

Improved identification of membrane proteins by MALDI-MS/MS using matrix pre-deposited MALDI chips

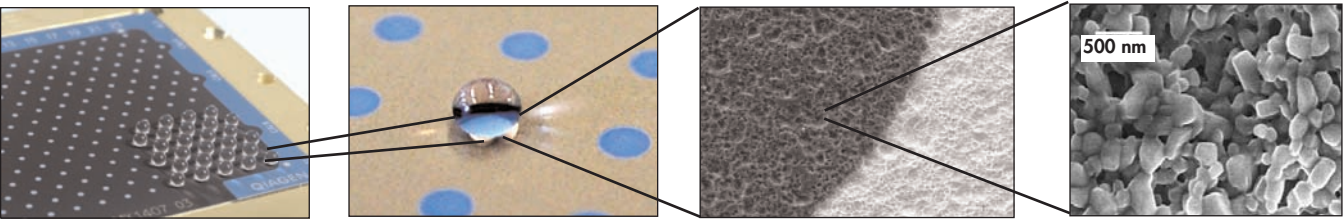


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Introduction

Due to their low abundance and limited number of trypsin cleavage sites, integral membrane proteins are difficult to analyze by mass spectrometry. Our approach is based on a novel technology for peptide sample preparation that uses plates with pre-deposited matrix spots of sub-micron size CHCA crystals prepared by vacuum sublimation onto an ultraphobic surface (Mass-Spec-Turbo Chips, QIAGEN). This design allows analyte concentration in the top layers of the matrix thin layer providing highest MS sensitivity. To evaluate the performance of this system in membrane proteomics two model proteins (ATP synthase subunit IV(a) from spinach and bacteriorhodopsin from *Halobacterium salinarum*) containing several transmembrane domains were analyzed and spectra quality was compared to standard MALDI preparations. In a second step the chips were tested with a membrane protein preparation sample from the bacterium *Rhodococcus* sp. RHA1, a soil actinomycete. The proteomic workflow comprised the isolation of the membrane fraction and proteins were separated using the recently developed technology based on ion-exchange chromatography and SDS-PAGE (see Methods). The gel bands were analyzed by MALDI-TOF/TOF, resulting in the identification of cytosolic, membrane-associated, and integral membrane proteins. Our results clearly show that more integral membrane proteins were identified using the Mass-Spec-Turbo Chips, which proves their advantage for membrane proteomics.



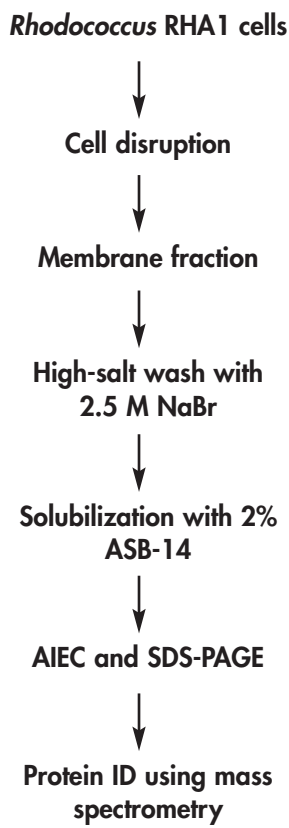
Methods

AIEC/SDS-PAGE

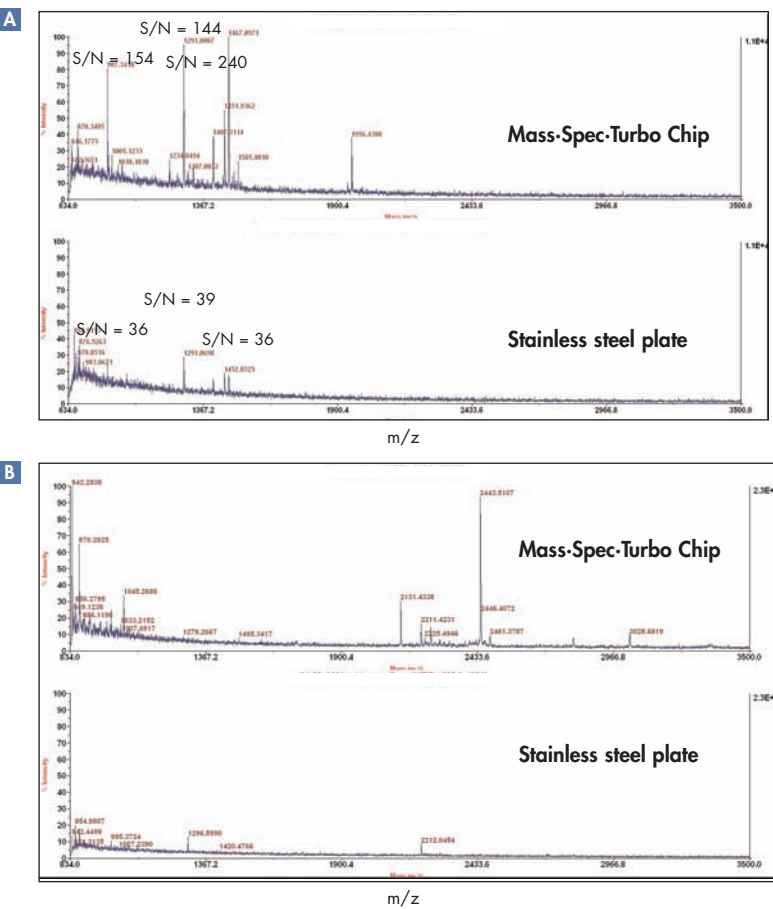
For details see Schluesener, 2005. Briefly, cells were disrupted by French pressure treatment and membranes washed with 2.5 M NaBr to remove membrane-associated proteins. The resulting membrane fraction was solubilized in buffer containing 2% (w/v) ASB-14 and applied onto an anion-exchange column (Poros20 HQ material, Applied Biosystems, Darmstadt, Germany). Fractions were precipitated by TCA and SDS-PAGE was performed according to Laemmli.

MALDI-MS/MS

After visualization with Coomassie blue, protein bands were excised from the SDS-polyacrylamide gel and prepared for overnight tryptic digestion according to standard protocols. To elute the peptides, 10 µl of 50% (v/v) acetonitrile, 0.5% (v/v) TFA was added to the samples and sonicated for 5 min. Aliquots of 1 µl were applied either onto the steel target plate and immediately mixed with an equal volume of α -cyano-hydroxycinnamic acid in 60% (v/v) acetonitrile, 1% (v/v) TFA or applied directly onto the matrix spots pre-deposited on the chip surfaces and washed with 1 µl finishing solution. Spectra were acquired on a 4800 MALDI-TOF-TOF instrument (Applied Biosystems) and analyzed using the Data Explorer software. Proteins were identified by searching extracted peak lists against the NCBI nr DB (Actinobacteria) using the Mascot search engine.



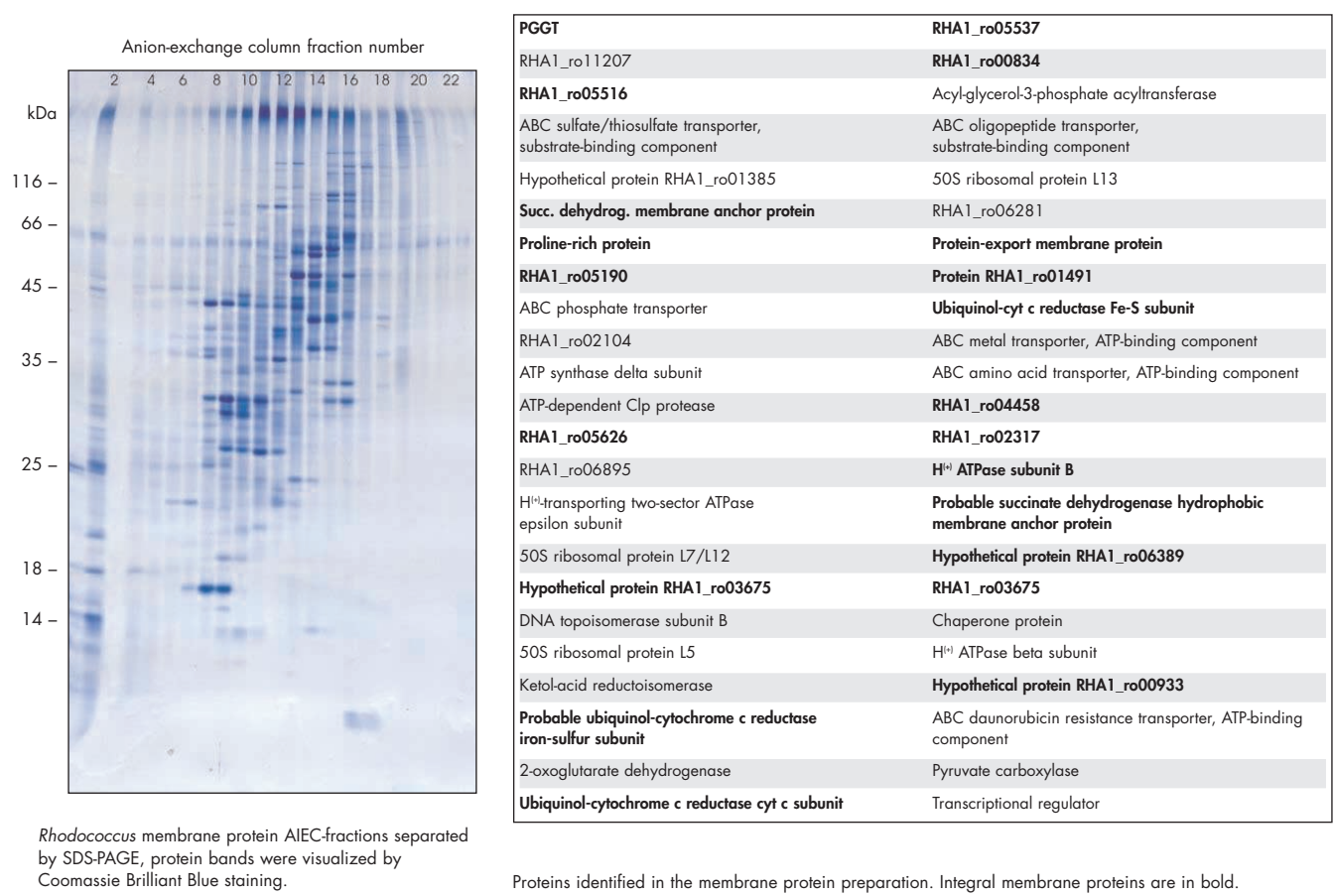
MALDI-MS spectra of model membrane proteins



- A direct comparison of the preparation methods shows that Mass-Spec-Turbo Chips deliver higher signal-to-noise ratios than stainless steel plates.
- In addition, the total number of peptides observed was higher in the case of samples processed using Mass-Spec-Turbo Chips. This was particularly the case for higher-mass peptides, typically found in membrane protein preparations, which could be analyzed with higher sensitivity.

MALDI-MS spectra of digested model membrane proteins obtained using an ABI Voyager DE Pro instrument. **A** bacteriorhodopsin from *Halobacterium salinarum* (7 transmembrane domains) **B** ATP synthase subunit IV(a) from *Spinacea oleracea* (5 transmembrane domains) **S/N** = signal to noise ratio.

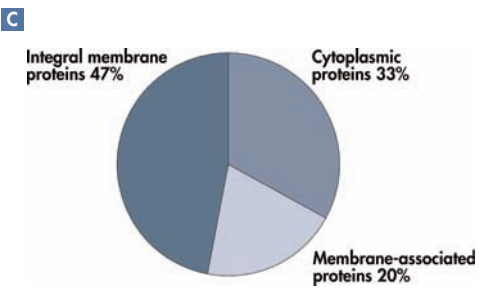
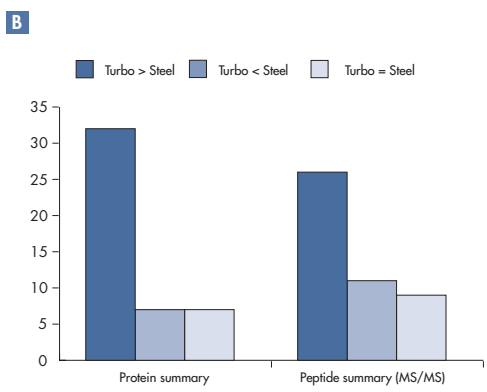
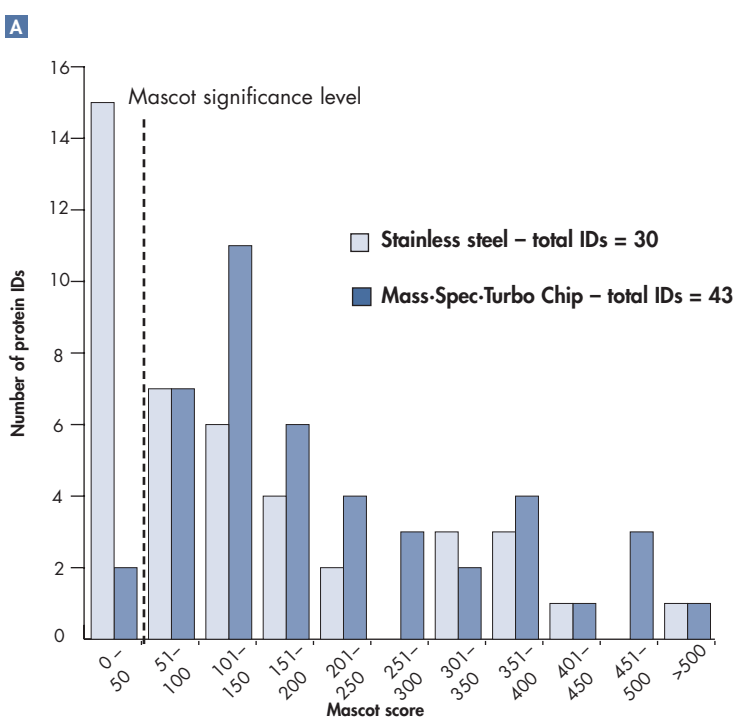
Separation of membrane protein extracts



Proteins identified in the membrane protein preparation. Integral membrane proteins are in bold.

Mascot scores: Stainless steel vs. Turbo Chip

- A direct comparison of the preparation methods shows that Mass-Spec-Turbo Chips typically deliver higher Mascot scores (and hence a more reliable identification) than stainless steel plates.



Comparative analysis of the two methods used to identify proteins by mass spectrometry using an ABI 4800 instrument. **A** Distribution of Mascot scores for each preparation method **B** Direct comparison of preparation methods showing which method delivered a higher Mascot score for individual proteins. **C** Cellular localization of identified proteins.

Summary

- Sample application on chips possessing sublimated CHCA matrix spots (Mass Spec Turbo Chips) resulted in the observation of a larger number of peptides and higher S/N peak ratios of spectra from model membrane protein digests, compared to classical dried-droplet sample preparation
- The recently developed 2-dimensional AIEC/SDS-PAGE technique provided an excellent tool for efficient separation of bacterial membrane proteomes
- In an exemplary membrane proteomics study of a *Rhodococcus* strain 2/3 of the proteins identified were annotated by sequence-based prediction of their cellular localization as integral or associated-membrane proteins
- Sample preparations on sublimated CHCA matrix delivered higher protein identification rates based on total Mascot scoring or MS/MS-based protein identification
- A comparison of the total Mascot score demonstrated on average a higher confidence of the identified proteins resulting from higher sequence coverage, however thusfar no peptide mass or fragment spectrum could be matched to putative transmembrane domains

Reference

Schluesener, D., Fischer, F., Kruij, J., Rogner, M., and Poetsch, A. (2005) Mapping the membrane proteome of *Corynebacterium glutamicum*. *Proteomics* **5**, 1317.