



## Chimera Tutorial

How to use Chimera and ChimeraX  
to visualize 3D RNA structures

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# Why should I care about 3D RNA structures?

- You don't *\*have\** to look at 3D structures to design RNA
- But sometimes things don't make sense otherwise . . .

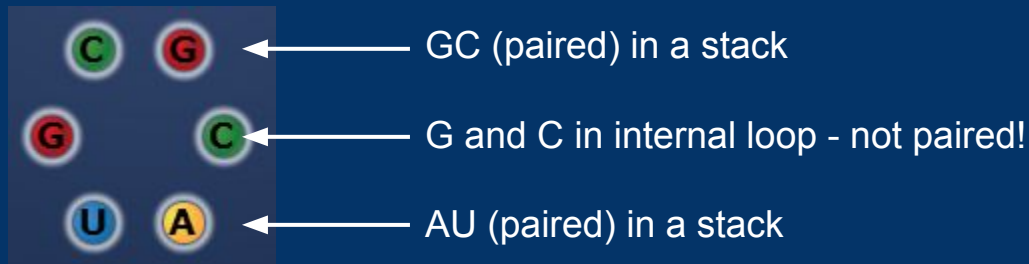
# Example: “Outsider”

- [Blog post](#) by Nando (ElNando888)
- Saw this in a real-life structure:



# Example: “Outsider”

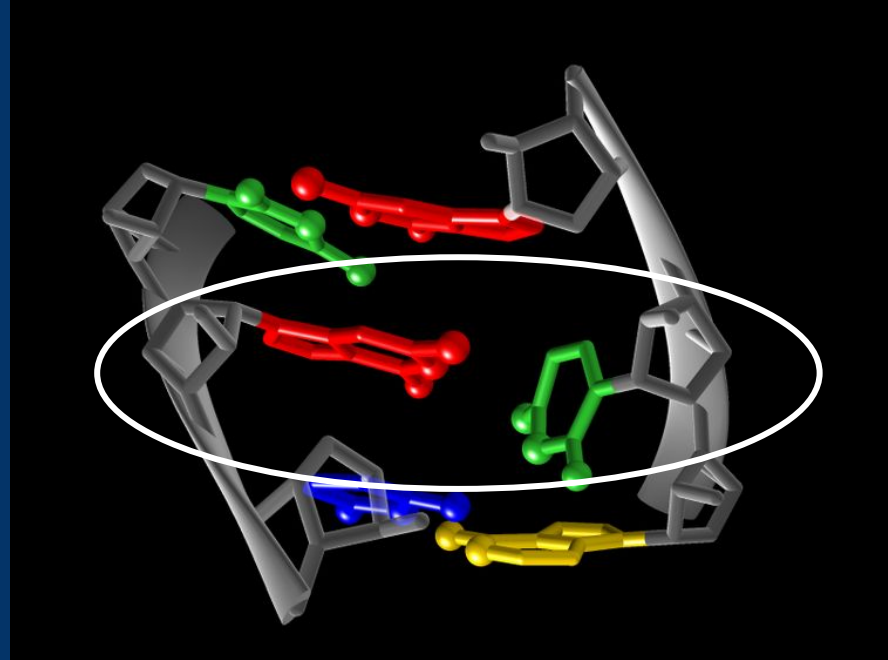
- [Blog post](#) by Nando (ElNando888)
- Saw this in a real-life structure:



- Our Eterna senses tell us this isn't right! What's going on?

# A 3D view lets us directly observe base interactions.

- Here, the middle G and C are “tilted” in relation to each other



(PDB accession [3J2A](#),  
residue 744 in chain N)

# Seeing someone else's 3D structures is great; how do I look at 3D structures myself?

- Wide variety of 3D viewing programs available
- Today I'll be showing ChimeraX (which is very similar to Chimera)
  - Both from UCSF (University of California San Francisco)
  - Free!
- Will revisit Nando's example and see if we can replicate it ourselves

# Installing ChimeraX (or Chimera)

- [ChimeraX](#) download page
  - I'll be using version 1.4 of ChimeraX
- [Chimera](#) download page
- Recommendation: use the production release for your operating system
  - If that doesn't work, try using a previously released version
- Note that this page also links to guides and tutorials
  - (Here, it's hidden under "Features")

Download UCSF ChimeraX

UCSF ChimeraX is the next-generation visualization program from the [Resource for Biocomputing, Visualization, and Informatics](#) at UC San Francisco.

- ChimeraX is **free for academic, government, nonprofit, and personal use**; commercial users, please see [commercial licensing](#)
- Please [cite ChimeraX](#) in publications for which it was used

[Latest Production Release](#)  
[Daily Build](#)  
[Older Releases](#)  
[Technology Preview Builds](#)

See also: [Common Platform Issues](#), [System Requirements](#), [Change Log](#), [Download & Citation Counts](#)

► Features  
► Missing Features

— Operating system detected as Windows 10 —

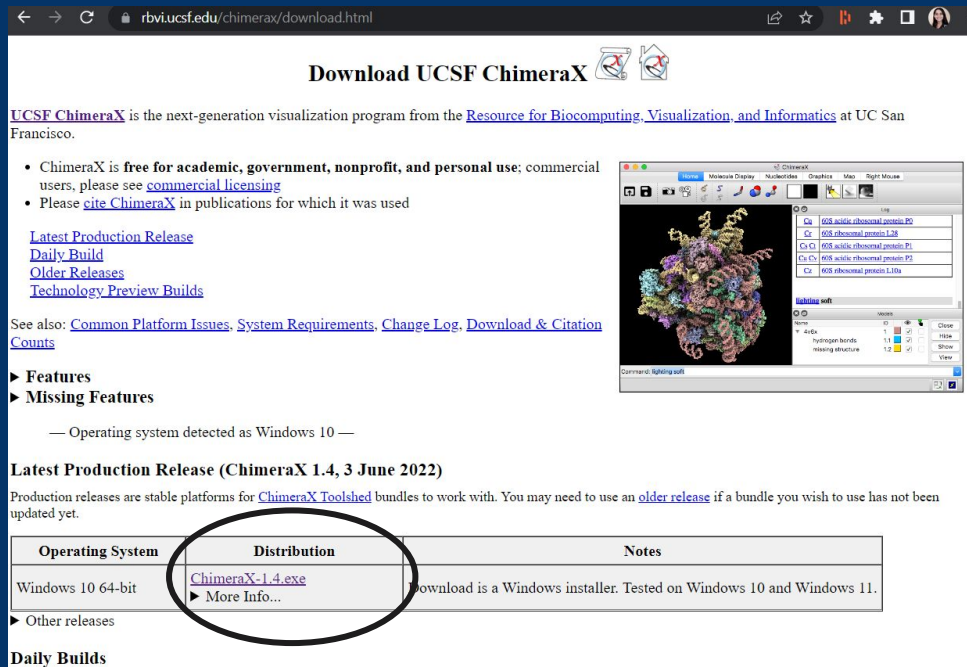
**Latest Production Release (ChimeraX 1.4, 3 June 2022)**

Production releases are stable platforms for [ChimeraX Toolshed](#) bundles to work with. You may need to use an [older release](#) if a bundle you wish to use has not been updated yet.

Operating System	Distribution	Notes
Windows 10 64-bit	<a href="#">ChimeraX-1.4.exe</a> ► More Info...	Download is a Windows installer. Tested on Windows 10 and Windows 11.

► Other releases

**Daily Builds**

The image shows a screenshot of a web browser displaying the UCSF ChimeraX download page. The page title is "Download UCSF ChimeraX" and it includes a description of the software as a next-generation visualization program. It lists features like being free for academic use and provides links for licensing, citing, and downloading. A table of production releases is shown, with the Windows 10 64-bit version highlighted. To the right, a small window shows the ChimeraX interface with a 3D molecular model and a list of loaded files.

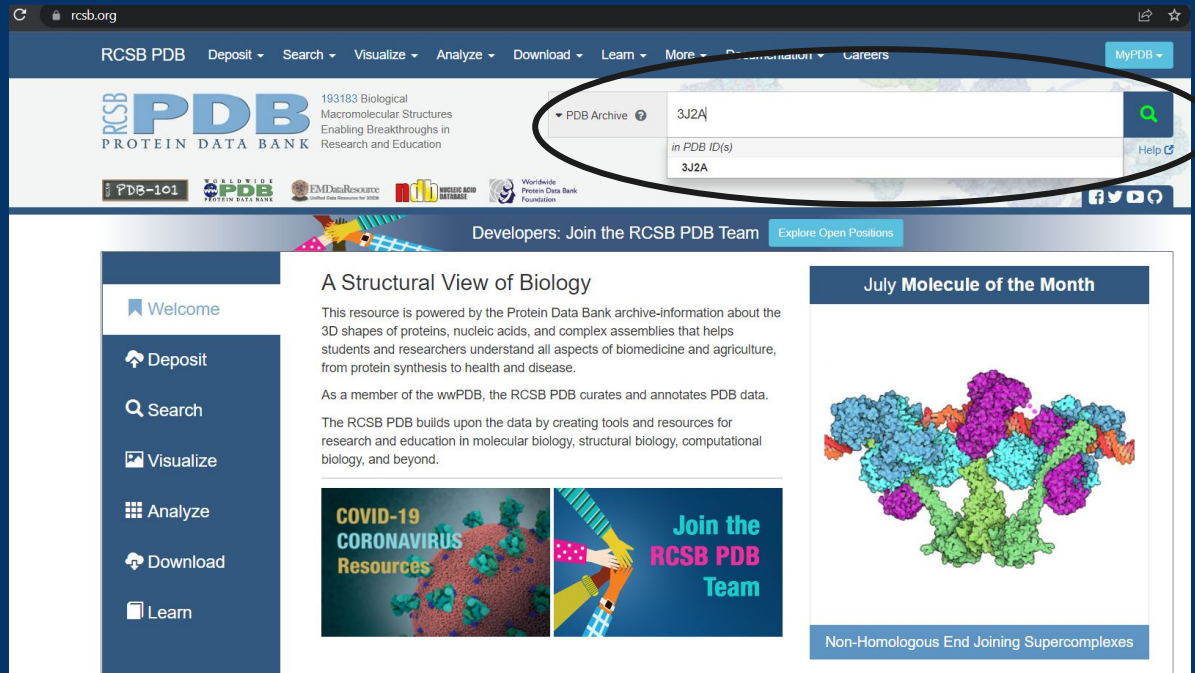
# Let's start. First, I have to find a 3D file.

- Remember what Nando said: “PDB accession 3J2A, residue 744 in chain N”
- .pdb is a common 3D structure format; you might also see “mmCIF” (.cif) files
  - Both should load within ChimeraX



# Finding the “3J2A” .pdb file

- RCSB PDB website
  - Type “3J2A” into search bar



The screenshot shows the RCSB PDB website interface. The search bar is highlighted with a black oval, and the text "3J2A" is entered. Below the search bar, the text "in PDB ID(s)" and "3J2A" are visible. The website header includes the RCSB PDB logo and navigation links. The main content area features a sidebar with links to Welcome, Deposit, Search, Visualize, Analyze, Download, and Learn. The central section is titled "A Structural View of Biology" and contains text about the resource. The right section is titled "July Molecule of the Month" and displays a 3D molecular structure. At the bottom, there are banners for COVID-19 Coronavirus Resources and Join the RCSB PDB Team.

RCSB PDB  
193183 Biological Macromolecular Structures  
Enabling Breakthroughs in Research and Education

PDB-101  
PDB  
EMDataResource  
EMDataResource  
Worldwide Protein Data Bank Foundation

Developers: Join the RCSB PDB Team  
Explore Open Positions

Welcome

Deposit

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Learn

A Structural View of Biology

This resource is powered by the Protein Data Bank archive-information about the 3D shapes of proteins, nucleic acids, and complex assemblies that helps students and researchers understand all aspects of biomedicine and agriculture, from protein synthesis to health and disease.

As a member of the wwPDB, the RCSB PDB curates and annotates PDB data.

The RCSB PDB builds upon the data by creating tools and resources for research and education in molecular biology, structural biology, computational biology, and beyond.

COVID-19 CORONAVIRUS Resources

Join the RCSB PDB Team

July Molecule of the Month

Non-Homologous End Joining Supercomplexes

# Finding the “3J2A” .pdb file

- This takes us to the 3J2A page
- Go to “Download Files” and choose “PDB Format”

The screenshot shows the RCSB PDB website interface for the 3J2A entry. The top navigation bar includes links for RCSB PDB, Deposit, Search, Visualize, Analyze, Download, Learn, More, Documentation, and Careers. The main header features the PDB logo and the text '193183 Biological Macromolecular Structures Enabling Breakthroughs in Research and Education'. A search bar is present with a 'PDB Archive' dropdown and a 'Help' link. The entry page for 3J2A is displayed, with tabs for Structure Summary, 3D View, Annotations, Experiment, Sequence, Genome, and Versions. The '3D View' tab is active, showing a 3D ribbon diagram of the protein structure. The right sidebar contains the entry title '3J2A', a description 'Dissecting the in vivo assembly of the 30S ribosomal subunit reveals the role of RimM', and various metadata including PDB DOI, EM Map, Classification (RIBOSOME), Organism(s) (Escherichia coli K-12), Mutation(s) (No), and Deposition/Release dates. The 'Experimental Data Snapshot' section provides details on the method (ELECTRON MICROSCOPY), resolution (13.1 Å), aggregation state (PARTICLE), and reconstruction method (SINGLE PARTICLE). The 'wwPDB Validation' section shows percentile ranks for Clashscore and RNA backbone, with a '3D Report' and 'Full Report' link. The 'Download Files' button is circled in red.

RCSB PDB | 193183 Biological Macromolecular Structures Enabling Breakthroughs in Research and Education

Advanced Search | Browse Annotations | Help

Structure Summary | 3D View | Annotations | Experiment | Sequence | Genome | Versions

Biological Assembly 1

## 3J2A

Dissecting the in vivo assembly of the 30S ribosomal subunit reveals the role of RimM

PDB DOI: 10.2210/pdb3J2A/pdb | EM Map EMD-5502: EMDB EMDataResource

Classification: **RIBOSOME**

Organism(s): Escherichia coli K-12

Mutation(s): No

Deposited: 2012-09-28 Released: 2013-01-16

Deposition Author(s): Guo, Q., Goto, S., Chen, Y., Muto, A., Himeno, H., Deng, H., Lei, J., Gao, N.

Experimental Data Snapshot

Method: ELECTRON MICROSCOPY

Resolution: 13.1 Å

Aggregation State: PARTICLE

Reconstruction Method: SINGLE PARTICLE

wwPDB Validation

3D Report | Full Report

Metric	Percentile Ranks	Value
Clashscore	10	
RNA backbone	0.37	

Worse | Better

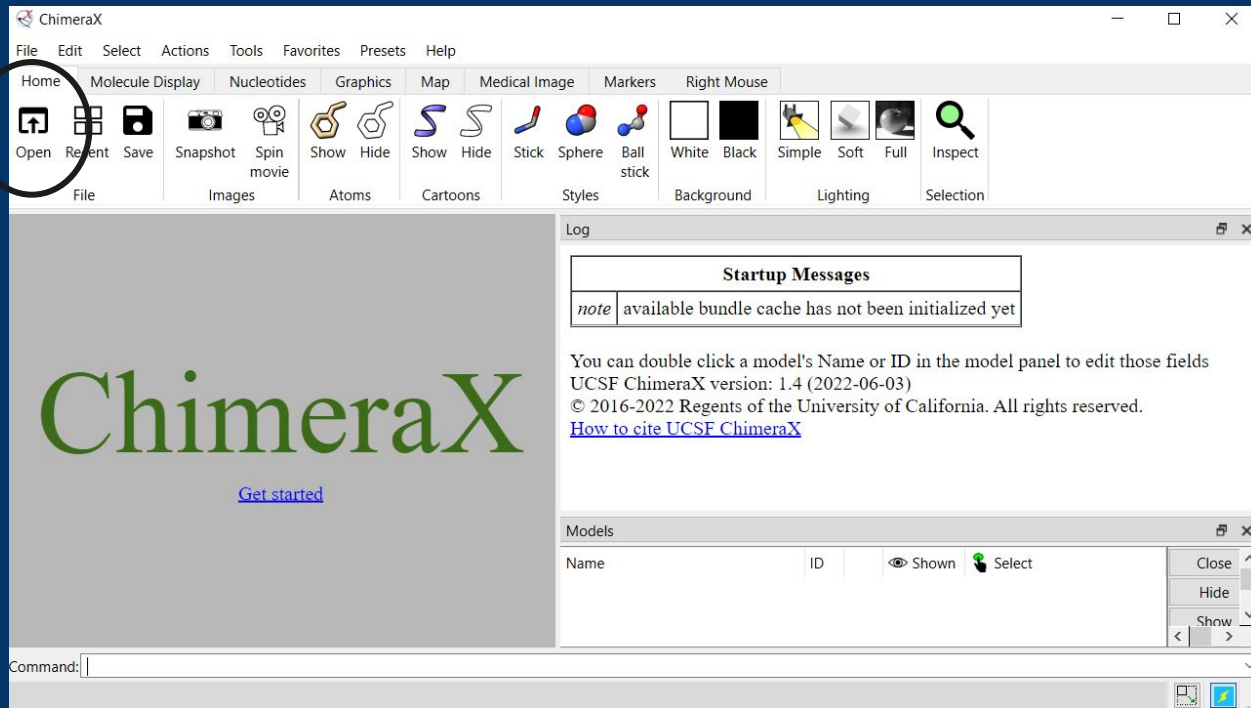
Percentile relative to all structures

Percentile relative to all EM structures

3D View: Structure | 1D-3D View | Electron Density | Validation Report

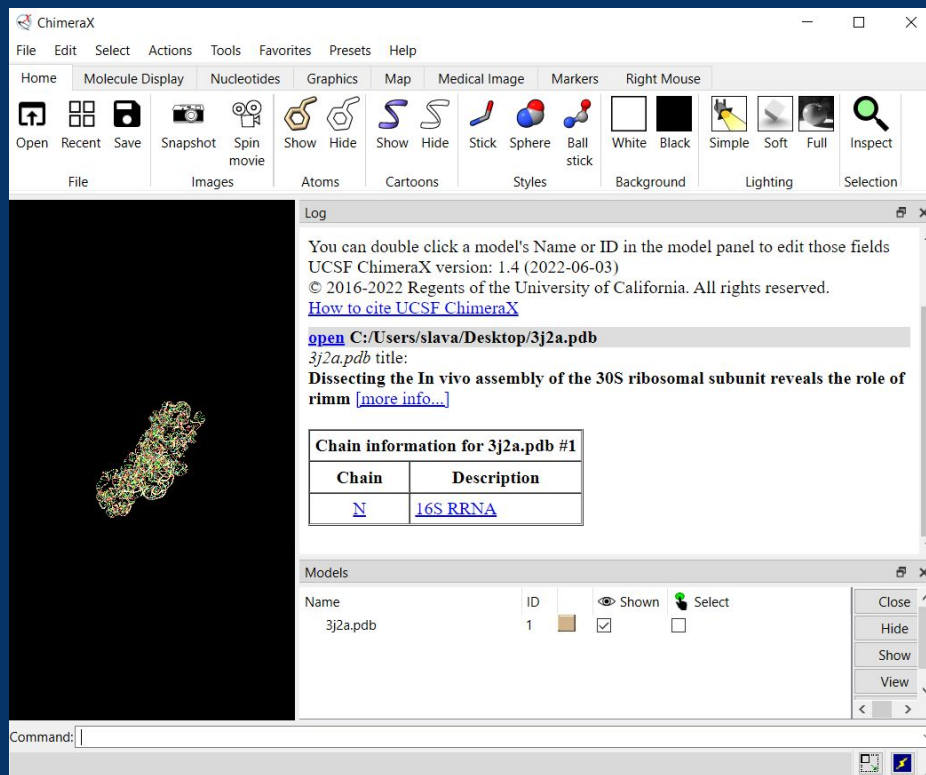
# How do I load the 3D file into ChimeraX?

- Choose the “Open” option and choose our .pdb file



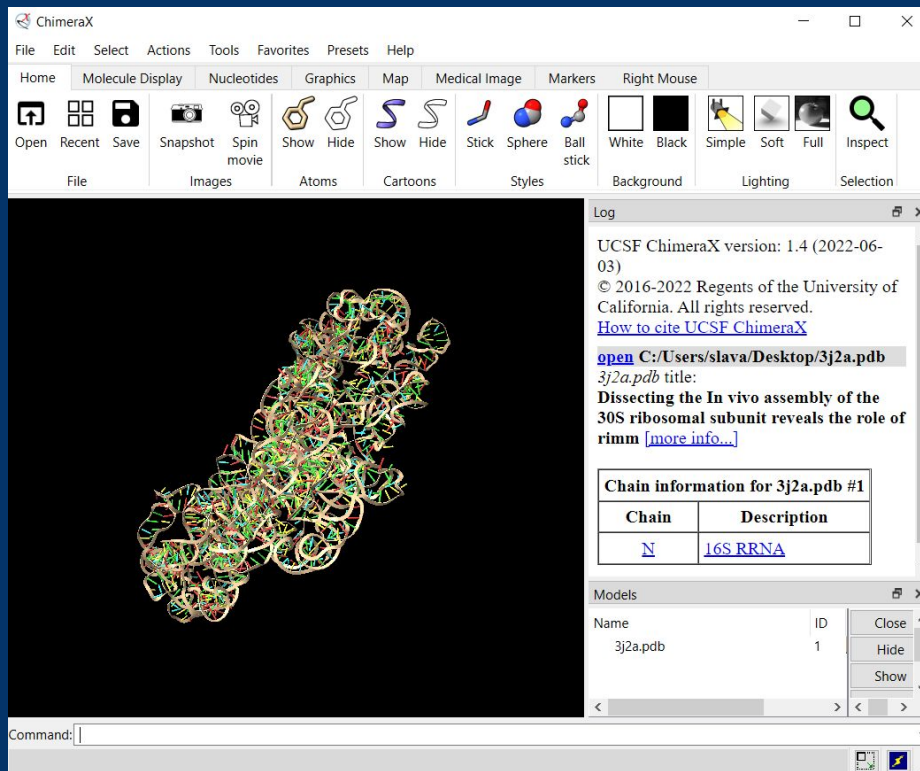
# Great! . . . Now what?

- Let's adjust the view so we can actually see the RNA
- Controls (on Windows):
  - Click and drag to rotate
  - Mouse wheel to zoom
  - Shift-click and drag to pan



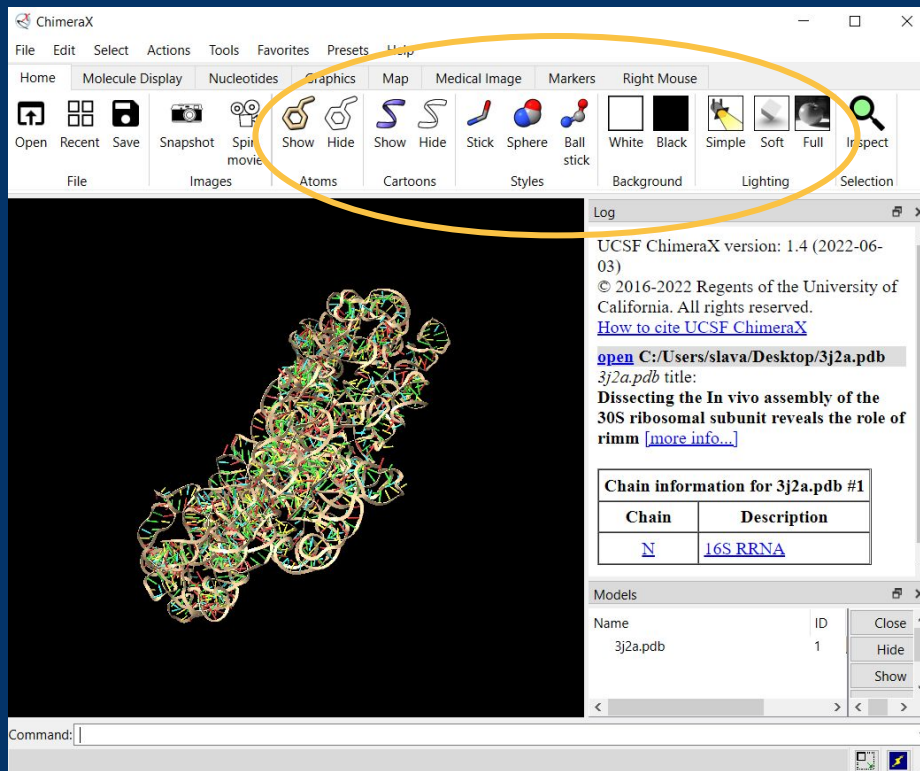
# Great! . . . Now what?

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- Controls (on Windows):
  - Click and drag to rotate
  - Mouse wheel to zoom
  - Shift-click and drag to pan
- Better!



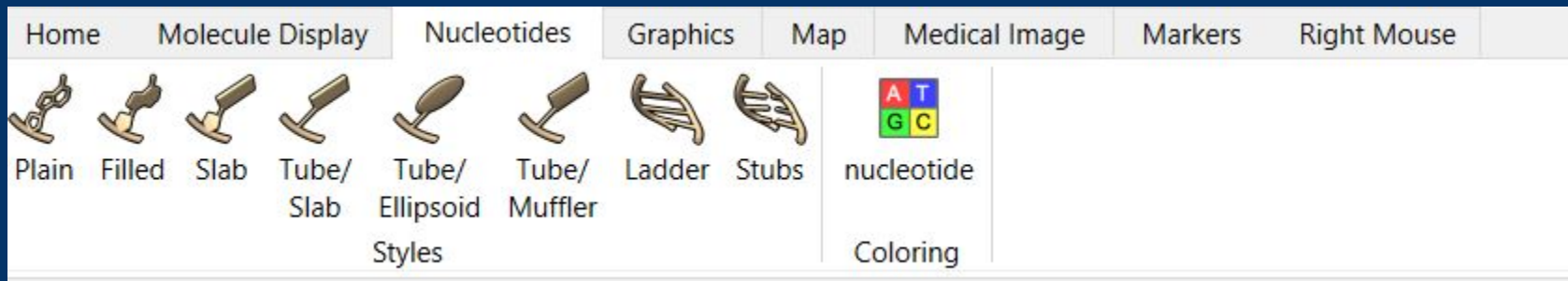
# Using visual style options to better interpret 3D

- Basic visual options shown in top menu: Atoms, Cartoons, Styles, Background, and Lighting
  - Note: some (like styles) will only be noticeable if you show atoms
- More detailed visual options available in dropdown menus and tabs
- Experiment with what works for you!



# Using visual style options to better interpret 3D

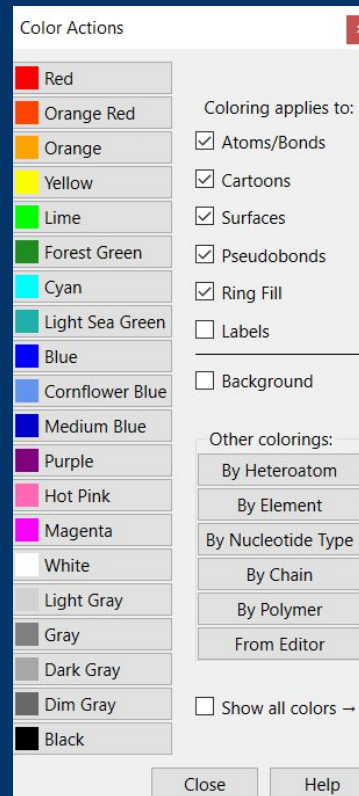
- I chose tube/slab style because I liked it



- Wanted to add color - but these don't match Eterna!
  - Let's do custom coloring

# Using visual style options to better interpret 3D

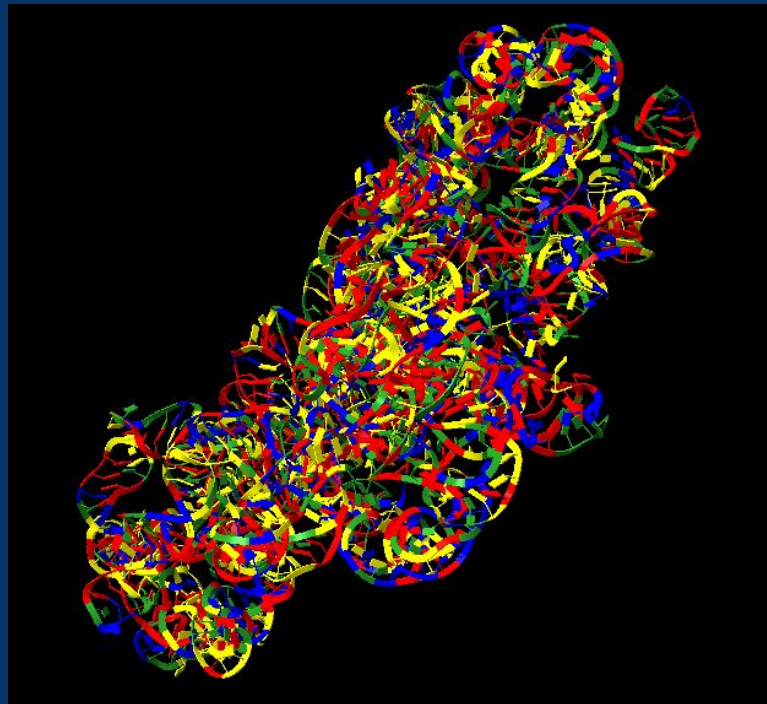
- Go to Actions → Color → All Options to load color menu (keep this open)
- To assign the colors we want to the correct bases:
  - Go to Select → Residues → (your choice of residue)
    - The matching bases will highlight
  - Then click the color you'd like in the color menu
  - Repeat until all bases are colored as you wish
  - Then unselect everything (Select → Clear)





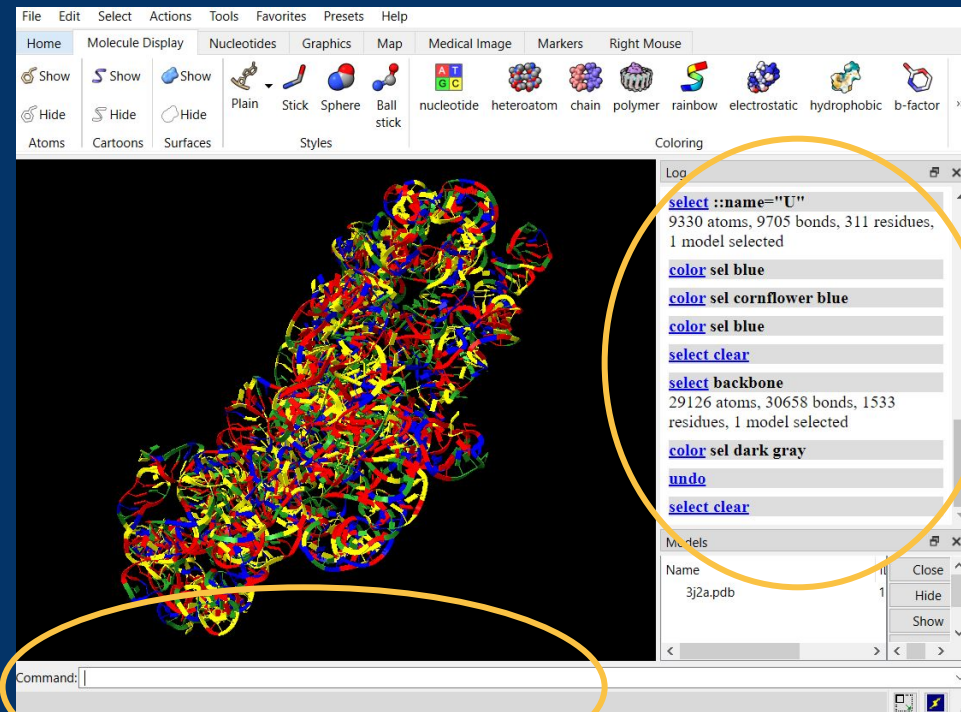
# Using visual style options to better interpret 3D

- Colors should match with Eterna now!
  - Confirm by hovering over base until it tells you the identity



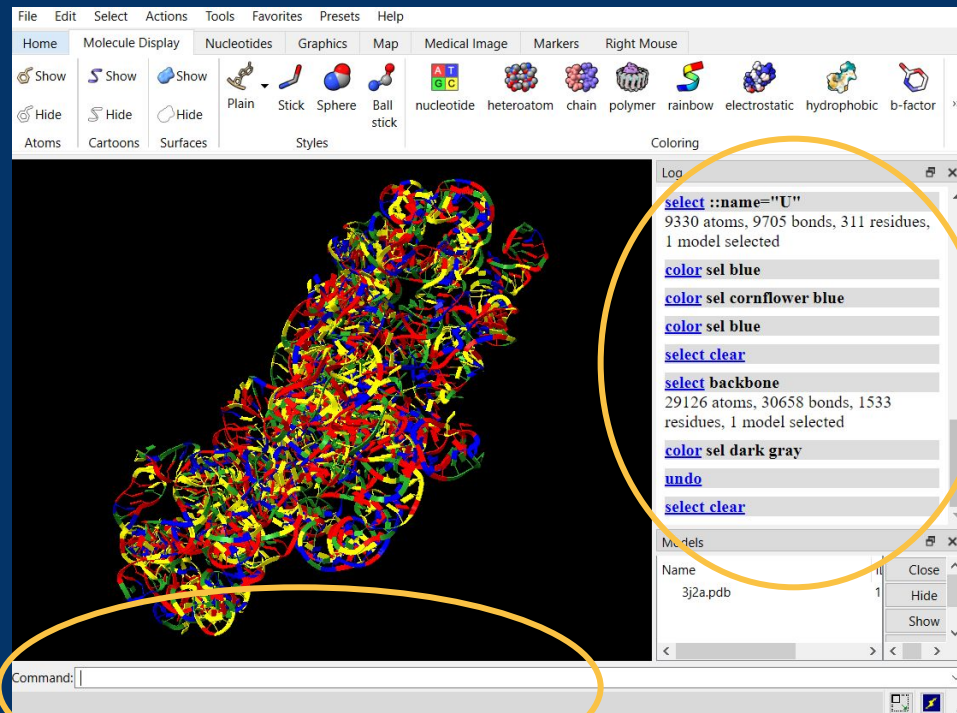
# Using the command line

- I'd like to find base 744, which Nando was looking at
- I could find it by hand . . .
- . . . or I could use command line shortcuts to find it
  - Command line can be used for a lot
  - When you do an action using menus, ChimeraX also tells you what the corresponding text command would be
  - Useful command: `undo` (reverses previous action)



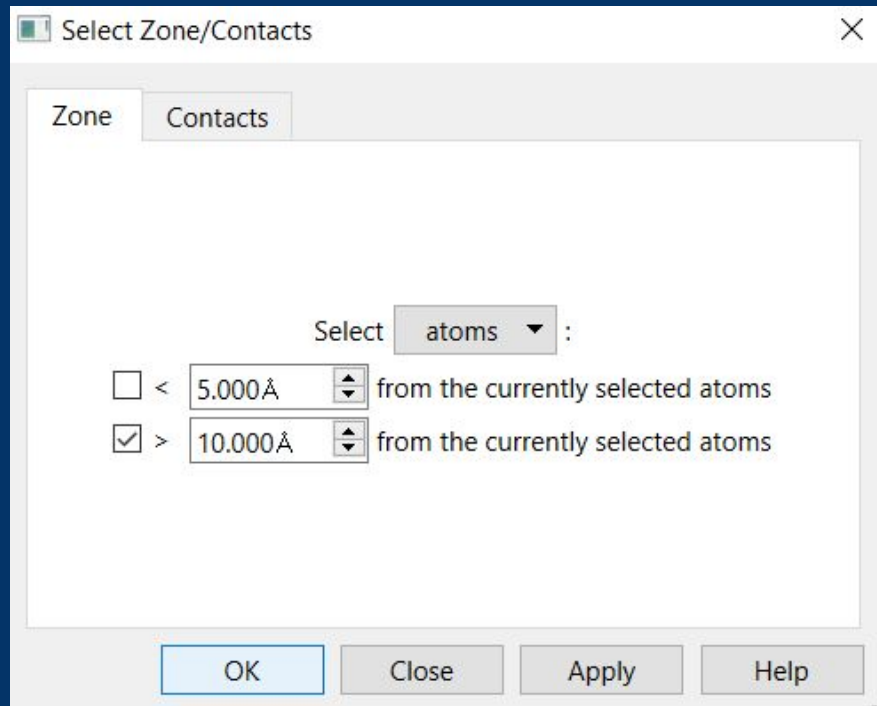
# Using the command line

- Type the following into the command line and press enter:
  - `sel :744`
- “sel” is short for select
- The “:” tells us we’re looking for a base (also called a residue)



# Focusing on a “zone”

- Still hard to find our base . . .
- Let's select everything a certain distance away . . .
  - Select → Zone/Contacts → fill in the settings you'd like, then click OK
  - I chose > 10 Angstroms away
- . . . and delete it
  - “del sel” in command line
  - Maybe not the most elegant option, but works for my purposes
  - Feel free to use hiding, transparency, etc. if you prefer



# Almost there . . .

- Much more manageable number of residues!
- After some rotation, I can see the bases Nando found (the G and C that weren't pairing):

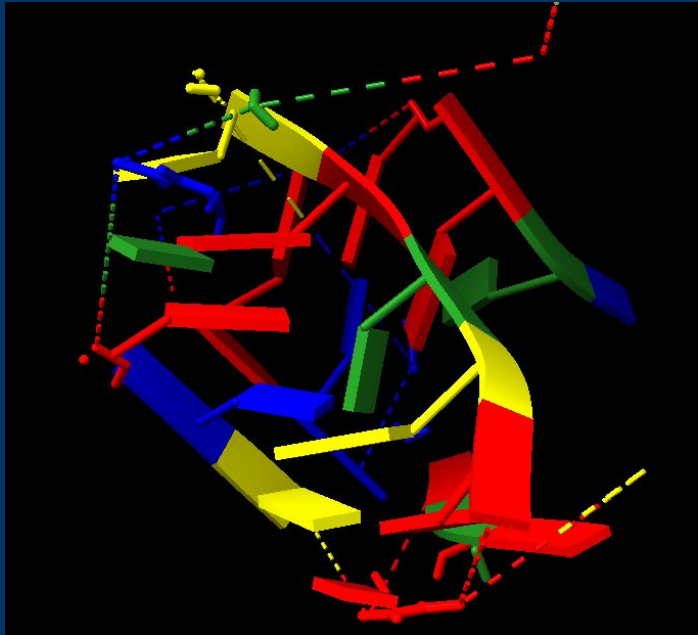


# What are the (predicted) hydrogen bonds?

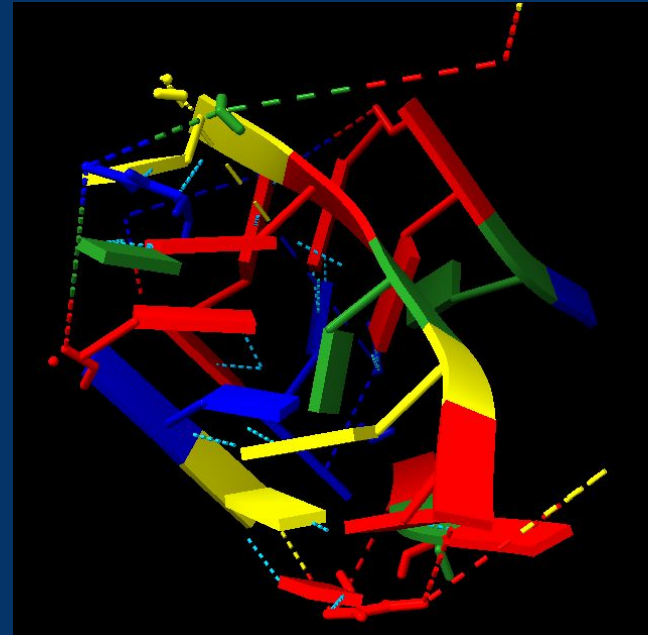
- Hydrogen bonding - the chemical interaction that causes base pairing (among other things)
  - Occurs when an electronegative atom, such as oxygen (O) or nitrogen (N), interacts with a hydrogen (H)
- We can visualize these interactions within ChimeraX
  - Type “hbond” in command line

# What are the (predicted) hydrogen bonds?

Before visualizing  
hydrogen bonds

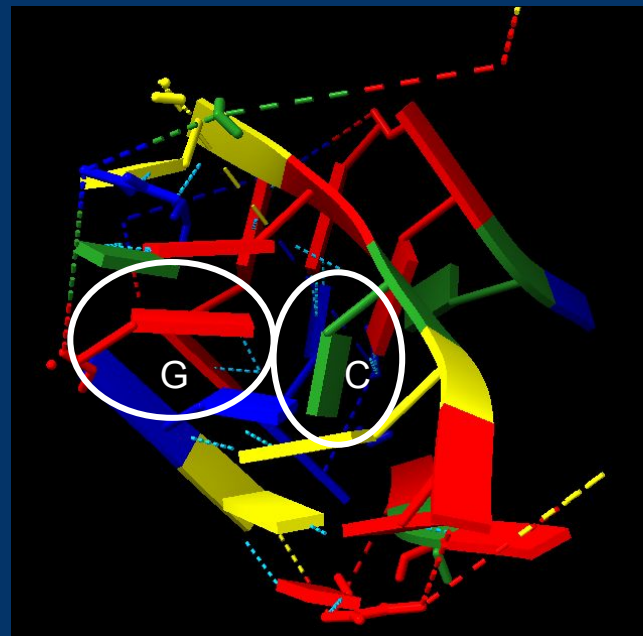


After visualizing hydrogen  
bonds



# Mission accomplished!

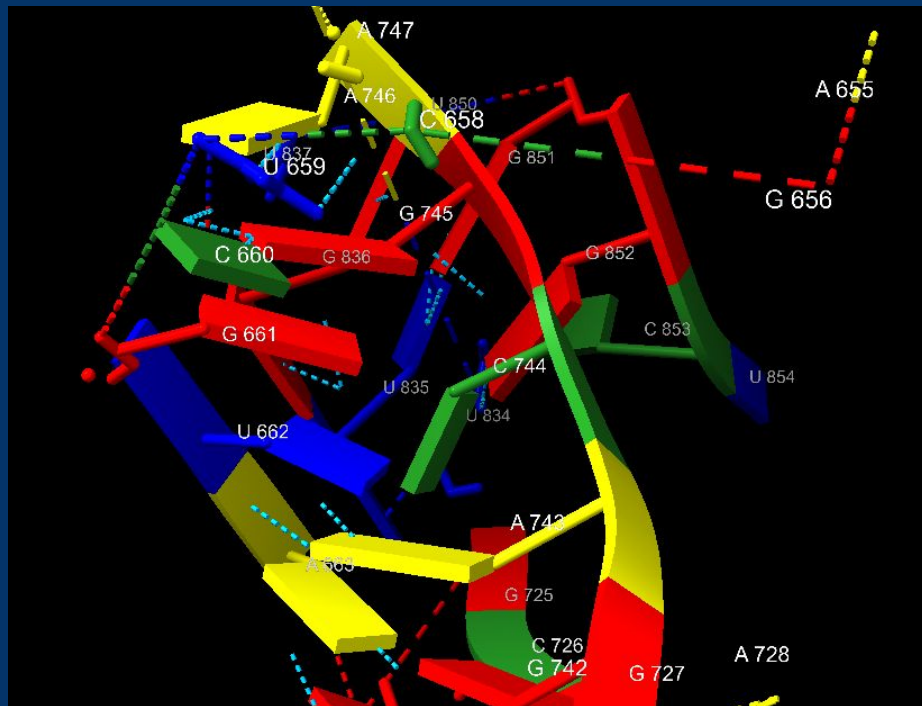
- We understand why this G and this C do not pair together
- They don't pair together because they don't have the right "shape" (angle, distance) to hydrogen bond
- Along the way, we learned some basics of how to use ChimeraX
  - Visual settings
  - Using the command line
  - Isolating a zone of interest
  - Visualizing hydrogen bonds





# Just one more thing . . .

- Adding labels!
- To get this picture, I did the following:
  - Actions → Label → Residues → Name and Number
- Good to see it's the correct base!



# More guides for Chimera(X)

- [Introduction to Chimera for EteRNA Players](#) by Omei and Eli
- [Bare Bones Guide to Creating 3D-RNA Structure Models Using UCSFs Chimera](#) by AndrewKae
- [ChimeraX Quick Start Guide](#) (provided by ChimeraX)
- [Command-Line Target Specification documentation](#) (provided by ChimeraX)
  - Useful for complicated structure files - for examples, ones with multiple RNAs
  - Also useful for knowing how to select individual atoms, etc.

# Questions?