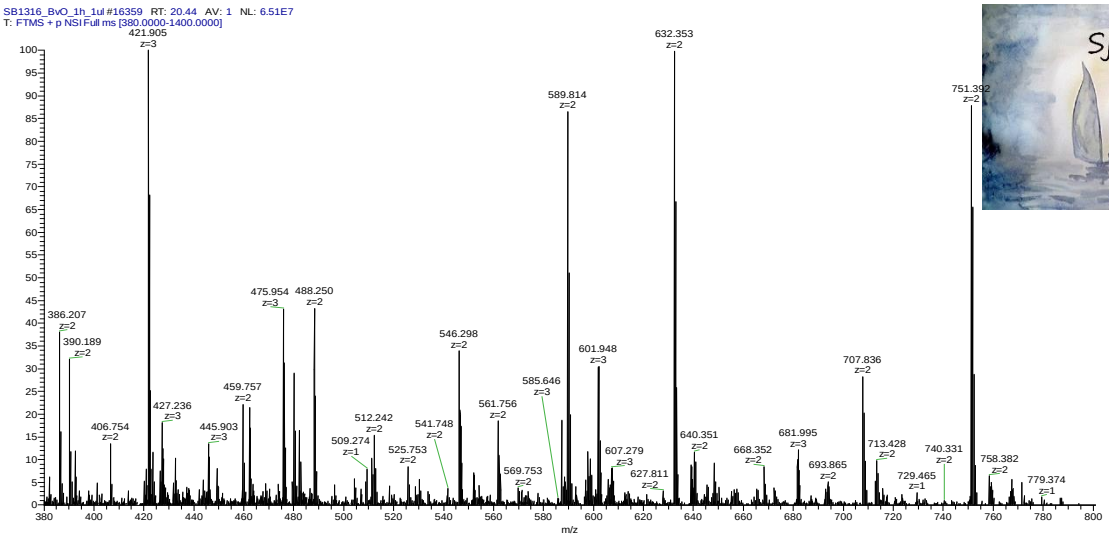
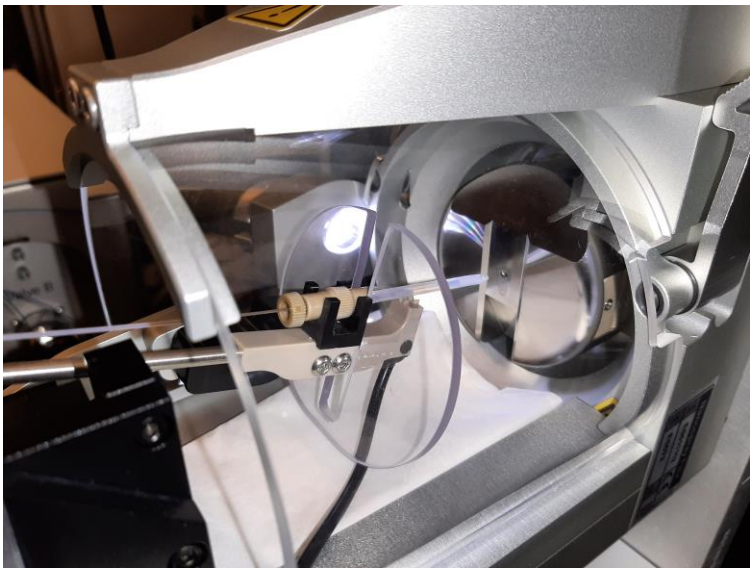
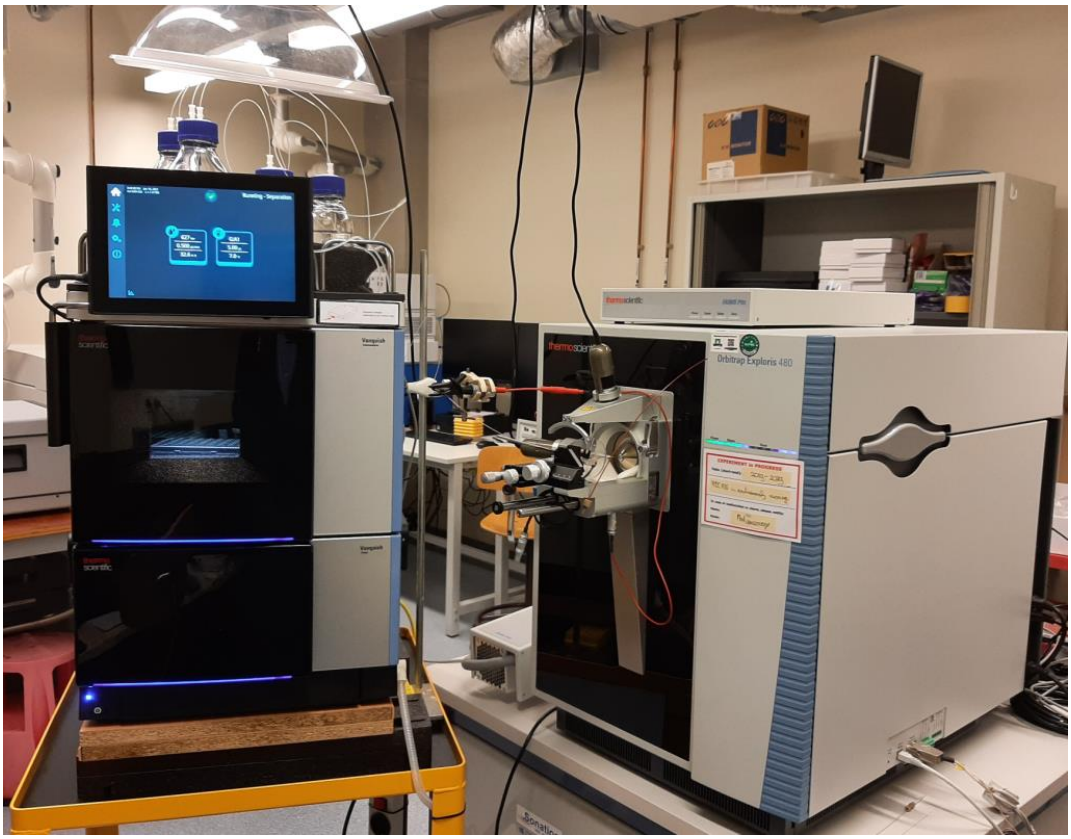
Proteomics at WUR Biochemistry



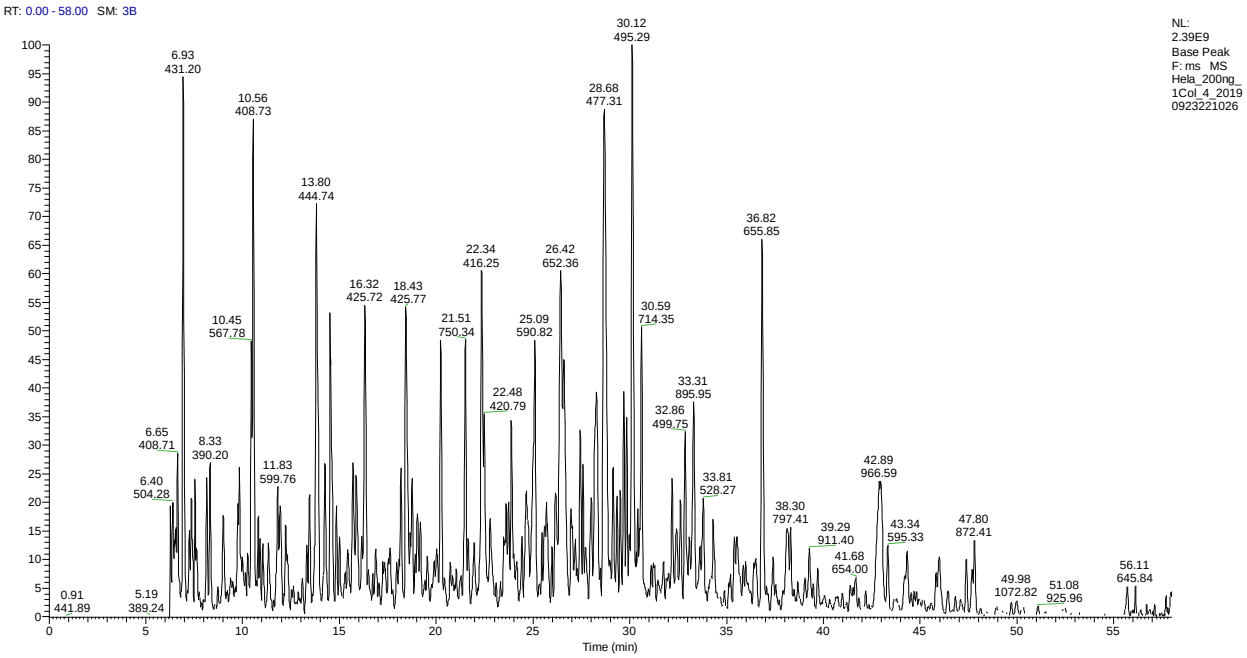
A) General information



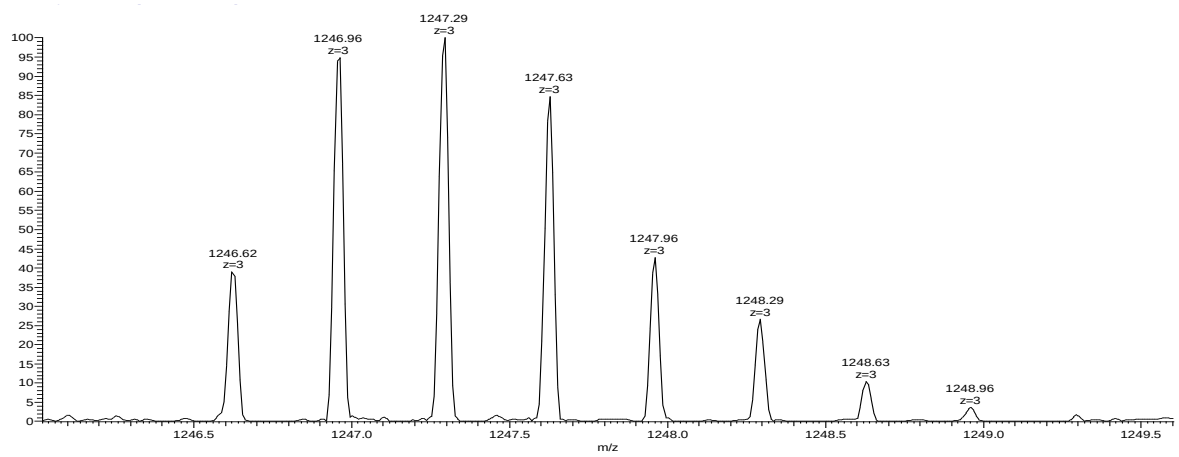
High quality protein identification as well as accurate relative protein quantitation is done by nanoLC-MSMS. Reversed phase nano LC (Thermo Vanquish Neo) using home made capillary columns (1.9 µm particles) results in peptide separations with a high resolution. MS spectra of the peptides are measured with an Orbitrap Exploris 480 at approximately 5 ppm deviation or less. After each MS scan, MSMS spectra of the peptides are acquired when enough peptide is available (ca 20 scans/s). All measurements combined yield optimal protein identifications and relative quantitation.



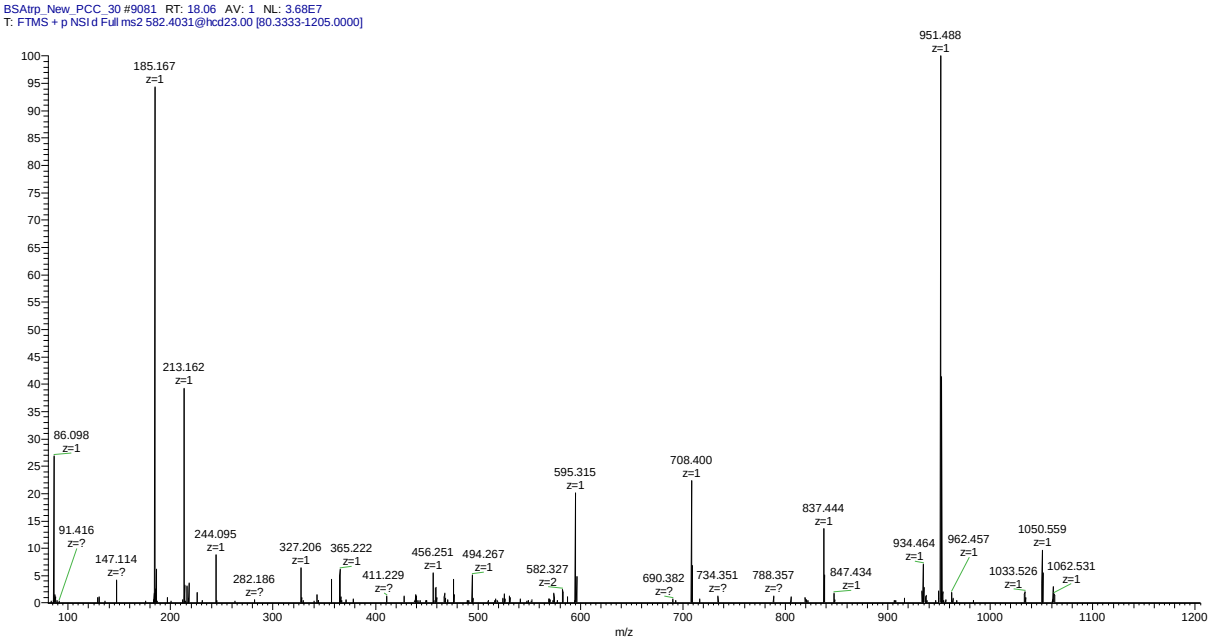
(Hela cell line) Peptide separations at a high chromatographic resolution:

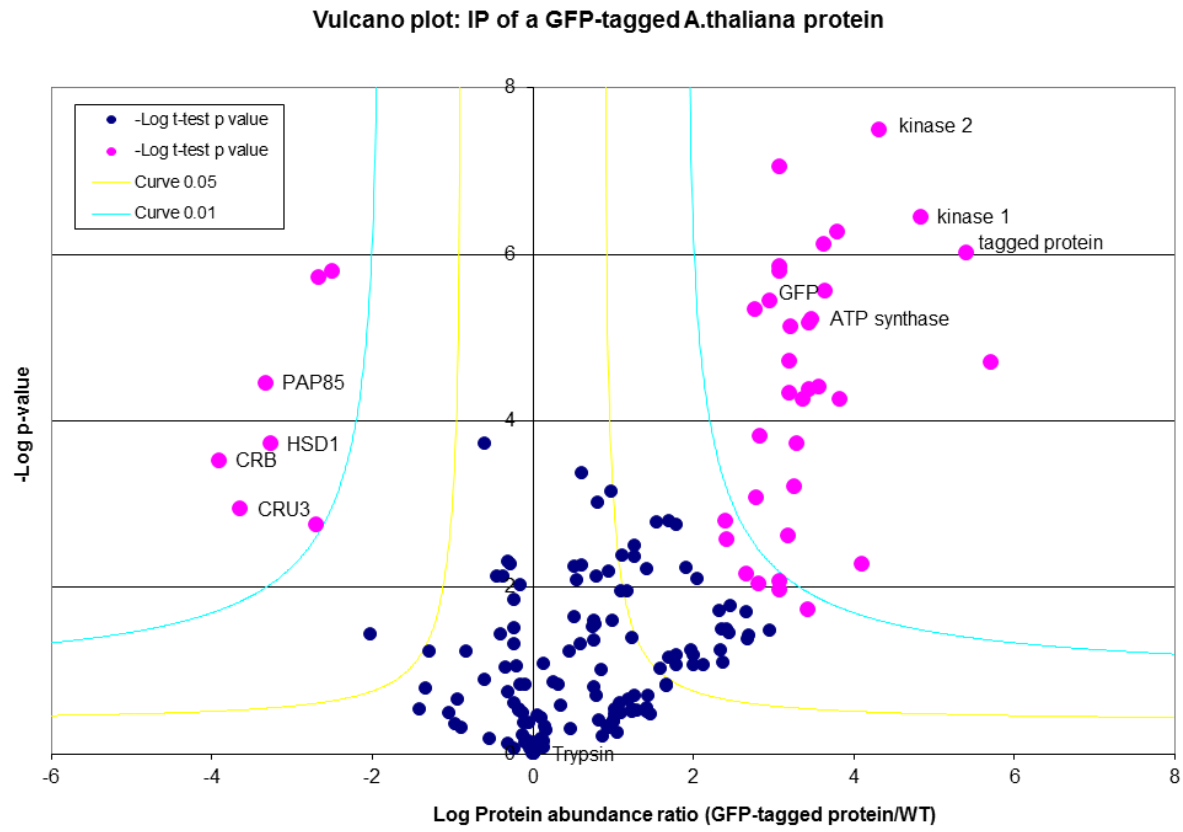


High resolution Orbitrap MS spectra at resolution 60.000

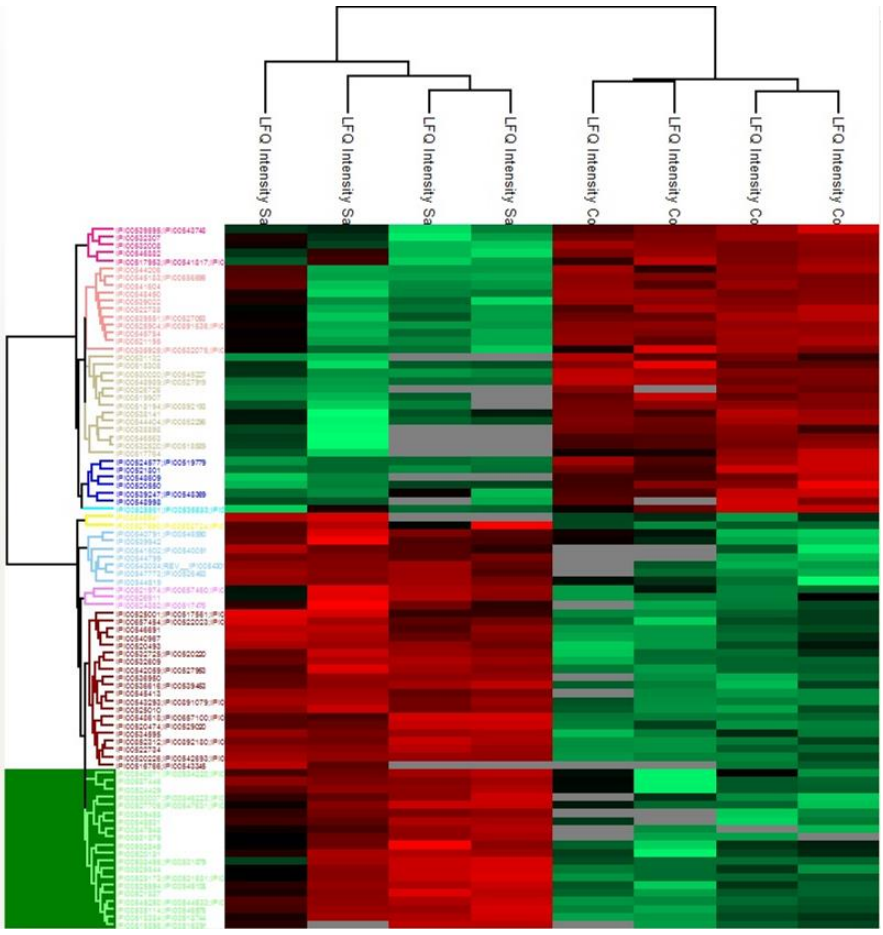


And high resolution MSMS peptide fragmentation spectra at resolution 15.000





Proteomics data clustering

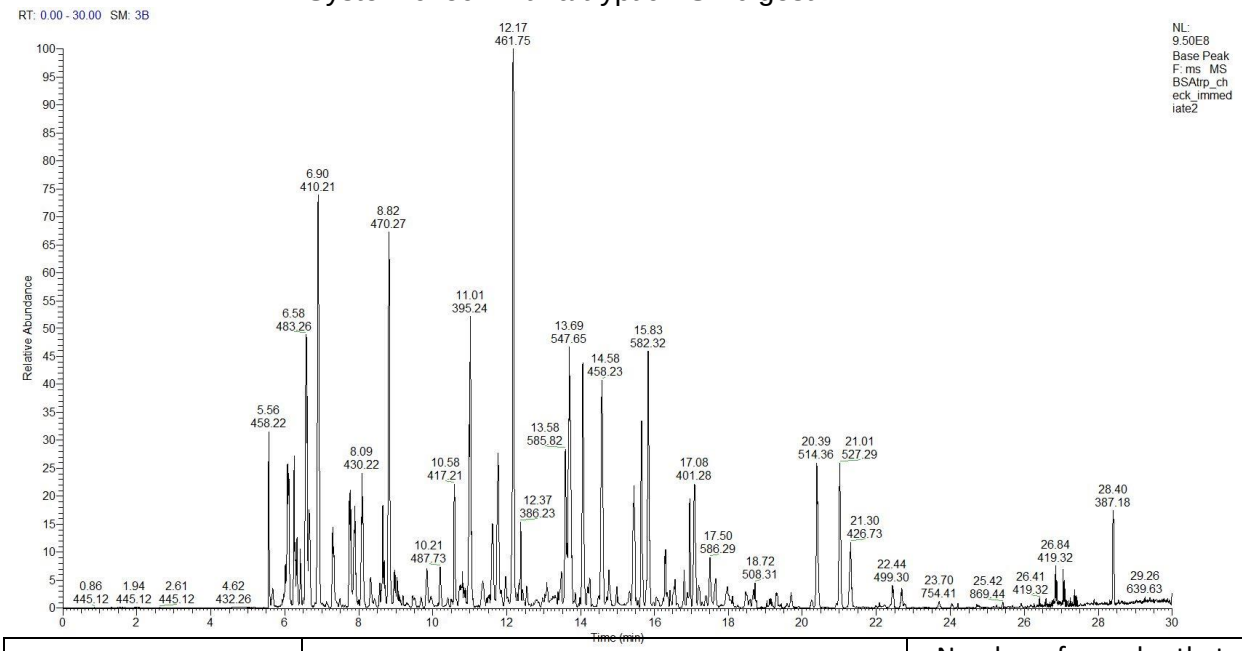


B) Practical proteomics information

Maximally 5 ul of sample will be injected per LC-MS run. Minimally 25 ul peptide solution has to be handed in optimally containing 0.1-0.2 ug/ul peptide. All peptide samples need to be prepared via the FASP, PAC or in-gel-digestion procedure or have to be cleaned via the µColumn cleanup procedure at pH 3. Please see our Proteomics Sample Preparation Protocol (see at bottom of: https://www.wur.nl/en/Research-Results/Chair-groups/Agrotechnology-and-Food-Sciences/Biomolecular-Sciences/Laboratory-of-Biochemistry/About-us.htm) for details how to prepare proteomic samples.

Before every measuring series, the system [nLC + MS + MSMS] is checked by measuring a standard BSA digest. Points that are checked include LC peak width as well as MS and MSMS sensitivity and spectral quality. Also, no peptides from previous injections should be visual. When everything is alright, and only then, the next sample set will be injected with a “fast” cleaning gradient directly after each measurement gradient.

System check with a tryptic BSA digest



Gradient	Sample type	Number of samples that can be measured per day
Fast 0.5h gradient	Simplified mixtures (e.g. Immuno Precipitations or In Gel Digests)	15
Standard 1h gradient	Complex mixtures (e.g. FASP/PAC prepared complete proteomes)	10

When the standard gradient is used, the total measuring time (including cleaning gradient) is 2 hours per sample. Because of the checks before and some cleaning gradients after each sample set, 10 samples can be measured per day using the standard gradient. It is advised to put the Controls/Blancs first in your sample list, followed by the Samples. When less than the maximal amount of samples is handed in, then the remaining time will be filled with cleaning gradients. Minimally one complete measurement day will therefore always be used and charged. When, due to dirty samples, the system is so heavily contaminated that it cannot be cleaned within the same day (e.g. because of high concentrations of detergents in the sample(s)), then an extra measurement day can be charged.

Large sample sets that need more than one week measurement time are advised to split into more smaller sample sets using the same reference samples in each set. How exactly to do this, needs to be discussed with Sjef.

For identification and relative quantitation, the MaxQuant software package will be used. When a database is not publicly available, than a database in fasta format has to be handed in before measurements will be done. Search times necessary to compare the data to the database

strongly depend both on the number of LCMS runs as well as on the database size. When a species specific database is used for trypsin digests, it will take about 0.5 to 1 hour per run. The MaxQuant search result (a table with identification + normalized intensities) will be filtered with a filtering and statistics software called Perseus leaving confident identifications only. When applicable, a example Vulcano plot will be added (see page 3), as well as significance info and e.g. hierarchical clustering when asked for. Significance info is only available for experiments done at least in triplicate. To be able to do it well, it is advised to do the experiments in fourfold with real biological replicates (not just technical replicates). The Perseus filtered data as well as the original MaxQuant data [+ the protein abundance ratio Graph] will be supplied to you.

An example Vulcano plot of a graph is shown on the top of page 3. In the plot, the p-values are shown on the Y-axis as –Log p (higher is more reliable). The X-axis shows the ratio of the average protein Label Free Quantitation intensities between each data set, e.g Sample versus Control (on a logarithmic scale as well). Proteins whose average concentration significantly differs between the two data sets are shown with pink dots. Proteins that do not vary between the two conditions are shown with blue dots.

One remark. Since MaxQuant uses peak intensities for its calculations, chromatographic column overloading will result in relatively lower peaks for the most abundant proteins and therefore always have a ratio of 1.

From nicely prepared samples with 0.5 ug peptide injected without interfering compounds you may get:

	Number of proteins quantified with a 1 hour gradient
Human blood serum	135
Human milk	185
Bacteria	800 - 1600
Bovine cell line	2500
Human cell line	2500
A. thaliana Plasma Membrane	2500

Maximum number of protein groups quantified with a 1 hour gradient and DDA: 5200
Using a sample prepared from a mix of 15 bacteria.

C) Pricing

See separate file: Costs Proteomics at BIC

Some published articles with a proteomics contribution:

2023

Bannenberg, J. W., S. Boeren, M. H. Zwietering, T. Abee and H. M. W. den Besten (2024). "Insight in lag phase of Listeria monocytogenes during enrichment through proteomic and transcriptomic responses." Food Research International **175**: 113609.

Bombelli, A., C. Araya-Cloutier, S. Boeren, J.-P. Vincken, T. Abee and H. M. W. d. Besten (2023). "Effects of the antimicrobial glabridin on membrane integrity and stress response activation in Listeria monocytogenes." Food Research International **175**.

Douwenga, S., B. van Olst, S. Boeren, Y. Luo, X. Lai, B. Teusink, J. Vervoort, M. Kleerebezem and H. Bachmann (2023). "The hierarchy of sugar catabolization in Lactococcus cremoris." Microbiol Spectr: e0224823.

Hendrickx, D. M., R. An, S. Boeren, S. K. Mutte, P. Chatchatee, A. Nowak-Wegrzyn, L. Lange, S. Benjaponpitak, K. W. Chong, P. Sangsupawanich, M. T. J. van Ampting, M. M. O. Nijhuis, L. F. Harthoorn, J. E. Langford, J. Knol, K. Knipping, J. Garssen, V. Trendelenburg, R. Pesek, C. M. Davis, A. Muraro, M. Erlewyn-Lajeunesse, A. T. Fox, L. J. Michaelis, K. Beyer, L. Noimark, G. Stiefel, U. Schauer, E. Hamelmann, D. Peroni, A. Boner, J. M. Lambert and C. Belzer (2023). "Assessment of infant outgrowth of cow's milk allergy in relation to the faecal microbiome and metaproteome." Scientific Reports **13**(1).

Hendrickx, D. M., R. An, S. Boeren, S. K. Mutte, J. M. Lambert and C. Belzer (2023). "Trackability of proteins from probiotic Bifidobacterium spp. in the gut using metaproteomics " Beneficial Microbes **14**(3): 269-280.

Huijboom, L., M. Tempelaars, M. Z. Fan, Y. R. Zhu, S. Boeren, E. van der Linden and T. Abee (2023). "L-tyrosine modulates biofilm formation of Bacillus cereus ATCC 14579." Research in Microbiology **174**(6).

Egas, R., J. Kurth, S. Boeren, D. Sousa, C. Welte and I. Sánchez-Andrea (2023 submitted). "A novel mechanism for dissimilatory nitrate reduction to ammonium in Acididesulfobacillus acetoxydans." mSystems 00967-23R1.

Zeng, Z., L. M. Wijnands, S. Boeren, E. J. Smid, R. A. Notebaart and T. Abee (2023). "Impact of vitamin B(12) on rhamnose metabolism, stress defense and in-vitro virulence of Listeria monocytogenes." Int J Food Microbiol **410**: 110486.

Zhang, C., S. Boeren, L. Zhao, E. Bijl and K. Hettinga (2023). "The impact of low temperature inactivation of protease AprX from Pseudomonas on its proteolytic capacity and specificity: A peptidomic study." Dairy **4**(1): 150-166.

2022

Dekker, P. M., S. Boeren, J. B. van Goudoever, J. J. M. Vervoort and K. A. Hettinga (2022). "Exploring Human Milk Dynamics: Interindividual Variation in Milk Proteome, Peptidome, and Metabolome." J Proteome Res 21(4): 1002-1016.

Dekker, P. M., M. B. Azad, S. Boeren, P. J. Mandhane, T. J. Moraes, E. S. , P. Subbarao, S. E. Turvey, E. Saccenti and K. A. Hettinga (2022 submitted). "The human milk proteome and allergy of mother and child: Exploring associations with protein levels and protein network connectivity." Frontiers in Immunology, section Nutritional Immunology.

Doloman, A., S. Boeren, C. D. Miller and D. Z. Sousa (2022). "Stimulating Effect of Trichococcus flocculiformis on a Coculture of Syntrophomonas wolfei and Methanospirillum hungatei." Applied and Environmental Microbiology **88**(13).

Feng, Y., T. P. N. Bui, A. J. M. Stams, S. Boeren, I. Sanchez-Andrea and W. M. de Vos (2022). "Comparative genomics and proteomics of Eubacterium maltosivorans: functional identification of trimethylamine methyltransferases and bacterial microcompartments in a human intestinal bacterium with a versatile lifestyle." Environmental Microbiology **24**(1): 517-534.

Kontopodi, E., S. Boeren, B. Stahl, J. B. van Goudoever, R. M. van Elburg and K. Hettinga (2022). "High-Temperature Short-Time Preserves Human Milk's Bioactive Proteins and Their Function Better Than Pasteurization Techniques With Long Processing Times." Frontiers in Pediatrics 9.

Kontopodi, E., B. Stahl, J. B. van Goudoever, S. Boeren, R. A. H. Timmermans, H. M. W. den Besten, R. M. Van Elburg and K. Hettinga (2022). "Effects of High-Pressure Processing,

UV-C Irradiation and Thermoultrasonication on Donor Human Milk Safety and Quality." Frontiers in Pediatrics 10.
Liu, C., S. Boeren, I. Miro Estruch and I. Rietjens (2022). "Intra-and Inter-individual Differences in the Human Intestinal Microbial Conversion of (-)-Epicatechin and Bioactivity of Its Major Colonic Metabolite 5-(3',4'-dihydroxyphenyl)-γvalerolactone in Regulating Nrf2-mediated Gene Expression." Frontiers in Nutrition 30.
Liu, C., S. Boeren and I. M. C. M. Rietjens (2022). "The gut microbial metabolite pyrogallol is a more potent regulator of Nrf2-associated gene expression than its parent compound green tea (-)-epigallocatechin gallate in in vitro cell models." Nutrients **14**: 3392.
Liu, Y., N. Charamis, S. Boeren, J. Blok, A. G. Lewis, E. J. Smid and T. Abee (2022). "Physiological Roles of Short-Chain and Long-Chain Menaquinones (Vitamin K2) in Lactococcus cremoris." Frontiers in Microbiology 13.
Liu, Y., M. H. Tempelaars, S. Boeren, S. Alexeeva, E. J. Smid and T. Abee (2022). "Extracellular vesicle formation in Lactococcus lactis is stimulated by prophage-encoded holin-lysin system." Microbial Biotechnology **15**(4): 1281-1295.
Malvestiti, M. C., M. B. F. Steentjes, H. G. Beenen, S. Boeren, J. A. L. van Kan and X. Shi-Kunne (2022). "Analysis of plant cell death-inducing proteins of the necrotrophic fungal pathogens Botrytis squamosa and Botrytis elliptica." Front Plant Sci **13**: 993325.
Nugroho, A. D. W., B. Van Olst, S. A. Bachtiar, S. Boeren, M. Kleerebezem and H. Bachmann (2022). "Manganese Modulates Metabolic Activity and Redox Homeostasis in Translationally Blocked Lactococcus cremoris, Impacting Metabolic Persistence, Cell Culturability, and Flavor Formation." Microbiology Spectrum **10**(3).
Sukarta, O., Q. Zheng, E. Slootweg, M. Mekken, M. Mendel, V. Putker, H. Overmars, R. Pomp, J. Roosien, S. Boeren, G. Smant and A. Goverse (2022). "Glycine-Rich RNA-Binding Protein 7 interacts with and potentiates effector-induced immunity by Gpa2 and Rx1 based on an intact RNA Recognition Motif " Plant Physiology: PP2021-RA-00883.
Zhang, L., S. Boeren, J. Heck, J. Vervoort, P. Zhou and K. Hettinga (2022). "First Insight into the Variation of the Milk Serum Proteome within and between Individual Cows." Dairy 2022 (3): 47-58.

2021

Bui, T. P. N., L. Manneras-Holm, R. Puschmann, H. Wu, A. D. Troise, B. Nijsse, S. Boeren, F. Backhed, D. Fiedler and W. M. deVos (2021). "Conversion of dietary inositol into propionate and acetate by commensal Anaerostipes associates with host health." Nature Communications **12**(1).
Chen, Y., E. van Pelt-KleinJan, B. van Olst, S. Douwenga, S. Boeren, H. Bachmann, D. Molenaar, J. Nielsen and B. Teusink (2021). "Proteome constraints reveal targets for improving microbial fitness in nutrient-rich environments." Mol Syst Biol **17**(4): e10093.
Dank, A., O. van Mastrigt, S. Boeren, S. K. Lillevang, T. Abee and E. J. Smid (2021). "Propionibacterium freudenreichii thrives in microaerobic conditions by complete oxidation of lactate to CO2." Environmental Microbiology **23**(6): 3116-3129.
Dank, A., Z. Zeng, S. Boeren, R. A. Notebaart, E. J. Smid and T. Abee (2021). "Bacterial Microcompartment-Dependent 1,2-Propanediol Utilization of Propionibacterium freudenreichii." Frontiers in Microbiology **12**.
Henderickx, J. G. E., R. D. Zwittink, I. B. Renes, R. A. van Lingen, D. van Zoeren-Grobben, L. J. G. Jebbink, S. Boeren, R. M. van Elburg, J. Knol and C. Belzer (2021). "Maturation of the preterm gastrointestinal tract can be defined by host and microbial markers for digestion and barrier defense." Scientific Reports **11**(1).
Koomen, J., L. Huijboom, X. C. Ma, M. H. Tempelaars, S. Boeren, M. H. Zwietering, H. M. W. den Besten and T. Abee (2021). "Amino acid substitutions in ribosomal protein RpsU enable switching between high fitness and multiple-stress resistance in Listeria monocytogenes." International Journal of Food Microbiology **351**.
Liu, Y., A. de Groot, S. Boeren, T. Abee and E. J. Smid (2021). "Lactococcus lactis Mutants Obtained From Laboratory Evolution Showed Elevated Vitamin K2 Content and Enhanced Resistance to Oxidative Stress." Frontiers in Microbiology **12**(3110).
Liu, Y. W., S. Boeren, L. N. Zhang, P. Zhou and K. Hettinga (2021). "Ultrasonication retains more milk fat globule membrane proteins compared to equivalent shear-homogenization." Innovative Food Science & Emerging Technologies **70**.
Mollaei, M., M. Suarez-Diez, V. T. Sedano-Nunez, S. Boeren, A. Stams and C. M. Plugge

(2021). "Proteomic analysis of a syntrophic coculture of Syntrophobacter fumaroxidans MPOBT and Geobacter sulfurreducens PCAT." Frontiers in Microbiology, section Microbial Physiology and Metabolism.

Mollaei, M., P. H. A. Timmers, M. Suarez-Diez, S. Boeren, A. H. v. Gelder, A. J. M. Stams and C. M. Plugge (2021). "Comparative proteomics of Geobacter sulfurreducens PCAT in response to acetate, formate and/or hydrogen as electron donor." Environmental Microbiology.

Moreira, J. P. C., M. Diender, A. L. Arantes, S. Boeren, A. J. M. Stams, M. M. Alves, J. I. Alves and D. Z. Sousa (2021). "Propionate Production from Carbon Monoxide by Synthetic Cocultures of Acetobacterium wieringae and Propionigenic Bacteria." Applied and Environmental Microbiology **87**(14).

Tieme A. Helderman, L. D., André Bertran, Sjef Boeren, Like Fokkens, Richard Kormelink, Matthieu H.A.J. Joosten, Marcel Prins, Harrold A. van den Burg * (2021). "An isoform of the eukaryotic Translation Elongation Factor 1A (eEF1a) acts as a pro-viral factor required for Tomato spotted wilt virus disease in Nicotiana benthamiana." Viruses-1390862.

Wang, P., H. Jiang, S. Boeren, H. Dings, O. Kulikova, T. Bisseling and E. Limpens (2021). "A nuclear-targeted effector of Rhizophagus irregularis interferes with Histone 2B mono-ubiquitination to promote arbuscular mycorrhization." New Phytologist NPH-MS-2020-34393.R1.

Xiong, L., S. Boeren, J. Vervoort and K. Hettinga (2021). "Effect of milk serum proteins on aggregation, bacteriostatic activity and digestion of lactoferrin after heat treatment." Food Chem **337**: 127973.

Zeng, Z., S. Boeren, V. Bhandula, S. H. Light, E. J. Smid, R. A. Notebaart and T. Abee (2021). "Bacterial Microcompartments Coupled with Extracellular Electron Transfer Drive the Anaerobic Utilization of Ethanolamine in Listeria monocytogenes." Msystems **6**(2).

Zeng, Z., S. Li, S. Boeren, E. J. Smid, R. A. Notebaart and T. Abee (2021). "Anaerobic Growth of Listeria monocytogenes on Rhamnose Is Stimulated by Vitamin B12 and Bacterial Microcompartment-Dependent 1,2-Propanediol Utilization." mSphere **6**(4): e0043421.

Some selected older papers from before 2021

Sanchez-Andrea, I., I. A. Guedes, B. Hornung, S. Boeren, C. E. Lawson, D. Z. Sousa, A. Bar-Even, N. J. Claassens and A. J. M. Stams (2020). "The reductive glycine pathway allows autotrophic growth of Desulfovibrio desulfuricans." Nature Communications **11**(1).

Göertz, G. P., J. v. Bree, A. Hiralal, B. M. Fernhout, C. Steffens, S. Boeren, T. M. Visser, C. B. Vogels, C. J. Koenraadt, M. M. v. Oers and G. P. Pijlman (2019 accepted). "Subgenomic flavivirus RNA binds the mosquito DEAD/H-box helicase ME31B and determines Zika virus transmission by Aedes aegypti." Proceedings of the National Academy of Sciences of the United States of America **116**(38): 19136-19144.

Houbaert, A., C. Zhang, M. Tiwari, K. Wang, A. D. Serrano, D. V. Savatin, M. J. Urs, M. K. Zhiponova, G. E. Gudesblat, I. Vanhoutte, D. Eeckhout, S. Boeren, M. Karimi, C. Betti, T. Jacobs, C. Fenoll, M. Mena, S. de Vries, G. De Jaeger and E. Russinova (2018). "POLAR-guided signalling complex assembly and localization drive asymmetric cell division." Nature **563**(7732): 574-+.

Mishev, K., Q. Lu, B. Denoo, F. Peurois, W. Dejonghe, J. Hullaert, R. De Rycke, S. Boeren, M. Bretou, S. De Munck, I. Sharma, K. Goodman, K. Kalinowska, V. Storme, L. S. L. Nguyen, A. Drozdzecki, S. Martins, W. Nerinckx, D. Audenaert, G. Vert, A. Madder, M. S. Otegui, E. Isono, S. N. Savvides, W. Annaert, S. De Vries, J. Cherfils, J. Winne and E. Russinova (2018). "Nonselective Chemical Inhibition of Sec7 Domain-Containing ARF GTPase Exchange Factors." Plant Cell **30**(10): 2573-2593.

Sousa, D. Z., M. Visser, A. H. van Gelder, S. Boeren, M. M. Pieterse, M. W. H. Pinkse, P. D. E. M. Verhaert, C. Vogt, S. Franke, S. Kummel and A. J. M. Stams (2018). "The deep-subsurface sulfate reducer Desulfotomaculum kuznetsovii employs two methanol-degrading pathways." Nature Communications **9**.

Ottman, N., J. Reunanen, M. Meijerink, T. E. Pietila, V. Kainulainen, J. Klievink, L. Huuskonen, S. Aalvink, M. Skurnik, S. Boeren, R. Satokari, A. Mercenier, A. Palva, H. Smidt, W. M. de Vos and C. Belzer (2017). "Pili-like proteins of Akkermansia

muciniphila modulate host immune responses and gut barrier function." Plos One **12**(3).
Wendrich, J. R., S. Boeren, B. K. Möller, D. Weijers and B. D. Rybel (2017). "In Vivo Identification of Plant Protein Complexes Using IP-MS/MS." Methods in Molecular Biology **1497**(Plant Hormones: Methods and Protocols): 147-158.
Bui, T. P., J. Ritari, S. Boeren, P. de Waard, C. M. Plugge and W. M. de Vos (2015). "Production of butyrate from lysine and the Amadori product fructoselysine by a human gut commensal." Nat Commun 6: 10062.
Hettinga, K. A., F. M. Reina, S. Boeren, L. N. Zhang, G. H. Koppelman, D. S. Postma, J. J. M. Vervoort and A. H. Wijga (2015). "Difference in the Breast Milk Proteome between Allergic and Non-Allergic Mothers." Plos One 10(3).
Zhang, L., S. Boeren, J. A. Hageman, T. van Hooijdonk, J. Vervoort and K. Hettinga (2015). "Bovine milk proteome in the first 9 days: protein interactions in maturation of the immune and digestive system of the newborn." PLoS One 10(2): e0116710.
De Rybel, B., Moller, B., Yoshida, S., Grabowicz, I., de Reuille, P.B., Boeren, S., Smith, R.S., Borst, J.W. and Weijers, D. (2013) A bHLH Complex Controls Embryonic Vascular Tissue Establishment and Indeterminate Growth in Arabidopsis. Developmental Cell 24(4), 426-437.
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