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Progress in the direct structural characterization of fibrous amphiphilic supramolecular assemblies in solution by transmission electron microscopic techniques

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ABSTRACT

The self-assembly of amphiphilic molecules into fibrous structures has been the subject of numerous studies over past decades due to various current and promising technical applications. Although very different in their head group chemistry many natural as well as synthetic amphiphilic compounds derived from carbohydrates, carbocyanine dyes, or amino acids tend to form fibrous structures by molecular self-assembly in water predominantly twisted ribbons or tubes. Often a transition between these assembly structures is observed, which is a phenomenon already theoretically approached by Wolfgang Helfrich and still focus point in current research. With the development of suitable sample preparation and electron optical imaging techniques, cryogenic transmission electron microscopy (cryo-TEM) in combination with three-dimensional (3D) reconstruction techniques has become a particular popular direct characterization technique for supramolecular assemblies in general. Here we review the recent progress in deriving precise structural information from cryo-TEM data of particularly fibrous structures preferably in three dimensions.

Contents

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1. Introduction

The emergence of life on earth is to a great extent based on the ability of certain molecular building blocks to generate structurally precise and functional supramolecular architectures in a molecular self-assembly process [1]. The significance of a precise structure for a determined functionality therefore defines the superior scientific goal: the assignment of supramolecular structures at the highest possible spatial resolution with the objective of understanding the interdependency with their function.

Nature has generated a number of different molecular players, which show self-assembling behavior, most importantly lipids, nucleic acids, proteins and carbohydrates. Depending on the individual molecular composition several driving forces determine the eventual three-dimensional structural organization of supramolecular assemblies, which are solvophobic effects, inter- and intramolecular hydrogen bonding, π–π-stacking, multivalent ionic interactions (complexation), and chirality [2,3]. The multitude of potential molecular interactions is one of the reasons why often hierarchically organized complex structures form and it also clarifies the difficulties in understanding corresponding structure formation processes or in designing molecules for the construction of predetermined supramolecular architectures.

A strategy to cope with this complexity is to create simpler molecular building blocks, which allow studying the impact of each of the above factors on structure formation either independently or at least in restricted combinations. In this context, a most suitable class of building blocks are amphiphilic molecules, which in their simplest form assemble by the interplay of hydrophilic and hydrophobic molecular domains due to the solvophobic or, in particular, the hydrophobic effect in an aqueous environment [4]. The interest in amphiphilic molecules and their ability to form supramolecular architectures by self-assembly dates back more than a hundred years and was initially focused on soaps, fats and oils [5] but continues up to date.

Perhaps the most important naturally occurring amphiphiles are phospholipids. A balanced volume ratio of water-exposed hydrophilic head groups and hydrophobic tails, respectively, initiates the formation of molecular bilayers, which constitute the membranes of living cells. At first glance, one would expect that extended two-dimensional membranes or large spherical vesicles with minimum curvature would generally represent the lowest-energy state of amphiphilic bilayers. The variety of observed structures, however, is much more diverse and with the development of suitable preparation and imaging techniques structural investigations (which took its course in the 1980s) revealed various uncommon morphologies, including ring- and disk-like, as well as extended fibrous structures. By evaluating publications of recent decades which were dealing with self-assembly of amphiphilic compounds it was found that an overwhelming number of them reported on fibrous structures, i.e. threads, ribbons, tapes or hollow tubes [6–9]. This is noteworthy given that the studied materials significantly differed in their molecular building blocks and their dominating intermolecular forces. Particularly supramolecular fibrous assemblies exhibiting helical ultrastructural features predominantly formed by chiral molecules became of interest. Chirality is certainly one of nature's key strategies to trigger more complex ultrastructures [10] and, most importantly, enables stereospecific molecular recognition processes [11], but also plays a prominent role in pharmaceutical therapies [12].

A most-favored phenomenon with chiral amphiphiles is the interdependent formation of twisted ribbons, wound ribbons and tubules. These structures often coexist in preparations or their transition can be successively monitored by temperature or time-dependent preparation techniques. These observations have initiated a vivid interest to explain the relation between different types of anisotropic intermolecular interactions and the development of corresponding morphological features [13]. Theoretical descriptions of the forces driving the formation of helical ribbons and their maturation towards closed tubes were especially promoted by Helfrich [14,15]. Due to the importance of this phenomenon and the fact that morphological transitions from ribbons to tubes are frequently observed independently of the molecular structure, we will particularly highlight systems in this contribution, that favor such kind of assembly process.

The allocation of precise experimental data is of fundamental importance to understand self-assembly processes. Moreover, accurate structural characterization is the precondition for a reproducible manufacture of molecular assemblies for technical applications. Due to the limitation that a vast majority of assemblies never crystallize (or adopt a different spatial or conformational organization in the crystalline state, see Section 3.2.), which, however, is a prerequisite for X-ray structure analysis, electron microscopy (TEM as well as SEM), especially in combination with cryo-fixating preparation techniques, has gained increased significance for the elucidation of supramolecular structures. Thanks to image processing and three-dimensional (3D) reconstruction techniques, including cryo-electron tomography, and in synergy with complementary analytical methods like NMR spectroscopy, scanning probe microscopy (AFM, STM), optical microscopy, X-ray fiber diffraction or various spectroscopic methods the characterization of supramolecular assemblies on the molecular and even the atomic level is in closer reach (see Section 2).

From this point of view we review on the latest advances in the fabrication and structural characterization of defined fibrous supramolecular assemblies and provide an update on the technical progress which became available in recent years to elucidate 3D molecular architectures with high resolution. Appropriate examples of amphiphilic glycolipids, dyes, amino acids, and peptides are chosen. Studies on biological materials (in particular protein structures), however, would go far beyond the scope of this review and are therefore not included.

2. TEM imaging, image processing and 3D reconstruction techniques

2.1. Cryo-TEM — a direct characterization method for native supramolecular architectures

The goal of this section is not to provide a general introduction to the field of electron microscopy, covering detailed aspects of electron optics, image formation, etc. For these aspects, the reader is referred to dedicated monographs or reviews cited below. In fact, the synergistic effects comprising novel approaches of sample preparation, instrumentation and image analysis techniques are the focus of this review, all of which have significantly improved our knowledge about structural aspects of supramolecular assembly processes in recent years. It should be noted that these strategies have long been successfully implemented in the methodical canon of structural biology. With the progress in synthetic supramolecular chemistry, which has produced a growing number of structurally precise supramolecular architectures, these established methods become applicable and promise to provide a much better understanding of the factors governing molecular self-assembly.

Transmission electron microscopy has the huge advantage over all alternative direct and indirect structure characterization methods that it can provide structure information at very high spatial resolution in the native assembly state by employing suitable preparation and imaging methods. Moreover, due to the fact that electrons transmit the sample, projection images (comparable to the recording of a radiography where all densities of a 3D object are accumulated in a 2D image) are generated from which internal structural information can be elucidated by the application of image processing and 3D reconstruction methods. A more detailed description of corresponding strategies is given in Section 2.2.1.

Low contrast and radiation sensitivity are the main obstacles to overcome in transmission electron microscopy in order to obtain high resolved structural information of biological or organic supramolecular architectures. The use of contrast agents, which aims to embed objects in an electron dense matrix of heavy metal salts, helps to increase