EMBL-EBI

EMBO Practical Course: Computational structural biology from data to structure to function

NMR Practical
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1. Introduction

This tutorial will focus on the use of the PDBe NMR resources to visualise and validate protein structures determined by NMR spectroscopy. Depositions of NMR structures mostly consist of an ensemble of multiple structures (typically 20).

In this tutorial, PDBe Vivaldi (Visualisation and Validation Display) is used to show most of the validation of PDB entries. It combines data from other resources into interactive graphs and tables and presents the molecule within a 3D viewer. Validation of general structure quality, chemical shifts, distance restraints and residual dipolar couplings are easily accessible within this web service.

2. Accessing NMR data at PDBe

The home page of the PDBe is located at www.pdbe.org and can be used to search the whole PDB archive for relevant structural data. The data summary page for a specific PDBe entry is available using their four-character PDB code at www.pdbe.org/*1xyz* (for PDB code *1xyz*).

An overview of NMR experimental data is accessed by clicking the "experiment" link in the menu-tree on the left-hand side of the data summary page. Alternatively the "NMR" icon on top of the page can be used. This icon will be coloured in green if any experimental data is available. Otherwise the "NMR" icon will appear grey.

Links on the experimental data webpage for every entry are provided to the data, i.e. VASCO chemical shift reports, original and processed (BMRB) restraints, clustering information and structure validation by NRG-CING. Additionally, links to the PDBe Vivaldi web service are provided ("View") for the interactive visualization and validation of the experimental NMR data.

Alternatively, this data can also be accessed from main webpage of the PDBe Vivaldi web service, www.pdbe.org/vivaldi. Here you can enter the four-character PDB code of your interest and either display the entry in its "Default View" or use the "Wizard". An extensive manual is available using the link on the top-right of any PDBe Vivaldi webpage.

3. Browsing within PDBe Vivaldi

To explore the possibilities of and the navigation in PDBe Vivaldi, we will use PDB code *2k4v*. Go to www.pdbe.org/vivaldi, enter "2k4v" into the text field and press the "Default View" button. The PDBe Vivaldi visualization of this entry will load and the progress is shown on the top-left of the screen. Make sure your browser allows the execution of JavaScript and Java applets. You might be prompted to confirm the execution of the OpenAstexViewer Java Applet by your web browser.

The main commands for handling molecules in the 3D viewer are "Left Click-Drag" (rotate), "Shift-Drag" (zoom) and "Ctrl-Drag" (pan). Press "r" to reset the applet to its default view and "c" to zoom and centre on a selection. More OpenAstexViewer actions can be found at www.openastexviewer.net.

Most of the functionality of PDBe Vivaldi is available from four dropdown menus as shown in Figure 1. Some features will be explored in the following step-by-step tutorial.

Figure 1. Dropdown menus in PDBe Vivaldi
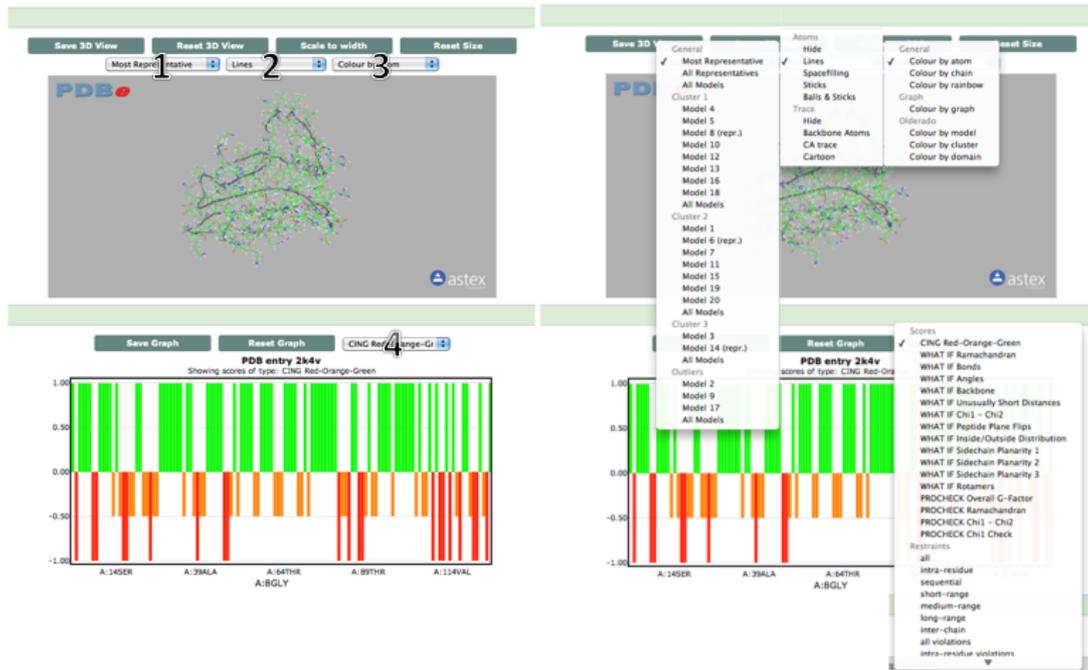
PDBe Vivaldi is developed to run under most popular web browsers although performance issues are known to exist when using Internet Explorer, please use **Firefox 5.0.1** for this tutorial.

Viewer

- Select the "All models" option from the "General" section in dropdown menu 1 (See Figure 1) to display all 20 models of the protein.
- Select the "Hide" option from the "Atoms" section in dropdown menu 2 (See Figure 1) to hide individual atoms. The schematic display will remain visible.
- Select the "Colour by cluster" option from dropdown menu 3 (See figure 1). Molecules will be coloured according to the PDBe OLDERADO clustering information.
- Select the "Colour by graph" option from dropdown menu 3 (See figure 1). Residues will now be coloured according to the data presented in the graph.
- Select the "Cartoon" option from the "Trace" section in dropdown menu 2 to display secondary structure elements.
- Hide all but the most representative model by selecting the "Model 8 (repr.)" option in dropdown menu 1. (The representative model of the largest cluster is always the most representative model)
- Show individual atoms by selecting "Lines" from dropdown 2.
- Click the "Scale to width" button above dropdown menus 1-3 to make the 3D viewer fit to the size of your web browser.
- Click the "Reset size" button above dropdown menus 1-3 to reset the applet size to its original value

Scores

- Display the WHATIF scores for unusual short distances in the graph by selecting the "WHAT IF Unusually Short Distances" option from dropdown menu 4 (See figure 1).
- Notice the change of colouring in the 3D viewer. Select the "Colour by graph" option from dropdown menu 3 (See figure 1) if no change in colour occurred.
- Click on the graph at residue "A:98MET" to zoom to this residue in the 3D viewer. Note that no major artefact can be seen. This might be caused by a slight difference in force field parameters used by the modelling and validation software.
- Display the WHATIF scores for angles in the graph by selecting the "WHAT IF Angles" option from dropdown menu 4.
- Note that all but one outliers are histidine residues.

Chemical Shifts

- Click the "Reset 3D View" button above dropdown menus 1-3 to reset the clipping planes and zoom.
- Visualise all hydrogen chemical shift outliers by selecting "CS Outliers: H" from dropdown 4.
- Click and drag in the graph to select a zone around residue A:71ASP. The graph will be updated to only show the selected zone.
- Click on the graph at "A:71ASP:HB3" to centre and zoom the 3D viewer to this atom.
- Notice the nearby aromatic ring in residue A:58PHE. A ring-current in this ring can explain the unusual chemical shift of the HB protons of A:71ASP.
- Click the "Reset Graph" button to the left of dropdown menu 4 to show the full graph.

Distance Restraints

- Click the "Reset 3D View" button above dropdown menus 1-3 to reset the clipping planes and zoom.
- Select "all" from the "Restraints" section in dropdown menu 4. This will update the graph to show the amount of distance restraints per residue.
- Note that there is little experimental data available for residues 84 to 97.
- Click on a residue on the graph (e.g. A:SER57) to display all restraints involving that residue.
- Select "all violations" from the "Restraints" section in dropdown menu 4. This will update the graph to show the amount of distance restraint violations per residue.
- The black points show the amount of violations in the model displayed, while the coloured lines indicate the average amount of violations over the NMR ensemble.
- Click in the graph on residue A:49ILE to zoom to this residue in the 3D viewer and to display the violations.
- Click on the "orange" violation and scroll down to the info section of the webpage. This section will provide information about the selected restraint (Figure 2).

Figure 2. Information about a distance restraint violation

Restraint information

Subrestraint		Lower Limit	Upper Limit	Model 8	
A:52LYS:HA	A:49ILE:H	1.800	5.000	5.356	●

4. Case 1 - PDB entry 2k8v

- Load entry "2k8v" at www.pdbe.org/vivaldi/2k8v
- Take a look at the structure of the most representative model. Display cartoons of strands and helices. What do you think about the general quality of this entry?
- Take a look at the overall, ramachandran, bond and angle WHATIF and at the different PROCHECK graphs. Which graphs indicate a specific problem in the structure of this protein? Which is the problematic residue? Would you expect other validation graphs to have picked up on the same problem?
- Browse through all models of this entry and take a look at that residue. Is the flaw present in all models? What could be the cause of this? What is the implication to the overall quality of the structure?
- How well do the deposited chemical shifts fit the structure? Could the unusual chemical shift of any of the outliers be explained? (Use the connected BMRB with id 15964)
- Visualise all distance restraint violations on the most representative model. How well does the structure comply with the experimental restraints?
- Can you find another structure of the same protein in the PDB that does not show the imperfection indicated by the WHATIF graphs? (Use other PDBe resources)

5. Case 2 - PDB entry 2kn1

- Load entry "2kn1" at www.pdbe.org/vivaldi/2kn1
- Take a look at the structure of the most representative model. Display cartoons of strands and helices. What do you think about the general quality of this entry?
- Look at the C terminal residues of the most representative model and model 11.
- Look through the available graphs of WHATIF and PROCHECK. Can you find any indication that something is wrong in model 11? Which WHATIF score would you expect to be indicative of the error in model 11?
- Visualise all distance restraint violations on the most representative model. How well does the structure comply with the experimental restraints?

6. Case 3 - PDB entries 2joq and 2h9z

PDB entries 2joq and 2h9z are both NMR structures of the same protein. Looking at the structure and validation data on both entries might help to choose the most appropriate entry to use for further research.

- Load entries "2joq" and "2h9z" in different tabs of the web browser.
- Take a look at the structure of the most representative models. Display cartoons of strands and helices. Hide all atoms and display all models. What do you think about the general quality of both entries?
- Look through the available graphs of WHATIF and PROCHECK. Can you find specific or general issues with these entries?
- Compare the number of all, medium and long-range restraints. Which entry was modelled using the most non-redundant experimental distance restraints? Compare the number of distance restraint violations.
- How well do the deposited chemical shifts fit the structure of entry 2joq? Could the unusual chemical shift of any of the outliers be explained?