Part III

Development and Application of Bicelles for Use in Biological NMR and Other Biophysical Studies

Charles R. Sanders
Department of Biochemistry and Center for Structural Biology, Vanderbilt University, Nashville, TN 37232-8725, U.S.A.

The term "bicelles" was first proposed in 1995 to describe aqueous assemblies of detergent and lipid that were believed to be "**bi**nary, **bi**layered mixed mic**elles** bearing a resemblance to the classical model for **bi**le salt-phosphatidylcholine aggregates" [1]. At that time, bicellar mixtures had already been in use for several years as magnetically alignable model membranes in solid-state NMR studies of membrane-associated molecules (Figure 1). Since 1995, there have been dramatic advances both in the applications of bicelles and in our understanding that bicellar mixtures are morphologically more complex than originally thought. Here, we trace the development and application of bicelles from their initial development in the late 1980s. Other reviews of bicelles and related developments in NMR and sample alignment methods are also available [2–10].

Bicelle Roots

That phospholipid/detergent mixtures believed to form discoidal bilayered mixed micelles can be aligned in a high magnetic field was first demonstrated in the lab of James Prestegard at Yale University. Preetha Ram, a graduate student, observed magnetic alignment of anionic bile salt-phosphatidylcholine mixtures in 1988 [11]. Leading to this achievement were earlier developments in disparate fields:

1. It was known that phospholipid vesicles could be aligned using a strong magnetic field in a way that distorted the (ideally) spherical vesicles to permit a large fraction of the lipid bilayers present to be preferentially aligned with the magnetic field (with bilayer normals orthogonal to the magnetic field) [12–16]. In other words, it was already established that lipid bilayers have significant anisotropy of diamagnetic susceptibility. The magnetic susceptibility tensor of hydrocarbon-based phospholipids is aligned within the molecular frame such that lipids prefer to align with their long axes orthogonal to the direction of the applied magnetic field. Lipid bilayers, therefore, prefer to be uniaxially aligned such that bilayer normals are perpendicular to the field.
2. Through many years of effort by membrane biophysicists, it was already believed that lipids sometimes form discoidal aggregates with detergents of the digestive system (bile salts) [17–19] and with certain amphipathic proteins [20–24]. Thus, bicelles were already known entities, although these systems had not been exploited as model membrane media or subjected to magnetic alignment.
3. By 1988 there were already a number of abiological aqueous lyotropic and nematic liquid crystals that were believed to be either bilayered-discoidal or tubular in morphology and that were known to align in the presence of a strong magnetic field [25–28]. For those believed to be disk-like, systems were available that aligned either with their bilayer normals orthogonal or parallel to the applied field. Several papers were published in the late 1980s in which some of these systems were used as model membrane media for NMR studies of biomolecules trapped in the aligned phases [29–31].
4. The utility of using aligned samples to facilitate measurement of solid-state NMR spectra was already well established (c.f. [32, 32–38]). However, there were limitations and drawbacks associated with existing methods of alignment, providing impetus for bicelle development.
5. The need for methods to attain weak magnetic alignment of molecules for NMR was already manifest. It had been demonstrated in the 1960s that even for highly mobile molecules yielding sharp line widths, induction of too high a degree of molecular alignment leads to NMR spectra of almost unfathomable complexity [39,40]. Later, Bothner-By and co-workers demonstrated the marginal unimolecular alignment of small molecules by very high magnetic fields [41], while MacLean had explored the use of electric fields for the same purpose [42]. Under conditions of marginal alignment, spectral complexity is manageable, so that structurally useful anisotropic parameters such as dipolar couplings can easily be measured. This set the stage not so much for initial bicelle development as for the later application of bicelles as a matrix for soluble protein alignment introduced in 1997 by Tjandra and Bax (see below).

Graham A. Webb (ed.), Modern Magnetic Resonance, 1–7.

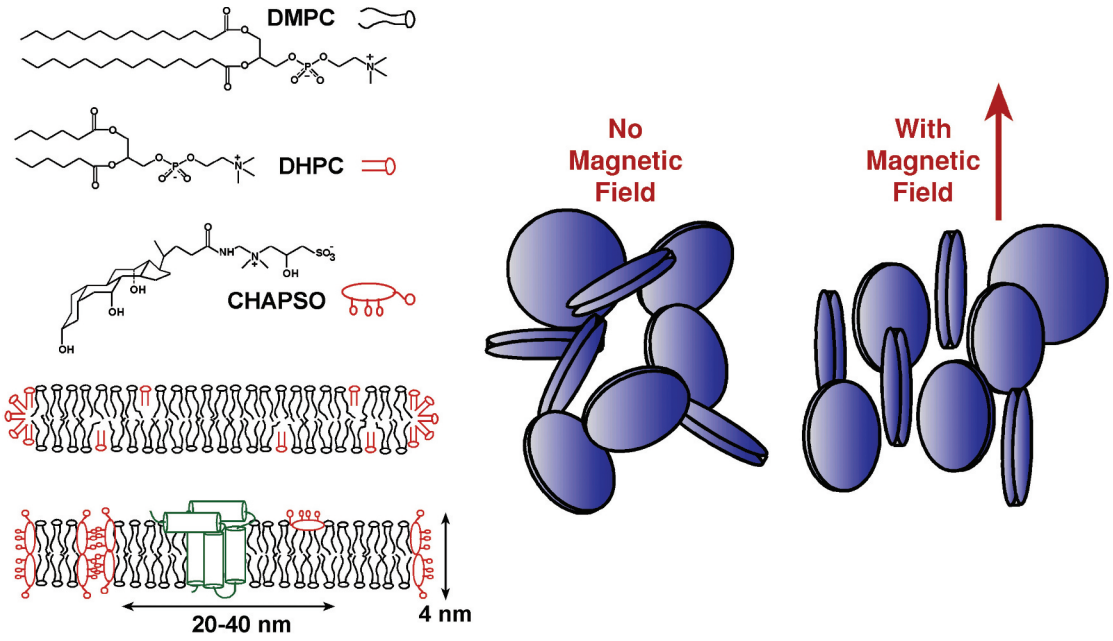

Fig. 1. Classical model for bicellar DMPC-CHAPSO and DMPC-DHPC assemblies (left) and for magnetic alignment of bicelles (right). This figure is reprinted from Sanders CR, Prosser RS. *Structure*. 1998;6:1227–1234 [6] with permission from Elsevier.

Early 1990s

Following the original report of bicelle alignment, anionic bile salts were replaced with a mild zwitterionic bile salt derivative, CHAPSO, to yield CHAPSO-dimyristoylphosphatidylcholine (DMPC) mixtures that were observed to be magnetically alignable over a wide range of compositions and temperatures [43]. Moreover CHAPSO-DMPC mixtures were non-denaturing toward soluble proteins. This system was soon employed as a model membrane medium in studies of a number of lipids [44–49]. It was also observed that the usual 90° orientation of CHAPSO-DMPC bicelles in a magnetic field could be flipped to align the bilayer normals with the field by adding certain amphiphilic aromatic hydrocarbons [50].

In 1992, the nascent Sanders lab at Case Western Reserve University introduced bicelles in which bile salt derivatives used in the original bicelle system were replaced by dihexanoyl-PC (DHPC) [51]. Part of the motivation for developing DHPC/DMPC bicelles arose from the fact that the NMR facilities then present at Case were 1970s-vintage. The bicelle project was among the most interesting NMR projects the author could think of that was feasible using such out-of-date instrumentation! This work built upon previous characterization of short-chain phosphatidylcholine micelles and their interactions with phospholipids carried out by the lab of Mary Roberts in Boston [52–55]. DHPC/DMPC mixtures were observed to undergo magnetic alignment over a wide range of compositions. Similar to CHAPSO/DMPC bicelles, alignment was observed to occur only above the gel-to-liquid crystalline phase transition of DMPC—below this temperature both CHAPSO/DMPC and DHPC/DMPC bicelles become isotropic.

^{31}P, ^{13}C, and ^{2}H NMR studies indicated that the dynamics and conformations of the phosphatidylcholine molecules present in the bilayered domain of aligned bicelles are quite similar to those present in bilayered L_α-phase vesicles [56]. It was also demonstrated that the activity of an integral membrane enzyme, diacylglycerol kinase (DAGK), could be supported when this protein was reconstituted into bicelles [1,57]. Most of the DAGK molecule is bilayer-embedded and one of its substrates is a lipid. Moreover, its active site is believed to lie at the water-membrane interface. Therefore, DAGK's functional reconstitution provided a strong biochemical validation of the use of bicelles as model membranes.

In the mid-/late 1990s, the first reports of membrane protein alignment using bicelles were reported for surface-associated membrane proteins [1,58–61] as well as for integral membrane proteins [1,62]. For surface-associating membrane proteins, motion, and orientational disorder are often quite high, such that NMR spectra of very high quality (narrow lines) have been obtained. For

transmembrane proteins, the number of spectra reported using aligned bicelles of both the conventional and flipped (see below) variety remains fairly small (cf. [63–65]), although there seems to be renewed interest in exploring the potential of bicelles as a medium for solid-state NMR studies of transmembrane proteins [66].

Late 1990s

Vold, Prosser, and and co-workers made two seminal contributions to bicelle development in the late 1990s. First, they introduced the use of paramagnetic lanthanide ions to induce a change in sign of the anisotropy of magnetic susceptibility of bicelles, such that magnetic alignment takes place with bilayer normals parallel to the field direction [67]. This was an important development because it provided a means by which oriented-sample spectra could be obtained from bicelle-associated molecules even in the absence of rapid rotation around the bilayer normal. Not only was it shown that lanthanide ions confer parallel alignment, but ion-chelating lipids were developed in order to sequester the bicelle-associated ions to prevent unwanted free radical or oxidative chemistry that might damage bicellar molecules [68,69]. A second important contribution of Vold and Prosser was to explore and advocate the use of isotropic bicelles as a medium for solution state NMR of membrane proteins [59,70], which continues to be an area of interest [71]. Isotropic bicelles form below the gel-to-liquid crystalline phase transition of the lipid component of bicelles and at relatively high detergent-to-lipid ratios.

By 1997, it had been demonstrated that biomacromolecules could be magnetically aligned to a degree which allowed many small dipolar couplings to be measured [72–75], heralding the now widespread acquisition and utilization of residual dipolar couplings in solution NMR-based structural analyses. However, unimolecular alignment required both that very high magnetic fields be employed and (usually) that the protein of interest must contain a tightly associated paramagnet in order to provide sufficient magnetic susceptibility. It was therefore a very important development in the field of biomolecular NMR when Bax and Tjandra showed that bicelles could be used as an alignment matrix for water soluble biomolecules [75,76]. Not only was this method widely applicable, it also provided a means by which the degree of alignment could be tuned by varying bicelle/buffer composition. Following this breakthrough were several developments: (1) Classical bicelle mixtures were improved by extending their temperature range and enhancing their chemical and morphological stability [77–83]. (2) The use of additives to bicelles was investigated for a variety of purposes [67–69, 84–93]. For example, by varying bicelle surface charge it is sometimes possible to vary the alignment tensor of the guest proteins, a great advantage for downstream structural calculations. (3) The use of various membranes and liquid crystals has been explored or re-explored, leading to the introduction of alternative membrane-like systems for achieving magnetic alignment of biomolecules [78,84,93–98]. (4) Radically different methods for attaining molecular alignment were developed [95,99–109] such as the use of magnetically aligned phage particles and the use of strained polyacrylamide gels. These methods have been particularly significant for those biomolecules that interact with bicelles and related systems in such a manner that the degree of molecular alignment is too high for solution NMR. Alternative methods of alignment are also welcome in cases where the bicelle matrices are disrupted by the biomolecular solute, or where the structure of the solute of interest is perturbed by bicelles.

2000–2005

The past few years have seen three particularly interesting developments. First, bicelles have been employed in biostructural studies extending beyond the realm of NMR spectroscopy. Lorigan and co-workers have embarked upon a continuing exploration of bicelles as a medium in which to conduct EPR spectroscopy [110–118]. Moreover, James Bowie's lab has shown that polytopic membrane proteins can be crystallized from bicellar mixtures, leading to high-resolution X-ray crystal structures of polytopic integral membrane proteins [119]. The bicelle crystallization approach offers a distinct alternative to crystallization using classical detergent or lipidic cubic phases as the host model membrane medium for the membrane protein of interest. It will be extremely interesting to see whether a range of membrane proteins can be crystallized from bicelles or whether only a few membrane proteins prove to be susceptible to this approach.

A second innovation associated with bicelles is the exploration by several labs of the potential of combining the use of bicelles with rapid sample spinning methods at various sample rotation angles with respect to the magnetic field [120–127]. This work builds upon previous work by a number of labs in which the physics and spectroscopy of liquid crystals under conditions of rapid sample spinning have been explored [128]. The use of rapid sample spinning methods in conjunction with bicelles offers exciting possibilities for manipulating sample orientation and effective orientational order in conjunction with the application of sophisticated solid-state NMR pulse technology.

The other major development in the years leading to 2005 is that the classical "bilayered-disk" model for bicelle aggregate morphology has been challenged. Early characterization of bicelles was almost exclusively NMR

in nature. An "Occam's razor" approach was used to argue that the bilayered-disk model (Figure 1) was the most reasonable model that could account for the available NMR data for magnetically alignable bicelles (review in [6,9]). However, the bilayered-disk model could not easily explain the very high viscosity of bicelle mixtures under conditions where magnetic alignment can take place. It was also hard to explain why, for a given bicelle composition, aligned bicelle order remains fairly constant over a wide range of total lipid + detergent content (5–40%, by weight).

Based on more recent, often non-NMR data, from the labs of Katsaras and others it now appears clear that the bilayered-disk model is inadequate to describe all of the detergent-lipid assemblies that fall within compositions typically described as being bicellar—particularly for compositions and temperatures at which magnetic alignment can take place [71,129–137]. While this work is ongoing, it appears that at higher lipid-to-detergent ratios, bicelle morphology can be likened to that of Swiss cheese-like perforated bilayered-lipid sheets. As the detergent-to-lipid ratio is increased, the sheets most likely break up into interconnected bilayered tape-like strands. Continuing to increase detergent content then leads to discoidal bilayer fragments and, finally, to classical mixed micelles (Figure 2).

Recently, Sligar and co-workers have developed bilayered-discoidal aggregates referred to as "nanodiscs" that are composed of mixtures of lipids with amphipathic lipoprotein mimetics [138–140]. These aggregates appear to conform quite closely to the classical bicelle morphology over a wide temperature range. To our knowledge, the potential that nanodisc mixtures can be magnetically aligned has yet to be tested.

Conclusion: How Good are Bicelles as Model Membranes?

The above emerging model for bicelles in which several different morphologies are possible, depending on exact composition and temperature, is very tentative. It will take much time and effort to use multiple techniques to systematically explore the very wide range of temperature and composition space that is inhabited by mixtures falling within the "bicelle" regime. In the meantime, it is likely that bicelles will continue to be employed as model membranes for biophysical studies of membrane-associated molecules. For any class of model membrane, it is reasonable to consider to what extent "native membrane" conditions are reflected by the model system. This question can be experimentally addressed in four ways. First, for a molecule of interest, structural measurements may be repeated in more than one type of bicelle [141]. If the same structural conclusion is reached in multiple systems, the notion that structure is native-like is supported. Second,

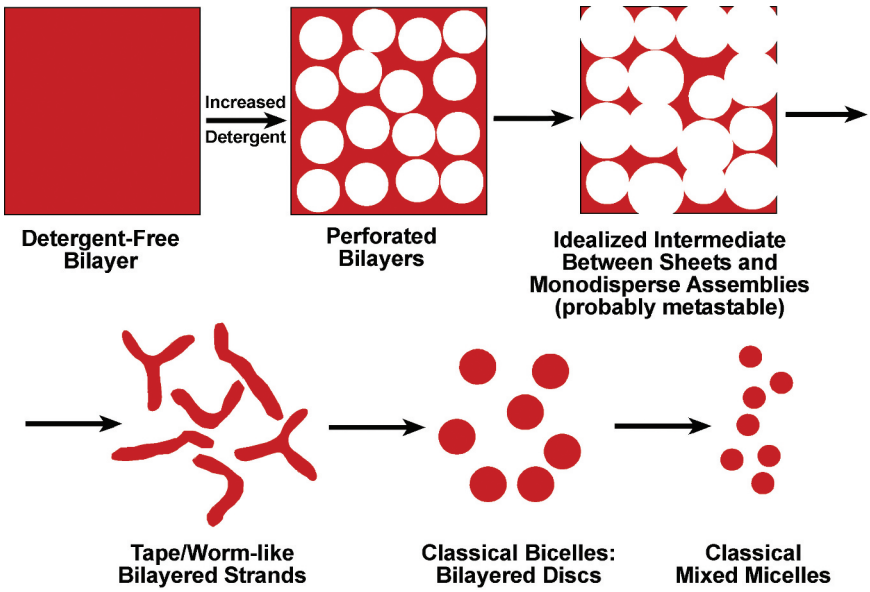

Fig. 2. Emerging model for morphological transitions occurring in bicelle mixtures as detergent is added to a fixed amount of lipid. The view is looking down onto the bilayer surface. The highly tentative nature of this model is emphasized.

measurements may be repeated as the lipid:detergent ratio is increased [1,141]. By extrapolating anisotropic parameters to a detergent-free limit, it is possible to estimate structural parameters for the solute of interest in lipid bilayers in the absence of detergent. Third, if a solute has an assayable function, a test for native-like function may be carried out under bicellar conditions [57]. Finally, direct verification that a bicelle-derived structure is the same as the structure solved under non-bicellar conditions may sometimes be possible [119]. The limited data generated thus far from these types of control experiments are extremely encouraging in affirming that bicelles typically maintain the native-like structure and dynamics of guest molecules.

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