Jalview 2.10.1

Manual and Introductory Tutorial

David Martin, James Procter

Andrew Waterhouse, Saif Shehata, Nancy Giang

Mungo Carstairs, Charles Ofoegbu, Kira Mourão

Suzanne Duce and Geoff Barton

School of Life Sciences, University of Dundee

Dundee, Scotland DD1 5EH, UK

Manual Version 1.9

20th February 2017

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Chapter 1

Basics

1.1 Introduction

1.1.1 Jalview

Jalview is a multiple sequence alignment viewer, editor and analysis tool. Jalview is designed to be platform independent (running on Mac, MS Windows, Linux and any other platforms that support Java). Jalview is capable of editing and analysing large alignments (thousands of sequences) with minimal degradation in performance, and able to show multiple integrated views of the alignment and other data. Jalview can read and write many common sequence formats including FASTA, Clustal, MSF(GCG) and PIR.

There are two types of Jalview program. The **Jalview Desktop** is a standalone application that provides powerful editing, visualization, annotation and analysis capabilities. The **JalviewLite** applet has the same core visualization, editing and analysis capabilities as the desktop, without the desktop's webservice and figure generation capabilities. It is designed to be embedded in a web page,[1] and includes a javascript API to allow customisable display of alignments for web sites such as Pfam.[2]

The Jalview Desktop in this version provides access to protein and nucleic acid sequence, alignment and structure databases, and includes the Jmol[3] viewer for molecular structures, and the VARNA[4] program for the visualization of RNA secondary structure. It also provides a graphical user interface for the multiple sequence alignment, conservation analysis and protein disorder prediction methods provided as **Ja**va **B**ioinformatics **A**nalysis **W**eb **S**ervices (JABAWS). JABAWS[5] is a system for running bioinformatics programs that you can download and run on your own machine or cluster, or install on compute clouds.

[1] A demonstration version of Jalview (Jalview Micro Edition) also runs on a mobile phone but the functionality is limited to sequence colouring.

[2] `http://pfam.xfam.org`

[3] Provided under the LGPL licence at `http://www.jmol.org`

[4] Provided under GPL licence at `http://varna.lri.fr`

[5] released under GPL at `http://www.compbio.dundee.ac.uk/jabaws`

1.1.2 Jalview's Capabilities

Figure 1.1 gives an overview of the main features of the Jalview desktop application. Its primary function is the editing and visualization of sequence alignments, and their interactive analysis. Tree building, principal components analysis, physico-chemical property conservation and sequence consensus analyses are built into the program. Web services enable Jalview to access online alignment and secondary structure prediction programs, as well as to retrieve protein and nucleic acid sequences, alignments, protein structures and sequence annotation. Sequences, alignments, trees, structures, features and alignment annotation may also be exchanged with the local filesystem. Multiple visualizations of an alignment may be worked on simultaneously, and the user interface provides a comprehensive set of controls for colouring and layout. Alignment views are dynamically linked with Jmol and UCSF Chimera[6] structure displays, a tree viewer and spatial cluster display, facilitating interactive exploration of the alignment's structure. The application provides its own Jalview project file format in order to store the current state of an alignment and analysis windows. Jalview also provides WYSIWIG[7] style figure generation capabilities for the preparation of alignments for publication.

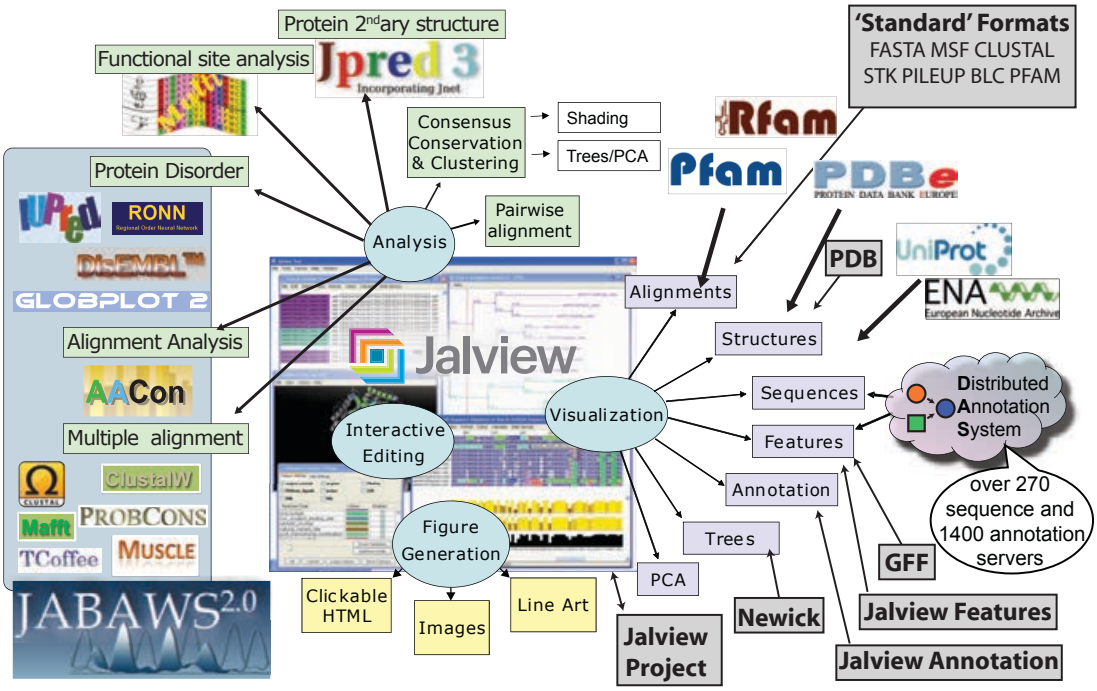


Figure 1.1: **Capabilities of the Jalview Desktop.** The Jalview Desktop Application provides a stable environment for the creation, editing and analysis of alignments and the generation of figures.

[6]UCSF Chimera needs to be installed separately. It is available free for academic use from `https://www.cgl.ucsf.edu/chimera/download.html`.

[7]WYSIWIG: What You See Is What You Get.

Jalview History

Jalview was initially developed in 1996 by Michele Clamp, James Cuff, Steve Searle and Geoff Barton at the University of Oxford and then the European Bioinformatics Institute. Development of Jalview 2 was made possible with eScience funding from the BBSRC[8] in 2004, enabling Andrew Waterhouse and Jim Procter to re-engineer the original program to introduce contemporary developments in bioinformatics and take advantage of the latest web and Java technology. Jalview's development has been supported from 2009 onwards by BBSRC funding, and since 2014 by a Wellcome Trust Biomedical Resource grant[9]. In 2010, 2011, and 2012, Jalview benefitted from the Google Summer of Code, when Lauren Lui and Jan Engelhardt introduced new features for handling RNA alignments and secondary structure annotation, in collaboration with Yann Ponty.[10]

Citing Jalview

If you use Jalview in your work you should cite:
"Jalview Version 2 - a multiple sequence alignment editor and analysis workbench"
Waterhouse, A.M., Procter, J.B., Martin, D.M.A, Clamp, M. and Barton, G. J. (2009)
Bioinformatics doi: 10.1093/bioinformatics/btp033

This paper supersedes the original Jalview publication:
"The Jalview Java alignment editor"
Michele Clamp, James Cuff, Stephen M. Searle and Geoffrey J. Barton (2004)
Bioinformatics **20** 426-427.

1.1.3 About this Tutorial

This tutorial is written in a manual format with short exercises where appropriate, typically at the end of each section. The first few sections concerns the basic operation of Jalview and should be sufficient for those who want to launch Jalview (Section 1.2), open an alignment (Section 1.4), perform basic editing (Section 2), colouring (Section 3.1), and produce publication and presentation quality graphical output (Section 3.2).

The remaining sections of the manual cover the visualization and analysis techniques available in Jalview. These include working with the embedded Jmol molecular structure viewer (or UCSF Chimera), building and viewing trees and Principal Components Analysis (PCA) plots, and using trees for sequence conservation analysis. An overview of the Jalview Desktop's webservices is given in Section 10, and the alignment and secondary structure prediction services are described in detail in Sections 5 and 8.1 respectively. Section 4 details the creation and visualization of sequence and alignment annotation. Section 9.1 discusses specific features of use when working with nucleic acid sequences, such as translation and linking to protein coding regions, and the display and analysis of RNA secondary structure.

[8] Biotechnology and Biological Sciences Research Council grant *"VAMSAS: Visualization and Analysis of Molecules, Sequence Alignments and Structures"*, a joint project to enable interoperability between Jalview, TOPALi and AstexViewer.
[9] Wellcome grant number 101651/Z/13/Z
[10] `http://www.lix.polytechnique.fr/~ponty/`

Typographic Conventions

Keystrokes using the special non-symbol keys are represented in the tutorial by enclosing the pressed keys with square brackets (*e.g.* [RETURN] or [CTRL]).

Keystroke combinations are denoted with a '-' symbol (*e.g.* [CTRL]-C means press [CTRL] and the 'C' key simultaneously).

Menu options are given as a path from the menu that contains them - for example *File* ⇒ *Input Alignment* ⇒ *From URL* means to select the 'From URL' option from the 'Input Alignment' submenu of a window's 'File' dropdown menu.

1.2 Launching the Jalview Desktop Application

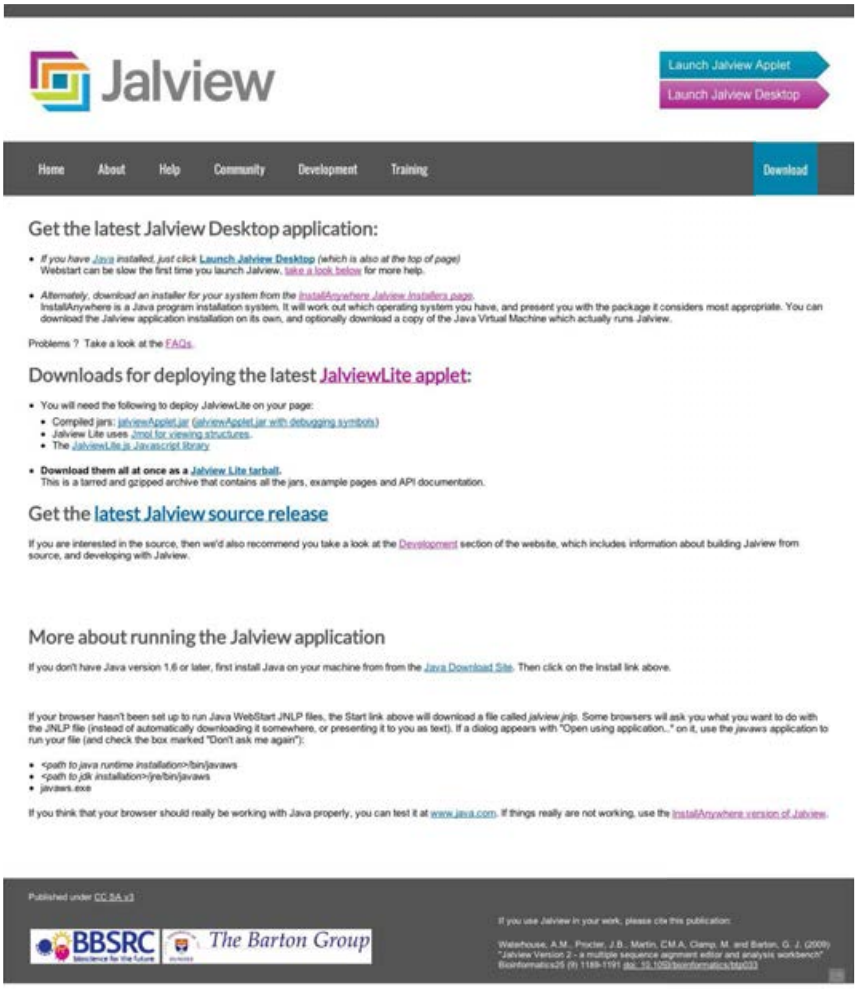


Figure 1.2: **Download page on the Jalview web site at www.jalview.org.**

This tutorial is based on the Jalview Desktop application. Much of the information will also be useful for users of the JalviewLite applet, which has the same core editing, analysis and visualization

capabilities (see the JalviewLite Applet Examples page for examples). The Jalview Desktop, however, is much more powerful, and includes additional support for interaction with external web services, and production of publication quality graphics.

The Jalview Desktop can be run in two ways; as an application launched from the web *via* Java webstart, or as an application loaded onto your hard drive. The webstart version is launched from the pink 'Launch Jalview Desktop button' at the top right hand side of pages of the website (www.jalview.org). To download the locally installable version, follow the links on the download page (www.jalview.org/download) (Figure 1.2). These links will launch the latest stable release of Jalview.

When the application is launched with webstart, two dialogs may appear before the application starts. If your browser is not set up to handle webstart, then clicking the launch link may download a file that needs to be opened manually, or prompt you to select the program to handle the webstart file. If that is the case, then you will need to locate the **javaws** program on your system[11]. Once java webstart has been launched, you may also be prompted to accept a security certificate signed by the Barton Group.[12] You can always trust us, so click trust or accept as appropriate. The splash screen (Figure 1.3) gives information about the version and build date that you are running, information about later versions (if available), and the paper to cite in your publications. This information is also available on the Jalview web site at `http://www.jalview.org`.

Figure 1.3: **Jalview splash screen.**

When Jalview starts it will automatically load an example alignment from the Jalview site. This behaviour can be switched off in the Jalview Desktop preferences dialog by unchecking the open file option. This alignment will look like the one in Figure 1.4 (taken from Jalview version 2.10.1).

Jalview News RSS Feed

Announcements are made available to users of the Jalview Desktop *via* the Jalview Newsreader. This window will open automatically when new news is available, and can also be accessed *via* the Desktop's *Tools* ⇒ *Show Jalview News* menu entry.

[11] The file that is downloaded will have a type of **application/x-java-jnlp-file** or **.jnlp**. The **javaws** program that can run this file is usually found in the **bin** directory of your Java installation

[12] On some systems, the certificate may be signed by 'UNKNOWN'. In this case, clicking through the dialogs to look at the detailed information about the certificate should reveal it to be a Barton group certificate.

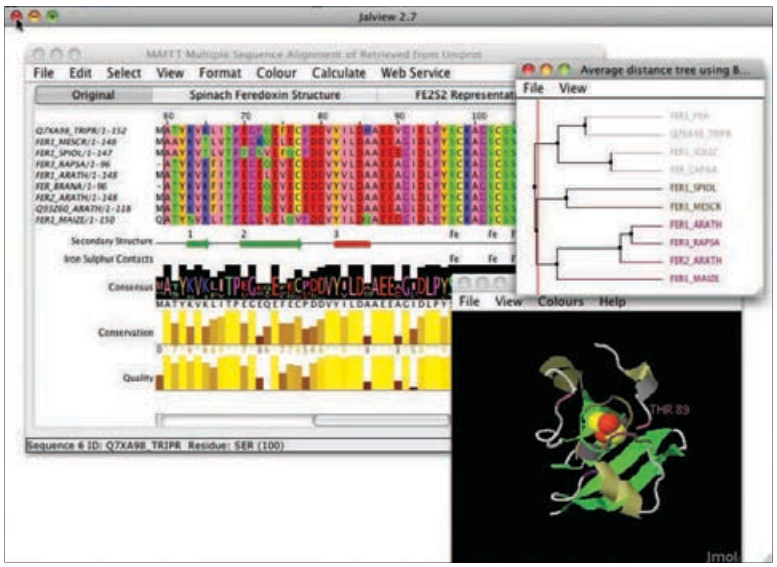


Figure 1.4: **Default startup for Jalview.**

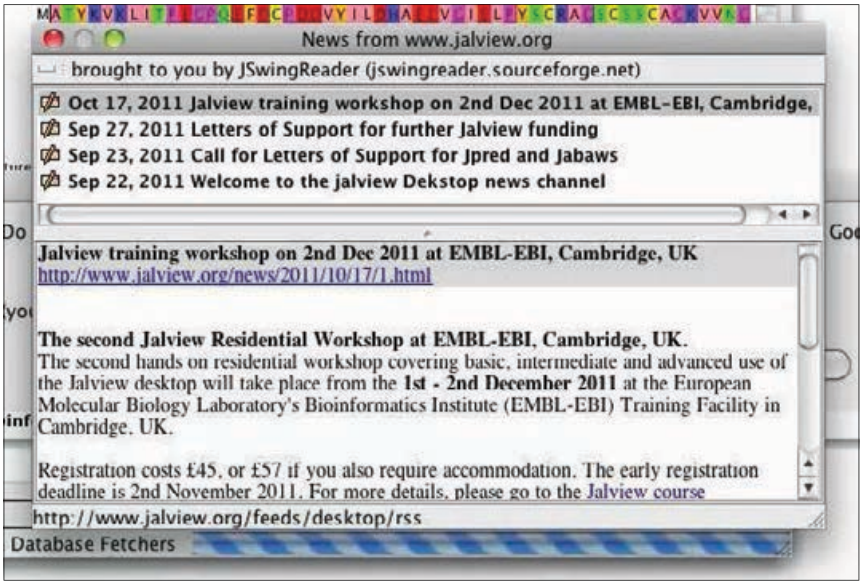


Figure 1.5: **The Jalview News Reader.** The newsreader opens automatically when new articles are available from the Jalview Desktop's news channel.

Exercise 1: Launching Jalview from the Jalview Website

1.a. Open the Jalview web site (www.jalview.org) in your web browser. Launch Jalview by clicking on the pink 'Launch Jalview' Desktop button in the top right hand corner. This will download and open a jalview.jnlp webstart file.

1.b. Dialog boxes will open and ask if you want to open the jalview.jnlp file as the file is an application downloaded from the Internet, click *Open*. (Note you may be asked to update Java, if you agree then it will automatically update the Java software). As Jalview opens, four demo Jalview windows automatically load.

1.c. If you are having trouble, it may help changing the browser you are using, as the browsers and its version may affect this process.

1.d. To deactivate the opening of the 4 demo sequences during the launch, go to the *Tools* ⇒ *Preferences...* menu on the desktop. A 'Preference' dialog box opens, untick the box adjacent to the 'Open file' entry in the 'Visual' preferences tab. Click *OK* to save the preferences.

1.e. Launch another Jalview workbench from the web site by clicking on the pink Launch button. The example alignment should not be loaded as Jalview starts up.

1.f. To reload the original demo file select the *File*⇒ *From URL* entry in the Desktop menu. Click on the URL history button (a downward arrow on the right hand side of the dialog box) to view the files, select exampleFile_2_7.jar, then click *OK*.

Note: Should you want to load your own sequence during the launch process, then go to the *Tools* ⇒ *Preferences...* menu on the desktop. The tick the 'Open file' entry of 'Visual' preferences tab, type in the URL of the sequence you want to load.

As the jalview.jnlp file launches Jalview on your desktop, you may want to move this from the downloads folder to another folder. Opening from the jnlp file will allow Jalview to be launched offline.

See the video at: `http://www.jalview.org/Help/Getting-Started`**.**

1.2.1 Getting Help

Built in Documentation

Jalview has comprehensive on-line help documentation. Select *Help* ⇒ *Documentation* from the main window menu and a new window will open (Figure 1.6). The appropriate topic can then be selected from the navigation panel on the left hand side. To search for a specific topic, click the 'search' tab and enter keywords in the box which appears.

Email Lists

The Jalview Discussion list `jalview-discuss@jalview.org` provides a forum for Jalview users and developers to raise problems and exchange ideas - any problems, bugs, and requests for help should be raised here. The `jalview-announce@jalview.org` list can also be subscribed to if you wish to be kept informed of new releases and developments.

Archives and mailing list subscription details can be found in the Jalview web site's community section.

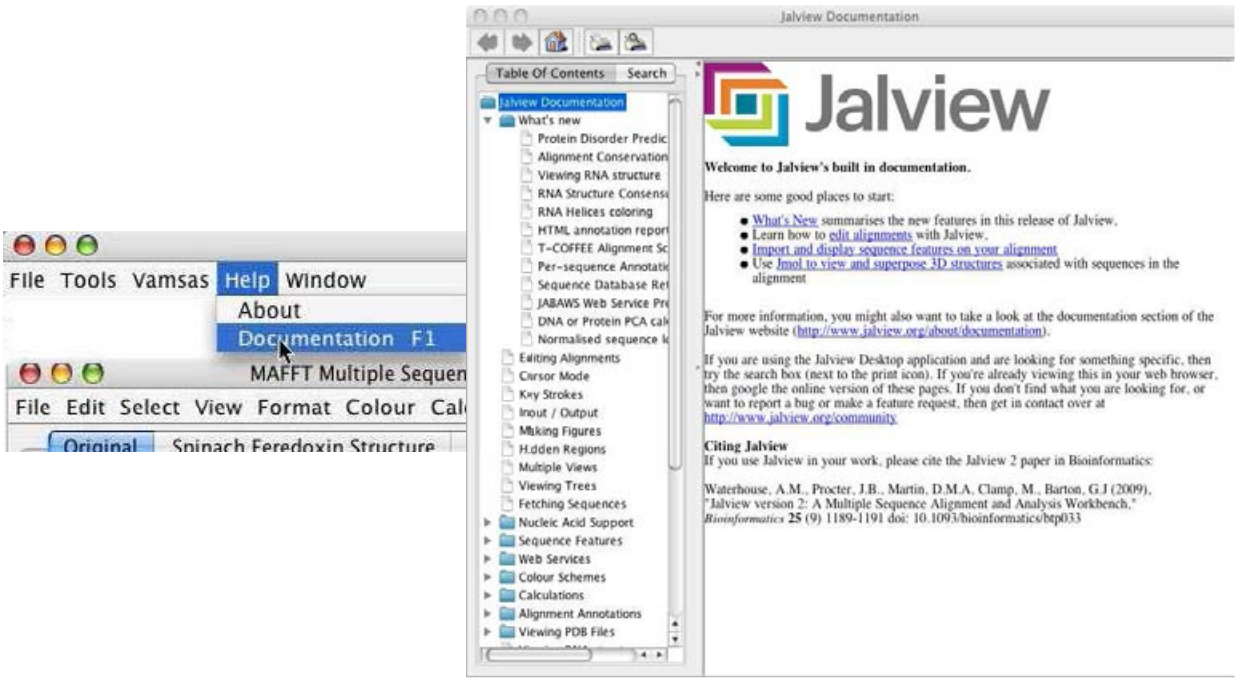


Figure 1.6: **Accessing the built in Jalview documentation.**

1.3 Navigation

The major features of the Jalview Desktop are illustrated in Figure 1.7. The alignment window is the primary window for editing and visualization, and can contain several independent views of the alignment being worked with. The other windows (Trees, Structures, PCA plots, etc) are linked to a specific alignment view. Each area of the alignment window has a separate context menu accessed by clicking the right mouse button.

Jalview has two navigation and editing modes: **normal mode**, where editing and navigation is performed using the mouse, and **cursor mode** where editing and navigation are performed using the keyboard. The **F2 key** is used to switch between these two modes.

Note: On MacBooks and other laptops with compact keyboards, you may need to press the **function key [Fn]** when pressing any of the numbered function keys. So to toggle between keyboard and normal mode, press [Fn]-[F2].

1.3.1 Navigation in Normal Mode

Jalview always starts up in Normal mode, where the mouse is used to interact with the displayed alignment view. You can move about the alignment by clicking and dragging the ruler scroll bar to move horizontally, or by clicking and dragging the alignment scroll bar to the right of the alignment to move vertically. If all the rows or columns in the alignment are displayed, the scroll bars will not be visible.

Each alignment view shown in the alignment window presents a window onto the visible regions of

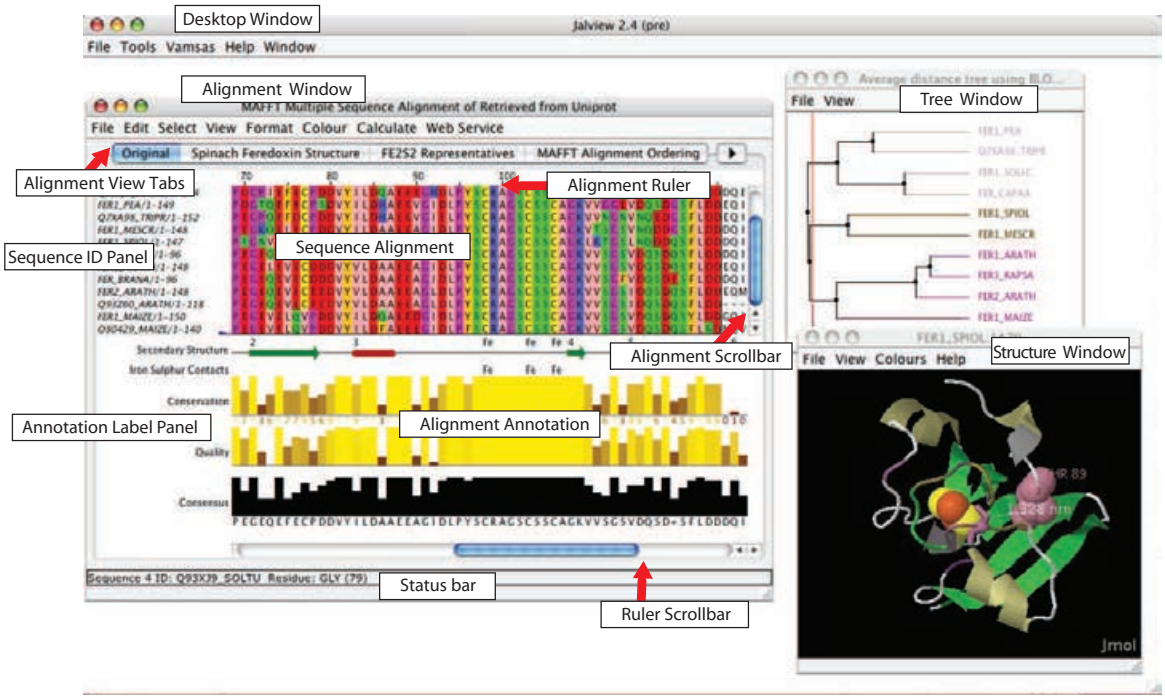


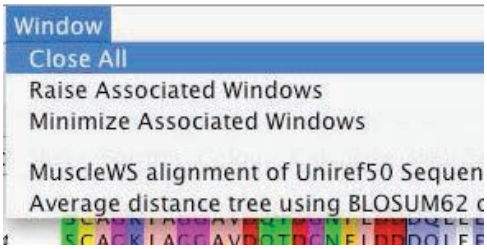
Figure 1.7: **The anatomy of Jalview.** The major features of the Jalview Desktop Application are labeled.

the alignment. This means that with anything more than a few residues or sequences, alignments can become difficult to visualize on the screen because only a small area can be shown at a time. It can help, especially when examining a large alignment, to have an overview of the whole alignment. Select *View* ⇒ *Overview Window* from the Alignment window menu bar (Figure 1.8[13]).

The red box in the overview window shows the current view in the alignment window. A percent identity histogram is plotted below the alignment overview. Shaded parts indicate rows and columns of the alignment that are hidden (in this case, a single row at the bottom of the alignment - see Section 2.5). You can navigate around the alignment by dragging the red box.

Alignment and analysis windows are closed by clicking on the usual 'close' icon (indicated by arrows on Mac OS X). If you want to close all the alignments and analysis windows at once, then use the *Window* ⇒ *Close All* option from the Jalview desktop.

Warning: Make sure you have saved your work because this cannot be undone!



[13] the menu shown in this figure is from Jalview 2.2, later versions have more options.

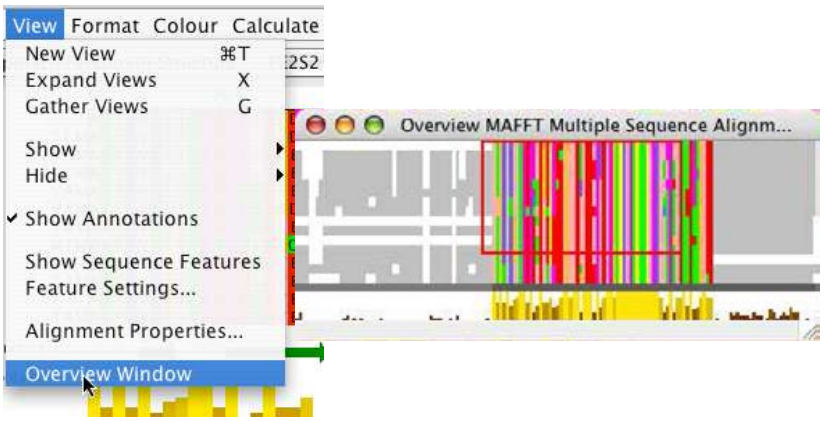
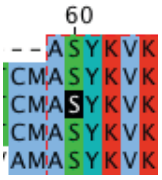


Figure 1.8: **Alignment Overview Window.** The overview window for a view is opened from the *View* menu.

1.3.2 Navigation in Cursor Mode



Cursor mode navigation enables the user to quickly and precisely navigate, select and edit parts of an alignment. On pressing F2 to enter cursor mode the position of the cursor is indicated by a black background and white text. The cursor can be placed using the mouse or moved by pressing the arrow keys (↑, ↓, ←, →).

Rapid movement to specific positions is accomplished as listed below:

- **Jump to Sequence** *n***:** Type a number *n* then press [S] to move to sequence (row) *n*.
- **Jump to Column** *n***:** Type a number *n* then press [C] to move to column *n* in the alignment.
- **Jump to Residue** *n***:** Type a number *n* then press [P] to move to residue number *n* in the current sequence.
- **Jump to column** *m* **row** *n***:** Type the column number *m*, a comma, the row number *n* and press [RETURN].

1.3.3 The Find Dialog Box

A further option for navigation is to use the *Select* ⇒ *Find...* function. This opens a dialog box into which can be entered regular expressions for searching sequences and sequence IDs, or sequence numbers. Hitting the [Find next] button will highlight the first (or next) occurrence of that pattern in the sequence ID panel or the alignment, and will adjust the view in order to display the highlighted region. The Jalview Help provides comprehensive documentation for this function, and a quick guide to the regular expressions that can be used with it.

Exercise 2: Navigation
Jalview has two navigation and editing modes: **normal** mode (where editing and navigation are via the mouse) and the **cursor** mode (where editing and navigation are via the keyboard). The **F2 key** is used to switch between these two modes. With a Mac, the key combination **Fn key and F2** is needed, as button is often assigned to screen brightness. Jalview always starts up in normal mode.

2.a. Load an example alignment from its URL (`http://www.jalview.org/examples/exampleFile_2_7.jar`) via the Desktop using *File* ⇒ *Input Alignment* ⇒ *From URL* dialog box. (The URL should be stored in its history and clicking on the *down arrow* on the dialog box is an easy way to access it.)

2.b. Scroll around the alignment using the alignment (vertical) and ruler (horizontal) scroll bars.

2.c. Find the Overview Window, *Views* ⇒ *Overview Window* and open it. Move around the alignment by clicking and dragging the red box in the overview window.

2.d. Return to the alignment window, look at the status bar (lower left hand corner of the alignment window) as you move the mouse over the alignment. It indicates information about the sequence and residue under the cursor.

2.e. Press [F2] key, or [Fn]-[F2] on Mac, to enter Cursor mode. Use the direction keys to move the cursor around the alignment.

2.f. Move to sequence 7 by pressing **7 S**. Move to column 18 by pressing **1 8 C**. Move to residue 18 by pressing **1 8 P**. Note that these can be two different positions if gaps are inserted into the sequence. Move to sequence 5, column 13 by typing **1 3 , 5** [RETURN].

Note: To view Jalview's comprehensive on-line help documentations select *Help* in desktop menu, clicking on *Documentation* will open a Documentation window. Select topic from the navigation panel on the left hand side or use the Search tab to select specific key words.
See the video at: `http://www.jalview.org/Help/Getting-Started`**.**

1.4 Loading Sequences and Alignments

1.4.1 Drag and Drop

In most operating systems you can just drag a file icon from a file browser window and drop it on an open Jalview application window. The file will then be opened as a new alignment window. Drag and drop also works when loading data from a URL - simply drag the link or url from the address panel of your browser on to an alignment or the Jalview desktop background and Jalview will load data from the URL directly.

1.4.2 From a File

Jalview can read sequence alignments from a sequence alignment file. This is a text file, **not** a word processor document. For entering sequences from a wordprocessor document see Cut and Paste (Section 1.4.4) below. Select *File* ⇒ *Input Alignment* ⇒ *From File* from the main menu (Figure 1.9). You will then get a file selection window where you can choose the file to open. Remember to select the appropriate file type. Jalview can automatically identify some sequence file formats.

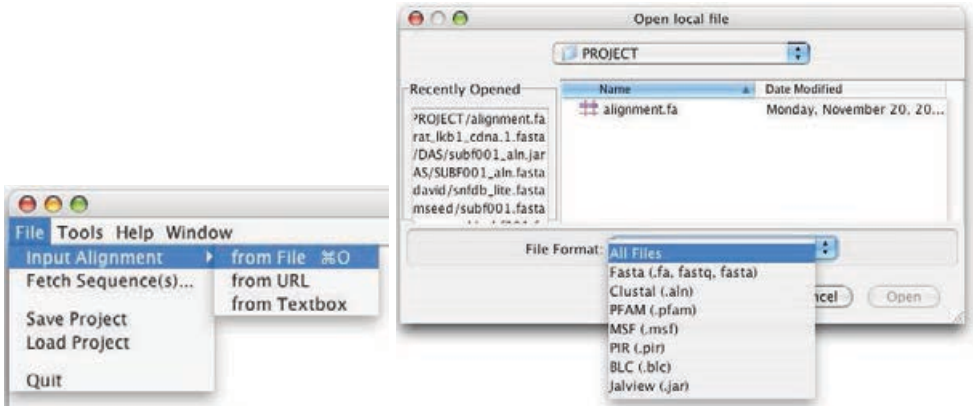


Figure 1.9: **Opening an alignment from a file saved on disk.**

1.4.3 From a URL

Jalview can read sequence alignments directly from a URL. Please note that the files must be in a sequence alignment format - an HTML alignment or graphics file cannot be read by Jalview. Select *File* ⇒ *Input Alignment* ⇒ *From URL* from the main menu and a window will appear asking you to enter the URL (Figure 1.10). Jalview will attempt to automatically discover the file format.

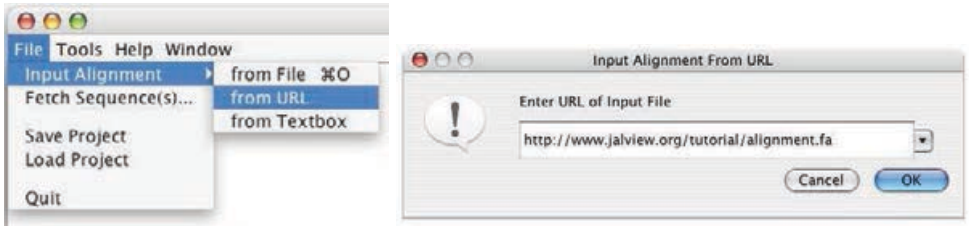


Figure 1.10: **Opening an alignment from a URL.**

1.4.4 Cut and Paste

Documents such as those produced by Microsoft Word cannot be readily understood by Jalview. The way to read sequences from these documents is to select the data from the document and copy it to the clipboard. There are two ways to do this. One is to right-click on the desktop background, and select the 'Paste to new window' option in the menu that appears. The other is to select *File* ⇒ *Input Alignment* ⇒ *From Textbox* from the main menu, paste the sequences into the text window that will appear, and select *New Window* (Figure 1.11). In both cases, presuming that they are in the right format, Jalview will happily read them into a new alignment window.

1.4.5 From a Public Database

Jalview can retrieve sequences and sequence alignments from the public databases housed at the European Bioinformatics Institute, including Uniprot, Pfam, Rfam and the PDB. Jalview's sequence fetching capabilities allow you to avoid having to manually locate and save sequences from a web

Figure 1.11: **Opening an alignment from pasted text.**

page before loading them into Jalview. It also allows Jalview to gather additional metadata provided by the source, such as annotation and database cross-references. Select *File* ⇒ *Fetch Sequence(s)* . . . from the main menu and a window will appear (Figure 1.12). Pressing the database selection button in the dialog box opens a new window showing all the database sources Jalview can access (grouped by the type of database). Once you've selected the appropriate database, hit OK close the database selection window, and then enter one or several database IDs or accession numbers separated by a semicolon and press OK. Jalview will then attempt to retrieve them from the chosen database. Example queries are provided for some databases to test that a source is operational, and can also be used as a guide for the type of accession numbers understood by the source.

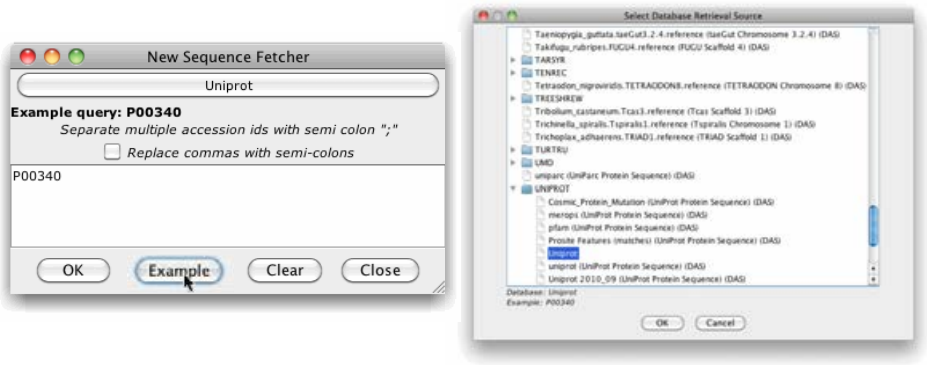


Figure 1.12: **Retrieving sequences from a public database.**

1.4.6 Memory Limits

Jalview is a Java program. One unfortunate implication of this is that Jalview cannot dynamically request additional memory from the operating system. It is important, therefore, that you ensure that you have allocated enough memory to work with your data. On most occasions, Jalview will warn you when you have tried to load an alignment that is too big to fit in to memory (for instance, some of the PFAM alignments are **very** large). You can find out how much memory is available to Jalview with the desktop window's ⇒ *Tools* ⇒ *Show Memory Usage* function, which enables the display of the currently available memory at the bottom left hand side of the Desktop window's background. Should you need to increase the amount of memory available to Jalview, full instructions are given

in the built in documentation (opened by selecting *Help* ⇒ *Documentation*) and on the JVM memory parameters page (`http://www.jalview.org/jvmmemoryparams.html`).

Exercise 3: Loading Sequences

3.a. Use *Window* ⇒ *Close All* from the Desktop window menu to close all windows.

3.b. **Loading sequences from URL:** Selecting File ⇒ *Input Alignment* ⇒ *From URL* from the Desktop and enter http://www.jalview.org/tutorial/alignment.fa in the box. Click *OK* to load the alignment.

3.c. **Loading sequences from a file:** Close all windows using the *Window* ⇒ *Close All* menu option from the Desktop. Then type the same URL (`http://www.jalview.org/tutorial/alignment.fa`) into your web browser and save the file to your desktop. Open the file you have just saved in Jalview by selecting *File* ⇒ *Input Alignment* ⇒ *From File* from the desktop menu and selecting this file. Click *OK* to load.

3.d. **Loading sequences by 'Drag and Drop' / 'Cut and Paste':**
(i) Select *Desktop* ⇒ *Window* ⇒ *Close All*. Then drag the alignment.fa file from the desktop and drop it onto the Jalview window, the alignment should open.
(ii) Test the differences between (a) dragging the sequence onto the Jalview desktop and (b) dragging the sequence onto an existing alignment window.
(iii) Open `http://www.jalview.org/tutorial/alignment.fa` in a web browser. Drag the URL directly from browser onto Jalview desktop. (If the URL is downloaded, then locate the file in your download directory and open it in a text editor.)

3.e. **The text editor:** (i) Open the alignment.fa file using text editor. Copy the sequence text from the file into the clipboard and paste it into the desktop background by right-clicking and selecting the *Paste to New Window* menu option.
(ii) In the text editor, copy the sequence text from alignment.fa into the clipboard (usually *via* the browser's *Edit* ⇒ *Copy* menu option).
(iii) In the Desktop menu, select *File* ⇒ *Input Alignment* ⇒ *From Textbox*. Paste the clipboard into the large window using the *Edit* ⇒ *Paste* text box menu option. Click *New Window* and the alignment will be loaded.

3.f. **Loading sequences from Public Database:** (i) Select *File* ⇒ *Fetch Sequence(s)...* from the Desktop. The *Select Database Retrieval Source* dialog will open showing all the database sources. Select the **PFAM seed** database and click *OK*.
(ii)Once a source has been selected, the *New Sequence Fetcher* window will open. Enter the accession number **PF03460** and click *OK*. An alignment of about 174 sequences should load.

3.g. These can be viewed using the Overview window accessible from *View* ⇒ *Overview Window*. Several database IDs can be loaded by using semicolons to separate them.

See the video at: `http://www.jalview.org/Help/Getting-Started`

1.5 Saving Sequences and Alignments

1.5.1 Saving Alignments

Jalview allows alignments to be saved to file in a variety of formats so they can be restored at a later date, passed to colleagues or analysed in other programs. From the alignment window menu select *File* ⇒ *Save As* and a dialog box will appear (Figure 1.13). You can navigate to an appropriate

directory in which to save the alignment. Jalview will remember the last filename and format used to save (or load) the alignment, enabling you to quickly save the file during or after editing by using the *File* ⇒ *Save* entry. The *File* ⇒ *Output To Textbox* menu option allows the alignment to be copied and pasted into other documents or web servers.

Jalview offers several different formats in which an alignment can be saved. Of these, only the jalview project format (.jar or .jvp) will preserve the colours, groupings and other additional information in the alignment. The other formats produce text files containing just the sequences with no visualization information, although some allow limited annotation and sequence features to be stored (e.g. AMSA). Unfortunately, as far as we are aware only Jalview can read Jalview project files.

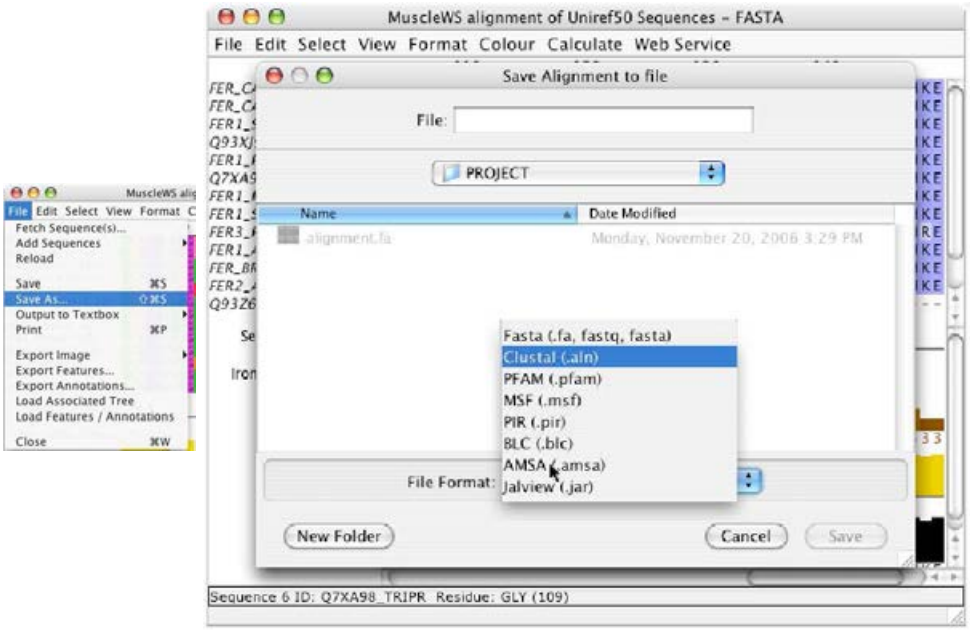


Figure 1.13: **Saving alignments in Jalview to disk.**

1.5.2 Jalview Projects

If you wish to save a complete Jalview session rather than just a single alignment (e.g. because you have calculated trees or multiple different alignments) then save your work as a Jalview Project file (.jvp).[14] From the main menu select *File* ⇒ *Save Project* and a file save dialog box will appear. Loading a project will restore Jalview to exactly the view at which the file was saved, complete with all alignments, trees, annotation and displayed structures rendered appropriately.

[14]Tip: Ensure that you have allocated plenty of memory to Jalview when working with large alignments in Jalview projects. See Section 1.4.6 for how to do this.

Exercise 4: Saving Alignments

4.a. Launch Jalview afresh, or use *Desktop* ⇒ *Window* ⇒ *Close all* .

4.b. Load the ferredoxin alignment (**PF03460**) from **PFAM (seed)** (see Exercise 3).

4.c. Select *File* ⇒ *Save As* from the alignment window menu. Choose a location into which to save the alignment and select your preferred format. All formats except *Jalview* jvp can be viewed in a normal text editor (e.g. Notepad) or in a web browser. Enter a file name and click *Save*.

4.d. Check this file by closing all windows and opening it with Jalview, or by browsing to it with your web browser.

4.e. Repeat the previous steps saving the files in different file formats.

4.f. Select *File* ⇒ *Output to Textbox* ⇒ *FASTA*. Select and copy this alignment to the clipboard using the textbox menu options *Edit* ⇒ *Select All* followed by *Edit* ⇒ *Copy*. The alignment can then be pasted into any application of choice, e.g. a word processor or web form.

4.g. Ensure at least one alignment window is active in Jalview. Open the overview window *View* ⇒ *Overview Window* and scroll red box to any part of the alignment. Select *File* ⇒ *Save Project* from the main menu and save the project in a suitable folder.

4.h. Close all windows and then load the project *via* the *File* ⇒ *Load Project* menu option. Observe how all the windows and positions are exactly as they were when they were saved.

See the video at: `http://www.jalview.org/Help/Getting-Started`**.**

Chapter 2

Selecting and Editing Sequences

Jalview makes extensive use of selections - most of the commands available from its menus operate on the *currently selected region* of the alignment, either to change their appearance or perform some kind of analysis. This section illustrates how to make and use selections and groups.

2.1 Selecting Parts of an Alignment

Selections can be of arbitrary regions in an alignment, one or more complete columns, or one or more complete sequences. A selected region can be copied and pasted as a new alignment using the *Edit* ⇒ *Copy* and *Edit* ⇒ *Paste* ⇒ *To New Alignment* in the alignment window menu options. **To clear (unselect) the selection press the [ESC] (escape) key.**

2.1.1 Selecting Arbitrary Regions

To select part of an alignment, place the mouse at the top left corner of the region you wish to select. Press and hold the mouse button and drag the mouse to the bottom right corner of the chosen region then release the mouse button. A dashed red box appears around the selected region (Figure 2.1).

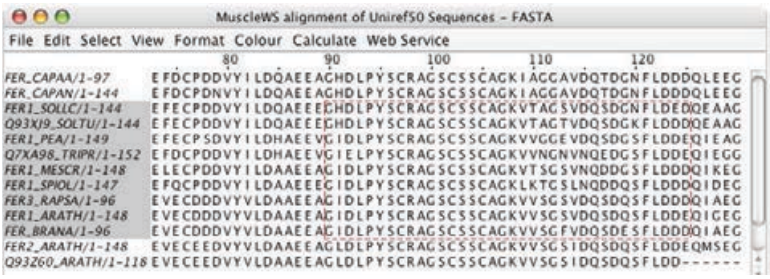


Figure 2.1: **Selecting a region in an alignment.**

2.1.2 Selecting Columns

To select the same residues in all sequences, click and drag along the alignment ruler. This selects the entire column of the alignment. Ranges of positions from the alignment ruler can also be selected by clicking on the first position and then holding down the [SHIFT] key whilst clicking the other end of the selection. Discontinuous regions can be selected by holding down [CTRL] and clicking on positions to add to the column selection. Note that each [CTRL]-Click (PC) or [CMD]-Click (Mac) changes the current selected sequence region to that column, but adds to the column selection. Selected columns are indicated by red highlighting in the ruler bar (Figure 2.2).

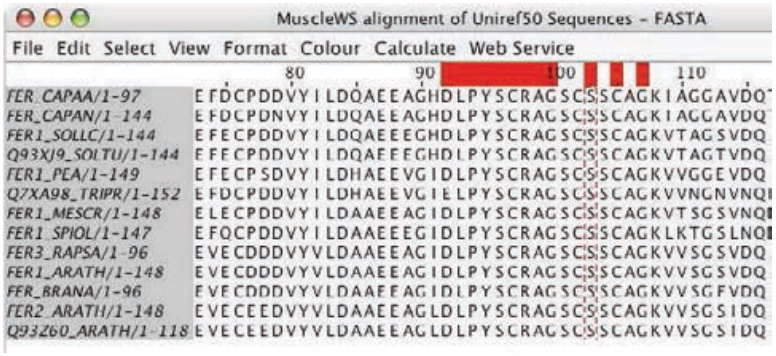


Figure 2.2: **Selecting multiple columns in an alignment.** The red highlighting on the alignment ruler marks the selected columns. Note that only the most recently selected column has a dashed-box around it to indicate a region selection.

2.1.3 Selecting Sequences

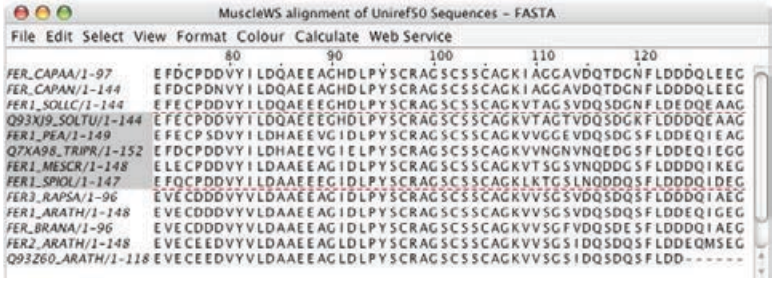


Figure 2.3: **Selecting multiple sequences in an alignment.** Use [CTRL] or [SHIFT] to select many sequences at once.

To select multiple complete sequences, click and drag the mouse down the sequence ID panel. The same techniques as used for columns (above) can be used with [SHIFT]-Click for continuous and [CTRL]-Click to select discontinuous ranges of sequences (Figure 2.3).

2.1.4 Making Selections in Cursor Mode

To define a selection in cursor mode (which is enabled by pressing [F2] when the alignment window is selected), navigate to the top left corner of the proposed selection (using the mouse, the arrow

keys, or the keystroke commands described in Section 1.3.2). Pressing the [Q] key marks this as the corner. A red outline appears around the cursor (Figure 2.4).

Navigate to the bottom right corner of the proposed selection and press the [M] key. This marks the bottom right corner of the selection. The selection can then be treated in the same way as if it had been created in normal mode.

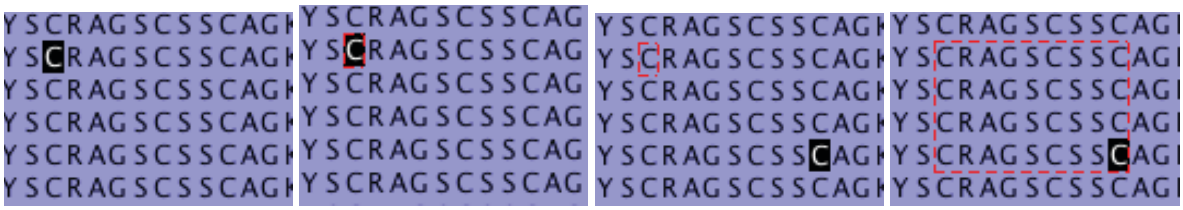


Figure 2.4: **Making a selection in cursor mode.** Navigate to the top left corner (left), press [Q], navigate to the bottom right corner and press [M] (right).

2.1.5 Inverting the Current Selection

The current sequence or column selection can be inverted, using *Select* ⇒ *Invert Sequence/Column Selection* in the alignment window. Inverting the selection is useful when selecting large regions in an alignment, simply select the region that is to be kept unselected, and then invert the selection. This may also be useful when hiding large regions in an alignment (see Section 2.5 below). Instead of selecting the columns and rows that are to be hidden, simply select the region that is to be kept visible, invert the selection, then select *View* ⇒ *Hide* ⇒ *Selected Region*.

2.2 Creating Groups

Selections are lost as soon as a different region is selected. Groups can be created which are labeled regions of the alignment. To create a group, first select the region which is to comprise the group. Then click the right mouse button on the selection to bring up a context menu. Select *Selection* ⇒

Figure 2.5: **Creating a new group from a selection.**

Group ⇒ *Edit name and description of current group*[1] then enter a name for the group in the dialog box which appears.

By default the new group will have a box drawn around it. The appearance of the group can be changed (see Section 3.1 below). This group will stay defined even when the selection is removed.

Exercise 5: Making Selections and Groups

5.a. Close windows.
Load the ferredoxin alignment (**PF03460** from **PFAM (seed)**).

5.b. Selecting an arbitrary region. Choose a residue and place the mouse cursor on it (residue information will show in alignment window status bar). Click and drag the mouse to the bottom-right to create a selection. As you drag, a red box will 'rubber band' out to show the extent of the selection. Release the mouse button and a red box borders the selected region. Press [ESC] to clear this.

5.c. Select one sequence by clicking on the sequence ID panel. Note that the sequence ID takes on a highlighted background and a red box appears around the selected sequence. Hold down [SHIFT] and click another sequence ID a few positions above or below. Note how the selection expands to include all the sequences between the two positions on which you clicked. Hold down [CTRL] and then click on several sequences' IDs - both selected and unselected. Note how unselected IDs are individually added to the selection and previously selected IDs are individually deselected.

5.d. Select columns by clicking on the Alignment Ruler. Note that the selected column is marked with a red box. Hold down [SHIFT] and click a column beyond. Note the selection expands to include all the sequences between the two positions on which you clicked.

5.e. Enter Cursor mode using [F2], or [Fn]-F2 for Macs. Navigate to column 59, row 1 by pressing **5 9 , 1 [RETURN]**. Press **Q** to mark this position. Navigate to column 65, row 8 by pressing **6 5 , 8 [RETURN]**. Press **M** to complete the selection. Note to clear the selection press the [ESC] key.

5.f. To create a group from the selected the region, click the right mouse button when mouse is on the selection, this opens a context menu in the alignment window.
Open the *Selection* ⇒ *Edit New Group* ⇒ *Group Colour* menu and select *Percentage Identity*. This will turn the selected region into a group and colour it accordingly.

5.g. Hold down [CTRL] and use the mouse to select and deselect sequences in the alignment by clicking on their Sequence ID label. Note how the group expands to include newly selected sequences, and the Percentage Identity colouring changes.

5.h. Another way to resize the group is by using the mouse to click and drag the right-hand edge of the selected group.

5.i. The current selection can be exported and saved by right clicking the mouse when on the text area to open the Sequence ID context menu. Follow the menus and pick an output format (eg BLC) from the *Selection* ⇒ *Output to Textbox . . .* submenu.

5.j. In the Alignment output window that opens, try manually editing the alignment before clicking the *New Window* button. This opens the edited alignment in a new alignment window.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

[1] In earlier versions of Jalview, this entry was variously 'Group', 'Edit Group Name', or 'JGroupXXXXX' (Where XXXXX was some serial number).

2.3 Exporting the Current Selection

The current selection can be copied to the clipboard (in PFAM format). It can also be output to a textbox using the output functions in the pop-up menu obtained by right clicking the current selection. The textbox enables quick manual editing of the alignment prior to importing it into a new window (using the *New Window* button) or saving to a file with the *File* ⇒ *Save As* pulldown menu option from the text box.

2.4 Reordering an Alignment

Sequence reordering is simple. Highlight the sequences to be moved then press the up or down arrow keys as appropriate (Figure 2.6). If you wish to move a sequence up past several other sequences it is often quicker to select the group past which you want to move it and then move the group rather than the individual sequence.

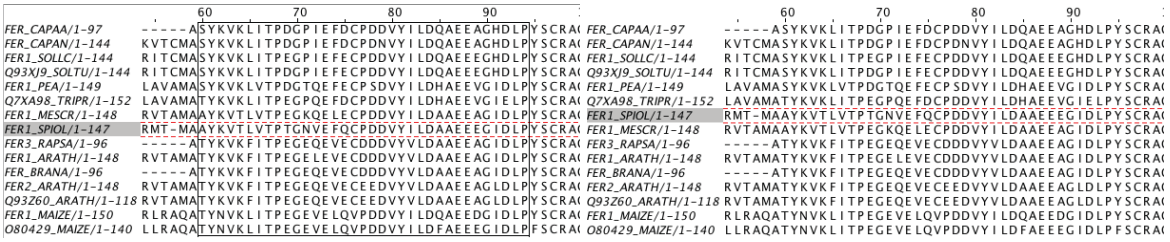


Figure 2.6: **Reordering the alignment.** The selected sequence moves up one position on pressing the ↑ key.

Exercise 6: Reordering the Alignment

6.a. Close windows.
Load the ferredoxin alignment (**PF03460** from **PFAM (seed)**).

6.b. Select one of the sequence in the sequence ID panel, use the up and down arrow keys to alter the sequence's position in the alignment. (Note that this will not work in cursor mode)

6.c. To select and move multiple sequences, use hold [SHIFT], and select two sequences separated by one or more un-selected sequences, repeat using the [CTRL] key. Note how multiple sequences are grouped together when they are re-ordered using the up and down arrow keys.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

2.5 Hiding Regions

It is sometimes convenient to exclude some sequences or residues in the alignment without actually deleting them. Jalview allows sequences or alignment columns within a view to be hidden, and this facility has been used to create the several different views in the example alignment file that is loaded when Jalview is first started (See Figure 1.4).

To hide a set of sequences, select them and right-click the mouse on the selected sequence IDs to bring up the context pop-up menu. Select *Hide Sequences* and the sequences will be concealed, with a small blue triangle indicating their position (Figure 2.7). To unhide (reveal) the sequences, right click on the triangle and select *Reveal Sequences* from the context menu.

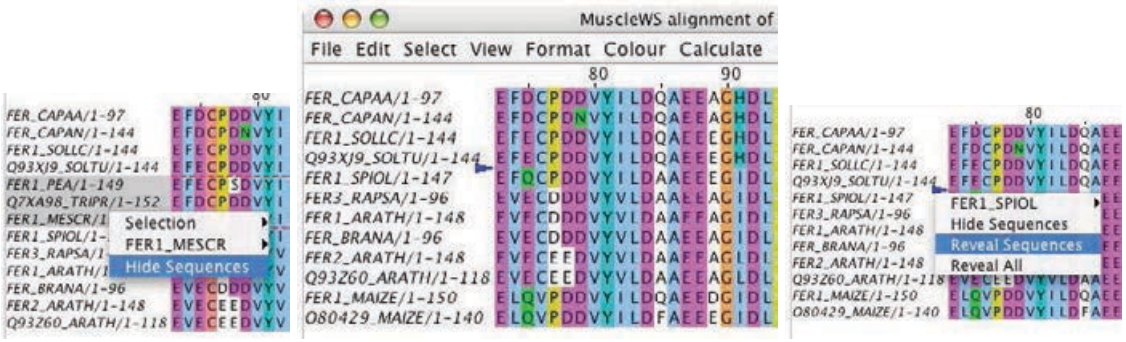


Figure 2.7: **Hiding Sequences** Hidden sequences are represented by a small blue triangle in the sequence ID panel.

A similar mechanism applies to columns (Figure 2.8). Selected columns (indicated by a red marker) can be hidden and revealed in the same way *via* the context pop-up menu by right clicking on the ruler bar. The hidden column selection is indicated by a small blue triangle in the ruler bar.

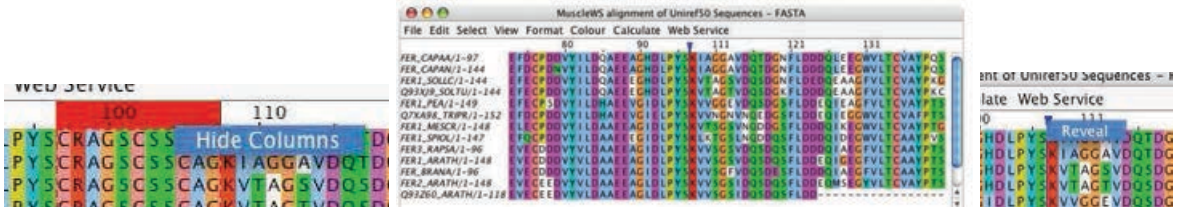


Figure 2.8: **Hiding Columns** Hidden columns are represented by a small blue triangle in the ruler bar.

It is often easier to select the region that you intend to work with, rather than the regions that you want to hide. In this case, select the required region and use the *View* ⇒ *Hide* ⇒ *All but Selected Region* menu entry, or press [Shift]+[Ctrl]+H to hide the unselected region.

2.5.1 Representing a Group with a Single Sequence

Instead of hiding a group completely, it is sometimes useful to work with just one representative sequence. The <*Sequence ID*> ⇒ *Represent group with* <*Sequence ID*> option from the sequence ID pop-up menu enables this variant of the hidden groups function. The remaining representative sequence can be visualized and manipulated like any other. However, any alignment edits that affect the sequence will also affect the whole sequence group.

Exercise 7: Hiding and Revealing Regions

7.a. Close windows.
Load the ferredoxin alignment (**PF03460** from **PFAM (seed)**).

7.b. Select a contiguous set of sequences by clicking and dragging on the sequence ID panel. Right click on the selected sequence IDs to bring up the sequence ID context menu, select *Hide Sequences.*

7.c. Right click on the blue triangle indicating hidden sequences and select *Reveal Sequences* in the panel. (If you have hidden all sequences then you will need to use the alignment window menu option *View* ⇒ *Show* ⇒ *All Sequences.*)

7.d. Repeat the process but use a non-contiguous set of sequences. Note that when multiple regions are hidden you can select either *Reveal Sequences* to reveal the hidden sequences that were clicked, or *Reveal All.*

7.e. Repeat the above using columns to hide and reveal columns instead of sequences.

7.f. Select a region of the alignment, and experiment with the *Hide all but selected region* option in *View* ⇒ *Hide* ⇒ *All but selected region.*

7.g. Select some sequences, pick one to represent the rest by hovering the mouse over this sequence. Bring up the Sequence ID context menu by right clicking and select *(Sequence ID name)* ⇒ *Represent group with (Sequence ID name).* To reveal these hidden sequences, right click on the Sequence ID and in the context menu select *Reveal All.*

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

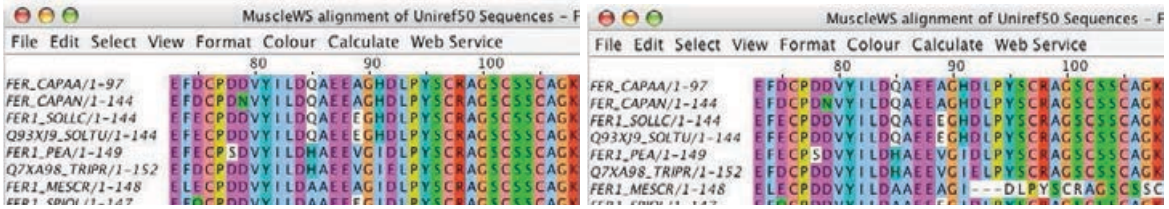


Figure 2.9: **Introducing gaps in a single sequence.** Gaps are introduced as the selected sequence is dragged to the right while pressing and holding [SHIFT].

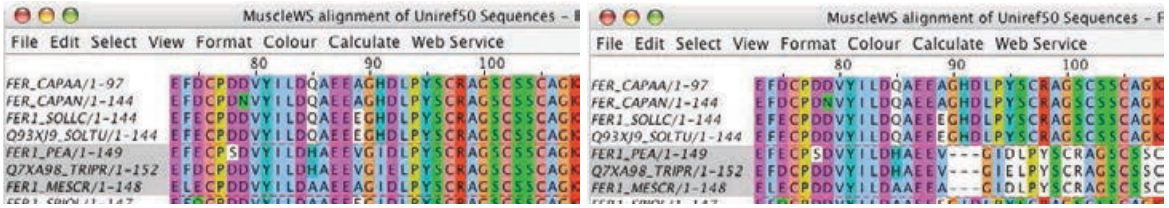


Figure 2.10: **Introducing gaps in a group.** Gaps are introduced as the selected group is dragged to the right with [CTRL] pressed.

2.6 Introducing and Removing Gaps

The alignment view provides an interactive editing interface, allowing gaps to be inserted or deleted to the left of any position in a sequence or sequence group. Alignment editing can only be performed whilst in keyboard editing mode (entered by pressing [F2]) or by clicking and dragging residues with the mouse when [SHIFT] or [CTRL] is held down (which differs from earlier versions of Jalview).

2.6.1 Undoing Edits

Alignment edits can be undone *via* the *Edit* ⇒ *Undo Edit* alignment window menu option, or CTRL-Z. An edit, if undone, may be re-applied with *Edit* ⇒ *Redo Edit*, or CTRL-Y. Note, however, that the *Undo* function only works for edits to the alignment or sequence ordering. Colouring of the alignment, showing and hiding of sequences or modification of annotation that only affect the alignment's display cannot be undone.

2.6.2 Locked Editing

The Jalview alignment editing model is different to that used in other alignment editors. Because edits are restricted to the insertion and deletion of gaps to the left of a particular sequence position, editing has the effect of shifting the rest of the sequence(s) being edited down or up-stream with respect to the rest of alignment. The *Edit* ⇒ *Pad Gaps* option can be enabled to eliminate 'ragged edges' at the end of the alignment, but does not avoid the 'knock-on' effect which is sometimes undesirable. However, its effect can be limited by performing the edit within a selected region. In this case, gaps will only be removed or inserted within the selected region. Edits are similarly constrained when they occur adjacent to a hidden column.

2.6.3 Introducing Gaps in a Single Sequence

To introduce a gap, first select the sequence in the sequence ID panel and then place the cursor on the residue to the immediate right of where the gap should appear. Hold down the SHIFT key and the left mouse button, then drag the sequence to the right until the required number of gaps has been inserted.

One common error is to forget to hold down [SHIFT]. This results in a selection which is one sequence high and one residue long. Gaps cannot be inserted in such a selection. The selection can be cleared and editing enabled by pressing the [ESC] key.

2.6.4 Introducing Gaps in all Sequences of a Group

To insert gaps in all sequences in a selection or group, select the required sequences in the sequence ID panel and then place the mouse cursor on any residue in the selection or group to the immediate right of the position in which a gap should appear. Hold down the CTRL key and the left mouse button, then drag the sequences to the right until the required number of gaps has appeared.

Gaps can be removed by dragging the residue to the immediate right of the gap leftwards whilst holding down [SHIFT] (for single sequences) or [CTRL] (for a group of sequences).

Exercise 8: Editing Alignments

You are going to manually reconstruct part of the example Jalview alignment available at http://www.jalview.org/examples/exampleFile.jar.

Mac Users: *Please use the Apple or [CMD] key in place of [CTRL] for key combinations such as [CTRL]-A.*

Remember to use [CTRL]-Z to undo an edit, or the *File* ⇒ *Reload* function to revert the alignment back to the original version if you want to start again.

8.a. Load the URL http://www.jalview.org/tutorial/unaligned.fa which contains part of the ferredoxin alignment from PF03460.

8.b. Select the first 7 sequences, and press H to hide them (or right click on the sequence IDs to open the sequence ID context menu, and select *Hide Sequences*).

8.c. Select FER3_RAPSA and FER_BRANA. Slide the sequences to the right so the initial residue A lies at column 57 using the ⇒ key.

8.d. Select FER1_SPIOL, FER1_ARATH, FER2_ARATH, Q93Z60_ARATH and O80429_MAIZE

Hint: you can do this by pressing [CTRL]-I to invert the sequence selection and then deselect FER1_MAIZE), and use the ⇒ key to slide them to so they begin at column 5 of the alignment view.

8.e. Select all the visible sequences (those not hidden) in the block by pressing [CTRL]-A. Insert a single gap in all selected sequences at column 38 of the alignment by holding [CTRL] and clicking on the R at column 38 in the FER1_SPIOL, then drag one column to right. Insert another gap at column 47 in all sequences in the same way.

8.f. Correct the ferredoxin domain alignment for FER1_SPIOL by inserting two additional gaps after the gap at column 47. First press [ESC] to clear the selection, then hold [SHIFT] and click and drag on the G and move it two columns to the right.

8.g. Now complete the alignment of FER1_SPIOL with a locked edit by pressing [ESC] and select columns 47 to 57 of the FER1_SPIOL row. Move the mouse onto the G at column 50, hold [SHIFT] and drag the G in column 47 of FER1_SPIOL to the left by one column to insert a gap at column 57.

8.h. In the next two steps you will complete the alignment of the last two sequences.

Select the last two sequences (FER1_MAIZE and O80429_MAIZE), then press [SHIFT] and click and drag the initial methionine of O80429_MAIZE 5 columns to the right so it lies at column 10.

Keep holding [SHIFT] and click and drag to insert another gap at the proline at column 25 (25C in cursor mode). Remove the gap at column 44, and insert 4 gaps at column 47 (after AAPM).

8.i. Hold [SHIFT] and drag the I at column 39 of FER1_MAIZE 2 columns to the right. Remove the gap at FER1_MAIZE column 49 by [SHIFT]-click and drag left by one column. Press [ESC] to clear the selection, and then insert three gaps in FER1_MAIZE at column 47 by holding [SHIFT] and click and drag the S in FER1_MAIZE to the right by three columns. Finally, remove the gap in O80429_MAIZE at column 56 using [SHIFT]-drag to the left on 56C.

8.j. Use the *Edit* ⇒ *Undo Edit* and *Edit* ⇒ *Redo Edit* menu option, or their keyboard shortcuts ([CTRL]-Z and [CTRL]-Y) to step backwards and replay the edits you have made.

2.6.5 Sliding Sequences

Pressing the [←] or [→] arrow keys when one or more sequences are selected will "slide" the entire selected sequences to the left or right (respectively). Slides occur regardless of the region selection - which, for example, allows you to easily reposition misaligned subfamilies within a larger alignment.

2.6.6 Editing in Cursor mode

Gaps can be easily inserted when in cursor mode (toggled with [F2]) by pressing [SPACE]. Gaps will be inserted at the cursor, shifting the residue under the cursor to the right. To insert *n* gaps type *n* and then press [SPACE]. To insert gaps into all sequences of a group, use [CTRL]-[SPACE] or [SHIFT]-[SPACE] (both keys held down together).

Gaps can be removed in cursor mode by pressing [BACKSPACE]. First make sure you have everything unselected by pressing ESC. The gap under the cursor will be removed. To remove *n* gaps, type *n* and then press [BACKSPACE]. Gaps will be deleted up to the number specified. To delete gaps from all sequences of a group, press [CTRL]-[BACKSPACE] or [SHIFT]-[BACKSPACE] (both keys held down together). Note that the deletion will only occur if the gaps are in the same columns in all sequences in the selected group, and those columns are to the right of the selected residue.

Exercise 9: Keyboard Edits
This continues on from the previous exercise, and recreates the final part of the example ferredoxin alignment from the unaligned sequences using Jalview's keyboard editing mode.
Window users: Please *only use* [SHIFT]-[SPACE] in this exercise.
Mac users: [CTRL]-[SPACE] can also be used instead of [SHIFT]-[SPACE].

9.a. Load the sequence alignment at http://www.jalview.org/tutorial/unaligned.fa, or continue using the edited alignment. If you continue from the previous exercise, first right click on the sequence ID panel and select *Reveal All.* Enter cursor mode by pressing [F2].

9.b. Insert 58 gaps at the start of the sequence 1 (FER_CAPAA). Press *58* then *[SPACE].*

9.c. Go down one sequence and select rows 2-5 as a block. Click on the second sequence ID (FER_CAPAN). Hold down shift and click on the fifth (FER1_PEA).

9.d. Insert 6 gaps at the start of this group. Go to column 1 row 2 by typing *1,2* then press *[RETURN].* Now insert 6 gaps in all the sequences. Type *6* then hold down *[SHIFT]* and press *[SPACE].*

9.e. Now insert one gap at column 34 and another at 38. Insert 3 gaps at 47. Press *34C* then *[SHIFT]-[SPACE].* Press *38C* then [SHIFT]-[SPACE]. Press *47C* then *3 [SHIFT-SPACE]* the first through fourth sequences are now aligned.

9.f. The fifth sequence (FER1_PEA) is poorly aligned. We will delete some gaps and add some new ones. Press *[ESC]* to clear the selection. Navigate to the start of sequence 5 and delete 3 gaps. Press *1,5 [RETURN]* then *3 [BACKSPACE]* to delete three gaps. Go to column 31 and delete the gap. Press *31C [BACKSPACE]* .

9.g. Similarly delete the gap now at column 34, then insert two gaps at column 38. Press *34C [BACKSPACE] 38C 2 [SPACE].* Delete three gaps at column 44 and insert one at column 47 by pressing *44C 3 [BACKSPACE] 47C [SPACE].* The top five sequences are now aligned.

Chapter 3

Colouring Sequences and Figure Generation

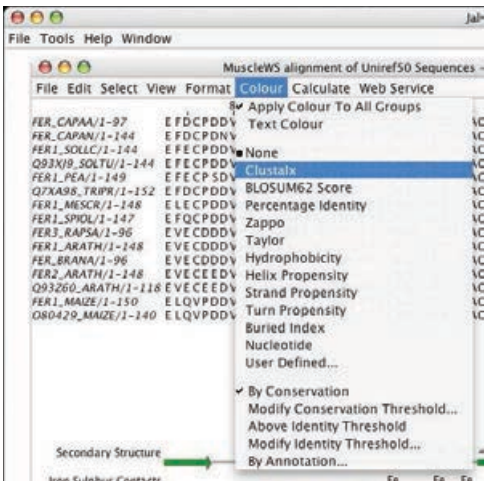
3.1 Colouring Sequences

Colouring sequences is a key aspect of alignment presentation. Jalview allows you to colour the whole alignment, or just specific groups. Alignment and group colours are rendered *below* any other colours, such as those arising from sequence features (these are described in Section 4). This means that if you try to apply one of the colourschemes described in this section, and nothing appears to happen, it may be that you have sequence feature annotation displayed, and you may have to disable it using the *View* ⇒ *Show Features* option before you can see your colourscheme.

There are two main types of colouring styles: **simple static residue** colourschemes and **dynamic schemes** which use conservation and consensus analysis to control colouring. **Hybrid colouring** is also possible, where static residue schemes are modified using a dynamic scheme. The individual schemes are described in Section 3.1.6 below.

3.1.1 Colouring the Whole Alignment

The alignment can be coloured *via* the *Colour* menu option in the alignment window. Selecting the colour scheme causes all residues to be coloured. The menu is divided into three sections. The first section gives options for the behaviour of the menu options, the second lists static and dynamic colourschemes available for selection. The last gives options for making hybrid colourschemes using conservation shading or colourscheme thresholding.



3.1.2 Colouring a Group or Selection

Selections or groups can be coloured in two ways. The first is *via* the Alignment Window's *Colour* menu as stated above, after first ensuring that the *Apply Colour To All Groups* flag is **not** selected. This must be turned *off* specifically as it is *on* by default. When unticked, selections from the Colours menu will only change the colour for residues in the current selection, or the alignment view's "background colourscheme" when no selection exists.

The second method is to select sequences and right click mouse to open pop-up menu and select *Selection* ⇒ *Edit New Group* ⇒ *Group Colour* from context menu options (Figure 3.1). This only changes the colour of the current selection or group.

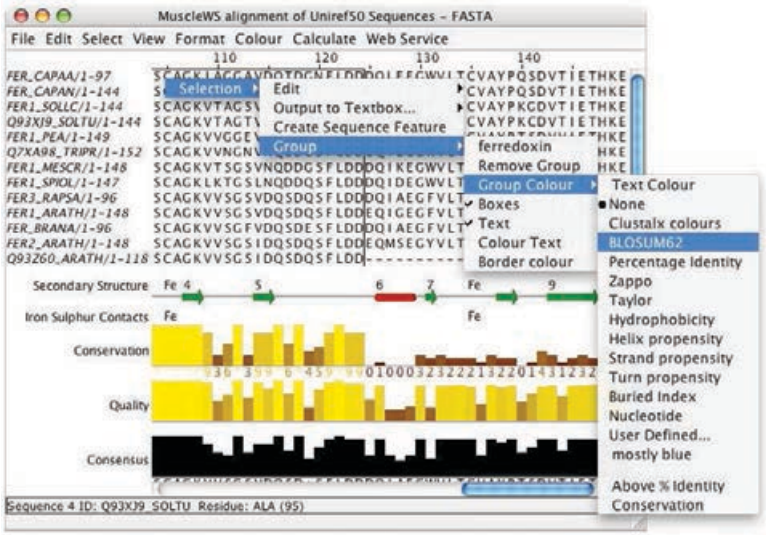


Figure 3.1: **Colouring a group via the context menu.**

3.1.3 Shading by Conservation

For many colour schemes, the intensity of the colour in a column can be scaled by the degree of amino acid property conservation. Selecting *Colour* ⇒ *By Conservation* enables this mode, and *Modify Conservation Threshold...* brings up a selection box (the *Conservation Colour Increment* dialog box) allowing the alignment colouring to be modified. Selecting a higher value limits colouring to more highly conserved columns (Figure 3.2).

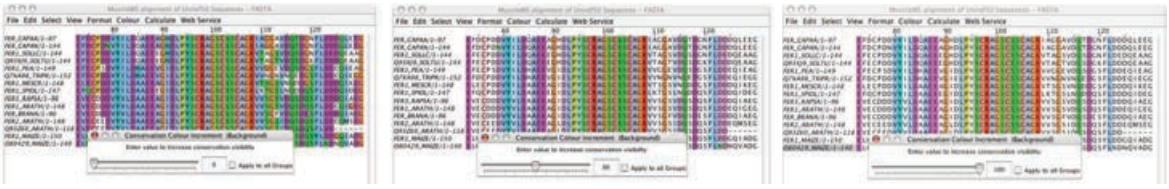


Figure 3.2: **Conservation Shading** The density of the ClustalX style residue colouring is controlled by the conservation threshold. The effect of 0% (left), 50% (center) and 100% (right) thresholds are shown.

3.1.4 Thresholding by Percentage Identity

'Thresholding' is another hybrid colour model where a residue is only coloured if it is not excluded by an applied threshold. Selecting *Colour* ⇒ *Above Identity Threshold* brings up a selection box with a slider controlling the minimum percentage identity threshold to be applied. Selecting a higher threshold (by sliding to the right) limits the colouring to columns with a higher percentage identity (as shown by the Consensus histogram in the annotation panel).

3.1.5 Colouring by Annotation

Any of the **quantitative** annotations shown on an alignment can be used to threshold or shade the whole alignment.[1]
The *Colour* ⇒ *By Annotation* option opens a dialog which allows you to select which annotation to use, the minimum and maximum shading colours or whether the original colouring should be thresholded (the 'Use original colours' option). Default settings for minimum and maximum colours can be set in the Jalview Desktop's preferences.

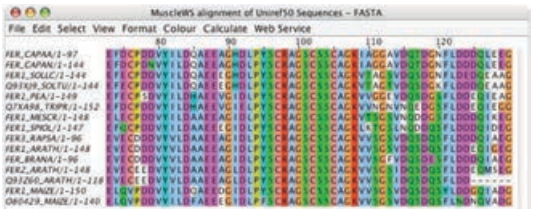
The **per Sequence** option in the **Colour By Annotation** dialog allows each sequence to be shaded according to sequence associated annotation rows, such as protein disorder scores. This functionality is described further in Section 8.2.

3.1.6 Colour Schemes

Full details on each colour scheme can be found in the Jalview on-line help. A brief description of each one is provided below:

ClustalX

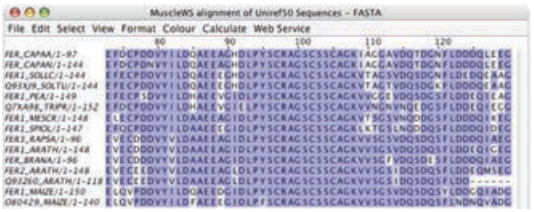
This is an emulation of the default colourscheme used for alignments in ClustalX, a graphical interface for the ClustalW multiple sequence alignment program. Each residue in the alignment is assigned a colour if the amino acid profile of the alignment at that position meets some minimum criteria specific for the residue type.



[1] Please remember to turn off Sequence Feature display to see the shading

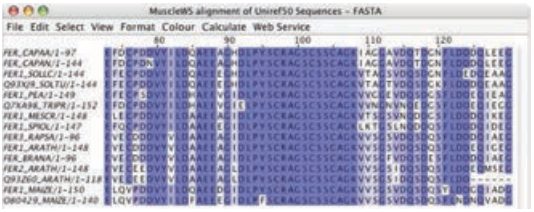
Blosum62

Gaps are coloured white. If a residue matches the consensus sequence residue at that position it is coloured dark blue. If it does not match the consensus residue but the Blosum62 matrix gives a positive score, it is coloured light blue.



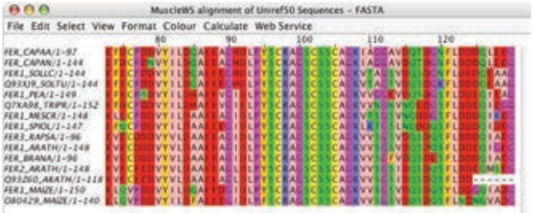
Percentage Identity

The Percent Identity option colours the residues (boxes and/or text) according to the percentage of the residues in each column that agree with the consensus sequence. Only the residues that agree with the consensus residue for each column are coloured.



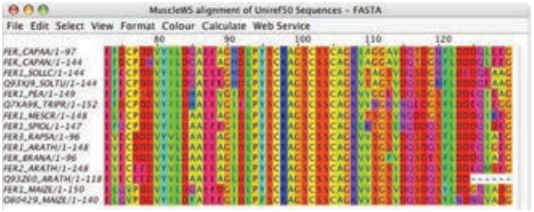
Zappo

The residues are coloured according to their physicochemical properties. The physicochemical groupings are Aliphatic/hydrophobic, Aromatic, Positive, Negative, Hydrophillic, conformationally special, and Cyst(e)ine.



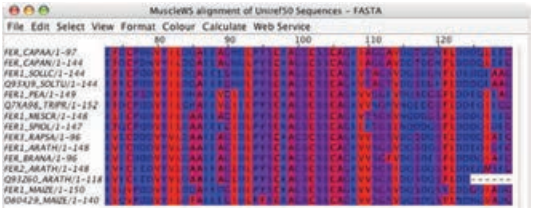
Taylor

This colour scheme was devised by Willie Taylor and an entertaining description of its origin can be found in Protein Engineering, Vol 10 , 743-746 (1997).



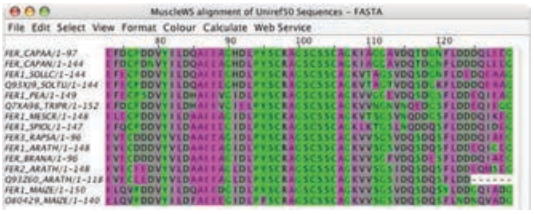
Hydrophobicity

Residues are coloured according to the hydrophobicity table of Kyte, J., and Doolittle, R.F., J. Mol. Biol. 1157, 105-132, 1982. The most hydrophobic residues are coloured red and the most hydrophilic ones are coloured blue.



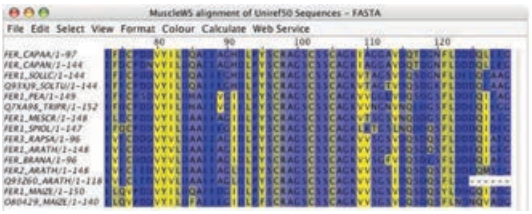
Helix Propensity

The residues are coloured according to their Chou-Fasman[2] helix propensity. The highest propensity is magenta, the lowest is green.



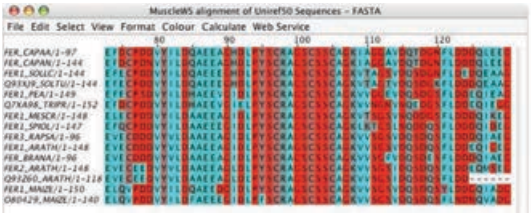
Strand Propensity

The residues are coloured according to their Chou-Fasman[2] Strand propensity. The highest propensity is Yellow, the lowest is blue.



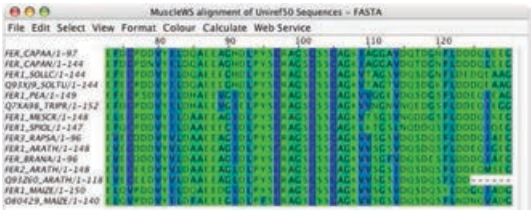
Turn Propensity

The residues are coloured according to their Chou-Fasman[2] turn propensity. The highest propensity is red, the lowest is cyan.



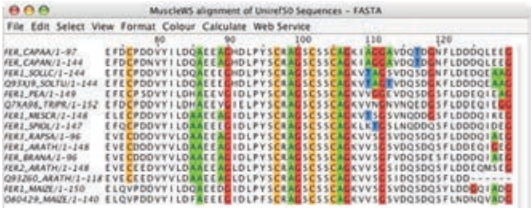
Buried Index

The residues are coloured according to their Chou-Fasman[2] burial propensity. The highest propensity is blue, the lowest is green.



Nucleotide

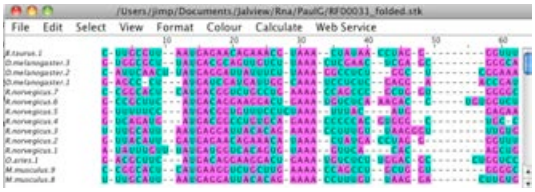
Residues are coloured with four colours corresponding to the four nucleotide bases. All non ACTG residues are uncoloured. See Section 9.1 for further information about working with nucleic acid sequences and alignments.



[2] Chou, PY and Fasman, GD. Annu Rev Biochem. 1978;47:251-76.

Purine Pyrimidine

Residues are coloured according to whether the corresponding nucleotide bases are purine (magenta) or pyrimidine (cyan) based. All non ACTG residues are uncoloured. For further information about working with nucleic acid sequences and alignments, see Section 9.1.



RNA Helix Colouring

Columns are coloured according to their assigned RNA helix as defined by a secondary structure annotation line on the alignment. Colours for each helix are randomly assigned, and option only available when an RNA secondary structure row is present on the alignment.

User Defined

This dialog allows the user to create any number of named colour schemes at will. Any residue may be assigned any colour. The colour scheme can then be named. If you save the colour scheme, this name will appear on the Colour menu (Figure 3.3).

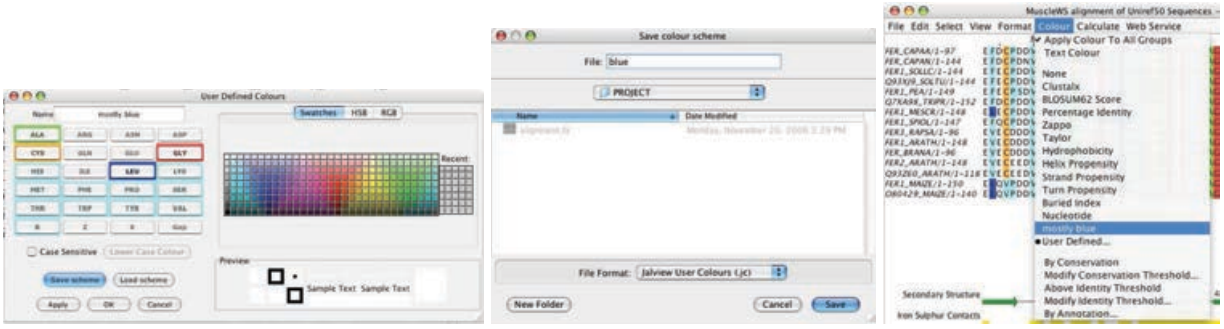


Figure 3.3: **Creation of a user defined colour scheme.** Residue types are assigned colours (left). The profile is saved (center) and can then be accessed *via* the *Colour* menu (right).

Exercise 10: Colouring Alignments

Note: Before you begin this exercise, ensure that the *Apply Colour To All Groups* flag is not selected in *Colour* menu in the alignment window.

10.a. Open a sequence alignment, for example the PFAM domain PF03460 in PFAM seed database. Select the alignment menu option *Colour* ⇒ *ClustalX* and note the colour change. Now try all the other colour schemes in the *Colour* menu. Note that some colour schemes do not colour all residues.

10.b. Colour the alignment using *Colour* ⇒ *Blosum62*. Select a group of around 4 similar sequences. Use the context menu (right click on the group) option *Selection* ⇒ *Edit New Group* ⇒ *Group Colour* ⇒ *Blosum62* to colour the selection. Notice how some residues which were not coloured are now coloured. The calculations performed for dynamic colouring schemes like Blosum62 are based on the selected group, not the whole alignment (this also explains the colouring changes observed in exercise 5 during the group selection step).

10.c. Keeping the same selection as before, colour the complete alignment except the group using *Colour* ⇒ *Taylor*. Select the menu option *Colour* ⇒ *By Conservation*. Slide the selector in the Conservation Colour Increment dialog box from side to side and observe the changes in the alignment colouring in the selection and in the complete alignment.

Note: Feature colours overlay residue colouring. The features colours can be toggled off by going to *View* ⇒ *Show Sequence Features*.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

Exercise 11: User Defined Colour Schemes

11.a. Load a sequence alignment. Select the alignment menu option *Colour* ⇒ *User Defined*. A dialog window will open.

11.b. Click on an amino acid button, then select a colour for that amino acid. Repeat till all amino acids are coloured to your liking.

11.c. Insert a name for the colourscheme in the appropriate field and click *Save Scheme*. You will be prompted for a file name in which to save the colour scheme. The dialog window can now be closed.

11.d. The new colour scheme appears in the list of colour schemes in the *Colour* menu and can be selected in future Jalview sessions.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

3.2 Formatting and Graphics Output

Jalview is a WYSIWIG alignment editor. This means that for most kinds of graphics output, the layout that is seen on screen will be the same as what is outputted in an exported graphics file. It is therefore important to pick the right kind of display layout prior to generating figures.

3.2.1 Multiple Alignment Views

Jalview is able to create multiple independent visualizations of the same underlying alignment - these are called *Views*. Because each view displays the same underlying data, any edits performed in one view will update the alignment or annotation visible in all views.

Alignment views are created using the *View* ⇒ *New View* option of the alignment window or by Pressing [CTRL]-T. This will create a new view with the same groups, alignment layout and display options as the current one. Pressing G will gather together Views as named tabs on the alignment window, and pressing X will expand gathered Views so they can be viewed simultaneously in their own separate windows. To delete a group, press [CTRL]-W.

3.2.2 Alignment Layout

Jalview provides two screen layout modes, unwrapped (the default) where the alignment is in one long line across the window, and wrapped, where the alignment is on multiple lines, each the width of the window. Most layout options are controlled by the Format menu option in the alignment window, and control the overall look of the alignment in the view (rather than just a selected region).

Wrapped Alignments

Wrapped alignments can be toggled on and off using the *Format* ⇒ *Wrap* menu option (Figure 3.4). Note that the annotation lines are also wrapped. Wrapped alignments are great for publications and presentations but are of limited use when working with large numbers of sequences.

If annotations are not all visible in wrapped mode, expand the alignment window to view them. Note that alignment annotation (see Section 4) cannot be interactively created or edited in wrapped mode, and selection of large regions is difficult.

Fonts

The text appearance in a view can be modified *via* the *Format* ⇒ *Font...* alignment window menu. This setting applies for all alignment and annotation text except for that displayed in tool-tips. Additionally, font size and spacing can be adjusted rapidly by clicking the middle mouse button and dragging across the alignment window.

Figure 3.4: **Wrapping the alignment.**

Numbering and Label Justification

Options in the *Format* menu are provided to control the alignment view, and provide a range of options to control the display of sequence and alignment numbering, the justification of sequence IDs and annotation row column labels on the annotation rows shown below the alignment.

Alignment and Group Colouring and Appearance

The display of hidden row/column markers and gap characters can be turned off with *Format* ⇒ *Hidden Markers* and *Format* ⇒ *Show Gaps*, respectively. The *Text* and *Colour Text* option controls the display of sequence text and the application of alignment and group colouring to it. *Boxes* controls the display of the background area behind each residue that is coloured by the applied colourscheme.

Highlighting Nonconserved Symbols

The alignment layout and group sub-menu both contain an option to hide conserved symbols from the alignment display (*Format* ⇒ *Show nonconserved* in the alignment window or *Selection* ⇒ *Group* ⇒ *Show Nonconserved* by right clicking on a group). This mode is useful when working with alignments that exhibit a high degree of homology, because Jalview will only display gaps or sequence symbols that differ from the consensus for each column, and render all others with a '.'.

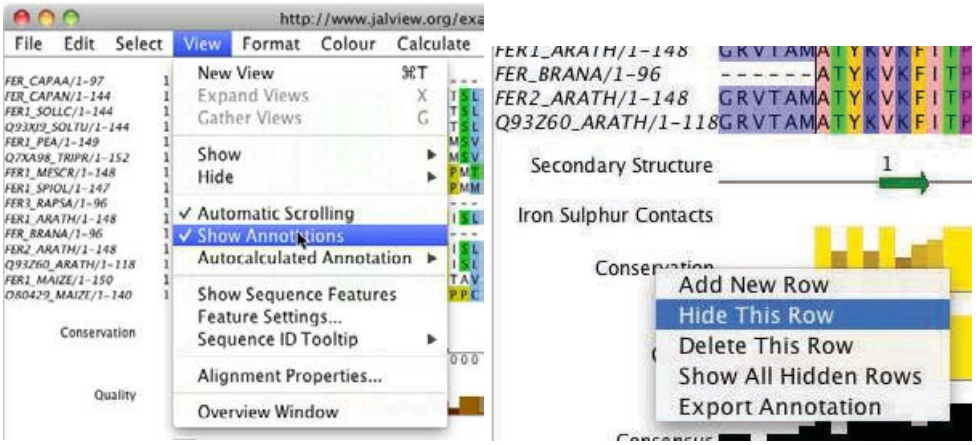


Figure 3.5: **Hiding Annotations** Annotations can either be hidden from the *View* menu (left) or individually from the context menu (right).

3.2.3 Annotation Ordering and Display

The annotation lines which appear below the sequence alignment are described in detail in Section 4. They can be hidden by toggling the *View* ⇒ *Show Annotations* menu option. Additionally, each annotation line can be hidden and revealed in the same way as sequences *via* the pop-up context menu on the annotation name panel (Figure 3.5). Annotations can be reordered by dragging the annotation line label on the annotation label panel. Placing the mouse over the top annotation label brings up a resize icon on the left. When this is displayed, Click-dragging up and down provides more space in the alignment window for viewing the annotations, and less space for the sequence alignment.

Exercise 12: Alignment Layout

12.a. Start Jalview and open the URL http://www.jalview.org/examples/exampleFile.jar. Select *Format* ⇒ *Wrap* from the alignment window menu. Experiment with the various options from the *Format* menu, for example adjust the ruler placement, sequence ID format and so on.

12.b. Hide all the annotation rows by toggling *Annotations* ⇒ *Show Annotations* from the alignment window menu. Reveal the annotations by selecting the same menu option.

12.c. Deselect *Format* ⇒ *Wrap*. Right click on the annotation row labels to bring up the context menu, then select *Hide This Row*. Bring up the context menu again and select *Show All Hidden Rows* to reveal them.

12.d. Annotations can be reordered by clicking and dragging the row to the desired position. Click on the *Consensus* row and drag it upwards to just above *Quality*. The rows should now be reordered. Features and annotations are covered in more detail in Section 4.

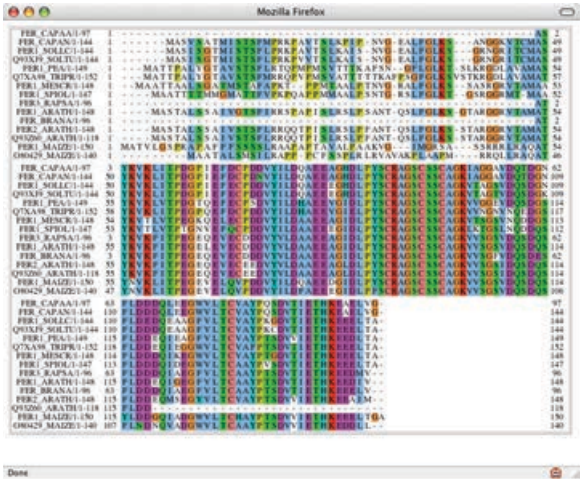
12.e. Move the mouse to the top left hand corner of the annotation labels - a grey up/down arrow symbol should appear - when this is shown, the height of the *Annotation Area* can be changed by clicking and dragging this icon up or down.

3.2.4 Graphical Output

Jalview allows alignments figures to be exported in three different formats, each of which is suited to a particular purpose. Image export is *via* the *File* ⇒ *Export Image* ⇒ ... alignment window menu option.

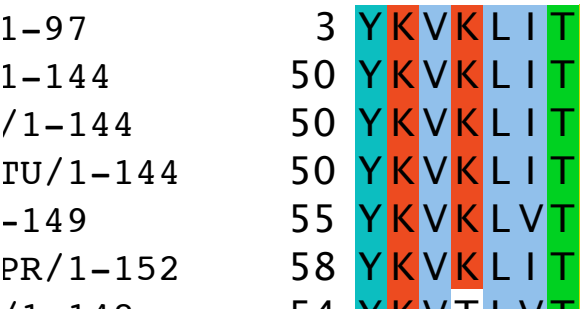
HTML

HTML is the format used by web pages. Jalview outputs the alignment as an HTML table with all the colours and fonts as seen. Any additional annotation will also be embedded as sensitive areas on the page, such as URL links for each sequence's ID label. This file can then be viewed directly with any web browser. Each residue is placed in an individual table cell. Unwrapped alignments will produce a very wide page.



EPS

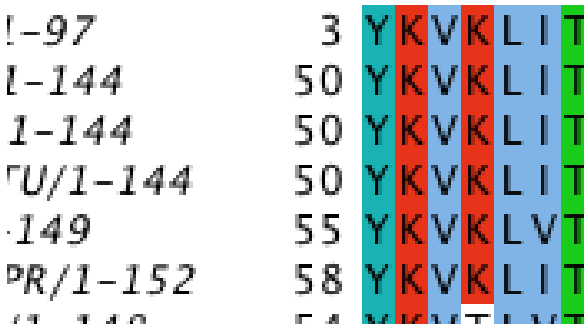
EPS is Encapsulated Postscript. **It is the format of choice for publications and posters** as it gives the highest quality output of any of the image types. It can be scaled to any size, so will still look good on an A0 poster. This format can be read by most good presentation and graphics packages such as Adobe Illustrator or Inkscape.



Zoom Detail of EPS image.

PNG

PNG is Portable Network Graphics. This output option produces an image that can be easily included in web pages and incorporated in presentations using e.g. Powerpoint or Open Office. It is a bitmap image so does not scale and is unsuitable for use on posters, or in publications.
For submission of alignment figures to journals, please use EPS[3].



Zoom Detail of PNG image.

Exercise 13: Graphical Output

13.a. Load the example Jalview Jar file in Exercise 12. Customise it how you wish but leave it unwrapped. Select *File* ⇒ *Export Image* ⇒ *HTML* from the alignment menu. Save the file and open it in your favoured web browser.

13.b. Wrap the alignment and export the image to HTML again. Compare the two images. (Note that the exported image matches the format displayed in the alignment window but **annotations are not exported**).

13.c. Export the alignment using the *File* ⇒ *Export Image* ⇒ *PNG* menu option. Open the file in an image viewer that allows zooming such as Paint or Photoshop (Windows), or Preview (Mac OS X) and zoom in. Notice that the image is a bitmap and it becomes pixelated when zoomed. (Note that the **annotation lines are included** in the image.)

13.d. Export the alignment using the *File* ⇒ *Export Image* ⇒ *EPS* menu option. Open the file in a suitable program such as Photoshop, Illustrator, Inkscape, Ghostview, Powerpoint (Windows), or Preview (Mac OS X). Zoom in and note that the image has near-infinite resolution.

[3] If the journal complains, *insist*.

Chapter 4

Annotation and Features

Annotations and features are additional information that is overlaid on the sequences and the alignment. Generally speaking, annotations reflect properties of the alignment as a whole, often associated with columns in the alignment. Features are often associated with specific residues in the sequence.

Annotations are shown below the alignment in the annotation panel, the properties are often based on the alignment. Conversely, sequence features are properties of the individual sequences, so they do not change with the alignment, but are shown mapped on to specific residues within the alignment.

Features and annotation can be interactively created, or retrieved from external data sources. Webservices like JPred (see 8.1 above) can be used to analyse a given sequence or alignment and generate annotation for it.

4.1 Conservation, Quality and Consensus Annotation

Jalview automatically calculates several quantitative alignment annotations which are displayed as histograms below the multiple sequence alignment columns. Conservation, quality and consensus scores are examples of dynamic annotation, so as the alignment changes, they change along with it. The scores can be used in the hybrid colouring options to shade the alignments. Mousing over a conservation histogram reveals a tooltip with more information.

These annotations can be hidden and deleted via the context menu linked to the annotation row; but they are only created on loading an alignment. If they are deleted then the alignment should be saved and then reloaded to restore them. Jalview provides a toggle to autocalculate a consensus sequence upon editing. This is normally selected by default, but can be turned off for large alignments *via* the *Calculate* ⇒ *Autocalculate Consensus* menu option if the interface is too slow.

Conservation Annotation

Alignment conservation annotation is quantitative numerical index reflecting the conservation of the physico-chemical properties for each column of the alignment. The calculation is based on AMAS method of multiple sequence alignment analysis (Livingstone C.D. and Barton G.J. (1993) CABIOS Vol. 9 No. 6 p745-756), with identities scoring highest, and amino acids with substitutions in the same physico-chemical class have next highest score. The score for each column is shown below the histogram. The conserved columns with a score of 11 are indicated by '*'. Columns with a score of 10 have mutations but all properties are conserved are marked with a '+'.

Consensus Annotation

Alignment consensus annotation reflects the percentage of the different residue per column. By default this calculation includes gaps in columns, gaps can be ignored via the Consensus label context menu to the left of the consensus bar chart. The consensus histogram can be overlaid with a sequence logo that reflects the symbol distribution at each column of the alignment. Right click on the Consensus annotation row and select the *Show Logo* option to display the Consensus profile for the group or alignment. Sequence logos can be enabled by default for all new alignments *via* the Visual tab in the Jalview desktop's preferences dialog box.

Quality Annotation

Alignment quality annotation is an ad-hoc measure of the likelihood of observing the mutations (if any) in a particular column of the alignment. The quality score is calculated for each column in an alignment by summing, for all mutations, the ratio of the two BLOSUM 62 scores for a mutation pair and each residue's conserved BLOSUM62 score (which is higher). This value is normalised for each column, and then plotted on a scale from 0 to 1.

Group Associated Annotation

Group associated consensus and conservation annotation rows reflect the sequence variation within a particular group. Their calculation is enabled by selecting the *Group Conservation* or *Group Consensus* options in the *Annotation* ⇒ *Autocalculated Annotation* submenu of the alignment window.

4.1.1 Creating User Defined Annotation

To create a new annotation row, right click on the annotation label panel and select the *Add New Row* menu option (Figure 4.1). A dialog box appears. Enter the label to use for this row and a new row will appear.

To create a new annotation, first select all the positions to be annotated on the appropriate row. Right-clicking on this selection brings up the context menu which allows the insertion of graphics for secondary structure (*Helix* or *Sheet*), text *Label* and the colour in which to present the annotation

(Figure 4.2). On selecting *Label* a dialog box will appear, requesting the text to place at that position. After the text is entered, the selection can be removed and the annotation becomes clearly visible[1]. Annotations can be coloured or deleted as desired.

Figure 4.1: **Creating a new annotation row.** Annotation rows can be reordered by dragging them to the desired place.

Figure 4.2: **Creating a new annotation.** Annotations are created from a selection on the annotation row and can be coloured as desired.

4.1.2 Automated Annotation of Alignments and Groups

On loading a sequence alignment, Jalview will normally[2] calculate a set of automatic annotation rows which are shown below the alignment. For nucleotide sequence alignments, only an alignment consensus row will be shown, but for amino acid sequences, alignment quality (based on BLOSUM 62) and physicochemical conservation will also be shown. Conservation is calculated according to Livingstone and Barton[3]. Consensus is the modal residue (or + where there is an equal top residue). The inclusion of gaps in the consensus calculation can be toggled by right-clicking on the Consensus label and selecting *Ignore Gaps in Consensus* from the pop-up context menu located with consensus annotation row. Quality is a measure of the inverse likelihood of unfavourable mutations in the alignment. Further details on these calculations can be found in the on-line documentation.

[1] When annotating a block of positions, the text can be partly obscured by the selection highlight. Pressing the [ESC] key clears the selection and the label is then visible.

[2] Automatic annotation can be turned off in the *Visual* tab in the *Tools* ⇒ *Preferences* dialog box.

[3] *"Protein Sequence Alignments: A Strategy for the Hierarchical Analysis of Residue Conservation."* Livingstone C.D. and Barton G.J. (1993) *CABIOS* **9**, 745-756

Exercise 14: Annotating Alignments

14.a. Load the alignment at http://www.jalview.org/tutorial/alignment.fa. Right-click on the *Conservation* annotation row to bring up the context menu and select *Add New Row*. A dialog box will appear asking for *Annotation Name* and *Annotation Description*. Enter "Iron binding site" and click *OK*. A new, empty, row appears.

14.b. Navigate to column 97. Move down and on the new annotation row called "Iron binding site, select column 97. Right click at this selection and select *Label* from the context menu. Enter "Fe" in the box and click *OK*. Right-click on the selection again and select *Colour*. Choose a colour from the colour chooser dialog and click *OK*. Press [ESC] to remove the selection.

Note: depending on your Annotation sort settings, your newly created annotation row might 'jump' to the top or bottom of the annotation panel. Just scroll up or down to find it again - the column you marked will still be selected.

14.c. Select columns 70-77 on the annotation row. Right-click and choose *Sheet* from the context menu. You will be prompted for a label. Enter "B" and press *OK*. A new line showing the sheet as an arrow appears. The colour of the label can be changed but not the colour of the sheet arrow.

14.d. Right click on the title text of annotation row that you just created. Select *Export Annotation* in context menu and, in the Export Annotation dialog box that will open, select the Jalview format and click the *[To Textbox]* button.

The format for this file is given in the Jalview help. Press [F1] to open it, and find the "Annotations File Format" entry in the "Alignment Annotations" section of the contents pane.

14.e. Export the file to a text editor and edit the file to change the name of the annotation row. Save the file and drag it onto the alignment view.

14.f. Add an additional helix somewhere along the row by editing the file and re-importing it.

Hint: Use the Export Annotation function to view what helix annotation looks like in a Jalview annotation file.

14.g. Use the *Alignment Window* ⇒ *File* ⇒ *Export Annotations...* function to export all the alignment's annotation to a file.

14.h. Open the exported annotation in a text editor, and use the Annotation File Format documentation to modify the style of the Conservation, Consensus and Quality annotation rows so they appear as several lines on a single line graph.

Hint: You need to change the style of annotation row in the first field of the annotation row entry in the file, and create an annotation row grouping to overlay the three quantitative annotation rows.

14.i. **Homework for after you have completed exercise 27:**

Recover or recreate the secondary structure predictions that you made from JPred. Use the *File* ⇒ *Export Annotation* function to view the Jnet secondary structure prediction annotation row.

Note the SEQUENCE_REF statements surrounding the row specifying the sequence association for the annotation.

4.2 Importing Features from Databases

Jalview supports feature retrieval from public databases. It includes built in parsers for Uniprot and ENA (or EMBL) records retrieved from the EBI. Sequences retrieved from these sources using the

sequence fetcher (see Section 1.4.5) will already possess features.

4.2.1 Sequence Database Reference Retrieval

Jalview maintains a list of external database references for each sequence in an alignment. These are listed in a tooltip when the mouse is moved over the sequence ID when the *View* ⇒ *Sequence ID Tooltip* ⇒ *Show Database Refs* option is enabled. Sequences retrieved using the sequence fetcher will always have at least one database reference, but alignments imported from an alignment file generally have no database references.

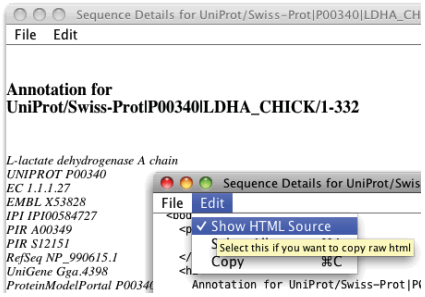
Database References and Sequence Coordinate Systems

Jalview displays features in the local sequence's coordinate system which is given by its 'start' and 'end'. Any sequence features on the sequence will be rendered relative to the sequence's start position. If the start/end positions do not match the coordinate system from which the features were defined, then the features will be displayed incorrectly.

Viewing and Exporting a Sequence's Database Annotation

You can export all the database cross references and annotation terms shown in the sequence ID tooltip for a sequence by right-clicking and selecting the *[Sequence ID]* ⇒ *Sequence details . . .* option from the popup menu. A similar option is provided in the *Selection* sub-menu allowing you to obtain annotation for the sequences currently selected.

The *Sequence Details . . .* option will open a window containing the same text as would be shown in the tooltip window, including any web links associated with the sequence. The text is HTML, and options on the window allow the raw code to be copied and pasted into a web page.



Automatically Discovering a Sequence's Database References

Jalview includes a function to automatically verify and update each sequence's start and end numbering against any of the sequence databases that the *Sequence Fetcher* has access to. This function is accessed from the *Webservice* ⇒ *Fetch DB References* sub-menu in the Alignment window. This menu allows you to query either the set of *Standard Databases*, which includes EMBL, Uniprot, the PDB, or just a specific datasource from one of the submenus. When one of the entries from this menu is selected, Jalview will use the ID string from each sequence in the alignment or in the currently selected set to retrieve records from the external source. Any sequences that are retrieved are matched against the local sequence, and if the local sequence is found to be a sub-sequence of the retrieved

sequence then the local sequence's start/end numbering is updated. A new database reference mapping is created, mapping the local sequence to the external database, and the local sequence inherits any additional annotation retrieved from the database sequence.

The database retrieval process terminates when a valid mapping is found for a sequence, or if all database queries failed to retrieve a matching sequence. Termination is indicated by the disappearance of the moving progress indicator on the alignment window. A dialog box may be shown once it completes which lists sequences for which records were found, but the sequence retrieved from the database did not exactly contain the sequence given in the alignment (the *"Sequence not 100% match" dialog box*).

The Fetch Uniprot IDs Dialog Box

If any sources are selected which refer to Uniprot coordinates as their reference system, then you may be asked if you wish to retrieve Uniprot IDs for your sequence. Pressing OK instructs Jalview to verify the sequences against Uniprot records retrieved using the sequence's ID string. This operates in much the same way as the *Web Service* ⇒ *Fetch Database References* function described in Section 4.2.1. If a sequence is verified, then the start/end numbering will be adjusted to match the Uniprot record.

Rate of Feature Retrieval

Feature retrieval can take some time if a large number of sources are selected and if the alignment contains a large number of sequences. As features are retrieved, they are immediately added to the current alignment view. The retrieved features are shown on the sequence and can be customised as described previously.

4.2.2 Colouring Features by Score or Description Text

Sometimes, you may need to visualize the differences in information carried by sequence features of the same type. This is most often the case when features of a particular type are the result of a specific type of database query or calculation. Here, they may also carry information within their textual description, or most commonly for calculations, a score related to the property being investigated. Jalview can shade sequence features using a graduated colourscheme in order to highlight these variations. In order to apply a graduated scheme to a feature type, select the 'Graduated colour' entry in the Sequence *Feature Type*'s popup menu, which is opened by right-clicking the *Feature Type* or *Color* in the *Sequence Feature Settings* dialog box. Two types of colouring styles are currently supported: the default is quantitative colouring, which shades each feature based on its score, with the highest scores receiving the 'Max' colour, and the lowest scoring features coloured with the 'Min' colour. Alternately, you can select the 'Colour by label' option to create feature colours according to the description text associated with each feature. This is useful for general feature types - such as Uniprot's 'DOMAIN' feature - where the actual type of domain is given in the feature's description.

Graduated feature colourschemes can also be used to exclude low or high-scoring features from the alignment display. This is done by choosing your desired threshold type (either above or below),

using the drop-down menu in the dialog box. Then, adjust the slider or enter a value in the text box to set the threshold for displaying this type of feature.

The feature settings dialog box allows you to toggle between a graduated and simple feature colourscheme using the pop-up menu for the feature type. When a graduated scheme is applied, it will be indicated in the colour column for that feature type - with coloured blocks or text to indicate the colouring style and a greater than (>) or less than (<) symbol to indicate when a threshold has been defined.

4.2.3 Using Features to Re-order the Alignment

The presence of sequence features on certain sequences or in a particular region of an alignment can quantitatively identify important trends in the aligned sequences. In this case, it is more useful to re-order the alignment based on the number of features or their associated scores, rather than simply re-colour the aligned sequences. The sequence feature settings dialog box provides two buttons: 'Seq sort by Density' and 'Seq sort by Score', that allow you to reorder the alignment according to the number of sequence features present on each sequence, and also according to any scores associated with a feature. Each of these buttons uses the currently displayed features to determine the ordering, but if you wish to re-order the alignment using a single type of feature, then you can do this from the *Feature Type*'s popup menu. Simply right-click the type's style in the Sequence Feature Settings dialog box, and select one of the *Sort by Score* and *Sort by Density* options to re-order the alignment. Finally, if a specific region is selected, then only features found in that region of the alignment will be used to create the new alignment ordering.

4.2.4 Creating Sequence Features

Sequence features can be created simply by selecting the area in a sequence (or sequences) to form the feature and selecting *Selection* ⇒ *Create Sequence Feature* from the right-click context menu (Figure 4.3). A dialog box allows the user to customise the feature with respect to name, group, and colour. The feature is then associated with the sequence. Moving the mouse over a residue associated with a feature brings up a tool tip listing all features associated with the residue.

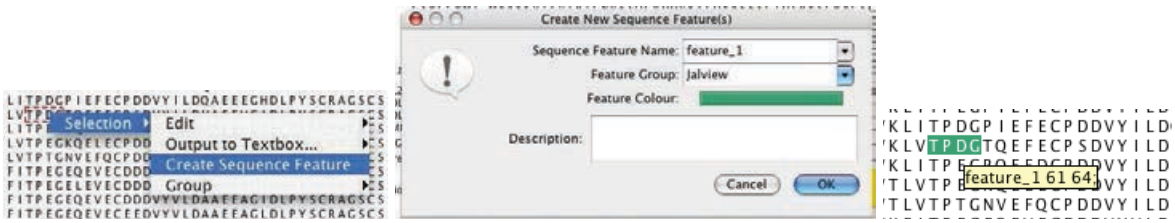


Figure 4.3: **Creating sequence features.** Features can readily be created from selections via the context menu and are then displayed on the sequence.

Creation of features from a selection spanning multiple sequences results in the creation of one feature per sequence. Each feature remains associated with its own sequence.

4.2.5 Customising Feature Display

Feature display can be toggled on or off by selecting the *View* ⇒ *Show Sequence Features* menu option. When multiple features are present it is usually necessary to customise the display. Jalview allows the display, colour, rendering order and transparency of features to be modified *via* the *View* ⇒ *Feature Settings...* menu option. This brings up a dialog window (Figure 4.5) which allows the visibility of individual feature types to be selected, colours changed (by clicking on the colour of each sequence feature type) and the rendering order modified by dragging feature types to a new position in the list. Dragging the slider alters the transparency of the feature rendering. The Feature Settings dialog also includes functions for more advanced feature shading schemes and buttons for sorting the alignment according to the distribution of features. These capabilities are described further in sections 4.2.2 and 4.2.3.

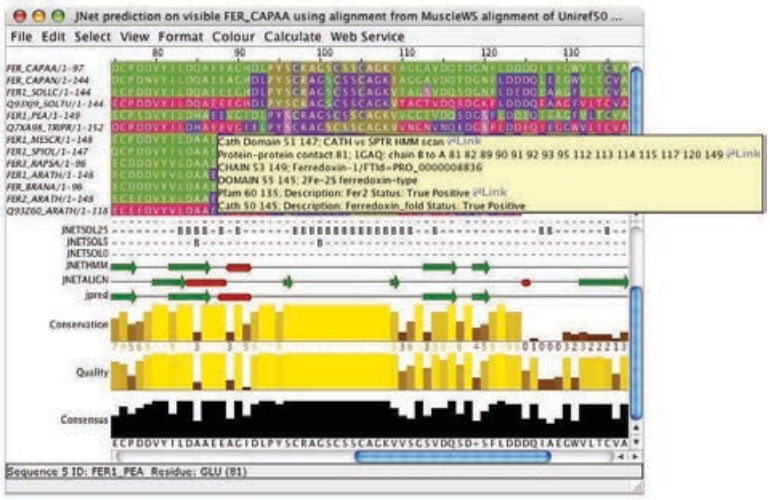


Figure 4.4: **Multiple sequence features.** An alignment with JPred secondary structure prediction annotation below it, and many sequence features overlaid onto the aligned sequences. The tooltip lists the features annotating the residue below the mouse-pointer.

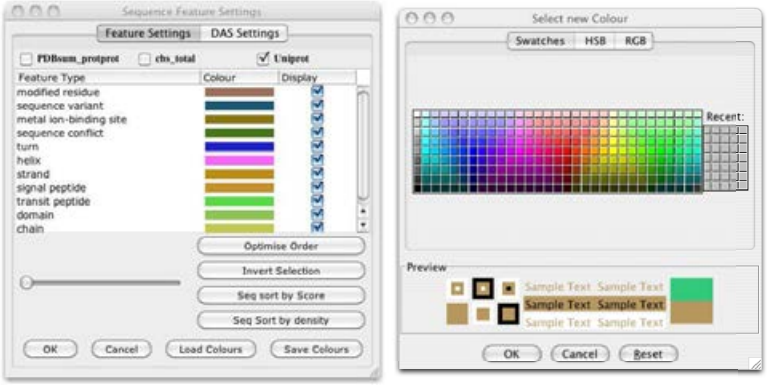


Figure 4.5: **Customising sequence features.** Features can be recoloured, switched on or off and have the rendering order changed.

4.2.6 Sequence Feature File Formats

Jalview supports the widely used GFF tab delimited format[4] and its own Jalview Features file format for the import of sequence annotation. Features and alignment annotation are also extracted from other formats such as Stockholm, and AMSA. URL links may also be attached to features. See the online documentation for more details of the additional capabilities of the Jalview features file.

Exercise 15: Creating Features

15.a. Open the alignment at http://www.jalview.org/tutorial/alignment.fa. We know that the Cysteine residues at columns 97, 102, 105 and 135 are involved in iron binding so we will create them as features. Navigate to column 97, sequence 1. Select the entire column by clicking in the ruler bar. Then right-click on the selection to bring up the context menu and select *Selection* ⇒ *Create Sequence Feature*. A dialog box will appear.

15.b. Enter a suitable Sequence Feature Name (e.g. “Iron binding site") in the appropriate box. Click on the Feature Colour bar to change the colour if desired, add a short description (“One of four Iron binding Cysteines") and press *OK*. The features will then appear on the sequences.

15.c. Roll the mouse cursor over the new features. Note that the position given in the tool tip is the residue number, not the column number. To demonstrate that there is one feature per sequence, clear all selections by pressing [ESC] then insert a gap in sequence 3 at column 95. Roll the mouse over the features and you will see that the feature has moved with the sequence. Delete the gap you created.

15.d. Add a similar feature to column 102. When the feature dialog box appears, clicking the Sequence Feature Name box brings up a list of previously described features. Using the same Sequence Feature Name allows the features to be grouped.

15.e. Select *View* ⇒ *Feature Settings...* from the alignment window menu. The Sequence Feature Settings window will appear. Move this so that you can see the features you have just created. Click the check box for “Iron binding site" under *Display* and note that display of this feature type is now turned off. Click it again and note that the features are now displayed. Close the sequence feature settings box by clicking *OK* or *Cancel*.

[4] see http://www.sanger.ac.uk/resources/software/gff/spec.html

Chapter 5

Multiple Sequence Alignment

Sequences can be aligned using a range of algorithms provided by JABA web services, including ClustalW[1], Muscle[2], MAFFT[3], ProbCons,[4] T-COFFEE[5] and Clustal Omega.[6] Of these, T-COFFEE is slow but accurate. ClustalW is historically the most widely used. Muscle is fast and probably best for smaller alignments. MAFFT is probably the best for large alignments, however Clustal Omega, released in 2011, is arguably the fastest and most accurate tool for protein multiple alignment.

5.1 Performing a multiple sequence alignment

To run an alignment web service, select the appropriate method from the *Web Service* ⇒ *Alignment* ⇒ ... submenu (Figure 5.1). For each service you may either perform an alignment with default settings, use one of the available presets, or customise the parameters with the '*Edit and Run ..*' dialog box. Once the job is submitted, a progress window will appear giving information about the job and any errors that occur. After successful completion of the job, a new alignment window is opened with the results, in this case an alignment. By default, the new alignment will be ordered in the same way as the input sequences. Note: many alignment programs re-order the input during their analysis and place homologous sequences close together, the MSA algorithm ordering can be recovered using the 'Algorithm ordering' entry within the *Calculate* ⇒ *Sort* sub menu.

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[1] *"CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice."* Thompson JD, Higgins DG, Gibson TJ (1994) *Nucleic Acids Research* **22**, 4673-80

[2] *"MUSCLE: a multiple sequence alignment method with reduced time and space complexity"* Edgar, R.C. (2004) *BMC Bioinformatics* **5**, 113

[3] *"MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform"* Katoh, K., Misawa, K., Kuma, K. and Miyata, T. (2002) *Nucleic Acids Research* **30**, 3059-3066. and *"MAFFT version 5: improvement in accuracy of multiple sequence alignment"* Katoh, K., Kuma, K., Toh, H. and Miyata, T. (2005) *Nucleic Acids Research* **33**, 511-518.

[4] PROBCONS: Probabilistic Consistency-based Multiple Sequence Alignment. Do, C.B., Mahabhashyam, M.S.P., Brudno, M., and Batzoglou, S. (2005) *Genome Research* **15** 330-340.

[5] T-Coffee: A novel method for multiple sequence alignments. (2000) Notredame, Higgins and Heringa *JMB* **302** 205-217

[6] Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Sievers F, Wilm A, Dineen DG, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG (2011) *Molecular Systems Biology* **7** 539 doi:10.1038/msb.2011.75

5.1.1 Realignment to add sequences to an existing alignment

The re-alignment option is currently only supported by Clustal Omega and ClustalW. When performing a re-alignment, Jalview submits the current selection to the alignment service complete with any existing gaps. Realignment with ClustalW is useful when one wishes to align additional sequences to an existing alignment without any further optimisation to the existing alignment. ClustalO's realignment works by generating a probabilistic model (a.k.a HMM) from the original alignment, and then realigns **all** sequences to this profile. For a well aligned MSA, this process will simply reconstruct the original alignment (with additonal sequences), but in the case of low quality MSAs, some differences may be introduced.

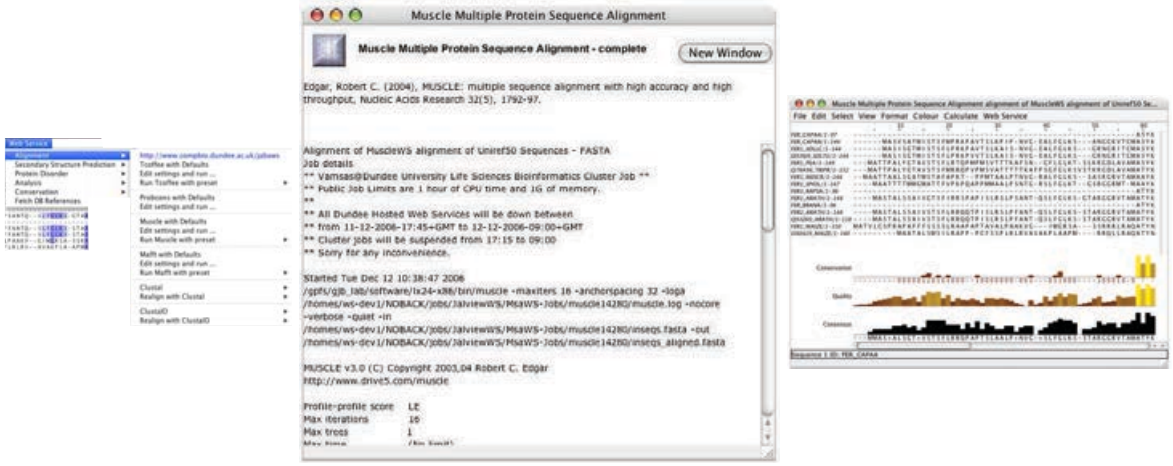


Figure 5.1: **Multiple alignment via web services** The appropriate method is selected from the menu (left), a status box appears (centre), and the results appear in a new window (right).

5.1.2 Alignments of Sequences that include Hidden Regions

If the view or selected region submitted for alignment contains hidden regions, then **only the visible sequences will be submitted to the service**. Furthermore, each contiguous segment of sequences will be aligned independently (resulting in a number of alignment 'subjobs' appearing in the status window). Finally, the results of each subjob will be concatenated with the hidden regions in the input data prior to their display in a new window. This approach ensures that 1) hidden column boundaries in the input data are preserved in the resulting alignment - in a similar fashion to the constraint that hidden columns place on alignment editing (see Section 2.6.2 and 2) hidden columns can be used to preserve existing parts of an alignment whilst the visible parts are locally refined.

5.1.3 Alignment Service Limits

Multiple alignment is a computationally intensive calculation. Some JABA server services and service presets only allow a certain number of sequences to be aligned. The precise number will depend on the server that you are using to perform the alignment. Should you try to submit more sequences than a service can handle, then an error message will be shown informing you of the maximum

number allowed by the server.

Exercise 16: Multiple Sequence Alignment

16.a. Close all windows and open the alignment at http://www.jalview.org/tutorial/unaligned.fa. Select *Web Service* ⇒ *Alignment* ⇒ *Muscle with Defaults*. A window will open giving the job status. After a short time, a second window will open with the results of the alignment.

16.b. Return to the first sequence alignment window by clicking on the window, and repeat using *ClustalO* (Omega) and *MAFFT*, from the *Web Service* ⇒ *Alignment* menu, using the same initial alignment. Compare them and you should notice small differences.

16.c. Select the last three sequences in the MAFFT alignment, and de-align them with *Edit* ⇒ *Remove All Gaps*. Press [ESC] to deselect these sequences. Then submit this view for re-alignment with *ClustalO*.

16.d. Return to the alignment window in section (c), use [CTRL]-Z (undo) to recover the alignment of the last three sequences in this MAFFT alignment. Once the ClustalO re-alignment has completed, compare the results of re-alignment of the three sequences with their alignment in the original MAFFT result.

16.e. Select columns 60 to 125 in the original MAFFT alignment and hide them, by right clicking the mouse to bring up context menu. Select *Web Service* ⇒ *Alignment* ⇒ *Mafft with Defaults* to submit the visible portion of the alignment to MAFFT. When the web service job pane appears, note that there are now two alignment job status panes shown in the window.

16.f. When the MAFFT job has finished, compare the alignment of the N-terminal visible region in the result with the corresponding region of the original alignment.

16.g. If you wish, select and hide a few more columns in the N-terminal region, and submit the alignment to the service again and explore the effect of local alignment on the non-homologous parts of the N-terminal region.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

5.2 Customising the Parameters used for Alignment

JABA web services allow you to vary the parameters used when performing a bioinformatics analysis. For JABA alignment services, this means you are usually able to modify the following types of parameters:

- Amino acid or nucleotide substitution score matrix
- Gap opening and widening penalties
- Types of distance metric used to construct guide trees
- Number of rounds of re-alignment or alignment optimisation

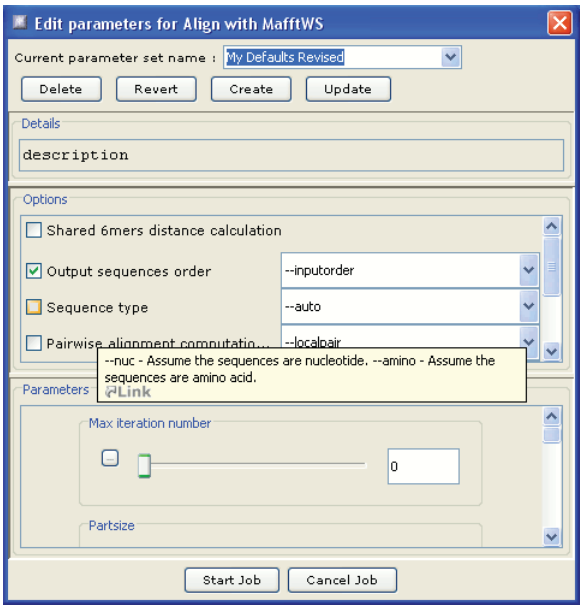


Figure 5.2: **Jalview's JABA alignment service parameter editing dialog box**.

5.2.1 Getting Help on the Parameters for a Service

Each parameter available for a method usually has a short description, which Jalview will display as a tooltip, or as a text pane that can be opened under the parameter's controls. In the parameter shown in Figure 5.3, the description was opened by selecting the button on the left hand side. Online help for the service can also be accessed, by right clicking the button and selecting a URL from the pop-up menu that will open.

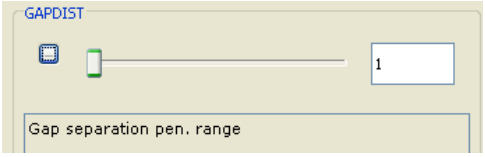


Figure 5.3: **ClustalW parameter slider detail**. From the ClustalW *Clustal* ⇒ *Edit settings and run ...* dialog box.

5.2.2 Alignment Presets

The different multiple alignment algorithms available from JABA vary greatly in the number of adjustable parameters, and it is often difficult to identify what are the best values for the sequences that you are trying to align. For these reasons, each JABA service may provide one or more presets – which are pre-defined sets of parameters suited for particular types of alignment problem. For instance, the Muscle service provides the following presets:

- Large alignments (balanced)
- Protein alignments (fastest speed)

- Nucleotide alignments (fastest speed)

The presets are displayed in the JABA web services submenu, and can also be accessed from the parameter editing dialog box, which is opened by selecting the '*Edit settings and run ...*' option from the web services menu. If you have used a preset, then it will be mentioned at the beginning of the job status file shown in the web service job progress window.

5.2.3 User Defined Presets

Jalview allows you to create your own presets for a particular service. To do this, select the '*Edit settings and run ...*' option for your service, which will open a parameter editing dialog box like the one shown in Figure 5.2.

The top row of this dialog allows you to browse the existing presets, and when editing a parameter set, allows you to change its nickname. As you adjust settings, buttons will appear at the top of the parameters dialog that allow you to Revert or Update the currently selected user preset with your changes, Delete the current preset, or Create a new preset, if none exists with the given name. In addition to the parameter set name, you can also provide a short description for the parameter set, which will be shown in the tooltip for the parameter set's entry in the web services menu.

Saving Parameter Sets

When creating a custom parameter set, you will be asked for a file name to save it. The location of the file is recorded in the Jalview user preferences in the same way as a custom alignment colourscheme, so when Jalview is launched again, it will show your custom preset amongst the options available for running the JABA service.

5.3 Protein Alignment Conservation Analysis

The *Web Service* ⇒ *Conservation* menu controls the computation of up to 17 different amino acid conservation measures for the current alignment view. The JABAWS AACon Alignment Conservation Calculation Service, which is used to calculate these scores, provides a variety of standard measures described by Valdar in 2002[7] as well as an efficient implementation of the SMERFs score developed by Manning et al. in 2008.[8]

5.3.1 Enabling and Disabling AACon Calculations

When the AACon Calculation entry in the *Web Service* ⇒ *Conservation* menu is ticked, AACon calculations will be performed every time the alignment is modified. Selecting the menu item will enable or disable automatic recalculation.

[7] Scoring residue conservation. Valdar (2002) *Proteins: Structure, Function, and Genetics* **43** 227-241.
[8] SMERFS Score Manning et al. *BMC Bioinformatics* 2008, **9** 51 doi:10.1186/1471-2105-9-51

5.3.2 Configuring which AACon Calculations are Performed

The *Web Service* ⇒ *Conservation* ⇒ *Change AACon Settings ...* menu entry will open a web services parameter dialog for the currently configured AACon server. Standard presets are provided for quick and more expensive conservation calculations, and parameters are also provided to change the way that SMERFS calculations are performed. AACon settings for an alignment are saved in Jalview projects along with the latest calculation results.

5.3.3 Changing the Server used for AACon Calculations

If you are working with alignments too large to analyse with the public JABAWS server, then you will most likely have already configured additional JABAWS servers. By default, Jalview will chose the first AACon service available from the list of JABAWS servers available. If available, you can switch to use another AACon service by selecting it from the *Web Service* ⇒ *Conservation* ⇒ *Switch Server* submenu.

Chapter 6

Analysis of Alignments

Jalview provides support for sequence analysis in two ways. A number of analytical methods are 'built-in', these are accessed from the *Calculate* alignment window menu. Computationally intensive analyses are run outside Jalview *via* web services - and found under the *Web Service* menu. In this section, we describe the built-in analysis capabilities common to both the Jalview Desktop and the JalviewLite applet.

6.1 PCA

Principal components analysis calculations create a spatial representation of the similarities within the current selection or the whole alignment if no selection has been made. After the calculation finishes, a 3D viewer displays each sequence as a point in 3D 'similarity space'. Sets of similar sequences tend to lie near each other in this space. Note: The calculation is computationally expensive, and may fail for very large sets of sequences - because the JVM has run out of memory. Memory issues, and how to overcome them, were discussed in Section 1.4.6.

What is PCA?

Principal components analysis is a technique for examining the structure of complex data sets. The components are a set of dimensions formed from the measured values in the data set, and the principal component is the one with the greatest magnitude, or length. The sets of measurements that differ the most should lie at either end of this principal axis, and the other axes correspond to less extreme patterns of variation in the data set. In this case, the components are generated by an eigenvector decomposition of the matrix formed from the sum of pairwise substitution scores at each aligned position between each pair of sequences. The basic method is described in the 1995 paper by *G. Casari, C. Sander* and *A. Valencia* [1] and implemented at the SeqSpace server at the EBI.

Jalview provides two different options for the PCA calculation: SeqSpace and Jalview mode. In SeqSpace mode, PCAs are computed using the identity matrix, and gaps are treated as 'the unknown

[1] *Nature Structural Biology* (1995) **2**, 171-8. PMID: 7749921

residue' (this actually differs from the original SeqSpace paper, and will be adjusted in a future version of Jalview). In Jalview mode, PCAs are computed using the chosen score matrix - which for protein sequences, defaults to BLOSUM 62, and for nucleotides, is the DNA identity matrix that also treats Us and Ts as identical, to support analysis of both RNA and DNA alignments. The *Change Parameters* allows the calculation method and score models to be changed.[2]

The PCA Viewer

PCA analysis can be launched from the *Calculate* ⇒ *Principal Component Analysis* menu option. **PCA requires a selection containing at least 4 sequences**. A window opens containing the PCA tool (Figure 6.1). Each sequence is represented by a small square, coloured by the background colour of the sequence ID label. The axes can be rotated by clicking and dragging the left mouse button and zoomed using the ↑ and ↓ keys or the scroll wheel of the mouse (if available). A tool tip appears if the cursor is placed over a sequence. Sequences can be selected by clicking on them. [CTRL]-Click can be used to select multiple sequences.

Labels will be shown for each sequence by toggling the *View* ⇒ *Show Labels* menu option, and the plot background colour changed *via* the *View* ⇒ *Background Colour..* dialog box. A graphical representation of the PCA plot can be exported as an EPS or PNG image *via* the *File* ⇒ *Save As* ⇒ ... submenu.

Exercise 17: Principal Component Analysis

17.a. Load the alignment at http://www.jalview.org/tutorial/alignment.fa.

17.b. Select the menu option *Calculate* ⇒ *Principal Component Analysis*. A new window will open. Move this window within the desktop so that the tree, alignment and PCA viewer windows are all visible. Try rotating the plot by clicking and dragging the mouse on the plot in the PCA window. Note that clicking on points in the plot will highlight the sequences on the alignment.

17.c. Select *Calculate* ⇒ *Calculate Tree* ⇒ *Neighbour Joining Using BLOSUM62*. A new tree window will appear. Place the mouse cursor on the tree window so that the tree partition location will divide the alignment into a number of groups, each of a different colour. Note how the colour of the sequence ID label matches both the colour of the partitioned tree and the points in the PCA plot.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

PCA Data Export

Although the PCA viewer supports export of the current view, the plots produced are rarely suitable for direct publication. The PCA viewer's *File* menu includes a number of options for exporting the PCA matrix and transformed points as comma separated value (CSV) files. These files can be imported by tools such as **R** or **gnuplot** in order to graph the data.

[2] See `http://www.jalview.org/help/html/calculations/pca.html`.

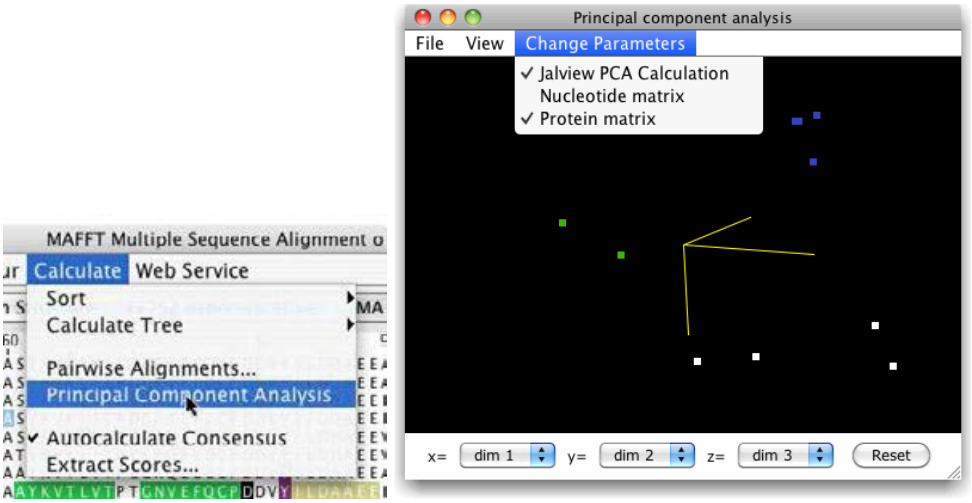


Figure 6.1: **PCA Analysis.**

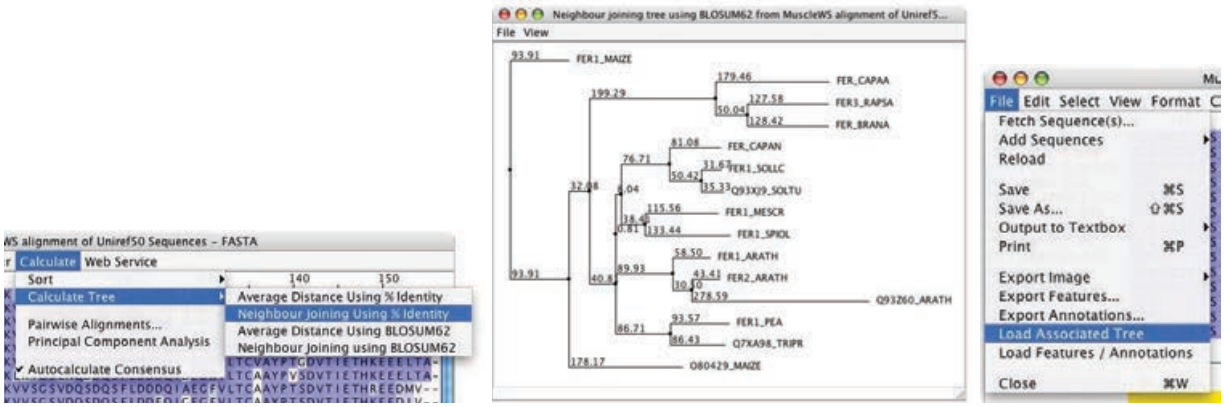


Figure 6.2: **Calculating Trees** Jalview provides a range of options for calculating trees. Jalview can also load precalculated trees in Newick format (right).

6.2 Trees

Jalview can calculate and display trees, providing interactive tree-based grouping of sequences though a tree viewer. All trees are calculated *via* the *Calculate* ⇒ *Calculate Tree* ⇒ . . . submenu. Trees can be calculated from distance matrices determined from % identity or aggregate BLOSUM 62 score using either *Average Distance* (UPGMA) or *Neighbour Joining* algorithms. The input data for a tree is either the selected region or the whole alignment, excluding any hidden regions.

On calculating a tree, a new window opens (Figure 6.2) which contains the tree. Various display settings can be found in the tree window *View* menu, including font, scaling and label display options. The *File* ⇒ *Save As* submenu contains options for image and Newick file export. Newick format is a standard file format for trees which allows them to be exported to other programs. Jalview can also read in external trees in Newick format *via* the *File* ⇒ *Load Associated Tree* menu option. Leaf names on imported trees will be matched to the associated alignment - unmatched leaves will still be displayed, and can be highlighted using the *View* ⇒ *Mark Unlinked Leaves* menu option.

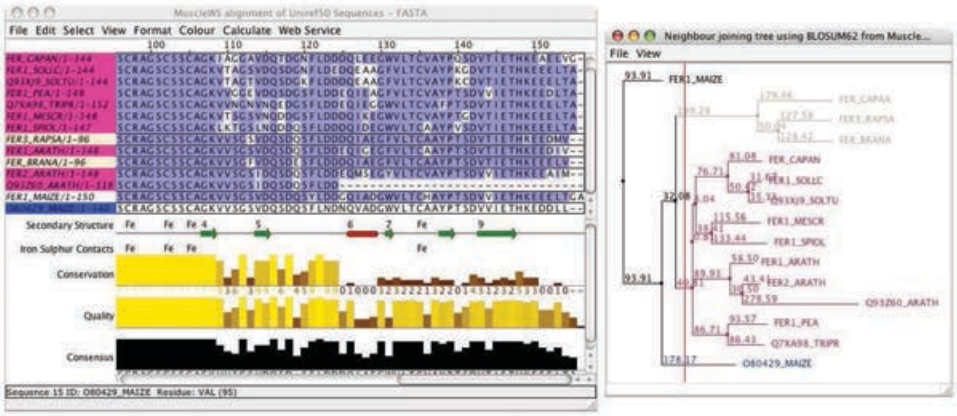
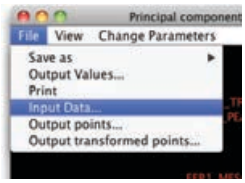


Figure 6.3: **Interactive Trees** The tree level cutoff can be used to designate groups in Jalview.

Clicking on the tree brings up a cursor across the height of the tree. The sequences are automatically partitioned and coloured (Figure 6.3). To group them together, select the *Calculate* ⇒ *Sort* ⇒ *By Tree Order* ⇒ *...* alignment window menu option and choose the correct tree. The sequences will then be sorted according to the leaf order currently shown in the tree view. The coloured background to the sequence IDs can be removed with *Select* ⇒ *Undefine Groups* from the alignment window menu. Note that tree partitioning will also remove any groups and colourschemes on a view, so create a new view ([CTRL-T]) if you wish to preserve these.

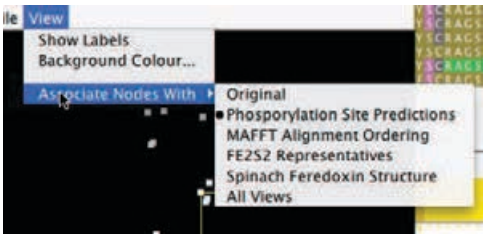
Recovering input Data for a Tree or PCA Plot Calculation

The *File* ⇒ *Input Data* option will open a new alignment window containing the original data used to calculate the tree or PCA plot (if available). This function is useful when a tree has been created and then the alignment subsequently changed.



Changing the associated View for a Tree or PCA Viewer

The *View* ⇒ *Associated Nodes With* ⇒ *..* submenu is shown when the viewer is associated with an alignment that is involved in multiple views. Selecting a different view does not affect the tree or PCA data, but will change the colouring and display of selected sequences in the display according to the colouring and selection state of the newly associated view.



6.2.1 Tree Based Conservation Analysis

Trees reflect the pattern of global sequence similarity exhibited by the alignment, or region within the alignment, that was used for their calculation. The Jalview tree viewer enables sequences to

be partitioned into groups based on the tree. This is done by clicking within the tree viewer window. Once subdivided, the conservation between and within groups can be visually compared in order to better understand the pattern of similarity revealed by the tree and the variation within the clades partitioned by the grouping. The conservation based colourschemes and the group associated conservation and consensus annotation (enabled using the alignment window's *View* ⇒ *Autocalculated Annotation* ⇒ *Group Conservation* and *Group Consensus* options) can help when working with larger alignments.

Exercise 18: Trees

Ensure that you have at least 1G memory available in Jalview.

(Start with link: http://www.jalview.org/services/launchApp?jvm-max-heap=1G, or in the Development section of the Jalview web site (http://www.jalview.org/development/development-builds) in the table, go to "latest official build" row and "Webstart" column, click on "2G".)

18.a. Open the alignment at http://www.jalview.org/tutorial/alignment.fa. Select *Calculate* ⇒ *Calculate Tree* ⇒ *Neighbour Joining Using BLOSUM62*. A tree window opens.

18.b. Click on the tree window, a cursor will appear. Note that placing this cursor divides the tree into a number of groups by colour. Place the cursor to give about 4 groups.

18.c. In the alignment window, select *Calculate* ⇒ *Sort* ⇒ *By Tree Order* ⇒ *Neighbour Joining Tree using BLOSUM62 from...* . The sequences are reordered to match the order in the tree and groups are formed implicitly. Alternatively in the tree window, select *View* ⇒ *Sort Alignment by Tree*.

18.d. Select *Calculate* ⇒ *Calculate Tree* ⇒ *Neighbour Joining Using % Identity*. A new tree window will appear. The group colouring makes it easy to see the differences between the two trees calculated by the different methods.

18.e. Select from sequence 2 column 60 to sequence 12 column 123. Select *Calculate* ⇒ *Calculate Tree* ⇒ *Neighbour Joining Using BLOSUM62*. A new tree window will appear. The tree contains 11 sequences. It has been coloured according to the already selected groups from the first tree and is calculated purely from the residues in the selection.

Comparing the location of individual sequences between the three trees illustrates the importance of selecting appropriate regions of the alignment for the calculation of trees.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

Exercise 19: Pad Gaps in an Alignment

19.a. Open the alignment at http://www.jalview.org/tutorial/alignment.fa. In alignment window, ensure that the *Edit* ⇒ *Pad Gaps* option is *not* ticked, and insert one gap anywhere in the alignment.

19.b. Select *Calculate* ⇒ *Calculate Tree* ⇒ *Neighbour Joining Using BLOSUM62*.
A warning dialog box **"Sequences not aligned"** appears because the sequences input to the tree calculation are of different lengths.

19.c. Select *Edit* ⇒ *tick Pad Gaps* and perform the tree calculation again. This time a new tree should appear - because padding gaps ensures all the sequences are the same length after editing.

Pad Gaps option can be set in Preferences using *Tool* ⇒ *Preference* ⇒ *Editing*.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

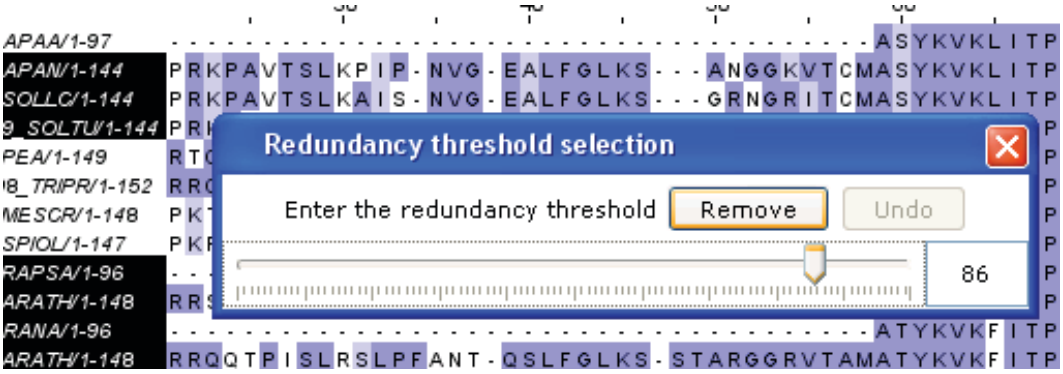


Figure 6.4: The Redundancy Removal dialog box opened from the edit menu. Sequences that exceed the current percentage identity threshold and are to be removed are highlighted in black.

Exercise 20: Tree Based Conservation Analysis

20.a. Load the PF03460 PFAM seed alignment using the sequence fetcher. Select *Colour* ⇒ *Taylor* ⇒ *By Conservation*, set *Conservation* shading threshold at around 20.

20.b. Build a Neighbour joining tree using Select *Calculate* ⇒ *Calculate Tree* ⇒ *Neighbour Joining Using BLOSUM62.*

20.c. Use the mouse cursor to select a point on the tree to partition the alignment into several sections.

20.d. Select *View* ⇒ *Sort Alignment By Tree* option in the tree window to re-order the sequences in the alignment using the calculated tree. Examine the variation in colouring between different groups of sequences in the alignment window.

20.e. You may find it easier to browse the alignment if you first uncheck the *Annotations* ⇒ *Show Annotations* option. Open the Overview Window within the View menu to aid navigation.

20.f. Try changing the colourscheme of the residues in the alignment to BLOSUM62 (whilst ensuring that *Apply Colour to All Groups* is selected).

Note: You may want to save the alignment and tree as a project file, since it is used in the next set of exercises.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

6.2.2 Redundancy Removal

The redundancy removal dialog box is opened using the *Edit* ⇒ *Remove Redundancy...* option in the alignment menu. As its menu option placement suggests, this is actually an alignment editing function, but it is convenient to describe it here. The redundancy removal dialog box presents a percentage identity slider which sets the redundancy threshold. Aligned sequences which exhibit a percentage identity greater than the current threshold are highlighted in black. The [Remove] button can then be used to delete these sequences from the alignment as an edit operation[3].

[3]Which can usually be undone. A future version of Jalview may allow redundant sequences to be hidden, or represented by a chosen sequence, rather than deleted.

6.2.3 Subdividing the Alignment According to Specific Mutations

It is often necessary to explore variations in an alignment that may correlate with mutations observed in a particular region; for example, sites exhibiting single nucleotide polymorphism, or residues involved in substrate recognition in an enzyme. One way to do this would be to calculate a tree using the specific region, and subdivide it in order to partition the alignment. However, calculating a tree can be slow for large alignments, and the tree may be difficult to partition when complex mutation patterns are being analysed. The *Select* ⇒ *Make groups for selection* function was introduced to make this kind of analysis easier. When selected, it will use the characters in the currently selected region to subdivide the alignment. For example, if a single column is selected, then the alignment (or each group defined on the alignment) will be divided into groups based on the residue or nucleotide found at that position. These new groups are annotated with the characters in the selected region, and Jalview's group based conservation analysis annotation and colourschemes can then be used to reveal any associated pattern of sequence variation across the whole alignment.

6.3 Pairwise Alignments

Jalview can calculate optimal pairwise alignments between arbitrary sequences *via* the *Calculate* ⇒ *Pairwise Alignments...* menu option. Global alignments of all pairwise combinations of the selected sequences are performed and the results returned in a text box.

Exercise 21: Remove Redundant Sequences

21.a. Using the alignment generated in the previous exercise (exercise 20). In the alignment window, you may need to deselect groups using Esc key.

21.b. In the *Edit* menu select *Remove Redundancy* to open the Redundancy threshold selection dialog. Adjust the redundancy threshold value, start at 50 and increase the value to 65. Sequences selected will change colour in the Sequence ID panel. Select "Remove" to remove the sequences that are more than 65% similar under this alignment.

21.c. From the tree window, select *View* ⇒ *Mark Unlinked Leaves* option, and note that the removed sequences are now prefixed with a * in the tree view.

21.d. Use the [Undo] button in the Redundancy threshold selection dialog box to recover the sequences. Note that the * symbols disappear from the tree display.

21.e. Experiment with the redundancy removal and observe the relationship between the percentage identity threshold and the pattern of unlinked nodes in the tree display.

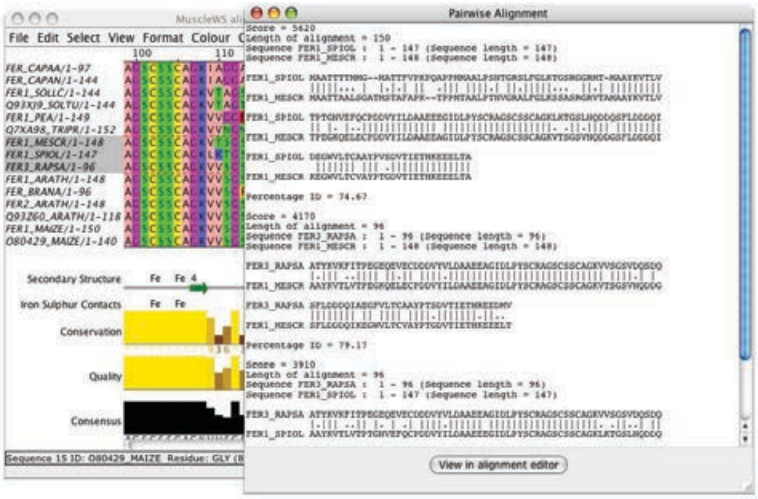


Figure 6.5: **Pairwise alignment of sequences.** Pairwise alignments of three selected sequences are shown in a textbox.

Exercise 22: Group Conservation Analysis

22.a. Re-use or recreate the alignment and tree which you worked with in the tree based conservation analysis exercise (exercise 20).

22.b. In the *View* menu in the alignment window, select *New View* to create a new view. Ensure the annotation panel is displayed (*Show annotation* in *Annotations* menu). Enable the display of *Group Consensus* option by checking *Group Consensus* in the *Annotation* ⇒ *Autocalculated Annotation* submenu in the alignment window.

22.c. Displaying the sequence logos will make it easier to see the different residue populations within each group. Activate the logo by right clicking on the Consensus annotation row to open the context menu and select the *Show Logo* option.

22.d. In the column alignment ruler, select a column exhibiting about 50% conservation that lies within the central conserved region of the alignment. (Column 74 is used in the Tree video).

22.e. Subdivide the alignment according to this selection using *Select* ⇒ *Make groups for selection.*

22.f. Re-order the alignment according to the new groups that have been defined by selecting *Calculate* ⇒ *Sort* ⇒ *By Group.*

Click on the group annotation row IDs to select groups exhibiting a specific mutation.

22.g. Select another column exhibiting about 50% conservation overall, and subdivide the alignment further. Note that the new groups inherit the names of the original groups, allowing you to identify the combination of mutations that resulted in the subdivision.

22.h. Clear the groups, and try to subdivide the alignment using two non-adjacent columns. *Hint: You may need to hide the intervening columns before you can select both of the columns that you wish to use to subdivide the alignment.*

22.i. Switch back to the original view, and experiment with subdividing the tree groups made in the previous exercise.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

Chapter 7

Working with 3D structures

Jalview facilitates the use of 3D structure data for the analysis of alignments by providing a linked view of structures associated with the aligned sequences. It also allows sequence, secondary structure and B-factor data to be imported from structure files, and supports the use of the EMBL-EBI's SIFTS database to construct accurate mappings between UniProt protein sequences and structures retrieved from the PDB.

7.1 Molecular graphics systems supported by Jalview

Jalview can interactively view 3D structure using Jmol, a Java based molecular viewing program[1] integrated with Jalview.[2] It also supports the use of UCSF Chimera, a powerful molecular graphics system that needs separate installation. Jalview can also read PDB and mmCIF format files directly to extract sequences and secondary structure information, and retrieve records from the European Protein Databank (PDBe) using the Sequence Fetcher (see 1.4.5).

7.1.1 Configuring the default structure viewer

To configure which viewer is used when creating a new structure view, open the Structures preferences window *via Tools ⇒ Preferences...* and select either JMOL or CHIMERA as the default viewer. If you select Chimera, Jalview will search for the installed program, and if it cannot be found, you will be prompted to locate the Chimera binary, or alternately, open the UCSF Chimera download page to obtain the software.

[1] See the Jmol homepage `http://www.jmol.org` for more information.

[2] Earlier versions of Jalview included MCView - a simple main chain structure viewer. Structures are visualized as an alpha carbon trace and can be viewed, rotated and coloured using the sequence alignment.

7.2 Automatic Association of PDB Structures with Sequences

Jalview will attempt to automatically determine which structures are associated with a sequence via its ID, and any associated database references. To do this for a particular sequence or the current selection, open the Sequence ID popup menu and select *View 3D Structure*, to open the 3D Structure Chooser.

When the structure chooser is first opened, if no database identifiers are available, Jalview will automatically perform a database reference retrieval (See 4.2.1) to discover identifiers for the sequences to use to search the PDB. This can take a few seconds for each sequence and will be performed for all selected sequences.[3]

Once the retrieval has finished, the structure chooser dialog will show any available PDB entries for the selected sequences.

7.2.1 Drag-and-Drop Association of PDB Files with Sequences by Filename Match

If you have PDB files stored on your computer named the same way as the sequences in the alignment, then you can drag them from their location on the file browser onto an alignment window. Jalview will search the alignment for sequences with IDs that match any of the files, and offer a dialog like the one in Figure 7.1.

If no associations are made, then sequences extracted from the structure will be simply added to the alignment. However, if only some of the PDB files are associated, Jalview will raise another dialog box giving you the option to add any remaining sequences from the PDB structure files not present in the alignment. This allows you to easily decorate sequences in a newly imported alignment with any corresponding structures you've already collected in a directory accessible from your computer.[4]

After associating sequencesÂăwith PDB files, you can view the PDB structures by opening the Sequence ID popup menu and selecting *View 3D Structure*. The PDB files you loaded will be shown in the **Cached Structures** view, after selecting it from the drop down menu in the dialog box.

7.3 Viewing Structures

The structure viewer is launched via the Sequence ID context menu. To view structures associated with a sequence or a selected set of sequences in the alignment, simply right click the mouse to open the context menu, and select *3D Structure data ...* to open the Structure Chooser dialog box.

If any of the **currently selected** sequences have structures in the PDB, they will appear in the Structure Chooser dialog box. The structures can be ranked by different parameters, but are by

[3] After this is done, you can can see the added database references in a tool tip by mousing over the sequence ID. You can use the *View* ⇒ *Sequence ID Tooltip* ⇒ *Show Db References* submenu option to enable or disable these data in the tooltip.

[4] We plan to extend this facility in future so Jalview will automatically search for PDB files matching your sequence within a local directory. Check out Jalview issue 801

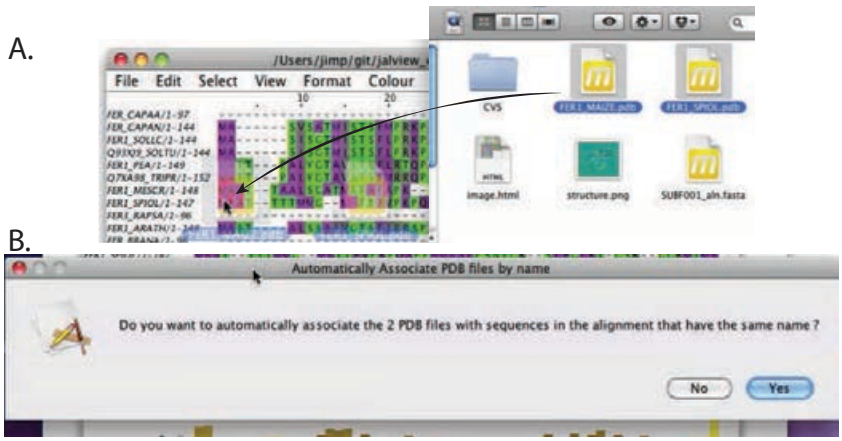


Figure 7.1: **Associating PDB files with sequences by drag-and-drop.** Dragging PDB files onto an alignment of sequences with names matching the dragged files names (A), results in a dialog box (B) that gives the option to associate each file with any sequences with matching IDs.

default ordered according to their PDB quality score.

To view one or more structures, simply click *View* to open a structure viewer containing the structures selected in the dialog. If several structures were picked, these will be shown superposed according to the alignment. You may find Jalview has already picked the best structure - using one of the criteria shown in the dropdown menu (e.g. 'Best Quality', which is picked by default). However, you are free to select your own.

The structure(s) to be displayed will be downloaded or loaded from the local file system, and shown as a ribbon diagram coloured according to the associated sequence in the current alignment view (Figure 7.2 (right)). The structure can be rotated by clicking and dragging in the structure window. The structure can be zoomed using the mouse scroll wheel or by [SHIFT]-dragging the structure.

Moving the mouse cursor over a sequence to which the structure is linked in the alignment view highlights the respective residue's sidechain atoms. The sidechain highlight may be obscured by other parts of the molecule. Similarly, moving the cursor over the structure shows a tooltip and highlights the corresponding residue in the alignment. Clicking the alpha carbon or phosphorous backbone atom will toggle the highlight and residue label on and off. Often, the position highlighted in the sequence may not be in the visible portion of the current alignment view and the sliders will scroll automatically to show the position. If the alignment window's *View* ⇒ *Automatic Scrolling* option is not selected, however, then the automatic adjustment will be disabled for the current view.

7.3.1 Customising Structure Display

Structure display can be modified using the *Colour* and *View* menus in the structure viewer. The background colour can be modified by selecting the *Colours* ⇒ *Background Colour...* option.

By default, the structure will be coloured in the same way as the associated sequence(s) in the align-

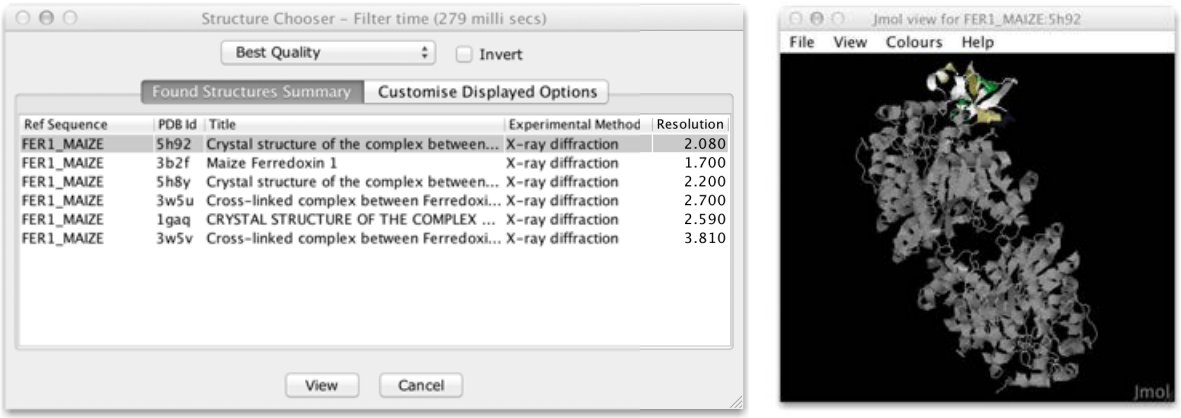


Figure 7.2: **Structure visualization** Structure viewers are launched from the 3D Structure chooser dialog (left). Jalview shows the displayed structures coloured according the alignment view (right).

ment view from which it was launched. The structure can be coloured independently of the sequence by selecting an appropriate colour scheme from the *Colours* menu. It can be coloured according to the alignment using the *Colours* ⇒ *By Sequence* option. The image in the structure viewer can be saved as an EPS or PNG with the *File* ⇒ *Save As* ⇒ ... submenu, which also allows the raw data to be saved as PDB format. The mapping between the structure and the sequence (how well and which parts of the structure relate to the sequence) can be viewed with the *File* ⇒ *View Mapping* menu option.

Using the Jmol Visualization Interface

Jmol has a comprehensive set of selection and visualization functions that are accessed from the Jmol popup menu (by right-clicking in the Jmol window or by clicking the Jmol logo). Molecule colour and rendering style can be manipulated, and distance measurements and molecular surfaces can be added to the view. It also has its own "Rasmol[5]-like" scripting language, which is described elsewhere[6]. Jalview utilises the scripting language to interact with Jmol and to store the state of a Jmol visualization within Jalview archives, in addition to the PDB data file originally loaded or retrieved by Jalview. To access the Jmol scripting environment directly, use the *Jmol* ⇒ *Console* menu option.

If you would prefer to use Jmol to manage structure colours, then select the *Colours* ⇒ *Colour with Jmol* option. This will disable any automatic application of colour schemes when new structure data is added, or when associated alignment views are modified.

[5]See `http://www.rasmol.org`

[6]Jmol Wiki: `http://wiki.jmol.org/index.php/Scripting`
Jmol Scripting reference: `http://www.stolaf.edu/academics/chemapps/jmol/docs/`

Exercise 23: Viewing Structures with the integrated Jmol Viewer

23.a. Load the alignment at http://www.jalview.org/examples/exampleFile.jar.

23.b. Right-click on the sequence ID label of *FER1_SPIOL* to open the ID popup menu and select *3D Structure*. After a short pause, a Structure Chooser dialog will open for the sequence, listing available structure data from the PDB. Select *1A70* from the list and click *View*.
The Structure Chooser dialog presents available PDB structures by querying the EMBL-EBI's PDBe web API. Extra information can be including in this window by checking boxes in the columns of the "Customise Displayed Options" tab.

23.c. By default the Jmol structure viewer opens in the Jalview desktop. Rotate the molecule by clicking and dragging in the structure viewing box. Zoom with the mouse scroll wheel.

23.d. Roll the mouse cursor along the *FER1_SPIOL* sequence in the alignment. Note that if a residue in the sequence maps to one in the structure, a label will appear next to that residue in the structure viewer.

23.e. Move the mouse over the structure. In the Jmol viewer, placing the mouse over a part of the structure will bring up a tool tip indicating the name and number of that residue. In the alignment window, the corresponding residue in the sequence is highlighted in black.

23.f. Clicking the alpha carbon toggles the highlight and residue label on and off. Try this by clicking on a set of three or four adjacent residues so that the labels are persistent, then finding where they are in the sequence.

23.g. In the structure viewer menu, select *Colours* ⇒ *Background Colour...* and choose a suitable colour. Press *OK* to apply this.

23.h. Select *File* ⇒ *Save As* ⇒ *PNG* and save the image. On your computer, view this with a suitable program.

23.i. Select *File* ⇒ *View Mapping* from the structure viewer menu. A new window opens showing the residue by residue alignment between the sequence and the structure.

23.j. Select *File* ⇒ *Save* ⇒ *PDB file* and choose a new filename to save the PDB file. Once the file is saved, open the location in your file browser (or explorer window) and drag the PDB file that you just saved on to the Jalview desktop (or load it from the *Jalview Desktop* ⇒ *Input Alignment* ⇒ *From File* menu). Verify that you can open and view the associated structure from the sequence ID context menu's *3D Structure* submenu in the new alignment window.

23.k. In the Jmol window, right click on the structure window and explore the menu options. Try to change the style of molecular display - for example by using the *Jmol* ⇒ *Select (n)* ⇒ *All* command (where *n* is the number of residues selected), and then the *Jmol* ⇒ *Style* ⇒ *Scheme* ⇒ *Ball and Stick* command.

23.l. In the alignment window, use the *File* ⇒ *Save As..* function to save the alignment as a Jalview Project. Now close the alignment and the structure view, and load the project file you just saved. Verify that the Jmol display is as it was when you just saved the file.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

Exercise 24: Setting Chimera as the default 3D Structure Viewer
Jalview supports molecular structure visualization using both Jmol and Chimera 3D viewers. Jmol is the default viewer, however Chimera can be set up as the default choice from Preferences.

24.a. First, Chimera must be downloaded and installed on the computer. Chimera program is available on the UCSF web site https://www.cgl.ucsf.edu/chimera/download.html.

24.b. In the desktop menu, select *Tool* ⇒ *Preferences*. In the "*Structure*" tab set *Default structure viewer* as *Chimera*; then click *OK*.

24.c. Close the Jalview program, from the *Desktop menu* select *Jalview* ⇒ *Quit Jalview*. Then reopen Jalview, Chimera should open as the default viewer.

Note: The Jmol structure viewer sits within the Jalview desktop. However the Chimera structure viewer sits outside the Jalview desktop and a Chimera view window sits inside the Jalview desktop.

See the video at: `http://www.jalview.org/training/Training-Videos`**.**

7.3.2 Superimposing Structures

Many comparative biomolecular analysis investigations aim to determine if the biochemical properties of a given molecule are significantly different to its homologues. When structure data is available, comparing the shapes of molecules by superimposing them enables substructure that may impart different behaviour to be quickly identified. The identification of optimal 3D superposition involves aligning 3D data rather than sequence symbols, but the result can still be represented as a sequence alignment, where columns indicate positions in each molecule that should be superposed to recreate the optimal 3D alignment.

Jalview can employ Jmol's 3D fitting routines[7] to recreate 3D structure superpositions based on the correspondences defined by one or more sequence alignments involving structures shown in the Jmol display. Superposition based on the currently displayed alignment view happens automatically if a structure is added to an existing Jmol display using the *3D Structure* option in the Sequence ID popup menu to open the Structure Chooser dialog box. Select the structures required and select *View*. A new Jmol view opens containing superposed structures if the current selection contains two or more sequences with associated structures.

Obtaining the RMSD for a Superposition

The RMSD (Root Mean Square Deviation) is a measure of how similar the structures are when they are superimposed. Figure 7.3 shows a superposition created during the course of Exercise 25. The parts of each molecule used to construct the superposition are rendered using the cartoon style, with other parts of the molecule drawn in wireframe. The Jmol console, which has been opened after the superposition was performed, shows the RMSD report for the superposition. Full information about the superposition is also reported on the Jalview console.[8] This output also includes the precise atom pairs used to superpose structures.

[7] See http://chemapps.stolaf.edu/jmol/docs/?ver=12.2#compare for more information.

[8] The Jalview Java Console is opened from *Tools* ⇒ *Java Console* option in the Desktop's menu bar

Choosing which part of the Alignment is used for Structural Superposition

Jalview uses the visible part of each alignment view to define which parts of each molecule are to be superimposed. Hiding a column in a view used for superposition will remove that correspondence from the set, and will exclude it from the superposition and RMSD calculation. This allows the selection of specific parts of the alignment to be used for superposition. Only columns that define a complete set of correspondences for all structures will be used for structural superposition, and as a consequence, the RMSD values generated for each pair of structures superimposed can be directly compared.

In order to recompute a superposition after changing a view or editing the alignment, select the *Jmol* ⇒ *Align Structures* menu option. The *Jmol* ⇒ *Superpose with ..* submenu allows you to choose which of the associated alignments and views are to be used to create the set of correspondences. This menu is useful when composing complex superpositions involving multi-domain and multi-chain complexes, when correspondences may be defined by more than one alignment.

Note that these menu options appear when you have two or more structures in one Jmol viewer.

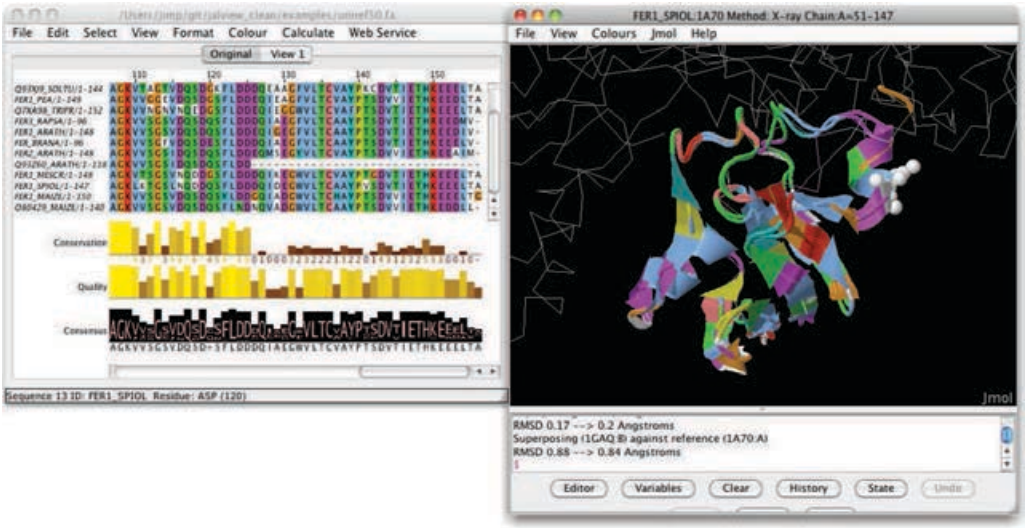


Figure 7.3: **Superposition of two ferredoxin structures.** The alignment on the left was used by Jalview to superpose structures associated with the FER1_SPIOL and FER1_MAIZE sequences in the alignment. Parts of each structure used for superposition are rendered as a cartoon, the remainder rendered in wireframe. The RMSD between corresponding positions in the structures before and after the superposition is shown in the Jmol console.

7.3.3 Colouring Structure Data Associated with Multiple Alignments and Views

Normally, the original view from which a particular structure view was opened will be the one used to colour structure data. If alignments involving sequences associated with structure data shown in a Jmol have multiple views, Jalview gives you full control over which alignment, or alignment view, is used to colour the structure display. Sequence-structure colouring associations are changed *via* the

View ⇒ *Colour by ..* menu, which lists all views associated with data shown in the embedded Jmol view. A tick is shown beside views currently used as colouring source, and moving the mouse over each view will bring it to the front of the alignment display, allowing you to browse available colour sources prior to selecting one. If the *Select many views* option is selected, then multiple views can be selected as sources for colouring the structure data. *Invert selection* and *Select all views* options are also provided to quickly change between multi-view selections.

Note that the *Select many views* option is useful if you have different views that colour different areas or domains of the alignment. This option is further explored in Section 25.

Exercise 25: Aligning Structures using the Ferredoxin Sequence Alignment

25.a. Continue with the Jalview project created in exercise 23

25.b. Open the 3D Structure chooser dialog from the popup menu for FER1_SPIOL by right-clicking its ID (CMD-click on Macs), and selecting ⇒ *3D Structure Data . . .*

25.c. Pick 1A70 from the Structure Chooser dialog, and click the **View** button. Jalview will give you the option of aligning the structure to the one already open. To superimpose the structure associated with FER1_MAIZE with the one associated with FER1_SPIOL, press *Yes*.
The Jmol view should update to show both structures, and one will be moved on to the other. If this doesn't happen, use the Align function in the Jmol submenu.

25.d. Create a new view on the alignment, and hide all but columns 121 through to 132 (you can do this via *View* ⇒ *Hide* ⇒ *All but selected region*).

25.e. Select the newly created view in the *Jmol* ⇒ *Superpose With* submenu, and then recompute the superposition with *Jmol* ⇒ *Align Structures*.
Note how the molecules shift position when superposed with only a small region of the alignment.

25.f. Compare RMSDs obtained when superimposing molecules with columns 121-132 and with the whole alignment.

25.g. The RMSD report can be viewed by right clicking the mouse on Jmol window, and select *Console* from the menu (if nothing is shown, recompute the superposition after displaying the console).
Which view do you think give the best 3D superposition, and why ?

Colouring Complexes

The ability to control which multiple alignment view is used to colour structural data is essential when working with data relating to multidomain biomolecules and complexes.

In these situations, each chain identified in the structure may have a different evolutionary history, and a complete picture of functional variation can only be gained by integrating data from different alignments on the same structure view. An example of this is shown in Figure 7.5, based on data from Song et. al.[9]

[9]Structure of DNMT1-DNA Complex Reveals a Role for Autoinhibition in Maintenance DNA Methylation. Jikui Song, Olga Rechkoblit, Timothy H. Bestor, and Dinshaw J. Patel. *Science* 2011 **331** 1036-1040 DOI:10.1126/science.1195380

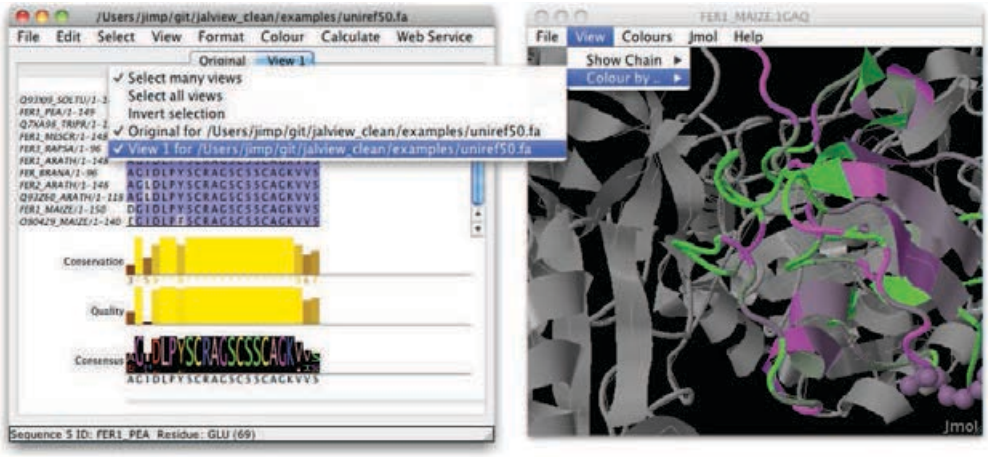


Figure 7.4: **Choosing a different view for colouring a structure display** Browsing the *View* ⇒ *Colour by ..* menu provides full control of which alignment view is used to colour structures when the *Colours* ⇒ *By Sequence* option is selected.

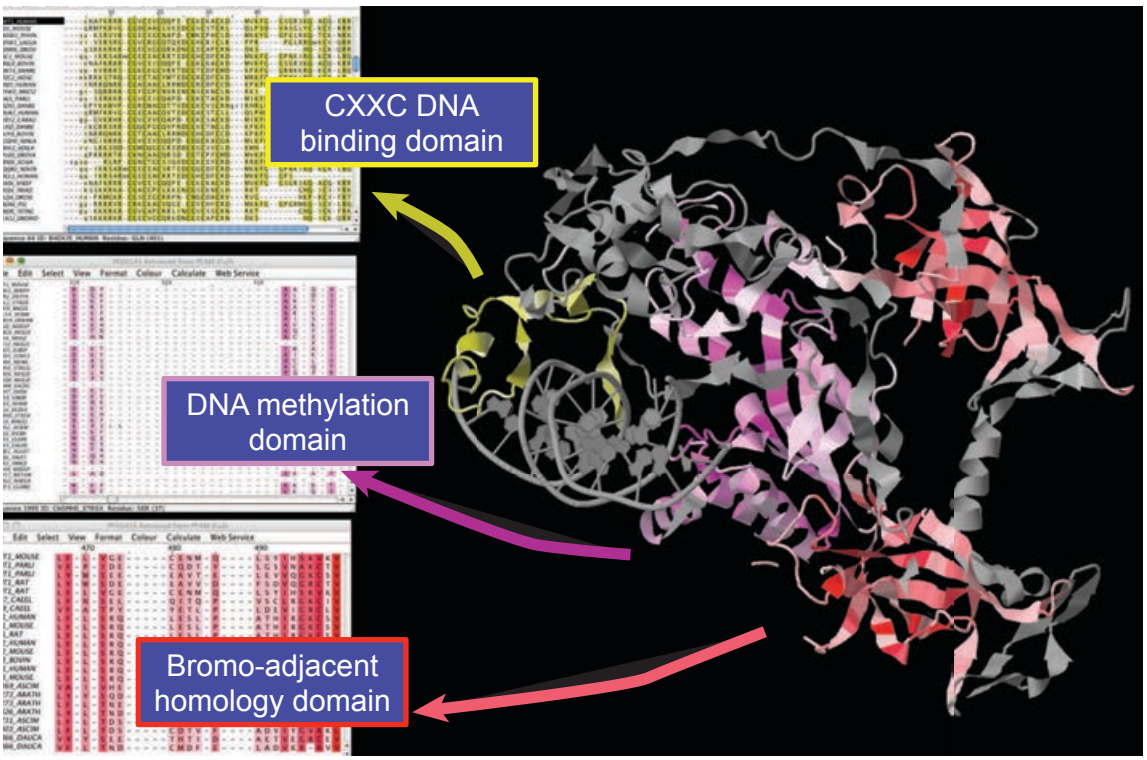


Figure 7.5: **The biological assembly of Mouse DNA Methyltransferase-1 coloured by Pfam alignments for its major domains** Alignments for each domain within the Uniprot sequence DNMT1_MOUSE have been used to visualise sequence conservation in each component of this protein-DNA complex. Instructions for recreating this figure are given in exercise 26.

Exercise 26: Colouring a Protein Complex to Explore Domain-Domain Interfaces

26.a. Download the PDB file at `http://www.jalview.org/tutorial/DNMT1_MOUSE.pdb` to your desktop. This is the biological unit for PDB ID 3pt6, as identified by the PDBe's PISA server.

26.b. Launch the Jalview desktop and ensure you have at least 1G of free memory available.
See section 1.4.6 for how to do this or click the following link:
`http://www.jalview.org/services/launchApp?jvm-max-heap=2G`

26.c. Retrieve the following PFAM alignments from the **PFAM (full)** source : PF02008 PF01426 PF00145 (enter all three - they will each be retrieved into their own alignment window).

26.d. Drag the URL or file of the structure you downloaded in step 1 onto one of the alignments to associate it with the mouse sequence in that Pfam domain family.

26.e. Use the Find dialog to locate every DNMT1_MOUSE sequence in the alignment and for each one, open the Structure Chooser via the ID popup menu (⇒ *3D Structure Data* . Select the DNMT1_MOUSE.pdb structure from the 'Cached Structures' view, and click **View**.
Part of the newly opened structure will be coloured the same way as the associated DNMT1_MOUSE sequence is in the alignment view.
WARNING: do not select all sequences and open the Structure Chooser ! *This will cause Jalview to attempt to discover all structures for sequences in the alignment.*

26.f. Repeat the previous two steps for each of the other alignments. In each case, after selecting the DNMT1_MOUSE.pdb structure and hitting the 'View' button on the Structure Chooser dialog, Jalview will ask if you wish to create a new Jmol view. Respond **'Yes'** each time. This will ensure ensure each sequence fragment is associated with the **same** Jmol view.

26.g. Pick a different colourscheme for each alignment, and use the *Colour by ..* submenu to ensure they are all used to colour the complex shown in the Jmol window.
The different shading schemes will allow regions of strong physicochemical conservation are highlighted on the domains in the structure.

26.h. The final step needed to reproduce the shading in Figure 7.5 is to use the *Colour* ⇒ *By Annotation...* option in each alignment window to shade the alignment by the **Conservation** annotation row (introduced in section 3.1.5).
Ensure that you first disable the *View* ⇒ *Show Features* menu option, or you may not see any colour changes in the associated structure.
Examine the regions strongly coloured at the interfaces betweeen each protein domain, and the DNA binding region. What do you think these patterns mean ?

26.i. Save your work as a Jalview project and verify that it can be opened again by starting another Jalview Desktop instance, and dragging the saved project into the desktop window.

Chapter 8

Protein sequence analysis and structure prediction

Many of Jalview's sequence feature and annotation capabilities were developed to allow the results of sequence based protein structure prediction methods to be visualised and explored. This chapter introduces services integrated with the Jalview Desktop for predicting protein secondary structure and protein disorder.

8.1 Protein Secondary Structure Prediction

Protein secondary structure prediction is performed using the Jpred[1] server at the University of Dundee[2]. The behaviour of this calculation depends on the current selection:

- If nothing is selected, Jalview will check the length of each alignment row to determine if the visible sequences in the view are aligned.
  - If all rows are the same length (often due to the application of the *Edit* ⇒ *Pad Gaps* option), then a JPred prediction will be run for the first sequence in the alignment, using the current alignment as the profile to use for prediction.
  - Otherwise, just the first sequence will be submitted for a full JPred prediction.
- If just one sequence (or a region in one sequence) has been selected, it will be submitted to the automatic JPred prediction server for homolog detection and prediction.
- If a set of sequences are selected, and they appear to be aligned using the same criteria as above, then the alignment will be used for a JPred prediction on the first sequence in the set (that is, the one that appears first in the alignment window).

[1] *"The Jpred 3 Secondary Structure Prediction Server"* Cole, C., Barber, J. D. and Barton, G. J. (2008) *Nucleic Acids Research* **36**, (Web Server Issue) W197-W201

"Jpred: A Consensus Secondary Structure Prediction Server" Cuff, J. A., Clamp, M. E., Siddiqui, A. S., Finlay, M. and Barton, G. J. (1998) *Bioinformatics* **14**, 892-893

[2] http://www.compbio.dundee.ac.uk/www-jpred/

Figure 8.1: **Secondary Structure Prediction** Status (left) and results (right) windows for JPred predictions.

Jpred is launched in the same way as the other web services. Select *Web Service* ⇒ *Secondary Structure Prediction* ⇒ *JPred Secondary Structure Prediction*[3] from the alignment window menu (Figure 8.1). A status window opens to inform you of the progress of the job. Upon completion, a new alignment window opens and the Jpred predictions are included as annotations. Consult the Jpred documentation for information on interpreting these results.

8.1.1 Hidden Columns and JPred Predictions

Hidden columns can be used to exclude parts of a sequence or profile from the input sent to the JNet service. For instance, if a sequence is known to include a large loop insertion, hiding that section prior to submitting the JNet prediction can produce different results. In some cases, these secondary structure predictions can be more reliable for sequence on either side of the insertion[4]. Prediction results returned from the service will be mapped back onto the visible parts of the sequence, to ensure a single frame of reference is maintained in your analysis.

[3] JNet is the Neural Network based secondary structure prediction method that the JPred server uses.
[4] This, of course, cannot be guaranteed.

Exercise 27: Secondary Structure Prediction
Note: The annotation panel can get quite busy during this exercise. Try hiding some annotations rows by right clicking the mouse in the annotation label panel and select the "Hide this row" option. The Annotations dropdown menu on the alignment wndow also provides options for reording and hiding autocalculated and sequence associated annotation.

27.a. Open the alignment at `http://www.jalview.org/tutorial/alignment.fa`. Select the sequence *FER_MESCR* by clicking on the sequence ID. Then select *Web Service* ⇒ *Secondary Structure Prediction* ⇒ *JPred Secondary Structure Prediction* from the alignment window menu. A status window will appear and after some time (about 2-4 min) a new window with the JPred prediction will appear. Note that the number of sequences in the results window is many more than in the original alignment as JPred performs a PSI-BLAST search to expand the prediction dataset. The results from the prediction are visible in the annotation panel. JPred secondary structure prediction annotations are examples of sequence-associated alignment annotation.

27.b. Select a different sequence and perform a JPred prediction in the same way. There will probably be minor differences in the predictions.

27.c. Select the sequence used in the second sequence prediction by clicking on its name in the sequence ID panel, and copy *([CTRL] or [CMD]-C)* and then paste it *[CTRL] or [CMD]-V)* into the first prediction window. You can now compare the two predictions as the annotations associated with the sequence has also been copied across.

27.d. Select and hide some columns in one of the alignment profiles that were returned from the JNet service, and then submit the profile for prediction again.

27.e. When you get the result, verify that the prediction has not been made for the hidden parts of the profile (by clicking the mouse on column ruler and right click to open the context menu and select *Reveal All*), and that the JPred reliability scores differ from the prediction made on the full profile.

27.f. In the original alignment that you loaded in step 1, select **all** sequences, then open the *Sequence ID* ⇒ *Selection* submenu by right clicking the mouse to open the context menu, and select the *Add Reference Annotation* option.
All the JPred predictions for the sequences will now be visible in the original alignment window.

Homework: Go back to the last step of exercise 14 and follow the instructions to view the Jalview annotations file created from the annotations generated by the JPred server for your sequence.

8.2 Protein Disorder Prediction

Disordered regions in proteins were classically thought to correspond to "linkers" between distinct protein domains, but disorder can also play a role in function. The *Web Service* ⇒ *Disorder* menu in the alignment window allows access to protein disorder prediction services provided by the configured JABAWS servers.

8.2.1 Disorder Prediction Results

Each service operates on sequences in the alignment to identify regions likely to be unstructured or flexible, or alternately, fold to form globular domains. As a consequence, disorder predictor re-

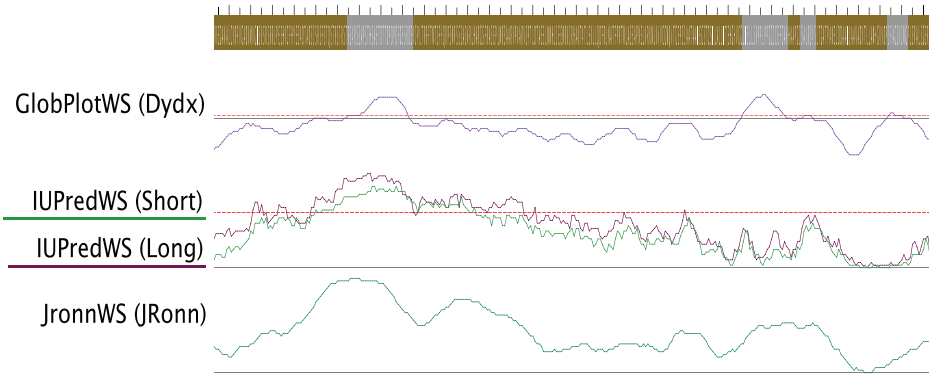


Figure 8.2: **Annotation rows for several disorder predictions on a sequence**. A zoomed out view of a prediction for a single sequence. The sequence is shaded to highlight disordered regions (brown and grey), and the line plots below the Sequence show the raw scores for various disorder predictors. Horizontal lines on each graph mark the level at which disorder predictions become significant.

sults include both sequence features and sequence associated alignment annotation rows. Section 4 describes the manipulation and display of these data in detail, and Figure 8.3 demonstrates how sequence feature shading and thresholding (described in Section 4.2.2) can be used to highlight differences in disorder prediction across aligned sequences.

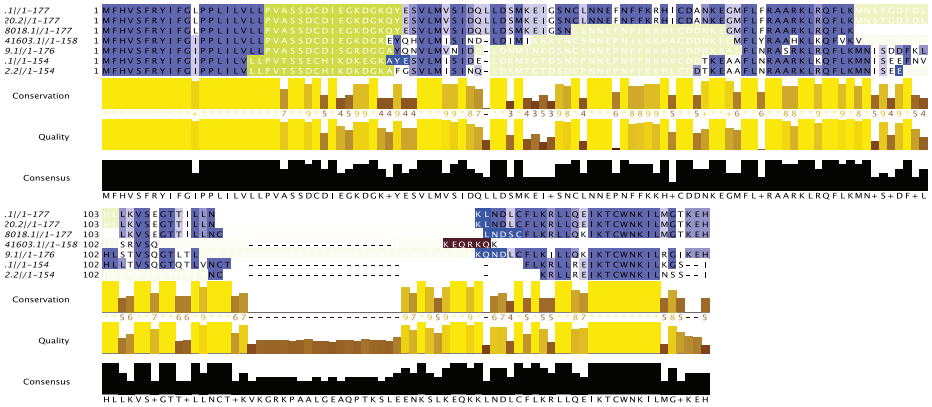


Figure 8.3: **Shading alignment by sequence disorder**. Alignment of Interleukin IV homologs coloured with Blosum62 with protein disorder prediction sequence features overlaid, shaded according to their score. Borderline disordered regions appear white, reliable predictions are either Green or Brown depending on the type of disorder prediction.

8.2.2 Navigating Large Sets of Disorder Predictions

Figure 8.2 shows a single sequence annotated with a range of disorder predictions. Disorder prediction annotation rows are associated with a sequence in the same way as secondary structure prediction results. When browsing an alignment containing large numbers of disorder prediction

annotation rows, clicking on the annotation row label will highlight the associated sequence in the alignment display, and double clicking will select that sequence.

8.2.3 Disorder Predictors provided by JABAWS 2.0

For full details of each predictor and the results that Jalview can display, please consult Jalview's protein disorder service documentation. Short descriptions of the methods provided in JABAWS 2.0 are given below:

DisEMBL

DisEMBL (Linding et al., 2003) is a set of machine-learning based predictors trained to recognise disorder-related annotation found on PDB structures.

COILS Predicts loops/coils according to DSSP definitions[5]. Features mark range(s) of residues predicted as loops/coils, and annotation row gives raw value for each residue. Value over 0.516 indicates loop/coil.

HOTLOOPS constitute a refined subset of **COILS**, namely those loops with a high degree of mobility as determined from Cα temperature factors (B factors). It follows that highly dynamic loops should be considered protein disorder. Features mark range(s) of residues predicted to be hot loops and annotation row gives raw value for each residue. Values over 0.6 indicates hot loop.

REMARK465 "Missing coordinates in X-ray structure as defined by remark465 entries in PDB. Nonassigned electron densities most often reflect intrinsic disorder, and have been used early on in disorder prediction." Features give range(s) of residues predicted as disordered, and annotation rows gives raw value for each residue. Values over 0.1204 indicates disorder.

RONN *a.k.a.* Regional Order Neural Network

RONN employs an approach known as the 'bio-basis' method to predict regions of disorder in sequences based on their local similarity with a gold-standard set of disordered protein sequences. It yields a set of disorder prediction scores, which are shown as sequence annotation below the alignment.

JRonn[6] Annotation Row gives RONN score for each residue in the sequence. Scores above 0.5 identify regions of the protein likely to be disordered.

[5]DSSP Classifications of secondary structure are: α-helix (H), 310-helix (G), β-strand (E) are ordered, and all other states (β-bridge (B), β-turn (T), bend (S), π-helix (I), and coil (C)) considered loops or coils.

[6]JRonn denotes the score for this server because JABAWS runs a Java port of RONN developed by Peter Troshin and distributed as part of Biojava 3

IUPred

IUPred employs an empirical model to estimate likely regions of disorder. There are three different prediction types offered, each using different parameters optimized for slightly different applications. It provides raw scores based on two models for predicting regions of 'long disorder' and 'short disorder'. A third predictor identifies regions likely to form structured domains.

Long disorder Annotation rows predict context-independent global disorder that encompasses at least 30 consecutive residues of predicted disorder. A 100 residue window is used for calculation. Values above 0.5 indicates the residue is intrinsically disordered.

Short disorder Annotation rows predict for short, (and probably) context-dependent, disordered regions, such as missing residues in the X-ray structure of an otherwise globular protein. Employs a 25 residue window for calculation, and includes adjustment parameter for chain termini which favors disorder prediction at the ends. Values above 0.5 indicate short-range disorder.

Structured domains are marked with sequence Features. These highlight likely globular domains useful for structure genomics investigation. Post-analysis of disordered region profile to find continuous regions confidently predicted to be ordered. Neighbouring regions close to each other are merged, while regions shorter than the minimal domain size of at least 30 residues are ignored.

GLOBPLOT

GLOBPLOT defines regions of globularity or natively unstructured regions based on a running sum of the propensity of residues to be structured or unstructured. The propensity is calculated based on the probability of each amino acid being observed within well defined regions of secondary structure or within regions of random coil. The initial signal is smoothed with a Savitzky-Golay filter, and its first order derivative computed. Residues for which the first order derivative is positive are designated as natively unstructured, whereas those with negative values are structured.

Disordered region sequence features are created marking mark range(s) of residues with positive first order derivatives, and **Globular Domain** features mark long stretches of order. **Dydx** annotation rows give the first order derivative of smoothed score. Values above 0 indicates residue is disordered.

Smoothed Score and Raw Score annotation rows give the smoothed and raw scores used to create the differential signal that indicates the presence of unstructured regions. These are hidden by default, but can be shown by right-clicking on the alignment annotation panel and selecting **Show hidden annotation**.

Exercise 28: Protein Disorder Prediction

Before starting this exercise, make sure you enable the 'Add Temperature Factor' option in your **Structures** *preferences.*

28.a. Open the alignment at: `http://www.jalview.org/tutorial/interleukin7.fa`.

28.b. Run the DisEMBL disorder predictor the Service ⇒ Disorder Prediction submenu.

28.c. Select all the sequences, and open the Structure Chooser via the *Sequence ID* ⇒ *3D Structure Data...* popup menu. Hit the **View** button to retrieve and show all PDB structures for the sequences.

28.d. Compare the disorder predictions to the structure data by mapping any available temperature factors to the alignment *via* the *Sequence ID Popup* ⇒ *Selection* ⇒ *Add reference annotation* option.

28.e. Apply the IUPred disorder prediction method. Use the *Per sequence option* in the *Colour* ⇒ *By annotation ...* dialog to shade the sequences by the long and short disorder predictors. *Note how well the disordered regions predicted by each method agree with the structure.*

Chapter 9

DNA and RNA Sequences

9.1 Working with DNA

Jalview was originally developed for the analysis of protein sequences, but now includes some specific features for working with nucleic acid sequences and alignments. Jalview recognises nucleotide sequences and alignments based on the presence of nucleotide symbols [ACGT] in greater than 85% of the sequences. Built in codon-translation tables can be used to translate ORFs into peptides for further analysis. ENA nucleotide records retrieved *via* the sequence fetcher (see Section 1.4.5) are also parsed in order to identify codon regions and extract peptide products. Furthermore, Jalview records mappings between protein sequences that are derived from regions of a nucleotide sequence. Mappings are used to transfer annotation between nucleic acid and protein sequences, and to dynamically highlight regions in one sequence that correspond to the position of the mouse pointer in another.

9.1.1 Alignment and Colouring

Jalview provides a simple colourscheme for DNA bases, but does not apply any specific conservation or substitution score model for the shading of nucleotide alignments. However, pairwise alignments performed using the *Calculate* ⇒ *Pairwise Alignment . . .* option will utilise an identity score matrix to calculate alignment score when aligning two nucleotide sequences.

Aligning Nucleic Acid Sequences

Jalview has limited knowledge of the capabilities of the programs that are made available to it *via* web services, so it is up to you, the user, to decide which service to use when working with nucleic acid sequences. The table shows which alignment programs are most appropriate for nucleotide alignment. Generally, all will work, but some may be more suited to your purposes than others. We also note that none of these include support for taking RNA secondary structure prediction into account when aligning sequences (but will be providing services for this in the future!)

Program	NA support	Notes
ClustalW	Yes	Default is to autodetect nucleotide sequences. Editable parameters include nucleotide substitution matrices and distance metrics.
Muscle	Yes (treat U as T)	Default is to autodetect nucleotide sequences. Editable parameters include nucleotide substitution matrices and distance metrics.
MAFFT	Yes	Will autodetect nucleotide sequences and use a hardwired substitution model (all amino-acid sequence related parameters are ignored). Unknown whether substitution model treats Uracil specially.
ProbCons	No	ProbCons has no special support for aligning nucleotide sequences. Whilst an alignment will be returned, it is unlikely to be reliable.
T-COFFEE	Yes	Sequence type is automatically detected and an appropriate parameter set used as required. A range of nucleotide specific score models are available.

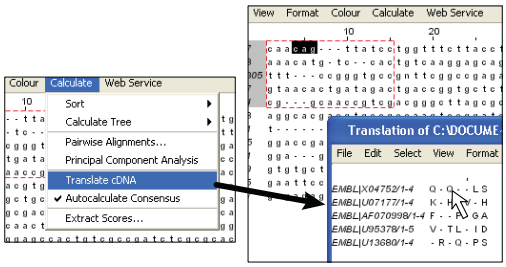
Table 9.1: **JABAWS Alignment programs suitable for aligning nucleic acid sequences.** All JABAWS alignment services will return an alignment if provided with RNA or DNA sequences, with varying reliability.

9.1.2 Translate cDNA

The *Calculate* ⇒ *Translate cDNA* function in the alignment window is only available when working with a nucleic acid alignment. It uses the standard codon translation table given in the online help documentation to translate a nucleotide alignment, or the currently selected region, into a set of aligned peptide sequences. Any features or annotation present on the nucleotide alignment will also be translated, allowing DNA alignment analysis results to be transferred on to peptide products for further investigation.

9.1.3 Linked DNA and Protein Views

Views of alignments involving DNA sequences are linked to views of alignments containing their peptide products in a similar way to views of protein sequences and views of their associated structures. Peptides translated from cDNA that have been fetched from ENA records for DNA contigs are linked to their 'parent' coding regions. Mousing over a region of the peptide highlights codons in views showing the original coding region.



9.1.4 Coding Regions from ENA Records

Many ENA records that can be retrieved with the sequence fetcher contain exons. Coding regions will be marked as features on the ENA nucleotide sequence, and Uniprot database cross references will be listed in the tooltip displayed when the mouse hovers over the sequence ID. Uniprot database cross references extracted from ENA records are sequence cross references, and associate a Uniprot sequence's coordinate system with the coding regions annotated on the ENA sequence. Jalview utilises cross-reference information in two ways.

Retrieval of Protein or DNA Cross References

The *Calculate* ⇒ *Get Cross References* function is only available when Jalview recognises that there are protein/DNA cross-references present on sequences in the alignment. When selected, it retrieves the cross references from the alignment's dataset (a set of sequence and annotation metadata shared between alignments) or using the sequence database fetcher. This function can be used for ENA sequences containing coding regions to open the Uniprot protein products in a new alignment window. The new alignment window that is opened to show the protein products will also allow dynamic highlighting of codon positions in the ENA record for each residue in the protein product(s).

Retrieval of Protein Features on Coding Regions

The Uniprot cross-references derived from ENA records can be used by Jalview to visualize protein sequence features directly on nucleotide alignments. This is because the database cross references include the sequence coordinate mapping information to correspond regions on the protein sequence with that of the nucleotide contig. Jalview will use the Uniprot accession numbers associated with the sequence to retrieve features, and then map them onto the nucleotide sequence's coordinate system using the coding region location.

Exercise 29: Visualizing Protein Features on Coding Regions

29.a. Use the sequence fetcher to retrieve ENA record D49489.

29.b. Ensure that *View* ⇒ *Show Sequence Features* is checked and change the alignment view format to *Wrapped* mode so the distinct exons can be seen.

29.c. Open the *DAS Settings* tab in the *Sequence Feature Settings...* window *View* ⇒ *Features setting* and fetch features for D49489 from the Uniprot reference server, and any additional servers that work with the Uniprot coordinate system.

29.d. Mouse over the features retrieved, note that they have been mapped onto the coding regions, and in some cases broken into several parts to cover the distinct exons.

29.e. Open a new alignment view containing the Uniprot protein product with *Calculate* ⇒ *Get Cross References* ⇒ *Uniprot* and examine the database references and sequence features. Experiment with the interactive highlighting of codon position for each residue.

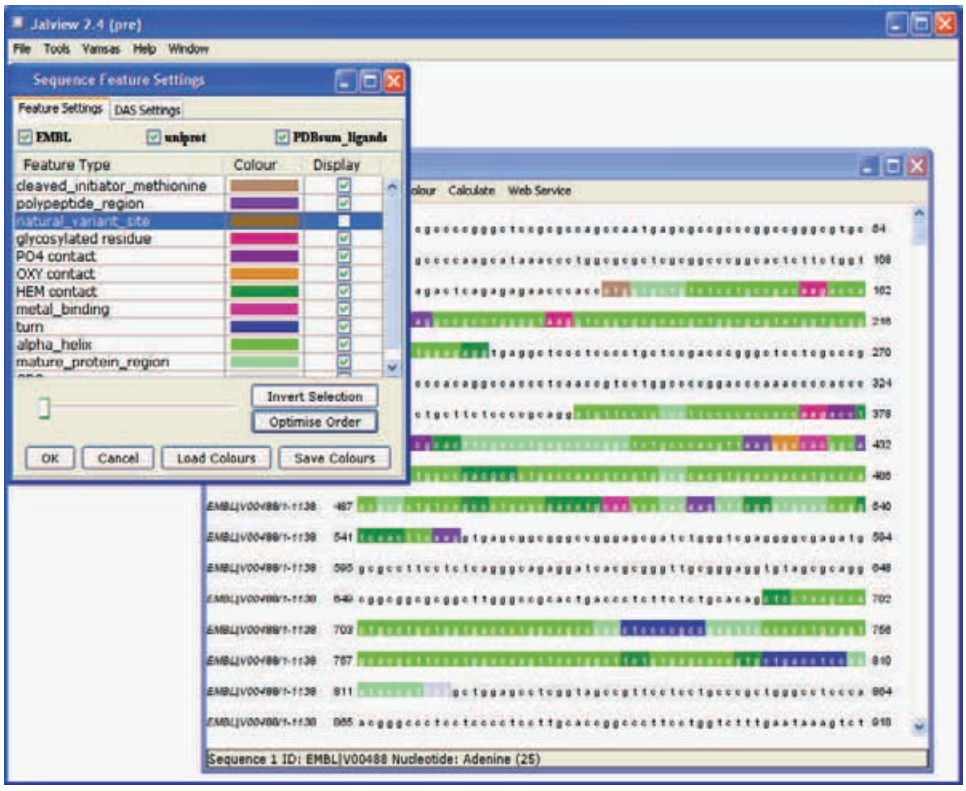


Figure 9.1: Uniprot and PDB sum features retrieved and mapped onto coding regions of ENA record V00488 (an earlier version of Jalview is shown here).

9.2 Working with RNA

Jalview allows the creation of RNA secondary structure annotation, and includes the VARNA secondary structure viewer for the display of RNA base pair diagrams. It also allows the extraction of RNA secondary structure from 3D data when available.

9.2.1 Performing RNA Secondary Structure Predictions

Secondary structure consensus calculations can be performed by enabling the VIENNA service *via* the *Web Service* ⇒ *Secondary Structure* menu. These consensus structures are created by analysing the covariation patterns in all visible sequences on the alignment. For more information see the VIENNA documentation.

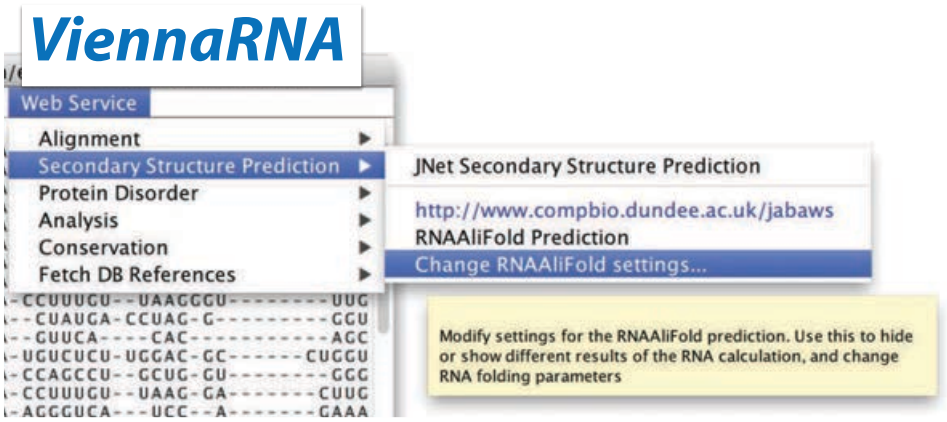


Figure 9.2: Secondary structure consensus calculations can be performed by enabling the VIENNA service *via* the *Web Service* ⇒ *Secondary Structure* menu.

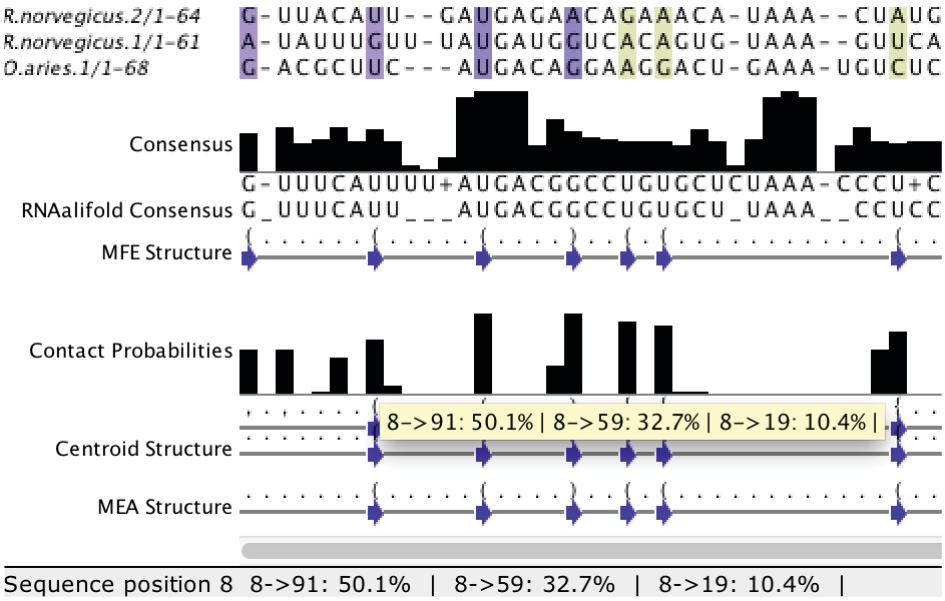


Figure 9.3: VIENNA can calculate alternate RNA base pairing probabilities. These are shown in Jalview as tool-tips on the RNA secondary structure probability score.

Exercise 30: Viewing RNA Structures

30.a. Import RF00162 from the Rfam (Seed) source using *Fetch sequence(s)* from the Desktop's File menu.

30.b. Select *Colour by RNA Helices* to shade the alignment by the secondary structure annotation provided by Rfam.

30.c. Open VARNA with *Structure* ⇒ *View Structure* ⇒ *RNA Secondary Structure*. In the VARNA Structures Manager toggle between (i) secondary structure (alignment) (with gaps) and (ii) trimmed secondary structure (alignment). Explore the difference between trimmed and untrimmed views. Click on different residues in the VARNA diagram - you should also see them highlighted and selected in the sequence alignment window.

30.d. In the VARNA Structures Manager, right click on display window to bring up the pop up context menu. Explore the options within the File, Export, Display and Edit sections.

VARNA views are stored in Jalview project files, in the same way as 3D structure views produced by Jmol and Chimera.

30.e. Enable the calculation and display of an RNAAliFold secondary structure prediction for the alignment by selecting *Web Service* ⇒ *Secondary Structure Prediction* ⇒ *RNAAliFold* .

30.f. Edit the RNAAliFold calculation settings to show Base Pair probabilities. Explore how editing the alignment affects the consensus calculation.

30.g. Import 2GIS from the PDB database into a new window with *Fetch sequence(s)*.

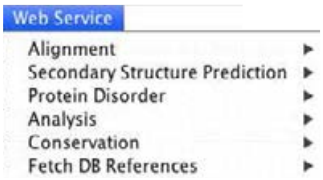
30.h. Click on a sequence in Sequence ID panel and select *Structure* ⇒ *View Structure* ⇒ *2GIS*, to view the structure in Jmol window. Click on different residues and located them in the sequence alignment window.

Chapter 10

Webservices

The term “Webservices” refers to a variety of data exchange mechanisms based on HTTP.[1]

Jalview can exploit public webservices to access databases remotely, and also submit data to public services by opening pages with your web browser. These types of services are ‘one-way’, *i.e.* data is either sent to the webservice or retrieved from it by Jalview. The desktop application can also interact with ‘two-way’ remote analysis services in order to offload computationally intensive tasks to High Performance Computing facilities. Most of these two-way services are provided by **Ja**va **B**ioinformatics **A**nalysis **W**eb **S**ervice (JABAWS) servers[2], which provides an easily installable system for performing a range of bioinformatics analysis tasks.



10.0.2 One-Way Web Services

There are two types of one way service in Jalview. Database services, which were introduced in in Section 1.4.5, provide sequence and alignment data. They can also be used to add sequence IDs to an alignment imported from a local file, prior to further annotation retrieval, as described in Section 4.2.

10.0.3 Remote Analysis Web Services

Remote analysis services enable Jalview to use external computational facilities. There are currently three types of service - multiple sequence alignment, protein secondary structure prediction, and alignment analysis. Many of these are provided by JABA servers, which are described at the end of this section. In all cases, Jalview will construct a job based on the alignment or currently selected sequences, ask the remote server to run the job, monitor status of the job and, finally, retrieve the results of the job and display them. The Jalview user is kept informed of the progress of the job

[1] HTTP: Hyper-Text Transfer Protocol.

[2] See http://www.compbio.dundee.ac.uk/jabaws for more information and to download your own server.

through a status window.

Currently, web service jobs and their status windows are not stored in Jalview Project Files[3], so it is important that you do not close Jalview whilst a job is running. It is also essential that you have a continuous network connection in order to successfully use web services from Jalview, since it periodically checks the progress of running jobs.

10.0.4 JABA Web Services for Sequence Alignment and Analysis

JABA stands for "JAva Bioinformatics Analysis", which is a system developed by Peter Troshin and Geoff Barton at the University of Dundee for running computationally intensive bioinformatics analysis programs. A JABA installation typically provides a range of JABA web services (JABAWS) for use by other programs, such as Jalview.

Exercises in the remainder of this section will demonstrate the simplest way of installing JABA on your computer, and configuring Jalview so it can access the JABA services. If you need any further help or more information about the services, please go to the JABAWS home page.

10.0.5 Changing the Web Services Menu Layout

If you are working with a lot of different JABA services, you may wish to change the way Jalview lays out the web services menu. You can do this from the Web Services tab of the *Preferences* dialog box.

Exercise 31: Changing the Layout of the Web Services Menu

31.a. Make sure you have loaded an alignment into Jalview, and examine the current layout of the alignment window's *Web Service* menu.

31.b. Open the preferences dialog box and select the web services tab.

31.c. Ensure the *Enable JABAWS services* checkbox is selected, and unselect the *Enable Enfin Services* checkboxes.

31.d. Hit *Refresh Services* to update the web services menu – once the progress bar has completed, open the *Web Service* menu to view the changes.

31.e. Select the *Index by host* checkbox and refresh the services once again.
Observe the way the layout of the JABAWS Alignment submenu changes.

31.f. Do the same with the *Index by type* checkbox.

Jalview provides these options for configuring the layout of the *Web Service* menu because different Jalview users may have access to a different number of JABA services, and each will have their own preference regarding the layout of the menu.

[3]This may be rectified in future versions.

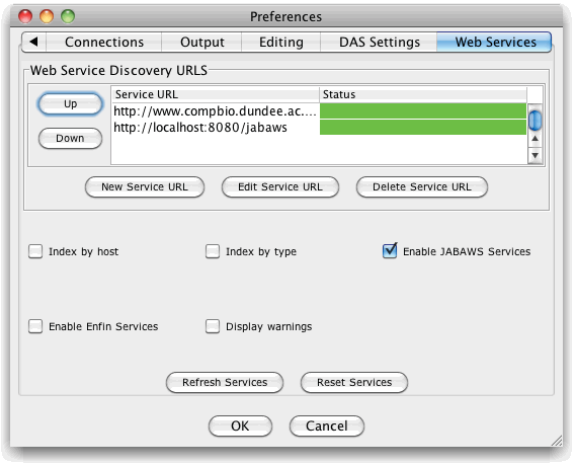


Figure 10.1: **The Jalview Web Services preferences panel.** Options are provided for configuring the list of JABA servers that Jalview will use, enabling and disabling Enfin services, and configuring the layout of the web services menu.

Testing JABA services

The JABAWS configuration dialog shown in Figure 10.1 has colour codes to indicate whether the Desktop can access the server, and whether all services advertised by the server are functional. The colour codes are:

- Red - Server cannot be contacted or reports a connection error.
- Amber - Jalview can connect, but one or more services are non-functional.
- Green - Server is functioning normally.

Test results from JABAWS are reported on Jalview's console output (opened from the Tools menu). Tests are re-run every time Jalview starts, and when the [Refresh Services] button is pressed on the Jalview JABAWS configuration panel.

Resetting the JABA Services Setting to their Defaults

Once you have configured a JABAWS server and selected the OK button of the preferences menu, the settings will be stored in your Jalview preferences file, along with any preferences regarding the layout of the web services menu. If you should ever need to reset the JABAWS server list to its defaults, use the 'Reset Services' button on the Web Services preferences panel.

10.0.6 Running your own JABA Server

You can download and run JABA on your own machine using the 'VMWare' or VirtualBox virtual machine environments. If you would like to do this, there are full instructions at the JABA web site.

Exercise 32: Installing a JABA Virtual Machine on your Computer

This tutorial will demonstrate the simplest way of installing JABA on your computer, and configuring Jalview so it can access the JABA services.

Prerequisites

You will need a copy of VMWare Player/Workstation/Fusion on your machine.

32.a. If you do not have VMWare player installed, download it from www.vmware.com (this takes a few minutes – you will need to register and wait for an email with a download link).

32.b. Download the JABA virtual appliance archive called 'jaba-vm.zip' from http://www.compbio.dundee.ac.uk/jabaws/archive/jabaws-vm.zip
WARNING: This is large (about 300MB) and will take some time to download.

32.c. Unpack the archive's contents to a place on your machine with at least 2GB of free space (On Windows, right click on the archive, and use the 'Extract archive..' option).

32.d. Open the newly extracted directory and double click the VMWare virtual machine configuration file (jabaws.vcf). This will launch the VMWare player.

32.e. Once VMWare player has started up, it may ask the question "Did you move or copy this virtual appliance?" – select 'Copy'.

32.f. You may be prompted to download the VMWare linux tools. These are not necessary, so close the window or click on 'Later'.

32.g. You may also be prompted to install support for one or more devices (USB or otherwise). Say 'No' to these options.

32.h. Once the machine has loaded, it will display a series of IP addresses for the different services provided by the VM. Make a note of the JABAWS URL – this will begin with 'http:' and end with '/jabaws".

Exercise 33: Configuring Jalview to Access your new JABAWS Virtual Appliance

33.a. Start Jalview (If you have not done so already).

33.b. Enable the Jalview Java Console by selecting its option from the Tools menu.
Alternately, use the System Java console if you have configured it to open when Jalview is launched, via your system's Java preferences (under the 'Advanced' tab on Windows).

33.c. Open the *Preferences* dialog and locate the Web Services tab.

33.d. Add the URL for the new JABAWS server you started in Exercise 32 to the list of JABAWS urls using the 'New Service URL' button.

33.e. You will be asked if you want to test the service. Hit 'Yes' to do this – you should then see some output in the console window.
Take a close look at the output in the console. What do you think is happening?

33.f. Hit OK to save your preferences – you have now added a new JABA service to Jalview!

33.g. Try out your new JABA services by loading the ferredoxin sequences from http://www.jalview.org/tutorial/alignment.fa

33.h. Launch an alignment using one of the JABA methods provided by your server. It will be listed under the JABAWS Alignment submenu of the *Web Service* menu on the alignment window.
Note: You can watch the JABA VM appliance's process working by opening the process monitor on your system. (On Windows XP, this involves right-clicking the system clock and opening the task manager – then selecting the 'Processes' tab and sort by CPU).