CHEM 498Q / CHEM 630Q: Molecular Modelling of Proteins
TUTORIAL #2a: Homology Modelling 1

INTRODUCTION

The goal of this tutorial is to learn how to perform comparative protein modeling using the `automodel` command of the program MODELLER.

To carry out this tutorial, you will need a working installation of MODELLER, which you can get from http://salilab.org/modeller/download_installation.html, and either a text editor such as `emacs` or `vi` or a word processor that you will use as a text editor—by always making sure you save all your files as "plain text".

MODELLER is a "command line interface" program, which means that it interacts with the user only through text files. The user writes specific instructions into a text file and "feeds" them to the program, which then writes the results into another text file:

Instructions ("`model.py`") → MODELLER ("`mod9.15`") → Results ("`model.log`")

These instructions are written in a computer language called Python, which has very strict syntactic rules. The "`.py`" files themselves are called "Python scripts".

In Steps 1, 2, 3, and 4 of the Tutorial, you will go through the procedure using the example scripts provided by the developers of MODELLER. You will then use the same techniques in Step 5 to produce a homology model of your own sequence and evaluate it using the DOPE score.

REQUIRED PRE-LAB READING

The first two sections of the MODELLER manual:
("Introduction" and "Automated comparative modeling with `automodel`")

http://salilab.org/modeller/manual

PRE-LAB REPORT

✦ Perform a BLAST alignment of your query sequence with the template sequence you have chosen from Tutorial #1 (see example next page).

✦ Prepare a "PIR" text file describing the alignment of your query sequence with the template sequence. The "PIR" file should have the following format (see MODELLER manual and example next page):

```
>P1;pdb_code
structureX:pdb_code:start_res:chain_id:end_res:chain_id::::
TEMPLATESEQUENCEWITH--GAPANDMOREAMINOACIDS*
>P1;query_code
sequence:query_code:start_res: :end_res: ::::
---QUERYSEQUENCEWITHNOGAP------------------*
```

For MODELLER to understand the alignment, the two sequences should be made the same lengths using dashes (-) and should be terminated with an asterisk (*).

✦ Send this file to yourself as an email. (You will need it for STEP 5 of the tutorial.)

✦ Your pre-lab report should contain both the BLAST alignment report and the content of the PIR file. (Use a `fixed-width font`, as in the example.)

Example

Using BLOSUM62 and a gap cost of 11 (the default value), the query sequence

AYVINDSCIACGACKPECPVNIIQGSIYAIDADSCIDCGSCASVCPVGAPNPED

aligns with template sequence "pdb|5fd1" as follows:

```
>pdb|5FD1|  Chain  , Crystal Structures Of Oxidized And Reduced Azotobacter
Vinelandii Ferredoxin At Ph 8 And Ph 6
Length=106

 Score = 33.1 bits (74),  Expect = 5e-07, Method: Compositional matrix adjust.
 Identities = 23/58 (39%), Positives = 29/58 (50%), Gaps = 4/58 (6%)

Query  1   AYVINDSCIACGA--CKPECPVN-IIQG-SIYAIDADSCIDCGSCASVCPVGAPNPED  54
           A+V+ D+CI C    C   CPV+   +G +   I  D CIDC  C   CP  A   ED
Sbjct  1   AFVVTDNCIKCKYTDCVEVCPVDCFYEGPNFLVIHPDECIDCALCEPECPAQAIFSED  58
```

The PIR file corresponding to this alignment would be:

```
>P1;5fd1
structureX:5fd1:1    :A:58   :A::::
AFVVTDNCIKCKYTDCVEVCPVDCFYEGPNFLVIHPDECIDCALCEPECPAQAIFSED*
>P1;query
sequence:query:1    : :54   : ::::
AYVINDSCIACGA--CKPECPVN-IIQG-SIYAIDADSCIDCGSCASVCPVGAPNPED*
```

READING

On homology modeling:
Chapter 7 of Tramontano ("Homology Modeling"): All sections.
Chapter 13 of Zvelebil & Baum ("Modeling Protein Structure"): Sections 13.3 to 13.6.

PROCEDURE

STEP 1: Run the "`model-default.py`" example

Create a directory "`tutorial2`" in your work folder (example: "`work_guillaume/tutorial2`") and download the Python script `model-default.py` (http://salilab.org/modeller/examples/automodel/model-default.py) and the file `alignment.ali` (http://salilab.org/modeller/examples/automodel/alignment.ali). The two files should be put in that directory.

Download the PDB file of the template sequence ("`5FD1.pdb`") and put it in the same directory as the other two files.

From the MODELLER command prompt, type:

```
mod9.15 model-default.py
```

MODELLER will read the Python script and write the following files (among other):

`model-default.log`	"log" file (contains the summary of the results)
`1fdx.B99990001.pdb`	3D model (can be opened in PyMOL)
`1fdx.V99990001`	Violation profiles for the model

Inspect the log file (`model-default.log`) and signal any error or warning message to the instructor (search for "`_E>`" and "`_W>`").

STEP 2: Visualize the template and the model produced by MODELLER

Open the two PDB files ("`5FD1.pdb`" and "`1fdx.B99990001.pdb`") in PyMOL and render them as cartoons. Display the amino acid sequences ("Display > Sequence On" menu) and color **in blue** the amino acids of the template that participate in the alignment, and **in red** the amino acids of the model that participate in the alignment. Leave all other amino acids (including those in gaps) their original colors.

Align the two structures using the backbone atoms of the matching residues:

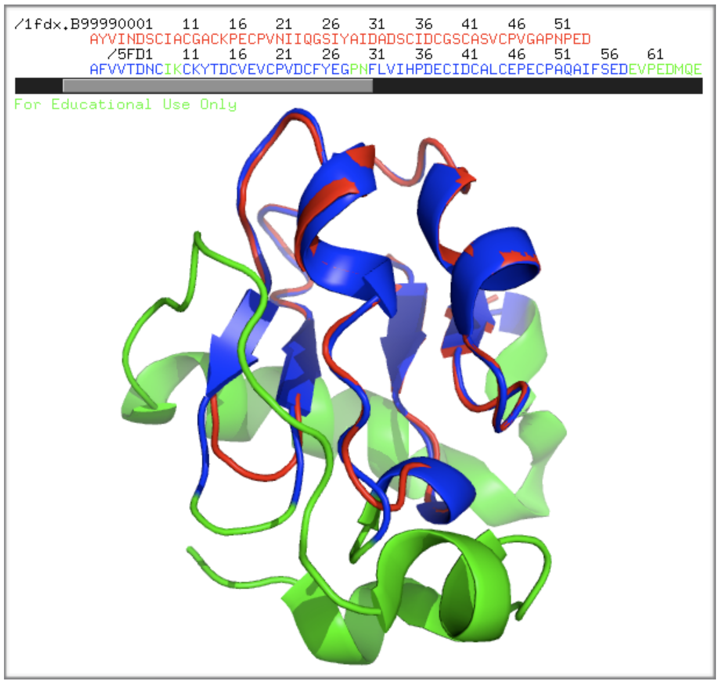
```
PyMOL> align 1fdx.B99990001 & resi 1-54 and name n+ca+c+o, 5FD1 &
       resi 1-8+11-28+31-58 and name n+ca+c+o
```

Note that the model ("`1fdx`") is selected from residues 1 to 54 and the template ("`5FD1`") is selected from residues 1 to 58 but excluding residues 9, 10, 29, and 30 (because they are gaps).

The PyMOL log should contain something like this:

```
Match: read scoring matrix.
Match: assigning 54 x 54 pairwise scores.
MatchAlign: aligning residues (54 vs 54)...
ExecutiveAlign: 216 atoms aligned.
ExecutiveRMS: 13 atoms rejected during cycle 1 (RMS=0.67).
ExecutiveRMS: 10 atoms rejected during cycle 2 (RMS=0.35).
ExecutiveRMS: 8 atoms rejected during cycle 3 (RMS=0.28).
ExecutiveRMS: 3 atoms rejected during cycle 4 (RMS=0.25).
ExecutiveRMS: 2 atoms rejected during cycle 5 (RMS=0.24).
Executive: RMS =    0.235 (180 to 180 atoms)
```

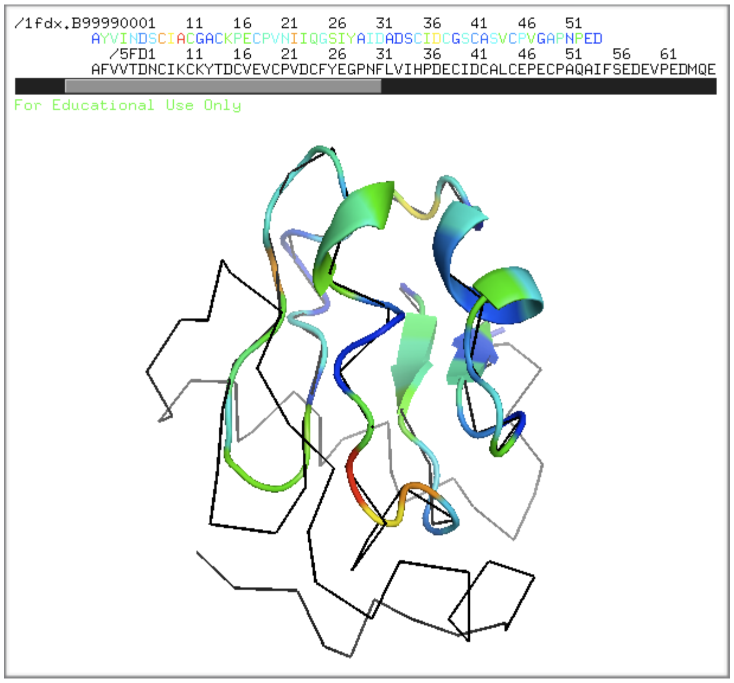
You should get something like this:



```
/1fdx.B99990001   11   16   21   26   31   36   41   46   51
        AYVINDSCIACGACKPECPVNIIQGSIYAIDADSCIDCGSCASVCPVGAPNPED
            /5FD1   11   16   21   26   31   36   41   46   51   56   61
        AFVVTDNCIKCKYTDCVEVCPVDCFYEGPNFLVIHPDECIDCALCEPECPAQAIFSEDEVPEDMQE
```

Change the rendering of structure “5FD1” to “ribbon” and make it black. Change the color of the “1fdx” model by clicking on the “C” button and by selecting “spectrum > b-factors”. This colors each residue according to the “B-factor” information written into file “`1fdx.B99990001.pdb`” by MODELLER.

You should get the following image:



```
/1fdx.B99990001   11   16   21   26   31   36   41   46   51
          AYVINDSCIACGACKPECPVNIIQGSIYAIDADSCIDCGSCASVCPVGAPNPED
            /5FD1   11   16   21   26   31   36   41   46   51   56   61
          AFVVTDNCIKCKYTDCVEVCPVDCFYEGPNFLVIHPDECIDCALCEPECPAQAIFSEDEVPEDMQE
```

The number written in place of the “B-factors” actually correspond to the total “violation” of the model at each position along the sequence: blue means a lower level of violation and red means a higher level of violation.

STEP 3: Assess the model using the DOPE score

Modify the Python script as follows:

```
a = automodel(env,
              alnfile  = 'alignment.ali',     # alignment filename
              knowns   = '5fd1',              # codes of the templates
              sequence = '1fdx',              # code of the target
              assess_methods = (assess.DOPE))
```

✦ Run the modified script (by typing “`mod9.15 model-default.py`”) and inspect the new log file. What is the value of the DOPE score for the final model? (Note that a lower DOPE score means a better model.)

✦ Keep a copy of the file "`model-default.log`" by renaming it "`model-default-ali.log`". In particular, make sure you keep a copy of the "Summary of the restraint violations":

```
Summary of the restraint violations:

   NUM     ... number of restraints.
   NUMVI   ... number of restraints with RVIOL > VIOL_REPORT_CUT[i].
   RVIOL   ... relative difference from the best value.
   NUMVP   ... number of restraints with -Ln(pdf) > VIOL_REPORT_CUT2[i].
   RMS_1   ... RMS(feature, minimally_violated_basis_restraint, NUMB).
   RMS_2   ... RMS(feature, best_value, NUMB).
   MOL.PDF ... scaled contribution to -Ln(Molecular pdf).

 #                     RESTRAINT_GROUP      NUM   NUMVI  NUMVP   RMS_1   RMS_2         MOL.PDF     S_i
----------------------------------------------------------------------------------------------------------
 1 Bond length potential              :     379       0      0   0.005   0.005      2.8674       1.000
 2 Bond angle potential               :     520       0      0   2.409   2.409      58.247       1.000
 3 Stereochemical cosine torsion poten:     244       0     11  54.040  54.040      100.17       1.000
 4 Stereochemical improper torsion pot:     146       0      0   1.402   1.402      5.7486       1.000
 5 Soft-sphere overlap restraints     :     637       0      0   0.003   0.003     0.47098       1.000
 6 Lennard-Jones 6-12 potential       :       0       0      0   0.000   0.000      0.0000       1.000
 7 Coulomb point-point electrostatic p:       0       0      0   0.000   0.000      0.0000       1.000
 8 H-bonding potential                :       0       0      0   0.000   0.000      0.0000       1.000
 9 Distance restraints 1 (CA-CA)      :     804       0      0   0.324   0.324      24.673       1.000
10 Distance restraints 2 (N-O)        :     837       0      1   0.468   0.468      38.091       1.000
11 Mainchain Phi dihedral restraints  :       0       0      0   0.000   0.000      0.0000       1.000
12 Mainchain Psi dihedral restraints  :       0       0      0   0.000   0.000      0.0000       1.000
13 Mainchain Omega dihedral restraints:      53       0      0   4.703   4.703      13.824       1.000
14 Sidechain Chi_1 dihedral restraints:      43       0      0  76.876  76.876      11.203       1.000
15 Sidechain Chi_2 dihedral restraints:      26       0      0  73.033  73.033      11.449       1.000
16 Sidechain Chi_3 dihedral restraints:       9       0      0  46.929  46.929      4.0291       1.000
17 Sidechain Chi_4 dihedral restraints:       1       0      0   9.151   9.151     0.27906       1.000
18 Disulfide distance restraints      :       0       0      0   0.000   0.000      0.0000       1.000
19 Disulfide angle restraints         :       0       0      0   0.000   0.000      0.0000       1.000
20 Disulfide dihedral angle restraints:       0       0      0   0.000   0.000      0.0000       1.000
21 Lower bound distance restraints    :       0       0      0   0.000   0.000      0.0000       1.000
22 Upper bound distance restraints    :       0       0      0   0.000   0.000      0.0000       1.000
23 Distance restraints 3 (SDCH-MNCH)  :     553       0      0   0.486   0.486      11.315       1.000
24 Sidechain Chi_5 dihedral restraints:       0       0      0   0.000   0.000      0.0000       1.000
25 Phi/Psi pair of dihedral restraints:      52       5      5  28.044  60.583      24.070       1.000
26 Distance restraints 4 (SDCH-SDCH)  :     100       0      0   0.870   0.870      7.4694       1.000
27 Distance restraints 5 (X-Y)        :       0       0      0   0.000   0.000      0.0000       1.000
...
38 SAXS restraints                    :       0       0      0   0.000   0.000      0.0000       1.000
39 Symmetry restraints                :       0       0      0   0.000   0.000      0.0000       1.000
```

STEP 4: Try to model the sequence based on an alternative alignment

Create a copy of the file "`alignment.ali`", called "`alignment2.ali`", that corresponds exactly to the alignment presented in the "PRE-LAB REPORT" section, containing the sequences:

AFVVTDNC**IKCKYTDCVEVCPVDCFYEGPN**FLVIHPDECIDCALCEPECPAQAIFSEDEVP...
AYVINDSC**IACGA--CKPECPVN-IIQG-S**IYAIDADSCIDCGSCASVCPVGAPNPED------

instead of:

AFVVTDNC**IKCKYTDCVEVCPVDCFYEGPN**FLVIHPDECIDCALCEPECPAQAIFSEDEVP...
AYVINDSC**--IACGACKPECPVNIIQGS--**IYAIDADSCIDCGSCASVCPVGAPNPED------

✦ Modify the Python script so that it reads file "`alignment2.ali`" instead of "`alignment.ali`" and run the script. How does the DOPE score for "`ali2`" compare to the one for "`ali`"?

✦ Keep a copy of the file “`model-default.log`” by renaming it “`model-default-ali2.log`”. Based on the “Summary of the restraint violations”, how do the violations for “`ali2`” compare to those for “`ali`”?

STEP 5: Redo STEP 1, STEP 2, and STEP 3 for your sequence

Save the Python script used in previous step (including the “DOPE” assessment) under the name “`model.py`”. Modify that script file to use your sequence and structure:

- Change the value of variable “`alnfile`” from “`alignment.ali`” to whatever name you gave your PIR file.
- Change the value of variable “`knowns`” from “`5fd1`” to the four-character PDB ID of your template protein and change the value of variable “`sequence`” from “`1fdx`” to “`query`” (or whatever you have put in the PIR file).

✦ Run the script (by typing “`mod9.15  model.py`”) and inspect the log file “`model.log`” for error and warning messages. You are now using your own PIR file, so pay special attention to the output from the alignment checking command (search for “`check_ali___>`”) and correct your PIR file if necessary.

✦ What is the DOPE score of your model?

✦ Produce figures similar to those of STEP 2 for your model. Based on the alignment of the two structures, what is the RMS distance for the backbone atoms (“N+CA+C+O”)? (Note that the RMS distances are given in Angstroms.)

✦ Perform the same structure alignment using only the alpha-carbons (using “`name  ca`” instead of “`name  n+ca+c+o`” in the “`align`” command). What is the alpha-carbon RMS distance?

✦ How well are the two structures overlapping? Discuss any significant deviation of the model with respect to the template.

✦ Inspect the “`.V99990001`” file for your model. Which amino acids have the largest total violation, and which “restraints groups” are they violating the most? (Note: There are 39 columns, which correspond to the 39 “restraint groups” from the “Summary of the restraint violations” in the log file.)

Back up your files

Back up your files as explained in Tutorial #1c.