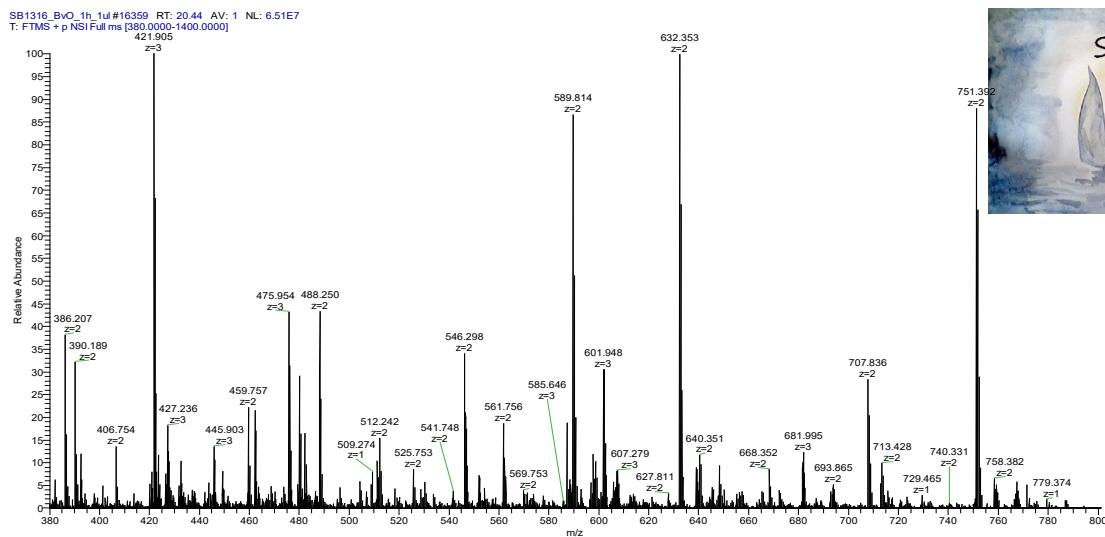
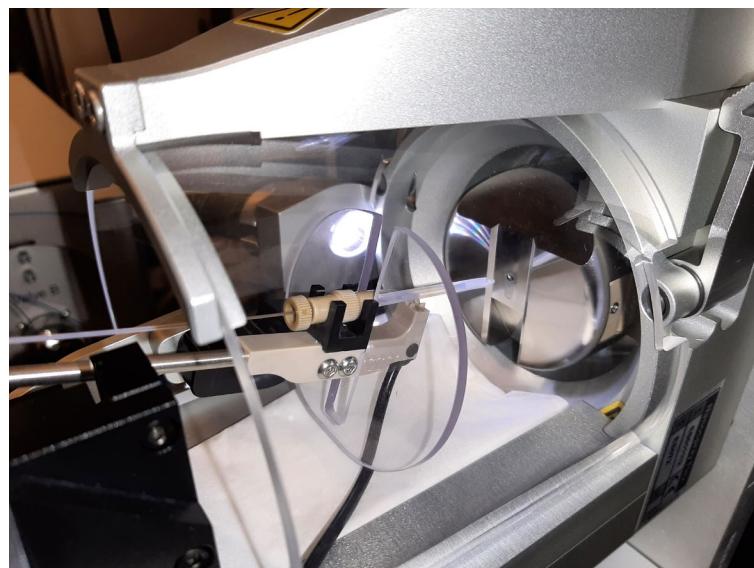


Proteomics at WUR Biochemistry

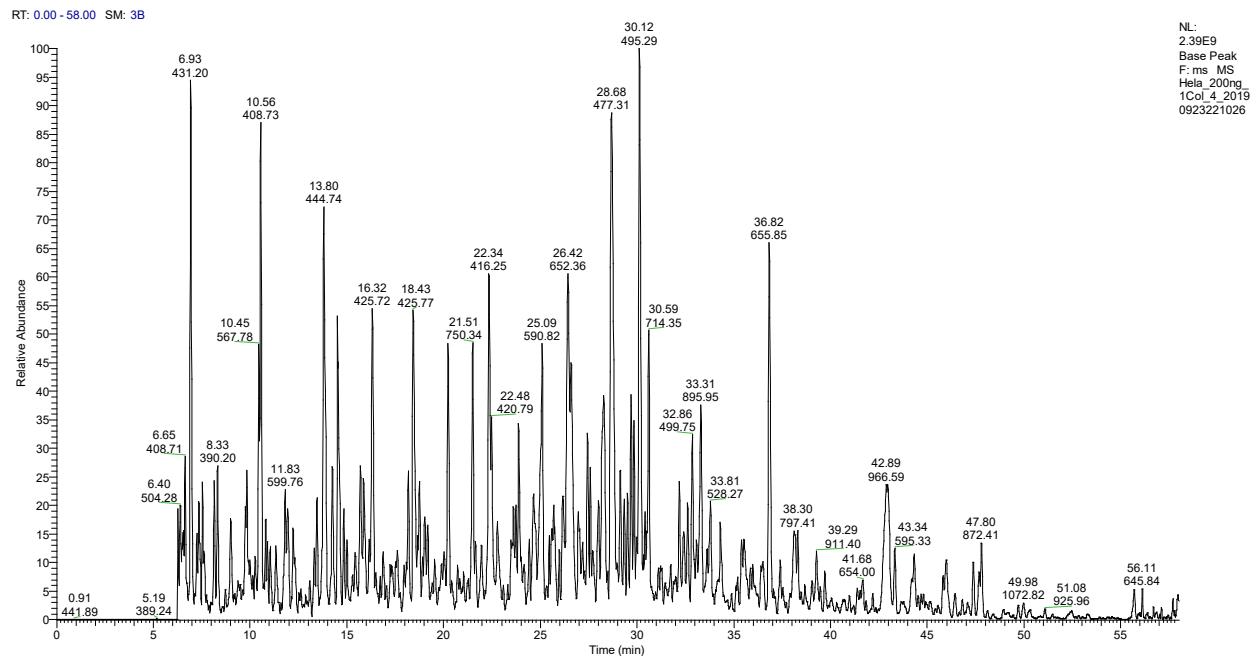


A) General information

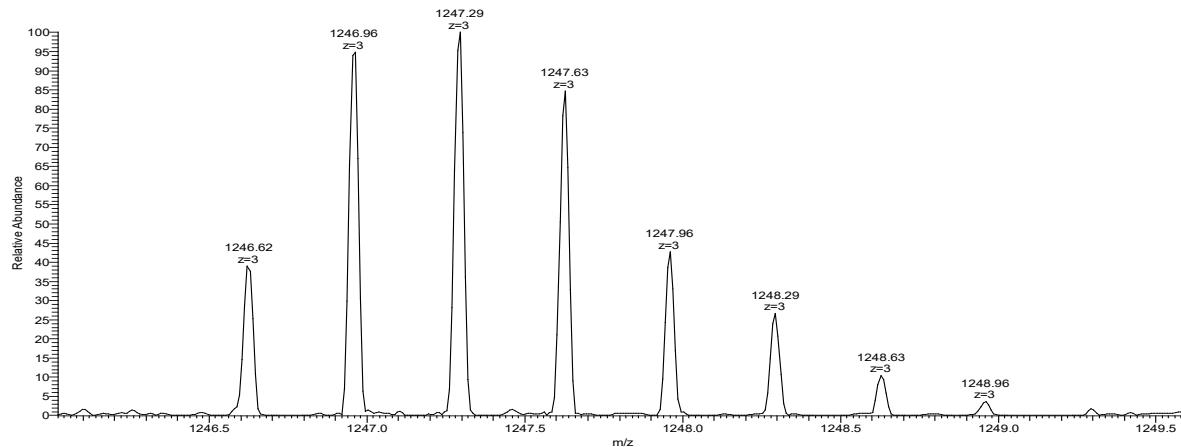
High quality protein identification as well as accurate relative protein quantitation is done by nanoLC-MSMS. Reversed phase nano LC (Thermo nLC1000) using home made capillary columns ($1.9\text{ }\mu\text{m}$ particles) results in peptide separations with a high resolution. MS spectra of the peptides 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:

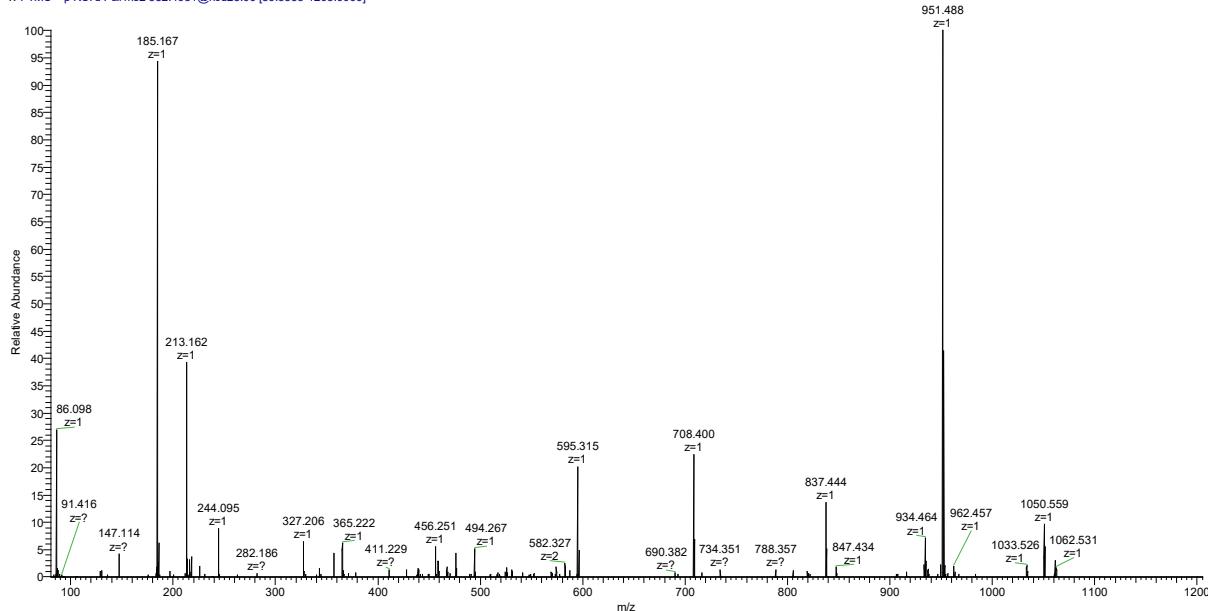


High resolution Orbitrap MS spectra

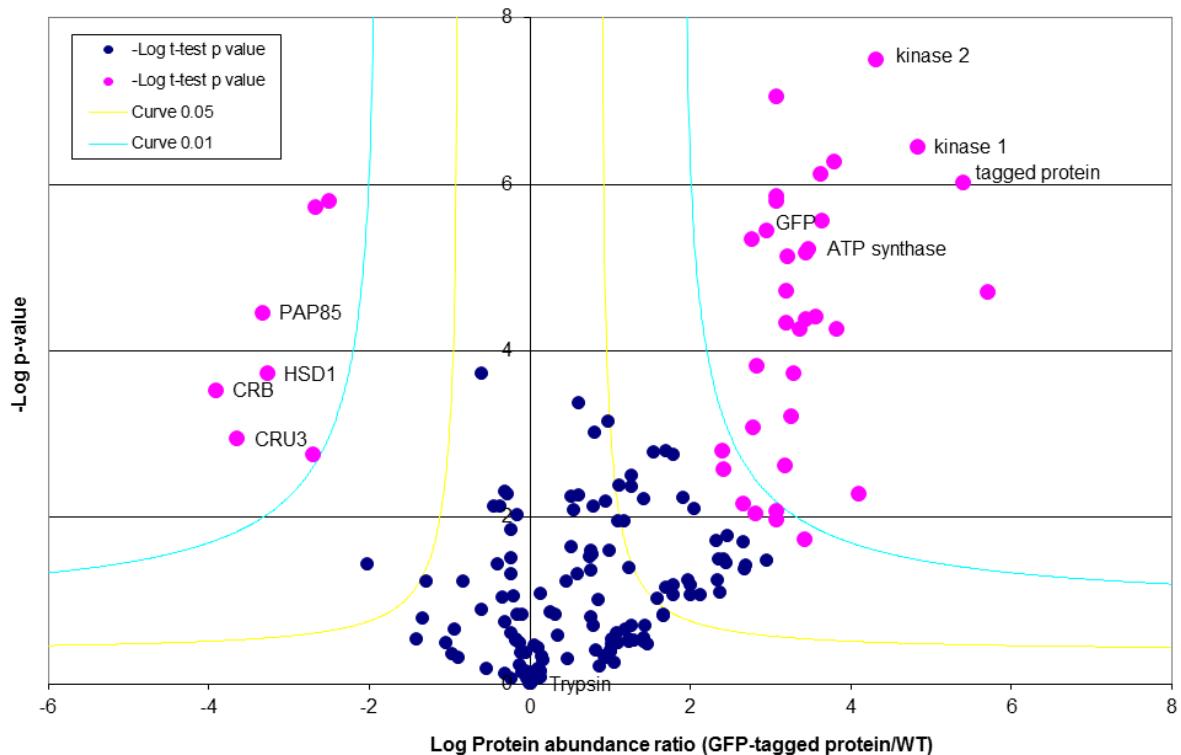


And high resolution MSMS peptide fragmentation spectra

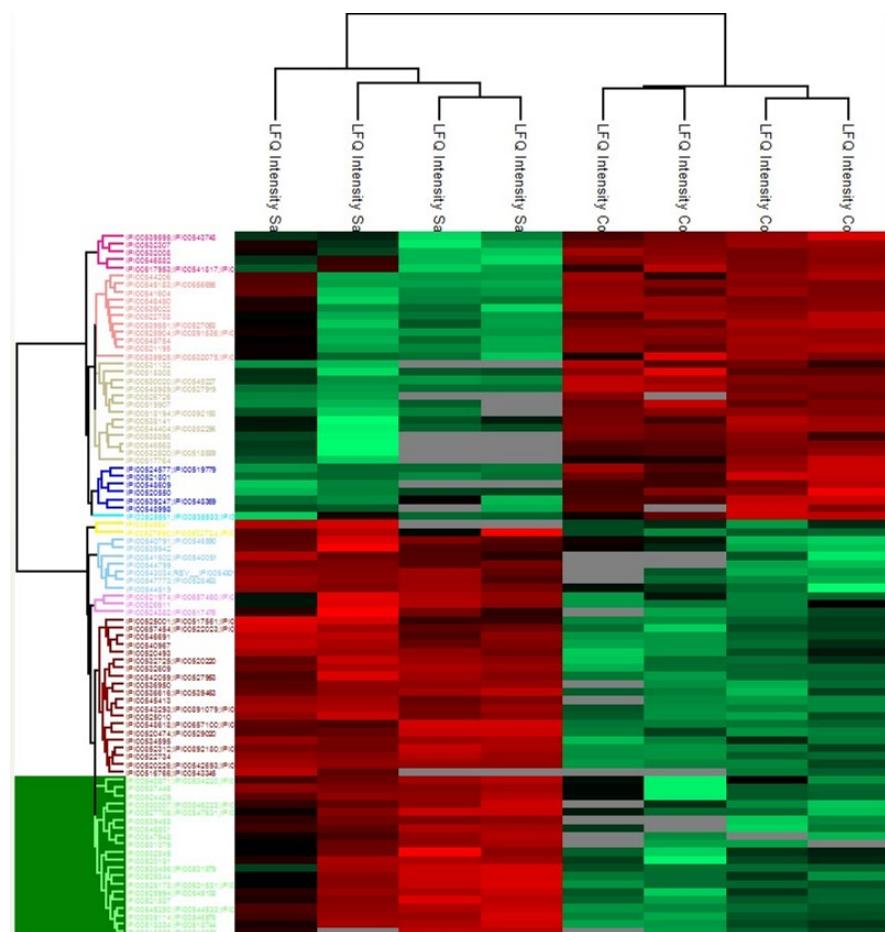
BSAtrp_New_PCC_30 #9081 RT: 18.06 AV: 1 NL: 3.68E7
T: FTMS + p NS1 d Full ms2 582.4031@hcd23.00 [80.3333-1205.0000]



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



Proteomics data clustering

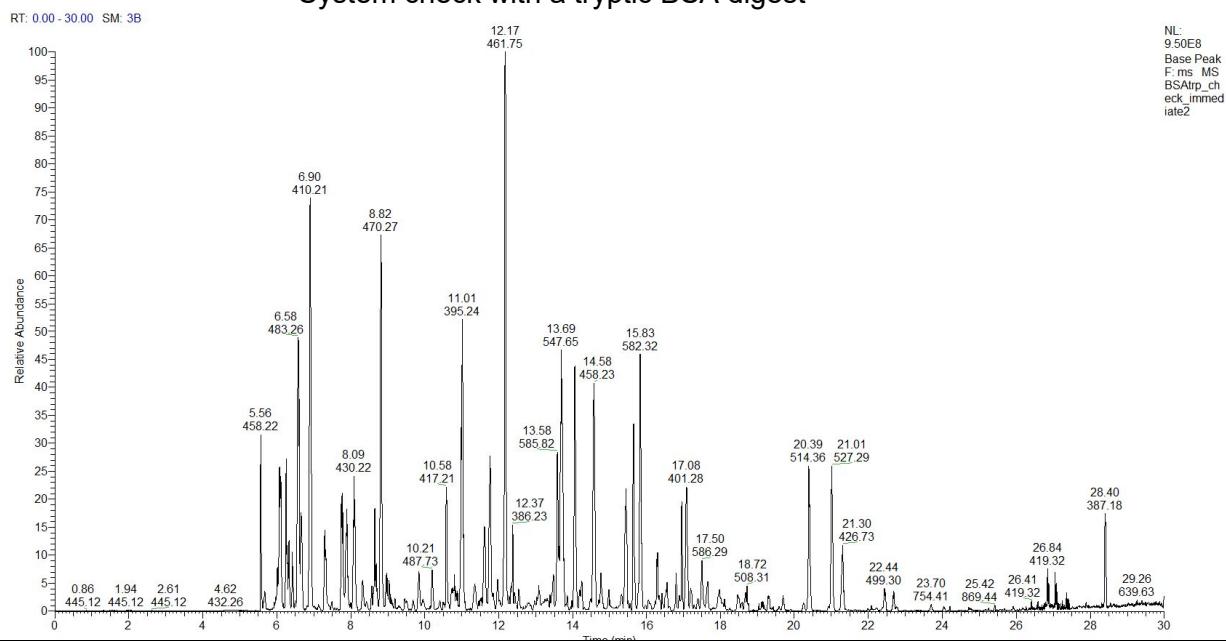


B) Practical proteomics information

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 procedure or have to be cleaned via the μ Column cleanup procedure at pH 3. Please see our Proteomics Sample Preparation Protocol (<https://www.wur.nl/en/Expertise-Services/Chair-groups/Agrotechnology-and-Food-Sciences/Laboratory-of-Biochemistry/Facilities.htm>) for details how to prepare proteomic samples.

Before every measuring series, the system [nLC1000 (Thermo EASY 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 gradient	Simplified mixtures (e.g. Immuno Precipitations or In Gel Digests)	16
Standard 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/Blanks 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 the system is so heavily contaminated that it cannot be cleaned within the same day (e.g. due to high concentrations of detergents) 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 Volcano 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 Volcano 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 0.1-0.2ug/ul peptide 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)
MS rental and usage (SRF)	WUR: € 1060,- per day External: € 1378,- per day	€ 2120,- per day
nLC rental and usage	€ 85,- per day	€ 170,- 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:

2021

- Bui, T. P. N., L. Manneras-Holm, R. Puschmann, H. Wu, A. D. Troise, B. Nijssse, 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. Abbe 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. Abbe (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. Abbe (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. Abbe 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. Abbe (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. Abbe (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.

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- Dekker, P., S. Boeren, A. H. Wijga, G. Koppelman, J. Vervoort and K. Hettinga (2020). "Maternal allergy and the presence of non-human proteinaceous molecules in human milk." *Nutrients* 12(4): 1169.
- Elwakiel, M., S. Boeren, W. D. Wang, H. A. Schols and K. A. Hettinga (2020). "Degradation of Proteins From Colostrum and Mature Milk From Chinese Mothers Using an in vitro Infant Digestion Model." *Frontiers in Nutrition* 7: 162.
- Vries, R. d., S. Boeren, K. Holtenius, J. Vervoort, H. Lindmark-Måansson and K. Hettinga (2020). "Influence of dry period length of Swedish dairy cows on the proteome of colostrum." *Dairy* 1: 313-325.
- 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.
- Maio, F., T. Helderman, 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.
- Xiong, L., C. Li, S. Boeren, J. Vervoort and K. Hettinga (2020). "Effect of heat treatment on the proteome composition and the bacteriostatic activity of bovine milk serum." *Food Research International* 127: 108688.
- Zenker, H. E., J. Raupbach, S. Boeren, H. J. Wickers 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. Wickers, 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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- Zeng, Z., E. Smid, S. Boeren, R. A. Notebaart and T. Abbe (2019). "Bacterial microcompartment-dependent 1,2-propanediol utilization stimulates anaerobic growth of *Listeria monocytogenes* EGDe." *Frontiers in Microbiology, section Microbial Physiology and Metabolism* 10(2660).
- Elwakiel, M., S. Boeren, J. A. Hageman, I. M. Szeto, H. A. Schols and K. A. Hettinga (2019). "Variability of Serum Proteins in Chinese and Dutch Human Milk during Lactation." *Nutrients* 11(3).

- Florentino, A. P., I. A. C. Pereira, S. Boeren, M. van den Born, A. J. M. Stams and I. Sanchez-Andrea (2019). "Insight into the sulfur metabolism of Desulfurella amilsii by differential proteomics." *Environmental Microbiology* **21**(1): 209-225.
- 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.
- Mora, D., R. Filardi, S. Arioli, S. Boeren, S. Aalvink and W. M. de Vos (2019). "Development of omics-based protocols for the microbiological characterization of multi-strain formulations marketed as probiotics: the case of VSL#3." *Microbial Biotechnology*.

Some selected older papers < 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-+.
- 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. Nericke, 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.
- Smaczniak, C., Li, N., Boeren, S., America, T., van Dongen, W., Goerdayal, S.S., de Vries, S., Angenent, G.C. and Kaufmann, K. (2012) Proteomics-based identification of low-abundance signaling and regulatory protein complexes in native plant tissues. *Nature Protocols* **7**(12), 2144-2158.
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