ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 8, Issue, 06, pp.33189-33195, June, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

STRUCTURAL, FUNCTIONAL INSIGHTS OF ALSIN PROTEIN USING HOMOLOGY MODELING AND DOCKING STUDIES

***Ruchi Yadav**

AMITY Institute of Biotechnology, AMITY University, Uttar Pradesh Lucknow, UP, India

ARTICLE INFO

Article History:
Received 04th March, 2016
Received in revised form
23rd April, 2016
Accepted 26th May, 2016
Published online 30th June, 2016

Key words:

ALS, Binding site,
Homology Modeling,
Docking,
Neuronal Diseases,
Sclerosis.

ABSTRACT

Amyotrophic lateral sclerosis (ALS), also known as "Lou Gehrig's disease". It is a neurodegenerative disease associated with degeneration of motor neurons in the cerebral cortex, brain stem, and spinal cord characterized by distal muscle weakness, atrophy, normal sensation, pyramidal signs and progressive muscular paralysis reflecting. ALS2 is a juvenile autosomal recessive disorder, slowly progressive, that maps to chromosome 2q33 and is associated with mutations in the alsin gene, a putative GTPase regulator. In this paper we have done homology modeling of alsin2 protein using multiple templates (3KCI_A, 4LIM_A, 402W_A, 4D9S_A, and 4DNV_A) designed using the Prime program in Schrödinger software. Structural and function analysis is done by using Prosite and ExPASy server that gives insight into conserved domains and motifs that can be used for protein classification. Further modeled structure is used to identify effective binding sites on the basis of structural and physical properties using sitemap program in Schrödinger software. Docking of Resveratrol with alsin protein evaluates the binding site and potential role of antioxidant in neurological disease. This paper summarizes the structural, functional, binding site, docking on alsin protein that gives insight into functional role of alsin protein in neurological disease this study can be used in drug discovery process.

Citation: Ruchi Yadav, 2016. "Structural, Functional Insights of Alsin Protein Using Homology Modeling and Docking Studies", *International Journal of Current Research*, 8, (06), 33189-33195.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a paralytic disorder caused by motor neuron degeneration (Devon *et al*., 2003). Motor neurons are the cells in the brain and spinal cord which are responsible for conscious muscle contraction (i.e., function). These nerve cells are the primary target for degeneration in ALS (Eymard-Pierre *et al*., 2002). The ALS2 gene provides instructions for making a protein called alsin. Alsin is produced in a wide range of tissues, with highest amounts in the brain. This protein is particularly abundant in motor neurons, the specialized nerve cells in the brain and spinal cord that control the movement of muscles (Hong *et al*., 2012). ALS is a motor neuron disease that is differentiated into two type in first type of ALS there is progressive degeneration of motor neurons and in second type there is damage to upper and lower motor neurons these neurons receive signals from brain to spinal cord and damage in these neurons result in loss of muscle and hence disfunctioning of muscle that leads to death of neurons (Matthew C. Kiernan *et al*., 2011; Shaw, 2001).

****Corresponding author: Ruchi Yadav,***
AMITY Institute of Biotechnology, AMITY University, Uttar Pradesh Lucknow, UP, India.

Mutation in ALS2 gene cause neurological disease but function how loss of alsin protein is involved in amyotrophic lateral sclerosis is not well understood .Current research interest are in to signify the role of alsin protein in neurological diseases (Mintchev *et al*., 2009).

Alsin Protein

Alsin protein is highly present in motor neurons but its function is not clearly known (Cronin *et al*., 2007). Homology study shows that alsin protein is involved in regulation of survival and growth of spinal motoneurons and have role in GTPase regulator (Fallis and Hardiman, 2009). Alsin protein is involved in neurological diseases mutation in alsin protein result in amyotrophic lateral sclerosis 2 (ALS2), juvenile primary lateral sclerosis (JPLS), infantile-onset ascending spastic paralysis (IAHSP) (Ringel *et al*., 1993).

Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic Lateral Sclerosis is also known as Lou Gehrig's disease the cause of this disease is due to mutation in ALS2 gene .Alsin protein is encoded by ALS2 gene which is highly

expressed in brain cells and in cells of motor neurons, nerve cells in spinal cord and functions mainly in regulation of muscle movement by passing signal from brain to muscle (Leigh *et al.,* 2003). Mutation in this gene leads to abnormal production of alsin protein that is deformed and very unstable due to which it is easily degraded. Loss of alsin protein in nerve cells leads to severe neurological disorders (Neumann *et al*., 2006). ALS is not curable and progressive degenerative neurological disorder the actual mechanism underlying Amyotrophic Lateral Sclerosis is under study. Major difference between ALS and other sclerosis is that in ALS only voluntarily moved muscles are impaired such as mouth, arm, tongue and does not affect other involuntarily movement such as heart beating, digestion it does not affect the patients memory and senses of taste, smell, hearing capabilities remains same (Lomen-Hoerth *et al*., 2002; Bian *et al*., 2014; Choudhary *et al*., 2009; Rigbolt *et al*., 2011)

Juvenile Primary Lateral Sclerosis (JPLS)

Juvenile primary lateral sclerosis is also called as primary lateral sclerosis, is rare disorder, caused by damage in motor neurons which functions signal transport from brain to spinal cord and in muscle movement (Rigbolt *et al*., 2011). JPLS occurs in childhood and progresses with age symptom of JPLS in stiffness in muscles of many parts of body including face, leg and arms as diseases progresses there is weakness in muscle that continues with age and finally death of motor neurons .studies reported that any Mutations in the *ALS2* gene (2q33-q35) encoding alsin protein causes this disease but function of alsin protein is still under study (Mayya *et al*., 2009; Cantin *et al*., 2008).

Infantile-Onset Ascending Spastic Paralysis (IAHSP)

Infantile-Onset Ascending Spastic Paralysis (IAHSP) is hereditary neurological disorder characterized by progressive spasticity and weakness of limbs (Eymard-Pierre *et al*., 2002). It is also known as hereditary spastic paraplegias. Infantine spastic paralysis is because of degeneration in motor neurons condition called as atrophy, these motor neurons function as signal transport from brain to muscle loss of these neurons result in paralysis (The MGC Project Team, 2004). Hereditary spastic paraplegias are of two types in one form that is called as pure IAHSP there is paralysis only in lower limbs due to loss of motor neurons while in complicated IAHSP there is loss of motor neurons in various body organs including arms, legs, face (Nakajima *et al*., 2002). Studies reported that infantile-onset ascending hereditary spastic paralysis is caused by mutations in the ALS2 gene. Due to loss of alsin protein there is loss in GTPase functions and hence degradation in development of axons and dendrite that leads to loss of motor neurons and result in atrophy. Still it is very unclear how alsin protein is involved in Infantile-Onset Ascending Spastic Paralysis (Aiken *et al*., 2011)

MATERIALS AND METHODS

[1]Alsin protein sequence is retrieved from UniProt database with id Q96Q42 (http://www.uniprot.org/uniprot/Q96Q42). Fig.1 shows the query sequence of alsin protein that was modeled using Schrödinger software using prime tool (Halgren, 2007) colored region in fig1 shows the position of sequence that was modeled. Since sequence similarity of alsin sequence is less that 30% for homology modeling Multitemplate homology modeling is used .Five templates were selected to build consensus model based on selected template table 1 shows the list of templates that are used to build consensus model and Prime STA method (Bell *et al*., 2012) is chosen for alignment between query sequence and template sequence because sequence similarity ranges from 28-35 %. Fig 2 shows the alignment of alsin sequence with template sequence colored regions represent the alignment between residues of query sequence and template sequence. Functional analysis of alsin protein is done using Scan Prosite tool at expasy (http://prosite.expasy.org/scanprosite/) and motif is mapped to secondary structure of alsin protein. For docking studies modeled protein structure Alsin_A is used since it is more reliable and binding site identification is done using sitemap tool of Schrödinger software, these sites are evaluated using docking of Resveratrol with Alsin_A protein using Glide dock tool in Schrödinger software (Friesner *et al*., 2006; Halgren *et al*., 2004)

RESULTS AND DISCUSSION

Homology Modeling

Multitemplate homology modeling is done using prime program in Schrödinger software two structures were modeled designated as Alsin_A and Alsin_B

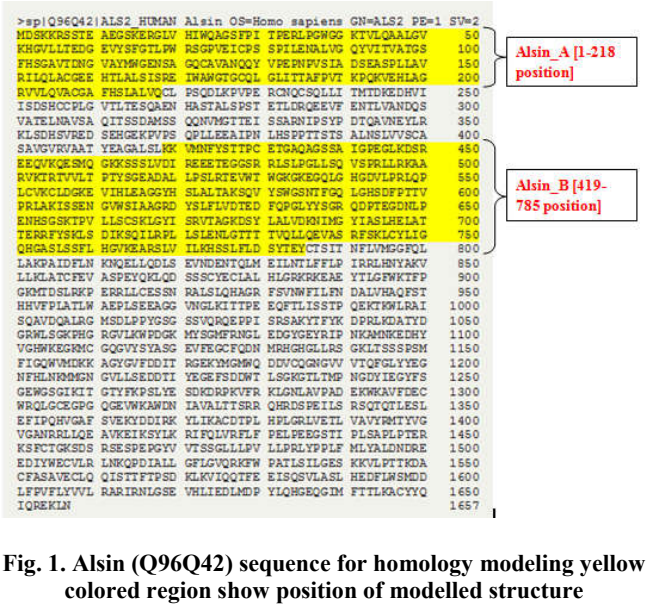

Fig. 1. Alsin (Q96Q42) sequence for homology modeling yellow colored region show position of modelled structure

Two structures were modeled from position 1-218 and 419-785. These regions represent the highest similarity in PDB database. Templates from PDB database was selected on the basis of identity percentage and query cover .Homology studies reveals that conserved region belongs to RCC_1domain as it share similarity with RLD and RCC domain of HERC2 with PDB ID: 3KCI, 4L1M, 402W, and can have function similar to RCC domain .Function of alsin protein is further studied and evaluated using detailed analysis of motif and domains.

Table 1. List of templates selected for Multitemplate homology modeling

S.No	Template id	Description	Identity (%)	Query cover (%)
1	3KCI_A	3rd RLD domain of HERC2	35	26
2	4L1M_A	1st RCC-1 like domain of HERC2	31	20
3	402W_A	3rd RCC domain of HERC1	28	23
4	4D9S_A	crystal structure of Arabidopsis thaliana Uvr8	31	31
5	4DNV_A	Crystal Structure Of The W285f Mutant Of Uvb-resistance Protein Uvr8	31	31

Table 2. Motif and domain analysis of alsin protein

S.No	POSITIONS	PROFILE	MOTIF	DESCRIPTION
1	60 - 109	REPEAT	β turn	RCC1_1 Regulator of chromosome condensation (RCC1) repeat profile
2	110 - 168	REPEAT	β turn, β hairpin	RCC1_2 Regulator of chromosome condensation (RCC1) repeat profile
3	169 - 219	REPEAT	β turn, β hairpin	RCC1_3 Regulator of chromosome condensation (RCC1) repeat profile
4	526 - 577	REPEAT	β turn, β hairpin	RCC1_4 Regulator of chromosome condensation (RCC1) repeat profile
5	578 – 628	REPEAT	β γ turn	RCC1_5 Regulator of chromosome condensation (RCC1) repeat profile
6	690 - 885	DOMAIN	β turn, β hairpin	Dbl homology (DH) domain profile
7	1513 - 1657	DOMAIN	Unmodelled	VPS9 domain profile

Table 3. List of binding sites predicted in alsin_A modeled protein

Site Map	Site Score	Dscore	Volume	Residues
sitemap_1_site_1	0.950439	0.802679	179.389	Chain A: 24,72,73,74,76,77,52,79,132,133,134,80,81,82,84,16,85,115,17,86,87,64,65,66,67,68,69
sitemap_1_site_3	0.93561	0.698928	89.866	Chain A: 146,147,137,148,138,139,192,150,172,140,141,152,174,164,143,144,145
sitemap_1_site_2	0.928715	0.961163	160.867	Chain A: 193,195,196,197,173,198,174,175,177,178,179,212,213,180,214,181,215,182,183,161,163,189
sitemap_1_site_5	0.900671	0.737503	98.441	Chain A: 51,53,1,54,55,33,35,10,36,8,9,83,18,63,64,65,41,44,45,20,46
sitemap_1_site_4	0.617906	0.557547	67.571	Chain A: 68,69,116,117,70,71,72,130,73,131,74,132

Fig. 2. Alignment between template and alsin sequence

Prime STA method is used for alignment of query alsin sequence with template sequence this method is used for low sequence similarity fig 2 shows the selection of template on the basis of query coverage and identity percentage and fig 3 shows the secondary structure assessment to query alsin sequence on the basis of template secondary structure

Alsin_A (1-218 position)

Alsin_A structure is modeled from position 1-218 and evaluation is done using ramachandran plot Fig 4 shows the Alsin_A structure and Fig 5 shows the ramachandran plot that shows this structure is more reliable as compare to Alsin_B. This structure is further used for binding site identification and docking studies

Fig. 3. Secondary structure alignment of template and alsin sequence

Rmachandran plot analysis is done using rampage tool (Lovell, *et al*., 2002),(http://mordred.bioc.cam.ac.uk/~rapper/rampage.php) shows that percentage of residues in favoured region is

86.1% and percentage of residues in allowed region 9.7% and in outlier region is 4.2% this suggest that modeled structure is good as compared to Alsin_B structure.

Fig. 4. Modeled structure Alsin_A from 1-218 position

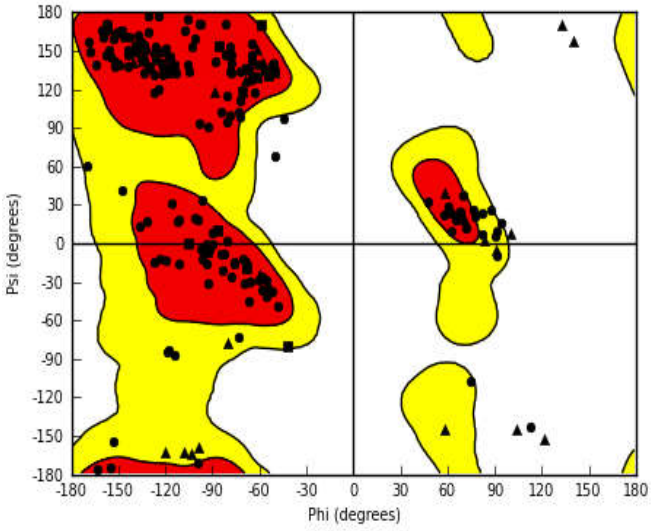

Fig. 5. Ramachandran plot of Alsin_A

Alsin_B (419-785position)

Alsin_B structure is modeled from position 419-785 fig 6 shows the modeled structure visualized in Schrödinger and protein structure evaluation is done using rmachandran plot that shows the quality of modeled structure in Fig 7.

Fig. 6. Modeled structure Alsin_B from 419-785position

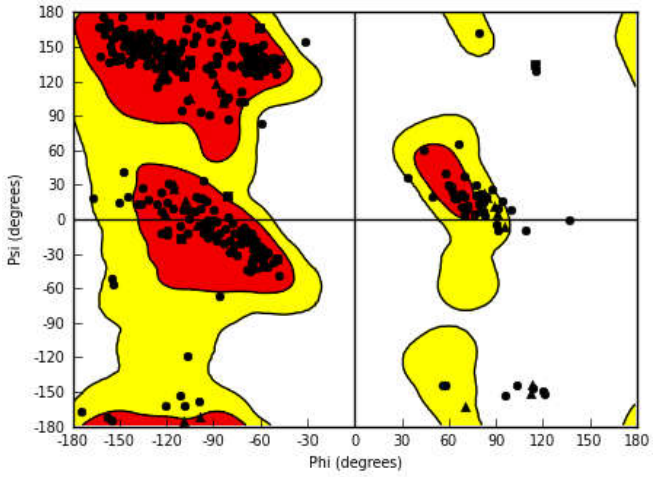

Fig. 7. Ramachandran plot of Alsin_B

Rmachandran plot study shows that 86.6 % residues lies in favoured region, 7.7% in allowed region and 5.7% in outlier region which is higher than Alsin_A structure. Evaluation of Alsin_A and Alsin_B suggest that alsin _a structure is more reliable as compared to Alsin_B structure on this basis alsin_A structure is used for further analysis and docking studies

Structure –Function Relationship

Functional analysis is done using Scan Prosite tool at expasy fig 8 shows the motif and domains identified in alsin sequence detailed analysis is shown in table 2 that shows motif that is present is alsin sequence is RCC1_1, RCC1_2, RCC1_3, RCC1_4, RCC1_5, that have a function in regulation of chromosome condensation .Probable function of alsin protein can be regulation of muscle condensation in spinal cord or in brain.

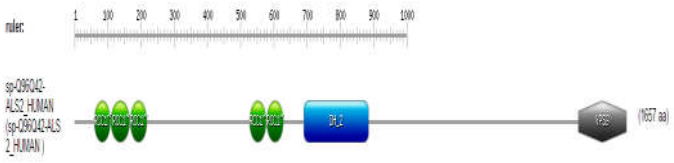

Fig. 8. Motif and domain analysis using scanprosite tool at expasy

Predicted motif and domain are mapped to secondary structure of alsin protein table2 shows the positions of conserved motif, structure of motif and function of motif .Functional analysis suggest that alsin protein belongs to RCC family and have similar function. Motifs and domain are mapped to topology of modeled structure that shows the position of RCC1 in secondary structure fig 9 shows the mapped motif to secondary structure that shows the protein structure and function relationship.

Binding Site Prediction

Alsin_A structure is used for docking analysis binding sites are identified using sitemap tool of Schrödinger software (Friesner *et al*., 2004; Zhu *et al*., 2014) five binding sites were predicted and further used as binding sites in docking.

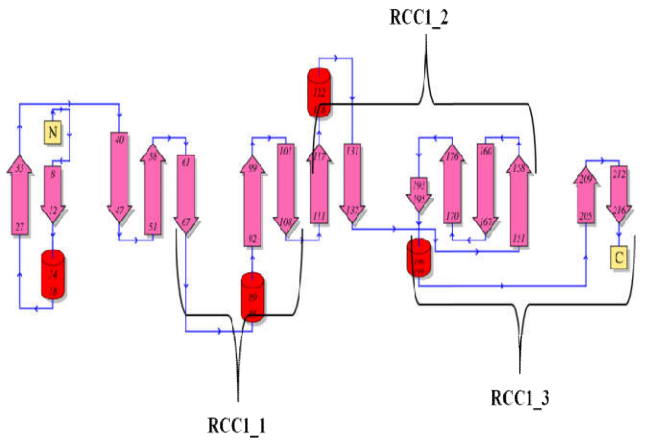

Fig. 9. Topological information of motif

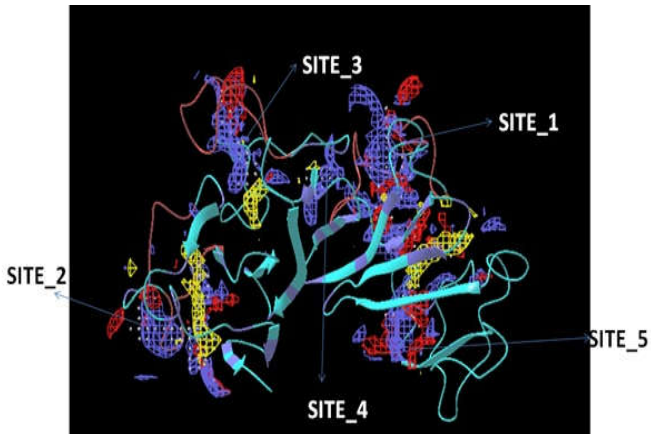

Fig. 10. Five binding sites predicted on Alsin_A protein

Table 3 shows the summarized list of binding sites ordered on the basis of site score and position in protein structure. These sites were verified and evaluated using docking studies.

Docking Result

Docking of Resveratrol (PubChem ID: 445154) (https://pubchem.ncbi.nlm.nih.gov/compound/445154) with alsin_A structure is done. Five binding sites were studied using docking. Only sitemap_1_site_2 shows interaction with Resveratrol ligand .Fig 11 shows the generation of grid on the basis of site_2 and fig 12 shows the interaction of Resveratrol ligand with alsin_A protein in two positions serine 213 and leucine 180. Binding site_2 surface is studied fig 13 shows the ligand surface region along with protein and fig 14 shows the surface of binding site_2 and interaction with alsin_A protein ligand –protein interaction analysis is done using Schrodinger software(31)-(32) fig 15 shows the Resveratrol and alsin_A protein interaction .Interaction plot shows two hydrogen bond with two residues LEU 180 and SER 213 position. Docking result shows the binding sites on modeled alsin protein and verified positions for ligand docking.

Conclusion

Alsin protein is important protein under study to understand the cause of neurological disorders .Protein structure and function plays a crucial role to uncover the mechanism of disease progression and its preventive measures.

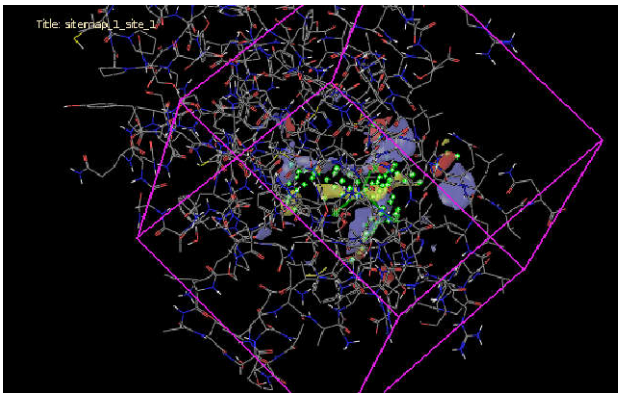

Fig. 11. Grid generation on site_2 of Alsin_A

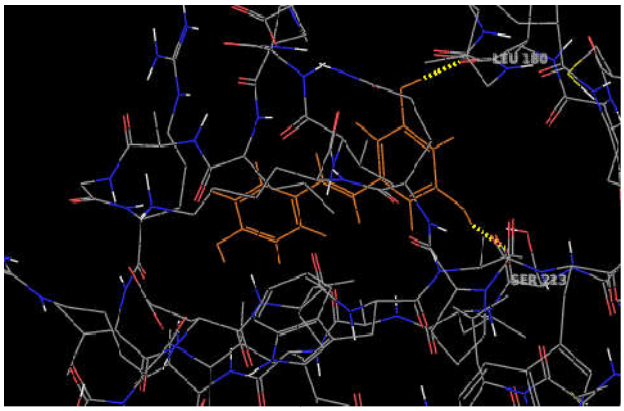

Fig. 12. Interaction of Resveratrol with alsin_A yellow dotted line represent the hydrogen bond between ligand and protein

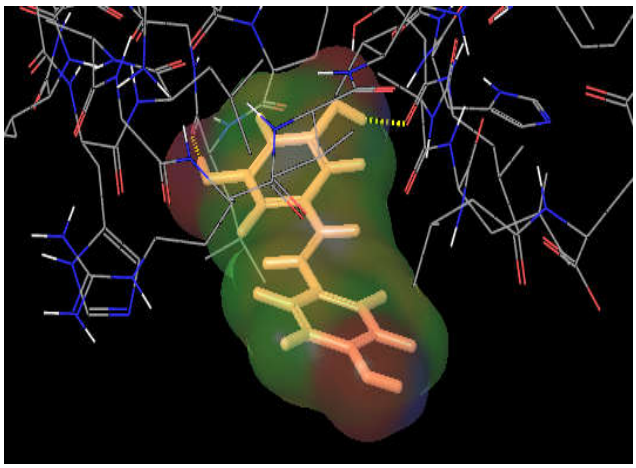

Fig. 13. Docking result showing ligand surface

This paper describes the structure ,function and interaction capability of alsin protein with chemical compound this study will help researchers working in field of amyotrophic lateral sclerosis and other neurological disease for better perceptive of neurological diseases mechanism and to identify potent drug for cure of neurological diseases.

Acknowledgement

I acknowledge to all those who are directly or indirectly helped me in this work. Further I wish to acknowledge Bioinformatics analysis tools and databases for conducting this study.

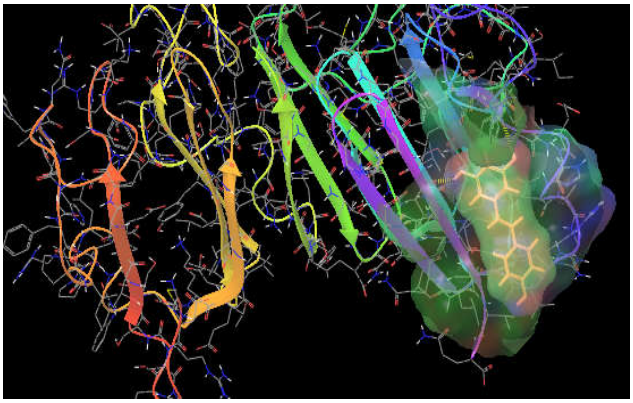

Fig. 14. Docking result showing binding site_2 region along with ligand

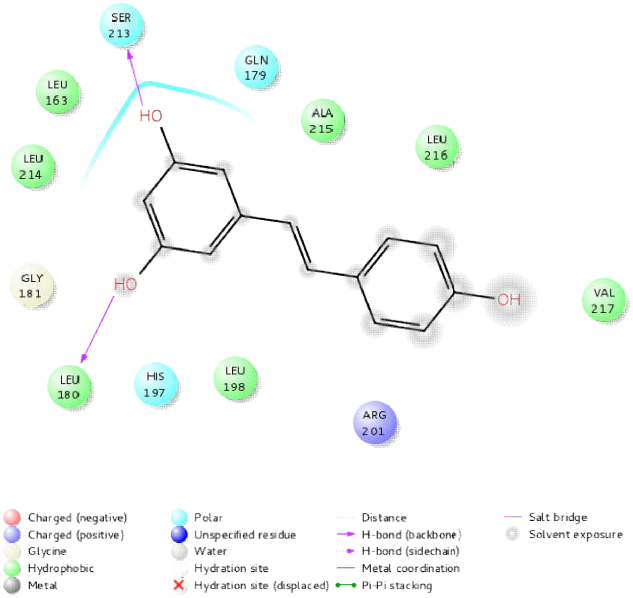

Fig. 15. Ligand –protein interaction map showing hydrogen bond between two residues LEU 180 and SER 213

REFERENCES

Aiken, C.T., Kaake, R.M. and Wang, X. *Oxidative stress-mediated regulation of proteasome complexes*, Huang, L. Mol. Cell Proteomics, 2011.

Beard, H., Cholleti, A., Pearlman, D., Sherman, W. and Loving, K.A. 2013. *Applying physics-based scoring to calculate free energies of binding for single amino acid mutations in protein-protein complexes*, *PLoS ONE, 8(12),* e82849.

Bell, J. A., Cao, Y., Gunn, J. R., Day, T., Gallicchio, E., Zhou, Z., Levy, R. and Farid, R. 2012. "*PrimeX and the Schrödinger computational chemistry suite of programs," International Tables for Crystallography, Volume F: Crystallography of Biological Macromolecules, 18, 534-538.*

Bian, Y., Song, C., Cheng, K., Dong, M., Wang, F., Huang, J., Sun, D., Wang, L. and Ye, M. 2014. *An enzyme assisted RP-RPLC approach for in-depth analysis of human liver phosphoproteome. J. Proteomics.,* 96:253-262.

Cantin, G.T., Yi, W., Lu, B., Park, S.K., Xu, T. and Lee, J. D., 2008. *Combining protein-based IMAC, peptide-based IMAC, and MudPIT for efficient phosphoproteomic analysis.* J. Proteome Res. 7:1346-1351.

Choudhary, C., Kumar, C., Gnad, F., Nielsen, M.L., Rehman, M., Walther, T.C., Olsen, J.V. and Mann, M. 2009. *Lysine acetylation targets protein complexes and co-regulates major cellular functions*. Science 325:834-840.

Cronin, S., Hardiman, O. and Traynor, B.J. 2007. *Ethnic variation in the incidence of ALS: a systematic review Neurology,* 68, pp. 1002–1007

Devon, R.S., Helm, J.R., Rouleau, G.A., Leitner, Y., Lerman-Sagie, T., Lev, D. and Hayden, M.R. 2003. *The first nonsense mutation in alsin results in a homogeneous phenotype of infantile-onset ascending spastic paralysis with bulbar involvement in two siblings. Clin Genet.,* Sep; 64(3):210-5

Eymard-Pierre, E., Lesca, G., Dollet, S., Santorelli, F.M., di Capua, M., Bertini, E. and Boespflug-Tanguy, O. 2002. *Infantile-onset ascending hereditary spastic paralysis is associated with mutations in the alsin gene. Am J Hum Genet.* Sep;71(3):518-27. Epub 2002 Jul 26

Eymard-Pierre, E., Lesca, G., Dollet, S., Santorelli, F.M., di Capua, M., Bertini, E., Boespflug-Tangu,y O., 2002. *Infantile-onset ascending hereditary spastic paralysis is associated with mutations in the alsin gene*. Am. J. Hum. Genet. 71:518-527.

Fallis, B.A. and Hardiman, O. 2009. *Aggregation of neurodegenerative disease in ALS kindreds Amyotroph Lateral Scler,* 10, pp. 95–98

Friesner, R. A., Banks, J. L., Murphy, R. B., Halgren, T. A., Klicic, J. J., Mainz, D. T., Repasky, M. P., Knoll, E. H., Shaw, D. E., Shelley, M., Perry, J. K., Francis, P. and Shenkin, P. S. 2004. "*Glide: A New Approach for Rapid, Accurate Docking and Scoring. 1. Method and Assessment of Docking Accuracy*," *J. Med. Chem., 47,* 1739–1749.

Friesner, R.A., Murphy, R.B., Repasky, M.P.,Frye, L.L., Greenwood, J.R., Halgren, T.A., Sanschagrin, P.C. and Mainz, D.T. 2006. "*Extra Precision Glide: Docking and Scoring Incorporating a Model of Hydrophobic Enclosure for Protein-Ligand Complexes," J. Med. Chem., 49, 6177–6196.*

Halgren, T. A. 2007. New *Method for Fast and Accurate Binding-site Identification and Analysis, Chem. Biol. Drug Des,* 69, 146.

Halgren, T. A., Murphy, R. B., Friesner, R. A., Beard, H. S., Frye, L. L., Pollard, W. T. and Banks, J. L. 2004. *Glide: A New Approach for Rapid, Accurate Docking and Scoring. 2. Enrichment Factors in Database Screening*, *J. Med. Chem., 47, 1750–1759.*

Hong, H., Branham, W.S., Dial, S.L., Moland, C.L., Fang, H., Shen, J., Perkins, R., Sheehan, D. and Tong, W. 2012. *Rat α-Fetoprotein Binding Affinities of a Large Set of Structurally Diverse Chemicals Elucidated the Relationships between Structures and Binding Affinities*

http://mordred.bioc.cam.ac.uk/~rapper/rampage.php

http://prosite.expasy.org/scanprosite/

http://www.uniprot.org/uniprot/Q96Q42

https://pubchem.ncbi.nlm.nih.gov/compound/445154

Leigh, P.N., Abrahams, S. Al-Chalabi, A. *et al.* 2003. *The management of motor neurone disease J Neurol Neurosurg Psychiatry,* 74, pp. S32–S47

Lomen-Hoerth, C., Anderson, T. and Miller, B. 2002. *The overlap of amyotrophic lateral sclerosis and fronto-temporal dementia*, Neurology, 59, pp. 1077–1079

Lovell, S.C., Davis, I.W., Arendall, W.B., de Bakker, III, P.I.W., Word, J.M., Prisant, M.G., Richardson, J.S. and Richardson D.C. 2002. *Structure validation by Calpha geometry: phi,psi and Cbeta deviation*. Proteins: Structure, Function & Genetics. 50: 437-450.

Matthew C. Kiernan, Steve Vucic, Benjamin C. Cheah, *et al*. 2011.*"Amyotrophic lateral sclerosis"*, *Lancet.* 377 (9769), 942–55

Mayya, V., Lundgren, D.H., Hwang, S. I., Rezaul, K., Wu, L., Eng, J.K., Rodionov, V. and Han, D.K. 2009. *Quantitative phosphoproteomic analysis of T cell receptor signaling reveals system-wide modulation of protein-protein interactions*. Sci. Signal. 2:RA46-RA46.

Mintchev, N., Zamba-Papanicolaou, E., Kleopa, K. A., Christodoulou, K., *A novel ALS2 splice-site mutation in a Cypriot juvenile-onset primary lateral sclerosis family.* Neurology 72: 28-32, 2009

Nakajima, D., Okazaki, N., Yamakawa, H., Kikuno, R., Ohara, O. and Nagase, T. 2002. *Construction of expression-ready cDNA clones for KIAA genes: manual curation of 330 KIAA cDNA clones*. DNA Res. 9:99-106.

Neumann, M. Sampathu, D.M. and Kwong, L.K. *et al.* 2006. *Ubiquitinated TDP-43 in fronto-temporal lobar degeneration and amyotrophic lateral sclerosis*, Science, 314, pp. 130–133

Rigbolt, K.T., Prokhorova, T.A., Akimov, V., Henningsen, J., Johansen, P.T., Kratchmarova, I., Kassem, M., Mann M., Olsen, J.V., Blagoev, B. 2011. *System-wide temporal characterization of the proteome and phosphoproteome of human embryonic stem cell differentiation*., Sci. Signal. 4: RS3-RS3.

Ringel, S.P., Murphy, J.R., Alderson, M.K. *et al.* 1993. *The natural history of amyotrophic lateral sclerosis Neurology*, 43,pp. 1316–1322

Salam, N.K., Adzhigirey, M., Sherman, W. and Pearlman, D.A. 2014. *Structure-based approach to the prediction of disulfide bonds in proteins, Protein Eng. Des. Sel., 27(10),* 365-74.

Shaw, P. J. 2001, *Genetic inroads in familial ALS*. Nature Genet. 29: 103-104.

Talbot, K. 2009. *Motor neuron disease: the bare essentials* Pract Neurol, 9, pp. 303–309

The MGC Project Team, 2004. *The status, quality, and expansion of the NIH full-length cDNA project: the Mammalian Gene Collection (MGC).* Genome Res. 14:2121-2127.

Van Damme, P., Dewil, M., Robberecht, W. and Van Den Bosch, L. 2005. *Excitotoxicity and amyotrophic lateral sclerosis Neurodegener* Dis, 2, pp. 147–159

Zhu, K., Day, T., Warshaviak, D., Murrett, C., Friesner, R. and Pearlman, D. 2014. *Antibody structure determination using a combination of homology modeling, energy-based refinement, and loop prediction, Proteins, 82(8),* 1646–1655.

****