Standardized solubilization and purification of different membrane proteins from *E. coli*, rat, and human

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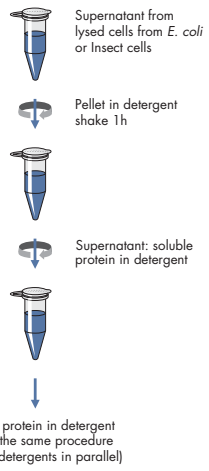
Membrane proteins — interesting, but challenging

Approximately 30% of genes encode for membrane proteins, which in turn represent most of the investigated drug targets. Membrane proteins constitute ~50% of possible targets for novel drugs and ~70% of currently marketed drugs act on G protein-coupled receptors and ion channels alone.

However, handling membrane proteins is challenging, with very few membrane protein structures solved to-date. Membrane proteins have large hydrophobic areas and tend to aggregate; hence, detergents are needed to solubilize them. However, the variability of membrane proteins makes it difficult to predict the right detergent for solubilization and purification. Factors such as size, number of transmembrane domains, and ionic strength are crucial for effective solubilization and purification of a specific protein. Therefore, different detergents are suitable for solubiliziation of different membrane proteins.

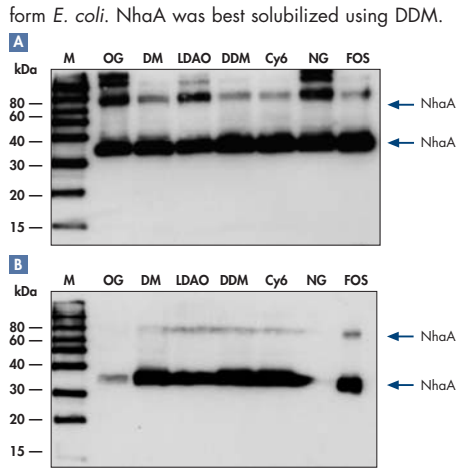
To facilitate the screening for the optimal detergent for protein solubilization and purification, we have developed the Ni-NTA Membrane Protein Kit. This kit with a set of 7 well selected detergents enables standardized handling of membrane proteins. The complete procedure of solubilization and purification can be reproducibly scaled up (data not shown) using Ni-NTA Superflow and the same detergents in bulk.

Solubilization procedure



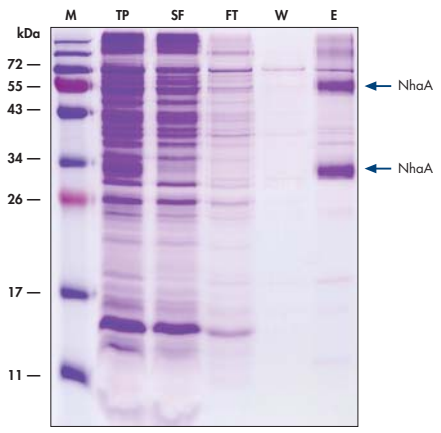
Purification of NhaA from *E. coli*

Screening for the optimal detergent for solubilization and purification of a homologue-expressed protein NhaA form *E. coli*. NhaA was best solubilized using DDM.



The indicated detergents were used to solubilized proteins from A the total protein fraction and B the detergent-soluble membrane fraction from cells overexpressing the 35 kDa *E. coli* membrane protein NhaA (arrowed). Fractions were separated on an SDS-PAGE and proteins visualized by immunodetection of the His-tag after western blotting. **M**: His-tagged marker proteins. Part B shows that DM, LDAO, DDM, Cy6 and Fos-16 are suitable for solubilization of this protein. The band at 70 kDa is a protein dimer.

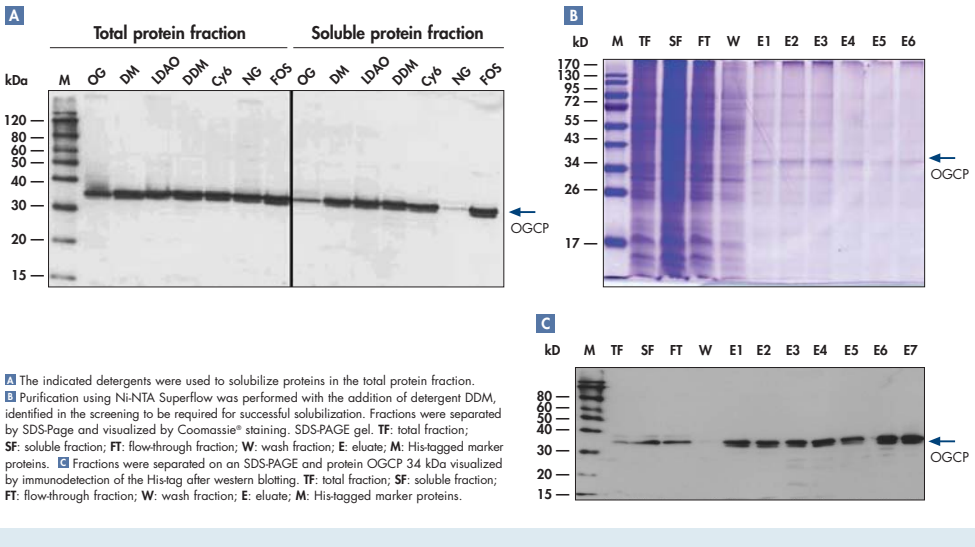
Subsequent purification was performed using Ni-NTA Superflow with the addition of DDM. Fractions were separated on an SDS-PAGE gel and proteins visualized by Coomassie® staining.



TP: total protein; **SF**: soluble fraction; **FT**: flow-through fraction; **W**: wash fraction; **E**: eluate; **M**: markers.

Purifying mammalian OGCP from insect cell (Sf9 cells) culture

Rat mitochondrial-2-oxoglutarate/malate Carrier Protein (OGCP, 34 kDa) expressed in insect cell was purified using Ni-NTA Superflow and buffers containing DDM. The Ni-NTA Membrane Protein kit was used to screen for the most suitable detergent. Fractions were separated on an SDS-PAGE gel and proteins visualized by immunodetection of the His-tag after western blotting. Purification with Ni-NTA Superflow was performed with the detergent DDM.

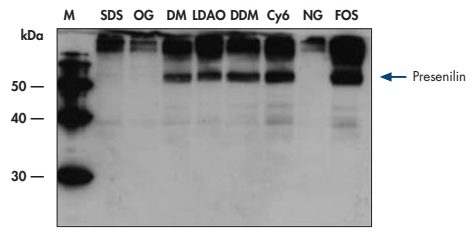


A The indicated detergents were used to solubilize proteins in the total protein fraction. B Purification using Ni-NTA Superflow was performed with the addition of detergent DDM, identified in the screening to be required for successful solubilization. Fractions were separated by SDS-Page and visualized by Coomassie® staining. SDS-PAGE gel. **TF**: total fraction; **SF**: soluble fraction; **FT**: flow-through fraction; **W**: wash fraction; **E**: eluate; **M**: His-tagged marker proteins. C Fractions were separated on an SDS-PAGE and protein OGCP 34 kDa visualized by immunodetection of the His-tag after western blotting. **TF**: total fraction; **SF**: soluble fraction; **FT**: flow-through fraction; **W**: wash fraction; **E**: eluate; **M**: His-tagged marker proteins.

Solubilization of the human membrane protein Presenilin

The membrane protein, Presenilin, is involved in important cellular processes in humans and defects are implicated in the development of Alzheimer's disease. The detergents DM, LDAO, DDM Cy6 and FOS are suitable for solubilization of Presenilin. The Ni-NTA Membrane Protein Kit was used to screen for the most suitable detergent for solubilization of the human membrane protein Presenilin. The protein was expressed in *E. coli* C41(DE3) solo cell culture using an expression-optimized QIAgenes® *E. coli* construct.

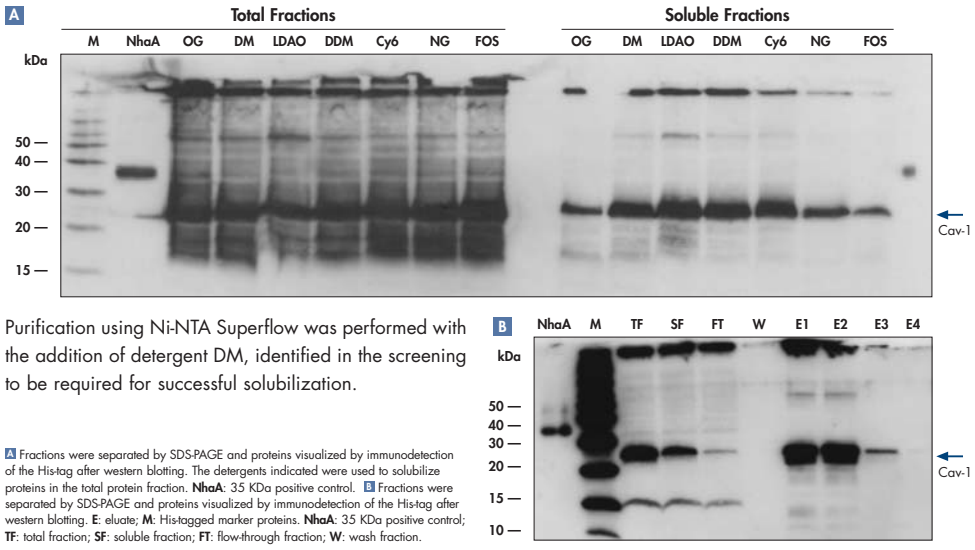
The 52 kDa protein Presenilin was expressed in TB-medium for 16 hours at 18°C and induced using 1 mM isopropyl-beta-D-thiogalactopyranoside (IPTG). The protein expressed well and could be best solubilized using the detergent Fos-choline 16. Scale-up of expression and protein purification is currently ongoing.



Fractions were separated on an SDS-PAGE gel and proteins visualized by immunodetection of the His-tag after western blotting. The indicated detergents were used to solubilize proteins.

Cell-free expression and solubilization of Cav-1 from human

The His-tagged human Caveolin-1, a 20 kDa membrane-anchored protein, was expressed cell-free using an expression-optimized QIAgenes Insect/Mammalia construct in EasyXpress Insect Kit II lysates. Detergent screen and purification were performed using the Ni-NTA Membrane Protein Kit.



Purification using Ni-NTA Superflow was performed with the addition of detergent DM, identified in the screening to be required for successful solubilization.

A Fractions were separated by SDS-PAGE and proteins visualized by immunodetection of the His-tag after western blotting. The detergents indicated were used to solubilize proteins in the total protein fraction. **NhaA**: 35 KDa positive control. B Fractions were separated by SDS-PAGE and proteins visualized by immunodetection of the His-tag after western blotting. **E**: eluate; **M**: His-tagged marker proteins. **NhaA**: 35 KDa positive control; **TF**: total fraction; **SF**: soluble fraction; **FT**: flow-through fraction; **W**: wash fraction.

Conclusions

We analyzed the literature with regard to detergents used for solubilization and purification of recombinant His-tagged membrane proteins. Approximately 50 detergents were experimentally screened for recovery of more than 10 prokaryotic and eukaryotic targets of different membrane proteins types (α-helical, β-barrel etc.) and from the membrane fraction of various expression systems. We have identified a panel of 7 high-quality detergents, at least one of which allowed membrane protein purification in each test case. Based on the methodology a kit has been developed which comprises the following features:

- Membrane proteins from different origin expressed in *E. coli*, insect cells, or cell-free can be successfully solubilized and purified.
- A standardized solution for membrane protein solubilization and purification is available and eliminates tedious evaluation of a large set of detergents.
- All proteins were expressed using QIAgenes (optimized expression constructs) as templates.
- The complete process can be reproducibly scaled-up.
- Detergents in bulk amounts – with the same quality as in the Ni-NTA Membrane Protein Kit are also available.

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