Simple, safe and efficient sample preparation using micro dialysis

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Introduction

- Medium exchange, desalting and concentration without selective but comprehensive recovery are urgent needs with the very small sample volumes and the vast sample quantities of common proteomic platforms.
- Suitable configured dialysis tools may offer non-selectivity, miniaturization, and parallelization and therewith good reproducibility and high throughput in contrast to hydrophobic tools generally used for these purposes, *e.g.* RP-chromatography and ZipTips.
- Xpress MicroDialyzer® (Scienova) has been developed to realize this configuration for dialysis of sample volumes down to 10 µl compatible to microplate format, and equipped with low molecular weight cut-off membranes applicable to peptide analysis (Rhode *et al*., WO002008106960, DE102007011866).

Summary

- Several features favor Xpress MicroDialyzer® not simply as dialysis tools but also as multifunctional tools to high-throughput proteomic applications. These features imply parallelization to SBS-format and parallelized handling, high recovery rates, speed and efficiency of medium change, low protein binding, non-selectivity of peptide preparation, applicability to small sample volumes as well as to sample reconcentration.

- These tools provide practically complete recovery rates of proteins and peptides from aqueous samples. Salt and detergent content may be sufficiently reduced within short times. Tryptic digests and proteins show improved mass spec signal quality and sequence coverage already after 30 min desalting. Moreover, these tools may be applied to very quick sample re-concentration, *i.e.* simply making use of evaporation. Sample reconcentration of at least two orders of magnitude may be easily combined with desalting within the same vessel.

Methods

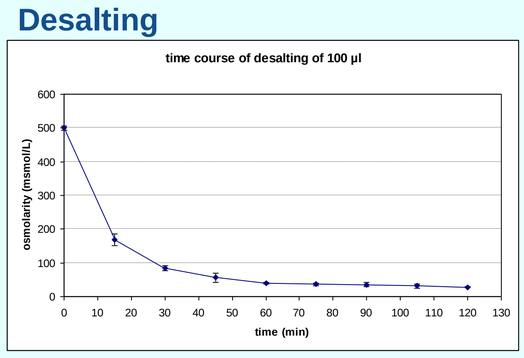
Desalting: sample: 100 µl 50 mM phosphate buffer, pH 7.4, 150 mM NaCl, dialysis buffer: 1.8 ml Aqua dest., dialysis condition: 20 ° C, measurements: Wescor Osmometer Vapor 5520
Detergent removal: sample: 100 µl BSA (1 mg/ml) with 1% CHAPS or 0.1% SDS (w/v) in PBS; dialysis buffer: 1.8 ml Aqua dest., MS: Agilent HP1100/MSD, positive mode (CHAPS), negative mode (SDS) quantification: sample diluted to linear range (SDS: 100 % MeOH, CHAPS: 2 % MeOH, 0.05 % formic acid in water) were directly injected in triplicates. Concentrations of CHAPS and SDS were calculated from peak areas at 614.5 ± 1.5 and 265.2 ±1 m/z, respectively, in comparison to detergent calibrators.
Protein binding: sample: 100 µl BSA in PBS (20 and 100 µg/ml), n= 5 - 6 dialysis buffer: 1,8 ml of Aqua. dest. 4h at room temperature; protein determination according to Bradford, Tecan Sunrise photometer.

Time course for re-concentration: All 8 microdialyzers of one bar were filled with 100 µl water. Weight loss was followed either with or without cold air flow produced by a hair dryer at temperatures and relative humitity of air (RHA) as given.
Desalting of proteins and peptides: 100 µl of samples, dialysis buffer: 1.8 ml 20 mM NH_4HCO_3, change of dialysis buffer with each data point;
Tryptic digest: albumin and transferrin containing human serum fractions (2D, Kreusch *et al*. J Chromatogr. B 2008, 875, 567) and pure BSA (5-20 µM, 20 mM NH_4HCO_3), 2 M guanidine/HCl, protein modification by DTT and iodoacetamide, digestion with trypsin.
MALDI-MS: Voyager DP (Applied Biosystems) all samples in quadruplicate and quantitation using an internal standard peptide and normalized peak height sums of all matching peptides (Bublitz *et al*.: Proteomics 2006, 6, 3909).
Peptide analysis: ExPASy, Proteomics Tools: ProtParam

PERFORMANCE RESULTS APPLICATIONS

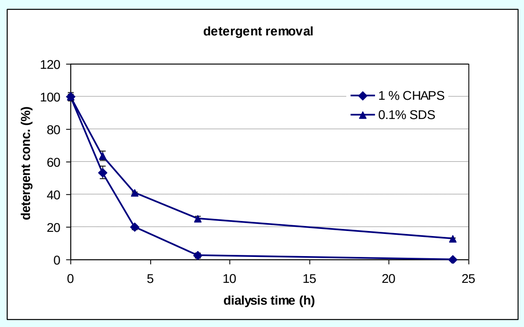
1. Medium change

Desalting



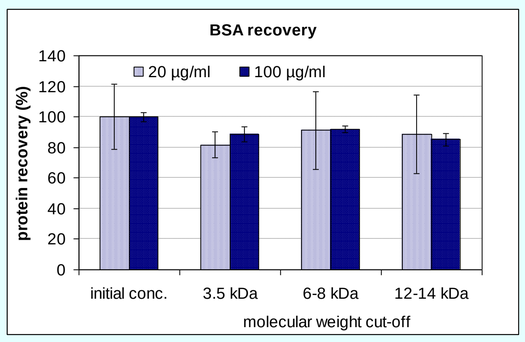
sufficient desalting within one hour

Removal of detergents

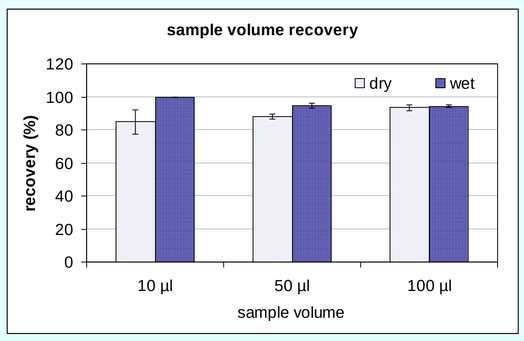


98% of CHAPS and 88% of SDS can be removed within 8 and 24 h, respectively

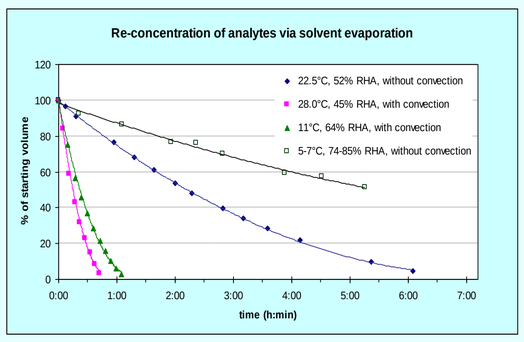
Recovery



~ 90% of sample protein can be recovered from capillaries of MD



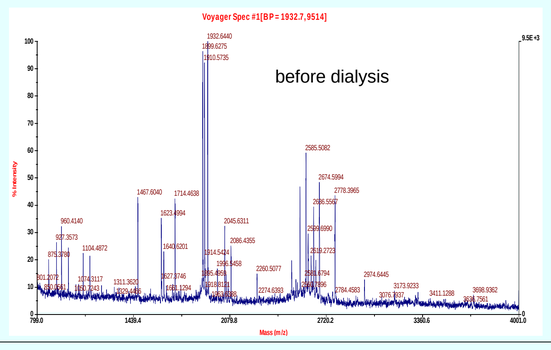
2. Sample reconcentration

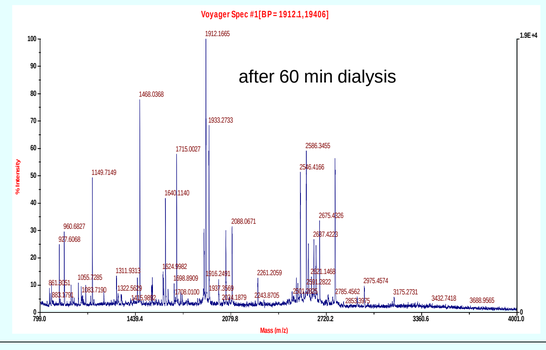


Solvent evaporation according to time and stability requirements

1. Desalting of digests

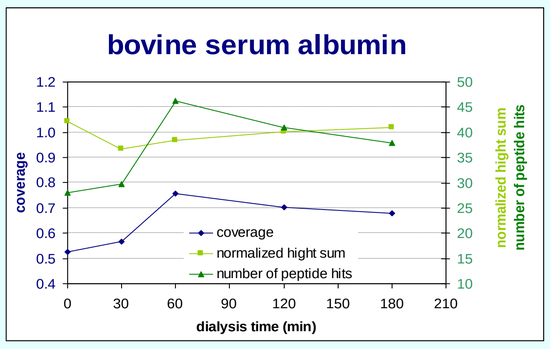
Improving quality of MALDI-MS spectra of peptides

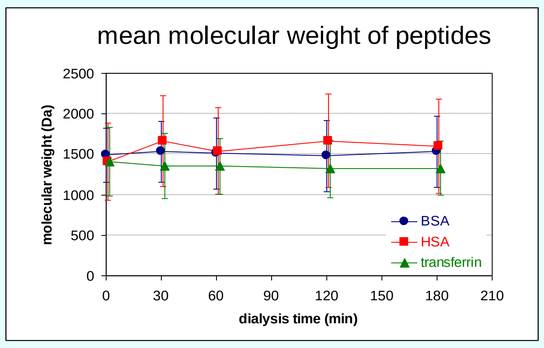




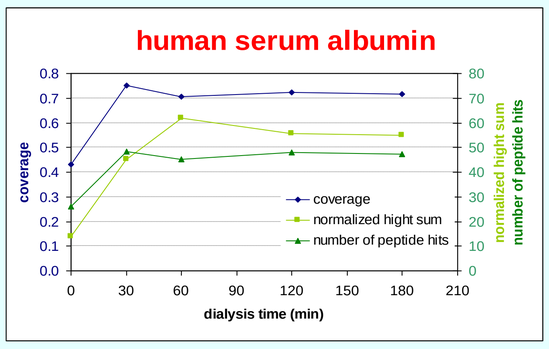
Tryptic digest of BSA, before and after dialysis using cut-off of ~7 kDa

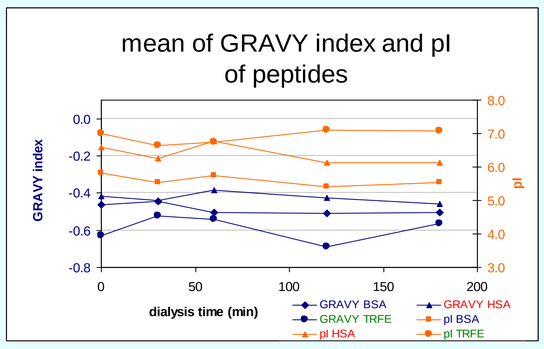
Characteristics of peptides identified



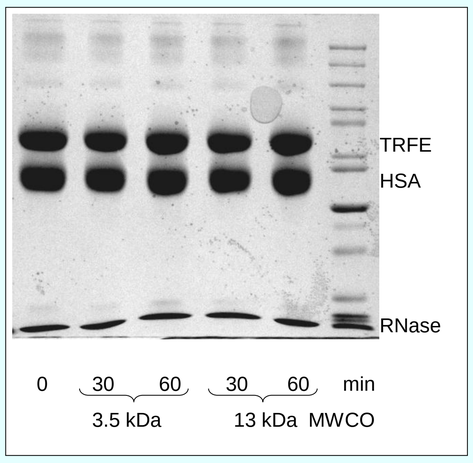


- parallel, easy and very fast preparation
- much better spectra quality (higher coverage, higher signal intensity, more matching peptides)
- no peptide loss (mean molecular weight of peptide hits remains constant)
- no selectivity (mean gravy index and pI of peptide hits remain constant) with 3.5 kDa and even with 7 kDa cut-off membranes under conditions used



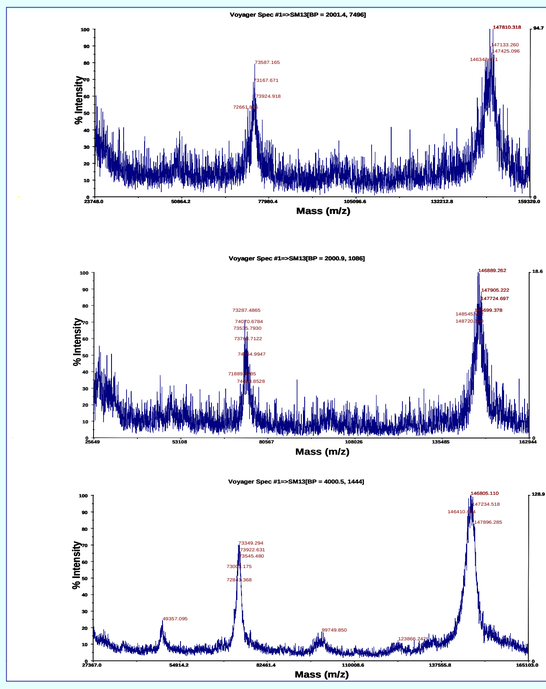


2. Desalting of small proteins



SDS-PAGE :
Sample: mixture of HSA (67 kDa), transferrin (80 kDa) and RNase (13.7 kDa, 0.2 mg/ml each) in 0.9% NaCl before (0) and after dialysis against 10 mM Tris/HCl, pH 7.4) for 30 and 60 min. Membranes with different molecular weight cut-off (MWCO) were used.

3. Reconcentration and desalting



MALDI-MS spectra of proteins

Sample 1: bovine IgG (Serva 22550, 0.3 mg/ml, 2.0 µM in 10 mM Tris/HCl, pH 7,4).
Sample 2: IgG as in sample 1, concentrated 23-fold by a hair-dryer.
Sample 3: sample 2 desalted by dialysis against 10 mM Tris/HCl, pH 7.4. MALDI-MS of samples

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