*Journal of Biomolecular NMR*, **13:** 387–391, 1999.
*KLUWER/ESCOM*



# Improved low pH bicelle system for orienting macromolecules over a wide temperature range

Silvia Cavagnero, H. Jane Dyson* & Peter E. Wright*
*Department of Molecular Biology MB-2 and Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.*

Received 16 December 1998; Accepted 25 January 1999

*Key words:* bicelle, ether-lipid, liquid crystal, protein alignment, residual dipolar coupling

**Abstract**

We have prepared and characterized a novel bicelle system composed of 1,2-di-O-dodecyl-*sn*-glycero-3-phosphocholine (DIODPC) and 3-(chloramidopropyl)dimethylammonio-2-hydroxyl-1-propane sulfonate (CHAPSO). At the optimal DIODPC/CHAPSO molar ratio of 4.3:1, this medium becomes magnetically oriented from pH 6.5 down to pH 1.0. Unlike previously reported bicelle preparations, these bicelles are chemically stable at low pH and are capable of inducing protein alignment, as illustrated by the large residual dipolar couplings measured for rusticyanin from *Thiobacillus ferrooxidans* at pH 2.1. The DIODPC/CHAPSO system is particularly useful for measuring residual dipolar couplings of macromolecules that require very acidic conditions.

*Abbreviations:* DIODPC, 1,2-di-O-dodecyl-*sn*-glycero-3-phosphocholine; CHAPSO, 3-(chloramidopropyl)dimethylammonio-2-hydroxyl-1-propane sulfonate; DMPC, 1,2-di-tetradecanoyl-*sn*-glycero-3-phosphocholine; DHPC, 1,2-di-hexanoyl-*sn*-glycero-3-phosphocholine; DLPC, 1,2-di-dodecanoyl-*sn*-glycero-3-phosphocholine; k, kinetic rate constant; HSQC, heteronuclear single quantum coherence; IPAP, in-phase/antiphase.

Conventional solution structure determination methods based on the NOE rely primarily on multiple broadly defined distance constraints. A recently developed methodology, which has the potential to supplement conventional NOE methods, involves the measurement of residual dipolar couplings (Kung et al., 1995; Tolman et al., 1995; Tolman and Prestegard, 1996; Tjandra et al., 1996, 1997; Tjandra and Bax, 1997b; Prestegard, 1998). These allow mapping of the orientation of several covalent bond vectors with respect to an alignment tensor or magnetic susceptibility tensor fixed in the molecular frame. In sufficiently rigid macromolecular systems, this procedure may improve the accuracy of the structure and serve as a powerful complement to the more traditional distance-based methods for structure determination in solution (Tjandra and Bax, 1997a).

*To whom correspondence should be addressed.
E-mail: wright@scripps.edu, dyson@scripps.edu

In order to measure residual dipolar couplings, it is necessary for the molecule to attain a certain degree of alignment in the presence of a magnetic field. With the exception of paramagnetic systems (Tolman et al., 1995), this is not usually spontaneously achieved to a significant extent, although a modest degree of alignment has been observed and studied in solution in cases where the magnetic susceptibility tensor is sufficiently anisotropic (Tjandra et al., 1997). An alternative way of achieving a far greater and more adjustable degree of alignment in the presence of a magnetic field is by exploiting the ability of dilute liquid crystalline media to orient macromolecules dissolved in their aqueous domain (Bax and Tjandra, 1997; Tjandra and Bax, 1997a). Provided that the liquid crystalline medium is dilute, the protein or nucleic acid remains sufficiently mobile to preserve sharp NMR resonances, while at the same time achieving a partial degree of alignment which gives rise to large and accurately measurable dipolar couplings (Tjandra

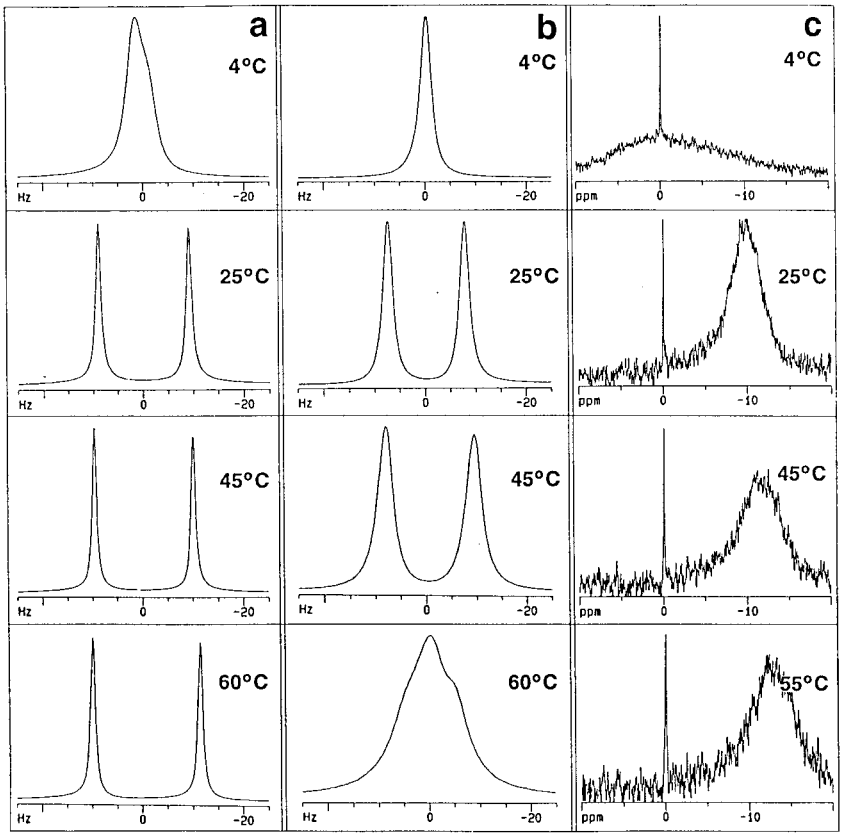


*Figure 1.* 1D deuterium NMR spectra of 5% total weight/volume DIODPC/CHAPSO bicelles at (a) pH 2.0 in 10 mM sodium phosphate buffer and (b) pH 4.1 in 10 mM sodium acetate buffer acquired at different temperatures on a 750 MHz Bruker spectrometer; (c) 1D $^{31}$P spectra of 5% DIODPC/CHAPSO bicelles at pH 4.1 in 10 mM sodium acetate buffer acquired at different temperatures on a 500 MHz NMR spectrometer. All of the NMR samples contain 10% $D_2O$. The molar ratio of DIODPC/ CHAPSO was 4.3:1. This ratio was found to yield optimal results at 5% total bicelle concentration. NMR samples were prepared by combining the required amounts of lipid, detergent and solvent followed by thorough mixing. The resulting solutions were then subjected to a few alternate equilibration cycles at 4 °C and 35 °C prior to the NMR experiments. CHAPSO was purchased from Sigma and DIODPC was purchased from Avanti Polar Lipids.

and Bax, 1997a). The most common medium used for these purposes consists of disk-shaped bicelles (Sanders and Schwonek, 1992), i.e., a binary mixture of two lipids which exhibits nematic liquid crystalline properties when exposed to strong magnetic fields. A relatively well-characterized bicelle system, a mixture of dimyristoyl phosphatidyl choline (DMPC), and dihexanoyl phosphatidyl choline (DHPC) has been the most popular choice so far, both for applications at high concentrations as a biomembrane mimetic (Vold and Prosser, 1996) and at lower concentrations for structural studies of water-soluble proteins and nucleic acids (Bax and Tjandra, 1997; Tjandra and Bax, 1997a).

A significant problem with phospholipid ester-based bicelles is the rapid irreversible degradation which occurs at low pH (Ottiger and Bax, 1998), due to the hydrolytic cleavage of the carboxyl ester linkages in positions *sn*-1 and *sn*-2 (Kemps and Crommelin, 1988; Grit and Crommelin, 1993). This issue is especially relevant for structural studies that require low pH conditions, such as NMR experiments on proteins derived from acidophilic organisms. This communication describes the preparation and NMR characterization of a new bicelle system suitable for structural studies of water-soluble biomolecules at low pH. These bicelles can also be employed over a wide temperature range.

In order to circumvent degradation of bicelles at low pH, we have replaced the carboxyl ester sites of the long chain lipid component by the considerably more acid-stable ether functionalities which are found in 1,2-di-O-dodecyl-*sn*-glycero-3-phosphocholine (DIODPC). Interestingly, a similar

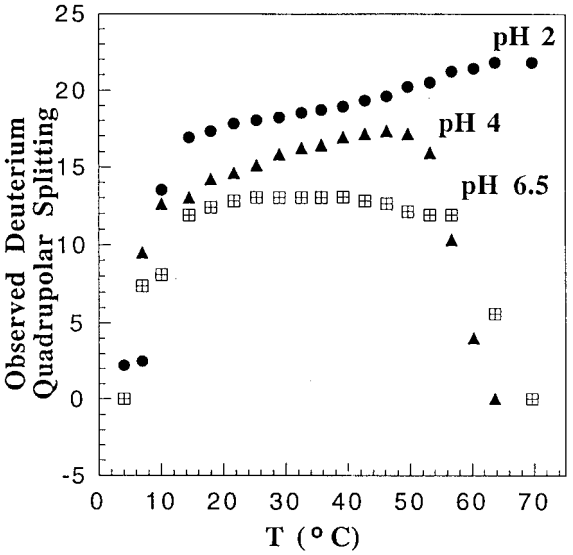


*Figure 2.* Temperature dependence of the observed deuterium quadrupolar splitting of 5% w/v DIODPC/CHAPSO bicelles. The buffers for the pH 2.0 (●) and pH 4.1 (▲) experiments are as described in Figure 1. The pH 6.5 sample (□) was in 10 mM sodium phosphate buffer.

strategy has been adopted by nature for stabilizing biomembranes under harsh environmental conditions; most of the complex lipids belonging to extreme halophilic and thermoacidophilic microorganisms contain alkyl ether derivatives (Smith, 1988). We use the water-soluble and acid-stable zwitterionic detergent CHAPSO as a replacement for the short acyl chain lipid component (Sanders et al., 1994; Wang et al., 1998). The resulting bicelles, denoted as DIODPC/CHAPSO (optimal molar ratio 4.3:1), display a high chemical stability over a wide pH range, being stable to hydrolysis for long times (> days or weeks) from neutral pH down to pH values as low as 1. This system is therefore an excellent complement to the currently available liquid crystalline media.

The degree of magnetically induced alignment of the bicelles can be conveniently assessed by the deuterium quadrupolar splitting of the HDO signal in liquid crystalline preparations containing a small percentage of $D_2O$ (typically about 10%). This signal is a dynamic average of isotropic free and oriented bicelle-bound HDO molecules and, as such, the observed splitting is a measure of the degree of magnetic alignment achieved by the system of interest. The spectra of Figure 1 (a and b columns) illustrate the magnitude of the observed deuterium quadrupolar splitting for the 5% DIODPC/CHAPSO bicelles at pH 2.1 and

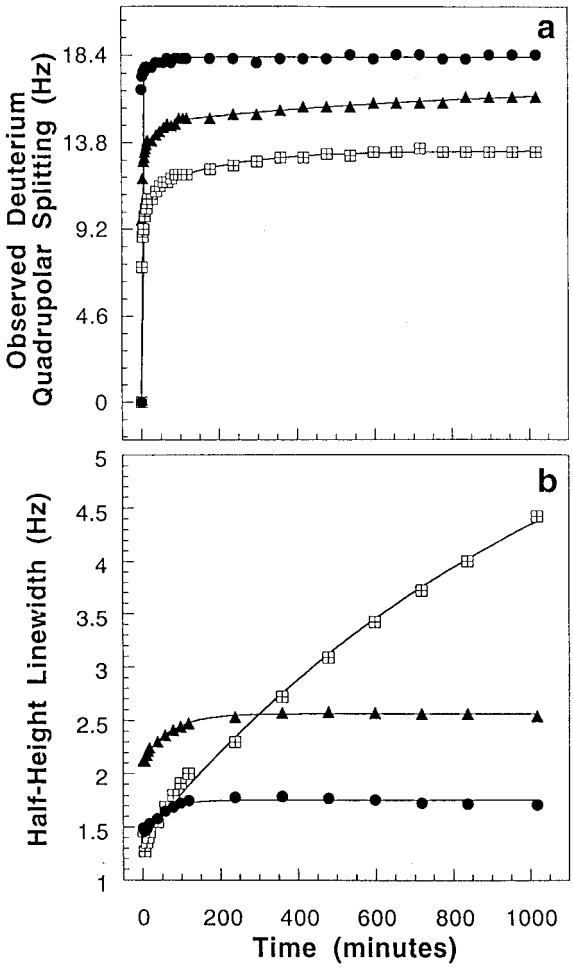


*Figure 3.* Effect of (a) observed deuterium quadrupolar splitting and (b) half-height deuterium linewidths as a function of time after placement in the magnetic field, at pH 2.0 (●), pH 4.1 (▲) and pH 6.5 (□). The temperature is 28.9 °C. Buffers and bicelle concentrations are as described in Figures 1 and 2. All the NMR samples contain 10% $D_2O$. Solid lines are fitted by the method of least squares to either 1 exponential or to the sum of 2 or 3 exponentials (see text).

4.0. At these low pH values, both peak splittings and lineshapes compare favorably with those observed for analogous DMPC/DHPC (Ottiger and Bax, 1998) and DLPC/CHAPSO (Wang et al., 1998) bicelles at neutral pH.

The orientation of the DIODPC/CHAPSO bicelles with respect to the applied field is indicated by the $^{31}P$ 1D spectra of Figure 1c. The upfield shift (about 11 ppm) of the broad $^{31}P$ resonance due to the oriented DIODPC component (i.e. at 35 °C) is typical of bicelles whose normal is perpendicular to the applied magnetic field (Seelig et al., 1985).

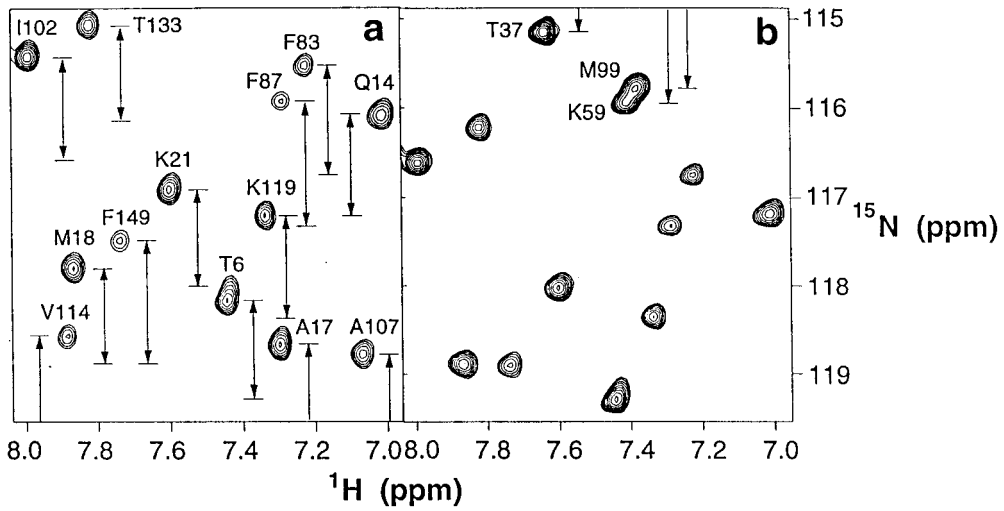


*Figure 4.* Expanded region of the (a) upfield and (b) downfield doublet components of the IPAP-[$^{1}$H-$^{15}$N]-HSQC spectrum (Ottiger et al., 1998) of $^{15}$N-$^{13}$C-labeled Cu(I) rusticyanin from *Thiobacillus ferrooxidans* in DIODPC/CHAPSO bicelles, 1 mM $H_2SO_4$ and 5 mM ascorbic acid at pH 2.1. The data were collected at 28.9 °C on a 750 MHz Bruker NMR spectrometer. Sample preparation involved mixing of a 7.5% bicelle preparation with the necessary amount of protein stock solution to reach a final protein concentration of 0.5 mM and a final total bicelle concentration of 5% w/v. Peak assignments (Hunt et al., 1994) are indicated on the spectrum. Doubly labeled rusticyanin was prepared as described previously (Casimiro et al., 1995).

A more comprehensive illustration of the wide range of conditions under which the DIODPC/CHAPSO bicelles can be oriented is provided by Figure 2. Satisfactory bicelle alignment is achieved over a wide pH range spanning from 2 to 6.5. We have observed orientation at even lower pH values (down to pH 1, data not shown). The DIODPC/CHAPSO bicelles are stable when stored for long periods at either 4 °C or at room temperature.

The kinetic behavior of these bicelles reveals some additional peculiar features of the liquid crystalline medium. The deuterium quadrupolar splitting of 5% DIODPC/CHAPSO bicelles at pH 2.1 reaches its final equilibrium value rapidly and with double exponential behavior, with the second phase having a much smaller amplitude ($k_1 = 1.5 \pm 0.1$ min$^{-1}$, $k_2 = 2.3 \pm 0.5 \times 10^{-2}$ min$^{-1}$, Figure 3a). On the other hand, the time course for reaching the equilibrium linewidth (Figure 3b) follows simple single exponential kinetics. The corresponding rate constant ($k = 1.9 \pm 0.3 \times 10^{-2}$ min$^{-1}$) is identical within error to that measured for the second kinetic phase of the quadrupolar splitting at the same pH. This observation suggests a field alignment mechanism for the DIODPC/CHAPSO bicelles consisting of an initial fast magnetic alignment followed by a slower broadening of the distribution of different field orientations concurrent with a further optimization of the overall alignment.

The time course for achieving alignment at pH 4.0 and 6.5 is generally slower and follows more complicated kinetics (Figure 3a), which can only be adequately fitted by 3 or more exponentials. As for the lower-pH measurements, the corresponding linewidth profile follows simple single exponential kinetics at these pH values (Figure 3b). The slow line broadening at pH 6.5 ($k = 9 \pm 2 \times 10^{-4}$ min$^{-1}$) may be due to unfavorable electrostatic effects such as the approach of the net charge on the bicelles to zero near neutral pH (Losonczi and Prestegard, 1998). As a consequence, the DIODPC/CHAPSO bicelles are not suitable for NMR structural studies at pH close to 6.5, despite their liquid crystalline properties under these conditions. The line broadening is reversible by a simple pH change, and is reproducible if the sample is returned to pH 6.5; it cannot therefore be ascribed to chemical degradation.

The unique ability of the DIODPC/CHAPSO system to promote alignment of macromolecules at low pH is demonstrated by measurements made with rusticyanin from *Thiobacillus ferrooxidans.* Figure 4 shows a section of the IPAP-[$^{1}$H-$^{15}$N]-HSQC (Ottiger et al., 1998) spectrum of $^{15}$N-$^{13}$C-labeled Cu(I) rusticyanin in 5% w/v DIODPC/CHAPSO bicelles at pH 2.1. The data are of good quality and the peaks are very similar to those of a reference spectrum collected in the absence of bicelles. The experimentally de-

termined $^{1}H$-$^{15}N$ residual dipolar couplings are quite large for a total bicelle concentration of 5% w/v, ranging from −13 Hz to +14 Hz.

In conclusion, we have prepared and characterized a new bicelle system which extends the pH range of applicability of NMR experiments based on the measurements of residual dipolar couplings in liquid crystalline media. This bicellar preparation, denoted DIODPC/CHAPSO, also performs extremely well over a wide range of temperatures (from 10 °C to at least 55 °C). The DIODPC/CHAPSO bicelles are particularly suited for NMR studies of proteins in acidic media, between pH 1 and pH 5.

## Acknowledgements

We wish to thank Tai-Huang Huang, Linda Tennant, John Chung and David Eliezer for stimulating comments and useful suggestions, Leiming Zhu and Vickie Tsui for help with the technical aspects of data collection and analysis, and Steven W. Fesik for a preprint. This work was supported by grants GM 34909 (P.E.W.) and GM 57374 (H.J.D.) from the National Institutes of Health, and in part by the Wills Foundation (S.C.).

## References

Bax, A. and Tjandra, N. (1997) *J. Biomol. NMR*, **10**, 289–292.

Casimiro, D.R., Toy-Palmer, A., Blake II, R.C. and Dyson, H.J. (1995) *Biochemistry*, **34**, 6640–6648.

Grit, M. and Crommelin, D.J.A. (1993) *Chem. Phys. Lipids*, **64**, 3–18.

Hunt, A.H., Toy-Palmer, A., Assa-Munt, N., Cavanagh, J., Blake II, R.C. and Dyson, H.J. (1994) *J. Mol. Biol.*, **244**, 370–384.

Kemps, J.M.A. and Crommelin, D.J.A. (1988) *Pharm. Weekbl.*, **123**, 457–469.

Kung, H.C., Wang, K.Y., Goljer, I. and Bolton, P.H. (1995) *J. Magn. Reson.*, **B109**, 323–325.

Losonczi, J.A. and Prestegard, J.H. (1998) *J. Biomol. NMR*, **12**, 447–451.

Ottiger, M. and Bax, A. (1998) *J. Biomol. NMR*, **12**, 361–372.

Ottiger, M., Delaglio, F. and Bax, A. (1998) *J. Magn. Reson.*, **131**, 373–378.

Prestegard, J.H. (1998) *Nat. Struct. Biol.*, **5**, 517–522.

Sanders, C.R., Hare, B.J., Howard, K.P. and Prestegard, J.H. (1994) *Prog. NMR Spectrosc.*, **26**, 421–444.

Sanders, C.R. and Schwonek, J.P. (1992) *Biochemistry*, **31**, 8898–8905.

Seelig, J., Borle, F. and Cross, T.A. (1985) *Biochim. Biophys. Acta*, **814**, 195–198.

Smith, P.F. (1988) In *Microbial Lipids* (Eds, Ratledge, C. and Wilkinson, S.G.) Harcourt Brace Jovanovich, London, pp. 489–501.

Tjandra, N. and Bax, A. (1997a) *Science*, **278**, 1111–1114.

Tjandra, N. and Bax, A. (1997b) *J. Magn. Reson.*, **124**, 512–515.

Tjandra, N., Grzesiek, S. and Bax, A. (1996) *J. Am. Chem. Soc.*, **118**, 6264–6272.

Tjandra, N., Omichinski, J.G., Gronenborn, A.M., Clore, G.M. and Bax, A. (1997) *Nat. Struct. Biol.*, **4**, 732–738.

Tolman, J.R., Flanagan, J.M., Kennedy, M.A. and Prestegard, J.H. (1995) *Proc. Natl. Acad. Sci. USA*, **92**, 9279–9283.

Tolman, J.R. and Prestegard, J.H. (1996) *J. Magn. Reson.*, **B112**, 245–252.

Vold, R.R. and Prosser, R.S. (1996) *J. Magn. Reson.*, **B113**, 267–271.

Wang, H., Eberstadt, M., Olejniczak, E.T., Meadows, R.P. and Fesik, S.W. (1998) *J. Biomol. NMR*, **12**, 443–446.