BBA - Biomembranes 1860 (2018) 1625–1638

Contents lists available at ScienceDirect

BBA - Biomembranes

journal homepage: www.elsevier.com/locate/bbamem

# Amyloid growth and membrane damage: Current themes and emerging perspectives from theory and experiments on Aβ and hIAPP

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A R T I C L E I N F O

*Keywords:*
Amyloid
Model membrane
Alzheimer
Type II diabetes mellitus
Amylin
Abeta

A B S T R A C T

Alzheimer's Disease (AD) and Type 2 diabetes mellitus (T2DM) are two incurable diseases both hallmarked by an abnormal deposition of the amyloidogenic peptides Aβ and Islet Amyloid Polypeptide (IAPP) in affected tissues. Epidemiological data demonstrate that patients suffering from diabetes are at high risk of developing AD, thus making the search for factors common to the two pathologies of special interest for the design of new therapies. Accumulating evidence suggests that the toxic properties of both Aβ or IAPP are ascribable to their ability to damage the cell membrane. However, the molecular details describing Aβ or IAPP interaction with membranes are poorly understood. This review focuses on biophysical and in silico studies addressing these topics. Effects of calcium, cholesterol and membrane lipid composition in driving aberrant Aβ or IAPP interaction with the membrane will be specifically considered. The cross correlation of all these factors appears to be a key issue not only to shed light in the countless and often controversial reports relative to this area but also to gain valuable insights into the central events leading to membrane damage caused by amyloidogenic peptides. This article is part of a Special Issue entitled: Protein Aggregation and Misfolding at the Cell Membrane Interface edited by Ayyalusamy Ramamoorthy.

## 1. Introduction

An increasingly large number of proteins are known to lack a stable structure under physiological conditions [1].These intrinsically disordered proteins (IDPs) are thus present as highly dynamic ensembles characterized by significantly variable conformations over time. Their structural adaptability provides IDPs with unique functional capabilities that cannot be accomplished by folded proteins [2]. Due to their delicate biological tasks, IDPs are involved in a large number of human diseases [3] and represent one of the most attractive (and challenging) drug target of the last decade. Important examples of unstructured polypeptides which form pathogenic amyloid aggregates in vivo include: the Aβ peptide, present in brain of patients affected by Alzheimer's Disease (AD); the Prion protein, responsible for the “mad cow” disease and IAPP, which is the protein component of type 2 diabetes-associated islet amyloid [4]. Upon interacting with other cytosolic partners such as proteins or membranes, IDPs can partly (mis)fold into (dys)functional conformations [5]: therefore, the mechanism of amyloid formation in vivo may be quite different from that observed in dilute aqueous solution and studies of IDPs assembly in an heterogeneous water/membrane environment are expected to provide more significant advances in our understanding of these pathogenic mechanisms. Many in vitro studies have shown that several IDPs become structured when bound to membrane surfaces, and that small-sized intermediates play a significant role in amyloid-mediated membrane damage and toxicity [6–8]. Three major membrane damage models have been proposed so far: (i) generation of stable transmembrane protein pores (poration); (ii) membrane destabilization via a “carpet model” or iii) removal of lipid components from the bilayer by a detergent-like mechanism (see Fig. 1) [9]. Whether these three models are mutually exclusive or if (and how) they may cooperate in triggering membrane damage remains to be established. However, several factors have been suggested to come into play. First, electrostatic forces are supposed to lead to an effective migration of the positive IDP from the aqueous environment to the negative membrane surface. Second, association of IDP with the membrane surface would result to an increased local peptide concentration and membrane-bound IDP monomeric units interact one another in a two-, rather than in a three dimensional space. Despite the wealth of information that can be obtained on IDP-induced changes in membrane properties and on structural rearrangements of membrane-bound IDP, a thorough description of the conformational dynamics sampled by IDP/membrane systems

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https://doi.org/10.1016/j.bbamem.2018.02.022
Received 22 December 2017; Received in revised form 21 February 2018; Accepted 21 February 2018
Available online 01 March 2018
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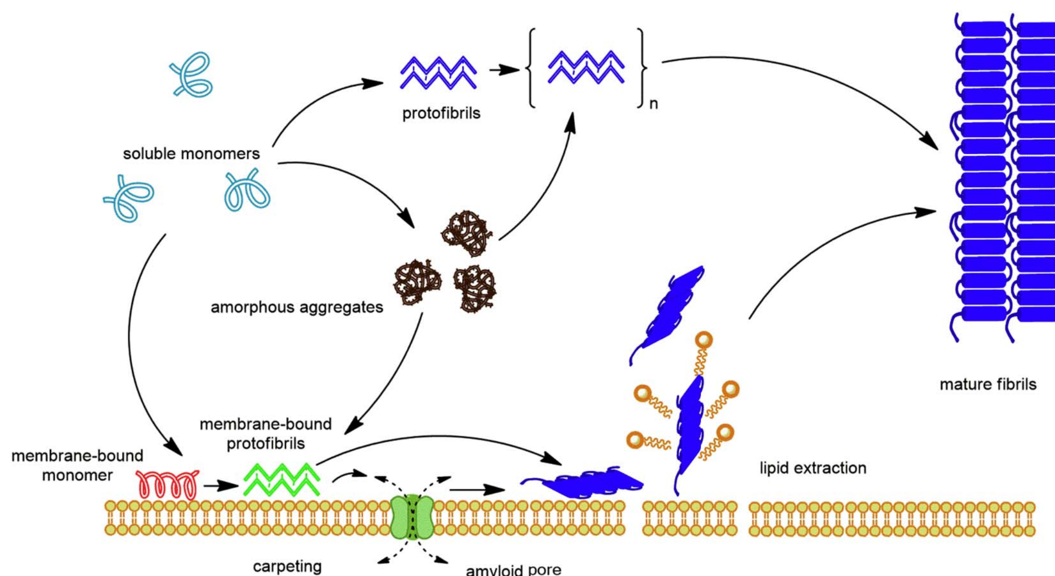


**Fig. 1.** Schematic representation of the amyloid formation and membrane disruption process. Misfolding of soluble peptide monomers starts aggregation via the formation of β-sheet rich protofibrils. and/or amorphous aggregates. Consequently, in the water phase, amyloid growth and fibril formation may occur. Peptide monomers and/or amorphous aggregates may also bind lipid membrane surfaces where they acquire an α-helix structure. Next, clustering of proteins on the membrane surface induces their assembling into β sheet-rich aggregates. Peptides may also assemble to form a pore thus enabling membrane leakage. Concomitant with this mechanism, peptide protofibrils may assemble in solution and permeabilize the membrane (pore-formation mechanism). Moreover, amyloid aggregates may grow at the membrane surface and induce lipid extraction by a detergent-like mechanism.

remains often unapproachable by conventional experimental techniques. On these bases, in silico methods play an increasingly central role in helping to figure out the atomistic implications of experimental evidences, as well as in generating de novo predictions.

The link between amyloid forming proteins and membrane disruption is largely documented. However, our understanding of the molecular details by which subtle changes in the microenvironment surrounding amyloid-membrane interfaces affect amyloid membrane interactions is still far from being complete. Indeed, such knowledge would be of great importance since it is required for a better comprehension of the role played by some risk factors such as lifestyle, diet, aging and the occurrence of other pathologies. For instance, several important issues remain concerning the variations of the membrane disruption mechanism depending on i) the presence on unbalanced levels of metal ions, ii) abnormal concentration of cholesterol and iii) changes in lipid membrane composition.

A first aim of the present review is to recapitulate the latest literature focusing on these specific issues. In particular, we present the current knowledge concerning the effect of metal ions, cholesterol and lipid composition on amyloid-induced membrane leakage. In silico studies including experimental results supporting each proposed model will be specifically considered in this review. This Review addresses the most recent results obtained for Aβ and IAPP highlighting, if possible, the common mechanistic aspects that are related to the pathogenesis of Alzheimer's Disease and Diabetes Mellitus. Studies addressing both IAPP and Aβ are of particular interest given recent epidemiological data showing that diabetic patients have an increased risk to have shown that patients suffering from T2DM have an increased risk to develop AD [10] supporting the attractive hypothesis that both Aβ and IAPP may share the same mechanism of toxicity [11].We hope that this Review will provide novel perspectives for a better of the mechanisms of amyloid toxicity and inspire further, multidisciplinary research in this area.

### *1.1. The Aβ peptide and the “amyloid hypothesis”*

In the early years of the 20th century, Alois Alzheimer reported for the first time the presence of plaques and fibrillary tangles in neuronal tissues of a 51 years old woman which have experienced during her life a progressive decline of cognitive function and memory failure [12]. This is the first scientific description of age-related dementia, a neurological pathology that will be later on commonly recognized as Alzheimer's Disease. A detailed analysis of those brain tissues allowed Alzheimer to characterize the main components in the senile plaques which were termed “amyloids” for their properties bordering on the staining capacities of starch with iodine. After those pioneering reports, over 8 decades have passed before the protein aggregates (Aβ peptide and tau aggregates) that form amyloids present in senile plaques [13] and NFTs [14] respectively were fully characterized. Based on those reports, the aggregation of Aβ peptides has been considered for many years a major target to develop effective therapeutic strategies for AD. Although the “amyloid hypothesis” is considered by many prominent scientists [15], the prevalent issue to describe the underlying molecular mechanisms involved in AD development [16,17], a large part of the scientific community has raised severe concerns on this assumption [18]. Certainly, several weak points of the amyloid hypothesis have been described and reverberate into the long list of amyloid-targeting drugs that were unsuccessful at clinical trials [19,20]. Surely, other factors contributing to Aβ proteotoxicity have to be considered and membranes have been called out as one of the other main checkpoints of the disease [21].

### *1.2. Aβ amyloids and membranes*

In vivo, the Aβ peptide originates from larger amyloid precursor proteins (APPs) which are integral membrane glycoproteins of 695, 714, 751, and 770 amino acids [22]. The amyloidogenic Aβ peptide encompasses 28 residues of the extracellular and 11–15 residues of the APPs transmembrane domain. In pathological conditions, APP is first cleaved by a β-secretase thus releasing a soluble extracellular domain (sAPPβ), and an intracellular segment that is further cleaved by the γ-secretase to form Aβ and the APP intracellular domain [23,24]. Based on this evidence, several biophysical studies have been carried out to describe the effect of membranes on the conformational properties of Aβ peptides in tube tests. As an example, Circular Dichroism (CD) spectroscopy, calorimetry and ultracentrifugation assays have been used to assess the binding capacities of Aβ to negatively charged lipid membranes. In particular, it was shown that the addition of anionic

lipid micelles to Aβ may induce a conformational shift from a prevalent random coil to a β-sheet rich structure. On this basis, peptide binding and penetration to the lipid membrane were proposed as the major driving force to explain the Aβ conversion from a soluble, unstructured conformation to a potentially toxic β-sheet rich form [25]. Other studies suggested that addition of dipolar compounds as phloretin or exifone that shield the negative charges on the surface of the lipid membranes may prevent both binding to the lipid bilayer and toxicity [26] thus pointing to a nonspecific interaction with membranes as the main reason of peptide pathogenicity. Aβ peptide, when incorporated into lipid membranes, forms calcium permeable channels that were suggested to induce cell death [27]. Consistently with this "channel hypothesis" of AD, formation of calcium permeable channels by Aβ depends on the presence of anionic lipids and is favored by acidic solutions. Next, calcium permeable channels are reversibly blocked by zinc ions and small molecules like Congo Red [28]. The aggregation state of Aβ may also influence the peptide-induced membrane damage. The self-assembly of Aβ results in a decreased membrane fluidity and, as a consequence, deleterious consequences on cellular functioning [29]. It was also found that incorporation of gangliosides on artificial bilayers induce Aβ peptide to adopt a mixed α/β conformation in aqueous solution at neutral pH [30]. Several studies have later on pointed to the close relationship existing between Aβ amyloid growth and membrane damage. In particular, biophysical studies including tapping mode atomic force microscopy techniques addressed the interaction of soluble monomeric Aβ with planar bilayers of total lipid brain extract. Those results suggested that the fibrillogenic properties of the amyloid peptide are partly associated membrane composition, peptide sequence, and modes of assembly within the membrane [31]. Aβ was also observed to impact cell cycle by forming heterogeneous or "chaotic" ion channels. These abnormal modifications of membrane integrity were observed before plaque formation and were associated to the early steps of neuronal impairment [32]. This proposed mechanism of neuronal toxicity hereinafter referred as to the "channel hypothesis", was supposed to damage the plasma membrane by modifying the membrane potential and/or allowing uncontrolled raise of intracellular levels of $Ca^{2+}$ ions. The channel mechanism was also proposed to explain damage of intracellular membranes such as mitochondrial and lysosomal membranes via release of harmful enzymes prompting apoptosis. Ion permeable channels are also formed by microbial toxins, and are proposed as a general cytoxic mechanism relevant to all amyloid diseases as Alzheimer's, Huntington's Parkinson's [33]. Oxidative stress is also known to play a key role in AD development. In particular, there are evidences of free radical overproduction in those AD brain areas in which Aβ levels are more abundant. Consistent with this hypothesis, Aβ was shown to induce oxidation of neuronal lipids, possibly modifying membrane composition and altering membrane fluidity [34]. By the way, oxidized lipid membranes are known to trigger misfolding and aggregation of Aβ fostering a vicious circle in which amyloid promote free radical production, lipid peroxidation and, in turn, formation of amyloid plaques [35,36]. Later on, it was demonstrated that small sized, soluble Aβ aggregates were the real cause of neuronal damage and memory loss in the early stages of AD [37]. However, the hypothesis that mature Aβ fibrils are inert was not fully confirmed by studies aimed at evaluating the neuronal toxicity of different Aβ assemblies. In particular, it was shown that both fibrils and oligomeric Aβ assemblies induced a decrease of mitochondrial membrane potential in neuron extracted from the brains of transgenic mice. This effect, however, was not observed when monomeric Aβ was added to neurons [38]. In general, the diverse effects of Aβ assemblies on cells depend on the ability of different membrane types to bind amyloid peptides with a consequent fiber growth resulting in membrane damage and, eventually, cell death. This issue has been investigated by a wide variety of experimental techniques all highlighting that amyloid-membrane interactions may be considered from a twofold viewpoint: on one hand, the lipid membrane may influence peptide misfolding and aggregation; on the other hand, peptide aggregates may affect membrane integrity and permeability [39]. Although it is believed that amyloid cytotoxicity is mainly ascribable to small Aβ oligomers, the real nature of toxic peptide assemblies is still object of controversy. Actually, freshly dissolved Aβ oligomers were shown to strongly associate with lipid bilayers causing leakage of model membranes. Conversely, it was observed that fibrils bind membranes with a two-fold reduced affinity if compared to oligomeric Aβ. In turn, their addition caused a two-fold less effective leakage of the membrane [40]. In an attempt to solve this apparent contradiction, some of us have previously demonstrated that Aβ causes membrane damage by a two-step mechanism which implies both binding of small-sized peptide oligomers to the bilayer to form heterogeneous ion channels and fibrillogenesis on the membrane surface which causes lipid extraction from the bilayer by a detergent-like mechanism [41]. It was also shown that the affinity of Aβ peptides for membranes is increased in the presence of gangliosides [42] and negatively charged phosphatidylserine (PS) membranes [43–45]. Notably, when the negatively charged lipids are clustered, peptide binding to the membrane results fostered [46]. Therefore, adverse environmental factors may promote membrane-Aβ interactions by recruiting Aβ-competent lipids on the membrane surface. Due to their importance in AD pathogenesis, here we will focus on two of these factors: $Ca^{2+}$ ions and cholesterol.

### 1.3. The role of calcium ions in Aβ/membranes interactions

The notion that calcium dyshomeostasis is correlated to the development of AD dates back to 1984 [47]. Later on, it was also shown that intracellular Calcium levels of lymphocytes and fibroblasts are elevated in AD patients when compared in age-matched controls [48,49]. Several authors also reported that the observed increase in intracellular calcium is ascribable to the formation of Calcium channels subsequent to the incorporation of Aβ amyloids into lipid membranes [44]. Actually, these findings suggest that there is a close correlation between calcium dysregulation, abnormal interaction of Aβ amyloids with the membrane and, eventually, cytotoxicity. $Ca^{2+}$ ions, as an example, promote lipid phase segregation by clustering negatively charged lipids on the membrane/water interface [50,51]. Next, they affect many membrane properties including the structure of membrane domains, [52] and vesicles fusion [53]. In previous papers some of us demonstrated that $Ca^{2+}$ ions may promote the interaction of hIAPP with the hydrophobic core of PS-enriched membranes [54] and favor lipid loss via a detergent-like mechanism [55]. However, both membrane-damage mechanisms (i.e. channel formation and detergent-like lipid loss) are shared by Aβ and IAPP; it is therefore plausible that calcium effects on amyloid-induced membrane damage may be part of a general mechanism underlying amyloid cytotoxic properties. This last hypothesis, however, still needs to be confirmed.

### 1.4. Cholesterol, lipid components and Aβ-activated membrane damage: a double faced partnership?

The effects of high levels of cholesterol on the accumulation of intracellular Aβ amyloids were first examined by feeding rabbits with dietary cholesterol [56]. Long-term therapy with statins, agents widely used in the prevention of myocardial infarction by a strict control of cholesterol levels, were also shown to decrease the risk of developing AD [57]. Those early reports, however did not addressed the causal link of high cholesterol levels, amyloid accumulation and dementia. Using fluorescent dye-labeled human Aβ, it was observed that gangliosides and also cholesterol content enhances peptide ability to bind membranes inducing a conformational transition from an α-helix to a β-sheet rich structure. Moreover, those results suggested that Aβ has a preferential affinity for "clusters" of gangliosides which are favored by cholesterol [58]. Further studies also underscored the important role played by cholesterol-enriched lipid rafts in accelerating plaque

formation and membrane disruption [59]. Additional evidence obtained from mouse models of excess cholesterol has shown that a cholesterol-induced perturbation of mitochondrial membrane makes neurons more sensitive to Aβ cytotoxic effects [60]. Despite accumulating evidence suggests that the presence of cholesterol in the plasma membrane plays a pivotal role in AD, the exact mechanism of cholesterol-dependent Aβ aggregation, membrane damage and toxicity remains unclear [61]. It has been shown that cholesterol-induced alteration of the biophysical properties of membranes including bilayer thickness, membrane curvature, surface hydrophobicity and lipid dynamics promote Aβ adhesion to lipid membranes [62]. Notably, in the same paper it was proposed that Calcium ions may also favor peptide-membrane binding, by optimizing the hydrophobic forces stabilizing the lipid-peptide complexes. All these data point to a significant role played by cholesterol in promoting peptide membrane interactions. Consistent with this hypothesis, other authors have examined the effects of cholesterol and Aβ on membrane fluidity [63]. In particular, it was shown that neuronal membranes containing high levels of cholesterol may incorporate Aβ more effectively. However, membrane components are not indiscriminately dispersed but, rather, they are organized in different domains. Each of these lipid domains has a specific role in signal transduction, lipid trafficking and cell metabolism. These domains consist of gangliosides, negatively charged pools of lipids, and cholesterol-rich lipid rafts. In particular, it was observed that in neuronal membranes there is an age-dependent increase of cholesterol distributed in the outer leaflet of the lipid bilayer that is consistent with the observed accumulation of Aβ with cholesterol-rich lipid rafts [64]. Metal ions are also known to play a pivotal role in protein misfolding [65] in general and, in particular, in Aβ toxicity [21]. Electron Paramagnetic Resonance (EPR) and CD spectroscopy have underscored the key role played by copper and zinc ions in modulating the interactions of Aβ peptides with membranes [66]. Of note, if cholesterol content is increased up to 0.2 mol fraction insertion of peptide into the membrane resulted inhibited in all the conditions investigated. Further studies revealed that Aβ peptide insertion into the lipid bilayer is strictly dependent on the cholesterol membrane content [67]. In particular, at low cholesterol concentrations the peptide remains on the membrane surface and adopts a predominantly β-sheet structure. Conversely, at high cholesterol levels (higher than 30% mole) the peptide inserts into the hydrocarbon core of the lipid bilayer and adopts an α-helical structure. The notion that low levels of cholesterol in the brain are associated to a lower risk of AD pathogenesis is contradicted by pharmacological evidences pointing to neuroprotective role for cholesterol [35]. This apparent contradiction was only in part solved by studies evidencing an enhanced amyloid deposition in membrane domains in which there is an increased colocalization of APP and cholesterol. Later on, biochemical studies have evidenced that specific components of membrane, including cholesterol, may accelerate the accumulation of Aβ on the plasma membranes in the presence of gangliosides [68]. In particular, it was shown that Aβ monomers, and not fibrillar Aβ was able to attack membrane causing toxicity [69]. However, this effect of Aβ was abolished when cholesterol levels are decreased of below 30%. These findings support the hypothesis that cholesterol may have multiple effects on membrane architecture and, in turn, on its ability to bind Aβ. It is likely that, depending on the composition of the host membrane, cholesterol acts as a switch between different mechanism of membrane damage i.e. pore formation or fibril growth on the membrane surface. The next sections will describe the presence of similar phenomena in IAPP/membrane systems. The whole of these observations is summarized in Table 1.

### *1.5. Brief history of hIAPP and its involvement in the development of type II diabetes mellitus*

Although amyloid deposits in pancreatic islets of a diabetic subject were firstly described more than 100 years ago [70], only in 1987 Cooper and Westermark, independently, isolated a novel protein called Human Islet Amyloid Polipeptide (hIAPP also known as Amylin) from pancreas tissues of T2DM patients [71,72]. The presence of these proteinaceous deposits in pancreas is one of the most common pathological features of T2DM, which affects more than 150 million individuals worldwide [73,74]. Despite the scientific community put an extraordinary effort to uncover the biochemical properties of hIAPP, considered the principal actor in the development of T2DM, the causal factors that contribute to diabetes onset are largely unknown. These include both the relationship of hIAPP aggregation with the development of diabetes as well as the causative factors at the root of hIAPP fibrillogenesis. Improved understanding of pancreatic islet function in diabetes and development of biophysical methods to investigate protein misfolding processes as well as the extensive study of the interaction of hIAPP with phospholipid membranes, have resulted in new approaches to the problem of how amyloid forms and how it affects islet function [75].

### *1.6. Mechanism of hIAPP toxicity*

Over the last decade several mechanisms to explain IAPP toxicity have been proposed. It has been suggested that IAPP aggregates might be toxic to pancreatic β-cells by forming pores that permeabilize not only plasma membranes but also those of the organelles involved in protein synthesis and secretory pathways (i.e. the ER, secretory vesicles) [76]. Several studies have focused on the interpretation of molecular mechanisms underlying T2DM, in particular by investigating the interaction between hIAPP and phospholipid membranes, a process that seems to be correlated to the loss of pancreatic β-cells [77–80]. Although some recent papers [81] suggest that the water/membrane interface is critical for influencing hIAPP amyloid aggregation, the mechanisms by which abnormal hIAPP/membrane interactions cause cytotoxicity remains unsolved.

It is known that human IAPP is toxic to islet cultured cells [82–84] and that the mechanism of cytotoxicity is mediated by apoptosis. Initially these effects have been attributed to the ability of mature hIAPP fibrils to bind cell membranes, create pores and affect normal transmembrane ionic currents [85]. Similar cytotoxic effects have been already described for fibrillar synthetic Aβ peptide [83,86] but only when fibrils are formed with aging since Aβ is not toxic when freshly prepared [87–89]. Indeed, it was initially supposed that the appearance of extracellular amyloid fibrils and apoptosis were correlated, and that the extracellular amyloid fibrils had induced apoptosis [82]. However, other studies did not evidence cytotoxicity despite the appearance of hIAPP fibrils [90,91]. The relationship between small non fibrillar hIAPP aggregates and apoptosis was studied by treating cells with freshly prepared solution of hIAPP [90]. These data suggest that the most toxic species which induce apoptosis are not mature amyloid fibrils but probably hIAPP protofibril or small oligomers, rapidly formed in aqueous solution [92]. The nature of the toxic form of hIAPP and the mechanism of induction of cell damage are still object of intense debate. It has been reported that IAPP oligomers are able to form pore-like structures in the membrane resulting in pro-apoptotic $Ca^{2+}$ dysregulation [93,94]. Of note, similar mechanisms have been reported for Aβ, α-synuclein and PrP oligomers (Fig. 2) [95,96]. It has been suggested that the composition of lipid bilayers may promote IAPP aggregation. For example, the presence of negatively charged phospholipids, such as phosphatidylserine, promotes the interaction of the positively charged IAPP monomer with the membrane [97]. Binding of negatively charged heparan sulfate proteoglycan, present on the cell surface, to positively charged N-terminal of IAPP and pro-IAPP, have also been shown to promote the aggregation of the peptide [98,99].

### *1.7. The two step mechanism*

Variation in bilayer fluidity, pore formation, carpet mechanism or

**Table 1**
Effects of Aβ peptides on different model membranes.

| Peptide | Membrane | Technique used | Effects | Ref |
|---|---|---|---|---|
| $A\beta_{1-40}$ | POPC/POPG (3:1) | Circular Dichroism (CD), Isothermal Titration Calorimetry (ITC) | Random-coil to beta-sheet structure transition induced by negatively charged lipids. | [25] |
| $A\beta_{1-42}$ $A\beta_{25-35}$ | POPC/POPG | HS-ITC | Dipolar compounds prevent peptide-membrane interactions. | [70] |
| $A\beta_{1-42}$ | Anionic lipid membranes (PS) POPG:POPE (1:3) | Conductance measurements | Formation of Aβ ion channels, inhibited by the presence of $Al^{3+}$ or $Zn^{2+}$ and Congo Red. | [27] [28] |
| $A\beta_{1-39}$ $A\beta_{1-40}$ | POPC:POPG POPC:POPE:POPS:chol POPC:POPE:POPS:chol:gangl | Dynamic Light Scattering (DLS), Fluorescence, Electron Microscopy (EM) | Membrane fluidity decrease as consequences of Aβ self-assembly. | [29] |
| $A\beta_{25-35}$ $A\beta_{1-40}$ $A\beta_{1-42}$ | mixtures of bovine brain PG, PS, PA, PC mixed ganglioside fraction of bovine brain pure gangliosides | Dye-Release (Fluorescence), CD | Gangliosides induce mixed α/β conformation of Aβ peptides and increase affinity with membrane. | [30] [42] |
| $A\beta_{1-28}$ $A\beta_{1-40}$ | DMPC TLBE | Atomic Force Microscopy (AFM), CD, EM | Tight correlation between amyloid growth and membrane damage. | [31] |
| $A\beta_{1-40}$ | DLPC DOPC | CD, AFM, ThT-assay, Trasmission Electron Microscopy (TEM), NMR | Oxidized lipids promote peptide misfolding and aggregation. | [36] |
| $A\beta_{1-42}$ | DMPC:DMPE:DMPG:DMPS:Chol:GM1:biotine-PE | Surface Plasmon Field-Enhanced (SPR) Fluorescence, AFM | Aβ oligomeric species bind lipid bilayer and cause stronger membrane disruption. | [40] |
| $A\beta_{1-40}$ | TLBE POPC:POPS 7:3 POPC:POPS:GM (15%) | ThT assay. Dye-leakage assay, Fura-2 assay, NMR. | Aβ causes membrane damage with a two-steps mechanism. | [41] |
| $A\beta_{1-40}$ | GM:chol:SM, POPE:POPS, GM1:chol:SM. GM1, GM2 | SEC analysis Fluorescence CD Conductance measurements | Gangliosides, anionic lipids membranes and cholesterol increase Aβ interaction with membranes. Plaque formation rate is directly proportional to gangliosides concentration. | [42–44] [58] [59] |
| $A\beta_{1-40}$ $A\beta_{1-42}$ | GM1:SM:chol (2:4:4) GM:PC (2:8) | CD, Confocal Fluorescence Microscopy, TEM | Aβ peptide prefers to bind GM that occur clustered, not homogeneously distributed. | [46] |
| $A\beta_{22-35}$ | Membranes containing cholesterol | Lamgmuir Blodgett (LB) experiments | The presence of cholesterol induces alteration of biophysical properties, such as bilayer thickness, membrane curvature, surface hydrophobicity and membrane fluidity. | [62] [63] |
| $A\beta_{1-40}$ $A\beta_{1-42}$ | POPC:POPS | EPR CD | $Cu^{2+}$ and $Zn^{2+}$ ions modulate interaction between Aβ peptide and model membranes. | [66] |
| $A\beta_{1-40}$ $A\beta_{1-28}$ | DMPC:DPPC:chol | Monolayer Surface Pressure Measurementes, MALDI-TOF MS, CD, EM | The peptide ability to penetrate the bilayer is influenced by cholesterol concentration. | [67] |
| $A\beta_{1-40}$ | DOPC | CD, Gel electrophoresis, Ion channel current measurements, AFM | Formation of ion-channel-like structures is a common mechanism of different amyloidogenic proteins. | [96] |

detergent-like mechanisms are some of the mechanisms hypothesized to shed light on membrane damage induced by hIAPP [101]. Recent studies have shown that membrane disruption induced by hIAPP may be described as a two-step process. The first step occurs after the insertion of monomeric or oligomeric species inside the membrane hydrophobic core and is correlated with pores formation. The second step, which is independent from the first step, is ascribable to fibers growth onto the membrane surface, which causes membrane dissolution through a

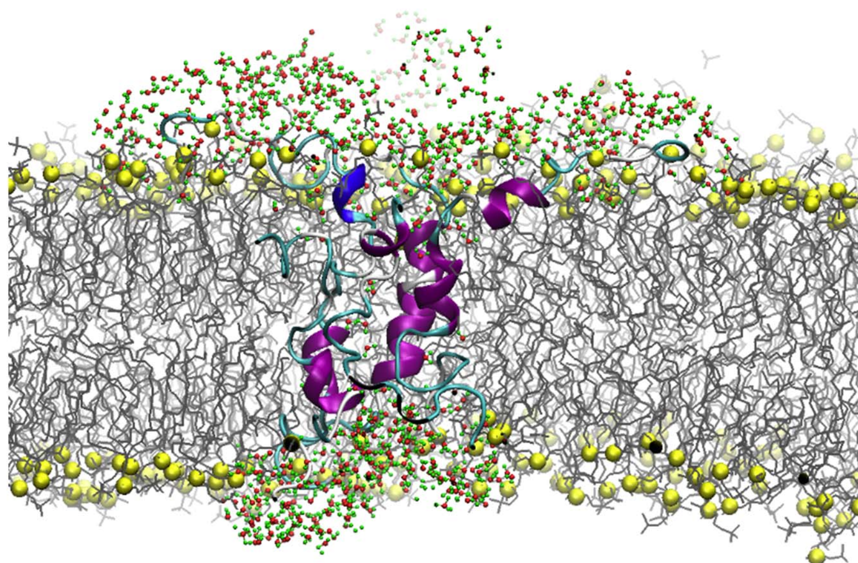

**Fig. 2.** Cartoon representation of a amyloid-induced aspecific ion channel-like pore. Peptide α-helices (purple), random coil (cyan) and β-turns (blue) are represented in a POPC model bilayer. Hydrocarbon tails are reported as grey lines, phosphate head-groups are in yellow. Water molecules surrounding the pore are also evidenced (red and green atoms). Coordinates were obtained from ref. [8]. The snapshots were created by using the software VMD [100] (W. Humphrey, A. Dalke and K. Schulten, VMD: Visual molecular dynamics, J. Mol. Graph., 14, (1996), 33–38).

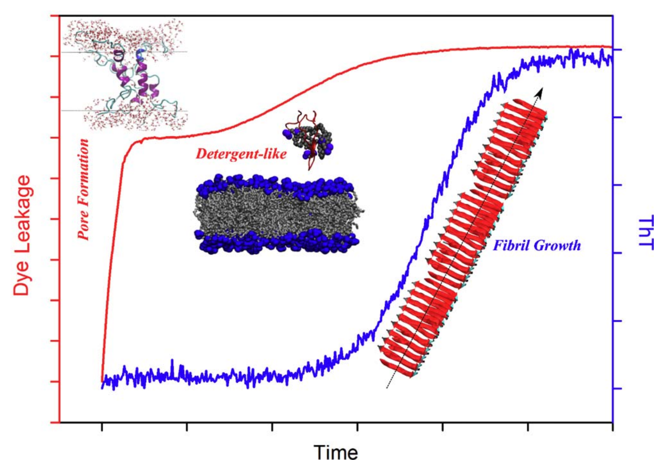


**Fig. 3.** Schematic representation of two-state mechanism of hIAPP-mediated membrane disruption derived from dye leakage assay, ThT assay, MD simulation and AFM.

detergent-like mechanism (see Fig. 3) [41,78]. It has also been shown that the formation of amyloid fibers occurs independently of membrane leakage and that membrane composition and the presence of ions may have a major influence on amyloid-mediated membrane damage [55]. The membrane lipid composition is an important factor which regulates the hIAPP-mediated membrane disruption. For example it was demonstrated that the presence of phosphatidylethanolamine headgroup lipids modulate the entity of the first and second step of membrane disruption [78]. Moreover, the presence of cholesterol perturbs the interaction of hIAPP with the membrane and its insertion into the bilayer.

Therefore, the membrane disruption mechanism can be regulated by several factors, such as metal ions balance, increased hIAPP secretion, the presence of free fatty acid, etc. which could arise from genetic predisposition, lifestyle, and diet [102].

### 1.8. Environmental factors influencing the two-step mechanism

#### 1.8.1. Calcium ions

Patients affected by familial hypercalciuric hypercalcemia are more prone to develop T2DM [103]. An increase in the intracellular calcium ion concentration ($[Ca^{2+}]_i$) was reported for diabetic patients [104,105]. Aging and obesity, which are common risk factors for T2DM, both induce dysregulation of $[Ca^{2+}]_i$ in the cells [106–108]. Dyshomeostasis of intracellular $Ca^{2+}$ affect domain organization, vesicular trafficking, and membrane adhesion/fusion [52,53]. $Ca^{2+}$ dyshomeostasis and hIAPP–membrane interaction have been already shown to be correlated [54,55]. hIAPP penetration into membrane to form pores was shown to be enhanced by high $Ca^{2+}$ concentration [55]. Amyloid peptides can destabilize $Ca^{2+}$ homeostasis in cells by inducing the formation of non-selective cation channels [109,110]. According to the cation channel hypothesis, it was observed a significant $[Ca^{2+}]_i$ elevations in GT1-7 cell lines induced by hIAPP [86]. $Ca^{2+}$ ions may also differently affect the binding properties of hIAPP and rIAPP to model lipid membranes [54]. Thus it is evident a correlation among $Ca^{2+}$ dyshomeostasis, abnormal hIAPP–membrane interaction, and, eventually, impaired hIAPP trafficking.

#### 1.8.2. Lipid composition: the role of cholesterol

Several reports have shown dietary type as a risk factor of T2DM [111]. Hyperlipidemia contributes to the pathogenesis of T2DM [112]. Several data show that an abnormal lipid metabolism may induce hyperglycemia and β-cells failure [113]. Plasma cholesterol is often elevated in obese patients [114] and a high consumption of foods rich in cholesterol are normally considered a risk factor in T2DM [115,116]. Normally cholesterol constitutes 30–50 mol% of mammalian plasma and internal cell membranes composition [117,118]. It plays a key role in regulating glucose metabolism in adipocytes [119] by activating several transcription factors and altering the chemico-physical properties of membrane rafts [120]. Cholesterol significantly affects membrane properties, participates in lipid bilayer packing, and contributes to the formation of lipid rafts which float in the more fluid lipid domains enriched in unsaturated hydrocarbon chains [121], which are involved in the regulation of many cell signaling pathways [121]. Cholesterol catalyzes IAPP amyloid aggregation on plasma membranes of PC12 cells [122]. Transgenic mice lacking Abca1 (ATP-binding cassette transporter 1, which removes excess cholesterol from cells) [123] specifically in β-cells were generated [124]. These transgenic mice have shown a decreased cholesterol efflux, an accumulation of intracellular cholesterol at the plasma membrane and a decreased insulin exocytosis. Moreover it was demonstrated the presence of lipid rafts in pancreatic β-cells [125] and in human extracellular amyloid fibrils [126]. All these evidences suggest that membrane cholesterol is involved in the damaging mechanism of pancreatic β-cells.

In a recent study hIAPP-membrane interactions were investigated by using two different model lipid membrane systems: 7:3 POPC:POPS and raft-forming 1:2 DOPC:DPPC large unilamellar vesicles [127]. It was shown that high concentrations of cholesterol, in 7:3 POPC:POPS non-raft membranes, do not affect IAPP fibril growth kinetics but significantly reduce pore formation. On the contrary, cholesterol enhances both fiber and pore formation in raft-like 1:2 DOPC:DPPC model membranes.

The interaction of cholesterol with the bilayer lipid tails was investigated by using the “nearest-neighbor recognition” (NNR) methodology [128]. The authors have proposed a “*push and pull*” mechanism in which the interaction between cholesterol and phospholipid containing a saturated acyl-chain are repulsive (push) and phospholipid containing saturated acyl-chain shows attractive forces (pull). Thus the effect of cholesterol on hIAPP interaction with membranes depends on a delicate equilibrium between i) peptide-membranes electrostatic interactions, ii) the better match of the peptide hydrophobic domain with the bilayer thickness, and iii) the rigidifying effect of cholesterol on the lipid bilayer. The balance of all these forces is strictly dependent on the lipid composition and it is thus not surprising that many studies have reported a variety of different effects of cholesterol on different model membranes. Studies describing the interaction of hIAPP with model membranes reported in this review are also summarized in Table 2.

### 1.9. Molecular dynamics of membrane/amyloids systems

Recent experimental evidences have shown that the species responsible of cell membrane damaging and toxicity are oligomeric species. These species are formed in the so-called lag-phase. The oligomers are transient and unstructured species in solution. NMR measurements showed the amyloidogenic proteins in solution give rise to chemical equilibrium between monomeric species in random coil conformation and large alpha-helix oligomeric aggregates, of approximately 50 nm, off-pathway [129]. Indeed, it has been hypothesized that toxic and damaging cell membranes species are small oligomeric ones [130,131]. In addition, experimental QCM-D measurements [79] have shown that amyloidogenic proteins interact with the membrane immediately, without any lag-phase. Therefore, it can be deduced from these experimental facts that the protein-membrane interaction must take place in a very short time, of the order of magnitude of seconds. Therefore, in order to study the early stage of oligomers formation it is crucial to refine the investigated event time scale observed. From an experimental point of view, the time required for sample preparation (protein solutions and model membranes), and all needed operations to carry out the acquisition data, are of the order of ten minutes. It follows from these considerations that the early stages of the protein-protein and membrane-protein interaction are not accessible by experimental techniques because the observer is out of the experimental time scale resolution.

**Table 2**
A summary of biophysical investigations of hIAPP/membrane interactions.

| Peptide | Membrane | Technique used | Remark | ref |
|---|---|---|---|---|
| hIAPP<br>rIAPP | DPPC<br>DPPS<br>DPPC:DPPS(3:1)<br>POPC:POPS | DSC<br>CD<br>NMR<br>AFM<br>Dye-leakage experiments<br>QCM-D | $Ca^{2+}$ ions inhibit membrane damage and enhance fibrils growth [55]. | [54]<br>[55] |
| hIAPP | POPC:POPS(7:3)<br>POPC:POPS:POPE(3:4:3)<br>POPC:POPS:LysoPC | ThT-assay<br>Dye-leakage assay<br>NMR<br>CD | Membranes containing PE strongly modulate bilayer disruption and influence the fibrils formation kinetic. | [78] |
| hIAPP<br>rIAPP | POPC | AFM<br>ThT-assay | Membrane disruption and fibrils formation are related phenomena. | [79] |
| hIAPP<br>rIAPP | Egg PG<br>DOPC:DOPS (7:3) | ThT-assay<br>Permeabilization assay<br>CD<br>Dye-leakage assay<br>negative-stain EM | Peptide permeabilizing activity disappears when fibrils appear. Oligomeric IAPP is the main responsible of membrane permeabilization. Membrane leakage is directly related to fibrils formation. | [92]<br>[97] |
| hIAPP | PC12 cells | Differential Interference Contrast imaging<br>Fluorescence | Gangliosides act as platform for the amyloid plaque formation. | [122] |
| Cryosections of murine AA amyloid-laden spleen | | hpTLC<br>Light microscopy<br>Polarized light microscopy<br>Confocal laser scanner microscopy | Tissue deposits of amyloid fibrils contain different kind of lipids, such as cholesterol, polar lipids (phosphatidylcoline and phosphatidyletanolamine), sphingomyelin, glycosphingolipids, FFA. | [126] |
| hIAPP<br>rIAPP | POPC:POPS(7:3)<br>DOPC:DPPC (1:2) | ThT-assay<br>Dye-leakage assay<br>CD measurements<br>AFM | Cholesterol does not influence the kinetics of IAPP fibers formation, but suppresses pore formation and enhances fiber-dependent membrane disruption in the case of POPC:POPS raft-free bilayer. On the contrary, cholesterol increases fiber growth and pore formation in containing-raft bilayer DOPC:DPPC. | [127] |

An atomistic description of the early steps of amyloid-membrane interactions may be conveniently obtained by molecular dynamics (MD) [132].

MD is still limited by some challenges: high computer demands, the force field used require further refinements and liquid water and hydrophobic parametrization should be described in more accurately way, especially when protein aggregation is investigated.

As already mentioned, although the mechanism underlying amyloid toxicity of amyloidogenic proteins is not fully understood, it was evidenced the disruption and pore formation of the cell membrane by proteins as being the primary toxic behavior. Some representative snapshots of membrane-bound hIAPP are reported in Fig. 4.

Thus, to understand the mechanism of proteotoxicity it is important a thorough description of the protein transfer from an aqueous environment to the membrane hydrocarbon core. IAPP and Aβ are the most studied amyloidogenic proteins. These two amyloid-prone proteins, as recently defined [133], have about 25% of shared sequence identity and about 50% of similarity. Moreover, IAPP and Aβ show similarity among sequences of critical importance for amyloid self-assembly into fibrils (see Fig. 5) [134–138].

### 1.9.1. *Aβ polypeptides*

Aβ is a 39 to 43 residues polypeptide cleaved from amyloid precursor protein (APP). The polypeptide structure and behavior in solution were widely investigated through computational methods. Here, we focus on the interaction of model membranes with residues 1–40 and 1–42, the most abundant and neurotoxic fragments [140,141]. Brown and Bevan [142] have used MD simulation to monitor the formation of tetramer of Aβ (1–42) and their interaction of lipid bilayer, starting from water solution. MD simulations showed that four Aβ (1–42) monomer form a stable tetramer with an ellipsoid shape. This tetramer was simulated with pure POPC and cholesterol-rich raft model membrane. For both model membranes, during the interaction between tetramer and each membrane surface, the oligopeptide structure changes. Simulations show an elongation of the tetramer when it is adsorbed onto lipid surface, leading to more rod-like structure, which is

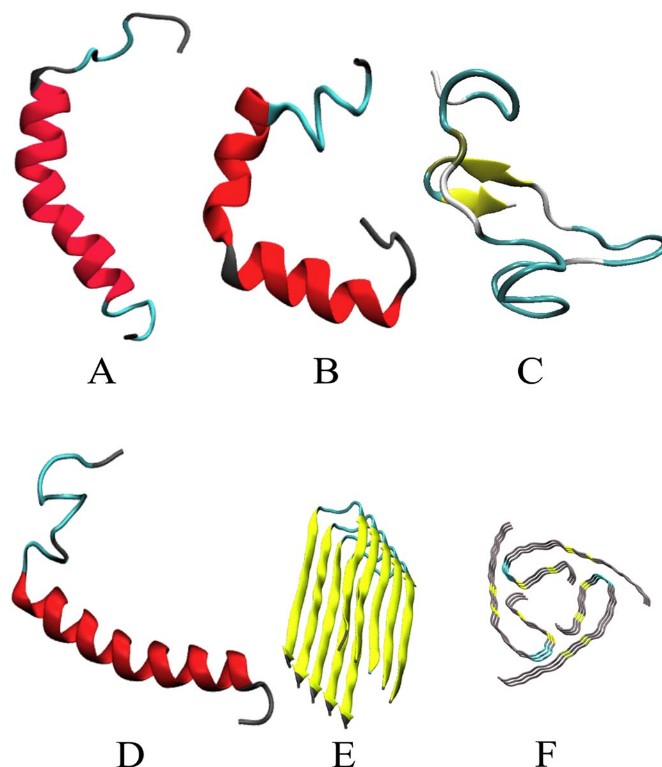


**Fig. 4.** Representative snapshots of membrane interacting hIAPP structures (A [80], B [152], C) and Aβ (1–40) (D [145], E [141], F [142]) interacting with various phospholipid membranes. Structures F and G is derived from solid state NMR. Structures A,B,E was derived from micellar solution NMR and structure D was obtained from MD simulation starting from solution NMR.

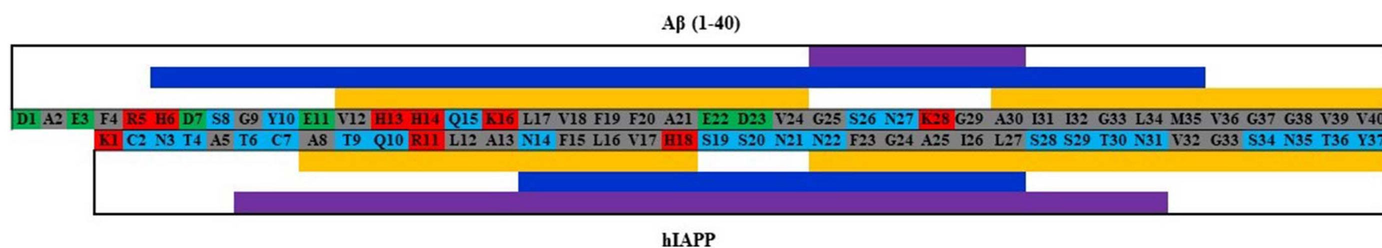


**Fig. 5.** Comparison of amino acid sequences of Aβ (1–40) and hIAPP. Amino acids color code: grey, hydrophobic; red, hydrophilic positively charged; light blue, neutral hydrophilic; green, hydrophilic negatively charged. Yellow bars: amyloidogenic regions. Blue bars: amino acids in α-helix conformation of n-mer aggregates within membrane [8,139]. Purple bars: amino acids in α-helix conformation of monomer within membrane [8,139].

considered on pathway intermediate in Aβ fibril-like structure. This effect is more evident in raft-like membranes. MD simulations show also that the physic-chemical properties of the membranes change, becoming more rigid and ordered. Nussinov and co-workers [143], using explicit solvent molecular dynamics (MD) simulations, observed that an Aβ (17–42) pentamer with fibril-like structure (U-shaped) is able to penetrate the membrane, through amphiphatic interaction. Instead, monomers and dimers are not able to insert into bilayer in U-shaped conformations. β-harpin structure favors this process, and after the insertion it may convert in a U-shaped form. These simulations show that the U-shaped motif is significantly present both in fibril like and dimer structure. Furthermore, they hypothesize that the trimer is the smallest oligomer with U-shaped conformations that is capable to insert into membrane. The role of cholesterol in binding of Aβ (1–42) onto the bilayer surface has been studied by Yu and Zheng [61], using molecular dynamics simulations. This work is based on the simulations of Aβ (1–42) monomer onto POPC bilayer with different mole fractions of cholesterol. The results show that an increasing mole fraction of cholesterol modifies the structural properties of membrane bringing to a thicker bilayer, more ordered hydrocarbon chain, more hydrophobic surface, and lower lipid mobility. This effect causes a stronger affinity of the peptide for the lipid bilayer because of electrostatic interactions and hydrophobic contacts. Furthermore, calcium ions stabilize the protein adsorption onto bilayer and anα-helix conformation is adopted when peptide is adsorbed onto the surface. Haddadian and co-workers [144] investigated the interaction of unstructured Aβ (1–40) both in form of monomer and fibril-like structure (PDB ID 2M4J) with different lipid bilayers (POPC/Cholesterol, ratio 4:1; POPC/POPG/Cholesterol, 1:1:1; POPC/POPE/POPG 1:1:1), using molecular dynamic simulations, for a total of 20 μs long simulation. Their computational studies start with peptides close to lipid surface. They found that the lipid bilayer accelerates the rate of conversion from random coil to β-sheet structures. Furthermore, also the oligomerization process has a faster kinetic compared to the bulk simulations. The simulation of fibril structure shows that fibrils reduce the lipid fluidity of about 40%. Chahinian and co-workers [62] performed MD simulations of a short Aβ peptide (22–35) in membrane environment to study the role of cholesterol in the oligomerization process. Results show that cholesterol alterates the topology of the peptide tilting α-helix and leading to enhanced interpeptide hydrogen bond network involving Asn27 and Lys28 residues, until the formation of octameric annular-channel structure in a phosphatidylcholine membrane. Nguyen and co-workers [145], using replica exchange molecular dynamic simulations, pointed out the structural property change of fibril-like Aβ (11–40) trimer, interacting with model bilayer. During the interaction between trimer and DPPC model membrane, the secondary structure observed is 44% β-sheet, 54% random coil, 2% turn, and absent α-helix structure. Simulations show two domains in trimer structure: one is deeply inserted into bilayer and contains β-structure (residue 14–19 and 31–37) and the second one is close to lipid head and contains random coil region (residue 11–13, 20–30 and 38–40). Furthermore, free energy calculations underscore that Van der Waals interactions have a prevalent role binding between the Aβ trimer and the membrane. Starting from partially inserted peptide, the behavior of a single Aβ (1–40) molecule within a dipalmitoylphosphatidylcholine (DPPC) bilayer was studied using all-atom MD simulations by Fazli and coworkers [146]. Simulations were performed at three different temperatures (323, 310, and 300 K), the authors found that the peptide remains embedded in the bilayer for all simulation time (at all temperatures). The percentage of the residues inserted into bilayer is higher at 320 K and 310 K than 300 K. This could be due to changes in the bilayer fluidity induced by temperature. The results also show that Aβ (1–40) peptide decreases bilayer thickness and it brings to water insertion into bilayer. This perturbed bilayer, increasing the local fluidity. Goñi and co-workers [147] used Aβ(1–42) monomer and three lipid bilayers composed by palmitoyl-sphingomyelin, dimyristoylphosphatidic acid, and cholesterol in three different proportions to understand how lipid composition (neutral, low and high charged) changes the binding mode and peptide structure onto membrane surface. Binding among lipids and protein are different for the low and high charged bilayers. They found that the effect of charge percentage on both the binding and the structure is not linear. The charged lipids increase this binding, but the highest effect is observed in the case of low charged bilayer, and furthermore it significantly changes protein secondary structure compared to the other kind of bilayers, leading to loss of helix propensity and β-structure appearance. Strodel [148] and coworkers performed long time molecular dynamics simulation of Aβ (1–42) embedded in different phospholipid membrane containing zwitterionic DPPC in the liquid crystalline state or DOPC and negatively charged POPG. They simulate transmembrane β-sheet monomer and tetramer optimized by MD and α-helix structure obtained from NMR measurements. Simulations have evidenced the role played by surface charge and the lipid tail type are determinant for transmembrane stability of Aβ (1–42) with zwitterionic surfaces and unsaturated lipids promoting stability. From the obtained structures, the β-sheet tetramer is most stable as a result of interpeptide interactions. In addition, fast water permeation thought bilayer was observed mainly governed by the lipid type, simulation temperature and Aβ (1–42) conformation. The β-sheet tetramer allows more water molecules to penetrate the bilayer respect to monomeric Aβ. These results support the idea that in the permeation process oligomers rather than monomers play a fundamental role. Also, Strodel group [149] found the same behaviour by simulating membrane bound monomers and tetramers of Aβ (1–42) mutants (wild-type and E22G, D23G, E22G/D23G, K16 M/K28 M and K16 M/E22G/D23G/K28 M). Increased stability for the E22G Aβ(1–42) peptide and a decreased stability for D23G compared to wild-type Ab42, while D23G has the largest membrane-disruptive effect. Water permeation and the role of electrostatic interaction was observed by other authors using long time multiscale molecular dynamic simulations. Some of us [8] used simulations and analytical theory to unveil the early stage of spontaneous self-assembling of 36 molecules of Aβ (1–40) as monomer embedded within POPC lipid matrix. In addition, the authors develop a simple analytical model describing the electrostatic repulsions among water-exposed charged residues, able to predict the presence of distorted structures called

"*frustrated helices*". Large scale (20 ms) Coarse Grained (CG) simulations of 36 replicas confirmed the formation of supramolecular assemblies which resemble a twisted ribbon and fully atomistic simulations have demonstrated the stability of these helical structures. Moreover, CG and atomistic simulations have evidenced membrane curvature operated by 24-mer assembly having cone-shaped inside the membrane. Interestingly, Aβ(1–40) forming 24-mer aggregate assume α-helix conformation with in internal organization by 2-mer and 3-mer units with chiral structure. Head-Gordon and co-worker [150] used a CG model of Aβ (1–42) and model membranes containing phospholipids (POPC, DOPE and POPS) and cholesterol with asymmetric distributions between the two leaflets. The authors found that a highly asymmetric cholesterol distribution of the model lipid membrane thermodynamically favors membrane retention of a fully embedded Aβ (1–42). Particularly, high concentration of cholesterol in the *exo*-leaflet favor the extrusion of the C-terminus into the extracellular space. Matsuzaki and co-workers [151] explored the influence of ganglioside GM1 on the interaction Aβ (1–42) from water phase to model membrane (GM1:Sfingomielin:Cholesterol molar ratio 1:2:2). The authors evidence the role of aromatic side chains of Aβ(1–42) and GM1 oligosaccharide assisted the polypeptide adhesion. Amine group of Lys28 side chain act as anchor to link protein and membrane surface. Moreover, after a first Aβ is adsorbed on the membrane surface a second molecule form a dimer and a third molecule is added a complex forming a 3-mer is formed. We have discussed different scientific works about Aβ polypeptide, a brief summary is reported above in order to underline the most important results. Cholesterol presence changes membrane properties and, consequently, binding affinity between membrane and peptide, leading to structural changes in peptide and even at octamerization process in phosphatidylcholine. Cholesterol leaflets asymmetry brings to increasing peptide retention into membrane. Compared to bulk simulations, membrane foster oligomerization process and its own composition (in particular the charge of polar head) influence the binding with peptide, but this effect is not linearly dependent on the number of charges. Lipids type influences even trans-membrane retention of peptide and water permeability. Insertion of peptide in membrane leads to water permeation, such as has been shown both for monomer and especially for oligomers (4-mer, 8-mer and 24-mer). Oligomers, particularly 8-mer and 24-mer, show alpha-helical structure. Furthermore, simulations have shown that both initial conformation and oligomers dimension are crucial factors for the insertion process. All these observations are summarized in Table 3.

#### 1.9.2. Amylin

In silico simulations of IAPP have been reviewed by different authors [152,153]. Here we discuss latest simulations of IAPP interacting with membrane. In general, the transfer of hIAPP into bilayer can be described, at a first approximation, as divided into three steps: in the first step, the protein assumes a proper optimal conformation and is adsorbed on the membrane surface, in the second step it inserts into bilayer and rearranges its secondary structure; and in the third step aggregates into the bilayer. Mittal and coworkers [154] performed atomistic molecular dynamics simulations on multiple systems containing a full-length amylin monomer and a lipid bilayer, in order to study the changes induced by the presence of membrane. The authors used as simulation starting point the protein conformation in the aqueous phase. They found that IAPP is adsorbed into bilayer surface assuming an α-helix conformation on zwitterionic DOPC membranes, reducing helical propensity on negatively charged DOPS and enhancing the stability of extended helical conformation if the bilayer surface is composed of a DOPC:DOPS mixture (7:3). These results fit with previous work reporting that negatively charged phospholipids induce protein to assume an α-helix conformation. The authors suggest that the average depth profiles of amylin for pure DOPC and mixed membrane are quite similar, especially in the 15–28 region, implying that the only adsorption is not sufficient for imposing helical stability. Considering that the localization of each lipid type around specific residues in the mixed membrane ensemble, it is likely that each residue of the peptide selectively forms contacts with specific head groups, resulting in conditions more favorable for both insertion and helical stabilization.

Schiøtt [155] and coworkers, using all-atom molecular dynamics simulations, studied the effect of pH on amylin interacting with mixture of negatively and zwitterionic charged phospholipids (molar ratio 1:3). In this set of simulations, the authors adopt as a starting point amylin as monomer in the aqueous environment by considering two possible configurations. One configuration is amylin as α-helix with histidine 18 pointing to the membrane surface and a second configuration where His18 is located in the opposite side with respect to the membrane surface. These simulations suggest that the pH acts as switch on fibrils formation. A low pH, where His18 is protonated, strong interactions of the C-terminal part of the peptide with the membrane are observed, with resulting immobilization of the C-terminal part on the membrane surface. This might constitute a mechanism by which low pH inhibits fibrils formation. Instead, at neutral pH the C-terminal part of amylin, displays a highly dynamic, unfolded state, which interacts with the

**Table 3**
Main remarks, model membrane used in molecular dynamics investigation of Aβ polypeptides.

| Peptide | Membrane | Remark | Ref |
|---|---|---|---|
| $A\beta_{1-40}$ | POPC | Early steps of self-Assembly of 36 replicas of monomer (alpha-helical conformation) in POPC environment. Presence of distorted structures called "frustated helices" is predicted based on an analytical model. | [8] |
| $A\beta_{1-42}$ | POPC/cholesterol | Cholesterol brings to more rigid membrane and increases the binding with peptide. Helix propensity is taken into account. | [61] |
| $A\beta_{22-35}$ | PC/cholesterol | Formation of octameric channel-like structure | [62] |
| $A\beta_{1-42}$ 4-mer | POPC and cholesterol-rich raft membrane | Tetramer structure changes from ellipsoid-shape to rod-like structure. Rigidity of membrane is influenced by tetramer | [142] |
| $A\beta_{17-42}$ | DOPC | Topology influences the capability of protein in insertion process | [143] |
| $A\beta_{1-40}$ | POPC/Cholesterol(4:1) POPC/POPG/Cholesterol (1:1:1) POPC/POPE/POPG (1:1:1) | Speed-up of oligomerization process. Adsorption of fibril-like structure leads to less bilayer fluidity of 40%. | [144] |
| $A\beta_{11-40}$ 3-mer | DPPC | DPPC leads to random-coil and B-sheet structure. B-sheet fragment stays embedded and random-coil fraction stays out of membrane | [145] |
| $A\beta_{1-40}$ | DPPC | Peptide insertion into membrane changes bilayer thickness and water permeability. This process is temperature dependent | [146] |
| $A\beta_{1-42}$ | Neutral/low and high charge bilayer | Charge fraction influences the binding between protein and membrane surface. This effect is not linear with charge. | [147] |
| $A\beta_{1-42}$ | DPPC (or DOPC and POPG) | Tetramer is most stable than monomer into bilayer. Tetramer bring to more water permeation | [148] |
| $A\beta_{1-42}$ | Mix of POPC, DOPE and POPS with asymmetric leaflets cholesterol fraction | Peptide retention is favored by high cholesterol concentration asymmetry | [150] |
| $A\beta_{1-42}$ | GM1, myelin and cholesterol in ratio 1:2:2 | Aromatic interaction between peptide side-chain and GM1 promotes protein adsorption onto lipid surface. Adsorbed peptide acts as a nucleation seed. | [151] |

membrane significantly less than the N-terminal part and fibrils formation are favored. Zheng and coworkers [156] performed all-atom MD simulations to study the adsorption, orientation, and surface interaction of IAPP with membranes. Starting from water solution, different aggregates sizes were used such as monomer to tetramer and protein conformations as monomer with α-helix and tetramer with β-sheet-rich U-turn upon adsorption on the lipid bilayers composed of both pure zwitterionic POPC and mixed zwitterionic POPC/POPE (3:1) phospholipids. Authors found a stronger interaction of IAPP and POPC/POPE mixed bilayer surface rather than for pure POPC. This result is explained considering the electrostatic interactions between of Lys1 and Arg11, and negatively charged lipid head group phosphate. The same sites of interactions were found by Wei and coworkers [81] using double negatively charged lipid POPG interacting with monomer and dimer of full length amylin. Authors used all-atom MD simulations, choosing as initial configurations the one where the N-terminal region of hIAPP is pre-inserted in POPG bilayer. During peptide-lipid interaction process, peptide dimerization occurs mostly through the C-terminal 20–37 region containing the amyloidogenic 20–29-residue segment NNFGAIL. Phe23 side chain of this region seems to have a crucial role in the aggregation process [157–161]. This finding is also consistent with simulations of IAPP in presence of model membranes interacting with some small molecules, such as resveratrol. Resveratrol significantly perturbs the interaction of IAPP with negatively charged membranes by anchoring specific hydrophobic regions (23FGA25 and 32VGS34) of the peptide and forming a stable 1:2 IAPP:resveratrol complex at the water/membrane interphase [162]. Also Phe15 side chain of IAPP is not required for aggregation process and membrane damage but influences the kinetic of self-assembling process [80,163]. These results support a general theory [164] suggesting that aromatic residues, although capable of affecting the self-assembly kinetics of small peptides and peptide-membrane interactions, are not essential either for amyloid formation or membrane leakage, and indicate that other factors such as β-sheet propensity, size and hydrophobicity of the side chain act synergistically to determine peptide properties. Amyloid formation is also influenced by disulfide bridge. In fact, systems containing the Cys2-Cys7 disulfide bridge exhibited a greater stability and a decreased tendency to evolve into β-sheet rich structures if compared to the disulfide-depleted variants. Conversely, the stability of assemblies constituted by the rat isoforms was shown to be independent from the presence of the disulfide bridge [165]. On other hand, 1–19 IAPP residue transfers from bulk water solution as random coil to membrane-water interface, displaying high helix propensity especially in the L12-L16 region [166]. The key role played by Arg11 and Phe15 self-assembling process was evidenced using inhibitor derived from insulin which are able to block the two sides chain [167].

Now we discuss simulations results using as starting point protein embedded in model membrane. IAPP as monomer inserted within membrane is stable as α-helix, tilted towards to membrane surface. This configuration remains stable also if an hydrophobic side chain (Ile26) is replaced with hydrophilic amino acid (Pro) [168,169]. Yang [170] and coworkers using all-atom bias-exchange meta-dynamics (BE-Meta) and unbiased molecular dynamics simulations investigated the behavior of IAPP dimers in model membrane containing DOPC:DOPS molar ratio 7:3 which is consistent with the observed ratio of neutral to anionic phospholipids in β-cell membranes. Comparison among free energy profiles simulation data shows that the 20–29 region plays a key role in the aggregation of IAPP on the membrane environments. The formation of β-sheet structure on this region would guide the formation of toxic species. Moreover, during simulation, different intermediate conformations were evidenced, including α-helix, β-sheet and full-disordered ones. The role played of N-terminus region in the interaction of IAPP and membrane was evidenced using both experimental and simulation investigation. De Pablo [171] and coworkers investigated the amyloidogenicity and cytotoxicity mechanism of IAPP using model membranes. These authors suggest a “detergent-like” mechanism operated by N-terminus of IAPP on lipid membrane and the kinetically competition between poration and aggregation process with consequent inhibition of (pore formation due to aggregation phenomena). Karttunen and coworkers [168] have used long time (μs time) multi-scale simulation and theory to investigate IAPP aggregation into membranes. The authors in this report used36 human IAPP as monomers embedded in POPC membrane employing as control the same system containing rat IAPP. The different electrostatic dipolar interactions cause the formation of pentameric aggregates with the hydrophobic residues facing the membrane core and stabilizing water-conducting pores. These results, according previous experimental data, give predictions about pore sizes, the number of hIAPP molecules, and aggregate morphology. Moreover, it was evidenced the importance of curvature-induced stress during the early stages of hIAPP assembly and the α-helical structures over β-sheets of IAPP self-assembling. Finally, Strodel and coworkers [172] take into account as starting configuration point the case where preformed IAPP aggregates came from NMR measurements and these are embedded into model membranes and then simulated. They tested the orientation and stability of U-shaped IAPP aggregates containing β-sheet embedded DPPG model membranes. The authors considered trimer and tetramer species interacting with mono and bilayer and they found that both aggregates are stable into hydrophobic region bilayer, assuming a 60° tilted configuration. Moreover, although the small size of aggregates, ion channel activity for water and $Na^+$ ion was evidenced. Zheng and coworkers [173] studied large (12–36 mer) preformed IAPP ion channels embedded into DOPC bilayer. Authors used NMR-derived β-strand-turn-β-strand motif as a building block in order to computationally construct a series of annular-like hIAPP structures with different sizes and topologies. Interestingly, authors showed that the channels' shapes, morphologies and dimensions are consistent with what was found performing atomic force microscopy experiments. Also, the latter was found to be comparable with the modeled channels for Aβ, the β2-microglobulin-derived K3 peptides, and the β-hairpin-based channels of antimicrobial peptide PG-1. In this section we have reported many computational studies about amylin and we think that it would be useful to summarize the most important results. In presence of membrane there are different structural properties that influence protein aggregation. Simulations show that pH acts (on Hys18) as an aggregation switch. Peptide region 20–37 (20–29 in particular) seems to have a key role in aggregation process in membrane environment. Furthermore, simulations underscore the role of Arg11, Phe15 and Phe23 in self-assembly. Secondary structure propensity changes depending on lipid composition and presence of disulfide bridge. Simulations of trimer and tetramer in DPPG have demonstrated ion channel activity and good agreement between AFM data and simulations about some annular-like structure it was shown. Other results have shown that self-assembly process from many replicas lead to pentameric channel-like structure with water permeability and alpha-helical structure. Detergent-Like mechanism was proposed from N-terminus residue. The whole of the results in this section are summarized in Table 4.

## 2. Conclusions

Current literature consistently suggests that the detrimental effects of amyloiodogenic peptides as Aβ or hIAPP on plasma membrane integrity could be one of the most relevant molecular mechanisms underlying amyloid toxicity. In the last two decades several distinct mechanisms have been proposed to explain the amyloid-induced membrane damage. The “channel hypothesis”, fibril mediated stress to the lipid bilayer and the detergent-like effect just to mention a few of the many proposed theories, have been alternatively used to interpret the observed effects of differently assembled amyloid peptides on membrane structure. However, despite the wealth of data collected so far, the real nature of the toxic lipid/peptide complex is still object of intense debate. Recently, some of us have contributed to propose a

**Table 4**
Summary of all the works here reviewed focusing on the Simulation of membrane boun hIAPP.

| Peptide | Membrane | Remark | Ref |
|---|---|---|---|
| Amylin 1–37 1-mer and 2-mer | POPG | Dimerization process is mediated by 20–37 peptide region. A special role is played by Phe23. | [81] |
| Amylin 1–37 1-mer | DOPC and DOPS | Charge of lipids influences the structure property of peptide adsorbed onto lipid surface. Negative lipids reduce helix propensity. | [154] |
| Amylin 1–37 1-mer | DVPS and DVPC | pH is a switch for peptide aggregation. | [155] |
| Amylin 1–37 1-mer and 4-mer | POPC and POPE | Lipid composition affects the strength of interaction between peptide and lipid surface. Lys1 and Arg11 play major role in this interaction. | [156] |
| Amylin 1–37 1-mer | POPG | Role of resveratrol in peptide - membrane surface interaction. | [162] |
| Amylin 12–18 andAmylin 21–27 | DMPC | Role of different fragments in aggregation process of peptide embedded into a bilayer | [163] |
| Amylin 1–37 | No membrane | Influence of Cys2-Cys7 disulfide bridge in amyloids formation. | [165] |
| Amylin 1–19 | POPG | Role of 12–16 region in conformational change (helical propensity) at water-membrane interface. | [166] |
| Amylin 1–37 | DOPC, POPC | Thermodynamic model to predict the morphology and stability of Amylin aggregates. In addition, formation of pentameric channel-like structure and water permeation. | [168] |
| Amylin 1–37 | DOPC | Role of I26P mutation of protein embedded into bilayer by free energy calculation. | [169] |
| Amylin 1–37 2-mer | DOPC:DOPS 7:3 | Role of 20–29 region in the dimerization process by free energy profile. Different intermediate conformations include α-helix, β-sheet and full-disordered ones. | [170] |
| hIAPP 1–20, hIAPP 23–37, rIAPP 1–20 | POPC | Free energy profile of insertion in bilayer and role of different fragments in insertion depth | [171] |
| Amylin 1–37 3-mer and 4-mer | DPPG | Trimer and tetramer are stable in lipid environment. Ions and water channel activity was evidenced. | [172] |
| Amylin 1–37 12–36 mer With channel-like structure | DOPC | Morphology and shape of channel structure was fitted with AFM experimental results. | [173] |

"two-step" mechanism involving pore formation and, subsequently, fibril-assisted lipid extraction from the bilayer as a new, general paradigm to describe aberrant lipid/amyloid interactions. Undoubtedly, the two-step mechanism has proven to be helpful in explaining some puzzling and apparently controversial experimental observations. Nevertheless, a full understanding of the mutual interference of the two steps and the role played by external factors as well as membrane composition remains elusive. Here we have reviewed, also in the light of this new hypothesis, the latest advances in our knowledge concerning the effects of cations (Calcium in particular), lipid composition and cholesterol on the two steps of membrane leakage. All data collected so far suggest that both $Ca^{2+}$ ions and cholesterol, although by means of different driving forces (i.e. electrostatic and hydrophobic) may contribute to recruit amyloid-competent lipids within the lipid bilayer by inducing abnormal phase segregation. Computer simulations, due to their higher spatial and time resolution have also allowed to depict the early stages of amyloid-membrane interactions evidencing that both Aβ and hIAPP may permeate the membrane only after their assembly in dimeric, trimeric or pentameric forms. In particular, pentameric assemblies, are responsible for the unspecific passage of ions across the membrane. Next, in the very early stages of membrane adhesion, peptides adopt a mainly α-helical conformation, in apparent contrast with experimental data evidencing β-sheet rich proteins when embedded in the lipid matrix. The process of membrane permeation by peptides, however, escapes a description by current molecular simulation engines. It would be advisable in the next future, considering the expected improvements in the computational resources, to extend simulations to a larger time scale in order to achieve a full match between computational and experimental results. In conclusion, our increased knowledge of the molecular events at the roots of amyloid toxicity shifts the goal of the research towards the design of molecules that can hinder both peptide assembly and membrane damage. Unfortunately, we are still missing a number of details about the cross correlations linking the many concurring adverse factors (including those reviewed in the present report) in triggering harmful membrane interactions. Hopefully, parallel in silico and biophysical studies performed using more sophisticated membrane-mimicking systems will help to fill this gap allowing, in turn, a better control of amyloid toxicity.

## Conflict of interest

The authors declare no conflict of interest.

## Transparency document

The Transparency document associated with this article can be found, in online version.

## Acknowledgements

This work was financially supported by the Italian MIUR program PRIN 20157WZM8A.

## References

[1] P.E. Wright, H.J. Dyson, Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm, J. Mol. Biol. 293 (1999) 321–331.
[2] V.N. Uversky, A.K. Dunker, Understanding protein non-folding, Biochim. Biophys. Acta Protein Proteomics 1804 (2010) 1231–1264.
[3] V.N. Uversky, C.J. Oldfield, A.K. Dunker, Intrinsically disordered proteins in human diseases: introducing the D2 concept, Annu. Rev. Biophys. 37 (2008) 215–246.
[4] A. Abedini, D.P. Raleigh, A critical assessment of the role of helical intermediates in amyloid formation by natively unfolded proteins and polypeptides, Protein Eng. Des. Sel. 22 (2009) 453–459.
[5] C.M. Dobson, Protein misfolding, evolution and disease, Trends Biochem. Sci. 24 (1999) 329–332.
[6] R.J. Perrin, W.S. Woods, D.F. Clayton, J.M. George, Interaction of human α-synuclein and Parkinson's disease variants with phospholipids structural analysis using site-directed mutagenesis, J. Biol. Chem. 275 (2000) 34393–34398.
[7] D. Eliezer, E. Kutluay, R. Bussell, G. Browne, Conformational properties of α-synuclein in its free and lipid-associated states11Edited by P E Wright, J. Mol. Biol. 307 (2001) 1061–1073.
[8] M. Pannuzzo, D. Milardi, A. Raudino, M. Karttunen, C. La Rosa, Analytical model and multiscale simulations of Aβ peptide aggregation in lipid membranes: towards a unifying description of conformational transitions, oligomerization and membrane damage, Phys. Chem. Chem. Phys. 15 (2013) 8940–8951.
[9] B. Cheng, H. Gong, H. Xiao, R.B. Petersen, L. Zheng, K. Huang, Inhibiting toxic aggregation of amyloidogenic proteins: a therapeutic strategy for protein misfolding diseases, Biochim. Biophys. Acta Gen. Subj. 1830 (2013) 4860–4871.
[10] A. Ott, R.P. Stolk, F. Van Harskamp, H.A.P. Pols, A. Hofman, M.M.B. Breteler, Diabetes mellitus and the risk of dementia the Rotterdam study, Neurology 53 (1999) (1937–1937).
[11] G. Cheng, C. Huang, H. Deng, H. Wang, Diabetes as a risk factor for dementia and mild cognitive impairment: a meta-analysis of longitudinal studies, Intern. Med. J. 42 (2012) 484–491.
[12] R.A. Stelzma, H.N. Schnitzlein, F.R. Murllagh, VIEWPOINT an English I'ranslation of Alzheimer's 1907 paper, "ijber eine eigenartige Erlranliung der Hirnrinde", Clin. Anat. 8 (1995) 429–443.
[13] G.G. Glenner, C.W. Wong, Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein, Biochem. Biophys. Res. Commun. 120 (1984) 885–890.
[14] V.M. Lee, B.J. Balin, L. Otvos, J.Q. Trojanowski, A68: a major subunit of paired helical filaments and derivatized forms of normal Tau, Science 251 (1991) 675–678.
[15] D.J. Selkoe, J. Hardy, The amyloid hypothesis of Alzheimer's disease at 25 years,

EMBO Mol. Med. 8 (2016) 595–608.
[16] R.A. Armstrong, A critical analysis of the "amyloid cascade hypothesis", Folia Neuropathol. 52 (2014) 211–225.
[17] J. Hardy, N. Bogdanovic, B. Winblad, E. Portelius, N. Andreasen, A. Cedazo-Minguez, H. Zetterberg, Pathways to Alzheimer's disease, J. Intern. Med. 275 (2014) 296–303.
[18] M. Luczkowski, "No screams and cries will convince us that white is white and black is black", an ode to the defenders of amyloid cascade hypothesis of Alzheimer's disease, Coord. Chem. Rev. 327–328 (2016) 35–42.
[19] M. Rowinska-Zyrek, M. Salerno, H. Kozlowski, Neurodegenerative diseases – understanding their molecular bases and progress in the development of potential treatments, Coord. Chem. Rev. 284 (2015) 298–312.
[20] W.J. Geldenhuys, A.S. Darvesh, Pharmacotherapy of Alzheimer's disease: current and future trends, Expert. Rev. Neurother. 15 (2015) 3–5.
[21] G. Grasso, A.M. Santoro, V. Lanza, D. Sbardella, G.R. Tundo, C. Ciaccio, S. Marini, M. Coletta, D. Milardi, The double faced role of copper in Aβ homeostasis: a survey on the interrelationship between metal dyshomeostasis, UPS functioning and autophagy in neurodegeneration, Coord. Chem. Rev. 347 (2017) 1–22.
[22] D. Goldgaber, M.I. Lerman, O.W. McBride, U. Saffiotti, D.C. Gajdusek, Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease, Science 235 (1987) 877–880.
[23] E. Buoso, C. Lanni, G. Schettini, S. Govoni, M. Racchi, β-Amyloid precursor protein metabolism: focus on the functions and degradation of its intracellular domain, Pharmacol. Res. 62 (2010) 308–317.
[24] S.S. Sisodia, Beta-amyloid precursor protein cleavage by a membrane-bound protease, Proc. Natl. Acad. Sci. 89 (1992) 6075–6079.
[25] E. Terzi, G. Hölzemann, J. Seelig, Self-association of β-amyloid peptide (1–40) in solution and binding to lipid membranes, J. Mol. Biol. 252 (1995) 633–642.
[26] C. Hertel, E. Terzi, N. Hauser, R. Jakob-Rotne, J. Seelig, J.A. Kemp, Inhibition of the electrostatic interaction between beta-amyloid peptide and membranes prevents beta-amyloid-induced toxicity, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 9412–9416.
[27] H.B. Pollard, E. Rojas, N. Arispe, A new hypothesis for the mechanism of amyloid toxicity, based on the calcium channel activity of amyloid beta protein (A beta P) in phospholipid bilayer membranes, Ann. N. Y. Acad. Sci. 695 (1993) 165–168.
[28] Y. Hirakura, M.C. Lin, B.L. Kagan, Alzheimer amyloid abeta1-42 channels: effects of solvent, pH, and Congo Red, J. Neurosci. Res. 57 (1999) 458–466.
[29] J.J. Kremer, M.M. Pallitto, D.J. Sklansky, R.M. Murphy, Correlation of beta-amyloid aggregate size and hydrophobicity with decreased bilayer fluidity of model membranes, Biochemistry 39 (2000) 10309–10318.
[30] J. McLaurin, A. Chakrabartty, Membrane disruption by Alzheimer beta-amyloid peptides mediated through specific binding to either phospholipids or gangliosides implications for neurotoxicity, J. Biol. Chem. 271 (1996) 26482–26489.
[31] C.M. Yip, J. McLaurin, Amyloid-beta peptide assembly: a critical step in fibrillogenesis and membrane disruption, Biophys. J. 80 (2001) 1359–1371.
[32] J.I. Kourie, Mechanisms of amyloid beta protein-induced modification in ion transport systems: implications for neurodegenerative diseases, Cell. Mol. Neurobiol. 21 (2001) 173–213.
[33] B.L. Kagan, Y. Hirakura, R. Azimov, R. Azimova, The channel hypothesis of Huntington's disease, Brain Res. Bull. 56 (2001) 281–284.
[34] D.A. Butterfield, J. Drake, C. Pocernich, A. Castegna, Evidence of oxidative damage in Alzheimer's disease brain: central role for amyloid beta-peptide, Trends Mol. Med. 7 (2001) 548–554.
[35] J. Abad-Rodriguez, M.D. Ledesma, K. Craessaerts, S. Perga, M. Medina, A. Delacourte, C. Dingwall, B.D. Strooper, C.G. Dotti, Neuronal membrane cholesterol loss enhances amyloid peptide generation, J. Cell Biol. 167 (2004) 953–960.
[36] K.J. Korshavn, C. Satriano, Y. Lin, R. Zhang, M. Dulchavsky, A. Bhunia, M.I. Ivanova, Y.-H. Lee, C. La Rosa, M.H. Lim, A. Ramamoorthy, Reduced lipid bilayer thickness regulates the aggregation and cytotoxicity of amyloid-β, J. Biol. Chem. 292 (2017) 4638–4650.
[37] P.N. Lacor, M.C. Buniel, P.W. Furlow, A.S. Clemente, P.T. Velasco, M. Wood, K.L. Viola, W.L. Klein, Abeta oligomer-induced aberrations in synapse composition, shape, and density provide a molecular basis for loss of connectivity in Alzheimer's disease, J. Neurosci. 27 (2007) 796–807.
[38] A. Eckert, S. Hauptmann, I. Scherping, J. Meinhardt, V. Rhein, S. Dröse, U. Brandt, M. Fändrich, W.E. Müller, J. Götz, Oligomeric and fibrillar species of beta-amyloid (A beta 42) both impair mitochondrial function in P301L tau transgenic mice, J. Mol. Med. 86 (2008) 1255–1267.
[39] M. Bucciantini, C. Cecchi, Biological membranes as protein aggregation matrices and targets of amyloid toxicity, Methods Mol. Biol. 648 (2010) 231–243.
[40] T.L. Williams, B.R.G. Johnson, B. Urbanc, A.T.A. Jenkins, S.D.A. Connell, L.C. Serpell, Aβ42 oligomers, but not fibrils, simultaneously bind to and cause damage to ganglioside-containing lipid membranes, Biochem. J. 439 (2011) 67–77.
[41] M.F. Sciacca, S.A. Kotler, J.R. Brender, J. Chen, D. Lee, A. Ramamoorthy, Two-step mechanism of membrane disruption by Aβ through membrane fragmentation and pore formation, Biophys. J. 103 (2012) 702–710.
[42] A. Kakio, S. Nishimoto, K. Yanagisawa, Y. Kozutsumi, K. Matsuzaki, Interactions of amyloid beta-protein with various gangliosides in raft-like membranes: importance of GM1 ganglioside-bound form as an endogenous seed for Alzheimer amyloid, Biochemistry 41 (2002) 7385–7390.
[43] K. Ikeda, K. Matsuzaki, Driving force of binding of amyloid beta-protein to lipid bilayers, Biochem. Biophys. Res. Commun. 370 (2008) 525–529.
[44] 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 (1993) 567–571.
[45] T.-L. Lau, E.E. Ambroggio, D.J. Tew, R. Cappai, C.L. Masters, G.D. Fidelio, K.J. Barnham, F. Separovic, Amyloid-beta peptide disruption of lipid membranes and the effect of metal ions, J. Mol. Biol. 356 (2006) 759–770.
[46] K. Matsuzaki, How do membranes initiate Alzheimer's disease? Formation of toxic amyloid fibrils by the amyloid β-protein on ganglioside clusters, Acc. Chem. Res. 47 (2014) 2397–2404.
[47] C. Peterson, J.E. Goldman, Alterations in calcium content and biochemical processes in cultured skin fibroblasts from aged and Alzheimer donors, Proc. Natl. Acad. Sci. U. S. A. 83 (1986) 2758–2762.
[48] A. Adunsky, D. Baram, M. Hershkowitz, Y.A. Mekori, Increased cytosolic free calcium in lymphocytes of Alzheimer patients, J. Neuroimmunol. 33 (1991) 167–172.
[49] G.E. Gibson, L. Toral-Barza, Cytosolic free calcium in lymphoblasts from young, aged and Alzheimer subjects, Mech. Ageing Dev. 63 (1992) 1–9.
[50] S. Tamamizu-Kato, M.G. Kosaraju, H. Kato, V. Raussens, J.-M. Ruysschaert, V. Narayanaswami, Calcium-triggered membrane interaction of the α-synuclein acidic tail, Biochemistry 45 (2006) 10947–10956.
[51] S. Ohnishi, T. Ito, Calcium-induced phase separations in phosphatidylserine-phosphatidylcholine membranes, Biochemistry 13 (1974) 881–887.
[52] V. Gerke, C.E. Creutz, S.E. Moss, Annexins: linking $Ca^{2+}$ signalling to membrane dynamics, Nat. Rev. Mol. Cell Biol. 6 (2005) 449–461.
[53] U. Rescher, V. Gerke, Annexins–unique membrane binding proteins with diverse functions, J. Cell Sci. 117 (2004) 2631–2639.
[54] M.F. Sciacca, M. Pappalardo, D. Milardi, D.M. Grasso, C. La Rosa, Calcium-activated membrane interaction of the islet amyloid polypeptide: implications in the pathogenesis of type II diabetes mellitus, Arch. Biochem. Biophys. 477 (2008) 291–298.
[55] M.F. Sciacca, D. Milardi, G.M. Messina, G. Marletta, J.R. Brender, A. Ramamoorthy, C. La Rosa, Cations as switches of amyloid-mediated membrane disruption mechanisms: calcium and IAPP, Biophys. J. 104 (2013) 173–184.
[56] D.L. Sparks, S.W. Scheff, J.C. Hunsaker, H. Liu, T. Landers, D.R. Gross, Induction of Alzheimer-like β-amyloid Immunoreactivity in the brains of rabbits with dietary cholesterol, Exp. Neurol. 126 (1994) 88–94.
[57] R.W. Haley, J.M. Dietschy, Is there a connection between the concentration of cholesterol circulating in plasma and the rate of neuritic plaque formation in Alzheimer disease? Arch. Neurol. 57 (2000) 1410–1412.
[58] A. Kakio, S. Nishimoto, K. Yanagisawa, Y. Kozutsumi, K. Matsuzaki, Cholesterol-dependent formation of GM1 ganglioside-bound amyloid β-protein, an endogenous seed for Alzheimer amyloid, J. Biol. Chem. 276 (2001) 24985–24990.
[59] M. Molander-Melin, K. Blennow, N. Bogdanovic, B. Dellheden, J.-E. Mansson, P. Fredman, Structural membrane alterations in Alzheimer brains found to be associated with regional disease development; increased density of gangliosides GM1 and GM2 and loss of cholesterol in detergent-resistant membrane domains, J. Neurochem. 92 (2005) 171–182.
[60] A. Colell, A. Fernández, J.C. Fernández-Checa, Mitochondria, cholesterol and amyloid β peptide: a dangerous trio in Alzheimer disease, J. Bioenerg. Biomembr. 41 (2009) 417–423.
[61] X. Yu, J. Zheng, Cholesterol promotes the interaction of Alzheimer β-amyloid monomer with lipid bilayer, J. Mol. Biol. 421 (2012) 561–571.
[62] C. Di Scala, J.-D. Troadec, C. Lelièvre, N. Garmy, J. Fantini, H. Chahinian, Mechanism of cholesterol-assisted oligomeric channel formation by a short Alzheimer β-amyloid peptide, J. Neurochem. 128 (2014) 186–195.
[63] S.V. Chochina, N.A. Avdulov, U. Igbavboa, J.P. Cleary, E.O. O'Hare, W.G. Wood, Amyloid β-peptide1-40 increases neuronal membrane fluidity: role of cholesterol and brain region, J. Lipid Res. 42 (2001) 1292–1297.
[64] W.G. Wood, F. Schroeder, U. Igbavboa, N.A. Avdulov, S.V. Chochina, Brain membrane cholesterol domains, aging and amyloid beta-peptides, Neurobiol. Aging 23 (2002) 685–694.
[65] M. Palmieri, G. Malgieri, L. Russo, I. Baglivo, S. Esposito, F. Netti, A. Del Gatto, I. De Paola, L. Zaccaro, P.V. Pedone, Structural Zn (II) implies a switch from fully cooperative to partly downhill folding in highly homologous proteins, J. Am. Chem. Soc. 135 (2013) 5220–5228.
[66] C.C. Curtain, F.E. Ali, D.G. Smith, A.I. Bush, C.L. Masters, K.J. Barnham, Metal ions, pH, and cholesterol regulate the interactions of Alzheimer's disease amyloid-β peptide with membrane lipid, J. Biol. Chem. 278 (2003) 2977–2982.
[67] S.-R. Ji, Y. Wu, S. Sui, Cholesterol is an important factor affecting the membrane insertion of beta-amyloid peptide (A beta 1-40), which may potentially inhibit the fibril formation, J. Biol. Chem. 277 (2002) 6273–6279.
[68] M.-S. Lin, L.-Y. Chen, S.S.S. Wang, Y. Chang, W.-Y. Chen, Examining the levels of ganglioside and cholesterol in cell membrane on attenuation the cytotoxicity of beta-amyloid peptide, Colloids Surf. B: Biointerfaces 65 (2008) 172–177.
[69] C. Hertel, E. Terzi, N. Hauser, R. Jakob-Rotne, J. Seelig, J.A. Kemp, Inhibition of the electrostatic interaction between beta-amyloid peptide and membranes prevents beta-amyloid-induced toxicity, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 9412–9416.
[70] E.L. Opie, The relation OE diabetes mellitus to lesions of the pancreas hyaline degeneration of the islands OE langerhans, J. Exp. Med. 5 (1901) 527.
[71] G.J. Cooper, A.C. Willis, A. Clark, R.C. Turner, R.B. Sim, K.B. Reid, Purification and characterization of a peptide from amyloid-rich pancreases of type 2 diabetic patients, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 8628–8632.
[72] P. Westermark, C. Wernstedt, E. Wilander, D.W. Hayden, T.D. O'Brien, K.H. Johnson, Amyloid fibrils in human insulinoma and islets of Langerhans of the diabetic cat are derived from a neuropeptide-like protein also present in normal islet cells, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 3881–3885.
[73] H. King, R.E. Aubert, W.H. Herman, Global burden of diabetes, 1995-2025:

prevalence, numerical estimates, and projections, Diabetes Care 21 (1998) 1414–1431.
[74] P.Z. Zimmet, K.G.M.M. Alberti, Epidemiology of diabetes—status of a pandemic and issues around metabolic surgery, Diabetes Care 39 (2016) 878–883.
[75] E.T.A.S. Jaikaran, A. Clark, Islet amyloid and type 2 diabetes: from molecular misfolding to islet pathophysiology, Biochim. Biophys. Acta (BBA) - Mol. Basis Dis. 1537 (2001) 179–203.
[76] T. Gurlo, S. Ryazantsev, C. Huang, M.W. Yeh, H.A. Reber, O.J. Hines, T.D. O'Brien, C.G. Glabe, P.C. Butler, Evidence for proteotoxicity in beta cells in type 2 diabetes: toxic islet amyloid polypeptide oligomers form intracellularly in the secretory pathway, Am. J. Pathol. 176 (2010) 861–869.
[77] J. Zhao, R. Hu, M.F.M. Sciacca, J.R. Brender, H. Chen, A. Ramamoorthy, J. Zheng, Non-selective ion channel activity of polymorphic human islet amyloid polypeptide (amylin) double channels, Phys. Chem. Chem. Phys. 16 (2014) 2368–2377.
[78] M.F.M. Sciacca, J.R. Brender, D.-K. Lee, A. Ramamoorthy, Phosphatidylethanolamine enhances amyloid fiber-dependent membrane fragmentation, Biochemistry 51 (2012) 7676–7684.
[79] S. Scalisi, M.F.M. Sciacca, G. Zhavnerko, D.M. Grasso, G. Marletta, C. La Rosa, Self-assembling pathway of HiApp fibrils within lipid bilayers, Chembiochem 11 (2010) 1856–1859.
[80] D. Milardi, M.F.M. Sciacca, M. Pappalardo, D.M. Grasso, C. La Rosa, The role of aromatic side-chains in amyloid growth and membrane interaction of the islet amyloid polypeptide fragment LANFLVH, Eur. Biophys. J. 40 (2011) 1–12.
[81] Y. Zhang, Y. Luo, Y. Deng, Y. Mu, G. Wei, Lipid interaction and membrane perturbation of human islet amyloid polypeptide monomer and dimer by molecular dynamics simulations, PLoS One 7 (2012) e38191.
[82] A. Lorenzo, B. Razzaboni, G.C. Weir, B.A. Yankner, Pancreatic islet cell toxicity of amylin associated with type-2 diabetes mellitus, Nature 368 (1994) 756.
[83] S. Janciauskiene, B. Ahrén, Fibrillar islet amyloid polypeptide differentially affects oxidative mechanisms and lipoprotein uptake in correlation with cytotoxicity in two insulin-producing cell lines, Biochem. Biophys. Res. Commun. 267 (2000) 619–625.
[84] P.C. May, L.N. Boggs, K.S. Fuson, Neurotoxicity of human amylin in rat primary hippocampal cultures: similarity to Alzheimer's disease amyloid-beta neurotoxicity, J. Neurochem. 61 (1993) 2330–2333.
[85] P.K. Wagoner, C. Chen, J.F. Worley, I.D. Dukes, G.S. Oxford, Amylin modulates beta-cell glucose sensing via effects on stimulus-secretion coupling, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 9145–9149.
[86] M. Kawahara, Y. Kuroda, N. Arispe, E. Rojas, Alzheimer's beta-amyloid, human islet amylin, and prion protein fragment evoke intracellular free calcium elevations by a common mechanism in a hypothalamic GnRH neuronal cell line, J. Biol. Chem. 275 (2000) 14077–14083.
[87] C. Behl, J.B. Davis, F.G. Klier, D. Schubert, Amyloid beta peptide induces necrosis rather than apoptosis, Brain Res. 645 (1994) 253–264.
[88] P.C. May, B.D. Gitter, D.C. Waters, L.K. Simmons, G.W. Becker, J.S. Small, P.M. Robison, β-amyloid peptide in vitro toxicity: lot-to-lot variability, Neurobiol. Aging 13 (1992) 605–607.
[89] D. Schubert, C. Behl, R. Lesley, A. Brack, R. Dargusch, Y. Sagara, H. Kimura, Amyloid peptides are toxic via a common oxidative mechanism, Proc. Natl. Acad. Sci. U. S. A. 92 (1995) 1989–1993.
[90] J. Janson, R.H. Ashley, D. Harrison, S. McIntyre, P.C. Butler, The mechanism of islet amyloid polypeptide toxicity is membrane disruption by intermediate-sized toxic amyloid particles, Diabetes 48 (1999) 491–498.
[91] B. Konarkowska, J.F. Aitken, J. Kistler, S. Zhang, G.J.S. Cooper, The aggregation potential of human amylin determines its cytotoxicity towards islet β-cells, FEBS J. 273 (2006) 3614–3624.
[92] M. Anguiano, R.J. Nowak, P.T. Lansbury, Protofibrillar islet amyloid polypeptide permeabilizes synthetic vesicles by a pore-like mechanism that may be relevant to type II diabetes, Biochemistry 41 (2002) 11338–11343.
[93] C. Huang, T. Gurlo, L. Haataja, S. Costes, M. Daval, S. Ryazantsev, X. Wu, A.E. Butler, P.C. Butler, Calcium-activated calpain-2 is a mediator of beta cell dysfunction and apoptosis in type 2 diabetes, J. Biol. Chem. 285 (2010) 339–348.
[94] T.A. Mirzabekov, M.C. Lin, B.L. Kagan, Pore formation by the cytotoxic islet amyloid peptide amylin, J. Biol. Chem. 271 (1996) 1988–1992.
[95] Y. Hirakura, W.W. Yiu, A. Yamamoto, B.L. Kagan, Amyloid peptide channels: blockade by zinc and inhibition by Congo red (amyloid channel block), Int. J. Experiment. Clin. Investig. 7 (2000) 194–199.
[96] A. Quist, I. Doudevski, H. Lin, R. Azimova, D. Ng, B. Frangione, B. Kagan, J. Ghiso, R. Lal, Amyloid ion channels: a common structural link for protein-misfolding disease, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 10427–10432.
[97] M.F.M. Engel, L. Khemtémourian, C.C. Kleijer, H.J.D. Meeldijk, J. Jacobs, A.J. Verkleij, B. de Kruijff, J.A. Killian, J.W.M. Höppener, Membrane damage by human islet amyloid polypeptide through fibril growth at the membrane, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 6033–6038.
[98] A. Abedini, S.M. Tracz, J.-H. Cho, D.P. Raleigh, Characterization of the heparin binding site in the N-terminus of human pro-islet amyloid polypeptide: implications for amyloid formation, Biochemistry 45 (2006) 9228–9237.
[99] S. Jha, S.M. Patil, J. Gibson, C.E. Nelson, N.N. Alder, A.T. Alexandrescu, Mechanism of amylin fibrillization enhancement by heparin, J. Biol. Chem. 286 (2011) 22894–22904.
[100] W. Humphrey, A. Dalke, K. Schulten, VMD: visual molecular dynamics, J. Mol. Graph. 14 (1996) 33–38 (27–28).
[101] J.A. Hebda, A.D. Miranker, The interplay of catalysis and toxicity by amyloid intermediates on lipid bilayers: insights from type II diabetes, Annu. Rev. Biophys. 38 (2009) 125–152.
[102] D. Milardi, M.F.M. Sciacca, L. Randazzo, A. Raudino, C. La Rosa, The role of calcium, lipid membranes and islet amyloid polypeptide in the onset of type 2 diabetes: innocent bystanders or partners in a Crime? Front. Endocrinol. 5 (2014).
[103] E. Ohkubo, K. Aida, J. Chen, J.-I. Hayashi, K. Isobe, M. Tawata, T. Onaya, A patient with type 2 diabetes mellitus associated with mutations in calcium sensing receptor gene and mitochondrial DNA, Biochem. Biophys. Res. Commun. 278 (2000) 808–813.
[104] M. Barbagallo, R.K. Gupta, L.M. Resnick, Cellular ions in NIDDM: relation of calcium to hyperglycemia and cardiac mass, Diabetes Care 19 (1996) 1393–1398.
[105] L.M. Resnick, Cellular calcium and magnesium metabolism in the pathophysiology and treatment of hypertension and related metabolic disorders, Am. J. Med. 93 (1992) 11S–20S.
[106] T.C. Squier, D.J. Bigelow, Protein oxidation and age-dependent alterations in calcium homeostasis, Front Biosci 5 (2000) D504–526.
[107] J.A. Nitahara, W. Cheng, Y. Liu, B. Li, A. Leri, P. Li, D. Mogul, S.R. Gambert, J. Kajstura, P. Anversa, Intracellular calcium, DNase activity and myocyte apoptosis in aging Fischer 344 rats, J. Mol. Cell. Cardiol. 30 (1998) 519–535.
[108] A. Astrup, The role of calcium in energy balance and obesity: the search for mechanisms, Am. J. Clin. Nutr. 88 (2008) 873–874.
[109] S.P. Fraser, Y.-H. Suh, M.B.A. Djamgoz, Ionic effects of the Alzheimer's disease β-amyloid precursor protein and its metabolic fragments, Trends Neurosci. 20 (1997) 67–72.
[110] M.P. Mattson, B. Cheng, D. Davis, K. Bryant, I. Lieberburg, R.E. Rydel, Beta-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity, J. Neurosci. 12 (1992) 376–389.
[111] I. Shai, D. Schwarzfuchs, Y. Henkin, D.R. Shahar, S. Witkow, I. Greenberg, R. Golan, D. Fraser, A. Bolotin, H. Vardi, O. Tangi-Rozental, R. Zuk-Ramot, B. Sarusi, D. Brickner, Z. Schwartz, E. Sheiner, R. Marko, E. Katorza, J. Thiery, G.M. Fiedler, et al., Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet, N. Engl. J. Med. 359 (2008) 229–241.
[112] R.H. Unger, Lipotoxicity in the pathogenesis of obesity-dependent NIDDM: genetic and clinical implications, Diabetes 44 (1995) 863–870.
[113] G.C. Yaney, D.B.E. Corkey, Fatty acid metabolism and insulin secretion in pancreatic beta cells, Diabetologia 46 (2003) 1297–1312.
[114] M. Hao, W.S. Head, S.C. Gunawardana, A.H. Hasty, D.W. Piston, Direct effect of cholesterol on insulin secretion a novel mechanism for pancreatic β-cell dysfunction, Diabetes 56 (2007) 2328–2338.
[115] R. Tajima, S. Kodama, M. Hirata, C. Horikawa, K. Fujihara, Y. Yachi, S. Yoshizawa, K.T. Iida, H. Sone, High cholesterol intake is associated with elevated risk of type 2 diabetes mellitus - a meta-analysis, Clin. Nutr. Edinb. Scotl. 33 (2014) 946–950.
[116] F.M. Sacks, M.P. Hermans, P. Fioretto, P. Valensi, T. Davis, E. Horton, C. Wanner, K. Al-Rubeaan, R. Aronson, I. Barzon, L. Bishop, E. Bonora, P. Bunnag, L.-M. Chuang, C. Deerochanawong, R. Goldenberg, B. Harshfield, C. Hernández, S. Herzlinger-Botein, H. Itoh, et al., Association between plasma triglycerides and high-density lipoprotein cholesterol and microvascular kidney disease and retinopathy in type 2 diabetes mellitus: a global case-control study in 13 countries, Circulation 129 (2014) 999–1008.
[117] D.E. Warnock, C. Roberts, M.S. Lutz, W.A. Blackburn, W.W. Young, J.U. Baenziger, Determination of plasma membrane lipid mass and composition in cultured Chinese hamster ovary cells using high gradient magnetic affinity chromatography, J. Biol. Chem. 268 (1993) 10145–10153.
[118] M. Hao, S.X. Lin, O.J. Karylowski, D. Wüstner, T.E. McGraw, F.R. Maxfield, Vesicular and non-vesicular sterol transport in living cells the endocytic recycling compartment is a major sterol storage organelle, J. Biol. Chem. 277 (2002) 609–617.
[119] S. Le Lay, S. Krief, C. Farnier, I. Lefrère, X. Le Liepvre, R. Bazin, P. Ferré, I. Dugail, Cholesterol, a cell size-dependent signal that regulates glucose metabolism and gene expression in adipocytes, J. Biol. Chem. 276 (2001) 16904–16910.
[120] M.S. Brown, J.L. Goldstein, The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor, Cell 89 (1997) 331–340.
[121] K. Simons, E. Ikonen, Functional rafts in cell membranes, Nature 387 (1997) 569–572.
[122] M. Wakabayashi, K. Matsuzaki, Ganglioside-induced amyloid formation by human islet amyloid polypeptide in lipid rafts, FEBS Lett. 583 (2009) 2854–2858.
[123] M.V. Chakravarthy, C.F. Semenkovich, The ABCs of β-cell dysfunction in type 2 diabetes, Nat. Med. 13 (2007) 241–242.
[124] L.R. Brunham, J.K. Kruit, T.D. Pape, J.M. Timmins, A.Q. Reuwer, Z. Vasanji, B.J. Marsh, B. Rodrigues, J.D. Johnson, J.S. Parks, C.B. Verchere, M.R. Hayden, Beta-cell ABCA1 influences insulin secretion, glucose homeostasis and response to thiazolidinedione treatment, Nat. Med., 13 (2007) 340–347.
[125] F. Xia, X. Gao, E. Kwan, P.P.L. Lam, L. Chan, K. Sy, L. Sheu, M.B. Wheeler, H.Y. Gaisano, R.G. Tsushima, Disruption of pancreatic β-cell lipid rafts modifies Kv21 channel gating and insulin exocytosis, J. Biol. Chem. 279 (2004) 24685–24691.
[126] G.P. Gellermann, T.R. Appel, A. Tannert, A. Radestock, P. Hortschansky, V. Schroeckh, C. Leisner, T. Lütkepohl, S. Shtrasburg, C. Röcken, M. Pras, R.P. Linke, S. Diekmann, M. Fändrich, Raft lipids as common components of human extracellular amyloid fibrils, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 6297–6302.
[127] M.F.M. Sciacca, F. Lolicato, G. Di Mauro, D. Milardi, L. D'Urso, C. Satriano, A. Ramamoorthy, C. La Rosa, The role of cholesterol in driving IAPP-membrane interactions, Biophys. J. 111 (2016) 140–151.
[128] M.R. Krause, T.A. Daly, P.F. Almeida, S.L. Regen, Push–pull mechanism for lipid raft formation, Langmuir 30 (2014) 3285–3289.
[129] R. Soong, J.R. Brender, P.M. Macdonald, A. Ramamoorthy, Association of highly compact type II diabetes related islet amyloid polypeptide intermediate species at

physiological temperature revealed by diffusion NMR spectroscopy, J. Am. Chem. Soc. 131 (2009) 7079–7085.
[130] M. Iljina, G.A. Garcia, A.J. Dear, J. Flint, P. Narayan, T.C.T. Michaels, C.M. Dobson, D. Frenkel, T.P.J. Knowles, D. Klenerman, Quantitative analysis of co-oligomer formation by amyloid-beta peptide isoforms, Sci. Rep. 6 (2016).
[131] A. Laganowsky, C. Liu, M.R. Sawaya, J.P. Whitelegge, J. Park, M. Zhao, A. Pensalfini, A.B. Soriaga, M. Landau, P.K. Teng, D. Cascio, C. Glabe, D. Eisenberg, Atomic view of a toxic amyloid small oligomer, Science 335 (2012) 1228–1231.
[132] M. Carballo-Pacheco, B. Strodel, Advances in the simulation of protein aggregation at the atomistic scale, J. Phys. Chem. B 120 (2016) 2991–2999.
[133] M. Lella, R. Mahalakshmi, Metamorphic proteins: emergence of dual protein folds from one primary sequence, Biochemistry 56 (2017) 2971–2984.
[134] Atomic Structures of IAPP (amylin) Fusions Suggest a Mechanism for Fibrillation and the role of insulin in the process - Wiltzius - 2009 - Protein Science - Wiley Online Library, (n.d.).
[135] S. Luca, W.-M. Yau, R. Leapman, R. Tycko, Peptide conformation and supramolecular organization in amylin fibrils: constraints from solid-state NMR, Biochemistry 46 (2007) 13505–13522.
[136] A.T. Petkova, Y. Ishii, J.J. Balbach, O.N. Antzutkin, R.D. Leapman, F. Delaglio, R. Tycko, A structural model for Alzheimer's β-amyloid fibrils based on experimental constraints from solid state NMR, Proc. Natl. Acad. Sci. 99 (2002) 16742–16747.
[137] S.-H. Shim, R. Gupta, Y.L. Ling, D.B. Strasfeld, D.P. Raleigh, M.T. Zanni, Two-dimensional IR spectroscopy and isotope labeling defines the pathway of amyloid formation with residue-specific resolution, Proc. Natl. Acad. Sci. 106 (2009) 6614–6619.
[138] Y. Mazor, S. Gilead, I. Benhar, E. Gazit, Identification and characterization of a novel molecular-recognition and self-assembly domain within the islet amyloid polypeptide, J. Mol. Biol. 322 (2002) 1013–1024.
[139] M. Pannuzzo, A. Raudino, D. Milardi, C. La Rosa, M. Karttunen, α-Helical structures drive early stages of self-assembly of amyloidogenic amyloid polypeptide aggregate formation in membranes, Sci. Rep. 3 (2013) 2781.
[140] J. Nasica-Labouze, P.H. Nguyen, F. Sterpone, O. Berthoumieu, N.-V. Buchete, S. Coté, A. De Simone, A.J. Doig, P. Faller, A. Garcia, A. Laio, M.S. Li, S. Melchionna, N. Mousseau, Y. Mu, A. Paravastu, S. Pasquali, D.J. Rosenman, B. Strodel, B. Tarus, et al., Amyloid β protein and Alzheimer's disease: when computer simulations complement experimental studies, Chem. Rev. 115 (2015) 3518–3563.
[141] A. Morriss-Andrews, J.-E. Shea, Computational studies of protein aggregation: methods and applications, Annu. Rev. Phys. Chem. 66 (2015) 643–666.
[142] A.M. Brown, D.R. Bevan, Molecular dynamics simulations of amyloid β-peptide (1-42): tetramer formation and membrane interactions, Biophys. J. 111 (2016) 937–949.
[143] H. Jang, L. Connelly, F. Teran Arce, S. Ramachandran, B.L. Kagan, R. Lal, R. Nussinov, Mechanisms for the insertion of toxic, fibril-like β-amyloid oligomers into the membrane, J. Chem. Theory Comput. 9 (2013) 822–833.
[144] S.R. Natesh, K.F. Freed, E.J. Haddadian, Membrane bilayers help to stabilize and are affected by Aβ-fibrils on the surface: a molecular dynamics study, Biophys. J., 112 (2017) 363a.
[145] S. Tung Ngo, H. Minh Hung, K. Nhat Tran, M. Tho Nguyen, Replica exchange molecular dynamics study of the amyloid beta (11–40) trimer penetrating a membrane, RSC Adv. 7 (2017) 7346–7357.
[146] F. Kargar, S. Emadi, H. Fazli, The molecular behavior of a single β-amyloid inside a dipalmitoylphosphatidylcholine bilayer at three different temperatures: an atomistic simulation study: Aβ interaction with DPPC: atomistic simulation, Proteins: Struct., Funct., Bioinf. 85 (2017) 1298–1310.
[147] H. Ahyayauch, M. Raab, J.V. Busto, N. Andraka, J.-L.R. Arrondo, M. Masserini, I. Tvaroska, F.M. Goñi, Binding of β-amyloid (1-42) peptide to negatively charged phospholipid membranes in the liquid-ordered state: modeling and experimental studies, Biophys. J. 103 (2012) 453–463.
[148] A. Porzoor, J.M. Caine, I.G. Macreadie, Pretreatment of chemically-synthesized Aβ42 affects its biological activity in yeast, Prion 8 (2014) 404–410.
[149] C. Poojari, B. Strodel, Stability of transmembrane amyloid β-peptide and membrane integrity tested by molecular modeling of site-specific Aβ42 mutations, PLoS One 8 (2013) e78399.
[150] N. Liguori, P.S. Nerenberg, T. Head-Gordon, Embedding Aβ42 in heterogeneous membranes depends on cholesterol asymmetries, Biophys. J. 105 (2013) 899–910.
[151] T. Hoshino, M.I. Mahmood, K. Mori, K. Matsuzaki, Binding and aggregation mechanism of amyloid β-peptides onto the GM1 ganglioside-containing lipid membrane, J. Phys. Chem. B 117 (2013) 8085–8094.
[152] A. Morriss-Andrews, J.-E. Shea, Simulations of protein aggregation: insights from atomistic and coarse-grained models, J. Phys. Chem. Lett. 5 (2014) 1899–1908.
[153] L. Nagel-Steger, M.C. Owen, B. Strodel, An account of amyloid oligomers: facts and figures obtained from experiments and simulations, Chembiochem 17 (2016) 657–676.
[154] G.L. Dignon, G.H. Zerze, J. Mittal, Interplay between membrane composition and structural stability of membrane-bound hIAPP, J. Phys. Chem. B 121 (2017) 8661–8668.
[155] K.K. Skeby, O.J. Andersen, T.V. Pogorelov, E. Tajkhorshid, B. Schiøtt, Conformational dynamics of the human islet amyloid polypeptide in a membrane environment: toward the aggregation prone form, Biochemistry (Mosc) 55 (2016) 2031–2042.
[156] M. Zhang, B. Ren, Y. Liu, G. Liang, Y. Sun, L. Xu, J. Zheng, Membrane interactions of hIAPP monomer and oligomer with lipid membranes by molecular dynamics simulations, ACS Chem. Neurosci. 8 (2017) 1789–1800.
[157] R. Azriel, E. Gazit, Analysis of the minimal amyloid-forming fragment of the islet amyloid polypeptide an experimental support for the key role of the phenylalanine residue in amyloid formation, J. Biol. Chem. 276 (2001) 34156–34161.
[158] D. Boyd-Kimball, H. Mohmmad Abdul, T. Reed, R. Sultana, D.A. Butterfield, Role of phenylalanine 20 in Alzheimer's amyloid β-peptide (1-42)-induced oxidative stress and neurotoxicity, Chem. Res. Toxicol. 17 (2004) 1743–1749.
[159] E. Gazit, A possible role for π-stacking in the self-assembly of amyloid fibrils, FASEB J. 16 (2002) 77–83.
[160] V. Singh, R.K. Rai, A. Arora, N. Sinha, A.K. Thakur, Therapeutic implication of L-phenylalanine aggregation mechanism and its modulation by D-phenylalanine in phenylketonuria, Sci. Rep. 4 (2014).
[161] R. Cukalevski, B. Boland, B. Frohm, E. Thulin, D. Walsh, S. Linse, Role of aromatic side chains in amyloid β-protein aggregation, ACS Chem. Neurosci. 3 (2012) 1008–1016.
[162] F. Lolicato, A. Raudino, D. Milardi, C. La Rosa, Resveratrol interferes with the aggregation of membrane-bound human-IAPP: a molecular dynamics study, Eur. J. Med. Chem. 92 (2015) 876–881.
[163] M.F.M. Sciacca, M. Pappalardo, F. Attanasio, D. Milardi, C. La Rosa, D.M. Grasso, Are fibril growth and membrane damage linked processes? An experimental and computational study of IAPP 12–18 and IAPP 21–27 peptides, New J. Chem. 34 (2010) 200–207.
[164] C. La Rosa, D. Milardi, E. Amato, M. Pappalardo, D. Grasso, Molecular mechanism of the inhibition of cytochrome c aggregation by Phe-Gly, Arch. Biochem. Biophys. 435 (2005) 182–189.
[165] D. Milardi, M. Pappalardo, M. Pannuzzo, D.M. Grasso, C. La Rosa, The role of the Cys2-Cys7 disulfide bridge in the early steps of islet amyloid polypeptide aggregation: a molecular dynamics study, Chem. Phys. Lett. 463 (2008) 396–399.
[166] C. Guo, S. Côté, N. Mousseau, G. Wei, Distinct helix propensities and membrane interactions of human and rat IAPP1–19 monomers in anionic lipid bilayers, J. Phys. Chem. B 119 (2015) 3366–3376.
[167] H. Figueroa, D. Peddi, J.M. Osborne, B.M. Wilson, R.R. Pesaru, B. Kurva, S. Ramaraju, M.C. Milletti, D.L. Heyl, Modeling the interface between islet amyloid polypeptide and insulin-based aggregation inhibitors: correlation to aggregation kinetics and membrane damage, J. Chem. Inf. Model. 52 (2012) 1298–1307.
[168] M. Pannuzzo, A. Raudino, D. Milardi, C. La Rosa, M. Karttunen, α-Helical structures drive early stages of self-assembly of amyloidogenic amyloid polypeptide aggregate formation in membranes, Sci. Rep. 3 (2013) 2781.
[169] S. Jalili, A. Maleki, M. Akhavan, B. Najafi, J. Schofield, Free energy simulations of amylin I26P mutation in a lipid bilayer, Eur. Biophys. J. 44 (2015) 37–47.
[170] N. Liu, M. Duan, M. Yang, Structural properties of human IAPP dimer in membrane environment studied by all-atom molecular dynamics simulations, Sci. Rep. 7 (2017) 7915.
[171] A. Martel, L. Antony, Y. Gerelli, L. Porcar, A. Fluitt, K. Hoffmann, I. Kiesel, M. Vivaudou, G. Fragneto, J.J. de Pablo, Membrane permeation versus amyloidogenicity: a multitechnique study of islet amyloid polypeptide interaction with model membranes, J. Am. Chem. Soc. 139 (2017) 137–148.
[172] C. Poojari, D. Xiao, V.S. Batista, B. Strodel, Membrane permeation induced by aggregates of human islet amyloid polypeptides, Biophys. J. 105 (2013) 2323–2332.
[173] J. Zhao, Y. Luo, H. Jang, X. Yu, G. Wei, R. Nussinov, J. Zheng, Probing ion channel activity of human islet amyloid polypeptide (amylin), Biochim. Biophys. Acta Biomembr. 1818 (2012) 3121–3130.