

Membrane protein production & structural biology capabilities at Evotec

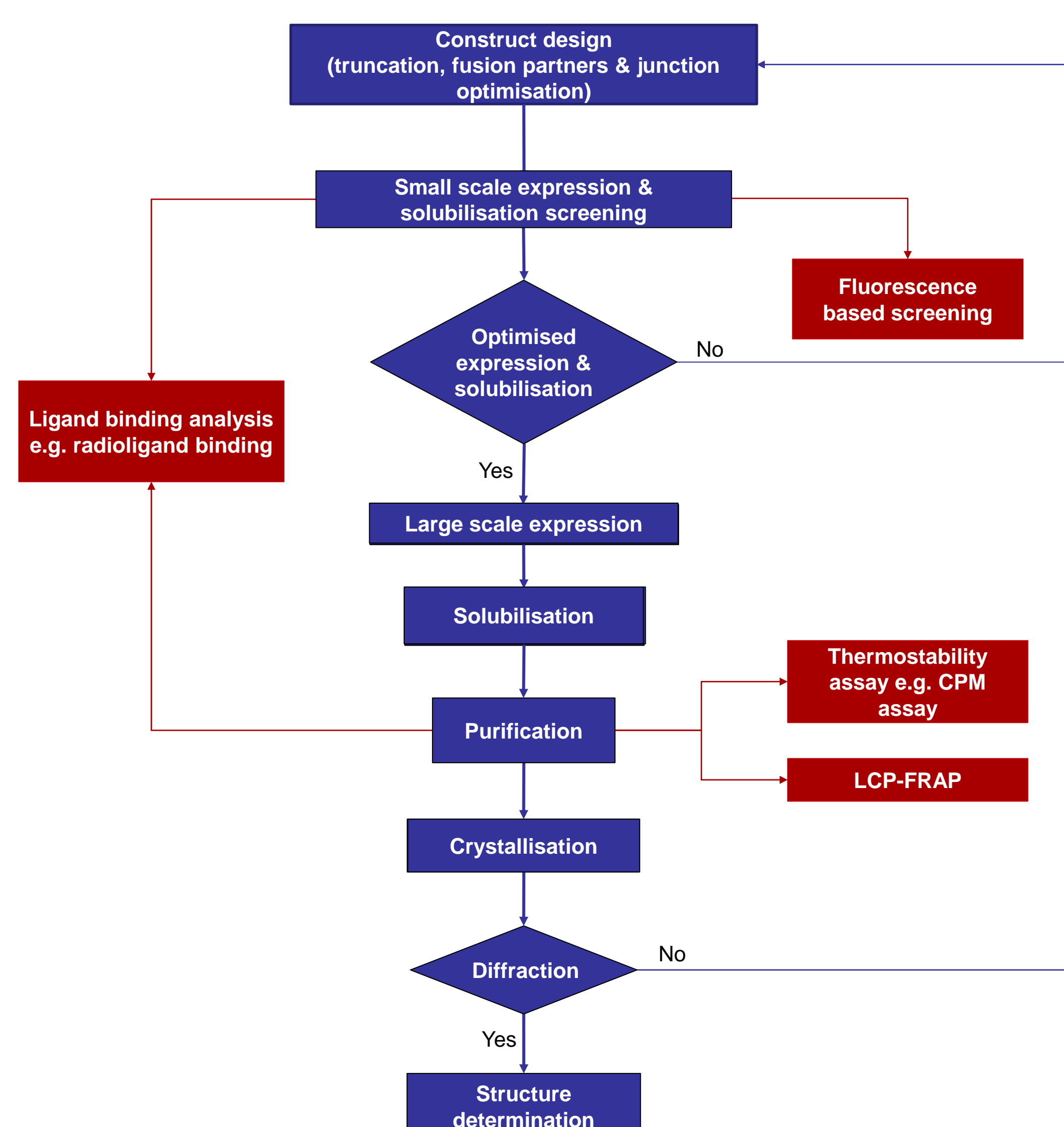


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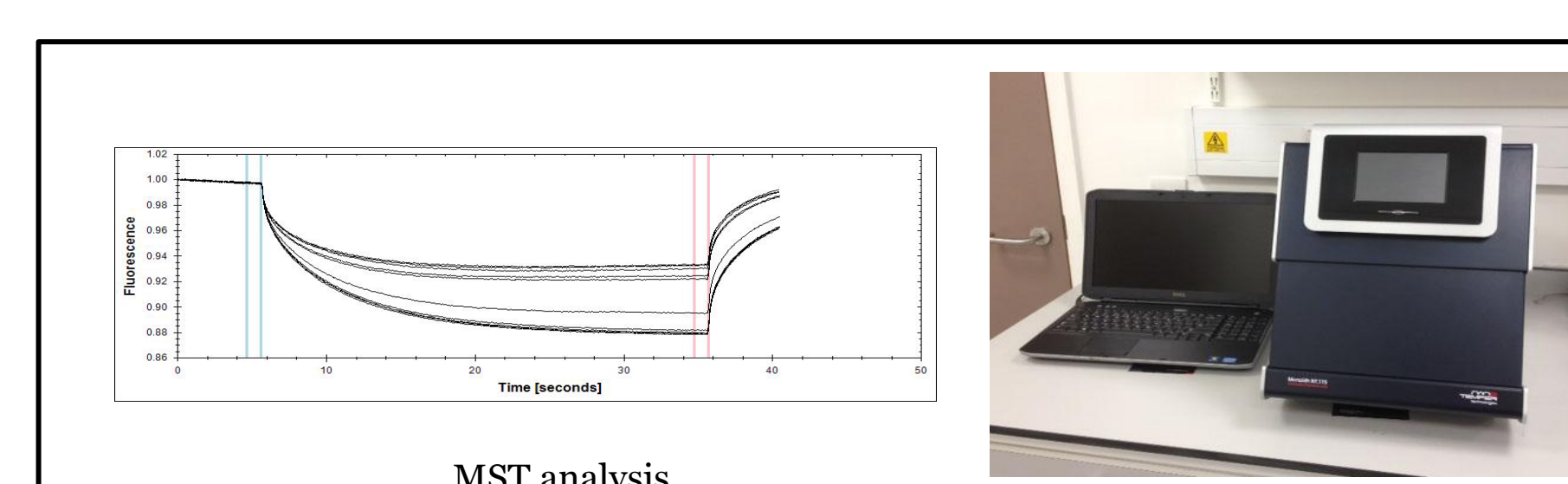
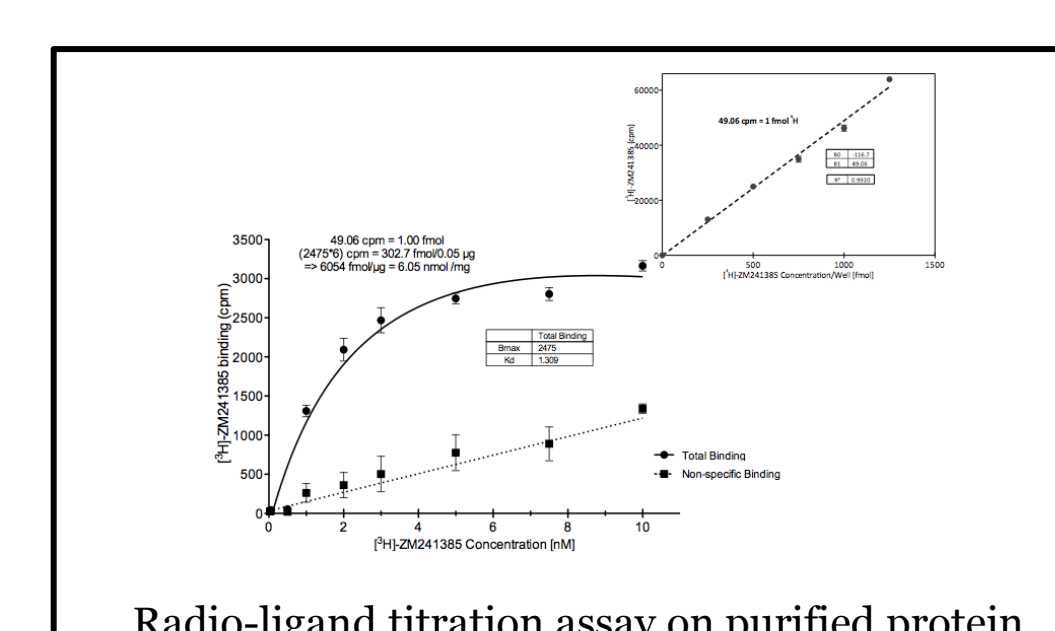
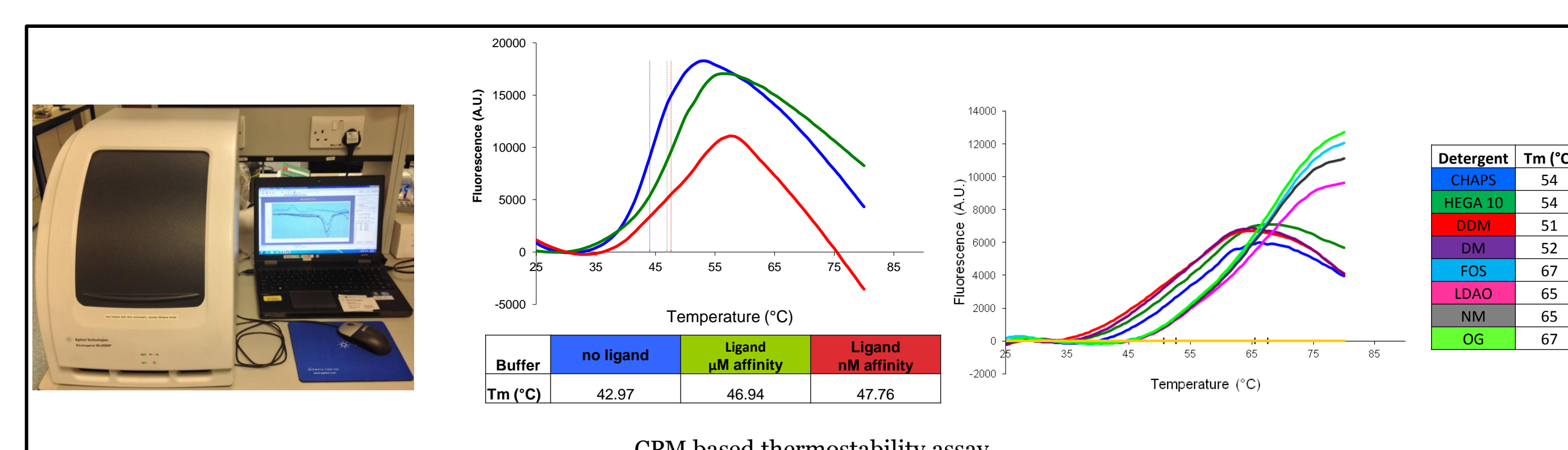
Executive Summary

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 within 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 module 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.



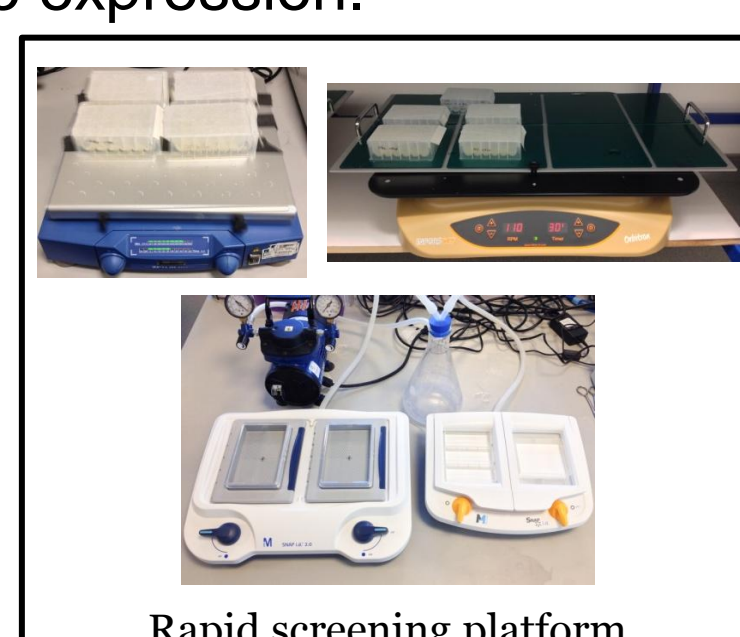
Biochemical and Biophysical studies

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.

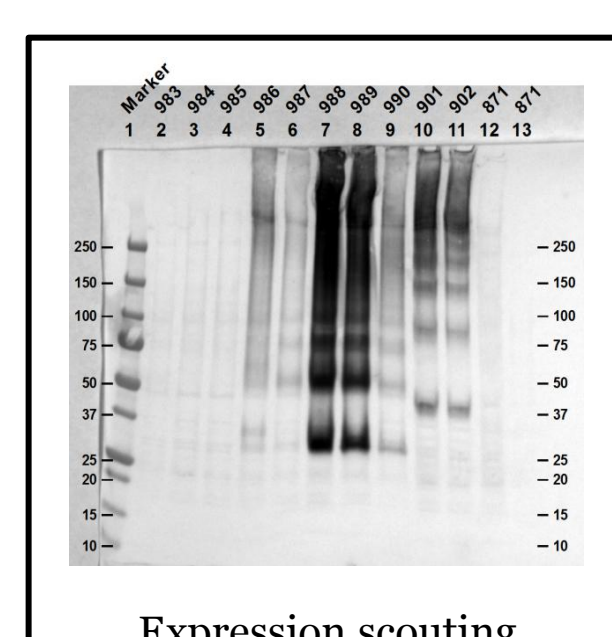


High-throughput small-scale protein production

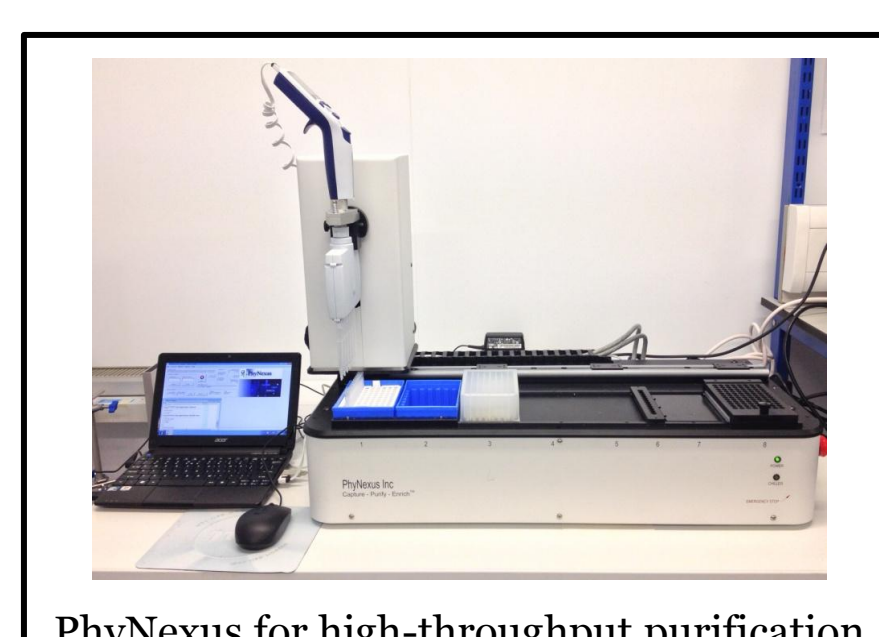
Evotec routinely runs campaigns for expression and purification of multiple targets in mammalian and insect cells. Virus generation and expression in insect cells is carried out in a 24-well block. Total expression and solubilised protein yields are determined through SDS-PAGE and densitometry. Time and reagent requirements are significantly reduced by designing a workflow around semi-automated devices such as iBlot™ for protein transfer and SNAP ID™ for immunodetection. PhyNexus™ robot allows isolation of pure, concentrated sample from small-scale expression.



Rapid screening platform



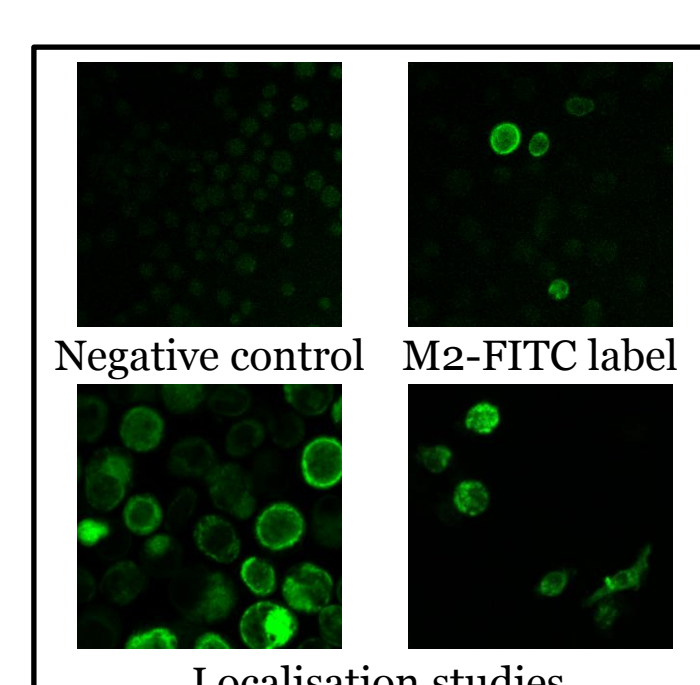
Expression scouting



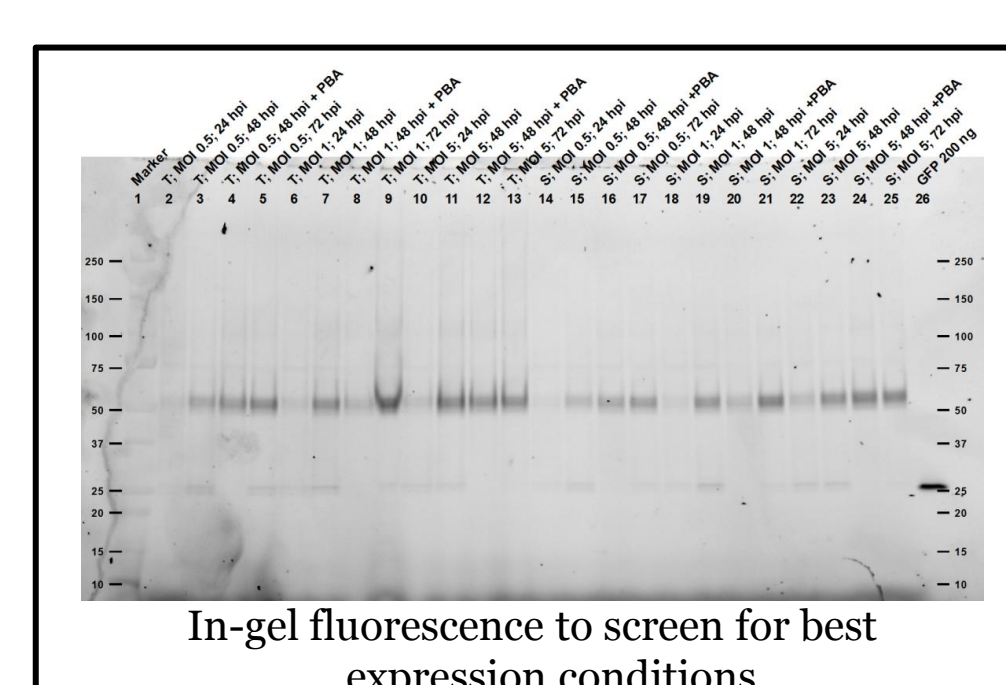
PhyNexus for high-throughput purification

Fluorescence based screening

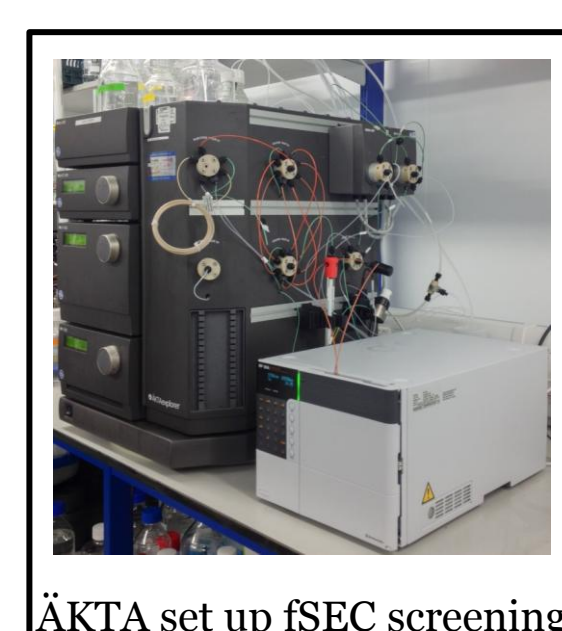
A screening platform is available to identify optimal protein constructs, detergents and buffer formulation for solubilisation and purification using fluorescence as a marker. The workflow is exemplified by but not limited to localisation studies in whole cells, protein profile on SDS-PAGE, SEC to determine yield and oligomeric state of protein in test conditions. The protein can be specifically probed with fluorophores interacting with Flag tag, His tag or target specific antibody or fluorescently labelled ligand. I.P. free fluorescent proteins are being assessed to allow expression of fusion proteins. A modified ÄKTA Explorer allows automated sequential analysis of up to thirty different samples per week.



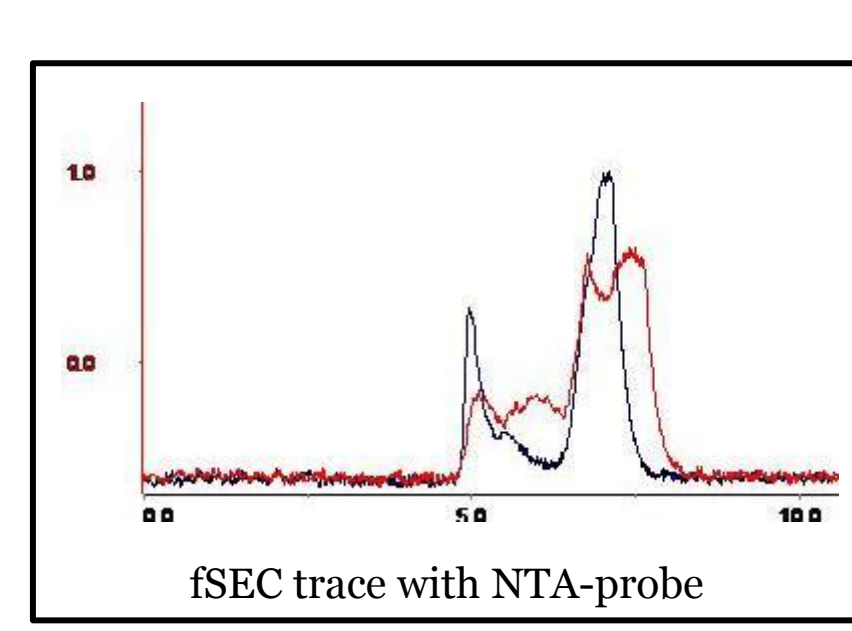
Localisation studies



In-gel fluorescence to screen for best expression conditions



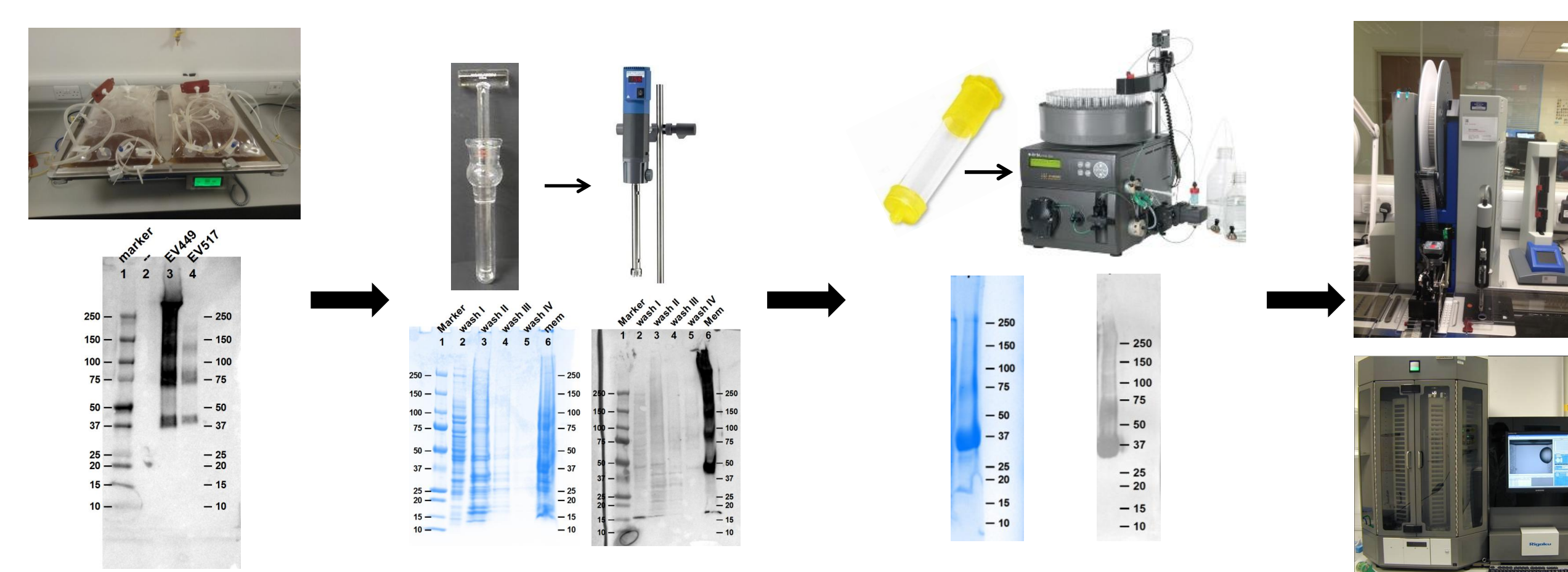
ÄKTA set up for SEC screening



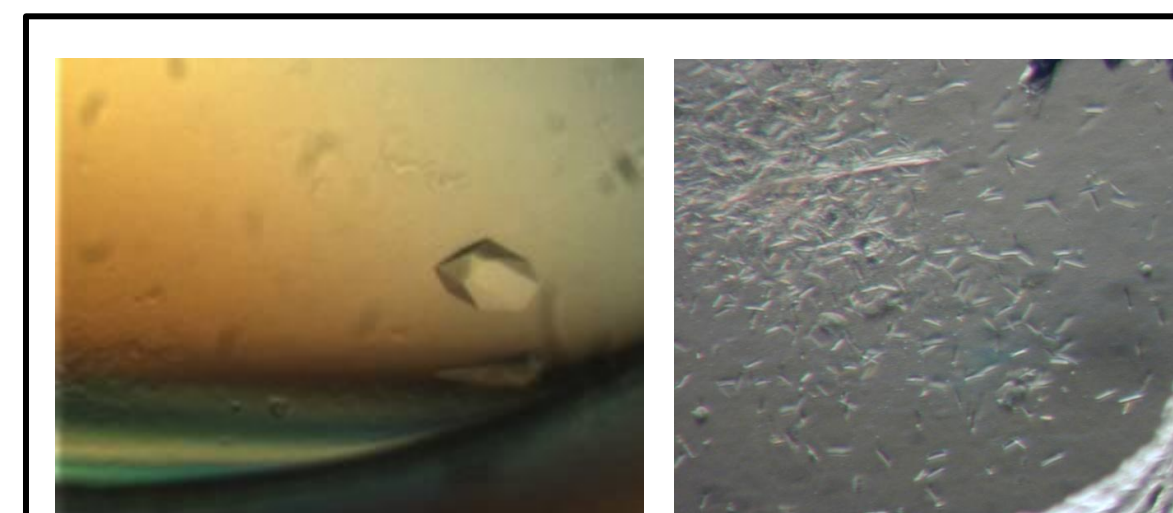
FSEC trace with NTA-probe

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 for membrane preparation and differential high-speed centrifugation is also available. The protein production facility includes 12 ÄKTA 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. Minstrel UV imager available at 4 °C & 20 °C allows for efficient inspection and management of crystal hits. Proximity and biweekly slots at Diamond, UK allows quick screening and data collection.



Membrane protein production and crystallisation workflow



Membrane protein crystals



Crystals under white & UV light

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.