Homology Modeling of Nitrogenase Iron Protein of Nitrogen Fixing Actinomycete *Arthrobacter* sp

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ABSTRACT

Nitrogenase is an important enzyme associated with nitrogen fixation. Nitrogenase iron protein (Nif H) is one of the vital genes contributing to the nitrogen fixation. *Arthrobacter* sp. is one of the actinomycetes fixing atmospheric nitrogen. Nitrogenase iron protein of *Arthrobacter* sp. was retrieved from NCBI in FASTA format and a good template was selected by Basic Local Alignment Search tool and it was found to be *Azotobacter vinelandi*i. The modeling of 3D structure of the protein was performed by Swiss model, GENO3D and ModWeb. The modeled 3D structure was evaluated by ProSA, VERIFY3D, PROCHECK, PROVE and ERRAT. The nitrogenase iron protein 3D structure modeled by ModWeb was found to be the best reliable model based on the above mentioned evaluation. The structural dynamics were performed by WEBnm@ and elNemo.3D structure of the nitrogenase iron protein was visualized by Rasmol.

Keywords:

Nitrogenase iron protein, Homology modeling, *Arthrobacter* sp., Geno 3D, Mod Web, Swiss model, Rasmol

1. INTRODUCTION

Nitrogen is the main constituent (~78%) of the earth's atmosphere and it is an important component of many compounds, like nucleic acids, proteins which forms the basis of life. However, nitrogen cannot be utilized directly by biological systems for their growth and reproduction; nitrogen must be converted to ammonia by combining with hydrogen. This process is referred as "nitrogen fixation" and this is done by physical, chemical or biological methods.

The biological conversion of atmospheric dinitrogen to ammonia is catalyzed by oxygen labile and highly conserved enzyme named nitrogenase that is hardly distributed among bacteria and archaea [1] [2]. NifH is one of the three structural genes for Fe protein, a core of nif genes (nifH, nifD, nifK, nifB, nifE, nifN, nifX, nifU, nifS, nifV, nifW, nifZ) are required for nitrogenase synthesis, and catalysis is conserved in all diazotrophs [3] [4] [5] [6] [7] [8]. For a long time it was believed that the ability to fix atmospheric nitrogen was limited to the actinomycetes *Frankia*, but lately, the number of nitrogen fixation (nifH) genes was identified in other non-*Frankia* actinomycetes like *Arthrobacter* [9].

Arthrobacter sp. is gram-positive obligate aerobes, commonly found in soil. The characteristic feature of this actinomycete is that, they look like rods in exponential phase and cocci in its stationary phase. Structural insights on NifH proteins are available for certain microorganisms like *Azotobacter*, *Clostridium* etc. [10] [11] [12] [13], but absent for *Arthrobacter* sp. This is due to the absence of X-ray crystallographic studies of *Arthrobacter* nitrogenase iron protein. The availability of the genome sequences for three *Frankia* strains [9] [14] now unlocked the possibility for the use of computational techniques to predict three-dimensional (3D) structure of NifH protein.

The 3D structure of a protein gives essential information of its function. Although it is hard to establish a protein structure experimentally from X-ray crystallography or NMR spectroscopy studies, computational techniques have become very well accepted tools for generating of 3D structures [15]. Homology modeling is a reliable method that can predict the 3D structure of a protein with exactly similar as obtained at low-resolution by experimental means [16].

2. MATERIALS AND METHODS

2.1. Retrieval of target sequences

Protein sequence of nitrogenase iron protein of *Arthrobacter* sp. was retrieved from National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov (Accession number: JF776688, Protein Id: AET12084.1) in FASTA format and used for further analysis. The 3D structure of nitrogenase iron protein of the following actinomycete was absent in Protein Data Bank (http://www.rcsb.org/pdb/home/home.do), hence the current work was started.

2.2. Template selection and alignment of the target

In homology modeling technique, the first task was to make out the protein structures related to the target sequence and the subsequent selection of template was made [17]. BLAST (Basic Local Alignment Search Tool) was carried out against Protein Data Bank available at National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/) and an appropriate template was selected

2.3. Construction of the models

Homology modeling was performed for nitrogenase iron protein
sequence using alignment mode by Swiss model server (http://swissmodel.expasy.org/). In the alignment mode of SWISS MODEL, aligned sequence was submitted and it was specified as the target sequence and a structurally known protein chain from the PDB library was taken as template. The server builds a model based on the given alignment of target and template.

GENO3D (http://geno3d-pbil.ibcp.fr) is an automated modeling tool for protein three dimensional structures [18]. The protein sequence was submitted and the GENO 3D server

performs the homology modeling by identifying homologous protein using PSI-BLAST as template and ten models were created.
ModWeb(http://modbase.compbio.ucsf.edu/ModWeb20.html/ modweb.html) is a relative protein structure modeling server and it depends on ModPipe for its functionality [19]. The ModPipe has a group of non-redundant protein chains which were picked out from structures in PDB [20]. Significant alignments with E-value greater than 1.0 covering minimum of 30 amino acids were selected for modeling and the models were built by sequence-structure matches using comparative modeling and satisfaction of spatial restraints as applied in Modeller [20]. Lastly, the resulting models were assessed using some model assessment schemes and the finest scoring models were sent.

2.4. Evaluation of the constructed model

For evaluation, the protein model was subjected to ProSA (https://prosa.services.came.sbg.ac.at/prosa.php)analysis[21] and was performed to assess accuracy and reliability of the modeled structure. SAVES (http://nihserver.mbi.ucla.edu/SAVS/) was used to carry out the verifications of the model with PROVE and ERRAT. The overall qualities of the modeled structures were evaluated using ERRAT [22]. RMS and Z score mean value were assisted by PROVE PLOT [23]. The packing quality of the refined structures was calculated by the PROCHECK [24] quality control value. Verify 3D [25] was used to validate the refined structure. The 3D structure of the protein was compared to its own amino-acid sequence taking into consideration a 3D profile calculated from the atomic coordinates of the structures of correct proteins [25]. The constructed model was evaluated for its backbone conformation using a Ramachandran plot [26]. The stereochemical quality of the modeled proteins is assessed from Ramachandran validation score for favoured and unfavoured regions [27].

2.5. Studying intrinsic dynamics of the protein models and visualization of the modeled protein

Understanding structural dynamics of proteins is essential for gaining greater insights into their biological functions [28]. Studies on the structural dynamics were performed by (http://www.bioinfo.no/tools/normalmodes) WEBnm@ [29] to calculate the slowest modes and related deformation energies and elNemo (http://igs-server.cnrs mrs.fr/elnemo/index.html) to calculate the normal mode analysis of the proteins contributing to the corresponding protein movement [30]. Normal mode analysis forecasts the probable movements of the proteins and is the method of selection for exploring the slowest activity of choice in [29]. Structural visualization of the modeled protein was performed by RasMol.

2.6. Protein Quality Prediction

ProQ (http://www.sbc.su.se/˜bjornw/ProQ/ProQ.html) is a neural network based predictor which is based on a number of structural characters calculates the quality of a protein model and it is optimized to find correct models to find native structures [31]. Two quality measures which predicted were LG score and MaxSub. LG score is a negative logarithm of P value and MaxSub ranges from 0-1, were 0 is insignificant and 1 very significant [31].

3. RESULTS AND DISCUSSION

3.1. Retrieval of target sequences

Protein sequence of nitrogenase iron protein of *Arthrobacter* sp. was retrieved from National Center for Biotechnology Information by giving its accession number. The nitrogenase protein sequences were retrieved in FASTA format and were saved as ".txt" file.

3.2. Templates selection and alignment of the target

BLAST search found the crystal structure of the *Azotobacter vinelandii* iron protein (1NIP) to be the best template. This template had an E value of 2e-69 and an X ray crystallographic resolution of 2.9 angstroms. The nitrogenase iron protein of *Azotobacter vinelandii* and the A and B chains of this protein revealed 82% sequence similarity with the *Arthrobacter* nitrogenase iron protein.

3.3. Construction of the models

The modeling of three dimensional structure of nitrogenase iron protein was performed by three homology modeling programs GENO3D, Swiss model and ModWeb.
In Swiss model, the alignment mode, nitrogenase iron protein of *Arthrobacter* was taken as target and *Azotobacter vinelandii* was selected as best template. QMEAN Z score gives an estimate of the degree of nativeness of the structural features observed in a model and indicates whether the model is of comparable quality to experimental structures [32]. Z score QMEAN for *Arthrobacter* was -4.15 and the QMEAN score of the nitrogenase iron protein was 0.4. The QMEAN score proved that the model predicted by SWISS MODEL was reliable because the score falls between 0 and 1.
The y-axis of the plot signified GROMOS empirical force field energy and ANOLEA atomic empirical mean force potential for each amino acid in the protein chain represented favourable energy environment in green and unfavourable energy environment in red for a given amino acid. Anolea assessed the packing quality of the model as a good model.
The GENO3D created ten 3D protein models based on distance geometry, repeated annealing and minimization of energy algorithms [18] and out of which the best model was selected based on least e value.
In ModWeb, template for the nitrogenase iron protein had more than 30% of sequence identity and the proposed model was found to be reliable. Percentage of identical residues in the alignment between the target and the template was 83% for *Arthrobacter* sp. during the template search. The ModPipe Protein Quality Score for *Arthrobacter* nitrogenase iron protein was 1.9016 (MPQS > 1.1) therefore the model created by ModWeb was reliable.

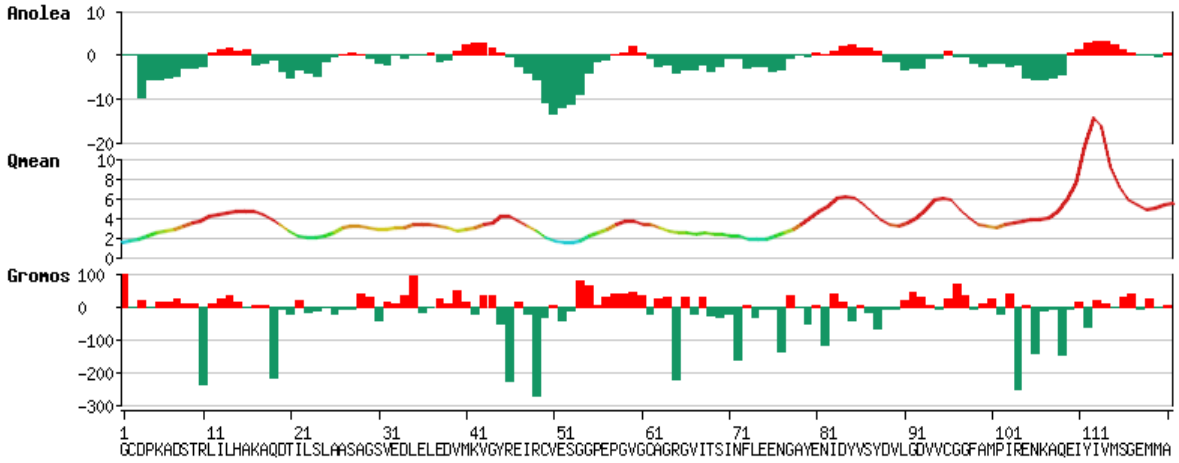


Figure 1: Gromos and Anolea of *Arthrobacter* sp. 3D model

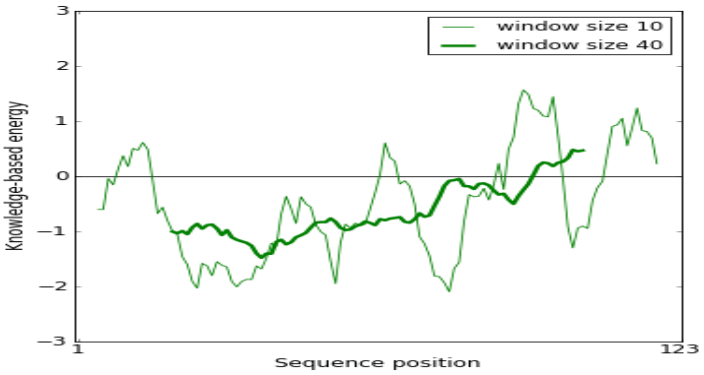


Figure 2: Screen shot showing the ProSA web plot of residues scores of nitrogenase iron protein of *Arthrobacter* modeled by ModWeb

3.4. Evaluation of refined models

3.4.1. ProSA

ProSA is a tool used extensively to check 3D models of protein structures for possible errors [21]. The Z score of *Arthrobacter* nitrogenase iron protein made by SWISS MODEL was -3.55, -3.53 for GENO3D model and -3.73 for ModWeb model. The web plot of residue scores displayed local model quality using plotting energy as a function of amino acid sequence location [21]. In general, positive values correspond to problematic or flawed parts of the input structure. Here, large parts of energy plot show highly negative values for *Arthrobacter* nitrogenase iron protein proved that all the three models were good and reliable models.

3.4.2. ERRAT for validating 3D model

ERRAT is a protein structure verification algorithm that is particularly suitable for estimating the progress of crystallographic model building and refinement [25]. The program worked by analyzing the statistics of non-bonded interactions between different atom types [33]. ERRAT analysis revealed the overall quality factor of nitrogenase iron protein from *Arthrobacter* was 72.816 for SWISS MODEL, 98.990 for GENO3D and 72.072 for ModWeb. These results implied that nitrogenase iron protein model made by GENO3D overall quality was very good and the other two models were fairly good.

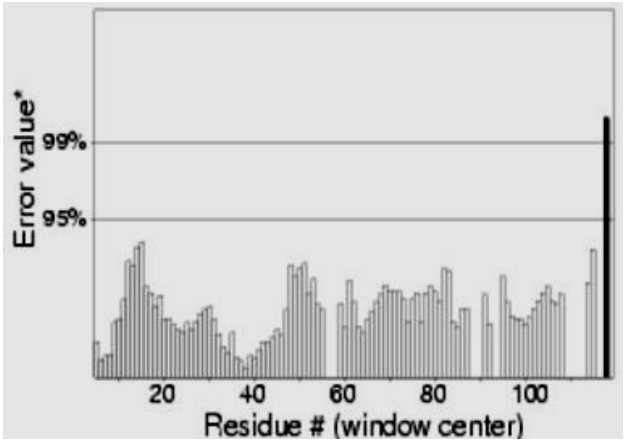


Figure 3: ERRAT for validating 3D model from GENO3D

3.4.3. VERIFY 3D for validating 3D model

VERIFY3D was used to validate the refined structure. The 3D structure of the protein was compared to its own amino acid sequence taking into consideration a 3D profile calculated from the atomic coordinates of the structures of correct proteins [25]. The constructed models were corroborated by VERIFY 3D to estimate correctness. VERIFY 3D reveals that 64.52% of the residues had an average 3D-1D score >0.2 for

Arthrobacter nitrogenase iron protein made by SWISS MODEL, 62.90% of the residues had an average 3D-1D score > 0.2 for *Arthrobacter* nitrogenase protein made byGENO3D, 75.81% of the residues had an average 3D-1D score > 0.2 for *Arthrobacter* nitrogenase iron protein made by ModWeb.

3.4.4. PROVE for validating 3D model

PROVE calculated the volume of atoms in macromolecules using an algorithm which treated the atoms like hard spheres and considered a statistical Z score deviation for the model from highly resolved and refined PDB deposited structures. The Z score mean was 0.426 and the Z score RMS was 1.960 for *Arthrobacter* nitrogenase iron protein made by SWISS MODEL. The PROVE Z score mean was 0.750 and Z score RMS was 1.825 for *Arthrobacter* nitrogenase protein made by GENO3D. The PROVE Z score mean was 0.221 and Z score RMS was 1.577 for *Arthrobacter* nitrogenase iron protein made by ModWeb. The PROVEPLOT proved the three models were good and reliable models.

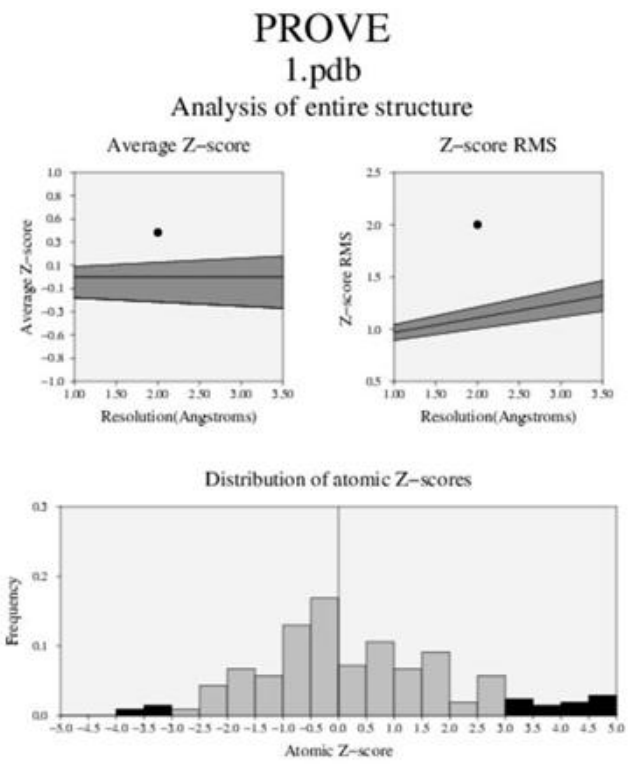
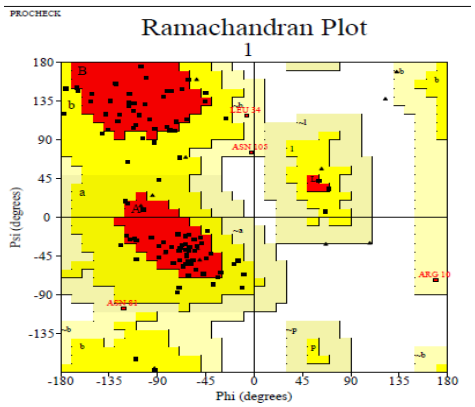


Figure 4: PROVE for validating 3D model from Swiss model server

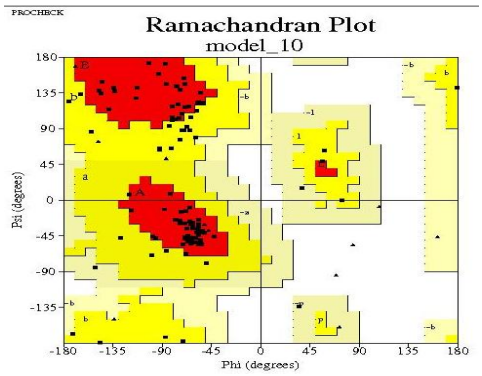
3.4.5. PROCHECK for validating 3D model

The stereo chemical quality of the predicted models and accurateness of the protein model was estimated after the refinement process using Ramachandran map calculations computed with PROCHECK [34] [35]. In the Ramachandran plot analysis, the residues were categorized according to its regions in the quadrangle. The red portions in the graph indicate the most allowed regions. Glycine is represented by triangles and other residues are represented in squares [35]. The result revealed that the modeled structure for SWISS MODEL nitrogenase iron protein for *Arthrobacter* was 71.2% residue in allowed region. The result revealed that the modeled structure for GENO3D nitrogenase iron protein for *Arthrobacter* had 73.1%, residue in allowed region. The result revealed that the modeled structure for Mod Web nitrogenase iron protein for *Arthrobacter* had 91.3%, residue respectively in allowed region. The distribution of the main chain bond lengths and bond angles were found confines within these proteins. The overall result of Ramachandran plot analysis by three modeling servers predicted that the *Arthrobacter* nitrogenase iron protein made by ModWeb was a very good, reliable model than other two models.

a) Modeled protein by SWISS MODEL



b) Modeled protein by GENO 3D



c) Modeled protein by ModWeb

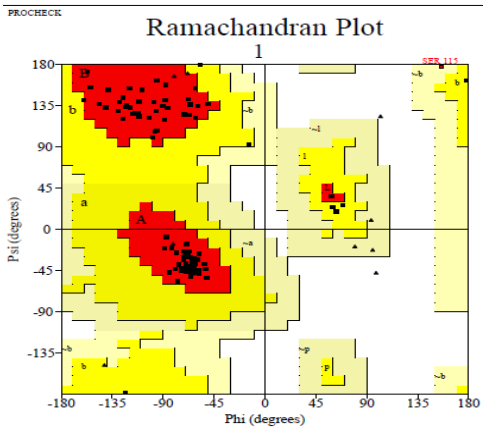


Figure 5: Ramachandran plot for nitrogenase iron protein by Swiss Model, Geno3D and Mod Web

Table 1: Ramachandran plot calculation and Comparative analysis of the models from GENO3D, Swiss model and ModWeb computed with the PROCHECK program

Server	Ramachandran plot	NifH
GENO3D	Residues in the most favoured region	73.1
	Residues in additionally allowed region	23.1
	Residues in generously allowed region	3.8
	Residues in disallowed region	0
Swiss model	Residues in the most favoured region	71.2
	Residues in additionally allowed region	25
	Residues in generously allowed region	3.8
	Residues in disallowed region	0
ModWeb	Residues in the most favoured region	91.3
	Residues in additionally allowed region	7.7
	Residues in generously allowed region	1
	Residues in disallowed region	0

3.5. Studying intrinsic dynamics of the protein models

3.5.1. WEBnm@

In normal mode analysis (NMA) first six modes matching with global rotation and translation of the system are generally ignored [9] [29] and hence lowest frequency mode of concern is the seventh one. Normal Mode Analysis of the NifH protein demonstrated that low deformation energies were associated with relatively rigid regions in the protein [9]. NMA indicated the vibrational and thermal properties of a protein at the atomic level. The nitrogenase iron protein from *Arthrobacter* had lowest deformation energies of 4122 in the seventh mode. It implied that the seventh mode with large rigid regions had a superior probability of describing domain motions.

Table 2: Deformation energies

Mode Index	Deformation energy
7	465.50
8	1027.22
9	1519.63
10	3001.61
11	3536.87
12	3675.51
13	5059.12
14	4122.00
15	6867.92
16	7096.73
17	7299.79
18	7119.50
19	8629.58
20	7784.94

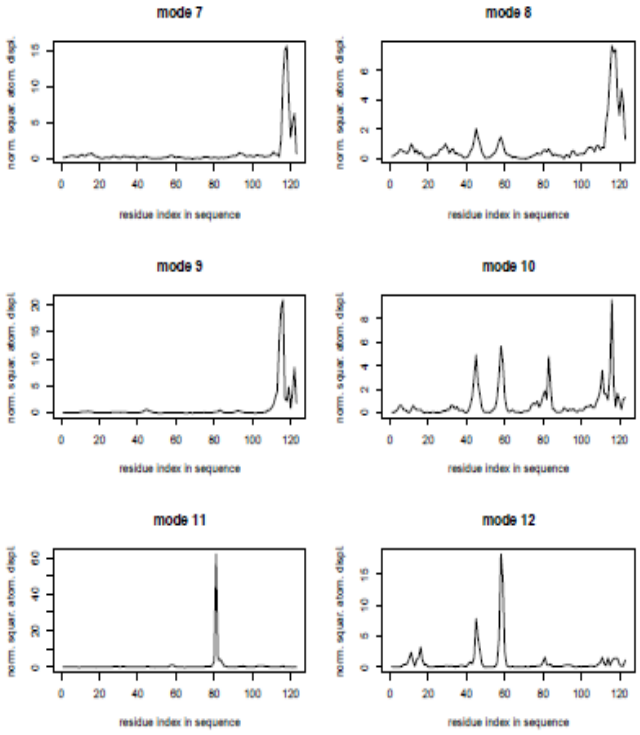


Figure 6: Deformation energies and Normalized atomic displacement plot

3.5.2. elNemo

B factors calculated from elNemo analysis were based on the first 100 normal modes and were scaled to match the overall B factors. The B factor has got very low negative correlations for the C-alpha atoms of the NifH proteins between the computed and observed B factors. It signifies that the models contain enough rigid regions and are less flexible. Figure 7 shows the plot of the normalized square atomic displacements calculated for modes 7 to 12. It represents the square of displacement of each C alpha atom, normalized as a result the sum over all residues is equal to 100. The highest values corresponded to the most displaced regions [14]. The correlation value was 0.325 for 123 C-alpha atoms.

3.6. Protein Quality Prediction

LG score predicts the quality of the model. LG score of *Arthrobacter* sp. was 4.318 and it was predicted as extremely good model.

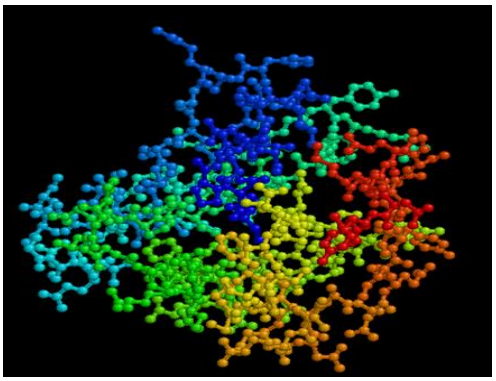


Figure 7: 3D structure of nitrogenase iron protein of *Arthrobacter* sp.

4. CONCLUSION

The 3D structure of the protein is very important to understand its molecular functions. Structural analysis of nitrogenase iron protein (NifH) was done using three molecular modeling programs. Ramachandran plot, minimized energy value, errat, verify 3D, Prove plot, prosa, webnm@, elnemo results revealed the rigidity and quality of the model. ProQ helped to optimize the protein model and to find correct models to find native structures. The postulations made in this model may be confirmed experimentally using X ray crystallography or Nuclear magnetic resonance spectroscopy for superior understanding of the biochemistry of *Arthrobacter* nitrogenase iron protein. Further conformational and computational analysis of nitrogenase iron protein, need to be studied to understand its functional mechanisms.

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