Nano-Analytics of Cellular Systems

From molecular dynamics, recognition and organization to membrane transport and motility

The **scientific goal** of this graduate program is to gain insight into the dynamics and the molecular assembly of cellular molecules, about their recognition on the membrane surface, and on the initiation and performance of cellular processes, such as membrane transport, motility, and differentiation. Functional and structural investigations are carried out on both model and native systems, from single proteins in reconstituted environments to sub-cellular and cellular samples. The major aim is to span the gap between the processes of **expression, structural arrangements,** and **molecular recognition** on one side, and **membrane transport, cell motility**, and **differentiation** on the other side. As the program focuses on frontier research in life science and cellular nano-research, it integrates numerous fields encompassing biophysics, cell biology, nanotechnology, soft matter physics, molecular and structural biology, genetics, bio-organic and inorganic chemistry, theoretical physics, mathematical modelling, and scientific computing, giving the Ph.D. students lifelong flexibility for continued professional growth.

Well established **nano-analytical** and **nanoscopic techniques** with a resolution ranging from sub-nanometer to micrometer cover the entire scale, from single molecules to molecular assemblies and living cells. Novel approaches in biophysics, cellular biology and data analysis provide a solid basis for the education and the training of the students within the NanoCell program. These methodologies are subsequently exploited and mutually linked.

This **highly interdisciplinary** graduate **research program** involves 12 faculty members from seven Institutes at Johannes Kepler University Linz (JKU) and two additional research centers: The Institutes of Biophysics, Applied Physics, Organic Chemistry, Inorganic Chemistry, Soft Matter Physics, and Theoretical Physics from the Johannes Kepler University Linz (JKU), the Institute of Science and Technology (IST), and the Institute of Applied Physics from the Vienna University of Technology (TUW).

The **main goal** is to provide a stimulating environment, which supports excellent training of talented graduate students in competitive research projects. The students will be trained in current concepts of of biophysics, biochemistry, molecular and cell biology, imaging, spectroscopy, macromolecular modelling, molecular recognition and mechanics, cellular transport and motility, as well as in a range of advanced bio-analytical methodologies (bulk-surface sensing, electrophysiology, nuclear magnetic resonance, optical spectroscopy, macromolecular crystallography), nanoscopy (optical super-

resolution and scanning probe microscopy), next generation sequencing, and data-analysis. An **integrated interdisciplinary teaching program** and international exposure at stays abroad will prepare them for a successful career as a scientist. The consortium of the supervisors and the student community will guarantee a broad range of training experience and continuous incorporation of expertise.

Faculty members and projects

Project leader ,PI (Affiliation)	**Project no. and title**	**Education/Expertise/Methods**
Michael Sixt (IST)	DK1: Probing the actin cortex and membrane organization of dendritic cells	*Cell Biologist* Cell Biology / Immunology Biology of Single Cell Motility Live Cell Imaging
Gerhard Schütz (TUW)	DK2: Nanostructural characterization of the plasma membrane	*Biophysicist* Superresolution Microscopy Single Molecule Fluorescence Microscopy
Irene Tiemann-Boege (JKU)	DK3: Functional analysis of driver mutations in the FGRR3 expanding with paternal age	*Molecular Geneticist* Evolutionary Genetics Mutagenesis, Single molecule PCR, Ultrasensitive sequencing
Thomas A. Klar (JKU)	DK4: STED lithography for immunology	*Experimental Physicist* Nanophotonics STED Microscopy
Ingrid Graz (JKU)	DK5: Biostretch – exercising cells: effects of complex mechanical forces on adhesion, growth and migration	*Experimental Physicist* Stretchable electronics and soft robotics Force-driven cell functionalities
Carl-Philipp Heisenberg (IST)	DK6: Cell sorting in development	*Developmental Biologist* Embryo morphogenesis in development Atomic Force Microscopy Micropipette aspiration Laser Cutting
Peter Pohl (JKU)	DK7: The mechanism of protein translocation through the bacterial translocon	*Biophysicist* Membrane Transport Scanning Electrochemical Microscopy, Electrophysiology, Fluorescence
Peter Hinterdorfer (JKU)	DK8: Forces and dynamics in protein translocation through the bacterial translocon	*Biophysicist* Single Molecule Interactions Molecular Recognition Scanning Probe Microscopy
Günther Knör (JKU)	DK9: Photochemical control of cellular processes and protein translocation	*Bio-Inorganic Chemist* Coordination Chemistry Photochemistry Synthesis, Spectroscopy, Photolysis

Thomas Renger (JKU)	DK10: Modelling of conformational transitions of translocon: from structure to function	*Theoretical Biophysicist* Structure-function Relationships of Biomolecules Theory and Modeling
Christoph Romanin (JKU)	DK11: STIM/Orai coupling and CRAC activation	*Biophysicist* Ca2+ Signaling Patch-Clamp FRET Microscopy
Norbert Müller (JKU)	DK12: Conformational dynamics at the STIM/Orai interface using NMR	*Bio-Organic Chemist* NMR Intermolecular Interactions Expression

The combined expertise of JKU, IST, and TUW will lead to valuable educational and research collaborations in biophysics, cellular biology, genetics, and nanomedicine, using high-end nanoanalytical and nanoscopic techniques, combined with data processing and analysis methods. The immediate activities and long-term aims of the **project areas** for the next funding period are surveyed below:

(i) Identifying the molecular mechanisms for membrane protein organization live cells. The recycling of adhesion and chemokine receptors (Sixt), the responsible trafficking pathways as well the effect of age related mutations of membrane receptors (Tiemann-Boege) will be followed with optical methods, such as fast confocal, Total Internal Reflection (Schütz, Sixt), single molecule microscopy (Schütz), and FCS (Pohl). *Long term aim*: Elucidate the biophysics of the spatial organization of membrane receptors, and of recycling pathways responsible for adaptation of adhesive and directional responses during cell migration.

(ii) Adhesion strength and spatial distributions of cytoskeletal and adhesion components underlying cellular adhesion in the development of multicellular organisms (Heisenberg) and cells (Graz) will be investigated using dual micropipette aspiration (Heisenberg), AFM methods (Hinterdorfer, Heisenberg), nano-optical imaging modes (Klar, Sixt, Schütz) and stretchable electronics (Graz). *Long term aim:* To describe progenitor cell-cell adhesion and in particular the role of tension in modulating cell-cell adhesion and contact formation through mechanosensitive mechanisms.

(iii) Mounting evidence suggests that the energetic costs for (i) protein insertion from the aqueous lumen of the bacterial protein translocation channel (SecYEG) into the membrane interior and (ii) partitioning from the aqueous bulk solution into the membrane are different. We explore whether the phenomenon is caused by (i) differences in the physico-chemical properties of water in the confined environment of the SecYEG pore or by (ii) the restricted residence time of the nascent chain in the channel that deprives the nascent chain from the possibility to

sufficiently sample the different environments. Here we will probe the position of the nascent polypeptide chain in the translocation channel by both light microscopy (single molecule FRET) and electrophysiology (Pohl), manipulate it by single molecule force spectroscopy (Hinterdorfer) and assess the corresponding intraluminal water mobility (Pohl). The studies benefit from using caged and photoswitchable compounds (Knör) and from support by molecular modeling (Renger). *Long term aim*: To understand the molecular mechanism of protein translocation through the bacterial and/or eukaryotic translocon.

(iv) While in the first period of the doctorate programme the topic has been refocused to a disease-related gain of function mutation within STIM1, now we aim at visualizing the dynamic interaction of wild-type STIM1 and its extended form STIM1L with the Orai channel in living cells and characterizing their impact on Orai/CRAC currents. For this, we will use a combination of FRET and patch-clamp (Romanin), STED (Klar), fast confocal (Sixt), single molecule microscopy (Schütz), molecular biology (Romanin, Müller), NMR spectroscopy (Müller), force spectroscopy (Hinterdorfer). *Long term aim*: To visualize and compare the dynamic interaction between wild-type STIM1 and STIM1L with the Orai channel in living cells, to estimate respective affinities and conformational rearrangements from STIM1/Orai-derived fragments and complement it with electrophysiological experiments in an attempt to fully characterize their distinct impact on Orai/CRAC currents.