Comments to the Editor

Modeling the Membrane Environment for Membrane Proteins

Increasingly, the membrane protein community is recognizing the sensitivity of protein structure and function to the protein's environment. Höfer et al. (1), in their recent Letter in the *Biophysical Journal*, aim to develop a crystallization protocol that will have increased biological relevance. The approach utilizes "lipidic mesophases" to deliver protein to the crystal interface via cubic phase lipids. We applaud the authors for attempting to develop such an approach that could potentially generate more nativelike membrane protein structures. This approach has been previously demonstrated for large membrane proteins (2), and here Höfer et al. (1) have used this approach to determine a structure of gramicidin A (gA). However, the main point of this Biophysical Letter, that the observed crystal structure of gA demonstrates that the in meso approach is a physiologically relevant environment, is misleading for the readership of the *Biophysical Journal.* The observed gA structure is not the native membrane conformation and, therefore, does not validate the in meso approach as a more physiologically relevant environment for small membrane protein crystallography.

The approach for crystallization that Höfer et al. (1) described is one in which monoolein and gramicidin A are cosolubilized in trifluoroethanol followed by drying the organic solvent, hydrating (40% by weight water), and then adding a precipitant, in this case, polyethylene glycol. Crystals of a double-stranded (DS), left-handed, antiparallel structure were obtained, similar to several other structures of gA that have been previously characterized (3–7).

Höfer et al. state that "controversy exists as to whether this (native structure) is a head-to-head single-stranded dimer or a left- or right-handed intertwined parallel or anti-parallel double helix." Note that this controversy was put to rest more than a decade ago—first with structures in detergent micelles (8) and later with a high-resolution structure solved by solid-state NMR spectroscopy in lamellar phase lipid bilayers (9,10). These structures were single-stranded (SS), right-handed structures. In 1999, when a gA structure crystallized from glacial acetic acid was then touted as the physiologically relevant conformation (11), 14 research groups from seven countries identified arguments from the fields of NMR spectroscopy, x-ray crystallography, and other biophysical methods as well as the field of physiology verifying the SS right-handed structure as the correct gA conformation in lipid bilayers (11,12). Unfortunately, the structure of gA achieved by Höfer et al. is the same nonphysiological structure as that which spawned the international outcry in 1999.

In a lipid bilayer, the minimum energy conformer of gA is SS because of the four Trp residues. This amphipathic side chain is well known to prefer the hydrophobic/hydrophilic interfacial region of lipid bilayers based on numerous crystal structures of proteins and experiments by Yau et al. (13), White and von Heijne (14), and Killian and von Heijne (15). The SS conformation has all of the indole groups in the interfacial region, whereas in DS conformations several of these residues would obligatorily reside in the middle of the lipid bilayer (16,17), which is energetically unfavorable. This biophysical foundation is further supported by experiments involving the purposeful insertion of a parallel DS conformation into a lipid bilayer and measurements of DS-SS interconversion rates (18–20), which show that the DS conformation is unstable in lipid bilayers and converts readily to the SS conformation. It has also been shown that, if the Trp residues are replaced by Phe residues (known as gM), the DS conformation becomes stable in a lipid bilayer (21), supporting an important role for a specific interactions between Trp residues with the lipid/water interfacial region for adopting the SS conformation.

Consequently, Höfer et al. are attempting to develop a more physiologically relevant membrane mimetic environment for crystallization, but their protocol has not succeeded in stabilizing the physiological conformation of the peptide used for this demonstration and, therefore, has not validated this in meso method as being more physiologically relevant. This is not to say that the protocol will not work for other small membrane proteins. For gA, a wealth of structural and electrophysiological data clearly shows that the DS structure is not the native membrane conformer.

Frances Separovic,[†] J. Antoinette Killian,[‡]
Myriam Cotten,[§] David D. Busath,[¶]
and Timothy A. Cross[‖*]
[†]*School of Chemistry, Bio21 Institute, University of Melbourne, Melbourne, Australia;*
[‡]*Institute of Biomembranes, Utrecht University, Utrecht, The Netherlands;*

Submitted Oct 12, 2010, and accepted for publication February 25, 2011.

*Correspondence: cross@magnet.fsu.edu

0006-3495/11/04/2073/2 $2.00

doi: 10.1016/j.bpj.2011.02.058

[§]*Department of Chemistry, Hamilton College, Clinton, New York;*
[¶]*Department of Physiology and Developmental Biology, Brigham Young University, Provo, Utah;*
[‖]*Department of Chemistry and Biochemistry, Institute of Molecular Biophysics & National High Magnetic Field Lab, Florida State University, Tallahassee, Florida*

REFERENCES

1. Höfer, N., D. Aragão, and M. Caffrey. 2010. Crystallizing transmembrane peptides in lipidic mesophases. *Biophys. J.* 99:L23–L25.
2. Caffrey, M. 2009. Crystallizing membrane proteins for structure determination: use of lipidic mesophases. *Annu. Rev. Biochem.* 38:29–51.
3. Burkhart, B. M., N. Li, …, W. L. Duax. 1998. The conducting form of gramicidin A is a right-handed double-stranded double helix. *Proc. Natl. Acad. Sci. USA.* 95:12950–12955.
4. Langs, D. A., G. D. Smith, …, M. Hospital. 1991. Monoclinic uncomplexed double-stranded, antiparallel, left-handed β 5.6-helix (increases decreases β 5.6) structure of gramicidin A: alternate patterns of helical association and deformation. *Proc. Natl. Acad. Sci. USA.* 88:5345–5349.
5. Langs, D. A. 1988. Three-dimensional structure at 0.86 Å of the uncomplexed form of the transmembrane ion channel peptide gramicidin A. *Science.* 241:188–191.
6. Pascal, S. M., and T. A. Cross. 1993. High-resolution structure and dynamic implications for a double-helical gramicidin A conformer. *J. Biomol. NMR.* 3:495–513.
7. Bystrov, V. F., and A. S. Arseniev. 1988. Diversity of the Gramicidin A spatial structure: two dimensional ^{1}H NMR study in solution. *Tetrahedron.* 44:925–940.
8. Arseniev, A. S., I. L. Barsukov, …, Ovchinnikov YuA. 1985. ^{1}H-NMR study of gramicidin A transmembrane ion channel. Head-to-head right-handed, single-stranded helices. *FEBS Lett.* 186:168–174.
9. Ketchem, R. R., W. Hu, and T. A. Cross. 1993. High-resolution conformation of gramicidin A in a lipid bilayer by solid-state NMR. *Science.* 261:1457–1460.
10. Ketchem, R. R., B. Roux, and T. A. Cross. 1997. High-resolution polypeptide structure in a lamellar phase lipid environment from solid state NMR derived orientational constraints. *Structure.* 5:1655–1669.
11. Cross, T. A., A. Arseniev, …, B. A. Wallace. 1999. Gramicidin channel controversy—revisited. *Nat. Struct. Biol.* 6:610–611, discussion 611–612.
12. Andersen, O. S., H. -J. Apell, …, A. Woolley. 1999. Gramicidin channel controversy—the structure in a lipid environment. *Nat. Struct. Biol.* 6:609–612.
13. Yau, W. -M., W. C. Wimley, …, S. H. White. 1998. The preference of tryptophan for membrane interfaces. *Biochemistry.* 37:14713–14718.
14. White, S. H., and G. von Heijne. 2005. Transmembrane helices before, during, and after insertion. *Curr. Opin. Struct. Biol.* 15:378–386.
15. Killian, J. A., and G. von Heijne. 2000. How proteins adapt to a membrane-water interface. *Trends Biochem. Sci.* 25:429–434.
16. Cornell, B. A., F. Separovic, …, R. Smith. 1988. Conformation and orientation of gramicidin A in oriented phospholipid bilayers measured by solid state carbon-13 NMR. *Biophys. J.* 53:67–76.
17. Separovic, F., J. Gehrmann, …, R. Smith. 1994. Sodium ion binding in the gramicidin A channel. Solid-state NMR studies of the tryptophan residues. *Biophys. J.* 67:1495–1500.
18. Cotten, M., F. Xu, and T. A. Cross. 1997. Protein stability and conformational rearrangements in lipid bilayers: linear gramicidin, a model system. *Biophys. J.* 73:614–623.
19. Arumugam, S., S. Pascal, …, T. A. Cross. 1996. Conformational trapping in a membrane environment: a regulatory mechanism for protein activity? *Proc. Natl. Acad. Sci. USA.* 93:5872–5876.
20. Killian, J. A., K. U. Prasad, …, D. W. Urry. 1988. The membrane as an environment of minimal interconversion. A circular dichroism study on the solvent dependence of the conformational behavior of gramicidin in diacylphosphatidylcholine model membranes. *Biochemistry.* 27:4848–4855.
21. Cotten, M., R. Fu, and T. A. Cross. 1999. Solid-state NMR and hydrogen-deuterium exchange in a bilayer-solubilized peptide: structural and mechanistic implications. *Biophys. J.* 76:1179–1189.