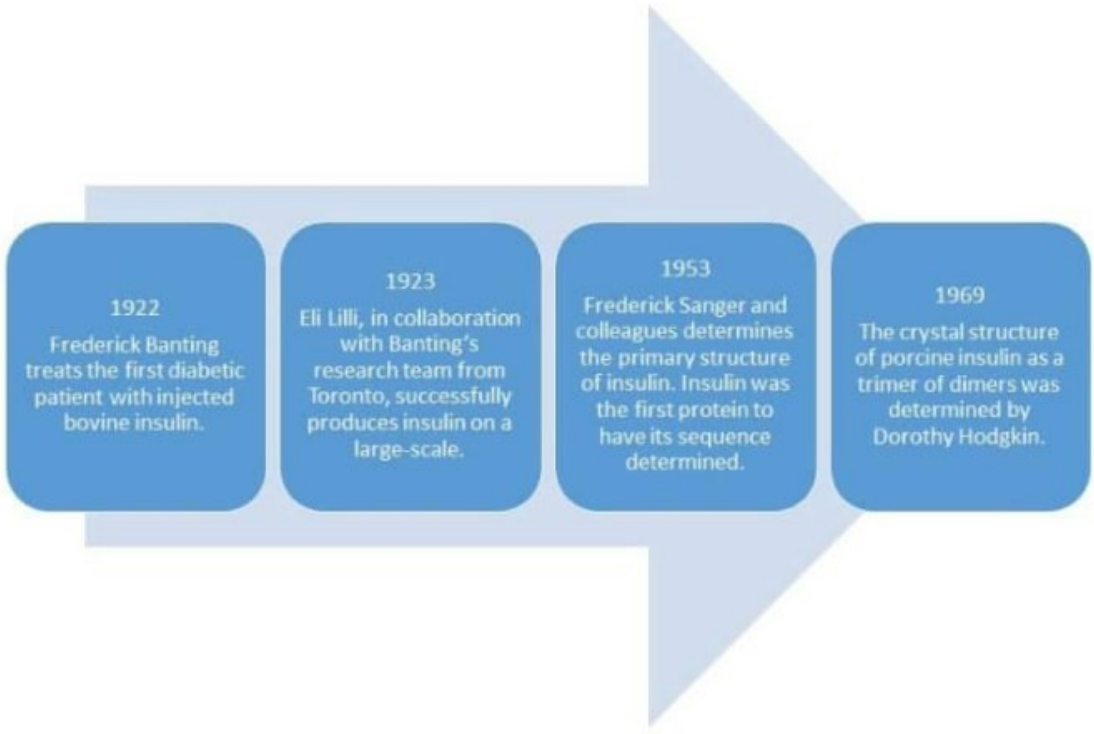
JUDE TUTORIAL 1

PART 1: UNWRAPPING THE STORY OF INSULIN

Diabetes has been known as a disease for a long time. Type 1 diabetes was considered incurable and a major cause of juvenile death before 1922. Life expectancy after diagnosis in children was typically less than one year. As a result, research on diabetes has been happening for a long time. To see the progression of knowledge about the disease check out the PDB 101 timeline here.

In a path to finding treatments for patients with diabetes a recurring theme of understanding structure and function has emerged. Scientists have continually sought to understand the structure of insulin to better understand its function in order to identify potential treatments. Some key events in this timeline include:



In order to understand how insulin operates as a trimer of dimers, we are going to start by exploring the active monomeric form of insulin in the molecular visualization software, Jmol.

1. ACCESSING JUDE:

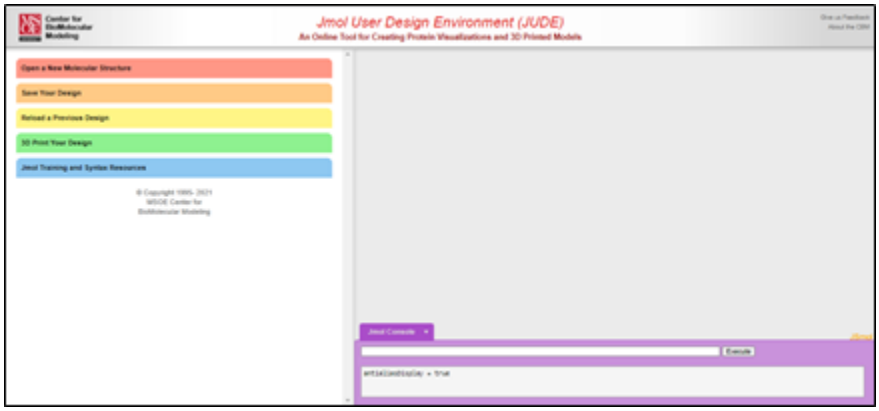
JUDE is an online form of Jmol, an open source molecular visualization program that runs on a Java platform. To access JUDE open the following URL:

https://www.centerforbiomolecularmodeling.org/modelingResources/jmolUserDesignEnvironment/#forward

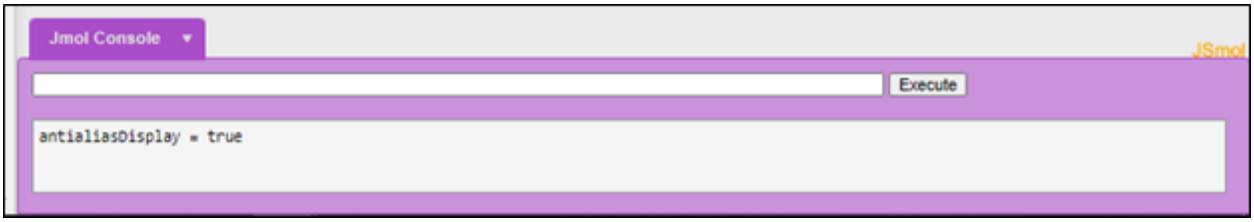
Note: Unfortunately, there is currently no UNDO function in JUDE (they're working on it!). Save early and save often! Each time you save, number your structure so you know which file is your most recent version.

2. JUDE WINDOWS

Once launched, JUDE will display one window that looks like this:

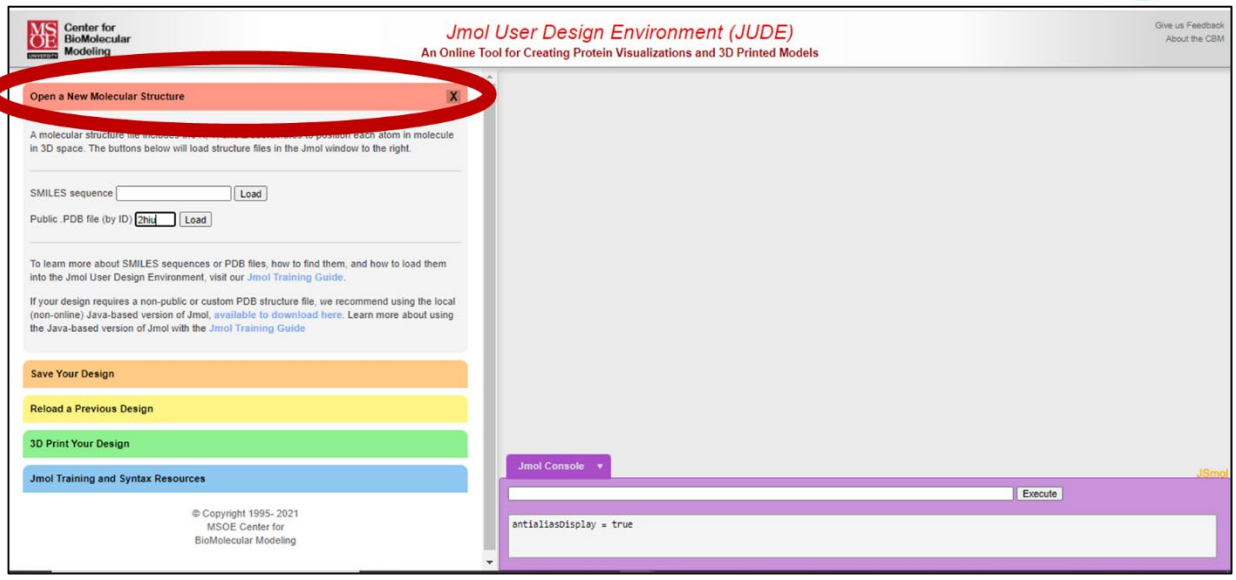


The console window is located at the bottom right of the page. All commands (i.e., syntax) is input through the "Jmol Console" window.

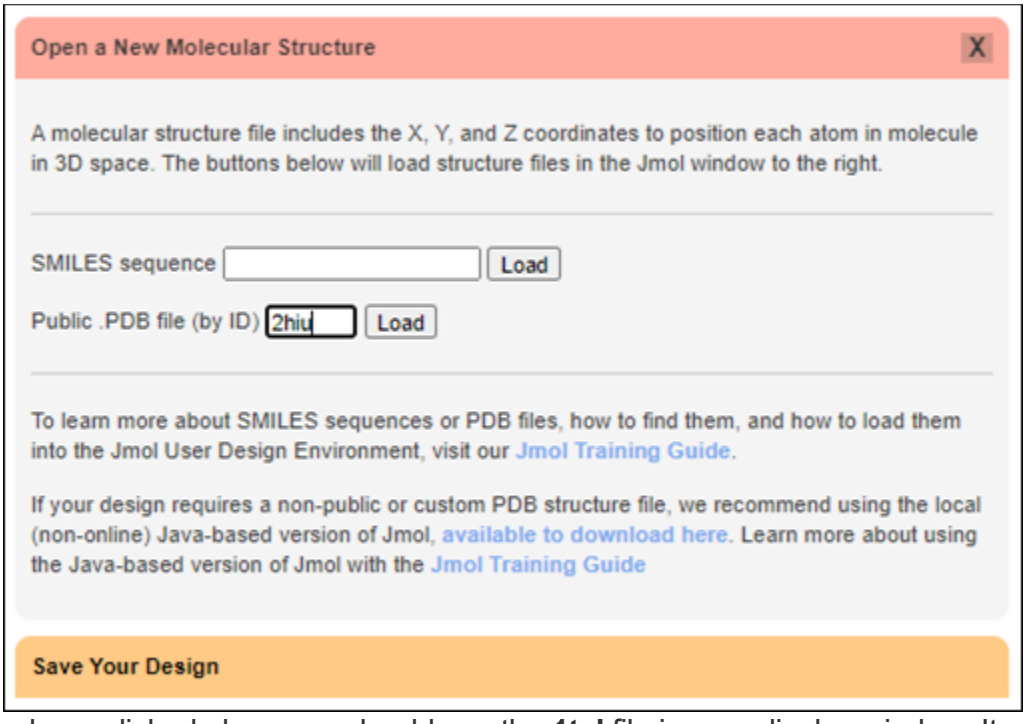


3.OPENING YOUR INSULIN .PDB FILE

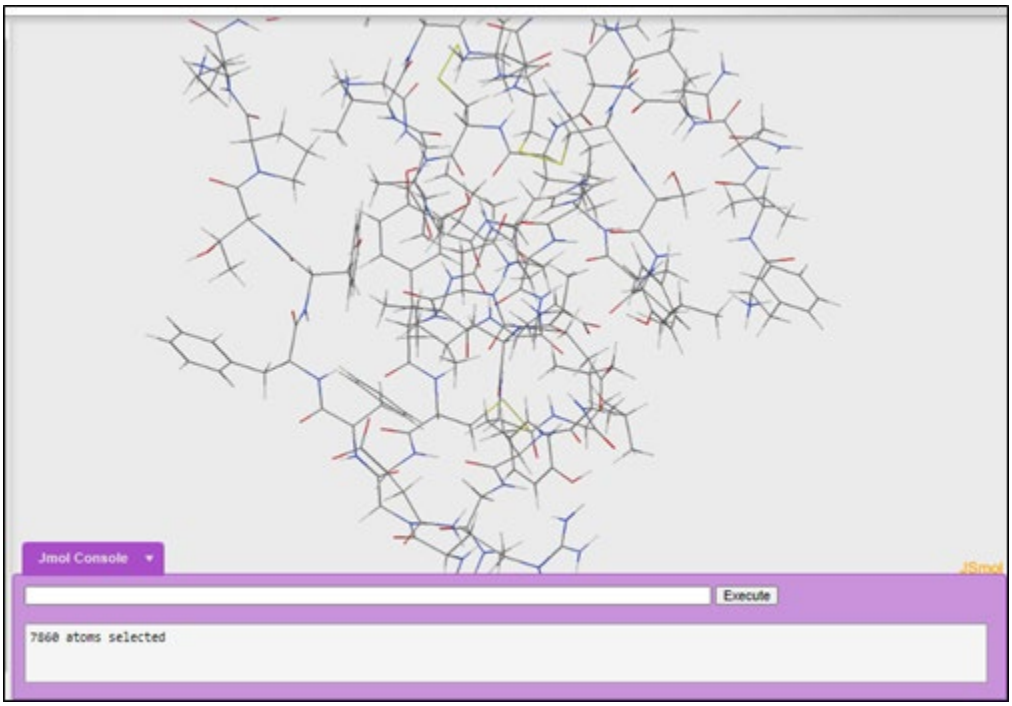
We will be using the **2hiu** pdb file. To open this file, click the red "Open a New Molecular Structure" bar on the left side of the screen.



Then type 2hiu into the “Public .PDB file (by ID)” bar. Click “Load”.



Once you have clicked okay, you should see the **1tyl** file in your display window. It will look like this:



If you want to zoom in and out, scroll up and down on your mouse. To zoom in/out without a mouse, hold the shift-key, then click and drag up to zoom in or click and drag to zoom out.

4. DECIDING HOW TO DISPLAY YOUR MOLECULE:

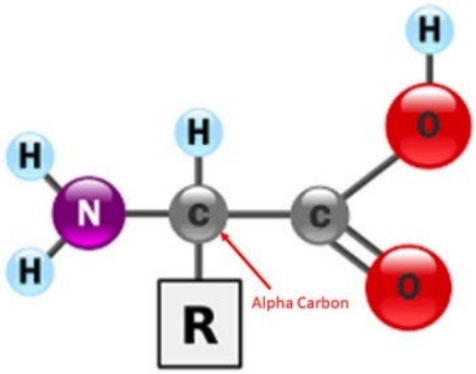
One of the most powerful features of JUDE is the ability to completely customize how your structure is displayed. Every protein or molecular structure can be visualized in an endless variety of ways, each highlighting different features of the structure. When designing your model you may want to consider the following:

- Amino acids involved in binding a substrate or ligand.
- Protein co-factors, ATP binding sites, DNA binding sites.
- Does the protein function in a dimeric (or multimeric) state? If so do you want to display the dimer? And if you model the dimer, do you want to show different things in the two monomers?
- Are there structural features you want to emphasize in the model?
    - Beta sheet
    - Alpha helices
    - A loop that undergoes a major conformational change when activates
    - Disulfide bonds
    - Individual chains in a multichain protein
    - An unusual gorge or pocket or domain that is important to protein function
    - An amino acid that, when mutates, impacts protein function
- Any ligands associated with the protein in ynour pdb file? (You will need to know how to call these up in JUDE; the pdb webpage will include a list of ligands and their abbreviations.) (CREST Jumol Resources, n.d.).

5. BACKBONE DISPLAY FORMATS"

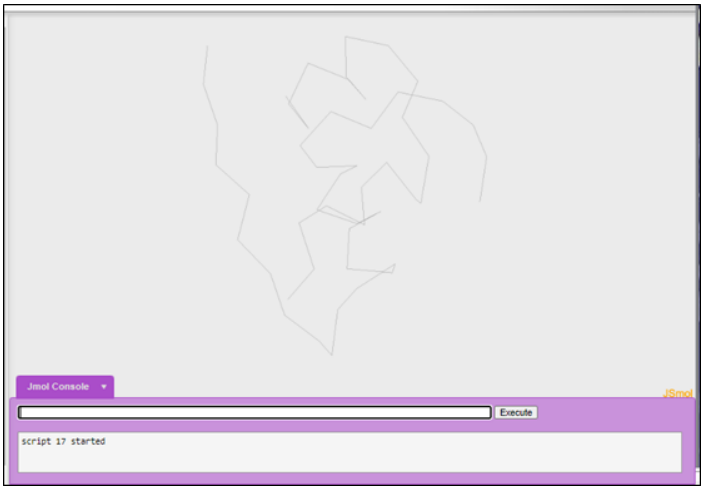
Your current view of the insulin protein does not show you much about the structure of the protein. By using some simple commands, you can change the way the molecule is displayed and highlight different structures within the protein.

In order to clearly see the secondary structures within the protein you can use the backbone format. The backbone format displays the position of the alpha carbon in each amino acid by a bend in the backbone. All other atoms within the amino acid are not displayed.



To turn all other formats off and turn only backbone on:

```
backbone only
```



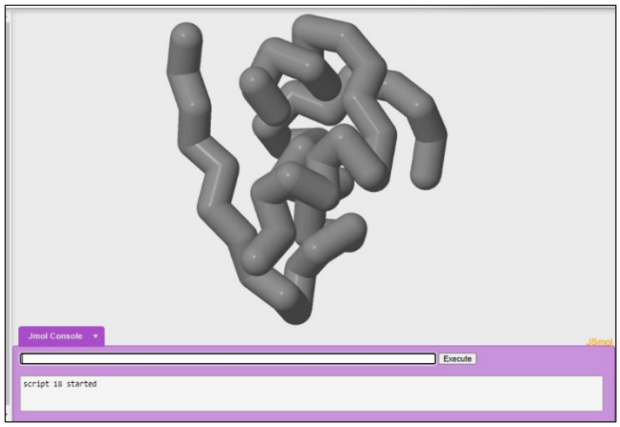
There are other display formats and commands that you can use when modeling in JUDE. To explore those formats and associated commands, check the JUDE commands cheat sheet here (you can use Jmol commands).

6. ADDING THINKNESS TO DISPLAY FORMATS:

Many of the display formats can be further altered by changing the thickness. To do this, simply add a number after any display format when typing the command into the console. The number added after the display format must include a decimal.

To display the backbone format with 1.5 angstrom (a unit of length equal to one hundred-millionth of a centimeter, 10^-10) thickness:

```
backbone 1.5
```



7. SELECTING CHAINS AND COLORING SELECTED CHAINS:

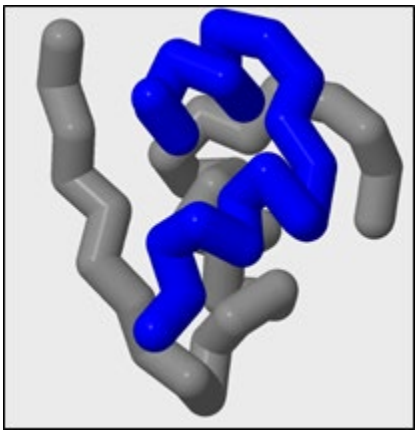
Many protein structures have more than one polypeptide chain. When a protein has more than one polypeptide chain, the protein is considered to have quarternary structure. These chains are labeled with single letter identifiers in the .pdb file and can be selected by entering a colon (:) followed by the letter identifier. **Only the atoms in chain you select will be affected by future commands.**
The protein insulin has two chains. Chain A and Chain B.

To select for chain A:

```
select :a
```

To color chain A blue:

```
color blue
```

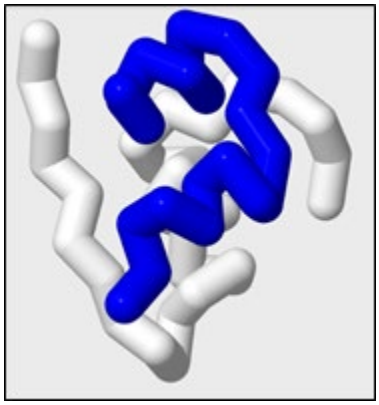


To select for chain B on the first monomer:

```
select :b
```

To color chain B on the first monomer white:

```
color white
```



You may decide to highlight secondary structure instead of the two polypeptide chains that make up the insulin protein. You would use the color structure command to color the model by secondary structures. Helices will be colored magenta, beta sheets will be colored yellow, and loop regions will be colored white.

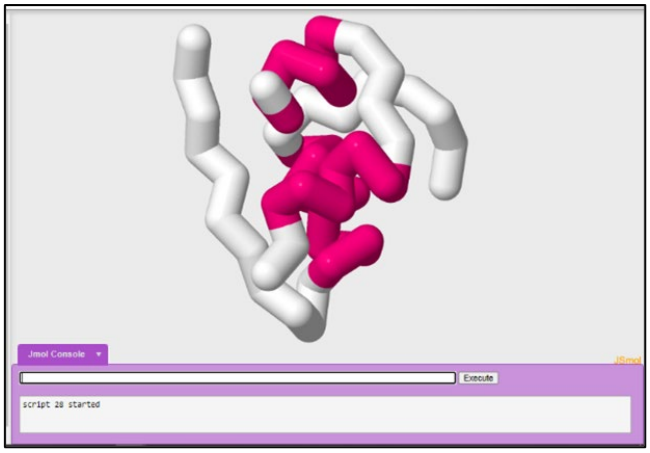
Since you currently have your B chain selected you will have to select all again to color the entire protein by structure.

To select all:

```
select all
```

To color by structure:

```
color structure
```



8. ADDING BONDS

JUDE has the ability to render different bonds within the protein like Hydrogen Bonds and Disulfide Bonds between Cysteine amino acids. If you wanted to 3D print your protein, you could also add additional structural supports called Struts.

One important feature of our insulin protein is the presence of disulfide bonds. In order to show all of the disulfide bonds in insulin, you will need to select all.

To select all:

```
select all
```

To show your disulfide bonds at 1.0 angstrom thickness:

```
ssbonds 1.0
```

Disulfide bonds occur between two cysteine amino acids. Your disulfide bonds currently look like they are floating, because the backbone format only shows the position of the alpha carbon.

To set your disulfide bonds in the backbone:

```
set ssbonds backbone
```

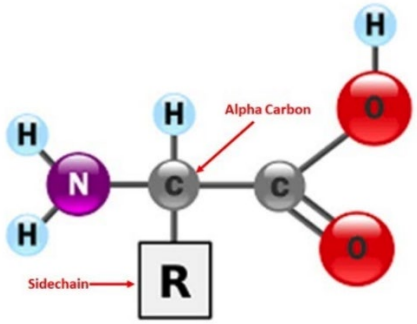
To color the disulfide bond yellow:

```
color ssbonds yellow
```

To explore how to add and format hydrogen bonds, check the JUDE commands cheat sheet here.

9. ADDING SIDECHAINS:

We are currently using the backbone format. This format only displays the position of the alpha carbon in each amino acid by a bend in the backbone. All of the other atoms within the amino acid are not displayed, including the sidechain (also known as an R-Group).



Sometimes you may wish to display an amino acid's sidechain with the rest of your protein structure in backbone format, because it is crucially important to telling the story of your protein. For example, instead of highlighting the disulfide bonds, you may wish to show the cysteine sidechains that form the disulfide bonds in your insulin protein (CREST Jmol Resources, n.d.).

To select the particular sidechain, you can use the amino acid sidechain three letter abbreviation and the number of the amino acid in the protein. You need to be careful here because there can be more than one amino acid with a given number if there are more than one polypeptide chains in a structure file (CREST Jmol Resources, n.d.).

Remember this map of the insulin protein from the Insulin: mRNA to Protein activity:

Image from 3D Molecular Designs: https://www.3dmoleculardesigns.com/Teacher-Resources/Insulin-mRNA-to-Protein-Kit.htm

This map shows that Cys7 of the B-chain forms a disulfide bond with Cys7 of the A-chain. Cys19 of the B-chain forms a disulfide bond with Cys20 of the A-chain. A third disulfide bond forms between Cys6 and Cys11, both from the A-chain.

To add your sidechains you are going to do a series of commands in JUDE. You will use the select, spacefill, and wireframe commands.

To select for Cys7 to show a clean sidechain (note that this command selects both the Cys7 of the A-chain and Cys7 of the B-chain):

```
Select cys7 and sidechain
```

The use of and in this command is a boolean operator. This command selects for atoms in cys7 and the sidechain (R-group) of the amino acids. Remember an 'and' boolean operator has to fulfill both conditions.

To add the spacefill format with a 1.25 Angstrom thickness:

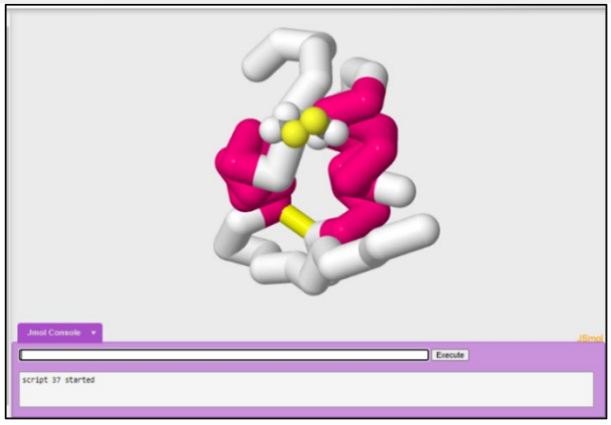
```
spacefill 1.25
```

To add the wireframe format with a 1.0 Angstrom thickness:

```
wireframe 1.0
```

To color the carbon's gray, the hydrogen's white, oxygen's red, nitrogen's blue, and sulfur's yellow:

```
color cpk
```



You can select for multiple sidechains at the same time.

To select for cys19 and cys20, spacefill to 1.25 angstrom thickness, wireframe to 1.0 angstrom thickness, and color cpk:

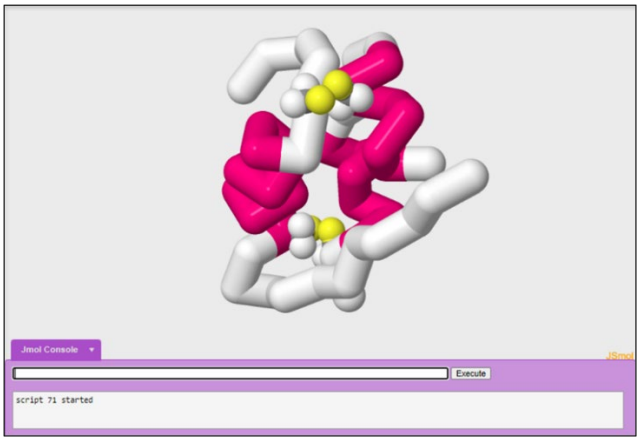
```
select cys19, cys20 and sidechain
spacefill 1.25
wireframe 1.0
color cpk
```

If you play with the viewer on JUDE, you may notice that we have not yet added sidechains to one of the disulfide bonds. What is the argument to not showcase the disulfide bond between cys 6 from the A chain and cys11 from the A chain?

If you choose to not highlight this bond and did not want to show the bond at all, you can turn the disulfide bonds off.

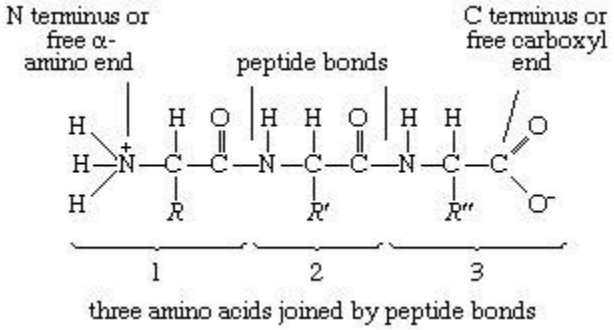
To turn disulfide bonds off:

```
select all
ssbonds off
```



10. COLORING THE N-TERMINUS AND THE C-TERMINUS

You may want to highlight where the N-terminus and C-terminus on each sidechain is. Remember, due to the way that amino acids link together, all proteins show the same pattern in connection. Look at the image below and see if you can find the pattern. This means that your polypeptide chain has directionality.



Using the protein data bank sequencing page for 2hiu (https://www.rcsb.org/pdb/explore/remediatedSequence.do?structureId=2HIU), you can find the N-terminus and C-terminus for each polypeptide.
The sequence for chain A looks like this:

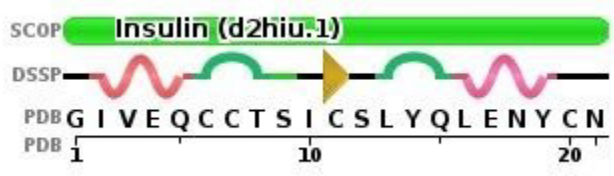


Image from The Protein Data Bank: https://www.rcsb.org/pdb/explore/remediatedSequence.do?structureId=2HIU
The N-terminus is found on the first amino acid in the chain. In this chain, the first amino acid is G or Gly. The protein databank only uses one letter abbreviations, you can find a great amino sidechain chart here.

To select for Gly:

```
select gly1
```

To color blue:

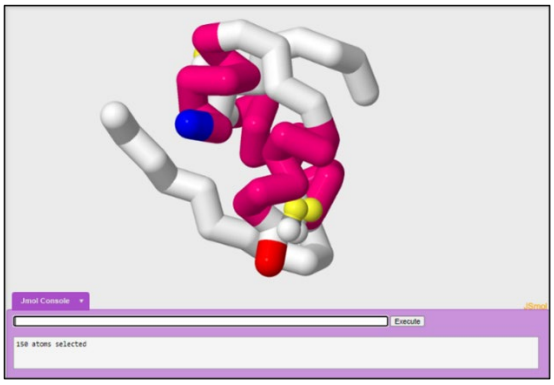
```
color blue
```

To select for the C-terminus:

```
select asn21
```

To color red:

```
color red
```



The sequence for chain B looks like this:

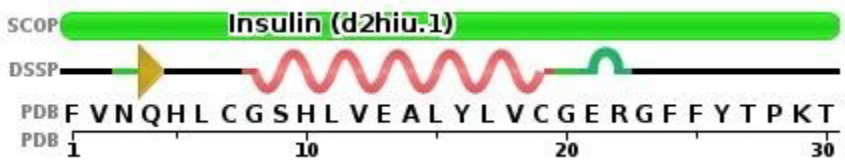


Image from The Protein Data Bank: https://www.rcsb.org/pdb/explore/remediatedSequence.do?structureId=2HIU

To select for the N-terminus on chain B:

```
select phe1
```

To color blue:

```
color blue
```

To select for the C-terminus on chain B:

```
select thr30
```

To color red:

```
color red
```

You could simplify this code and select for both of your N-termini at the same time:

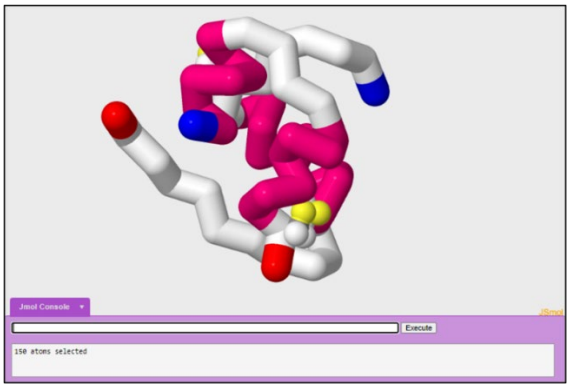
```
select phe1, gly1
```

To color blue:

```
color blue
```

The same applies for your c-termini:

```
select thr30 or asn21
color red
```



11. SAVING YOUR FILE TO 3D PRINT

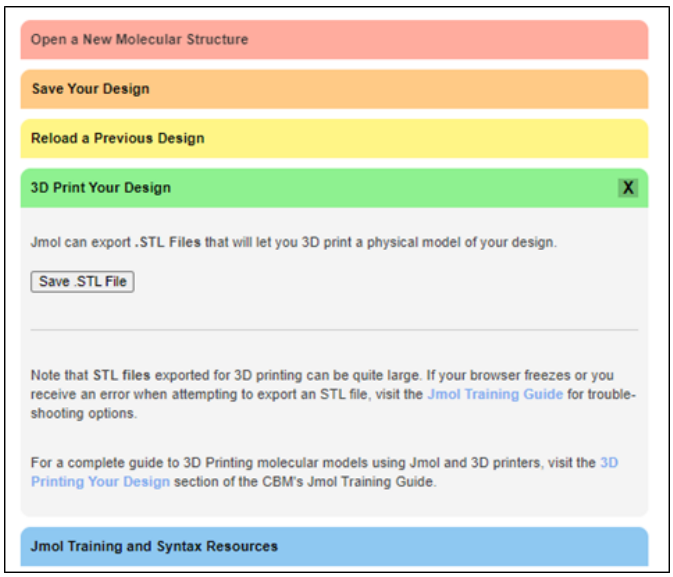
Once you have the file that you want in JUDE, you can export your file to an STL file. STL files store information about 3D models by describing only the surface geometry of a three-dimensional object without any representation of color, texture, or other common model

attributes. Your saved STL file will then be used in conjunction with a 3D slicer to 3D print your model.

While any design would work for creating a printable image or interactive webpage, certain design specifications work better for 3D printing than others. Below are some suggested sizes, formats, and design specifications from the Milwaukee School of Engineering Center for Biomolecular Modeling.

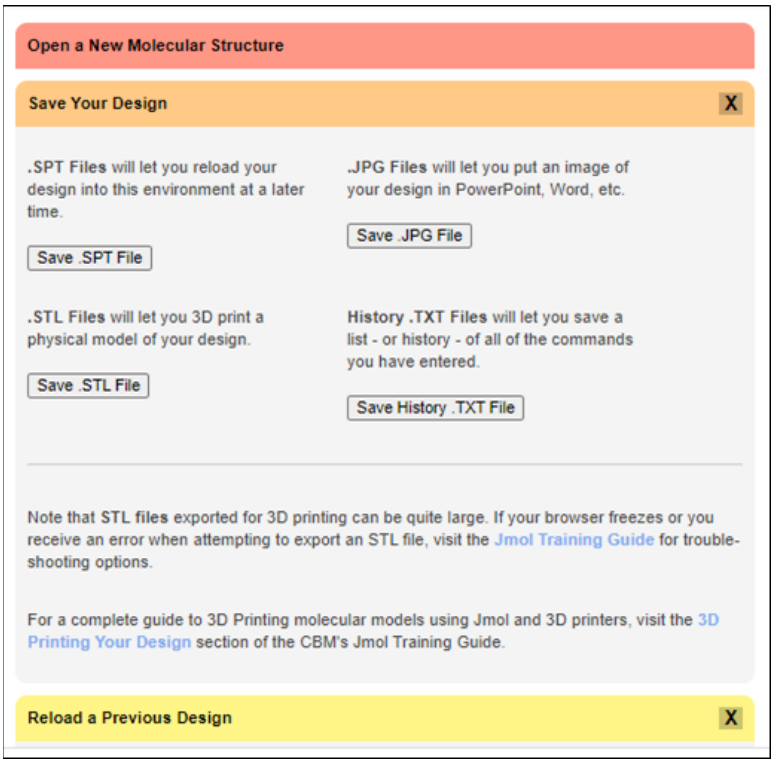
- backbone 1.5
- wireframe 1.0
- spacefill 1.25
- hbond 1.0
- strut 1.0
- ssbond 1.0
-

To save your model of 2hiu at an STL file, click “3D Print Your Design” on the left side of the page.



12. SAVING YOUR JUDE SESSION FOR LATER:

JUDE allows you to save your modeling sessions as .SPT files to view or edit them at another time. An .SPT file saves all aspects of your model (i.e., coloring and structure format). To save your session as an .SPT file, click the “Save Your Design” bar then click “Save .SPT File”. The file will be saved to your downloads folder.



To load a saved session simply drag the .SPT file into the modeling window. You can view a set of detailed instructions by clicking on the “Reload a Previous Design” bar.

***Tip: When working in your SMART Teams, you will have to create images of your models to present the molecular story. Once you have identified what idea you want to show, immediately create an .SPT file after the .PNG file. Minor tweaks are often made to the model to perfect the original idea/image and saving the exact image as an .SPT file allows you to make the minor changes that you desire quickly. This will save a MASSIVE amount of time.

13. OTHER COMMANDS YOU MAY FIND USEFUL:

Some molecular structures include additional small molecules that are not proteins. These molecules are given a three-character alpha-numeric identifier that can be used to select them. The file 2hiu.pdb does not contain any small and unique molecules. Some of the .pdb files you will be looking at later today may contain small and unique molecules that you want to select for and highlight in your model (CREST Jmol Resources, n.d.).

To determine the three character identifier, you can click on the JUDE display window or review the structure summary page for the .pdb file on the Protein Databank (www.pdb.org) website (CREST Jmol Resources, n.d.). When looking at the structure summary page of the .pdb file, you can scroll down to see if it contains any small molecules. Let's use hemoglobin (1a3n) as an example. The structure summary will look like this:

SEPA SCIENCE EDUCATION PARTNERSHIP AWARD
SUPPORTED BY THE NATIONAL INSTITUTES OF HEALTH
PennState College of Medicine
PennState Eberly College of Science
PennState College of Education
CSATS RESEARCH & EDUCATION

Macromolecules

Find similar proteins by: Sequence | Structure

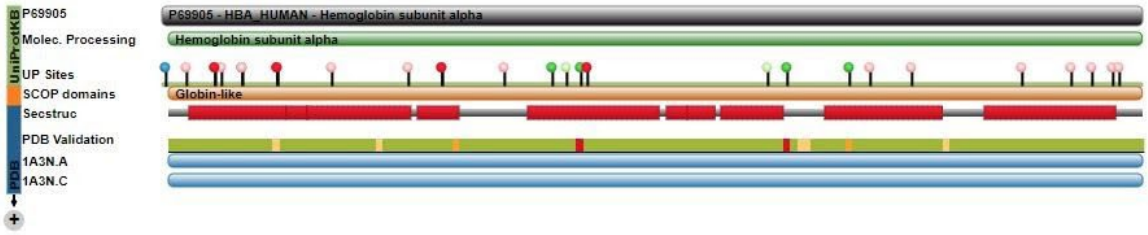
Entity ID: 1

Molecule	Chains	Sequence Length	Organism	Details
HEMOGLOBIN (ALPHA CHAIN)	A, C	141	Homo sapiens	**Mutation(s)**: 0 **Gene Names**: HBA1, HBA2

Find proteins for P69905 *(Homo sapiens)* Go to Gene View: HBA1 HBA2 Go to UniProtKB: P69905

Protein Feature View

Full Protein Feature View for P69905



Entity ID: 2

Molecule	Chains	Sequence Length	Organism	Details
HEMOGLOBIN (BETA CHAIN)	B, D	146	Homo sapiens	**Mutation(s)**: 0 **Gene Names**: HBB

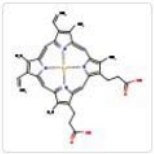
Find proteins for P68871 *(Homo sapiens)* Go to Gene View: HBB Go to UniProtKB: P68871

Protein Feature View

Full Protein Feature View for P68871

Small Molecules

Ligands 1 Unique

ID	Chains	Name / Formula / InChI Key	2D Diagram & Interactions	3D Interactions
HEM Query on HEM Download SDF File Download CCD File	A, B, C, D	**PROTOPORPHYRIN IX CONTAINING FE** *HEME* $C_{34}H_{32}FeN_4O_4$ KABFMIBPWCXCRK-RGGAHWMASA-L		Ligand Interaction

To select for the small molecule HEM:

```
select hem
```

You may also want to restrict or only show listed residues or small molecules.

To only show the hem group listed above:

```
restrict not hem
```

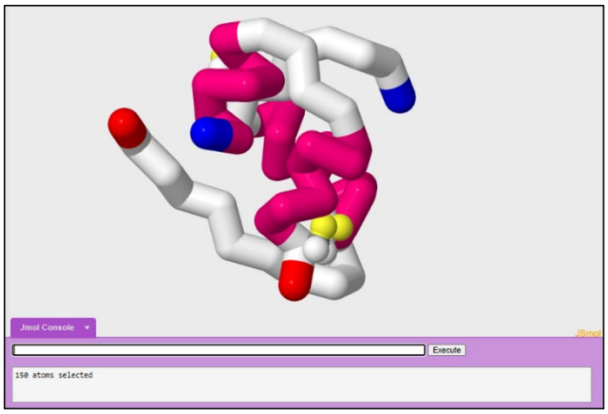
REFERENCES:

CREST Jmol Resources. (n.d.). Retrieved from http://cbm.msoe.edu/crest/crestJmolResources.php

JUDE TUTORIAL 2

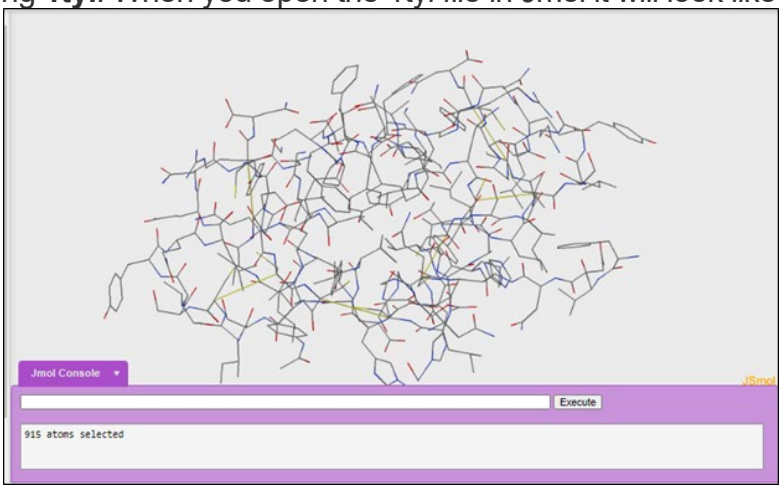
PART 2: EXPLORING A INSULIN DIMERS

In tutorial one, we started off by looking at the active monomeric form of insulin in order to understand how insulin forms a trimer of dimers. In this tutorial we are going to look at another PDB file in order to look at a different form of insulin. In tutorial one, we modeled the insulin monomer to look like the figure below:



1. PDB FILE

2. We will be using **1tyl**. When you open the 1tyl file in Jmol it will look like this:



2. INITIAL EXPLORATION OF STRUCTURE

When exploring a structure for the first time, a scientist's goal is to obtain as much information as possible about the structure of a protein as fast as possible in order to better

understand how structure leads to function. You can explore the structure in different display formats. Many scientists will first look at the cartoon display format.

To change your display format into cartoon:

```
cartoon only
```

To color the structure to highlight the secondary structures of the protein:

```
color structure
```

QUESTIONS:

1. How is the form of insulin in the 1tyl file different from the form of insulin in the 2hiu file we looked at in the first tutorial?

2. What information can you get by looking at the cartoon display format?

3. What features of the protein does this model show and what are the model's limitations?

3.WIREFRAME FORMAT

After looking at the cartoon display format, a scientist may switch to look at a different display format like the wireframe format.

To change your display format into wireframe:

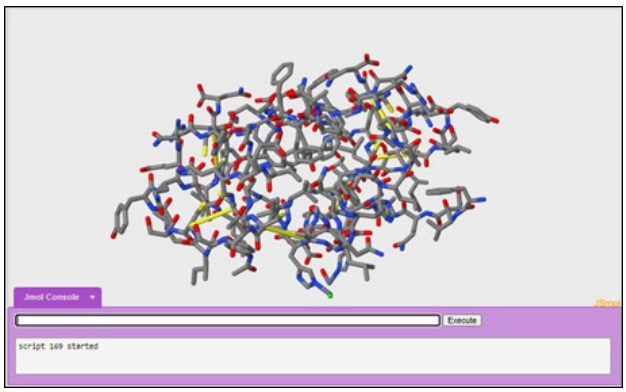
```
wireframe only
```

To increase the thickness of the wireframe to 0.25 angstroms:

```
wireframe 0.25
```

To color the carbon's gray, the hydrogen's white, oxygen's red, nitrogen's blue, and sulfur's yellow:

```
color cpk
```



QUESTIONS:

4. What information can you get by looking at this display format?

5. What features of the protein does this model show and what are the model's limitations?

4. EXPLORING HYDROPHYLLIC AND HYDROPHOBIC INTERACTIONS

n this section we are going to compare and contrast two different 1tyl models in order to draw conclusions about hydrophilic and hydrophobic interactions within the insulin protein. We will make this comparison by highlighting with different colors the hydrophobic portions of the protein in yellow and the hydrophilic portions of the protein in white.

For our first model, we are going to use the cartoon format.

To change your display format to cartoon only:

```
cartoon only
```

To color your display format grey:

```
color grey
```

To select the hydrophobic portions of the protein:

```
select hydrophobic
```

To color the hydrophobic portions yellow:

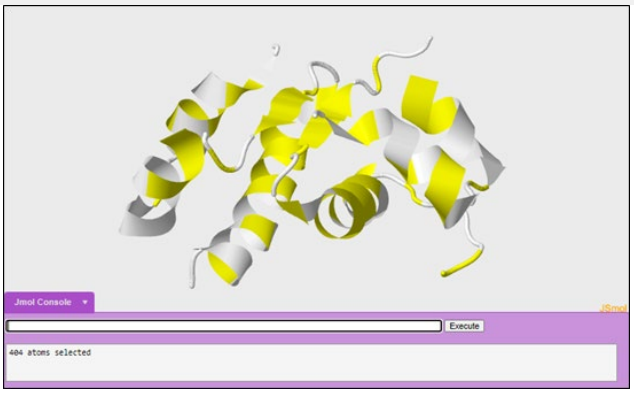
```
color yellow
```

To select the hydrophilic or polar portions of the molecule:

```
select polar
```

To color the polar portions of the molecule white:

```
color white
```



To make the second molecule, open a new JUDE display window. Then, open another 1tyl molecule.

To change the display to wireframe only:

```
wireframe only
```

To increase the thickness to 0.25 angstroms:

```
wireframe 0.25
```

To select the hydrophobic portions of the protein and the sidechains:

```
select hydrophobic and sidechains
```

The use of and in this command is a boolean operator. This command selects for the hydrophobic portions of the protein and the sidechains. Remember an 'and' boolean operator has to fulfill both conditions.

To color yellow:

```
color yellow
```

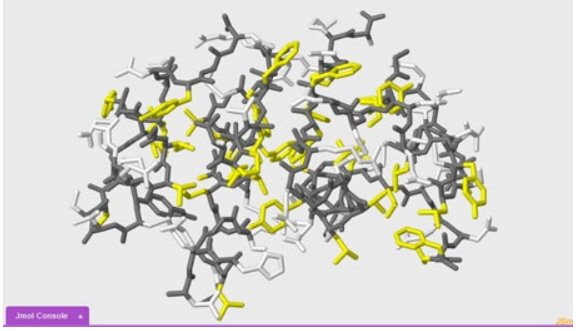
To select the polar portions of the protein and the sidechains:

```
select polar and sidechains
```

The use of and in this command is a boolean operator. This command selects for the hydrophobic portions of the protein and the sidechains. Remember an 'and' boolean operator has to fulfill both conditions.

To color white:

```
color white
```



QUESTIONS:

6. Compare the two images that you just made side by side. What do you notice?

7. As you observe the model, what questions about insulin structure come to mind?

5. A DEEPER LOOK AT SECONDARY STRUCTURE

One advantage to looking at the wireframe format is that you can explore secondary structure in more detail by exploring one alpha helix in the molecule and adding in hydrogen bonds. We are going to explore the alpha helix on chain b.

To make sure your entire molecule is selected:

```
select all
```

To change the display to wireframe:

```
wireframe only
```

To increase the thickness to 0.25 angstroms:

```
wireframe 0.25
```

To color the carbon's gray, the hydrogen's white, oxygen's red, nitrogen's blue, and sulfur's yellow:

```
color cpk
```

To only display the helix on chain b:

```
restrict :b and helix
```

Notice how the restrict command removes the display of everything except what was restricted. The use of and in this command is a boolean operator. This command selects for chain b and the alpha helix. Remember an 'and' boolean operator has to fulfill both conditions.

To display hydrogen bonds:

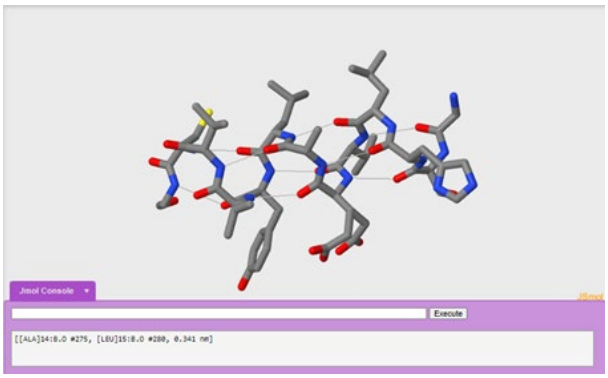
```
calculate hbonds
```

To set hbond solids:

```
set hbonds solid
```

To color hbonds white:

```
color hbonds white
```



QUESTIONS:

8. Which amino acids are contributing to secondary structure? (Hint: if you click on the structure, the console window will tell you what amino acid and atom it is.)

9. What pattern do you see with hydrogen bonding in the alpha helix?

10. How does hydrogen bonding contributes to secondary structure? Be specific!

11. Which bonding interactions occur first: the covalent bonds between amino acids or hydrogen bonding? Why?

12. Try highlighting the hydrophobic sidechains and the polar sidechains with different colors, like we did in the previous section. What do you notice?

13. Based upon your observations from question 12, what questions about insulin structure do you now have?

6. COMAPRING SECONDARY AND TERTIARY STRUCTURE

We are now going to compare secondary and tertiary structure using the wireframe format. You will need to open a new blank JUDE display window. Then, open another 1tyl molecule.

To change the display to wireframe only:

```
wireframe only
```

To increase the thickness to 0.25 angstroms:

```
wireframe 0.25
```

To hide the sidechains of each amino acid:

```
hide sidechains
```

To calculate hbonds:

```
calculate hbonds
```

To color hbonds white:

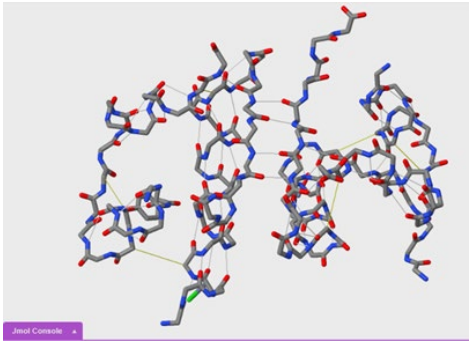
```
color hbonds white
```

To turn on disulfide bonds:

```
ssbonds on
```

To show ssbonds with backbone only selected:

```
set ssbonds backbone
```



QUESTIONS:

14. Based on what you observed, which bonds form first, the hydrogen bonds of an alpha helix or the disulfide bonds between helices? Why?

15. Order the formation of disulfide bonds in the insulin protein. Explain.

16. Based on what you observed, which bonding interactions occur first, hydrogen bonds between beta sheets or disulfide bonds? Why?

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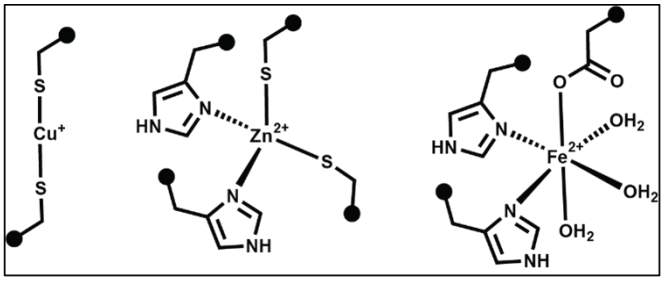
JUDE TUTORIAL 3

PART 3: EXPLORING QUATERNARY STRUCTURE – THE INSULIN HEXAMER

1. IDENTIFYING ZINC

Metal ions form coordination bonds with amino acids. These interactions are similar in strength to covalent and ionic bonds. The amino acid side chain needs to have a pair of electrons – O, N, or S atom – to coordinate a metal ion.

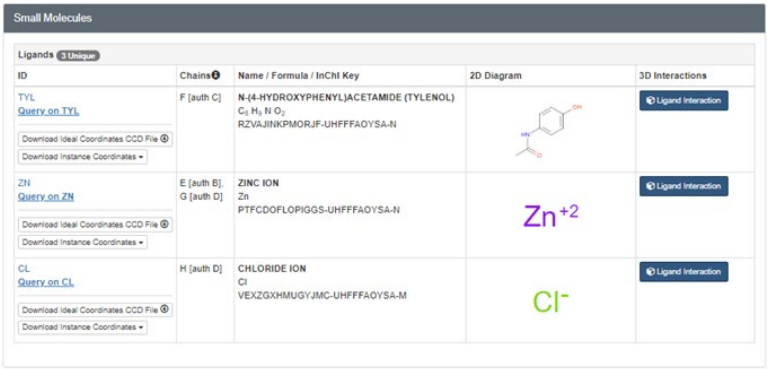
Each type of metal ion prefers to bind certain amino acids and in certain bonding arrangements. Some examples are shown below.



How would you know that insulin uses a small molecule (Zinc) to stabilize its quaternary structure? You can check out the Protein Data Banck (www.rcsb.org) to find this information.

Go to www.rcsb.org. In the search bar on the top right of the webpage, search for the PDB file: **1tyl**.

The search will take you to the Structure Summary page. Scroll down the page to find the box labeled "Small Molecules." This show you the other molecules that are important for the overall structure of the insulin hexamer. In this case, you can see that one of the important small molecules is zinc. Insulin forms important coordination interactions with the metal ion, Zn(II).



Next, you will identify the zinc binding sites in the PDB file 1TYL and show zinc in a spacefill format.

To access JUDE, go to:
https://www.centerforbiomolecularmodeling.org/modelingResources/jmolUserDesignEnvironment/#forward

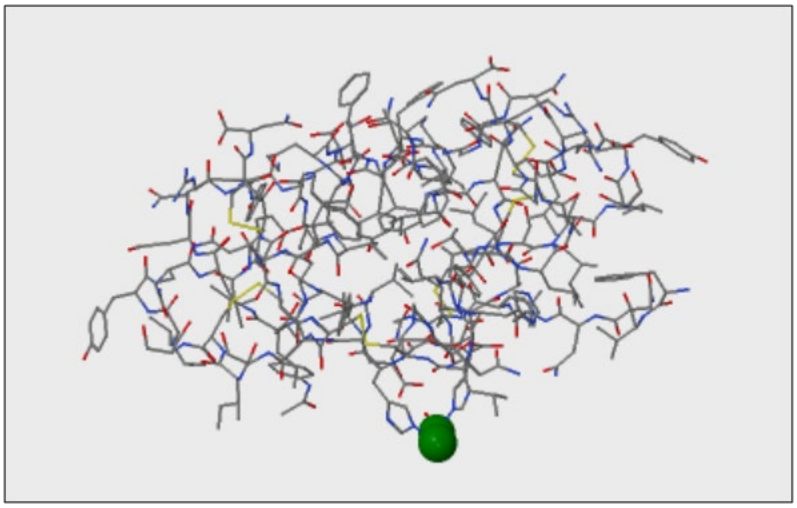
Load public PDB file: 1tyl.

To select the Zinc ion and color it green:

```
select Zn
```

```
spacefill 1.25
```

```
color green
```



Zoom in on each zinc site and identify all coordination partners. Mouse over each bonding partner to view amino acid name, atom name, chain ID, etc.

QUESTIONS:

1. Which chains do the zinc-coordinating amino acids originate from?

2. Which atom of the side chain interacts with zinc?

3. Is there anything else bound to the metal?

4. Measure the distance between the side chain and metal ion for each zinc site. Record the distances in Angstroms. How do these distances compare to typical values for covalent bonds, ionic bonds, H-bonds, etc.?

5. Double-click on the zinc ion. Mouse over to the bonding partner and click the atom coordinated to zinc. Given what we know about the requirements for zinc coordination bonds – do the interactions between zinc and insulin match our predictions for this metal?

2. EXPLORING INSULIN QUATERNARY STRUCTURE

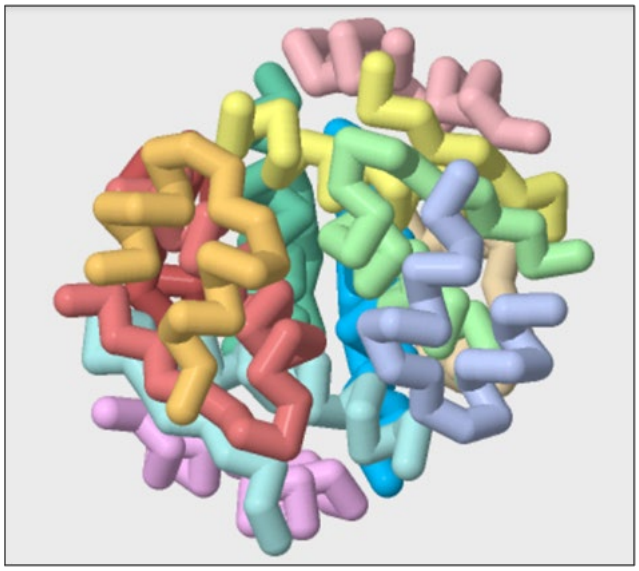
Insulin can adopt different quaternary structures. The quaternary structure of a protein is defined by the number of protein chains and how they are arranged. In this tutorial, we will view a common insulin structure used for storage of the protein in backbone format using the commands below and color each chain a different color.

On JUDE, Load public PDB file 1EV6.

```
Backbone only
```

```
Backbone 1.5
```

```
Color chains
```



QUESTIONS:

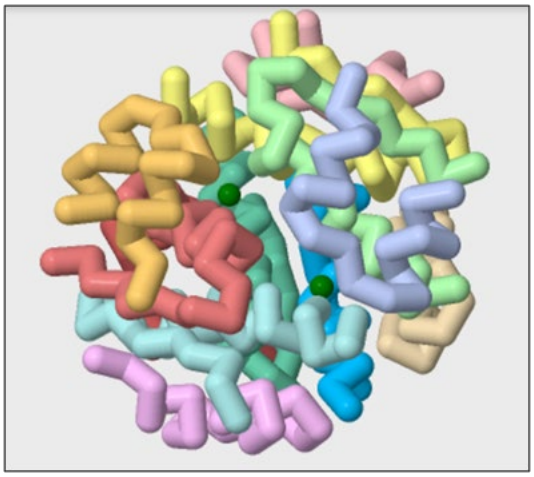
6. Count the number of A and B chains and describe how they are arranged in space.

7. Identify the zinc sits in this form of insulin.

```
select Zn
```

```
spacefill 1.25
```

```
color green
```



QUESTIONS:

8. Describe the number of bound metal ions and their location in the structure.

9. Zoom in on each zinc site and identify all coordination partners. Mouse over each bonding partner to view amino acid name, atom name, chain ID, etc.

10. Describe or draw the geometry of the Zn(II) binding site.

11. Make a prediction – does zinc stabilize or destabilize the hexameric quaternary structure of insulin? Why or why not?

12. Could another metal substitute for zinc in this function?

13. What would happen if one of the amino acids coordinated to zinc were mutated to another type of side chain?

14. Compare and contrast zinc in insulin to the function of a disulfide bond? Could a disulfide bond substitute for the metal ion in insulin? Why or why not?