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Molecular modeling approach and RMSD calibration for superimposed 3D structure of DHFR from *Pneumocystis jiroveci* (PCP)

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ABSTRACT

The research illuminates DHFR from *Pneumocystis jiroveci* as a newly potential drug target against pneumonia. *P. jiroveci* DHFR sequence Q9UUP5 was obtained from Swiss-Prot database and deployed for 3-dimensional structure prediction. Sequence similarity templates searching found between *P.j* DHFR against 1CD2, 1VJ3 and 1DR1 paved the modeling with high confidence. The superimposition of the predicted template structures revealed the sequence identity of more than 30% and RMSD values of 4vs.1, 4vs.2, 4vs.3 and 4vs.5 and RMSD values 0.094, 0.093, 0.094 and 0.108 respectively; it comes under the expected range of <2Å. The structure showed overall conservation domains involved in binding affinity, energy minimization value, as well as inter-subunit interactions. Our results provided a basis of structural modeling (threading), energy minimization, RMSD value, structural validation and evaluation, to compare the overall structure and functional amino acids dependent on *P.j* DHFR in *Pneumocystis*. Further analysis to show the differences found between the inter and intra species of *P.j* DHFR is a leeway to design inhibitors targeted specifically against *Pneumocystis jiroveci* pneumonia (PJP).

Keywords: Threading, RMSD value, Templates, Superimposition and Pneumonia.

Abbreviations: Pneumocystis carinii Pneumonia (PCP), Pneumocystis jiroveci Pneumonia (PJP), Dihydrofolate reductase (DHFR), Root Mean Square Deviation (RMSD).

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INTRODUCTION

Pneumocystis pneumonia (PCP) or pneumocystosis is a form of pneumonia, caused by the *Pneumocystis jiroveci* yeast-like fungi. This pathogen is specific to humans; no evidence has vindicated to infect other animals, in contrast to other species of *Pneumocystis* that parasitize animals have not equally been shown to infect humans [1] *Pneumocystis* is commonly found in the lungs of healthy people and being a source of opportunistic infection could cause lung infection in people with a compromised immune system. *Pneumocystis* pneumonia is especially seen in people with cancer, HIV/AIDS and the victims under medication affecting the immune system[2]

Pneumonia is an inflammatory condition of the lung, especially inflammation of the alveoli or when the lungs fill with fluid[3] There are many causes of Pneumonia, majorly bacteria, viruses, fungi and parasites [4] Chemical burns or physical injury to the lungs can also produce pneumonia [5]. Pneumonia is a common disease that occurs in all age groups with vaccines to prevent certain types of pneumonia are available. The prognosis depends on the type of pneumonia, the treatment, any complications, and the person's underlying health. Some forms of pneumonia are responsible for specific symptoms. PCP can also develop in patients who are taking immunosuppressive drugs. Prior to the development of more effective treatments, PCP was a common and rapid cause of death in persons living with AIDS, further in populations deprived access to preventive treatment and continues to be a major cause of death in AIDS patients.

The most common signs and symptoms include progressive dyspnea, non-productive cough, and low-grade fever, fast heartbeat and trouble breathing lasting for two to four weeks[6.] Other warning signs encompasses persistent dry cough that does not produce any phlegm, occasional pain or tightness in the chest and also production or non-production of sputum is also noteworthy. While PCP typically causes a dry, non-productive cough, bacterial pneumonia is often associated with the production of thick, purulent (pus-containing) sputum[7] Haemoptysis has also been recorded as a presenting feature. Clinical examination often reveals an increased respiratory rate, tachycardia, cyanosis and fine crackles on auscultation of the chest[8] Chest pain, coughing up sputum (phlegm), fast breathing, getting tired very easily, weight loss, malaise and diarrhea are also frequently associated with PCP development.

Dihydrofolate reductase (DHFR) is an enzyme encoded by DHFR gene constituted of 798 bp and is located in the q11→q22 region of chromosome 5[12], where it has a critical role in regulating the amount of tetrahydrofolate in the cell of all organisms. DHFR reduces dihydrofolic acid to tetrahydrofolic acid, using NADPH as an electron donor, which is converted to the kinds of

tetrahydrofolate co-factors involved in 1-carbon transfer. Finally, dihydrofolate is reduced to tetrahydrofolate and NADPH is oxidized to NADP+ [9-11]. Tetrahydrofolate and its derivatives are vital for purine and thymidylate synthesis [13.] In humans, folate is the active form of tetrahrdrofolate. Folic acid is essential for growth and maturation of sporozoites in *Pneumocystis*, [14] and thus, the principle targeting the folic acid metabolic pathway is crucial for vaccine development against *Pneumocystis*[15] Inhibition of DHFR can cause functional folate deficiency as pointed out by megablostic anemia with dihydrofolate reductase deficiency [11] Further studies into inhibitors of DHFR can lead to more ways of treatment with reduced forms of folic acid in *Pneumocystis.* Thus far, DHFR structure has not been deposited in PDB expository and hence structure prediction is imperative to check the efficiency of chemical compounds against *P. jiroveci* dihydrofolate reductase.

MATERIALS AND METHODS

Sequence retrieval of the *Pneumocystis jiroveci* DHFR

P. jiroveci DHFR sequence ID number Q9UUP5 were obtained from Swiss-Prot database (http://www.expasy.ch/sprot. BlastP accredited to its sensitivity and balanced speed was used for template selection against PDB structures and the best three were selected to aid modeling.

Structure prediction and Template selection

Modeller is used for homology or comparative modeling of protein three-dimensional structures[16, 17] MODELLER implements comparative protein structure modeling by satisfaction of spatial restraints[18,19] and can perform many additional tasks, including de novo modeling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures, etc. Python is a programming language that helps work more quickly and integrates systems more effectively. PyMOL Viewer was used to view all the PDB structures and saved in PDB format. The PDB structure from the file menu was opened and all structures were saved and compared with the 'Publication' mode of the preset menu.

Modeller 9v8 version was used to predict the structure using the three selected structures (1CD2, IVJ3 and 1DR1) from the blastP against DHFR (Q9UUP5) structure enumeration. Salign () command in MODELLER was used to generate multiple alignment of the family (salign.py) followed by query sequence alignment against the template structures (Align2d_mult.py). The resultant sequence information was used for final DHFR sequence (Model_mult.py) and DOPE evaluated the potential new model candidates (evaluate_model.py).

Salign

```
# illustrates the SALIGN multiple structure/sequence alignment
From modeller import *
log.verbose()
env = environ()
env.io.atom_files_directory = './:../atom_files/'
aln = alignment(env)
for (code, chain) in (('1CD2', 'A'), ('1VJ3', 'A'), ('1DR1', 'A')):
   mdl = model(env, file=code, model_segment=('FIRST:'+chain, 'LAST:'+chain))
   aln.append_model(mdl, atom_files=code, align_codes=code+chain)
for (weights, write_fit, whole) in (((1., 0., 0., 0., 1., 0.), False, True),
                                    ((1., 0.5, 1., 1., 1., 0.), False, True),
                                    ((1., 1., 1., 1., 1., 0.), True, False)):
aln.salign(rms_cutoff=3.5, normalize_pp_scores=False,
rr_file='$(LIB)/as1.sim.mat', overhang=30,
gap_penalties_1d=(-450, -50),
gap_penalties_3d=(0, 3), gap_gap_score=0, gap_residue_score=0,
dendrogram_file='fm00495.tree',
alignment_type='tree', # If 'progresive', the tree is not
# computed and all structues will be
# aligned sequentially to the first
feature_weights=weights, # For a multiple sequence alignment only
# the first feature needs to be non-zero
improve_alignment=True, fit=True, write_fit=write_fit,
write_whole_pdb=whole, output='ALIGNMENT QUALITY')
aln.write(file='fm00495.pap', alignment_format='PAP')
aln.write(file='fm00495.ali', alignment_format='PIR')
aln.salign(rms_cutoff=1.0, normalize_pp_scores=False,
rr_file='$(LIB)/as1.sim.mat', overhang=30,
gap_penalties_1d=(-450, -50), gap_penalties_3d=(0, 3),
gap_gap_score=0, gap_residue_score=0, dendrogram_file='1is3A.tree',
alignment_type='progressive', feature_weights=[0]*6,
improve_alignment=False, fit=False, write_fit=True,
```

write_whole_pdb=False, output='QUALITY')

Next alignment was query sequence to the template structures. For that task again used the salign() command (file `align2d_mult.py'). Only sequence information was used for the final DHFR sequence.

Align2d_mult

```
from modeller import *
log.verbose()
env = environ()
env.libs.topology.read(file='$(LIB)/top_heav.lib')
# Read aligned structure(s):
aln = alignment(env)
aln.append(file='fm00495.ali', align_codes='all')
aln_block = len(aln)
# Read aligned sequence(s):
aln.append(file='Q9UUP5.ali', align_codes='Q9UUP5')
# Structure sensitive variable gap penalty sequence-sequence alignment:
aln.salign(output='', max_gap_length=20,
gap_function=True,   # to use structure-dependent gap penalty
alignment_type='PAIRWISE', align_block=aln_block,
feature_weights=(1., 0., 0., 0., 0., 0.), overhang=0,
gap_penalties_1d=(-450, 0),
gap_penalties_2d=(0.35, 1.2, 0.9, 1.2, 0.6, 8.6, 1.2, 0., 0.),
similarity_flag=True)
aln.write(file='Q9UUP5-mult.ali', alignment_format='PIR')
aln.write(file='Q9UUP5-mult.pap', alignment_format='PAP')
```

The new model was built for the DHFR target sequence based on the alignment against the multiple templates using the `model_mult.py' file.

Model_mult

```
from modeller import *
from modeller.automodel import *
env = environ()
a = automodel(env, alnfile='Q9UUP5-mult.ali',
```

knowns=('1CD2A','1VJ3A','1DR1A'), sequence='Q9UUP5',assess_methods=(assess.DOPE, assess.GA341))

a.starting_model = 1

a.ending_model = 5

a.make()

Finally, DOPE was used to evaluate the potential of the new model coordinates using the evaluate_model.py' file.

Evaluate_model

```
from modeller import *
from modeller.scripts import complete_pdb
log.verbose()    # request verbose output
env = environ()
env.libs.topology.read(file='$(LIB)/top_heav.lib') # read topology
env.libs.parameters.read(file='$(LIB)/par.lib') # read parameters
# read model file
mdl = complete_pdb(env, 'Q9UUP5.B99990001.pdb')
# Assess all atoms with DOPE:
s = selection(mdl)
s.assess_dope(output='ENERGY_PROFILE NO_REPORT', file='Q9UUP5.profile',
normalize_profile=True, smoothing_window=15)
```

Energy Minimization of the Modeled protein

The steepest descent method was used for energy minimization of the molecule by Swiss-PDB Viewer. The minimization cycle was repeated till the molecule attained its minimum energy level.

Structure validation and evaluation

The modeled protein structure was submitted to the structure validation evaluation server. Swiss-Model was used to check for quality of the models. Protein structure and model assessment tool mode was selected. Uploaded a model in PDB format and the local model quality estimation, global model quality estimation, Pro-check and What-check for stereochemical quality check with Ramachandran plot, Promotif for analysis of protein structure motifs were satisfied. Helix, beta strand, random coil, most favored regions, favored regions, allowed regions and disallowed regions were notified.

RESULTS AND DISCUSSION

Templates identification by BlastP

Homology modeling needs template identification. A template is a homologous protein with known experimental protein structure. The first parameter to pay attention in template identification is the percentage of sequence identity between the protein and the template. This simply means that for template identification for longer sequences (>100 amino acids) needs to have more than 30% sequence identity and notably 33% [21] and the template for the homology model reliability [20]. Our template similarities showed more than 33% between the template and protein (Table 1). 1DR1 with 38% identity to the query sequence was considered in order to perform multiple template modeling on the grounds the sequences not represented by the other two templates could be supported by the less identity template as evidenced by modeling [21]

Table 1. Identified templates with their chemical properties

Name	**1CD2**	**1VJ3**	**1DR1**
Properties			
Fasta ID	>gi|7245416	>gi|42543914	>gi|157830864
Chain	A	A	A
Length	206	205	189
Weight (KD)	25103.51	25000.88	22702.82
Molecule	Dihydrofolate Reductase	Dihydrofolate Reductase	Dihydrofolate Reductase
Organism	*Pneumocystis Carinii* DHFR complexes with Folate and Nadp+	*Pneumocystis Carinii* DHFR Cofactor complex with tab, A highly selective Antifolate	*Chicken Liver* DHFR complex with Nadp+ and Biopterin
Identity	127/206 (61%)	126/203 (62%)	79/205 (38%)
Positives	157/206 (76%)	155/203 (76%)	121/205 (59%)
Gaps	0	0	11%
Classification	Oxidoreductase	Oxidoreductase	Oxidoreductase
Sequence	MNQQKSLTLIVALTTS YGIGRSNSLPWKLKK EISYFKRVTSFVPTFDS FESMNVVLMGRKTW ESIPLQFRPLKGRINV VITRNESLDLGNGIHS AKSLDHALELLYRTY GSESSVQINRIFVIGGA QLYKAAMDHPKLDRI MATIIYKDIHCDVFFP LKFRDKEWSSVWKK EKHSDLESWVGTKVP HGKINEDGFDYEFEM WTRDL	NQQKSLTLIVALTTSY GIGRSNSLPWKLKKEI SYFKRVTSFVPTFDSF ESMNVVLMGRKTWE SIPLQFRPLKGRINVVI TRNESLDLGNGIHSA KSLDHALELLYRTYG SESSVQINRIFVIGGAQ LYKAAMDHPKLDRI MATIIYKDIHCDVFFP LKFRDKEWSSVWKK EKHSDLESWVGTKVP HGKINEDGFDYEFEM WTRDL	VRSLNSIVAVCQNM GIGKDGNLPWPPLRN EYKYFQRMTSTSHV EGKQNAVIMGKKTW FSIPEKNRPLKDRINI VLSRELKEAPKGAH YLSKSLDDALALLDS PELKSKVDMVWIVG GTAVYKAAMEKPIN HRLFVTRILHEFESDT FFPEIDYKDFKLLTEY PGVPADIQEEDGIQY KFEVYQKSVLAQ
Conserved regions	Identical sites: 66 (31.1%), Pairwise identity: 54.8%		

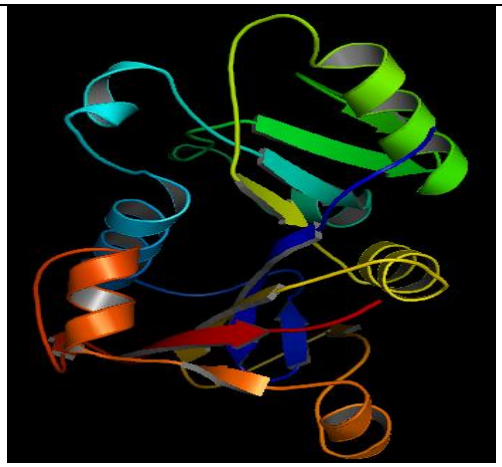
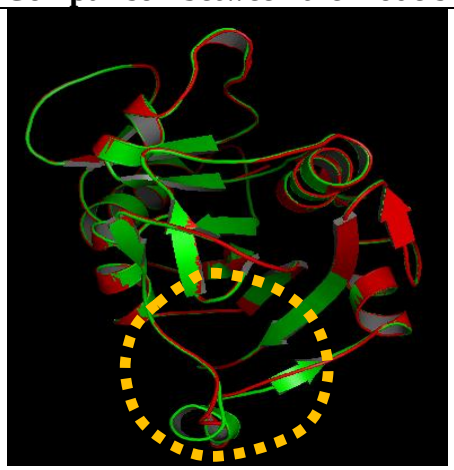
The templates 1cd2 (206 amino acids) from DHFR of *Pneumocystis carinii*, 1vj3 (205 amino acids) from DHFR of *Pneumocystis carinii*, and 1dr1 (189 amino acids) from the DHFR of *Chicken Liver* chosen possess 'A' chain molecules under oxidoreductases that catalyzes the transfer of electrons from one molecule to another utilizing NADP or NAD as cofactors. The review by Fernandez-Fuentes *et al* [22] revealed the use of multi-template yields accurate modeling, our templates merged crucial for multi-template to model the structure.

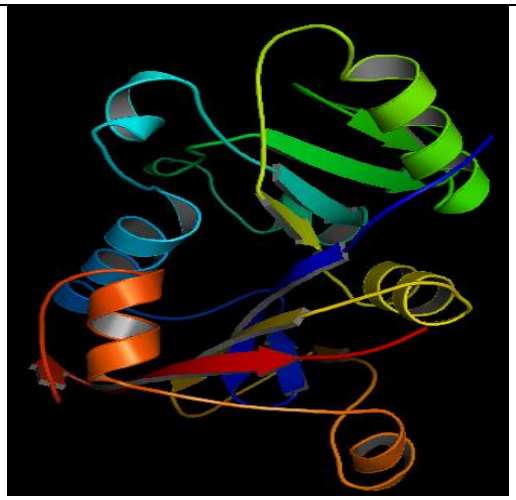
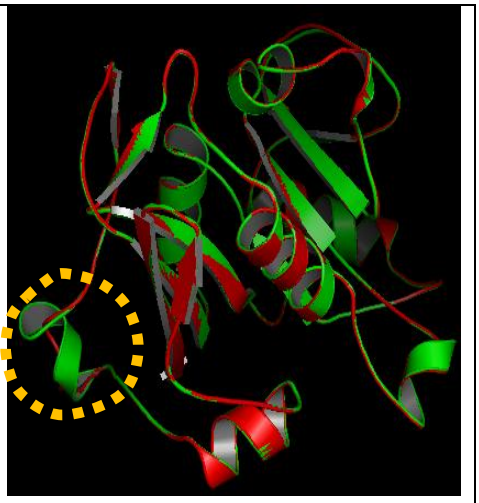
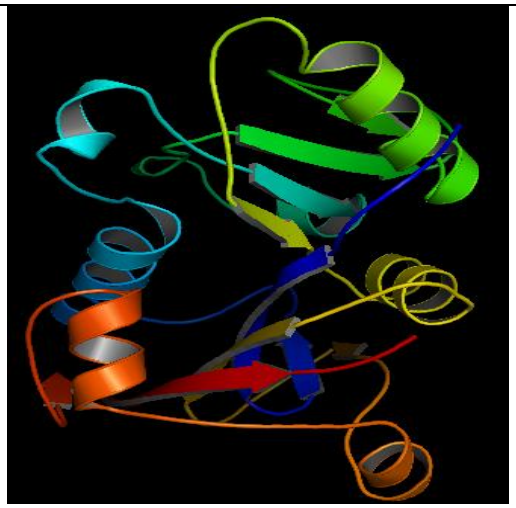
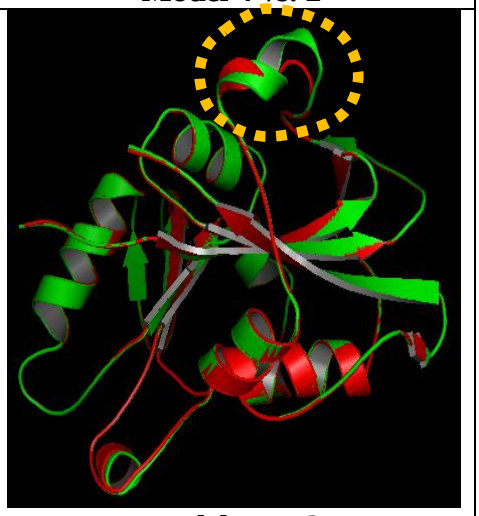
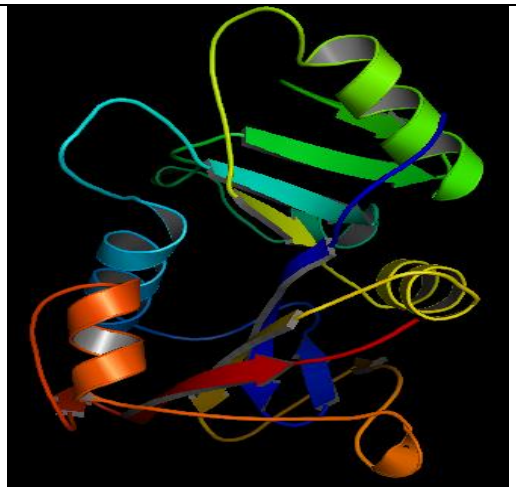
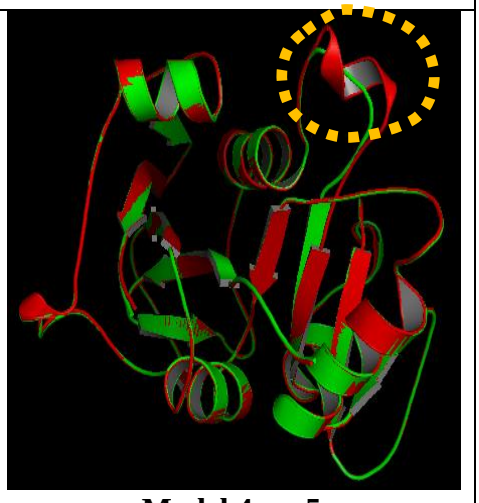
Totally, five models were generated for *P. jiroveci*, the fourth was selected as best one for docking based upon the lesser Dope score and Molpdf model values -24597.46094 and 8092.32031 respectively. It has more variations in loops and helixes as highlighted in Table 2.

Table 2. Identification of the best model

No	File name	Molpdf	DOPE score
1.	Q9UUP5.B99990001	8170.31445	-24404.26367
2.	Q9UUP5.B99990002	8348.48145	-24055.08789
3.	Q9UUP5.B99990003	8158.10010	-24309.00781
4.	Q9UUP5.B99990004	8092.32031	-24597.46094
5.	Q9UUP5.B99990005	8296.11719	24185.03125

The selected model 4th (red color) was compared with other models (green color); values influenced the structural variations, were then compared through the PyMOL Viewer (Fig. 1) and highlighted with yellow dashed lines; which indicated the divergence of model 4 vs. 1 showed the difference of turn and helix in position 163-Lysine to 168-Valine at model 1; model 4 vs. 2 and model 4 vs. 3 showed dissimilarity in same positions 163-Lysine to 168-Valine (helix), model 4 vs. 5 had a variation in position 45-Thyrosine to 51-Serine; hence, models 2, 3, 5 contain helix structure in the revealed positions. Each amino acid substitutions may affect and change the protein function [23]

Model	Modeled Structure	Comparison between the models
Q9UUP5.B99990001 **Dope score: -24404.26367**	 **Model 1**	 **Model 4 vs. 1**

Q9UUP5.B99990002 **Dope score: 24055.08789**	 **Model 2**	 **Model 4 vs. 2**
Q9UUP5.B99990003 **Dope score: 24309.00781**	 **Model 3**	 **Model 4 vs. 3**
Q9UUP5.B99990005 **Dope score: 24185.03125**	 **Model 5**	 **Model 4 vs. 5**

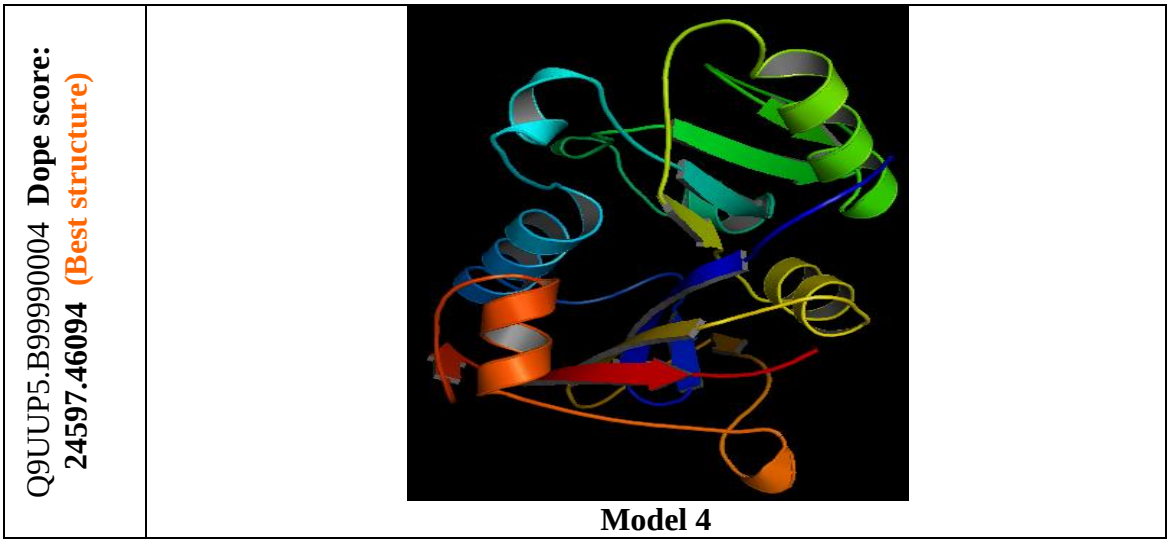


Figure. 1 Structure comparison of modeled structures

Table 3. Structure comparison tables of five modeled structures (Model 1, 2, 3, 5 compared with the 4th Model)

		Model 4 vs. 1	Model 4 vs. 2	Model 4 vs. 3	Model 4 vs. 5
Match		assigning 206 x 206 pairwise scores	assigning 206 x 206 pairwise scores	assigning 206 x 206 pairwise scores	assigning 206 x 206 pairwise scores
Match align		aligning residues (206 vs. 206)	aligning residues (206 vs. 206)	aligning residues (206 vs. 206)	aligning residues (206 vs. 206)
Executive RMS	**Cycle 1**	10 atoms rejected during cycle 1 (RMS=0.15)	9 atoms rejected during cycle 1 (RMS=0.24)	7 atoms rejected during cycle 1 (RMS=0.25)	9 atoms rejected during cycle 1 (RMS=0.17)
	Cycle 2	10 atoms rejected during cycle 2 (RMS=0.11)	9 atoms rejected during cycle 2 (RMS=0.11)	11 atoms rejected during cycle 2 (RMS=0.13)	9 atoms rejected during cycle 2 (RMS=0.12)
Executive RMS		0.094 (186 to 186 atoms)	0.093 (188 to 188 atoms)	0.094 (188 to 188 atoms)	0.108 (188 to 188 atoms)

Cycle 1 and Cycle 2 showed the RMS differentiations of the DHFR Modeled structures.

DOPE (Discrete Optimized Protein Energy) is a statistical potential used to assess homology models in protein structure prediction. DOPE is based on an improved reference state that corresponds to non-interacting atoms in a homogeneous sphere with the radius dependent on a sample native structure; it thus accounts for the finite and spherical shape of the native structures. It is implemented in the popular homology modeling program MODELLER and used to assess the energy of the protein model generated through many iterations MODELLER, which produces homology models by the satisfaction of the spatial restraints. The models returning the minimum molpdfs can be chosen as best probable structures and can be further used for

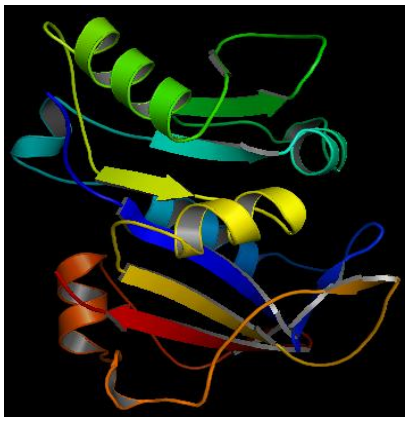
evaluating with the DOPE score. DOPE is implemented in Python and is applied within the MODELLER environment. Alternatively, DOPE can also generate a residue-by-residue energy profile for the input model, making it possible for the user to spot the problematic region in the structure model[24]. Structural errors can change the Dope score, at present errors are highlighted in Table 3.

Energy Minimization

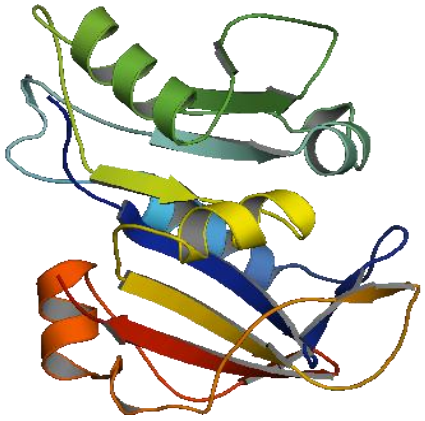
Energy minimization was used to estimate the sizes of features on the protein potential energy surface [25.] The selected model was energy minimized by using the Swiss-PDB revealed E-value of10672.023 after completion of 100 cycles against Initial E-value of -2819.186.

RMSD value

The root mean square deviation (RMSD) is the measure of the average distance between the atoms (usually the backbone atoms) of superimposed proteins. A widely used way to compare the structures of biomolecules or solid bodies is to translate and rotate one structure with respect to the other to minimize the RMSD [26]. Typically RMSD was used to make a quantitative comparison between the structure of a partially folded protein and the structure of the native state. Also some scientists who study protein folding simulations used RMSD as a reaction coordinate to quantify whether the protein is between the folded state and the unfolded state. Since, the result in the present model showed more than 0.5 Å differences which is one of the determining factors for stabilizing the structure [27] could suggest minimization of energy for stable structure (Figure. 2).



Before energy minimization



After energy minimization

Figure. 2 Modeled DHFR structure before and after the energy minimization

Structure validation and evaluation

SWISS - MODEL was used to validate the DHFR structure by exploring tools like Pro Check and What_ Check and Ramachandran Map.

The predicted 3-D structures were evaluated using the PROCHECK and WHATCHECK Verify 3D programs with the Ramachandran plot. The Ramachandran plot showed that around 170 residues were in most favored region as indicated by [A, B, L] respectively, accounting 91.9% residue score, whereas those in allowed region [a, b, l, p] accounting 7.0% with 13 residues, 2 amino acids in the generously allowed region [~a, ~b, ~l, ~p] were noted and no residues were observed in the disallowed region. Our model proved to be the best with more than 80% in most favored region as supported by Ayadi *et al* [28]. Further analysis by Residues property diagram showed the number of non-glycine and non-proline residues revealing 185 (100.0%), number of end-residues (excluding Glycine and Proline) were 2, number of glycine residues (shown as triangles) 12 and 7 proline residues totalling 206 (Fig. 3a). Structure resolution was less than 2.0 Angstroms and R-factor not greater than 20% at most favored regions and that accounted to 91.9% in Ramachandran plot of 91.9% and Residues property diagram (Fig. 3b) identified the model to be the best according to Laskowski and co-workers [29]. R-factors are measures of the extent to which a crystallographic model accounts for the original experimental data specifically, the measured intensities of reflections in the diffraction pattern. As such, R-factors are important indicators of progress in refining models, and the final values of R-factors are important criteria of model quality (http://spdbv.vital-it.ch/TheMolecularLevel/ModQual/#R-Equation). As the R-factor itself is minimized in the structure refinement process and is comprehensible, implications for its value as a structure-quality indicator [30]

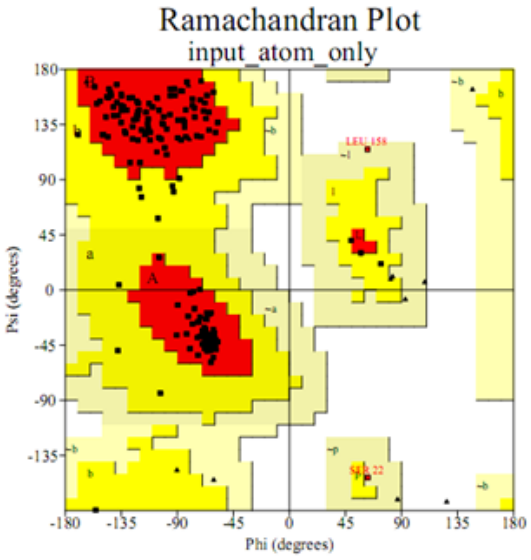
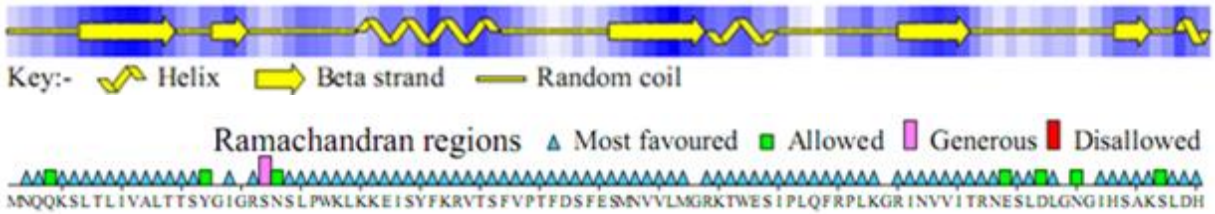


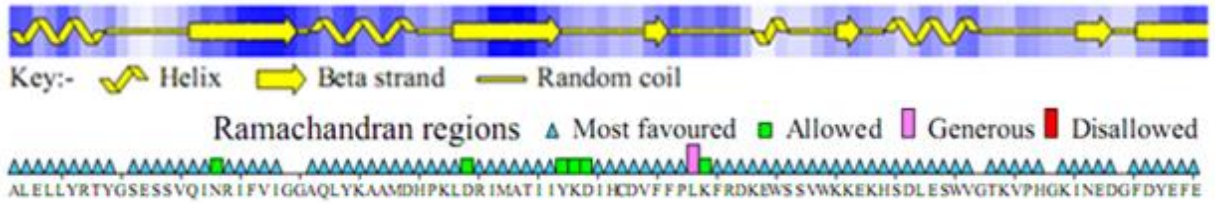
Figure. 3 (a). Ramachandran Plot

No residues (■ ▲) are presented in the disallowed regions (white color division) of the Ramachandran Plot.

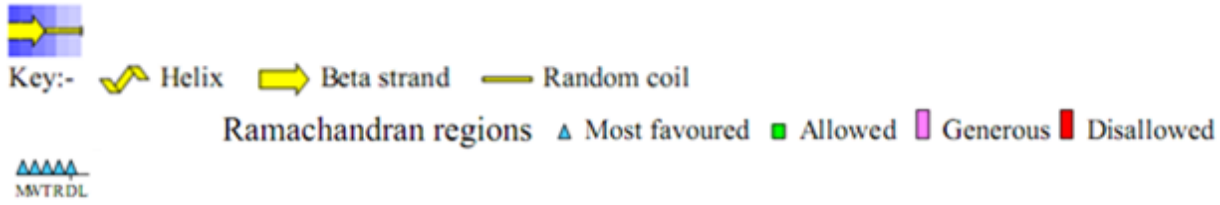
Secondary structure and estimated accessibility for regions 1-100 amino acid.



Secondary structure and estimated accessibility for regions 101-200 amino acid.



Secondary structure and estimated accessibility for regions 201-206 amino acid.



3 (b). Residues property diagram

CONCLUSION

The endeavor of the work was to model the DHFR to halt its functions in opportunistic disease due to *Pneumocystis jiroveci* and the work was executed successfully whereby the models could be used for further research.

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