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Development of a lipidic-sponge phase screen for membrane protein crystallization

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Currently, the major deficit in structural biology is a lack of high-resolution structures of mammalian membrane proteins, many of which are key drug targets for the treatment of human disease. Numerous membrane proteins, particularly those of mammalian origin, require specific lipids for their stability and activity. To address this issue we have developed a sparse matrix crystallization screen consisting of 48 different lipidic-sponge phase conditions. Sponge phases consist of lipid bilayers with intersecting water channels. The water channels allow membrane proteins with large aqueous domains to be incorporated with their hydrophobic domains reconstituted into the membrane, mimicking their native environment and thus facilitating crystallization. The sponge phases are liquid at room temperature and the most obvious practical advantage of this approach is that they can be used directly in vapour diffusion experiments. This liquid property is also compatible with crystal drop dispensing using crystallization robots and greatly facilitates the mounting of protein crystals in nylon loops. Furthermore it allows optimization using additives as well as other techniques such as seeding. The sponge phase screen was designed to contain different solvents, salts and pH to accommodate the requirements of many membrane proteins. In some cases, other lipids such as cholesterol were incorporated into the phases to provide extra stability for the proteins. This approach yielded crystals of the photosynthetic core complex of Blastochloris viridis. The screen's effectiveness was further proven by crystallization experiments using protein from other bacteria as well as from higher plants. Crystals were obtained for 8 out of 12 proteins and are currently undergoing optimization.

Keywords: membrane protein crystallization, lipidic-sponge phase, macromolecular crystal growth

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Crystal growth of multicopper oxidase CueO Δ α 5-7 mutant

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CueO is a multicopper oxidase (MCO) involved in Cu-homeostasis of *E.coli.*. CueO has the four catalytic copper binding sites, a type 1 Cu, a type 2 and a pair of type 3 Cu's, in a single chain protein molecule consisting of 484 amino acids. CueO has fifth Cu-binding site as the substrate-binding site, which is isolated from bulk waters by the Met-rich α-helical segment. The high cuprous oxidase activity of CueO is realized by the presence of this fifth Cu-binding site. Furthermore, CueO exhibits enhanced oxidizing activities for organic substrates in the presence of Cu^{2+} ion at the fifth Cu-biding site. Nevertheless, it has been recently reported that CueO receives electrons directly from electrodes even in the absence of the fifth copper and oxygen reduction current is very large. Especially, a recombinant protein (Δα 5-7 mutant) of which the Met-rich α-helical segment was genetically removed has been considered to be an important candidate as a catalyst of the cathode in biofuel cell because the distance between electrodes and the type I Cu site becomes shorter. Although the structures of the CueO and Δα5-7 mutant have already been revealed by X-ray crystallography, it remains unclear whether the present structures were fully oxidized form or not. It is impossible to characterize the state of the obtained structure without knowing the protonation state of bridging oxygen or amino acid residues in the vicinity of the oxygen reduction site. Neutron diffraction experiment is an essential technique for observing those protonation states. We have succeeded in crystal growth of the Δα5-7 mutant in D_2O environment based on the obtained crystal phase diagram under H_2O environmental condition. So far, the size of which is 0.8mmx0.8mmx0.3mm, is obtained.

Keywords: MCO, biofuel cell, Δα5-7 mutant

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Influence of polytypism on polymorphism in n-alkanes: Cryatallization and thermodynamic stability

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Even-numbered n-alkanes exhibit a variety of crystalline phases depending on crystallization conditions and also thermal process. These crystal structures can be classified with two categories, polymorphism and polytypism, although the latter has not been fully taken into consideration in the polymorphic studies of n-alkanes. For the comprehensive understanding of the relationship between polymorphism and polytypism in n-alkanes, we have studied the condition that a specific polymorphic phase exhibits polytypism and also the influence of polytypism on thermodynamic stability and phase transition behavior by means of X-ray diffraction, IR, and inelastic neutron scattering. We followed the solution crystallization of the M011 phase of n-$C_{36}H_{74}$ using a micro-FTIR system. For this purpose, we developed an obliquely incident optical system, which makes it possible to identity the polytype of a growing single crystal. We found that a polytypic transformation takes place on a growing single crystal. At the initial stage, a single crystal of M011 appeared as the single-layer polytype. The overgrowth of the double-layer polytype occurred subsequently through heterogeneous nucleation on the (001) face of the single crystal. After that the single crystal gradually transformed to a complete single crystal of the double-layer polytype through a solution-mediated phase transition. The