

Class 1 Lab *

Bioinformatics Databases and Key Online Resource

Barry Grant

Version 220919

Instructions

Save this document to your computer and open it in a PDF viewer such as Preview (available on every mac) or Adobe Acrobat Reader ([free for PC and Linux](#)). Be sure to add your name and UC San Diego personal identification number (PID) and email below before answering all questions in the space provided.

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Overview:

The purpose of this lab session is to introduce a range of bioinformatics databases and associated services available online whilst investigating the molecular basis of a common human disease.

Sections [1](#) and [2](#) deal with querying and searching GenBank, GENE and OMIM databases at **NCBI**. Sections [3](#) and [4](#) provide exposure to **EBI** resources for comparing proteins and visualizing protein structures. Finally, section [5](#) provides an opportunity to explore these and other databases further with additional examples.

Side-note: The Web is a dynamic environment, where information is constantly added and removed. Servers “go down”, links change without warning, etc. This can lead to “broken” links and results not being returned from services. Don’t give up - give it a second go and try a search engine using terms related to the page you are trying to access.

*<http://thegrantlab.org/teaching/>

Section 1

The following transcript was found to be abundant in a human patient's blood sample.

```
>example1
```

```
ATGGTGCACTGACTCCTGTGGAGAAGTCTGCCGTTACTGCCCTGTGGGCAAGGTGAACGTGGATGAAG  
TTGGTGGTGAGGCCCTGGCAGGCTGCTGGTGGTCAACCTTGGACCCAGAGGTTCTTGAGTCCTTGG  
GGATCTGCACTCCTGATGCAGTTATGGGCAACCCTAACGGTAAGGCTCATGGCAAGAAAGTGCCTCGGT  
GCCTTAGTGTGATGGCCTGGCTCACCTGGACAACCTAACGGCACCTTGCCACACTGAGTGAGCTGCACT  
GTGACAAGCTGCACGTGGATCCTGAGAACTTCAGGCTCTGGCAACGTGCTGGTCTGTGCTGGCCA  
TCACCTGGCAAAGAATTCAACCCCACCAAGTGCAGGCTGCCTATCAGAAAGTGGTGGCTGGTGTGGCTAAT  
GCCCTGGCCCACAAGTATCACTAAGCTGCTTCTGCTGTCCAATT
```

The only information you are given is the above sequence so you must begin your investigation with a sequence search - for this example we will use NCBI's **BLAST** service at: <http://blast.ncbi.nlm.nih.gov/>

Side-note: There are several different "basic BLAST" programs available at NCBI (including nucleotide BLAST (**BLASTn**), protein BLAST (**BLASTp**), and translated nucleotide nucleotide (**BLASTx**)). We will explore using all of these in the coming sections.

Q1 Which BLAST program should we use in this case?

Tip

What type of sequence are you provided with?

[Nucleotide Blast \(BLASTn\)](#)

Searching against the "**Nucleotide collection**" (**NR database**) that includes GenBank is a good place to start your investigation of this sequence.

Q2 What are the names and accession numbers of the top four hits from your BLAST search?

1. *Homosapiens hemoglobinsubunitbeta (HBB)*, mRNA, NM_000518.5
2. *PREDICTED: Pan paniscus hemoglobin subunit beta (LOC100976465)*, mRNA, XM_003819029.5
3. *PREDICTED: Pan troglodytes hemoglobin subunit beta (HBB)*, mRNA, XM_508242.5
4. *Homo sapiens hemoglobin beta chain variant Hb S-Wake (HBB)* mRNA, complete cds, AY136510.1

Q3. What are the percent identities, coverage and E-values for these top few hits?

Tip

These three metrics (E-value, coverage and identities) are the most important for us to consider at this stage. I suggest you have a discussion with your neighbor and Barry to make sure you have a firm grasp of these concepts as you will need them later in your project.

1. percent identities: 99.57% Coverage: 100% E-value: 0.0
2. percent identities: 99.36% Coverage: 100% E-value: 0.0
3. percent identities: 99.36% Coverage: 100% E-value: 0.0
4. percent identities: 99.36% Coverage: 100% E-value: 0.0

To investigate these results further click on the **Alignments** section (tab) of your BLAST result page (Figure 1). This will give you more details on matched nucleotides and important links to “Related information” about a given “*subject sequence*”.

Side-note: In BLAST terminology we talk about *query sequence* and *subject sequence*. The *query* being the input sequence you searched with and the *subject* being the identified hit sequence from the database.

Figure 1: The BLAST Alignemnts tab contains more detailed information about your results

Q4. How many identical and non identical nucleotides are there in your top hit compared to your last reported hit?

Tip

Scroll down to the end of the Alignments page to lower ranked hits?

Top Hit: Identical: 466/468(99%), non identical: 2
 Last hit: Identical: 401/438(92%), non identical: 37

From the results of your BLAST search you can link to the **GENE** entry for one of your top hits. This link is located under the “Related Information” heading at the right hand side of each displayed alignment on the “Alignments” tab (Figure 1).

Q5. What is the “Official Symbol” and “Official Full Name” for this gene?

HBB, hemoglobin subunit beta

Q6. What chromosome is this gene located on?

chromosome 11

Note that there is a rather basic schematic diagram of neighboring genes and their orientations in the “Genomic context” section that you can use for answering the next question (Figure 2).

Our HBB gene is in maroon. All other gene arrows can be hovered over for full names and clicked on to link to that specific GENE page to find out more. We will explore more full featured genome browsers at ENSEMBLE and UCSC in an upcoming lab but basically it is the same idea here in a more simplified form.

Q7. What are the names of neighboring genes on this chromosome?

The genes near HBB(beta-globin) on chromosome 11 include HBD(delta-globin), HBG1, HBG2 (gamma-globins), and HBE1 (epsilon-globin). These are all part of the beta-globin gene cluster.

Q8. How many exons and introns are annotated for this gene?

Genomic context

Location: 11p15.4 See HBB in [Genome Data Viewer](#)

Exon count: 3

Annotation release	Status	Assembly	Chr	Location
110	current	GRCh38.p14 (GCF_000001405.40)	11	NC_000011.10 (5225464..5227071, complement)
110	current	T2T-CHM13v2.0 (GCF_009914755.1)	11	NC_060935.1 (5284832..5286439, complement)
105.20220307	previous assembly	GRCh37.p13 (GCF_000001405.25)	11	NC_000011.9 (5246694..5248301, complement)

Chromosome 11 - NC_000011.10

[5199735 ➤ ➤ 5239383]

OR51V1 ← LOC107133510 → HBD ←
HBB-3'HS1 → LOC110006319 → LOC106099063 →
LOC106099062 → HBB ← LOC109951029 →

Figure 2: NCBI GENEs Genomic context section indicating location, structure and neighboring genes

There are 3 exons and 2 introns.

Q9 What is the function of the encoded protein?

Tip

In addition to reading the abstract like text on a given GENE entry I encourage you to explore the linked “**Gene Ontology**” information and discuss with your neighbor and Barry the advantages you think such controlled terms might have over free text?

The HBB gene is involved in making the b-globin part of hemoglobin, which helps red blood cells carry oxygen from the lungs to the body. Mutations can cause blood disorders like sickle cell disease.

Q10. Does the protein have a role in human disease(s)? If so what diseases?

Tip

Scroll down to the “Phenotypes” section of the GENE entry page and also explore the link to the **OMIM** database

Yes. Mutations in HBB cause sickle cell disease, beta-thalassemia, and related blood disorders.

Section 2

By now you should be aware that there are a number of human diseases linked to particular variants of the beta-globin gene. In this case our example sequence corresponds to human sickle cell beta-globin mRNA with this disease resulting from a point mutation in the beta globin gene. In the following section, you will compare sickle cell and normal beta globin sequences to reveal the nature of the sickle cell mutation at the protein level.

To do this you need to find at least one sequence representing the normal beta globin gene. Open a new window and visit the NCBI home page (<http://www.ncbi.nlm.nih.gov>) and select “Nucleotide” from the drop menu associated with the top search box. Then enter the search term: **HBB** (Figure 3).



Figure 3: The main Google like search tool for searching across all NCBI databases is called **Entrez**. Note that productive use often requires the use of additional “filters” as we will explore later.

Note that lots of often irrelevant results are returned so lets apply some “Filters” (available by clicking in the left-hand sidebar) to focus on **RefSeq** entries (under “Source databases”) for **Homo sapiens** (under “Results by taxon” on the right-hand sidebar in this later case).

Side-note: Boolean operators (NOT, AND, OR) as well as fielded queries (i.e. “HBB[Gene Name] AND Human[Organism]”) can be used directly in ENTREZ searches to filter results for more efficient searching.

Remember that we are after mRNA so we can compare to the mRNA sequence from section 1 above.

Q11. What is the ACCESSION number of the “Homo sapiens hemoglobin, beta (HBB), mRNA” entry?
[NM_000518.5](#)

Select “Homo sapiens hemoglobin, beta (HBB), mRNA” from the results and scroll down to the “FEATURES” section to answer the following. You can also find some of this same information from selecting the “GRAPHICS” display format and, for example, placing your mouse over the first exon (see Figure 4).

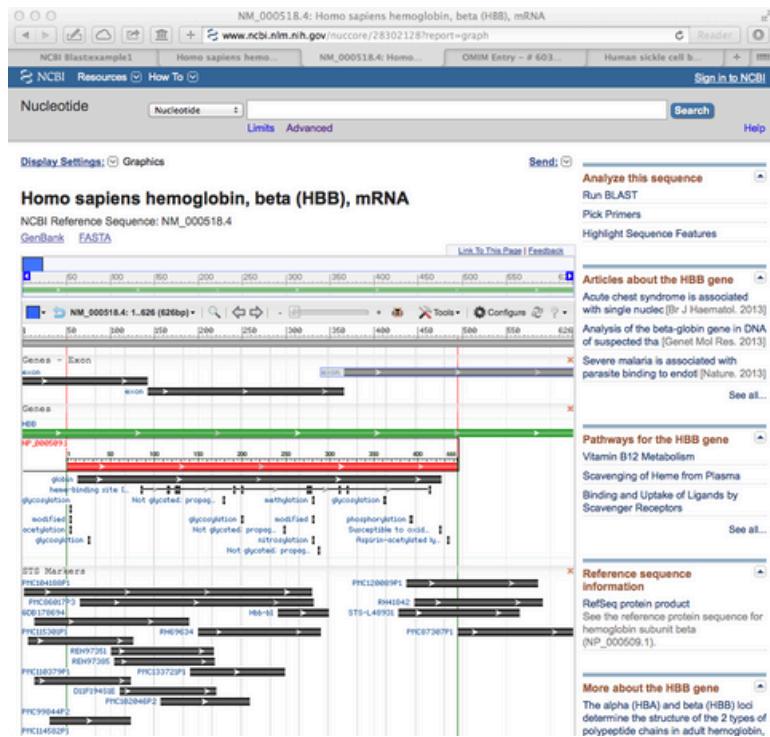


Figure 4: The GRAPHICS view of a GenBank entry can be more user friendly than the traditional text of the corresponding GenBank format display.

Q12. What are the numbers of the first and last base positions of exon 1 of this entry?

1 to 142

Q13. What are the numbers of the first and last base positions of the CDS?

Tip

CDS or “coding sequence” refers to the portion of a genomic DNA sequence that is translated, from the start codon to the stop codon. Successful translation of a CDS results in the synthesis of a protein.

positions: 51 to 494

Section 3

Here we will compare the retrieved sequences by creating a sequence alignment. This will make the difference between the two sequences easy to spot.

To generate the alignment, we will use **MUSCLE** available on the EBI website at: <http://www.ebi.ac.uk/Tools/msa/muscle/>

Select the FASTA display for the “Homo sapiens hemoglobin, beta (HBB), mRNA” (NM_000518) entry from section 2.

Now copy-and-paste this FASTA format sequence and also the **example1** sequence from section 1 into the input box of the **MUSCLE** page. Then click the submit button (see red circle in Figure 5).

Side-note: If your alignment is incomplete, please wait until the page refreshes.

If the job appears to be in an undefined state try clicking refresh until a result is returned.

The two sequences should now be aligned. Where the aligned sequences are identical, an * is placed under the alignment. Examine the results and note that your sequences are nearly identical. However, being much shorter, the sickle cell sequence has many padding gap characters (-----) to bring equivalent regions into the correct register (Figure 6).

When inspecting alignments (especially those with lots of sequences) it can be helpful to use a **graphical user interface** (or GUI) to display colored, interactive and scrollable versions of your alignment. One such GUI is the **seaview** program.

From your muscle results web page click **Download Alignment File** (red highlight in Figure 6). Note that if a download does not automatically begin then you may need to save the resulting plain text page from your web browser via **File > Save As...**

The screenshot shows a web browser window for the EBI MUSCLE server. The URL is www.ebi.ac.uk/Tools/msa/muscle/. The page title is "MUSCLE". The main content area is titled "Multiple Sequence Alignment". A sub-section "Input form" is active. The first step, "STEP 1 - Enter your input sequences", contains a text area with multiple FASTA sequences pasted in. The sequences begin with:

```

ATGGGTGCACTCTGACTCCCTGTGAGAGAATCTGCCGTACTGCCCTGTGGGGCAAGGTGAACCTGATGAAG
TTGGTGTTGAGGCCCCCTGGGCAGGGTGTGTGGGTTACCGTGTACCGTGTACCGCAGAGGTTCTTTGAGGTCCTTTGG
GGGGTGTCTGCTCTGAGAAAGGGTGGGCACTGAGGTAAAGGCTCATGGCAAGAAAGGTCTCGGT
GCCTTAACTGATGCGCAGGCTCACCTGCAACCTCAAGGGCACCTTGGCACACTGAGTGAGGTGACT
GTGACAAAGCTGCTGAGTCTCTGAGAAACTTCAGGCTGGGCAACGCTGCTGTCTGAGTGAGGTGACT
TCACTTTGCGCAAGAAATTCAACCCACCAAGTGGAGGCTGCTTATCAGAAAGTGGTGGCTGTTGCGCTAA
GCCCTGGCCACAAAGTATCACTTAAGCTCGCTTCTGCTGTCCTAAATT

```

Below the text area is a file upload field: "Or upload a file: Choose File | No file chosen". The second step, "STEP 2 - Set your Parameters", has "OUTPUT FORMAT: ClustalW" selected. A link "More options..." is present. The third step, "STEP 3 - Submit your job", contains a checkbox "Be notified by email" and a red-outlined "Submit" button.

Figure 5: To use the EBI MUSCLE server you must paste multiple FASTA format sequences that include their ID lines.

Results for job muscle-I20220915-202341-0706-86356419-p2m

Alignments | Result Summary | Phylogenetic Tree | Results Viewers | Submission Details
[Download Alignment File](#)

CLUSTAL multiple sequence alignment by MUSCLE (3.8)

```

patient reference      ATGGTGCACT
ACATTTGCTTCTGACACAACGTGTTACTAGCAACCTAAACAGACACCATGGTCATC
*****  

patient reference      TGACTCTGTGGAGAAGCTGCCGTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAG
*****  

patient reference      TTGGTGGTGGAGGCCCTGGCAGGCTGCTGGTGTACCCCTGGACCCAGAGGTTCTTG
*****  

patient reference      AGTCTTGGGATCTGCCACTCTGATGCAGTTATGGCAACCCTAAGGTGAAGGCTC
*****  

patient reference      ATGGCAAGAAAAGTGCCTGGCCTTAGTGTGATGCCCTGGCTCACCTGGACAACCTCAAGG
ATGGCAAGAAAAGTGCCTGGCCTTAGTGTGATGCCCTGGCTCACCTGGACAACCTCAAGG
*****  

patient reference      GCACCTTGGCACACTGAGTGAAGCTGCAGTGCAGTTATGGCAACCTGGATCTGAGAAGCT
GCACCTTGGCACACTGAGTGAAGCTGCAGTGCAGTTATGGCAACCTGGATCTGAGAAGCT
*****  

patient reference      TCAGGCTCTGGCAACGTGCTGGTCTGTGTGCTGGCCCATCTTGGCAAAGAATTCA
TCAGGCTCTGGCAACGTGCTGGTCTGTGTGCTGGCCCATCTTGGCAAAGAATTCA
*****  

patient reference      CCCACCACTGCAAGCTGCCTATCAGAAAGTGGTGGCTGGTGTGGCTAATGCCCTGGCC
CCCACCACTGCAAGCTGCCTATCAGAAAGTGGTGGCTGGTGTGGCTAATGCCCTGGCC
*****  

patient reference      ACAAGTATCAACTAAGCTCGCTTCTGCTGTCCAATTTC
ACAAGTATCAACTAAGCTCGCTTCTGCTGTCCAATTTCATAAAGGTTCTTGTCC
*****
```

Figure 6: Alignment of patient and reference HBB sequence

Next download **seaview** for your computer from: <http://doua.prabi.fr/software/seaview>

Once downloaded open seaview by double clicking on it's icon (most likely in your Downloads folder) and then select **File > Open >** and select your muscle alignment results. A colored version of your alignment should now be displayed (Figure 7).

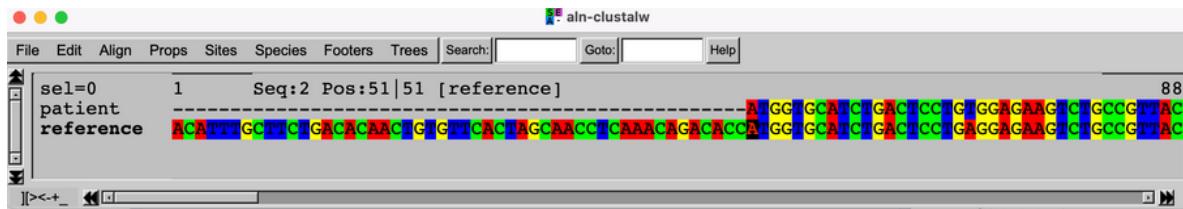


Figure 7: Programs like SEAVIEW are most useful when you have large many sequence alignments

See if you can now use seaview to answer the following 3 questions:

Q14. How many gap characters (-) are added to the beginning of the sickle cell beta-globin sequence in order to align it with the beta globin sequence? How might you have

guessed this number from information you read in the GenBank annotation?

Tip

See section 2, Q13.

50 gaps found

Q15. Ignoring ambiguity codes (Y and N), what are the differences between the two sequences?

Tip

There may be more than one

The main difference is one nucleotide change in codon 6 (GAG to GTG), which changes the amino acid from glutamic acid to valine. There are also gaps at the start and end of the sickle cell sequence because the sequence is shorter than the full beta globin mRNA.

Q16. Which codon position from the start of the sickle cell sequence would this difference affect? What amino acid would the different codons encode in the two sequences?

Tip

Use the codon table below to help (Figure 8).

This difference affects codon 6 of the sickle cell sequence. The normal codon (GAG) codes for glutamic acid, while the sickle cell codon (GTG) codes for valine.

		Second base					
		U	C	A	G		
First base	U	UUU Phenyl-alanine F UUC UUA UUG	UCU Serine S UCC UCA UCC	UAU Tyrosine Y UAC UAA Stop codon UAG Stop codon	UGU Cysteine C UGC UGA Stop codon UGG Tryptophan W	UCAG	
	C	CUU Leucine L CUC CUA CUG	CCU Proline P CCC CCA CCG	CAU Histidine H CAC CAA Glutamine Q CAG	CGU CGC Arginine R CGA CGG	UCAG	
	A	AUU Isoleucine I AUC AUA AUG M Methionine start codon	ACU Threonine T ACC ACA ACG	AAU Asparagine N AAC AAA Lysine K AAG	AGU Serine S AGC AGA Arginine R AGG	UCAG	
	G	GUU Valine V GUC GUA GUG	GCU Alanine A GCC GCA GCG	GAU Aspartic D GAC GAA Glutamic E GAG	GGU Glycine G GGC GGA GGG	UCAG	
						UCAG	

Figure 8: Codon table for use in Q16

Section 4

In this section we will retrieve and visualize the 3D protein structure of sickle cell haemoglobin. The aim here is to ascertain how the Glu6 → Val6 mutation might cause the mutant molecules to oligomerise into fibers, hence deforming erythrocytes. This will require you to examine the structural context of the mutation in the beta globin chains.

We could find sickle cell haemoglobin structures via a text search of main PDB website @

<http://www.rcsb.org/>. However, as we know the nucleotide sequence from our previous work, lets use BLASTx to search the PDB database from the NCBI site.

To do this visit <http://blast.ncbi.nlm.nih.gov/> select the appropriate BLAST program and make sure the database you are searching against is set to “**Protein Data Bank (pdb)**”.

Note the accession numbers and alignment statistics for the top few hits.

Q17. Are there any PDB structures with 100% identity to your *example1* query sequence? Give the PDB codes for these entries.

Tip

Note that this might not be the top listed hit

1HBS

To further examine these structures we will jump over to the main PDB database as it has more annotation data and more full featured **3D molecular viewers** than NCBI.

Visit <http://www.rcsb.org/> and use the 4 character PDB accession code you found previously in your BLASTx search to pull up each PDB entry you listed in Q17.

From scrolling through this entry you can find out information about the “Experimental Data” (such as the resolution of the structure and quality of the data collected), “Literature” links (i.e. associated publications), “Macromolecules” (i.e. protein chains present) and “Small Molecules” (i.e. any ligands or co-factors that might be present).

From the “Macromolecules” section notice that the hemoglobin structure is composed of multiple alpha and beta globin molecules corresponding to gene names HBA and HBB.

Q18. Which four chain identifiers in the 1HBS structure represent beta globin?

The four chain identifiers that represent beta globin in the 1HBS structure are B, D, F, and H.

At the top of the PDB entry page click the **3D View** tab to pull up an interactive 3D structure view (Figure 9).

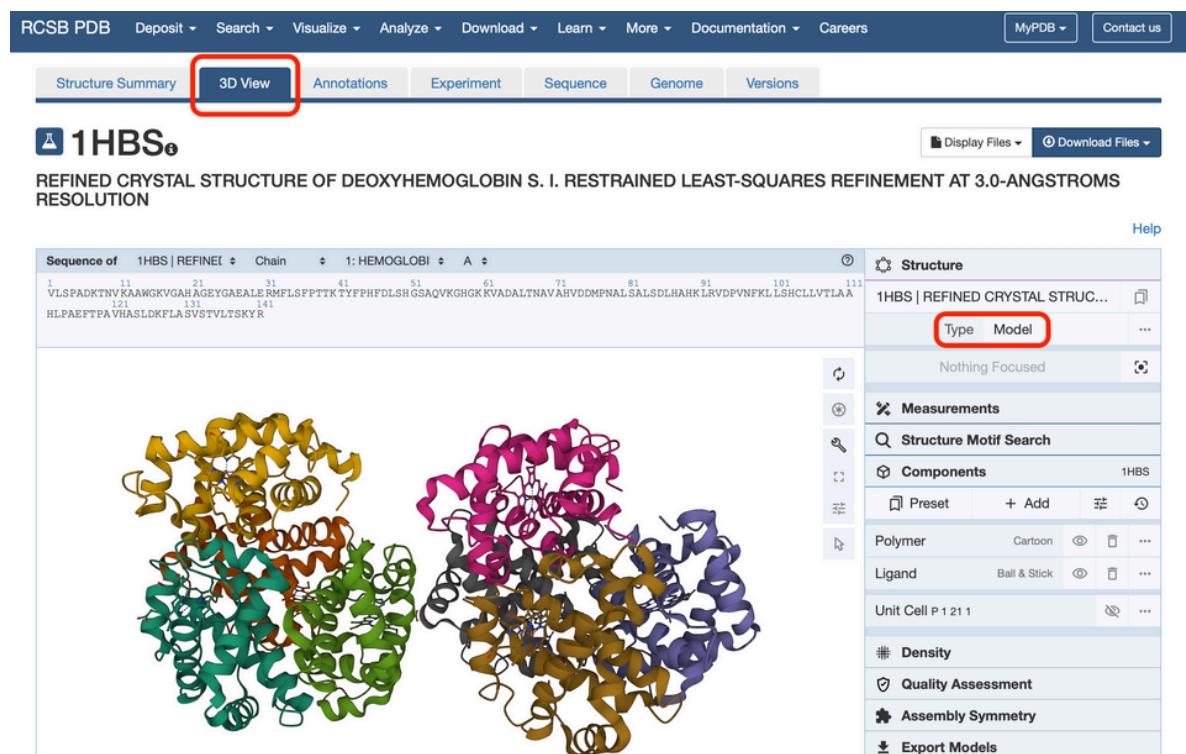


Figure 9: Using the molecular viewer at the PDB database

Under the “Structure” section of the right side control panel change the “Type” of view from “Assembly” to “Model” (see second red rectangle in Figure 9). This will now display the model observed in the asymmetric unit of the crystal (i.e. the packing of chains observed in the actual experiment) rather than the simplified default biological assembly view of the minimal functional form.

Side-note: This is the relatively new Mol* 3D viewer. This viewer also features

at a number of other major databases including UniProt. The user interface is still somewhat clunky and limited when compared to stand-alone software like PyMol, Chimera or VMD. However, these sand-alone viewes require consideral download and installion time so we will stick with Mol* for this lab. Essentially there are 3 major components to the viewer (Figure 10):

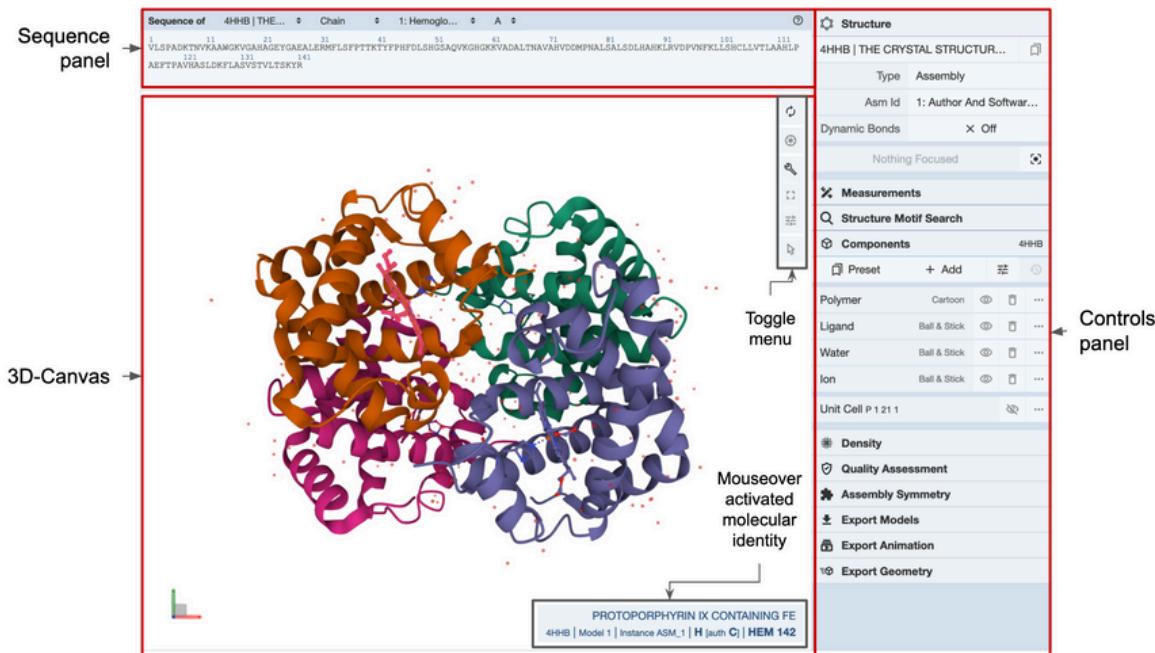


Figure 10: The three major components of the Mol* viewer include the Sequence panel, 3D-canvas and Control panel.

Notice by rotating the molecule in the 3D Canvas that there are now two hemoglobin molecules displayed rather than the previous single molecule. Notice that each is comprised of four distinctly colored chains with two alpha and two beta chains in each.

To highlight our beta globin amino acid of interest toggle the sequence display to the **beta chain** (Figure 11) and specifically chain **H** (Figure 12) in the top “Sequence panel”.

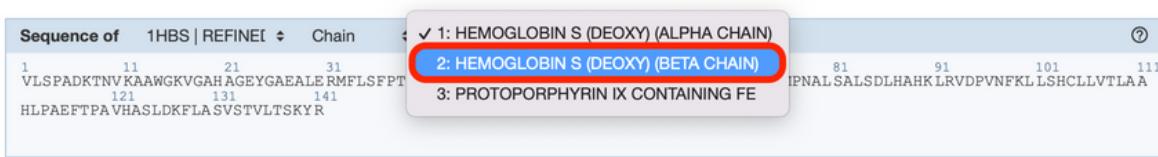


Figure 11: Display the sequence of beta globin chains in the PDB entry

Find and click on amino acid V 6 in the sequence view to highlight this amino acid in the 3D



Figure 12: Focus on beta globin chain H

view (Figure 13). Notice that the right side control panel now lists VAL 6 in several locations (purple highlight in Figure 13).

Try alternating the zoom level by clicking on these side panel entries (purple in Figure 13) to get a better feel for the location of our Valine amino acid in the overall structure.

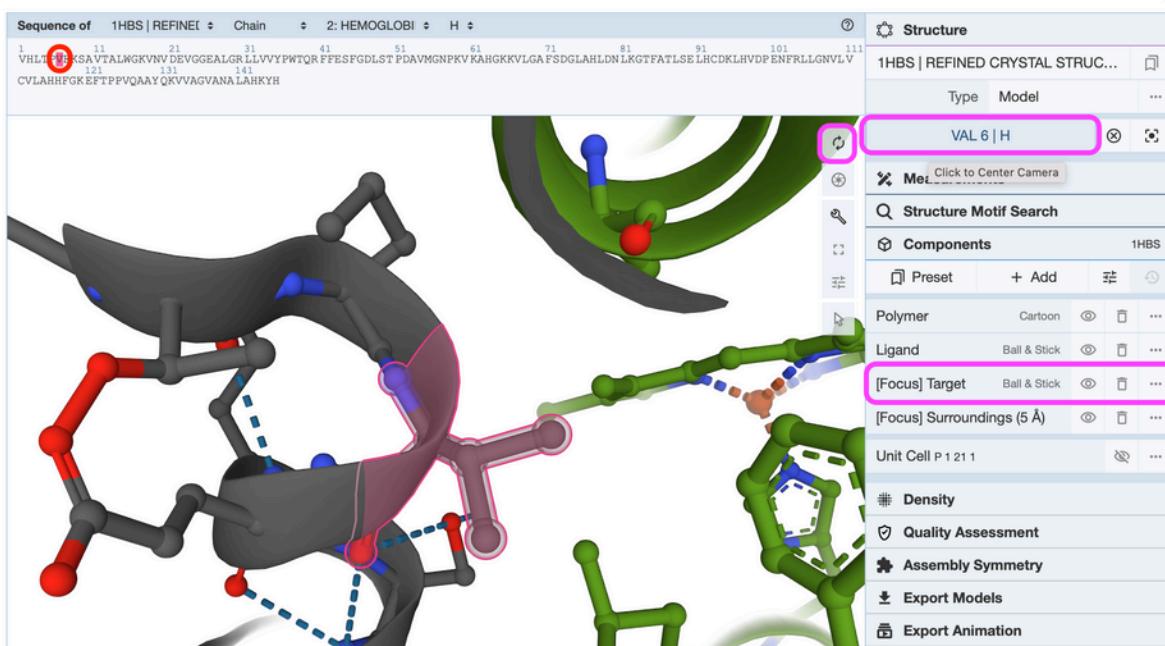


Figure 13: Highlighting Val 6 in the sequence and structure of 1HBS chain H

Using the “Control panel” we can add a new “*Representation*” to more clearly display the mutated Valine residue. First click on the 3 dots “action” menu icon beside the **[Focus] Target** component of the side panel. This should correspond to our Valine amino acid (Figure 14). Then click “Add Representation” and select **spacefill** from the dropdown list of options. This will result in all atoms of our entry being displayed as so called “*spacefill spheres*” with different atom types in different colors (e.g. oxygens in red, carbons in gray etc.) Play around with the settings from the spacefill menu and others in this section until you have a reasonable feel for how the program works. Can you clearly see our mutated residue

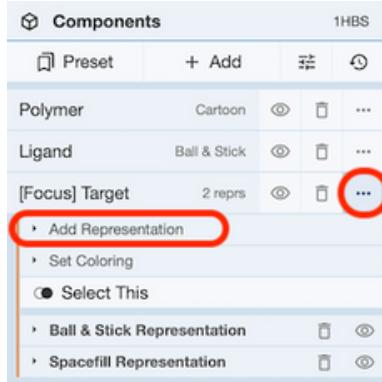


Figure 14: Adding a spacefill representation to our Val 6 residue

position?

Try zooming (via scrolling up and down) and rotating (via clicking and moving your mouse). You can always “reset” the view by clicking the reset like circular arrows icon. Also experiment with different settings and views. You can render a

Q19. What do you notice about the location of the Val6 residue in chain H of the 2HBS structure in relation to porphyrin?

Tip

See Figure below where I have used a white “spacefill” representation for Val6 and changed the viewer background to black.

Val6 in chain H is close to the porphyrin but not touching it, it appears to be on the outside of the beta-globin chain.

In general black backgrounds can help with interactive visualization but should not be used for publication quality figures. You can download a high resolution image with transparent background of your final representation using the iris like screenshot icon.

Side note: Some folks have reported issues using the Mol* viewer with older versions of the Edge, IE and Chrome browsers. The workaround is to use a different web browser. If, the structure is still not displayed correctly for you, download

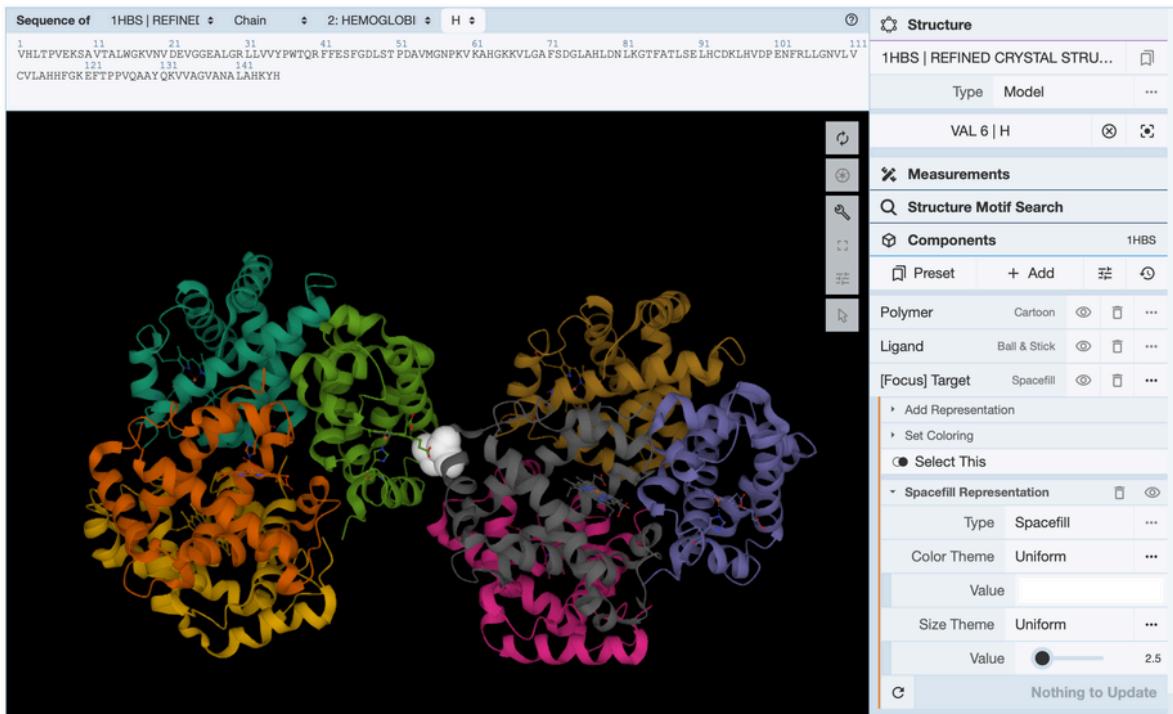


Figure 15: In this representation, the background has been changed to black and E6V (i.e. Val6) mutation is highlighted in white spacefill representation and the porphyrin prosthetic group in ball and stick representation).

its coordinates from the **PDB** database at: <http://www.rcsb.org/> and **ask for assistance.**

Q20: What one part of this exercise or associated lecture material is still confusing?
If appropriate please also indicate the question number from this document and answer the question in the following anonymous form: [**Muddy Point Assessment Form**](#)
Your comments will let us know which material needs to be further clarified and will help us gain stronger control of the material in this course. Thank you!

Discussion

The original paper discussing the 1HBS and 2HBS crystal structures is available online:

<http://www.sciencedirect.com/science/article/pii/S0022283697912535>

In this article, Figure 3 demonstrates how the Glu6->Val6 mutation could result in the characteristic “sickle” phenotype. The charged Glu6 mutating to Val6 creates a superficial hydrophobic patch on one HbS molecule that interacts with hydrophobic surface residues of another. The molecules thus polymerize, creating extended fibers that distort the shape of the red blood cell.

Assessment of the disparate biochemical properties of normal and sickle haemoglobin, together with microscopy studies showing long crystal fibres inside sickle cells, led Linus Pauling (1949) to (correctly) predict the morphological effects of these changes. The abnormal sickle form causes the cells to clump together, hampering their passage through blood vessels, depriving tissues of oxygen. See this YouTube video for an illustration: <http://www.youtube.com/watch?v=Qd0HrY2NlwY>

The sickled blood cells have a short lifetime and cannot be replaced fast enough, leading to chronic anaemia. Sickle cell anemia was one of the first diseases to be linked to a defect at the molecular level, providing a clear demonstration that a single base mutation can change a single amino acid, which in turn can result in a defective protein.

Bluebird Bio as well as Vertex Pharmaceuticals and CRISPR Therapeutics have ongoing **sickle-cell gene therapy trials**. As noted in this [excellent recent easy in the New Yorker](#): “There is something of a paradox in the fact that patients with sickle-cell disease — a population that has faced extraordinary levels of bias, neglect, and marginalization - may be among the first to have their illnesses transformed by the most cutting-edge of medical technologies”.

Appendix

>gi|179408|gb|M25079.1|HUMBETGLA Human sickle cell beta-globin mRNA
ATGGTNCAYTNACNCCNGTGGAGAACGTCYGCYGTNACNCNCT
NTGGGGYAAGGTNAAYGTGGATGAAGYYGGYGGYGAGGCCCTGG
GCAGNCTGCTNGTGGTCTACCCTGGACCCAGAGGTTCTNGAN
TCNTTYGGGATCTGNNNACNCNGANGCAGTTATGGCAACCC
TAAGGTGAAGGCTCATGGCAAGAAAGTGCCTGGTGCCTTAGTG
ATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTGCCACA
CTGAGTGAGCTGACTGTGACAAGCTNCAYGTGGATCCTGAGAA
CTTCAGGCTNCTNGGCAACGTGYTNGTCTGYGTGCTGGCCCATC
ACTTGGCAAAGAATTCAACCCACCAAGTGCANGCNGCCTATCAG
AAAGTGGTNGCTGGTGTNGCTAATGCCCTGGCCCACAAGTATCA
CTAAGCTNGCYTTTGTCCAATT

>gi|28302128|ref|NM_000518.4| Homo sapiens hemoglobin, beta (HBB), mRNA
ACATTTGCTCTGACACAACGTGTTCACTAGCAACCTCAAACA
GACACCATGGTGCATCTGACTCCTGAGGAGAACGTCGCCGTAC
TGCCCTGTGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGGAGG
CCCTGGGCAAGGTCTGGTGGTCTACCCCTGGACCCAGAGGTTTC
TTTGAGTCCTTGGGATCTGCCACTCCTGATGCTGTTATGGG
CAACCCCTAAGGTGAAGGCTATGGCAAGAAAGTGCCTGGTGCCT
TTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTT
GCCACACTGAGTGAGCTGCACTGTGACAAGCTGCACGTGGATCC
TGAGAACTTCAGGCTCTGGCAACGTGCTGGTCTGTGCTGG
CCCATCACTTGGCAAAGAATTCAACCCACCAAGTGCAGGCTGCC
TATCAGAAAGTGGTGGCTGGTGGCTAATGCCCTGGCCCACAA
GTATCACTAAGCTCGCTTCTGCTGTCCAATTCTATAAAGG
TTCCTTGTCCCTAAGTCCAACACTAAACTGGGGGATATTAT
GAAGGGCCTTGAGCATCTGGATTCTGCCTAATAAAAACATTATTTCAATTGC

<http://www.rcsb.org/pdb/files/2hbs.pdb>

The mutation causing sickle cell anemia is a single nucleotide substitution (A to T) in
The codon for glutamic acid (GAG) to
a valine codon (GTG). Changing a hydrophilic amino acid to a hydrophobic one, see
<http://themedicalbiochemistrypage.org/sicklecellanemia.php>

Note there is also a T -> A difference at position 162 ($162/3 \Rightarrow$ codon 54 GCT -> GCA). This is in the third position of the codon and hence does not change the corresponding amino-acid.