Sickle Cell Mutation, Protein Structure and Chromosomal Location of Gene

**Instructions: Complete the worksheet. This is an INDIVIDUAL assignment.**

Learning objective:

At the end of this worksheet, students should be able to:

* Use and identify structures within the protein sequence
* Identify the location of specific amino acids
* Identify information regarding the genetics, inheritance and hemoglobin structure

Sickle cell anemia is caused by a DNA point mutation (a missense mutation) in the ß-globin gene that leads to an alteration in the amino acid sequence (E6V - after processing (removal) of the N-terminal methionine). In 1949, Linus Pauling described sickle cell anemia as the first “molecular disease” (Pauling *et al*., 1949), ushering in the age of molecular medicine. Initial characterization of the hemoglobin polypeptides associated with sickle cell anemia indicated that there was a charge difference, detected as a change in the electrophoretic mobility of the ß-globin polypeptide.

The hemoglobin protein, with a mutant ß-globin (sickle cell allele), binds normally to O2, but when the O2 is released (deoxyhemoglobin), the protein sticks together in the cell. This leads to changes in red blood cell shape and can cause additional problems in the individual. Oxygenation leads to a return to normal cell shape.

Before continuing, review general protein structure information.



Image is from <http://www.ncbi.nlm.nih.gov/Structure/mmdb/mmdbsrv.cgi?uid=1183>

1. Look at the tertiary structure of α globin subunit (1hho.pdb) by going to the website <http://bioinformatics.org/firstglance/fgij//fg.htm?mol=http%3A//www.umass.edu/molvis/bme3d/materials/structures/1hho_a.pdb.gz&>.
	1. The image that you are looking at (as long as the program needed to view the image is working on your computer) should be rotating. This is a part of the crystal structure of hemoglobin (Shaanan, 1983).
		1. You will see the tertiary structure of the polypeptide, including alpha-helical secondary structures.
		2. You should also see the heme (gray) prosthetic group and iron (brown) ion associating with the globin subunit.
	2. In the top left panel, click on the ‘views’ button.
	3. Then click on the ‘cartoon’ button and you will see the α helical domains of the polypeptide. This is a version of the polypeptide, with the amino acid side chains stripped away and focusing on the secondary structure.

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| How many alpha-helical domains are shown? | 7-8 (depending on size of alpha-helix) |
| What types of bonds stabilize alpha helices and what structures interact? | H-bonding between backbone structures |

* 1. Click on the ‘solid’ button to see what the polypeptide looks like with the amino acid side chains present.
	2. Click on the ‘charge’ button.

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| What do the different colors on the surface of the polypeptide represent? | The different colors represent different charges on the side chains. Blue is basic (+) and red is acidic (-). |

* 1. Click on ‘tools’ and then ‘salt bridges’. There should be three red and three blue spheres. These represent ionic interactions in the polypeptide chain. *Stop the ‘spin’ of the molecule by clicking on the ‘spin’ box.*

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| Click on a blue sphere and find Lys7. With what amino acid does Lys7 interact (click on red sphere)? | Asp74 |
| Find Glu116. With what amino acid does this side chain interact?  | Lys16 |
| Find Arg31. With what amino acid does this side chain interact? | Glu27 |

1. Look at the quaternary structure of hemoglobin by going to https://bioinformatics.org/firstglance/fgij//fg.htm?mol=http%3A//www.umass.edu/molvis/bme3d/materials/structures/1hho\_quat.pdb.gz&. (*The image should pop up. If an error message occurs, click until the image appears.)* This structure is oxyhemoglobin (hemoglobin bound to oxygen molecules).
	1. Clicking on ‘views’ you can look at the secondary and cartoon structure as you did with the alpha-globin polypeptide.
	2. Click on ‘hydrophobic/polar’ to view the surface of the protein. While on this view, click ‘find’ and search for Glu6. *Hint: Click ‘Spin’ to stop the rotation of the structure. You can manually rotate using your mouse.*

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| On the protein, there should be 2 locations marked that correspond to Glu6, both in the ß-globin subunits (there are 2). Do these residues appear to be on the surface? | Yes |
| For sickle cell anemia, this amino acid is altered to valine. How does this change the surface of the protein? | Introduces a hydrophobic residue onto the surface where there once was a hydrophilic, charged amino acid. |

1. Why do scientists believe that the sickle cell anemia allele has persisted in the population? *To answer this question, please watch the 15 min video found at* [*http://www.hhmi.org/biointeractive/making-fittest-natural-selection-humans*](http://www.hhmi.org/biointeractive/making-fittest-natural-selection-humans)*.*

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**Reference**:

Pauling, L., H.A. Itano, S.J. Singer, and I.C. Wells. 1949. Sickle cell anemia, a molecular disease. Science **110**: 543-548.

Shaanan, B. 1983. Structure of human oxyhaemoglobin at 2.1 Å resolution. J. Mol. Biol. **171**: 31.

**Online Mendelian Inheritance in Man**

Online Mendelian Inheritance in Man (OMIM) is a website (<http://www.omim.org>) that is continuation of volumes of work compiled by Victor McKusick and has been maintained online. As of August 2018 there are more than 24,000 entries, including almost 16,000 genes and over 8,000 phenotypes. OMIM provides a resource to find out much about the nature and cause of diseases (when known) as well as information about genes that may influence disease. The database has been organized (and revised) as shown in Table 1 and further annotation of the entries is shown in Table 2.

Table 1. Organization of OMIM entries by the first number (of a six-digit identification number).

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| **OMIM first number** | **Category** |
| 1, 2 | Autosomal locus or phenotypes (created before May 15, 1994) |
| 3 | X-linked locus or phenotypes |
| 4 | Y-linked locus or phenotypes |
| 5 | Mitochondrial locus or phenotypes |
| 6 | Autosomal locus or phenotypes (entries created after May 15, 1994) |

Table 2. Additional notation on the OMIM entries.

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| **Symbol before an entry** |  |
| Asterisk (\*) | Indicates a gene |
| Number symbol (#) | A descriptive entry, usually a phenotype (does not represent a unique locus) |
| Plus sign (+) | Entry contains the description of a gene of known sequence and phenotype |
| Percent sign (%) | The entry describes a confirmed Mendelian phenotype or phenotypic locus, but the molecular basis is unknown |

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Sickle cell anemia has an entry in OMIM. You can gain much information from this entry.

1. Go to the OMIM website (<http://www.omim.org>) and search for the sickle cell anemia entry. Enter ‘sickle cell anemia’ and search the database. The first entry should be the disease entry.
2. You can obtain some basic information regarding the disease and locus from this page.

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| What is the OMIM number for sickle cell anemia? | 603903 |
| What is the chromosomal location associated with the disease (in this case, the ß-globin gene)? | 11p15.4 |
| What does the first number indicate? | Chromosome 11 |
| What does the letter indicate? | The p (petite/short) arm of the chromosome |
| What is the OMIM number of the gene (HbB) associated with this disease? | 141900 |
| What is the name of the first person reported to have been diagnosed with sickle cell anemia? | Walter Clement Noel |

1. Near the top of the OMIM entry for sickle cell anemia, there is a link (top right) for the protein. Click on ‘Protein’ and then ‘Uniport’ to get another window to open. On this page (Uniport), you can find more information about the HbB polypeptide.

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| What are the two amino acid residues (positions) that are responsible for binding heme? | His64, His93 |
| What are the four amino acid residues (positions) that are responsible for binding 2,3 bisphosphoglycerate? | 2, 3, 83, 144 |