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Review

Amyloid fibrils compared to peptide nanotubes

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ABSTRACT

Prefibrillar oligomeric states and amyloid fibrils of amyloid-forming proteins qualify as nanoparticles. We aim to predict what biophysical and biochemical properties they could share in common with better researched peptide nanotubes. We first describe what is known of amyloid fibrils and prefibrillar aggregates (oligomers and protofibrils): their structure, mechanisms of formation and putative mechanism of cytotoxicity. In distinction from other neuronal fibrillar constituents, amyloid fibrils are believed to cause pathology, however, some can also be functional. Second, we give a review of known biophysical properties of peptide nanotubes. Finally, we compare properties of these two macromolecular states side by side and discuss which measurements that have already been done with peptide nanotubes could be done with amyloid fibrils as well.

1. Introduction

Amyloid fibrils are highly stable insoluble self-assembled protein deposits usually regarded as a characteristic of neural or systemic pathologies. Under diverse conditions they form an ordered β-sheet structure [1–3]. The phase states displayed by these deposits include liquid crystals, hydrogel, and nanotubes. Besides special mechanical properties each protein in amyloid fibril may also have a biological function. Therefore, the potential applications of amyloid fibrils exceed those of synthetic polymers.

Amyloid fibrils (Fig. 1) are associated with various diseases known as amyloidoses. Currently there are more than 25 proteins with non-homologous amino acid sequences that are known to form amyloid deposits extra- and/or intracellularly. This is a hallmark of neurodegenerative diseases such as Alzheimer's, Parkinson's and spongiform encephalopathies but also of type 2 diabetes mellitus. These diseases are progressive and associated with decreased quality of life and high mortality of the aging population.

In the scientific community there is now a consensus that it is not the amyloid fibrils themselves that are responsible for those diseases [4]. Instead, smaller soluble aggregates, the prefibrillar and globular oligomers, of the order of 10–60 protein chains are thought to be responsible for cellular dysfunction and death [5,6]. However, at least in some species amyloid fibrils as such are known to have a physiological role [7].

Peptide nanotubes (Fig. 2)—an axially-symmetric ring-shaped system of protein molecules—have received a considerable amount of experimental interest in the recent years. Self-assembly, their ferroelectric and optical properties along with quantum confinement phenomena have been thoroughly studied [8,9]. A range of nanotechnological applications has been proposed, especially in the field of medicine and electrochemistry [9]. Here we review some of the studies that led to those applications and compare peptide nanotubes to amyloid fibrils.

2. Structure and properties of amyloid fibrils

Amyloid fibrils are ordered protein aggregates that are characterized by a β-sheet rich secondary structure, Thioflavin T dye binding, consequently enhanced fluorescence, and Congo red dye birefringence. The ability of proteins to form amyloid fibrils is not limited to specific range of peptide lengths or any particular primary structure. By choosing appropriate solution conditions, amyloid fibrils can also be produced efficiently in vitro.

2.1. Protein folding and misfolding

Native conformation of a protein is determined by its primary structure—the amino acid sequence. Natively folded proteins may occasionally partially unfold and adapt a non-native conformation. Mutations,

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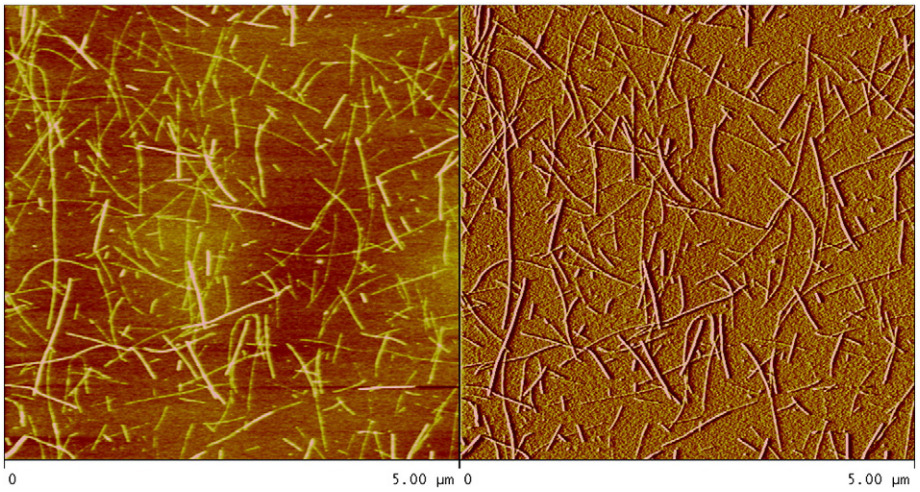

Fig. 1. An AFM image of amyloid fibrils formed by human stefin A (at pH 2.4 upon heating to about 90 °C for 2 h). Melting temperature of stefin A is 94 °C. We can see thicker and thinner type of amyloid fibrils of heights 2.8 and 5.6 nm [100]. We thank Dr. Miha Škarabot from Soft-Matter Physics (JSI) for his help in acquiring the image.

metal interactions, acidic pH, elevated temperature, oxidation and proteolysis or increase in protein concentration can all drive protein misfolding [10]. A set of protein structures, however, is natively unfolded (NUP), intrinsically disordered (IDP) [11]. These too, among them α-synuclein and tau, are prone to form amyloid fibrils [12].

With or without assistance of chaperone proteins misfolded proteins eventually refold back to their native conformation, get labeled for degradation via the ubiquitin proteasome pathway or start to oligomerize. Oligomerization is thermodynamically favorable either because of formation of new intermolecular hydrogen bonds of the edge strands or due to hydrophobic effect, which shields hydrophobic parts of protein chains from the solvent. Additionally, lipid bilayers of cell membranes can change protein conformation and increase the propensity of amyloid-forming proteins to aggregate [13].

2.2. Structure and morphology of amyloid fibrils

The main structural characteristics of amyloid fibrils is the cross β structure, where β-strands run penperdicularly to the fibril axis, which is revealed by the double concentric pattern in X-ray diffraction, where characteristic lengths can be observed: a meridional signal at 4.76 Å 4.7 Å (represents the distance between β-strands connected by hydrogen bonds) and an equatorial signal at 10.6 Å (represents the distance between β-sheets). The antiparallel β-sheets are zipped together by π-bonds between adjacent phenylalanine rings and salt-bridges between charge pairs (glutamic acid–lysine), thus controlling and stabilizing the structure [14]. Whereas the strands are parallel or anti-parralel depends on each case. For example, solid-state NMR studies [15–17] of amyloid-β peptide usually gave an in register, parralel arrangement of the β-strands. An in-register parallel β-sheet seems more favorable as it maximizes favorable hydrophobic interactions for any amino acid sequence. It also aligns Gln and Asn residues with themselves, maximizing favorable "polar zipper" interactions. Antiparallel β-sheets have also been found in some amyloid fibrils, but until recently only in fibrils formed by short peptides. From 2005 many solid-state structures of amyloid fibrils have been solved as reviewed in Tycko [16]. Of interest, HET-$s_{218–289}$, formed a β-helix resembling motif that has been observed in crystal structures of certain monomeric proteins, such as the pertactin [18]; supporting suggestions that β-helices might be important structural motifs within amyloid fibrils [16 and refs therein].

An amyloid fibril (if it grows not agitated) appears to be made of several filaments (4–6), twisting helically around the fibril axis [2]. However, before the filaments or fibrils form (several micrometers long), usually less ordered and shorter structures (200 nm), the so called protofibrils, which are slightly bended, form. Also, smaller globular oligomers, which compose the granular aggregate, already have similar structural features [16].

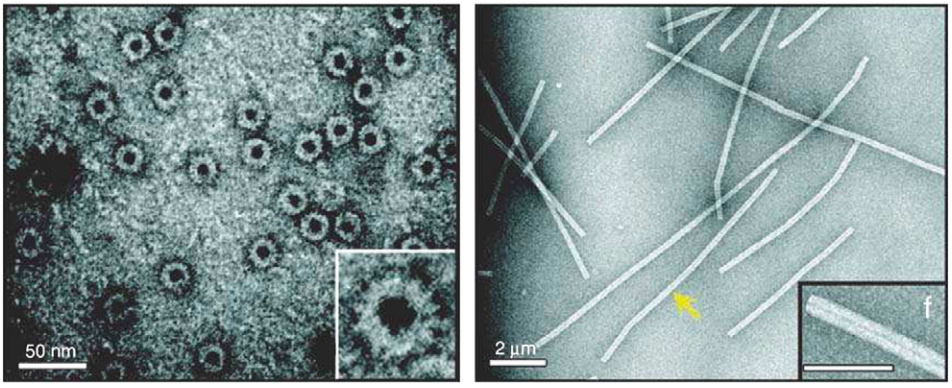

Fig. 2. Transmission electron microscope (TEM) image of representative peptide nanodisks (left) and nanotubes (right) generated from modified TMV coat protein. TMV nanodisks are layered heptadecameric structures. (Copyright 2007 American Chemical Society), web page: http://wires.wiley.com/WileyCDA/WiresArticle/articles.html?doi=10.1002%2Fwnan.1180.

In prions and also other proteins, amyloid fibrils polymorphism has been observed. The molecular basis of this phenomenon was documented by detailed AFM studies [19] and was shown to arise from differences in structure already on the level of the pre-fibrillar aggregates. Interestingly, the morphological differences proved as key factors in the prion strain propagation. Recent progress in understanding the dynamic polymorphism, twisting behavior, and handedness of amyloid fibrils has been reviewed [20].

Of interest, studies of α-synuclein revealed that fibrils exhibit polymorphism in response to changes in the solution conditions during self-assembly; aggregates formed at pH 7.0 and 6.0 were fibrillar, while those formed at pH 5.0 and 4.0 were amorphous [21]. In stefin B, also extensively used as a model amylodogenic protein, something similar was observed, namely, that fibrils at pH 3 were like protofibrils, whereas fibrils at pH 4.8 differed, depending on TFE and temperature, with more amorphous aggregates observed at higher temperatures and in presence of TFE [22].

2.3. Small amyloidogenic peptides can form nanotubes

Some insight about the propensity to form amyloid conformation can be gained by revealing the nature of amyloidogenic motif sequences. Following these studies, bioinspired nanostructures with diverse properties can be designed based on amyloidal recognition motifs. Generally, stability of amyloid structures is retained through non-covalent bonds, especially hydrogen bonds, hydrophobic interactions and π-π stacking interactions [23,24]. These interactions occur both between side chains and main chains. Amyloid fibril formation is essentially a process of intermolecular interactions that leads to protein aggregation and precipitation. Therefore, the importance of hydrophobic interactions in amyloid fibril formation is rather explicit.

Aromatic peptides occur quite frequently in short amyloid-related peptides [25,26]. This observation suggests that π-stacking may be important for accelerating the self-assembly process that leads to formation of amyloid fibrils. This can be explained by the fact that π-stacking interactions provide geometrical restrictions that promote directionality and orientation of the growing fibril. Additional contribution to a decrease of potential energy comes from the stacking itself.

For example, if phenylalanine residues at the C-terminus of Aβ1-42 are replaced by hydrophobic residues, peptide aggregation becomes somewhat slower. In addition, a mutant variant that contains four phenylalanine residues at the C-terminus of Aβ1-42 displays faster amyloidogenesis. The contribution of aromatic amino acid residues to the rate of amyloid fibril assembly is also substantiated by high-resolution X-ray and solid-state NMR analyses [14,27]. These findings correlate well with stacking of aromatic rings between layers of adjacent β-sheets. Another experimental evidence that supports the explanation about the importance of aromatic amino acid residues for the rate of amyloid fibril formation is the observation that phenylalanine dipeptide, an important structural motif of Aβ peptide (Phe19–Phe20) can spontaneously self-assemble into long and stiff nanotubes [28,29].

3. Amyloid self-assembly mechanisms

The mechanism of amyloid fibril formation has not been completely resolved. It may well be that a whole range of mechanisms (as in protein folding) apply [10,30]. In simple terms, as globular proteins start to oligomerize and fibrillize from an intermediate state, formed after partial unfolding, natively unfolded proteins have first to fold to an amyloidogenic intermediate [31]. Examples of natively unfolded proteins are, among others, Aβ, amylin, N-terminal region of prion, Tau.

In some studies it has been shown that early stages of oligomerization, i.e. accumulation in a nucleus, proceed quite slowly until a critical concentration of higher oligomers is reached [32]. From this point on aggregation progresses rapidly. The rate of aggregation can be increased by acidic pH, high protein concentration and seeding [33]. Poor reproducibility of the results of early experiments [34] has hinted that protein aggregation is a highly variable process. Therefore, a precise control of experimental conditions is critical [35], as is the composition of monomers and oligomers in the starting solution.

The mechanism of amyloid fibril formation can be regarded on the first glance as similar to the processes of crystallization and gelation. It obeys most principles of colloidal physics yet is more complicated due to variable composition and properties of amino-acids. In broad terms the following processes contribute to protein aggregation [36]: (i) nucleation and fragmentation that increase the number of aggregates, (ii) growth that leads to increase in the size of existing aggregates and (iii) dissociation, de-aggregation or degradation of the aggregates. The kinetics of such processes is complicated and global fitting is needed to obtain microscopic description.

In our review [30] we classified the models describing the mechanism of amyloid fibril formation into one of the three groups: templating and nucleation, linear colloid-like assembly of spherical oligomers, and domain-swapping. What follows is a short description of each model.

Nucleated polymerization model [37] was used to describe the formation of amyloid fibrils of Aβ. The model predicts a lag phase that decreases exponentially with protein concentration and disappears completely upon seeding. Above critical protein concentration micelles are formed and fibrils grow by binding of monomers to fibril ends. *Nucleation-dependent polymerization model* suggests that at low concentrations protein dissociation rate is smaller than the association rate, which causes a lag phase until a critical nucleus size is reached. The rate of fibrillation is again highly concentration-dependent [38]. On the other hand, *nucleated conformational conversion model* [39] describes cases where the growth of fibrils does not depend significantly on protein concentration. This can be achieved by almost constant concentration of oligomers that are ready to assemble. Increasing protein concentration results mostly in concentration increase of assembly-incompetent complexes. Oligomer growth is not a rate-determining step. Instead, the rate of growth depends on a conformational change in the oligomer nucleus. The three above-described models form a group of templating and nucleation models [40].

Model of "critical oligomers" [41,42] is an example of linear colloid-like assembly of spherical oligomers. In this two-step model, the first step can be characterized by the formation of "critical oligomers," which grow linearly into protofibrils in the second step. Both steps are irreversible and based on generalized diffusion–collision processes. However, a similar *dipole assembly model* proposes that in the first step uniform-sized spherical nuclei form due to electrostatic repulsion between the nuclei and monomers. Aggregation is driven by dipole interactions and proceeds linearly [41].

As opposed to the described models *double-concerted fibrillization model* [43,44] suggests that firstly monomers associate into oligomers, which then linearly form protofibrils in the absence of a template. The growth of fibrils is governed by shear stress, which induces structural rearrangement within oligomers.

The mechanism of formation of amyloid fibrils of stefins, cystatins, also likely β2-microglobulin and prion, involves domain-swapping. If the conditions that trigger fibrillization favor population of folding intermediates, then domain-swapped oligomers are more likely to form than oligomers that arise in a two-state folding manner. Monomer–monomer interaction is usually prevented by the strands on the protein edge. Denaturation exposes internal strands and intermolecular forces become important. Domain-swapping requires that proteins first unfold almost fully, which presents a very high energy barrier [45,46]. Only then can the two chains rearrange to swap strands. Therefore, in this model amyloid fibril formation starts with dimerization. Rate of fibril formation is also influenced by the formation of off-pathway oligomers which cannot convert directly to fibrils and thus decrease the rate of fibril formation.

In the case of Aβ peptide [47] and in some other cases there also is a secondary nucleation observed. In a study of Aβ1-42 fibrillization, it was shown that secondary nucleation occurred on certain types of fibrils and that this process shaped the final morphology of the fibrils. There actually were five types of fibrils observed, depending on initial ratio of the monomer, oligomers and protofibrils. Some formed by lateral association (type 3 fibrils), some by twisting of the filaments (types 4 and 5 fibrils), whereas only type 2 fibrils showed spots for secondary nucleation, leading to branched fibrils [47].

Protofilaments associate into multistranded ribbon-like fibrils, due to electrostatic repulsion between polar and charged side chains these ribbons twist along the fibril axis, resulting in a periodic pitch [48].

3.1. Peptide nanotubes assembly mechanisms

Peptide nanotubes can be prepared by spontaneous growth of heterogenic populations in bulk solutions. The assembly mechanisms of amyloid fibrils, prions and peptide nanotubes [49–51] seem rather similar. A nucleation occurs before filaments can start growing. When growth rate increases, the break-down (fibril fragmentation) is also important as this provides new ends for fibril growth [52].

As discussed under morphology, for the same protein sequence different polymorphic amyloid fibrils exist in parallel and they can form twisted and helical ribbons, precisely, as was observed for peptide nanotubes [53,54].

Another self-assembly technique for preparation of peptide nanotubes was developed that is based on vapor deposition [55]. In this approach peptide nanotubes grow perpendicularly to the surface with the density of 10^9 cm^{-2}, diameter of about 100 nm, and length in the range of 1–50 μm. High-resolution SEM images show that peptide nanotubes have an open tip and crystallize into a hexagonal structure, quite uncommon in the biological world.

4. Pathological function of protein aggregates

It is surprising that cellular defense mechanisms such as the chaperones, ubiquitin proteasome degradation system and autophagy do not always take care of misfolded proteins. While these mechanisms may be working properly it is possible that they cannot always handle the amount of misfolded proteins. This point may represent the onset of disease. It is also possible that β-sheet aggregates insert themselves into cellular membranes and become inaccessible to chaperones and ubiquitin-proteasome pathway [4]. Alternatively, oligomerization can proceed so rapidly that larger aggregates cannot be handled by the cell defense mechanisms. It has been found that protein aggregates use the microtubules-driven cargo transport and accumulate in special compartments by the nucleus, called aggresomes [56].

However, it has been proposed that the aggregation is actually a defense mechanism that sequesters misfolded proteins from the vital machinery of the cell. There is some ground to this claim as amyloid fibrils have been found to have little, if any, cytotoxicity while smaller oligomers appear to be considerably more toxic [57].

Apart from the proteins that are associated with amyloid diseases it has been found [58] that under appropriate conditions other proteins too can form amyloid fibrils. Although the structure of proteins in early stages of oligomerization cannot be easily observed the results obtained with electron and atomic force microscopy have hinted that smaller aggregates may be spherical in shape. These globular particles then combine themselves into chain- or ring-like structures referred to as protofibrils. It is these protofibrils that appear to be much more cytotoxic than the fibrils themselves (Fig. 3).

A detailed explanation of molecular mechanism of amyloid cytotoxicity is still unclear. Loss of protein function, oxidative stress and channel-forming hypothesis are a few possible models to explain this phenomenon.

4.1. Cytotoxicity of amyloid aggregates

Loss of function hypothesis postulates that toxicity to cells comes from a loss of native protein function due to misfolding and aggregation. However, there are many proteins with non-homologous amino acid sequences associated with amyloid diseases and it seems unlikely that loss of protein function would be the main reason for cytotoxicity. In fact transgenic mouse models show that inserting amyloid protein-encoding gene also produces amyloid-related disease. Amyloid peptides may directly induce oxidative stress by producing free radicals which in turn causes mitochondrial dysfunction and death [59]. It has not been confirmed that free radicals can be generated by peptides alone but it was clearly shown that oxidative stress does occur in cells affected by amyloid proteins. This explanation is referred to as the *oxidative stress hypothesis* [60].

There is a growing consensus in the scientific community that the *channel hypothesis* [61] provides an adequate explanation of the most probable molecular mechanism to amyloid protein cytotoxicity. Most, maybe even all, amyloid peptides can aggregate to β-sheet structures which can spontaneously insert themselves into lipid membranes. When inserted these peptide aggregates function as relatively stable ion channels [6,61,62].

It has been shown that such channels have devastating consequences for cells. They have a large ring diameter, are non-selective, heterogeneous, voltage-independent, irreversible, can be inhibited by aggregation-

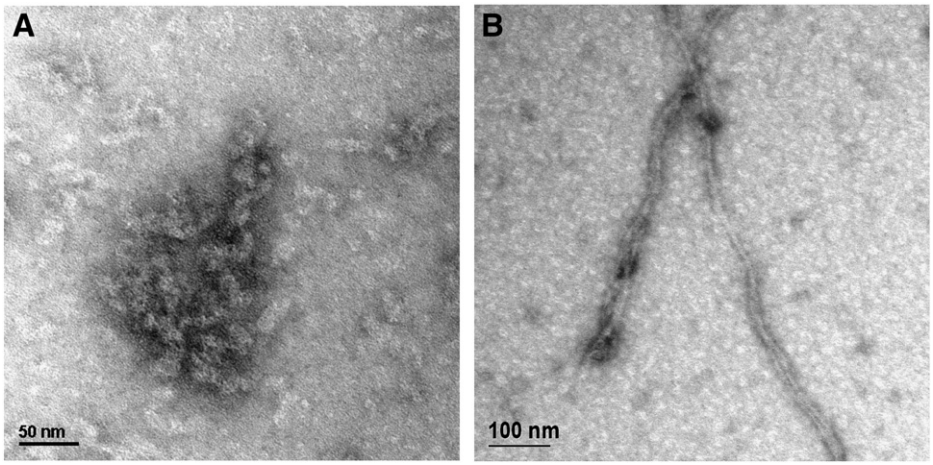

Fig. 3. TEM images of the protofibrils (A) and fibrils (B). Model protein human stefin B. Thanks are due to Dr. Magda Tušek Žnidarič (National Institute of Biology, Ljubljana).

preventing agents such as Congo red dye and are blocked by zinc ions. It is likely that such channels form a pathway for ions across the membrane. Increased flux of ions decreases trans-membrane potential and ion concentration gradients. The concentration of vital intracellular ions, such as K^+ and Mg^{2+}, is reduced. This in turn increases the influx of toxic Ca^{2+} ions. Consequently, ion pumps work harder and consume more energy. There is also quite some experimental evidence that supports the channel-forming hypothesis [61].

Although channel-forming is necessary for cytotoxicity, it is not sufficient. In fact, Lin has noted the existence of two channel-forming variants of Aβ25-35 that do not cause cell death [63,64]. Channel activity can be increased by lipids carrying a net-negative surface charge and decreased by higher concentration of salts. Introduction of membrane-stiffening agents, such as cholesterol, decreases ion channel activity. This observation confirms that the membrane plays a critical role in Aβ toxicity. In order to form channels Aβ25-35 need to be at least 10 residues long which corresponds to 30 Å lipid bilayer thickness. Though even a shorter variant of Aβ31-35 has been reported to form membrane channels it is not clear whether such a channel is comprised of two hemi-channels. Fibroblasts can be killed by both Aβ1-40 and Aβ1-42. Toxicity can be inhibited by antibodies but not by antioxidants again confirming the channel-forming hypothesis over the oxidative stress hypothesis. Whether mitochondria or endocytic trafficking are involved is being looked at [65,66].

Some properties of amyloid induced channels (pores) may resemble the pore-forming toxins [67,68].

All putative mechanisms leading toward cytotoxicity by the "amyloid toxins" are drawn in the scheme (Fig. 4).

4.2. Studies of peptide nanotube toxicity

Peptide nanotubes as typical nanoparticles are toxic depending on their size, shape and surface properties, however, many studies were performed on carbon nanotubes. Carbon nanotube toxicity studies have been performed due to the need for safety as these particles are intended for usage as a delivery agent or for diagnostic means in biomedicine [69-71]. In general, the entrance into cells is thought to occur either via active or passive endocytosis. Inside cells some of the smaller-sized nanotubes are collected by macrophages, while the bigger ones cause more problems, floating around. Reactive oxygen species are produced and consequently inflammation, due to traces of metal ions or by electron donor and acceptor sites on nanotube surface, promoting production of superoxide radicals O_2^-. Increased oxidative stress can finally lead to apoptosis [72].

5. Functional amyloid structures

5.1. Function in vivo systems

Functional amyloids are spread over bacteria, plant and animal systems [73]. In bacteria amyloid fibrils are involved in biofilm formation and cell/cell cell/substrate adhesion. Curli proteins in *Escherichia coli* and *Salmonella* are able to form amyloid structures [74]. Spider silk [75] and silk-worm silk are networks of amyloid [76,77].

In humans, a functional amyloid is made up of a protein called pmel17 which is involved in polymerization of the pigment melanin. The melanin precursors are sequestered inside melanosomes by pmel17 amyloid to avoid cellular toxicity of free melanin molecules [78]. Amyloids also serve as natural storage of peptide hormones [79].

5.2. Bio-nanomaterials on the basis of amyloids and possible applications

Amyloidogenic motifs are attractive for the design of new nanostructures and nanomaterials [80].

Exceptional stability, mechanical strength and increased resistance to degradation are hallmark of many proteins in their amyloidal state. Additional desirable properties are high melting-point temperature and increased resistance to the presence of sodium dodecyl sulfate. Highly amyloidogenic proteins, especially the smaller ones, are capable of rapid self-assembly.

Many potential technological applications rely on the remarkable properties of amyloid fibrils. By using the bottom-up approach for the fabrication of molecular bionanomaterials, amyloid fibrils offer themselves as excellent candidates for self-assembly of wires, layers, gels,

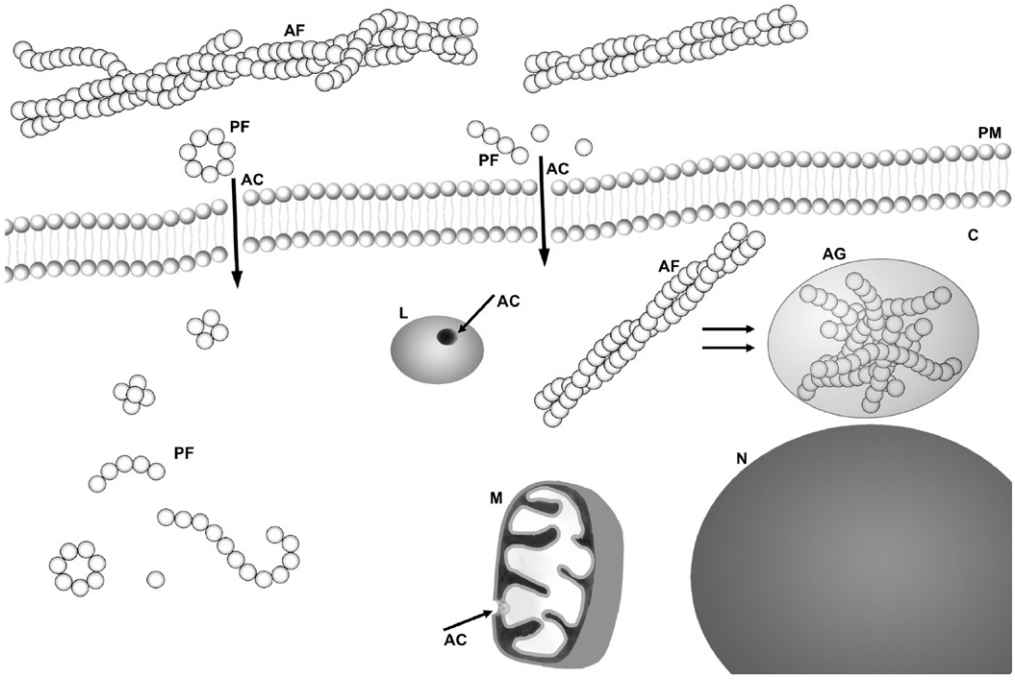

Fig. 4. Scheme: globular oligomers with β-sheet conformation and protofibrils (beads of such oligomers) formed by amyloid forming proteins induce cytotoxicity via membrane interactions and actual channel (pore) formation. AF: amyloid fibril, PF: protofibril, AC: amyloid channel, PM: plasma membrane, C: cytoplasm, L: lysosome, AG: aggresome, M: mitochondrion, N: nucleus. We thank Janja Širovnik from the Vetsuisse Faculty, University of Bern for help with the drawing.

scaffolds, templates and liquid crystals. Structural compatibility, nanoscale dimensions, efficient self-assembly into well-defined ultrastructures, ease of production and low cost are the most important reasons why amyloid fibrils are so desirable for nanotechnological applications. An additional advantage is that the properties of the formed nanostructures can be varied considerably by making changes in amino acid sequences of amyloidogenic proteins. The perspective paper in ACS Nano [20] discusses the promising future of self-assembled structures comprising amyloid fibrils as advanced functional materials with applications in nanotechnology and biomedicine.

Several studies have been done showing the use of amyloid fibrils as functional templates [28,81,82]. For example, it has been demonstrated that conductive nanowires can be fabricated by coating amyloid fibrils with gold. First, a genetic modification of N-terminal and middle region of yeast prion Sup35 was done to include a cysteine residue on its surface. After fibrillization cysteine residues were used to covalently link gold particles to amyloid fibrils. The coated fibrils were 80–200 nm wide and showed conductance competitive to solid conducting wires [81].

In tissue engineering, in the efforts to produce a material capable of mimicking real bone, the most frequent approach was based on collagen self-assembled structures. However, besides collagen-based extracellular matrices, many non-collagenous nanofibrous scaffolds, among them amyloid fibrils. These proved perfect to replace collagen. They possess exceptional mechanical and physical properties, they can be made from affordable protein sources, and their growth can be accurately controlled in-vitro. Most importantly, they strongly adhere to both hydrophobic and hydrophilic surfaces and exhibit excellent biocompatibility, therefore, can be used for cell attachment and proliferation [83].

Amyloid fibrils also serve as a biofunctional material. Graphene is a hydrophobic molecule which limits its use in biological applications. New composite materials have been composed from graphene and amyloid fibrils.This biodegradable composite material has interesting properties; from high conductance to reversible change in shape with variation in humidity, this latter is used to design biosensors for measuring the activity of enzymes [84]. Other applications of amyloid for biosensors are being reported [85].

Besides many polymers, liquid crystals can be formed by amyloidogenic proteins. Hen lysozyme is known to efficiently form amyloid fibrils at low pH and elevated temperatures. A network of lysozome fibrils can be described by a liquid-crystal phase [86]. This is a large number of liquid crystal domains that form a liquid crystal glass. The reason for domains is the lack of a long-range order in a system with a large number of amyloid fibrils. Additional studies revealed that pH can serve as a trigger for reversible self-assembly of peptides into β-sheet tapes, ribbons, fibrils and fibers. This can be thought of as a controlled polymerization of monomeric peptides from isotropic fluids up to nematic gel states. By exploiting this property self-assembled polymers with controlled mechanical properties and new diverse soft solid-like nanostructured materials that are biocompatible and biodegradable can be designed.

5.3. Biomaterials based on peptides; hydrogels and nanotubes

By combining the 20 proteinogenic amino acids a range of nanostructures can be synthesized from hydrogel to nanotubes. Amphiphilic sequences have been designed that fold into β-hairpins in aqueous solution. With self-assembly into a network of fibrils a hydrogel is formed. If the primary amino acid sequence is slightly modified the process can be triggered and reversibly controlled by changes in pH, ionic strength, temperature and by UV irradiation. Such amino acid sequences are likely to find their application in the construction of predictably responsive materials.

It has been reported recently [87] that a hydrogel can be formed from very short peptide conjugate building blocks. Namely, naphtalene (Nap)–Phe–Phe conjugates can polymerize into biocompatible hydrogels. First, the peptide conjugates formed nanofibers which later self-assembled into hydrogel. Stability of the hydrogel structure was achieved by a network of π-π and hydrogen-bonding interactions. The Fmoc derivative of the Aβ diphenylalanine core motif can form a rigid hydrogel as well. It is composed of a fibrous network with fibril diameter on the interval between 10 and 100 nm. Considerable stability at a broad range of temperatures and pH values is characteristic of this hydrogel. The Fmoc-diphenylalanine hydrogel is remarkably strong and rigid in comparison to other peptide hydrogels. Thus it is a very promising candidate for several applications. It can be used as a scaffold for cell growth or it can get fabricated into a solid biocompatible mold that is suitable for encapsulation and controlled drug release.

Experimental evidence shows that certain amphiphilic β-sheet peptides with 7–17 amino acid residues composed of repeating pairs of hydrophilic and hydrophobic amino acid residues can efficiently self-assemble at the air–water interface and form a highly ordered two-dimensional β-sheet crystalline layer. The formed layer provides a planar scaffold and promises numerous nanoscale applications due to its exceptional one-dimensional elastic characteristics.

Furthermore, cell culture scaffolds have been produced [88] by designing artificial amphiphilic peptides based on various models of alternating polar and non-polar segments. A perforated hydrogel with more than 99% of water content can be formed from chemically customized peptides that undergo ordered self-assembly into 10-nm-wide fibrils in aqueous solution. Because of extremely high water content in hydrogel such peptides are suitable for the fabrication of scaffolds. Potential applications include a range of novel cell growth supports for applications in three-dimensional cell cultures, controlled cell differentiation, tissue engineering, and regenerative medicine.

Ghadiri et al. [89] noticed that β-sheet-like stacked cyclic peptides can form peptide nanotubes (Fig. 2). Stabilized by hydrogen bonds such structures show large axial symmetry. Studies by Reches and Gazit have shown [28,90] that peptide nanotubes with six-fold crystalline symmetry and nanospheres can be self-assembled from synthetic dipeptide H–Phe–Phe–OH. Gorbitz [91,92] has analyzed conformational packing of 160 dipeptides in order to establish a link between amino acid sequence and peptide nanotube structure. Based on his model peptide nanotubes with desired dimensions, shape, crystalline symmetry and wettability properties can be predicted and designed.

6. Biophysical properties of peptide nanotubes

6.1. Optical properties

Peptide nanomaterials can form various types of structure, such as nanotubes, nanobelts and nanospheres. If dimensions of material are comparable to de Broglie wavelength of confined charges quantum confinement phenomena can be observed [93]. Order of quantum confinement can range from two-dimensional quantum wells and one-dimensional quantum wires to quantum dots of zero dimension. This phenomenon gives rise to bioluminescence [93,94].

Density of electron states as a function of energy in three dimensions is quasi-continuous. By confining such system in one dimension, we obtain a quantum well and density of states changes dramatically, i.e. we get a step-like behavior. Density of states becomes a piecewise-constant function. By confining the system in two dimensions or three dimensions we get a quantum wire or a quantum dot. In both cases the density of states shows spikes. Changes in density of states result in remarkable optical properties that are used in light-emitting diodes and lasers.

Absorption spectra provide a confirmation that confinement phenomena really occur and allow for determination of the length of confining dimension. Experiments show [9] that this length approximately equals 2 nm which corresponds to two to three crystal unit cells. Excitons that are so tightly bound are responsible for pronounced

photoluminescence at room temperature. Therefore, peptide nanotubes are a promising material of biological origin for development of a new generation of quantum well lasers.

6.2. Piezoelectricity

Analysis of a wide range of bioinspired dipeptides shows that they are packed into a crystalline arrangement lacking a center of symmetry [92]. A study of diphenylalanine and dileucine [29] shows that both dipeptides can self-assemble into peptide nanotubes and crystallize into asymmetric crystal structure. Piezoelectric phenomenon is a linear dielectric effect. By using atomic force microscopy the longitudinal component of piezoelectric coefficient can be measured. Its value for diphenylalanine peptide nanotubes was found to be 60 pm/V [95], well above the typical values measured in biological tissues.

6.3. Second harmonic generation

Another effect that can be observed in ordered structures with no center of symmetry is second harmonic generation. Intrinsic and highly noncetrosymmetric protein nanotubes have been intensely studied [9] by two-photon spectroscopy to assess the presence of second harmonic generation. By using a pulsed power density of 1 GW/cm^2 a narrow second harmonic peak is obtained for diphenylalanine peptide nanotubes. Detailed studies show that for low power the intensity of second harmonic generation is a quadratic function of the excitation light power density. Measured non-linear optical susceptibility of peptide nanotubes is 20 pm/V, comparable to the best inorganic non-linear materials.

7. Biophysical properties of amyloid fibrils

If one compares the peptide nanotubes and amyloid fibrils the aromatic or charged amino-acid side chains get positioned at finite, regular distances along the axis because of the repetitive oligomeric structure of the filaments. From the repetition of aromatic and ionic side chain amino-acid residues, putative special optical and electrical properties can be anticipated. However, not much research has been performed about the optical, electrical and even less quantum properties of amyloid fibrils.

Here we have collected some data on biophysical properties of peptide nanotubes (previous section). We suggest that most of them could hold also for amyloid fibrils. For example, to see amyloid structures in living organisms without any label the effect of bioluminescence, similar to the one observed with nanotubes [93,94], has been used [96].

We propose some new experiments, such as measuring coherence length in liquid crystals and optical properties of amyloid fibrils, either directly or as a function of some external parameter. Studies of optical absorption and photoluminescence would reveal if, like peptide nanotubes, also amyloid fibrils exhibit quantum confinement effects. If so, we could calculate characteristic length of confinement and speculate where in the structure of amyloid fibrils the confinement actually occurs.

Another possible study is to measure non-linear optical susceptibility in second harmonic generation experiment. From the obtained value we could determine symmetry properties of amyloid fibrils.

Optical spectra further lead to the possibility of quantitatively evaluating the van der Waals interactions between amyloid fibrils just as it is done for e.g. carbon nanotubes or other filamentous structures [97]. This means that the frequency-dependent dielectric permittivity could be used in the Lifshitz formula wherefrom one could extract forces and torques between amyloid fibrils in various mutual geometric arrangements.

Lastly, already now it is known that amyloid-fibril-based films can function as protective coats for some species. This opens a whole new area of potential applications. Such amyloid fibril films could be used as a functionalized coating to improve properties of the coated material, for example increase electrical capacity of electrodes in batteries.

8. In perspective

In this review we left out from consideration ATP-driven self-assembly processes of actin filaments, which occur in the cell dynamics, linked to cell shape, transport and signaling, even shaping and strengthening synapses in the process of consolidation memories [98]. Cells contain two types of actin networks. One type, associated with the plasma membrane, is planar or two-dimensional, like a web; the other type, associated with the cytosol, is three-dimensional, giving cytosol gel-like properties. In both, the bundles and networks, the filaments are held together by actin cross-linking proteins such as fascin and gelsolin. It is of note that gelsolin is a known amyloidogenic protein itself. The F-actin filaments (negatively stained by uranyl acetate) as observed by transmission electron microscopy, appear as twisted strings of beads with diameter from 7 to 9 nm [99].

We suggest that biophysical properties of the actin filaments and amyloid fibrils may not be so distinct, however, we did not explore implications of such a possibility any further.

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References

[1] T. Fukuma, A.S. Mostaert, L.C. Serpell, S.P. Jarvis, Revealing molecular-level surface structure of amyloid fibrils in liquid by means of frequency modulation atomic force microscopy, Nanotechnology 19 (2008) 384010.
[2] L.C. Serpell, Alzheimer's amyloid fibrils: structure and assembly, Biochim. Biophys. Acta 1502 (2000) 16–30.
[3] M. Sunde, L.C. Serpell, M. Bartlam, P.E. Fraser, M.B. Pepys, C.C. Blake, Common core structure of amyloid fibrils by synchrotron X-ray diffraction, J. Mol. Biol. 273 (1997) 729–739.
[4] J.D. Sipe, The β-Pleated Sheet Conformation and Protein Folding: A Brief History, in: J.D. Sipe (Ed.), Amyloid Proteins, Wiley-VCH verlag GmbH & Co.KGaA, Weinhaim, 2005, pp. 49–61.
[5] M. Bucciantini, et al., Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases, Nature 416 (2002) 507–511.
[6] S. Ceru, et al., Size and morphology of toxic oligomers of amyloidogenic proteins: a case study of human stefin B, Amyloid 15 (2008) 147–159.
[7] D.M. Fowler, A.V. Koulov, W.E. Balch, J.W. Kelly, Functional amyloid–from bacteria to humans, Trends Biochem. Sci. 32 (2007) 217–224.
[8] A. Handelman, E. Mishina, A. Kudriavstev, N. Amdursky, G. Rosenman, Bioinspired Peptide Nanotubes: Ferroelectricity at Nanoscale, Integr. Ferroelectr. 134 (2012) 48–49.
[9] G. Rosenman, P. Beker, I. Koren, M. Yevnin, B. Bank-Srour, E. Mishina, S. Semin, Bioinspired peptide nanotubes: deposition technology, basic physics and nanotechnology applications, J. Pept. Sci. 17 (2011) 75–87.
[10] E. Zerovnik, Amyloid-fibril formation. Proposed mechanisms and relevance to conformational disease, Eur. J. Biochem. 269 (2002) 3362–3371.
[11] V.N. Uversky, Seven lessons from one IDP structural analysis, Structure 18 (2010) 1069–1071.
[12] E. Zerovnik, Oligomerization preceding amyloid fibril formation: a process in common to intrinsically disordered and globular proteins, Network 22 (2011) 154–161.
[13] J. McLaurin, T. Franklin, P.E. Fraser, A. Chakrabartty, Structural transitions associated with the interaction of Alzheimer beta-amyloid peptides with gangliosides, J. Biol. Chem. 273 (1998) 4506–4515.
[14] O.S. Makin, E. Atkins, P. Sikorski, J. Johansson, L.C. Serpell, Molecular basis for amyloid fibril formation and stability, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 315–320.
[15] R. Tycko, R.B. Wickner, Molecular Structures of Amyloid and Prion Fibrils: Consensus versus Controversy, Acc. Chem. Res. 46 (2013) 1487–1496.
[16] R. Tycko, Solid-State NMR Studies of Amyloid Fibril Structure, Annu. Rev. Phys. Chem. 62 (62) (2011) 279–299.
[17] R. Tycko, Characterization of amyloid structures at the molecular level by solid state nuclear magnetic resonance spectroscopy, Amyloid, Prions, and Other Protein Aggregates, Pt C 413, 2006. 103–122.
[18] J. Jenkins, R. Pickersgill, The architecture of parallel beta-helices and related folds, Prog. Biophys. Mol. Biol. 77 (2001) 111–175.

[19] I. Usov, J. Adamcik, R. Mezzenga, Polymorphism complexity and handedness inversion in serum albumin amyloid fibrils, ACS Nano 7 (2013) 10465–10474.
[20] L.R. Volpatti, M. Vendruscolo, C.M. Dobson, T.P. Knowles, A clear view of polymorphism, twist, and chirality in amyloid fibril formation, ACS Nano 7 (2013) 10443–10448.
[21] W. Hoyer, T. Antony, D. Cherny, G. Heim, T.M. Jovin, V. Subramaniam, Dependence of alpha-synuclein aggregate morphology on solution conditions, J. Mol. Biol. 322 (2002) 383–393.
[22] E. Zerovnik, M. Skarabot, K. Skerget, S. Giannini, V. Stoka, S. Jenko-Kokalj, R.A. Staniforth, Amyloid fibril formation by human stefin B: influence of pH and TFE on fibril growth and morphology, Amyloid 14 (2007) 237–247.
[23] I. Cherny, E. Gazit, Amyloids: not only pathological agents but also ordered nanomaterials, Angew. Chem. Int. Ed. Engl. 47 (2008) 4062–4069.
[24] N. Amdursky, P. Beker, J. Schklovsky, E. Gazit, G. Rosenman, Ferroelectric and related phenomena in biological and bioinspired nanostructures, Ferroelectrics 399 (2010) 10.
[25] E. Gazit, Mechanistic studies of the process of amyloid fibrils formation by the use of peptide fragments and analogues: implications for the design of fibrillization inhibitors, Curr. Med. Chem. 9 (2002) 1725–1735.
[26] E. Gazit, A possible role for pi-stacking in the self-assembly of amyloid fibrils, Faseb J. 16 (2002) 77–83.
[27] E. Jack, M. Newsome, P.G. Stockley, S.E. Radford, D.A. Middleton, The organization of aromatic side groups in an amyloid fibril probed by solid-state 2H and 19F NMR spectroscopy, J. Am. Chem. Soc. 128 (2006) 8098–8099.
[28] M. Reches, E. Gazit, Casting metal nanowires within discrete self-assembled peptide nanotubes, Science 300 (2003) 625–627.
[29] C.H. Gorbitz, Nanotube formation by hydrophobic dipeptides, Chemistry 7 (2001) 5153–5159.
[30] E. Zerovnik, V. Stoka, A. Mirtic, G. Guncar, J. Grdadolnik, R.A. Staniforth, D. Turk, V. Turk, Mechanisms of amyloid fibril formation–focus on domain-swapping, FEBS J. 278 (2011) 2263–2282.
[31] V.N. Uversky, A.L. Fink, Conformational constraints for amyloid fibrillation: the importance of being unfolded, Biochim. Biophys. Acta 1698 (2004) 131–153.
[32] J.D. Harper, P.T. Lansbury Jr., Models of amyloid seeding in Alzheimer's disease and scrapie: mechanistic truths and physiological consequences of the time-dependent solubility of amyloid proteins, Annu. Rev. Biochem. 66 (1997) 385–407.
[33] Y. Hirakura, Y. Satoh, N. Hirashima, T. Suzuki, B.L. Kagan, Y. Kirino, Membrane perturbation by the neurotoxic Alzheimer amyloid fragment beta 25–35 requires aggregation and beta-sheet formation, Biochem. Mol. Biol. Int. 46 (1998) 787–794.
[34] C.J. Pike, D. Burdick, A.J. Walencewicz, C.G. Glabe, C.W. Cotman, Neurodegeneration induced by beta-amyloid peptides in vitro: the role of peptide assembly state, J. Neurosci. 13 (1993) 1676–1687.
[35] M.M. Ramirez Alvarado, C. Sanchez Roitz, A. Perez Diaz, E. Millan Brito, Effect of a high saturated fatty acids load on serum concentrations of C-reactive protein, alpha1-antitrypsin, fibrinogen and alpha1-acid glycoprotein in obese women, Nutr. Hosp. 25 (2010) 72–79.
[36] S.I. Cohen, M. Vendruscolo, C.M. Dobson, T.P. Knowles, From macroscopic measurements to microscopic mechanisms of protein aggregation, J. Mol. Biol. 421 (2012) 160–171.
[37] J.T. Jarrett, P.T. Lansbury Jr., Seeding "one-dimensional crystallization" of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? Cell 73 (1993) 1055–1058.
[38] S.J. Wood, J. Wypych, S. Steavenson, J.C. Louis, M. Citron, A.L. Biere, alpha-synuclein fibrillogenesis is nucleation-dependent. Implications for the pathogenesis of Parkinson's disease, J. Biol. Chem. 274 (1999) 19509–19512.
[39] T.R. Serio, A.G. Cashikar, A.S. Kowal, G.J. Sawicki, J.J. Moslehi, L. Serpell, M.F. Arnsdorf, S.L. Lindquist, Nucleated conformational conversion and the replication of conformational information by a prion determinant, Science 289 (2000) 1317–1321.
[40] J.W. Kelly, Mechanisms of amyloidogenesis, Nat. Struct. Biol. 7 (2000) 824–826.
[41] S. Xu, B. Bevis, M.F. Arnsdorf, The assembly of amyloidogenic yeast sup35 as assessed by scanning (atomic) force microscopy: an analogy to linear colloidal aggregation? Biophys. J. 81 (2001) 446–454.
[42] A.J. Modler, K. Gast, G. Lutsch, G. Damaschun, Assembly of amyloid protofibrils via critical oligomers–a novel pathway of amyloid formation, J. Mol. Biol. 325 (2003) 135–148.
[43] G. Bhak, Y.J. Choe, S.R. Paik, Mechanism of amyloidogenesis: nucleation-dependent fibrillation versus double-concerted fibrillation, BMB Rep. 42 (2009) 541–551.
[44] G. Bhak, J.H. Lee, J.S. Hahn, S.R. Paik, Granular assembly of alpha-synuclein leading to the accelerated amyloid fibril formation with shear stress, PLoS One 4 (2009) e4177.
[45] R. Jerala, E. Zerovnik, Accessing the global minimum conformation of stefin A dimer by annealing under partially denaturing conditions, J. Mol. Biol. 291 (1999) 1079–1089.
[46] S. Jenko Kokalj, et al., Essential role of proline isomerization in stefin B tetramer formation, J. Mol. Biol. 366 (2007) 1569–1579.
[47] J.S. Jeong, A. Ansaloni, R. Mezzenga, H.A. Lashuel, G. Dietler, Novel mechanistic insight into the molecular basis of amyloid polymorphism and secondary nucleation during amyloid formation, J. Mol. Biol. 425 (2013) 1765–1781.
[48] S. Bolisetty, J. Adamcik, R. Mezzenga, Snapshots of fibrillation and aggregation kinetics in multistranded amyloid beta-lactoglobulin fibrils, Soft Matter 7 (2011) 493–499.
[49] R.C. Elgersma, D.T. Rijkers, R.M. Liskamp, pH controlled aggregation morphology of Abeta(16–22): formation of peptide nanotubes, helical tapes and amyloid fibrils, Adv. Exp. Med. Biol. 611 (2009) 239–240.
[50] J. Madine, H.A. Davies, C. Shaw, I.W. Hamley, D.A. Middleton, Fibrils and nanotubes assembled from a modified amyloid-beta peptide fragment differ in the packing of the same beta-sheet building blocks, Chem. Commun. (Camb.) 48 (2012) 2976–2978.
[51] A. Rawat, R. Nagaraj, Peptide Self-assembly: From Toxins to Amyloid Fibrils and Nanotubes, Curr. Top. Med. Chem. (2014).
[52] J.E. Gillam, C.E. MacPhee, Modelling amyloid fibril formation kinetics: mechanisms of nucleation and growth, J. Phys. Condens. Matter 25 (2013).
[53] C. Lara, J. Adamcik, S. Jordens, R. Mezzenga, General Self-Assembly Mechanism Converting Hydrolyzed Globular Proteins Into Giant Multistranded Amyloid Ribbons, Biomacromolecules 12 (2011) 1868–1875.
[54] J. Adamcik, V. Castelletto, S. Bolisetty, I.W. Hamley, R. Mezzenga, Direct Observation of Time-Resolved Polymorphic States in the Self-Assembly of End-Capped Heptapeptides, Angew. Chemie.Int. Ed. 50 (2011) 5495–5498.
[55] L. Adler-Abramovich, D. Aronov, P. Beker, M. Yevnin, S., S., L., B., G. Rosenman, E. Gazit, Self-assembled arrays of peptide nanotubes by vapour deposition, Nat. Nanotechnol. 4 (2009) 849–854.
[56] R.R. Kopito, Aggresomes, inclusion bodies and protein aggregation, Trends Cell Biol. 10 (2000) 524–530.
[57] 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.
[58] M. Fandrich, V. Forge, K. Buder, M. Kittler, C.M. Dobson, S. Diekmann, Myoglobin forms amyloid fibrils by association of unfolded polypeptide segments, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15463–15468.
[59] D.A. Butterfield, A.I. Bush, Alzheimer's amyloid beta-peptide (1–42): involvement of methionine residue 35 in the oxidative stress and neurotoxicity properties of this peptide, Neurobiol. Aging 25 (2004) 563–568.
[60] V.P. Patel, C.T. Chu, Nuclear transport, oxidative stress, and neurodegeneration, Int. J. Clin. Exp. Pathol. 4 (2011) 215–229.
[61] N. Arispe, H.B. Pollard, E. Rojas, Giant multilevel cation channels formed by Alzheimer disease amyloid beta-protein [A beta P-(1–40)] in bilayer membranes, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 10573–10577.
[62] S. Rabzelj, G. Viero, I. Gutierrez-Aguirre, V. Turk, M. Dalla Serra, G. Anderluh, E. Zerovnik, Interaction with model membranes and pore formation by human stefin B: studying the native and prefibrillar states, FEBS J. 275 (2008) 2455–2466.
[63] H. Lin, R. Bhatia, R. Lal, Amyloid beta protein forms ion channels: implications for Alzheimer's disease pathophysiology, Faseb J. 15 (2001) 2433–2444.
[64] Y.J. Lin, G.J. Greif, J.E. Freedman, Permeation and block of dopamine-modulated potassium channels on rat striatal neurons by cesium and barium ions, J. Neurophysiol. 76 (1996) 1413–1422.
[65] S. Treusch, et al., Functional links between Abeta toxicity, endocytic trafficking, and Alzheimer's disease risk factors in yeast, Science 334 (2011) 1241–1245.
[66] J.W. Lustbader, et al., ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease, Science 304 (2004) 448–452.
[67] G. Anderluh, E. Zerovnik, Pore formation by human stefin B in its native and oligomeric states and the consequent amyloid induced toxicity, Front. Mol. Neurosci. 5 (2012) 85.
[68] H.A. Lashuel, P.T. Lansbury, Are amyloid diseases caused by protein aggregates that mimic bacterial pore-forming toxins? Q. Rev. Biophys. 39 (2006) 167–201.
[69] S. Lanone, P. Andujar, A. Kermanizadeh, J. Boczkowski, Determinants of carbon nanotube toxicity, Adv. Drug Deliv. Rev. 65 (2013) 2063–2069.
[70] P.P. Simeonova, Update on carbon nanotube toxicity, Nanomedicine (Lond) 4 (2009) 373–375.
[71] C.W. Lam, J.T. James, R. McCluskey, S. Arepalli, R.L. Hunter, A review of carbon nanotube toxicity and assessment of potential occupational and environmental health risks, Crit. Rev. Toxicol. 36 (2006) 189–217.
[72] A.A. Shvedova, A. Pietroiusti, B. Fadeel, V.E. Kagan, Mechanisms of carbon nanotube-induced toxicity: focus on oxidative stress, Toxicol. Appl. Pharmacol. 261 (2012) 121–133.
[73] D.M. Fowler, A.V. Koulov, C. Alory-Jost, M.S. Marks, W.E. Balch, J.W. Kelly, Functional amyloid formation within mammalian tissue, Plos Biol. 4 (2006) e6.
[74] Y. Zhou, D. Smith, B.J. Leong, K. Brannstrom, F. Almqvist, M.R. Chapman, Promiscuous cross-seeding between bacterial amyloids promotes interspecies biofilms, J. Biol. Chem. 287 (2012) 35092–35103.
[75] J.M. Kenney, D. Knight, M.J. Wise, F. Vollrath, Amyloidogenic nature of spider silk, Eur. J. Biochem. 269 (2002) 4159–4163.
[76] S.J. Hamodrakas, A. Hoenger, V.A. Iconomidou, Amyloid fibrillogenesis of silkmoth chorion protein peptide-analogues via a liquid-crystalline intermediate phase, J. Struct. Biol. 145 (2004) 226–235.
[77] S.J. Ling, C.X. Li, J. Adamcik, S.H. Wang, Z.Z. Shao, X. Chen, R. Mezzenga, Directed Growth of Silk Nanofibrils on Graphene and Their Hybrid Nanocomposites, ACS Macro Lett. 3 (2014) 146–152.
[78] D.C. Harper, A.C. Theos, K.E. Herman, D. Tenza, G. Raposo, M.S. Marks, Premelanosome amyloid-like fibrils are composed of only golgi-processed forms of Pmel17 that have been proteolytically processed in endosomes, J. Biol. Chem. 283 (2008) 2307–2322.
[79] S.K. Maji, et al., Functional amyloids as natural storage of peptide hormones in pituitary secretory granules, Science 325 (2009) 328–332.
[80] R. Wetzel, S. Shivaprasad, A.D. Williams, Plasticity of amyloid fibrils, Biochemistry 46 (2007) 1–10.
[81] T. Scheibel, R. Parthasarathy, G. Sawicki, X.M. Lin, H. Jaeger, S.L. Lindquist, Conducting nanowires built by controlled self-assembly of amyloid fibers and selective metal deposition, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 4527–4532.
[82] Y. Song, et al., Synthesis of peptide-nanotube platinum-nanoparticle composites, Chem. Commun. (Camb.) 1044–5 (2004).

[83] C. Li, A.K. Born, T. Schweizer, M. Zenobi-Wong, M. Cerruti, R. Mezzenga, Amyloid-Hydroxyapatite Bone Biomimetic Composites, Adv. Mater. (2014).
[84] C.X. Li, J. Adamcik, R. Mezzenga, Biodegradable nanocomposites of amyloid fibrils and graphene with shape-memory and enzyme-sensing properties, Nat. Nanotechnol. 7 (2012) 421–427.
[85] L. Sasso, S. Suei, L. Domigan, J. Healy, V. Nock, M.A.K. Williams, J.A. Gerrard, Versatile multi-functionalization of protein nanofibrils for biosensor applications, Nanoscale 6 (2014) 1629–1634.
[86] A.M. Corrigan, C. Muller, M.R. Krebs, The formation of nematic liquid crystal phases by hen lysozyme amyloid fibrils, J. Am. Chem. Soc. 128 (2006) 14740–14741.
[87] Z. Yang, G. Liang, L. Wang, B. Xu, Using a kinase/phosphatase switch to regulate a supramolecular hydrogel and forming the supramolecular hydrogel in vivo, J. Am. Chem. Soc. 128 (2006) 3038–3043.
[88] X. Zhao, S. Zhang, Designer self-assembling peptide materials, Macromol. Biosci. 7 (2007) 13–22.
[89] M.R. Ghadiri, J.R. Granja, R.A. Milligan, D.E. McRee, N. Khazanovich, Self-assembling organic nanotubes based on a cyclic peptide architecture, Nature 366 (1993) 324–327.
[90] M. Reches, E. Gazit, Controlled patterning of aligned self-assembled peptide nanotubes, Nat. Nanotechnol. 1 (2006) 195–200.
[91] C.H. Gorbitz, Structures of dipeptides: the head-to-tail story, Acta Crystallogr. B 66 (2010) 84–93.
[92] C.H. Gorbitz, Microporous organic materials from hydrophobic dipeptides, Chemistry 13 (2007) 1022–1031.
[93] N. Amdursky, M. Molotskii, D. Aronov, L. Adler-Abramovich, E. Gazit, G. Rosenman, Blue Luminescence Based on Quantum Confinement at Peptide Nanotubes, Nano Lett. 9 (2009) 3111–3115.
[94] N. Amdursky, I. Koren, E. Gazit, G. Rosenman, Adjustable Photoluminescence of Peptide Nanotubes Coatings, J. Nanosci. Nanotechnol. 11 (2011) 9282–9286.
[95] A. Kholkin, N. Amdursky, I. Bdikin, E. Gazit, G. Rosenman, Strong piezoelectricity in bioinspired peptide nanotubes, ACS Nano 4 (2010) 610–614.
[96] J.C. Watts, K. Giles, S.K. Grillo, A. Lemus, S.J. DeArmond, S.B. Prusiner, Bioluminescence imaging of A beta deposition in bigenic mouse models of Alzheimer's disease, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 2528–2533.
[97] R.H. French, et al., Long range interactions in nanoscale science, Rev. Mod. Phys. 82 (2010) 1887–1944.
[98] W. Huang, et al., mTORC2 controls actin polymerization required for consolidation of long-term memory, Nat. Neurosci. 16 (2013) 441–448.
[99] H.F. Lodish, olecular cell biology, Freeman, Basingstoke, New York, NY, 2000.
[100] S. Jenko, M. Skarabot, M. Kenig, G. Guncar, I. Musevic, D. Turk, E. Zerovnik, Different propensity to form amyloid fibrils by two homologous proteins-Human stefins A and B: searching for an explanation, Proteins 55 (2004) 417–425.