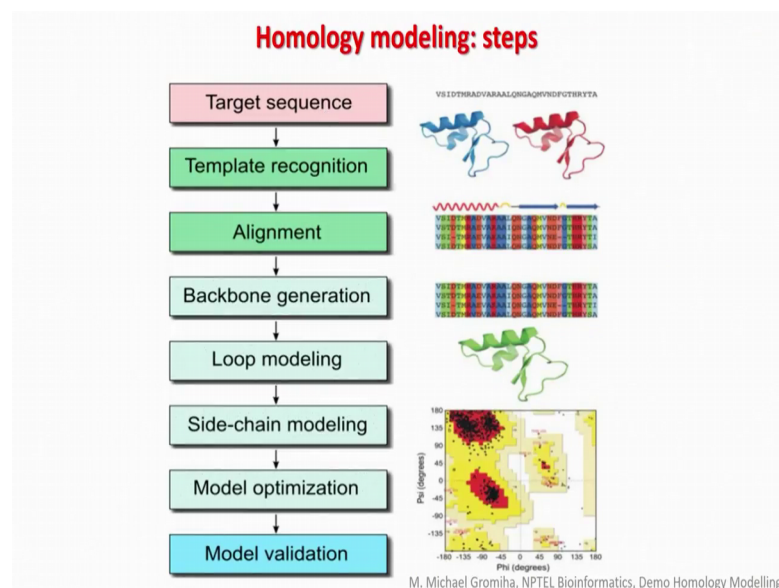


Bioinformatics
Prof. M. Michael Gromiha
Department of Biotechnology
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Lecture - 44
Demo on Homology Modeling

Demo on Homology Modeling. So, here we will demonstrate how to build a model for a protein structure from any specific amino acid sequence.

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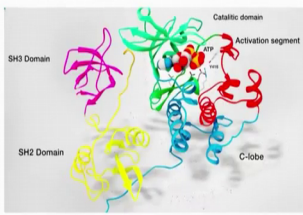
As I discussed earlier the homology model requires several steps, such as target sequence. This sequence which one you like to get the structure and one scenario get that completes, then alignment backbone generation, loop modeling, side chain modeling, model optimization and validation.

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Workflow

Target protein : Human c-Yes tyrosine kinase (Uniprot id: P07947)

- Search template using BLAST
- Build Kinase domain of c-Yes kinase
- Generate maximum of 10 models
- Calculate DOPE Score (lowest denotes the best model)
- Evaluate the model using Ramachandran plot and report the statistics
- Report Structure similarity and visualize the structures



Prerequisites

- Installation and registration of Modeller (Recent version 9.19)
- Internet for template search and model validation
- Tool for visualization, RMSD calculation, etc.,

M. Michael Gromiha, NPTEL Bioinformatics, Demo Homology Modelling

For example if you have a protein called human c-Yes kinase tyrosine kinase that the Uniprot id, uniprot is the database for protein sequences P07947. So, in our sequence now the task is to get the structure. So, there are various steps involved to get the structure, first you have to get the template right using the blast, then build kinase domain of the c-Yes kinase and we generate a maximum 10 models and calculate (Refer Time: 01:26) score, which these lowest denotes the best model. Then evaluate the model using Ramachandran plot and report the structure similarity and visualize the structures.

So, to get the structures their prerequisites are first install and register modeller this software used for modeling the structure cc homology modeling and we had internet for the template search as well as validating the model, and we meet the tools suggest primal for visualization and RMSD calculation and so on. Now, we will see how to build a model from the sequence of the human c-Yes tyrosine kinase.

Now, I will give demonstration on prediction of protein structure using modeller software. Using modeller software we can do a homology modeling and here the task is predicting the structure of human c-Yes tyrosine kinase and its uniprot sequence id is P07947.

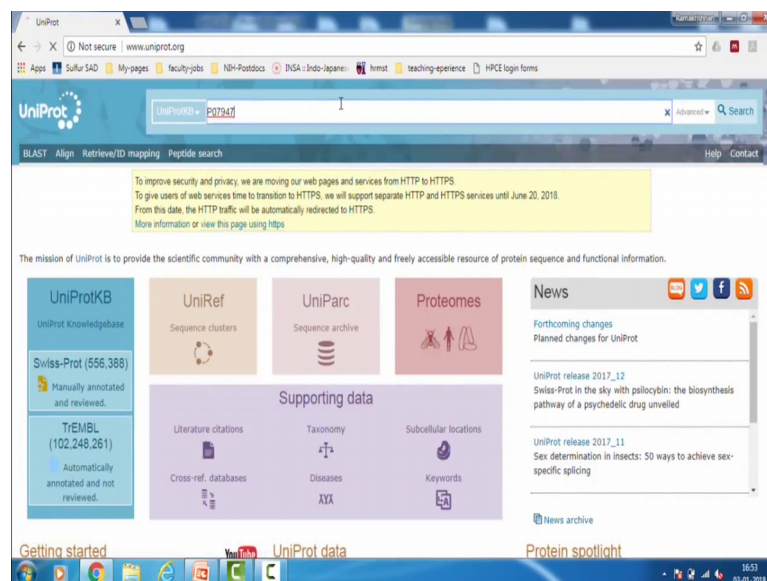
So, it includes few steps like retrieving protein sequence from uniprot, and searching template using blast and building the structure based on the template.

So, these are all the 3 essential steps which I am going to give demo and to do these we have to understand how the structure structural organization of the protein tyrosine kinase. Here the protein c-Yes kinase which belongs to the SRC kinase family has 3 domains, one is the kinase domain and then SH 2 and SH 3 domain. But the task given is building the kinase in domain alone. So, accordingly we have to retrieve the sequence and start doing modeling.

So, if the prerequisites are the model software installed and then the internet facility for template search and model validation and the tools visualization tools for visualizing the target and templates and to make a comparative statements on it using matrix likes RMSD.

So, first I will explain how to start searching the template for given protein sequence.

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So, to retrieve the sequence go to uniprot and type the id and it will show the full annotation.

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The screenshot shows the UniProtKB entry for YES1 (P07947). The left sidebar contains a 'Display' menu with options like Entry, Publications, Feature viewer, and Feature table. The main content area features a diagram of a cell with various organelles labeled: Cytoskeleton, Centrosome, Plasma membrane, Cell membrane, and Cytosol. A note states: 'Note: Newly synthesized protein initially accumulates in the Golgi region and traffics to the plasma membrane through the exocytic pathway.' Below the diagram, there are sections for 'Keywords - Cellular component', 'Pathology & Biotech', 'Mutagenesis', and 'Keywords - Disease'.

And you search for the sequence belong to the kinase domain.

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The screenshot shows the UniProtKB entry for YES1 (P07947) with the title 'UniProtKB - P07947 (YES_HUMAN)'. The left sidebar is similar to the previous slide. The main content area displays protein details: 'Protein Tyrosine-protein kinase Yes', 'Gene YES1', 'Organism Homo sapiens (Human)', and 'Status Reviewed - Annotation score: 5.00 - Experimental evidence at protein level'. The 'Function' section describes the protein as a non-receptor protein tyrosine kinase involved in cell growth, survival, apoptosis, cell-cell adhesion, cytoskeleton remodeling, and differentiation. It mentions that stimulation by receptor tyrosine kinases (RTKs) including EGF, PDGFR, CSF1R, and FGFR leads to recruitment of YES1 to the phosphorylated receptor, and activation and phosphorylation of downstream substrates. Upon EGF activation, it promotes the phosphorylation of PARO3 to favor epithelial tight junction assembly. It also mentions participation in the phosphorylation of specific junctional components such as CTNND1 by stimulating the FYN and FER tyrosine kinases at cell-cell contacts. Upon T-cell stimulation by CXCL12, it phosphorylates collagen response mediator protein 2 (DPYSL2) and induces T-cell migration. It also mentions participation in the CD95L/FASLG signaling pathway and mediates AKT-mediated cell migration. It plays a role in cell cycle progression by phosphorylating the cyclin-dependent kinase.

So, for that if you search kinase domain or SH 2 or SH 3 whatever it is.

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UniProt P07947 protein entry page. The 'Family & Domains' section is expanded, showing a table of domains. The third domain, 'Protein kinase', is selected, and a tooltip shows the 'BLAST subsequence' for the region 277-530. The 'Sequence similarities' section indicates it belongs to the protein kinase superfamily.

Feature key	Position(s)	Description	Actions	Graphical view	Length
Domain ¹	91 - 152	SH3 # PROSITE-ProRule annotation	Add BLAST		62
Domain ¹	158 - 255	SH2 # PROSITE-ProRule annotation	Add BLAST		98
Domain ¹	277 - 530	Protein kinase # PROSITE-ProRule annotation	Add BLAST		254

Sequence similarities¹
Belongs to the protein kinase superfamily, Tyr protein kinase family, SRC subfamily. # PROSITE-ProRule annotation

Keywords - Domain¹
SH2 domain, SH3 domain

Phylogenomic databases
eggNOG² KOG0197, Eukaryota, COG0515, LUCA.
GeneTree³ ENSGT00760000118938.
HOGENOM⁴ HOG000233858.

So, here you can see the region the sequence belongs to protein kinase that starts from 277 to 530.

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UniProt BLAST search page. The 'How to use this tool' section is visible, showing instructions for using the BLAST tool. The 'Target database' is set to 'with 3D structure (PDB)'. The 'E-Threshold' is set to 10, 'Matrix' is set to Auto, 'Filtering' is set to None, 'Gapped' is set to yes, and 'Hits' is set to 250.

How to use this tool

The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between sequences, which can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families.

1. Enter either a protein or nucleotide sequence or a UniProt Identifier (e.g. P00750 or A4_HUMAN or UP1000000001) into the form field.
2. Optionally, change the program parameters with the dropdown menus under the form.
3. Click the Run BLAST button.

Target database¹ E-Threshold¹ Matrix¹ Filtering¹ Gapped¹ Hits¹

with 3D structure (PDB) 10 Auto None yes 250

Run BLAST in a separate window.

If you click on this link, you will get the sequence exactly that is belongs to the kinase domain of the protein kinase. You can copy this and save it as the fasta file save as. So, use the same id as a file name dot f a s t a fasta and save it in your preferred working directory, just make homology or modeler.

So, now we have retrieve the kinase domain of the sequence of kinase domain and now as per the need for the modeller software, this fasta file should be converted into the PIR format.

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Modeller example, step 1

- Example: build a model of one chain of the GroEL
- Step 1: put the sequence in PIR format:

'align code': an identifier used to identify the sequence.
Often PDB code + chain ID (e.g. 1xyzA)

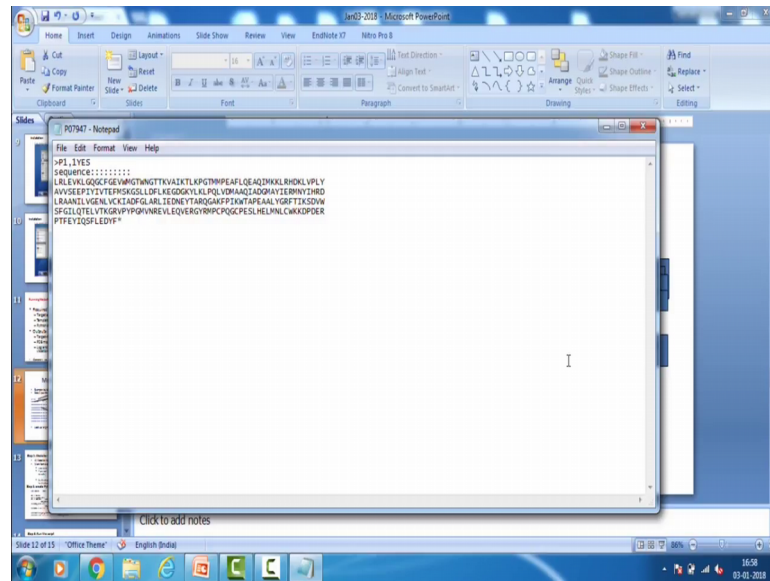
```
>P1:1oel
sequence:1oel:  1  ::522  ::undefined::undefined: 1.00: 1.00
AKDVKFGNDAGVQMLRGVNLADAVKVTLGPKGRNVLDKSPGAPFITKDGVSAREIELEDKPEVMGSAQWKEV
ASKKQDAAGDGTITATVLAQAIITEGLKAVAAAGNPMDLKRGIDKAVTVAVEELKALSVPCSDSKAIAQVGTISA
NSDETGVGLIAEAMDKVKEGVIITVEDGTGLQDELVDVVEGQDFRGVLSPPYFINKPETGAVELESPPILLADKKI
SNIREMLFVLEAVAKAGKFLIIAEDVEGALATAVNTIRGIVKVAAKAGFGDRRKAMLDQIATLTGGTVIS
EEIGMELEKATLEDLGQAKRVVINKDTTIIIDGVGEAAIQGRVQIRQITEATSDDYDREKLQERVAKLGGVA
VIKVGATEVEVMEKKARVEDALEATRAAVEEGVVAGGGVALIRVASKLADLRGQNEQNVGIKVALRAEAPLR
QIVLNCGEPSVAVNTVKGDDGNYGNAATEEYGNMIDMGILDPTKVTRESALQYAA SVAGLMIITECMVTDL*
```

- Look up 'alignment file format' in the Modeller manual index for more information

So, you know. So, this is the typical PIR file format shown in blue color, it starts with the comment line followed by the sequence description and the actual sequence and it ends with the star. Actually the first line gives the detail about the align code, which is very essential for modeller to read the sequence.

And the same ID should be used throughout the process and so, accordingly the user can specify any id in 4 letter alpha numeric 4 letter to identify the sequence. And followed by the second line the first term sequence are denotes this is belongs to the target sequence and in case of the template, it will be structure x that is explained here. And followed by this it has 10 fields hills related to the structural detail of the protein, but for sequence it will be null.

(Refer Slide Time: 08:04)



And so, accordingly we have to modify the fasta file to make the PIR. So, you just modify this P 1 comma you can give this CYES as a keyword or 1YES followed by this is the sequence target sequence.

So, it has to be specified with sequence, sequence followed by 10 fields 1 2 3 4 5 6 9 9 10 fields separated by 9 colons. So, we can leave these fields blank because for which the structure is not known and it has to be ended up with the asterisks then you can save it as PIR.

So, this is the first essential step to get ready with the target sequence. So, now, we can start with the typical workflow involved in homology modeling.

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The screenshot shows the NCBI BLAST web interface. The main heading is 'BLAST® » blastp suite'. Below it, the 'Standard Protein BLAST' form is displayed. The 'Enter Query Sequence' section has a text area containing a FASTA-formatted protein sequence. The 'Database' dropdown is set to 'Non-redundant protein sequences (nr)'. The 'Program Selection' section shows 'blastp' as the selected algorithm. The 'BLAST' button is visible at the bottom of the form.

Now, in order to do the first step, we have to search template using the blast do protein blast against the bpdb database. So, that the homology will be searched in the pdb database use the fasta formatted sequence, and here you have to select the database the structure database pdb and do a blast. And you play around this parameters also in order to get some more number of hits, and do blast.

(Refer Slide Time: 10:33)

The screenshot shows the 'Algorithm parameters' section of the NCBI BLAST web interface. The 'Max target sequences' is set to 100, 'Short queries' is set to 100, 'Expect threshold' is set to 500, 'Word size' is set to 5000, and 'Max matches in a query range' is set to 20000. The 'BLAST' button is visible at the bottom of the form.

(Refer Slide Time: 10:44)

BLAST[®] » blast suite » RID-4TFN4DVP015

Format Request Status

Job Title: sp|P07947|277-530

Request ID	4TFN4DVP015
Status	Searching
Submitted at	Wed Jan 3 06:29:40 2018
Current time	Wed Jan 03 06:29:43 2018
Time since submission	00:00:02

This page will be automatically updated in 2 seconds

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So, as a result you will get the number of homology the homologues proteins, with protein structure existing the pdb.

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Descriptions

Sequences producing significant alignments:

Select: All None Selected 0

Alignments

Description	Max score	Total score	Query cover	E value	Ident	Accession
Chain A: Src in Complex With Dose-Dependent Macrocyclic Inhibitor M-4p	481	481	100%	3e-174	90%	2J46W_A
Chain A: Human Src Kinase Bound To Kinase Inhibitor Boudinib	481	481	100%	4e-174	89%	4X0D_A
Chain A: Crystal Structure Of Chicken C-Src Kinase Domain In Complex With The Cancer Drug Imatinib	481	481	100%	4e-174	90%	2Q6Q_A
Chain B: Structural Basis For The Recognition Of C-src By Its Inactivator Csp	481	481	100%	4e-174	90%	3Q7U_B
Chain A: Src Kinase In Complex With Inhibitor Ap2451	481	481	100%	5e-174	89%	2B0F_A
Chain A: Crystal Structure Of The L1173 Mutant Of The Chicken C-Src Tyrosine Kinase Domain Complexed With Imatinib	480	480	100%	9e-173	89%	3X6Z_A
Chain A: Structure of JMB-P21 bound to anaplastic-sensitive Src kinase	479	479	100%	1e-173	89%	4LGG_A
Chain A: Human Src A4573 Mutant Bound To Kinase Inhibitor Boudinib	479	479	100%	1e-173	89%	4H0X_A
Chain A: C-Src Kinase Domain In Complex With Sap-95	479	479	100%	2e-173	90%	5T0P_A
Chain A: C-Src V281s Kinase Domain In Complex With Sap-95	479	479	100%	2e-173	89%	5S0H_A
Chain A: Crystal Structure Of Src Kinase Domain In Complex With Crizotinib	479	479	100%	3e-173	89%	1Y0L_A
Chain A: Src The388le Inhibited In The City-Apo-Out Conformation	479	479	100%	3e-173	89%	3Q6H_A
Chain A: Crystal Structure Of Src Kinase Domain In Complex With Covalent Inhibitor	479	479	100%	3e-173	89%	2H0Q_A
Chain A: Crystal Structure Of T330s C-Src Covalently Bound To Vinylphosphonate-Paracetamolamide 9	479	479	100%	4e-173	89%	3B0V_A
Chain A: Star 12 bound to anaplastic-sensitive Src kinase	478	478	100%	4e-173	89%	4M0V_A
Chain A: Structural Basis For The Chemical Rescue Of Src Kinase Activity	478	478	100%	4e-173	89%	3X6Z_A
Chain A: Crystal structure of HMM-P21 bound to anaplastic-sensitive Src kinase	478	478	100%	6e-173	89%	4LGG_A

So, in this list you can see the first hit that comes from the Src family even the c-Yes kinases also belongs to the same family and it has the score and query coverage good score query coverage of 100 percent and identities about 90 percent.

So, you can note down this pdb id and see how the structure exist. Go to the pdb and give it. The template search is a very important task because the modeller will build based on

template only. If template is not chosen properly the result will also be ended up with error.

So, it is very important to choose the template appropriately. So, even though the score and query coverages are good for the first hit the ranked one based on the A value.

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Macromolecules

Classification: TRANSFERASE / TRANSFERASE INHIBITOR

Total Structure Weight: 33950.75

Molecule	Chains	Length	Organism	Details
Proto-oncogene tyrosine-protein kinase Src	A	275	Gallus gallus	EC: 2.7.10.2 Fragment: Src kinase domain (UNP residues 259-533)

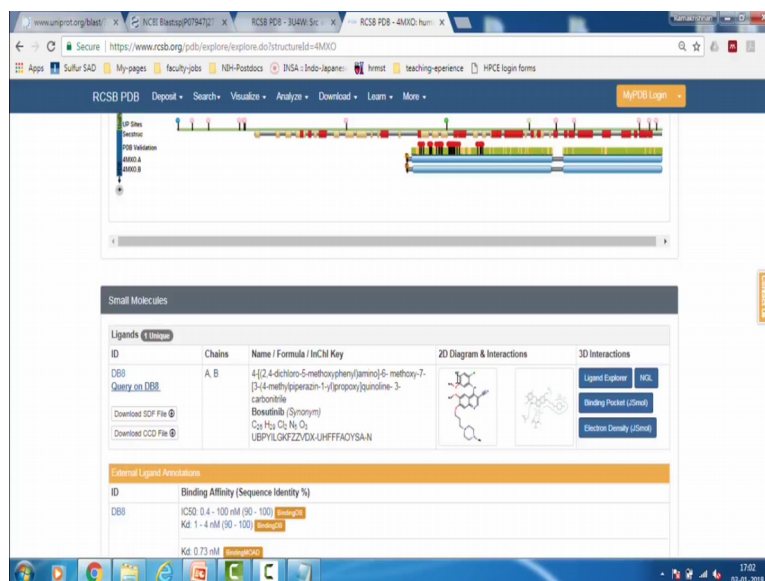
Protein Feature View: UniProtKB AC: P05522

This is belongs to Gallus gallus, but the task given for us is building structure for the human protein kinase human c-Yes kinase.

So, in this case we have to see carefully whether any of the other hits the blast belongs to human are exist. If you see the second hit which is also equivalently good, but only the identity percentage identity is less, but still the 89 percentage is good.

So, if you go to MXO and search go to PDB and see how this structure exists. See here it has two chains A and B belongs to the protein kinase domain and it has the ligand bounder conformation and this is also equivalently good.

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So, in this case which one we will take whether the template has the good identity the ranked first as per the blast result or the second one which belongs to the human.

(Refer Slide Time: 13:34)

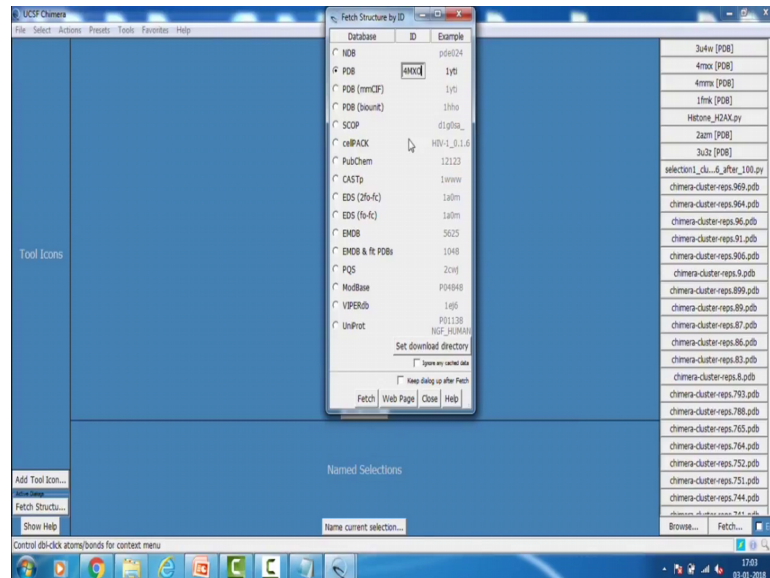


So, both are equivalently good not for model building, but still if you visualize using some visualizer tool for example, chimera if you take.

The visual inspection is very very important and in addition to the blast search results. Because this sequence this is blast search is based on the sequence alignment, while even the PDB the chances for having missing residual PDB structure is high. So, in that case if

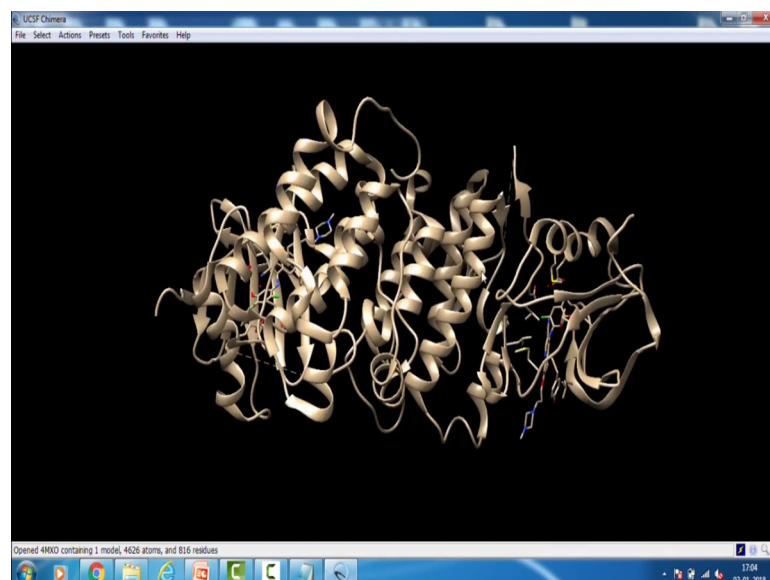
you visualize the structure, you can see whether the structure structural information for the given template is complete or not.

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So, here for a for example, if you see MXO that is belongs to the human, you can retrieve you can retrieve the structure 4 MXO 4 MXO the fetch.

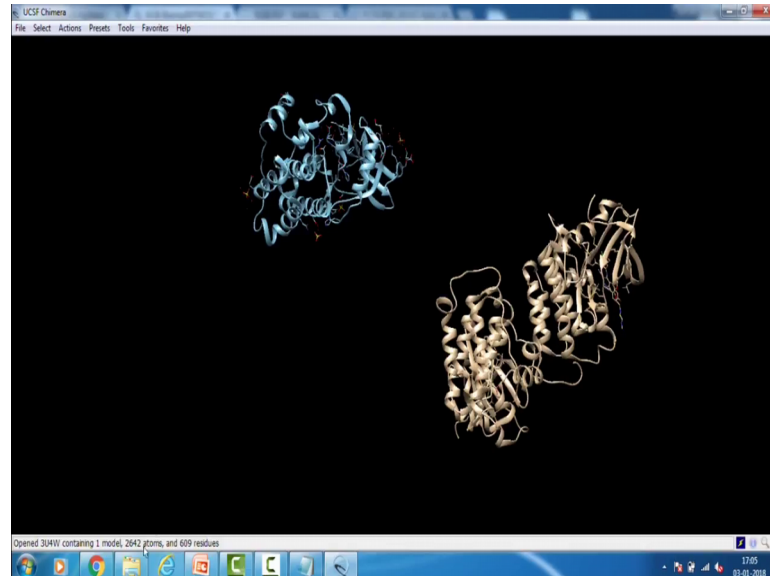
(Refer Slide Time: 15:12)



This is the simplest way to see whether the structure is having any missing residues. See in the chimera will easily which will tell you through the dotted lines see here you can see the dotted lines that is that is belongs to the missing segments.

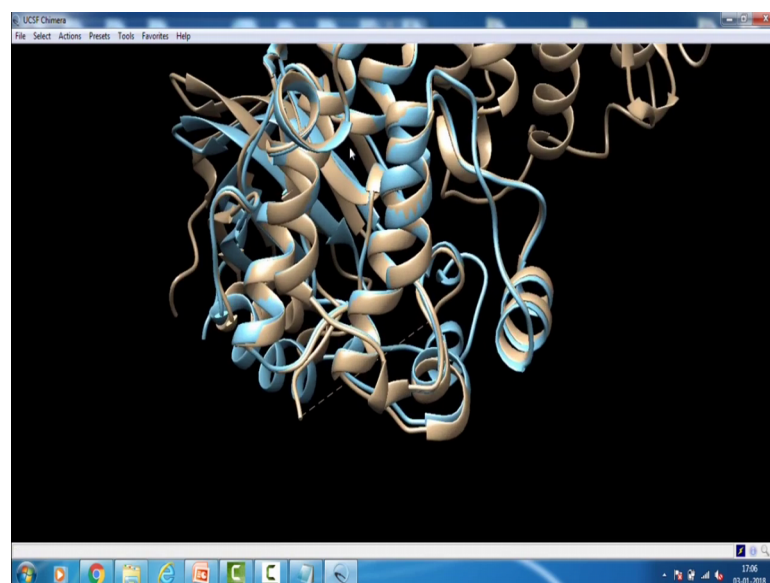
So, this 4 MXO is not the complete one. So, we do not choose it. If you go if you check for 3 u 4 w even though that is belongs to the Gallus gallus, fetch.

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See you can compare now, see here for in Gallus gallus you do not see any missing residues, there is no dotted lines. Here you can now compare and see both the structures how identity both are using the chimera tool matchmaker. This is the reference structure and one another one can be the structure to match you just click ok.

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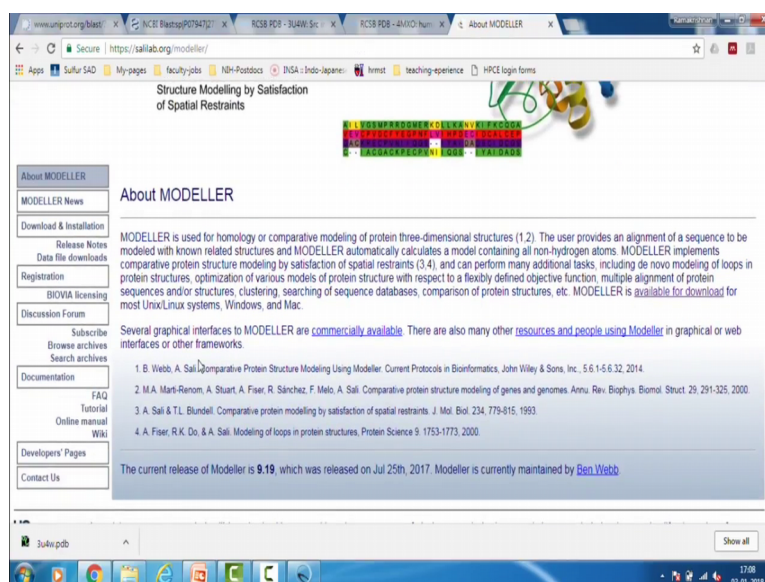
See here the both the structures are identical and you can see here the segment, which is missing in human protein is present in the chicken protein.

So, in this case we do not mind the one percent difference in the identity, here the structural information is very important for model building. So, we will take the c-Yes kinase of Gallus gallus instead of human for this task, and start doing the homology modeling.

So, now we have collected retrieved sequence from uniprot and we have retrieved the appropriate template from PDB. Now target and template both are ready to do the homology modeling.

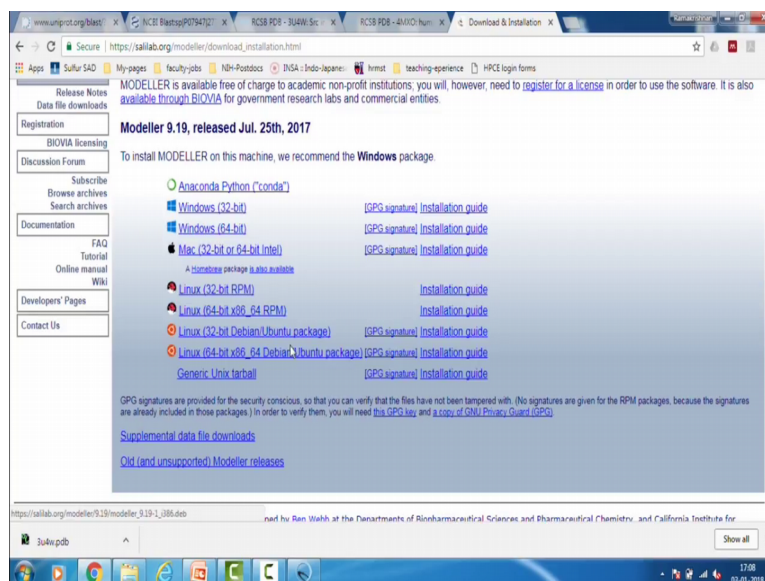
So, if you go to your working directory, you will be having three files first one is corresponding to the structure in PDB file format and the two files fasta and PIR for formats belongs to the sequence. Now, you can start working with the modular software, which we have already installed in our computer using the procedure given in the modular website.

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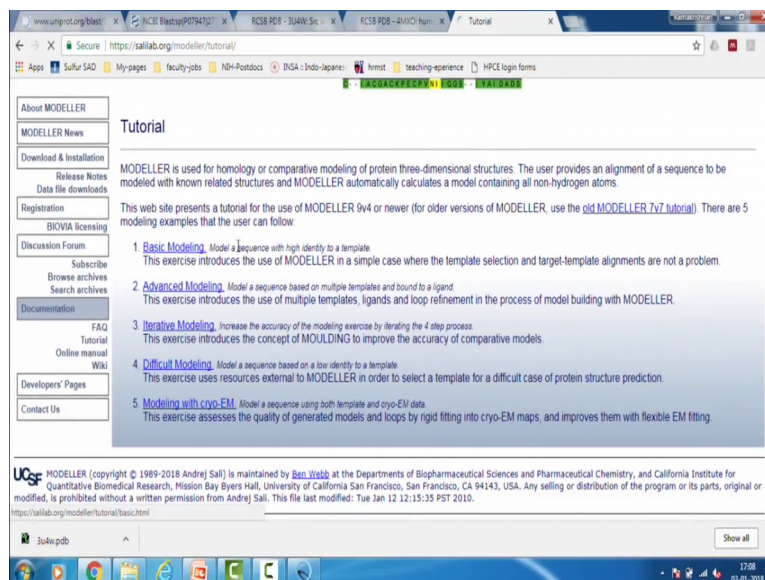
If you go to the modular website you can see the documentation for downloading and installing the software, and to do registration also. Here in the documentation download and installation instruction is given here you can download the software which is so, compatible for your computer and install it

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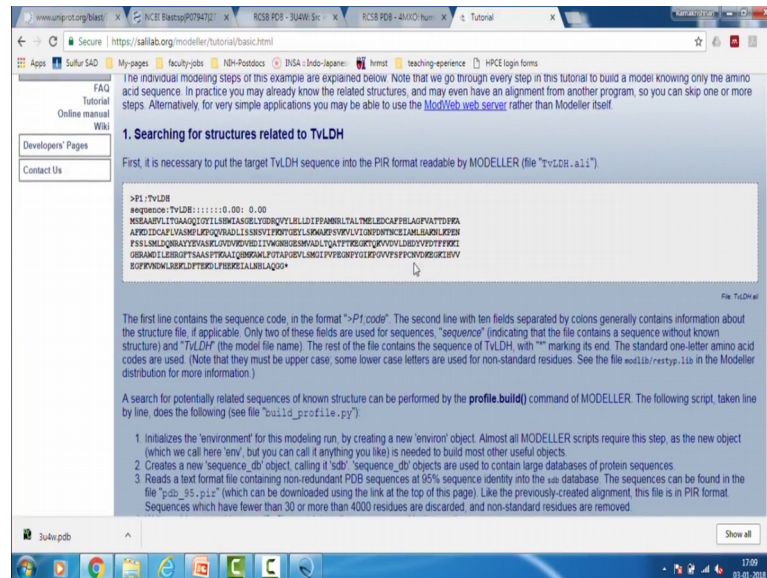
And the current version of the modular is 9.19 and this is command mode. And in the documentation you can see the help manual to perform the modeling.

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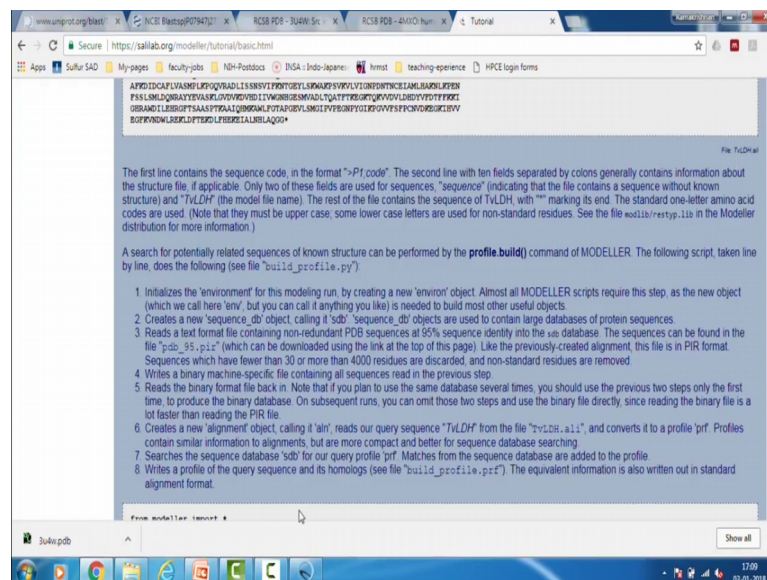
You can follow this simple instruction to perform the homology modeling as we are going to discuss now.

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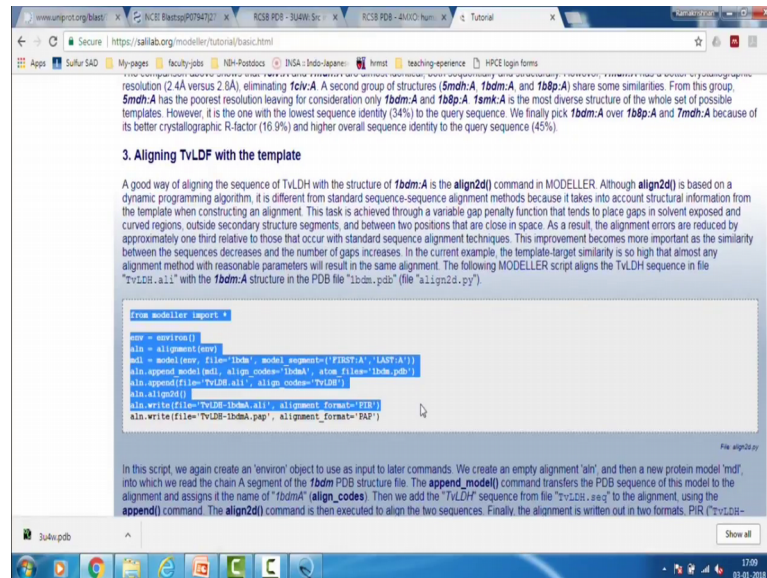
So, in model building there are two essential steps, which is which is very important to discuss here is first the alignment of protein sequence with the template structure.

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We will call it as target template alignment, to do this we can get the script modular script from their website [here](#).

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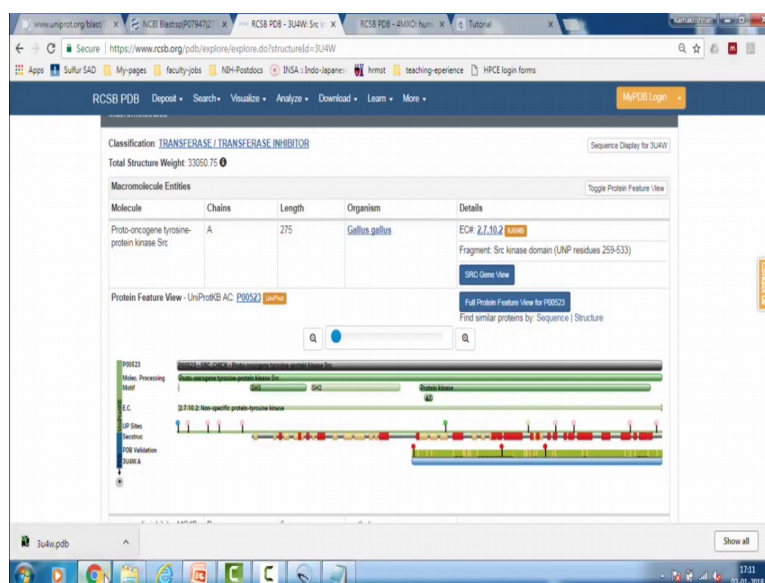


This is the script we have to use to perform the target template alignment; so, that the sequence of our target sequence will be aligned to those sequence of the template structure.

So, you can save it save this script as the model dot py because this is the python step the first step is more alignment dot py. So, if you look at this script, the first 3 4 lines corresponds to setting up the environment variable and improve importing the necessary modules from modular and action to perform the alignment.

So, here in the next line if you see the value to the variable file, that is belongs to the template. So, here what template we have chosen is 3 u 4 w that has to be given here carefully 3 u 4 w and this has only chain a if you go to the pdb side there you can see you can confirm how many chains the protein this protein has.

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It has only one chain A so, you have to specify the starting and ending chain names so, here only one. So, in both first and last will have the same value A and in the in the alignment. This the next line corresponds to the sequence where you have to specify the align code for the sequence of the template structure. So, here we will give the same id 3 u 4 w followed by A. In order to identify the sequence that is belongs to the chain A of the protein tyrosine kinase structure, and followed by the atom file name is 3 u 4 w dot pdb.

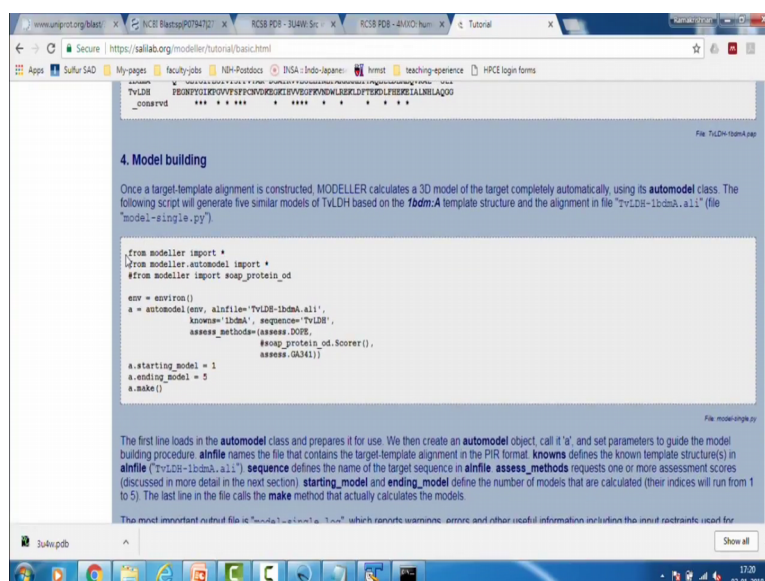
So, that these two lines until modular to consider the chain A of the template structure and for the alignment, and in the next line we will give the target sequence information that is P07497 dot PIR and the align code corresponding to the target protein is 1YES that is what we have mentioned here the same thing should be given here 1YES, and here also you specify the same.

So, that modeller will read these two codes and identify which is the template and which is the target. So, the output alignments file will be like target template target template dot ali is the output file in PIR format we can save this.

So, now, the script for target template alignment is ready and we can perform it using modeller. So, start modeller command mode and change the working directory using the cd command in windows. Now here you can use the command mod 9.18 this is the command here for this version followed by one dot alignment the python script file.

So, it will start doing the alignment.

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The screenshot shows a web browser window with the URL <https://salilab.org/modeller/tutorial/basic.html>. The page is titled "4. Model building" and contains the following text:

Once a target-template alignment is constructed, MODELLER calculates a 3D model of the target completely automatically, using its **automodel** class. The following script will generate five similar models of TvLDH based on the **1bda** template structure and the alignment in file "TvLDH-1bda.ali" (file "model-single.py").

```
from modeller import *
from modeller.automodel import *
from modeller import soap_protein_od

env = environ()
a = automodel(env, alnfile='TvLDH-1bda.ali',
              knowns='1bda', sequence='TvLDH',
              assess_method=(assess.DOPE,
                           #soap_protein_od.Scorer(),
                           assess.GA341))

a.starting_model = 1
a.ending_model = 5
a.make()
```

The first line loads the **automodel** class and prepares it for use. We then create an **automodel** object, call it 'a', and set parameters to guide the model building procedure. **alnfile** names the file that contains the target-template alignment in the PIR format. **knowns** defines the known template structure(s) in **alnfile** ("TvLDH-1bda.ali"). **sequence** defines the name of the target sequence in **alnfile**. **assess_methods** requests one or more assessment scores (discussed in more detail in the next section). **starting_model** and **ending_model** define the number of models that are calculated (their indices will run from 1 to 5). The last line in the file calls the **make** method that actually calculates the models.

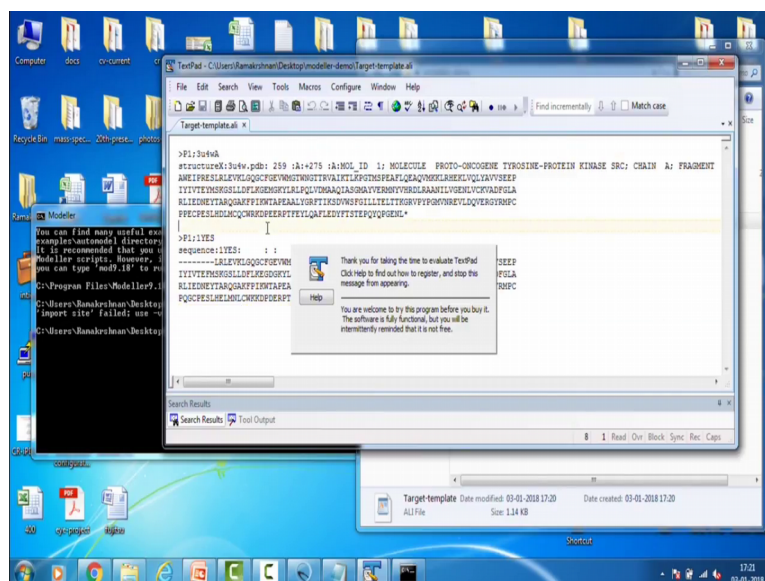
The most important output file is "model-single.pdb", which contains warnings, errors and other useful information including the input restraints used for

So, once the alignment is completed we can start doing with the model building. In the same help page you can find the second script that is belongs to model building and you can copy the script and save it similarly.

So, in this script if you see similarly the first 4 line corresponding to the modular libraries and the next line belongs to the specification of the target template alignment file, and then the corresponding the align codes. So, from the result file of step 1 we can note down the align codes and then specify here to perform model building.

So, the target template alignment is the output file we go it now.

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And if you open and see here in the alignment file you can see the alignment in the PIR format, and the align codes that is corresponding to the structure and the alignment align codes that is corresponding to the sequence and both the codes you can note down or even for say if you can copy and paste it to the new script the next script.

Here the knowns is corresponding to the structure id. So, here what we used is 3 u 4 w a and for sequence that is target that is 1YES. And the next line tells the what method is going to be used for model building and scoring. Here the dope scoring scheme is used to evaluate the models and here this line corresponds to the number of models to be generated.

So, this is the file called the second step model building, dot py yeah and we have to specify the correct alignment file also, that is target template dot ali. This is the file actually we have we have got from the previous run target template dot ali that is specified now and now you can perform model building, using the same command two underscore.

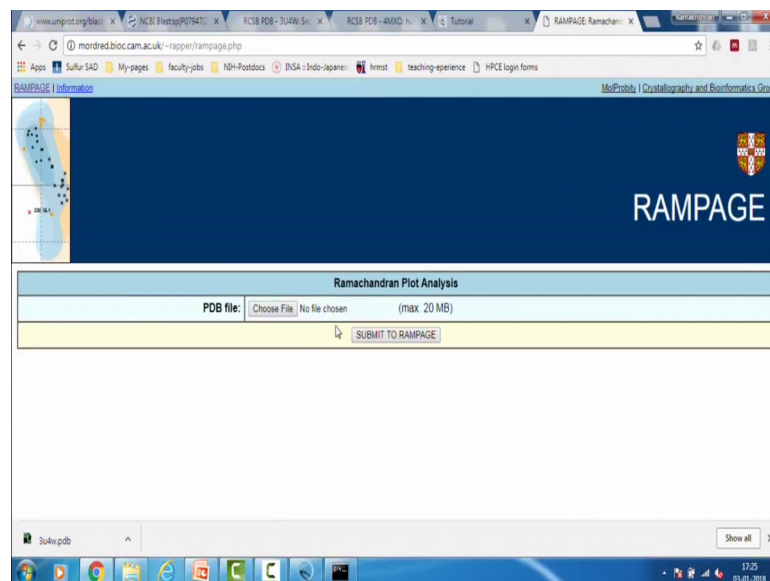
So, these are the two important scripts to be edited carefully in order to perform target template alignment and model building. Once this is done and you will be getting the 10 kinase structures for the given target sequence and you can evaluate the correctness of the model in the next step. So, we will wait for a while until this generates the 10 structures.

So, now, you can see the progress, it generated the two structures so far, that has the file name starts with the align code of the target sequence followed by some alphanumeric numbers. So, once all these 10 structures are built, we can start evaluating the models based on dope score, that will be written in the log file of the present running presently running script.

So, so far we have completed the model building, in the workflow we have retrieved the target sequence and retrieved the template structure and perform the alignment, and perform the model building also that includes backbone generation, loop modeling everything will be taken care by model build step that is called auto model next we have to evaluate the model.

So, to perform this we need a software to generate the Ramachandran plot, we can use some online tools called rampage for example.

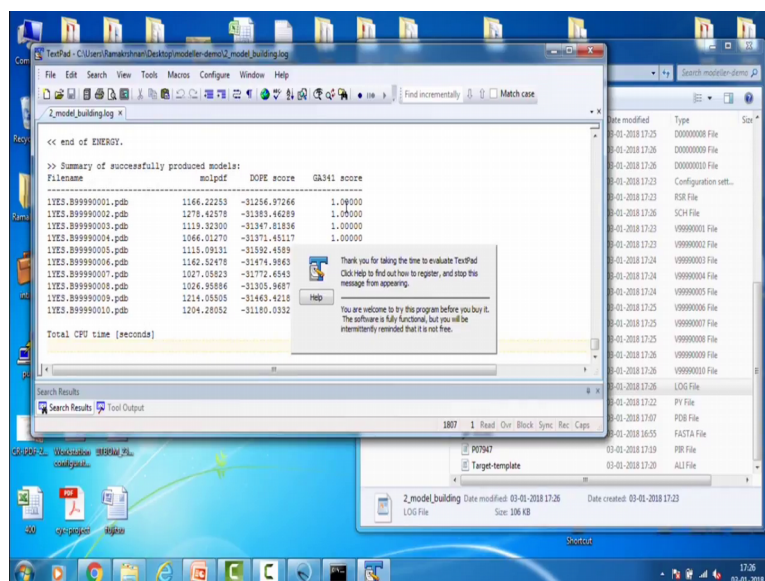
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So, here we have to upload the selected model based on dope score and see how many residues are in allowed and disallowed regions in order to finalize the model for further work.

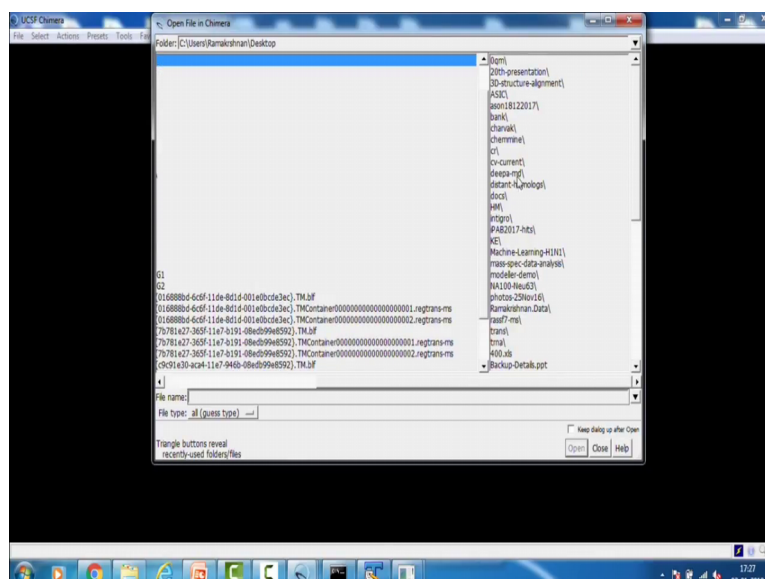
So, the model building is almost done now we will see the log file of the model building and choose the model based on the dope score.

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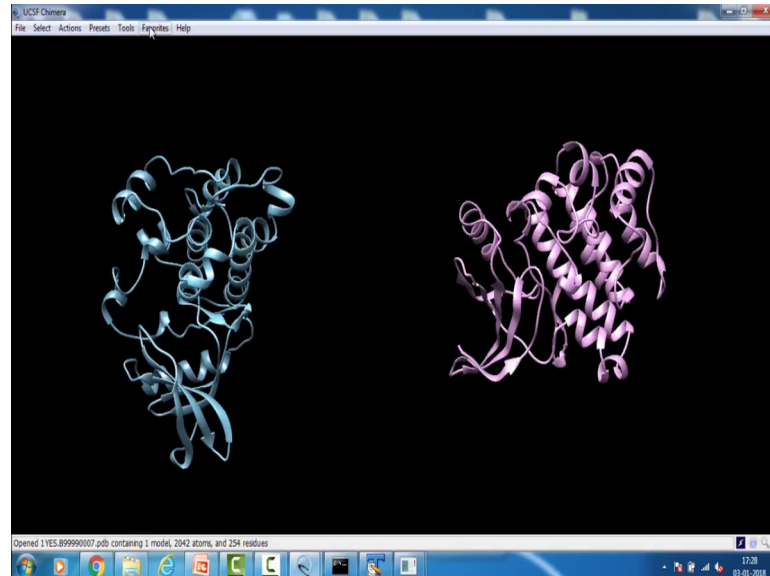
That is written at the end of this log file, here you if you see for the given for the 10 structures generated the least dope score is the best one, that is somehow 3 5 7 yeah the 7th model is the best as per the dope score. If you take the 7th structure now to visualize using primal or chimera whatever you have, you can see the model built using primal here and you can compare it with the template already we have the template loaded in the chimera.

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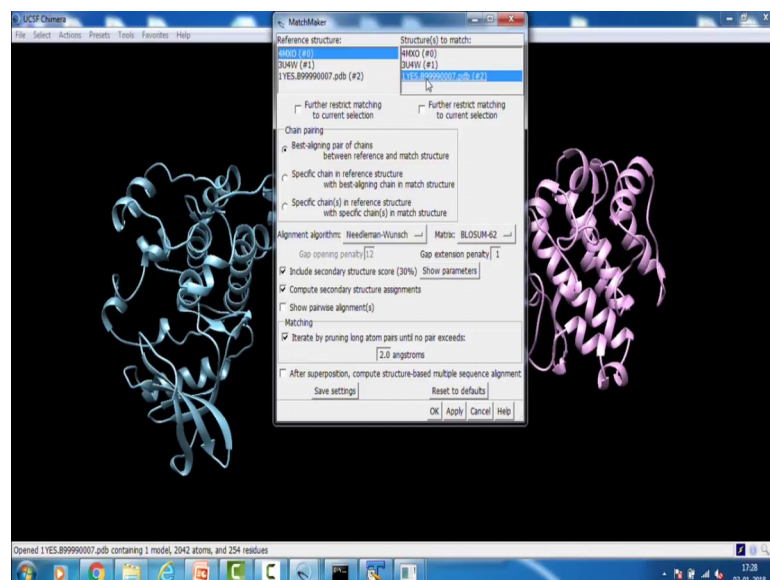
You just visualize our template alone and load the selected build structure based on the dope score.

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The seventh one is the best one open it and align using a chimera the reference structure is your template and the structure to match must be your the build structure.

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Now, you can see the alignment how perfectly the order built, the structure of your target based on the template with the RMSD of 0.1, which is very low and means the both the structures are not deviating much.

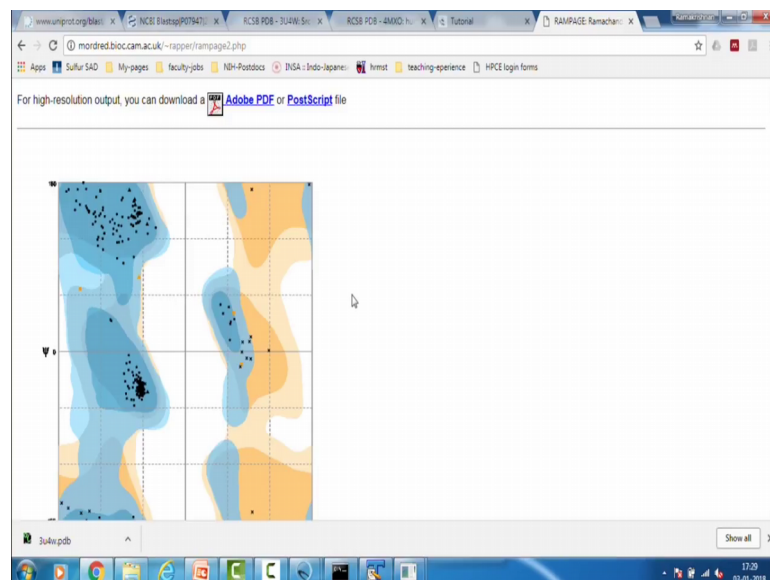
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So, once this is confirmed the structure the model building is confirmed, you can evaluate the same using the Ramachandran plot also.

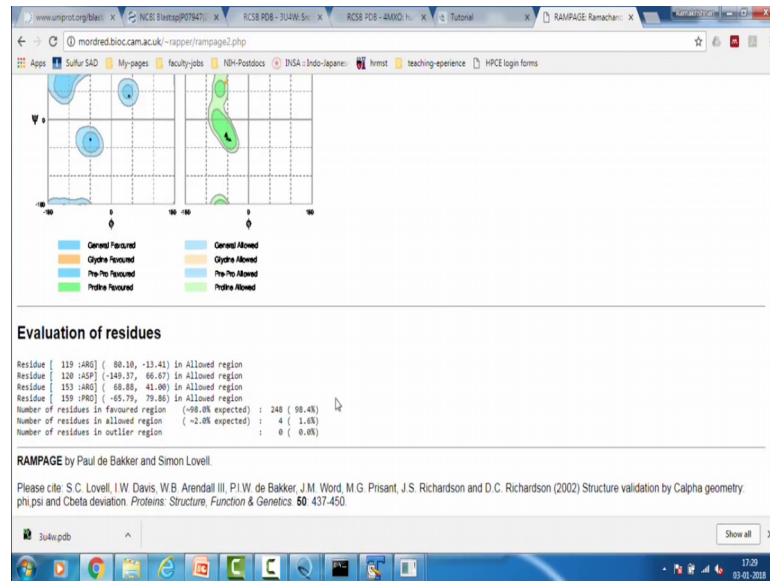
The modeller demo you can load the 7th model and submit.

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Here you can see the Ramachandran plot with the phi and psi angles specified for each and every amino acids and the statistics.

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So, here the model was built with 98 percent 0.4 percent of the residues are in favored region and 4 percent are in 4 residues are in; that means, 1.6 percent are in allowed region and there is no even single residue in the disallowed region means, the model built the 10 confirmations and we have selected the correct one and evaluate and evaluated the correctness using the Ramachandran plot.

So, it means the model what we built is very perfect and we can take it for further structure based web designing.