query cover to the query sequence. The program generated 18 different models for both wild type and mutant out of which one was selected for each category and the results were analyzed on the basis of dope energy. Less the energy, better stability to the structure. The dope energy after minimization for the wild type came out to be -6871.8828 and for mutant type came out to be -8847.7402.Then both the structures were further submitted for refinement which was done through Rosetta server.

3.3 Structural validation

The generated structure by homology modelling was analyzed using the SAVES server "Structural Analysis and Verification Server" (www.nihserver.mbi.ucla.edu)[25]. (**Figure2**). Procheck tool was used. PROCHECK checks the stereo chemical quality of a protein structure by analyzing residue-by-residue geometry and overall structural geometry**.** Errat analyzes the statistics of non-bonded interactions between different atom types and plots the value of the error function versus position of a 9-residue sliding window, calculated by a comparison with statistics from highly refined structures. Also ProSA was used which calculated z scores and the local alignment of the structure (**Figure3**).The green speck in between is the 81st location containing the cysteine residue in the wild type Hrt1p **(Figure4**).

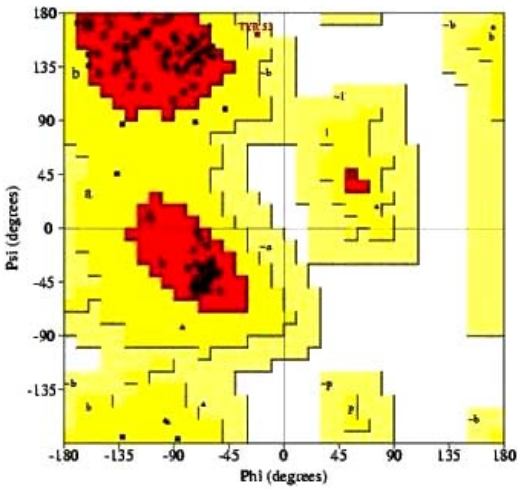


Figure 2(A): Ramachandran plot for wild type Hrt1p showed: - 91.6% core region, 7.5% allow and 0.0% disallowed region.

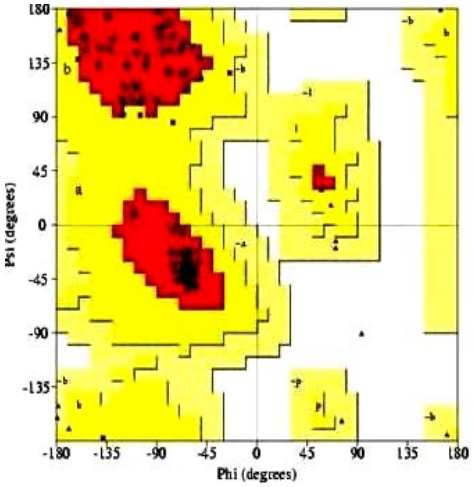


Figure 2(B) Ramachandran plot for mutant Hrt1p 93.1% core region, 6.9% allow and 0.0% disallowed region, Errat value – 91.5.

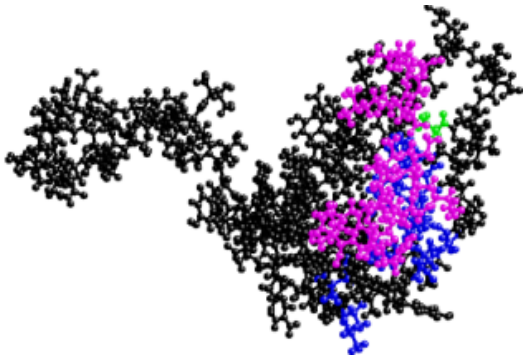
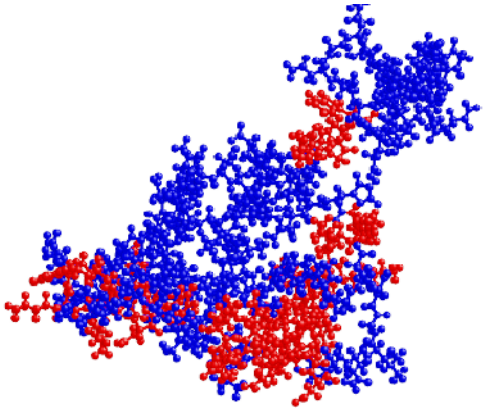


Figure 3: 3D structures of a) wild type Hrt1p. The green spec represents 81st position where C81Y change carried out.



(b) 3D structure of mutant Hrt1p. The red region shows the helical region.

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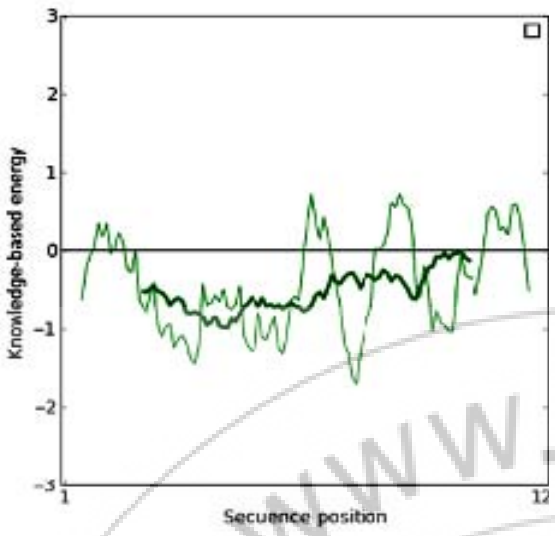
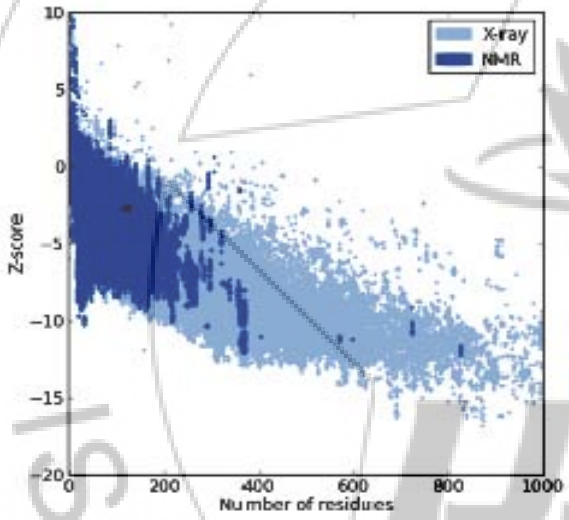


Figure 4 (a) ProSA analysis of mutant Hrt1p, showed graph between knowledge based energy and the energy of our structure. A positive value depicts errors and negative values are relevant.



(b) Z score based on X ray and NMR data. Z score represents the accuracy of the structure of proteins.

4. Conclusion

In this study the 3D structure of both wild type and mutant (C81Y) Hrt1p was generated using modeller. The template taken was same for both the structure queries. The template taken was of Cullin Ring Ligase which is also an important complex of SCF complex. SCF complex consists of 4 Subunits; Skp1, Cullin, F box Protein and Hrt1. Skp1 forms part of the horseshoe-shaped complex, along with Cullin (cul1). Skp1 is essential in the recognition and binding of the F-box. The cullin forms the major structural scaffold of the SCF complex, linking the Skp1 domain with the Rbx1/Hrt1 domain. Hrt1p contains a small zinc-binding domain called the RING Finger, to which the E2-ubiquitin conjugate binds, allowing the transferral of the ubiquitin to a lysine residue on the target protein.

Hrt1p forms an important interactive partner as it contains the ring finger domain (zinc binding), to which the E2 ubiquitin conjugate binds, allowing transfer of Ubiquitin to a lysine residue of target protein. On visualizing the structure of wild type it was found that majority of secondary structure was composed of helix followed by sheets. It was observed that it exhibited an open structure which has active residues from 2-91 position and the mutation was carried out on position 81, replacing C with Y. In case of wild type both helical atoms and sheet were present but in case of mutant, it was observed that the structure got compacted and the number of helical atoms showed an increase while the sheet atoms were reduced to zero.

It was observed that there was a major difference between the 3D structures of the Hrt1p and Hrt1p. The wild type initially consisted of 251 helical atoms and 188 sheet atoms labelling it as 50% helical and other part consisting of sheets and turns. On the other hand, the mutant type was found to have 597 helical atoms, almost double of the previous wild type. Another major change observed was the lack of beta sheets suggesting that during the conversion from wild type to mutant, all the sheets got converted into helical state owing to the compacted structure of the molecule. Also the amino acids involved in the substitution played a major role in the outcome. As cysteine is involved in thioester bond formation it forms a conjugate with cofactor. In case of Hrt1p a zinc binding domain called ring finger was involved in binding with E2. Thus on substitution, this interactions might get affected leading to the loss of ubiquitination activity. It might be said that the structure showed transition from an open to closed state. It can be hypothesized that tyrosine being hydrophobic tends to lie on the inner side, thereby leading to compact nature of the mutant protein.

5. Future Scope of study

The present study with the Hrt1p which is functionally conserved from yeast to human has potential to develop the inhibitors against it for controlling the activity of this molecule.

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