Current Issues of Nano-Bio-Science

CeNS Winterschool 2003

Mauterndorf, Austria
24-28 February 2003
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in cooperation with SFB 486 and SFB 513

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Internal Students’ Seminar: Schedule

Saturday, 22\textsuperscript{nd} February

7:30 pm  Meeting in front of the castle’s lower gate to go the “Skialm” together

8:00 pm  Get-together at the „Skialm“ in Mauterndorf (in the Skiing center) (kitchen closes at 9:30 pm)

Sunday, 23\textsuperscript{rd} February

9:45 am  Introduction (W. Parak, F. Simmel)

10:00 am Simon Keller (AG Rädler)

10:30 am Stefan Griessl (AG Heckl)

\textbf{11:00 am}  \textbf{Coffee break}

11:30 am Christine Meyer (AG Kotthaus)

12:00 pm Michael Sindel (AG von Delft)

\textbf{12:30 pm}  \textbf{Lunch (not provided), informal discussions}

5:00 pm  Ferdinand Kühner (AG Gaub)

5:30 pm  Stefan Beyer (NG Simmel)
         Teresa Pellegrino (NG Parak)

6:00 pm  Stefan Kowarik (AG Feldmann)

\textbf{6:30 pm}  \textbf{Break}

7:00 pm  Niklolay Petkov (AG Bein)

7:30 pm  Sebastian Gritschneider \textsuperscript{(AG Reichling)}

8:00 pm  Ralf Bausinger / Johanna Kirstein (AG Bräuchle)

8:30 pm  Conclusion
Program

Monday, 24th February 2003

8.30 – 8.45 Opening

8.45 – 9.45 Cees Dekker, Delft University of Technology
Carbon nanotubes as model systems for nanoelectronics and nanosensors

9.45 – 10.45 Ulf Diederichsen, Universität Göttingen
Molecular architecture with biooligomers

10.45 – 11.15 Coffee Break

11.15 – 12.15 Wolfgang Parak, LMU München
Biological Applications of Colloidal Nanoparticles

12.15 Lunch & informal discussions

17.00 Coffee

17.15 – 18.15 Viola Vogel, University of Washington
Mechano-Chemical Sensing

18.15 – 19.15 Stefan Thalhammer, LMU München
Atomic force microscopy and laser microdissection as tools for life sciences

19.30 Conference Dinner

Tuesday, 25th February 2003

8.45 – 9.45 Ned Seeman, New York University
Structural DNA Nanotechnology

9.45 – 10.45 Jens Michaelis, UC Berkeley
Viral DNA packaging - Single molecule studies of a unique molecular motor

10.45 – 11.15 Coffee Break

11.15 – 12.15 Ulrich Zülicke, Universität Karlsruhe
Spintronics with semiconductor quantum wires

12.15 Lunch & informal discussions

17.00 Coffee

17.15 – 18.15 Hans Hennig von Grünberg, Universität Konstanz
Electrostatic interactions in soft-matter systems: membranes and charged colloids

18.15 1st Poster Session
Wednesday, 26th February 2003

8.45 – 9.45 Uri Sivan, Technion Haifa
Molecular Electronics – the Gap between Devices and Circuits plus Some Lessons from Biology

9.45 – 10.45 Christof Niemeyer, Universität Dortmund
Semisynthetic DNA-Protein Conjugates: Synthesis, Characterization and Applications in NanoBiotechnology

10.45 – 11.15 Coffee Break

11.15 – 12.15 Siegfried Engelbrecht, Universität Osnabrück
ATP Synthase – a molecular machine

12.15 Lunch & informal discussions

17.00 Coffee

17.15 – 18.15 Julio Fernandez, Columbia University
Protein mechanics: a new paradigm for understanding protein function

18.15 – 19.15 Marileen Dogterom, AMOLF, Niederlande
Force generation by single microtubules

Thursday, 27th February 2003

8.45 – 9.45 Stephen Quake, CalTech USA
Sequence and Structure in DNA: From Knots to Genomes

9.45 – 10.45 Bianca Hermann, Universität Basel
Self-Assembled and Self-Ordered Monolayers of Large Molecules on Surfaces Investigated with STM

10.45 – 11.15 Coffee Break

11.15 – 12.15 Petra Schwille, TU Dresden
Confocal detection and beyond: On the look-out for single molecules

12.15 Lunch & informal discussions

17.00 Coffee

17.15 – 18.15 Joachim Spatz
Title to be announced

18.15 2nd Poster Session
Friday, 28th February 2003

8.45 – 9.45  Gerhard Schütz, Universität Linz
Ultra-sensitive Microscopy to Image Molecular Processes in Living Cells

9.45 – 10.45  Roland Netz, LMU München
Static and Dynamic Aspects of Charged Surfaces

10.45 – 11.15  Coffee Break

11.15 – 12.15  -

12.15  Lunch & informal discussions

17.00  Coffee

17.15 – 18.15  Silvano de Franceschi, TU Delft
Semiconductor Quantum Dot Structures

18.15 – 19.15  Peter Fromherz, MPI für Biochemie
Interfacing of Ion Channels and Electron Channels
Lectures
Abstracts
This talk will give an overview of some of the work of our group on electrical transport through carbon nanotubes which are long cylindrical all-carbon molecules with unprecedented electrical and mechanical properties. I will in particular describe recent electron-transport and STM results obtained on individual carbon nanotube molecules. Nanotubes can be semiconducting or metallic. The atomic structure and molecular orbitals can be studied by STM spectroscopy in nanotubes of finite length. Electrical transport has been studied through individual nanotube molecules between nanofabricated metal contacts. Nanotubes appear to be excellent coherent conductors. We have realized a variety of single-molecule devices (SETs, FETs, ..) that operate at room temperature. Recently we demonstrated the use of nanotubes as biosensors. Finally I will discuss our efforts to couple nanotubes with DNA ends.
If time allows, I may also mention some of the new single-molecule biophysics efforts of our group.
The formation of secondary structures, their aggregation and structural organization is essential for the biological function of proteins and oligonucleotides. Helices, β-sheets and turns form tertiary structures within the folding process of proteins. DNA double strands are known in different types of helices, whereas RNA-folding is even more complex.

The reversible organization of secondary structures by specific hydrogen bond recognition will be presented. Complementarity between the canonical nucleobases can be used in order to get the desired specificity. The organization of peptide helices as well as peptides with linear backbone will be presented in the α- and β-peptide series.\[1\],\[2\] Double strand and quadruplex formation can be obtained next to higher aggregates.\[3\]

Furthermore, alanyl-PNA represents a model system for a DNA nucleobase stack, that lacks the dynamic behaviour of DNA because of its linear and highly rigid topology.\[4\] Alanyl-PNA is an oligomer based on a regular peptide backbone with alternating configuration of alanyl amino acids and nucleobases covalently attached to the side chains. The base stack is rigid and well defined. It can be varied by side chain homology, different nucleobase pairing modes, and intercalation.\[5\] Interactions with the base stack or nucleobase mediated electron transfer can be studied.

Finally, a concept will be presented about how a conformational switch in proteins can be induced by hydrogen bonding. In oligonucleotide chemistry the transition from B-DNA to Z-DNA might also be facilitated by recognition of small molecules.

Discovering how mechanical force can switch the functions of proteins, and in many cases regulate cell signaling, is of fundamental importance in proteomics and medicine. It will ultimately reveal the molecular basis of many diseases where mechanical forces play critical roles in their onset or progression. Structural insights will be provided illustrating how protein function can be switched by stretching proteins into non-equilibrium states. E. coli, for example, binds more tightly to surfaces under shear-flow made possible by a nanoscale switch located at the outer tip of their long fimbriae. This adhesin switches from low to high affinity if mechanically stretched. Other mechanical switches exist in the multimodular adhesion proteins of mammalian cells. We used fluorescence resonance energy transfer (FRET) to probe the conformational states of the adhesion protein fibronectin in cell culture. Displaying protein unfolding events as visible color changes allowed us to prove that cells can apply sufficient tension to fibronectin to induce partial unfolding of its modules. These biological systems also provide new insights how biology addresses the issue of systems integration using nanocomponents. Finally, we assembled a picoNewton force probe meter from molecular building blocks to study receptor-ligand interactions under physiological loading rates.

**Literature:**


The combination of high resolution microscopy, such as atomic force microscopy (AFM), and laser-based microdissection provides a direct approach for the investigation and isolation of biological specimen. We show a non-contact isolation method, *laser microdissection and pressure catapulting* (LMPC), in combination with AFM for the isolation of cell clusters, single cells and cell compartments. We present results on different biological and medical applications: a) isolation of single chloroplasts for evolutionary studies in botany; b) detection of virus particles in infected tissue sections; c) isolation of *ancient* DNA d) isolation of single metaphase chromosomes and chromosomal parts for studies in molecular cytogenetics. Besides the isolation of fixed material we present the possibility to isolate single living cells to establish homogeneous tissue cultures.

**Literature:**


STRUCTURAL DNA NANOTECHNOLOGY

Nadrian C. Seeman, Department of Chemistry, New York University, New York, NY 10003, USA, ned.seeman@nyu.edu.

DNA nanotechnology uses reciprocal exchange between DNA double helices or hairpins to produce branched DNA motifs, like Holliday junctions, or related structures, such as double crossover (DX), triple crossover (TX), paranemic crossover (PX) and parallelogram motifs. We combine DNA motifs to produce specific structures by using sticky-ended cohesion, or, more recently, forms of paranemic and edge-sharing cohesion. From simple branched junctions, we have constructed DNA stick-polyhedra, such as a cube and a truncated octahedron, several deliberately designed knots, and Borromean rings. We have used two DX molecules to construct a DNA nanomechanical device by linking them with a segment that can be switched between left-handed Z-DNA with right-handed B-DNA. PX DNA has been used to produce a robust sequence-dependent device that changes states by varied hybridization topology; sequence-dependent devices can provide the diversity of structures necessary for nanorobotics.

A central goal of DNA nanotechnology is the self-assembly of periodic matter. We have constructed micron-sized 2-dimensional DNA arrays from DX, TX and parallelogram motifs. We can produce specific designed patterns visible in the AFM from DX and TX molecules. We can change the patterns by changing the components, and by modification after assembly. In addition, we have generated 2D arrays from DNA parallelograms. These arrays contain cavities whose sizes can be tuned by design. In studies complementary to specific periodic self-assembly, we have performed algorithmic constructions, corresponding to XOR operations.
Viral DNA packaging

Single molecule studies of a unique molecular motor

J. Michaelis¹, Y. Chemla¹, Aathavan¹, D. E. Smith², S.J. Tans³, S. Grimes⁴, P. J. Jardine⁴, D.L. Anderson⁴ and C. Bustamante¹

¹Department of Physics, UC Berkeley, Berkeley Ca; ²Department of Physics, UC San Diego, San Diego Ca; ³ FOM-Institute for Atomic and Molecular Physics, Amsterdam; ⁴Department of Microbiology & Oral Science, Univ. of Minnesota, Minneapolis.

The packaging of the viral DNA into a capsid protein shell is an essential part of the viral infection cycle. In the case of bacteriophage Φ29, this active process is facilitated by a complex multi-component molecular machinery, which hydrolysis ATP in order to do the mechanical work required for packaging the 6.6µm long DNA into the 42x54nm small capsid. We have studied the properties of this molecular motor using single molecule force measurements and single molecule fluorescence.

We have developed an in vitro assay to study the motor dynamics using optical tweezers. The DNA is packaged through a portal complex against an increasing internal force, by the use of an extremely strong molecular motor, that can work against loads of up to 57pN. ATP dependent studies of the packaging have allowed us to gain further insight into the nature of the mechano-chemical cycle. Moreover we are also investigating the effect of non-hydrolysable ATP analogs on the dynamics of the motor to formulate a molecular mechanism of packaging.

In order to understand the conformational changes that occur in the protein complex during the packaging process, we have fluorescently labeled a single monomer of the portal domain. A single fluorescent molecule can be used as a reporter for the molecular dynamics during DNA packaging. It is commonly believed that the translocation of the DNA is coupled to a rotation of (at least) part of the packaging complex. Experiments are currently being performed to test this hypothesis using single molecule fluorescence polarization.

Spintronics with semiconductor quantum wires

Ulrich Zülicke
Institut für Theoretische Festkörperphysik, Universität Karlsruhe, Germany

The study of spin–dependent transport phenomena has become a very active field of research recently. It started in the late eighties with the investigation of magnetoresistance effects induced by the manipulation of ferromagnetic electrodes. Called magnetoelectronics, this research effort has provided the basis for commercially viable applications as, e.g., hard–disk read heads, and nonvolatile computer memory.

These days, spin effects in electron transport due to quantum–mechanical phase coherence are intensely studied. In particular, proposals for spin–controlled field–effect transistors have fuelled a lot of spintronics research. Their design is based on the coherent spin precession of electrons in two–dimensional systems. We review its underlying physics, i.e., the Rashba spin–orbit coupling of electrons in asymmetric quantum wells, and discuss novel spin effects arising from the interplay of spin–orbit coupling and quantum confinement in nanowires. As an intriguing example, we show how spin–polarized currents can be created in the absence of magnets and magnetic fields; realizing a Stern–Gerlach experiment simply by turning on a voltage.

How does it work? Consider two parallel quantum wires that are coupled by an extended uniform barrier. (See Fig. 1a.) Translational invariance implies conservation of canonical momentum in a single tunneling event. Together with energy conservation, this results in severe limitations of phase space for tunneling. Only states with wave numbers where the energy dispersion curves of the two wires cross can tunnel. In the typical case depicted in Fig. 2a, no such crossing occurs, and electrons injected into the upper wire by reservoirs 1 and 2 remain there (Fig. 1b).

Tunneling can be enabled by applying a magnetic field or a voltage bias which lead to a relative shift of the dispersion curves in momentum and energy direction, respectively. (See Figs. 2b and c.) This mechanism makes it possible to selectively allow tunneling, e.g., for spin–up right–movers (Fig. 2b) when Rashba spin–orbit coupling is different in the two wires. A spin–polarized current will then flow from reservoir 2 to reservoir 4 (Fig. 1c). Without any magnetic field, simply by applying a voltage, tunneling can be enabled for spin–up right–movers and spin–down left–movers (Fig. 2c). A spin–unpolarized current flowing in the upper wire is then divided into spin–up and spin–down parts which end up in lead 3 and 4, respectively.

Figure 1.

Figure 2.
I present a study investigating the effect of lipid demixing on the electrostatic interaction of two oppositely charged membranes in solution. Contact between oppositely charged surfaces in an electrolyte is possible only if the two surfaces have exactly the same charge density. If this condition is not fulfilled the surfaces can repel each other, even though they are oppositely charged. In our model of a membrane the lipodic charge distribution on the membrane surface is not homogeneous and frozen, but the lipids are allowed to freely move within the plane of the membrane. We show that lipid demixing allows contact between membranes even if there is a certain charge mismatch, and that in certain limiting cases, contact is always possible. We furthermore find that of the two interacting membranes, only one membrane shows a major rearrangement of lipids while the other remains in exactly the same state it has in isolation, and that at zero disjoining pressure the electrostatic mean-field potential between the membranes follows a Gouy-Chapman potential from the more strongly charged membrane up to the point of the other, more weakly charged membrane.

I will then discuss the interaction between charged colloidal particles. Because the interactions among colloids are mediated by microions, the total interaction of a system of more than two colloids is not simply the sum over pair-potentials. In a recent experiment the three-body interaction between charged colloids has been measured directly. The experimental findings are compared to Poisson-Boltzmann calculations and show good agreement. Measured colloid pair-potentials are finally used to calculate the equation of state of a charge-stabilized colloidal suspension and the three-point correlation function between the colloids. We show explicitly that the triplet correlation function obtained from the experimental data satisfies the well-known Born-Green equation.
Practical implementation of molecular electronics depends on the development of concepts and tools for the assembly of an immense number of individual molecules into a functional circuit. DNA molecules and related proteins provide a particularly promising avenue in that direction. In the talk I will review our attempts to construct DNA-templated electronics, to harness the biological machinery of homologous recombination for sequence-specific molecular lithography, and to develop a programmed synthesis of the large variety of DNA molecules needed for the construction of an elaborate substrate. I'll present the insight gained in that process together with the obstacles we have encountered and the standing challenges.

Christof M. Niemeyer *

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The „bottom-up“ biomimetic assembly of programmed molecular building blocks provides a novel strategy for the generation of nanometer scaled functional devices and materials. Due to their evolutionary optimized recognition capabilities, biomolecules, such as DNA and proteins, are currently investigated as building blocks for the self-assembly of nanostructured architecture [1]. As an example, we have developed novel classes of semisynthetic DNA-protein conjugates, self-assembled oligomeric networks consisting of streptavidin (STV) and double-stranded DNA [2], which can be converted to well-defined supramolecular nanocircles [3]. The DNA-STV conjugates are applicable as modular building blocks for the generation of novel immunological reagents for the ultra sensitive trace analysis of proteins and other antigens [2, 4], ion-switchable nanoparticle networks [5], nanometer-scaled "soft materials" calibration standards for scanning probe microscopy [6], and other fields of nanobiotechnology.

Other developments concern covalent conjugates of single-stranded DNA oligomers and STV [7], which can be utilized as biomolecular adapters for the immobilization of biotinylated macromolecules at solid substrates via nucleic acid hybridization. This „DNA-directed immobilization“ proceeds with high immobilization efficiencies and allows for reversible and site-selective functionalization of solid substrates with proteins, metal and semiconductor nanoparticles, and other compounds [8]. In addition, the covalent DNA-STV conjugates are also convenient for constructions at the nanometer-scale. For instance, they have been used for the DNA-directed functionalization of gold nanoparticles with immunoglobulins [9]. The combination of specific antibodies and DNA-stabilized colloidal gold is applicable for the biochip-based detection of antigens. Moreover, the covalent DNA- STV conjugates allow for selective positioning of biotin-derivatized molecular components along a single-stranded nucleic acid carrier molecule. Examples include the spatially controled assembly of enzymes to form artificial multienzyme complexes [10], and the fabrication of functional biometallic nanostructures from gold nanoparticles and antibodies, applicable as tools in bioanalytics [11].

ATP is the universal energy currency of living organisms driving many processes like chemical syntheses, motion, and information processing.

It is synthesized from ADP and phosphate by ATP synthase (aka F$_0$F$_1$-ATPase). This membrane-bound enzyme uses a proton motive (sometimes a sodium motive) force as free energy source for the highly endergonic reaction. The enzyme is found in mitochondria, bacteria, and chloroplasts and invariably consists of two portions, F$_0$ and F$_1$, with well conserved functions. F$_0$ is inserted into the respective coupling membrane and acts as H$^+$- or Na$^+$-channel. It is connected by two stalks with the extrinsic F$_1$ portion which contains the catalytic sites and catalyses substrate turnover. F$_1$ after solubilization catalyses ATP hydrolysis. The enzyme from bacteria like E. coli (EF$_0$EF$_1$) has the simplest subunit structure of ATP synthases, i.e. $ab_2c_{10}$ for F$_0$ and $\alpha_3\beta_3\gamma\delta\varepsilon$ for F$_1$.

It has been shown by cross-linking, spectroscopy, and micro videography of single molecules, that the enzyme functions as a rotary engine with subunits $c_{10}\gamma\varepsilon$ rotating relatively to the remaining subunits $ab_2\alpha_3\beta_3\delta$. The available data suggest that ion flux through F$_0$ causes rotation of the subunit c assembly and thus co-rotation of F$_1$ subunits $\gamma$ and $\varepsilon$, which are tightly connected with $c_{10}$. The $\gamma$ subunit is located in the center of a hexagon consisting of three $\alpha\beta$ pairs, thus forming a central shaft within F$_1$. Rotation of $\gamma$ consecutively opens the catalytic sites located at the interfaces of subunits $\alpha$ and $\beta$ thereby liberating tightly trapped ATP, 3 molecules per full rotation. The entire sequence is reversible, causing pumping of ions upon ATP hydrolysis. ATP synthase thus converts electrochemical into mechanical and then into chemical energy. The initial free energy input is not only used to drive the chemical synthesis reaction of ATP from ADP and phosphate but also to concomitantly release product from another catalytic site and to bind substrates at the third.

Exciting details of the catalytic mechanism have been clarified by sophisticated single molecule analysis leaving but little doubt about the basic rotary mechanism of ATP synthase. The sequence of events following binding of substrate into one catalytic site and ultimately resulting in a 120° rotation of $\gamma\delta\epsilon_{10}$, however, remains unknown. Also the enzyme is far more robust towards protein structural manipulations than one would expect in analogy with a macroscopic machine. In other words, the “screen resolution of the movie” of ATP synthesis still is rather low.

W. Junge, O. Pänke, D. A. Cherepanov, K. Gumbiowski, M. Müller & S. Engelbrecht (engel@uos.de)
Inter-subunit rotation and elastic power transmission in F$_0$F$_1$-ATPase (Minireview)
F E B S L e t t r . 5 0 4 , 1 5 2 – 1 6 0 (2 0 0 1 )

Left, a sketch of EF$_0$EF$_1$ with one $\alpha\beta$ pair removed
Sequence and Structure in DNA: From Knots to Genomes

Stephen Quake

Applied Physics and Physics, Caltech

I will discuss recent results from our group using single molecule methods to investigate sequence and structure of DNA. On the sequence side, we have developed a single molecule assay to study DNA polymerase, and have used that assay to demonstrate the first proof of principle DNA sequencing experiments. On the structure side, we have used optical tweezers to tie DNA molecules into transient knots, and have systematically investigated the dynamics properties of such structures.
Building self-assembled/ordered monolayers is the most elegant but also the most challenging way to bring large molecules onto surfaces for possible applications in coatings, catalysts, photo-active materials, molecular electronics, and more. The investigation of such molecular architectures is important for the basic understanding of both intermolecular and molecule-substrate interactions. Besides potential high-resolution images achieved with scanning tunneling microscopy (STM), self-assembled (SAM) or self-organized monolayers (SOM) also provide large statistics for the study of individual molecules.

The deposition techniques to promote the growth of a SAM or SOM can vary from solution-casting, spin-coating, Langmuir-Blodgett techniques in a wet-chemical environment to electro-spraying in high vacuum or organic molecular beam epitaxy (OMBE) (e.g. through thermal evaporation) in ultra-high vacuum (UHV).

Studying the self-assembly and self-ordering with STM is possible under various conditions: ambient in a dry environment, at the solid-liquid interface (with or without applying an electrochemical potential), or in vacuum. Working at low temperatures can further increase image resolution.

Assembly/ordering strategies can be based on bonding to the substrate (e.g. SH-bonds to noble metal surfaces), hydrogen bonds between molecules, alkane or other chains for stabilization and linking, complexing on the surface, or π-stacking. Depending on the bond-strength, dynamic processes like defect hopping in the 2D molecular ‘lattices’ can be studied.

Most published studies limit themselves to stiff and flat (macro)molecules specially designed for STM/atomic force microscopy (AFM) investigations. However, also remarkable results can be obtained with self-ordering of flexible and non-planar molecules despite the lack of well-controlled conditions (OMBE).
Confocal detection and beyond: On the look-out for single molecules

Petra Schwille
Biophysics/BioTec, TU Dresden

Fluorescence imaging is presently considered to be one of the most powerful tools to elucidate cellular structures and processes, as both sensitivity and time resolution of scanning microscopes and CCD cameras are being constantly improved. However, if molecular dynamics on time scales of ms and below are to be observed, the combination of microscopic with spectroscopic techniques appears to be particularly promising. Steadily “parking” a confocal volume element at an intracellular site of interest, dynamics and interactions of minute quantities down to the single molecule level can be observed in situ with maximum precision. Molecules can be identified and their properties observed as they pass the detection volume due to diffusion or active transport, and interactions between different molecular species can be quantitatively analyzed by dual- or multi-color cross-correlation or coincidence analysis.

On the other hand, the possibility to identify subpopulations with respect to a certain molecular property renders it highly attractive to take advantage of that information and sort the molecules, i.e. isolating particularly “good” or particularly “bad” ones out of a large heterogeneous ensemble. To achieve this in fluid phase, microfluidic elements have been constructed that allow particle sorting with high efficiency (99%) at throughput rates of 1-10/s, which can easily be combined with ultrasensitive confocal detection.
Ultra-sensitive Microscopy to Image Molecular Processes in Living Cells

Manuel Mörtelmaier¹, Nina Kieberger¹, Karel Drbal², Hannes Stockinger², Hans-Günter Knaus³, Bernt Pragl³, Gerhard J. Schütz¹

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A detailed understanding of molecular processes is the basic requirement for the description of cellular function. These processes typically involve the interplay of different proteins, but also of proteins and the lipids in the cell membrane. While large-scale rearrangements of the protein distribution proceed on seconds up to minutes time scales, local changes are much faster. New ultra-sensitive methodologies for imaging single molecules in living cells allow the direct observation of molecular rearrangements on millisecond time scales. Such rearrangements are of particular importance during the stimulation of immune cells. We followed the motion of individual molecules during different phases of T-cell stimulation in the area of the immunological synapse. The mobility of different proteins, as they pass the synapse, reveals information upon the microstructure of such cell-to-cell contact areas. Such studies shed further light on the structural relevance of lipid microdomains for T-cell stimulation.

In a second example, a view onto the distribution and mobility of a voltage gated ion channel, Kv1.3, in human T-lymphocytes will be presented. Single ion-channels, labeled with the high affinity ligand Hongotoxin-Cy5, are observed as individual fluorescent peaks along the cell membrane. For determination of the full 3-dimensional distribution of Kv1.3, consecutive images of different optical cross-sections were recorded from each cell, which allowed positioning each molecule in 3 dimensions, with an accuracy of 50nm in x/y and 150nm in z-direction.

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References
Static and Dynamic Aspects of Charged Surfaces

Roland Netz

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Charged nanomaterials (colloids or polymers) tend to be water-soluble. Biological and technological systems function mainly in aqueous environments, therefore the statistical mechanics of charged systems is of importance.

No true understanding of charged colloidal and polymeric systems can be achieved without first understanding the behavior of simple charged model systems. Accordingly, in the first part I will talk about some recent results on rather basic systems, namely charged planar walls with counter ions only. Since some time it is known that similarly charged surfaces attract each other for sufficiently large surface-charge densities and/or in the presence of multivalent ions, a phenomenon not explicable within standard (Poisson-Boltzmann) approaches. I discuss the so-called strong-coupling theory, which is valid in the limit of large surface charge densities and for multi-valent ions and which yields attraction between similarly charged walls\(^1\), in quantitative agreement with Monte-Carlo simulations.\(^2\)

The electrophoretic mobility of charged particles can be described by the Helmholtz-Smoluchowski equation, which relates the electrostatic potential at the shear plane, the so-called Zeta-Potential, to the osmotic solvent flux. It is shown that deviations between theoretical predictions and experimental results are expected because of specific and unspecific ion adsorption at substrates which show some lateral structure at the nanoscale.\(^3\) The findings can be rationalized in terms of an electrostatic friction between charged surfaces and the first layer of bound counterions. The static concept of the Stern-layer is carried over to the dynamic behavior and used to distinguish the first layer of rather immobile ions from the bulk ions.

A second topic concerns the so-called hydrophobic attraction between hydrophobic walls which cannot be explained with standard dispersion theory. This interaction seems to be dominant in the biological milieu, unless the surfaces are strongly charged. I discuss various possible mechanisms involving water cavitation (or density depression) and non-local dielectric effects.

\(^1\)Roland R. Netz, European Physical Journal E 5, 557 (2001)
\(^3\)Andre G. Moreira and Roland R. Netz, Europhysics Letters 57, 911 (2002)
Semiconductor Quantum Dot Structures

Silvano De Franceschi, TU Delft, The Netherlands

Semiconductor quantum dots consist of a small electronic island connected via tunnel barriers to large electron reservoirs acting as source and drain leads. The repulsive Coulomb interaction between electrons results in a well-defined number of electrons on the island or, under proper conditions, in a one-by-one flow of electrons from source to drain via the island (single-electron tunneling).

Several fabrication techniques have been developed to form semiconductor quantum dots. The most widespread approach (the split-gate technique) takes advantage of a semiconductor heterostructure with a high-mobility two-dimensional electron gas (2DEG) located ~100 nm below the surface of the chip. Metal gate electrodes defined on top of chip by e-beam lithography are used to deplete the 2DEG underneath and isolate a small puddle of electrons. The same gate electrodes can also be used to finely control the tunnel coupling between the quantum dot and the nearby reservoirs, which in this case are formed by extended regions of the same 2DEG.

In the first part of this lecture, I will review some transport experiments done with split-gate quantum dots with an emphasis on the physical phenomena related to the electron spin.

In strongly coupled quantum dots (i.e. tunnel resistances comparable to the quantum resistance), a nonzero local spin on the dot can be effectively screened by the anti-ferromagnetic exchange interaction with the delocalized electrons in the leads. This is a manifestation of the well-known Kondo effect, where the quantum dot itself can be regarded as a single artificial magnetic impurity. The high control over the quantum dot parameters has offered a new grip to this fundamental many-body phenomenon. I intend to provide an introduction to the subject and highlight some of the most important experimental results.

In weakly coupled quantum dots (i.e. tunnel resistances much larger than the quantum resistance) Kondo correlations are suppressed. A local spin-1/2 behaves as a well-defined two-level system that could be exploited as elementary quantum bit in a solid-state quantum computer, as originally proposed by Loss and DiVincenzo in 1998. In principle, any quantum dot with an odd number of confined electrons could provide the desired spin-1/2 ground state. However, having only one electron on the dot is highly preferable for a practical and reliable implementation of a spin quantum bit. I will discuss an approach to few-electron quantum dots with controllable coupling and illustrate the most recent progress in the field of quantum computing with electron spins in quantum dots.

In the second part of the lecture, I shall present an alternative approach to the realization of quantum dot systems which makes use of chemically synthesized semiconductor nanowires. These recently developed nanostructures present a number of attractive properties, such as high aspect ratios (i.e., 10-100 nm diameters and lengths even larger than 100 µm), and a wide range of materials (group IV, III-V, and II-VI semiconductors), with the possibility to form nanowire heterostructures by sequential growth of different semiconductors. Nanowires can be deposited on oxidized Si substrates and contacted individually by e-beam fabricated metal electrodes. In Delft we have used n-doped InP nanowires grown at Philips to make single-wire field-effect devices. Low-temperature transport measurements have revealed single-electron tunneling and energy quantization resulting from the confinement in the wire. These results represent an important premise for a deeper study of quantum phenomena and the development of controllable quantum devices based on semiconductor nanowires.
The fundamental electrical elements of brain and computer are ion channels in cell membranes and electron channels in semiconductor microstructures. First, the problem of direct electrical interaction of these two devices is discussed. Two experiments are described that elucidate the structure and electrical nature of membrane-semiconductor contacts using optical methods. Then two other crucial experiments demonstrate the gating of transistors by ion channels and the gating of ion channels from silicon chip. Finally, the implications with respect to a direct electrical communication of nerve cells, neuronal networks and brains with semiconductor chips is considered.

Posters
Abstracts

1st Session
Tuesday, February 25th
Molecular Addressing of Single Chromophores using lyotropic Lipid/DNA Networks

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Our research aims to address single perylene molecules using specific DNA hybridization. The general problem is to overcome the incompatibility of solvents, since perylene is strongly hydrophobic, while nucleic acid chemistry is water based. To this end we investigate complexes of DNA and cationic lipids solubilized in oil phases. We show that DNA/Lipid/water emulsions form inverse tubular structures in alcanes and impedance spectroscopy measurements clarify that these microemulsions can alter the insulator behaviour between electrodes. We can prove hybridization between novel synthesized Perylene-di-Oligonucleotides (PON) and the complementary oligonucleotide sequences. Moreover, FCS measurements show evidence for the amphiphilic behaviour of PON.

We aim to use PONs and DNA in fluorescence spectroscopy as a single chromophore system which can be easily addressed and modified by external potentials. In addition, Perylene-tri-Oligonucleotides will help in the future to establish three dimensional lyotropic Lipid/DNA Networks. The question arises, if it is possible to gate ion transfer within theses networks.

Cell-adhesion on micro-structured surfaces

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Cell to cell adhesion has been scrutinized by many techniques in the past decades. Cell adhesion often is an interplay of many molecular processes and signal cascades. Even with new methods the complex adhesion processes remain difficult to analyze. With force spectroscopy we directly measure the influence of certain parameters on the de-adhesion force when lifting adherent cells.

The de-adhesion forces of various cells and cells in various states were measured. The calcium dependency of certain adhesion molecules was measured by adding EDTA to the solution.

The increase of the de-adhesion force with contact time was investigated by varying the force and the time of a contact. First results on the influence of surface functionalization and of the micro patterning of a surface on cell adhesion were obtained with this technology. The force spectroscopy turns out to be a powerful technique to directly measure the de-adhesion forces down to the single molecular level. Moreover the influence of drugs and other environmental entities can be investigated in a direct way with respect to the cell adhesion force.
Complexes formed between charged polymers and oppositely-charged spheres are a common pattern in chemistry, physics and biology. Such complexes are formed between DNA, a rather stiff, strongly negatively charged biopolymer, and histones, which are basic globular proteins in the cell. DNA-histone complexation serves to package DNA in a very effective way, making it possible to store the roughly 1 m of human DNA in a cell nucleus of a few micrometers in diameter. We investigate complexation behavior and interaction between two complexes of semiflexible charged polymers with oppositely-charged spheres with parameters appropriate for the DNA-histone system. We determine the ground state of the system numerically by minimizing a free energy expression, which includes electrostatic effects on a linear level. We obtain the effective interaction potential between two complexes for various system parameters, namely, the charge valency of the spheres and the salt concentration. It is shown that within this model, complexes can attract each other at short distances depending on the system parameters, which arises from an interplay between electrostatics, bending rigidity and conformational behavior of the charged polymers. We examine different phases of the system based on symmetry arguments, which include bridging and mosaic-binding phases. Finally, we present the behavior of the second virial coefficient of this system in terms of the salt concentration, which exhibits a non-monotonic behavior with a minimum at moderate salt concentrations.

Onset of DNA Aggregation in Presence of Multivalent Counterions

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We address theoretically aggregation of DNA segments by multivalent counterions such as the polyamines spermine and spermidine. In experiments, aggregation occurs above a certain threshold concentration of multivalent counterions. We show that the dependence of this threshold on DNA concentration is of a simple form and is related to the counterion density profiles around each DNA chain.

Our analysis agrees very well with recent detailed measurements on DNA aggregation in the presence of spermine [1]. From the fit to the experimental data several conclusions are reached on the number of condensed spermine counterions surrounding the chains, at the onset of aggregation.

G-quadruplex DNA are cyclic arrays of four hydrogen bond guanine bases in which each base acts as both donor and acceptor of two hydrogen bonds with other guanines, and the pairing between bases is Hoogsteen type.

Self-assembled DNA networks have been shown for G-rich oligonucleotides, where then exhibit semiconductor characteristics. First experiments were carried out to study the self-assembled formation dependence with DNA concentration and ions concentration.

Furthermore, we used AFM images to elucidate specific G-rich oligonucleotide structures under physiological conditions in order to use it as a potential target for therapeutic treatments. Once, these structures have been investigated due to their possible relevance to the recombinational events at the immunological effects.

Self-Assembly Behavior of DNA Polyplexes

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The influence of various polycations on the complexation behavior in DNA polyplexes was investigated. Synchrotron small-angle x-ray scattering (SAXS) experiments were used to determine the internal structure in the polyplexes and their dependence on various parameters.

Similar hexagonal packing of DNA was observed for all systems with wide variation in the internal spacing and degree of ordering dependent on the specific polycation used. An electrostatically induced continuous phase transition was observed in the polyplexes with increasing added monovalent salt concentration. Salt-induced phase transitions were highly dependent on the polycation and scale with the approximate binding energies of the polycation to the DNA. At high salt, dissolution occurs where all the electrostatic interactions between the polypeptide and the DNA are screened by the added salt. Influence of mixing ratios and pH were investigated. In addition, compressibility was investigated in these systems by osmotic stress measurements as a function of salt.
Single Virus Tracing:
Observation of A Single Dye Labeled Virus on its Infectious Entry Pathway into a Living Cell

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Single molecule experiments were conducted to follow the migration of single adeno-associated viruses on their infectious entry pathway into living HeLa cells. Only one fluorescent dye molecule (Cy5) was attached to the viruses in order not to influence their physiological behavior. For the first time we could obtain diffusion trajectories of single viruses in four different stages of the infection:
1.) Diffusion of the virus in front of the cell membrane and receptor mediated adsorption
2.) Formation of the endosome with inclusion of the virus
3.) Transport of the endosome in the cell plasma and release of the virus
4.) Penetration and diffusion of the virus in the cell nucleus
From the trajectories of the individual viruses diffusion coefficients were obtained for all four stages. A detailed picture of the processes involved was modeled. Adeno-associated viruses show promising prospects for the use in human gene therapy. For this purpose a detailed understanding of the interactions of the virus and the target cell is important. The actual investigations of our single virus tracing method are experiments with synthetic viruses (used in gentherapy) and various important virus systems like Baculo Virus and at least HIV.

Adsorption of charged objects on charged membranes

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In the first part we consider a stiff membrane consisting of mobile surface groups whose state of charge depends on the pH and on the ionic composition of the adjacent electrolyte solution. To calculate the mean-filed interaction between a charged object and such a model membrane, one needs to solve a Poisson-Boltzmann boundary value problem. We derive and discuss the boundary condition at the membrane surface, a condition that is generally appropriate for biological membranes where two charge-regulation mechanisms are present at the same time: the pH dependent chemical charge regulation and a regulation through the in-plane mobility of the surface groups. As an application of this general formalism, we consider the specific example of a single DNA molecule, approximated by a cylinder with smeared-out surface charges, interacting with such a model membrane. We find that, at short DNA-membrane distances, membrane fluidity can have a considerable impact on the DNA adsorption. In the second part we address the question of how a charged colloid adsorbs on a now flexible fluid membrane by investigating the system on a scaling level. In the low salt regime the wrapping of the spherical colloid by the membrane is dominated by the membrane-membrane repulsion, whereas in the high salt regime it is mainly the mechanical bending rigidity of the membrane which controls the wrapping state of the system. For homogenous charged membranes these results agree well with our numerically found results for the ground state of the membrane/colloid system. For mobile charges in the membrane we find a different scaling behavior due to the enhancement of the electrostatic attraction between the colloid and the membrane in such a way that the membrane now more easily wraps the colloid, as one would expect regarding the results presented in the first part.
Desorption of Single Polyelectrolyte Chains from Various Substrates Studied by Single Molecule Force Spectroscopy

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AFM based force spectroscopy allows the measurement of intra- and intermolecular forces of single molecules. It has recently been used to investigate the desorption of polyvinylamine, a positively charged polyelectrolyte with Amine groups from silica, mica, gold and calcite surfaces. Our work is focused on the investigation of negatively charged polyelectrolytes, namely Polyacrylic Acid, which carries COOH-groups and DNA.

As Polyelectrolytes normally show weak interactions with high exchange rates with the surfaces our measurements take place in thermal equilibrium and we observe desorption plateaus in the force spectra. For various substrates we identified salt dependent electrostatic contributions as well as salt-independent constant contributions for the desorption force.

Another aspect is to investigate transitions from non-equilibrium to equilibrium (i.e. the transition from ruptures to desorption plateaus in the force spectra) which can be observed for certain substrates. Beside this it is a major goal to model the affinity of Polyelectrolytes to metal surfaces by applying an external potential.

A self-assembled template structure of small organic molecules

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The adsorption of Trimesic Acid (TMA) to various single crystal surfaces has been studied under Ultra High Vacuum and ambient conditions. The self-assembled structure is characterized by periodic non-dense-packing of the molecules. Depending on the preparation method, two different network structures could be realized. In both cases, induced by directed hydrogen bonding, the organic molecules built a two-dimensional grid architecture with molecular caves - both able to store guest molecules at specified adsorption sites.

As a first step TMA molecules (Fig. 3) themselves were inserted as guest molecules into the host structure. The guest molecules could be identified in two different vertical and 6 different horizontal adsorption sites. In the horizontal case STM induced switching of a single guest molecule to six stable positions was observed. The states of this molecular switch have a distance of 0.15 nm. The calculated energy barriers indicating that the switch is stable at room temperature are consistent with the experiment.

Furthermore Bucky-Balls (Fig. 2) could be inserted in the hollow sites. With a diameter of about 0.7nm C60 fits easily in the pores of the TMA structure which have a diameter of about 1.5 nm. By means of STM the Bucky-Balls could be imaged within the template structure and be directly kicked from one cell to another. As a third example coronene molecules (Fig. 3) were co-crystallized after the host structure was formed. With a diameter of about 1nm coronene molecules also fit quite well into the open pores. Closed layers of TMA with every cavity filled by a coronene molecule could be imaged. Even the submolecular structure of coronene can be seen in the STM images. By applying voltage pulses the guests molecules could controllable be kicked out of the cavities.

Fig. 1 Trimesic Acid - C₉H₆O₆  Fig. 2 Buckminsterfullerene - C₆₀  Fig. 3 Coronene - C₂₄H₁₂
Simultaneous force and optical spectroscopy of single molecules

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In the drive towards single molecule manipulation, combined techniques such as atomic force spectroscopy and optical fluorescence microscopy can achieve more than the sum of their parts.

Atomic force spectroscopy offers the unique possibility to study the mechanics of polymer molecules on a single molecule level, thus circumventing ensemble-averaging effects. The usefulness of this method has been demonstrated on numerous occasions, e.g. biologically significant molecules such as DNA and sugars or on organic polymers.

On the other hand, optical detection provides an intriguing method to resolve the position of said molecules and gain information on their fluorescent properties.

By examining the fluorescence of intercalating dyes and simultaneously stretching single DNA strands with an AFM tip, new insights into the interaction mechanics of these dyes with DNA can be gained. Furthermore, the real-time observation and manipulation of relevant single polymers, such as DNA promises to open up a whole host of new possibilities, with far reaching consequences in the nano-technology field.

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Single Molecule Microscopy and Spectroscopy in Nanostructured Molecular Sieves

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Nanometre-scale sized structures and defects in the bulk volume of molecular sieves can be characterized using confocal fluorescence microscopy. In addition to that, the capability to detect and characterize single molecules in the volume of molecular sieves allows us to gain access to a more detailed characterization of nanostructured porous materials. Investigations of static and dynamic systems exemplify the high potential of single molecule microscopy in material science.

First the incorporation of three differently sized dye molecules (Oxazine 1, 170 and 750) in an AlPO₄-5 (AFI) crystal was used to investigate the formation of nanostructures built within molecular sieves. In all three cases the molecular dimensions are bigger than the diameter of the pores. The smallest of these molecules aligns to the pore orientation and causes only minor defects, whereas the bulkier dyes generate distorted structures but do not affect the macroscopic habitus of the crystal.

In a second experiment we studied the diffusion of single terylene diimide (TDI) molecules incorporated into the channels of a mm-sized M41S-body. This molecular sieve was synthesized using a micellar liquid crystalline template. Our results reveal a high tortuosity of the channels. X-Ray diffraction measurements will only point out the existence of a hexagonal order of the pores in an otherwise amorphous solid body.

Literature:
**Optimizing dynamical Parameters for the AFM-Microdissection**

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In the presented work, the dependency of the AFM Microdissection on dynamical Parameters were investigated. It could be shown, that the dissection result is essentially dependent on the cutting speed, but is nearly untouched by the modulation amplitude.

These results have been compared with different visco-plastic models. Additionally, the effect of different tip surfaces on the amount of extracted material was investigated.

Here the results indicate, that even minor changes of the tip surface can increase the amount of extracted material essentially.

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**Manipulation of Single DNA Molecules on Nanostructured Surfaces**

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Cationic lipid layers prepared on hydrophobic or hydrophilic surfaces force DNA molecules to adsorb onto the lipid plane. On fluid lipid layers the DNA is horizontally mobile allowing the use of fluorescence microscopy to study the dynamic behavior of single DNA molecules. Therefore, the DNA molecules serve as model 2D-polymers and their behavior has been compared to different polymer theories.

Moreover, this system can be expanded to a molecular workbench for the manipulation of single molecules if one finds the means to relocate, position, stretch, separate, and analyse DNA molecules. The first goal, relocation, can be achieved with electric fields. The movement of single DNA molecules under the influence of electric fields has been studied previously.

Currently, nanostructured surfaces are used to force long DNA threads (50 000 bps) to keep one direction and form a straight linear molecule. A theory has been developed to explain this phenomenon. In the future, this will allow to study the dynamic interaction of DNA molecules with single proteins by fluorescence microscopy.
Light action on mechanical oscillators

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Since the introduction of atomic force microscopy this technique was refined such that it is possible to detect single electrons or to pull on single molecules. Nonetheless it is still desirable to make force detection even more sensitive for a broad range of applications. This can be achieved for example by fabricating very soft AFM cantilevers. However, these cantilevers become difficult to handle.

We succeeded in optically tuning the mechanical rigidity of cantilevers with initial spring constants of about 0.008 N/m. Using the gold coated back side of the cantilever as one mirror of a Fabry-Pérot microcavity it is possible to change its spring constant in a controlled way due to photon pressure or other light-induced force gradients.

Here we present measurements of the shift in the resonance frequency of the lever in proportion with its spring constant. The behaviour can be understood and modelled by the action of the photon gas in the system. This effect allows us to tune the mechanics of nano-opto-mechanical systems and can be used in order to detect ultra-low forces.

Low-dimensional electron systems in suspended nanostructures

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Freely suspended nanostructures with a thickness in the range of 100 nm enable to build ‘phonon cavities’ with a discrete spectrum of lattice vibrations in the constricted dimension. A two-dimensional electron gas (2DEG) can be embedded within the free-standing membrane [1] allowing to investigate the modified electron-phonon interaction and its effects on dissipation and dephasing processes. Starting from a GaAs/AlGaAs heterostructure containing a 2DEG as well as an additional sacrificial layer we fabricated freely suspended beams sustaining a fully gate-tunable electron system. To this end, two subsequent steps of electron beam lithography are used followed by a combination of dry and wet etching techniques in which the sacrificial layer is removed beneath the nanostructures [2]. Applying negative voltages to the gate electrodes the electron system of the resulting devices can be successively depleted. In particular, the dimension of the electron system can be reduced from to form quantum point contacts and quantum dots [3]. Hence, continuous operation is permitted from 2D to 0D allowing detailed analysis of the electron-phonon interaction in phonon cavities.

Desorption of Single Polyelectrolyte Chains from Solid Supports
Studied by Single Molecule Force Spectroscopy

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AFM-based force spectroscopy measures intra- and intermolecular forces of single molecules. It recently was used to investigate the desorption of single polymer chains from silica, mica and calcite surfaces. In first experiments the desorption of polyelectrolytes from an equilibrated physisorbed polymer film on silica was investigated. These experiments show that the Coulomb interaction between polymer and substrate depends linearly on the Debye screening length and the polymer's line charge density. Besides, a non-Coulombic contribution was found, which did not depend on any charges in the system [1]. Further experiments were performed with tip-attached polymers on bare mica and calcite surfaces. They confirm the findings on silica and show that the non-Coulombic interactions (van der Waals, H-bonds, coordinative interactions, etc.) can by far outweigh the Coulombic interaction. A better understanding of these non-Coulombic interactions opens new ways to control surface adhesion. In addition, with the tip-attached polymers a study of the desorption dynamics of single polymer chains seems possible.


Coupling electronic and nuclear spins

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Spin states in semiconductor quantum dots are known to have many properties desired for qubit base states. As long as more than one electron is present, however, the quantum dot level spectra can be quite complex. An example of such a situation is given by spin blockade - where internal spin coupling changes between subsequent electron numbers, leading to a large spin difference and a suppression of SET tunneling. As can be seen, perturbation terms of the Hamiltonian such as spin-orbit coupling and hyperfine coupling play an important role in explaining the data.

The spin blockade scenario enables control of the electronic spin and its transitions by the externally applied gate voltage. In addition, the perturbation terms couple the electronic spin system selectively to the nuclear lattice spins, as predicted and observed in similar situations.

We present data on the detection of a nuclear spin polarization via the single electron tunneling through a quantum dot. Since we are working at extremely low external magnetic fields orthogonal to the 2DES (B⊥< 0.25T ) and since the phenomenon does not depend on a magnetic field parallel to the 2DES (tested for 0T < B∥ < 1T), orbital momentum states seem to play an important role.
Investigating transport processes with fluorescence correlation spectroscopy

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Fluorescence Correlation Spectroscopy (FCS) was used to probe the dynamics of λ-phage DNA in aqueous solution labeled with the randomly intercalating dye TOTO. The linear macromolecules (i) carry more than one chromophore and (ii) are larger than the waist of the focal volume. The correlation function decays significantly faster than expected for a stiff globule of corresponding size, but is in good agreement with the dynamic model of semiflexible chains including hydrodynamic interactions. As the chromophore density is lowered the correlation time decreases in accordance with this model.

In addition first measurements of short rod-like molecules in a DNA network are presented.

Unfolding Bacteriorhodopsin Helix By Helix

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We used atomic force microscopy to extract individual molecules of Bacteriorhodopsin (BR) out of purple membrane (PM) patches in native environment. In high resolution images that were taken before and after the extraction of BR, we clearly can see that individual proteins were extracted. The force versus distance curves recorded during the extraction of the proteins provide detailed insight into the possible unfolding pathways of Bacteriorhodopsin. At the unfolding of Helix D and E and the adjacent loops, we can distinguish between four different pathways. The same phenomena occur with the unfolding of Helix B and C respectively. The probability of these different pathways is not dependent on pH in the range from pH 4.2 to pH 10.0.

Here we present a theory to explain our experimental results, that seems to fit quite well to current structural models of BR.
Posters
Abstracts

2nd Session
Thursday, February 27th
Investigation of GaAs / organosilicate interfaces with grazing incidence X-ray reflectivity

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GaAs is a semiconductor with interesting electrical and optical attributes. The chemical modification of its surface provides many opportunities for tuning the electronic properties of the underlying bulk semiconductor material and for creating surfaces with tailored physicochemical properties, e.g., for the design of chemical or biophysical sensors. (Seker, Meeker et al. 2000).

The present work focuses on the structural analysis of covalently bound organosilicate layers for the long term functionalization of the GaAs surface in physiological environments (Kirchner, George et al. 2002). The nature of the GaAs-organosilicate interface is discussed by means of results from X-ray reflectivity measurements under grazing incidence (GIXRR). Surface chemistry on GaAs usually requires an etching step prior to functionalization (Seker, Meeker et al. 2000), to remove the native oxide of GaAs. For the case of 3-mercaptopropyltrimethoxysilane we show the redundance of the etching step, which leads to layers with a simplified deposition procedure and higher quality.

The Organic Microcavity Photodiode: Tailoring Linear and Nonlinear Photocurrent Response

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Polymeric photodiodes show many features that may lead to cheap and versatile optoelectronic devices. We use a bilayer structure comprised of a conjugated polymer and a C60-type electron accepting layer sandwiched between electrodes that also act as semitransparent mirrors, forming a resonant microcavity structure. By changing the organic bilayer thickness, we can control the spectral position of the cavity resonances. Photocurrent spectra of these devices show a sharp peak at the cavity resonance even in a spectral region of very low absorption of the polymer. We attribute this additional photocurrent to the field enhancement at the cavity resonance. This leads to an increased probability of photon absorption through secondary mechanisms like Anti-Stokes-excitation, absorption at defect sites or the weak normal absorption in the C60 layer.

The narrow response and the enhancement of the photocurrent in the microcavity may engender future applications in the field of cheap and simple spectrometer or colour-detector devices and provide improved spectral characteristics of organic solar cells. Additionally, we observe an enhancement of two-photon induced photocurrent by a factor of over 500 in organic microcavity photodiodes. This is also due to the high field enhancement leading to a higher probability for the low cross-section process of nonlinear photon absorption. We demonstrate a simple autocorrelation setup without any need for additional nonlinear optical elements.
Conjugation of Biomolecules to Silanized Colloidal Semiconductor Nanocrystalline Quantum Dots

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Water soluble, highly fluorescent semiconductor nanocrystals are synthesized, coated with a glass shell, and functionalized with biological molecules. The functionalization is achieved by incorporating thiol, amino, or carboxyl groups to the outermost shell of the crystals.

In a next step biological macromolecules are coupled to this shell via commercially available crosslinkers. When fed to cells one can observe the regions to which the biomolecules are transported by the bright fluorescence of the attached nanocrystals. This offers great possibilities for observing dynamic transport processes in living cells. Due to the small bandwidth of the nanocrystals’ fluorescence line the location of several biomolecules inside the cell can be observed in parallel.

Affinity measurements between Aptamers and Proteins with an AFM

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Aptamers are small, in vitro selectable single stranded nucleic acids that are capable of binding proteins and other molecules with specificity and dissociation constants similar or better to those of antibodies. Aptamers can be used for protein biosensors or for drug development. The aptamer D17.4ext (ext is the abbreviation for an extended D17.4) has a length of 45 nucleic acids and forms a stem-loop structure, which has a dissociation constant (Kd) of about 3.6nM for human-IgE(1). We are using an AFM to specify the unbinding force. The force sensor (cantilever) is functionalised with the aptamer via a polymerspacer, to avoid unspecific bindings. The human-IgE is immobilised in the same way to the substrate. Measurements show that the peak of the unbinding force distribution is at 70pN for a loading rate of 1000pN/sec. Because of multiple binding of the polymerspacer to the cantilever, different spacer lengths are obtained. A new method, which fits the summation, weighted by the length distribution of the spacer, of the force probability function of a discrete spacer to the experiments, was used to determine dissociation rates and potential widths.

We found that most of the distributions for the rupture forces and their loading rates can be fitted consistently by the developed fit functions and deliver values for the dissociation rates between 0.21 s⁻¹ and 0.23 s⁻¹ and for the potential widths between 3.2 Å and 3.4 Å. Due to this potential width and to the high force, we assume that the 3-dimensional structure of the Aptamer seems to stay preserved.
Bacteria are the simplest free-living organisms. This quality makes them excellent models to study the physical mechanism and control of molecular motors in vivo. We studied the mechanism of force generation in a bacterium that glides over inert surfaces as well as on human host cells using polymer fibers called pili. Force production by type IV pilus retraction is critical for infectivity of *Neisseria gonorrhoeae* and DNA transfer. A strain was used that naturally lacked pili but upon induction expressed a variable number of pili. We investigated the roles of pilus number and the retraction motor, PilT, in force generation in vivo at the single molecule level and found that individual retraction events are generated by a single pilus fiber and only one PilT complex appears to power retraction. Retraction velocity is constant at low forces but decreases at forces greater than 40pN, giving a remarkably high average stall force of 110±30pN. Novel insight into the molecular mechanism of force generation is gained from the effect of ATP-depletion, which reduces the rate of retraction but not the stall force. Energetic considerations suggest that more than one ATP is involved in the removal of a single pilin subunit from a pilus. The results are most consistent with a model in which the AAA ATPase PilT forms an oligomer that disassembles the pilus by a cooperative conformational change.

Polymers and weakly charged polyelectrolytes at interfaces

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A new theoretical approach is used to study neutral and weakly charged polymers at interfaces. It emphasises the role played by the loops and tails formed by the adsorbed polymers in the internal structure and the free energy of the adsorbed layer.

The issue of the dependence of the surface tension of neutral polymer liquids on molecular weight is then revisited: the role tensio-activity of chain-ends and the entropy associated to the loop size distribution are enlightened. This theory yields also an explanation for the inversion charge process in the case of polyelectrolyte adsorption from semi-dilute solutions.
Nanotweezers: Investigation of their deflection

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Nowadays there is growing interest in nano-electromechanical systems and their applications. The nanotweezers structures presented here are designated to rearrange nanometer-sized objects, which are in general difficult to position. Our structures are based on SOI-material. Electron beam deposited tips are grown on top of lithographically defined silicon prongs.

In order to detect the deflection, it seems evident to study the structure with an electron microscope while applying a bending bias across the prongs. However, the movement as a reaction to an applied DC voltage of the nanotweezers presented here, could not be visualized by electron microscopy. Firstly the cantilevers get contaminated by carbon and moreover, a probing electron beam strongly interferes with the electric fields applied, leading to a modified actuation and a perturbed image. Additionally, calculations suggest that the resolution of a SEM may be not good enough to resolve the expected deflections for some special cases investigated.

Here we introduce a new optical technique allowing to detect 200 nm wide tweezers as well as their deflection according to an applied low frequency AC voltage using a laser of 633 nm wavelength (HeNe) and lock-in technique. The measurement takes advantage of scattering phenomena and should work for most nanomechanic applications.

Effective interactions between DNA molecules

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Electrostatic interactions play a prominent role in many soft-matter and biological systems as a huge class of macromolecules are water-soluble and thus bear electric charges in aqueous solutions. An important example is the interactions between DNA molecules, which are rather stiff, strongly negatively charged biopolymers. In recent years, there has been a considerable evidence from both experiments and numerical simulations, which shows that similarly-charged macromolecules can attract each other via effective forces of electrostatic origin. Such attraction are believed to be responsible in formation of dense packages of DNA molecules (DNA condensates). Here we present results of the strong-coupling (SC) calculation for effective interaction between two similarly-charged rods at large electrostatic couplings, that is when the strength of electrostatic interactions is much larger than thermal fluctuations (the usual case in DNA solutions). The SC theory predicts a long-ranged attraction between the rods when single-rod charge parameter (the so-called Manning parameter) is larger than 2/3. In this regime, rods can also form a stable bound state. However, by approaching the threshold Manning parameter from larger values, the bound state becomes unstable and a continuous binding transition occurs, which may be characterized by a scaling exponent. The results are in reasonable agreement with previous numerical simulations. We also present the results obtained for SC interaction between two charged plates and two charged spheres and compare them with results from numerical simulations on charged membranes and charged colloidal particles.
Single molecule motor driven by light

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The coupling of optical excitation into the mechanical AFM experiment seems to be a most attractive approach for exploring energy transductions at the single molecule level.

The reversible, optical switching of individual polymer molecules was observed using molecular force spectroscopy in combination with optical excitation in total internal reflection. We synthesized a polypeptide with multiple photoactive azobenzene groups incorporated in the backbone. The cis to trans configurational transition induced by ultraviolet light resulted in a measurable change in polymer contour length. The ability to shorten the polymer against an external load is the first demonstration of photomechanical energy conversion in an individual molecule.

In an extension of this concept, chemomechanical systems could also be driven indirectly by the use of photoactivated compounds. For instance, metal ions may modulate the elasticity of a polyamine chain via coordinative interactions. Photoswitchable receptor molecules based on azobenzene were synthesized for the reversible optical release and uptake of metal ions. Their potential use as "chemical fuel" in the indirect optical operation of chemomechanical molecular machines will be discussed.

A Novel 2d Invasion Assay Using Quantum Dot Layers

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The ability of tumor cells to invade surrounding tissues and to metastasize to different sites is related with the cell motility. To test invasive capacities of different cancer cell lines we have developed a two dimensional invasion assay using water-soluble CdSe/ZnS nanocrystals. Semiconductor nanocrystals are inorganic fluorophores: excitation with UV light stimulates fluorescence in the visible range, whereby the color of fluorescence can be controlled by the size of the particles.

It has been shown that cells are able to engulf nanocrystals in non-specific way. When cancer cells are seeded on top of a homogenous nanocrystals layer and are incubated at 37 °C for 24 h, they create trails free of nanocrystals that are no longer fluorescent. The size and the shape of these trails are related with the potential of invasiveness of the cells. We have compared the behavior of six different human cancer cell lines including colon, breast, lung and bone cancer cells. With this test it is possible to discriminate between non-invasive and invasive cancer cell lines.
Combining photonic and mechanical nanomanipulation for the collection of minimal amounts of biological material

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In order to manipulate material at the nanometer scale new methods and devices have to be developed. A nanomanipulator interface was designed and implemented into a commercial atomic force microscope (AFM) system. With the aid of a positioning joystick, direct positioning of the AFM probe with nanometer precision is possible. A commercial force feedback joystick serves as haptic interface and provides the user with real-time feeling of the tip-sample interactions. Due to the open design the manipulator interface can be used with other microscopes of the SPM family using tip-sample interactions like tunnelling current, interatomic magnetic force or electrostatic forces as haptic feedback signal. In addition, the nanomanipulator and a 337 nm nitrogen UV-laser microbeam for photoablation were combined on an inverted optical microscope.

To test the nanomanipulator, human metaphase chromosomes were dissected using both techniques, photoablation and mechanical AFM manipulation. The experimental results show that by combining both methods biological material can be manipulated on different size scales in one integrated instrument. The effects of manipulation on human metaphase chromosome were studied in detail by atomic force microscopy. Sub-400-nm cuts were achieved by photonic ablation. Chromosomal fragments of a size less than of 500 nm could be isolated. By means of mechanical microdissection different cut size ranging from 80 nm to 500 nm can be easily obtained applying different load forces.

Nanomechanical Electron Transport

D V Scheible et al
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We manufactured electromechanical single electron shuttles featuring a mechanically oscillating single electron island. The shuttle consists of a nano-machined cantilever with a metallic island on the tip. The island vibrates between source and drain contact, establishing electron transport at radio-frequency. The enhanced precision of this concept, due to the absence of co-tunneling effects, promises many applications of the device, ranging from metrology to quantum limited displacement detection. We discuss the mechanism behind nano-mechanical charge transport and give an introduction to theoretical considerations. Actual samples and their recent experimental results are presented as well.
Optical properties of charged excitons in quantum dots

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A semiconductor quantum dot (QD) represents an ideal system for the investigation of quantum mechanical electron-electron interactions. This is because Coulomb blockade allows electrons to be added one by one simply by a field effect [1,2]. As a result, the electrical [2], optical [3] - [6] and magnetic properties [7] are charge-tunable. An exciton complex consists of a hole bound to the electrons in the QD. The spatial extent of the excitonic wave function reflects the joint effects of the QD’s confinement potential and Coulomb interactions. This can be probed applying a magnetic field, B. For neutral excitons, the energy increases quadratic in B (diamagnetic shift) with a curvature proportional to the area of the wave function. The linear dependence on the magnetic field leads to the characteristic Zeeman splitting of the spin degenerated branches. However, the behaviour of charged excitons is less well known and potentially much more interesting because of many-particle interactions.

Here we present the experimental results of low temperature (4.2 K) magneto-luminescence measurements performed on charge-tunable InGaAs self-assembled QDs. The photoluminescence generated by recombining single, twofold and threefold charged excitons in a single QD within a magnetic field ramped up to 9 Tesla is investigated. While the diamagnetic shift is found to be dependent on the charging state, the Zeeman splitting is measured to be charge-independent. For excitons involving more than one excess charge, the singlet and triplet state lead to a fine structure splitting. We report here on their magnetic field dispersion. In particular, we highlight the suppression of the diamagnetic shift in the dispersion of the singlet line of doubly charged excitons within the full range of 0 - 9 Tesla as well as the collapse of Hund’s rule for the threefold charged excitons at higher magnetic field. Above a critical magnetic field the fine structure splitting can no longer be observed which is a consequence of the Hund’s rule, and a new broad line appears in the spectrum. The intensity of this line oscillates with a period in 1/B, revealing well-defined anticrossings with the Landau levels.

Force Induced Unfolding Intermediate in an Ig Domain

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The Dictyostelium discoideum gelation factor (Ddfilamin, ABP 120) is an actin-crosslinking protein that, in addition to an N-terminal actin-binding domain, has a rod domain constructed from six tandem repeats of a 100-residue motif that has an Ig fold [1]. We have used Force Microscopy to study the mechanical properties of this molecule. The force extension curves exhibit the well known periodic sawtooth pattern due to the unfolding of the Ig domains [2]. However consistently one domain shows during the extension an additional unfolding peak which can be explained by the existence of an “unfolding intermediate”. Although the hydrophobic stabilisation of the intermediate due to its size of 50 amino acids is expected to be very low, it can resist force over 50 pN over a timescale of several 100 ms. We have expressed engineered constructs in order to investigate the structure of this intermediate. From experiments with Ddfilamin/Titin constructs we were able to study the unfolding of individual Ddfilamin domains separately and identify domain 4 as the domain unfolding via an intermediate. We investigated mutant proteins containing five extra glycine residues added into different loops of the folded core of domain 4 and could identify the structure of the intermediate.


Material specific mapping of nanosystems using visible and infrared s-SNOM

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Scattering-type scanning near-field optical microscopy (s-SNOM) uses the optical near-field interaction between an illuminated metal probe tip and the sample surface[1-5]. Its spatial resolution is not limited by diffraction but rather by the actual size of the scattering probe tip (< 20 nm). We describe the principles of contrast formation and compare the material specific measurements with two s-SNOMs, one operating at 633 nm wavelength, the other at 10 µm.

In both microscopes we show examples of material-specific imaging of the same nanostructured, three-component sample consisting of Au, Si and PS. The achieved resolution is similar at both widely different wavelengths, reaching down to 10 nm. The observed brightness agrees with an electrostatic model which predicts a simple analytical relation to the local refractive index of the sample material [3].

Our results are evidence that the imaging process of s-SNOM is wavelength-independent, namely that the resolution is mainly determined by the tip's properties, and that the contrast is given by the refractive index of the sample. This categorizes s-SNOM contrast into the material classes of metals, semiconductors, and polymers enabling a simple, high resolution material-specific mapping of nanosystems [4,5].

In this study, we present a new technique for the structural analysis of the collagen compound and for the isolation of DNA in historic tissues. We therefore used atomic force microscopy (AFM), a new high resolution technique which offers significant information on the fibrillar assembly and ultrastructure of collagen fibrils, which may provide insight into both the physiological and eventually pathogenic pattern of collagen fibrils, but also into possible diagenetic destructive changes of those fibrils. AFM figures three-dimensionally the surface of a sample with high resolution down to a nanometer scale. In our investigation we used the AFM to image paraffin embedded tissue sections from femoral bone tissue of a recent case and an age-determined historic sample (600 - 800 AD) in ambient conditions. With this technique we were able to identify unambiguously collagen bundles and to determine their diameter. These results led us to differentiate the bundling pattern of collagen type I from that of collagen type II. In addition, we identified collagen type I in the historic sample, which provided a fibrillar pattern as that of recent bone. The results can be compared to standard immunohistochemical staining techniques of the respective collagen types.

For the isolation of DNA samples we performed the non-contact laser pressure catapulting technique. Paraffin embedded sections of bone samples from ancient Egypt (New Kingdom, appr. 1500 – 1000 BC) were mounted on a supporting membrane and bone sections of 50 µm in diameter were isolated for DNA analysis. This non-contact isolation procedure decreases the problem of contamination. To confirm specificity of the isolated material PCR for β-actin and amelogenin DNA was performed.

In conclusion, our study presents circumstantial evidence that AFM analysis as a novel morphological technique can successfully be applied to historic and forensic tissue specimen. Laser based microdissection presents a non-contact method for the isolation of contamination free DNA material out of ancient bone samples. Consequently, these methods can be used for clinical forensic applications, such as the evaluation of diagenetic processes in tissue.

Charge transfer through a quantum-dot-interferometer

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We examine the charge transfer through quantum dots arranged to form a triangular geometry the center of which is pierced by a localized magnetic flux. Here, quantum dots are coupled each other via junctions through which electrons can tunnel from one dot to the other, manifesting interference effect according to the flux.

It is found that the conductance depends on the spin configuration of the dots due to a two-particle (destructive) interference between spin-up electrons which does not occur between spin up and spin-down electron.

Further, the periodicity of the conductance oscillation depends on the electron occupation in the dots, which is discussed with basis on the large gauge transformation.
**Effects of charge fluctuation on flexible membrane**

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Biological membranes contain a number of different charged lipids and macromolecules, which play unique roles in membrane conformational transitions during the life cycle of cells. Motivated by this perspective, we investigate the conformation and stability of flexible membranes with fluctuating charge distributions in an ionic solution. The salient features incorporated here are the conformational flexibility characteristic of soft matter, thermal fluctuation, and collective interplay among fluctuating degrees of freedom.

At first, we study electrostatic effects on undulation of flexible charged membranes, with and without added salts. A single membrane becomes unstable to a long wavelength undulation due to Coulomb repulsion between excess charges on membrane. This instability is suppressed both by charge fluctuation which induces an effective attraction and by free ions in solution which screen the Coulomb repulsion. The charge fluctuation-induced attraction, unless screened by the free ions, softens the membrane by reducing bending rigidity.

For two adjacent charged membranes, we consider the coupling of undulation with hydrodynamic modes of ambient fluid as well as the fluctuating charges. When the charge fluctuations are coupled to the membrane undulations in a cooperative manner, the dynamical instability to undulation with a finite wavelength can occur. This result implies a possible mechanism for the intriguing effects of multivalent cations on the destabilization during cell fusions.

**Self-oriented boron nanowire junctions for chemical and biological sensors**

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Due to substantial limitation of carbon nanotubes for integrated nanoelectronics, the possible use of semiconducting nanowires has aroused considerable expectations. For such applications it is important to be able to connect the nanowires. The complex multi-point nanowire junctions have been proposed as the building blocks of nanoelectronics. To be realized, however, the synthesis of self-assembled semiconducting nanowire junctions is obviously demanded.

Here we use a simple chemical vapor transfer method with modification to grow semiconducting boron nanowire Y-type junction arrays. Our approach to grow semiconducting boron nanowire junctions is based on a hybrid conventional vapor-liquid-solid and oxide assisted growth mode. Junction types were controlled by varying processing parameters, such as processing temperature, heating and cooling rate, thickness of catalytic gold layer, substrate materials, and distance between vapor source and substrate. We suggest a potential use of chemical and biological sensors using boron nanowire Y-junction arrays.
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<tr>
<td>Time</td>
<td>Monday, 24th</td>
<td>Tuesday, 25th</td>
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<tr>
<td>8.30 – 8.45</td>
<td>Opening</td>
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<td>8.45 – 9.45</td>
<td>Cees Dekker</td>
<td>Ned Seeman</td>
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<td></td>
<td>Carbon nanotubes as model systems for nanoelectronics and nanosensors</td>
<td>Structural DNA Nanotechnology</td>
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<td>9.45 – 10.45</td>
<td>Ulf Diederichsen</td>
<td>Jens Michaelis</td>
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<td></td>
<td>Molecular architecture with biooligomers</td>
<td>Viral DNA packaging – Single molecule studies of a unique molecular motor</td>
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<td>10.45 – 11.15</td>
<td>Coffee Break</td>
<td>Coffee Break</td>
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<td>11.15 – 12.15</td>
<td>Wolfgang Parak</td>
<td>Ulrich Züllicke</td>
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<td>Biological Applications of Colloidal Nanoparticles</td>
<td>Spintronics with semiconductor nanowires</td>
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<td>12.15</td>
<td>Lunch &amp; informal discussions</td>
<td>Lunch &amp; informal discussions</td>
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<td>17.00</td>
<td>Coffee</td>
<td>Coffee</td>
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<td>17.15 – 18.15</td>
<td>Viola Vogel</td>
<td>Hans Hennig von Grünberg</td>
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<td>Mechano-Chemical Sensing</td>
<td>Electrostatic interactions in soft-matter systems: membranes and charged colloids</td>
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<td>18.15 – 19.15</td>
<td>Stefan Thalhammer</td>
<td>Posters I</td>
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<td>Atomic force microscopy and laser microdissection as tools for life sciences</td>
<td>Force generation by single microtubules</td>
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<tr>
<td>Evening</td>
<td>19.30 Conference Dinner</td>
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