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Use of IMAC magnetic beads for simplified detergent screening for optimization of purification workflows of histidine-tagged membrane proteins

Karin Torstenson, Inger Salomonsson, Erik Mascher, Marianne Carlsson, Pia Liljedahl, and Anna Heijbel
GE Healthcare Bio-Sciences AB, SE-751 84 Uppsala, Sweden

Background

Integral membrane proteins are main targets for development of new pharmaceuticals.

Affinity purification is a powerful tool that has become the prime choice as capture step in membrane protein purification. Recombinant membrane proteins are commonly purified by use of polyhistidine tags and immobilized metal affinity chromatography (IMAC).

The choice of detergent is the major key factor for successful solubilisation and further purifications of integral membrane proteins to avoid protein loss and inactivation. Therefore a detergent screen is often necessary to find the optimal detergent for each purpose.

The use of the Membrane Protein Purification Kit allows for rapid, small-scale screening of optimal purification conditions for histidine-tagged membrane proteins. The kit contains IMAC magnetic beads pre-charged with Ni^{2+}-ions (His Mag Sepharose™ Ni), selected detergents, buffers and an easy-to-follow protocol.

Screening for optimal detergent(s) for purification conditions of some integral membrane proteins was performed using the Membrane Protein Purification Kit.

The detergents screen workflow is outlined in Figure 1.

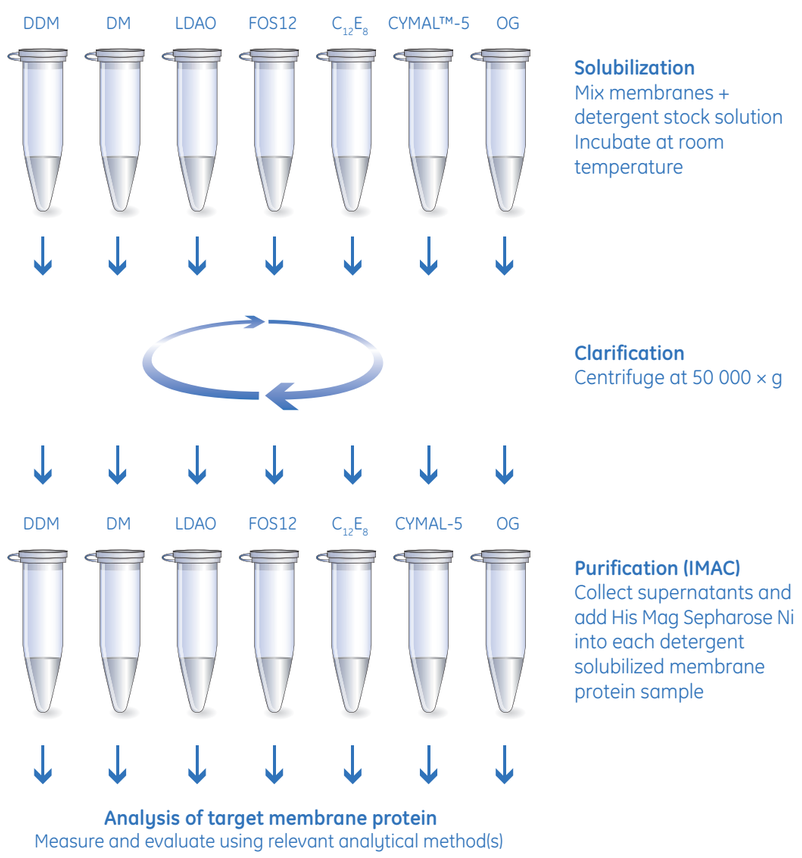

Fig 1. Detergent screening workflow.

Conclusions

Detergent screening using Membrane Protein Purification Kit:

- Allows efficient and rapid screening for simultaneous evaluation of solubilization and purification efficency for membrane proteins
- Gives useful information and guidance for scale up attempts
- Provides convenient handling due to the magnetic bead format and detergents in aqueous solution

Screen for optimal detergent(s) for solubilization/purification of $(His)_9$-cytochrome bo_3 ubiquinol oxidase

Seven detergents (see Table 1) were used for screening of optimal detergent(s) for $(His)_9$-cytochrome bo_3 ubiquinol oxidase (expressed in *E. coli*). Analyses were done by Western blot (Fig 2A) using enhanced chemoluminescence for quantitative evaluations (Fig 2B) and gel filtration (Fig 2C) was used to determine size homogeneity and purity of the membrane protein.

This detergent screen (Fig 2A and 2B) shows that DDM, DM or LDAO are suitable for solubilization and purification of histidine tagged cytochrome bo_3 ubiquinol oxidase.

FOS12 might also be considered since this detergent seems to give a slightly purer and a more homogeneous preparation (Fig 2C).

Table 1. Concentrations of the respective detergents for solubilization and purification

Detergents	Solubilization	Purification
n-Dodecyl-β-D-maltoside (DDM)	1%	0.1%
n-Decyl-β-D-maltoside (DM)	1%	0.2%
Lauryldimethylamine-N-oxide (LDAO)	1%	0.2%
n-Dodecylphosphocholine (FOS12)	1%	0.1%
Dodecyl octaethyleneglycol ether ($C_{12}E_8$)	1%	0.1%
Cyclohexyl-1-pentyl-β-D-maltoside (CYMAL-5)	1%	0.2%
n-Octyl-β-D-glucoside (OG)	2%	1.0%

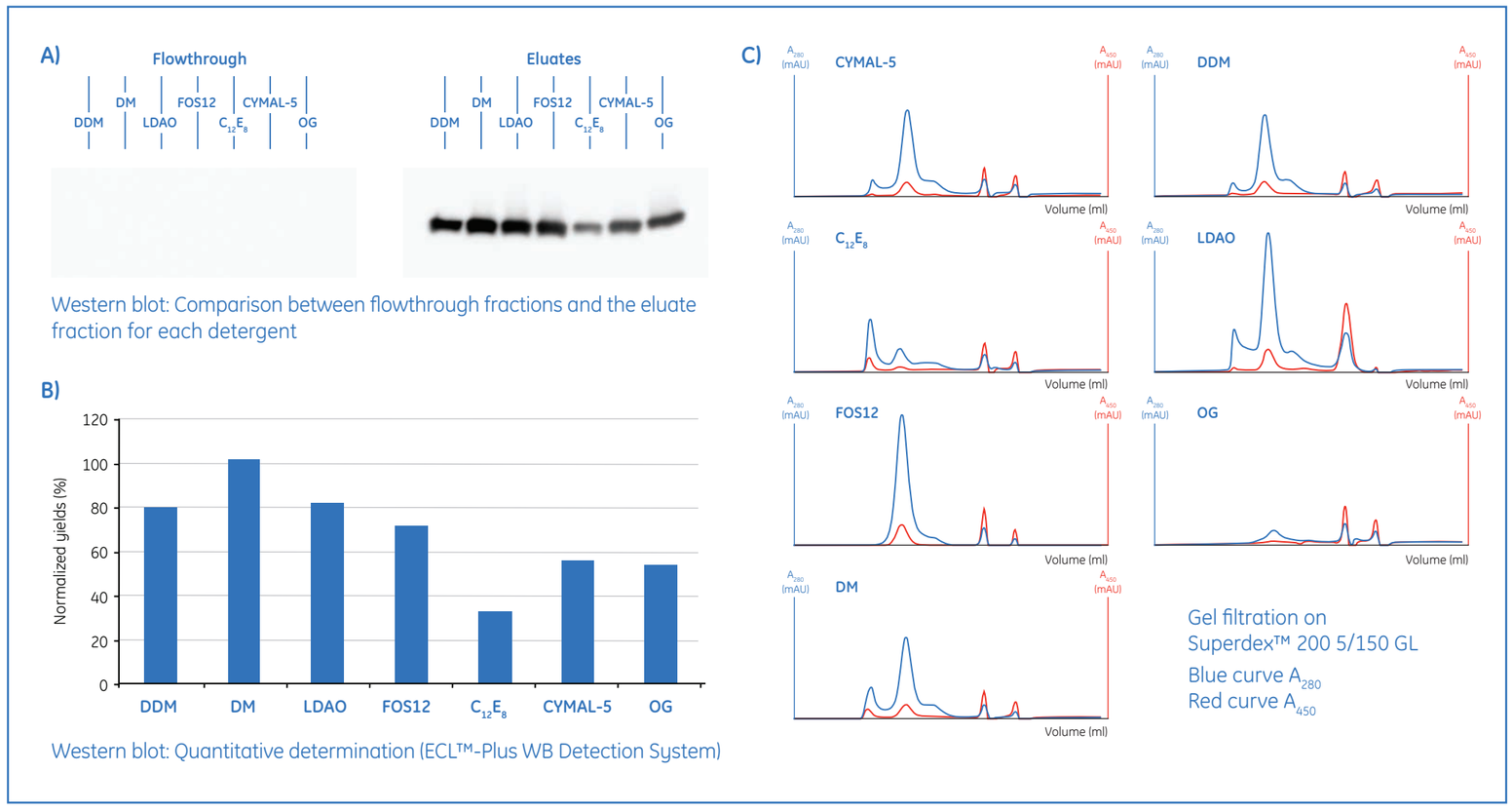

Fig 2. Analyses of purified cytochrome bo_3 ubiquinol oxidase.

Scale up preparation of YedZGFP-$(His)_8$

A detergent screen for YedZGFP-$(His)_8$ (expresssed in *E. coli*) had identified DM as a suitable candidate for solubilization/purification of YedZGFP-His (data not shown).

A scale up preparation in the presence of 0.2% DM, using a 1-mL HisTrap™ HP column, was done of material solubilized in 1% DM.

The main component in the purified fraction (Fig 3A) was the YedZGFP-His target protein. The SDS-PAGE profile (Fig 3B) suggests that a second purification step is required to obtain highly pure YedZGFP-His.

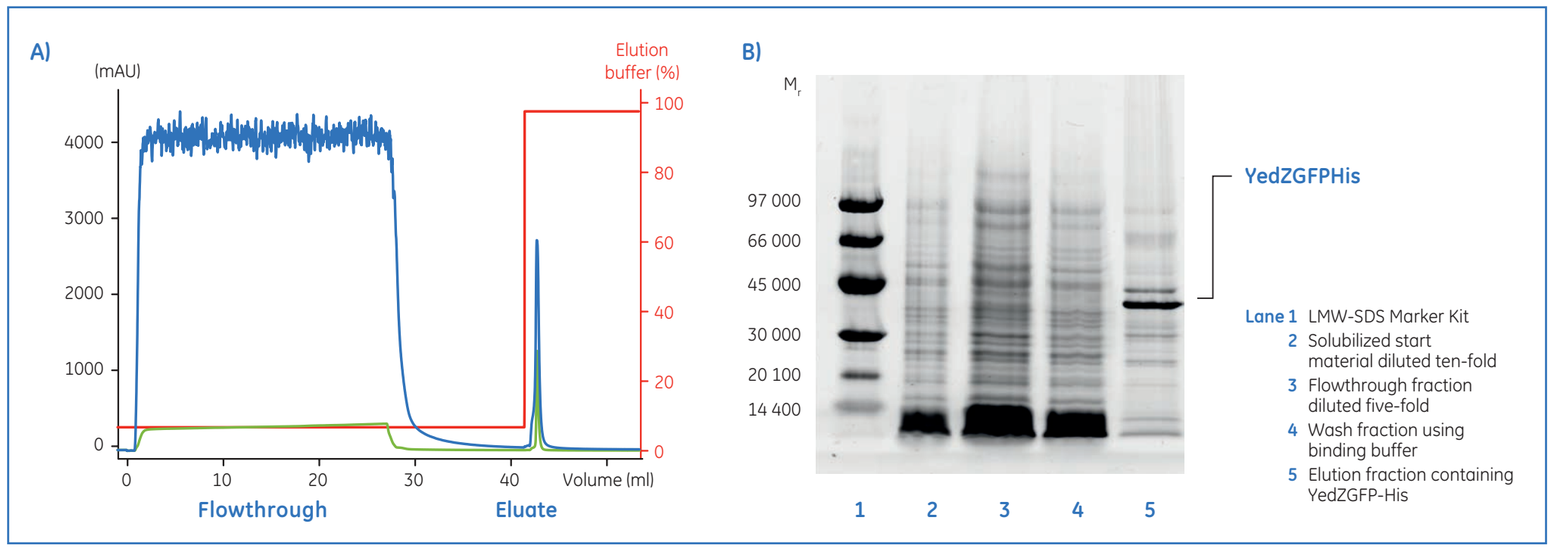

Fig 3. Preparation of YedZGFP-His on 1-mL HisTrap HP in 0.2% DM. **A)** Chromatographic profile; (blue curve A_{280} and green curve A_{490}) **B)** Deep Purple™-stained SDS-PAGE image.

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