

Übung: Protein Classifications

Übung für Grundlagen der Strukturanalyse

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1. Introduction and Aims

Classifying proteins is a popular pastime and the classifications are used for problems ranging from structure to function prediction. Sometimes it is well automated, but some of the classifications are largely built by human beings. Sometimes it is treated a conventional clustering problem, but sometimes even the clusters are largely defined by humans. Here, we will look at some of the classifications which are very heavily based on human decisions.

The classifications to be used are

CATH: This defines 4 levels of hierarchy

Class, architecture, topology, homologous proteins

At the lowest level (homology) the members are very similar to each other and have easily detected sequence homology. This "H" level is often further divided up into different levels of sequence similarity (S35, S60, ..). Within a "topology" family, the proteins have a similar shape, but their sequences may be rather different. For example, the globin family includes haemoglobins, but also includes a domain from diphtheria toxin which has a very similar shape, but no obvious sequence or functional similarity.

SCOP: This usually defines 4 hierarchical levels, but uses different names

Class, fold, superfamily, family

As with CATH, proteins clustered together at the lowest level are sequence similar and usually have the same function. By the "fold" level, proteins will have a similar shape, but maybe no detectable sequence or functional similarity.

PFAM: is a classification based on sequences using hidden markov models. It does not impose a hierarchy on the proteins

Aims

- To understand the principles of structure classifications.
- To become familiar with the common classifications.
- To obtain and interpret the structural annotation for a protein.

2. Addresses

PDB	www.rcsb.org
SCOP	scop.mrc-lmb.cam.ac.uk/scop
CATH	www.cathdb.info
PFAM	www.sanger.ac.uk/pfam
Multiprot	bioinfo3d.cs.tau.ac.il/MultiProt

3. Tasks

Chimera: looking at the structures

Fetch the coordinates for

1ftt

Unfortunately, this coordinate file has 20 models (it was solved by NMR). The easiest way to look at this structure is to delete most of them:

Favourites

model panel

then select everything except the first model and click on "close" on the right hand side.

Fetch the second coordinates, for

1apl

To make the picture clearer, you might try

- select all coordinates (in the Model Panel)
- trace chains
- Actions -> ribbons -> show

To look for similarity (at the sequence level):

In the model panel

- Select both sets of coordinates
- Match

In the "MatchMaker" dialog

- Select one protein on the left and the second protein on the right. You may play with the other options, but the defaults should work fine.
- Turn off "Include secondary structure score"
- Turn on "Show pairwise alignment"
- apply

You should see the structural similarity between the proteins. You should also look in the panel "MultAlignViewer". Look under

- Tools
- Percent Identity

You should find an estimate of sequence similarity at the bottom of the MultAlignViewer.

A more interesting case

Proteins can be very similar, even when sequence methods cannot show the similarity. In this case, one needs a method which can align two structures without using their sequences. One should use either "multiport" or "SSAP".

Close the existing coordinates or restart chimera.

Load the coordinates for

1gxw

1i1i (note – the third character is the digit 1, not the character "ell").

To simplify the picture, select both coordinates, trace chains and show ribbons.

In the Model Panel, select both coordinates and then the "match" button. In the Matchmaker dialog, repeat the alignment with

- "include secondary structure score" off
- "Show pairwise alignment" on

At this point, is there any point to think these coordinates are similar ?

Note down the names of the files and whether they look at all similar to you.

Using Multiprot for the superposition

Visit the multiprot web page (see page 2).

Give it the PDB identifiers, "1i1i" and "1gxw" and submit. In a few seconds, this should offer you more than one alignment. Pick the larger. Save the coordinates it offers you *aligned.pdb*. The coordinates will have the first chain proteins labelled as model 1 and 2.

Using SSAP for the superposition

On the CATH web page, near the bottom, follow the link to "Pairwise structure comparison (SSAP)" Enter the names of the coordinates: 1gxw and 1i1i. Click on the continue button twice. This will start a calculation trying to align the two molecules. Now, wait, drink coffee or look at the next part of this text. This calculation will take a few minutes. When the results come back, you should see a page describing the structural alignment.

At the bottom of the page is the corresponding sequence alignment and you should be able to see that only the second half of 1gxw has been aligned to 1i1i.

Go back to the top of the page and follow the link "View/Save superposed PDB".

Scroll down and make sure this file has lines that begin with "ATOM". From the browser save this file as plain text with a name like super.pdb. This file contains the coordinates of 1gxw (chain A) and 1i1i (chain P).

Looking at the superposition

If you used SSAP to superimpose the coordinates, you can colour them with a command like

```
color red :.P
color blue :.A
```

If you used Multiprot, you can colour them with commands like

```
color red #0.1
color blue #0.2
```

This superposition should look very different to the one that you originally made in chimera for 1gxw and 1i1i. Try to follow the secondary structure elements (strands and helices) in the smaller structure and see if there is a corresponding element in the second structure.

Stop and make notes:

- Could you see similarity between 1i1i and 1gxw using chimera's built in sequence alignment ?
- Using the superposition from SSAP (the file, super.pdb) was the similarity clear ?

b) Homeodomain: 1ftt and 1apl

In both SCOP and CATH, search for the proteins 1ftt and 1apl.

In each classification, try to move around the classification tree (level) by going up from the leaf representing each protein.

Which classification level is common to both 1ftt and 1apl ?

Compare the number of homeodomain proteins that are classified in SCOP and CATH.

c) Immunoglobulins

Use this as a keyword to search both classifications. Both SCOP and CATH dedicate a branch of the classification tree to these "greek key" domains. Try to correspond (or match) the terminology (family, superfamily, fold) that SCOP uses, to the names used for CATH's last two levels and its S35 and S95 divisions.

d) Differences of opinion (1gxw and 1i1i)

There is a group of proteins known as metalloproteases or neutral proteases. In both SCOP and CATH, find the entry for 1gxw. Note down the number of domains, that each classification claims to find. Note down the broad hierarchical description (hydrolase, β -roll, ...). Where there is more than one domain, note down the domain boundaries.

What is the biggest difference between SCOP and CATH viewpoints ?

For the classification which claims to recognise more than one domain, note down the boundaries (start and end residues) of the domains.

Given two different opinions, one may look for another point of view.

Visit the PFAM web site and look up 1gxw. How many domains does it find ? Note down the domain boundaries.

Now, repeat the steps for 1i1i. You should have enough information to fill out a table like..

		SCOP	CATH	PFAM
1gxw	Number of domains			
	Name type of each domain		note the CATH codes as well	
	Boundaries of domains			
1i1i	Number of domains			
	Name type of each domain		note the CATH codes as well	
	Boundaries of domains			

4. Assignment

Please answer the following questions in a brief written report (1 page), and hand it in not later than 9. Dez 2009 with your name. From n students, there should be n different reports.

- Which classification level is common to both 1ftt and 1apl in CATH and SCOP ?
- For the immunoglobulins, describe the hierarchy in CATH and SCOP that leads to a protein such as 1bww.
- Fill out a table comparing the 1ili and 1gxw as outlined above.
 - Note down whether you could see any similarity between the two proteins when using the initial sequence-based alignment (chimera) and the superposition based on structure.
 - For the classifications which believe in more than one domain, are the domain boundaries similar ?