

IB-course 2011
Lectures march 21-25

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**A course for
newly appointed PhD candidates
and post-docs**

**PhD programme Biomembranes
GS-LS, UU
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. Prof. dr. Bernd Helms succeeded Gerrit van Meer as programme director of the IB.

As of 1-1-2009, the IB runs the PhD programme of the Graduate School of Biomembranes at Utrecht University.

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.*

The IB board/programme committee is composed of:

- Dr. Mark Roest (Faculty of 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)

Programme director: Prof. dr. Bernd Helms (Faculty of Veterinary Medicine)

Programme coordinator: Dr. Willie Geerts, Tel. 253 2885; e-mail: W.J.C.Geerts@uu.nl

Secretariat: Antje Feitsma, Tel: 253 3184; e-mail: a.j.feitsma@uu.nl

The 2011 PhD committee (since 2-2010)

- Pauline Krijgsheld (Microbiology, Fac. of Science) 030-253 3041
p.krijgsheld@uu.nl
- Remko van Vught (Biology, Fac. of Science) 030-253 5512
r.vanvught@uu.nl
- Emma Martinez Sanchez (Cell Biology, Medicine) 088 7556479
e.martinezsanchez@umcutrecht.nl
- Oliver Wicht (Virology, Fac. of Vet. Medicine) 030-253 5094
O.Wicht@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 EC (1.5 EC = 1 week, or 1 EC = 28 hrs). For more information and more courses available at the GS-LS, see the following link: <http://www.uu.nl/lifesciences>. Requests for a certificate of the GS-LS can be sent to the secretary of the Graduate School of Life Sciences:

Dr. Saskia Ebeling, Bureau Faculteit Betawetenschappen
Androclusgebouw kamer W.241 | Yalelaan 1 | 3584 CL Utrecht
| T. 030 253 8756 / 06 3800 3792

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

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

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

Preparation for lectures, workshops & site visits

During this course several lectures, workshops and site visits will require preparation by all participants by reading the indicated literature (see program).

For the two workshops after the IB seminar, a subgroup (see last page) will prepare a short presentation (10 min max) mentioning the most important points of the papers as well as a set of questions/discussion points that will serve as a start for further discussions with the rest of the group and IB speaker.

Project presentation march 23 & 25

All participants will present shortly an outline of their own research project (**± 7 min each!**):

1. Short outline & major research questions
2. How to approach (short)
3. Which techniques (why & where in the IB)

Recommended textbooks:

Stryer's "Biochemistry"

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

Program

- The participants will have lunch (provided by IB) together in the restaurant of de Kruytbuilding (first floor), which is served between 12.15 and 13.00 h.
- Between lectures, coffee and tea is served
- Locations
 - HK: H.R. Kruytbuilding, Padualaan 8
 - DG: Nieuw Gildesteijn, Yalelaan 2
 - UMC: UMCU, Heidelberglaan 100

Program IB course, 21-3/25-3 2010

Selected lectures & workshops to be prepared by PhD students:

Tuesday 22-3 IB lecture Ian Brewis

Friday 25-3 IB lecture Mary Munson

Program IB Course 2011, 21-3/25-3 2011			
Date	name	Activity	Location
Mon, March 21			
<i>Morning</i>			
9.00-9.45	Programmaleader/coord IB AiO committee	Welcome Introduction & activities	O110 HK „
9.45-10.30	Bernd Helms	Biochem & Cell Biol	„
Pause			„
10.45-11.30	Judith Klumperman	Cell Biology/UMCU	„
11.30-12.15	Jan Tommassen	Prokaryotic Microbiology	„
<i>Afternoon</i>			
13.00-13.45	Richard Wubbolts	Biochem & Cell Biol	O108 HK
13.45-14.30	Fulvo Reggiori	Cell Biology/UMCU	„
Pause			
14.45-15.30	Joost Holthuis	Membrane Enzymology	O108 HK
15.30-16.15	G Posthuma/D Egan	<u>Site visit CMC</u>	G.02.532/ UMC
Tu, March 22			
<i>Morning</i>			
9.00-9.45	Stefan Rüdiger	Cell. Prot. Chemistry	O310 HK
9.45-10.30	Berend Jan Bosch	Virology	„
Pause			
10.45-11.30	Jan Tommassen	Prokaryotic Microbiology	„
11.30-12.15	Toon de Kroon	Membrane Enzymology	„
<i>Afternoon</i>			
13.00-14.00	Ian Brewis	IB seminar	O123 HK
14.15-15.00	„	Workshop with IB speaker	O310 HK
Pause			
15.15-16.00	Eefjan Breukink	Biochemistry of Membranes	„
16.15-16.45	J Ballering/J Doux	<u>Site Visit Biochemistry of Membranes</u>	West 8 HK
Wed, March 23			
<i>Morning</i>			
9.00-9.45	Presentations participants	Project presentations	O110 HK
9.45-10.30	Han Wösten	Eukaryotic Microbiology	„
Pause			
10.45-11.30	Mark Roest	Haematology	„
11.30-12.15	Harry Heijnen	Haematology	„
<i>Afternoon</i>			
13.00-13.45	Anna Akhmanova	Cell Biology	„
13.45-14.30	Casper Hoogenraad	Cell Biology	„
Pause			
14.45-15.30	J Brouwer	<u>Site Visit Lipidomics &FACS facility; lectures</u>	C201 DG
15.45-16.30	B Gadella		(second floor)
Thu, March 24			
<i>Morning</i>			
9.00-9.45	Peter van der Sluijs	Cell Biology/UMCU	O108 HK
9.45-10.30	Madelon Maurice	Cell Biology/UMCU	„
Pause			
10.45-11.30	Thomas Schwend	Mass Spectrometry	„
11.30-12.15	Bas Vaandrager	Biochem & Cell Biol	„
<i>Afternoon</i>			
13.00-13.45	Eric Huizinga	Crystallography	„
13.45-14.15	Paul van Bergen en Henegouwen	Cell Biology	„
Pause			
14.45-15.30	Jan Andries Post	Biomolecular Imaging	„
15.30-16.15	JA Post	<u>Site visit Biomolecular Imaging</u>	West 5 HK

Fri, March 25 <u>Morning</u> 9.00-12.15	Presentations participants	Project presentations	O110 HK
Afternoon 13.00-14.00	Mary Munson	IB seminar	O123 HK
14.15-15.00	„	Workshop with IB speaker	O110 HK
Closing drinks			„

Information on site visits and papers to be studied

21-3-2011

Site Visit Cell Biology & Cell Microscopy Center, UMCU,

Location: G.02-532 (UMCU)

Organizers: George Posthuma and David Egan

Subjects to be discussed/demonstrated: Cryosectioning & Immunogoldlabeling; correlative light-electron microscopy; automated screening methods/imaging based screening

Papers to be studied:

NATURE PROTOCOLS | VOL.2 NO.10 | 2007 | 2481

Microsc Res Tech. 2010 Mar;73(3):215-24.

Nature methods | VOL.5 NO.11 | NOVEMBER 2008 | 973

NATURE Reviews | Drug Discovery volume 8 | july 2009 | 567

NATURE Vol 464| 11 March 2010

NATURE CHEMICAL BIOLOGY VOLUME 4 NUMBER 1 JANUARY 2008

22-3-2011

Site Visit Biochemistry of Membranes, Chemistry, Faculty of Science

Location: West 8 (H Kruytgebouw)

Organizers:

Joost Ballering and Jacques Doux

Subjects to be discussed/demonstrated: circular dichroism; calorimetry; monolayers; solid state NMR

23-3-2011

Site Visit Lipidomics & FACS facility, Biochemistry & cell Biology, Veterinary Sciences

Location: second floor room C201 (DG)

Organizers: Jos Brouwer & Bart Gadella

Subjects to be discussed/demonstrated: MS of lipids, FACS

lecture by Bart Gadella: **Signal transduction pathways in sperm activation and mammalian fertilization**

24-3-2011

Site visit Biomolecular Imaging, Biology, Faculty of Science

Location: West 5 (H Kruytgebouw)

Organizers: Jan Andries Post

Subjects to be discussed/demonstrated: Scanning EM

Papers to be studied: Journal of Microscopy, Vol. 235, Pt 3 2009, pp. 336–347

Abstracts

March 21

Bernd Helms,

Dept. Biochemistry and Cell Biology, Veterinary Medicine, UU

Of Fatty acid transport and Caveolins..... and cows

Papers to read

Kamp F, Hamilton JA. (1992) pH gradients across phospholipid membranes caused by fast flip-flop of un-ionized fatty acids. Proc Natl Acad Sci U S A. 89:11367-70.

Meshulam T, Simard JR, Wharton J, Hamilton JA, Pilch PF. (2006) Role of caveolin-1 and cholesterol in transmembrane fatty acid movement. Biochemistry. 45:2882-93.

Simard JR, Meshulam T, Pillai BK, Kirber MT, Brunaldi K, Xu S, Pilch PF, Hamilton JA. (2010) Caveolins sequester fatty acids on the cytoplasmic leaflet of the plasma membrane, augment triglyceride formation and protect cells from lipotoxicity. J Lipid Res. In press

Contact information: j.b.helms@uu.nl

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.

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

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.

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.

Joost Holthuis

Membrane Enzymology, 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

Site visit at Cell Microscopy Center, Cell Biology, UMCU, Fac. of Medicine

March 22

Stefan Rüdiger

Cellular Protein Chemistry, 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

Berend Jan Bosch

Virology Division, Faculty of Veterinary Sciences

Cell Entry of Coronaviruses.

For a successful infection, viruses need to enter the host cell. Enveloped viruses - like coronaviruses - use membrane fusion for cell entry. Specialized viral proteins have evolved that mediate the fusion of the viral and cellular membrane, leading to the delivery of the genetic material of the virus to the cytosol. For coronaviruses, this process is mediated by the spike glycoprotein, a large trimeric type I protein responsible for both receptor binding and membrane fusion. During membrane fusion, the spike protein undergoes dramatic structural reorganization in a coordinated fashion. The refolding pathway and requirements for refolding of the coronavirus spike protein will be discussed.

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.

Toon de Kroon

Membrane enzymology, Chemistry, Faculty of Science

Membrane lipids in yeast

Topics:

- lipid biosynthesis and intracellular transport in yeast
- regulation of membrane lipid composition
- lipid remodeling by acyl chain exchange
- detection of lipid-protein interactions in biomembranes

Techniques:

- stable isotope labeling of lipids and tandem mass spec: "dynamic lipidomics"
- photoactivatable and clickable lipid analogues

IB seminar Ian Brewis & Workshop

Paper workshop

Molecular Human Reproduction, Vol.16, No.2 pp. 68–79, 2010

Sperm surface proteomics: from protein lists to biological function

Ian A. Brewis^{1,4} and Barend M. Gadella^{2,3}

¹Department of Infection, Immunity and Biochemistry, Henry Wellcome Building, School of Medicine, Heath Park, Cardiff University, Cardiff CF14 4XN, UK ²Department of Biochemistry and Cell Biology, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 2, 3584 CM Utrecht, The Netherlands ³Department of Farm Animal Health, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 2, 3584 CM

abstract:

Proteomics technologies have matured significantly in recent years and proteomics driven research articles in reproductive biology and medicine are increasingly common. The key challenge is to move from lists of identified proteins to informed understanding of biological function. This review introduces the range of proteomics workflows most commonly used for protein identification before focusing on the mammalian sperm cell at fertilization as an exemplar for proteomic studies. We review the work of others on entire cells but then argue that proper subcellular fractionation and proper solubilization strategies offers critical advantages to achieving increased biological understanding. In relation to understanding initial gamete recognition events at fertilization (capacitation, zona binding and acrosomal exocytosis) it is imperative to study the sperm surface proteome by using purified plasma membrane fractions. Although this task is challenging there are now strategies at our disposal to achieve comprehensive coverage of the proteins at the sperm surface. Within this context it is also important to understand the milieu of the sperm cell during transit from the testis to the oviduct as proteins (or other entities) from the genital tract epithelia and fluids may also affect the composition and organization of proteins on the sperm surface. Finally the arguments presented for studying the cell plasma membrane proteome to understand the role of the cell surface equally apply to all cell types with important roles in reproductive function.

Suggested reading also:

Molecular & Cellular Proteomics 9.6 p1324

Proteomics Analysis of Bladder Cancer Exosomes

Joanne L. Welton *et al.*

Eefjan Breukink

Biochemistry of Membranes, Chemistry, Fac. of Science

Membrane interactive peptides and how to kill bacteria

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

Site visit at Biochemistry of Membranes, Chemistry, Faculty of Science

March 23

Presentations by PhD students

Each PhD student will present ***shortly*** the PhD research project.

Han Wösten

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.

Mark Roest

Department of Clinical Chemistry and Haematology

Thrombosis and Haemostasis Laboratory

UMC Utrecht, Fac. of Medicine

Translational Research

Platelets play a crucial role in acute coronary heart disease (CHD). Causality is proven in randomized clinical trials, which have shown that the incidence of acute CHD is 25-30% reduced by inhibition of platelets with aspirin or clopidogrel with regard to placebo. Little is understood about the mechanisms of platelet involvement in cardiovascular disease. Therefore a translation of the knowledge, skills and techniques from fundamental research to clinical applications is urgently needed. We have adapted two techniques from the fundamental research field and optimized them for applied clinical studies. (1) A standardized quantification procedure for real time measurements of platelet function under physiological flow conditions has been developed. This test is validated on several in house platelet function assays and will be used to get a study the different steps in platelet function in haemostatic and bleeding disorders and to study the effects of medical interventions on platelet functions. Furthermore, real time platelet function assay will be used to validate plasma markers of platelet function to study the relation of platelet function the risk of Atherothrombotic disease.

(2) A point of care test for high throughput platelet sensitivity measurements has been developed to offers the unique opportunity to study high throughput platelet function in patients.

Both techniques will form a bridge to overcome the gap between fundamental research and clinical practice

Harry Heijnen

Department of Clinical Chemistry and Haematology

Thrombosis and Haemostasis Laboratory

UMC Utrecht, Fac. of Medicine

The dynamic life cycle of a platelet

Blood contains ~ 150,000-350.000 platelets/mm³. 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.

Anna Akhmanova

Cell Biology, Biology, Faculty of Science

Regulation of microtubule dynamics.

Microtubules are polymeric cylinders that are essential for fundamental cellular processes including mitosis, intracellular transport, cell motility and the regulation of cell organization. Microtubules are composed of $\alpha\beta$ -tubulin dimers and are intrinsically polarized. Microtubule plus-ends are the fast-growing ends *in vitro* and the only ends that grow in cells. The opposite (minus) ends can slowly grow *in vitro*, while in cells they are usually stabilized or serve as the sites of disassembly. Microtubules are highly dynamic: they continuously switch between phases of growth and shrinkage. This phenomenon, termed dynamic instability, is essential for formation and remodelling of microtubule networks and is tightly controlled by numerous cellular factors both spatially and temporally. A number of specialised proteins and protein complexes that are conserved in all eukaryotes specifically accumulate at growing microtubule plus ends, and are collectively referred to as plus-end-tracking proteins (+TIP). Recent studies have provided important advances in understanding how +TIPs interact with microtubule ends and with each other, and

how they regulate microtubule dynamics and microtubule interactions with different cellular structures.

Techniques: live cell imaging, in vitro reconstitution of microtubule dynamics, single molecule assays, biochemical analysis of protein-protein interactions, mouse knockout and knock-in technology

Casper Hoogenraad

Cell Biology, Biology, Faculty of Science

Cell Biology of the Neuron

Neuronal cells represent a unique model system for addressing fundamental questions in molecular and cellular biology. Progress in recent years has provided evidence that remodeling of synapses is a fundamental mechanism for information storage and processing in the brain. The primary goal of the lab is to understand how basic cell biological mechanisms underlie synaptic development and function. By studying the basic cellular mechanisms acting during development of single neurons and at individual synapses, we can get insight into how the nervous system develops and functions in an entire animal. At the same time, fundamental understanding of neuronal and synapse plasticity has major implications for the treatment of neurodegenerative diseases. We particularly focus on the areas of microtubule cytoskeleton, synaptic cargo trafficking and synaptic plasticity. Our research over the last years can roughly be divided in three themes

- “The Roadmap” - Cytoskeleton dynamics during neurodevelopment and synaptic plasticity

- “Traffic Rules” - Motor proteins and adaptors as regulators of synaptic transport

- “Accidents on the Highway” – Brain diseases linked to intracellular transport

We particularly focus on the areas of the cytoskeleton, synaptic cargo trafficking and synaptic plasticity. Our research relies on combining different genetics, biochemistry, molecular, and cellular biology methods in in vitro (neuron cultures), ex vivo (brain slices), and in vivo (mice) systems. In addition we employ immunofluorescent confocal microscopy, high-resolution live cell imaging (spinning disc microscopy and total internal reflection fluorescence microscopy, TIRF) and quantitative analysis using advanced high-resolution microscopy (photo-activated localization microscopy, PALM).

Site visit at Site visit at Biochemistry & Cell Biology, Veterinary Sciences

March 24

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 : p.vandersluijs@umcutrecht.nl

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 discuss our approaches and findings on 1) how proximal Wnt signaling events are controlled and 2) how dysregulation of protein function in the Wnt pathway leads to cancer.

Ad 1) We focus on a number of key questions in the transmission of the Wnt signal in receiving cells: How do Wnt-activated transmembrane receptors recruit and talk to their cytoplasmic effectors? What is the role of endocytosis in Wnt pathway activation?

Ad 2) 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.

Techniques:

Cell culture, protein-protein interactions, BN-PAGE, luciferase gene reporter analysis, gene silencing, imaging (confocal, live cell, immuno-EM microscopy), peptide libraries, recombinant protein purification, fluorescence spectroscopy,

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Thomas Schwend

Biomolecular Mass Spectrometry, Chemistry

What is this “proteomics” that everybody is talking about?

Bas Vaandrager

Dept Biochemistry and Cell Biology, Veterinary Medicine, UU

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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Crystal & Structural Chemistry, Chemistry

Crystal

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 over expressing 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.

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Jan Andries Post

Biomolecular Imaging, Biology, Faculty of science

Cell biology at high resolution in 3 dimensions by means of electron microscopy

Electron microscopy (EM) is of crucial importance for our understanding of cell biological processes. Visualisation of these cell biological processes at high resolution is of great importance not only for understanding disease and development of intervention/treatment, but also for validation of biomarkers and of tools to detect these biomarkers in in vivo imaging. This can be achieved by state of the art electron microscopy in combination with the detection of molecules of interest. Of current and future importance are especially: obtaining 3 dimensional information at EM level, correlative microscopy and automated image analysis.

In recent years developments to obtain 3 dimensional information at EM level have and are been further developed and include electron tomography and slice and view using the FIB-SEM [1]. Correlative microscopy allows one to “find the needle in the haystack” or find specific cells in a sea of not affected cells and subsequently zoom in at high resolution at those specific cells. By coupling different imaging modalities this can be achieved. For instance the in our group developed iLEM (an laser microscope integrated in an EM [Biol Cell. 2009:287]) is an excellent example, but also other approaches are available and under development. Next to hardware improvement, improving sample preparation and image analyses are topics of importance.

[1] L.H.P. Hekking, M.N. Lebbink, D.A.M. de Winter, C.T.W.M. Schneijdenberg, C.M. Brand, B.M. Humbel, A.J. Verkleij and J.A. Post. Focused ion beam-scanning electron microscope: exploring large volumes of atherosclerotic tissue *J. Microscopy* 235: 336-347 (2009).

[2] Agronskaia AV, Valentijn JA, van Driel LF, Schneijdenberg CT, Humbel BM, van Bergen en Henegouwen PM, Verkleij AJ, Koster AJ, Gerritsen HC. Integrated fluorescence and transmission electron microscopy. *J Struct Biol.* 164(2):183-189 (2008).

March 25

Morning: presentations by PhD students

Each PhD student will present *shortly* the PhD research project.

IB seminar & Workshop

Mary Munson

Paper Workshop

Cell 128, 183–195, January 12, 2007 (Plus supplemental data)

Selective Activation of Cognate SNAREpins by Sec1/Munc18 Proteins

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SUMMARY

Sec1/Munc18 (SM) proteins are required for every step of intracellular membrane fusion, but their molecular mechanism of action has been unclear. In this work, we demonstrate a fundamental role of the SM protein: to act as a stimulatory subunit of its cognate SNARE fusion machinery. In a reconstituted system, mammalian SNARE pairs assemble between bilayers to drive a basal fusion reaction. Munc18-1/nSec1, a synaptic SM protein required for neurotransmitter release, strongly accelerates this reaction through direct contact with both t- and v-SNAREs. Munc18-1 accelerates fusion only for the cognate SNAREs for exocytosis, therefore enhancing fusion specificity.

Participants IB course 2011

Participants IB course 2011			
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Preparation for the workshops

Group 1: even numbers (2, 4 etc)	Workshop Ian Brewis 22-3-2011
Group 2: odd numbers (1, 3 etc)	Workshop Mary Munson 25-3-2011