

# Self-organization of Short Peptide Fragments: From Amyloid Fibrils to Nanoscale Supramolecular Assemblies

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*Received (in Austin, USA) 21 July 2004; Accepted 9 September 2004*

Numerous supramolecular protein assemblies have been demonstrated to have either physiological or pathological activities. The most significant case of disease-associated self-organized structures is that of amyloid fibrils. The formation of these fibrils is the hallmark of major human disorders, including Alzheimer's disease and type II diabetes. In this review we illustrate the molecular properties of the amyloid fibrils as supramolecular assemblies in the nanometric scale. We present recent advances in the elucidation of the molecular recognition and self-assembly processes that lead to the formation of these toxic structures, and we describe how the mechanistic study of amyloid formation process has led to unexpected discoveries of peptide-based nanostructures.

*Keywords:* Amyloid fibrils; Molecular recognition; Peptide nanostructures; Self-assembly; Supramolecular biochemistry

## INTRODUCTION: SUPRAMOLECULAR ASSEMBLIES IN BIOLOGY

The formation of supramolecular protein structures is common to all living systems. The level of complexity of these structures ranges from bimolecular receptor–ligand complex formation, which may be regarded as a “host-guest” interaction, through the self-assembly of microscopic virus particles, up to the formation of macroscopic self-assembled fibrils such as silk. Many of the cellular proteinous assemblies are considered to have a “quaternary structure”, which refers to the non-covalent assembly of folded, tertiary-structured, protein subunits. Such quaternary structures include the oxygen carrier, hemoglobin, several enzymes and other “cellular machines”. In this review, we focus on

a specific class of quaternary structure formation, the self-assembly of polypeptides into highly ordered and large fibrillar structures that play a major pathological role, specifically the disease-related amyloid assemblies. We further explore the connection between these assemblies and novel bioinspired nanomaterials.

The natural fibrillar supramolecular structures that are mostly related to amyloid fibrils, as discussed below, are those of spider and worm silk. These fibrils are composed predominantly of two protein molecules that create macroscopic filaments by precise molecular recognition and self-assembly processes [1,2]. The notable properties of silk fibers are their outstanding strength and high flexibility. Despite its noncovalent supramolecular nature, spider silk is considered, on the one hand, to be considerably stronger than a steel filament of the same diameter but, on the other hand, highly flexible. These unusual mechanical properties are the basis for the stability and functionality of the spider-net and cocoon. This serves as a vivid example of the unique properties of supramolecular biomaterials, and their potential superiority over traditional organic and inorganic materials.

## AMYLOID FIBRILS: NATURAL NANOSCALED SUPRAMOLECULAR STRUCTURES

Another key example of noncovalent fibrillar structures that occurs in biological systems is found in the disease-related amyloid fibrils. The formation of amyloid fibrils is associated with a large number of major diseases. A partial list includes Alzheimer's

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disease, type II diabetes, prion diseases, Parkinson's disease and various familial and systemic amyloidosis disorders [3–6]. Interestingly, in the various diseases, different proteins with no structural or functional homology are found to form amyloid fibrils with similar chemical and physical properties. Unlike the case of silk, amyloid fibrils do not represent the native conformation of the protein but rather an alternative one that seems to be formed spontaneously and is thermodynamically favorable.

At the chemical level, the amyloid fibrils are large and highly ordered self-organized structures with a clear X-ray diffraction pattern with a 4.6–4.8 Å diffraction on the meridian and an average diameter of 7–10 nm [3–5,7–9]. They are rigid and organized in bundles. The secondary structure of the fibrils is also very uniform and consists mainly of cross- $\beta$ -sheet elements. In cross-section, amyloid fibrils appear as hollow cylinders or ribbons [10–12]. Hence, they were referred to as water-filled nanotubes by the late Max Perutz [13]. The mechanism of amyloid formation, although not fully understood, resembles the processes of crystallization or gelation [8,14]. These processes begin with the slow formation of “nuclei” or “seeds” from which larger assemblies are being rapidly assembled. Similarly, the process of amyloid formation begins with the formation of intermediates that are small oligomers that serve as seeds for fibril development. The nuclei are formed during a kinetic lag-phase whose length is highly concentration dependent [6,14,15]. The regularity of the formed fibrils along their long axis, together with the classical nucleation and growth mechanism, has led to the referral of the process of amyloid formation as “one-dimensional crystallization” [14].

### SHORT PEPTIDES CAN FORM AMYLOID-LIKE STRUCTURES

As the formation of amyloid fibrils is associated with major human disease, significant research efforts are being devoted towards understanding the mechanism of amyloid assembly. Genuine mechanistic insight into the molecular mechanism may pave the way toward the design of agents that will inhibit this process [9,16–18]. A key direction of such studies involves the search for the recognition elements within amyloidogenic proteins. These elements mediate the processes of molecular recognition and self-assembly that lead to the formation of the fibrils [19–22]. Peptide fragments as short as tetrapeptides were found to form fibrillar structures that resemble the biochemical and ultrastructural nature of the fibrils formed by much larger polypeptides. This approach reduces significantly the complexity of the system in the search for

common molecular denominators that mediate the formation of similar structures by a diverse group of proteins.

The most evident example of the use of small peptide models to understand the basis of amyloid fibril self-assembly is the study of the islet amyloid polypeptide (IAPP) [19,21,23,24]. The IAPP is a 37-amino-acid peptide hormone that is found to form amyloid fibrils in the pancreas of individuals with type II diabetes. Westermark *et al.* [23] have shown that typical amyloid fibrils can be formed by the central specie-specific decapeptide sequence IAPP<sub>20–29</sub> (SNNFGAILSS; all the sequences are presented in a one-letter code). Tenidis *et al.* [19] later showed that even shorter peptide fragments within this region, the penta- and hexapeptide sequences hIAPP<sub>23–27</sub> (FGAIL) and hIAPP<sub>22–27</sub> (NFGAIL), can form fibrillar structures (Fig. 1a). Later studies identified two other peptide sequences, hIAPP<sub>14–18</sub> (NFLVH) and hIAPP<sub>15–19</sub> (FLVHS), that form well-ordered fibrillar structures. These peptides were shown to be part of a central molecular recognition module within the IAPP hormone [21].

Another study has demonstrated the formation of amyloid-like fibrils by a pentapeptide and a tetrapeptide, DFNKF and DFNK, derived from the human calcitonin (hCT) polypeptide hormone [20]. Amyloid fibrils composed of hCT are found to be associated with medullary carcinoma of the thyroid. This is the first reported case in which a peptide as short as a tetrapeptide was shown to form amyloid-related fibrils. It was also shown that the KLVFFAE heptapeptide fragment of the  $\beta$ -amyloid, the main constituent of amyloid plaques in the brains of Alzheimer's disease patients, forms amyloid fibrils [22]. These fibrils were characterized by solid-state NMR and were shown to have highly organized antiparallel  $\beta$ -sheet assemblies. Finally, a recent study has demonstrated that the NFGSVQ hexapeptide fragment of Medin, the constituent of amyloid deposits found in all individuals above the age of 60 [25], forms fibrillar structures that are remarkably similar to those formed by the full-length polypeptide [26].

When all the various peptide fragments are compared (Fig. 1a), the existence of aromatic residues within the fragments is quite evident. We have previously hypothesized that aromatic residues play a key role in the formation of amyloid fibrils through  $\pi$ -stacking interactions [27]. We speculate that stacking interactions, rather than mere hydrophobicity, may provide an energetic contribution as well as order and directionality in the self-assembly of amyloid structures. The stacking hypothesis suggests a new approach to understanding the self-assembly mechanism that governs amyloid formation, enables the identification of novel motifs, and indicates possible ways to control this process.

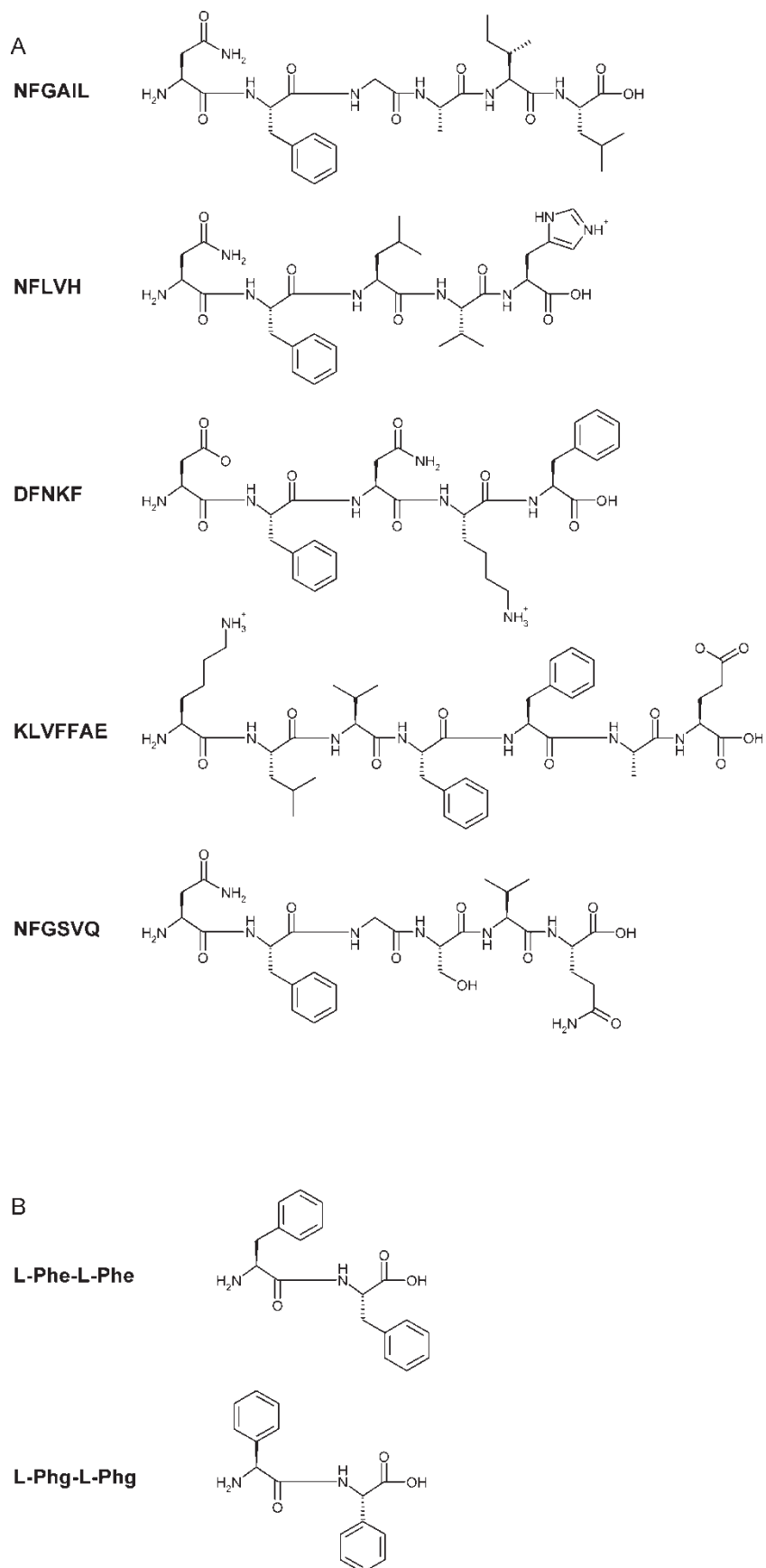


FIGURE 1 Chemical structures of self-assembling peptides. (a) Short peptides, fragments of amyloidogenic proteins that were shown to form typical amyloid-like structures. (b) Dipeptides that were shown to form tubular and spherical nanostructures.

The examples above demonstrate that amyloid fibrils can be formed by very short peptide fragments, and not only by intact proteins. This indicates that short linear sequences within a protein contain all the molecular information that is needed to facilitate amyloid formation. Although amyloid-forming peptides are variable in their sequences, they are thought to have a common denominator that enables them to form fibrils. Following the identification of short but potent amyloidogenic regions, many studies have focused on the determination of important elements within these regions by systematic amino acid modifications and position switching [24,28–31]. Of interest are the variant morphologies formed by similar peptides [19,21,30]. For example, while the NFLVHSSNN peptide, derived from the IAPP, forms long thin coiling filaments, the NFLVHSS peptide forms large broad ribbon-like fibrils [21]. However, there is no difference in secondary structure between the peptide fibers. This emphasizes the importance and contribution of each amino acid in amyloid forming peptides. Another peptide-nanoscale field developing in parallel to the one described above is the production of nanotubes and nanostructures composed of peptide building blocks.

## PEPTIDE-BASED NANOSTRUCTURES

Peptide nanostructures provide an interesting example of the well-organized structures that can be formed by simple peptide building blocks. We have found a clear connecting line between amyloid structures and a family of novel nanometric peptide assemblies (discussed below). The advantages of peptides over other materials used for nanostructures are their complex biological properties, which can be used to manipulate different structures, their biocompatible nature and their ability to be biodegradable. One central advantage is their spontaneous formation through a process of self-organization. A number of peptide-based nanomaterials have been prepared using different building blocks, including cyclic peptides with alternating D- and L-amino acids [32,33], peptide amphiphiles and peptide bolaamphiphiles, which undergo different chemical modifications [34–37], short peptides with alternating negatively and positively charged residues and surfactant-like peptides [38,39], aromatic dipeptides [40–42] and hydrophobic dipeptides [43].

A pioneering study has demonstrated the formation of hollow tubular nanostructures by the self-assembly of cyclic peptides, designed with an even number of alternating D- and L-conformation amino acids [32,33]. This unique architecture resulted in flat ring-shaped subunits that are stacked together through intermolecular hydrogen bonds in

a  $\beta$ -sheet conformation, similar to the one suggested for amyloid fibrils. The closed cycle and the alternating D- and L-conformations direct the side chains outwards of the ring and the backbone amides approximately perpendicular to the ring plane. The self-assembly process and the geometry of the nanotubes could be controlled by rationally changing the number of amino acids in the ring and the amino acid identity.

Discrete nanofibers were also shown to be assembled from peptide amphiphiles (PAs) [34]. These are amphiphile molecules composed of a long hydrophobic alkyl chain tail and a hydrophilic peptide chain head. The assumed structure of the nanotube formed is of a cylindrical micelle in which the PA is perpendicular to the fiber axis with the hydrophobic tail in the fiber center and the hydrophilic head at the external part of the fiber. After the nanofibers were formed, they underwent different manipulations such as cross-linking and mineralization. These were achieved by designing the PAs to contain cysteine residues that are oxidized to form intermolecular disulfide bonds and residues that interact with metal ions. Similar nanofibers were also formed by peptide bolaamphiphiles, which are molecules with hydrophilic heads in both sides of a hydrophobic chain [35]. In this case, the fiber center is hydrophilic as well as the external surface. Hypothetically, this alteration from the PA nanotubes results in the formation of hollow nanofibers. If so, it is possible that mineralization could also be achieved in the interior of the fiber, thus creating a new variety of nanomaterials.

Nanostructures can serve as templates for nanoparticle fabrication in the bottom-up approach, as in the mineralization example above. Peptide nanotubes have been used as templates for the production of Au nanowires and nanocrystals [36,37]. The template nanowires were self-assembled from peptide bolaamphiphile monomers, and then a histidine-rich peptide was immobilized on the nanowires [36]. Au nanowires were formed by the addition of Au ions that were reduced and immobilized on the nanotube. The use of histidine-rich peptide leads to a uniformly coating with monodisperse Au nanocrystals. Recently, in a resembling procedure, Au nanocrystals were grown inside the cavities of doughnut-shaped peptide nanoassemblies [37].

Another direction of the use of peptide nanostructures is self-assembling peptide scaffolds for biological systems. Hydrogel scaffolds made of peptides with alternating hydrophilic and hydrophobic amino acids were used as template for tissue-cell attachment, extensive neurite outgrowth, and formation of active nerve connections [38]. It was also revealed that surfactant-like peptides undergo self-assembly to form nanotubes and nanovesicles. The peptide monomer contains 7–8

residues and has a hydrophilic head composed of aspartic acid and a tail of aliphatic hydrophobic amino acids. The surfactant-like peptides formed a network of open-ended nanotubes with remarkable size uniformity [39].

One study of amyloid fibrils formation has led to the unexpected discovery of a novel type of nanomaterial. In our path to discover the shortest peptide fragment that can form amyloid fibrils, we discovered that the diphenylalanine recognition motif of the Alzheimer's  $\beta$ -amyloid polypeptide self-assembles into ordered and discrete peptide nanotubes with micro-scale persistence length (Fig. 1b) [40]. It was also shown by our group and others [41] that these peptide nanotubes can serve as a mold for the fabrication of nanoscale inorganic materials. A parallel independent study has demonstrated that the KLVFFAE heptapeptide fragment of the  $\beta$ -amyloid polypeptide mentioned above can also form very similar tubular structures under the right assembly conditions [44].

A previous study on the crystallization of the diphenylalanine peptide, formed by evaporation of aqueous solution at 80°C, showed the formation of aligned and elongated channels within the crystalline lattice [42]. These structures were also referred to as peptide nanotubes. However, the crystal arrangement of the dipeptide is completely different from the molecular organization of the self-assembled discrete tubular structures. Later studies of the crystallization of hydrophobic dipeptides also revealed similar crystal packing [43].

We later revealed that diphenylglycine, a highly similar analogue and the simplest aromatic peptide, forms spherical nanometric assemblies (Fig. 1b) [45]. Both the nanotubes and nanospheres assemble efficiently and have remarkable stability. The introduction of a thiol group into the diphenylalanine peptide alters its assembly from tubular to spherical particles similar to those formed by diphenylglycine. The formation of either nanotubes or closed cages by fundamentally similar peptides is

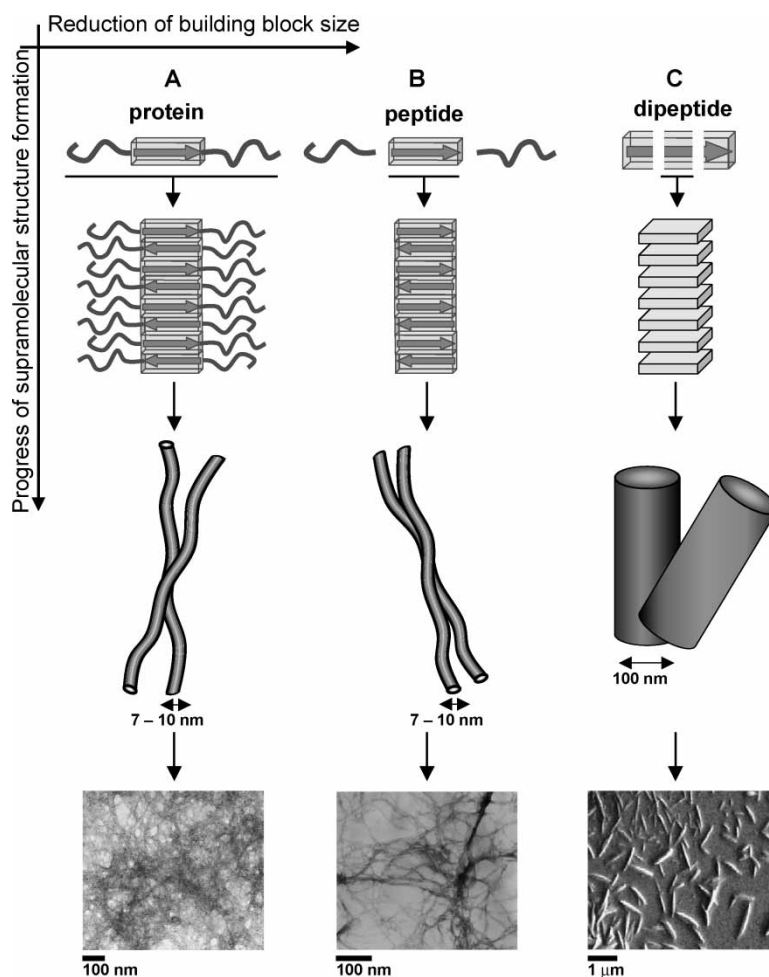


FIGURE 2 The molecular linkage between amyloid fibrils and novel types of peptide nanostructures. Various types of supramolecular nanostructures are formed by peptidic building blocks derived from similar origin. The monomeric building block (upper panel) is stacked and self-assembles to a supramolecular structure (bottom panel depicts electron microscopic photographs). (a) Amyloid fibril formation by a natural whole protein. (b) Amyloid-like fibrils formation by a synthetic short peptide recognition fragment. (c) Peptide nanotubes formation by the shortest motif within the recognition fragment.

consistent with a two-dimensional layer closure, as described for both carbon and inorganic nanotubes and for their corresponding buckminsterfullerene and fullerene-like structures [46].

## DISCUSSION

In this short review we have described recent studies in two branches of supramolecular biochemistry that are investigating the formation of nanoscale objects by the self-assembly of various polypeptide molecules into highly ordered structures. One branch is dealing with the pathological phenomenon of amyloid fibril formation and is driven by a medical motivation to prevent the formation of these toxic assemblies. The other branch is focused on the development of novel materials and devices as part of the emerging nanotechnology field. Apparently, these two branches have no common root. However, similar building blocks are used and comparable nanostructures are formed. It appears that common general principles of geometrically restricted interactions, and specific and coordinated assemblies, are being shared by both groups. Indeed, understanding the mechanism of the self-organization processes has led to the discovery of novel peptide-based nanostructures (Fig. 2). A key theme is the hypothesis regarding the role of  $\pi$ -stacking interactions in amyloid fibril formation, which has led us to the production of peptide nanotubes and nanospheres.

Peptide nanostructures represent an intriguing example of an interdisciplinary research characteristic of recent modern science. Applications, methodologies and theories that were applied to the study of carbon and inorganic nanostructures should be of great importance for future exploration and utilization of the peptide nanostructures. The properties of the peptide nanostructures, taken together with their biological compatibility and remarkable thermal and chemical stability, may provide very important tools for future nanotechnology applications.

## Acknowledgements

We thank the members of the Gazit group for helpful communications. Support from the Israel Science Foundation (Bikura program) is gratefully acknowledged.

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