



University of
Stavanger

Faculty of Science and Technology

MASTER'S THESIS

Study program/Specialization: Biological Chemistry	Spring semester, 2017 Open / Restricted access
Writer: Anyanwu, Ferdinand Eziokwu (Writer's signature)
Faculty supervisor: Pro. Dr. Lutz Eichacker External supervisor(s):	
Thesis title: Alpha-Synuclein and Synucleinopathies as related to Parkinson's disease	
Credits (ECTS): 60	
Key words: Alpha-Synuclein, Synucleinopathies Parkinson's disease, Aggregation and Fibrillation, Post translational Modifications, Neurodegenerations	Pages: 62 + Enclosure Stavanger, 15/06/2017 Date/year

ABSTRACT

Parkinson's disease (PD) is a chronic, progressive, complex, age-related and movement neurodegenerative disorder, caused by the loss of dopaminergic neurons in a small portion of cells within the mid brain known as the substantia nigra, with its actual etiology undiscovered. Alpha-Synuclein (α -Syn), a minute soluble protein that is primarily expressed at the presynaptic terminal or regions of the central nervous system with a yet unknown function is termed the central or key component to the pathogenesis of this disorder. Thus, Parkinson's disease is the most commonly diagnosed and observed synucleinopathies. Alpha-Synucleinopathies are neurodegenerative disorders featured by the aberrant accretion or deposition of aggregates of alpha-synuclein in the neurons, glial cells or nerve fibres. There are three main types of synucleinopathies; Parkinson's disease (PD), Dementia with Lewy bodies (DLB) and Multiple system atrophy (MSA). Not minding the similarities in their progressions and expansion, there are pathological differences that are significant between multiple system atrophy and Lewy body diseases, along with the cell types that are involved (in Parkinson's disease, oligodendroglia in Multiple System Atrophy and Dementia with Lewy bodies) and the extent of neuronal loss in selected regions in Parkinson's disease but spread all over the regions in Multiple System Atrophy.

Alpha-synuclein, as abundantly found in various regions of the brain, having two intimately related homologs; Beta-synuclein and Gamma-synuclein, was recognised as the dominant component of amyloid fibrils observed in Lewy bodies and Lewy neurites which are the diagnostic hallmark of Parkinson's disease. Aggregation of alpha-Synuclein is assumed to be the key or major event in the pathogenesis of synucleinopathies. Although, several alpha-Synuclein reformations and modifications have been thought to be responsible for early steps of aggregation.

In this thesis work, I will be able to summarize and review the data so far available on Alpha-synuclein, its structure, aggregation and fibrillation, the modifications and synucleinopathies.

ACKNOWLEDGEMENTS

I am sincerely grateful to God Almighty for the gift of life, favour, strength, endurance and good health throughout my stay in UIS and the period of this thesis work. My deep heart gratitude and appreciation goes to my supervisor; Prof. Dr. Lutz Eichacker for his mentorship, advice and encouragement, patience, love and tolerance, his academic contribution towards this work is immeasurable and inestimable. I whole heartedly say ‘thank you Sir’.

To Dr. Johannes Lange, your academic input to this work is well acknowledged and cherished, thank you for tolerating and humbly correcting my errors in the laboratory, you are truly a mentor.

I thank all my past and present lecturers here in University of Stavanger, my colleagues and friends at Centre for Organelle Research (CORE) I thank all of you. I am indubitably and very pleased to say a big thanks to all my friends here in Norway especially Ifedinma Okeoma Njoku for making my study and stay in this country a cozy, may God reward you.

To my loving parents, Mr/Mrs Joel C. Anyanwu and my siblings, I appreciate you all for your prayers, financial contributions and for believing in me.

Finally, to the queen of my heart, my most cherished and alluring wife, Mrs Chidinma Francisca Anyanwu, your incalculable care, love, understanding, patience and prayers towards this achievement is next to none. I heart you forever my beauty.

Gracias.

CONTENTS

ABSTRACT	ii
ACKNOWLEDGEMENTS	iii
ABBREVIATIONS.....	vi
TABLE OF FIGURE	viii
1 INTRODUCTION	1
2 HISTORICAL REVIEW.....	2
2.1 ALPHA SYNUCLEIN (A- SYN)	2
2.2 FACTS ABOUT ALPHA-SYNUCLEIN.....	3
3 STRUCTURE AND PHYSIOLOGICAL FUNCTION OF A -SYN.....	4
4 INTRACELLULAR POOLS OF ALPHA-SYNUCLEIN	10
5 AGGREGATION OF ALPHA-SYNUCLEIN.....	12
5.1 FACTORS THAT CONTRIBUTE TO AGGREGATION OF A-SYN	14
5.1.1 GENETIC FACTORS	14
5.1.2 MOLECULAR CROWDING	15
5.1.3 ANIONS AND SALTS	15
5.1.4 POLYANIONS AND POLYCATIONS	15
5.2 ENVIRONMENTAL FACTORS	16
5.2.1 PESTICIDES AND HERBICIDES.....	16
5.2.2 HEAVY METALS	16
5.2.3 ORGANIC SOLVENTS	16
5.2.4 OXIDATIVE STRESS.....	17
5.2.5 REACTIVE OXYGEN SPECIES (ROS)	19
5.2.6 PROTEIN INTERACTION WITH OTHER PROTEINS.....	20
5.2.7 INTERACTION WITH PARAQUAT	20
5.2.8 INTERACTION WITH TUBULIN	21
5.2.9 INTERACTION WITH LEUCINE-RICH REPEAT KINASE2 (LRRK2)	21
5.2.10 PARKIN INTERACTION WITH A-SYN.....	22
5.2.11 DOPAMINE RECEPTOR INTERACTION.....	22
5.2.12 INTERACTION WITH PHOSPHOLIPASES	22
5.3 ALPHA-SYNUCLEIN INTERACTION WITH MEMBRANE.....	23
5.4 INTERACTION WITH SMALL MOLECULES	24
5.5 SMOKING	24
5.6 INHIBITORS OF A-SYN FIBRILLATION.....	25

6	POST-TRANSLATIONAL MODIFICATION OF ALPHA-SYNUCLEIN	27
6.1	PHOSPHORYLATION OF ALPHA-SYNUCLEIN	27
6.2	NITRATION OF A-SYN	28
6.3	DOPAMINE MODIFICATION.....	29
6.4	UBIQUITINATION OF A-SYN.....	31
6.4.1	FUNCTION OF UBIQUITIN IN AGGREGATION OF A-SYN	32
6.5	SUMOYLATION OF A-SYN	34
7	ALPHA SYNUCLEIN AND SYNUCLEINOPATHIES	36
7.1	PARKINSON’S DISEASE	36
7.2	DEMENTIA WITH LEWY BODIES	41
7.3	MULTIPLE SYSTEM ATROPHY (MSA)	43
8	CONCLUSION	44
9	REFERENCES	45

ABBREVIATIONS

A-syn	Alpha-Synuclein
AD	Alzheimer's disease
AFM	Atomic force microscopy
ALS	Amyotrophic Lateral sclerosis
DA	Dopamine
DAQ	Dopamine Quinone
DAT	Dopamine Transporter
DDC	Dopamine decarboxylase
DLB	Dementia Lewy Bodies
GAG	Glycosaminoglycan
GSHPx	Glutathione Peroxidase
GSHRd	Glutathione Reductase
HPLC	High Performance Liquid Chromatography
Hsp	Heat shock protein
LB	Lewy Bodies
L-DOPA	Levodopa
LN	Lewy Neurites
LRRK2	Leucine-rich repeat kinase2
MDA	Malondialdehyde
MSA	Multiple System Atrophy
NAC	Non-A β component of AD plaques
NBIA	Neurodegeneration with brain iron accumulation
NMR	Nuclear Magnetic Resonance
NSF	
PAF	Pure Autonomic Failure
PD	Parkinson's Disease
PG	

PTM	Post Translational Modification
RBC	Red Blood Cells
ROS	Reactive Oxygen Species
SAXS	Small angle x-ray scattering
SEC	Size Exclusive Chromatography
SEC-HPLC	Size Exclusive Chromatography High Performance Liquid Chromatography
sHsp	Small heat shock proteins
SNARE	Soluble N-ethylmaleimide-sensitive factor attachment Protein receptor
SOD	Superoxide dismutase
SUMO	Small ubiquitin-like modifiers
SUV	Small Unilamellar Vesicles
VMAT2	Vesicular Monoamine Transporter2
WT	Wild Type

TABLE OF FIGURE

Figure 1-1: How the structure and shape of α -syn in Parkinson's might cause disorder. The protein monomer, when assembled in groups of four (tetramers) can always protect itself from being disrupted or damaged. However unfolded single strands (monomers) are at risk for disruption which leads to clumping in Parkinson's disease (Nyström, Nordström and Nordström, 2016)	1
Figure 3-1: Amino-acid sequence of α -synuclein. The N-terminal region is blue, the aggregation-prone (NAC) domain is in green, and the C-terminal region is in red. The seven imperfect repeats are indicated in bold type. Protein fragments are schematically shown as bars above (synthetic small peptides) or below (chemically truncated protein). For clarity, the N-terminal truncated mutants are omitted (Bartels et al., 2010).....	5
Figure 3-2: Schematic representation of micelle-bound α -synuclein. The N-terminal region, the non-amyloid- β component of Alzheimer's disease amyloid plaques (NAC) region and the C-terminal part are coloured blue, orange and red, respectively. Numbers refer to amino acid residues flanking the different regions (Lashuel et al., 2013).....	6
Figure 3-3: Western blot identifying α syn in brain homogenates from control and Lewy body disease (LBD) cases that were divided into cytosolic and particulate fractions. α syn migrated to 57–60 kDa as well as to 14 kDa in the particulate but not cytosolic fraction owing to the different conformational states of the protein (Lashuel et al., 2013)	7
Figure 3-4: α -Synuclein domain structure. Upon binding to lipid membranes, the N-terminal domain of α -synuclein folds into two amphipathic helices; the C-terminal tail of α -synuclein does not contribute to membrane binding. The lipid binding domain can be divided into seven highly conserved 11-mer sequences. Helix 2 contains the aggregation-prone NAC-domain. All disease-linked mutations of α -syn are in the second and fourth 11-mer stretch (Burré, 2015).	8
Figure 4-1: Physiological and pathological conformations of α -synuclein at the synapse. Cytosolic α -synuclein is monomeric and natively unfolded. Upon binding to synaptic vesicles, the N-terminal residues of α -synuclein adopt a helical structure (Burré, 2015)	10
Figure 5-1: Mechanisms of α -synuclein aggregation and propagation (Lashuel et al., 2013)	13
Figure 5-2: Multiple pathways for α -Syn aggregation. where Nu represents the natively unfolded α -synuclein monomer. (Hong, Fink and Uversky, 2009).....	14
Figure 5-3: Schematic illustration on possible oxidative stress pathways in a dopaminergic neuron. (1) Dopamine up take by the dopamine transporter (DAT) into the dopaminergic neuron (purple circle); (2) Dopamine uptake by the vesicular monoamine transporter (VMATS) into synaptic vesicle2; (3) Dopamine release from the synaptic vesicle by a-Syn (red triangle); (4) Dopamine oxidation to dopamine quinone (DAQ); (5) DAQ production of potential mitochondrial inhibitors such as metabolites or 5cysDAQ conjugates; (6) Mitochondrial production of oxidative stress; (7) Oxidation of a-Syn; (8) Tagging of a-Syn by ubiquitin and subsequent degradation by the proteasome; (9) Alpha-syn oligomerization; (10) Toxic interaction of a-Syn with the proteasome; (11) Interactions of oxidation by products such as 4-hydroxynonenol with the proteasome; (12) Oxidative stress produced by the surrounding glial cells; (13) Induction of programmed cell (Andersen, 2004).	18

Figure 5-4: Reactive Oxygen Species (Andersen, 2004)	20
Figure 6-1: Schematic representation of the main sites of α -Syn.	28
Figure 6-2: A schematic representation of the potential cycle connecting α -Syn with elevated cytoplasmic dopamine (Barrett and Timothy Greenamyre, 2015).....	30
Figure 6-3: Inclusion body formation in Parkinson's disease cell models	33
Figure 7-1: Symptoms of PD are caused by insufficient dopamine	37
Figure 7-2: Schematic illustration of anxious frozen face and flexed posture of a Parkinson's disease patient (Lees, Hardy and Revesz, 2009).....	39
Figure 7-3: L-DOPA is converted to Dopamine by enzyme DOPA decarboxylase (DDC).....	41
Figure 7-4: Microscopic image of a Lewy body surrounded by α -Syn as its major component (National Institute of Neurological Disorders and Stroke, 2015).....	42

1 INTRODUCTION

In recent times, more than several findings or researches have been carried out about this protein alpha-synuclein (a-syn) by several researchers and research institutions. I think the tangible discovery of this protein defect linked to Parkinson's disease (PD) in a family was a ground-breaking discovery and has opened a door to a flood of studies investigating the genetic base of the disorder. The actual role it plays or how it is genetically linked to these disorders has vehemently remained a misery but with the speed of research on alpha synuclein and these disorders, I think with more effort on scientific research, the actual understanding of the role of this protein will be established. This protein is a naturally occurring protein of the nerve cells just like other cells of the body, but when there are unwanted changes in shape or conformation (mutation), it leads to abnormalities. The actual cause of the mutation is yet to be known. One can hardly write on alpha synuclein without correlating the protein with neurodegenerative disorders, but for this thesis, I will be glad to explain the context of a-syn and synucleinopathies with its relationships with some neurodegenerative disorders focussing attention on its relationship with Parkinson's disease, since it has been identified as the major contributor of this neurodegenerative disorder.

According to Dr. Dennis J. Selkoe (a Prof. of Neurologic Diseases at Harvard Medical School), researchers are working hard over the years to ascertain what normal a-Syn in the brain looks like, with reference to its shape or structure. He said it was believed initially that strands of normal a-Syn appear as individual molecule (standing alone) as a monomer; however, in recent times, it has been observed that normal a-Syn is always folded in a group made up of four molecules that are held together natively in a cluster known as tetramer, as illustrated in figure 1.1 (Nyström, Nordström and Nordström, 2016).

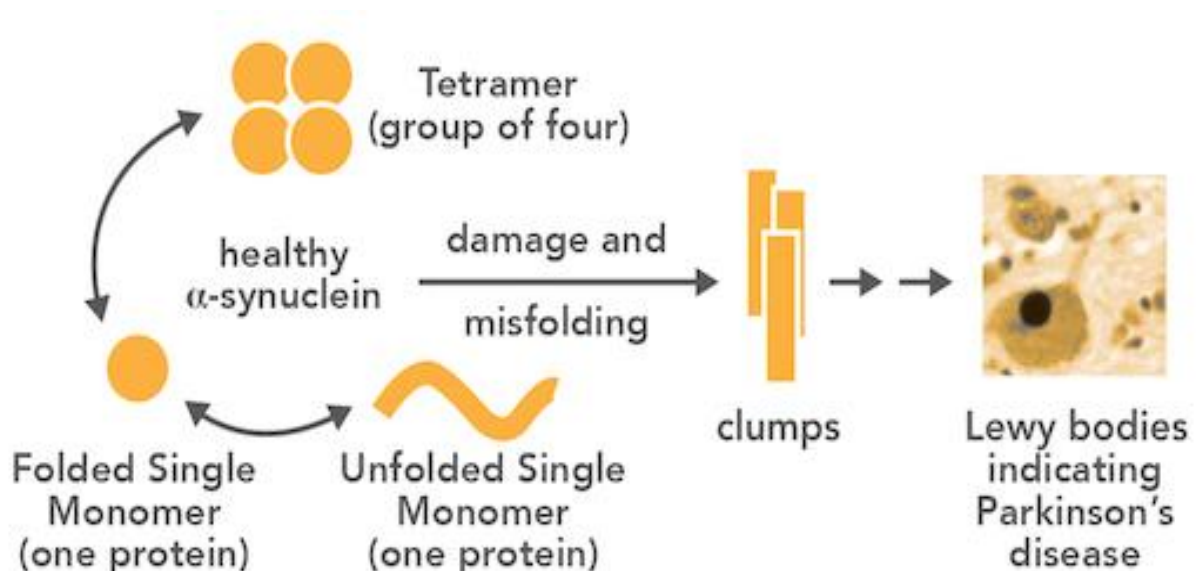


Figure 1-1: How the structure and shape of α-syn in Parkinson's might cause disorder. The protein monomer, when assembled in groups of four (tetramers) can always protect itself from being disrupted or damaged. However unfolded single strands (monomers) are at risk for disruption which leads to clumping in Parkinson's disease (Nyström, Nordström and Nordström, 2016)

2 HISTORICAL REVIEW

This theoretical review of Alpha Synuclein and synucleinopathies as related to Parkinson disease would not be a complete review work without a word or sentence on any or all of these: Lewy bodies, Alzheimer's Disease, Dementia and other forms of neurodegenerative maladies.

Alpha-synuclein is a protein that is abundant in the human brain. Fewer quantity are seen in the muscles, heart and other tissues. In the brain, a-syn is found mainly at the tips of nerve cells (neurons) in specialized structures called presynaptic terminals (Federoff *et al.*, 2015), it is good to know that α -synuclein is never seen in all terminals of the synaptic vesicle, and, surprisingly, not all terminals contain the protein in neurodegenerative diseases (Burré, 2015). The name of the protein a-Syn was coined from its place of abode (presynaptic vesicles), and nuclear region of the brain (Maroteaux, Campanelli and Scheller, 1988). a-Syn was in parallel identified as the non-amyloid- β component (NAC) found in amyloid plaques of Alzheimer's disease patients. After the discovery of a-Syn, followed the identification of its close homologs β - and γ -synuclein (Uéda *et al.*, 1993). Since the discovery of this protein, it has been linked to several devastating diseases, in which Parkinson's disease and dementia with Lewy bodies are inclusive (Galvin *et al.*, 1999), multiple system atrophy (Spillantini *et al.*, 1998), Alzheimer's disease (Lewis *et al.*, 2010), Pick's disease, diffuse Lewy body disease (Nishioka *et al.*, 2010), amyotrophic lateral sclerosis (ALS) (Doherty, Bird and Leverenz, 2004), frontotemporal dementia (Wilhelmsen *et al.*, 2004), progressive supranuclear palsy (Judkins *et al.*, 2002), corticobasal degeneration (Yamashita *et al.*, 2014), Krabbe disease (Smith *et al.*, 2014). These diseases are collectively termed synucleinopathies, but the physiological function of a-Syn and its contribution towards these disorders has remained unknown.

2.1 ALPHA SYNUCLEIN (A- SYN)

Alpha-Synuclein (a-Syn) is a 14 kD (140 amino acids) acidic presynaptic protein with sequence number: **MDVFMKGLSK AKEGVVAAA E KTKQGVAAEAA GKTKEGVLYV GSKTKEGVVH GVATVAEKT E QVTNVGGAV VTGVTAVAQK TVEGAGSIAA ATGFVKKDQL GKNEEGAPQE GILEDMPVDP DNEAYEMPSE EGYQDYEPEA**. It is a major component of Parkinson's disease aggregates and is implicated in the pathogenesis of Parkinson's Disease and related neurodegenerative disorders. a-Syn accumulates in the brains of sporadic Parkinson's disease patients as a major component of Lewy bodies, which are cytoplasmic inclusions features of Parkinson's disease (rPeptide), (Kempuraj *et al.*, 2015). a-Syn appears to associate with other proteins that aggregates and is found in β -amyloid plaques and neuritic tangles in Alzheimer's disease. (Masliah *et al.*, 2001). Proteins are the building blocks of the

machinery inside the cell that makes the brain to work. The protein must be folded into a desired shape in order to do its job properly at the bud or early stage. a-syn are much in the brain, about one percent of the total protein that surround or move around the brain or the central nervous system (Dieriks *et al.*, 2017). In a well functional brain cell, a-Syn are found beneath the membrane covering the cell body and their branch ends or tips that originated from the cells called presynaptic terminal which passes messages between brain cells (Shults, 2006). Alpha-Synuclein as one of the synuclein family that is made up of beta Synuclein (β -Syn) and gamma Synuclein (γ -Syn). They are observed majorly within the neurones mainly at the synaptic terminals, they may increase the functions of the synaptic vesicles, regulate the release and transport of dopamine (Lashuel, Overk and Oueslati, 2013). Alpha-Synuclein is a minute protein of the presynaptic vesicle that plays a wide role in neurodegenerative maladies also called synucleinopathies.

2.2 FACTS ABOUT ALPHA-SYNUCLEIN

- Alpha-Synuclein misfolds to form protein aggregates in PD and in various invitro and in vivo models of synucleinopathies.
- Alpha-Synuclein is associated with both familial and idiopathic PD.
- The precise function of a-Syn is yet unknown.
- Alpha-Synuclein is the major constituent of Lewy bodies.
- Alpha-Synuclein is a member of the Synuclein family proteins.
- Alpha-Synuclein is all over in various brain parts in PD patients.
- It is present in a number of other synucleinopathies, such as multiple system atrophy (MSA), dementia with Lewy bodies (DLBs), Some Alzheimer's disease (AD) cases, neurodegeneration with brain iron accumulation (NBIA) type I, pure autonomic failure (PAF), and various others, (Kahle, 2007)

3 STRUCTURE AND PHYSIOLOGICAL FUNCTION OF A -SYN

Because the protein's sequence and structure of a-Syn are linked to its function, there is need to characterise the sequence and structural determinants that govern its cellular properties and its aberrant behaviour in PD and other synucleinopathies. a syn is a 14KDa protein, with 140 amino acids and pKa value of 4.7. It is characterised by an amphipathic lysine-rich amino terminal (aa 1-65) with several imperfect KTKEGV which modulates its interactions with the membrane, a hydrophobic central region which comprises amino acid residues (aa 66-90) known as the non-amyloid- β component (NAC) and a disordered, highly negatively charged carboxy-terminal tail C-terminus (aa 96-140) which regulates its nuclear localization and interactions with metals, small molecules and proteins (Lashuel *et al.*, 2013) and (Jakes, Spillantini and Goedert, 1994). It is also known to be a target to various post translational modification (Oueslati, Fournier and Lashuel, 2010) and also protects a-Syn from aggregation (Hoyer *et al.*, 2004)

Various mutations clinically if not all are seen in this region which portrays the usefulness of this N-terminal domain to all the activities of a-Syn including its pathological dysfunctions. The central portion which is unique to this presynaptic protein is called the NAC region with its stretch of amino acid residues , takes care of the aggregation features of a-Syn through hindering and promoting its degradation and fibrillation respectively (Xu and Pu, 2016). Surprisingly, all known changes or mutations that are linked with synucleinopathies originate from this domain: A30P, E46K, H50Q, G51D, A53E, and A53T (Burré, 2015). Many studies however have been focused on N-terminal portion, not much findings has been done on C-terminal may be in the nearer findings in future needs to put C-terminal region into consideration based on the fact that that some modifications like truncation is assumed to take place there (Games *et al.*, 2014). Represented in *figure 3-1* is the amino acid sequence of a-syn.

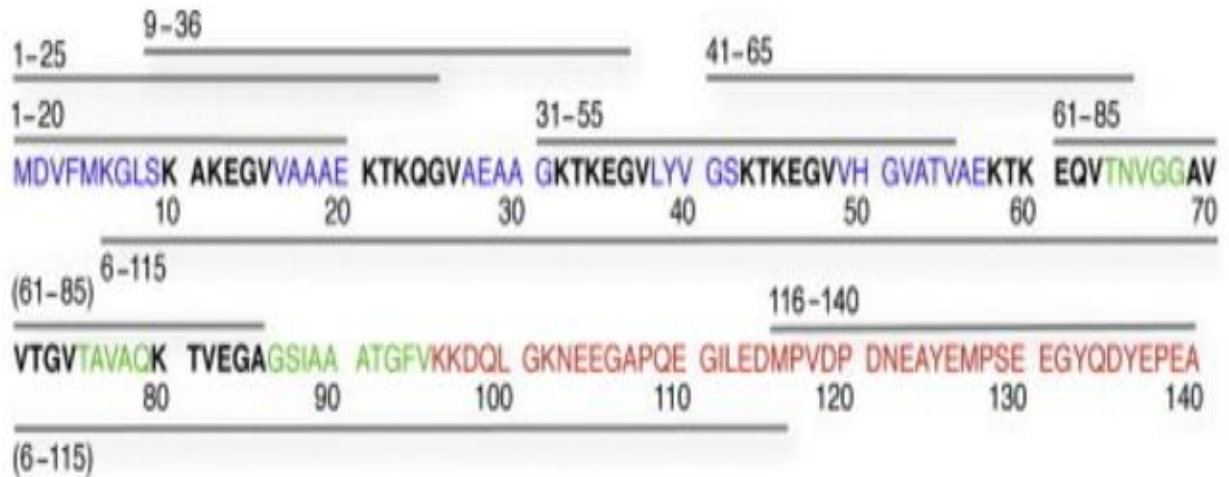


Figure 3-1: Amino-acid sequence of α -synuclein. The N-terminal region is blue, the aggregation-prone (NAC) domain is in green, and the C-terminal region is in red. The seven imperfect repeats are indicated in bold type. Protein fragments are schematically shown as bars above (synthetic small peptides) or below (chemically truncated protein). For clarity, the N-terminal truncated mutants are omitted (Bartels *et al.*, 2010)

Numerous clinical, pathological or environmental factors such as oxidative stress (Hashimoto *et al.*, 1999), Post-translational modification (Paleologou *et al.*, 2010), proteolysis (Dufty *et al.*, 2007), and the concentration of fatty acids (Karube *et al.*, 2008), ionic metals and phospholipids (Oueslati, Fournier and Lashuel, 2010) induce or modulate in vitro conformational modifications of a-Syn as influenced by these factors.. It is believed that a-Syn adapts to several changes when in contact with several compositions of biological membranes other protein complexes (Lashuel *et al.*, 2013). However, when a-Syn binds in vivo to a membrane either biological or synthetic, it adopts an α -helical conformation (Eliezer *et al.*, 2001a).

Studies by different research groups using several biophysical methods like; light scattering, circular dichroism Nuclear Magnetic Resonance (NMR) often showed that a-Syn under denaturing or natural condition subsist unfolded (Eliezer *et al.*, 2001b). But can be migrated as 57-60 KDa proteins mainly when gotten from Lewy bodies dementia (LBD) patients under non denaturing environment or Size Exclusive Chromatography (SEC) columns, but migrated as 14KDa proteins under native conditions, figure 3-3 (Luk *et al.*, 2009). From the beginning, the evident size of a-Syn in Native and SEC made it easier for researchers to assume that this protein exist as oligomer; now subsequent biophysical studies detailed revealed that the over-size or bigger size than hoped of a-Syn was because of the fact that monomeric a-Syn got an unfolded and elongated conformation (Fauvet *et al.*, 2012). Due to the fact that a-Syn got the ability to change easily or flexibly, which permits the protein to assume several conformational changes when interacting with different compositions of biological membranes, other proteins or protein complexes, a-Syn might exhibit multifunctional features (Muthu

Ramakrishnan, Poul H. Jensen and Derek Marsh*, 2006)(Ullman, Fisher and Stultz, 2011), though as earlier said it adopts an α -helical conformation only upon synthetic or biological membrane binding. α -Syn might may portray different conformation in several sections or partitions of the cell meanwhile that has not been detailed including the actual function of this oligomeric proteins in the membranes and other cell compartments.

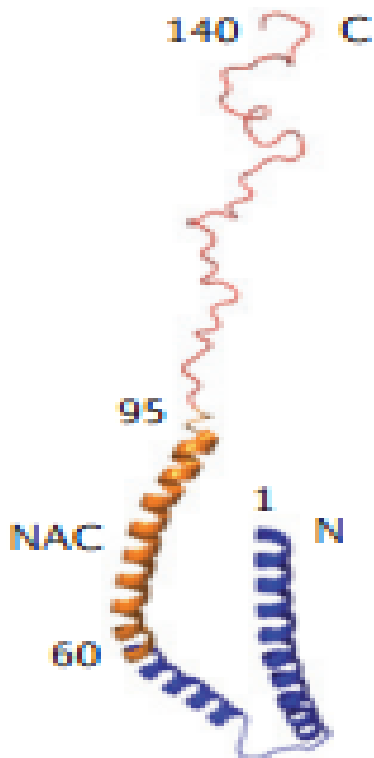


Figure 3-2: Schematic representation of micelle-bound α -synuclein. The N-terminal region, the non-amyloid- β component of Alzheimer's disease amyloid plaques (NAC) region and the C-terminal part are coloured blue, orange and red, respectively. Numbers refer to amino acid residues flanking the different regions (Lashuel et al., 2013)

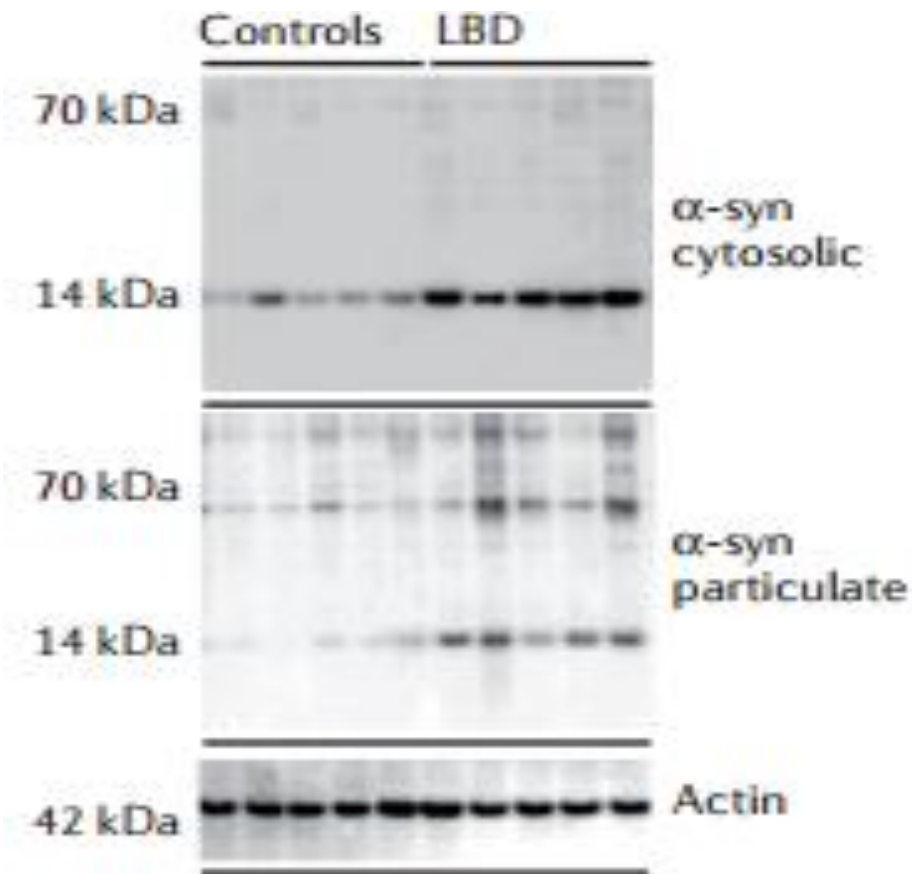


Figure 3-3: Western blot identifying α syn in brain homogenates from control and Lewy body disease (LBD) cases that were divided into cytosolic and particulate fractions. α syn migrated to 57–60 kDa as well as to 14 kDa in the particulate but not cytosolic fraction owing to the different conformational states of the protein (Lashuel et al., 2013)

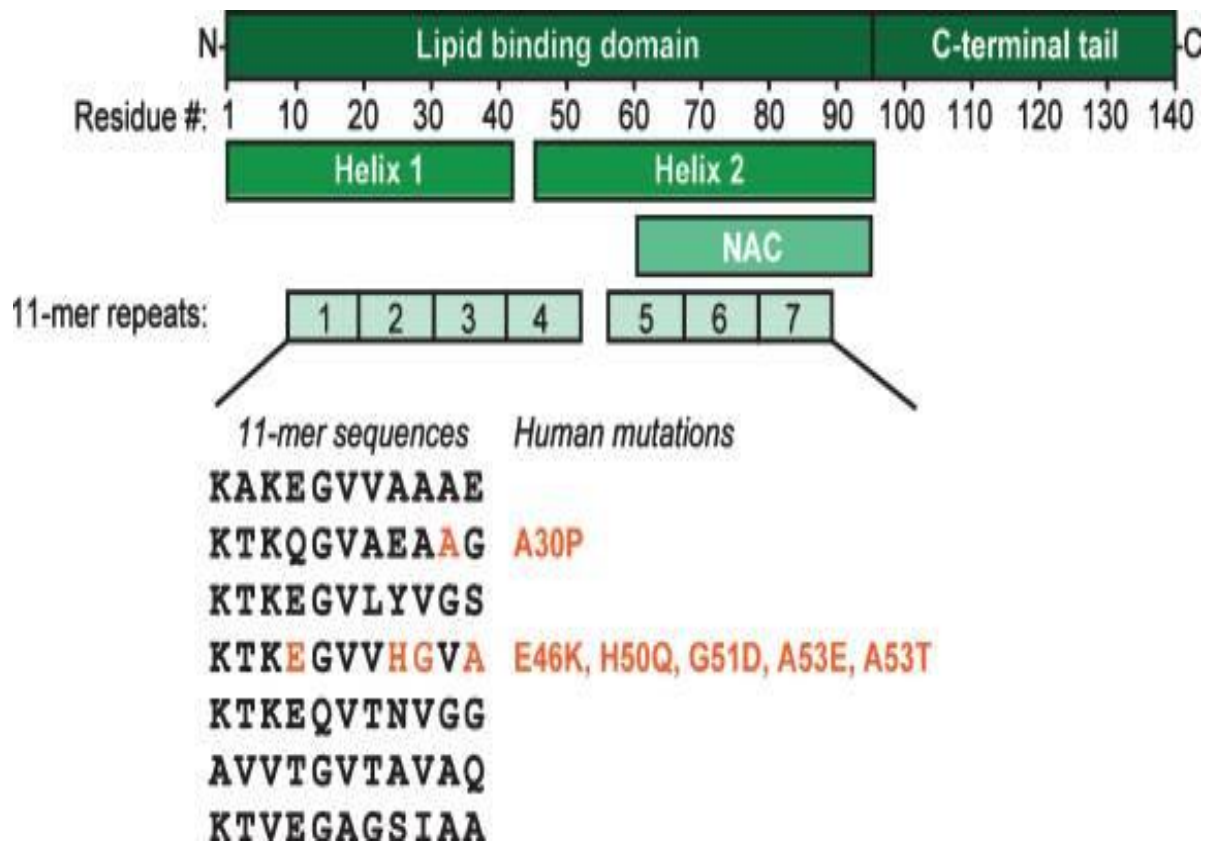


Figure 3-4: α -Synuclein domain structure. Upon binding to lipid membranes, the N-terminal domain of α -synuclein folds into two amphipathic helices; the C-terminal tail of α -synuclein does not contribute to membrane binding. The lipid binding domain can be divided into seven highly conserved 11-mer sequences. Helix 2 contains the aggregation-prone NAC-domain. All disease-linked mutations of α -syn are in the second and fourth 11-mer stretch (Burré, 2015).

There are two hypotheses concerning the native state of a-Syn; the monomeric shape and an α -helical folded tetramer. Initial findings of a-Syn extracted from bacterial expression system or mouse tissue showed that it is monomeric, with minimal secondary structure (Mor *et al.*, 2016). But according to (Bartels, Choi and Selkoe, 2011), identified the state of a-Syn in living human cells by freshly examining collected human red blood cells (RBC) indicated that natively, endogenous cellular a-Syn exist largely as an α -helical folded 58KDa tetramer. Recent findings now show that the normal physiological function of a-Syn involves roles in compartmentalization, storage and recycling of neurotransmitters (Allen Reish and Standaert, 2015). It is also linked with the physiological regulation of some enzymes and increase the number of dopamine transporter molecules (Xu and Pu, 2016), release of neurotransmitter (Nemani *et*

al., 2010) and interaction with the synaptic SNARE complex are partly mediated by its action as molecular chaperon (Xu and Pu, 2016),(Burré *et al.*, 2010)

4 INTRACELLULAR POOLS OF ALPHA-SYNUCLEIN

This explains in details the intracellular existence and reactions of a-Syn within the cells at different environmental conditions be it denaturing condition or non-denaturing. Also, the behavioural and structural appearance of the protein when in association with or found in such environment. Alpha-synuclein either exist in a soluble state or a membrane bound state with its secondary structure depending on its environment. Alpha-synuclein's interaction with lipid surfaces serves as a mediator for its cellular functions in synaptic regulations and impacts the formation of fibrils which is associated with PD, this is illustrated schematically on figure 4-1

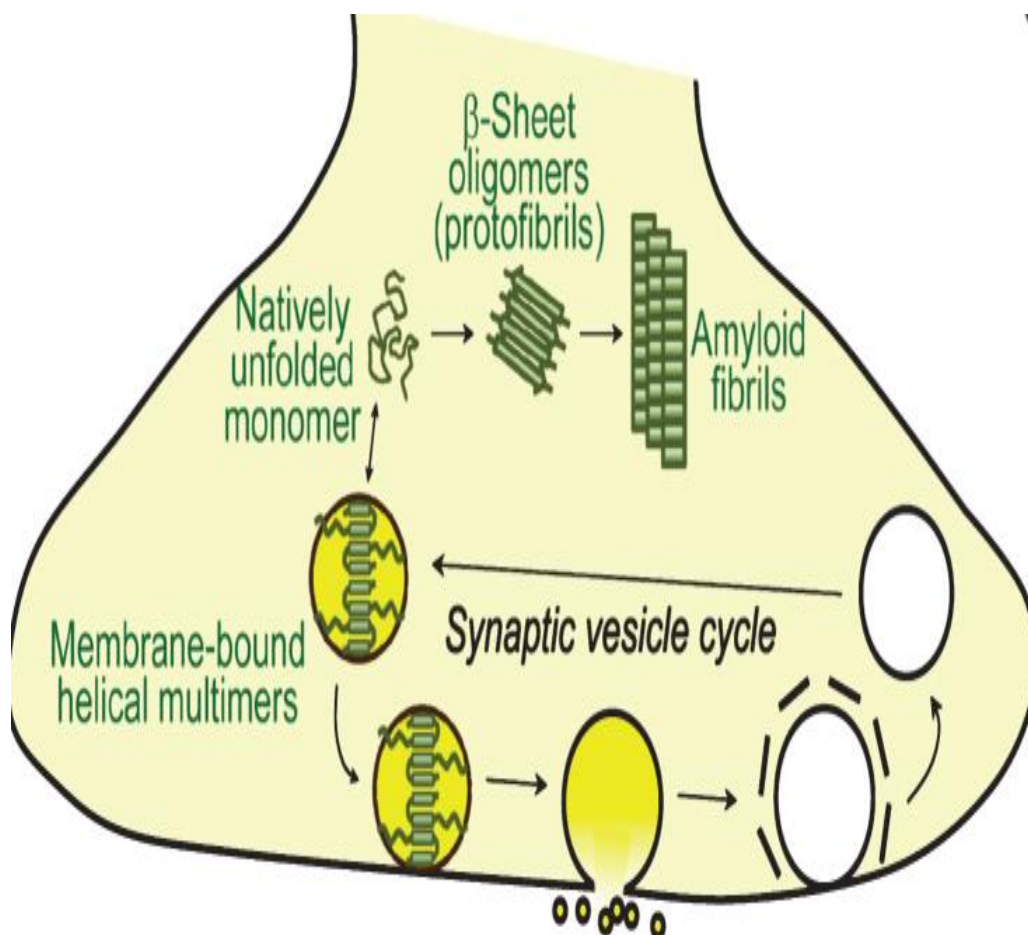


Figure 4-1: Physiological and pathological conformations of α -synuclein at the synapse. Cytosolic α -synuclein is monomeric and natively unfolded. Upon binding to synaptic vesicles, the N-terminal residues of α -synuclein adopt a helical structure (Burré, 2015)

The N-terminal residue of α -Syn adopts an α -helical structure in the presence of lipid membranes such as artificial liposomes, lipid droplets and lipid rafts and this α -helical structure mediates the binding of α -Syn to the membranes (Bussell and Eliezer, 2003). Membrane binding is a cooperative effect of the 11-mer sequences, as truncation of N-terminal domain hinders lipid binding drastically. So, acid heads such as phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol are required (Middleton and Rhoades, 2010). This suggests the interaction of the membrane head groups with lysine's found on opposite sides of α -Syn helix, based on the size or curvature of the membrane, α -Syn can transition between two states (Ulmer *et al.*, 2005). In that regards, α -Syn adopts an elongated helix conformation upon binding to membranes with larger diameter, and adopts a broken helix conformation in the presence of small and highly curved vesicles (Chandra *et al.*, 2003).

Acetylation of N-terminal α -Syn is seen both in healthy and PD individuals, it helps to increase its helical folding propensity, its affinity for membranes and its resistance to aggregation (Kang *et al.*, 2012). With these, one can suggest that N-terminal acetylation of α -Syn could be of great importance for both the native and pathological structures and functions of α -Syn (Trexler and Rhoades, 2012). Not forgetting that phosphorylation of α -Syn which is a post translational modification regulates its membrane binding, its fibril formation, its structure, protein interaction, neurotoxicity and oligomerization (Fujiwara *et al.*, 2002a), whereas other forms of post translational modification may lead to changes in protein charge and structure which may also alter the binding affinities with other proteins and lipids. The folding of α -Syn stabilizes and protects α -Syn from aggregation (Lokappa *et al.*, 2014), though under oxidative stress condition, membrane binding accelerates α -Syn aggregation (Lee, Choi and Lee, 2002a).

5 AGGREGATION OF ALPHA-SYNUCLEIN

Aggregation of proteins has been a shared feature of many human neurodegenerative disorders, and it has portrayed to be an inevitable consequence of excessive accumulation of misfolded proteins. Several factors like molecular chaperones, protein degradation systems and free radicals of which are under the control of normal mitochondrial functions, can either suppress or promote protein misfolding or aggregation *in vivo*; this aggravates the speculation that mitochondrial dysfunction might lead to accumulation of protein aggregates. Defects in mitochondria can therefore lead to a-Syn aggregation and aggregated forms of a-Syn has been identified to be more toxic than the monomers (Lee, 2003).

The pathological formation of a-Syn aggregates in different brains of PD patients is still a misery, but however it has been proposed that some of the symptoms of these neurodegenerative disorders might be related to the widespread pathology of a-Syn aggregation in different nuclei of the central and peripheral nervous system. This simply means that PD and other neurodegenerative diseases are associated with abnormal neuronal aggregation of a-Syn which either takes place in the cytoplasmic region or together with the membrane of the cells. In the cytoplasmic region, there is interaction between unfolded monomers first to form inconsistent dimers that slowly grow to produce to produce different morphologies of oligomers like ring-like and globular oligomers that eventually metamorphous to fibrils through a mechanism called nucleated polymerization which accumulates with time to form Lewy bodies as intracellular inclusions. Membrane bound monomeric a-Syn adopts an alpha-helical conformation, but when the concentration of protein is high, it undertakes a conformational innovation to form β -sheet-rich structures that associate self to produce oligomer, amyloid pores and fibrils (Lashuel *et al.*, 2002). Throughout the process of a-Syn aggregation and fibrillogenesis, amyloid pores and oligomers which are the intermediate species show high level of toxicity that affect the functions of the mitochondria, Endoplasmic reticulum - Golgi trafficking, transmission of the synaptic vesicles and degradation of proteins. All these intracellular reactions are believed to instigate or generate neurodegenerative disorders. Neuronal toxicity may also be contributed by transmembrane pores which also disrupt the integrity of the membrane, intracellular calcium homeostasis and signalling. It is interesting to know that alpha-synuclein oligomers/fibrils and monomers can move between cells to generate spreading of diseases to other brain areas, there are several mechanisms of spreading; direct penetration, endocytosis, membrane receptors or trans-synaptic transmission and once it enters the cell of the host, its aggregates nucleate aggregation and expand through this mechanism. Figure 5-1 portrays the schematic illustration.

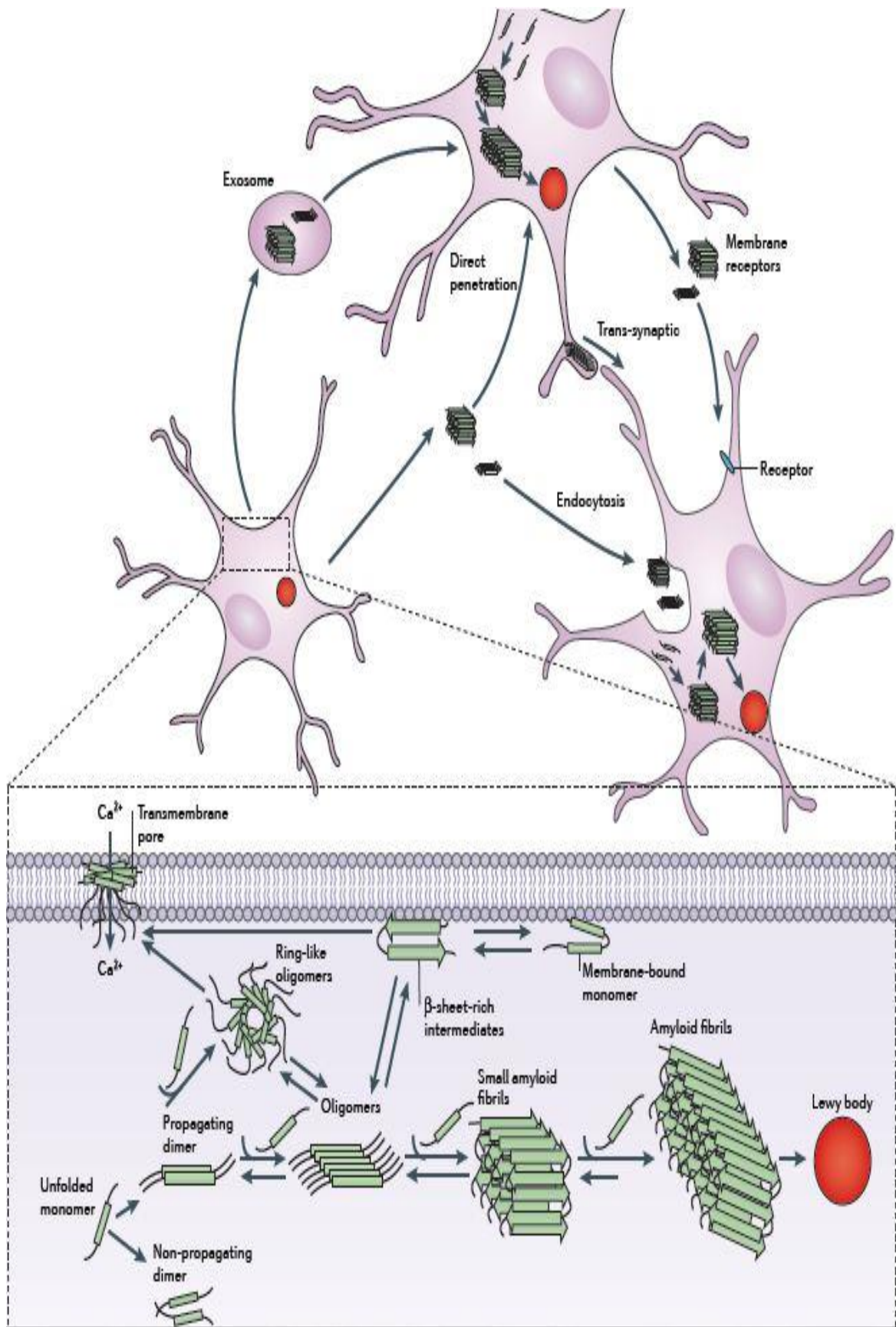


Figure 5-1: Mechanisms of α -synuclein aggregation and propagation (Lashuel et al., 2013)

The pathways of α -Syn aggregation starts with negatively unfolded α -Syn monomer, that folds partially to form an aggregation-prone intermediate which is environmentally conditioned dependent. The formed intermediate produces three separate products; soluble oligomers, insoluble fibrils or insoluble amorphous aggregates (Uversky, 2007a). The adoption state of α -Syn is dependent on the changes associated with the environmental conditions like increase in temperature or decrease in pH which evolved to form partial folded intermediate, figure 5-2.

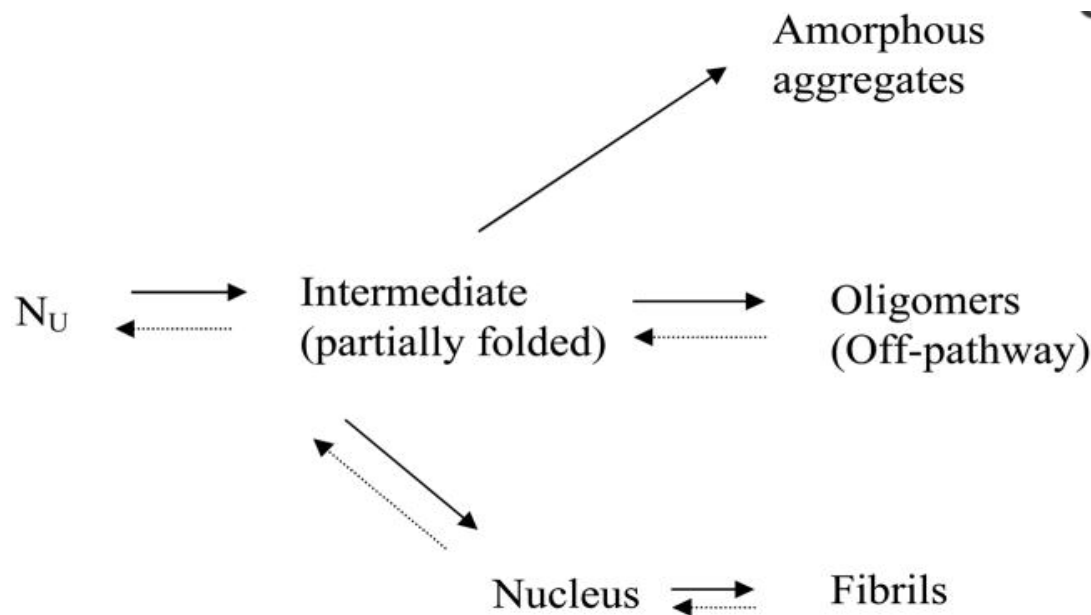


Figure 5-2: Multiple pathways for α -Syn aggregation. where Nu represents the natively unfolded α -synuclein monomer. (Hong, Fink and Uversky, 2009)

5.1 FACTORS THAT CONTRIBUTE TO AGGREGATION OF A-SYN

There are several contributing factors to the aggregation and fibrillation of α -Syn in the central or peripheral nervous systems, but for the sake of this theoretical review, I will be mentioning these few.

5.1.1 GENETIC FACTORS

There are two main genetic abnormalities that link α -Syn directly with neurodegenerative disorders; overexpression of α -Syn may be lead to that because the α -Syn gene locus was doubled or tripled (Singleton *et al.*, 2004), a kind of point mutation in which a single nucleotide change result to a code, coding different amino acids of α -Syn which correspond to E46K, A30P and A53T substitutions in the α -Syn protein (Zarranz *et al.*, 2004). Though it will be good to know that without adding any factor, there will be equilibrium existence amongst

the naturally existed one that is not folded and the one that is folded partially in shape. Increased protein concentration will also elevate the whole collection of amyloidogenic intermediate thereby increasing fibrillation (Uversky and Eliezer, 2009)

Studies from different familial PD patients clearly revealed that no matter how minute an alteration is at the α -Syn gene of human, it is enough to induce Parkinson's disease, therefore the above mentioned PD related gene alterations immensely contributed to the in vitro aggregating not fibrillating features of α -Syn (Li, Uversky and Fink, 2002). It has been known via high resolution NMR analysis that alteration at A30P distorts or destroys a portion of the protein helical shape (Eliezer *et al.*, 2001c), while the mutation of A53T results in extension of minute portion around the mutation site (Bussell and Eliezer, 2001). Therefore, increase in vulnerability of A53T and A30P internally to generate β -sheet may not change the protein structure rather it might alter the α -Syn mutant aggregation behaviour (Li, Uversky and Fink, 2001), for this reason, the α -Syn mutant fibrillation is more or faster at A53T. As for E46K, because of its location in KTKEGV at the region of α -Syn, enhances fibrillation (Uversky and Eliezer, 2009),(Greenbaum *et al.*, 2005).

5.1.2 MOLECULAR CROWDING

The proteins natural environment inside the living cells is densely crowded with different concentrations of cellular components such as nucleic acids, carbohydrates, small solutes and macromolecules. In this environment, the space taken or occupied by one of the molecules will never be available to another molecule which gives rise to what is called excluded volume effect (Uversky and Eliezer, 2009). Suggestions made it clear that volumes exclusion in media physiologically will likely enhance the rate of in vivo amyloid formation in vivo (Minton, 2000). Metals and other substances like pesticides which also increase the rate of PD through epidemiological studies, when present under the crowding condition, accelerate the aggregation or fibrillation of α -Syn (Munishkina *et al.*, 2004), (Munishkina *et al.*, 2008).

5.1.3 ANIONS AND SALTS

Folding of α -Syn at neutral PH to amyloidogenic intermediate can be caused by various anions. The magnitude at which these anions inspire fibrillation depends on the anion and the position of the anion in the lyotropic or Hofmeister series, which suggests that anions modulates protein-water interactions as its own contribution to α -Syn fibrillation. Because of this finding, it has been deduced that the increased α -Syn fibrillation amidst anions is the outcome of the folding partially regulated by non-compensating charge loss and hydration increase preferentially that boosts aggregation and partial folding by empowering interaction hydrophobically (Uversky and Eliezer, 2009).

5.1.4 POLYANIONS AND POLYCATIONS

Glycosaminoglycan's (GAGs) and polyglycans (PGs) contribute to the formation

of proteinaceous deposits in several human diseases (McLaurin *et al.*, 2000). These polysaccharides especially GAG (heparin, heparin sulphate) and other highly sulphated polymers like dextran sulphate, an extracellular matrix and trans-membrane heparin sulphate known as agrin were observed to bind to a-Syn and initiate its *in vitro* fibrillation in the central nervous system (Cohlberg *et al.*, 2002), (Liu *et al.*, 2005)

Agrin and a-Syn were observed to be closely located in the LB and LN of PD patients found in their substantia nigra, this indicates that PG may have contributed to the origin of Parkinson's disease by regulating a-Syn aggregation state in the brain (Liu *et al.*, 2005). Several other polycations like spermidine, spermine, polyethyleneimine, polyArg and polyLys also interacted with a-Syn to originate or instigate the folding of this protein partially, also encourage the fibrillation and oligomerization (Uversky and Eliezer, 2009).

5.2 ENVIRONMENTAL FACTORS

Exposure to the environment has been a potential contributing factor to neurodegenerative disorders that is one of the reasons why Parkinson's disease is now considered as an environmental disease (Tanner, 1989). Many factors contribute to this environmental factor some of which I will mention.

5.2.1 PESTICIDES AND HERBICIDES

The result gotten by analysing some pesticides and herbicides that are daily used, on a-Syn structure and aggregation revealed that they (pesticides and herbicides) induce a conformational change in a-Syn which significantly accelerates its fibrillation, (Manning-Bog *et al.*, 2002). These structural change and fibrillation acceleration feature or property was due to their potential to stick and balance the amyloidogenic partially folded conformation (Uversky and Eliezer, 2009)

5.2.2 HEAVY METALS

Subjection to some kinds of heavy metals has proven to be a potential risk factor to neurodegenerative maladies PD in particular (Dexter *et al.*, 1991).

From the studies of disease spreading and the result of post-mortem analysis of the brain of a PD patient, it is shown that a number of monovalent, divalent, and trivalent ionic metals can potentially elevate a-Syn fibrillation process, (Uversky, Li and Fink, 2001). The rate at which these metal cations induce this reaction can be traced to their correlation with increasing density of charged ions, also their capacity to modulate amyloidogenic species that are folded partially due to masking of intracellular charge-charge repulsion in natively a-Syn molecule (Sung, Rospigliosi and Eliezer, 2006).

5.2.3 ORGANIC SOLVENTS

Exposure to organic solvent like lacquer thinner was identified to increase the incidence of PD by analysing the propensity of a-Syn aggregation or fibrillation in water-organic solvent and its structural features (Uversky, 2007b) a-syn fibrillation has been identified to be favoured by several organic solvent even at

their low concentration which induce partially amyloidogenic folded conformation (Munishkina *et al.*, 2003).

5.2.4 OXIDATIVE STRESS

Oxygen plays a vital role in human existence and other animals, in the other words, oxygen is life and no life without oxygen but paradoxically the by-product of oxygen metabolism produces reactive oxygen species (ROS) which are identified to be highly toxic to the cells. Oxidative stress can either enhance a-Syn aggregation or inhibit the aggregation of a-Syn.

Oxidative stress as a result of the by-product of oxygen metabolism in which the production of ROS out powers anti-oxidant defences has acceleratively been observed in several brain maladies characterized by depositing affected proteins in the affected brains of PD, AD, LBD, ALS, Huntington's Disease HD, and Picks Disease (Wang *et al.*, 2003). ROS induces peroxidation of lipids to generate highly reactive aldehydes, amongst which are the highly reactive aldehyde-4-hydroxy-2-nonenal (HNE) and Malondialdehyde (MDA). These aldehydes have been seriously sported out in early development of brain maladies (Dalfó *et al.*, 2005). Although oxidatively modified proteins can also accumulate during normal aging (Smith *et al.*, 1991). All the neurodegenerative disorders risk factor that have been considered, are strong generators of a-Syn fibrillation, though amyloid fibril was believed to be harmful on its own. According to (Arrasate *et al.*, 2004), some proteinaceous inclusions deposited like Lewy neurites or Lewy bodies deposited in PD, senile plaques deposited in AD are not harmful but are rather protective to the survival of the cells. The information above led to the proposal of an alternative amyloid hypothesis (the oligomer hypothesis) which states that during the assembling of fibrils or fibrillation process, some components or species formed may instigate cell damage since matured amyloid fibrils might not be toxic. Protofibrils or small oligomers formed during the process of fibrillation were presumed to be accountable for neurotoxicity (Uversky and Eliezer, 2009),(Sokolov *et al.*, 2006)

Oxidative stress has therefore consistently been associated with these neurodegenerations. But there is no evidence not withstanding that oxidative stress is directly involved in the degeneration that is associated with these maladies. The cells have different defence and repair mechanisms (antioxidants) to handle the issues of oxidative stress and associated oxidative damage but when injurious effects of ROS out power the defensive effects of these defence mechanisms, there will be deposition of unwanted proteins in the affected brain regions that leads to aggregation of fibrillation of a-Syn. The antioxidant enzymes Superoxide dismutase (SOD), Catalase, Glutathione peroxidase (GSHPx) and Glutathione reductase (GSHRd) showed reduced effects within the affected regions of the brain in AD (Pappolla *et al.*, 1992). Citric acid concentrations which potentially scavenge ONOO⁻ the activities of the enzyme methionine sulfoxides reductase which reverses oxidation at protein methionine residues are also

reduced (Gabbita *et al.*, 1999). Methionine oxidized α -Syn showed high unfolded conformation compared to the one that is not oxidized (Glaser *et al.*, 2005) and they are less prone to aggregate, and are also capable of inhibiting the fibrillation of non-modified α -Syn (Vladimir N Uversky *et al.*, 2002). Here the number of oxidized methionine determines the strength of the inhibition of α -Syn fibrillation by methionine oxidation (Hokenson *et al.*, 2004) but this inhibitory effect of methionine oxidation can be overcome by certain metals like (Ti^{3+} , Zn^{2+} , Al^{3+} and Pb^{2+}) (Yamin *et al.*, 2003). This basically suggests that under conditions of industrial pollution where some metals like the above mentioned are exposed, the protective anti fibrillation effect of methionine residue in α -Syn will likely reduce or fail totally (Uversky and Eliezer, 2009).

Generally, one can say that disorders that involved oxidative stress can be observed because of ineffective scavenging system, low or insufficient antioxidant concentrations, overproduction of free radicals and other oxidants.

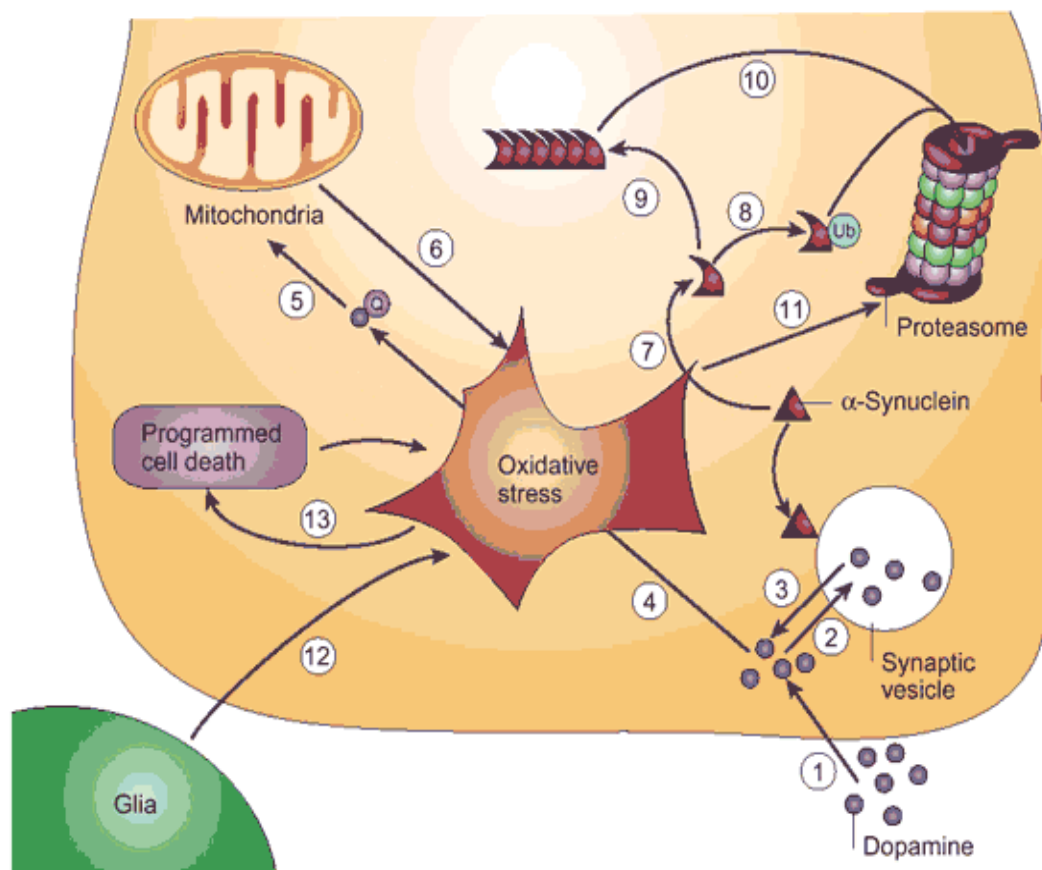


Figure 5-3: Schematic illustration on possible oxidative stress pathways in a dopaminergic neuron. (1) Dopamine up take by the dopamine transporter (DAT) into the dopaminergic neuron (purple circle); (2) Dopamine uptake by the vesicular monoamine transporter (VMAT2) into synaptic vesicles; (3) Dopamine release from the synaptic vesicle by α -Syn (red triangle); (4) Dopamine oxidation to dopamine quinone (DAQ); (5) DAQ production of potential mitochondrial inhibitors such as metabolites or 5cysDAQ conjugates; (6) Mitochondrial production of oxidative stress;

(7) Oxidation of a-Syn; (8) Tagging of a-Syn by ubiquitin and subsequent degradation by the proteasome; (9) Alpha-syn oligomerization; (10) Toxic interaction of a-Syn with the proteasome; (11) Interactions of oxidation by products such as 4-hydroxynonenol with the proteasome; (12) Oxidative stress produced by the surrounding glial cells; (13) Induction of programmed cell (Andersen, 2004).

5.2.5 REACTIVE OXYGEN SPECIES (ROS)

These are molecular bodies that interact with components of cells leading to their functional defective effect. Reactive oxygen species are made up of free radicals that contain very high reactive unpaired electrons like superoxide, hydroxyl radicals (OH^\cdot) and nitric oxide (NO) with various molecular species like Peroxynitrite (ONOO^-) and hydrogen peroxide (H_2O_2). Mostly, majority of cellular reactive oxygen species are initiated when oxygen is incompletely reduced to water metabolically. Oxygen undergoes one transfer of electron to generate (H_2O_2), H_2O_2 undergoes another electron transfer to produce H_2O . Nitric oxide synthase converts arginine to citrulline and NO, this NO, can react with O_2^- to yield ONOO^- figure 5-2.

Ferrous iron (Fe^{2+}) which is a reduced metal ion can convert to H_2O_2 to OH^\cdot through a reaction process known as Fenton reaction. All these redox species can combine or interact with surrounding cell components like protons, DNA and lipids. Oxidation of proteins produces a wide range of defective or damaging effects like interrupting the active sites of enzymes or affecting the structural conformation of proteins like a-Syn. (Andersen, 2004)

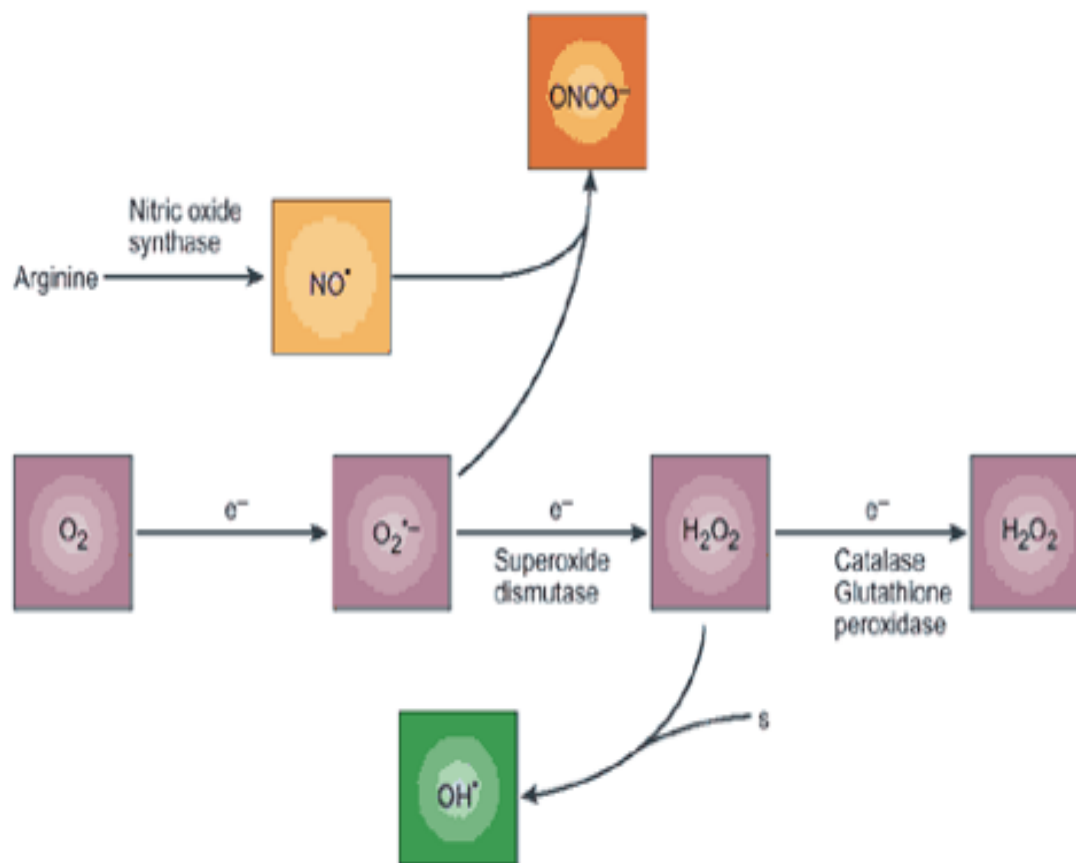


Figure 5-4: Reactive Oxygen Species (Andersen, 2004)

5.2.6 PROTEIN INTERACTION WITH OTHER PROTEINS

The interaction of α -Syn with various other proteins in the brain region has a lot of influence or effect on the proteins aggregation and fibrillation. Addition of other α -Syn binding proteins to it or protein-protein interaction may either enhance α -Syn aggregation and fibrillation or inhibit it depending on the nature and conformation of the proteins. For instance, when any of the other members of the synuclein family beta-synuclein (β -syn) or gamma-synuclein (γ -syn) that is natively unfolded interacts with α -Syn in a concentration dependent manner, it inhibits the fibrillation of this protein (Sung and Eliezer, 2007). Therefore, the two homologous proteins β -syn and γ -syn may be termed to function potentially as chaperone by regulation the in vitro fibril formation of α -Syn. The inability of fibril formation by β -syn is as a result of unavailability of hydrophobic stretch residue from the middle portion or area of the protein (Vladimir N. Uversky *et al.*, 2002).

5.2.7 INTERACTION WITH PARAQUAT

Interactions or administration of paraquat (N, N'-dimethyl 4, 4'-bipyridilium) with α -Syn activates α -Syn upregulation followed by the translocation of this protein into the nucleus to generate histone- α -Syn- complexes, this mechanism helped to sustain or activates the normal response of α -Syn (Goers *et al.*, 2003). Going by the words of (Makwana and Sundd, 2016) on chaperone, outlined thus, chaperones like heat shock proteins (Hsp) can regulate α -Syn aggregation. Alpha-

B-crystallin which is a Hsp seen in Lewy body hinders the fibrillation of a-Syn by relating with the oligomeric species and fibrils (Waudby *et al.*, 2010). Minute or Small heat shock proteins (sHsp) such as Hsp20, Hsp27, HspB2B3 and HspB8 after passing through in vitro test for their inhibitive ability on a-Syn aggregation was found that all small heat shock proteins reacts with monomeric a-Syn temporarily, showing that Hsp B2B3 cannot hinder or inhibit the aggregation of A53T and E46k but it can hinder that of A30P and wild type mutant which suggests that it hinders the aggregation of slowly or gently aggregating proteins (Cox, Carver and Ecroyd, 2014), Contrarily, Hsp20 inhibits the fibrillation of E46K and A53T and not that of A30P and wild type (Bruinsma *et al.*, 2011), whereas Hsp27 inhibit in vitro a-Syn aggregation (Huggins *et al.*, 2011).

Heat shock inhibitor (Hsp90) that upregulates or boost Hsp70 levels also inhibit the oligomerization of a-Syn and its associated toxicity (Putcha *et al.*, 2010). From the findings of (Gade, Kardani and Roy, 2014), heat shock protein (Hsp104) portrayed deleterious or negative effect on yeast cells , Recently, (Jones, Moussaud and McLean, 2014) discussed in details the roles or effects of Hsp in preventing the toxicity associated with a-Syn.

DJ-2 is another a-Syn protein binding protein though malfunctional in nature but have the activities of a chaperone by inhibiting a-Syn aggregation (Zhou *et al.*, 2006),(Shendelman *et al.*, 2004).

The inhibitive effect of this malfunctional protein (DJ-1) is oxidation state related, unoxidized or native DJ-1 neither interact with a-Syn nor hinders its aggregation or fibrillation but when DJ-1 is oxidized through the formation of sulfinic acid of Cys106, it interacts with a-Syn and prevent its in vivo fibrillation effectively (Uversky and Eliezer, 2009)

5.2.8 INTERACTION WITH TUBULIN

In Parkinson's disease brain extracts including LB and LN was identified the combination of a-Syn and tubulin. In vitro studies revealed that addition of small quantity or amount of tubulin into a media that contains a-Syn can not only begin but also increase the aggregation of a-Syn into fibrils (Alim *et al.*, 2002). Going by the findings of (Chen *et al.*, 2007), it said that polymerization of tubulin within the dopaminergic neurons which lead to cell death can be reduced by extracellular oligomeric a-Syn, which suggests that such interaction between a-Syn and tubulin might be responsible for Lewy bodies formation. Therefore, the disruption of assembled tubulin by environmental toxins into microtubules to create a lot of free cytosolic tubulin for a-Syn interaction could initiate the aggregation of a-Syn.

5.2.9 INTERACTION WITH LEUCINE-RICH REPEAT KINASE2 (LRRK2)

As a synaptic protein, a-Syn might gather within the cytosol when the synaptic assemblies have been destroyed by the mutations of parkin and LRRK2 which distributes a-Syn unwantedly within the cytosol. It has been shown that overexpression of LRRK2 destroys tubulin polymerization and can also affect a-Syn trafficking. A-Syn phosphorylation at S129 which propagates polymerisation may be induced by mutant LRRK2, however a-Syn phosphorylation is not directly

caused by or due to the activity of LRRK2 but possibly 14-3-3 which mediates between LRRK2 and a-Syn. LRRK2 is also associated with improper function of mitochondria which leads to superoxide production and aggregation of a-Syn subsequently. Moreover, mutation of a-Syn A53T or mutation of LRRK2 can destroy chaperone-mediated autophagy and the activity of ubiquitin-proteasome system. Therefore, both proteins mutation at the same time can worsen the condition leading to a very high protein accumulation within the neurons (Liu, Aliaga and Cai, 2012). It will be necessary in future to embark on more detailed studies to specify the actual role of LRRK2 and a-Syn in regulating the mitochondrial functions biogenetically.

5.2.10 PARKIN INTERACTION WITH A-SYN

The degradation of disordered proteins is accelerated by parkin which is a ubiquitin-ligase found in proteasome system. This means that mutation in parkin that affects that affects the activity of its ligase could form insoluble aggregates. One of the most common cause of familial Parkinson's disease is the mutation of parkin that damages its functions, also it is possible that a-Syn parkin interaction under stress conditions creates a site for alpha-tubulin deposition which triggers neuronal cytoskeleton change leading to neuronal dysfunction (Kawahara *et al.*, 2008). However, parkin and LRRK2 are the modulators secretory pathway which assumes their involvement in development of synapses, the regulation of the dynamics of this synaptic vesicle is necessary for proper activity of the neuron and the mutations of both proteins may be related to synucleinopathies (Plowey and Chu, 2011).

5.2.11 DOPAMINE RECEPTOR INTERACTION

The reuptake of dopamine to and from the synaptic terminal of the neuronal cells is the responsibility of dopamine transporter (DAT), it has been revealed that in the brains of PD patients, a-Syn through its NAC sequence binds to the terminal portion of dopamine transporter which increases dopamine levels of the neurons by facilitating the clustering of DAT within the membrane thereby its activities. Enhanced dopamine levels in neurons can destroy cells because of increased metabolic reactions of dopamine which leads to oxidative stress (Lee *et al.*, 2001). In normal brain, however, a-Syn decreases the activities of dopamine transporter thereby controlling the neuronal dopamine levels. The suggestion from this finding is that a-Syn aggregate could be toxic to the dopaminergic cells by creating exposure to dopamine oxidative metabolism free radical products (Wersinger and Sidhu, 2003)

5.2.12 INTERACTION WITH PHOSPHOLIPASES

A hydrolytic enzyme present in the plasma membrane that hydrolyses phosphatidylcholine to PA is known as PLD. PA which is a signalling lipid regulates several processes mainly those processes involved in vesicular transport

and cellular morphology. In the brain, PA might be involved in differentiation, growth and neurotransmitters. The activity of PLD is inhibited by a-Syn. Therefore a-Syn regulates cell signalling by controlling the production or formation of PA (Emamzadeh, 2016).

5.3 ALPHA-SYNUCLEIN INTERACTION WITH MEMBRANE

Different studies have portrayed the association of a-Syn with lipids found mostly in cytosol and presynaptic parts of the neuron associating with the membranes. This membrane-bound a-Syn has been found to show high aggregation property with the power of seeding the aggregation of the cytosolic form (Lee, Choi and Lee, 2002b). The interaction between a-Syn and different sizes, compositions and effects of lipid vesicles on fibrillation kinetics and protein conformation were analysed. Several factors like the vesicle size, a-Syn ratio to phospholipids, phospholipid composition were observed to be effective in modulating the interaction of a-Syn vesicle, this means that high concentration of lipid produced alpha-helical structure and prevented its fibrillation whereas at low concentration of lipid, partial folding of a-Syn was induced by acidic phospholipids which encourage its fibril formation (Zhu and Fink, 2003). A strong relationship was seen between the introduction of alpha-helix in a-Syn and the prevention of fibrillation which suggests that alpha-helical protein-bound a-Syn is not likely to bestow to fibrillation and aggregation (Zhu, Li and Fink, 2003).

According to the reference above, this association may lead to destruction of membrane and significantly affect the fibrillation of a-Syn kinetics. This disruptive ability of a-Syn to membrane was shown to be due to the protein alpha-helicity and proteins binding affinity, also protofibrillar a-Syn showed more destructive effects on membrane than the soluble monomeric protein, portraying vividly that protofibrils (oligomers) are probably neurotoxic. Recently, there has been reports that a-Syn can associate with caveolae and lipid rafts to produce a substantial alpha-helical structure and prevent totally the fibril formation of a-Syn mainly when it interacts the GM1 ganglioside that are present in the caveolae as its molecular marker and this outcome is also dependent on the amount of GM1 present in the caveolae (Martinez *et al.*, 2007). Caveolae are special membranes of the neuron enriched in proteins caveolins family and sphingolipids mainly ganglioside and sphingomyelins whereas lipid rafts are distinguished microdomains of plasma membrane that are boosted with sphingolipids and cholesterol with lipid composition like caveolae. The interaction of a-Syn and GM1 that contains small unilamellar vesicles was followed by the formation a-Syn oligomer. Familial. A53T mutation showed no effect on this interaction while A30P mutation inhibited this interaction dramatically. This recruitment of a-Syn to caveolae and lipid rafts by GM1-SUV explains why this protein is preferentially localized to presynaptic membrane (Martinez *et al.*, 2007).

5.4 INTERACTION WITH SMALL MOLECULES

The interaction of α -Syn with small molecules like drugs can either promote the formation of α -Syn fibrillation, hinder its fibrillation or disaggregate already formed fibrils. The *in vivo* interaction of dopamine and α -Syn aggregation as a usual pathway for familial and idiopathic Parkinson's disease causes disruption and further loss or disappearance of dopaminergic neurons followed by the production of α -Syn which contains Lewy bodies and Lewy neurites in the pars compacta of substantia nigra (Maguire-Zeiss and Federoff, 2003). Assorted catecholamine's that include dopamine and Levodopa (L-DOPA) were observed to prohibit α -Syn fibril formation and also to dissociate already formed fibrils *in vitro*, mainly the oxidized products from catechol's portray more inhibitive effects to α -Syn fibrillation than the non-oxidized products from catecholamine's (Doherty, Bird and Leverenz, 2004). Some drugs like rifampicin that is used for the treatment of leprosy, are found to lower the aggregation of amyloid-beta ($A\beta$) and inhibit deposition of amyloid. This implies that leprosy patients that are under the treatment of rifampicin and other drugs closely related to it for some years, have less or zero probability of acquiring AD or its equivalents (Tomiyama *et al.*, 1996). The fact is that rifampicin and other drugs closely related to it prevent *in vitro* neurotoxicity and aggregation of $A\beta$ and also eliminate or inhibit α -Syn fibril formation *in vitro* (Jie Li *et al.*, 2004). These drugs in a concentration dependent manner were also found to disengage or dissociate already formed α -Syn fibrils and encourage the production of soluble oligomers made up of partially folded α -Syn. From the analysis of several biophysical techniques such as SEC-HPLC and AFM, revealed that numerous other drugs like baicalein with its unique structural propensities and spherical shaped, stabilize oligomers, inhibit α -Syn fibril formation and dissociate already formed fibrils *in vitro* (Hong, Fink and Uversky, 2008).

5.5 SMOKING

According to the findings of (Hong, Fink and Uversky, 2009), it deduced that smoking can minimize the incidence of PD showing that smoke might contain neuro protective chemicals. Based on this findings, the relationship between α -Syn fibrillation and five separate compound found in cigarette smoke; cotinine, anabasine, hydroquinone, nor nicotine and nicotine was studied using gel electrophoresis, thioflavin T assays, atomic force microscopy (AFM) and size exclusive chromatography-high performance liquid chromatography (SEC-HPLC) to check the rate of α -Syn fibril formation and the inhibitory effects or actions of the components of the cigarette smoke, the result portrayed that hydroquinone and nicotine inhibit the formation of α -Syn fibrils in a concentration dependent manner with more effects from nicotine. SEC-HPLC analysis showed that hydroquinone and nicotine also stabilize soluble oligomers which gives insight to the production of three stable oligomers with average height of 16nm, 10nm, 4nm as evaluated by AFM, instead of producing insoluble fibrils. In

accordance with the above studies or findings, similar results were also gotten for the smoke components on A53T mutant fibril formation, this outcome showed that hydroquinone and nicotine inhibit fibrillation of α -Syn and make stable its oligomers. This information made it clearer the molecular mechanism of using hydroquinone and nicotine for developing Parkinson's disease therapeutic solutions. This can be expressed thus that the risk of Parkinson's disease in non-smokers is about two times that of cigarette smokers (Fratiglioni and Wang, 2000).

5.6 INHIBITORS OF A-SYN FIBRILLATION

Numerous α -Syn associated with Lewy bodies has been modified or innovated covalently by tyrosine nitration but whether this happens before fibrillation or after fibrillation, whether the monomeric fibril nitration enhance or distorts fibril formation is still under controversy (Ischiropoulos and Beckman, 2003). The in vitro examination of tyrosine nitration effects on the tendency of α -Syn to fibrils portrayed that nitration totally inhibits fibrillation because it forms stable oligomers (soluble) (Uversky *et al.*, 2005), also non-modified α -Syn fibril formation was prevented by the presence of nitrated α -Syn that is substoichiometrically concentrated (Yamin, Uversky and Fink, 2003b). Homologous proteins β -Syn and γ -Syn are abundantly found in the brain with γ -Syn produces fibrils much slower than α -Syn whereas β -Syn fibrils not at all under both in vivo and in vitro conditions due to lack of hydrophobic stretched residues from the central portion of the protein and generation of stable oligomers; this suggests that β -Syn negatively be used to regulate α -Syn fibril formation (Fink, 2006).

Dopamine and catecholamine can inhibit the fibrillation of α -Syn and catecholamine's also can dissolve existing α -Syn fibrils in vitro (J. Li *et al.*, 2004). Several flavonoids and polyphenols can prevent α -Syn fibril formation and disaggregate already made fibrils in some cases by binding to monomeric α -Syn to form a stable oligomeric one (Jie Li *et al.*, 2004), (Zhu *et al.*, 2004)

Numerous chemicals both natural and synthetic have shown inhibitive features to α -Syn. Polyphenol compounds such as baicalein, dopamine chloride, delphinidin, exifone, epigallocatechin gallate, gossypetin, hypericin, hinokiflavone, procyanidin B1 and B2, theaflavin and rosmarinic acid inhibit the aggregation of α -Syn strongly with a minimal concentration. More compounds like porphyrin ferric dehydroporphyrin ix, alpha-tocopherol (vitamin E), Congo red, 1-bromo-2,5-bis(3-carboxystyryl) benzene, also portrayed a huge reduction to aggregation of α -Syn (Masuda *et al.*, 2006). Also rifamycin, phenothiazine, porphyrins and polyene of same polyphenolic compounds hindered amyloid fibril formation (Shahpiri *et al.*, 2016). Sighting the words of (Makwana and Sundd, 2016), Gallic acid that is found on the skin extract of grape, tea leaves and gall nuts inhibit α -Syn aggregation by stabilization of its monomer as proven by ion mobility mass

spectroscopy techniques and NMR (Liu *et al.*, 2014), Gallic acid does not only show inhibitive effect to α -Syn, but also disentangles already formed fibrils, it was also observed to bind to α -Syn soluble (non-toxic) oligomers.

Polyamidoamine dendrimer (PAMAM), made up of ethylenediamine core and unit branch gotten from ethylenediamine and acrylate can prohibit α -Syn aggregation but its mechanism is unclear (Milowska, Malachowska and Gabryelak, 2011). Combination of tryptophan analogues and naphthoquinone, both as amyloid formation inhibitors also inhibited α -Syn aggregation (Scherzer-Attali *et al.*, 2012).

6 POST-TRANSLATIONAL MODIFICATION OF ALPHA-SYNUCLEIN

The cellular and molecular determinants that regulate Parkinson's disease pathology when understood, will be of great relevant to develop effective preventive, diagnostic and therapeutic strategies to handle this devastating maladies. A-Syn undergoes several post translational modifications which influences or regulates its aggregation and toxicity, though, the exact contribution of these several modifications to its disease mechanism remains unclear. Amongst several or numerous post translational modification of a-Syn such as Phosphorylation, Ubiquitylation, Nitration, Acetylation, Sumoylation, Truncation, Covalent cross-linking, Oxidation, to mention but a few. For the sake of this work, I will review the mostly observed Post translational modification that is associated with disorders.

6.1 PHOSPHORYLATION OF ALPHA-SYNUCLEIN

Phosphorylation of a-Syn is the most important and likely the most common post translational modification of protein and the most widely studied, from the words of (Fujiwara *et al.*, 2002b), the major make up of Lewy bodies found in brain tissues of Parkinson's disease patients was made up of a-Syn phosphorylated at serine residues 129 (S129). It is interesting to know that a-Syn observed in the non-fibrillar tissues of Parkinson's disease patient was phosphorylated also at S129 (Anderson *et al.*, 2006). This triggered the concept that PTM of a-Syn could change its ability to either aggregate or produce oligomeric species and might initiate PD or progresses it, figure 6-1, presents the sites for phosphorylation of a-Syn.

Other residues that can be phosphorylated within a-Syn include: Serine 87 (S87), or the tyrosine residues Y125, Y133, and Y136 (Oueslati, Fournier and Lashuel, 2010). Phosphorylation at S129 appeared pathologically as most relevant to Parkinson's disease but the actual effects of it is yet to be determined either encourages the formation inclusion bodies and toxicity in different models of cells (Sugeno *et al.*, 2008) or been protective according to the studies carried out with rat models and yeast (da Siveira *et al.*, 2009; Mbefo *et al.*, 2010), notwithstanding the facts that these studies were carried out in varying animal systems with each having unique neuropathological mechanism. In both rat and fly modes, S129A mutant produced more inclusions that are insoluble and smaller or fewer oligomers (Chen and Feany, 2005, Chen *et al.*, 2009), this support the hypothesis that increased fibril formation of this mutant reduces the level of toxic oligomers that leads to the protection of fly models, while the actual function of this S129 phosphorylation is yet unclear but might be relevant and have some roles to play in Parkinson's disease. In addition to inducing toxicity, posttranslational modification might also link a-Syn with impaired mitochondrial function in Parkinson's disease. Several results have shown phosphorylation incidents as useful for targeting proteins to mitochondria (Luth *et al.*, 2014). Whereas

unproven one could guess or gamble that phosphorylation of a-Syn elevates its trafficking to mitochondria where it is portrayed to damage or distort mitochondrial function (Barrett and Timothy Greenamyre, 2015). More research work is needed to ascertain the actual impact of a-Syn phosphorylation on the dysfunction of mitochondria and how it is related to Parkinson's disease.

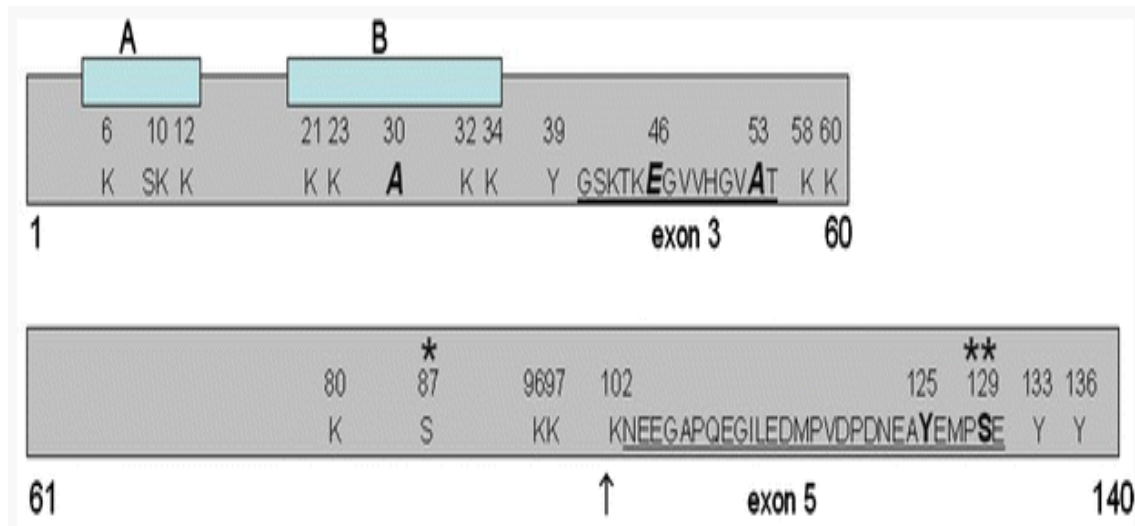


Figure 6-1: Schematic representation of the main sites of a-Syn.

Showing exons three and five, with numbered amino acid codons. Three mutations (pathological) are boldly shown in italics. Corresponding sites of modification are shown by correspondent amino acid residues with their positions shown above each residue. Potential sites for phosphorylation is represented by serine residue (S). ** represent sites for primary phosphorylation and * represent sites for secondary phosphorylation. A-Syn can either be oxidized or nitrated at its four residues of tyrosine (Y). The potential substrate of sumoylation and ubiquitylation are Lysine residues (K). First box represents fibrillar a-Syn ubiquitylation and the second box portrays in vitro a-Syn ubiquitylation sites. The major a-Syn sumoylation site is the Lysine residue at 102 codons shown by the arrow ↑

6.2 NITRATION OF A-SYN

Oxidative injury sets in when the reactive oxygen species (ROS) outpowers the antioxidant effect or capacity of the brain cells. The commonly known outcome of elevated oxidative stress is the tyrosine residue nitration by peroxynitrite which resulted from the reaction of nitric oxide and oxygen. Just like the phosphorylation, various forms of 3-nitrotyrosine-modified a-Syn in LB have been recognised by specific antibodies. Advanced studies have also portrayed four tyrosine nitration locations, which include: Y125, Y133, Y136, Y139 (Barrett and Timothy Greenamyre, 2015). The nitration role of a-Syn toxicity on Parkinson's disease is yet unclear but according to (Souza, 2000), a major effect of tyrosine

nitration is the production of α -Syn oligomers and dimers through dityrosine crosslinking (Yamin, Uversky and Fink, 2003a). It will be interesting to know that nitration of α -Syn prevents fibrillation and nitration at Y39 has also been observed to prevent the propensity of α -Syn to bind to lipids. Combining the fibrillation inhibition result or stabilization of oligomers and the lipid binding reduction, suggests that posttranslational modification (nitration) perform similar action with the A30P mutation that resulted to early PD outbreak (Hodara *et al.*, 2004).

Several factors unite to accelerate oxidative stress dopaminergic neurons. Nigral neuron do not only welcome oxidative stress but it also produces reactive oxidative species (Sanders *et al.*, 2014 ; Horowitz *et al.*, 2011). Mitochondria function decline which is age-related and antioxidant defences elevate oxidative injury with protein nitration included. Not minding the fact that α -Syn tyrosine nitration might imitate some A30P mutation aspects, it might help to maintain or prolong the disease process.

6.3 DOPAMINE MODIFICATION

Inside the dopaminergic neurons of substantia nigra, dopamine (DA) usually gather and concealed in synaptic vesicles. Dopamine with its high reactive properties, can oxidize automatically at neutral pH to produce a toxic dopamine Quinone (DAQ), hydrogen peroxide and superoxide radicals (Graham, 1978). The production of these species together with the antioxidant reduction or decrease like glutathione mostly seen in PD and with aging overtime can lead to oxidative stress with detrimental effects to the cells. In addition to the negatively generated oxidative environment, there might also be elevated levels of cytoplasmic dopamine in Parkinson's disease (Lotharius *et al.*, 2002). Dopamine, under normal environmental condition is stored safely within the synaptic vesicles the spontaneous production of DAQ is prevented by the low pH. Earlier studies have connected α -Syn oligomeric forms, as well as A30P and A53T mutations, with increment of synaptic vesicle permeability (Volles and Lansbury, 2002), potentially connecting toxic functions of α -Syn with elevated cytoplasmic dopamine levels in Parkinson's disease patients. Consistency of these findings is a prove that A53T mutant form of α -Syn reduces the levels of a protein known as VMAT2 that is responsible for dopamine uptake into vesicles. This reduction in the level of VMAT2 could contribute to poor cytoplasmic dopamine (Lotharius *et al.*, 2002). Over expression of the rate limiting enzyme in production dopamine (tyrosine hydroxylase) inhibits α -Syn aggregation and increase the toxicity of the cells in SH-SY5Y connecting the elevated dopamine levels with changes or innovations in aggregation of α -Syn (Mazzulli *et al.*, 2006). The potential cycle connecting α -Syn to increased or elevated cytoplasmic dopamine is represented in figure 6-2

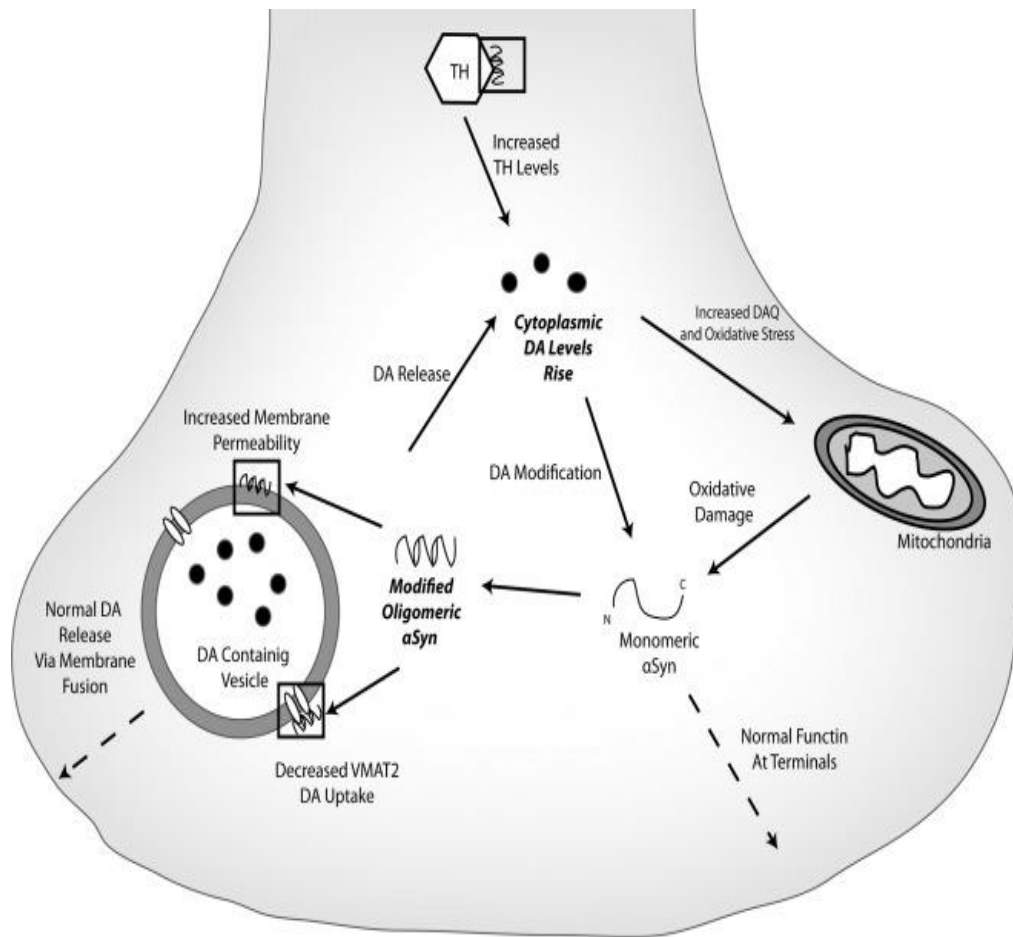


Figure 6-2: A schematic representation of the potential cycle connecting α -Syn with elevated cytoplasmic dopamine (Barrett and Timothy Greenamyre, 2015)

Elevated cytoplasmic dopamine is a strong driving factor in progression of Parkinson's disease. Dopamine containing vesicles intermix with plasma membrane under normal environmental condition at the synaptic terminal for release of extracellular dopamine, and α -Syn exists at the terminal; but these functions changes under Parkinson's disease conditions forming modified oligomeric α -Syn in abundance. These α -Syn formed, have been seen to distort the function of dopamine vesicle both increasing permeability of the membrane and reducing dopamine uptake by VMAT2, both elevates the level of cytoplasmic dopamine. Elevated TH level prompted by modified oligomeric α -Syn might also increase the levels of cytoplasmic DA. Increased levels of cytoplasmic dopamine can promote α -Syn oligomerization, modify monomeric α -Syn or react to produce dopamine Quinone (DAQ) that increases oxidative stress through the mitochondria. This elevated oxidative stress might encourage oligomerization and modification of α -Syn, increase further the cytoplasmic dopamine levels and finally increase oxidative stress within the cell (Barrett and Timothy Greenamyre, 2015)

Cytoplasmic dopamine level increment goes beyond the deleterious products formed by dopamine oxidation. Hypothesis have that dopamine specifically can

innovate α -Syn and change its aggregation propensity. Dopamine Quinone can stabilize α -Syn protofibrils kinetically and can stop its aggregation into bigger fibrils (Conway *et al.*, 2001). This finding was supported later by Li *et al.*, 2004 whose findings was that dopamine modification of α -Syn also degenerated already existing fibrils by making weak the intermolecular forces existing within the fibrils which leads to oligomeric (stable) species. The suggestions by many studies have it that dopamine modification was covalent in nature as modification by dopamine yielded oligomers that are SDS resistant (Cappai, 2005); Moreover, there was argument that the result was more temporary and took place within YEMP sequence of C-terminal domain (Norris *et al.*, 2005). These outcomes were possible or obtained as dopamine still change α -Syn even when its major residues (His, Met and Tyr) were mutated. Later it was observed that YEMPS portion was not needed for modification as truncation inhibited fibril formation (Leong *et al.*, 2009). Recently, it has been proven that modification by dopamine occurs via dopamine cross-linking in a stoichiometric reaction between dopamine polymers and α -Syn through Tyrosine and Lysine residues. The above reactions made the formation of α -Syn dopamine Trimers stable as revealed by small angle x-ray scattering(SAXS) analysis to possess a worm-like structure not like the traditional beta-sheet structure that is associated with α -Syn fibrils (Rekas *et al.*, 2010). Just like other posttranslational modification, it is difficult to ascertain if α -Syn dopamine modification is either a causative effect or a by-product from other events that ultimately assist promote Parkinson's disease progression. Dopamine modification of α -Syn occurs sorely at high oxidative stress condition or when the cytoplasmic level of dopamine is high and does not occur in the areas of the neurons containing low levels dopamine or the areas that are not susceptible to oxidative stress. In addition to α -Syn modification, dopamine has also been associated with changes in mitochondrial functions and proteins (Barrett and Timothy Greenamyre, 2015).

6.4 UBIQUITINATION OF A-SYN

In PD, A-Syn is mutated and is mostly found in cytosolic inclusions known as Lewy bodies. A portion of α -Syn extracted from Lewy bodies is monoubiquitinated in sporadic form of Parkinson's disease though the role of monoubiquitination here is quite unclear. SIAH which is an E3 ubiquitin-ligase is found in Lewy bodies where E3 monoubiquitinates α -Syn at Lysine that has already been monoubiquitinated in LB.

Within dopaminergic cells, increment of inclusion formation and α -Syn aggregation into amorphous aggregate is promoted by monoubiquitination by SIAH. Even when the monoubiquitination level is low, such effects will be felt or observed which suggests that α -Syn (monoubiquitinated) might serve as an aggregation seed. Production of cytosolic inclusion and collection of monoubiquitinated α -Syn is boosted or stimulated by inhibition of autophagy and inhibition of lysosomal and proteasomal to a lesser extent. Inclusion of monoubiquitinated α -Syn are highly toxic to cells, recruit proteins that are related

to Parkinson's disease such as UCHL1 and Synphilin-1. Meanwhile, monoubiquitinated α -Syn might play a vital role in formation of Lewy body. Decreasing monoubiquitination of α -Syn by inhibiting the function of SIAH or encouraging autophagy will constitute a Parkinson's disease therapeutic strategy (Engelender, 2008).

6.4.1 FUNCTION OF UBIQUITIN IN AGGREGATION OF A-SYN

Reports have it that some portion of α -Syn extracted from LB is monoubiquitinated (Hasegawa *et al.*, 2002) (Anderson *et al.*, 2006) but how α -Syn aggregation is regulated by monoubiquitination is unclear and how α -Syn monoubiquitination is caused by E3 ubiquitin-ligase also remain a misery. It was shown that E3 ubiquitin-ligase reacts with and in vitro monoubiquitinates α -Syn (Liani *et al.*, 2004). Additionally, the presence of this enzyme SIAH as mentioned by the above reference have been shown in Lewy bodies of Parkinson's disease patients, this portrays that SIAH might stand as a novel part of ubiquitin proteasome involved in Parkinson's disease. The monoubiquitination of α -Syn is also in vivo (Rott *et al.*, 2008), (Lee *et al.*, 2008). Analysis from spectrometry revealed that monoubiquitination of α -Syn by SIAH takes place at several lysine residues including 12, 21, and 23 (Rott *et al.*, 2008). The observation that a little fraction of α -Syn about 10% is ubiquitinated in LB makes it difficult to believe the role of monoubiquitination of α -Syn in Lewy bodies formation (Hasegawa *et al.*, 2002).

However, monoubiquitination of α -Syn encouraged by SIAH leads to a seen increase in in-vivo and in vitro α -Syn aggregation. α -Syn ubiquitination induces a strong amorphous α -Syn aggregate formation, *figure 6-3*. Ubiquitination of α -Syn produces a notable inclusion formation in dopaminergic cells which suggests that monoubiquitinated α -Syn may primarily function in Lewy bodies formation. Parkinson's disease mutations A53T, A30P and E46K do not alter α -Syn monoubiquitination by SIAH (Rott *et al.*, 2008) but however within the dopaminergic cells, monoubiquitinated α -Syn A53T mutant forms more aggregates than proteins wild type which goes in line with the increased power A53T mutant α -Syn to aggregate. (Conway, Harper and Lansbury, 1998). This probably means that monoubiquitination serves as a common mechanism to elevate the aggregation of α -Syn with or without mutations. According to the findings of (Lee *et al.*, 2008) A30P mutant α -Syn was never ubiquitinated efficiently or effectively by SIAH with no clear reason. Different Parkinson's disease cell models develop cyto protective inclusions (Ross and Poirier, 2005), for example Synphilin-1 which is α -Syn binding protein form cytosolic inclusions in the presence or absence of overexpressed α -Syn which safeguard cells from death. (Liani *et al.*, 2004), (Tanaka *et al.*, 2003). An isoform of synphilin-1 known as synphilin-1A forms neuroprotective inclusions also (Eyal *et al.*, 2006). However, inclusions that are mainly produced monoubiquitinated α -Syn are always toxic to the cells which could be as a result of the amorphous nature or state of the monoubiquitinated α -Syn aggregates. Protofibrillar and oligomeric forms of α -Syn plays a toxic function in the disease as against the α -Syn fibrillar forms (Caughey and Lansbury, 2003).

Having amorphous protein aggregated within the core of LB and fibrils present at their periphery entails that Lewy bodies might be too toxic to cells at their early stages or initial stages of production. According to (Periquet *et al.*, 2007), depending on stages of maturation, Lewy bodies may either promote the death of cells or protection of the cells. The involvement of lysosomal, proteasomal and autophagic pathways in Parkinson's disease have been supported by several studies. The activities of proteasome are reduced in the substantia nigra of Parkinson's disease patients (McNaught and Jenner, 2001), and a lysosomal storage disease called Gaucher is related with a higher incidence of Parkinson's disease (Aharon-Peretz, Rosenbaum and Gershoni-Baruch, 2004). Several storage diseases of lysosome elevate autophagic dysfunction and ubiquitinated protein inclusion accumulation (Settembre *et al.*, 2007). Moreover, autophagic cell death is induced by expression of α -Syn A53T mutant (Stefanis *et al.*, 2001). These three proteolytic pathways seemed to be associated with α -Syn degradation (Rott *et al.*, 2008). Autophagic inhibition prevented the degradation of α -Syn more than the lysosomal and proteasomal pathways which suggest autophagy to be a predominant pathway seen in α -Syn clearance. The above is supported by the observation that α -Syn and autophagy morphology were detected within or inside vesicle where rapamycin (autophagy activator) stimulate α -Syn clearance (Webb *et al.*, 2003). Inhibition of autophagy elevates the accumulation of monoubiquitinated α -Syn and its subsequent aggregation. In this work, it is proposed that interference to α -Syn monoubiquitination and macro autophagy stimulation may represent a strategy to prevent PD progression.

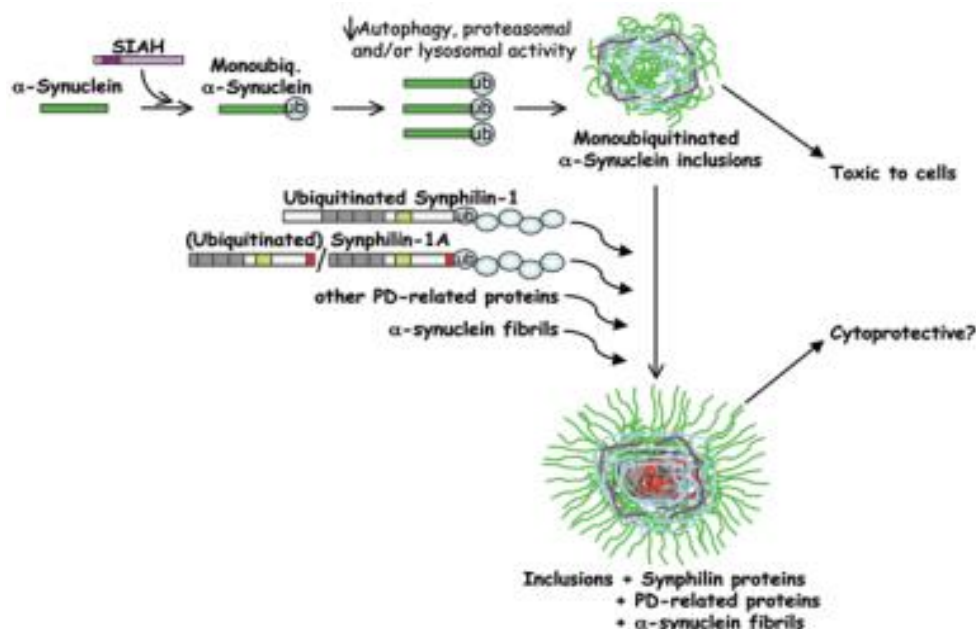


Figure 6-3: Inclusion body formation in Parkinson's disease cell models

A-Syn monoubiquitination by SIAH leads to inclusion body formation and a-Syn aggregation within the dopaminergic cells. Autophagic, lysosomal and proteasomal pathways inhibition further promote the aggregation and accumulation of monoubiquitinated a-Syn within the cells. Inclusions of a-Syn are toxic to the cells, suggestions from the protective nature from several Parkinson cell models having synphilin proposed that the coaggregation of synphilin-1 and synphilin-1A ubiquitinated or not might prevent the mediated toxicity by inclusions of monoubiquitinated a-Syn. Building up of a-Syn fibrils and accumulation of other related PD proteins like Parkin and UCH-L1 might also reduce the toxicity of monoubiquitinated a-Syn inclusions. Lewy bodies might promote or encourage several extents of cell toxicity (Engelender, 2008).

6.5 SUMOYLATION OF A-SYN

As a posttranslational modification, where a small ubiquitin-like modifier (SUMO) is covalently coupled to target proteins. This modification process is involved in several cellular processes like: protein stability, apoptosis, nuclear transport, regulation of transcription and stress response. Sumoylation takes place by a process that is reversibly dependent on ATP, where thioester bond covalently attach SUMO to lysine side chains of the proteins target. Many isopeptidases of SUMO can detach the modifier which makes this reversible sumoylation a dynamic process, and several proteins undergo rapid sumoylation and SUMO deconjugation (Melchior, Schergaut and Pichler, 2003).

All eukaryotes express SUMO (Meulmeester and Melchior, 2008), and mammals are made of three SUMO proteins at least; SUMO1 to SUMO3. Sumoylation is important to neurodegenerative disorders (Dorval and Fraser, 2007), (Eckermann, 2013). SUMO co-localised or seen together with the neural inclusions that is associated with different neurodegenerative maladies, co-localize like ubiquitin, this elevated the question to identify the actual target of SUMO within the aggregates. The findings of (Dorval and Fraser, 2006) demonstrated that SUMO1 monosumoylated a-Syn in vitro. SUMO conjugation takes place at the two main sites of sumoylation K96 and K102, this shows that sumoylation hindered in vitro fibrillation of a-Syn and further suggested that aggregation of a-Syn is negatively regulated by sumoylation by activating its solubility.

Yeast models has recently been used to investigate the sumoylation effect of a-Syn on cellular toxicity (Shahpasandzadeh *et al.*, 2014). This showed that cellular sumoylation mechanism is preserved from yeast to men. A30P mutant as well as wild type a-Syn are sumoylated in vivo in yeast also at the same K96 and K102 sumoylation sites which are the major sumoylation sites in humans also. This posttranslational modification of a-Syn protects yeast cells against toxicity mediated by a-Syn and inclusion formation. Sumoylation impairment in yeast resulted in alarming increase in the number of cells with inclusions. The analysis of amino acid substitution portrayed that the protective duty of SUMO needed a-Syn modification at the two major sites of sumoylation which confirms the

function of SUMO modification in regulating the toxicity and aggregation of α -Syn which support the use of yeast to study the impact or effect of SUMO on cells that need to cope with high concentration α -Syn (Popova, Kleinknecht and Braus, 2015).

7 ALPHA SYNUCLEIN AND SYNUCLEINOPATHIES

A vital group of neurodegenerative maladies that are that are linked to a-Syn, they are featured by the illicit deposition or accumulation of aggregates of a-Syn within the neurons, glial cells or nerve fibres around the neuronal portion of the body and they are age related. These disorders commonly share a pathologic inclusion made up of aggregated a-Syn that are deposited in strategic or selective portions or areas of the neuron and glia cells. Synucleinopathy as a term was introduced in 1998 after a deposition of filamentous a-Syn were seen not only in Parkinson's disease but also in Dementia with Lewy Bodies (DLB) and multiple system atrophy (MSA) (Spillantini *et al.*, 1998). These maladies are characterized by progressive or gradual aggregation of fibrillary insoluble a-Syn in glia or nerve fibres and neurons. There are three major types of synucleinopathies, they are Parkinson's disease, Dementia with Lewy bodies and multiple system atrophy. Though there are other rare maladies that are associated with a-Syn pathologies. Symptoms of these disorders significantly overlap with each other which hinders diagnosis, they include reduction in motor and cognitive duties and general behavioural changes.

7.1 PARKINSON'S DISEASE

Parkinson's disease is the most commonly diagnosed and observed in clinical activities among the three major types of synucleinopathies. As the most widely seen neurodegenerative disorder after Alzheimer's disease, PD is an age-related, commonly known movement disorder that progresses slowly due to nerve cell loss or deterioration of the nerve cells in the mid-brain portion called substantia nigra compacta (SNc). These substantia nigra cells oversee the production of chemical messenger that is responsible for coordination of movement known as Dopamine (DA).

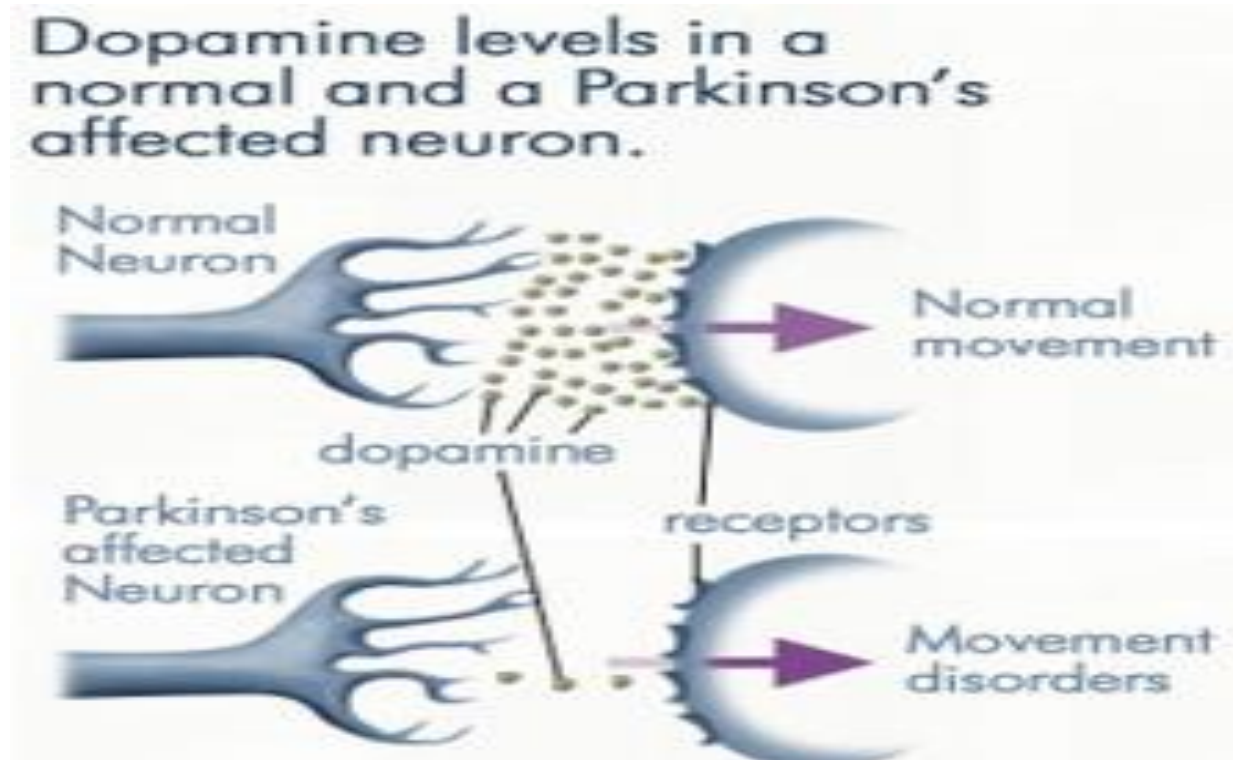


Figure 7-1: Symptoms of PD are caused by insufficient dopamine

When these substantia nigra cells destroyed or damaged, which reduces which reduces the concentration of dopamine within the substantia nigra, there is abnormal neuron functioning which reduces the ability to control the movement of the body, Parkinson's disease develops, the *figure7-0-1* represented above

According to (National Institute of Neurological Disorders And Stroke, 2014), a-Syn was the first gene to be identified among several genes that are linked to Parkinson's disease. The National Institute of Health researchers with other institutions found out after genetically studying the profiles of three Greek families and a large Italian family suffering from familial PD; that their disorder was related to a-Syn gene mutation, the second a-Syn gene mutation was found in a German family suffering from PD also. These findings activated the studies of a-Syn role in Parkinson's disease. In the cause of these findings, it was discovered the Lewy bodies that was seen in every PD cases is made up of a-Syn protein which revealed the relationship between sporadic and familial forms of PD. Also from the above referenced, researchers in 2003, found out that this disorder in one big family was because of normal a-Syn gene triplication of a copy of chromosome 4. This triplication led to the production of too much of normal a-Syn by all the people within the affected family. This outcome portrayed that too much of normal a-Syn could be as risky as abnormal a-Syn and result to Parkinson's disease, with various signs which ranges from cardinal motor or movement signs examples; cardinal feature, bradykinesia, resting tremor, postural instability, dysphagia, akinesia, bradyphemia and micrographia (Gelb, Oliver and

Gilman, 1999). *Figure 7-0-2*, represents the flexed posture of a Parkinson's disease patient with a frozen face.



Figure 7-2: Schematic illustration of anxious frozen face and flexed posture of a Parkinson's disease patient (Lees, Hardy and Revesz, 2009)

Non-motor/movement signs includes: olfactory disturbances, hallucinations, sleep disturbances, pain, constipation, depression and dementia (McCann et al., 2014). Many of these non-movement signs or non-motor symptoms (NMS) can come up many years before the emergence or appearance of the motor or movement symptoms which are termed the earliest clinical Parkinson's disease symptoms especially olfactory dysfunction that is seen in about 90% of patients is often used as a useful tool or material for early diagnosis (Bonnet et al., 2012).

The surviving dopaminergic neurons of the substantia nigra is made up of a specific inclusion of protein known as Lewy Bodies and Lewy neurites which constitutes majorly of α -Syn that are seen also in several other neurodegenerative disorders. Hallucination and dementia are observed later in the cause of this ill health. In patients that were followed longitudinally, after a duration of twenty years, 74% were hallucinated visually and 83% were demented (Hely *et al.*, 2008). In Parkinson's disease, aging is considered as a major risk factor, the onset of this ailment is averaged at about 60years with about 15 years life expectancy from diagnosis except for those with strong positive family history of Parkinson's disease that might be diagnosed under 40years of age though about 10% but older diagnosed patients from 70years and above portray a shorter duration of the malady with more rapid progression of the disease (van Rooden *et al.*, 2010).

In diagnosis of PD, there are two pathological features that are essential and required; Loss of dopaminergic neurons (pigmented) within the substantia nigra pars compacta of the mid brain, and presence of α -Syn cytoplasmic inclusions and exons called Lewy bodies and Lewy neurites (Lewy-related α -Syn pathologies). α -Syn as a protein found within the synaptic vesicle of the brain is of great importance or interest to PD's research because it majorly constitutes Lewy bodies, clumps of protein that are pathologically the hallmark of Parkinson's disease. Since the discovery of this, there have been more questions than answers as to the role α -Syn play in causing PD and why it is a potential target for neuroprotective therapies. According to Michael J. Fox Foundation for PD Research, recent studies have compelling evidence that α -Syn might contribute in the development of both sporadic PD (more common) and familial PD (rarely). Here in familial form of PD, the gene of α -Syn either produces abnormal form of the protein or two much proteins of α -Syn which are usually believed or understood by some researchers to be brain cell toxic that lead to dysfunction of the neuron. Aggregation of α -Syn plays a major duty in sporadic PD as well since α -Syn primarily is the structural component of LB. With all these findings, there is a total support to develop a Parkinson's disease therapies that minimize or reduce the expression of α -Syn or totally block its aggregation. Such therapies potentially could prevent the onset of Parkinson's disease, delay the onset of PD, slow or totally block its progression.

Parkinson's disease does not have exert cure but some medications and common drugs for Parkinson's disease is dopamine precursors like Levodopa which is a natural chemical that is converted to dopamine when passed through the blood-brain barrier; the equation for the conversion of Levodopa to Dopamine when passed through the brain is represented in *figure 7-0-3*, and surgery (Pallidotomy

and Thalamotomy, Deep Brain Stimulation) can assist to control the motor symptoms. This medication is administered usually at the early onset of the disease (MAYO CLINIC, 2017)

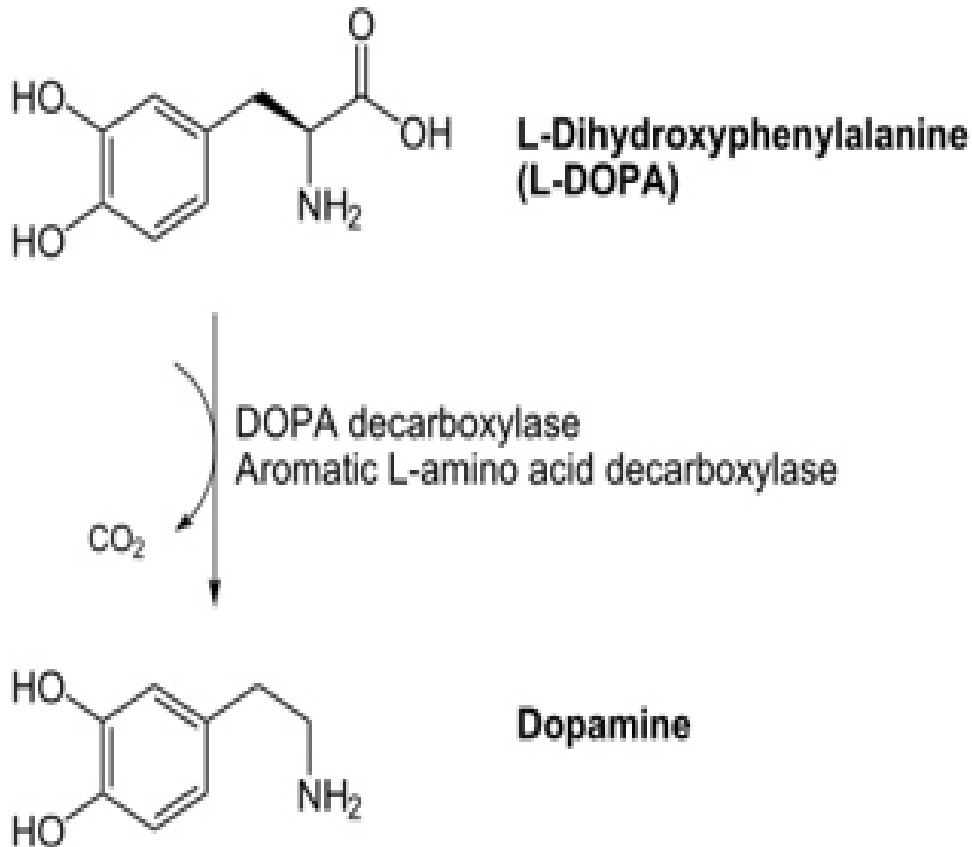


Figure 7-3: L-DOPA is converted to Dopamine by enzyme DOPA decarboxylase (DDC).

7.2 DEMENTIA WITH LEWY BODIES

This is a neurodegenerative malady that featured the same deposits of abnormal protein called Lewy bodies, that is also found in Parkinson's disease, but its widespread throughout the brain areas.

This synucleinopathy was named for a German neurologist Dr. Friedrich Lewy who in 1912, discovered the deposits of abnormal proteins that disrupt the normal functioning of the brain in Parkinson's disease patients. The abnormal protein deposits are called Lewy bodies and they majorly contain α -Syn. In DLB, α -Syn produces clumps within the neurons, commencing from a region or area of the brain which makes the affected neurons less effective and eventually leads to

death of the brain cells. This will also affect the activities of the brain chemicals and it is not restricted to a region rather it causes widespread damage to several regions of the brain and a total decline in the activities of the affected areas (National Institute of Neurological Disorders And Stroke, 2015).

The brain regions affected by Dementia with Lewy bodies include:

- The cerebral cortex, that regulates several brain functions including processing of information, thought, perception and language.
- The hippocampus, that is relevant to producing new memories
- The limbic cortex that regulates emotions and behaviour.
- The midbrain, that includes the substantia nigra which is essential in movement.
- The brain stem that regulates sleep and alert maintenance.
- The brain region that recognizes smell (the olfactory pathway)

The disorder portrays fluctuating symptoms which might range primarily from that of PD like rigidity, tremor, bradykinesia to that like Alzheimer's disorder which include confusion, loss of memory and poor judgement (Institute on Aging, 2015)

The first symptoms of this condition are usually visual hallucination and the patient might suffer other psychiatric turmoil like depression and delusions. Dopamine precursors like levodopa and other PD medications can alleviate the motor symptoms of DLB but they might worsen hallucination and delusion that will make the patient require a typical antipsychotic treatment (medications) (National Institute of Neurological Disorders And Stroke, 2015)

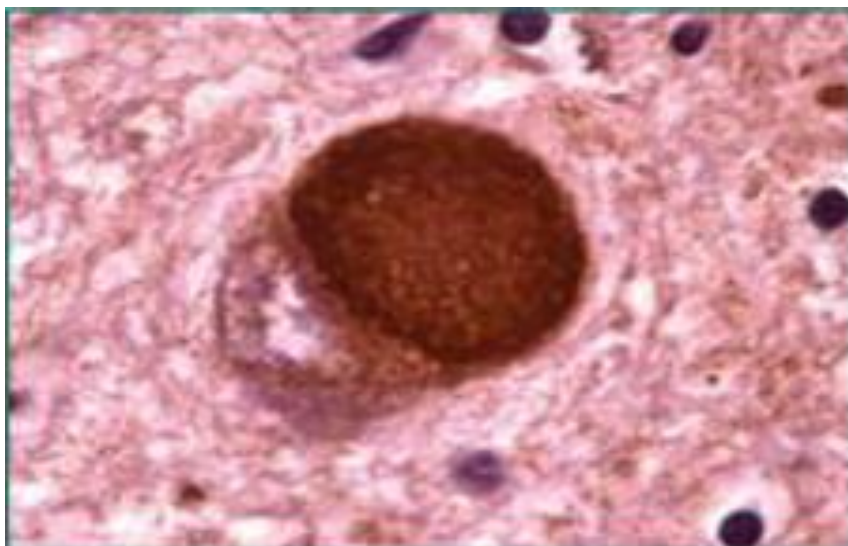


Figure 7-4: Microscopic image of a Lewy body surrounded by α -Syn as its major component (National Institute of Neurological Disorders and Stroke, 2015)

Dementia with Lewy bodies can be of two related conditions:

Lewy bodies dementia and Parkinson's disease dementia. The actual difference between both conditions lies in the cognitive timing and movement symptoms.

7.3 MULTIPLE SYSTEM ATROPHY (MSA)

This synucleinopathy can be said to be an adult onset neurodegenerative malady that is progressive with an unknown etiology. It is clinically characterized by different severity of Parkinson's disease features (features like PD), the autonomic failure and cerebellar ataxia.

The spreading of this disorder encompasses or involves three functional systems within the central nervous system; the striatonigral system, olivopontocerebellar structures of the brain and the spinal cord impacting on movement, blood pressure, muscle control bladder function and heart rate (Kim, Kågedal and Halliday, 2014), associated with excessive discrete argyrophilic fibrillary inclusions within the oligodendrocytes and glial cytoplasmic inclusions. These inclusions are also referred to as Papp-Lantos bodies and they are formed by α -Syn proteins (Uversky and Eliezer, 2009). However, like Dementia with Lewy bodies and Parkinson's disease, the dominant histopathology of Multiple system atrophy is the presence or availability of fibrillar and misfolded α -Syn in the cytoplasm but the principal deposition site of α -Syn which is in the oligodendrocytes varies from that of PD and DLB where α -Syn are deposited in the neurons (Kim, Kågedal and Halliday, 2014).

Moreover, according to (Institute of Neurological Disorders, 2014), there are no drugs or medications that are able to alleviate the progression of this disease and it has no cure. This disorder includes disorders that had been referred historically as Shy-Drager syndrome, striatonigral degeneration and olivopontocerebellar atrophy. Initial symptoms of this disorder are difficult to differentiate from the initial symptoms of Parkinson's disease, and they include: tremor, slowness of movement, impaired speech, inability to coordinate, clumsiness and bladder control problems. MSA may be divided into two distinct types based on the most prominent symptoms when the patient is evaluated.

The Parkinsonian type MSA (MSA-P), comprises of symptoms like that of PD such as tremor, stiffness and slow movement.

The Cerebellar type MSA (MSA-C), with primary symptoms such as balance and coordination problem (ataxia), speech problem or quavering voice, difficult swallowing and abnormal movement of the eye. Multiple system atrophy tends to rapidly progress more than Parkinson's disease and most patients with MSA require an aid like walker for walking within few years of emergence of symptoms (Institute of Neurological Disorders, 2014).

8 CONCLUSION

Deposition of a-Syn has been considered as the basis for neurodegeneration in all alpha-synucleinopathies. Not minding the similarities in their progressions and expansion, there are pathological differences that are significant between multiple system atrophy and Lewy body diseases, along with the cell types that are involved (in Parkinson's disease, oligodendroglia in MSA and Dementia Lewy bodies) and the extent of neuronal loss in selected regions in Parkinson's disease but spread all over the regions in MSA.

These cardinal disparities portray the involvement of different pathological mechanisms in the advancement of both disorders, possibly another member of the synuclein family might be involved though that has not been investigated fully. There is need for further studies on clinical phenotypes of all synucleinopathies to understand their full pathological and clinical associations more, the actual role of effect of a-Syn within the brain and neurodegenerative disorders respectively. Particularly, it is essential that the complexities of the pathological variations observed clinically in Parkinson's disease phenotype be classified further to establish more treatment that befit the effective symptoms. Another challenge in this area is the need for an advanced research on the activities of the identified proteins by the susceptibility genes, the interactions of these disorders with normal aging and the environmental conditions that triggers or awaken the progress of these diseases need to be cared for by developing reliable and appropriate biomarkers to ameliorate the progression of these disorders.

9 REFERENCES

- Aharon-Peretz, J., Rosenbaum, H. and Gershoni-Baruch, R. (2004) 'Mutations in the Glucocerebrosidase Gene and Parkinson's Disease in Ashkenazi Jews', *New England Journal of Medicine*, 351(19), pp. 1972–1977. doi: 10.1056/NEJMoa033277.
- Alim, M. A., Hossain, M. S., Arima, K., Takeda, K., Izumiyama, Y., Nakamura, M., Kaji, H., Shinoda, T., Hisanaga, S. and Ueda, K. (2002) 'Tubulin Seeds α -Synuclein Fibril Formation', *Journal of Biological Chemistry*, 277(3), pp. 2112–2117. doi: 10.1074/jbc.M102981200.
- Allen Reish, H. E. and Standaert, D. G. (2015) 'Role of α -Synuclein in Inducing Innate and Adaptive Immunity in Parkinson Disease', *Journal of Parkinson's Disease*. IOS Press, 5(1), pp. 1–19. doi: 10.3233/JPD-140491.
- Andersen, J. K. (2004) 'Oxidative stress in neurodegeneration: cause or consequence?', *Nature Reviews Neuroscience*, 10(7), pp. S18–S25. doi: 10.1038/nrn1434.
- Anderson, J. P., Walker, D. E., Goldstein, J. M., de Laat, R., Banducci, K., Caccavello, R. J., Barbour, R., Huang, J., Kling, K., Lee, M., Diep, L., Keim, P. S., Shen, X., Chataway, T., Schlossmacher, M. G., Seubert, P., Schenk, D., Sinha, S., Gai, W. P. and Chilcote, T. J. (2006) 'Phosphorylation of Ser-129 Is the Dominant Pathological Modification of α -Synuclein in Familial and Sporadic Lewy Body Disease', *Journal of Biological Chemistry*, 281(40), pp. 29739–29752. doi: 10.1074/jbc.M600933200.
- Arrasate, M., Mitra, S., Schweitzer, E. S., Segal, M. R. and Finkbeiner, S. (2004) 'Inclusion body formation reduces levels of mutant huntingtin and the risk of neuronal death', *Nature*, 431(7010), pp. 805–810. doi: 10.1038/nature02998.
- Barrett, P. J. and Timothy Greenamyre, J. (2015) 'Post-translational modification of α -synuclein in Parkinson's disease', *Brain Research*, 1628, pp. 247–253. doi: 10.1016/j.brainres.2015.06.002.
- Bartels, T., Ahlstrom, L. S., Leftin, A., Kamp, F., Haass, C., Brown, M. F. and Beyer, K. (2010) 'The N-terminus of the intrinsically disordered protein α -synuclein triggers membrane binding and helix folding.', *Biophysical journal*. Elsevier, 99(7), pp. 2116–24. doi: 10.1016/j.bpj.2010.06.035.
- Bartels, T., Choi, J. G. and Selkoe, D. J. (2011) ' α -Synuclein occurs physiologically as a helically folded tetramer that resists aggregation.', *Nature*. NIH Public Access, 477(7362), pp. 107–110. doi: 10.1038/nature10324.
- Bonnet, A. M., Jutras, M. F., Czernecki, V., Corvol, J. C. and Vidailhet, M. (2012) 'Nonmotor Symptoms in Parkinson's Disease in 2012: Relevant Clinical Aspects', *Parkinson's Disease*. Hindawi Publishing Corporation, 2012, pp. 1–15. doi: 10.1155/2012/198316.
- Bruinsma, I. B., Bruggink, K. A., Kinast, K., Versleijen, A. A. M., Segers-Nolten, I. M. J., Subramaniam, V., Bea Kuiperij, H., Boelens, W., de Waal, R.

- M. W. and Verbeek, M. M. (2011) 'Inhibition of α -synuclein aggregation by small heat shock proteins', *Proteins: Structure, Function, and Bioinformatics*, 79(10), pp. 2956–2967. doi: 10.1002/prot.23152.
- Burré, J. (2015) 'The Synaptic Function of α -Synuclein.', *Journal of Parkinson's disease*. IOS Press, 5(4), pp. 699–713. doi: 10.3233/JPD-150642.
- Burré, J., Sharma, M., Tsetsenis, T., Buchman, V., Etherton, M. R. and Südhof, T. C. (2010) ' α -Synuclein Promotes SNARE-Complex Assembly in Vivo and in Vitro', *Science*, 329(5999). Available at: <http://science.sciencemag.org/content/329/5999/1663> (Accessed: 27 March 2017).
- Bussell, R. and Eliezer, D. (2001) 'Residual Structure and Dynamics in Parkinson's Disease-associated Mutants of α -Synuclein', *Journal of Biological Chemistry*, 276(49), pp. 45996–46003. doi: 10.1074/jbc.M106777200.
- Bussell, R. and Eliezer, D. (2003) 'A structural and functional role for 11-mer repeats in alpha-synuclein and other exchangeable lipid binding proteins.', *Journal of molecular biology*, 329(4), pp. 763–78. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12787676> (Accessed: 28 March 2017).
- Cappai, R. (2005) 'Dopamine promotes α -synuclein aggregation into SDS-resistant soluble oligomers via a distinct folding pathway', *The FASEB Journal*. doi: 10.1096/fj.04-3437fje.
- Caughey, B. and Lansbury, P. T. (2003) 'PROTEIN FIBRILS, PORES, FIBRILS, AND NEURODEGENERATION: Separating the Responsible Protein Aggregates from The Innocent Bystanders', *Annual Review of Neuroscience*. Annual Reviews 4139 El Camino Way, P.O. Box 10139, Palo Alto, CA 94303-0139, USA, 26(1), pp. 267–298. doi: 10.1146/annurev.neuro.26.010302.081142.
- Chandra, S., Chen, X., Rizo, J., Jahn, R. and Südhof, T. C. (2003) 'A Broken α -Helix in Folded α -Synuclein', *Journal of Biological Chemistry*, 278(17), pp. 15313–15318. doi: 10.1074/jbc.M213128200.
- Chen, L. and Feany, M. B. (2005) ' α -Synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease', *Nature Neuroscience*, 8(5), pp. 657–663. doi: 10.1038/nn1443.
- Chen, L., Jin, J., Davis, J., Zhou, Y., Wang, Y., Liu, J., Lockhart, P. J. and Zhang, J. (2007) 'Oligomeric α -synuclein inhibits tubulin polymerization', *Biochemical and Biophysical Research Communications*, 356(3), pp. 548–553. doi: 10.1016/j.bbrc.2007.02.163.
- Chen, L., Periquet, M., Wang, X., Negro, A., McLean, P. J., Hyman, B. T. and Feany, M. B. (2009) 'Tyrosine and serine phosphorylation of α -synuclein have opposing effects on neurotoxicity and soluble oligomer formation', *Journal of Clinical Investigation*, 119(11), pp. 3257–65. doi: 10.1172/JCI39088.
- Cohlberg, J. A., Li, J., Uversky, V. N. and Fink, A. L. (2002) 'Heparin and other glycosaminoglycans stimulate the formation of amyloid fibrils from alpha-synuclein in vitro.', *Biochemistry*, 41(5), pp. 1502–11. Available at:

<http://www.ncbi.nlm.nih.gov/pubmed/11814343> (Accessed: 30 March 2017).

Conway, K. A., Harper, J. D. and Lansbury, P. T. (1998) 'Accelerated in vitro fibril formation by a mutant α -synuclein linked to early-onset Parkinson disease', *Nature Medicine*, 4(11), pp. 1318–1320. doi: 10.1038/3311.

Conway, K. A., Rochet, J. C., Bieganski, R. M. and Lansbury, P. T. (2001) 'Kinetic Stabilization of the alpha-Synuclein Protofibril by a Dopamine-alpha-Synuclein Adduct', *Science*, 294(5545), pp. 1346–1349. doi: 10.1126/science.1063522.

Cox, D., Carver, J. A. and Ecroyd, H. (2014) 'Preventing α -synuclein aggregation: The role of the small heat-shock molecular chaperone proteins', *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, 1842(9), pp. 1830–1843. doi: 10.1016/j.bbadis.2014.06.024.

Dalfó, E., Portero-Otín, M., Ayala, V., Martínez, A., Pamplona, R. and Ferrer, I. (2005) 'Evidence of oxidative stress in the neocortex in incidental Lewy body disease.', *Journal of neuropathology and experimental neurology*, 64(9), pp. 816–30. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/16141792> (Accessed: 31 March 2017).

Dexter, D. T., Carayon, A., Javoy-Agid, F., Agid, Y., Wells, F. R., Daniel, S. E., Lees, A. J., Jenner, P. and Marsden, C. D. (1991) 'Alterations in the levels of iron, ferritin and other trace metals in Parkinson's disease and other neurodegenerative diseases affecting the basal ganglia.', *Brain : a journal of neurology*, pp. 1953–75. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/1832073> (Accessed: 30 March 2017).

Dieriks, B. V., Park, T. I.-H., Fourie, C., Faull, R. L. M., Dragunow, M. and Curtis, M. A. (2017) ' α -synuclein transfer through tunneling nanotubes occurs in SH-SY5Y cells and primary brain pericytes from Parkinson's disease patients.', *Scientific reports*, 7, p. 42984. doi: 10.1038/srep42984.

Doherty, M. J., Bird, T. D. and Leverenz, J. B. (2004) '?-Synuclein in motor neuron disease: an immunohistologic study', *Acta Neuropathologica*, 107(2), pp. 169–175. doi: 10.1007/s00401-003-0790-2.

Dorval, V. and Fraser, P. E. (2006) 'Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and alpha-synuclein.', *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology, 281(15), pp. 9919–24. doi: 10.1074/jbc.M510127200.

Dorval, V. and Fraser, P. E. (2007) 'SUMO on the road to neurodegeneration', *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, 1773(6), pp. 694–706. doi: 10.1016/j.bbamcr.2007.03.017.

Dufty, B. M., Warner, L. R., Hou, S. T., Jiang, S. X., Gomez-Isla, T., Leenhouts, K. M., Oxford, J. T., Feany, M. B., Masliah, E. and Rohn, T. T. (2007) 'Calpain-Cleavage of α -Synuclein', *The American Journal of Pathology*, 170(5), pp. 1725–1738. doi: 10.2353/ajpath.2007.061232.

Eckermann, K. (2013) 'SUMO and Parkinson's Disease', *NeuroMolecular Medicine*, 15(4), pp. 737–759. doi: 10.1007/s12017-013-8259-5.

- Eliezer, D., Kutluay, E., Bussell, R. and Browne, G. (2001a) ‘Conformational properties of α -synuclein in its free and lipid-associated states’, *Journal of Molecular Biology*, 307(4), pp. 1061–1073. doi: 10.1006/jmbi.2001.4538.
- Eliezer, D., Kutluay, E., Bussell, R. and Browne, G. (2001b) ‘Conformational properties of α -synuclein in its free and lipid-associated states’, *Journal of Molecular Biology*, 307(4), pp. 1061–1073. doi: 10.1006/jmbi.2001.4538.
- Eliezer, D., Kutluay, E., Bussell, R. and Browne, G. (2001c) ‘Conformational properties of α -synuclein in its free and lipid-associated states’, *Journal of Molecular Biology*, 307(4), pp. 1061–1073. doi: 10.1006/jmbi.2001.4538.
- Emamzadeh, F. N. (2016) ‘Alpha-synuclein structure, functions, and interactions.’, *Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences*. Medknow Publications, 21, p. 29. doi: 10.4103/1735-1995.181989.
- Engelender, S. (2008) ‘Ubiquitination of α -synuclein and autophagy in Parkinson’s disease’, *Autophagy*, 4(3), pp. 372–374. doi: 10.4161/auto.5604.
- Eyal, A., Szargel, R., Avraham, E., Liani, E., Haskin, J., Rott, R. and Engelender, S. (2006) ‘Synphilin-1A: An aggregation-prone isoform of synphilin-1 that causes neuronal death and is present in aggregates from α -synucleinopathy patients’, *Proceedings of the National Academy of Sciences*, 103(15), pp. 5917–5922. doi: 10.1073/pnas.0509707103.
- Fauvet, B., Mbefo, M. K., Fares, M.-B., Desobry, C., Michael, S., Ardah, M. T., Tsika, E., Coune, P., Prudent, M., Lion, N., Eliezer, D., Moore, D. J., Schneider, B., Aebischer, P., El-Agnaf, O. M., Masliah, E. and Lashuel, H. A. (2012) ‘ α -Synuclein in Central Nervous System and from Erythrocytes, Mammalian Cells, and Escherichia coli Exists Predominantly as Disordered Monomer’, *Journal of Biological Chemistry*, 287(19), pp. 15345–15364. doi: 10.1074/jbc.M111.318949.
- Federoff, M., Schottlaender, L. V., Houlden, H. and Singleton, A. (2015) ‘Multiple system atrophy: the application of genetics in understanding etiology’, *Clinical Autonomic Research*, 25(1), pp. 19–36. doi: 10.1007/s10286-014-0267-5.
- Fink, A. L. (2006) ‘The Aggregation and Fibrillation of α -Synuclein’, *Accounts of Chemical Research*, 39(9), pp. 628–634. doi: 10.1021/ar050073t.
- Fratiglioni, L. and Wang, H. X. (2000) ‘Smoking and Parkinson’s and Alzheimer’s disease: review of the epidemiological studies.’, *Behavioural brain research*, 113(1–2), pp. 117–20. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10942038> (Accessed: 13 April 2017).
- Fujiwara, H., Hasegawa, M., Dohmae, N., Kawashima, A., Masliah, E., Goldberg, M. S., Shen, J., Takio, K. and Iwatsubo, T. (2002a) ‘alpha-Synuclein is phosphorylated in synucleinopathy lesions.’, *Nature cell biology*, 4(2), pp. 160–4. doi: 10.1038/ncb748.
- Fujiwara, H., Hasegawa, M., Dohmae, N., Kawashima, A., Masliah, E., Goldberg, M. S., Shen, J., Takio, K. and Iwatsubo, T. (2002b) ‘alpha-Synuclein

is phosphorylated in synucleinopathy lesions.’, *Nature cell biology*, 4(2), pp. 160–4. doi: 10.1038/ncb748.

Gabbita, S. P., Aksenov, M. Y., Lovell, M. A. and Markesbery, W. R. (1999) ‘Decrease in peptide methionine sulfoxide reductase in Alzheimer’s disease brain.’, *Journal of neurochemistry*, 73(4), pp. 1660–6. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10501213> (Accessed: 3 April 2017).

Gade, V. R., Kardani, J. and Roy, I. (2014) ‘Effect of endogenous Hsp104 chaperone in yeast models of sporadic and familial Parkinson’s disease.’, *The international journal of biochemistry & cell biology*, 55, pp. 87–92. doi: 10.1016/j.biocel.2014.08.013.

Galvin, J. E., Uryu, K., Lee, V. M. and Trojanowski, J. Q. (1999) ‘Axon pathology in Parkinson’s disease and Lewy body dementia hippocampus contains alpha-, beta-, and gamma-synuclein.’, *Proceedings of the National Academy of Sciences of the United States of America*, 96(23), pp. 13450–5. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10557341> (Accessed: 22 March 2017).

Games, D., Valera, E., Spencer, B., Rockenstein, E., Mante, M., Adame, A., Patrick, C., Ubhi, K., Nuber, S., Sacayon, P., Zago, W., Seubert, P., Barbour, R., Schenk, D. and Masliah, E. (2014) ‘Reducing C-Terminal-Truncated Alpha-Synuclein by Immunotherapy Attenuates Neurodegeneration and Propagation in Parkinson’s Disease-Like Models’, *Journal of Neuroscience*, 34(28), pp. 9441–9454. doi: 10.1523/JNEUROSCI.5314-13.2014.

Gelb, D. J., Oliver, E. and Gilman, S. (1999) ‘Diagnostic criteria for Parkinson disease.’, *Archives of neurology*, 56(1), pp. 33–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9923759> (Accessed: 12 May 2017).

Glaser, C. B., Yamin, G., Uversky, V. N. and Fink, A. L. (2005) ‘Methionine oxidation, α -synuclein and Parkinson’s disease’, *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1703(2), pp. 157–169. doi: 10.1016/j.bbapap.2004.10.008.

Goers, J., Manning-Bog, A. B., McCormack, A. L., Millett, I. S., Doniach, S., Di Monte, D. A., Uversky, V. N. and Fink, A. L. (2003) ‘Nuclear Localization of α -Synuclein and Its Interaction with Histones[†]’, *Biochemistry*, 42(28), pp. 8465–8471. doi: 10.1021/bi0341152.

Graham, D. G. (1978) ‘Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones.’, *Molecular pharmacology*, 14(4), pp. 633–43. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/98706> (Accessed: 24 April 2017).

Greenbaum, E. A., Graves, C. L., Mishizen-Eberz, A. J., Lupoli, M. A., Lynch, D. R., Englander, S. W., Axelsen, P. H. and Giasson, B. I. (2005) ‘The E46K Mutation in α -Synuclein Increases Amyloid Fibril Formation’, *Journal of Biological Chemistry*, 280(9), pp. 7800–7807. doi: 10.1074/jbc.M411638200.

Hasegawa, M., Fujiwara, H., Nonaka, T., Wakabayashi, K., Takahashi, H., Lee, V. M.-Y., Trojanowski, J. Q., Mann, D. and Iwatsubo, T. (2002) ‘Phosphorylated α -Synuclein Is Ubiquitinated in α -Synucleinopathy Lesions’,

Journal of Biological Chemistry, 277(50), pp. 49071–49076. doi: 10.1074/jbc.M208046200.

Hashimoto, M., Hsu, L. J., Xia, Y., Takeda, A., Sisk, A., Sundsmo, M. and Masliah, E. (1999) 'Oxidative stress induces amyloid-like aggregate formation of NACP/alpha-synuclein in vitro.', *Neuroreport*, 10(4), pp. 717–21. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10208537> (Accessed: 22 March 2017).

Hely, M. A., Reid, W. G. J., Adena, M. A., Halliday, G. M. and Morris, J. G. L. (2008) 'The Sydney multicenter study of Parkinson's disease: The inevitability of dementia at 20 years', *Movement Disorders*, 23(6), pp. 837–844. doi: 10.1002/mds.21956.

Hodara, R., Norris, E. H., Giasson, B. I., Mishizen-Eberz, A. J., Lynch, D. R., Lee, V. M.-Y. and Ischiropoulos, H. (2004) 'Functional Consequences of - Synuclein Tyrosine Nitration: DIMINISHED BINDING TO LIPID VESICLES AND INCREASED FIBRIL FORMATION', *Journal of Biological Chemistry*, 279(46), pp. 47746–47753. doi: 10.1074/jbc.M408906200.

Hokenson, M. J., Uversky, V. N., Goers, J., Yamin, G., Munishkina, L. A. and Fink, A. L. (2004) 'Role of Individual Methionines in the Fibrillation of Methionine-Oxidized α -Synuclein[†]', *Biochemistry*, 43(15), pp. 4621–4633. doi: 10.1021/bi049979h.

Hong, D.-P., Fink, A. L. and Uversky, V. N. (2008) 'Structural Characteristics of α -Synuclein Oligomers Stabilized by the Flavonoid Baicalein', *Journal of Molecular Biology*, 383(1), pp. 214–223. doi: 10.1016/j.jmb.2008.08.039.

Hong, D.-P., Fink, A. L. and Uversky, V. N. (2009) 'Smoking and Parkinson's disease: does nicotine affect alpha-synuclein fibrillation?', *Biochimica et biophysica acta*. NIH Public Access, 1794(2), pp. 282–90. doi: 10.1016/j.bbapap.2008.09.026.

Horowitz, M. P., Milanese, C., Di Maio, R., Hu, X., Montero, L. M., Sanders, L. H., Tapias, V., Sepe, S., van Cappellen, W. A., Burton, E. A., Greenamyre, J. T. and Mastroberardino, P. G. (2011) 'Single-Cell Redox Imaging Demonstrates a Distinctive Response of Dopaminergic Neurons to Oxidative Insults', *Antioxidants & Redox Signaling*, 15(4), pp. 855–871. doi: 10.1089/ars.2010.3629.

Hoyer, W., Cherny, D., Subramaniam, V. and Jovin, T. M. (2004) 'Impact of the Acidic C-Terminal Region Comprising Amino Acids 109–140 on α -Synuclein Aggregation in Vitro[†]', *Biochemistry*, 43(51), pp. 16233–16242. doi: 10.1021/bi048453u.

Huggins, K. N. L., Bisaglia, M., Bubacco, L., Tatarek-Nossol, M., Kapurniotu, A. and Andersen, N. H. (2011) 'Designed hairpin peptides interfere with amyloidogenesis pathways: fibril formation and cytotoxicity inhibition, interception of the preamyloid state.', *Biochemistry*. NIH Public Access, 50(38), pp. 8202–12. doi: 10.1021/bi200760h.

Institute of Neurological Disorders, N. (2014) 'Multiple System Atrophy', p. 16. Available at: <https://catalog.ninds.nih.gov/pubstatic//15-5597/15-5597.pdf> (Accessed: 23 May 2017).

Institute on Aging, N. (2015) 'LEWY BODY DEMENTIA', p. 44. Available at: <https://catalog.ninds.nih.gov/pubstatic/15-7907/15-7907.pdf> (Accessed: 22 May 2017).

Ischiropoulos, H. and Beckman, J. S. (2003) 'Oxidative stress and nitration in neurodegeneration: cause, effect, or association?', *The Journal of clinical investigation*. American Society for Clinical Investigation, 111(2), pp. 163–9. doi: 10.1172/JCI17638.

Jakes, R., Spillantini, M. G. and Goedert, M. (1994) 'Identification of two distinct synucleins from human brain', *FEBS Letters*, 345(1), pp. 27–32. doi: 10.1016/0014-5793(94)00395-5.

Jones, D. R., Moussaud, S. and McLean, P. (2014) 'Targeting heat shock proteins to modulate α -synuclein toxicity.', *Therapeutic advances in neurological disorders*. SAGE Publications, 7(1), pp. 33–51. doi: 10.1177/1756285613493469.

Judkins, A., Forman, M., Uryu, K., Hinkle, D., Asbury, A., Lee, V. and Trojanowski, J. (2002) 'Co-occurrence of Parkinson's disease with progressive supranuclear palsy', *Acta Neuropathologica*, 103(5), pp. 526–530. doi: 10.1007/s00401-001-0483-7.

Kahle, P. J. (2007) ' α -Synucleinopathy models and human neuropathology: similarities and differences', *Acta Neuropathologica*, 115(1), pp. 87–95. doi: 10.1007/s00401-007-0302-x.

Kang, L., Moriarty, G. M., Woods, L. A., Ashcroft, A. E., Radford, S. E. and Baum, J. (2012) 'N-terminal acetylation of α -synuclein induces increased transient helical propensity and decreased aggregation rates in the intrinsically disordered monomer', *Protein Science*, 21(7), pp. 911–917. doi: 10.1002/pro.2088.

Karube, H., Sakamoto, M., Arawaka, S., Hara, S., Sato, H., Ren, C.-H., Goto, S., Koyama, S., Wada, M., Kawanami, T., Kurita, K. and Kato, T. (2008) 'N-terminal region of α -synuclein is essential for the fatty acid-induced oligomerization of the molecules', *FEBS Letters*, 582(25–26), pp. 3693–3700. doi: 10.1016/j.febslet.2008.10.001.

Kawahara, K., Hashimoto, M., Bar-On, P., Ho, G. J., Crews, L., Mizuno, H., Rockenstein, E., Imam, S. Z. and Masliah, E. (2008) ' α -Synuclein Aggregates Interfere with Parkin Solubility and Distribution: ROLE IN THE PATHOGENESIS OF PARKINSON DISEASE', *Journal of Biological Chemistry*, 283(11), pp. 6979–6987. doi: 10.1074/jbc.M710418200.

Kempuraj, D., Thangavel, R., Yang, E., Pattani, S., Zaheer, S., Santillan, D. A., Santillan, M. K. and Zaheer, A. (2015) 'Dopaminergic Toxin 1-Methyl-4-Phenylpyridinium, Proteins α -Synuclein and Glia Maturation Factor Activate Mast Cells and Release Inflammatory Mediators', *PLOS ONE*. Edited by D. Holowka, 10(8), p. e0135776. doi: 10.1371/journal.pone.0135776.

Kim, W. S., Kågedal, K. and Halliday, G. M. (2014) 'Alpha-synuclein biology in Lewy body diseases.', *Alzheimer's research & therapy*. BioMed Central, 6(5), p. 73. doi: 10.1186/s13195-014-0073-2.

- Lashuel, H. A., Hartley, D., Petre, B. M., Walz, T. and Lansbury, P. T. (2002) 'Neurodegenerative disease: Amyloid pores from pathogenic mutations', *Nature*, 418(6895), pp. 291–291. doi: 10.1038/418291a.
- Lashuel, H. A., Overk, C. R., Oueslati, A. and Masliah, E. (2013) 'The many faces of α -synuclein: from structure and toxicity to therapeutic target', *Nature Reviews Neuroscience*, 14. doi: 10.1038/nrn3406.
- Lashuel, H., Overk, C. and Oueslati, A. (2013) 'The many faces of α -synuclein: from structure and toxicity to therapeutic target', *Nature Reviews*. Available at: <http://www.nature.com/nrn/journal/v14/n1/abs/nrn3406.html> (Accessed: 21 March 2017).
- Lee, F. J., Liu, F., Pristupa, Z. B. and Niznik, H. B. (2001) 'Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis.', *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 15(6), pp. 916–26. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/11292651> (Accessed: 9 May 2017).
- Lee, H.-J., Choi, C. and Lee, S.-J. (2002a) 'Membrane-bound α -Synuclein Has a High Aggregation Propensity and the Ability to Seed the Aggregation of the Cytosolic Form', *Journal of Biological Chemistry*, 277(1), pp. 671–678. doi: 10.1074/jbc.M107045200.
- Lee, H.-J., Choi, C. and Lee, S.-J. (2002b) 'Membrane-bound α -Synuclein Has a High Aggregation Propensity and the Ability to Seed the Aggregation of the Cytosolic Form', *Journal of Biological Chemistry*, 277(1), pp. 671–678. doi: 10.1074/jbc.M107045200.
- Lee, J. T., Wheeler, T. C., Li, L. and Chin, L.-S. (no date) 'Ubiquitination of α -synuclein by Siah-1 promotes α -synuclein aggregation and apoptotic cell death'. doi: 10.1093/hmg/ddm363.
- Lee, S.-J. (2003) ' α -Synuclein Aggregation: A Link Between Mitochondrial Defects and Parkinson's Disease?', *Antioxidants & Redox Signaling*, 5(3), pp. 337–348. doi: 10.1089/152308603322110904.
- Lees, A. J., Hardy, J. and Revesz, T. (2009) 'Parkinson's disease', *The Lancet*, 373(9680), pp. 2055–2066. doi: 10.1016/S0140-6736(09)60492-X.
- Leong, S. L., Cappai, R., Barnham, K. J. and Pham, C. L. L. (2009) 'Modulation of α -Synuclein Aggregation by Dopamine: A Review', *Neurochemical Research*. Springer US, 34(10), pp. 1838–1846. doi: 10.1007/s11064-009-9986-8.
- Lewis, K. A., Su, Y., Jou, O., Ritchie, C., Foong, C., Hynan, L. S., White, C. L., Thomas, P. J. and Hatanpaa, K. J. (2010) 'Abnormal Neurites Containing C-Terminally Truncated α -Synuclein Are Present in Alzheimer's Disease without Conventional Lewy Body Pathology', *The American Journal of Pathology*, 177(6), pp. 3037–3050. doi: 10.2353/ajpath.2010.100552.
- Li, J., Uversky, V. N. and Fink, A. L. (2001) 'Effect of familial Parkinson's disease point mutations A30P and A53T on the structural properties,

aggregation, and fibrillation of human alpha-synuclein.’, *Biochemistry*, 40(38), pp. 11604–13. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/11560511> (Accessed: 29 March 2017).

Li, J., Uversky, V. N. and Fink, A. L. (2002) ‘Conformational behavior of human alpha-synuclein is modulated by familial Parkinson’s disease point mutations A30P and A53T.’, *Neurotoxicology*, 23(4–5), pp. 553–67. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12428728> (Accessed: 29 March 2017).

Li, J., Zhu, M., Manning-Bog, A. B., Di Monte, D. A. and Fink, A. L. (2004) ‘Dopamine and L-dopa disaggregate amyloid fibrils: implications for Parkinson’s and Alzheimer’s disease’, *The FASEB Journal*, 18(9), pp. 962–4. doi: 10.1096/fj.03-0770fje.

Li, J., Zhu, M., Rajamani, S., Uversky, V. N. and Fink, A. L. (2004) ‘Rifampicin Inhibits α -Synuclein Fibrillation and Disaggregates Fibrils’, *Chemistry & Biology*, 11(11), pp. 1513–1521. doi: 10.1016/j.chembiol.2004.08.025.

Liani, E., Eyal, A., Avraham, E., Shemer, R., Szargel, R., Berg, D., Bornemann, A., Riess, O., Ross, C. A., Rott, R. and Engelender, S. (2004) ‘Ubiquitylation of synphilin-1 and alpha-synuclein by SIAH and its presence in cellular inclusions and Lewy bodies imply a role in Parkinson’s disease.’, *Proceedings of the National Academy of Sciences of the United States of America*, 101(15), pp. 5500–5. doi: 10.1073/pnas.0401081101.

Liu, G., Aliaga, L. and Cai, H. (2012) ‘ α -synuclein, LRRK2 and their interplay in Parkinson’s disease.’, *Future neurology*. NIH Public Access, 7(2), pp. 145–153. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/22563296> (Accessed: 9 May 2017).

Liu, I.-H., Uversky, V. N., Munishkina, L. A., Fink, A. L., Halfter, W. and Cole, G. J. (2005) ‘Agrin binds α -synuclein and modulates α -synuclein fibrillation’, *Glycobiology*, 15(12), pp. 1320–1331. doi: 10.1093/glycob/cwj014.

Liu, Y., Carver, J. A., Calabrese, A. N. and Pukala, T. L. (2014) ‘Gallic acid interacts with α -synuclein to prevent the structural collapse necessary for its aggregation’, *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1844(9), pp. 1481–1485. doi: 10.1016/j.bbapap.2014.04.013.

Lokappa, S. B., Suk, J.-E., Balasubramanian, A., Samanta, S., Situ, A. J. and Ulmer, T. S. (2014) ‘Sequence and Membrane Determinants of the Random Coil–Helix Transition of α -Synuclein’, *Journal of Molecular Biology*, 426(10), pp. 2130–2144. doi: 10.1016/j.jmb.2014.02.024.

Lotharius, J., Barg, S., Wiekop, P., Lundberg, C., Raymon, H. K. and Brundin, P. (2002) ‘Effect of Mutant alpha -Synuclein on Dopamine Homeostasis in a New Human Mesencephalic Cell Line’, *Journal of Biological Chemistry*, 277(41), pp. 38884–38894. doi: 10.1074/jbc.M205518200.

Luk, K. C., Song, C., O’Brien, P., Stieber, A., Branch, J. R., Brunden, K. R., Trojanowski, J. Q. and Lee, V. M.-Y. (2009) ‘Exogenous α -synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells’, *Proceedings of the National Academy of Sciences*, 106(47), pp. 20051–20056. doi: 10.1073/pnas.0908005106.

- Luth, E. S., Stavrovskaya, I. G., Bartels, T., Kristal, B. S. and Selkoe, D. J. (2014) 'Soluble, Prefibrillar α -Synuclein Oligomers Promote Complex I-dependent, Ca^{2+} -induced Mitochondrial Dysfunction', *Journal of Biological Chemistry*, 289(31), pp. 21490–21507. doi: 10.1074/jbc.M113.545749.
- Maguire-Zeiss, K. A. and Federoff, H. J. (2003) 'Convergent pathobiologic model of Parkinson's disease.', *Annals of the New York Academy of Sciences*, 991, pp. 152–66. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12846984> (Accessed: 14 April 2017).
- Makwana, P. K. and Sundd, M. (2016) 'Alpha-synuclein structure, aggregation and modulators'. Available at: http://jpp.org.in/public/site/aop-articles/JPPR202016-5eM_Sundd_FINAL.pdf (Accessed: 19 April 2017).
- Manning-Bog, A. B., McCormack, A. L., Li, J., Uversky, V. N., Fink, A. L. and Di Monte, D. A. (2002) 'The Herbicide Paraquat Causes Up-regulation and Aggregation of α -Synuclein in Mice', *Journal of Biological Chemistry*, 277(3), pp. 1641–1644. doi: 10.1074/jbc.C100560200.
- Maroteaux, L., Campanelli, J. T. and Scheller, R. H. (1988) 'Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 8(8), pp. 2804–15. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/3411354> (Accessed: 22 March 2017).
- Martinez, Z., Zhu, M., Han, S. and Fink, A. L. (2007) 'GM1 Specifically Interacts with α -Synuclein and Inhibits Fibrillation [†]', *Biochemistry*, 46(7), pp. 1868–1877. doi: 10.1021/bi061749a.
- Masliah, E., Rockenstein, E., Veinbergs, I., Sagara, Y., Mallory, M., Hashimoto, M. and Mucke, L. (2001) '-Amyloid peptides enhance -synuclein accumulation and neuronal deficits in a transgenic mouse model linking Alzheimer's disease and Parkinson's disease', *Proceedings of the National Academy of Sciences*, 98(21), pp. 12245–12250. doi: 10.1073/pnas.211412398.
- Masuda, M., Suzuki, N., Taniguchi, S., Oikawa, T., Nonaka, T., Iwatsubo, T., Hisanaga, S., Goedert, M. and Hasegawa, M. (2006) 'Small Molecule Inhibitors of α -Synuclein Filament Assembly [†]', *Biochemistry*, 45(19), pp. 6085–6094. doi: 10.1021/bi0600749.
- MAYO CLINIC (2017) 'Parkinson's disease Treatments and drugs - Mayo Clinic'. Available at: <http://www.mayoclinic.org/diseases-conditions/parkinsons-disease/basics/treatment/con-20028488> (Accessed: 31 May 2017).
- Mazzulli, J. R., Mishizen, A. J., Giasson, B. I., Lynch, D. R., Thomas, S. A., Nakashima, A., Nagatsu, T., Ota, A. and Ischiropoulos, H. (2006) 'Cytosolic Catechols Inhibit -Synuclein Aggregation and Facilitate the Formation of Intracellular Soluble Oligomeric Intermediates', *Journal of Neuroscience*, 26(39), pp. 10068–10078. doi: 10.1523/JNEUROSCI.0896-06.2006.
- Mbefo, M. K., Paleologou, K. E., Boucharaba, A., Oueslati, A., Schell, H., Fournier, M., Olschewski, D., Yin, G., Zweckstetter, M., Masliah, E., Kahle, P. J., Hirling, H. and Lashuel, H. A. (2010) 'Phosphorylation of Synucleins by

- Members of the Polo-like Kinase Family’, *Journal of Biological Chemistry*, 285(4), pp. 2807–2822. doi: 10.1074/jbc.M109.081950.
- McCann, H., Stevens, C. H., Cartwright, H. and Halliday, G. M. (2014) ‘ α -Synucleinopathy phenotypes’, *Parkinsonism & Related Disorders*, 20, pp. S62–S67. doi: 10.1016/S1353-8020(13)70017-8.
- McLaurin, J., Yang, D.-S., Yip, C. M. and Fraser, P. E. (2000) ‘Review: Modulating Factors in Amyloid- β Fibril Formation’, *Journal of Structural Biology*, 130(2–3), pp. 259–270. doi: 10.1006/jsbi.2000.4289.
- McNaught, K. S. and Jenner, P. (2001) ‘Proteasomal function is impaired in substantia nigra in Parkinson’s disease.’, *Neuroscience letters*, 297(3), pp. 191–4. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/11137760> (Accessed: 4 May 2017).
- Melchior, F., Schergaut, M. and Pichler, A. (2003) ‘SUMO: ligases, isopeptidases and nuclear pores’, *Trends in Biochemical Sciences*, 28(11), pp. 612–618. doi: 10.1016/j.tibs.2003.09.002.
- Meulmeester, E. and Melchior, F. (2008) ‘Cell biology: SUMO’, *Nature*, 452(7188), pp. 709–711. doi: 10.1038/452709a.
- Middleton, E. R. and Rhoades, E. (2010) ‘Effects of Curvature and Composition on α -Synuclein Binding to Lipid Vesicles’, *Biophysical Journal*, 99(7), pp. 2279–2288. doi: 10.1016/j.bpj.2010.07.056.
- Milowska, K., Malachowska, M. and Gabryelak, T. (2011) ‘PAMAM G4 dendrimers affect the aggregation of α -synuclein’, *International Journal of Biological Macromolecules*, 48(5), pp. 742–746. doi: 10.1016/j.ijbiomac.2011.02.021.
- Minton, A. P. (2000) ‘Implications of macromolecular crowding for protein assembly.’, *Current opinion in structural biology*, 10(1), pp. 34–9. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/10679465> (Accessed: 29 March 2017).
- Mor, D. E., Ugras, S. E., Daniels, M. J. and Ischiropoulos, H. (2016) ‘Dynamic structural flexibility of α -synuclein’, *Neurobiology of Disease*, 88, pp. 66–74. doi: 10.1016/j.nbd.2015.12.018.
- Munishkina, L. A., Ahmad, A., Fink, A. L. and Uversky, V. N. (2008) ‘Guiding Protein Aggregation with Macromolecular Crowding [†]’, *Biochemistry*, 47(34), pp. 8993–9006. doi: 10.1021/bi8008399.
- Munishkina, L. A., Cooper, E. M., Uversky, V. N. and Fink, A. L. (2004) ‘The effect of macromolecular crowding on protein aggregation and amyloid fibril formation’, *Journal of Molecular Recognition*, 17(5), pp. 456–464. doi: 10.1002/jmr.699.
- Munishkina, L. A., Phelan, C., Uversky, V. N. and Fink, A. L. (2003) ‘Conformational Behavior and Aggregation of α -Synuclein in Organic Solvents: Modeling the Effects of Membranes [†]’, *Biochemistry*, 42(9), pp. 2720–2730. doi: 10.1021/bi027166s.
- Muthu Ramakrishnan, ‡, Poul H. Jensen, § and and Derek Marsh*, ‡ (2006)

‘Association of α -Synuclein and Mutants with Lipid Membranes: Spin-Label ESR and Polarized IR†’. American Chemical Society . doi: 10.1021/BI052344D.

National Institute of Neurological Disorders And Stroke (2014) *Parkinson’s Disease: Hope Through Research | National Institute of Neurological Disorders and Stroke*. Available at: <https://www.ninds.nih.gov/Disorders/Patient-Caregiver-Education/Hope-Through-Research/Parkinsons-Disease-Hope-Through-Research#Parkinsons> from neuro disorders (Accessed: 16 May 2017).

Nemani, V. M., Lu, W., Berge, V., Nakamura, K., Onoa, B., Lee, M. K., Chaudhry, F. A., Nicoll, R. A. and Edwards, R. H. (2010) ‘Increased Expression of α -Synuclein Reduces Neurotransmitter Release by Inhibiting Synaptic Vesicle Reclustering after Endocytosis’, *Neuron*, 65(1), pp. 66–79. doi: 10.1016/j.neuron.2009.12.023.

Nishioka, K., Wider, C., Vilariño-Güell, C., Soto-Ortolaza, A. I., Lincoln, S. J., Kachergus, J. M., Jasinska-Myga, B., Ross, O. A., Rajput, A., Robinson, C. A., Ferman, T. J., Wszolek, Z. K., Dickson, D. W. and Farrer, M. J. (2010) ‘Association of α -, β -, and γ -Synuclein With Diffuse Lewy Body Disease’, *Archives of Neurology*, 67(8), pp. 970–5. doi: 10.1001/archneurol.2010.177.

Norris, E. H., Giasson, B. I., Hodara, R., Xu, S., Trojanowski, J. Q., Ischiropoulos, H. and Lee, V. M.-Y. (2005) ‘Reversible inhibition of alpha-synuclein fibrillization by dopaminochrome-mediated conformational alterations.’, *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology, 280(22), pp. 21212–9. doi: 10.1074/jbc.M412621200.

Nyström, H., Nordström, A. and Nordström, P. (2016) ‘Risk of Injurious Fall and Hip Fracture up to 26 y before the Diagnosis of Parkinson Disease: Nested Case–Control Studies in a Nationwide Cohort’, *PLOS Medicine*. Edited by A. J. M. de Craen, 13(2), p. e1001954. doi: 10.1371/journal.pmed.1001954.

Oueslati, A., Fournier, M. and Lashuel, H. A. (2010) ‘Role of post-translational modifications in modulating the structure, function and toxicity of α -synuclein’, in *Progress in brain research*, pp. 115–145. doi: 10.1016/S0079-6123(10)83007-9.

Paleologou, K. E., Oueslati, A., Shakked, G., Rospigliosi, C. C., Kim, H.-Y., Lamberto, G. R., Fernandez, C. O., Schmid, A., Chegini, F., Gai, W. P., Chiappe, D., Moniatte, M., Schneider, B. L., Aebischer, P., Eliezer, D., Zweckstetter, M., Masliah, E. and Lashuel, H. A. (2010) ‘Phosphorylation at S87 Is Enhanced in Synucleinopathies, Inhibits α -Synuclein Oligomerization, and Influences Synuclein-Membrane Interactions’, *Journal of Neuroscience*, 30(9), pp. 3184–3198. doi: 10.1523/JNEUROSCI.5922-09.2010.

Pappolla, M. A., Omar, R. A., Kim, K. S. and Robakis, N. K. (1992) ‘Immunohistochemical evidence of oxidative [corrected] stress in Alzheimer’s disease.’, *The American journal of pathology*, 140(3), pp. 621–8. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/1372157> (Accessed: 3 April 2017).

Periquet, M., Fulga, T., Myllykangas, L., Schlossmacher, M. G. and Feany, M.

- B. (2007) ‘Aggregated α -Synuclein Mediates Dopaminergic Neurotoxicity In Vivo’, *Journal of Neuroscience*, 27(12), pp. 3338–3346. doi: 10.1523/JNEUROSCI.0285-07.2007.
- Plowey, E. D. and Chu, C. T. (2011) ‘Synaptic dysfunction in genetic models of Parkinson’s disease: A role for autophagy?’, *Neurobiology of Disease*, 43(1), pp. 60–67. doi: 10.1016/j.nbd.2010.10.011.
- Popova, B., Kleinknecht, A. and Braus, G. (2015) ‘Posttranslational Modifications and Clearing of α -Synuclein Aggregates in Yeast’, *Biomolecules*. Multidisciplinary Digital Publishing Institute, 5(2), pp. 617–634. doi: 10.3390/biom5020617.
- Putcha, P., Danzer, K. M., Kranich, L. R., Scott, A., Silinski, M., Mabbett, S., Hicks, C. D., Veal, J. M., Steed, P. M., Hyman, B. T. and McLean, P. J. (2010) ‘Brain-Permeable Small-Molecule Inhibitors of Hsp90 Prevent α -Synuclein Oligomer Formation and Rescue α -Synuclein-Induced Toxicity’, *Journal of Pharmacology and Experimental Therapeutics*, 332(3), pp. 849–857. doi: 10.1124/jpet.109.158436.
- Rekas, A., Knott, R. B., Sokolova, A., Barnham, K. J., Perez, K. A., Masters, C. L., Drew, S. C., Cappai, R., Curtain, C. C. and Pham, C. L. L. (2010) ‘The structure of dopamine induced α -synuclein oligomers’, *European Biophysics Journal*. Springer-Verlag, 39(10), pp. 1407–1419. doi: 10.1007/s00249-010-0595-x.
- van Rooden, S. M., Heiser, W. J., Kok, J. N., Verbaan, D., van Hilten, J. J. and Marinus, J. (2010) ‘The identification of Parkinson’s disease subtypes using cluster analysis: A systematic review’, *Movement Disorders*, 25(8), pp. 969–978. doi: 10.1002/mds.23116.
- Ross, C. A. and Poirier, M. A. (2005) ‘Opinion: What is the role of protein aggregation in neurodegeneration?’, *Nature Reviews Molecular Cell Biology*, 6(11), pp. 891–898. doi: 10.1038/nrm1742.
- Rott, R., Szargel, R., Haskin, J., Shani, V., Shainskaya, A., Manov, I., Liani, E., Avraham, E. and Engelender, S. (2008) ‘Monoubiquitylation of α -Synuclein by Seven in Absentia Homolog (SIAH) Promotes Its Aggregation in Dopaminergic Cells’, *Journal of Biological Chemistry*, 283(6), pp. 3316–3328. doi: 10.1074/jbc.M704809200.
- rPeptide, P. P. S. (no date) *Alpha-Synuclein | rPeptide*. Available at: <https://www.rpeptide.com/products/proteins/synuclein/s-1001-1> (Accessed: 4 April 2017).
- Sanders, L. H., McCoy, J., Hu, X., Mastroberardino, P. G., Dickinson, B. C., Chang, C. J., Chu, C. T., Van Houten, B. and Greenamyre, J. T. (2014) ‘Mitochondrial DNA damage: Molecular marker of vulnerable nigral neurons in Parkinson’s disease’, *Neurobiology of Disease*, 70, pp. 214–223. doi: 10.1016/j.nbd.2014.06.014.
- Scherzer-Attali, R., Shaltiel-Karyo, R., Adalist, Y., Segal, D. and Gazit, E. (2012) ‘Generic inhibition of amyloidogenic proteins by two naphthoquinone-tryptophan hybrid molecules’, *Proteins: Structure, Function, and*

Bioinformatics, 80(8), p. n/a-n/a. doi: 10.1002/prot.24080.

Settembre, C., Fraldi, A., Jahreiss, L., Spampinato, C., Venturi, C., Medina, D., de Pablo, R., Tacchetti, C., Rubinsztein, D. C. and Ballabio, A. (2007) 'A block of autophagy in lysosomal storage disorders', *Human Molecular Genetics*, 17(1), pp. 119–129. doi: 10.1093/hmg/ddm289.

Shahpasandzadeh, H., Popova, B., Kleinknecht, A., Fraser, P. E., Outeiro, T. F. and Braus, G. H. (2014) 'Interplay between sumoylation and phosphorylation for protection against α -synuclein inclusions.', *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology, 289(45), pp. 31224–40. doi: 10.1074/jbc.M114.559237.

Shahpiri, Z., Bahramsoltani, R., Hosein Farzaei, M., Farzaei, F. and Rahimi, R. (2016) 'Phytochemicals as future drugs for Parkinson's disease: a comprehensive review', *Reviews in the Neurosciences*, 27(6). doi: 10.1515/revneuro-2016-0004.

Shendelman, S., Jonason, A., Martinat, C., Leete, T. and Abeliovich, A. (2004) 'DJ-1 Is a Redox-Dependent Molecular Chaperone That Inhibits α -Synuclein Aggregate Formation', *PLoS Biology*. Edited by Huda Y. Zoghbi, 2(11), p. e362. doi: 10.1371/journal.pbio.0020362.

Shults, C. (2006) 'Lewy bodies', *Proceedings of the National Academy of*. Available at: <http://www.pnas.org/content/103/6/1661.short> (Accessed: 21 March 2017).

Singleton, A., Gwinn-Hardy, K., Sharabi, Y., Li, S.-T., Holmes, C., Dendi, R., Hardy, J., Singleton, A., Crawley, A. and Goldstein, D. S. (2004) 'Association between cardiac denervation and parkinsonism caused by α -synuclein gene triplication', *Brain*, 127(4), pp. 768–772. doi: 10.1093/brain/awh081.

Smith, B. R., Santos, M. B., Marshall, M. S., Cantuti-Castelvetri, L., Lopez-Rosas, A., Li, G., van Breemen, R., Claycomb, K. I., Gallea, J. I., Celej, M. S., Crocker, S. J., Givogri, M. I. and Bongarzone, E. R. (2014) 'Neuronal inclusions of α -synuclein contribute to the pathogenesis of Krabbe disease', *The Journal of Pathology*, 232(5), pp. 509–521. doi: 10.1002/path.4328.

Smith, C. D., Carney, J. M., Starke-Reed, P. E., Oliver, C. N., Stadtman, E. R., Floyd, R. A. and Markesbery, W. R. (1991) 'Excess brain protein oxidation and enzyme dysfunction in normal aging and in Alzheimer disease.', *Proceedings of the National Academy of Sciences of the United States of America*, 88(23), pp. 10540–3. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/1683703> (Accessed: 31 March 2017).

Sokolov, Y., Kozak, J. A., Kaye, R., Chanturiya, A., Glabe, C. and Hall, J. E. (2006) 'Soluble Amyloid Oligomers Increase Bilayer Conductance by Altering Dielectric Structure', *The Journal of General Physiology*, 128(6), pp. 637–647. doi: 10.1085/jgp.200609533.

Souza, J. M. (2000) 'Dityrosine Cross-linking Promotes Formation of Stable α -Synuclein Polymers. IMPLICATION OF NITRATIVE AND OXIDATIVE STRESS IN THE PATHOGENESIS OF NEURODEGENERATIVE SYNUCLEINOPATHIES', *Journal of Biological*

Chemistry, 275(24), pp. 18344–18349. doi: 10.1074/jbc.M000206200.

Spillantini, M. G., Crowther, R. A., Jakes, R., Cairns, N. J., Lantos, P. L. and Goedert, M. (1998) 'Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies.', *Neuroscience letters*, 251(3), pp. 205–8. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/9726379> (Accessed: 22 March 2017).

Stefanis, L., Larsen, K. E., Rideout, H. J., Sulzer, D. and Greene, L. A. (2001) 'Expression of A53T mutant but not wild-type alpha-synuclein in PC12 cells induces alterations of the ubiquitin-dependent degradation system, loss of dopamine release, and autophagic cell death.', *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 21(24), pp. 9549–60. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/11739566> (Accessed: 4 May 2017).

Sugeno, N., Takeda, A., Hasegawa, T., Kobayashi, M., Kikuchi, A., Mori, F., Wakabayashi, K. and Itoyama, Y. (2008) 'Serine 129 Phosphorylation of α -Synuclein Induces Unfolded Protein Response-mediated Cell Death', *Journal of Biological Chemistry*, 283(34), pp. 23179–23188. doi: 10.1074/jbc.M802223200.

Sung, Y. and Eliezer, D. (2007) 'Residual Structure, Backbone Dynamics, and Interactions within the Synuclein Family', *Journal of Molecular Biology*, 372(3), pp. 689–707. doi: 10.1016/j.jmb.2007.07.008.

Sung, Y., Rospigliosi, C. and Eliezer, D. (2006) 'NMR mapping of copper binding sites in alpha-synuclein', *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1764(1), pp. 5–12. doi: 10.1016/j.bbapap.2005.11.003.

Tanaka, M., Kim, Y. M., Lee, G., Junn, E., Iwatsubo, T. and Mouradian, M. M. (2003) 'Aggresomes Formed by α -Synuclein and Synphilin-1 Are Cytoprotective', *Journal of Biological Chemistry*, 279(6), pp. 4625–4631. doi: 10.1074/jbc.M310994200.

Tanner, C. M. (1989) 'The role of environmental toxins in the etiology of Parkinson's disease.', *Trends in neurosciences*, 12(2), pp. 49–54. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/2469210> (Accessed: 30 March 2017).

The Mayo clinic (no date) 'Parkinson's disease Treatments and drugs - Mayo Clinic'. Available at: <http://www.mayoclinic.org/diseases-conditions/parkinsons-disease/basics/treatment/con-20028488> (Accessed: 15 May 2017).

Tomiyama, T., Shoji, A., Kataoka, K., Suwa, Y., Asano, S., Kaneko, H. and Endo, N. (1996) 'Inhibition of amyloid beta protein aggregation and neurotoxicity by rifampicin. Its possible function as a hydroxyl radical scavenger.', *The Journal of biological chemistry*, 271(12), pp. 6839–44. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8636108> (Accessed: 14 April 2017).

Trexler, A. J. and Rhoades, E. (2012) 'N-terminal acetylation is critical for forming α -helical oligomer of α -synuclein', *Protein Science*, 21(5), pp. 601–605. doi: 10.1002/pro.2056.

- Uéda, K., Fukushima, H., Masliah, E., Xia, Y., Iwai, A., Yoshimoto, M., Otero, D. A., Kondo, J., Ihara, Y. and Saitoh, T. (1993) 'Molecular cloning of cDNA encoding an unrecognized component of amyloid in Alzheimer disease.', *Proceedings of the National Academy of Sciences of the United States of America*, 90(23), pp. 11282–6. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/8248242> (Accessed: 22 March 2017).
- Ullman, O., Fisher, C. K. and Stultz, C. M. (2011) 'Explaining the structural plasticity of α -synuclein.', *Journal of the American Chemical Society*. American Chemical Society, 133(48), pp. 19536–46. doi: 10.1021/ja208657z.
- Ulmer, T. S., Bax, A., Cole, N. B. and Nussbaum, R. L. (2005) 'Structure and Dynamics of Micelle-bound Human α -Synuclein', *Journal of Biological Chemistry*, 280(10), pp. 9595–9603. doi: 10.1074/jbc.M411805200.
- Uversky, V. N. (2007a) 'Neuropathology, biochemistry, and biophysics of α -synuclein aggregation', *Journal of Neurochemistry*, 0(0), p. 070710052154012–???. doi: 10.1111/j.1471-4159.2007.04764.x.
- Uversky, V. N. (2007b) 'Neuropathology, biochemistry, and biophysics of α -synuclein aggregation', *Journal of Neurochemistry*. Blackwell Publishing Ltd, 0(0), p. 070710052154012–???. doi: 10.1111/j.1471-4159.2007.04764.x.
- Uversky, V. N. and Eliezer, D. (2009) 'Biophysics of Parkinson's disease: structure and aggregation of alpha-synuclein.', *Current protein & peptide science*. NIH Public Access, 10(5), pp. 483–99. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19538146> (Accessed: 3 April 2017).
- Uversky, V. N., Li, J. and Fink, A. L. (2001) 'Metal-triggered Structural Transformations, Aggregation, and Fibrillation of Human α -Synuclein: A POSSIBLE MOLECULAR LINK BETWEEN PARKINSON'S DISEASE AND HEAVY METAL EXPOSURE', *Journal of Biological Chemistry*, 276(47), pp. 44284–44296. doi: 10.1074/jbc.M105343200.
- Uversky, V. N., Li, J., Souillac, P., Millett, I. S., Doniach, S., Jakes, R., Goedert, M. and Fink, A. L. (2002) 'Biophysical Properties of the Synucleins and Their Propensities to Fibrillate', *Journal of Biological Chemistry*, 277(14), pp. 11970–11978. doi: 10.1074/jbc.M109541200.
- Uversky, V. N., Yamin, G., Munishkina, L. A., Karymov, M. A., Millett, I. S., Doniach, S., Lyubchenko, Y. L. and Fink, A. L. (2005) 'Effects of nitration on the structure and aggregation of α -synuclein', *Molecular Brain Research*, 134(1), pp. 84–102. doi: 10.1016/j.molbrainres.2004.11.014.
- Uversky, V. N., Yamin, G., Souillac, P. O., Goers, J., Glaser, C. B. and Fink, A. L. (2002) 'Methionine oxidation inhibits fibrillation of human alpha-synuclein in vitro.', *FEBS letters*, 517(1–3), pp. 239–44. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12062445> (Accessed: 3 April 2017).
- Volles, M. J. and Lansbury, P. T. (2002) 'Vesicle permeabilization by protofibrillar alpha-synuclein is sensitive to Parkinson's disease-linked mutations and occurs by a pore-like mechanism.', *Biochemistry*, 41(14), pp. 4595–602. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/11926821> (Accessed: 24 April 2017).

Wang, D.-S., Iwata, N., Hama, E., Saido, T. C. and Dickson, D. W. (2003) 'Oxidized neprilysin in aging and Alzheimer's disease brains.', *Biochemical and biophysical research communications*, 310(1), pp. 236–41. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/14511676> (Accessed: 31 March 2017).

Waudby, C. A., Knowles, T. P. J., Devlin, G. L., Skepper, J. N., Ecroyd, H., Carver, J. A., Welland, M. E., Christodoulou, J., Dobson, C. M. and Meehan, S. (2010) 'The interaction of alphaB-crystallin with mature alpha-synuclein amyloid fibrils inhibits their elongation.', *Biophysical journal*. The Biophysical Society, 98(5), pp. 843–51. doi: 10.1016/j.bpj.2009.10.056.

Webb, J. L., Ravikumar, B., Atkins, J., Skepper, J. N. and Rubinsztein, D. C. (2003) '-Synuclein Is Degraded by Both Autophagy and the Proteasome', *Journal of Biological Chemistry*, 278(27), pp. 25009–25013. doi: 10.1074/jbc.M300227200.

Wersinger, C. and Sidhu, A. (2003) 'Attenuation of dopamine transporter activity by alpha-synuclein.', *Neuroscience letters*, 340(3), pp. 189–92. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12672538> (Accessed: 9 May 2017).

Wilhelmsen, K. C., Forman, M. S., Rosen, H. J., Alving, L. I., Goldman, J., Feiger, J., Lee, J. V., Segall, S. K., Kramer, J. H., Lomen-Hoerth, C., Rankin, K. P., Johnson, J., Feiler, H. S., Weiner, M. W., Lee, V. M.-Y., Trojanowski, J. Q. and Miller, B. L. (2004) '17q-Linked Frontotemporal Dementia–Amyotrophic Lateral Sclerosis Without Tau Mutations With Tau and α -Synuclein Inclusions', *Archives of Neurology*, 61(3), p. 398. doi: 10.1001/archneur.61.3.398.

Xu, L. and Pu, J. (2016) 'Alpha-Synuclein in Parkinson's Disease: From Pathogenetic Dysfunction to Potential Clinical Application', *Parkinson's Disease*. Available at: <https://www.hindawi.com/journals/pd/2016/1720621/abs/> (Accessed: 5 April 2017).

Yamashita, S., Sakashita, N., Yamashita, T., Tawara, N., Tasaki, M., Kawakami, K., Komohara, Y., Fujiwara, Y., Kamikawa, M., Nakagawa, T., Hirano, T., Maeda, Y., Hasegawa, M., Takeya, M. and Ando, Y. (2014) 'Concomitant accumulation of α -synuclein and TDP-43 in a patient with corticobasal degeneration', *Journal of Neurology*, 261(11), pp. 2209–2217. doi: 10.1007/s00415-014-7491-8.

Yamin, G., Glaser, C. B., Uversky, V. N. and Fink, A. L. (2003) 'Certain Metals Trigger Fibrillation of Methionine-oxidized -Synuclein', *Journal of Biological Chemistry*, 278(30), pp. 27630–27635. doi: 10.1074/jbc.M303302200.

Yamin, G., Uversky, V. N. and Fink, A. L. (2003a) 'Nitration inhibits fibrillation of human alpha-synuclein in vitro by formation of soluble oligomers.', *FEBS letters*, 542(1–3), pp. 147–52. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/12729915> (Accessed: 20 April 2017).

Yamin, G., Uversky, V. N. and Fink, A. L. (2003b) 'Nitration inhibits fibrillation of human α -synuclein in vitro by formation of soluble oligomers', *FEBS Letters*, 542(1–3), pp. 147–152. doi: 10.1016/S0014-5793(03)00367-3.

Zarranz, J. J., Alegre, J., Gómez-Esteban, J. C., Lezcano, E., Ros, R., Ampuero,

I., Vidal, L., Hoenicka, J., Rodriguez, O., Atarés, B., Llorens, V., Tortosa, E. G., del Ser, T., Muñoz, D. G. and de Yébenes, J. G. (2004) 'The new mutation, E46K, of α -synuclein causes parkinson and Lewy body dementia', *Annals of Neurology*, 55(2), pp. 164–173. doi: 10.1002/ana.10795.

Zhou, W., Zhu, M., Wilson, M. A., Petsko, G. A. and Fink, A. L. (2006) 'The Oxidation State of DJ-1 Regulates its Chaperone Activity Toward α -Synuclein', *Journal of Molecular Biology*, 356(4), pp. 1036–1048. doi: 10.1016/j.jmb.2005.12.030.

Zhu, M. and Fink, A. L. (2003) 'Lipid Binding Inhibits α -Synuclein Fibril Formation', *Journal of Biological Chemistry*, 278(19), pp. 16873–16877. doi: 10.1074/jbc.M210136200.

Zhu, M., Li, J. and Fink, A. L. (2003) 'The Association of α -Synuclein with Membranes Affects Bilayer Structure, Stability, and Fibril Formation', *Journal of Biological Chemistry*, 278(41), pp. 40186–40197. doi: 10.1074/jbc.M305326200.

Zhu, M., Rajamani, S., Kaylor, J., Han, S., Zhou, F. and Fink, A. L. (2004) 'The Flavonoid Baicalein Inhibits Fibrillation of α -Synuclein and Disaggregates Existing Fibrils', *Journal of Biological Chemistry*, 279(26), pp. 26846–26857. doi: 10.1074/jbc.M403129200.