Proteomics Facility

Operator and User Regulations

The Core Facility "Proteomics" (PF) of the IZKF Aachen provides all members of the medical faculty of RWTH Aachen with access 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 experimental set-ups and workflows based on the customer's individual scientific question. This includes advice and suggestions for the process of appropriate sample generation. Acquired data is subsequently discussed with the researchers. Furthermore, the PF offers 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:

Researchers should 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 submit a signed "sample submission form" describing the nature and state of the individual samples, the planned sample analysis and an agreement to cover the analysis costs. Furthermore, a signed certificate of compliance regarding S1 regulations is to be provided. Samples that require S2 handling or contain radioactivity will not be analysed.

The Proteomics Facility is open to the entire medical faculty. 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 if the investigator lacks financial means.

2. Cost of sample analysis:

Individual groups cover all expenses for 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 invoiced at an hourly rate ("hours of instrument time"), depending on the number of samples.

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) not at the facility itself.

Samples that are "ready-to-use" (these may include Coomassie stained protein gels, protein lysates/solutions with or without sample buffer, organs, tissues or similar) may be submitted to the PF. The samples are then prepared for their respective analysis.in the PF

4. Order of sample analysis:

Samples are usually analysed in order of the date of submission. Exceptions to this order (at the discretion of the Director of the PF) are made for samples that must be measured for manuscript revisions or similar deadlines.

5. Working in the PF laboratory:

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 must 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 PF lab, this is to be discussed in advance. All personnel working in the lab are required to adhere to federal health and safety regulations and obey S1 regulations. It is also necessary to participate in IZKF safety training before working in the Proteomics facility.

6. Instrument operation:

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

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 significant scientific input is carried out in the form of a collaboration between the respective research groups and the Proteomics Facility.

Please note:

IZKF Core Facility users are obliged to refer to the support provided by the facility as an acknowledgement in their publications. Please use the following wording:

This work was supported by the Core Facility "Proteomics", a core facility of the Interdisciplinary Center for Clinical Research (IZKF) Aachen within the Faculty of Medicine at RWTH Aachen University.

OR

Diese Arbeit wurde unterstützt durch die Core Facility "Proteomics, eine Core Facility des Interdisziplinären Zentrums für Klinische Forschung (IZKF) Aachen der Medizinischen Fakultät der RWTH.

Experimental approaches offered by the facility

Sample preparation:

Depending on the "state" of the submitted sample.

- Gel electrophoresis of protein samples
- Proteolytic digestion of protein samples in-solution, in-gel or on-bead (proteases: Trypsin, LysC, GluC,…)
- Sample purification and desalting (C18); small (zip-tips) to large scale (C18 cartridges)
- Sample labelling: if protein quantitation is required within the particular project, peptides can be isotopically labelled using the dimethyl stable isotope labelling method. Cells that have been grown in SILAC medium can be used as an alternative.

Sample enrichment - peptide chromatography:

- The facility provides separation using strong cation exchange (SCX) or similar techniques (weak anion exchange, (hydrophilic) strong anion exchange (hSAX), immobilized metal ion affinity chromatography (IMAC), among others.
- The facility also provides phosphopeptide enrichment using TiO2 or Ti-IMAC based chromatographic technology (Ser, Thr) as well as antibody-based phosphotyrosine peptide enrichment and subsequent quantification of phosphorylation changes.

Mass spectrometry:

- Analysis of purified samples by nanoLC-MS/MS. All samples are currently analysed on the Orbitrap Elite system.

Data analysis:

- Analysis of raw data is performed in-house using the MaxQuant software package. This includes protein (and PTM) identification and their respective quantification.
- Storage of raw data in-house (IZKF cloud) on separate hard drives for future access

Special services:

- The facility can also provide Western Blot analysis for groups that are not equipped with the required instrumentation.

- We also provide 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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