

## Übung: Homology Modeling

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### 1. Introduction

This is about building a model for a protein when you only know its sequence and using the structure of related proteins. We use the program MODELLER. A major point of the assignment is to see how the quality of the results depends on the initial alignments.

### 2. What is MODELLER?

Constructing a model for a protein, using its homology to other proteins means taking structural features from the parent structure(s) based on sequence alignment. These are then combined with restraints based on the sequence being modeled. MODELLER attempts to automate the homology modeling process. It can use multiple sequence alignments. It can use multiple templates, which were not discussed in lectures.

The program uses distance geometry and energy minimisation. Given a sequence and template structure(s), it can make an alignment and decide which parts of a template structure are probably conserved in the sequence. This alignment is used to build a set of distance restraints, which is then used by a distance geometry routine. The initial structures are then refined by energy minimization.

MODELLER balances the physical requirements of the modeled sequence against the geometric relations obtained from the template structures. The code and even more information are at: <http://salilab.org/modeller/modeller.html>.

### 3. Using MODELLER

MODELLER reads a script of instructions including run-time variables and procedural function calls. The variables are lists of flags, or strings, and set like this:

*Simplemodel.py*

```
from modeller.automodel import * # Load the automodel class
log.verbose()                   # request verbose output
env = environ()                 # create a new MODELLER environment
# directories for input atom files
env.io.atom_files_directory = './../atom_files'
a = automodel(env,
               alnfile = 'fdxhomologs.pir', # alignment filename
               knowns   = ('lfxd', 'lfdn'),  # codes of the templates
               sequence = 'ferodox')        # code of the target
a.auto_align() # get an automatic alignment
a.make()       # do the actual homology modeling
```

The `knowns` variable is a list of structure names, and `sequence` is a string containing the name of the sequence to be modeled. These names correspond to the entries for each protein in the alignment file:

*fdxhomologs.pir: A PIR file*

```
# A Comment
# This is the .pir file entry for lfdn
>P1;lfdn
structure:lfdn:FIRST:@:55:@:~::~~..
*
>P1;lfxd
structure:lfxd:FIRST:@:58:@:~::~~..
*
# This entry doesn't get used by simplemodel.py
>P1;5fd1
structure:5fd1:FIRST:@106:@:ferredoxin:Azotobacter vinelandii: 1.90.0.92
*
>P1;ferodox
sequence:lfdx:1: :54: :ferredoxin:Peptococcus aerogenes: 2.00:-1.00
AYVINDSCIACGACKPECPVNIIQGSIIYADADSCIDCGSCASVCPVGAPNPED*
# The * indicates the end of the sequence entry
```

The alignment file is like a flat-file database. Each '>P1;blah' line is a tag for the protein data called 'blah'. MODELLER looks for tags with the same names as those given in the `sequence` and `knowns` variables defined in the `.py` file.

The line after a tag is a ':' separated set of fields defining the protein data. For 5fd1 the first field is 'structure', and the next is the structure's PDB name. MODELLER uses this to search for the protein's PDB at each of the paths defined by another runtime variable called `env.io.atom_files_directory`. The next four fields are for the beginning and end residue number and chain ID that define the stretch of coordinates which MODELLER should read. In this case, 'FIRST:@' means the first residue in the first chain of the PDB file, and '60:@'

means the residue 60 (in PDB file numbering) in the same chain. The rest of the fields are typical sequence database entries (the protein name, and species), and the last two are the resolution and R factor for the X-ray structure. These are defined as a wildcard ('.') in most of the other entries, and the extra information is not important for this example.

The 'ferodox' entry is different, because it is the sequence to be modeled. The first field is 'sequence' - indicating that there is no structure. The second is a filename that will be used if a model is generated, and the rest give the numbering and amino acid chain code that will be used when the model is written.

The final line(s) give sequence information for the protein, as bounded by the start and end positions given in the fields in the previous line. Sequence entries must be in upper case, single letter amino acid codes, where the '\*' is the terminator for the sequence. Any whitespace characters are ignored. When the 'START:@:XX:@' field is used, where XX is some number, no sequence is necessary. However, for 'ferodox', the amino acid sequence must be defined, and is given as shown. It is important that there are precisely the same number of amino acids as is given in the preceding header by the start and end amino-acid numbering (54 a.a. characters in this case). The PIR format allows the representation of multiple alignments. A '-' character can be used for the sequences to indicate a gap. An example of this will be shown later.

The alignment filename is given to MODELLER via the `alnfile` variable, and any extra PDB file paths are defined in `env.io.atom_files_directory` - the filenames and paths are all relative to the current working directory in this example. 'a.auto\_align()' makes a multiple alignment of all the protein sequences. This is written out as a new .pir file called `fdxhomologs.pir.ali`. The final command 'a.make()' runs the automatic modeling function, which results in the following:

1. A log file is created, called *simplemodel.log*.
2. MODELLER reads *fdxhomologs.pir*, finds the unknown sequence and also reads in the structures specified in `knowns`.
3. A multiple alignment of all the sequences is made and written to a new .pir file called *fdxhomologs.pir.ali*.
4. The known structures are analysed based on the alignment, and used to make a set of distance restraints.
5. A model is made by distance geometry and refined.
6. The model is written out as *ferodox.B99990001.pdb*.

To run MODELLER using this script, make a new directory, change to it, and enter the following shell commands:

```
> setenv PATH "/home/schenk/bin:$PATH"
> cp -R /home/schenk/teaching/WS_2008_2009/GST/modelling/ferodox .
> cd ferodox
> mod9v5 simplemodel.py
```

to set up the path for MODELLER, and run the fully automatic modeling script file.

If everything has gone well, the MODELLER command will finish after a few minutes. The directory listing should then look something like:

```
ferodox> ls
atom files          ferodox.D00000001  ferodox.rsr
fdxhomologs.pir     ferodox.V99990001  ferodox.sch
fdxhomologs.pir.ali ferodox.ini         ferodox.B99990001.pdb
family.mat          simplemodel.log     simplemodel.py
```

The *ferodox.\** files are related to the modeled sequence. The others are the log file *simplemodel.log* written by MODELLER, and the multiple alignment that was actually used to generate the model (*fdxhomologs.pir.ali*).

*fdxhomologs.pir.ali* : Describing a multiple sequence alignment to match the *ferodox* sequence to two homologs:

```
>P1;lfxd
structure:lfxd:FIRST:@:58   :@:..: 0.00: 0.00
PIEVNDDCMACEACVEIC--PDVFEMNEEGDKAVVINP--DSD-LDCVEEAIDSCPAEAI-VRS*

>P1;lfdn
structure:lfdn:FIRST:@:55   :@:..: 0.00: 0.00
AYVINEACISCGACEPECPV-NAISSG-D-DRYVID-ADT-CID---CGACAGVCPVDAP-VQA*

>P1;ferodox
sequence:lfxd:1      : :54   : :ferredoxin:Peptococcus aerogenes: 2.00:-1.00
AYVINDSCIACGACKPECPV-NIIQ----GSIIY AIDADS--CID---CGSCASVCPVGAPNPED*
```

The alignment also yields a distance matrix: *family.mat*. This is the 'distance' between each sequence, as defined by MODELLER's own multiple alignment (the multiple sequence alignment is shown in the *.pir.ali* file).

The most important files are the generated coordinates:

*ferodox.B99990001.pdb*: The PDB coordinates for the model. The 9999 indicates that the coordinates are modeled rather than 'measured', and 0001 indicate that this is model number 1. In addition to 'ATOM' records, there are two informational entries in the file:

```
EXPDTA MODEL, MODELLER Version 7v7 2006/04/....
REMARK MODELLER OBJECTIVE FUNCTION: 1391.2858
```

The REMARK line gives the 'energy' value or objective function for the model (more negative is better).

The rest of the files give detailed output from the steps of model construction and minimization.

*ferodox.ini*: The initial model generated by distance geometry.

*ferodox.rsr*: The restraints applied to the atoms in the model, during construction and minimization. It lists pairs of atoms and the ideal distances.

*ferodox.sch*: The energy function schedule used in the optimization.

*ferodox.V99990001*: Heavy atom violations' for each residue.

*ferodox.D00000001*: The progress of optimization from model 0 (*ferodox.ini*) to model 1 (*ferodox.B99990001.pdb*).

That was a very simple example. In the next section, we see how one can use better alignments from outside the MODELLER program to make better models.

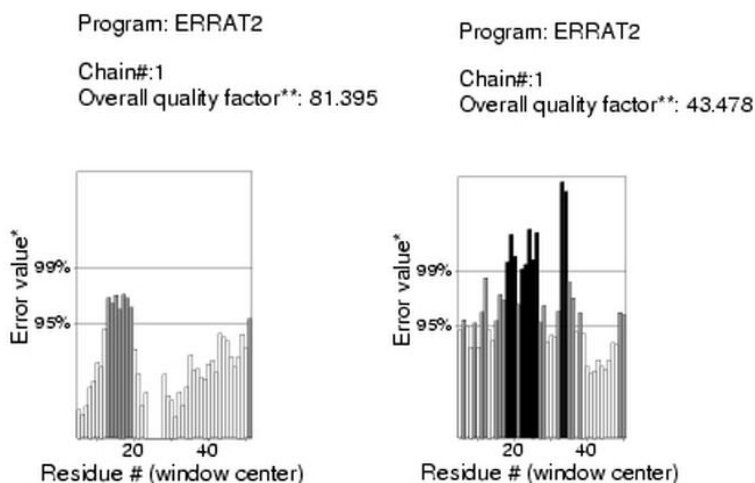
#### **4. Measuring Model Quality**

The protein sequence used in this exercise is from a known structure (PDB ID 1dur), so it is possible to determine if a model is good or bad simply by comparing it to the real protein structure. In a real homology modeling experiment, the true structure is not known, so it is necessary to use tools for analyzing the quality of the models. Such a measurement of model quality can be based on physical energy functions - which give a measure of structural quality, but it can be hard to estimate the absolute quality with these. The alternative is to assess the backbone conformations and side-chain packing using statistical observations of the typical geometry of proteins (knowledge based energy functions). One of these protein structure validation programs, called ERRAT, will be used in this exercise. The program examines a PDB file, and generates a score based on the quality of the local structure surrounding each residue, as compared to the typical ranges of dihedral angles and side chain contacts observed in real proteins. You can find the webserver for the program at:

<http://nihserver.mbi.ucla.edu/ERRATv2/>

To use the server, upload your PDB file using the web form. The program generates a plot which gives a measure of the structure error at each residue in the protein. It also

calculates an overall score for the structural quality. You can read more information about the method by following the links on the ERRAT server page. *Figure 1* shows two ERRAT assessments - one for the model generated by the *simplemodel.py* script, and along side it is the plot for the real structure for the sequence. The overall measure of quality is given at the top of the plot and each bar in the histogram is shaded according to the significance of the local structural error.



*Figure 1. ERRAT output for the Ferredoxin structure and model.*

*The validation plot for the real structure (PDB code 1dur) is on the left, and the model generated by MODELLER is on the right. The overall quality factor is given just above the plot of structure error.*

## 5. Exercise: Modelling Ferredoxin

In this exercise, you will reproduce the ferredoxin model and structural quality analysis which were described in the last two sections. You will then try to generate a better model, as assessed by ERRAT, by modifying the alignment. This will involve making a multiple sequence alignment with a variety of sequences and gap penalties, in order to get a better mapping of the sequence on to the template structures.

Your report should include an introduction, methods, results, and conclusion section. It should be brief. Your introduction should be a paragraph explaining what you have investigated, and what proteins you have used.

The methods section should be one or two paragraphs explaining how a model of a protein's structure can be generated by using its homology to some known structures. Finally,

you should summarize the procedure you have followed, and briefly describe the tools you have used to generate the alignments, homology models, and for measurement of model quality.

The results section should contain a short description of how each model's alignment was made, along with the alignment, and the quality of the model that was generated from it. The quality should be described by the ERRAT plot, its overall quality measurement, and the MODELLER objective function value. For each alignment, try to give a reason why there is a poor region in the model structure.

In your conclusion, you should discuss the reasons why the different alignments have produced models of better quality, and describe which alignment was most effective for obtaining a good model, and why. The aspects that you should discuss will be shown later.

If you have followed all the steps described, and sent *ferodox.B99990001.pdb* to ERRAT server, you should get a result which is similar to the left one on *figure 1*. Save it as a .jpg or postscript file for your report.

Examine the generated model (*ferodox.B99990001.pdb* with chimera), its ERRAT plot, and the alignment file (*fdxhomologs.pir.ali*). This shows the parts of the other ferredoxin structures which were used to make the model. Comment on the quality, using these aspects as a guide: Find the residues that ERRAT suggests are badly modelled (grey or black in histogram). Using the viewer program, see if these are  $\alpha$ -helical,  $\beta$ -sheet or not regular structure. Are the poorly modelled regions the result of gaps or insertions?

### **Making a better alignment**

Use the EMBNet ClustalW web form to make an alignment between the ferodox sequence, and the two templates (1fxd and 1fdn):

<http://www.ch.embnet.org/software/ClustalW.html>

1. Make a copy of the *fdxhomologs.pir.ali* file, and change it to a FASTA file by removing the ':' separated fields line and the 'P1;' from the tags.
2. Remove the '\*' and any '-' symbols from the sequence entries.
3. Change the tag of the ferodox entry to ferodoxM.
4. Save the FASTA file as *clustalM.fasta* as you will use it again later.
5. Paste the file into the form. Change the output format to 'PIR' but leave the alignment parameters at their defaults (BLOSUM matrix, 10 for gap opening and end gap penalties, and

0.05 for extension).

6. Select the 'RunClustalW' button to make the alignment.

7. The PIR file will be presented shortly, as a link ('PIR') amongst a number of links to other alignment file formats. Save the file as *fdxCWM.pir*.

8. Edit the new alignment so it can be used by MODELLER:

- Copy the ':' separated fields line for each protein into the new alignments so that MODELLER can match the structure files to the sequence entries.
- Save it.

9. Make a copy of *simplemodel.py*, called *cwMmodel.py*. Edit this new *.py* file so it will read the ClustalW alignment and make a new model:

- Change the ALNFILE variable:

```
alnfile = 'fdxCWM.pir'
```

ALNFILE is the variable that defines the filename for an alignment that is to be used by the modelling routine.

- Change the SEQUENCE variable assignment so that SEQUENCE is set to ferodoxM.

This is the tag that you used in the FASTA file you submitted to ClustalW.

- Comment out "a.auto\_align()" - put a hash (#) at the start of the line

- Save the updated file.

10. Run the MODELLER script with *cwMmodel.py*. It should finish in about a minute. The new model will be called 'ferodoxM', after the tag used in the new PIR file.

11. Send *ferodoxM.B9990001.pdb* to ERRAT and save the plot as *ferodoxM.ps* or *ferodoxM.jpg*. Note which parts of the model have been improved due to the more accurate alignment.

### Finding better gap penalties

Repeat the multiple sequence alignment and model generation that you did in the last section, but use the following alignment parameters:

BLOSUM matrix (unchanged).

Gap Opening and End Gap = 3

Extension and Separation = 0.05

Remember to save this alignment as a PIR file under a new name, and add the extra tag lines. You also need to modify your *cwMmodel* script so that it uses this new alignment file



rather than *fdxCWM.pir*, and include the following lines before the call to the 'model' procedure.

```
a.starting_model = 2
a.ending_model = 2
```

These statements set the first and last 'model number' for the generated models. By default, modeller makes one model. If the variables above are defined, then modeller enters a loop:

```
i = a.starting_model
do {
  generate a new distance geometry structure
  optimize the structure
  write the i'th model (as name.B(99990000 + i))
  i++
} while (i < a.ending_model)
```

### **Adding more homologs to the multiple alignment**

This section is optional:

The two template sequences are reasonably good homologs for the sequence that you are modeling, but they are not the only ones that could be used. Open the file *fdxfamily.fasta*. You will see some additional members of the ferredoxin family.

1. Add the extra sequences to the sequences as input to ClustalW - and submit them to the server using the same gap penalties as in the last section.
2. Save the new, wider multiple sequence alignment as *fdxCWW.pir*.
3. Examine the file *fdxfamily.pir* to find the tags and extra information fields needed to complete the entries in your new alignment.
4. Modify your *fdxCWM* script again for this new alignment, and assign 3 to the *a.starting\_model* and *a.ending\_model*.
5. Generate the new model and assess its quality.

### **Your Conclusions**

Look at your results.

Were there any patterns?

Were good/bad regions associated with secondary structure, high/low sequence homology?

Did the results change much with different alignment parameters?

Please hand in a report by **February 13<sup>th</sup>, 2009** to [schenk@zbh.uni-hamburg.de](mailto:schenk@zbh.uni-hamburg.de).