AKB 50 Imaging and Microscopy

Zeit: Montag 16:00–18:15 Raum: TU H2013

AKB 50.1 Mo 16:00 TU H2013

FRET Studies of the Mobility of TBP-DNA Complexes upon Binding of NC2 — •Don C. Lamb[1,2,3], Peter Schlüsche[1,3], Christoph Bräuchle[1,3], Gertrard Stelzer[4], and Michael Meisterernst[4] — [1]Physical Chemistry, LMU Munich, Munich, Germany — [2]Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL, USA — [3]Center for Nanoscience, LMU, Munich, Germany — [4]National Research Center for Environment and Health, Munich, Germany

Transcription of DNA into RNA is an essential process in life and the initiation of the transcription processes is a popular target for gene regulation. Transcription is initiated with binding of the TATA box binding protein (TBP) to the promoter site on the DNA and recruiting other elements of the transcription complex. When negative cofactor 2 (NC2) is present, transcription is down regulated. NC2 binds to TBP with high affinity. Biochemical evidence suggests that upon binding, the TBP-NC2 complex diffuses along the DNA. Hence, transcription is down regulated because the TBP is not longer located at the promoter site. To verify this hypothesis and to investigate the mobility of the TBP-NC2 complex upon NC2 binding, we have performed fluorescence resonance energy transfer (FRET) experiments on single molecules. TBP was labeled specifically with a donor molecule while a short piece of double-stranded DNA containing a TATA box in the center was labeled with an acceptor. The FRET efficiency was measured before NC2 and after addition of NC2 to solution to investigate the dynamics of individual TBP-NC2 complexes.

AKB 50.2 Mo 16:15 TU H2013

Multi Focal 2-Photon Laser Scanning Microscopy of Cells and Biological Tissue — •Jörg Martini, Katja Tönsing, and Dario Anselmetti — University of Bielefeld, Experimental Biophysics, 33615 Bielefeld, Germany

Near infrared 100fs laser pulses focussed through a high numerical aperture objective lens provide an energy density, that is high enough to induce 2-photon excitation of native fluorophors and fluorescent dyes in the focal volume. By scanning the back aperture of the objective lens with the laser beam, confocal like collection of the fluorescence signal from a single optical plane up to hundreds of μm inside the sample is possible. Varying the distance between the lens and sample, i.e. measuring a depth dependent stack of optical planes, produces a 3D fluorescence scan of the sample with sub μm resolution. The output of today's Ti:Sa-lasers in the focal volume is much higher than the destruction threshold of almost all biological samples. By splitting up the laser power and directing several beams into the objective lens, we create up to 64 foci in the sample. This setup allows for short acquisition times while minimizing the photo damage to the sample. We will present our results on the 3D-distribution of fluorescence and second harmonic generation signal in single cells and biological tissue and their spectral properties.

AKB 50.3 Mo 16:30 TU H2013

Single fluorescent molecules imaged by the near-field of a metal tip — •Heinrich Gotthard Frey[1] and Reinhard Guckenberger[2] — [1]Universität Bielefeld, Fakultät für Physik, Universitätsstr. 25, 33615 Bielefeld, Germany — [2]Max-Planck-Institut für Biochemie, Abt. Molekulare Strukturbiologie, Am Klopferspitz 18, 82152 Martinsried, Germany

We show experimentally that a sharp metal tip, illuminated by a close-by aperture, can be used as high resolution optical near-field probe with an unique combination of good qualities [1]:

Single Cy3 molecules covalently bound to DNA were imaged as test sample. The fluorescence images of such single molecules show patterns with one or two peaks. The width of these peaks can be as small as 10 nm. A simple model allows to explain these patterns. By fitting model calculations to the data, the positions of the dye molecules can be determined with an accuracy better than 1 nm. The 3D orientation of the dyes is also provided by the fit. The positions of two single molecules with only 12 nm distance and overlapping fluorescence patterns could still be determined. The metal tip also provides a topographical signal simultaneously to the optical one. The topographical and optical images have nearly the same resolution and the lateral shift between these images is smaller than the resolution. So, optical and topographical information can be measured simultaneously at the same point, what is of high importance for time dependent measurements.

[1] H.G. Frey, S. Witt, K. Felderer, and R. Guckenberger, Phys. Rev. Lett., in press

AKB 50.4 Mo 16:45 TU H2013

Infrared-spectroscopic mapping of a single virus by near-field microscopy — •Markus Brehm, Thomas Taubner, and Fritz Keilmann — MPI für Biochemie, 82152 Martinsried (München)

Infrared fingerprint spectroscopy has traditionally been a powerful tool for chemical and structural analysis, but because of diffraction could not solve problems requiring $< 5\mu m$ microscopic resolution.
Scattering scanning near-field optical microscopy (s-SNOM) overcomes this limit by exploiting the near-field coupling between a sharp tip and the sample, allowing resolutions of 20 nm even at mid-infrared wavelengths [1,2].
Here we show infrared-spectroscopic mapping of a single virus within the spectral range of the protein amide-I band ($\approx 1600 - 1700\,\mathrm{cm}^{-1}$) demonstrating a resolution of $\approx$ 100 times better compared to conventional infrared microscopy. We therefore believe that this method can be of significant use to some problems in biology.
1. T. Taubner, R. Hillenbrand, and F. Keilmann, "Performance of visible and mid-infrared scattering-type near-field optical microscopes,"Journal of Microscopy, vol. 210, pp. 311-314, 2003.
2. T. Taubner, R. Hillenbrand, and F. Keilmann, "Nanoscale polymer recognition by spectral signature in scattering infrared near-field microscopy", Applied Physics Letters, vol. 85 (22), 2004.

AKB 50.5 Mo 17:00 TU H2013

Imaging of molecular interactions with Photonic Force Microscopy — •Alexander Rohrbach[1], Ernst Stelzer[1], Holger Kress[1], and Nils Becker[2] — [1]European Molecular Biology Laboratory (EMBL), Meyerhofstr. 1, 69117 Heidelberg — [2]Max-Planck-Institut für Physik komplexer Systeme, Nöthnitzer Straße 38, 01187 Dresden

Molecular interactions are controlled by the thermal environment of the binding partners. The resulting energy fluctuations offer a broad spectrum of orientations, distances and kinetics to the molecules enabling an optimal interaction. This concept is exploited in photonic force microscopy: An optically trapped probe fluctuates in its position as a function of the trapping parameters and the probes local environment. The probe position can be tracked interferometrically in the MHz range with a precision of 1 - 5 nm in three dimensions. The fluctuations are altered by external enthalpic or entropic forces acting on the probe. These interactions can be visualized by recording the particles three-dimensional trajectories, resulting in an interaction profile with nm resolution. Two applications illustrate the potential of this technique: single molecule experiments with the protein Myosin II to determine its nano-mechanics; and experiments resolving the temporal and spatial binding of particles to macrophage membranes.

AKB 50.6 Mo 17:15 TU H2013

Subdiffraction Fluorescence Imaging with photoswitchable fluorescent proteins — •M. Hofmann, C. Eggeling, S. Jakobs, and S.W. Hell — MPI für biophysikalische Chemie, Dep. NanoBiophotonics

The resolution of optical imaging in conventional far-field microscopy is limited by the diffraction of light. We present fluorescence imaging beyond this barrier by controlling the light driven transition between the dark and fluorescent state of a photoschwitchable protein. Fluorescence emission in the outer region of a diffraction-limited excitation spot is deactivated in a saturated manner, thereby reducing the effective fluorescence volume. Scanning images of protein stained structures exhibit an increased resolution. This can be described on the basis of a photophysical model and its underlying rate constants, which were determined from spectroscopic experiments of the fluorescence emission. The data shows that a reversible saturable optical fluorescence transition of a protein can be utilized to achieve optical imaging beyond the diffraction limit.

Hell, S. W., Nature Biotechnol. 21(11): 1347-1355 (2003)

M. Hofmann, C. Eggeling, S. Jakobs, S. W. Hell "Subdiffraction Imaging with the photoswitchable fluorescent protein asCP"(in preparation)

AKB 50.7 Mo 17:30 TU H2013

Applications of Pulsed Interleaved Excitation — •Barbara K. Müller, Christoph Bräuchle, and Don C. Lamb — Department Chemie und Biochemie, LMU München, Butenandtstr. 5-13, 81377 München

Pulsed interleaved excitation (PIE) is used in multi-color experiments, whereby the excitation source of a detected photon is known. In our approach, we use a two channel confocal setup, pulsed lasers as excitation sources and a single photon counting card for data storage. The excitation pulses are delayed with respect to each other such that the fluorescence photons from one excitation source arrive before the other excitation pulse and vice versa. Hence, this technique enables one to eliminate crosstalk or to enhance the sensitivity of fluorescence resonance energy transfer (FRET) experiments. We show that this technique increases the sensitivity of fluorescence cross-correlation spectroscopy (FCCS) by removing the spectral cross talk as well as provides the possibility of accurate FCCS in presence of FRET. Moreover, PIE can be used in wide field spectroscopy or laser scanning microscopy, where multi-color detection with one detector is possible. In addition to the economic benefits, this allows higher precision in distance measurements between different fluorophores because both colors are imaged with the same optics. This technique also promises new possibilities in single-pair FRET measurements. With sub-nanosecond pulses, not only stoichiometric information are available, but also the fluorescence lifetime from the same measurements. Thus, the FRET-efficiency can be calculated from either the intensities of donor and acceptor dyes or from their lifetimes.

AKB 50.8 Mo 17:45 TU H2013

Single particle fluorescence microscopy enlights active and diffusive transport processes of nanoparticles in living cells — •Ralf Bausinger[1], Katharina von Gersdorff[2], Manfred Ogris[2], Christoph Bräuchle[1], Ernst Wagner[2], and Andreas Zumbusch[1] — [1]LMU München, Department of Chemistry, Butenandtstr. 5-13, Building E, D-81377 München — [2]LMU München, Department of Pharmacy, Butenandtstr. 5-13, Building D, D-81377 München

Biochemical experiments demonstrated the general applicability of cationic polyethyleneimine-DNA complexes for the delivery of genetic material into the cell nucleus. However only few mechanistic details about this transfection process are known so far. We use single-molecule sensitive fluorescence video microscopy for the tracking of individual nanoparticles inside the cell in combination with structured wide-field illumination for the imaging and three-dimensional reconstruction of labelled cellular structures. Our observations include the interaction of the polyplexes with parts of the cytoskeletton, namely the actin stress fibers and the microtubules. We further analyse the role of mitosis for the delivery of gene carriers into the cell nucleus.

AKB 50.9 Mo 18:00 TU H2013

Single Virus Tracing: Real-time visualization of the membrane attachment and cellular uptake of individual HIV particles — •Thomas Endress[1], Stefan Riegelsberger[1], Marko Lampe[2], Barbara Müller[2], Hans-Georg Käusslich[2], Don Lamb[1], and Christoph Bräuchle[1] — [1]Physikalische Chemie, Universität München, Butenandtstr. 11, 81377 München — [2]Virologie, Unversität Heidelberg, Im Neuenheimer Feld 324, 69120 Heidelberg

Viruses play a major role in biology and medicine. A detailed analysis of the different steps of a viral infection is not only necessary for understanding viral biology, but also for the development of efficient antiviral drugs. Single Virus Tracing (SVT) allows visualization of the infection pathway of an individual virus labelled with fluorescent dye molecules. The fluorescence of the marker molecule is imaged and used to follow the pathway of the virus with high spatial (40 nm) and temporal (10 ms) resolution (*Science, 294 (2001)1929*).
HIV was labeled in the shell with its matrix protein (MA) using eGFP and in the core via its viral protein (Vpr) with mRFP. This allows simultaneous observation of the outer shell and subviral core of the individual HIV in real time. Analysis of virus-cell interactions by SVT revealed a detailed picture of the membrane interactions and cellular surface factors like HSPG involved in typical membrane attachment of HIVs. We were able to distinguish between the very rapid entry ($\leq$ 1 min) of subviral particles by membrane fusion and a slower endosomal uptake of HIV ($\geq$ 12 min). Furthermore, two types of intracellular trafficking were observed.