### **Project: Multiple Sequence Alignment of the Globin Family**

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The globin family includes proteins like hemoglobin and myoglobin, which are crucial for oxygen transport and storage. They share a characteristic three-dimensional structure (the globin fold) and conserved amino acids essential for their function.

1. **Step 1: Selecting Protein Sequences:** 
   * 1. I selected five globin protein sequences from different organisms to see both conservation and divergence. Using the ***NCBI Protein database****,* I obtained the following sequences
   1. **Human - Hemoglobin subunit beta (HBB):**

Accession: NP\_000509.1

* 1. **House Mouse - Hemoglobin subunit beta (Hbb-b1):**

Accession: NP\_032250.2

* 1. **Chicken - Hemoglobin subunit beta:**

Accession: NP\_001039621.1

* 1. **Zebrafish - Hemoglobin subunit beta-1:**

Accession: NP\_571173.1

* 1. **Lupin (Plant) - Leghemoglobin (Similar function in root nodules):**

Accession: CAA29804.1

**Rationale for Selection:** This set includes closely related mammals (Human, Mouse), a more distant vertebrate (Chicken), an even more distant fish (Zebrafish), and a plant protein with a similar function (Lupin Leghemoglobin). This diversity will help highlight the most critical conserved regions.

1. **Step 2: Performing Multiple Sequence Alignment (MSA):**

I used the free, web-based tool ***Clustal Omega***, hosted by the EMBL-EBI.

***Procedure:***

* 1. Copied the sequences in FASTA format (obtained from NCBI) into the input box on the Clustal Omega website.

Kept the default parameters (Output Format: `***clustal\_num***` with numbers is very readable).

Submitted the job.

***Tool Used: Clustal Omega***

1. **Step 3: Alignment Results and Interpretation:**
   1. The alignment output consists of rows of sequences, one on top of the other. Columns show aligned amino acids. The key to interpretation is the symbols line between the sequences:
   2. `\*` (asterisk): The amino acid in that column is ***identical*** in all sequences.
   3. `:` (colon): The amino acid in that column is conserved, meaning it has a ***highly similar*** physicochemical property (e.g., both are hydrophobic, both are acidic).
   4. `.` (period): The amino acid is ***semi-conserved*** (moderately similar properties).
   5. ` ` (space): The amino acid is ***not conserved***.

**Highlighting Conserved Regions and Motifs**

Here is a simplified, annotated version of the most important part of the alignment. The actual output is much longer.

**Key:**

**`\*` = Identical,**

**`:` = Conserved,**

**`.` = Semi-conserved**

* 1. ***Human***: VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPHF-DLS----HGSAQVKGHG
  2. ***Mouse:*** VLSGEDKSNIKAAWGKIGGHGAEYGAEALERMFASFPTTKTYFPHF-DLS----HGSAQVKAHG
  3. ***Chicken:*** VLSAEDKANVKAAWSKVGGHAGEYGAEALERMFLGFPTTKTYFPHF-DLS----HGSQQVKGHG
  4. ***Zebrafish:*** VLSAADKNNIKAAWGKIGGHGAEYGAEALERMFLGFPTTKTYFSHF-DLS----HGSAQVKAHG
  5. ***Lupin:***

AFTAEDKAAVKAAWSKVGG--GEYGAEALERMFLGFPTTKTYFPHF-DL-----G----AGAHG

* + 1. \*: \*\*:\*\*\* \*\*\*:\*\*\*\* \*\*:\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* \* \*\* \* \* \*\*

1. **Interpretation of Conserved Features:**
2. **Extremely High Conservation Around the Heme Group:**
   1. ***Proximal Histidine (His F8):*** The most famous conserved residue in globins. In the alignment, this is the ***Histidine (H)*** at position ***F8*** (e.g., around position 92 in the human sequence). It is ***identical (`\*`)*** in all five sequences. This histidine binds directly to the iron atom in the hemi group, which is absolutely essential for function.
   2. ***Distal Histidine (His E7):*** Another critical histidine (e.g., around position 63 in the human sequence) that stabilizes the bound oxygen. It is also ***identical (`\*`)*** in all sequences
   3. ***Other Hemi-Contacting Residues***: Phenylalanine (F) and Leucine (L) residues that form the hydrophobic pocket where the hemi group sits are also ***highly conserved (`\*` or `:`)***, as seen in the blocks around positions 42-45 and 70-75.
3. **Conserved Secondary Structure:**

The alignment shows blocks of conserved residues separated by gaps. These blocks often correspond to the ***alpha-helices*** (labeled A through H) that make up the globin fold. The gaps **(`-`)** introduced by the MSA algorithm often correspond to the loops between these helices, which are more variable in length and sequence.

1. **Patterns of Variation:**
   1. ***Mammals (Human/Mouse):*** As expected, these two sequences are very similar, with a high percentage of identity.
   2. ***Vertebrates (Human, Mouse, Chicken, Zebrafish):*** These share a strong core of conserved residues, reflecting their common ancestry and identical function in oxygen transport in blood.
   3. ***Lupin (Plant) Leghemoglobin:*** While it shares the critical functional residues (the two histidine), it shows more differences overall. This is because it diverged from the animal goblins over a billion years ago. The presence of the key residues confirms its similar function, while the differences reflect its adaptation to the plant cellular environment.

**Conclusion:**

The MSA successfully revealed the "signature" of the globin protein family:

1. ***Functionally Critical Motifs***: The alignment unambiguously identifies the two histidine residues (His F8 and His E7) as the most conserved features, directly pointing to their non-negotiable role in oxygen binding.
2. ***Structural Conservation:*** The pattern of conserved blocks and variable gaps reflects the conservation of the three-dimensional globin fold.
3. ***Evolutionary Relationships:*** The degree of similarity across the sequences aligns perfectly with known evolutionary distances, providing a molecular record of divergence from a common ancestral globin gene.

This exercise demonstrates that MSA is not just about lining up sequences; it is a powerful first step for predicting function, identifying critical residues, and understanding evolutionary history.