INTRODUCTION

Alpha-synuclein is a soluble, 140-residue, predominantly presynaptic protein that is highly conserved in vertebrates and has been implicated in Parkinson's Disease as well as other eponymously named "synucleinopathies" such as dementia with Lewy bodies and multiple system atrophy [1-7]. Several rare mutations in the alpha-synuclein gene (SNCA), have been identified in cases of familial Parkinson's Disease (A53T, A30P, E46K, and most recently H50Q and G51D), and SNCA gene duplications and triplications similarly lead to familial PD [8-15]. Lewy Bodies and Lewy Neurites, pathological hallmarks of the synucleinopathies, are composed largely of beta-sheet rich alpha-synuclein amyloid fibrils [3]. Alpha synuclein's contribution to such disorders could in principle result either from a toxic gain of function resulting from synuclein oligomerization and/or aggregation, or from a loss or perturbation of normal synuclein function (or possibly from a combination of the two). Unfortunately, the normal functions of alpha-synuclein remain elusive, though in general it has been implicated in synaptic plasticity [16] and learning [17], neurotransmitter release [18,19], and synaptic vesicle pool maintenance [2,20,21].

Alpha synuclein is intrinsically disordered when free in solution [22-24]. The N-terminal ~100 residues of the protein constitute a lipid-binding domain that contains 7 imperfect 11-residue repeats, each centered on a variation of a KTKEGV core consensus sequence. Similar repeat sequences are found in the exchangeable apolipoproteins, and as for many apolipoproteins, the N-terminal lipid-binding domain of alpha synuclein adopts an amphipathic helical structure upon binding to detergent micelles.
or phospholipid vesicles. Residues 61-95 of the N-terminal domain constitute a hydrophobic region referred to as the NAC domain (for non-Aβ component of senile plaques) that may contribute critically to synuclein oligomerization and aggregation [1]. The acidic C-terminal ~40 residues of the protein, often referred to as the C-terminal domain or tail, remain disordered even in the presence of membranes, although evidence exists for limited interactions of this region with membranes [1,25-29]. The membrane-induced disorder-to-order transition of the N-terminal lipid-binding domain is considered functionally important and has been characterized in a wide variety of contexts. Several helical membrane-bound conformations have been observed, featuring amphipathic helices that lie along the surface of the membrane with their apolar face embedded as deep as the C3 or C4 acyl chain carbons [30-32], and interfacial lysine residues may “snorkel” from the membrane interior to interact with negatively charged lipid headgroups [33,34]. An extended-helix conformation binds to the membrane surface via an ~100 residue long amphipathic alpha helix [32,35-39] with an unusual 11/3 periodicity [25,30-32]. A broken-helix conformation has also been observed in which the extended-helix is broken into two distinct helices separated by a non-helical linker region spanning residues 39-45 [25,40,41]. Both conformations have been observed in the context of both detergent micelles and lipid vesicles [25,30-32,35,37,41-46]. Additional binding modes observed on phospholipid vesicles involve a shorter helix at the N-terminus of the lipid-binding domain with the remainder of the domain remaining unbound. These include an SL1 binding mode involving the 25 N-terminal residues [28,47] and a binding mode where residues up to 19 are bound, but residues beyond 69 are not [48]. Structures comparable to these partly helical binding modes have also been observed in mixtures of organic and aqueous solvents [49] and on n-octyl-β-glucopyranoside (BOG) detergent micelles [50], and one of the most recently reported PD-associated mutations also seems to favor such binding modes [51]. Though such conformational states have been posited to contribute to synuclein’s putative functions, the detailed relationship between synuclein membrane binding, structure, and function remains an important open avenue of investigation [2]. Likewise, membrane interactions may mediate pathological roles of alpha synuclein, either through membrane effects on synuclein aggregation or through synuclein effects on membrane structure and integrity [1,2,52]. Below we delineate possible normal functions of synuclein at the membrane and discuss a number of possible membrane-associated targets; we then consider the role that membranes might play in synuclein dysfunction and how this ultimately contributes to disease.

**Recent Developments in Synuclein/Membrane Biophysics at the Synapse**

A detailed mechanistic understanding of synuclein function will ultimately require additional characterization of its structure and dynamics at the membrane surface as well as of the membrane determinants of synuclein binding. These will be critical for generating and verifying hypotheses regarding mechanisms of action and relevant in vivo binding target(s) – ie. specific cellular membrane(s) at which synuclein exerts its functions. Synaptic vesicles are considered the “classic” cellular membrane binding target for alpha synuclein. Synuclein localizes to the presynaptic terminal and specifically to synaptic vesicles, to which it can directly bind [17,53-58]. It has become clear, however, that alpha-synuclein may in fact interact with a wider variety of cellular membranes than previously expected and that these interactions may contribute to alpha synuclein function, pathology, or both. Efforts to characterize the membrane properties that favor synuclein binding indicate that both electrostatic interactions and hydrophobic interactions contribute to binding [1,59]. Membrane curvature also plays a key role, with enhanced binding to membranes of increased curvature [60-63]. This likely results from an increased size and number of so-called packing defects in more highly curved membranes [64,65]. Packing defects are regions where the hydrophobic acyl chain interior of the membrane is transiently exposed, and they likely act as effective protein binding sites [60-62,64,66-68]. Lipid headgroup composition, which can influence both charge and curvature, also modulates synuclein binding. An increased percentage of conical lipids such as phosphatidylethanolamine (PE) increases binding, perhaps through enhanced formation of packing defects [66]. Finally, electrostatic interactions with positively charged synuclein residues (in particular the many lysines) are enhanced by increasing the membrane negative charge density [27,60,61,66,67,69-71]. Notably, synaptic vesicles present a highly curved, negatively charged membrane surface [72], making them an optimal target for synuclein binding.

Synuclein’s preference for more highly curved membranes has led to its classification as a “curvature sensing” protein [34,62,64]. In addition to sensing membrane curvature, alpha-synuclein is also able to actively alter membrane shape/curvature [28,36,73,74]. Such direct manipulation of the membrane could play a role in synaptic vesicle homeostasis and/or exocytosis, though whether, when, and how synuclein might actively model membranes in vivo remains unclear.

Multiple post-translational modifications of alpha-synuclein have been reported, including N-terminal acetylation [75],
serine/threonine and tyrosine phosphorylation [75-79], tyrosine nitration [80], ubiquitination [75,81,82], sumoylation [83], transglutamination [84-86], and methionine oxidation [87]. Many of these impact synuclein interaction with membranes, indicating that such modifications will influence synuclein behavior in ways that must be characterized. As an example, it was recently reported that alpha-synuclein is N-terminally acetylated [75], probably by the acetyltransferase NatB [88,89], and that this modification increased the transient helical propensity of the N-terminal ~10 residues in the free state [50,89-91]. Conflicting results were initially reported regarding the effects of N-terminal acetylation on membrane binding, with enhanced binding observed in some cases and a negligible impact in others [89,90]. These differences were likely due to the differing liposome compositions and sizes used by the different groups, and a more recent study examining a larger set of liposome sizes and compositions showed that N-terminal acetylation has a pronounced effect on binding to highly curved membranes of moderate charge, but less of an effect on more highly charged membranes [50]. Importantly, such highly curved vesicles of moderate negative charge approximate the properties of synaptic vesicles; thus, the impact of N-terminal acetylation appears greatest for liposomes most closely comparable to a known synuclein binding target in vivo [50].

Alpha-synuclein can be phosphorylated at multiple serine, threonine and tyrosine residues in vivo (including Y39, S87, Y125, and S129) [75-79,92]. The structural and functional consequences of such modifications have begun to be addressed but remain incompletely understood, in part because generating phosphorylated proteins for in vitro studies remains a challenging task [93-95]. Several recent studies have examined the impact of phosphorylation on synuclein membrane binding. Phosphorylation of S87 was found to reduce binding to membranes and alter the detergent micelle bound conformation, as well as expand the free state of the protein and increase its conformational flexibility [94]. Phosphorylation of S129 in contrast has little effect on membrane association by wild type alpha-synuclein [95], although some effect on the membrane-binding of PD-linked synuclein mutants was reported [96]. This modification was also shown to have little impact on the SDS-micelle bound conformation [95]. Phosphorylation of other residues is only just beginning to be characterized and further work is clearly needed to more completely elucidate how phosphorylation impacts membrane binding, and how its perturbation of synuclein structure, dynamics, and membrane binding might contribute to both function and pathology.

Other post-translational modifications that have been shown to influence membrane binding of alpha-synuclein include methionine oxidation [87,97] and tyrosine nitrosylation [29], but as with phosphorylation, the effects of these and other modifications remain to be more fully characterized. Interestingly, lysine acetylation was recently shown to occur in the Alzheimer’s protein tau and to be associated with tau pathology [98-100], and it may be interesting to see whether this or other less commonly reported types of modification may be discovered to occur on synuclein as well.

FUNCTIONAL IMPLICATIONS OF SYNUCLEIN INTERACTIONS WITH OTHER PROTEINS AT PRESYNAPTIC MEMBRANES

Alpha-synuclein structure/function relationships remain enigmatic but are perhaps best characterized in the context of presynaptic function and, more specifically, synaptic vesicle homeostasis. An area of focus is the role that synuclein/membrane and synuclein/protein interactions play in synuclein’s contribution to synaptic vesicle docking, priming, clustering, fusion, and/or recycling. Importantly, it is likely that synuclein/membrane interactions at the synapse are modulated by additional proteins. Indeed, it has been shown that dissociation of membrane-bound synuclein depends on brain-specific cytosolic proteins [101], though none were specifically identified in this particular study. Synuclein also binds to calmodulin [102-104], a protein that is thought to regulate secretory processes at the synapse in a variety of ways, including by interacting with protein targets such as calcium-CaM-dependent kinase II and by inhibiting SNARE-mediated membrane fusion. Binding to calmodulin is mediated by the N-terminal amphipathic helical region of alpha synuclein (ie. the membrane binding domain), and an NMR structure of N-terminally acetylated synuclein bound to Ca-bound calmodulin has been recently described [104]. Membrane-bound synuclein is released upon interacting with calmodulin, suggesting competition for synuclein between membranes and calmodulin [105]. This leads to a model in which calcium bound calmodulin mediates presynaptic depolarization-dependent dissociation of alpha-synuclein from the membrane surface. Conversely, GTP-Rab3a may stabilize synuclein on synaptic vesicles, as antibodies to Rab3a and RabGDI abrogated synuclein membrane binding, while inhibition of Rab3a recycling increased synuclein sequestration on intracellular membranes. Rab3a is a presynaptic Rab that interacts with the synapse-organizing complex of RIMalpha/Munc13/α-liprin, and so its contribution to synuclein membrane interactions is intriguing, but the functional consequences of such interactions are as of yet unknown [106]. Rab8a, a Rab GTPase that modulates post–Golgi vesicle trafficking, also interacts with synuclein in a Ser129-phosphorylation dependent manner.
[107]. As discussed below, Rab-mediated cellular trafficking is often perturbed by synuclein; thus, aberrant synuclein/Rab interactions could in principle contribute to neurodegeneration in synucleinopathies.

Synaptic vesicle fusion is mediated by three SNARE proteins – syntaxin-1, SNAP-25, and synaptobrevin-2 – whose SNARE motifs zipper into a four-helix bundle. Recent evidence suggests a contribution of alpha-synuclein to SNARE complex assembly through a direct interaction between alpha-synuclein and synaptobrevin-2 [18,19]. A potential role for synuclein in SNARE assembly first came from the observation that expression of synuclein rescues CSPalpha deficient mice in a phospholipid-binding dependent fashion [18]. CSPalpha is an abundant presynaptic chaperone, and deletion of CSPalpha inhibits SNARE complex assembly. Synuclein was subsequently reported to directly bind to synaptobrevin-2 and was proposed as a potential nonclassical chaperone facilitating SNARE complex assembly [19]. This raised the question of how synuclein might affect SNARE-mediated synaptic vesicle docking, priming, and/or fusion, and whether membrane binding could play a role in this function. Indeed, it has been proposed that the broken helical conformation of synuclein could span both the synaptic vesicle and plasma membranes and so help stabilize docked vesicles at the presynaptic membrane [2,108], and it was recently shown that synaptosomal preparations of plasma-membrane associated docked synaptic vesicles are enriched for synuclein when compared with preparations of unattached undocked vesicles [109]. Synuclein overexpression in cultured chromaffin cells inhibits catecholamine release by blocking a late step in the exocytosis process and, specifically, by inhibiting the fusion of docked vesicles [110]. In addition, synuclein has been implicated to function in maintenance of SV pool size [111], and it may enhance vesicle clustering, again perhaps through a membrane-bridging mode. This hypothesis is suggested by initial observations in yeast [112-115], and by more recent work showing that synuclein induces clustering of synaptic vesicle mimics [116]. In the latter study, clustering depended on synuclein interactions with both synaptobrevin-2 and with anionic lipids in phospholipid bilayers. Synuclein also inhibits SNARE-mediated vesicle fusion in in vitro lipid mixing assays, likely through inhibition of docking rather than of the fusion step itself [117,118]. Inhibition requires membrane binding by synuclein and may or may not require SNARE binding; it is possible that the requirement for SNARE-binding may in fact depend on synuclein’s oligomeric state [109,118,119]. Indeed, in some contexts, synuclein fails to interact with SNARE proteins and instead might modulate SNARE-mediated exocytosis through the more indirect sequestration of arachinoic acid, which can itself stimulate SNARE complex formation and exocytosis [120]. Synuclein additionally appears to promote clathrin-mediated endocytosis [121], and it is required for the fast kinetics of SV endocytosis through some impact on the early steps of SV endocytosis [122]. Finally, synuclein reportedly contributes to SV mobilization through its inhibition of SV reclustering after endocytosis [123]. All together, these observations point to some direct role for synuclein/membrane and synuclein/protein interactions in multiple steps of the synaptic vesicle cycle; however, open questions remain, particularly given the multitude of steps and apparent discrepancies observed across multiple studies.

As mentioned above, synuclein may directly generate membrane curvature and so remodel membranes, an activity that could also have a impact on synaptic vesicle fusion with the plasma membrane. Additional evidence for some role for synuclein in membrane remodeling processes comes from the early observation that synuclein may interact with and inhibit phospholipase D2, which catalyzes the hydrolysis of phosphatidylcholine to phosphatidic acid [112,124,125]. The synuclein/PLD2 interaction has been contested as well, however [126]. In vitro, PLD binding requires both the helical membrane binding N-terminal domain and the disordered C-terminal tail and can be modulated by synuclein phosphorylation [125,127]. PLD2 likely acts on the plasma membrane and is implicated in the regulation of secretory vesicle budding and/or fusion: phosphatidic acid may mediate processes involved in vesicular transport and changes in cell morphology by modulating membrane curvature and by regulating phosphatidylinositol-4-phosphate 5 kinase activity [128]. Interestingly, synuclein also displays higher affinity for membranes rich in PA [59,60,129], and so a feedback mechanism could be envisioned in which high levels of PA recruit synuclein, which then inhibits PLD2 and so reduces PA levels. The enzyme CTP:phosphocholine cytidyltransferase (CCT) seems to represent an interesting parallel to synuclein in this regard. Similarly to alpha-synuclein, CCT also contains 11-residue repeats that are capable of binding to membranes in a helical conformation. CCT catalyzes the rate-limiting step in phosphatidylcholine synthesis. Further, it may preferentially bind membranes deficient in PC lipids, insofar as PC deficiency increases the relative proportion of negatively charged (PS) headgroups and conical (PE) headgroups, both of which could enhance binding. Though the CCT amphipathic helix is covalently attached to its relevant enzymatic domain, a similar feedback cycle emerges wherein PC deficiency enhances CCT binding, which catalyzes PC synthesis [130-139].

Finally, beyond potential contributions to the synaptic vesicle cycle, alpha-synuclein may also play a role in dopamine synthesis.
First, synuclein reportedly binds to and inhibits tyrosine hydroxylase, the rate-limiting enzyme in dopamine biosynthesis [140]. In addition, synuclein binds to a 14-3-3 protein that binds to and activates tyrosine hydroxylase [141]; homology between synuclein and 14-3-3 proteins has also been noted [140,141]. Importantly, synuclein is also reported to bind directly to the human dopamine transporter hDAT [142] and to inhibit its reuptake of dopamine from the synapse [142]. Decreased uptake required neither the N-terminal half of the membrane binding domain nor the C-terminal region, but was absolutely dependent on the NAC region of synuclein [143,144]. hDAT represents another potential functional target for synuclein at the membrane, though how membrane binding per se contributes to hDAT regulation remains unclear. More generally, synuclein is also reported to regulate other monoamine transporters, including those for serotonin and norepinephrine [143,145,146]. Finally, synuclein has been reported to interact with the signaling proteins ARPP16/19 in a membrane dependent fashion [147]. ARPP16/19 belong to the same family as DARPP32, a phosphoprotein involved in signaling networks that mediate responses to the binding of dopamine (and other neurotransmitters) to the postsynaptic receptors. Thus, synuclein-ARPP16/19 interactions may be involved in regulation of dopamine signaling pathways.

SYNUCLEIN MAY BIND TO AND FUNCTION AT OTHER CELLULAR MEMBRANES

Alpha-synuclein clearly localizes to synaptic terminals, contributes to synaptic vesicle homeostasis and synaptic plasticity, and binds to synaptic vesicles. However, a number of other potential cellular membrane targets have been proposed and studied, including the plasma membrane, lipid rafts, the inner nuclear membrane, and mitochondrial membranes (Fig. 1); further, synuclein binds fatty acids and may contribute to fatty acid metabolism. These interactions, which may be functional, pathological, or both, are included here because until greater certainty is achieved regarding synuclein’s precise normal functions, all such observations should be considered as potentially relevant.

The plasma membrane represents a target that could cooperate with synaptic vesicle binding in facilitating synuclein’s function at the presynapse. As discussed above, it has been proposed that synuclein may span the synaptic vesicle and plasma membranes through its broken helical conformation [2,108] and recent data supports this possibility [109]. Interestingly, synuclein has been reported to associate specifically with lipid rafts and caveolae [148,149], and membrane association is specifically enhanced by gangliosides including, among others, GM1 and GM3 [149-153]. GM1 also seems to specifically enhance the binding and helical folding of N-terminally acetylated relative to non-acetylated synuclein [154]. The synuclein/GM1 association is likely due to a specific interaction between helical alpha synuclein and both the sialic acid and carbohydrate moieties of GM1, and it may contribute to synuclein’s presynaptic localization [149,150]. Notably, gangliosides are considered to reside on the outer leaflet of the plasma membrane, making it unclear how a cytosolic protein like synuclein could interact with them. However, recent interest in cell-to-cell transmission of synuclein pathology has suggested a role for extracellular synuclein in disease (see below), and this context may provide an opportunity for synuclein to interact with outer leaflet lipids and proteins.

Synuclein was initially discovered as a protein localized to both the presynaptic nerve terminal and to the nucleus, and indeed its name is derived from this observation [53]. Its nuclear localization has remained contentious, however, and it remains unclear whether synuclein really is enriched in the nucleus, whether it might function there, and whether aberrant nuclear localization might contribute to pathology. Later studies have also observed nuclear localization, though this appears to be antibody-dependent and could in part result from non-specific binding by certain antibodies [53,155-163]. Synuclein has, however, additionally been noted to interact with histones and with DNA, though these interactions may be pathologic rather than functional [155,164]. Thus, the nuclear membrane could represent an additional membranous target for alpha-synuclein, though synuclein-nuclear membrane interactions have not, to our knowledge, been clearly established in any study to date.

Synuclein has also been reported to interact with mitochondria, which, combined with strong evidence that oxidative damage contributes to Parkinson’s disease, has spurred interest in the interplay between alpha-synuclein and lipid oxidation. Parkinson’s Disease is characterized by selective degeneration of dopaminergic neurons of the substantia nigra. These neurons may be particularly sensitive to oxidative stress, as dopamine metabolism generates a number of toxic species; eventually these cells could lose the capacity to handle continuously generated reactive oxygen species [165]. Interestingly, red blood cells contain large amounts of alpha-synuclein and have a high oxygen/oxidative load [166]. One emerging theory suggests that alpha synuclein functions as an antioxidant that prevents oxidation of unsaturated membrane lipids. Monomeric synuclein has in fact been shown to prevent lipid oxidation, and membrane binding is required for this function [97,167]. Further, increased alpha-synuclein content is associated with neuroprotection from oxidative stress [168]. The N-terminal methionine residues of synuclein (particularly M1
and M5) become oxidized upon binding to vesicles containing peroxidized lipids, and these methionine residues have been established as substrates for methionine sulfoxide reductase when synuclein is in its free, soluble form. Interestingly, methionine sulfoxide reductase A (MsrA) protects dopaminergic cells from toxic, disease-related insults, including expression of mutant alpha-synuclein, by repairing methionine-oxidized proteins [169]. MsrA’s participation in these cycles of methionine oxidation and reduction serves to ultimately consume reactive oxygen species [169]. From these observations, a cycle of synuclein membrane binding, methionine oxidation, release from membrane, and methionine reduction by Msr has been proposed [97,167]. It is not currently clear on which cellular membrane(s) such a cycle might optimally occur, or even whether this might be a general or membrane specific function, and the effects of methionine oxidation on synuclein binding to membranes of differing biophysical properties remain incompletely characterized.

Mitochondria represent a particularly intriguing potential binding partner for synuclein, given the established roles of mitochondrial dysfunction and oxidative stress in the pathogenesis of Parkinson’s Disease. Oxidative stress associated with high levels of dopamine and/or mitochondrial dysfunction leads to elevated levels of lipid peroxides in the neuronal tissue of PD patients [97,170]. Synuclein has also been associated with mitochondrial function [171], and is reported to localize and bind to mitochondria [172-178] as well as to vesicles that mimic mitochondrial membranes [179]. Notably, cardiolipin – an inner mitochondrial membrane lipid - appears able to enhance synuclein membrane binding and to alter its behavior on and at the membrane [178,179].

There are thus clear links between alpha-synuclein and lipid oxidation, and between oxidized lipids, oxidative damage and neurodegenerative diseases. Polyunsaturated fatty acids (PUFAs) normally serve as both an energy reservoir and as intra-and extracellular second messengers that contribute to signaling pathways. Their unsaturated acyl chain bonds, however, represent a target for lipid oxidation in disease states [180]. The effects of PUFAs on alpha-synuclein properties and the effect of synuclein on PUFA metabolism have thus received considerable attention. Alpha-synuclein was noted to have homology to fatty acid binding proteins, and it reportedly interacts with free fatty acids (although it does not bind them like a classical fatty acid binding protein)
as well as phospholipid bilayers [180,181]. Binding to free PUFAs such as arachidonic acid or docosahexaenoic acid (but not to saturated fatty acids) is mediated by the N-terminal lipid-binding domain, which adopts a helical conformation upon such binding; binding to PUFAs reportedly prevented their micellar formation [182,183]. It was initially suggested that synuclein may transport fatty acids between cytosolic and membrane bound cellular compartments [180-184]. It was later proposed that synuclein and PUFAs may act together to enhance clathrin-mediated endocytosis and thus play a role in SV recycling after neuronal stimulation [121]. Indeed, synucleins were very recently shown to be required for the fast kinetics of SV endocytosis [122].

Alpha-synuclein also appears to contribute to fatty acid uptake and metabolism. Alpha-synuclein deficiency leads to: (1) disrupted astrocyte fatty acid uptake and trafficking, with increased trafficking to cholesteryl esters and triacylglycerols (ie. neutral lipid pools) and decreased trafficking to phospholipids [185]; (2) increased (whole brain) neutral lipid mass [186]; (3) decreased (whole brain) incorporation rate and fractional turnover of 16:0 acyl chains in a number of phospholipid classes albeit without direct binding to 16:0 (but note that synuclein deficiency led to increased incorporation rate and fractional turnover of 16:0 acyl chains in choline glycerophospholipids [187]); (4) reduced (brain) arachidonate (20:4n-6) turnover through modulation of endoplasmic reticulum-localized acyl-CoA synthetase, possibly because synuclein may play a role in substrate presentation to acetyl coa synthetase (and not substrate removal) [188]; and (5) a likely compensatory increase in 22:6n-3 incorporation and turnover, with a low level of synuclein/22:6n-3 binding. Such compensation makes sense insofar as 20:4n-6 and 22:6n-3 are two major PUFAs in the brain [189].

SYNUCLEIN/MEMBRANE INTERACTIONS IN THE MULTIFACETED PATHOLOGY OF PD

Alpha-synuclein represents the primary component of Lewy Bodies and Lewy Neurites, which are pathological hallmarks of Parkinson’s Disease and, more generally, the synucleinopathies [3,6,7]. Synuclein is genetically linked to Parkinson’s Disease as well: the synuclein point mutants A53T, A30P, E46K, H50Q and G51D have been linked to rare familial cases of Parkinson’s Disease, as have SNCA gene duplications and triplications [8-15]. Intense effort has focused on the aggregation propensity and properties of synuclein because of the clear and extensive accumulation of mature beta-sheet rich amyloid fibrils in the brains of synucleinopathy patients. In theory, alpha-synucleins contribution to the neuronal degeneration observed in the synucleinopathies could arise from a toxic gain of function, or from a loss of synuclein’s normal function – the latter occurring either as a consequence of synuclein modification through e.g. familial mutations or post-translational modifications, or from sequestration into non-functional oligomeric or fibrillar aggregated forms. The alpha-synuclein knockout phenotype in mice is mild with only moderate electrophysiological anomalies, perhaps due to compensation by beta- and gamma-synucleins [21]. While alpha/beta synuclein double knockout mouse phenotypes are similarly moderate [190], a synuclein triple knockout mouse does displays age-dependent neurological impairments, including decreased SNARE-complex assembly and decreased life span [19]. It may be worth noting that because gamma synuclein expression is largely orthogonal to that of alpha- and beta-synuclein, closer reexamination of the alpha/beta knockout mouse may be warranted.

Systematic examination of the role of distinct regions of the synuclein primary sequence on its physiological vs. pathological activities suggested that the normal and neuropathogenic effects of synuclein may be molecularly distinct and separable. The N-terminal and C-terminal sequences were required for synuclein function as a SNARE complex chaperone but were dispensable for its toxic function; conversely, the central NAC region was more essential for synuclein neurotoxicity but played a negligible role in SNARE-complex assembly [191]. These data favor a model in which some toxic gain of function primarily contributes to neurodegeneration in the synucleinopathies, particularly given the potential toxicity of synuclein oligomeric aggregates (see below). However, some caution is warranted as the model for toxicity used in these studies relied on overexpression and such models have not succeeded in completely capturing the features of human disease.

If a toxic gain of function causes neurodegeneration, this raises the critical question: what are the toxic species? Intense study has focused on defining the specific mechanisms and pathways of synuclein aggregation and on defining the toxic contributions of any and all species along such a pathway, from monomeric synuclein, to intermediate oligomeric or prefibrillar aggregates, and ultimately to mature amyloid fibrils. Oligomeric synuclein intermediates are increasingly favored as the key contributor to cellular dysfunction and death. Oligomeric or protofibrillar synuclein species have been reported to permeabilize membranes by acting as a pore or channel [192,193]. One or more molecules of monomeric synuclein, too, may at times form membrane permeabilizing channels [194]. Such pore formation could clearly alter membrane potential and ion distribution across the membrane and thus contribute to cellular toxicity [194]. Interestingly, membrane binding modulates synuclein aggregation,
though results are conflicting as to whether membrane-bound synuclein is more or less prone to aggregation; membrane composition and relative concentration of protein vs. lipid are likely key factors determining the contribution of the membrane to synuclein aggregation [195-199]. Indeed, depending on protein:lipid (P:L) ratios, membrane binding could either protect against aggregation by isolating monomeric synuclein (at lower P:L ratios), or favor self-association and aggregation by raising the effective local concentration of synuclein on a reduced dimensionality (2D) surface [70,108,200,201].

The behavior and properties of the PD-linked synuclein mutants have also been intensely studied in the hopes that recurring themes might emerge that would shed light on PD pathogenesis. Notably, there is considerable variety in the effects of the mutants on aggregation. A53T, E46K and H50Q [202-204] enhance mature fibril formation [202-206], A30P enhances oligomer formation but retards mature fibril formation [205-207], while G51D retards aggregation [51,204], suggesting different mechanisms of toxicity. Different mutants also have differing effects on membrane binding affinity: Of the original three PD mutations, A30P clearly perturbs binding [47,55,66,69], A53T has no effect [47,59,129], and E46K shows enhanced binding [2,47,61,108,208]. Despite differing effects on overall membrane affinity, the membrane binding behavior of these mutants was unified by the observation that they all increase the population of the aggregation prone partly helical SL1 binding mode in which the N-terminal 25 residues are bound, while the remainder of the protein remains free. Enhanced concentration, due to the reduced dimensionality of the membrane surface, of an exposed hydrophobic NAC region in the SL1 binding mode could help nucleate synuclein aggregation [47]. A conformation similar to the SL1 binding mode has also been observed on BOG micelles [50]. The H50Q and G51D mutations have emerged much more recently and so have not yet been as extensively studied. G51D appears to decrease membrane binding affinity, but promotes the formation of partly helical states [51], while H50Q did not alter binding affinity nor obviously alter the bound-state structure [202].

Cellular studies of synuclein overexpression, oligomerization, and aggregation have also proven informative. Studies in yeast suggested that synuclein overexpression could contribute to trafficking defects [113,114]. In light of synuclein’s potential interactions at the membrane with Rab and SNARE proteins during synaptic vesicle cycling and homeostasis, it is plausible that these interactions may go awry in disease states. Upon overexpression, synuclein accumulates in yeast cells, leads to ER stress and cytotoxicity, and blocks ER to golgi trafficking. Synuclein toxicity can be rescued by the Rab GTPase Ypt1p, which also functions at this trafficking step. Rab1, the mammalian YPT1 homolog, protected against synuclein-induced dopaminergic cell loss [113]. The effects of synuclein on trafficking result from a direct effect on the transport machinery, as vesicles bud efficiently from the ER but fail to dock and/or fuse to the Golgi membrane [113,114]. Cytoplasmic synuclein accumulations are associated with clusters of vesicles originating from the ER-Golgi transport step of the secretory pathway, further implying that synuclein expression impairs vesicular transport [115]. Rab3a and Rab8a – localized to presynaptic termini and post-Golgi vesicles, respectively – also suppress toxicity in synuclein-based neuronal models of PD, implying that synuclein overexpression can affect multiple membrane trafficking steps [114]. Overexpression of synuclein in mammalian kidney and neuroendocrine cells similarly delayed ER to Golgi transport, and this was rescued by expression of SNARE proteins, implying that the overexpressed synuclein antagonized SNARE function [209]. Purified A53T synuclein inhibited COPII vesicle docking and fusion at a pre-Golgi step, and soluble A53T bound ER/Golgi SNAREs to inhibit SNARE complex assembly [209]. This particular observation represents a possible toxic perturbation of synuclein’s normal function as a putative SNARE complex chaperone contributing to SV exocytosis [19].

Aggregation of synuclein in yeast has also been associated with defects in endosomal trafficking and phospholipid biosynthesis. Synuclein aggregation was enhanced in the presence of higher levels of acidic phospholipids, colocalized with yeast membranes enriched for phosphatidic acid, and induced the aggregation of many yeast Rab GTPase proteins [210]. Synuclein expression further induced sensitivity to perturbations in retrograde endosome-Golgi transport [210]. Finally, direct synuclein/Rab interactions have been observed in disease or disease-related situations. Abnormal rab3a/synuclein interactions have been observed in brains of patients with multiple system atrophy, Parkinson’s Disease [211], and Lewy Body Disease [212]. Rab8a interacts with synuclein in a Ser129 phosphorylation dependent manner [107] and Rab8a enhanced synuclein aggregation and reduced synuclein induced cellular toxicity [107]. Rab3a, Rab5, and RAB8 are associated with synuclein aggregates in transgenic mice overexpressing wild type or A30P synuclein [213]. These results suggest that synuclein aggregates may sequester a subset of Rab proteins, and that synuclein overexpression could perturb neuronal vesicular trafficking (particularly at docking and fusion steps) and so contribute to cellular toxicity.

Mitochondrial dysfunction has been extensively linked to PD pathogenesis. First, mitochondrial toxins such as MPTP 6-OHDA, and others have been used to mimic PD symptoms in the absence
of Lewy Body formation. MPTP can arise as an accidental impurity in heroin manufacture and produces PD-like symptoms in people exposed to the toxin. MPP+, the active metabolite of MPTP, can enter neurons via the dopamine transporter and inhibit mitochondrial complex I. Inherited mitochondrial DNA mutations can cause familial Parkinson's disease, and a number of proteins with roles in mitochondrial function have been linked to PD, including Parkin (a ubiquitin E3 ligase that functions in mitophagy-related pathways), and the mitochondrial kinase PINK1 (which functions together with Parkin). The PD-linked kinase LRRK2, and the oxidative stress response protein DJ-1 have also been linked to mitochondria. Mitochondrial dysfunction could enhance cellular reactive oxygen species (ROS), which can exert toxicity in many ways. An interesting example is that ROS may alter membrane fusion and transmitter exocytosis by impacting the SNARE proteins [180]. Thus, synuclein/mitochondria interactions are of particular interest in the context of PD pathophysiology. Synuclein can directly interact with mitochondria [177] and overexpression of synuclein increases cellular susceptibility to mitochondrial toxins and inhibits mitochondrial complex I activity [214]. Synuclein can increase intra-mitochondrial ROS, nitric oxide, and Ca\(^{2+}\) levels and thereby lead to cytochrome c release and apoptosis [174,180,215,216].

Synuclein has been shown to inhibit mitochondrial fusion and drive mitochondrial fission in a cardiolipin-dependent manner [178,217]. Synuclein thus has a direct effect on mitochondrial morphology, and synuclein-associated mitochondrial fragmentation is followed by a decline in cellular respiration and ultimately neuronal death. These mitochondrial effects depend on the direct interaction between synuclein and mitochondrial membranes and so establish a role for this interaction in PD-associated mitochondrial toxicity [178]. It should be noted, however, that synuclein has also at times been shown to have an anti-apoptotic, protective role against mitochondrial-mediated cell death [180]. Interestingly, the G51D mutant was found to enhance mitochondrial fragmentation [51].

Increased oxidative stress can reportedly lead to the translocation of alpha-synuclein from the cytoplasm into the nucleus and perhaps generate a devastating positive feedback cycle: oxidative stress could disrupt the nuclear membrane, lead to synuclein translocation into the disrupted nucleus (specifically of a C-terminal fragment of synuclein), and then enhance cellular susceptibility to further oxidative stress (although the specific mechanisms for this last step are not presently clear) [218,219]. Iron has also been shown to alter mitochondrial morphology, disrupt the nuclear membrane, and cause the translocation of synuclein from the perinuclear region into the disrupted nucleus [220]. Synuclein may somehow mediate neurotoxicity in the nucleus, perhaps through some effect on histones, or on DNA itself [155,164]; synuclein can directly bind both DNA and histones, and was reported to reduce the level of acetylated histone H3 while also inhibiting histone acetylation [155,164,221]. It has also been suggested that nuclear synuclein accumulation is mediated by importin alpha, and that this promotes neurotoxicity through cell cycle acceleration [222]. Finally, the PD-linked G51D mutant was found to be enriched in the nuclear compartment [51].

As discussed above, synuclein may interact with a wide variety of cellular membranes, and so a number of membrane properties and membrane lipid components may contribute to synuclein/membrane binding and to synuclein dysfunction on the membrane surface (Fig. 2). First, synuclein may bind to lipid rafts, and this interaction may localize synuclein to synapses, which contain cholesterol rich lipid microdomains [148]. Synuclein also binds directly to artificial lipid raft membrane mimetics; this binding requires acidic phospholipids - particularly phosphatidylserine(PS) and, more specifically, a combination of PS with oleic (18:1) and polyunsaturated (20:4 or 22:6) fatty acyl chains. Binding was particularly enhanced with PS on the polyunsaturated fatty acyl chain (vs. the oleoyl chain), suggesting that synuclein membrane interactions are subject to a strict "combinatorial code" [223]. Interestingly, synuclein redistributed away from synapses upon raft disruption [148]. The membrane-binding impairing A30P synuclein mutation similarly exhibited impaired localization at the synapse [148]. If lipid rafts mediate synuclein localization in some functionally requisite fashion, perturbation of lipid rafts or of this association could lead to synuclein mislocalization and so contribute to disease [148].

In yeast, synuclein binds to lipid rafts [88] and inhibition of sterol synthesis led to decreased plasma membrane association by synuclein, increased (aberrant) vesicular association, and increased cellular toxicity [224]. Thus, higher membrane sterol concentrations favor plasma membrane binding of synuclein, though whether this is of functional or pathological relevance remains to be determined [224]. Finally, given the interactions between synuclein and fatty acids discussed above, it is of interest that PUFAs are able to directly promote synuclein oligomerization both in vitro and in vivo [180,198]. In each of these cases a contribution of specific lipids, membrane properties, and membrane compositions to synuclein function vs. dysfunction has been established yet remains incompletely understood.

Thus far, discussion of synuclein function and toxicity has focused on the view that the effects of alpha-synuclein will be cell autonomous. Recently, it has become apparent that this may not be the case, as cell-to-cell spread of synuclein oligomers/aggregates...
has become an attractive model for how neurodegeneration may spread through the synucleinopathy-diseased brain [5,225-227]. First, synuclein has been detected in extracellular biological fluids of both healthy and PD subjects, including the CSF and blood plasma [153,228-230]. A key observation resulted from an experimental Parkinson’s Disease treatment in which patients received fetal ventral mesencephalic tissue transplants. Lewy body-like inclusions were found in these exogenously grafted nigral neurons in the brains of multiple patients with Parkinson’s Disease; this implies spread of synuclein from endogenous neurons into disease-free, grafted neurons in a potentially prion-like manner [231-233]. Experimentally, both synuclein exocytosis and endocytosis have been observed. A portion of cellular synuclein is present in the lumen of vesicles, and this intravesicular synuclein is more aggregation prone than cytosolic protein [234]. Also, a small percentage of newly synthesized synuclein is rapidly secreted via ER/Golgi-independent exocytosis, and aggregated synuclein is also secreted [234]. Interestingly, both monomeric and aggregated synuclein secretion and transmission are elevated upon proteasomal and mitochondrial dysfunction [235], suggesting that synuclein exocytosis may increase in synucleinopathy patients. In general, extracellular synuclein is cytotoxic in culture media [236], and aggregated extracellular synuclein can induce microglial activation, dopaminergic neurotoxicity [237], and production of pro-inflammatory factors from astrocytes and astrocytoma cells [238,239]. Released synuclein acts as an endogenous agonist for Toll-like receptor 2, which activates microglial inflammatory responses [240]. Exogenous synuclein further induced neuronal cell death through Rab5A-dependent endocytosis [236], though the mechanism of synuclein internalization appears to be
synuclein assembly-state specific: aggregated fibrils or oligomers display receptor mediated endocytosis, while monomeric synuclein passively diffuses across the plasma membrane [241]. Some exogenous synuclein can also be resecreted by recycling endosomes; this process is regulated by rab11a. Hsp90 interacts with rab11a and is critical for the toxicity of exogenous synuclein [242]. Alternately, endocytosed synuclein aggregates can be degraded by lysosomes [241], which is particularly interesting given that impaired autophagy and lysosomal function have been implicated in Parkinson’s pathogenesis [226,243]. Impaired autophagy could enhance synuclein accumulation, and increased exophagy of both synuclein monomers and aggregates has been observed upon manipulations that perturb autophagosomes (either by increasing the pool of autophagosomes/amphisomes through e.g. lysosomal disturbance, or by altering the polarity of vesicular transport of autophagosomes on microtubules) [244]. Finally, a dysfunctional interaction between alpha-synuclein and the Gaucher’s Disease linked lysosomal glucocerebrosidase (GCase) has recently emerged. Functional loss of GCase causes synuclein accumulation, and synuclein inhibits the lysosomal activity of GCase [245]. This suggests that GCase and synuclein form a bidirectional pathogenic loop in the synucleinopathies and that GCase depletion contributions to synucleinopathy pathogenesis. Further, the substrate of GCase, glucosylceramide, stabilized soluble synuclein oligomeric intermediate species [245]. Reduced GCase with synuclein accumulation has been observed in PD brain tissue as well [246]. Failure of cellular protein quality control systems (particularly lysosomes) also promotes the accumulation of transmitted synuclein (i.e. that spread from adjacent cells) and inclusion formation [235]. Indeed, synuclein aggregates appear to spread from cell to cell through a cycle of external aggregate uptake, co-aggregation with endogenous synuclein, and coaggregate exocytosis [247]. GCase depletion promotes this propagation of synuclein aggregates [247], and the enhanced spread of synuclein could further contribute to mechanism(s) by which GCase mutations contribute to PD and to increased cognitive impairment [247]. Clearly, then, as synuclein monomers and aggregates are secreted, endocytosed, and trafficked through endosomal, autophagosomal, or lysosomal compartments, they will contact a wealth of proteins and cellular membranes whose relevance to synuclein function or dysfunction was not previously considered or appreciated. The putative contribution, if any, of synuclein/membrane interactions within such compartments has not been extensively considered or examined, though it now appears likely that these pathways and interactions could contribute to the synucleinopathies.

The newly established cell-to-cell spread and endosomal/lysosomal trafficking of synuclein requires consideration of an increasing number of extracellular and intravesicular binding targets, including both proteins – as in the case of glucocerebrosidase - and novel cellular membrane binding partners such as the outer plasma membrane leaflet or inner endosomal and lysosomal leaflets. In this context, synuclein/sphingolipid and, more specifically, synuclein/gangliocide interactions become particularly relevant, as gangliocides are found primarily exposed to the cell exterior. Of note, gangliocides are negatively charged and so would be expected to interact favorably with synuclein [150]. Extracellular synuclein is internalized into microglia via the monosialoganglioside GM1 in a lipid raft-dependent (but clathrin-, caveolae, and dynamin-independent) manner [153]. Gangliocides also appear to have interesting effects on the biophysical behavior of synuclein at the membrane. Residues 34-50 of synuclein have been identified as a putative ganglioside-binding domain [152]. It has thus been proposed that synuclein first interacts with a cell surface glycopingolipid such as GM3 (in astrocytes) or GM1 (in neurons) through these residues; tyrosine 39 appears particularly critical for this interaction [150,151]. This binding then induces the helical folding of synuclein, including of residues 67–78, which then form a so-called “tilted peptide” with high affinity for cholesterol. The tilted geometry of the cholesterol/synuclein complex then facilitates the formation of an oligomeric channel with potential dysfunctional consequences [150]. It has been shown that GM1 (and to a lesser extent GM2 and GM3) can induce the formation of helical synuclein oligomers yet inhibit amyloid fibrillation [152].

CONCLUSION

While both the normal functions of alpha-synuclein and the specific mechanisms by which it leads to cell death and disease remain elusive, it is clear that the interactions of alpha-synuclein with membranes play an important role in both synuclein biology and synuclein pathology. Here we have covered much of the information currently available regarding the structural and biophysical aspects of synuclein-membrane interactions, how these are influenced by post-translational modifications, how they relate to synuclein’s interactions with other proteins, which organelles they may involve and how they may influence synuclein aggregation and dysfunction. Much remains to be learned in each of these areas, but it is hoped that this review will help to provide both current and future investigators in this topic area with a snapshot of some of the most promising directions to pursue in order to fill in critical gaps in our knowledge. It is clearer than ever that alpha-synuclein is perhaps the most important single protein in...
the etiology of Parkinson’s disease, and we posit that advances in our understanding of synuclein-membrane interactions will help bring us closer to improved treatments.

ACKNOWLEDGMENTS

This work was supported by NIH grants R37AG019391 (DE), F30MH101982 (DS) and MSTP GM07739 (DS). This publication was made possible by NPRP grant no 4-1371-1-223 from the Qatar National Research Fund (a member of Qatar Foundation). The statements made herein are solely the responsibility of the authors.

REFERENCES

1. Pfefferkorn CM, Jiang Z, Lee JC (2012) Biophysics of α-synuclein membrane interactions. Biochim Biophys Acta 1818:162-171.
2. Dikiy I, Eliezer D (2012) Folding and misfolding of alpha-synuclein on membranes. Biochim Biophys Acta 1818:1013-1018.
3. Spillantini MG (1999) Parkinson’s disease, dementia with Lewy bodies and multiple system atrophy are alpha-synucleinopathies. Parkinsonism Relat Disord 5:157-162.
4. Moore DJ, West AB, Dawson VL, Dawson TM (2005) Molecular pathophysiology of Parkinson’s disease. Annu Rev Neurosci 28:57-87.
5. Luk KC, Lee VM (2014) Modeling Lewy pathology propagation in Parkinson’s disease. Parkinsonism Relat Disord 20 Suppl 1:S85-S87.
6. Irwin DJ, Lee VM, Trojanowski JQ (2013) Parkinson’s disease dementia: convergence of α-synuclein, tau and amyloid-β pathologies. Nat Rev Neurosci 14:626-636.
7. Norris EH, Giasson BI, Lee VM (2004) Alpha-synuclein: normal function and role in neurodegenerative diseases. Curr Top Dev Biol 60:17-54.
8. Polymerosopoulos MH, Lavedan C, Leroy E, Ide SE, Deheja A, Dutra A, Pike B, Root H, Rubenstein J, Boyer R, Stenroos ES, Chandrasekharappa S, Athanassiadou A, Papapetropoulos T, Johnson WG, Lazzarini AM, Duvoisin RC, Di Iorio G, Golbe LI, Nussbaum RL (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson’s disease. Science 276:2045-2047.
9. Krüger R, Kuhn W, Müller T, Woitalla D, Graeber M, Kösel S, Przuntek H, Epplen JT, Schös L, Riess O (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson’s disease. Nat Genet 18:106-108.
10. Zarranz JJ, Alegre J, Gómez-Esteban JC, Lezcano E, Ros R, Ampuero I, Vidal L, Hoenicka J, Rodriguez O, Atarés B, Lorens V, Gomez Tortosa E, del Ser T, Muñoz DG, de Yebenes JG (2004) The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. Ann Neurol 55:164-173.
11. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, Kachergus J, Hulihan M, Peuralinna T, Dutra A, Nussbaum R, Lincoln S, Crawford A, Hanson M, Maraganore D, Adler C, Cookson MR, Muenter M, Baptista M, Miller D, Blanca G, Hardy J, Gwinn-Hardy K (2003) alpha-Synuclein locus triplication causes Parkinson’s disease. Science 302:841.
12. Chartier-Harlin MC, Kachergus J, Roumier C, Mouroux V, Douay X, Lincoln S, Leveque C, Larvor L, Andrieux J, Hulihan M, Waucquier N, Defebrl E, Amouyel P, Farrer M, Destée A (2004) Alpha-synuclein locus duplication as a cause of familial Parkinson’s disease. Lancet 364:1167-1169.
13. Appel-Cresswell S, Vilarino-Guell C, Encarnacion M, Sherman H, Yu I, Shah B, Weir D, Thompson C, Szu-Tü C, Trinh J, Aasly JO, Raaput A, Raupert AH, Jon Stoessl A, Farrer MJ (2013) Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson’s disease. Mov Disord 28:811-813.
14. Proukakis C, Dudzik CG, Brier T, MacKay DS, Cooper JM, Millhauser GL, Houlden H, Schapira AH (2013) A novel α-synuclein missense mutation in Parkinson disease. Neurology 80:1062-1064.
15. Lesage S, Anheim M, Letournel F, Bousset L, Honoré A, Rozas N, Pieri L, Madiona K, Dürr A, Melki R, Verny C, Brice A; French Parkinson’s Disease Genetics Study Group (2013) G51D α-synuclein mutation causes a novel parkinsonian-pyramidal syndrome. Ann Neurol 73:459-471.
16. Watson JB, Hatami A, David H, Maslih E, Roberts K, Evans CE, Levine MS (2009) Alterations in corticostriatal synaptic plasticity in mice overexpressing human α-synuclein. Neuroscience 159:501-513.
17. George JM, Jin H, Woods WS, Clayton DF (1995) Characterization of a novel protein regulated during the critical period for song learning in the zebra finch. Neuron 15:361-372.
18. Chandra S, Gallardo G, Fernández-Chacón R, Schlüter OM, Südhof TC (2005) α-Synuclein cooperates with CSPα in preventing neurodegeneration. Cell 123:383-396.
19. Burre J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Südhof TC (2010) α-Synuclein promotes SNARE-complex assembly in vivo and in vitro. Science 329:1663-1667.
20. Murphy DD, Rueter SM, Trojanowski JQ, Lee VM (2000) Synucleins are developmentally expressed, and alpha-synuclein regulates the size of the presynaptic vesicular pool...
in primary hippocampal neurons. J Neurosci 20:3214-3220.
21. Abeliovich A, Schmitz Y, Farina I, Choi-Lundberg D, Ho WH, Castillejo PE, Shinsky N, Verdugo JM, Armanini M, Ryan A, Hykes M, Phillips H, Sulzer D, Rosenthal A (2000) Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. Neuron 25:239-252.
22. Eliezer D, Kutluay E, Bussell R Jr, Browne G (2001) Conformational properties of alpha-synuclein in its free and lipid-associated states. J Mol Biol 307:1061-1073.
23. Weinreb PH, Zhen W, Poon AW, Conway KA, Lansbury PT Jr. (1996) NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. Biochemistry 35:13709-13715.
24. Mantsyzov AB, Maltsev AS, Ying J, Shen Y, Hummer G, Bax A (2014) A maximum entropy approach to the study of residue-specific backbone angle distributions in alpha-synuclein, an intrinsically disordered protein. Protein Sci 23:1275-1290.
25. Bussell R Jr, Eliezer D (2003) A structural and functional role for 11-mer repeats in a-synuclein and other exchangeable lipid binding proteins. J Mol Biol 329:763-778.
26. Ulmer TS, Bax A (2005) Comparison of structure and dynamics of micelle-bound human alpha-synuclein and Parkinson disease variants. J Biol Chem 280:43179-43187.
27. Davidson WS, Jonas A, Clayton DF, George JM (1998) Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes. J Biol Chem 273:9443-9449.
28. Bodner CR, Dobson CM, Bax A (2009) Multiple tight phospholipid-binding modes of alpha-synuclein revealed by solution NMR spectroscopy. J Mol Biol 390:775-790.
29. Sevcik E, Trexler AJ, Dunn JM, Rhoades E (2011) Allostery in a disordered protein: oxidative modifications to alpha-synuclein alter distally to regulate membrane binding. J Am Chem Soc 133:7152-7158.
30. Bussell R Jr, Ramlall TF, Eliezer D (2005) Helix periodicity, topology, and dynamics of membrane-associated alpha-synuclein. Protein Sci 14:862-872.
31. Jao CC, Der-Sarkissian A, Chen J, Langen R (2004) Structure of membrane-bound alpha-synuclein studied by site-directed spin labeling. Proc Natl Acad Sci U S A 101:8331-8336.
32. Jao CC, Hegde BG, Chen J, Haworth IS, Langen R (2008) Structure of membrane-bound alpha-synuclein from site-directed spin labeling and computational refinement. Proc Natl Acad Sci U S A 105:19666-19671.
33. Segrest JP, Jones MK, De Loof H, Brouillette CG, Venkatachalanpathi YV, Anantharamaiah GM (1992) The amphipathic helix in the exchangeable apolipoproteins: a review of secondary structure and function. J Lipid Res 33:141-166.
34. Drin G, Antonny B (2010) Amphipathic helices and membrane curvature. FEBS Lett 584:1840-1847.
35. Georgieva ER, Ramlall TF, Borbat PP, Freed JH, Eliezer D (2008) Membrane-bound alpha-synuclein forms an extended helix: long-distance pulsed ESR measurements using vesicles, bicelles, and rodlike micelles. J Am Chem Soc 130:12856-12857.
36. Georgieva ER, Ramlall TF, Borbat PP, Freed JH, Eliezer D (2010) The lipid-binding domain of wild type and mutant alpha-synuclein: compactness and interconversion between the broken and extended helix forms. J Biol Chem 285:28261-28274.
37. Borbat P, Ramlall TF, Freed JH, Eliezer D (2006) Inter-helix distances in lysophospholipid micelle-bound alpha-synuclein from pulsed ESR measurements. J Am Chem Soc 128:10004-10005.
38. Trexler AJ, Rhoades E (2009) a-Synuclein binds large unilamellar vesicles as an extended helix. Biochemistry 48:2304-2306.
39. Ferreon AC, Gambin Y, Lemke EA, Deniz AA (2009) Interplay of alpha-synuclein binding and conformational switching probed by single-molecule fluorescence. Proc Natl Acad Sci U S A 106:5645-5650.
40. Chandra S, Chen X, Rizo J, Jahn R, Südhof TC (2003) A broken alpha-helix in folded alpha-Synuclein. J Biol Chem 278:15313-15318.
41. Ulmer TS, Bax A, Cole NB, Nussbaum RL (2005) Structure and dynamics of micelle-bound human alpha-synuclein. J Biol Chem 280:9595-9603.
42. Lokappa SB, Ulmer TS (2011) Alpha-synuclein populates both elongated and broken helix states on small unilamellar vesicles. J Biol Chem 286:21450-21457.
43. Bortolus M, Tombolato F, Tessari I, Bisaglia M, Mammi S, Bubacco L, Ferrarini A, Maniero AL (2008) Broken helix in vesicle and micelle-bound alpha-synuclein: insights from site-directed spin labeling-ESR experiments and MD simulations. J Am Chem Soc 130:6690-6691.
44. Drescher M, Veldhuis G, van Rooijen BD, Milikisyants S, Subramaniam V, Huber M (2008) Antiparallel arrangement of the helices of vesicle-bound alpha-synuclein. J Am Chem Soc 130:7796-7797.
45. Robotta M, Braun P, van Rooijen B, Subramaniam V, Huber M, Drescher M (2011) Direct evidence of coexisting horseshoe and extended helix conformations of membrane-bound alpha-synuclein. ChemPhysChem 12:267-269.
46. Ferreon AC, Deniz AA (2007) a-Synuclein multistate folding thermodynamics: implications for protein misfolding and
aggregation. Biochemistry 46:4499-4509.
47. Bodner CR, Maltsev AS, Dobson CM, Bax A (2010) Differential phospholipid binding of α-synuclein variants implicated in Parkinson’s disease revealed by solution NMR spectroscopy. Biochemistry 49:862-871.
48. Drescher M, Godschalk F, Veldhuis G, van Rooijen BD, Subramaniam V, Huber M (2008) Spin-label EPR on α-synuclein reveals differences in the membrane binding affinity of the two antiparallel helices. Chembiochem 9:2411-2416.
49. Anderson VL, Ramllall TF, Rospigliosi CC, Webb WW, Eliezer D (2010) Identification of a helical intermediate in trifluoroethanol-induced alpha-synuclein aggregation. Proc Natl Acad Sci U S A 107:18850-18855.
50. Dikiy I, Eliezer D (2014) N-terminal acetylation stabilizes N-terminal helicity in lipid- and micelle-bound α-synuclein and increases its affinity for physiological membranes. J Biol Chem 289:3652-3665.
51. Fares MB, Ait-Bouziad N, Dikiy I, Mbefo MK, Jovičić A, Kiely A, Holton JL, Lee SJ, Gitter AD, Eliezer D, Lashuel HA (2014) The novel Parkinson’s disease linked mutation G51D attenuates in vitro aggregation and membrane binding of α-synuclein, and enhances its secretion and nuclear localization in cells. Hum Mol Genet 23:4491-4509.
52. Trexler AJ, Rhoades E (2013) Function and dysfunction of α-synuclein: probing conformational changes and aggregation by single molecule fluorescence. Mol Neurobiol 47:622-631.
53. Maroteaux L, Campanelli JT, Scheller RH (1988) Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. J Neurosci 8:2804-2815.
54. Clayton DF, George JM (1999) Synucleins in synaptic plasticity and neurodegenerative disorders. J Neurosci Res 58:120-129.
55. Jensen PH, Nielsen MS, Jakes R, Dotti CG, Goedert M (1998) Binding of alpha-synuclein to brain vesicles is abolished by familial Parkinson’s disease mutation. J Biol Chem 273:26292-26294.
56. Irizarry MC, Kim TW, McNamara M, Tanzi RE, George JM, Clayton DF, Hyman BT (1996) Characterization of the precursor protein of the non-A beta component of senile plaques (NACP) in the human central nervous system. J Neuropathol Exp Neurol 55:889-895.
57. Kahle PJ, Neumann M, Ozmen L, Muller V, Jacobsen H, Schindzielorz A, Okochi M, Leimer U, van Der Putten H, Probst A, Kremmer E, Kretzschmar HA, Haass C (2000) Subcellular localization of wild-type and Parkinson’s disease-associated mutant alpha-synuclein in human and transgenic mouse brain. J Neurosci 20:6365-6373.
58. Iwai A, Masliah E, Yoshimoto M, Ge N, Flanagan L, de Silva HA, Kittel Saitoh T (1995) The precursor protein of non-Aβ component of Alzheimer’s disease amyloid is a presynaptic protein of the central nervous system. Neuron 14:467-475.
59. Bussell R Jr, Eliezer D (2004) Effects of Parkinson’s disease-linked mutations on the structure of lipid-associated alpha-synuclein. Biochemistry 43:4810-4818.
60. Rhoades E, Ramllall TF, Webb WW, Eliezer D (2006) Quantification of α-synuclein binding to lipid vesicles using fluorescence correlation spectroscopy. Biophys J 90:4692-4700.
61. Middleton ER, Rhoades E (2010) Effects of curvature and composition on α-synuclein binding to lipid vesicles. Biophys J 99:2279-2288.
62. Pranke IM, Morello V, Bigay J, Gibson K, Verbavatz JM, Antonny B, Jackson CL (2011) α-Synuclein and ALPS motifs are membrane curvature sensors whose contrasting chemistry mediates selective vesicle binding. J Cell Biol 194:89-103.
63. Kjaer L, Giehm L, Heimburg T, Otzen D (2009) The influence of vesicle size and composition on alpha-synuclein structure and stability. Biophys J 96:2857-2870.
64. Jensen MB, Bhatia VK, Jao CC, Rasmussen JE, Pedersen SL, Jensen KJ, Langen R, Stamou D (2011) Membrane curvature sensing by amphipathic helices: a single liposome study using α-synuclein and annexin B12. J Biol Chem 286:42603-42614.
65. Cui H, Lyman E, Voth GA (2011) Mechanism of membrane curvature sensing by amphipathic helix containing proteins. Biophys J 100:1271-1279.
66. Jo E, Fuller N, Rand RP, Ste George-Hyslop P, Fraser PE (2002) Defective membrane interactions of familial Parkinson’s disease mutant A30P α-synuclein. J Mol Biol 315:799-807.
67. Nuscher B, Kamp F, Mehnert T, Odoy S, Haass C, Kahle PJ, Beyer K (2004) Alpha-synuclein has a high affinity for packing defects in a bilayer membrane: a thermodynamics study. J Biol Chem 279:21966-21975.
68. Kamp F, Beyer K (2006) Binding of α-synuclein affects the lipid packing in bilayers of small vesicles. J Biol Chem 281:9251-9259.
69. PERRIN RJ, Woods WS, Clayton DF, George JM (2000) Interaction of human alpha-synuclein and Parkinson’s disease variants with phospholipids. Structural analysis using site-directed mutagenesis. J Biol Chem 275:34393-34398.
70. Zhu M, Li J, Fink AL (2003) The association of alpha-
synuclein with membranes affects bilayer structure, stability, and fibril formation. J Biol Chem 278:40186-40197.
71. Ramakrishnan M, Jensen PH, Marsh D (2003) α-Synuclein association with phosphatidylglycerol probed by lipid spin labels. Biochemistry 42:12919-12926.
72. Takamori S, Holt M, Stenius K, Lemke EA, Gronborg M, Riedel D, Urlaub H, Schenck S, Brügger B, Ringler P, Müller SA, Ramminger B, Gräter F, Hub JS, De Groot BL, Mieskes G, Moriyama Y, Klingauf J, Grubmüller H, Heuser J, Wieland F, Jahn R (2006) Molecular anatomy of a trafficking organelle. Cell 127:831-846.
73. Varkey J, Isas JM, Mizuno N, Jensen MB, Bhatia VK, Jao CC, Petrlova J, Voss JC, Stamou DG, Steven AC, Langen R (2010) Membrane curvature induction and tubulation are common features of synucleins and apolipoproteins. J Biol Chem 285:32486-32493.
74. Mizuno N, Varkey J, Kegulian NC, Hegde BG, Cheng N, Langen R, Steven AC (2012) Remodeling of lipid vesicles into cylindrical micelles by α-synuclein in an extended α-helical conformation. J Biol Chem 287:29301-29311.
75. Anderson JP, Walker DE, Goldstein JM, de Laat R, Banducci K, Caccavello RJ, Barbour R, Huang J, Kling K, Lee M, Diep L, Keim PS, Shen X, Chataway T, Schloessmacher MG, Seubert P, Schenk D, Sinha S, Gai WP, Chilcote TJ (2006) Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. J Biol Chem 281:29739-29752.
76. Okochi M, Walter J, Koyama A, Nakajo S, Baba M, Iwatsubo T, Meijer J, Kahle PJ, Haass C (2000) Constitutive phosphorylation of the Parkinson's disease associated alpha-synuclein. J Biol Chem 275:390-397.
77. Pronin AN, Morris AJ, Surguchov A, Benovic JL (2000) Synucleins are a novel class of substrates for G protein-coupled receptor kinases. J Biol Chem 275:26515-26522.
78. Ellis CE, Schwartzberg PL, Grider TL, Fink DW, Nussbaum RL (2001) alpha-synuclein is phosphorylated by members of the Src family of protein-tyrosine kinases. J Biol Chem 276:3879-3884.
79. Nakamura T, Yamashita H, Takahashi T, Nakamura S (2001) Activated Fyn phosphorylates alpha-synuclein at tyrosine residue 125. Biochem Biophys Res Commun 280:1085-1092.
80. Giasson BI, Duda JE, Murray IV, Chen Q, Souza JM, Hurtig HI, Ischiropoulos H, Trojanowski JQ, Lee VM (2000) Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. Science 290:985-989.
81. Hasegawa M, Fujiwara H, Nonaka T, Wakabayashi K, Takahashi H, Lee VM, Trojanowski JQ, Mann D, Iwatsubo T (2002) Phosphorylated alpha-synuclein is ubiquitinated in alpha-synucleinopathy lesions. J Biol Chem 277:49071-49076.
82. Tofarisi GK, Razzaq A, Ghetti B, Lilley KS, Spillantini MG (2003) Ubiquitination of alpha-synuclein in Lewy bodies is a pathological event not associated with impairment of proteasome function. J Biol Chem 278:44405-44411.
83. Dorval V, Fraser PE (2006) Small ubiquitin-like modifier (SUMO) modification of natively unfolded proteins tau and alpha-synuclein. J Biol Chem 281:9919-9924.
84. Citron BA, Suo Z, SantaCruz K, Davies PJ, Qin F, Festoff BW (2002) Protein crosslinking, tissue transglutaminase, alternative splicing and neurodegeneration. Neurochem Int 40:69-78.
85. Junn E, Ronchetti RD, Quezado MM, Kim SY, Mouradian MM (2003) Tissue transglutaminase-induced aggregation of alpha-synuclein: Implications for Lewy body formation in Parkinson's disease and dementia with Lewy bodies. Proc Natl Acad Sci USA 100:2047-2052.
86. Andringa G, Lam KY, Chegary M, Wang X, Chase TN, Bennett MC (2004) Tissue transglutaminase catalyzes the formation of alpha-synuclein crosslinks in Parkinson's disease. FASEB J 18:932-934.
87. Uversky VN, Yamin G, Souillac PO, Goers J, Glaser CB, Fink AL (2002) Methionine oxidation inhibits fibrillation of human alpha-synuclein in vitro. FEBS Lett 517:239-244.
88. Zabrocki P, Bastiaens I, Delay C, Bammens T, Ghillebert R, Pellens K, De Virgilio C, Van Leuven F, Winderickx J (2008) Phosphorylation, lipid raft interaction and traffic of alpha-synuclein in a yeast model for Parkinson. Biochim Biophys Acta 1783:1677-1680.
89. Fauvet B, Fares MB, Samuel F, Dikiy I, Tandon A, Eliezer D, Lashuel HA (2012) Characterization of semisynthetic and naturally Nα-acetylated α-synuclein in vitro and in intact cells: implications for aggregation and cellular properties of α-synuclein. J Biol Chem 287:28243-28262.
90. Maltsev AS, Ying J, Bax A (2012) Impact of N-terminal acetylation of α-synuclein on its random coil and lipid binding properties. Biochemistry 51:5004-5013.
91. Kang L, Moriarty GM, Woods LA, Ashcroft AE, Radford SE, Baum J (2012) N-terminal acetylation of α-synuclein induces increased transient helical propensity and decreased aggregation rates in the intrinsically disordered monomer. Protein Sci 21:911-917.
92. Mahul-Mellier AL, Fauvet B, Gysbers A, Dikiy I, Oueslati A, Georgeon S, Lamontanara AJ, Bisquertt A, Eliezer D,
Masliah E, Halliday G, Hantschel O, Lashuel HA (2014) c-Abl phosphorylates α-synuclein and regulates its degradation: implication for α-synuclein clearance and contribution to the pathogenesis of Parkinson’s disease. Hum Mol Genet 23:2858-2879.

93. Hejjaoui M, Butterfield S, Fauvet B, Vercruysse F, Cui J, Dikiy I, Prudent M, Olschewski D, Zhang Y, Eliezer D, Lashuel HA (2012) Elucidating the role of C-terminal post-translational modifications using protein semisynthesis strategies: α-synuclein phosphorylation at tyrosine 125. J Am Chem Soc 134:5196-5210.

94. Paleologou KE, Oueslati A, Shakked G, Rospigliosi CC, Kim HY, Lamberto GR, Fernandez CO, Schmid A, Chegini F, Gai WP, Chiappe D, Moniatte M, Schneider BL, Aebischer P, Eliezer D, Zweckstetter M, Masliah E, Lashuel HA (2010) Phosphorylation at S87 is enhanced in synucleinopathies, inhibits alpha-synuclein oligomerization, and influences synuclein-membrane interactions. J Neurosci 30:3184-3198.

95. Paleologou KE, Schmid AW, Rospigliosi CC, Kim HY, Lamberto GR, Fredenburg RA, Lansbury PT Jr, Fernandez CO, Eliezer D, Zweckstetter M, Lashuel HA (2008) Phosphorylation at Ser-129 but not the phosphomimics S129E/D inhibits the fibrillation of α-synuclein. J Biol Chem 283:16895-16905.

96. Visanji NP, Wislet-Gendebien S, Oschipok LW, Zhang G, Aubert I, Fraser PE, Tandon A (2011) Effect of Ser-129 phosphorylation on interaction of α-synuclein with synaptic and cellular membranes. J Biol Chem 286:35863-35873.

97. Maltsev AS, Chen J, Levine RL, Bax A (2013) Site-specific interaction between α-synuclein and membranes probed by NMR-observed methionine oxidation rates. J Am Chem Soc 135:2943-2946.

98. Irwin DJ, Cohen TJ, Grossman M, Arnold SE, Xie SX, Lee VM, Trojanowski JQ (2012) Acetylated tau, a novel pathological signature in Alzheimer’s disease and other tauopathies. Brain 135:807-818.

99. Irwin DJ, Cohen TJ, Grossman M, Arnold SE, McCarty-Wood E, Van Deerlin VM, Lee VM, Trojanowski JQ (2013) Acetylated tau neuropathology in sporadic and hereditary tauopathies. Am J Pathol 183:344-351.

100. Cohen TJ, Guo JL, Hurtado DE, Kwong LK, Mills IP, Trojanowski JQ, Lee VM (2011) The acetylation of tau inhibits its function and promotes pathological tau aggregation. Nat Commun 2:252.

101. Wislet-Gendebien S, D’Souza C, Kawarai T, St George-Hyslop P, Westaway D, Fraser P, Tandon A (2006) Cytosolic proteins regulate alpha-synuclein dissociation from presynaptic membranes. J Biol Chem 281:32148-32155.

102. Martinez J, Moeller I, Erdjument-Bromage H, Tempst P, Lauring B (2003) Parkinson’s disease-associated alpha-synuclein is a calmodulin substrate. J Biol Chem 278:17379-17387.

103. Bertini I, Gupta YK, Luchinat C, Parigi G, Peana M, Sgheri L, Yuan J (2007) Paramagnetism-based NMR restraints provide maximum allowed probabilities for the different conformations of partially independent protein domains. J Am Chem Soc 129:12786-12794.

104. Gruschus JM, Yap TL, Pistolesi S, Maltsev AS, Lee JC (2013) NMR structure of calmodulin complexed to an N-terminally acetylated α-synuclein peptide. Biochemistry 52:3436-3445.

105. Lee D, Lee SY, Lee EN, Chang CS, Paik SR (2002) alpha-Synuclein exhibits competitive interaction between calmodulin and synthetic membranes. J Neurochem 82:1007-1017.

106. Chen RH, Wislet-Gendebien S, Samuel F, Visanji NP, Zhang G, Marsilio D, Langman T, Fraser PE, Tandon A (2013) α-Synuclein membrane association is regulated by the Rab3a recycling machinery and presynaptic activity. J Biol Chem 288:7438-7449.

107. Yin G, Lopes da Fonseca T, Eibach SE, Anduaga AM, Breda C, Orcellet ML, Szegő EM, Guerreiro P, Lazaro DF, Braus GH, Fernandez CO, Griesinger C, Becker S, Goody RS, Itzen A, Giorgini F, Outeiro TF, Zweckstetter M (2014) α-Synuclein interacts with the switch region of Rab8a in a Ser129 phosphorylation-dependent manner. Neurobiol Dis 70:149-161.

108. Eliezer D (2008) Protein folding and aggregation in vitro models of Parkinson’s disease: structure and function of alpha-synuclein. In: Parkinson’s disease: molecular and therapeutic insights from model systems (Nass R, Prezedborski S, eds), pp 575-598. Elsevier/Academic Press, New York, NY.

109. Burré J, Sharma M, Südhof TC (2014) α-Synuclein assembles into higher-order multimers upon membrane binding to promote SNARE complex formation. Proc Natl Acad Sci U S A 111:E4274-E4283.

110. Larsen KE, Schmitz Y, Troyer MD, Mosharov E, Dietrich P, Quazi AZ, Savalle M, Nemani V, Chaudhry FA, Edwards RH, Stefanis L, Sulzer D (2006) Alpha-synuclein overexpression in PC12 and chromaffin cells impairs catecholamine release by interfering with a late step in exocytosis. J Neurosci 26:11915-11922.

111. Cabin DE, Shimazu K, Murphy D, Cole NB, Gottschalk W, McIlwain KL, Orrison B, Chen A, Ellis CE, Paylor R, Lu B,
Nussbaum RL (2002) Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking α-synuclein. J Neurosci 22:8797-8807.

112. Outeiro TF, Lindquist S (2003) Yeast cells provide insight into alpha-synuclein biology and pathobiology. Science 302:1772-1775.

113. Cooper AA, Gitler AD, Cashikar A, Haynes CM, Hill KJ, Bhullar B, Liu K, Xu K, Strathearn KE, Liu F, Cao S, Caldwell KA, Caldwell GA, Marsischky G, Kolodner RD, Labaer J, Roche J, Bonini NM, Lindquist S (2006) Alpha-synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson’s models. Science 313:324-328.

114. Gitler AD, Bevis BJ, Shorter J, Strathearn KE, Hamamichi S, Su LJ, Caldwell KA, Caldwell GA, Roche JC, McCaffrey JM, Barlowe C, Lindquist S (2008) The Parkinson’s disease protein α-synuclein disrupts cellular Rab homeostasis. Proc Natl Acad Sci U S A 105:145-150.

115. Soper JH, Roy S, Stieber A, Lee E, Wilson RB, Trojanowski JQ, Burd CG, Lee VM (2008) Alpha-synuclein-induced aggregation of cytoplasmic vesicles in Saccharomyces cerevisiae. Mol Biol Cell 19:1093-1103.

116. Diao J, Burré J, Vivona S, Cipriano DJ, Sharma M, Kyoung M, Südhof TC, Brunger AT (2013) Native α-synuclein induces clustering of synaptic-vesicle mimics via binding to phospholipids and synaptobrevin-2/VAMP2. Elife 2:e00592.

117. DeWitt DC, Rhoades E (2013) α-Synuclein can inhibit SNARE-mediated vesicle fusion through direct interactions with lipid bilayers. Biochemistry 52:2385-2387.

118. Lai Y, Kim S, Varkey J, Lou X, Song JK, Diao J, Langen R, Shin YK (2014) Nonaggregated α-synuclein influences SNARE-dependent vesicle docking via membrane binding. Biochemistry 53:3889-3896.

119. Choi BK, Choi MG, Kim JY, Yang Y, Lai Y, Kweon DH, Lee NK, Shin YK (2013) Large α-synuclein oligomers inhibit neuronal SNARE-mediated vesicle docking. Proc Natl Acad Sci U S A 110:4087-4092.

120. Darios F, Ruipérez V, López I, Villanueva J, Gutierrez LM, Davletov B (2010) Alpha-synuclein sequesters arachidonic acid to modulate SNARE-mediated exocytosis. EMBO Rep 11:528-533.

121. Ben Gedalya T, Loeb V, Israeli E, Altschuler Y, Selkoe DJ, Sharon R (2009) α-Synuclein and polyunsaturated fatty acids promote clathrin-mediated endocytosis and synaptic vesicle recycling. Traffic 10:218-234.

122. Vargas KJ, Makani S, Davis T, Westphal CH, Castillo PE, Chandra SS (2014) Synucleins regulate the kinetics of synaptic vesicle endocytosis. J Neurosci 34:9364-9376.

123. Nemani VM, Lu W, Berge V, Nakamura K, Onoa B, Lee MK, Chaudhry FA, Nicoll RA, Edwards RH (2010) Increased expression of α-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle re clustering after endocytosis. Neuron 65:66-79.

124. Jenco JM, Rawlingson A, Daniels B, Morris AJ (1998) Regulation of phospholipase D2: selective inhibition of mammalian phospholipase D isoenzymes by α- and β-synucleins. Biochemistry 37:4901-4909.

125. Ahn BH, Rhim H, Kim SY, Sung YM, Lee MY, Choi JY, Wolozin B, Chang JS, Lee YH, Kwon TK, Chung KC, Yoon SH, Hahn SJ, Kim MS, Jo YH, Min DS (2002) α-Synuclein interacts with phospholipase D isoenzymes and inhibits pervanadate-induced phospholipase D activation in human embryonic kidney-293 cells. J Biol Chem 277:12334-12342.

126. Rappley I, Gitler AD, Selvy PE, LaVoie MJ, Levy BD, Brown HA, Lindquist S, Selkoe DJ (2009) Evidence that α-synuclein does not inhibit phospholipase D. Biochemistry 48:1077-1083.

127. Payton JE, Perrin RJ, Woods WS, George JM (2004) Structural determinants of PLD2 inhibition by alpha-synuclein. J Mol Biol 337:1001-1009.

128. McDermott M, Wakedama MJ, Morris AJ (2004) Phospholipase D. Biochem Cell Biol 82:225-253.

129. Jo E, McLaurin J, Yip CM, St George-Hyslop P, Fraser PE (2000) alpha-Synuclein membrane interactions and lipid specificity. J Biol Chem 275:34328-34334.

130. Lee J, Taneva SG, Holland BW, Tieleman DP, Cornell RB (2014) Structural basis for autoinhibition of CTP:phosphocholine cytidylyltransferase (CCT), the regulatory enzyme in phosphatidylcholine synthesis, by its membrane-binding amphipathic helix. J Biol Chem 289:1742-1755.

131. Chong SS, Taneva SG, Lee JM, Cornell RB (2014) The curvature sensitivity of a membrane-binding amphipathic helix can be modulated by the charge on a flanking region. Biochemistry 53:450-461.

132. Arnold RS, Cornell RB (1996) Lipid regulation of CTP: phosphocholine cytidylyltransferase: electrostatic, hydrophobic, and synergistic interactions of anionic phospholipids and diacylglycerol. Biochemistry 35:9917-9924.

133. Cornell RB, Taneva SG (2006) Amphipathic helices as mediators of the membrane interaction of amphitropic proteins, and as modulators of bilayer physical properties. Curr Protein Pept Sci 7:539-552.
134. Davies SM, Epand RM, Kraayenhof R, Cornell RB (2001) Regulation of CTP: phosphocholine cytidylyltransferase activity by the physical properties of lipid membranes: an important role for stored curvature strain energy. Biochemistry 40:10522-10531.

135. Johnson JE, Xie M, Singh LM, Edge R, Cornell RB (2003) Both acidic and basic amino acids in an amphitropic enzyme, CTP: phosphocholine cytidylyltransferase, dictate its selectivity for anionic membranes. J Biol Chem 278:514-522.

136. Johnson JE, Rao NM, Hui SW, Cornell RB (1998) Conformation and lipid binding properties of four peptides derived from the membrane-binding domain of CTP: phosphocholine cytidylyltransferase. Biochemistry 37:9509-9519.

137. Ding Z, Taneva SG, Huang HK, Campbell SA, Semenc L, Chen N, Cornell RB (2012) A 22-mer segment in the structurally pliable regulatory domain of metazoaan CTP: phosphocholine cytidylyltransferase facilitates both silencing and activating functions. J Biol Chem 287:38980-38991.

138. Friesen JA, Campbell HA, Kent C (1999) Enzymatic and cellular characterization of a catalytic fragment of CTP:phosphocholine cytidylyltransferase alpha. J Biol Chem 274:13384-13389.

139. Yang W, Boggs KP, Jackowski S (1995) The association of lipid activators with the amphipathic helical domain of CTP: phosphocholine cytidylyltransferase accelerates catalysis by increasing the affinity of the enzyme for CTP. J Biol Chem 270:23951-23957.

140. Perez RG, Waimire JC, Lin E, Liu JJ, Guo F, Zigmond MJ (2002) A role for α-synuclein in the regulation of dopamine biosynthesis. J Neurosci 22:3090-3099.

141. Osterova N, Petruccelli L, Farrer M, Mehta N, Choi P, Hardy J, Wolozin B (1999) alpha-Synuclein shares physical and functional homology with 14-3-3 proteins. J Neurosci 19:5782-5791.

142. Wersinger C, Sidhu A (2003) Attenuation of dopamine transporter activity by alpha-synuclein. Neurosci Lett 340:189-192.

143. Oaks AW, Sidhu A (2011) Synuclein modulation of monoamine transporters. FEBS Lett 585:1001-1006.

144. Lee FJ, Liu F, Pristupa ZB, Niznik HB (2001) Direct binding and functional coupling of alpha-synuclein to the dopamine transporters accelerate dopamine-induced apoptosis. FASEB J 15:916-926.

145. Wersinger C, Rusnak M, Sidhu A (2006) Modulation of the trafficking of the human serotonin transporter by human alpha-synuclein. Eur J Neurosci 24:55-64.

146. Wersinger C, Jeannotte A, Sidhu A (2006) Attenuation of the norepinephrine transporter activity and trafficking via interactions with alpha-synuclein. Eur J Neurosci 24:3141-3152.

147. Woods WS, Boettcher JM, Zhou DH, Kloepper KD, Hartman KL, Ladró N, Qi Z, Rienstra CM, George IM (2007) Conformation-specific binding of alpha-synuclein to novel protein partners detected by phage display and NMR spectroscopy. J Biol Chem 282:34555-34567.

148. Fortin DL, Troyer MD, Nakamura K, Kubo S, Anthony MD, Edwards RH (2004) Lipids mediate the synaptic localization of alpha-synuclein. J Neurosci 24:6715-6723.

149. Martinez Z, Zhu M, Han S, Fink AL (2007) GM1 specifically interacts with alpha-synuclein and inhibits fibrillation. Biochemistry 46:1868-1877.

150. Fantini J, Yahni N (2013) The driving force of alpha-synuclein insertion and amyloid channel formation in the plasma membrane of neural cells: key role of ganglioside- and cholesterol-binding domains. Adv Exp Med Biol 991:15-26.

151. Fantini J, Yahni N (2011) Molecular basis for the glycosphingolipid-binding specificity of α-synuclein: key role of tyrosine 39 in membrane insertion. J Mol Biol 408:654-669.

152. Di Pasquale E, Fantini J, Chahinian H, Maresca M, Taieb N, Yahni N (2010) 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 397:202-218.

153. Park JY, Kim KS, Lee SB, Ryu JS, Chung KC, Choo YK, Jou I, Kim J, Park SM (2009) On the mechanism of internalization of alpha-synuclein into microglia: roles of ganglioside GM1 and lipid raft. J Neurochem 110:400-411.

154. Bartels T, Kim NC, Luth ES, Selkoe DJ (2014) N-acetylation of α-synuclein increases its helical folding propensity, GM1 binding specificity and resistance to aggregation. PLoS One 9:e103727.

155. Goers J, Manning-Bog AR, McCormack AL, Millett IS, Doniach S, Di Monte DA, Uversky VN, Fink AL (2003) Nuclear localization of alpha-synuclein and its interaction with histones. Biochemistry 42:8465-8471.

156. Huang Z, Xu Z, Wu Y, Zhou Y (2011) Determining nuclear localization of alpha-synuclein in mouse brains. Neuroscience 199:318-332.

157. Yu S, Li X, Liu G, Han J, Zhang C, Li Y, Xu S, Liu C, Gao Y, Yang H, Uéda K, Chan P (2007) Extensive nuclear localization of alpha-synuclein in normal rat brain neurons revealed by a novel monoclonal antibody. Neuroscience 145:539-555.
158. Vivacqua G, Casini A, Vaccaro R, Fornai F, Yu S, D’Este L (2011) Different sub-cellular localization of alpha-synuclein in the C57BL/6 mouse s central nervous system by two novel monoclonal antibodies. J Chem Neuroanat 41:97-110.

159. Zhong SC, Luo X, Chen XS, Cai QY, Liu J, Chen XH, Yao ZX (2010) Expression and subcellular location of alpha-synuclein during mouse-embryonic development. Cell Mol Neurobiol 30:469-482.

160. Wakamatsu M, Ishii A, Ukai Y, Sakagami J, Iwata S, Ono M, Matsumoto K, Nakamura A, Tada N, Kobayashi K, Iwatsubo T, Yoshimoto M (2007) Accumulation of phosphorylated alpha-synuclein in dopaminergic neurons of transgenic mice that express human alpha-synuclein. J Neurosci Res 85:1819-1825.

161. Zhang L, Zhang C, Zhu Y, Cai Q, Chan P, Ueda K, Yu S, Yang H (2008) Semi-quantitative analysis of alpha-synuclein in subcellular pools of rat brain neurons: an immunogold electron microscopic study using a C-terminal specific monoclonal antibody. Brain Res 1244:40-52.

162. Specht CG, Tigaret CM, Rast GF, Thalhammer A, Rudhard Y, Schoepfer R (2005) Subcellular localisation of recombinant alpha- and gamma-synuclein. Mol Cell Neurosci 28:326-334.

163. Schell H, Hasegawa T, Neumann M, Kahle PJ (2009) Nuclear and neuritic distribution of serine-129 phosphorylated alpha-synuclein in transgenic mice. Neuroscience 160:796-804.

164. Guerrero E, Vasudevaraju P, Hegde ML, Britton GB, Rao KS (2013) Recent advances in α-synuclein functions, advanced glycation, and toxicity: implications for Parkinson’s disease. Mol Neurobiol 47:525-536.

165. Lotharius J, Brundin P (2002) Impaired dopamine storage resulting from alpha-synuclein mutations may contribute to the pathogenesis of Parkinson’s disease. Hum Mol Genet 11:2395-2407.

166. Barbour R, Kling K, Anderson JP, Banducci K, Cole T, Diep L, Fox M, Goldstein JM, Soriano F, Seubert P, Chilcote TJ (2008) Red blood cells are the major source of alpha-synuclein in blood. Neurodegener Dis 5:55-59.

167. Zhu M, Qin ZJ, Hu D, Munishkina LA, Fink AL (2006) Alpha-synuclein can function as an antioxidant preventing oxidation of unsaturated lipid in vesicles. Biochemistry 45:8135-8142.

168. Quilty MC, King AE, Gai WP, Pountney DL, West AK, Vickers JC, Dickson TC (2006) Alpha-synuclein is upregulated in neurones in response to chronic oxidative stress and is associated with neuroprotection. Exp Neurol 199:249-256.

169. Liu F, Hindupur J, Nguyen JL, Ruf KJ, Zhu J, Schieler JL, Bonham CC, Wood KV, Davission VJ, Rochet JC (2008) Methionine sulfoxide reductase A protects dopaminergic cells from Parkinson’s disease-related insults. Free Radic Biol Med 45:242-255.

170. Dexter DT, Carter CJ, Wells FR, Javoy-Agid F, Agid Y, Lees A, Jenner P, Marsden CD (1989) Basal lipid peroxidation in substantia nigra is increased in Parkinson’s disease. J Neurochem 52:381-389.

171. Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443:787-795.

172. Martin IJ, Pan Y, Price AC, Sterling W, Copeland NG, Jenkins NA, Price DL, Lee MK (2006) Parkinson’s disease alpha-synuclein transgenic mice develop neuronal mitochondrial degeneration and cell death. J Neurosci 26:41-50.

173. Cole NB, Dieuliiis D, Leo P, Mitchell DC, Nussbaum RL (2008) Mitochondrial translocation of alpha-synuclein is promoted by intracellular acidification. Exp Cell Res 314:2076-2089.

174. Devi L, Raghavendran V, Prabhuj BM, Avadhani NG, Anandatheerthavarada HK (2008) Mitochondrial import and accumulation of alpha-synuclein impair complex I in human dopaminergic neuronal cultures and Parkinson disease brain. J Biol Chem 283:9089-9100.

175. Li WW, Yang R, Guo JC, Ren HM, Zha XL, Cheng JS, Cai DF (2007) Localization of alpha-synuclein to mitochondria within midbrain of mice. Neureport 18:1543-1546.

176. Shavali S, Brown-Borg HM, Ebadi M, Porter J (2008) Mitochondrial localization of alpha-synuclein protein in alpha-synuclein overexpressing cells. Neurosci Lett 439:125-128.

177. Nakamura K, Nemani VM, Wallender EZ, Kaehlcke K, Ott M, Edwards RH (2008) Optical reporters for the conformation of alpha-synuclein reveal a specific interaction with mitochondria. J Neurosci 28:12305-12317.

178. Nakamura K, Nemani VM, Azarbal F, Skibinski G, Levy JM, Egami K, Munishkina L, Zhang J, Gardner B, Wakabayashi J, Sesaki H, Cheng Y, Finkbeiner S, Nussbaum RL, Masliah E, Edwards RH (2011) Direct membrane association drives mitochondrial fission by the Parkinson disease-associated protein α-synuclein. J Biol Chem 286:20710-20726.

179. Zigoneanu IG, Yang YJ, Krois AS, Haque E, Pielak GJ (2012) Interaction of α-synuclein with vesicles that mimic mitochondrial membranes. Biochim Biophys Acta 1818:512-519.

180. Ruipérez V, Darias F, Davletov B (2010) Alpha-synuclein, lipids and Parkinson’s disease. Prog Lipid Res 49:420-428.

181. Sharon R, Goldberg MS, Bar-Josef I, Betensky RA, Shen J, Selkoe DJ (2001) Alpha-Synuclein occurs in lipid-rich high
molecular weight complexes, binds fatty acids, and shows homology to the fatty acid-binding proteins. Proc Natl Acad Sci U S A 98:9110-9115.

182. Broersen K, van den Brink D, Fraser G, Goedert M, Davletov B (2006) Alpha-synuclein adopts an alpha-helical conformation in the presence of polyunsaturated fatty acids to hinder micelle formation. Biochemistry 45:15610-15616.

183. De Franceschi G, Frare E, Bubacco L, Mammi S, Fontana A, de Laureto PP (2009) Molecular insights into the interaction between alpha-synuclein and docosahexaenoic acid. J Mol Biol 394:94-107.

184. Karube H, Sakamoto M, Arawaka S, Hara S, Sato H, Ren CH, Goto S, Koyama S, Wada M, Kawanami T, Kato T (2008) N-terminal region of alpha-synuclein is essential for the fatty acid-induced oligomerization of the molecules. FEBS Lett 582:3693-3700.

185. Castagnet PI, Golovko MY, Barceló-Coblijn GC, Nussbaum RL, Murphy EJ (2005) Fatty acid incorporation is decreased in astrocytes cultured from alpha-synuclein gene-ablated mice. J Neurochem 94:839-849.

186. Barceló-Coblijn G, Golovko MY, Weinhofer I, Berger J, Murphy EJ (2007) Brain neutral lipids mass is increased in alpha-synuclein gene-ablated mice. J Neurochem 101:132-141.

187. Golovko MY, Faergeman NJ, Cole NB, Castagnet PI, Nussbaum RL, Murphy EJ (2005) Alpha-synuclein gene deletion decreases brain palmitate uptake and alters the palmitate metabolism in the absence of alpha-synuclein palmitate binding. Biochemistry 44:8251-8259.

188. Golovko MY, Rosenberger TA, Faergeman NJ, Feddersen S, Cole NB, Pribill I, Berger J, Nussbaum RL, Murphy EJ (2006) Acyl-CoA synthetase activity links wild-type but not mutant alpha-synuclein to brain arachidonate metabolism. Biochemistry 45:6956-6966.

189. Golovko MY, Rosenberger TA, Feddersen S, Faergeman NJ, Murphy EJ (2007) Alpha-synuclein gene ablation increases docosahexaenoic acid incorporation and turnover in brain phospholipids. J Neurochem 101:201-211.

190. Chandra S, Fornai F, Kwon HB, Yazdani U, Atasoy D, Liu X, Hammer RE, Battaglia G, German DC, Castillo PE, Südhof TC (2004) Double-knockout mice for alpha- and beta-synucleins: effect on synaptic functions. Proc Natl Acad Sci U S A 101:14966-14971.

191. Burré J, Sharma M, Südhof TC (2012) Systematic mutagenesis of α-synuclein reveals distinct sequence requirements for physiological and pathological activities. J Neurosci 32:15227-15242.

192. Volles MJ, Lee SJ, Rochet JC, Shitlberman MD, Ding TT, Kessler JC, Lansbury PT Jr. (2001) Vesicle permeabilization by proteofibrillar alpha-synuclein: implications for the pathogenesis and treatment of Parkinson’s disease. Biochemistry 40:7812-7819.

193. Volles MJ, Lansbury PT Jr. (2002) Vesicle permeabilization by proteofibrillar alpha-synuclein is sensitive to Parkinson’s disease-linked mutations and occurs by a pore-like mechanism. Biochemistry 41:4595-4602.

194. Zakharov SD, Hulmeen JD, Dutseva EA, Antonenko YN, Rochet JC, Cramer WA (2007) Helical α-synuclein forms highly conductive ion channels. Biochemistry 46:14369-14379.

195. Narayanan V, Scarlata S (2001) Membrane binding and self-association of alpha-synucleins. Biochemistry 40:9927-9934.

196. Zhu M, Fink AL (2003) Lipid binding inhibits alpha-synuclein fibril formation. J Biol Chem 278:16873-16877.

197. Lee HJ, Choi C, Lee SJ (2002) Membrane-bound alpha-synuclein has a high aggregation propensity and the ability to seed the aggregation of the cytosolic form. J Biol Chem 277:671-678.

198. Sharon R, Bar-Joseph I, Frosch MP, Walsh DM, Hamilton JA, Selkoe DJ (2003) The formation of highly soluble oligomers of alpha-synuclein is regulated by fatty acids and enhanced in Parkinson’s disease. Neuron 37:583-595.

199. Jo E, Darabie AA, Han K, Tandon A, Fraser PE, McLaurin J (2004) alpha-Synuclein-synaptosomal membrane interactions: implications for fibrillogenesis. Eur J Biochem 271:3180-3189.

200. Necula M, Chirita CN, Kuret J (2003) Rapid anionic micelle-mediated alpha-synuclein fibrillization in vitro. J Biol Chem 278:46674-46680.

201. Perrin RJ, Woods WS, Clayton DF, George JM (2001) Exposure to long chain polyunsaturated fatty acids triggers rapid multimerization of synucleins. J Biol Chem 276:41958-41962.

202. Khalaf O, Fauvet B, Oueslati A, Dikiy I, Mahul-Mellier AL, Ruggeri FS, Mbefo MK, Vercruyssse F, Dietler G, Lee SJ, Eliezer D, Lashuel HA (2014) The H50Q mutation enhances α-synuclein aggregation, secretion, and toxicity. J Biol Chem 289:21856-21876.

203. Ghosh D, Mondal M, Mohite GM, Singh PK, Ranjan P, Anoop A, Ghosh S, Jha NN, Kumar A, Maji SK (2013) The Parkinson’s disease-associated H50Q mutation accelerates α-Synuclein aggregation in vitro. Biochemistry 52:6925-6927.

204. Rutherford NJ, Moore BD, Golde TE, Giasson BI (2014)
Divergent effects of the H50Q and G51D SNCA mutations on the aggregation of α-synuclein. J Neurochem (in press).

205. Giasson BI, Uryu K, Trojanowski JQ, Lee VM (1999) Mutant and wild type human alpha-synucleins assemble into elongated filaments with distinct morphologies in vitro. J Biol Chem 274:7619-7622.

206. Narhi L, Wood SJ, Steavenson S, Jiang Y, Wu GM, Anafi D, Kaufman SA, Martin E, Sitney K, Denis P, Louis JC, Wypych J, Biere AL, Citron M (1999) Both familial Parkinson's disease mutations accelerate alpha-synuclein aggregation. J Biol Chem 274:9843-9846.

207. Conway KA, Lee SJ, Rochet JC, Ding TT, Williamson RE, Lansbury PT Jr. (2000) Acceleration of oligomerization, not fibrillization, is a shared property of both alpha-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy. Proc Natl Acad Sci USA 97:571-576.

208. Choi W, Zibae S, Jakes R, Serpell LC, Davletov B, Crowther RA, Goedert M (2004) Mutation E46K increases phospholipid binding and assembly into filaments of human alpha-synuclein. FEBS Lett 576:363-368.

209. Thayani D, Helm JR, Nycz DC, Bentley M, Liang Y, Hay JC (2010) Alpha-synuclein delays endoplasmic reticulum (ER)-to-Golgi transport in mammalian cells by antagonizing ER/Golgi SNAREs. Mol Biol Cell 21:1850-1863.

210. Soper JH, Kehm V, Burd CG, Bankaitis VA, Lee VM (2011) Aggregation of α-synuclein in S. cerevisiae is associated with defects in endosomal trafficking and phospholipid biosynthesis. J Mol Neurosci 43:391-405.

211. Dalló E, Ferrer I (2005) Alpha-synuclein binding to rab3a in multiple system atrophy. Neurosci Lett 380:170-175.

212. Dalló E, Barrachina M, Rosa JL, Ambrosio S, Ferrer I (2004) Abnormal alpha-synuclein interactions with rab3a and rabphilin in diffuse Lewy body disease. Neurobiol Dis 16:92-97.

213. Dalló E, Gómez-Isla T, Rosa JL, Nieto Bodelón M, Cuadrado Tejedor M, Barrachina M, Ambrosio S, Ferrer I (2004) Abnormal alpha-synuclein interactions with Rab proteins in alpha-synuclein A30P transgenic mice. J Neuropathol Exp Neurol 63:302-313.

214. Loeb V, Yakunin E, Saada A, Sharon R (2010) The transgenic overexpression of alpha-synuclein and not its related pathology associates with complex I inhibition. J Biol Chem 285:7334-7343.

215. Parihar MS, Parihar A, Fujita M, Hashimoto M, Ghaffourifar P (2008) Mitochondrial association of alpha-synuclein causes oxidative stress. Cell Mol Life Sci 65:1272-1284.

216. Saha AR, Ninkina NN, Hanger DP, Anderton BH, Davies AM, Buchman VL (2000) Induction of neuronal death by alpha-synuclein. Eur J Neurosci 12:3073-3077.

217. Kamp F, Exner N, Lutz AK, Wender N, Hegemann J, Brunner B, Nuscher B, Bartels T, Giese A, Beyer K, Eimer S, Winklhofer KF, Haass C (2010) Inhibition of mitochondrial fusion by α-synuclein is rescued by PINK1, Parkin and DJ-1. EMBO J 29:3571-3589.

218. Xu S, Zhou M, Yu S, Cai Y, Zhang A, Uéda K, Chan P (2006) Oxidative stress induces nuclear translocation of C-terminus of alpha-synuclein in dopaminergic cells. Biochem Biophys Res Commun 342:330-335.

219. Zhou M, Xu S, Mi J, Uéda K, Chan P (2013) Nuclear translocation of alpha-synuclein increases susceptibility of MES23.5 cells to oxidative stress. Brain Res 1500:19-27.

220. Sangchot P, Sharma S, Chetsawang B, Porter J, Govitrapong P, Ebadi M (2002) Deferoxamine attenuates iron-induced oxidative stress and prevents mitochondrial aggregation and alpha-synuclein translocation in SK-N-SH cells in culture. Dev Neurosci 24:143-153.

221. Kontopoulos E, Parvin JD, Feany MB (2006) Alpha-synuclein acts in the nucleus to inhibit histone acetylation and promote neurotoxicity. Hum Mol Genet 15:3012-3023.

222. Ma KL, Song LK, Yuan YH, Zhang Y, Han N, Gao K, Chen NH (2014) The nuclear accumulation of alpha-synuclein is mediated by importin alpha and promotes neurotoxicity by accelerating the cell cycle. Neuropharmacology 82:132-142.

223. Kubo S, Nemani VM, Chalkley RJ, Anthony MD, Hattori N, Mizuno Y, Edwards RH, Fortin DL (2005) A combinatorial code for the interaction of α-synuclein with membranes. J Biol Chem 280:31664-31672.

224. Valastyan JS, Termine DJ, Lindquist S (2014) Splice isoform and pharmacological studies reveal that sterol depletion relocals α-synuclein and enhances its toxicity. Proc Natl Acad Sci USA 111:3014-3019.

225. Eisbach SE, Outeiro TF (2013) Alpha-synuclein and intracellular trafficking: impact on the spreading of Parkinson's disease pathology. J Mol Med (Berl) 91:693-703.

226. Vekrellis K, Xilouri M, Emmanouilidou E, Rideout HJ, Stefanis L (2011) Pathological roles of α-synuclein in neurological disorders. Lancet Neurol 10:1015-1025.

227. Lee SJ, Lim HS, Masliah E, Lee HJ (2011) Protein aggregate spreading in neurodegenerative diseases: problems and perspectives. Neurosci Res 70:339-348.

228. Borghi R, Marchese R, Negro A, Marinelli L, Forloni G, Zaccheo D, Abbuzzese G, Tabaton M (2000) Full length alpha-synuclein is present in cerebrospinal fluid from
Parkinson’s disease and normal subjects. Neurosci Lett 287:65-67.

229. El-Agnaf OM, Salem SA, Paleologou KE, Cooper LJ, Fullwood NJ, Gibson MJ, Curran MD, Court JA, Mann DM, Ikeda S, Cookson MR, Hardy J, Allsop D (2003) Alpha-synuclein implicated in Parkinson’s disease is present in extracellular biological fluids, including human plasma. FASEB J 17:1945-1947.

230. El-Agnaf OM, Salem SA, Paleologou KE, Curran MD, Gibson MJ, Court JA, Schlossmacher MG, Allsop D (2006) Detection of oligomeric forms of alpha-synuclein protein in human plasma as a potential biomarker for Parkinson’s disease. FASEB J 20:419-425.

231. Li JY, Englund E, Holton JL, Soulet D, Hagell P, Lees AJ, Lashley T, Quinn NP, Rehncrona S, Björklund A, Widner H, Revesz T, Lindvall O, Brundin P (2008) Lewy bodies in grafted neurons in subjects with Parkinson’s disease suggest host-to-graft disease propagation. Nat Med 14:501-503.

232. Kordower JH, Chu Y, Hauser RA, Freeman TB, Olanow CW (2008) Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson’s disease. Nat Med 14:504-506.

233. Kordower JH, Chu Y, Hauser RA, Olanow CW, Freeman TB (2008) Transplanted dopaminergic neurons develop PD pathologic changes: a second case report. Mov Disord 23:2303-2306.

234. Lee HJ, Patel S, Lee SJ (2005) Intravesicular localization and exocytosis of alpha-synuclein and its aggregates. J Neurosci 25:6016-6024.

235. Desplats P, Lee HJ, Bae EJ, Patrick C, Rockenstein E, Crews L, Spencer B, Masliah E, Lee SJ (2009) Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. Proc Natl Acad Sci U S A 106:13010-13015.

236. Sung JY, Kim J, Paik SR, Park JH, Ahn YS, Chung KC (2001) Induction of neuronal cell death by Rab5A-dependent endocytosis of alpha-synuclein. J Biol Chem 276:27441-27448.

237. Zhang W, Wang T, Pei Z, Miller DS, Wu X, Block ML, Wilson B, Zhang W, Zhou Y, Hong JS, Zhang J (2005) Aggregated alpha-synuclein activates microglia: a process leading to disease progression in Parkinson’s disease. FASEB J 19:533-542.

238. Klegeris A, Giasson BI, Zhang H, Maguire J, Pelech S, McGeer PL (2006) Alpha-synuclein and its disease-causing mutants induce ICAM-1 and IL-6 in human astrocytes and astrocytoma cells. FASEB J 20:2000-2008.

239. Lee HJ, Kim C, Lee SJ (2010) Alpha-synuclein stimulation of astrocytes: potential role for neuroinflammation and neuroprotection. Oxid Med Cell Longev 3:283-287.

240. Kim C, Ho DH, Suk JE, You S, Michael S, Kang J, Joong Lee S, Masliah E, Hwang D, Lee HJ, Lee SJ (2013) Neuron-released oligomeric α-synuclein is an endogenous agonist of TLR2 for paracrine activation of microglia. Nat Commun 4:1562.

241. Lee HJ, Suk JE, Bae EJ, Lee HJ, Paik SR, Lee SJ (2008) Assembly-dependent endocytosis and clearance of extracellular alpha-synuclein. Int J Biochem Cell Biol 40:1835-1849.

242. Liu J, Zhang JP, Shi M, Quinn T, Bradner J, Beyer R, Chen S, Zhang J (2009) Rab11a and HSP90 regulate recycling of extracellular alpha-synuclein. J Neurosci 29:1480-1485.

243. Zhang H, Duan C, Yang H (2014) Defective autophagy in Parkinson’s disease: lessons from genetics. Mol Neurobiol (in press).

244. Ejlerskov P, Rasmussen I, Nielsen TT, Bergström AL, Tohyama Y, Jensen PH, Vilhardt F (2013) Tubulin polymerization-promoting protein (TPPP/p25α) promotes unconventional secretion of α-synuclein through exophagy by impairing autophagosome-lysosome fusion. J Biol Chem 288:17313-17335.

245. Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, Caldwell GA, Sidransky E, Grabowski GA, Krainc D (2011) Gaucher disease glucocerebrosidase and α-synuclein form a bidirectional pathogenic loop in synucleinopathies. Cell 146:37-52.

246. Murphy KE, Gysbers AM, Abbott SK, Tayebi N, Kim WS, Sidransky E, Cooper A, Garner B, Halliday GM (2014) Reduced glucocerebrosidase is associated with increased α-synuclein in sporadic Parkinson’s disease. Brain 137:834-848.

247. Bae EJ, Yang NY, Song M, Lee CS, Lee JS, Jung BC, Lee HJ, Kim S, Masliah E, Sardi SP, Lee SJ (2014) Glucocerebrosidase depletion enhances cell-to-cell transmission of α-synuclein. Nat Commun 5:4755.