MS6-P6 The role of active humidity control in successful membrane protein crystallization with mosquito® Crystal and mosquito® LCP

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Membrane proteins are involved in a wide range of physiological functions and abnormalities in the structures of these proteins can lead to many known diseases such as heart disease, depression, cancer and many others. However, growing crystals of membrane proteins which are suitable for x-ray diffraction remains a challenge for crystallographers. The use of liquid handling robots such as TTP labtech's mosquito® Crystal and mosquito® LCP has increased throughput and repeatability allowing for many more conditions to be easily screened. They also offer the ability to accurately dispense nanolitre volumes of both protein and screen solutions, which saves valuable protein and reduces reagent costs. The mosquito LCP is capable of automating both microbatch and vapour diffusion methods of protein crystallography (sitting drop, hanging drop) as well as crystallisation of membrane proteins using the bicelle and the highly viscous lipidic cubic phase (LCP) methods. This can be achieved without instrument configuration changes and provides significant flexibility in the crystallisation workflow. This poster describes the features of mosquito Crystal and mosquito LCP, showing their ability to successfully overcome inherent issues in the automated set-up of membrane protein crystallisation screen trials. It also demonstrates the effective use of an active humidity chamber with mosquito LCP to crystalise a GPCR, the b_1-adrenoceptor (b_1AR). Interestingly, a 12% increased yield of crystals was observed when using the mosquito active humidity chamber compared to the same cubic-phase experiment set up in its absence. In summary, whilst mosquito LCP can rapidly set up a crystallisation screen, the use of TTP Labtech's active humidity chamber ensures there is minimal evaporation of the LCP drops which ultimately yields significant increases in both reproducibility and success rate.

Keywords: membrane protein, humidity control, crystallisation drop set-up

MS6-P7 Structural and functional characterization of Ybr137wp implicate its involvement in the targeting of tail-anchored proteins to membranes

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Nearly 5% of membrane proteins are guided to nuclear, endoplasmic reticulum, mitochondrial, Golgi, or peroxisome membranes by their C-terminal transmembrane domain and are classified as tail-anchored (TA) membrane proteins. In *Saccharomyces cerevisiae,* the Guided Entry of TA-protein (GET) pathway has been shown to function in delivery of TA proteins to the ER. The sorting complex for this pathway is comprised of Sgt2, Get4, and Get5 and facilitates the loading of nascent tail-anchored proteins onto the Get3 ATPase. Multiple pull-down assays also indicated that Ybr137wp associates with this complex *in vivo*. Herein, we report a 2.8-Å resolution crystal structure for Ybr137wp from *Saccharomyces cerevisiae*. The protein is a decamer in the crystal and also in solution as observed by size-exclusion chromatography and analytical ultracentrifugation. In addition, isothermal titration calorimetry indicated that the C-terminal acidic motif of Ybr137wp interacts with the tetratricopeptide repeat (TPR) domain of Sgt2. Moreover, an *in vivo* study demonstrated that Ybr137wp is induced in yeast exiting the log-phase culture and ameliorates the defect of TA-protein delivery and cell viability derived by the impaired GET system under starvation condition. Therefore, this study might suggest a possible role for Ybr137wp related to targeting of tail-anchored proteins.

Keywords: endoplasmic reticulum, tail-anchored membrane proteins, Ybr137wp