# Automated sample preparation workflows for quantitative proteomics applications

Oliver Popp[1], Lucas Luethy[2], Tamara Kanashova[1], HaAn Nguyen[1], Julia Kikuchi[1], Guenter Boehm[2], Thomas Blenkers[3], Andreas Bruchmann[3], Gunnar Dittmar[1]

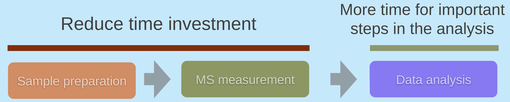
[1]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, MDC, Berlin, Germany; [2]CTC Analytics, Zwingen, Switzerland; [3]Axel Semrau GmbH, Sprockhövel, Germany

## Overview

Three reasons for automating workflows in a proteomic laboratory:

- Reduce time spent on repetitive work
- Increase consistency
- Increase reproducibility

**WORKFLOW in a PROTEOMICS lab**

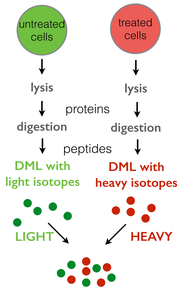



- In-solution digest √
- In-gel digest √
- **Isotopic dimethyl labelling**
- **Phospho-peptide enrichment**

Recently, we presented the successful automation of in-solution and in-gel digestion on a PAL setup (CTC, Axel Semrau; presented on the ASMS 2014). We achieved medium-high throughput with high reproducibility and very good quality data.
Here, we extended our automated sample preparation repertoire by the implementation of isotopic dimethyl labelling, and, more recently, a fast phospho-peptide enrichment strategy.

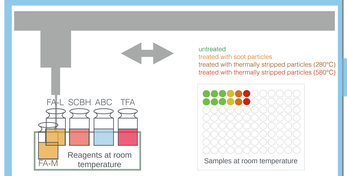
## Isotopic dimethyl labelling

Similar to a SILAC approach, dimethyl labelling (DML) is used for quantitative shotgun proteomics when SILAC is not applicable (*e.g.* tissue samples are difficult to be metabolically labelled). DML is a post-lysis labelling where peptides are modified with light (L), medium-heavy (M) or heavy (H) isotope variants of formaldehyde. This introduces a mass shift of 28 Da (L), 32 Da (M) or 36 Da (H), which can be analysed similarly to a SILAC sample.
Here, the change in protein abundance of mouse macrophages was determined upon treatment of the cells with particles from ship engine exhaust fumes.




Heavy and light versions are combined and analysed together. Isotopical peptide species elute together. Quantitation is done on MS level.

Automation:
Schematics of the RTC PAL setup for the dimethyl labeling.



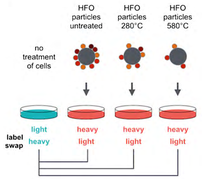

FA-L: formaldehyde "light"
FA-M: formaldehyde "medium-heavy"
SCBH: sodium cyanoborohydride
ABC: ammonium bicarbonate
TFA: trifluoroacetic acid

A representation of an RTC-PAL setup. Each module is variable and thus the robotic setup can be extended by introducing new tools.

Transfer of liquids is achieved by a syringe tool. Syringes of different volumes can be automatically exchanged during the process and are washed in a washing station with organic solvent and water.

## Application of the automated dimethyl labelling

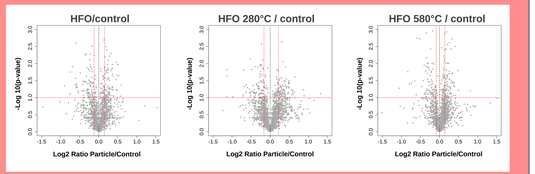
***Impact of heavy fuel oil derived combustion particles on macrophages***
Tamara Kanashova & Oliver Popp & Jürgen Orasche & Erwin Karg & Horst Harndorf & Benjamin Stengel & Martin Sklorz & Thorsten Streibel & Ralf Zimmermann & Gunnar Dittmar
*Analytical and Bioanalytical Chemistry 2015*




Mouse macrophages were exposed to heavy-fuel oil combustion particles (HFO) and particles that have been thermally stripped of their organic surface.
The composition of the particles strongly defines their ecological impact and the effect on human health.

Treated cells were labelled with heavy and untreated samples with light formaldehyde in the automated DML procedure and subsequently pooled. A label swap experiment was carried out in parallel.

### Quantification




Heavy/light ratios were calculated and statistically analysed. Outliers represent potentially regulated candidate proteins.
Our quantitative data from the dimethyl labelling experiment was - in part - complementary to a SILAC data set which was acquired in parallel.

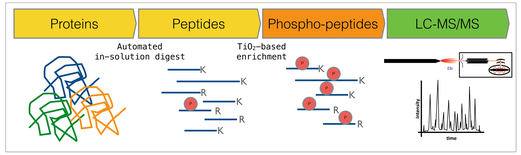
g e n e  o n t o l o g y  a n a l y s i s

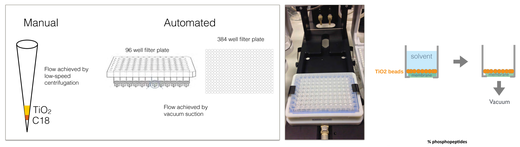
- Regulation of catabolic processes and reactive oxygen species metabolic processes.
- Induction of RNA-related processes, electron transport chain processes, and regulated a number of mitochondria-associated processes.
- Mainly regulation of proteins associated with intracellular trans- port and macromolecular organization.

## Phospho-peptide enrichment

Protein function can then be regulated by covalent modification of amino acids. These post-translational modifications (PTMs), *i.e.* the addition of small chemical groups like phosphorylation, methylation or acetylation, can serve as a switch of a protein's activity or binding ability. Phosphorylation is the best studied and probably most common PTM. Phosphorylation occurs on serine, threonine and tyrosine residues and is implicated in all cellular processes. The phosphorylation status of proteins strongly regulates their function (especially in intracellular signalling) and is therefore studied thoroughly especially to understand signalling-dependent diseases. Recent developments in the field of proteomics allow the large scale identification of phospho-peptides leading to sophisticated models of regulatory networks within the cell (Olsen et al., Cell, 2006). Most of the studies are hampered by the lack of large scale, automated enrichment techniques making this step one of the most time consuming steps in the analysis. Enrichment-strategies are diverse and involve the digestion of the proteins and the binding and release of the resulting peptides to affinity matrices. We adapted the titanium dioxide-based technique for an automation approach.

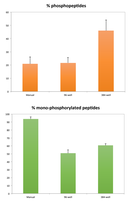
**Titanium dioxide-based method**
*Modified from Kelstrup et al. (Protocol prime XS)*




We compared a manual, stage-tip based procedure with the automated procedure where a 96-well format was compared with a 384-well format. While in the manual procedure centrifugation is used for enabling solvent flow, in the automated version on the PAL, a vacuum chamber is used.

We obtained comparable numbers of phospho-peptides comparing the manual with the automated 96-well setup but higher numbers by using the 384-well plates. Also, the number of multi-phosphorylated peptides was higher in the automated procedure.




## Summary

The PAL system provides an affordable and reliable platform optimised for medium-throughput peptide preparation for shotgun-proteomics based mass spectrometry.