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Biophysics of Parkinson's Disease: Structure and Aggregation of α-Synuclein

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Abstract

Parkinson's disease (PD) is a slowly progressive movement disorder that results from the loss of dopaminergic neurons in the *substantia nigra*, a small area of cells in the mid-brain. PD is a multifactorial disorder with unknown etiology, in which both genetic and environmental factors play important roles. Substantial evidence links α-synuclein, a small highly conserved presynaptic protein with unknown function, to both familial and sporadic PD. Rare familial cases of PD are associated with missense point mutations in α-synuclein, or with the hyper-expression of the wild type protein due to its gene duplication/triplication. Furthermore, α-synuclein was identified as the major component of amyloid fibrils found in Lewy body and Lewy neurites, the characteristic proteinaceous deposits that are the diagnostic hallmarks of PD. α-Synuclein is abundant in various regions of the brain and has two closely related homologs, β-synuclein and γ-synuclein. When isolated in solution, the protein is intrinsically disordered, but in the presence of lipid surfaces α-synuclein adopts a highly helical structure that is believed to mediate its normal function(s). A number of different conformational states of α-synuclein have been observed. Besides the membrane-bound form, other critical conformations include a partially-folded state that is a key intermediate in aggregation and fibrillation, various oligomeric species, and fibrillar and amorphous aggregates. A number of intrinsic and extrinsic factors that either accelerate or inhibit the rate of α-synuclein aggregation and fibrillation *in vitro* are known. There is a strong correlation between the conformation of α-synuclein (induced by various factors) and its rate of fibrillation. The aggregation process appears to be branched, with one pathway leading to fibrils and another to oligomeric intermediates that may ultimately form amorphous deposits. The molecular basis of Parkinson's disease appears to be tightly coupled to the aggregation of α-synuclein and the factors that affect its conformation. This review focuses on the contributions of Prof. Anthony L. Fink to the field and presents some recent developments in this exciting area.

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1. PARKINSON'S DISEASE AS A TYPICAL SYNUC-LEINOPATHY

Parkinson's disease, PD, is the most common aging-related movement disorder and the second most common neurodegenerative disorder after Alzheimer's disease (AD). It is estimated that ~1.5 million Americans are affected by PD. The probability of sporadic PD development increases with age, with only a small percentage of patients diagnosed before the age of 50 [1]. The prevalence of PD is much greater among those who are at least 65 years old [2]. Approximately 1% of the population at 65–70 years of age is affected by PD, whereas the number of PD patients increases to 4–5% in 85-year-olds [3]. Based on these facts, PD is now considered an aging-related disease.

PD is a slowly progressive malady that affects the neurons in the *substantia nigra*, a small area of cells in the midbrain. Gradual degeneration of these cells causes a reduction in the dopamine content. This, in turn, can produce one or more of the classic signs of PD: resting tremor on one (or both) side(s) of the body; generalized slowness of movement or difficulty initiating movement (bradykinesia/akinesia); stiffness of limbs (rigidity); and gait or balance problems (postural instability). The *substantia nigra* consists of ~400,000 nerve cells, which begin to pigment after birth and are fully pigmented at age 18. The symptoms of PD become apparent after more than ~70% of the dopaminergic neurons die. The "normal" rate of nigral cell loss is ~2,400 per a year. Thus, if an unaffected person lives to be ~120 years old he (she) will probably develop PD. In clinical PD, the neuron loss is accelerated. Epidemiological studies and pathological analyses revealed a mean age of onset of 70 years in sporadic PD [4]. The detailed mechanism of the neuronal death is unknown. It is also unknown why the loss of the nerve cells is accelerated in PD. However, this acceleration seems to be determined by a combination of genetic susceptibility and environmental factors. Sporadic (or idiopathic) forms of this disease account for about 95% of PD patients [4, 5]. In addition to the sporadic form, there are multiple familial forms of the disease linked to mutations in a number of genes. These hereditary forms account for ~4% of PD patients, who develop early-onset disease before the age of 50 [6, 7].

In PD, some surviving nigral dopaminergic neurons contain cytosolic filamentous inclusions known as Lewy bodies (LBs) and Lewy neurites (LNs) [8, 9]. Besides the *substantia nigra,* LBs and LNs also are found in other brain regions, such as the *dorsal motor nucleus* of the vagus, the *nucleus basalis* of Meynert, and the *locus coeruleus* [9]. Abundant LBs and LNs in the cerebral cortex are also neuro-phathological hallmarks of dementia with LBs (DLB), a common late-life dementia that is clinically similar to AD [10], in LB variant of AD [11], diffuse LB disease [12], and multiple system atrophy (MSA) [11, 13]. Structurally, typical LBs appear as intracytoplasmic inclusions, 5–25 μm in diameter with a dense eosinophilic core and clearer surrounding halo. Ultra-structurally, they are composed of a dense core of filamentous and granular material that is surrounded by radially oriented filaments [14].

Several observations implicate α-synuclein in the pathogenesis of PD. A direct involvement of α-synuclein in the neurodegenerative processes in PD was demonstrated by genetic evidence. Autosomal dominant early-onset Parkinson's disease is present in a small number of kindreds as a result of three different missense mutations in the α-synuclein gene, corresponding to A30P, E46K, and A53T substitutions in the α-synuclein protein sequence [15–17], or as a result of the hyper-expression of wild type α-synuclein due to the gene

duplication/triplication [18–20]. Antibodies to α-synuclein detected this protein in LBs and LNs, the hallmark lesions of PD [21]. A substantial portion of fibrillar material in these specific inclusions was shown to be α-synuclein, and insoluble α-synuclein filaments were recovered from purified LBs [21, 22]. The production of wild type α-synuclein in transgenic mice [23] or of WT, A30P, and A53T in transgenic flies [24], led to motor deficits and neuronal inclusions reminiscent of PD. Cells transfected with α-synuclein develop LB-like inclusions under certain conditions. Other important observations correlating α-synuclein and PD pathogenesis, being reviewed in more detail elsewhere [25–32], are briefly outlined below. Numerous studies from different laboratories have established that recombinant α-synuclein easily assembles into amyloid-like fibrils *in vitro* and this process is modulated by familial point mutations. α-Synuclein is abnormally phosphorylated, ubiquitinated, and nitrated in pathology-related inclusions. Co-expression of chaperones or β-synuclein with α-synuclein in transgenic animals suppresses neurodegeneration. α-Synuclein-positive proteinaceous deposits were shown to accumulate in several animal models where Parkinsonism was induced by exposure to different neurotoxicants. Based on all these observations it is clear that PD is a typical synucleinopathy; i.e., a neurode-generative disorder whose pathogenesis is somehow linked to α-synuclein aggregation.

2. α-SYNUCLEIN AND SYNUCLEINOPATHIES

α-Synuclein is involved not only in pathogenesis of PD, but is also commonly associated with a diverse group of neurodegenerative diseases, known as synucleinopathies. These maladies share common pathologic inclusions composed of aggregated α-synuclein, which are deposited in selectively vulnerable neurons and glia [25, 33–35]. The term synucleinopathy was introduced in 1998 after filamentous α-synuclein deposits were found not only in PD, but also in MSA and DLB [36]. Since that discovery, α-synuclein-containing inclusions have been observed in increasing numbers of neurodegenerative diseases. Growing evidence associates the onset and progression of clinical symptoms, as well as the degeneration of affected brain regions, in all these neurodegenerative disorders with the formation of abnormal filamentous aggregates containing α-synuclein. Some of these maladies include: neurodegeneration with brain iron accumulation type 1 (NBIA1), pure autonomic failure, Down's syndrome, complex of Guam, and several Lewy body disorders, such as diffuse Lewy body disease (DLBD), the Lewy body variant of Alzheimer's disease (LBVAD), and PD dementia (PDD) [11–13, 21, 36–40]. All the aforementioned disorders are brain amyloidoses unified by pathological intracellular inclusions containing α-synuclein as a major component [22, 25, 33–36, 40, 41]. Some of the synucleinopathies are briefly outlined below.

2a. Dementia with Lewy Bodies

DLB is the second most frequent late-onset dementia after AD. It exists either in a pure form, or overlaps with the neuropathological features of AD. Neurophathological hallmarks of DLB are numerous α-synuclein-positive LBs and LNs in the *substantia nigra* [21], and in the cortical brain area [42]. In most DLB cases, LBs are frequently associated with typical AD-associated lesions: neurofibrillary tangles and amyloid plaques [43, 44].

2b. PD Dementia

The incidence of dementia in PD is higher than expected from aging alone [45]. In fact, dementia affects ~40% of PD patients [46], and the prevalence of dementia in PD patients is up to six times greater than observed in normal age matched controls [47]. It is believed that the LB diseases are characterized by a clinical continuum [48], from pure PD cases characterized solely by motor dysfunction, to numerous PD patients diagnosed clinically as having PD dementia (PDD), to DLB cases that combine movement dysfunction with

dementia. DLB and PDD, being different in the temporal course of the disease, share most of the same clinical and neuropathologic features and are often considered as belonging to a spectrum of the same disease [49–51].

2c. Amyotrophic Lateral Sclerosis-Parkinsonsim-Dementia Complex of Guam

Guam disease (Guam ALS/PDC) frequently affects the Chamorro people of Guam [52–54] and has been proposed to be caused by seeds of the neurotoxic plant *Cycas circinalis,* commonly used by the Chamorro people as a traditional source of food and medicine [54], although more recent studies have questioned this conclusion [55, 56]. In general it appears that the three neurodegenerative diseases, ALS, dementia, and PD, co-occur within families more often than expected by chance, suggesting that there may be a shared genetic susceptibility to these maladies [57]. As might therefore be expected, α-synuclein pathology has been associated with Guam ALS/PDC [58].

2d. Other LB Diseases

LB deposition can affect several peripheral and central areas of the nervous system, such as the substantia nigra, hypothalamic nuclei, nucleus basalis of Meynert, dorsal raphe, locus ceruleus, dorsal vagus nucleus, and intermedio-lateral nucleus [59]. A ‘neuritic’ form of LB was also described in the dorsal vagus nucleus, sympathetic ganglia, and in intramural autonomic ganglia of the gastrointestinal tract, and cases are also known with extensive cortical and basal ganglia involvement [42, 60]. This broad spectrum of nervous system regions affected by LB deposition produces great variability in disease manifestation [27].

2e. Alzheimer’s Disease

AD is the most common aging-related neurological disorder. It is characterized by slow, progressive memory loss and dementia due to a gradual neurodegeneration in the cortex and hippocampus [61]. The pathological hallmarks of AD are neuronal loss, extracellular senile plaques containing the peptide Aβ, and neurofibrillary tangles composed of a hyperphosphorylated form of the microtubule associated protein tau [62]. α-Synuclein-positive inclusions resembling LBs and LNs were found in ~50% cases of sporadic AD [63]. LB-like intra-cytoplasmic inclusions were found in the amygdala, the temporal cortex, the parahippocampal gyrus, and in the parietal cortex, whereas LN-like inclusions were abundant in the amygdala, the CA2/3 region of hippocampus formation, parahippocampal gyrus, the temporal cortex, substantia nigra, locus ceruleus, the frontal cortex, and in the parietal cortex [63].

2f. Down’s Syndrome

Down’s syndrome is a genetic disorder characterized by an extra chromosome 21 (trisomy 21). Numerous LBs and LNs were found in the neurons of the limbic areas, predominantly of the amygdala, of Down’s syndrome patients with AD pathology [64, 65].

2g. Multiple System Atrophy

MSA is an adult-onset progressive neurodegenerative disorder of unknown etiology. It is characterized by any combination of parkinsonian, autonomic, cerebellar or pyramidal symptoms, and pathologically by the variable neuron loss in the *striatum*, *substantia nigra pars compacta*, *cerebellum*, pons, inferior olives and intermediolateral column of the spinal cord [66]. The histological hallmarks of MSA include argyrophilic fibrillary inclusions in the oligodendrocytes and glial cytoplasmic inclusions (GCIs), which are also known as Papp-Lantos bodies [67]. Fibrillar inclusions are also found in the neuronal somata, axons, and nucleus and neuronal cytoplasmic inclusions are frequently seen in the pontine and

inferior olivary nuclei [68]. A major component of these MSA-specific glial and neuronal inclusions is α-synuclein [36, 68].

2h. Neurodegeneration with Brain Iron Accumulation Type 1

NBIA1, formerly know as Hallervorden-Spatz disease (HSD) or adult neuroaxonal dystrophy, is a rare neurodegenerative malady that occurs in both sporadic and familial forms. Clinically, NBIA 1 is characterized by rigidity, dystonia, dyskinesia, and choreoathetosis [69–72], together with dysarthria, dysphagia, ataxia, and dementia [72–74]. Histopathologically, NBIA1 is characterized by neuronal loss, neuraxonal spheroids, and iron deposition in the *globus pallidus* and *substantia nigra pars compacta*, as well as by the presence of LB-like and GCI-like inclusions and dystrophic neuritis [73]. These LB-like inclusions throughout the cortex and brainstem, axonal swellings, and the rare GCI-like inclusions of the midbrain clearly possess α-synuclein immunoreactivity [75–77]. Importantly, axonal spheroids were also shown to contain α-synuclein [77, 78].

3. STRUCTURAL PROPERTIES AND CONFORMATIONAL BEHAVIOR OF α-SYNUCLEIN

α-Synuclein is an abundant brain protein of 140 residues, lacking both cysteine and tryptophan residues. This protein is present in high concentration at presynaptic terminals and is found in both soluble and membrane-associated fractions of the brain. α-Synuclein was estimated to account for as much as 1% of the total protein in soluble cytosolic brain fractions [79]. Several possible functions have been suggested, including synaptic vesicle release and trafficking, fatty acid binding and physiological regulation of certain enzymes, transporters, and neurotransmitter vesicles, as well as roles in neuronal survival [29]. α-Synuclein was shown to physically interact with at least 50 ligands including >30 proteins [29, 31, 80]. Recently, a proteomic analysis identified 324 α-synuclein-interacting proteins in the dopaminergic cells of the MES cell line. Almost half of these interactors (141 proteins) experienced a significant change in their relative abundance (increase or decrease) after the MES cell were treated with rotenone [81], a pesticide that produces parkinsonism in animals and induces LB-like inclusions in the remaining dopaminergic neurons [82]. The involvement of α-synuclein in the control of the neuronal apoptotic response and in the protection of neurons from various apoptotic stimuli was demonstrated [83].

At the level of amino acid sequence, the structure of α-synuclein can be divided into three regions: residues 1–60, which contain four 11-amino acid imperfect repeats (coding for amphipathic helices) with a conserved motif (KTKEGV); residues 61–95, which contain the highly amyloidogenic NAC region and two additional repeats; and the highly charged C-terminal region, residues 96–140. The first two regions comprise a lipid-binding domain, whereas the C-terminal tail is thought to be a protein-protein interaction motif.

In 1996, Weinreb *et al.* [84] showed that when isolated in solution, α-synuclein (known at that time as NACP for the “non-Aβ component of Alzheimer’s disease amyloid plaque” (NAC) precursor protein) had a much larger Stokes radius (34 Å) but sedimented more slowly ($s_{20,w}$ = 1.7S) than globular proteins of similar molecular weight suggesting that it is elongated. According to circular dichroism (CD) in the far-UV region and Fourier-transform infrared spectroscopy (FTIR), NACP/α-synuclein was shown to be devoid of significant amounts of secondary structure. The structure of this protein was practically unaffected by boiling, pH, salt, and chemical denaturants and was independent of protein concentration. All this indicated that NACP/α-synuclein likely belongs to a class of proteins referred to as natively unfolded proteins [84].

3a. Basic Structural Properties of α-Synuclein

Subsequent studies supported this conclusion and added some new features to the structural description of this protein. Fig. (1) presents some of the data obtained in the laboratory of Prof. Anthony L. Fink (Tony) by the middle of 2000 [85]. At neutral pH, α-synuclein possessed far-UV CD (Fig. (1A)) and FTIR (amide I region, Fig. (1B)) spectra typical of an unfolded polypeptide chain. In fact, the far-UV CD spectrum was characterized by a minimum in the vicinity of 196 nm and the absence of bands in the 210–230 nm region, whereas a broad peak at 1650 cm^{-1} in FTIR spectrum suggested that the majority of the molecule (~70%) was disordered [85]. The hydrodynamic properties of α-synuclein analyzed by size exclusion chromatography and small-angle X-ray scattering (SAXS) were in agreement with the results of far-UV CD and FTIR studies. α-Synuclein was shown to be slightly more compact than expected for a random coil [85, 86], as the Stokes radius measured for α-synuclein by size-exclusion chromatography was notably lower than that calculated for a completely unfolded polypeptide chain of the appropriate molecular mass. NMR diffusion measurements confirmed these results as well [87] and R_g values calculated for α-synuclein at neutral pH from SAXS data using the Guinier approximation (40±1 Å, Fig. (1C)) were also significantly smaller than those estimated for a random coil polypeptide of the same length (52 Å) [85]. The analysis of SAXS data in the form of Kratky plots provides information on the globularity (packing density) and the conformation of a polymer molecule, being able to distinguish between a compact globular conformation and a chain-like extended conformation [88–98]. The scattering curve for a globular conformation follows Porod's law — $I(Q) \infty Q^{-4}$ — at large values of Q and the scattering intensity from the expanded chain molecule is proportional to Q^{-2} in the moderate Q region and to Q^{-1} in the high Q region. Thus, the Kratky plot for a globular conformation shows a clear peak, whereas the plot for a chain-like conformation has a plateau and then rises monotonically [95]. Fig. (1D) shows that the profile of the Kratky plot of α-synuclein at neutral pH was typical for a random coil conformation. Thus, at neutral pH α-synuclein was shown to be essentially disordered, but slightly more compact than a random coil [85]. The compactness of α-synuclein was also later confirmed by NMR studies, which posited [99] and subsequently demonstrated [100–102] the presence of transient long-range contacts within the protein. Thus, α-synuclein definitely belongs to the family of intrinsically disordered proteins, and more specifically to the subfamily of its most disordered members, known as natively unfolded proteins, which are characterized by a unique combination of low overall hydrophobicity and high net charge [103–107].

3b. Structure-Forming Effects of pH and Temperature

At the logical next step, the effects of basic environmental factors on structural properties of α-synuclein were evaluated in Tony's lab. This was a fundamental question at the time, as very little was known about the conformational behavior of structureless proteins. An educated guess, based on the assumption that the unfolded nature of α-synuclein is determined by low overall hydrophobicity and high net charge, posited that any alterations in the protein environment leading to an increase in hydrophobicity and/or decrease in net charge should be accompanied by at least partial folding of the intrinsically disordered protein. At a basic level, these two parameters, charge and hydrophobicity, can be modulated via changes in the protein environment: the excess negative charge of α-synuclein at neutral pH (pI = 4.7) would be neutralized at low pH values, and the overall hydrophobicity of a protein will increase with increasing temperature. Therefore, the protein was expected to partially fold under conditions of low pH or high temperature [85].

Data presented in Fig. (1) support this hypothesis. According to Fig. (1A–1D), α-synuclein became more ordered at pH 3.0 or at high temperature. The protein clearly gained some ordered secondary structure (Fig. (1A) and (1B)), became a bit more compact (Fig. (1C),

and developed the beginnings of a tightly packed core (Fig. (1D)). This meant that at acidic pH natively unfolded α-synuclein was transformed into a partially folded conformation with a significant amount of β-structure [85]. Furthermore, Fig. (1E) shows that a decrease in pH led to a noticeable blue shift of the ANS fluorescence maximum (from ~515 to ~475 nm, open triangles in Fig. (1C)) and that the pH-induced structural transitions monitored by ANS fluorescence and CD occur simultaneously in a co-operative manner and were completely reversible, excluding aggregation. This meant that protonation of α-synuclein resulted in the transformation of this natively unfolded protein into a partially folded conformation with a significant amount of ordered secondary structure, some compactness, the beginnings of a tightly packed core, and a high affinity for ANS [85].

Comparable results were obtained for the protein at high temperatures (Fig. (1)). The temperature-dependence of $[\theta]_{222}$ shown in Fig. (1F) revealed that an increase in temperature was also sufficient to induce the reversible formation of some ordered secondary structure in α-synuclein [85]. The major spectral changes occurred over the range of 3 to 50°C, with subsequent heating leading to a less pronounced effect. Analyses of these results indicated that high temperatures induced a reversible transition of α-synuclein into a partially folded intermediate with spectral properties similar to those of the species found at low pH [85].

Taken together, the results of this study indicated that α-synuclein, while being highly unstructured at neutral pH, is nevertheless not a random coil and has some residual structure [85]. Either a decrease in pH, or an increase in temperature is sufficient to transform α-synuclein into a partially folded pre-molten globule-like conformation. The structure-forming effects of low pH were attributed to the effective minimization of the high net charge of this protein. This net charge minimization decreased intramolecular charge-charge repulsion and therefore promoted the hydrophobic-driven collapse to a partially folded intermediate. The effect of elevated temperatures was attributed to the increased strength of the hydrophobic effect at higher temperatures, leading to a stronger hydrophobic driving force for folding [85].

3c. Effect of Other Factors on α-Synuclein Structure

Detailed analysis using a combination of low resolution techniques, such as CD, FTIR, fluorescence, and several hydrodynamic approaches [108–117], revealed that the PD-related point mutations A30P, E46K, and A53T do not affect the overall structure of human α-synuclein, which remains natively unfolded [108, 116, 117]. However, high resolution solution NMR spectroscopy revealed that the A30P mutation strongly attenuates the helical propensity found in the N-terminal region of wild type α-synuclein [99, 126]. The A53T mutation was found to exert a more modest influence on local structural propensity, resulting in a slightly enhanced preference for extended conformations in a small region around the site of mutation [99]. The E46K mutation results in subtle changes in the conformation of the monomeric protein [118]. These mutations were also proposed to modify long-range transient structure in α-synuclein [119] although this conclusion remains controversial [102]

Human α-synuclein was shown to bind specifically to synthetic vesicles containing acidic phospholipids [120, 121]. This binding was accompanied by a dramatic increase in α-helical content [120, 121] and was attributed to the formation of two curved antiparallel but non-contacting α-helices (Val3-Val37 and Lys45-Thr92) connected by a well ordered, extended linker [122-125], whereas the acidic, glutamate-rich C-terminal region (Asp98-Ala140) was shown to behave as a highly mobile tail; i.e., it remained unstructured even in the presence of membranes [125–127]. More recently, the helical structure of α-synuclein was shown to be dependent on the size of the lipid particle it is bound to [128] with the two helices able to

fuse into a single long helix [129], and this transition has been proposed to be functionally important [130].

In general, the conformation of α-synuclein is highly pliable and sensitive to modification by numerous environmental factors. Although in the majority of cases such factors were shown to induce partial folding, the non-compact natively unfolded conformation was effectively stabilized via methionine oxidation [131–133]. In addition to low pH and high temperature [85], a pre-molten globule-like partially folded conformation was stabilized in α-synuclein by low concentrations of organic solvents [134] and TMAO [135], by different metal ions [136], salts [137], pesticides/herbicides [138–140], heparin and other glycosoaminoglycans [132], some polycations [141], or by spontaneous oligomerization both *in vitro* and *in vivo* [142]. The addition of high concentrations of different alcohols dramatically increased the degree of folding of α-synuclein [134], with simple alcohols inducing a β-sheet-enriched conformation and fluorinated alcohols promoting α-helix-rich species [134]. Alcohol-induced α-helical and β-structural species were initially monomeric but readily underwent association over longer time scales [134]. High concentrations of TMAO induced α-synuclein to form oligomeric α-helical globular species with rigid tertiary structure [135].

Prolonged incubation of α-synuclein at elevated temperatures results in a progressive aggregation, with pre-molten globule-like dimers formed first [142]. Under conditions of oxidative stress, the formation of oxidative dimers with dityrosine cross-links was also reported [143]. Besides covalent and non-covalent dimers, α-synuclein is able to form morphologically different soluble oligomers. For example, nitrated α-synuclein assembles into oligomeric spheroids [144]. The incubation of α-synuclein with different metals for 24 hours at 4° gave rise to three different classes of oligomers: Cu^{2+}, Fe^{3+} and Ni^{2+} yielded 0.8–4 nm spherical particles, similar to α-synuclein incubated without metal ions; Mg^{2+}, Cd^{2+} and Zn^{2+} gave larger, 5–8 nm spherical oligomers; Co^{2+} and Co^{2+} produced annular (doughnut-like) oligomers, 70–90 nm in diameter with Ca^{2+} and 22–30 nm in diameter with Co^{2+} [145]. Finally, under appropriate conditions, α-synuclein was shown to form amorphous aggregates and fibrils (see Fig. (2)), with the appearance of each type of insoluble aggregate being determined by the environmental conditions. Based on this astonishing conformational behavior, the concept of a protein-chameleon was proposed, according to which the structure of α-synuclein is mainly determined by the peculiarities of its environment [146].

4. AGGREGATION OF α-SYNUCLEIN: MOLECULAR MECHANISMS OF FATAL ATTRACTION

4a. Critical Amyloidogenic Partially Folded Intermediate

The formation of amyloid fibrils, amorphous aggregates or various oligomers induces different degrees of α-synuclein folding. Early stages of fibril formation by α-synuclein involve the formation of a pre-molten globule-like partially folded intermediate that is critical for α-synuclein fibrillation [85]. Fig. (1) shows that a decrease in pH or an increase in temperature forced α-synuclein to gain some ordered structure. As these same conditions led to the dramatic acceleration of α-synuclein fibrillation, it has been concluded that the pre-molten globule-like partially structured conformation is a key intermediate in the fibril-forming pathway [85]. This suggests a simple kinetic model of fibrillation involving conversion of monomeric α-synuclein into the critical partially folded intermediate, which leads to formation of the amyloidogenic nucleus and subsequent propogation into fibrils. Such intermediates typically have sizable nonpolar patches (i.e., spatial clusters of hydrophobic side chains) on their surface, which lead to hydrophobic interactions between

molecules, resulting in intermolecular interactions and aggregation. These hydrophobic patches are absent in the natively unfolded monomeric α-synuclein. Factors that increase the concentration of such intermediates will favor aggregation [147]. This model has strong predictive power, as all intrinsic or extrinsic factors shifting the equilibrium in favor of this monomeric partially folded conformation are expected to facilitate the fibrillation process. As α-synuclein is a natively unfolded protein whose structure is extremely sensitive to its environment, such folding-promoting factors seem to be wide-ranging. Obvious instances include point mutations, non-polar molecules including some pesticides (that might preferentially bind to the partially-folded intermediate), cations (that might mimic the effect of low pH/high proton concentration), as well as factors that result in an increase in the concentration of α-synuclein itself or cause modifications of the protein (e.g., via oxidative damage, etc.) (reviewed in [31, 32, 146, 147]). Consistent with this model, the rate of α-synuclein fibril formation *in vitro* was shown to be highly dependent on the conditions used. *In vitro* α-synuclein fibril formation kinetics are described by a typical sigmoid curve that shows an initial lag phase followed by an exponential growth phase and a final plateau, usually attributed to a nucleation-dependent polymerization. Agitation is a strong accelerator of the fibril formation process: typically fibrillation of 70 FM (1 mg/mL) α-synuclein is completed within 3 days with agitation, whereas without agitation it takes months [147]. Many endogenous and exogenous factors were shown to increase the fibrillation rates. This acceleration can be attributed to the increased concentration of the amyloidogenic intermediate caused by the various conditions. Certain environmental factors are also known to inhibit fibril formation by α-synuclein. This inhibition of fibrillation occurs when either the monomer or non-fibrillogenic oligomers are stabilized [31, 32, 146, 147]. Some of these rate-modulating conditions are considered below.

4b. Genetic Factors

There are two major genetic abnormalities that directly link α-synuclein with neurodegeneration in familial early onset PD: overexpression of α-synuclein due to duplication or triplication of the α-synuclein gene locus [18–20] and missense mutations in the α-synuclein gene, corresponding to A30P, E46K, and A53T substitutions in α-synuclein protein [15–17]. Even in the absence of additional factors, α-synuclein exists in an equilibrium between the natively unfolded and the partially folded conformations. Although the overall probability for intermediate formation is normally low, increased protein concentrations will necessarily raise the total concentration of amyloidogenic intermediates, thus increasing accordingly the rate of fibril formation. In agreement with this argument, increases in α-synuclein concentration have been shown to result in decreased lag times and increased fibril elongation rates *in vitro* [85]. This observation is consistent with and may underlie the fact that autosomal-dominant PD (average age of onset, 34 years), ranging clinically from DLB to typical PD, is associated with the duplication/triplication of α-synuclein locus, which potentially enhances the level of α-synuclein production [18–20].

Studies of several kindreds with familial PD clearly show that a single mutation in the human α-synuclein gene is sufficient to cause PD. Importantly, all three PD-related point mutations, A30P, E49K, and A53T, were shown to accelerate α-synuclein aggregation (but not necessarily fibrillation) *in vitro* [108, 109, 111, 112, 114–117, 148]. The absence of major structural differences (at least those detectable by low resolution techniques, such as CD, FTIR, fluorescence, and hydrodynamics) between groups of natively unfolded and partially folded conformations of all these α-synuclein variants raised the question of how the mutations affect the aggregation propensity of the protein. Analysis of hydrophobicity and propensities to form β-sheet or α-helix for WT, A30P and A53T revealed that both mutations reduced hydrophobicity in the vicinity of the substitution, but the propensity to form α-helical structure was somewhat diminished in the N-terminal region of both mutants,

whereas the predisposition to form β-structure was predicted to be slightly enhanced [116]. In agreement with these observations, high-resolution solution NMR analysis established that the A30P mutation disrupts a region of residual helical structure that exists in the wild type protein [126], whereas the A53T mutation results in a slight enhancement of a preference for extended conformation in a small region around the mutation site [99]. All this suggests that the increased internal susceptibility of A30P and A53T to form β-sheets may not be strong enough to alter the structure of the monomeric proteins, but may affect the aggregation behavior of the α-synuclein mutants through specific stabilization of an intermolecular β-structure [116]. As a result, the mutants show a faster rate of fibrillation (A53T) or amorphous aggregation (A30P).

As far as the E46K mutation is concerned, it is located in the fourth KTKEGV-type repeat in the amino-terminal region of α-synuclein. It has been emphasized that a Glu residue similar to E46 is present in five of the seven degenerative repeats in α-synuclein, and the only repeat that does not have such a residue (repeat 2) has Glu residues adjacent to each side of the repeat [117]. Based on these observations it has been suggested that the N-terminal region of α-synuclein and, more specifically, Glu residues in the repeats may be important in regulating the ability of α-synuclein to polymerize into amyloid fibrils [117].

4c. Molecular Crowding

The natural environment of any protein inside a living cell is extremely crowded, as the concentration of macromolecules, including proteins, nucleic acids, carbohydrates, and small solutes within a living cell is estimated to be as high as 400 g/L [149]. The volume occupied by solutes is unavailable to other molecules, giving rise to a phenomenon known as “excluded volume effects” [149, 150]. It has been suggested that volume exclusion in physiological media could modulate the rate and the extent of the amyloid formation *in vivo* [151]. The validity of this hypothesis was confirmed for the *in vitro* fibrillation of human α-synuclein [152, 153]. Pesticides and metals, which are linked to increased risk of PD via the epidemiological studies (see below), were also shown to further accelerate α-synuclein fibrillation when present under conditions of molecular crowding [154–156].

4d. Anions and Salts

Partial folding of α-synuclein at neutral pH to the potentially amyloidogenic intermediate can be induced by various anions. However, the magnitude of the fibrillation accelerating effect varied significantly between anions and generally followed the position of the anions in the Hofmeister series, suggesting that the major role of anions in fibrillation is their modulation of protein-water interactions [154]. Based on these observations it has been concluded that the enhanced fibrillation of α-synuclein in the presence of anions is the result of partial folding induced by the loss of uncompensated charge and an increase in preferential hydration, which promotes partial folding and aggregation by strengthening hydrophobic interactions [154].

4e. Polyanions and Polycations

In several human diseases, glycosaminoglycans (GAGs) and proteoglycans (PGs) are involved in the formation of proteinaceous deposits [157, 158]. Different GAGs (heparin, heparan sulfate) and other highly sulfated polymers (dextran sulfate) as well as agrin (an extracellular matrix and trans-membrane heparan sulfate proteoglycan in the central neuronal system [159–161]) were shown to bind to α-synuclein and stimulate its fibrillation *in vitro* [132, 162]. Agrin and α-synuclein were shown to be co-localized in LBs and LNs found in the *substantia nigra* of PD patients, indicating that this PG may contribute to the etiology of PD by modulating the aggregation state of α-synuclein in dopaminergic neurons [162]. Similarly, several unstructured polycations such as polyethyleneimine, spermine,

spermidine, polyLys, and polyArg were shown to interact with α-synuclein, to induce partial folding of this protein and to accelerate its oligomerization and fibrillation [141, 163].

4f. Environmental PD Risk Factors

PD is now considered as an "environmental" disease, since several lines of evidence point to environmental exposures as potential contributing factors in the pathogenesis of this malady [164–170]

4f.i. Pesticides and Herbicides—In a search for a potential explanation of the increased risk of PD associated with exposure to pesticides, the *in vitro* effect of several commonly used pesticides and herbicides on the structure and aggregation of human α-synuclein was analyzed. These studies revealed that several pesticides and herbicides induced a conformational change in α-synuclein and significantly accelerated its fibrillation [138–140]. These structure-forming and fibrillation-accelerating effects of hydrophobic pesticides were ascribed to their ability to bind and stabilize the amyloidogenic partially folded conformation [138, 139]. Importantly, a noticeable up-regulation of α-synuclein production was induced by the administration of paraquat to mice, which also led to the accumulation of α-synuclein containing fibrilar aggregates within the neurons of the *substantia nigra* [140]. These observations provide a rationale for using both *in vitro* and *in vivo* approaches for the screening of putative neurotoxicants, which, by affecting α-synuclein conformation and aggregation, may play a role in the pathogenesis of synucleinopathies [140].

4f.ii. Heavy Metals—Exposure to heavy metals is another potential PD risk factor [171–178]. Motivated by the results of epidemiological studies and postmortem analyses of the brain tissues of PD patients, Tony's *in vitro* analysis established that a number of mono-, di-, and trivalent metal ions are able to accelerate the α-synuclein fibrillation process [136]. The effectiveness of metal cations in inducing fibrillation was shown to correlate with increasing ion charge density and with their ability to induce amyloidogenic partially folded species due to the masking of intramolecular Coulombic charge-charge repulsion in the natively unfolded α-synuclein molecule [136, 179].

4f.iii. Organic Solvents—Because of the increased incidence of PD resulting from the exposure to organic solvents [180–184], the structural properties and aggregation/fibrillation propensities of α-synuclein in water-organic solvent mixtures were also analyzed [134]. At low concentrations, all organic solvents studied effectively induced the amyloidogenic partially folded conformation and favored very rapid α-synuclein fibrillation [134].

4f.iv. Oxidative Stress: Aggregation Enhancers—Oxidative injury has been implicated in the pathogenesis of several disorders, such as AD [185, 186], PD [187], DLB [188], amyotrophic lateral sclerosis [189], Huntington's disease [190], atherosclerosis and inflammatory diseases [191], chronic renal failure [192], cataractogenesis [193], and brain ischemia and carcinogenesis [194], and oxidatively modified proteins are known to accumulate during normal aging [195–197]. As α-synuclein does not have cysteines and tryptophanes, the primary targets for oxidative modifications are its methionine and tyrosine residues. Under mild oxidative conditions (1–2% H_2O_2), all four methionines of α-synuclein can be oxidized to the methionine sulfoxide, MetO [198]. Methionine oxidized α-synuclein was found to be more highly unfolded than the non-oxidized protein [131, 133, 198], less prone to oligomerize and aggregate, and even able to inhibit the fibrillation of non-modified α-synuclein [198]. The inhibition α-synuclein fibrillation by methionine oxidation was shown to be proportional to the number of oxidized methionines [199]. The presence of certain metals (Ti^{3+}, Zn^{2+}, Al^{3+} and Pb^{2+}) could overcome this inhibition [131]. This suggests that the balance between the protective anti-oxidant role of the methionine residues

and the protective anti-fibrillation effect of oxidized methionine residues in α-synuclein may fail under conditions of industrial pollution due to exposure to lead, aluminum, zinc, titanium, and other metals. In the presence of enhanced concentrations of such industrial pollutants, toxic insult-induced up-regulation of α-synuclein no longer plays a protective role; rather, it may represent a new risk factor, leading to the effective metal-triggered fibrillation of the methionine-oxidized protein [131].

4f.v. Oxidative Stress: Aggregation Inhibitors—All PD risk factors considered thus far are strong accelerators of α-synuclein fibrillation. Although for a long time it was believed that amyloid fibrils themselves were harmful, a novel emerging paradigm favors the idea that the deposited proteinaceous inclusions (such as senile plaques in AD or LBs and LNs in PD, etc.) are not cytotoxic [200] and even may be protective [201] favoring cell survival. As a result, an alternate amyloid hypothesis, the oligomer hypothesis, was proposed which claims that although mature amyloid fibrils may not be toxic, some species formed during the fibril assembly process may be responsible for cell damage. As fibrillation is known to involve oligomeric species, the formation of some small oligomers, known as protofibrils, was proposed to be responsible for neurotoxicity [202–209]. One of the potential mechanisms of this cytotoxicity is the ability of oligomeric intermediates populated during the conversion of proteins from a monomeric state into amyloid fibrils to permeabilize lipid bilayers and cell membranes [204–206, 208, 209]. Paragraphs below consider several PD risk factors potentially linked to the formation of such toxic oligomers.

One of the most frequent oxidative modifications of tyrosines is their nitration [210–212]. The selective and specific nitration of α-synuclein in PD and other synucleinopaties has been proposed to directly link oxidative and nitrative damage to the onset and progression of these maladies [213]. Nitration of α-synuclein *in vitro* was shown to be accompanied by partial folding and oligomerization of this protein [144, 214]. The formation of these soluble oligomers completely inhibited the fibrillation of nitrotyrosinated α-synuclein at neutral pH, and the addition of nitrated α-synuclein substantially inhibited the fibrillation of the non-modified protein in a concentration-dependent manner [144, 214].

In addition to various protein oxidative modifications, lipid oxidation is frequently induced as a result of the oxidative stress. One of the most crucial products of lipid oxidation is 4-hydroxy-2-nonenal (HNE), which has been implicated in PD pathogenesis. Recently, it has been shown that the co-incubation of HNE with α-synuclein led to covalent modification of the protein, with up to six HNE molecules incorporated as Michael addition products [215]. Highly increased β-sheet content was found by CD and FTIR in the HNE-modified α-synuclein, whereas fibrillation of this protein was inhibited in an HNE concentration-dependent manner. This inhibition was caused by the formation of soluble oligomers, which were compact and tightly packed according to SAXS analysis. The HNE-induced oligomers were very stable and their addition to primary mesencephalic cultures caused marked neurotoxicity [215].

4g. Protein-Protein Interactions

α-Synuclein is known to interact with various proteins (see above). Such interactions might have a significant influence on the aggregation and fibrillation of this protein. Therefore, several illustrative examples were studied in Tony's lab. For example, the addition of either β- or γ-synuclein (two natively unfolded members of the synuclein family [86, 102]) inhibited α-synuclein fibrillation in a concentration-dependent manner [86]. As α-synuclein effectively inhibited α-synuclein aggregation in animal models [216], it has been suggested that β- and γ-synucleins may act as regulators of α-synuclein fibrillation *in vivo*, potentially acting as chaperones [86].

Later, core histones were shown to physically interact with α-synuclein and to accelerate α-synuclein fibrillation [163]. It also has been shown that paraquat administration triggers an upregulation of α-synuclein in the mouse brain [140] and is accompanied by α-synuclein translocation into the nucleus [163]. Nuclear translocation of α-synuclein and the formation of histone-α-synuclein complexes was suggested to provide a mechanism by which α-synuclein-related neuronal response may be activated or sustained. In this model, α-synuclein-histone complexes have a regulatory role by decreasing the pool of free histones available for DNA binding. The subsequent destabilization of nucleosomes and enhanced manifestation of the DNA matrix activity could lead to increased transcription, and ultimately to the production of proteins in response to a variety of stimuli, including toxic insults [163].

Another α-synuclein-binding protein analyzed in Tony's lab was DJ-1 [217], a multifunctional protein known to prevent protein aggregation and to serve as a molecular chaperone [218]. The fibrillation inhibition efficiency of DJ-1 was shown to be directly related to its oxidation state: native (unoxidized) DJ-1 did not interact with α-synuclein and did not inhibit its fibrillation, whereas being oxidized via the sulfinic acid of Cys106 formation, DJ-1 very effectively prevented α-synuclein fibrillation *in vitro* [217].

4h. Interaction with Membranes

Several studies have demonstrated α-synuclein's association with lipids [79, 121, 219–225]. In fact, this protein is found both in the cytosol and in association with membranes in the presynaptic region of neurons. The membrane-bound state of α-synuclein has also been suggested to play an important role in fibril formation [226]. The N-terminal region (approximately residues 1–95) of α-synuclein contains six 11-amino acid imperfect repeats with a highly conservative hexamer motif (KTKEGV), resulting in a variation in hydrophobicity with a strictly conserved periodicity of 11 residues [122, 124, 127]. Such a periodicity is characteristic of the amphipathic lipid-binding α-helical domains of apolipoproteins [223, 227]. In 2003, the interaction of α-synuclein with lipid vesicles of different composition and sizes and the effects of these interactions on the protein conformation and the fibrillation kinetics were analyzed [228]. Different factors, such as the phospholipid composition, the size of the vesicles, and the ratio of α-synuclein to phospholipid, were shown to play a role in modulation of α-synuclein-vesicle interaction. High lipid concentrations induced α-helical structure in α-synuclein and inhibited its fibrillation. At relatively low lipid concentrations, acidic phospholipids were shown to induce the partial folding of α-synuclein and to accelerate its fibrillation. A strong correlation was observed between the induction of α-helix in α-synuclein and the inhibition of fibril formation. This suggested that α-helical, membrane-bound α-synuclein is unlikely to contribute to aggregation and fibrillation [228].

Of great importance was Tony's related finding that α-synuclein-membrane interactions affect both protein and membrane [229]. The association of the protein with the bi-layer led to disruption of the membrane, and the kinetics of α-synuclein fibril formation were significantly affected by the protein association and subsequent membrane disruption. The ability of α-synuclein to disrupt membranes was shown to be correlated with the protein's binding affinity and the protein's α-helicity. In these experiments, protofibrillar α-synuclein caused a much more rapid destruction of the membrane than soluble monomeric protein, clearly showing that protofibrils (oligomers) are likely to be significantly neurotoxic [229].

It has recently been reported that α-synuclein can be associated with lipid rafts and caveolae [230–232]. Caveolae are specialized neuronal membrane domains that are enriched in the caveolin family of proteins and sphingolipids, mostly gangliosides and sphingomyelin. Lipid rafts are specialized plasma membrane microdomains that are enriched in cholesterol and

sphingolipids, with similar lipid composition to caveolae. GM1 ganglioside is a molecular marker for these regions and is specifically enriched in the caveolae. Interaction of α-synuclein with ganglioside GM1-containing small unilamellar vesicles (SUVs,) induced substantial α-helical structure in this protein and inhibited or completely eliminated α-synuclein fibril formation, depending on the amount of GM1 present [233]. The interaction of α-synuclein with GM1-containing SUVs was accompanied by α-synuclein oligomer formation. The familial A53T mutation had no effect on the interaction between α-synuclein and GM1-containing SUVs, whereas the A30P mutation dramatically inhibited this interaction [233]. This GM1-modulated recruitment of α-synuclein to caveolae and lipid rafts could explain the preferential localization of this protein to presynaptic membranes [233].

4i. Small Molecules

α-Synuclein aggregation and dopamine are obviously brought together *in vivo*, as the common pathway for both idiopathic and familial PD is the damage and subsequent loss of dopaminergic neurons accompanied by the formation of α-synuclein containing LBs and LNs in the *substantia nigra pars compacta* [234]. Several catecholamines, including L-dopa and dopamine, were shown to inhibit α-synuclein fibrillation and even to dissolve the preformed fibrils *in vitro* [235]. The oxidation products derived from these catecholamines were more potent inhibitors of the α-synuclein fibrillation than the non-oxidized catecholamines [235, 236].

According to epidemiological studies, leprosy patients have a significantly lower probability of developing AD if they had been undergoing antileprosy treatment with rifampicin and closely related drugs for the preceding several years [237–239]. Therefore, it has been suggested that these antileprosy drugs might inhibit Aβ aggregation and prevent amyloid deposition [240]. In fact, rifampicin and its analogs were shown to inhibit Aβ aggregation and neurotoxicity *in vitro* [240–242]. Similarly, rifampicin eliminated α-synuclein fibrillation *in vitro* [243]. Furthermore, it is was able to disaggregate preformed α-synuclein fibrils in a concentration-dependent manner and led to the formation of soluble oligomers comprised of partially folded α-synuclein [243].

The flavonoid baicalein is the main component of the traditional Chinese herbal medicine *Scutellaria baicalensis*, also known as Chinese skullcap or Huang Qin, which has multiple biological activities including antiallergic, anticarcinogenic, antiviral, antibacterial, anti-inflammatory, and anti-HIV properties [114, 244–247]. Micromolar concentrations of baicalein or its oxidized forms were shown to inhibit the formation of α-synuclein fibrils, and to disaggregate pre-formed fibrils *in vitro* giving rise to soluble oligomers [248]. Recently, the structural properties of the baicalein-stabilized α-synuclein oligomers, purified by SEC-HPLC, and their conformational stability was analyzed using a set of biophysical techniques such as CD, FTIR, SEC-HPLC, SAXS, EM, and AFM [249]. This analysis revealed that the baicalein-stabilized oligomers possess very specific structural features, being spherical in shape (according to the AFM and EM data), having relatively globular structure with packing density intermediate between that of pre-molten globules and typical globular proteins (according to the Kratky plot analysis of the SAXS data), and having relatively well-developed secondary structure (according to the FTIR and far-UV CD analysis). These oligomers were characterized by high thermodynamic stability (as evidenced by the results of the unfolding analyses and by the fact that their shape did not change after the prolonged incubation) and were able to inhibit fibrillation of non-baicalein-treated α-synuclein. The most important finding was the fact that these highly stable and fibrillation-inhibiting oligomers did not disrupt the integrity of biological membrane (at least they are not more disruptive than the monomeric protein) [249]. This important observation demonstrated for the first time that formation of soluble oligomers is not always harmful and

can be beneficial. These findings have paved the road for the development of novel therapies for PD, whose molecular mechanism of action could be similar to that of baicalein – specific stabilization of the thermodynamically stable, non-fibrillating soluble oligomers that can inhibit α-synuclein fibrillation and do not cause the inappropriate membrane permealization [249].

4j. Smoking and PD

Interestingly, there is an apparent negative association between smoking or caffeine intake and PD [250, 251] (the risk of PD in nonsmokers is about twice that of smokers, cigarette smokers are about 50% less likely to have PD and patients with PD are about 50% less likely to have smoked cigarettes during their lifetime [252, 253]). This association is again correlated with *in vitro* studies, where the presence of nicotine and caffeine was shown to have no accelerating effect on the kinetics of α-synuclein fibrillation [139]. Recently, more detailed analysis of the fibrillation of α-synuclein in the presence of five different compounds found in cigarette smoke: anabasine, cotinine, hydroquinone, nicotine and nornicotine, revealed that nicotine and hydroquinone effectively inhibited α-synuclein fibrillation and stabilized soluble oligomeric forms [254]. In fact, instead of insoluble amyloid-like fibrils, three stable soluble oligomeric forms were formed, which according to AFM imaging were mostly spherical species with heights of ~16 nm, ~10 nm, and ~4 nm. Furthermore, it was shown that nicotine and hydroquinone successfully inhibited A53T fibrillation as well [254]. The fact that nicotine and hydroquinone effectively inhibited α-synuclein fibrillation and stabilized soluble oligomeric forms, combined with the results of epidemiological studies showing that smoking and PD incidence are negatively correlated, suggested that the nicotine- or hydroquinone-stabilized α-synuclein oligomers might be similar to those stabilized by baicalein (see above). These studies supported the hypothesis that protein aggregates (including oligomers) are highly heterogeneous. This heterogeneity might be caused either by heterogeneous starting materials or by multiple pathways of assembly, or by both these factors. Therefore, it is unlikely that all soluble oligomers, considering their astonishing morphological variability, will be cytotoxic to the same degree [254].

V. CONCLUDING REMARKS

The contributions of Tony Fink to understanding the structure of α-synuclein and the molecular mechanisms of its aggregation are truly remarkable. His research in this area was extremely productive: out of 1216 ISI Web of Knowledge entries dealing with α-synuclein aggregation, 44 papers were from Tony's lab. On average, each of his 44 papers was cited 56 times, which is almost twice as high as the average citation rate of the remaining 1172 papers. As of January 7, 2009, his first paper on the structure and fibrillation of human α-synuclein [85], being cited 230 time, occupies position 18 among the 1216 papers on α-synuclein aggregation. Out of 189 highly cited α-synuclein-related papers (i.e., papers that are cited 50 or more times), 18 are from his lab. In other words, 40.9% of Tony's papers are highly cited, whereas this parameter is equal to 14.6% for all other papers in this field. These numbers provide a formal basis for our opinion that Tony's research on α-synuclein structure and aggregation was very productive and his work exerted a significant impact on this field. This review includes an overview of some of his papers, covering a very broad range of topics, from meticulous descriptions of α-synuclein structure and conformational behavior, to elucidation of the molecular mechanisms of α-synuclein aggregation and fibrillation *in vitro*, to detailed evaluation of the various PD risk factors in the simplest *in vitro* model of the highly complex syndrome known as PD, i.e., in physiological solutions containing purified α-synuclein.

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ABBREVIATIONS USED

AD	Alzheimer’s disease
PD	Parkinson’s disease
DLB	Dementia with Lewy bodies
MSA	Multiple system atrophy
DLBD	Diffuse Lewy body disease
LBVAD	Lewy body variant of Alzheimer’s disease
HSD	Hallervorden-Spatz disease
NBIA1	Neurodegeneration with brain iron accumulation type 1
PDD	PD dementia
LB	Lewy body
LN	Lewy neurite
NAC	Non-Aβ component of AD plagues
Aβ	Amyloid beta peptide
MetO	Methionine sulfoxide
GAG	Glycosaminoglycan
PG	Proteoglycan
HNE	4-Hydroxy-2-nonenal

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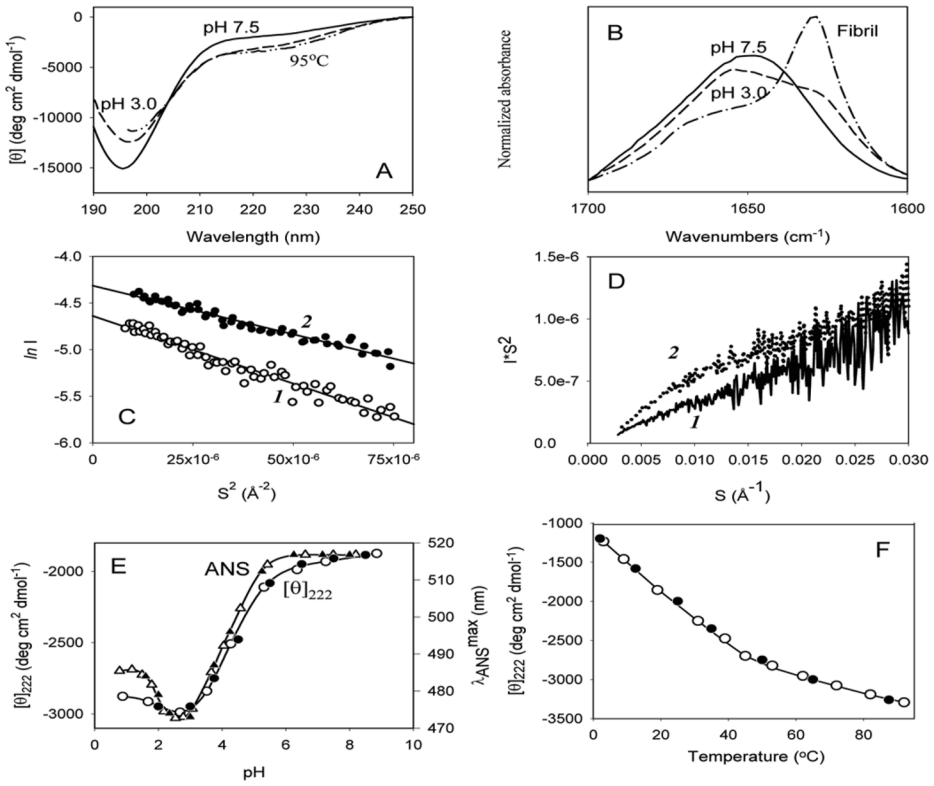

Fig. 1.

Structural properties and conformational behavior of human α-synuclein. (**A).** Far-UV CD spectra measured under different conditions. (**B).** FTIR spectra measured for natively unfolded, partially folded and fibrillar forms of α-synuclein. Guinier (**C**) and Kratky plot (**D**) representation of the results of SAXS analysis of human α-synuclein at different experimental conditions: 1 – pH 7.5; (**E).** pH-Induced partial folding of α-synuclein. (**F).** Temperature-induced folding of the natively unfolded α-synuclein.

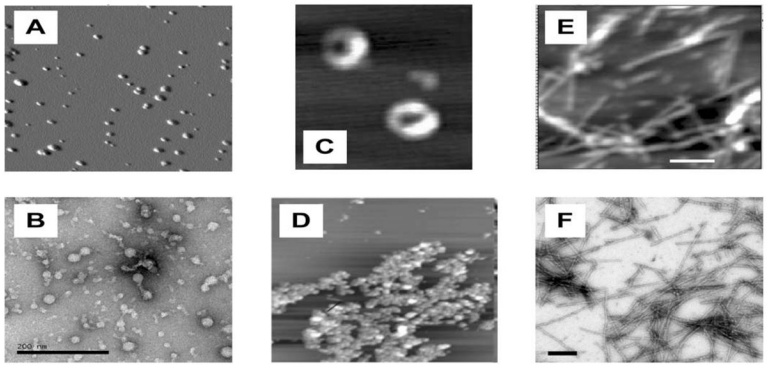

Fig. 2.

Various aggregated forms of human α-synuclein. Spherical oligomers as seen by AFM (**A**) and EM (**B**); annular oligomers (**C**) and amorphous aggregates (**D**) visualized by AFM; amyloid fibrils as seen by AFM (**E**) and EM (**F**).