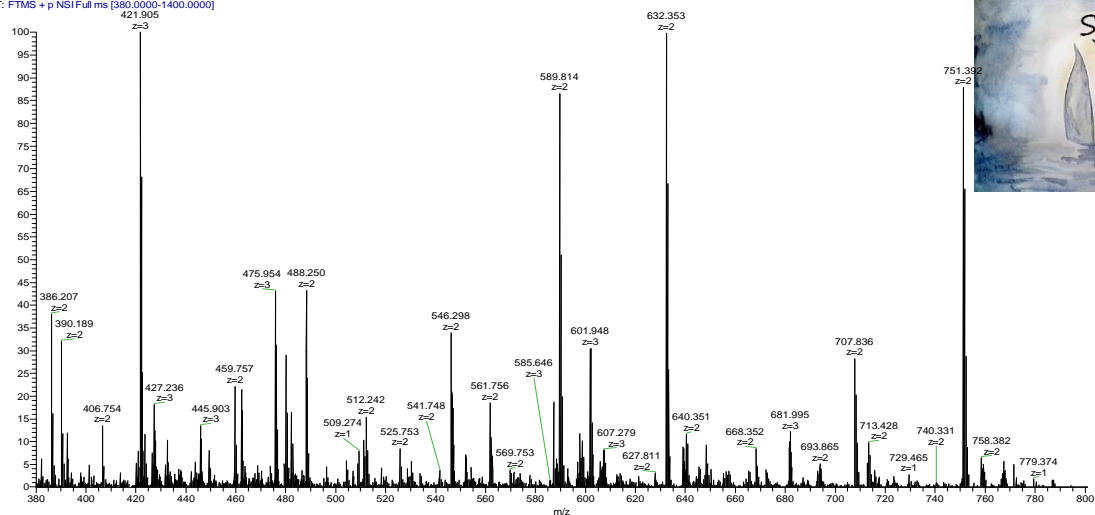


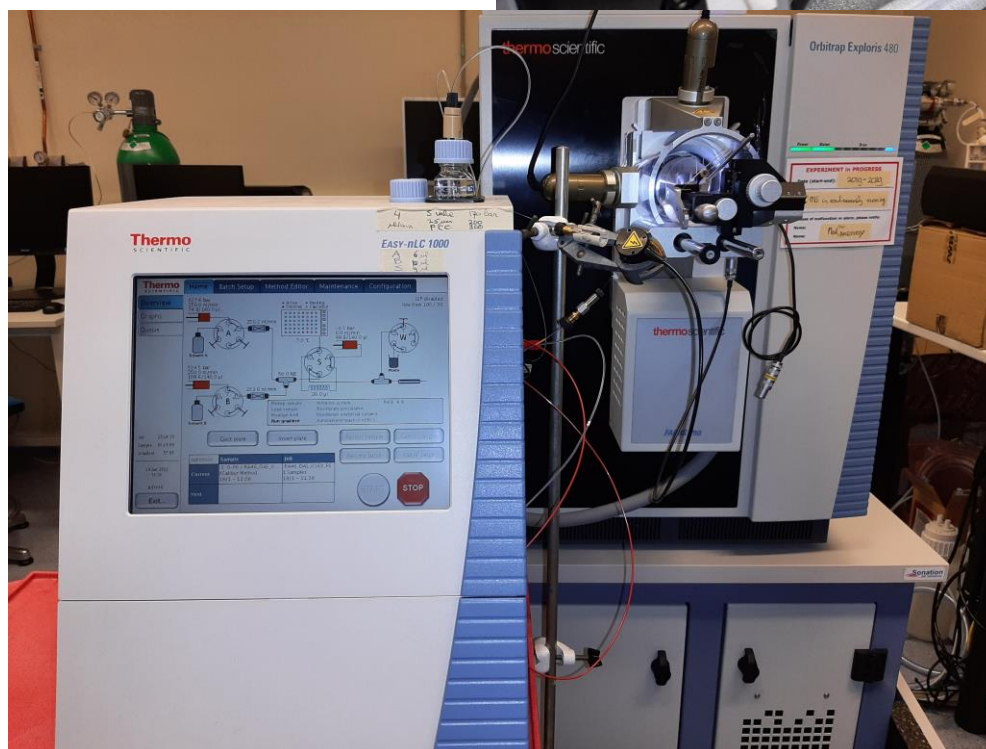
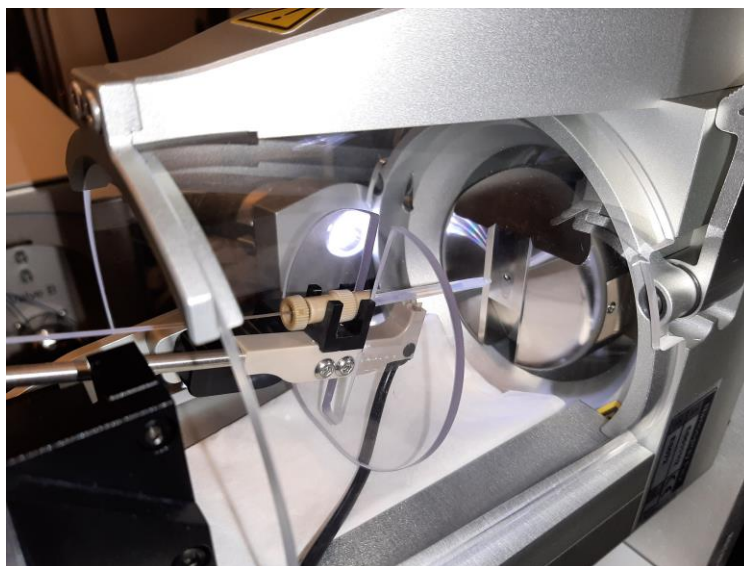
Proteomics at WUR Biochemistry

SB1316, BvO_1h_1uf #16359 RT: 20.44 AV: 1 NL: 6.51E7
T: FTMS + p NSI Full ms [380.0000-1400.0000]

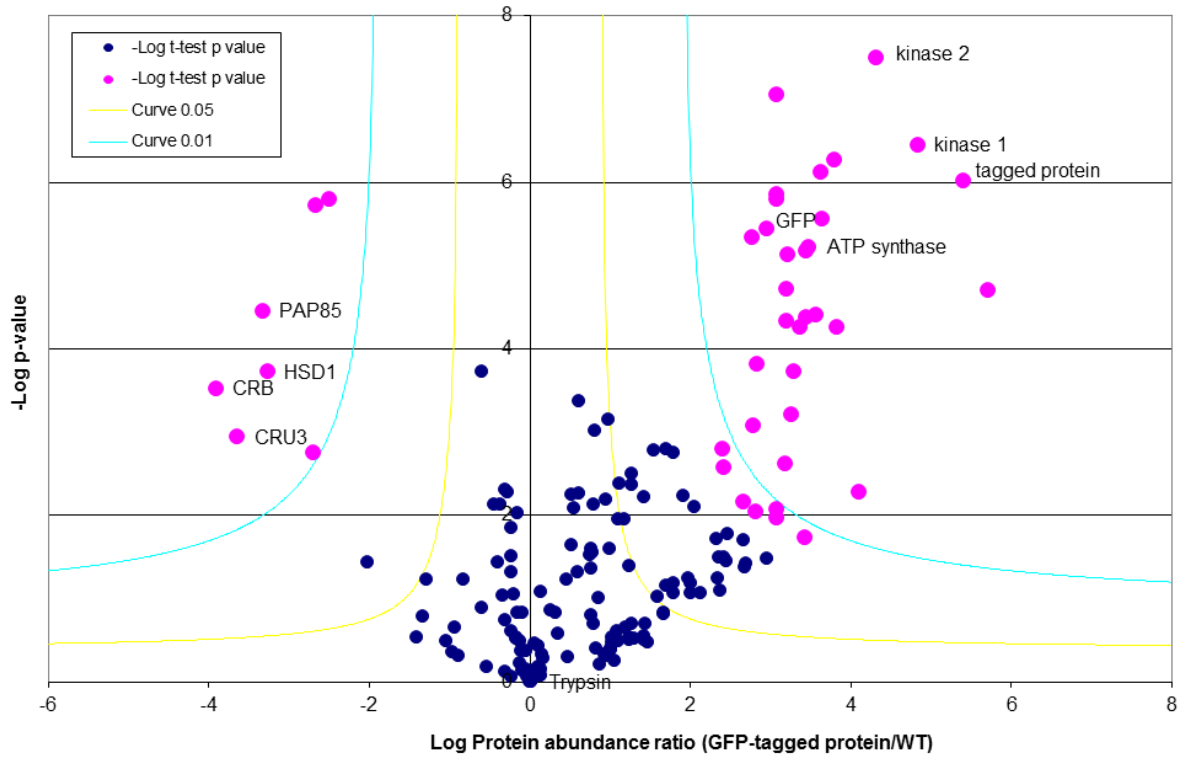


A) General information

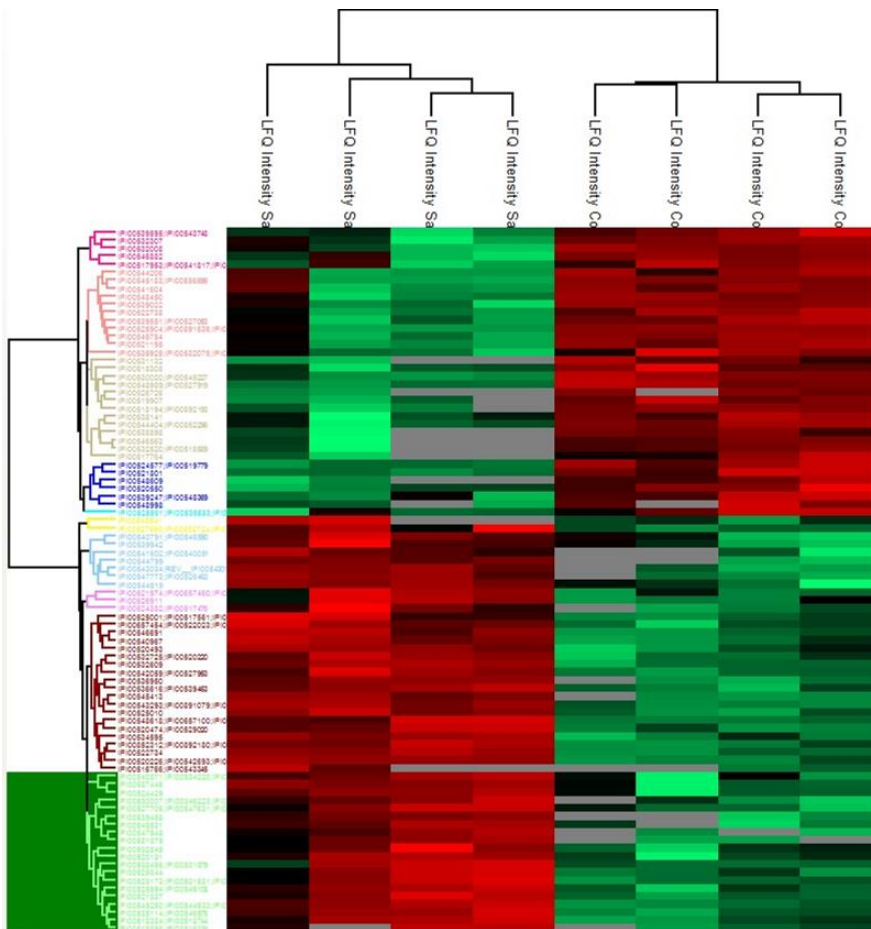
High quality protein identification as well as accurate relative protein quantitation is done by nanoLC-MS/MS. Reversed phase nano LC (Thermo Vanquish Neo) using home made capillary columns (1.9 μm particles) results in peptide separations with a high resolution. MS spectra of the peptides are measured with an Orbitrap Exploris 480 at approximately 5 ppm deviation or less. After each MS scan, MS/MS 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.



Vulcano plot: IP of a GFP-tagged A.thaliana protein



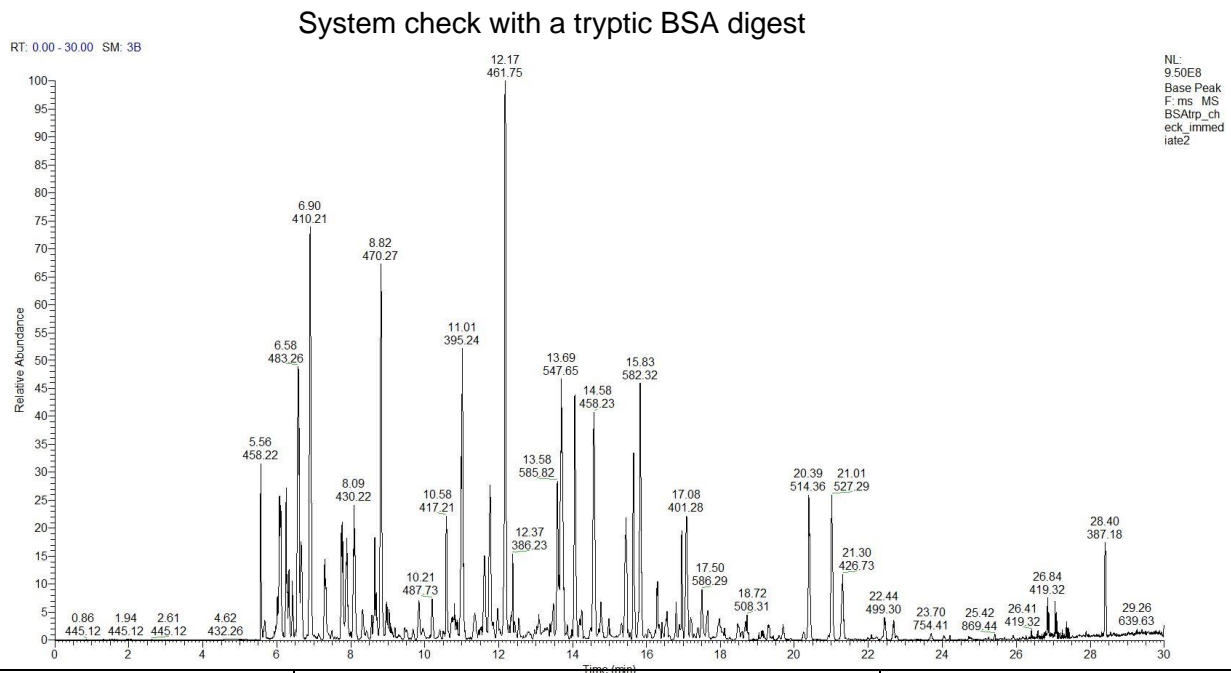
Proteomics data clustering



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 μ Column 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.



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 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

C) Pricing

	Academic research	Non-academic research (Excluding VAT taxes)
nLC-MS rental and usage (SRF)	WUR: € 1153,- per day External: € 1500,- per day	€ 3000,- per day
Sample preparation assistance	€ 325,- per half day *	€ 650,- per half day
Data analysis consisting of: database search, result filtering, prepare for and perform T-test(s) when applicable.	€ 400,- per measured day *	€ 1200,- per measured day
Report	Result will be handed in as a set of excel tables when data analysis was included	€ 1200,- for a full written report including set of excel tables

* Price reductions are possible for academic users when there is mutual agreement to consider the proteomics part as a scientific collaboration that may lead to a joined publication.

Some published articles with a proteomics contribution:

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)- γ -valerolactone 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. Bachtar, 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.
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- 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.
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- 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**.
- Mollaie, 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.
- Mollaie, 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.
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- Tieme A. Helderma, L. D., André Bertran, Sief 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-**13**90862.
- 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 monoubiquitination to promote arbuscular mycorrhization." New Phytologist NPH-MS-2020-34393.R1.
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- Kostopoulos, I., N. Ottman, J. Elzinga, B. Blijenberg, S. Aalvink, S. Boeren, M. Mank, J. Knol, W. M. d. Vos and C. Belzer (2020). "AKKERMANSIA MUCINIPHILA USES HUMAN MILK OLIGOSACCHARIDES TO THRIVE IN THE EARLY LIFE CONDITIONS IN VITRO." Scientific Reports 10(1): 14330.
- Lee, I. C., S. van, II, S. Boeren, J. Vervoort, M. Meijerink, N. Taverne, M. Starrenburg, P. A. Bron and M. Kleerebezem (2020). "Lipoproteins Contribute to the Anti-inflammatory Capacity of Lactobacillus plantarum WCFS1." Front Microbiol 11: 1822.
- Liu, Y. W., L. Xiong, E. Kontopodi, S. Boeren, L. N. Zhang, P. Zhou and K. Hettinga (2020). "Changes in the milk serum proteome after thermal and non-thermal treatment." Innovative Food Science & Emerging Technologies 66: 102544.
- Maiorino, F., T. Helderma, M. Arroyo-Mateos, M. v. d. Wolf, S. Boeren, M. Prins and H. A. V. d. Burg (2020). "Identification of tomato proteins that interact with Replication initiator protein (Rep) of the geminivirus TYLCV." Frontiers in Plant Science, section Virology 11(online).
- Mollaei, M., P. H. A. Timmers, M. Suarez-Diez, S. Boeren, A. H. v. Gelder, A. J. M. Stams and C. M. Plugge (2020). "Comparative proteomics of Geobacter sulfurreducens PCAT in response to acetate, formate and/or hydrogen as electron donor." Environmental Microbiology.
- 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).
- Shetty, S. A., S. Boeren, T. P. N. Bui, H. Smidt and W. M. de Vos (2020). "Unravelling lactate-acetate and sugar conversion into butyrate by intestinal Anaerobutyricum and Anaerostipes species by comparative proteogenomics." Environ Microbiol 22(11): 4863-4875.
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- Zenker, H. E., J. Raupbach, S. Boeren, H. J. Wichers and K. A. Hettinga (2020). "The effect of low vs. high temperature dry heating on solubility and digestibility of cow's milk protein." Food Hydrocolloids 109.
- Zenker, H. E., H. J. Wichers, M. M. M. Tomassen, S. Boeren, N. W. D. Jong and K. A. Hettinga (2020). "Peptide release after simulated infant in vitro digestion of dry heated cow's milk protein and transport of potentially immunoreactive peptides across the Caco-2 cell monolayer." Nutrients ID: nutrients-882168.

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- 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.

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Some selected older papers from before 2019

- 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-+.
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- 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).
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