Lipid Tubules: A Paradigm for Molecularly Engineered Structures

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The use of molecular self-assembly to fabricate microstructures suitable for advanced material development is described. Templating techniques that transform biomolecular self-assemblies into rugged and stable nano- and microstructures are described. By using a lipid-based microcylinder (tubule) as a paradigm, the path followed from research and development to emerging technological applications is detailed. This process includes modification of the lipid molecular structure, the formation and subsequent characterization of cylindrical microstructures, the use of these structures as templates for metallization, and the characterization and assessment of these hollow metal microcylinders for several potential applications.

There has been much excitement about the possibility of using nanosized structures for real world applications (*1*). In fact, there are now a number of techniques for fabricating ultrasmall structures. Atomic force and scanning tunneling microscopy (*2*) techniques are now being used for atomic- and molecular-scale manipulations. Molecular self-assembly is being used to fabricate microstructures that may have potential for material applications (*3*). The development of characterization techniques in the regime below 1000 Å coupled with enhanced understanding of the role of molecular architecture in determination of material properties suggests that this area is ripe for technological breakthroughs.

Recent advances in biology have given us insights into the way in which nature uses self-assembly to construct microstructures for use in the functioning of living organisms. Living systems are constructed through the hierarchal assembly of these microstructures on many different size scales. In order to use biomolecular self-assembly techniques to design, engineer, and fabricate nano- and microstructures for the development of advanced materials and sensors, several complex issues must be addressed. The precise relation between the molecule that is the building block of the microstructure and the microstructure itself must be clearly understood. Methods must be developed to modify the structure of the molecule in order to optimize the microstructure for particular applications. In most cases, the resulting microstructures will not be sturdy and will be unable to survive wide extremes in environmental conditions. Techniques must be developed to render these self-assembled nano- and microstructures rugged and stable; the problem of cost and mass production must then be solved.

This article describes one approach based upon the molecular self-assembly of lipids to form submicrometer diameter tubules (*6*) with the potential for real world applications in the manufacture of electroactive composites and for use as microvials for long-term release applications. Biologically based self-assembly is used to fabricate a microstructure, and the structure is then made functional with templating techniques.

This process involves a number of distinct steps, including:

1) Synthesis and modification of the self-assembling molecule.

2) Self-assembly of the microstructure.

3) Characterization of the microstructure.

4) Use of the microstructure as a template or scaffold for ruggedization and function modification.

5) Characterization of the templated structures.

6) Assessment for potential applications.

This approach (like many in the material sciences) requires the expertise of a number of different disciplines (for example, biology, biochemistry, organic chemistry, inorganic chemistry, physics, and materials engineering). This article describes a coherent effort across all of these disciplines in which lipid molecules are used to form self-assembled hollow cylinders that are subsequently coated with metal for materials applications. The results of this effort can be viewed as a paradigm for the use of biologically based self-assembly for the development of advanced materials in general.

Molecular and Kinetic Aspects of Tubule Formation

Any serious attempt to use molecular self-assembly for advanced material development must begin with a rational understanding of the relation between the molecular structure and the microstructure derived from it. A schematic representation of some of the structures that have been observed from lipid-based self-assembly is shown in Fig. 1.

Phospholipids are the basic building blocks of biological membranes. The fundamentals of the self-assembly of microstructures formed by amphiphilic molecules have been discussed in several recent books and review articles (*5*). An early report of helical self-assembled microstructures was published by Nakashima *et al.* (*6*). Previous studies by Kunitake *et al.* (*7*) on some amino acid–derived amphiphiles gave an indication of self-assembled microstructures with remarkably high circular dichroism. Nakashima *et al.* (*8*) and Ihara *et al.* (*9*) have also reported observing helical microstructures as well as tubular structures in single-chain amphiphiles. Similar microstructures have been formed from amphiphiles with different head groups (*10*) such as poly(L-aspartic acid) derivatives (*11*), monosaccharides (*12*), and diacetylenic phosphocholines (*4, 13, 14*).

At about the same time Nakashima *et al.* (*6*) reported helical structures, Yager and Schoen (*4*) observed the formation of a hollow cylindrical structure in copious quantities while studying the properties of the polymerizable diacetylenic phospholipid, 1,2-bis (tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine ($DC_{8,9}PC$). These tubules appear to be similar in shape to a paper soda straw (Fig. 2). The tubules that Yager and Schoen observed had a diameter of the order of 0.5 μm, with lengths varying from a few to several hundred micrometers and wall thickness of several to many bilayers (100 to 600 Å or more). In most cases, the tubules appeared to consist of helically wrapped bilayers where the edges of the bilayer are packed together.

There are many molecular features that appear to be relevant to the self-assembly of tubular microstructures. These are shown in Fig. 3 along with a molecular graphic representation of $DC_{8,9}PC$. Singh has begun a study to synthesize a number of molecules to assess the effect of variation of these features on the self-assembly of cylindrical microstructures. His first efforts were the synthesis of diacetylenic positional isomers of $DC_{8,9}PC$ (*15*). Detailed microscopic, calorimetric, and Fourier transform infrared

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(FTIR) spectroscopy studies have been conducted on the 27-carbon acyl chain series ($DC_{m,n}PC$) with m varying from 4 to 15 and n varying from 6 to 17 (*16*).

The most salient results of these studies were:

1) The nondiacetylenic versions of these molecules did not form tubules.

2) Molecules with nonsymmetrical positions of the diacetylenes in the acyl chain did not form tubules ($m \neq n$).

3) The position of the diacetylene group had little or no effect on the observed structure of tubules formed from these substances.

4) The acyl chains had very high *trans* conformational order.

In all of the cases above, the diameter of the tubule is ~0.5 μm with very little variation. FTIR and Raman studies on these materials suggest that the conformational order of acyl chains in the tubule morphology is very high (*17*). X-ray diffraction and electron scattering studies have also been performed on wet tubules, on the polycrystalline powder, and on Langmuir films made from $DC_{8,9}PC$ (*18*). The results are consistent with minimal interdigitation (interleaving) of acyl chains and with the high *trans* conformational order observed in the vibrational studies discussed above. These structural studies suggest that tubules have significantly more in-plane order than typically found in lipid-based microstructures. Taken together, the studies described above suggest that the structure of the acyl tails is a significant feature for the formation of tubules.

Because of the recent theoretical discussions of phase-mediated microstructure formation, it is important to know if tubules have truly long-range in-plane crystal order. Recent research by Thomas *et al.* (*20*) has begun to answer this question. They suggest that it is likely that the in-plane order observed in tubules is either that of a two-dimensional crystal with a finite correlation or a highly ordered smectic liquid crystal phase with hexatic order. More detailed studies will be required before the exact structure and the nature and extent of the long-range order in these systems are determined.

After the effects of diacetylenic positional isomerization were studied and the materials characterized, the next step was to investigate the role of the head group in tubule formation. An early study by Singh *et al.* (*20*) showed that a nonchiral head group attached to similar acyl tails, as in the $DC_{8,9}PC$ molecule, did not form tubules but did form sheet-like structures that curled. Subsequently, a number of diacetylenic lipids in which the chirality, size, and charge of the head group were systematically varied was synthesized (*21*). Cylinders were not initially observed in the case of chiral charged head groups (*22*). When metallic counterions were used in the solution, a number of cylindrical structures with diameters of ~0.05 or ~0.6 μm were observed (Fig. 4) (*23–25*). These structures do not form in the absence of metal cations. From these results, it would seem that a highly ordered acyl tail, some degree of chirality, and an "appropriate head group" are requirements for tubule formation.

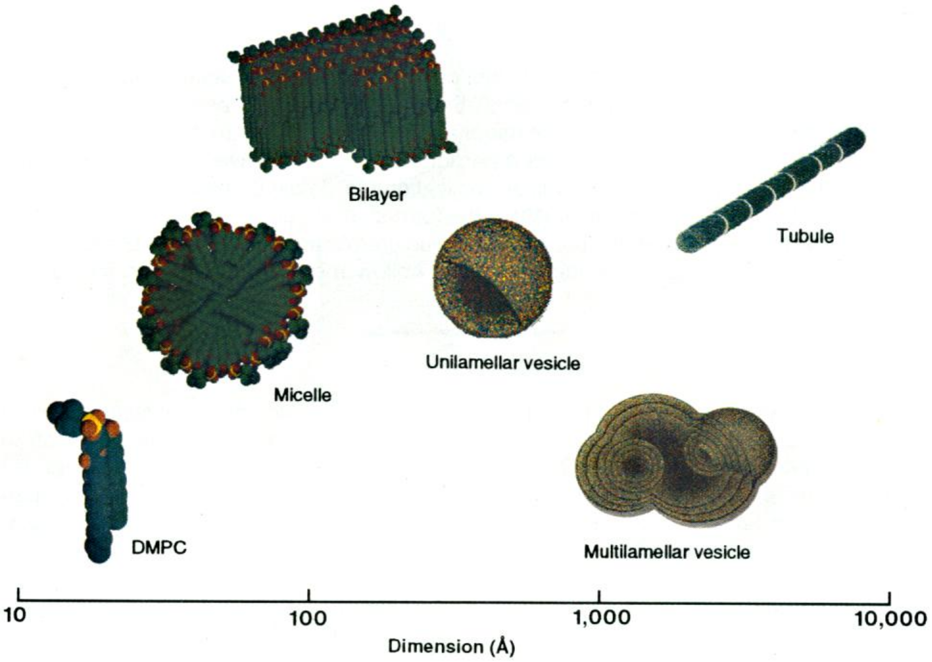


Fig. 1. Schematic representation of some lipid-based microstructures. [Courtesy of R. W. Light and B. Gaber]

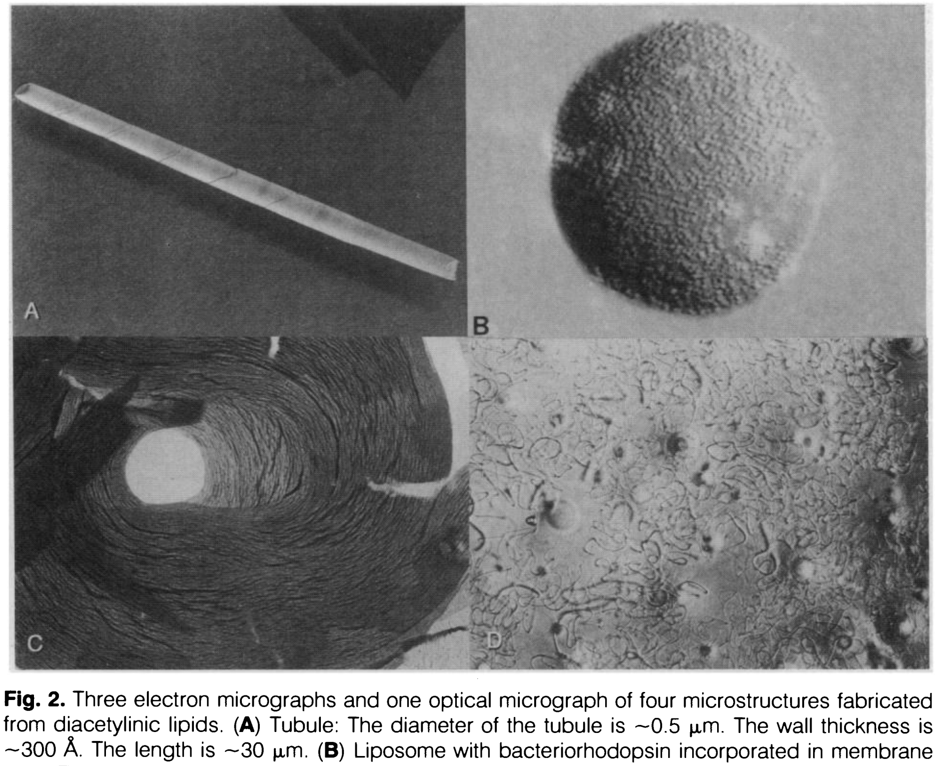


Fig. 2. Three electron micrographs and one optical micrograph of four microstructures fabricated from diacetylinic lipids. (**A**) Tubule: The diameter of the tubule is ~0.5 μm. The wall thickness is ~300 Å. The length is ~30 μm. (**B**) Liposome with bacteriorhodopsin incorporated in membrane (*65*). The diameter is ~0.7 μm. [Reprinted from (*65*) with permission © Elsevier] (**C**) A ring-like microstructure prepared by A. Singh and M. Markowitz. The inner diameter of the ring is ~1 μm. (**D**) Optical micrograph of fibrils prepared by cooling a diacetylenic lipid (*13*). Typical diameter of fibril is ~0.3 μm. [Reprinted from (*13*) with permission © Macmillan]

We have seen that by varying the geometry and the nature of the intermolecular interactions between the molecule and the solution, some changes in the structure of the resulting tubule could be reproducibly obtained. It is, of course, impossible to separate the relation between the molecule and the formation of its self-assembled microstructure from the conditions of formation. From a technological point of view, this question is also of extreme importance. The question of cost-effective large-scale production is critical in determining the ultimate technological viability of a new material.

Yager and Schoen (*4*) first observed tubule formation as they cooled multilamellar vesicles through the L_α (a fluid lamellar phase)-L_c (a lamellar phase having crystal-like order) transition point. Tubules formed rapidly as the phase transition was passed. Rudolph, Burke, and co-workers improved the formation efficiency by cycling the process (*26*). This process led to almost 100% conversion of lipids into tubules.

Although the thermal process is attractive, it does not seem likely that this approach can lead to the easy production of large (kilograms to tons) amounts of material because of the difficulty in controlling the required homogeneous temperature variation with the proper precision in very large vats. In order to overcome this problem, a process was developed for the formation of tubules that used an ethanol/lipid/water solution at constant temperature or over a narrow temperature range (*27*). Two approaches have been used: (i) adding water to an ethanol solution of the diacetylenic lipid; and (ii) lowering the temperature of the ethanol/water/lipid solution until tubules precipitate. By varying the initial conditions, some of the morphological properties of the resulting microstructures can be controlled. When a racemate is used, both left- and right-handed helices are formed as well as tubules (*28*) (Fig. 5). The average lengths of the tubules formed were also found to be a function of the ethanol-water ratio. However, there was always a wide variation (greater than a factor of 2) in the lengths at all concentrations, with the median length controllable from 12 to 170 μm. If the temperature is precisely controlled, median lengths of 500 μm can be obtained. Indeed, a tubule >1200 μm long was observed in the latter case (Fig. 6). These tubules also exhibit wide variation of the number of bilayers formed (for example, from 2 to more than 30). We have used this process for the production of kilogram quantities of tubules (*29*).

Ratna *et al.* (*30*) have conducted an extensive study of the role of solvent on the structure of the formed tubules. Tubules precipitated from a methanol/water solution were only one bilayer thick, with a wall thickness of ∼80 Å. This product is quite different from that obtained from ethanol-water or water, which have multiple bilayers. The observation by Rudolph *et al.* (*31*) that tubules of a somewhat different morphology will precipitate from acetonitrile suggests that bulk water is not a prerequisite for tubule formation. In contrast, liposomes require a water solution for formation, which suggests a different mechanism of formation. These results provide further evidence that features of the resulting microstructures can be controlled by varying the formation conditions.

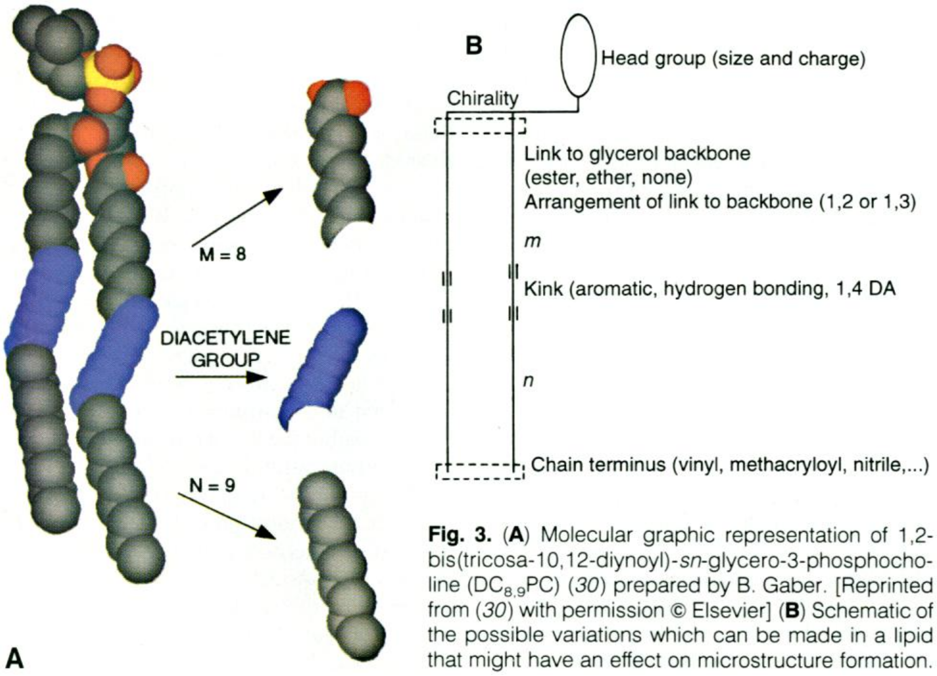
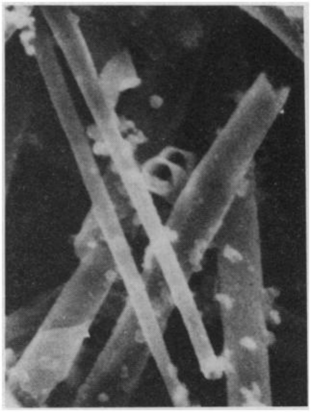


Fig. 3. (**A**) Molecular graphic representation of 1,2-bis(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine (DC$_{8,9}$PC) (*30*) prepared by B. Gaber. [Reprinted from (*30*) with permission © Elsevier] (**B**) Schematic of the possible variations which can be made in a lipid that might have an effect on microstructure formation.



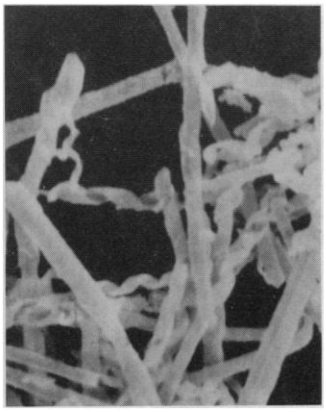


Fig. 4 (**left**). Electron micrograph of lipid-based cylinders formed with metallic counterions. The larger diameter is ∼0.6 μm, and the smaller diameter is ∼0.2 μm (*22*). [Reprinted from (*22*) with permission © American Chemical Society] **Fig. 5** (**right**). Electron micrograph of helical structures (diameters of ∼0.5 μm) formed from a racemic mixture of D-L diacetylenic phospholipids (*28*). [Reprinted from (*28*) with permission © Elsevier]

Theoretical Considerations for Tubule Formation

In order to seriously consider the use of molecularly assembled microstructures in a general sense, it is important to understand the relation between the structure of the individual molecules comprising the microstructure and the geometry of the microstructures. If this is well understood, then it should be possible to fine tune the geometry (for example, changing the diameter in the case of tubules) for a particular application. Unfortunately, the level of our understanding has not yet reached this detailed molecular level in the case of tubule formation. However, there has been progress in a more macroscopic, continuum theory of tubules.

There have been three general approaches to the theory of tubule formation. First, de Gennes has argued that a narrow strip of a tilted chiral bilayer will buckle to form a tubule because of its spontaneous electric polarization (*32*). This theory leads to a prediction that the tubule diameter will vary as a function of the electrolyte concentration in the solvent, which screens the electrostatic attraction. However, the experimental results of Chappell and Yager show little correlation between tubule diameter and electrolyte concentration (*33*). This finding suggests that the electrostatic attraction predicted by de Gennes is not the dominant cause of tubule formation. Second, Lubensky and Prost have developed a general phase diagram for nonchiral vesicles, which shows a number of morphologies, including hollow cylinders (*34*). This theory predicts that the dimensions of the cylinders are determined by competition between edge and curvature energy. However, the predicted scaling of the diameter as a function of the length is not observed experimentally.

In a third approach, Helfrich and Prost (*35*) have argued that a tilted chiral bilayer will form a cylinder because of an intrinsic bending force strictly due to chirality. Ou-Yang and Liu (*36*) have also pursued this approach using an analogy with cholesteric liquid crystals. Nelson and Powers (*37*) have further developed this approach by including the effects of thermal fluctuations. Selinger and Schnur (*38*) have recently advanced similar arguments to explain the helical substructure commonly seen in tubules (Fig. 2A). All of these calculations predict that the tubule diameter is independent of both electrolyte concentration and tubule length, in agreement with the experimental data. The fundamental basis of this theoretical approach is that the interaction between molecules in the bilayer is chiral. This chirality normally results from the three-dimensional chirality of the molecules, but it can also result from chiral symmetry breaking in the arrangement of nonchiral molecules in the bilayer (*20*) (the letter F, for example, meets this criterion in two dimensions). Because of this chirality, the molecules do not pack parallel to each other; each molecule instead packs at some favored angle with respect to its nearest neighbors. This favored twist from molecule to molecule leads the bilayer to twist out of the plane and thus to form a tubule.

From the perspective of microstructure engineering, the continuum theory presented in (*35–38*) makes two predictions. First, the formation of tubules is driven by the chirality of the bilayer, and the tubule diameter depends on the magnitude of the chirality, that is, the magnitude of the favored twist in the molecular packing. Second, tubule formation also requires tilting of the molecules with respect to the bilayer, and the tubule diameter diverges as the untilted phase is approached. Thus, the diameter can be varied by changing the magnitude of the chirality and the molecular tilt. These two predictions have yet to be experimentally verified. Although these results are useful, they still leave the synthetic chemist in a quandary as to what molecule to synthesize to obtain a particular self-assembled structure. It is likely that other lines of research will be required to establish specific connections between the actual molecular structure and the dimensions of the self-assembled microstructure. It is interesting to note that tubular and helical structures seem to be rather common in nature. The recent flurry of activity in the area of nanotubes is a case in point (*39*). It would not be surprising if some of the theoretical issues being addressed in lipid tubule formation were relevant to other cases of tubule or helix formation. Very recently, structures strikingly similar to lipid tubules have been observed to form from cholesterol-based solutions found in bile (*40*). These observations suggest that the chiral packing factors that affect tubule formation lead to molecular self-assembly of similar microstructures in a number of other systems.

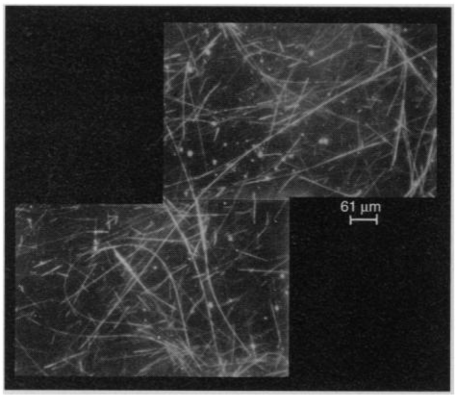


Fig. 6. Optical micrograph of a very long tubule. Length is >1200 µm. [Micrograph prepared by R. Price]

Templating Concepts: Metallization for Applications

Although the cylindrical structures described above are extremely interesting, their technological relevance to advanced materials development may not be obvious, except perhaps in the medical arena. After all, these structures are fabricated from lipids. They are essentially "globs of fat" with little inherent strength. Thermal variations of a few tens of degrees Celsius will destroy them.

The technological utility of ultrasmall cylinders is a strong function of their mechanical robustness and electromagnetic properties. The electronics and communications industries are among those most interested in miniaturization. Structures that are frail will not survive low-cost mass production assembly techniques. In order to use the microstructures formed by molecular self-assembly in most advanced materials applications, these microstructures must first be made rugged and conducting.

We have solved this problem by using the lipid tubule as a mold, pattern, or scaffolding. We use the word templating to describe this process. By using this approach, a nonconducting, weak structure with an inherently interesting size and structure made of "fat" can be converted into a rugged, conducting microstructure with the same geometry. This approach is particularly attractive for the case of self-assembled microstructures. Kresge *et al.* (*41*) have used the templating approach to fabricate molecular sieves from a liquid crystal template. We have used a number of templating strategies in our laboratory, including: (i) coating tubules with sol-gels to make 0.5-µm diameter ceramic rods or hollow cylinders or (ii) coating tubules with silanes that change the chemical nature of the tubule's surface. One of the most direct approaches to technological applications has been cladding tubules with a metal (in this case, the inside as well as the outside of the tubule is coated). This process converts a lipid tubule into a hollow metal cylinder. This section describes some of the metallization techniques that have been developed, the properties of the metallized tubules, and the electroactive composites fabricated from them.

The electroless metallization techniques developed by Brenner and Ridell (*42*) and Shipley (*43*) have been modified to clad tubules with nickel, copper, and magnetic alloys (*44*). In order for electroless metallization to work, a surface must be modified (sensitized) to encourage metallization. Shipley used small colloidal particles of palladium and platinum to sensitize a surface for subsequent metallization. In the case of phospholipid-based tubules, the polar head groups help provide a suitable substrate for adsorption of the colloid. Colloidal suspensions of palladium in a matrix of tin and chloride ions have been used to prepare lipid tubules for subsequent electroless metallization by copper and nickel (*45*). Both polymerized and nonpolymerized lipid tubules have been used as substrates for metallization. Typically, palladium-tin colloidal particles are used for bulk preparations. The catalyst is first added to an acid solution, followed by the appropriate Shipley plating solutions (such as Niposit 468 for nickel). These electroless plating solutions are formulated to be metal stable, that is, they are thermodynamically unstable but are stable enough to prevent uncatalyzed precipitation of metal. When the metal ion solutions encounter a catalyzed surface, however, the activation barrier is lowered and the reaction proceeds. The reducing agent in the copper bath is formaldehyde. The reducing agent used in the first nickel plating of tubules was hypophosphite (this made Ni-P coatings); the most recent nickel baths contain dimethylamine-borane (DMAB) as the reducing agent and make Ni-B coatings. The repeated gentle agitation (including pouring, stirring, and settling) required for this process causes substantial breakage of the lipid tubules. In our lab, the median length of metallized tubules is about half that of the precursor lipid tubules. Metal tubules with lengths of 70 to 90 μm have been produced with good yields. Typical median lengths are ~40 μm. The thickness of the coating can be controlled within 15% from ~200 Å to >1000 Å. Scanning electron micrographs of metallized tubules are shown in Fig. 7 (a collage of scanning electron micrographs of gold-, copper-, nickel- and permalloy-coated tubules). Clusters of metal 50 to 300 Å in size combine to form a rather rough surface with many boundaries between particles. The process and the exposed surface area also cause substantial oxidation and incorporation of the solutes present in the bath. These factors combine to produce metal tubules with a conductivity a factor of 1000 lower than the conductivity of the pure metal. Markowitz *et al.* (*24*) have shown that diacetylenic lipids with charged head groups with palladium counterions can self-assemble to form tubules. The counterion appears to act as a catalyst for electroless metallization of gold and nickel, thus avoiding the use of a colloidal catalyst. Recently, Pazirendeh *et al.* (*46*) have demonstrated that this templating process can be used to metallize proteinaceous, tubular microstructures called rhapidosomes found in bacteria, producing metal cylinders a factor of 20 or so smaller than those fabricated from lipid tubules.

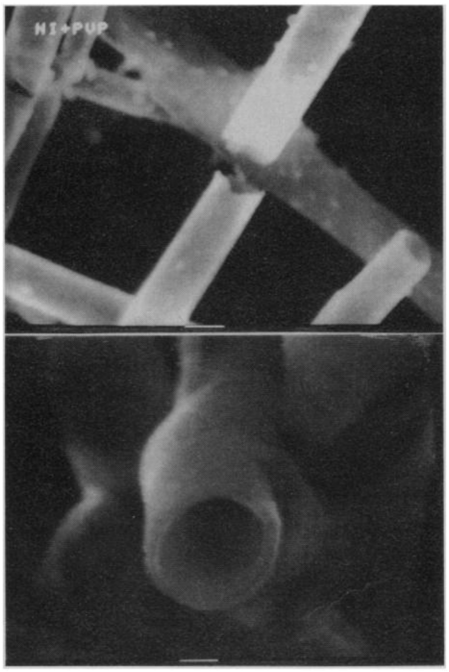


Fig. 7. Electron micrograph of nickel-coated tubules prepared by R. Price. In both pictures, the inner diameter of the tubules is ~0.4 μm and the wall thickness of the nickel is ~500 Å.

It is known that for conductors smaller in size than one-fourth of the wavelength of an incident electromagnetic field, an increased aspect ratio leads to an increase in the magnetic and electric polarizability (*47*). Metallized tubules offer an attractive geometry for microwave applications based upon this property. Most of the applications associated with the electrical properties of ultrasmall cylinders require the insertion of these structures into nonconductive matrices to form electroactive composites. There are many applications where high dielectric materials formed in this way provide a path for circuit miniaturization and reduced interchip interference. Although high aspect ratio structures with diameters of a few micrometers and lengths in excess of several hundred micrometers interact with radiation strongly, they are much too large for a number of applications requiring thin films. Cylinders with diameters of less than 0.5 μm offer the possibility of fabrication of thin electroactive composites. Metal-clad tubules could be one possible route to the fabrication of such composites. Stockton *et al.* (*48*) and Behroozi *et al.* (*49*) have fabricated and characterized electroactive tubule-based composites. Behroozi *et al.* inserted permalloy-coated tubules in an epoxy (74% Epon 815 epoxy + 26% Ancamide 507b curing agent). This composite was then cast into rods and sheets. Magnetic fields were used to align the tubules in the matrix in one direction, and the permittivity and permeability of these samples were studied at microwave frequencies. Classical electrodynamical models were used successfully to interpret the results for dilute concentrations of tubules. The results clearly demonstrated that the high aspect ratios of the metal cylinders led to high dielectric constants that were extremely anisotropic. Stockton *et al.* (*48*) conducted further investigations over a wider range of concentrations and tubule lengths and also studied conventional fibers. Their results were in substantial agreement with those of Behroozi *et al.* They demonstrated that the small diameter (<0.5 μm) provides opportunities for high aspect ratio conductors to be placed in composites, yielding materials with very high anisotropy and dielectric constants. Such materials can be used to substantially reduce the size of microwave electronics.

Tubule-based composites are being evaluated for applications as variable phase shifters. Lackner *et al.* (*50*) have dispersed nickel-coated tubules in liquid crystal mixtures. Initial studies suggested that field alignment of tubules was higher during ultraviolet radiation when using liquid crystal prepolymers than in isotropic optical cements. It was also found that nickel-coated tubules in liquid crystal mixtures had a much higher birefringence in the microwave regime than dispersions in an isotropic solvent.

Shashidhar and co-workers have pioneered the fabrication and characterization of magnetic tubules (*51*). The past few years have seen a number of investigations of the properties of thin magnetic films (*52*). The tubule geometry has been used as a template to fabricate bulk composite structures that exhibit the magnetic properties of thin films. Permalloy was coated on lipid tubules with thicknesses varying between 200 and 500 Å, and these permalloy tubules were placed in an epoxy matrix. During the curing process, a 450-Oe magnetic field was used to align the tubules. The magnetic properties of the resulting composites were measured by vibrating sample magnetometry (VSM) as well as ferromagnetic resonance (FMR). The results showed a very high magnetic anisotropy that arises from the inherent shape anisotropy. The coercivity was found to be similar to that found in thin magnetic films. In other words, by using tubules as a template for magnetic coatings, it was possible to fabricate a large bulk sample exhibiting the properties of a very thin film.

Applications: Two Specific Examples

The previous sections have shown how self-assembly can be used to fabricate 0.5-μm hollow metal cylinders. There are many potential applications for these structures. We are pursuing several applications based on their use as electroactive composites. This section will highlight two specific examples where tubules have been used. The first is the fabrication of a cathode for vacuum field emission. The second is the use of these hollow cylinders as a microvial for controlled release applications. In both cases, the lipid plays no part in the final application and can be removed after metallization has been completed.

Field emitting cathodes. There is considerable interest in the development of high current density, high brightness, survivable cathodes as microwave sources (*53*), for communications (*54*), and in electron beam processing of materials (*55*). The generation of large electron beam currents through vacuum field emission from a large number of emission sites requires a surface with a complex microstructure. Theoretical considerations and subsequent modeling suggest that 0.5-μm hollow cylinders terminating in edges with a very small radius of curvature (≤300 Å) may offer significant advantages in terms of the brightness of the emission (*56*). Given these predictions, it seemed reasonable to see if the metal tubules described above could be used to construct a vacuum field emission cathode.

A schematic of the fabrication process (*57*) developed by Chow, Kirkpatrick, and co-workers for this cathode is shown in (Fig. 8A). Nickel-coated tubules were prepared as described above. The tubules were then suspended in acetone and mixed with an epoxy resin (Epon 815) to form a composite. The nickel tubule–epoxy mixture was cast in a cylindrical mold and then placed in a magnetic field with a density of ~500 G to align the tubules. A microtome was used to cut 50-μm thin sections. These sections were then etched to expose the aligned nickel tubules. The exposed surfaces were alternately coated with gold (200 Å) and silver (2000 Å). This assembly was then mounted in an anodized cathode holder (Fig. 8B). Measurements were obtained with a microanode to ensure that any observed emission came from the tubule microstructures. Although this harsh treatment destroyed a number of tubules, a sufficient number survived to produce stable (*56*) emission for more than 10 hours.

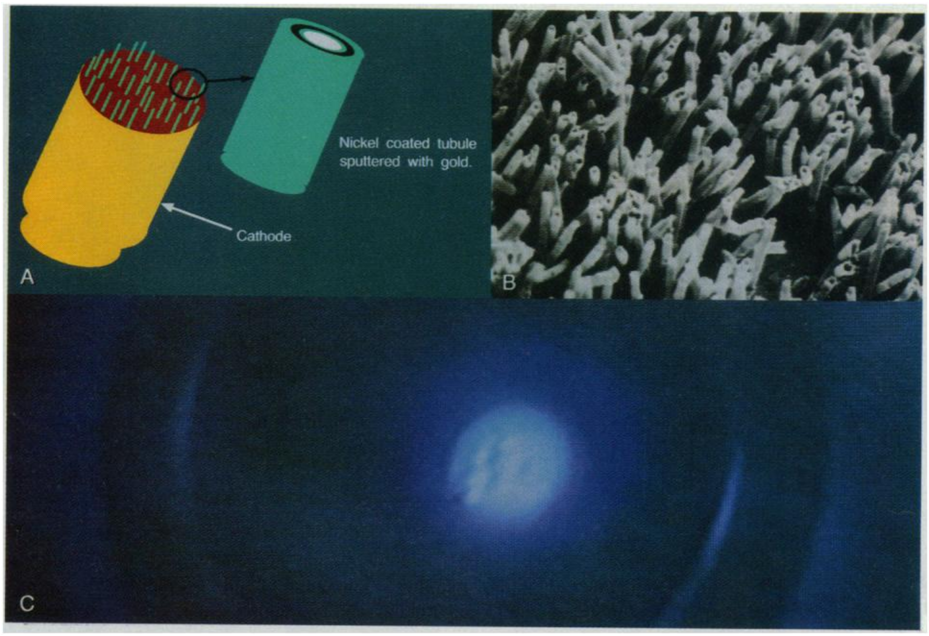


Fig. 8. Tubule-based field emission. (**A**) Schematic of design for tubule-based field emitting cathode. (**B**) Electron micrograph of a tubule-based cathode. The total area of the emitting surface is ~0.75 cm^2. (**C**) Photograph of the emission from tubule-based cathode. A phosphor plate intercepts the beam current and produces the image where emission nonuniformities are readily noted. For the conditions shown in the photograph, the phosphor response is nonlinear. [Photo courtesy of Science Applications International Corporation]

From a number of points of view, this experiment can be considered a substantial success. Self-assembled lipid tubules were used as templates to fabricate metal cylinders that produced field emission. The results are consistent with models for enhanced brightness from cylindrical structures. Thus, the use of metallized tubules provided an excellent route to demonstrate the validity of the theory and to suggest opportunities for the development of field emitters based upon this concept. Because the fabrication techniques were exceedingly difficult and did not lead to homogeneous arrays, new fabrication techniques are required. Based on these initial measurements of vacuum field emission from submicrometer diameter, narrow-edge cylinders, we are continuing to investigate alternative approaches to produce cylinders with similar morphology and improved electrical and thermal characteristics (*58*).

Controlled release. When tubules are considered to be microvials into which materials can be placed, a number of nonelectronic applications come to mind. Many of these are related to the problem of controlling the release of desired materials into the environment at a specified rate over long periods of time.

Most approaches to controlled release involve the incorporation of a chemical species into a polymer or lipid sphere (*59*). The spheres are then placed into a polymer matrix. By controlling the diffusivity in the matrix and the diffusion through the walls of the sphere, some degree of control can be achieved. Because the surface area of the spheres is a major factor in the rate of release, control can be difficult to achieve. This is due to the relation between the concentration of the entrapped species, the osmotic pressure, and the diameter of the sphere. The osmotic pressure decreases as a function of solute release, which then decreases the diameter. The use of small hollow cylinders with rigid nonporous walls offers several potential advantages over a sphere. Because the length and diameter are fixed and not a function of osmotic pressure, they will not change as the contents are released. These parameters can thus be more easily optimized for the desired release parameters (*60*). Because the diameter is less than 1 μm, tubules should offer no mechanical or size barrier to their incorporation in controlled-release coatings. In addition, it is possible to incorporate the target species into a polymer that is subsequently entrapped in the cylinder. The filled cylinder can then be placed in another matrix (or paint). By varying the diameter of the cylinder, the length of the tubule, and the two matrices, excellent control of the release parameters should be obtained.

Price and co-workers (*61, 62*) have applied tubule-based controlled release to the problem of marine fouling. Copper-coated tubules with inner diameters of ~0.4 μm with lengths varying between 40 and 120 μm were prepared. After metallization, the tubules were dried so that they would act as microcapillary tubes. Potential antifouling agents (such as a crude methanol extract of the sea pansy, *Renilla reniformis*) were mixed with an epoxy and slurried with the tubules. The tubules were extracted from the slurry and the incorporated epoxy-antifoulant mixture was cured. These tubules were then studied in free solution for their release properties as a function of time (*63*). Similar tubules were mixed into a final paint matrix for application testing. The process was then scaled up from grams to kilograms for marine testing. The cost for this scale-up was significantly decreased by recycling the lipid used to fabricate the tubules.

This paint was used to coat fiberglass rods for testing in the marine environment. Paints were prepared with various concentrations of antifoulants and tubules to determine the effect of this process. At the first sign of fouling, the rods were withdrawn and examined to determine the composition and relative percentage cover of fouling organisms.

The first studies used tetracycline (*62*) as a model, because it is easily monitored by spectrophotometric analysis. Initial findings indicated that the release of tetracycline could be sustained for many months. Recent modeling of this system suggests that a long-term exponential release can be anticipated; the nature of the diffusion is strongly affected by the length of the encapsulating tubule (*64*). In contrast, tetracycline mixed directly into the paint is totally released in a few hours. By varying the matrix cross-linking inside the tubule and the wettability, Price has demonstrated excellent control of the release parameters.

Fig. 9. Test rods from off-shore testing in Hawaii after 6 months of exposure. The rod with little fouling was coated with a paint that included tubules containing *Renilla* extract. The rod with the greater amount of fouling had only the base paint. [Photo by R. Price]

Price then analyzed the use of this system for marine applications. He has extensively studied two possible antifouling agents, isothiazolone, and 2-fural-*n*-pentyl ketone, a structural analog of the active component in the *R. reniformis* extract.

A pair of test rods taken from a sample exposed at Coconut Island, Hawaii, for 6 months are shown in Fig. 9. One has a tubule-based release system with *Renilla* extract, and the other has the antifouling additives simply free-associated in the paint. The experimental rod successfully repelled fouling during the test period with concentrations of 2% by weight of active agent.

These results suggest that these small hollow cylinders formed using molecular-based self-assembly can be very useful in the development of long-term sustained release systems. Numerous potential applications in agriculture, environmental quality, and medicine require sustained release over a period of months to years. For most of these applications, the feasibility of scale-up and the price of the ultimate system will determine marketplace acceptance.

Summary

The development of advanced materials based on molecular self-assembly will require the sustained interaction of fundamental research scientists and material engineers. Initial microstructures and material concepts will have to be tested at the prototype level and then tuned and optimized at the lab bench to solve problems as they arise. As we learn more about the relation between the structure of a molecule and the relation of the molecule to its environment, it should be possible to actually design and engineer that molecule to form self-assembled structures optimized for specific applications.

The size of these structures may range from micrometers (as in the case of long tubules) to as small as a few hundred angstroms. The templating examples given above show how these structures could then be metallized for particular functions. As the structures are evaluated, design changes will have to be made, new molecules will be synthesized, and the process will be repeated and optimized for a particular application. It should also be possible to decorate these structures with other molecules (antibodies, for example) to exploit different aspects of each particular microstructure. It should be possible to use the templating approach on some of the new molecular architectures being generated in new polymers and their mixtures. These structures should lead to the solution of material problems requiring advanced electroactive or smart composite materials. Applications in catalysis, separation systems, and smart materials also seem likely. The field of nanoscience is just now being tested for its potential technological applications. Molecular self-assembly will be an important tool as nanoscience matures into a number of nanotechnologies:

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66. The work described in this paper is the result of several years of research on a coherent theme by many people in a number of different laboratories. Space limitations make it impossible to thank each of them in this section. I thank T. Coffey for his sense of vision and foresight that led to the establishment of a multidisciplinary research group in which this type of work could be pursued. I. Skurnick was a source of excellent advice and provided a sounding board for many of the concepts. The former DARPA, the former ONT, and ONR all provided financial support. R. Shashidhar, B. Ratna, A. Singh, J. Selinger, and S. L. Schnur were especially helpful in their careful reading and critical comments.

Biological Diversity, Soils, and Economics

Michael Huston

Terrestrial biological diversity is supported by solar energy captured by plants growing in soil. This soil-based plant productivity also provides the foundation for human societies through production of food and renewable forms of energy. Variations in plant productivity, resulting from differences in inherent soil fertility, variations in climate and weather, and differences in chemical inputs and agricultural practices, produce patterns of biological diversity that are associated with the agricultural component of economic productivity. Ecological processes lead to a generally negative relation between the diversity of plant species and potential agricultural productivity at both local and global scales. One implication of this negative relation is that preservation of areas of high plant biodiversity does not require the sacrifice of productive agricultural lands.

The relation of biological diversity to economics is generally seen as an impediment imposed on economic development or as a difficult-to-value externality determined by the opportunity-cost of extinction. Both of these views miss the true economic significance of biological diversity, and their perpetuation has negative implications both for the preservation of biodiversity and for rational economic development. Biological diversity can be understood as the consequence of organisms struggling for survival in the same environment in which humans, as individuals and societies, struggle for survival.

The fundamental unit of biodiversity is the species. Extinction of species, as well as of subspecies and populations, is of international concern for many reasons, including the loss of genetic material of potential value for medicine and agriculture (*1*). Biodiversity is considered to be highest where there are the most species living in the same area, as exemplified by tropical rain forests. Thus, biodiversity is an issue of coexistence, and extinction can be seen as the extreme manifestation of a failure to coexist. Extinction can result from (i) the failure of organisms to survive natural disasters such as floods, droughts, ice ages, and asteroid impacts; (ii) the failure of organisms to coexist with one another as a result of competition, predation, or disease; and (iii) the failure of organisms to coexist with humans as the result of overharvesting, habitat destruction, or direct competition.

Local Patterns of Biodiversity

The processes leading to "survival of the fittest" form the basis for understanding the economic implications of biological diversity. The "fittest" species, which in many cases are the best competitors, are able to dominate more rapidly and completely under some environmental conditions than under others. Dominance by a single species often occurs more rapidly under favorable conditions, where all organisms can potentially grow rapidly. It is under these conditions that the best competitors are able to eliminate most other species by monopolizing an essential resource. Abiotic disturbances, such as fires or hurricanes, as well as predators or herbivores can prevent competitive dominance by killing some proportion of the dominant competitors, particularly under favorable conditions when dominance would otherwise occur rapidly (*2*). Thus, under the most favorable conditions, diversity among competing species can be reduced by competition. However, under somewhat poorer conditions, the superior competitors are not able to dominate as rapidly, if at all, and many species are able to coexist, leading to higher diversity among individuals that are smaller in size, fewer in number, or both. Of course, under extremely poor conditions, few or no species are able to survive, and biodiversity drops to very low levels.

The pattern described above appears most strongly among plants, primarily because of the importance of a single limiting resource, sunlight. The conclusion from the above argument is that plant biodiversity should be higher on unproductive, poor soils than on fertile, productive soils where plants are taller and total plant mass is higher. This pattern of highest plant diversity on poor soils and low plant diversity on the best soils is found throughout the world under a wide variety of conditions (*3–5*). The diversity of plant species in temperate prairies and grasslands (Fig. 1, A to C) (*3–6*) shows the same pattern in relation to soil fertility that is found among trees in tropical forests (Fig. 1, D to F) (*4, 7–9*). Addition of nutrients to plant communities nearly always leads to a reduction in plant diversity (*3–6, 10*), as demonstrated by fertilization experiments in temperate pastures and grasslands (*10*) and tropical forests (*11*). Anthropogenic nutrient pollution also reduces plant diversity, both in aquatic systems, where the effects of eutrophication are well known, and in terrestrial systems, such as the European heathlands that are being converted to grasslands by atmospheric deposition of nitrogen (*12*). A variety of theoretical models predict this pattern and explain why there are exceptions to the general rule (*2, 3, 5, 6, 12*).

Agriculture, Biodiversity, and Conservation

Soil is also the key to explaining much of the variation in agricultural productivity. Situations where agricultural productivity is in-

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