Keywords: Membrane proteins, high-throughput, green fluorescent protein

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Methods to get high quality membrane protein crystals are in high demand and in a constant development. A preferred method of choice is to maintain low levels of detergent, near the Critical Micelle Concentration (CMC), to shield the hydrophobic regions throughout the purification and crystallization process. This approach typically results in ‘Type II’ crystals that are built up through interactions between the hydrophilic surfaces of the molecules [1]. In contrast, ‘Type I’ crystals (often described as stacked 2D crystals) [2, 3] are characterized by continuous bilayers, formed by a lipid/detergent saturated environment, in which the proteins are packed. We present an improved method to induce growth of bilayer membrane protein crystals in high concentrations of lipids and detergent. The straightforward procedure includes a systematic screening approach for lipidation and crystallization, and subsequent improvement of diffraction properties of membrane protein crystals by optimization of added amounts of detergents and lipids.


Keywords: Membrane proteins, crystallization, bilayers, type I crystals

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Membrane transport proteins are classified into different groups. However, the common molecular mechanism of all of them is based on the alternating access model [1]. Mhp1 belongs to the nucleobase–cation–symport-1 family of secondary active transporters with indolyl methyl- and benzyl-hydantoin as substrates in M. liquefaciens. Two crystal structures of this protein were already solved and present the outward facing and occluded forms [2]. Recently, a crystal structure in a third conformation, inward facing, was solved and revealed detailed insights into the alternate access model. The structure was first solved by molecular replacement and later on by SAD and refined at 3.8 Å resolution to R=27.3% and Rfree=31.3.1% [3].

Mhp1 comprises a five-helix inverted repeat, a common motif among secondary transporters. This new crystal structure is complementing its previously described structures in outward-facing and occluded states. From analyses of the three structures and molecular dynamics simulations, a mechanism for the transport cycle in Mhp1 could be proposed. The switch from the outward- to the inward-facing state, to effect the inward release of sodium and benzylhydantoin, is primarily achieved by a rigid body movement of transmembrane helices 3, 4, 8, and 9 relative to the rest of the protein. This forms the basis of an alternating access mechanism applicable to many transporters of this emerging superfamily.


Keywords: Membrane transport; membrane protein structure; membrane protein X-ray structure determination