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Review

Interaction of α-synuclein with biomembranes in Parkinson's disease —role of cardiolipin

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ABSTRACT

One of the key molecular events underlying the pathogenesis of Parkinson's disease (PD) is the aberrant misfolding and aggregation of the α-synuclein (αS) protein into higher-order oligomers that play a key role in neuronal dysfunction and degeneration. A wealth of experimental data supports the hypothesis that the neurotoxicity of αS oligomers is intrinsically linked with their ability to interact with, and disrupt, biological membranes; especially those membranes having negatively-charged surfaces and/or lipid packing defects. Consequences of αS–lipid interaction include increased membrane tension, permeation by pore formation, membrane lysis and/or leakage due to the extraction of lipids from the bilayer. Moreover, we assert that the interaction of αS with a liquid-disordering phospholipid uniquely enriched in mitochondrial membranes, namely cardiolipin (1,3-diphosphatidyl-*sn*-glycerol, CL), helps target the αS oligomeric complexes intracellularly to mitochondria. Binding mediated by CL may thus represent an important pathomechanism by which cytosolic αS could physically associate with mitochondrial membranes and disrupt their integrity. Impaired mitochondrial function culminates in a cellular bioenergetic crisis and apoptotic death. To conclude, we advocate the accelerated discovery of new drugs targeting this pathway in order to restore mitochondrial function in PD.

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1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative condition, affecting around 2% of the population above the age of 65 [1]. It is characterized clinically by bradykinesia (slowness in initiating movements), rigidity and a resting tremor. Most of the clinical manifestations of the disease are due to a progressive loss of dopaminergic neurons in the substantia nigra (SN) *pars compacta* in the midbrain [2]. One of the principal molecular events in the pathogenesis of PD is thought to involve the aberrant folding and aggregation of the α-synuclein (αS) protein, which leads to neuronal dysfunction and degeneration in the affected brain areas [3]. Genomic duplication and triplication of the *SNCA* gene as well as several missense mutations (including A53T, A30P and E46K) have been identified in rare cases of inherited PD. Patients harboring mutations in *SNCA* have a clinical phenotype that resembles sporadic PD, although onset is typically earlier and the disease more aggressive [4–7].

αS is a 140-residue soluble protein and its primary sequence is typically divided into three distinct regions: (i) a positively charged N-terminal region (residues 1–60) made up of a series of seven imperfect KTKEGV repeats, and which are characteristic of amphipathic helices of apolipoproteins [8]; (ii) a central hydrophobic region (residues 61–95) also referred to as the NAC (non-amyloid-β-component) domain composed of nonpolar side-chains and therefore having a high propensity for adopting β-sheet secondary structure [9]; and (iii) a highly acidic C-terminal domain rich in proline residues (residues 96–140) that remains disordered in both the oligomeric and fibrillar state [10,11]. αS is abundantly expressed throughout the human central nervous system and is highly conserved in vertebrates [12]. Although its exact physiological function is unknown, it has been recently suggested that αS acts as a soluble NSF attachment protein receptor (SNARE) complex chaperone to promote the assembly of large complexes at the presynaptic plasma membrane, required for neurotransmission [13,14].

It is now widely accepted that in aqueous solution as well as in the cellular milieu, αS exists mainly as an intrinsically disordered protein [15]. During the aggregation process, several factors promote the misfolding and self-assembly of αS into toxic β-sheet-rich oligomeric structures (for further detailed discussion of the mechanisms underlying αS aggregation and modulating factors, the reader is referred to Paleologou and El-Agnaf [16]. Oligomers reorganize into mature amyloid fibrils, which form cytoplasmic deposits termed Lewy bodies (Fig. 1) [17]. This is a key feature in the neuropathology of PD [18]. Extensive data support the hypothesis that a partially-folded intermediate precursor is critical for αS aggregation. Such intermediates are assumed to expose hydrophobic domains on their surface, allowing intermolecular interaction and aggregation. Thus, a wide range of endogenous and exogenous factors that increase the concentration of this critical amyloidogenic intermediate will alter the kinetics of αS fibrillation, favoring aggregation [19]. It is important to recognize that there exist a multiplicity of pathways for the self-assembly of αS, and a heterogeneous mixture of oligomeric species with different β-sheet arrangements are formed early during the self-assembly process. Typically, oligomers with antiparallel β-sheets within their core structures represent a stable, toxic oligomeric species, while those oligomers harboring parallel β-sheets are able to elongate efficiently into fibrils [20].

Acceleration of αS aggregation is promoted by environmental and genetic conditions known to be important in the pathogenesis of PD: overexpression of αS (resulting from duplication or triplication of the *SNCA* gene locus) [4,5]; missense mutations such as A53T, A30P, and E46K [21,22]; occupational exposure to pesticides (e.g. paraquat and rotenone); and specific heavy metal ions (e.g. iron, copper, lead and manganese) [23–26]. Other factors influencing the formation of the critical amyloidogenic precursor in the intracellular environment include: macromolecular crowding, low pH, phosphorylation of Ser129 and oxidative modifications of the αS protein [27,28]. More recently, the formation of oligomeric αS species has been demonstrated in live cells using fluorescence correlation spectroscopy. It was found that the threshold cytoplasmic concentration needed for αS to form oligomers is ~90 nM [29].

A prevailing hypothesis in the pathogenesis of PD relates to the ability of oligomeric intermediates formed during the aggregation of αS to disrupt cellular membranes [30]. Although this would provide a straightforward explanation for the cytotoxicity of αS, the exact molecular mechanisms are still unclear. Suggested mechanisms of membrane damage range from a generalized increase in membrane conductance, to discrete channel or pore formation, and complete membrane disruption [31].

2. α-Synuclein interaction with biological membranes

2.1. Membrane binding properties of αS

Binding of αS with biomembranes has been extensively studied [31]. Typically, experiments make use of planar bilayers, liposome vesicles and micelles containing physiologically relevant phospholipids [32]. Although several distinct binding modes have been shown for αS, there is a general consensus that upon binding to a membrane, αS forms an amphipathic α-helical structure involving the N-terminal and NAC regions (Fig. 1) [33]. Membrane binding of monomeric αS appears to involve two steps: first, anchoring by the N-terminal residues 3–25, followed by a coil-to-helix transition of residues 26–97 which act as a membrane sensor and which determine the affinity of αS binding; the C-terminal domain exhibits only weak interactions with the membrane surface [34–36]. As revealed by solution NMR spectroscopy of micelle-bound αS, the axis of the N-terminal α-helical structure lies parallel to the membrane interface and is actually made up of two curved α-helices, helix N (residues 3–37) and helix C (residues 45–92) connected by a well-ordered linker. Positively-charged lysine side chains protruding from the helices make electrostatic interactions with the negatively-charged membrane surfaces [37]. The presence of

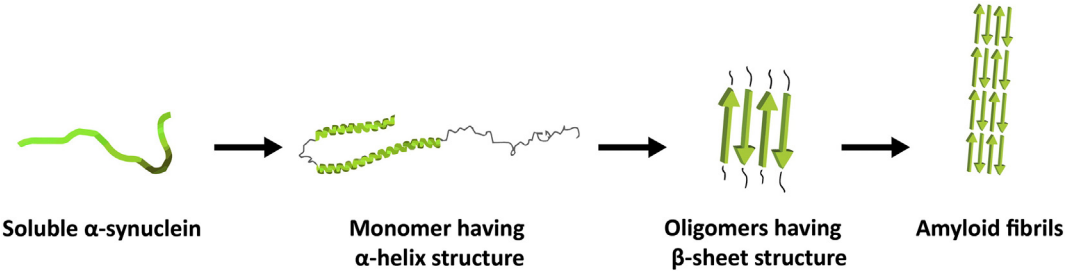

Fig. 1. A simplified representation of α-synuclein aggregation process. Alpha-synuclein (αS; in green) normally exists as a monomeric, intrinsically unstructured protein. Upon interaction with membranes containing anionic phospholipids, the N-terminus of αS adopts an α-helical structure. Increased local concentration of membrane-associated monomers exposes disordered domains and facilitates self-association into dimers and β-sheet-rich intermediate oligomeric aggregates. A suggested mechanism of neurotoxicity would involve permeabilization of cellular and organelle membranes by oligomers with membrane permeation properties. Further intermolecular association between oligomers leads to stable higher-order aggregates and cross-β-strand fibril formation. The amyloid-like fibrils deposit in brain parenchyma as Lewy bodies and Lewy neurites.

multiple lysine residues in the N-terminal region of αS may therefore partly explain the preferential binding of αS to vesicles containing anionic (e.g. phosphatidylinositol [PI], phosphatidylglycerol [PG] and phosphatidylserine [PS]), but not zwitterionic (e.g. phosphatidylcholine [PC]), lipids [38,39]. It has been estimated that each molecule of αS can bind to a lipid bilayer patch composed of not more than 85 acidic lipid molecules [40].

Also favored is the binding of αS to membrane lipids with small head groups (e.g. phosphatidic acid [PA]) and polyunsaturated acyl chains. This is likely due to the presence of packing defects and the availability of more space for αS to embed in such membranes [41,42]. In this manner, αS may insert and be accommodated more easily into the bilayer [43,44]. The protein penetrates deeply in between the lipid head groups, inducing lateral expansion of lipid molecules which in turn leads to a reduction of the average thickness of the bilayer (membrane thinning) [45–47]. Moreover, bilayer defects are present in small, highly curved vesicles, and indeed αS shows a binding preference for small unilamellar vesicles (SUVs) over large unilamellar vesicles (LUVs) [48–50]. Nevertheless, αS has been observed to bind to anionic LUVs and planar bilayers as well [40,51,52]. In conclusion, the composition and physical properties (especially curvature, charge, and packing defects) of the phospholipid membrane bilayer markedly influence its interaction with αS [53,54]. Furthermore, in addition to electrostatic interactions, hydrophobic interactions are important in the association of αS with membranes [55]. Importantly, binding of the amphipathic helix of αS to stressed membranes causes a stabilization of the lipid packing which reduces their propensity to fuse [50,56].

2.2. Aggregation of αS at the membrane surface

Lipid binding by αS can be associated with enhanced fibrillogenicity by promoting the formation of aggregates at the membrane surface. In the presence of lipidic molecules and detergents, the aggregation rate of αS was found to increase several-fold [57–63]. The lipid:protein ratio appears to be a critical determining factor: while low concentrations of lipid tend to drive aggregation, high concentrations of lipid tend to prevent aggregation [64]. One mechanism by which lipids facilitate αS aggregation is by crowding the protein at the two-dimensional liquid/lipid interface on the surface of the vesicle, thereby increasing its effective local concentration [65,66]. This in turn facilitates the formation of stable αS dimers on the membrane surface, which represents the first "core" step and initiates oligomerization [67]. In fact, on-surface nucleation of αS has been observed to occur at low nanomolar concentrations in the presence of negatively charged lipid bilayers [68]. It has been recently calculated that lipid vesicles enhance the rate of the primary nucleation of αS by a thousand-fold, compared to bulk solution [69]. Use of surface plasmon resonance has shown formation of a high affinity lipid-binding intermediate [70]; thus, by driving aggregation, lipids favor αS adopting the partially-folded critical intermediate state involved in the pathway of conversion into β-sheet fibrils [69]. In the presence of phospholipid vesicles, αS undergoes significant perturbations in the N-terminal domain and the NAC domain [71]. Such perturbations lead to increased accessibility of hydrophobic surfaces and an oligomeric state with mostly β-sheet secondary structure [72]. Recently, a detailed characterization of the structure of 'on-pathway' oligomeric species revealed small, ellipsoidal oligomers consisting of ~30 monomers and having β-sheet structure with a high degree of flexibility at the solvent interface [73]. Of note, the β-sheet structure of toxic soluble oligomeric αS species was found to be distinct from that of fibrils; in these experiments, oligomers were reported to adopt an antiparallel β-sheet organization, differing from the parallel arrangement of the fibrillar structure [74].

The essential role of hydrophobic interactions in the association of αS with membranes is supported by analysis of the effects of familial PD-linked αS mutations on membrane binding. In general, it appears that the A30P mutation decreases the affinity of the protein for lipid surfaces, lacking binding activity to rat brain vesicles [75] and to phospholipid vesicles in vitro [38,76,77]. On the other hand, the A53T mutant displays membrane-binding activity at least comparable to wild-type (WT) αS [76,77], while the E46K mutant shows enhanced binding [38]. According to molecular dynamics simulation of the membrane interactions of a range of αS species, the region including residues 39–45 may have maximum membrane penetration. Increased oligomerization ability was associated with a greater propensity to penetrate the membrane. The E57K (artificial) and A53T mutants had the highest frequency of interaction with the membrane, WT in-between and A30P the last [78]. Nonetheless, although the A30P mutation has a reduced membrane affinity, prolonged incubation with SUVs resulted in membrane-induced aggregation of A30P αS into oligomers on the membrane surface as a result of exposure of the central hydrophobic region [79]. Collectively, these findings suggest that interactions between the membrane-bound αS molecules themselves are also relevant for self-assembly on the bilayer.

2.3. Membrane perturbation and neurotoxicity by oligomeric αS

As with native αS, the two major factors driving oligomer–membrane interactions are net negative charge and loose packing of the bilayer. Oligomers of αS bind preferentially to lipids in the liquid-disordered phase and show significant less affinity for liquid-ordered bilayers. Liquid-ordered membranes are much more densely packed; conversely, a disordered bilayer allows exposed hydrophobic patches in the oligomeric structure to make hydrophobic interactions with the membrane interior [80]. As with monomers, the N-terminus of αS plays a major role in determining oligomer–membrane interactions. For instance, deletion of residues 2–11 abolishes permeabilization of LUVs by αS oligomers [81]. Similarly, N-terminal deletions in αS correlated with reduced vesicle affinity and toxicity towards yeast [82]. It is expected that the simultaneous binding of a large number of adjacent N-terminals having juxtaposed hydrophobic regions in the oligomeric structure would evoke a high degree of membrane instability. On the other hand, monomers would be widely distributed over a membrane, while in the case of fibrils most of the hydrophobic patches are buried in the highly ordered structure. This provides an explanation why it has been consistently observed that αS oligomers exhibit a higher potency for permeabilizing membranes than either monomers or fibrils [73,83]. In general, oligomers have a stronger membrane affinity compared to monomers [84] and unlike monomers may not necessarily require membranes with high curvature or defects in lipid packing [85].

Interestingly, many other amyloidogenic proteins apart from αS have been shown to form membrane-active oligomers; these include the amyloid-beta (Aβ) peptide [86], tau [87], the islet amyloid polypeptide [88], the prion protein [89], salmon calcitonin [90] and the N-terminal domain of Hyp-F from *Escherichia coli* [91]. A common underlying theme is the key role of acidic lipids in accelerating aggregation and recruiting oligomers [92]. Oligomerization has also been suggested to be the basis for induction of membrane leakage by antimicrobial peptides [93].

Neurotoxicity of αS oligomers is intrinsically linked with their ability to interact with and permeabilize lipid bilayers, and this has been amply demonstrated both in cellular and membrane model systems [30,83,92]. Various mechanisms of permeabilization have been put forward, such as: membrane disruption, membrane thinning, permeabilization via pore formation, and leakage induced by the extraction of lipids. Downstream consequences of membrane permeabilization include dysregulation of cellular ion homeostasis, increased levels of intracellular Ca^{2+}, generation of reactive oxygen species (ROS), altered signal transduction, and ultimately triggering of apoptotic neurodegeneration. The increased generation of ROS is especially relevant to αS-induced toxicity of dopaminergic neurons, in view of the fact that dopamine (DA) itself is highly prone to oxidation [94]. DA oxidation generates further reactive intermediates, which increase αS aggregation into

oligomers while slowing the conversion of protofibrils to fibrils [95]. In the presence of DA, αS forms SDS-resistant soluble oligomers [96]. At the same time, αS may catalyze the formation of free radicals, like the hydroxyl radical and hydrogen peroxide, further increase αS oligomer formation by impairment of cellular quality control mechanisms and by increasing the amount of ferric iron [26,97]. The treatment of dopaminergic cells with both DA and the radical-generating compound paraquat enhanced αS-induced membrane conductance and toxicity [98]. The formation of DA-induced αS oligomers may also alter membrane binding and result in disruption of its normal function in synaptic vesicle trafficking [99,100]. Thus, it is clear that the complex interplay between DA, oxidative stress, αS-oligomerization and membrane binding enhances the vulnerability of dopaminergic neurons to cell death.

2.3.1. Amyloid pore mechanism

The first indication of a pore-like mechanism for membrane permeabilization by αS came from studies in which leakage of encapsulated dye from LUVs exposed to protofibrillar αS was strictly dependent upon the size of the marker, with a strong preference for low-molecular weight molecules [101]. More direct evidence for pore formation has been obtained from electrophysiological studies. Soluble 'on-pathway' oligomers of αS, but not monomers or fibrils, formed channel-like pores when reconstituted in planar lipid bilayers in the presence of an applied trans-negative potential. The pores had well-defined conductance states and were cation-selective [102]. The PD-linked E46K mutant also formed channels with a higher selectivity for cations [103]. Formation of distinct and uniform pores has also been demonstrated using iron-induced αS oligomers [104]. These oligomers, termed "intermediate II" aggregates, have been extensively characterized and are formed in a multi-step aggregation pathway that includes micromolar concentrations of ferric iron (Fe^{3+}) similar to the concentration found in physiological conditions [26,104,105]. Importantly, the oligomers share properties with αS aggregates isolated from post-mortem human and animal brains with regard to SDS-resistance and anti-oligomer antibody epitopes [26]. Electrophysiological characterization of "intermediate II" oligomers indicated stable and uniform pores exhibiting quantized and step-wise changes in bilayer conductance [106]. This suggests the formation of distinct transmembrane oligomeric pores, rather than non-specific damage to bilayer integrity. Oligomeric pores can also be formed from membrane-bound monomeric αS. Under applied voltage, the peptide helices of WT, E46K and A53T αS immerse into the bilayer and adopt a transmembrane orientation to form a 'barrel-stave' pore. Well-defined conductance states are again observed, reminiscent of pore-forming peptides like alamethicin [107]. Multilevel channel conductances could be due to conformational changes in the pore structure, or could reflect the difference in the number of subunits that form a single pore [108].

Consistent with the electrophysiological data, structural studies further support the notion of pore formation by αS. For instance, atomic force microscopy (AFM) and transmission electron microscopy (TEM) have enabled visualization of annular structures with a central pore formed by aggregated αS [109,110]. A small-angle X-ray scattering (SAXS) study showed annular oligomers in the shape of a wreath, with a central channel. The potential for membrane permeabilization by the wreath-shaped oligomers was confirmed by demonstrating liposome disruption, hence strongly suggesting cytotoxic properties [111]. Computer modeling has simulated the perforation of membrane bilayers by αS oligomeric ring-like structures, with immersion of mutant A53T occurring more rapidly than for WT αS [67,112].

Thus, αS oligomer binding can create a membrane pore through which small molecules permeate the lipid bilayer. A pore size of ~10 nm (outer diameter) and ~2 nm (inner diameter) would be sufficient for most types of ions to pass through. An unregulated flux of ions and other molecules would provide an evident mechanism for oligomer-induced cytotoxicity. A crucial role is likely to be played by disruption of calcium homeostasis, since elevated intracellular Ca^{2+} levels are known to activate caspases and protein phosphorylation, leading to synaptic degeneration and neuronal cell death [113]. Extracellular oligomeric αS increased intracellular Ca^{2+} levels in neurons by inducing Ca^{2+} influx, independent of cobalt-sensitive calcium channels. Furthermore, the neuronal membrane potential was significantly depolarized after oligomer treatment [114]. Similarly, high plasma membrane ion permeability and raised intracellular Ca^{2+} levels were observed in neuronal cell lines stably expressing WT or mutant αS, suggesting the possibility of excessive Ca^{2+} influx through large non-selective pores formed by αS [115]. Notably, αS was displayed by electron microscopy to be present in close proximity to the plasma membrane and in association with other organelles [112]. Thus, interventions aims at preventing pore formation or blocking existing pores may represent a novel strategy for treatment of PD.

2.3.2. Carpet mechanism and toroidal pores

Yet other studies on the permeabilization of lipid membranes by soluble αS oligomers have reported a generalized increase in bilayer conductivity, i.e. without any evidence of discrete channel or pore formation [116,117]. It is envisaged that oligomeric assemblies bind onto the surface of the target membrane and cover it in a "carpet-like" manner. Once a threshold concentration is reached, the lipid packing is disturbed and the hydrophobic core of the bilayer thins out. This lowers the dielectric barrier, thereby facilitating the transport of small molecules and ions across the membrane [117,118]. In the presence of high concentrations of αS monomers, a lateral expansion of lipid molecules that progressed to membrane thinning was also observed, again without amyloid pore formation [45].

Membrane thinning may however lead to the formation of finite-sized, toroidal lipidic pores. Essentially, the membrane-inserted protein gives rise to a local curvature stress in the membrane, which decreases the curvature elastic energy for poration. At a high protein concentration, the tendency is for the formation of well-ordered toroidal pores stabilized by the inserted proteins, while more stochastic transient pores are formed at lower concentrations [119]. Such a mechanism, also known as the membrane tension model, is again shared by antimicrobial peptides [120].

2.3.3. Lipid extraction mechanism

Growth of membrane-adsorbed amyloid aggregates, and the related co-aggregation of proteins and lipids, can lead to membrane damage by the uptake of phospholipids from the bilayer. Aggregation of αS on anionic membranes has been reported to involve extraction of lipids from the bilayer and clustering of lipids around the growing αS aggregates [121,122]. This leads to membrane disintegration and leakage of dye-filled vesicles [123]. Furthermore, as a result of membrane fragmentation, soluble complexes of αS and lipid are formed [124].

An elegant study has convincingly demonstrated that αS oligomers are toxic in vivo. Recombinant E35K and E57K αS variants that had an increased ability to form oligomers in vitro, correlated well with a higher affinity to bind liposomes, triggering of Ca^{2+} influx in neuronal cell lines, and greater toxicity in cell-based assays. Importantly, rats expressing the E35K and E57K membrane-associated αS oligomers also suffered the most severe dopaminergic loss in the SN [125]. In other words, a qualitative correlation was established between the ability of αS oligomers to cause in vitro toxicity by disrupting cellular membranes, and their in vivo toxicity to nigral neurons.

3. α-Synuclein interaction with mitochondrial membranes

3.1. Mitochondrial phospholipids and cardiolipin

Mitochondria are dynamic organelles enclosed by a complex double membrane. They are essential for cell viability, not least because they provide most of the chemical energy required by the cell (adenosine triphosphate, ATP). The functional integrity of these organelles is

intricately linked with the maintenance of intact membranes having a proper phospholipid composition [126]. Mitochondrial membrane lipids are involved in a plethora of physiological processes, ranging from the organization and function of respiratory complexes, to the regulation of mitochondrial dynamics and apoptosis [42]. The major phospholipids in mitochondrial membranes are PC and phosphatidylethanolamine (PE), making up ~40% and ~30% by weight of total mitochondrial phospholipids, respectively. PI and PS together comprise another ~10–15%, whereas cardiolipin (1,3-diphosphatidyl-*sn*-glycerol, CL) accounts for ~10% of the total mitochondrial phospholipids [127]. The most notable difference in the relative abundance of phospholipids between the outer (OMM) and inner (IMM) mitochondrial membranes concerns CL, with ~5% abundance in the OMM and ~20% in the IMM [128]. Apart from the IMM, CL is a major component of mitochondrial contact sites, regions of close apposition between the outer and inner membranes [129]. Contact sites allow CL to diffuse from the IMM to the OMM. Indeed, CL represents the signature lipid species of mitochondrial membranes and plays multiple roles in organization of multi-subunit oxidative phosphorylation complexes, the maintenance of membrane integrity in response to environmental stress, and the regulation of programmed cell death [130]. CL peroxidation and depletion have been reported in a variety of pathological conditions associated with energy deficiency, and CL has been lately identified as a target for drug development [126,131].

Uniquely, CL is a divalent lipid having two connected phosphatidylglycerol moieties and four acyl chains. This chemical structure results in a small anionic headgroup and a large hydrophobic tail, thereby giving CL its characteristic conical shape. In turn, the structure of CL imparts distinctive biophysical properties to the phospholipid membrane. Firstly, CL is likely to disturb lipid packing and thus the mechanical stability of the membrane [132,133]. Secondly, the 'hinged' acyl chains induce negative curvature strain in CL microdomains, as in the cristae of inner mitochondrial membranes [130]. Thirdly, CL can potentially carry two negative charges. The biological inference of these three biophysical characteristics of CL, i.e. lipid packing defects, high curvature and divalent anionic charge, is that CL-rich sites could represent primary targets for permeabilization of mitochondrial membranes by pathological αS oligomers.

3.2. Direct association of αS with mitochondria

A robust, direct interaction of αS with isolated mitochondria, which is not affected by loss of the inner membrane potential, has been observed in several studies [134,135]. The αS–mitochondria interaction is mediated by the N-terminal region of αS, which adopts an α-helical conformation [136]. The N-terminal 32 amino acids contain a cryptic mitochondrial targeting sequence [137] and mitochondrial binding was abolished upon deletion of residues 2–11 [138]. It is possible that the N-terminal motif facilitates translocation of αS across the OMM by the mitochondrial import machinery, thus allowing αS to gain access to the IMM [137]. Consistent with these studies, fluorescently-labeled αS was imported into isolated mitochondria and localized to the IMM [139]. Moreover, αS has been shown on electron micrographs to be localized to the mitochondrial membrane in normal dopaminergic neurons of the SN *pars compacta* from human and mouse brain [137,140]. Recent experiments suggest that monomeric αS interacts with the voltage-dependent anion channel (VDAC), a major channel of the OMM, and reversibly blocks the channel pore [141]. A direct consequence for mitochondrial function would involve steric hindrance by αS of ATP/ADP fluxes between mitochondria and the cytosol. Further, at high voltages of more than 40 mV, the whole αS molecule was able to translocate through the channel. Thus, VDAC could serve as a route for αS to access the intermembrane space and impair the respiratory chain complexes of the IMM [142].

Mitochondrial model membranes are more prone to oligomer-induced damage compared to plasma membrane model systems. In fact, liposomes with physiologically relevant lipid mixtures that mimic biological membranes of mitochondria undergo robust permeabilization [143]. Both wild-type αS oligomers and monomers exhibited a preference for the CL-enriched IMM over the OMM, although monomers perturbed the packing density of the bilayer but did not trigger permeabilization [139,144]. Soluble A53T and A30P mutant oligomers, however, were equally damaging to both membrane types [144].

4. α-Synuclein causes deficits in mitochondrial function and dynamics

4.1. α-Synuclein and mitochondrial dysfunction — role of cardiolipin

The effects and consequences on mitochondrial morphology, dynamics and function, of a direct interaction of αS with mitochondrial membranes have been intensively investigated. Treatment of mitochondria isolated from rat brain or neuronal cell lines with soluble prefibrillar αS oligomers, but not with monomeric or fibrillar αS, caused mitochondrial swelling, loss of mitochondrial membrane potential, release of the respiratory protein cytochrome *c* (cyto *c*), elevated levels of ROS, and an increase of mitochondrial Ca^{2+} [134,144–146]. Similar detrimental effects of αS on mitochondria were seen in dopaminergic cell lines and primary neurons overexpressing αS intracellularly. Mitochondrial impairment was associated with bioenergetic failure, an increase in oxidative stress, and a decrease in cell viability [134, 146,147]. Overexpression of αS in neuronal cells also evoked the fragmentation of mitochondria [56,148]. Remarkably, the hallmarks of mitochondrial dysfunction and oxidative stress induced by αS are recapitulated in the unicellular eukaryote *Saccharomyces cerevisiae*. In yeast cells expressing high levels of αS, the mitochondria fragment, swell, and produce ROS [149,150]. This observation lends support to a direct, deleterious interaction of αS with mitochondria. An important role for direct lipid binding in mediating the detrimental effects of αS on mitochondria is further evident by the fact that deletion of amino acids 1–65 abrogated mitochondrial damage [147]. The latter region includes the N-terminal KTKEGV repeats that bind phospholipid vesicles. Taken together, these studies point to a novel, direct effect of oligomeric αS on mitochondrial membranes.

CL may account for the selective effect of pathological αS oligomers on mitochondrial membranes (Fig. 2). Given the central role of CL in the formation and stability of respirasomes composed of complex I/III/IV [151], the physical association of αS oligomers with CL and perturbation of mitochondrial membrane phospholipids might disrupt electron transfer between the respiratory complexes [135,152,153]. It has also been discovered that αS oligomers bound to CL form a triple complex with cyto *c* and thereby act as substrates for peroxidase activity of cyto *c*. Peroxidative activity of αS–CL–cyto *c* complexes may favor permeabilization of mitochondrial membranes, apart from contributing to a constant source of oxidative stress in dopaminergic SN neurons [154]. Another consequence of αS–CL binding could be loss of function of the mitochondrial ADP/ATP carrier, which is normally stabilized by CL molecules [155,156]. Moreover, analysis of mitochondria from PD brain and αS-expressing dopaminergic neurons confirmed the association of αS with complex I [137]. Thus, a feed-forward cycle of increasing ROS generation and reduced mitochondrial respiratory chain enzyme function would be set up [145]. Consistent with this concept, a decline in mitochondrial CL content correlated with bioenergetic deficits in mitochondria of neurons overexpressing the N-terminal domain of αS [147]. Furthermore, α-synuclein knockout mice were reported to have qualitative and quantitative mitochondrial lipid abnormalities including a decrease in CL content, which was associated with a reduction in complex I/III activity [157].

Interestingly, aggregated forms of various amyloidogenic proteins, including αS, Aβ and tau, have shown a preference to compromise the integrity of membranes with a high content of CL [144]. In a paper published during preparation of this manuscript, the authors attributed cytotoxic properties of cobra venom toxins to their ability to bind CL and

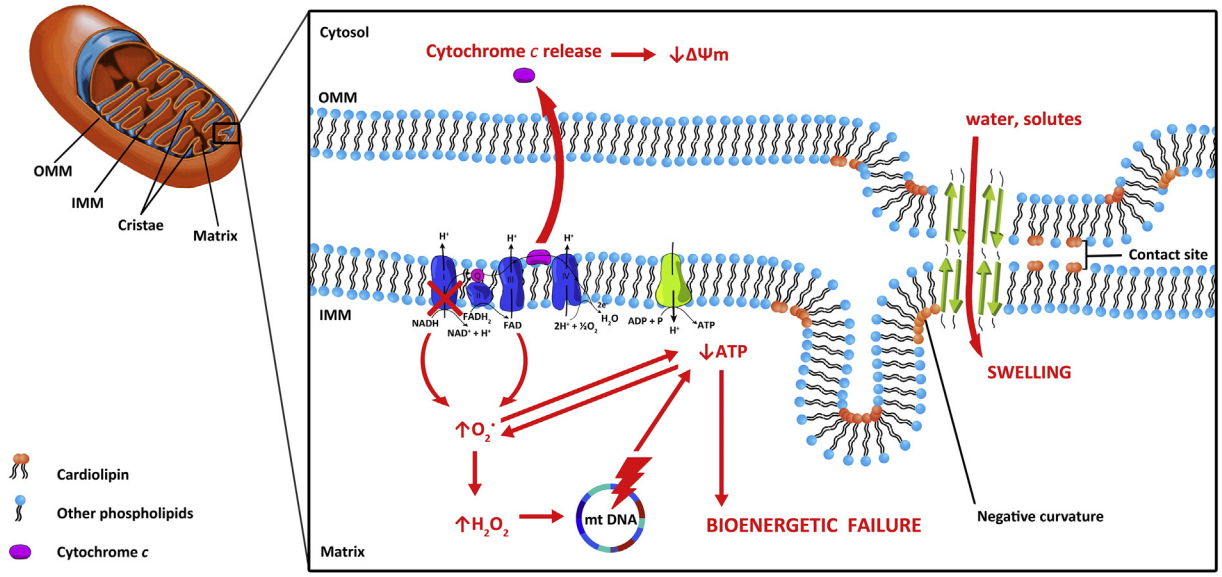

Fig. 2. α-Synuclein-induced mitochondrial dysfunction prompted by disruption of membrane lipids at contact sites. Mitochondria contact sites are enriched in cardiolipin, the signature phospholipid of energy-generating membranes. Cardiolipin imparts biophysical characteristics that make OM contact sites a prime target for αS oligomers. Destabilization of contact sites leads to permeabilization of both outer and inner mitochondrial membranes (OMM, IMM) because the two membranes are closely juxtaposed at this site. Permeabilization may be induced by insertion of oligomeric pore complexes, or by an increase in membrane curvature stress prompting the formation of a toroidal protein–lipid pore with its walls partially covered by lipid head groups. Mitochondrial *outer membrane* permeabilization permits release of cyto *c* and other apoptogenic proteins into the cytosol. Loss of *inner membrane* integrity allows entry of water, and hence matrix swelling with damage to mitochondrial cristae and further mobilization of cyto *c*. Inevitably, there is a fall in mitochondrial membrane potential (Δψm) which compromises mitochondrial respiratory function. Increased mitochondrial levels of αS in PD have also been associated with reduced complex I activity and increase free-radical generation. Superoxide ($O_2^{\bullet -}$) is converted to hydrogen peroxide (H_2O_2) by mitochondrial superoxide dismutase (MnSOD) in the matrix. The free radicals will further decrease respiratory activity and promote a fall in Δψm. Finally, oxidative damage to mitochondrial DNA (mtDNA) sets up a vicious cycle of αS-induced mitochondrial damage culminating in bioenergetic failure.

thus physically associate with mitochondrial membranes. Moreover, they suggested formation of toroidal-like pores by the cytotoxins leading to disruption of mitochondrial structural integrity [158]. It is undoubtedly intriguing that amyloidogenic proteins and natural cytotoxins might share a common poration mechanism involving the thrilling of mitochondria via CL binding. It is plausible that amphipathic peptide-induced electrostatic clustering of CL increases membrane tension in CL-containing membranes, which is then released by the formation of toroidal pores [159]. A similar mechanism has been recently described for the formation of toroidal protein–lipid pores involving cyto *c*/H_2O_2-induced peroxidation of mitochondrial cardiolipin. Electrostatic binding of cyto *c* to CL phospholipids in the bilayer is followed by conformational changes of the protein leading to the induction of peroxidase activity, CL oxidation and formation of permeating protein–lipid pores [160].

4.2. Mitochondrial dysfunction induced by αS in animal models of PD

Mitochondrial dysfunction in relation to αS toxicity has also been demonstrated in vivo. Overexpression of human WT αS in mice was associated with brain mitochondria having decreased mitochondrial membrane potential, higher ROS production, oxidative mitochondrial DNA damage and reduced ATP production [161,162]. In one of these studies, mainly monomeric full-length N-terminally acetylated αS was found to accumulate in mitochondria. Thus, brain mitochondria can also been impaired by a threshold amount of a soluble, monomeric form of human αS [161]. In another study, inhibition of mitochondrial function by overexpressed αS was linked to defective mitochondrial import machinery, specifically, a decrease in TOM40 (translocase of the OMM) protein levels [162]. Overexpression of human A53T mutant αS in mice resulted in a severe motor disorder, with destruction of motor neurons in the spinal cord leading to paralysis and death [163]. Intraneuronal αS-positive inclusions were seen by electron microscopy to consist of degenerating mitochondria [163]. Mitochondria had a depolarized membrane potential and showed impaired maximum respiratory capacity [164]. Similar mitochondrial deficits were observed in the SN of rats overexpressing human WT αS; for instance, a reduction in the mitochondrial membrane potential was associated with the in vivo accumulation of αS within mitochondria [165]. A particularly impressive finding in the latter study is that AFM analysis revealed a high frequency of pore-like structures on the outer membrane of mitochondria isolated from the αS-overexpressing rats. These findings thus support a mechanism involving direct formation of a pore complex by αS in mitochondrial membranes.

5. Conclusion and future perspectives

There is now compelling evidence that the reciprocal interaction between the amyloidogenic αS protein and lipid membranes may represent a key pathological mechanism in PD [166]. There are two major consequences of such an interaction. Firstly, the biomembrane interface favors aggregation of αS and the generation of a pathogenic, intermediate oligomeric species on the two-dimensional membrane surface. Secondly, interaction of soluble αS oligomers with membranes evokes various models for permeabilization; these include a generalized membrane thinning and increase in membrane tension, insertion of oligomeric amyloid pores or formation of lipidic toroidal pores, and leakage induced by lipid extraction. Highly charged, anionic membranes play a particularly important role, since they strongly favor αS binding via electrostatic interactions and self-assembly of αS on the bilayer. Another critical parameter is decreased lipid packing and instability of the bilayer itself, which enhances hydrophobic protein–membrane interactions. The aforementioned membrane characteristics are distinctive of membranes that are enriched in the dimeric, conical-shaped phospholipid CL. We therefore propose that pathological αS oligomers have an intrinsic affinity to target and harm mitochondria due to an abundance of CL, particularly at the mitochondrial inner membrane and contact sites. Thus, while the interaction of αS with the bilayer surface is aided by electrostatic interactions with multiple lysine residues on the N-terminus, its ability to penetrate deeper into the membrane

is favored by the higher exposure of hydrophobic surfaces in the oligomeric structure.

Association of αS with CL could lead to one or more of the following [167]: decline in CL content and inhibition of respiratory complexes; mitochondrial ROS generation; CL peroxidation and release of pro-apoptotic factors like cyto *c*; and formation of amyloid or lipidic pores in the membrane bilayer. In this manner, pathological αS interactions with mitochondrial membranes possibly represent an upstream event that feeds a vicious cycle contributing to a cellular bioenergetic crisis and apoptotic death. More generally, the mechanism bears a strong similarity to that observed in apoptosis triggered by the pro-apoptotic members of the Bcl-2 family such as Bax, Bak and tBid. Specific cooperation between tBid and CL has been suggested to promote Bax or Bak oligomerization at contact sites [168,169]. This leads to subsequent permeabilization of the OMM, cyto *c* release and final execution of apoptosis [170].

To date, a significant effort in drug discovery has been made to develop compounds that attenuate mitochondrial ROS in PD, typically using mitochondria-targeted antioxidants like Coenzyme Q10 and Vitamin E. However, these compounds have largely failed to improve mitochondrial bioenergetics [171]. A novel effective therapeutic approach is potentially one in which compounds are screened for their effectiveness in protecting mitochondrial membrane integrity and preserving mitochondrial bioenergetics. This can be achieved by drugs such as anle138b that act as αS oligomer modulators and are enriched in lipophilic environments [172]. In addition, CL would represent a prime drug target for improving the bioenergetic robustness of mitochondria. Protecting CL would also be expected to hinder the insertion of αS oligomeric pores in mitochondrial membranes. In this respect, clinical trials are presently being conducted to evaluate the first-in-class CL-protective compound, the Szeto–Schiller (SS) peptide Bendavia™ (also referred to as SS-31 in the literature) as a therapeutic agent to restore mitochondrial bioenergetics in a host of age-associated diseases, including neurodegenerative disease [131]. SS-31 targets CL and improves mitochondrial respiration and ATP synthesis, while protecting the structure of the mitochondrial cristae [173].

Focusing on the development of drugs that target mitochondrial lipids, and in particular cardiolipin, in order to recharge the mitochondrial cellular powerhouses thus represents a novel approach that should be earnestly validated by further experimental, pharmacological and clinical studies.

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References

[1] Elbaz A, Manubens-Bertran JM, Baldereschi M, Breteler MM, Grigoletto F, Lopez-Pousa S, et al. Parkinson's disease, smoking, and family history. EUROPARKINSON Study Group. J Neurol 2000;247:793–8.
[2] Goedert M. Alpha-synuclein and neurodegenerative diseases. Nat Rev Neurosci 2001;2:492–501.
[3] Eschbach J, Danzer KM. Alpha-synuclein in Parkinson's disease: pathogenic function and translation into animal models. Neurodegener Dis 2014;14:1–17.
[4] Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997;276:2045–7.
[5] Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, et al. Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. Lancet 2004;364:1167–9.
[6] Kruger R, Kuhn W, Muller T, Woitalla D, Graeber M, Kosel S, et al. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. Nat Genet 1998;18:106–8.
[7] Zarranz JJ, Alegre J, Gomez-Esteban JC, Lezcano E, Ros R, Ampuero I, et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 2004;55:164–73.
[8] Mishra VK, Palgunachari MN. Interaction of model class A1, class A2, and class Y amphipathic helical peptides with membranes. Biochemistry 1996;35:11210–20.
[9] Giasson BI, Murray IV, Trojanowski JQ, Lee VM. A hydrophobic stretch of 12 amino acid residues in the middle of alpha-synuclein is essential for filament assembly. J Biol Chem 2001;276:2380–6.
[10] Eliezer D, Kutluay E, Bussell Jr R, Browne G. Conformational properties of alpha-synuclein in its free and lipid-associated states. J Mol Biol 2001;307:1061–73.
[11] Chen M, Margittai M, Chen J, Langen R. Investigation of alpha-synuclein fibril structure by site-directed spin labeling. J Biol Chem 2007;282:24970–9.
[12] Iwai A, Masliah E, Yoshimoto M, Ge N, Flanagan L, de Silva HA, et al. The precursor protein of non-A beta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. Neuron 1995;14:467–75.
[13] Burre J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Sudhof TC. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science 2010;329:1663–7.
[14] Burre J, Sharma M, Sudhof TC. Alpha-synuclein assembles into higher-order multimers upon membrane binding to promote SNARE complex formation. Proceedings of the National Academy of Sciences of the United States of America, 111. 2014. p. E4274–83.
[15] Fauvet B, Mbefo MK, Fares MB, Desobry C, Michael S, Ardah MT, et al. Alpha-synuclein in central nervous system and from erythrocytes, mammalian cells, and *Escherichia coli* exists predominantly as disordered monomer. J Biol Chem 2012;287:15345–64.
[16] Paleologou KE, El-Agnaf OM. Alpha-synuclein aggregation and modulating factors. Subcell Biochem 2012;65:109–64.
[17] Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature 1997;388:839–40.
[18] Cheng B, Gong H, Xiao H, Petersen RB, Zheng L, Huang K. Inhibiting toxic aggregation of amyloidogenic proteins: a therapeutic strategy for protein misfolding diseases. Biochim Biophys Acta 1830;2013:4860–71.
[19] Fink AL. The aggregation and fibrillation of alpha-synuclein. Acc Chem Res 2006;39:628–34.
[20] Chen SW, Drakulic S, Deas E, Ouberai M, Aprile FA, Arranz R, et al. Structural characterization of toxic oligomers that are kinetically trapped during alpha-synuclein fibril formation. Proceedings of the National Academy of Sciences of the United States of America, 112. 2015. p. E1994–2003.
[21] Kara E, Lewis PA, Ling H, Proukakis C, Houlden H, Hardy J. Alpha-synuclein mutations cluster around a putative protein loop. Neurosci Lett 2013;546:67–70.
[22] Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, et al. Alpha-synuclein locus triplication causes Parkinson's disease. Science 2003;302:841.
[23] Binolfi A, Rasia RM, Bertoncini CW, Ceolin M, Zweckstetter M, Griesinger C, et al. Interaction of alpha-synuclein with divalent metal ions reveals key differences: a link between structure, binding specificity and fibrillation enhancement. J Am Chem Soc 2006;128:9893–901.
[24] Huls S, Hogen T, Vassallo N, Danzer KM, Hengerer B, Giese A, et al. AMPA-receptor-mediated excitatory synaptic transmission is enhanced by iron-induced alpha-synuclein oligomers. J Neurochem 2011;117:868–78.
[25] Santner A, Uversky VN. Metalloproteomics and metal toxicology of alpha-synuclein. Metallomics: Integrated Biometal Science 2010;2:378–92.
[26] Levin J, Hogen T, Hillmer AS, Bader B, Schmidt F, Kamp F, et al. Generation of ferric iron links oxidative stress to alpha-synuclein oligomer formation. J Park Dis 2011;1:205–16.
[27] Nubling GS, Levin J, Bader B, Lorenzl S, Hillmer A, Hogen T, et al. Modelling Ser129 phosphorylation inhibits membrane binding of pore-forming alpha-synuclein oligomers. PLoS ONE 2014;9, e98906.
[28] Lundvig D, Lindersson E, Jensen PH. Pathogenic effects of alpha-synuclein aggregation. Brain Res Mol Brain Res 2005;134:3–17.
[29] Plotegher N, Gratton E, Bubacco L. Number and brightness analysis of alpha-synuclein oligomerization and the associated mitochondrial morphology alterations in live cells. Biochim Biophys Acta 1840;2014:2014–24.
[30] van Rooijen BD, Claessens MM, Subramaniam V. Membrane interactions of oligomeric alpha-synuclein: potential role in Parkinson's disease. Curr Protein Pept Sci 2010;11:334–42.
[31] Snead D, Eliezer D. Alpha-synuclein function and dysfunction on cellular membranes. Exp Neurol 2014;23:292–313.
[32] Beyer K. Mechanistic aspects of Parkinson's disease: alpha-synuclein and the biomembrane. Cell Biochem Biophys 2007;47:285–99.
[33] Bussell Jr R, Eliezer D. A structural and functional role for 11-mer repeats in alpha-synuclein and other exchangeable lipid binding proteins. J Mol Biol 2003;329:763–78.
[34] Fusco G, De Simone A, Gopinath T, Vostrikov V, Vendruscolo M, Dobson CM, et al. Direct observation of the three regions in alpha-synuclein that determine its membrane-bound behaviour. Nat Commun 2014;5:3827.
[35] Bodner CR, Maltsev AS, Dobson CM, Bax A. Differential phospholipid binding of alpha-synuclein variants implicated in Parkinson's disease revealed by solution NMR spectroscopy. Biochemistry 2010;49:862–71.
[36] Bartels T, Ahlstrom LS, Leftin A, Kamp F, Haass C, Brown MF, et al. The N-terminus of the intrinsically disordered protein alpha-synuclein triggers membrane binding and helix folding. Biophys J 2010;99:2116–24.
[37] Ulmer TS, Bax A, Cole NB, Nussbaum RL. Structure and dynamics of micelle-bound human alpha-synuclein. J Biol Chem 2005;280:9595–603.

[38] Stockl M, Fischer P, Wanker E, Herrmann A. Alpha-synuclein selectively binds to anionic phospholipids embedded in liquid-disordered domains. J Mol Biol 2008; 375:1394–404.
[39] Davidson WS, Jonas A, Clayton DF, George JM. Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. J Biol Chem 1998;273: 9443–9.
[40] Rhoades E, Ramlall TF, Webb WW, Eliezer D. Quantification of alpha-synuclein binding to lipid vesicles using fluorescence correlation spectroscopy. Biophys J 2006;90:4692–700.
[41] McMahon HT, Gallop JL. Membrane curvature and mechanisms of dynamic cell membrane remodelling. Nature 2005;438:590–6.
[42] Osman C, Voelker DR, Langer T. Making heads or tails of phospholipids in mitochondria. J Biol Chem 2011;192:7–16.
[43] Kubo S, Nemani VM, Chalkley RJ, Anthony MD, Hattori N, Mizuno Y, et al. A combinatorial code for the interaction of alpha-synuclein with membranes. J Biol Chem 2005;280:31664–72.
[44] Perrin RJ, Woods WS, Clayton DF, George JM. Exposure to long chain polyunsaturated fatty acids triggers rapid multimerization of synucleins. J Biol Chem 2001; 276:41958–62.
[45] Ouberai MM, Wang J, Swann MJ, Galvagnion C, Guilliams T, Dobson CM, et al. Alpha-synuclein senses lipid packing defects and induces lateral expansion of lipids leading to membrane remodeling. J Biol Chem 2013;288:20883–95.
[46] Hahl H, Moller I, Kiesel I, Campioni S, Riek R, Verdes D, et al. Alpha-synuclein insertion into supported lipid bilayers as seen by in situ X-ray reflectivity. ACS Chem Neurosci 2015;6:374–9.
[47] Shi Z, Sachs JN, Rhoades E, Baumgart T. Biophysics of alpha-synuclein induced membrane remodelling. Phys Chem Chem Phys 2015;17:15561–8.
[48] Shvadchak VV, Falomir-Lockhart LJ, Yushchenko DA, Jovin TM. Specificity and kinetics of alpha-synuclein binding to model membranes determined with fluorescent excited state intramolecular proton transfer (ESIPT) probe. J Biol Chem 2011; 286:13023–32.
[49] Kjaer L, Giehm L, Heimburg T, Otzen D. The influence of vesicle size and composition on alpha-synuclein structure and stability. Biophys J 2009;96: 2857–70.
[50] Kamp F, Beyer K. Binding of alpha-synuclein affects the lipid packing in bilayers of small vesicles. J Biol Chem 2006;281:9251–9.
[51] Trexler AJ, Rhoades E. Alpha-synuclein binds large unilamellar vesicles as an extended helix. Biochemistry 2009;48:2304–6.
[52] Haque F, Pandey AP, Cambrea LR, Rochet JC, Hovis JS. Adsorption of alpha-synuclein on lipid bilayers: modulating the structure and stability of protein assemblies. J Phys Chem B 2010;114:4070–81.
[53] Middleton ER, Rhoades E. Effects of curvature and composition on alpha-synuclein binding to lipid vesicles. Biophys J 2010;99:2279–88.
[54] Pfefferkorn CM, Jiang Z, Lee JC. Biophysics of alpha-synuclein membrane interactions. Biochim Biophys Acta 1818;2012:162–71.
[55] Pirc K, Ulrih NP. Alpha-synuclein interactions with phospholipid model membranes: key roles for electrostatic interactions and lipid-bilayer structure. Biochim Biophys Acta 2015.
[56] Kamp F, Exner N, Lutz AK, Wender N, Hegermann J, Brunner B, et al. Inhibition of mitochondrial fusion by alpha-synuclein is rescued by PINK1, Parkin and DJ-1. EMBO J 2010;29:3571–89.
[57] Rivers RC, Kumita JR, Tartaglia GG, Dedmon MM, Pawar A, Vendruscolo M, et al. Molecular determinants of the aggregation behavior of alpha- and beta-synuclein. Protein Sci PublProten Soc 2008;17:887–98.
[58] Lee HJ, Choi C, Lee SJ. Membrane-bound alpha-synuclein has a high aggregation propensity and the ability to seed the aggregation of the cytosolic form. J Biol Chem 2002;277:671–8.
[59] Necula M, Chirita CN, Kuret J. Rapid anionic micelle-mediated alpha-synuclein fibrillization in vitro. J Biol Chem 2003;278:46674–80.
[60] Zhu M, Li J, Fink AL. The association of alpha-synuclein with membranes affects bilayer structure, stability, and fibril formation. J Biol Chem 2003;278:40186–97.
[61] Sharon R, Bar-Joseph I, Frosch MP, Walsh DM, Hamilton JA, Selkoe DJ. The formation of highly soluble oligomers of alpha-synuclein is regulated by fatty acids and enhanced in Parkinson's disease. Neuron 2003;37:583–95.
[62] Cole NB, Murphy DD, Grider T, Rueter S, Brasaemle D, Nussbaum RL. Lipid droplet binding and oligomerization properties of the Parkinson's disease protein alpha-synuclein. J Biol Chem 2002;277:6344–52.
[63] De Franceschi G, Frare E, Pivato M, Relini A, Penco A, Greggio E, et al. Structural and morphological characterization of aggregated species of alpha-synuclein induced by docosahexaenoic acid. J Biol Chem 2011;286:22262–74.
[64] Zhu M, Fink AL. Lipid binding inhibits alpha-synuclein fibril formation. J Biol Chem 2003;278:16873–7.
[65] Aisenbrey C, Borowik T, Bystrom R, Bokvist M, Lindstrom F, Misiak H, et al. How is protein aggregation in amyloidogenic diseases modulated by biological membranes? Eur Biophys J 2008;37:247–55.
[66] Hardt SL. Rates of diffusion controlled reactions in one, two and three dimensions. Biophys Chem 1979;10:239–43.
[67] Tsigelny IF, Sharikov Y, Miller MA, Masliah E. Mechanism of alpha-synuclein oligomerization and membrane interaction: theoretical approach to unstructured proteins studies. Nanomed: Nanotechnol, Biol Med 2008;4:350–7.
[68] Rabe M, Soragni A, Reynolds NP, Verdes D, Liverani E, Riek R, et al. On-surface aggregation of alpha-synuclein at nanomolar concentrations results in two distinct growth mechanisms. ACS Chem Neurosci 2013;4:408–17.
[69] Galvagnion C, Buell AK, Meisl G, Michaels TC, Vendruscolo M, Knowles TP, et al. Lipid vesicles trigger alpha-synuclein aggregation by stimulating primary nucleation. Nat Chem Biol 2015;11:229–34.
[70] Smith DP, Tew DJ, Hill AF, Bottomley SP, Masters CL, Barnham KJ, et al. Formation of a high affinity lipid-binding intermediate during the early aggregation phase of alpha-synuclein. Biochemistry 2008;47:1425–34.
[71] Sood N, Jayaram G. Cytology of papillary adenoma of the nipple: a case diagnosed on fine-needle aspiration. Diagn Cytopathol 1990;6:345–8.
[72] Comellas G, Lemkau LR, Zhou DH, George JM, Rienstra CM. Structural intermediates during alpha-synuclein fibrillogenesis on phospholipid vesicles. J Am Chem Soc 2012;134:5090–9.
[73] Lorenzen N, Nielsen SB, Buell AK, Kaspersen JD, Arosio P, Vad BS, et al. The role of stable alpha-synuclein oligomers in the molecular events underlying amyloid formation. J Am Chem Soc 2014;136:3859–68.
[74] Celej MS, Sarroukh R, Goormaghtigh E, Fidelio GD, Ruysschaert JM, Raussens V. Toxic prefibrillar alpha-synuclein amyloid oligomers adopt a distinctive antiparallel beta-sheet structure. Biochem J 2012;443:719–26.
[75] Jensen PH, Nielsen MS, Jakes R, Dotti CG, Goedert M. Binding of alpha-synuclein to brain vesicles is abolished by familial Parkinson's disease mutation. J Biol Chem 1998;273:26292–4.
[76] Jo E, Fuller N, Rand RP, St George-Hyslop P, Fraser PE. Defective membrane interactions of familial Parkinson's disease mutant A30P alpha-synuclein. J Mol Biol 2002; 315:799–807.
[77] Bussell Jr R, Eliezer D. Effects of Parkinson's disease-linked mutations on the structure of lipid-associated alpha-synuclein. Biochemistry 2004;43:4810–8.
[78] Tsigelny IF, Sharikov Y, Kouznetsova VL, Greenberg JP, Wrasidlo W, Overk C, et al. Molecular determinants of alpha-synuclein mutants' oligomerization and membrane interactions. ACS Chem Neurosci 2015;6:403–16.
[79] Ysselstein D, Joshi M, Mishra V, Griggs AM, Asiago JM, McCabe GP, et al. Effects of impaired membrane interactions on alpha-synuclein aggregation and neurotoxicity. Neurobiol Dis 2015;79:150–63.
[80] van Rooijen BD, Claessens MM, Subramaniam V. Membrane binding of oligomeric alpha-synuclein depends on bilayer charge and packing. FEBS Lett 2008;582: 3788–92.
[81] Lorenzen N, Lemminger L, Pedersen JN, Nielsen SB, Otzen DE. The N-terminus of alpha-synuclein is essential for both monomeric and oligomeric interactions with membranes. FEBS Lett 2014;588:497–502.
[82] Vamvaca K, Volles MJ, Lansbury Jr PT. The first N-terminal amino acids of alpha-synuclein are essential for alpha-helical structure formation in vitro and membrane binding in yeast. J Mol Biol 2009;389:413–24.
[83] Caruana M, Neuner J, Hogen T, Schmidt F, Kamp F, Scerri C, et al. Polyphenolic compounds are novel protective agents against lipid membrane damage by alpha-synuclein aggregates in vitro. Biochim Biophys Acta 1818;2012:2502–10.
[84] Stockl M, Claessens MM, Subramaniam V. Kinetic measurements give new insights into lipid membrane permeabilization by alpha-synuclein oligomers. Mol BioSyst 2012;8:338–45.
[85] Hogen T, Levin J, Schmidt F, Caruana M, Vassallo N, Kretzschmar H, et al. Two different binding modes of alpha-synuclein to lipid vesicles depending on its aggregation state. Biophys J 2012;102:1646–55.
[86] Askarova S, Yang X, Lee JC. Impacts of membrane biophysics in Alzheimer's disease: from amyloid precursor protein processing to abeta peptide-induced membrane changes. Int J Alzheimers Dis 2011;2011:134971.
[87] Flach K, Hilbrich I, Schiffmann A, Gartner U, Kruger M, Leonhardt M, et al. Tau oligomers impair artificial membrane integrity and cellular viability. J Biol Chem 2012; 287:43223–33.
[88] Engel MF. Membrane permeabilization by islet amyloid polypeptide. Chem Phys Lipids 2009;160:1–10.
[89] Chich JF, Chapuis C, Henry C, Vidic J, Rezaei H, Noinville S. Vesicle permeabilization by purified soluble oligomers of prion protein: a comparative study of the interaction of oligomers and monomers with lipid membranes. J Mol Biol 2010;397: 1017–30.
[90] Diociaiuti M, Macchia G, Paradisi S, Frank C, Camerini S, Chistolini P, et al. Native metastable prefibrillar oligomers are the most neurotoxic species among amyloid aggregates. Biochim Biophys Acta 1842;2014:1622–9.
[91] Relini A, Torrassa S, Rolandi R, Gliozzi A, Rosano C, Canale C, et al. Monitoring the process of HypF fibrillization and liposome permeabilization by protofibrils. J Mol Biol 2004;338:943–57.
[92] Andreasen M, Lorenzen N, Otzen D. Interactions between misfolded protein oligomers and membranes: a central topic in neurodegenerative diseases? Biochim Biophys Acta 2015.
[93] Brogden KA. Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat Rev Microbiol 2005;3:238–50.
[94] Maguire-Zeiss KA, Short DW, Federoff HJ. Synuclein, dopamine and oxidative stress: co-conspirators in Parkinson's disease? Brain Res Mol Brain Res 2005; 134:18–23.
[95] Rochet JC, Outeiro TF, Conway KA, Ding TT, Volles MJ, Lashuel HA, et al. Interactions among alpha-synuclein, dopamine, and biomembranes: some clues for understanding neurodegeneration in Parkinson's disease. J Mol Neurosci 2004; 23:23–34.
[96] Cappai R, Leck SL, Tew DJ, Williamson NA, Smith DP, Galatis D, et al. Dopamine promotes alpha-synuclein aggregation into SDS-resistant soluble oligomers via a distinct folding pathway. FASEB J 2005;19:1377–9.
[97] Turnbull S, Tabner BJ, El-Agnaf OM, Moore S, Davies Y, Allsop D. Alpha-synuclein implicated in Parkinson's disease catalyses the formation of hydrogen peroxide in vitro. Free Radic Biol Med 2001;30:1163–70.
[98] Feng LR, Maguire-Zeiss KA. Dopamine and paraquat enhance alpha-synuclein-induced alterations in membrane conductance. Neurotox Res 2011;20:387–401.
[99] Pham CL, Cappai R. The interplay between lipids and dopamine on alpha-synuclein oligomerization and membrane binding. Biosci Rep 2013;33.

[100] Follmer C, Coelho-Cerqueira E, Yatabe-Franco DY, Araujo GD, Pinheiro AS, Domont GB, et al. Oligomerization and membrane-binding properties of covalent adducts formed by the interaction of alpha-synuclein with the toxic dopamine metabolite 3,4-dihydroxyphenylacetaldehyde (DOPAL). J Biol Chem 2015.
[101] Volles MJ, Lansbury Jr PT. Vesicle permeabilization by protofibrillar alpha-synuclein is sensitive to Parkinson's disease-linked mutations and occurs by a pore-like mechanism. Biochemistry 2002;41:4595–602.
[102] Kim HY, Cho MK, Kumar A, Maier E, Siebenhaar C, Becker S, et al. Structural properties of pore-forming oligomers of alpha-synuclein. J Am Chem Soc 2009;131:17482–9.
[103] Di Pasquale E, Fantini J, Chahinian H, Maresca M, Taieb N, Yahi N. Altered ion channel formation by the Parkinson's-disease-linked E46K mutant of alpha-synuclein is corrected by GM3 but not by GM1 gangliosides. J Mol Biol 2010;397:202–18.
[104] Kostka M, Hogen T, Danzer KM, Levin J, Habeck M, Wirth A, et al. Single particle characterization of iron-induced pore-forming alpha-synuclein oligomers. J Biol Chem 2008;283:10992–1003.
[105] Hillmer AS, Putcha P, Levin J, Hogen T, Hyman BT, Kretzschmar H, et al. Converse modulation of toxic alpha-synuclein oligomers in living cells by N′-benzylidene-benzohydrazide derivates and ferric iron. Biochem Biophys Res Commun 2010;391:461–6.
[106] Schmidt F, Levin J, Kamp F, Kretzschmar H, Giese A, Botzel K. Single-channel electrophysiology reveals a distinct and uniform pore complex formed by alpha-synuclein oligomers in lipid membranes. PLoS ONE 2012;7, e42545.
[107] Zakharov SD, Hulleman JD, Dutseva EA, Antonenko YN, Rochet JC, Cramer WA. Helical alpha-synuclein forms highly conductive ion channels. Biochemistry 2007;46:14369–79.
[108] Tosatto L, Andrighetti AO, Plotegher N, Antonini V, Tessari I, Ricci L, et al. Alpha-synuclein pore forming activity upon membrane association. Biochim Biophys Acta 1818;2012:2876–83.
[109] Lashuel HA, Petre BM, Wall J, Simon M, Nowak RJ, Walz T, et al. Alpha-synuclein, especially the Parkinson's disease-associated mutants, forms pore-like annular and tubular protofibrils. J Mol Biol 2002;322:1089–102.
[110] Quist A, Doudevski I, Lin H, Azimova R, Ng D, Frangione B, et al. Amyloid ion channels: a common structural link for protein-misfolding disease. Proceedings of the National Academy of Sciences of the United States of America, 102. 2005. p. 10427–32.
[111] Giehm L, Svergun DI, Otzen DE, Vestergaard B. Low-resolution structure of a vesicle disrupting alpha-synuclein oligomer that accumulates during fibrillation. Proceedings of the National Academy of Sciences of the United States of America, 108. 2011. p. 3246–51.
[112] Tsigelny IF, Sharikov Y, Wrasidlo W, Gonzalez T, Desplats PA, Crews L, et al. Role of alpha-synuclein penetration into the membrane in the mechanisms of oligomer pore formation. FEBS J 2012;279:1000–13.
[113] Demuro A, Mina E, Kayed R, Milton SC, Parker I, Glabe CG. Calcium dysregulation and membrane disruption as a ubiquitous neurotoxic mechanism of soluble amyloid oligomers. J Biol Chem 2005;280:17294–300.
[114] Danzer KM, Haasen D, Karow AR, Moussaud S, Habeck M, Giese A, et al. Different species of alpha-synuclein oligomers induce calcium influx and seeding. J Neurosci 2007;27:9220–32.
[115] Furukawa K, Matsuzaki-Kobayashi M, Hasegawa T, Kikuchi A, Sugeno N, Itoyama Y, et al. Plasma membrane ion permeability induced by mutant alpha-synuclein contributes to the degeneration of neural cells. J Neurochem 2006;97:1071–7.
[116] Kayed R, Sokolov Y, Edmonds B, McIntire TM, Milton SC, Hall JE, et al. Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases. J Biol Chem 2004;279:46363–6.
[117] Stockl MT, Zijlstra N, Subramaniam V. Alpha-synuclein oligomers: an amyloid pore? Insights into mechanisms of alpha-synuclein oligomer–lipid interactions. Mol Neurobiol 2013;47:613–21.
[118] Sokolov Y, Kozak JA, Kayed R, Chanturiya A, Glabe C, Hall JE. Soluble amyloid oligomers increase bilayer conductance by altering dielectric structure. J Gen Physiol 2006;128:637–47.
[119] Last NB, Miranker AD. Common mechanism unites membrane poration by amyloid and antimicrobial peptides. Proceedings of the National Academy of Sciences of the United States of America, 110. 2013. p. 6382–7.
[120] Lee MT, Chen FY, Huang HW. Energetics of pore formation induced by membrane active peptides. Biochemistry 2004;43:3590–9.
[121] Reynolds NP, Soragni A, Rabe M, Verdes D, Liverani E, Handschin S, et al. Mechanism of membrane interaction and disruption by alpha-synuclein. J Am Chem Soc 2011;133:19366–75.
[122] Hellstrand E, Nowacka A, Topgaard D, Linse S, Sparr E. Membrane lipid co-aggregation with alpha-synuclein fibrils. PLoS ONE 2013;8, e77235.
[123] Chaudhary H, Stefanovic AN, Subramaniam V, Claessens MM. Membrane interactions and fibrillization of alpha-synuclein play an essential role in membrane disruption. FEBS Lett 2014;588:4457–63.
[124] van Maarschalkerweerd A, Vetri V, Langkilde AE, Fodera V, Vestergaard B. Protein/lipid coaggregates are formed during alpha-synuclein-induced disruption of lipid bilayers. Biomacromolecules 2014;15:3643–54.
[125] Winner B, Jappelli R, Maji SK, Desplats PA, Boyer L, Aigner S, et al. In vivo demonstration that alpha-synuclein oligomers are toxic. Proceedings of the National Academy of Sciences of the United States of America, 108. 2011. p. 4194–9.
[126] Monteiro JP, Oliveira PJ, Jurado AS. Mitochondrial membrane lipid remodeling in pathophysiology: a new target for diet and therapeutic interventions. Prog Lipid Res 2013;52:513–28.
[127] Colbeau A, Nachbaur J, Vignais PM. Enzymic characterization and lipid composition of rat liver subcellular membranes. Biochim Biophys Acta 1971;249:462–92.
[128] Klingenberg M. Cardiolipin and mitochondrial carriers. Biochim Biophys Acta 1788;2009:2048–58.
[129] Ardail D, Privat JP, Egret-Charlier M, Levrat C, Lerme F, Louisot P. Mitochondrial contact sites. Lipid composition and dynamics. J Biol Chem 1990;265:18797–802.
[130] Mileykovskaya E, Dowhan W. Cardiolipin membrane domains in prokaryotes and eukaryotes. Biochim Biophys Acta 1788;2009:2084–91.
[131] Szeto HH. First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. Br J Pharmacol 2014;171:2029–50.
[132] Unsay JD, Cosentino K, Subburaj Y, Garcia-Saez AJ. Cardiolipin effects on membrane structure and dynamics. Langmuir ACS Surf Colloid 2013;29:15878–87.
[133] Zeczycki TN, Whelan J, Hayden WT, Brown DA, Shaikh SR. Increasing levels of cardiolipin differentially influence packing of phospholipids found in the mitochondrial inner membrane. Biochem Biophys Res Commun 2014;450:366–71.
[134] Parihar MS, Parihar A, Fujita M, Hashimoto M, Ghafourifar P. Mitochondrial association of alpha-synuclein causes oxidative stress. Cell Mol Life Sci 2008;65:1272–84.
[135] Nakamura K, Nemani VM, Wallender EK, Kaehlcke K, Ott M, Edwards RH. Optical reporters for the conformation of alpha-synuclein reveal a specific interaction with mitochondria. J Neurosci Off J Soc Neurosci 2008;28:12305–17.
[136] Robotta M, Gerding HR, Vogel A, Hauser K, Schildknecht S, Karreman C, et al. Alpha-synuclein binds to the inner membrane of mitochondria in an alpha-helical conformation. Chembiochem Eur J Chem Biol 2014;15:2499–502.
[137] Devi L, Raghavendran V, Prabhu BM, Avadhani NG, Anandatheerthavarada HK. Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. J Biol Chem 2008;283:9089–100.
[138] Robotta M, Hintze C, Schildknecht S, Zijlstra N, Jungst C, Karreman C, et al. Locally resolved membrane binding affinity of the N-terminus of alpha-synuclein. Biochemistry 2012;51:3960–2.
[139] Zigoneanu IG, Yang YJ, Krois AS, Haque E, Pielak GJ. Interaction of alpha-synuclein with vesicles that mimic mitochondrial membranes. Biochim Biophys Acta 1818;2012:512–9.
[140] Li WW, Yang R, Guo JC, Ren HM, Zha XL, Cheng JS, et al. Localization of alpha-synuclein to mitochondria within midbrain of mice. Neuroreport 2007;18:1543–6.
[141] Lu L, Zhang C, Cai Q, Lu Q, Duan C, Zhu Y, et al. Voltage-dependent anion channel involved in the alpha-synuclein-induced dopaminergic neuron toxicity in rats. Acta Biochim Biophys Sin 2013;45:170–8.
[142] Rostovtseva TK, Gurnev PA, Protchenko O, Hoogerheide DP, Yap TL, Philpott CC, et al. Alpha-synuclein shows high-affinity interaction with voltage-dependent anion channel suggesting mechanisms of mitochondrial regulation and toxicity in Parkinson disease. J Biol Chem 2015.
[143] Stefanovic AN, Stockl MT, Claessens MM, Subramaniam V. Alpha-synuclein oligomers distinctively permeabilize complex model membranes. FEBS J 2014;281:2838–50.
[144] Camilleri A, Zarb C, Caruana M, Ostermeier U, Ghio S, Hogen T, et al. Mitochondrial membrane permeabilisation by amyloid aggregates and protection by polyphenols. Biochim Biophys Acta 1828;2013:2532–43.
[145] Luth ES, Stavrovskaya IG, Bartels T, Kristal BS, Selkoe DJ. Soluble, prefibrillar alpha-synuclein oligomers promote complex I-dependent, Ca^{2+}-induced mitochondrial dysfunction. J Biol Chem 2014;289:21490–507.
[146] Banerjee K, Sinha M, Pham Cle L, Jana S, Chanda D, Cappai R, et al. Alpha-synuclein induced membrane depolarization and loss of phosphorylation capacity of isolated rat brain mitochondria: implications in Parkinson's disease. FEBS Lett 2010;584:1571–6.
[147] Shen J, Du T, Wang X, Duan C, Gao G, Zhang J, et al. Alpha-synuclein amino terminus regulates mitochondrial membrane permeability. Brain Res 2014;1591:14–26.
[148] Nakamura K, Nemani VM, Azarbal F, Skibinski G, Levy JM, Egami K, et al. Direct membrane association drives mitochondrial fission by the Parkinson disease-associated protein alpha-synuclein. J Biol Chem 2011;286:20710–26.
[149] Flower TR, Chesnokova LS, Froelich CA, Dixon C, Witt SN. Heat shock prevents alpha-synuclein-induced apoptosis in a yeast model of Parkinson's disease. J Mol Biol 2005;351:1081–100.
[150] Su LJ, Auluck PK, Outeiro TF, Yeger-Lotem E, Kritzer JA, Tardiff DF, et al. Compounds from an unbiased chemical screen reverse both ER-to-Golgi trafficking defects and mitochondrial dysfunction in Parkinson's disease models. Dis Model Mech 2010;3:194–208.
[151] Mileykovskaya E, Dowhan W. Cardiolipin-dependent formation of mitochondrial respiratory supercomplexes. Chem Phys Lipids 2014;179:42–8.
[152] Fry M, Green DE. Cardiolipin requirement for electron transfer in complex I and III of the mitochondrial respiratory chain. J Biol Chem 1981;256:1874–80.
[153] Zhang M, Mileykovskaya E, Dowhan W. Gluing the respiratory chain together. Cardiolipin is required for supercomplex formation in the inner mitochondrial membrane. J Biol Chem 2002;277:43553–6.
[154] Bayir H, Kapralov AA, Jiang J, Huang Z, Tyurina YY, Tyurin VA, et al. Peroxidase mechanism of lipid-dependent cross-linking of synuclein with cytochrome C: protection against apoptosis versus delayed oxidative stress in Parkinson disease. J Biol Chem 2009;284:15951–69.
[155] Claypool SM, Oktay Y, Boontheung P, Loo JA, Koehler CM. Cardiolipin defines the interactome of the major ADP/ATP carrier protein of the mitochondrial inner membrane. J Cell Biol 2008;182:937–50.
[156] Beyer K, Nuscher B. Specific cardiolipin binding interferes with labeling of sulfhydryl residues in the adenosine diphosphate/adenosine triphosphate carrier protein from beef heart mitochondria. Biochemistry 1996;35:15784–90.
[157] Ellis CE, Murphy EJ, Mitchell DC, Golovko MY, Scaglia F, Barcelo-Coblijn GC, et al. Mitochondrial lipid abnormality and electron transport chain impairment in mice lacking alpha-synuclein. Mol Cell Biol 2005;25:10190–201.

[158] Gasanov SE, Shrivastava IH, Israilov FS, Kim AA, Rylova KA, Zhang B, et al. *Naja naja oxiana* cobra venom cytotoxins CTI and CTII disrupt mitochondrial membrane integrity: implications for basic three-fingered cytotoxins. PLoS ONE 2015;10, e0129248.
[159] Xu J, Vanderlick TK, Beales PA. Lytic and non-lytic permeabilization of cardiolipin-containing lipid bilayers induced by cytochrome C. PLoS ONE 2013;8, e69492.
[160] Firsov AM, Kotova EA, Korepanova EA, Osipov AN, Antonenko YN. Peroxidative permeabilization of liposomes induced by cytochrome c/cardiolipin complex. Biochim Biophys Acta 1848;2015:767–74.
[161] Sarafian TA, Ryan CM, Souda P, Masliah E, Kar UK, Vinters HV, et al. Impairment of mitochondria in adult mouse brain overexpressing predominantly full-length, N-terminally acetylated human alpha-synuclein. PLoS ONE 2013;8, e63557.
[162] Bender A, Desplats P, Spencer B, Rockenstein E, Adame A, Elstner M, et al. TOM40 mediates mitochondrial dysfunction induced by alpha-synuclein accumulation in Parkinson's disease. PLoS ONE 2013;8, e62277.
[163] Martin LJ, Pan Y, Price AC, Sterling W, Copeland NG, Jenkins NA, et al. Parkinson's disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. J Neurosci Off J Soc Neurosci 2006;26:41–50.
[164] Li L, Nadanaciva S, Berger Z, Shen W, Paumier K, Schwartz J, et al. Human A53T alpha-synuclein causes reversible deficits in mitochondrial function and dynamics in primary mouse cortical neurons. PLoS ONE 2013;8, e85815.
[165] Zhu Y, Duan C, Lu L, Gao H, Zhao C, Yu S, et al. Alpha-synuclein overexpression impairs mitochondrial function by associating with adenylate translocator. Int J Biochem Cell Biol 2011;43:732–41.
[166] Relini A, Marano N, Gliozzi A. Probing the interplay between amyloidogenic proteins and membranes using lipid monolayers and bilayers. Adv Colloid Interf Sci 2014;207:81–92.
[167] Pope S, Land JM, Heales SJ. Oxidative stress and mitochondrial dysfunction in neurodegeneration; cardiolipin a critical target? Biochim Biophys Acta 1777;2008:794–9.
[168] Lutter M, Fang M, Luo X, Nishijima M, Xie X, Wang X. Cardiolipin provides specificity for targeting of tBid to mitochondria. Nat Cell Biol 2000;2:754–61.
[169] Gonzalvez F, Pariselli F, Jalmar O, Dupaigne P, Sureau F, Dellinger M, et al. Mechanistic issues of the interaction of the hairpin-forming domain of tBid with mitochondrial cardiolipin. PLoS ONE 2010;5, e9342.
[170] Raemy E, Martinou JC. Involvement of cardiolipin in tBID-induced activation of BAX during apoptosis. Chem Phys Lipids 2014;179:70–4.
[171] Jin H, Kanthasamy A, Ghosh A, Anantharam V, Kalyanaraman B, Kanthasamy AG. Mitochondria-targeted antioxidants for treatment of Parkinson's disease: preclinical and clinical outcomes. Biochim Biophys Acta 1842;2014:1282–94.
[172] Wagner J, Ryazanov S, Leonov A, Levin J, Shi S, Schmidt F, et al. Anle138b: a novel oligomer modulator for disease-modifying therapy of neurodegenerative diseases such as prion and Parkinson's disease. Acta Neuropathol 2013;125:795–813.
[173] Birk AV, Liu S, Soong Y, Mills W, Singh P, Warren JD, et al. The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin. J Am Soc Nephrol 2013;24:1250–61.