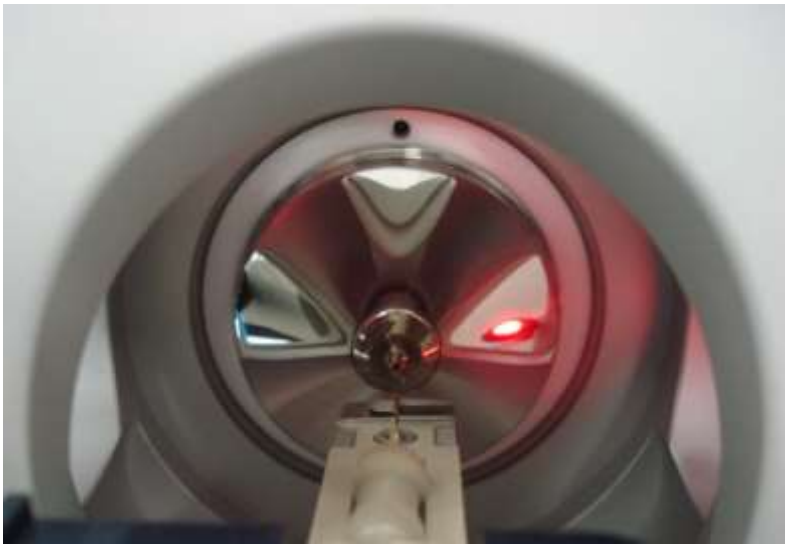
Annual report 2012-2013

Mass Spectrometry Core Facility



Interfaculty Mass Spectrometry Center

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26 November 2013

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1. Introduction

This first annual report of the Mass Spectrometry Core Facility covers a time period of almost two years, starting from January 2012. In fact, the years 2012 and 2013 marked an important transition for the MSCF: the move to a brand new building and laboratory, merging our equipment and expertise with those of the Analytical Biochemistry (RuG) and former Medical Biomics (UMCG) groups. The move also marked the transition to the Interfaculty Mass Spectrometry Center (IMSC), in which we have joined forces with MS experts of both RuG and UMCG. The ERIBA building provides an open, interdisciplinary work environment which we expect to facilitate fruitful collaborations and service projects.

Figure 1. Views of the IMSC open lab as seen from the open office (left) and from inside (right)

After extensive preparation in 2012 and early 2013 the physical move was carried out smoothly in June and July 2013, with very little down-time and interruption of service. The floor plan below shows the new premises of the MSCF/IMSC (ERIBA building 6th floor). It comprises 3 MS labs of about 115 m^2 each, with all the required specialized infrastructure (gases, temperature, electricity) and flexible space for 7-8 MS setups per lab. The open lab space of 150 m^2 provides ample bench space for wet lab activities. Lastly, there are four separate labs for sample preparation and storage, and utility labs of 85 m^2 in total.

On the office side, 24 desks are available for PhDs, postdocs, students and guests in an open office environment. In addition, there are 4 staff offices as well as quiet discussion cubicles and a large lounge area which is also suitable for small presentations and work discussions. The building has a separate server room and high-speed connections to the university network and storage, to enable handling the ever larger amounts of data generated on modern LC-MS systems.

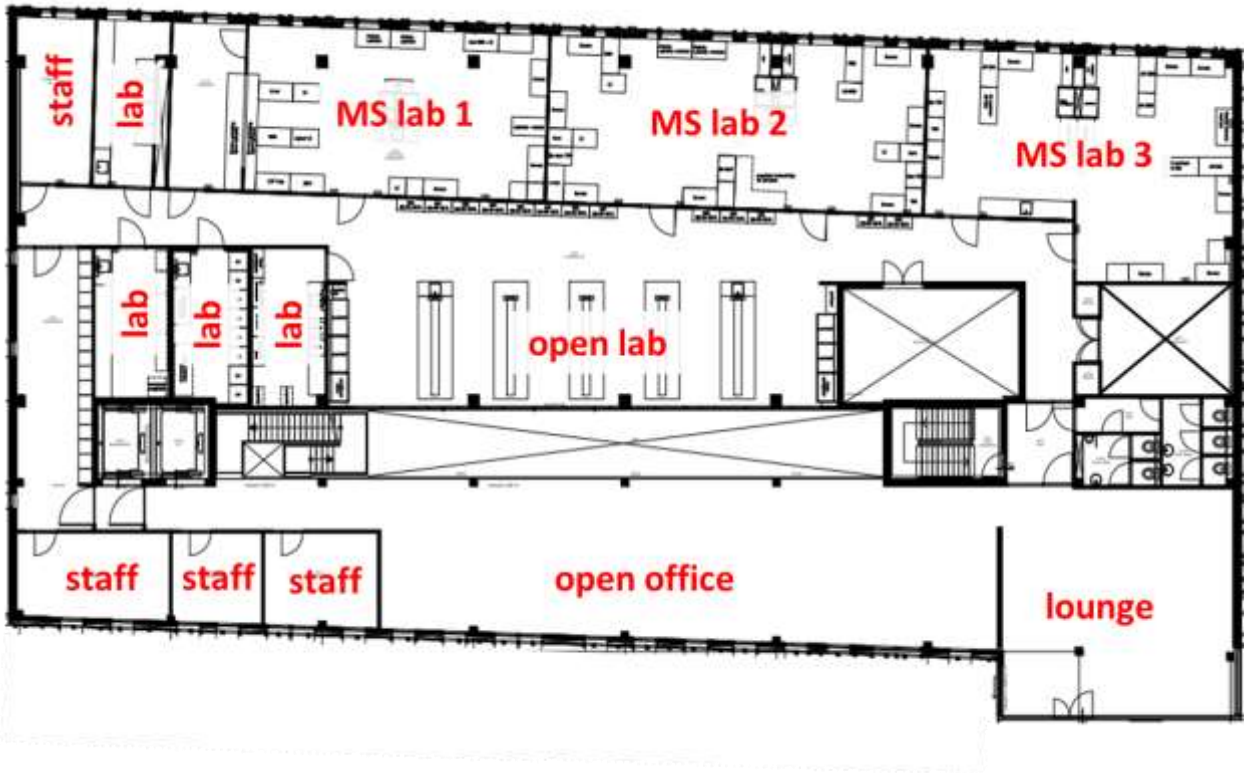

Figure 2. Floor plan of the 6th floor of ERIBA (building 3226), housing the Interfaculty Mass Spectrometry Center (Mass Spectrometry Core Facility), and the Analytical Biochemistry group.

2. Equipment & facilities

With the fusion of the laboratories of Analytical Biochemistry and Medical Biomics, more equipment and expertise has become available for the MS Center. Important additions are high-resolution mass spectrometers, sample preparation equipment (various gel-based methods, SPE, preparative LC), and bioinformatics facilities. All equipment housed and supported by the MS Center is intended to be accessible to researchers from RuG and UMCG, as well as for external customers. Instrument use is charged based on an hourly fee or on lump sum payments for larger projects (see section 3).

Mass spectrometry equipment

The most commonly associated HPLC systems and ion sources are listed per MS; HPLCs are often interchangeable, ion sources less commonly so. Broadly speaking, high-resolution MS systems and ion traps are most useful for identification and characterization of compounds, while triple quadrupole MS systems are mostly used for targeted quantification. In combination with LC separation both types are suitable for analysis of complex samples. Different ionization methods are used for specific compound classes but the bulk of the analyses are performed with electrospray ionization (ESI). NanoLC and nanoESI are used for small sample amounts, primarily for proteomics applications. MALDI and SELDI-TOF are suitable for fast analysis of single compounds and simple mixtures, particularly of large molecules (intact biomacromolecules and polymers).

High resolution MS

1. LTQ-Orbitrap XL (Thermo)
    - Ultimate 3000 nanoHPLC (Dionex)
    - Shimadzu LC20 UFLC
    - ESI, nanoESI
2. QToF 6510 (Agilent)
    - 1200 series nanoHPLC (Agilent)
    - ESI, nanoChipCubeESI
3. Synapt G2-Si (Waters)
    - Acquity UPLC (Waters)
    - ESI

Triple quadrupole MS

4. Xevo-TQ-S (Waters)
    - Acquity UPLC (Waters)
    - ESI
5. TSQ Vantage (Thermo)
    - Ultimate 3000 nanoUPLC (Dionex)
    - ESI, nanoESI
6. TSQ Vantage (Thermo)
    - Accela UPLC (Thermo)
    - ESI, nanoESI, APCI
7. TSQ Quantum AM (Thermo)
    - Surveyor HPLC (Thermo)
    - LC Packings Ultimate nanoLC (Dionex)
    - ESI, APCI, APPI
8. API3000 (PE Sciex)
    - Shimadzu LC20 HPLC

        - ESI, nanoESI, APCI, APPI
    9. API365 (PE Sciex), upgraded EP10+ (Ionics)
        - Ultimate 3000 UPLC (Dionex)
        - ESI
    10. API365 (PE Sciex) , upgraded EP10+ (Ionics)
        - LC Packings Ultimate nanoLC (Dionex)
        - ESI
    11. 6410 (Agilent)
        - 1200 series HPLC (Capillary pump, Nano pump, Micro Autosampler) (Agilent)
        - ESI, nanoChipCubeESI

Ion trap MS

12. HCTultra ETDII (Bruker)
    - LC
    - nanoChipCubeESI
13. Ion Trap LCMS MSD (Agilent)
    - LC
    - ESI, nanoESI, AP-MALDI

MALDI-TOF MS

14. Voyager DE-Pro (Applied Biosystems)
15. ProteinChip SELDI System Enterprise Edition (Bio-Rad)

Sample preparation equipment

- On-line SPE (Solid phase extraction)
    - Spark Integrity
    - Spark Symbiosis
- Preparative LC
    - Äkta design (Amersham)
- Stand-alone HPLC
    - Shimadzu LC20 with fraction collector
    - 1290 Infinity UPLC (Agilent)
    - 1100 series HPLC (Agilent)
- Gel electrophoresis
    - 1D Mini gel systems (Bio-Rad)
    - Molecular Imager (gel imager, Bio-Rad)
- Solution electrophoresis
    - 3100 Offgel fractionator (Agilent)
- Capillary electrophoresis
    - CE 3D (Hewlett Packard)

Figure 3. Mass spectrometry setups installed in the IMSC at ERIBA (photos: 8 October 2013). The numbers refer to the list above. Setups 1-13 are complete LC-MS systems.

3. Personnel & organization

Personnel

The MSCF staff members, now fully part of the IMSC, are:

- Hjalmar Permentier, head of MS Center, 1.0 fte (RuG)
  Room 3226.0609; tel +31-50-3633262; h.p.permentier@rug.nl
- Margot Jeronimus-Stratingh, technician, 0.5 fte (RuG)
  Room 3226.0613; tel +31-50-3633170/8081; c.m.jeronimus-stratingh@rug.nl
- Annie van Dam, technician, 0.5 fte (RuG)
  Room 3226.0613; tel +31-50-3633170/8081; a.van.dam@rug.nl

The head of the scientific board of the IMSC is Prof Rainer Bischoff. After the merger of the IMSC in the ERIBA building the scientific board will consist of Prof Rainer Bischoff (RuG) and Prof Ido Kema (Clinical Chemistry, UMCG). Marcel de Vries, from the Medical Biomics group (UMCG), is detached to the IMSC (1.0 fte) after the move to ERIBA. Technician Jos Hermans of Analytical Biochemistry (1.0 fte) is responsible for the MS instruments in his group which are also available within the IMSC in ERIBA. Further technical support is provided by technician Natalia Govorukhina (1.0 fte) of Analytical Biochemistry, in particular for sample preparation related to proteomics. Data analysis and bioinformatics support is also provided as part of the IMSC, headed by Prof Peter Horvatovich (Computational Mass Spectrometry).

Figure 4. Group photo taken during combined lab day of Mass Spectrometry, Analytical Biochemistry and Pharmaceutical Analysis groups, October 3rd 2013.
Top row, left-to-right: Hjalmar Permentier, Tao (Larry) Zhang, Kees Bronsema, Jos Hermans, Peter Horvatovich, Maciej Skolimowski, Gert Salentijn, Maciej Grajewski, Rainer Bischoff, Turan Gül, Daniël Wilffert, Patty Mulder.
Bottom row, left-to-right: Natalia Govorukhina, Pieter Oomen, Tao Yuan, Margot Jeronimus-Stratingh, Annie van Dam, Najib Bawary, Sara Ongay Camacho.

Organization and policy

All MS instruments and associated equipment listed above are supported by one or more technicians. The technicians all have broad MS experience but each has developed in-depth knowledge on specific subjects or methods. The general policy of the IMSC is to provide high-level service to every eligible scientist from the University of Groningen or the UMCG within its possibilities. Exploitation of the Facility, notably repair and maintenance of MS equipment, and costs of MS-specific consumables is covered by levying user fees (currently 30 euro per hour). For external customers (non-RuG or UMCG) overhead costs are added to the standard user fee (currently 150 euro per hour). This financial model has been sustainable for more than 15 years.
The type of service, contribution and collaboration depend on the type of research project and input of the research group:

1. Scientists that wish to have samples analyzed on an occasional basis with questions that do not require extensive methods development will usually interact directly with the technical staff of the Center and have their analyses done based on an hourly user fee.
2. Scientists that have research questions which require extensive methods development discuss first with the head of the IMSC and, if necessary, the scientific board, to define the approach and workload. Users will have to contribute financially to the methods development work at least on a partial basis, by paying the regular hourly user fee. In the case of long-term developments, which will also enhance the overall competence of the Center, a contract can be concluded over a longer time period (e.g. 3 months) and the fee will be fixed according to agreed-upon criteria but will be lower than just adding the hourly user fee. It is strongly recommended that users foreseeing such a use of the Center discuss their project needs prior to applying for funding and include the cost in their regular project budget.
3. Scientists that have research questions, where the use of cutting-edge biological mass spectrometry and related techniques is required on a structural basis (e.g. biomarker discovery projects, systems biology projects) collaborate with the IMSC on a scientific level. This implies, in general, that the IMSC is already involved at the stage of the grant application and that cost for the use of instrumentation, personnel and consumables are part of the project budget. Such projects may also request dedicated instrumentation in their grants, which will be housed in the IMSC and reserved with priority for these users.
4. Scientists and commercial parties from outside the University of Groningen or the UMCG will be classified in the same way as internal academic users, but the user fees include salary cost and instrument depreciation (currently 150 Euro), or agreed-upon contract criteria as mentioned above.

4. Overview of projects

The report lists two types of MS analyses, namely service work and scientific projects, although the distinction is fading, since many service questions expand into larger projects. Scientific projects as described below are those were a researcher is working for extended times or permanently in the MS facility, in the case of PhD students and postdocs usually supervised by our staff. Service work is categorized by department and a brief description of the type of analyses and techniques is given. Very minor or brief analyses are omitted. For project background information please refer to the respective research groups or their websites.

Service work and projects

University of Groningen

- **Pharmaceutical Biology**[4, 18]
  - quantitative and qualitative LC-MS en MS/MS on (natural) metabolites and proteins
  - MALDI-TOF and (LC-)MS of proteins and peptides
- **Pharmacokinetics, Toxicology and Targeting**[11]
  drug metabolism, LC-MS/MS quantification of drugs and metabolites
- **Pharmaceutical Gene Modulation**
  (LC-)MS and MALDI-TOF of proteins and protein reagents
- **Pharmaceutical Technology**[1, 12]**, Innocore**
  LC-MS of peptides, quantification of proteins by LC-MS/MS
- **Stratingh Institute for Chemistry & Membrane Enzymology**
  - MALDI-TOF and (LC)-MS, mainly samples that cannot be measured in-house on own instruments
  - ESI-MS of organometallic complexes
- **Biophysical Chemistry**
  MALDI-TOF of proteins and peptides
- **Polymer Chemistry**
  MALDI-TOF and LC-MS of polymers and modified proteins
- **Molecular Genetics & Molecular Microbiology**
  MALDI-TOF and LC-MS, mainly of peptides

University Medical Center Groningen

- **Cell biology**[2, 5]**, Clinical Pharmacology**
  sphingolipids, LC-MS/MS quantification
- **Nuclear Medicine and Molecular Imaging**
  (LC)-MS of drugs and contrast compounds
- **Biomedical Engineering**
  MALDI-TOF of proteins
- **Internal Medicine/Allergology**
  peptides**,** LC-MS/MS quantification

- **Cardiology**
  LC-MS/MS quantification of metabolites

External

- **Biomade**
  MALDI-TOF and LC-MS, mainly of peptides
- **Synvolux**
  MALDI-TOF of intact proteins
- **Citeq**
  MALDI-TOF of protein digests

Scientific projects and collaborations

Apart from its primary task of providing MS service, the IMSC is closely involved in several larger scientific projects. In these projects, equipment and/or PhDs and postdocs are placed in the IMSC and make direct use of the infrastructure and expertise.

Electrochemistry-mass spectrometry[7, 8, 14, 15, 20]
A research line that has been running in the MSCF/IMSC for more than a decade involves the coupling of electrochemistry (EC) with mass spectrometry. This line has resulted in two STW-funded projects, the first of which ended in 2011 with two PhD theses. A follow-up STW-project has started in 2012, with two PhD students employed at the RuG, co-supervised by Hjalmar Permentier and Rainer Bischoff, and a third PhD student having his own funding (CSC grant). The projects use the existing, older LC-MS equipment of the MSCF as well as dedicated EC equipment acquired as part of the project. The current research focuses on (1) the specific synthesis by EC of drug metabolites and their analysis by LC-MS and (2) the specific digestion of proteins by EC followed by enrichment and analysis of resulting peptides by LC-MS. The methods are potentially interesting for drug development, metabolism research and proteomics, but not immediately applicable to other IMSC users. However, valorisation of the EC-MS methods is one of the aims of the project.
In the beginning of 2012 an NWO-CW-ECHO project entitled 'Electrochemically-assisted redox enzyme reactors by cofactor immobilization' was funded. This project is supervised by Prof Marco Fraaije (Biochemistry, RuG), and co-written by Hjalmar Permentier and Rainer Bischoff, who will also co-supervise the PhD student. In the course of this project a significant amount of work will be performed in the IMSC in collaboration with the PhD students of the STW EC-MS project.

Targeted proteomics and metabolomics of ageing[9,10, 19]
Starting from 2010, the Systems Biology Centre for Energy Metabolism and Ageing (SBC-EMA) has 1 dedicated LC-MS instrument and two postdocs working on quantitative proteomics (Karin Wolters) and metabolomics (David Siegel, started 2012) projects. New LC-MS/MS methods based on SRM analysis are developed for large-scale high-throughput quantitative analyses of complex proteome and metabolome samples of yeast and mice model systems. Direct supervision is shared between Rainer Bischoff and Hjalmar Permentier. The expertise and methods developed in this project are also very useful for many other groups outside SBC-EMA and these types of analyses can now also be offered to other research groups.

Metabolomics in metabolic disease

In October 2013, a state-of-the-art high-resolution LC-MS instrument with ion mobility capability has been placed in the MS Center by the Laboratory Medicine department (Prof Dirk-Jan Reijngoud, UMCG). High-resolution screening of complex metabolite samples, notably lipids, will be developed in close collaboration with the MS Center.

Ionization methods for mass spectrometry

Also in October 2013, a mass spectrometer has been placed in the MS Center by the Pharmaceutical Analysis group (Prof Sabeth Verpoorte, RuG), for development of new ionization methods, notably paper spray ionization.

5. Publications, presentations, teaching

In this section papers and activities are included of IMSC-employed personnel (Hjalmar Permentier, Annie van Dam, and Margot Jeronimus-Stratingh); names in author lists are underlined. Papers with explicit mention of IMSC personnel in the acknowledgements are separately listed. For other (unpublished) contributions refer to section 4. Teaching comprises both lectures and practical work. The two-day mass spectrometry course is organized yearly to inform, educate, and interest students and researchers on the practical and theoretical aspects of MS and how they can make use of the Facility.

Peer-reviewed papers

2013

1. Avanti C, Hinrichs WLJ, Casini A, Eissens AC, van Dam A, Kedrov A, Driessen AJM, Frijlink HW, Permentier HP. **2013.** The formation of oxytocin dimers is suppressed by the zinc-aspartate-oxytocin complex. *Journal of Pharmaceutical Sciences* 102(6): 1734-1741.
2. Bouma HR, Mandl JN, Strijkstra AM, Boerema AS, Kok JW, van Dam A, IJzerman A, Kroese FG, Henning RH. **2013**. 5'-AMP impacts lymphocyte recirculation through activation of A2B receptors. *Journal of Leukocyte Biology* 94(1): 89-98.
3. Budnik LT, Preisser AM, Permentier H, Baur X. **2013.** Is specific IgE antibody analysis feasible for the diagnosis of methylenediphenyl diisocyanate-induced occupational asthma? *International Archives of Occupational and Environmental Health* 86(4): 417-430.
4. Happyana N, Agnolet S, Muntendam R, van Dam A, Schneider B, Kayser O. **2013**. Analysis of cannabinoids in laser-microdissected trichomes of medicinal *Cannabis sativa* using LCMS and cryogenic NMR. *Phytochemistry* 87: 51–59.
5. Meszaros P, Klappe K, van Dam A, Ivanovac PT, Milnec SB, Myers DS, Brown HA, Permentier H, Hoekstra D, Kok JW. **2013.** Long term myriocin treatment increases MRP1 transport activity. *The International Journal of Biochemistry & Cell Biology* 45(2):326-334.
6. Ourailidou ME, van der Meer JY, Baas BJ, Jeronimus-Stratingh M, Gottumukkala AL, Poelarends GJ, Minnaard AJ, Dekker FJ. **2013**. Aqueous oxidative Heck reaction as a protein-labeling strategy. *ChemBioChem* DOI: 10.1002/cbic.201300714.
7. Roeser J, Alting NFA, Permentier HP, Bruins AP, Bischoff R. **2013.** Chemical labeling of electrochemically cleaved peptides. *Rapid Communications in Mass Spectrometry* 27: 546-552.
8. Roeser J, Alting NFA, Permentier HP, Bruins AP, Bischoff R. **2013.** Boron-doped diamond electrodes for the electrochemical oxidation & cleavage of peptides. *Analytical Chemistry* 85(14): 6626-6632.
9. Siegel D, Permentier H, Bischoff R. **2013.** Controlling detrimental effects of metal cations in the quantification of energy metabolites via UPLC-(-)ESI-MS/MS by employing acetylacetone as a volatile eluent modifier. *Journal of Chromatography A* 1294: 87–97.
10. Siegel D, Permentier H, Reijngoud, DJ, Bischoff R. **2013.** Chemical and technical challenges in the analysis of central carbon metabolites by liquid-chromatography mass spectrometry. *Journal of Chromatography B* DOI:10.1016/j.jchromb.2013.11.022.
11. Siissalo S, de Waard H, de Jager MH, Frijlink HW, Hinrichs W, Dinter H, van Dam A, Groothuis GMM, de Graaf IAM. **2013.** Nanoparticle formulation of a poorly soluble CB-1 antagonist

improves absorption by rat and human intestine. *Drug Metabolism and Disposition* 41: 1557-1565.

2012

12. Avanti C, Permentier HP, van Dam A, Poole P, Jiskoot W, Hinrichs WLJ, Frijlink HW. **2012.** Stabilization of the disulfide bond of oxytocin in citrate buffered solution by divalent metal ions. *Molecular Pharmaceutics* 9(3): 554-562.
13. Gandhi T, Puri P, Fusetti F, Breitling R, Poolman B, Permentier HP. **2012.** The effect of iTRAQ labeling on relative abundance of peptide fragment ions produced by MALDI. *Journal of Proteome Research* 11(8): 4044-4051.
14. Nouri-Nigjeh E, Bruins AP, Bischoff R, Permentier HP. **2012.** Electrocatalytic oxidation of hydrogen peroxide on a platinum electrode in the imitation of oxidative drug metabolism by Cytochrome P450s. *Analyst* 137: 4698-4702.
15. Nouri-Nigjeh E, de Vries MP, Bruins AP, Bischoff R, Permentier HP. **2012.** Electrochemical oxidation of quaternary ammonium electrolytes: unexpected side reactions in organic electrochemistry. *Electrochemistry Communications* 21: 54-57.

Publications with IMSC acknowledgement

16. Handayani N, Loos K, Wahyuningrum D, Buchari, Zulfikar MA. **2012.** Immobilization of *Mucor miehei* lipase onto macroporous animated polyethersulfone membrane for enzymatic reactions. *Membranes* 2(2): 198-213.
17. de Jong W, Vijgenboom E, Dijkhuizen L, Wösten HAB, Claessen D. **2012.** SapB and the rodlins are required for development of *Streptomyces coelicolor* in high osmolarity media. *FEMS Microbiology Letters* 329(2): 154-159.
18. Zandvoort E, Geertsema EM, Baas BJ, Quax WJ, Poelarends GJ. **2012.** An unexpected promiscuous activity of 4-oxalocrotonate tautomerase: the *cis–trans* isomerisation of nitrostyrene. *ChemBioChem* 13: 1869-1873.

Non peer-reviewed papers

19. Wolters K, Permentier H, Bischoff R. **2012.** Targeted proteomics as a tool to study aging in yeast. *NPC Highlights* May, 16-19.

PhD thesis defence

20. Roeser J. **2013.** Electrochemical oxidation and cleavage of peptides in bioanalysis. 8 March 2013, University of Groningen.

Presentations at international conference/workshop

21. COST Nano IBCT Tutorial Workshop on Complex Targets, 31 May - 1 June 2012, Groningen, The Netherlands Invited tutorial lecture: 'Electrospray ionization: Transferring ions from liquid to gas phase', H.P. Permentier
22. 19th International Mass Spectrometry Conference, 16-21 September 2012, Kyoto, Japan Poster: 'Novel approaches in the imitation of oxidative drug metabolism by electrochemistry-mass spectrometry', E. Nouri, H.P. Permentier, A.P. Bruins, R. Bischoff.

Guest researcher

- Miina Ruokolainen, PhD student, University of Helsinki, May 2013, 4 weeks project on EC-MS of drug compounds: “Imitation of phase I metabolism reactions by photocatalysis and electrochemical reactions”.

Teaching, (under)graduate courses

April 2012:	Proteomics / Genomics course WLB07041
May 2012:	Medical Genomics & Proteomics course WLB07090
December 2012:	2-day course on Mass Spectrometry, 18-19 December
March 2013:	Industrial Bioanalysis course, 7 March
April 2013:	Proteomics / Genomics course WLB07041
May 2013:	Medical Genomics & Proteomics course WLB07090
November 2013:	2-day course on Mass Spectrometry, 4-5 November