Refolding of GPCRs and ion channels for structural studies

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Introduction

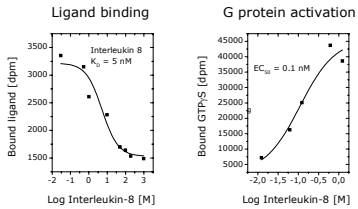
Although human membrane proteins account for two thirds of the known drug targets, their three-dimensional structures remain elusive. To date, no structure of any eukaryotic membrane protein produced in recombinant form has been solved. Common bottlenecks are inefficient expression systems, heterogenous post-translational modification, insufficient stability of the detergent-solubilized protein at high concentration and a low tendency of membrane proteins to form ordered crystals.

Production of protein in *E. coli* inclusion bodies followed by in-vitro refolding is an alternative to functional expression, but has mostly been applied to soluble proteins in the past.

Here we describe a detergent-based refolding method, M-FOLD™, able to deliver stable, purified GPCRs and ion channels in multi-milligram amounts. The high quality of the refolded protein is not only supported by analytical results, but, more important, by the successful crystallization of several GPCRs and one ion channel.

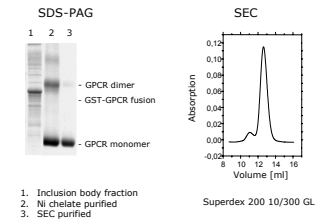
Results - GPCRs

1. Refolded GPCRs are native



Refolding of the chemokine receptor CXCR1. Ligand binding (B_{max}, left panel) reveals that ~80% of the refolded protein is active. When reconstituted with Gi/o, refolded CXCR1 activates the G protein in a ligand-dependent manner (GTPγS binding, right panel). The EC_{50} is shifted to high affinity in the presence of the G protein.

2. Refolded GPCRs are pure and monodisperse



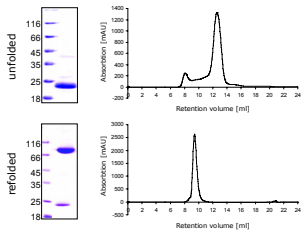
The refolded CXCR1 is >95% pure after two chromatography steps (SDS-PAG, left).
Size exclusion chromatography (SEC) confirms the electrophoresis result: >85% of the chromatogram area belong to a single peak at the expected molecular mass of the receptor/detergent micelle. Similar results are obtained for other GPCRs.

3. Refolded GPCRs form crystals

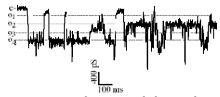
Crystals of two different refolded GPCRs. Scale bar: 50 µm

Results – ion channels

1. Refolded voltage-gated potassium channel is native

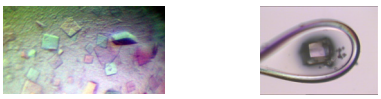


Refolding of an archaebacterial 6 TM potassium channel. Tetramer formation has been analyzed by SDS-PAGE (left) and SEC (right).



The refolded potassium channel has been reconstituted into planar bilayers for electric recording. The trace shows open/close events of four individual channels.

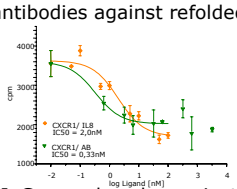
2. Refolded ion channel forms crystals



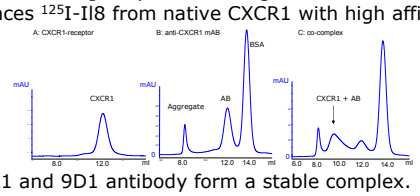
Crystals of refolded ion channel. Loop size: 100 µm
First trials have shown diffraction up to 12 Å.

Results – Antibodies

1. High affinity antibodies against refolded GPCRs



monoclonal IgG produced against refolded CXCR1 displaces ^{125}I-Il8 from native CXCR1 with high affinity.



CXCR1 and 9D1 antibody form a stable complex.

Summary

- M-FOLD™ delivers multi-milligram quantities of GPCRs and ion channels from bacterial inclusion bodies.
- Refolded protein shows native-like pharmacology.
- High purity, monodispersity and stability resulted in successful crystallization.
- *E. coli* expression system allows parallel experiments on multiple targets and variants.
- To date over 150 GPCRs have been expressed and purified.

To Do's

- Improve diffraction: variants, co-crystallization with antibody fragments , ligands and binding proteins.