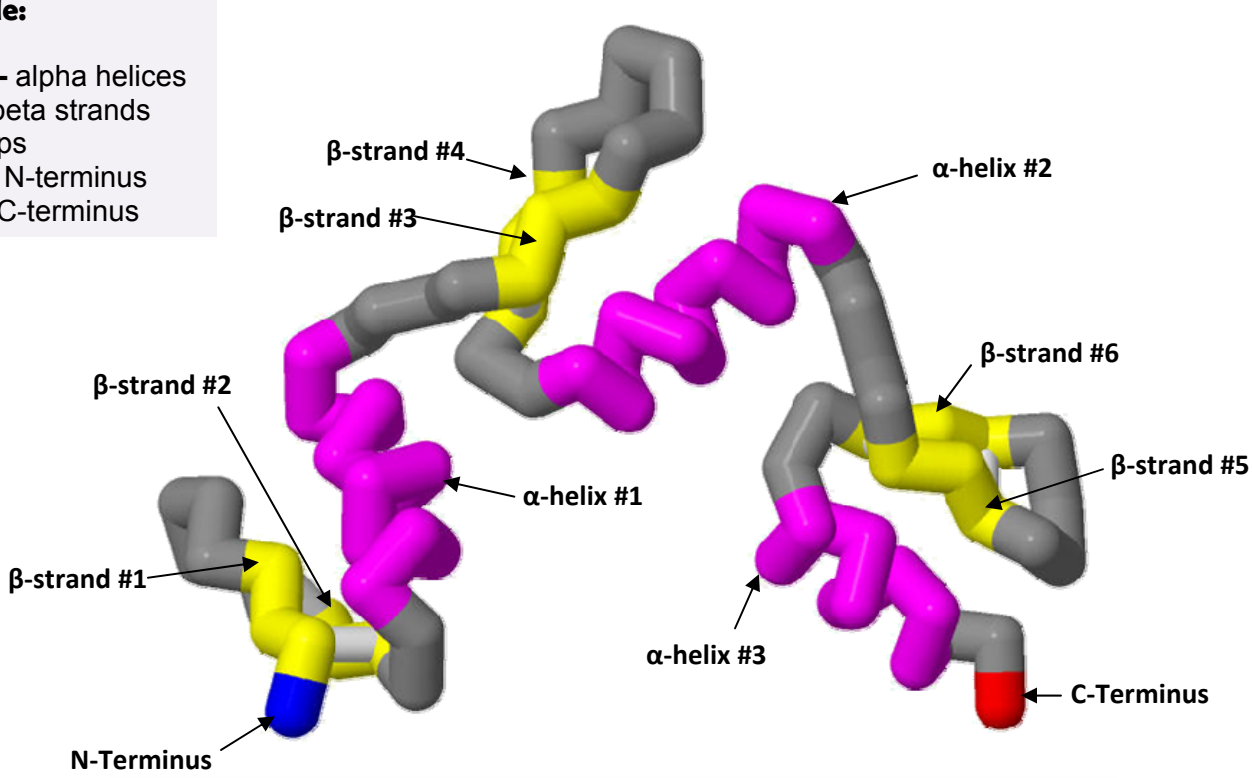
Guide to Using the Rubric to Score the Klf4 PREBUILD Model for Science Olympiad 2010-2011 National Competitions

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 2011 Science Olympiad **National Pre-Build** Mini-Toober models of Klf4, based on **2wbu.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 Molecule:

Color Code:

Magenta – alpha helices
Yellow – beta strands
Gray - loops
Blue tip – N-terminus
Red tip – C-terminus

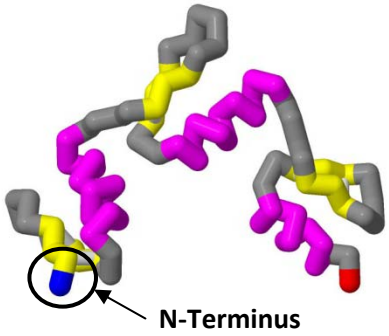


Order of Secondary Structures:

- N-terminus → β-strand #1 → turn
- β-strand #2 →Turn
- α-helix #1 →turn
- β-stand #3 →turn
- β-strand #4 →turn
- α-helix #2 →turn
- β-strand #5 →turn
- β-strand #6 → turn
- α-helix #3 →C-terminus

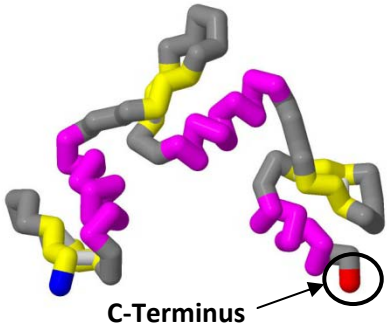
1. Blue Cap on N-terminal Amino Acid (Thr399) (0.5 pt)

- To receive credit, the blue cap needs to be located at the N-terminus of the protein, which is located at the beginning of beta strand #1. Please see the figure to the right for the correct positioning of the blue end cap.
- If the blue end cap is by a helix, then the model does not receive credit for this feature.



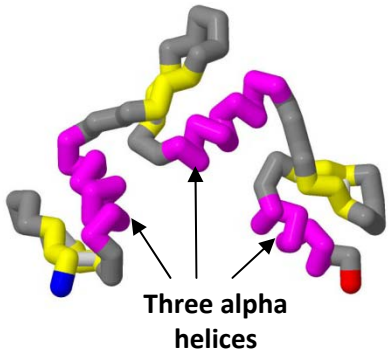
2. Red Cap on C-terminal Amino Acid (Phe483) (0.5 pt)

- To receive credit, the red cap needs to be located at the C-terminus of the protein, which is located at the end of helix #3. Please see the figure to the right for the correct positioning of the red end cap.
- If the red end cap is positioned on a β-strand, then the model does not receive credit for this feature.



3. Model has 3 alpha helices according to Jmol selection criteria (0.5 pt per helix for a total of 1.5 points)

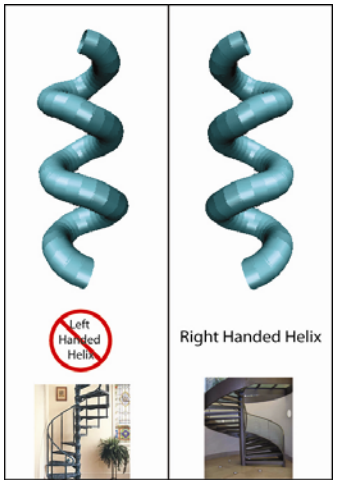
- To receive these points, there should be 3 helices within the model. Please see figure to the right for the correct location of these helices. **(Helices are colored magenta on the model and on the figure to the right.)**
- Deduct 0.5 points for each extra helix. For example, if a model has 4 helices,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



4. 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. Pretend that you are climbing that staircase and you need to have a hand-rail and the helix is the hand-rail, which is always on the outside edge of the staircase. If you would put your right hand on the toober as you go up the staircase, you have a right-handed helix. If you would put your left hand on the toober, you have a left-handed helix and the modeled helix would not receive credit.



Left-handed vs right-handed helices

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

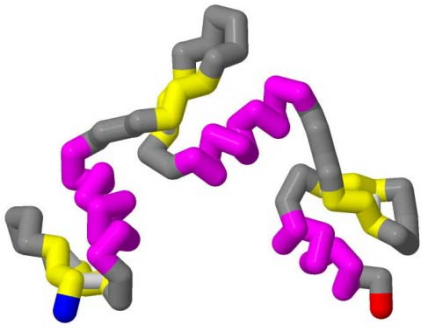
- Helix #1, Helix #2 and Helix #3 are approximately the same length. Each helix should have approximately 3-4 turns. Each helix that is the correct length should receive 0.5 points, for a total of 1.5 points.

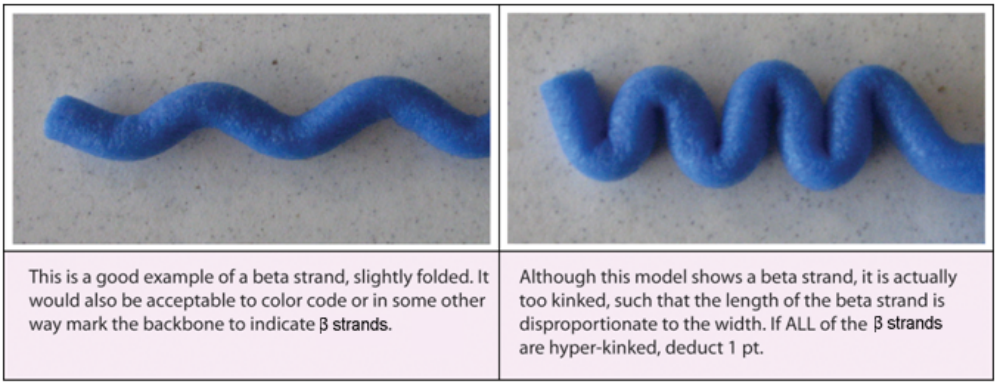
6. Model has 6 β-strands according to Jmol selection criteria (0.5 pt per β-strand for a total of 3 points)

- To receive these points, there should be 6 β-strands within the model. Please see figure to the right for the correct location of these beta strands. **(β-strands are colored yellow on the model and on the figure to the right.)**

- 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 beta strands to distinguish them from loops or write on the toober indicating the location of the β-strands. Please see photos below depicting β-strands modeled using toobers.

- If there are more than 6 β-strands in the model, 0.5 pt should be deducted for each extra strand. For example, if the model has 7 β-strands, the model should receive 2.5 points, rather than the full 3 points.

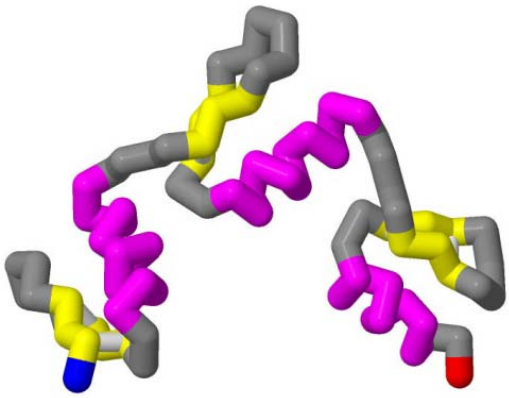




This is a good example of a beta strand, slightly folded. It would also be acceptable to color code or in some other way mark the backbone to indicate β strands.	Although this model shows a beta strand, it is actually too kinked, such that the length of the beta strand is disproportionate to the width.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

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

- To receive these points, the sequence of the secondary structures should be in the following order:
  - N-terminus→ β-strand #1→Turn
  - β-strand #2→ Turn
  - α-Helix #1 → Turn
  - β-strand #3→Turn
  - β-strand #4→ Turn
  - α-Helix #2 → Turn
  - β-strand #5→Turn
  - β-strand #6→ Turn
  - α-Helix #3 → C-terminus



- Each secondary structure (helix or β-strand), 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.
- Please refer to the figure at right, or to the physical model, to assist in scoring the model.

Items #8 - #12 refer to the 3-dimensional shape of the model.

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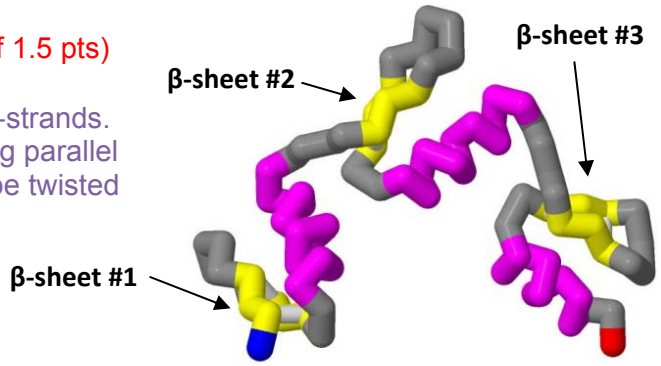
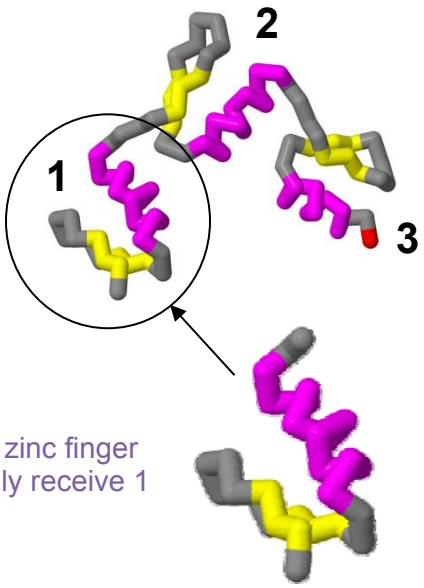
8. Model has 3 β-sheets (0.5 pt for each sheet for a total of 1.5 pts)

- This protein has two β-sheets arranged from the 6 β-strands. A β-sheet is composed of two or more β-strands lying parallel to each other, though the plane of the β-sheet may be twisted instead of flat.
- β-sheet #1 is composed of β-strands #1 and #2.



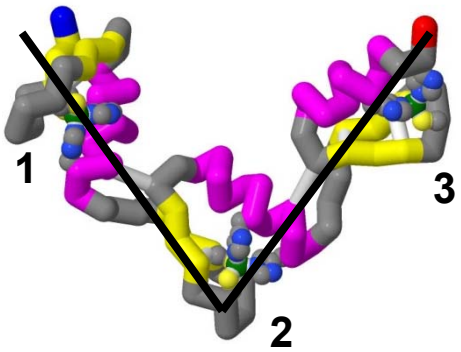
- β-sheet #2 is composed of β-strands #3 and #4.
- β-sheet #3 is composed of β-strands #5 and #6.
- To receive credit, the model should have three distinct β-sheets.
- If the model has more than three β-sheets, then deduct 0.5 pt for each additional sheet. For example, if the model has four β-sheets, the model should receive 1 pt, rather than the full 1.5 pts.

9. Model has three distinguishable zinc fingers
(0.5 pt each; for a total of 1.5 pts)



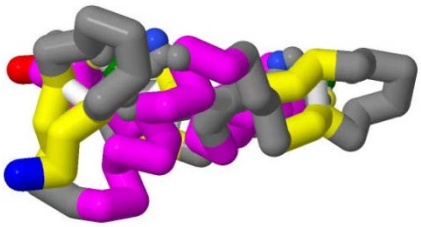
- A zinc finger is a common structural motif stabilized by the presence of a zinc ion. In Klf4, this motif is characterized by 1 helix and 1 sheet. Please see figure to the right illustrating a single zinc finger motif found in the Klf4 protein.
- To receive these points, the model should have 3 distinguishable zinc finger motifs. Each zinc finger present in the model should receive 0.5 pt, up to 1.5 pts. Please see physical model and figure to the right to identify the three zinc fingers.
- If a model has more than 3 zinc fingers, deduct 0.5 for each additional zinc finger motif. For example, if a model has 4 zinc fingers, the model should only receive 1 pt, rather than the full 1.5 pts.

10. Zinc fingers are arranged to form a "V" shape (1 pt)



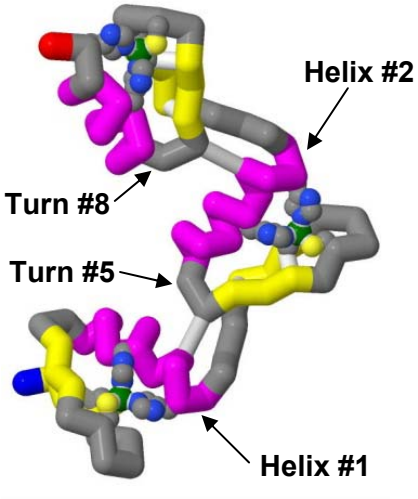
- Hold the model so that the N and C-termini are pointing upward. Pleas see figure to the right for proper orientation.
- The three zinc finger motifs should be arranged so that the model has a "V" shape. Please see physical model or image on the right.
- The N-terminus and the C-terminus should form the tips of the "V", with the 2nd zinc finger forming the apex of the angle.
- If a model has all three zinc fingers in a straight line, the model will not receive the points. If the N and C termini are directly adjacent to one another (forming a hairpin loop, rather than a V), the model will not receive credit for this feature.

11. The protein is compact and flat (1pt)



- The Klf4 protein is arranged such that the three zinc fingers lay in the same basic plane (see physical model or figure to the right).
- If you place the model on a flat surface with the N-terminus positioned facing toward you and the C-terminus is behind the N-terminus (see figure above), then the model should not have any portions of the protein extending far above the table or cause the model not to lie flat on the table.
- If a model has regions that project above or below the table when lying on the surface, the model should not receive credit for this feature.

12. The zinc fingers are positioned correctly next to one another (2 pts)



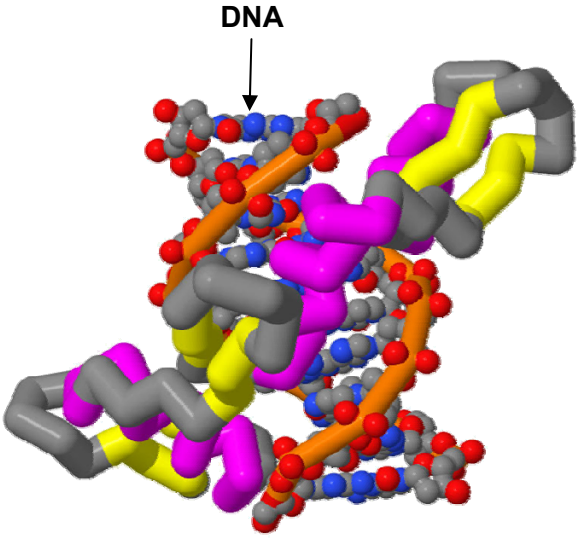
- The zinc fingers are positioned so that the turn between the sheet and helix of each zinc finger are pointing towards the helices of the previous motif.
    - Turn #5 should be pointing towards helix #1
    - Turn #8 should be pointing towards helix #2
- Award 1 pt for each correctly positioned zinc finger.

13. Students submitted a 3x5 card with explanation of the model (2 pts)

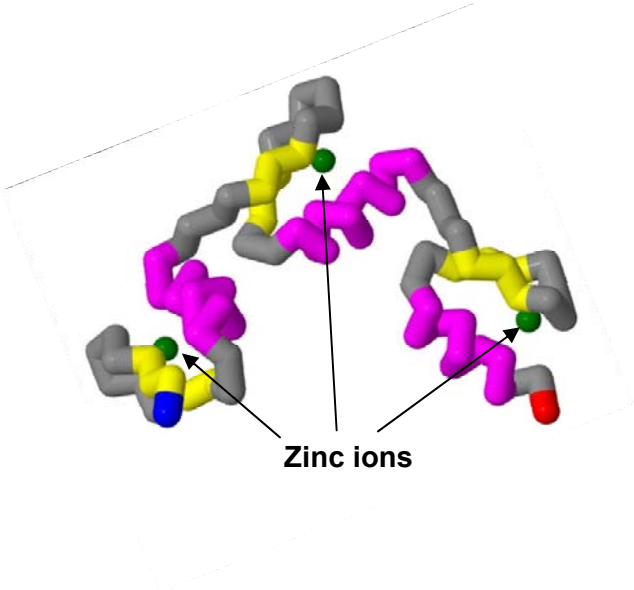
- The 3x5 card submitted with the model should describe the model in terms of what additional features have been added to the model so that the judge is not left guessing what the model represents.

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

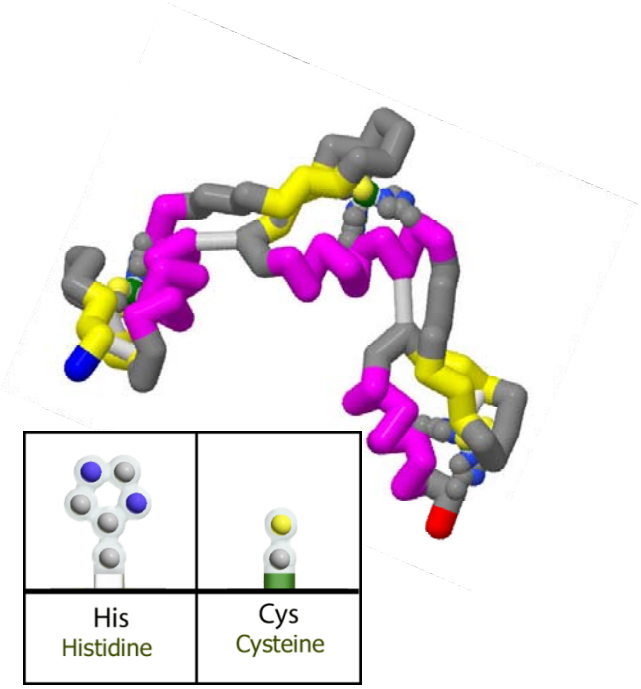
- **DNA** (4 pts)
    - Klf4 is a transcription factor, which means that it has the ability to bind to DNA. Teams may elect to add DNA to their model of Klf4 to reflect this function. The PDB file (2wbu.pdb) that was used to develop the models includes data on how Klf4 interacts with DNA. Please see the figure to the right, which is based on the PDB file 2wbu, to see how the protein interacts with the DNA.
    - Klf4 binds in the major groove of the DNA.



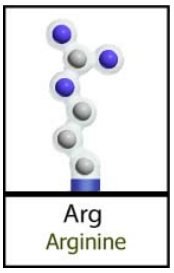
- **Zinc Atoms** (4 pts)
    - The zinc finger motif is stabilized by the presence of a zinc ion. Teams may elect to include zinc atoms in their model to illustrate the stabilizing property of this ion. On the figure to the right, and on the physical model, the zinc ions are shown in spacefill and are colored green.
    - Each finger should have a zinc ion associated with it. To receive full credit, the model must display all three zinc ions. If only one or two atoms are displayed, deduct 2 pt.

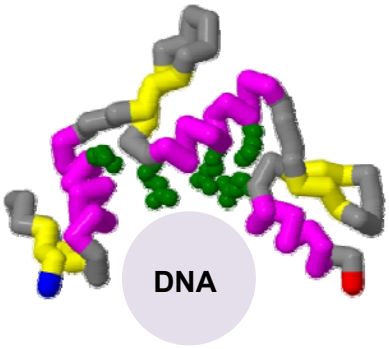


- **Zinc Atom Coordination** (4 pts)
    - The zinc ions are held in place through coordination with sidechains of the protein. In Klf4, the zinc ions interact with two histidine and two cysteine sidechains in each zinc finger motif.
    - To receive credit, each finger should have 2 histidine and 2 cysteines sidechains displayed and interacting with the zinc ion.
    - Please see the figure to the right, and the physical model, to see where these sidechains are positioned relative to the zinc ions.



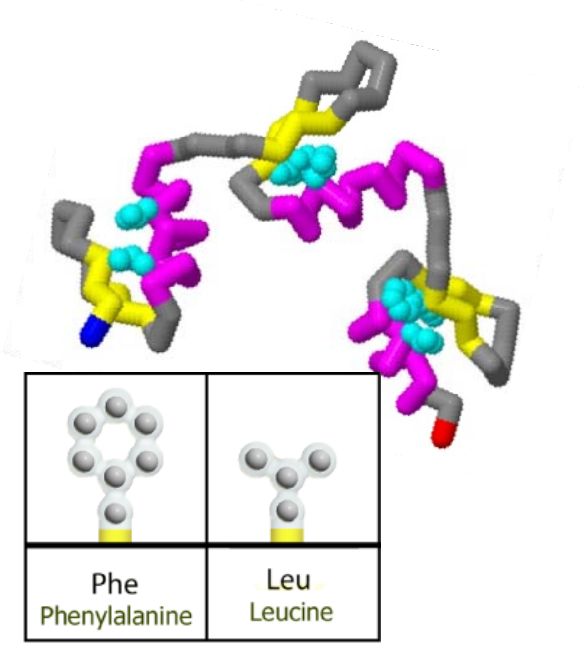
- **Sidechains that interact with DNA** (4 pts)
    - In order to function as a transcription factor, Klf4 has to interact with the DNA; this is accomplished through sidechain interaction with the nucleic acid.
    - Award 1pt to the model if the model has at least 2 arginine residues positioned on the Klf4 protein that would enable the sidechains to interact directly with the DNA. Arginine is shown in green in the figure to the right.





    - Most frequently, this arginine is positioned at the top of the helix. Klf4 has 3 arginine residues on the 2nd zinc finger on the helix that interact with the DNA. The model may have as many as 5 arginines indicated, but the model should have at least 2 to receive this point.

- **Sidechains that stabilize the structure of the protein** (4pts)
    - Hydrophobic amino acids stabilize the fold of this protein.
    - Award 1 pt if the model has a hydrophobic core shown on the 3 zinc fingers – this core is typically composed of at least one phenylalanine and one leucine residue. These amino acids should be positioned to stabilize the alpha helix near the beta sheet. Please see figure to the right (hydrophobic amino acids shown in cyan).



- Additional features that are not listed above may be added to the protein. It is up to the event supervisor to determine whether the addition to the model is appropriate and accurate. A model cannot receive more than 16 pts total for this section.

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

- To receive credit for this feature, the creative additions need to be relevant to telling the functional story of the protein. Credit should be awarded to those proteins that meet the following criteria:
    - Model has creative additions
        - Models that are just the toober will not receive credit
    - Additions are appropriate to the function of the protein
        - Models that have ALL sidechains displayed should not receive credit. 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, credit should not be awarded if amino acids outside of those that play a specific functional role in the protein are displayed.