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"An In-silico Analysis to find potent Inhibitors of Alpha synuclein aggregation for the treatment of Parkinson's disease"

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MAY, 2022

DECLARATION OF THE CANDIDATE

I, **Mayank Sagar**, 2K20/MSCBIO/14, henceforth declare that this research work which I submitted as a Major Project entitled **"An In-silico Analysis to Find Potent Inhibitors of Alpha Synuclein Aggregation for the Treatment of Parkinson's Disease"** in requirements for the degree for the award of the Masters of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, It is a genuine document through my own work, which I completed under **Prof. Pravir Kumar'**s mentorship from January to May of 2022. I have not submitted the literature in this dissertation for academic award from this or any other institute.

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SUPERVISOR CERTIFICATE

To the best of my knowledge, the above work entitled **"An In-silico Analysis to Find Potent Inhibitors of Alpha Synuclein Aggregation for the Treatment of Parkinson's Disease"** has never been presented in full or in part for a diploma or degree at this institution or anywhere else. I also certify that the student's publication and indexing information is accurate.

Place: DTU, Delhi

Date: 5th of May, 2022

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ABSTRACT

Parkinson's disease is amongst the most common neurodegenerative diseases (NDD) in elders. It involves gradual degeneration of the dopamine-producing neurons, and is a main neuropathological characteristic of PD. Alpha synuclein plays a significant role in the etiology of Parkinson's disease (PD). Parkinson's disease impacts about one percent of individuals over the age of 60 and as much as 5% of the persons over the age of 85 [1,2]. Parkinson's disease (PD) is characterized by clinical manifestations such as slowness of movement, stiffness, shivering, and walking difficulties, furthermore uncontrollable symptoms such as problems in sleep disturbance, loss of smell, cognitive loss, and anxiety [3]. Our research includes the molecular docking investigation of the three-dimensional structure of the human alfa-synuclein collected out from Protein Data Bank along with their chemical ligands. Auto Dock 4.2.6 was used in our molecular docking the human alpha synuclein against certain drugs (ligands). Our molecular docking experiment sheds light on an in-silico drug reproposing methodology to alfa-synuclein (αS) modulating as a hopeful PD treatment for patients.

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INTRODUCTION

Parkinson's disease is amongst the highest prevalent neurodegenerative disorder (NDD) which affects neurons in regions of brain like basal ganglia & basal ganglia is majorly responsible for controlling locomotion. Parkinson's disease impacts about one percent of individuals over the age of 60 and as much as 5% of the persons over the age of 85 [1,2]. Parkinson's disease (PD) is characterized by clinical manifestations such as slowness of movement, stiffness, shivering, and walking difficulties, furthermore uncontrollable symptoms such as problems in sleep disturbance, loss of smell, cognitive loss, and anxiety [3]. Recent research suggests the addition of acetyl group onto lysine & removal of the acetyl group on a variety of proteins, including histones non-histones, and also N-acetylation of Alpha-synuclein, are all linked to the molecular mechanisms that underpin PD development [2,3]. PD, like all other neurodegenerative illnesses like Alzheimer's and prion disorders, is marked by neurodegeneration and the production of fibrillar protein aggregates. Protein aggregates [54, 55], as well as organized prefibrillar, oligomer, or protofibrils [56], may cause neurodegeneration. The fibrils could be neuroprotective in this circumstance [56]. In whatsoever event, the chemical mechanism of fibrillation is mostly unclear at the moment.

Alpha synuclein is a neural peptide that is ubiquitous and naturally unfolded. It's very permeable, and it is also found between the cytosol and the nucleus. It's more abundant in nerve terminals, in which it's linked to nerves terminals. It's an unorganized molecule by nature, although it has a lot of structural flexibility (depending on the place). Alpha Synuclein, it occurs in solution as an unorganized monomer and therefore can stay disorganized, produce oligomer and monomeric forms, as well as form amyloidogenic fibrils [6]. The monomeric form is in balance with surface variants exhibiting increased helix composition within the cells. Dopamine acts as a dynamical stabilizer for oligomeric form as well as other metabolites (DA). All of those are, ultimately, transitory entities that eventually assemble into matured fibers. Fibrillization is thought to be the cause of something like the creation of Lewy bodies. The binding of ubiquitin, for example, is considered to occur after the early agglomeration and deposit mechanisms [7]. Alpha-Synuclein is a crucial constituent of Lewy bodies in both acquainted and sporadic Parkinson's disease [39],

implying that its agglomeration and fibrillation play a significant part in the Parkinson's disease onset and development progression. Both as result, the importance of Alpha Synuclein in neurodegenerative disease has been recognized, and therapeutic initiatives have focused on targeting linked to Alpha Synuclein conformational changes and its fibrillization. Alpha-Synuclein is however observed in the Lewy body variety of AD, cognitive impairment with Lewy bodies, all of which have been referred to as alpha-synucleinopathies [61].

Parkinson’s Disease

Parkinson’s disease impacts about one percent of individuals over the age of 60 and as much as 5% of the persons over the age of 85 [1,2]. Parkinson's disease (PD) is characterized by clinical manifestations such as slowness of movement, stiffness, shivering, and walking difficulties, furthermore uncontrollable symptoms such as problems in sleep disturbance, loss of smell, cognitive loss, and anxiety [3]. The main neuropathological agent in Parkinsons disease is aggregation of alpha synuclein. Alpha-Synuclein is a crucial constituent of Lewy bodies in both acquainted and sporadic Parkinson's disease [39], implying that its agglomeration and fibrillation play a significant part in the Parkinson's disease onset and development progression. Both as result, the importance of Alpha Synuclein in neurodegenerative disease has been recognized, and therapeutic initiatives have focused on targeting linked to Alpha Synuclein conformational changes and its fibrillization.

Pathogenesis of Parkinson's Disease (PD).

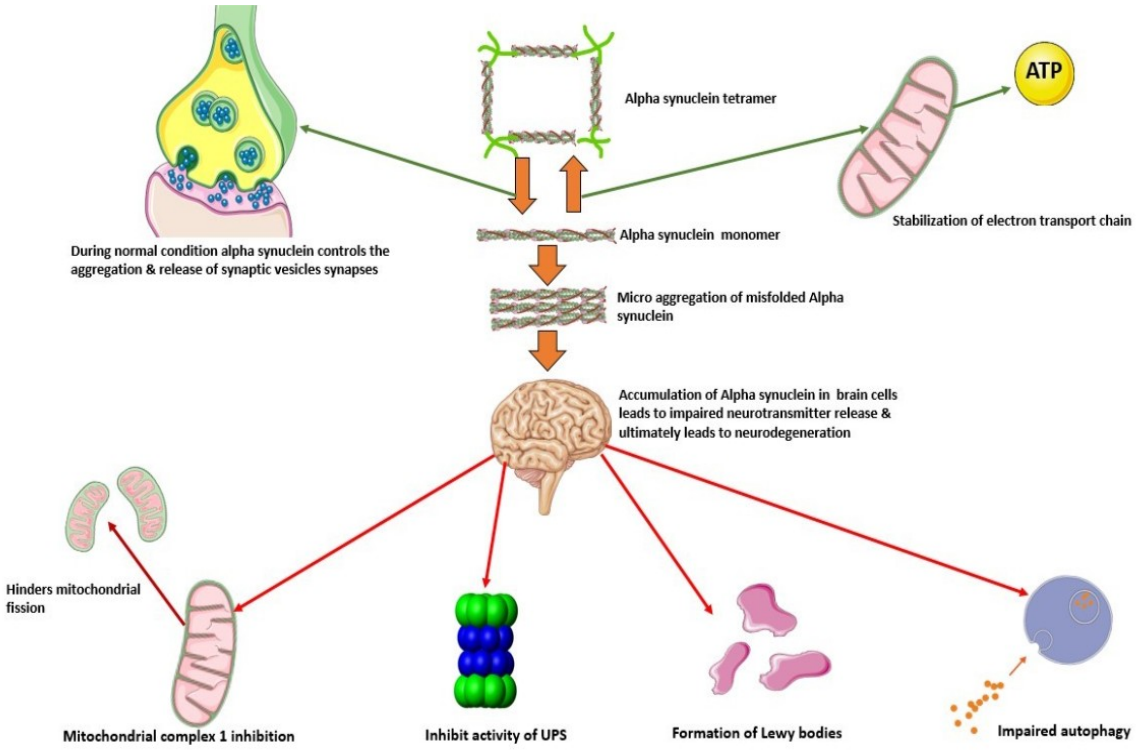

Fig. 1. Figure shows the activity of Alpha-synuclein during normal and neuropathology modifications in the PD. AS is known to occur mostly in the configuration of monomeric and tetrameric forms, in normal physiological conditions. alpha-synuclein, in collaboration with cardiolipin, also alpha-synuclein regulates both aggregation and as well as the discharge of synaptic vesicles synapses and stabilizes the ETC proteins on the mitochondrial matrix and membrane. Alpha-synuclein when misfolded severely affects the action of the UPS mediated protein degradation and autophagy as the alpha-synuclein inhibits mitochondria fusion and also impairs autophagy.

Reasons that are thought to play a significant role PD; **Protein misfolding and clumping (i), mitochondria dysfunction (ii), and oxidative damage (iii)** are all important factors in the advancement of both kinds of diseases.

(i) Protein misfolding and clumping; PD, like all other neurodegenerative illnesses like Alzheimer's and prion disorders, is marked by neurodegeneration and the production of fibrillar protein aggregates. Protein aggregates [54, 55], as well as organized prefibrillar, oligomer, or protofibrils [56], may cause neurodegeneration. The fibrils could be neuroprotective in this circumstance [56]. In whatsoever event, the chemical mechanism of fibrillation is mostly unclear at the moment.

(ii) mitochondria dysfunction; The mitochondria complex I(MC-1) affect the MRC is reduced within the autopsy of Parkinson's Disease affected human brains [40]. This finding of several of the familial PARKINSONS'S DISEASE genes added to the notion that mitochondrial malfunction plays a significant role in PD. Mitochondrial malfunction is a road to parkinsonism, according to the finding of IPINK1, HtrA, and DJ1 alterations. The DJ-1 molecule is predominantly cytosolic in cell organization, by a minor collection of mitochondria and several nuclear-linked proteins. DJ1 polymorphisms also alter the activity of enzymes by destabilizing the protein or changing its intracellular location. The nuclear location among all DJ-1 PD-linked alterations is diminished due to mitochondrial activity [30]. However, it’s unclear that the toxic behavior linked with the enhanced mitochondria localization has been caused by the denial of access for receptor proteins in various cellular divisions or by mitochondria increase of functioning [58].

(iii) oxidative damage; Numerous aspects involved in the pathogenesis of PD juncture towards the contribution of ROS generation in the disorder: A. inhibiting complex increases generating ROS; B. Dopamine producing nerve cells might just be important breeding surroundings about the induction of Oxidative stress, like dopamine (DOP) metabolic activity generates superoxide and hydrogen peroxide(H202) radical; & C. the DJ1 might act as antioxidants in against various reactive oxygen species.

Alpha-synuclein and dopamine fibrillization inhibitor

Alpha-Synuclein is a crucial constituent of Lewy bodies(LB) in both acquainted and sporadic Parkinson's disease [39], implying that its agglomeration and fibrillation play a significant part in the Parkinson's disease onset and development progression. Both as result, the importance of Alpha Synuclein in neurodegenerative disease has been recognized, and therapeutic initiatives have focused on targeting linked to Alpha Synuclein conformational changes and its fibrillization. Alpha-Synuclein is however observed in the Lewy body variety of AD, cognitive impairment with Lewy bodies, all of which have been referred to as alpha-synucleinopathies [61].

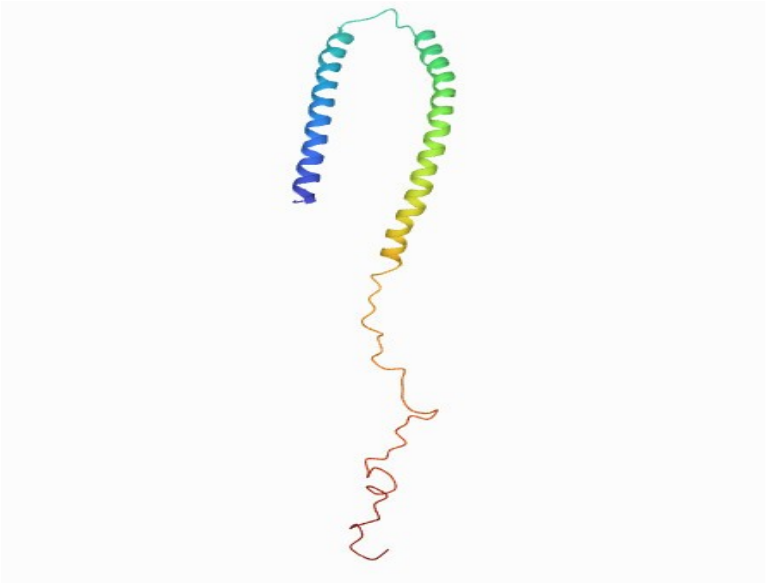

Fig 2; Human micelle-bound alpha-synuclein structure downloaded from Protein Data Base ID: 1XQ8

Alpha synuclein is a neural peptide that is ubiquitous and naturally unfolded. It's very permeable, and it is also found between the cytosol and the nucleus. It's more abundant in nerve terminals, in which it's linked to nerves terminals. It's an unorganized molecule by nature, although it has a lot of structural flexibility (depending on the place). Alpha Synuclein, it occurs in solution as an

unorganized monomer and therefore can stay disorganized, produce oligomer and monomeric forms, as well as form amyloidogenic filament [62]. The monomeric form is in balance with surface variants exhibiting increased helix composition within the cells. Dopamine acts as a dynamical stabilizer for oligomeric form as well as other metabolites (DA). All of those are, ultimately, transitory entities that eventually assemble into matured fibers. Fibrillization is thought to be the cause of something like the creation of Lewy bodies. The binding of ubiquitin, for example, is considered to occur after the early agglomeration and deposit mechanisms [53].

Alpha-synuclein belongs to a group of 3 synaptic peptides that have a lot of similarities: alpha-synuclein, beta-synuclein, and gamma-synuclein. These Synucleins share an evolutionary conserved -helical lipid-binding domain that is similar to the interchangeable apo-lipoproteins category of lipid-binding domains. The proteins alpha and beta-synucleins are generally present in the central nervous system, where they could be mostly located in presynaptic neurons. The alpha-synuclein molecules are identified mainly in the peripheral nerves and the photoreceptors; it is indeed an indicator of cancer development in breast tumors. Even though normal biological activities for any of the synuclein molecules were found, there's really an indication that beta-synuclein activity regulates the amounts or metabolic activity of alpha-synuclein, since beta-synuclein suppresses -synuclein aggregation in transgenic animals [63]. Similarly, recent research suggests that increasing - beta synuclein activity can lower alpha-synuclein amounts via processes that don't seem to alter alpha-synuclein messenger RNA amounts, although it's possible to function beta-synuclein roles go far beyond controlling alpha-synuclein activity. The alpha synuclein motif is 140 amino acids long and excludes cysteine and tryptophan residue. It could be split into 3 major zones [65, 66]: I The positive charge amino-terminus region, amino acids 1 to 60, which is made up of 7 incomplete 11-amino-acid repetitions with the predetermined standard. (ii) The nonamyloid components [67, 53, 68]. (iii) The negative charged Carboxyl-terminus area comprising of 96-140, which includes multiple post-translational alterations and metal-binding affinity [70, 69].

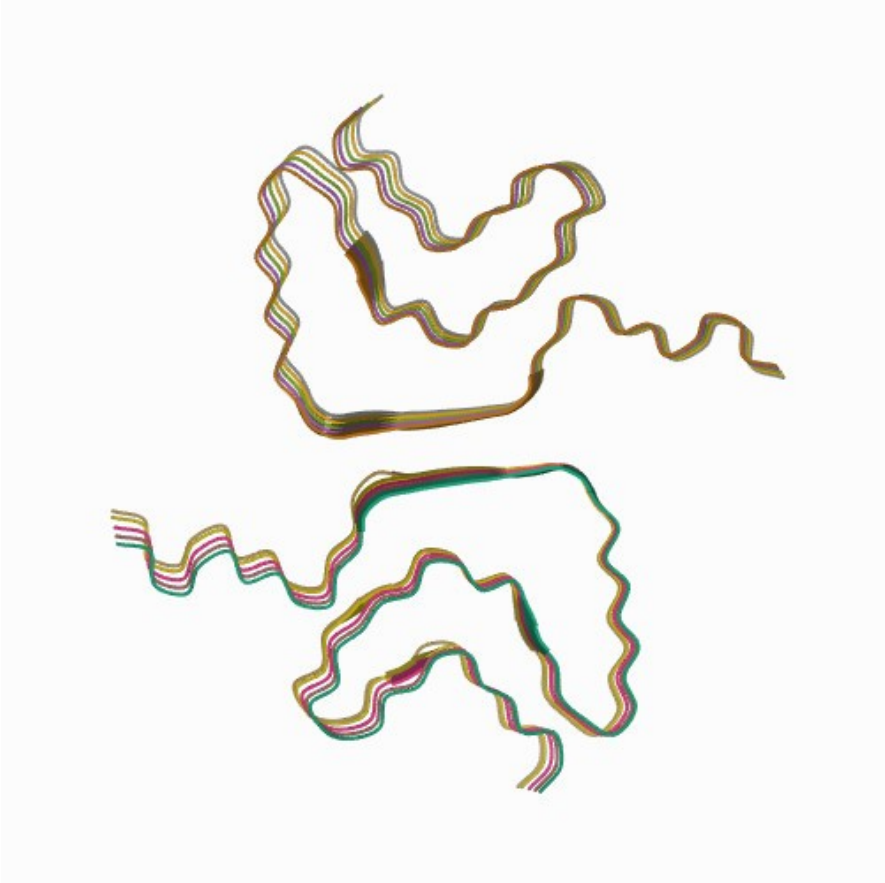

Fig 3., Structure of alpha-synuclein fibrils PDB ID: 6FLT

The NAC area was already discovered in misfolded proteins linked to AD, & multiple publications indicated somehow this particular section is a major factor for alpha synuclein fibrillation [71], and that this portion of the proteins may enhance the development of beta-amyloid in vivo [72, 73]. This area appears to be the most amyloidogenic section of the protein, by several indicators. Numerous phosphorylation sites can be found in the molecule's acidic C-terminal tail. The missense exon V and a calpain I cleave domain are also found at the C-terminus. Proteins clumping is reduced by the acidity terminal, while agglomeration is promoted by a hydrophobic area near the faulty repeats. The gene for hereditary Parkinson's disease in alpha synuclein first was discovered on a gene called 4q21–q23 [74]. The A53T genetic variant, the A30-P variant, & the E46K modification are modifications implicated in the maturation of Parkinson's Disease. Furthermore, chromosomal triplication of a region covering the alpha

synuclein genes has been linked to Parkinson's disease [45]. Individuals having gene duplications are also observed, although they have a milder appearance and develop sickness at an older period than those with triplications, suggesting that alpha synuclein levels significantly can predispose patients to disorder [77]. Consanguineous Parkinson's disease caused by alpha synuclein missense mutations, on the other hand, is extremely uncommon.

Structure: NMR-derived morphology for the structure of the monomers is accessible and is rapidly has been used to research and model alpha synuclein accumulation and interactions with several other molecules [78].

Alpha synuclein occurs as an ensemble of disorganized morphologies in an aqueous medium. Based on PRE impacts, NMR experiments yielded an ensemble of 4,000 molecules with transitory Carboxy-terminus and Ammino-terminus contacts. Alpha-Synuclein acquires a partly helical shape upon attachment to sodium dodecyl sulfate micelle & negative charge artificial vesicle, with both helixes (AA 1 to 38 & AA 44 to 94) forming inside the nonpolar surroundings, and indeed rest of these peptides being unstructured, as demonstrated by Spectroscopic methods [66]. At high doses or when exposed to different chemical and biological conditions, the monomers seem susceptible to aggregating forming amyloid-like formations (for example., shaking). Initial sphere protofibrils, pore-like, chain-like aggregation, and other beta-sheet-rich aggregation precursors, such as early sphere protofibrils, vanish after amyloidosis fibers are produced [79, 80]. Initial circular protofibrils of Alpha Synuclein are succeeded by pore-like protofibrils, & ultimately fast transformation into the fibrils mostly during in vitro fibrillization phase of alpha synuclein. Both the A53-T & A30-P Parkinsons disease-linked modifications boost Alpha Synuclein aggregates formation, whereas A53T promotes fibril development extremely quickly, whereas A30P promotes the development of sphere protofibrils [81] with gradual maturation into fibrillar [79, 82]. This finding generated the first hypothesis that the protofibril, rather than the fibril, is harmful in Parkinson's disease [83, 84].

Alpha-synuclein has been shown to attach surfaces, which could have been connected to the molecule's unknown physiology [11, 8]. Furthermore, the attraction of alpha synuclein for walls increases while the condition for the protofibril & decreases when the shape of the fibril. Moreover, Proto fibrillar arrangement uses a pore-like method to permeabilize artificial vesicles [85, 86]. In the Parkinson's disease-associated variants protofibrils portions, such amyloid channel forms are substantially more common.

Regardless of the actual architecture of the amyloidogenic molecule, all fibrils share the same cross-structure comprised of beta-sheets with beta-strands vertical to the fibril plane. To achieve this architecture, the molecules must experience major structural changes in addition to allowing for the required topographical modifications. It provides a simple fibrillation kinetic model converting monomeric alpha-synuclein further into crucial, partially folded intermediates, that opens up the possibility of accessible fibrils, intractable crystalline clumps, or insoluble fibrils, depending on the application.

Acceleration and Inhibition of alpha synuclein fibrillation in PD.

Acceleration of alpha synuclein fibrillation in PD: Divalent and trivalent ions speed up the fibrillization process. Al (III), Cu (II), Fe (III), and lead are by far the most potent icons in situ (II). This process is thought to be that metals link to negative charge carboxylates, concealing repulsive forces and allowing the partly curled configuration to fall.

Inhibition: Flavanols and polyphenol have been shown to prevent the production of alpha synuclein fibrils and, in certain circumstances, disentangle preexisting fibrils [88]. Such chemicals attach to monomeric alpha synuclein and cause robust alpha synuclein oligomers to develop. Dopaminergic and related adrenal hormones are yet another fascinating family of drugs that can reduce alpha synuclein fibrillization. Although some variables are said to impact alpha synuclein clustering and toxic effects in situ, such as protein modification, oxidative stress, and interactions with toxins and metals [69, 70, 89, 90, 91], the underlying molecular basis of Alpha-synuclein accumulation and toxicological effects in situ, as well as the connections among accumulation and neuronal loss, remain unclear.

Dopamine is fibrillation Alpha-synuclein suppressor:

A involvement of dopamine (DOP) in triggering alpha-synuclein toxic effects is a feasible explanation again for comparative destruction of Neuronal cells in Parkinson's disease. Similarly, alpha synuclein appears to be preferential for DA toxicity [92], (ii) alpha synuclein can engage with as well as boost the function of the neurotransmitter dopamine [93], and (iii) mutated alpha-synuclein expression could also suppress the vesicular monoamine transporters 2 [11]. Numerous pieces of evidence also suggest to alpha synuclein getting involved in dopamine biosynthesis control upon many levels: (i) alpha synuclein hinders dopamine formation thru the interplay to tyrosine hydroxylase (TH) [94] (see Appendix A.1), (ii) alpha synuclein is engaged in vesicular accumulation and reusing thru the interplay with PLD2 [95] and adheres lipids in vacuoles to the high affinity between many pathophysiological variants [10], (iii) alpha synuclein A53T over-expression reduces VMAT2 transcription [96], and (iv) In situ catechol compounds, particularly dopamine is been found to suppress alpha Synuclein fibrillogenic, resulting in an oligomer component buildup in cell lines. All findings imply dopaminergic oxidation might contribute to controlling alpha synuclein accumulation and neurotoxicity and that it is associated with dopamine neurons' specific sensitivity to PARKINSONS' DISEASE [101]. Dopamine generates DOP-AS compounds that are covalently [98, 99] and/or non-covalent [16, 17]. Relationships among dopaminergic and the C-terminal residue 125YEMPS129 in the C-terminal section of the AS motif have been found to be involved in the non-covalent interaction complexes [16].

Native composition and function of alpha-synuclein

After much research, the specific natural structure of alpha synuclein remains unknown. It's now been labeled as fundamentally unstructured [28, 29], helix [30, 31], or a mix of both [32]. Within the vicinity of membrane proteins, a helical structure has already been demonstrated to become more efficiently inhibited [33, 34] (Fig. 1), suggesting one putative vital role for the molecule.

The absence of information on a definite activity for the proteins, the interacting proteins, or having a particular characteristic change must have surely complicated determining the approximate native structure. The bulk of research has refused to take responsibility for these characteristics. A multitude of papers has attempted to investigate the architecture in a plethora of buffering environments, spanning salinity, pH, and fatty acid content alterations [35]. Many have lately looked into diverse structural alterations (such as phosphorylated, glycation, glycosylation, and acetylation) and their impact on protein expression [29, 36, 37]. To uncover and comprehend putative effects for PTMs and the surrounding conditions on pathophysiology, some researchers explored protein levels and aggregating in disorder mammal experimental models.

Alpha synuclein promotes membrane cell membrane curvature, which helps synapse transport and vesicle releasing, according to the existing arrangement [38, 39]. Considering alpha-synuclein interaction with postsynaptic terminals SNARE structures [40], it could be significant, implying an involvement for alpha synuclein in dopaminergic production modulation. As a result, a variety of investigations have been conducted to look into the molecule's communication via nerve vesicles. More data supports a 'prion-like' concept, in which oligomeric alpha synuclein can travel across synapses and promote the creation of Lewy bodies all throughout the forebrain and then into extranigral locations. studies both individually demonstrated that alpha synuclein can wrap together into a durable helix configuration by interacting to generate defining feature

complexes. Because multimers can break down following cell damage to yield accumulation subunits, this discovery was hard to emulate in culture [41]. Researchers later discovered that adding triglycerides to the construct might recreate it [42], resulting in spiral oligomers & support for the aboriginal function for synuclein linkage in transmembrane contacts, particularly vesicles bursting. A comparable effect was found when the N-terminus was acetylated [43] or when the N-terminus was extended by Ten amino acid residues [31, 44], resulting in the development of a stable protein complex through the exclusion of lipid. Changes to the ammino-terminus stand recognized to play a key role in promoting folds forward towards the helix form of alpha synuclein [31], which has a subsequent effect on aggregates [45].

Amusingly, based on the measured characteristics of an artificial homotetramer constituted from four counterparts of a simple Glu/Gln rich protein intentionally gathered in comparison on a synthetic substratum, a comparable experiments concept for amyloidogenesis like a core proposition was already suggested prior [46, 47]. Whenever the peptides got along in a similar alignment, establishing a homo-tetrameric configuration, the protein become substantially more -helical and endlessly durable at pH 7. Acidity, on the other hand, changed the alpha-helical aggregates, leading to tetramer agglomeration as well as a further extension onto different strands, spawning beta-sheet aggregating and oligomerization into tangled fibril fibrils through a somewhat more lengthened 4(310) helical bundled [47]. The significant discovery seemed to be that, like other molecules, the oligomeric alpha-helix bundled was sustained in freshwater due to the hydrophobic center and polar hydrophilic periphery. The alpha-helix, on the other hand, is in steady-state including its longer 310 helical counterparts, and the shift to a 4(310) alpha-helix bundles occurs in acidity because of the nucleophilic attack of hydrophilic residue (Glu). Clustering is aided by the relocation of hydrophilic Glu/Gln residue to the inside of the helical center as well as some hydrophobic residue (Leu) to the outer surface. The within the interactions developed among morphologically coupled nucleophile Glu residue (carboxylic acid dimers) or coupled Gln residue resulted in inner instability and an alpha-helix to 4(310) helix transformation (hydrogen-bonded carboxamides). These connections served as a catalyst, causing the demand curve to shift forward towards stable strand/sheet creation and isomeric amyloidosis production. By returning the pH to 7, the procedure could've been radically

changed, resulting in robust helical tetrads for just that specific target protein. Acid reflux has been linked to the buildup of alpha synuclein molecules in the past [48, 49]. Regional acidity happens at inflammatory locations and during systemic inflammation (glycolysis and lactic acidosis), however, it is unclear if these amyloidogenesis models involve partial glutamate nucleophilic attack or crosslinks linking of polarity side-chains is important to alpha synuclein oligomerization and PD.

According to current understanding, alpha synuclein exists in practice as an optimal blend of unorganized monomers and numerically unfavorable coiled oligomer(s), possibly factor in order at surfaces via lipid contacts. The molecule's alpha-helical configuration may well be necessary for such an undisclosed native role and yet is unlikely toward being harmful, contributing to the concept of stabilizing helix alpha synuclein as a potential section that allows for Parkinson's disease. This could be comparable to Kelly and colleagues' strategy to stabilize the native transthyretin form but use tiny compounds to bind the molecule [50].

Alpha-Synuclein Misfolding: Consequences for Parkinson's Disease

The SNCA gene encodes alpha synuclein, and hence has been implicated as a major contributor to pathogenesis in hereditary types of Parkinson's' disease [20,21,22], and it has also been demonstrated to become the dominant molecule identified within Lewy bodies [19]. The protein mis-folding & aggregating of alpha synuclin into the fibrils were discovered to really be dependent on an inner hydrophobic interior of the molecule correlating to residue 71–82. Also, this amino acid sequence was indeed discovered to be responsible for alpha synuclein aggregate in solitude [51], with the removal of all these positions (residues 71–82 [51] or 66–74 [52]) inhibiting particle agglomeration and indicating them as critical areas in misfolded proteins and perhaps amyloidosis induction. Tuttle et al. subsequently used ssNMR to show that organization of alpha synuclein through its fibrillar beta-sheet configuration have a sinuous Greek key geometry [24]. The analysis confirmed the relevance of the 71-82 domain in stabilizing the problematic orientation of alpha synuclein, but it often revealed a crucial and necessary area that would be highly linked to rapidly progressive variants. Within filament structure, the area encompassing residue 45–57 is critical for facilitating beta-strand to beta strand linkages. Analysis revealed a surface layer on the fibrillar spanning positions 46 and 57, indicating that this area of alpha synuclein is approachable in the fibril (see below).

Two research organizations [56,75] have recently solved a variety of cryo-EM architectures of matured fibrillar arrangements of proteins with numerous parallels to the ssNMR structure [25,26,27, 53]. Two of the structures had a Greek-key topology, while the other two have a water-insoluble gap anchored by inter-molecular hydrophobic interaction and other contacts within the N-acetylcysteine and the ammino-terminus. Fibrils produce bidentate filaments with rotation about just the shaft throughout all Cryogenic electron microscopy formations. The apparent uncovered 45–57 area of the fibrillated peptide involves a systematic approach in the first two. This area may thus act as a hydrophobic steric barrier connecting neighboring Fuzzy c-

means, clustering, as previously discovered in amyloid fibers by Eisenberg and colleagues [54], facilitating the creation of something more robust doubly strand fibril construction [25, 55].

There is genetic evidence for alpha synuclein in Parkinson's disease pathogenesis.

The whole first evidence of a link's inheritance with the PD was uncovered in the year 1990 after descendants of an Immigrant family were realized to have acquired initial Parkinson's disease. After dissection, scientists found Lewy body abnormalities [21], & indeed the known variant for hereditary young on-set Parkinson's disease were discovered in the alpha synuclein gene (SNCA) on chromosome 4 [20]. The alteration in the problem was indeed an inherited base-pair alteration in SNCA, which resulted in the A53T replacement in Alpha synuclein [20]. Since these findings, numerous autosomal dominant variants in the Coding region that cause familial Parkinson's disease have already been discovered. G51D is by far the most effective of the identified variants, causing the disorder to manifest at an early stage. Remarkably, deny the reality that every one of these specific amino acid alterations causes early stages of PD, they all have very varying implications mostly on rates of alpha synuclein agglomeration and the oligomers that form.

The E46 K & A53 T variants, for example, substantially enhance fibril formation, while the A53 E mutant tends to lower the fibril nucleation rate. These alterations should indeed lead to increases in the aggregating frequency, a shift within the oligomeric stage or orientation that's also populated upon agglomeration, and a reduction in the typical tetramer:monomer percentages that promote those alterations. The variants show that the aggregate of synuclein causes rapidly progressive parkinsons disease in general, whereas others show the prefibrillar fibrils are far more hazardous than matured clustered fibrils in particular. Alterations in the aggregate dynamics of variant synuclein variations have already been reported, as well as alterations in their affinity with the cellular membrane. G51-D, A30P [68, 69], and A53E [70] variations, for instance, have decreased lipid attachment as nothing more than a result of variations. E46K and A53T, on the other hand, result in enhanced lipid adherence [58]. Such findings point to a physiological link's synuclein and lipid adherence that can still be jeopardized in early-onset mutants due to alterations in association and morphology. Autosomal dominant hereditary

Parkinson's disease was already ascertained once the SNCA gene is recreated or conditions that affect, in furthermore towards the point mutation mentioned above [71, 72]. Triplication of the alpha synuclein gene has been linked to more severe types of Parkinson's disease than gene duplication, which is consistent with the involvement of Synuclein in PD. This emphasizes the role of intracellular intensities in promoting alpha synuclein misfolding, spawning, and, finally, a family history of the point mutation compared to familial cases of PD.

HAT and HDAC Involvement in Pathogenesis of PD.

At least 22 human HATs with acetyltransferase activity have been found so far; these HATs are classified in a few primary families: the GNAT histone acetylase family, & the MYST histone acetyltransferases family moreover the p300/CBP histone acetyltransferase. HAT target specificity is mostly influenced by the subcellular location, interaction associates, the specific presence concerning Lys in the protein side-chain [17]. The majority of HATs are present in the nucleus of the cell, from where these HATs tend to control activities such as acetyl group transfer to histones resulting in their acetylation [16]. HDAC are the crucial players in metabolic regulation. Any disruption in the function of these protein molecules as well as its interactors can result in illness. Posttranslational modifications are a type of secret regulatory overlay that can alter a protein's functionality, location, or associations in a short period of time - possibly by themselves or by interacting with certain other Posttranslational modifications. SIRT1 overexpression shielded SK-N-SH neuroblastoma cell line cells against cell necrosis, & this camouflage remained somewhat self-sufficient of SIRT1's deacetylation action and that remained linked to nuclear factor-κB protein expression control. Sirtuin 1 inhibited the significant generation of alpha-synuclein, however, it was only localized by alpha-synuclein in a minor way. CNS cells from cases with PD, dementia with and without aggregates of alpha-synuclein were examined [17,18]. Sirtuin 1 affects the activities of these important molecules through its deacetylation activity, demonstrating its critical and complex involvement in cell physiological functions [18].

Alpha-synuclein PTM's.

Since Alpha-synuclein is been discovered and it undergoes a diverse range of PTMs in situ, implying that they are important in the pathogenesis of Parkinson's disease. Thus recently, N-acetylation, nitration& phosphorylation is already been found to influence in production various molecules & fibril development rates. In cell cultures, N-acetylation is quite common PTM of alpha synuclein [65]. It's already been demonstrated to boost helicity & reduce aggregating ratios within N - side of a protein [86], as well as a two-fold increase in attraction to liposomes [87]. Li et al employed this PTM in the peptide they used to determine the Cryogenic electron microscopy composition of grown fibrils. Histone acetylation is among the most well-studied processes in the Brain and by far the most visible epigenetic signature. By reducing the engagement with the negatively charged phosphate groups of deoxyribonucleic acid, PTM acetylation decreases the total positive charge onto histones & promotes open chromatin architecture. The transcription of transcripts within this locus is enabled by such alterations in chromatin structure. Furthermore, the acetyl group tags operate as docking sites for transcription activators and epigenetic readers. The inhibition of HATs & HDACs controls the overall histone acetylation status of chromatin under physiological circumstances.

The half-life of the several essential regulatory factors-like tp53, it's been proved that post acetylation of specific sites, their half-life of these regulatory factors has been increased. p53 is altered at several places by ubiquitination, acetylation, and other PTMs, and thus serves as a model for studying how acetylation interacts with ubiquitination [78]. The most thoroughly studied acetylated non-histone protein is p53, which is regulated by acetylation. HDAC1, HDAC3, SIRT1, and SIRT7 are four deacetylases that have been found to deacetylate p53 [94].

The main reason behind this is competition among lysine ubiquitination and acetylation has been surfaced as the main reason which prevents ubiquitination and degradation by UPS. Certainly, in the situations of Tp53, the identical lysine sites do propose to remain directed on lysine

acetylation and lysine ubiquitination. Here it remarks greatly suggests a shielding function for acetylation via inhibiting more alterations concerning here particular Lys-residue of side-chain of protein. In this respect, Histone acetyltransferases particularly acetylating of the aimed lysine then enhance the protein stability also, in opposition, particular Histone deacetylases then will fasten their degeneration [100]. Tumour protein 53 accurately represents this distinct modulatory mechanism. Acetyl group acceptor positions of the tp53 do further are the positions concerning lysine ubiquitination by the ubiquitin ligase activity of the murine double minute 2 gene. Also, the mdm2 gene did prove to be correlated with histone deacetylase 1, which causes deacetylation of the tp53 & causes the release of aimed lysine's toward succeeding ubiquitination [81]. Furthermore, acetylation of Runt-related transcription factor 3 through Histone acetyltransferase p300, inhibits degeneration by Smurf ubiquitin ligases & Histone deacetylase 5 has implied to invert this outcome of Histone acetyltransferase p300.

Ubiquitination; Lysine residue can also get modified by ubiquitination, which involves the ubiquitin transfer onto a lysine residue of the target protein. Specifically, the major role of ubiquitination is the degeneration of unfolded/toxic protein through the UPS [61]. This molecule of ubiquitin then becomes a destination for more ubiquitination and then this method proceeds to eventually form a polyubiquitin chain and thus the tagged Ubiquitin protein is finally degraded by UPS [45,63]. It is known that all ubiquitin enzymes, act together to trigger and finally transport a molecule from ubiquitin to substrate [69]. The engagement of genetic mutations in the gene parkin (PRKN2) together inherited parkinsonism about ten years earlier [70], and the successive presentation through few self-supporting categories that parkin acts as a ubiquitin ligase linked with degraded proteasomal, provided proof and suggested a direct role for the UPS in PD [52,81].

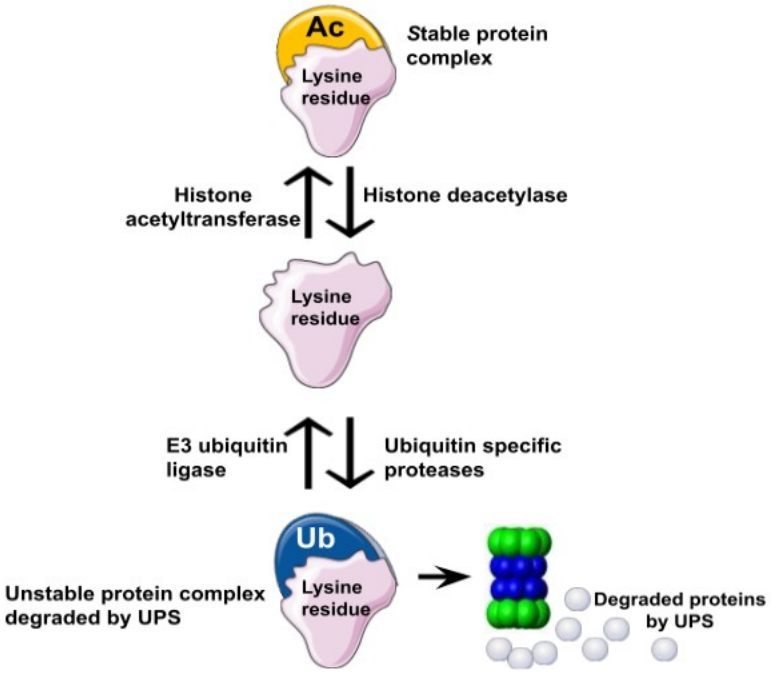

Fig. 4. Protein stabilization based upon the opposition between lysine ubiquitination & lysine acetylation happens on lysine residues,

The main connection between the ubiquitination and acetylation is based the target protein, which in both the scenarios is lysine [24]. Before acetylation of lysine residue of target protein, would then affect succeeding ubiquitination. This leads to acetylation-based preservation of proteins, which are usually tagged for its degradation through the UPS, in this way target proteins evades from UPS. The interaction between these two posttranslational modifications, acetylation and ubiquitination does go far from the normal competitive process and this involves a complicated interplay these signaling pathways. Along with the control of protein stability, ubiquitination does also control numerous cellular functions, including transcription and intracellular trafficking of several molecules. It is hence obvious to uncover a regulatory nexus within the addition of acetyl group and ubiquitination in cells function that is previously known to involve in either of these signaling pathways [4,7].

Phosphorylation is known to occur on residue S8,Y125,Y133 & Y136, however, position S129 seems to be among the most neurotically significant phosphorylation site. This PTM was detected in around ninety percent of the alpha-synuclein in LB, whereas only four percent of alpha synuclein, was recovered from Parkinson's disease patient's neuronal cells [89], and it was demonstrated to accelerate the pace of alpha synuclein fibrilization in cell cultures. Exact role of Phosphorylation is unknown, along both harmful and defensive outcomes documented in various studies [87], further complicating the situation. When S129 phosphorylation was elevated, toxic effects hastened in the alpha synuclein overexpressing SHSY5Y cells lines[90], whereas neuronal loss was reduced in the D.melanogaster when S 129 phosphorylation were blocked [79]. In yeast and rat investigations, knockouts that block S 129 phosphorylates were reported to increase alpha synuclein toxic affects [82, 96], as well as the development of betasheet affluent aggregation [83]. As a result, it appears plausible that Phosphorylation recreates toxic effects in alpha synuclein, although, essence of this function is still unknown at this time and appears to be model dependent.

Nitration, which is induced by elevated amounts of oxidative stress inside neurons, has also been discovered on tyrosine residues Y39,Y125,Y133, & Y136 [36]. Its undefined if these signs of Parkinson's disease or simply a condition that contributes to this disease. The nitration at Y39 seems to be the most intriguing and physiologically significant since its been demonstrated to hinders fibrils formation & stabilize these oligomers through tyrosine cross linking [94]. Its been established to block alpha synuclein affinity with artificial vesicle [99], presumably mimicking activities of the A 30P [66] premature-onset conversion, suggesting that the dangerous form of alpha synuclein is an oligomer rather than a fibril. To sustain their unmyelinated axonal arbor, DOP neurons in the par's compacta have a high requirement [96], which leads to the generation of reactive oxygen species and susceptibility to them [97], which might explain why these neuronal cells were the first to succumbed to alpha synuclein poisoning.

METHODOLOGY

Ligand preparation; Our research includes the blind molecular docking investigation of the three-dimensional structure of the human alfa-synuclein collected out from Protein Data Bank along with their chemical ligands. Auto Dock 4.2.6 was used in our molecular docking the human alpha synuclein against certain drugs (ligands). Our molecular docking experiment sheds light on an in-silico drug reproposing methodology to alfa-synuclein (αS) modulating as a hopeful PD treatment for patients.

Docking of molecules; Utilizing Auto dock 4.2.6 the prepared alpha synuclein had been docked against with the prepared alpha synuclein where all water molecules were removed and just hydrogen being given to the alpha synuclein protein. In order to incorporate every one of the amino acids found in alpha synuclein, the grids size is adjusted to 125 Å,100 Å & 100 Å (x, y, & z axes), & as well as the distance among grids cells has been adjusted toward the default setting of 0.55. The primary goal of the investigation would have been to interpret the docking site to search of the particular drug, so, molecular dock was done.

RESULTS AND DISCUSSION

Our In-silico experiment alpha synuclein aggregation inhibitor discovery is being accomplished using molecular docking using Auto Dock 4.2.6, the results were as follows.

Compound	**Free energy of the Binding in Kcal/mol**
Amento flavone	-5.76
Resorcinol	-4.27
Epigallocatechin	-4.18
L dopa	-3.68
Rosmerinic acid	-1.87

Computational Bioinformatics computational tools has always played a pivotal job in today's virtual drug repurposing approach. Molecular docking always provides an easy to use and effective method to study molecule configuration, and also in several things like drug discovery, because it provides a cost-effective and time-effective alternative to in vitro & in-vivo assays experiments, as well as lowering, perfecting, and substituting laboratory experimentations in the initial research stages. Molecular docking investigation of three-dimensional protein of human alpha synuclein extracted from Protein data base (PDB) with various molecules is part of our research. AutoDock4.2.6 was used for docking the protein alpha synuclein against several ligands. This research sheds light on an in-silico pharmaceutical design technique for alpha synuclein stimulators, which can be a feasible Parkinson's disease therapy.

For greater understanding of drug-protein interaction, computer aided drug design is typically use docking studies techniques. Previous research has also shown that such bioinformatics computational tools aid with the development and discovery of new, effective antagonists

through elucidating the mechanics of drug target engagement. Our, experiment, the Amento flavone was found to be a more acceptable antagonist against alpha synuclein aggregation in PD. Such findings indicated how Amento flavone can specifically bind domain of the alpha synuclein & block the alpha synuclein from self-associating to generate the pathogenesis of PD. Amentoflavone has a wide range of therapeutic actions, according to previous research. past researchers, the clinical application on the cardiac and nervous systems too was documented. 17-19 Because alpha synuclein is a defining feature of PD, & it plays a significant role in the early identification and treatment of a PD.

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I, **Mayank Sagar**, 2K20/MSCBIO/14, henceforth declare that this research work which I submitted as a Major Project entitled **"An In-silico Analysis to Find Potent Inhibitors of Alpha Synuclein Aggregation for the Treatment of Parkinson's Disease"** in requirements for the degree for the award of the Masters of Science in Biotechnology and submitted to the Department of Biotechnology, Delhi Technological University, It is a genuine document through my own work, which I completed under **Prof. Pravir Kumar**'s mentorship from January to May of 2022. I have not submitted the literature in this dissertation for academic award from this or any other institute.

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