JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Communication

pubs.acs.org/JACS

A Positively Charged Liquid Crystalline Medium for Measuring Residual Dipolar Couplings in Membrane Proteins by NMR

Pallavi Thiagarajan-Rosenkranz,[‡] Adrian W. Draney,[‡] Sean T. Smrt, and Justin L. Lorieau*

Department of Chemistry, University of Illinois at Chicago, 845 West Taylor Street, Chicago, Illinois 60607, United States

Supporting Information

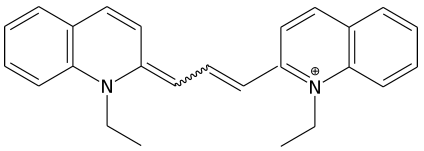
ABSTRACT: Residual Dipolar Couplings (RDCs) are integral to the refinement of membrane protein structures by NMR since they accurately define the orientation of helices and other structural units. Only a small set of liquid crystals used for RDC measurements are compatible with the detergents needed in membrane protein studies. The available detergent-compatible liquid crystals are negatively charged, thus offering effectively only one of five orthogonal components of the alignment Saupe matrix. In this communication, we present a robust liquid crystalline medium that is positively charged, pinacyanol acetate (PNA), for the determination of orthogonal sets of RDCs in membrane proteins. This new medium promises to enhance the accuracy of membrane protein structures and the measurement of dynamics based on RDCs.

Membrane proteins are an important class of biomolecules involved in cell signaling, communication, and transport, and they represent a significant fraction of expressed proteins in genomes.[1] Structural refinement of membrane proteins is a challenge to structural biology, and membrane protein structures remain under-represented in the protein databank.[2] NMR has made useful contributions in the determination of membrane protein structures in detergent micelles and isotropically tumbling bicelles.[3] Membrane proteins solubilized in detergent micelles tumble rapidly and produce high-resolution NMR spectra, from which chemical shift and NOE structural information are readily measured for these samples. Supplementing these measurements with Residual Dipolar Couplings (RDCs) further refines the orientation of secondary structure units and domains within the molecule, which is particularly important in ascertaining the structure of trans-membrane helices and helical bundles.[4]

RDCs measure the orientations of bonds from magnetic dipole−dipole interactions in a global frame of alignment of the structure.[5,6] RDCs result from the partial alignment of biomolecules in a liquid crystalline (LC) medium or from the attachment of a paramagnetic tag.[4,7,8] LC media are routinely used in RDC measurements, as they do not require the chemical modification of the protein and are readily dissolved in protein samples. Several LC media have been used for the measurement of RDCs of soluble proteins, including Pf1 phage,[9,10] aligned bicelles,[5] polyethylene glycol mixtures,[11] collagen,[12] and squalamine.[13] However, only a small subset of available LC media is compatible with the detergents needed for membrane protein structural studies. Compressed and stretched acrylamide gels (SAG) are compatible with detergents,[14,15] yet proteins aligned in gels can have lower rates of rotational diffusion and, consequently, lower resolution NMR spectra. The available LC media that are compatible with detergents, including DNA nanotubes,[16] guanosine dinucleotides,[17] fd bacteriophage,[18] and cellulose nanocrystals,[19] are negatively charged and thus offer only one type of protein−LC interaction.

RDCs have an inherent orientational degeneracy such that a single RDC data set for one dipolar coupling is consistent with a continuum of bond vector orientations on two arched cones of a sphere.[20] A total of five orthogonal alignment tensors are needed to form a complete set of RDCs, thus reducing the uncertainty to a subset of distinct, degenerate mirror-image orientations.[21] Orthogonal tensor orientations are achieved by varying the nature of the interaction between the protein and the LC medium. The available detergent-compatible LC media, however, are negatively charged, presenting effectively only one of five alignment tensors when the protein interaction with the LC is dominated by electrostatics.[17] Consequently, significant additional information is needed to resolve ambiguity introduced from only a single RDC data set. This limitation can lead to incorrect membrane protein structures,[22] and it underscores the need for detergent-compatible LC media that can produce orthogonal sets of RDCs. PNA is an inexpensive LC medium that aligns in a magnetic field (Scheme 1).[23] In this communication, we show that PNA is a robust and, to our knowledge, the first positively charged LC medium for measuring orthogonal sets of RDCs for membrane proteins solubilized in detergents.

Scheme 1. Structure of Pinacyanol at Neutral pH



The PNA liquid crystal aligns in a magnetic field with and without 100 mM dodecylphosphocholine (DPC) detergent (Figure 1). Alignment is achieved with an LC concentration as low as 0.75% (w/v), producing partial alignment of the solvent ^2H_2O resonance and a residual quadrupolar coupling (RQC) splitting of ca. 10 Hz resolved to the baseline. Alignment in the

Received: July 18, 2015
Published: September 8, 2015

DOI: 10.1021/jacs.5b07515
J. Am. Chem. Soc. 2015, 137, 11932−11934

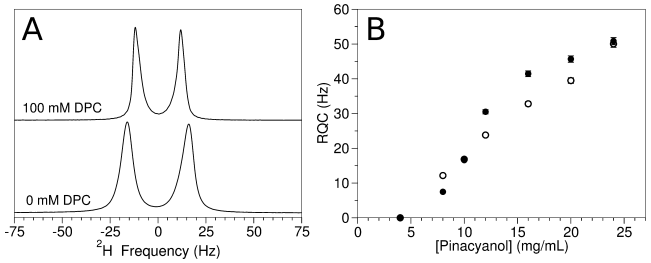


Figure 1. ^2H_2O RQCs in the PNA LC medium. (A) ^2H spectra of 12 mg/mL PNA in 25 mM Tris pH 7.4 with 100 mM DPC (RQC = 23.7 Hz) and without DPC (RQC = 32.1 Hz). (B) Dependence of the ^2H_2O RQC with the PNA concentration in 25 mM Tris pH 7.4, with 100 mM DPC (○) and without DPC (●). Errors are comparable to the symbol size. Experimental and sample details are included in the SI.

magnetic field reaches equilibrium within 2−3 h (Supporting Information (SI)) and remains stable for a period of weeks to months. The LC medium is resistant to large deviations in pH and temperature, maintaining homogeneous alignment between pH 3−11 and at temperatures between 15 and 60 °C (SI). PNA is resistant to NaCl concentrations below 50 mM, and exceeding this concentration begins to precipitate the PNA.

We demonstrated the orthogonal RDC data set achieved from alignment in PNA using the influenza hemagglutinin fusion peptide (HAfp) in DPC micelles. HAfp is an amphipathic membrane protein critical to influenza membrane fusion and infection. The published HAfp structure in DPC at pH 7.4 (PDB: 2KXA[24]) was refined using chemical shifts, NOEs, and RDCs from K-d(GpG) and SAG doped with 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS). K-d(GpG) and SAG have alignment tensor products of −0.956,[25] indicating that these two negatively charged alignment media are highly correlated and effectively contain the same information. We further refined the 2KXA structure with the $^1H^{-15}N$ RDCs from the PNA LC medium (Table 1). The PNA medium produces an RDC data set with an alignment tensor that is highly orthogonal to K-d(GpG) and a tensor product of −0.165 (see SI for a correlation plot).

A representative region of the In-Phase, Anti-Phase Heteronuclear Single-Quantum Coherence (IPAP-HSQC) spectrum[29] of ^{15}N-labeled HAfp in PNA is shown in Figure 2. The sample aligned in PNA produces high-resolution spectra with accurate and well-resolved splittings. The fit RDCs show excellent agreement to the refined structure using PNA. The fit

Table 1. Liquid Crystal Alignment Tensor Parameters for HAfp in DPC Further Refined with the PNA RDCs

medium[a]	D_a (Hz)	R_h	$\langle A^1 \vert A^2 \rangle$[b]	Q-factor[c]
K-d(GpG)	−10.6	0.22	1.000	8.2%
SAG	8.0	0.13	−0.956	15.5%
PNA	−11.9	0.49	−0.165	7.1%

[a]The K-d(GpG) and SAG RDC data sets were previously published and reproduced for comparison.[24] Each data set had 20 $^1D_{NH}$ couplings. The PNA sample had 0.35 mM ^{15}N-HAfp in 25 mM ^2H-Tris pH 7.2, 150 mM ^2H-DPC, 10% ^2H_2O in 15.4 mg/mL PNA. [b]Normalized tensor scalar products[25] calculated relative to K-d(GpG). Tensor alignment orientations are (70.4°, 25.0°, 21.4°), (76.6°, 32.9°, −4.2°), and (8.7°, −117.9°, 88.2°) for K-d(GpG), SAG, and PNA, respectively, in XYZ-format.[26] [c]Q-factors[27,28] calculated as previously described. Structural refinement details are in the SI.

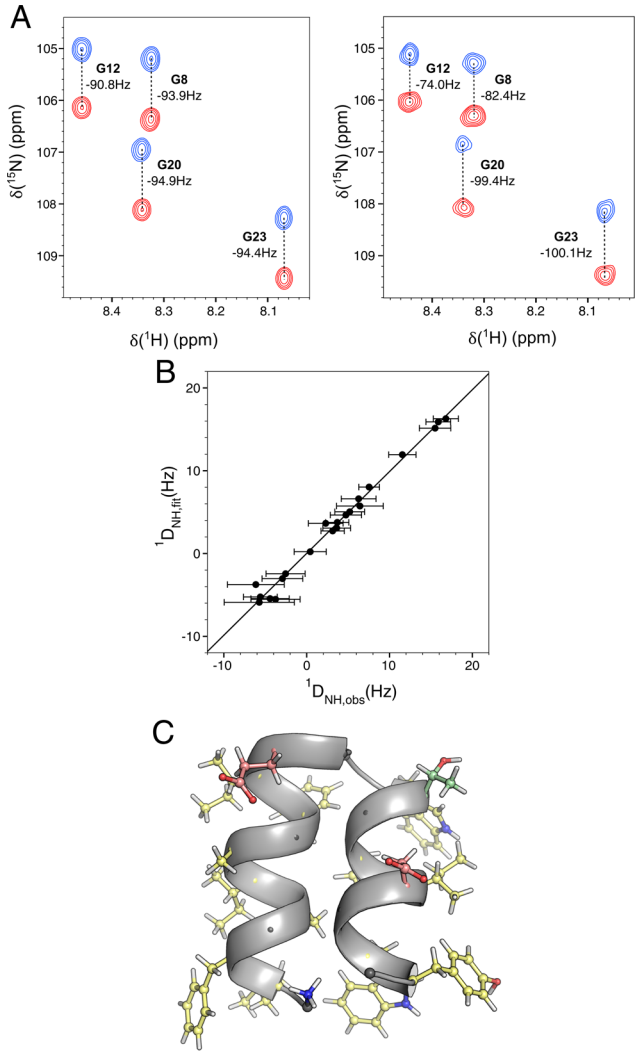


Figure 2. RDCs for 0.35 mM ^{15}N-HAfp in 150 mM ^2H-DPC, 25 mM ^2H-Tris pH 7.2, and 10% ^2H_2O using 15.4 mg/mL PNA. (A) Representative IPAP-HSQC spectra in the glycine region showing the upfield (blue) and downfield (red) components. The isotropic $^1J_{NH}$-couplings (left panel) and aligned $^1J_{NH} + {}^1D_{NH}$-couplings (right panel) are shown. Spectra were measured at 800 MHz. (B) The PNA RDCs were used to further refine the previously published HAfp structure solved using NOEs, chemical shifts, and RDCs from the K-d(GpG) and SAG alignment media (PDB: 2KXA[24]). The refined PNA RDCs are shown, and the fit Q-factor is 7.1%. The PNA RDC free Q-factor against the reference 2KXA structure is 19.1%, and the heavy-atom backbone RMSD between the newly refined structure and 2KXA is 0.13 Å. (C) The newly refined structure is shown in cartoon representation.

Q-factor[27,28] for the PNA $^1H^{-15}N$ RDCs is 7.1%, whereas the free Q-factor of these couplings to the reference 2KXA structure is 19.1%. The refined structure maintains the unique helical-hairpin fold of the 2KXA structure with a backbone RMSD of 0.13 Å.

PNA presents a promising new LC medium for measuring orthogonal sets of RDCs in proteins and membrane proteins. PNA maintains stable alignment over a large pH and temperature range in the presence and absence of DPC micelles. RDCs collected with PNA are readily integrated into NMR structural refinement protocols and will further improve

DOI: 10.1021/jacs.5b07515
J. Am. Chem. Soc. 2015, 137, 11932−11934

the accuracy of structural and dynamic information in membrane proteins.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b07515.

Protocol for the preparation of PNA LC protein samples; plots of the equilibration time, pH, and temperature dependence of alignment (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*justin@lorieau.com

Author Contributions

‡P.T.-R. and A.W.D. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was supported by funds from the Department of Chemistry at the University of Illinois at Chicago. Data were collected using high-field NMR spectrometers at the Center for Structural Biology at the University of Illinois at Chicago.

■ REFERENCES

(1) Krogh, A.; Larsson, B.; von Heijne, G.; Sonnhammer, E. L. *J. Mol. Biol.* **2001**, *305*, 567−580.
(2) Berman, H. M.; Westbrook, J.; Feng, Z.; Gilliland, G.; Bhat, T. N.; Weissig, H.; Shindyalov, I. N.; Bourne, P. E. *Nucleic Acids Res.* **2000**, *28*, 235−242.
(3) Poget, S. F.; Girvin, M. E. *Biochim. Biophys. Acta, Biomembr.* **2007**, *1768*, 3098−3106.
(4) Bax, A. *Protein Sci.* **2003**, *12*, 1−16.
(5) Tjandra, N.; Bax, A. *Science* **1997**, *278* (5340), 1111−1114.
(6) Prestegard, J. H.; Al-Hashimi, H. M.; Tolman, J. R. *Q. Rev. Biophys.* **2000**, *33*, 371−424.
(7) Veglia, G.; Opella, S. J. *J. Am. Chem. Soc.* **2000**, *122*, 11733−11734.
(8) Kamen, D. E.; Cahill, S. M.; Girvin, M. E. *J. Am. Chem. Soc.* **2007**, *129*, 1846−1847.
(9) Clore, G. M.; Starich, M. R.; Gronenborn, a M. *J. Am. Chem. Soc.* **1998**, *120*, 10571−10572.
(10) Hansen, M. R.; Rance, M.; Pardi, A. *J. Am. Chem. Soc.* **1998**, *120*, 11210−11211.
(11) Rückert, M.; Otting, G. *J. Am. Chem. Soc.* **2000**, *122*, 7793−7797.
(12) Ma, J.; Goldberg, G. I.; Tjandra, N. *J. Am. Chem. Soc.* **2008**, *130*, 16148−16149.
(13) Maltsev, A. S.; Grishaev, A.; Roche, J.; Zasloff, M.; Bax, A. *J. Am. Chem. Soc.* **2014**, *136*, 3752−3755.
(14) Tycko, R.; Blanco, F.; Ishii, Y. *J. Am. Chem. Soc.* **2000**, *122*, 9340−9341.
(15) Sass, H. J.; Musco, G.; Stahl, S. J.; Wingfield, P. T.; Grzesiek, S. *J. Biomol. NMR* **2000**, *18*, 303−309.
(16) Douglas, S. M.; Chou, J. J.; Shih, W. M. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 6644−6648.
(17) Lorieau, J.; Yao, L.; Bax, A. *J. Am. Chem. Soc.* **2008**, *130*, 7536−7537.
(18) Park, S. H.; Son, W. S.; Mukhopadhyay, R.; Valafar, H.; Opella, S. J. *J. Am. Chem. Soc.* **2009**, *131*, 14140−14141.
(19) Denisov, A. Y.; Kloser, E.; Gray, D. G.; Mittermaier, A. K. *J. Biomol. NMR* **2010**, *47*, 195−204.
(20) Ramirez, B. E.; Bax, A. *J. Am. Chem. Soc.* **1998**, *120*, 9106−9107.
(21) Hus, J.; Salmon, L.; Bouvignies, G.; Lotze, J.; Blackledge, M.; Brüschweiler, R. *J. Am. Chem. Soc.* **2008**, *130*, 15927−15937.
(22) Shi, L.; Traaseth, N. J.; Verardi, R.; Gustavsson, M.; Gao, J.; Veglia, G. *J. Am. Chem. Soc.* **2011**, *133*, 2232−2241.
(23) Rodríguez-Abreu, C.; Torres, C. A.; Tiddy, G. J. T. *Langmuir* **2011**, *27*, 3067−3073.
(24) Lorieau, J. L.; Louis, J. M.; Bax, A. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 11341−11346.
(25) Sass, J.; Cordier, F.; Hoffmann, A.; Rogowski, M.; Cousin, A.; Omichinski, J. G.; Löwen, H.; Grzesiek, S. *J. Am. Chem. Soc.* **1999**, *121*, 2047−2055.
(26) Delaglio, F.; Grzesiek, S.; Vuister, G. W.; Zhu, G.; Pfeifer, J.; Bax, A. *J. Biomol. NMR* **1995**, *6*, 277−293.
(27) Clore, G. M.; Garrett, D. S. *J. Am. Chem. Soc.* **1999**, *121*, 9008−9012.
(28) Cornilescu, G.; Marquardt, J. L.; Ottiger, M.; Bax, A. *J. Am. Chem. Soc.* **1998**, *120*, 6836−6837.
(29) Ottiger, M.; Delaglio, F.; Bax, A. *J. Magn. Reson.* **1998**, *131*, 373−378.

DOI: 10.1021/jacs.5b07515
J. Am. Chem. Soc. 2015, 137, 11932−11934