f·g·c·z proteomics

newsletter

Technologies, Applications, and Access to Support

Proteomics at the Functional Genomics Center Zurich

OVERVIEW

INTRODUCTION

The Functional Genomics Center Zurich (FGCZ) is operational since 2002 as a joint research and training facility of the ETH Zurich and the University of Zurich. The center is located on the Irchel campus of the University of Zurich. The FGCZ offers access to technologies, provides training, and collaboratively develops new analytical approaches in the areas of genomics, transcriptomics, proteomics, and metabolomics. There are several access modes to perform research projects at FGCZ.
The Analytical Service mode (www.fgcz.ch, Support Modes) provides fee-based sample analysis for all research groups at University of Zurich and ETH Zurich. The User Lab mode provides teaching and access to specific analytical technologies and data analysis tools and strategies. Project application is handled via the FGCZ web platform called B-Fabric and it is open to all academic institutions in Zurich.

WHY PROTEOMICS?

Proteomics aims to elucidate the entire content of proteins in cells, organs or organisms, including protein abundances, protein modifications and protein-protein interactions, at a given time. This is a challenging approach because the overall protein content differs in different organs or cell types, and it changes over time and conditions. In addition, proteins appear in multiple splice variants, vary in abundance, and almost all proteins carry post-translational modifications (PTMs) such as e.g. acetylation, glycosylation or phosphorylation. The rapid development of new instrumentation and bioinformatic tools have made mass spectrometry (MS)-based proteomics an essential technology to study complex molecular and cellular processes in living cells and organisms. Currently there are two analytical approaches that are widely used in proteomics namely the discovery (or shotgun) and the targeted proteomics methods. The discovery analysis offers a first estimation of sample complexity and protein dynamic range enabling the identification of several thousands of proteins in any cell type or organism and comprehensive quantification of some hundreds of proteins. If measured across multiple samples, the obtained abundances provide key information about chemical or biologically induced perturbations and pinpoint pathways affected over time or across conditions.
The targeted proteomics workflow is optimized to detect and quantify a smaller set of proteins of interest (i.e protein network in central metabolism or biomarker candidates), but with a higher sample throughput (n> 100) than discovery proteomics. Targeted proteomics has recently emerged as a powerful tool in systems biology studies and biomedical research, since large cohorts of samples need to be analysed in a high throughput manner to validate prior biological findings.

Do you want to know more about Proteomics at FGCZ, or about FGCZ in general?
Do not hesitate to contact us at:
proteomics@fgcz.ethz.ch

PROTEOMICS AT FGCZ

Protein identification

What is the protein complexity and identities of my sample? This is one of the most common questions a biologist has to answer. The scope could be heterogeneous (i.e.: protein characterization, protein-protein interaction studies, proteome-wide identifications), but the workflows for protein identification are few and well established, and act as fundamental tools for the researchers seeking their answers. Protein identification is normally achieved through enzymatic digestion of proteins into peptides, subsequently analysed by liquid chromatography-mass spectrometry (LC-MS). A preliminary fractionation of the protein sample (at the protein or at the peptide level) must be performed for a deeper coverage of the proteome, despite the development of always faster and more sensitive MS technologies.
The FGCZ can support you in every step of analysis of your protein sample, from protein/proteome identification to the characterization of purified proteins, from the amino acid analysis to Edman degradation sequencing.

Relative and absolute protein quantification

Relative as well as absolute protein quantification can effectively be performed using stable isotope labeling. Isotopes can be introduced metabolically at protein level, or chemically at peptide level (i.e., SILAC (Stable isotope labeling by amino acids in cell culture)[1], or iTRAQ (Isobaric tag for relative and absolute quantitation)[2]). These labeling approaches allow the analysis of multiple conditions in one single LC-MS run, minimizing the technical variation introduced.
Alternatively, protein abundances can be obtained using an approach named label free quantification, a technique that relies on comparison of consecutive sample data acquisitions. This approach has the advantage of being less labor intensive and more flexible when comparing multiple conditions, but requires high reproducibility between the LC-MS/MS analyses. The immediate access to quantification data and its low cost makes it a favorable approach for initial proteome analysis.
If a specific subset of proteins are of interest, a targeted analysis could be a better option, with its higher quality

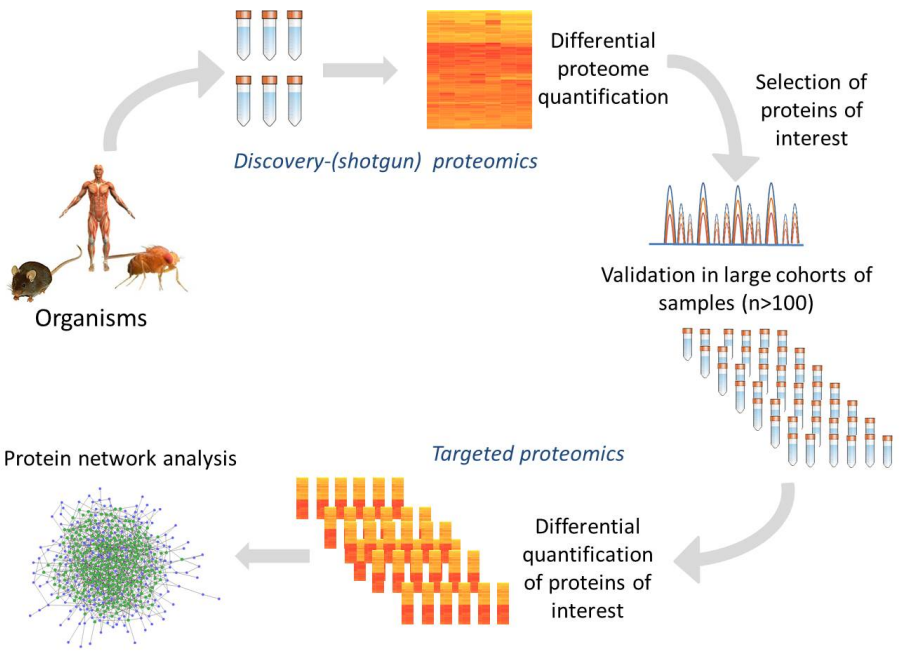


Fig. 2: general workflow for the idenfication and quantification of proteins differentially expressed in 2 or more conditions, followed by MS validation of the results.

quantification data output. Selected Reaction Monitoring (SRM [3]) is a well established technology and several other emerging techniques are now more widely applied, foremost: DIA (Data Independent Acquisition) [4], SWATH (Sequential Window Acquisition of All Theoretiocal fragment ion spectra [5])) and PRM (Parallel Reaction Monitoring [6]).
At FGCZ, multiple software and workflows are supported to fully make use of label-based or label-free experiments as well as targeted approaches to promote generation of accurate and meaningful quantitative proteomics data to the research community.

Post translational modifications

Post-translational modifications (PTMs) are known to have pivotal roles in cellular physiology and diseases, but only a few of the over 300 currently known modifications are extensively investigated in proteomics studies. Some of the most common PTMs are phosphorylation, glycosylation, ubiquitination, methylation, acetylation and disulfide bridge formation. PTMs can be identified by mass spectrometric techniques and database searching of tandem MS data. Since PTMs are generally present at low stoichiometry level, modified proteins or peptides often have to be enriched.
To facilitate the identification and the localization of the modification site in case of labile PTMs, a combination of different MS fragmentation techniques (HCD, ETD, CID) has to be applied. With such state of the art technologies, the study of PTMs on both single protein or proteome level is possible.

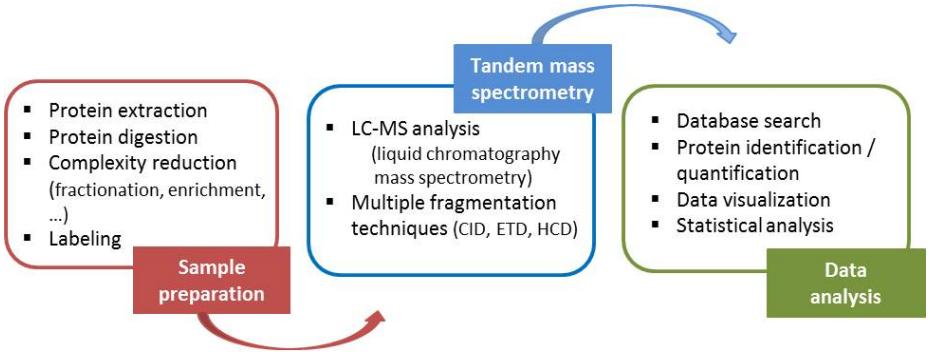
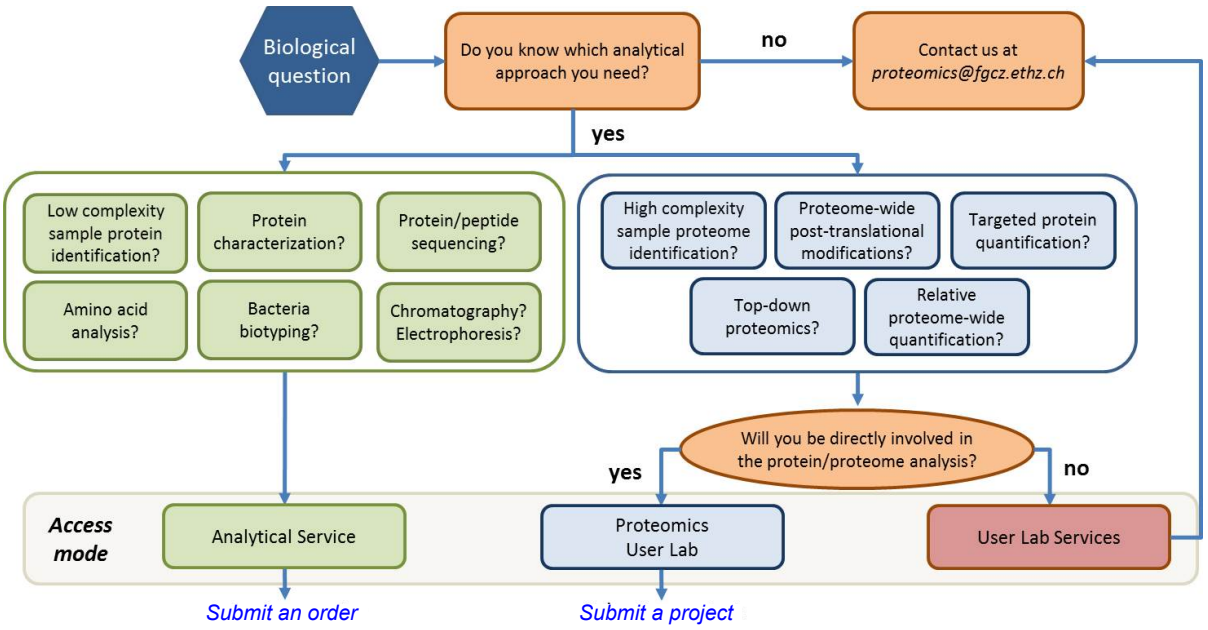


Fig. 3: overview of the main proteomics sample preparation, mass spectrometry and data analysis steps

HOW TO ACCESS THE FGCZ PROTEOMICS FACILITY



HIGHLIGHTED CURRENT PROTEOMICS PROJECTS AT FGCZ

Global analysis of proteolysis in wound healing (Werner S., Auf dem Keller U. / ETH D-BIOL)

Proteases are crucial mediators of cellular responses at every stage of cutaneous wound healing. To better understand proteolysis in wound repair, the researchers use Terminal Amine Isotopic Labeling of Substrates (TAILS), a novel system-wide quantitative technology for the identification of protease substrates in complex samples using mass spectrometry.

"Cumulomics": A mass spectrometric approach for non-invasive assessment of single oocytes' developmental competence (Walter J., Bleul U. / UZH Vetsuisse Faculty)

The focus of this study is to investigate the metabolism of oocytes and their surrounding cumulus cells during maturation. Novel biomarkers in cumulus cells shall be identified to predict the developmental competence of the corresponding oocyte. Proteomic analysis are performed by liquid chromatography followed by tandem mass spectrometry.

Investigation of heme toxicity and protective pathways (Schaer, D. / UZH University Hospital)

Free hemoglobin and heme have multiple toxic and pro-inflammatory activities and may be an important determinant of multiple disease phenotypes. In this project we examine the global response of cells and tissues to heme exposure in different disease models. The aim of the studies is to identify the key adaptive and maladaptive pathways that may be targeted in future therapeutic interventions.

Circadian clock mediated regulation of the Arabidopsis proteome and transcriptome (Gruissem W., Baerenfaller K. / ETH D-BIOL)

In this project the group investigates how the circadian clock regulates metabolic pathways and influences growth in the model plant Arabidopsis thaliana. In addition to other parameters, changes in the proteome and transcriptome are measured in response to day-length (photoperiod) and in mutants that modify clock function.

Establishing Targeted Proteomics in Drosophila by SWATH-MS (Basler K., Brunner E. / UZH Science Faculty)

SWATH-MS [5] is a data independent acquisition (DIA) mass spectrometric method that generates, in a single measurement, a complete recording of the fragment ion spectra of all the analytes detectable in a biological sample. The capabilities of SWATH-MS and selected reaction monitoring (SRM [3]) mass spectrometry to quantify proteins in Drosophila melanogaster are investigated

Establishment of a method for the detection of ADP-ribosylated proteins during cellular stress (Hottiger, M. / UZH Vetsuisse Faculty)

A comprehensive understanding of the molecular mechanism and cellular functions of ADP-ribosylation, a reversible protein post-translational modification, is still missing. The aim of this project is to establish fast and reliable assays for the identification and quantification of specific ADP-ribosylated proteins in physiological and pathological conditions by using an MS/MS approach.

Measuring protein turnover by dynamic stable isotope labeling and targeted mass spectrometry (Aebi M. / ETH D-BIOL)

The group developed a sensitive mass spectrometry method to monitor turnover of subunits in protein complexes in the yeast Saccharomyces cerevisiae. The method applies SILAC-based pulse-chase experiments followed by selected reaction monitoring (SRM [3]) mass spectrometry.

Applying selected reaction monitoring to determine the effect of brain specific microRNAs on potential target proteins in the mouse hippocampus (Mansuy, I. / ETH D-HEST)

The formation of long-term memory is highly dependent on protein synthesis, a process tightly regulated by a variety of regulators, including microRNAs. The effect of hippocampal microRNA infusions in mice on the expression of target proteins is investigated using selected reaction monitoring (SRM [3]). The aim of this project is to experimentally validate predicted miR target proteins, and compare mRNA and protein expression levels to further elucidate the mechanisms underlying miR induced regulation.

MASS SPECTROMETRY PARK

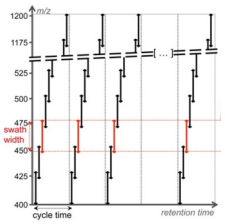
for more details check our website: *www.fgcz.ch/equipment/massspecs*

Instrument	Main applications
LTQ-Orbitrap XL ETD *(Proteomics User Lab)*	• Protein identification • Post translation modification (PTM) analysis
LTQ-Orbitrap Velos *(Proteomics User Lab)*	• Protein identification • Relative protein quantification
LTQ-Orbitrap Velos ETD *(Proteomics User Lab)*	• Protein identification • Relative protein quantification • Post translation modification (PTM) analysis • Middle-Down Proteomics
Q-Exactive *(Proteomics User Lab)*	• Protein identification • Relative protein quantification • Post translation modification (PTM) analysis
Q-Exactive *(Proteomics User Lab & Metabolomics)*	• Protein identification • Relative protein quantification • Targeted quantification • Data independent proteomics (DIA) • Metabolomics
Orbitrap Fusion *(Proteomics User Lab)*	• Top/middle-down proteomics • Post translation modification (PTM) analysis • Data independent proteomics (DIA) • Quantitative proteomics
4800 MALDI ToF ToF *(Proteomics User Lab)*	• Glycan analysis • Protein identification • Sample Quality checks
QTRAP 5500 *(Proteomics User Lab)*	• Selected Reaction Monitoring (SRM) for absolute / relative protein quantification
TripleTOF 5600 *(Proteomics User Lab)*	• Protein identification-quantification • SWATH analysis
TSQ Quantum Ultra EMR *(Proteomics User Lab)*	• Selected Reaction Monitoring (SRM) for absolute / relative protein quantification
TSQ Vantage *(Proteomics User Lab & Metabolomics)*	• Selected Reaction Monitoring (SRM) for absolute / relative protein quantification
Synapt G2 HDMS QTOF *(Metabolomics)*	• ion mobility MS (IMS) analysis of isobaric compounds lipids /oligosaccharides • data dependent-independent acquisition (MS2,MS3,MSe) • metabolite analysis in positive or negative mode • polar, ionic, non-polar metabolites
Micromass GCT Premier *(Metabolomics)*	• automated scheduled derivatization and injection of metabolite extract
MALDI Autoflex *(Protein Service Analysis)*	• Rapid identification of bacteria (Biotyping, protein-profiling) • Analysis of intact proteins • Oligonucleotides
MALDI-ToFToF UltrafleXtreme *(Protein Service Analysis)*	• Protein identification • Post translation modification (PTM) analysis • Rapid identification of bacteria (Biotyping, protein-profiling) • intact protein characterization: e.g., N- and C-terminal sequencing using topdown-sequencing, disulfide bond alignement in proteins • Oligonucleotides
Synapt G2 HDMS QTOF *(Protein Service Analysis)*	• Characterization of intact proteins
Q-Exactive *(Protein Service Analysis)*	• Protein identification • Relative protein quantification • Post translation modification (PTM) analysis

TOP NOTCH FGCZ TECHNOLOGIES AND USER LAB WORKFLOWS

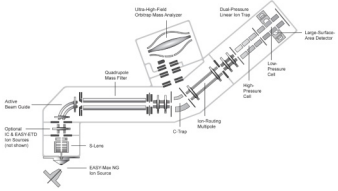
MS/MS ALL with SWATH (TripleTOF)

TripleTOF 5600
(AB Sciex)



Versatility and sensitivity (Orbitrap Fusion)

Orbitrap Fusion
(Thermo Scientific)



Targeted quantitative analysis

Q Exactive
(Thermo Scientific)

QTRAP 5500
(AB Sciex)

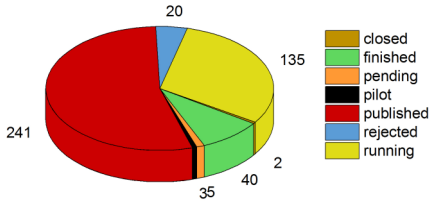
TSQ Vantage
(Thermo Scientific)

FGCZ PROTEOMICS AT A GLANCE:

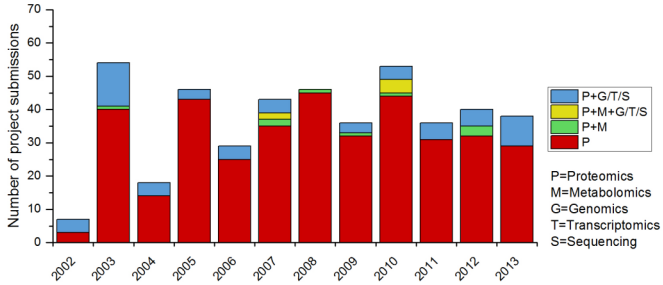
PROTEOMICS USER LAB (2002-2013)

- Total number of projects: 446
- Currently running projects: 135
- Organizations: 21
- Departments: 58
- Institutes: 114
- Published projects 241
- Projects including other OMICs technologies 73

Status of FGCZ projects



Proteomics project submissions per year



Project submissions by:

Organization	#
University of Zurich	220
ETH Zurich	159
University Hospital Zurich	34
ETHZ/UZH	12
University of Basel	3
Paul Scherrer Institut (PSI)	2
University Children's Hospital of Zurich	2
Others:	*14*

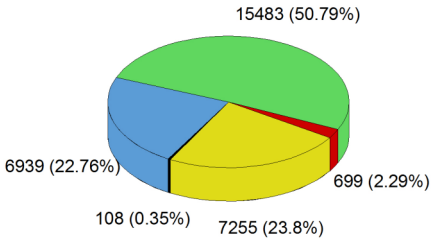
Department	#
D-BIOL	106
Faculty of Science (MNF)	94
Faculty of Medicine (MED)	60
CHAB	32
University Hospital Zurich	29
Vetsuisse Faculty (VET)	19
Brain Research Insitute	11
Dep. of Internal Medicine	10
Others:	*85*

Institute	#
Plant Sciences	33
Organic Chemistry	27
Institute of Plant Biology	24
Microbiology	21
Institute of Biochemistry	20
Cell Biology	18
Biochemistry	17
Institute of Molecular Biology	15
Institute of Molecular Cancer Research	14
Institute of Zoology	14
Molecular Systems Biology	14
Brain Research Institute	13
Pharmaceutical Sciences	10
Institute of Veterinary Biochemistry and Molecular Biology	9
Molecular Biology and Biophysics	9
Physiology	9
Others:	*228*

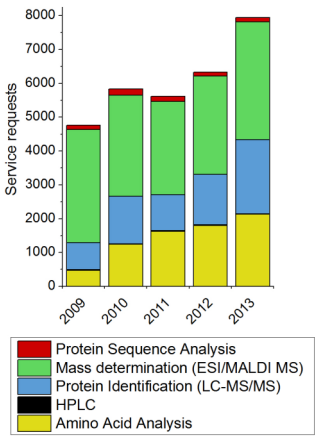
PROTEIN SERVICE (2009-2013)

- Total number of samples analysed: 30484
- Organizations: 50
- Departments: 107
- Institutes: 176

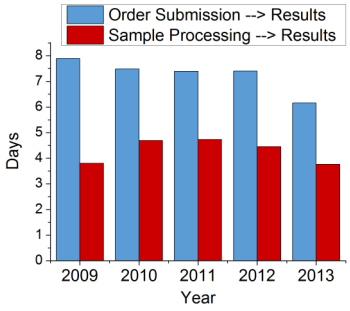
Types of protein services requested



Protein services by year



Average sample processing time



GET YOUR RESULTS RAPIDLY!
2013 results:
from Order submission to Results: 6.15 days
from Sample processing to Results: 3.76 days

Orders submitted by:

Organization	#
University of Zurich	2024
ETH Zurich	1503
University Hospital Zurich	84
University of Geneva	84
University of Basel	51
ETHZ/UZH	45
University of Konstanz	44
Paul Scherrer Institute (PSI)	78
EPFL	20
Others:	189

Department / Faculty	#
Faculty of Science (MNF)	1154
D-BIOL	1150
Faculty of Medicine (MED)	438
D-CHAB	194
Institute of Organic Chemistry	98
Vetsuisse Faculty (VET)	58
D-AGRL	57
Department of Biochemistry	56
Faculty of Biology	49
Others:	868

Institute	#
Molecular Biology and Biophysics (D-BIOL)	953
Institute of Biochemistry (MNF)	520
Biochemistry (MED)	267
Institute of Physical Chemistry	218
Organic Chemistry (MNF)	199
Organic Chemistry (D-CHAB)	149
Inorganic Chemistry (MNF)	128
Institute of Physiology (MNF)	102
Medical Microbiology	80
Others	1506

FGCZ Proteomics projects in Switzerland and Europe

http://primexs.eu/

FGCZ is part of the transnational network program named Prime-XS. The project is funded under the 7th Framework Programm of the European Union. The Prime-XS initiative aims to develop new technologies to better support the research community in answering scientific questions and to provide access to state-of-the-art proteomics technologies to the European research community. The twelve partners of Prime-XS, all well known in the field of proteomics, are distributed over Europe and will provide access to their technology at six access facilities. Prime-XS is organizing a wide range of meetings, courses and training events to share knowledge and expertise. Special emphasis will be placed on extending this knowledge to new member states of the European Union and other regions of Europe with less privileged availability of proteomics facilities.

http://www.hecatos.eu/

The HeCaToS project (Hepatic and Cardiac Toxicity Systems modelling) aims at developing integrative in silico tools for predicting human liver and heart toxicity. The objective is to develop an integrated modeling framework, by combining advances in computational chemistry and systems toxicology, for modelling toxic perturbations in liver and heart across multiple scales. This framework will include vertical integrations of representations from drug(metabolite)-target interactions, through macromolecules /proteins, to (sub-)cellular functionalities and organ physiologies, and even the human whole-body level.
In view of the importance of mitochondrial deregulations and of immunological dysfunctions associated with hepatic and cardiac drug-induced injuries, focus will be on these particular Adverse Outcome Pathways. Models will be populated with data from innovative in vitro 3D liver and heart assays challenged with prototypical hepato- or cardiotoxicants; data will be generated by advanced molecular and functional analytical techniques retrieving information on key (sub-)cellular toxic events.

http://www.battlex.ch/

The goal of the BattleX project is to combine accurate genome-scale models of both host and pathogen that will pave the way for an integrated comprehensive understanding of infectious diseases as a rational basis to develop urgently needed control strategies. It is a Research, Technology and Development (RTD) project, funded by SystemsX.ch, the Swiss Initiative in Systems Biology. We aim to develop novel strategies to combat infections; specifically, to identify novel targets/target combinations in metabolism for control of shigellosis, focusing on one infection model, intracellular Shigella infection of human cells. This model is medically relevant and offers unique advantages for a systems approach.

DIFFERENTIAL LABEL-FREE PROTEIN QUANTIFICATION AS A SERVICE

NEW

In the last years, the FGCZ Proteomics team invested a lot of efforts in the development of reproducible, sensitive and accurate methods for the label-free quantification of proteins in different sample conditions. Due to the challenges typically faced during this type of proteome-wide quantification studies, these experiments were up to now performed only in the user-lab mode. Since a few months, we developed a pipeline that allows us to offer label-free protein quantification as a service. Contact us at *proteomics@fgcz.ethz.ch*!

REFERENCES

[1] Ong SE, *et al.* Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. **Mol Cell Proteomics. 2002; 1(5):376-86**

[2] Ross PL, *et al.* Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents. **Mol Cell Proteomics. 2004;3(12):1154-69.**

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[4] Egertson JD, *et al.* Multiplexed MS/MS for improved data-independent acquisition. **Nat Methods. 2013;10(8):744-6.**

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[6] Gallien S, *et al.* Targeted proteomic quantification on quadrupole-orbitrap mass spectrometer. **Mol Cell Proteomics. 2012;11(12):1709-23**.

FURTHER INFORMATION

USER LAB AND SERVICES:
More information on the general setup of the FGCZ and its access modes can be found at www.fgcz.ch

PROTEOMICS CONTACT:
proteomics@fgcz.ethz.ch

SEMINARS:
Should we come to your institute for a seminar on our technologies and applications?
Please contact us:
coordinator@fgcz.ethz.ch

ETH Eidgenössische Technische Hochschule Zürich Swiss Federal Institute of Technology Zurich

University of Zurich[UZH]