Membrane protein production & structural biology capabilities at Evotec

Executive Summary
Evotec is a recognised leader in the field of HTS, combinatorial and medicinal chemistry and structural biology services to the pharmaceutical industry. An extensive range of capabilities in structural biology have been integrated into the company workflow to facilitate rapid delivery of structural data in support of drug discovery activities. Evotec appreciates the growing need for elucidation of membrane protein structures. Current membrane protein targets include GPCRs, channels and porins. The work is offered as an integrated package or as independent modules for clients and for internal R&D activities. A platform for membrane protein structural biology has been established using GPCRs as a proof of concept. The workflow is illustrated below and outlines incorporation of key methodologies.

Bioc hemical and Biophysical studies
Successful membrane protein production platforms rely on a systematic screening and validation methodology at every stage of the process. It is important to rapidly identify robust constructs and stabilising buffer formulations from a large array of conditions. Classical radio-ligand binding studies help understand the interaction of protein with the ligand and are a gold standard for SAR studies. Surface plasmon resonance and micro scale thermophoresis are emerging as complementary techniques to understand ligand-binding events. Determination of thermostability of protein in different conditions is a valuable method for screening reagents or targets amenable to crystallisation.

Optimised process to produce crystal grade membrane protein
A dedicated facility with high capacity for large-scale expression in insect, mammalian, yeast and bacterial cells is available. Membrane fractions for GPCRs are routinely prepared through repeated homogenisation and high salt washes. Nitrogen decompression membrane preparation and differential high-speed centrifugation is also available. The protein production facility includes 12 ÅKTA FPLC systems including 6 Xpress systems allowing development of quick and reproducible purification protocols. LCP, bicelle and vapour diffusion crystallisation are routinely performed for internal and client projects. Minstar UV imager available at 4 °C & 20 °C allows for efficient inspection and management of crystal hits. Proximity and bioxenic slots at Diamond, UK allows quick screening and data collection.

Conclusion
Evotec is well-equipped to provide membrane protein production and structural biology activities as a service. Over the past few years Evotec has seen increased client demand for membrane proteins including pores, receptors or channels for biophysics and crystallography. Following this trend, a robust platform has been developed to rapidly triage constructs and deliver high quality proteins for multiple projects. Evotec has acquired expertise in screening of targets, reagent production and structural characterisation and offers integrated and stand-alone projects to meet client requirements. Our flexible and modular approach sets us apart from our competitors.