https://doi.org/ 10.33472/AFJBS.6.5.2024.183-194

African Journal of Biological Sciences

Homology Modeling and DockingInvestigations of Polyglutamine (PolyQ) and Non-PolyQ Peptides for the Treatment of Huntingtin's Disease.

Indulekha John[1], R.Remya[2],Salma Kausar M[3], Ram Kumar Chenthur Pandian[4],K.Valarmathy[5*]

1. Assistant Professor, Department of Biotechnology, The Oxford College of Engineering,Bangalore,indulekhajohn@gmail.com.
2. Associate Professor,Department of Electronics and Communication Engineering,VeltechRangarajDrSagunthala R & D Institute of Science & Technology,Chennai , remiamernath@gmail.com
3. Assistant Professor, Department of Biotechnology, The Oxford College of Engineering,Bangalore,msalmakausar@gmail.com.
4. Professor,Department of Electronics and Communication Engineering,Shri Krishna College of Technology,Coimbatore,India,profcramkumar@gmail.com
5. Associate Professor, Department of Biotechnology, The Oxford College of Engineering, Bangalore, dr.valarmathyk@gmail.com

*Corresponding author: Associate Professor,
Department of Biotechnology,
The Oxford College of Engineering, Bangalore,
dr.valarmathyk@gmail.com
Phone no: 9739672705

Article History
Volume 6, Issue 5, Apr 2024
Received: 01 Apr 2024
Accepted: 08 Apr 2024
doi: ***10.33472/AFJBS.6.5.2024.183-194***

ABSTRACT

The first exon of the HTT (Huntingtin) gene has an aberrant increase of CAG repeats, which causes Huntingtin's disease, a neurological illness disease called Huntingtin. This genetic anomaly leads to creation of an aberrant protein of Huntingtin protein called as (Htt) which has extended poly-glutamine sequences of varying lengths, which aggregate and become toxic to the brain, causing significant damage. Although the exact role of the HTT gene is unknown, it is known to be essential for the prenatal development of neurons in the brain. While thorough reversal for the damage of brain is currently beyond our capabilities, there is promising research engrossed treatments based on peptide for Huntingtin, including both polyglutamine and non-polyQ peptides such as QBP1, P42, ED11, and BIP.By focusing on different areas of the Htt protein, these peptides—QBP1 and P42 in particular—avoid aggregation. P42 ties to residues between 480 and 502, while QBP1 interacts with several locations within the Htt protein's N-terminal section.

Furthermore, the disease-associated protein caspase 6 is the target of ED11. Additionally, the Htt protein interacts with other proteins such as calpain, caspase 3, and p42 peptide.Many databases and bioinformatic tools, including as NCBI, PDB, SWISSMODEL, CABSDock, and Procheck, were used to further this research.These databases were employed to locate the pertinent target protein, validate and model it, and finally dock it with the chosen therapeutic peptides. The peptide-protein combination with the lowest value of energy among those that displayed the lowest energy values during docking was chosen as the most advantageous. There is hope for the development of future Huntingtin's disease therapeutics because to this research.

KEYWORDS: Huntingtin, Htt protein,anomaly,aggregation,peptide

1. INTRODUCTION

Uncontrollably dynamic movements, behavioral and cognitive problems, and memory loss are the hallmarks of disease named Huntingtin(HD), a rare neurodegenerative illness that affects the central nervous system (Roos, 2010). An enlarged CAG trinucleotide repeat found in the IT-15 gene on chromosome 4 is the cause of this disorder, which is inheritedautosomally. The result of this genetic mutation is the synthesis of a distortedHuntingtin protein (mHTT) that has an abnormally lengthy polyglutamine replicate (polyQ) sequence (Caltaneo*et al.*, 2005). Individuals who have 36 to 39 CAG repeats are less likely to develop the condition than those who have more than 39 repeats (Colgan*et al.*, 2018;Schelling *et al.*, 1995).

The transmutation in HD results in an abnormal and extended polyQextension in the protein of Huntingtin (Htt), that confers more than one toxicproperty to the mutant Htt, causes neurodegeneration. The accumulation and aggregation of Htt with the expanded polyQ segment are believed to be linked to the disease pathogenesis, and strategies to reduce protein misfolding are being explored (Finkbeiner, 2011). Symptoms typically appear around the age of 40, however there are also young (onset below 20 years) and aged (onset above70 years) forms of the disease (Novak *et al.*, 2010).

The precise commonpurpose of Htt remains unclear, however it is believed to play roles in intracellular signaling, preserving the protein binding site of cyclic adenosine monophosphate and guarding against neurondamage(Nucifora*et al.*, 2001).While there is currently no known cure for HD, some individuals may see improvements in their quality of life, motor function, and safety with symptomatic treatments that address the Neurochemical imbalances linked to chorea. Clinicians may also investigate therapies for non-motor components of HD, dystonia, and other movement disorders (Aharony*et al.*, 2015).

Usually, background in the family of the disease and the development of distinctive motor

symptoms are necessary for the diagnosis of HD. In symptomatic individuals, a DNA test can confirm the diagnosis by detecting the anomalous CAG extension in the gene (Htt)(Belwal*et al.,* 2020).For people who run the risk of having HD, genetic testing can be performed with the guidance of skilledphysicians and with appropriate inherent counseling upon the Patient's appeal. In managing behavioral aspects of the disease, cognitive approaches may sometimes be more beneficial than pharmaceuticals, even though research in this area is limited. Some clinical trials have explored impact of medications that improve cognition on HD patients, such as Atomoxetine, Rivastigmine, and donepezil(Marelli*et al.,* 2016).

3,144 amino acids make up the big molecule known as the human protein (Htt). Starting at amino acid position 18, the polyQ domain has between 11 and 34 glutamine residues in healthy people and up to 37 glutamine residues in HD patients. Two proline-rich domains (polyP) containing 11 and 10 prolines, respectively, come after the polyQ domain(Chen *et al.,* 2017).Htt has relatively few motifs sequence with established functions in spite of its vastness. HEAT (Huntingtin, elongation factor 3, a subunit of protein phosphatase 2A, and TOR1) repeats are one structural feature of Htt (Novak *et al.,* 2010). The function of these HEAT repeats, which are sequences of about 40 amino acids and are present in a number of proteins involved in chromosomal segregation and intracellular transport, is currently unknown (Li & Li ,2004).

The disease called Huntingtinhas no recognized treatment, and the problems it creates in the brain cannot currently be stopped or reversed.As such, the management of symptoms is the main goal of treatment. A group of international experts suggests the following as first-line treatments for three of the disease's most bothersome symptoms:Involuntary motions, or chorea: Atypical antipsychotics such as olanzapine are recommended as the first prescription for treating Huntingtin's disease by some doctors, whereas tetrabenazine - a drug that the US Food and Drug Administration (FDA) recently approved is preferred by others.Irritability: Professionals agree that the best way to treat severe anger and violent behaviour is with atypical antipsychotic drugs (Kohli*et al.*,2021). Therefore, the primary focus of treatment is on managing the symptoms.

2. MATERIALS AND METHODOLOGY

2.1 THE DETERMINATION OF THE PROTEIN CAUSATIVE

Using search terms like "Huntingtin's disease," "Huntingtin protein," and "Peptide," a large number of research publications, review papers, and books published between 2003 and 2021 were examined. There is a spectrum of movement impairments associated with Huntingtin's disease, including both intentional and involuntary movement deficiencies.

These manifestations include inadvertentshuddering or squirmingactions, known as chorea, as well as muscle issues such as inflexibility or dystonia. Patients may experience abnormal or slow eye movements, and their pace, position, and stability can be significantly affected. Dialogue and swallowing problems are also common. It's worth noting that voluntary movement impairments can devise a more substantial impression on an individual's capability to accomplish daily tasks, converse effectively, and maintain independence than involuntary movement impairments (Colgan*et al.,* 2018).

In addition to motor difficulties, individuals with Huntingtin's disease often face cognitive challenges. These may manifest as trouble with organization, focus, and task completion. Patients might exhibit a lack of flexibility, becoming fixated on certain thoughts, behaviors, or actions, a condition known as perseveration. Impaired impulse control can lead to outbursts, impulsive actions, and sexual indiscretion. Patients may also lack awareness of their own behaviors and capabilities and may exhibit sluggishness in handlingopinions and discovering words. Acquiring fresh knowledge be particularly challenging for them (Chu *et al.,*2021)

One of the most prevalent mental illnesses connected to Huntingtin's disease is depression.Depression has been thought to be caused by brain damage and the ensuing changes in brain function rather than just being a reaction to learning that you have Huntingtin's disease.Signs of depression can comprisestate of mind of bad temper, grief,social disengagement, sleeplessness, weakness, and loss of vitality. Individuals with Huntingtin's disease may experience persistent ideas of suicide, death and dying. Other mental illnesses that can be linked to the condition include mania, which can cause an elevated mood, anxiety, aggressive behavior, and inflated self-esteem, bipolar disorder, which alternates between periods of depression and mania, and obsessive-compulsive disorder, which is characterized by recurrent intrusive thoughts and repeated behaviors. Loss of weight is also frequent, particularly as the illness worsens(Finkbeiner,2011).

With respect to context of Huntingtin, various treatments hadbeen explored, including therapies involving QBP1, BIP, P42, and ED11. These investigations through journal articles and research papers shed light on potential interventions for this challenging condition.

2.2 THE DETERMINATION OF TARGET PROTEIN

Synchronize files pertaining to biological compounds make up the majority of the central data in the Protein Data Bank database. Through the use of online resources such as the RCSB Protein Data Bank web page, we were able to perform extensive searches and explore the abundance of data included in the PDB header. This data includes specifics of the experimental methods used in the study as well as the complex chemistry and protein biology

being investigated. After obtaining this important data from the PDB entries, we used visualization tools to examine the protein's structural arrangement in visual form. This stage was crucial in exposing important structural features and traits of the protein.

A FASTA sequence for 3IO6 is as follows: >3IO6_1|Chains A, B, C| Huntingtin Fusion Protein, Escherichia coli K-12 (83333), maltose-binding periplasmic protein KIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGP DIIFWAHDRFGGYAQSGITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNK DLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGK YDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPW AWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTD EGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYAV RTAVINAASGRQTVDAALAAAQTNAAAATLEKLMKAFESLKSFQQQQQQQQQQQQ QQQQQPPPPPPPPPPQLPQPPPQAQPLLPQQSYQITAGKLGTGR RFTTS

2.3.1. MODELLING OF PROTEINSTRUCTURE HOMOLOGY

For the purpose of protein structure homology modeling, we employed SWISS-MODEL, a completely programmed server known for its capabilities in this domain. This tool is easily accessible through the database Expasy otherwise by using DeepView programmed database. Primary function with respect to homology modeling is to predict the 3Dassemblythrough aligning the protein sequence, which is that is relevant to template proteins (Li *et al.*, 2010).Usually, there are four main processes in this process: building models, model refining, genome alignment, and identifying the target. Users of the SWISS-MODEL platform can interact at different levels via the platform's Web interface. In "first mode of approach," users only need to enter the protein's amino acid sequence that they want to simulate. The server then takes on the tasks of template selection, alignment, and model creation in an entirely automated fashion. In practice, this means that we pasted the FASTA sequence of the target protein into SWISS-MODEL, which initiated the modeling process. Following the completion of this process, we carefully examined the results to select the most suitable model. This selection was further validated to ensure its accuracy and reliability.

2.3.2 THE VERIFICATION OF PRIMARY MODELS

In the realm with respect to homology modeling, the validation of initial models holds a paramount importance. To ensure the reliability and accuracy of the model created by SwissModel, we employed a validation tool known as PROCHECK. PROCHECK conducts an in-depth analysis of a protein structure's geometry, both in terms of its overall configuration and the geometry of individual residues. This analysis results in the generation of several PostScript graphs, offering a comprehensive view of the structure's

stereochemistry. The suite of tools within PROCHECK is highly effective in scrutinizing the fine details of a protein structure.PROCHECK produces a range of Post-Script-format charts and meticulously detailed residue-by-residue listing. The outputs have two purposes: first, they offer an evaluationof the total quality of the structure relative to other highly refined structures at the equivalent resolution.This comprehensive toolanalyzes the quality of the structure of protein that are being unraveled other than those that are already in existence and those that are modelled using known structural information.In our case, the PROCHECK analysis was conducted by uploading file from Protein Data Bank with extension 4wth.2A, and the specific valid results were meticulously reviewed. Fig. 1 visually represents the 3-D structure of 4wth.2A, shedding light on its spatial characteristics and providing valuable insights into its structural integrity.

2.3.3. DOCKING AT MOLECULAR LEVELS

Peptides and proteins are essential for the proper operation of cells, as well as understanding their interactions is essential, particularly when it comes to identifying potential new drugs. This task, though challenging, is pivotal. To aid in this endeavor, we turned to the Web server of CABS-dock,that offers a platform for modeling interfaces between proteins and peptides. This service uses a quick and adaptable docking method,allowing the proteins to dock the peptides with. An interesting aspect of CABS-dock is that it doesn't require prior data of the site of binding, a feature that sets it apart from other docking methods.CABS-dock operates by simulating a look for the binding site inside a particular peptide sequence and protein receptor structure.This method is very useful since it begins with peptides conformations and positions that are random, giving peptides a great degree of flexibility and permitting small variations in the backbone of the receptor. This flexibility is particularly advantageous in the protein-peptide interaction modeling. By means of CABS-dock, we conducted docking experiments using a validated protein molecule. In this process, we docked the protein molecule with various peptides of interest that wereidentified through literature mining.The process involved uploading the protein data bank file with extension 4wth.2A, the protein receptor structure, and the sequences retrieved from FASTAs of the target peptides, which were obtained from the Research articles. These components are then submitted individually by uploading for the docking simulations, enabling the exploration of potential binding interactions between the protein and the target peptides.

3. RESULTS AND DISCUSSION

3.1 RECOGNITION OFCAUSATIVE PROTEIN

After a thorough literature review, it became evident that the Huntingtin protein is the primary factor responsible for the aggregation process. Under normal, healthy conditions, the

Htt protein typically contains a range of 6 to 35 glutamine residues. However, in the context of the disease, it was observed that there are more than 36 repeats of glutamine residues, indicating a substantial increase in glutamine length within the protein.

3.2 TARGET PROTEIN IDENTIFICATION

A set of promising peptides, including QBP1 and p42, function by impeding the aggregation process through their specific interactions with N-terminal part of the protein of Huntingtin(Htt). In contrast, ED11 target caspase 6, which is also associated with the disease. Other proteins like caspase 3, calpain, and the P42 peptide are knownfor binding to the protein of Huntingtinas well. Notably, QBP1 has the capacity to interact with multiple sites within the N-terminal portion of the Htt protein, while p42's binding is concentrated within the residues spanning from 480 to 502. Given that the most of these possible peptides interrelate with N-terminal segment of the Htt protein, a suitable protein structure for this region was selected from the Protein Data Bank (PDB), specifically with protein of accord ID: 3IO6. This choice provides a foundation for further investigations into the interactions of these peptides with the Htt protein's N-terminal domain.

3.3 MODELLING HOMOLOGY

Utilizing web-based modeling tool SWISSMODEL for homology identification, protein with ID 3IO6 was subjected to modeling, guided by insights regarding the aggregation sites. Subsequently, the models generated were rigorously assessed through the Procheck server's validation process. From the array of models produced, the one that exhibited the highest level of suitability and alignment with the aggregation sites was identified and selected as the most fitting model, labeled as 4wth.2A. This meticulous selection process expected to ensure the precision and relevance between the chosen protein model for further investigations and analysis.

3.4 VALIDATION OF MODEL

The Procheck tool plays a critical role in validating the stereochemical integrity of a protein structure. It accomplishes this by meticulously examining both the geometry of individual residues and the overall structural geometry. In this particular case, the model denoted as 4wth.2A was singled out as the optimal choice due to its superior performance across various key validation parameters. These included the preeminent 3D-1D score, G-factor, Quality factor, and alignment of its structures within the acceptable sections of the Ramachandran plot. This thorough evaluation process ensured that 4wth.2A stood out as the most suitable model, meeting the necessary criteria for further analysis and research.

3.5 ANALYSIS OF DOCKING

To conduct the docking of all the peptides, the CABS-dock tool was employed, and as a

result, data of energy including energy values from receptor, ligand, interaction, and total energy were acquired. A total often distinct modelwas generated and subsequently related based on their values of total energy. Within the tabulated results, the model with the lowest energy value for each of the target peptides, signifying the most favorable binding interaction (superlative model), had been prominently emphasized. The findings from Table 2 present the best models selected for each of the specific peptides, based on their minimal energy values. This approach aids in identifying the most energetically favorable configurations, which are of particular significance for further analysis and research.

4. DISCUSSION

An uncommon neurological condition called Huntingtin's illness appears in adulthood and is caused by an increase of CAG triplet repeats in the coding area of the Htt gene. A mutant Htt (mHtt) protein wasproduced when the number of glutamine repeats in the Htt gene increases beyond the normal range of 35–36. Due to its enlarged polyglutamine repeat, this mHtt protein is extremely brittle and prone to aggregation. Mutant Htt aggregates become increasingly important in the pathophysiology of the disease as it advances. Htt interacts with a wide range of proteins, including CREBBP, BDNF, caspase, HDAC3, Hsp90, HIP (Huntingtin interacting protein), and HAP (Huntingtin associated protein). Nevertheless, mutant Htt is unable to interact with HDAC3, which adds to the molecular mechanisms causing neurodegeneration. It's interesting to note that Huntingtin increases BDNF via an unidentified transcriptional pathway. Peptides QBP1, P42, ED11, and P42 have shown promise as treatments for Huntingtin's illness by focusing on mutant Htt and blocking its interactions with other proteins. The most promising candidate among these peptides is ED11, which was selected due to its greater docking performance and lowest overall energy. The search for new and efficient treatment approaches is becoming more and more important given the high death rates linked to Huntingtin.

5. CONCLUSION

Our research outcomes indicate that peptide ED-11 effectively interrelates with the active spot of the Htt protein, a crucial factor in mitigating the neuronal cell degeneration associated with Huntingtin's disease (HD). It's noteworthy that ED-11 exhibits a highvalue of energy compared to other peptides, including BIP, QBP1, and P42, as observed in our investigations. Specifically, ED-11 possesses an energy value of -3851.30 kcal/mol, while P42, QBP1, and BIP were measured at -3831.12 kcal/mol, -3699.11 kcal/mol, and 3592.80 kcal/mol, correspondingly. This significant shift in total energy, coupled with its ability to bind effectively to the energeticconcise of the Htt protein, positions ED-11 as a promising candidate for alleviating HD symptoms and warrants further exploration for its potential

application in the study of other polyglutamine-related disorders. Consequently, ED-11 merits further investigation concerning its docking and aggregation capabilities.

6.REFERENCES

1. Aharony, I., Ehrnhoefer, D. E., Shruster, A., Qiu, X., Franciosi, S., Hayden, M. R., &Offen, D. (2015). A Huntingtin-based peptide inhibitor of caspase-6 provides protection from mutant Huntingtin-induced motor and behavioral deficits. Human molecular genetics, 24(9), 2604-2614.
2. Belwal, V. K., Datta, D., & Chaudhary, N. (2020). The β- turn- supporting motif in the polyglutamine binding peptide QBP1 is essential for inhibiting Huntingtin aggregation. FEBS letters, 594(17), 2894-2903.
3. Cattaneo, E., Zuccato, C., &Tartari, M. (2005). Normal huntingtin function: an alternative approach to Huntingtin's disease. Nature Reviews Neuroscience, 6(12), 919-930.
4. Chen, M., &Wolynes, P. G. (2017). Aggregation landscapes of Huntingtin exon 1 protein fragments and the critical repeat length for the onset of Huntingtin's disease. Proceedings of the National Academy of Sciences, 114(17), 4406-4411.
5. Chu-Qiao Liang, Yan-Mei Li, Peptides for disrupting and degrading amyloids, Current Opinion in Chemical Biology, 10.1016/j.cbpa.2021.05.011, 64, (124-130),
6. Finkbeiner S. (2021). Huntingtin's Disease. Cold Spring HarbPerspect Biol. 2011 Jun 1;3(6):a007476
7. Frank S. Treatment of Huntingtin's disease. (2014).Neurotherapeutics. Jan;11(1):153-60. doi: 10.1007/s13311-013-0244-z.
8. Kohli, H., Kumar, P., &Ambasta, R. K. (2021). In silico designing of putative peptides for targeting pathological protein Htt in Huntingtin's disease. Heliyon, 7(2), e06088.
9. Li, S. H., & Li, X. J. (2004). Huntingtin–protein interactions and the pathogenesis of Huntingtin's disease. *TRENDS in Genetics*, *20*(3), 146-154.
10. Li, Y., Yokota, T., Gama, V., Yoshida, T., Gomez, J. A., Ishikawa, K., ...& Matsuyama, S. (2007). Bax-inhibiting peptide protects cells from polyglutamine toxicity caused by Ku70 acetylation. Cell Death & Differentiation, 14(12), 2058-2067.
11. Marelli, C., &Maschat, F. (2016). The P42 peptide and Peptide-based therapies for Huntingtin's disease. Orphanet journal of rare diseases, 11(1), 1-18.
12. McColgan, P., &Tabrizi, S. J. (2018). Huntingtin's disease: a clinical review. European journal of neurology, 25(1), 24-34.

13. Novak MJ, Tabrizi SJ. Huntingtin's disease. BMJ. 2010 Jun 30;340:c3109. doi: 10.1136/bmj.c3109.
14. Nucifora FC Jr, Sasaki M, Peters MF, Huang H, Cooper JK, Yamada M, Takahashi H, Tsuji S, Troncoso J, Dawson VL, Dawson TM, Ross CA. Interference by huntingtin and atrophin-1 with cbp-mediated transcription leading to cellular toxicity. (2001). Science. Mar 23;291(5512):2423-8. doi: 10.1126/science.1056784.
15. Roos, R. A. (2010). Huntingtin's disease: a clinical review.Orphanet journal of rare diseases, 5(1), 1-8.
16. Schilling, G., Sharp, A. H., Loev, S. J., Wagster, M. V., Li, S. H., Stine, O. C., & Ross, C. A. (1995). Expression of the Huntingtin's disease (IT15) protein product in HD patients. Human molecular genetics, 4(8), 1365-1371.

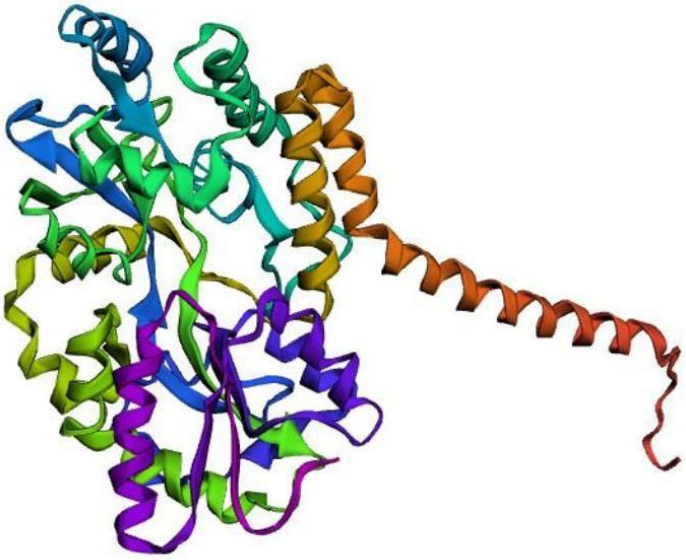

Figure 1: 3D Structure of 4 WTH.2A Structure

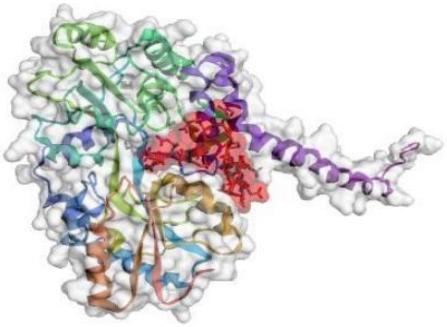

Figure 2: Model 4 of QBP1

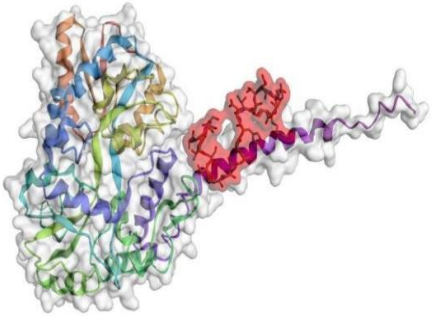

Figure 3: Model 10 of P42

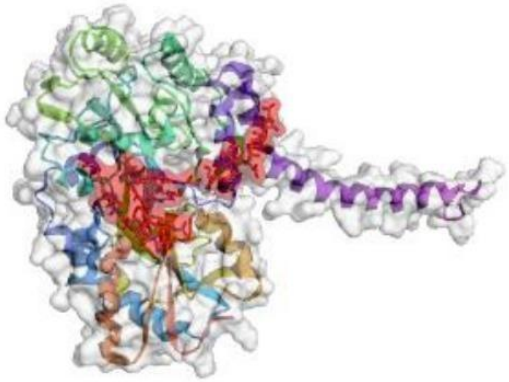

Figure 4: Model 2 of ED11

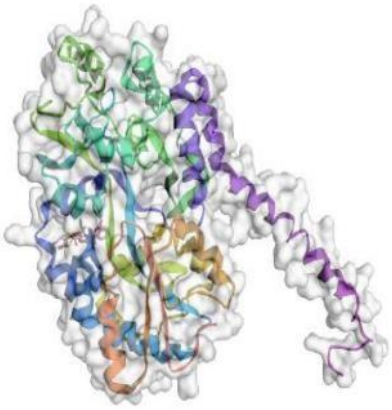

Figure 5: Model 3 of BIP

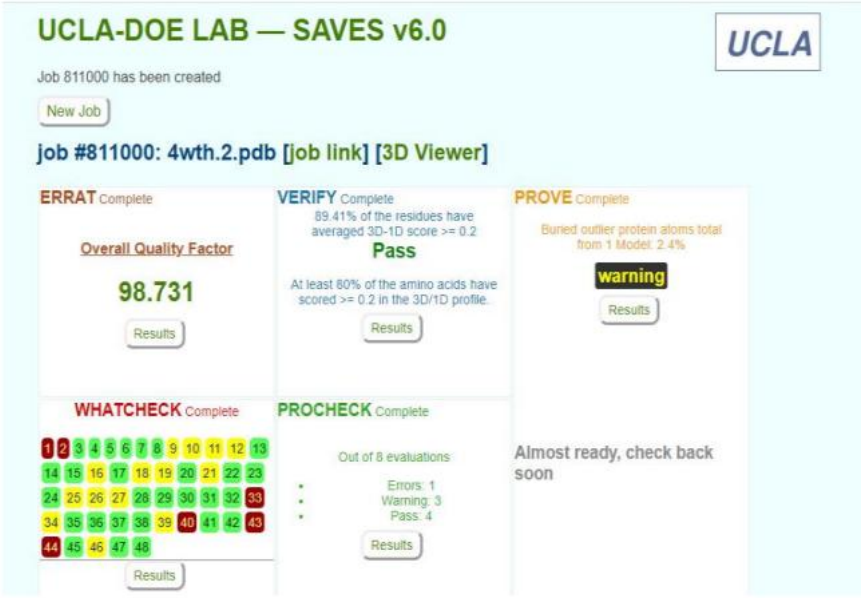

Figure 6: Results of Procheck Validation

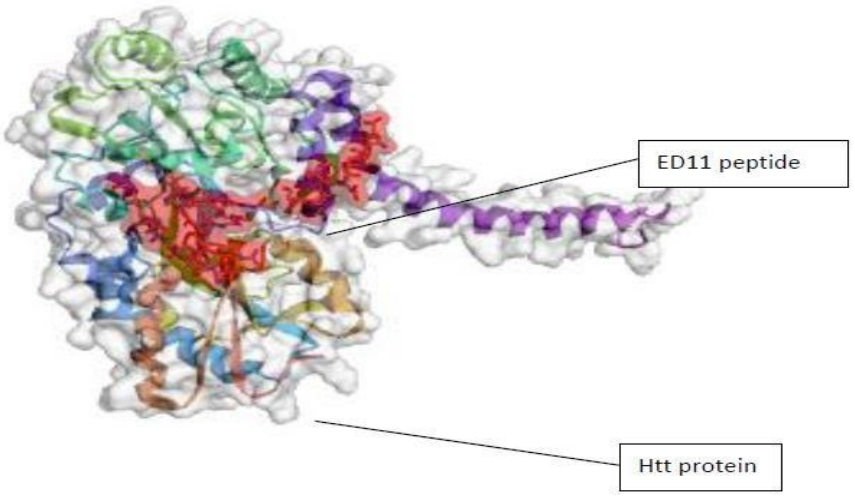

Figure 6:Model 2 with ED11

Peptides	Sequence of Peptide	Target of Therapeutics
QBP1	SNWKWWPGIFD	PolyQ Aggregtion
P42	AASSGVSTPGSAGHDIITEQPRS	N17 (htt domain)
ED11	GRKKRRQRRRPPQSSEIVLDGTDN	Caspase 6 inhibitor
BIP	VPMLK/VPTLK	Apoptosis induced by Bax

Table1: Target Peptide sequences

Peptide Names	Model No	$E_{receptor}$	E_{ligand}	$E_{interaction}$	E_{total}
QBP1	4	-3498.38	-18.53	1.00	-3699.11
P42	10	-3488.43	-94.34	1.05	-3831.12
ED11	2	-3441.60	-55.45	1.00	-3851.30
BIP	3	-3476.23	-1.07	1.00	-3592.80

Table 2: Values of Energy