



Automated native sample preparation for proteome analysis in microplates



Proteomic biomarker search requires both comprehensiveness (avoiding depletion) and high resolution/sensitivity ...



sample



control



High comprehensiveness requires a lot of time and labour with many opportunities to fail...



...due to insufficient precision and coverage

...due to the heterogeneity of methods

...due to unreasonable demands

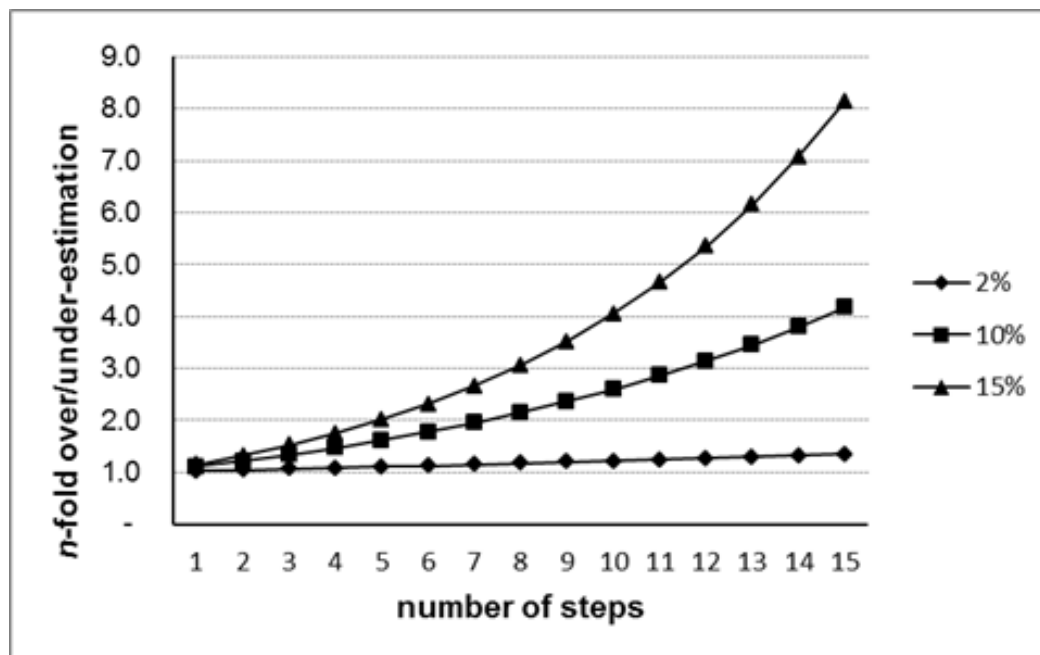
...like with depletion, enrichments,
labelling, medium exchange, 2D-PAGE,
modifications, concentration, denaturation,
digest...

CVs of liquid handling = pipetting: 1-3%

H. Rhode *et al.* J. Biomol. Screening, 2004, 9, 726



High resolution needs high precision and smart separation,



and a lot of preparation steps with many opportunities to deviate...

Krüger *et al.*,
Anal. Chim. Acta, 2013, 776, 1



Comprehensiveness may be achieved by chromatographic distribution applying multidimensional native chromatography

without any depletion and without loss of any sample constituent

Native fractionation of proteins:



1D: Size exclusion chromatography (SEC)

2D: Anion exchange chromatography (AEC)

3D: Lectin affinity chromatography (LAC)

Pre-analytic fractionation of tryptic peptides:



4D: LC-ESI-MS/MS

Kreusch et al.,
J. Chromatogr. B, 2008, 875, 567

Challenges:

How to prepare/analyse thousands of sub-fractions
with high throughput and high reproducibility?

medium exchange

native SEC → AEC

LAC → digest, mass spectrometry

modifications → digest, mass spectrometry

determination of protein concentration

digest

suitable micro columns

mixing of small volumes



S. Kreusch *et al.* *Anal. Biochem.* , 2003, 313, 208

A. Horn *et al.* *Proteomics*, 2006, 6, 559

S. Kreusch *et al.* *J. Chromatogr. B*, 2008, 875, 567

E. Mitre *et al.* *J. Biomol. Screening* , 2007, 12, 361



High comprehensiveness and precision requires a lot of labour and time with many opportunities to fail...



...that requires help.





Maximum precision by parallelization
and maximum throughput
by automation
using
microplates

for

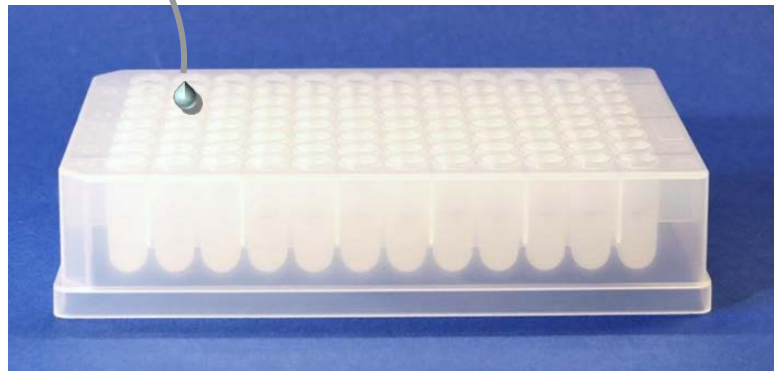
sample fractionation
sample preparation
analytics
storage





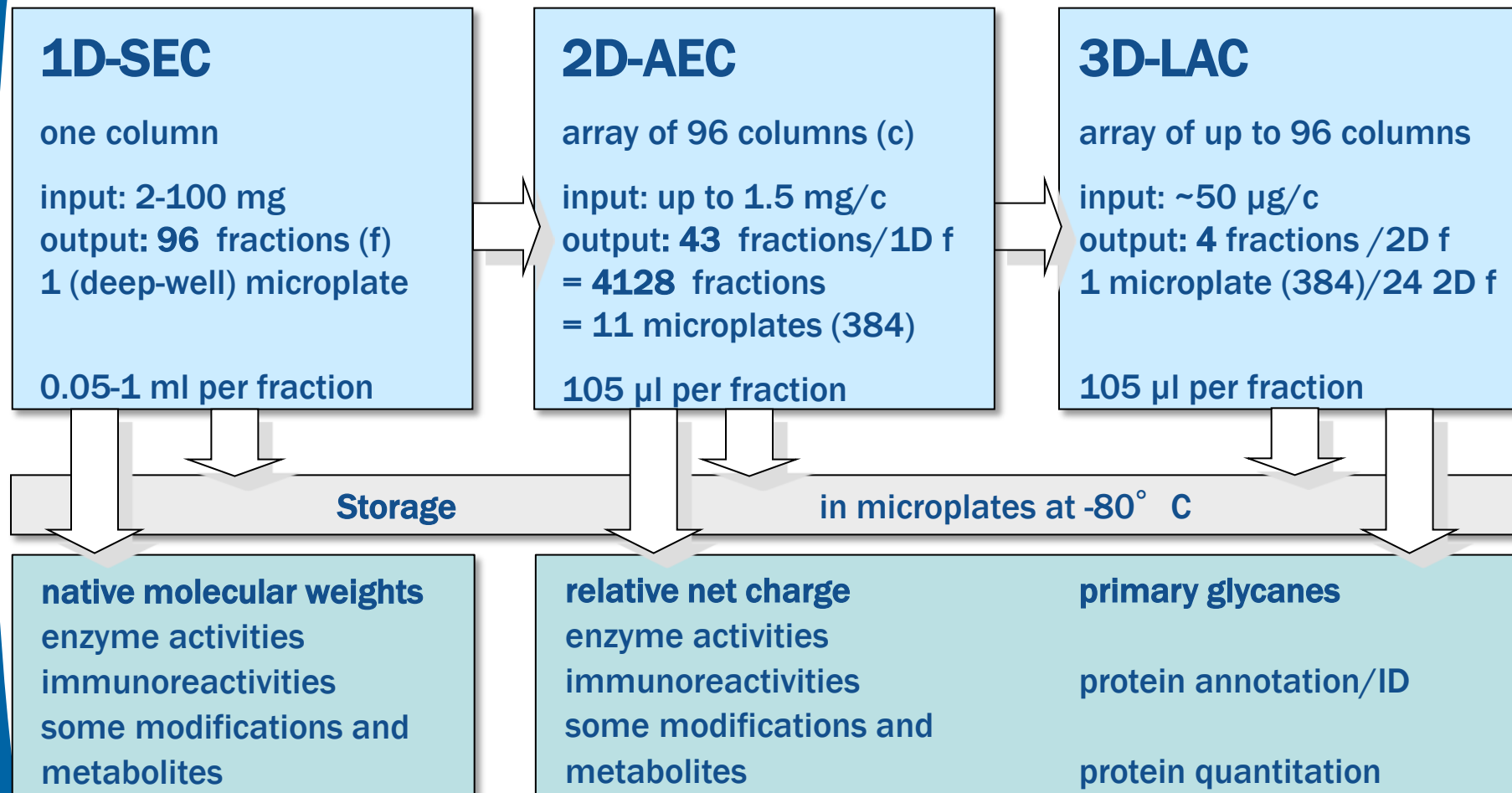
1D - Size exclusion chromatography

- serial
- defines scale
- output into microplates





Process characteristics - overview



Information - overview

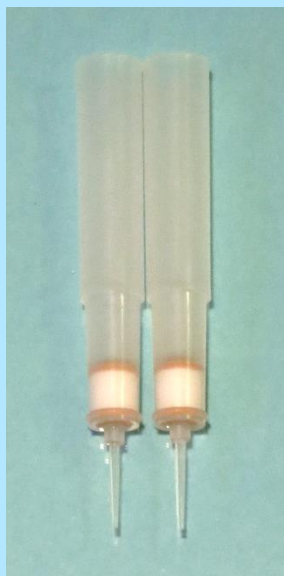


Tools for parallel chromatography

2D-AEC

100 μ l

Toyopearl DEAE-650M

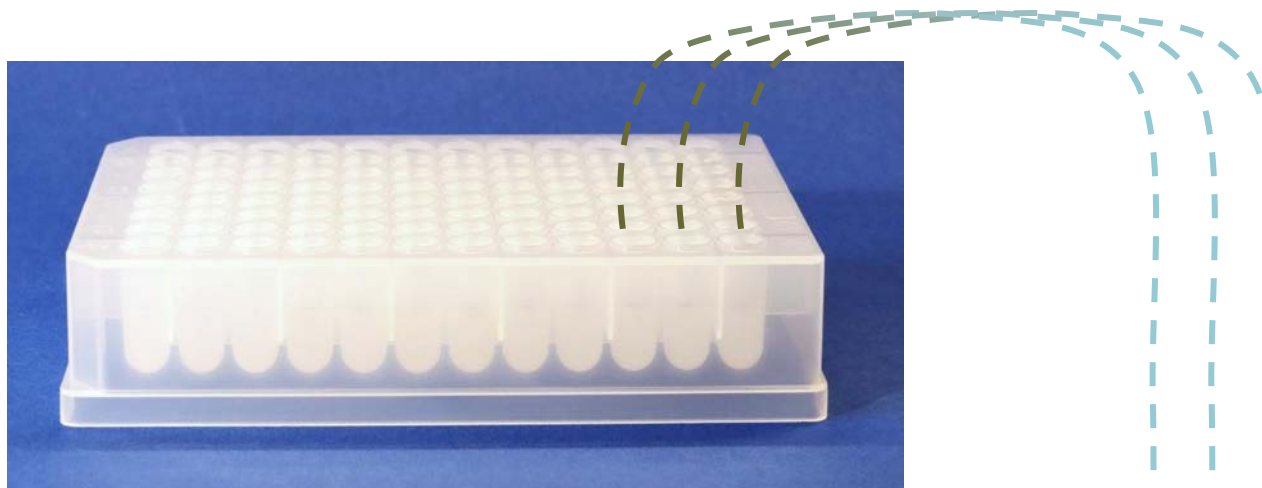


3D-LAC

70 μ l

ConA- or WGA-modified Toyopearl AF-Tresyl 650M





simultaneous transfer and
chromatography

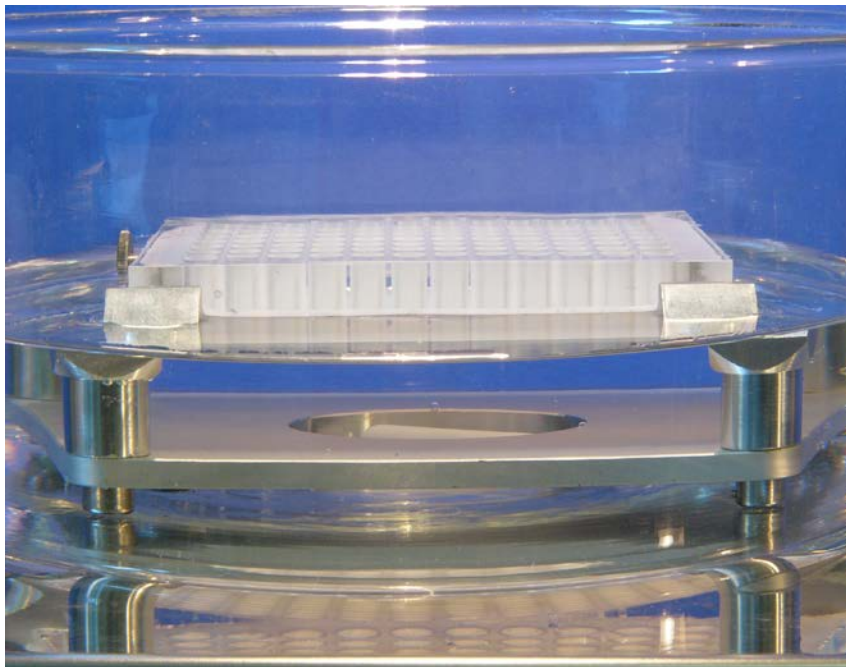




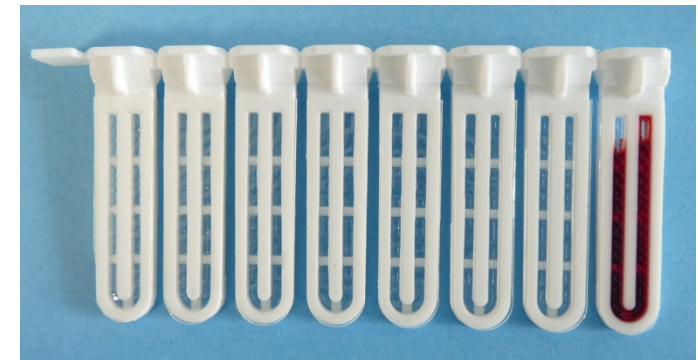
Tools for parallel medium exchange

create compatibility during fractionation,
to digestion, and to mass spectrometry

 **scienova**TM



Central scientific workshop



Rhode *et al.*, W0002008106960, Maischak
et al., *Anal. Biochem.*, 2012, 424, 184



Automated workstation for parallel chromatography



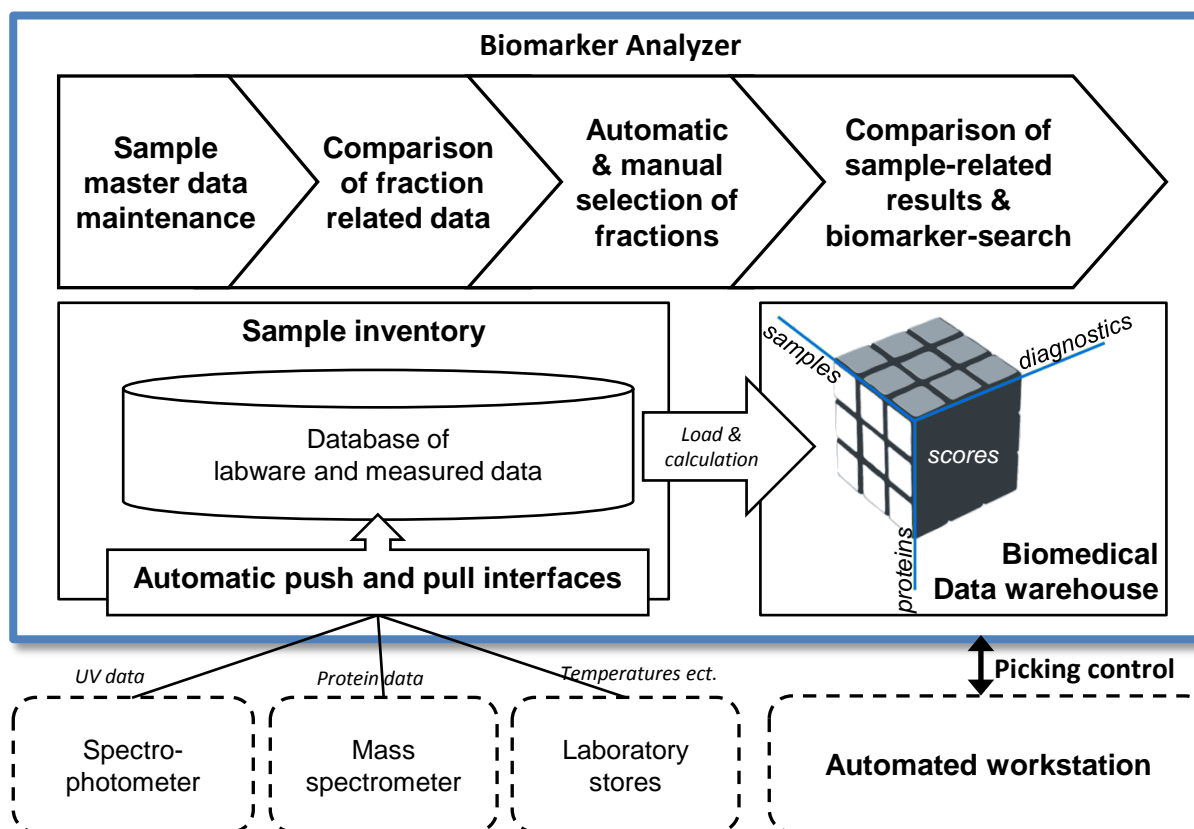


Time scale:

1 day:	1D-SEC, 4 samples, serial
1 day:	2D-AEC, 4 samples, parallel
~1 day:	3D-LAC, 10-30 fractions per sample, parallel
~10 days:	digest, 96 fractions per day, parallel
~8 weeks:	mass spectrometry, 96 fractions per week, serial
some weeks:	final data analysis

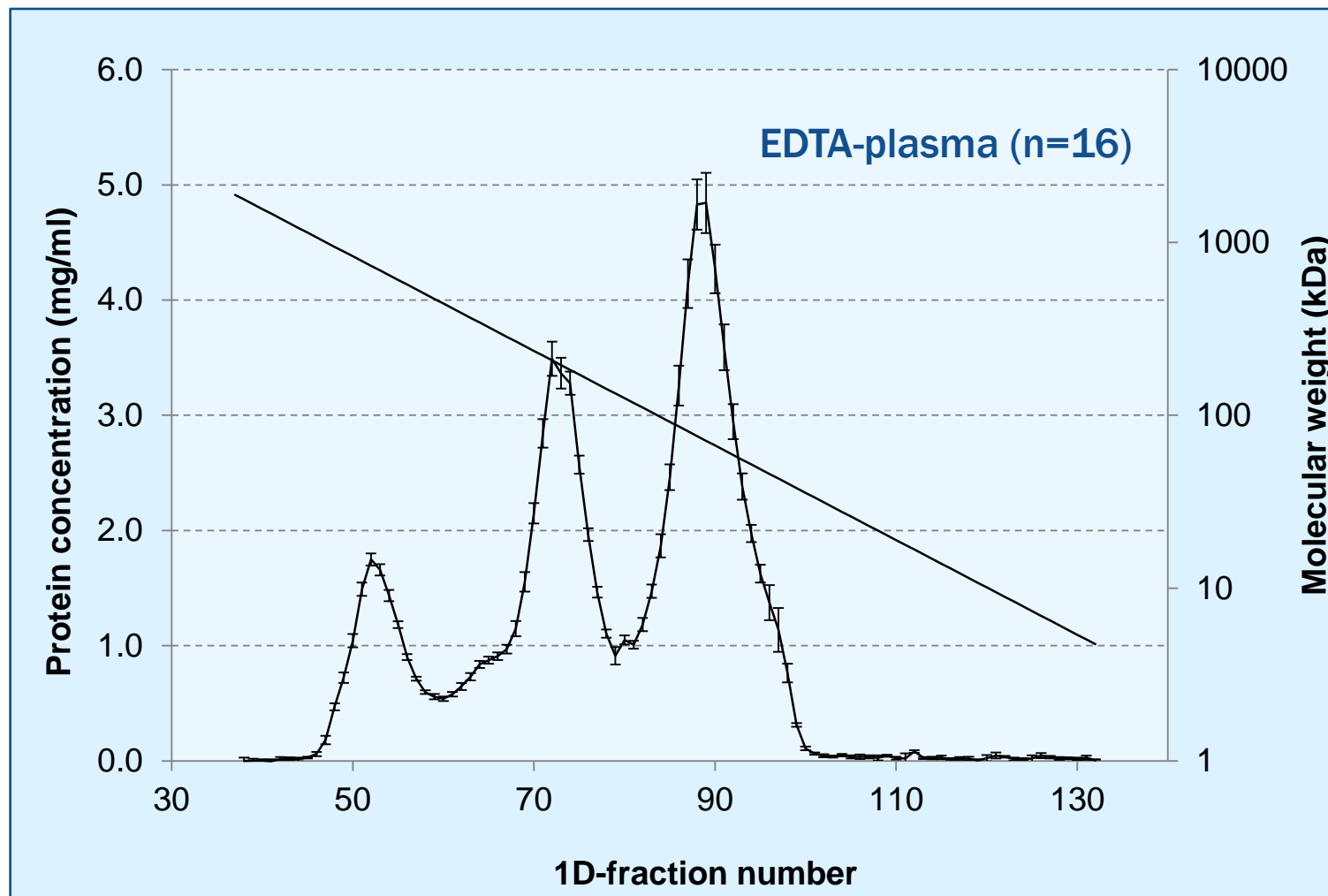


Software package “Biomarker Analyzer” for process control and analytics:

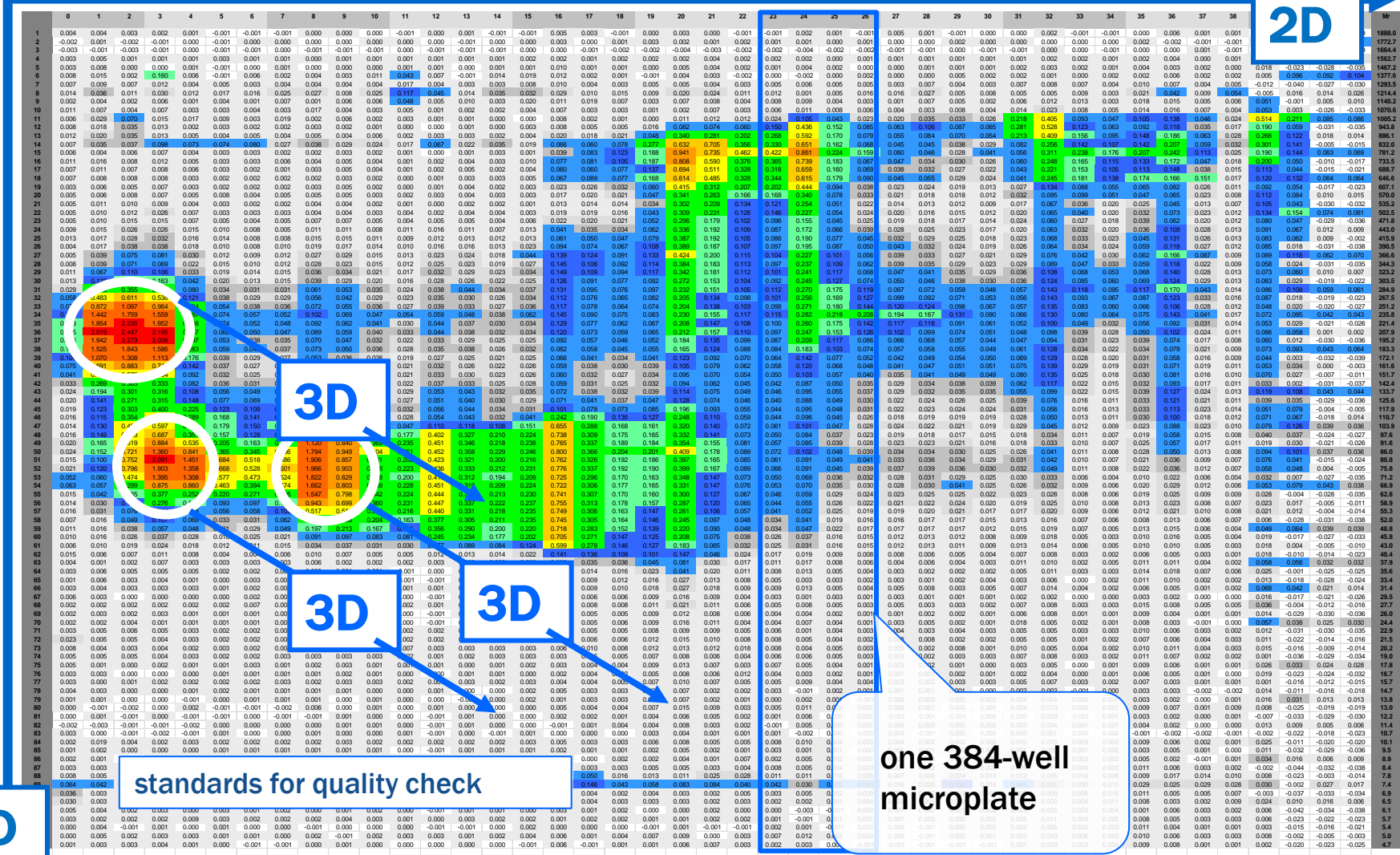




Precision of 1D-fractionation: mean and SD of protein concentrations of 1D-fractions

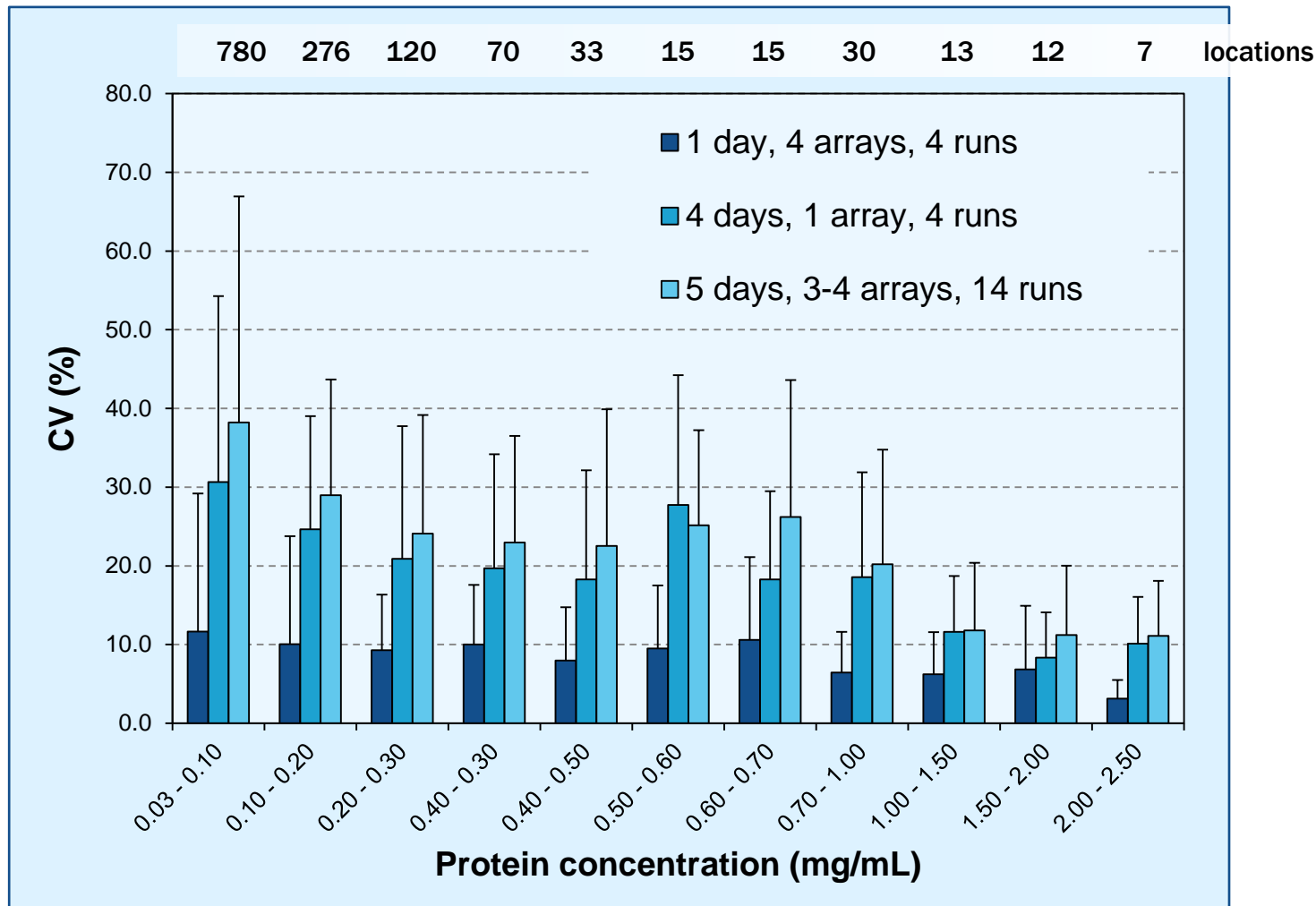


4128 2D-fractions - distribution of protein concentrations from human EDTA-plasma





Precision of 2D-fractionation: mean and SD of CV of 2D-fractions





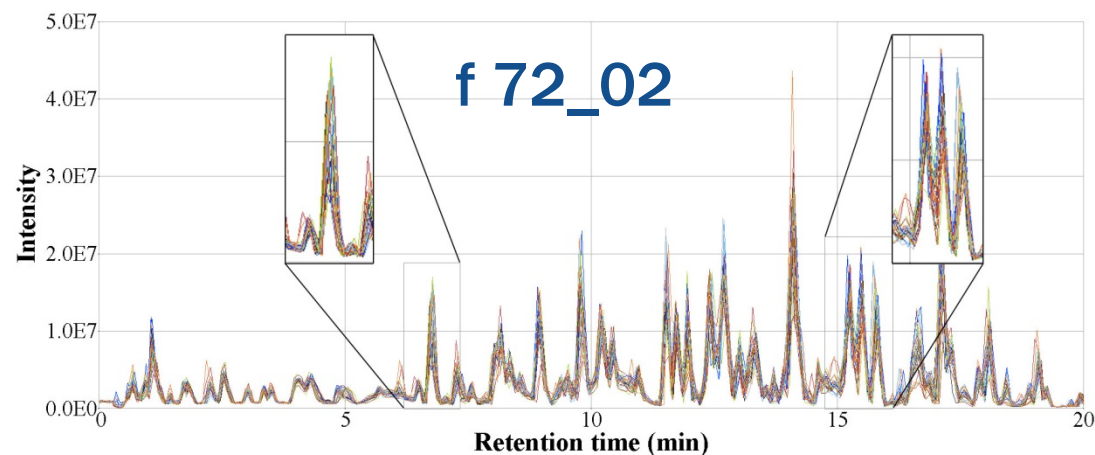
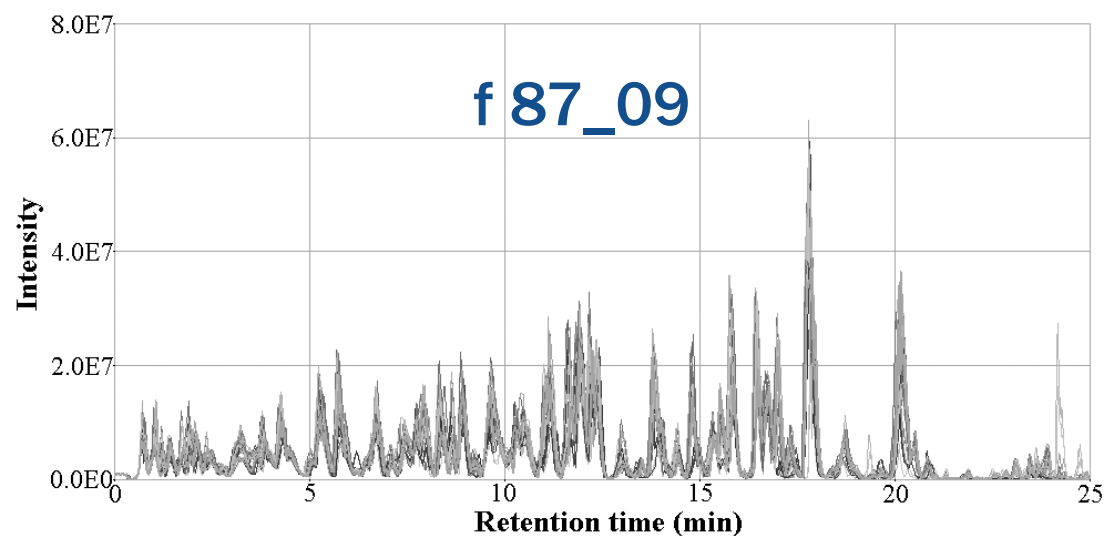
Precision of mass spec analysis

14 individual 2D runs

two randomly selected locations

14 individual digestion runs with
each location

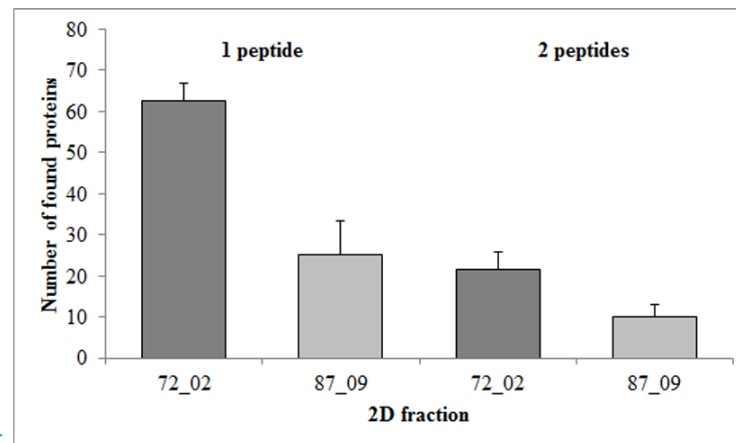
2 LC-MS runs with each fraction =
28 runs with each location





Precision of mass spec analysis

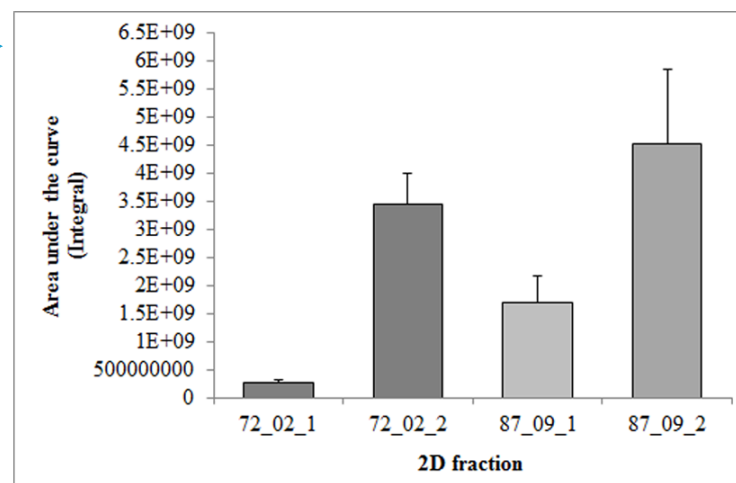
Number of identified proteins



AUC of two randomly selected
ion current peaks



CV: 24.3 15.5 28.6 28.9%

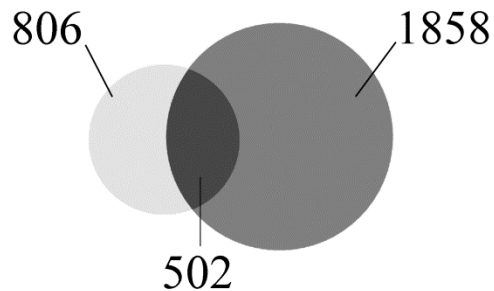


for both locations



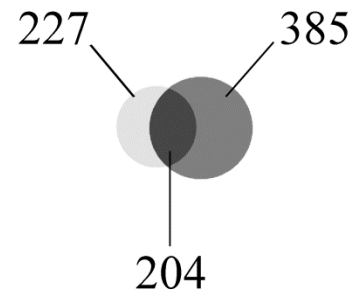
Identified non-redundant proteins:

≥1 peptide



3166

≥ 2 peptides



816

human plasma (two samples)

1370

408

human CSF (two pools)

1151

262

bovine plasma (one sample)

1044

218

goat plasma (one sample)

378

111

mouse serum (one pool)



Numbers of identified biomarker candidates

Severe sepsis/sepsis: 36

validated:

apparently low molecular weight albumin, CRISP/A1B-GP

Baum *et al.* J. Chrom. B, 2008, 876, 31

Alport syndrome: 23

validated:

several biomarker combinations

Pohl *et al.* Ped. Nephrol. 2009, 24, 903

Pohl *et al.* Ped. Nephrol. 2013, in press

End stage renal disease: 29

Hallbauer *et al.* Proteomics Clin. Appl., 2010, 4, 953

Psoriasis arthritis (in progress): up to now 10

Amyotrophic lateral sclerosis (in progress): up to now 305



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ご清聴ありがとうございました。

