Review

Cédric Eichmann, Stefan Bibow* and Roland Riek*

α-Synuclein lipoprotein nanoparticles

DOI 10.1515/ntrev-2016-0062
Received July 28, 2016; accepted November 12, 2016; previously published online January 20, 2017

Abstract: Apolipoprotein nanodiscs are a versatile tool in nanotechnology as membrane mimetics allowing, for example, the study of membrane proteins. It has recently been discovered that the Parkinson's disease associated protein α-synuclein (α-Syn) can also form discoid-like lipoprotein nanoparticles. The present review highlights the observation that α-Syn has the properties to define stable and homogeneous populations of nanoparticles with diameters of 7–10 nm and 19–28 nm by modifying lipid vesicles or encapsulating lipid bilayers in a nanodisc-type fashion, respectively. In contrast to apolipoprotein nanodiscs, α-Syn nanoparticles can incorporate entirely negatively charged lipids emphasizing their potential use in nanotechnology as a negatively charged membrane mimetic.

Keywords: lipoprotein; nanodiscs; NMR; Parkinson's disease; α-synuclein.

1 Introduction

The protein α-synuclein (α-Syn) is associated with Parkinson's disease (PD) [1, 2]. Its conformational plasticity appears to be of key importance for the physiological functions. Whereas monomeric α-Syn is largely disordered [3–5] in an aqueous solution, membrane mimicking environments containing anionic detergents [3] or phospholipids [5–7] trigger a disorder-to-helix transition. The structural transition towards an α-helical state is mediated by seven imperfect 11 amino acid long amphipathic repeats in the N-terminal region of α-Syn that bear resemblance to the amphipathic helical repeats found in apolipoproteins [3, 6, 8]. Notably, the C-terminal residues remain unstructured during *in vitro* experiments since they do not seem to interact with anionic membranes [5, 9]. Still highly debated is the *in vivo* structure of α-Syn. In 2011, two groups reported that cellular α-Syn exists as a helical tetramer when purified under non-denaturing conditions [10, 11]. However, these results were recently challenged by in-cell nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR) studies of α-Syn electroporated into mammalian cells [12]. The in-cell NMR spectra showed protein signals that overlapped with those NMR signals observed for disordered *in vitro* α-Syn, therefore excluding the possibility of a major tetrameric folded species under these experimental conditions [12, 13].

A structure-function relationship has not yet been established because the physiological role of α-Syn is still unknown [14–16]. There is, however, growing evidence that α-Syn-membrane interactions form the basis of multiple *in vivo* functions such as synaptic vesicle pool maintenance [17, 18], regulation of dopamine neurotransmission [19, 20], transport of lipids and fatty acids [21–25], membrane trafficking [26–28], synaptic plasticity [29, 30], and assistance in SNARE complex formation [31–34]. Moreover, membranes also seem to influence the pathological aggregation of α-Syn towards amyloid fibrils with β-sheet structure, the hallmark of PD [35–43]. Although the physiological function(s) of α-Syn remain elusive, the seven imperfect 11 amino acid long amphipathic repeats in the N-terminal region of α-Syn and their capability to interact both with negatively charged and zwitterionic phospholipids allows for the *in vitro* formation of discoid-like lipoprotein nanoparticles, so-called α-Syn lipoprotein nanoparticles [44–46]. These α-Syn lipoprotein nanoparticles are the focus of this review with an emphasis on their potential use in nanotechnology as a membrane container comprising negatively charged lipids or a membrane mimetic for the study of membrane proteins with properties distinct from the usually used apolipoprotein nanodiscs.

***Corresponding authors: Stefan Bibow,** Laboratory of Physical Chemistry, Swiss Federal Institute of Technology, ETH-Hönggerberg, CH-8093 Zürich, Switzerland, e-mail: stefan.bibow@phys.chem.ethz.ch; and Biozentrum, University of Basel, Klingelbergstr. 50/70, CH-4056 Basel, Switzerland; and **Roland Riek,** Laboratory of Physical Chemistry, Swiss Federal Institute of Technology, ETH-Hönggerberg, CH-8093 Zürich, Switzerland, e-mail: roland.riek@phys.chem.ethz.ch
Cédric Eichmann: In-Cell NMR Laboratory, Department of NMR-supported Structural Biology, Leibniz Institute of Molecular Pharmacology (FMP Berlin), Robert-Rössle Strasse 10, 13125 Berlin, Germany

2 Formation of stable α-synuclein lipoprotein particles

Incubation of monomeric α-Syn with preformed negatively charged lipid vesicles at high protein-to-lipid ratios (1 : 10 and higher) results in reshaping of negatively charged giant lipid vesicles and small unilamellar vesicles (SUVs, diameter ~ 25 nm) into discoid-like lipoprotein nanoparticles with a diameter of 7–10 nm [44, 45]. Interestingly, incubation of α-Syn with multilamellar vesicles composed of lipids commonly used to mimic mitochondrial membranes leads to a disruption of the vesicles and subsequent formation of lipid nanoparticles, indicating a link between overexpressed α-Syn and loss of mitochondrial membrane integrity [45]. In these particles the protein-to-lipid molar mass ratio is found to be in the range of 1 : 1.4 (protein-to-lipid molar ratio of 1 : 20–25), and EPR data show that α-Syn adopts a broken helical state with a partially disordered second helix [45]. Notably, this approach does not allow the formation of α-Syn nanoparticles with zwitterionic phosphatidylcholine-containing vesicles [45].

Recently, another method was established to generate α-Syn lipoprotein nanoparticles with negatively charged as well as zwitterionic phospholipids using a low protein-to-lipid ratio of 1 : 40 [46]. Mixing 500 μM α-Syn with 2 mM of the desired lipids dissolved in sodium cholate followed by detergent removal results in the formation of discoid-like α-Syn lipid nanoparticles of 19–28 nm diameter. A protein-to-lipid ratio higher than 1 : 40 leads to residual amounts of free monomeric α-Syn when incubated with negatively charged lipids. Importantly, using the nanodisc approach with the same protein-to-lipid ratio, stable α-Syn lipoprotein particles of similar size can also be formed in the presence of the natural zwitterionic lipid sphingomyelin. Remaining residual amount of monomeric α-Syn in the latter sample preparation, as evidenced by size exclusion chromatography, indicates that the α-Syn-derived lipoprotein particles with negatively charged lipids are more stable than particles comprising zwitterionic lipids (Figure 1A). The circular dichroism (CD) spectrum of α-Syn 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (DOPS) lipoprotein particles (Figure 1B) shows the characteristics of α-helical proteins, with two negative $[\Theta]_{MRW}$ peaks at ~ 210 and 221 nm, respectively, and one positive $[\Theta]_{MRW}$ peak at ~ 195 nm, resembling CD spectra of α-Syn bound to anionic lipid vesicles and anionic detergents [3, 5, 6].

Cryo-electron microscopy images of these α-Syn lipoprotein nanoparticles show a low-density inner region (~ 10 nm) attributed to the lipid bilayer, which is surrounded by a 6–7 nm wide higher density belt of α-Syn molecules (Figure 1C) [46]. Similar to the architecture of lipoprotein particles formed by the apolipoprotein A-1 (ApoA-1) [47–49], the higher density features at the periphery of the discs are compatible with the interpretation that α-Syn molecules are wrapped around the lipids in a ring-like manner. The core structure of these particles is formed by the first ~ 100 amino acid residues of α-Syn in a helical conformation (Figure 1B), while ~ 40 C-terminal residues remain flexible and do not interact with the lipid bilayer (Figure 1D) [45, 46], as previously documented for α-Syn in the presence of sodium dodecyl sulfate micelles or SUVs containing anionic phospholipids [5, 9].

An exact mass and composition determination of the α-Syn DOPS lipoprotein particles using size exclusion coupled multiangle static light scattering (MALS) combined with refraction index measurements indicates a total molecular weight of ~ 982 kDa for the α-Syn-lipid entity, ~ 865 kDa for the DOPS lipids, and ~ 116 kDa for the protein component (Figure 2A). Moreover, chemical cross-linking experiments with the disuccinimidyl glutarate (DSG, spacer length 7.7 Å) linker show at higher DSG concentration a predominant single ~ 150 kDa species (Figure 2B). These findings suggest that α-Syn DOPS lipoprotein particles are composed of approximately 8–10 α-Syn and ~ 1070 DOPS molecules with a protein-to-lipid molar mass ratio of ~ 1 : 8–10 [46] in line with theoretical calculations following a procedure established for membrane scaffold protein (MSP) nanodiscs [50]. By comparison, ~ 160 lipid molecules are observed in nanodiscs made of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) and two copies of the protein MSP1D1 (Table 1), a truncated version of ApoA-1 [47–52]. In contrast to α-Syn lipoprotein nanoparticles, fully negatively charged lipids cannot be incorporated into MSP nanodiscs (Table 1).

3 Biophysical and physiological relevance of α-synuclein lipoprotein nanoparticles

The apolipoprotein-like lipid-binding capabilities, sequence similarities to apolipoproteins, and the seven imperfect 11 amino acid long amphipathic repeats stimulated the speculation that α-Syn might be capable of forming lipid-protein nanoparticles, but only recent studies confirmed these speculations with experimental evidence as discussed above [44–46].

From a biological point of view, α-Syn nanoparticles might be involved in lipid transport and storage [45, 46].

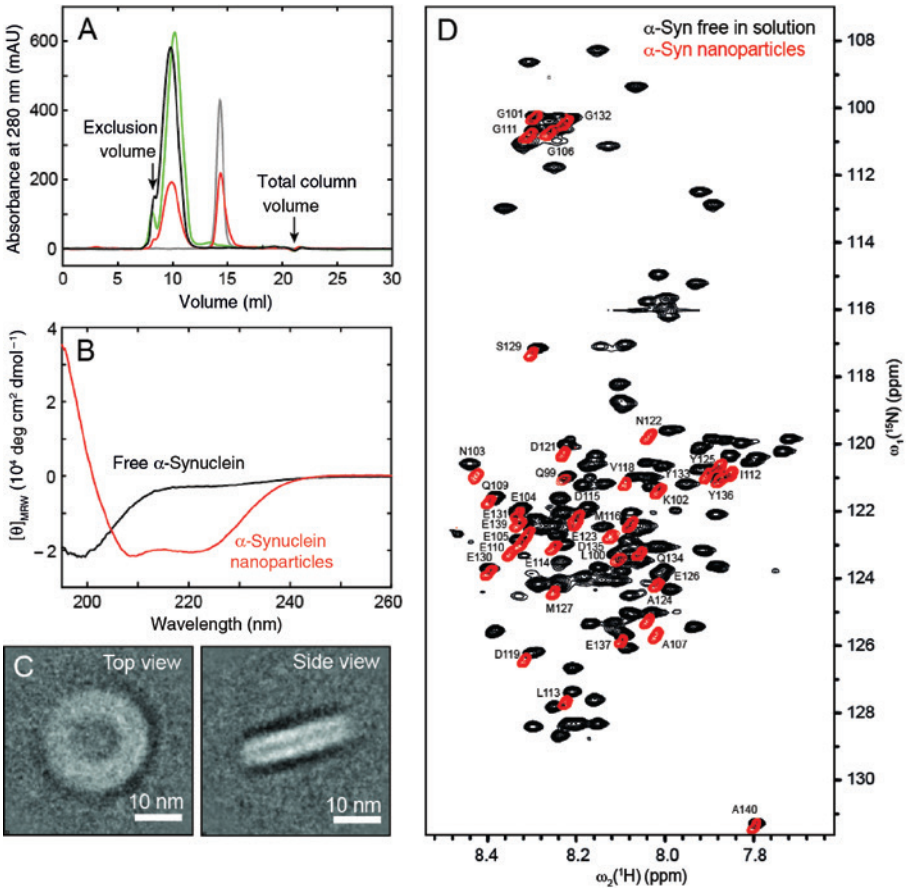

Figure 1: Structural characterization of α-Syn lipoprotein particles. (A) Size-exclusion gel chromatography (Superdex 200 10/300GL) of α-Syn DOPS (black), α-Syn POPS (green), and α-Syn sphingomyelin (red) lipoprotein particles. Monomeric (gray) α-Syn elutes at ~14.3 ml. (B) CD indicates that α-Syn adopts a helical secondary structure within α-Syn DOPS lipoprotein particles. (C) Cryo-electron microscopy images (top and side view) of α-Syn DOPS lipoprotein particles. (D) Solution state NMR shows that the ~40 C-terminal residues of α-Syn are flexible in α-Syn DOPS lipoprotein particles (red). Figure adapted from ref. [46].

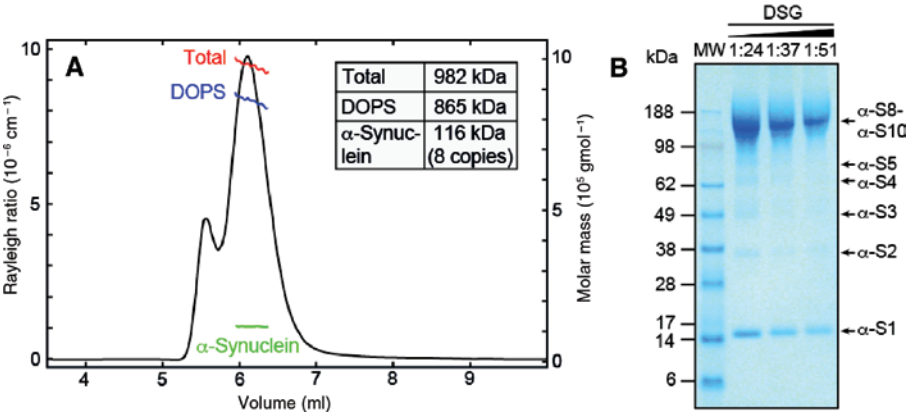

Figure 2: Protein-lipid composition of α-Syn lipoprotein particles. (A) Molecular weight analysis of the α-Syn DOPS lipoprotein complex by MALS coupled with size-exclusion gel chromatography and refractive index measurements. The black line corresponds to the static light scattering signal at 454 nm of DABMI-labeled α-Syn(C141) in the presence of DOPS lipids; red, blue, and green lines show average molar masses of the complex, the lipid component, and the protein component in the lipoprotein particle, respectively. Following these investigations, the protein mass is ~116 kDa indicating that α-Syn is of octameric nature in DOPS lipoprotein particles. (B) Cross-linking studies of α-Syn DOPS lipoprotein particles. Lane 1, molecular weight marker (MW, SeeBlue plus2 prestained standard, Invitrogen). Lanes 2–4, cross-linked α-Syn DOPS lipoprotein particles (final concentration 83 μM) with increasing concentrations of DSG as indicated. Presumed α-Syn monomer and oligomers are indicated by arrowheads. Figure adapted from ref. [46].

Table 1: Properties of α-Syn lipoprotein particles and MSP nanodiscs [45–47, 51, 52].

	Protein			
	α-Syn	**α-Syn**	**MSPΔH5**	**MSP1D1**
Notes	Formation using MSP nanodisc approach	Formation using preformed lipid vesicles	Truncated version of MSP1D1, deletion of residues 121–142 (helix 5)	Residues 44–243 from ApoA-1
Zwitterionic lipids	Yes	No	Yes	Yes
Negatively charged lipids	Yes	Yes	Partly[a]	Partly[a]
Positively charged lipids	No	No	–	–
	Nanodisc type			
	α-Syn DOPS	**α-Syn POPG**	**MSPΔH5 DMPC**	**MSP1D1 DMPC**
Number of proteins	8–10	3–4	2	2
Number of lipids	1070	80–100	100	160
Diameter	19–28 nm	7–10 nm	8.4 nm	9.5 nm
Molecular weight	982 kDa	135 kDa	108 kDa	158 kDa
Protein-to-lipid molar mass ratio	1 : 8	1 : 1.4	1 : 1.7	1 : 2.5

[a]From our experience, loading the nanodiscs with 100% negatively charged lipids results in highly unstable nanodiscs, whereas a composition of 33% negatively charged lipids with 66% zwitterionic lipids yields stable nanodiscs.

The reviewed *in vitro* reconstitution of α-Syn nanoparticles may allow to test, under experimentally controlled conditions, whether and how α-Syn nanoparticles are involved in lipid transport, lipid metabolism with the help of (unknown) enzymes, synaptic plasticity, synaptic vesicle pool maintenance, SNARE complex formation or mitochondrial membrane disruption, etc.

From a biophysical perspective, α-Syn lipoprotein particles may serve as a complementary tool to study membrane proteins in a native-like bilayer environment since α-Syn lipoprotein particles allow the incorporation of negatively charged lipids that are incompatible with other self-assembling lipid bilayer nanodiscs.

Acknowledgments: This work was supported by a Swiss National Science Foundation (SNSF) Grant/Award Number: "154461 and 160979" Sinergia grant and the SNSF grant P300PA_160979 to C.E.

References

[1] Ulusoy A, Di Monte DA. α-Synuclein elevation in human neurodegenerative diseases: experimental, pathogenetic, and therapeutic implications. *Mol. Neurobiol.* 2013, 47, 484–494.
[2] Spillantini MG, Goedert M. The α-synucleinopathies: Parkinson's disease, dementia with Lewy bodies, and multiple system atrophy. *Ann. NY Acad. Sci.* 2000, 920, 16–27.
[3] Weinreb PH, Zhen W, Poon AW, Conway KA, Lansbury PT. NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry (Mosc.)* 1996, 35, 13709–13715.
[4] Uversky VN. A protein-chameleon: conformational plasticity of α-synuclein, a disordered protein involved in neurodegenerative disorders. *J. Biomol. Struct. Dyn.* 2003, 21, 211–234.
[5] Eliezer D, Kutluay E, Bussell R, Browne G. Conformational properties of α-synuclein in its free and lipid-associated states. *J. Mol. Biol.* 2001, 307, 1061–1073.
[6] Davidson WS, Jonas A, Clayton DF, George JM. Stabilization of α-synuclein secondary structure upon binding to synthetic membranes. *J. Biol. Chem.* 1998, 273, 9443–9449.
[7] Jo E, McLaurin J, Yip CM, George-Hyslop PS, Fraser PE. α-Synuclein membrane interactions and lipid specificity. *J. Biol. Chem.* 2000, 275, 34328–34334.
[8] Bussell R, Eliezer D. A structural and functional role for 11-mer repeats in α-synuclein and other exchangeable lipid binding proteins. *J. Mol. Biol.* 2003, 329, 763–778.
[9] Chandra S, Chen X, Rizo J, Jahn R, Südhof TC. A broken α-helix in folded α-synuclein. *J. Biol. Chem.* 2003, 278, 15313–15318.
[10] Bartels T, Choi JG, Selkoe DJ. α-Synuclein occurs physiologically as a helically folded tetramer that resists aggregation. *Nature* 2011, 477, 107–110.
[11] Wang W, Perovic I, Chittuluru J, Kaganovich A, Nguyen LT, Liao J, Auclair JR, Johnson D, Landeru A, Simorellis AK, Ju S, Cookson MR, Asturias FJ, Agar JN, Webb BN, Kang C, Ringe D, Petsko GA, Pochapsky TC, Hoang QQ. A soluble α-synuclein construct forms a dynamic tetramer. *Proc. Natl. Acad. Sci. USA* 2011, 108, 17797–17802.
[12] Theillet FX, Binolfi A, Bekei B, Martorana A, Rose HM, Stuiver M, Verzini S, Lorenz D, van Rossum M, Goldfarb D, Selenko P. Structural disorder of monomeric alpha-synuclein persists in mammalian cells. *Nature* 2016, 530, 45–50.
[13] Alderson TR, Bax A. Parkinson's disease: disorder in the court. *Nature* 2016, 530, 38–39.
[14] Snead D, Eliezer D. Alpha-synuclein function and dysfunction on cellular membranes. *Exp. Neurobiol.* 2014, 23, 292–313.
[15] Bendor JT, Logan TP, Edwards RH. The function of α-synuclein. *Neuron* 2013, 79, 1044–1066.

[16] Lashuel HA, Overk CR, Oueslati A, Masliah E. The many faces of α-synuclein: from structure and toxicity to therapeutic target. *Nat. Rev. Neurosci.* 2013, 14, 38–48.
[17] Murphy DD, Rueter SM, Trojanowski JQ, Lee VMY. Synucleins are developmentally expressed, and α-synuclein regulates the size of the presynaptic vesicular pool in primary hippocampal neurons. *J. Neurosci.* 2000, 20, 3214–3220.
[18] Cabin DE, Shimazu K, Murphy D, Cole NB, Gottschalk W, McIlwain KL, Orrison B, Chen A, Ellis CE, Paylor R, Lu B, Nussbaum RL. Synaptic vesicle depletion correlates with attenuated synaptic responses to prolonged repetitive stimulation in mice lacking α-synuclein. *J. Neurosci.* 2002, 22, 8797–8807.
[19] Abeliovich A, Schmitz Y, Fariñas I, Choi-Lundberg D, Ho WH, Castillo PE, Shinsky N, Verdugo JMG, Armanini M, Ryan A, Hynes M, Phillips H, Sulzer D, Rosenthal A. Mice lacking α-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron* 2000, 25, 239–252.
[20] Nemani VM, Lu W, Berge V, Nakamura K, Onoa B, Lee MK, Chaudhry FA, Nicoll RA, Edwards RH. Increased expression of α-synuclein reduces neurotransmitter release by inhibiting synaptic vesicle reclustering after endocytosis. *Neuron* 2010, 65, 66–79.
[21] Halliday GM, Ophof A, Broe M, Jensen PH, Kettle E, Fedorow H, Cartwright MI, Griffiths FM, Shepherd CE, Double KL. α-Synuclein redistributes to neuromelanin lipid in the substantia nigra early in Parkinson's disease. *Brain* 2005, 128, 2654–2664.
[22] Golovko MY, Barceló-Coblijn G, Castagnet PI, Austin S, Combs CK, Murphy EJ. The role of α-synuclein in brain lipid metabolism: a downstream impact on brain inflammatory response. *Mol. Cell. Biochem.* 2009, 326, 55–66.
[23] Sharon R, Goldberg MS, Bar-Josef I, Betensky RA, Shen J, Selkoe DJ. α-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. USA* 2001, 98, 9110–9115.
[24] Sharon R, Bar-Joseph I, Mirick GE, Serhan CN, Selkoe DJ. Altered fatty acid composition of dopaminergic neurons expressing α-synuclein and human brains with α-synucleinopathies. *J. Biol. Chem.* 2003, 278, 49874–49881.
[25] Barceló-Coblijn G, Golovko MY, Weinhofer I, Berger J, Murphy EJ. Brain neutral lipids mass is increased in alpha-synuclein gene-ablated mice. *J. Neurochem.* 2007, 101, 132–141.
[26] 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, Rochet JC, Bonini NM, Lindquist S. α-Synuclein blocks ER-Golgi traffic and Rab1 rescues neuron loss in Parkinson's models. *Science* 2006, 313, 324–328.
[27] Gitler AD, Bevis BJ, Shorter J, Strathearn KE, Hamamichi S, Su LJ, Caldwell KA, Caldwell GA, Rochet JC, McCaffery JM, Barlowe C, Lindquist S. The Parkinson's disease protein α-synuclein disrupts cellular Rab homeostasis. *Proc. Natl. Acad. Sci. USA* 2008, 105, 145–150.
[28] Lee HJ, Kang SJ, Lee K, Im H. Human α-synuclein modulates vesicle trafficking through its interaction with prenylated Rab acceptor protein 1. *Biochem. Biophys. Res. Commun.* 2011, 412, 526–531.
[29] Clayton DF, George JM. The synucleins: a family of proteins involved in synaptic function, plasticity, neurodegeneration and disease. *Trends Neurosci.* 1998, 21, 249–254.
[30] Hartman VN, Miller MA, Clayton DF, Liu WC, Kroodsma DE, Brenowitz EA. Testosterone regulates α-synuclein mRNA in the avian song system. *Neuroreport* 2001, 12, 943–946.
[31] Chandra S, Gallardo G, Fernández-Chacón R, Schlüter OM, Südhof TC. α-Synuclein cooperates with CSPα in preventing neurodegeneration. *Cell* 2005, 123, 383–396.
[32] Burré J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Südhof TC. α-Synuclein promotes SNARE-complex assembly *in vivo* and *in vitro*. *Science* 2010, 329, 1663–1667.
[33] Burré J, Sharma M, Südhof TC. α-Synuclein assembles into higher-order multimers upon membrane binding to promote SNARE complex formation. *Proc. Natl. Acad. Sci. USA* 2014, 111, E4274–4283.
[34] Lai Y, Kim S, Varkey J, Lou X, Song JK, Diao J, Langen R, Shin YK. Nonaggregated α-synuclein influences SNARE-dependent vesicle docking via membrane binding. *Biochemistry (Mosc.)* 2014, 53, 3889–3896.
[35] Zhu M, Fink AL. Lipid binding inhibits α-synuclein fibril formation. *J. Biol. Chem.* 2003, 278, 16873–16877.
[36] Necula M, Chirita CN, Kuret J. Rapid anionic micelle-mediated α-synuclein fibrillization in vitro. *J. Biol. Chem.* 2003, 278, 46674–46680.
[37] Martinez Z, Zhu M, Han S, Fink AL. GM1 specifically interacts with α-synuclein and inhibits fibrillation. *Biochemistry (Mosc.)* 2007, 46, 1868–1877.
[38] Haque F, Pandey AP, Cambrea LR, Rochet JC, Hovis JS. Adsorption of α-synuclein on lipid bilayers: modulating the structure and stability of protein assemblies. *J. Phys. Chem. B* 2010, 114, 4070–4081.
[39] Reynolds NP, Soragni A, Rabe M, Verdes D, Liverani E, Handschin S, Riek R, Seeger S. Mechanism of membrane interaction and disruption by α-synuclein. *J. Am. Chem. Soc.* 2011, 133, 19366–19375.
[40] Galvagnion C, Buell AK, Meisl G, Michaels TC, Vendruscolo M, Knowles TP, Dobson CM. Lipid vesicles trigger α-synuclein aggregation by stimulating primary nucleation. *Nat. Chem. Biol.* 2015, 11, 229–234.
[41] Heise H, Hoyer W, Becker S, Andronesi OC, Riedel D, Baldus M. Molecular-level secondary structure, polymorphism, and dynamics of full-length α-synuclein fibrils studied by solid-state NMR. *Proc. Natl. Acad. Sci. USA* 2005, 102, 15871–15876.
[42] Vilar M, Chou HT, Lührs T, Maji SK, Riek-Loher D, Verel R, Manning G, Stahlberg H, Riek R. The fold of α-synuclein fibrils. *Proc. Natl. Acad. Sci. USA* 2008, 105, 8637–8642.
[43] Gath J, Bousset L, Habenstein B, Melki R, Böckmann A, Meier BH. Unlike twins: an NMR comparison of two α-synuclein polymorphs featuring different toxicity. *PLoS One* 2014, 9, e90659.
[44] Mizuno N, Varkey J, Kegulian NC, Hegde BG, Cheng N, Langen R, Steven AC. Remodeling of lipid vesicles into cylindrical micelles by α-synuclein in an extended α-helical conformation. *J. Biol. Chem.* 2012, 287, 29301–29311.
[45] Varkey J, Mizuno N, Hegde BG, Cheng N, Steven AC, Langen R. α-Synuclein oligomers with broken helical conformation form lipoprotein nanoparticles. *J. Biol. Chem.* 2013, 288, 17620–17630.
[46] Eichmann C, Campioni S, Kowal J, Maslennikov I, Gerez J, Liu X, Verasdonck J, Nespovitaya N, Choe S, Meier BH, Picotti P, Rizo J, Stahlberg H, Riek R. Preparation and characterization of stable α-synuclein lipoprotein particles. *J. Biol. Chem.* 2016, 291, 8516–2857.
[47] Bibow S, Polyhach Y, Eichmann C, Chi CN, Kowal J, Albiez S, McLeod RA, Stahlberg H, Jeschke G, Güntert P, Riek R. The 3D solution structure of engineered discoidal high-density lipoprotein particles. *Nat. Struct. Mol. Biol.* 2016. DOI: 10.1038/nsmb.3345.

[48] Murray SC, Gillard BK, Ludtke SJ, Pownall HJ. Direct measurement of the structure of reconstituted high-density lipoproteins by cryo-EM. *Biophys J.* 2016, 110, 810–816.
[49] Blanchette CD, Cappuccio JA, Kuhn EA, Segelke BW, Benner WH, Chromy BA, Coleman MA, Bench G, Hoeprich PD, Sulchek TA. Atomic force microscopy differentiates discrete size distributions between membrane protein containing and empty nanolipoprotein particles. *Biochim Biophys Acta* 2009, 1788, 724–731.
[50] Denisov IG, Grinkova YV, Lazarides AA, Sligar SG. Directed self-assembly of monodisperse phospholipid bilayer nanodiscs with controlled size. *J. Am. Chem. Soc.* 2004, 126, 3477–3487.
[51] Bayburt TH, Sligar SG. Membrane protein assembly into Nanodiscs. *FEBS Lett.* 2010, 584, 1721–1727.
[52] Hagn F, Etzkorn M, Raschle T, Wagner G. Optimized phospholipid bilayer nanodiscs facilitate high-resolution structure determination of membrane proteins. *J. Am. Chem. Soc.* 2013, 135, 1919–1925.