Structure determination of membrane proteins remains an important but difficult research target. Solution NMR offers a number of experimentally accessible parameters that can aid in structural studies, including interproton distances derived from NOEs, torsion angles from J-couplings and chemical shift data, and vector orientations from residual dipolar couplings (RDCs). RDCs, NOEs, torsion angles from J-couplings and chemical shift data, and structural studies, including interproton distances derived from a number of experimentally accessible parameters that can aid in important but difficult research target. Solution NMR offers a basepair "scaffold" and 42-basepair "staple" DNA molecules. They the first detergent-compatible liquid crystal suitable for the NMR size of the coordinating monovalent cations, with K⁺ of the dinucleotide 2'-deoxyguanylyl-(3',5')-2'-deoxyguanosine, d(GpG). Guanosine mono-, di-, and larger oligonucleotides form G-tetrad structures, where hydrogen bonds link four G bases in a C₄-symmetric planar arrangement, with cations on the C₄ axis coordinating the C₆ carbonyl groups of G. Indeed, above ca. 10 mg/ml d(GpG) forms a cholesteric LC phase. As has long been known, structure it adopts a hexagonal LC phase.12 As has long been known, the first detergent-compatible liquid crystal suitable for the NMR study of larger proteins, where measurement at or temperature of ca. 40 °C is done, the **K**-d(GpG) maintains a liquid crystalline phase in the presence of 1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine (DPC) and 30 mM sodium dodecylsulfate (SDS). However, the columns orthogonal to the field. K-d(GpG) alignment is dominated by causing the long axis of the phage filament to align parallel with the applied magnetic field.18 K-d(GpG) alignment is dominated by the diamagnetic purine bases, which have lowest energy when the external magnetic field is parallel to the plane of the base, orienting the columns orthogonal to the field. K-d(GpG) maintains a liquid crystalline phase in the presence of DPC detergent. Furthermore, the liquid crystalline phase is maintained at moderately acidic pH (Figure 1) as well as in the presence of 1-palmitoyl-2-hydroxy-sn-glycerol-3-phosphocholine (LPPC) detergent or the more membrane-like mixed micelles,19,20 a small region of the 1H-coupled 1H-15N HSQC NMR spectrum is included as Supporting Information, and the alignment tensors in K-d(GpG) and Pf1 media are compared in Table 1. The normalized scalar product17 of the protein alignment tensors in d(GpG) and Pf1 is −0.983, confirming that protein alignments in these two media are simply related by a scale factor. The opposite sign of the two alignments results from the different orientations of the rod-shaped particles in the magnetic field. The magnetic susceptibility anisotropy of Pf1 is dominated by that of its major coat protein helices, causing the long axis of the phage filament to align parallel with the applied magnetic field.18 K-d(GpG) alignment is dominated by the diamagnetic purine bases, which have lowest energy when the external magnetic field is parallel to the plane of the base, orienting the columns orthogonal to the field. K-d(GpG) maintains a liquid crystalline phase in the presence of DPC detergent. Furthermore, the liquid crystalline phase is maintained at moderately acidic pH (Figure 1) as well as in the presence of 1-palmitoyl-2-hydroxy-sn-glycerol-3-phosphocholine (LPPC) detergent or the more membrane-like mixed micelles,19,20 often referred to as small bicelles (data not shown). Simply dissolving the K-d(GpG) overnight, followed by addition of KCl, results in a sample with a stable residual quadrupole coupling (RQC) for the 2H lock signal (Figure 1). The transition temperature to the isotropic phase depends on the d(GpG) concentration and deprecitates by ~10 °C when the concentration is lowered from 20 to 10 mg/ml; the threshold for liquid crystal formation increases to ca. 22 mg/ml, whereupon the DPHC concentration reaches 200 mM (Supporting Information).

Table 1. Alignment Tensor Parameters in Liquid Crystalline Media for Uracil-[15N]-[K19E,D40N,V42E]-GB3

<table>
<thead>
<tr>
<th>LC medium</th>
<th>D₁ (Hz)</th>
<th>R</th>
<th>tensor orientation</th>
<th>Q-factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pf1</td>
<td>−7.12</td>
<td>0.321</td>
<td>(154°, 90°, 220°)</td>
<td>0.083</td>
</tr>
<tr>
<td>K-dGpG</td>
<td>8.30</td>
<td>0.434</td>
<td>(156°, 90°, 232°)</td>
<td>0.096</td>
</tr>
</tbody>
</table>

| Tensor parameters based on 48 NH RDC measurements. Data recorded in 25 mM K₂HPO₄ pH 7.4, 35 mM KCl, 22 mg/ml dGpG (Sigma) at 25 °C. The Pf1 sample contained 22 mg/ml Pf1 bacteriophage [Asla Biotech], 25 mM NaH₂PO₄ pH 6.7, 50 mM NaCl. Tensor orientation expressed as the Rose convention Euler angles between the GB3 molecular frame (2OED) and the alignment frame. Stacked columns of G-tetramers, which have a diameter of ~2.5 nm (vs 6.5 nm for Pf1) carry a high net negative charge of ca. 0.4 e/nm² (~0.5 e/nm² for Pf1)13. Their similarity in shape and charge to Pf1 suggests that alignment tensors for proteins compatible with both types of liquid crystals will be directly related to one another.14 We first demonstrate this similarity for a mutant of GB3, previously extensively characterized by RDCs. A small region of the 1H-coupled 1H-15N HSQC NMR spectrum is included as Supporting Information, and the alignment tensors in K-d(GpG) and Pf1 media are compared in Table 1. The normalized scalar product of the protein alignment tensors in d(GpG) and Pf1 is −0.983, confirming that protein alignments in these two media are simply related by a scale factor. The opposite sign of the two alignments results from the different orientations of the rod-shaped particles in the magnetic field. The magnetic susceptibility anisotropy of Pf1 is dominated by that of its major coat protein helices, causing the long axis of the phage filament to align parallel with the applied magnetic field. K-d(GpG) alignment is dominated by the diamagnetic purine bases, which have lowest energy when the external magnetic field is parallel to the plane of the base, orienting the columns orthogonal to the field. K-d(GpG) maintains a liquid crystalline phase in the presence of DPC detergent. Furthermore, the liquid crystalline phase is maintained at moderately acidic pH (Figure 1) as well as in the presence of 1-palmitoyl-2-hydroxy-sn-glycerol-3-phosphocholine (LPPC) detergent or the more membrane-like mixed micelles, often referred to as small bicelles (data not shown). Simply dissolving the K-d(GpG) overnight, followed by addition of KCl, results in a sample with a stable residual quadrupole coupling (RQC) for the 2H lock signal (Figure 1). The transition temperature to the isotropic phase depends on the d(GpG) concentration and deprecitates by ~10 °C when the concentration is lowered from 20 to 10 mg/ml; the threshold for liquid crystal formation increases to ca. 22 mg/ml, whereupon the DPHC concentration reaches 200 mM (Supporting Information).

Figure 2 shows 1H-[15N]-IPAP-HSQC20 and J_C,Cα-coupled HNCA spectra for the U-[15C,15N-gly,ala] hemagglutinin fusion peptide (HAfp) solubilized in DPC, in the absence and presence of K-d(GpG). Partial alignment of the fusion peptide in DPC...
Figure 1. Residual $^{2}$H$_2$O quadrupole coupling in the presence of d(GpG). (A) Spectra of $^{2}$H$_2$O with and without DPC and (B) the dependence of the $^{2}$H$_2$O RQC on the concentration of d(GpG). (A) RQCs are 21.9 Hz (DPC, pH 5.8), 20.6 Hz (DPC, pH 6.6), 21.9 Hz (DPC, pH 7.4) and 25.0 Hz (pH 7.4). The pH titration was carried out on a sample of 3 mM U-[^13]C,[^15]N-gly,ala]-HAfp and 28 mg/ml d(GpG), 23 mM K$_2$HPO$_4$, pH 7.4. The pH titration was carried out on a sample of GB3 in d(GpG), 23 mM K$_2$HPO$_4$, 81 mM DPC on a Bruker 500 MHz DDMX NMR spectrometer at 32°C. See footnote to Table 1 for sample details on the sample without DPC. (B) Concentration dependence of the $^{2}$H$_2$O residual quadrupolar coupling.

Figure 2. $^1$H-[^15]N IPAP-HSQC$^{20}$ and J$_{C}$$^N$H$_{15}$-coupled HNCA spectra for 2.1 mM U-[^13]C,[^15]N-gly,ala]-HAfp [Biopeptide, sequence: GLFGAIAG FIENGWEG MIDG-OH] in 81 mM DPC [Anatrace], 23 mM K$_2$HPO$_4$, pH 7.4. J$_{NH}$ and J$_{CH}$ splittings are shown for the isotropic sample. RDCs (D$_{NH}$ and D$_{CH}$), obtained from the change in splitting are shown for the spectra recorded after addition of 28 mg/ml d(GpG). Spectra containing the upfield (blue) and downfield (red) ^15$N-[^1]H$ doublt components of the $^1$H-[^15]N IPAP-HSQC spectra are superimposed. Measurements conducted on a Bruker 500 MHz DDMX NMR spectrometer at 32°C. Micelles adds a RDC to the amide $^1$J$_{NH}$ and $^1$J$_{C}$$^N$H$_{15}$ splittings.$^{2}$ Studies of uniformly enriched fusion domain are currently underway to obtain a more detailed characterization of the pH-induced structural transition, which triggers fusion of the virus with host cell membranes.$^{21}$

G-tetrad formation previously has been studied extensively by a range of physical and biophysical chemistry methods. Although its biological role has been subject to extensive debate, G-tetrad formation now is increasingly recognized as a critical element in the functioning of biological macromolecules. Like Pf1, the negative charge of G-tetrad polymers makes it best suited for the study of negatively charged biological macromolecules. However, with peptide-nucleic-acid chemistry well established, adjustment of the charge characteristics of G-tetrad-based liquid crystals is likely to be feasible too, a subject we are currently exploring.

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Supporting Information Available: Protocol for NMR sample preparation; plots of (A) GB3 $^{1}$D$_{NH}$ RDCs in Pf1 vs d(GpG), (B) a region of the IPAP-HSQC $^{1}$H-[^15]N spectrum of GB3 in d(GpG), $^{2}$H RQC as a function of temperature at 100 and 214 mM DPC. This material is available free of charge via the Internet at http://pubs.acs.org.

References

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