

Doctoral School - Letter of Intend

1. Name of proposed Doctoral School:

Doctoral School "Translational Molecular and Cellular Biosciences".

Doktoratsschule "Medizinisch Angewandte (Translationale) Molekulare und Zelluläre Biowissenschaften"

2. Speaker:

Speaker: W. Schreibmayer (Institute for Biophysics)

Deputy Speakers: C. Windpassinger (Institute for Human Genetics); T. Bauernhofer (Oncology Department).

3. Description and rationale of the programme:

Modern Medicine and the understanding of physiological and pathophysiological phenomena in terms of molecular and cellular bioscience (as e.g.: medical biophysics & biochemistry, cell biology, molecular and cellular physiology, medical genetics) are intimately related. In order to effectively improve human health, scientific discoveries at the molecular and cellular level must be translated into practical application. Typically, such discoveries begin at "the bench" with basic research – then progress to the clinical level, or the patient's "bedside". Scientists are increasingly aware that this bench-to-bedside approach of translational molecular and cellular bioscience research is indeed a bidirectional process: basics scientists provide clinicians with new tools for use in patients and for assessment of their impact and on the other side clinical researchers make novel observations about the nature and progression of disease – that often stimulates basic investigations. Translational research, especially at the molecular and cellular level, has proven to be a powerful process that drives the clinical, as well as the preclinical research engine. Therefore, the establishment of a corresponding doctoral school at the Medical University in Graz is timely and required.

The Curriculum is intended to forge a uniquely transformative, novel and integrative academic home for clinical and translational bioscience at the molecular and cellular level that has the consolidated resources to: (i) captivate, advance and nurture a cadre of well-trained multi- and interdisciplinary investigators and research teams; (ii) create an incubator for innovative research tools and technologies; (iii) synergize multi-disciplinary and inter-disciplinary clinical and translational bioscience. This curriculum will provide a platform to catalyze this process in order to create this added value. The students will be enrolled in demanding and novel scientific research projects within the Medical University, including faculty of relevant clinical institutions. Using the existing expertise and infrastructure, the programme will be focused on basic research but also on possible practical applications in the clinical situation (translational component).

4. Names of proposed faculty¹:

Thomas Bauernhofer (Department of Oncology)
Gottfried Dohr (Institute of Cell Biology, Histology and Embryology)
Günther Fauler (Clinical Institute for Medical and Chemical Laboratory
Diagnostics (KIMCL))
Klaus Groschner (Institute of Pharmacology; Graz University)
Berthold Huppertz (Institute of Cell Biology, Histology and Embryology)
Manfred Kollroser (Institute for Forensic Medicine)
Hans Jörg Leis (Department of Pediatrics)
Ernst Malle (Institute for Medical Biochemistry and Molecular Biology)
Eduard Paschke (Department of Pediatrics)
Erwin Petek (Institute for Human Genetics)
Stefan Quasthoff (University Clinics of Neurology)
Andreas Sandner-Kiesling (Department of Anesthesiology)
Wolfgang Schreiber (Institute for Biophysics)
Thomas Schwarzbraun (Institute for Human Genetics)
Peter Sedlmayr (Institute of Cell Biology, Histology and Embryology)
Manfred Windischhofer (Department of Pediatrics)
Christian Windpassinger (Institute for Human Genetics)

5. Resources:

Resources as laboratory space and equipment, and the financial basis of the Doctoral School are described in **Appendix II**.

The costs for the conduction of a *single* Thesis project (only salary and consumables), as outlined under 6., are estimated to range between € 104. 010.- to € 158. 680.- (FWF salary for a doctoral student plus € 3.000.- to € 8.000.- for consumables for the projected duration of 3 – 4 years). As further outlined in Appendix II, the number of individual funded research projects, were faculty members of the projected Doctoral School were able to attract funding as principal investigators (within the last 5 years) is as follows: 2 EC projects, 3 SFB sub-projects (within 2 different SFB's), 6 FWF projects, 1 FFG project, 3 OENB projects and 1 direct grant from the Austrian Ministry of Science. Hence the projected Doctoral School is standing on a solid financial basis and seems realistic.

6.: Individual Thesis proposals projected:

6.1.: Thesis projects that are already performed (Dr. sci. med., old version), but will be enrolled in the new Doctoral School:

#01.: Abundance and function of GIRK proteins in breast cancer cell lines. The role of G-Protein activated receptors (e.g.: cytokine receptors, Ca²⁺ sensing receptors) in several signalling pathways, involved in breast cancer became evident during the last decade. Much less is known about the molecular targets of these G-protein mediated actions. The proposal aims to elucidate the role of a family of direct G-Protein effectors, G-Protein activated potassium channels in pathophysiological signalling, related to invasion, homing, dormancy

¹ : This list comprises the *present* faculty members that are committed to the Doctoral School on “Translational Molecular and Cellular Bioscience”. This list is open and will be extended during the course of the Doctoral School as other researchers of MUG express their interest and join.

and metastatic colonization of breast tumour metastasis. Our findings might help in establishing GIRK channels as new candidates to serve as prognostic and/or predictive factors in addition to already established factors in patients with breast cancer. The proposal is performed in close interaction between clinicians and basic scientists as it represents a combination of analytical and functional molecular approaches that will be combined with the clinical curriculum of the disease.

Supervisors: T. Bauernhofer, W. Schreibmayer, C. Windpassinger.

#02.: Single cell DNA fingerprint analysis as a gold standard of sex-independent confirmation of fetal identity of cells present in the circulation of pregnant women and as a target for non-invasive genetic diagnosis. Numerous reports describe presence of various types of fetal or placental cells (stem cells, erythroblasts, trophoblast cells etc.) in the circulation of pregnant women. These cells are potential targets for non-invasive genetic diagnosis. So far, however, this has not been achieved on a routine basis. Our approach includes detection of these rare cells on slides on basis immunocytochemistry or FISH, followed by laser catapulting and single cell DNA fingerprint analysis. In addition, we plan to do whole genome amplification of single cells and pooling of genetic material of cells of confirmed fetal origin for analysis of allelic diseases and chromosome disorders (This is done in collaboration with the Institute of Medical Genetics).

Supervisors: G. Dohr, B. Huppertz, P. Sedlmeyer.

#03.: Influence of HLA-G on trophoblast invasion. The MHC- I molecule HLA-G is selectively expressed on the invasive extravillous cytotrophoblast, which invades into the maternal decidua in some respects similar to a malignant tumor. We assess invasiveness using 2 types of confrontation assays. (1) Spheroids of the HLA-G negative choriocarcinoma cell line JAR is compared to a HLA-G transfectant of JAR in terms of invasivity into spheroids and fresh pieces of first trimester decidua. (2) Explant cultures of first trimester villous explants are loaded with antisense-oligonucleotides of HLA-G and confronted with pieces of first trimester decidua.

Supervisors: G. Dohr, B. Huppertz, P. Sedlmeyer.

6.2.: Thesis projects, to be started with the Doctoral School:

#04.: Possible Pathophysiological role of GIRK gene splice variants in breast cancer cell lines. Recently we succeeded in the isolation of different splice variants of the GIRK1 gene, the identification of GIRK1 proteins with different size from various breast cancer cell lines (MCF7, MCF10A, SKBR3, MDA453, T47D) with Western blotting and the functional characterization of different K⁺ channels in the MCF7 and the MCF10A cell line. In order to investigate, whether the different GIRK1 variants play a role in the progress and pathophysiology of cancer, fusions of the GIRK1 or the GIRK4 protein with the yellow variant of the green fluorescence protein (GFP) were produced and transfected into MCF7 cells. Stably expressing cells were selected using a cell sorter (BD Aria FACS) and cloned². Migration and mobility of these MCF7 cells, stably expressing the GIRK1 or GIRK4 proteins will be monitored using the Zeiss Axiovert 200M cell observer microscope with Incubator XL and Heated stage Pecon 24 well and the Achroplan 20X objective (ZMFI). Effects on vital parameters, e.g. proliferation and/or cell motility will be assessed. An extension of this experimental paradigm to other cell lines, e.g. breast cancer cell lines with different malignant properties, cell lines resembling healthy breast tissue and also overexpression/knockout of

² : these results were obtained during the Masters thesis of 2 students of the curriculum "Human Medicine". At least one of them (in fact both) are eager to proceed with a Doctoral thesis.

splice variants, specifically detected in MCF7 cells, is projected. This study is aimed to elucidate a possible pathophysiological role of GIRK channels in cancerogenesis and metastasis.

Supervisors: T. Bauernhofer, W. Schreibmayer, E. Malle.

#05.: Identification of the causative disease gene for distal hereditary motor neuropathy type V in a multigenerational family of German origin. Distal hereditary motor neuropathy type V (dHMN-V, i.e. distal spinal muscular atrophy = dSMA) is genetically heterogeneous group of motor neuron disorders with predominant degeneration of the second motor neurons. Recently we were able to identify *BSCL2* as the causing gene for one variant of dHMN-V (Windpassinger, Nature Genetics, 2004). Latest findings could prove the existence of one or more further disease genes for dHMN-V (Rohkamm, Journal of the Neurological Findings, 2007).

The main goal of this project is to identify the genetic locus and disease causing genetic determinant in a hitherto genetically uncharacterised multigenerational dHMN-V family.

In the first phase of the project we have already excluded all known genes (*BSCL2*, *GARS*, *HSPB1* and *HSPB8*) and loci associated with dHMN-V and no pathogenic mutation could be found in these genes.

SNP-array technology and subsequent linkage analysis will be applied in order to identify and narrow down the genetic locus which harbours the disease gene. Within this locus all genes and transcripts will be characterised and the genomic organisation will be determined. Genes which are expressed in the peripheral and central nervous system and map within the region will be chosen for mutation analysis. Subsequently, all exons and flanking intronic regions of these functional candidate genes will be sequenced. All possible pathogenic genetic alterations will then have to be tested for segregation within the family, followed by screening of control DNAs. After identification of the pathogenic mutation, functional genetic approaches and localisation studies will be performed.

The identification of this dHMN gene will add important knowledge to the understanding of novel disease mechanisms and *underlying* cellular processes responsible for inherited motor neuron disorders.

Supervisors: E. Petek, T. Schwarzbraun, C. Windpassinger.

#06.: Functional analysis of proteins involved in cell cycle control and their effects on genome stability. Although it is one of the most investigated topics over the past decades, the detailed individual steps necessary for cancer initiation are still controversially discussed. A common consensus at the moment is that virtually all tumor cells have at some stage an underlying genomic instability. The term genomic instability includes all forms of unstable genetic information e.g. chromosomal instability (CIN), microsatellite instability (MIN) and epigenetic instability, but is often used in an ambiguous context in the literature. A current model for tumour initiation and progression is that an underlying genomic instability accelerates the accumulation of mutations necessary for the malignant transformation of a cell.

Recent data suggests that potential CIN acting mutations and the consequences of CIN and aneuploidy do not follow a simple black & white pattern. For example it was recently confirmed that aneuploidy can act oncogenically as well as a tumour suppressor depending on the level of genetic instability (Weaver et al. 2007-A; Weaver et al. 2007-B).

In this context, a special interest lies on the mechanisms of chromosome segregation and the involved factors when investigating genomic instability and CIN. With the recent identification of PICH (Plk1-interacting checkpoint helicase), a centromere-associated SNF2 family ATPase, as an essential component of the spindle assembly checkpoint, present theories about the mitotic process and sister chromatid separation need to be updated. PICH

was found to localize to kinetochores, inner centromeres, and most interestingly it decorated thin threads connecting separating chromosomes even until late anaphase (Baumann et al. 2007).

The main goal of this project is to analyze in detail the function of PICH during mitosis, to elucidate the functional role of DNA-threads in anaphase and the proteins involved, and to establish a correlation between PICH mutations and cancer. To this end a combination of molecular biological methods (e.g. sequencing, cloning) and cell biological methods will be applied (e.g. cell culture, FISH, immunofluorescence). The results will represent important contributions to cell cycle and mitosis and all related areas such as ageing and especially tumour biology.

Supervisors: E. Petek, T. Schwarzbraun, *C. Windpassinger*.

#07.: Involvement of group VI calcium-independent phospholipases A₂ in immediate and delayed prostaglandin synthesis by osteoblasts. Group VI calcium-independent phospholipases A₂ (iPLA₂) are well described in many cell types. Their main function elucidated so far is providing lysophospholipids for re-acylation of fatty acids, in particular arachidonic acid (AA), into membrane phospholipids. A role in AA liberation has also been described. AA is the precursor fatty acid of prostaglandins, formed by prostaglandin-endoperoxide synthases (PGHS) 1 and 2. In this project, the involvement of iPLA₂s in AA substrate release and sequestration shall be investigated. A possible differential role in both, immediate and delayed prostaglandin formation (catalysed by PGHS-1 and PGHS-2, respectively) shall be studied. Signal transduction of these effects are to be elucidated as well. Supervisors: *H.J.Leis*, E. Malle, M. Windischhofer.

#08: Calcium ionophores and cellular prostaglandin production. Calcium ionophores, such as ionomycin and A23187, act as specific calcium transporters across cellular membranes. They are widely used as pharmacological tools for studying calcium-dependent cellular events. However, preliminary data from our laboratory (along with published research) suggest considerable side-activities, specific and non-specific, which are not attributed to their calcium transport properties. As thousands of published studies discuss their findings mostly in the light of ionophoric action, a thoroughly investigation of the cellular effects is desirable. These studies are to be conducted in this project. Supervisors: *H.J.Leis*, E. Malle, M. Windischhofer.

#09: Expression Analysis in COS-1 cells of Missense Alleles of the GLB1 (Acid β -Galactosidase) Gene with presumptive influence on catalytical enzyme function.

Mutations in the β -D-galactosidase (β -gal) gene (GLB1) can result in GM1 gangliosidosis (GM1), a neurodegenerative disease of varying onset and extraneural affection, or Morquio B disease (MBD), with entirely normal CNS function. In most infantile GM1 genotypes gene products are absent, while MBD may be caused by impaired catalytic function of normally synthesized and transported enzyme precursors. In juvenile or adult GM1, precursor stability may be reduced and thus be sensitive to chaperone treatment. Mutations and gene products of the GLB1 gene have therefore recently gained particular interest.

Novel and known missense mutations with presumptive influence on the catalytical activity were expressed in COS-1 cells, the increase of β -gal activities measured and the amount of material monitored with specific antibodies. According to expression analysis and clinical phenotypes several domains of the protein may be catalytically active on the various natural substrates of the enzyme. The expressed proteins derived from selected mutants shall permanently expressed in fibroblasts and characterized for their intracellular transport, assembly in to the lysosomal multienzyme complex and kinetic properties against natural substrates in the presence of natural saposins and phospholipids.

Supervisors: G. Fauler, E.Paschke, T. Wrodnigg.

#10.: Biochemical characterization of novel galactose analogues with presumptive chaperone activity in fibroblasts from patients with GM1 gangliosidosis and Morquio B disease.

Morquio B disease (MBD; OMIM #253010) and GM1-gangliosidosis (GM1; OMIM +23500) both evolve from defects in the function of human acid β -galactosidase (β -gal; EC 3.2.1.23) encoded by the GLB1 gene. While in MBD cases, a normally localized, stable, but catalytically impaired enzyme was detected, several GM1 mutants were shown to be caused by premature degradation of the mutant β -gal precursor. In cells of these patients, the addition of competitive inhibitors, mostly galactose analogues, to the culture media increased their residual β -gal activities, presumably due to stabilization and normalized trafficking. This was proposed as a therapeutic option though the knowledge on structural requirements of presumptive “pharmacological chaperones” and the spectrum of sensitive β -gal mutations is limited.

In the course of a cooperation with the Institute of Organic Chemistry TU Graz, novel 1-deoxynojirimycin derivatives were characterized for their inhibitory effects on β -gal activity of normal fibroblasts and different mutants from patients with GM1 or MBD. Further experiments shall be done to characterize the turnover of natural substrates, like glycosphingolipids and glycosaminoglycans, in natural and transgenic cell lines with GLB1 mutations without or under the influence of potential chaperones. Further work shall correlate these results with data on subcellular localization, transport and catalytic properties of mutant β -galactosidase. This may be useful to obtain an improved mutation-specific design of molecules used in chaperone therapies.

Supervisors: G. Fauler, E.Paschke, T. Wrodnigg.

#11.: Influence of potential pharmacological chaperones on the activity of α -galactosidase and the concentration of globotriaosylsphingosine in Fabry fibroblasts.

Fabry disease, a severe lysosomal disorder leading to cardiomyopathy and lethal chronic kidney disease is caused by the deficiency of α -galactosidase, an enzyme degrading the glycosphingolipid globotriaosylceramide (Gb3) in the lysosomes of affected cells and is excreted in large amounts with the urine of Fabry patients. Recently the lyso-derivative of Gb3, globotriaosylsphingosine has been proposed to be a main player in the pathogenesis of Fabry disease. However, very little is known on the metabolism of this compound and its relation to the primary enzyme defect in Gb3 degradation. We currently test a number of substances which have the potential to stabilize mutants of a lysosomal β -galactosidase from being prematurely degraded by ER proteases. Some of these substances are currently under investigation as therapeutic drugs for Fabry disease but have also been shown to modulate also the residual activity of α -galactosidase in Fabry cells. We furthermore established novel methods for the quantitation of LysoGb3, Gb3 and other glycosphingolipids in cultured fibroblasts by tandem mass spectrometry together with the KIMCL laboratory. The metabolic fate and the effects of potential chaperones on the residual activity and protein stability of α -galactosidase shall be tested and correlated with the concentrations of Gb3 and its lyso-derivative.

Supervisors: G. Fauler, E.Paschke, T. Wrodnigg.

#12.: Hypochlorite, oxidized (lipo)proteins and cation-channels – the trio infernal in atherogenesis? The main weapon of phagocytes, the first line of defense in the human immune system, is NADPH-oxidase, a supramolecular complex that generates substantial amounts of superoxide free radicals ($O_2^{\cdot-}$). Subsequently, this relatively harmless $O_2^{\cdot-}$ is converted to other reactive oxygen species, like H_2O_2 . Via myeloperoxidase (MPO) catalyzed

halogenations H_2O_2 is converted to hypochlorite (HOCl) that is used to kill and initiate disintegration of the engulfed pathogens. As these reactive oxygen species are extremely aggressive, potentially also for the phagocytotic cell itself, their homeostasis is based on a delicate balance of signalling events, that are currently under extensive investigation by several groups. Interestingly, voltage-dependent H^+ channels (but also structurally related voltage dependent protein phosphatases and K^+ channels) have been reported to play a crucial role by triggering NADPH-oxidase. A major cause for the differentiation of phagocytes is the engulfment of HOCl-oxidized low- and high-density lipoproteins that in contrast to the corresponding non-oxidized lipoproteins seemingly paralyze the phagocytotic abilities, leading to the formation of foam cells (that in turn form the atherosclerotic plaque). Aim of the proposal is to identify the role of voltage dependent H^+ channels in this pathophysiological course, i.e. to study (i) expression, (ii) isoform heterogeneity, (iii) function and (iv) modulation of the function of these channels via reactive oxygen species and oxidized/reduced lipoproteins.

Supervisors: E. Malle, W. Schreibmayer, C. Windpassinger.

7.: Specific Lectures and Courses³:

a) "Pflichtfach":

i.: "Methodisch/naturwissenschaftliche Grundlagen für Mediziner": 4 SWS⁴:

1,33 SWS: "Molecular Physiology of the Cell", *Gaffari-Tabrizi, Graier, Groschner, Holzer, Schreibmayer*

0,53 SWS: "Cellular Physiology and Communication": *Bauernhofer, Holzer, Kresse, Quasthoff, Schreibmayer.*

0,53 SWS: "Translational Molecular and Cellular Physiology": *Jeglitsch, Kokskämper, Quasthoff, Sandner-Kiesling.*

0,67 SWS: "Cellular Physiology and Biochemistry": *Graier, Malle, Sattler, Schreibmayer.*

0,80 SWS: "Microanatomy of the Cell": *Leitinger*

0,13 units: "Mathematical Modelling in Molecular and Cellular Bioscience": *Plank, Zorn-Pauly*

ii.: "Medizinische Grundlagen für Nawi u. Technikerinnen": 4 SWS: for the time being, we intend to use the corresponding course of the former Dr. sci. med. curriculum. If required, we will plan a substitute, using synergisms with the other doctoral schools.

iii.: "Wissenschaftliche Grundlagen und allgemeine Fähigkeiten": 4 SWS: distinguished experts, partially from the private sector, will introduce this next generation of scientists into : complementary skills, entrepreneurship, intellectual property rights, public-private partnership, translational research, contacts with the private sector, project management, time management, acquisition of funding, presentation (oral or posters), ethics, communication, technology transfer. Career development will be an important aspect with future planning, applications for grants and the importance of mobility. On demand, this course can also be used by other Doctoral Schools. Specifically we plan:

0,27 – 0,53 SWS: "Good Scientific Practice", *P. Holzer.*

2,67 – 3,4 SWS: "Project management, time management, acquisition of funding, presentation (oral or posters), ethics, communication, technology transfer, intellectual property rights, public-private partnership, complementary skills, translational research and entrepreneurship." *A. Meir (CSO, Alomone Labs. Ltd., Jerusalem), G. Jeglitsch (Study Coordinator, Biotronik, Vienna),* other founders and/or CSO's⁵ of local biotechnology/pharmacology related enterprise.

0,07 – 0,13 SWS: Ethik and Ethikkommission, (*P. Rehak*)

0,13 – 0,33 SWS: Quality control in the laboratory (N.N.)

0,13 – 0,33 SWS: Planning and managing clinical trials (*A. Berghold*)

³ : the proposed teaching is based on the curriculum "Doktoratsstudium der Medizinischen Wissenschaften" as published in the MTBl. from 02.04.2008, StJ 2007/08, 18. Stk.

⁴ : 1 SWS ("Semesterwochestunde") corresponds to 15 units, each 45 minutes.

⁵ : the negotiations with prospective lecturers are in good progress.

iv.: “Dissertationsseminare“: 8 SWS:

2,67 – 6,00 SWS: Journal Club.

1,33 SWS: Thesis Symposium.

3,67 – 0,67 SWS: selected specific courses, as described in appendix I.

b) “Wahlfach“: 4 SWS:

1,00 – 3,00 SWS: Journal Club.

1,00 – 3,00 SWS: selected specific courses, as described in appendix I, but complementary to the “Pflichtfach“.

c) “Stammtisch”⁶: in order to facilitate social contacts and promote the exchange of ideas and technologies, a ”Stammtisch” will be organized on a 2 week basis (faculty is allowed once a month).

⁶ : non-compulsory. Not an official part of the curriculum.

Appendix I.: A wealth of smart courses that will contribute to the “Dissertationsseminare” and to the “Wahlfach”⁷.

P. Sedlmayr.: *Flow and slide-based cytometry. principle and applications*
(1 SWS).

G. Fauler, H.J. Leis, N.N.: *Mass Spectrometry and other Analysis Techniques in Biomedical Research*.
(1 SWS).

T. Bauernhofer, T. DeVaney, N.N.: *Introduction into Cell Culture*.
(1 SWS).

E. Hofer, W. Schreibmayer, N.N.: *Principles of Electronic Measurement Techniques in Cellular Physiology Research*.
(1 SWS).

W. Schreibmayer, N.N.: *Single Channel Recording Techniques*.
(1 SWS).

W. Schreibmayer, N.N.: *Basic Voltage Clamp Technology*
(1 SWS).

K. Groschner, W. Schreibmayer, N.N.: *Dynamic Confocal and TIRF Microscopy in vivo*.
(1 SWS).

Ch. Windpassinger, W. Schreibmayer, N.N.: *Practical Genetic Engineering*.
(1 SWS). Courses on different Techniques are projected (e.g.: subcloning, site directed mutagenesis, recombinant protein production etc..). Students bring samples and problems along.

K. Groschner, W. Schreibmayer, N.N.: *Heterologous Expression Techniques*.
(1 SWS). Courses on different Techniques are projected (e.g.: HEK cells, Xenopus oocytes, CHO cells, genomic integration of DNA etc..). Students bring samples and problems along.

A. Sandner-Kiesling, N.N.: *Translational Research in Clinical Practice*.
(1 SWS). Introduction and case studies, prepared for students of the programme.

⁷: as faculty and requirements change, this list will be adequately adjusted.

Appendix II.: Resources of the projected Doctoral School

5.1.: Basic resources for teaching:

It is expected that the Medical University of Graz will provide the required infrastructure (seminar rooms, lecture halls etc..) and the remuneration for the lecturers.

5.2.: Places where the research projects will be conducted:

Pediatric Laboratory of KIMCL (G. Fauler): The Pediatric Laboratory of KIMCL is working in close cooperation with the pediatric institutes, on the field of diagnosis and inherited and metabolic disorders.

Technology established and available: 1.: *gas chromatography*. 2.: *mass spectrometry*. 3. *liquid chromatography*. 4. *tandem mass spectrometry*. 5. *synthesis of internal standards*. 6. *sample preparation from different biological materials*. 7. *derivatization of target molecules*.

Biophysics and Molecular Physiology laboratory (Klaus Groschner): The main research topic of this research group is biophysics and molecular physiology of non selective cation channels with particular focus on transient receptor potential (TRP) channels. The laboratory provides expertise in classical electrophysiology (patch-clamp) including methods for characterization of channel functions at the single molecule level as well as equipment and experience for standard protein biochemistry and molecular biology. Particular know-how is available in terms of experimental strategies for the analysis of protein-protein interactions (immunoprecipitation, GST-pulldown), subunit composition/ stoichiometry and dynamic assembly of ion channel complexes. Methods for culture and characterization of cells (FACS) and for transfection of cardiovascular cells (freshly isolated cardiac cells from mouse and rat as well as cell lines such as HL-1) are established. Methods and technology for characterization of signaling events in living cells including visualization of rapid trafficking and dynamic assembly of signal complexes by high-resolution fluorescence microscopy (TIRF and TIRF/FRET microscopy) as well as infrastructure for functional measurements such as characterization of cardiac excitation (ECG) and electrical properties in isolated mouse hearts (Langendorff) are available

Molecular Physiology Laboratory (W. Schreibmayer): The Molecular Physiology Laboratory is working in the field of voltage dependent sodium channels, G-protein activated potassium channels and, more generally, in the field of signal transduction.

Technology established and available: 1. *cell culture*: *Xenopus laevis* oocytes; adult cardiomyocytes, all sort of cell lines 2. *genetic engineering*: heterologous expression (*Xenopus laevis* oocytes; mammalian cells); site directed mutagenesis; RNA isolation and synthesis; all sorts of DNA constructs; production and high-scale purification of recombinant protein 3. *biochemistry*: protein interaction using the pull-down assay; in-vitro phosphorylation (Phosphorimager); 4. *cell biology*: confocal laser scanning microscopy for the study of synthesis and trafficking of signal transduction molecules in living cells. Protein-protein interaction in-vivo using FRET. Phosphorylation and back-phosphorylation in-vivo in living cells. 5. *electrophysiology*: Two electrode voltage clamp of whole cells; Patch Clamp techniques (whole cell clamp; macropatch recording; single channel recording; perforated patch).

Laboratory of Peter Sedlmayr: The laboratory is mainly involved in 2 fields of research: placental immunology and non-invasive prenatal diagnosis.

Established and available technology: flow and slide based cytometry, rare cell detection in context to single cell microdissection and laser catapulting (patent pending), PCR including single cell PCR, immunohistochemistry, in situ-hybridization, cell and tissue culture including confrontation culture, confocal laser scanning microscopy. Electron microscopy, Western blotting.

Laboratory of Metabolic Diseases at the Department of Pediatrics (E. Paschke): Our main task is research on and pre- and postnatal diagnosis of inherited metabolic diseases. We originally started together with what is now the Pediatric Laboratory of KIMCL (W.Erwa; organic acidurias, peroxisomal diseases). Meanwhile the KIMCL laboratory mainly provides chromatographic techniques and we offer the molecular analyses as well as the entire enzymology of lysosomal diseases, including cell culture and in vivo assays. We furthermore cooperate worldwide in the molecular analyses for lysosomal and other genes (e.g. GLB1, ALDH7A1 and others). Research currently concentrates on phenotype-genotype analysis of lysosomal diseases, the molecular genetics of pyridoxin-dependent epilepsy (PDE) and, together with the KIMCL laboratory, on clinical case finding studies in Fabry disease. A large number of proven and mostly genotyped cell lines (e.g. I-cell disease, Niemann-Pick type C, sphingolipidoses, mucopolysaccharidoses etc.) is available for research purposes.

Available and established techniques are: enzymology of lysosomal enzymes using natural and synthetic substrates, cell culture assays (e.g. pulse chase labeling experiments of phospholipids, sphingolipids, glycosaminoglycans etc.) and immunohistochemical methods, transient and stable expression of mutant alleles in COS-1, CHO-cells and fibroblasts, characterization of mutants at the protein level (Western blots etc.), comprehensive characterization of gene mutations.

Institute of Human Genetics (T. Schwarzbraun, C. Windpassinger): The Institute of Human Genetics is working in the broad field of human molecular genetics with a focus on tumourbiology (Schwarzbraun) and neurogenetics (Windpassinger).

Technology established and available: 1. *molecular genetics*: DNA extraction, amplification of low amounts or few cells, PCR methods including real-time PCR, sequencing 2. *genetic engineering*: DNA constructs, site directed mutagenesis; RNA isolation 3. *biochemistry*: Western Blot, protein detection 4. *cell biology*: live-cell microscopy, FISH, immunofluorescence 5. *cell culture*: basic cell culture methods with a broad range of cell lines – mainly tumour derived, transient and stable transfection of mammalian cells 6. *DNA-Array based technologies*: SNP-array based linkage mapping, LOH analysis, genome wide detection of gains and losses of DNA 7. *Linkage mapping*: family-based identification of new genetic loci of monogenic inherited diseases, microsatellite marker analysis, haplotype analysis.

Research unit of osteology and analytical mass spectrometry at the department of pediatrics (HJ Leis): The research activities of our group are focussed mainly on two areas: 1) mass spectrometry: development of ultrasensitive detection methods based on GC-MS using various ionisation techniques. Validation of analytical methods. Synthesis of stable isotope labelled compounds. 2) Signal transduction involved in prostaglandin synthesis of bone cells. Technologies available are: gas chromatography, mass spectrometry, basic techniques in biochemistry and molecular biology (western blotting, PCR, etc), cell culture.

Equipment is in place and methods are established and running.

Laboratory of Ernst Malle: Ernst Malle is an established expert in cellular interaction of native and modified lipoproteins. He has extensively investigated the consequences of MPO-mediated modification on physiological properties of low- and high-density lipoproteins in

healthy and disease states. His research group is interested in inflammation, atherosclerosis and cancer and underlying inter/intracellular mechanisms, upregulation of co-stimulatory molecules, receptors, proinflammatory cytokines, as well as reactive oxygen products. His expertise on lipid/lipoprotein-mediated signal transduction in inflammation and atherosclerosis is most valuable to the Doctoral school. Standard techniques in molecular biology and biochemistry, standard cell culture techniques, transfection of cells. *Models & Tools*: A variety of CHO- or HEK293 cells overexpressing different receptors are available

Clinical Oncology Group of the Division of Oncology (Th. Bauernhofer): The Clinical Oncology Group is interested in research upon basic pathophysiological mechanisms of cancerogenesis and progression and translation of the outcome to the clinical situation.

Technology established and available: Laboratory to perform sample preparation for ELISA, flow cytometry, immunohistochemistry, PCR. Fully equipped PCR facility, flow cytometry facility (LSRII (11 colours) and Calibur (4 colours) Becton Dickinson. Automax for cell separation procedures

5.3.: Funding of the research projects:

The existing infrastructure⁸ as described under 5.2 will be used for the proposals. Materials like e.g. disposables are financed by the university and grants of the responsible faculty, that is committed to raise third-party funding in order to sustain the doctoral school projects. The Doctoral School on “Translational Molecular and Cellular Bioscience” is currently and was recently supported by the following grants (funding within last 5 years):

European Commission:

SAFE: Network of Excellence of the EC <http://safenoe.org/cocoon/safeorg/>

EMBIC: Network of Excellence of the EC <http://www.embic.org/>

Austrian Research Foundation:

FWF P15404-B05: “HOCL-HDL impairs endothelium-dependent dilatation“

FWF P17103-B05: “Serum amyloid A-activating factor-1 and HOCL-HDL”

FWF P19074-B05: “Receptor-mediated signalling events and myeloperoxidase-modified HDL”

FWF SFB-F3007: “Myeloperoxidase-mediated lipotoxicity in the central nervous system“

FWF SFB007/708: “Molecular Aspects of Ion Channels”

FWF SFB007/F715: “Crosstalk of nitric oxide synthase and ion transport systems”

FWF P14950: “Functional and molecular organization of vascular smooth muscle TRP channels”

FWF P18280: “Characterization of cardio-vascular TRPC3 channels”

FWF 19820: “TRPC proteins as determinants of endothelial proliferation”

Austrian Research Foundation for enterprise related research:

FFG 814315/Bridge: “Mechanisms by which components of bare metal stents contribute to in-stent restenosis”

Research Foundation of the Austrian National Bank:

OENB 12575: “On a putative role of GIRK in breast cancer”.

⁸ : This infrastructure is financed from the university, governmental foundations as well as from private companies and organisations.

OENB 9962: “PPAR γ -dependent activities in human choriocarcinoma cells”

OENB 8216: “Characterization of diabetes-associated changes in endothelial progenitor cells”

Austrian Ministry of Science and Research:

“Investigations on the Early Diagnosis and Prognosis of Lysosomal Storage Disorders”.