

**IB-course 2009**  
**March 23-April 1**

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**A course**  
**for**  
**Master students BMS**  
**newly appointed graduate students**  
**and post-docs**

**at the Institute of Biomembranes**

## **A short introduction to the Institute of Biomembranes**

From the very beginning of the Institute, its scientific mission has been focused on the following three themes:

- 1) Structure, function and organization of lipids and proteins in biological membranes
  - 2) Biogenesis of membranes and the transport and sorting of lipids and proteins
  - 3) The role of biomembranes in signal transduction via membrane receptors, cell-cell, and cell-matrix interactions.
- Later the topic vascular membrane biology was added.

The Institute of Biomembranes was officially inaugurated on October 4, 1991. One year thereafter the University Board formally recognized the institute as a Research Institute; the Faculty of Biology was appointed as the corresponding faculty. The Institute of Biomembranes was recognized as Graduate School by the Royal Netherlands Academy of Sciences (KNAW) in August 1994, a recognition that was renewed in June 1999 and 2004. The original number of eleven research groups that participated in the Institute / Graduate School of Biomembranes grew to a total of sixteen from three Faculties: Science (with departments of Biology & Chemistry), Medicine and Veterinary medicine.

In 2004 the IB graduate school has received re-accreditation for the forthcoming six years from the ECOS committee of the KNAW. At that time Prof. dr. Gerrit van Meer (Membrane Enzymology, Chemistry, UU) succeeded Prof. dr. Arie Verkleij, cofounder of the IB (Cell Biology, Biology, UU), as Scientific Director. Since 2009 the Institute of Biomembranes is a PhD programme in the Graduate School of Life Sciences.

### *Mission of IB*

*The Institute of Biomembranes (IB) is a multi-disciplinary research-training PhD programme at Utrecht University. Its mission is:*

- *To perform cutting-edge research on biological membranes and thereby increase our insight into important processes of life.*
- *To apply the newly acquired knowledge for solving societal problems.*
- *To provide an optimal infrastructure and training for young researchers.*

### **In 2009 the IB board/programme committee is composed of:**

- Prof. dr. Flip de Groot (Faculty of Medicine)
- Prof. dr. Bernd Helms (Faculty of Veterinary Medicine)
- Prof. dr. Antoinette Killian (Dept. of Chemistry, Faculty of Science)
- Prof. dr. Judith Klumperman, chair (Faculty of Medicine)
- Dr. Paul van Bergen en Henegouwen (Dept. of Biology, Faculty of Science)

Director/programme leader: Prof. dr. Gerrit van Meer; e-mail: [g.vanmeer@uu.nl](mailto:g.vanmeer@uu.nl)

Managing director: Dr. Hans de Cock, Tel. 2536616; e-mail: [ib.decock@uu.nl](mailto:ib.decock@uu.nl) or [h.decock@uu.nl](mailto:h.decock@uu.nl)

Secretariat: Antje Feitsma, Tel: 2533184; e-mail: [a.j.feitsma@uu.nl](mailto:a.j.feitsma@uu.nl)

## The 2009 PhD committee (since 2-2009)

- **Elsa Berends** (Membrane Enzymology & Microbiology, Fac. of Science) 030-253 3622 [e.berends@uu.nl](mailto:e.berends@uu.nl)
- **Bram Dorresteijs** (Biology, Fac. of Science) 030-253 3257 [b.dorresteijs@uu.nl](mailto:b.dorresteijs@uu.nl)
- **Emma Martinez Sanchez** (Cell Biology, Fac. of Medicine) 088 7556479 [e.martinezsanchez@umcutrecht.nl](mailto:e.martinezsanchez@umcutrecht.nl)
- **Johanna Schaefer** (Biochemistry & Cell Biology., Fac. of Vet. Medicine) 030-253 2563 [j.m.schaefer@uu.nl](mailto:j.m.schaefer@uu.nl)

The goal of the IB-AIO/OIO committee is to promote the interests of all graduate students united in the Institute of Biomembranes. If Ph.D. students have problems with, or questions or suggestions about the daily practise of the IB, they can pose these to the IB-AIO/OIO committee. Subsequently, one of the committee members will inform the IB board about problems or useful ideas. Another major goal of the committee is to strengthen the scientific and social contacts between graduate students of the different research fields. To promote these goals as good as possible, a meeting of two days will be held at which Ph.D. students can participate (no staff members allowed). During this meeting, graduate students can get to know each other on friendly terms, regardless of their research field or graduate year. Half a day will be reserved for the annual IB-AIO/OIO meeting, in which subjects regarding the interests of all Ph.D. students within the IB can be discussed and voted upon.

## **PhD-training programme & CV**

The Graduate School of Life Sciences (GS-LS) will evaluate the education of the PhD students, based on the CV prepared by the student and the signature of the thesis advisor. The minimum program size required to obtain your certificate of the GS-LS is 20 ECTS (1.5 ECTS = 1 week, or 1 ECTS = 28 hrs). For more information and more courses available at the GS-LS, see the following link: <http://www.uu.nl/lifesciences>. Requests for an education certificate of the GS-LS can be sent to the secretary of the Graduate School of Life Sciences:

**Dr. Saskia Ebeling**, Bureau Faculteit Betawetenschappen  
kamer 001b, Budapestlaan 6, 3584 CD Utrecht, Tel: 253 7843 /  
063800 3792, e-mail [s.ebeling@uu.nl](mailto:s.ebeling@uu.nl)

PhD students will receive the following ECTS credit points for the indicated IB courses

1. IB course (3 ECTS)
2. Day of the Graduate students (1.5 ECTS)
3. IB seminar program (5 ECTS)
4. IB evenings for Graduate students (4 ECTS)
5. IB AIO retreat (3 ECTS)
6. IB Conference on Biomembranes (1.5 ECTS)

A total of 18 ECTS over 4 years can be obtained via the education program of the IB. PhD students can obtain additional ECTS credit point by following additional courses, for example summer schools and conferences.

## **Set up of the course**

During the course the participants will receive theoretical and some practical insights (site visits) in the area of biomembrane research by experts of the Institute of Biomembranes. Detailed information of techniques and technology present in the IB will be discussed. PhD students and Post docs receive in this way a basic training in the field of biomembrane research. Your participation will be evaluated.

## **Project presentation**

The project presentation will consist of two parts.

**Part I:** Summary of special techniques used and present within IB groups within specific IB themes

**Part II:** Presentation of your own project.

### **Part I:**

The participants will form 4 equally sized groups, which will be named according to the 4 IB themes, **BTS**, **MP**, **ST**, and **VMB + all site visits**. Each group will summarize all special techniques available and/or discussed by speakers within this theme. **It is therefore important to pay special attention to special techniques during this course and ask questions to all speakers which techniques they use, how it works and have indeed available in their lab.**

The group members will prepare a short summary of Part I indicating: Available technique/which IB group/ advantages or disadvantages (deadline, end of the afternoon March 31: send to H. de Cock)

### **Part II:**

All participants will present an outline of their own research proposal/project and will emphasize the use of new techniques or approaches that were discussed during this IB course.

This research proposal will need to include:

1. Research question
2. Some background information

3. The general outline of the approach (short)
4. Which techniques (why, where in the IB)

Group presentations of Part I and II will be on April 1 (max 40 min/group including discussion)

Recommended textbooks:

Stryer's "Biochemistry"

Alberts, *et al.* "The Molecular Biology of the Cell"

### **Coordinators of the course**

**BTS** (Biogenesis, Translocation & Sorting)

Hans de Cock en Judith Klumperman

**MP** (Membrane Proteins)

Gerrit van Meer en Antoinette Killian

**VMB** (Vascular Membrane Biology)

Flip de Groot

**ST** (Signal Transduction)

Bernd Helms

**General coordination:**

**Hans de Cock 030-253 6616/ IB.decock@uu.nl**

## The four IB themes

### ***"Biogenesis, Translocation & Sorting"*** (BTS)

- Mechanisms of protein- and lipid-insertion in-, and translocation through membranes in prokaryotic and eukaryotic cells (included are cytoplasmic- and outer-membranes, ER, mitochondria, or chloroplasts).
- Biogenesis of membrane-, secretion- and organelle-proteins (included are aspects like folding, co- and post-translational modifications, and the assembly in macro molecular complexes).
- Mechanisms of addressing, sorting, transport and translocation of proteins and lipids in the cell.

### ***"Membrane proteins"*** (MP)

- Structural and topographic analysis of membrane-proteins/enzymes, by way of Röntgen-analysis, EM, and chemical- and genetic techniques;
- How do these proteins work (biochemical characterisation, ligand-binding sites, specificity);
- Regulation of membrane protein functions by way of membrane structure/-components (effects of membrane lipids/lipid-organisation on the activity of membrane enzymes and / or the characteristics of membrane proteins).

### ***"Signal transduction"*** (ST)

Localisation and function of:

- Membrane-associated proteins like phospholipases, kinases, receptors and pore- and transport proteins.
- Signal transduction components, like ras, MAP-kinase, etc.
- Phospholipid transport proteins.
- Cell cycle regulation: cdks, cyclins, etc.

### ***"Vascular Membrane Biology"*** (VMB)

- Cardiovascular risk factors (hypertension, uremia) and endothelial cell function and the role of progenitor cells.
- Aging of the vessel wall and endothelial cells by way of genomics, proteomics, electron microscopy and cell biological techniques.
- Interaction between haemostatic proteins and cell surface receptors on platelets, blood cells and endothelial cells, implications for haemostasis and thrombosis.

## Program

- The participants will have lunch together in the restaurant of de Wentbuilding (first floor), which is served between 12.00 and 13.15 h.
- Between lectures, coffee and tea is served
- Nearly all activities are organized in the Wentbuilding, Sorbonnelaan 16, Utrecht (Exceptions are indicated)

## **Program IB course, March 23-March 27**

<b>Date</b>	<b>Group</b>	<b>Activity</b>	<b>Location</b>
Mo, March 23 <b>Morning</b> 9.15-10.00 10.15-11.00 11.15-12.00 <i>Afternoon</i> 13.15-14.00 14.15-15.00	Van Meer/de Cock Gerrit van Meer Jan Tommassen  Han Wösten Richard Wubbolts	Welcome Lecture on BTS* " " Lecture on BTS "	Went N017 " " " "
Tu, March 24 <b>Morning</b> 9.15-10.00 10.15-11.00 11.15-12.00 <i>Afternoon</i> 13.15-14.00 14.15-15.00	Stefan Rüdiger Joost Holthuis Judith Klumperman  Fulvio Reggiori Peter van der Sluijs	Lecture on BTS " " Lecture on BTS "	Went N020 " " " "
We, March 25 <b>Morning</b> 9.15-10.00 10.15-11.00 11.15-12.00 <i>Afternoon</i> 13.15-14.00 14.15-15.00	Peter Rottier Antoinette Killian Kees Rodenburg  Paul van Bergen en Henegouwen Group Killian	Lecture on MP* " " Lecture ST <b>Site visit</b> CD/NMR	Went N018 " " N022 Kruytbuilding Floor 8 West wing
Thu, March 26 <b>Morning</b> <b>9.00 - 9.45</b> <b>10.00-10.45</b> <b>11.00-11.45</b> <i>Afternoon</i> 13.15-14.00 14.15-15.00	Marcel van der Heyden Eefjan Breukink Jan Tommassen  Jos Brouwers Richard Wubbolts	Lecture on MP " " <b>Site visits</b> Lipidomics Multiphoton microscopy	Went N018 " Went N017 N. Gildestein Colloquim- zaal 201
Fi, March 27 <b>Morning</b> 9.15-10.00 10.15-11.00 11.15-12.00 <i>Afternoon</i> <b>13.00-14.00</b> 14.15-15.00	Mark Roest Harry Heijnen Jan-Willem Akkerman  <b>IB seminar</b> <i>Meeting with speaker</i>	Lecture on VMB* " " " <b>A. Kocer</b> Discussion papers	Went N018 " " Kruyt O128 Kruyt O104

### Program IB course, March 30 – april 1

<b>Date</b>	<b>Group</b>	<b>Activity</b>	<b>Location</b>
Mo, March 30 <b>Morning</b> 9.15-10.00 10.15-11.00 11.15-12.00  <i>afternoon</i> 13.15-14.00	Ger Strous Madelon Maurice Bart Gadella  Willie Geerts	Lecture on ST* ” ”  <b>Site visit EM</b>	Went N018 ” ”  Kruytbuilding West 511
Tu, March 31 <b>Morning</b> 9.15-10.00 10.15-11.00 11.15- <b>11.45</b>  <i>afternoon</i>	Bas Vaandrager BerndHelms IB AiO committee  All Participants	Lecture on ST ” Introduction  Prepare presentations	Went N018 ” ”  
We, April 1 <b>9.00-11.45</b>	All participants	<b>Project presentations</b>	Went N018

### **Building**

H.R. Kruytbuilding  
F.A.F.C. Wentbuilding  
Nieuw Gildestein

### **Address**

Padualaan 8, Utrecht  
Sorbonnelaan 16, Utrecht  
Yalelaan 2, Utrecht

## **Abstracts**

**March 23**

### **Gerrit van Meer**

Membrane Enzymology, Chemistry

#### **The biosynthesis, transport and sorting of membrane lipids and proteins**

The various cellular membranes have different protein and lipid compositions. In view of the rapid transport between these membranes via vesicles, this must be selective. The basis for the selective transport of both proteins and lipids resides in their aggregation into microdomains. For proteins this generally requires an interaction with coat proteins on the cytosolic surface of the membrane. Since lipids do not span the bilayer and since there are many more lipids than coat proteins, their aggregation must be based on their self-aggregating properties. Lipid "rafts" are thought to be membrane domains of a specific lipid composition that are enriched in specific proteins. They are thought to be involved in protein sorting and in signal transduction at the plasma membrane. An additional level of specificity is found in the transbilayer movement of the lipids by flippases.

### **Jan Tommassen**

Microbiology, Biology

#### **Outer membrane biogenesis in Gram-negative bacteria**

Gram-negative bacteria are surrounded by a double membrane, an inner and an outer membrane, which are separated by the periplasm. Due to its composition, the outer membrane provides a rigid barrier to the influx of many antibacterial compounds. This membrane consists of phospholipids, lipopolysaccharides (LPS), integral membrane proteins, and lipoproteins. These components are all synthesized in the cytoplasm or at the inner leaflet of the inner membrane and have to be transported across the inner membrane and through the periplasm to assemble eventually in the correct membrane. One of the major research areas in our laboratory is to understand the molecular details of these transport and assembly processes, with emphasis on how outer membrane proteins and LPS are inserted in the outer membrane. Furthermore, our insights into the appearance of the bacterial cell surface are applied for vaccine development for human pathogens such as *Neisseria meningitidis* and *Bordetella pertussis*

Techniques used:

- general microbiology techniques
- molecular biology: construction of mutants, expression systems etc.
- protein refolding and structural analysis
- biochemical techniques: protein purification by chromatography
- immunological techniques: ELISA, bactericidal assays

### **Han Wosten**

Microbiology, Biology

#### **Differentiation in the fungal colony**

Within our research group we study fungal growth and differentiation. We use both industrial and pathogenic model systems. In this presentation I will focus on one of our research lines as an example how we approach our research questions.

Filamentous fungi form colonizing mycelia. The hyphae making up the fungal mycelium are interconnected by porous septa. In other words the cytoplasm can be considered a continuous system. We recently showed that the fungal colonizing mycelium is highly differentiated. Expression profiles in the centre and the periphery of the mycelium are distinct and can be explained by nutrient dependent and nutrient independent mechanisms. Even within the periphery we have observed differentiation. For instance, part of the hyphae highly expressed the glucoamylase gene *glaA* while others did it to a low extent. We also observed temporal changes in the expression profile at the periphery despite the fact that the medium composition did not change in this part of the colony. Future studies will aim at the identification of the mechanisms underlying differentiation in the fungal mycelium. Within our research group we (will) use the following techniques: genome wide expression analysis, massive parallel sequencing, reporter studies, fluorescence light and confocal microscopy, and laser dissection.

### **Richards Wubbolts**

Department of Biochemistry and Cell Biology

#### **Membrane Transport by Specialised Mammalian Cells**

Within the group of Willem Stoorvogel in the department of Biochemistry and Cell Biology we are interested in the intracellular and intercellular traffic of membrane embedded molecules. We focus on endosomal sorting mechanisms that are highly developed in specialized immune cells, dendritic cells. Especially the traffic of MHC class II molecules in dendritic cells is studied with fluorescence microscopy, electron microscopy and biochemical methods. We use retroviral transfer to modify sorting pathways in these dendritic cells and assay by combining advanced imaging techniques (multiphoton live cell imaging) with classical biochemical techniques. Next to endosomal trafficking paths, intercellular transfer of membrane embedded molecules is studied. Such molecular micro-domains of immune cells can be transferred from for example an antigen-presenting cell towards T cells. We are studying when and how endosomal-derived membranes termed exosomes can perform these functions.

**March 24**

**Stefan Rüdiger**

CPC, Chemistry

**Protein folding and protein sorting in the cell**

The Central Dogma states that DNA makes RNA makes protein. The least understood step in this basis sequence is how the folding of proteins into their three-dimensional structure is controlled inside the cell. This fundamental question is the focal point in the section Cellular Protein Chemistry, which consists of three research groups. The work of the group of Ineke Braakman focuses on protein folding in the endoplasmic reticulum, where the maturation of secretory and membrane proteins such as the CFTR or influenza haemagglutinine is controlled. The group of Henk Tabak investigates the origin of peroxysomes, for which it emerged only recently that the text book assumption that they are self-replicating organelles was wrong. The group of Stefan Rüdiger analyses protein folding assisted by the molecular chaperone Hsp90, which has a mysterious preference to assist folding of oncogenes.

We address our questions by using various methods to study protein folding and sorting in vivo and in vitro. Our methods range includes cell culture, pulse chase experiments, semi-permeable cell systems, fluorescence microscopy, yeast genetics, protein purification, in vitro stability analysis of proteins and fluorescence spectroscopy. In collaboration with other groups in the IB or the Bijvoet Center, we use proteomics approaches, mass spectroscopy, EM and NMR spectroscopy

**Joost Holthuis**

Chemistry

**The emerging role of lipid flippases in membrane trafficking**

Cells display asymmetric lipid distributions across their plasma membranes with the aminophospholipids concentrated in the inner leaflet. How this asymmetry is established and what purpose it serves for the functioning of cells is not well understood. We identified two P-type ATPases required for aminophospholipid transport across the plasma membrane in yeast and uncovered a functional link between ATPase-dependent lipid pumping and endocytic vesicle formation. Recently, we found that the yeast Golgi contains similar ATPases with a critical role in secretory vesicle budding. Our current work aims to unravel the mechanism of ATPase-dependent lipid transport in relation to membrane deformation and vesicular trafficking.

***Techniques:***

yeast genetics; subcellular fractionation; lipid transport assays; flow cytometry; membrane protein purification and reconstitution.

**Judith Klumperman**

CMC, Department of Cell Biology, University Medical Center Utrecht

**Microscopy approaches in cell biology**

The Cell Microscopy Center is an internationally renowned expertise center for the application of advanced microscopy studies in cutting edge bio-medical research. The

main microscopy techniques available are immuno-electron microscopy, live cell imaging, correlative live cell – immuno electron microscopy. We also do quite a lot of 3D tomography in collaboration with the group of Arie Verklei.

The CMC presently houses a Leica confocal microscope, a Zeiss LSM510-Meta confocal and a Zeiss Axiovert 200MBP epifluorescence system. The CMC further houses microtomes to prepare ultrathin (cryo)sections, 3 JEOL transmission EMs and Leica high pressure freezing equipment for the fastest way to fix specimen for microscopy assays.

The CMC is also equipped with a Drosophila lab to exploit the power of genetics and developmental biology and combine it with the microscopy facilities described above.

The general research theme in the CMC concerns intra- and extracellular communication events in relation to disease and development. Presently, there are three main research lines:

- Lysosome biogenesis in healthy and diseased cells (PI: Judith Klumperman)
- The exocytic pathway in relation to Drosophila development (PI: Catherine Rabouille)
- Cellular autophagy from yeast to mammals (PI: Fulvio Reggiori).

I will illustrate the use of the technology in the lab in the research done in my group.

### **Fulvio Reggiori**

CMC, Department of Cell Biology, University Medical Center Utrecht

#### **Exploring the molecular mechanism of autophagy**

The conserved catabolic pathway of autophagy plays a key role in eukaryotic organisms because directly implicated in several physiological functions and triggered in numerous pathological situations. The basic mechanism of autophagy entails the sequestration of cytoplasmic material, including proteins and organelles, inside double-membrane transport vesicles called autophagosomes that are targeted to the lysosome/vacuole for breakdown and recycling. Important progresses have been achieved with the identification and preliminary characterization of the Atg proteins, the factors specifically involved in the formation of autophagosomes. The study of the precise function of these proteins as well as the mechanism of autophagy, however, has been hampered by the lack of information regarding the membrane dynamics during this process, e.g., how autophagosomes are generated. Our research group is studying that as well as the function of various Atg proteins in both yeast and mammalian cells. In this lecture, I will present the different experimental approaches that we use in these two systems.

Techniques: yeast genetics, cell lines, molecular biology, biochemical approaches, life-cell imaging, fluorescence microscopy, electron microscopy, immuno-electron microscopy, proteomics, lipidomics.

### **Peter van der Sluijs**

CMC, Cell Biology, UMCU, Fac. of Medicine

#### **Regulation of secretory lysosome function in haematopoietic cells**

Haematopoietic cells combine the functions of lysosomes and secretory granules into a hybrid organelle, otherwise known as secretory lysosome. Secretory lysosomes are

distinct from conventional lysosomes in that signaling routes originating from the cell surface of haematopoietic cells trigger the release of biological effectors like granzymes in CTLs, and of histamine in mast cells. It is not understood how immune receptor signaling is wired into the machinery that actually accomplishes the fusion of secretory lysosomes with the plasma membrane. In this lecture I will discuss our strategies to find and functionally characterize proteins important for this pathway.

#### *Techniques*

Proteomic screens for identification novel proteins involved in membrane transport  
Genetic biochemical and light microscopy-based protein interaction assays  
Recombinant protein expression and purification  
Creation of transfected cell lines by various methods  
Fluorescence techniques including live cell imaging and FACS analysis  
Transport assays in endocytic and exocytic pathways in higher eukaryotes

#### *Contact information*

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### **March 25**

#### **Peter J.M. Rottier**

Virology Division, Faculty of Veterinary Sciences.

**The biology of coronaviruses, with emphasis on how these viruses enter their target cells.**

The presentation will have 2 parts. In the first part a general overview will be given about coronaviruses. Coronavirus infections will be discussed at three levels: the level of the cell, which involves the complete infection cycle going from the penetration of these viruses into cells to the stage where new viral particles are massively being produced; the level of the organism, where aspects of virulence and pathogenesis will be discussed; the level of the population, with emphasis on the development of vaccines.

In the second part of my talk I will go into more detail regarding the process of coronavirus entry. In this part I will review our work on the viral spike protein, which is responsible for the fusion of the viral and cellular membrane that leads to the delivery of the viral genome into the cell's cytoplasm

#### **Antoinette Killian**

Chemical Biology, Chemistry, Faculty of Science

**Understanding membrane proteins**

#### *Topics:*

- short introduction of research themes within the dept. Biochemistry of Membranes
- model membrane systems
- architecture of membrane proteins
- use of synthetic peptides

#### *Techniques:*

- solid state NMR on peptides and lipids in membranes

- circular dichroism and FT-IR as tools to determine the secondary structure of (membrane) proteins

### **Kees Rodenburg**

Endocrinology and Metabolism, Biology, Faculty of Science

#### **Lipids and energy generation: cellular control of lipid storage and release**

The research program addresses animal metabolism, and is particularly focused on regulation of energy generation and lipid metabolism. Underlying questions lead to fundamental knowledge, whereas spin-off is applied:

- ✓ How can animal metabolism sustain exercise for longer periods of time (e.g. flight activity of insects)? Main topics are lipid storage, mobilization, transport and uptake.
- ✓ Neutral lipids are stored in dynamic intracellular organelles called lipid droplets: where in the cell and how do lipid droplets form? What is the molecular machinery, which proteins and protein-lipid interactions are involved, and what are the underlying biophysical principles?
- ✓ What is the molecular and structural basis of lipoprotein functioning in lipid transport? Insect lipoproteins are used as a model system for the structure and metabolism of human lipoproteins. Latter lipoproteins are involved in transport of lipids such as cholesterol, but also in metabolic disorders (obesity, cardiovascular disease).
- ✓ How are evolutionary data on lipoprotein receptor functioning applicable for a better understanding of this process in human tissues? Many aspects of lipoprotein receptors in lower animals differ from those in man.
- ✓ Is defective recycling of a fatty acid transporter in the heart involved in diabetes type-2, and does this transporter provide a target for treatment?

### **Paul van Bergen en Henegouwen**

Cellular Architecture and Dynamics, Biology, Fac. of Science

#### **How to stop signal transduction?**

EGFR and its family members are strongly implicated in the development and progression of different human tumors including breast-, lung-, prostate-, colorectal-, head and neck cancer and glioma. The epidermal growth factor (EGF) receptor (EGFR or ErbB1) is the prototype of a family of four related receptor tyrosine kinases (RTK: ErbB1-4), which are activated by trans- or cross-phosphorylation of the intracellular domain. This receptor is an attractive target for therapy: signaling can be blocked by prevention of ligand binding, stimulation of receptor degradation or by targetting small molecule inhibitors to tumor cells overexpressing EGFR. For these purposes we have developed Llama antibodies that recognize the extracellular domain of the receptor. The variable region of the heavy chain of the Llama antibodies is the the smallest antigen-binding unit (15 kDa) which is called nanobody. Development and analysis of therapeutical application of anti-EGFR nanobodies will be presented in this lecture.

*Contact information*

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## **March 26**

### **Marcel van der Heyden**

UMCU, Fac. of Medicine

#### **Physiology of membrane protein channels**

##### **Topics:**

- Short introduction of research themes in Medical Physiology
- Stem cell models for studying ion and gap junction channel regulation.
- Patch Clamp, a tool to study expression and function of ion channel proteins in embryonic and adult heart progenitor cells.

##### **Techniques:**

- Stem cell differentiation
- Ectopic ion channel expression
- Gene silencing
- Patch Clamp / Whole Cell Voltage Clamp
- (PCR, immuno-cytochemistry / Western blot)

### **Eefjan Breukink**

Chemical Biology, Chemistry, Fac. of Science

#### **Interaction of antibiotics with membranes**

##### **Topics:**

- Antibiotics, focussed on nisin
- bacterial cell wall synthesis,
- interaction of antibiotics with membranes

##### **Techniques:**

- Use and preparation of different model membrane systems
- Binding assays: biochemical assays, isothermal titration calorimetry
- Fluorescence spectroscopy: Trp fluorescence, quenching, FRET

### **Jan Tommassen**

Molecular Microbiology, Biology, Fac. of Science

#### **Transport proteins in the bacterial outer membrane**

The outer membrane of Gram-negative bacteria is not energized by a proton gradient and energy-rich compounds, such as ATP, are not available in the periplasm. An intriguing question then is how transport processes, such as the uptake of nutrients and the secretion of proteins, are energized. The bacteria have found many different solutions to this problem. In this lecture, several membrane proteins involved in these processes will be described, ranging from simple diffusion porins, which allow for the influx of nutrients by passive diffusion, to highly complex, multi-component machineries, which inject proteins directly from the bacterial cytoplasm into the cytoplasm of eukaryotic target cells.

## **March 27**

### **Mark Roest**

Department of Clinical Chemistry and Haematology  
Thrombosis and Haemostasis Laboratory  
UMC Utrecht, Fac. of Medicine

#### **Real time platelet function**

Platelets play a crucial role in acute coronary heart disease (CHD). Causal involvement for this is proven in clinical trials, which have shown that the incidence of acute CHD is 25-30% reduced by inhibition of platelets with aspirin or clodipogrel. There is an urgent need for accurate measurements of platelet function in blood. We have developed a real time assay to measure platelet function. This test will be validated with several in house platelet function assays. In the near future, we will use the real time platelet function assay to get a clear view on the different steps in platelet function in haemostatic and bleeding disorders.

In addition the real time platelet function assay will be used to validate plasma markers of platelet function. Reliable plasma markers of platelet function can be used to study the relation of platelet function the risk of Atherothrombotic disease.

### **Harrie Heijnen**

Department of Clinical Chemistry and Haematology  
Thrombosis and Haemostasis Laboratory  
UMC Utrecht, Fac. of Medicine

#### **Morphology of megakaryocyte and blood platelet**

Blood contains ~ 150,000-350.000 platelets/mm<sup>3</sup>. Platelets are non-nucleated discoid cells, 2-5 um diameter large, that derive from the megakaryocyte in the bone marrow. The life span of a circulating blood platelet is 8-11 days.

In this introduction we will discuss MK differentiation and maturation, the ultimate formation of platelets, and their function. Issues that will be addressed: morphology of MK and platelet, biosynthesis, endocytosis, organelle biogenesis, platelet birth, adhesion and cell dynamics.

Functional aspects: Adhesion to damaged endothelium and exposed collagen induces cytoskeletal rearrangement, platelet shape change, and release of storage granules. These actions lead to the promotion of the coagulation cascade and the formation of a stable clot and the arrest of bleeding. Cellular responses upon adhesion and activation, cytoskeletal rearrangements, microtubule organization

Electron microscopy: Ultrastructure, (trans) location of macromolecules, EM-Tomography.

Light microscopic imaging: Visualization of platelet dynamics in real time (i.e. adhesion under flow conditions. Different microscopical methods will be shown (confocal microscopy, interference reflection contrast microscopy), including the localization of receptors, visualization of cytoskeletal dynamics.

### **Jan-Willem Akkerman**

Department of Clinical Chemistry and Haematology  
Thrombosis and Haemostasis Laboratory  
UMC Utrecht, Fac. of Medicine

## **Signal transduction in blood platelets**

Blood platelets are anucleate cytoplasts that are vital in the arrest of bleeding but also form thrombi in the arteries. Its surface is covered with thousands of receptors that recognize signals that initiate platelet functions or suppress them. Platelets are formed by the megakaryocyte in a process of proplatelet formation and budding. Its circulates about ten days and is then removed by spleen and liver through destruction by macrophages. Under normal conditions platelets remain dormant. Factors released from the endothelium start signaling sequences that result in platelet inhibition. When the vessel wall is disturbed, the platelet adheres to adhesive proteins in the wound, starts shape change, secretion, platelet-platelet interaction and forms a surface that assists the coagulation scheme in formation of a fibrin clot. These reactions prevent excessive blood loss. A loss of inhibition by the vessel wall together with overproduction of activating factors results in an overreaction and platelets clump together even without major bleeding. The results is arterial thrombosis. Thus, a proper balance between activating and inhibitory signals keep the platelets in a resting state under normal conditions.

Platelets have an excess of activating and inhibitory receptors resulting in a complicated network of signaling sequences. Seven transmembrane receptors may start activating or inhibitory routes, cytokine receptors may prime the platelet for later activation and receptor-mediated ion channels contribute to adjustment of signal generating systems. This makes the platelet a cell that responds to changes in the surrounding medium within milliseconds.

### **IB seminar**

Friday March 27, 13:00, *H.R. Kruytgebouw, Room O128*

### **Armağan Kocer**

Dept. of Biochemistry, University of Groningen

## **"Membrane protein tailoring: new approaches to study membrane proteins"**

### **Abstract**

During the last decade there have been significant progress in engineering the membrane proteins<sup>1,2</sup>. The ability to manipulate these proteins brings exciting opportunities to both fundamental and applied research.

In the first part of this talk the recent engineering techniques will be presented. In the second part, engineering of Mechanosensitive channel of large conductance (MscL) from *Escherichia coli* will be discussed as an example of engineering of a protein in order to go beyond the limits of the currently available techniques and gaining new insights on its functioning.

References:

<sup>1</sup>Molecular Membrane Biology, 2004, 21, 209\_ 220. "Functional engineered channels and pores"

<sup>2</sup> Mol. BioSyst., 2007, Issue 3 "Understanding and Manipulating Channels and Pores"

### **Papers to be studied (will be provided as PDF)**

I would be very happy if students could read the papers (and the supporting information). They are interesting membrane protein activity

detection methods alternative to patch clamp and planar bilayer techniques. It could be nice if they think about potential applications of them and compare them with the patch clamp and planar bilayer techniques.

#### **Asymmetric Droplet Interface Bilayers**

William L. Hwang, Min Chen, Brid Cronin, Matthew A. Holden, and Hagan Bayley  
*J. Am. Chem. Soc.*, **2008**, 130 (18), 5878-5879 • DOI: 10.1021/ja802089s • Publication  
Date (Web): 26 March 2008

#### **Simultaneous Measurement of Ionic Current and Fluorescence from Single Protein Pores**

Andrew J. Heron, James R. Thompson, Bríd Cronin, Hagan Bayley, and Mark I. Wallace  
*J. Am. Chem. Soc.*, **2009**, 131 (5), 1652-1653 • DOI: 10.1021/ja808128s • Publication  
Date (Web): 15 January 2009

#### **Screening Blockers Against a Potassium Channel with a Droplet Interface Bilayer Array**

Ruhma Syeda, Matthew A. Holden, William L. Hwang, and Hagan Bayley  
*J. Am. Chem. Soc.*, **2008**, 130 (46), 15543-15548 • DOI: 10.1021/ja804968g •  
Publication            Date            (Web):            24            October            2008

## **March 30**

### **Ger Strous**

Department of Cell Biology, UMCU, Fac. of Medicine

#### **Growth Hormone Receptor studies**

GH and its downstream effector, IGF1, are major regulators of metabolic processes. Defects result in either metabolic syndromes (obesity, cachexia, and immune-deficiency) or unwanted growth (acromegaly and cancer). Fighting these conditions requires detailed knowledge of the GH receptor (GHR) signal transduction and degradation. We have identified the ubiquitin ligase SCF(TrCP) as a cargo selector for both endocytosis and endosomal sorting towards the lysosomes. In addition, our studies have revealed that the kinase (Jak2) is a negative factor in the degradation of the GHR. Both enzymes act via a direct interaction with the Ubiquitin Endocytosis (UbE) motif and box-1 in its cytosolic tail, respectively.

Although both SCF(TrCP) and Jak2 are common factors in many key regulatory processes, their interactions with the dimerized cytosolic tails of the GHR are unique. This insight has triggered a drug finding expedition to find small molecules in compound libraries that can either inhibit increase the activity of the GHR.

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### **Madelon Maurice**

CMC, Cell Biology, UMCU, Fac. of Medicine

#### **Mechanisms of Wnt signaling initiation in development and cancer**

Wnt protein secretion and signal reception are critical events in tissue patterning during development and in adult tissue homeostasis. Misregulation of Wnt signalling is a hallmark of cancer. Despite their central biological roles, remarkably little is known about how Wnts initiate productive signalling through their receptors.

In this lecture we will address how proximal Wnt signaling events are controlled and how dysregulation of protein function in the Wnt pathway leads to cancer. We focus on a number of key mechanistic aspects in the transmission of the Wnt signal in receiving cells. In an interdisciplinary approach, linking biophysics and *in vivo* cell biology, we aim to determine how mutational damage of Wnt cascade regulatory proteins affects their structural stability and how this relates to their physiological function in the cell.

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### **Bart Gadella**

Dept. Biochemistry and Cell Biology, Veterinary Medicine, UU

#### **Signal transduction pathways in sperm activation and mammalian fertilization**

Fertilization is a decisive moment in life and enables the combination of the DNA of two gametes to ultimately form a new organism. Intriguingly the identity and spatial ordering of molecules at the sperm surface involved in mammalian fertilization is essentially unknown. This lecture aims to address the question: Which sperm molecules are involved in fertilization and how are they reordered at the sperm's surface to achieve fertilization?

The sperm surface is highly heterogeneous and specific membrane domains are respectively involved in; sperm adhesion to the extra-cellular matrix of the egg, a secretion process required for sperm penetration, and the final fertilization at the egg plasma membrane. In mammals these processes only become apparent when sperm enters the oviduct or during in vitro fertilization incubation. A sperm specific signalling cascade induces clustering of membrane proteins involved in fertilization. The protein complexes emerge exclusively at the sperm surface area involved in fertilizing the egg. The described sperm surface ergonomics might be the key to fertilization.

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## **March 31**

### **Bas Vaandrager**

Dept Biochemistry and Cell Biology, Veterinary Medicine, UU

#### **Lipid droplets in health and disease.**

In eukaryotic cells the excess of hydrophobic molecules are stored in special organelles named, lipid bodies or lipid droplets. The formation and enlargement of lipid droplets is thought to be involved in various high incidence pathologies including obesity, liver disease, and atherosclerosis. For instance, a pathological accumulation of lipid droplets in hepatic cells is observed in obese and type II diabetic persons, after excessive alcohol consumption or during infections with hepatitis c virus. Interestingly, during the process of liver repair, hepatic stellate cells lose their retinyl esters-containing lipid droplets upon activation. Typically, lipid droplets contain triacylglycerides, cholesteryl esters and/or retinyl esters, depending on the function of the cell in which they reside. The hydrophobic content is shielded from the cellular interior by a monolayer of phospholipids and cholesterol, containing various specific proteins. Lipid droplets have traditionally been regarded as inert storage vessels. However, recent identification of proteins involved in lipid metabolism, signaling and membrane traffic suggests a more active role of these organelles in metabolism. In this presentation we will focus on the regulation of the formation and breakdown of lipid droplets and how this is affected in various pathologies.

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### **Bernd Helms,**

Dept. Biochemistry and Cell Biology, Veterinary Medicine, UU

#### **Host-pathogen Interactions: Hijacking intracellular signaling pathways**

Successful establishment of infection by bacterial pathogens requires adhesion to host cells and subsequent cellular invasion. This process is followed by intracellular multiplication, dissemination to other tissues, or persistence. Bacteria use sophisticated strategies to establish a complex host/pathogen molecular crosstalk that leads to subversion of cellular functions and establishment of disease. In many cases, pathogens divert existing intracellular signaling and membrane transport pathways to escape the immune response. We will focus on strategies of some intracellular pathogens and discuss how they target specific host cell lipids and proteins. Especially the modification of signaling pathways appears effective in changing their temporal and spatial distribution, making them ideal modulators of local and transient cellular mechanisms.

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### **IB AiO commissie**

They will inform you about all kind of IB activities.

## Participants IB course 2009

**Participants** **e-mail**

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