Proteomics Facility

Operator and User Regulations

The Proteomics Core Facility (PF) of the IZKF Aachen provides access for all members of the RWTH Aachen University to proteomic technologies in order to facilitate the analysis and investigation of protein function on a molecular level in health and disease. The facility provides advice and support for the generation of such experimental setups and workflows based on the individual scientific question of the customer. This includes advice and suggestions for the process of appropriate sample generation. The acquired data is subsequently discussed with the researchers. Furthermore, the PF contributes to the explanation and advice on data interpretation and possible follow-up experiments. The PF also offers experimental support in protein enrichment strategies such as co-IPs and affinity purification as well as PTM-specific protein and peptide enrichment strategies (such as phosphopeptide analysis).

1. **Sample analysis requests:**

All researchers have to contact the PF via email or phone (see contact details below) if they are interested in any form of proteomic analysis. The PF will then discuss the biological question, the necessary sample preparation, the required analysis procedure and the estimated costs with the respective researchers. After a decision has been made on a particular form of sample analysis, the respective researchers have to submit a signed "sample submission form" describing the nature and state of the individual samples (available on request), the planned sample analysis and an agreement to cover the costs for the analysis. Furthermore, a signed certificate of compliance regarding S1 regulations has to be provided. Samples that require S2 handling or contain radioactivity will not be analysed.
The PF is open to the entire RWTH Aachen University. However, the PF has the right to refuse sample analysis if the project is ill-defined, too time-intensive (e.g. 2-3 months of consecutive measurement time for one large experiment) or lacks the required funding.

2. **Cost of sample analysis:**

The individual groups have to carry the expenses for all the reagents required for sample analysis. These include - not exclusively - proteases (mainly trypsin and LysC), labelling reagents (formaldehyde (+/- Deuterium and ^{13}C) and cyanoborohydride (+/- Deuterium)), nanoLC columns, chromatography material (e.g. TiO_2 and/or Ti-IMAC for phosphopeptide enrichment), precasted gradient gels and others. Since the PF is required to employ refinancing measurements, the subsequent sample analysis will be charged at a time dependent rate ("hours of instrument-time"), depending on the number of samples (see table below). The current fee is 25 €/hour for members of the RWTH Aachen University (according

to DFG-guidelines), 37.5 €/hour for external users from other universities and 50 €/hour for commercial users. The cost for bioinformatics analysis is also 25 €/hour for members of the RWTH Aachen University (according to DFG-guidelines), 37.5 €/hour for external users from other universities and 50 €/hour for commercial users.

3. Sample submission:

The experimental work required for sample generation is usually carried out in the individual laboratories (including the expression of recombinant proteins in bacteria or eukaryotic host systems and their application in *in vitro* assays, the growing and potential treatment of cells in cell culture, treatment of transgenic mice vs control animals and isolation of the relevant organs/tissues, amongst others), not the facility itself.
Samples that are “ready-to-use” (these may include Coomassie stained protein gels, protein lysates/solutions with or w/o sample buffer, organs, tissues or similar) can be submitted to the PF. All users submitting samples have to employ quality control measurements and procedures to ensure sufficient sample quality. Samples meeting the quality criteria will be prepared in the PF for their respective analysis.

4. Order of sample analysis:

Samples are usually analysed in a timely order depending on their date of submission. Exceptions to this order (at the discretion of the head of the PF) are made for samples that have to be measured for manuscript revisions or similar deadlines.

5. Working in the laboratory of the PF:

As described above, most of the work for sample generation is carried out in the individual labs of the respective researchers. These experiments cannot be performed in the PF lab. The PF has to operate in a very clean environment in order to minimize potential sources of contamination (such as dust, hair or skin particles) and can therefore not be considered a “standard wet lab”. In case participating researchers need to perform specific parts of their sample generation within the lab of the PF, this needs to be discussed in advance. All personnel working in the lab is required to adhere to federal health and safety regulations and obey S1 regulation rules. It is also necessary to participate in the IZKF safety instruction lecture before working in the PF lab.

6. Instrument operation:

The PF harbours expensive equipment that requires a significant long-term experience for operation. Therefore, both the chromatography systems as well as the mass spectrometers are exclusively operated by the staff members of the PF.

7. Core facility contribution:

Routine analysis in the PF is performed as a service (such as repeated measurements of a particular protein in a number of plasma samples). All work requiring a significant scientific input is carried out in form of a collaboration between the respective research groups and the PF.

Hinweis / please note:
All users of an IZKF core facility have to acknowledge the support of the respective facility in all relevant publications. Please use the following wording:

„Diese Arbeit wurde unterstützt durch die Proteomics core facility eine Core Facility des Interdisziplinären Zentrums für Klinische Forschung (IZKF) Aachen der Medizinischen Fakultät der RWTH.“
“This work was supported by the Proteomics core facility, a core facility of the Interdisciplinary Center for Clinical Research (IZKF) Aachen within the Faculty of Medicine at RWTH Aachen University.”

Experimental approaches offered by the facility

- Gel electrophoresis of protein samples

- Lysis and proteolytic digestion of protein samples in-solution, in-gel or on-bead (proteases: Trypsin, LysC, GluC,…); the PF uses standard lysis conditions such as urea or the FASP protocol. Alternative protocols (such as the SP3 or iST methods) will be used for the analysis of samples with low concentrations (sub µg-range, <100000 cells, etc.).

- Sample purification and desalting (C18); small (zip-tips) to large scale (C18 cartridges)

- Sample concentration

- Sample depletion (plasma/serum)

- Sample labelling: If protein quantitation is required within the particular project, peptides can be modified using isobaric tags (TMT or iTRAQ; maximum 8-plex). Furthermore, peptides can also be isotopically labelled using the dimethyl stable isotope labelling method. Cells that have been grown in SILAC medium can be used as an alternative. However, the label-free quantification approach is usually the preferred choice of quantitation for most analyses.

Sample fractionation/enrichment – peptide chromatography:

- The PF provides peptide separation using high pH reversed phase chromatography or strong anion/cation exchange (SAX/SCX) or similar techniques (such as HILIC, WAX) in both small scale (pipette tip format) as well as large scale (HPLC).

- The PF also provides phosphopeptide enrichment using either a TiO_2 based chromatographic approach (mainly for peptides phosphorylated on serine and threonine residues) or anti-phosphotyrosine antibody mediated immunoprecipitation of peptides phosphorylated on tyrosine residues, followed by subsequent mass spectrometry analysis and quantification of phosphorylation changes.

Mass spectrometry:

- Analysis of desalted/purified samples by nanoLC-MS/MS using standardized methods and gradients. All samples are analysed on either the Q Exactive Plus or the Orbitrap Elite system. Both mass spectrometers are coupled to a nano-UPLC system.

Data analysis:

- Analysis of the raw data is performed by the PF in house using designated software packages (MaxQuant (with the built in Andromeda search engine) and Perseus). This includes protein (and PTM) identification and their respective quantification. The resulting

data is explained to and discussed with the researchers. Furthermore, the facility provides advice on data interpretation and possible follow-up experiments.

- Storage of raw and analysis data in house (IZKF cloud) on separate hard drives for access at later time points.

Special services:

- The PF can also provide Western Blot analysis for groups that are not equipped with the required instrumentation. The PF also provides technical assistance and expertise for “unusual” biological questions in the field of protein biochemistry, including non-common protein chromatography and in vitro assays.

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Table 1: Price list for reagents, experimental approaches and instrument time in the Proteomics Facility

Type	Service	Quantity	Details	Price (€)		
User				**RWTH**	**Extern academia**	**Extern commercial**
Sample preparation	SDS Page gradient gel	1	Separation of samples on a 4-20% gradient gel	20	30	40
	In-gel digest	1 >10 >30	Tryptic digest of a single gel band/region and desalting	12/pc. 10.8/pc. 9/pc.	18 16.2 13.5	24 21.6 18
	In-solution digest	1 >10	Tryptic digest of a protein lysate (100 µg)	22 16.5/digest	33 24.8	44 33
	On-bead digest	1	Tryptic digest of proteins enriched on agarose/sepharose beads	18	27	36
	Peptide fractionation	1 >10	Fractionation using high pH C18 chromatography (8 fractions)	44 33/fractionation	66 49,5	88 66
	Phosphopeptide enrichment	1	Enrichment of phosphorylated peptides (residues: serine and threonine), 1 mg; incl. in-solution digest	58	87	116
	Phosphopeptide enrichment (pTyr)	1	Enrichment of phosphorylated peptides (phosphotyrosine) from 6 mg protein lysate; incl. in-solution digest and immunoenrichment using 4G10 antibodies	320	480	640
	Isobaric labelling		Isobaric labelling of peptides (TMT/iTRAQ)	On request	On request	On request
	Serum depletion	1	Depletion of 12 most abundant serum proteins (10 µl sample)	48	72	96
	Peptide preparation from low sample amount	1	Samples with < 1E06 cells (e. FACS-derived material), preparation of tryptic peptides using the iST protocol	42	63	84
MS measurement	Protein identification	1 hour >100 hours	Protein identification(s) with an Orbitrap MS instrument coupled to a nano-LC system	25 20/hour	37.5 30	50 40

Other information (prices of dimethyl labelling reagents or alternative proteases (such as GluC), amongst others) is available on request.