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LETTERS

Magnetic Field-Induced Ordering of a Polymer-Grafted Biomembrane−Mimetic Hydrogel

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A biomembrane−mimetic complex fluid that spontaneously orients in the presence of a magnetic field to yield a highly ordered lamellar structure is described. Macroscopically oriented lamellae were produced by exploiting the inverted thermoreversible phase transition of the material, that is, by aligning the sample below the phase transition temperature (<16 °C) (i.e., in the fluid, hexagonal micellar phase) and warming to produce the lamellar gel phase in a 7.05 T magnetic field. The in situ field-induced alignment was studied by deuterium NMR. The lamellar domains were found to preferentially orient perpendicular to the applied field (negative order). Characterization of the magnetic field-induced anisotropy by polarized optical microscopy and small-angle X-ray scattering/diffraction (SAXS) indicates that it persists even upon field termination. The directional alignment was flipped by 90°, with the lamellar domains oriented parallel to the field (positive order), simply by modifying the composition through the addition of a lanthanide ion (Eu^{3+}). The system offers the opportunity to spatially organize both membrane and aqueous soluble proteins in an anisotropic matrix, thereby facilitating structure and dynamic studies using a range of techniques, including magnetic resonance (both NMR as well as EPR), optical spectroscopy, and small-angle neutron and X-ray scattering.

Recently, there has been considerable interest in the development of biomembrane−mimetic materials (e.g., bicelles, liposomes, liquid crystals) that can provide an ordered matrix in which biomolecules such as proteins and peptides can be spatially organized, thereby facilitating the determination of their structure and dynamics.[1−9] The development of such systems is particularly important for conducting physicochemical studies of proteins that have eluded crystallization or of biomolecules in their native environments (i.e., at physiological pH and ionic strength and in the presence of an excess of water or in a lipid membrane environment). Much effort to date has been directed at the development and application of magnetically aligned bicelles (oblate bilayer micelles) consisting of dimyristoylphosphatidylcholine (DMPC) and dihexanoylphosphatidylcholine (DHPC).[2,4,5,9] These systems, while offering an improvement over simple solutions of field-oriented proteins[10,11] and alternatives requiring more elaborate sample preparation or not providing a realistic hydration environment for proteins (e.g., isopotential spin-dry ultracentrifugation,[12,13] squeezed and/or stretched polymer gels,[14,15] or multilamellar dispersions sandwiched between[16] or spin coated onto thin glass substrates[17]), suffer from several limitations. For example, in these bicelle systems, stable alignment can be induced only over a narrow, elevated temperature range (35−45 °C).[7] In addition, undesirable bicelle surface−protein interactions/association can occur, leading to phase separation and poor temporal stability.[18] Recognition of these drawbacks has spawned interest in the development of other systems, including liquid crystalline media such as cetylpyridinium chloride/*n*-hexanol/brine mixtures,[7] and colloidal suspensions of rod-shaped viruses.[18] These approaches have been developed primarily for use in macromolecular structure determination by magnetic resonance spectroscopy.

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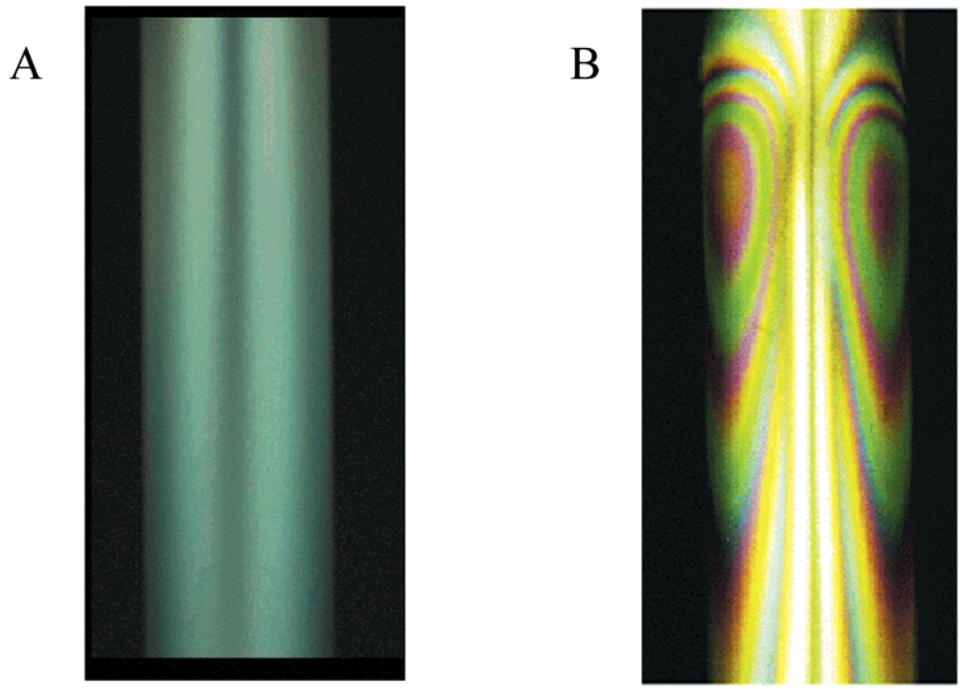

Figure 1. Polarized optical micrograph of a quaternary mixture of DMPC/DMPE-EO_{99}/LDAO hydrated in 10 mM Tris 100 mM NaCl buffer pH 7.8 in a 5 mm NMR tube between crossed polarizers (A) at 23 °C prior to magnetic field exposure and (B) after magnetic field processing.

Less effort has been directed at the development of biocompatible media that are capable of providing an organized anisotropic environment for structure−function studies and that could be adapted for use in a variety of techniques to yield information on a range of length scales and dynamic ranges (including not only NMR and EPR, but also techniques such as small-angle neutron or X-ray diffraction and optical spectroscopy). Ideally, such a medium would not only address the limitations of current approaches but would also provide magnetic field-induced alignment that is both long-range and persistent, even upon removal of the field.

In this report, we describe the magnetic field-induced alignment of a recently developed polymer-grafted lipid-based complex fluid,[19,20] consisting of a quaternary mixture of water, a phospholipid (dimyristoylphosphatidylcholine), a lipopolymer comprising poly(ethylene) oxide terminally grafted onto the headgroup of dimyristoylphosphatidylethanolamine, and a zwitterionic surfactant (*N*,*N*-dimethyldodecylamine *N*-oxide), detailing our investigations of the nature of the alignment process by polarized optical microscopy (POM), deuterium NMR (^{2}H NMR), and small-angle X-ray scattering (SAXS). In previous reports, we described the preparation and structural characterization of this complex fluid employing a variety of spectroscopic and scattering techniques.[19,20] These studies revealed that at room temperature, the molecular components of this system self-assemble to form ordered microdomains of lamellae, while below the phase transition (16 °C), the material exists as a 2-D hexagonal array of micellar cylinders. Our subsequent work has demonstrated that membrane- and aqueous-soluble proteins can be readily introduced into the cold phase of this material, transferred into the ordered lamellar phase (liquid crystalline state), and organized in the alkane and water domains, respectively, by simply warming the sample to above the phase transition temperature.[21] In the present work, we explore the use of an applied magnetic field as a means of aligning the lamellar microdomains, thereby eliminating unfavorable orientation and defects in this material and extending the order into macroscopic dimensions. In addition, we demonstrate that unlike previously reported systems (i.e., bicelles), the induced structural changes and enhanced asymmetry persist even upon cessation of the magnetic field.

As a first step in the structural characterization of magnetic field-induced order in this material,[22] polarized light microscopy was employed to determine the anisotropy of the medium.[23] Under polarized light, liquid crystalline mesophases produce characteristic optical patterns arising from a variety of defect structures associated with them. These patterns can be useful for the identification of phase morphologies and for qualitative assessment of the extent of order (i.e., domain size). As shown in Figure 1A, prior to magnetic field exposure, the polarized light microscopy image exhibits a coarse mosaic texture indicative of a high-defect lamellar structure with poor cooperative alignment between microdomains.[19,27] After magnetic field processing[31] (Figure 1B), however, dramatic changes in the optical birefringence pattern of the sample are observed. The increasing intensity and complexity of the pattern, in particular, the emergence of a range of polarization (retardation) colors[24] of several orders that propagate perpendicular to the long axis of the NMR tube, indicate that the magnetic field dramatically enhances the anisotropic alignment of the phospholipid chains perpendicular to the field axis. This directional alignment extends uniformly over the length scale of the NMR tube, indicating macroscopic cooperative alignment/orientation of the domains in the gel phase material.

To determine the nature of the alignment process and to establish the factors governing the magnetic field-induced ordering, the time-averaged local aggregate structure was examined by deuterium (^{2}H) NMR spectroscopy. The in situ alignment process was evaluated from the shape of the ^{2}H NMR spectra.[25] Figure 2 shows the spectra collected on a sample below, at, and above the phase transition temperature ($T_c = 16$ °C). Below the transition temperature, that is, in the fluid micellar phase, a broad, single-line resonance is observed, indicative of isotropic motion presumably arising from rapid molecular movement occurring within the anisotropic environment, thereby producing a time-averaged quadrupolar interac-

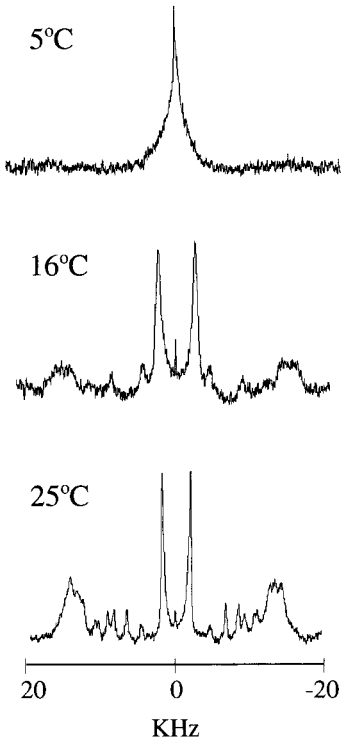

Figure 2. Variable-temperature deuterium NMR of chain-perdeuterated, d_{54}-DMPC in deuterium-depleted water compositions of the polymer-grafted lipid mesophase. Spectra collected below (5 °C) at (16 °C), and above (25 °C) the phase transition temperature.

tion. When the sample temperature is raised to 25 °C, a well-resolved spectrum reflecting a uniaxially oriented sample with the lipid bilayer normal perpendicular to the applied field is observed. The series of symmetric doublets arise from the individual DMPC-d_{54} acyl chain segments, with the intense doublet having the smallest coupling constant arising from the mobile terminal methyl groups (C_{14}) and the broad doublets with the largest splittings resulting from the superposition of resonances from the less mobile methylene groups adjacent to the ester linkage.[5,26] The intermediate, less intense doublets result from those methylene groups between the ester linkage and the terminal position of the acyl chain. The narrow isotropic peak in the center of the spectrum may be attributed to a small portion of the sample that remains in the H_1 phase at 25 °C.[19] The shape of the ^{2}H NMR spectrum does not change discernibly upon further magnetic field exposure (12 h), indicating that the sample orients rapidly and is aligned within minutes at this field strength. Note too that the alignment process is reversible (i.e., the induced orientation can be erased) by cycling through the phase transition temperature.

The molecular anisotropy (i.e., degree of acyl chain anisotropy) can be determined quantitatively from the quadrupolar splitting, $\Delta\nu$, and can be related to the average local order parameter, S_{CD}, of the C−^{2}H bonds.[26,28] After brief (5−10 min) equilibration of the sample at 5 °C, followed by rapid warming to 25 °C in the 7.05 T field, a quadrupolar splitting of 25.36 kHz is observed for the plateau regions (i.e., the headgroup end of the acyl chain), yielding a S_{CD} of 0.20.[29] The magnitude of this order parameter corresponds closely with those reported for well-aligned bicelles.[4]

The changes associated with magnetic field exposure were further assessed by ex situ small-angle X-ray scattering (SAXS),[30] which probes structural changes on the mesoscopic scale (nanometers to micrometers). The two-dimensional SAXS pattern and the corresponding plot of the azimuthally averaged intensity as a function of scattering vector for the gel phase prior to magnetic field exposure are presented in Figure 3B,C, respectively. The positions of the six Bragg peaks, which occur at integer multiples of the first-order reflection ($Q = 0.037$ Å^{-1}), are indicative of a lamellar structure with a periodicity of 170 Å. (The large background scattering on which the Bragg peaks are superimposed has been shown previously to arise from a small, residual contribution of the cold phase structure, H_1, at 25 °C[19]). The isotropic 2-D intensity pattern observed for the gel phase prior to magnetic field exposure, however, indicates that it is a mosaic structure consisting of lamellar microdomains in which all spatial orientations are present (i.e., polydomain, Figure 3A). After magnetic field alignment below the phase transition temperature followed by warming in the presence of the field,[31] a strong anisotropic 2-D scattering pattern is observed (Figure 3E). No anisotropic scattering is observed for samples that are aligned solely by employing temperatures at or above the phase transition temperature ($T >$ 16 °C), even after lengthy magnetic field exposure (24 h). The anisotropy about the equatorial axis indicates that the lamellae preferentially orient perpendicular to the capillary axis; that is, the lamellar domains lie perpendicular to the direction of the applied magnetic field (Figure 3D). Integration of the scattered X-ray intensities along a wedge in the equatorial direction indicates that the lamellar morphology and lattice dimensions are preserved upon magnetic-field exposure (Figure 3F). The "smearing" of the intensity around the equatorial axis may arise from some degree of residual mosaic spread (i.e., average orientational disorder) that remains after magnetic-field alignment or may be produced by removal of the sample from the field and its subsequent storage until SAXS analysis. Analysis of the azimuthal arcs of the (001) Bragg reflection (2-D scattering data shown in Figure 3E) indicates that the data can be fit to a Gaussian distribution of domain orientations with a fwhm of 29 ± 2°. This value compares favorably with those reported for other magnetic field-aligned liquid-crystalline systems examined after field cessation and represents a dramatic improvement relative to the DMPC/DHPC bicelles (mosaic spread 90°, an apparent relaxation to an isotropic state).[32] This value may not represent the best achievable mosaic spread for this system, however, and optimization of alignment parameters (time, temperature, etc.) by careful study of in situ kinetics will determine conditions under which maximum persistent anisotropy can be achieved.

As is the case for previously reported bicelle systems, the magneto-orientation effect observed here arises from the diamagnetic susceptibility ($\Delta\chi$) of phospholipids. This value is negative for phospholipids such as DMPC, and thus, the principal axis of the tensor lies along the average direction of the acyl chain long axis. Therefore, the energetically favorable orientation of organized assemblies composed of these molecules is one in which the principal axis is perpendicular to the magnetic field (Figure 3D). This system differs from the DMPC/DHPC bicelles in that a high degree of cooperative alignment of the lamellar domains can be achieved (6 orders of diffraction with a narrow distribution of intensity around the equatorial axis). Moreover, the alignment persists after removal of the magnetic field.

It has been postulated that the primary limitation to achieving magnetic field-induced orientation in liquid crystals is slow kinetics arising from both the formation of extended multilamellar domains that cannot readily align independently and opposing viscoelastic forces that dictate the extent and rate of macroscopic alignment.[33,34] Another factor impeding both the

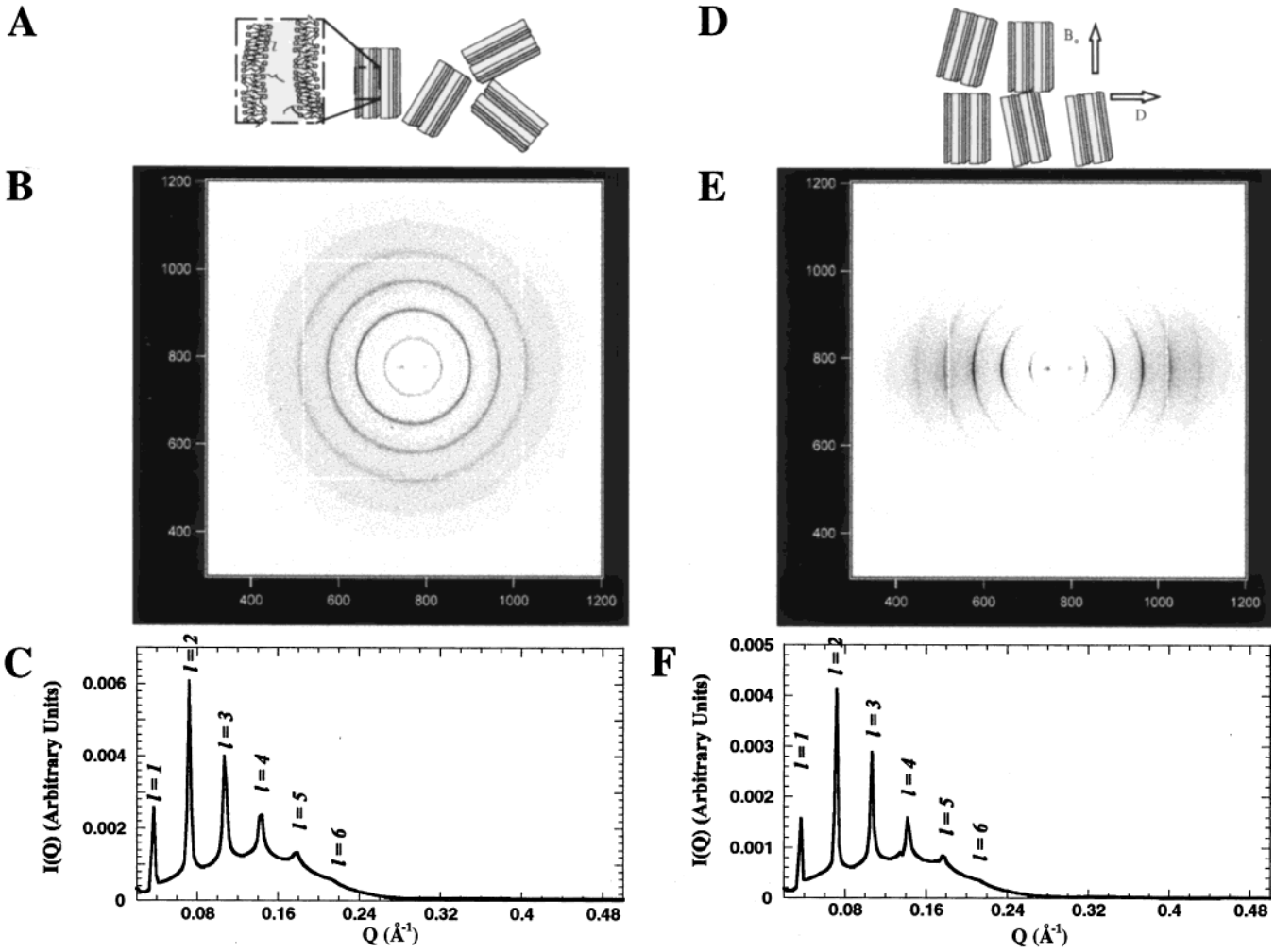

Figure 3. (A) Schematic representation of lamellar liquid crystalline domains prior to magnetic field alignment. (B) Two-dimensional small-angle X-ray diffraction patterns from the unoriented gel phase sample. (C) Azimuthal integration of the scattering data presented in (B). (D) Schematic illustrating the lamellar domains oriented with their directors, D, perpendicular the applied magnetic field, $\mathbf{B}_o$, direction (magnetic field direction is coincident with the long axis of the capillary). (E) 2-D SAXS image obtained after alignment in a 7.05 T magnetic field. The sample was aligned in a 1.5 mm quartz capillary held in a 5 mm NMR tube that was placed in the magnet for 1 h at 2 °C, then warmed to 25 °C in the presence of the field, and held for an additional 1 h. The sample was removed from the field and stored at 23 °C for 20 h prior to SAXS studies. (F) Integrated scattering data presented in (E).

production of highly ordered liquid crystalline arrays or bicelles and the retention of the induced alignment external to the magnetic field is thermal disordering induced by Brownian motion. These problems have limited the utility of most lyotropic liquid crystalline materials, which typically require lengthy (days) exposure to high magnetic fields (11.7−13.5 T) to achieve alignment.[33,34] In contrast, macroscopic sample alignment is easily attained in this complex fluid by exploiting its unique phase transition characteristics. That is, the ability to convert the sample into a hexagonal array of short micellar tubes (i.e., a low viscosity state) by a modest reduction in sample temperature allows for facile alignment of the micelles (believed to orient with the long axis of the micelles parallel to the field direction)[41] in the presence of a magnetic field. Warming the sample through the phase transition allows for coalescence of the aligned tubular micelles into extended ordered lamellar domains, which is accompanied by the onset of a highly viscous gel phase that acts to "lock in" the field-induced sample asymmetry.

To be useful in structure−function studies of biomolecules, a magnetic field-alignable phospholipid-based matrix should possess the ability to encapsulate and preferentially orient a variety of biological macromolecules, including, for example, α-helical proteins. These proteins have been shown, however, to possess large positive magnetic susceptibilities that may act to cancel and/or limit attainable bicelle alignment,[35] a limitation that can be addressed by producing positive aligned lamellar domains ($\Delta\chi > 0$). In the bicelle systems, it has recently been demonstrated that incorporation of a variety of paramagnetic lanthanide ions can be used as a means of switching the alignment of phospholipid based micelles in a magnetic field from perpendicular to parallel with respect to the field.[5,32,35,36] These studies have explored the use of both simple doping of the composition with trivalent paramagnetic ions (e.g., addition of Eu(III), Er(III), Tm(III), or Yb(III) to bicellar systems) and incorporation of lanthanide−phospholipid chelate complexes (for model membrane systems). To examine the possible use of lanthanide addition as a means of controlling the direction of domain orientation upon magnetic field exposure, 140 mM Eu^{3+} was introduced into the buffer component of the composition prior to magnetic field alignment. As shown in the two-dimensional small-angle diffraction image (Figure 4B), this addition leads to strong anisotropic scattering about the meridional axis, indicating bilayer orientation parallel to the applied field direction (Figure 4A). Integration of the small-angle intensity along a wedge in the meridional direction (Figure 4C)

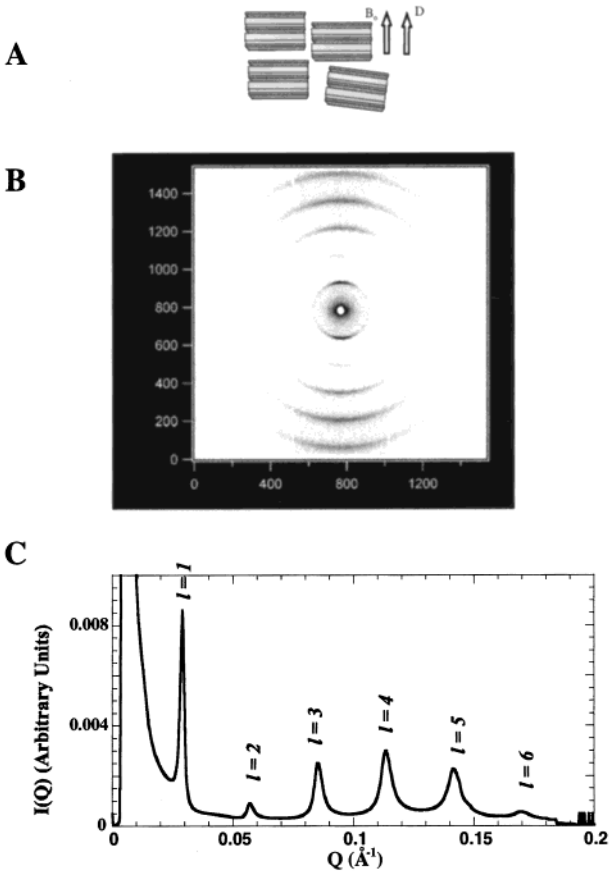

Figure 4. (A) Schematic illustration of lamellar domains oriented with their directors, D, parallel to the applied magnetic field directions, $\mathbf{B}_o$. (B) 2-D SAXS diffraction pattern collected after magnetic field alignment (7.05 T). The sample was aligned in a 1.5 mm quartz capillary held in a 5 mm NMR tube that was placed in the magnet for 1 h at 3 °C and warmed to 25 °C in the presence of the field and maintained for 1 h. Upon field removal, the sample was stored at 23 °C for 60 h before being taken for SAXS analysis. (C) Integrated scattering intensity presented in (B).

reveals six diffraction peaks with a *d* spacing ratio of integral order, confirming retention of the lamellar structure upon introduction of the lanthanide reagent. The change in orientation upon Eu^{3+} addition is believed to arise from a change in the bilayer director from a negative to positive order parameter (i.e., changing the sign of the anisotropy of the magnetic susceptibility) and is likely the result of association of the paramagnetic Eu(III) ions with the DMPC headgroups.[37]

Diffraction data collected (data not shown) over a wider scattering vector range ($Q = 0.030-0.50$ Å^{-1}) indicate that 10 orders of diffraction can be resolved, four more than the six reflections resolved in the undoped, magnetically aligned samples. Consequently, unlike the previously reported lanthanide-doped bicelle systems, which frequently undergo phase separation and/or are accompanied by the coexistence of an isotropic phase,[38] incorporation of Eu^{3+} ions into the complex fluid actually serves to enhance the lamellar structural ordering. The position of the first-order Bragg peak ($Q = 0.029$ Å^{-1}) indicates that addition of Eu^{3+} is accompanied by a significant increase in the lattice spacing by ca. 50 Å (from 170 to 225 Å). In addition, no alterations in lattice parameters are observed between doped-unaligned and aligned samples. The increased lattice spacing may arise from electrostatic interaction of the Eu^{3+} with the bilayer and/or formation of poly(ethylene oxide) chelates of the metal cation, which may also induce an apparent electrostatic effect on the bilayer.[39] Determination of the exact nature of the bilayer−ion interaction awaits further experimentation. Although preliminary studies indicate that positive bilayer alignment can be achieved at a 1:1 lipid to Eu^{3+} ion ratio, it may be possible to lower the paramagnetic ion concentration (which at a 1:1 ratio may lead to severe paramagnetic shifts and line broadening in solid-state NMR studies) by using lanthanides with larger $\Delta\chi$, such as Tm^{3+}, which possesses the largest $\Delta\chi$ of the lanthanides. The ability to switch the orientation of the anisotropic aligned lamellar domains (and hence proteins encapsulated within the complex fluid) is expected to prove to be of considerable importance in our efforts to adapt the complex fluid to structure studies of biomembrane-associated peptides and proteins.

In summary, a polymer-grafted biomembrane-mimetic hydrogel has been shown to be macroscopically oriented by application of a moderate (7.05 T) magnetic field.[40] The field-induced anisotropy of the gel phase persists even after removal from the field. The high degree of persistent anisotropy is achievable due to the inverted, thermoreversible phase transition characteristic of this complex fluid. That is, macroscopic alignment is achieved by the nucleation of highly aligned cylindrical micelles from the low-viscosity micellar phase into extended liquid crystalline domains of the lamellar gel phase. While the material itself preferentially aligns with the lamellar domains oriented perpendicular (negative order) to the direction of the magnetic field, this domain orientation can be switched to parallel (positive order) by simple addition of paramagnetic reagents. The biomimetic liquid-crystalline medium described here offers several other important advantages over existing materials, among them, ease of preparation, a high-level hydration environment (80% water), and rapid and persistent macroscopic alignment induced by moderate magnetic fields. These features, together with previously established physicochemical properties of the complex fluid (e.g., excellent optical transparency and the ability to encapsulate a wide range of molecules,[42] including membrane proteins) suggest that this material offers enormous potential as a medium for the study of a range of biomolecules by magnetic resonance, optical spectroscopy, and small-angle diffraction.

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(23) Polarized light microscopy images were obtained using a Wild M400 microscope by placing the sample between crossed sheet polarizers. The images were recorded using a 35 mm camera and/or a CCD color camera (Diagnostic Instruments, Sterling Heights, MI).

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(30) Ex situ magnetic field studies were carried out on samples in 1.5 mm quartz capillaries that were then inserted into a 5 mm NMR tube. Magnetic field alignment of the sample was achieved in the superconducting magnet (7.05 T) of a GE Omega 300 NMR spectrometer. The sample temperature was controlled via dry ice-cooled N_2 gas flow. In the presence of the field, the sample was cooled to 2 °C and held for 1 h, then gradually (over a 30 minute period) warmed to 25 °C, held an additional 1 h, and then removed from the magnet. Aligned samples were stored at room temperature until taken for X-ray studies. Synchrotron small-angle X-ray diffraction measurements were preformed on the BESSRC undulator beam line (12ID) of the Advanced Photon Source at Argonne National Laboratory. The scattering profiles were recorded with a mosaic detector composed of 9 CCD chips with an imaging area of 15 × 15 cm, with 1536 × 1536 pixel resolution. The area detector images were corrected for background scattering of water by subtracting from the recorded images an area detector image of a water sample obtained with the same total exposure time as for the liquid crystalline sample (5 s). The collected low-angle scattering data were calibrated on the basis of the known positions of silver behenate powder Bragg reflections.

(31) The timing profile for the alignment procedure was 1 h at 3 °C followed by slow warming (0.5 h) through the phase transition (16 °C) to 25 °C and held in the field for 1 h.

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