IMAGING, DETECTING AND DIRECTING MOLECULAR INTERACTIONS TO DRIVE BIOLOGICAL MACHINES AND PROCESSES

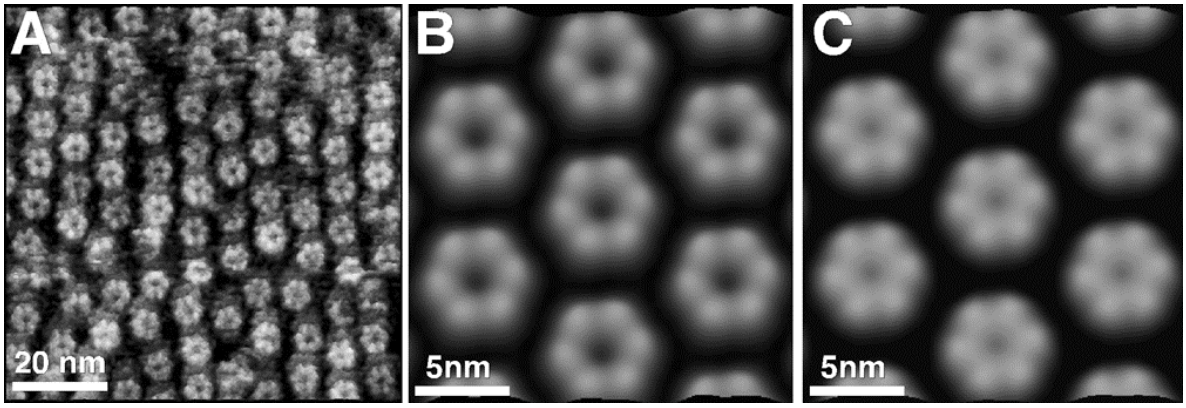
Daniel J. Müller

Center of Biotechnology, University of Technology Dresden, Germany
daniel.mueller@biotec.tu-dresden.de

Live has created molecular machineries which have the dimension of a few nanometers only and which perform their highly specific work at an efficiency and effectivity, which is unreached from any machine designed so far by human hands. Being of fundamental importance for all living organisms, malfunction of these machineries cause major diseases. Besides studying their structure-function relationship for health sciences these machineries borough a great potential to be applied in future an existing biotechnological applications. In this lecture we will present various approaches to characterize these molecular machines of the biological cell on a molecular scale applying bionanotechnological tools. It will become clear that scanning probe microscopy (SPM) techniques applied in biology and medicine will help to discover and unravel pertinent questions in biotechnological, pharmaceutical and medical research and application.

Observing biological machines at subnanometer resolution: The exceptional signal-to-noise ratio of the atomic force microscope (AFM) allows individual proteins to be imaged under physiological relevant conditions at a lateral resolution of 0.4 - 1 nm and a vertical resolution of 0.1 - 0.2 nm. This capability is reviewed on various native cells, compartments of the cells and of the cellular machines such as proteins. Being applied in molecular and cell biology the AFM can be addressed to many more topics than solely high resolution imaging of native proteins.

Simultaneous detection of biochemical information: Lately, it became possible to observe molecular processes at the single-molecule level using SPM techniques. Examples observing function, variability, and assembly of single proteins are discussed. Recent developments of SPM techniques enable measuring simultaneously multiple biochemical signals on individual macromolecules. Recorded with submolecular resolution these signals can be directly assigned to structural details of individual proteins of a cellular membrane. Examples discussed are the ***detection of structural variability and flexibility***, of ***surface charges***, and of ***electrostatic potentials*** of cellular machines.



Ca^{2+} induced conformational change of the extracellular connexon surface. **A**, Extracellular connexon surface imaged in buffer solution ((5 mM Tris, 1 mM EGTA and 1 mM PMSF). **B** and **C**, averaged topographs of extracellular connexon surfaces recorded in absence of (**B**) and with 0.5 mM Ca^{2+} (**C**). The contact mode AFM topographs were recorded using applied forces of 50 pN, a line frequency of 5.5 Hz and were displayed with a vertical scale of 2 nm. (Müller et al. EMBO J. 2002)

Observing the dynamic assembly of cellular machineries: In recent approaches we combined above techniques to gain insights into vesicle transport, fusion and signal trafficking of native cell biological systems. First results of the experiments will be presented showing dynamics of membrane protein assembly at molecular resolution.

Characterizing folding and unfolding of native membrane proteins**:** Cellular machines are only functional if their polypeptide precisely adopts a given three-dimensional conformation. A pertinent question that arises in biology, medicine and pharmacology is how the polypeptide find its three-dimensional confromation to be functional. Behind this question are various diseases based on protein destabilization and malfunction in which interactions that destabilze and misfolded a polypeptide builds must be understood. The combination of single-molecule imaging and force-spectroscopy enables the controlled manipulation of single cellular machines to detect their inter- and intramolecular interactions. Delicate experiments allow observing the unfolding pathways and forces of secondary structural elements of biological macromolecules such as -helices, -sheets and, most surprisingly, of polypeptide loops. Examples unfolding of antiporters, of light-driven proton pumps and of water channels are discussed. Direct observations of the folding process of a single cellular machine are reported as well. In future these and forthcoming methods will provide novel molecular biological insights into factors determining structure, stability and function of individual biological machineries and of their assemblies.

Directing biological assemblies and pathways: The AFM stylus can be used as a 'molecular spinning and knitting machine' to orientate the assembly of individual biological molecules into well-defined, two-dimensional patterns with feature sizes of a few nm to several hundreds of µm. The resulting nanostructured scaffolds are stable for several months without loss of orientation or functionality. Our results directly demonstrate the plasticity of biological assemblies, and give insight into the physical mechanisms by which biological systems may be organized by cells *in vivo*. Examples show that these nanoscaffolds can serve as platforms on non-biological surfaces to direct molecular and cellular processes.

Observing cytoskeleton assembly: An AFM *in vitro* system is introduced that has been established to study the assembly and dynamics of individual filaments in cell extracts. The approach allows observing the polymerization of filaments, the formation of their networks, to study their length distribution and their formation of different types of junctions and branches. The approach builds an avenue to study *in vitro* assays of complex biological systems.

Observing cellular surfaces and diseases: By combining AFM with advanced light microscopy techniques the cell surfaces can be not only directly observed at high resolution, but also their compartments can be identified by specific labeling using modern cell biological techniques. Examples shown are processes of cell surface structuring which are related to the cell function and diseases such as skin cancer.

Detecting cellular interactions at the molecular scale: Here we report on a novel development of an SPM which can be applied to detect single biomolecular interactions of living cells and simultaneously to detect their fluorescence signals.