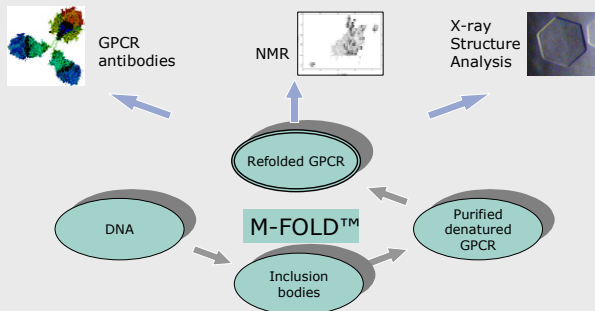


Introduction

M-fold Biotech's proprietary platform, M-FOLD™, allows to produce purified membrane proteins on a proteome-wide scale. This opens up new opportunities in drug discovery with regard to GPCR and ion channel targets. Currently, the main areas of focus are the generation of GPCR antibodies for diagnosis and therapy, the use of GPCRs and ion channels in structure analysis by X-ray crystallography and NMR and the development of novel assay formats based on purified membrane proteins.

M-FOLD™ is protected by several patents in Europe, the USA and Japan.



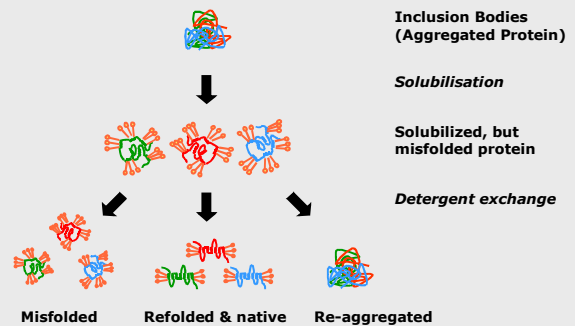
M-fold Biotech' technology platform M-FOLD™: source of purified membrane proteins in multi-milligram amounts.

How does M-FOLD™ work?

Membrane proteins are expressed in *E. coli* with N-terminal tags to enhance expression levels. The protein is deposited in intracellular inclusion bodies. Typically, 5-50 mg of purified recombinant protein can be obtained from one litre of cell culture. Multiple targets or multiple variants of a given membrane protein can be easily produced in parallel. To date, more than 200 different GPCRs and ion channels, not counting variants, have been over-produced and purified successfully.

M-fold Biotech's proprietary M-FOLD™ technology uses a harsh, denaturing detergent to solubilize the inclusion body protein. After proteolytic removal of the tag, the protein is purified under denaturing conditions. Refolding is induced by replacing the harsh detergent with a mixture of mild detergent and phospholipid.

The refolding conditions must be carefully optimized for each new protein by systematically varying parameters such as pH, salt concentration, additives and the type of detergent and lipid used.

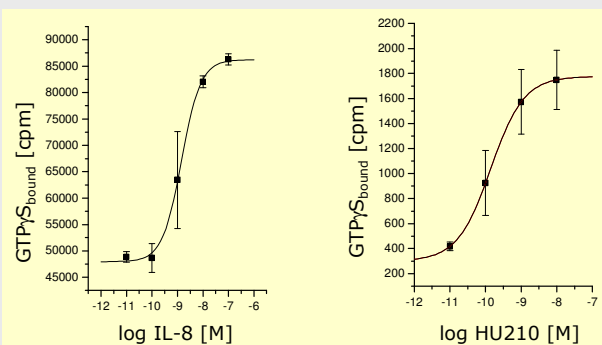


M-FOLD™ process: Inclusion body protein is solubilized in a strong detergent followed by exchange with a mild detergent that induces refolding. Multiple experiments are required to identify refolding conditions.

Identifying refolding conditions

Optimization requires a method able to quantify the amount of refolded protein in each experiment so that the influence of the parameter settings on the refolding yield can be modelled and predicted. In the case of GPCRs this is achieved by ligand binding: The B_{max} allows to calculate the efficiency of refolding while the K_D is indicative of the native conformation of the refolded GPCR.

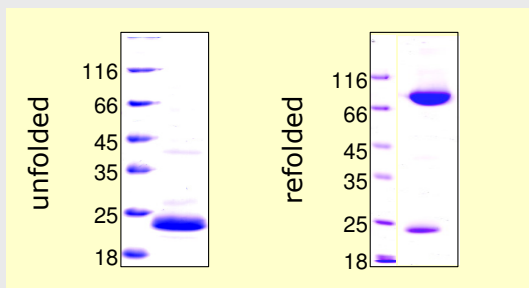
In several cases, the refolded GPCR was reconstituted with the cognate G protein and ligand-dependent G protein activation was demonstrated.



G protein activation of refolded and reconstituted chemokine receptor CXCR1 (left) and cannabinoid receptor CB1 (right). The results show that the GPCRs not only bind ligand, but also activate G proteins in a ligand-dependent manner.

For ion channels, the amount of refolded protein is measured in a different way: Active channels form tetramers that can be detected by appropriate

electrophoresis systems. The amount of tetramer is quantified and used to optimize refolding conditions.



Tetramer formation of a potassium channel is detected by electrophoresis.

A Generic Technology

The success rate of M-FOLD™ has been estimated from a large-scale experiment where 150 different GPCRs were expressed and a selected set of 40 subjected to refolding. Expression reached levels exceeding 1 mg of purified GPCR per litre of cell culture in 95% of the cases. Refolding, as confirmed by functional analysis, could be achieved for 80% of the GPCRs attempted, yielding an overall success rate of 76%. Similar results were obtained for ion channels. It is likely that in the future, more extensive screening will further increase the probability of success, making M-FOLD™ a generic technology for membrane protein production.

Applications

GPCR antibodies

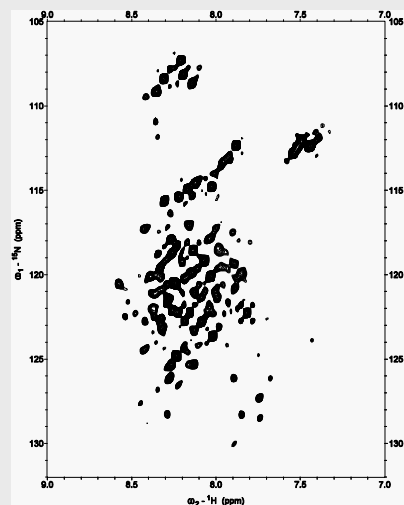
Refolded GPCRs have been used in collaboration with partner companies to generate monoclonal antibodies both by immunization and phage display technology. The resulting antibodies recognize native GPCRs on the cell surface with high specificity and nano-molar affinity. Therapeutic and diagnostic potential is currently being evaluated. Cf. "Fact sheet GPCR antibodies"

NMR spectroscopy

The efficient expression system allows to introduce isotope labels in recombinant membrane proteins at affordable cost.

The example below shows a spectrum of a uniformly labelled GPCR. Another collaborative project with Stan Opella's group at UCSD aimed at determining GPCR structures by solid-state NMR has recently been awarded with a major NIH grant. Details are published in a UCSD press release *).

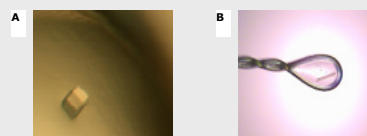
*) <http://ucsdnews.ucsd.edu/newsrel/science/partnership06.asp>



¹H/¹⁵N HSQC spectrum of a GPCR labeled with ¹⁵N and deuterium.

X-ray structure analysis

M-fold Biotech has programs for X-ray structure analysis of both GPCRs and ion channels. Membrane proteins have been purified to a level of at least 90% chemical purity, monodispersity and specific activity, making them ideal candidates for crystallization trials. The diffraction quality of the resulting crystals has been optimized through various approaches, such as truncation of flexible portions or co-crystallization with ligands and binding proteins. It is expected that further optimization will lead to crystals diffracting to atomic resolution, allowing to solve ligand/target structures suitable for SAR analyses.



Different crystal forms of a potassium channel. The crystals were grown by vapour diffusion. Loop size 100 μm. The crystals diffract to 12 Å.

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