Nanoxis
Membrane protein solutions

Application Note

# Benefits of immobilizing your membrane proteins

## Use of multistep digestion protocols to improve the information output from your membrane protein sample analysis

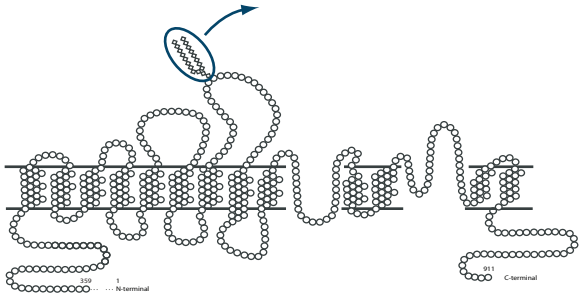


Figure 1. Step 1 - Sugar removed by PGNaseF.

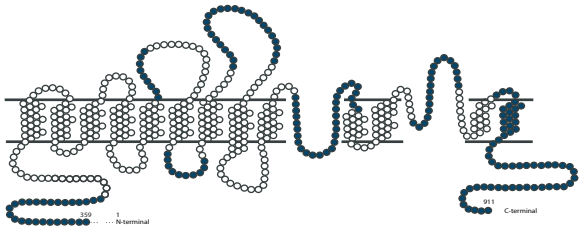


Figure 2. Step 2 - Extramembrane peptides identified in the trypsin fraction.

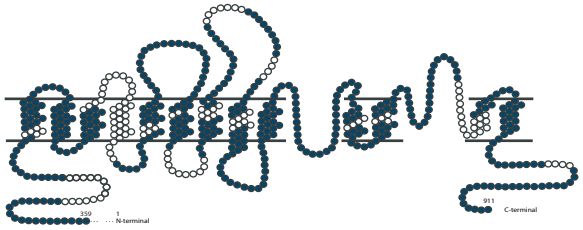


Figure 3. Step 3 - Transmembrane peptides identified in the pepsin fraction.

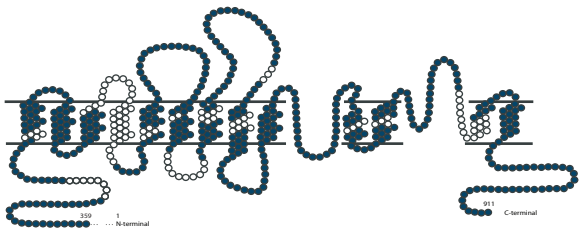


Figure 4. Total sequence coverage.


## Summary

This study demonstrates a multistep digestion protocol on proteoliposomes immobilized in LPI™ FlowCell. The multistep procedure enables the collection of several eluates containing different information from a sample without dilution. Applications include studies of PTMs (post-translational modifications), such as glycosylations, topology and sequence coverage.


## Method

A three step enzymatic digestion protocol was applied to proteoliposomes from red blood cells immobilized in LPI™ FlowCell. As a first step, PNGaseF was used to cleave off sugar groups, demonstrating the use for glycosylation studies. Further, trypsin was added to cleave the extramembrane parts of the membrane protein, demonstrating the use for finding potential epitope candidates. To access and digest the transmembrane parts of the protein, pepsin was added in a specific solution aimed at loosening the membrane structure, demonstrating that a combination of proteases can be used to achieve high sequence coverage and also for topology studies.

## Results

Peptide fractions from the trypsin and pepsin digestions were analyzed separately using LC-MS/MS. For the target membrane protein (Anion exchanger 1, AE1) in the preparation of red blood cells, sequence coverage of above 80% was achieved with less than ten cycles of MS analysis. Figure 1 shows the sugar group removed from AE1 in step 1. Figure 2 and 3 shows the peptides found in the trypsin and the pepsin fraction, respectively (dark blue circles). Figure 4 shows the total peptide coverage.

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