New and Improved Features of the Lipid Cubic Phase (*In Meso*) Method for Crystallizing Membrane and Soluble Proteins and Complexes

Martin Caffrey

Membrane Structural & Functional Biology Group, School of Medicine and School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin 2. Ireland

The lipid cubic phase (*in meso*) method for crystallizing membrane proteins has been in use now for two decades (1). In part because the cubic mesophase in which crystals grow is extremely viscous and sticky, the method in its infancy was adopted with great hesitancy and only by the truly adventurous. As the structures to emerge from the method grew in number and profile, so too did interest in the method (2). This was due in no small measure to advances in making the method more efficacious, versatile, user-friendly and high-throughput. Advances continue to be made and three of the more recent ones will be summarized in this presentation. The first addresses the many challenges faced by needing to open the glass sandwich plate used for crystallization in order to harvest crystals. This led to the *in meso in situ* serial crystallography (IMISX) method which enables data collection in the mesophase where and as crystals grow without the need to directly harvest crystals (3). It is a simple, robot-compatible method that requires miniscule (ng – single digit μg) amounts of protein and can be used for data collection at room and at cryo temperatures. A distinctly different '*in situ*' approach has proven successful in the area of serial femtosecond crystallography. In this case, crystals grown in the cubic mesophase are extruded across a micrometer-sized free-electron laser X-ray beam for data collection at ambient temperature, again without the need for direct crystal harvesting (4). The third advance refers to unstable proteins that cannot be concentrated in functional form by standard methods to values required for crystallization. The new Cubicon method solves this problem by using the cubic mesophase itself as a medium in which to concentrate the membrane protein through sequential rounds of reconstitution for direct use in *in meso* crystallization trials.

These few examples bear witness to the fact that the *in meso* method, whilst mature and robust, continues to evolve and to improve. It has a bright future and not only in its application to membrane proteins. Increasing attention is being paid to the method and to the mesophase itself for use with soluble proteins (2).

Supported, in part, by a grant from Science Foundation Ireland (12/IA/1255).

1. Landau, E.M., Rosenbusch, J.P. 1996. Lipidic cubic phases: a novel concept for the crystallization of membrane proteins. Proc Natl Acad Sci U S A. 93:14532-5.

2. Caffrey. M. 2015. A comprehensive review of the lipid cubic phase or *in meso* method for crystallizing membrane and soluble proteins and complexes. Acta Cryst. F71, 3-18.

3. Huang C.-Y., Olieric V., Ma P., Howe N., Vogeley L., Liu X., Warshamanage R., Weinert T., Panepucci E., Kobilka B., Diederichs K., Wang M., Caffrey M. 2016. *In meso in situ* serial X-ray crystallography of soluble and membrane proteins at cryogenic temperatures. Acta Cryst. D72, 93-112.

4. Liu W, Wacker D, Gati C, Han GW, James D, Wang D, Nelson G, Weierstall U, Katritch V, Barty A, Zatsepin NA, Li D, Messerschmidt M, Boutet S, Williams GJ, Koglin JE, Seibert MM, Wang C, Shah ST, Basu S, Fromme R, Kupitz C, Rendek KN, Grotjohann I, Fromme P, Kirian RA, Beyerlein KR, White TA, Chapman HN, Caffrey M, Spence JC, Stevens RC, Cherezov V. 2013. Serial femtosecond crystallography of G protein-coupled receptors. Science 342:1521-4.