than it should be, indicating the presence of a second state. With the above expression for the energy, we find that there are two states near 100 meV: the $n = 0$, $\ell = 2$ state (108 meV) and a doubly degenerate $n = 2$, $\ell = 1$ state (93 meV). These two states are not resolvable at room temperature by STS. A simulation of the image in Fig. 5B shows that it can be fitted by a superposition of two-state densities: about 80% of the (2, 1) and 20% of the (0, 2) state, in good agreement with the expectations from the simple circular-box model.

Concerning the use of metallic structures such as those studied here as models for the study of electron confinement, we note that in the simplicity of their generation and stability, such structures have advantages over corrals formed by atomic manipulation at liquid-helium temperatures (*7*). Atomic manipulation, however, can be implemented in conjunction with the natural structures. It can be used to modify these structures or dope them with specific atoms to, for example, model scattering during electrical transport processes. The charge-density distributions and field gradients resulting from confined states, such as in a growing interface, are different from those at an extended terrace. These factors are likely to influence surface processes that depend on them, such as the sticking, diffusion, and spatial distribution of adatoms and the growth of islands. The STS methods provide a powerful tool to start exploring these issues.

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28 January 1994; accepted 30 March 1994

Diacetylenic Lipid Tubules: Experimental Evidence for a Chiral Molecular Architecture

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Molecular self-assembly is of key importance for the rational design of advanced materials. To investigate the causal relation between molecular structure and the consequent self-assembled microstructure, self-assembled tubules of diacetylenic lipids were studied. Circular-dichroism studies give experimental evidence that the formation of tubules is driven by chiral molecular packing, in agreement with recent theories of tubules. On the basis of these results, a molecular mechanism for the formation of tubules is proposed.

Molecular self-assembly has the potential to enable the fabrication of technologically useful microstructures, because it may be possible to "engineer" these micrometer-scale structures by "tweaking" the structure of their constituent molecules (*1, 2*). To develop rational design rules for self-assembled materials, one must understand the causal relation between the molecular structure and the resulting microstructure. As a first step in understanding this relation, several research groups have studied the self-assembly of cylindrical tubules. Tubules are composed of membranes of amphiphilic molecules wrapped in a cylinder, as shown in Fig. 1 (*3, 4*). These structures are unusual, because most organic membranes form either flat sheets or nearly spherical vesicles. Tubules have been observed in a number of systems of chiral organic molecules, including diacetylenic lipids (*3, 5*), surfactants (*6*), bile (*7*), and glutamates (*8*). In diacetylenic lipid systems, they have a typical diameter of 0.5 μm and a typical length of 50 to 200 μm. They have been used for a variety of technological applications, including electroactive composites and controlled-release systems (*1*).

Several investigators have developed theories that explain the formation of tubules in terms of an intrinsic bending force in a chiral membrane (*7, 9–13*). Although the details of these theories differ, they are all based on the same physical principle of chiral packing. The essential point is that long chiral molecules do not pack parallel to their nearest neighbors but rather at a non-zero twist angle with respect to their nearest neighbors. This favored twist angle can be visualized by packing hard screws together. In liquid crystals, the favored twist angle gives the pitch of a cholesteric phase. In systems of organic membranes, where the chiral molecules are confined to bilayers, the effects of the twist are more subtle. If the membrane is in a tilted phase, with the molecules tilted with respect to the local layer normal, the favored twist from neighbor to neighbor leads the whole membrane to twist into a cylinder, as illustrated in Fig. 2. The diameter of the cylinder can be calculated in terms of the favored twist and the molecular tilt.

So far, experiments on tubules have given only limited support to this theoretical model. Although there is strong evidence against competing theories (*1, 13*), there has been only indirect evidence in favor of chiral molecular packing in tubules. This indirect evidence comes from the observation that most tubules exhibit a helical substructure or have helical microstructures as precursors to tubule formation. For example, diacetylenic lipid tubules formed in water and in ethanol-water systems commonly show helical patterns (Fig. 1A) (*14–16*). However, diacetylenic lipid tubules formed in methanol-water systems show no indication of

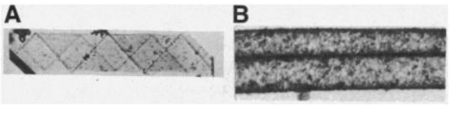

Fig. 1. Transmission electron micrographs of (**A**) an ethanol tubule with helical markings, the helical markings being decorated by Pd catalyst particles, compared to (**B**) a methanol tubule with no visible helical markings.

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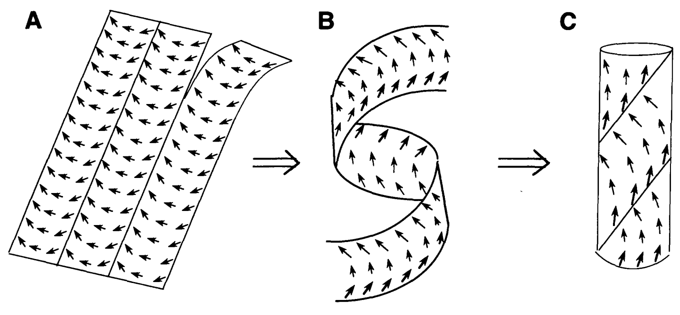

Fig. 2. Schematic illustration of a chiral bilayer (**A**), with the molecules tilted with respect to the local layer normal. (The arrows indicate the direction of the molecular tilt, projected into the layer plane.) The favored twist between chiral molecules leads the whole membrane to curve into (**B**) a wound ribbon, then fuse into (**C**) a cylindrical tubule. The observed CD spectra come from the chiral molecular packing common to all three figures, not from the micrometer-scale helical structure in (B) and (C).

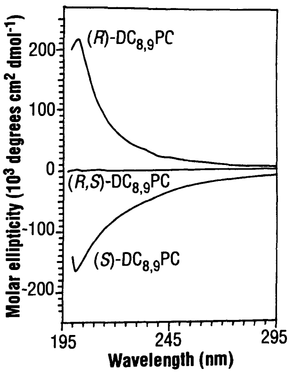

Fig. 4. CD spectra of (*R*)-DC$_{8,9}$PC, (*S*)-DC$_{8,9}$PC, and a racemic mixture in methanol containing 20% water. Concentration of the lipid was 0.33 mg/ml.

such a helical pattern (Fig. 1B). These results have left open the question of whether all of these microstructures can be understood in terms of chiral molecular packing.

To answer this question, we studied the circular dichroism (CD) spectra of diacetylenic lipid tubules formed from a number of different conditions (*17*). Figure 3 shows the CD spectra for tubules formed in ethanol-water and methanol-water mixtures and those formed in water by cooling (*15, 16, 18*). The lipid dissolved in ethanol had only a weak CD signal, but the tubules in ethanol-water showed a strong CD signal at a wavelength around 200 nm (Fig. 3A). (Because of the limitations of the instrument, the exact peak of the CD absorption band could not be determined but is probably below 200 nm.) A similar behavior was observed for tubules formed in methanol-water (Fig. 3B). Finally, in pure water the lipid formed spherical multilamellar vesicles at 45°C, which transform into tubules when the system is cooled to 25°C. The spherical vesicles had only a weak CD signal, but the CD signal became strong when the lipid formed tubules (Fig. 3C). Note that the CD signal is similar for all of these methods of tubule formation, regardless of whether the helical substructure is visible in electron micrographs of tubules. Thus, the CD results show that the molecular packing is chiral in tubules formed through all of these methods and that the molecular packing is not chiral in spherical vesicles.

For a further test of the effects of chirality, we measured the CD spectra for the *R* and *S* enantiomers and for a racemic mixture of DC$_{8,9}$PC [1,2-bis(tricosa-10,12-diynoyl)-*sn*-glycero-3-phosphocholine]. The CD signal had the opposite sign for the *R* and *S* enantiomers (Fig. 4). This result indicates that tubules of the two enantiomers have a chiral molecular architecture with opposite handedness, as would be expected. The racemic mixture had a CD signal of approximately 0 (Fig. 4). Previous electron microscope studies (*14*) of the racemic mixture of (*R*)- and (*S*)-DC$_{8,9}$PC showed that this racemic mixture forms tubules with the same dimensions as tubules formed from pure enantiomers. The electron micrographs suggested that there were left- and right-handed helices on different tubules. Thus, we can infer that a racemic mixture of *R* and *S* molecules phase-separates into chiral microstructures of opposite handedness, which give no net CD effect.

The CD spectra of tubules can be contrasted with those of cholesteric liquid crystals, which are familiar chiral systems with a strong CD effect. In cholesteric liquid crystals, the dominant signal normally comes from the selective reflection of one sense of circularly polarized light by the periodic helical structure. This mechanism leads to a peak at a wavelength corresponding to the pitch of the helix. As an example, Fig. 5A shows a series of CD spectra for a cholesteric liquid crystal in which the peak shifts as a function of temperature as the cholesteric pitch shifts (*19*). By contrast, in our experiment on tubules we can rule out this mechanism because the peak is not observed in the 400 to 500 nm range, which is the pitch of the helical pattern as determined by electron microscopy, and does not change wavelength as a function of temperature (Fig. 5B). This mechanism is also ruled out by the fact that the signal is seen in methanol-formed tubules that do not exhibit any helical substructure. In our experiment, the CD signal is probably caused by the selective absorption of one circular polarization because of the presence of a dichroic chromophore in a chiral environment, which leads to a CD peak at the absorption peak of the chromophore. Indeed, ultraviolet absorption studies of these and related molecules show that

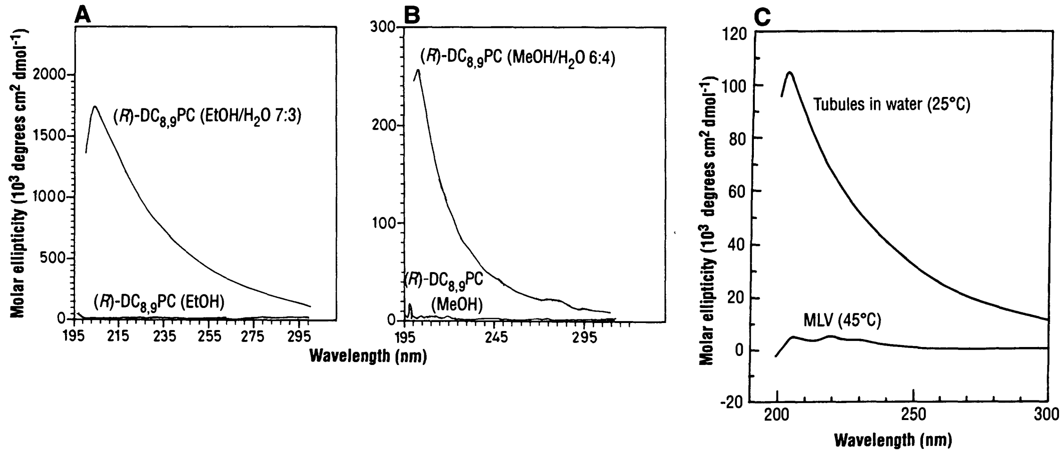

Fig. 3. CD spectra of (*R*)-DC$_{8,9}$PC tubules formed (**A**) in ethanol (EtOH) containing 30% water with a reference CD spectrum of lipid in 100% ethanol, or (**B**) in methanol (MeOH) containing 40% water with a reference CD spectrum of lipid dissolved in 100% methanol, and (**C**) thermally formed tubules in water at 25°C and multilamellar vesicles (MLV) at 45°C. The concentration of the lipid in all cases was 0.33 mg/ml.

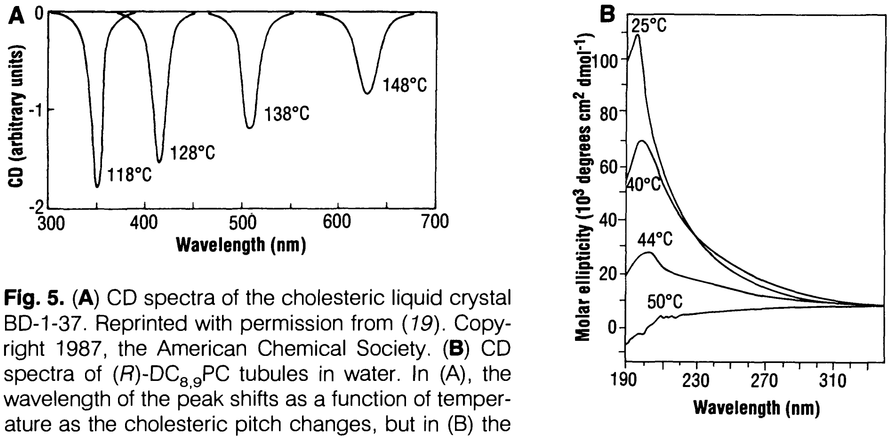

Fig. 5. (**A**) CD spectra of the cholesteric liquid crystal BD-1-37. Reprinted with permission from (*19*). Copyright 1987, the American Chemical Society. (**B**) CD spectra of (*R*)-DC$_{8,9}$PC tubules in water. In (A), the wavelength of the peak shifts as a function of temperature as the cholesteric pitch changes, but in (B) the peak wavelength is approximately constant as a function of temperature.

both the phosphatidylcholine head group and the diacetylene group absorb in the 200-nm region. The CD signal could also be caused by circular intensity differential scattering (*20*), which would also arise from the chiral environment of the chromophore. In either case, the observed CD effect must be related to the chirality of the underlying molecular architecture (*21*). Hence, we can conclude that the packing of lipid molecules in tubules must be chiral.

Our CD results thus give clear experimental support for the theories of tubules based on chiral molecular packing (*7*, *9*–*13*). With this experimental support, we can use these theories to propose a molecular mechanism for the formation of tubules. Our hypothesis is based on the theory of Selinger and Schnur (*13*), which shows that chiral packing leads both to stable tubular structures and to helical patterns in the orientation of molecules within the lipid bilayers of the tubules (Fig. 2). This theory can also be applied to helically wound ribbons. In this theory, a wound ribbon resembles a single stripe of the helical pattern on a tubule, with a modulation in the molecular orientation across the width of the ribbon. On the basis of this approach, we can predict that the optimum width of the ribbon is approximately $K/(Kq - E_{edge})$, where K is the Frank constant (*22*) for variations in the molecular orientation, E_{edge} is the edge energy per unit length, and q is the chirality parameter, which gives the favored twist between the orientations of neighboring molecules divided by the distance between their centers of mass.

The experimental evidence for little (if any) chiral packing in spherical vesicles and substantial chiral packing in tubules, coupled with the theoretical approach described above, permits us to propose a mechanism for the formation of tubules. Our hypothesis is that large vesicles or lipid bilayer aggregates first develop stripes in the molecular orientation, separated by sharp domain walls (Fig. 2A). The vesicles or lipid aggregates then break up along these domain walls to form ribbons (Fig. 2B). [This breaking-up process should be intensified because each stripe in the molecular orientation leads to a ripple in the membrane curvature (*23*)]. Each ribbon is free to twist into a helix in solution. Once the helix is formed, the ribbon may be stable, or it may fuse into a cylindrical tubule to reduce its edge energy (Fig. 2C). The most important point about this proposed mechanism is that it can happen only if the initial vesicle or aggregate is larger than the favored ribbon width. If the initial vesicle or aggregate is too small, it cannot transform into a tubule. This prediction is consistent with the experimental results of Rudolph and Burke (*24*), who found that large multilamellar vesicles (with outer diameters >1 μm) form tubules directly upon cooling, whereas small unilamellar vesicles (with diameters <0.05 μm) do not. It is also consistent with the light-scattering data of Ratna (*25*), who found that methanol-water solutions formed tubules only after large micrometer-sized aggregates were observed.

In order to understand the nature of the chiral molecular interactions more fully, similar studies could involve other tubule-forming systems (*7*, *8*) and related organic and polymeric materials, such as chiral smectic liquid crystals (*26*) and chiral polymeric structures (*27*). Simulations can also be used to understand the nature of the molecular interactions leading to helix and tubule formation. The increased knowledge resulting from these studies should enable the design and fabrication of molecules that will form hollow cylinders of predicted diameters.

Note added in proof: Preliminary time-resolved, high-resolution microscopic studies of ethanol-formed tubules are consistent with the model proposed here and provide clear evidence of helix formation leading to tubules (*28*).

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29. The authors wish to acknowledge the support of the Office of Naval Research under the auspices of its Indo-American Collaboration Program. The insightful comments and suggestions of A. Rudolph, J. Brauman, and R. Shashidhar were very helpful during the preparation of the manuscript.

22 December 1993; accepted 4 April 1994