Computational study of intermolecular interactions between α-synuclein fibrils and Tau protein propagating Tau aggregation

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α-Synuclein is the principal component responsible for the onset of Parkinson's disease, a neurodegenerative disorder. It has been recently suggested that α-synuclein fibrils probably interact with Tau protein, inhibit its function to stabilize microtubules, and also promote Tau aggregation, leading to dysfunction of neuronal cells. Here, we have studied the interactions between α-synuclein fibrils and Tau protein. The results show that the basic region of Tau protein strongly interacts with the C-terminal acidic regions of α-synuclein fibrils, and undergoes conformational change resulting in the formation of seed for assembly of Tau into amyloid-like fibrils.

Keywords: Fibrils, microtubules, intermolecular interactions, neurodegenerative disorders.

PARKINSON'S disease (PD) is one of the familiar neurodegenerative diseases like Alzheimer's disease (AD), that is characterized by progressive tremor, bradykinesia, muscular rigidity and postural-reflex impairment. It affects about 1–2% of the population above the age of 65 years. PD is signified by the loss of dopaminergic neurons in the substantia nigra of the brain and the presence of intracellular inclusion bodies, i.e. Lewy bodies (LB) and Lewy neuritis (LN). LB and LN are the pathological hallmark of PD. Till now, several missense mutations (A30P, A53T, E46K, H50Q and G51D) have been associated with PD and also with dementia with Lewy bodies (DLB)[1–6]. The main component present in LB and LN is α-synuclein protein, which consists of 140 amino acid residues[7–9]. It is expressed in the presynaptic nerve terminals of several parts of the brain region comprising 1% of total cytosolic protein[10]. Reports have suggested that α-synuclein proteins assemble to form a fibril structure which exhibits the same morphology as in LB[11]. The fibril structure consists of cross-β fibre diffraction patterns which are analogous to those of amyloid fibrils[12]. Many studies have highlighted the probable toxic effect of α-synuclein fibrils and intermediated proto-fibrils on the neuronal cells[6,13,14]. It has been reported that the truncated form of α-synuclein in the C-terminal shows an increased fibrillation rate, which suggests that the C-terminal region inhibits oligomerization and aggregation to a positive extent[11]. α-Synuclein fibrils have been shown to interact with Tau protein through the C-terminal region, thereby affecting the microtubule assembly and stabilization[15]. α-Synuclein fibrils upon binding to Tau protein inhibit the tau-promoting microtubule self-assembly. This leads to the disruption of microtubules, which thereby results in neuronal dysfunction. This abnormal activity is due to the structural conformer of the fibrils. This effect was not prominent in the monomeric form of α-synuclein[15]. The abnormal function responsible for the cytotoxicity of α-synuclein is due to the transformation of monomeric form of α-synuclein to filamentous form, which is considered to be the core event[15]. Several *in vitro* studies reported that α-synuclein has the capacity to induce Tau protein fibrillation, and that co-incubation of Tau and α-synuclein promotes mutual fibrillation synergistically[16,17]. Recent studies have suggested that various compounds bind to the C-terminal region of α-synuclein due to its acidic nature that inhibits filament formation[18], which increases the possibility that the reactivity of the C-terminal is reduced by binding to such compounds. The C-terminal has a strong affinity for proteins that are positively charged and may contribute to pathogenic interactions with other compounds. These compounds may suppress the cytotoxicity also and may inhibit filament formation of α-synuclein fibrils[19]. Further studies should be more focused for a better understanding of the pathogenesis of PD. Also it is seen from the literature that various missense mutants such as A53T and E46K form fibrils at an increased rate[20,21] and that A30P mutation can promote the oligomerization[22] of α-synuclein and form fragile fibrils[23]. However, advanced studies are required to define *in vivo*, whether α-synuclein or Tau fibrils may interact through an ionic interaction at the initial or early stage of fibrillation propensity. Figure 1 provides a schematic representation of the formation of Tau seed structure on α-synuclein fibril.

In this computational study, we have used PDBSum[24], on-line server for an overview of the interaction between the chains, interactions across any selected interface and

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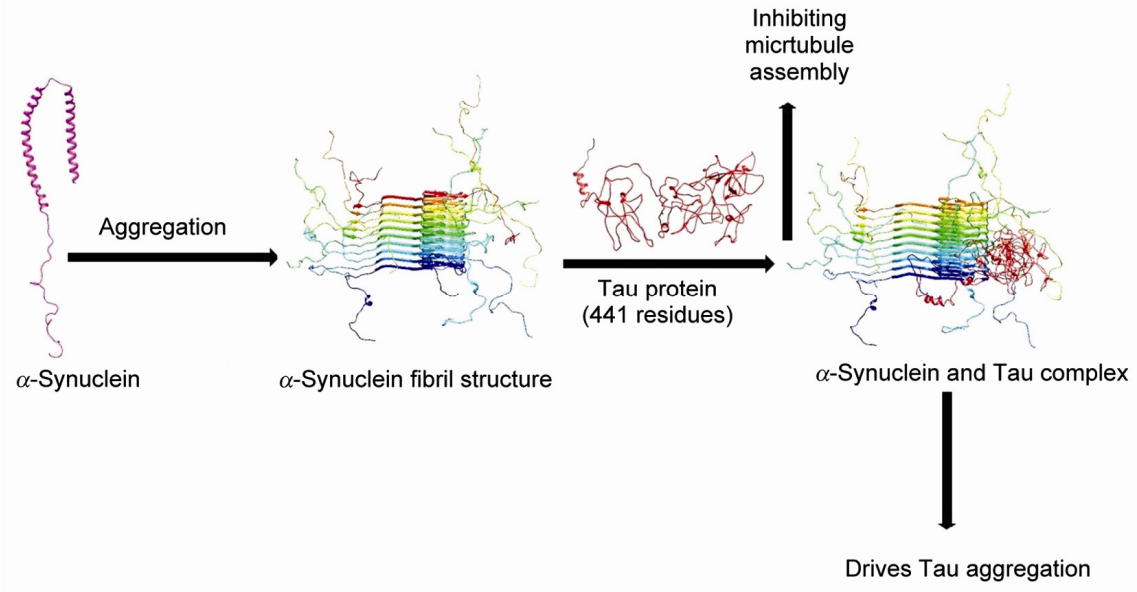

Figure 1. Schematic representation of formation of Tau seed structure on α-synuclein fibrils.

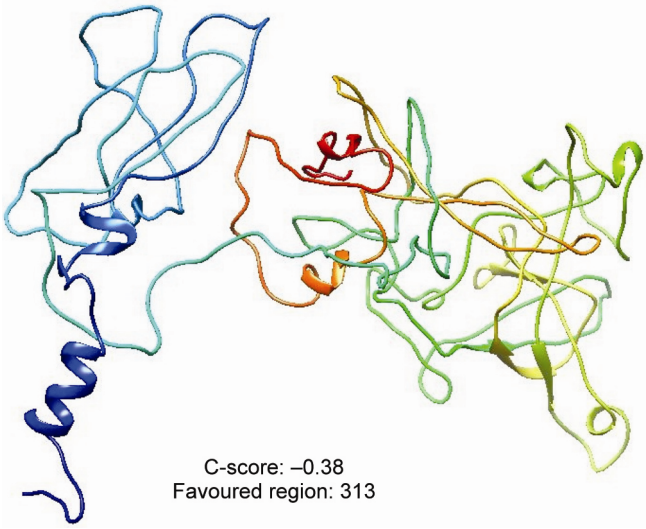

Figure 2. The best model structure of Tau protein obtained from the I-TASSER (Iterative Threading ASSEmbly Refinement) server.

residues actually interacting across the interface of Tau protein with α-synuclein fibril and with monomer structure. We observed that α-synuclein monomer and fibril structure interact with Tau protein through the C-terminal acidic regions, which may be considered to affect the microtubule assembly and stabilization. It has been suggested in an earlier study that abnormal interaction of Tau protein with α-synuclein arises because of conformational change of the latter[15]. This might lead not only to the dysfunction of Tau and α-synuclein, but also propagate Tau aggregation. From our interaction study, we noticed that α-synuclein fibrils possess a unique conformation in which both the N- and C-terminals are exposed as reported earlier[15]. The results of the present study show that the basic region of Tau protein strongly interacts with the C-terminal acidic regions of α-synuclein fibrils and undergoes conformational change resulting in the formation of seed for assembly of Tau into amyloid-like fibrils. We can thus consider that the monomeric and filamentous forms are the core events for appearance of abnormal α-synuclein function that is responsible for cytotoxicity. Consequently, designing inhibitors for α-synuclein monomeric and fibril forms might be the potential target for therapeutic intrusion in PD.

Methodology

Structure modelling and PDB structures

We used the α-synuclein (1XQ8) structure and high-resolution NMR α-synuclein amyloid fibril structure[25] (PDB ID: 2N0A) from the Protein Data Bank (PDB)[26] to study the intermolecular interactions between α-synuclein monomer and Tau protein, as well as α-synuclein fibril and Tau protein. The 3D structure of full-length Tau protein is not available. Therefore, we modelled the 3D structure of Tau protein by submitting its amino acid sequence to the I-TASSER server (Iterative Threading ASSEmbly Refinement)[27]. The secondary structure of the protein using Profile–Profile Alignment (PPA) threading techniques is predicted in the server. From I-TASSER, the best modelled structure was identified based on the *C*-score value. In this study, the C-score value for the best modelled structure of Tau protein was –0.38. Figure 2 shows the best modelled structure of Tau protein obtained from the I-TASSER server.

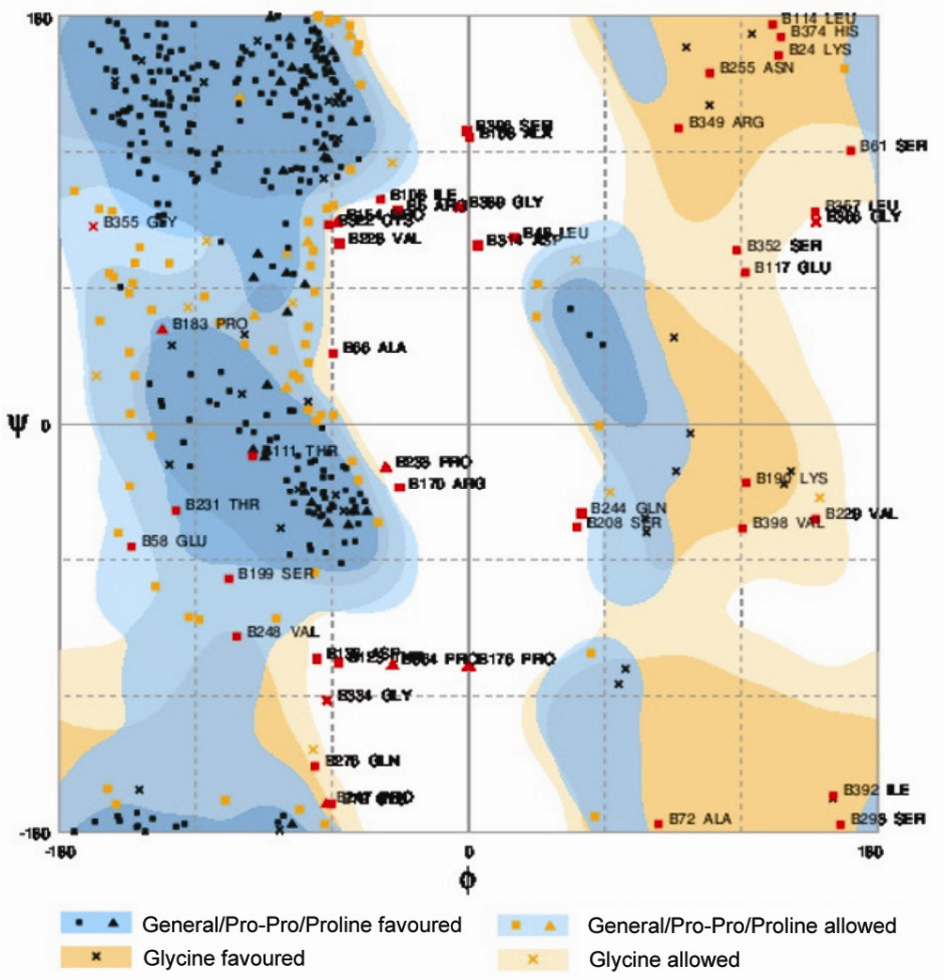

Figure 3. Ramachandran plot of the best model conformer of Tau protein. Black dots and crosses denote the residues in the most favoured regions and orange dots and crosses indicate the residues in allowed regions.

Table 1. Secondary structure details of modelled Tau protein as predicted by YASARA

Secondary contents	Percentage (%)
α-helix	2.7
3_{10} helix	0.9
π-helix	0.00
Sheets	7.5
Turn	14.5
Coil	74.4

Rigid docking

We used PatchDock[28] on-line docking server to advocate a rigid docking model for α-synuclein (monomer/fibril structure) – Tau protein. From a set of two molecules, the on-line server computes the 3D transformations of one of them with respect to the other, such that surface shape complementarity would maximize and number of steric clashes minimize. PatchDock[28] is based on the complementarity principles and geometry docking algorithm. The server forms a receptor–ligand complex and depending on the atomic contact energy (ACE), scores the top conformers. It, however, finds the optimum candidate solutions and removes redundant solutions using RMSD (root mean square deviation) clustering. Each candidate solutions is given a particular score based on the atomic desolvation energy[29] and geometric fit. In this study, the RMSD value was kept as 4 Å for clustering the solutions.

Refinement

For refinement of the α-synuclein (monomer/fibril structure)–Tau protein conformers obtained from PatchDock[28], we used the FireDock[30] server which is based on the global energy algorithm. The FireDock[30] server refines and scores the candidate solutions according to an energy function from a set of transformations. The individual candidate solutions are refined by rigid-body orientation and optimization of side-chain conformers. The side-chain flexibility of the protein is modelled by a rotamer library. The optimal combination of rotamers for the

interface residues is found by solving an integer linear programming (LP) problem[31]. The LP minimizes a partial energy function consisting repulsive van der Waals and rotamer probabilities terms. After arranging the side chains, the relative position of the docking partner is thereby refined by Monte Carlo minimization of the scoring function. The obtained refined candidates are then ordered according to an energy-based score. This score consists of atomic contact energy[29], electrostatics, hydrogen-bonding, softened van der Waals interactions and additional estimations of the binding free energy. The server by fast rigid-body docking algorithms, targets the flexibility problem and solutions scoring formed. It refines according to an energy function by spending about 3.5 sec per candidate solution from a total of 1000 potential docking candidate solutions.

Protein–protein interaction studies

To check the protein–protein interaction and evaluate the interface residues in the complex structures of α-synuclein (monomer/fibril structure)–Tau protein, the solution conformer having largest surface contact area, ACE value and geometrical shape complementarity score obtained from PatchDock[28] was submitted to the PDBSum server[24]. Here the interface residues of a protein are described as those residues whose contact distances from the interacting protein partner are less than 6 Å.

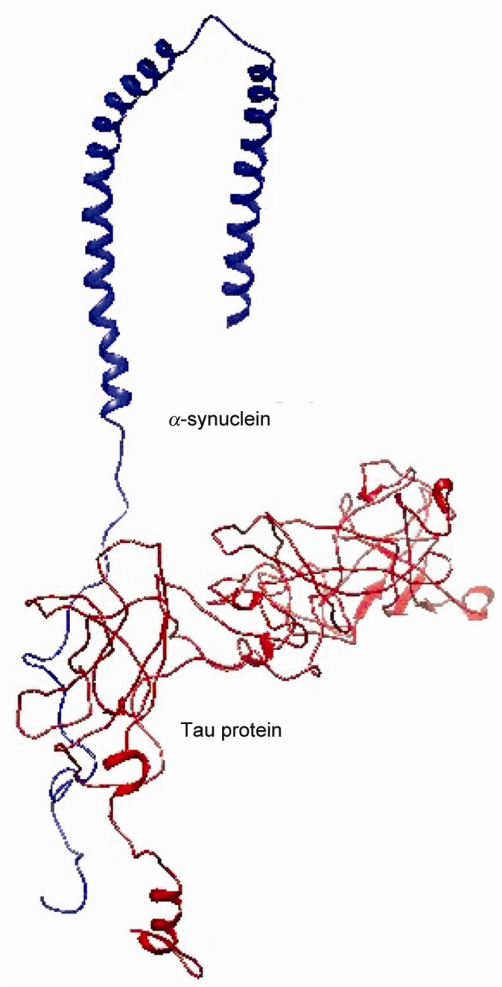

Figure 4. The complex structure of α-synuclein monomer and modelled Tau protein obtained from PatchDock based on geometric shape complementarity score, atomic contact energy and surface contact area.

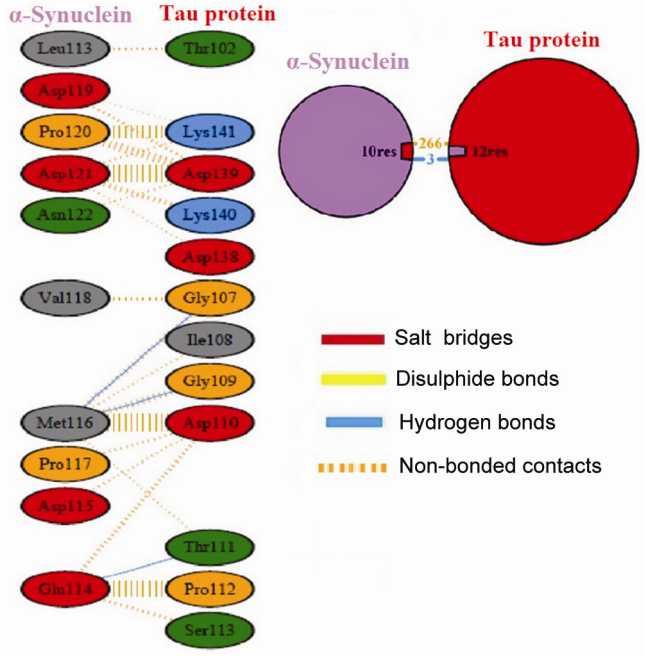

Figure 5. Summary of the interface and possible interacting residues across the interface of α-synuclein monomer/Tau complex obtained from the PDBSum server.

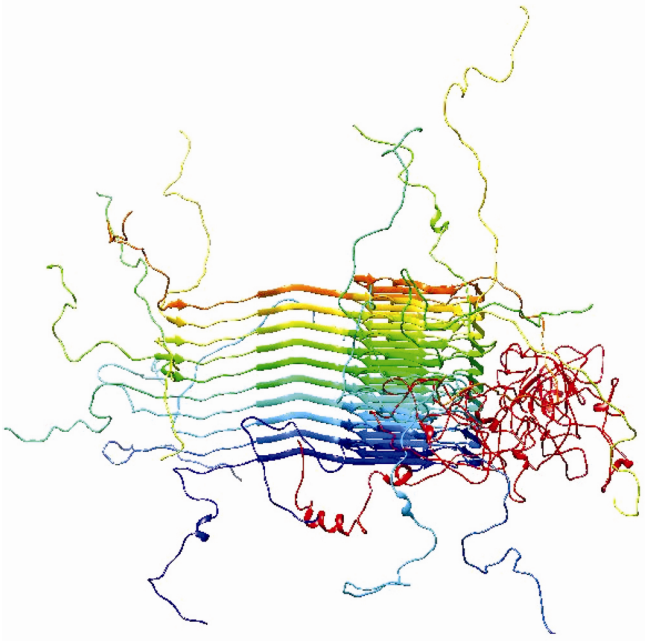

Figure 6. Schematic representation of the complex obtained from FireDock showing the α-synuclein fibril and Tau protein.

Table 2. Summary of interface statistics of protein–protein interactions for α-synuclein monomer and modelled Tau protein showing interface area, number of interface residues, salt bridges, disulphide bonds, hydrogen bonds and non-bonded contacts

Chain	No. of interface residues	Interface area (Å)^2	No. of salt bridges	No. of disulphide bonds	No. of hydrogen bonds	No. of non-bonded contacts
A	10	608	–	–	3	266
B	12	522	–	–		

Table 3. Summary of interface statistics of protein–protein interactions for α-synuclein fibril structure and modelled Tau protein showing interface area, number of interface residues, salt bridges, hydrogen bonds and non-bonded contacts

Chain	No. of interface residues	Interface area (Å)^2	No. of salt bridges	No. of disulfide bonds	No. of hydrogen bonds	No. of non-bonded contacts
A><B	71 : 70	2789 : 2787	1	–	73	430
B><C	70 : 69	2792 : 2811	1	–	71	419
C><D	75 : 74	2947 : 2964	1	–	72	455
D><E	74 : 72	2958 : 2970	1	–	72	426
E><F	73 : 71	2916 : 2951	1	–	71	422
F><G	75 : 73	2889 : 2906	1	–	74	439
G><H	76 : 72	2997 : 3021	1	–	72	430
H><I	72 : 69	2818 : 2863	1	–	72	433
I><J	75 : 70	2916 : 2962	3	–	72	438
A><Tau	5 : 2	283 : 258	–	–	3	36
B><E	2 : 1	37 : 50	–	–	–	3
B><Tau	14 : 17	918 : 687	–	–	4	443
C><Tau	21 : 30	1576 : 1295	1	–	5	543
D><F	1 : 1	48 : 50	–	–	–	1
D><Tau	23 : 40	1842 : 1400	2	–	8	838
E><G	2 : 2	132 : 128	–	–	–	5
E><Tau	13 : 22	1004 : 844	–	–	2	355
F><Tau	8 : 10	386 : 380	–	–	–	80
G><Tau	4 : 4	224 : 248	–	–	–	24
H><Tau	4 : 4	216 : 225	–	–	1	36
J><Tau	18 : 20	1013 : 861	2	–	–	350

Results and discussion

Structure modelling and validation

We modelled the Tau structure (Figure 2) from I-TASSER[27]. The server generated five models using threading programs. Based on *C*-score, the best model was selected. We carried out structural validation for the model Tau structure by constructing the Ramachandran plot using RAMPAGE server. In the modelled structure of Tau, we noticed that 71.3% of the residues were in the most favoured region and 10.5% in the disallowed region (Figure 3).

Secondary structure analysis of Tau protein

The secondary structure analysis of modelled Tau protein was carried out using the YASARA software[32]. The secondary structure content of the Tau protein was obtained from the 3D structure of the protein which gives details about the total percentage of secondary structure contents. Table 1 provides details about the secondary structure percentage contents of Tau protein. From the table, we can observe that the number of random coils in Tau protein is predominant compared to the other secondary structure contents (β-turns, extended strand, α-helix, 3_{10} helix and π-helix). The percentage of random coils was observed to be around 60.32, which suggests that coils are the main predominating factor for stabilizing the Tau protein.

Interaction of monomeric α-synuclein with modelled Tau protein

To investigate the effect of monomeric α-synuclein on Tau protein, we prepared the model complex structure from PatchDock[28]. The complex structure was prepared by submitting the monomeric α-synuclein (receptor) and modelled Tau protein (ligand) to the PatchDock[28] on-line server. Figure 4 shows the complex structure of α-synuclein and modelled Tau protein based on geometric shape complementarity score, ACE value and surface contact area obtained from PatchDock[28]. The conformers obtained from PatchDock[28] were then refined using

FireDock[30] that is ranked based on global energy, ACE and attractive van der Waals, repulsive van der Waals and hydrogen bonding.

For the complex model structure of α-synuclein/Tau having maximum interface area (4466.70 Å^2), we analysed the interacting residues using PDBSum[24]. From this, the interface and possible interacting residues across the interface of α-synuclein/Tau complex were obtained (Figure 5). Table 2 summarizes the interface statistics results.

From Table 2, it can be seen that the highly acidic C-terminal region of α-synuclein binds to Tau protein (basic region: 164–226) which leads to strong intermolecular interactions, thereby promoting Tau aggregation as reported in the earlier studies[15]. This unusual interaction of α-synuclein with Tau protein is due to the conformational change of α-synuclein inducing both dysfunction of Tau protein and its propagation.

Interaction of fibril structure of α-synuclein with modelled Tau protein

We investigated the effect of α-synuclein fibrils on Tau protein that might promote aggregation of Tau by protein–protein interaction studies. For this, we prepared the complex structures of α-synuclein fibril with Tau protein from the PatchDock[28] on-line server.

The complex structures obtained were refined using FireDock[30]. They were ranked based on global energy, atomic contact energy, attractive van der Waals, repulsive van der Waals and hydrogen bonding. The conformer (Figure 6) having maximum interface area (7894 Å^2), was analysed to check the interacting residues using PDBSum[24] server. From this server, the protein–protein interactions between the fibril structure of α-synuclein and Tau protein were studied (Figure 7). Table 3 summarizes the interface statistics.

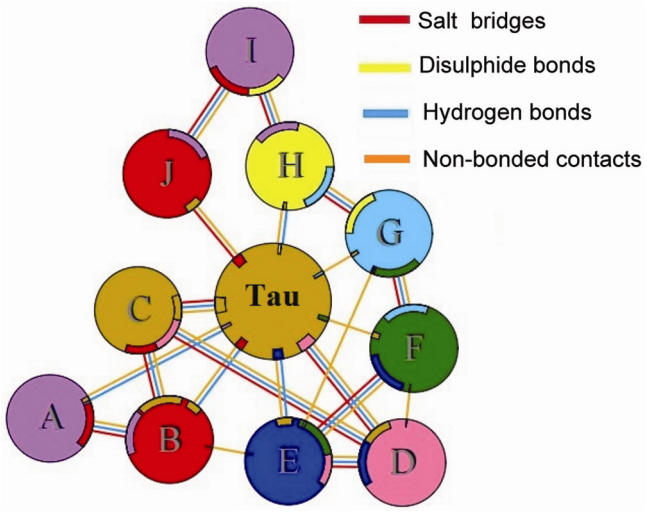

Figure 7. Summary of the protein–protein interactions between the fibril structure of α-synuclein (chain: A–J) and Tau protein (centre) obtained from the PDBSum server.

We found that α-synuclein fibril interacts with the Tau protein through the C- and N-terminal regions. Thus, we observed that the α-synuclein fibril possesses a distinct conformation wherein the N- and C-terminal regions get exposed. This is due to the unique conformer of α-synuclein fibril structure. This change in the conformer for the fibril structure of α-synuclein resulted in the abnormal interaction of Tau with the α-synuclein fibril structure. This intermolecular interaction strongly affects the microtubule assembly and stabilization. As reported in earlier studies, the increase in toxicity is due to the structural features of the α-synuclein fibril[15]. However results of the present study strongly suggest that Tau binds to α-synuclein at the highly acidic C-terminal, region which is crucial for inhibition of Tau-promoted microtubule assembly and also for aggregation of the Tau protein.

Conclusion

In this study, we have demonstrated the intermolecular interactions between α-synuclein (monomer/fibril structure) and Tau protein. We observed that α-synuclein interacts with Tau protein mainly through the N- and C-terminal regions. Also we found that the basic region of Tau protein interacts with C-terminal acidic region of α-synuclein which may lead to toxic functioning of α-synuclein, thereby promoting Tau aggregation and inhibiting Tau-promoted microtubule assembly. The non-bonded contacts, interface area and interface residues between α-synuclein (fibril structure) and Tau protein were however, found to be more than those between α-synuclein (monomer) and Tau protein. The findings of this study suggest that due to the binding of the C-terminal region of α-synuclein with suitable compounds, the filamentous formation of α-synuclein can be inhibited and also the cytoxicity of α-synuclein fibrils can be suppressed. Hence, research focusing on this approach may open up new avenues for the treatment of PD.

Competing interests: We hereby declare that there are no competing interests.

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ACKNOWLEDGEMENTS. We thank Tezpur University and UGC, New Delhi for the start-up grant. We acknowledge the DBT-funded Bioinformatics Infrastructure facility in the Department of Molecular Biology and Biotechnology, Tezpur University for providing computational facility to carrying out this work. We also thank Dr Sanjib Kumar Borkakoti, Associate Professor, ADP College, Nagaon, Assam for editing the manuscript.

Received 21 October 2016; revised accepted 4 January 2017

doi: 10.18520/cs/v112/i11/2219-2225