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INSIGHT INTO INTERACTION OF γ-SYNUCLEIN INHIBITING α-SYNUCLEIN OLIGOMERS – A POSSIBLE STRATEGY TO CURE PARKINSON'S DISEASE

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ABSTRACT

The α-synuclein oligomers are believed to be involved in the pathogenesis of Parkinson's disease. Single nucleotide polymorphism in α-synuclein is found to be genetically linked for misfolding of the protein into aggregates. Mutants of α-synuclein A30P, A53T and E46K were docked to get homodimer to mimic the initial stages of aggregation using the molecular modelling techniques. E46K mutant was found to be the most involved in aggregation as the electrostatic energy of docking was the lowest, although, number of residues involved in the interaction was comparatively less. Further, γ-synuclein monomer was docked to the membrane bound E46K mutant of α-synuclein to show that γ-synuclein blocks the further propagation of α-synuclein into the oligomers via preventing fibrillation.

Keywords: *α-synuclein; γ-synuclein; oligomers; POPC (1-palmitoyl-2-oleoyl-sn-glysero-3-phosphocholine); Parkinson's disease; molecular docking*

[I] INTRODUCTION

Parkinson's disease (PD) is the most common aging dependent neurodegenerative movement disorder; affecting approximately four million individuals worldwide, it constitutes to be a remarkable societal burden[1]. PD is characterized by the death of neurons of the substantianigra pars compacta, accompanied by the appearances of Lewy bodies and Lewyneurites[2].Biochemical and histologicalstudies suggest that the insoluble abnormal assemblies ofα-synucleinasthe major component of lewy bodies and neuritis in Parkinson's disease and dementia with lewy bodies[3]. Genome wide association studies has suggested three specificsingle nucleotide polymorphisms in the α-synuclein gene lead to rare autosomal dominant forms of PD[4].A30P substitution has been identified in a German family, E46K in a Spanish family and A53T in large kindred of Italian and Greek families[5, 6, 7].Thesepathogenic point mutations cause helicalα-synuclein to develop a beta-sheet structure, giving rise to oligomer formation and higher ordered fibrillization. Nuclear Magnetic Resonance spectroscopic data has revealed the proposed fold in the fibril core to be composed offive layers ofanti-parallelβ-strands comprising residues 30-110[8].The ability of α-synuclein to form large oligomers is accompanied by increased level of toxicity*in*

vivo and it might also interact with and potentially disrupt membranes[9]. Therefore disease modifying therapy involving either reduction of threshold level of toxicity in cells or inhibition of mutant α-synuclein aggregation into oligomers is needed. The novel idea 'proteinopathy' - targeting the development of protein aggregates, would possibly be a fruitful approach to disease modification [10].

Molecular modelling and dynamics simulation studies have showed that α-synuclein dimers propagate on the membrane by incorporating additional monomers into a ring-like structure and β-synucleininhibits the aggregation of α-synuclein into pore-like oligomers[11].Like β-synuclein, a stoichiometric excess of γ-synuclein has been reported to inhibit α-synuclein fibril formation *in vitro*. Also, γ-synuclein may counteract α-synuclein toxicity by activating the ELK-1 pathway[12]. Therefore, γ-synuclein may also act as endogenous regulator of α-synucleinoligomerization by stabilizing oligomeric intermediates[13].But there is not much evidencethroughmolecular basison how γ-synuclein interacts with α-synucleinand inhibits its formation into oligomers. Therefore, the present study was aimed on molecular docking of γ-synuclein with α-synuclein monomer and dimmers and the docked structureswere analysed for interactions involving the amino acid residues. Also interaction between γ-synuclein and membrane bound α-synuclein was studied, as fibrillation of α-synuclein occurs only at cell membranes.

[II] METHODS AND COMPUTATIONAL DETAILS

The sequence of human γ-synuclein protein was obtained from NCBI [www.ncbi.nlm.nih.gov]database and BLASTP was performed to obtain a suitable template having the lowest E value, maximum query coverage and score[14, 15]. The 3D structure of the protein was modeled using the modeler tool 9 version8[16, 17]. Ramchandran's plot were obtained using SAVES server in order to validate the structure[18]. The α–synuclein structural file (PDB ID:1XQ8) was downloaded from RCSB-PDB [http://www.rcsb.org/pdb/home/home.do]database[19, 20], and variants were constructed by substituting alanine, glutamic acid and alanine residues at positions 30, 46 and 53 with proline, lysine and threonine respectively using the Build Mutant tool of Discovery Studio module 3.1[21].All resulted pdb files were repaired using 'Prepare PDB file for docking programs' tool of WHATIF online server[http://swift.cmbi.ru.nl/servers/html/index.html][22].

Theoretical study of interaction between two α-synuclein monomers and monomers of γ-synuclein with α-synuclein was done using the molecular docking tool Hex 6.3 with 0.6 grid dimensions and 3 Å RMS thresholds[23, 24]. Correlation type was set to shapeandelectrostatics and post processing was done by Molecular Mechanics (MM) minimization. A total of 500 clusters were analyzed for post processing out of 2000 solutions (Table 1).

Correlation Type	**Shape + Electrostatics**		
FFT Mode	**3D**		
Post Processing	**MM Minimization**		
Grid Dimension	0.6	Solutions	2000
Receptor Range	45	Step Size	7.5
Ligand Range	45	Step Size	7.5
Twist Range	360	Step Size	5.5
Distance Range	40	Scan Step	0.8
		Substeps	2
Steric Scan	18	Final Search	25

Table 1: Docking Parameters used in the study

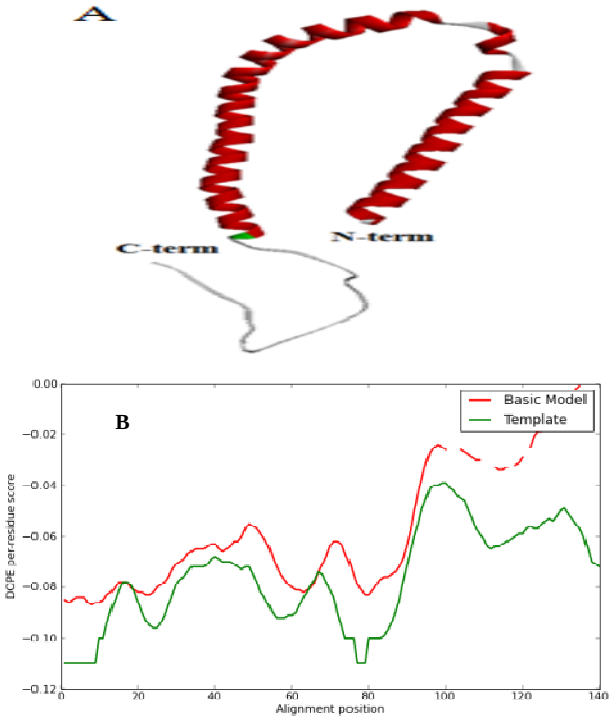
As previous studies suggested that α-synuclein fibrillation occurs at the membrane, we also dockedα-synucleindimer molecules on POPC(1-palmitoyl-2-oleoyl-sn-glysero-3-phosphocholine)lipid bi-layer representing the

plasma membrane. The POPCmembrane was constructed using 'Membrane Builder' tool of VMD 1.9 [http://www.ks.uiuc.edu/Research/vmd/] using 150Å X-length and 100 Å Y-length[25, 26].The γ-synucleinwas docked on the POPC membrane bound E46K mutant of α-synuclein using the online server of docking module Hex 6.3[27].

[III] RESULTS AND DISCUSSION

3.1 Homology modelling of γ-synuclein

The sequence of γ-synuclein was assessed using Protein BLAST to find the template having the most sequence similarities in Protein Data Base RCSB. 2KKW-A was chosen as the template because of its maximum score of 116, lowest E value (2e-34) and maximum sequence coverage. The 3D structure was modelled using a comparative homology modelling technique using Swift Modeller9v8 software.Discrete optimized protein energy (DOPE) and modeller objective function (MOF) were used to identify the most reliable model. DOPE scores per residues obtained from the homology model was plotted against alignment position to get the gnuplot (Fig.1).



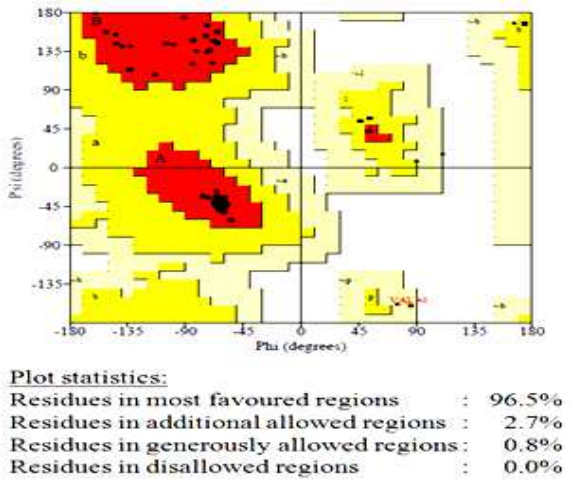


Fig. 1: (A) Homology model of γ-synuclein, (B) gnuplot of homology modelled γ-synuclein with template 2KKW-A and (C) Ramchandran's plot obtained from SAVES server.

The obtained homology model of γ-synuclein was submitted to online SAVES server to check the validity of structure. The Ramchandran's plot showed 96.5 % residues in most favoured region and 0% residues in disallowed region indicating the suitability of the structure for further studies.

3.2 Construction of various mutants of α-synuclein

Mutants of wild type α-synuclein A30P, E46K and A53Twere constructed at positions 30, 46 and 53 by substituting alanine, glutamic acid and alanineresidues with proline, lysine and threonine respectively (Fig. 2).

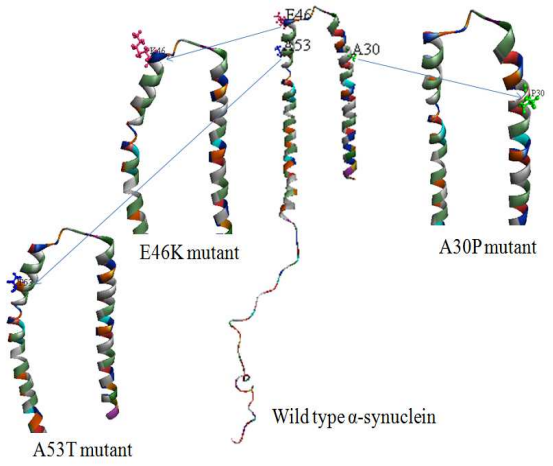


Fig. 2: Wild type α-synuclein has been mutated by substitutions at positions 30, 46 and 53 with proline (P), lysine (K) and threonine (T) respectively.

All three mutations of α-synuclein are in the N-terminusand residues of thisregion are responsiblefor the membrane binding, so these mutationsmight have an effect ontheir interactions with the membrane[28]. The A30P α-synuclein mutation wherein proline replaces alaninedisrupts the helical structure of α-synuclein more in comparison with A53T mutation[29]. However, there is no significant disruption in the structure of membrane-associated α-synuclein due to A30P mutation.The protein to lipid ratio is higher when E46K mutant of α-synuclein binds to synaptic vesicles compared to wild-type α-synuclein*in vivo*[30].

3.3 Interactions of α-synucleinhomodimer predict the residues involved in aggregation

The first study of molecular docking was performed to obtain wild type α-synucleinhomodimerwithout any specific constrains. Mutants of α-synuclein were also docked to obtain homodimers of A30P, A53T and E46K (Fig. 3).

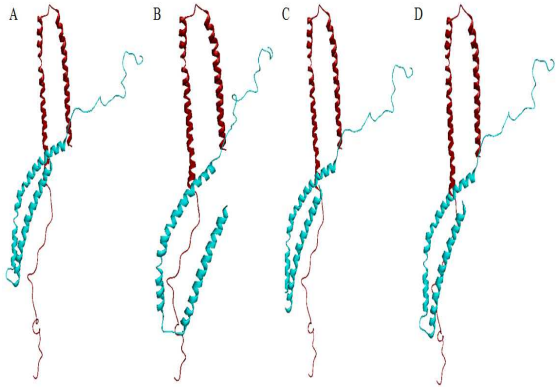


Fig. 3: Homodimers of α-synuclein. (A) Wild type 1XQ8 α-synuclein dimer, (B) mutant A30P α-synuclein dimer, (C) mutant A53T α-synuclein dimer and (D) mutant E46K α-synuclein dimer. Monomer-1 (in red) and monomer 2 (in blue) displayed as solid ribbon.

The total energy of all the homodimers was compared to check which interaction is thermodynamically more favourable (Table 2). E46K homodimer has the lowest energy of -456.8 Kcal/mol, suggested itto bethermodynamically more favourable over the other three interactions. Despite its lowest energy, the number of residues interacting was higher in the case of A30P homodimer of α-synuclein (Table 2).

S. No.	Docked complex		E_{total}	Interacting residues of Monomer-2
	Monomer-1	Monomer-2	(Kcal/mol)	
1	1XQ8 α-synuclein	1XQ8 α-synuclein	-345.4	T81, V82, T92, G93, F94, K96, D98, G101
2	A30P α-synuclein	A30P α-synuclein	-356.4	Y39, K45, F94, K97, D98, Q99, L100, G101, K102, N103
3	A53T α-synuclein	A53T α-synuclein	-319.4	T81, V82, K96
4	E46K α-synuclein	E46K α-synuclein	-456.8	V48, V49, A78, V82, G93, K96, D98
5	1XQ8 α-synuclein	γ-synuclein	-561.6	N75, T76, T79, K80, E86, A89, V90, G93, V94, R96, D127
6	A30P α-synuclein	γ-synuclein	-573.4	S73, T76, K80, E83, E116
7	A53T α-synuclein	γ-synuclein	-579.2	T76, T79, K80, V82, E83, E86, A89, V90, G93, V94, G125, G126, D127
8	E46K α-synuclein	γ-synuclein	-569.5	N75, T76, T79, K80, E86, A89, V90, G93, V94, R96, G126, D127

Table 2: Docked structure showing their energies and the residues involved in interaction within a sphere of radius 3 Å (E_{total} is the total energy obtained after docking)

Also, molecular docking was performed between a monomer of α-synuclein and γ-synuclein to get heterodimeric interactions (Fig. 4). The comparison between energies of α-α synuclein and α-γ synuclein docked complexes shows the lesser energy in case of α-γ Synuclein docking suggesting itsthermodynamically more favourable interaction over the other. Heterodimer of A53T α-synucleinwith γ-synucleinshows the lowest energy of -579.2 Kcal/mol after docking. However, very minimal difference of around 15 Kcal/mol in energy values was detected between all the heterodimers.

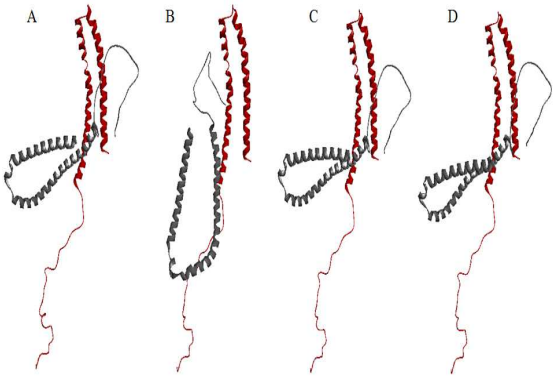


Fig. 4: Heterodimers of different mutants of α-synuclein displayed as solid ribbon (A) wild- type 1XQ8 α-synuclein, (B) mutant A30P α-synuclein, (C) mutant A53T α-synuclein and (D) mutant E46K α-synuclein with γ-synuclein. α-synuclein (in red) and γ-synuclein (in grey).

3.4 γ-synuclein blocks the formation of α-synuclein oligomers

It has been reported that γ-synuclein may also act as endogenous regulator of α-synucleinoligomerization by stabilizing oligomeric intermediates[13]. However, the molecular basis for the interaction between these two molecules is still unknown. Thus, molecular docking was performed between α-synucleinhomodimer and a monomer of γ-synuclein to get hetero-trimeric interactions (Fig. 5). The docking energy obtained from the heterotrimer of E46K α-synucleinhomodimer with γ-synuclein was -601.3 Kcal/mol, whichis low in comparison with that of the homotrimer of E46K α-synuclein (Table 3).

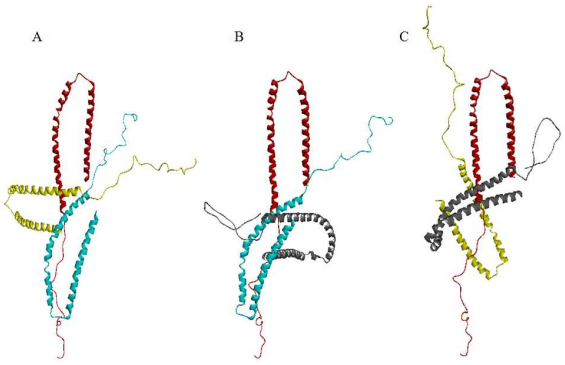


Fig. 5: Heterotrimer docked complexes displayed as solid ribbon, γ-synuclein (grey), α-synuclein Monomer-1 (red), 2 (blue) and 3 (yellow). (A) homotrimer of E46K mutant α-synuclein, (B) γ-synuclein docked with E46K α-synucleinhomodimer and (C) E46K α-synuclein docked to heterodimer of E46K α-synuclein with γ-synuclein.

Heterotrimer					
S.No.	**Monomer-1**	**Monomer-2**	**Monomer-3**	**E_{total} (Kcal/Mol)**	**Interacting Residues**
1	E46K α-synuclein	E46K α-synuclein	E46K α-synuclein	-407.9	F4, A85, I88, I112
2	E46K α-synuclein	E46K α-synuclein	γ-synuclein	-601.3	M1, D2, V3, K20, K79, V81, E82, E83, E85, I87, A88, R95
3	E46K α-synuclein	γ-synuclein	E46K α-synuclein	-516.7	M1, V3, F4, M5, K6, L8, G84, S87, I88, A90, A91, F94, V95

Table 3: Heterotrimers with their docking energies and residues involved in the interactions within a sphere of radius 3 Å

Lower energy suggests it to be a stable and thermodynamically more favourable conformation in comparison with the homotrimer. Also, to check whether binding of γ-synuclein blocks or enhances the further aggregation of α-synuclein into oligomers, monomer of mutant E46K α-synucleinwas docked to heterodimers of E46K α-synuclein and γ-synuclein.The docked complex wasthermodynamically not favourable, due to its higher docking energy, as compared to the previous one. So here it can be concluded that due to the most possible lower docking energy of the heterotrimer of E46K homodimer with γ-synuclein over the other complexes, γ-synuclein possesses the ability to inhibit the further development of α-synuclein into the oligomers.

3.5 α-synucleinhomodimer docked on the membrane

Disorders with α-synuclein aggregation such as Parkinson's disease and LewyBodies Dementia, it is possible that propagating dimers might be present in a large proportion.Association of small oligomers of α-synucleinhas been reported with lipids and cell membranes[31]. However, the conformations of α-synucleinmultimers have been difficult to study due to their inability to crystallize the oligomeric form of this protein.

The monomer of mutant E46K α-synuclein had been docked on the POPC membrane surface in order to obtain membrane bound protein structure. The docking energy for monomer bound to membrane was found to be -94.67 Kcal/mol. Further, γ-synuclein was docked to membrane bound monomer of E46K α-synuclein (Fig. 6).

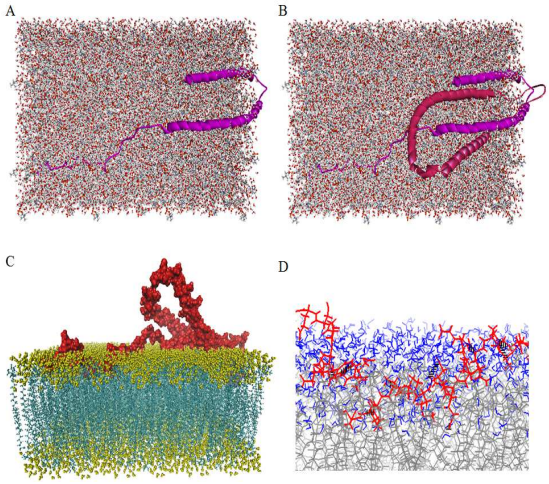


Fig. 6: (A) Docked complex showing E46K α-synuclein (in violet) monomer embedded into POPC membrane, (B) γ-synuclein (in Pink) docked to membrane bound monomer of E46K α-synuclein (in violet), (C) penetration of E46K α-synuclein bound γ-synuclein heterodimers (in red) into POPC membrane and (D) residues (in red) of heterodimer involved in hydrogen bonding (in black) with residues of membrane (in grey) and water (in blue).

The docking energy obtained was -132 Kcal/mol. Residues involved in the hydrogen bonding when E46K mutant of α-synuclein docked on POPC membrane were found to be A107, P108, E114, D115, E123, Y125 and E126. This showed the ability of C-terminal residues in membrane binding. Presence of water molecules had made the hydrogen bonding possible with the polar head groups of the lipid bi-layer.

[III] CONCLUSIONS

We demonstrated that *in silico* approach can be used to mimic the interaction involving the γ-synuclein inhibiting the development of α-synuclein into oligomers docked on the membrane *in vivo*. Mutants were highly interactive and involved in the misfolding of protein leading to oligomers formation. A30Phomodimer of α-synuclein showed highest number of non-polar residues involved in the interactions; still the docking energy was lower for the E46K homodimer of α-synuclein.The γ-synuclein interaction with E46Khomodimer of α-synuclein docked on POPC membrane showed the high degree of residues involved in the interactions. By studying the molecular mechanism of interactions between γ-synuclein and membrane bound α-synuclein and thereby residues involved in the interactions, structural based therapies can be developed with an aim to inhibit α-synucleinoligomerization. The identified involvement of γ-synuclein can serve as an effective inhibitor of α-synuclein oligomers and therefore aid in future Parkinson's disease therapy.

[IV] ACKNOWLEDGEMENTS

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