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 picture of how cellular molecules are recognized on the membrane surface, of how they are organized into molecular assemblies, and about how cellular processes such as membrane transport and motility are initiated and performed. Functional and structural investigations are carried out on both model and native systems, starting with single proteins in an artificial (reconstituted) environment and ending with sub-cellular and cellular samples. The major aim is to span the gap between the processes of molecular recognition and structural rearrangements on one side, and membrane transport and cell motility on the other side. As it focuses on frontier research in life- and cellular nano-science, the program will be applicable to many scientific and technological fields related to biophysics, cell biology, nanotechnology, applied physics, theoretical physics, bioorganic and inorganic chemistry, structural and molecular biology, mathematical modelling, and scientific computing, giving the Ph.D. students lifelong flexibility for continued professional growth.

Well established nano-analytical and nano-scopic techniques with a resolution ranging from sub-nanometer to micrometer cover the entire scale, from single molecules to molecular assemblies and living cells. Novel cellular biology and data analysis approaches complement it and provide a solid basis for the education and the training of the students within the NanoCell program. These methodologies will consequently be exploited and connected to each other.

This highly interdisciplinary graduate research program involves faculty from six Institutes at Johannes Kepler University Linz (JKU) and three additional research centers. These include: The Institutes of Biophysics, Applied Physics, Organic Chemistry, Inorganic Chemistry, and Theoretical Physics from Johannes Kepler University Linz (JKU), the Institute of Science and Technology (IST), the Institute of Applied Physics from the Vienna University of Technology (TUW), and the Research Group “Computational Mathematics for Direct Field Problems” at the Johann Radon Institute for Computational and Applied Mathematics (RICAM) from the Austrian Academy of Sciences.

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 biophysics and biochemistry, cell and structural biology, molecular recognition, membrane transport, and motility as well as in a range of advanced bio-analytical, nano-scopic, and data-analytical methodologies. 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**

<table>
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<tr>
<th>Project leader, PI (Affiliation)</th>
<th>Project no. and title</th>
<th>Education/Expertise/Methods</th>
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| Michael Sixt (IST)               | DK1: Probing receptor recycling in migrating leukocytes using photoactivation and single molecule imaging techniques. | Medical Doctor  
Cell Biology / Immunology  
Biology of Single Cell Motility  
Live Cell Imaging |
| Gerhard Schütz (JKU)             | DK2: Nanostructural Characterization of the Dendritic Cell Plasma membrane | Biophysicist  
Superresolution Microscopy  
Single Molecule  
Fluorescence Microscopy |
| Thomas A. Klar (JKU)             | DK3: Optical Nanoscopy | Experimental Physicist  
Nanophotonics  
STED Microscopy |
| Carl-Philipp Heisenberg (IST)    | DK4: Cell sorting in Development | Developmental Biologist  
Embryo Morphogenesis in Development  
Atomic Force Microscopy  
Micropipette Aspiration, Laser Cutting |
| Peter Pohl (JKU)                 | DK5: The Mechanism of protein translocation through the bacterial translocon | Biophysicist  
Membrane Transport  
Scanning Electrochemical Microscopy, Electrophysiology, Fluorescence |
| Peter Hinterdorfer (JKU)         | DK6: Forces and Dynamics in protein translocation through the bacterial translocon | Biophysicist  
Single Molecule Interactions  
Molecular Recognition  
Scanning Probe Microscopy |
| Günther Knör (JKU)               | DK7: Photochemical Control and Nanoscopic imaging of Protein Translocation | Bio-Inorganic Chemist  
Coordination Chemistry  
Photochemistry  
Synthesis, Spectroscopy, Photolysis |
| Thomas Renger (JKU)              | DK8: Modelling of conformational transitions of fluorescent labelled proteins | Theoretical Biophysicist  
Structure-function  
Relationships of Biomolecules  
Theory and Modeling |
| Johannes Kraus (RICAM)           | DK9: Electrostatic computations of FRET rate constants in fluorescent labeled proteins | Mathematician  
Numerical Methods for Partial Differential Equations  
Subspace Correction Methods |
| Christoph Romanin (JKU)          | DK10: STIM/Orai coupling and CRAC activation | Biophysicist  
Ca2+ Signaling  
Patch-Clamp  
FRET Microscopy |
The combined expertise of JKU, IST, RICAM, and TUW will lead to valuable educational and research collaborations in biophysics, cellular biology, and nanomedicine, using high-end nanoanalytical and nanoscopic techniques, combined with data processing and analysis methods. The immediate research activities and aims of the project areas are surveyed below:

(i) Recycling of integrin receptors from the back to the front of migrating leukocytes and the involved transport processes will be followed with optical methods, such as fast confocal (Sixt), single molecule microscopy (Schütz), and FCS (Pohl). Aim: Elucidate the cell biological and biophysical principles that drive leukocyte motility.

(ii) Adhesion strength and spatial distributions of cytoskeletal and adhesion components underlying cellular adhesion in the development of multicellular organisms will be investigated using dual micropipette aspiration (Heisenberg), AFM methods (Hinterdorfer, Heisenberg), and nano-optical imaging modes (Klar, Sixt, Schütz). Aim: To describe progenitor cell sorting both in vitro and within the gastrulating embryo, and determine the specific function of critical processes, such as differential cell adhesion, cell attraction, repulsion, and migration, therein.

(iii) The molecular organization of the reconstituted protein translocation channel in its resting state, the molecular rearrangements accompanying its activation, as well as distinct steps of nascent chain translocation will be examined by light microscopy (FRET, LRET) (Pohl), electrophysiological techniques (Pohl), single molecule force spectroscopy (Hinterdorfer, Gruber), and TREC (Hinterdorfer). The studies benefit from using caged and photoswitchable compounds (Knör), and from support by molecular modeling (Renger, Kraus). Aim: To understand the molecular mechanism of protein translocation through the bacterial and/or eukaryotic translocon.

(iv) 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 will be done using a combination of FRET and patch-clamp (Romanin), LRET (Gruber), STED (Klar), fast confocal (Sixt), single molecule microscopy (Schütz), molecular biology (Romanin, Müller), NMR (Müller), force spectroscopy (Gruber, Hinterdorfer). 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.