



Review

Interactions between misfolded protein oligomers and membranes: A central topic in neurodegenerative diseases?☆



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ABSTRACT

The deposition of amyloid material has been associated with many different diseases. Although these diseases are very diverse the amyloid material share many common features such as cross- β -sheet structure of the backbone of the proteins deposited. Another common feature of the aggregation process for a wide variety of proteins is the presence of prefibrillar oligomers. These oligomers are linked to the cytotoxicity occurring during the aggregation of proteins. These prefibrillar oligomers interact extensively with lipid membranes and in some cases leads to destabilization of lipid membranes. This interaction is however highly dependent on the nature of both the oligomer and the lipids. Anionic lipids are often required for interaction with the lipid membrane while increased exposure of hydrophobic patches from highly dynamic protein oligomers are structural determinants of cytotoxicity of the oligomers. To explore the oligomer lipid interaction in detail the interaction between oligomers of α -synuclein and the 4th fasciclin-1 domain of TGF β 1p with lipid membranes will be examined here. For both proteins the dynamic species are the ones causing membrane destabilization and the membrane interaction is primarily seen when the lipid membranes contain anionic lipids. Hence the dynamic nature of oligomers with exposed hydrophobic patches alongside the presence of anionic lipids could be essential for the cytotoxicity observed for prefibrillar oligomers in general. This article is part of a Special Issue entitled: Lipid–protein interactions.

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1. Introduction

Today more than 40 different diseases have been linked to the deposition of proteinaceous amyloid material in various tissues [1]. The deposits are mainly composed of protein fibrils and each disease usually has one major protein associated with it. Certain components are regularly found bound to the protein deposits including glucosaminoglycans, collagen, chaperones, serum amyloid P component, and apolipoprotein E [2,3]. The deposited material can be intra- or extra-cellular or both [1,4,5]. The diseases can be familial, sporadic or even transmissible and they can affect a single tissue or be systemic [6]. Some amyloid-associated diseases, such as type II diabetes, Alzheimer's disease (AD) and Parkinson's disease (PD), have great socio-economic consequences due to their extensive prevalence. Others are rare and only affect few people.

Despite the many differences in this group of diseases, the proteinaceous deposits share many common features. The protein fibrils are all arranged in a so-called cross- β ultra structure composed of hydrogen-bonded β -strands where the backbone of the individual proteins is arranged in an extended conformation with the individual protein strands running perpendicular to the long axis of the fibril, with inter-strand hydrogen bonds parallel to the fibril axis [7,8]. This arrangement gives rise to amyloid-specific features like the binding of amyloid specific dyes, β -sheet secondary structure signature and signals at 4.8 and 10 Å in small angle X-ray diffraction patterns [9,10]. This regular packing of protein backbone strands into fibrils is very precise, with only one misplaced strand pr. 30,000 strands [11]. Typically, fibrils are straight and unbranched and consist of one or more protofilaments wrapping around each other [12]. The fibrils can be up to several μ m long. Fibrils can however display various different morphologies like branching or curly appearance. Although many features are shared by *in vivo* isolated amyloid material and *in vitro* produced fibrils, seeding recombinant protein with *in vivo* isolated material can give rise to fibrils with a structure different from that reported for fibrils composed of purely recombinant material [13]. In addition to pathology-associated proteins, many others have been found to form β -sheet rich amyloid-like aggregates, and indeed it has been suggested that proteins have a generic ability to form fibrillar amyloid-like aggregates with cross- β sheet structure [14]. The propensity to do so is dependent on the specific protein's hydrophobicity, electrostatic charge and propensity to form secondary structural elements [15,16]. Remarkably, the cross- β sheet fold also acts as a functional entity e.g. for storage of peptide hormones [17] and in the production of melanin [18].

The amyloid cascade theory proposed early on stated that the fibrils in the deposited amyloid material were the main culprit causing the pathology of amyloid diseases [19]. In the case of systemic amyloidosis this is indeed the case, but for other amyloid diseases the attention has shifted towards prefibrillar species, particularly prefibrillar oligomers. This was mainly due to the observation of amyloid material in the brains of healthy elderly individuals [20] and the observation that the severity of neurodegenerative diseases did not correlate with the amount of amyloid material deposited in the brain of AD patients [21–23]. Now the prevailing view is that fibrils represent a non-toxic molecular dumping ground for toxic prefibrillar oligomers formed during the aggregation of the involved protein [24,25]. This correlates with the observation of elevated levels of prefibrillar oligomers in patients suffering from neurodegenerative disease [26,27].

2. Prefibrillar oligomers

The aggregation pathway can be very complex, with multiple species in equilibrium with each other. For some proteins, partial unfolding is necessary for the aggregation to occur, other proteins need to monomerize from native multimers in order to oligomerize into aggregation prone species, and yet others can form fibrils which maintain some of the native structure of the monomer. Despite the many differences, prefibrillar oligomers of both disease-associated and non-disease associated proteins and peptides have been identified [24,28,29] and they do indeed share common features. The prefibrillar oligomers are generally thought to contain more β -sheet than the corresponding monomer but less than the resulting mature fibrils, display hydrophobic patches on the surface and are transient in nature, i.e. gradually transform to other more organized aggregates over time, though isolated oligomers can be remarkably stable [30]. Many of these oligomers are doughnut-shaped according to atomic force microscopy (AFM) [31], cf. Fig. 1. An antibody raised against prefibrillar oligomers of A β also binds prefibrillar oligomers of many different proteins [32]. Given the high degree of similarity between the mature fibrils, it is not surprising that many commonalities are also seen among the prefibrillar oligomers. Nevertheless, it is still unclear if the doughnut shaped oligomers identified so far are on- or off-pathway species. For α -synuclein (α SN) the doughnut-shaped oligomer has been shown to be off-pathway and is most likely the cytotoxic component [33]; another co-existing oligomeric species is suggested to be on-pathway and hence transform to fibrils over time [34].

Despite these shared features, nucleation events leading to the formation of oligomers are not likely to adhere to a universal mechanism, in which a single well-defined nucleus from a specific protein gives rise to a single down-stream polymerization reaction resulting in fibrils with a specified morphology. Given the multiple types of conformations that can stabilize different aggregates and the lack of evolutionary optimization of this process, nucleation is probably a messy business, in which multiple structurally different nuclei can form and give rise to structurally distinct fibrils. This is supported by the observation that fibril seeds isolated from different AD patients with different disease histories give rise to structurally distinct fibrils which differ not only from each other but also from fibrils previously formed by A β *in vitro* [13]. This could also explain the fibril polymorphism seen for many aggregating proteins. Differences in the resulting fibril structure are seen for many different proteins. This polymorphism is induced by differences in the physico-chemical properties of the environment and this can for some proteins be overcome by the presence of fibril seeds of a single morphology [35,36]. The morphology of the prefibrillar oligomers could be important for their mechanism of cytotoxicity.

3. Proposed modes of oligomer toxicity

Different mechanisms of cytotoxicity exerted by prefibrillar oligomers have been proposed based on various reports. The oligomers or aggregates might sequester cellular entities essential for cell viability and indeed transcription factors, proteasomal and, cytoskeletal components have been located in amyloid aggregates [37,38]. By interacting with cellular receptors, the oligomers might activate signal transduction pathways leading to apoptosis [39]. The prefibrillar oligomers might also induce oxidative stress by production of free radicals. These in

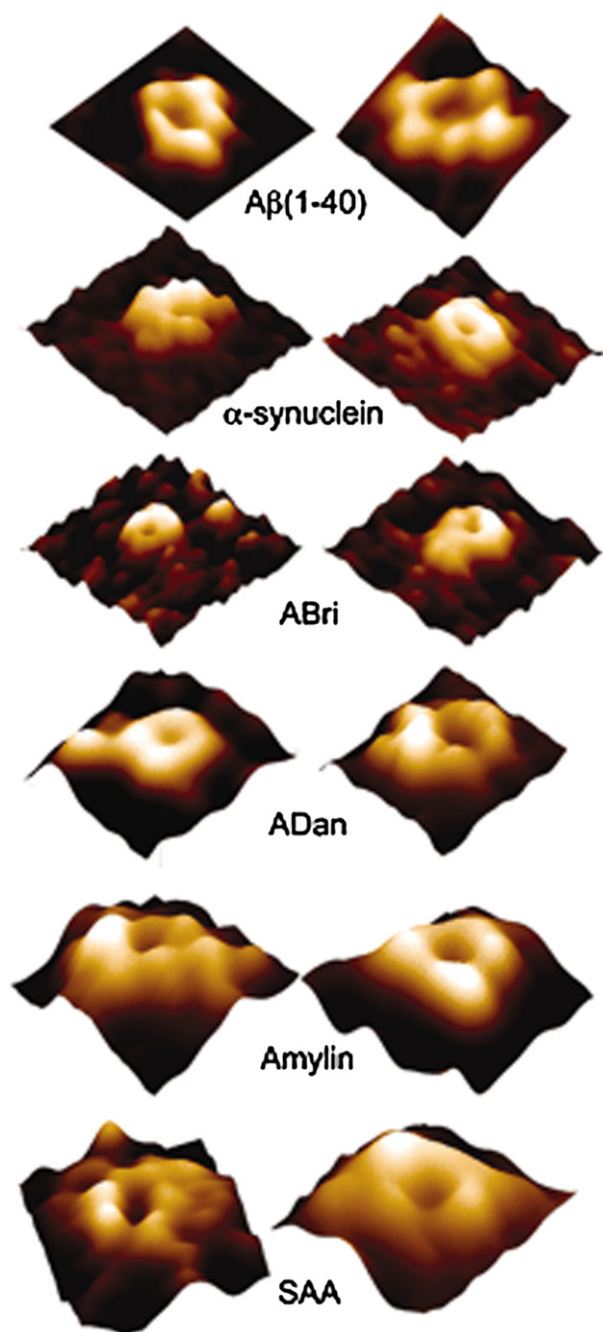


Fig. 1. Doughnut-shaped pore-like structures of prefibrillar oligomers imaged using atomic force microscopy.

Reprinted with permission from [31].

turn lead to lipid and protein oxidation, mitochondrial dysfunction and increased levels of intracellular calcium [40,41]. In fact a long-standing link has also been made between increased levels of oxidative stress (by the production of intracellular reactive oxygen species) and aggregating proteins implicated in neurodegenerative diseases like PD [42–45]. Another widespread hypothesis is that cytotoxicity is caused by interaction between the prefibrillar oligomers and the lipid bilayer of the cell membranes, leading to membrane disruption or even pore formation. The formation of an actual ion channel could lead to membrane disruption and depolarization, eventually resulting in dysregulation of signal transduction and changes in ion homeostasis [39]. This is consistent with the original pore formation theory which is supported by the oligomeric structures observed in Fig. 1. However, a more likely

scenario is that direct interaction between the membrane and the prefibrillar oligomers destabilizes the membrane. Annular oligomers have only been detected using transmission electron microscopy (TEM) and AFM imaging techniques on dried samples and not in solution, so it cannot be excluded that they are drying artifacts.

Understanding oligomer–membrane interactions is a challenge involving both biochemists, biophysicists and structural biologists. Table 1 lists a number of biophysical and biochemical methods used to study oligomer–membrane interactions.

All of the abovementioned mechanisms are based on a toxic gain-of-function of the oligomeric species involved in the aggregation pathway. It is likely that the cytotoxicity does not arise from a single mechanism but that several of the proposed mechanisms are involved in the cellular degeneration observed in amyloid diseases. Furthermore, different mechanisms might be involved depending on whether the aggregation occurs intra- or extra-cellularly.

Another way in which prefibrillar oligomers can interact with lipid membranes is by extracting lipids from the membrane and incorporating them into the aggregates [55]. Many different aggregation-prone proteins have been shown to form membrane-active prefibrillar oligomers, including α SN [33], A β [56], prion protein [57], islet amyloid polypeptide (IAPP) [58] and the fasciclin1-4 (Fas1-4) fragment of transforming growth factor- β induced protein (TGFB1p) [59]. Two of these protein oligomeric systems (α SN and Fas1-4) will be reviewed in detail below. Membrane-active oligomers have also been reported for oligomers of non-disease related protein such as the yeast prion protein Ure2 [60] and the N-terminal fragment of Hyp-F from *Escherichia coli* [61]. Anionic surfaces, in particular anionic lipids, are known to play a significant role in the nucleation of aggregation by unfolding proteins and recruiting oligomers [62].

4. Structural determinants of oligomer cytotoxicity

The link between structural determinants and the cytotoxicity of oligomers has been investigated by formation of stable oligomers of HypF-N, a non-disease associated protein from *E. coli* [61]. This protein forms two different oligomers that are indistinguishable from each other in terms of morphology and ThT binding, yet differ markedly in cytotoxicity as monitored by MTT reduction and Hoechst staining. One oligomer is completely non-toxic and the other is just as toxic as A β ₄₂ oligomers. The two different oligomers are formed under different conditions (50 mM acetate buffer, 12% (v/v) trifluoroethanol, 2 mM DTT, pH 5.5 for non-toxic oligomers and 20 mM trifluoroacetic acid, 330 mM NaCl, pH 7 for toxic oligomers) but both of them are stable for the duration of the various measurements (at least 24 h). Importantly, hydrophobic regions in the toxic oligomers are more solvent-exposed (and thus less well organized) than those in the non-toxic oligomers. Both types of oligomers interact with the cell membrane, but only the toxic ones with increased exposure of hydrophobic patches penetrate

Table 1

List of analytical methods used to investigate oligomer–membrane interactions. References refer to studies with α SN.

Method	Measure	References
Asymmetrical flow field-flow fractionation	Binding	[46,47]
Circular dichroism	Binding and folding	[48]
Confocal microscopy	Binding, permeabilization and effect on vesicles	[47–49]
Centrifugation experiments	Binding	[50]
Differential scanning calorimetry	Binding	[47]
Dynamic light scattering	Binding and effect on vesicles	[47]
Tryptophan fluorescence	Binding	[51]
Ion-channel current measurements	Permeabilization	[31]
Isothermal titration calorimetry	Binding	[47]
Vesicle leakage/influx	Permeabilization	[46,52–54]

the membrane. It is very likely that the increased plasticity and solvent-exposure of the toxic oligomer facilitate this membrane disruption and cell penetration.

The correlation between cytotoxicity and increased exposure of hydrophobic patches as well as less dense packing of the hydrophobic core has also been reported for A β ₄₀ protofibrils when compared to mature fibrils [63]. Interestingly, for the protein α SN two different oligomeric species have also been detected during the aggregation process. In one study, the cytotoxic form was found to be more highly structured (as measured by proteinase K resistance and exposure of hydrophobic side chains to the solvent) than the non-toxic oligomer [64], in striking contrast to the HypF-N oligomers. The cytotoxic oligomers were proposed to originate from the interconversion of the non-toxic oligomers at later time-points during the aggregation. The cytotoxic oligomers were also found to be released from mature fibrils by dissociation, suggesting that mature fibrils could be metastable storage states for cytotoxic oligomers (though release of oligomers is likely confined to the ends of the fibrils).

In another study of α SN oligomers two oligomeric species were found to co-exist [34]. Type I oligomers were more protected against hydrogen/deuterium exchange in the backbone and data suggested that they could revert back to the monomer and also forms mature fibrils, whereas type II oligomers were less protected against hydrogen/deuterium exchange and hence more flexible. Type II oligomers formed amorphous aggregates and are thought to be off-pathway in the aggregation process [30]. Type I oligomers were only found at low levels and the highly dynamic equilibrium with the oligomer and the on-pathway aggregation of this species could lead to the mistaken assumption that the oligomeric fractions previously obtained for α SN are homogeneous. Oligomer size heterogeneities have also been reported [33]. Although the authors could not directly identify which oligomer is cytotoxic, the abundance of type II oligomers alongside the decreased backbone protection against hydrogen/deuterium exchange suggests increased flexibility compared to type I oligomers, in common with the cytotoxic HypF-N oligomer, and indicates that the off-pathway oligomer is the one responsible for the α -synuclein oligomer cytotoxicity.

Overall, a high degree of flexibility and less dense packing of the hydrophobic core in the oligomer structure together with increased exposure of hydrophobic patches on the surface of prefibrillar oligomers appear to be important for oligomer cytotoxicity – though this might not be the case for all oligomers (*cf.* [64]).

5. α -Synuclein oligomers and their mode of action on the membrane

α SN has now for more than a decade been one of the most investigated proteins within the fields of neurodegenerative diseases and protein misfolding. α SN became linked to Parkinson's disease (PD) in the late 1990s due to two important findings: (1) certain mutations in the α SN gene led to early onset PD [65] and (2) α SN is the primary constituent of the amyloid material, so-called Lewy bodies, which are found in the brains of PD patients [66]. α SN is an intrinsically disordered protein of 140 residues. It has no persistent structure at physiological conditions, but long-range interactions reduce the hydrodynamic volume compared to a fully extended protein [67,68]. The primary sequence of α SN is normally divided into three regions: (1) the N-terminal region (residues 1–60) which is highly basic, (2) the NAC (non-amyloid- β component) region (residues 61–95) which is hydrophobic having only few charged residues, and (3) the C-terminal (residues 96–140) which is highly acidic and is disordered in both the amyloid fibril [69] and oligomer state [34,70].

5.1. Binding of α SN monomers to membranes

The association of the α SN monomer with membranes is believed to be important for its physiological role [31,71]. Upon binding to a membrane, the N-terminal and NAC regions fold into an amphipathic

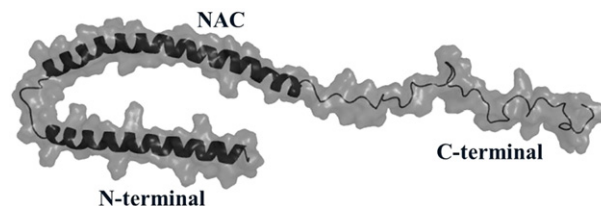


Fig. 2. Structure of α SN bound to sodium dodecyl sulfate micelles. The figure is made from PDB file 1XQ8 [72].

α -helical structure. The NMR-based structure of α SN in association with sodium dodecyl sulfate micelles (Fig. 2), reveals two α -helices, helix N (residues 3–37) and helix C (residues 45–92), the latter containing both N-terminal and NAC regions. The two helices are linked by an ordered linker in an anti-parallel arrangement (residues 38–44). The C-terminus remains disordered in the membrane-bound structure [72]. There is compelling data that membrane binding is a two-step process initiated by the N-terminal residues 3–25, followed by a subsequent coil–helix transition of residues 26–97 [73,74]. A recent solid-state NMR study on the membrane interaction of the α SN monomer with anionic small unilamellar vesicles (SUVs) demonstrated how residues 6–25 function as a rigidly bound membrane anchor independent of lipid composition. They further demonstrated how the membrane binding of residues 26–98 is dependent on lipid composition and therefore defines the affinity of the α SN monomer towards membranes. The disordered conformation of the C-terminus in the membrane bound form was confirmed, however transient interactions with the membrane surface were observed [75]. The α SN monomer is curvature sensing and binds preferentially to smaller vesicles such as SUVs and large unilamellar vesicles (LUVs) [76,77]. Upon binding of the monomer to SUVs and LUVs, membrane remodeling has been observed [73,78].

Though intrinsically disordered, α SN is able to form amyloid fibrils and oligomeric structures which are ordered aggregates with well-defined intermolecular contacts. As discussed previously in this review, different types of α SN oligomers have been identified. Here we will focus on one type of α SN oligomers. These oligomers were initially purified and characterized by Lansbury and co-workers, who also demonstrated how the oligomers were able to permeabilize lipid vesicles [79,80]. In the discussion of these oligomers and their interaction with membranes we will primarily focus on data published by the Subramaniam group and the authors of the present review, as the work from these two groups is in many ways complementary.

5.2. Structure of an α SN oligomer

The average oligomer consists of ~30 monomers according to small angle X-ray scattering (SAXS), size-exclusion chromatography coupled with multi angle laser light scattering (SEC-MALLS) and single-molecule fluorescence [33,81]. The oligomer is shaped like an prolate ellipsoid according to SAXS measurements [33,82], *cf.* Fig. 3, and its dimensions agree well with those obtained from dynamic light scattering [33,47] and TEM [30]. The oligomers consist of β -sheet structure, likely forming the oligomer core surrounded by a disordered outer shell

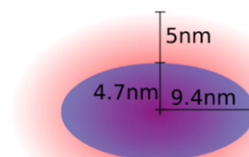


Fig. 3. Model of the major α SN oligomer based on SAXS [33]. The purple ellipse indicates the structured core, while the pink halo represents the surrounding shell of disordered protein.

(Fig. 3) [33,83,84]. According to hydrogen/deuterium exchange mass spectrometry (HDX-MS), the NAC region together with parts of the N-terminal region builds up the core of the oligomer whereas the C-terminal and the early N-terminal are disordered [34] in good agreement with complementary studies [47,51]. These oligomers are ideal as a model system to elucidate the mechanism of oligomer–membrane interaction and permeabilization because of their high stability towards temperature, pH and long-term incubation [30] combined with the relative simple protocol to produce these oligomers [85]. Furthermore, these oligomers are not chemically modified or covalently linked, in fact they can dissociate in the presence of high concentrations of the chemical denaturant urea [30,83].

5.3. Lipid requirements for membrane interactions

Considering the generic property of the α SN primary sequence to fold into membranes, it is not surprising that the oligomers show affinity towards membranes. The question is rather why the oligomer form is more toxic than the monomer form? First, how much more potent is the oligomer compared to the monomer in permeabilization of membranes? Based on vesicle leakage experiments we have recently shown that 1 α SN oligomer (consisting of ~30 monomers) induce as much vesicle leakage as ~500 monomer molecules. As for the α SN monomer [86], the oligomer also selectively binds to anionic lipids and most oligomer–membrane studies have been carried out with anionic phospholipids [46,54,87]. In one study, the authors systematically varied the lipid composition of giant unilamellar vesicles (GUVs) and observed oligomer binding to different anionic lipids [87]. No binding was detected to zwitterionic POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) GUVs, however lipid mixtures of POPC and the anionic lipid POPG (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoglycerol) led to oligomer binding. A key observation in this study was that binding did not *per se* induce permeabilization of the vesicles as measured with dye leakage [87].

5.4. Mechanisms of membrane perturbation by oligomers

As discussed previously in this review, there is a general trend that oligomers have more hydrophobic surface exposed area (or at least larger surface exposed hydrophobic patches) than the monomer and amyloid fibril form [88]. This is believed to be one of the key reasons for their potent membrane permeabilization. As for the monomer [86] the oligomer preferentially binds to the liquid disordered domains rather than the liquid ordered domains [87]. This might be due to the higher degree of accessibility of the hydrocarbon core of the lipid bilayer in the liquid disordered domain whereas the lipid bilayer is tightly packed in the liquid ordered phase. Thus, hydrophobic interactions between the oligomer and lipid bilayer is more favored when the lipids are in the liquid disordered phase. Increased headgroup spacing, leading to increased accessibility of the lipid bilayers hydrocarbon core, also led to increased permeabilization, again highlighting the importance of hydrophobic interactions [54].

5.5. Role of the N-terminus in membrane interactions

In addition to hydrophobic interactions, N-terminal interactions appear to be equally important for monomer and oligomer association with membranes. A simple and elegant fluorescence study, using selected single tryptophan mutant of α SN, gave the first indication of the importance of the N-terminal in oligomer–membrane interactions [51]. α SN has a net negative charge so intuitively we would also expect the basic N-terminal to take part in the interactions with anionic lipids. Furthermore, deletions of the early N-terminal of the α SN sequence led to decreased α SN induced toxicity in yeast [89]. We therefore investigated how deletions in the first 13 N-terminal residues affected membrane interactions. Our data clearly showed that the interaction of

both monomer and oligomer was almost completely abolished upon deletion of residues 2–11, suggesting that the extreme N-terminal part is essential for both monomer–membrane and oligomer–membrane interactions. In the oligomeric state, the early N-terminal only seems to be partly protected towards H/D exchange [34] and therefore it might be able to initiate binding and act as a rigid anchor as described for the monomer. This might include the formation of a structure similar to helix N, in good agreement with the slight increase in α -helical structure upon membrane binding [48]. The binding mode of the oligomer will naturally differ from that of the monomer, as most of the sequence that forms helix C is involved in the oligomer core. However, we find it likely that the initiation of the interaction happens in a similar way. We speculate that the potency of α SN oligomers to permeabilize membranes stems from the possible fixation of several N-terminals in proximity (*i.e.* increased avidity) combined with hydrophobic interactions which lead to disturbance of the hydrophobic core of the lipid bilayer.

These oligomers have also been demonstrated to be more cytotoxic towards neuronal cells than monomers [47]. While recent data suggest that the membrane permeabilization observed with negatively charged vesicles might not be as dramatic in more complex membrane systems [48], more research is needed in this direction. Interestingly, the oligomers can also bind to membranes without inducing membrane disruption [87], thus there might be several binding modes dependent on the chemistry of the lipids and the ionic strength and pH of the solution.

Currently, no solution based structural methods show an inner pore in the α SN oligomer structure. Rather, our recent structural model (Fig. 3) suggests a solid core [33]. Until we obtain a higher-resolution oligomer structure, it will be difficult to achieve a complete description of how α SN oligomers permeabilize membranes.

6. Keeping an eye on Fas1-4: different oligomers in different pathways

Another example of an aggregation prone protein forming membrane-disruptive prefibrillar oligomers is provided by the protein Fas1-4. Fas1-4 is the C-terminal domain of the full-length protein transforming growth factor- β induced protein (TGFB1p). This domain shows promise as a model system for TGFB1p-related diseases, since mutations destabilize the full-length protein and the Fas1-4 domain to the same degree [90]. Several genetic analyses have linked TGFB1p to corneal dystrophies, in which proteinaceous deposits in the cornea lead to loss of vision [91–93]. TGFB1p is an extracellular multi-domain protein consisting of an N-terminal cysteine-rich region, four consecutive and homologous fasciclin-1 domains (of which Fas1-4 is the last) and a C-terminal RGD integrin-binding motif [94]. TGFB1p constitutes the majority of the protein inclusions found in the cornea of corneal dystrophy patients, and abnormal turnover of the protein has been associated with various mutants [95,96]. More than 30 mutations in TGFB1p have been linked to the disease, which is inherited in an autosomal-dominant manner and is phenotypically heterogeneous with different mutations giving rise to different phenotypes [92]. Interestingly, some disease-linked mutations enhance TGFB1p stability while other disease-linked mutations destabilize it [90]. Apart from one mutational hotspot in residue 124, all disease-causing mutations are found in Fas1-4 (residues 502–632 of TGFB1p) [92,97].

6.1. Concentration-dependent changes in A546T Fas1-4 aggregation behavior

The mutation A546T in TGFB1p leads to a very aggressive form of corneal dystrophy with an age of onset of 35 to 40 years and results in lattice corneal dystrophy with amyloid deposits found in the stroma of the cornea [98]. A546T Fas1-4 is significantly destabilized as compared to wild type [59,90] and forms polymorphic fibrils *in vitro*. Aggregation at high protein concentrations gives rise to short and curly fibrils with a

mixture of α -helical and β -sheet structure, while aggregation at lower concentrations results in long and straight fibrils with pure β -sheet structure [59]. These two fibril morphologies are preceded by different species of prefibrillar oligomers with distinct membrane activity. The oligomers formed during aggregation at high concentrations are well defined with a single size distribution and can permeabilize phospholipid vesicles. In contrast, aggregation at lower concentrations gives rise to a range of different oligomers with different sizes, some of which are membrane active but less so than the well-defined oligomers formed at higher concentrations (Fig. 4).

Membrane permeability requires the vesicles to contain anionic lipids; purely zwitterionic lipids elicit no activity. As Fas1-4 aggregation proceeds, the membrane activity decreases. At low Fas1-4 concentrations, this decrease is more rapid than at higher concentrations, where membrane activity is still observed at the endpoint of fibrillation. This could indicate that the oligomers responsible for the membrane permeation disappear either by conversion into fibrils or by dissociation to monomers which can then be incorporated into the growing fibrils.

The membrane active oligomer species seen at higher concentrations are thought to be formed through very rapid aggregation. This leads to oligomers of the native monomers being formed and incorporated into the fibrils, which also explains the α -helical and β -sheet structure seen in the final fibrils. At lower concentrations, it might be that the slower kinetics allows for monomer rearrangements, possibly through partial unfolding before oligomerization occurs. These oligomers composed of rearranged monomers do not display membrane activity. The rearrangement of the monomers could also explain the purely β -sheet structure seen in the fibrils formed at

lower concentrations. This indicates that in this specific example the kinetics of aggregation can also play a central role in the production of membrane active oligomers during fibrillation. SAXS has revealed multimers of full-length TGFBIp at high concentrations, which may be precursors for the well-defined oligomer species formed at high concentrations [99].

6.2. Formation of aggregation-prone TGFBIp fragments *in vivo*

The *in vitro* oligomers may have parallels *in vivo*. Lack of blood vessels in the cornea leads to slow protein turnover in the cornea [100] and may allow prefibrillar oligomers to accumulate in the cornea of CD patients. These oligomers could even be formed by fragments of TGFBIp; C-terminal fragments of TGFBIp have been identified in the cornea of patients suffering from lattice corneal dystrophy with amyloid deposits [101]. These fragments are thought to arise from the catalytic activity of the serine protease HtrA1. The release of these C-terminal fragments could lead to oligomers by accelerating the aggregation and hence posing a threat to corneal cells. Interestingly wild type TGFBIp has not been observed to form higher order structures *in vivo*; this has been attributed to the low effective concentrations of free TGFBIp in the cornea of healthy individuals [102].

In this specific case the oligomers formed during the aggregation are distinct in both size, distribution, and membrane activity, indicating that the aggregation pathways for the two different fibril types are distinct. This is thus an example of how fibrillar polymorphism arises from differences in the aggregation pathway and different oligomeric species.

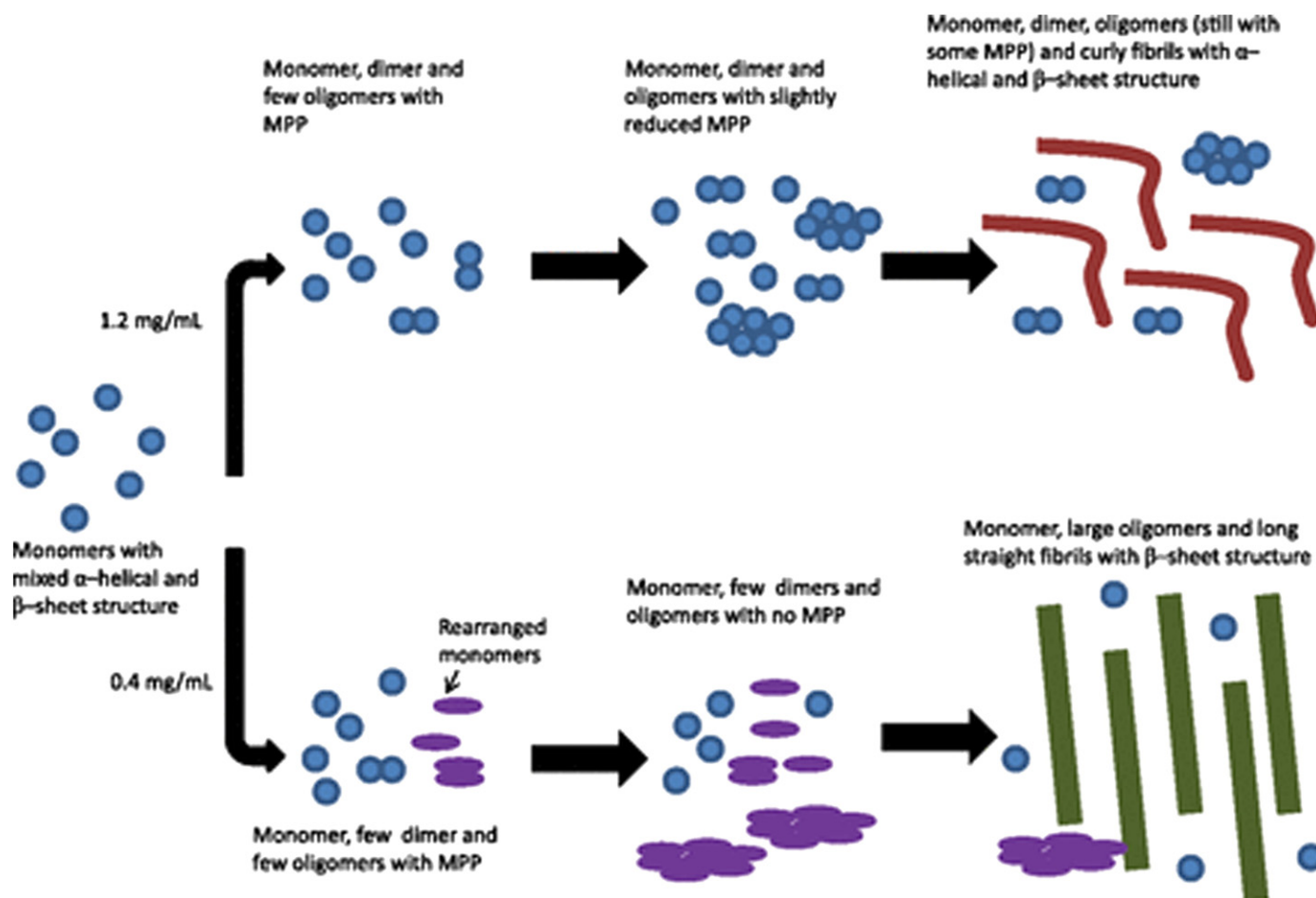


Fig. 4. Polymorphic fibrillation can lead to the formation of different oligomeric species with distinct membrane permeation properties during fibrillation. Reprinted from [59] with permission.

7. Inhibiting oligomer–lipid interaction

The simplest way to circumvent oligomer lipid interactions is to prevent the formation of oligomers altogether. Numerous approaches have been undertaken in this direction, mostly focusing on the more general (but not identical) goal of inhibiting amyloid formation. These have met with various degrees of success.

7.1. Immunotherapy

One approach has been antibody-mediated immunotherapy using antibodies raised against the fibrillating protein. Antibodies against A β raised against early-stage A β aggregates were indeed found to inhibit fibril formation and even disaggregate existing fibrils [103]. Furthermore, the concentration of toxic oligomers in the brain following immunotherapy can possibly be reduced [104]. Indeed the level of A β oligomers in the brains of mice treated with an antibody raised against A β was reduced [105]. Another clever way to indirectly target amyloid material with immunization strategies is to target proteins that associate with amyloid material. With this strategy the physiological role of the monomer form of the amyloid forming protein will not be affected. Targeting the serum amyloid P component, which binds amyloid material formed by different proteins, leads to depletion of amyloid material as shown with A β [106,107]. This is potentially also a useful strategy for oligomers, provided binding partners for oligomers can be identified. However, while amyloid represents a stable and inert state which can readily be localized and treated, oligomer interactions are likely more dynamic and may therefore be more challenging to target.

7.2. Small molecule aggregation inhibitors

Small molecules are also attractive as inhibitors of the formation of fibrils and possibly oligomers. Unlike antibodies, many small molecules can cross the blood–brain barrier, and this makes them attractive for the treatment of neurodegenerative diseases. The amyloid binding dye Congo Red (and derivatives thereof) is known to inhibit the aggregation of several proteins including A β , IAPP and polyQ stretches from huntingtin [108–111]. Many flavonoids can inhibit protein aggregation, but in general they do so by stabilizing small oligomers [112–115]. One of the most studied flavonoids is epigallocatechin gallate (EGCG), which has been shown to bind to both the intrinsically disordered monomeric state of α SN and also oligomeric states [116]. EGCG inhibits the aggregation of α SN by forming higher molecular weight oligomers that are non-toxic and off-pathway [117]. Interestingly it has recently been shown that Δ^9 -tetrahydrocannabinol (THC), the active compound from marijuana, inhibits A β aggregation by reducing the levels of soluble A β and also reduces cytotoxicity, possibly suppressing aggregation altogether through the reduction in monomeric precursors [118]. Even though small molecules are a promising drug discovery platform for amyloid diseases, it may be a challenge to find small molecules that are highly specific towards single proteins.

7.3. Peptide mimetics of aggregation cores

Peptide based ligands designed to correspond to the central fibrillating core have also been found to inhibit fibril formation and reduce cytotoxicity of various aggregating proteins [119–123]. The inhibitory effect is achieved by binding to the growing ends of fibrils or oligomers. To aid the inhibitory effect, β -sheet breakers like proline or backbone N-methylations can be added to the peptide ligand, thereby inhibiting further growth of the aggregates [122,124]. Peptide ligands which are able to distinguish between monomers and higher order structures have also been developed. A peptide that specifically recognizes a conformational epitope of oligomeric A β and fibrils but not monomeric A β has been shown to reduce the level of higher-ordered structures when used in combination with an antibody raised against the peptide

ligand [125]. Furthermore, a bivalent peptide ligand for A β fused to linker specifically recognize early oligomers of A β and displays very low binding to monomeric and mature fibrils of A β [126].

7.4. Small molecule aggregation promoters

A different approach to reduce the appearance and cytotoxicity of oligomers is to speed up the aggregation reaction and thereby reduce the amount of free toxic oligomers by rapid incorporation and sequestering into non-toxic mature fibrils. Various compounds enhance the aggregation kinetics of A β [127], for example the molecule O4 binds to the hydrophobic amino acids of A β , thereby stabilizing self-assembly into fibrils. Furthermore O4 decreased the concentration of toxic oligomers and suppressed the inhibition of long-term potentiation in hippocampal brain slices [128].

7.5. Reduction of oligomers' membrane affinity

All of the abovementioned efforts to inhibit oligomer cytotoxicity focus on interference with the aggregation process and hence formation of the oligomers. A different approach is to change the membrane affinity. We recently demonstrated that this is how the flavonoid EGCG modulates the cytotoxicity of oligomers of α SN [47] (Fig. 5). Addition of EGCG rescued the toxicity of oligomers in both cell-based and vesicle-based experiments. The presence of EGCG did not change the secondary structure or the size of the isolated α SN oligomers but significant changes to the flexibility of the C-terminus were observed upon binding of EGCG to the oligomer as seen by NMR. This was ascribed to induction of structure in the otherwise flexible C-terminal upon binding of EGCG to the oligomers. The oligomers still bound to the membrane of vesicles in the presence of EGCG but to a lesser extent than in the absence of the flavonoid and no permeation or disruption of the vesicles was seen in the presence of EGCG. The oligomer–membrane interaction is thus inhibited but not abolished by EGCG, suggesting that the oligomer acts by destabilizing the membrane rather than by forming an actual pore. The loss of the C-terminal flexibility by binding of EGCG to the oligomers could be key to the change in the membrane interaction seen in the presence of EGCG. This line of work demonstrates that it is possible to directly change the membrane interactions of prefibrillar oligomers using small molecules.

8. Amyloid fibril–membrane interactions

In this review the focus is on the interaction of soluble oligomers with membranes. However, as mentioned in the introduction, there are many different amyloid fibrils which are also able to perturb membranes e.g. fibrils formed by α SN [33,129], Sup35 [130], IAPP [131] and β_2 m-microglobulin [132]. Since fibrils are often easier to form and obtain in high yields compared to oligomers they are potentially easier to study in membrane interaction experiments. The large size of fibrils is a challenge for some biophysical methods as e.g. NMR, however for some experimental approaches this can also be an advantage. One excellent example of this is the cryoelectron tomography study by the Saibil and Radford groups, where they present a 3D description of membrane disruption by β_2 m-microglobulin fibrils [133]. Their visualization of fibril–membrane interactions revealed that this interaction can indeed be strong and in this case spherical vesicles were reorganized into teardrop-shaped structures, cf. Fig. 6. Small vesicles were also observed, suggesting that fibrils are able to extract lipids from lipid bilayers. Also, they show how the ends of the β_2 m-microglobulin fibrils induce most vesicle damage, stabilized by the sides of the fibrils which also interact with the membrane. Fibril ends are the reactive site in fibril elongation and perturbation of lipid bilayers is perhaps caused by higher hydrophobicity and flexibility in these regions. As for oligomer–membrane interactions, an anionic lipid composition is essential for the interaction of β_2 m-microglobulin fibrils [132].

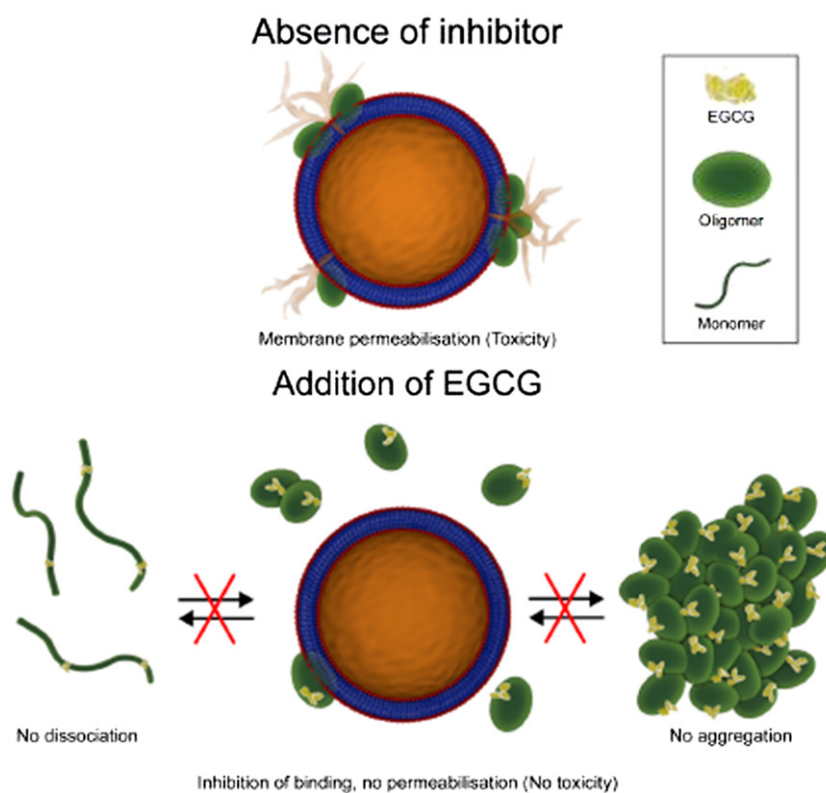


Fig. 5. Modulation of cytotoxicity of α -synuclein oligomers by inhibiting the lipid specificity of the toxic oligomers through the presence of the flavonoid EGCG. Reprinted from [47] with permission.

Another remarkable but different mechanism of fibril induced membrane damage stems from the observation that the deleterious effects of fibrils can also be viewed as a consequence of membrane templated

amyloid formation *via* the process of fibrillation *per se*. Membrane surfaces, particularly those containing anionic lipids [134], are able to bind monomeric amyloid precursors and thus increase their local

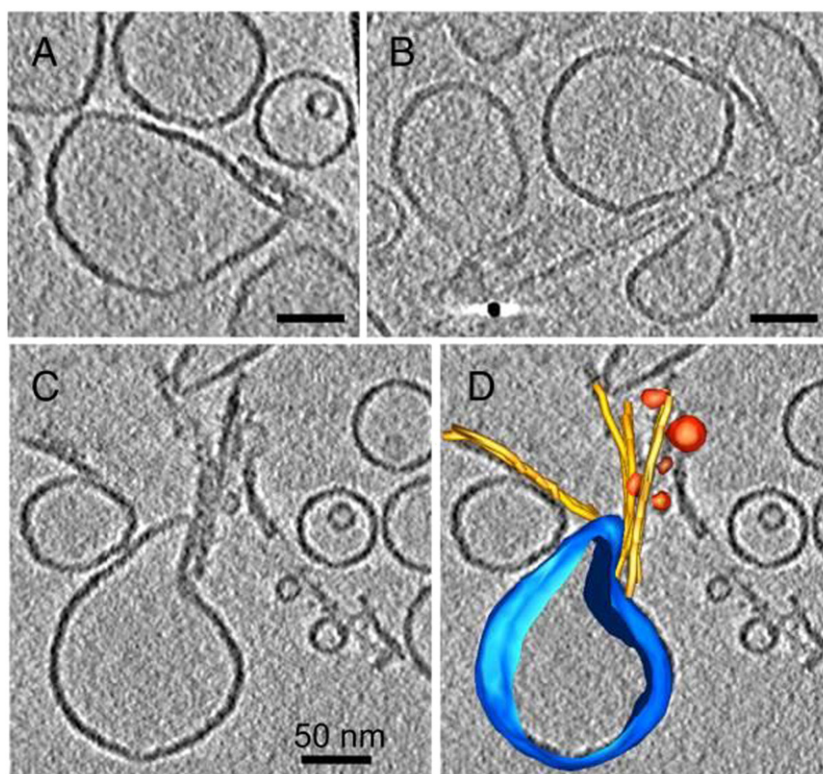


Fig. 6. Examples of membrane reorganization by β_2 m-microglobulin fibrils as pictured in 2D (A–C) and rendered in 3D (D) using cryoelectron tomography. Reprinted from [133] with permission.

concentration [131,135]. This can kick-start amyloid formation and the resulting conformational rearrangements during fibril elongation can perturb membranes sufficiently to permeabilize and even radically reorganize them [55,133,136,137]. One of the best-studied examples is provided by the hormone IAPP, which forms amyloid fibrils in the pancreas of patients with type II diabetes. Fibrillation of IAPP is templated by phospholipids, and sub-stoichiometric amounts of anionic lipids accelerate fibrillation considerably more than fibril inducers such as heparin and HFIP, suggesting that the energy barrier of the rate limiting step of fibril nucleation is lowered. Monomeric IAPP binds to the membrane and fibril formation proceeds from there. Strikingly mature amyloid fibrils do not interact with the membrane, suggesting that it is really the fibril growth on the membrane which leads to membrane permeabilization [135,137]. Again anionic lipids are essential for the binding of IAPP and initiation of fibrillation [138]. Recently it has also been demonstrated how α SN forms amyloid clusters at the membrane surface. These structures consist of both protein and lipids extracted from the vesicles and they compromise the membrane integrity and leads to a decrease in the lateral diffusion of both protein and lipids [139,140].

9. Conclusion

The analysis of oligomer–membrane interactions is a highly challenging task. One of the fundamental challenges in these studies is the preparation of oligomeric samples which are sufficiently stable and well-characterized. For now, α SN oligomers appear to be a solid model system for these studies as they are well-characterized and highly stable. However, for a better understanding of the mechanisms for interaction and perturbation more model systems are required together with the advancement of the biophysical and structural methods used to analyze these mechanisms.

The “amyloid pore” hypothesis was proposed at an early stage, but while conceptually appealing it is still not underpinned by direct structural evidence. Rather, recent data suggest that membrane perturbation may stem from other destabilization events. Clearly lipid-aggregate interactions will remain an exciting field of investigation for years to come.

Transparency document

The [Transparency document](#) associated with this article can be found, in the online version.

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References

- [1] F. Chiti, C.M. Dobson, Protein misfolding, functional amyloid, and human disease, *Annu. Rev. Biochem.* 75 (2006) 333–366.
- [2] G.M. Hirschfield, P.N. Hawkins, Amyloidosis: new strategies for treatment, *Int. J. Biochem. Cell Biol.* 35 (12) (2003) 1608–1613.
- [3] A.T. Alexandrescu, Amyloid accomplices and enforcers, *Protein Sci.* 14 (1) (2005) 1–12.
- [4] M. Stefani, Protein misfolding and aggregation: new examples in medicine and biology of the dark side of the protein world, *Biochim. Biophys. Acta* 1739 (1) (2004) 5–25.
- [5] M. Stefani, C.M. Dobson, Protein aggregation and aggregate toxicity: new insights into protein folding, misfolding diseases and biological evolution, *J. Mol. Med. (Berl)* 81 (11) (2003) 678–699.
- [6] C.M. Dobson, Protein aggregation and its consequences for human disease, *Protein Pept. Lett.* 13 (3) (2006) 219–227.
- [7] E.D. Eanes, G.G. Glenner, X-ray diffraction studies on amyloid filaments, *J. Histochem. Cytochem.* 16 (11) (1968) 673–677.
- [8] L.C. Serpell, P.E. Fraser, M. Sunde, X-ray fiber diffraction of amyloid fibrils, *Methods Enzymol.* 309 (1999) 526–536.
- [9] R. Nelson, D. Eisenberg, Structural models of amyloid-like fibrils, *Adv. Protein Chem.* 73 (2006) 235–282.
- [10] J.D. Sipe, A.S. Cohen, Review: history of the amyloid fibril, *J. Struct. Biol.* 130 (2–3) (2000) 88–98.
- [11] T.P.J. Knowles, et al., Spatial persistence of angular correlations in amyloid fibrils, *Phys. Rev. Lett.* 96 (2006) 238301.
- [12] L.C. Serpell, et al., The protofilament substructure of amyloid fibrils, *J. Mol. Biol.* 300 (5) (2000) 1033–1039.
- [13] J.-X. Lu, et al., Molecular structure of β -amyloid fibrils in Alzheimer's disease brain tissue, *Cell* 154 (6) (2013) 1257–1268.
- [14] C.M. Dobson, Protein misfolding, evolution and disease, *Trends Biochem. Sci.* 24 (9) (1999) 329–332.
- [15] K.F. DuBay, et al., Prediction of the absolute aggregation rates of amyloidogenic polypeptide chains, *J. Mol. Biol.* 341 (5) (2004) 1317–1326.
- [16] F. Chiti, et al., Rationalization of the effects of mutations on peptide and protein aggregation rates, *Nature* 424 (6950) (2003) 805–808.
- [17] S.K. Maji, et al., Functional amyloids as natural storage of peptide hormones in pituitary secretory granules, *Science* 325 (5938) (2009) 328–332.
- [18] J.F. Berson, et al., Proprotein convertase cleavage liberates a fibrillogenic fragment of a resident glycoprotein to initiate melanosome biogenesis, *J. Cell Biol.* 161 (3) (2003) 521–533.
- [19] J.A. Hardy, G.A. Higgins, Alzheimer's disease: the amyloid cascade hypothesis, *Science* 256 (5054) (1992) 184–185.
- [20] D.W. Dickson, et al., Identification of normal and pathological aging in prospectively studied nondemented elderly humans, *Neurobiol. Aging* 13 (1) (1992) 179–189.
- [21] R.D. Terry, et al., Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment, *Ann. Neurol.* 30 (4) (1991) 572–580.
- [22] C.A. Lemere, et al., Sequence of deposition of heterogeneous amyloid β -peptides and APO E in Down syndrome: implications for initial events in amyloid plaque formation, *Neurobiol. Dis.* 3 (1) (1996) 16–32.
- [23] D.W. Dickson, et al., Correlations of synaptic and pathological markers with cognition of the elderly, *Neurobiol. Aging* 16 (3) (1995) 285–298 (discussion 298–304).
- [24] B. Caughey, P.T. Lansbury, Protofibrils, pores, fibrils, and neurodegeneration: separating the responsible protein aggregates from the innocent bystanders, *Annu. Rev. Neurosci.* 26 (2003) 267–298.
- [25] M.J. Volles, P.T. Lansbury Jr., Zeroing in on the pathogenic form of alpha-synuclein and its mechanism of neurotoxicity in Parkinson's disease, *Biochemistry* 42 (26) (2003) 7871–7878.
- [26] K.E. Paleologou, et al., Detection of elevated levels of soluble alpha-synuclein oligomers in post-mortem brain extracts from patients with dementia with Lewy bodies, *Brain* 132 (Pt 4) (2009) 1093–1101.
- [27] T. Tokuda, et al., Detection of elevated levels of alpha-synuclein oligomers in CSF from patients with Parkinson disease, *Neurology* 75 (20) (2010) 1766–1772.
- [28] M. Bucciantini, et al., Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases, *Nature* 416 (6880) (2002) 507–511.
- [29] I. Sirangelo, et al., Fibrillogenesis and cytotoxic activity of the amyloid-forming apomyoglobin mutant W7FW14F, *J. Biol. Chem.* 279 (13) (2004) 13183–13189.
- [30] W. Paslawski, et al., High stability and cooperative unfolding of alpha-synuclein oligomers, *Biochemistry* 53 (39) (2014) 6252–6263.
- [31] A. Quist, et al., Amyloid ion channels: a common structural link for protein-misfolding disease, *Proc. Natl. Acad. Sci. U. S. A.* 102 (30) (2005) 10427–10432.
- [32] R. Kaye, et al., Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis, *Science* 300 (5618) (2003) 486–489.
- [33] N. Lorenzen, et al., The role of stable alpha-synuclein oligomers in the molecular events underlying amyloid formation, *J. Am. Chem. Soc.* 136 (10) (2014) 3859–3868.
- [34] W. Paslawski, et al., Co-existence of two different alpha-synuclein oligomers with different core structures determined by hydrogen/deuterium exchange mass spectrometry, *Angew. Chem. Int. Ed. Engl.* 53 (29) (2014) 7560–7563.
- [35] A.T. Petkova, et al., Self-propagating, molecular-level polymorphism in Alzheimer's beta-amyloid fibrils, *Science* 307 (5707) (2005) 262–265.
- [36] K. Yamaguchi, et al., Seeding-dependent propagation and maturation of amyloid fibril conformation, *J. Mol. Biol.* 352 (4) (2005) 952–960.
- [37] C.J. Cummings, et al., Chaperone suppression of aggregation and altered subcellular proteasome localization imply protein misfolding in SCA1, *Nat. Genet.* 19 (2) (1998) 148–154.
- [38] K. Ii, et al., Immunocytochemical co-localization of the proteasome in ubiquitinated structures in neurodegenerative diseases and the elderly, *J. Neuropathol. Exp. Neurol.* 56 (2) (1997) 125–131.
- [39] C. Soto, Unfolding the role of protein misfolding in neurodegenerative diseases, *Nat. Rev. Neurosci.* 4 (1) (2003) 49–60.
- [40] C. Behl, et al., Hydrogen peroxide mediates amyloid beta protein toxicity, *Cell* 77 (6) (1994) 817–827.
- [41] L.J. Hsu, et al., Alpha-synuclein promotes mitochondrial deficit and oxidative stress, *Am. J. Pathol.* 157 (2) (2000) 401–410.
- [42] P. Jenner, C.W. Olanow, Understanding cell death in Parkinson's disease, *Ann. Neurol.* 44 (3 Suppl 1) (1998) S72–S84.
- [43] P. Jenner, Oxidative stress in Parkinson's disease, *Ann. Neurol.* 53 (Suppl. 3) (2003) S26–S36 (discussion S36–8).
- [44] B.J. Tabner, et al., Production of reactive oxygen species from aggregating proteins implicated in Alzheimer's disease, Parkinson's disease and other neurodegenerative diseases, *Curr. Top. Med. Chem.* 1 (6) (2001) 507–517.

- [45] M.S. Parihar, et al., Alpha-synuclein overexpression and aggregation exacerbates impairment of mitochondrial functions by augmenting oxidative stress in human neuroblastoma cells, *Int. J. Biochem. Cell Biol.* 41 (10) (2009) 2015–2024.
- [46] N. Lorenzen, et al., The N-terminus of alpha-synuclein is essential for both monomeric and oligomeric interactions with membranes, *FEBS Lett.* 588 (3) (2014) 497–502.
- [47] N. Lorenzen, et al., How epigallocatechin gallate can inhibit alpha-synuclein oligomer toxicity in vitro, *J. Biol. Chem.* 289 (31) (2014) 21299–21310.
- [48] A.N. Stefanovic, et al., Alpha-synuclein oligomers distinctively permeabilize complex model membranes, *FEBS J.* 281 (12) (2014) 2838–2850.
- [49] C.M. van Rooijen BD, V. Subramaniam, Membrane permeabilization by oligomeric α -synuclein: in search of the mechanism, *PLoS ONE* 5 (2010).
- [50] M.J. Volles, P.T. Lansbury Jr., Relationships between the sequence of alpha-synuclein and its membrane affinity, fibrillization propensity, and yeast toxicity, *J. Mol. Biol.* 366 (5) (2007) 1510–1522.
- [51] B.D. van Rooijen, et al., Tryptophan fluorescence reveals structural features of alpha-synuclein oligomers, *J. Mol. Biol.* 394 (5) (2009) 826–833.
- [52] M.J. Volles, S.-J. Lee, J.-C. Rochet, M.D. Shitlerman, T.T. Ding, J.C. Kessler, P.T. Lansbury, Vesicle Permeabilization by Protofibrillar α -Synuclein: Implications for the Pathogenesis and Treatment of Parkinson's Disease, *Biochemistry* 40 (26) (2001) 7812–7819.
- [53] M. Stockl, M.M. Claessens, V. Subramaniam, Kinetic measurements give new insights into lipid membrane permeabilization by alpha-synuclein oligomers, *Mol. Biosyst.* 8 (1) (2012) 338–345.
- [54] B.D. van Rooijen, M.M. Claessens, V. Subramaniam, Lipid bilayer disruption by oligomeric alpha-synuclein depends on bilayer charge and accessibility of the hydrophobic core, *Biochim. Biophys. Acta* 1788 (6) (2009) 1271–1278.
- [55] E. Sparr, et al., Islet amyloid polypeptide-induced membrane leakage involves uptake of lipids by forming amyloid fibers, *FEBS Lett.* 577 (1–2) (2004) 117–120.
- [56] N. Arispe, E. Rojas, H.B. Pollard, Alzheimer disease amyloid beta protein forms calcium channels in bilayer membranes: blockade by tromethamine and aluminum, *Proc. Natl. Acad. Sci.* 90 (2) (1993) 567.
- [57] M.C. Lin, T. Mirzabekov, B.L. Kagan, Channel formation by a neurotoxic prion protein fragment, *J. Biol. Chem.* 272 (1) (1997) 44–47.
- [58] T.A. Mirzabekov, M. Lin, B.L. Kagan, Pore formation by the cytotoxic islet amyloid peptide amylin, *J. Biol. Chem.* 271 (4) (1996) 1988.
- [59] M. Andreassen, et al., Polymorphic fibrillation of the destabilized fourth fasciclin-1 domain mutant A546T of the transforming growth factor- β -induced protein (TGFB β) occurs through multiple pathways with different oligomeric intermediates, *J. Biol. Chem.* 287 (41) (2012) 34730–34742.
- [60] L. Pieri, et al., The yeast prion Ure2p native-like assemblies are toxic to mammalian cells regardless of their aggregation state, *J. Biol. Chem.* 281 (22) (2006) 15337–15344.
- [61] S. Campioni, et al., A causative link between the structure of aberrant protein oligomers and their toxicity, *Nat. Chem. Biol.* 6 (2) (2010) 140–147.
- [62] M. Stefani, Generic cell dysfunction in neurodegenerative disorders: role of surfaces in early protein misfolding, aggregation, and aggregate cytotoxicity, *Neuroscientist* 13 (5) (2007) 519–531.
- [63] I. Kheterpal, R. Wetzel, Hydrogen/deuterium exchange mass spectrometry—a window into amyloid structure, *Acc. Chem. Res.* 39 (9) (2006) 584–593.
- [64] N. Cremades, et al., Direct observation of the interconversion of normal and toxic forms of alpha-synuclein, *Cell* 149 (5) (2012) 1048–1059.
- [65] M.H. Polymeropoulos, et al., Mutation in the alpha-synuclein gene identified in families with Parkinson's disease, *Science* 276 (5321) (1997) 2045–2047.
- [66] M.G. Spillantini, et al., Alpha-synuclein in Lewy bodies, *Nature* 388 (6645) (1997) 839–840.
- [67] M.M. Dedmon, et al., Mapping long-range interactions in alpha-synuclein using spin-label NMR and ensemble molecular dynamics simulations, *J. Am. Chem. Soc.* 127 (2) (2005) 476–477.
- [68] J.R. Allison, et al., Determination of the free energy landscape of alpha-synuclein using spin label nuclear magnetic resonance measurements, *J. Am. Chem. Soc.* 131 (51) (2009) 18314–18326.
- [69] C. Del Mar, et al., Structure and properties of alpha-synuclein and other amyloids determined at the amino acid level, *Proc. Natl. Acad. Sci. U. S. A.* 102 (43) (2005) 15477–15482.
- [70] S. Mysling, et al., Characterizing the dynamics of alpha-synuclein oligomers using hydrogen/deuterium exchange monitored by mass spectrometry, *Biochemistry* 52 (51) (2013) 9097–9103.
- [71] W.S. Davidson, et al., Stabilization of alpha-synuclein secondary structure upon binding to synthetic membranes, *J. Biol. Chem.* 273 (16) (1998) 9443–9449.
- [72] T.S. Ulmer, et al., Structure and dynamics of micelle-bound human alpha-synuclein, *J. Biol. Chem.* 280 (10) (2005) 9595–9603.
- [73] C.R. Bodner, et al., Differential phospholipid binding of alpha-synuclein variants implicated in Parkinson's disease revealed by solution NMR spectroscopy, *Biochemistry* 49 (5) (2010) 862–871.
- [74] T. Bartels, et al., The N-terminus of the intrinsically disordered protein alpha-synuclein triggers membrane binding and helix folding, *Biophys. J.* 99 (7) (2010) 2116–2124.
- [75] G. Fusco, et al., Direct observation of the three regions in alpha-synuclein that determine its membrane-bound behaviour, *Nat. Commun.* 5 (2014) 3827.
- [76] J. Varkey, et al., Membrane curvature induction and tubulation are common features of synucleins and apolipoproteins, *J. Biol. Chem.* 285 (42) (2010) 32486–32493.
- [77] M.B. Jensen, et al., Membrane curvature sensing by amphipathic helices: a single liposome study using alpha-synuclein and annexin B12, *J. Biol. Chem.* 286 (49) (2011) 42603–42614.
- [78] M.M. Ouberaï, et al., Alpha-synuclein senses lipid packing defects and induces lateral expansion of lipids leading to membrane remodeling, *J. Biol. Chem.* 288 (29) (2013) 20883–20895.
- [79] M.J. Volles, P.T. Lansbury Jr., Vesicle permeabilization by protofibrillar alpha-synuclein is sensitive to Parkinson's disease-linked mutations and occurs by a pore-like mechanism, *Biochemistry* 41 (14) (2002) 4595–4602.
- [80] H.A. Lashuel, et al., Neurodegenerative disease: amyloid pores from pathogenic mutations, *Nature* 418 (6895) (2002) 291.
- [81] N. Zijlstra, et al., Molecular composition of sub-stoichiometrically labeled alpha-synuclein oligomers determined by single-molecule photobleaching, *Angew. Chem. Int. Ed. Engl.* 51 (35) (2012) 8821–8824.
- [82] L. Giehm, S.D., D.E. Otzen, B. Vestergaard, Low-resolution structure of a vesicle disrupting β alpha-synuclein oligomer that accumulates during fibrillation, *Proc. Natl. Acad. Sci. U. S. A.* 108 (8) (2011) 3246–3251.
- [83] J.I. Gallea, M.S. Celej, Structural insights into amyloid oligomers of the Parkinson disease-related protein alpha-synuclein, *J. Biol. Chem.* 289 (39) (2014) 26733–26742.
- [84] M.S. Celej, et al., Toxic prefibrillar alpha-synuclein amyloid oligomers adopt a distinctive antiparallel beta-sheet structure, *Biochem. J.* 443 (3) (2012) 719–726.
- [85] W. Paslawski, N. Lorenzen, D.E. Otzen, Formation and characterization of α -synuclein oligomers, *Methods Mol. Biol.* (2015) (in press).
- [86] L. Kjaer, et al., The influence of vesicle size and composition on alpha-synuclein structure and stability, *Biophys. J.* 96 (7) (2009) 2857–2870.
- [87] B.D. van Rooijen, M.M. Claessens, V. Subramaniam, Membrane binding of oligomeric alpha-synuclein depends on bilayer charge and packing, *FEBS Lett.* 582 (27) (2008) 3788–3792.
- [88] B. Bolognesi, et al., ANS binding reveals common features of cytotoxic amyloid species, *ACS Chem. Biol.* 5 (8) (2010) 735–740.
- [89] K. Vamcava, M.J. Volles, P.T. Lansbury, The first N-terminal amino acids of α -synuclein are essential for α -helical structure formation in vitro and membrane binding in yeast, *J. Mol. Biol.* 389 (4755) (2009) 413–424.
- [90] K. Runager, et al., Human phenotypically distinct TGF β 1 corneal dystrophies are linked to the stability of the fourth FAS1 domain of TGF β 1p, *J. Biol. Chem.* 286 (7) (2011) 4951–4958.
- [91] F.L. Munier, et al., Kerato-epithelin mutations in four 5q31-linked corneal dystrophies, *Nat. Genet.* 15 (3) (1997) 247–251.
- [92] C. Kannabiran, G.K. Klintworth, TGF β 1 gene mutations in corneal dystrophies, *Hum. Mutat.* 27 (7) (2006) 615–625.
- [93] G.K. Klintworth, The molecular genetics of the corneal dystrophies—current status, *Front. Biosci.* 8 (2003) d687–d713.
- [94] J. Skonier, et al., cDNA cloning and sequence analysis of beta ig-h3, a novel gene induced in a human adenocarcinoma cell line after treatment with transforming growth factor-beta, *DNA Cell Biol.* 11 (7) (1992) 511–522.
- [95] B.W. Streeten, et al., Immunolocalization of beta ig-h3 protein in 5q31-linked corneal dystrophies and normal corneas, *Arch. Ophthalmol.* 117 (1) (1999) 67–75.
- [96] E. Korvatska, et al., Amyloid and non-amyloid forms of 5q31-linked corneal dystrophy resulting from kerato-epithelin mutations at Arg-124 are associated with abnormal turnover of the protein, *J. Biol. Chem.* 275 (15) (2000) 11465–11469.
- [97] E. Korvatska, et al., Mutation hot spots in 5q31-linked corneal dystrophies, *Am. J. Hum. Genet.* 62 (2) (1998) 320–324.
- [98] P. Dighiero, et al., A new mutation (A546T) of the beta-ig-h3 gene responsible for a French lattice corneal dystrophy type IIIA, *Am. J. Ophthalmol.* 129 (2) (2000) 248–251.
- [99] R.V. Basaiawmoit, et al., SAXS models of TGF β 1 reveal a trimeric structure and show that the overall shape is not affected by the Arg124His mutation, *J. Mol. Biol.* 408 (3) (2011) 503–513.
- [100] D.C. Beebe, Maintaining transparency: a review of the developmental physiology and pathophysiology of two avascular tissues, *Semin. Cell Dev. Biol.* 19 (2) (2008) 125–133.
- [101] H. Karring, et al., Composition and proteolytic processing of corneal deposits associated with mutations in the TGF β 1 gene, *Exp. Eye Res.* 96 (1) (2012) 163–170.
- [102] R.B. Andersen, et al., Purification and structural characterization of transforming growth factor beta induced protein (TGFB β) from porcine and human corneas, *Biochemistry* 43 (51) (2004) 16374–16384.
- [103] B. Solomon, et al., Disaggregation of Alzheimer beta-amyloid by site-directed mAb, *Proc. Natl. Acad. Sci. U. S. A.* 94 (8) (1997) 4109–4112.
- [104] C. Janus, et al., A beta peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease, *Nature* 408 (6815) (2000) 979–982.
- [105] C.A. Hawkes, et al., Small molecule beta-amyloid inhibitors that stabilize protofibrillar structures in vitro improve cognition and pathology in a mouse model of Alzheimer's disease, *Eur. J. Neurosci.* 31 (2) (2010) 203–213.
- [106] K. Bodin, et al., Antibodies to human serum amyloid P component eliminate visceral amyloid deposits, *Nature* 468 (7320) (2010) 93–97.
- [107] M. Botto, et al., Amyloid deposition is delayed in mice with targeted deletion of the serum amyloid P component gene, *Nat. Med.* 3 (8) (1997) 855–859.
- [108] A. Lorenzo, B.A. Yankner, Beta-amyloid neurotoxicity requires fibril formation and is inhibited by Congo red, *Proc. Natl. Acad. Sci. U. S. A.* 91 (25) (1994) 12243–12247.
- [109] V. Heiser, et al., Inhibition of huntingtin fibrillogenesis by specific antibodies and small molecules: implications for Huntington's disease therapy, *Proc. Natl. Acad. Sci. U. S. A.* 97 (12) (2000) 6739–6744.
- [110] W.E. Klunk, et al., Chrysamine-G, a lipophilic analogue of Congo red, inhibits A beta-induced toxicity in PC12 cells, *Life Sci.* 63 (20) (1998) 1807–1814.
- [111] F. Yang, et al., Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo, *J. Biol. Chem.* 280 (7) (2005) 5892–5901.
- [112] F.E. Herrera, et al., Inhibition of alpha-synuclein fibrillization by dopamine is mediated by interactions with five C-terminal residues and with E83 in the NAC region, *PLoS ONE* 3 (10) (2008) e3394.

- [113] E.H. Norris, et al., Reversible inhibition of alpha-synuclein fibrillization by dopaminochrome-mediated conformational alterations, *J. Biol. Chem.* 280 (22) (2005) 21212–21219.
- [114] J. Li, et al., Dopamine and L-dopa disaggregate amyloid fibrils: implications for Parkinson's and Alzheimer's disease, *FASEB J.* 18 (9) (2004) 962–964.
- [115] S.L. Leong, et al., Formation of dopamine-mediated alpha-synuclein-soluble oligomers requires methionine oxidation, *Free Radic. Biol. Med.* 46 (10) (2009) 1328–1337.
- [116] D.E. Ehrnhoefer, et al., EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers, *Nat. Struct. Mol. Biol.* 15 (6) (2008) 558–566.
- [117] J. Bieschke, et al., EGCG remodels mature alpha-synuclein and amyloid-beta fibrils and reduces cellular toxicity, *Proc. Natl. Acad. Sci. U. S. A.* 107 (17) (2010) 7710–7715.
- [118] C. Cao, et al., The potential therapeutic effects of THC on Alzheimer's disease, *J. Alzheimers Dis.* 42 (3) (2014) 973–984.
- [119] O.M. El-Agnaf, et al., A strategy for designing inhibitors of alpha-synuclein aggregation and toxicity as a novel treatment for Parkinson's disease and related disorders, *FASEB J.* 18 (11) (2004) 1315–1317.
- [120] K. Muthusamy, et al., Design and study of peptide-based inhibitors of amylin cytotoxicity, *Bioorg. Med. Chem. Lett.* 20 (4) (2010) 1360–1362.
- [121] R. Shaltiel-Karyo, et al., Inhibiting alpha-synuclein oligomerization by stable cell-penetrating beta-synuclein fragments recovers phenotype of Parkinson's disease model flies, *PLoS ONE* 5 (11) (2010) e13863.
- [122] A.M. Bodles, et al., Inhibition of fibril formation and toxicity of a fragment of alpha-synuclein by an N-methylated peptide analogue, *Neurosci. Lett.* 359 (1–2) (2004) 89–93.
- [123] B.M. Austen, et al., Designing peptide inhibitors for oligomerization and toxicity of Alzheimer's beta-amyloid peptide, *Biochemistry* 47 (7) (2008) 1984–1992.
- [124] C. Soto, et al., Inhibition of Alzheimer's amyloidosis by peptides that prevent beta-sheet conformation, *Biochem. Biophys. Res. Commun.* 226 (3) (1996) 672–680.
- [125] P. Hoogerhout, et al., A cyclic undecamer peptide mimics a turn in folded Alzheimer amyloid beta and elicits antibodies against oligomeric and fibrillar amyloid and plaques, *PLoS ONE* 6 (4) (2011) e19110.
- [126] A.A. Reinke, et al., Chemical probes that selectively recognize the earliest Abeta oligomers in complex mixtures, *J. Am. Chem. Soc.* 132 (50) (2010) 17655–17657.
- [127] A.D. Williams, et al., Structural properties of Abeta protofibrils stabilized by a small molecule, *Proc. Natl. Acad. Sci. U. S. A.* 102 (20) (2005) 7115–7120.
- [128] J. Bieschke, et al., Small-molecule conversion of toxic oligomers to nontoxic beta-sheet-rich amyloid fibrils, *Nat. Chem. Biol.* 8 (1) (2012) 93–101.
- [129] L. Bousset, et al., Structural and functional characterization of two alpha-synuclein strains, *Nat. Commun.* 4 (2013).
- [130] M. Bucciantini, et al., Toxic effects of amyloid fibrils on cell membranes: the importance of ganglioside GM1, *FASEB J.* 26 (2) (2012) 818–831.
- [131] J.D. Knight, A.D. Miranker, Phospholipid catalysis of diabetic amyloid assembly, *J. Mol. Biol.* 341 (5) (2004) 1175–1187.
- [132] S.C. Goodchild, et al., β_2 m-Microglobulin amyloid fibril-induced membrane disruption is enhanced by endosomal lipids and acidic pH, *PLoS ONE* 9 (8) (2014).
- [133] L. Milanesi, et al., Direct three-dimensional visualization of membrane disruption by amyloid fibrils, *Proc. Natl. Acad. Sci. U. S. A.* 109 (50) (2012) 20455–20460.
- [134] H. Zhao, E.K.J. Tuominen, P.K.J. Kinnunen, Formation of amyloid fibers triggered by phosphatidylserine-containing membranes, *Biochemistry* 43 (3610) (2004) 10302–10307.
- [135] M.F. Engel, et al., Islet amyloid polypeptide inserts into phospholipid monolayers as monomer, *J. Mol. Biol.* 356 (3) (2006) 783–789.
- [136] M.F. Sciacca, et al., Two-step mechanism of membrane disruption by Abeta through membrane fragmentation and pore formation, *Biophys. J.* 103 (4) (2012) 702–710.
- [137] M.F. Engel, et al., Membrane damage by human islet amyloid polypeptide through fibril growth at the membrane, *Proc. Natl. Acad. Sci. U. S. A.* 105 (16) (2008) 6033–6038.
- [138] J.D. Knight, A.D. Miranker, Phospholipid catalysis of diabetic amyloid assembly, *J. Mol. Biol.* 341 (2004) 1175–1187.
- [139] A. Iyer, et al., Amyloids of alpha-synuclein affect the structure and dynamics of supported lipid bilayers, *Biophys. J.* 106 (12) (2014) 2585–2594.
- [140] H. Chaudhary, et al., Membrane interactions and fibrillization of alpha-synuclein play an essential role in membrane disruption, *FEBS Lett.* 588 (23) (2014) 4457–4463.