



Using Jalview to look inside the blood of a Woolly Mammoth

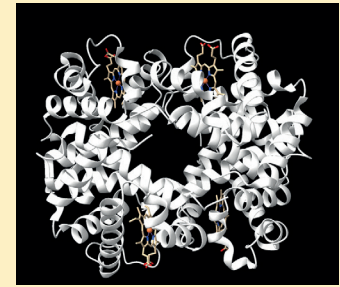


University
of Dundee

(1) Viewing DNA in a gene of the Woolly Mammoth

Background: Woolly mammoths are huge, shaggy beasts with tusks up to 15 feet in length. They are ancestors of the elephant and are roughly the same size. They appeared on the earth about 700,000 years ago, roaming across the ice plains of northern Eurasia and North America. About 10,000 years ago, changes in the climate and vegetation after the Ice Age, along with the presence of humans, lead to their extinction. A few isolated communities survived, such as the one on Wrangel Island in the Arctic Ocean off Siberia, however they died out about 4,000 years ago.

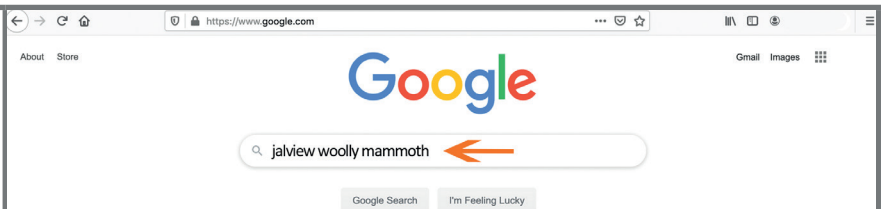
A few woolly mammoths were preserved, nearly intact, in solid blocks of ice in the permafrost. This has allowed scientists to extract fragments of their DNA and gradually piece together the woolly mammoth's genome. Scientists have used the genetic code to recreate the haemoglobin of the extinct woolly mammoth using recombinant gene expression. Haemoglobin (right image) is the protein in the blood responsible for delivering oxygen to the body.



Goal: The aim of this workbook is to examine woolly mammoth DNA, identify the HBA-T2 gene and the protein it codes. Exercise 1 examines the HBA-T2 gene in the woolly mammoth genome that is responsible for the production of the haemoglobin alpha subunit. Exercise 2 views the protein sequences and 3D structure of woolly mammoth haemoglobin recreated from the genetic code using recombinant genes expression. Exercise 3 & 4 compares the haemoglobin alpha subunit of the woolly mammoth with that of other animals, including its nearest living relative the elephant.

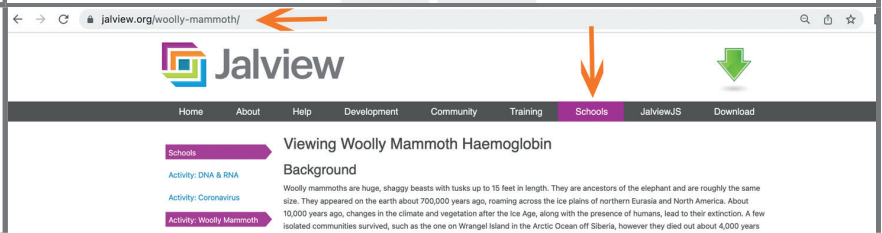
1. **Open a web browser** such as Chrome or Firefox.

We suggest you avoid Internet Explorer as JalviewJS does not always work in older browsers.

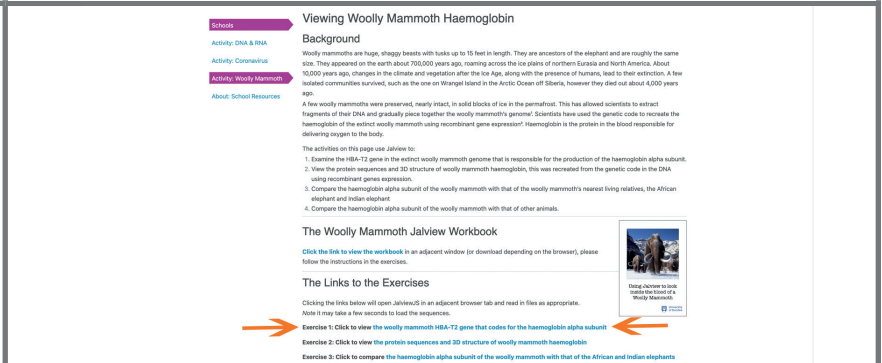


2. **Search** using keywords '*Jalview Woolly Mammoth*'.

From the list of results, select www.jalview.org.school/woolly-mammoth to open the web page.

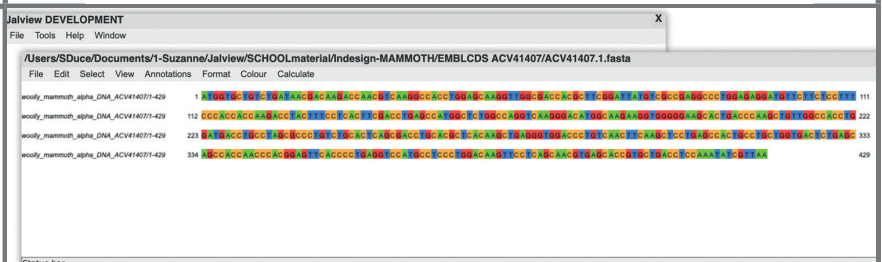


3. In 'The Links to the Exercises' section, **click the link 'Exercise 1: Click to view the woolly mammoth HBA-T2 gene that codes for the haemoglobin alpha subunit'**.

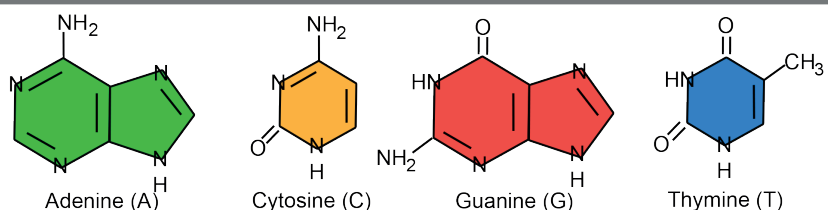


4. **JalviewJS viewer opens** containing a small part of the woolly mammoth genome.

This is the **HBA-T2 gene** that codes for the haemoglobin alpha protein subunit. The sequence is made up of 429 bases.



The Jalview nucleotide colour scheme: adenine bases are green, cytosine bases are yellow, guanine bases are red and thymine bases are blue.



(2) Viewing Woolly Mammoth Haemoglobin Protein

1. Return to the **Woolly Mammoth web page** opened in Step 3 in Exercise 1.
In 'The Links to the Exercises' section, **click the link 'Exercise 2: Click to view the protein sequences and 3D structure of woolly mammoth haemoglobin'**.

The Woolly Mammoth Jalview Workbook

Click the link to view the workbook in an adjacent window (or download depending on the browser), please follow the instructions in the exercises.

The Links to the Exercises

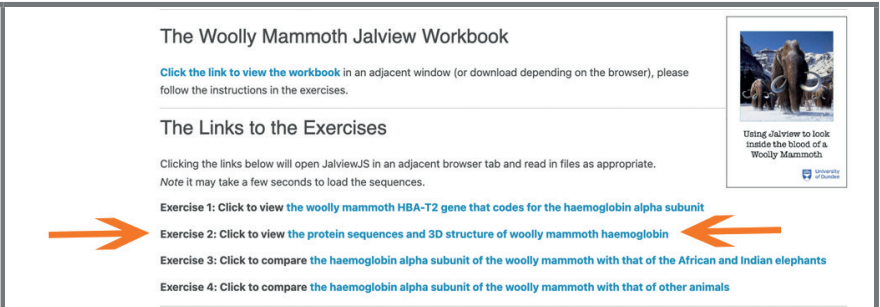
Clicking the links below will open JalviewJS in an adjacent browser tab and read in files as appropriate. Note it may take a few seconds to load the sequences.

Exercise 1: Click to view the [woolly mammoth HBA-T2 gene that codes for the haemoglobin alpha subunit](#)

Exercise 2: Click to view [the protein sequences and 3D structure of woolly mammoth haemoglobin](#)

Exercise 3: Click to compare [the haemoglobin alpha subunit of the woolly mammoth with that of the African and Indian elephants](#)

Exercise 4: Click to compare [the haemoglobin alpha subunit of the woolly mammoth with that of other animals](#)



2. In an adjacent tab of the web browser, **JalviewJS viewer opens containing Woolly Mammoth haemoglobin**.

The alignment window contains the **4 protein sequences** that make up haemoglobin.
The 3D window contains the **3D structure** of haemoglobin.




3. **Move the 3D structure window**, by placing the mouse on the title panel on the top of the window, then click-and-drag. **Enlarge the window**, by placing the mouse on the lower right-hand corner of the window, then click-and-drag.


Top Tip: If the Jmol window disappears it is probably behind the other window. Go to the **Window menu** in the desktop window and select **Jmol view** to bring it to the front.


4. **Rotate the 3D structure** by placing the mouse cursor on the structure, then **click-and-drag** the mouse.


Zoom in and out by pressing the **shift key**, then **click-and-drag** the mouse.

Mouse Movements -- for Jmol

Rotate on the X-Y axes: 

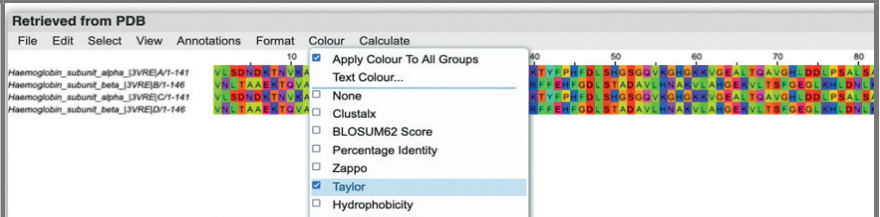
Zoom in and out: 

Translate the Molecule: 

Rotate on the Z axis: 

5. Colour the residues, **select the Colour menu** in the alignment window.

Select the **Taylor** colour scheme.



Top Tip: Each amino acid has its own colour as shown in this table.

Note: The name of the amino acids is given on the next page.

A	V	I	L
M	F	Y	W
H	R	K	N
Q	E	D	S
T	G	P	C

A haemoglobin molecule is made up of 2 alpha (blue and pink strands) and 2 beta (green and yellow strands) subunits.

Q. Using the sequences in the alignment window, how many amino acids are there in the alpha (α) and beta (β) subunits?

Top Tip: Either scroll to the end of the sequence and check the number ruler on the top or look at the sequence ID name as the sequence length is given at the end of the sequence name.

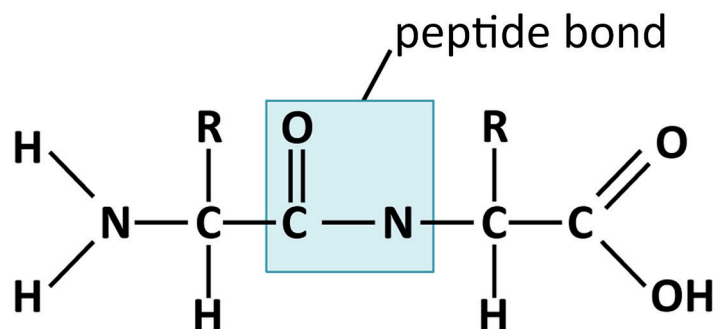
(2) Viewing Woolly Mammoth Haemoglobin Protein

There are 20 common amino acids found in nature:

Amino Acid	One letter	Molecular Formula
Alanine	A	$C_3H_7NO_2$
Arginine	R	$C_6H_{14}N_4O_2$
Asparagine	N	$C_4H_8N_2O_3$
Aspartic acid	D	$C_4H_7NO_4$
Cysteine	C	$C_3H_7NO_2S$
Glutamic acid	E	$C_5H_9NO_4$
Glutamine	Q	$C_5H_{10}N_2O_3$
Glycine	G	$C_2H_5NO_2$
Histidine	H	$C_6H_9N_3O_2$
Isoleucine	I	$C_6H_{13}NO_2$

Amino Acid	One letter	Molecular Formula
Leucine	L	$C_6H_{13}NO_2$
Lysine	K	$C_6H_{14}N_2O_2$
Methionine	M	$C_5H_{11}NO_2S$
Phenylalanine	F	$C_9H_{11}NO_2$
Proline	P	$C_5H_9NO_2$
Serine	S	$C_3H_7NO_3$
Threonine	T	$C_4H_9NO_3$
Tryptophan	W	$C_{11}H_{12}N_2O_2$
Tyrosine	Y	$C_9H_{11}NO_3$
Valine	V	$C_5H_{11}NO_2$

Proteins are polymers made up of amino acid subunits. The amino acids are joined together by peptide bonds between the COOH and NH_2 of neighbouring amino acids.



(3) Comparing Woolly Mammoth with Elephant Haemoglobin

1. Return to the **Woolly Mammoth web page** opened in Step 3 of Exercise 1.

In 'The Links to the Exercises' section, **click the link 'Exercise 3: Click to compare the haemoglobin alpha subunit of the woolly mammoth with that of the African and Indian elephants'.**

3. Compare the haemoglobin alpha subunit of the woolly mammoth with that of the woolly mammoth's nearest living relatives, the African elephant and Indian elephant
 4. Compare the haemoglobin alpha subunit of the woolly mammoth with that of other animals.

The Woolly Mammoth Jalview Workbook

Click the link to view the workbook in an adjacent window (or download depending on the browser), please follow the instructions in the exercises.

The Links to the Exercises

Clicking the links below will open JalviewJS in an adjacent browser tab and read in files as appropriate. Note it may take a few seconds to load the sequences.

Exercise 1: Click to view the woolly mammoth HBA-T2 gene that codes for the haemoglobin alpha subunit
 Exercise 2: Click to view the protein sequences and 3D structure of woolly mammoth haemoglobin
 Exercise 3: Click to compare the haemoglobin alpha subunit of the woolly mammoth with that of the African and Indian elephants
 Exercise 4: Click to compare the haemoglobin alpha subunit of the woolly mammoth with that of other animals

2. In an adjacent tab of the web browser, **JalviewJS viewer opens containing the haemoglobin alpha subunit of the woolly mammoth (top), African elephant and Indian elephant.**

ClustalOWS alignment of ClustalOWS alignment of Retrieved from Uniprot

Woolly_Mammoth_Hemoglobin_alpha_D3U1H8_MAMPR 1 M V L S D N D K T N V K A T W S K V G D H A S D Y V A E A L E R M F F S F P T T K T Y F P H F D L S H G S G Q V K 95
 Indian_Elephant_Hemoglobin_alpha_HBA_ELEMA 1 - V L S D N D K T N V K A T W S K V G D H A S D Y V A E A L E R M F F S F P T T K T Y F P H F D L S H G S G Q V K 94
 African_Elephant_Hemoglobin_alpha_HBA_LOXAF 1 - V L S D N D K T N V K A T W S K V G D H A S D Y V A E A L E R M F F S F P T T K T Y F P H F D L S H G S G Q V K 94

Conservation

Consensus

Occupancy

Woolly_Mammoth_Hemoglobin_alpha_D3U1H8_MAMPR 95 P V N F R L L S H C L L V T S S H Q P T F F E V H A S L D K F L S N V S T V L T S K Y R 142
 Indian_Elephant_Hemoglobin_alpha_HBA_ELEMA 95 P V N F R L L S H C L L V T S S H Q P T F F E V H A S L D K F L S N V S T V L T S K Y R 141
 African_Elephant_Hemoglobin_alpha_HBA_LOXAF 95 P V N F R L L S H C L L V T S S H Q P T F F E V H A S L D K F L S N V S T V L T S K Y R 141

Conservation

Consensus

Occupancy

Q. There are 4 places where the woolly mammoth sequence is different from the sequences of the elephants. Can you spot these differences?

Top Tip: Look at the Conservation row below the sequences or the changes in the letters and colours on the sequences.

ClustalOWS alignment of ClustalOWS alignment of Retrieved from Uniprot

Woolly_Mammoth_Hemoglobin_alpha_D3U1H8_MAMPR 1 M V L S D N D K T N V K A T W S K V G D H A S D Y V A E A L E R M F F S F P T T K T Y F P H F D L S H G S G Q V K 142
 Indian_Elephant_Hemoglobin_alpha_HBA_ELEMA 1 - V L S D N D K T N V K A T W S K V G D H A S D Y V A E A L E R M F F S F P T T K T Y F P H F D L S H G S G Q V K 141
 African_Elephant_Hemoglobin_alpha_HBA_LOXAF 1 - V L S D N D K T N V K A T W S K V G D H A S D Y V A E A L E R M F F S F P T T K T Y F P H F D L S H G S G Q V K 141

Conservation

Consensus

Q. Which of the two elephant sequences is most similar to that of the woolly mammoth sequence?

3. Go to the **Calculate** menu and select **Calculate Tree or PCA...** in the drop down menu.

ClustalOWS alignment of ClustalOWS alignment of Retrieved from Uniprot

File Edit Select View Annotations Format Colour Calculate

Woolly_Mammoth_Hemoglobin_alpha_D3U1H8_MAMPR
 Indian_Elephant_Hemoglobin_alpha_HBA_ELEMA
 African_Elephant_Hemoglobin_alpha_HBA_LOXAF

Conservation

Calculate

- Sort
- Calculate Tree or PCA...
- Pairwise Alignment
- Get Cross-References
- AutoCalculate Consensus
- Sort Alignment With New Tree
- Show flanking regions

4. Click **Calculate** in the box that opens to produce a **similarity tree**.

Choose Calculation

Principal Component Analysis

Tree

Neighbour Joining Average Distance

BLOSUM62

Calculate Close

ClustalOWS alignment of ClustalOWS alignment of Retrieved from Uniprot

Neighbour Joining Using BLOSUM62 from ClustalOWS alignment of ClustalOWS alignment of Retrieved from Uniprot

African_Elephant_Hemoglobin_alpha_HBA_LOXAF

Woolly_Mammoth_Hemoglobin_alpha_D3U1H8_MAMPR

Indian_Elephant_Hemoglobin_alpha_HBA_ELEMA

Q. Using the tree, is the woolly mammoth sequence more like the Indian or African elephant sequence?

Top Tip: If the Tree window disappears. It is probably behind the alignment window.

Either move the windows or go to the **Window** menu and select **Tree window (Neighbour Joining Using Blosum62)** from the drop down menu.

File Tools Help Window

- Close all
- Raise Associated Windows
- Minimize Associated Windows
- ClustalOWS alignment of ClustalOWS alignment of Retrieved from Uniprot
- Neighbour Joining Using BLOSUM62 from ClustalOWS alignment of ClustalOWS alignment of Retrieved from Uniprot

Woolly_Mammoth_Hemoglobin_alpha_D3U1H8_MAMPR
 Indian_Elephant_Hemoglobin_alpha_HBA_ELEMA
 African_Elephant_Hemoglobin_alpha_HBA_LOXAF

Conservation

Consensus

(4) Comparing Woolly Mammoth with Other Animal Haemoglobins

1. Return to the **Woolly Mammoth web page** opened in Step 3 of Exercise 1.

In 'The Links to the Exercises' section, **click the link 'Exercise 4: Click to compare the haemoglobin alpha subunit of the woolly mammoth with that of other animals'**.

The Woolly Mammoth Jalview Workbook

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The Links to the Exercises

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Exercise 1: Click to view the woolly mammoth HBA-T2 gene that codes for the haemoglobin alpha subunit

Exercise 2: Click to view the protein sequences and 3D structure of woolly mammoth haemoglobin

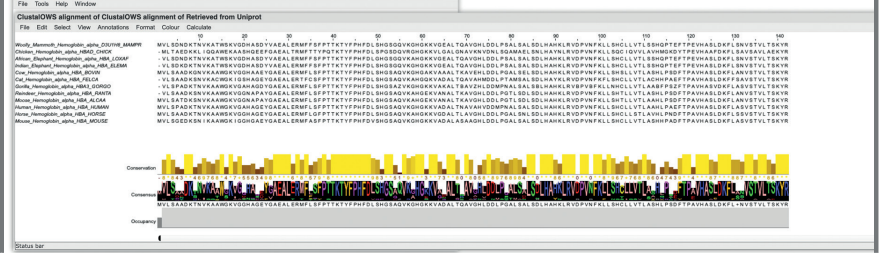
Exercise 3: Click to compare the haemoglobin alpha subunit of the woolly mammoth with that of the African and Indian elephants

Exercise 4: Click to compare the haemoglobin alpha subunit of the woolly mammoth with that of other animals

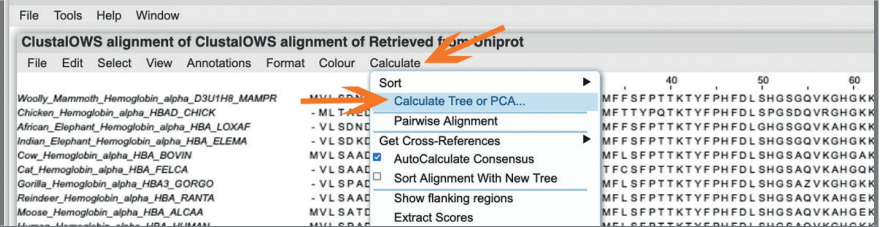


Using Jalview to look inside the blood of a Woolly Mammoth

2. In an adjacent tab of the web browser, JalviewJS viewer opens containing the haemoglobin alpha (α) subunits from a woolly mammoth and a range of other animals.



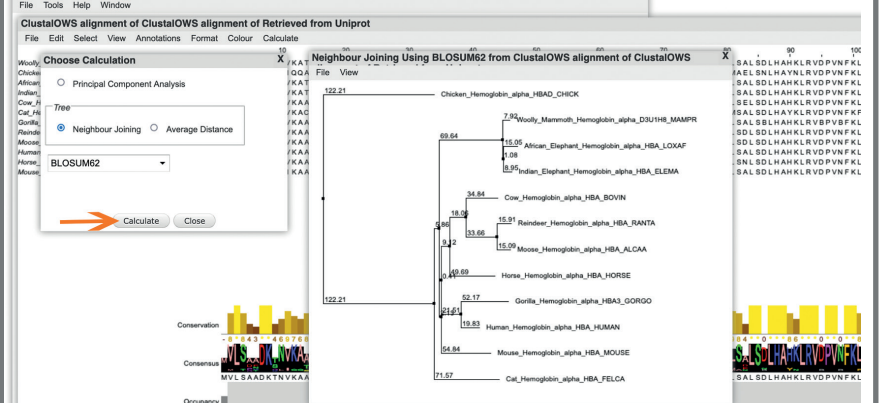
3. Go to the **Calculate** menu and select **Calculate Tree or PCA...** in the drop down menu.



4. Click **Calculate** in the 'Choose Calculation' box that opens to produce a **similarity tree**.

Close the 'Choose Calculation' box once the tree has appeared.

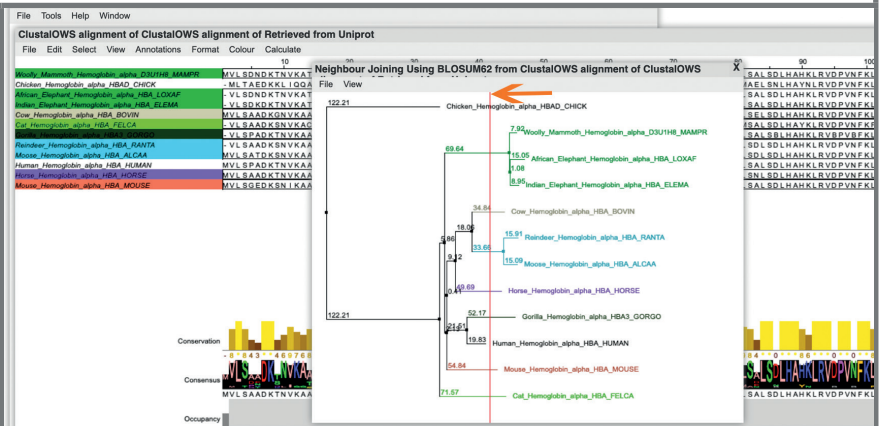
(By default, Neighbour Joining and Blosum62 should be selected, and the Principal Component Analysis option is not selected)



The tree reflects the similarity between the protein sequences. The numbers on the trees are distance scores. They can be added together to allow comparison. The smaller the distance scores between animals, the greater the similarity between the sequences.

5. Click the mouse cursor on the tree, and a red vertical line appears.

The red line groups sequences. Each group has its own randomly generated colour.



- Q. Which haemoglobin sequence is most similar to the Woolly Mammoth haemoglobin α -subunit?
- Q. Which haemoglobin sequence is most similar to the Human haemoglobin α -subunit?
- Q. Which haemoglobin sequence is most dissimilar to that of Woolly Mammoth haemoglobin α -subunit?

(4) Comparing Woolly Mammoth with Other Animal Haemoglobins

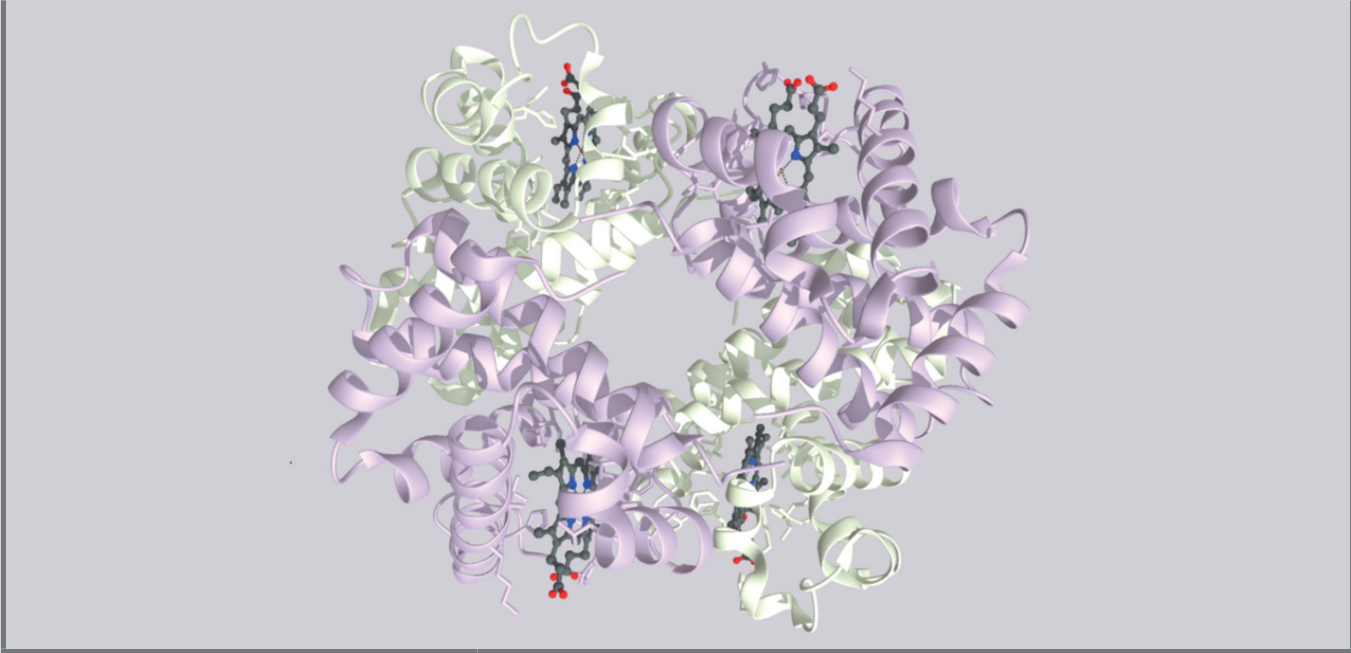
6. Click the mouse at different locations in the tree, this will change the grouping and the colours of groups within the tree.

9. Select the View menu in the tree window. Select Sort Alignment by Tree.

10. The sequences in the alignment window are reordered and coloured to reflect the sequence order and groupings in the tree.

Note: The sequence names in the alignment window have the same colours as those in the tree window.

Q. Which animals are grouped together? Is this what you would have expected?



The extinct woolly mammoth haemoglobin is made up of two α -subunits strands (cream ribbons) and two β -subunits strands (lavender ribbons). The heme molecule is represented by a stick-and-ball model. The iron atoms are coloured orange. The carbon, nitrogen and oxygen atoms are coloured grey, blue and red respectively. The 3D model can be viewed in Sketchfab (<https://skfb.ly/otnl>).

Q. How many iron-containing heme molecules (coloured grey) are present in the haemoglobin 3D model of woolly mammoth haemoglobin?

If you have a question or comment, please drop the Jalview team a line at schools@jalview.org. Also check the comments on the Jalview Discussion Forum. Feel free to contribute, there is a school's section <https://discourse.jalview.org>.

Background Information

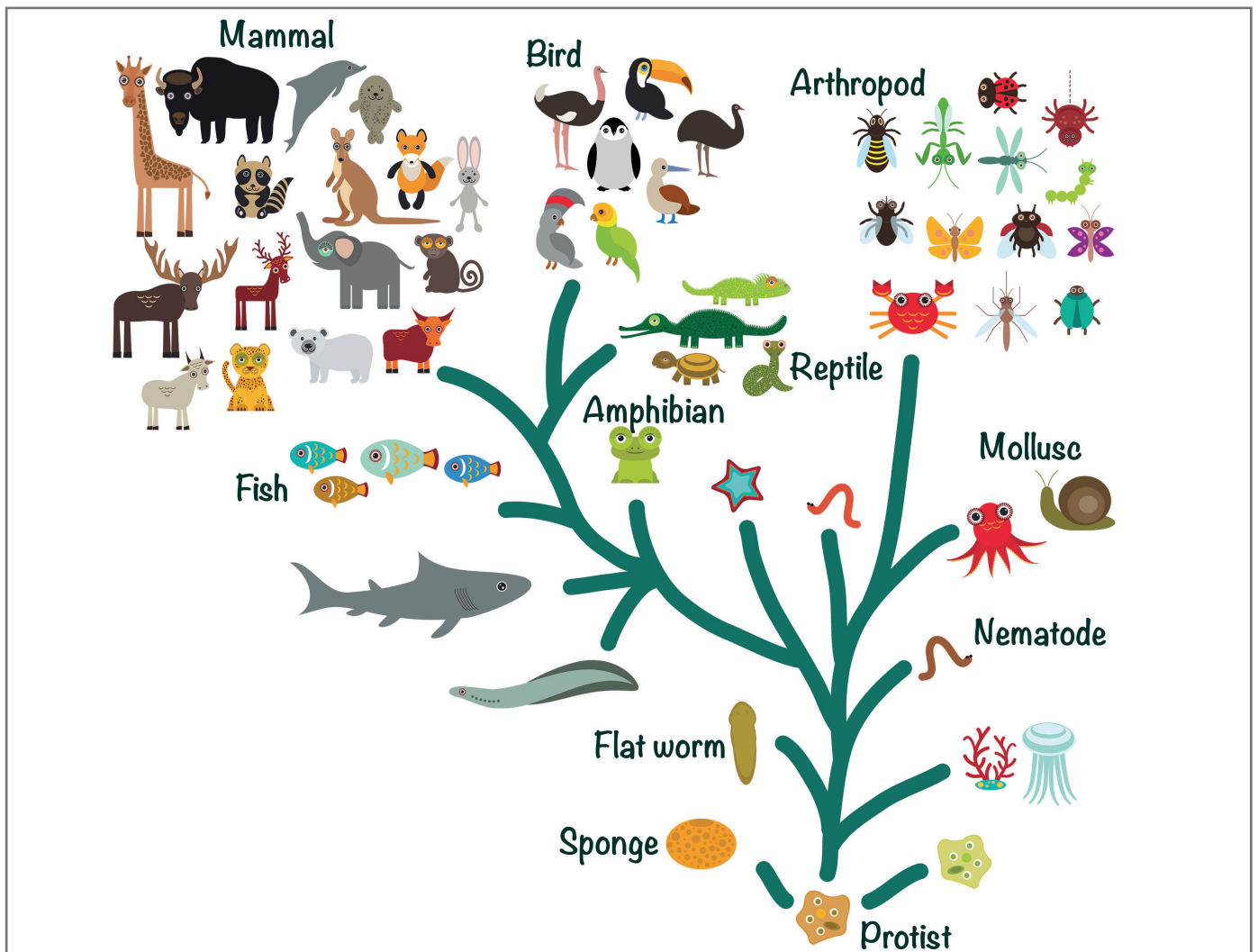
The Windows in the Jalview Desktop

The screenshot displays the Jalview desktop environment with several windows open:

- Desktop window:** The main window showing a ClustalOVS alignment of hemoglobin sequences from various species. It includes a 'Sequence ID Panel' on the left, an 'Alignment window' with a color-coded sequence, a 'Conservation' bar chart below it, and an 'Annotation Label Panel' at the bottom.
- Tree window:** A phylogenetic tree titled 'Neighbour Joining Using BLOSUM62 from ClustalOVS alignment of'. It shows the evolutionary relationships between the species, with bootstrap values at the nodes.
- Structure window:** A 3D ribbon model of a protein structure, likely hemoglobin, rendered in various colors (blue, green, yellow, pink, purple).
- Overview window:** A smaller window showing a condensed overview of the sequence alignment.

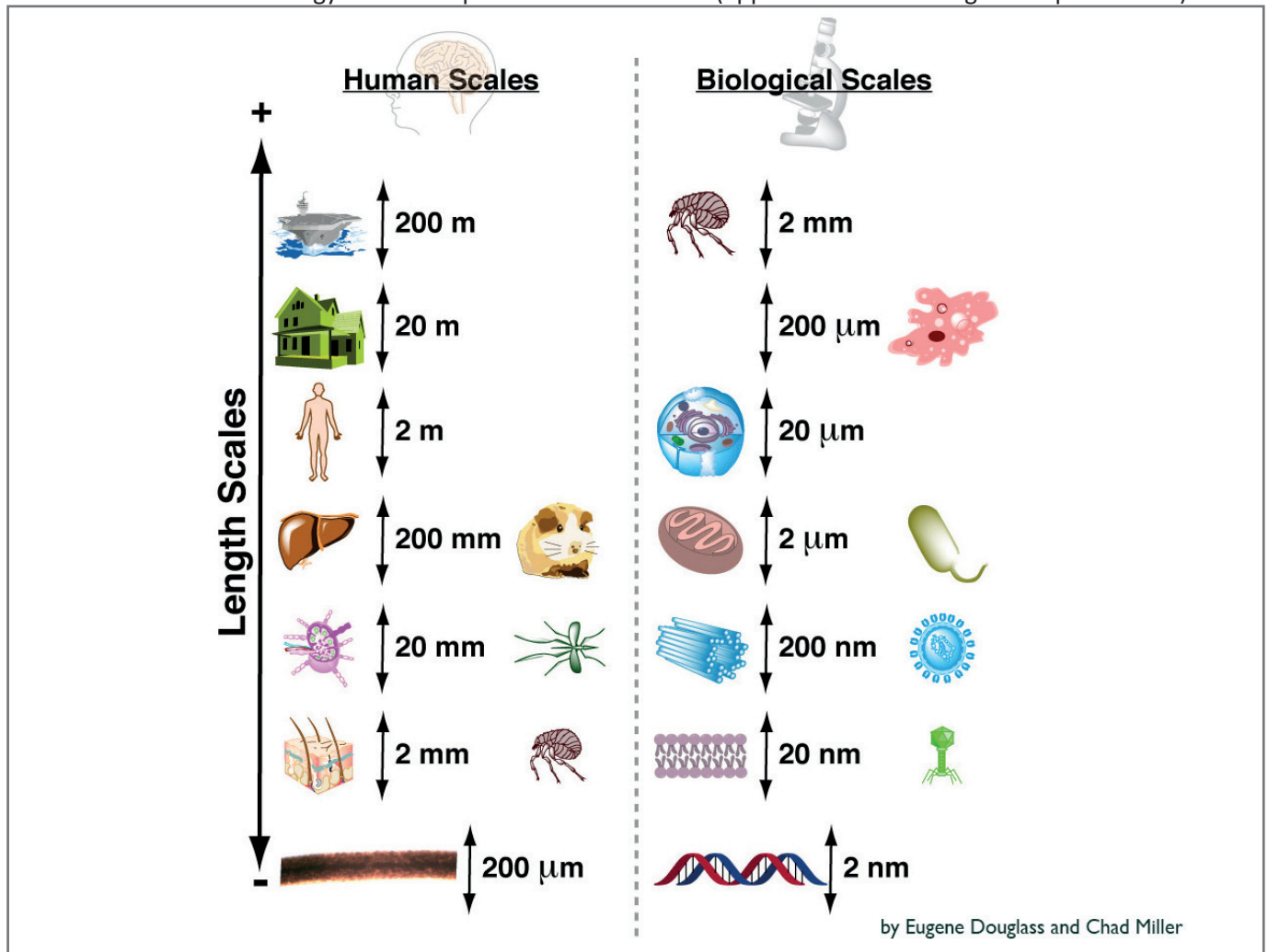
Animal Kingdom Tree

Evolution scale from unicellular organisms to mammals



Guide to Biological and Human Scales

Molecular and cellular biology scales compared to human scales (approx 5 orders of magnitude per column)



Human Scale Key: Aircraft Carrier (200m), 3-story House (20m), Human (2m), Liver/Gerbil (200mm), Lymph Node/Mosquito (20mm), Skin/Flea (2mm), Hair thickness (200um)

Biological Scale Key: Flea (2mm), Amoeba (200um), Eukaryotic Cell (20um), Mitochondria/Bacteria (2um), Centriol/Large Virus (200nm), Cell Membrane/Small Virus (20nm), dsDNA thickness (2nm)

<https://www.practicalscience.com/intuiting-biological-scales-using-human-scales/#more-53>

Glossary

Amino acid:-the basic building block molecules of peptides and proteins.

DNA (deoxyribonucleic acid):- the molecule that encodes genetic information. It carries the instructions for the organism to function such as growth, metabolism and reproduction.

Gene:- a region of DNA that encodes a specific protein or protein subunit.

Genetic code:- sets of triplet nucleotides that encode specific amino acids.

Genome:- all the genetic material in the chromosomes of a particular organism.

Protein:- large biological molecule composed of a long string of amino acids.

Protein sequence:- the linear sequence of amino acids in a protein.

Multiple sequence alignment:- an alignment involving three or more sequences.

Nucleotide bases:- DNA building blocks contain nitrogenous base, sugar molecule and phosphoric acid.

Sequence alignment:- a comparison between protein sequences to determine sequence similarity.

Tree:- reflects the similarity between sequences from different species.

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For more information, please visit the Jalview website at www.jalview.org

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