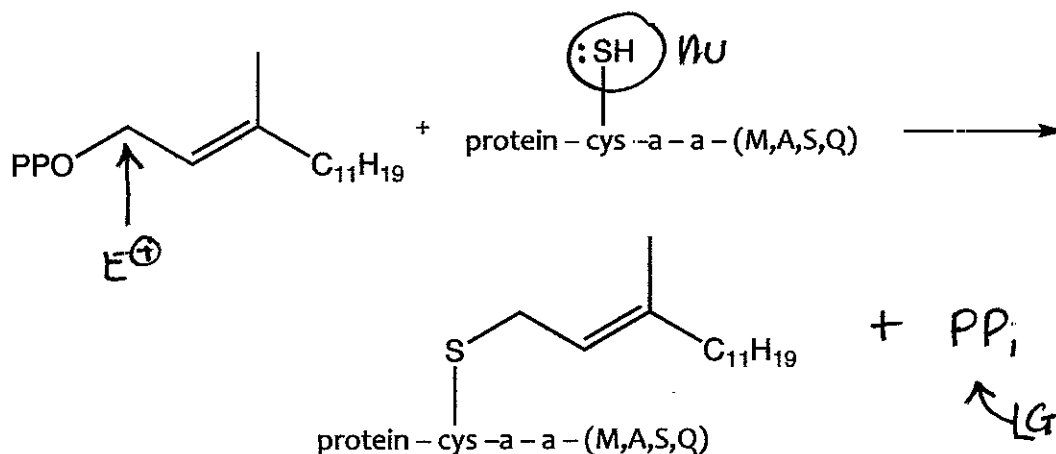


Study Question 2: Chimera Exploration of Protein Farnesyl Transferase

Go to the PDB site (Protein Data Bank; www.rcsb.org/pdb/home/home.do) and find the entry 1JCQ. Download it to your computer. This is the structure of protein farnesyl transferase which adds a farnesyl group to the cysteine side chain of certain protein sequences. The reaction is shown below. Whether or not a protein is farnesylated is important the initiation of certain cancers.



Answer the following questions about the structure by exploring with Chimera. Most of the tools you will need will be under the **Select** or **Action** menus; explore the various options and suboptions available. It is also handy to know that the mouse handles rotation, translation and scaling. Details of how the mouse works are at www.cgl.ucsf.edu/Outreach/Tutorials/GettingStarted.html - MenuMouse where you can also access all the Chimera tutorials and reference materials. Open the file you downloaded and select **Preset** → **Interactive 1** to get started.

- What kind of reaction is occurring? What are the possible mechanisms that should be considered? *nucleophilic substitution SN1 or SN2*
 - Balance the reaction above (don't worry about protons or charge in balancing), and label any nucleophiles, electrophiles and leaving groups. *see above*
- What is the general structure of this protein? One subunit, more than one, if more than one, are they identical, are there β sheets and/or α helices?
- How many farnesyl pyrophosphates are bound to this protein? Give the complete structure of FPP.
- This particular structure contains an inhibitor that resembles typical peptide substrates but does not react. The inhibitor in this case is called 739.
 - Draw the structure of this inhibitor.
 - On your drawing from (a), draw in several amino acid side chains that interact with the inhibitor to bind it to the active site. Give their names and identifiers. Show the intermolecular forces accurately.
 - What 3 amino acids appear to be represented in this inhibitor, that is, what are -a-a-(M,A,S,Q) by analogy to the typical peptide sequence given above?

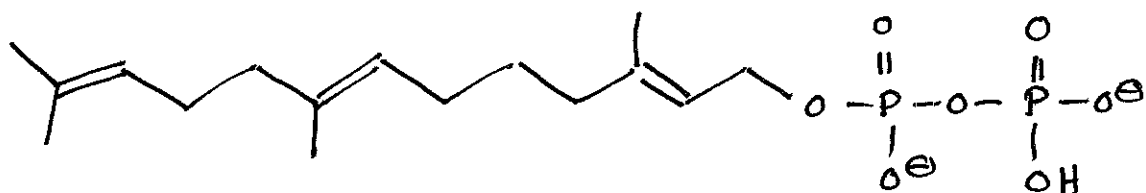
5. Give the names and identifiers of 3 amino acid side chains that stabilize the PP of FPP.
6. There is an ion at the active site.
 - a. What metal is it?
 - b. List all the amino acid side chains that are complexed to the ion (name and identifier).
 - c. What appears to be the geometry of these ligands around the metal? State how you determined this, preferably with supporting data.
7. Examine the location of the nucleophile relative to the electrophile in this reaction.
 - a. Comment on what you see.
 - b. Give the distance, in Å between these two groups.
8. There appears to be a cation- π interaction adjacent to the active site. Give the names and identifiers of the amino acids involved in this interaction.

2. There are 2 subunits.

The blue one is composed of many more or less parallel α -helices that form two layers.

The red one is almost all α -helices, but not organized in any obvious way. There is a very small β -sheet too.

3. One FPP is present (Select \rightarrow Residue \rightarrow FPP)



Note: unless you go checking bond lengths individually, you would not know where the double bonds are in FPP.

5. tyr 300.B — this would interact by hydrogen bonding

lys 294.B

lys 164.A

} these would be (+) charged to interact w/ the (-) charge on (P)(P)

this is a combination of hydrogen bonding + electrostatic (ion-ion) attractions

6. a. Zn^{2+}

b. -SH of inhibitor (739)

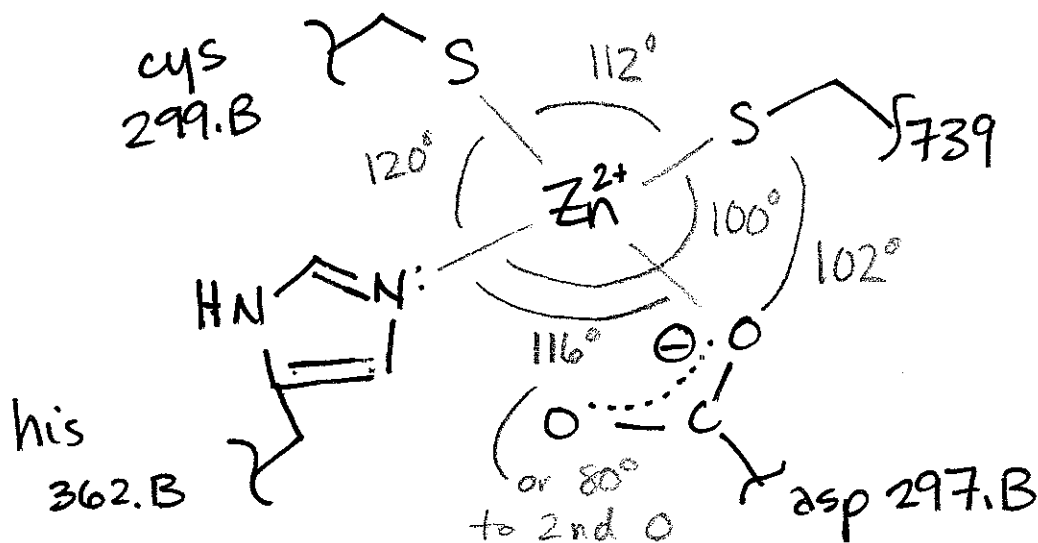
-CO₂[⊖] of asp 297.B

-SH of cys 299.B

ring N of his 362.B

c. tetrahedral, due to the following bond angles

(Tools → Structure Analysis → Angles/Torsions)



looks approximately tetrahedral

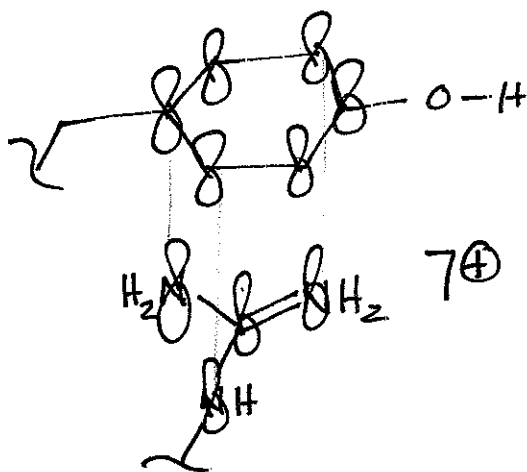
7 a. The ~~the~~ nucleophile is the $-SH$ on the end of 739.
 The electrophile is $-\overset{\uparrow}{CH_2}-O^{\oplus}P^{\oplus}$. They do not look to be in a good orientation to react

b. Tools \rightarrow Structure Analysis \rightarrow Distance

They are about 7.5 \AA apart - quite far

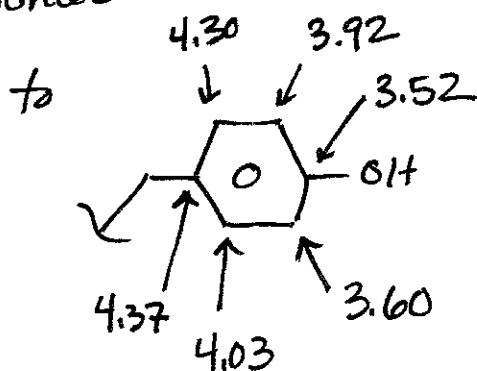
(it is an inhibitor after all)

8. tyr 116.A + arg 202.B



much like base stacking, the 2 π clouds can interact, but one is (+) charged

distances from center C of arg



So the 2 groups are not quite centered

typical base stacking distance

is $\sim 3.8 \text{ \AA}$ or even a little less