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Amyloid Aggregation: Role of Biological Membranes and the Aggregate−Membrane System

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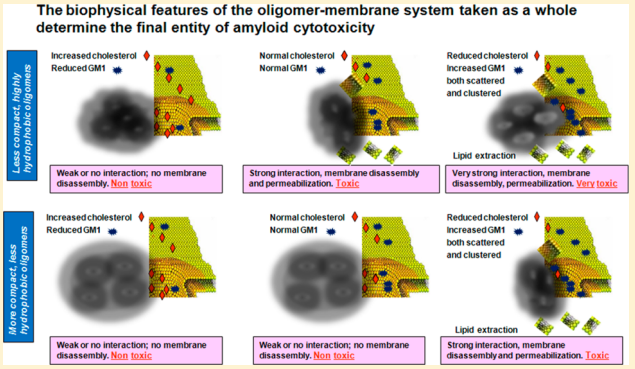
ABSTRACT: Several human degenerative diseases involve amyloidogenic peptides/proteins with high conformational plasticity and propensity to self-aggregate into polymeric fibrillar assemblies sharing the cross-β structure and endowed with cytotoxic potential. Although the mechanisms of amyloid growth and toxicity are not fully understood, a common property of amyloids is their ability to interact with lipid bilayers disturbing membrane integrity. Lipid bilayers can also act as conformational catalysts, favoring protein misfolding and inducing the growth of aggregation nuclei, early oligomers, and mature fibrils with specific biophysical, structural, and toxicity features. This Perspective will highlight these effects in the context of a membrane−oligomer system where the conformational/biophysical features of either component affect those of the other. In this context, we will highlight the modulation of the protein−cell surface interaction by the content of membrane cholesterol and gangliosides, notably GM1. In particular, we will discuss data that indicate how these interactions affect the structural and stability properties of both protein and bilayers as well as the final cytotoxic effect. Our goal is to propose shared membrane-based mechanisms that could apply to any amyloidogenic peptide/protein, providing a biochemical background for amyloid growth and toxicity.



Amyloid fibrils are polymeric fibrillar assemblies of specific protein/peptides where each monomer displays a shared, β-structure-enriched, amyloid fold that can be quite different from that displayed in the normally folded, biologically active form.[1] Amyloid fibrils are long (in the μm range), narrow (most often 5−10 nm in width), unbranched assemblies typically resulting from 2−4 intertwined protofilaments, each consisting of a double β-sheet that runs along the fibril axis, where each monomeric unit provides a number of parallel or antiparallel β-strands (the "cross-β" structure[2]).

The general emerging picture is that oligomers cannot be described as a finite number of peptide/protein structures defined by a specific set of parameters. Rather, they are best described as a number of conformational ensembles, each comprising an indefinite number of highly dynamic different assemblies.

Amyloid fibrils of one out of a number of peptides or proteins (around 25) are the main intracellular or extracellular components of tissue and organ deposits that are considered the main hallmarks of several systemic or neurological degenerative diseases. The amyloidoses include type-II diabetes, some systemic amyloidoses, Alzheimer's, Huntington's, and Parkinson's diseases (Table 1), each characterized by the presence of fibrillar deposits of a specific protein/peptide.[3−5] Presently, amyloid fibrils and their precursors (see later) are considered among the main species responsible for the biological and functional impairment, which eventually culminates with the appearance of the clinical symptoms of each specific disease; accordingly, the study of the structural and biophysical features of amyloid fibrils and their precursors as well as of the molecular basis of their growth and cytotoxicity has become a leading theme in protein science.

Why and how does a natively folded protein or a natively unfolded protein/peptide adopt the basic alternative fold that characterizes its amyloid aggregates? Past and ongoing research to answer these questions has been carried out mainly in vitro, even though in vivo data are also starting to appear. Until

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Table 1. Main Amyloid Diseases and Components of Their Associated Fibrillar Deposits

clinical syndrome	fibril component
Alzheimer's disease	Aβ peptides (wild-type or mutant); Tau (wild-type or mutant)
spongiform encephalopathies	prion protein (full-length or fragments)
Parkinson's disease	α-synuclein (wild-type or mutant)
fronto-temporal dementias	Tau (wild-type or mutant)
familial Danish dementia	ADan peptide
familial British dementia	ABri peptide
hereditary cerebral hemorrhage with amyloidosis	cystatin-C (minus a 10-residue fragment); Aβ peptides
amyotrophic lateral sclerosis	superoxide dismutase (wild-type or mutant)
dentatorubro-pallido-luysian atrophy	atrophin 1 (polyQ expansion)
Huntington disease	huntingtin (polyQ expansion)
cerebellar ataxias	ataxins (polyQ expansion)
Kennedy disease	androgen receptor (polyQ expansion)
spino cerebellar ataxia 17	TATA-box binding protein (polyQ expansion)
primary systemic amyloidosis	Ig light chains (full-length or fragments)
secondary systemic amyloidosis	serum amyloid A (fragments)
familial Mediterranean fever	serum amyloid A (fragments)
hemodialysis-related amyloidosis (DRA)	β2-microglobulin
familial amyloid polyneuropathy (FAP)	transthyretin (over 45 variants or fragments thereof)
familial amyloid cardiomyopathy (FAC)	
senile systemic amyloidosis	transthyretin (wild type or fragments thereof)
Finnish hereditary systemic amyloidosis	gelsolin (fragments of the mutant protein)
type-II diabetes	pro-islet amyloid polypeptide (fragments)
inclusion body myositis (IBM)	Aβ peptides
familial non-neuropathic systemic amyloidosis	lysozyme (mutant)

recently, it was generally thought that the globular native fold of a typical protein, which is mainly dictated by intramolecular hydrophobic and, to a lesser extent, secondary interactions, was the lowest free-energy state that the polypeptide chain could reach.[6] However, the structural characterization of amyloid fibrils, mainly stabilized by intermolecular (intersubunit) secondary interactions, and the study of their biophysical properties have shown that, typically, the amyloid fold is the most stable one and that amyloid fibrils appear to populate an energy-minimum state that is remarkably deeper than that pertaining to the natively folded state[4] (Figure 1).

It has also been shown that both the globular and the amyloid states of a protein are stabilized by the same forces (secondary and hydrophobic interactions); however, for any peptide/protein, the folding-to-aggregation shift requires conditions where an alteration of the residue−water interactions does occur with a profound modification of the balance between hydrophobic and hydrogen bonding contacts.[5]

Therefore, why would a natural polypeptide chain typically fold into a biologically active globular protein instead of aggregating into nonfunctional, potentially harmful, amyloid assemblies? It must be noted that the number of all known or still unknown polypeptide chains in nature, though huge, is only an infinitesimal fraction of those arising from any combination of the 20 different amino acids; for example, the number of different polypeptide chains of 300 amino acid residues, the average length of a folded protein, is 20^{300}. It results that natural polypeptide chains are the product of a careful selection by the biological evolution that faced many constraints, some of which were highlighted by the study of amyloid aggregation. Those constraints arose primarily from the need to generate polypeptide chains able to fold into compact, stable structures able to perform specific molecular functions at specific working conditions. However, the need was also mandatory to select polypeptide chains with the exact conformational stability suitable not only to ensure the plasticity needed for their biological function but also to minimize unfolding. Accordingly, these chains must resist the possibility to undertake the path alternative to folding, resulting in the growth of amyloid assemblies not only devoid of biological activity (loss of function) but also endowed with new cytotoxic properties (gain of function).[6]

That protein evolution has been very efficient in such a choice is testified to by the fact that, under normal conditions, each of the different proteins encoded by the 30 000 genes in the human genome folds correctly at any time inside of the cells, displaying a negligible tendency to misfold, unfold, and aggregate. However, the polypeptide chains selected by peptide/protein evolution still maintain the basic tendency to enter the aggregation path; this is why biological evolution has also provided cells with specific tools that monitor the quality of protein folding, repairing or destroying any misfolded molecule as soon as it appears, thus reducing the risk of aggregation. As a result, only a very limited number of protein molecules can escape those controls priming aggregation, and this usually happens in aged organisms, where the efficiency of those controls can decline and other environmental modifications favoring aggregation may appear.[2]

In general, protein aggregation becomes favored over correct folding by all of those factors that reduce protein stability and/or induce a monomer−monomer interaction to form aggregation nuclei.[2] Typically, these include mutations, chemical modifications, alterations of the medium conditions, increased protein expression, and the presence of surfaces with proaggregation physicochemical features. A mutation (any amino acid replacement or modification of the polypeptide chain length) can affect the thermodynamic stability of a folded protein, thus favoring the increase of partially folded species already present at equilibrium with the fully folded molecules as a result of the molecular dynamics. At proper conditions, the unstable partially folded molecules can interact with each other instead of refolding, thus initiating the aggregation path. Any increase of expression of a given protein can work similarly, increasing the net amount of molecules that, at a given time, are present in a partially folded state in equilibrium with the natively folded one.[7] Chemical modifications (oxidation, phosphorylation, glycosylation, presence, or lack of specific ligands) as well as any alteration of the medium conditions (including pH, temperature, medium polarity, macromolecular crowding, and the presence of modified surfaces) can also affect the conformational stability of a polypeptide chain in vitro and in vivo.[3] A mutation can also favor aggregation kinetically, when it does not affect the protein conformational stability but speeds the reciprocal interaction of partially folded species. This can be the case for mutations that reduce the net charge or increase the mean hydrophobicity or the tendency to adopt the β-structure of the polypeptide chain.[8]

The presence of surfaces can modulate profoundly protein folding and the stability of a folded protein. Peptides and

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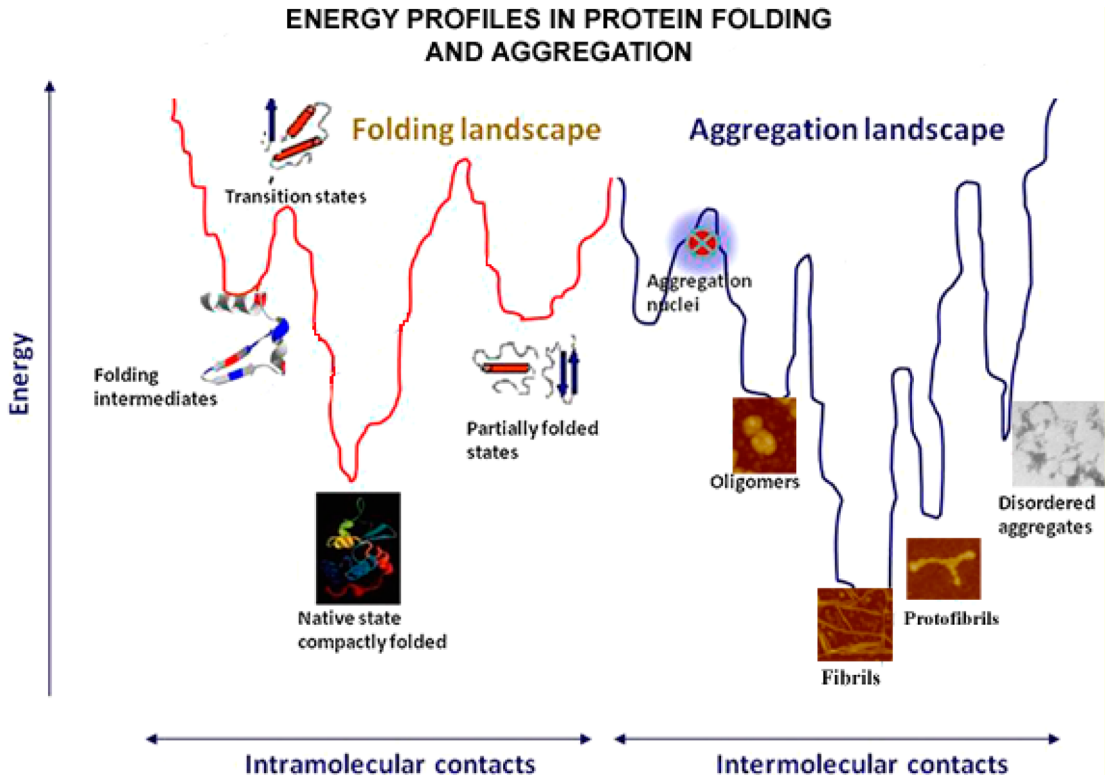

Figure 1. The protein folding and aggregation energy landscape. As far as the unfolded chain (at the top of the energy landscape) establishes intramolecular contacts, it lowers its free energy, and the number of conformations it can sample is rapidly reduced, thus eliminating the need for a global search. At the end of the path, it reaches an energy minimum, providing the conformational stability of the native state ensemble. The aggregation side of the protein energy landscape can be experienced by an unfolded polypeptide chain or a polypeptide chain undergoing misfolding or partial unfolding due to modified environmental cues or molecular properties favoring intermolecular, rather than intramolecular, contacts and the β-structure. The two sides of the same landscape highlight the competition between intramolecular (folding) and intermolecular (aggregation) interactions, showing that any polypeptide chain can undergo either pathway depending on a combination of its structural and physicochemical features, medium conditions, and the presence of surfaces. (Modified from Silva, J. L.; et al. Ligand Binding and Hydration in Protein Misfolding: Insight from Studies of Prion and p53 Tumor Suppressor Proteins. *Acc. Chem. Res.* **2010**, *43*, 271−279.

proteins can interact with, and be actively recruited by, inorganic, synthetic, or biological surfaces, with modifications of their conformational states into non-native, aggregation-prone conformations. These considerations account for the increasing interest to investigate the physicochemical features of protein interaction with, and aggregation on, artificial or natural surfaces, particularly lipid bilayers, even in relation to the structure and lipid composition of the latter. This Perspective will focus on the importance of phospholipid bilayers (i) as modulators of protein misfolding and aggregation able to induce the growth of aggregates with conformational and physicochemical features different from those displayed by comparable aggregates arising in free solution and (ii) as key interaction sites of prefibrillar and fibrillar amyloid aggregates. The importance of both membrane and aggregate biophysical features for these effects and the ensuing membrane damage and cytotoxicity will also be considered. We will then propose a new view featuring amyloid cytotoxicity as a property that is not inherent to specific amyloids but, rather, that emerges from the reciprocal effects of the biophysical properties of both lipid bilayers and amyloid aggregates taken as a whole.

Amyloid Oligomers Preceding the Appearance of Mature Fibrils and Endowed with the Highest Cytotoxicity. Amyloid aggregation is a hierarchical process starting with misfolded/unfolded protein/peptide monomers whose structural features, molecular dynamics, and extent of exposed charged or hydrophobic surface depend, to a large extent, on the environmental conditions; the latter also determine monomer arrangement in the growing supramolecular structures. Amyloid fibrils appear as the end products of a sequential path that, similarly to folding, progresses through several variously defined steps. At variance with folding, intermediate oligomeric/polymeric entities can be stable enough to be monitored and, in the most favorable cases, studied (Figure 2).

The heterogeneous and more or less defined entities that precede the appearance of well-defined mature fibrils are generically indicated as prefibrillar aggregates. Prefibrillar assemblies of amyloid type occur in vitro and/or in vivo in the aggregation path of many proteins and peptides associated or not with amyloid diseases. Prefibrillar aggregates include the aggregation nuclei, whose formation is needed for aggregation to start and is often the rate-limiting step of fibril growth, explaining the delay time of polymer appearance recorded in in vitro protein aggregation experiments. In most cases, amyloid oligomers represent a more advanced stage even though they can also coincide with the aggregation nuclei. Higher-order entities including tiny annular assemblies (when present), curvy beaded protofibrils, or short fibrils appear later and precede the appearance of mature fibrils. In most cases, different amyloids at various stages of maturation can coexist in the same sample. Even though most of these intermediates occur in the path of fibril formation, others, such as small annular oligomers and curvy beaded protofibrils, can be off-pathway dead end products of the process or, at any rate, structurally and

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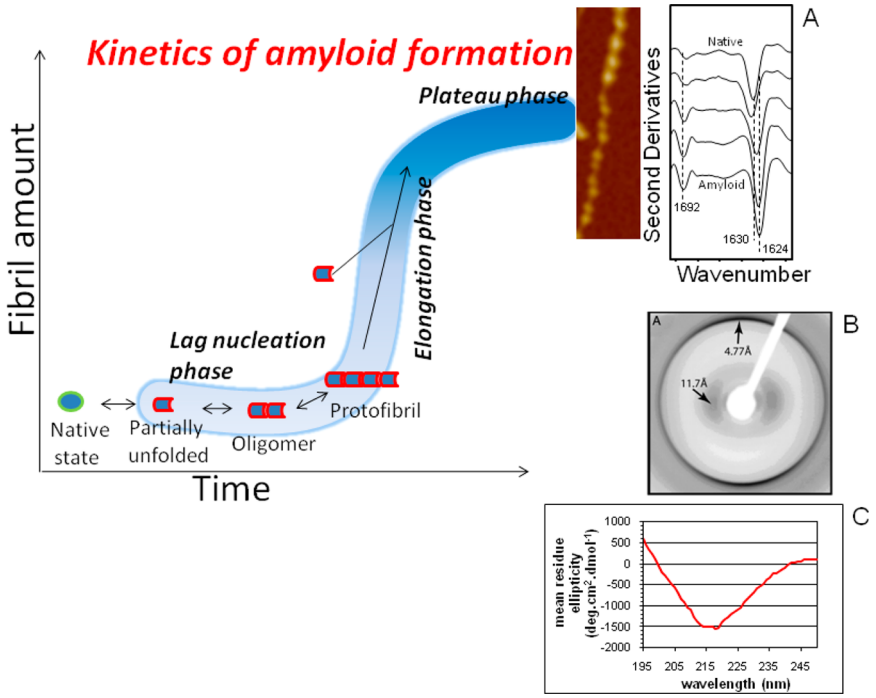

Figure 2. (Left) The sigmoidal profile of amyloid fibrils growth. Amyloid growth consists of two phases, (i) a lag nucleation phase, in which monomers undergo a conformational change/misfolding and associate to form oligomeric nuclei, and (ii) an elongation phase, in which the nuclei rapidly grow by further addition of monomers and form larger fibrils until saturation. The lag nucleation phase is thermodynamically disfavored and occurs gradually, whereas the elongation phase is much more favored and proceeds quickly. Thus, the kinetics of amyloid formation are best represented by an S-shaped curve with a lag phase followed by a fast growth phase. (Right) (A) Second derivatives of the absorption spectra of (i) a native protein with amide I maxima at around 1630 cm^{-1} arising from the β-sheet structure of the native protein and (ii) a typical amyloid fibril with amide I maxima down-shifted to ~1624 cm^{-1}. (B) X-ray diffraction of a mature amyloid fibril. (C) Circular dichroism spectrum of amyloid fibrils with a high content of β-structure.

functionally distinct types of amyloids often devoid of cytotoxicity with no further evolution.[2]

Soluble amyloid oligomers of several peptides and proteins have been repeatedly detected in, and purified from, cultured cells and tissues where the monomeric precursors are expressed;[10−16] these findings have reinforced the idea that those species are really present in vivo and that their cytotoxicity is directly associated with cell/tissue impairment (see later). However, differently from mature fibrils, a severe lack of knowledge on the overall structural features of fibril precursors still remains, even though significant progress in the elucidation of their biophysical and conformational features has been recently made.[17] Actually, some of the energy minima occurring in the protein aggregation energy landscape are expected to be scarcely defined due to the broad heterogeneity and instability of rapidly interconverting oligomeric states with similar free energies. Conversely, the energy minima of the more structurally defined and stable higher-order polymers (protofilaments and mature fibrils) can be much more easily identifiable. Finally, the same protein can organize into oligomers with different conformations growing into fibrils with differing morphologies and structural features under variable solution conditions both in vitro and in tissue (amyloid polymorphism; see later).[18,19]

In the past few years, a number of studies have started to shed light on the conformational and physicochemical properties of prefibrillar aggregates, and it can be expected that these studies will open the way to more intense efforts. For example, a quantitative kinetic model for the amyloid aggregation of β-lactoglobulin has been reported recently, together with a proposed aggregation energy landscape for this protein.[20] The study was a simulation exploiting a combination of atomic force microscopy, particle size distribution, 1-anilino-8-naphthalene sulfonate, thioflavin T, and dynamic light scattering measurements. Moreover, a recent SAXS study carried out on α-synuclein oligomers has shown the presence of wreath-like assemblies with a central channel; those data suggest the possibility that a pore can be formed, thus explaining the ability of these oligomers to permeabilize synthetic or biological membranes.[21] Finally, a recent paper on a segment of the amyloid-forming protein αB crystallin describes at the atomic level a toxic, amyloid-related, oligomeric, cylindrically shaped β-barrel (cylindrin) likely to be grown off-pathway to fiber formation.[22] More recently, other authors used the Rosetta-Profile method to check whether other segments of amyloid proteins could be threaded to assume the cylindrin structure.[23] The X-ray-derived atomic structure, showing a supramolecular assembly resulting from six antiparallel β-strands, was proposed by the authors as a common structural core of amyloid oligomers.[22] Finally, significant data on the biophysical features of different types of amyloid oligomers have been reported in the past few years (see later).[24,25]

Phospholipid Bilayers and Biological Membranes Promoting Protein Aggregation. The huge surface extension in the intra/extracellular environment (macromolecules, supramolecular assemblies, lipid bilayers) can influence profoundly protein folding, favoring, in some cases, the tendency of a protein to self-organize into alternative conformations, resulting in aggregate nucleation.[26] Indeed, in the adsorbed state, the

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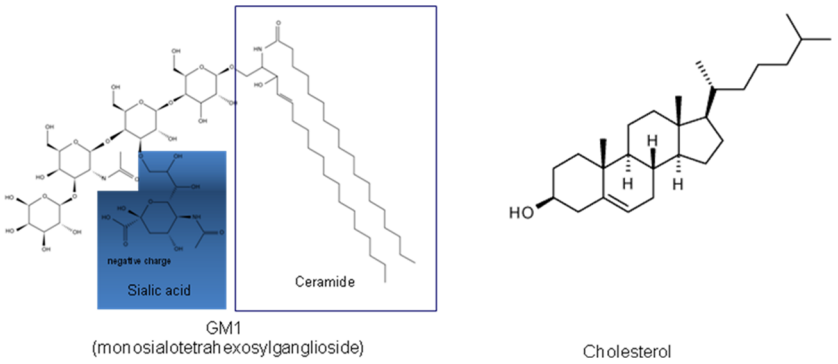

Figure 3. Structure of ganglioside GM1 and cholesterol. The sialic acid portion of the GM1 molecule carrying the negative charge is highlighted.

interaction of specific residues of a folded protein with surface-exposed hydrophobic or charged groups can induce local or more extensive unfolding, populating non-native, aggregation-prone conformations.[9] The structure of many protein molecules is loosened at these conditions, where native electrostatic contacts can be weakened, the peptide backbone becomes exposed, and/or normally buried nonpolar groups can interact with surface-exposed hydrophobic clusters. The resulting misfolded/partially unfolded polypeptide chain can be different and more favored energetically with respect to that present in the bulk solution or resulting from exposure to mild destabilizing conditions. Such a view has led to propose surfaces as conformational catalysts that can recruit proteins and then favor their misfolding, amyloid nucleation, and fibril growth.[27,28]

A large body of studies has shown that basic physicochemical properties of a surface, such as net electrostatic charge and hydrophilicity/hydrophobicity, can affect the conformational properties of the interacting peptide/protein.[29−31] In the case of lipid bilayers, the factors inherently favoring aggregation of a peptide/protein are modulated by the lipid composition, which can generate a wide range of different surface environments differing in terms of hydrophilicity/hydrophobicity, electrostatic charge, polarity, fluidity, curvature, and lateral pressure, which can result in lipid asymmetry and the formation of ordered phase systems embedded into a disordered lipid environment.[32] Actually, protein fibrillization can be modulated by a variety of factors, in particular, by lipid−protein interactions. A wealth of experimental evidence provides support to the notion that amyloid fibril assembly and the toxicity of the resulting prefibrillar aggregates are either closely related or membrane-associated phenomena. Depending on its lipid content, the membrane environment can enhance fibril formation in several ways, (i) by actively recruiting a protein, thus increasing its local concentration, (ii) by favoring its structural conversion into a partially folded/misfolded conformation, (iii) with an aggregation-prone orientation, and (iv) with a modulation of the depth that it penetrates in the bilayer, which affects its nucleation propensity (reviewed in ref 33). In particular, several reports have highlighted the key role played by anionic surfaces and anionic phospholipid-rich synthetic or biological membranes as triggers of protein/peptide misfolding and aggregation,[29,34−36] promoting the formation of β-sheet-rich structures and acting as conformational catalysts for amyloids.[37,38] Accordingly, it has been proposed that membranes rich in anionic phospholipids such as phosphatidylserine (PS), phosphatidylglycerol, phosphatidylinositol, and cardiolipin can interact with amyloid aggregates possibly by recognizing a shared fold.[38] These and other data have led to the conclusion that both electrostatic and hydrophobic protein−lipid interactions appear to modulate the aggregation behavior of peptides/proteins when bound to lipid bilayers, as shown for lysozyme.[39]

Finally, membrane fluidity can also affect the efficiency with which a polypeptide chain interacts with, misfolds, and aggregates on a bilayer.[40] Fluidity depends not only on the content of cholesterol, which acts as a "glue" stiffening the bilayer, but also on fatty acid composition, being increased by unsaturated fatty acid chains that reduce lipid packing and increase lipid disorder. This is clearly shown by several studies including two recently reported on α-synuclein and the Aβ peptide. In the case of α-synuclein, it was shown that the perturbation of the acyl chain packing resulting from protein adsorption favors its association with unsaturated phospholipids.[41] Another study reported that, in addition to the surface charge, the lipid tail type is also a key determinant of Aβ42 transmembrane stability, which is promoted by zwitterionic surfaces and unsaturated lipids.[42] Finally, it must be considered that membrane functional activities can be modulated by protein−lipid interactions and that amyloid growth into[43] or amyloid adsorption to lipid bilayers can heavily damage membranes by extracting membrane lipids and modifying profoundly membrane stability and order, lipid packing, and hydration at the interfacial region, as was shown in the case of β2-microglobulin[44] and lysozyme.[45]

Lipid Composition Affecting the Efficiency with Which a Lipid Bilayer Promotes Misfolding and Aggregation of a Polypeptide Chain. GM1 (Figure 3), the most abundant ganglioside found in lipid rafts, is particularly enriched in neuronal cell membranes. In normal conditions, it is the most abundant negatively charged lipid of the outer leaflet of the membrane bilayer of most cells, particularly neuronal cells. Gangliosides found in lipid rafts and the rafts themselves are involved in several neurodegenerative diseases, including prion, Parkinson's, Huntington, and Alzheimer's diseases.[46,47] The role of GM1 not only in maintaining the proper functional state of neuronal cells but also as a factor favoring protein aggregation and amyloid cytotoxicity has been widely studied.[48,49] In particular, it has been recently reported that Aβ is bound to GM1 in the brains of Alzheimer's disease patients, leading to propose that the GM1−Aβ complex could act as a seed for Aβ aggregation.[50] The negative charge carried by the sialic acid moiety of GM1 plays an important role in the stabilization of the monomeric and clustered GM1 conformation;[38] cholesterol seems to affect GM1 arrangement into the membrane and to favor its tendency to cluster into patches with intense negative

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electrostatic potential, stabilized by the formation of an extensive hydrogen bond network among the lipid head groups.[50−52] Recent data indicate that changes in ganglioside composition can alter cell behavior and that a reduction of the content of cholesterol results in increased GM1 expression,[53] thus contributing to explain the increased vulnerability to amyloids of cholesterol-poor cells.[54]

Clustered GM1 has been shown to favor the formation of the GM1−Aβ complex[55,56] and to be a potent inductor of protein misfolding and aggregation;[47,50] it has also been described as a major site of amyloid interaction with the cell membrane.[56,57] Intriguingly, a recent molecular dynamics simulation study has shown that in GM1 clusters on membrane, the negatively charged sialic acid component appears at regular intervals of around 1.4 nm, a value that is double the distance between the amide nitrogen atoms of the β-strands in the amyloid fold.[51] These data suggest that Aβ peptides in the β-strand conformation recognize the negative charges carried by the sialic acid residues aligned at a proper interval and/or that Aβ peptides in the native disordered conformation acquire a regular conformation enriched in β-structure upon interaction with such a pattern of regularly spaced negative charges. This consideration is supported by recent data indicating that negatively charged glycosaminoglycans (GAGs), such as heparin, are potent inductors of β-strand conformation and aggregation for a number of peptides and proteins.[58−60] A negatively charged sulfate group spacing in the 0.61−0.73 nm range, complementary to that (0.58−0.67 nm) of positively charged groups in aggregating amylin[61] and TTR,[62] has been related to the ability of heparin to promote the aggregation of these polypeptides. A scaffolding effect of the GAG polymer on oligomer recruitment has also been suggested, and the conformational plasticity of GAG chains could facilitate such a template effect.[63] A similar mechanism can be supposed for the reported proaggregation power of nucleic acids.[64]

Another important lipid player affecting membrane efficiency to recruit and unfold proteins/peptides and to nucleate amyloid growth[65] is cholesterol (Figure 3), a major membrane component particularly enriched in lipid rafts where, among others, it modulates the conformational state of GM1 (see above). Even though some data suggest that Aβ toxicity is associated with the formation of oligomeric-Aβ/cholesterol domain complexes in the cell membrane,[56,66,67] several pieces of evidence support a protective role of increased cholesterol against toxicity of preformed amyloids. Cholesterol also results in higher membrane rigidity, increased membrane insertion of the interacting peptide with reduced fibrillization, and inhibition of β-sheet formation.[68−70] Moreover, reduced levels of cholesterol are found in brains from Alzheimer's disease (AD) patients, and a loss of cholesterol in the brain leads to neurodegeneration.[71] Recent reports also show that loss of cholesterol in neuronal membranes enhances amyloid peptide generation[72] and that membrane enrichment in cholesterol reduces the toxic interaction of cells with prefibrillar aggregates supplemented to the culture media.[54]

The relation among cholesterol, protein aggregation, and aggregate toxicity is quite complex and is the subject of ongoing discussion; in fact, it includes several different effects with contrasting outcomes in terms of amyloid cytotoxicity.[73,74] However, where the cytotoxicity of preformed amyloids is concerned, it appears likely that the interaction with, and insertion into, the cell membrane of prefibrillar species, the first event of amyloid toxicity, is hindered by a high membrane rigidity provided by cholesterol. This theme is made even more complex considering that other lipids, often associated with cholesterol in lipid rafts, such as gangliosides, notably GM1 (see above), may have opposing effects, favoring amyloid aggregation, aggregate recruitment at the cell membrane, and cytotoxicity; furthermore, it must be considered that, as specified above, in the cell membrane, cholesterol affects GM1, conformational features,[50−52] and levels.[53]

The data on the aggregation-promoting effect of cholesterol and GM1 depict a scenario where the two lipids, taken separately, display somehow opposite effects at least in terms of amyloid recruitment at the cell membrane (see also later). Our recent data confirm this view, clearly supporting GM1 content as a major modulator of amyloid aggregate recruitment at the cell membrane and cytotoxicity;[46,75,76] conversely, amyloid interaction with GM1, notably with its negatively charged sialic acid moiety, reduces its lateral mobility and clustering and induces abnormal accumulation and overstabilization of raft domains.[56] These data support the view describing the interaction of fibrillar assemblies with the cell membrane as the contact of two surfaces driven by electrostatic forces, resulting in mild lateral displacement of molecules and domains within the membrane fluid mosaic. Taken together, the data on the effect of membrane cholesterol and gangliosides on protein/peptide misfolding and aggregation and oligomer interaction with the cell membrane further support the importance of lipid rafts as key sites where these processes occur and hence as major players in neurodegeneration.[46] They also provide a possible rationale for the acute vulnerability of neuronal cells to amyloids, considering the large surface area of these cells and the raft enrichment in their plasma membranes.

Amyloid Polymorphisms. Despite the increasing awareness that mature amyloid fibrils can display a direct or indirect toxic potential, both in tissue and to cultured cells,[46,77−79] amyloid oligomers, either grown from monomers or leaked from mature fibrils, are still considered the amyloid entities endowed with the highest direct cytotoxicity. Therefore, the ability of differing conditions to generate conformationally different oligomers with different biophysical features and variable cytotoxicity (oligomer polymorphism) appears of particular importance.

In the case of the Aβ peptides, the most studied system, many data support the idea that Aβ interaction with the cell membrane and the way that it occurs[9] imply peptide conformational changes and are important in determining its toxicity;[80] therefore, it has been proposed that the toxicity of any Aβ species can, at least in part, be related to its ability to change structure and to aggregate on, or within, the cell membrane and, hence, ultimately, to the flexibility and relative stability of both the peptide and the bilayer. Similar conclusions have been drawn in a study concerning the conformational features and toxicity of cultured cells and animal tissue of two types of amyloid assemblies grown at two different temperatures from the peptide encoded by the huntingtin exon-1 with an expanded polyglutamine stretch.[81] The study suggests that the same protein in different brain areas experiences conditions that modulate differently its stability, favoring aggregation into fibrils with variable biophysical features and biological properties. Following this line of evidence, it has been reported that soluble 30−50 nm sized annular α-synuclein oligomers are released by mild detergent treatment of glial inclusions from the brain tissue of people affected by a specific α-synucleinopathy, whereas comparable aggregates of recombi-

nant α-synuclein yielded only spherical oligomers upon similar treatment.[82]

This new view supports the concept that, although relying on basic physicochemical properties of amyloid oligomers and implying the initial interaction with the cell membrane, cytotoxicity is not a property inherent to a specific type of aggregate but, rather, an emerging one, stemming from the characteristics of the oligomer−membrane complex taken as a whole.

Our recent results on HypF-N, a bacterial protein not associated with any amyloid disease, further confirm and extend the generality of these considerations, providing clues on the structural features of amyloid oligomers and their relationship to toxicity. In fact, at different destabilizing conditions (acidic pH or reduced medium polarity), HypF-N generates apparently similar, yet conformationally different, oligomers with differing relative stability, biophysical features, and toxicity.[83] The more hydrophobic, less stable oligomers (arising in the presence of trifluoroethanol) grow into mature fibrils, whereas the less hydrophobic, more stable ones (grown in the presence of trifluoroacetic acid) eventually assemble into stable curvy protofibrils with no further evolution. The two types of oligomers display differing cytotoxicity and the ability to interact with, to permeabilize, and to cross the plasma membrane of exposed cells both in culture and in tissue;[24,83] however, toxicity and the ability to trigger apoptosis are associated only with the less compact, less stable, and more hydrophobic assemblies.[24] Our findings have been supported and, possibly, generalized by more recent reports on Aβ peptides,[84,85] α-synuclein,[86,87] and Sup35, a yeast prion.[88] Taken together, all of these data establish a direct link between protein aggregation conditions, general structural features, stability, and cytotoxicity of the resulting prefibrillar assemblies and their ability to grow into distinct, stable amyloid fibrils. They also provide clues on the relationship between oligomer conformational (molecular dynamics), biophysical (hydrophobic or charged surface exposure, flexibility), and stability (compactness) properties and their ability to stick to, to disassemble, and to permeabilize the cell membrane impairing cell viability.

However, it is important to emphasize that, in general, toxicity does not reside in one or a limited number of oligomeric forms of a given protein; in fact, different oligomers grown from the same protein have been shown to affect, to some degree, cell viability (see above). The general emerging picture is that oligomers cannot be described as a finite number of peptide/protein structures defined by a specific set of parameters. Rather, they are best described as a number of conformational ensembles, each comprising an indefinite number of highly dynamic different assemblies. As far as aggregation proceeds, the increasing size, stability, compactness, β-sheet regularity, and hydrophobic burial reduce dynamic fluctuations, hydrophobic exposure, and thus oligomer structural heterogeneity and toxicity.[24,87−90]

Oligomer−Cell Membrane System. From the data reported in the previous sections, it emerges that the role of surfaces, notably phospholipid bilayers and biological membranes, in amyloid growth and cytotoxicity is double; from one side, membranes can recruit, misfold, and aggregate peptides and proteins with ensuing membrane damage; from the other side, preformed amyloids generated elsewhere can interact with membranes, determining their disassembly and permeabilization. The data so far reported have clearly provided two lines of evidence describing the molecular basis of amyloid interaction with cell membranes. The first indicates that the conformational and biophysical properties of amyloid oligomers and other prefibrillar aggregates are major determinants of their toxicity, which basically results from the initial interaction of the aggregates with the cell membrane; the latter observation appears true for aggregates grown either from different peptides/proteins or arisen from the same peptide/protein at different environmental conditions and hence with polymorphic features. The second line of evidence highlights a strict dependence of the ability of amyloids to interact with phospholipid bilayers on basic physicochemical features of the bilayer itself; these arise from the lipid composition of the bilayer and include the extent of exposed charged surface, fluidity, curvature, lipid disorder, lateral pressure, and others. Taken together, the two bodies of data described above suggest that there should be a comprehensive overall effect resulting from both the aggregate and the membrane biophysical features that determines the final outcome in terms of cell sufferance and death.

This Perspective is supported by our most recent data on the relative cytotoxicity of the two types of HypF-N oligomers described above, when administered to cultured cells treated so as to display different membrane properties arising from normal, increased, or reduced content of cholesterol.[57] We found that the actual level of cytotoxicity of either oligomer was indeed modulated by the lipid composition and biophysical features of the cell plasma membrane.[57] In particular, increasing membrane cholesterol made safe the oligomers toxic to untreated cells, whereas in cells with decreased membrane cholesterol, the oligomers nontoxic to untreated cells became cytotoxic (Table 2). Opposite effects were found following modulation of the content of membrane GM1. Finally, increasing both cholesterol and GM1 resulted in a net effect that was substantially related to the GM1 (but not the cholesterol) content, suggesting that this lipid plays a remarkable role.[57] These data agree with recent findings showing that GM1, mainly in its clustered form, is a major binding site of β-sheet-rich amyloids but that its clustering, conformational features, and binding efficiency depend on the physicochemical properties of the bilayer and are modulated by the presence of cholesterol and other lipids.[51,56]

Besides establishing a more complex link between oligomer/membrane conformational and biophysical properties and the resulting toxic effects to exposed cells, these data also provide a rationale contributing a possible explanation of the different vulnerability to the same amyloids of different cell types.[25,54,91] These ideas also provide new clues to describe the molecular determinants of sporadic amyloid diseases, where the aggregating proteins/peptides do not display any mutation, as well as the known lack of a direct relationship between amyloid load in tissue and the severity of the clinical signs in

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Table 2. Oligomer Toxicity that Depends on the Membrane Biophysical Features and Is Mainly Determined by the Content of GM1[a]

Oligomer type	Membrane cholesterol / Membrane GM1 / Both: Low	Basal	High
A. *Loose, with high hydrophobic exposure*	Highly toxic	Toxic	Non-toxic
	Non-toxic	Toxic	Higly toxic
	Non-toxic		Higly toxic
B. *Compact, with low hydrophobic exposure*	Toxic	Non-toxic	Non-toxic
	Non-toxic	Non-toxic	Toxic
	Non-toxic		Toxic

[a]Membrane lipid composition is a key determinant of the relative toxicity of amyloid oligomers. In fact, membranes poor or rich in cholesterol tend to be highly vulnerable or resistant, respectively, to amyloid oligomers independently of the structural features of the latter. The opposite is true for GM1, which, in addition, appears to dictate the main effect (see squares). Conversely, the vulnerability of membranes with a physiological content of cholesterol and GM1 appears to depend on oligomer structural features.

neurodegenerative diseases.[92] This new view supports the concept that, although relying on basic physicochemical properties of amyloid oligomers and implying the initial interaction with the cell membrane, cytotoxicity is not a property inherent to a specific type of aggregate but, rather, an emerging one, stemming from the characteristics of the oligomer−membrane complex taken as a whole.

We are still at the beginning of the way to describe in detail the oligomer conformations, how they are reached, the effect of different protein structure and environmental conditions on their polymorphism, and the biochemical, biophysical, and cellular basis of their cytotoxicity. However, the intense research carried out both in vitro, with cultured cells, and in tissue is likely to provide further information in this field in the near future. A more solid knowledge of the oligomer−cell membrane system will represent an important contribution to establish new therapeutic approaches and to suggest rational drug design against oligomer growth and cytotoxicity.

Discussion and Future Directions. The body of knowledge accumulated in the last 15 years has provided some solid knowledge on key aspects of amyloid aggregation, aggregate growth, and structural features as well as on the ability of amyloid aggregates to impair cell function and viability. The hallmarks of such information include, among others, the notion that amyloid toxicity resides mainly in the assemblies arising early in the path of aggregation, notably oligomeric entities (even though mature fibrils can also be toxic in several ways, comprising leakage of toxic oligomers); the idea that oligomers are toxic no matter whether they have been grown from peptides/protein associated or not associated with disease is also of importance. Thus, amyloid cytotoxicity appears generic, depending on shared conformational/biophysical features of the supramolecular structure of the aggregates rather than on some specific property of the amino acid sequence of aggregated monomers. Another step forward in the amyloid science was the awareness that amyloid polymorphism can be a key issue to explain the relation between the aggregation conditions and the conformational, biophysical, and cytotoxicity features of the resulting amyloid assemblies. More recently, data have started to appear providing significant information on some conformational and biophysical features of amyloid oligomers. However, getting solid information on the latter is made difficult by the heterogeneity, remarkable instability, and intrinsically disordered nature of these species. An important contribution has been provided by the use of antibodies raised against amyloid fibrils and their precursors, which are able to recognize specific structural features displayed by different forms of these assemblies and hence to discriminate among them.[93] More recently, molecular dynamics simulations coupled to solid-state NMR and recently developed biophysical and single-molecule techniques have expanded the tools available to get new and more reliable data on the amyloid conformational features and their relation to cytotoxicity. These seminal studies are providing key information on the relation between the biophysical and conformational features of amyloid assemblies, even when they were grown from the same molecule at different conditions, and the ensuing ability to impair cell function and viability. More knowledge in this field is still needed, but intense research warrants increased information to be obtained in the near future.

Recently reported seminal data highlight the importance of the content of cholesterol and gangliosides, notably GM1; the two lipids appear to exert opposite effects where cell vulnerability to the same amyloid is concerned.

The information on the effect of surfaces, notably membranes, on protein/peptide misfolding and aggregation, as well as on aggregate cytotoxicity has undergone parallel growth. Presently, convincing data on the relation between the composition of a phospholipid bilayer and its ability to misfold and to aggregate polypeptide chains as well as to recruit assemblies grown elsewhere are clearly depicting the important role of the cell membrane in amyloid growth and cytotoxicity. However, even in this case, a deeper knowledge is needed. For example, it is not clear in many cases whether amyloid toxicity at the cell membrane is mediated by a mere phospholipid disassembly or by some interaction with, and deregulation of, specific ion (particularly Ca^{2+}) channels, signaling receptors, or both. It appears that in different cases, differing mechanisms can be involved; however, the relative contribution of each of them to the final functional and viability derangement of the exposed cells is not clearly established. Finally, recent results indicate that a direct relation does exist between the biophysical properties of the interacting membrane, arising from its lipid composition, and those of toxic amyloids resulting from their conformational features. Data are starting to appear showing that amyloid aggregates display a toxic power that is not absolute but depends strictly on the properties of the membrane bilayer with which they interact, featuring the oligomer−membrane system as a whole complex whose overall physicochemical properties determine the extent of the toxic effect. Recently reported seminal data highlight the importance of the content of cholesterol and gangliosides, notably GM1; the two lipids appear to exert opposite effects where cell vulnerability to the same amyloid is concerned. In particular, an

increasing body of data on the effect of GM1 and its clusters of negative charge on protein/peptide destabilization, oligomer recruitment, and aggregation promotion has been accumulating together with increasing information on the modulation by cholesterol and other membrane lipids of GM1 clustering and binding behavior. These data, when added to those relative to the aggregation-promoting effect of negatively charged GAGs and nucleic acids, suggest a basic shared molecular mechanism of charge-induced protein/peptide aggregation relying on proper content and ordered spacing of the negative charges on the polymer or, in the case of GM1, its clusters. However, more information on these effects as well as on those provided by other important membrane lipids such as sphingomyelins and ceramides, and their importance in disease pathogenesis, is needed together with a more comprehensive knowledge of the role of the ordered lipid domains, including lipid rafts and ceramide platforms, as modulators of oligomer cytotoxicity.

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REFERENCES

(1) Glabe, C. G. Structural Classification of Toxic Amyloid Oligomers. *J. Biol. Chem.* **2008**, *283*, 29639−29643.

(2) Stefani, M.; Dobson, C. M. Protein Aggregation and Aggregate Toxicity: New Insights into Protein Folding, Misfolding Diseases and Biological Evolution. *J. Mol. Med.* **2003**, *81*, 678−699.

(3) Stefani, M. Protein Misfolding and Aggregation: New Examples in Medicine and Biology of the Dark Side of the Protein World. *Biochim. Biophys. Acta* **2004**, *1739*, 5−25.

(4) Baldwin, A. J.; Knowles, T. P.; Tartaglia, G. G.; Fitzpatrick, A. W.; Devlin, G. L.; Shammas, S. L.; Waudby, C. A.; Mossuto, M. F.; Meehan, S.; Gras, S. L.; Christodoulou, J.; Anthony-Cahill, S. J.; Barker, P. D.; Vendruscolo, M.; Dobson, C. M. Metastability of Native Proteins and the Phenomenon of Amyloid Formation. *J. Am. Chem. Soc.* **2011**, *133*, 14160−14163.

(5) Fitzpatrick, A. W.; Knowles, T. P. J.; Waudby, C. A.; Vendruscolo, M.; Dobson, C. M. Inversion of the Balance Between Hydrophobic and Hydrogen Bonding Interactions in Protein Folding and Aggregation. *PLoS Comput. Biol.* **2011**, *7*, e1002169.

(6) Dobson, C. M. Protein Folding and Misfolding. *Nature* **2003**, *426*, 884−890.

(7) Tartaglia, G. G.; Vendruscolo, M. Proteome-Level Interplay between Folding and Aggregation Propensities of Proteins. *J. Mol. Biol.* **2010**, *462*, 919−928.

(8) Chiti, F.; Dobson, C. M. Amyloid Formation by Globular Proteins under Native Conditions. *Nat. Chem. Biol.* **2009**, *5*, 15−22.

(9) Bokvist, M.; Lindstrom, F.; Watts, A.; Grobner, G. Two types of Alzheimer's β-Amyloid (1−40) Peptide−Membrane Interactions: Aggregation Preventing Trans Membrane Anchoring versus Accelerated Surface Fibril Formation. *J. Mol. Biol.* **2004**, *335*, 1039−1049.

(10) Bucciantini, M.; Giannoni, E.; Chiti, F.; Baroni, F.; Formigli, L.; Zurdo, J.; Taddei, N.; Ramponi, G.; Dobson, C. M.; Stefani, M. Inherent Toxicity of Aggregates Implies a Common Mechanism for Protein Misfolding Diseases. *Nature* **2002**, *416*, 507−511.

(11) Goldberg, A. L. Protein Degradation and Protection against Misfolded or Damaged Proteins. *Nature* **2003**, *426*, 895−899.

(12) Paravastu, A. K.; Qawash, I.; Leapman, R. D.; Meredith, S. C.; Tycko, R. Seeded Growth of β-Amyloid Fibrils from Alzheimer's Brain-Derived Fibrils Produces a Distinct Fibril Structure. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *105*, 7443−7448.

(13) Walsh, D. M.; Klyubin, I.; Fadeeva, J. V.; Cullen, W. K.; Anwy, L. R.; Wolfe, M. S.; Rowan, M. J.; Selkoe, D. J. Naturally Secreted Oligomers of Amyloid β Protein Potently Inhibit Hippocampal Long-Term Potentiation In Vivo. *Nature* **2002**, *416*, 535−539.

(14) Billings, L. M.; Oddo, S.; Green, K. N.; McGaugh, J. L.; LaFerla, F. M. Intraneuronal Aβ Causes the Onset of Early Alzheimer's Disease-Related Cognitive Deficits in Transgenic Mice. *Neuron* **2005**, *45*, 675−688.

(15) Koffie, R. M.; Meyer-Luehmann, M.; Hashimoto, T.; Adams, K. W.; Mielke, M. L.; Garcia-Alloza, M.; Micheva, K. D.; Smith, S. J.; Kim, M. L.; Lee, V. M. Oligomeric Amyloid β Associates with Postsynaptic Densities and Correlates with Excitatory Synapse Loss near Senile Plaques. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 4012−4017.

(16) Gurlo, T.; Ryazantsev, S.; Huang, C.-J.; Yeh, M. W.; Reber, H. A.; Hines, O. J.; O'Brien, T. D.; Glabe, C. G.; Butler, P. C. Evidence for Proteotoxicity in β Cells in Type 2 Diabetes, Toxic Islet Amyloid Polypeptide Oligomers form Intracellularly in the Secretory Pathway. *Am. J. Pathol.* **2009**, *176*, 861−869.

(17) Fändrich, M. Oligomeric Intermediates in Amyloid Formation: Structure Determination and Mechanisms of Toxicity. *J. Mol. Biol.* **2012**, *421*, 427−440.

(18) Relini, A.; Torrassa, S.; Rolandi, R.; Gliozzi, A.; Rosano, C.; Canale, C.; Bolognesi, M.; Plakoutsi, G.; Bucciantini, M.; Chiti, F. Monitoring the Process of HypF Fibrillization and Liposome Permeabilization by Protofibrils. *J. Mol. Biol.* **2004**, *338*, 943−957.

(19) Poirier, M. A.; Li, H.; Macosko, J.; Cail, S.; Amzel, M.; Ross, C. A. Huntingtin Spheroids and Protofibrils as Precursors in Polyglutamine Fibrillization. *J. Biol. Chem.* **2002**, *277*, 41032−41037.

(20) He, X.; Giurleo, J. T.; Talaga, D. S. Role of Small Oligomers in the Amyloidogenic Aggregation Free-Energy Landscape. *J. Mol. Biol.* **2010**, *395*, 134−154.

(21) Giehm, L.; Svergun, D. I.; Otzen, D. E.; Vestergaard, B. Low-Resolution Structure of a Vesicle Disrupting Alpha-Synuclein Oligomer that Accumulates During Fibrillation. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 3246−32451.

(22) Laganowsky, A.; Liu, C.; Sawaya, M. R.; Whitelegge, J. P.; Park, J.; Zhao, M.; Pensalfini, A.; Soriaga, A. B.; Landau, M.; Teng, P. K.; Cascio, D.; Glabe, C.; Eisenberg, D. Atomic View of a Toxic Amyloid Small Oligomer. *Science* **2012**, *335*, 1228−1231.

(23) Goldschmidt, I.; Teng, P. K.; Riek, R.; Eisenberg, D. Identifying the Amylome Proteins Capable of Forming Amyloid-Like Fibrils. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 3487−3492.
(24) Campioni, S.; Mannini, B.; Pensalfini, A.; Zampagni, M.; Parrini, C.; Evangelisti, E.; Relini, A.; Stefani, M.; Dobson, C. M.; Cecchi, C.; Chiti, F. The Causative Link Between the Structure of Aberrant Protein Oligomers and Their Ability to Cause Cell Dysfunction. *Nat. Chem. Biol.* **2010**, *6*, 140−147.
(25) Bolognesi, B.; Kumita, J. R.; Barros, T. P.; Esbjorner, E. K.; Luheshi, L. M.; Crowther, D. C.; Wilson, M. R.; Dobson, C. M.; Favrin, G.; Yerbury, J. J. ANS Binding Reveals Common Features of Cytotoxic Amyloid Species. *ACS Chem. Biol.* **2010**, *5*, 735−740.
(26) Stefani, M. Structural Features and Cytotoxicity of Amyloid Oligomers: Implication in Alzheimer's Disease and Other Diseases with Amyloid Deposits. *Prog. Neurobiol.* **2012**, *99*, 226−245.
(27) Sethuraman, A.; Belfort, G. Protein Structural Perturbation and Aggregation on Homogeneous Surfaces. *Biophys. J.* **2005**, *88*, 1322−1333.
(28) Zhu, J.; Nathan, C.; Jin, W.; Sim, D.; Ashcroft, G. S.; Wahl, S. M.; Lacomi, L.; Erdjument-Bromage, H.; Tempst, P.; Wright, C. D.; Ding, A. Conversion of Proepithelin to Epithelins: Roles of SLPI and Elastase in Host Defense and Wound Repair. *Cell* **2002**, *111*, 867−878.
(29) Necula, M.; Chirita, C.; Kuret, J. Rapid Anionic Micelle-Mediated α-Synuclein Fibrillization In Vitro. *J. Biol. Chem.* **2003**, *278*, 46674−46680.
(30) Hoyer, W.; Cherny, D.; Subramaniam, V.; Jovin, T. M. Impact of the Acidic C-Terminal Region Comprising Amino Acids 109−140 on Alpha-Synuclein Aggregation In Vitro. *Biochemistry* **2011**, *43*, 16233−16242.
(31) Arce, F. T.; Jang, H.; Ramachandran, S.; Landon, P. B.; Nussinov, R.; Lal, R. Polymorphism of Amyloid β Peptide in Different Environments: Implications for Membrane Insertion and Pore Formation. *Soft Matter* **2011**, *7*, 5267−5273.
(32) Kinnunen, P. K. Amyloid Formation on Lipid Membrane Surfaces. *Open Biol. J.* **2009**, *2*, 163−175.
(33) Gorbenko, G. P.; Kinnunen, P. K. The Role of Lipid−Protein Interactions in Amyloid-Type Protein Fibril Formation. *Chem. Phys. Lipids* **2006**, *141*, 72−82.
(34) Engel, M. F. M.; Yiggitop, H. A.; Eigersma, R. C.; Rijkers, D. T. S.; Liskamp, R. M. J.; de Kruijff, B.; Höppener, J. W. M.; Killian, A. Islet Amyloid Polypeptide Inserts into Phospholipid Monolayers as Monomer. *J. Mol. Biol.* **2006**, *356*, 783−789.
(35) Lee, G.; Pollard, H. B.; Arispe, N. Annexin 5 and Apolipoprotein E2 Protect against Alzheimer's Amyloid-β-Peptide Cytotoxicity by Competitive Inhibition at a Common Phosphatidylserine Interaction Site. *Peptides* **2002**, *23*, 1249−1263.
(36) Kayal, T. A.; Russo, E.; Pieri, L.; Caminati, G.; Berti, D.; Bucciantini, M.; Stefani, M.; Baglioni, P. Interactions of Lysozyme with Phospholipid Vesicles: Effects of Vesicle Biophysical Features on Protein Misfolding and Aggregation. *Soft Matter* **2012**, *8*, 9115−9126.
(37) Jayakumar, R.; Jayaraman, M.; Koteeswarl, D.; Gomath, K. Cytotoxic and Membrane Perturbation Effects of a Novel Amyloid Forming Model Peptide Poly(leucine-glutamic acid). *J. Biochem.* **2004**, *136*, 457−462.
(38) Zhao, H.; Tuominen, E. K. J.; Kinnunen, P. K. J. Formation of Amyloid Fibers Triggered by Phosphatidylserine-Containing Membranes. *Biochemistry* **2004**, *43*, 10302−10307.
(39) Gorbenko, G. P.; Ioffe, V. M.; Kinnunen, P. K. Binding of Lysozyme to Phospholipid Bilayers: Evidence for Protein Aggregation upon MembraneAssociation. *Biophys. J.* **2007**, *93*, 140−53.
(40) Kim, Y. J.; Takahashi, R. Role of Polyunsaturated Fatty Acids for Misfolding Protein Aggregations: Implication for Neurodegenerative Diseases. *Ann. N.Y. Acad. Sci.* **2006**, *1086*, 11−20.
(41) Hellstrand, E.; Grey, M.; Ainalem, M. L.; Ankner, J.; Forsyth, V. T.; Fragneto, G.; Haertlein, M.; Dauvergne, M. T.; Nilsson, H.; Brundin, P.; Linse, S.; Nylander, T.; Sparr, E. Adsorption of α-Synuclein to Supported Lipid Bilayers: Positioning and Role of Electrostatics. *ACS Chem. Neurosci.* **2013**, *4*, 1339−1351.
(42) Poojari, C.; Kukol, A.; Strodel, B. How the Amyloid-β Peptide and Membranes Affect Each Other: an Extensive Simulation Study. *Biochim. Biophys. Acta* **2013**, *1828*, 327−339.
(43) Yip, C. M.; Mclaurin, J. Amyloid-β Peptide Assembly: A Critical Step in Fibrillogenesis and Membrane Disruption. *Biophys. J.* **2001**, *80*, 1359−1371.
(44) Milanesi, L.; Sheynis, T.; Xue, W.-F.; Orlova, E. V.; Hellewell, A. L.; Jelinek, R.; Hewitt, E. W.; Radford, S. E.; Saibil, H. R. Direct Three-Dimensional Visualization of Membrane Disruption by Amyloid Fibrils. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 20455−20460.
(45) Kastorna, A.; Trusova, V.; Gorbenko, G.; Kinnunen, P. K. Membrane Effects of Lysozyme Amyloid Fibrils. *Chem. Phys. Lipids* **2012**, *165*, 331−337.
(46) Schengrund, C. L. Lipid Rafts: Keys to Neurodegeneration. *Brain Res. Bull.* **2010**, *82*, 7−17.
(47) Sanghera, N.; Correia, B. E.; Correia, J. R.; Ludwig, C.; Agarwal, S.; Nakamura, H. K.; Kuwata, K.; Samain, E.; Gill, A. C.; Bonev, B. B.; Pinheiro, T. J. Deciphering the Molecular Details for the Binding of the Prion Protein to Main Ganglioside GM1 of Neuronal Membranes. *Chem. Biol.* **2011**, *18*, 1422−1431.
(48) Maglione, V.; Marchi, P.; Di Pardo, A.; Lingrell, S.; Horkey, M.; Tidmarsh, E.; Sipione, S. Impaired Ganglioside Metabolism in Huntington's Disease and Neuroprotective Role of GM1. *J. Neurosci.* **2010**, *30*, 4072−4080.
(49) Yamamoto, N.; Matsubara, E.; Maeda, S.; Minagawa, H.; Takashima, A.; Maruyama, W.; Michikawa, M.; Yanagisawa, K. A Ganglioside-Induced Toxic Soluble Aβ Assembly. Its Enhanced Formation from Aβ Bearing the Arctic Mutation. *J. Biol. Chem.* **2007**, *282*, 2646−2655.
(50) Matsuzaki, K.; Kato, K.; Yanagisawa, K. Aβ Polymerization through Interaction with Membrane Gangliosides. *Biochim. Biophys. Acta* **2010**, *1801*, 868−877.
(51) Mori, K.; Mahmood, M. I.; Neya, S.; Matsuzaki, K.; Hoshino, T. Formation of GM1 Ganglioside Clusters on the Lipid Membrane Containing Sphingomyeline and Cholesterol. *J. Phys. Chem. B* **2012**, *116*, 5111−5121.
(52) Hayashi, H.; Kimura, N.; Yamaguchi, H.; Hasegawa, K.; Yokoseki, T.; Shibata, M.; Yamamoto, N.; Michikawa, M.; Yoshikawa, Y.; Terao, K.; Matsuzaki, K.; Lemere, C. A.; Selkoe, D. J.; Naiki, H.; Yanagisawa, K. A Seed for Alzheimer Amyloid in the Brain. *J. Neurosci.* **2004**, *24*, 4894−4902.
(53) Petro, K. A.; Schengrund, C. L. Membrane Raft Disruption Promotes Axonogenesis in n2a Neuroblastoma Eells. *Neurochem. Res.* **2009**, *34*, 29−37.
(54) Cecchi, C.; Baglioni, S.; Fiorillo, C.; Pensalfini, A.; Liguri, G.; Nosi, D.; Rigacci, S.; Bucciantini, M.; Stefani, M. Insights into the Molecular Basis of the Differing Susceptibility of Varying Cell Types to the Toxicity of Amyloid Aggregates. *J. Cell Sci.* **2005**, *118*, 3459−3470.
(55) Kakio, A.; Nishimoto, S.; Yanagisawa, K.; Kozutsumi, Y.; Matsuzaki, K. Interactions of Amyloid β-Protein with Various Gangliosides in Raft-Like Membranes: Importance of GM1 Ganglioside-Bound Form as an Endogenous Seed for Alzheimer Amyloid. *Biochemistry* **2002**, *41*, 7385−7390.
(56) Bucciantini, M.; Nosi, D.; Forzan, M.; Russo, E.; Calamai, M.; Pieri, L.; Formigli, L.; Quercioli, F.; Soria, S.; Pavone, F.; Savistchenko, J.; Melki, R.; Stefani, M. Toxic Effects of Amyloid Fibrils on Cell Membranes: The Importance of Ganglioside GM1. *FASEB J.* **2012**, *26*, 818−831.
(57) Evangelisti, E.; Cecchi, C.; Cascella, R.; Sgromo, C.; Becatti, M.; Dobson, C. M.; Chiti, F.; Stefani, M. Membrane Lipid Composition and Its Physicochemical Properties Define Cell Vulnerability to Aberrant Protein Oligomers. *J. Cell Sci.* **2012**, *125*, 2416−2427.
(58) Motamedi-Shad, N.; Garfagnini, T.; Penco, A.; Relini, A.; Fogolari, F.; Corazza, A.; Esposito, G.; Bemporad, F.; Chiti, F. Rapid Oligomer Formation of Human Muscle Acylphosphatase Induced by Heparan Sulfate. *Nat. Struct. Mol. Biol.* **2012**, *19*, 547−554 ; S1−2..
(59) Suk, J. Y.; Zhang, F.; Balch, W. E.; Linhardt, R. J.; Kelly, J. W. Heparin Accelerates Gelsolin Amyloidogenesis. *Biochemistry* **2006**, *45*, 2234−2242.

(60) Monsellier, E.; Ramazzotti, M.; Taddei, N.; Chiti, F. A Computational Approach for Identifying the Chemical Factors Involved in the GAGs-Mediated Acceleration of Amyloid Fibril Formation. *PLoS One* **2010**, *5*, e11363.
(61) Jha, S.; Patil, S. M.; Gibson, J.; Nelson, C. E.; Alder, N. N.; Alexandrescu, A. T. Mechanism of Amylin Fibrillization Enhancement by Heparin. *J. Biol. Chem.* **2011**, *286*, 22894−22904.
(62) Bourgault, S.; Solomon, J. P.; Reixach, N.; Kelly, J. W. Sulfated Glycosaminoglycans Accelerate Transthyretin Amyloidogenesis by Quaternary Structural Conversion. *Biochemistry* **2011**, *50*, 1001−1015.
(63) Valle-Delgado, J. J.; Alfonso-Prieto, M.; de Groot, N. S.; Ventura, S.; Samitier, J.; Rovira, C.; Fernàndez-Busquets, X. Modulation of Aβ42 Fibrillogenesis by Glycosaminoglycan Structure. *FASEB J.* **2010**, *24*, 4250−4261.
(64) Di Domizio, J.; Zhang, R.; Stagg, L. J.; Gagea, M.; Zhuo, M.; Ladbury, J. E.; Cao, W. Binding with NucleicAcids or GAGs Converts Soluble Protein Oligomers to Amyloid. *J. Biol. Chem.* **2012**, *287*, 736−747.
(65) Yip, C. M.; Elton, E. A.; Darabie, A. A.; Morrison, M. R.; McLaurin, J. Cholesterol, a Modulator of Membrane-Associated Aβ-Fibrillogenesis and Neurotoxicity. *J. Mol. Biol.* **2001**, *311*, 723−734.
(66) Qiu, L.; Lewis, A.; Como, J.; Vaughn, M. W.; Huang, J.; Somerharju, P.; Virtanen, J.; Cheng, K. H. Cholesterol Modulates the Interaction of β-Amyloid Peptide with Lipid Bilayers. *Biophys. J.* **2009**, *96*, 4299−42307.
(67) Koudinov, A. L.; Koudinova, N. V. Cholesterol Homeostasis Failure as a Unifying Cause of Synaptic Degeneration. *J. Neurol. Sci.* **2005**, *229*−*230*, 233−240.
(68) Ji, S. R.; Wu, Y.; Sui, S. F. Cholesterol Is an Important Factor Affecting the Membrane Insertion of β-Amyloid Peptide (Aβ 1−40), which May Potentially Inhibit the Fibril Formation. *J. Biol. Chem.* **2002**, *277*, 6273−6279.
(69) Micelli, S.; Meleleo, D.; Picciarelli, V.; Gallucci, E. Effect of Sterols on β-Amyloid Peptide (AβP 1−40) Channel Formation and Their Properties in Planar Lipid Membranes. *Biophys. J.* **2004**, *86*, 2231−2237.
(70) Qiu, L.; Buie, C.; Reay, A.; Vaughn, M. W.; Cheng, K. H. Molecular Dynamics Simulations Reveal the Protective Role of Cholesterol in β-Amyloid Protein-Induced Membrane Disruptions in Neuronal Membrane Mimics. *J. Phys. Chem. B* **2011**, *115*, 9795−9812.
(71) Arispe, N.; Doh, M. Plasma Membrane Cholesterol Controls the Cytotoxicity of Alzheimer's Disease Aβ(1−40) and (1−42) Peptides. *FASEB J.* **2002**, *16*, 1526−1536.
(72) Da Silva, J. S.; Hasegawa, T.; Miyagi, T.; Dotti, C. G.; Abad-Rodriguez, J. Asymmetric Membrane Ganglioside Sialidase Activity Specifies Axonal Fate. *Nat. Neurosci.* **2005**, *8*, 606−615.
(73) Stefani, M.; Liguri, G. Cholesterol in Alzheimer's Disease: Unresolved Questions. *Curr. Alzheimer Res.* **2009**, *6*, 15−29.
(74) Shobab, L. A.; Hsiung, G. Y.; Feldman, H. H. Cholesterol in Alzheimer's Disease. *Lancet Neurol.* **2005**, *4*, 841−852.
(75) Cecchi, C.; Nichino, D.; Zampagni, M.; Bernacchioni, C.; Evangelisti, E.; Pensalfini, A.; Liguri, G.; Gliozzi, A.; Stefani, M.; Relini, A. A Protective Role for Lipid Raft Cholesterol against Amyloid-Induced Membrane Damage in Human Neuroblastoma Cells. *Biochim. Biophys. Acta* **2009**, *1788*, 2204−2216.
(76) Cecchi, C.; Evangelisti, E.; Cascella, R.; Zampagni, M.; Benvenuti, S.; Luciani, P.; Deledda, C.; Cellai, I.; Wright, D.; Saccardi, R.; Peri, A.; Stefani, M. Neuronal Differentiation of Human Mesenchymal Stromal Cells Increases Their Resistance to Aβ42 Aggregate Toxicity. *J. Alzheimer's Dis.* **2011**, *27*, 651−664.
(77) Gharibyan, A. L.; Zamotin, V.; Yanamandra, K.; Moskaleva, O. S.; Margulis, B. A.; Kostanyan, I. A.; Morozova-Roche, L. A. Lysozyme Amyloid Oligomers and Fibrils Induce Cellular Death via Different Apoptotic/Necrotic Pathways. *J. Mol. Biol.* **2007**, *365*, 1337−1349.
(78) Novitskaya, V.; Bocharova, O. V.; Bronstein, I.; Baskakov, I. V. Amyloid Fibrils of Mammalian Prion Protein Are Highly Toxic to Cultured Cells and Primary Neurons. *J. Biol. Chem.* **2006**, *281*, 13828−13836.
(79) Engel, M. F.; Khemtémourian, L.; Kleijer, C. C.; Meeldijk, H. J.; Jacobs, J.; Verkeij, A. I.; de Kruijff, B.; Killan, J. A.; Höppener, J. K. Membrane Damage by Human Islet Amyloid Polypeptide through Fibril Growth at the Membrane. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 6033−6038.
(80) Mclaurin, J.; Franklin, T.; Fraser, P. E.; Chakrabarty, A. Structural Transitions Associated with the Interaction of Alzheimer's β-Amyloid Peptides with Gangliosides. *J. Biol. Chem.* **1997**, *273*, 4506−4515.
(81) Nekooki-Machida, Y.; Kurosawa, M.; Nukina, N.; Ito, K.; Oda, T.; Tanaka, M. Distinct Conformations of In Vitro and In Vivo Amyloids of Huntingtin-Exon 1 Show Different Cytotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 9679−9684.
(82) Pountney, D. L.; Voelcker, N. H.; Gai, W. P. Annular α-Synuclein Oligomers Are Potentially Toxic Agents in Alpha-Synucleinopathy. Hypothesis. *Neurotoxic. Res.* **2005**, *7*, 59−67.
(83) Tatini, F.; Pugliese, A. M.; Traini, C.; Niccoli, S.; Maraula, G.; Ed Dami, T.; Mannini, B.; Scartabelli, T.; Pedata, F.; Casamenti, F.; Chiti, F. Amyloid-β Oligomer Synaptotoxicity is Mimicked by Oligomers of the Model Protein HypF-N. *Neurobiol. Aging* **2013**, *34*, 2100−2109.
(84) Iijima, K.; Chiang, H. C.; Hearn, S. A.; Hakker, I.; Gatt, A.; Shenton, C.; Granger, L.; Leung, A.; Iijima-Ando, K.; Zhong, Y. Aβ42 Mutants with Different Aggregation Profiles Induce Distinct Pathologies in Drosophila. *PLoS ONE* **2008**, *3*, e1703.
(85) Ladiwala, A. R. A.; Litt, J.; Kane, R. S.; Aucoin, D. S.; Smith, S. O.; Ranjan, S.; Davis, J.; Van Nostrand, W. E.; Tessier, P. M. Conformational Differences between Two Amyloid β Oligomers of Similar Size and Dissimilar Toxicity. *J. Biol. Chem.* **2012**, *287*, 24765−24773.
(86) Cremades, N.; Cohen, S. I.; Deas, E.; Abramov, A. Y.; Chen, A. Y.; Orte, A.; Sandal, M.; Clarke, R. W.; Dunne, P.; Aprile, F. A.; Bertoncini, C. W.; Wood, N. W.; Knowles, T. P.; Dobson, C. M.; Klenerman, D. Direct Observation of the Interconversion of Normal and Toxic Forms of α-Synuclein. *Cell* **2012**, *148*, 1048−1058.
(87) Guo, J. L.; Covell, D. J.; Daniels, J. P.; Iba, M.; Stieber, A.; Zhang, B.; Riddle, D. M.; Kwong, L. K.; Xu, Y.; Trojanowski, J. Q.; Lee, V. M. Distinct α-Synuclein Strains Differentially Promote Tau Inclusions in Neurons. *Cell* **2013**, *154*, 103−117.
(88) Krishnan, R.; Goodman, J. L.; Mukkopadhyay, S.; Pacheco, C. D.; Lemke, E. A.; Deniz, A. A.; Lindquist, S. Conserved Features of Intermediates in Amyloid Assembly Determine Their Benign or Toxic States. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 11172−11177.
(89) Bemporad, F.; Chiti, F. Protein Misfolded Oligomers: Experimental Approaches, Mechanism of Formation, and Structure−Toxicity Relationships. *Chem. Biol.* **2012**, *19*, 315−327.
(90) Fantini, J.; Yahi, N.; Garmy, N. Cholesterol Accelerates the Binding of Alzheimer's β-Amyloid Peptide to Ganglioside GM1 through a Universal Hydrogen-Bond-Dependent Sterol Tuning of Glycolipid Conformation. *Front. Physiol.* **2013**, *4*, 120.
(91) Capetillo-Zarate, E.; Staufenbiel, M.; Abramowski, D.; Haass, C.; Escher, A.; Stadelmann, C.; Yamaguchi, H.; Wiestler, O. D.; Tha, D. R. Selective Vulnerability of Different Types of Commissural Neurons for Amyloid β-Protein-Induced Neurodegeneration in APP23 Mice Correlates with Dendritic Tree Morphology. *Brain* **2006**, *129*, 2992−3005.
(92) Dickson, D. W.; Crystal, H. A.; Bevona, C.; Honer, W.; Vincent, I.; Davies, P. Correlations of Synaptic and Pathological Markers with Cognition of the Elderly. *Neurobiol. Aging* **1995**, *16*, 285−298.
(93) Kayed, R.; Canto, I.; Breydo, L.; Rasool, S.; Lukacsovich, T.; Wu, J.; Albay, R., 3rd; Pensalfini, A.; Yeung, S.; Head, E.; Marsh, J. L.; Glabe, C. Conformation Dependent Monoclonal Antibodies Distinguish Different Replicating Strains or Conformers of Prefibrillar Aβ Oligomers. *Mol. Neurodegener.* **2010**, *5*, 57−66.