University of Amsterdam
Faculty of Science
Swammerdam Institute for Life Sciences
Annual report 2010

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Science Park 904, photos by Muus de Haan
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Preface by the director

The Swammerdam Institute for Life Sciences: developments in 2010

The most important development in 2010 was the relocation of the SILS research groups that were still housed at the Roeterseiland to the new building of the Faculty of Science at the Science Park Amsterdam. Finally, ten years after the establishment of SILS, all personnel is now housed together in state-of-the-art laboratory facilities and offices, which will definitively lead to more extensive sharing of knowledge, facilities and equipment between the different groups. All eight research institutes of the Faculty of Science, its teaching organisation and its directorate are now housed together in a building having a length of 260 meters, a width of 50 to 100 meters and a height of 26 meters.

In 2010 the number of bachelors that enrolled for the first time in one of the programmes of the Faculty of Science increased to over 1000. Fifty five percent of them have chosen Biomedical Sciences, Psychobiology or Biology, which are bachelor programmes to which SILS staff contributes substantially. The number of students that chose one of the master programmes that are organised by SILS or have a substantial SILS involvement, i.e. Biomedical Sciences, Brain & Cognition, Biology and Life Sciences/Systems Biology, increased substantially as well. Noteworthy is the success of the new master track Psychopharmacology and Pathophysiology that attracted 20 students at the start. Income of SILS out of educational activities in 2010 reached M€ 1,8.

Ten PhD students E. Lee, C. Oomen, S. Arisz, D. Vis, C. Rubingh, R. Orij, E. van Velzen, M. Nessen, L. Smit-Rigter and W. Huijbers who were employed by SILS and supervised by one of the full professors of SILS, defended their thesis in 2010 while two PhD students, V. Peperzak and C. Maas, were supervised by Prof. dr. J. Borst of the Netherlands Cancer Institute, who is a SILS professor by special appointment.

Next to 28 novel externally funded projects in 2010, personal grants were also awarded to four SILS scientists. Dr. M. Rep obtained a NWO VICI grant of k€ 1500 while dr. C. Fitzsimons, dr. M. Postma and dr. H. van den Burg each obtained a NWO VIDI grant of k€ 800. In total SILS staff obtained M€ 8.6 out of external research grants in 2010 on a total budget of M€ 19.

SILS’ output regarding scientific publications reached an impressive 150 peer-reviewed publications. The highlights of 2010 were publications in Nature Methods (Th. Gadella, J. Goedhart, L. van Weeren, M. Hink and N. Vischer), Nature (M. Rep, C. van der Does, P. Houterman, and W. Jonkers), Science (R. Orij and G. Smits), Science (A. Otte) and Cell (T. den Blaauwen, B. van den Berg van Saparoea and J. Verheul).

Other activities in 2010 were the annual SILS Research Day organised by the SILS PhD/PD Council in June and, at the end of 2010, the SILS Retreat that was attended by 34 staff members. The research programmes of all groups were discussed, improvements of the organisation of SILS were suggested and developments in the life sciences in The Netherlands and the position of SILS in this exciting field evaluated. Overall it was concluded that in the coming years there are enough opportunities to further improve SILS’ performance and promise a bright future.

Finally, in July dr. H.D. Veldhuis, left SILS after 5 years of being in charge of the institute.

Prof. dr. W.J. Stiekema
director
Research groups within the Swammerdam Institute for Life Sciences

Systems Biology of the Living Cell

Molecular Microbial Physiology  Prof.dr. K.J. Hellingwerf
Molecular Biology and Microbial Food Safety  Prof.dr. S. Brul
Nuclear Organisation  Prof.dr. R. van Driel
Epigenetic Regulation of Gene Expression  Prof.dr. A.P. Otte
Molecular Cytology  Prof.dr. Th.W.J. Gadella

Plant Signalling

Plant Physiology  Prof.dr. M.A. Haring
Molecular Plant Pathology  Prof.dr. B.J.C. Cornelissen

SILS Center for NeuroScience

Cognitive and Systems Neuroscience  Prof.dr. C.M.A. Pennartz
Cellular and Systems Neurobiology  Prof.dr. W.J. Wadman
Structural and functional plasticity of the nervous system  Prof.dr. P.J. Lucassen

Life Science Technologies

Mass Spectrometry of Biomacromolecules  Prof.dr. C.G. de Koster
BioSystems Data Analysis  Prof.dr. A.K. Smilde
Micro Array Department and Integrated Bioinformatics Unit  Dr. T.M. Breit
Molecular Microbial Physiology

Chairholder: Prof. dr. K.J. Hellingwerf
Prof. dr. M.J. Teixeira de Mattos Professor
Prof. dr. J. Hugenholtz Special Chair

Introduction
The impact that microbes exert on life on Earth and indeed on Earth itself has been well-recognised now for many decades. The activity of microorganisms is not seen only at the global levels of evolution, the cycle of elements, ecological interactions and so on, but also affects human life directly both for better and for worse. Our knowledge about the biochemistry and physiology of microbial species is extensive but in many 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 apply their many beneficial capacities, that is, transforming fundamental insights into applications. The Molecular Microbial Physiology Group (MMPG) has recognised the need for the "new" quantitative microbiology and has in the past years shifted its research accordingly to what may be called Systems Microbiology. Our research deals with integrating the properties of biochemical/biophysical networks such as glycolysis, photosynthesis and respiration with signal transduction and signal processing, with structure-function relations and with physiological strategies for survival and growth. This integrative 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 the chemotrophic cell). The work aims at understanding how their life style endows microbes with the capacity to successfully cope with often severe and ever-changing environments.

The broad diversity of microbial genera, and the large genetic, biochemical and physiological differences between the genera, makes it unavoidable to focus the research on more than one species. We study both chemoheterotrophs, including the Gram-positive, endospore-forming Bacillus subtilis, industrially relevant Lactic Acid Bacteria and the metabolically extremely versatile Gram-negative Escherichia coli, and the model organism for oxygenic photosynthesis: Synechocystis. For a large part our interest in Lactic Acid Bacteria stems from the expertise of Professor Hugenholtz. This year he moved from the Dutch Institute for Dairy Research (NIZO) to the Coca Cola Company where he will be the Director of the Fermentation Research Center in Germany. Nevertheless his activities with respect to teaching and supervision of research within our group will be continued.

Research Highlights
- Our work on the significance of the respiratory chain of E. coli, being branched and partially uncoupled from energy conservation, has attracted much attention. New collaborations have been initiated to confirm the challenging suggestion that alternative catabolic routes are of much more importance than expected to
date. Furthermore, the role of the various quinone species as key redox signals has become apparent.

- The SUMO-2 project (a second NWO-grant as part of the transnational SysMo-2 initiative) has started this year: the physiological and biochemical characterisation of relevant mutants is now being carried out to provide data for a kinetic model of the respiratory chain that is being constructed in collaboration with Dr F. Bruggeman.

- Another follow-up Sysmo project, SysmoLAB has been awarded. Here, the aim is to model catabolism in lactic acid bacteria. The most challenging finding was made that the well-known catabolic switch from homolactic to mixed acid fermentation, invoked by the availability of external energy resources, is mainly controlled by the allosteric regulators Fructose- bis-phosphate and Glyceraldehyde-phosphate rather than by changes in gene expression.

- An important observation has been made related to the functional photocycle of Photoactive Yellow Protein. For the first time direct evidence has been provided that the photo-isomerization of the ethylene bond of the chromophore of PYP has to be accompanied by additional single-bond rotation. A multidisciplinary comparison (using a.o. picosecond time-resolved IR studies and molecular dynamics simulations) of the photochemistry of a set of mutant proteins has resulted in a consistent picture of the photo-isomerization event: as the carbonyl group of the chromophore is held more and more strongly, e.g. through the formation of multiple hydrogen bonds, the quantum yield of photo-isomerization of PYP decreases progressively. By the use of another set of mutant PYP proteins, which allow tryptophan fluorescence to monitor the entry of hydrated protons into the main hydrophobic core of the protein, we are resolving the sequential events of proton transfer to the chromophore, a key step that has to take place before the signalling state of PYP can be formed. This work is carried out in close collaboration with Profs. Groot and Van Grondelle (Vrije University Amsterdam), Larsen (UC Davis, Ca, USA) and Boelens (University of Utrecht).

- The general stress response (GSR) of the chemotrophic Gram-positive Bacillus subtilis can be activated by diverse input signals ranging from energy limitations to salt-, ethanol- or heat-shock. Our group has shown that blue light also acts as an activator. Induction is under control of a photoreceptor protein called YtvA, which exerts its function through a large protein complex called the stressosome. We have discovered a (chronologically) second effect of light on the activation of the GSR, regulated by the RsbP/Q pathway that also relays information on energy stress to the cells.

- Our research on the use of photosynthetic cyanobacteria for the production of a variety of products from CO₂ continues steadily. Site-directed insertions of gene-cassettes from fermentative organisms into the photosynthetic Synechocystis sp. PCC 6803 have been obtained resulting in organisms that are able to produce ethanol. The same approach was used successfully for lactate and ethene. Now we will extend this to other products like butanol. In addition, we focus on elucidating how environmental conditions and intracellular metabolic regulation affect the flux to these products, at the genetic, biochemical and physiological level.

Other Highlights

- The core patent that describes the technology to engineer photosynthetic cyanobacteria for the production of valuable chemicals has been registered.

- A research proposal entitled: “Expanding society’s toolbox to harvest solar energy: Creating multi-scale computational models to optimize oxygenic photosynthesis” (coordinator: Prof. Hellingwerf and submitted under the call “Towards BioSolar Cells” of FOM/ALW) has been granted to a consortium
of UvA, VUA, WUR, AMOLF and CWI. We anticipate that a group of five PhD students will take up this challenging task.
- A grant application by Photanol BV for the "Bioraffinage" theme financed by the Ministries of Economic Affairs and Agriculture, Nature and Food (LNV) was ranked as #1 in the Netherlands and awarded as of August 2010. The grant will be applied for the design, construction and running of a pilot plant. The plant will be housed in the greenhouse of the Faculty at Science Park.
- Prof. Hellingwerf, as a co-applicant with Prof. Van Grondelle (VUA) has been invited by FOM/NOW to submit a grant proposal to found a national Focus Group for research on “Fuel by Photosynthesis”.

Research aims for the coming year
- The two Sysmo projects will continue to integrate the physiological and biochemical experimental data with the modelling. For SUMO, a kinetic model will be constructed that includes kinetic data on components of the respiratory chain from literature and obtained from our own experiments. Data will include non-steady state analysis of fluxes and metabolites. The model will include proton gradients and eventually the proton motive force. Finally, such a kinetic model will be linked to existing models and models under construction that describe other catabolic units, such as transport, glycolysis and tca cycle. Related to this subject will be the investigation on the role of alternative glycolytic pathways/enzymes to further unravel the energetic efficiency of catabolism. For SysmoLAB, we will aim at pinpointing the biochemical and genetic control points that define the differences between different lactic acid bacteria.
- Photofermentation research will not just continue along the current lines but be strengthened by the start of the TBSC initiative. Besides focus on photofermentative production of butanol and related intermediates, fundamental research that includes a systems biological analysis of the effects of inserting heterologous pathways in primary metabolic routes will be enhanced. As for the previous period, pathway expression will be optimized through gene-amplification strategies.
- Site-directed mutagenesis, combined with high resolution time-resolved spectrometric technologies will continue to be an important tool in our studies on microbial photoreceptor and photosignalling structures, functions and mechanisms.
- Mutant strains of Lactobacillus johnsonii, lacking one or more of the hydrogen peroxide producing systems will be made available by our collaborators at Nestlé, Zurich, Switzerland. This will be very helpful in the NIZO-UvA research collaboration on stress response, population heterogeneity and survival of this probiotic organism. Time-resolved transcriptome analyses will be carried out on perturbed (e.g. anaerobic/aerobic transitions) steady state cultures to describe the events that precede and accompany the stress response.
Molecular Biology and Microbial Food Safety

Chairholder: Prof. dr. S. Brul

Dr. J.C. van der Spek  Assistant Professor
Dr. G.J. Smits  Assistant Professor
Dr. F.M. Klis  Senior scientist (former Associate Professor)
Dr. B. Ter Kuile  Researcher Dutch Food & Drug Authority (VWA)

Introduction
2010 saw a strengthening of the group in relation to our efforts on fundamental understanding of stress response mechanisms in (micro)organisms. We focus on the bioenergetics of the response as well as on pH homeostasis. Both are topical and relate to homeostasis in yeast and the simple multicellular eukaryote Caenorhabditis elegans. The latter is used where single cell eukaryotes are insufficient to explain a stress phenotype. In the McGillavry application round for excellent young talent we were successful in attracting a young PI from Stanford University who will reinforce the C. elegans team.

A prime topic is the development of reactive oxygen species-derived damage as a consequence of energy stress. Some 10 years ago, as a spin-off of the yeast work, we started studies in the field of bacterial spore formers starting from the same questions on bioenergetics and pH maintenance driven by demands from practice where weak-organic acids are used as prime food preservatives. Such application-driven research also include elevated temperature and antibiotic stress. Finally, in the framework of a large EU consortium we focus on putting our knowledge of Candida cell wall proteins to use for the development of vaccines. In 2010, we converted our work into 15 publications most of which were published before the end of the year. Most notably, Dr. Gertien Smits and our PhD student Rick Orij were co-authors of a paper in Science. The introduction of the thesis has meanwhile been converted into a review for BBA on the behaviour of the intracellular pH in eukaryotic cells focusing on yeast. Furthermore, in all studies a systems approach is advocated. We contributed with 4 authors together with the researchers of the Free University and the TU-Delft to a paper in FEBS journal (van Eunen, 2010) that set standards for the measurement of enzyme activities under in vivo-like conditions for systems biology. The paper won a prestigious prize of the European Federation

Future Prospects & Societal impact
Spin-off of our studies in society aims at contributing to improved food safety in close collaboration with the Dutch Food Safety Authority (Havelaar et al., 2010). To this end a new 4-year contract for sponsoring of research on antibiotic resistance was secured. Secondly, the group continues to deliver data with the STW-sponsored post-doctoral fellow Alex Ter Beek to the benefit of the discovery of the Achilles heel of food spoilage bacterial spore formers. Thirdly, we are key players in a large European project on vaccine development against an important group of infectious diseases caused by Candida albicans. In this context, group leader prof. Brul was asked to organise a symposium at the large 2011 FEMS European Congres of Microbiology in Geneve. We actively pursue patent opportunities in the application areas of our research.

Research Highlights
- A collaboration with the Canadian research group of Dr. Chris Loewen resulted in co-authorships on a Science publication describing the role of the intracellular pH in regulating the inositol biosynthesis and thus tuning lipid metabolism to the availability of sugar substrate (Young et al. 2010). The paper was part of the PhD thesis of our PhD student Rick Orij who successfully defended his thesis. The introduction of the thesis has meanwhile been converted into a review for BBA on the behaviour of the intracellular pH in eukaryotic cells focusing on yeast. Furthermore, in all studies a systems approach is advocated. We contributed with 4 authors together with the researchers of the Free University and the TU-Delft to a paper in FEBS journal (van Eunen, 2010) that set standards for the measurement of enzyme activities under in vivo-like conditions for systems biology. The paper won a prestigious prize of the European Federation
for Biochemical Societies as the best paper published in 2010 in the journal by a young first author scientist.

- In 2010 our group was successful in a collaborative effort with the de Mass Spectrometry of Biomacromolecules Group of de Koster and the Molecular Microbial Physiology Group of Hellingwerf in the quantification of the *Candida albicans* cell wall proteome in response to the environmental pH, crucial to health and disease (Sosinska et al., 2010). Work in our group in collaboration with the de Koster group at SILS has subsequently focused on establishing a comprehensive quantitative proteomics analysis of the hyphal and yeast form of *Candida* in response to a wide array of hyphal inducers (Heilmann et al. 2011, under final review). In addition, we have covered the secretome of the pathogenic yeast (Sorgo et al., 2010), providing markers for monitoring the presence of *Candida* cells in body fluids. A fully updated review of the presence and importance of yeast cell wall proteins was published (Klis et al., 2010). The proteomics analysis of microbial cell walls was extended to the bacterial spores by ERASMUS MUNDUS fellow Wishwas Abhyankar (Brul et al. appeared in 2011 Food Microbiology; Abhyankar et al. Proteomics under revision).

- In our line of research on bacterial spore-formers we continued our studies on bacterial spore germination and outgrowth inhibition in the framework of an STW-sponsored project. A prime focus is on enhancing the efficacy of weak organic acids for this purpose (Ter Beek and Brul, 2010). Furthermore, we established experimental systems to analyse the stress resistant coat of Bacillus spores (above) as well as to assess at single spore level germination and outgrowth, using live imaging techniques (Ter Beek et al., appeared in 2011 Food Microbiology; Pandey, 2011 submitted). Both sponsored by ERASMUS Mundus and the latter in close collaboration with the Centre for Advanced Microscopy of SILS (Prof. E. Manders).

- The bacterial research-line focusing on antibiotic resistance development directly linked to microbial food safety, continued to run in close collaboration with the Dutch Food Safety Authority (VWA). The data show that resistance acquisition of bacteria to fluoroquinolone exposure can be very rapid (Schuurmans et al. 2010). Such resistance can be both adaptation-driven as well as occur de novo and is not limited to this type of antibiotics (van der Horst et al. 2011). Genome sequencing studies were successfully initiated with the AMC group of Frank Baas. Challenges to microbial food preservation were extensively discussed (Havelaar et al., 2010).

### 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; STW fellowship started; FES funds (Nanonext) secured; VWA grant applied for (meanwhile assured); member of the STW VICI committee and EU FP7 project reviews.

**F.M. Klis**: Editor Eukaryotic Cell, FEMS Yeast Research, Yeast.

**McGillavry fellow**: Y. Budovskaya from Stanford reinforces our *Caenorhabditis elegans* model work.

### Research aims for the coming year

- **Weak organic acid stress mechanisms; what is the role of energy metabolism and which are the efficient long term responses.** In the study of yeast and *Bacillus* energy metabolism and short term stress response against these compounds we focus on both second scale time-resolved measurements of intracellular pH perturbation, verification of membrane potential as well as membrane structural long-term adaptations. With the department of Mass Spectrometry of Biomacromolecules we will set up a system in yeast and *C. elegans* to assess the energy stress induced ROS-dependent cellular damage (see also ageing stress).

- **The mechanisms of temperature stress response and resistance.** This research line includes vegetative stress response to thermal stress as well as bacterial spore response towards extreme thermal challenge. The line on vegetative stress will be concluded with the PhD defense by student Jarne Postmus...
(fulfilled 2011). Further efforts in the field will focus on an analysis of bacterial spore thermal resistance. We will analyse heat damage repair systems and characterise the primary protein composition of the spore (coat). Single spore germination and outgrowth data will be gathered using live imaging.

- **Antibiotic / mycotic stress response.** Here we will continue our work on the detailed proteomic analysis of the *Candida* cell wall proteins for vaccine generation against this medically relevant yeast in collaboration with Dr. Mihai Netea (Nijmegen). In a preventative setting the studies on antibiotic resistance development will be continued and expanded to include genome wide sequence data.

- **Mechanisms of ageing (stress).** This new line of research by the McGillavry fellow will be affirmed. We will initiate a collaborative project to study the molecular mechanisms of ageing in *C. elegans* together with the Center for Advanced Microscopy (prof. E. Manders) and the department of Mass Spectrometry of Biomacromolecules (prof. C. de Koster). In particular we are planning to establish methodology to study effect of ageing, diet, and stress on protein oxidation throughout the life span of nematodes using quantitative proteomics.
Nuclear Organisation

Chairholder: Prof.dr. R. van Driel

Dr. P.F. Fransz  Assistant Professor
Dr. M.E. Stam  Assistant Professor
Dr. P.J. Verschure  Assistant Professor

Introduction

Nuclear Organisation Group (NOG)

Gene expression is controlled by different mechanisms, including epigenetic modifications, intra-and interchromosomal interactions and chromatin folding. Our mission is to understand how these mechanisms control genome activity together. We concentrate on the dynamics of chromatin structure in relation to gene expression and DNA repair. Our methodology involves a multi-disciplinary approach combining microscopic, molecular, biochemical and genetic analyses, and mathematical modelling. Predictive mathematical modelling is used to develop precise quantitative working hypotheses that constitute the basis of our experiments. The functional relationship between gene regulation, nuclear organisation and chromatin structure is evolutionary conserved in eukaryotic cells. Combining information from different model systems gives us unique insights into structure-function relationships of the eukaryotic genome inside the nucleus.

Research Highlights

Paul Fransz and colleagues published the intriguing phenomenon of light-induced reorganisation of chromatin, which is a reversible process and regulated by the photoreceptors CRY2 and PHYB. A model is proposed in which light controls chromatin decompaction via CRY2 and PHYB. The data support the concept that stress and developmental changes trigger responses in the global organisation of chromatin.

Maike Stam and colleagues published findings providing insight into the mechanisms underlying paramutation and tissue-specific regulation at the chromatin structure level in maize. Their results indicate a role for DNA methylation in the establishment and heritable maintenance of a silenced chromatin state, while histone modifications appear mainly involved in tissue-specific regulation of gene expression.

Roel van Driel and coworkers continued the systems biology projects on (i) in vivo assembly and functional behaviour of chromatin-associated multi-protein complexes, and (ii) folding of the chromatin fibre in the interphase nucleus in relation to local transcriptional activity. In both cases key predictions made by recently published quantitative models were tested experimentally. Results are translated to updated models, creating new insights into the functioning and underlying principles of these nuclear systems.

Pernette Verschure and colleagues created computational models explaining stochastic gene activity as well as ‘spreading’ of the epigenetic state over nucleosomes to predict the behaviour of epigenetically toggled synthetic mammalian cell systems. The experimental measurements of the epigenetically modulated state within these systems are ongoing.

Other Highlights

Roel van Driel is director of the national NGI-funded research programme Netherlands Consortium for Systems Biology (NCSB) and advisor of several German national systems biology programmes. Pernette Verschure chaired the executive board of the Women in the Faculty network (WiF). Maike Stam was independent member of the appointment advisory committee on the MacGillavry tenure track for woman at the FNWI.


Verschure, P.J. (2010). Mammalian Synthetic Biology from tools to therapies. Organisation of
Mini-symposium (SILS-NISB): Amsterdam, the Netherlands (2010, December 09).

**Research aims for the coming year**

*Fransz and colleagues*

Our aim is to assess the relationship between chromosome folding and nuclear reprogramming and to investigate long-range chromosome interactions. The research in 2011 will concentrate on (1) tracking an entire chromosome during interphase and (2) setting up experiments to study chromosome looping in collaboration with M. Stam.

*Stam and colleagues*

The general aim is to get insight into the functional relation between gene activity, epigenetic mechanisms and chromosomal interactions. Together with P. Fransz, in 2011 a study in Arabidopsis will be started in which the generated data will be used in mathematical modelling to establish quantitative relationships between chromatin folding and gene activity (collaboration with Prof. Heermann, Heidelberg, Germany).

*Van Driel and coworkers*

The two systems biology projects will be continued, focusing on experimental results that do not agree with the present versions of the predictive models. Updating the models in collaboration with the Heermann and the Höfer groups, both in Heidelberg, will have priority. In particular, we will put effort in unveiling novel emerging system properties.

*Verschure and colleagues*

We expand our research to understand design principles of epigenetic regulation: (1) real-time transcription measurements, (2) single-cell/molecule transcript counting combined with mathematical modelling to study a set of variably regulated mammalian genes (3) (epi)genetic network behaviour of Huntington’s disease onset using bioinformatics and computational models (HD) (collaboration Dr. A. Kremer ErasmusMC).

**Fluorescent DNA halo.**

Upon permeabilization and treatment of nuclei with high-salt buffers, supercoiled DNA loops unwind and form a halo (red) around an insoluble nuclear scaffold (yellow). The technique is used for estimating the average length of DNA loops in interphase nuclei.
Epigenetic Regulation of Gene Expression

Chairholder: Prof. dr. A.P. Otte
Dr. Ir. J.A. Verhees Assistant Professor

Introduction
It is our aim to understand aspects of epigenetic regulation of gene expression. Research is focused on genomic elements that have a powerful, positive influence on promoter activity. These elements are employed to facilitate the production of therapeutic proteins in mammalian cell lines. However, the increased protein expression levels have a negative influence on cell growth. Very high protein expression levels force cells to stop growing, which is an undesirable phenomenon from a practical point of view. We attempt to understand this inverse relationship between protein expression levels and cell growth at a quantitative level. Furthermore, we develop inducible gene expression systems in which we can reversibly modulate protein expression levels and cell growth.

Research Highlights
We have identified novel genomic elements that are initiation points for high levels of transcription. These elements probably provide a more ‘open’ chromatin state in which a transfected gene that is flanked by these elements becomes more open for transcription. In order to develop novel inducible expression systems in which we can reversibly modulate protein expression levels and cell growth, we devised a novel set of selection markers. These markers include the Zeocin resistance protein, as well as markers that restore the synthesis of essential metabolic components that cells normally lack.

Application of these markers warrants both high protein expression levels as well as a high degree of stability of protein expression over prolonged periods of time.

Other Highlight
Director of a biotechnology company, Cellagenics, a spin-off company, emerging from SILS, FNWI and the UvA Holding in 2008. Cellagenics works in the research area of expression of therapeutic proteins and cell growth.

Future Prospects
In the coming year we will focus on further developing expression systems in which protein expression, growth rates of the cells and secretion of the proteins can be coordinately modulated to achieve an optimal expression platform for therapeutic proteins. As such we aim to investigate and modulate in a comprehensive way:
1) the role of novel genomic elements in expression and stability of protein expression;
2) an inverse relationship between cell growth and protein expression levels.
We are also in the process of pursuing collaborations with industrial parties to evaluate our findings and to test our newly developed protein expression platform at an industrial scale.
Molecular Cytology

Chairholder: Prof.dr. T.W.J. Gadella

Dr. T. den Blaauwen  Assistant Professor
Dr. Ir. J. Goedhart  Assistant Professor
Dr. E. M. M. Manders  Assistant Professor
Dr. Ir. M.A. Hink  Assistant Professor

Introduction

Molecular Cytology & Centre for Advanced Microscopy (CAM):

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 into 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) Spatial organisation of sub-cellular signalling (group leaders prof. dr. T.W.J. Gadella, dr. J. Goedhart & dr. M.A. Hink). 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) and 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-alfaQ to PLC activation triggering downstream calcium, kinase signalling and small GTPase (Rho/Rac/Cdc24) signalling. The close intertwining of several signalling cascades and our quantitative microscopy approach both necessitates and permits the generation of quantitative predictive modelling, which will effectively integrate this research line with Systems Biology approaches.

2) Molecular dynamics of the bacterial cycle (group leader dr. T. den Blaauwen). The morphology of rod shaped bacteria is achieved through two very dynamic synthetic complexes: the elongasome and the divisome. The elongasomes use the actin-like cytoskeleton MreB helix underneath the plasma membrane as a tracking device to elongate the cell envelope whereas the divisome is responsible for the division and synthesis of new cell poles. Cell division is directed by the FtsZ ring (a tubulin homolog), which exerts a small force on the bacterial envelope. The assembly and the dynamics of the elongasome and divisome are studied in vivo using immunofluorescence and fluorescence microscopy techniques (FRET, FRAP, localisation) and in vitro using state of the art biochemical and biophysical techniques. By aiming to obtain quantitative data, we hope to model the measured and observed interactions.

3) These research themes heavily depend on advanced microscopy technology organised within the Centre for Advanced Microscopy (CAM, 2004). The goal of CAM (em. prof. dr. G.J. Brakenhoff, prof. dr. T.W.J. Gadella, dr. E.M.M. Manders and dr. M. Hink) is to boost Life Sciences research using & developing (optical) microscopy techniques. Current most prominent developments are Controlled Light Exposure Microscopy (CLEM) (dr. Manders), multimode Fluorescence Lifetime Imaging Microscopy (FLIM)
Research Highlights

- Main scientific achievements in 2010 were the publication in Nature Methods of mTurquoise, the brightest cyan fluorescent protein to date, and the elucidation of a regulation mechanism for peptidoglycan synthesis in the cell wall of *E. coli*, published in Cell.

- In 2010 the van Leeuwenhoek Centre for Advanced Microscopy (LCAM) was founded as a formal collaboration between the Centre for Advanced Microscopy (SILS), the Centre for Microscopy Research (CMO) at the AMC (prof. C. J. van Noorden) and the Cell Biophysics group at the NKI (dr. K. Jalink).

- In 2010 we successfully implemented super resolution microscopy (photoactivated localisation microscopy, PALM). Hereby we achieved an accuracy of 7 nm resolution in light microscopic objects. This is 30x below the diffraction-limited resolution.

- In 2010 a new high-end microscope was installed (funded by NWO middelgroot) allowing single-molecule analysis by advanced spectroscopic techniques.

Other Highlights

- Erik Manders was appointed as guest professor Microscopic Techniques at the faculty of Bioscience Engineering of the University of Ghent (Belgium).

- Marten Postma was awarded an NWO VIDI grant on “Computational modelling of Cnidarian embryogenesis” and joined the section of Molecular Cytology in April 2010. His activities are an integral part of the spatiotemporal systems biology research efforts of the section of Molecular Cytology.

Research aims for the coming year

- To start a new master trajectory Cell Biology and Advanced Microscopy in the Biomedical Sciences Master programme with the LCAM partners.

- To publish on enhanced sensors for signal transduction based on mTurquoise

- To publish a new labelling method to study the growth of peptidoglycan in living cells and publish on the temporal interaction of the elongasome and divisome.

- To publish on the characterisation and application of new large Stokes-shift fluorescent proteins

Hela cell with fluorescently stained actin, mitochondria and nuclei.
**Research Programme**

**Plant Physiology**

*Chairholder: Prof. dr. M.A. Haring*

Dr. Ir. R. C. Schuurink  
Associate Professor

Dr. T. Munnik  
Associate Professor

Dr. C. Testerink  
Assistant Professor

**Introduction**

The Plant Physiology group investigates plant signalling at the cellular level and at the level of the whole plant. Our phospholipid signalling research is focused on the biological function of phosphatidic acid (PA) and polyphosphoinositides (PPIs). To visualise lipid signalling at the cellular level, we have developed genetically encoded-lipid biosensors for PA, PI3P, PI4P, DAG, PS and PI(4,5)P2. Knockout lines of individual PIK (11), PIPK (11), PLC (9), DGK (7) and PLD (12) genes in Arabidopsis plants, are used to elucidate their role in stress signalling and development. An important research goal is to elucidate how PA modulates protein function and downstream plant responses. To this end we are studying the effect of PA on several protein kinases, including CTR1, SnRKs, PDK1 and PID. To study the biochemistry of scent of Petunia flowers we investigate transcription factors involved in regulating volatile benzenoid and phenylpropanoid synthesis and emission. Because plant volatiles that are important for interactions with insects are produced in leaf-hairs (trichomes) we are dissecting the metabolism of volatile terpenes in trichomes. RNA-Seq of trichome-ESTs from wild and cultivated tomato plants has provided us with a wealth of candidate genes. We are engineering the production of terpenoids in tomato trichomes in such a way that they become repellent for pest insects. Finally, we use Arabidopsis for transcriptomics and forward genetic screens to identify genes important in the response to the wound-induced C6-volatile E-2-hexenal and the subsequent signal GABA.

**Research Highlights**

*Phospholipid research:*

Recently, we discovered that heat stress (40°C) triggers two lipid signalling pathways, one through phospholipase D (PLD), generating phosphatidic acid (PA), and another via phosphatidylinositol 4-phosphate 5-kinase (PIPK), generating PIP2. 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 tobacco BY-2 cells, we found that heat stress triggers the formation of PIP2 at the plasma membrane within minutes. Slightly later, unknown punctate structures appear in the cytosol that, after about 30 minutes, end up at the nuclear membrane. For PA, no such probe is yet available, so we have started to develop one. DNA constructs encoding 9 different PA-binding regions have been fused to an FP, while an additional 8 constructs have been made for controls, containing point mutations crucial for binding PA. Using T-DNA insertion KO-lines of Arabidopsis thaliana, we identified two PLDIs that are involved in heat stress signalling. Moreover, double-mutants completely lost their heat stress-triggered PA response while the PIP2 response was still intact. Microarray experiments and physiological assays will be used to establish PLD’s function in the heat stress response, while an RNAi strategy will applied for the 3 remaining PIPKs for which no homozygous insertion lines could be found, to see whether they are
Plant Signalling

responsible for the heat stress-induced PIP2 response.

We study intracellular signalling pathways linking salinity to root development and direction of root growth. These involve perception of high cytosolic Na+ concentrations in the root, activation of lipid signalling and protein kinase activation, and modulation of endocytic pathways. We also investigate natural variation between Arabidopsis accessions, with the aim to identify novel loci contributing to optimal root growth in the presence of salinity or osmotic stress. One of the key players in stress signalling is the lipid second messenger phosphatidic acid (PA). Osmotic stress and salinity have been shown to induce the rapid and transient accumulation of PA. We have identified several protein kinases, including CTR1, SnRKs, PDK1 and PID, which selectively bind PA in vitro. Currently, we are investigating the relevance of PA-binding for function of these kinases in vivo, using mutant versions that can no longer bind PA. To increase our understanding of how lipid signals can affect downstream responses, we also study the molecular basis and structure of lipid-protein interactions (collaboration with Dr. E.E. Kooijman, Kent State, Ohio). In 2010, we have published our work on how anionic phospholipids, including PA, modulate activity of the phosphoenolpyruvate carboxylase enzyme of C4 plants.

Plant volatiles research:
We focus on the role of C6 volatiles as signalling and priming molecules and have identified through forward genetic screens several E-2-hexenal response (her) Arabidopsis mutants. The her2 mutant has now been mapped, cloned and shown to encode an oxido-reductase in the mitochondria, of which we are trying to identify its specific activity. We are continuing our efforts to identify volatile terpenoids important in tomato-herbivore interactions. Through NMR and X-ray analysis we discovered that S. habrochaites makes actually 7-epizingiberene instead of alpha-zingiberene that is present in zinger. This 7-epizingiberene acts as a repellent to whiteflies while alpha-zingiberene does not. In order to identify transcription factors in the glandular trichomes of tomato that are involved in the regulation of terpene biosynthesis, two approaches are used: (1) Yeast one-hybrid and (2) Trichome RNA-Seq (GS FLX Titanium, 454 Life Sciences, USA). The Y1H screen yielded a putative TF binding to the Monoterpene synthase 1 (MTS1) promoter. This TF can transactivate MTS1p:GUS in leaves of N. benthamiana and we are currently constructing transgenic plants to study effects on the volatile terpenoid metabolome. Finally we try to identify the enzymes involved in synthesising sesquiterpenoid carboxylic acids toxic for spider mites. In Petunia we have been able to identify motifs in the promoter of the R2R3-MYB ODORANT1 (ODO1) gene that determine volatile production in non-fragrant and fragrant petunias. We have identified the Petunia EOBII transcription factors as one of the regulators of ODO1. A specific target gene of ODO1 encodes an ABC transporter. RNAi lines for this gene show minor changes in volatile emission.

Other Highlights
Robert Schuurink: Keygene collaborative project (post-doc), 3 yrs: Sucking Insect Resistance (500k€).
Christa Testerink: NWO-ALW (post-doc) 3 yrs: Novel salt stress-induced signals that control the direction of root growth (250 k€).
Christa Testerink: NGI Horizon Breakthrough grant, 1,5 yrs: Synthesis of phosphatidic acid (PA) on demand for a proteome-wide membrane recruitment screen (100 k€).

Future Prospects
- Identification of the PA-binding site of the SnRK2, PID and PDK1 protein kinases
- To investigate the role of lipid signalling and protein kinases in the response of Arabidopsis roots to salt
- To analyse the results of a proteome-wide membrane recruitment screen of salt-induced PA-binding proteins
- To explore natural variation in Arabidopsis accessions for salt tolerance
- Develop and validate PA-FP biosensors
- Investigate the effects of altered terpene emission of transgenic tomato lines on insect behaviour
- Elucidate the functional role of the petunia ABC transporter regulated by ODO1
*Agrobacterium tumefaciens*-mediated transient assays in petunia petals of the fragrant petunia Mitchell (white) show that the 1.2kbp promoter of Mitchel (M12:GUS) is 4-times more active than the 1.2kbp promoter of the non-fragrant (red) petunia R27 (R12:GUS).
Molecular Plant Pathology

Chairholder: Prof. dr. B.J.C. Cornelissen
Dr. Ing. F. L. W. Takken  Assistant Professor
Dr. M. Rep  Assistant Professor

Introduction
Adapted pathogens cause disease by evading host defences that are aimed to restrict microbial proliferation. To reveal the molecular basis of resistance and susceptibility in plants we focus on the interaction between the fungus *Fusarium oxysporum* and susceptible and resistant tomato (*Solanum esculentum*) plants that carry the I-2 resistance (*R*) gene. Besides I-2 we also study other (*R*) proteins that belong to the same family. Our specific interests are basal and induced defence mechanisms of the host, and virulence and avirulence factors of the pathogen.

The ability of a pathogen to colonise its host depends on ‘general’ pathogenicity genes as well as on specific, secreted ‘effector’ proteins. Effectors are called ‘avirulence factors’ when they are recognised by a matching *R* protein, thereby triggering disease resistance. For example, the *R* protein I-2 provides resistance of tomato to strains of *F. oxysporum* producing the effector Avr2 (Avirulence factor 2).

Our research aims at: (1) the identification and dissection of the protein complex(es) involved in *R* protein-mediated resistance. This work includes the functional analysis of individual complex-components and conformational changes in *R* proteins; (2) uncovering the role of pathogenicity genes and effector proteins (including avirulence factors) of *F. oxysporum* and identification of their targets in tomato; (3) unravelling the dynamics of genome evolution and the mechanisms of horizontal chromosome transfer in *F. oxysporum*.

Research Highlights
- This year a new project was started supported by a NWO-VICI grant to investigate the mechanism of chromosome transfer between fungi and the consequences of this remarkable phenomenon for the evolution of host-specific pathogenicity in the *F. oxysporum* species complex. A post-doc and two PhD students started on this project in September-November. The post-doc has established the melon-*F. oxysporum* system, with the aim of identifying effectors of *F.o. melonis* through melon xylem proteomics.
  - First yeast two-hybrid (Y2H)-interactors of Avr1 and Avr2 have been identified in an effort to identify plant targets of this effector.
  - After transient expression of *SIX6* in *N. benthamiana* leaves or after incubation of soybean roots with GFP-tagged Six6 protein, the protein was found to accumulate in plant nuclei.
  - Surprisingly, deletion of *SIX5*, like deletion of *AVR2*, breaks I-2-mediated resistance. In the yeast two-hybrid system, Six5 interacts with Avr2, whereas Avr2 interacts with Avr1. The significance of these observations is unclear at present and under investigation.
  - Transgenic tomato lines stably expressing *AVR2* show a distinct phenotype, possibly reflecting a change in hormone homeostasis.
  - Transgenic Arabidopsis lines stably expressing *AVR2, SIX6* and *SIX8* show distinct phenotypes indicative for the presence of an effector-target in this non-host plant.
  - Fol lines expressing fluorescently tagged Avr2 have been created to monitor the fate of Avr2 during infection. Preliminary data indicates that AVR2 is strongly induced upon root contact and is also expressed during colonisation of xylem vessels. RFP-labelled Avr2 was found to localise inside host cells confirming its uptake from the xylem sap.
  - Full-length MLA23, a barley powdery mildew resistance protein, was found to specifically co-purify with ADP, but not ATP, supporting our molecular “switch” model for NB-LRR proteins.
  - Most *R* proteins are multi-domain proteins. Structure-function analyses of the tomato Mi-1 nematode resistance protein revealed that its extended N-terminal domain exerts both
negative and positive regulatory functions. The minimal Mi-1 fragment able to trigger HR is the NT-2NB-LRR domain.

- Analysis of Arabidopsis plants in which SUMO (small ubiquitin-like modifier) isoforms were either silenced, knocked-out or over-expressed revealed that the different SUMO isoforms have non-redundant and specific functions in plant development and SA-mediated plant defences.

**Other Highlights**

Ben Cornelissen: (1) Panellist for the US National science foundation to serve in the advisory panel Symbiosis, Defense and Self-recognition, (2) Panelist for the Dutch NWO - ALW programme.

**Research aims for the coming year**

- To test the interaction of Avr1 with Y2H-identified interactors with other approaches: cytotrap, pull-down with *E.coli*-produced effector proteins.
- To initiate plant transformation to produce transgenic tomato plants expressing AVR1 or AVR3.
- To make a first set of targeted gene deletions in *F. oxysporum* to determine which processes are required for chromosome transfer.
- To identify transferrable chromosomes in *F. oxysporum* f.sp. *lycopersici* through a newly developed genetic screen.
- To make transgenic *F. oxysporum* strains expressing fluorescent proteins (split GFP system) to allow a first detailed analysis of cellular and nuclear processes occurring during hyphal fusion.
- To assemble novel *F. oxysporum* genome sequences in collaboration with Li-Jun Ma (Broad, Amherst) and explore new strategies for assignment of contigs to chromosomes.
- To identify effectors of *F. oxysporum* f.sp. *melonis* (Fom)
- To identify additional effector genes on transferrable pathogenicity chromosome (chr14) of *F. oxysporum* f.sp. *lycopersici* through manual genome annotation, transcript detection and gene knock-out
- To delete and over-express the regulator gene SGE1 in different *formae speciales* of *F. oxysporum* and determine the effect on the transcriptome (RNAseq), secretome and cell wall proteome.
- To test the interaction of Avr1 with Y2H-identified interactors with other approaches: cytotrap, pull-down with *E.coli*-produced effector proteins.
- To initiate plant transformation to produce transgenic tomato plants expressing AVR1 or AVR3.
- To make a first set of targeted gene deletions in *F. oxysporum* to determine which processes are required for chromosome transfer.
- To identify transferrable chromosomes in *F. oxysporum* f.sp. *lycopersici* through a newly developed genetic screen.
- To finalise structure-function analysis of the Mi-1 N-terminus.

Confocal images of *Fusarium* colonising a xylem vessel of a susceptible tomato root.

In locus Avr2 replacement in a *Fusarium* strain containing PSix1:GFP with PAvr2:RFP reveals that Avr2 and Avr3 (Six1) are co-expressed during infection.
Research programme    SILS - Center for Neuroscience

Cognitive and Systems Neuroscience

Chairholder: Prof. dr. C.M.A. Pennartz
Dr. W.E.J.M. Ghijsen  Assistant Professor
Dr. F.P. Battaglia  Assistant Professor
Dr. T. Kalenscher  Assistant Professor

Introduction
The group’s global research aim is to elucidate how neuronal networks distributed across the prefrontal cortex, sensory neocortex, hippocampus and ventral striatum, cooperate in cognitive processes, including learning and memory consolidation, perception and multisensory integration. 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 physiology. General research topics include:

- The consolidation of memorised information of recent experiences. A promising candidate mechanism for mediating this process is spontaneous “off-line” reactivation of stored information. 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 this phenomenon for memory consolidation by electrical interventions, and how replay is orchestrated amongst different brain areas, such as the hippocampus and ventral striatum. Technically, this project is carried out in animals by performing ensemble recordings using ‘tetrode arrays’ and by state-dependent deep brain stimulation techniques.

- We are also studying memory consolidation from theoretical and computational viewpoints. We are developing new computational models of memory consolidation and the formation of semantic memories, by making use of concepts from computational linguistics and Bayesian inference. Furthermore we develop new analytic methods to study coherence within and between cell assemblies in the brain, and how this coherence supports memory formation.

- Another main question in the field of learning and memory is how networks of cells collectively learn to generate predictions about upcoming rewards, based on sensory cues that precede reward delivery. Learning-related changes in rhythmic neural activity and network coherence are highlighted. We investigate which neurotransmitters and receptors influence the formation of neural representations of reward predictions. This line of research has been recently augmented by studying how stress hormones influence memory formation.

- We investigate relationships between genes, learning and memory capacities as measured in behaviour, and the systems physiology which forms the interface between gene expression and overt behaviour. These relationships are studied in the context of spatial navigation, conditioned place preference and cognitive flexibility in targeted knockout mice, e.g. regionally restricted NMDA receptor deletions in hippocampus and deletions of the Arc gene, which is involved in synaptic plasticity and AMPA receptor trafficking. This research line has been supplemented with a genetic mouse model of mental retardation (fragile-X syndrome).

- We investigate how neural assemblies in the brain cooperate to generate conscious or
unconscious multisensory representations, and how sensory inputs from different modalities are combined to achieve such integrated representations. A theoretical framework to understand consciousness as a process of multimodal integration is under construction, and we are moving to test its experimental predictions using in vivo 2-photon Calcium imaging and multi-area ensemble recordings. For instance, visual processing and tactile-visual integration are compared under awake and anaesthetised conditions. Moreover, the impact of the dimension of motivation and reward prediction on visual representations is studied using 2-photon-imaging of neural ensemble activity.

**Research Highlights**

- 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 sensory cues that predict reward elicit a switch in hippocampal representation. This is visible as an increase or decrease of firing rate of individual neurons for a variable time period, often locked to the duration of the sensory cues. Moreover, ventral striatal ensembles appear to switch concurrently with the hippocampus. This indicates that a cue-elicited change in attentive-motivational state of the animal causes coordinated, robust changes in neural representations distributed over multiple brain areas.

- New measures of phase synchronisation between spikes and EEG (in relation to oscillatory field potentials) were developed. We showed that these measures are more robust in the face of volume-conduction, noise and sample-size bias than previously published methods.

- 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. NMDA receptors are thought to be key elements in the neural mechanisms for remembering and learning to predict future rewards. We examine the role of these receptors in neural coding processes in the orbitofrontal cortex by combining tetrode recordings with local infusion of NMDA-R antagonist using reverse microdialysis. NMDA receptors were found to play an enhancing role in discrimination between and representation of different odours coupled to positive or negative trial outcomes, and in keeping firing rates under control to prevent premature responses and disinhibition.

- We studied the interactions between the hippocampus and the prefrontal cortex in rats during performance of a decision making task, and during the subsequent sleep. During behaviour, hippocampal/cortical coherence manifested itself in the form of oscillatory coherence and neural ensemble synchronisation. During sleep, transient replay events were observed in the prefrontal cortex, simultaneously with hippocampal sharp wave/ripples.

- We carried out an extensive set of experiments involving tetrode ensemble recordings in the hippocampus of control and NMDA receptor knockout mice, in a series of tasks, involving food search in a star-shape maze and running in a circular task, with the purpose of analysing the activity of hippocampal place cells when the NMDA receptor is functionally impaired. This study sheds new light on the role of synaptic plasticity in the plasticity of spatial representations and mechanisms underlying spike timing relative to theta rhythm. Similar ensemble recordings in mouse models related to mental retardation and Arc-dependent memory deficits have been conducted, and the results are currently under analysis.

- A 2-photon imaging setup was used to visualise the spatially and temporally ordered structure of neuronal population activity in the living mouse brain. To examine the characteristics of neural processing in conscious conditions, we compared how this structure differs between the awake and anaesthetised state. In the anaesthetised state, correlations between neural resting activity were on average higher and spatially spread out further than in the awake state. Functional clustering of neurons, manifested by spontaneous activity correlations, appears sparser and more sharply
delineated during consciousness than under anaesthesia.

- To investigate how neural assemblies in brain areas belonging to different sensory modality interact, we developed a new 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). The results from this unique recording approach are currently being analysed.

- 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 if it may also function in representing state parameters of other agents in the environment, e.g. conspecifics. A new behavioural protocol was developed to study whether rats can discriminate positions and movements of robotic agents in the environment. This was indeed the case. Preliminary results indicate that the hippocampus harbours cells sensitive to the position of external agents.

Other Highlights

- The group published 5 papers in high-impact journals, viz. J. Neuroscience (2X Van Wingerden et al.), Neuroimage (Vinck et al.; Daselaar et al.) and Neuron (Benchenane et al.).
- Cyriel Pennartz was appointed Chairman of the committee on Medium-size Equipment Investments in the Medical Sciences (Zon-Mw).
- Cyriel Pennartz was appointed Board member of the NWO committee on multidisciplinary Top grant reviews, Medical and Life Sciences (“TOP-GO”).
- Cyriel Pennartz acquired a Collaborative Grant from the Cognition Spearhead Programme of the University of Amsterdam (PhD student fellowship).
- Cyriel Pennartz acquired a grant from the NWO Programme for Excellence ‘Brain and Cognition’ together with Prof. Dr. P. R. Roelfsema, € 499.000.

- Cyriel Pennartz and Francesco Battaglia each acquired a grant from the Open Programme in the Earth & Life Sciences (NWO), for a PhD studentship and postdoc fellowship, respectively.
- Francesco Battaglia was appointed Associate Editor at Brain Research and Cyriel Pennartz functions as Associate Editor at the European Journal of Neuroscience.
- The Master Curriculum in Brain and Cognitive Sciences received a very good/excellent evaluation from the NVAO. In this curriculum, Battaglia is member of the teaching committee and Pennartz (co-)director of the track ´Cognitive Neuroscience´.

Research aims for the coming year

- We aim to disrupt memory consolidation and extra-hippocampal replay by electrical and optogenetic intervention of hippocampal processing in rats. The results will be used to develop novel methods for intervening with anxiety and PTSD-like disorders.
- The in vivo 2-photon imaging technique, combined with bulk labelling of neurons with Calcium-indicator dyes, will permit us to study multimodal interactions in the population dynamics of sensory neurons in the rat neocortex. Bulk labelling methods will be supplemented with genetically encoded Calcium dyes, e.g. GCamp-3. We further aim to study ensemble activity at high spatial resolution in a more evolved type of cortex, i.e. of ferrets.
- We aim to develop a dual visual task for rats that will allow scientists to establish whether rodents or ferrets have perceived a stimulus or not. Multi-area recordings are planned to examine ensemble interactions during visual perception.
- We aim to make further ensemble recordings from mutant mouse brains, yielding indications about the neural mechanisms of spatial memory, self-localisation, short- and long-term consolidation. Recordings from several genetically modified mouse lines will be completed (e.g. Arc & FMR-1 genes).
recordings from mice with hippocampal NMDA-receptor deletions will be completed. This project will also be the test-bed for the development of a wireless electrophysiology recording system.

- We plan a new series of experiments investigating the interaction between the hippocampus and prefrontal cortex during sleep, by using Local Field Potential and Current Source Density Analysis methods.
- Using ensemble recording techniques applied to several neocortical and hippocampal recording areas simultaneously, we will study how information from the tactile and visual sensory modalities is integrated along the sensory neocortical-to-hippocampal hierarchy, using optogenetic techniques in combination with electrophysiology.
- A similar approach will be followed in a novel project on audio-visual integration applied to the problem of how the brain localises multimodal sources in the environment.

- Based on the newly developed behavioural protocol for studying the effects of stress hormones on decision-making and learning (place preference), we will conduct ensemble recordings from hippocampus and orbitofrontal cortex to examine how these hormones affect the neural mechanisms underlying the behavioural changes.
- New technological possibilities will be utilized to identify the cell types and intracellular correlates of a number of processes indicated above. These techniques range from optogenetics and in vivo 2-photon imaging to in vivo patch-clamp in the awake state. Particularly sub(spike) threshold intracellular phenomena resulting from multimodal interactions between sensory inputs will be studied.
Cellular and Systems Neurobiology

Chairholder: Prof.dr. W. J. Wadman

Dr. J. A. van Hooft Assistant Professor
Dr. J. A. Gorter Assistant Professor
Dr. T. R. Werkman Assistant Professor
Dr. N. L. M. Cappaert Assistant Professor

Introduction

Excitability is still the most prominent property of the nervous system. How ion-channels are organised and quantitatively balanced in the neuronal membrane, how they lead to neuron specific firing patterns and neuronal activity is modulated at different times scales (plasticity) belong to the most exciting questions in neuroscience. We approach them from a multidisciplinary angle. Neurons communicate with each other through a variety of synapses. To provide minimal functionality, neurons need to be combined in small circuits. New techniques allow us to slowly shift the focus of our research from the single cell to the (small) circuit level. Our research is organised around a few well defined topics in the realm of neuronal excitability. Our core approach is a functional electrophysiological one (from patch-clamping to in vivo). State-of-the-art optical techniques (Ca-imaging, Voltage Sensitive Dyes) and various multi-contact electrode recordings allow the analysis of population activity.

Research Highlights

The first of our three major research lines studies the fundamental properties of the 5-HT3 receptor and investigates its functional role in local circuits, development and behaviour. Molecular techniques produced mice in which the 5-HT3 receptor expressing neurons are labelled with GFP and can be studied efficiently. This has opened a wide range of possibilities to investigate the role of this receptor in functionally connected neurons and also its highly specific role in cortical column formation.

The second research line studies epilepsy e.g. seizure generation, epileptogenesis and pharmacoresistance. The latter topic we approach from two sides: a) (non-)penetration of drugs via the blood-brain-barrier and b) modification of drug targets, mainly sodium channels. These studies are of high clinical relevance and we strengthen them through side appointments at the Academic Hospital in Ghent and intense collaboration with the epilepsy center in Heemstede (SEIN). The therapeutic potential of deep brain stimulation is investigated in patients and in animal models.

The third research line concentrates on specific pharmacological modulation of neuronal circuits. We have a continuous collaboration with Solvay Pharmaceuticals in Weesp on the interactions of serotonin and dopamine in the Ventral Tegmental Area and the Substantia Nigra, two areas highly relevant for schizophrenia. Within the context of Top Institute Pharma we investigate the role of the endocannabinoids at the cellular and the circuit level in the PreFrontal Cortex (PFC).

Most of our experiments are supported by computer modelling, focusing on single cell excitability in relation to the direct chemical surrounding of the neuron as well as on the adaptive strategies for excitability that optimize the working range of active neurons. In the latter case we try to extrapolate the consequences of single cell strategies to larger neuronal networks. The combination of theoretical and experimental work has proven to be very fruitful in the scientific setting of SILS and the FNWI.

Other Highlights

On May 28th, Erwin van Vliet received the Meinardi Award for best thesis in epilepsy, 2007-2010. The prize is awarded by the National Dutch Epilepsy Foundation. The research for his thesis: The role of the blood-brain barrier and multidrug
transporters in pharmacoresistant epilepsy was performed in our group under the guidance of Jan Gorter and Wytse Wadman and financed by a grant from the Stichting Epilepsie Instellingen Nederland (SEIN, Heemstede).

A new grant awarded to Wytse Wadman from the Platform for alternatives in Animal Experimentation was started (Luuk van der Velden), with the aim of making tests for Anti-Epileptic Drugs more efficient and less dependent on laboratory animals. A new set-up based on a Multi Electrode Array (64-128 channels) was configured for that aim and successfully tested on slices from VTA and SN.

We contributed to a management book BREIN@WORK, relating fundamental properties of the brain to best management principles in human resource management. The book made it to a respectable second place in the Dutch management book of the year competition.

Jan Gorter acquired a new NEF project that will employ a PhD student and concentrate on the way inflammation underlies epileptogenesis.

Future Prospects

The coming year, most of our PhD students will finish their projects and round up results. The downfall of the Dutch pharma industry, with whom we have collaborated intensely and successfully over the last 10 years will require a reorientation of some of the research lines. The success of the collaboration with Philips Eindhoven, will lead to strengthening of the research on fundamental principles of Deep Brain Stimulation.

We have found a very successful synthesis between experiments and theory and were able to underpin many experimental studies by insightful computational modelling.

Unfortunately, to a large extent such a success builds on the expertise of only a very few temporary collaborators and is therefore volatile.
Structural and functional plasticity of the nervous system

**Chairholder:** Prof. dr. P.J. Lucassen

**Dr. H. Krugers** Assistant Professor

**Dr. C.P. Fitzsimons** Assistant Professor

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**Introduction**

**Group Lucassen/Krugers**

We study structural and functional plasticity in the brain and focus on adult neurogenesis and synaptic plasticity in relation to stress, learning and memory and diseases like depression, epilepsy and dementia.

**Current status**

The past year, the group has gone through a transitional phase following the leave of Marian Joels and her group members to Utrecht. The remaining group of Lucassen and Krugers was expanded with VIDI laureate Fitzsimons, technician Meerhoff and assistant professor Korosi, as well as with several new PhD students who started their projects. Furthermore the research line on stress has shifted more to, in particular, early life stress, and the subsequent consequences for learning and memory and the development of (psycho)pathology later in life.

With Fitzsimons joining the group, more molecular and viral tools will be available that will also allow us to focus on molecular mechanisms of such stressors like epigenetics and microRNA regulation, while Korosi will strengthen behavioural, epigenetic and early life stress expertise.

In 2010, the teaching and management load has further increased due to the strong rise in bachelor student numbers and the introduction of a newly developed master curriculum, also in terms of committee work. As such, the senior members face increasing challenges in dividing their time between teaching, management and research in the very competitive and rapidly developing research fields of neurogenesis and AMPA receptor biology. Despite this, the group has kept up a very good output in 2010 and managed to publish 18 high quality papers this year, a.o. in J. Neuroscience and Nature Rev Neurosci.

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**Research Highlights**

We tested if severely adverse early-life stress reduces structural and functional plasticity in adult life. After maternal deprivation at postnatal day 3, reduced levels of adult hippocampal neurogenesis and an altered dendritic tree organisation were found in adult animals. These structural changes were not only paralleled by impaired learning of a spatial learning task, but also by improvements in network properties and emotional learning under high-stress conditions. Hence, adversity early on in life is not always 'bad', but can even improve emotional forms of memory and thereby prepare the organism to perform optimally under stressful conditions in adulthood (Oomen et al., J Neurosci, 2010, Oomen et al., Psychopharmacology, 2011).

**Other highlights**

The group has improved its visibility, and the visibility of SILS/UVA, by organising the International NEURAD Alzheimer meeting (PL) in Amsterdam, as well as several master classes and sessions at scientific meetings (ENP, HSN publieksdag, ECNP, FENS, ISAO workshop), and by membership of the boards of ALW-NWO (HK), the Neurofederation (HK, board secretary) and the International Alzheimer Society ISAO (chairman of the Scientific Advisory Board, PJL).

Group members were also successful in obtaining external funding from NWO (CF, HK, PL), the Alzheimer (ISAO to HK) and Parkinson Foundation (IPF to PL) and industry Corcept (PL with Joels).

Group members received numerous invitations to give lectures or write papers and chapters and to act as invited reviewer for major journals like J Neuroscience, Biological Psychiatry, PNAS, Nature and Science.

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**Research aims for the coming years**

- To further develop our research line into the consequences of stress during early life for
structural and synaptic plasticity, AMPA receptor dynamics and cognition at adult age.
- To extend these lines into models of diseases like depression, anxiety, epilepsy and dementia.
- To extend the research on neurogenesis in rodent models to other brain areas like the amygdala and cortex and also in a translational perspective to human brain.
- To develop translational approaches to monitor and measure neurogenesis in the live human brain using MRS spectroscopy in relation to development and depression.

To incorporate and develop tools to manipulate adult neurogenesis in vivo using molecular and viral tools.
- To increase our understanding of the molecular mechanisms underlying stress-mediated regulation of learning and memory through e.g. AMPA receptor trafficking and spinogenesis.
- To determine how proteins that are critically involved in dementia regulate synaptic function and cognition.

Adult neurogenesis as identified by retroviral mediated labeling of newborn neurons (white) in the adult hippocampus (blue cells). Picture: Paul Lucassen & Boldizsar Cze
Mass Spectrometry of Biomacromolecules

Chairholder: Prof. dr. C.G. de Koster
Dr. L. de Jong
Dr. L.J. de Koning

Introduction
In the context of the UVA priority area Systems Biology, Mass Spectrometry of Biomacromolecules focuses on three research themes that adhere to the study of cellular response to external signals. (1) We study post-transcriptional regulation of gene expression by quantitatively analysing protein concentrations, synthesis and degradation rates and mapping post-translational modifications. (2) We develop new analytical strategies for the experimental evaluation of models of the 3-D structure of protein complexes. (3) We aim at insight in adaptation of the cell surface proteome of fungi and bacteria. SILS-MS is developing advanced and innovative, mass spectrometry-based proteomics technology that is designed for these research areas. Our technology is not confined to the field of molecular systems 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.

Research Highlights
Enzyme reprofiling in bacteria during adaptation from one environmental condition to another may be regulated by both transcription and translation. However, little is known about the contribution of translational regulation. Recently, we have developed a pulse labelling method using the methionine analog azidohomoalanine to determine the relative amounts of proteins synthesised by Escherichia coli in a brief time frame upon a change in environmental conditions. We extended our analytical strategy.

The improved method entails measuring changes in total protein levels on the same time scale as new protein synthesis for the first time. This allows identification of stable and labile proteins and demonstrates that altered levels of most newly synthesised proteins are the result of a change in translation rate rather than degradation rate. With this extended strategy, average relative translation rates of 10 minutes immediately after a switch from aerobiosis to anaerobiosis were determined. The majority of proteins with increased synthesis rates upon an anaerobic switch are involved in glycolysis and pathways aimed at preventing glycolysis grind to a halt by a cellular redox imbalance. Our method can be used to compare relative translation rates with relative mRNA levels at the same time. Discrepancies between these parameters may reveal genes whose expression is regulated by translation rather than by transcription. This may help unravel molecular mechanism underlying changes in translation rates, e.g. mediated by small regulatory RNAs.

Other Highlights
We organised an international three-day course Fundamentals of Mass Spectrometry and Proteomics for the participants of the EU Marie Curie Consortium FINSysB at Science Park Amsterdam from October 4th to October 6th 2010.
Research aims for the coming year

Focal points in our MS research programme are as mentioned above (1) post-transcriptional regulation of gene expression, (2) systematic analysis of protein-protein interactions, and (3) proteomics at the cell surface. (1) In 2011, we will finalise our method development for estimation of protein degradation rates from azhal pulse labelling MS data sets. We will move in this research line to the study of post translational modifications that affect function and activity of proteins. Oxidation affects the activity of many proteins and their loss by degradation and aggregation. The latter may lead to cellular dysfunction and eventual death. Protein oxidation by reactive oxidative species comprises a plethora of reactions. We will map in vivo protein modification caused by oxidative stress imposed on cells and correlate this map with biological function and dysfunction. We will start with the reactivity of hydroxyl radicals towards proteins in the mitochondrion. Another important reaction in this organelle is carbonylation of proteins. In programme (2) we will map interaction sites of initiation and elongation factors that modulate the activity of RNA polymerase and we will quantitate cross-links formed during initiation and elongation, in an effort to detect conformational changes. (3) The MS group 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 the framework of the EC FINSysB project, we will focus on the quantitative cell wall protein composition of Candida albicans to identify new leads for novel anti-Candida vaccines, drugs and diagnostic markers. In collaboration with Prof. Brul, we will carry out functional characterisation of spore coat proteins of B. subtilis. We will continue the productive collaborations with the groups of the SILS-plant programme, directed to characterisation of proteins secreted by the cell upon host-pathogen interactions, and with our external national and international partners.

Changes in protein synthesis by Escherichia coli compared with changes in protein levels upon onset of anaerobiosis. Light gray or coloured circles, relative change of newly synthesised proteins; dark gray or coloured diamonds at the same position on the x axis as a circle are relative total protein levels of the same protein 10 min after the switch to anaerobiosis. Proteins are ordered from most induced to most repressed synthesis. In a, proteins having a high turnover rate are marked, namely Grca (purple circle and diamond) and ClpA, KatG, AhpC, MetN, and AhpF (orange circles and diamonds). In b, coloured symbols are used to represent proteins with a low turnover rate in the same data set. Red circles and diamonds, proteins involved in anaerobic respiration and mixed acid fermentation; green circles and diamonds, glycolysis; blue circles and diamonds, ribosomal proteins. Prot., protein.
Biosystems Data Analysis

Chairholder: Prof. dr. A.K. Smilde

Dr. H.C.J. Hoefsloot  Associate Professor
Dr. J.A. Westerhuis  Assistant Professor
Prof. dr. A.H.C. van Kampen  Special Chair

Introduction

General goal
Developing and validating methods for organising, summarising and visualising complex biological data.

The research is divided in three connected themes: Semantic Biosystems, Data Fusion and Networks & Dynamics. We apply our methods in diverse areas of systems biology focusing mainly on microbiology, nutrition and medical biology.

Semantic Biosystems (Antoine van Kampen)
Well-structured, accessible and integrated information is crucial for disciplines like genomics and systems biology. To support these disciplines, we develop novel information management approaches based on cutting-edge Semantic Web standards in a framework that allows the construction of high quality domain-specific knowledge bases.

Data Fusion (Johan Westerhuis)
To understand the functionality of complex biological systems, different types of measurements have to be combined with systems information stored in a knowledge base. We develop data analysis methods that are able to find biologically relevant patterns in these data that on the one hand match the systems information and on the other hand generate new insights.

Networks & Dynamics (Huub Hoefsloot)
In a biological system molecules interact. These interactions, the network, causes the system to change over time. We develop methods to reverse engineer networks from time-resolved functional genomics data. The networks can be metabolic networks, protein-protein interaction networks, gene-regulatory networks or association networks.

Research highlights

General
We have been able to fill all vacant positions in the group and have established new international collaborations, e.g. with Aarhus University in the area of Nutrition and Production Physiology; with University College Dublin in the area of multicomartment modelling; with Oxford University in the area of BioPax and Citation Typing Ontology. This gives us the opportunity to obtain data sets and validate our methods on real-life problems.

Semantic Biosystems
Interest in knowledge publication, management and integration is growing rapidly, and the BioExpert project is ideally placed to take advantage of this. We have accumulated a great deal of experience in the application of Semantic Web technologies, and methodologies that support the creation and publication of new knowledge base content. The Peroxisome Knowledge Base has a new portal on the web (www.peroxisomekb.nl), which has allowed us to publish the knowledge base content already captured in concept maps in an open, machine-readable way, known as Linked Data. The peroxisome vocabulary that forms the core of the knowledge base has been significantly improved and extended and now includes over 1600 peroxisome related concepts, with ~1000 of these linked to a public resource and many now having definitions and several labels. The knowledge base also contains over 4000 peroxisome related publications as RDF converted from MedLine. We have used the vocabulary to ‘text-mine’ these publications to identify peroxisomal concepts mentioned in the titles and abstracts, giving over 60,000 links between the vocabulary and the publications. In order to support ongoing work on the
vocabulary, and further involve experts from the field, we have developed a new application for editing SKOS vocabularies that are in an RDF triple store. We started research to implement a polyphenol degradation knowledgebase and to integrate this with grey statistical modelling. Collaboration with Peter Barth and Bwee-Tien Poll-Thé (Pediatric Neurology, AMC) is yielding a very detailed description of concepts related to Zellweger Syndrome, on which he is a leading figure. We have also begun collaborations with partners in NCSB, TNO and VU to construct new knowledge bases on Metabolic Syndrome and Yeast Glycolysis. Collaborations are also ongoing to develop and deploy standards for knowledge publication (nanopublications), standards for pathway data on the Semantic Web (BioPAX) and standards for metadata about publications (Citation Typing Ontology, CiTO).

**Data Fusion**

Repeatability of metabolomics measurements reveals that each metabolite may have a different measurement error that depends on its concentration. A new measurement error model was developed that also takes into account the correlation between measurement errors of different metabolites. Biological variation between human individuals gives rise to two data analysis problems. Small treatment effects cannot be observed and treatment effects may be different for the individuals. A new data analysis method (MLPLSDA) was introduced that is much better able to find the individual treatment effects than conventional methods. Johan Westerhuis received the EAS Award for Outstanding Achievements in Chemometrics for his contributions to the field of chemometrics.

**Research Highlight**

In a recent PNAS paper we described the metabolic faith of polyphenols in humans. The figure below shows a compartmental model depicting the nutrikinetic processes of phenolic compounds across the human system. Three nutrikinetic processes are illustrated; (A) fast systemic absorption of phenolic compounds via the upper digestive system, (B) delayed and slow systemic absorption of gut-mediated metabolites derived from phenolic components (x), and (C) biphasic absorption of resveratrol due to enterohepatic cycling between liver and intestine. Constants $k_a(A)$, $k_a(B)$, and $k_a(C)$ are the absorption rate constants; $k_e(A)$, $k_e(B)$, and $k_e(C)$ are the elimination rate constants; $F_1$ and $F_2$ are the absorbed fractions of native and conjugated compounds, and $FC$ is the fraction of resveratrol absorbed in the bile.
Networks & Dynamics
In 2010, a paper on the reverse engineering of metabolic networks is accepted. It showed that it is difficult to reconstruct metabolic nets because the time constants involved in metabolic reactions vary over a large range. To capture the dynamics of the network fast sampling is necessary to capture the fast reactions. This sampling turned out to be faster than most systems for metabolomics are capable of. For endocrine regulation, association networks were built from time-resolved hormonal data (collaboration with the LUMC). These regulatory networks show differences between control and diseased persons, and between patients before and after treatment in concise and biologically interpretable way.

Research aims for the coming year
General
Bringing together the fields of biostatistics and bioinformatics.

Semantic Biosystems
Our new collaborations with partners within NCSB, TNO and Vrije Universiteit present the opportunity to employ the technologies and methodologies for constructing knowledge bases in different areas. We will develop knowledge bases and new tools that support the needs of researchers in these organisations wishing to capture and use their expert knowledge. Our knowledge base architecture will be extended in order to support the tasks of pathway modelling (VU) and the interpretation and annotation of datasets derived from system-level experimentation (TNO). The existing PeroxisomeKB will be extended and important as an established Semantic Web framework in which new ideas for information management can be validated. We expect first results with the integration using prior knowledge, from the polyphenol knowledgebase, in grey statistical modelling.

Data Fusion
In the following years the focus will be on the analysis of functional genomics data obtained from challenge tests. In such tests the individual is brought out of homeostasis and its resilience is observed. The goal is to develop data analysis methodology that can describe data obtained from such tests for improved mechanistic information and to provide better prediction of the health status of individuals.

Networks & Dynamics
In the next few years we are going to develop tools to analyse the interactions of bio-molecules and the changes of these interactions over time. For this we will use and adapt concepts for the analysis of covariation- and correlation matrices. Especially the changes in covariance and correlation over time will have our attention. A first paper on this has already been submitted. Together with our collaborators at the Amsterdam Free University we will estimate protein protein interaction networks in the synaps. The data is measured using immuno precipitation experiments. Methods will be developed to extract information on protein complexes in the synaps.
Micro Array Department and Integrative Bioinformatics Unit

Group leader: Dr. T.M. Breit

Dr. Ir. R.A. Wittink  Project management “wet-lab”
Drs. O. Bruning  Project management “dry-lab”

Introduction

MicroArray Department (MAD) & Integrative Bioinformatics Unit (IBU):

Microarray technology is a well-established tool for genome-wide gene expression, i.e. transcriptomics studies. The ultimate goal of a microarray experiment is to simultaneously investigate the expression of all genes of a specific organism, in a cell type, during specific growth or stress conditions. This enables the study of complex cellular mechanisms or identification and use of biomarkers.

Transcriptomics biomarkers are genes whose expression profile can be used for diagnostic purposes or to monitor and predict cellular processes. Recently, innovative next-generation sequencing technologies have also become available for gene-expression analysis. Because transcriptomics experiments produce a vast amount of data, extensive bioinformatics infrastructure, methods and expertise are needed to cope with these data effectively.

Bioinformatics for transcriptomics comprises: data-handling (storage and exchange), data-preprocessing (normalisation and validation), data-analysis (clustering, biomarker selection, etc.), and e-infra (high-performance computing, grid, cloud).

The MAD-IBU consists of a microarray technology section (Wet-lab) with ~5 specialists that provides transcriptomics service and support and performs microarray and next-gen. sequencing technology R&D; a transcriptomics data-analysis section (Dry-lab) with ~9 bioinformaticians and informaticians that provides transcriptomics data analysis service and support, performs bioinformatics R&D, and builds e-infra. Together, the MAD operates as a transcriptomics technology and bioinformatics expertise centre and core facility for UvA scientists, as well as external academic and industrial customers.

The focus of the Wet-lab R&D is to improve the microarray technology for transcriptomics with a strong focus on array controls and sample size reduction. We aim to eventually analyse various types of single cells by microarray technology such as (un)fertilised eggs. The focus of the Dry-lab is on the bioinformatics and e-bioscience methods, tools and infrastructure necessary to perform advanced transcriptomics data-analysis starting from array design to publication. Another important focal point for the whole group is design-for-experimentation. Performing well-designed range-finding experiments should elucidate the role of time and space in transcriptomics experiments. To this end, MAD-IBU participates in six nation-wide projects: BioRange, a NBIC bioinformatics research project; BioAssist, a NBIC bioinformatics support programme; Virtual Lab for e-Science (VL-e); e-Science Center (eSR); and e-BioGrid -BiG Grid-, Virtual Lab for Plant Breeding (VLPB), all four being Dutch Bsiik or NWO e-science projects in the field of e-infrastructure and methods.

Research Highlights

The Wet & Dry-lab:

- First major microRNA project with AMC.
- Established a strategic collaboration with the Center of reproductive medicine at the AMC.
- Started to build the analysis pipeline for next-generation sequencing data.
- Start of the first 3 PhD students at the MAD.
- Performed over 70 collaborative experiments for biologists in 2010 alone.

Other highlights

The whole group further strengthened the strategic collaborations with several external research organisations: Laboratory for Health Protection Research, RIVM, (Bilthoven); Medical Microbiology, UMC (Utrecht); ACTA, VU-AMC (Amsterdam); Molecular Cell biology, UL, (Leiden); Center of reproductive medicine, AMC, Amsterdam.
Research aims for the coming year

- To develop ultimate unbiased microarray gene-expression and CGH protocols.
- To analyse maternal RNAs in unfertilised Zebrafish and human eggs.
- To analyse the transcriptomics of the earliest stages of Zebrafish and human embryogenesis.
- To analyse a hallmark time-axis microarray experiment on Zebrafish.

- To perform several range-finding experiments in the context of design-for-experimentation.
- To extend the Problem-Solving Environment (PSE) for microarray data analysis and interpretation.
- To become a central player in the national e-bioscience domain, such as the UvA zwaartepunt e-science and the e-Science Research Centre.
- To file at least 1 patent on microarray technology innovations.
- To write > 15 (collaborative) research articles.
Management

Finance

The integrated results for 2010 show an operating profit of 143 k€ where a negative result of 859 k€ was budgeted. This is partially due to delayed employment of new personnel.

<table>
<thead>
<tr>
<th>Year</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
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<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
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<tr>
<td>University funding</td>
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<td>7364</td>
<td>8987</td>
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<td>12795</td>
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<td>14561</td>
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<tr>
<td>External funding</td>
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<td>3883</td>
<td>4474</td>
<td>6167</td>
<td>4515</td>
<td>4701</td>
<td>4952</td>
<td>5489</td>
<td>5084</td>
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<tr>
<td>total revenues</td>
<td>9690</td>
<td>10014</td>
<td>11838</td>
<td>15154</td>
<td>12092</td>
<td>17935</td>
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<td>18645</td>
<td>19297</td>
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<td>Personnel costs</td>
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<td>7465</td>
<td>8919</td>
<td>9626</td>
<td>9122</td>
<td>12816</td>
<td>13918</td>
<td>14448</td>
<td>11191</td>
<td>10342</td>
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<tr>
<td>Bench fees**</td>
<td>2236</td>
<td>2450</td>
<td>3310</td>
<td>4989</td>
<td>2729</td>
<td>5362</td>
<td>4138</td>
<td>5240</td>
<td>7795</td>
<td>8812</td>
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<tr>
<td>total costs</td>
<td>9332</td>
<td>9915</td>
<td>12229</td>
<td>14614</td>
<td>11851</td>
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<td>19154</td>
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<tr>
<td>result</td>
<td>358</td>
<td>99</td>
<td>-391</td>
<td>540</td>
<td>241</td>
<td>-243</td>
<td>-309</td>
<td>-351</td>
<td>-342</td>
<td>143</td>
</tr>
</tbody>
</table>

Table 1: Representation of revenues and costs of the Swammerdam Institute for Life Sciences, in k€, for the years 2001-2010. In 2006, the university changed to a new financial system in which budgets were increased and full costs were calculated. ** as of 2009, costs that were included in “personnel costs” were transferred to “bench fees”

Revenues and costs over 2010 can not be compared directly with previous years and higher numbers in the “funding” part not necessarily reflect more money for research. This is due to ongoing changes in the financial methodologies used by the UvA. Therefore, to assess the capacity of the institute the number of employees may provide a better insight.

<table>
<thead>
<tr>
<th>Year</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
</tr>
</thead>
<tbody>
<tr>
<td>FTE university funded</td>
<td>95.6</td>
<td>90.6</td>
<td>90.6</td>
</tr>
<tr>
<td>FTE (NWO/FOM funded)</td>
<td>24.1</td>
<td>20.0</td>
<td>33.3</td>
</tr>
<tr>
<td>FTE (EU, contracts)</td>
<td>42.5</td>
<td>38.1</td>
<td>37.3</td>
</tr>
<tr>
<td>FTE (scholarships)</td>
<td>1</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Total number of FTE*</td>
<td>163.1</td>
<td>159.7</td>
<td>171.6</td>
</tr>
</tbody>
</table>

| Total number of employees | 172 | 167 | 180 |

Table 2: number of FTE and employees on December 31st of each year.
Funding

The funding system of Dutch universities distinguishes three different kinds of funding resources. These are referred to as so called “funding sources” and are numbered one to three. Resources originating from the university itself are referred to as the first funding source. External funding is divided into funding from the Netherlands Organisation for Scientific Research (second funding source) and money originating from all other resources such as EU and contract research (third funding source).

<table>
<thead>
<tr>
<th></th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
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</thead>
<tbody>
<tr>
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<td>8987</td>
<td>7577</td>
<td>13234</td>
<td>12795</td>
<td>13848</td>
<td>13580</td>
<td>14561</td>
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<tr>
<td>Costs</td>
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<td>-346</td>
<td>-464</td>
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<td>-265</td>
<td>493</td>
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Table 3: representation of income and costs in the 1st funding source, in k€, for the years 2003-2010.

<table>
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<th></th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
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</thead>
<tbody>
<tr>
<td>Revenues</td>
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<td>2303</td>
<td>2160</td>
<td>2032</td>
<td>2299</td>
<td>2434</td>
<td>1821</td>
<td>1884</td>
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<tr>
<td>Costs</td>
<td>2279</td>
<td>2303</td>
<td>2160</td>
<td>2048</td>
<td>2226</td>
<td>2436</td>
<td>1713</td>
<td>2195</td>
</tr>
<tr>
<td>Result</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-16</td>
<td>73</td>
<td>-2</td>
<td>108</td>
<td>-311</td>
</tr>
</tbody>
</table>

Table 4: representation of income and costs in the 2nd funding source, in k€, for the years 2003-2010.

<table>
<thead>
<tr>
<th></th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
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<th>2009</th>
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<tbody>
<tr>
<td>Revenues</td>
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<td>3864</td>
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<td>2669</td>
<td>2653</td>
<td>3055</td>
<td>3244</td>
<td>2851</td>
</tr>
<tr>
<td>Costs</td>
<td>1659</td>
<td>3409</td>
<td>2334</td>
<td>2550</td>
<td>2571</td>
<td>3097</td>
<td>3429</td>
<td>2891</td>
</tr>
<tr>
<td>Result</td>
<td>107</td>
<td>455</td>
<td>21</td>
<td>119</td>
<td>82</td>
<td>-42</td>
<td>-185</td>
<td>-40</td>
</tr>
</tbody>
</table>

Table 5: representation of income and costs in the 3rd funding source, in k€, for the years 2003-2010.

Table 4 shows an exceptional negative result of -311 k€ in the 2nd funding source where a result of ~0 is expected. This is caused by a change in the financial methodologies related to two KNAW (Royal Dutch Academy of Arts and Sciences). The funds for these projects were received in previous years and were sufficient.

Personnel

The university aims at a more equal division of males and females in the staff at all levels. On December 31st, 2010 the PhD student male: female ratio was 41:59. At the post doctoral level this was 83:17. Of the assistant professors 71% was male. Age-wise our staff is spread over the full range from starting PhD, to people who are (close to) retiring.

Infrastructure

In 2010, the entire Swammerdam Institute for Life Sciences was located in the new building of the faculty. Positive aspects of the new infrastructure are the brand new labs in a beautiful building.
Appendix 1

Research Programme  Systems Biology of the Living Cell

Molecular Microbial Physiology

Patent application
M. Bekker, M.J. Teixeira de Mattos, K.J. Hellingwerf: Production of L-lactic acid by photofermentation. P6030715PCT

Academic publications


Membership editorial board

Invited lectures
Hellingwerf, K.J. (2010, December 14). Biophysics and Biochemistry of Photo-perception. Amsterdam, Free University, Dr.R.Frese.

Hellingwerf, K.J. (2010, November 15). The molecular basis of blue-light sensing in bacteria. Karlsruhe, Germany, Botanical Inst.of the Karlsruhe Inst. of Technology.


Hellingwerf, K.J. (2010, June 13). Biofuel production with Cyanobacteria using the Photanol approach. Lake Arrowhead, USA, 10th Cyanobacterial Molecular Biology Workshop.

Hellingwerf, K.J. (2010, April 21). The role of the protein in the isomerization reaction of photoactive yellow protein. Il Ciocco, Italy, Gordon Conference on Photosensory Receptors and Signal Transduction.

Hellingwerf, K.J. (2010, April 21). Biophysical methods as applied to photosense. II Ciocco, Italy, Gordon Conference on Photosensory Receptors and Signal Transduction.


Hugenholtz, J. (2010, October 20). Fermentation for increased health benefits of beverages. Davis,California, USA, University of Davis.

Hugenholtz, J. (2010, June 08). SysMO-LAB1. Noordwijkhout, the Netherlands, Meeting SysMO-LAB.


Teixeira de Mattos, MJ (June, 2010). Lecture on "From idea to spin-off: using engineered cyanobacteria for clean productions" Honours program, UvA.

Teixeira de Mattos, MJ (June, 14, 2010). Lecture on "Sustainable production of chemicals by engineered cyanobacteria" Department of Biophysics, Free University of Amsterdam, host: Prof v Grondelle)

Teixeira de Mattos, MJ (October, 2010). Lecture on "Biofuel production by engineered cyanobacteria" University of Delft


Molecular Biology and Microbial Food Safety

PhD Thesis

Patent application
Brul, S., Waal, S.V. van der, Soet, J.J. de & Sluis, L.W.M. van der (18-02-2010). Disinfectant composition and its use in dental treatment. no P6028544US.

Academic publications


spores; putative indicator microorganism for optimisation of thermal processes in food sterilisation. Food research international, 43(7), 1895-1901.


Non refereed publication

Book Chapter

Invited lectures


Membership Editorial board

Academic publications

Nuclear Organisation

**Book Chapters**


**Membership Editorial Board**


**Prize**


**Invited lectures**


**Epigenetic Regulation of Gene Expression**

**Academic publications (refereed)**


Molecular Cytology

Patent

Academic publications

- 43 -
Appendix 1

conditions in adulthood. *Journal of Neuroscience*, 30(19), 6635-6645.

**Membership editorial board**

**Invited lectures**

Gadella, T.W.J. (2010, June 3). *New probe strategies for quantitative imaging of subcellular signalling dynamics*. Amsterdam. SILS research day, University of Amsterdam.
Appendix 1


Appendix 2

Research Programme  Plant Signalling

Plant Physiology

PhD Thesis

Academic publications

Book Chapters
Appendix 2

Book editing

Membership editorial board

Invited lectures
Munnik, T. (2010, July 02). Polyphosphoinositides in membrane trafficking during plant stress and development. Amsterdam, the Netherlands, EPS workshop - 'Endomembranes in plants'.

Molecular Plant Pathology

Academic publications

Testerink, C. (2010, May 22). SnRK2 protein kinases directly bind the lipid second messenger phosphatidic acid and are involved in the response of Arabidopsis roots to salinity. Valencia, Spain, The third meeting of FAO605 "Plant Abiotic Stress: from signalling to crop improvement".
Munnik, T. (2010, July 02). Polyphosphoinositides in membrane trafficking during plant stress and development. Amsterdam, the Netherlands, EPS workshop - 'Endomembranes in plants'.


**Invited lectures**


Rep, M. (2010, June 16). *Pathogenicity chromosomes of Fusarium oxysporum (Keynote lecture)*. Wageningen, the Netherlands, Spring meeting of the Royal Dutch Phytopathological Society (KNPV).


Rep, M. (2010, March 22). *Genome dynamics and host-specific virulence in plant pathogenic fungi*. University of Turin, Italy, Mycological Snapshots, workshop organised by the PhD School of Science and High Technology, Course Biology and Biotechnology of Fungi, Plant Biology Department.


Takken, F.L.W. (2010, October 08). *Molecular co-evolution in the tomato-Fusarium pathosystem*. Giessen, Germany, Justus Liebig University, Giessen, Germany.

Appendix 3

Research Programme  SILS Center for NeuroScience

Cognitive and Systems Neuroscience

PhD Thesis


Academic publications (refereed)


Huijbers, W., Pennartz, C.M.A. & Daselaar, S.M. (2010). Dissociating the “retrieval success”
regions of the brain: Effects of retrieval delay.  
*Neuropsychologia*, 48(2), 491-497.

*Neurolmage*, 51(1), 112-122.

*Journal of Neurophysiology*, 103(3), 1658-1672.

*Neurobiology of Learning and Memory*, 93(3), 422-427.

*Neurolmage*, 49(1), 1045-1054.

**Book chapters**


**Membership editorial board**


**Invited lectures**


Cellular and Systems Neurobiology

PhD Thesis


Academic publications


Noam, Y. & Baram, T.Z. (2010). Hyperpolarized views on the roles of the


**Proceedings**


**Book Chapter**


**Membership editorial board**


**Prizes**

Vliet, E.A. van (2010). The role of the blood-brain barrier and multidrug transporters in pharmaco-resistant epilepsy'. Prijs uitgereikt tijdens het Nationaal Epilepsie Symposium: Nieuwegein,
Erkenning.

**Other result**

**Invited lectures**
Hooft, J.A.van (November 2010). 5-HT3 receptors are growing up: a critical role in cortical development. Invited lecture at the Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii, USA.

Wadman, W.J. (2010, May 21). *Neurostimulation, where do we go?* Int.Epilepsy Workshop, Ghent, Belgium
Wadman, W.J. (2010, June 21). *We zijn ons brein*. The magic machine, Museum van der Togt, Amstelveen
Wadman, W.J. (2010, August 7). *Brain@Work*. Van Ditmar, Amsterdam
Wadman, W.J. (2010, September, 24). *The future of Neuroscience*. Dean special session, Ghent, Belgium
Wadman, W.J. (2010, December 9). University Maastricht
Structural and functional plasticity of the nervous system

PhD Theses


Academic publications


**Book Chapters**


**Membership editorial board**


**Invited lectures**


Korosi, A. (2010, August). Resilience to stress-related disorders as a result of augmented maternal care involves reduced excitation onto CRH expressing neurons in the hypothalamus. 7th World Congress on Stress. Leiden, the Netherlands.


Lucassen, P.J. (2010, March 15). Structural plasticity in dementia. Amsterdam, the Netherlands, Lecture at the International NEURAD meeting.


Lucassen, P.J. (2010, November 11). Mouse models for Alzheimer; where have we come from and are we there yet? San Diego, USA, Invited Lecture at the ISAO workshop.


Appendix 4

Research programme

Life Science Technologies

Mass Spectrometry of Biomacromolecules

PhD Theses


Academic publications (refereed)


Invited lectures


BioSystems Data Analysis

PhD Theses


Academic publications


paired data analysis: multilevel PLSDA versus OPLSDA. *Metabolomics*, 6(1), 119-128.

**Membership editorial board**


**Invited lectures**
Smilde, A.K. (2010, September 02). *Analyzing metabolomics data: from simple to complex*. Vienna, Austria, University of Vienna.

**Micro Array Department and Integrated Bioinformatics Unit**

**Academic publications (refereed)**


Proceedings

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