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Membrane protein crystallization from lipidic phases

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Membrane protein structural biology is enjoying a steady acceleration in the rate of success. Nevertheless, numerous membrane protein targets are resistant to the traditional approach of directly crystallizing detergent solubilized and purified protein and the 'niche market' of lipidic phase crystallization is emerging as a powerful complement. These approaches, including lipidic cubic phase, lipidic sponge phase, and bicelle crystallization methods, all immerse purified membrane protein within a lipid rich matrix before crystallization. This environment is hypothesized to contribute to the protein's long-term structural stability and thereby favor crystallization. Spectacular recent successes include the high-resolution structures of the β_2-adrenergic G-protein-coupled receptor, the A_{2A} adenosine G-protein-coupled receptor, and the mitochondrial voltage dependent anion channel. In combination with technical innovations aiming to popularize these methods, lipidic phase crystallization approaches can be expected to deliver an increasing scientific impact as the field develops.

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Introduction

Membrane proteins are fickle entities and repeatedly resist even the most determined efforts to overproduce, purify, and crystallize them for structural studies. Despite this, the field of membrane protein structural biology is undergoing a period of rapid expansion with accelerating rates of successful structural determination, in part due to the incorporation of technical innovations developed for high-throughput structural biology [1]. Detergent based crystallization protocols, whereby detergent solubilized and purified membrane protein is crystallized using the vapor-diffusion method essentially as if it were a soluble protein, are by far the most popular and successful approach developed to date [2•]. Despite this, it has almost become a truism that the improvement of initial membrane protein crystals is painstakingly slow and frequently the quality of crystals can plateau at a point unsuitable for structural determination. In other cases no crystal leads whatsoever emerge despite success in recovering pure, stable protein. When faced with such disappointments, it is attractive to explore other crystallization protocols. A celebrated approach to improve the chances of recovering high quality crystals is to enhance the crystal contacts by enlarging the soluble domains through the addition of proteins specific antibodies [3], although this typically falls outside the capabilities of most laboratories. A variation of this theme is to increase the membrane protein's soluble domains by protein engineering [4••] but this may potentially introduce other complications.

Another approach, the crystallization of membrane proteins in lipidic environments, explicitly recognizes and addresses the fact that membrane proteins are most stable in lipid bilayers. Detergent solubilization, the extraction of membrane proteins from their native membrane, often causes structural lipids and weakly bound subunits to be lost and potentially impairs protein integrity. As a consequence, membrane proteins frequently display reduced activity and poor stability in detergent solution and this role of lipids in membrane protein structural biology has been extensively discussed [5]. Alternatively, as originally conceived and demonstrated by Landau and Rosenbusch [6], purified membrane proteins can be reconstituted into lipidic bilayer environments before crystallization. By reintroducing proteins into a lipidic bilayer it was hypothesized that enhanced protein stability would be achieved, thus aiding the crystallization process.

In this review we focus on recent progress using lipidic phase environments as vehicles aiding membrane protein crystallization. We first sketch the underlying ideas of lipidic phases and describe their application to the methods of lipidic cubic phase (LCP) crystallization [6], its offspring lipidic sponge phase (LSP) crystallization [7], and the conceptually related approach of bicelle crystallization [8]. We emphasize the recent structural results to emerge using each of these methods and highlight efforts to increase their popularity. In closing we speculate upon the future role likely to be played by lipidic phase crystallization protocols within the broader discipline of membrane protein structural biology.

Lipidic phases

Amphipathic lipid molecules consist of both hydrophobic and hydrophilic moieties. When exposed to aqueous environments they spontaneously form well-defined structures so as to maximize energetically favorable interactions of the hydrophilic head group with water and to minimize the exposure of hydrophobic tail group. The lipidic phases that emerge from the optimal enthalpy/entropy balance depend upon the specific geometry of the lipid itself, its concentration, its temperature, as well as the presence of other additives including ions and amphiphiles. The spontaneous formation of detergent micelles and unilamellar vesicles are used daily by membrane protein biochemists, but other less familiar lipidic aggregates are also easily accessed including reverse micelles and various other lipidic bilayer structures.

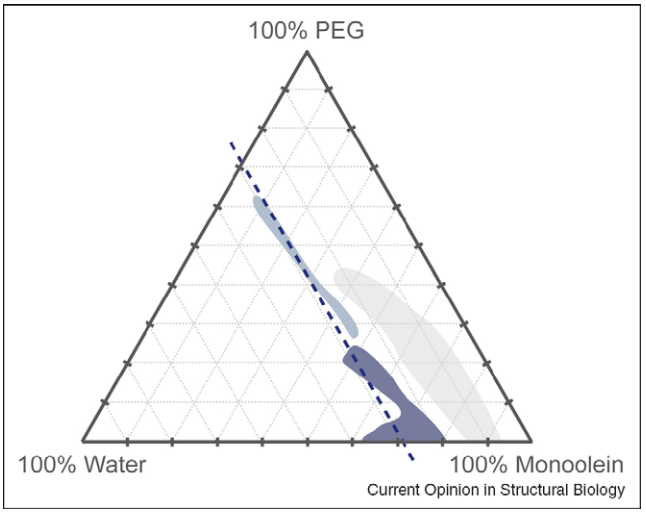
Lipidic bilayer phases encompass vesicles, lamellar phases (stacked bilayers) as well as the bicontinuous cubic and sponge phases, which can be traversed in any direction along either hydrophilic or hydophobic paths. These mesophases [9] are more ordered than a liquid but less ordered than a solid. LCPs are semi-solid in texture and their long-range order can be visualized using small-angle X-ray scattering which reveals scattering peaks corresponding to the characteristic lattice parameters of their cubic packing [7]. Sponge phases, by comparison, can be thought of as swollen cubic phases with aqueous pores up to three times larger than those of a cubic phase [10] and lower long-range order. Another characteristic distinguishing the lipidic cubic and sponge phases is that the latter are liquid in nature. Lamellar phases also have low viscosity, but they are anisotropic and are therefore birefringent, whereas the isotropic cubic and sponge phases are non-birefringent. Thus cross-polarized microscopy provides a useful and rapid diagnostic distinguishing the lamellar and sponge phases.

Bicelles can be thought of as solubilized lipidic bilayer disks. They are formed by the addition of detergent (or a short-chain lipid) to a long-chain lipid [8,11]. These mixtures spontaneously form disc-shaped aggregates of lipids and detergents, with the long-chain phospholipid forming a central planar bilayer which is surrounded by a rim of detergents that protect the bilayer from water. The physical diameter of the bicelle is controlled by the ratio of long-chain to short-chain amphiphiles. Moreover, membrane proteins reconstituted into bicelles maintain functionality [12].

Lipidic cubic phase crystallization

Monoolein has been the lipid of choice for membrane protein crystallization from LCPs [6], although some successes have also been reported using closely related lipids such as monovaccenin [13]. When mixed with water, monoolein spontaneously swells to form several

Figure 1



Phase diagram of the PEG:monoolein:water system illustrating the presence of LCP (dark blue), LSP (light blue) and lamellar (gray) phases. Sponge phases do not form in the absence of PEG (triangle base) but require approximately 30% water content (dashed line). The PEG (25–60%) and monoolein (10–45%) concentrations can vary over a broader range [10]. Phase diagrams recovered from PEGs of variable length are rather similar.

mesophases. At room temperature the *Pn*3*m* cubic phase is recovered at approximately 40% water concentration with the (larger cell) *Ia*3*d* cubic phase emerging at slightly lower water content [9]. The traditional starting point for any LCP crystallization experiment has been to incorporate membrane protein into either of these semi-solid cubic phases by mixing a buffer containing purified protein with monoolein in an approximate ratio of 2:3 by volume. As with any other batch crystallization experiment, this protein-containing LCP is then dispensed into tubes [6] or crystallization plates [14], overlaid with a crystallization agent and sealed. Dehydration of the cubic phase drives a phase-transition to a lamellar phase (Figure 1) and it is believed that this phase-transition is a key ingredient in successfully recovering crystals, a hypothesis which receives support from the observation of birefringence in the immediate vicinity of crystals grown in the LCP [15].

Bacteriorhodopsin was the first membrane protein to be successfully crystallized using LCP crystallization [6] and improvements in the crystal quality shortly later yielded high-resolution crystal structures of bacteriorhodopsin in its resting conformation [16–18]. LCP crystals of bacteriorhodopsin grow as stacked layers of 2D crystals and are functionally active, such that these crystals continue to diffract after illumination. Thus several conformational changes associated with the photo-cycle of bacteriorhodopsin have been convincingly demonstrated using light illuminated LCP crystals [19–23] and have contributed to