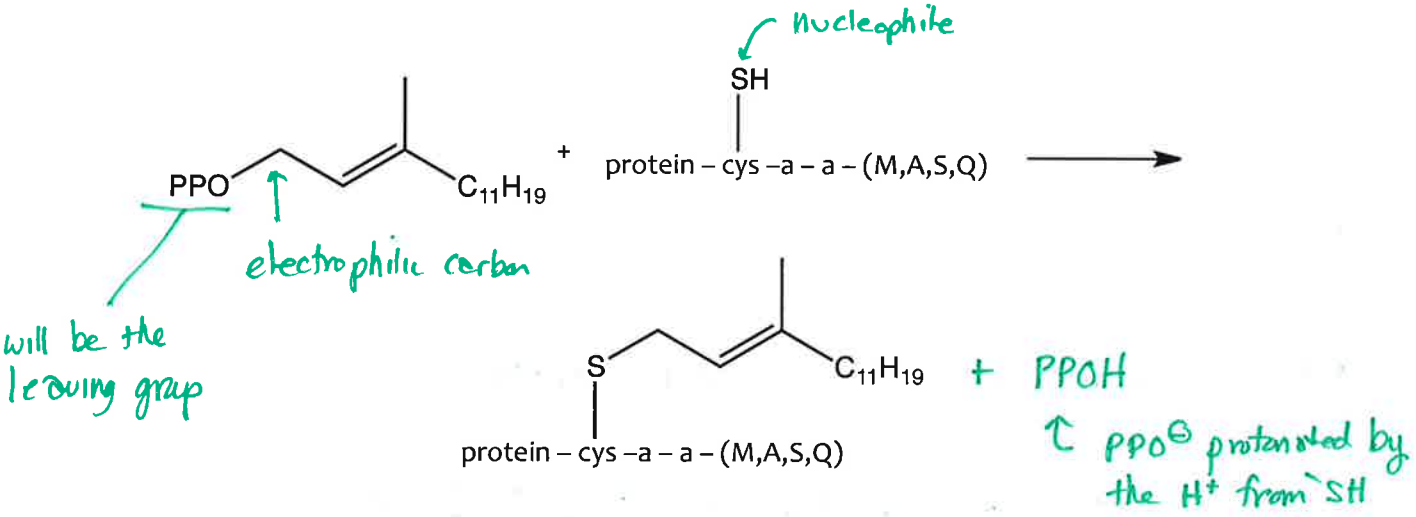
Study Question 3: Chimera Exploration of Protein Farnesyl Transferase

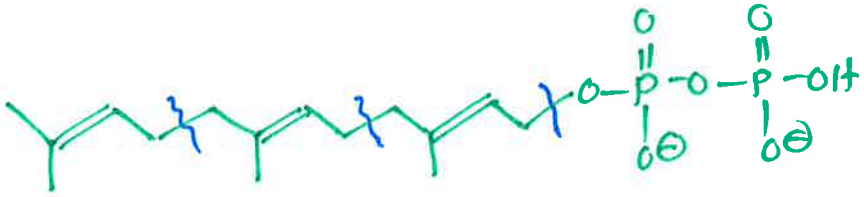
Go to the PDB site and fetch the entry 1JCQ. This is the structure of protein farnesyl transferase which adds a farnesyl group to the cysteine side chain of certain protein sequences. The reaction is shown below. Whether or not a protein is farnesylated is important in the initiation of certain cancers.



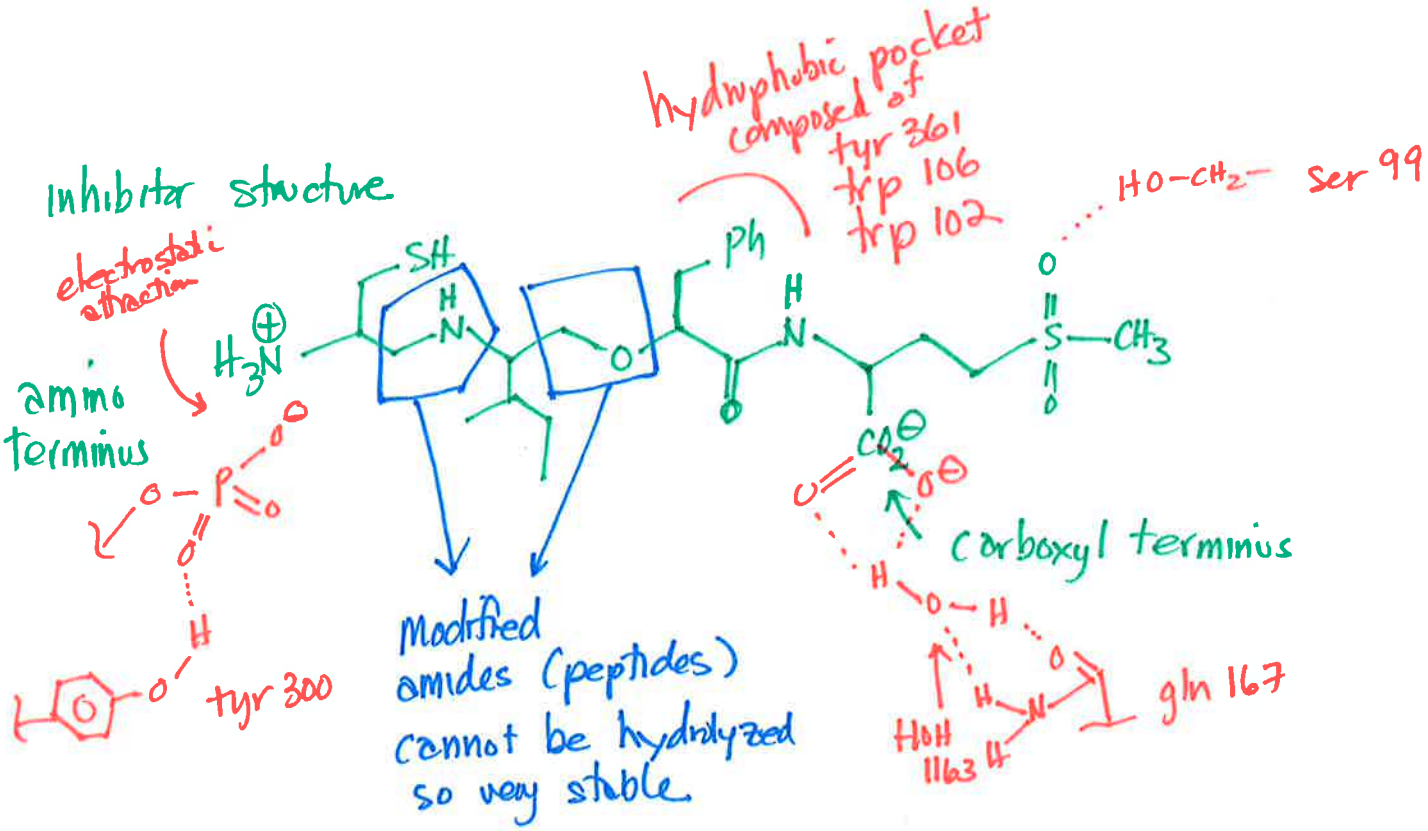
Answer the following questions about the structure by exploring with Chimera. Most of the tools you will need will be under the *Select* or *Action* menus; explore the various options and suboptions available. Open the file you downloaded and select *Preset* → *Interactive 1* to get started.

1. What kind of reaction is occurring? What are the possible mechanisms that should be considered? Nucleophilic substitution: both S_N1 & S_N2 are reasonable.
2. Balance the reaction above, and label any nucleophiles, electrophiles and leaving groups. see above
3. Describe 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? heterodimer; each subunit almost exclusively α-helices
4. How many farnesyl pyrophosphates are bound to this protein? 1 Give the complete structure of FPP. PTO
5. This particular structure contains an inhibitor that resembles typical peptide substrates but does not react. The inhibitor in this case is called 739.
   (a) Draw the structure of this inhibitor. PTO
   (b) 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. Include at least 2 amino acids that interact with the PP group, and 2 that interact with the rest of the inhibitor. PTO
   (c) 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? PTO
6. There is an ion at the active site.
   (a) What metal is it? Zn^{2+}
   (b) List all the amino acid side chains that are complexed to the ion (name and identifier). Asp 297, Cys 299, His 362
   (c) What appears to be the geometry of these ligands around the metal? State how you determined this, preferably with supporting data. It "looks" close to tetrahedral, but you have to average the asp position. S–Zn–S angle is 112.5°
7. Examine the location of the nucleophile relative to the electrophile in this reaction.
   (a) Comment on what you see. The nucleophile (SH) is facing the wrong way
   (b) Give the distance, in Å between these two groups. ~7.5 Å
8. There appears to be a cation-pi interaction adjacent to the active site. Give the names and identifiers of the amino acids involved in this interaction.
   Arg 200 is lying flat next to Tyr 166

FPP = farnesyl pyrophosphate = farnesyl diphosphate



C_{15}, composed of 3 C_5 isoprene units



Sequence is

cys-like ile-like phe-like · met-like

"2" "2" "M"

oxidized met