Membrane Microdomains and their Role in Human Disease
Technical University of Dresden,
University of Heidelberg
and
University of Regensburg

The concept of membrane microdomains, also referred as lipid rafts has significantly influenced the molecular cell biology of plasma membrane processes.

Rafts are defined as lateral assemblies of certain lipids and integral membrane proteins in the plane of biological membranes.

At the protein level TM-proteins such as influenza virus hemagglutinin and GPI-anchor proteins are found at the exoplasmic leaflet and double acetylated proteins such as Tyr-kinases of the src-family, Gα subunits of heterotrimeric G-proteins and eNOS at the inner leaflet have been identified as raft constituents.

In addition, scaffold proteins such as caveolins are involved that lead to the morphologically visible formation of caveolae.

A number of technologies for in situ study of lipid rafts, became available in recent years that promote the field of microdomain research.
- Rafts are small platforms of sphingolipids and cholesterol in the exoplasmic leaflet of the PM connected to phospholipids and cholesterol in the inner cytoplasmic leaflet of the lipid bilayer
- These assemblies are fluid but more ordered and more tightly packed than the surrounding bilayer
- The difference in packing is due to the saturation of the hydrocarbon chains in raft sphingolipids and phospholipids as compared with the unsaturated state of fatty acids of phospholipids in the liquid disordered state

**Physiological Function of Membrane Microdomains:**

Clustering of Rafts Triggers Signalling


Signal transduction processes involving rafts:
- Fc receptor
- T-cell & B-cell receptor
- EGF receptor
- Insulin receptor
- Ephrin B1 receptor
- Neurotrophin

Clustering of lipid microdomains is the physiological basis of raft microdomain function
- PM-rafts at basal conditions are principally small and contain only a few proteins
- Clustering induces raft fusion and leads to reassociation of molecules
- Clustering agents include ligands, antibodies and lectins
- Raft clustering induces at the interaction of src-kinases inner leaflet of the PM and subsequently phosphorylation and downstream signaling
- There is strong evidence, that numerous receptor cluster that are important for physiological all functions involve raft microdomains.
Transregio 6031 - Membrane Microdomains

Membrane Microdomains and Disease

- Atherosclerosis, diabetes
- Hypertension, hemodynamic regulation
- Alzheimer disease, Parkinson disease
- Muscular dystrophy, polyneuropathies, demyelinating diseases
- Autoimmune diseases, chronic inflammation, vaccine response
- B cell response, T cell response
- Asthma and allergic response
- Neoplasia
- Hyperparathyroidism
- Osteoarthritis
- Gastrointestinal ulceration
- Paroxysmal nocturnal hemoglobinuria
- Lysosomal storage disease, Niemann-Pick disease
- Tay-Sachs disease, Morbus Fabry, metachromatic leukodystrophy
- Pelizaeus-Merzbacher disease
- Postsqualene cholesterol biosynthesis disorders
- Pore-forming toxins (gas gangrene)
- Sepsis, septic shock, Bacterial infections
- Viral infections and other pathogens

Simons K & Ehehalt R, J Clin Invest 2002

- The complex regulation of clustering and dissociation and
- The sensitivity for cholesterol, glycosphingolipids and saturated FA renders Raft microdomains susceptible to diseases
- The list of disorders indicates that genetic disorders as well as environmental factors and inflammation interfere with the physiology of raft membrane microdomains
- The upcoming problems of Aging disorders are of great importance for our health care system and the major disorders in the elderly are related to atherosclerosis, diabetes and degenerative disorders of the brain which all involve membrane microdomains.

Long Term Research Goals (1)

Section A

- Molecular cell biology of membrane microdomains
  - What are the mechanisms of lipid raft formation?
    - Membrane compartments involved
    - Interaction of proteins and lipids
    - Clustering of small microdomains into large functional units
    - Role of cholesterol and glycolipids in the process
  (A1: Simons, Dresden)

  - What is the lipid and protein composition of different types of rafts?
    - Properties of rafts from different tissues
    - Role of (lipid-binding) proteins
    - Effect of lipid manipulation on raft stability
  (A2: Steinhem, Regensburg)

(A4: Hoflack, Dresden)
(A3: Drobnik & Schmitz, Regensburg)
Long Term Research Goals (2)

Section B

- Physiological functions of membrane microdomains
  - What are the major physiological functions of lipid rafts?
    - Mechanisms of raft activation
    - Role of cholesterol content on signal transduction
    - Regulation of signal processes within rafts
    - Correlation of the expression level of raft-associated proteins with raft function

(B1: Corbeil & Huttner, Dresden)
(B2: Kurzchalia, Dresden)
(B3: Kasper, Dresden)

Long Term Research Goals (3)

Section C

- Role of membrane microdomains in disease
  - How do alterations in raft structure and function cause disease?
    - Correlation of changes in raft composition (or properties) with pathological processes
    - Effect of mutations in raft-associated proteins
    - Raft lipid composition and raft function in disorders in
      - the processing and secretion of proteins
      - cholesterol and fatty acid metabolism
      - signal transduction and proliferation
      - cell adhesion and motility

(C1: Schwencke & Strasser, Dresden)
(C2: Hartmann & Beyreuther, Heidelberg)
(C3: Wieland & Brügger, Heidelberg)
(C4: Fackler, Heidelberg)

SFB/TR: Locations and Institutions

Dresden
- University Hospital Carl Gustav Carus
  - Institute of Anatomy
  - Department of Cardiology
- Technical University
  - Biotec
  - Max-Planck-Institute of Molecular Cell Biology and Genetics

Heidelberg
- University Hospital
  - Department of Gastroenterology
  - Department of Virology
- University
  - Joint Center for Molecular Biology
    - Center of Biochemistry

Regensburg
- University Hospital
  - Department of Dermatology
    - Institute for Clinical Chemistry and Laboratory Medicine
- University
  - Institute for Biophysics and Physical Biochemistry
  - Institute of Analytical Chemistry
Many questions of the 3 addressed research topics are unresolved and open a wide field for long term research.

In the still young field of membrane microdomain research Germany plays a leading role especially due to the fundamental work performed in the research group of Kai Simons.

The planned SFB-TR represents the first integrated research initiative in Germany in the field of membrane microdomain research and their clinical importance.

Also at the international level there exist no competitive activity.

The time frame is optimal and the three selected locations have the expertise to generate the necessary added value for the project.

The selection of the locations and the projects guarantee that the clinical projects will profit from the basic research of more than one location.

At the individual locations basic research and disease oriented research together with advanced technology are synergistic and foreseeably will generate important progress in this field of research.

Any other construction such as “DFG-Schwerpunkt" would neither lead to a local critical mass nor to the formation of Structures in this complicated field of research, that is full of controversy and contradictions.

In conclusion, the 3 locations represent a highly complementary scientific network with a short distance from bench to bedside and perfectly match the criteria for an SFB-TR project.
Section D: Technology

- Novel analytical technologies: (i) quantitative lipid analysis, (ii) fluorescent lipid probes, (iii) raft protein conformation
  - Multiple precursor ion-scanning on a hybrid quadrupole time-of-flight mass spectrometer: Quantitative profiling of complex mixtures of phospho- and glycosphingolipids (D1: Shevchenko)
  - Fluorescent fatty acid and sphingosine derivatives: subcellular distribution and movement of metabolically generated sphingo- and phospholipids (D2: Thiele)
  - Conformational structure of raft-associated proteins in presence of lipids: β-amyloid as a model (D3: Kalbitzer)

- Due to the high importance of new enabling technologies for the progress in membrane microdomain research we included a technology section as a separate structure for the Transregio-SFB
- The collaborative center for lipid microanalysis by mass-spectometry will bundle the expertise of the outstanding group of Felix Wieland and Britta Brügger with the expertise of the centers in Dresden and Regensburg. This will generate considerable added value in the field of lipidomics of raft microdomains.
The table shows the structure of the SFB/TR with its three thematic sections A-C as well as the technology section D. In addition to the primary contribution that each project makes towards the common research goals in its respective section, the major additional contribution of each project in one of the two other sections is indicated. The complementarity of the research at the three locations demonstrates their synergy within the SFB/TR.
### Support of Young Researchers

<table>
<thead>
<tr>
<th>General activities</th>
<th>Dresden</th>
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<tr>
<td>- Regular local seminars and workshops, a biannual symposium for the integration of the three locations</td>
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<td>- Support for recently established young investigator groups (e.g. B1, C4, D1, D2)</td>
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<td>- International Max-Plank-Research School for Molecular Cell Biology and Bioengineering</td>
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<td>- Graduate College „Molecular Cell Biology and Bioengineering“</td>
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<td>Heidelberg</td>
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<td>- International Masters Programme in Molecular and Cellular Biology (MCoB) of the ZMBH and several campus wide graduate-programs</td>
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<td>- Intramural funding of young investigators (Junior-Forschungsprogramm der Med. Fakultät)</td>
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<td>- MD/PhD initiative of the EMBL and the Faculty of Medicine</td>
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<td>Regensburg</td>
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<td>- Two DFG-funded graduate colleges with participants from the SFB/TR</td>
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<tr>
<td>- Joint MD/PhD programme of the Medical Faculty and the Faculty for Biology and Preclinical Medicine</td>
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<td>- ReForM programme for the intramural funding of young scientists</td>
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### Integration of the Three Locations

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<th>Exchange and Mobility</th>
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<tr>
<td>- Institutionalized exchange of scientists (e.g. providing guest apartments)</td>
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<td>- Funding for the exchange of scientists between locations</td>
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<td>- Joint PhD supervision bridging the locations</td>
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<td>- Specific funding for the access of central collaborative technical structures</td>
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<td>- Summer/winter courses for relevant technologies, molecular cell biology and microdomain related diseases</td>
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<tr>
<td>Telecommunication</td>
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<tr>
<td>- Intranet communication for the SFB/TR and a joint internet-platform</td>
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<td>- Teleconferencing to communicate between the three locations</td>
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### Summary of the projects, structured by project sectors:

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<th>Section</th>
<th>Project Title</th>
<th>Project Details</th>
<th>Project Leads</th>
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<td><strong>Section A: Molecular Cell Biology of Membrane Microdomains</strong>&lt;br&gt;A1</td>
<td>Lipid raft clustering in membrane trafficking</td>
<td>molecular cell biology</td>
<td>K. Simons (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden)</td>
</tr>
<tr>
<td><strong>Section B: Physiological Function of Membrane Microdomains</strong>&lt;br&gt;B1</td>
<td>Physiological function of the cholesterol-interacting, lipid raft-associated plasma membrane protein prominin: from cell biology to human disease</td>
<td>molecular cell biology, membrane microdomains</td>
<td>D. Corbeil/W. Huttner (Medical Faculty, Medical Clinic and Polyclinic I, Technical University Dresden and Max-Planck-Institute of Molecular Cell Biology and Genetics, Dresden)</td>
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<tr>
<td><strong>Section C: Role of Membrane Microdomains in Disease</strong>&lt;br&gt;C1</td>
<td>Pathophysiological role of Caveolae and caveolin in vascular proliferative disease</td>
<td>cardiovascular medicine</td>
<td>C. Schwenke/R. Strasser (Department of Cardiology, Medical Faculty, Technical University Dresden)</td>
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<tr>
<td><strong>Section D: Technology</strong>&lt;br&gt;D1</td>
<td>Quantitative profiling of phospholipids and glycolipids by quadrupole time-of-flight mass spectrometry</td>
<td>analytical biochemistry, mass spectrometry</td>
<td>A. Shevchenko (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden)</td>
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<tr>
<td><strong>Section C: Role of Membrane Microdomains in Disease</strong>&lt;br&gt;C2</td>
<td>Intracellular cholesterol-trafficking as a potential crossroad in A-beta-generation</td>
<td>cell and molecular biology, neurodegeneration, Alzheimer’s disease</td>
<td>T. Hartmann (ZMBH - Centre for Molecular Biology, University of Heidelberg)</td>
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<tr>
<td><strong>Section C: Role of Membrane Microdomains in Disease</strong>&lt;br&gt;C3</td>
<td>Specific lipid interactions of transmembrane segments of membrane proteins</td>
<td>biochemistry, molecular cell biology</td>
<td>T. Wieland/B. Brügger (Centre of Biochemistry, University of Heidelberg)</td>
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<tr>
<td><strong>Section D: Technology</strong>&lt;br&gt;D2</td>
<td>Lipid fluorescence microscopy</td>
<td>cell biology of lipids and lipid-protein interactions</td>
<td>C. Thiele (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden)</td>
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<td><strong>Section D: Technology</strong>&lt;br&gt;D3</td>
<td>Characterization of lipid-dependent conformational intermediates of raft-associated proteins using NMR spectroscopy</td>
<td>biochemistry, biophysics</td>
<td>H. Kalbitzer (Institute for Biophysics and Physical Biochemistry, University of Regensburg)</td>
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<td><strong>Section A: Molecular Cell Biology of Membrane Microdomains</strong>&lt;br&gt;A2</td>
<td>Raft formation in artificial membrane systems</td>
<td>biochemistry, membrane biophysics</td>
<td>C. Steinem (Institute for Analytical Chemistry, Chemo- and Biosensors, University of Regensburg)</td>
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<td>A3</td>
<td>Analysis of ABCA1 interactive proteins and raft domain association depending on genetic factors and pre-beta-HDL composition</td>
<td>cell and molecular biology, cellular lipid metabolism</td>
<td>W. Drobnik /G. Schmitz (Institute for Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg)</td>
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<td>A4</td>
<td>Lipid rafts and obesity: function of OBR-GRP and endospanin, two lipid raft-associated tetraspanins, in the leptin receptor trafficking.</td>
<td>Molecular cell biology, membrane microdomains</td>
<td>G. Hoflack (Biotec, Technical University Dresden)</td>
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<tr>
<td><strong>Section B: Physiological Function of Membrane Microdomains</strong>&lt;br&gt;B2</td>
<td>Investigation of molecular mechanisms responsible for the phenotype of caveolin-1 KO-mice</td>
<td>cell biology</td>
<td>T. Kurzchalia (Max Planck Institute of Molecular Cell Biology and Genetics, Dresden)</td>
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<td>B3</td>
<td>Caveolae as trafficking compartments to manage transcytosis within the alveolar epithelium</td>
<td>anatomy, cell biology</td>
<td>M. Kasper (Institute for Anatomy, Medical Faculty, Technical University Dresden)</td>
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Project A1

Lipid raft clustering in membrane trafficking

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Summary

Lipid rafts are assemblies of exoplasmic cholesterol most probably linked to inner leaflet lipids. They function as platforms for membrane sorting in intracellular transport in signal transduction as well as in generating cell surface polarity. We and others estimate the size of rafts in fibroblasts to be small, around 40-70 nm in diameter. Each individual raft of this size would not contain more than 10-20 proteins. This implies that out of tens of millions of proteins on the cell surface each raft would carry so few proteins that the raft-associated proteins would be segregated away from most other raft and non-raft proteins i.e. rafts are mobile storage platforms. For raft processes to be activated, individual rafts have to be specifically clustered to form larger platforms where the reactants meet each other. It is the specific aim of this project to analyze how raft clustering occurs. We will choose two experimental models: The first is the formation of the influenza virus envelope. Influenza virus hemagglutinins and neuraminidase (both raft-associated) become clustered by the viral M-protein during virus budding from the apical plasma membrane of epithelial cells. The second raft-clustering process is a complex one: the formation of apical transport contained in the trans-Golgi network of epithelial MDCK cells. The apical sorting machinery is key to understanding raft exocytosis, a central process of plasma membrane biogenesis and epithelial cell polarity.
**Project A2**

Membrane microdomain formation in artificial membrane systems

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**Summary**

Our objective is the *in situ* visualization of glycolipid-enriched membrane microdomains in artificial membranes. First, lipid membranes containing defined but varying concentrations of raft associated lipids such as glycosphingolipids, sphingomyelin and cholesterol will be investigated by means of fluorescence and scanning force microscopy with respect to their capability of forming raft domains and their dynamic behaviour. Special emphasis will be put on the influence of $\alpha$-hydroxylation and chain length of the glycosphingolipids on raft formation. Second, the cross talk between microdomains of the outer and inner leaflet will be studied. By Langmuir-Blodgett technique asymmetric lipid bilayers will be produced with one leaflet composed of a lipid mixture resembling that of the outer face, while the other one contains a lipid mixture representing the inner leaflet. Fluorescence microscopy on glass supports will allow us to visualize lipid domains in both, the outer and inner leaflet and its influence of each other. Thirdly, the directed interaction of proteins with and the formation of microdomains in fluid lipid bilayers will be visualized by scanning force microscopy. The focus will be on annexin A2, a protein that is found associated with cholesterol-rich domains at the cytoplasmic side of the plasma membrane and amyloid $\beta$-peptide that has been identified in cholesterol-enriched membrane domains.
Project A3

Crosstalk between the apoA-I/ABCA1 pathway and lipid microdomains

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Summary

ABCA1 is the major regulator of apoA-I mediated cholesterol and phospholipid efflux, and functional defects in ABCA1 result in complete HDL deficiency. Recent studies indicate that ABCA1 is rather a transport facilitator than a bona fide transporter, which affects and associates with cholesterol based membrane microdomains. Most interestingly, ABCA1 was partially localized in Lubrol detergent resistant membranes (DRM) in cholesterol loaded primary human monocyte derived macrophages but not in primary human fibroblasts indicating cell-type specific ABCA1 pathways. Moreover, depending on the cell type, Lubrol and/or Triton X-100 detergent resistant membranes (DRM) are depleted of cholesterol and choline-phospholipids upon overnight treatment with apoA-I. The objective of the proposed study is to provide a more detailed understanding of the relationship of the ABCA1 pathway with the lipid and protein composition of membrane microdomains. In the first part of the project we aim to further characterize membrane microdomains associated with the ABCA1 pathway. This will include the analysis of membrane microdomains isolated by different detergents, comparison of various cell types and characterization of the role of caveolin-1 as well as the identification of the plasma membrane domains that serve as initial donors for apoAI mediated lipid efflux. Further parts of the project are directed towards the relationship of ABCA1 function to vesicular transport pathways and the identification of factors regulating the cross talk between ABCA1 and membrane microdomains. These work packages will be complemented by the identification of novel proteins involved in the ABCA1 pathway, using the yeast two hybrid system and high density microarrays. The results of this project should provide a better understanding of how the ABCA1 pathway, which is of critical importance in the pathogenesis of atherosclerosis, is related to membrane microdomain homeostasis integrating cell signalling and cholesterol/phospholipid transport and the factors that regulate the proportion of ABCA1 inside and outside of these membrane domains.
Project A4

Lipid rafts and obesity

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Summary

This project focuses on two membrane proteins with four transmembrane domains (OBR-GRP and endospanin) mostly localized in the trans-Golgi network and present in lipid rafts. Complementation experiments in yeast indicate that they most likely function in lysosomal targeting. We want to analyze the protein and lipid composition of the microdomains containing these two tetraspanins and determine how the different components affect the stability of these membrane microdomains. We want to investigate further their function of OBR-GRP and endospanin in lysosomal targeting in mammalian cells, in particular their influence on the trafficking of the leptin receptor since OBR-GRP arises from alternative splicing of the leptin receptor gene. Such studies could unravel a key mechanism regulating the cell surface expression of the leptin receptor and the biological importance of lipid rafts in the trafficking of molecules controlling obesity.
Physiological function of the cholesterol-interacting, lipid raft-associated plasma membrane protein prominin: from cell biology to human disease

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Summary

The present research proposal concerns a novel family of pentaspan membrane glycoproteins called prominins. The prominins (i) occur throughout the animal kingdom, (ii) are expressed by a variety of cells including hematopoietic and neural stem cells, (iii) are specifically localized to plasma membrane protrusions, (iv) are associated with a novel membrane microdomain, and (v) specifically interact with membrane cholesterol. The physiological function of the prominins is not known. Mutations in prominin-1, however, which in the retina is concentrated in the plasma membrane evaginations of photoreceptor cells, cause retinal degeneration.

Here, we propose to determine the physiological function of the prominins by studying various prominin-expressing and prominin-deficient cell lines and animal models. Focussing on a possible role of the prominins in the formation and/or stabilization of plasma membrane protrusions and the membrane microdomains that are the building blocks of these protrusions, we will (i) characterize the prominin-containing membrane microdomains and the prominin–cholesterol interaction, (ii) investigate the consequences of prominin deficiency on membrane microdomains and plasma membrane protrusions, and (iii) study the cell biological basis of the retinal degeneration caused by mutations in the prominin-1 gene. These studies will not only contribute the characterization of a novel type of membrane microdomain but also provide insight into the pathogenesis of, as yet unexplained, human disease caused by impairment of plasma membrane protrusions.
**Summary**

Caveolae are plasma membrane invaginations that play an important role in numerous cellular processes including transport, signaling, and tumor suppression. According to a current view, caveolae are specific forms of rafts. By targeted disruption of caveolin-1, the main protein component of caveolae, we generated mice that lacked caveolae and displayed several physiological aberrations. In particular, the absence of this organelle impaired nitric oxide and calcium signaling in the cardiovascular system causing disregulation of endothelium-dependent relaxation, contractility, and maintenance of myogenic tone. In addition, the lungs of mutant animals displayed dramatic thickening of alveolar septa caused by uncontrolled endothelial cell proliferation and fibrosis. According to our working hypothesis, the absence of caveolin-1 leads to changes in lipid raft composition and to elevated production of NO. Our three major goals are as follows:

1. Investigation of biochemical differences between rafts derived from wild type and cav-1 deficient mice using methods for lipid and protein analysis;
2. Establishment of cellular model of caveolar deficiency;
3. Elucidation of the influence of elevated NO in cav-1 deficient mice on signalling processes. Caveolae are specific forms of rafts and hence our data will provide information of how rafts are involved in crucial signaling pathways. S-nitrosylation and nitration of proteins in wild type and mutant mice organs will be compared and major targets of S-nitrosylation will be identified. We will study if the same aberrant processes take place in KO-derived cell lines. High S-nitrosylation and nitration of proteins could be reason of fibrotic phenotype or deregulation of smooth muscle relaxation described in our publication. One of the possibilities to prove the role of eNOS hyperactivity is to rescue the phenotype by application of eNOS inhibitors.
Project B3

Caveolae as trafficking compartments to manage transcytosis within the alveolar epithelium

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Summary

Caveolae are omega-shaped invaginations of the plasmamembrane possessing a cytoplasmic membrane protein coat of caveolin. Although caveolae are present in most tissues, caveolae are most abundant in lung, particularly in microvascular endothelial cells, type I pneumocytes, fibroblasts and smooth muscle cells. From a pharmaceutical point of view the caveolae may represent important carriers of therapeutic macromolecules across the alveolar-capillary barrier. It would be important to realize a site-directed treatment of pulmonary and other diseases. For this we need the understanding of the role of caveolae in alveolar transport processes. By using slice cultures of lung tissues of rat lung and caveolin-1 knockout mice combined with in situ-tracer studies, vital microscopy, immunoelectron microscopy, paraffin embedding of slices and immunohistochemistry, our research will focus on the dynamics of caveolae in alveolar epithelium as well as in capillary endothelium. Two questions represent the main focus of our work: What is the function of caveolae in pulmonary epithelial cells? Is there a functional difference between caveolae from type I pneumocytes and capillary endothelial cells? In parallel we will adapt models of lung injury (bleomycin, CdCl₂) to the lung slice culture for the study of caveolae and caveolin protein under conditions of disease.
Project C1

Pathophysiologic role of caveolae and caveolin in vascular proliferative disease

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Summary

The recent identification of various signaling molecules in caveolae and their functional interaction with the integral membrane protein caveolin, a major structural component of caveolae, suggests that these membrane microdomains may participate in transmembrane signaling. So far, three members of the caveolin gene family (caveolin-1-α and -β, caveolin-2, and -3) have been identified which are present at their highest levels in terminally differentiated cells. Several lines of evidence suggest that caveolins might act as scaffolding proteins by directly interacting with and modulating the activity of caveolae-localized signaling molecules. Indeed, an inhibition of their enzymatic activity by a short cytosolic domain derived from the N-terminal region of caveolin, called the caveolin scaffolding domain, could be demonstrated. In effect, caveolin seems to act as a scaffolding protein, able to negatively regulate the activity of signaling molecules.

Interestingly, many of the signaling molecules that either interact with or are inhibited by caveolin-1 mediate mitogenic signals to the nucleus. Taken together with the fact that many tumor cells show down-regulation of caveolin-1, these observation lead to the hypothesis that caveolin-1 tonically inhibits cellular proliferation and hence that the loss of caveolin-1 is a hallmark of dedifferentiation and proliferation.

Cellular proliferation is involved in the pathogenesis of vascular proliferative diseases such as primary atherosclerosis and restenosis after angioplasty. Whereas early events in atherogenesis are characterized by an altered endothelial function and by the recruitment of mononuclear leucocytes to the intima, the progression of atheroma and restenosis after angioplasty involves the proliferation of VSMC, their migration from the underlying media to the intima and their production of extracellular matrix macromolecules.

On the background of the recently postulated role of caveolin-1 in the modulation of cell cycle progression the proposed project will focus on the investigation of the possible pathophysiologic significance of caveolin-1 for the proliferation of VSMC in vascular-proliferative diseases like primary atherosclerosis and restenosis after angioplasty.
**Project C2**

Intracellular lipid-trafficking as a potential crossroad in Abeta-generation

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**Summary**

Several lines of evidence link Alzheimer’s disease (AD) and AD proteins with lipids, lipid trafficking and especially raft biology. We aim to determine the cross talk between lipid metabolism and lipid trafficking with AD-proteins and AD-protein processing as a etiological factor in AD pathogenesis.

The extensive analogy between the proteolytic regulation of the cholesterol modulated SREBP/SCAP system and the proteolytic cleavage of APP by presenilines and b-secretase indicates the plausible assumption that a similar regulation cascade may exist for AD-proteins.

Our working hypothesis therefore is, that not only Aβ generation is cholesterol sensitive, but also that presenilin and other AD proteins have an active role in lipid metabolism and based on our experiments may be an important factor in cholesterol trafficking and lipid homeostasis.

Therefore, lipids and AD proteins would influence each other in both directions creating a crossroad of effects including Aβ production, raft function and lipid trafficking.

To facilitate this research a large array of well-established single and multiple AD-protein knock-out cell culture lines and transgenic or knock-out mice will be used. This will be further supplemented by available disease linked and functional mutated AD proteins which will be used to retransfect KO-cells in absence of endogenous background. The specific work tasks include:

1- Determination of cholesterol dependent presenilin function.
2- Is presenilin activity sensitive to cholesterol/lipid trafficking?
3- The influence of AD proteins on raft biology and further aspects of cellular lipid biology.
4- Verification of the observed effects in the in vivo model.
**Project C3**

**Role of Protein-Lipid-Interactions in APP Processing and Transport**

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**Summary**

The conformation and specific lipid environment of transmembrane segments (TMS) in membrane proteins is widely unknown. Knowledge of such interactions will be pivotal for the understanding of the specific partitioning of a membrane protein into specialized bilayer microdomains. In our project we plan to investigate a role of specific lipid-TMS interactions in the proper localization of membrane proteins within the bilayer. To this end, we chose APP and its proteolytic products as a model protein for protein-lipid photoaffinity labeling studies. Because of its relation to Alzheimer disease APP is a well studied protein with defined dynamics during its transport from the ER to the plasma membrane. Therefore, it offers a possibility to analyze its lipid interactions at the various stations of the secretory and endocytic pathway in a defined manner. Exploiting its processing combined with cell biological methods to trap it in various membranes along these pathways should allow to compare lipid-TMS interactions at various stages. Furthermore, APP is dynamically associated with lipid rafts, i.e. in an equilibrium between specialized plasma membrane microdomains. Photolabeling and subsequent analysis of these forms (APP_ran and APP_non-ran) will allow to study if this equilibrium depends on specific lipid-TMS interactions. In order to judge whether our results are of general character, we plan to extend this investigation to a type I transmembrane protein of similar behaviour, CD91.
**Project C4**

**Nef and Lipid Rafts: Functional Coordination of the HIV Pathogenicity factor during particle release and TCR Signaling**

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**Summary**

The Nef protein of the primate lentiviruses HIV and SIV is a key determinant for the pathogenicity of these viruses. Understanding the molecular mechanisms of action of Nef therefore is a primary goal towards the development of more efficient strategies to combat and prevent HIV infection. Nef expression alters intracellular sorting as well as signaling events in HIV target cells. Downregulation of the viral receptor CD4 and MHC class I molecules, activation of the T cell receptor signaling cascade as well as enhancement of virion infectivity have been recognized as primary effects of Nef. Partial T cell activation by Nef was shown to directly correlate with elevated levels of HIV replication in primary cells. All functions of Nef require its association with cellular interaction partners as well as cellular membranes, that is mediated by the N-terminal myristoylation of the protein. Our recent findings suggest that the incorporation of a subpopulation of Nef into lipid rafts is essential for its activity in signal transduction and infectivity enhancement. The overall goal of this proposal is therefore to unravel how Nef uses lipid rafts to exert and/or optimize its activities.

The first specific aim seeks to identify the mechanism of virion infectivity enhancement by Nef. We have shown previously that Nef recruits HIV budding structures into lipid rafts resulting in elevated infectivity of these particles. We postulate that this increase in infectivity is the consequence of lipid raft specific modifications of lipid and/or protein composition of the viral envelope. Thus, the aim will be to identify and subsequently characterize lipid and protein components that are enriched in highly purified HIV particles in the presence of Nef. In a last step, the functional consequence of the preferential incorporation of these components will be addressed.

The second specific aim of the proposal is targeted at the role of lipid rafts in the activation of the TCR cascade by Nef via the NAKC multiprotein complex. Via the recruitment of NAKC, Nef is phosphorylated and activates the TCR proximal tyrosine kinase Lck, which mediates further activation steps downstream. In preliminary studies we found that this activation is achieved via the recruitment of NAKC into lipid rafts. Furthermore, we have identified two novel components of NAKC, PKC theta and the diaphanous related formin FHOD1, that likely affect the polymerization state of actin. We therefore aim at the characterization of the role of lipid rafts in the function of NAKC and the functional consequences of NAKC recruitment. Given the importance of actin-mediated clustering of lipid rafts for the initiation of TCR signaling, effects of Nef on raft clustering and the formation of contacts with antigen presenting cells (immunological synapse, IS) will be a special emphasis of these studies.
Project D1

Quantitative Profiling of Phospholipids and Glycolipids by Quadrupole Time-of-Flight Mass Spectrometry

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Summary

We propose to develop a technology of multiple precursor ion scanning on a hybrid quadrupole time-of-flight mass spectrometer for quantitative profiling of complex unseparated mixtures of endogenous phospho- and glyco-lipids. By quantitative characterization of lipids as chemically individual species the technology will further our understanding of molecular structure and dynamics of cellular membrane microdomains. As the method allows us to detect multiple classes of lipids in parallel in a single mass spectrometric experiment, it is also expected to be more comprehensive, accurate and robust compared to conventional methods.
Project D2

Lipid fluorescence microscopy

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Summary

Although lipids are the major component of all biological membranes, their role in membrane function is largely unexplored. This missing knowledge is a direct consequence of a lack of adequate technologies to study lipid localization, lipid dynamics and lipid interactions. Fluorescence microscopy has become a central technology to study the cell biology of proteins but still fails to provide information on lipid distribution and its dynamics. This is due to a lack of hydrophobic fluorescent groups that can be integrated into natural lipids without disturbing their physico-chemical characteristics. Therefore we search for hydrophobic fluorescent groups that mimic natural fatty acids and can be observed in a fluorescence microscope. The most promising class of substances are linear hydrocarbon fatty acids containing a conjugated polyene as a fluorescent group. We synthesized both fatty acids and sphingosine derivatives containing fluorescent conjugated polyene moieties. Both are readily metabolically incorporated into sphingo- and glycerophospholipids. Metabolic pattern vary with the length of the fluorescent fatty acids and correspond to those of natural fatty acids of comparable length. Despite of the sensitivity of these fluorophores towards photobleaching, we demonstrated their usability in fluorescence microscopy, particularly using two-photon excitation. After optimizing both the specificity of incorporation into cellular lipids and the imaging procedures, we will use them to study the subcellular distribution, movements of membrane lipids and interactions of lipids with membrane proteins.
**Project D3**

**Characterization of lipid-dependent conformational intermediates of raft-associated proteins using NMR spectroscopy.**

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**Summary**

High-field high-pressure NMR spectroscopy is a novel method to observe conformational states occurring in low population at ambient pressures in atomic detail. High pressure in the range up to 200 MPa will be used to create and stabilize folding intermediates of \( \alpha \)-amyloid as a paradigmatic raft-associated protein, which do only exist in very low populations at normal conditions. The structure of these intermediates will be studied directly in the high pressure cell developed in our laboratory by multidimensional NMR-spectroscopy. Since the interaction between A\( \beta \) and lipid rafts affects Alzheimer pathogenesis, lipids such as cholesterol and small rafts < 10 nm in diameter will be added to the solution. Thus we will be able to investigate if raft binding causes a conformational change that promotes amyloid plaque formation. The folding intermediates of A\( \beta \) and structurally interesting mutants in the presence and absence of rafts can then be stabilized by tuning pressure and temperature. A detailed structural characterization of these intermediates will improve our understanding of the plaque formation process.