written using a 3-button mouse with a scroll wheel.

- 1. *Drawing Adenosine - p. 1***
- 2. *Creating Double stranded RNA - p. 6***
- 3. *Examining Enzymes - p. 9***
- 4. *Glossary of Terms - p. 13***

1. Drawing Adenosine

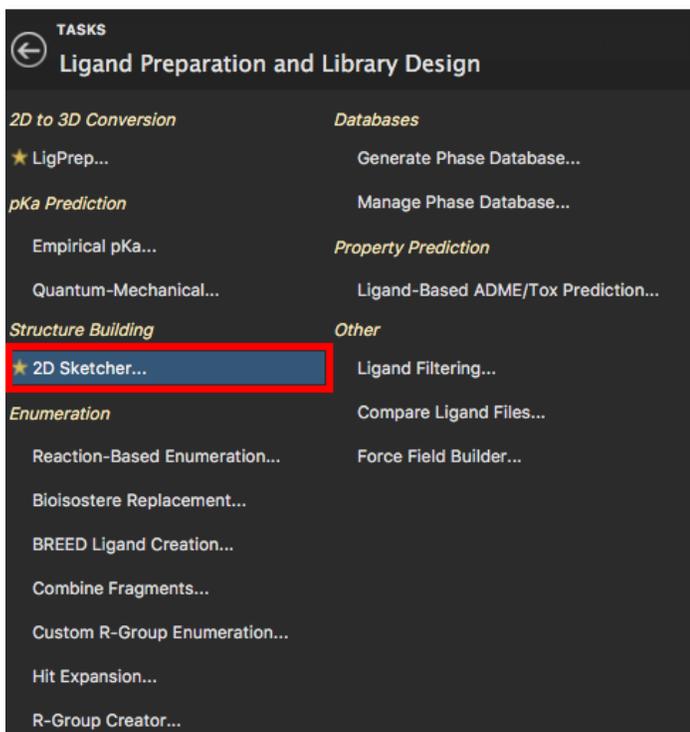


1. Double-click the **Maestro** icon



2. Go to **File > Save Project As**
3. Change the File name to **YourID_YourLastName_Adenosine**, click **Save**
 - The project is now named YourID_YourLastName_Adenosine.prl

Figure 1-2. Save Project panel.



4. Go to **Edit > 2D Sketcher (top tap)**
 - The 2D Workspace - 2D Sketcher opens

Figure 1-3. Open the 2D Sketcher.

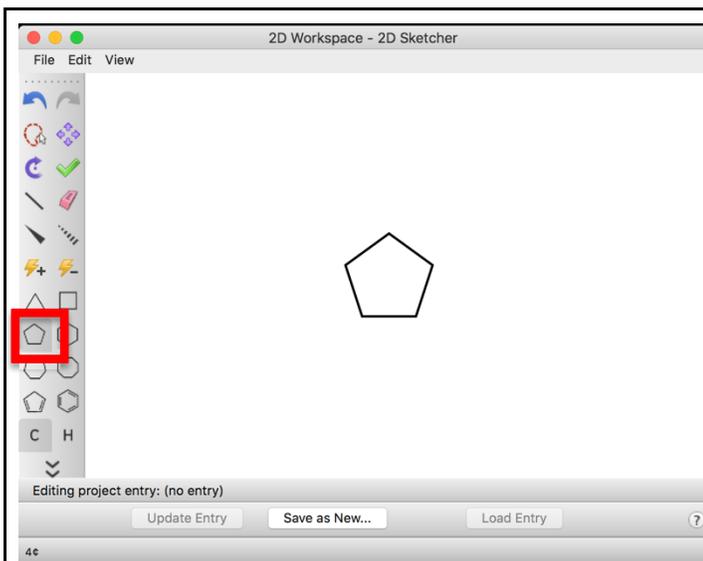


Figure 1-4. Draw cyclopentane.

5. In the 2D Sketcher, click **cyclopentane**

Note: Type Ctrl+Z (Cmd+Z on a Mac) to undo a previous action.

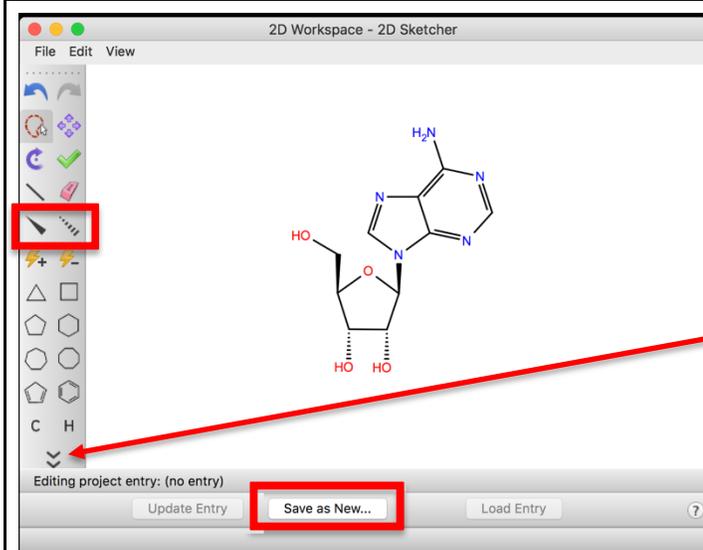


Figure 1-5. Draw adenosine.

6. Draw **Adenosine**, as shown in Figure 1-5, using wedge and dash bonds.

Wedge bonds (pointing to the front)

Dash bonds (pointing to the back)

Click this for adding oxygen and nitrogen atoms

7. Click **Save as New**

Note: Add bonds initially as straight lines, then adjust to wedges and dashes.

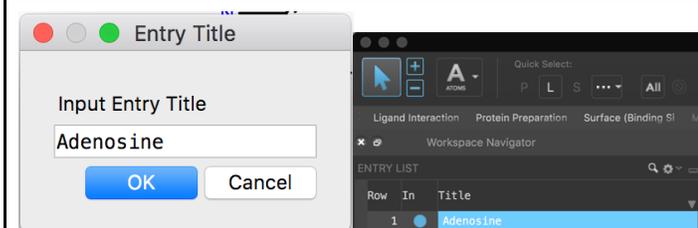


Figure 1-6. Add Adenosine to the Entry List.

8. In Entry Title, type **Adenosine** and click **OK**

- A new entry titled Adenosine is in the Entry List
- Adenosine is included in the Workspace

9. Exit 2D Sketcher: click x icon

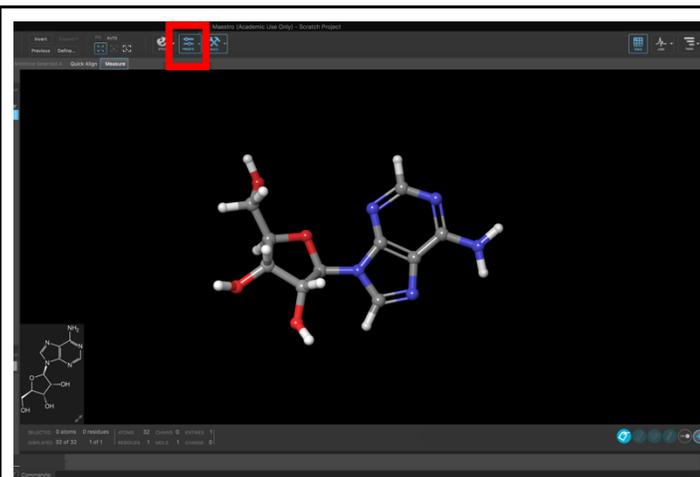


Figure 1-7. Render Adenosine in ball and stick.

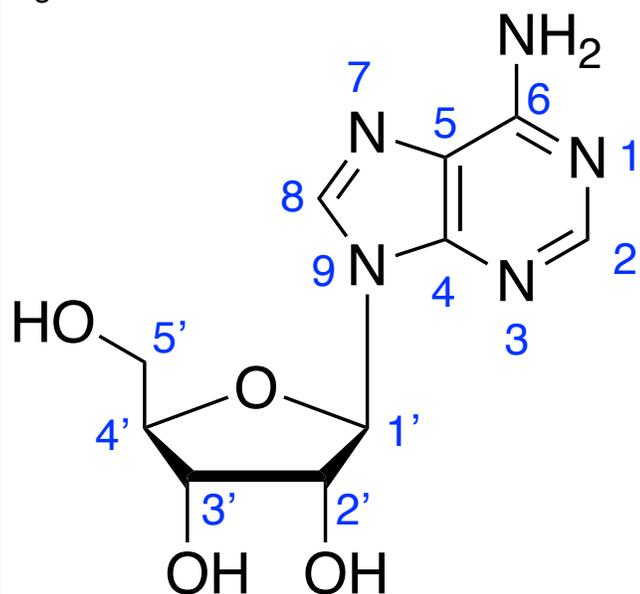


Figure 1-8. 2D structure of Adenosine

10. Click **Presets > Ball and Stick**

- Adenosine is rendered in ball and stick

Practice#1: you can choose others like maestro default, Bioluminate default, publication, etc.

Practice#2: you can rotate the structure (left click, then click and drag your scroll wheel, zoom in or out (using the wheel), and translate (using right click and drag)

Can you see the adenine ring is pointing to the upside, two hydroxyl groups (-OH) are pointing to the downside, and methylene hydroxy group (-CH₂OH) is pointing to the upside, or vice versa?

Each nucleotide in RNA contains a ribose sugar, with carbons numbered 1' through 5' as shown in the left figure. A base, adenine in adenosine, is attached to the 1' position of the ribose sugar and numbered 1 through 9.

A phosphate group (PO₃) is attached to the 3' position of one ribose and the 5' position of the next.

N1 (nitrogen 1) and NH₂ at the carbon 6 in adenine form hydrogen bond interactions with the uracil base of Uridine in RNA base-pairs.

(note: carbon atoms and hydrogen atoms connected to carbon atoms are not presented in the skeletal structure (Figure 1-8).

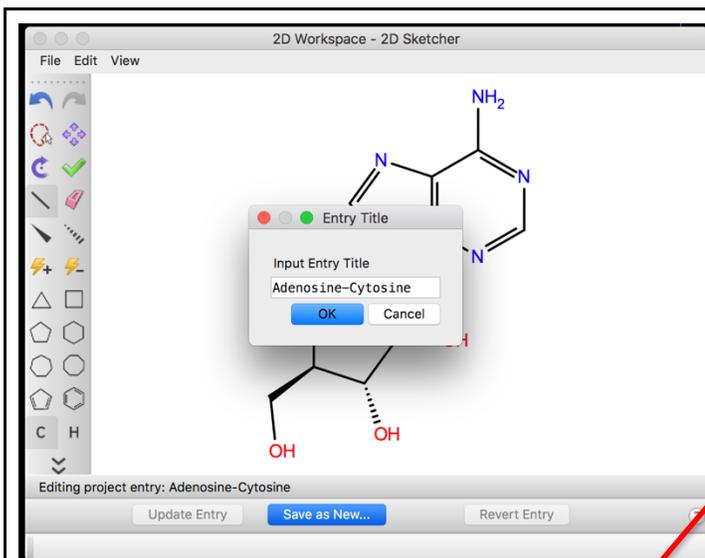


Figure 1-9. Include Adenosine-Cytidine in the Workspace.

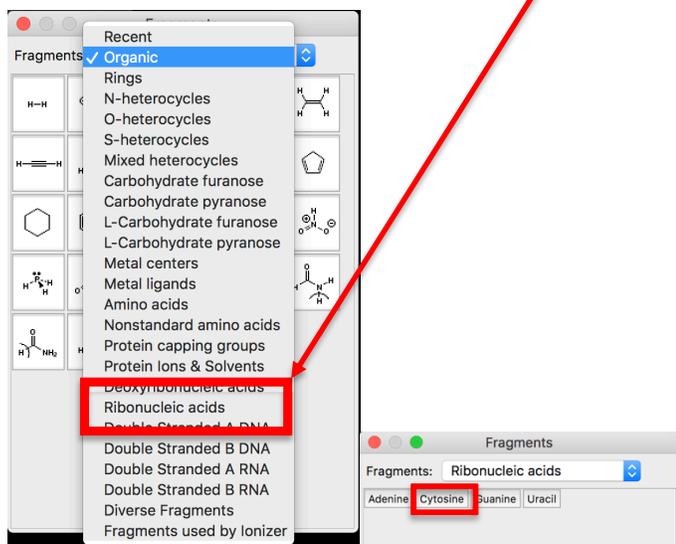
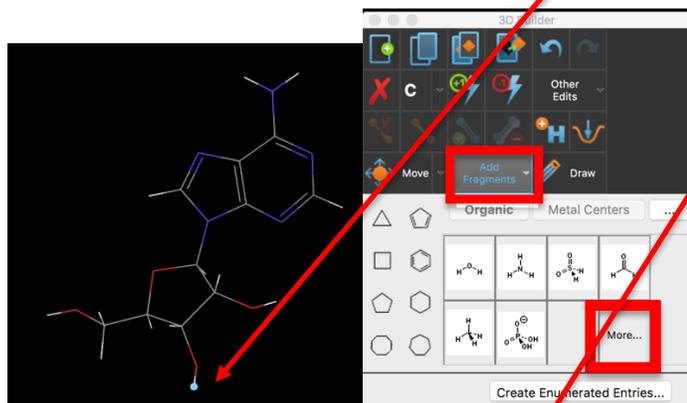


Figure 1-10. Add Cytosine to Adenosine

11. Open 2D sketch: **Edit > 2D sketcher**

12. Click Save as New, then save as Adenosine-Cytidine

- A new entry titled Adenosine-Cytidine is in the Entry List

13. Click the Hydrogen atom of OH at the 3' position: blue dot is shown.

14. Open 3D Builder: **Edit > 3D Builder** and 3D Builder panel opens.

15. Click Add Fragments and More: Fragments panel opens.

16. Select Ribonucleic acid in Fragments

17. Select Cytosine

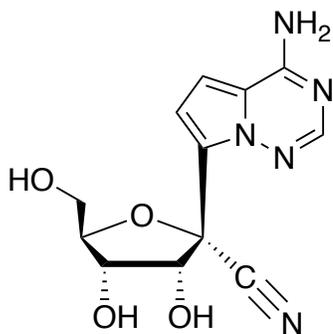
- Cytidine is added to the 3' position of Adenosine

18. Click **Presets > Ball and Stick**

- Adenosine-Cytidine is rendered in ball and stick

Can you see a phosphate group connected to 3'- position of Adenosine and 5'-position of Cytidine?

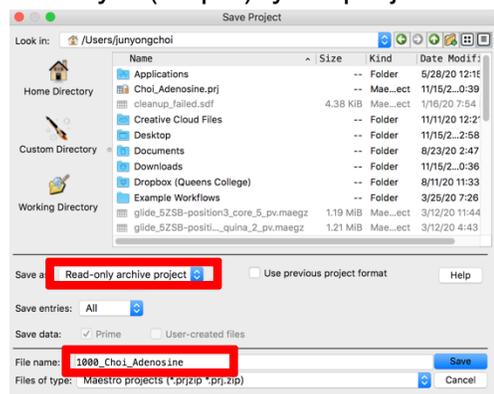
Activity 1 (10 pts): Draw the 3D structure of Remdesivir in maestro
(instructions: clear workspace: click workspace on the top tap > clear workspace
draw Remdesivir in 2D sketcher and save it as New. Entry title should be Remdesivir.)



Activity 2 (5 pts): How do the adenosine and Remdesivir differ?
(notes: After drawing the Remdesivir structure, include Adenosine and Remdesivir in the workspace.
Click circles in the entry list in the left panel by using Command (keyboard) and left click (mouse)
Then, type Command & L (Control & L for Win): the workspace is tiled and both Adenosine and Remdesivir can be seen)

Activity 3 (5 pts): How are they same?

Activity 4 (10 pts): your project file will be evaluated. Save your project as a read-only archive project.



1. Go to **File > Save Project As**
2. Click **Save as** and choose **Read-only archive project**.
3. Write the file name as **YourID_YourLastName_Adenosine**, click **Save**

The project is now named
YourID_YourLastName_Adenosine.prjzip

After saving your project file, close the project: **File > close project**

2. Creating Double Stranded RNA

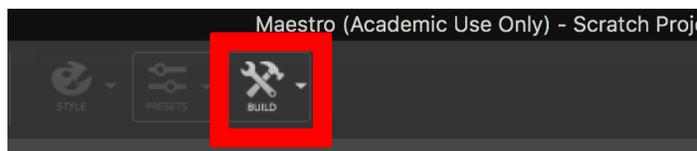


Figure 2-1. Open the Build panel.

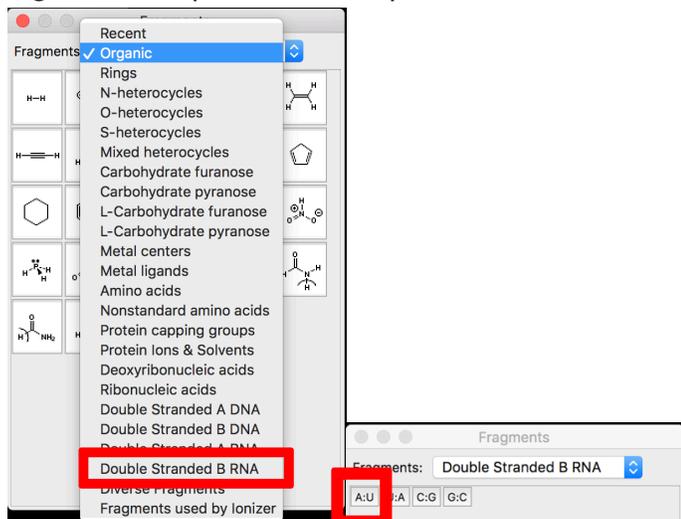


Figure 2-2. Build Adenosine : Uracil

1. Open 3D Builder: **Edit > 3D Builder** and 3D Builder panel opens.

Or click Build icon

2. Click Add Fragments and More: Fragments panel opens.

3. Select Double Stranded B RNA in Fragments

4. Select A:U

- Adenosine and Uracil are shown
- Two phosphate groups are added to 3'- and 5'-position of ribose

5. Rename Structure1 to A:U for the title in the Entry List

6. Click **Presets > Ball and Stick**

- Adenosine : Uracil are rendered in ball and stick

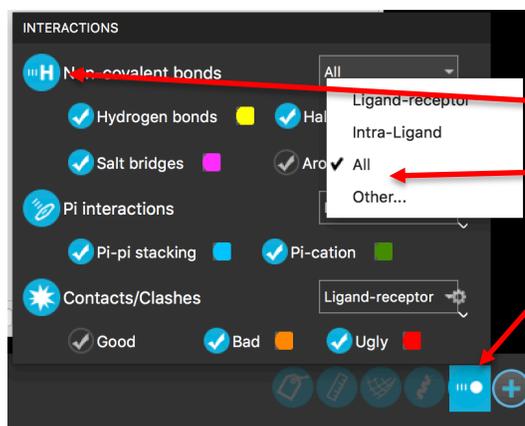
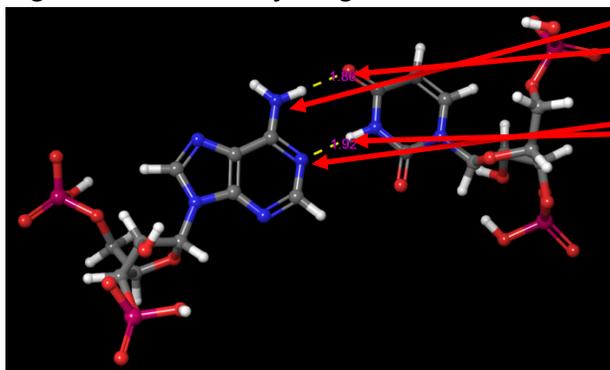


Figure 2-2. Show hydrogen bond interactions.



7. Click the icon on the right bottom window and three dot icon

8. Click the H icon (highlight it)

9. Select all

You can see yellow dot lines between adenosine and uracil bases, which shows hydrogen bonding interactions.

10. Click **Workspace > Measure**

Select the hydrogen atom (white) on nitrogen (blue) of adenine, then the oxygen atom (red) of Uracil.

Select the nitrogen atom (blue) of adenine and the hydrogen atom of Uracil.

Which numbers are shown?

The unit is an angstrom (10^{-10} meter)

11. Click OK on the measurement panel

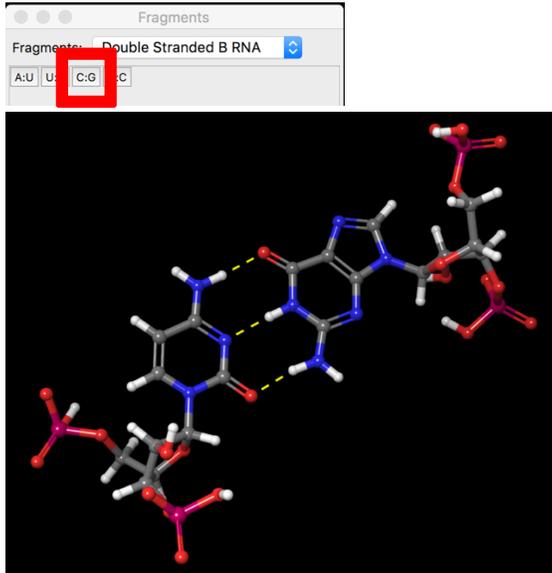


Figure 2-3. Build Cytosine : Guanine

12. Clear workspace: **workspace > clear workspace**
13. Click C:G icon on the Fragments panel
14. Rename Structure2 to C:G for the title in the Entry List

C stands for Cytidine, and G stands for Guanosine. Cytidine and Guanosine base pairs form three hydrogen bond interactions, while Adenosine and Uridine form two hydrogen bonds.

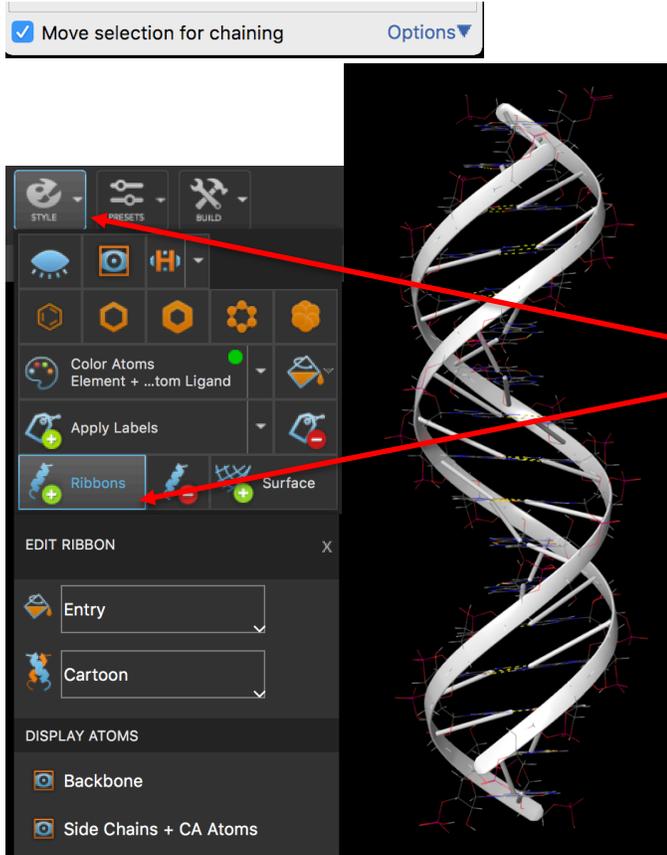


Figure 2-4. Build Double Stranded B RNA.

1. Clear workspace: **workspace > clear workspace**
2. Select “move selection for chaining” in the Fragments panel
3. Click A:U, U:A, C:G, and/or GC (at least 20 times)
4. Click **STYLE > Ribbons**, then choose Ribbon
5. Rename Structure3 to B-RNA for the title in the Entry List
6. *Note:* Type **Z** if you cannot see the whole RNA structure
7. You can exam the structure by rotating the structure and/or changing presentation types

Activity 5 (5 pts): Build double stranded B-RNA in the maestro: use at least 20 base pairs of ribonucleic acids and rename it to A-RNA. You can show the ribbon style of the RNA.

Activity 6 (10 pts): Compare and explain the shape of A-RNA with B-RNA.

Activity 7 (5 pts): Draw 2-D structures (or skeletal structures) of Adenosine – Uridine and Guanosine - Cytidine that show hydrogen bond interactions. Use 3D structures you built in maestro as a reference (you don't need to draw a phosphate group).

Activity 8 (10 pts): your project file will be evaluated. Save your project as a read-only archive project (**YourID_YourLastName_RNA**)

3. Examining an Enzyme – RNA – Remdesivir complex

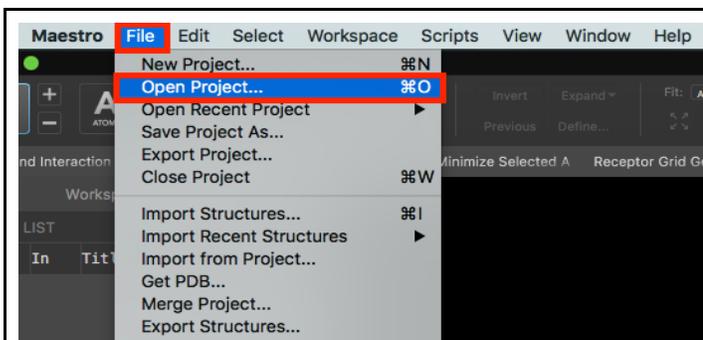


Figure 3-1. Open the KMSO2020.prjzip.

1. Go to **File > Open Project > KMSO2020.prjzip**
2. In the Save Scratch Project box, click **OK**
3. Go to **File > Save Project As**
4. Name the project
YourID_YourLastName_Design
5. Type **Z** to see the whole structure
6. Click and drag the **scroll wheel** to rotate the structure

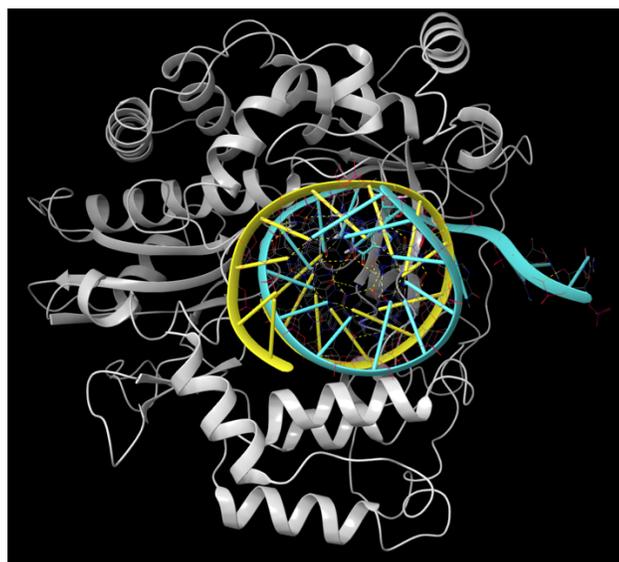
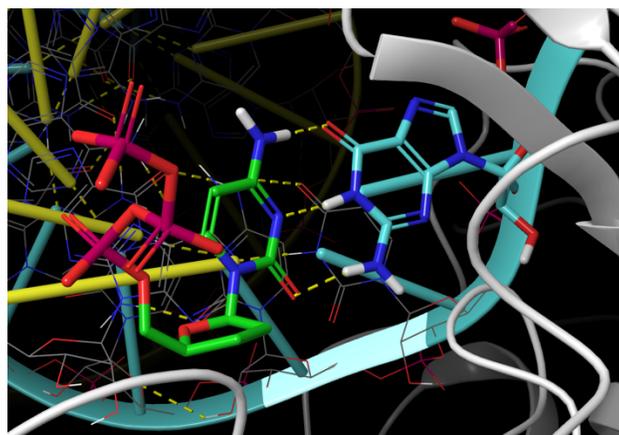


Figure 3-2.

7. Click **View > Restore Camera View > 1 whole view**
 - The enzyme (white) is called RNA-dependent RNA polymerase, or RNA replicase, which catalyzes the synthesis of the RNA strand complementary to a given RNA template (also called replication of RNA).
 - Cyan is a give RNA template, and yellow is a synthesized RNA strand.
8. Click **View > Restore Camera View > close view 2**

Activity 9 (5 pts): Which ribonucleotide is in the RNA template for the RNA replication (Adenosine, Guanosine, Cytidine, or Uridine), and which nucleotide needs to be added for RNA replication?



9. Include 3Dpol-RNA-CTP open in the Entry List
 - A nucleoside triphosphate with green carbon is added to the pocket.
 - The new nucleoside triphosphate form three hydrogen bond interactions with the nucleotide in the RNA template.
 - The OH group at the 3'-position of Adenosine in the synthesized RNA

Figure 3-3. A newly added nucleotide triphosphate and hydrogen bond interactions with a nucleotide in the RNA template.

(yellow) is close to the first phosphate of the new nucleoside triphosphate.

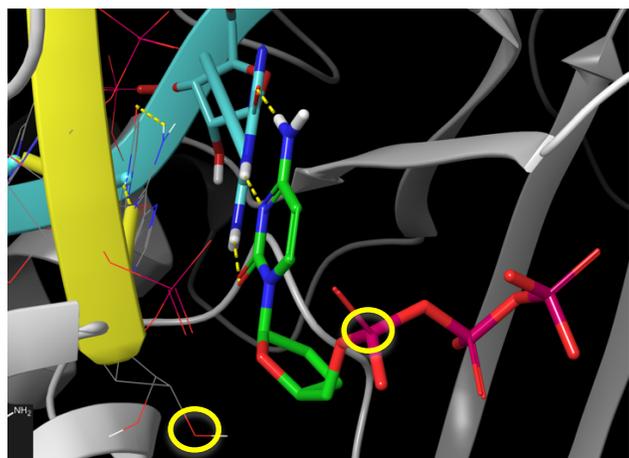


Figure 3-4. A new nucleotide triphosphate added to the pocket of the protein and RNA.

Activity 10 (5 pts): What is the distance between the oxygen atom at the 3'-position of the Adenosine in the yellow strand and the first phosphor atom (magenta) connected to the oxygen atom at the 5'-position of the new nucleoside triphosphate (two yellow circles in the Figure 3-4).

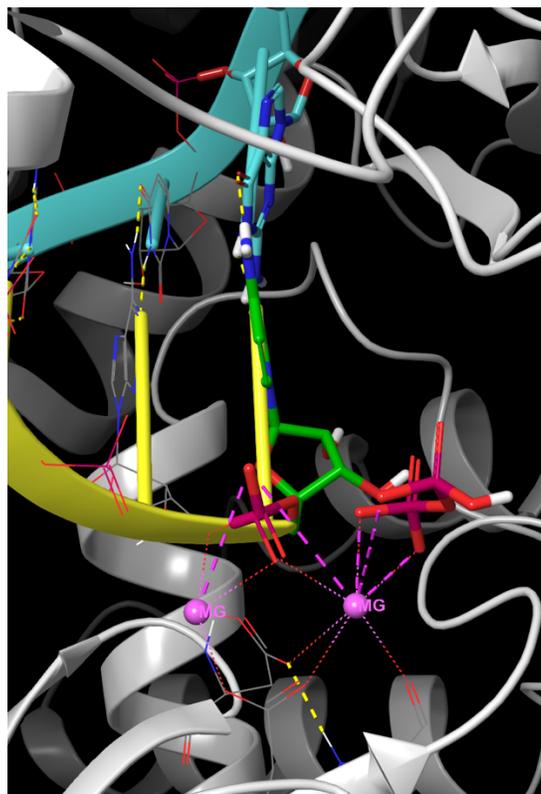


Figure 3-5. The new nucleotide is connected to the oxygen of the 3'-position of the previous nucleotide in replication.

10. Include 3Dpol-RNA-CTP closed in the Entry List

- A green nucleoside is connected to the Adenosine in the yellow strand in the process of RNA replication.
- Diphosphate is released from the nucleotide triphosphate, and two magnesium ions and Aspartic acids are involved in the synthesis of double stranded RNA.

11. Include Enzyme-RNA-inhibitor in the Entry List

12. Type **L** to zoom into the inhibitor

Activity 11 (10 pts): Explain what the inhibitor is and how it works to kill viruses.

Activity 12 (20 pts): RNA-dependent RNA polymerase is a crucial enzyme in the coronavirus life cycle and in other RNA viruses. This nezyme mediates the transcription and replication of the RNA genome during infection. Since this enzyme has no human counterpart, together with its essentiality for the virus's life cycle, it has been an interesting therapeutic target for the treatment of viruses including SARS-CoV and Covid-19. Remdesivir, an adenosine analogue with broad antiviral spectrum in RNA viruses, inhibits the RNA-dependent RNA polymerase. Although the FDA authorized the use of this drug for emergency treatment of Covid-19 patients, it was not associated with statistically significant clinical benefits for patients.

You are a team leader in a pharmaceutical biotech company, and your urgent job is to design therapeutic candidates for Covid-19 via computer-aided molecular modeling. Design two or more inhibitors in maestro and write rationale for your design in the blank below and next page.

Note: Include Inhibitor 1, then open 2D sketcher or 3D builder panel, and draw an inhibitor. Repeat this for Inhibitor 2.

If you want to design more, click Inhibitor 2 title in the entry list, then **right button > Duplicate > In place**. Include the duplicated inhibitor 2, then rename it to Inhibitor 3, then draw the third inhibitor.

Save your project as a read-only archive project (YourID YourLastName Design)



4. Glossary of Terms

Entry List - a simplified view of the Project Table that allows you to perform basic operations such as selection and inclusion

included - the entry is represented in the Workspace, the circle in the In column is blue

Project Table - displays the contents of a project and is also an interface for performing operations on selected entries, viewing properties, and organizing structures and data

Scratch Project - a temporary project in which work is not saved, closing a scratch project removes all current work and begins a new scratch project

selected - (1) the atoms are chosen in the Workspace. These atoms are referred to as "the selection" or "the atom selection". Workspace operations are performed on the selected atoms. (2) The entry is chosen in the Entry List (and Project Table) and the row for the entry is highlighted. Project operations are performed on all selected entries

Working Directory - the location that files are saved

Workspace - the 3D display area in the center of the main window, where molecular structures are displayed