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In Silico docking analysis of Amyloid Precursor Protein Intracellular Domain (AICD) with neuronal cytoplasmic and nuclear membrane proteins and its relevance in neuronal death and AD pathogenesis

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Abstract:

Recent evidences reveal the controlled intramembrane proteolysis of the C-terminal fragment of amyloid precursor-protein (APP) by gamma-secretases, yielding an additional 57 residue peptide fragment called APP intracellular domain (AICD). This AICD is known to interact with cytoplasmic and neuronal proteins, and trigger AD pathology. Appreciating the fact that, the key motifs in the c-terminal residues of AICD exhibit strong binding preferences, our study focuses on deciphering the modes of interactions with 16 select cytoplasmic and neuronal proteins via *in silico* methods. The results suggest that key residues of the AICD belonging to the YENTPY motif interact with most of the 16 functionally important neuronal proteins. Docking studies indicate that the proteins such as Lamin2, GRP78, ABAD, TOM20, TOM70, NUDC, HSPA8, TOG and neuroserpin interact very strongly with the AICD fragment. These computational results provide vital insights into the binding patterns of AICD with these crucial neuronal proteins, thus suggesting design and development of plausible inhibitors that could control the disease progression, neurodegeneration, and neuronal death, which are all potential hall marks of AD.

Keywords: Alzheimer's disease, Amyloid precursor-protein, AICD, Neurodegeneration, Neuronal proteins.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, occurring mostly in the age group greater than 65.[1] According to the World Alzheimer's Report 2015, the number of AD patients in India is an alarming 4.1 million, third only to China and USA. Pathological studies have revealed that AD brain comprises of 2 distinct proteins, the amyloid plaques formed by the proteolytic cleavage of Amyloid Precussor Protein (APP), and neurofibrillary tangles (NFTs) formed from tau protein, respectively.[2,3,4] The APP is proteolytically cleaved by different secretases via 2 distinct pathways, the amyloidogenic and the non amyloidogenic, as depicted in Fig.1.[5] Recent investigations have reveal that, controlled intramembrane proteolysis of the C-terminal fragment of APP by gamma-secretases, yield an additional 57 amino acid residue peptide called APP intracellular domain (AICD).[6] This AICD is in turn cleaved by secretases (epselon cleavage) and caspase 3 into JCASP (**VMLKKKQYTSIHHGVVEVD)** and C31 (A**AVTPEERHLSKMQQNGYENPTYKFFEQMQN**) fragments.[7] In spite of its small size and short half-life,[8] AICD has become the focus of studies as it has been reported in brains of patients with Alzheimer's disease (AD). In contrast to Aβ, the AICD is a physiological relevant protein domain, which modulates a diverse set of important APP functions including, trafficking and signal transduction.[9]

The full length AICD is known to interact with other proteins of the signalling pathway and up regulate the expression/ activity of APP, BACE1, LRP1, p53, and GSK3β.[10,11] This sequence of events finally leads to enhanced Aβ generation, apoptosis, Ca2+ signalling, cytoskeletal dynamics and tau phosphorylation,[12] which all results in neuronal and mitochondrial death.[11] Interestingly, both AICD and C31 can translocate to the nucleus and lead to apoptosis.[9] Though AICD is playing roles in neuronal death, the key motifs in C31(the c-terminal part of AICD) are known to be critical elements for triggering AD pathology.[13] The 757-YENPTY- 762 sequence is known to be a consensus motif for clathrin-mediated endocytosis and contains the consensus sequence for phosphotyrosine binding (PTB) domain interactions.[9] The other motif, 743-TPEE-746 is known to bind to other proteins aiding in translocation of the AICD into the nucleus.[9] It has been reported that the AICD binds to different intracellular binding partners ('adaptor protein'), which regulate its stability and cellular localization.[8] The adaptor proteins form complexes with the AICD influencing the production of Aβ.[9] AICD and its various adaptor proteins like nuclear adaptor protein Fe65 and transcription factor forkhead box O (FOX O) are thought to take part in various cellular events, including regulation of gene transcription, apoptosis, calcium signalling, growth factor, and NF-κB pathway activation, as well as the production, trafficking, and processing of APP, and the modulation of cytoskeletal dynamics.[14-18] Hence, there is a need to evaluate the C31 fragment to decipher the modes of binding of the motifs with key neuronal and cytoplasmic proteins, as this information could enable development of ligands as plausible drug molecules

which could disrupt the interactions and dissuade the AD events.

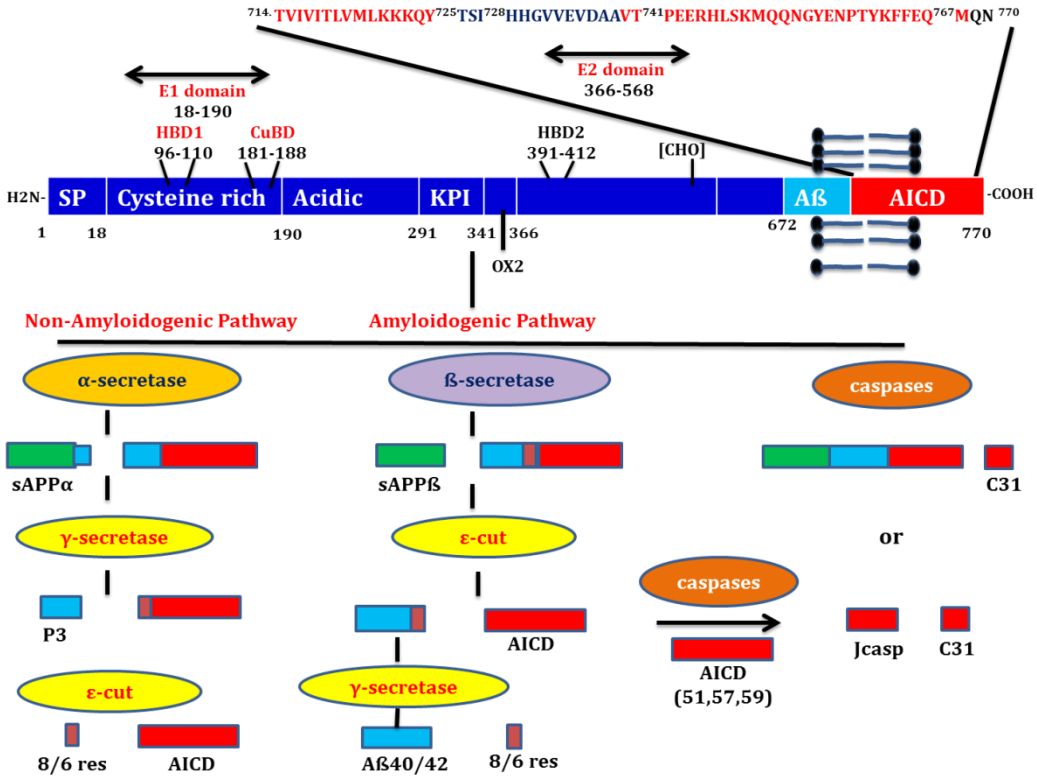

Fig. 1: Amyloidogenic and non-amyloidogenic pathways of APP. Amyloid precursor protein APP is a single pass transmembrane glycoprotein. APP may be cleaved by β–γ secretases (amyloidogenic) releasing amyloid Aβ peptide and AICD or by α–γ secretases (non-amyloidogenic) releasing AICD and P3.Thus formed AICD is instant cleaved by caspases yielding C31 and JCASP.[5]

Materials and Method

The protocol followed for the work is illustrated in Fig. 2.

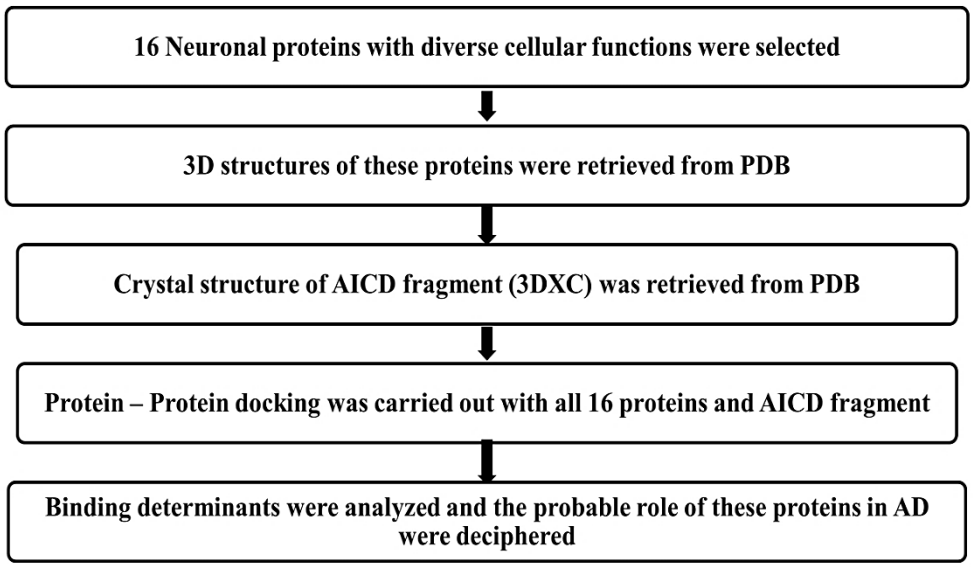

Fig. 2: Protocol for the studies

Based on the available literature, 16 cytoplasmic and neuronal proteins with diverse cellular functions were retrieved from Protein data bank (PDB).[19] The proteins were chosen looking at the location and its functional importance in neuronal cells. The functions of each of these proteins are indicated in Table 1. The PDB structure of the AICD fragment was retrieved from published sources.[20] Protein-peptide docking analysis was carried out using online tool Cluspro2.0.[21] The docking results were analysed using the visualization tool Discovery Studio.[22] Poses were saved for each docking combination, and the amino acid interactions were tabulated. Hydrogen bonds were computed around 5Angstroms distances from the interacting peptide atoms.

Table 1: Cellular locations of various proteins and their respective functions

Sl. No	Name of the protein	Uniprot ID	Location	PDB ID	Functions	Reference
1.	**Lamin B2**	Q03252	Nucleus inner membrane, Lipid-anchor, Nucleoplasmic side.	2LLL	Component of nuclear lamina.	Unpublished source
2.	**HSP90**	P14625	Endoplasmic reticulum lumen, Melanosome.	4NH9	Molecular chaperone with ATPase activity.	24
3.	**GRP78/HSPA5**	P11021	Endoplasmic reticulum lumen, Melanosome,	3IUC	Facilitates multimeric protein complex assembly inside ER.	25
4.	**ABAD**	Q2L8D9	Cytoplasm.	1U7T	ABAD is important for mitochondrial function, It facilitate the ketone body utilization by promoting the generation of acetyl coA to feed into TCA cycle an effect that is particularly important in situation of stress.	26
5.	**Cyclophilin D**	P30405	Mitochondria	2BIT	Involved in regulation of the mitochondrial permeability transition pore (mPTP).	27
6.	**TOM20**	Q62760	Mitochondrion matrix	3AX2	Central component of the receptor complex responsible for the recognition & translocation of cytosolically synthesized mitochondrial pre-proteins.	28
7.	**TOM70**	P07213	Mitochondrion outer membrane	2GW1	Component of the TOM (translocase of outer membrane) receptor complex responsible for the recognition & translocation of cytosolically synthesized mitochondrial pre-proteins.	29
8.	**Structure of the human voltage-dependent anion channel (VDAC-1)**	P21796	Mitochondrion outer membrane	2JK4	Forms a channel through the mitochondrial outer membrane and also the plasma membrane.	30
9.	**Structure of Human Bcl-XL at 1.95 Angstroms**	Q07817	Mitochondrion outer membrane	1R2D	Potent inhibitor of cell death. Inhibits activation of caspases. Appears to regulate cell death by blocking the voltage-dependent anion channel (VDAC) by binding to it and preventing the release of the caspase activator, CYC1, from the mitochondrial membrane. Also acts as a regulator of G2 checkpoint and progression to cytokinesis during mitosis.	31
10.	**NPD011/NUDC**	Q9Y266	Cytoplasm, Cytoskeleton, Nucleus.	3QOR	Neurogenesis & neuronal migration	32
11.	**APOA4**	P06727	Mitochondrion inner membrane, Cytoplasm, Nucleus membrane	3S84	May have a role in VLDL secretion and catabolism	33
12.	**HSPA8**	P11142	Cytoplasm, Cytoskeleton, Nucleus.	4H5R	Molecular chaperone	34
13.	**Solution structure of a pro-apoptotic protein BAX**	Q05655	Blood microparticle, Cytosol, Endoplasmic reticulum lumen	4BD2	Calcium-independent, phospholipid and diacylglycerol (DAG)-dependent serine/threonine-protein kinase that plays contrasting roles in cell death and cell survival by functioning as a pro-apoptotic protein during DNA damage-induced apoptosis, but acting as an anti-apoptotic protein during cytokine receptor-initiated cell death, is involved in tumor suppression as well as survival of several cancers.	35
14.	**Clip associating protein 1**	Q7Z460	Cytoplasm, Melanosome, Nucleus, Nucleolus, Cell membrane	4K92	Microtubule plus-end tracking protein that promotes the stabilization of dynamic microtubules	36
15.	**Galectin-1**	P09382	Cytoplasm, Nucleus, Mitochondrion	3T2T	May regulate apoptosis cell proliferation and differentiation	37
16.	**Neuroserpin**	Q99574	Extracellular space	3F5N	Involved in the formation or reorganization of synaptic connections as well as for synaptic plasticity in the adult nervous system.	38

Results and Discussions

The overall strengths of interactions and the set of residue interactions are tabulated in Table 2. Our results reveal the interaction of AICD with neuronal and cytoplasmic proteins. The docking poses are shown in Fig. 2. The active site was found using the online server tool fpocket 2.0 (38). The AICD binds to the active site and surrounding residues of all 16 proteins. Common interacting partners were analysed and are tabulated in Table 2. The pharmacophore pattern revealed that the residues E758, N759, T761 and Y757 of the AICD interact with 5/16, 8/16, 10/16 and 12/16 proteins respectively. Interestingly these residues form part of the YENPTY motif region in AICD (Table 3 and 4). It is also observed that, E745, R747, E766 and Q767 interact with 5/16, 7/16, 6/16, and 3/16 proteins respectively, indicating a common set of binding determinants across these 16 proteins.

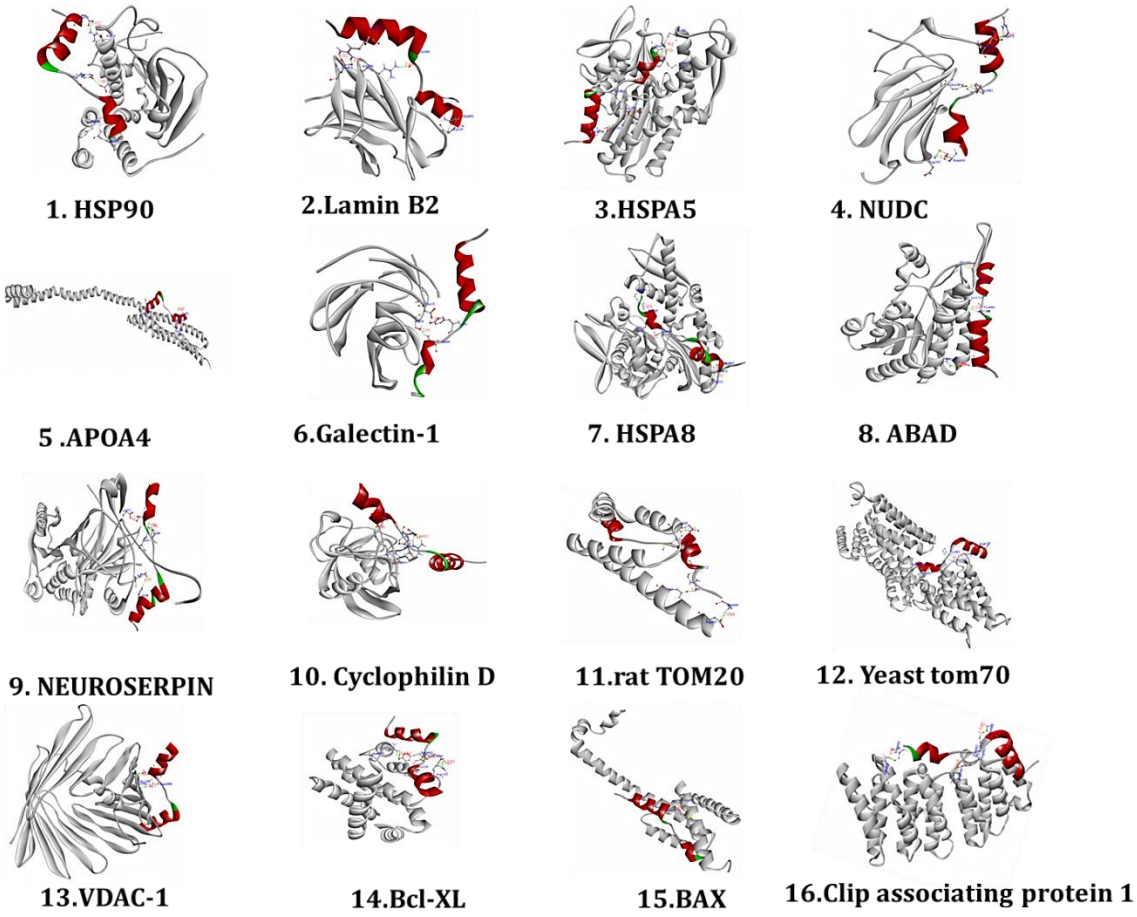

Fig. 3: AICD docking interactions with nuclear membrane and cytoplasmic neuronal protein

Thus this infers that proteins Lamin2, GRP78, ABAD, TOM20, TOM70, NUDC, HSPA8, TOG and neuroserpin interact with the AICD fragment, as indicated by the strength/number of interactions (refer Table 2). With strong evidences from literature (24, 25, 27, 28, 31, 33, 35, 37) that these proteins are important in neuronal functions, and binding of AICD to these proteins may disrupt their normal functions escalating the AD pathogenesis, our results reveal the probable interface of binding of AICD with these neuronal proteins, which provide clues towards their roles in disruption of normal functions and pathways leading to the neuronal death. These evidences will also help us to design suitable inhibitors that could prevent the AICD from binding to these key proteins, and triggering AD.

Table 2: AICD peptide protein interface interacting amino acid residues and their respective hydrogen bond distances

Proteins / AICD Residues	Lamin B2 (Aa)	HSP90Î²	GRP78/HSPA5	ABAD	Cyclophilin D	TOM20	TOM70	VDAC1	Human Bcl-XL	NPD011 / NUDC	APOA4	HSPA8	PROTEIN BAX	TOG	Galectin-1	Neuroserpin	No of Interacting proteins
745 E		R116 (2.56)	T247 (3.20)			Q67(4.02, 4.02)					L289 (4.35)	R322 (3.85)					5
747 R	D475 (2.93)			R130 (3.10, 3.10)		Q102 (3.22,3.24)	D378 (3.10, 2.86)			E272 (2.84)		E318 (2.65), D315 (3.20)				E278 (2.96, 3.20,2.82)	7
748 H										L178 (3.21)						E227 (3.30)	2
752 M						Q105 (3.17, 3.66)							Q32 (3.01), Q28 (3.35)				2
753 Q				D119 (4.01), Q115 (3.22)		Q105 (3.65)											2
755 N							R376(4.56, 5.00)							R482 (3.01)			2
757 Y	Q467 (2.18), T510 (3.01)	D110 (2.56)	D250 (3.56)	A170 (3.91)	H54 (4.10)		D375 (3.54)		E129 (3.56), R103 (3.01)	E180 (3.10)	E301(3.11),R304(4.11)			R452 (3.10)	D123 (3.01)	E227 (3.65)	12
758 E			Q110 (3.16)		R151 (3.01, 2.96)				R132(3.10, 2.29)					R444 (3.01)		Q369 (2.55)	5
759 N	E458 (3.10)		D111 (3.15)	S169 (3.00), A158 (3.00)				S196 (3.81)		D182 (3.01)		H89 (3.75)			S29(3.50)	R259(2.85,3.01,3.34)	8
761 T	E458 (2.86)	D110 (3.01)	D257 (3.01)	A158 (3.10)	R55 (3.10)		Q405 (3.10)	G195 (3.54)			E308 (3.54)	E231 (3.01)				D373 (3.1,2.5)	10
766 E			R289(3.40) N282 (3.54)				S541(3.01) S253 (3.25)		R139(3.01) R136(3.45)	T242 (3.01)	Y204 (3.01)			R367 (2.65, 2.65)			6
767 Q	A519 (3.55)	F195 (3.45)										N235, (3.03), N239,(3.01), R264,(2.56)					3
768 M										D237 (3.01)				Q364 (4.15)			2
Total residue interacting	6	4	7	7	3	4	5	2	4	6	5	8	2	5	2	6	
Strength	V.S	S	V.S	V.S	W	S	V.S	V.W	S	V.S	V.S	V.S	W	V.S	W	V.S	

7-5: Very Strong (V.S): 4: Strong(S): 3: WEAK (W) Very Weak (V.W)

Table 3: TPEE motif interface interacting amino acid residues and their respective hydrogen bond distances

PROTEINS	LAMIN B2 (Aa)	HSP90Î²	GRP78/ HSPA5	ABAD	TOM20	TOM70	NPD011/ NUDC	APOA4	HSPA8	Neuroserpin	Total
743 T											-
744 P									R322 (3.10)		1
745 E		R116 (2.56)	T247 (3.20)		Q67 (4.02, 4.02)			L289 (4.35)	R322 (3.85)		5
746 E											-
747 R	D475 (2.93)			R130 (3.10, 3.10)	Q102 (3.22 ,3.24)	D378(3.10,2.86)	E272 (2.84)		E318 (2.65), D315 (3.20)	E278 (2.96, 3.20, 2.82)	7

Table 4: YENPTY motif interface interacting amino acid residues and their respective hydrogen bond distances

PROTEINS	Lamin B2 (Aa)	HSP90Î²	GRP78/ HSPA5	ABAD	Cyclophilin D	TOM70	VDAC1	Human Bcl-XL	NPD011/ NUDC	APOA4	HSPA8	TOG	Galectin-1	Neuroserpin	Total
757 Y	Q467(2.18), T510 (3.01)	D110 (2.56)	D250 (3.56)	A170 (3.91)	H54 (4.10)	D375 (3.54)		E129 (3.56), R103 3.01)	E180 (3.10)	E301(3.11), R304 (4.11)		R452 (3.10)	D123 (3.01)	E227 (3.65)	12
758 E			Q110 (3.16)		R151 (3.01,2.96)			R132(3.10,2.29)				R444 (3.01)		Q369 (2.55)	5
759 N	E458 (3.10)		D111 (3.15)	S169 (3.00), A158 (3.00)			S196 (3.81)		D182 (3.01)		H89 (3.75)		S29 (3.50)	R259 (2.85, 3.01, 3.34)	8
760 P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
761 T	E458 (2.86)	D110 (3.01)	D257 (3.01)	A158 (3.10)	R55 (3.10)	Q405 (3.10)	G195 (3.54)			E308 (3.54)	E231 (3.01)			D373 (3.1, 2.5)	10
762 Y				G164 (3.21)											1

Conclusions

The present study uses an approach to analyze the AICD peptide and its influence on AD, in the context of its interaction with significant neuronal proteins and its plausible roles toward the disease pathogenesis. We deciphered a set of candidate proteins with diverse cellular functions, which can strongly interact with AICD. Our results indicate that the interaction of the key proteins encompassing the YENPTY domain correlates with earlier literature.[9] The binding of these proteins may demonstrate the cascade amplification of the pathologic signals. Taken together, these results reveal that the YENTPY domain may be a potential drug target towards preventing the binding of neuronal proteins to AICD, and offer clues towards designing suitable natural inhibitors to prevent the onset of AD pathogenesis.

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References

1. Kosik KS. "The Alzheimer's disease sphinx: a riddle with plaques and tangles". *J Cell Biol.* (1994), 127:1501-4.
2. Götz J. "Tau and transgenic animal models". *Brain Res Brain Res Rev*.(2001) 35(3):266-86.
3. Palmer AM. "Neurochemical studies of Alzheimer's disease". *Neurodegeneration.* (1996), 5(4):381-91.
4. von Rotz RC1, Kohli BM, Bosset J, Meier M, Suzuki T, Nitsch RM, Konietzko U." The APP intracellular domain forms nuclear multiprotein complexes and regulates thetranscription of its own precursor". *J Cell Sci. (*2004); 1: 4435-48.
5. Wang H, Li R, Shen Y. "β-Secretase: its biology as a therapeutic target in diseases". *Trends Pharmacol Sci*. (2013) 34(4):215-25.
6. Haass C, Kaether C, Thinakaran G, Sisodia S. Trafficking and proteolytic processing of APP. *Cold Spring Harb Perspect Med*. (2012), 2(5):a006270..
7. Park SA, Shaked GM, Bredesen DE, Koo EH. 'Mechanism of cytotoxicity mediated by the C31 fragment of the amyloid precursor protein". *Biochem Biophys Res Commun*. (2009) 16;388(2):450-5.
8. Raychaudhuri M, Mukhopadhyay D. 'AICD and its adaptors - in search of new players". *J Alzheimers Dis*. (2007) 11(3):343-58.
9. Chang KA, Suh YH. "Possible roles of amyloid intracellular domain of amyloid precursor protein". *BMB Rep.* (2010);43(10):656-63.
10. Słomnicki LP, Leśniak W. "A putative role of the Amyloid Precursor Protein Intracellular Domain (AICD) in transcription". *Acta Neurobiol Exp* (Wars).(2008);68(2):219-28.
11. Kim HS, Kim EM, Lee JP, Park CH, Kim S, Seo JH, Chang KA, Yu E, Jeong SJ, Chong YH, Suh YH. "C-terminal fragments of amyloid precursor protein exert neurotoxicity by inducing glycogen synthase kinase-3beta expression", *FASEB J*. (2003), 17(13):1951-3.
12. Müller T, Meyer HE, Egensperger R, Marcus K. "The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics-relevance for Alzheimer's disease". *Prog Neurobiol.* (2008), 85(4):393-406.
13. Lu DC, Soriano S, Bredesen DE, Koo EH. "Caspase cleavage of the amyloid precursor protein modulates amyloid beta-protein toxicity". *J Neurochem*. (2003), 87(3):733-41.
14. Wang X, Wang Z, Chen Y, Huang X, Hu Y, Zhang R, Ho MS, Xue L. "FoxO mediates APP-induced AICD-dependent cell death". *Cell Death Dis*. (2014), 15;5:e1233.
15. Chakrabarti, Arunabha, Chatterjee, Atri Sengupta, Mohor B. Chattopadhyay, Partha Mukhopadhyay, Debashis: "Altered Levels of Amyloid Precursor Protein Intracellular Domain-interacting Proteins in Alzheimer Disease". *Alzheimer Disease & Associated Disorders* (2014),28,283–290.
16. Arunabha Chakrabarti, Kasturi Roy, Debashis Mukhopadhyay. "Differential Expression of Neuroblastoma Cellular Proteome due to AICD Overexpression" *Journal of Alzheimer's disease* (2013)*:*38(4).
17. Mithu Raychaudhuri · Debashis Mukhopadhyay "AICD overexpression in neuro 2A cells regulates expression of PTCH1 and TRPC5" *International Journal of Alzheimer's Disease* (2011), 21.
18. Samir Das · Mithu Raychaudhuri · Udayaditya Sen · Debashis Mukhopadhyay "Functional Implications of the Conformational Switch in AICD Peptide upon Binding to Grb2-SH2 Domain" *Journal of Molecular Biology (*2011) 414(2):217-30.
19. Berman HM, Westbrook J, Feng Z, et al. "The Protein Data Bank". *Nucleic Acids Res*. (2000);28(1):235-42.
20. Radzimanowski, J., Simon, B., Sattler, M., Beyreuther, K., Sinning, I., Wild, K "Structure of the intracellular domain of the amyloid precursor protein in complex with Fe65-PTB2". *Embo Rep* (2008). 9: 1134-1140.
21. Stephen R. Comeau, David W. Gatchell, Sandor Vajda, and Carlos. Camacho: ClusPro: a fully automated algorithm for protein–protein docking" *Nucleic Acids Res*. (2004) 1; 32.
22. Discovery studio (DS) (Discovery Studio 3.5, Accelrys Inc., San Diego, California, (http://www.accelrys.com/). USA).
23. Ernst, J.T., Liu, M., Zuccola, H., Neubert, T., Beaumont, K., Turnbull, A., Kallel, A., Vought, B., Stamos D, "Correlation between chemotype-dependent binding conformations of HSP90 alpha / beta and isoform selectivity-Implications for the structure-based design of HSP90 alpha / beta selective inhibitors for treating neurodegenerative diseases". *Bioorg. Med. Chem. Lett.* (2014), 24: 204-208.
24. Wisniewska, M., Karlberg, T., Lehtio, L., Johansson, I., Kotenyova, T., Moche, M., Schuler, H. "Crystal structures of the ATPase domains of four human Hsp70 isoforms: HSPA1L/Hsp70-hom, HSPA2/Hsp70-2, HSPA6/Hsp70B', and HSPA5/BiP/GRP78" *Plos One*, (2010) 5: e8625-e8625.
25. Kissinger, C.R., Rejto, P.A., Pelletier, L.A., Thomson, J.A., Showalter, R.E., Abreo, M.A., Agree, C.S., Margosiak, S., Meng, J.J., Aust, R.M., Vanderpool, D., Li, B., Tempczyk-Russell, A., Villafranca, J.E, "Crystal structure of human ABAD/HSD10 with a bound inhibitor: implications for design of Alzheimer's disease therapeutics" *J. Mol. Biol* (2004). 342: 943-952.
26. Schlatter, D., Thoma, R., Kueng, E., Stihle, M., Mueller, F., Boroni, E., Hennig, M. "Crystal Engineering Yields Crystals of Cyclophilin D Diffracting to 1.7 A Resolution" *ActaCrystallogr., Sect. D* , (2005) 61: 513.
27. Saitoh, T., Igura, M., Miyazaki, Y., Ose, T., Maita, N., Kohda, D "Crystallographic snapshots of tom20-

mitochondrial presequence interactions with disulfide-stabilized peptides". *Biochemistry*.(2011) 50: 5487-5496.

28. Wu, Y., Sha, B "Crystal structure of yeast mitochondrial outer membrane translocon member Tom70p" *Nat. Struct. Mol. Biol.* (2006) 13:589-593.
29. Bayrhuber, M., Meins, T., Habeck, M., Becker, S., Giller, K., Villinger, S., Vonrhein, C., Griesinger, C., Zweckstetter, M., Zeth, K "Structure of the Human Voltage-Dependent Anion Channel", *Proc. Natl. Acad. Sci. USA* (2008) 105:15370-15375.
30. Manion, M.K., O'Neill, J.W., Giedt, C.D., Kim, K.M., Zhang, K.Y., Hockenbery, D.M "Bcl-XL mutations suppress cellular sensitivity to antimycin A", *J. Biol. Chem*.(2004). 279: 2159-2165.
31. Zheng, M., Cierpicki, T., Burdette, A.J., Utepbergenov, D., Janczyk, P.L., Derewenda, U., Stukenberg, P.T., Caldwell, K.A., Derewenda, Z.S "Structural Features and Chaperone Activity of the NudC Protein Family". *J. Mol. Biol*, (2011). 409: 722-741.
32. Deng, X., Morris, J., Dressmen, J., Tubb, M.R., Tso, P., Jerome, W.G., Davidson, W.S., Thompson, T.B "The Structure of Dimeric Apolipoprotein A-IV and Its Mechanism of Self-Association", *Structure*.(2012) 20: 767-779.
33. Zhang, Z., Cellitti, J., Teriete, P., Pellecchia, M., Stec, B "New crystal structures of HSC-70 ATP binding domain confirm the role of individual binding pockets and suggest a new method of inhibition". *Biochimie*.(2015) 108: 186-192.
34. Czabotar, P.E., Westphal, D., Dewson, G., Ma, S., Hockings, C., Fairlie, W.D., Lee, E.F., Yao, S., Robin, A.Y., Smith, B.J., Huang, D.C., Kluck, R.M., Adams, J.M., Colman, P.M 'Bax Crystal Structures Reveal How Bh3 Domains Activate Bax and Nucleate its Oligomerization to Induce Apoptosis". *Cell (Cambridge, Mass.)*.(2013) 152: 519.
35. J.B., Rogers, S.L., Slep, K.C "A cryptic TOG domain with a distinct architecture underlies CLASP-dependent bipolar spindle formation. Leano", *Structure*.(2013) 21: 939-950.
36. Collins, P.M., Oberg, C.T., Leffler, H., Nilsson, U.J., Blanchard, H "Taloside inhibitors of galectin-1 and galectin-3". *Chem. Biol. Drug Des* (2012). 79: 339-346.
37. Ricagno, S., Caccia, S., Sorrentino, G., Antonini, G., Bolognesi, M. "Human neuroserpin: structure and time-dependent inhibition" *J. Mol. Biol.* (2009) 388: 109-121.
38. BMC Bioinformatics. 2009; 10: 168.Published online 2009 Jun 2. doi: 10.1186/1471-2105-10-168 PMCID: PMC2700099 Fpocket: An open source platform for ligand pocket detection.