

# A Laboratory Course on Crystallizing Membrane and Soluble Proteins and Complexes by the Lipid Cubic Phase (*In Meso*) Method

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The lipid cubic phase or *in meso* method is, by now, a proven and robust method for crystallizing membrane proteins. It also works with soluble proteins. Already, it is responsible for over 300 entries in the Protein Data Bank, some corresponding to high profile targets such as the  $\beta_2$ -adrenoreceptor-G protein and the rhodopsin-arrestin complexes (1-3). Because crystallization takes place in a bicontinuous mesophase, the method in its execution requires manual dexterity to properly prepare, dispense, screen and harvest from this characteristically sticky and viscous hosting liquid crystal. Over the past decade, we have been active in running workshops worldwide with a view to passing along this specialized technique. In support of this outreach activity, several papers that include instructional videos have been made available online and open-access (4). Recently, the method was included as part of a laboratory module in a third year undergraduate biochemistry course at Trinity College Dublin. Given that the method works with soluble proteins, for reasons of ease and cost, lysozyme was used as the test protein following a protocol specifically designed to produce recognizable crystals *in meso* within an hour at room temperature (5). Eighteen students successfully completed the module that came in three parts. The first involved preparing the protein solution, forming and dispensing the protein-laden mesophase, setting up screens and monitoring crystal growth. A one-hour lecture on the theory and practice of the method was given the week preceding the laboratory exercise and students were advised to view the online video that describes the method well in advance of the lab. The second part began with a one-hour lecture on principles and practices of macromolecular crystallography. This was followed by laboratory work that involved evaluating the crystallization screening results, recording crystal characteristics (size, shape, density, distribution), discussing protein solubility and lipid/water temperature-composition phase diagrams, and viewing *in meso* robots, an imager/incubator and a rotating anode X-ray diffractometer in action. The final part consisted of a computer lab where the students were introduced to and trained in the rudiments of molecular modelling/graphics using Coot and PyMol. Because the students enrolled in the course were specializing in Molecular Medicine, insulin was used for the computer lab work. In this presentation, our experiences teaching the course, how it might be improved, and feedback from the students will be described.

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