

# Patenting Activity in Synthesis of Lipid Nanotubes and Peptide Nanotubes

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Received: September 11, 2006; Accepted: October 3, 2006; Revised: November 6, 2006

**Abstract:** Lipid nanotubes (LNTs) and peptide nanotubes (PNTs) are especially intriguing and noncovalent self-assemblies of amphiphiles. They have hydrophilically internal and external membrane surfaces, and can provide the wide scope for chemical modifications, in sharp contrast to carbon nanotubes. These unique properties make themselves as ideal candidates for a variety of applications in chemistry, biochemistry, materials science and medicine. Patenting the LNTs and PNTs is quite active recently. This mini-review provides a brief outline of patenting activity in synthesis of the LNTs and PNTs since 1980s. The key point of the present review aims to create an optimistic circulation between the basic research achievement and potential application of this sub-field of nanotechnology, promoting each other in their future development.

**Keywords:** Lipid, peptide, nanotube, self assembly and hollow cylinder.

## INTRODUCTION

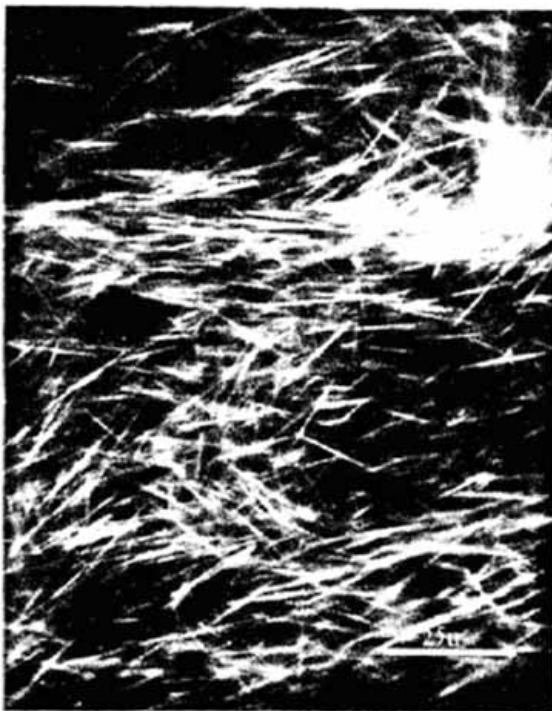
Nature has developed astonishingly efficient and sophisticated strategies for generating various nanostructures at room temperature and atmospheric pressure. Since 1980s, increasing interest has been focused on supramolecular self assembly of small organic molecules to give organized materials with macroscopically well-defined shapes and structures *via* molecular-recognition functions and non-covalent interactions such as hydrogen bonds,  $\pi$ -stacking, hydrophobic and van der Waals interactions [1]. This supramolecular assembly offers numerous opportunities for chemical variation and provides an important direction for the controlled fabrication of a new class of nanoscopic materials and devices [2]. Thus, the realization of self-assembly will have far-reaching significance for not only fundamental understanding but also important applications.

Lipid nanotubes (LNTs) and peptide nanotubes (PNTs) are especially intriguing assemblies, which represent a potentially powerful architecture generated through self-assembly of amphiphilic molecules [3]. They can be considered among the largest self-organized non-living structures yet observed. Such tubular structures may offer a variety of applications in chemistry, biochemistry, materials science and medicine. In this mini-review, we briefly assess the patents and the patent-interrelated papers concerning the preparation of LNTs and PNTs. The key point of the present mini-review aims to create an optimistic circulation between the basic research achievement and potential application of this sub-field of nanotechnology, promoting each other in their future development.

## PATENTING ACTIVITY IN LNTS

Schnur and co-workers first reported and patented the preparation of LNTs self-assembled from diacylenic phospholipids, when they investigated the phase behavior of 1,2-bis(10,12-tricosadiynoyl)-*sn*-glycero-3-phosphocholine (DC<sub>2,3</sub>PC) in 1984 [4-7]. They found that those formed tubules were morphologically analogous to soda straws with diameters of approximately 400-1000 nm, wall thickness of 2-10 bilayers (10-50 nm) and lengths from 1 to 200  $\mu$ m. Aging under suitable conditions may produce tubules in excess of 1.2 mm [8]. The optical micrograph of the LNT was shown in Fig. 1. Theories based on molecular chirality has explained the molecular architecture of tubules [9, 10], suggesting that the formation of tubular morphologies is driven by twisting of the amphiphile bilayer due to symmetry breaking in the packing of chiral molecules. A typical preparation process of the DC<sub>2,3</sub>PC-based LNT was described below. A chloroform solution of DC<sub>2,3</sub>PC was evaporated to dryness under nitrogen, then placed *in vacuo* for 18 h. Deionized water was subsequently added to the lipid, giving a concentration of approximately 4 mg ml<sup>-1</sup>. The lipid was then hydrated by heating the mixture to at least 20 K above the melting point of the lipid (*T*<sub>m</sub> for DC<sub>2,3</sub>PC is 43°C). Gentle agitation aided hydration. Finally, the dispersion was slowly cooled to room temperature at approximately 0.3°C min<sup>-1</sup>. The LNTs formed spontaneously as the temperature passed through the phase transition from liquid crystal to gel. Furthermore, an alternative preparation technique can be used to produce somewhat longer tubules. It involves the addition of lipid to ethanol at concentrations of approximately 0.3 mg ml<sup>-1</sup>. When water is slowly added, tubules are formed from the solution. The tubule structures produced can be polymerized to render them mechanical, thermal, and chemical stability by exposure to a UV lamp or gamma ray irradiation, or a suitable polymerization reaction. Much efforts have been contributed to understanding how

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**Fig. (1).** Optical micrograph of DC<sub>2,3</sub>PC tubules in distilled water taken with dark field illumination. Reproduced with permission from *Thin Solid Film*, **1987**, 152, 181. Copyright **1987** Elsevier Sci. B. V.

structural variation affects nanotube dimensions at the molecular level, which has facilitated a more efficient and systematic approaches to rationally generating these tubular libraries. For instance, the length and inner diameter of the LNTs may be adjusted by varying the molecular chirality and tilt, the cooling rate during the preparation [3].

Until now 17 different diacetylenic lipids have been prepared to form the LNT [11]. Moreover, besides the family of diacetylenic lipids, several other classes of amphiphiles have also been explored to create LNTs, including peptidic-lipid [12], bolaamphiphiles [13], and glycolipid conjugates [14].

It has been widely reported that the LNTs may act as an excellent template to create one-dimensional metal nanotubes by coating the template with metal precursors [2]. The most preferred method for the coating of metal is electroless plating, developed by Brenner and Ridell [15] and Shipley [16]. The electroless plating is a particularly rapid and effective means of producing thin, reasonably uniform metal coatings on the exterior and interior surfaces of the tubules, and the procedure mainly involves inexpensive reagents and do not require complicated or expensive processing equipment [17-19]. Schnur [20] and Zabetakis [21] patented the coating of the diacetylenic-based LNT with the electroless plating technique to convert the LNT into a hollow metal cylinder. The typical procedure for making metallic microcylinders from pretreated LNTs was described as below [20]. The aqueous dispersion of the LNT was acidified by dialysis against 0.1 M HCl. The protonated LNT solution was slowly added to a fourfold (by volume)

excess of commercial Pd-Sn colloidal activator, followed by being filtered and rinsed with excess HCl solution to remove unbound activator. The dispersion was rinsed with deionized water again until the filtrate reached pH 5, and concentrated to the original volume and then added to a fourfold excess of the desired metal plating bath. After the desired length of time in the plating bath, the metallized LNT were added to a twenty fold excess of water, filtered and rinsed copiously with water. The thickness of the metal layer is controllable by adjusting the plating time, typically ranging between about 20 nm to 100 nm. The results show that the metallization procedure of the electroless plating is substantially compatible with the chemical and mechanical constraints of the lipid microstructures so that the tubular morphology is preserved. However, the produced metal microcylinders typically have an aspect ratio of less than the employed LNTs [22]. Reduction of the aspect ratio of the metal microcylinders is indicative of considerable breakage of the tubules during metallization, probably attributed to the mechanical stress on the tubules from the centrifugation and resuspension procedures. It is deduced that the tubules can be metallized with any metals capable of being plated. By plating on the tubules an electrically conducting metal, such as copper, highly electrically conducting tubules can be formed; by plating on the tubules a magnetic metal, such as nickel, tubules of low electrical conductivity but of high magnetism can be obtained; by plating both an electrically conducting metal and a magnetic metal with an overplating process, i.e. deposit a coating of another metal on the initial metal coat, tubules can be produced with high electrical conductivity and high magnetism.

One of the most important applications of the produced metal nanotube is to be employed as antifouling agent. In marine field, many organisms create an environment attractive to free swimming marine larva. By creation of an environment inhospitable to these organisms fouling, free swimming larva may be reduced or eliminated. Price *et al* reported and patented metal tubule-based nanocomposite to solve the problem of marine fouling [23-26]. In their reports, the small copper or copper and zinc coated tubules are demonstrated to be excellent anti-microbial or pesticidal agents. The toxic potential of the metal tubules can be further increased by encapsulating biocides into the hollow cylinder of the tubule. The applied biocides include bactericides, herbicides, molluskicides, insecticides, pesticides. Encapsulation of the biocides was accomplished by dispersing the desired biocide into a fluidic carrier. The selection of the carrier is determined by the viscosity of the carrier and the solubility of the active agent in the carrier. The carrier must possess a sufficiently low viscosity so that it can fill the lumen of the tubule as a result of capillary action. This carrier may be a monomer, a linear polymer or a polymerizable cross-linking material. The release rate for a given agent is determined by the average inner diameter and length of the LNT, the viscosity of the carrier, the relative solubilities of the agent in the carrier and in the surrounding matrix (if present), and molecular weight of the active agents as well as that of the carrier. If the agent is soluble or is mobile in the carrier, then the rate of release will mainly depend on the diffusion rate and solubility of the agent in the carrier and in the external matrix. If the agent is insoluble or

immobile in the carrier, then the rate of release will mainly depend on the rate of release of the carrier itself from the tubule.

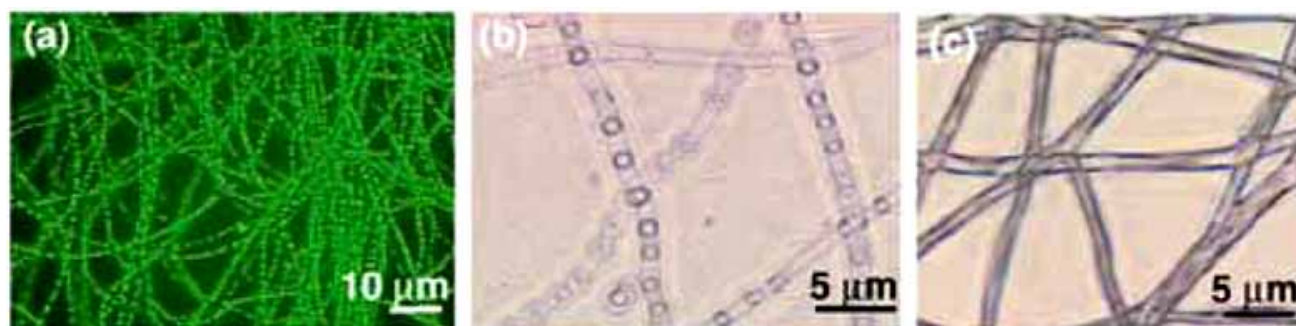
For extending the application of LNTs, Kulkarni patented the LNTs for topical delivery of drug into skin [27]. It is well known that skin is an excretory organ that often causes topical delivery of pharmacological or cosmetic agents difficult to penetrate against the natural excretory forces. Moreover, the skin surface is enriched with sweat, bacteria, and cells that have been damaged or killed by ultraviolet light, creating a harsh environment for drug molecules and making the drug susceptible to degradation before reaching their target. The delivery system with LNTs confers special advantages for topical delivery of agents to the skin over other delivery vehicles. The diameter of human skin pores has been estimated to about 40 nm [28]. Unlike traditional liposomal systems, LNTs have a significant size population under 100 nanometers in diameter, while still carrying significant quantities of active ingredient. These LNTs are therefore particularly useful as topical drug delivery vehicles because their small size permits rapid dermal penetration. In addition, the tubular delivery system described in Kulkarni's patent consists of lipids compatible with lipids in stratum corneum, which further facilitates skin penetration. Furthermore, the delivery system with LNTs is capable of transporting a multitude of active ingredients, including drugs, genetic material or cosmaceuticals deep into the skin.

Besides the pure tubular cylinder, Shimizu *et al* patented a new oligoglycine-appended peptide-lipid compounds forming an interesting microtubes containing spherical vesicles thereof [29-31]. The molecular structure of the lipid was formulated as following:  $\text{MO}-(\text{CO}-\text{CH}_2-\text{NH})_p-\text{CO}-(\text{CH}_2)_n-\text{CO}-(\text{NH}-\text{CH}_2-\text{CO})_q-\text{OM}$  (1), wherein M represents a hydrogen atom or an alkali metal, n is an integer of 6-18 and p and q each represent an integer of at least 1. The unique fibrous microtubes were obtained by allowing an aqueous solution of an alkali metal salt of the compound to stand, preferable quiescently, in air or in an atmosphere of an organic acid for a period of time sufficient to grow the fibrous microtubule, preferable from 3 days to 4 weeks. Fig. 2 shows a typical phase-contrast microscopic image of the vesicle-encapsulated microtubes. It can be seen that the tubular body generally has a uniform diameter of about 1-3  $\mu\text{m}$  with closed ends, a wall thickness of 10-100 nm and a length in the range from 200  $\mu\text{m}$  up to 5 mm. The stiff tube

may be linear or branched, and contain many multilamellar vesicular thereof. These smaller spherical vesicles around the ends of the fibrous were movable and undergo Brownian motion inside the tube. The tubular nanostructures are of strongly physical and thermal properties, which can stand a few minute of sonification, dehydration and subsequent drying in vacuum without destroying their morphology. The dried structure can be reconstructed by rehydration by addition of excess water and sonication.

It has been demonstrated that the tubular self-assembly of 1 is based on the multiple hydrogen-bonded networks provided by sugar and peptide moieties of the lipid molecules. The molecular structure plays crucial role on the formation of the vesicle-encapsulated microtube. Both the glycylglycine residue in oligopeptide headgroup and appropriate lengths of even-numbered oligo(methylene) spacers are indispensable for the microtube formation. The bolaamphiphiles containing no glycylglycine or glycylglycylglycine residues did not produce microtubular structures through self-assembly. In addition, it is preferred that p and q be the same and be in the range of 1 to 3 when n is smaller than 6. In contrast, when the total number of p and q is greater than 6, the peptide lipid is insoluble in water so that it is difficult to use the peptide lipid for the preparation of fibrous microtube assemblies. The concentration of the lipid also plays an important role on the formation of the unique superstructure. The aqueous solution of the alkali metal salt of the peptide lipid preferably has a lipid concentration of 5 mM to 15mM. Too high a concentration above the saturated point leads to produce an amorphous solid while at excessively low concentration a long period of time is required for the formation of molecular assemblies in the form of fibrous microtubes.

As mentioned above, magnetic LNTs can be produced by coating the LNT with metals exhibiting magnetic properties such as Fe, Ni, Ni-B, Ni-P, Ni-Fe-B, and Co-B alloys with the electroless plating technique [32]. Recently, Matsui patented another method for fabricating magnetic LNTs with the aid of magnetic bacteria [33,34]. The fabrication process consists of three steps: firstly magnetite ( $\text{Fe}_3\text{O}_4$ ) nanocrystals was produced by a bacteria [35], then the magnetic nanocrystals thus produced were extracted from the cells, and incorporated into the hollow cylinder of a LNT produced by self assembly of a glycylglycine-based bolaamphiphiles, bis(N- amidoglycylglycine)-1,7-heptane dicarboxylate,



**Fig. (2).** Vesicle-encapsulated microtubes observed using phase-contrast light microscopy: (a) and (b) in water at 25 °C (left and middle) and (c) after vacuum drying (right). Reproduced with permission from *Macromolecular Rapid Commun.* **2002**, 23, 311. Copyright **2002** VCH.

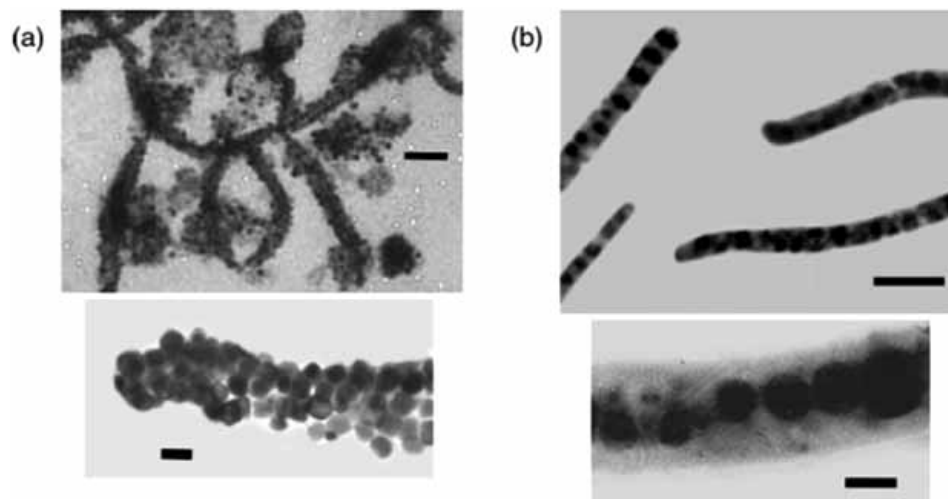
which was firstly synthesized by Shimizu and Kogiso *et al.* [12]. Fig. 3 shows a typical TEM image of the bacterial magnetic nanocrystals coated on the entire nanotube surface (a) and inside the LNT (b). The reports show that the bacterial magnetic nanocrystals can be selectively deposited only to the interior or to the exterior surfaces of the nanotubes when the concentration of the nanocrystal in the nanotube solution is optimized. At high concentration, the nanocrystal was found to deposit on the outer surface of the nanotube, and low concentration resulted in selective incorporation of the magnetic nanocrystals substantially inside the nanotube to form a linear chain. The produced linear chain of the magnetic nanoparticles introduces a uniaxial magnetic anisotropy, not present in the magnetic particles or assemblies commonly known. Compared to chemically produced  $\text{Fe}_3\text{O}_4$  nanocrystals, there are two advantages of using bacterial magnetic nanocrystals. First, the bacterial magnetic crystals are covered with lipid-bilayer membranes consisting of organic lipids and proteins. The proteins on the bacterial magnetic nanocrystals may have a particularly strong affinity toward the applied LNTs [36], which facilitates the contacting of the bacterial magnetic nanocrystals onto the surfaces of the LNT. Another advantage is that the mineral type, crystal size, and morphology of the magnetic nanocrystals can be controlled by the selection of the bacterial species or strain. It is expected that the invented approach offers a low-cost fabrication process to novel magnetic nanotubes.

Starting with the studies of McConnell and co-workers [37,38], it is now well documented that phospholipid vesicles fused spontaneously into planar membranes when incubated on treated solid surfaces. Smirnov *et al.* presented an invention related to coating of a lipid bilayer on the inner wall of a porous substrate to form LNT arrays [39]. The porous substrate may be composed of any suitable material including but not limited to carbon, glass, silicon, metals and metal oxides such as anodic aluminum oxide (AAO), polymers such as polycarbonate, or any other nonconductive, conductive or semiconductive material. In a particular

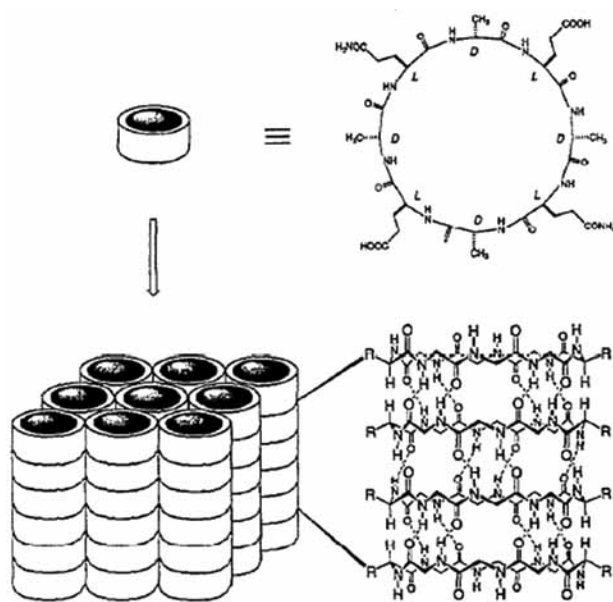
embodiment of AAO-supported phospholipid bilayers [40-42], the lipids were deposited by exposing treated AAO disks from one side to a 20% phospholipid aqueous dispersion. The lipids in fluid bilayers undergo a complex anisotropic motion including fast rotations around the long axis, lateral diffusion, and flip-flop, and finally self assemble into lipid nanotubes inside the AAO substrate. The substrate-supported LNT arrays have potential for building robust biochips and biosensors, in which the rigid substrates protect the bilayer surface from contamination.

#### PATENTING ACTIVITY IN PNTS

Ghadire *et al.* in 1993 first report the design, synthesis and characterization of a new class of PNTs based on rationally designed eight alternating D- and L-amino acid residue *cyclo*[-(D-Ala-Glu-D-Ala-Gln)<sub>2</sub>-] [43,44]. The schematic illustration of the chemical structure of the peptide subunit (D or L refers to the amino acid chirality) was presented in Fig. 4. The cyclic peptide is created by linking together the two terminal amino acids in the chain by an amide bond. This subunit has a flat ring-shaped conformation. It was found that by controlled acidification of the alkaline peptide solution, the cyclic peptide may be triggered to spontaneously stack on top of each other in an anti-parallel fashion and hold together by the formation of  $\beta$ -sheet hydrogen bonding, resulting in a hollow nanotube also shown in Fig. 4. In this conformation, all backbone amide functionalities of the cyclic peptide lie approximately perpendicular to the plane of the ring structure, facilitating the tubular assembly of individual cyclic peptide molecules. The PNT self assembled from the eight residue peptide has a uniform 0.75 nm internal diameter and hundreds of nanometers long and open-ends. The internal pore diameter of the peptide nanotube can be rigorously controlled simply by adjusting the ring size of the peptide subunit employed, i.e., the number of amino acid residues within the cyclic structure. The largest pore diameter of 1.3 nm peptide-based nanotube structure thus far was constructed by utilizing the thirty six-membered ring peptide subunit *cyclo*[-(Gln-D-Ala-



**Fig. (3).** TEM micrographs of (a) bacterial magnetic nanocrystals coated on the entire nanotube surface, and (b) bacterial magnetic nanocrystals coated inside the LNT. Scale bars are 300 nm. Lower images are at the higher magnification (Scale bars are 50 nm). Reproduced with permission from *Adv. Mater.* **2005**, *17*, 1128. Copyright **2005** VCH.

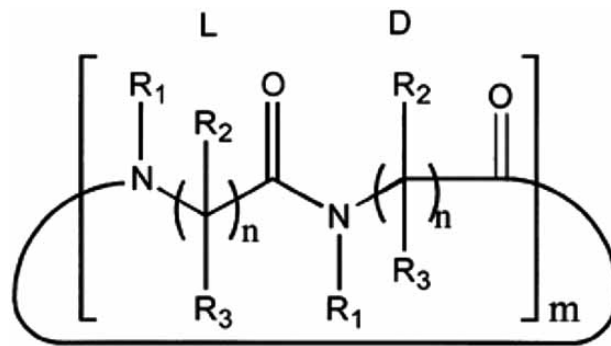


**Fig. (4).** Cyclic peptides with alternating D-, L-amino acids preferentially adopt flat disk-like conformations. Under the appropriate conditions, the cyclic subunits self assemble into highly ordered parallel arrays of nanotubular objects. The extensive intersubunit hydrogen bonding interactions between the backbone amides are responsible for the contiguous  $\beta$ -sheet structure (for clarity most side chains are omitted). Reproduced with permission from Mater. Sci. Engineering C, **1997**, 4, 207. Copyright **1997** Elsevier Sci. B. V.

Glu-D-Ala)<sub>3</sub>-] [45]. The further study reveals that too small of a ring structure of less than six residues may have prohibitively large ring strain to allow the peptide backbone to adopt the required geometry for stacking and intermolecular hydrogen-bonding interactions, and thus are not useful in the context of nanotube designs. Conversely, too large of a ring structure, due to the greater flexibility of the peptide backbone, may not adopt the flat ring-shaped conformation state to effectively take part in the nanotube self-assembly process. The studies reveal that the formed PNTs exhibit highly mechanical, chemical and thermal stabilities. The PNTs are stable for long periods of times in most common organic polar and non-polar solvents including DMF and DMSO, and can withstand repeated centrifugation and strong vortex mixing. The nanotube crystals are even stable to highly acidic (pH = 1) and strongly basic solutions (pH = 14) and can survive even boiling water [46]. The remarkable stability of the PNTs may be attributed to the highly cooperative nature of the self-assembled structure which simultaneously reinforces multiple noncovalent interactions throughout the lattice. The properties of the outer surface and the internal diameter of PNTs can be adjusted simply by the choice of the amino acid side chain functionalities. Cyclic peptides with appropriately chosen hydrophobic side chain can be inserted into lipid bilayers to form highly efficient transmembrane ion channels [47]. The channels allow ion transport at rates remarkably better than the performance of natural ion channels formed from Gramicidin A and Amphotericin B. Efficient channel-mediated transport of glucose has also been demonstrated for

a large pore transmembrane tubular structure [48]. The driving force for the self-assembly of the channel structure in lipid bilayers is primarily provided by the enthalpic contribution of a large number of hydrogen bonding interactions which are favored in the low dielectric constant medium of lipid bilayers and by the increase in the lipid chain entropy arising from side chain-lipid interactions.

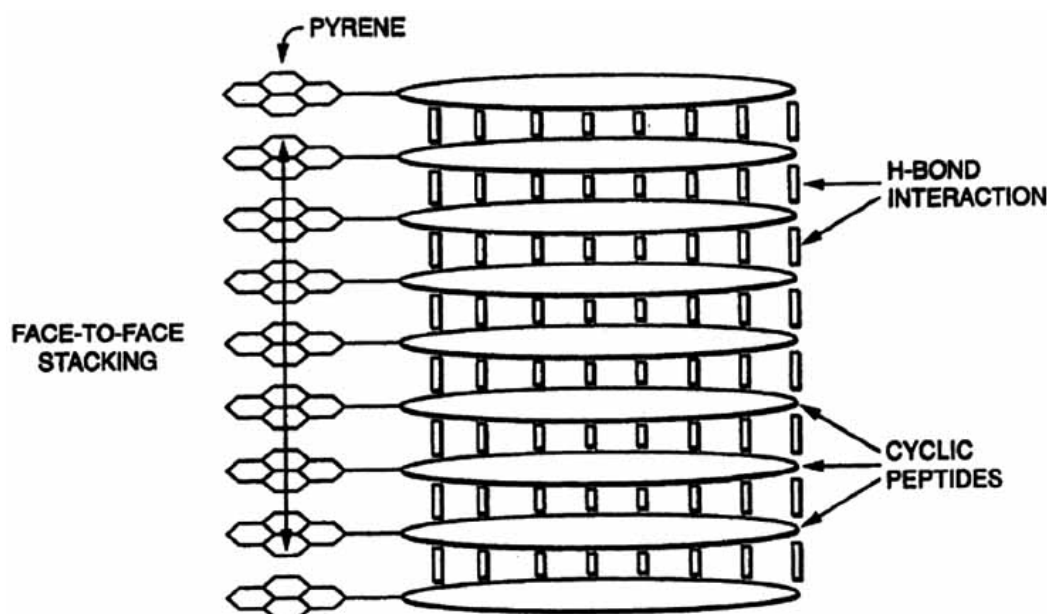
Recently, based on Ghadiri's work, McGimpsey *et al* reported an invention describing a new family of cyclic peptide monomers comprising chromophore residues which possess electronic conductivity and non-linear optical behavior for producing electronic and photonic devices with molecular scale [49]. The cyclic peptide is represented by the general formula, as shown in Fig. 5, wherein R<sub>1</sub> is H, CH<sub>3</sub> or alkyl; R<sub>2</sub> a chromophore or a rigid and flat  $\pi$ -conjugated system other than benzene, such as pyrene and pyrenylalanine; R<sub>3</sub> is H, CH<sub>3</sub> or a polar or non-polar organic functional group used for controlling peptide stacking and solubility; n equals 1 or 2; m equals 4 or 6; and a first adjacent amino acid residue has an alphacarbon chirality of L and a second adjacent amino acid residue has an alphacarbon chirality of D. The cyclic peptides have been shown to stack into PNTs in an anti-parallel fashion in which L-isomers in one cyclic peptide lie on top of L-isomers in another cyclic peptide (and the same for D-isomers).



**Fig. (5).** General formula of the stackable cyclic peptide.

Fig. 6 provide a schematic representation of a stacked array of di-pyrenyl cyclic peptide where the cyclic peptides form a nanotube structure through hydrogen bonding and the pyrene residues stack face-to-face to provide maximum  $\pi$ -orbital overlap for enhanced conduction. With having increasing numbers of pyrene residues of cyclic peptides, the formed PNTs will exhibit higher conductive behavior. The formed PNT containing pyrene can provide a conduct for the flow of charge over large distances, *i.e.*, a molecular scale wire. In addition to electronic conduction applications, cyclic PNT supramolecular assemblies containing alternating electron donor-electron acceptor groups provide a family of materials having precisely controlled non-linear optical behavior.

Besides the cyclic peptide, linear peptide molecules can also form PNTs. Zhang *et al.* describes a new class of surfactant-like peptides that can self-assemble to form regular nanotubes [50-52]. The sequences and molecular models of the several surfactant-like peptides are shown in



**Fig. (6).** Schematic representation of a stacked array of di-pyrenyl cyclic peptide where the cyclic peptides form a nanotube structure through hydrogen bonding and the pyrene residues stack. Reproduced with permission from *US20030144185A1* (2003).

Fig. 7. The designed peptide monomer contains 7-8 residues, and has a hydrophilic head group of negatively charged aspartic acid at the C terminus, thus containing two negative charges (one from the side chain carboxyl group and the other from the C terminus) and a lipophilic tail made of six consecutive hydrophobic amino acids such as alanine, valine, or leucine. The N terminus is acetylated, making it uncharged. The length of each peptide is about 2 nm, similar to that of biological phospholipids, and the overall hydrophobicity of these peptides can be fine-tuned by modifying the aliphatic side groups of the amino acids. When dissolved in water, this class of surfactant-like peptides undergo self-assembly to form nanotubes. The TEM image (Fig. 8) shows that the nanotubes with open ends is of an average diameter of 30-50 nm and the length to several

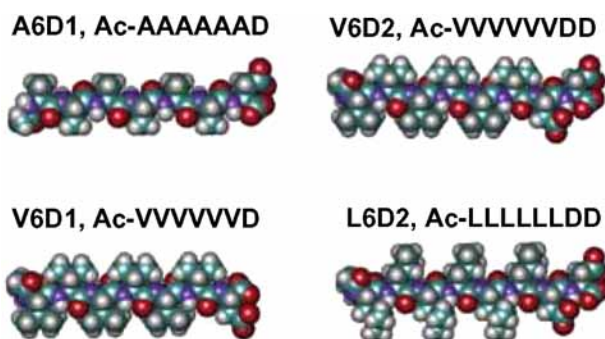
tens of micrometers. Cryo-TEM micrographs reveal numerous threefold junctions among the PNTs connecting the self-assembling nanostructures and thus leading to the formation of a rather dense network of entangled nanotubes. A possible structural organization is suggested by molecular modeling of the PNTs. It is proposed that two peptides initially form dimeric tail-to-tail packing to form a bilayer creating a unilamellar shell. Then, they grow into single subunit rings and multirings through continuous dynamic energy minimization. Finally, the cylindrical rings can subsequently stack together via non-covalent interactions to form continuous tubes.

## CURRENT & FUTURE DEVELOPMENTS

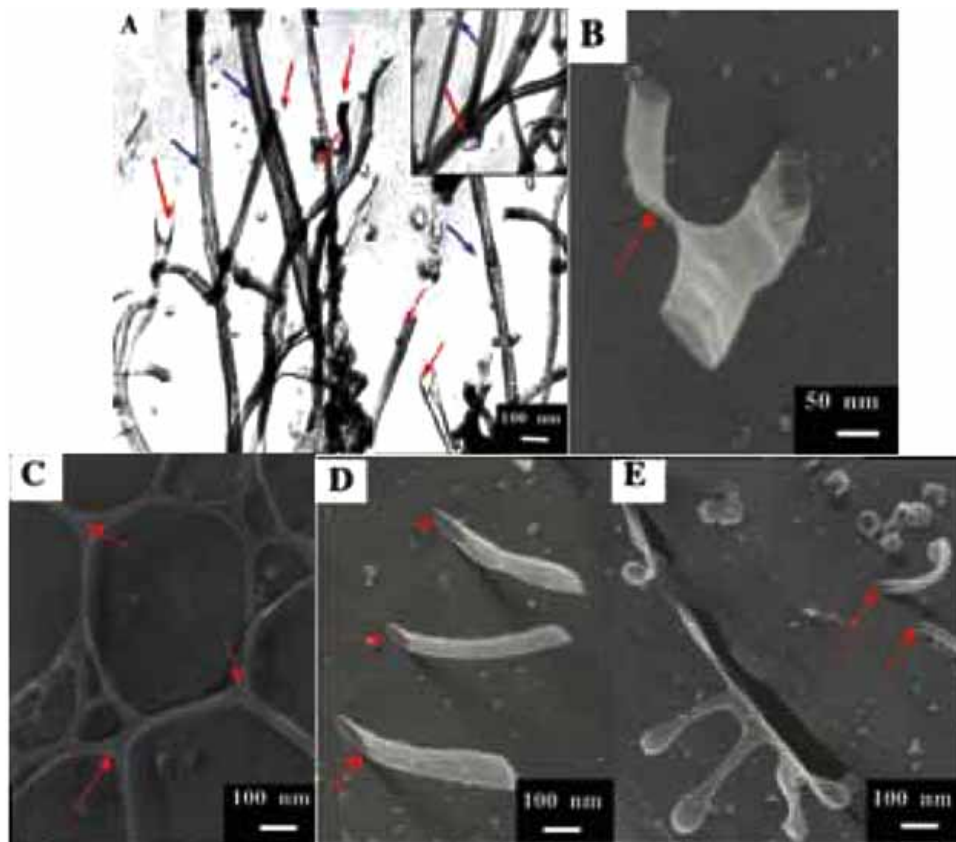
In past two decades, significant progress has been made in the realization of the controlled preparation and the understanding of formation mechanism of the LNT and PNT. Next stage is moving to explore their extensive application. One of the most prosperous applications of this one-dimensional nanostructure is to act as attractive carriers in delivery of drugs and controlled release. However, for practical consideration of this application, there are still many questions to remain to be addressed. For instance, firstly, there is a requisite for large-scale production of the LNT and PNT in a low-cost way. Secondly, convenient approaches should be explored to improve encapsulation yield of drug in the hollow cylinder of the LNT and PNT. Confidently, it is believed that as the field matures and moves closer to its lofty goal, the problems will be settled one by one, and it will be fascinating to see how these biocompatible microvials will be employed as smart vehicles for a greater variety of drug delivery and controlled release in practice.

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**Fig. (7).** Space-filling molecular models of surfactant peptide. (A) A6D. (B) V6D. (C) V6D2. (D) L6D2. D (aspartic acid) bears negative charges, and A (alanine), V (valine), and L (leucine) constitute the hydrophobic tails with increasing hydrophobicity. Reproduced with permission from *Proc. Natl. Acad. Sci. USA*, 2002, 99, 5355. Copyright 2002 the National Academy of Sciences.



**Fig. (8).** (A) Quick-freeze/deep-etch TEM image of A6D and V6D dissolved in water (4.3 mM at pH 7) at high-resolution. The images show the dimensions, 30–50 nm in diameter with openings of nanotube ends (red arrows). (*Inset*) Opening ends in more detail. Note some opening ends of the PNT may be cut vertically. The strong contrast shadow of the platinum coat also suggests the hollow tubular structure. *B* and *C* show a three-way junction and many three-way junctions, respectively. There are openings at the ends (*D* and *E*, indicated by red arrows), with the other ends possibly buried inside the replica. There also are some vesicles and nanotubes in the upper right corner (*E*, arrows point to the hollow opening at the ends). Micelles and vesicles are present also. (*E*) Example of vesicles that are budding off of a nanotube. Reproduced with permission from *Proc. Natl. Acad. Sci. USA* **2002**, 99, 5355. Copyright **2002** the National Academy of Sciences.

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