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Research Article

HOMOLOGY MODELLING AND STRUCTURAL ANALYSIS OF HER-2

Shailima RD Vardhini[1,2]*, Siddeshwari Ekke[2]
[1]Department of Biochemistry, St. Marys College, Hyderabad, Andhra Pradesh, India
[2]Celesta Research Lab, MBNR, Andhra Pradesh, India
*Corresponding Author Email: shailima.rampogu@gmail.com

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ABSTRACT
Homology modeling is an *in silico* technique which has an advantage of building the protein molecules. Her-2 is a protein which is considered has a validate drug target for Breast cancer. Besides Breast Cancer, its overexpression is significantly seen in colorectal cancers, prostate cancer, in the development of the human fetuses and many more. The present paper deals with the Homology Modeling of the Herstatin, a protein from Homo sapiens, its structural analysis and active site prediction. In this pursuit, the experiment proceeds *in silico.*
Keywords: HER-2, Homology Modelling, BLAST, SPDBV, Clustal X, Python, Modeller 9.12.

INTRODUCTION

Breast Cancer is one of the major causes of deaths in women and in most cases, the epidermal growth factor receptor-2 (HER-2) is over expressed[1-4]. The proliferation of the cancer cells is mediated by the signaling of the HER-2[5]. The epidermal growth factor receptor is an extracellular, transmembrane protein with tyrosine kinase domain[6,7]. In more than 45 % of the breast cancers, the detection is done by ELISA[8]. Besides its role in the development of Breast Cancer, the human epidermal growth receptor factor also plays a key role in the nervous system, muscle, skin, heart, lungs, intestinal epithelium and in the development of human fetuses[9,10]. The over expression of HER-2 is also seen in colorectal cancers, prostate cancers[11,12]. As HER-2 is present on the surface of the tumor cells, it is hence regarded as a validate drug target[13]. Earlier it was reported that the effected body compartments should be treated solely as biological units[14]. The protein functions are said by its structures[15]. Reports exists, proteins with the same structure and active sites performs different functions and proteins with different structures perform a similar kind of function[16,17]. Apart from having huge impact in the field of gene expression, prediction, experimental toxicology, personal health prediction, drug effects etc.[18], the homology modeling has a key role in the rational drug designing mechanism[19]. The homology modeling exhibits an advantage over the X-ray crystallographic method or the Nuclear Magnetic Resonance (NMR) by providing more accurate results and are also performed at less time and are cost effective[20]. The aim of the present experiment is to build the Homology Model of Her-2, its validation and structure analysis.

Methodology

Selection of the target protein:

The protein, whose structure is to be modeled, is selected from the swissprot/uniprot database. For the present investigation, HER-2 protein with the accession no. Q9UK79 was selected. It is a protein from Homosapien with the chain length of 419 amino acids. The protein and the gene name are Herstatin and HER-2 ERBB 2 hcg_ 28177. The sequence is downloaded and is saved on the notepad. Hereafter all the files and the information is to be saved on a folder.

Identification of similar sequences

The identification of the similar sequences was done using the Basic Local Alignment Search Tool (BLAST) an algorithm using which the biological information in the form of sequences is compared. The results showing the similar sequences are displayed.

Selection of the chain from the template

Knowing the BLAST results, the structure of the protein is to be loaded onto the SPDBV 4.10. Using this software, the chain with similarity has to be selected and the remaining chains are to be deleted. The sequence and the template layer are to be saved then.

Alignment

The alignment is done with the template sequence and the target sequence. Clustal X2 is used for this to accomplish. The alignment formats are generated in the folder with file names, ALN file, DND file and the PIR file. The PIR file then entered with the required information with regard to the amino acid chain length and the resolution. The file is saved in the ALI file format.

Protein modelling

Homology protein modeling of the target protein is performed with the Modeller 9.12 software. In its pursuance the file should be in the Python format. The Modeller (Python based) is run for the development of the structure and the structure is generated in the PBD format.

Validation

The validation of the protein structure is done by SAVS and RAMPAGE by analyzing the Ramachandran Plots.

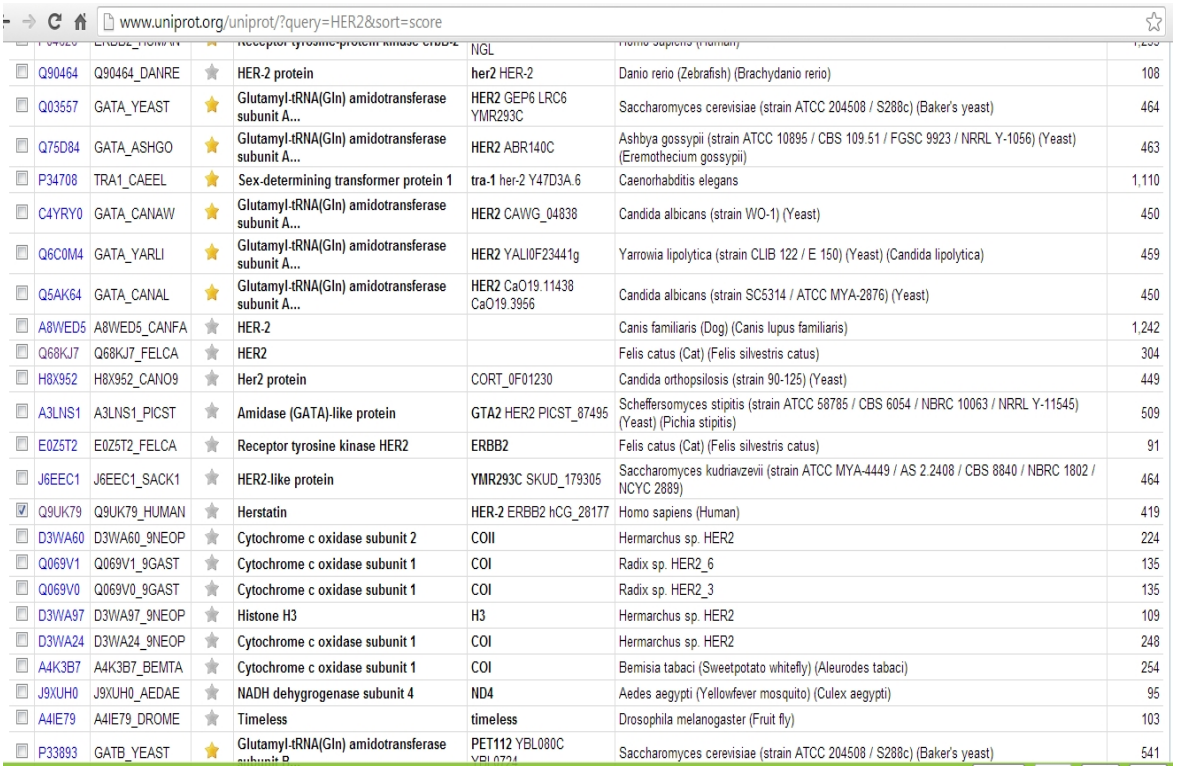
Structure analysis

The modeled protein structure was analyzed for the structural analysis. Further the structure of the protein was built and the amino acids present in the active site cavities were analyzed.

RESULTS AND DISCUSSION

Selection of the target protein

From the Swissprot database, the target protein is selected with the accession no. Q9UK79. The protein and the gene name are Herstatin and HER-2 ERBB 2 hcg_ 28177.



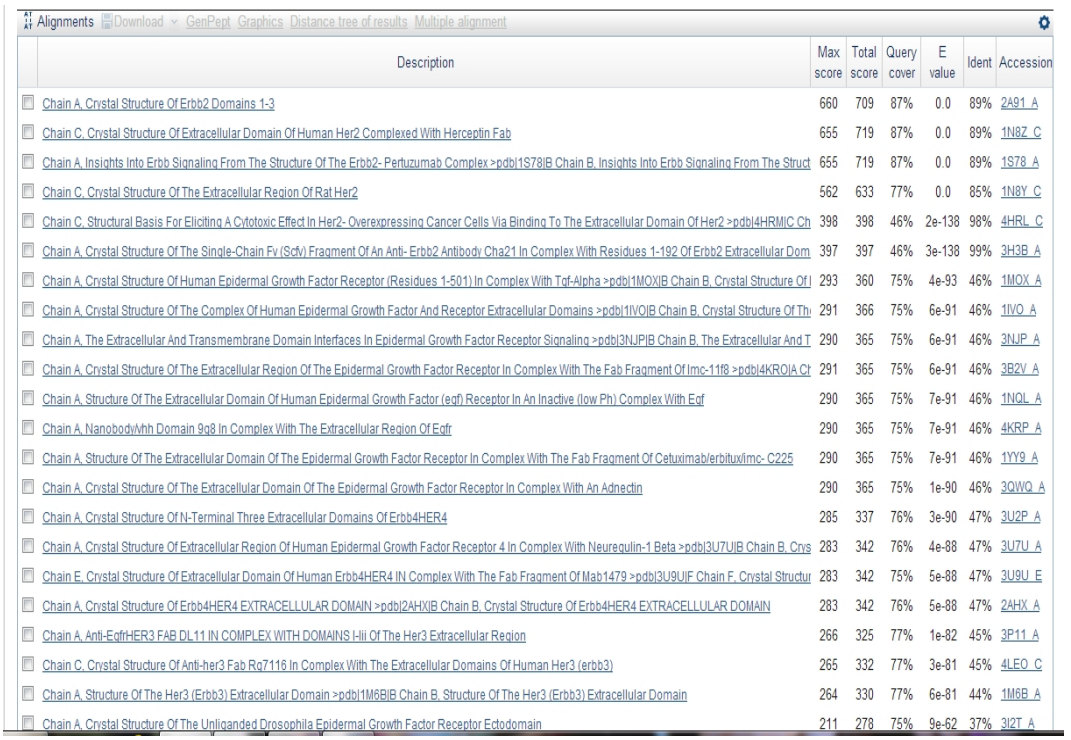
www.uniprot.org/uniprot/?query=HER2&sort=score

				NGL		
☐	Q90464	Q90464_DANRE	☆	**HER-2 protein**	**her2** HER-2	Danio rerio (Zebrafish) (Brachydanio rerio)	108
☐	Q03557	GATA_YEAST	★	**Glutamyl-tRNA(Gln) amidotransferase subunit A...**	**HER2** GEP6 LRC6 YMR293C	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	464
☐	Q75D84	GATA_ASHGO	★	**Glutamyl-tRNA(Gln) amidotransferase subunit A...**	**HER2** ABR140C	Ashbya gossypii (strain ATCC 10895 / CBS 109.51 / FGSC 9923 / NRRL Y-1056) (Yeast) (Eremothecium gossypii)	463
☐	P34708	TRA1_CAEEL	★	**Sex-determining transformer protein 1**	**tra-1** her-2 Y47D3A.6	Caenorhabditis elegans	1,110
☐	C4YRY0	GATA_CANAW	★	**Glutamyl-tRNA(Gln) amidotransferase subunit A...**	**HER2** CAWG_04838	Candida albicans (strain WO-1) (Yeast)	450
☐	Q6C0M4	GATA_YARLI	★	**Glutamyl-tRNA(Gln) amidotransferase subunit A...**	**HER2** YALI0F23441g	Yarrowia lipolytica (strain CLIB 122 / E 150) (Yeast) (Candida lipolytica)	459
☐	Q5AK64	GATA_CANAL	★	**Glutamyl-tRNA(Gln) amidotransferase subunit A...**	**HER2** CaO19.11438 CaO19.3956	Candida albicans (strain SC5314 / ATCC MYA-2876) (Yeast)	450
☐	A8WED5	A8WED5_CANFA	☆	**HER-2**		Canis familiaris (Dog) (Canis lupus familiaris)	1,242
☐	Q68KJ7	Q68KJ7_FELCA	☆	**HER2**		Felis catus (Cat) (Felis silvestris catus)	304
☐	H8X952	H8X952_CANO9	☆	**Her2 protein**	CORT_0F01230	Candida orthopsilosis (strain 90-125) (Yeast)	449
☐	A3LNS1	A3LNS1_PICST	☆	**Amidase (GATA)-like protein**	**GTA2** HER2 PICST_87495	Scheffersomyces stipitis (strain ATCC 58785 / CBS 6054 / NBRC 10063 / NRRL Y-11545) (Yeast) (Pichia stipitis)	509
☐	E0Z5T2	E0Z5T2_FELCA	☆	**Receptor tyrosine kinase HER2**	**ERBB2**	Felis catus (Cat) (Felis silvestris catus)	91
☐	J6EEC1	J6EEC1_SACK1	☆	**HER2-like protein**	**YMR293C** SKUD_179305	Saccharomyces kudriavzevii (strain ATCC MYA-4449 / AS 2.2408 / CBS 8840 / NBRC 1802 / NCYC 2889)	464
☑	Q9UK79	Q9UK79_HUMAN	☆	**Herstatin**	**HER-2** ERBB2 hCG_28177	Homo sapiens (Human)	419
☐	D3WA60	D3WA60_9NEOP	☆	**Cytochrome c oxidase subunit 2**	**COII**	Hermarchus sp. HER2	224
☐	Q069V1	Q069V1_9GAST	☆	**Cytochrome c oxidase subunit 1**	**COI**	Radix sp. HER2_6	135
☐	Q069V0	Q069V0_9GAST	☆	**Cytochrome c oxidase subunit 1**	**COI**	Radix sp. HER2_3	135
☐	D3WA97	D3WA97_9NEOP	☆	**Histone H3**	**H3**	Hermarchus sp. HER2	109
☐	D3WA24	D3WA24_9NEOP	☆	**Cytochrome c oxidase subunit 1**	**COI**	Hermarchus sp. HER2	248
☐	A4K3B7	A4K3B7_BEMTA	☆	**Cytochrome c oxidase subunit 1**	**COI**	Bemisia tabaci (Sweetpotato whitefly) (Aleurodes tabaci)	254
☐	J9XUH0	J9XUH0_AEDAE	☆	**NADH dehygrogenase subunit 4**	**ND4**	Aedes aegypti (Yellowfever mosquito) (Culex aegypti)	95
☐	A4IE79	A4IE79_DROME	☆	**Timeless**	**timeless**	Drosophila melanogaster (Fruit fly)	103
☐	P33893	GATB_YEAST	★	**Glutamyl-tRNA(Gln) amidotransferase subunit B**	**PET112** YBL080C YBL0724	Saccharomyces cerevisiae (strain ATCC 204508 / S288c) (Baker's yeast)	541

Figure 1: Target selection

Identification of similar sequences

The protein- protein BLAST has to be run with selecting the search set database as Protein Data Bank Proteins (PDB).



Alignments | Download | GenPept | Graphics | Distance tree of results | Multiple alignment

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Chain A, Crystal Structure Of Erbb2 Domains 1-3	660	709	87%	0.0	89%	2A91_A
☐	Chain C, Crystal Structure Of Extracellular Domain Of Human Her2 Complexed With Herceptin Fab	655	719	87%	0.0	89%	1N8Z_C
☐	Chain A, Insights Into Erbb Signaling From The Structure Of The Erbb2- Pertuzumab Complex >pdb|1S78|B Chain B, Insights Into Erbb Signaling From The Struct	655	719	87%	0.0	89%	1S78_A
☐	Chain C, Crystal Structure Of The Extracellular Region Of Rat Her2	562	633	77%	0.0	85%	1N8Y_C
☐	Chain C, Structural Basis For Eliciting A Cytotoxic Effect In Her2- Overexpressing Cancer Cells Via Binding To The Extracellular Domain Of Her2 >pdb|4HRM|C Ch	398	398	46%	2e-138	98%	4HRL_C
☐	Chain A, Crystal Structure Of The Single-Chain Fv (Scfv) Fragment Of An Anti- Erbb2 Antibody Cha21 In Complex With Residues 1-192 Of Erbb2 Extracellular Dom	397	397	46%	3e-138	99%	3H3B_A
☐	Chain A, Crystal Structure Of Human Epidermal Growth Factor Receptor (Residues 1-501) In Complex With Tgf-Alpha >pdb|1MOX|B Chain B, Crystal Structure Of I	293	360	75%	4e-93	46%	1MOX_A
☐	Chain A, Crystal Structure Of The Complex Of Human Epidermal Growth Factor And Receptor Extracellular Domains >pdb|1IVO|B Chain B, Crystal Structure Of Th	291	366	75%	6e-91	46%	1IVO_A
☐	Chain A, The Extracellular And Transmembrane Domain Interfaces In Epidermal Growth Factor Receptor Signaling >pdb|3NJP|B Chain B, The Extracellular And T	290	365	75%	6e-91	46%	3NJP_A
☐	Chain A, Crystal Structure Of The Extracellular Region Of The Epidermal Growth Factor Receptor In Complex With The Fab Fragment Of Imc-11f8 >pdb|4KRO|A Ch	291	365	75%	6e-91	46%	3B2V_A
☐	Chain A, Structure Of The Extracellular Domain Of Human Epidermal Growth Factor (egf) Receptor In An Inactive (low Ph) Complex With Egf	290	365	75%	7e-91	46%	1NQL_A
☐	Chain A, Nanobodyvhh Domain 9g8 In Complex With The Extracellular Region Of Egfr	290	365	75%	7e-91	46%	4KRP_A
☐	Chain A, Structure Of The Extracellular Domain Of The Epidermal Growth Factor Receptor In Complex With The Fab Fragment Of Cetuximab/erbitux/imc- C225	290	365	75%	7e-91	46%	1YY9_A
☐	Chain A, Crystal Structure Of The Extracellular Domain Of The Epidermal Growth Factor Receptor In Complex With An Adnectin	290	365	75%	1e-90	46%	3QWQ_A
☐	Chain A, Crystal Structure Of N-Terminal Three Extracellular Domains Of Erbb4HER4	285	337	76%	3e-90	47%	3U2P_A
☐	Chain A, Crystal Structure Of Extracellular Region Of Human Epidermal Growth Factor Receptor 4 In Complex With Neuregulin-1 Beta >pdb|3U7U|B Chain B, Crys	283	342	76%	4e-88	47%	3U7U_A
☐	Chain E, Crystal Structure Of Extracellular Domain Of Human Erbb4HER4 IN Complex With The Fab Fragment Of Mab1479 >pdb|3U9U|F Chain F, Crystal Structur	283	342	75%	5e-88	47%	3U9U_E
☐	Chain A, Crystal Structure Of Erbb4HER4 EXTRACELLULAR DOMAIN >pdb|2AHX|B Chain B, Crystal Structure Of Erbb4HER4 EXTRACELLULAR DOMAIN	283	342	76%	5e-88	47%	2AHX_A
☐	Chain A, Anti-EgfrHER3 FAB DL11 IN COMPLEX WITH DOMAINS I-Iii Of The Her3 Extracellular Region	266	325	77%	1e-82	45%	3P11_A
☐	Chain C, Crystal Structure Of Anti-her3 Fab Rg7116 In Complex With The Extracellular Domains Of Human Her3 (erbb3)	265	332	77%	3e-81	45%	4LEO_C
☐	Chain A, Structure Of The Her3 (Erbb3) Extracellular Domain >pdb|1M6B|B Chain B, Structure Of The Her3 (Erbb3) Extracellular Domain	264	330	77%	6e-81	44%	1M6B_A
☐	Chain A, Crystal Structure Of The Unliganded Drosophila Epidermal Growth Factor Receptor Ectodomain	211	278	75%	9e-62	37%	3I2T_A

Figure 2: BLAST Analysis

Based on the E- value and the highest identity %, the sequence producing the significant alignment is to be selected. From the BLAST search, the sequence with the Accession no. 2A91 A is selected and the similar chain is to be noted. In this case chain "A".

Selection of the chain from the template

Using the SPDBV software the structure and the sequence with similar identity to the target are identified and are saved on to a folder.

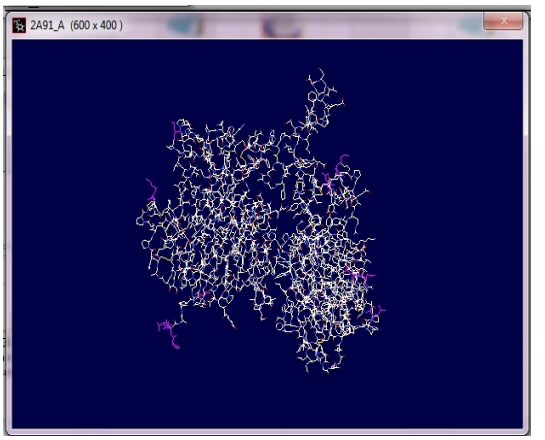


Figure 3: Similar chain A on SPDBV

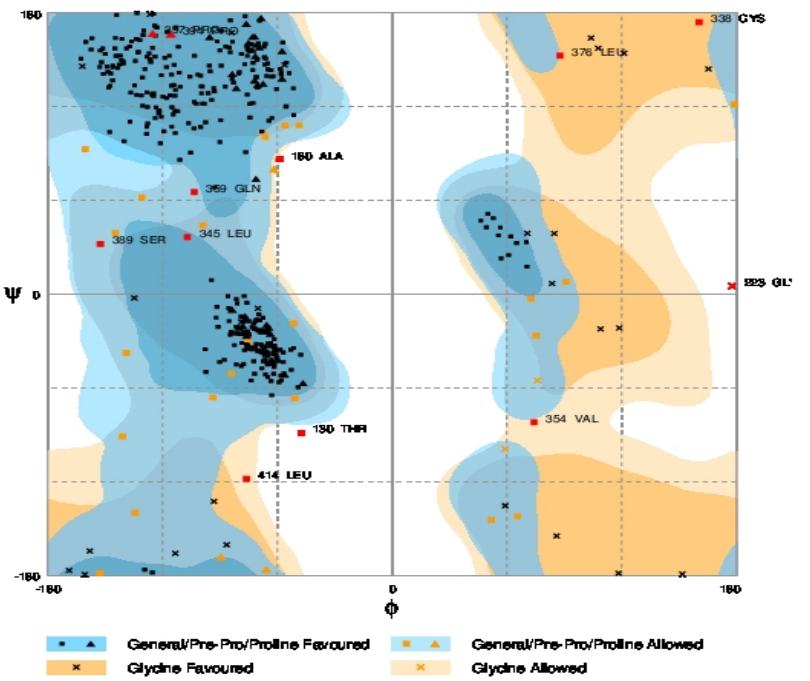
Alignment

The alignment of the target and the desired protein is performed and are saved into the folder with ALN and DND formats.

Validation

RAMPAGE[21] and Prochek were used to validate the proteins based on the Ramachandran Plot analysis.

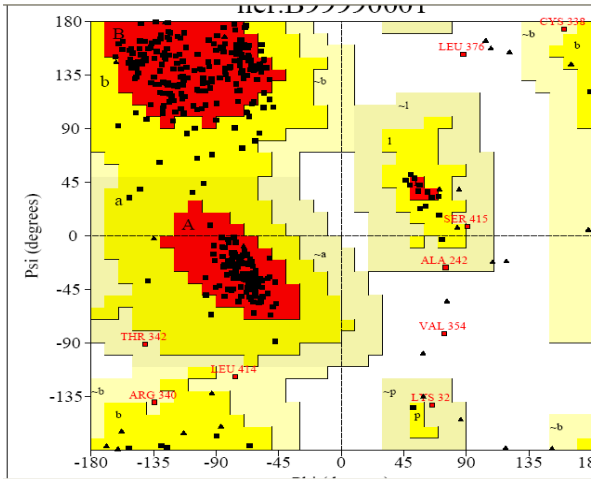
Rampage




```
Number of residues in favoured region     (~98.0% expected)  :  377 ( 90.4%)
Number of residues in allowed region      ( ~2.0% expected)  :   28 (  6.7%)
Number of residues in outlier region                         :   12 (  2.9%)
```

Figure 4: Ramachandran Plot

Procheck



Plot statistics

Residues in most favoured regions [A,B,L]	303	86.3%
Residues in additional allowed regions [a,b,l,p]	39	11.1%
Residues in generously allowed regions [~a,~b,~l,~p]	7	2.0%
Residues in disallowed regions	2	0.6%
Number of non-glycine and non-proline residues	351	100.0%
Number of end-residues (excl. Gly and Pro)	1	
Number of glycine residues (shown as triangles)	30	
Number of proline residues	37	
Total number of residues	419	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

Figure 5: Procheck Ramachandran Plot

Structure Analysis

The Modeller and cast P server[22] were used to identify the 3D structures and the active sites of the protein were predicted on the basis of highest Volume and largest area.

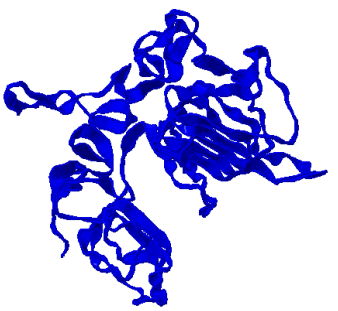


Figure 6: Modeled structure

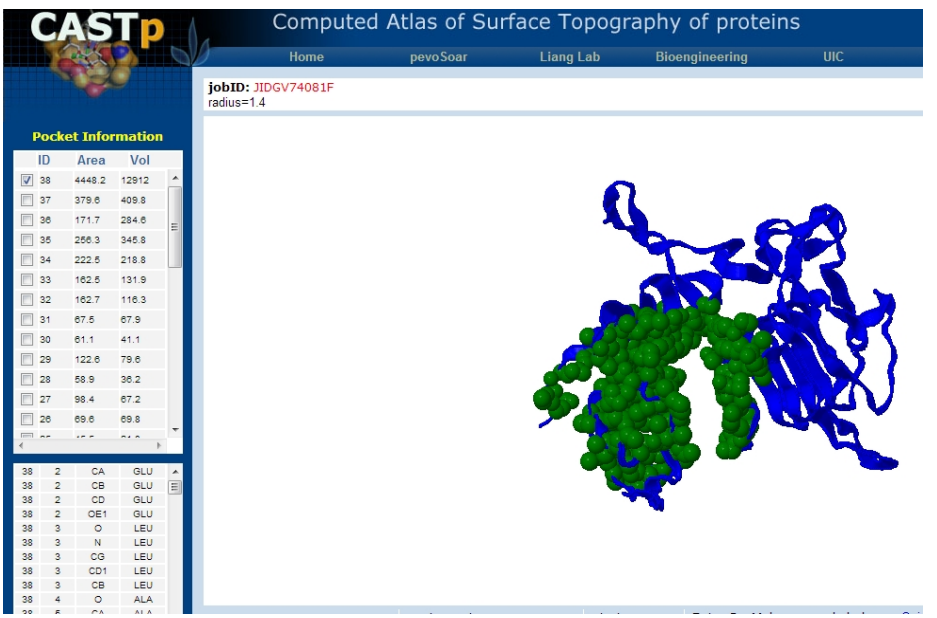


Figure 7: Modelled structure with 38 pocket information and the corresponding amino acids

CONCLUSION

The homology model of the Protein HER 2 was modeled using the Modeller software. The structure was predicted with the active site identification. The validation of the protein by Procheck and Rampage were promising. Hence this protein can be an alternative for the Researchers further studies.

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