

Swammerdam Institute for Life Sciences

**Faculty of Science
Annual Report 2013**

**Swammerdam Institute
for Life Sciences**

**Faculty of Science
University of Amsterdam**

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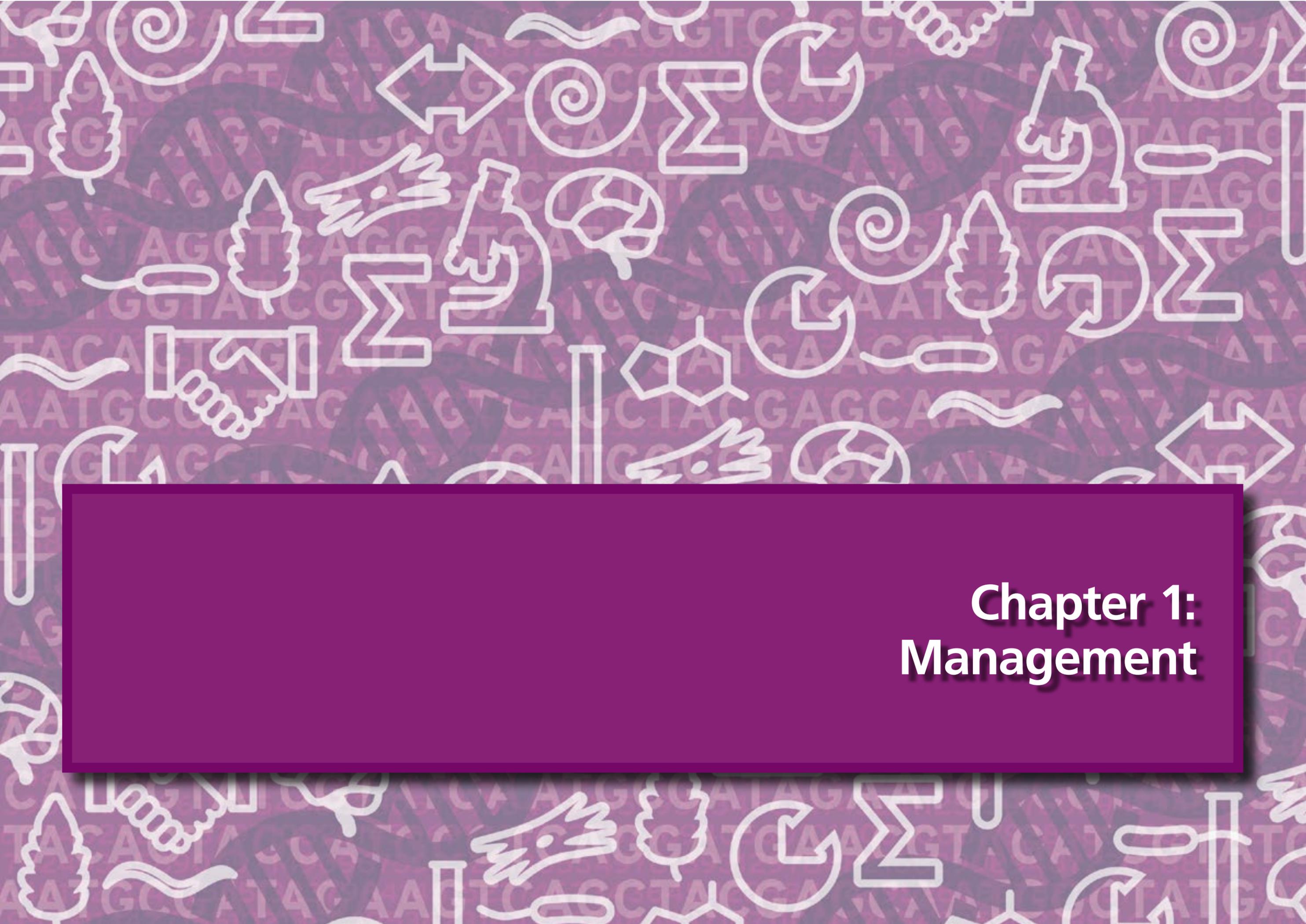
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Swammerdam Institute for Life Sciences (SILS)
Faculty of Science, University of Amsterdam
Annual Report 2013

Amsterdam, July 2014

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**Chapter 1:
Management**

2013 was an exciting year. Key appointments were made aimed at starting a new stem cell research programme, enforcing systems biology, reducing the teaching load of the neuroscience staff and intensifying our collaboration with the plant breeding industry. In total SILS appointed over 30 full-time employees mostly on external grants to



Prof. Willem Stiekema

new positions as tenure trackers, postdocs, PhD students or technicians. This shows that the earning capacity of SILS is still high in spite of diminishing government funding. This success was not easy to achieve, for in spite of being rated as very good to excellent many research proposals nevertheless failed to attract funding owing to the high number of proposals submitted leading to low success rates. I am proud of those SILS employees who were persistent and, rather than giving up, improved their rejected proposals and resubmitted them. I am proud of those SILS employees who keep spirits high and make SILS a fantastic place to be, do research and teach. And I am proud of those employees who inspire our young scientists to strive for their full potential. Let's continue along these lines.

Appointments

In 2013 professor Roel Nusse of Stanford University was named visiting professor of Stem Cells and Regenerative Biology. Roel is an expert in stem cells, regenerative biology and Wnt signalling, a rapidly growing field worldwide. There are significant implications for health research, including cancer research, but also for fundamental developmental biology. In addition, this subject closely relates to the research agenda of the University of Amsterdam's (UvA) Systems Biology research priority area. A further three professors by special appointment joined SILS. Dr Joost Keurentjes was appointed at the end of 2012 to the special chair in Applied Quantitative Genetics and he delivered his inaugural speech in June of this year. Dr Marcel Prins of Keygene NV in Wageningen was appointed in July to the special chair in Phytopathology, with

a special focus on plant virology. Both professors, who will be hosted by SILS's Molecular Plant Biology groups, will work on subjects closely related to the Faculty of Science's Green Life Sciences research priority area as well as the plant breeding industry. Dr Ole Jensen of the Donders Institute of Radboud University Nijmegen was appointed to the special chair in Human Cognition, and will work closely with the group of professor Cyriel Pennartz on subjects related to the UvA's Brain & Cognition research priority area. Dr Renée van Amerongen was appointed as one of the six MacGillavry Fellows who were selected by the Faculty in 2013 out of more than 200 international candidates. The MacGillavry Fellowship recruitment programme of the Faculty of Science targets ambitious talented female scientists who have excelled in their respective discipline, exhibit leadership potential and aspire to a tenure track position leading towards a full professorship. Renée's research focuses on Wnt-responsive stem cells in the mammary gland, work that puts her at the exciting interface of stem cell biology and fundamental cancer research.

Furthermore, three tenure trackers, Dr Matteo Barberis (Westerhoff group), Dr Filipe Branco dos Santos (Hellingwerf group) and Dr Umberto Olcese (Pennartz group), were appointed in addition to 22 PhD students, five postdocs and seven technicians/support staff members.

Research

A major breakthrough towards personalised medicine was achieved by an international team of systems biologists including professor Hans Westerhoff that published the first human metabolic road map in Nature Biotechnology. This biochemical survey can aid in elucidating the causes of metabolic disorders and improve the diagnosis and therapy of diseases such as obesity, diabetes and cancer.

Dr Gertien Smit studies the response of pH in environmental changes in yeast. She and others have shown how small changes in pH can affect cells on a global scale. Cancer induces changes in pH and this phenomenon needs to be incorporated much more in our thinking about the causes and development of diseases in general.

Dr Timo Breit's RNA Biology and Applied Bioinformatics group received a Medium Investment Grant from the Netherlands Organisation for Scientific Research (NWO) for a Next Generation Sequencing (NGS) Platform, the latest revolutionary technology to be developed in life sciences research. With this technology, the entire genome sequences of bacteria, plants animals and humans can be determined in

days at an affordable cost. Consequently, NGS is set to transform life sciences research, especially research into so-called non-model organisms. Moreover, this is the reason why the number of bioinformaticians cannot meet the demand for their skills, and why there are ample employment opportunities.

PhD Graduations

2013 saw the graduation of 14 SILS professor-supervised PhD students: Xin Qiau, Linda Holtman, Clemens Heilmann, Alice Sorgo, Marijn van Wingerden (cum laude), Diana Hendrickx, Marlies Oostland, Femke den Boon, Johan van Beilen, Poonam Sharma, Hansuk Buncherd, Hemi Malkki, Martin Vinck and Jeroen van der Steen. I would like to say a little more about one of them in particular, neuroscientist Dr Martin Vinck. He graduated cum laude while he also received the Scopus Young Scientist Award, an NWO Rubicon grant and a Human Frontiers Science Programme Fellowship to continue his career at Yale University. A further eight PhD students supervised by SILS professors appointed to special chairs also graduated in 2013: Rogier Rooswinkel (Borst, AvL-NKI), Niek Wit (Borst, AvL-NKI), Willem Jan Keune (Borst, AvL-NKI), Theresa Guhr (van Ham, Sanquin), Daniel Lam (Hordijk, Sanquin), Younes Zoughlami (Hordijk, Sanquin), Ilse Timmerman (Hordijk, Sanquin) and Marc Hogenbirk (Berns, AvL-NKI).

Research grants

In 2013 SILS obtained external grants with a total value of more than € 7 million. This is an all-time record for the institution. Most notable are the VIDI grant of € 800,000 awarded to Dr Petra Bleeker for her research proposal 'Defence in the wild: from trichome transcriptomes and metabolomes to breeding tools for defence markers in tomato' and the STW grant of € 3.3 million awarded to professor Michel Haring and colleagues for his research proposal 'Green Defence against Pests'. Together with colleagues in Utrecht, Dr Harm Krugers obtained a ZonMw TOP grant to study how early life experiences influence the function and sensitivity of the brain as well as a USA Military Operational Medicine Research grant to study post-traumatic stress syndrome. Dr Aniko Korosi received an NWO MEERVOUD grant to study the effects of early-life stress on the development of Alzheimer's disease. Dr Rob Schuurink received three research grants totalling more than € 1 million to allow him to further develop his research into the role of plant volatiles.

Awards and prizes

The SILS Research Day Presentation award was presented to PhD student Philipp Savakis who works on the production of biofuels and chemical feedstock from light, water and CO₂ using the cyanobacteria *Synechocystis sp.* Runner up in this category was Eva Nanninck who investigates how brain structure and function are programmed by stressful early-life experiences. The Poster Award was won by Willemieke Kouwenhoven who studies transcription factor Engrailed 1 in the embryonic development of dopaminergic neurons. The runner up in this category was Pascal Bielefeld who presented a poster on the role of the receptor 5HT_{3aR} on migration of new-born granule cells in the adult *Dentate Gyrus*.

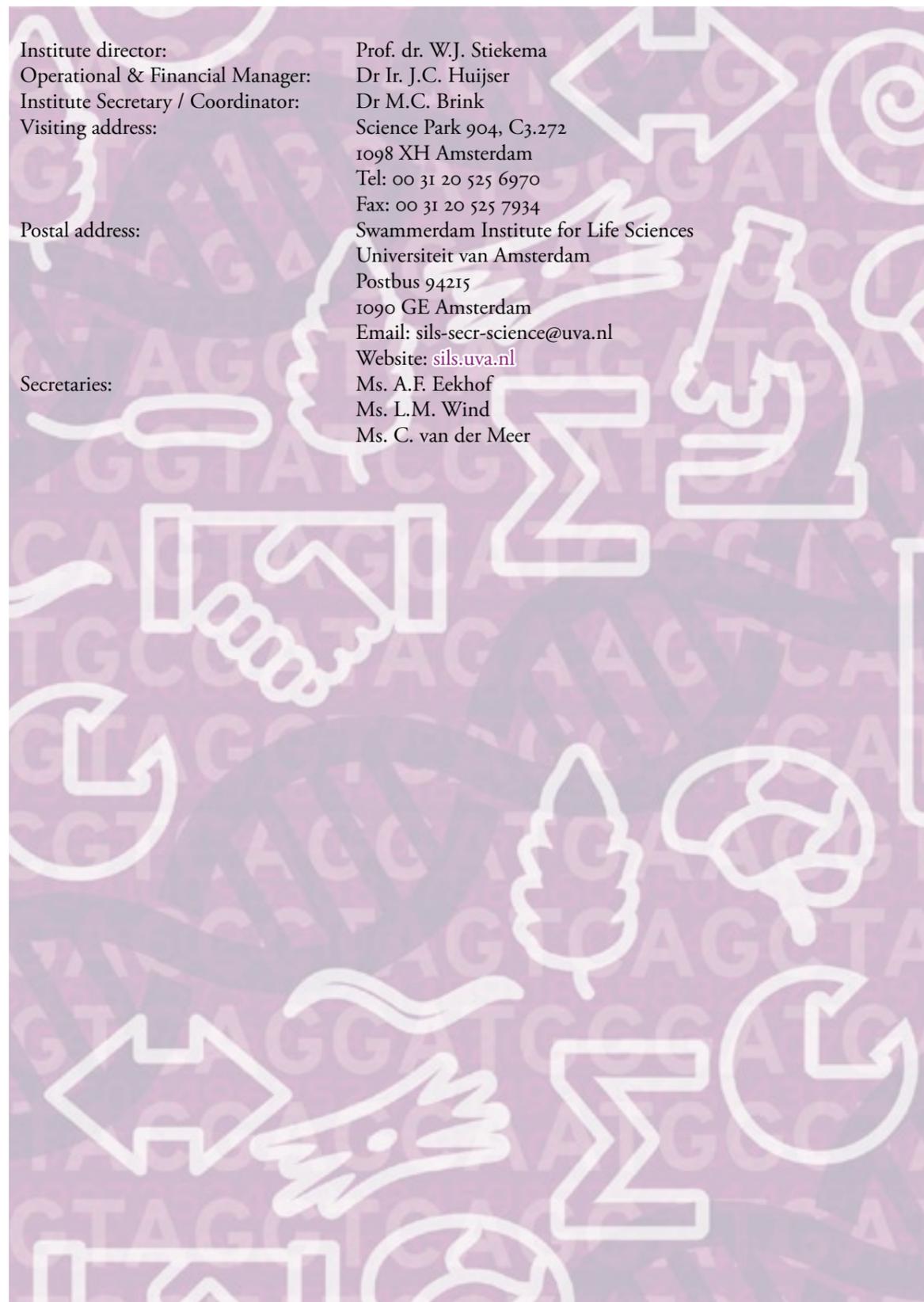
The 2013 SILS Overall (research, education, valorisation) Award was granted to the group of professor Paul Lucassen for their outstanding across-the-board performance. The Education Award was granted to Dr Natalie Cappaert and Dr Wim Ghijsen for their abiding dedication to teaching. Dr Rob Schuurink won the Valorisation Award for obtaining research grants from three different granting agencies amounting to more than € 1 million. Institution manager Dr Casper Huijser was awarded the 2013 Annual Support Staff Award for his everlasting efforts to make SILS the best place to teach and do research. The Metabolomics Society granted two awards to Dr Johan Westerhuis: 'Pre-2013 Best Paper Award' and '2013 Highest Download Award'.

Valorisation

Within the Framework of the Green Life Science Hub and in close collaboration with Naktuinbouw (the Netherlands Inspection Service for Horticulture), professor Michel Haring, Dr Timo Breit and professor Chris de Koster were awarded € 910,700 in grants for four subprojects: Green Forensics (€ 620,000), Vegetables Origin Tracer (€ 200,700), Green Innovation Counter (€ 40,000) and Green Student Lab (€ 50,000). These projects were endorsed by the Amsterdam Economic Board and funded by EU-EFRO, the Province of North Holland, and the Amsterdam Chamber of Commerce.

Willem

Willem Stiekema
Director



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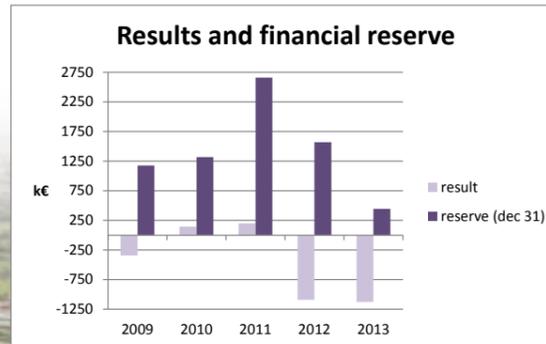
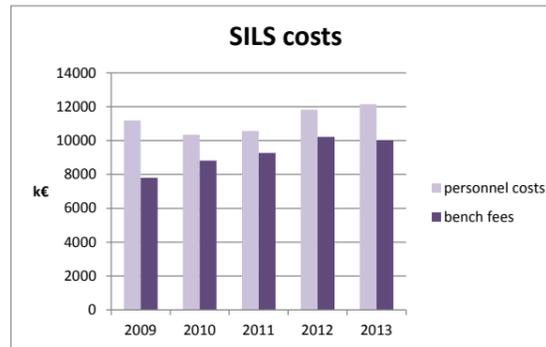
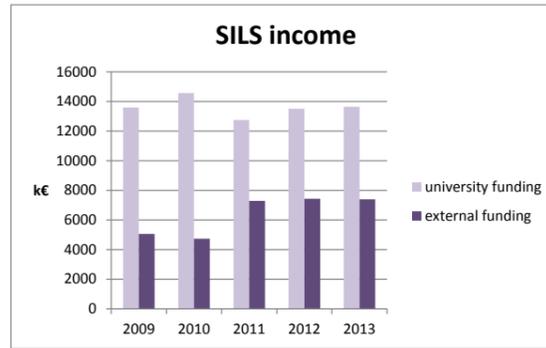
Special chairs

<i>Prof. E.M.A. (Eleonora) Aronica</i>	Chair: Pathology of the Nervous System Head Department of Pathology, Academic Medical Centre
<i>Prof. J. (Jannie) Borst</i>	Chair: Experimental Oncology Head Division Immunology, Netherlands Cancer Institute, Amsterdam
<i>Prof. S.M. (Marieke) van Ham</i>	Chair: Biological Immunology Manager Immunopathology, Sanquin Research, Amsterdam
<i>Prof. E.M. (Elly) Hol</i>	Chair: Biology of Glia and Neural Stem Cells Group Leader, Netherlands Institute for Neuroscience, Amsterdam
<i>Prof. P.L. (Peter) Hordijk</i>	Chair: Molecular Cell Biology of Cell Migration Head of the Dept. of Molecular Cell Biology, Sanquin Research, Amsterdam
<i>Prof. J. (Jeroen) Hugenholtz</i>	Chair: Industrial Molecular Microbiology Head Coca Cola Bioscience Department, Germany
<i>Prof. O. (Ole) Jensen</i>	Chair: Human Cognition Principal researcher of the Neuronal Oscillations group at the Donders Institute for Brain, Cognition and Behaviour, Nijmegen
<i>Prof. A.H.C. (Antoine) van Kampen</i>	Chair: Biological and Biomedical Information Head Bioinformatics Laboratory, Academic Medical Centre, Amsterdam
<i>Prof. J.J.B. (Joost) Keurentjes</i>	Chair: Applied Quantitative Plant Genetics Associate Professor Laboratory of Genetics Plant Genetics and Cytogenetics Wageningen University, Wageningen
<i>Prof. C.G. (Chris) Kruse</i>	Chair: Cellular and Systems Neurobiology Director Business Development/Scientific Advisor, Pharma Plexus Holland, Utrecht
<i>Prof. R. (Roel) Nusse</i>	Chair: Stem Cells and Regenerative Biology Head of the Nusse Laboratory, Howard Hughes Medical Institute, Department of Developmental Biology, Stanford University, School of Medicine, Stanford, USA
<i>Prof. M.S. (Melly) Oitzl</i>	Chair: Cognitive Neurobiology Associate Professor, Center for Drug Research, Medical Pharmacology, University of Leiden
<i>Prof. A.P. (Arie) Otte</i>	Chair: Valorisation in the Life Sciences
<i>Prof. M.W. (Marcel) Prins</i>	Chair: Phytopathology (in particular Plant virology) Vice President Vegetable Crops, KeyGene, Wageningen

The integrated results for 2013 show a deficit of 1.127 K€ where a deficit of K€ 974 was budgeted. This deficit was deducted from the financial reserve of SILS resulting in K€ 442 of reserve remaining on January 1, 2014.

The shortfalls in 2012 and 2013 owe mainly to the appointment of new teaching staff needed in view of the sharp increase in student numbers. The decision to employ new staff, and thus to overspend budgets, was made in consultation with the Faculty of Science (FNWI).

Over the longer term, this will serve to increase SILS' revenue as set out in the FNWI models used to allocate funds within the Faculty to the research institutes. This model is based (in part) on teaching load and the number of degrees conferred. However, as the initial investment was not made in accordance with these models, as aspected, the result has been negative for the last two years.



Right: overview of revenues and costs of the Swammerdam Institute for Life Sciences, in K€, for the years 2009-2013.



Amsterdam Science Park

Funding

The funding system for Dutch universities distinguishes between three different kinds of funding resources, which are generally referred to by number: one to three. Funding originating from the university itself are known as the first 'flow of funds' or 'moneystream'. External funding (e.g. from the Netherlands Organisation for Scientific Research (NWO) is known as the second flow of funds, and all other external funding such as from the EU and industry form the third flow of funds.

Personnel

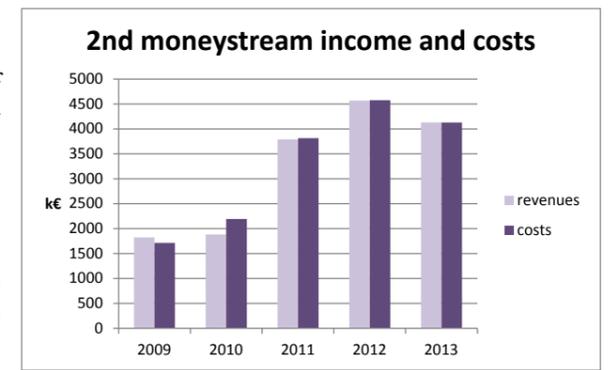
The University of Amsterdam (UvA) is committed to an equal balance between male and female staff at all levels. The figure below presents the male/female ratio on 31 December 2013 and shows that females are still underrepresented in the higher ranks. At this time, 30% of the total 232 SILS employees (including bursaries) were from abroad, most notably from Germany, China, Poland and Italy. In terms of age, SILS staff span the full range from new PhD students to employees near retirement.

Below: number of FTEs and total number of employees at SILS on December 31 (including FOM employees and PhD fellows with a scholarship).

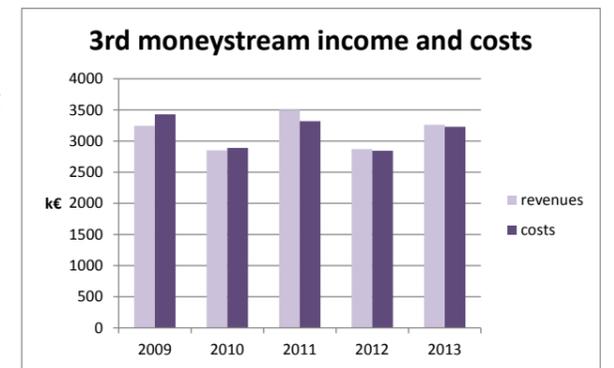
Year	2009	2010	2011	2012	2013
FTE university funded	90,6	90,6	103,9	101,4	103,3
FTE (NWO/FOM funded)	20	33,3	43,6	50,5	52,8
FTE (EU, contracts)	38,1	37,3	38,6	36,7	47,5
FTE (scholarships)	11	10	10	12	16
number of FTE	159,7	171,6	196,1	200,8	219,6
number of employees	167	180	208	211	232

Male/female ratio	2010		2011		2012		2013	
	M	F	M	F	M	F	M	F
PhD	0.41	0.59	0.45	0.55	0.48	0.52	0.51	0.49
Postdoc	0.82	0.18	0.72	0.28	0.67	0.33	0.56	0.44
Assistant prof	0.71	0.29	0.70	0.30	0.68	0.32	0.7	0.3
Associate prof	1.00	0.00	0.83	0.17	0.87	0.13	0.78	0.22
Professor	0.92	0.08	0.93	0.07	0.93	0.07	0.94	0.06

Left: ratio male/female staff at SILS on 31 December (including FOM employees, excluding PhD/postdoc fellows on a scholarship).



Overview of revenues and costs in the 2nd funding source, in K€, for the years 2009-2013.



Overview of revenues and costs in the 3rd funding source, in K€, for the years 2009-2013.



Chapter 2: Highlights

Interviews with Joost Keurentjes and Marcel Prins

SILS welcomed two new special chairs in 2013: Joost Keurentjes, professor of Applied Quantitative Genetics, and Marcel Prins, professor in Phytopathology with a special focus on plant virology.

What is the focus of your special chair?

'The chair of Applied Quantitative Genetics focuses on the application of large-scale genomic data-sets in modern plant breeding approaches. Over the last decade, breeding practices have shifted from selection strategies keyed on end products towards predictive breeding by design using DNA sequence information. This requires training a new generation of plant breeders.'

What is your incentive?

'I like reducing the complexity of genetic systems to a comprehensible scale without losing the possibility to explain global patterns. This requires a multidisciplinary approach and collaboration between research teams with very distinct areas of expertise. It's great to learn from different fields and to be able to integrate each other's contributions in a dedicated research project. Moreover, students get a chance to be involved in cutting-edge research, and it's very rewarding to have a hand in their perspective.'

How does this job connect to your other work?

'I head up a research group at Wageningen University that is working to elucidate the regulatory mechanisms of complex traits. We develop novel genetic resources to address questions about fundamental and applied aspects of genetic diversity and inheritance patterns. We try to understand why plant properties vary in nature and in crops and how we can use this information to improve our current breeding approaches.'

What will be your biggest challenge?

'The biggest challenge probably lies in increasing the number of students who would consider a career in the breeding industry to keep pace with the rapid growth of this sector and demand for highly specialised professionals. Besides that, the fast developments in the field of applied quantitative genetics need constant investments from the industry and funding agencies to keep up with the latest developments and to consolidate the leading position of the Dutch green industry.'

What do you want to achieve?

'I hope to convince students of the exciting challenges that lie ahead for using the wealth of genetic information currently being generated through advances in sequencing and other omics technologies. We hope to achieve a strong interaction between the breeding industry and the UvA in order to train the next generation of students and offer them attractive career perspectives.'



Prof. Joost Keurentjes. Photo: Jeroen Oerlemans.



Prof. Marcel Prins. Photo: Jeroen Oerlemans.

What is the focus of your special chair?

'I have been fascinated by plant viruses since the first lecture (early 1988) on this topic by the late Professor Rob Goldbach, later my PhD supervisor at Wageningen University. In my research chair, I focus on the interplay between plants and plant viruses. How can tiny viruses - often encoding not more than five proteins - carry out all the many complex functions that are needed for their own reproduction and spread and deal with plant defenses at the same time?'

How does this job connect to your other work?

'My other job is vice-president of vegetable crop research at KeyGene. KeyGene is the oldest plant biotech company in the Netherlands, where 150 people work on innovations for the plant breeding industry. I lead the unit responsible for a large part of our business with vegetable seed companies. Since KeyGene is an R&D company, this management job also has a big focus on content, primarily in the development and application of technologies for plant breeding, and involving next-generation sequencing applications for genomic breeding, mutation breeding and computational lead discovery.'

What is your incentive?

'On the professional side, the topic of plant virology relates to the work we do with plant viruses at KeyGene. At the same time, this combination of jobs provides me with the opportunity to 'dig deeper' into the basic principles of virus infections in plants without the urgency for short to mid-long term application. A personal motivation is to bring industry and academia closer together. Opportunities for future jobs in industry are typically under-illuminated in our academic curricula, whereas by far the most jobs are available there. I have discovered first-hand how interesting science can be in an industrial setting. In the USA it is very common for experts to flip back and forth between industry and academia, but in Europe we are much more conservative. I think that's a waste of talent and opportunity and it undermines our competitiveness in the world.'

What is your main goal?

'I aim to gain new insight into the plant-virus interactions. I hope to be able to supervise two or three PhD students and postdocs and bring them to the next level in their professional lives. Also, I want to educate a broader audience of students (and others) in the fascinating world of plant viruses and at the same time be inspiring as an industrial R&D leader for potential future colleagues.'

What will be your biggest challenge?

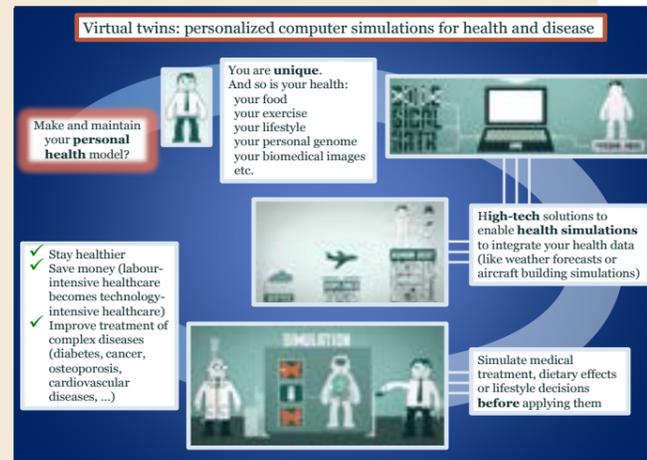
'Organising my time effectively. Besides the two jobs - which are impossible to fit into a 40 hour week - I want to spend quality time with my family. My wife has a flourishing career as a freelance journalist and it's important that she also has time to pursue her dreams. We have two young children that we also want to give our best attention. Fitting that all together has been a struggle and likely will continue to be for years to come. Then again, we are not the first to be in this situation and I consider myself extremely fortunate to have the opportunity to pursue it all!'

Research highlights

Research highlight 1: human metabolic road map

What are your chances of developing cancer, diabetes or heart disease? Are they determined just by genes ('nature'), by what you eat (or smoke) ('nurture'), or by what you should but don't do (lifestyle)? In a team of international systems biologists, Hans Westerhoff has drawn up a comprehensive 'roadmap' of human metabolism. This biochemical survey can elucidate causes of metabolic disorders and improve the diagnosis and therapy of diseases such as obesity, diabetes and cancer. Determining an individual's DNA sequence on the basis of a blood, skin or saliva sample is now even cheaper than six months of health care, whilst that DNA sequence persists for a lifetime.

By projecting an individual's particularities onto the map and computing possible flux patterns, potential malfunctions and diseases can be predicted for that individual. This applies to malfunctions that may arise from eating too much or too little of certain types of food, from lifestyle factors or from genetic predisposition. The same procedure can be applied to predict which drug therapies will treat diseases, and to greater effect than most current procedures, if only because they can now target individuals.



Research highlight 2: pH-researcher Gertien Smits

You study the response to environmental changes in yeasts. What do you want to know?

'I am fascinated by the flexibility of life: living cells all have to meet the same basic requirements, and they can do so in a number of environmental niches, as single cells, or in a multicellular context. How can this balanced network of chemical reactions and molecular interactions be maintained in such a diverse set of conditions?'

What is the most challenging part in this?

'We have to connect our knowledge and insight at many levels to understand how the biophysics and chemistry of life lead to cells and organisms that can display specific behaviors in response to specific environments. These networks have evolved into what they are now; they weren't designed with a specific function in mind. When we try to understand life, we always do so assuming a specific function or role for each adaptation, but we can only assume these to be the 'why' the cells have responded to.'

Intracellular pH has been gaining more attention in recent years, why is that?

'It's a combination of possibility and knowledge. We were convinced that the pH should be constant. The scant data available suggesting this might not be the case may have been at the back of a few people's minds, but for the general scientific community this was too difficult to fit with generally accepted knowledge. Recent technologies for monitoring pH in living cells are revealing additional conditions that affect pH, and we and others have shown how small changes in pH can affect an entire cell. This is going hand in hand with renewed interest in biochemistry, through systems biology. With all the approaches now using metabolic networks and genome scale analyses, it has become much more feasible to study disease. For instance, changes in pH have been observed in cancer. This needs to be incorporated into our thinking about the causes and development of disease.'



Research highlight 3: MacGillavry Fellow Renée van Amerongen

SILS welcomed Renée van Amerongen in 2013. She is a researcher in Molecular Cytology and works as a MacGillavry Fellow.

What is it like, being a MacGillavry Fellow?

'So far, so good! I get to set up my own line of independent research, but am still embedded in a larger group, the Molecular Cytology section. My first few months at SILS have been hectic: trying to get our experiments up and running, doing my first bit of teaching, writing applications for grants and permits, managing the transfer of my mice, etc. Everyone at SILS has been very supportive and I enjoy going to work. I was already used to designing and managing my own experiments. Now I also have to focus on building my research team and planning a bigger trajectory for the team. The MacGillavry Fellowship allowed me to hire my first PhD student right at the start of my tenure track, which was great.'

What is the main focus of your research?

'My team is interested in understanding the development and maintenance of complex, mammary tissue. How does this work in a three-dimensional context? Specifically, we are focusing on Wnt-responsive stem cells in the mammary gland, which puts us at the exciting interface of stem cell biology and fundamental cancer research.'

You're collaborating with the Dutch Cancer Society?

'I am funded by a personal fellowship from the Dutch Cancer Society. These fellowships are competitive and I am really excited that they're continuing to fund fundamental research like mine. There has been a shift in support towards more translational projects, but I am an avid proponent of fundamental research. Hopefully I will get the chance to contribute important basic knowledge about cell growth and differentiation that will ultimately benefit cancer patients, even if it's much further down the line.'

What would you advise other women who want to become MacGillavry Fellows?

'Go for it! Those positions are hard to come by. I wish we wouldn't have to create specific opportunities for women, especially since I don't think of myself as a female scientist, just as a good and passionate one. If I end up serving as a role model for others along the way, that's fine by me.'



Research highlight 4: Next Generation Sequencing

In 2013, the MAD Dutch Genomics Service & Support Provider was able to acquire a Next Generation Sequencing (NGS) platform thanks to an 'Investment Grant NWO Medium' awarded by the Netherlands Organisation for Scientific Research. NGS is the latest revolutionary technology in life sciences research, for instance making it possible to determine the entire DNA sequence of bacteria, plants and animals in just days and at relatively low costs. As such, NGS is set to transform life sciences research, particularly research into what are known as non-model organisms.

The Ion Proton system (produced by Life Technologies) was set up last year and is now available for researchers from SILS and other universities as well as for R&D departments in industry. The Ion Proton platform is ideal for sequencing transcriptomes (RNA) and genomes (DNA). Thanks to its patented protocol, it is perfect for small RNA sequencing of any organism. The system currently produces 14 Gbases in ~80 million reads per run of four hours, making it possible to go from library to primary data analysis results in less than 24 hours.

In the press

'Routeplanner' menselijk lichaam brengt revolutie teweeg in geneeskunde

Door: Maarten Keulemans - 04/03/13, 06:07



'Een van de grootste doorbraken'

© ANP. Foto ter illustratie.

Op maat gemaakte diëten ter vervanging van geneesmiddelen. Het einde van de hielpruk. Beter kankergeneesmiddelen. Volgens wetenschappers ligt het allemaal in het verschiet, nu systeembioologen voor het eerst een complete biochemische 'routekaart' van de mens hebben voltooid.

We begrijpen nu voor het eerst hoe een mens zichzelf precies maakt uit de voeding die hij tot zich neemt

Hans Westerhoff, hoogleraar systeembioologie

De kaart, die zondagavond werd onthuld in Nature Biotechnology, mag gerust 'een van de grootste wetenschappelijke doorbraken aller tijden' heten, vindt hoogleraar systeembioologie Hans Westerhoff. 'We begrijpen nu voor het eerst hoe een mens zichzelf precies maakt uit de voeding die hij tot zich neemt. Als het om alle huizen, dan is dit de wegenkaart die aangeeft hoe de verkeersstromen...

De kaart is... als Wester... hele geno...

Microbiologie

Genetisch veranderende blaaswalgen mikken een nog nooit vertoond botanische rechtstreeks om in waanzinnige producten als melkzuur. Een scala van andere stoffen ligt in het verschiet.

Geleende genen laten algen melkzuur afstaan

De kaart is... als Wester... hele geno... De kaart is... als Wester... hele geno... De kaart is... als Wester... hele geno...

UNIVERSITY OF AMSTERDAM nak!tuinbouw

Roelofarendsveen, 02 mei 2013
Ref: Com/13.74

Persbericht

Impuls voor gebruik techniek herkomstbepaling in tuinbouw

De provincie Noord-Holland en Kamer van Koophandel (KvK) Amsterdam hebben een subsidie toegekend om de techniek van herkomstbepaling van tuinbouwproducten op basis van isotopen te ontwikkelen. Het gaat om een subsidiebedrag van respectievelijk 0,2 miljoen en 10.000 euro. Het Swammerdam Instituut voor Levenswetenschappen (Universiteit van Amsterdam/ UvA) en Naktuinbouw in Roelofarendsveen gaan de techniek samen ontwikkelen.

Herkomstbepaling van tuinbouwproducten wordt steeds belangrijker. Dit bleek in het recente verleden tijdens de EHEC-crisis, maar ook tijdens de discussie op de najaarsconferentie van Greenport Holland (november 2012). De techniek van herkomstbepaling kan bijdragen aan de continuïteit van de tuinbouwsector te behouden. Ook blijkt keer op keer dat consumenten veel waarde hechten aan betrouwbare gegevens met betrekking tot de herkomst van voedingsmiddelen.

Forensisch onderzoek

Herkomstbepaling kan op basis van een zogeheten 'isotopenanalyse'. Dit is een beproefde methode om herkomstbepaling van producten te bewijzen. Isotopen komen in verschillende samenstellingen voor in planten en dieren. Deze samenstellingen worden vastgesteld door middel van isotopenanalyse.

Profiel Mark Post, hoogleraar vasculaire fysiologie

Vleesprofessor staat graag in de schijnwerpers

Mark Post, vader deze week in Londen bij de presentatie van zijn hamburger van kweekvlees. Foto: ANP

Mark Post, de Maastrichtse hoogleraar vasculaire fysiologie, was deze week wereldnieuws met zijn eerste hamburger van kweekvlees. Maar wie is de man achter de labburger?

De Maastrichtse hoogleraar vasculaire fysiologie Mark Post is wereldnieuws met zijn eerste hamburger van kweekvlees. Maar wie is de man achter de labburger?

Post is een wetenschapper met een passie voor vlees. Hij heeft een unieke manier gevonden om vlees te kweken in een laboratorium. Hij heeft een unieke manier gevonden om vlees te kweken in een laboratorium.

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SUBSIDIE VOOR NAKTUINBOUW

Naktuinbouw ontvangt een subsidie als impuls voor gebruik van een techniek voor herkomstbepaling in de tuinbouw.



De provincie Noord-Holland en Kamer van Koophandel (KvK) Amsterdam hebben een subsidie toegekend om de techniek van herkomstbepaling van tuinbouwproducten op basis van isotopen te ontwikkelen. Het gaat om een subsidiebedrag van respectievelijk 0,2 miljoen en 10.000 euro. Het Swammerdam Instituut voor Levenswetenschappen (Universiteit van Amsterdam/ UvA) en Naktuinbouw in Roelofarendsveen gaan de techniek samen ontwikkelen.

Hoogleraar Michel Haring, Universiteit van Amsterdam:

'Eerst gratis advies, dan zelf onderzoek doen'

Stel, een later wil een nieuwe bloem ontwikkelen die nog niemand anders heeft. Is dat technisch en financieel haalbaar? Is er onderzoek nodig? Hoe pak je dat aan? Advies van een onafhankelijk wetenschapper kan dan handig zijn. „En dat is precies waarvoor je bij de Griene Innovatiebank terecht kunt“, zegt hoogleraar Michel Haring van de UvA.

PLANTEN

Wortels van gewassen buigen weg van zout

AMSTERDAM. Planten mijden zout in de bodem door hun wortels de andere kant uit te laten groeien. Biologen van de Universiteit van Amsterdam ontdekten dat mechanisme met hulp van collega's van de Universiteit van Lyon. De groei van wortels wordt gestuurd door het plantenhormoon auxine. Het auxine in het uiteinde van de worteltopjes maakt dat de wortels gewoonlijk omlaag groeien. Maar als planten veel zout waarnemen, sturen ze het auxine in de worteltopjes actief naar de zijkant die het verst van het zout verwijderd is. De wortels groeien daardoor weg van het zout. Dat verschijnsel, dat de biologen 'halotropisme' noemen, is gisteren online gepubliceerd in *Current Biology*. (NRG)

RUNDEREN

Chinese koe k... het Midden-O...

AMSTERDAM. Oost-runderen stammen af van runderen uit het Midden-Ond rond 2500 voor Christus. Dit blijkt van mitochondriaal DNA uit de periode van 2500 voor Christus. Het genoom van runderen uit de Indus-vallei zijn gecreëerd. Het is opvallend dat de DNA-profielen voor de Midden-Oosten, die wijser op de continuïteit houden sinds de Bronstijd. *Journal of Archaeological Science* online 25 september 2013



End-of-Year Awards 2013



Research Day 2013 Awards

Presentations: 1st Prize: Philipp Savakis (Hellingwerf group)

'My research focuses on the production of biofuels and chemical feedstock from light, water and CO₂ using the cyanobacterium *Synechocystis sp.*'

**Presentations: 2nd Prize: Eva Nanninck (Lucassen group)**

'I investigate how brain structure and function are programmed by stressful early life experiences. Unravelling the mechanisms through which early life stress programs the brain may have clinical implications for the development of treatments to repair or prevent the lasting consequences of early life stress, such as cognitive impairments and increased vulnerability to psychopathology.'

**Posters: 1st Prize: Willemieke Kouwenhoven (Smidt group)**

'I investigate the role of transcription factor Engrailed 1 in the embryonic development of dopaminergic neurons.'

**Posters: 2nd Prize: Pascal Bielefeld (Lucassen group)**

'This study is focused on establishing the role of 5HT_{3aR} in the migration of newborn granule cells in the adult dentate gyrus. We have already shown that this receptor is differentially expressed over the different maturation stages of newborn granule cells, and that a loss of this receptor results in a loss of Reelin secretion and concomitant ectopic localisation of newborn cells. We are currently investigating the exact role of 5HT_{3aR} in this 'migratory process'.'



SILS staff additional jobs

Michel Haring -Director of Education, Faculty of Science, UvA

Teun Munnik -Associate Editor, Plant Physiology

Age Smilde -Professor (part-time), AMC-UvA
-Professor of Computational Systems Biology (part-time) University of Copenhagen

Johan Westerhuis -Professor by special appointment, Centre for Business Mathematics and Informatics, North-West University

Leendert Hamoen-Group leader, University of Newcastle

Hans Westerhoff -Professor of Molecular Cell Physiology (part-time), VU University Amsterdam

Stanley Brul -Chair, NIBI (Netherlands Institute for Biology)
-Programme Director bachelor's program in Bio-medical Sciences, University of Amsterdam
-Representative Federation of European Microbiological Societies, KNVM (Royal Netherlands Association for Microbiology)

Willem Stiekema -Member Board, Genootschap ter Bevordering van Natuur-, Genees- en Heelkunde
-Member, University Committee on Research, UvA
-Member, Steering Committee Biosolar Cells
-Member, Steering Committee, Netherlands Bioinformatics Centre

Dorus Gadella -Director, Van Leeuwenhoek Centre for Advanced Microscopy

Chris de Koster -Member, Works Council, Faculty of Science, UvA

Klaas Hellingwerf-Professor by Special Appointment of Physics, VU University Amsterdam
-Scientific adviser, Photanol BV

Timo Breit -Group leader, Bioinformatica Support Group (BioIT), Leiden University

Marten Smidt -Chair EC-PB, Programme Director, Master's programme in Education, UvA

Ben Cornelissen -Vice-Dean, Faculty of Science, UvA
-Member, Supervisory Board, Naturalis Biodiversity Center
-Chair, Graduate School Experimental Plant Sciences

Teun Munnik: 'I am an associate editor of Plant Physiology, an international journal established in 1926, devoted to the physiology, biochemistry, cellular and molecular biology, genetics, biophysics, and environmental biology of plants. Plant Physiology is one of the world's oldest and most well-respected plant science journals.'

'All manuscripts that are submitted will go through an associate editor. I am responsible for the section Signaling & Response. It is my job to find appropriate Managing Editors who will handle the manuscript and find reviewers for it. I get about 5 papers per week, which is about 1-2 hrs of work. This is all done in my own private time.'

Johan Westerhuis: 'My position as professor by special appointment at the North West University in Potchefstroom relates to me being the supervisor of a PhD student that is involved in the statistical analysis of metabolomics data. Over there we do a study on the metabolic effect of acute alcohol abuse, a major problem in South Africa. Together with a local expert on metabolic diseases we develop data analysis methods for the complex data that arises from such a study. Besides supervising the PhD student I also try to be involved with other projects at the metabolic centre over there. I try to visit Potchefstroom once or twice a year. During the rest of the year we have regular skype meetings.'



Interview with Martin Vinck

Martin Vinck received his PhD cum laude in 2013. He also won the Scopus Prize and was awarded an NWO Rubicon Fellowship and a Human Frontiers Science Program Fellowship.

What was your PhD research about?

'My PhD research dealt with the question of the purpose of brain rhythms, how they are generated, how they couple across brain areas that process different modalities, and how we should quantify coupling between different neuronal oscillators.'

What was your most remarkable finding?

'We found very pronounced gamma (i.e. fast: 40-90 Hz) oscillations in the barrel (whisking) cortex of awake rats, which are specifically carried by putative fast-spiking basket cells, a type of interneuron. These oscillations have been linked to attention and consciousness in primates but had not yet been observed in this particular brain area. Interestingly, we found these oscillations were not coupled to gamma oscillations in other connected areas, indicating that there is no global pacemaker or network for gamma oscillations and that gamma oscillations have a more local function in the barrel cortex.'

You are now a postdoc at Yale University. What is your research focus?

'At Yale I will study the primary visual cortex in awake mice. We'll use a combination of extracellular and intracellular recordings and optogenetics. The goal is to understand the ways in which cortical activity is shaped by different behavioral states like alertness and arousal, and the function of different inhibitory interneurons in shaping pyramidal cell output.'



What is the biggest challenge in your field?

'Neurophysiology is moving at an extremely rapid pace, both in terms of technological developments (e.g. optogenetics, whole-cell recordings in awake animals) and actual scientific progress. This especially holds for research on the rodent hippocampus or the primary visual cortex, for example. It's hard to keep up with these developments. Also, neurophysiology is an interdisciplinary science, meaning that it takes a lot of time to develop all the required skills.'

How do you look back on your time at the UvA?

'I was lucky to be around very knowledgeable and skilled researchers like Jeroen Bos, Jadin Jackson and Francesco Battaglia. These guys taught me lots of hands-on tricks in electrophysiology and animal handling. The Pennartz Lab is unique in the Netherlands in that it is a high-quality rodent electrophysiology lab. The project that Jeroen Bos and I did at the UvA, supervised by Cyriel Pennartz, was extremely challenging, involving recording single units from four different brain areas in task-performing rats, but I learned a lot from it! Plus, Science Park was really a fun place to work, also socially – I miss it!'

Grants & awards

Harm Krugers – € 675,000 – ZonMwTop and USA Military

Operational Medicine Research Program grant

Krugers: 'We study how early life experiences influence brain function and sensitivity of the brain. Early life experiences and the context in which we grow up play an important role in the development of the brain and brain processes related to memory and emotion. With the ZonMw grant that we received with Marian Joels and Casper Hoogenraad (Utrecht University) we want to study how early life experiences affect the mechanisms responsible for learning and storing emotional and other information.'



Johan Westerhuis – Two Metabolomics

Society publication awards

Johan Westerhuis received two publication awards from the Metabolomics Society in recognition of his outstanding scientific publications in the journal Metabolomics. Having previously received the Best Paper Award (recognising the most-cited paper) for 'Assessment of PLSDA cross validation', on a strategy to validate classification models, in 2013 Westerhuis was the recipient of the pre-2013 Paper Award (highest download) for his paper entitled 'Multivariate paired data analysis: multilevel PLSDA versus OPLSDA'. This paper demonstrates how clever use of the underlying structure of experimental design data facilitates the interpretation of differences between treatments.



Aniko Koroso - € 228.000 - Meervoud Award

Koroso: 'The aim of the Meervoud programme is to foster the appointment of more women to assistant professorships. This award provides four years' salary for me as assistant professor, securing my position at the UvA thereafter. I will use this grant for a study titled 'The role of nutritional and epigenetic programming in the early life stress-induced accelerated cognitive decline: relevance for Alzheimer's disease.'

Stanley Brul and Chris de Koster- 2 Erasmus Mundus PhD scholarships

Antibiotic-resistant bacteria are a major threat to public health, with some bacterial infections having become untreatable and the development of novel antibiotics lagging behind. In an effort to develop further options for treatment, two new PhD candidates have been appointed on Erasmus Mundus scholarships.

Petra Bleeker - € 800.000 - VIDI grant



Bleeker: 'My research focuses on defence in the wild; from trichome transcriptomes and metabolomes to breeding tools for defence markers in tomatoes. Cultivated tomatoes are hampered in their ability to effectively defend themselves against agricultural pests like insects. In the germplasms of wild-tomato relatives, however, the production of a wide variety of defence-related metabolites results in elevated resistance levels. By identifying specific metabolites and unravelling the genetics behind "green defence" compounds and the way they are regulated in wild ancestors, cultivated tomatoes can be naturally re-armed, reducing the need to use classic insecticides, which have become very controversial.'

Michel Haring – € 3.3 million STW program:

Green defence Against Pests



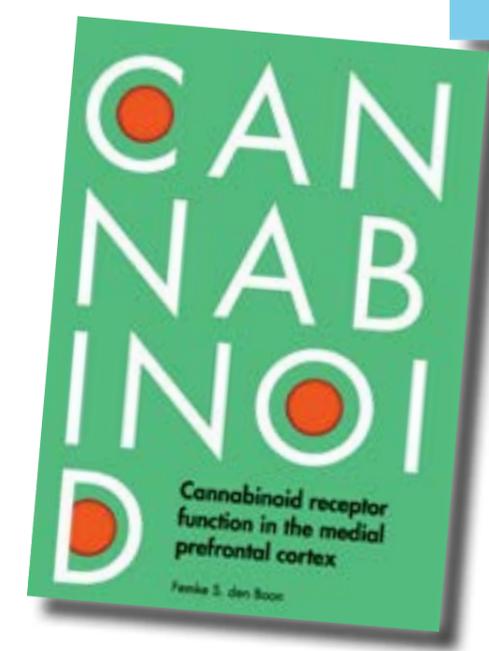
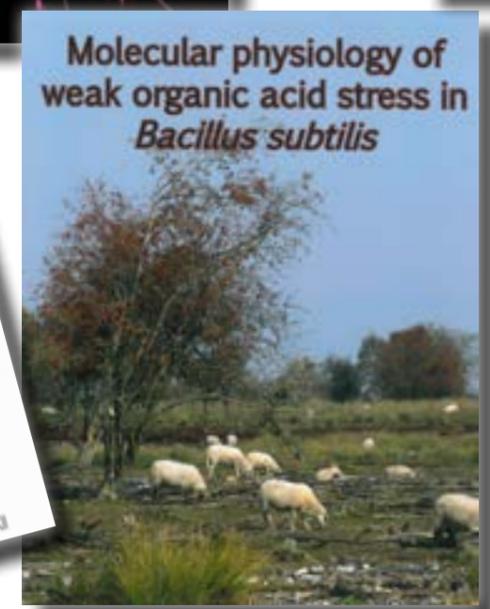
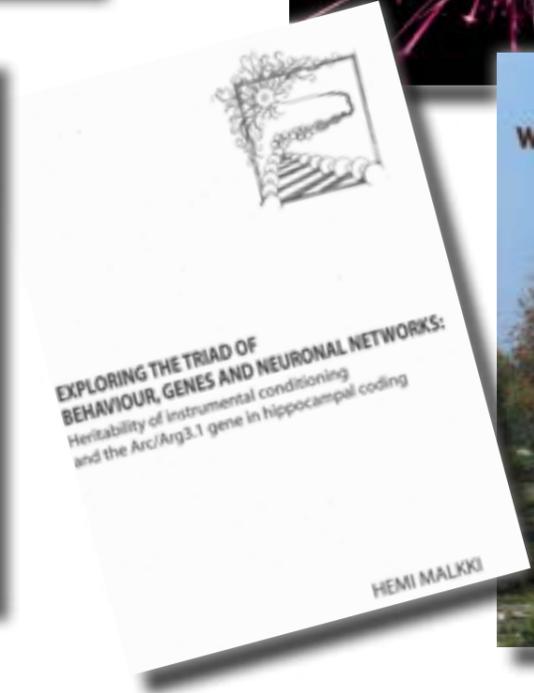
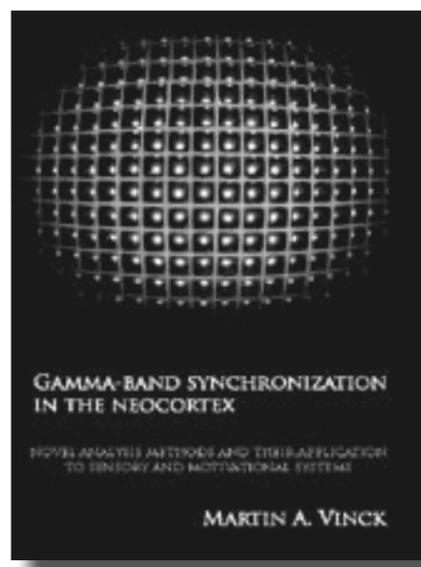
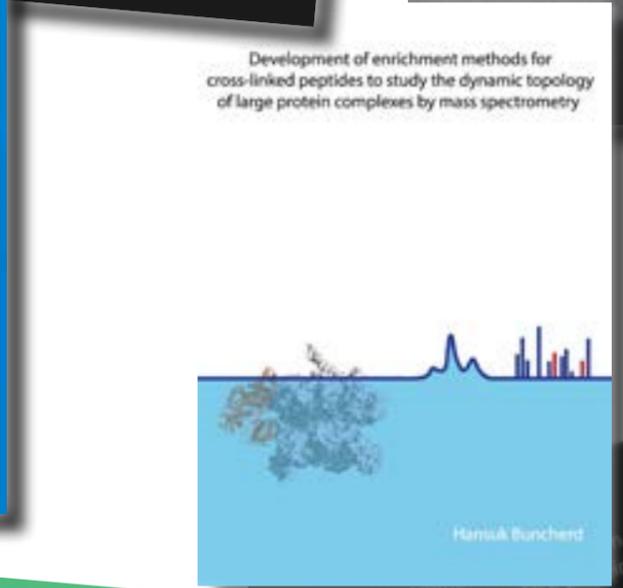
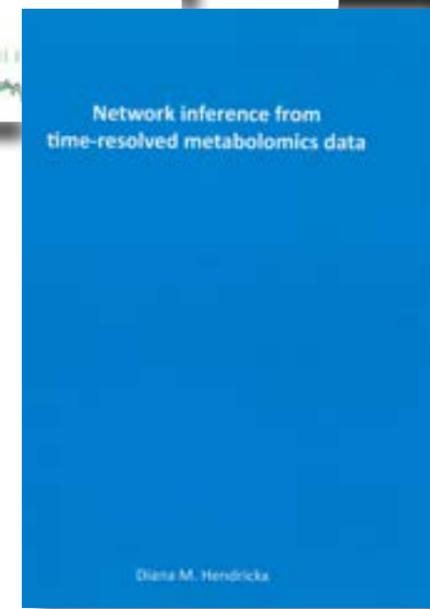
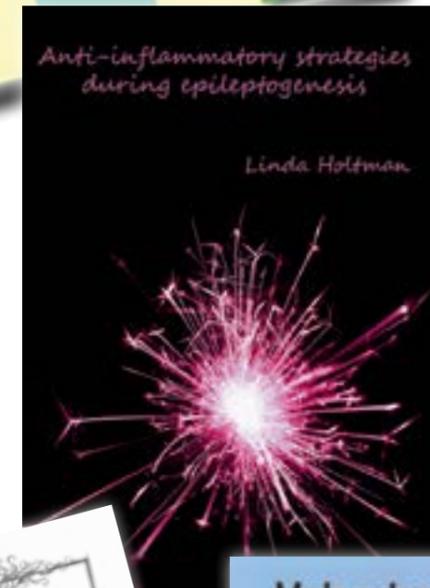
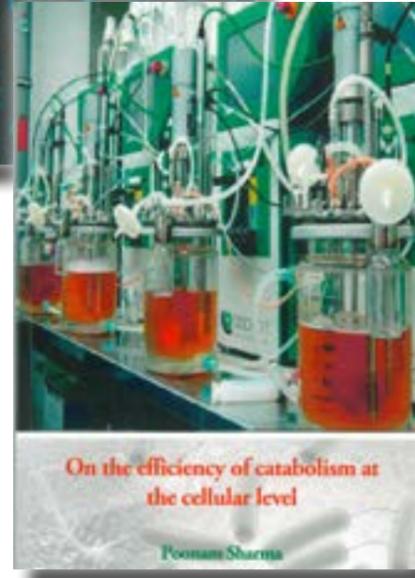
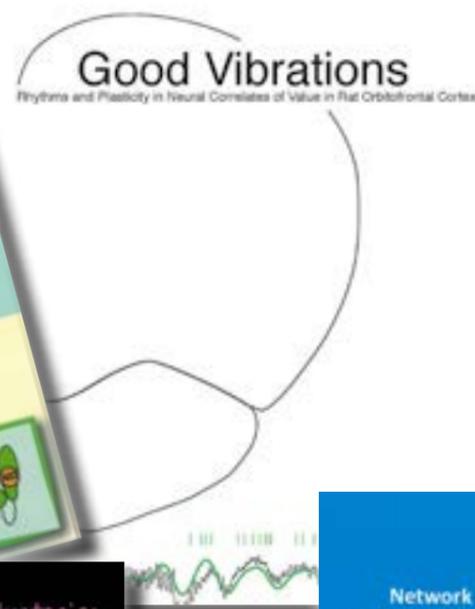
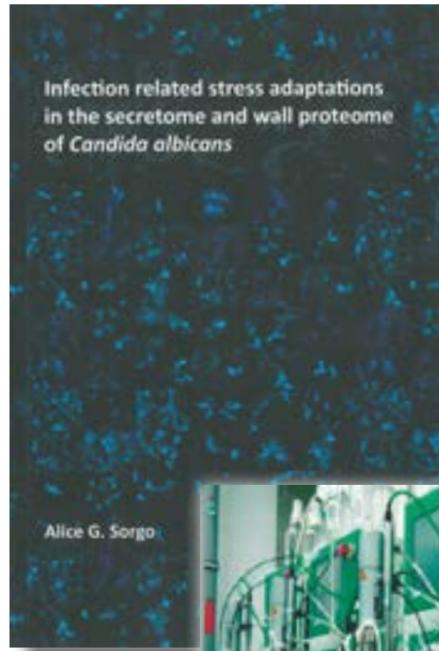
'This program aims to find natural and sustainable solutions for defence against plant pests. With the ban on several important pesticides due in 2020, vegetable breeders need to develop new and sustainable resistance mechanisms in their crops. With a focus on tomatoes and bell peppers, ten companies and six university groups have joined forces to address this issue. The STW GAP programme applies metabolomics, transcriptomics and genomics techniques to discover novel defensive metabolites. The identification of the genes involved allows targeted breeding of resistant crops.'

Stanley Brul and Gertien Smits - TNO grant K€ 240

'Using TNO's position in the field of microbial fermentation, host strains, fermentation and metabolic engineering, it has been possible to identify new opportunities for pathway engineering. Fungal metabolite production can be a suitable paradigm for microbial production processes. Our project, titled 'Synthetic biology for fungal metabolite production', focuses on organic acids that are industrial fungal metabolites and that play a pivotal role in the fungal core metabolism. This study brings together an extensive amount of published data. Several of the core processes of the pathway remain poorly understood, including compartmentalisation, transport and the presence of parallel (redundant) pathways. A systems biology approach will enable us to gain a more detailed insight into the generic processes related to the core metabolism, which is relevant not only for synthetic (rational) strain design, but also for understanding universal aspects of the core metabolism, which is present in all eukaryotic and prokaryotic organisms.'

Filipe Branco dos Santos - K€ 250 - VENI Grant

'The aim of my research is to understand the adaptive evolution strategies of microbes in dynamic environments through the use of laboratory evolution. Life continuously evolves in dynamic environments. Using mathematical modelling and laboratory evolution experiments extending over a thousand generations, I'll be exploring how these dynamics shape life.'



Green Life Science Hub

SILS carried out several projects as part of the Amsterdam Economic Board's Green Life Sciences Hub Icon programme: *Green Innovation Counter*, *Green Forensics* and *Traceability of horticultural products based on stable isotope composition*.

Green Innovation Counter

SILS carried out a number of projects as part of the Amsterdam Economic Board's Green Life Sciences Hub Icon programme. Green Forensics, Origin Tracing and Green Student Lab were three projects that received funding from the province of North-Holland or from EFRD (European Fund for Regional Development) grants.

The Green Innovation Counter was initiated by Prof. Michel Haring (SILS Plant Physiology) to stimulate innovation in the ornamental crop sector (flowers and potted plants) and aims to connect scientists working in the green life sciences to companies seeking to innovate in their product development (mostly plant breeding tools). The Green Innovation Counter offers companies free advice on their research and facilitates pilot projects in which companies can use SILS facilities to conduct experiments and explore the suitability of new techniques for their innovation projects. In 2013, six small enterprises participated in this programme: Dekker Chrysanten, CornBak, Agriom, Anthura, Sande and Blom Palmen. Projects focused on the generation of DNA markers for desirable traits, for example, and on the use of tissue culture techniques for crop improvement. Several projects have already resulted in technology transfer, whilst others will require further research by the participating companies. Having secured funding from the province of North-Holland and the Amsterdam Chamber of Commerce, this project will continue in 2014.

Green Forensics: using next-generation sequencing to detect fraud in the green sector

Creating a new plant variety for the consumer market takes a considerable R&D effort and an investment of at least several years. When plant breeders develop a new plant variety with unique characteristics, they can turn to the Naktuinbouw (Netherlands Inspection Service for Horticulture) centre of expertise to acquire breeders' rights for the variety, thereby formally protecting their investments against illegal cultivation by other parties. If there is a suspicion of a breach of these rights, breeders can apply to Naktuinbouw to conduct a DNA test to determine the relatedness of the suspect varieties. Work on improving existing DNA profiling techniques for the determination of genetic distinctness is currently being done by SILS researchers from the MAD Dutch Genomics Service and Support Provider, as part of the Green Forensics project. The project aims to set up a new DNA profiling technique based on next-generation sequencing (NGS) of whole plant genomes. For proof of principle this approach has to be cost-effective, generally applicable and more accurate than current DNA techniques in terms of resolution and representation of the whole genome. The project will develop a generic protocol that could eventually be applied to all crops, regardless of genome complexity, ploidy and heterozygosity. For each of the five market-relevant species selected for this project, a genome reference database will be created to enable reliable discrimination of plant varieties using NGS-based DNA profiling.

The Green Forensics project is a collaboration between SILS, Naktuinbouw and the Amsterdam Chamber of Commerce and is funded by the Amsterdam Economic Board/PRES. Set to run from 2013 to 2016, the first years will focus on developing the new method and testing the databases. The project draws on expertise in molecular biology, NGS technology, bioinformatics and statistics at SILS. In the final year, the focus will shift to transferring the acquired knowledge through the implementation of the newly developed method at Naktuinbouw.



The Ion Proton is being prepared for a sequencing run.

Traceability of horticultural products based on stable isotope composition

In April 2013, a project investigating the geographical origin of bell peppers, titled 'Traceability of horticultural products based on stable isotope composition', was launched in a partnership between the UvA, VU University Amsterdam, Helderman Peppers and Naktuinbouw. As SILS Green Life Sciences projects, it is subsidised by the EFRD and the Amsterdam Economic Board. The EU is the largest exporter and second largest importer of agricultural products worldwide – a world that is seeing increasing demand from consumers for guarantees regarding the labelling and origin of their food, partly as a result of recent food scandals. Most foods bear the fingerprint of the environment in which they were produced; that is, the stable isotopic composition of elements from which a plant is built. This fingerprint depends on regional geological and climatic patterns, leading to a direct correlation between regional geochemical and climatic factors and the properties of locally produced food. Many elements have 'heavy' and 'light' versions or isotopes, depending on the number of neutrons in the nucleus. In the EFRD Traceability project, isotope ratio mass spectrometry (IRMS) was used to link the isotopic composition of the water and the lipid composition present in fruit and vegetables to regional differences between products from different locations. This was done using isotopic maps of the regional water isotope composition showing natural isotope variation in hydrogen and oxygen. These isotope profiles can then be linked to the isotope composition of a product from a certain region and a map can be created to trace the origin of that product.

The first results of this project have revealed that the oxygen isotope profiles of the water in bell peppers show significant differences between those grown in Egypt and the Netherlands. The isotope composition of bell peppers could be linked directly to the isotope composition of the irrigation water, proving IRMS a promising tool for agricultural product traceability. This method will be developed further in collaboration with Naktuinbouw, with the final objective of the project being to offer origin determination as a service to Dutch bell pepper growers and traders.

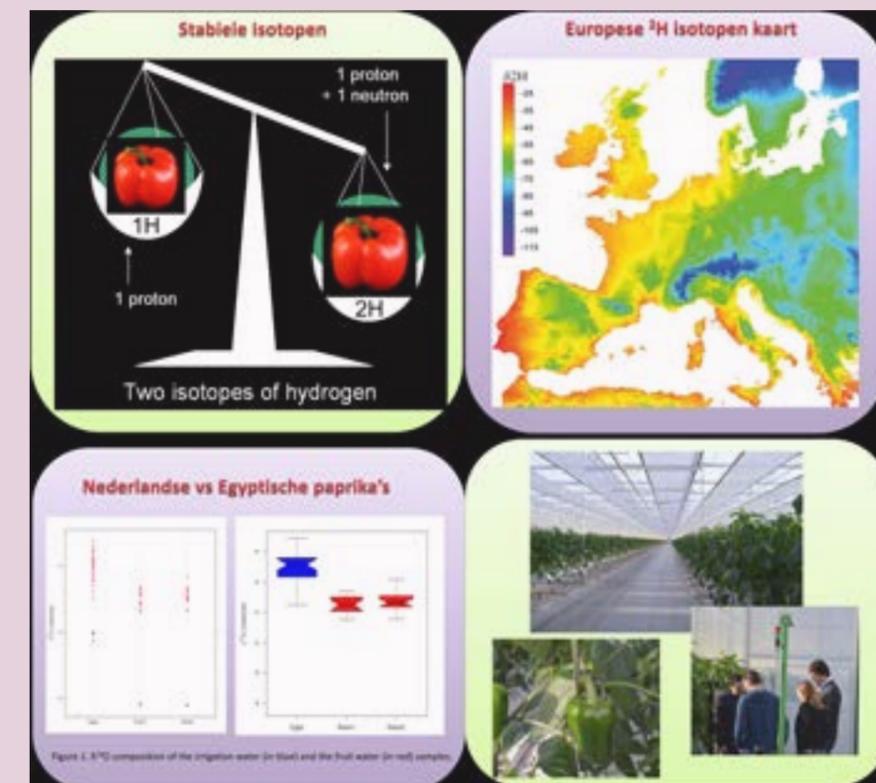


Figure 1. $\delta^{18}O$ composition of the irrigation water (in blue) and the fruit water (in red) samples.



Interview with Klaas Hellingwerf in the series 'UvA in the Spotlight'

Despite occasional accusations of it being a left-wing plot and the unfounded ramblings of soft-brained scientists, few today would deny that global warming and resource depletion are serious challenges facing our planet. Built on two centuries of cheaply available fossil fuels, the global industrial economy runs the risk of falling victim to its own success as it seeks to wean itself off its dependency on carbon-emitting fuels while at the same time relentlessly pursuing economic growth. As of late, alarmingly high levels of atmospheric carbon coupled with the impending effects of 'peak oil' have necessitated the search for clean, renewable energy in the form of biofuels and solar power. Initially hailed as viable alternatives, these energy sources have, however, proven to be less effective in terms of cost and efficiency than was hoped. Illustrating the law of unintended consequences, a number of biofuels have actually caused a spike in food prices, as agricultural land and crops are increasingly diverted for use in the biofuels industry. A truly clean and abundant energy source, it would seem, continues to elude us.

Or does it?

According to two of the UvA's brightest minds, a simple prokaryotic microorganism might be the answer to one of the world's most complex issues. Known as cyanobacteria, these primordial microorganisms have the same photosynthetic abilities as plants and can be found in almost every terrestrial and aquatic environment on earth. Indeed, so convinced are UvA researchers Klaas Hellingwerf and Joost Teixeira de Mattos of cyanobacteria's prospects as a source of clean energy, that – together with the University of Amsterdam – they have formed their own start-up company to develop and market this simple, yet effective type of algae.

In the December issue of 'UvA in the Spotlight', we speak to Klaas Hellingwerf, professor of General Microbiology at the Faculty of Science about bacteria, climate change and the possibility of clean energy.

Cyanobacteria can be found in most aquatic environments. Could you tell us more about the Photanol concept?

'Photanol offers a viable, clean and efficient alternative to current fossil fuels and biofuels by harnessing the photosynthetic power of cyanobacteria. Since the industrial revolution, the world has become dependent on a finite amount of fossil fuels, such as oil and coal, for use in transport and production of bulk chemicals in industry. Over the last two centuries, these fossil fuels have, however, managed to disturb the earth's carbon cycle, leading to an increase in atmospheric CO₂, with global warming and climate change as a direct result. Although recent times have seen the emergence of cleaner fossil alternatives, these sources of energy are not only complex to produce, but also wasteful and inefficient. At Photanol, a start-up created by my colleague and me in 2008/9, we've taken a more biological approach by using the natural photosynthetic properties of cyanobacteria to capture sunlight and directly convert CO₂ into valuable compounds, such as ethanol, butanol and propanediol. We do this by genetically altering the metabolism of cyanobacteria, thus allowing us to circumvent the traditional photosynthetic route in which sunlight is used to convert CO₂ into biomass. By introducing properties of fermentative bacteria, we create a shortcut in which cyanobacteria use solar power to immediately convert CO₂ into biofuels. The only by-product of this whole process is oxygen.'



Cyanobacteria can be found in most aquatic environments.

Photo: Flickr-CreativeCommons)

Are you the first to do so?

'Strictly speaking, no. The first to suggest the possibility of using cyanobacteria as a source for producing alcohol were two Canadian researchers, who published their idea in the 1990s. Despite the initial excitement following their article, nothing more was heard for several years. In 2008, we took a closer look at the idea and realised that instead of alcohol we could also genetically modify cyanobacteria to produce other energy carriers, including butanol, lactic acid and acetone. We then immediately set about patenting our idea, which enabled us to create a commercial spin-off. Since then, this spin-off has been the catalyst for further research and development.'

When do you hope to bring your product to the market?

'After forming Photanol and safeguarding our intellectual ownership over a number of products, we immediately went about proving that these products could indeed be manufactured within a laboratory setting. Once our hypothesis had been successfully confirmed, we were awarded a grant in 2010 to build a pilot plant at the Faculty of Science (Science Park 904 – ed.), which was recently completed. Now that this infrastructure is operational, our next step will be to increase the scale and efficiency of the production process to such an extent that our product becomes commercially viable. To do so, we'll need to expand our floor area from its current 60 square metres to about a hectare. Despite a number of growing pains, we're confident that we'll manage to do so in the near future.'

Aside from biofuels, are there any other products you might be able to produce using cyanobacteria?



Klaas Hellingwerf.

Photo: Jan Willem Steenmeijer

'When we received our grant in 2010, the world was experiencing an unprecedented surge in the price of oil and natural gas. Faced with the prospect of depleting oil resources and increasing energy consumption, the government encouraged investment in the biofuels industry, which was an incentive to us at the time to continue focusing on cyanobacteria-based energy carriers. Since then, the global energy landscape has shifted once again. Recent events such as the drop in oil price and the surge in fracking activities in the US have forced us to also think of other alternative products that could be synthesised using cyanobacteria. Within the field of biotechnology, *E. coli* and *Saccharomyces*, a yeast, are some of the most widely used organisms used in the manufacture of pharmaceutical and industrial products, such as washing powder enzymes and bulk plastics. The enzymes and biomolecules made by these organisms can, however, also be produced by cyanobacteria. Better still, cyanobacteria use only sunlight and CO₂ to produce the same products as *E. coli* and *Saccharomyces*, which need sugars. When one considers the abundance of CO₂ and decreasing amount of agricultural space to grow plants that will provide these sugars, cyanobacteria are a very clean and cost-efficient alternative.'

You've mentioned the increase in fracking activities in the US. Do you think that shale gas might be an answer to the world's energy needs?

'Quite the opposite. Shale gas, in my opinion, is an absolute disaster in the fight against climate change. If you closely examine shale gas fracking in its current form, you'll see that the cons far outweigh the pros. Hydraulic fracturing (fracking) is dependent on large amounts of water, an increasingly scarce commodity, especially in agriculture. If more water is needed for fracking, a conflict of interests will arise between energy and agriculture. Moreover, fracking has been known to contaminate water supplies, on which all living matter depends.

Also alarming is the appalling wastefulness of the fracking process. During fracking, only about 50 per cent of the gas that gets mined is actually captured, while the rest ends up in the atmosphere. Gas of course, consists of methane, which is a 20-fold more potent greenhouse gas than CO₂. If atmospheric methane concentrations continue to increase as a result of fracking, the outcome might turn out to be quite unpleasant.

Are you hopeful society will become cognisant of the need to fully embrace cleaner energy sources like cyanobacteria, instead of thinking in terms of short-term economic gain?

'Everything's possible. However, one usually only sees a change in mindset after something extreme happens, like the Fukushima nuclear disaster in 2011. As terrible as they are, recent natural disasters might make people understand that man-made global warming is a threat that requires a global response. Even if we reduce or totally stop pumping greenhouse gases into the atmosphere, it will still take years for atmospheric carbon levels to stabilise, which is why alternatives like cyanobacteria are so sorely needed. I'm hopeful, however, that current attitudes will change and that society will cross over to a more sustainable mode of energy consumption.'

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2 December 2013

PhD in the spotlight: Niek Wit

On 1 July, Niek Wit (b. 1984) was awarded a doctorate by the University of Amsterdam (UvA). His research at the Netherlands Cancer Institute (NKI) and the Swammerdam Institute for Life Sciences (SILS) examined damage tolerance mechanisms in DNA damage. He found that in mammals, including humans, this mechanism is more complex than had previously been thought.

What did you research?

'I looked at damaged DNA. External factors such as UV rays or cigarette smoke, can inflict so much damage on DNA that it can no longer replicate properly, which ultimately can cause cancer. Fortunately, there are special proteins, known as TLS polymerases, which temporarily allow replication to continue. I looked at how the TLS polymerase polk works. We already knew that in yeast cells this material is always regulated by the PCNA-Ub protein complex. Our lab bred mice in which that protein complex could no longer be made. You would expect those mutated mice to also be unable to make polk. But when damaged by the cancer medication MMS it turned out that they still could, just less efficiently. When the DNA damage came from other sources such as cigarette smoke, they couldn't produce it anymore. So it seems that polk regulation in mammals, including humans, doesn't just depend on the protein complex, as it does in yeast cells.'

Will those findings help in cancer treatment?

'Absolutely. The more we find out about how TLS polymerases work, the better we can treat cancer. Radiation treatments and many cancer drugs cause damage to DNA. Because tumours sometimes lack DNA repair mechanisms, medication will kill those cells more quickly than healthy cells. We now know that we can also make potential cancer cells more sensitive to DNA damage by blocking the formation of PCNA-Ub, or specific TLS polymerases, or both. We also know that this only works for certain types of DNA damage. More research is needed into other types of DNA damage.'



Will you be pursuing that research?

'I'm going to continue in cancer research but in a different area. As of 1 June, I have a post-doc position working with the researcher KJ Patel at the MRC Laboratory of Molecular Biology in Cambridge, on the regulation of DNA repair. Fortunately, my head of department had advised me to start looking for a new job more than a year before I was due to receive my doctorate. When KJ Patel gave a presentation at Leiden University last summer, I gave him my CV. Many months later, I was offered the position. The MRC is a renowned institution and they have their pick of applicants. When I heard just after Christmas that I'd been offered a place, I already had other applications in progress in Copenhagen and a few other cities in the UK such as Birmingham. I cancelled all of those.'

Was it a coincidence that you had applied for so many positions in the UK?

'No, I've always loved it there. We used to go there every year on our family holidays. As a student I didn't have much money to go on holiday, but as a PhD student I went as often as I could. It's somewhere I have always wanted to live. My new boss said all the Spaniards in his lab complain about the bad weather and the food. I won't be complaining, as I can't see any difference to the Netherlands. Another advantage is the high standard of scientific research in the UK, and the MRC is financially robust too. It also has strong links with the University of Cambridge. It will open up so many opportunities for me.'

Author: Carin Röst

Published on the website of the Faculty of Science

17 September 2013

PhD in the spotlight: Clemens Heilmann

Clemens Heilmann (1984) was awarded his doctorate degree at the UvA on 15 February. During his research at the Swammerdam Institute for Life Sciences (SILS) he mapped out the cell wall of the pathogenic *Candida* fungus. This information will make it easier to detect this fungus and, hence, to eliminate it.

What was the most remarkable result produced by your research?

'The cell wall proteome of the *Candida* fungus is more stable than we thought, which makes the fungus easier to detect. The cell wall of *Candida albicans* contains dozens of proteins. Until now, we had always thought that these proteins varied all the time because the circumstances of the fungus – for example, whether or not it is growing – determine which type of proteins it produces. I made use of mass spectrometry, a technique that can be used to identify molecules, to look at the proteins and discovered that five proteins on the cell wall and seven secreted proteins outside of it are always present. These permanent proteins can therefore be used to detect *Candida*.'

Is the fungus dangerous?

'*Candida* poses no imminent danger to healthy people. Around 80% of the human population is colonized by *Candida*. The fungus nestles itself in the mucous membranes – in the mouth, the intestines and the skin, for example. It can, for instance, cause vaginal infections. However, *Candida* can pose a serious threat to people with low immune status, such as intensive care patients. It can enter the blood stream and cause death within a week. At present it takes two to three days to detect the fungus. Accelerating the detection process will increase a patient's chances of survival. Perhaps a vaccine will be developed that focuses on the permanent proteins I discovered. There are medicines available to fight *Candida*, but the advantage of a drug or vaccine aimed at the cell wall is that it has very few side effects because humans cells have no cell walls.'



Did you enjoy conducting this research?

'One of the things I enjoyed most was being part of the FINSysB network. This is a network of European research groups specialised in fungal infections. Every year I also attended several international conferences, and researchers from other universities in the network came here to work with our mass spectrometry equipment, which is of very high quality. I contributed to their research, and in exchange I became co-author on their papers. All of this was tremendously helpful. Of course, there were also times when I wanted to throw everything out of the window. When that happens, the trick is not to give up and just keep going.'

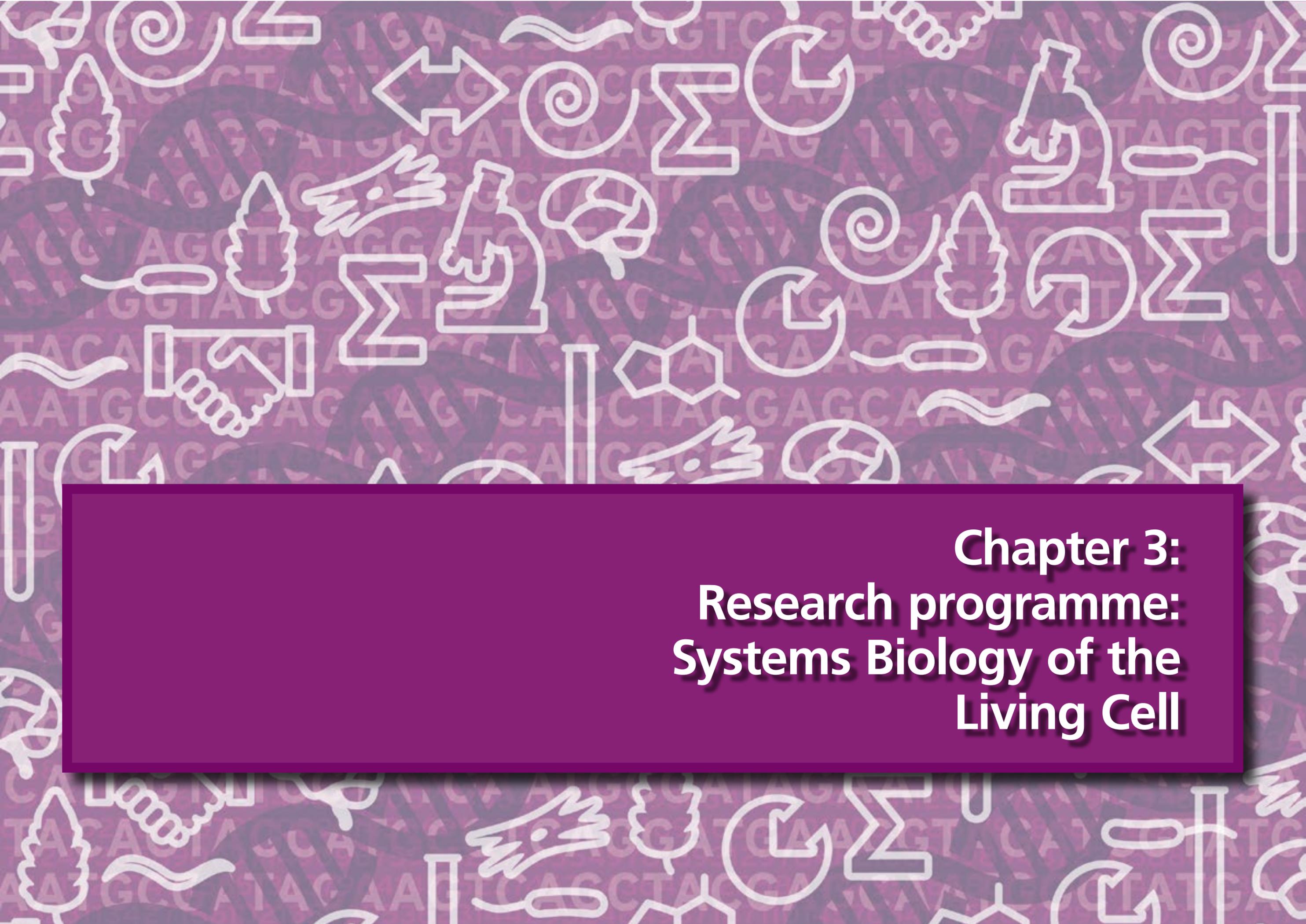
What will you be doing next?

'I may continue to do research on fungi, but I am also interested in the industrial side of the discipline. The research is being continued at the other universities in the network, so if I choose another path my results will still form the basis of extended research. The Netherlands was a great place to gain international work experience and I enjoyed my stay here very much, but I will probably return to Germany or move to another country nearby, such as Switzerland. I miss the good bread and the mountains.'

Author: Carin Röst

Published on the website of the Faculty of Science

11 February 2013



**Chapter 3:
Research programme:
Systems Biology of the
Living Cell**

Molecular Biology and Microbial Food Safety

Prof. S. Brul	Chairholder
Dr J.C. van der Spek	Assistant Professor
Dr G.J. Smits	Assistant Professor
Dr Y. Budovskaya	McGillavry fellow (tenure track Assistant Professor)
Dr B. Ter Kuile	Researcher Dutch Food & Drug Authority (VWA)
Dr. J.P.P.M. Smelt	Guest researcher (former Unilever senior Scientist)
Dr F.M. Klis	Senior scientist (former Associate Professor)
Prof. S.M. van Ham	Special Chair

In 2013 we capitalized on our analysis of stress response of microorganisms to the benefit of strengthening our molecular physiological understanding of their reaction against adverse environments.

Research highlights

► Our studies on *Candida albicans* have been very successful and led to two theses by now Dr. Alice Sorgo and Dr. Clemens Heilmann. Their work concluded the FINSysB Integrated Training Network contribution of our department together with the department of Mass Spectrometry of Biomacromolecules (prof. de Koster) and provided a framework for current trials on vaccine development against *Candida albicans*. In addition it shed further light on antimycotic and environmental (low pH, low iron) stress resistance and laid a foundation for the successful application of Dr. Karin Strijbis for an International Incoming Marie-Curie Fellowship (see below). We aim in this project at consolidating our basic understanding of the *Candida* wall structure and function as well as enter into new collaborations that focus on the role of *Candida* in host-microbe interactions that determine intestinal health. The conclusion of the two PhD projects by Sorgo and Heilmann under supervision of senior scientist (former associate professor) Dr. Frans Klis marked the end of his long career. This was celebrated with an honorary symposium with over 100 guests in attendance, six scientific lectures from scientists trained by Frans now active in academia and industry, and featuring Prof. Dr. Neil Gow from the University of Aberdeen (UK) as a keynote speaker.

► Our basic understanding of the molecular physiological basis of yeast stress response to low environmental pH mediated by weak organic acids was given a new boost by the appointment of a new PhD student and leveraged in a new PhD project funded by TNO-Zeist. The latter aims at understanding fungal stress response to organic acids, specifically itaconic acid, in biotechnological production settings of these industrial natural building blocks.

► Our bacterial studies on the physiology of antibiotic resistance, funded by the Netherlands Food and Consumer Product Safety Authority (NVWA), has resulted in 4 published papers (see illustration 1). This line of research emphasizes the molecular physiological quantitative aspects and yields results of fundamental scientific interest as well as useful for policy making at the NVWA. Furthermore we continued our successful collaboration with the Mass Spectrometry of Biomacromolecules group and the Molecular Cytology group at the Van Leeuwenhoek Centre for Advanced Microscopy (LCAM). This led to the establishment of an innovative live-imaging

Research highlights (continued)

analysis system with annexed image analysis, 'Spore Tracker' that is now fully operative and was used for analysis of *Bacillus subtilis* vegetative cells and spores as a case study. The work was done in the framework of the STW project Targeted Inhibition of Bacterial Spores as well as a company funded project on the presence of putative antimicrobial compounds in tea (Plos One paper Pandey et al. and Food Microbiology subject to minor revision). Dr. Jan Smelt was instrumental in the statistical analyses. In collaboration with the Mass Spectrometry group of prof. de Koster we have identified the main components of the bacterial spore coat in organisms ranging from food spoilers (*Bacillus subtilis*) to medically infectious/toxigenic (*Bacillus cereus* and *Clostridium difficile*) and reported on it in the Journal of Proteome Research (see Illustration 2). The data was extended with studies on spore coat protein cross-links and proposed for publication in Food Microbiology (under revision). Again host-microbe interaction spin-off analysis is foreseen in a renewed EU project proposal that is to be submitted in 2014. Initial spin-off already matured in a study on the use of Bacilli as food source for the simple multicellular eukaryotic model organism *Caenorhabditis elegans*. MacGillavry fellow Yelena Budovskaya showed that indeed *B. subtilis* may be used for this purpose and sustains a longer life-span. Meanwhile *B. subtilis* has been made usable for RNA-interference approaches in *C. elegans*. Finally, in collaboration with Dr. Erik Manders at LCAM and Prof. Dr. Winnok de Vos from Ghent University a highly innovative tool for the visualization of mitochondrial morphology in the normal and stressed state in *C. elegans* was set up (see illustration 3). We continue to use the nematode in eukaryote energy stress studies as a multicellular model.

► In 2013, 20 peer reviewed publications appeared, 3 PhD theses were defended and close to 10 lectures on invitation (amongst others a main 'Spoilers' congress, a Beilstein symposium and the Yeast Physiology meeting) were delivered by group members. We maintained our size at ~15-20 permanent and temporary staff members and provided internships to more than 15 bachelor and master students. We appointed a new research and teaching technician as well as host a new lecturer molecular biology practice who is fully funded from educational funds.

► Finally, our research led to new project grants from the Erasmus Mundus cooperation windows with South Africa and China. In addition we attracted funds from TNO-Zeist that cover a 4th year of one of the new PhD students as well as a fungal metabolism and (weak organic) acid production PhD project with questions in stress resistance. In collaboration with Leendert Hamoen from the Bacterial Cell Biology group an EU Marie-Curie reintegration grant was awarded and with post-doc Karin Strijbis from MIT a Marie-Curie International Incoming Fellowship was granted. Karin Strijbis also had an offer from Utrecht University with whom we now aim at executing the proposed work.

► We are evaluating a dual role of Wnt signaling pathway during aging in *Caenorhabditis elegans*, by utilizing RNA-sequencing technique to analyze gene expression changes in various components of Wnt signaling pathway mutants and wild type worms during aging. We discovered that over-activation of the Wnt signaling pathway during aging leading to metabolic changes that are highly resemble Warburg syndrome that preceding development of cancer and other age-related diseases.

► We developed expression system that allows expressing *C. elegans* dsRNA in non-pathogenic bacteria, *Bacillus subtilis*. This methodology would be instrumental in our future endeavors to identified pathways involved in regulation of aging in *C. elegans* via organismal interaction with gut microbiome.

► We are developing a better tools to characterize changes in organismal physiology (health) during natural aging in *C. elegans*. We established valuable collaborations with AMC and FOM Institute AMOLF in Amsterdam, as well as Virginia Commonwealth University in USA to use metabolomics, behavior assays, and high-resolution video microscopy to characterize how worm ages and how mutations in Wnt signaling pathway effect this process.

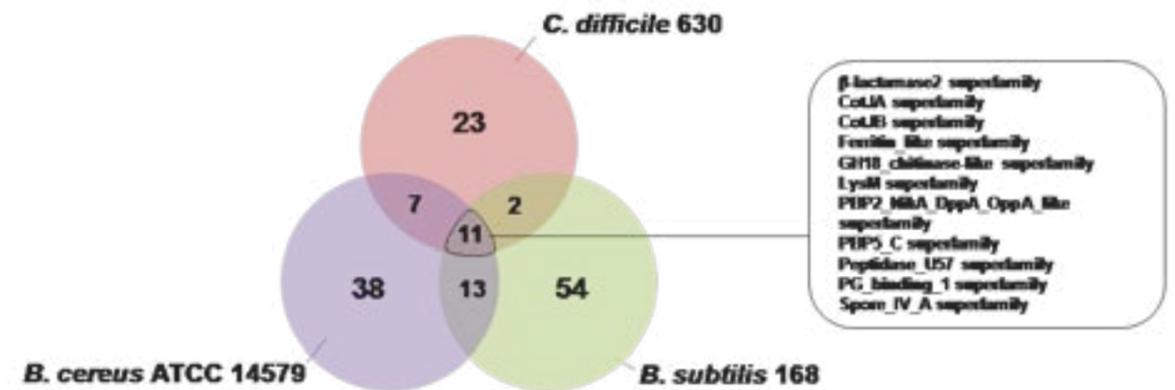
Future prospects and societal impact

► The group aims at a continued leveraging of its fundamental research with the application areas sustainability and human health. The fundamental understanding of intracellular pH signal transduction and its central role in cellular physiology (Laura Dolz Edo) will be coupled to the physiology of industrial fermentation. The newly hired PhD student (Abeer Hossain) in the unit of Dr. Gertien Smits builds on the work done by our former PhD students A. Ullah and Rick Orij.

► We leverage this focus in the area of sustainability in studies with a spin-off towards the production of industrial building blocks together with TNO, as well as in studies focusing on microbial food stability and safety with respectively industry and the Dutch Food and Consumer Products Safety Authority (VWA). The intracellular pH is a proxy for cellular stress resistance. Knowledge of stress resistance can be deployed both to enhance growth in fermentation (projects with TNO) as well as to inhibit growth with weak organic acid food preservatives (projects with Unilever, Friesland Campina and TNO) or antibiotics (VWA project). Intracellular pH is also a key marker of bacterial spores from food spoilage Bacilli regaining physiological activity. We will continue our work on cold-growth of the spore forming pathogen *Bacillus weihenstephanensis* in the framework of a FES sponsored project, Nanonext. The capability to respond to a weak organic depends on their ability to enter (germinating) spored as well as the thermal pre-treatment of spores. Against this background our group collaborates with the proteomics group and will continue to analyze the spore coat layer structure through joint PhD students. In addition we aim at deploying our newly developed live imaging spore germination and outgrowth tracking system SporeTracker which is able to analyze at single cell level the dynamics of intracellular pH. Thus population heterogeneity in stress response will be addressed. Finally we will continue to deploy our insight in cellular physiology of single celled organisms in its interaction with and extension to multicellular eukaryotes. Our knowledge of the *Candida albicans* yeast cell wall will be deployed through an IIF-Marie Curie fellow where the focus is on *Candida* and its interaction with a host in collaboration with the University Utrecht and the AMC. In the simple eukaryote model, *Caenorhabditis elegans*, we focus on the role of mitochondria in its homeostasis, as well as its perturbation, in situations where anti-HIV medication is administered, and in the ageing organism. The innovative tool for in vivo visualization and quantification of mitochondrial morphology in *C. elegans* that was set up will be deployed in further analyses focused on anti-HIV medication and alleviation of its side effects.

Other highlights

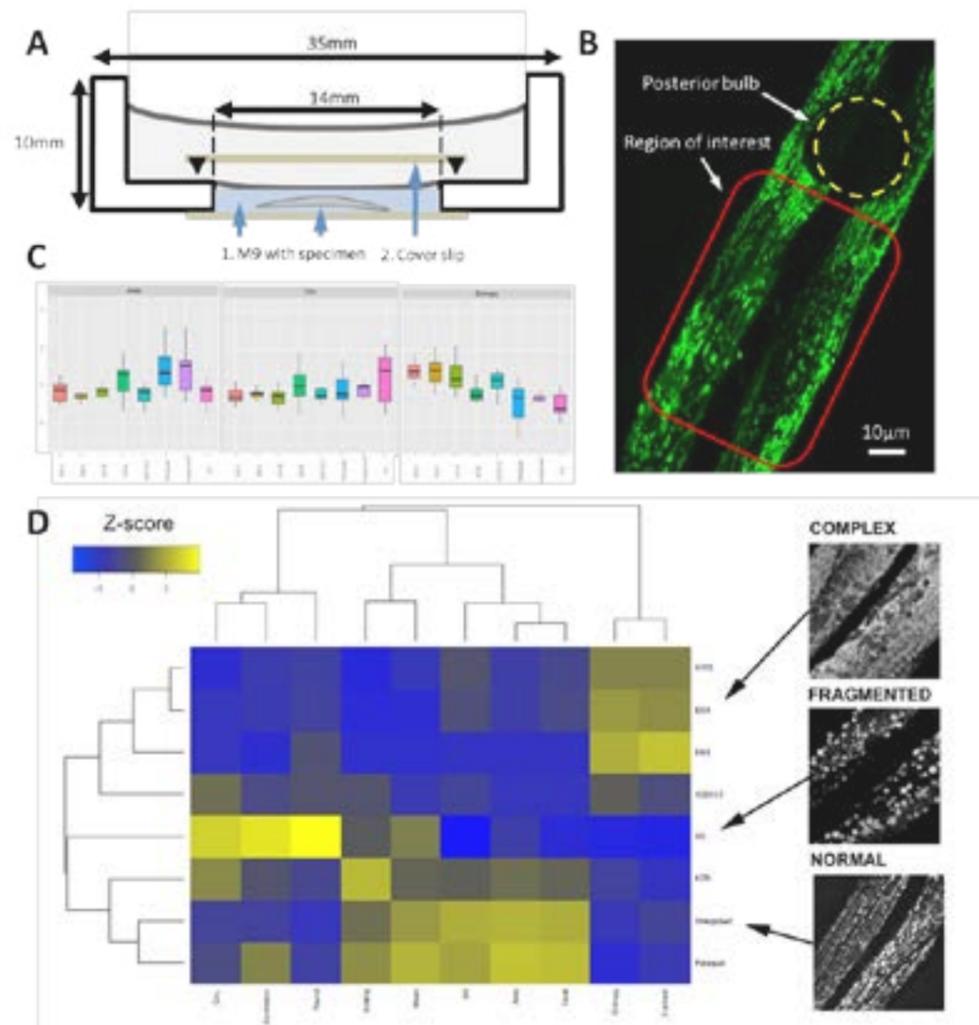
- S. Brul: FEMS representative of the Dutch Society for Microbiology as of 2009; Chair of the Dutch Institute for BioScience; editor Elsevier's Food Microbiology & Biomed. Research International; TNO & SILS investment in the Bio-based economy; Continuation of STW project TIBS and successful FES project Nanonext with Manders and de Koster group; Erasmus Mundus grants to support the work on novel antimicrobials for medical use with the AMC (Bas Zaat); F.M. Klis: Editor Eukaryotic Cell, FEMS Yeast Research, Yeast.
- F.M. Klis: Editor Eukaryotic Cell, FEMS Yeast Research, Yeast.



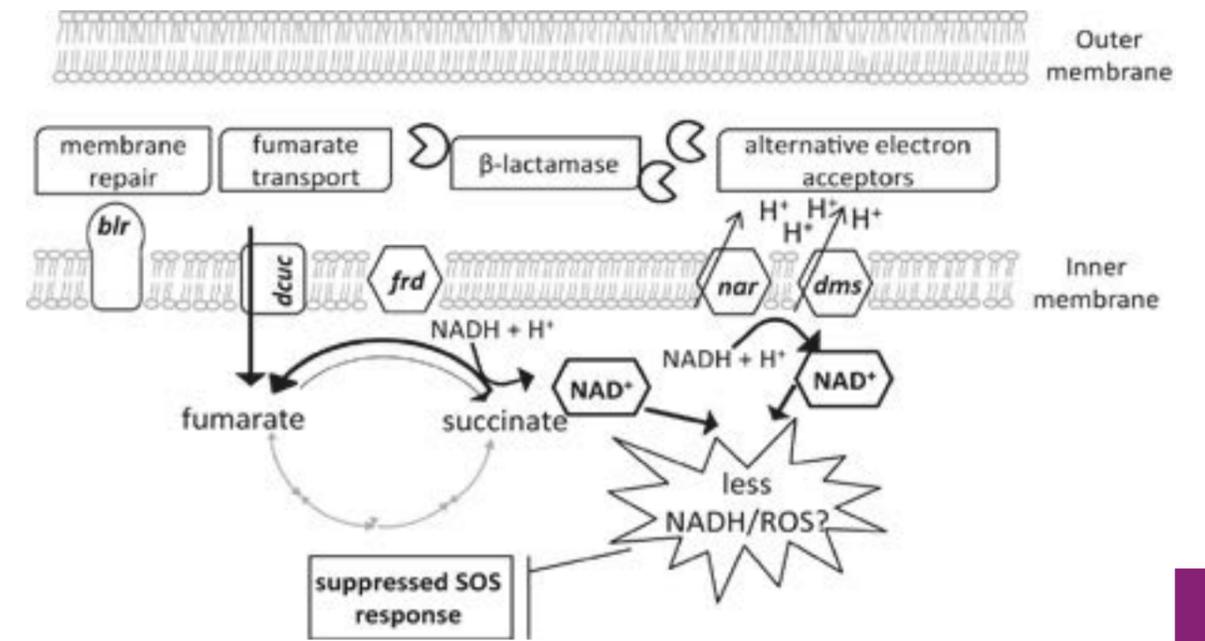
Venn diagram of conserved superfamily domains in the identified spore coat and exosporium proteins from *B. subtilis*, *B. cereus* ATCC 14579 and *Clostridium difficile* 630.

A total of 148 superfamily domain were assigned to coat protein identified from the three species listed. The numbers correspond to the superfamily domains from the identified proteins. Eleven superfamily domains common to the three organisms were identified and are listed in the box.

In collaboration with prof. Chris de Koster. Abhyankar et al. 2013 Journal of Proteome Research.



In vivo visualization and quantification of mitochondrial morphology in *C. elegans*
 A. Preparation of the MatTek glass bottomed petri dishes. 1. Pipette 120µL of an M9 worm suspension into a glass bottomed well. 2. Cover the well with a cover slip. B. Region of interest (red). Align the focal center just behind the clearly distinguishable area of the posterior bulb (yellow). C. Boxplots comparing selected metrics per condition (fltr): Area, Circularity & Entropy. D. Heatmap obtained after analysis of a set of images from *C. elegans* worms treated with different chemical compounds or RNAi. The columns represent different features of mitochondrial shape and intensity as well as image texture and the rows represent the different treatments. On the right, representative images are shown for the most dominant phenotypical patterns of mitochondrial networks (normal, complex, fragmented). Smith et al., *Mitochondrial Methods*, Elsevier (in press).
 In collaboration with Dr. Erik Manders (LCAM) and prof. Dr. Winnok de Vos (Ghent University).



Schematic model summarizing the main metabolic consequences of amoxicillin resistance in *E. coli*. In drug-exposed resistant cells, gene expression of alternative electron acceptors (*frdABCD*, *narGHJI*, and *dmsABC*) is induced, indicating a partial switch in metabolism from aerobic to anaerobic. Depletion of NADH may counter the elevated NADH-dependent superoxide production via the electron transport chain that was proposed by Kohanski and coworkers as a common mechanism of cell death induced by bactericidal antibiotics (20). Metabolic changes in amoxicillin-resistant cells include a suppressed SOS response compared to sensitive cells, regardless of the presence or absence of amoxicillin. Resistance is further enhanced by a mutation in the promoter region of *ampC*, resulting in increased expression of the β -lactamase (taken from Handel et al. *Antimicrob. Agents Chemother.* 2013).

Group members

Postdoctoral researchers

Alex ter Beek

PhD students

Laura Dolz Edo
 Yanfang Feng
 Nadine Händel
 Abeer Hossain
 Marco Lezzerini
 Rachna Pandey
 Reuben Smith
 Sacha Stelder

Group members (continued)

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Soraya Omardien
 Wishwas Abhyankar
 Linli Zheng

Docent

Richard de Boer (50%)

Technicians

Marjan de Jong
 Belinda Koenders-Vansintanneland
 Richard de Boer (50%)

Academic publications

Abhyankar, W., Hossain, A.H., Djajasaputra, A., Permpoonpattana, P., Beek, A. ter, Dekker, H.L., Cutting, S.M., Brul, S., Koning, L.J. de & Koster, C.G. de (2013). In pursuit of protein targets: proteomic characterization of bacterial spore outer layers. *Journal of Proteome Research*, 12(10), 4507-21.

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Kuile, B. ter & Brul, S. (2013). *Antibiotic resistance development and identification of response measures*. In J. Sofos (Ed.), *Advances in microbial food safety: volume 1* (Woodhead Publishing series in food science, technology and nutrition, 259) (pp. 157-169). Cambridge: Woodhead Publishing.

PhD theses

Beilen, J.W.A. van (17 October 2013). *Molecular physiology of weak organic acid stress in Bacillus subtilis*. Universiteit van Amsterdam (142 pag.). Prom./coprom.: prof. dr. S. Brul.

Heilmann, C.J. (15 February 2013). *Mass spectrometric quantification of Candida albicans surface proteins to identify new diagnostic markers and targets for vaccine development*. Prom./coprom.: prof. dr. C.G. de Koster, prof. dr. S. Brul, dr. F.M. Klis & dr. L.J. de Koning.

Sorgo, A.G. (22 February 2013). *Infection related stress adaptations in the secretome and wall proteome of Candida albicans*. Universiteit van Amsterdam (186 pag.). Prom./coprom.: prof. dr. S. Brul, prof. dr. C.G. de Koster, dr. F.M. Klis & dr. L.J. de Koning.

Invited lectures

Brul, S. (05 March 2013). *Functional genomics for optimal food preservation; Bacillus subtilis spore germination and outgrowth or 'the awakening of sleeping beauty'*. Norwich Institute for Food Research, Norwich, UK.

Brul, S. (25 March 2013). *Spoorzoecken in voedsel; van ongenode gast tot goede vriend*. FNWI Colleegetour, Amsterdam.

Brul, S. (10 April 2013). *Functional genomics for food preservation; Inhibiting bacterial spore germination*. Bacell, Newcastle.

Brul, S. (03 July 2013). *Omics steered risk assessment of food spoilage; Bacillus subtilis spore germination and outgrowth as a case study*. Microbial spoilers in food, Quimper, France.

Brul, S. (19 September 2013). *Modelling the rate of transfer of antibiotic resistance between E coli strains cultured under well controlled environmental conditions*. Paris, France. 8th International Congress Predictive Food Microbiology.

Budovskaya, Y. (11 March 2013). *A dual role of Wnt signaling pathway in aging*. The 8th European Congress of Biogerontology. Beer Sheva, Israel.

Budovskaya, Y. (22 April 2013). *Developmental drift: a new mechanism of aging in Caenorhabditis elegans*. European Research Institute for the Biology of Aging, University of Groningen, Groningen.

Budovskaya, Y. (25 May 2013). *Developmental Drift: a new mechanism of aging*. University Day, University of Amsterdam.

Smits, G.J. (19 September 2013). *Intracellular pH, A Metabolic Signal? Rudesheim, Germany, 6th Beilstein Symposium on Experimental Standard Conditions of Enzyme Characterizations (ESCEC)...Celebrating the 100th Anniversary of Michaelis-Menten Kinetics*.

Smits, G.J. (06 June 2013). *The smallest signal: protons as second messenger of environmental status in yeast*. Montpellier, France, 5th Conference on Physiology of Yeast and Filamentous Fungi.

Smits, G.J. (13 December 2013). *The simplest signal: protons as second messengers controlling cell division rate and more*. Kluiver Colloquium Series, Delft University.

Smits, G.J. (05 November 2013). *pH signaling to control growth and stress adaptation*. Groningen Biomolecular Sciences and Biotechnology Institute, Groningen.

Molecular Microbial Physiology

Prof. K.J. Hellingwerf
 Prof. M.J. Teixeira de Mattos
 Filipe Branco dos Santos
 Prof. J. Hugenholtz

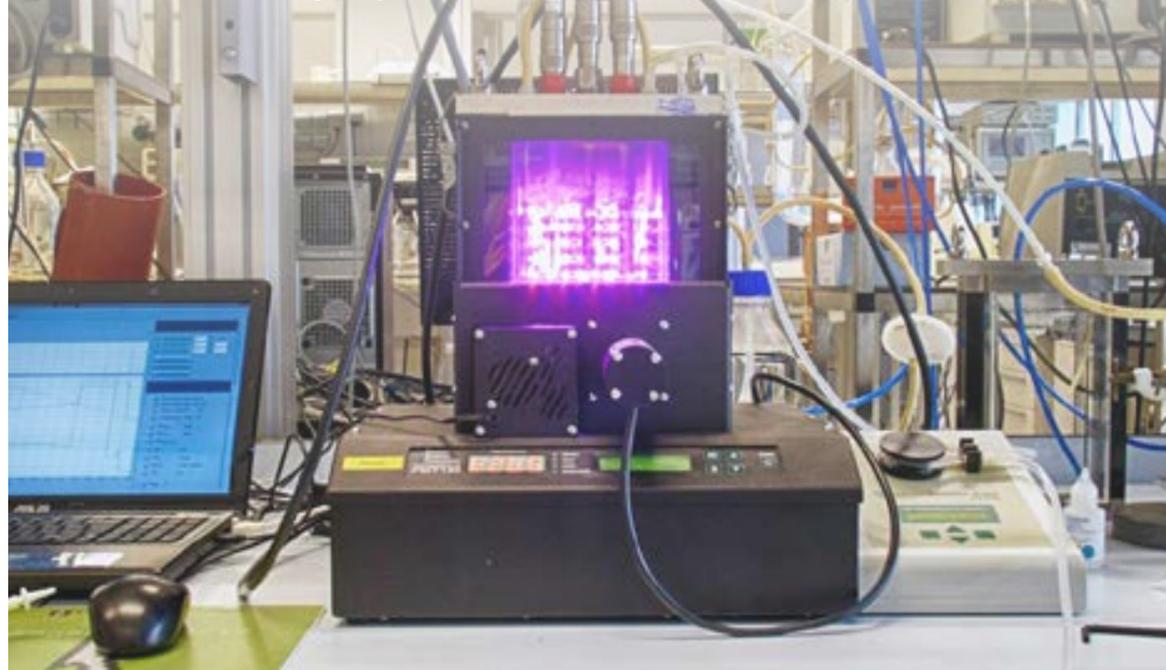
Chairholder
 Professor
 Assistant professor
 Special Chair

The impact that microbes have on life on Earth, and indeed on the Earth itself, has been well-recognized for many decades. The activity of microorganisms is seen not only at the global levels of evolution, the cycles of elements, ecological interactions and so on, but also in the direct effects on human life, both for better and for worse, through each person's microbiome. Our knowledge about the biochemistry and physiology of some microbial species is extensive but in most cases, however, only descriptive and qualitative. Further understanding of the impressive potential of microbes to adapt, proliferate and survive under a vast range of conditions, demands a quantitative and systems-analytical approach. This is even more so when it comes either to combating the adverse properties of microbes or to applying their many beneficial capacities, that is, transforming fundamental insights into applications.

The Molecular Microbial Physiology Group has organized its research accordingly, with a focus on the systems microbiology of lactic acid and cyanobacteria. Our research deals with integrating the biochemical/

biophysical properties of signal transduction and metabolic networks that function in fermentation, respiration and oxygenic photosynthesis, with physiological strategies for survival and growth. This integrative cellular approach goes hand in hand with studies on specific (sub)molecular events (e.g. in proteins involved in light sensing or the regulatory role of electron carriers in the redox chemistry of chemotrophic cells). Our work aims at furthering understanding of how their specific lifestyle endows microbes with the capacity to successfully cope with often severely-changing environments.

The broad diversity among micro-organisms, and the genetic and physiological differences between them, makes it worthwhile to focus research on more than a single species. For this reason we have selected for our studies both a chemo-heterotrophic organism - the industrially relevant lactic acid bacterium *Lactococcus lactis* - and the model organism for studies in oxygenic photosynthesis: *Synechocystis* PCC6803. Both organisms are highly relevant for the transition of our society towards a bio-based economy.



Growing cyanobacterial cell factories under well-controlled photoautotrophic conditions.

Research highlights

► In our work on the systems biology of the two most prominent prokaryotic model organisms, i.e. *Escherichia coli* and *Bacillus subtilis*, a number of research lines have been/will be completed with successful thesis defenses, i.e. those of Drs. Sharma, Van der Steen and Borirak. In the former a consistent description of the role of redox chemistry in the regulation of the an/aerobiosis transition could be formulated. Orawan Borirak has completed a project in which application of a range of omics techniques has allowed her to compile a list of genes that are subject to post-transcriptional regulation in one of the best-studied environmental responses of *E. coli*: carbon catabolite repression. This gene list is a rich source of information for the discovery of new regulatory processes in *E. coli*.

► The light-dependency of the environmental stress response of *Bacillus subtilis* has been exploited to provide a bottom-up systems biology description of this aspect of the organisms' physiology, in which the consequences of altering sub-molecular detail in a photosensory receptor can be read-out through the organisms' physiological responses.

► Drs. R. Hertzberger has successfully completed her studies of the aerobic physiology of the anaerobic, but aerotolerant and homolactic lactic acid bacterium *Lactobacillus johnsonii*. This bacterium produces high levels of hydrogen peroxide via two - newly identified - proteins that are essential for the organisms' aero-tolerance. Furthermore, growth of the organism is dependent on a supply of CO₂ and acetate. In the absence of an external source for these nutrients the pyruvate oxidase pathway can provide them, but only if O₂ is present.

► Stability of production strains is often a major bottleneck in biotechnological applications. We have initiated a proof-of-principle study in which the latter will be tackled from a radically different angle. Based on the analysis of genome-scale metabolic models, we are resorting to metabolic engineering to achieve metabolic networks in which product- and biomass formation is tightly coupled. This is followed by evolution experiments in which cells are selected for higher growth rates. This will on one hand result in a strain that produces a product much faster, but ultimately, will also shed light on the factors limiting growth rate in *Synechocystis* PCC6803. Within the framework of this project we have been able to secure additional funds to re-sequence the genome of 40 evolved isolates of *Synechocystis* (ZonMW Enabling Technologies Grant).

► For a varied spectrum of biofuel products and chemical commodities proof of principle has now been provided that these products can be synthesized from sunlight and CO₂ with *Synechocystis* PCC6803 and our 'Photanol' approach. This has shifted attention in the BioSolar Cell projects to the factors that govern distribution of the fixed carbon over the product vs. new cells. With lactate as the model substrate, during the past year we have achieved a ten-fold increase in this partitioning towards product, using a combination of molecular- (i.e. co-factor specificity), genetic- and physiological engineering.

Other highlights

► With the start of this new academic year Dr. Felipe Branco dos Santos has joined our group with his recently acquired NWO-VENI project, in which he aims at quantitatively understanding how dynamic, yet tightly controlled, nutrient feeding regimes in a chemostat can shape the evolution of microorganisms with different strategies of responding to variations in the environment. He will do this using a Systems Biology approach, i.e. his work will iteratively cycle between experimental and theoretical efforts. In essence the initial plan revolves around (i) prolonged cultivations under different regimes; (ii) characterization of evolved cells, and (iii) mathematical modeling of the evolutionary processes, starting off with using *Lactococcus lactis* as the model organism. Technical challenges that have already been encountered while implementing tightly controlled dynamic feeding regimes have already made it necessary to look for alternative ways to test hypotheses that would be immediately feasible experimentally.

► Prolonged cultivations in (a) photobioreactor(s) are a very suitable alternative for the chemostat since imposing dynamic environments there can be achieved by something as simple as controlling a light-intensity switch. Therefore, the focus in this VENI project will be expanded to include phototrophic bacteria, such as the model organism for oxygenic (i.e. plant-type) photosynthesis *Synechocystis* PCC 6803.

► In this first phase of the project, significant progress has already been achieved in the implementation of dynamic feeding regimes in both *L. lactis* and *Synechocystis*. Beyond that, we are now able to quantitatively predict the evolutionary outcome of selected competition experiments in dynamic environments, which is being used to design the feeding regimes imposed during the prolonged cultivations.

Research aims for the coming year

► A round-up is planned for the projects on *E. coli* and *B. subtilis*. For the former elucidation of the specific role of naphthoquinones in the regulation of the ArcB/A two-component system is foreseen, whereas in the general stress response of *B. subtilis* the visualization of stressosomes with advanced light microscopy will be completed.

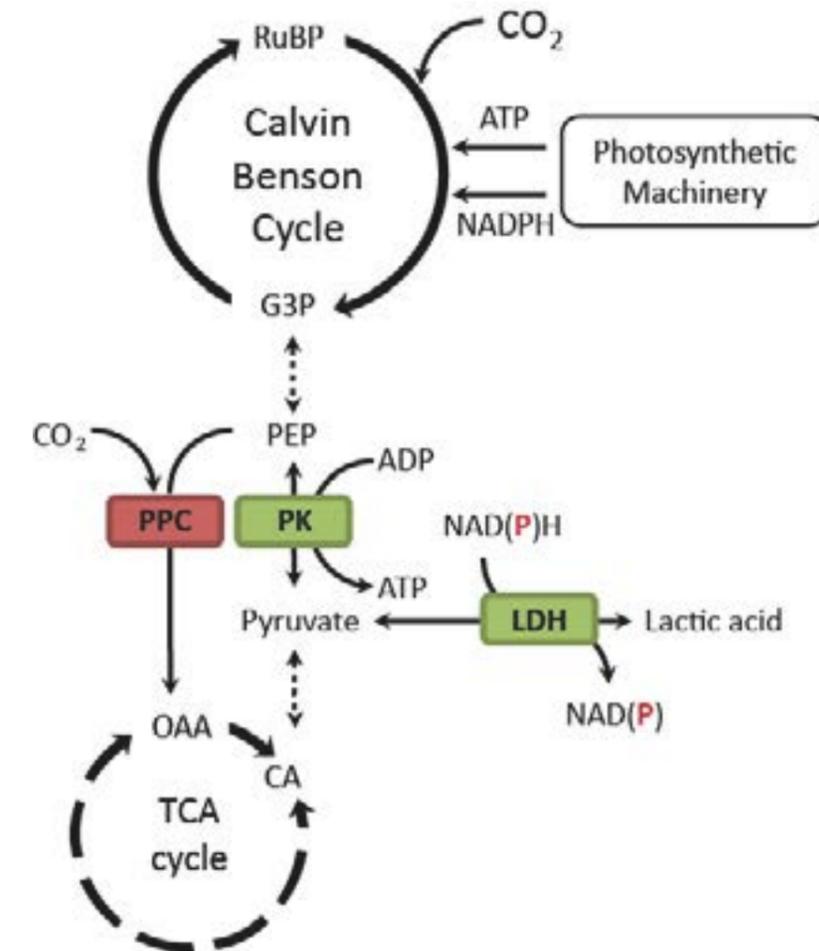
► Site-directed mutagenesis, combined with high resolution time-resolved spectrometric – and microscopy technologies will continue to be an important tool in the ongoing studies on microbial photoreceptor- and photo-signalling structures, functions and mechanisms, both in vitro and in vivo.

► In the BioSolar Cell projects the focus will be on (i) expanding the molecular toolbox for these organisms (i.e. marker-less deletions, modulated expression levels, etc.), (ii) improved production levels of ethylene, meso-2,3-butanediol, butanol, and cellulose, and (iii) furthering our understanding of energy- and intermediary metabolism of these organisms so that more detailed and therefore a more relevant computational simulation of the growth of these organisms can be provided.

Research aims for the coming year (continued)

► We are expanding our facilities to perform prolonged cultivation experiments in photobioreactors. These can be carried out applying different selection pressures under different cultivation regimes. We will focus on two specific questions at this stage: (i) what are the genetic determinisms limiting growth rate in *Synechocystis*? (ii) how do different illumination regimes shape the strategies of cells to deal with environmental fluctuations?

► In the upcoming years, we expect to unravel many of the genetic mechanisms underlying the adaptation of both *L. lactis* and *Synechocystis* to different environments. Two on-going projects focusing on these phylogenetically distant model organisms have adapted strains to specific feeding regimes and will now move on to the full genome re-sequencing of evolved isolates along with their extensive phylogenetic characterization.



Synthetic biology to construct a cyanobacterial cell factory for lactic acid: Next to high-level expression of a lactate dehydrogenase (LDH), lactic acid production from CO₂ can be increased by up-regulation of pyruvate kinase (PK) and down-regulation of PEP carboxylase (PPC).

Academic publications

Angermayr, S. & Hellingwerf, K.J. (2013). On the Use of Metabolic Control Analysis in the Optimization of Cyanobacterial Biosolar Cell Factories. *The journal of physical chemistry. B*, 117(38), 11169-11175.

Bitrian, M., Gonzalez, R.H., Paris, G., Hellingwerf, K.J. & Nudel, C.B. (2013). Blue-light dependent inhibition of twitching motility in *Acinetobacter baylyi* ADP1: Additive involvement of three BLUF domain-containing proteins. *Microbiology - SGM*, 159(9), 1828-1841.

Hertzberger, R.Y., Pridmore, R.D., Gysler, C., Kleerebezem, M. & Teixeira De Mattos, M.J. (2013). Oxygen relieves the CO₂ and acetate dependency of *Lactobacillus johnsonii* NCC 533. *PLoS One*, 8(2), e57235.

Hospes, M., Hendriks, J. & Hellingwerf, K.J. (2013). Tryptophan fluorescence as a reporter for structural changes in photoactive yellow protein elicited by photo-activation. *Photochemical & Photobiological Sciences*, 12(3), 479-488.

Hugenholtz, J. (2013). Traditional biotechnology for new foods and beverages. *Current Opinion in Biotechnology*, 24(2), 155-9.

Mensonides, F.I.C., Hellingwerf, K.J., Teixeira De Mattos, M.J. & Brul, S. (2013). Multiphasic adaptation

of the transcriptome of *Saccharomyces cerevisiae* to heat stress. *Food research international*, 54(1), 1103-1112.

Savakis, P.E., Angermayr, S.A. & Hellingwerf, K.J. (2013). Synthesis of 2,3-butanediol by *Synechocystis* sp. PCC6803 via heterologous expression of a catabolic pathway from lactic acid- and enterobacteria. *Metabolic Engin.*, 20, 121-30.

Sharma, P., Stagge, S., Bekker, M., Bettenbrock, K. & Hellingwerf, K.J. (2013). Kinase activity of ArcB from *Escherichia coli* is subject to regulation by both ubiquinone and demethylmenaquinone. *PLoS One*, 8(10), e75412.

Song, S.H., Madsen, D., Steen, J.B. van der, Pullman, R., Freer, L.H., Hellingwerf, K.J. & Larsen, D.S. (2013). Primary Photochemistry of the Dark- and Light-Adapted States of the YtvA Protein from *Bacillus subtilis*. *Biochemistry*, 52(45), 7951-63.

Steen, J.B. van der, Nakasone, Y., Hendriks, J.C. & Hellingwerf, K.J. (2013). Modeling the functioning of YtvA in the general stress response in *Bacillus subtilis*. *Molecular BioSystems*, 9, 2331-2343.

Vaughan, E. E. & Hugenholtz, J. (2013). Food biotechnology. *Current Opinion in Biotechnology*, 24(2), 121-123.

Wijffels, R.H., Kruse, O. & Hellingwerf, K.J. (2013). Potential of industrial biotechnology with

cyanobacteria and eukaryotic microalgae. *Current Opinion in Biotechnology*, 24(3), 405-413.

Zhu, J., Paparelli, L., Hospes, M., Arents, J., Kennis, J.T., Stokkum, I.H.M. van, Hellingwerf, K.J. & Groot, M.L. (2013). Photoionization and Electron Radical Recombination Dynamics in Photoactive Yellow Protein Investigated by Ultrafast Spectroscopy in the Visible and Near-Infrared Spectral Region. *The journal of physical chemistry. B*, 117(38), 11042-11048.

PhD theses

Sharma, P. (19 November 2013). *On the efficiency of catabolism at the cellular level*. Universiteit van Amsterdam (160 pag.). Prom./coprom.: prof.dr. K.J. Hellingwerf.

Steen, J.B. van der (12 December 2013). *The general stress response of Bacillus subtilis*. Universiteit van Amsterdam (174 pag.). Prom./coprom.: prof.dr. K.J. Hellingwerf.

Invited lectures

Branco dos Santos, F. (01 December 2013). *Fermentation for vitamin enrichment and (metabolic) modeling of industrial fermentation for better control and product improvement*. Beijing, China. Food Biotechnology Training Course for Developing Countries and the World Academy of Sciences.

Hellingwerf, K.J. (20 November 2013). *Engineering cyanobacteria into biosolar cell factories*. Porto, Portugal, Scientific Meeting Cyanofactory.

Hellingwerf, K.J. (16 January 2013). *Cyanobacteria: the 'plug-bugs' for CO₂*. DSM Research, Eindhoven.

Hellingwerf, K.J. (23 January 2013). *Cyanobacteria: the 'plug-bugs' for CO₂*. Brussels, Belgium. DEMA Kick-off meeting.

Hellingwerf, K.J. (07 February 2013). *Understanding of Microbial Oxygen-Dependent and Independent Catabolism (SUMO₂)*. Berlin, Germany. Closing meeting of the SUMO₂ consortium.

Hellingwerf, K.J. (14 March 2013). *Zonne-energie en CO₂ vastlegging: De biologische route*. Lecture Spui25, Amsterdam.

Hellingwerf, K.J. (18 March 2013). *Sustainable CCS: Cyanobacterial CO₂-based Synthesis, or: Cyanobacteria: the 'plug-bugs' for CO₂ - Exxon Mobile Research*, Amsterdam.

Hellingwerf, K.J. (08 April 2013). *CO₂ as the feedstock for a truly sustainable biotech industry* (Baseclear congress, Genomics in Business Conference, Beurs van Berlage, Amsterdam).

Hellingwerf, K.J. (12 April 2013). *Synthetische biologie: 60 jaar na Watson en Crick*. Contribution to 'Homo digitalis', organised by Expertise Centrum voor de Journalistiek, Hilversum

Hellingwerf, K.J. (19 April 2013). *Physiology and energetics of product formation from CO₂, water and sunlight by the cyanobacterium Synechocystis sp. PCC6803*. Plenary lecture at the ESF Meeting on Energetics of Cyanobacteria, Pultusk, Poland

Hellingwerf, K.J. (07 May 2013). *Cyanobacterial cell factories & CO₂ as the feedstock for ultimate sustainability*. TNO Research, Delft, The Netherlands

Hellingwerf, K.J. (09 May 2013). *Cyanobacteria: the 'plug-bugs' for CO₂*, Repsol Research, Madrid, Spain.

Hellingwerf, K.J. (13 June 2013) *Increasing productivity of phototrophic cell factories for energy, commodities and feedfood*. Ede. Annual Meeting TBSC.

Hellingwerf, K.J. (27 August 2013). *Synthetische biologie & de toekomst van de microbiële biotechnologie*, Scheveningen. Symposium Ministerie van Infrastructuur, Milieu en Ruimtelijke Ordening,

Hellingwerf, K.J. (05 September 2013). *Intrigued by light: 'Bacterial observations' were not enough!* Groningen. Lezing 4e lustrum Onderzoekschool GBB, .

Hellingwerf, K.J. 21 September 2013. *Cyanobacteria as the ultimate photo-catalysts of the conversion of CO₂ into chemical commodities and liquid fuel, driven by either sunlight or electricity*. Stockholm, Sweden. Breakthrough Meeting of the European Federation of Academies of Science (EASAC).



Synthetic biology with *Synechocystis* requires transformation, genome segregation, and growth of single cell on agar plates.

Hellingwerf, K.J. (02 November 2013). *Engineering microbial cell factories for the production of commodity chemicals and liquid fuel*. Lecture, Artis, Amsterdam

Hellingwerf, K.J. (03 November 2013). *Het gebruik van cyanobacterien als 'cell factories' voor biobrandstof productie*. Lezing Hogeschool Leiden.

Hellingwerf, K.J. (04 October 2013). *CO₂ as the ultimate substrate for a sustainable biotech industry. Conference on CO₂ as chemical feedstock: a challenge for sustainable chemistry*. Essen, Germany .

Hellingwerf, K.J. (15 October 2013). *Expanding society's toolbox to harvest solar energy: Creating multiscale computational models to optimize oxygenic photosynthesis*. Introduction to the 1st Toolbox-meeting on oxygenic photosynthesis, Amsterdam.

Hellingwerf, K.J. (20 October 2013). *Photofermentative product formation in Cyanobacteria: the 'plug-bugs' for CO₂*. DEMA Progress Meeting, Amsterdam.

Hellingwerf, K.J. (01 November 2013). *Het gebruik van cyanobacterien als 'cell factories' voor biobrandstof productie*. Den Haag. Provincie Zuid Holland.

Hellingwerf, K.J. (11 November 2013). *Het gebruik van cyanobacterien als 'cell factories' voor biobrandstof productie*. Rotterdam. OCAP.

Hellingwerf, K.J. (20 November 2013). *Engineering cyanobacteria into biosolar cell factories. Invited lecture at the EU project meeting of 'Cyanofactory'*, Porto, Portugal.

Other

Hellingwerf, K.J. (2013). website UvA, 'UvA in the spotlight', interview with...

Teixeira De Mattos, M.J. (2013). Commentaar interview 'Vleesprofessor'. *NRC Weekend*: (2013, 10 August - 11 August 2013).

Media performance

Hellingwerf, K.J. (expert) (24 November 2013). CO₂ als brandstof [television-broadcast]. In *Labyrint*. Nederland 2.

Group members

Postdoctoral researchers

Johan van Beilen
Koen van Grinsven
Johannes van Heerden

Maria Palmeros Parada
Vinod Puthan Veetil

PhD students

Pascal van Alphen
Andreas Angermayr
Orawan Borirak
Aleksandra Bury
Que Chen
Wei Du
Rosanne Herzberger
Philipp Savakis
Rosa Milou Schuurmans
Poonam Sharma
Jeroen van der Steen
Wei Du

Technicians

Jos Arents
Seyedparsa Mahallehyousefi

Amanuensis

Dennis Rijnsburger

Bacterial Cell Biology

Dr L. Hamoen
Dr T. den Blaauwen

Associate Professor (tenure track towards a full professorship)
Associate Professor

The new research group 'Bacterial Cell Biology' is rapidly growing with new and exciting projects that deal with morphogenetic protein function, drug efflux and antibiotic screening assay development. Several new members have been appointed using the STW-Vici grant (Terrens Saaki and Michaela Wenzel) and Marie Curie grant (Laura Bohorquez-Suarez) obtained by Leendert Hamoen, and Nils Meiresonne and Pranav Puri were appointed on an ALW-NWO grant and HFSP grant by Tanneke Den Blaauwen. Den Blaauwen's work is focussed on Gram-negative bacteria, using *Escherichia coli* as key model system, whereas Hamoen is working on Gram-positive bacteria, whereby *Bacillus subtilis* is the main 'work horse.' Bacterial Cell Biology deals with the study of bacteria at the level of the single cell. With the recent advancements in fluorescence light microscopy it

became apparent that the bacterial cell is surprisingly complex in its organization. Many proteins are only found at specific regions within the cell, such as at the cell pole, or at the middle of the cell, where division takes place. How this complex organization is achieved is in many cases unclear. One of the processes that we study is cell division. Most of the cell division proteins have been identified, but how exactly they accomplish cytokinesis is largely unknown. Not only is cell division an interesting biological research question, it is also a useful target for the development of novel antibiotics. This is important since the number of novel antibiotics that enter the clinic has dwindled over the last decades, and the development of new antibiotics is urgent. We use the knowledge emanating from bacterial cell biological research to set up new ways to develop novel antimicrobials.

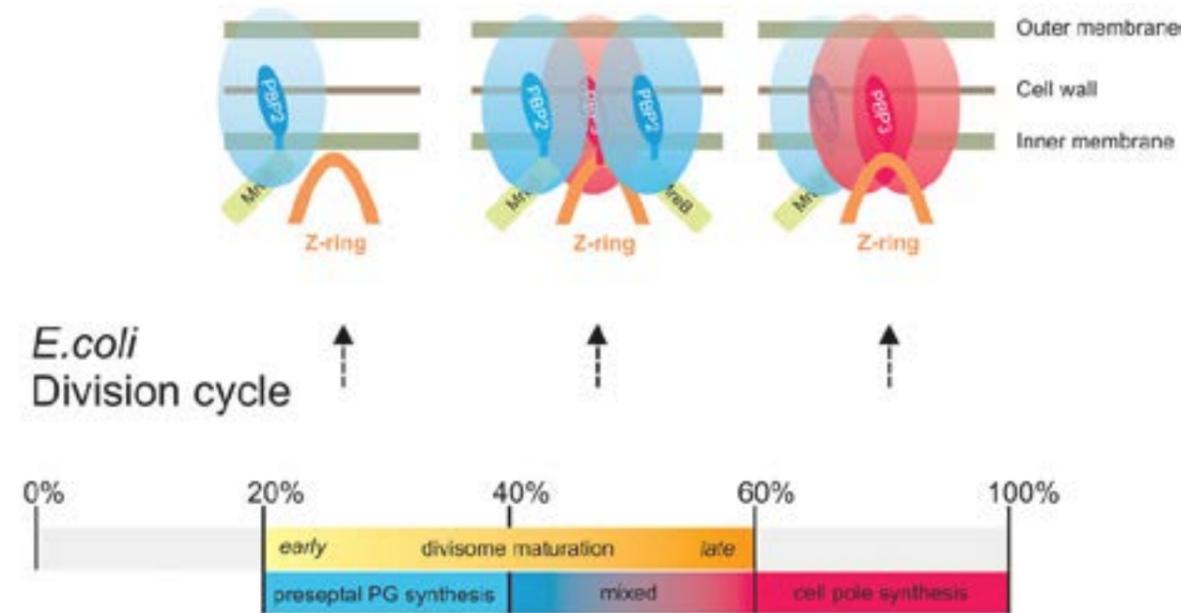
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Research highlights

- ▶ Tanneke Den Blaauwen and colleagues showed using image analysis and FRET that the protein machinery involved in bacterial length growth associates temporarily with the protein machinery that is essential for cell division. The interacting surface between FtsQ, FtsL, FtsB essential cell division proteins in *E. coli* were mapped. This accesses the proteins for in silico docking of small molecule libraries for antibiotic design.
- ▶ Leendert Hamoen and colleagues have discovered that the conserved protein WhiA is important for cell division in *B. subtilis*, furthermore they have identified different protein interaction domains of the morphogenetic protein DivIVA, and they identified a new membrane anchor for the essential cell division protein FtsZ. In collaboration with the MRC laboratory in Cambridge they have solved the structural domain of this protein (SepF).

Other highlights

- ▶ An HSFP grant in collaboration with groups in the UK, Germany and Japan was rewarded to study the activity of the multi-drug efflux pump in *E. coli*.
- ▶ Hamoen: coordinator of two Marie Curie ITN programmes that were awarded in 2012.



Model of midcell localization of PBP2 and PBP3 during cell division for cells that are grown with a mass doubling of 85 min. After 20% of the division cycle time FtsZ proteins start to accumulate at midcell. The Z-ring structure will be formed and stabilized to initiate divisome maturation. Simultaneously some elongasome complexes (including at least MurG, MreB and PBP2) move to midcell and synthesize pre-septal peptidoglycan. After about 40% of the division cycle the late proteins of the divisome complex begin to increase in concentration at midcell. More PBP3 proteins arrive and compete with PBP2 for peptidoglycan substrate. A mixed situation, in which elongasome and divisome PG synthases are both active, results in the slow decrease in midcell diameter of the cells between 40% and 60% of the division cycle. At 60% of the division cycle the divisome is mature and new cell pole synthesis occurs at a much faster rate. PBP2 is not present in the new cell poles and presumably does not contribute the new cell pole synthesis, although the presence of a minor amount of PBP2 at the leading edge of constriction cannot be excluded van der Ploeg, et al. (2013). Colocalization and interaction between elongasome and divisome during a preparative cell division phase in Escherichia coli. *Molecular Microbiology*, 87(5), 1074–1087.

Group members

Postdoctoral researcher

Pia Ehrentraut
René van der Ploeg

PhD students

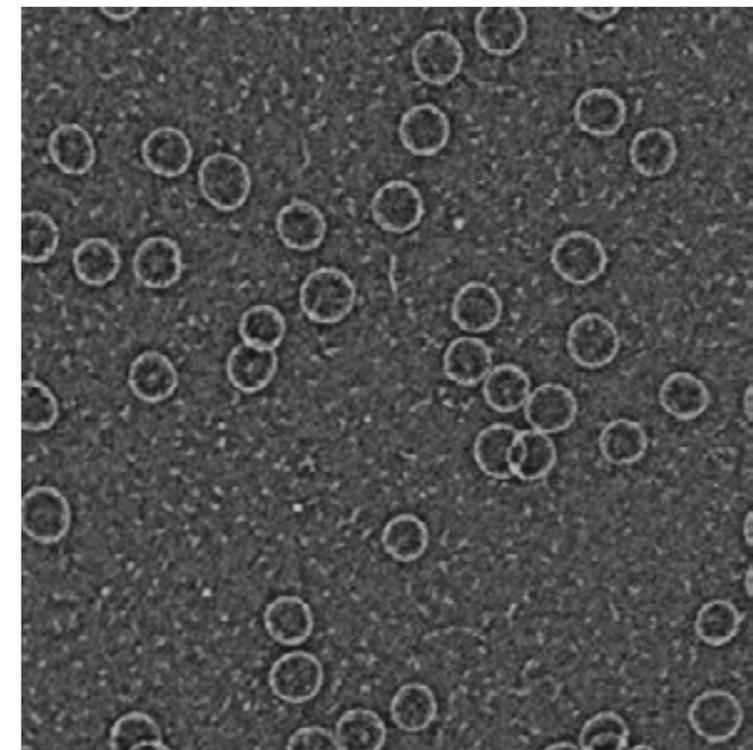
Laura Bohorquez
Nils Meiresonne
Sebastian Robalino-Espinosa
Terrens Saaki

Technicians

Tjalling Siersma
Jolanda Verheul

Research aims for the coming year

- Den Blaauwen: Publish papers on (i) The mode of growth of two newly discovered bacterial nematode symbionts (ii) the use of FRET for the screening of antibiotics that inhibit bacterial length growth, (iii) the identification of the interacting surfaces of the bacterial cell division proteins ZapA and FtsZ, (iv) On the function of YgbF in cell division on and (v) on the interaction between ZapA and Zap. In addition, we plan to develop a periplasmic FRET assay, to characterize Llama antibodies that recognize FtsZ, to study the polymerization behaviour of *Laxus oneistus* FtsZ.
- Hamoen: Publish papers on (i) the membrane organization by MreB, (ii) the effect of tetracycline on cell division, (iii) the systematic deletion of non-essential cell division genes in *B. subtilis*. In addition, we will optimize our screen for compounds targeted against SepF, FtsA and against persister cells.



Purified SepF from *Bacillus subtilis* forms large protein rings with a diameter of approximately 50 nm (related to Duman et al., 2013, PNAS).

Academic publications

Baarle, S. van, Celik, I.N., Kaval, K.G., Bramkamp, M., Hamoen, L.W. & Halbedel, S. (2013). Protein-protein interaction domains of Bacillus subtilis DivIVA. *Journal of Bacteriology*, 195(5), 1012-1021.

Berg van Saparoea, H.B. van den, Glas, M., Vernooij, I.G., Bitter, W., Blaauwen, T. den & Luirink, J. (2013). Fine-mapping the contact sites of the Escherichia coli cell division proteins FtsB and FtsL on the FtsQ protein. *The Journal of Biological Chemistry*, 288(34), 24340-50.

Blaauwen, T. den (2013). Prokaryotic cell division: flexible and diverse. *Current opinion in microbiology*, 16(6), 738-44.

Buddelmeijer, N., Aarsman, M.E.G. & Blaauwen, T. den (2013). Immunolabeling of Proteins in Situ in Escherichia coli K12 Strains. *BIO-PROTOCOL*.

Duman, R., Ishikawa, S., Celik, I., Strahl, H., Ogasawara, N., Troc, P., Lowe, J. & Hamoen, L.W. (2013). Structural and genetic analyses reveal the protein SepF as a new membrane anchor for the Z ring. *Proceedings of the National Academy of Sciences of the United States of America*, 110(48), E4601-10.

Ploeg, R. van der, Verheul, J., Vischer, N.O., Alexeeva, S., Hoogendoorn, E., Postma, M., Banzhaf, M., Vollmer, W. & Blaauwen, T. den (2013). Colocalization and interaction between elongasome and divisome during a preparative cell division phase in Escherichia coli. *Molecular Microbiology*, 87(5), 1074-87.

Surdova, K., Gamba, P., Claessen, D., Siersma, T., Jonker, M.J., Errington, J. & Hamoen, L.W. (2013). The Conserved DNA-Binding Protein WhiA Is Involved in Cell Division in Bacillus subtilis. *Journal of Bacteriology*, 195(24), 5450-60.

Verhoeven, G.S., Dogterom, M. & Blaauwen, T. den (2013). Absence of long-range diffusion of OmpA in E. coli is not caused by its peptidoglycan binding domain. *BMC Microbiology*, 13, 66.

Invited lectures

Blaauwen, T. den (07 September 2013). *Longitudinal fission in a rod-shaped Gammaproteobacterium*. Groningen, the Netherlands, Secretie Platform.

Blaauwen, T. den (29 May 2013). *Morphogenesis of E. coli*. Amsterdam, the Netherlands, VU Amsterdam, Medical MicroBiology.

Blaauwen, T. den (06 November 2013). *Morphogenesis of E. coli*. Santiago, Chili, University of Chili.

Blaauwen, T. den (21 October 2013). *Morphogenesis of E. coli*. Cambridge, UK, 2013 Kick off meeting multidrug efflux pumps HSFP.

Blaauwen, T. den (11 October 2013). *Morphogenesis of E. coli*. Vienna, Austria, Department of Ecogenomics and Systems Biology.

Hamoen, L. (17 May 2013). *Membrane curvature, charge, and fluidity influences protein localization*. Université Paul Sabatier, Toulouse, France.

Hamoen, L. (14 March 2013). *Membrane Curvature, Charge, and Fluidity Determine Protein Localization in Bacteria*. Leiden, Netherlands, toplecture series.

Hamoen, L. (10 April 2013). Organizer and session chair 2013. BACELL conference. Newcastle, UK.

Hamoen, L. (11 March 2013). Session chair VAAM meeting (German association for general and applied microbiology). Bremen, Germany.

Ploeg, R. van der & Blaauwen, T. den (12 November 2013). *Screening bacterial cell growth and cell division inhibitors by FRET (Förster Resonance Energy Transfer)*. Puerto Varas, Chili, PanAmerican Association of Biochemistry and Molecular Biology; XII PABMB.

Ploeg, R. van der & Blaauwen, T. den (25 November 2013). *Screening bacterial cell growth and cell division inhibitors by FRET (Förster Resonance Energy Transfer)*. Paris, France, EUR-INTAFAR meeting.

Media performance

Blaauwen, T. den & Maarseveen, J.H. van (guests) (24 February 2013). Antibiotica resistentie [radio-performance]. Radio programme *Swammerdam wetenschapsprogramma* radio Amsterdam FM.

Molecular Cytology & van Leeuwenhoek Centre for Advanced Microscopy

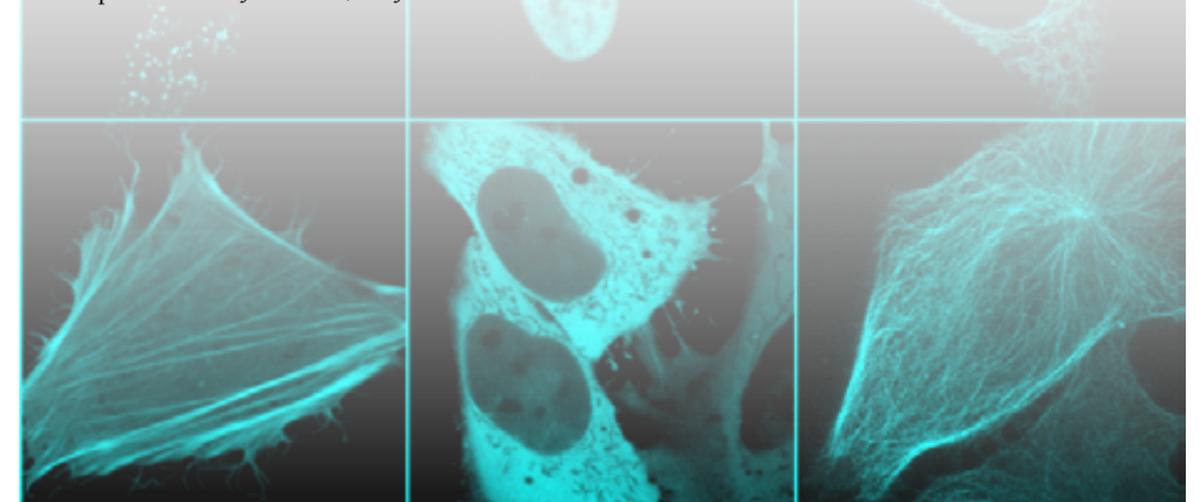
Prof. T.W.J. Gadella
Dr E.M.M. Manders
Dr Ir J. Goedhart
Dr Ir M.A. Hink
Dr M. Postma
Dr. R. van Amerongen
Prof. P.L. Hordijk

Chairholder
Associate Professor
Assistant Professor
Assistant Professor
Tenure Track Assistant Professor
Tenure Track Assistant Professor
Special Chair

Molecular Cytology is the study of the dynamic architecture of living cells. Our central theme is 'Self-organisation and signalling in living cells'. Self-organisation is the intrinsic property of matter to organise itself in a (dynamic) structure, whereas signalling implies the activity of gene-products to control a local activity, which can alter the local cellular architecture (e.g. driving morphogenesis). In order to achieve a certain 3D architecture in cells, these two important mechanisms work in concert. At Molecular Cytology both mechanisms are studied with emphasis on membrane-related architecture of living cells using advanced microscopy tools. The activities are connected to the Faculty of Science Spearhead programme on Systems Biology, where our contribution is on spatiotemporal systems biology of higher eukaryotes. The main research areas are:

- 1) Spatiotemporal organisation of cellular signalling and its relation tissue and organ development (group leaders prof. dr. T.W.J. Gadella, dr. J. Goedhart & dr. M.A. Hink, dr. M. Postma, dr. R. van Amerongen, prof. dr. P.J. Hordijk). By employing genetic encoded fluorescent biosensors we analyse the in situ molecular interactions between signalling molecules (phospholipid-second messengers, receptors, G-proteins and effector molecules) & flow of information across and in the plane of the membrane of living mammalian cells. We aim to understand how cells can achieve and maintain a local signal in the membrane (e.g. in order to drive morphogenesis, or to define new cytoskeletal anchorage or vesicle-docking sites). The main pathways under study involve histamine/P2Y GPCR receptors, G-αq to PLC activation triggering downstream calcium, kinase signalling and small GTPase (Rho/Rac/Cdc24) signalling (the last in collaboration with prof. dr. P.J. Hordijk/Sanquin).

More recently we have started a new activity on developing models that include gene regulation and biomechanics of *Nematostella vectensis*



embryogenesis. Hereby we use fluorescently labelled biomolecular markers to follow embryogenesis like adhesion molecules, cytoskeletal components (F-actin and Myosin) that are directly involved in cell shape changes and cell-cell interactions and also quantification of expression patterns. In November 2013 we were joined by dr. R. van Amerongen, a MacGillavry fellow of the Faculty of Science. She will set up a novel, independent research line focusing on the role of Wnt signaling in mammary gland stem cells and breast cancer.

The close intertwining of several signalling cascades, their spatial organisation both within and between cells and our quantitative microscopy approach both necessitates and permits the generation of quantitative predictive modelling, which effectively will integrate this research line with Systems Biology approaches.

2) Advanced microscopy organised within the van Leeuwenhoek Centre for Advanced Microscopy (LCAM-FNWI). The goal of LCAM-FNWI (em. prof. dr. G.J. Brakenhoff, prof. dr. T.W.J. Gadella, dr. E.M.M. Manders, dr. M. Hink, dr. Goedhart, dr. M. Postma) is to boost Life Sciences research by implementing & developing (optical) microscopy techniques. Current most prominent developments are new super-resolution microscopy techniques such as Re-scan Confocal Microscopy (RCM), Structured Illumination Microscopy (SIM), Multi-point Image Scanning Microscopy (ISM) (dr. Manders), PhotoActivated Localization Microscopy (PALM) and Stochastic Optical Reconstruction Microscopy (STORM) (Gadella, Postma and Manders). Several activities within super-resolution microscopy development are active collaborations within the Nikon Centre of Excellence for Super Resolution Microscopy Development.

Another main focus of LCAM-FNWI is centred around Functional Imaging Microscopy of living cells including multimode Fluorescence Resonance Energy Transfer (FRET), Fluorescence Spectral Imaging Microscopy (SPIM), Fluorescence Lifetime Imaging Microscopy (FLIM), Fluorescence Recovery After Photobleaching (FRAP) (dr. Gadella, dr. Goedhart & dr. Hink), & Fluorescence fluctuation microscopy, including Fluorescence (Cross) Correlation Spectroscopy (F(C)CS), Fluorescence Lifetime Correlation Spectroscopy (FLCS), Line-scan FCS, and Number & Brightness techniques (dr. Hink).

In each of the above approaches we invest strongly in quantitative data assessment (mostly driven by dr. M. Postma & coworkers), since processing and evaluation algorithms are becoming more important, sophisticated and difficult. The extension of our research area towards multicellular 3D systems: cross endothelial cell migration (prof. dr. Hordijk & coworkers), *Nematostella* embryonic development (dr. M. Postma and coworkers) and mammary gland development (R. van Amerongen & coworkers) imposes new technical challenges, especially to enable quantitative in situ imaging of cell behavior and signal transduction in a complex three dimensional context. Of increasing importance for advanced microscopy in biology is the development of fluorescent probes with enhanced properties and -derived molecular biosensors. We have activities for enhancing intrinsic fluorescent protein brightness by mutagenesis & screening (dr. Gadella, dr. Goedhart, dr. Hink), for development of new photoswitchable fluorescent proteins (dr. Gadella and dr. Hink) and for new FRET-based biosensors reporting on second messenger levels, and on heterotrimeric & small G-protein activities in live cells (Goedhart & Gadella).

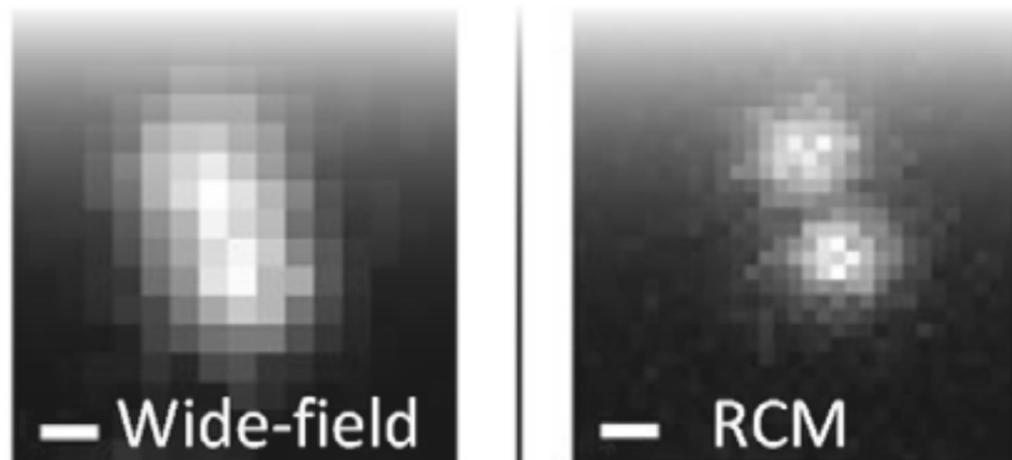


Image resolution enhancement by Re-scan Confocal Microscopy as compared to wide field imaging.

Research highlights

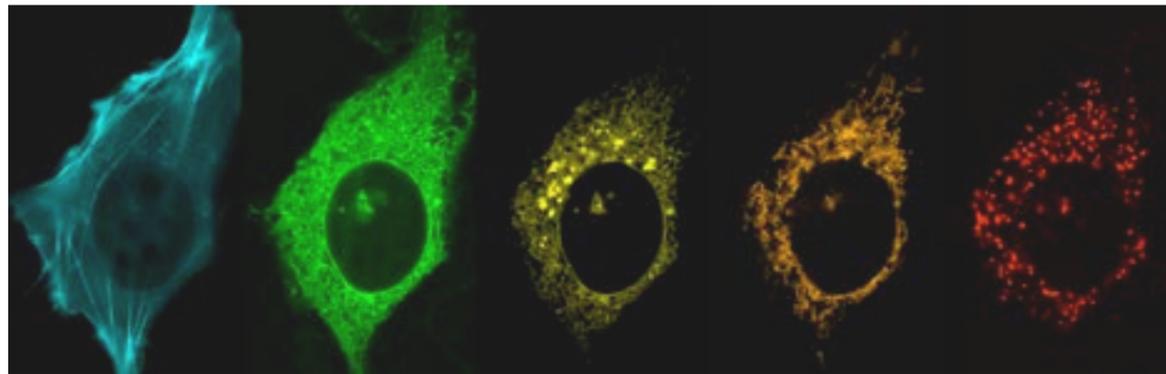
- ▶ In 2013 our LCAM expression of interest to become a Euro-BioImaging Flagship Node on Functional Imaging received the highest possible score 'Highly recommended'.
- ▶ In 2013 we published an extensive functional imaging analysis (number and brightness, FRET and FRAP analysis) of Annexin-oligomerization in membranes in *Biophys. J.*. This article was highlighted in a 2 page new & notable article within the same issue in which the study was regarded as 'a paradigm shift in the way membrane-associated macromolecular assembly and dynamics are measured'.
- ▶ A set of seriously enhanced monomeric red fluorescent proteins were developed displaying fluorescence lifetimes > 3 ns which are more than two-fold enhanced as compared to the currently most employed RFP mCherry (with a lifetime of 1.5 ns).
- ▶ A new robust analysis algorithm for stochastic localization microscopy was developed (PALM, STORM), part of this work, specifically focussing on background estimation has been submitted to Scientific Reports
- ▶ Manders and co-workers invented a new technique to improve the optical resolution (from 250 nm down to 170 nm, see illustration) and detection sensitivity of the confocal microscope. After building and testing the first prototype, results were published in *Biomedical Optics Express*. The 3-colour RCM prototype is now available for biological experiments.
- ▶ A large set of constructs was created to measure and validate the quantification of molecular interactions in the MAPK signalling pathway as will be measured by Fluorescence lifetime cross-correlation spectroscopy (FLCCS) and FCCS.
- ▶ We have successfully imaged F-actin and Tubulin dynamics in live *Nematostella vectensis* using the photostable mTurquoise2.
- ▶ We have developed and utilized a new set of FRET based biosensors (for RhoA, cAMP, Gai) allowing the quantitative analysis of GPCR activated pathways
- ▶ We have set up three-dimensional mammary organoid cultures and imaged these by confocal microscopy to reveal the clonal outgrowth of Wnt-responsive cells.
- ▶ We have imaged live *Nematostella vectensis* embryos using lifeact-mTq2 capturing details of the cell division during the cleavage stage.

Other highlights

- ▶ Dr. R. van Amerongen was awarded a MacGillavry Fellowship by the Faculty of Science of the University of Amsterdam. She joined the Section of Molecular Cytology and will set up a new independent research line on Wnt signalling in mammary gland development & tumorigenesis.
- ▶ Dr. R. van Amerongen was awarded a cancer research career award by KWF kankerbestrijding
- ▶ Dr. R. van Amerongen, was awarded an NWO Aspasia grant (declined)
- ▶ Dr. J. Goedhart, J. and prof. dr. T.W.J. Gadella, were awarded an NWO, CW-Echo grant (1 PhD student position) 'Visualizing the chemistry of cellular decision making with engineered fluorescent biosensors'.
- ▶ The proposal 'Netherlands-BioImaging Advanced Microscopy' (NL-BioImaging AM), coordinated by prof. TWJ Gadella on behalf of 18 participating microscopy centres in the Netherlands was included in the Netherlands' Roadmap for Large-Scale Research Infrastructures.

Research aims for the coming year

- ▶ Introduce the CRISPR technology to allow genetic engineering of endogenous genes in a variety of mammalian cell lines.
- ▶ Set up a robust three dimensional culture system allowing us to manipulate, image and quantify signal transduction in 3D.
- ▶ Publish on G-protein signalling pathways that remodel the (actin) cytoskeleton.
- ▶ Publish on a set of seriously enhanced red fluorescent proteins.
- ▶ Develop new Turquoise2-based FRET pairs and biosensors.
- ▶ Publish on enhanced algorithms for localization-based super-resolution microscopy.
- ▶ Publish on new super-resolution techniques including RCM, ISM and STED.
- ▶ Publish fluorescence lifetime correlation spectroscopy using multiple single-color fluorescent proteins.
- ▶ Publish about visualisation of live *Nematostella vectensis* embryos.
- ▶ Develop a high quantum yield variant of a monomeric red fluorescent protein.



Multicolour live cell confocal microscopy of a human skin cell expressing diverse GFP-colour variants (scale 45x60 μm). This example visualizes, all in the same living cell, the actin cytoskeleton (turquoise), the protein production machinery (Endoplasmic reticulum, green), the protein processing factory (Golgi apparatus, yellow), the cell's energy plant (mitochondria, orange) and the cellular waste-disposal and recycling plant (Lysosomes, red). The images feature the best and brightest "third-generation" fluorescent proteins available, the left 3 of which have been developed in the section of Molecular Cytology, SILS.

Academic publications

Adjobo-Hermans, M.J.W., Crosby, K.C., Putyrski, M., Bhageloe, A., Weeren, L. van, Schultz, C., Goedhart, J. & Gadella (jr.), T.W.J. (2013). PLCbeta isoforms differ in their subcellular location and their CT-domain dependent interaction with Galphaq. *CELL SIGNAL*, 25(1), 255-263.

Bilan, D.S., Pase, L., Joosen, L., Gorokhovatsky, A.Y., Ermakova, Y.G., Gadella, T.W.J., Grabher, C., Schultz, C., Lukyanov, S. & Belousov, V.V. (2013). HyPer-3: A Genetically Encoded H₂O₂ Probe with Improved Performance for Ratiometric and Fluorescence Lifetime Imaging. *ACS Chem Biol*, 8(3), 535-542.

Chu, B., Postma, M. & Hardie, R.C. (2013). Fractional Ca²⁺ currents through TRP and TRPL channels in *Drosophila* photoreceptors. *Biophysical Journal*, 104(9), 1905-16.

Crosby, K.C., Postma, M., Hink, M.A., Zeelenberg, C.H., Adjobo-Hermans, M.J. & Gadella, T.W. (2013). Quantitative analysis of self-association and mobility of annexin A4 at the plasma membrane. *Biophysical Journal*, 104(9), 1875-85.

Gillis, J., Schipper-Krom, S., Juenemann, K., Gruber, A., Coolen, S., Nieuwendijk, R. van den, Veen, H. van, Overkleeft, H., Goedhart, J., Kampinga, H.H. & Reits, E.A. (2013). The DNAJB6 and DNAJB8 protein chaperones prevent intracellular aggregation of polyglutamine peptides. *The Journal of Biological Chemistry*, 288(24), 17225-17237.

Goedhart, J., Unen, J. van, Adjobo-Hermans, M.J. & Gadella, T.W.J. (2013). Signaling efficiency of Galphaq through its effectors p63RhoGEF and GEFT depends on their subcellular location. *Scientific Reports*, 3, 2284.

Hink, M.A. & Postma, M. (2013). Monitoring receptor oligomerization by line-scan fluorescence cross-correlation spectroscopy. *Methods in Cell Biology*, 117, 197-212.

Lestini, R., Laptinok, S.P., Kuhn, J., Hink, M.A., Schanne-Klein, M.C., Liebl, U. & Myllykallio, H. (2013). Intracellular dynamics of archaeal FANCM homologue Hef in response to halted DNA replication. *Nucleic Acids Research*, 41(22), 10358-10370.

Lim, X., Tan, S.H., Koh, W.L., Chau, R.M., Yan, K.S., Kuo, C.J., Amerongen, R. van, Klein, A.M. & Nusse, R. (2013). Interfollicular epidermal stem cells self-renew via autocrine Wnt signaling. *Science*, 342(6163), 1226-1230.

Luca, G.M. De, Breedijk, R.M., Brandt, R.A., Zeelenberg, C.H., Jong, B.E. de, Timmermans, W., Azar, L.N., Hoebe, R.A., Stallinga, S. & Manders, E.M. (2013). Re-scan confocal microscopy: scanning twice for better resolution. *Biomedical optics express*, 4(11), 2644-2656.

Pandey, R., Beek, A. ter, Vischer, N.O., Smelt, J.P.P.M., Brul, S. & Manders, E.M.M. (2013). Live cell imaging of germination and outgrowth of individual *Bacillus subtilis* spores; the effect of heat stress quantitatively analyzed with SporeTracker. *PLoS One*, 8(3), e58972.

Pietraszewska-Bogiel, A., Lefebvre, B., Koini, A.M., Klaus-Heisen, D., Takken, F.L.W., Geurts, R., Cullimore, J.V. & Gadella, Th.W.J. (2013). Interaction of *Medicago truncatula* Lysin Motif Receptor-Like Kinases, NFP and LYK3, Produced in *Nicotiana benthamiana* Induces Defence-Like Responses. *PLoS One*, 8(6), e65055.

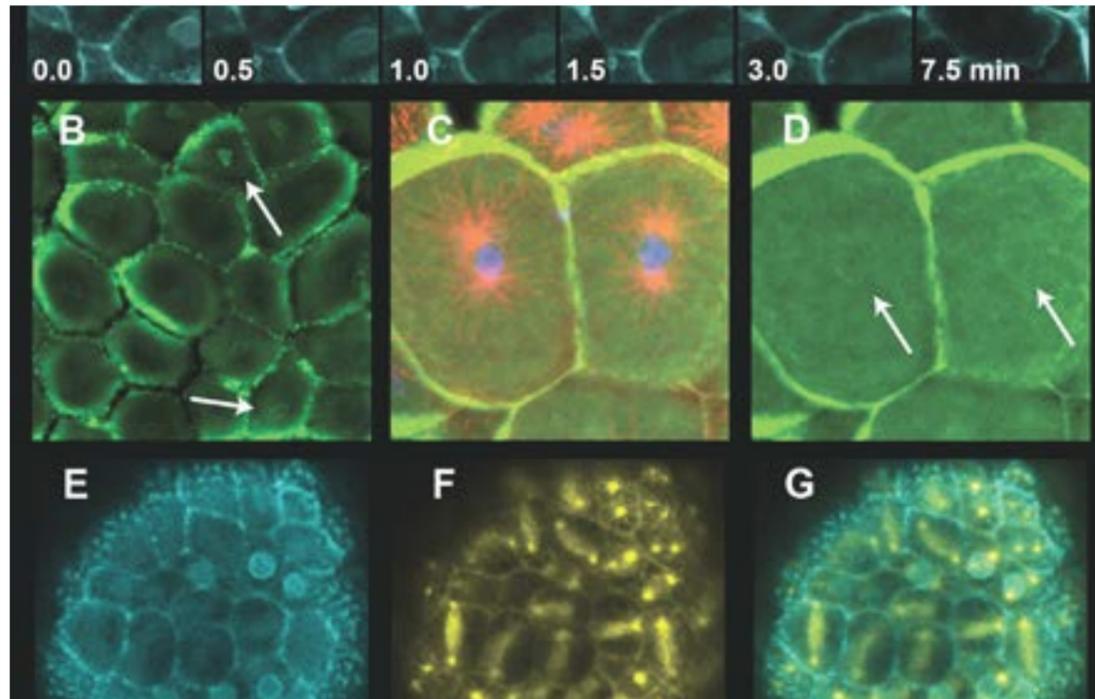
Ploeg, R. van der, Verheul, J., Vischer, N.O., Alexeeva, S., Hoogendoorn, E., Postma, M., Banzhaf, M., Vollmer, W. & Blaauwen, T. den (2013). Colocalization and interaction between elongasome and divisome during a preparative cell division phase in *Escherichia coli*. *Molecular Microbiology*, 87(5), 1074-87.

Verhoeven, G.S., Dogterom, M. & Blaauwen, T. den (2013). Absence of long-range diffusion of OmpA in *E. coli* is not caused by its peptidoglycan binding domain. *BMC Microbiology*, 13, 66.

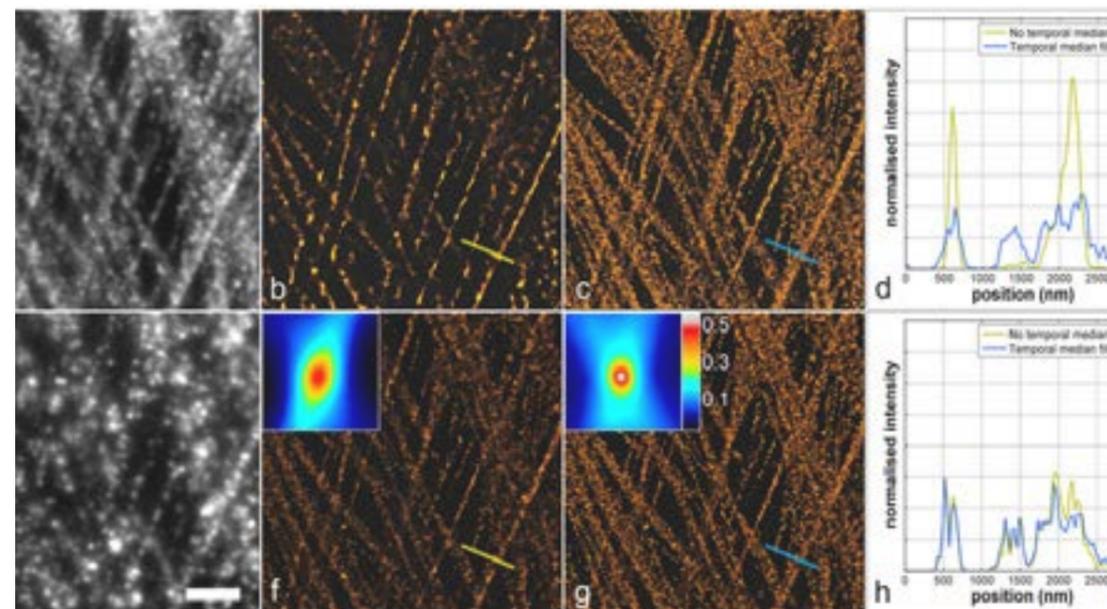
Phd theses

Lam, B.D. (01 March 2013). *Ready, set? Go! Microscopy and image analysis of cell signalling in cell migration*. Universiteit van Amsterdam (135 pag.). Prom./coprom.: prof.dr. P.L. Hordijk.

Timmerman, E. (03 July 2013). *Dynamic control of VE-cadherin-based junctions*. Universiteit van Amsterdam (164 pag.). Prom./coprom.: prof.dr. P.L. Hordijk & J.D. van Buul.



A) Lifeact-mTurquoise2 protein during cell division. B) Preserved embryos labelled with phalloidin-FL show various levels of nuclear f-actin staining. C-D) Preserved cells labelled with phalloidin-FL (green), Hoechst (blue), alpha-tubulin (red). E-G) Lifeact-mTurquoise2 localized to cell boundaries and around the nucleus at multicellular stage. EB1-mVenus reveals tubulin reveal spindle fibers of dividing cells in the same embryo during mitosis.



GSDIM imaging of myosinIIa independently labeled with Alexa532 (a-c) and Alexa647 (e-g). Without the utilization of the temporal median filter, the RapidSTORM reconstruction of the Alexa532 data set shows localizations that are skewed towards regions of high fluorescence (b) and exhibit poor colocalization with the Alexa647 (f) based on Pearson's cross-correlation analysis (f, inset). Use of the temporal median filter prior to running the localization eliminates these artifacts in the Alexa532 reconstruction (c) and shows higher correlation with the Alexa647 reconstruction (g, inset). Intensity traces (d, h) are normalized to the area under the trace. Scale bar: 3 μ m.

Zoughlami, Y. (25 April 2013). *New dimensions in CXCR4 and Rac1 regulation*. Universiteit van Amsterdam (137 pag.). Prom./coprom.: prof.dr. P.L. Hordijk, P.B. van Hennik & Drs. C. Voermans.

Invited lectures

Amerongen, R. van (01 November 2013). *R. Wnt-responsive stem cells and turnover of the mammary epithelium*. Amsterdam, the Netherlands, Nederlandse Vereniging voor Celbiologie, Trippenhuismeting 2013 'From Stem Cells to Aging'.

De Luca, G., Breedijk, R.M., Stallinga, S. & Manders, E.M.M. (08 September 2013). *Re-scan Confocal Microscopy: scanning twice for better resolution*. Genoa, Italy, Conference on Methods and Applications of Fluorescence, MAF 2013.

Gadella, T.W.J., Haarbosch, L., Bindels, D.S., Goedhart, J., Weeren, L. van, Joosen, L., Adjobo-Hermans, M.J.W. & Hink, M.A. (11 July 2013). *3rd generation fluorescent proteins with enhanced properties for FRET and for monitoring signaling in living cells*. St Petersburg, Russia, 38th FEBS congress.

Gadella, T.W.J., Goedhart, J., Weeren, L. van, Joosen, L., Adjobo-Hermans, M.J.W. & Hink, M.A. (14 July 2013). *Enhanced fluorescent proteins for FRET and for studying signaling across the membrane*. Lisbon, Portugal, 9th European Biophysics Conference.

Gadella, T.W.J. (26 June 2013). *FRET and FLIM*. Copenhagen, Denmark, Advanced Microscopy Course.

Gadella, T.W.J. (26 February 2013). *FRET-probes for FLIM*. University of Amsterdam, the Netherlands, Lambert Instruments FRET-FLIM symposium.

Gadella, T.W.J. (05 April 2013). *New frontiers in advanced fluorescence microscopy & their applications for the study of cellular signalling*. VU Amsterdam, the Netherlands, Keynote lecture 3rd Annual AIMMS meeting.

Gadella, T.W.J., Goedhart, J., Haarbosch, L., Bindels, D.S., Unen, J. van, Reinhard, N.R., Weeren, L. van, Joosen, L., Adjobo-Hermans, M.J.W., Hink, M.A., Hoogendoorn, E. & Postma, M. (06 November 2013). *New probes and (super-resolution) microscopy*

approaches for direct visualization of signaling in living cells. Heidelberg, Germany, EMBL.

Gadella, T.W.J. (30 January 2013). *The microscopy and bioimaging revolution: seeing believing*. AMC, Amsterdam, the Netherlands, department Pathology.

Goedhart, J. (24 March 2013). *An ABC of Fluorescent Proteins*. Maastricht, the Netherlands, Focus on Microscopy.

Goedhart, J. (09 July 2013). *Imaging signal transduction with a new generation of genetically encoded fluorescent probes*. Magdenburg, Germany, Leibniz Institute for Neurobiology.

Hink, M.A. (20 February 2013). *Introduction to Fluorescence Correlation Spectroscopy*. Berlin, Germany, 5th European Short Course on Time-Resolved Microscopy and Correlation Spectroscopy.

Hink, M.A. (23 October 2013). *Probing protein dynamics and interactions by Fluorescence Fluctuation Spectroscopy*. Oulu, Finland, EMBO course on Modern biophysical methods for protein-ligand interactions.

Hink, M.A. (19 March 2013). *Quantitative microscopy in cells using Fluorescence Fluctuation Spectroscopy*. Groningen, the Netherlands, Cellular Imaging Course 2013.

Hink, M.A. (16 May 2013). *Quantitative microscopy in cells using Fluorescence Fluctuation Spectroscopy*. Amsterdam, the Netherlands, Confocal Microscopy Course 2013.

Manders, E.M.M. (11 February 2013). *Improving sensitivity and resolution in confocal microscopy*. TU Delft, the Netherlands, IST-seminar in the Faculty of Applied Physics.

Manders, E.M.M. (26 February 2013). *Improving sensitivity and resolution in confocal microscopy: Advanced Optical Microscopy and Micromanipulation*. Münster, Germany, Symposium at the University of Münster.

Manders, E.M.M. (28 October 2013). *Re-scan Confocal Microscopy: scanning twice for better resolution*. Delft, the Netherlands, Symposium on Frontiers on Nanoscopy, NanoNextNL.

Manders, E.M.M. (03 October 2013). *Super-resolution Microscopy in the Netherlands*. Nieuwegein, Netherlands, STW-jaarcongres 2013.

Relevant positions

Hink, M.A. (2009-2015). Board member for the Dutch Society for Microscopy.

Manders, E.M.M. Board member (treasurer) for the Stichting Focus on Microscopy.

Awards

Amerongen, R. van (2013). Cancer research career award. by KWF kankerbestrijding: Recognition.

Group members

Postdoctoral researcher

Anna Pietraszewska

PhD students

Daphne Bindels

Rick Brandt

Ada Dattoli

Lindsay Haarbosch

Eelco Hoogendoorn

Giulia de Luca

Venkataraman Krishnaswami

Marieke Mastop

Nathalie Reinhard

Jakobus van Unen

Laura van Voorst Vader

Christiaan Zeelenberg

Technicians

Linda Joosen

Laura van Weeren

Amber Zeeman

Engineer

Ronald Breedijk

Synthetic Systems Biology and Nuclear Organisation

Prof. H.V. Westerhoff

Dr. M. Barberis

Dr P.F. Fransz

Dr M.E. Stam

Dr P.J. Verschure

Prof. R. van Driel

Chairholder

Assistant Professor

Assistant Professor

Assistant Professor

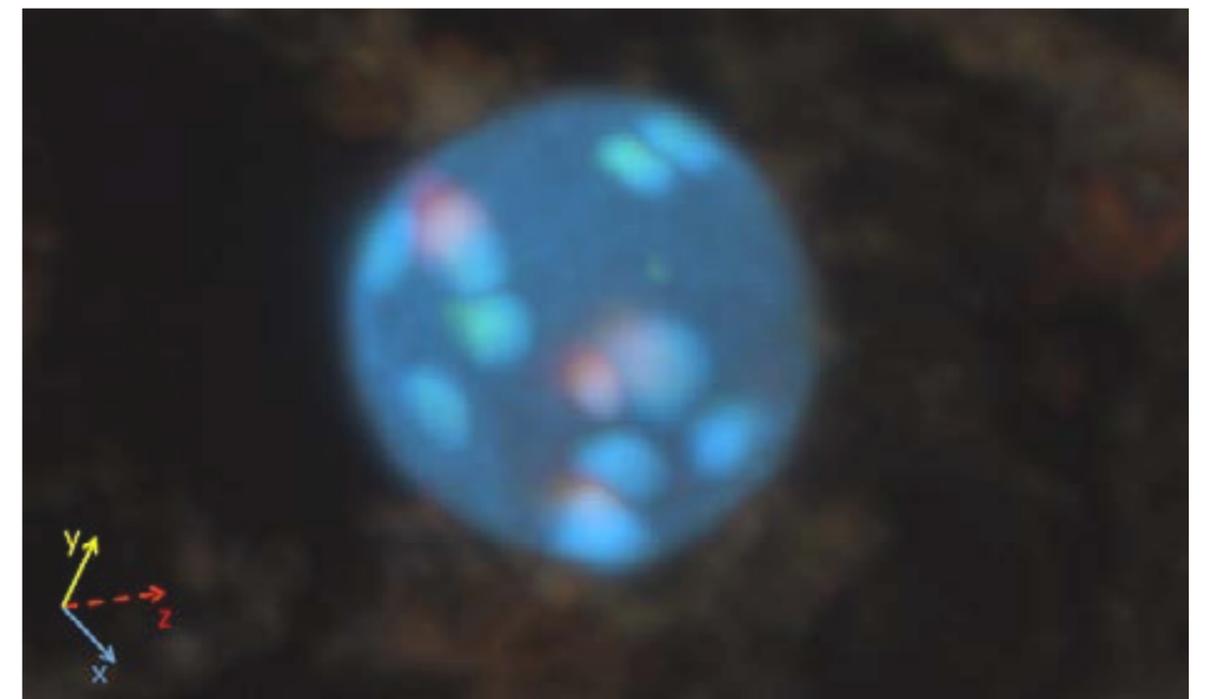
Associate Professor

Emeritus Professor

Systems Biology deciphers how biological functions emerge from interactions, many of which are molecular. Gene regulation is amongst the more germane examples of this. Whilst it controls how cells in living organisms are operating, it is itself controlled by what happens at the workplace of the cell in important processes such as cell cycle, metabolism, and differentiation. This cyclic control involves processes such as epigenetic regulation through intra- and inter-chromosomal contacts, the activation of transcription factors by proteins, signal transduction, and metabolic conversions. These molecular processes are controlled by complex networks of interacting components, which enable the cell to 'decide' which cellular state to commit to and how to respond to

environmental changes. To study these networks, we invoke a multi-disciplinary approach, combining microscopic, biochemical, genetic and molecular analyses, mathematical modelling, and systems theory. Combining information from different model systems helps elucidate principles underpinning the roles that both structural and catalytic interactions play. The group addresses the functional relationships between transcriptional dynamics, nuclear organisation and chromatin structure in metabolism, cell cycle and epigenetic gene regulation. As an embodiment of systems biology the group is associated with research groups at the VU University Amsterdam and the University of Manchester.

3



Confocal microscopical 3-D image of a leaf nucleus from *Arabidopsis thaliana* after fluorescence in situ hybridisation, showing euchromatin (blue-gray), heterochromatin (bright blue), centromere repeats (green) and ribosomal repeats (red).

Research highlights

► One of the 2013 highlights has been the arrival of the research group of Dr Matteo Barberis from the Max Planck Institute for Molecular Genetics and the Humboldt University in Berlin. The group constitutes new bridges between the systems biology of the cell cycle and DNA replication and topics studied in the SSB-NOG group such as metabolism, differentiation, chromatin organization and epigenetics.

► Matteo Barberis and colleagues set up their new lab and established experimental pipelines for studying protein-protein interactions, unravelling a number of unknown interactions between key components of the cell cycle, and examining epigenetic and DNA replication networks in the budding yeast *S. cerevisiae*. They identified a new potential link between metabolism and the cell cycle. They also developed a computational framework for the study of DNA replication dynamics that will be employed to investigate spatiotemporal chromatin dynamics integrating different layers of regulation including metabolism, cell cycle and epigenetics.

► Paul Fransz and colleagues have established 3D-analyses of chromosome 4 in intact organs of Arabidopsis using FISH and immunolabeling. The FISH data are being processed to reconstruct the interphase nucleus. The group has started to set up microscopic tools for single cell analysis of genes involved in flowering time (EpiTRAITS EU-consortium). This includes a protocol for whole-mount immunofluorescence (FISH). In collaboration with M.E. Chabouté from the IBMP CNRS in Strasbourg, aberrant chromosome configurations have been characterized at the nuclear envelope in the *gip* mutant. Continued research of the paracentric inversion in the short arm of chromosome 4 has discovered a number of genomic features with genetic and phylogenetic implications for Arabidopsis ecotypes worldwide, including haplotype formation, historical recombination events, phylogenetic relationship and geographic distribution.

► To study chromatin looping in gene regulation in Arabidopsis and maize, Maïke Stam and co-workers established the chromosome conformation capture-technique 4C in these organisms. In collaboration with Dr Keurentjes (WUR, UvA), Dr Angenent (WUR) and Dr Johannes (RUG) phenotypic screens were performed to determine the role of epigenetic mechanisms in hybrid vigour. For the STW project 'Epigenetics meets targeted mutagenesis' tools and techniques have been established to allow the identification of epigenetic drugs and mutants affecting oligo-directed mutagenesis. Projects have been started in collaboration with partners in the EU-FP7 Marie Curie ITN EpiTRAITS.

► Pernelle Verschure and colleagues established single molecule mRNA counting with single cell volume measurements in human cells. They showed gene location dependent transcription statistics and numbers of transcripts per cell were shown to vary proportional with cell volume. Using engineered mammalian cell systems the Verschure group discovered a function of the epigenetic regulatory protein MeCP2. This finding might impact the pathophysiology of Rett syndrome in which MeCP2 plays an important role. Moreover, engineered living cell assays were set-up to show transcription-coupled/global repair switching in living cells upon UV damage (NWO-ZonMW-TOP project). In a theoretical study, the systems behaviour of cellular reprogramming was captured in gene network motifs.

► Hans Westerhoff and co-workers integrated a control engineering approach that anticipated perfect adaptation in intracellular networks, with their hierarchical control analysis. They concluded that perfect adaptation requires zero-order degradation processes, which are rare. Westerhoff's 'watchmaker modelling approach' was brought to multiscale stage, both for glutathione-mediated drug detoxification and for cortisol/nuclear-hormone-receptor regulation of human stress. The publication of the consensus human metabolic map triggered new insights into how personalized medicine may be put in place and how Nature, Nurture and Lifestyle integrate. An integral understanding of the systems biology of nitrogen assimilation in *E. coli*, prepared together with VUA's Fred Boogerd and the late Wally van Heeswijk, saw the light in an authoritative journal. Together with Westerhoff, Roel van Driel conceived a way to organize the Infrastructure for Systems Biology Europe (ISBE), with prospective Modelling-Integration and Data-Stewardship nodes in Amsterdam.

Research aims for the coming year

► Barberis and colleagues will establish the quantitative relationship between the Substrate Inhibitor of Cyclin-dependent kinase (Sic1), ForKHead (Fkh1 and Fkh2) and SIRtuin (Sir2) proteins in cell cycle phasing and in the exchange of control between cell cycle and metabolism. They will use their mathematical model of the cell cycle to compute whether and how the differential control is distributed over the three cyclin-dependent kinase-defined phases, and where in the network the most powerful controller resides. Deregulation of cell cycle phasing may be crucial in certain diseases.

► Fransz and colleagues will continue to study chromosome folding in different (stress and developmental) conditions in order to understand the relation between 3D-organization and function of the genomic sequence. Whole-mount localization of the FT locus and several chromatin proteins will be examined during the floral transition in the framework of single cell variation of FT activity. Together with M. Stam the position and frequency of meiotic crossovers will be studied in tomato hybrids. In collaboration with F. Barneche from Institut de Biologie de l' Ecole Normale Supérieure (Paris) the Fransz group has started to investigate nuclear changes in germinating seedling under various light conditions.

► Stam and coworkers will deepen the understanding of the functional relationship between gene activity, epigenetic mechanisms and chromosomal interactions. Dr Stam will continue studying the role of chromatin looping in gene regulation in Arabidopsis and maize, the mechanisms underlying paramutation, and if epigenetic mechanisms are involved in hybrid vigour. In addition she focuses on improving targeted mutagenesis by modulating chromatin structure and together with P. Fransz she focuses on the identification of genes and sequences involved in homologous recombination in tomato.

► Verschure and colleagues will continue studying the mechanistic networking principles of functional genome organization, combining gene activity measurements at single cell/single molecule level of endogenous genes with the construction and analysis of designed mammalian epigenetic networks. This approach provides proofs-of-principle for biomedical applications. UV-induced DNA damage/repair will be studied in engineered cell systems to follow dynamic molecular behaviour during transcription-coupled/global repair switching. (Epi)genetic gene control during early disease onset will be examined in an induced Huntington's disease cell system, setting the scene to develop more advanced induced-pluripotency stem cell models (Charity funding Stichting Zeldzame ziekten/Huntington patient association and the Amsterdam University fund).

► Westerhoff and colleagues will further define both modelling-integration and model-driven data management nodes for the ISBE, and pay attention to how these should function in The Netherlands' Systems Biology community. They will also finalize an analysis method for the potentially debilitating effects of hardly-toxic xenobiotics and develop a corresponding new 'mountain drizzle' concept of ageing. An exciting development is the 'downward' extension of systems biology into macromolecular complex kinetics, where they will try to discover modularity in dynamic behaviour. Meanwhile the group is liaising with patient advocate organizations and physicians to improve awareness of the potential of post 2000 systems biology and genomics for society.

Academic publications

Belele, C.L., Sidorenko, L., Stam, M., Bader, R., Arteaga-Vazquez, M.A. & Chandler, V.L. (2013). Specific tandem repeats are sufficient for paramutation-induced trans-generational silencing. *PLoS Genetics*, 9(10), e1003773.

Brink, M.C., Piebes, D.G., Groote, M.L. de, Luijsterburg, M.S., Casas-Delucchi, C.S., Driel, R. van, Rots, M.G., Cardoso, M.C. & Verschure, P.J. (2013). A role for MeCP2 in switching gene activity via chromatin unfolding and HP1gamma displacement. *PLoS One*, 8(7), e69347.

Feldman-Salit, A., Hering, S., Messiha, H.L., Veith, N., Cojocar, V., Sieg, A., Westerhoff, H.V., Kreikemeyer, B., Wade, R.C. & Fiedler, T. (2013). Regulation of the activity of lactate dehydrogenases from four lactic acid bacteria. *The Journal of Biological Chemistry*, 288(29), 21295-306.

Geenen, S., Preez, F.B. du, Snoep, J.L., Foster, A.J., Sarda, S., Kenna, J.G., Wilson, I.D. & Westerhoff, H.V. (2013). Glutathione metabolism modeling: a mechanism for liver drug-robustness and a new biomarker strategy. *Biochimica et Biophysica Acta*, 1830(10), 4943-59.

Geenen, S., Yates, J.W., Kenna, J.G., Bois, F.Y., Wilson, I.D. & Westerhoff, H.V. (2013). Multiscale modelling approach combining a kinetic model of glutathione metabolism with PBPK models of paracetamol and the potential glutathione-depletion biomarkers ophthalmic acid and 5-oxoproline in humans and rats. *Integrative Biology*, 5(6), 877-88.

Kolodkin, A., Simeonidis, E. & Westerhoff, H.V. (2013). Computing life: Add logos to biology and bios to physics. *Progress in Biophysics & Molecular Biology*, 111(2-3), 69-74.

Kolodkin, A., Sahin, N., Phillips, A., Hood, S.R., Bruggeman, F.J., Westerhoff, H.V. & Plant, N. (2013). Optimization of stress response through the nuclear receptor-mediated cortisol signalling network. *Nature Communications*, 4, 1792.

Kouril, T., Esser, D., Kort, J., Westerhoff, H.V., Siebers, B. & Snoep, J.L. (2013). Intermediate instability at high temperature leads to low pathway efficiency for an in vitro reconstituted system of

gluconeogenesis in *Sulfolobus solfataricus*. *The FEBS Journal*, 280(18), 4666-80.

Mensonides, F.I., Bakker, B.M., Cremazy, F., Messiha, H.L., Mendes, P., Boogerd, F.C. & Westerhoff, H.V. (2013). A new regulatory principle for in vivo biochemistry: pleiotropic low affinity regulation by the adenine nucleotides—illustrated for the glycolytic enzymes of *Saccharomyces cerevisiae*. *FEBS Letters*, 587(17), 2860-2867.

Muraro, M.J., Kempe, H. & Verschure, P.J. (2013). Concise review: the dynamics of induced pluripotency and its behavior captured in gene network motifs. *STEM CELLS*, 31(5), 838-48.

Pietraszewska-Bogiel, A., Lefebvre, B., Koini, A.M., Klaus-Heisen, D., Takken, F.L.W., Geurts, R., Cullimore, J.V. & Gadella, Th.W.J. (2013). Interaction of *Medicago truncatula* Lysin Motif Receptor-Like Kinases, NFP and LYK3, Produced in *Nicotiana benthamiana* Induces Defence-Like Responses. *PLoS One*, 8(6), e65055.

Quinton-Tulloch, M.J., Bruggeman, F.J., Snoep, J.L. & Westerhoff, H.V. (2013). Trade-off of dynamic fragility but not of robustness in metabolic pathways in silico. *The FEBS Journal*, 280(1), 160-73.

Smallbone, K., Messiha, H.L., Carroll, K.M., Winder, C.L., Malys, N., Dunn, W.B., Murabito, E., Swainston, N., Dada, J.O., Khan, F., Pir, P., Simeonidis, E., Spasic, I., Wishart, J., Weichart, D., Hayes, N.W., Jameson, D., Broomhead, D.S., Oliver, S.G., Gaskell, S.J., McCarthy, J.E., Paton, N.W., Westerhoff, H.V., Kell, D.B. & Mendes, P. (2013). A model of yeast glycolysis based on a consistent kinetic characterisation of all its enzymes. *FEBS Letters*, 587(17), 2832-2841.

Thiele, I., Swainston, N., Fleming, R.M., Hoppe, A., Sahoo, S., Aurich, M.K., Haraldsdottir, H., Mo, M.L., Rolfsson, O., Stobbe, M.D., Thorleifsson, S.G., Agren, R., Bolling, C., Bordel, S., Chavali, A.K., Dobson, P., Dunn, W.B., Endler, L., Hala, D., Hucka, M., Hull, D., Jameson, D., Jamshidi, N., Jonsson, J.J., Juty, N., Keating, S., Nookaew, I., Novere, N. Le, Malys, N., Mazein, A., Papin, J.A., Price, N.D., Selkov E, S.r., Sigurdsson, M.I., Simeonidis, E., Sonnenschein, N., Smallbone, K., Sorokin, A., Beek, J.H. van, Weichart, D., Goryanin, I., Nielsen, J., Westerhoff, H.V., Kell, D.B., Mendes,

P. & Palsson, B.O. (2013). A community-driven global reconstruction of human metabolism. *Nature Biotechnology*, 31(5), 419-25.

Van Heeswijk, WC, Westerhoff, H.V., Boogerd, FC (2013) *Microbiol. Mol. Biol. Rev.*: 77 (2013), 628 – 695. Nitrogen Assimilation in *Escherichia coli*: *Putting Molecular Data into a Systems Perspective*.

Verma, M., Zakhartsev, M., Reuss, M. & Westerhoff, H.V. (2013). ‘Domino’ systems biology and the ‘A’ of ATP. *Biochimica et Biophysica Acta - Bioenergetics*, 1827(1), 19-29.

Verma, M., Karimiani, E.G., Byers, R.J., Rehman, S., Westerhoff, H.V. & Day, P.J. (2013). Mathematical modelling of miRNA mediated BCR.ABL protein regulation in chronic myeloid leukaemia vis-a-vis therapeutic strategies. *Integrative Biology*, 5(3), 543-54.

Book chapter

Zanten, M. van, Tessadori, F.G., Peeters, A.J.M. & Franz, P.F. (2013). Environment-Induced Chromatin Reorganisation and Plant Acclimation. In G. Grafi & N. Ohad (Eds.), *Epigenetic Memory and Control in Plants (Signaling and Communication in Plants, volume 8)* (pp. 21-40-IX). Berlin Heidelberg: Springer-Verlag.

Phd thesis

Groote, M.L. de (06 February 2013). *Potentials and pitfalls of epigenetic editing in gene-specific re-expression of epigenetically silenced genes*. Rijksuniversiteit Groningen (184 pag.) (Groningen: University Library Groningen). Prom./coprom.: dr. M.G. Rots & dr. P.J. Verschure.

Contribution magazine or newspaper

Verschure, P.J. (24 May 2013). ‘Simulaties ontrafelen genetische wirwar’ door Eveline Thoenes. *C2W (Chemisch2Weekblad)*, pp. 21, jg109, volume 9.

Driel, R. van (26 April 2013). Gigantische geldsommen. *C2W Life Sciences*, pp. 5-5.

Westerhoff, H.V. (04 March 2013). ‘Routeplanner’ menselijk lichaam brengt revolutie teweeg in geneeskunde. *de Volkskrant*.

Invited lectures

Franz, P.F. (23 April 2013). *Genetic, epigenetic, evolutionary and population dynamic consequences of an inversion in Arabidopsis*. Karlsruhe, Germany, Karlsruhe Institute of Technology (KIT). Seminar Series Plant Molecular Biology and Biochemistry.

Franz, P.F. (29 May 2013). *Identification, origin, evolutionary history and population dynamics of an inversion in Arabidopsis thaliana*. Gatersleben, Germany, Gatersleben, Institut für Pflanzengenetik und Kulturpflanzenforschung, Arbeitstagung der GPZ-AG Cytogenetik.

Franz, P.F. (13 December 2013). *A multidisciplinary view on an inversion in Arabidopsis thaliana*. Wageningen, the Netherlands, EPS Theme 4 Symposium Genome Plasticity.

Franz, P.F. (17 December 2013). *What are the consequences of a paracentric inversion? A multidisciplinary view*. Fijnaart, the Netherlands. Seminars Series Rijkzwaan.

Stam, M.E. (26 June 2013). *Gene regulation by epigenetics and chromosomal interaction: bi paramutation*. Paris-Sud, France, UMR de Génétique Végétale (INRA, Université Paris-Sud, CNRS) -invited by Dr. Clémentine Vitte.

Stam, M.E. (12 March 2013). *Gene regulation by epigenetics and chromosomal interaction: bi paramutation*. University of Padova, Padova, Workshop Environmental Epigenetics (AEnEAS Final Meeting).

Verschure, P.J. (04 September 2013). *Dynamic and stochastic epigenetic state switching studied in epigenetic engineered mammalian cell systems*. Bologna, Italy, 19th International Chromosome Conference.

Verschure, P.J. (01 May 2013). *Epigenetic state switching in higher eukaryotes: Engineered mammalian cell systems and stochastic modeling*. Langen, Germany, Paul Erlich Institute, hosted by Prof. Zoltan Ivics (Head of the division of Medical Biotechnology).

Verschure, P.J. (25 June 2013). *Epigenetic state switching in higher eukaryotes: Engineered mammalian cell systems and stochastic modeling*. Radboud University Nijmegen, the Netherlands, Chemistry of Life -

Engineering Biology (NBV Werkgroependag and the KNCV spring event).

Verschure, P.J. (19 November 2013). *Epigenetic state switching: Engineered mammalian cell systems and stochasticity*. Moscow, Russia, M.V.Lomonosov Moscow State University, A.N.Belozersky Institute of Physico-Chemical Biology, Guest of Prof. Igor Kireev (head of the Electron Microscopy Dept).

Verschure, P.J. (19 September 2013). *The dynamics and stochasticity of epigenetic state switching studied in engineered mammalian cell systems*. Budapest, Hungary, Conference on Transposition and Genome Engineering 2013.

Westerhoff, H.V. (19 April 2013). *Keynote Arts en Voeding congres*, Haarlem.

Westerhoff H.V. (24 July 2013). International Union of Physiological Sciences: *The Future of Medicine: putting the individual where it should be*. Birmingham

Westerhoff H.V. (30 September 2013). Keynote on Dutch Medicines Day (FIGON): *Mapping individualized medicines*.

Journal-refereeship

Barberis, M. (ed) *Frontiers in Biology*.

Fransz, P.F. member of the Editorial Advisory Board of *Chromosome Research*.

Fransz, P.F. member of the Editorial Advisory Board of *Frontiers in Plant Genetics and Genomics*.

Verschure, P.J. (ed.). *Frontiers in Bioengineering and Biotechnology*.

Verschure, P.J. (ed.). *The Journal of Histochemistry and Cytochemistry*.

Westerhoff, H.V. (ed). Molecular Systems Biology, Biochimica Biophysica Acta General Subjects, *Frontiers in Biology*.

Relevant positions

Stam, M.E. External expert at EU AEnEAS FP7 collaborative project.

Stam, M.E. Coordinator at Marie Curie ITN EpiTRAITS.

Verschure, P.J. member of the committee at NWO-ALW 'Investeren in Middelgroot.'

Verschure, P.J. Chief of the executive board at Women in the Faculty (WiF) network.

Westerhoff, H.V. Advisory Board MIP-DILI (IMI project).

Van Driel. Chair Infrastructure Systems Biology Europe-Nodes.

Westerhoff, H.V. Chair Infrastructure Systems Biology Europe-Connections.

Other

Verschure, P.J. (2013). *de toekomst van Synthetic Biology*. BE-Basic consortium - Athena Institute, VUA: Amsterdam, the Netherlands.

Media performance

Westerhoff, H.V. (guest) (04 March 2013). De voltooiing van de biochemische routekaart (television broadcast). *Pauw en Witteman*.

Westerhoff, H.V. (guest) (04 March 2013). *RTL-4 Evening news*.

Westerhoff, H.V. (guest) (04 March 2013). Radio programme 'Dit is de dag'.

Westerhoff, H.V. (guest) (09 March 2013). *Radio 3FM Stenders Late*.

Westerhoff, H.V. (guest) (25 March 2013). *Radio 5 OBA Live*; with Theo Holman.

Group members

Postdoctoral researcher

Esther de Boer
Christian Linke
Mariliis Tark
Paul Verbruggen

PhD students

Till Bey
Iris Hövel
Mannus Kempe
Maria-Anastasia Koini
Kathrin Lauss
Ton Muller (guest)
Diewertje Piebes
Ilona Vuist
Blaise Weber

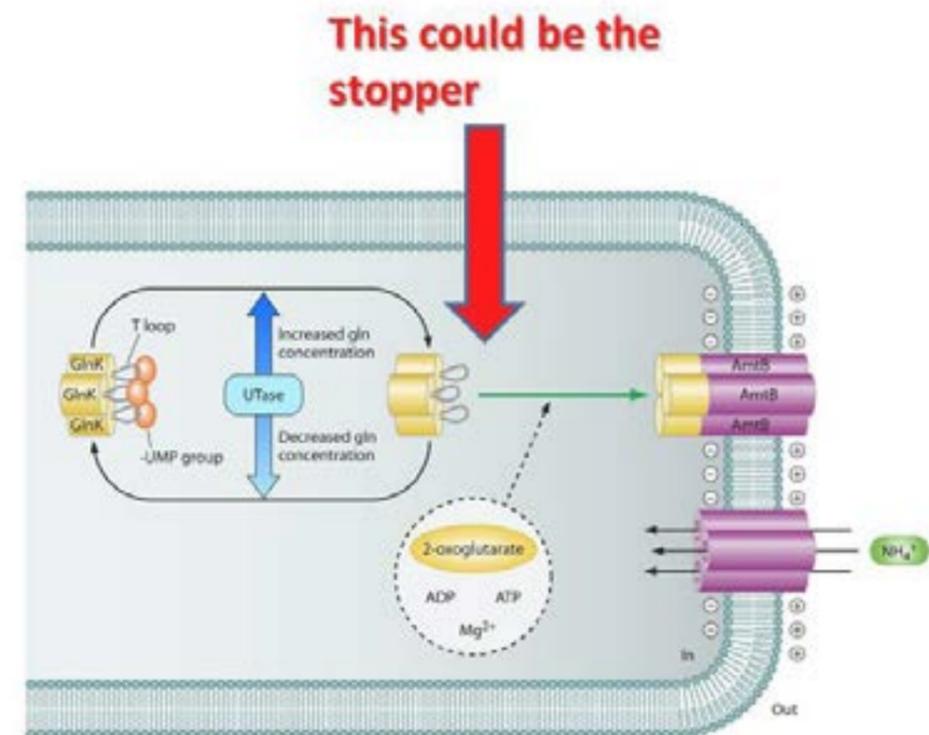
Group members (continued)

Technicians

Damar Anggoro
Rechien Bader
José Kiewiet

Project manager

Frans van Nieuwpoort



Biosystems Data Analysis

Prof. A.K. Smilde
 Dr H.C.J. Hoefsloot
 Dr J.A. Westerhuis
 Dr. G. Zwanenburg
 Prof. A.H.C. van Kampen

Chairholder
 Associate Professor
 Assistant Professor
 Assistant Professor
 Special Chair Medical Bioinformatics

General goal: developing and validating methods for organizing and summarizing complex biological data. The research is divided into two connected themes: Data Fusion and Networks & Dynamics. We apply our methods in diverse areas of systems biology, focusing mainly on microbiology, nutrition and medical biology.

Data Fusion

To understand the functionality of complex biological systems, different types of measurements have to be combined with systems information.

Networks & Dynamics

In a biological system molecules interact. These interactions, the network, causes the system to change over time. We develop methods to analyze and model data from time-resolved functional genomics data in a network context.

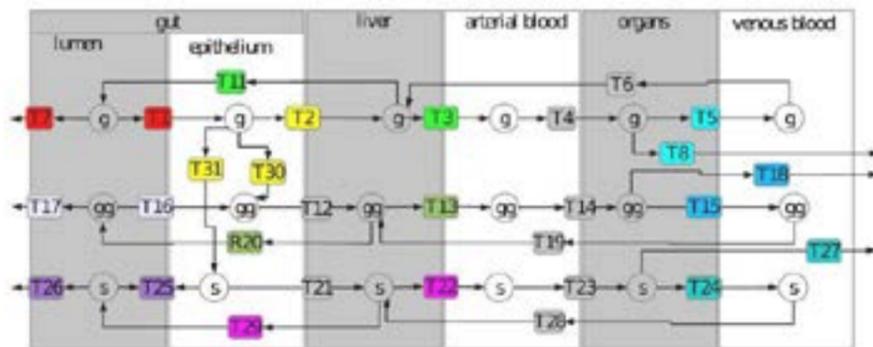


Figure of a PetriNet of the excretion pathway of Genistein.

Research highlights

- ▶ As of June 2013, A.K. Smilde is appointed as a part-time professor of Computational Systems Biology at the University of Copenhagen.
- ▶ In EU-project STATegra, the fusion of metabolomics data of different platforms is almost finished. Two papers will be submitted early 2014 on the fusion of metabolomics data for explorative and classification analysis. These papers describe different approaches to deal with the heteroscedastic measurement error that is observed in metabolomics data.
- ▶ In 2012, J.A. Westerhuis was appointed as extraordinary professor at North-West University in Potchefstroom, South Africa. The acute effect of alcohol abuse study was conducted and metabolomics analysis of the blood samples of 24 volunteers was started.
- ▶ In the collaboration with GSK and the KU Leuven progress has been made in identifying synergistic effects of vaccin-adjuvants. A paper is being prepared.
- ▶ A paper has been published on protein complex prediction from IP/MS data. The method that was developed proved to work well also for very large data sets. To obtain information of protein interactions at different levels the method was further developed to work in a hierarchical way.
- ▶ Resampling methods are extensively used in data analysis, but prove to be useful also in systems biology for exploring optimal model complexity. The paper describing this approach will be submitted in early 2014.
- ▶ Challenge tests are gaining popularity in medical and nutritional research. In collaboration with Unilever, strategies for individual phenotyping of linoleic and arachidonic acid metabolism by means of reaction parameters obtained from an oral glucose tolerance test were developed. A paper will be submitted in the beginning of 2014.
- ▶ Association networks are a popular tool to visualize and analyze metabolomics data. However, it is shown that correlated measurement error can severely hamper the reliability of such networks. A paper on this topic is submitted.
- ▶ Dynamic Flux Balance Analysis has been adapted to incorporate metabolite concentration profiles. This approach gives fluxes similar to an alternative approach based on ¹³C mass isotopomer measurements. Publication will be submitted early 2014.
- ▶ In collaboration with DSM, a hybrid Petri Net was built to determine the relative contribution of routes in the Genistein excretion pathway based on metabolite profiles from a nutritional study (see Figure). A publication will be submitted early 2014.

Research aims for the coming year

► Data Fusion

► For STATegra, we will write a conceptual paper that describes the use of figures of merit in various omics technologies. For the integration of different omics data sets it is important to have an idea of the quality of each of these sets. However the quality is defined in different ways in the various omics technologies. Some figures of merit focus on the technological aspects of the data while others refer to the identification or reproducibility of the data. In this paper we aim to describe the different aspects of quality in such data sets to improve their integration. A next step is to integrate metabolomics with RNA-seq data and with proteomics data obtained from B-cell differentiation experiments. New data fusion methods will be developed for the integration of these omics data sets.

► The metabolomics measurements of the acute alcohol abuse study in South Africa will be finalized. Two papers are in progress, one on the experimental design and measurement design issues involved in the study, and a second paper on the combination of bioinformatics and metabolomics in South Africa.

► Fusing data of different omics technologies will also be the next step in the collaboration with GSK and the KU Leuven. A conceptual paper about this topic – also including clinical measurements – is in progress.

► Networks & Dynamics

► A paper on hierarchical protein complex prediction is foreseen in the spring of 2014. The method that was developed is going to be tested on AMPA receptor data that was obtained at the lab of prof. A.B. Smit (VU). A review will be written on protein complex prediction and the rest of the year will be focused on finishing the thesis.

► Since the work on resampling for systems biology models gave such nice results, a review will be written on this topic in collaboration with prof. J. Snoep (University of Stellenbosch, South-Africa). The remainder of the year will be devoted to finishing the thesis.

► T and B cell immune responses can be modelled by differential equations and facilitate a better understanding of underlying biological processes, and to predict immune responses when changing parameters of the system. Several models, comprising various parts of the immune response have been developed in the past. We aim to extend these models in the context of (auto)immune disorders. These models will be integrated with experimental data such as RNAseq experiments for these lymphocytes.

► We developed a protocol to quantitatively measure the B cell repertoire (B cell receptors) with RNAseq (Next Generation Sequencing). This allows studying the process of antigen driven B cell selection (affinity maturation) in greater detail. From the RNAseq data it is possible to reconstruct lineage trees that reflect the affinity maturation process which involves B cell proliferation, somatic hypermutation and selection. To interpret the lineage trees we aim to integrate a mathematical model of affinity maturation (differential equations and simulated of B cell proliferation/mutation) with trees obtained from experimental data.

Academic publications

Abeln, S., Molenaar, D., Feenstra, K.A., Hoefsloot, H.C.J., Teusink, B. & Heringa, J. (2013). Bioinformatics and Systems Biology: bridging the gap between heterogeneous student backgrounds. *Briefings in Bioinformatics*, 14(5), 589-598.

Christin, C., Hoefsloot, H.C.J., Smilde, A.K., Hoekman, B., Suits, F., Bischoff, R. & Horvatovich, P. (2013). A critical assessment of feature selection methods for biomarker discovery in clinical proteomics. *Molecular & Cellular Proteomics*, 12(1), 263-276.

Cobos Jimenez, V., Bradley, E.J., Willemsen, A.M., Kampen, A.H.C. van, Baas, F. & Kootstra, N.A. (2013). Next-generation sequencing of microRNAs uncovers expression signatures in polarized macrophages. *Physiological Genomics*.

Doorenspleet, M.E., Klarenbeek, P.L., Hair, M.J.H. de, Schaik, B.D.C. van, Esveldt, R.E.E., Kampen, A.H.C. van, Gerlag, D.M., Musters, A., Baas, F., Tak, P.P. & Vries, N. de (2013). Rheumatoid arthritis synovial tissue harbours dominant B-cell and plasma-cell clones associated with autoreactivity. *Annals of the Rheumatic Diseases*, 73, 756-762.

González-Martínez, J.M., Westerhuis, J.A. & Ferrer, A. (2013). Using warping information for batch process monitoring and fault classification. *Chemometrics and Intelligent Laboratory Systems*, 127, 210-217.

Kutzer, J., Hoefsloot, H.C., Malovannaya, A., Smit, A.B., Mechelen, I. van & Smilde, A.K. (2013). Inferring protein inverted question mark protein interaction complexes from immunoprecipitation data. *BMC Research Notes*, 6(1), 468.

Madougou, S., Shahand, S., Santcroos, M., Schaik, B. van, Benabdelkader, A., Kampen, A.H.C. van & Olabarriaga, S. (2013). Characterizing workflow-based activity on a production e-infrastructure using provenance data. *Future Generation Computer Systems*, 29, 1931-1942.

Stobbe, M.D., Swertz, M.A., Thiele, I., Rengaw, T., Kampen, A.H.C. van & Moerland, P.D. (2013). Consensus and conflict cards for metabolic pathway databases. *BMC Systems Biology*, 7, 50.

Book chapter

Hoefsloot, H.C.J. (2013). Statistical Analysis and validation. In P. Horvatovich & R. Bischoff (Eds.), *Comprehensive Biomarker Discovery and Validation for Clinical Application*.

Smilde, A.K., Hendriks, M.M.W.B., Westerhuis, J.A. & Hoefsloot, H.C.J. (2013). Data Processing in Metabolomics. In *Metabolomics in Practice: Successful Strategies to Generate and Analyze Metabolic Data* (pp. 261-284). Wiley-VCH Verlag GmbH & Co. KGaA.

PhD thesis

Hendrickx, D.M. (18 April 2013). *Network inference from time-resolved metabolomics data*. Universiteit van Amsterdam (vi, 176 pag.). Prom./coprom.: prof.dr. A.K. Smilde, dr.ir. H.C.J. Hoefsloot & M.M.W.B. Hendriks.

Invited lectures

Kampen, A.H.C. van (18 June 2013). *Sequencing of the T-cell repertoire in Rheumatoid Arthritis*. London, United Kingdom, Next Generation Sequencing Data Congress.

Smilde, A.K. (10 June 2013). *Analyzing metabolomics data: univariate, multivariate or simplivariate?* Amiens, France, French Metabolomic and Fluxomic Network activities.

Smilde, A.K. (27 April 2013). *Data fusion in human systems biology*. Leiden, The Netherlands, KNAW International Conference on Data Fusion.

Westerhuis, J.A., Korobko, O.B., Waaijenborg, S., Batenburg, M.F. van & Smilde, A.K. (18 September 2013). *Towards maximum likelihood fusion of multiple platform metabolomics data*. Modena, Italy, CIADAG 2013.

Editorships

Van Kampen, A.H.C. Editorial board *Advances in Bioinformatics*.

Smilde, A.K. *Journal of Chemometrics*.

Westerhuis, J.A. *Metabolomics*.

Relevant positions

Van Kampen, A.H.C. Scientific Advisory Board, Graduate school Chemical Biology (KoRS-CB), University of Konstanz, Germany (September 2009 –).

Smilde, A. Scientific Advisory Board of the EU-project Mimomics.

Smilde, A. Part-time professor at the AMC

Smilde, A. Part time professor at the University of Copenhagen.

Awards

Westerhuis, J.A., Velzen, E.J.J. van, Hoefsloot, H.C.J. & Smilde, A.K. (2013). The 2013 Highest Download Award of Metabolomics Journal was given to the paper entitled 'Multivariate paired data analysis: multilevel PLSDA versus OPLSDA.' Recognition.

Westerhuis, J.A., Hoefsloot, H.C.J., Smit, S., Vis, D.J., Smilde, A.K., Velzen, E.J.J. van, Duijnhoven, J.P.M. van & Dorsten, F.A. van (2013). The Pre-2013 Best Paper Award recognising the most cited paper in Metabolomics Journal was given to 'Assessment of PLSDA cross validation.' Recognition.

Group members*Postdoctoral researcher*

Oksana Korobko
Edoardo Saccenti
Sandra Waaijenborg

PhD students

Dicle Hasdemir
Joachim Kutzera
Polina Reshetova

Technicians

Ishtiaq Ahmad

RNA Biology & Applied Bioinformatics

Dr. T.M. Breit

Dr. M.J. Jonker

Dr. R.J. Dekker

Dr. J. Rauwerda

Dr. E.J. Hoekstra/ Drs. S.M. van Leeuwen

Groupleader

Research line 'Transcriptome dynamics'

Research line 'RNA as genetic information carrier'

Research line 'Applied bioinformatics'

Support: MAD; Dutch Genomics Service & Support Provider

In recent years RNA has increasingly gained attention of life sciences researchers as a key regulator of molecular processes in living organisms. This renewed interest was mainly sparked by the discovery of miRNA and other important non-coding RNAs, as well as the development of transcriptomics technologies such as microarray technology and next-generation sequencing (NGS).

The importance of RNA is underlined by the fact that it is estimated that over 70% of the human genome is transcribed into RNA, while less than 2% of the total genomic sequences encode the ~25,000 protein-coding genes. This is evidence for a major role of RNA in many cellular processes, such as gene-expression regulation, epigenetic regulation, splicing, signal transduction, molecular transport and localization.

To gain insight in the regulatory roles of RNA, our research focuses on the characterization of maternal RNAs and cell-free RNAs combined with research to the dynamics of these transcriptome components. As we have our own NGS system, we will also try to discover new types of RNA.

This kind of research demands the use of advanced omics technologies and associated bioinformatics. Since omics and bioinformatics are still immature, we also do applied bioinformatics research on how to optimally design omics experiments, analyze data, and interpret results.

There are three research and one support lines:

Research line: Transcriptome dynamics

Although being the focus of much research worldwide, the temporal and spatial dynamics of the transcriptome are still largely unknown. The primary challenges here are the complexity of the transcriptome and the limitations of omics experimentation. For this we focus on two topics: Gene expression and degradation regulation and Non-coding RNA.

Research line: RNA as genetic information carrier
Though often ignored, most organisms inherit more RNA than DNA from their parents. It is unclear whether this RNA has any role in the transfer of genetic information. The same holds for cell-free or extracellular RNA. Just their (abundant) presence in eggs and for instance breast milk, respectively, hints on an intriguing role of RNA in development and evolution by transfer of genetic information between cells, organs and individuals. Here we focus on two topics: Maternal RNA and Cell-free RNA.

Research line: Applied bioinformatics
Bioinformatics is an interdisciplinary life-sciences domain that develops methods and software tools for storing, retrieving, organizing, analyzing, and interpreting biological data. Although often perceived as a pure technical domain, based on mathematics, informatics and computer sciences, an important bioinformatics subdomain deals with applied research into improving life sciences research by using appropriate experimental approaches. With the introduction of big data by the omics technologies, it becomes increasingly clear that only research approaches that cover the whole research chain from optimal design for experimentation to adequate computer infrastructure are effective. Also, the overwhelming complexity of biological systems demands not only a more collaborative as well as interdisciplinary approach, it also means that the experimental approaches in life sciences are drastically transforming. As such, applied bioinformatics deals with topics such as, design-for-experimentation, multidisciplinary collaboration, data and knowledge integration, advanced visualization of results, e-science methods, and so on. The aim here remains to improve biological knowledge, which renders applied bioinformatics an enabling science. We focus on three topics: Design for experimentation, Data-analysis workflows and Data-interpretation environments.

Support Line: MAD: Dutch Genomics Service & Support Provider (www.dutchgenomics.nl) Previously known as MicroArray Department, the MAD is the genomics and transcriptomics expertise center of the UvA. The MAD consists of a genomics technology section (Wet-Lab) with ~5 specialists that provides transcriptomics plus genomics service & support and performs microarray as well as next-generation sequencing technology R&D; a omics data-analysis section (Dry-Lab) with ~4 bioinformaticians and informaticians that provides omics data analysis

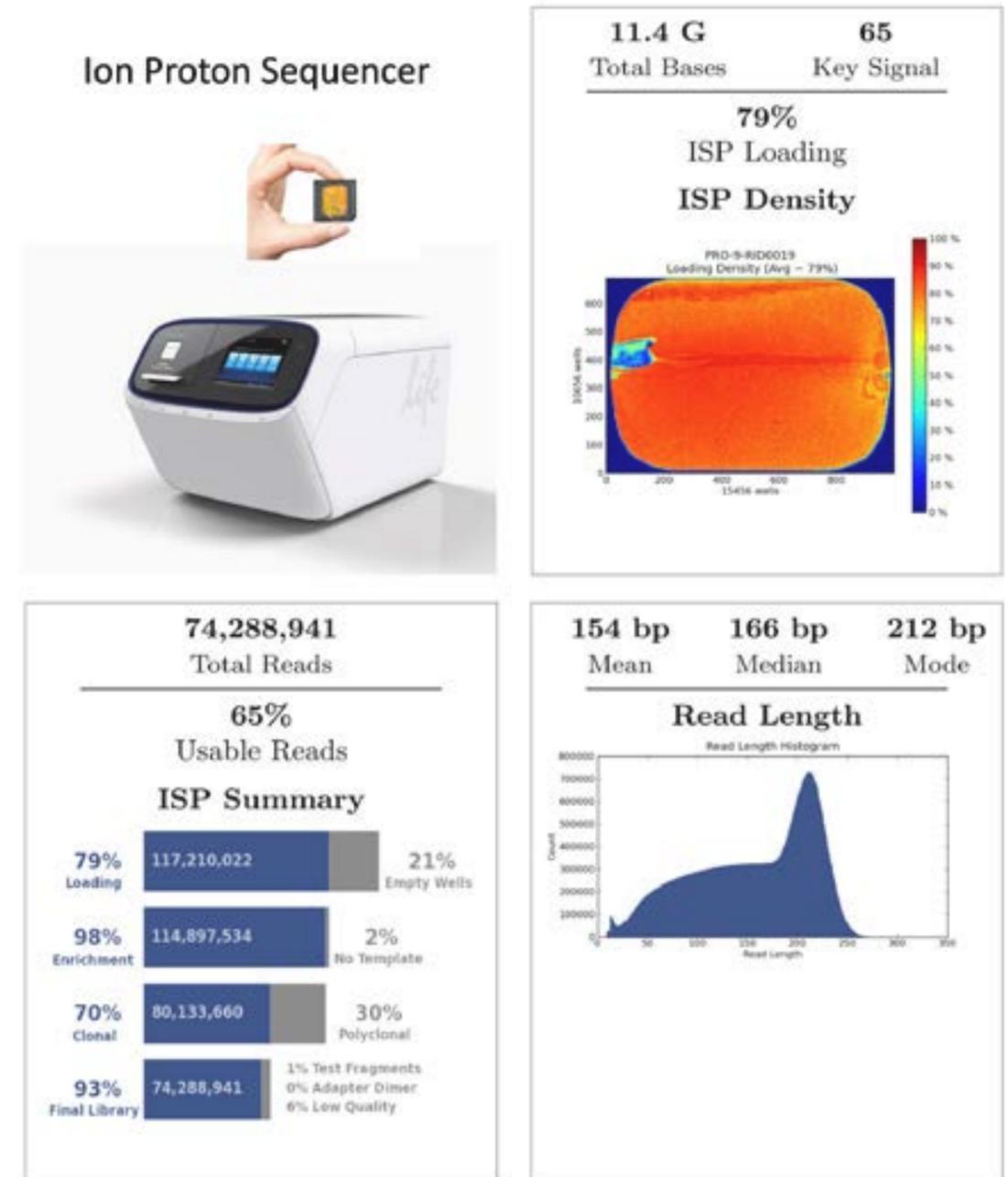
service and support, performs bioinformatics R&D, and builds e-infra. We perform omics (microarray Agilent & Affymetrix & next-generation sequencing IoProton) technology research as well as operate as worldwide transcriptomics service provider and support partner for academic and industrial research & development projects of UvA scientists plus external academic and industrial customers.

Research highlights

- ▶ IonProton technology set up for next-generation genome sequencing, RNAseq and smallRNAseq.
- ▶ The first whole prokaryote (*Bacillus subtilis*) and eukaryote (Tomato) genomes were sequenced.
- ▶ Two sets of spike-in controls for smallRNAseq were developed and tested.
- ▶ Several plants and mammal smallRNA samples were successfully sequenced.
- ▶ An extensive transcriptome study on murine aging was published and acknowledged as important by invitation for a 'Perspective' by another journal on aging.
- ▶ Analysis completed on dense-time course transcriptome analysis of Zebrafish embryogenesis.
- ▶ Next-generation sequencing data for oocyte small RNAs obtained.
- ▶ First maternal RNA experiment on individual Zebrafish oocytes finished.
- ▶ MAD has performed over 40 support projects for biologists in 2013.
- ▶ Publication of several collaborative manuscripts.
- ▶ MAD was awarded three DTL Hotel projects, two with SILS researchers, one with TNO.
- ▶ A NWO Cloud computing grant was awarded.

Other highlight

In 2013 the RB&AB research group was established and became considerably involved in the genomics and bioinformatics education of bachelor and master students. A grant was awarded by the City of Amsterdam for the set-up of an innovative educational environment for master students: the Green Student Lab.



Picture of the next-generation Ion Proton sequencer (Life Technologies) plus the hand holding a sequencing chip with 140 million pores. The other images show the output of a 4 hours DNA sequencing run, with a total output of 11,4 Gbases from over 74 million short reads of about 212 base pairs long.

Research aims for the coming year

- ▶ To obtain and implement the 3rd generation sequencing platform: Oxford Nanopore.
- ▶ To upgrade the IonProton to 50Gbases/run.
- ▶ To create all necessary next-generation sequencing bioinformatics pipelines.
- ▶ To support as many research groups as possible with NGS technology.
- ▶ To set-up a marker approach for plant breeding with low-coverage NGS technology.
- ▶ To achieve a proof of principle for plant identification via NGS technology (Green Forensics).
- ▶ To produce a PhD thesis on design for omics experimentation.
- ▶ To produce a PhD thesis on transcriptome dynamics.
- ▶ To analyse maternal RNAs in unfertilised Zebrafish and human eggs.
- ▶ To perform a hallmark transcriptomics study on p53 function in DNA damage.
- ▶ To produce >5 own and >10 collaborative research articles.
- ▶ To start a Genomics & Bioinformatics Support Group for the Amsterdam Faculty of Science.

Group members

Postdoctoral researcher

Irene Nooren
Rick Orij
Inez Terpstra

PhD students

Oscar Bruning
Mauro Locati

Technicians

Willem Ensink
Ilse van Leeuwen
Marina van Olst
Paul Wackers

ICT-developers

Linda Bakker - de Jong
Wim de Leeuw

Academic publications

Jonker, M.J., Melis, J.P.M., Kuiper, R.V., Hoeven, T.V. van der, Wackers, P.F.K., Robinson, J., Horst, G.T.J. van der, Dollé, M.E.T., Vijg, J., Breit, T.M., Hoeijmakers, J.H.J. & Steeg, H. van (2013). Life spanning murine gene expression profiles in relation to chronological and pathological aging in multiple organs. *Aging Cell*, 12(5), 901-909.

de Knecht, G.J., Bruning O., ten Kate MT, de Jong M, van Belkum A, Endtz H, Breit TM, Bakker-Woudenberg IAAJM, de Steenwinkel JEM (2013). Rifampicin-induced transcriptome response in rifampicin-resistant *Mycobacterium tuberculosis*. *Tuberculosis*, 93(1), 96-101.

Melis, J.P., Jonker, M.J., Vijg, J., Hoeijmakers, J.H., Breit, T.M. & Steeg, H. van (2013). Aging on a different scale - chronological versus pathology-related aging. *Aging (Albany NY)*, 5(10), 782-8.

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Surdova, K., Gamba, P., Claessen, D., Siersma, T., Jonker, M.J., Errington, J. & Hamoen, L.W. (2013). The Conserved DNA-Binding Protein WhiA Is Involved in Cell Division in *Bacillus subtilis*. *Journal of Bacteriology*, 195(24), 5450-60.

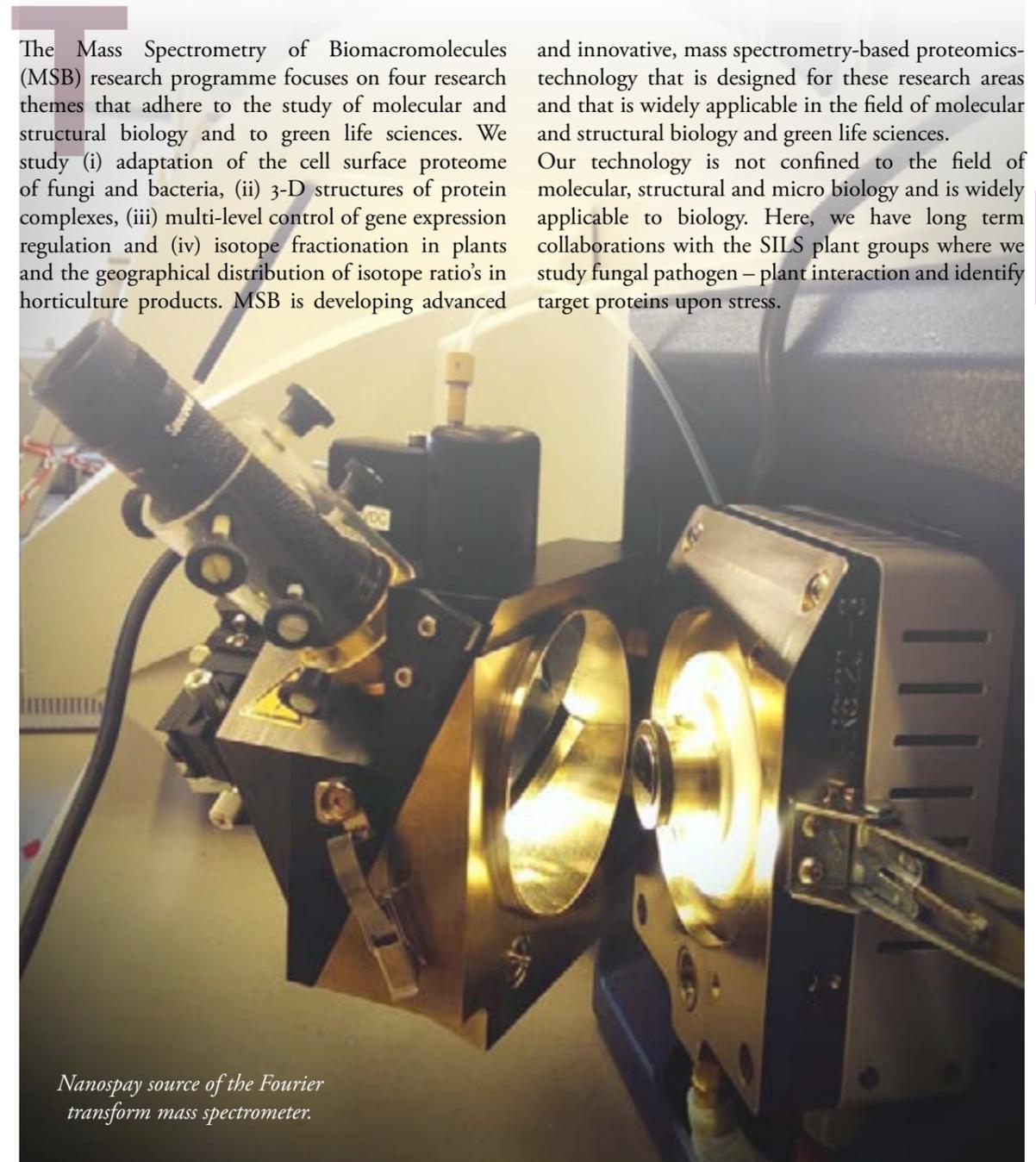
Mass Spectrometry of Biomacromolecules

Prof. dr. C.G. de Koster
Dr L.J. de Koning

Chairholder
Assistant Professor

The Mass Spectrometry of Biomacromolecules (MSB) research programme focuses on four research themes that adhere to the study of molecular and structural biology and to green life sciences. We study (i) adaptation of the cell surface proteome of fungi and bacteria, (ii) 3-D structures of protein complexes, (iii) multi-level control of gene expression regulation and (iv) isotope fractionation in plants and the geographical distribution of isotope ratios in horticulture products. MSB is developing advanced

and innovative, mass spectrometry-based proteomics-technology that is designed for these research areas and that is widely applicable in the field of molecular and structural biology and green life sciences. Our technology is not confined to the field of molecular, structural and micro biology and is widely applicable to biology. Here, we have long term collaborations with the SILS plant groups where we study fungal pathogen – plant interaction and identify target proteins upon stress.



Nanospay source of the Fourier transform mass spectrometer.

Research highlights

- ▶ *Bacillus cereus*, responsible for food poisoning and *Clostridium difficile*, causative agent of *Clostridium difficile*-associated diarrhoea (CDAD) are both spore forming pathogens involved in food spoilage, food intoxication and other infections in humans and animals.
- ▶ The proteinaceous coat and the exosporium layers from spores are important for their resistance and pathogenicity characteristics.
- ▶ The exosporium additionally provides an ability to adhere to surfaces eventually leading to spore survival in food. Thus studying these layers and identifying suitable protein targets for rapid detection and removal of spores is of utmost importance. In this study, we identified 100 proteins from *B. cereus* spore coat, exosporium and 54 proteins from the *C. difficile* coat insoluble protein fraction. In an attempt to define a universal set of spore outer layer proteins we identified 11 superfamily domains common to the identified proteins from two Bacilli and a *Clostridium* species. The evaluated orthologue relationships of identified proteins across different spore formers revealed a set of 13 coat proteins conserved across the spore formers and 12 exosporium proteins conserved in the *B. cereus* group. These protein sets could be tested for quick and easy detection or targeted in strategies aimed at removal of spores from surfaces.

Group members

Postdoctoral researcher

Eva de Rijke

PhD students

Wishwas Abhyankar
 Orawan Borirak
 Hansuk Buncherd
 Sacha Stelder
 Linli Zheng

Technicians

Henk Dekker
 Han Peeters
 Winfried Roseboom

Retired but still active personnel

Dr L. de Jong, Associate Professor

Research progress

▶ In course of 2013 we finalized the EC FINSysB project. In this project we quantitatively map the cell wall and secretome proteome of the human pathogen *Candida albicans*. We focus on the understanding at the molecular level of the surface cell wall response upon stress with the aim to identify new leads for novel anti-*Candida* vaccines, drugs and diagnostic markers. *Candida albicans* secretes a considerable number of proteins that are involved in biofilm formation, tissue invasion, immune evasion, and wall maintenance, as well as acquisition of nutrients including metal ions. The secretome of *C. albicans* is predicted to comprise 225 proteins. On a proteomic level, however, analysis of the secretome of *C. albicans* is incomplete as many secreted proteins are only produced under certain conditions. Interestingly, glycosyl-phosphatidylinositol proteins and known cytoplasmic proteins are also consistently detected in the growth medium. Importantly, a core set of seven wall polysaccharide-processing enzymes seems to be consistently present, including the diagnostic marker Mp65. We studied the importance of the secretome for virulence and reported potential targets for better and faster diagnostic methods.

▶ *Candida albicans* can grow at temperatures of up to 45°C. We showed that at 42°C substantially less biomass was formed than at 37°C. The cells also became more sensitive to wall-perturbing compounds, and the wall chitin levels increased, changes that are indicative of wall stress. Quantitative mass spectrometry of the wall proteome using ¹⁵N metabolically labeled wall proteins as internal standards revealed that at 42°C the levels of the β-glucan transglycosylases Phr1 and Phr2, the predicted chitin transglycosylases Crh11 and Utr2, and the wall maintenance protein Ecm33 increased. Consistent with our previous results for fluconazole stress, this suggests that a wall-remodeling response is mounted to relieve wall stress. Thermal stress as well as different wall and membrane stressors led to an increased phosphorylation of the mitogen-activated protein (MAP) kinase Mkc1, suggesting activation of the cell wall integrity (CWI) pathway. Furthermore, all wall and membrane stresses tested resulted in diminished cell separation. This was accompanied by decreased secretion of the major chitinase Cht3 and the endoglucanase Eng1 into the medium. Consistent with this, cht3 cells showed a similar phenotype. When treated with exogenous chitinase, cell clusters both from stressed cells and mutant strains were dispersed, underlining the importance of Cht3 for cell separation. We proposed that surface stresses lead to a conserved cell wall remodeling response that is mainly governed by Mkc1 and is characterized by chitin reinforcement of the wall and the expression of remedial wall remodeling enzymes.

▶ We have cross-linked for the first time the proteins of a HeLa cell nuclear extract. We have shown show that the use of bis(succinimidyl)-3-azidomethyl glutarate to cross-link proximate lysine residues provides a solution for two major analytical problems of cross-link mapping by peptide fragment fingerprinting (PFF) from complex sequence databases, i.e. low abundance of protease-generating target peptides and lack of knowledge of the masses of linked peptides. The relations between the sum of the masses of the cleavage products and the mass of the cross-linked peptide enables determination of the masses of candidate linked peptides with add of two software tools we have developed that support PFF from the entire human sequence database in single LC-MS/MS runs. We identified 272 intraprotein and 25 interprotein cross-links in a HeLa cell nuclear extract at a false discovery rate of 0.3%.

▶ The molecular physiology of carbon catabolite repression (CCR) in the model organism *Escherichia coli* is studied together with Hellingwerf (SILS-Molecular Microbial Physiology). A glucose-limited chemostat culture was used to create a CCR-free reference condition followed by an activation of CCR via an addition of a pulse of glucose that saturates the cells for at least one hour. During this hour we have accurately proteome-wide monitored the changes in the protein levels using a ¹⁵N metabolic-labelling quantification method. The proteomic studies were complemented with transcript analyses using micro arrays, to directly identify genes under transcriptional and post-transcriptional regulation. Novel dedicated statistical analysis to correlate the complex time resolved transcriptomics and proteomics datasets was developed (SILS-Biosystems Data Analysis).

Research aims for the coming year

► Focal points in our MS research programme are as mentioned above (i) adaptation of the cell surface proteome of fungi and bacteria, (ii) 3-D structures of protein complexes, (iii) multi-level control of gene expression regulation and (iv) isotope fractionation in plants and the geographical distribution of isotope ratios in horticulture products. In programme (i) we will continue to explore the question how mass spectrometry, in combination with novel purification strategies and bioinformatics tools, can provide detailed quantitative structural and functional information about proteins at the interface between the cell and its external environment. In collaboration with Prof. Brul, we aim at identification of the hitherto unknown germination receptors of *Clostridium difficile* spores. In a preliminary study to develop methodology and technologies to map spore inner membrane proteins we identified various *B. subtilis* integral membrane proteins including the known germination receptors GerAB, GerBA, GerKA and the DPA transporters SpoVAC and SpoVAF. In the framework of a Nanonext project we will extend our spore coat proteomics work to biomarker identification on the surface of *B. cereus* and *B. weihenstephanensis* spores. For this purpose we will sequence and annotate the *B. weihenstephanensis* genome following a proteogenomics strategy. To corroborate genome annotation we will acquire a complete proteome dataset of the psychrotolerant sporeformer *B. weihenstephanensis* grown and sporulated under warm and cold growing conditions. Heat resistance is developed in a late stage of sporulation and during maturation of released spores. We will use our gel-free spore proteomics technology and LC-FT-ICR-MS/MS analysis to probe the efficiency of the tryptic digestion of proteins in spore outer layers during spore maturation, using metabolically ¹⁵N labeled mature spores as reference to obtain insight in the development of heat resistance. In programme (ii) we will explore in vivo cross-linking of the *B. subtilis* proteome with our cross-linker BAMG. We will continue the productive collaborations with the groups of the SILS systems biology (iii) and plant programme, directed to characterization of proteins secreted by the cell upon host-pathogen interactions, and with our external national and international partners.

► In the scope of the project origin tracking and tracing of horticulture products through stable isotope analysis (iv) we will develop mass spectrometry methods to identify the geographic origin of horticulture products and propagation materials. The project is part of the Amsterdam research programme Green Forensics. We collect stable isotope data and extract geographical fingerprints of important horticulture products such as belly peppers at various production sites in Europe and Africa. We store the isotope data and geographical fingerprints in databases. Our mass spectrometry methods and isotope databases enable producers to provide objective, reliable data in a controlled chain on origin, production method, transport, authenticity, content and security of the product.

Academic publications

Abhyankar, W., Hossain, A.H., Djajasaputra, A., Permpoonpattana, P., Beek, A. ter, Dekker, H.L., Cutting, S.M., Brul, S., Koning, L.J. de & Koster, C.G. de (2013). In pursuit of protein targets: proteomic characterization of bacterial spore outer layers. *Journal of Proteome Research*, 12(10), 4507-21.

Heilmann, C.J., Sorgo, A.G., Mohammadi, S., Sosinska, G.J., Koster, C.G. de, Brul, S., Koning,

L.J. de & Klis, F.M. (2013). Surface stress induces a conserved cell wall stress response in the pathogenic fungus *Candida albicans*. *Eukaryotic Cell*, 12(2), 254-264.

Liu, Y. Solis, N.V., Heilmann, C.J., Phan, Q.T., Mithcell, A.P., Klis, F.M. & S.G. Filler. (2013). *Role of Retrograde Trafficking in Stress Response, Host Cell Interactions, and Virulence of Candida albicans*. *Eukaryotic Cell*.

McLoughlin, F., Arisz, S.A., Dekker, H.L., Kramer, G., Koster, C.G. de, Haring, M.A., Munnik, T. & Testerink, C. (2013). Identification of novel candidate phosphatidic acid-binding proteins involved in the salt-stress response of *Arabidopsis thaliana* roots. *Biochemical Journal*, 450(3), 573-581.

Sorgo, A.G., Heilmann, C.J., Brul, S., Koster, C.G. de & Klis, F.M. (2013). Beyond the wall: *Candida albicans* secret(e)s to survive. *FEMS Microbiology Letters*, 338(1), 10-17.

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Phd theses

Buncherd, H. (02 December 2013). *Development of enrichment methods for cross-linked peptides to study the dynamic topology of large protein complexes by mass spectrometry*. Universiteit van Amsterdam. Prom./coprom.: prof.dr. C.G. de Koster & dr. L. de Jong.

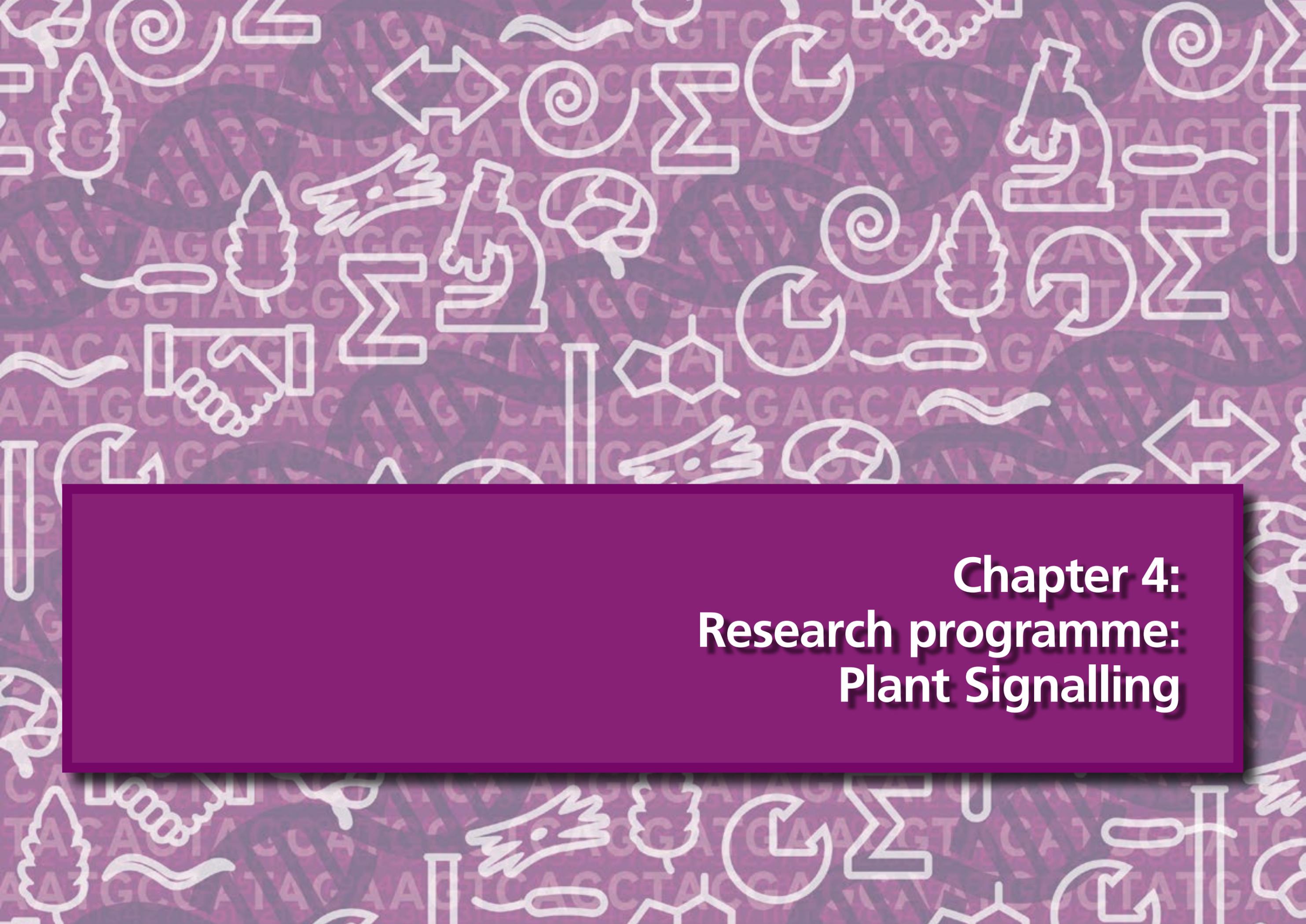
Heilmann, C.J. (15 February 2013). *Mass spectrometric quantification of Candida albicans surface proteins to identify new diagnostic markers and targets for vaccine development*. Prom./coprom.: prof.dr. C.G. de Koster, prof.dr. S. Brul, dr. F.M. Klis & dr. L.J. de Koning.

Sorgo, A.G. (22 February 2013). *Infection related stress adaptations in the secretome and wall proteome of Candida albicans*. Universiteit van Amsterdam (186 pag.). Prom./coprom.: prof. dr. S. Brul, prof. dr. C.G. de Koster, dr. F.M. Klis & dr. L.J. de Koning.

Invited lectures

Koster, C.G. de (22 November 2013). *Novel Enrichment methods of cross-linked peptides to study the dynamic topology of large protein complexes by mass spectrometry*. Prague, Czech Republic, 3rd Symposium on Structural Proteomics.

Heilmann, C.J. (15 February 2013). *Mass spectrometric quantification of Candida albicans surface proteins to identify new diagnostic markers and targets for vaccine development*. Universiteit van Amsterdam (180 pag.). Prom./coprom.: prof.dr. C.G. de Koster, prof.dr. S. Brul, dr. F.M. Klis & dr. L.J. de Koning.

The background features a repeating pattern of DNA base pairs (A, T, C, G) and various scientific icons such as a microscope, a leaf, a brain, a chemical structure, and a handshake, all rendered in white outlines on a purple background.

**Chapter 4:
Research programme:
Plant Signalling**

Plant Physiology

Prof. M.A. Haring
Dr C. Testerink
Dr ir R.C. Schuurink
Dr T. Munnik

Chairholder
Assistant Professor
Associate Professor
Associate Professor

The section Plant Physiology is interested in understanding the molecular mechanisms by which stress signalling and development occurs, on the cellular and whole plant level. Three groups are active. One of the groups focuses on phospholipid signalling. In particular, on the role of phosphatidic acid (PA) and polyphosphoinositides (PPIs) in the model plant system, *Arabidopsis thaliana*. To functionally characterise their role in stress and development, knockout lines of various genes encoding lipid kinases, phosphatases and phospholipases are used, including PIK (12), PIPK (11), PLC (9), DGK (7) and PLD (12). A second group aims to elucidate how plants can adjust and optimize their root system architecture (RSA) to deal with drought or high salinity of the soil. In this

framework, we study the role of intracellular signalling pathways, including lipid signalling and protein kinase pathways, in linking salinity to root development and direction of root growth. The other research group focuses on plant volatile signalling. This includes: 1) the scent of *Petunia* flowers, where the biosynthesis of volatile benzenoid and phenylpropanoids and the transcription factor network involved is being studied; 2) the biosynthesis of terpenes in tomato trichomes, proven to be important for plant-insect interactions, and the transcription factor network involved is being studied; 3) the role of green leaf volatiles in plant pathogen, plant-plant and plant-insect interactions. We currently focus on the isomerisation of Z-3-hexenal, which is an important cue for bodyguard insects.



Research highlights

► Phospholipid signalling research: salt stress triggers the formation of two lipid second messengers: phosphatidic acid (PA), which is generated via phospholipase D (PLD)- and/or diacylglycerol kinase (DGK) pathways, and PIP₂, which is generated through phosphatidylinositol 4-phosphate 5-kinase (PIP₅K) activation. Both lipids are important second messengers in eukaryotes, functioning as membrane-localised docking sites for various protein targets. Using a genetically encoded-PIP₂ biosensor, i.e. a fusion of a PIP₂-specific lipid-binding domain with a fluorescent protein (FP; e.g. YFP) expressed in *Arabidopsis* seedlings and tobacco BY-2 cells, we found that salt stress triggers PIP₂ formation at the plasma membrane within minutes. For PA, no such probe is available, so we have started developing one using different PA-binding regions. Using T-DNA insertion KO-lines, two PLD, two PIK and two PIPK genes have been identified that are involved in the salt stress response. Microarray experiments and physiological assays are being used to understand their role in salt stress tolerance. Meanwhile, we found that cold stress triggers a PA response within minutes, which was found to be completely generated via the DGK pathway (Arisz et al., 2013).

► How plant roots avoid salt: tropisms represent fascinating examples of how plants respond to environmental signals such as light and gravity, by adapting their growth and development. Recently, we have discovered that plants use a similar mechanism, which we call halotropism, to avoid excess salt (Galvan-Ampudia et al., 2013). *Arabidopsis*, tomato, and sorghum roots were found to actively prioritize growth away from salinity gradient assembled in agar media or soil, above following the gravity axis. Directionality of this response is established by an active redistribution of the plant hormone auxin in the root tip, which is mediated by the PIN-FORMED 2 (PIN2) auxin efflux carrier. We show that salt-induced phospholipase D activity stimulates clathrin-mediated endocytosis of PIN2 at the side of the root facing the higher salt concentration. Interestingly, in an independent biochemical screen for PA-binding proteins, several components of the clathrin machinery were identified (McLoughlin et al., 2013). The intracellular relocation of PIN2 allows for auxin redistribution and for the directional bending of the root away from the higher salt concentration. Our results thus identify a cellular pathway essential for the integration of environmental cues with auxin-regulated root growth that likely plays a key role in plant adaptive responses to salt stress (Galvan-Ampudia et al., 2013). That the PA-generating PLD enzyme is also implicated in gravity, water and osmotic-stress responses suggests that its fluctuations may serve to integrate multiple environmental signals to optimize root growth in response to changing conditions.

► Plant volatiles research: in our efforts to elucidate the roles of Green leaf volatiles (GLVs) in plants, we have purified a (Z₃):(E₂)-enal isomerase from *Manduca sexta*'s oral secretion and cloned the corresponding cDNA. This will enable us to modify the green leaf volatile profile of plants and to determine the consequences of this for the plant and caterpillar.

► For our ongoing investigations on the role of terpenes, produced by glandular trichomes of tomato, we have made transgenic plants expression two transcription factors (TFs) that can transactivate various terpene synthase promoters in a transient assay in *Nicotiana benthamiana*. These TFs are driven by glandular trichome specific promoters and our objective is to determine their effect on the terpenes produced and emitted.

Research highlights (continued)

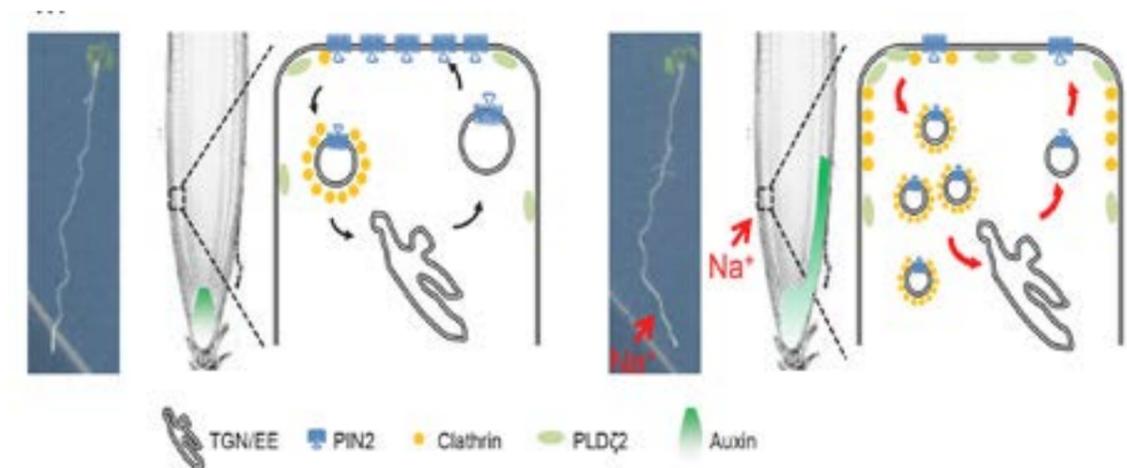
- ▶ *Petunia hybrida* is our model of choice to study volatile benzenoid and phenylpropanoid synthesis, emission and regulation. Our aim is to identify the remaining unknown steps in the biosynthetic pathway and the transcriptional network underlying the regulation of the
- ▶ Biosynthesis of these volatiles. We have identified a new enzyme in the biosynthetic pathway that upon silencing resulted in colored (red) plants. We thus unexpectedly hit on the colorful side of floral scent in *Petunia*, since under normal conditions both scent and color (anthocyanin) pathways are active at different developmental stages of the flower. This indicates that there is a (hidden) metabolic interconnection between color and scent production.
- ▶ In a relative new project we are trying to identify, together with the group of Merijn Kant (IBED), effectors of spider mites that manipulate the direct and indirect defenses of plants. Since some spider mites are well capable of manipulating these defenses, we hypothesize that this is done via effectors in their saliva. For this we take a transcriptomics approach followed by transient overexpression of candidates in various plants.

Research aims for the coming year

- ▶ Determine the role of *Manduca sexta*'s isomerase for the caterpillar and plant.
- ▶ To elucidate the role of lipid signalling in salt stress and development in *Arabidopsis*.
- ▶ Elucidate the role of vesicle transport in salt sensing and avoidance.
- ▶ Characterize genes underlying natural variation in *Arabidopsis* accessions for salt tolerance.
- ▶ Validate Lipid-FP biosensors for DAG and PA.
- ▶ Elucidation of upstream and downstream targets of SnRK2 protein kinases.
- ▶ Characterization of salt-induced changes in RSA and the halotropic response of crop plants.
- ▶ Develop mathematical models to describe root development and architecture.
- ▶ Characterize transgenic plants overexpressing transcription factors driving terpene synthases.
- ▶ Characterize the effect of spider mite effectors on plants.

Other highlights

- ▶ 2013 NWO Topsector (R. Schuurink): Whitefly effectors and their targets, 246.000 euro.
- ▶ 2013 EU ERA-CAPS (R. Schuurink): Homeostasis of Isoprenoids in Plants: understanding compartmentalization, flux and transport of isoprenoids in glandular trichomes for non-crop and crop species, 251.000 euro.
- ▶ 2013 STW Perspectief (M. Haring and R. Schuurink): Green defence Against Pests (GAP) 537.000 euro.
- ▶ 2013, 21 juni NWO-STW Vidi (P. Bleeker): Defence in the wild; from trichome transcriptomes and metabolomes to breeding tools for defence markers in tomato, 800.000 euro.



BYPASS THE SALT: Proposed model for the molecular and cellular mechanism regulating halotropism. Salt stress induces PLDζ₂-dependent recruitment of clathrin to the PM and PIN₂ internalization and recycling (thick red arrows) at one side of the root, causing differential auxin accumulation (green) and bending of the root away from the salt source. TGN/EE, trans-Golgi network/early endosomes.

Academic publications

McLoughlin, F. & Testerink, C. (2013). Phosphatidic acid, a versatile water-stress signal in roots. *Frontiers in Plant Science*, 4, 525.

Allmann, S., Späthe, A., Bisch-Knaden, S., Kallenbach, M., Reinecke, A., Sachse, S., Baldwin, I.T. & Hansson, B.S. (2013). Feeding-induced rearrangement of green leaf volatiles reduces moth oviposition. *eLife*, 2.

Arisz, S.A. & Munnik, T. (2013). Distinguishing phosphatidic acid pools from de novo synthesis, PLD, and DGK. *Methods in Molecular Biology*, 1009, 55-62.

Arisz, S.A., Wijk, R. van, Roels, W., Zhu, J.K., Haring, M.A. & Munnik, T. (2013). Rapid phosphatidic acid accumulation in response to low temperature stress in Arabidopsis is generated through diacylglycerol kinase. *Frontiers in Plant Science*, 4(January), 1.

Arisz, S.A. & Munnik, T. (2013). Use of phospholipase A2 for the production of lysophospholipids. *Methods in Molecular Biology*, 1009, 63-68.

Galvan-Ampudia, C.S., Julkowska, M.M., Darwish, E., Gandullo, J., Korver, R.A., Brunoud, G., Haring, M.A., Munnik, T., Vernoux, T. & Testerink, C. (2013). Halotropism is a response of plant roots to avoid a saline environment. *Current Biology*, 23(20), 2044-50.

Julkowska, M.M., Rankenberg, J.M. & Testerink, C. (2013). Liposome-binding assays to assess specificity and affinity of phospholipid-protein interactions. *Methods in Molecular Biology*, 1009, 261-71.

McLoughlin, F., Arisz, S.A., Dekker, H.L., Kramer, G., Koster, C.G. de, Haring, M.A., Munnik, T. & Testerink, C. (2013). Identification of novel candidate phosphatidic acid-binding proteins involved in the salt-stress response of Arabidopsis thaliana roots. *Biochemical Journal*, 450(3), 573-581.

McLoughlin, F. & Testerink, C. (2013). Lipid affinity beads: from identifying new lipid binding

proteins to assessing their binding properties. *Methods in Molecular Biology*, 1009, 273-280.

Munnik, T. (2013). Analysis of d3-,4-,5-phosphorylated phosphoinositides using HPLC. *Methods in Molecular Biology*, 1009, 17-24.

Munnik, T. & Zarza, X. (2013). Analyzing Plant Signaling Phospholipids Through (32)P i-Labeling and TLC. *Methods in Molecular Biology*, 1009, 3-15.

Munnik, T. & Wierchowicka, M. (2013). Lipid-binding analysis using a fat blot assay. *Methods in Molecular Biology*, 1009, 253-259.

Munnik, T. & Laxalt, A.M. (2013). Measuring PLD Activity In Vivo. *Methods in Molecular Biology*, 1009, 219-31.

Scala, A., Mirabella, R., Mugo, C., Matsui, K., Haring, M.A. & Schuurink, R.C. (2013). E-2-hexenal promotes susceptibility to Pseudomonas syringae by activating jasmonic acid pathways in Arabidopsis. *Frontiers in Plant Science*, 4, 74.

Scala, A., Allmann, S., Mirabella, R., Haring, M.A. & Schuurink, R.C. (2013). Green leaf volatiles: a plant's multifunctional weapon against herbivores and pathogens. *International Journal of Molecular Sciences*, 14(9), 17781-811.

Spyropoulou, E.A., Haring, M.A. & Schuurink, R.C. (2013). Expression of Terpenoids 1, a glandular trichome-specific transcription factor from tomato that activates the terpene synthase 5 promoter. *Plant Molecular Biology*.

Vermeer, J.E. & Munnik, T. (2013). Using genetically encoded fluorescent reporters to image lipid signalling in living plants. *Methods in Molecular Biology*, 1009, 283-289.

Book chapter

Schie, C.C.N. van, Haring, M.A. & Schuurink, R.C. (2013). *Prenyldiphosphate Synthases and Gibberellin Biosynthesis*. In T.J. Bach & M. Rohmer (Eds.), *Isoprenoid Synthesis in Plants and Microorganisms: New Concepts and Experimental*

Approaches (pp. 212-232-chapter 15). New York: Springer Science+Business Media.

Editorial book

Munnik, T. & Heilmann, C.J. (2013). *Plant Lipid Signaling Protocols (Methods in Molecular Biology, 1009)*. New York, Heidelberg, Dordrecht, London: Humana Press Springer

Invited lectures

Haring, M.A., (19 November 2013), *Neue Züchtungstechniken – in der Grauzone der Gentechnik*. ÖkoHaus Frankfurt, Germany.

Haring, MA, (22 June 2013), *Genkomposition oder Lebewesen?* Überlingen, Germany.

Haring, MA, (28 May 2013), *Gen-denken in der Pflanzenzüchtung: ist das Öko-Logisch?* TU München, Weihenstephan, Germany.

Testerink, C. (28 February 2013). *Halotropism - A novel response of plant roots to avoid salinity stress*. Galveston, TX, USA, Gordon Research Conference on Plant Lipids: Structure, Metabolism & Function.

Testerink, C. (08 June 2013). *Halotropism - A response of plant roots to avoid a saline environment*. Leiden, the Netherlands, Leiden University.

Testerink, C. (11 July 2013). *Novel functions for the lipid second messenger PA: shaping roots in response to salinity stress*. Vancouver, BC, Canada, University of British Columbia.

Testerink, C. (26 August 2013). *Signals and responses that allow roots to avoid salinity stress*. Utrecht, the Netherlands, 7th Utrecht PhD Summer School on Environmental Signaling.

Testerink, C. (22 February 2013). *Take it or leave it: cellular signaling pathways linking salinity stress to root growth*. Halle, Germany, IPB Halle.

Testerink, C. (15 March 2013). *Take it or leave it: cellular signaling pathways linking salinity stress to root growth*. Louvain-la-Neuve, Belgium, UCL Louvain-la-Neuve.

Testerink, C. (8 July 2013). *Take it or leave it: cellular signaling pathways linking salinity stress to root growth*. Vancouver, BC, Canada, International Symposium on Plant Signaling and Behavior.

Testerink, C. (22 April 2013). *Take it or leave it: cellular signaling pathways linking salinity stress to root growth*. Lunteren, the Netherlands, Annual Dutch Experimental Plant Science Meeting.

Testerink, C. (02 October 2013). *Underground strategies of plants to avoid salinity*. Wageningen, the Netherlands, 2nd Dutch Seed Symposium.

Munnik, T. (27 May 2013). *Lipid Signals at Work*. Würzburg, Germany, Julius-von-Sachs-Institut für Biowissenschaften.

Munnik, T. (23 September 2013). *Phospholipid signals in plant stress and development*. Metaponto, Mt, Italy, Agrobios.

Munnik, T. (21 June 2013). *Phospholipid signals in plant stress and development*. Norwich, UK, The Sainsbury Laboratory.

Munnik, T. (07 June 2013). *PLC meets Auxin: New data on an old Story*. Leiden, the Netherlands, Auxin Sailing 2013.

Munnik, T. (17 September 2013). *Stress-activated, phospholipid-based signaling pathways in plants*. Bari, Italy, Plenary speaker, 54th International Conference on the Biochemistry of Lipids (ICBL): Linking Transcription to Physiology in Lipidomics.

Munnik, T. (26 January 2013). *Visualizing phospholipid signals in plants*. Galveston, TX, USA, Gordon Research Conference (GRC) on Plant Lipids: Structure Metabolism and Function.

Schuurink, R.C. (31 August 2013). *Metabolic engineering of volatile production*. Münster, Germany, EU COST meeting Workshop Metabolic Pathway Engineering preceding the 61st International Congress and Annual Meeting (GA).

Schuurink, R.C. (18 January 2013). *Plants and insects, a volatile interaction*. Halle, Germany, Leibniz Institute of Plant Biochemistry.

Refereeship

Munnik, T., Associate Editor *Plant Physiology*.

Other

Haring, M.A. (2013). Groene onderwijs. *Vakblad Groen Onderwijs*: (12 July 2013).

Haring, M.A. (2013). Interview Groene Innovatie Toonbank. *Vakblad voor de Bloemisterij* 43.

Group members**Postdoctoral researchers**

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PhD students

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Mabel Castillo Blasco
Magdalena Julkowska
Dorota Kawa
Alessandra Scala
Fariza Shaipulah
Carlos Villarroel
Jiesen Xu
Xavier Cubero Zarza
Qianqian Zhang

Research assistant

Jiorgos Kourelis

Docent

Pieter van Egmond

Technicians

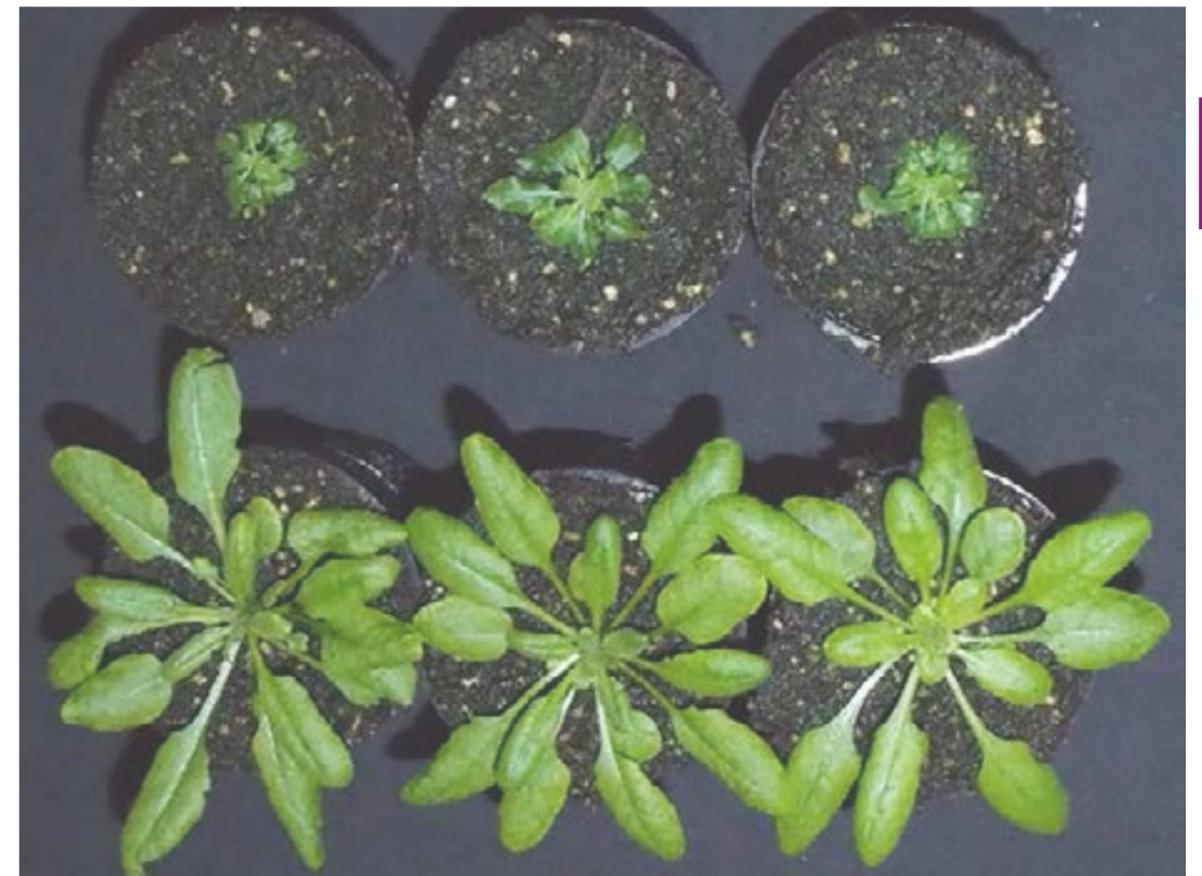
Selena Mol
Michel de Vries
Ringo van Wijk

Molecular Plant Pathology

Prof. B.J.C. Cornelissen
Dr M. Rep
Dr F.L.W. Takken
Dr H.A. van den Burg

Chairholder
Associate Professor
Assistant Professor
Assistant Professor (Tenure Track)

Microbial pathogens cause disease by evading host defences. Our aim is to reveal the molecular basis of resistance and susceptibility in plants, and of virulence in fungi. Our main model is the interaction between the fungus *Fusarium oxysporum* and tomato, but we also use *Arabidopsis* to answer fundamental questions of plant immunity. We study R proteins of the NBS-LRR family, such as the tomato *F. oxysporum* (Fo)-resistance protein I-2, basal and induced defence mechanisms of plants, and virulence and avirulence factors ('effectors') of the pathogen. Our research aims at: (i) the identification and dissection of protein complex(es) involved in R protein-mediated resistance. This work includes (i) the functional analysis of individual complex-components and conformational changes in R proteins; (ii) dissection of the role of SUMO (small ubiquitin-like modifier) isoforms in disease resistance and stress responses including heat acclimation and stability of immune receptors; (iii) uncovering the role of effector proteins of *F. oxysporum* and identification of their targets in host plants; (iv) unravelling the dynamics of genome evolution and the mechanisms of horizontal chromosome transfer in *F. oxysporum*.



The effect of expression of a Fol effector gene at Arabidopsis.

Research highlights

► RNAseq from *F. oxysporum* strains pathogenic to melon or tomato revealed that effector genes are among the most highly expressed genes in planta.

► DNA binding sites were identified for several transcription factors encoded on a mobile pathogenicity chromosome of tomato-infecting *F. oxysporum* – one of these sites was found earlier to be enriched in promoters of effector genes.

► Through comparative genomics the first candidate AVR gene was identified in *F. oxysporum* pathogenic towards melon.

► From the *F. oxysporum* f.sp. *lycopersici* (Fol) reference strain, only the ‘pathogenicity’ chromosome (number 14) can be independently transferred to another strain, sometimes accompanied by another chromosome.

► Transgenic tomato plants expressing Fol effector genes AVR1, AVR2 and AVR3 and Arabidopsis expressing Fol effector genes AVR1, AVR2, AVR3, SIX5, SIX6 and SIX8 have been obtained.

► In collaboration with the Cann group, Durham University (UK) we followed up on our findings that: a) some R proteins exert their function in the nucleus and b) that I-2 recognizes the *Fusarium* effector protein Avr2 in the nucleus. Using heterologously produced I-2 protein we found that I-2 can directly bind dsDNA and that its affinity for DNA binding depends on its nucleotide binding state. Besides I-2 also other NBS-LRR R proteins were found to be able to bind to DNA in vitro and in vivo.

► Activation of I-2 resistance responses in tomato not only requires the presence of Avr2, but also of Six5. SIX5 and AVR2 form a gene-pair whose expression is regulated by a shared promoter region and the encoded proteins were found to interact in both the Y2H system and in planta when co-expressed in *N. benthamiana*.

► The dynamic changes of the xylem sap composition upon infection with either wild type Fol or SIX1, SIX2, SIX3, SIX5 or SIX6 knockout mutants were determined using a quantitative, large-scale proteomics approach. This study showed that Six proteins perform both redundant and non-redundant functions and a set of xylem sap proteins was identified whose accumulation is exclusively affected by a specific Six protein.

► Six6, which is present in *F. oxysporum* and in *Colletotrichum*, was shown to be required for full virulence. Notably, Six6 specifically suppress I-2 mediated cell death (I2CD) in *N. benthamiana*, while it does not compromise the activity of other cell death-inducing genes. Yet, the I2CD suppressing activity does not allow the fungus to overcome I-2 resistance in tomato, suggesting that I-2 resistance is independent from cell death.

► SIX8 expression in Arabidopsis triggers a strong constitutive defence phenotype that is in agreement with the proposed activity of its putative target, which is a known negative regulator of plant defence.

► The Arabidopsis paralog SUMO3 cannot complement the knockdown of canonical SUMO genes. The SUMO machinery plays an important role in temperature acclimation, which appears to involve NO signalling.

► We screened an array of 1,300 Arabidopsis transcription factors for novel interactors of the SUMO machinery. The data suggest that the SUMO machinery does not directly interact with transcription factors despite such reports in other systems. We now hypothesize that these interactions require a protein interaction platform including co-factors such as chromatin remodelling and chromatin modifying enzymes.

► We have developed a tool to screen for SUMO modification on novel targets in response to resistance protein activation by the elicitors avrRPM1 and avrRPS2 from *Pseudomonas*.

Research research aims for the coming year

► Annotate one or more genomes of several newly sequenced isolates of *Fo melonis* and *Fo cucumerinum*, in combination with RNAseq analysis to identify common and unique effectors in these *formae speciales*.

► Determine the in vivo binding sites of three transcription factors of *F. oxysporum* for which there is evidence that they are involved in regulation of effector genes, using ChIPseq.

► Determine distribution of histone modifications across the genome of *F. oxysporum*, to see to what extent these are different between the core genome and the accessory genome.

► Determine the most likely evolutionary path(s) of the accessory genome within the *F. oxysporum* species complex using bioinformatics.

► Explore PacBio sequencing of Fol isolates to assess the extent to which this method leads to assembly of (near) complete accessory chromosomes rich in repetitive elements.

► Determine chromosome stability and chromosome transfer frequencies with fluorescence-activated cell sorting of fungal spores.

► Determine whether *Fo*-resistance genes in tomato recognize homologs of Fol Avr proteins from other *formae speciales*, to assess potential for trans-species transfer of R genes.

► Identify novel SUMO targets in defence signalling in Arabidopsis by screening a large yeast two-hybrid library with full-length arrayed cDNA clones (+13,000) and by performing a SUMO proteomics screen.

► Study the role of SUMO in protein folding, in specific immune receptor protein stability at elevated temperatures studying the consequence of SUMOylation of chaperones and heat shock factors.

► Determine the biologically active isoform of SUMO3 to see if it acts as novel protein modification or can only interact with the SUMO-network. In addition, we will determine the full range of the SUMO protein network using a yeast two-hybrid approach.

► Identify plant proteins/structures interacting with Six5 and Avr2 and elucidate their role in resistance and/or pathogenicity.

► Identify tomato genes whose expression is affected by the virulence and avirulence activity of Avr2.

► Create transgenic *F. oxysporum* strains expressing fluorescently labelled Six5 and Avr2 proteins to reveal the subcellular (co)localisation of the Six5/Avr2 complex upon natural infection.

► Further study the role of DNA binding by NLR proteins for the activation of plant defence.

► Compare genomes of strains of *F. oxysporum* f.sp. *melonis* to identify potential effector genes specific for this *forma specialis* or for races of this pathogen.

Academic publications

Gawehns, F., Cornelissen, B.J.C. & Takken, F.L.W. (2013). The potential of effector-target genes in breeding for plant innate immunity. *Microbial Biotechnology*, 6(3), 223-229.

Ma, L., Cornelissen, B.J.C. & Takken, F.L.W. (2013). A nuclear localization for Avr2 from *Fusarium oxysporum* is required to activate the tomato resistance protein I-2. *Frontiers in Plant Science*, 4, 94.

Pietraszewska-Bogiel, A., Lefebvre, B., Koini, A.M., Klaus-Heisen, D., Takken, F.L.W., Geurts, R., Cullimore, J.V. & Gadella, Th.W.J. (2013). Interaction of *Medicago truncatula* Lysin Motif Receptor-Like Kinases, NFP and LYK3, Produced in *Nicotiana benthamiana* Induces Defence-Like Responses. *PLoS One*, 8(6), e65055.

Schmidt, S.M., Houterman, P.M., Schreiver, I., Ma, L., Amyotte, S., Chellapan, B., Boeren, S., Takken, F.L.W. & Rep, M. (2013). MITEs in the promoters of effector genes allow prediction of novel virulence genes in *Fusarium oxysporum*. *BMC Genomics*, 14, 119.

Book chapter

Kistler, H.C., Rep, M. & Ma, L. (2013). *Structural dynamics of Fusarium genomes*. In D.W. Brown & R.H. Proctor (Eds.), *Fusarium: genomics, molecular and cellular biology* (pp. 46-60). Norwich, UK: Caister Academic Press.

Invited lectures

Rep, M. (25 August 2013). *Accessory genomes and host-specific pathogenicity in Fusarium oxysporum*. Beijing, China, 10th International Congress of Plant Pathology.

Rep, M. (29 September 2013). *Speculations on the evolution of host-specific pathogenicity in the Fusarium oxysporum species complex*. Karlsruhe, Germany, XI International Fungal Biology Conference.

Rep, M. (06 February 2013). *The genomic organization of virulence in the vascular wilt fungus Fusarium oxysporum*. Cologne, Germany, Max Planck Institute for Plant Breeding Research.

Takken, F.L.W. (28 October 2013). *De strijd tegen plantenziekten – het plant immuunsysteem*. Amsterdam, the Netherlands, Gyrimus natans symposium VU University Amsterdam.

Takken, F.L.W. (16 January 2013). *The role of pathogen effectors in NLR-mediated immunity: The molecular interplay between Fusarium and tomato*. Copenhagen, DK, University of Copenhagen.

Relevant positions

Takken, F.L.W. Member Scientific advisory board at New Phytologist.

Takken, F.L.W. Scientific Advisor at SciENZA Biotechnologies.

Other

Takken, F.L.W. (2013). *Goedgekeurde gentechnais is trage stap vooruit*. C2W Life Sciences actueel: (13 December 2013).

Group members**Postdoctoral researcher**

Lotje van der Does
Sarah Schmidt

PhD students

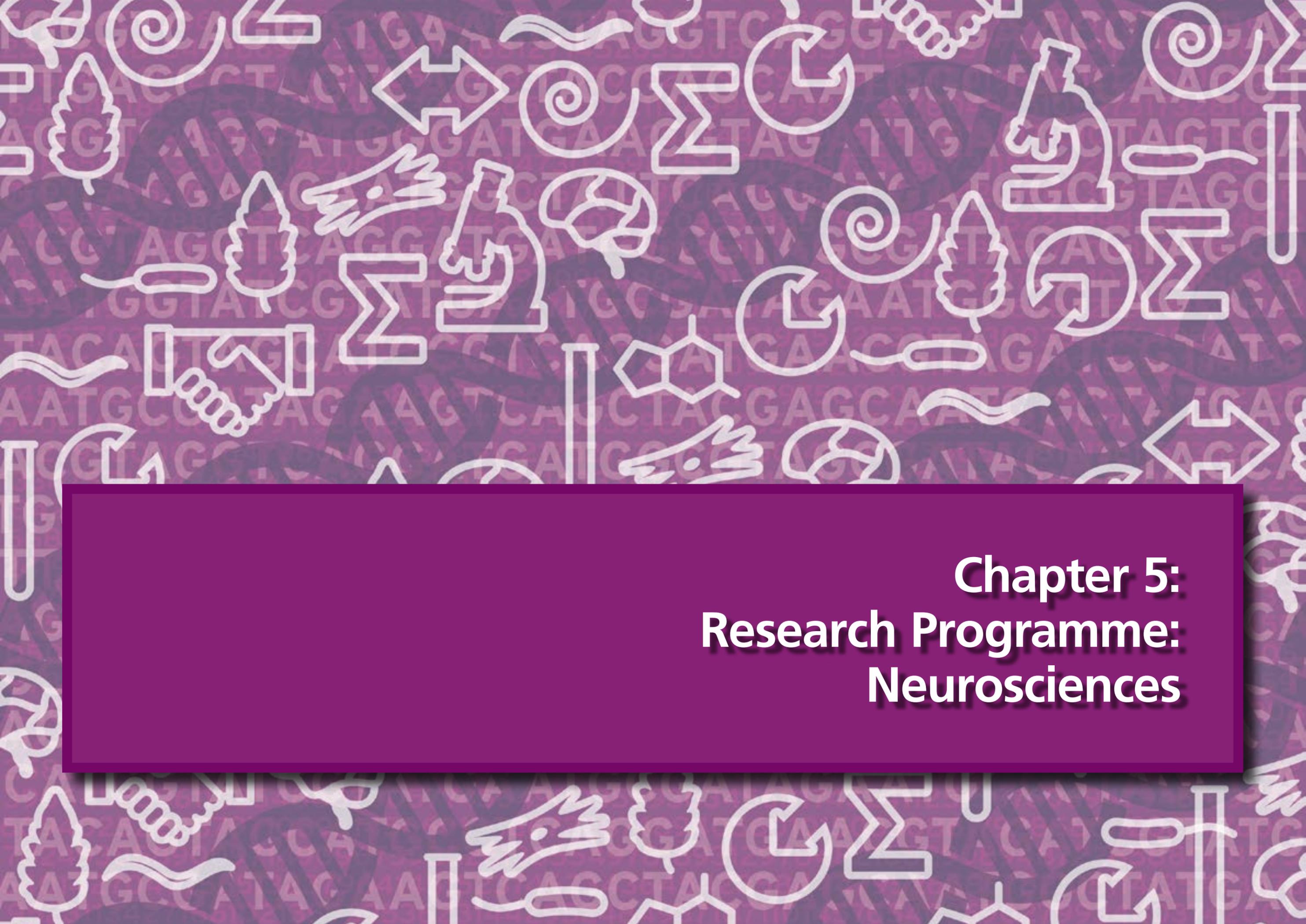
Biju Chellapan
Peter van Dam
Fleur Gawehns-Bruning
Valentin Hammoudi
Magdalena Mazur
Mara de Sain
Shermineh Shahi
Ido Vlaardingerbroek

Technicians

Bas Beerends
Lieke Fokkens
Petra Houterman
Patrick Mak
Hannah Richter
Georgios Vlachakis

Other highlights

- ▶ A ZonMW Hotel project was obtained by Takken to 'Deconstruct the plant immune system using a *Fusarium* effector protein as a probe'.
- ▶ An Indonesian PhD student started work in the Rep group on a fellowship from the Dutch KNAW-SPIN and Indonesian DIKTI programs in a project, coordinated by Dr. Gert Kema, to combat Panama disease of banana.
- ▶ A TKI-U topsector grant (Ministry of Economic affairs) was obtained by Van den Burg in conjunction with two Dutch plant breeding companies on disease in various Cabbage cultivars.
- ▶ Appointment of Dr. Marcel Prins as special chair Professor affiliated to Molecular Plant Pathology, with a focus on plant virology.
- ▶ SciENZA biotechnology, a spinout company founded by ENZA Zaden and hosted by the SILS, further expanded its activities and hired a third researcher or field crop breeding. Besides applying key technology from ENZA, the company uses the know-how from Takken and collaborates closely with the Molecular Plant Pathology group.



**Chapter 5:
Research Programme:
Neurosciences**

Cognitive and Systems Neuroscience

Prof. C.M.A. Pennartz
 Dr W.E.J.M. Ghijsen
 Dr C.S. Lansink
 Dr L.J. Gentet
 Dr. C. Bosman
 Dr. U. Olcese
 Prof. O. Jensen

Chairholder
 Assistant Professor
 Assistant Professor
 Assistant Professor
 Assistant Professor
 Assistant Professor
 Special Chair

The group's global research aim is to elucidate how neuronal networks distributed across cortical structures, most notably the sensory neocortex, prefrontal cortex and hippocampus, cooperate in cognitive processes, in particular conscious perception, multisensory integration and memory formation. This aim is pursued using a variety of techniques and at various aggregate levels, ranging from cellular to systems and behavioural levels. The research focuses on the level of systems neurophysiology.

We investigate how neural assemblies in the brain cooperate to generate conscious and unconscious representations, with a particular emphasis on multimodal integration. This process refers to the integration of multiple sensory modalities (e.g. vision, audition) as well as integration of sensory and memorized information. A theoretical framework to understand how multimodal integration contributes to consciousness has been constructed, and we are testing its experimental predictions using in vivo 2-photon Calcium imaging and multi-area neuronal ensemble recordings. An important aspect of these studies is to elucidate the role of oscillations and spike-phasing relative to these oscillations in network communication and integration. To illustrate research on conscious and unconscious representations, we compare visual processing under awake and anaesthetised conditions, and study how neuronal populations in visual cortex function differently when animals detect a visual stimulus or miss it. As an example of research on multimodal integration, we started a Veni project investigating how the integration of audio-visual stimuli is represented in the firing patterns of single units and populations of neurons at successive hierarchical nodes of the cortical system. Using whole-cell recordings in vivo, guided by 2-photon imaging, we are studying how GABAergic interneurons in the sensory cortices shape response patterns of principal (pyramidal) cells to sensory input, and how they control multisensory interactions

between brain areas. The team members contribute to this overall theme using their own strengths and expertise, e.g. in ensemble recordings, field potentials and oscillations, patch-clamp recordings and optogenetics and theoretical neuroscience and modelling. All of these disciplines are combined with behavioral-cognitive paradigms.

As indicated above, our research on multimodal integration also includes the interaction between sensory inputs and memory. The flow of sensory information into the medial temporal lobe, including hippocampus, is being tracked by ensemble recordings, but also the feedback from hippocampal output into higher sensory areas is investigated. After an initial experience, which is marked by highly specific firing patterns in brain structures involved in memory, a replay of these firing patterns can be observed, with preservation of temporally specific features such as the order in which brain cells fire. We pursue the causal relevance of replay phenomena for memory consolidation by electrical interventions, and study how replay is orchestrated amongst different brain areas, such as the hippocampus, sensory cortices and ventral striatum. This project is carried out in animals by performing multi-area ensemble recordings using 'tetrode arrays' and by state-dependent deep brain stimulation. Furthermore, we continue to develop new analytic methods to study coherence within and between cell assemblies in the brain. Furthermore, this line of research has been augmented by studying how stress hormones influence memory formation.

The current systems-level efforts to elucidate neural substrates of multimodal integration and consciousness are being complemented by novel techniques such as optogenetics. Using optogenetics we study the causal role specific cortical cell types play during sensory analysis and integration. These studies are integrated with 2-photon Ca²⁺ imaging results and extracellular recordings.

Research highlights

► We investigate relationships between genes, learning and memory capacities as measured in behaviour, and the systems physiology that forms the interface between gene expression and overt behaviour. These relationships are studied in the context of spatial navigation, multisensory integration and cognitive flexibility. We carried out experiments involving tetrode ensemble recordings in a mouse model of fragile-X mental retardation. This model shows a higher instability of spatial representation as gauged from the hippocampus, pointing to a neural substrate for deficient learning and memory processing as found in several intellectual disability disorders.

► The hippocampus is thought to play a crucial role in representation of an animal's location and, more in general, in representation of its internal and external state. However, it is unknown

► Whether it may also function in representing state parameters of other agents in the environment, e.g. conspecifics. We studied whether rats can discriminate positions and movements of robotic agents in the environment. This was indeed the case. The results indicate that the hippocampus harbours cells sensitive to the motion behavior of the robot.

► We used in vivo 2-photon Ca²⁺ imaging setup to examine the effects of a learning process (conditioning based on selective parts of the visual field) on the spatial organization of information processing in mouse visual cortex. One hypothesis held that a subspace of the visual field, which is important to the animal because its stimulation predicts reward, will be expanded upon learning. A contrasting hypothesis held that such retinotopically specific conditioning leads to more selectivity and efficiency in the corresponding part of cortex. Our results confirm the latter hypothesis.

► To investigate how neural assemblies in brain areas belonging to different sensory modality interact, we developed a behavioural task in which visual and tactile inputs can be separately manipulated. Next we applied ensemble recording techniques to record four neocortical and hippocampal areas simultaneously (i.e., visual cortex, somatosensory cortex, perirhinal cortex and hippocampus). First results from this project hold that (i) gamma oscillations do not serve as a common communication mechanism between these sensory and mnemonic temporal areas, as indicated by a lack of gamma coherence between areas; (ii) perirhinal cells have unique properties in representation an animal's global position in space, relative to hippocampus and sensory cortices.

► Joint ensemble recordings were made from Hippocampus and Ventral striatum simultaneously to study how animals learn to predict reward value based on discrete cues and locations in the environment. In a Y-maze task, we found that the hippocampus uses a new, special mode of information processing when the animal engages in goal-directed behavior. This mode is characterized by beta-oscillations (~ 18 Hz) and by an accelerated speed of processing relative to the regularly observed theta rhythm. Hippocampus and Ventral striatum generate coherent activity in the beta-range, pointing to a new communication mechanism by which the episodic memory system impacts on systems for motivation and behavioral output.

► A key question regarding the neural basis of reinforcement learning concerns the role of the orbitofrontal cortex in coding reward expectancy and in using this information to direct behaviour. Gamma (~60 Hz) and theta (~ 8 Hz) oscillations are thought to be key elements in the neural mechanisms for remembering and learning to predict future rewards. We examined the role of theta-gamma phase-amplitude coupling in neural coding processes in the orbitofrontal cortex by conducting tetrode recordings. We discovered phase-amplitude coupling of neural rhythms in orbitofrontal cortex and found this phenomenon to correlate with correct decision-making.

► Important technical progress was made in implementing new behavioral tasks to study multimodal integration, conscious visual processing, and memory-guided planning in rats, optogenetics combined with electrophysiology in vivo, and patch-clamp recordings from identified cell types during visual stimulation in vivo.

Other highlights

► The following UvA-based papers were accepted and/or published in high-impact review journals, including research papers in the Journal of Neuroscience, PNAS and Neuron.

► Stanisor L, Van der Togt C, Pennartz CMA, Roelfsema PR (2013) Relative value and selective attention in primary visual cortex: two sides of the same coin. *Proc. Natl. Acad. Sci. U.S.A.* 110: 9136-9141.

► Goltstein PM, Coffey EBJ, Roelfsema PR and Pennartz CMA (2013) In vivo two-photon Ca²⁺ imaging reveals selective reward effects on stimulus-specific assemblies in mouse visual cortex. *J. Neurosci.* 33: 11540-11555.

► Lopes da Silva FH (2013) EEG and MEG: relevance to neuroscience. *Neuron* 80: 1112-1128.

► Van Wingerden M, van der Meij R, Maris E, Pennartz CMA (2013) Phase-amplitude coupling in rat orbitofrontal cortex discriminates between correct and incorrect decisions during associative learning. *J. Neurosci.* (in press).

► Cabral H, Vinck MA, Fouquet C, Pennartz CMA, Rondi-Reig L Battaglia FP (2013) Oscillatory dynamics and place field maps reflect sequence and place memory processing in hippocampal ensembles under NMDA receptor control. *Neuron* (in press).

► Martin Vinck was awarded the Elsevier Scopus Award for best young scientist in the Life Sciences, Netherlands.

► Wim Ghijsen was member of the 'Rubicon' committee of the Dutch Organization for Scientific Research (NWO; for recently graduated PhD applying for postdoc fellowships abroad), and Cyriel Pennartz was member of the Earth & Life Sciences Review panel (Open program).

► Cyriel Pennartz acquired a grant for a 3-year postdoc fellowship from NWO Earth & Life Sciences.

► Cyriel Pennartz acted as Associate Editor at the European Journal of Neuroscience.

► Cyriel Pennartz acted as Guest Editor for a special issue of the Philosophical Transactions of the Royal Society B, 'The principles of goal-directed decision-making: from neural mechanisms to computation and robotics' (forthcoming).

Research aims for the coming years

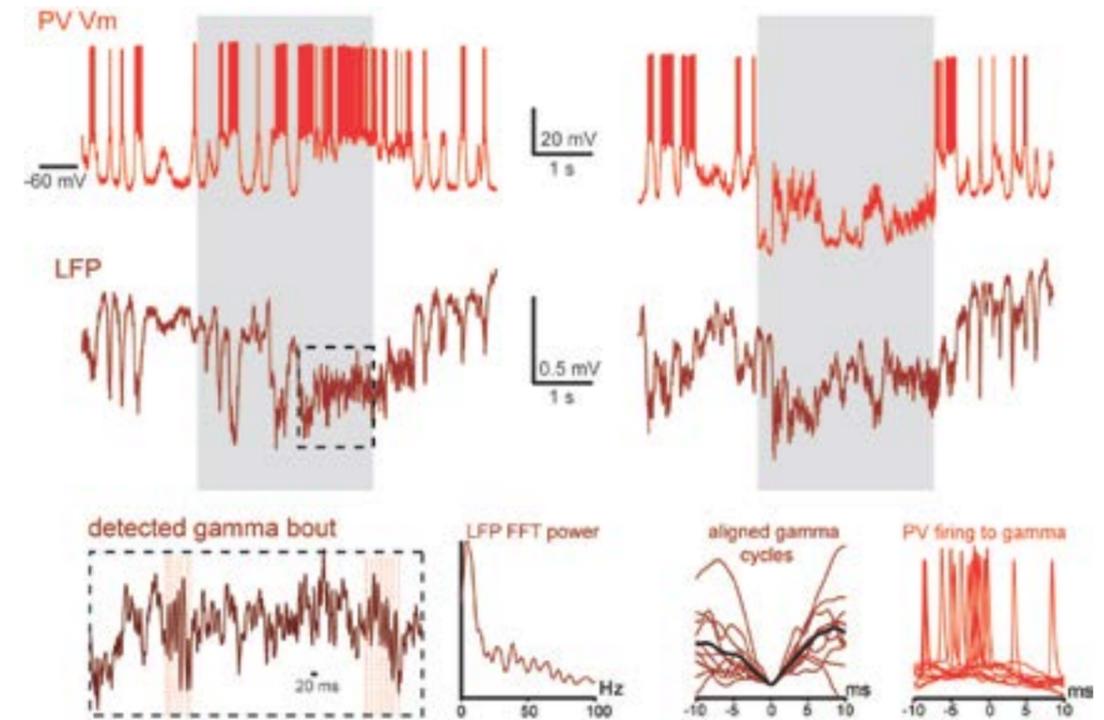
► We will elaborate our program aiming to disrupt memory consolidation and extra-hippocampal replay by electrical and optogenetic intervention of hippocampal processing in rats.

► We are setting up a new research line in ferrets, permitting us to study multisensory influences on the population dynamics and oscillatory activity of visual and parietal neurons in the neocortex.

► We will investigate neural mechanisms underlying audiovisual integration by recording the activity of neuronal ensembles simultaneously in various brain areas along the cortico-hippocampal hierarchy.

► We have set up whole-cell recordings in vivo will be performed under 2-photon visually guided microscopy, targeting both excitatory pyramidal and inhibitory GABAergic neurons in the superficial layer of primary visual cortex in transgenic mice. We will study the tuning properties of specific interneurons in primary visual cortex and their modulation by sensory stimulation from another modality.

► The two-photon Ca²⁺ imaging will be extended to the use of genetically coded Ca²⁺ indicators (gCaMP6) applied to problems of visual feature integration and stimulus detection.



In vivo electrophysiology: patch-clamp recordings during visual stimulation in mouse primary visual cortex. Top: Membrane potential fluctuations of a GABAergic Parvalbumin-positive interneuron during visual presentation of a grating stimulus (grey box), without (left) or with (right) concomitant hyperpolarizing current injection. Middle: Simultaneously recorded local field potential (LFP) traces with apparent gamma oscillations (dotted black box). Bottom: 1-2 s episodes of high gamma activity (gamma "bouts") are detected offline; fast-fourier-transform analysis of LFP trace reveals increased gamma power over a wide range of frequencies (25-80Hz); gamma cycles aligned at the trough of their oscillatory cycles allow phase-locking of PV cells to the gamma cycle to be analyzed (right). Unpublished data (Gentet, Perrenoud, Pennartz).

Academic publications

Brunet, N., Bosman, C.A., Roberts, M., Oostenveld, R., Womelsdorf, T., Weerd, P. de & Fries, P. (in press). Visual Cortical Gamma-Band Activity During Free Viewing of Natural Images. *Cerebral Cortex*.

Goltstein, P.M., Coffey, E.B.J., Roelfsema, P.R. & Pennartz, C.M.A. (2013). In vivo two-photon Ca²⁺ imaging reveals selective reward effects on stimulus-specific assemblies in mouse visual cortex. *Journal of Neuroscience*, 33(28), 11540-11555.

Lopes da Silva, F.H. (2013). EEG and MEG: relevance to neuroscience. *Neuron*, 80(5), 1112-1128.

Maroteaux, G., Loos, M., Sluis, S. van der, Koopmans, B., Aarts, E., Gassen, K., Geurts, A., Brussaard, A.B., Borst, J.G.G., Elgersma, Y., Galjart, N., Horst, G.T.J. van der, Levelt, C.N., Pennartz, C.M.A., Zeeuw, C.I. de, Largaespada, D.A., Spruijt, B.M., Stiedl, O., Smit, A.B. & Verhage, M. (2012). High-throughput phenotyping of avoidance learning in mice discriminates different genotypes and identifies a novel gene. *Genes Brain and Behavior*, 11(7), 772-784.

Nagtegaal, A.P., Spijker, S., Crins, T.T.H., Brussaard, A.B., Borst, J.G.G., Elgersma, Y., Galjart, N., Horst, G.T.J. van der, Levelt, C.N., Pennartz, C.M., Smit, A.B., Spruijt, B.M., Verhage, M. & Zeeuw, C.I. de (2012). A novel QTL underlying early-onset, low-frequency hearing loss in BXD

recombinant inbred strains. *Genes Brain and Behavior*, 11(8), 911-920.

Phillips, J.M., Vinck, M.A., Everling, S. & Womelsdorf, T. (in press). A Long-Range Fronto-Parietal 5- to 10-Hz Network Predicts "Top-Down" Controlled Guidance in a Task-Switch Paradigm. *Cerebral Cortex*.

Stefan, H. & Lopes da Silva, F.H. (2013). Epileptic neuronal networks: methods of identification and clinical relevance. *Frontiers in Neurology*, 4, 8.

Stănişor, L., Togh, C. van der, Pennartz, C.M.A. & Roelfsema, P.R. (2013). A unified selection signal for attention and reward in primary visual cortex. *Proceedings of the National Academy of Sciences of the United States of America*, 110(22), 9136-9141.

Vinck, M.A., Womelsdorf, T., Buffalo, E.A., Desimone, R. & Fries, P. (2013). Attentional modulation of cell-class-specific gamma-band synchronization in awake monkey area v4. *Neuron*, 80(4), 1077-1089.

Vinish, M., Elnabawi, A., Milstein, J.A., Burke, J.S., Kallevang, J.K., Turek, K.C., Lansink, C.S., Merchenthaler, I., Bailey, A.M., Kolb, B., Cheer, J.F. & Frost, D.O. (2013). Olanzapine treatment of adolescent rats alters adult reward behaviour and nucleus accumbens function. *International Journal of Neuropsychopharmacology*, 16(7), 1599-1609.

Wang, T., Rusu, S.I., Hruskova, B., Turecek, R. & Borst, J.G. (2013). Modulation of synaptic depression of the calyx of Held synapse by GABA(B) receptors and spontaneous activity. *Journal of Physiology*, 591(Pt 19), 4877-4894.

Proceedings

Velden, L. van der, Vinck, M., Werkman, T.R. & Wadman, W.J. (2012). A network of weakly coupled oscillators in the ventral tegmental area. In A. Stett & G. Zeck (Eds.), MEA Meeting 2012: July 10-July 13, 2012, Reutlingen, Germany: 8th International Meeting on Substrate-Integrated Microelectrode Arrays: proceedings (pp. 140-141). Reutlingen: NMI Natural and Medical Sciences Institute at the University of Tuebingen.

Book chapter

Womelsdorf, T., Bosman, C.A. & Fries, P. (2013). Selective Neuronal Synchronization and Attentional Stimulus Selection in Visual Cortex. In J.S. Werner & L.M. Chalupa (Eds.), *The New Visual Neurosciences* (pp. 1013-1030-chapter 71). MIT Press.

PhD theses

Malkki, H.A.I. (04 December 2013). Exploring the triad of behaviour, genes and neuronal networks: Heritability of instrumental conditioning and the Arc/Arg3.1 gene in hippocampal coding. Universiteit van Amsterdam (169 pag.). Prom./coprom.: prof.dr. C.M.A. Pennartz.

Vinck, M.A. (05 December 2013). Gamma-band synchronization in the neocortex: novel analysis methods and their application to sensory and motivational systems. Universiteit van Amsterdam (291 pag.) (BOXPress). Prom./coprom.: prof.dr. C.M.A. Pennartz.

Wingerden, E.J.M. van (27 March 2013). Good vibrations: Rhythms and plasticity in neural correlates of value in rat orbitofrontal cortex. Universiteit van Amsterdam (227 pag.). Prom./coprom.: prof.dr. C.M.A. Pennartz.

Refereeship

Bosman Vittini, C.A. *Deutsche Forschungsgemeinschaft*.

Bosman Vittini, C.A. *Frontiers in Neuroscience*.

Bosman Vittini, C.A. *Journal of Neurophysiology*.

Bosman Vittini, C.A. *Schizophrenia Research*.

Olcese, U. *PLoS One*.

Pennartz, C.M.A. *Cerebral Cortex*.

Pennartz, C.M.A. *Journal of Neuroscience*.

Pennartz, C.M.A. *Philosophical Transactions of the Royal Society B - Biological Sciences*.

Relevant positions

Pennartz, C.M.A. member of the Advisory Group at Amsterdam Brain & Cognition Center (formerly: Cognitive Science Center Amsterdam), UvA.

Pennartz, C.M.A. Member of the evaluation panel at Earth & Life Sciences program of the Netherlands Organization for Scientific research.

Pennartz, C.M.A. reviewer at Newton International Fellowships of the Royal Society UK.

Award

Vinck, M.A. (2013). Scopus Young Researcher Award. Dag van de Valorisatie: (11 June 2013). Recognition.

Group members

Postdoctoral researcher

Silviu Rusu

PhD students

Tara Arbab
Jeroen Bos
Gerben Klein
Guido Meijer
Paul Mertens
Ivana Milojevic
Jorrit Montijn
Martin Vinck
Zbigniew Zielinski

Technicians

Laura van Mourik-Donga

ICT developer

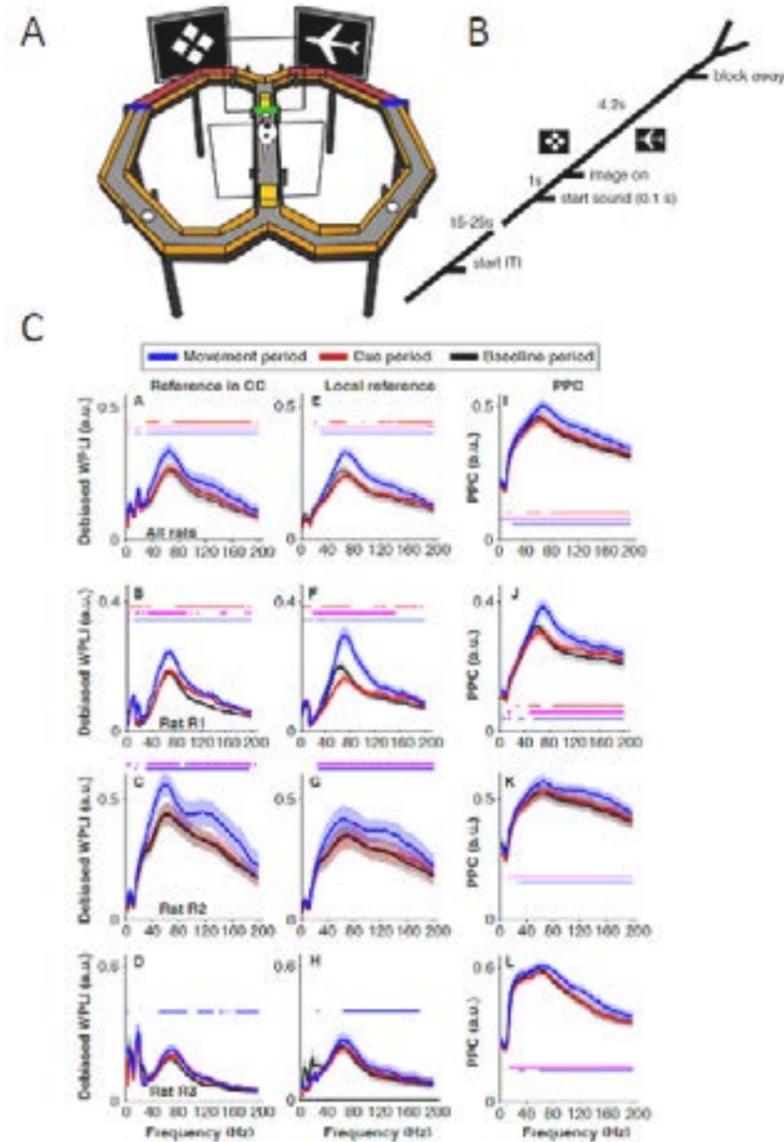
Jan Lankelma



Cellular and Systems Neurobiology

Prof. W.J. Wadman
 Dr J.A. van Hooft
 Dr J.A. Gorter
 Dr T.R. Werkman
 Dr N.L.M. Cappaert
 Prof. C. G. Kruse
 Prof. E. M. Hol
 Prof. E.M.A. Aronica

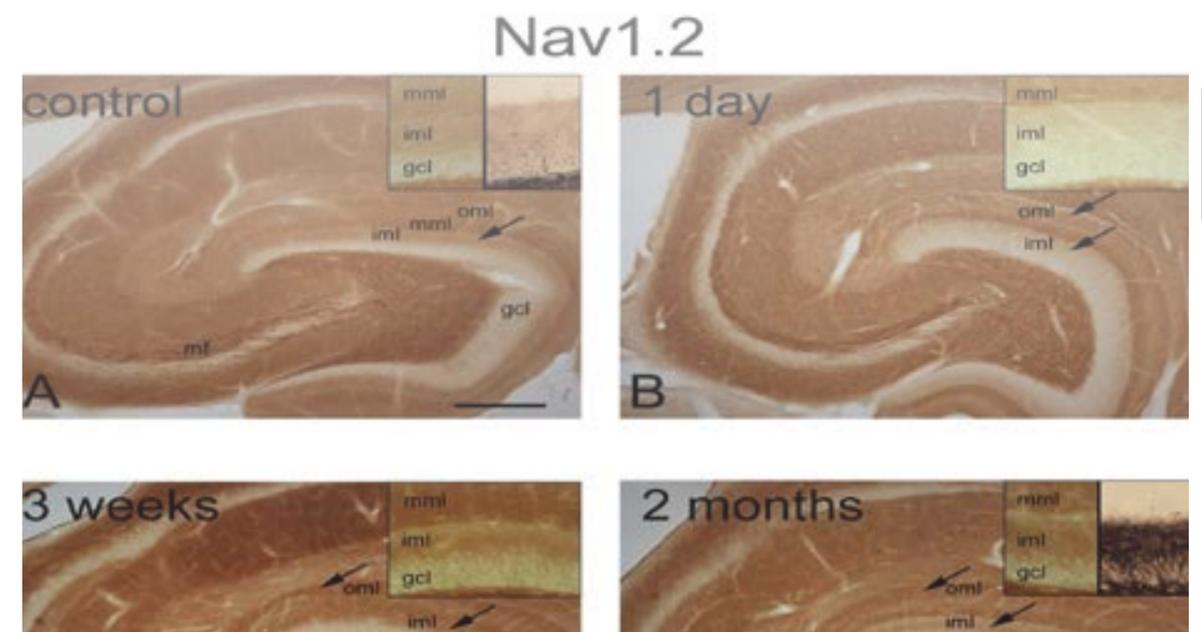
Chairholder
 Assistant Professor
 Assistant Professor
 Assistant Professor
 Assistant Professor
 Special Chair
 Special Chair
 Special Chair



In vivo electrophysiology: “Quadruple” tetrode recordings in four brain areas reveal gamma rhythm in rat barrel cortex. A: Layout of the conditional visual discrimination task. Rats learned to discriminate two isoluminant stimuli (CS+ and CS-) that could be displayed left or right on screens placed in front of the rat. On the figure-8 maze, the rat had to choose the side where the CS+ stimulus was displayed, and ignore the other route. Once the animal had entered the left or right hand track, he encountered differential tactile (sandpaper) cues informative of the upcoming reward amount (upon correct trials). B: The branch tree shows a time-line of task events (ITI: intertrial interval).

C: We used a measure (“debiased WPLI”) to compute the coherence between local-field potential (EEG) signals recorded from tetrodes all placed within the rat somatosensory cortex. This measure was chosen because it corrects for volume-conduction artifacts and noise effects. The plots show a broad but clear peak in the gamma-band range (50-90 Hz), consistently across different behavioral states and task conditions (blue: rat locomoting while executing the task, etc.). Different electrode referencing methods show similar frequency profiles. PPC: Pairwise phase consistency. These plots reveal a state-dependent occurrence of gamma-rhythms in rat barrel cortex. Unpublished data (Vinck, Bos, Pennartz et al.).

Excitability is the most prominent property of the nervous system. Nerve cells process information by integrating the thousands of inputs that they receive, and converting those signals into characteristic spatio-temporal firing patterns. One of the most exciting questions in neuroscience is how ionic-channels are organised in the neuronal membrane and how their activity is quantitatively balanced under the many circumstances that neurons have to operate. Neurons communicate with each other through a variety of synapses, that add additional flexibility and long term plasticity. Neuronal activity is also modulated at different time scales in order to allow processes like learning and memory, development and aging. Neurons need to be combined in small micro-circuits in order to provide minimal cognitive functionality. The fast development of new techniques has made us shift the focus of our research from the well understood single neuron to the more difficult micro circuit. We approach neuronal plasticity from a multidisciplinary angle. Our research is organised around a small number of well defined topics in the realm of neuronal excitability. Our core expertise is functional electrophysiology (from patch-clamping to *in vivo* recording). We combine this approach with molecular biology, immuno-cytochemistry, behavioural analysis and a large variety of imaging techniques (Voltage Sensitive Dye imaging, Multi-Electrode Array analysis and MRI imaging). Computational approaches are an integral element to investigate hypotheses, predict experiments and understand experimental results. Each research line is intricately linked to a pathological brain condition: epilepsy, schizophrenia, Alzheimer’s disease, Parkinson’s disease and Autism; these relations are further strengthened by links to several clinical institutions.



Research highlights

► Four PhD defenses in 2013 also marked the end of several successful research lines. Marlies Oostland defended her thesis on the role of the 5HT₃ receptors in the development of the cerebellum. Mid 2013 dr. Hans van Hooft decided to leave the research group and continue his work in the Molecular Neuroscience group of Marten Smidt. Femke den Boon defended her PhD thesis in which she describes a new intracellular location of the cannabinoid type II receptor in neurons in the prefrontal cortex. This successful research line, funded by the Dutch Top Institute Pharma, was long-lasting collaboration with Solvay Pharmaceuticals (prof.dr. C.Kruse; Weesp).

► The take-over and closing of Solvay Pharmaceuticals in the Netherlands ends this line. The exciting new functional perspectives of Femke's discoveries will further be exploited on a smaller scale, funding permitting. Xin Qiao and Linda Holtman both defended their theses on topics relating to epilepsy. Linda investigated the role of inflammation in epileptogenesis and the possibilities of therapeutical interference with epileptogenesis by suppressing early inflammation. Xin completed a huge investigation on the role of various sodium channel subunits as targets of anti-epileptic drugs. Although both research lines clearly indicate significant roles for inflammation and sodium channels in epilepsy and epileptogenesis, they also show that there is more to the complex problem of epilepsy. In a third approach Erwin van Vliet demonstrated that it is possible to use Gd imaging I MRI to follow blood-brain barrier damage during epileptogenesis. But also here, the chicken and egg problem has not yet been solved. Erwin will continue his work under the guidance of our extra-ordinary professor E.Aronica at the AMC. Two new lines of research, each supported by a PhD student, will continue our efforts epileptogenesis and pharmacoresistance in epilepsy.

Other highlights

► In 2012 dr E. Aronica (neuropathologist at the AMC) was appointed to the newly established chair for epilepsy (funded by the Dutch National Epilepsy Foundation). This consolidated a long standing collaboration with her on epilepsy research.

► The collaboration with prof. Elly Hol, whose major activities are at the NIN, was expanded and a joint project in which the modulation of synaptic connections by astrocytes, especially in the context of Alzheimer's disease, was started.

► Sabbaticals of two staff members, one in UCI California and one in Kuopio, Finland established new collaborations and brought new technologies to the lab. A grant acquired from the KNIP foundation allowed the start-up of a new line of research in which we will use optogenetic tools to investigate function in cortical and limbic micro-circuits.

Future prospects

► The direct future will show a gradual shift in focus from the cellular level to the micro-circuit level.

► Elaborate facilities have been generated to use optogenetic tools that can specifically activate genetically defined neuronal (sub) populations. New large scale recording/stimulation facilities for in-vivo recordings in epilepsy have been built. Three new PhD projects have been started that use these new technologies. A close collaboration with the newly appointed extra-ordinary professor dr. Elly Hol have broadened our disease spectrum with Alzheimer disease; we specifically concentrate on fundamental investigations on the role of astrocytes and neuron-glia interactions.

Academic publications

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Proceedings

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PhD theses

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Holtman, L. (13 February 2013). Anti-inflammatory strategies during epileptogenesis. Universiteit van Amsterdam (223 pag.). Prom./coprom.: prof.dr. W.J. Wadman & dr. J.A. Gorter.

Oostland, M. (19 June 2013). Serotonergic control of the developing cerebellum. Universiteit van Amsterdam (vii, 143 pag.). Prom./coprom.: prof.dr. W.J. Wadman & dr. J.A. van Hooft.

Qiao, X. (23 January 2013). Antiepileptic drugs targeting sodium channels: subunit and neuron-type specific interactions. Universiteit van Amsterdam (164 pag.). Prom./coprom.: prof.dr. W.J. Wadman & dr. T.R. Werkman.

Award

Vliet, E.A. van (2013). Education Award - Most Innovative Teacher. Recognition.

Group members

Postdoctoral researcher

Erwin van Vliet

PhD students

Lana Beex

Sicco de Knecht

Luuk van der Velden

Janske Willems

Technicians

Pascal Chameau



Structural and Functional Plasticity of the Nervous System

Prof. P.J. Lucassen
Dr H.J. Krugers
Dr C.P. Fitzsimons
Dr A. Korosi
Prof. M.S. Oitzl

Chairholder
Associate Professor
Assistant Professor
Assistant Professor
Special Chair

Mission of the group

We study structural and functional plasticity of the brain and focus on neurogenesis and synaptic plasticity in relation to (early) stress exposure, cognition and brain disorders like depression, PTSD, epilepsy and dementia.

Personel

In 2013, our group has further grown with 2 new PhD students, Lesuis (co-promotor Krugers) and Hoeijmakers (co-promotor Korosi) who study effects of early-life stress on the vulnerability to develop Alzheimer's disease (AD) in mouse models. In addition, Hesseling started as technician to work on the TNO and PRIOMED projects studying consequences of (early) exposure to Sarin or Blast or the psychopharmaca Ritalin and Fluoxetine for (adult) brain structure and function.

Research topics

Our research on plasticity partly focuses on the perinatal period with Krugers focusing on the molecular mechanisms underlying emotional memory formation, and early life effects on synaptic function and behavior, while Korosi studies effects of stress and nutrition during early life on epigenetics and the programming of adult brain structure and (dys) function. Korosi, Naninck, Yam and Lucassen in collaboration with pre-clinical, clinical and industry groups will investigate the role of early nutrients in programming of brain function and metabolism. Molecular tools, stem cell cultures and techniques for in vivo delivery of viral vectors in adult brain are operational in the Fitzsimons group to modulate brain plasticity and neurogenesis focusing on microRNAs, stress hormone rhythmicity and epilepsy.

Scientific output

Despite our teaching efforts, we kept up an excellent output for which we were awarded the *SILS Research Award 2013*. We published 14 papers, 1 book chapter, plus 4 additional high impact papers; 2x in *Mol Psychiatry* (IF 14,8), *PNAS* (IF 9,7) and one in *Trends in Neurosciences* (IF 13,5). The paper by Fitzsimons et al., 2013 further received the TOP Paper Award at the EndoNeuroPsycho meeting.

Grants/Academic activities/lectures/outreach

- Krugers obtained external funding from ZonMW-TOP and from USArmy.
- Korosi was awarded a personal MEERVOUD grant from NWO.
- Krugers is treasurer of the EBBS, board secretary of the Neurofederation, and member of Amsterdam Brain and Cognition (ABC). Korosi is a member of the ENP organizing board.
- Lucassen and Korosi organized a session on early stress in Munich and Korosi organized a symposium on early life programming in Barcelona and at the ENP meeting. Lucassen co-organized the TN2 conference on Alzheimer's disease and chaired the Alzheimer research symposium in Maastricht. He co-organized the first EUROGENESIS meeting in Bordeaux. Krugers (co-)organized (symposia on): 'Synapses under Stress' (Lunteren), the Publieksdag Hersenstichting Nederland, a Brainstorm session on 'PTSD' in Barcelona and a masterclass on 'The Stressed Brain' (Amsterdam). Fitzsimons organized a session on stress hormones and brain plasticity at the ENP meeting.
- Lucassen is chair of the SAB of the ISAO, is co-founder of the EUROGENESIS consortium and invited Fellow Member of the ECNP, of the Scientific Board of the TN2 meeting on Alzheimer's disease, Visiting Scientist at the Netherlands Institute for

Advanced Social Sciences (NIAS), board member of the Stichting 'Vrienden van het Herseninstituut' and chair of the jury for the ISAO Alzheimer Prize and Alzheimer Post-doc Prize. He joined the Editorial Boards of PlosOne, Neuroscience and Brain Plasticity and wrote an invited review for the LifeStyle Issue of *Acta Neuropathologica* (IF 9.7) and 2 book chapters. Korosi, Fitzsimons and Lucassen are invited guest editors of a Special Issue of the journal 'Neural Plasticity' on Adult Neurogenesis. Krugers wrote 2 invited reviews.

- The group has further delivered various (invited) lectures in; Groningen, Nijmegen, Amsterdam (NIN), Amersfoort, Nice, Barcelona, Regensburg, Yerevan (Krugers), Ketelhuis, VUMC, the Grand, Amsterdam, Bordeaux, ETH Zurich and ISS Rome (Lucassen), Leiden (Korosi), Oviedo, Bordeaux (Fitzsimons).
- Group members acted frequently as reviewer for; Nature Communications, Biol Psychiatry, J Neurosci, Frontiers, EJM, PloSOne, Neuroscience, Int J Dev NS, Exp Neur, J Endo, Behav Brain Res, J Neuroinflam, Cer Cortex, Brain Pathol, Brain Behav&Imm, Stress,

Hippocampus, Neurobiol Dis, and for the Welcome Trust, ISAO, NWO, Czech Science Foundation and Alzheimer Research UK.

- Group members acted as PhD opponent (10x Lucassen) (3x, Krugers) (1x Korosi).

- Group members participated in outreach activities (FNWI College tour Spui25 (Lucassen, Krugers), Alzheimer info days (Lucassen), High school lectures (Lucassen), World Alzheimer day (Lucassen), NEMO, Publieksdag Hersenstichting and ECNP newspaper (Krugers).

Teaching

2013 was a very busy year, our group was again 'Teaching champion' of SILS. Our master track 'Psychopharmaca and Pathophysiology', run for the third year by Korosi, Naninck and Lucassen, remained very popular and was again evaluated very well by the students, many of which have obtained a PhD position now, e.g. in Amsterdam, Utrecht, Nijmegen, Stockholm, Cambridge, USA or New Zealand. Krugers is Programme Director of the Research Master Brain and Cognitive Sciences.

Research highlights

- ▶ Glucocorticoid receptor distribution has been elucidated in various regions of the human brain. GR protein expression was found to be increased in the amygdala in depression.
- ▶ The glucocorticoid receptor regulates functional integration of newborn neurons in the adult hippocampus and fear-motivated behavior.
- ▶ Exercise modulates neurogenesis as well as neuropathology in Alzheimer mouse models.
- ▶ Nutrition and stress during early life are important in determining later cognition, neurogenesis and metabolism.

Research aims for the coming years

- ▶ To understand how the early environment can exert such lasting effects on brain structure and function, to identify the critical elements in that environment and to establish their influence on the risk to develop disorders like depression and dementia.
- ▶ To clarify how stress hormones, epigenetics and microRNAs can regulate adult neurogenesis and modify hippocampus-related cognition and (vulnerability to) epilepsy.
- ▶ To understand how early stress influences later synaptic plasticity, AMPA receptor dynamics, spinogenesis and emotional memory at an adult age.

Group members

Docent

Rob de Heus

PhD students

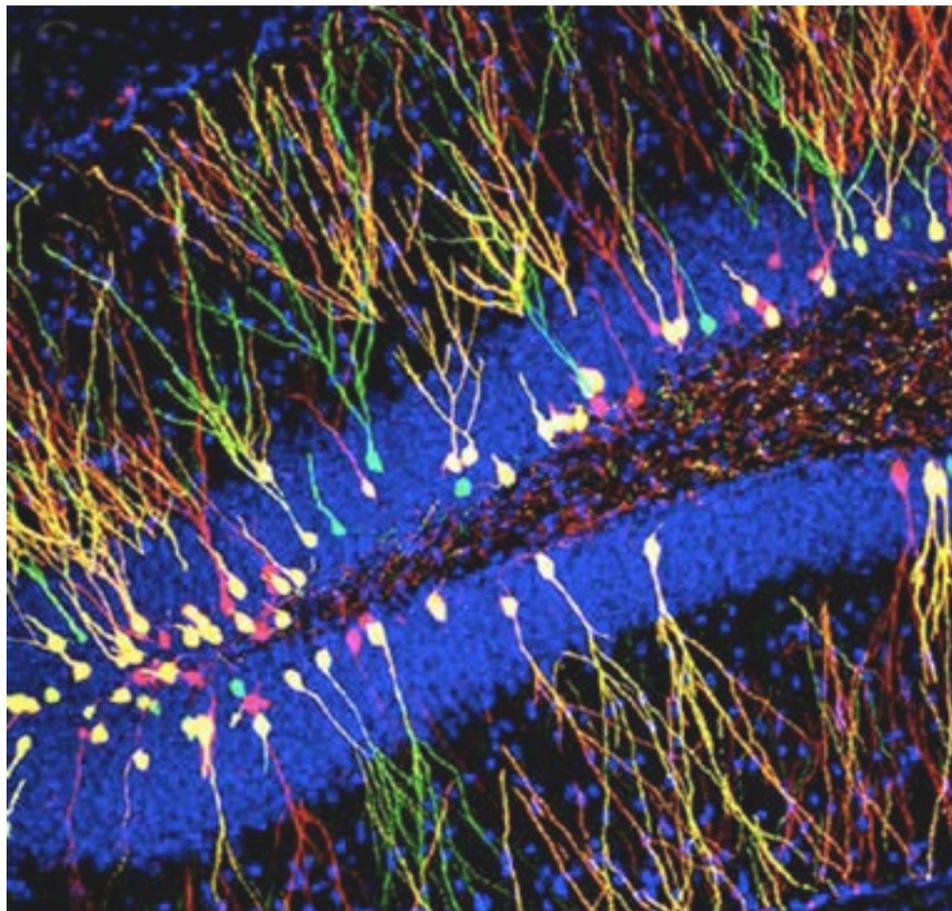
Marit Arp
Pascal Bielefeld
Karljin Doorn
Lianne Hoeijmakers
Sylvie Lesuis
Eva Naninck

Group members (continued)

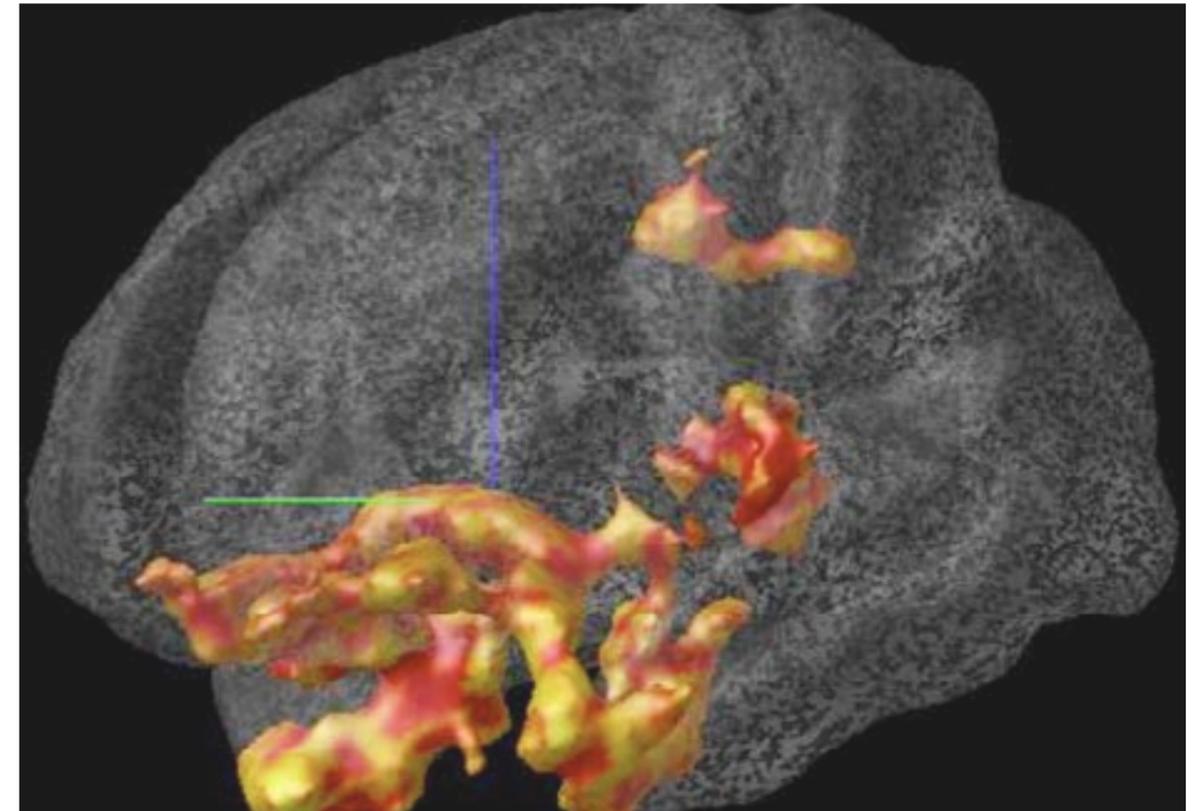
Marijn Schouten
Wendy Timmermans
Qian Wang
Hui Xiong

Technicians

Jan den Blaauwen
Gideon Meerhoff



Neurogenesis in the adult brain. Newly formed neurons are seen as white nerve cell bodies with their coloured branches (dendrites) in the adult hippocampus (blue), a brain region involved in learning and memory. Neurogenesis is influenced by stress and nutrition during early life (Korosi et al., *BBR* 2012; Lucassen et al., *TINS* 2013).



Human brain under stress. Red and yellow colours indicate the regions in the human brain that become activated when a person is exposed to stress. From; Lucassen et al., *Acta Neuropathol* 2014.

Academic publications

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Molecular Neuroscience

Prof. M.P. Smidt
Dr M.F.M. Hoekman
Dr. J.A. van Hooft

Chairholder
Assistant Professor
Assistant Professor

W

Within our laboratory we study the molecular biology that is behind developmental mechanisms in the central nervous system.

In our research team we endeavor to understand the molecular programming of specific neuronal groups within mdDA neurons and the cortex. To this end we have identified transcription factors that play key roles in these processes, such as Pitx3, Lmx1a, Engrailed1, Dlx1, Lmx1b and Nurr1 for mdDA neurons. To study developmental processes we use complex gene transfer models as in-utero electroporation and whole brain ex-vivo culturing, to track these neurons as they leave the ventricular zone and start differentiating and move to their final position where they send out axons and receive inputs from other systems. A combination of these techniques with more classical genetic models (Nurr1-ko, Pitx3-ko, Lmx1a-ko, Hdac2-ko, Pitx3-Cre driver etc.) provide us with the essential tools to answer our research questions.

Active research lines

1. Role of FoxO factors in Cortical development.
2. Regulation of "Clock" by FoxO factors.

3. Role of neurotransmitters in structural brain development.

4. Epigenetic mechanisms active during brain development.

5. Molecular programming of mesodiencephalic dopaminergic (mdDA) neurons.

A. Role of retinoids in the terminal differentiation of mdDA neurons.

B. Cross-talk of homeodomain factors with the Nurr1 transcriptional complex in steering the mdDA neuronal phenotype.

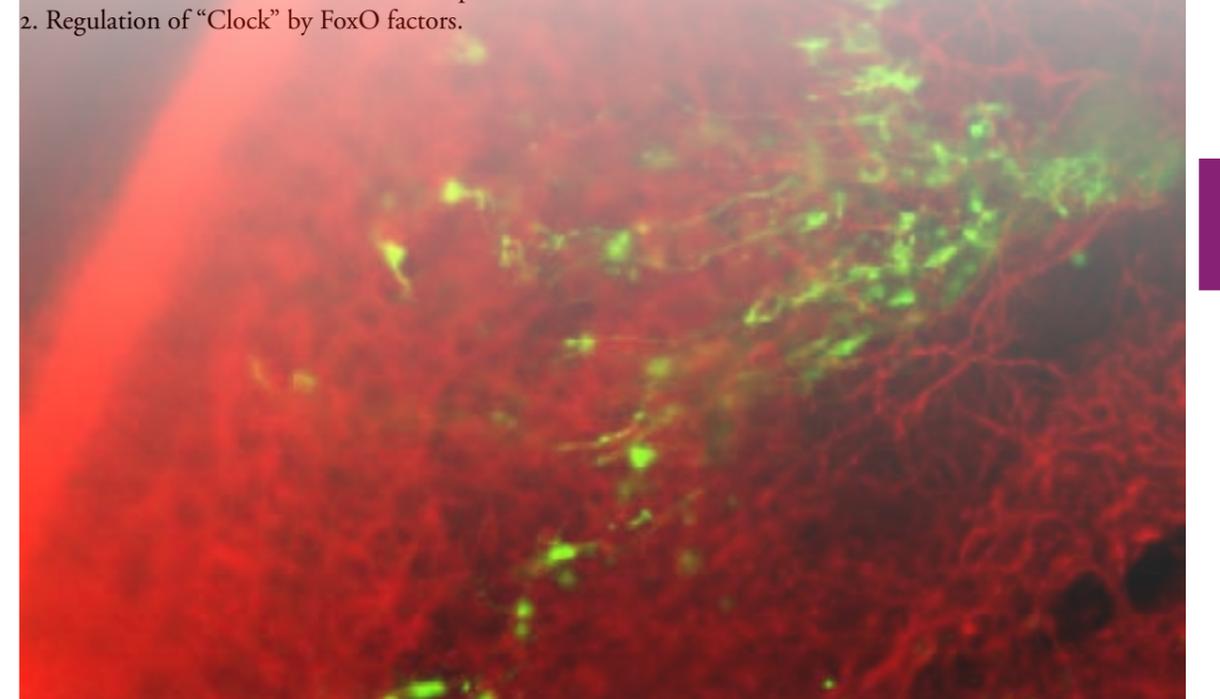
C. Ventricular zone coding as signaling center for mdDA subset specification.

D. Role of En1 in specifying the mdDA neuronal phenotype.

E. Tgf-beta signaling in mdDA neuronal development and survival.

F. Role of Lmx1a and Lmx1b in specification of the mesodiencephalon and subset specification within mdDA neurons.

G. Rostral/caudal genetic programming within mdDA neuronal subsets.



Academic publications

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Relevant positions

Smidt, M.P. Coordinator of the SILS art. 9 WOD course and part of the Dutch coordination program on animal research.

Group members

Postdoctoral researcher

Lars van der Heide
Elise Hoekstra (until 1 June 2013)

PhD students

Erik van Heesbeen
Willemieke Kouwenhoven
Simone Mesman
Ricardo Paap
Jesse Veenvliet
Iris Wever

Technicians

Lars van Oerthel
Saskia Oosterbroek

Research highlights

► This year was our first normal year of full functionality. We started with four new researchers (2 PhD student and 2 assistant professors). We still have 2 PhD positions open and 1 assistant professor position which will all be implemented in 2014.

► After discussion within the Neuroscience groups it was decided that Dr. Hans van Hooft moved from the Wadman group to the our group and he will be actively implementing advanced neurophysiology in the running research lines and will expand on this. In order to do so we have setup a system for performing optogenetics. Together with this setup we have developed mouse models where we introduce channelrhodopsins in mdDA neurons by our developed Pitx3-Cre driver. This setup has been validated and is fully functional. This approach will also be used by other researchers within SILS. In addition, we have setup an in-utero electroporation approach with two different constructs of channelrhodopsins coupled to either Gfp or mCherry. This setup will be used for studying cortical interneuron development and function. This last approach is complemented by specific interneuron-Cre drivers as VP-Cre (present in the lab).

► We have been active in terms of publications and have successfully published 9 papers in 2013 (see academic output).

► The main advancement is not restricted to academic output, we were also successful in implementing research techniques as in-utero and ex-vivo and focal electroporation. These "state of the art" transient in-vivo gene transfer methods provide a unique opportunity to study in depth the role of critical genetic modulators during brain development. Moreover, we have broadened our use of the FACS-Aria-3 machine (fluorescent cell sorter) in combination with advanced transcriptomics (in collaboration with the micro-array department).

► Finally, we have been active in applying for research grants: Zwaartekracht, M.J. Fox foundation, NWO-TOP (3*)(zonMW), NWO-ALW openprogramme (3*), MRA-grant (Impact), ZonMW-project (kennis met minder dieren (co-PI)), NWO-ALW open access publications, Autism speaks, Simons foundation and Hersenstichting. Of all these programs the ZonMw project, the open access publications grants (3*) and the MRA grant were successful up to writing this report.

Educational highlights

▶ We have been active in many aspects of education, some of the main activities are mentioned here:

▶ We have developed and started the new Master program “Molecular NeuroScience (MNS)” The courses (3) have been performed and are very well evaluated ranging from 7.8 to 8.1. The current students (17) are allocated to internships successfully and we hope to welcome the second cohort of students in 2014.

▶ We have been active as coordinators for the following programs:

▶ Bachelor Biomedical sciences: Neurobiology track biomedical sciences (dr. Hans van Hooft)

▶ Bachelor Psychobiology: Labvaardigheden course; Cell biology course (dr. Marco Hoekman)

▶ UvA masters: Molecular Neuroscience master program (dr. Lars van der Heide/Prof. dr. Marten Smidt).

▶ Moreover, we have been and are still active in the setup of the new bachelor program for Psychobiology (dr. Marco Hoekman). Next to these activities we are involved in teaching courses in year 1-3 of the bachelor program Psychobiology and Biomedical sciences.

Other highlights

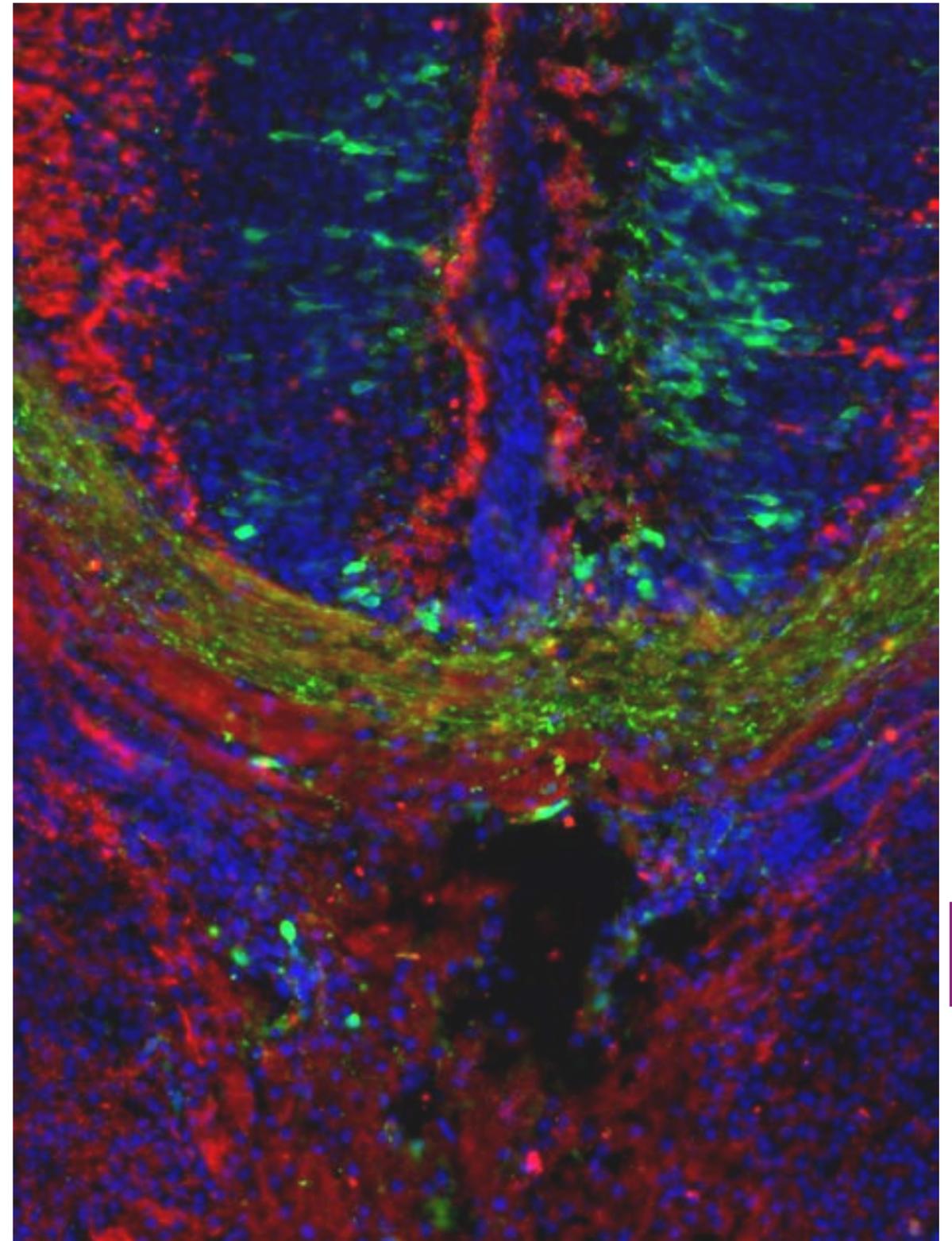
▶ Smidt is Chairman of the Exam-committee Psychobiology. The largest bachelor at the FNWI.

▶ Smidt is program director/developer of the neurobiology cluster of the UvA master program.

▶ Smidt has obtained a national accreditation to perform art9 WOD courses at the UvA. This course is setup now and is scheduled to run for the first time in June 2014 as part of the masters program.

▶ Smidt has been re-appointed as the Chairman of the NWO-VIDI committee for 2014.

▶ Smidt has been appointed coordinator of art 9 WOD and is part of the Dutch coordination program on animal research.



Coronal brain section with a triple staining of the developing corpus callosum. Green: Gfp, showing axons derived from in utero electroporated primary cortical neurons present in one of the hemispheres. ; Bleu: Dapi, indicating nuclei; Red: NeuN, neuronal marker (Molecular Neuroscience, experiment by Ricardo Paap).