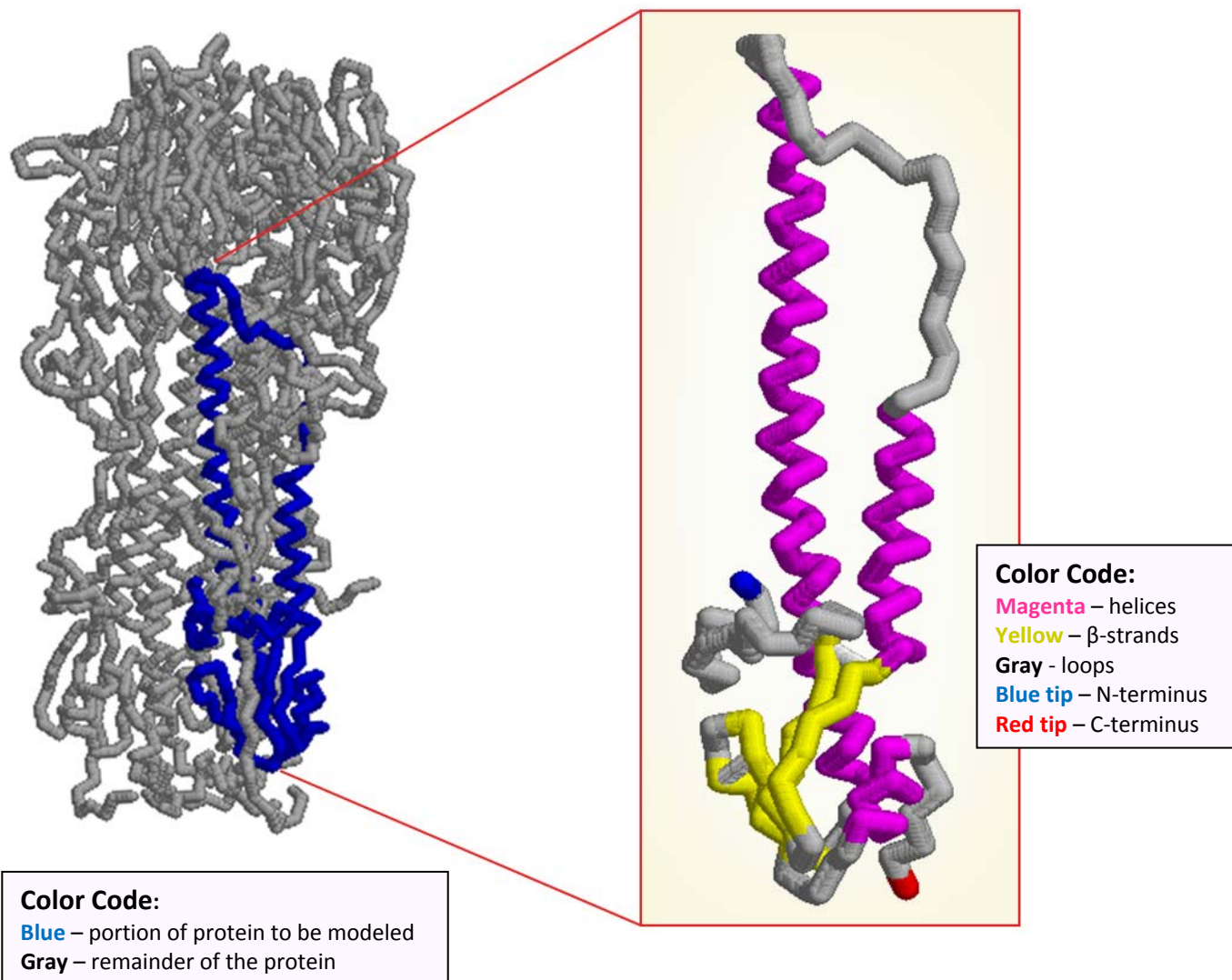


Guide to Using the Rubric to Score the Hemagglutinin **Pre-Build Model** for Science Olympiad 2010 National Competitions

These instructions are to help the event supervisor and scoring judges use the rubric developed by the MSOE Center for BioMolecular Modeling when scoring the 2010 Science Olympiad **National Pre-Build** Mini-Toober models of influenza hemagglutinin, based on [5hmg.pdb](#). Each category on the rubric is addressed within these instructions and is accompanied by a short description and picture, if appropriate.

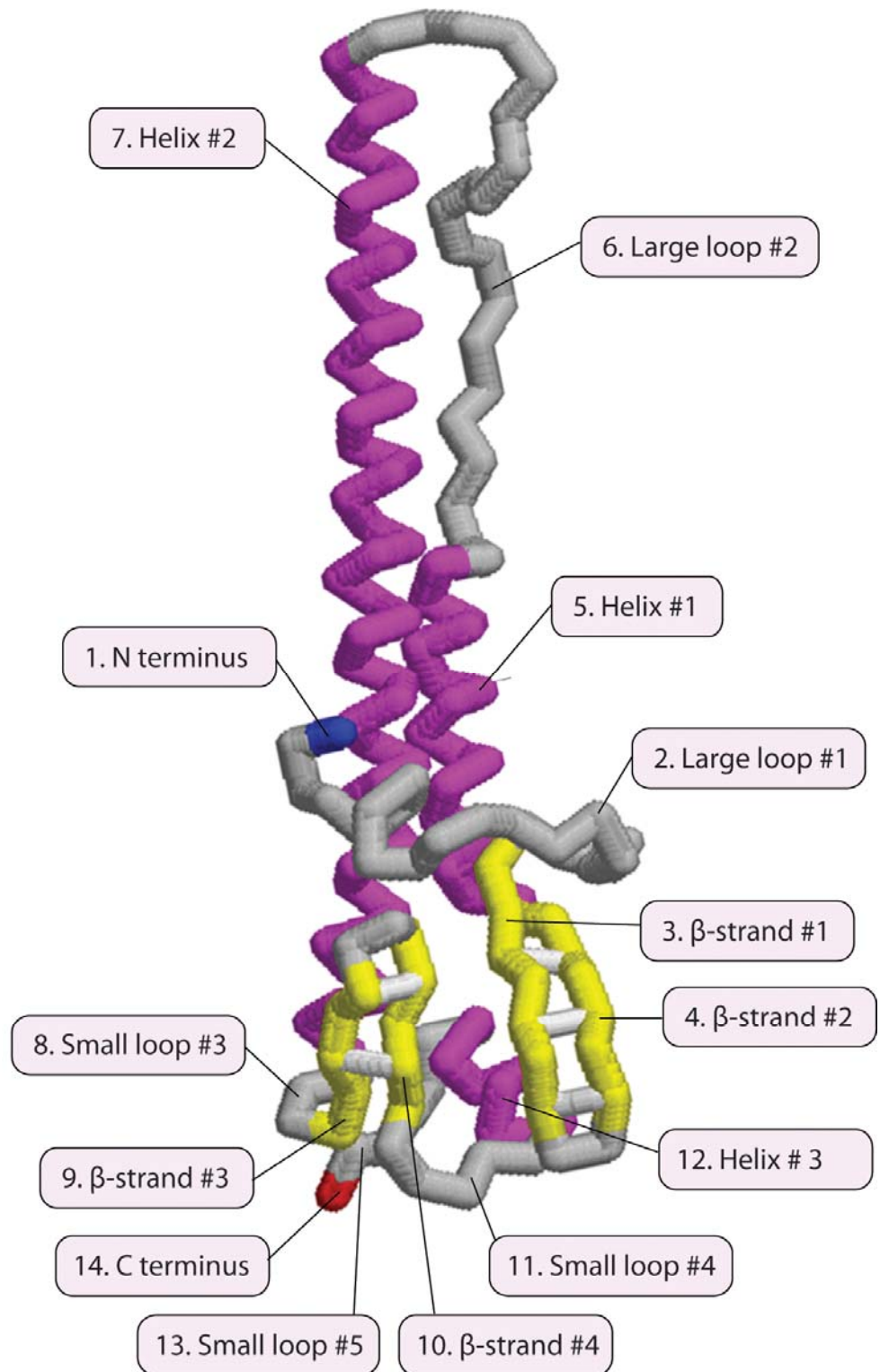
Overview of the Entire Molecule:

The Pre-build Molecule is based on amino acids 1-160 of chain B of 5hmg.pdb, which is shown in the image on the bottom right and is highlighted in blue in the image on the bottom left.



Order of Secondary Structures:

| |
|---------------------------------------|
| 1. N terminus |
| 2. Large loop #1 (short turn) |
| 3. β -strand #1 (short turn) |
| 4. β -strand #2 |
| 5. Helix #1 |
| 6. Large Loop #2 |
| 7. Helix #2 (long) |
| 8. Small loop #3 |
| 9. β -strand #3 (short turn) |
| 10. β -strand #4 |
| 11. Small loop #4 |
| 12. Helix #3 (short) |
| 13. Loop #5 |
| 14. C terminus |

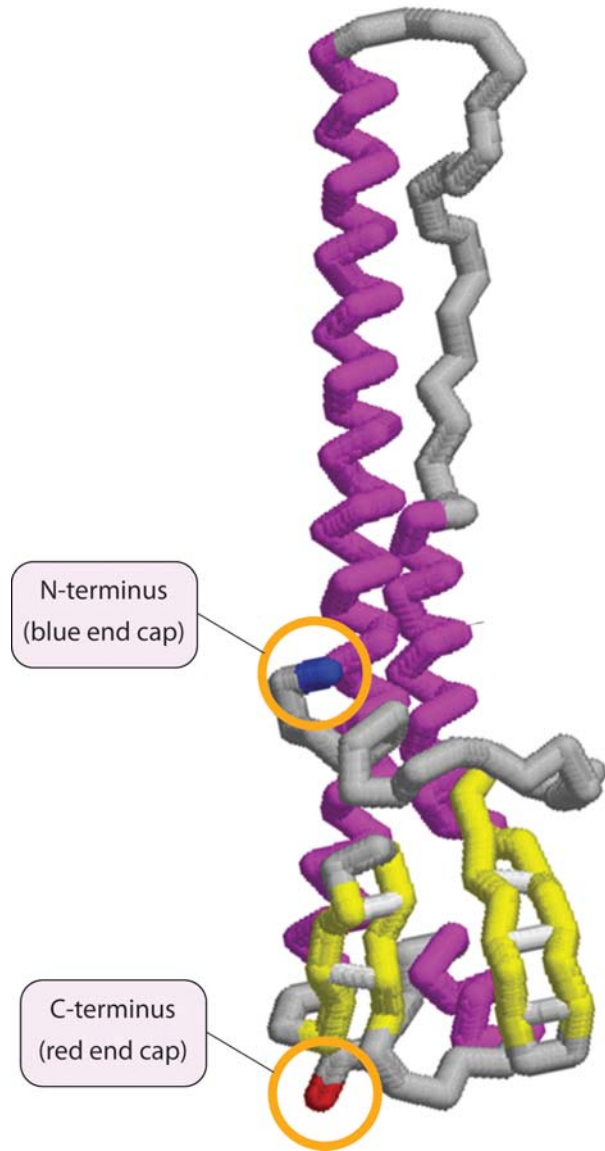


1. Blue Cap on N-terminal Amino Acid (Gly1) (0.5 pt)

- To receive this point, the blue cap needs to be located at the N-terminus of the protein, which is located near the protein's midsection. The blue end cap should be followed by a long unstructured region and then the first β -sheet. Please see the figure to the right and the physical model for the correct positioning of the blue end cap. If the blue end cap is near a short alpha helix, the model does not receive the 0.5 points for this feature.

2. Red Cap on C-terminal Amino Acid (Asp160) (0.5 pt)

- To receive this point, the red cap needs to be located at the C-terminus of the protein, which is at the base of the protein. The C-terminus is preceded by a short unstructured region lying next to an alpha helix. Please see the figure to the right and the physical model for correct positioning of the red end cap. If the red cap is adjacent to a β -sheet in the *primary structure*, the model does not receive the 0.5 points for this feature. (Note that the C terminus lies *near* a β -sheet in the folded structure, but is not linearly near the β -sheet.)



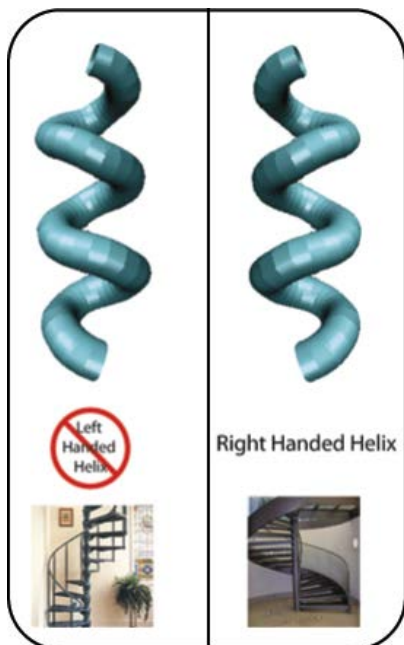
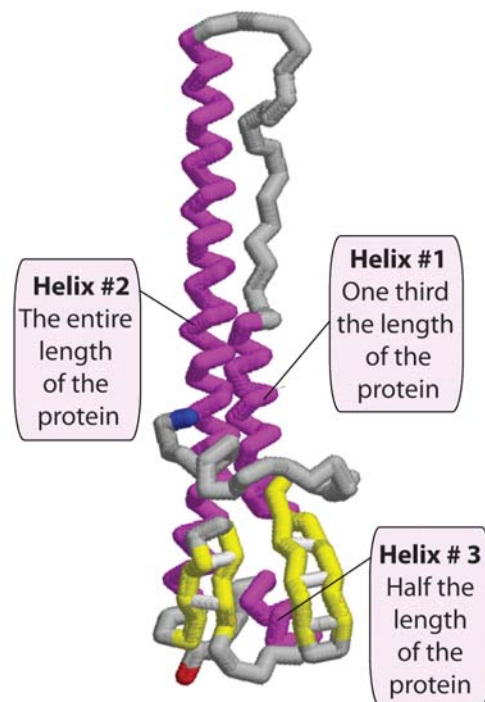
3. N and C termini are positioned correctly on the model. (1 pt)

- Hold the model so that the two long helices are vertical and the β -sheets (yellow in the plaster model) are forming a triangular base on the right.
 - The C-terminus (red end cap) should be on the left side at the base of the model. (0.5 pt)
 - The N-terminus should lie just above the triangular base and in line with the two long helices. (0.5 pt)
- Please see the figure above and the physical model for correct positioning of the caps.

4. Model has 3 alpha helices according to Jmol selection criteria

(0.5 pt per helix for a total of 1.5 pts)

- To receive these points, there should be 3 helices within the model. Please see figure to the right for the correct location of these helices. (Helices are colored magenta on the model and on the figure to the right.)
- Deduct 0.5 point for each extra helix. For example, if a model has 4 helices, then the model would receive 1 pt rather than the full 1.5 points.

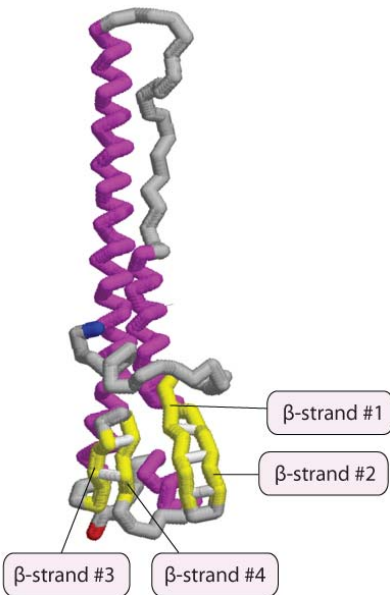


5. Alpha helices are right-handed (0.5 pt each; total of 1.5 pts)

- Alpha helices are right-handed. Check each alpha helix in the model to confirm that the helix is right-handed. For each right-handed helix, the model should receive 0.5 points, for a total of 1.5 points if all three helices are correct.
- To determine if the helix is right-handed, find one of the ends of the helix and imagine that the helix is a spiral staircase. Imagine walking up the staircase with your hand on the handrail on the outside of the staircase. If your right hand is on the handrail as you climb up the staircase, you have a right-handed helix. If your left hand is on the handrail as you climb up the staircase, you have a left-handed helix and the modeled helix would not receive credit.
- Please refer to figure at left for illustration of left-handed and right-handed helices.

6. Alpha helices are of the correct length. (0.5 pts each; total of 1.5 pts)

- Helix #1 should have approximately 3-5 turns and should be about 1/3 the length of helix #2.
- Helix #2 should have approximately 12-14 turns and should run almost the entire length of the model.
- Helix #3 should have approximately 2 turns and should be about 1/2 the length of helix #1.
- See figure above.



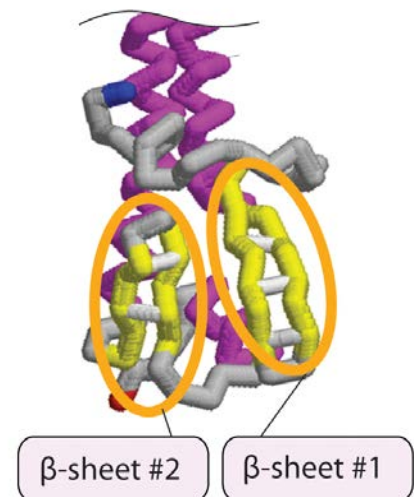
7. Model has 4 β -strands (0.5 pt each; total of 2 pts)

- This protein has 4 β -strands. Please see figure to the left.
- β -strands need to be clearly distinguishable from loops; there may be some slight 'zig-zag' folding of the toober to indicate the up-and-down positioning of the amino acids. Alternately, teams might color-code their β -strands to distinguish them from loops. Please see photos below depicting β -strands.
- If there are more than 4 strands in this model, 0.5 pt should be deducted for each extra strand. For example, if the model has 5 β -strands, the model should receive 1.5 points, rather than the full 2 points.
- Please see the figure at the left for location of the strands.

| | |
|--|--|
| | |
| <p>This is a good example of a beta strand, slightly folded. It would also be acceptable to color code or in some other way mark the backbone to indicate beta sheets.</p> | <p>Although this model shows a beta strand, it is actually too kinked, such that the length of the beta strand is disproportionate to the width. If ALL of the beta strands are hyper-kinked, deduct 1 pt.</p> |

8. Model has 2 β -sheets (0.5 pt for each sheet; total of 1 pt)

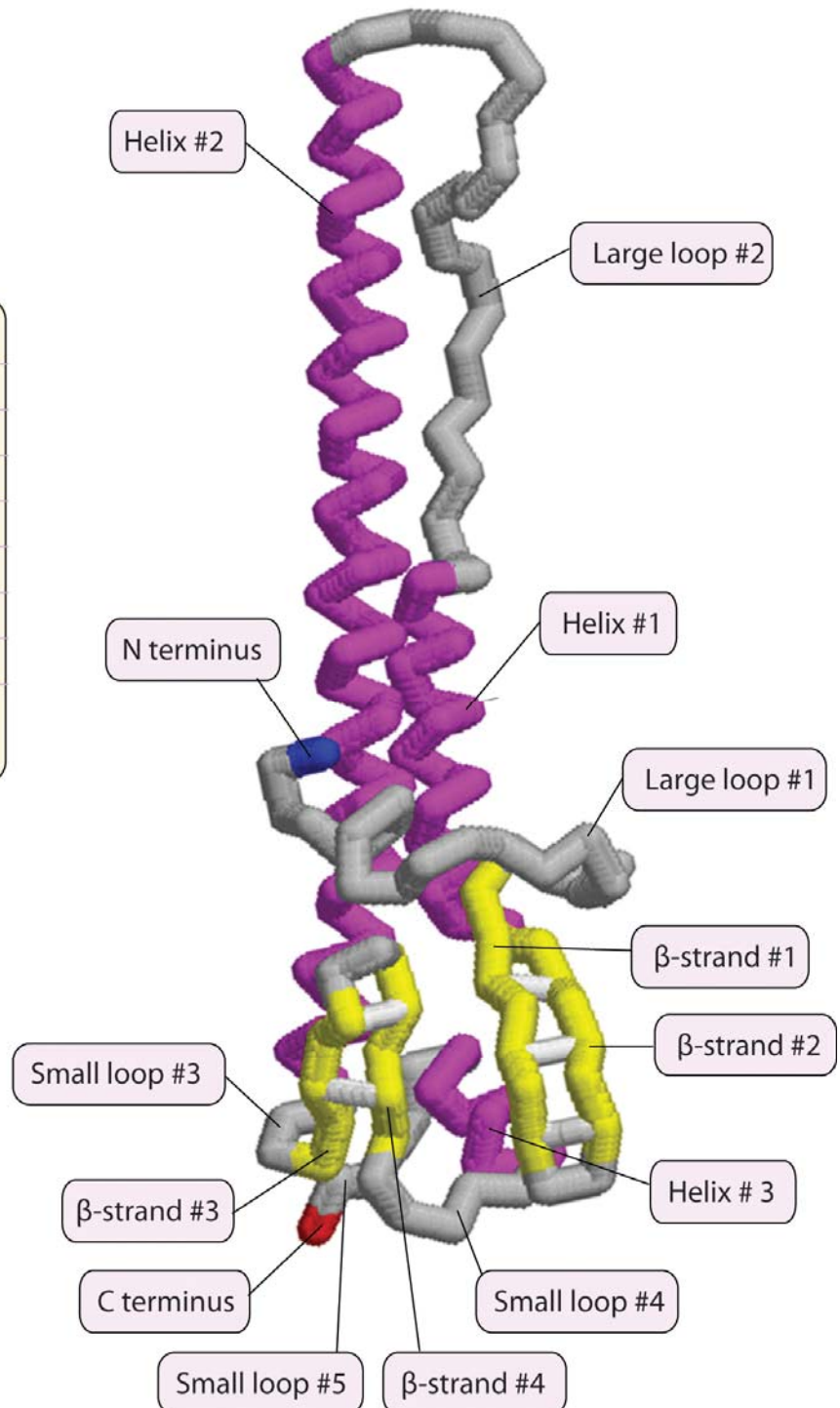
- This protein has two β -sheets arranged from the 4 β -strands. Each sheet consists of two β -strands.
- β -sheet #1 is comprised of β -strands #1 and #2.
- β -sheet #2 is comprised of β -strands #3 and #4.
- To receive full points, the model should have two clearly separate β -sheets. The β -strands should be positioned to create a sheet (β -strands are near one another). Note that the two sheets should be slightly separated – the β -strands do NOT form one continuous sheet, though they lie near each other. If all four strands form a single sheet, deduct 0.5 pt.



9. Positioning of secondary structures in proper order (0.5 pts each; for a total of 4.5 pts)

- To receive these points, the sequence of the secondary structures should be in the order shown in the bulleted box below.
- Each bulleted sequence below, if in the correct order, should receive 0.5 pt, for a total of 4.5 pts.
- Secondary structures that are out of order should not be counted.

- | |
|---|
| • N terminus --> loop #1 |
| • Short turn --> β -strand #1 |
| • Short turn --> β -strand #2 |
| • Helix #1 |
| • Large Loop #2 |
| • Helix #2 (long) --> Small loop #3 |
| • β -strand #3 --> Short turn |
| • β -strand #4 --> Small loop #4 |
| • Helix #3 (short) --> Loop #5 --> C terminus |

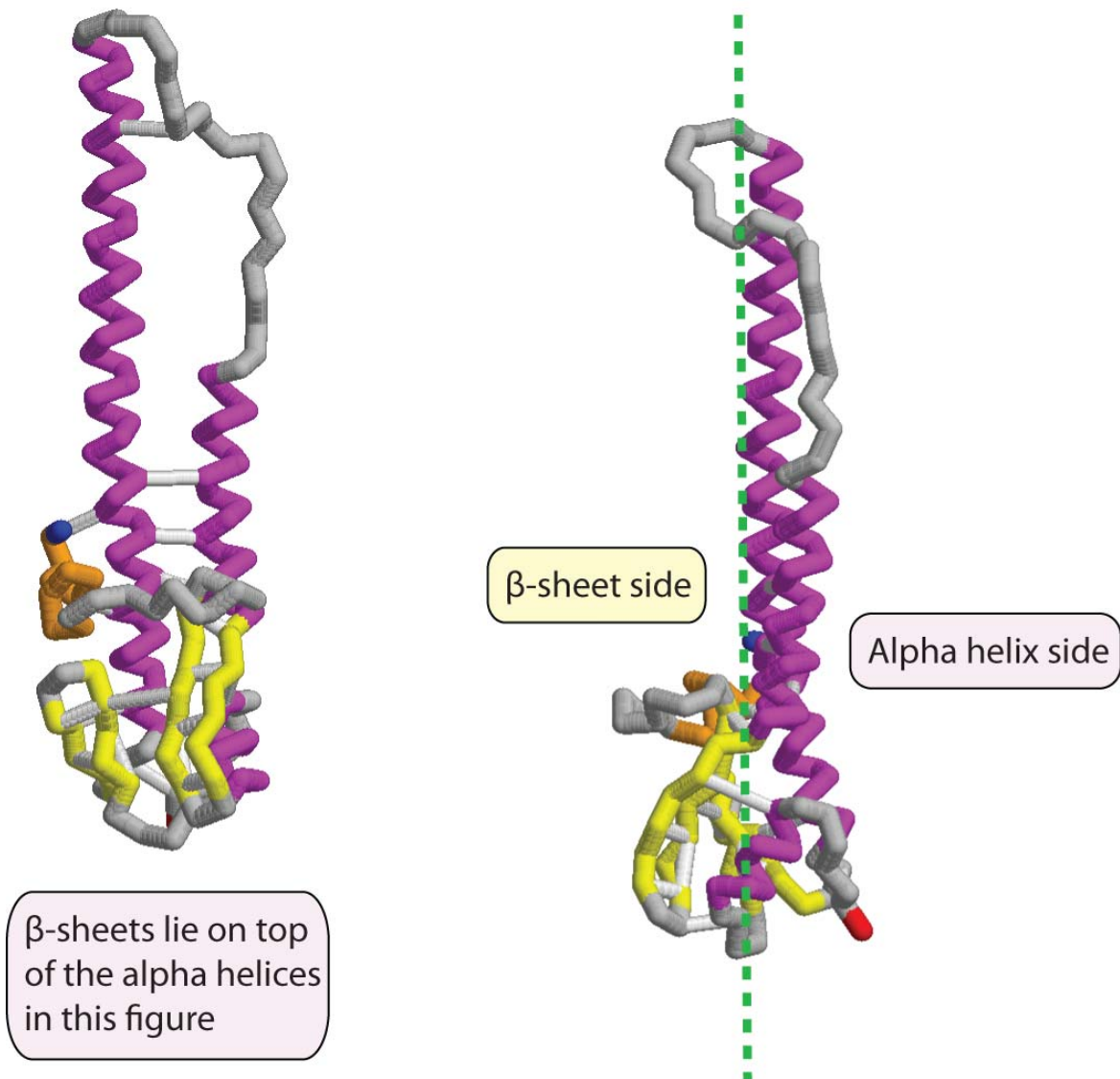


Items #10, 11 and 12 refer to the 3-dimensional shape of the model

- The positioning of the secondary structures is very important to the overall 3-dimensional fold of the protein. Proteins are not flat; therefore the model should have a 3-dimensional look to it.

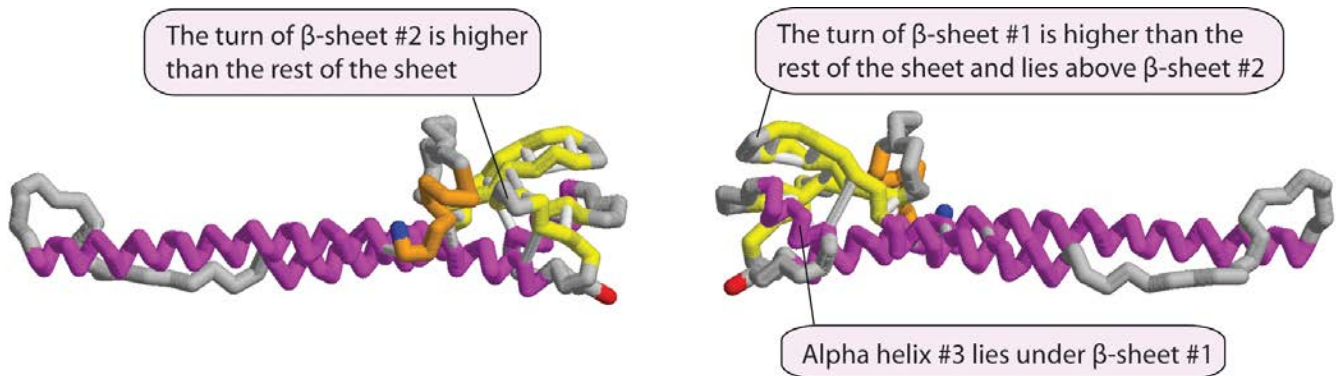
10. Alpha helices should be on the same side of the model (0.5 pts per helix for a total of 1.5 pts)

- All three helices are positioned on the same side of the model. If you divide the model in half, there should be an “alpha helix side” and a “ β -sheet side”. Please see figures below. Note that there is a slight twist in the axis separating the helices and sheets at the base of the protein where the β -sheets are located.
- Each helix should receive 1 pt if on the correct side of the model.



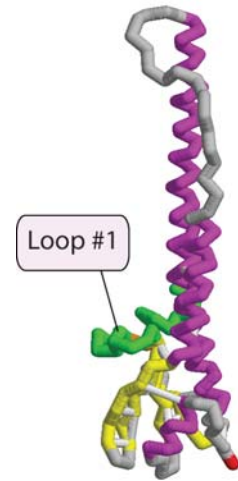
11. Orientation of the β -sheets. (3 pts)

- When the model is placed on a flat surface, with alpha helices #1 and #2 (two long helices) on the bottom, β -sheet #1 should be slightly higher than β -sheet #2, with short alpha helix #3 lying under β -sheet #2. Model earns 1 point for β -sheet #1 lying slightly higher than β -sheet #2, and 1 pt for alpha helix #3 lying under β -sheet #1.
- When the model is placed flat with alpha helices on the bottom, the turn joining the strands of each of the β -sheets should be the highest point of each of the β -sheets (though β -sheet #2 lies slightly above β -sheet #1). Award 0.5 pt for each β -sheet whose turn is the highest point of the sheet.
- See the plaster model and figures below; β -sheets are in yellow.

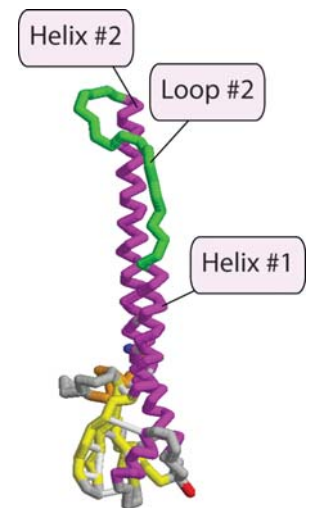


12. Position of loops.

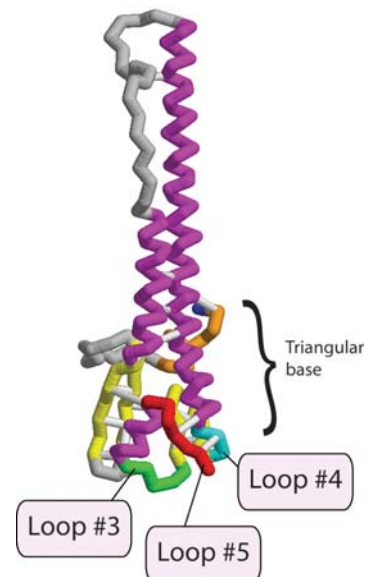
- Loop #1 (0.5 pt)
 - Loop #1 (highlighted in green to the right and beginning at the N terminus) should wrap almost halfway around the 'waist' of the protein and be about a third of the way up the length of the protein. It should be on the same side of the protein as the β -sheets.
 - Award 0.25 pt if loop #1 is on the β -sheet side of the protein.
 - Award 0.25 pt if loop #1 wraps approximately halfway around the protein.



- Loop #2 (0.5 pt)
 - Loop #2 (highlighted in green to the right) connects helix #1 to helix #2; half of loop #2 should lie in the same plane formed by these two helices. The other half of loop #2 should lie on the same side of the molecule as loop #1.
 - Award 0.25 pt if part of loop #2 lies in the same plane as helices #1 and #2.
 - Award 0.25 pt if part of loop #2 bulges on the same side of the protein as loop #1.



- Loops #3, #4, and #5 (0.5 pt each, for a total of 1.5 pts)
 - Loops #3 (highlighted green to the right), #4 (highlighted cyan) and #5 (highlighted red) should all lie in the triangular 'base' of the protein. Award 0.5 pt for each of the three helices lying in this region.

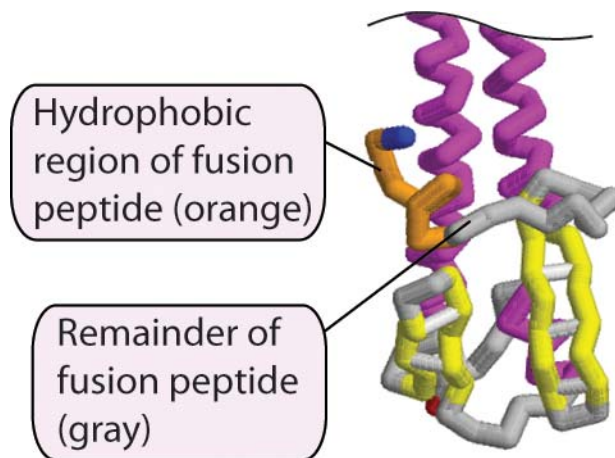


13. Students submitted a 3x5 card with explanation of the model (1 pt)

- The 3x5 card submitted with the model should describe the model in terms of what additional features have been added to the model so that the judge is not left guessing what the model represents.
- To receive full credit, the card should identify both the importance of ALL of the additional features (0.5 pt) as well as how the features have been depicted in the model (0.5 pt). If the card only describes some of the features, then full credit should not be awarded.

14. Creative additions to the model. (Note that there are many possible creative additions to this model; each creative addition is worth 4 pts; teams may have more than 4 creative additions, but may earn no more than 16 pts in this section. If you are using the electronic score sheet, it will automatically calculate sum the points and record a maximum of 16 points.)

- Fusion peptide is highlighted. (4 pts)
 - The fusion peptide is the harpoon that hooks the membrane of the endosome. It consists of amino acids 1-23 of the model and is equivalent to loop #1. The first 10 amino acids are hydrophobic, allowing this region to embed in the endosome membrane.
 - Teams may highlight either the first 10 hydrophobic amino acids, or the entire fusion peptide. Either is acceptable.
 - Award 1 pt for highlighting either the first 10 hydrophobic amino acids or the first 23 amino acids.
 - Award 2 pt if the FUNCTION of this region is included in the description on the 3 x 5 card.
 - Award 1 pt if they indicate (either on the model or the card) that the first 10 hydrophobic amino acids embed in the host membrane.
 - See the figure below – the first 10 hydrophobic amino acids are colored orange; the rest of the fusion peptide is in gray.



- Non-polar residues at top of coiled coil (4 pts)

- The three subunits of hemagglutinin interact along the longest helix in the model, forming a structure called a 'coiled coil' (the alpha helices twist together to form a left handed triple helix) which teams may describe in their 3x5 card. Note that teams do NOT need to show all three subunits to get credit. The structure is stabilized at the top of helix #2 with non-polar residues:

- Ile77
- Leu80
- Val84
- Leu91
- Leu98
- Leu99
- Leu102

- Note that credit is given for up to 4 of the 7 amino acids

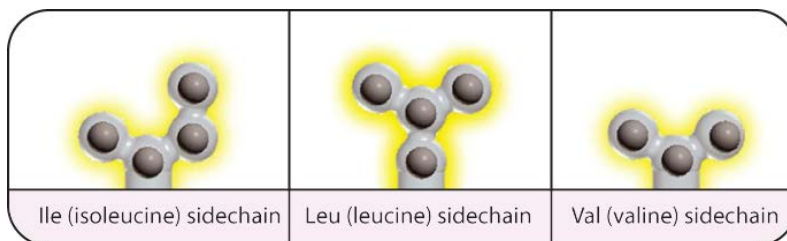
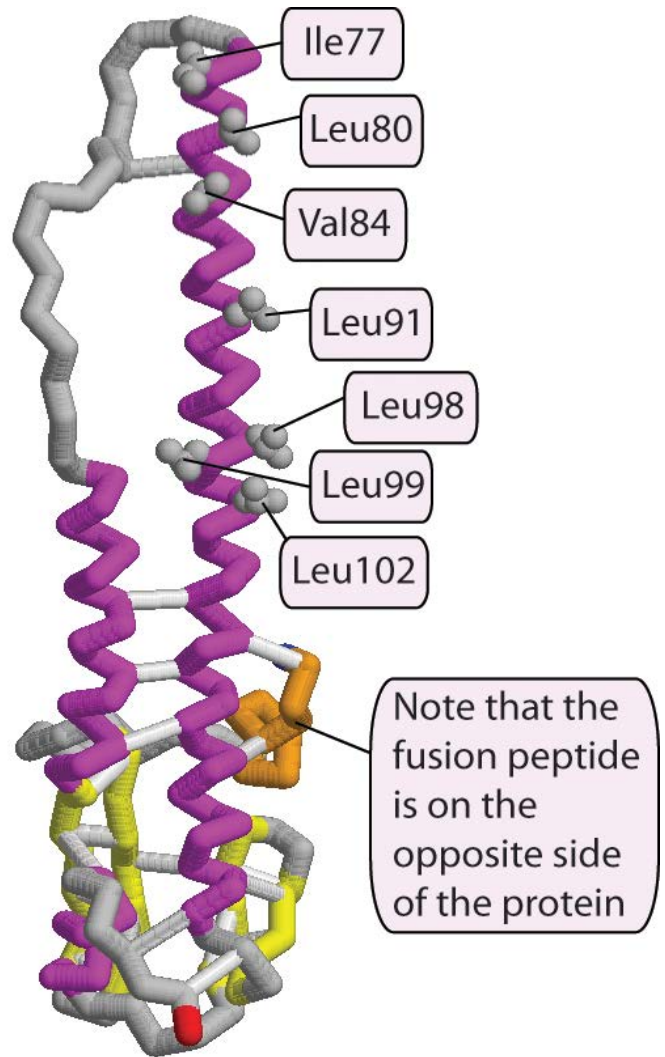
- The non-polar amino acids should be properly positioned along the helix #2. (0.5 pts each for a max total of 2 pt)

- If an amino acid is displayed, but is not in the correct location, it does not receive credit.

- All the amino acids should be on the same face of the helix, on the side of helix #2 opposite the side on which the non-planar portion of loop #2 resides. (0.5 pt each for a max total of 2 pt)

- If some of the amino acids lie on the back face of the helix, deduct 0.25 pt.
- If all of the amino acids are on the same side of the helix, but it is the same side as loop #1, deduct 0.25 pt.

- Figure below displays these amino acids in ball and stick. Teams do not have to have accurate depictions of the sidechains to receive credit, but they need to be either labeled on the model or identified on the 3x5 card.



- Polar and charged residues at base of coiled coil (4 pt)

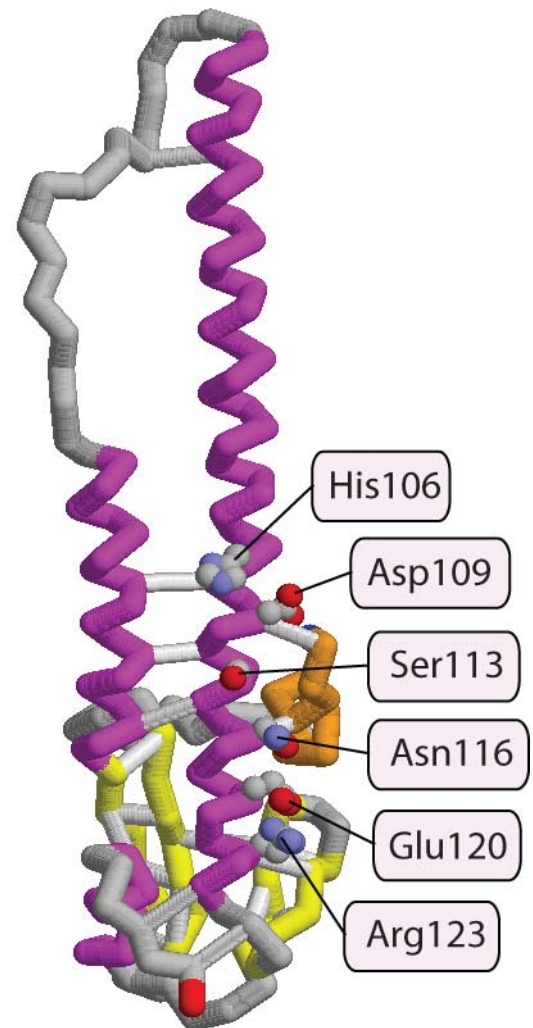
- The coiled coil is stabilized at the lower end of the long helix (#2) with polar and charged residues:

- His106
- Asp109
- Ser113
- Asn116
- Glu120
- Arg123

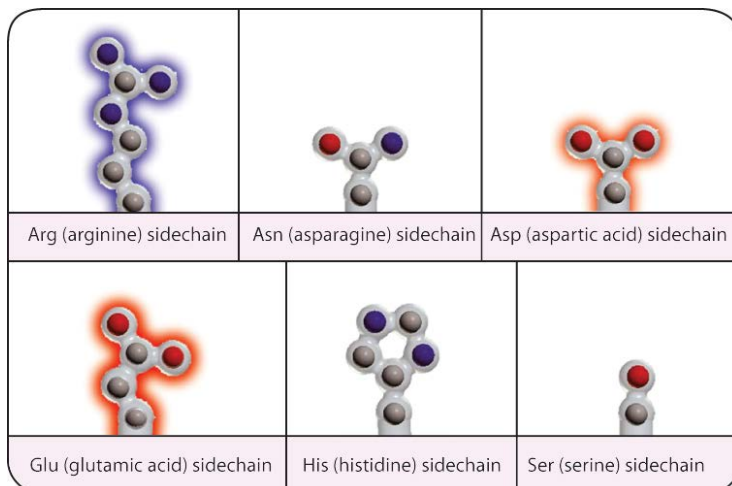
- The polar amino acids should be properly positioned along the long alpha helix. (0.5 pts each, 3 pt total):

- Addition is accurate (.5 pt)

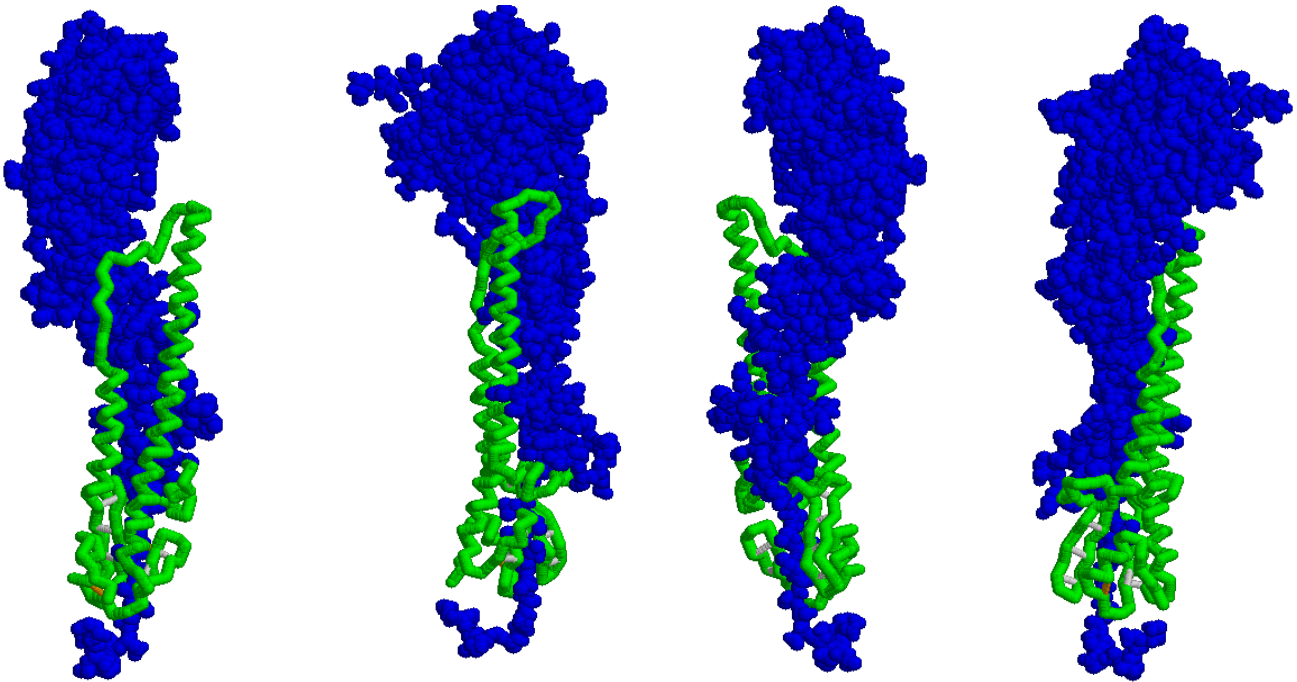
- With the β sheets facing down and back:
 - His106 should lie directly above Ser113. (0.25 pt);
 - The remaining 4 amino acids should stack above each other, but to the right of His106 and Ser113. (.25 pt).
- All the amino acids should be on the same face of the helix, where it would coil with the other two strands of the protein. (0.5 pt)



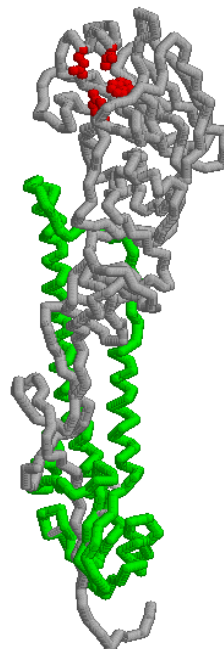
- Figure above indicates locations of these amino acids. These amino acids are shown in ball and stick in the figure below. Teams do not have to have accurate depictions of the sidechains to receive credit, but they need to be either labeled on the model or identified on the 3x5 card.



- Addition of HA₁ with sialic acid receptor (4 pt)
 - HA₂ (model) is part of a larger protein. HA₁ might be displayed in some fashion to show the remainder of the hemagglutinin subunit. Award 2 pts if HA₁ is appropriately displayed. To receive full credit, HA₁ should have a 'bulb' that extends above loop #2 and a long tail that becomes narrower as it extends through the base of HA₂. See figures below for an accurate depiction of HA₁ (in blue and spacefill) in association with HA₂ (in green backbone). Each image is rotated approximately 90° to the left of the previous image.



- Award 2 pt if sialic acid receptor, where hemagglutinin docks to the host cell, is indicated on the model. See figure at right – receptor is red, HA₁ is gray, HA₂ is green.

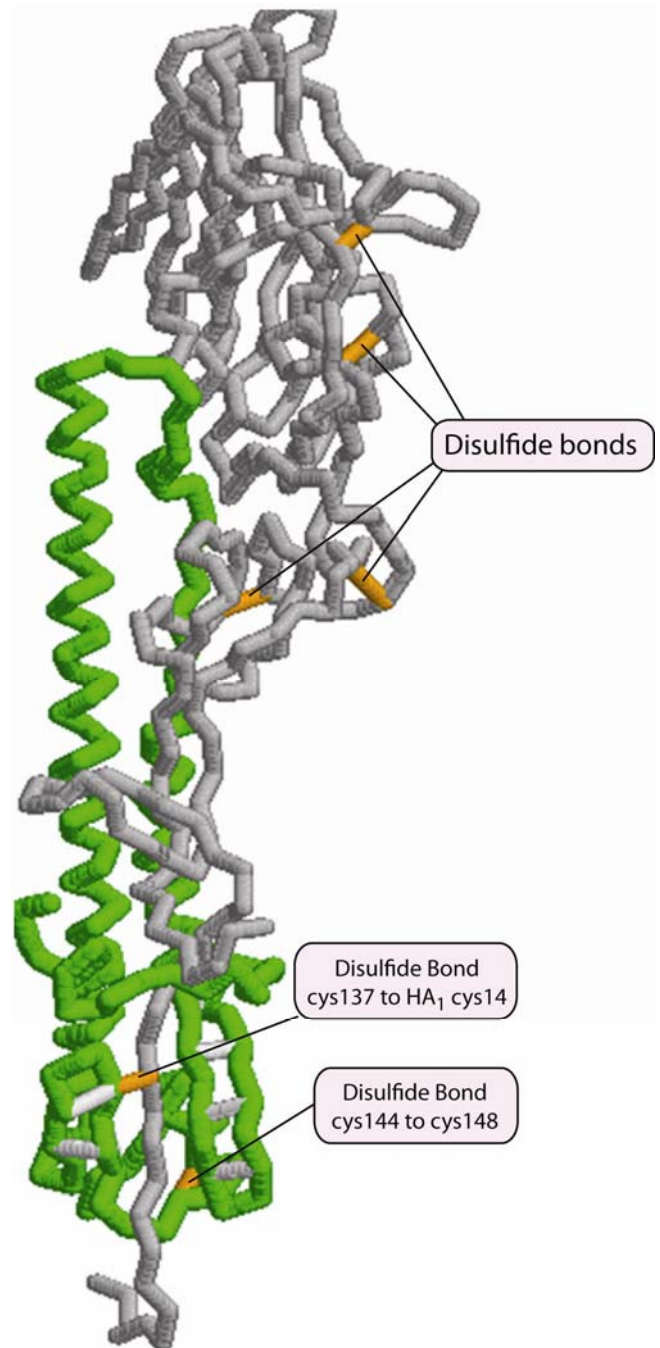


- Displaying disulfide bonds (2 pts per bond, for a maximum of 4 pts)

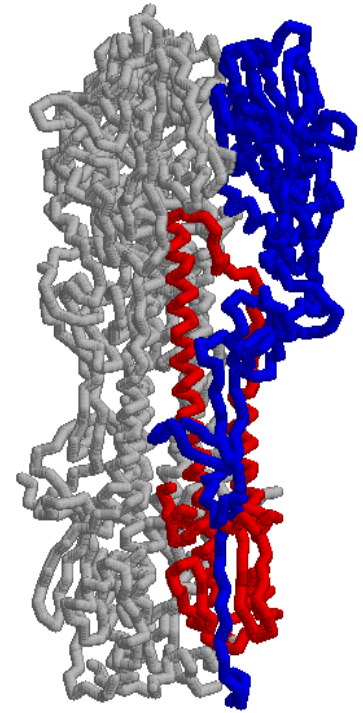
- Disulfide bonds stabilize proteins. There are two disulfide bonds in this portion of the protein that students may include in their model; additional disulfide bonds may be displayed if they include HA₁ as part of their model. To receive credit for a disulfide bond, it must be positioned correctly in the model.

- There is one disulfide bond within the model, between cys144 and cys148. This lies at the base of the model, joining loop 4 to helix 3. See base of figure at left for position (indicated in orange). Award 2 pts for this disulfide bond.
- There is a second disulfide bond, joining HA₂ cys137 to HA₁ cys14. Cys137 is located on β strand #4, just after the turn from β strand #3. See figure left at base for position (indicated in orange). Teams should receive full credit if they don't display HA₁, but mark cys137 correctly. Award 2 pts for this disulfide bond.
- If teams display HA₂, they may display 4 additional disulfide bonds within HA₂. Award 2 pts per disulfide bond in the HA₂ subunit. Please note that the model may receive a maximum of 4 pts for disulfide bonds. See figure at right for placement of these bonds. These are located at:

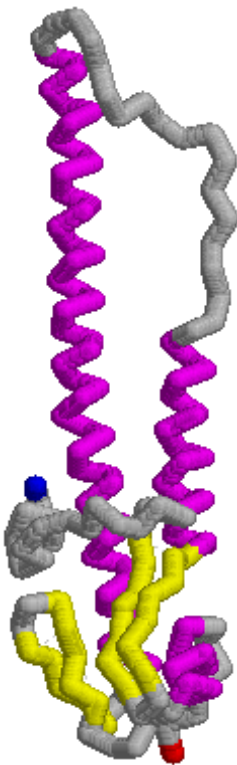
- Cys52-Cys277
- Cys64-Cys76
- Cys97-Cys139
- Cys281-Cys305



- Incorporation of model into the hemagglutinin trimer (4 pts)
 - Teams may choose to display the entire hemagglutinin trimer in some fashion. The overall appearance of the model should be a stalk with a bulb at the top. The image at the right shows the portion of the protein to be modeled in red, the associated HA₁ in blue, and the remainder of the trimer in gray. Note that the addition need not be perfectly accurate, but the overall shape of the protein should be reflected in the model.



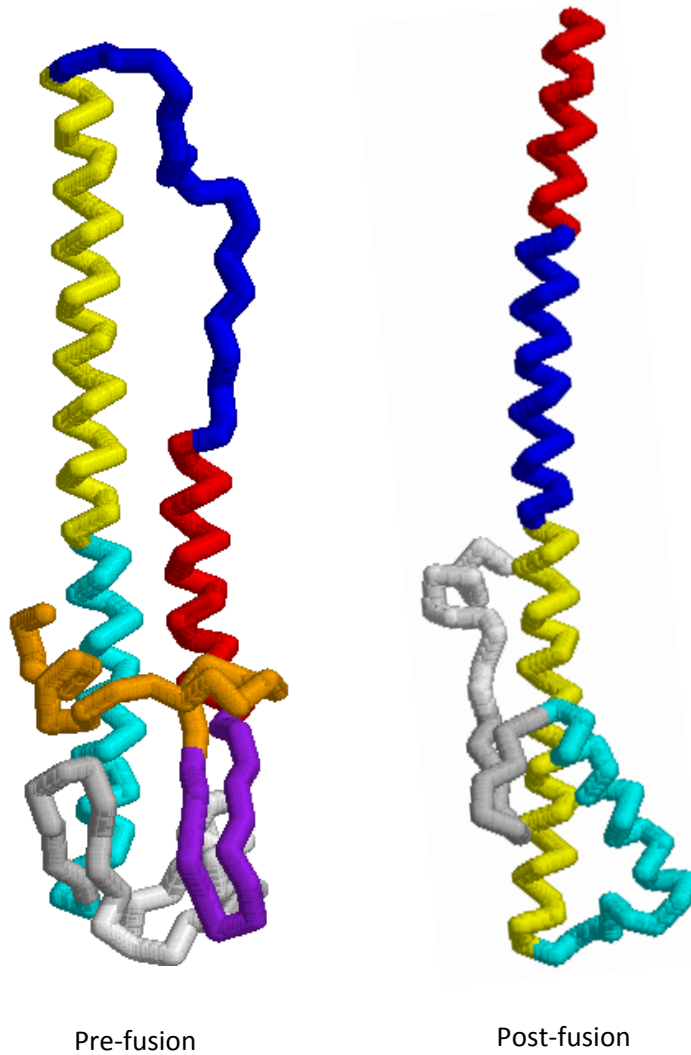
Protein would attach to virus here.



Protein would attach to virus here.

- Positioning model on viral capsule to represent the protein on the surface of a virus (4 pts)
 - Hemagglutinin is a protein that is attached to the surface of the virus particle.
 - Students may choose to depict hemagglutinin on the surface of the virus. It is not necessary that they display the entire protein to receive credit; however, if they show the entire protein, it should be oriented such that the stalk of the protein sits on the virus and the bulbous region is away from the virus. If they are only showing HA₂, the β sheet base of the protein should sit on the virus.
 - See figures above and at left for orientation of protein on surface of virus.

- Highlighting the region of the protein that changes conformation at pH 5 (4 pts)
 - Teams may choose to tell the story of the conformational change the protein undergoes within the endosome (discussed in detail in the Molecule of the Month). Although there are many ways they can depict these changes, the regions that change shape are color coded as follows:
 - Loop #2 (colored blue in the figure below) becomes a helix at pH5. (3 pts)
 - Award 1.5 pt if they indicate that loop #2 forms an alpha helix.
 - Award 1.5 pt if they indicate that loop #2, in combination with helix #1 and part of helix #2, forms a very long helix.
 - Part of helix #2 (colored cyan in the figure below) bends into a loop and a shorter helix at pH 5. Award 1 pt if they mention this change in structure.



- Any other added features that help to tell the story (4 pts)
 - To receive these points, the model should have more to it than just the tober we provided. Listed above are suggestions for inclusions that might appear based on the primary citation and the Online Macromolecular Museum website.
 - Additional features that are explained (on the 3x5 card or labeled on the model) that help to tell the story and are accurate are also acceptable. To receive full credit, the addition must be
 - Located correctly on the protein (1.5 pt)
 - Described on the 3 x 5 card (1 pt) and
 - The role the addition plays in the protein function must be appropriately described (1.5 pt)

15. Additions to model are appropriate to function of the protein (2 pts)

- To receive these points, the creative additions need to be relevant to telling the functional story of the protein. These points should be awarded to those proteins that meet the following criteria:
 - Model has creative additions
 - Models that are just the tober will not receive these points
 - Additions are appropriate to the function of the protein
 - Models that have ALL sidechains displayed should not receive these points. If all sidechains are displayed, the team did not recognize the significance of a select few amino acids to the enzyme function. The focus of adding the amino acids should be on the ones that play a role in the function of the protein.
 - Additionally, these points should not be awarded if amino acids outside of those that play a specific functional role in the protein are displayed.