February 2013- April 2013, Vol. 3, No. 2, 1389-1394. e- ISSN: 2249 –1929

Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

*Available online at*www.jcbsc.org

Section C: Physical science

CODEN (USA): JCBPAT

Research Article

A Study on Bhageerath and Bhageerath-H Software Tools

Ch. Lakshmi Padma[1]*, Sreedhara R. Voleti[2], A. Ranganadha Reddy[1], R.K.Kishore[1]

[1]School of Biotechnology, Vignan University, Vadlamudi, Guntur, 522213, India

[2]Institute of Life Sciences, University of Hyderabad, Hyderabad, 500046, India

Received: 2 March 2013; **Revised:** 15 March 2013; **Accepted:** 20 March 2013;

Abstract: Generally, to know the structure of a protein X-ray crystallography or NMR techniques are followed but in some cases such as Family B GPCRs these techniques are always not successful. So, we opt for the homology modeling techniques. There are many softwares available in the market which are both free for access and to be paid for their usage, two of those free softwares available in the market are Bhageerath and Bhageerath-H. Study of these two softwares was presented in the paper. Computational tools of SCHRÖDINGER software were used for molecular dynamics studies.

Keywords: Bhageerath, Bhageerath-H, Homology modeling, SCHRÖDINGER, GPCRs

INTRODUCTION

Bhageerath: Bhageerath is an Ab initio protein structure prediction web interface server. It is developed by Scfbio team in India at IIT Delhi by Prof. B. Jayaram's group; the web server predicts five native-like candidate structures for the given protein's primary sequence[1]. Inspite of these advantage it has a limitation for the input sequence i.e., query's length should be less than 100 amino acids.

Bhageerath-H: Bhageerath-H is hybrid web interface server which makes use of both template and Ab initio calculations for the prediction of a three dimensional structure of a given proteins primary sequence. It is also developed by the Scfbio team of IIT Delhi. Bhageerath-H also predicts five native like structures for the query sequence. It does not have limitations for the input sequence, so any number of residues could be submitted to this software for structure predictions.

Bhageerath and Bhageerath-H are the only two softwares coming out of India, which predicts the protein folding of a given primary sequence. The study of these two softwares was incorporated into the project.

COMPUTATIONAL TOOLS

All calculations were carried out in Maestro v9.2 installed in Cadd-WS3 machine under 64-bit centos operating system placed in CADD department, Institute of Life Sciences. The machine was built up with:

A) 4 cores and 8 processers with Intel Xeon CPU E5620 @ 2.40GHZ
B) 16 GB RAM
C) Nvidia Quadro fx3800 Graphical Process Unit (GPU)

PROCEDURE

For the verification of Bhageerath-H, a part of N-terminus sequence of Glucagon like Peptide -1 Receptor (28-128 amino acids, which also have 3 disulfide bonds) was downloaded from uniprot[2] and submitted as input to Bhageerath-H. Five models were obtained as output and their RMSD[3] values with the query sequence (3IOL) were calculated.

For the verification of Bhageerath, a part of N-terminus sequence of Glucagon like Peptide -1 Receptor (38-127) was submitted as input. Five models were obtained and their RMSDs with respect to the query sequence were calculated. As the results were not found to be satisfactory, two more queries were submitted. One of the queries was from 156 to 248 amino acid residues of GLP-1R i.e., the end of first helix to the starting of third helix. Another query was from 78-104 amino acid residues i.e., the beta sheet part of the GLP-1R sequence was also submitted. The obtained results were analyzed.

RESULTS AND DISCUSSION

Bhageerath-H: Of all those five models, one model showed maximum homology that maintained all the disulfide bonds as shown in the Figure-1(A). RMSDs of the obtained models resulted from Bhageerath-H with the 3IOL[4] (partially crystallized N-term structure of GLP-1R) were calculated. It is with a great degree of confidence, all models were found to be within 0.2 Å from the template 3IOL as shown in Figure-1(B).

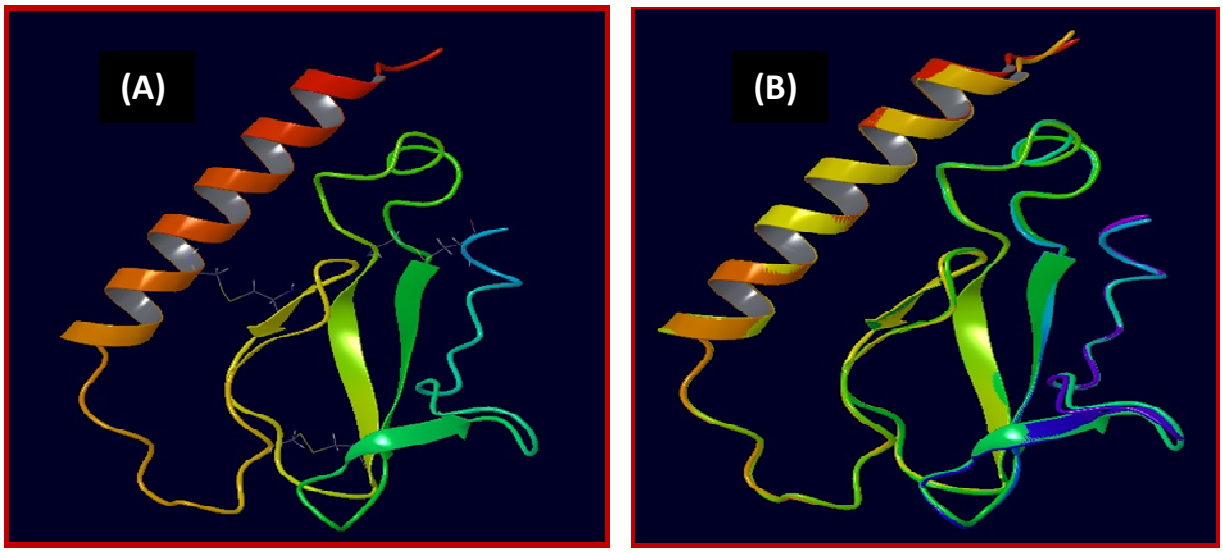


Figure-1: (A) The best model obtained from Bhageerath-H (B) Superposition of that model on 3IOL

It was also found that the disulfide bridges as expected were conserved as shown in Figure-6. Since Bhageerath-H uses template (in this case, 3IOL), the expected results turned out to be the predicted outcome.

Bhageerath: For the verification of Bhageerath software a 38-127 amino acid residues of 3IOL were submitted (38-127 instead of 28-128 due to the limitation of handling of number of residues in Bhageerath as input). Since Bhageerath doesn't use any template, the expected Ab initio protein folding from first principles would be less close to the crystal structure template, 3IOL. As observed, the obtained results (five models, as shown in Figure-2) are far from crystal structure, although some encouraging results bring confidence in use of Bhageerath.

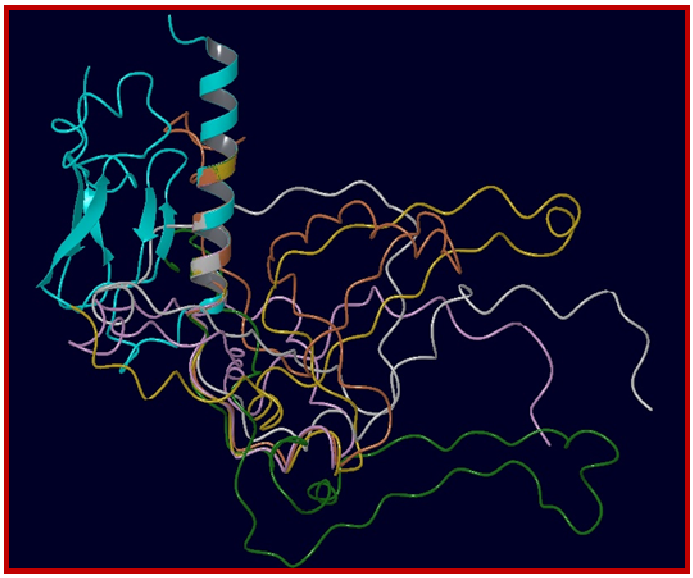


Figure-2: The superposition of all the Bhageerath results with 3IOL

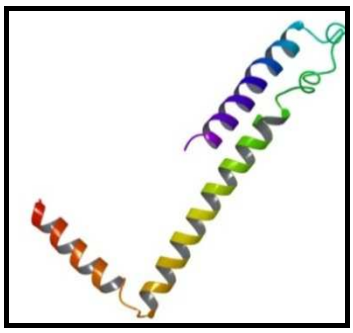
Table-1: The RMSDs of the structures obtained from Bhageerath with 3IOL

Models obtained from Bhageerath	RMSD (Å) with 3IOL
Model-1	18.7
Model-2	19.5
Model-3	17
Model-4	18.4
Model-5	18.5

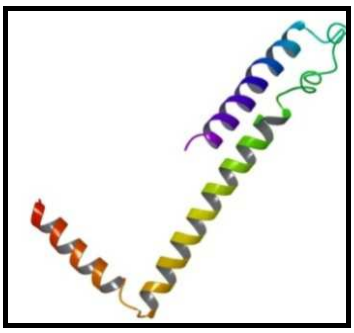
The resulted models did not maintain the disulfides which were expected to be maintained. Although, the RMSDs for predicted structures are far from the crystal structure, all models correctly predict the helical part of the sequence structure. To investigate further, we submitted a helix-loop-helix-loop-helix structure sequence of the same protein (GLP-1R) shown as below (Figure-3). To our surprise, predictions of tertiary structures were spot-on for the given primary sequence, although the models vary with respect to fold patterns giving us the current success of the Bhageerath in prediction of protein-structure folding.

Figure-3: The sequence of helix loop helix loop helix part of the TMs submitted to Bhageerath

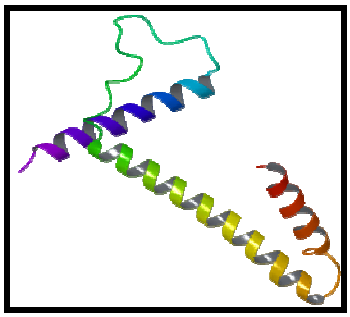
The 'helix-loop-helix-loop-helix' part of the given query was predicted pretty well. The results obtained are in good agreement with respect to RMS deviation as shown in the Figure-4 and Figure-5.



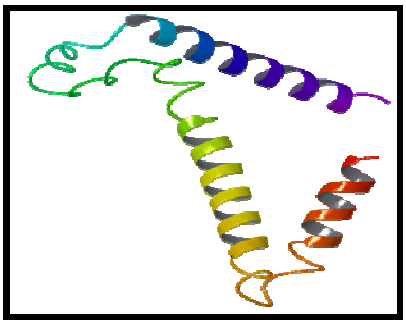
Model 1



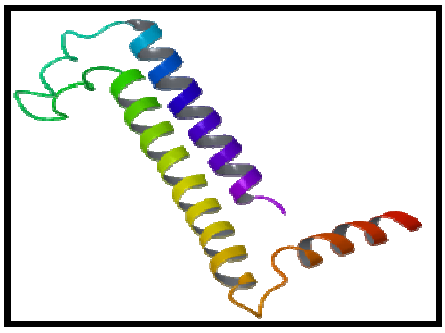
Model 2



Model 3



Model 4



Model 5

Figure- 4: Five models obtained from Bhageerath

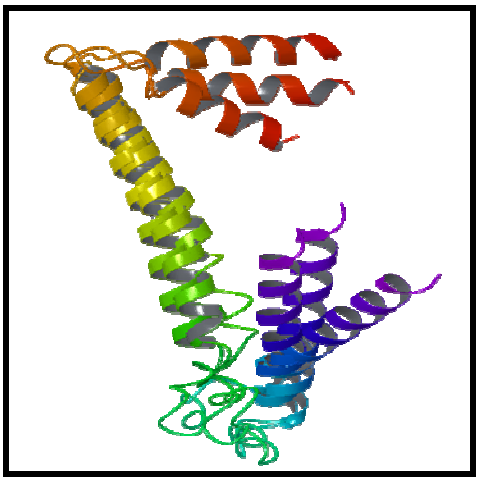


Figure-5: Superposition of obtained models

We then submitted few sequence lengths from crystal structure of 3IOL as β-sheets for a predictive fold. The sheet part of the N-terminus structure (27 amino acid sequence length i.e., from 78-104 amino acid residues) of the GLP-1R was submitted. The amino acid sequence that was submitted to Bhageerath is shown in Figure-6.

The sheet part present on the N-terminus of the GLP-1R was not predicted well by the Bhageerath software. The models obtained were like loops instead of sheets (Figure-7). With a hope, we expected whether molecular dynamics could convert these loops into sheets. Hence, a molecular dynamics of 10ns was implemented in MacroModel module of Schrodinger in gas phase with no constrains[5,6]. To our interest, we found that there is no change in the secondary structure from loops to sheet for the model.

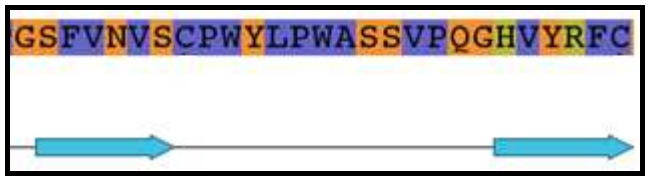
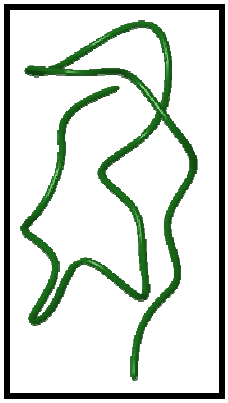
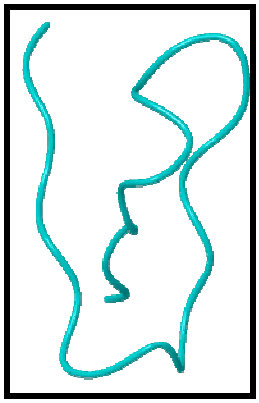


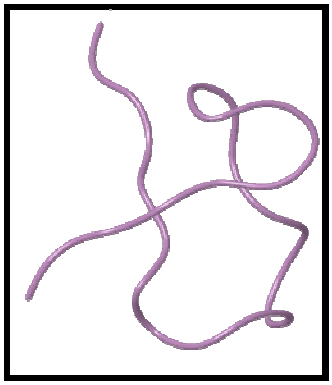
Figure-6: Sequence of 3IOL submitted for the sheet prediction in Bhageerath



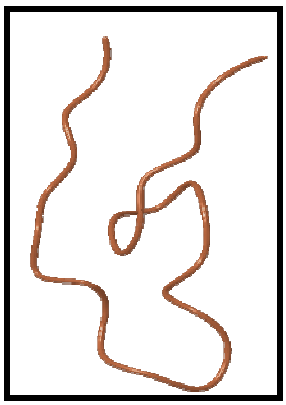
Model-1



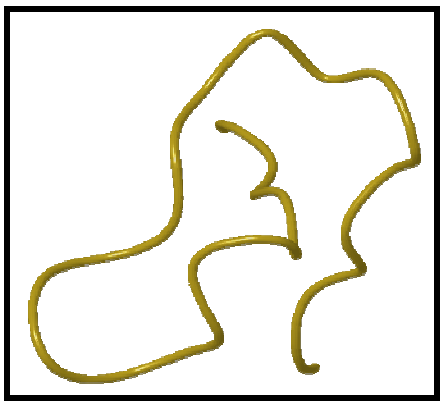
Model-2



Model-3



Model 4



Model 5

Figure-7: Five models obtained from Bhageerath protein structure prediction software

CONCLUSION

Bhageerath-H module predicted the homology model well, with the crystal structure template, while, the Bhageerath module predicted the structure well and the helix-loop-helix environments much more accurately. The need for improvement of the homology model lies on the evaluation of beta-sheet structures within the proteins.

REFERENCES

1. http://www.scfbio-iitd.res.in/bhageerath/bhageerath_h.jsp
2. http://www.uniprot.org/uniprot/P43220
3. Maestro 9.2 Command Reference Manual
4. http://www.rcsb.org/pdb/home/home.do
5. MacroModel, version 9.7, Schrödinger, LLC, New York, NY, 2009
6. MacroModel 9.9 User Manual, Schrödinger, LLC, New York, NY, 2009

Corresponding author: Ch. Lakshmi Padma; School of Biotechnology, Vignan University, Vadlamudi, Guntur, 522213, India