Proteome Sciences

Highly Sensitive Peptidomics Profiling of Native Plasma Samples by Efficient Tandem Mass Tag Labeling Avoiding Bias and Proteolytic Artifacts

Hans-Dieter Zucht; Petra Budde; Christian Baumann; Sasa Koncarevic; Karsten Kuhn; Thorsten Prinz; Peter Schulz-Knappe
Proteome Sciences R & D GmbH & Co. KG, Frankfurt , Germany ; Poster No.: 474

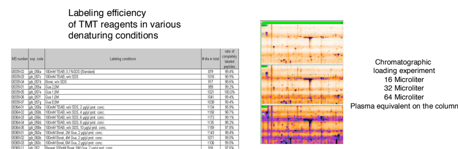
Overview

- **A routine workflow for quantitiative analysis of native peptides from blood plasma as well as from other sources has been developed**
- **Isobaric labeling with Tandem mass tags (TMT™) has been used for enabling quantification and preserving the native state of the peptidome**
- **The new peptidomics workflow follows a "*top-down*" proteomics paradigm dealing with "known" and "anonymous" common features**
- **Data integration is based on an alignment of MS/MS precursor m/z and RT properties**
- **Peptide annotation is done in a database merging biomarker statistics of the reporter ions with the sequence annotations of MS/MS consensus spectra**

Introduction

Biomarker research claims that peptides and proteolytically cleaved polypeptides carry a relevant portion of information. However, the analysis of native peptides in plasma is challenging due to its high complexity and sensitivity towards artificial proteolytic cleavage of abundant proteins. As a consequence, it has been difficult to differentiate analytical artifacts and truly regulated peptides. We have developed a novel peptidomics workflow enhancing the quantitative measurement of peptides by using TMT labeling. Since the initial steps are labeling and mixing of clinical samples the native peptide pattern is conserved quantitatively throughout the workflow. We introduce a highly efficient multi-step extraction protocol for peptides enabling the measurement of low abundant molecules such as hormones and cytokines.

Labeling efficiency of TMT reagents in various denaturing conditions



MS number	exp. code	Labeling conditions	# dta in total	ratio of completely labeled peptides
08339-02	gib_056a	100mM TEAB, 0,1%SDS (Standard)	879	99.4%
08339-03	gib_057c	100mM TEAB, w/o SDS	1018	98.9%
08339-04	gib_057d	Borat, w/o SDS	917	98.6%
08339-01	gib_055a	Gua 2,0M	959	99.2%
08339-05	gib_057e	Gua 1,5M	1021	100.0%
08339-06	gib_057f	Gua 1,0M	1041	99.4%
08339-07	gib_057g	Gua 0,5M	1038	99.4%
08364-01	gib_058a	100mM TEAB, w/o SDS, 2 µg/µl prot. conc.	1134	95.9%
08364-02	gib_058b	100mM TEAB, w/o SDS, 4 µg/µl prot. conc.	1158	98.7%
08364-03	gib_058c	100mM TEAB, w/o SDS, 6 µg/µl prot. conc.	1173	99.1%
08364-04	gib_058d	100mM TEAB, w/o SDS, 8 µg/µl prot. conc.	1135	98.2%
08364-05	gib_058e	100mM TEAB, w/o SDS, 10 µg/µl prot. conc.	1159	97.8%
08369-01	gib_060a	100mM Borat, 2M Gua, 2 µg/µl prot. conc.	1143	98.4%
08369-02	gib_060b	100mM Borat, 4M Gua, 2 µg/µl prot. conc.	1071	99.0%
08369-03	gib_060c	100mM Borat, 6M Gua, 2 µg/µl prot. conc.	1106	99.0%
08369-11	gib_062	Repeat 100mM Borat, 6M Gua, 2 µg/µl prot. conc.	916	97.6%

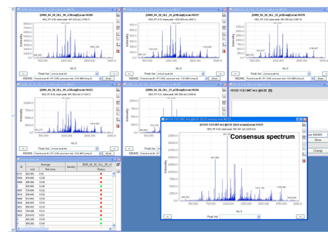
Chromatographic loading experiment
16 Microliter
32 Microliter
64 Microliter
Plasma equivalent on the column

Methods

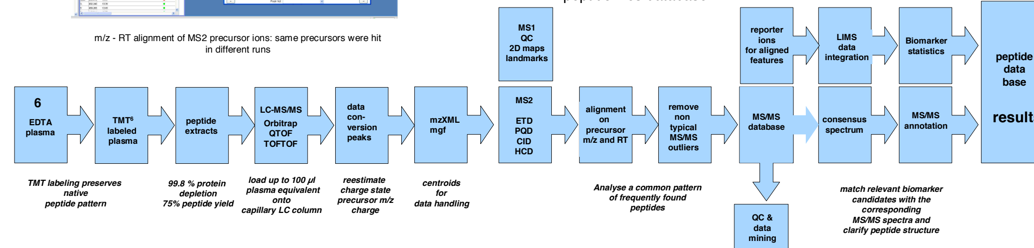
Native human plasma samples were labeled using TMTsixplex reagents under optimized conditions. After mixing of up to six samples, the extraction of peptides was achieved by using a newly developed multi-step procedure deploying a combination of membrane ultrafiltration, cation exchange as well as several reversed phase solid phase extraction steps. No trypsin digestion is performed unlike a proteomic workflow! Peptides were measured by means of nano-LC-MS/MS analysis using various electrospray and MALDI MS instrumentations.

To obtain a comprehensive peptide map the data were aligned for matching MS/MS precursor ions using the highly precise m/z-values as well as retention time and spectral similarity of the individual MS/MS spectra. For each aligned feature a consensus MS/MS spectrum was computed and the data related to the TMT-precursor ions was submitted to biostatistical analysis for a biomarker datamining. Individual MS/MS spectra were analysed for quality control.

Overall quality control was done by spiking the plasma samples with reference peptides enabling the validation of the analytical platform by MS, MS/MS and ELISA measurements. The efficiency of protein depletion was monitored by means of an albumin ELISA.

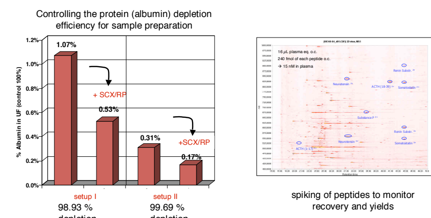


m/z - RT alignment of MS2 precursor ions: same precursors were hit in different runs



Results

Several plasma samples pools were created to investigate the typical peptide composition. We succeeded to develop a complete intact protein and peptide labelling protocol which allowed mixing of whole plasma proteome/peptidome prior to protein depletion. We further succeeded to reproducibly extract the LMW peptidome (peptides < 10 kDa) while almost completely depleting high molecular mass proteins. For the developed workflow it could be shown that the efficiency of protein depletion after ultrafiltration, cation exchange and reversed phase extraction is 99.8 % while retaining more than 75% of the spiked peptide standards. Using TMTsixplex samples the loading capacity of the LC column can be extended from sub-microliter to load a plasma equivalent volume of up to 100 microliters.



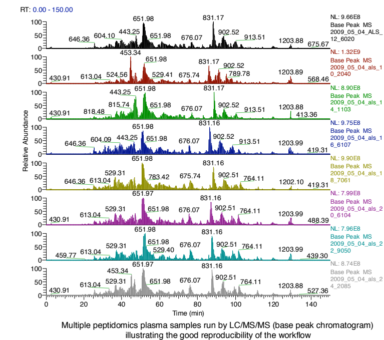
spiking of peptides to monitor recovery and yields

Consequently, for low abundant peptides of 1 pmol/liter concentration the total amount on the column is 100 attomoles. The results show the richness of the native human peptidome as well as robustness of the technology. The alignment of the MS/MS precursor ions yields typically a number of appr. 5,000 common features. The creation of consensus spectra facilitates sequence annotation and enables the creation of a comprehensive peptidomics database.

Conclusions

The analysis of native peptides in plasma is challenging due to its high complexity and sensitivity towards artificial proteolytic cleavage of abundant proteins. Therefore, we have developed a novel peptidomics workflow enhancing the quantitative measurement of peptides by using TMT labeling. Since the initial steps are labeling and mixing of clinical samples the native peptide pattern is conserved throughout the whole workflow.

Top down proteomic/peptidomic analysis requires dedicated tools for data integration and data handling, which have been successfully developed.



Multiple peptidomics plasma samples run by LC/MS/MS (base peak chromatogram) illustrating the good reproducibility of the workflow

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