Chapter 6

Identification of Active Sites and Calculation of Structural Parameters for the Her2 Target Receptor

K. Ramanathan

Abstract
The aim of this work is to identify the active sites and the calculations of structural parameters for the HER2 target receptor. The active site is the key factor where the ligand molecule can able to bind with the receptor. The stability of the interactions between the target receptor and the ligand are maintained by the structural parameters. HER2 receptor is associated with the breast cancer and it is the ideal target for the therapeutic activity. If the structural alterations occur in the HER2 it is failed to over expression and it leads to various types of tumours. The structural model could be constructive to recognize the hydrogen bonding and hydrophobic interactions with the ligand in the active sites of the receptor.

Keywords: Active sites, HER2 target receptor, structural parameters, ligand, hydrophobic interactions

Introduction

The active site is the region where the target protein is to be bind with the ligand and it consists of residues that form temporary bonds with the binding site and residues that catalyze a reaction. Identification of active sites is essential in the process of drug deigning. The three dimensional structure of the target protein is analyzed to identify active sites and design drugs where the ligand is to be bind with the receptor. HER2 breast cancers are aggressive and are associated with poor prognosis. The aim of this study was to develop the clinical grade Lu 177 trastuzumab and its preliminary evaluation for specific tumor targeting in HER2 positive breast cancer patients. Trastuzumab was conjugated to bifunctional chelator and characterized for integrity and the number of molecules conjugated and it has good antigen binding ability and specificity for HER2 receptor protein. **(Bhusari *et al.,* 2016).**

The fluorescence labeled therapeutic antibodies targeting human epidermal growth factor receptor (HER) family members and insulin-like growth factor-1 receptor (IGF1R) in combination with fluorescence imaging modalities to determine

Department of Bioinformatics, Thanthai Hans Roever College, Perambalur – 621 220, Tamil Nadu, India

S. Vignesh and A. Philip Arokiadoss (ed.), *Statistical Approaches on Multidisciplinary Research*, Volume I

tumor antigen expression, drug-target interaction, and biodistribution and tumor saturation kinetics. The tumor cell receptor status of HER1-3 and IGF1R, monitor the antibody target interaction and evaluate the receptor binding sites of anti-HER2-targeting antibodies. **(Dobosz *et al.,* 2016)**
The strength of binding between the receptor and the ligand in the active site plays a significant role in the process of drug designing **(Zuercher, 2008).** Active sites can be mapped to aid design of new drugs and this involves description of the size of an active site and the number and properties of sub sites such as details of the binding interaction. **(Powers, 2006)**.

Structural Parameters
The interactions that occur between the Carbon, Oxygen and amide groups on amino acids in a polypeptide chain to form alpha helix, beta sheets, turns, coils and other forms and that facilitate the folding into a three dimensional structure. The polypeptide chain of a protein forms a random coil. The alpha helix is the most abundant type of secondary structure in proteins. The alignment of the H bonds creates a dipole moment for the helix with a resulting partial positive charge at the amino end of the helix. Helices observed in the proteins can range from four to over forty residues long but a typical helix contains about ten amino acids.
The beta sheet is the second form of regular secondary structure in proteins. The higher level association of beta sheets has been implicated in formation of the protein aggregates and fibrils observed in many human diseases. The random coils show the alignment of monomer subunits with conformation. A region of secondary structure that is not an alpha helix and beta sheet or any recognizable turn is referred to as a coil.

Stability
In general, short polypeptides do not exhibit much alpha helical structure since he entropic cost associated with the folding of the polypeptide chain is not compensated for by a sufficient amount of stabilizing interactions. In general, the backbone hydrogen bonds of alpha helices are considered slightly weaker than those found in beta sheets and are readily attacked by the water molecules **(Hudgins *et al.,* 1999).**

HER 2 Receptor
The human epidermal growth factor receptor 2 (HER2) is a member of the epidermal growth factor receptor family and it plays a significant role in the development of many human adenocarcinomas. The HER2 extracellular domain is an ideal target for therapeutic approaches. The biochemical and structural analysis are to be obtained from the large quantities of active HER2 protein for detecting anti HER2 antibodies in serum **(Liu *et al.,* 2007).**
An over expression of this receptor plays a vital role in the development and progression of certain aggressive types of breast cancer. The HER2 target receptor has become an important biomarker and target of therapy for breast cancer patients **(Mitri *et al.,* 2012)**. HER2 has a similar structure to human epidermal growth factor receptor and an over expression of the receptor leads to breast cancers which is strongly associated with increased disease recurrence and a poor prognosis. Over expression is also known to occur in ovarian, stomach, and aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma **(Tan *et al.,* 2007).**

Variations in the HER2

The structural alterations in the HER2 cause ligand independent firing in the absence of over expression and it is found in a variety of tumors. Some of these tumors carry point mutations in the sequence specifying the transmembrane domain of HER2. Substitution of a valine for a glutamic acid in the transmembrane domain can result in the constitutive dimerization of this protein in the absence of a ligand. HER2 mutations have been found in non small cell lung cancers and can direct treatment **(Mazières *et al.,* 2003)**

Drugs targeting with HER2

A major goal of personalized medicine in oncology is the identification of drugs with predictable efficacy based on a specific trait of the cancer cell has been demonstrated with herceptin HER2 over expression. It requires identifying a cellular component that is altered in cancer but not normal cells and discovering a compound that specifically interacts with it **(Parkinson *et al.,* 2013)**

HER2 is the target of the monoclonal antibody trastuzumab marketed as Herceptin and it is effective only where HER2 is over expressed. One year of trastuzumab therapy is recommended for all patients with HER positive breast cancer who are also receiving chemotherapy **(Mates *et al.,* 2015)** An important downstream effect of trastuzumab binding to HER2 is an increase in p27, a protein that halts cell proliferation **(Le *et al.,* 2005)**. Another monoclonal antibody Pertuzumab which inhibits dimerization of HER2 and HER3 receptors was approved by the FDA for use in combination with trastuzumab.

Methodology

The target receptor HER2 was studied and the sequence was retrieved from the database. The identification of active sites for the target receptor was calculated using prosite tool. The location of the active sites was also identified where the ligand is to be bind with the target protein. The structural parameters were calculated and the compositions of the parameters were predicted by GOR tool. The sequence for the target protein was retrieved and the structural models were predicted by Swiss model tool.

Results

Identification of Active sites

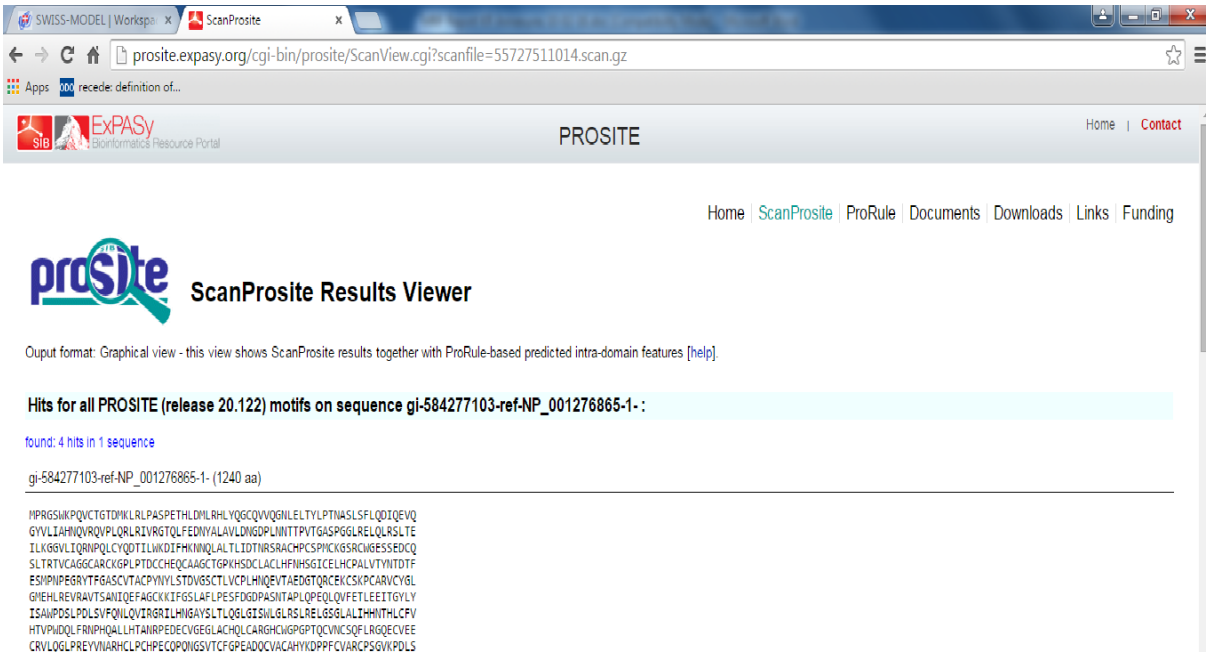


Fig 6.1: HER2 sequence submitted in to Prosite tool

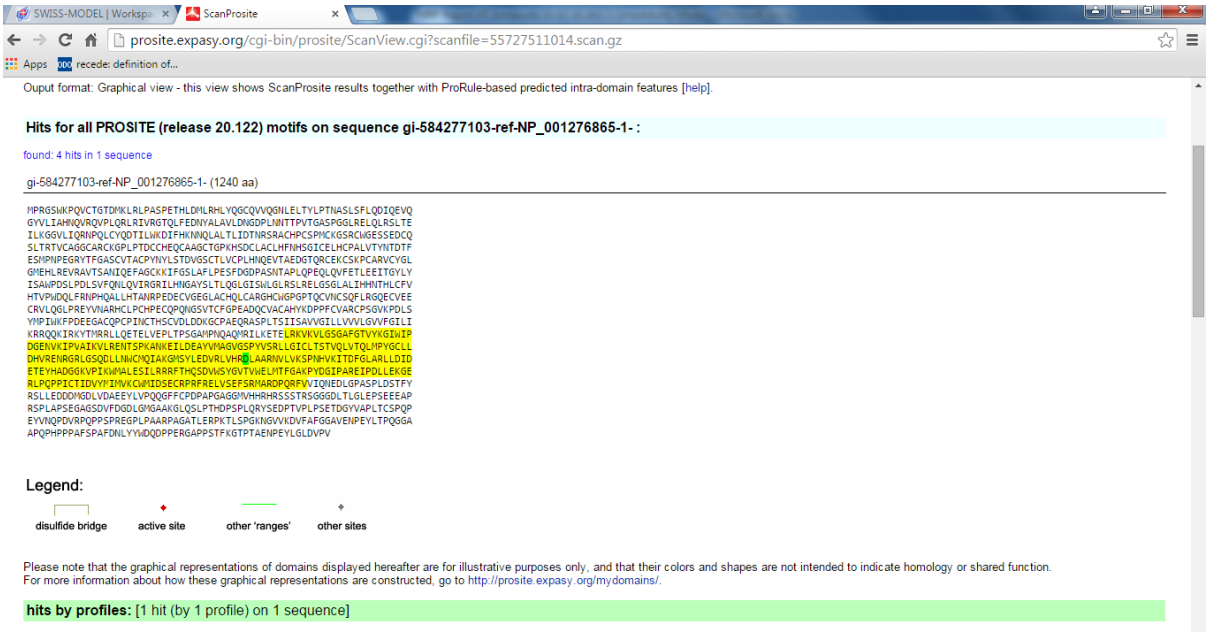


Fig 6.2: Identification of Active Sites for the HER2 target Protein

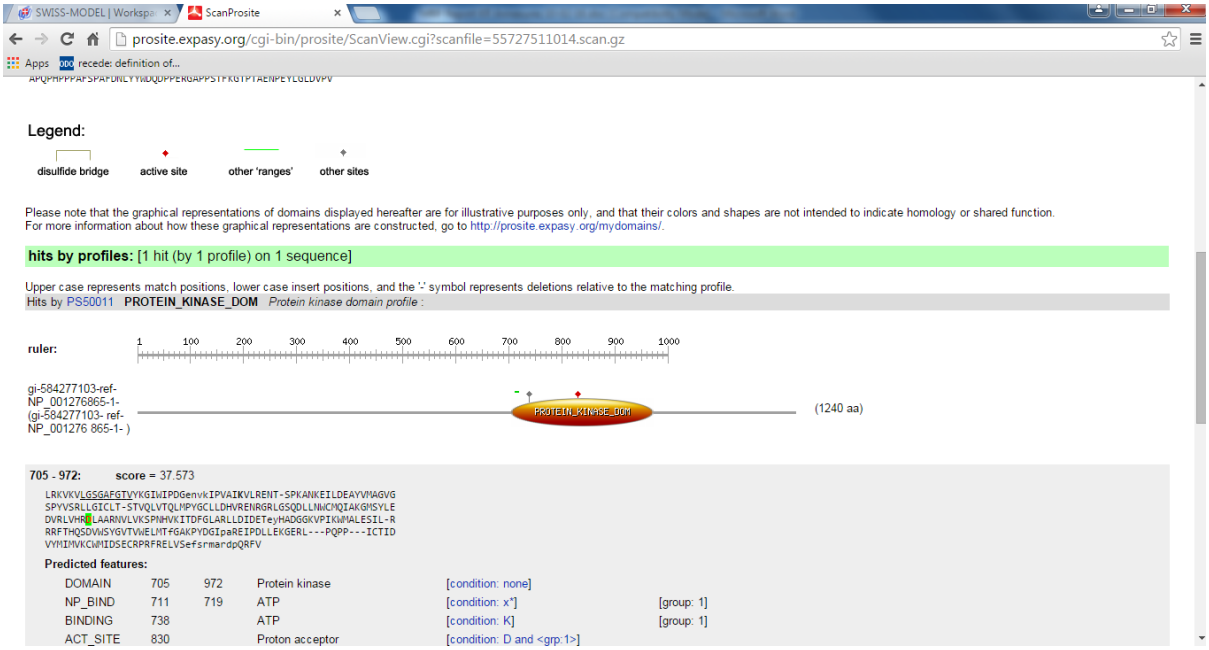


Fig 6.3: Four Active sites in the HER2 Protein, First active site present from the position 705 to 972

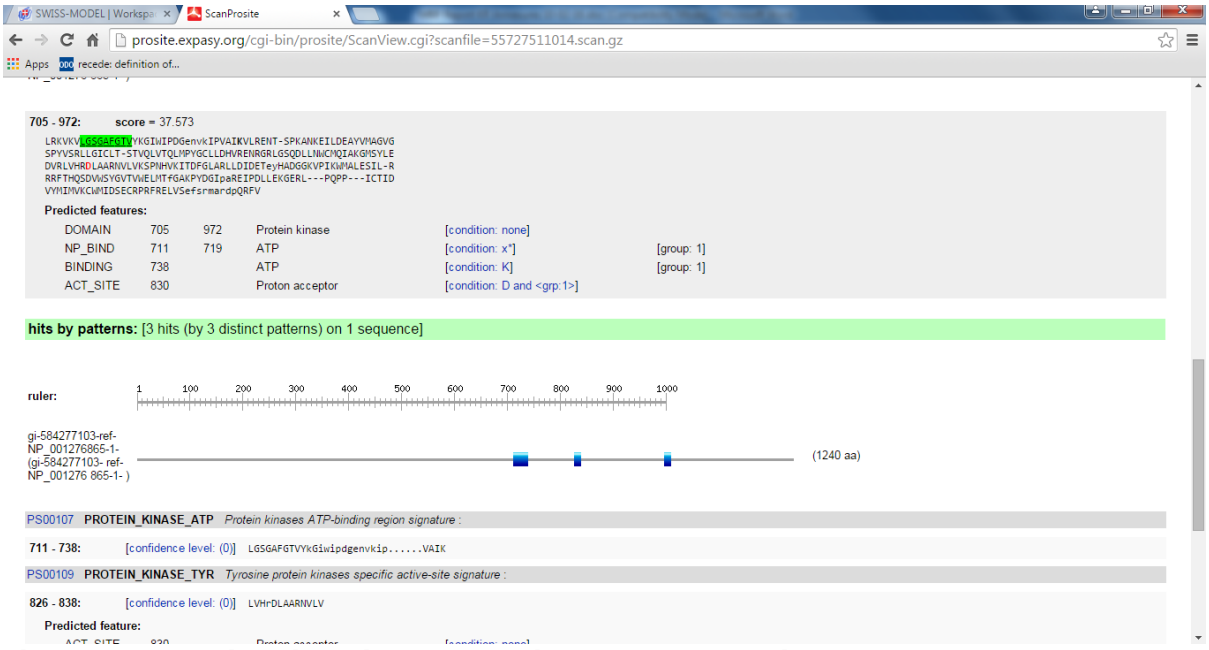


Fig 6.4: Second active site present between 711 and 738

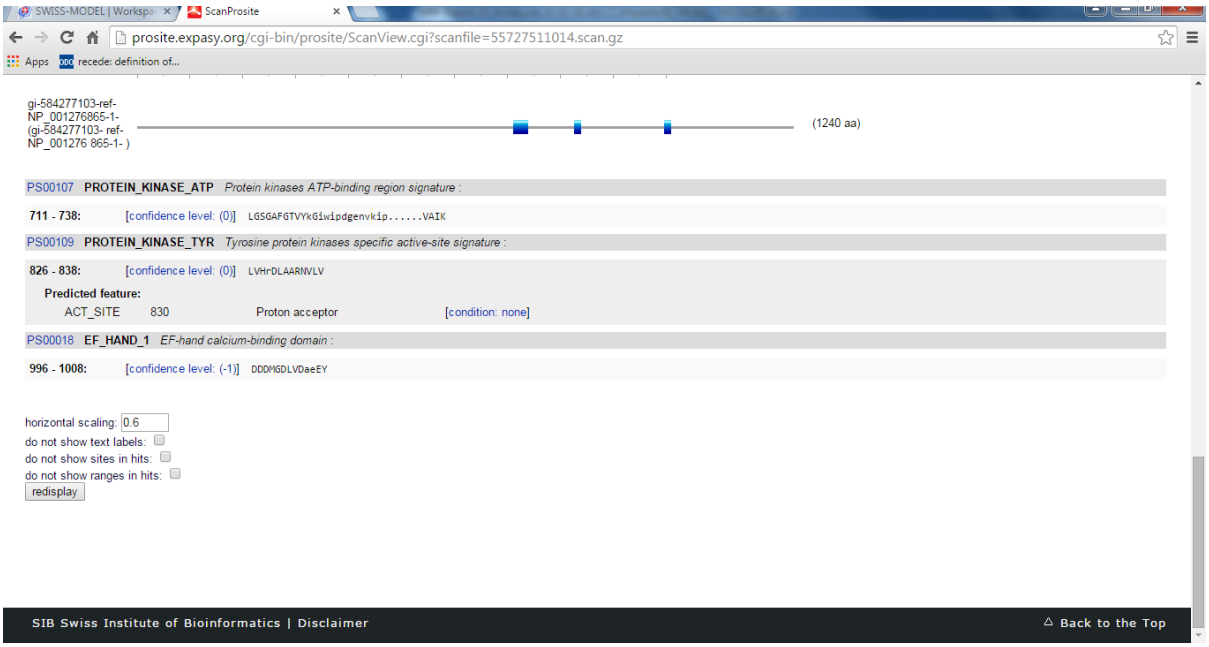


Fig 6.5: Third active site present from 826 to 838 and fourth active site falls present from 996 to 1008

Calculation of Structural parameters

Fig 6.6: HER2 Target Protein submitted in to GOR tool

Fig 6.7: Secondary structural parameters for the HER2 protein

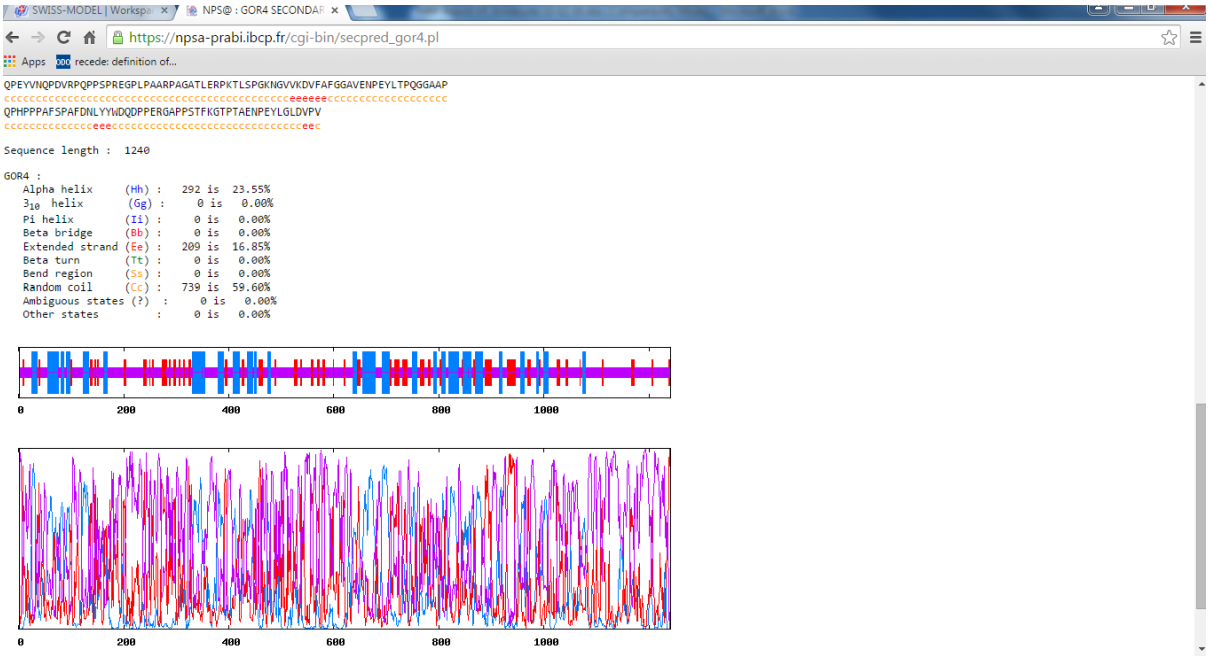
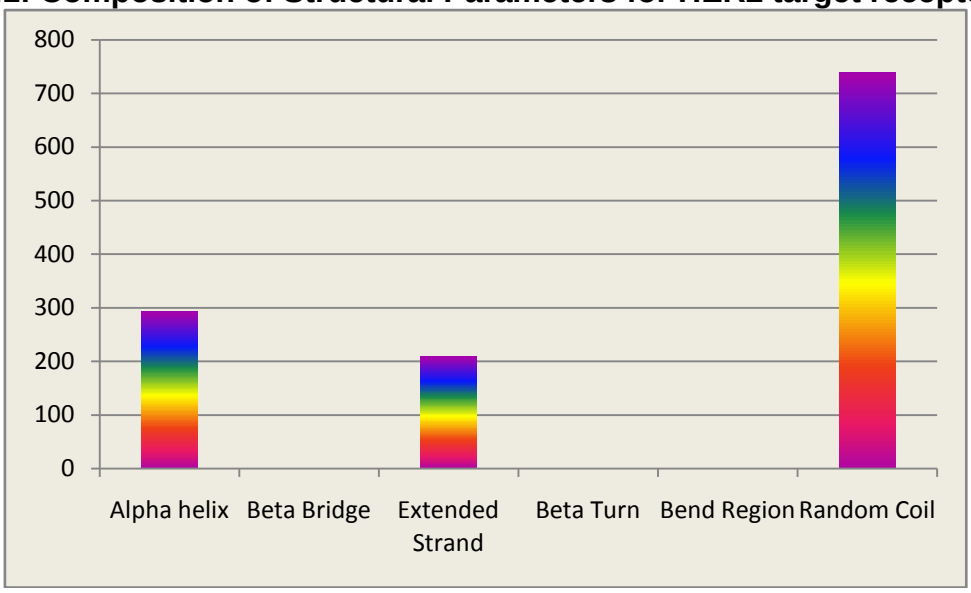


Fig 6.8: Composition of structural parameters in HER2 target protein

Structural Parameters	Composition
Alpha helix	292
Beta Bridge	0
Extended Strand	209
Beta Turn	0
Bend Region	0
Random Coil	739

Table 6.1: Composition of Structural Parameters for HER2 target receptor



Graph 6.1: Comparison of Structural Parameters for HER2 target receptor

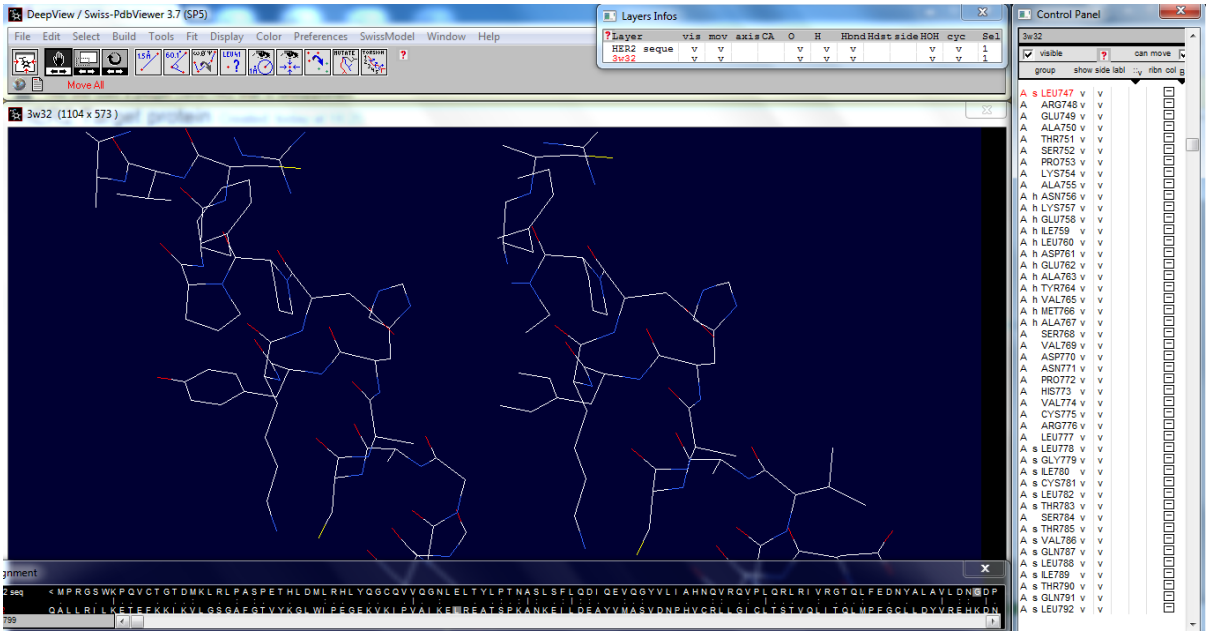


Fig 6.9: Residue analysis in the target protein HER2

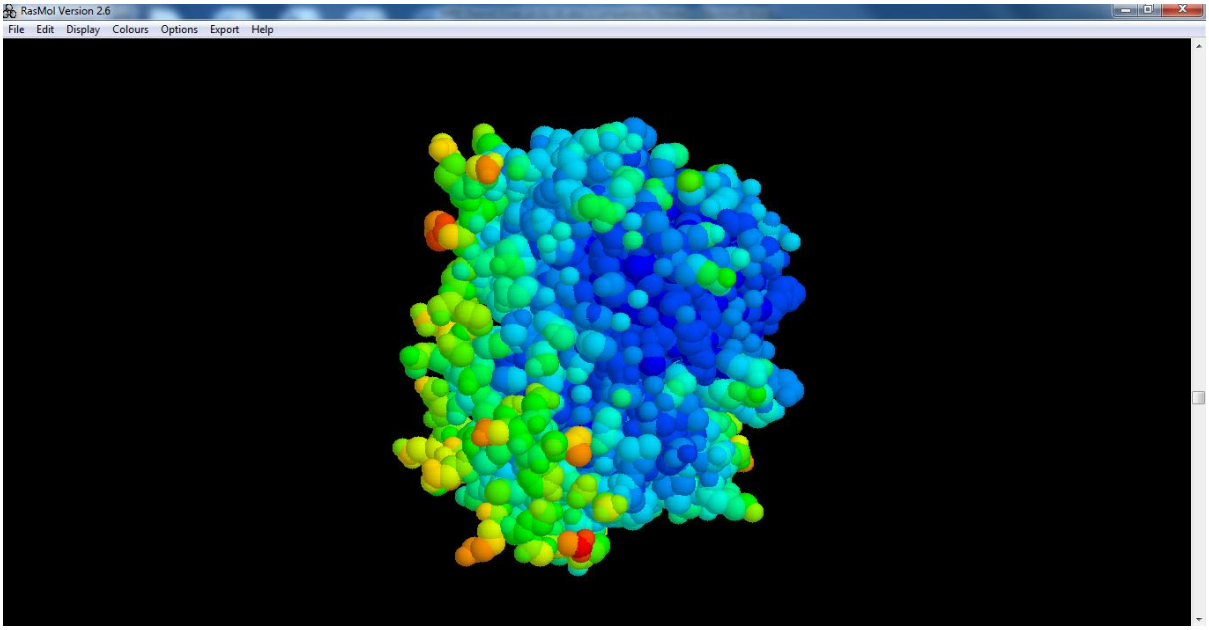


Fig 6.10: Structure Visualization for the target Protein HER2

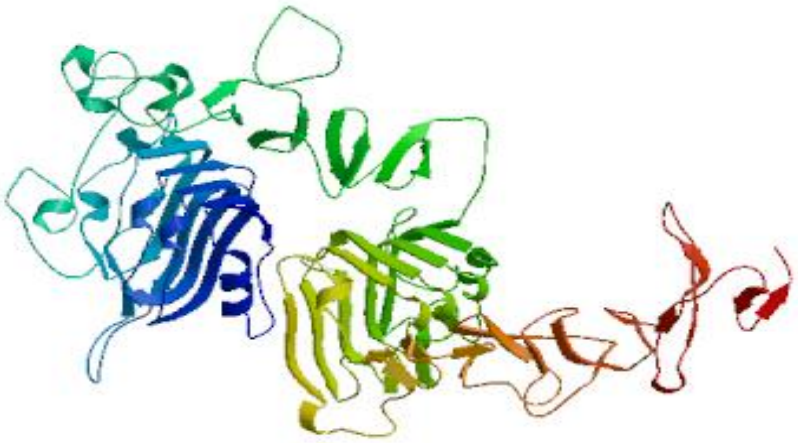


Fig 6.11: Structural model for the target protein HER2

Discussion

The target receptor was identified and the sequence was retrieved from the database and submitted in to prosite tool (Fig 6.1). The active sites were identified for the HER2 target protein in the Fig 6.2. Fig 6.3 shows that the four active site

regions present in the target protein and the first active site is present between the positions 705-972. The position of the second active is present between 711-738 represented in the Fig 6.4. The third and fourth active sites are present between the positions 826-838 and 996-1008 respectively where the ligand can bind with the target receptor (Fig 6.5). The sequence for the target receptor was submitted in to GOR tool (Fig 6.6). The total sequence length of the HER2 target receptor is 1240 and the structural parameters were calculated (Fig 6.7). Fig 6.8 shows that the composition of the alpha helix in the target protein is 292 (23.55%) and there are 209 extended strands (16.85%) with 739 random coils (59.60%) present in the target protein. The composition of random coils is very high in the target protein when compared with other structural parameters. The residues present in the target protein were analyzed and their location of the residues was identified. The residues present in the target protein were identified in Fig 6.9. The structure of the target protein was visualized in the space fill model Fig 6.10. The Structural model was predicted for the target Protein HER2 using Homology Modeling represented in the Fig 11.

Conclusion

The sequence for the HER2 target receptor associated with the breast cancer was retrieved from the database and the active sites were predicted by prosite tool. The four active site regions with their locations were identified in the HER2 target receptor. The ligand molecule can simply bind with the target receptor through the active sites. The compositions of the structural parameters were predicted for the target receptor. The highest composition of the random coils (739) is present in the HER2 target receptor. From these results, I observed that the HER2 target receptor shows a good binding affinity with various ligands because of four binding site regions and the highest composition of random coils present in the target receptor. . The Structural model was predicted and it could be possible to understand the study of hydrogen bonding and hydrophobic interactions with key residues in the active sites of the target receptor.

Acknowledgement

I express my sincere thanks to University Grants Commission (UGC) for providing financial assistance (Minor Research Project 2015 - 2017) to carry out this research and also extend my thanks to Thanthai Hans Roever College (Autonomous), Perambalur for providing necessary facilities.

References

Bhusari P, Vatsa R, Singh G, Parmar M, Bal A, Dhawan DK, Mittal BR, Shukla J. (2016) Development of Lu-177-trastuzumab for radioimmunotherapy of HER2 expressing breast cancer and its feasibility assessment in breast cancer patients. Int J Cancer. 15; 140(4):938-947.

Dobosz M, Haupt U, Scheuer W. (2016) Improved decision making for prioritizing tumor targeting antibodies in human xenografts: Utility of fluorescence imaging to verify tumor target expression, antibody binding and optimization of dosage and application schedule. MAbs. Sep 23:1-14.

Zuercher M (2008). "Structure-Based Drug Design: Exploring the Proper Filling of Apolar Pockets at Enzyme Active Sites". Journal of Organic Chemistry 73: 4345–4361.

Powers R (2006). "Comparison of protein active site structures for functional annotation of proteins and drug design". Proteins: Structure, Function, and Bioinformatics 65: 124–135.

Hudgins, RR; Jarrold MF (1999). "Helix Formation in Unsolvated Alanine-Based Peptides: Helical Monomers and Helical Dimers". Journal of the American Chemical Society 121 (14): 3494–3501.

Liu X, He Z, Zhou M, Yang F, Lv H, Yu Y, Chen Z. Purification and characterization of recombinant extracellular domain of human HER2 from Escherichia coli Protein Expr Purif. 2007 Jun; 53(2):247-54.

Mitri Z, Constantine T, O'Regan R (2012). "The HER2 Receptor in Breast Cancer: Pathophysiology, Clinical Use, and New Advances in Therapy". Chemotherapy Research and Practice 2012: 743193.

Tan M, Yu D (2007). "Molecular mechanisms of erbB2-mediated breast cancer chemoresistance". Advances in Experimental Medicine and Biology 608: 119–29.

Mazières J, Peters S, Lepage B, Cortot AB, Barlesi F, Beau-Faller M, Besse B, Blons H, Mansuet-Lupo A, Urban T, Moro-Sibilot D, Dansin E, Chouaid C,Wislez M, Diebold J, Felip E, Rouquette I, Milia JD, Gautschi O. (2013) Lung cancer that harbors an HER2 mutation: epidemiologic characteristics and therapeutic perspectives. J Clin Oncol. 1; 31(16):1997-2003.

Parkinson EI, Bair JS, Cismesia M, Hergenrother PJ. (2013) Efficient NQO1 substrates are potent and selective anticancer agents.ACS Chem Biol. 18;8 (10):2173-83.

Mates M, Fletcher GG, Freedman OC, Eisen A, Gandhi S, Trudeau ME, Dent SF (2015). "Systemic targeted therapy for her2-positive early female breast cancer: a systematic review of the evidence for the 2014 Cancer Care Ontario systemic therapy guideline". Current Oncology 22 (Suppl 1): S114–22.

Le XF, Pruefer F, Bast RC (2005). "HER2-targeting antibodies modulate the cyclin-dependent kinase inhibitor p27Kip1 via multiple signaling pathways". Cell Cycle 4 (1): 87–95.