5th International Symposium

Optical Tweezers in Life Sciences

A forum for applications of optical tweezers and related technologies in life sciences

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Program
09:00 am  Registration
10:00 am  Opening
10:10 am  Structure formation and dynamics studied with single-molecule techniques  
Dr Michael Schlierf  –  Technical University of Dresden, DE
10:40 am  Single-molecule manipulation of RNA structures: double-stranded helices and G-quadruplexes  
Dr J. Ricardo Arias-Gonzalez  –  IMDEA NanoScience, ES
11:10 am  Coffee Break and Poster Session
11:50 am  Energy landscape analysis of biomolecular folding: pathways, rates and transition times  
Prof Michael Woodside  –  University of Alberta, CA
12:20 am  Single colloid electrophoresis  
Prof Friedrich Kremer  –  University of Leipzig, DE
12:50 pm  Lunch Break and Poster Session
02:50 pm  Optical heating and manipulation of a single metallic nanoparticle  
Prof Lene B. Oddershede  –  University of Copenhagen, DK
03:20 pm  4π AFM using a Light Touch  
Prof Mervyn Miles  –  University of Bristol, UK
03:50 pm  Coffee Break and Poster Session
04:30 pm  DNA translocation through nanopores by optical tweezers  
Prof Dario Anselmetti  –  University of Bielefeld, DE
05:00 pm  Laser trapping in chemistry and materials science  
Dr Hiroshi Masuhara  –  National Chiao Tung University, TW
05:30 pm  Poster Award and Closing
07:00 pm  BBQ dinner at Deck 5 Skybeach
Talks
Strains of pathogenic bacteria that exhibit multiple resistances to common antibiotics pose a significant threat to human health. After their first discovery in the 1950s, it was shown that these strains developed faster than mere mutation and selective pressure on the population would allow. Further studies indicated that bacteria possessed a mechanism to exchange and collect genetic sections, which code for such resistances or other adaptive traits. This mechanism is implemented with integrons, genetic elements that provide the means for a gene cassette transfer system between bacteria of the same or even different species.

Crucial to the function and recognition of gene cassettes is most likely the formation of a secondary DNA structure, in which the initial double-stranded DNA of the bacterial genome opens and forms a cruciform structure. We employ a selection of single-molecule techniques to study the formation, structure, dynamics and recognition of this cruciform structure.
RNA are ubiquitous macromolecular platforms that develop multiple biological roles in the cell. Here, we present single-molecule investigations on double-stranded (ds) RNA and on RNA G-quadruplex forming sequences.

DsRNA is the genetic material of a variety of viruses and has been recently recognized as a relevant molecule in cells for its regulatory role. Despite that the elastic response of dsDNA has been thoroughly characterized in recent years in single-molecule stretching experiments, an equivalent study with dsRNA was still lacking. Here, we have engineered long dsRNA molecules for their individual characterization contrasting information with dsDNA molecules of the same sequence.\(^1\) It is known that dsRNA is an A-form molecule unlike dsDNA, which exhibits B-form in physiological conditions.\(^2\) These structural types are distinguished at the single-molecule level with atomic force microscopy and are the basis to understand their different elastic response. Force-extension curves of dsRNA with optical and magnetic tweezers manifest two main regimes of elasticity, an entropic regime whose end is marked by the A-form contour-length and an intrinsic regime that ends in a low-cooperative overstretching transition in which the molecule extends to 1.7 times its A-form contour-length. DsRNA does not switch between the A and B conformations in the presence of force. Finally, dsRNA presents both a lower stretch modulus and overstretching transition force than dsDNA, whereas the electrostatic and intrinsic contributions to the persistence length are larger.

Left, force-extension curves of dsRNA and dsDNA, normalized in the extension axis to the A and B forms, respectively. Right, Force-induced unfolding of RNA constructions of GGGUUA repeats by optical tweezers.
G-quadruplexes are nucleic acid sequences that are rich in guanine and are capable of forming a four-stranded conformation. These structures are rare from a biological point of view but it is believed that they play a role in gene expression and telomere regulation. Here, we have studied long human telomeric RNA (TERRA). These non-coding RNA molecules contain subtelomere-derived sequences and an average of 34 GGGUUA repeats at their 3’ end. TERRA acts as a scaffold for the assembly of telomeric proteins involved in telomere maintenance and telomeric heterochromatin formation. By using optical-tweezers and other biophysical techniques, we have found that long RNA constructions of up to 25 GGGUUA repeats form higher order structures comprised of single parallel G-quadruplex blocks, which unfold at lower forces than their DNA counterparts.

References

Energy landscape theory provides the conceptual framework for understanding the folding of proteins and nucleic acids. I describe approaches for measuring energy landscape profiles using force spectroscopy and mapping the network of states in the folding pathways.

Using these methods, we can determine some of the key properties describing the folding, such as the configurational diffusion constant and the time needed for the actual structural change to take place.

I discuss results for the folding of both nucleic acids and proteins, showing how we can explore questions like roughness in the landscape and internal friction.
Optical Tweezers are employed to study the electrophoretic and the electroosmotic motion of a single colloid immersed in electrolyte solutions of ion concentrations between $10^{-5}$ and 1 mol/l and of different valencies (KCl, CaCl2, LaCl3). The measured particle mobility in monovalent salt is found to be in agreement with computations combining primitive model molecular dynamics simulations of the ionic double layer with the standard electrokinetic model. Mobility reversal of a single colloid — for the first time — is observed in the presence of trivalent ions (LaCl3) at ionic strengths larger than $10^{-2}$ mol/l. In this case, our numerical model is in a quantitative agreement with the experiment only when ion specific attractive forces are added to the primitive model, demonstrating that at low colloidal charge densities, ion correlation effects alone do not suffice to produce mobility reversal.

References:

1) Semenov, I., O. Otto, G. Stober, P.Papadopoulos, U.F. Keyser, and F. Kremer

2) I. Semenov, P.Papadopoulos, G. Stober, and F.Kremer

3) Semenov, I., S. Raafatnia, M. Sega, V. Lobaskin, C. Holm, F. Kremer
2:50 pm – Optical heating and manipulation of a single metallic nanoparticle

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A precise control over individual nanoparticles has huge potential for nano-architectural purposes and for probing nano-scale interactions. However, the absorption and heating associated with resonant irradiation of an individual gold nanoparticle can be extreme.

Moreover, the heating of a nanoparticle cannot be theoretically predicted as the precise focal intensity distribution on the nanoscale is unknown and typically highly aberrated [1]. Utilizing a novel assay based on partitioning of lipophilic dyes between membrane phases we quantify the heating of an individual irradiated gold nanoparticle [2,3]. The heating of the particle is dependent on laser power, and for a nanorod, also on its orientation with respect to the laser polarization [4]. A dramatic and irreversible change in plasmonic behavior of a nanorod occurs at high illumination intensities as the nanorod restructures into a more spherical shape. Nanoparticles can be used inside living cells for precise force measurements [5], and the photo-thermal effect of metallic nanoparticles can be used to create localized heat gradients inside living cells thus investigating cellular responses to heat chocks.

3:20 pm – 4π AFM using a Light Touch

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We have used optical tweezers to develop a new type of atomic force microscope (AFM). AFM offers unique characteristics amongst microscopy techniques including high-resolution 3D imaging in many environments including liquids, which are clearly essential for most biological studies. However, there are limitations, one of which is the requirement that the sample should be approximately planar. This is a consequence of the AFM cantilever and tip scanning in a plane, essentially in 2D. The tip only ‘sees’ the sample from above with the sides and bottom surfaces of the sample being inaccessible.

Using holographically generated traps, we have overcome this limitation by steering the tip of a nanorod in a three dimensional scan, with six degrees of freedom, such that it is possible to scan around a sample from any direction. The holographic optical traps allow not only positioning of the optical traps in x and y but also in z by changing the effective focal length of the objective lens independently for each optical trap. We have used various probe types: including silica and cadmium selenide nanorods, rod-like living diatoms, and two-photon polymerized 3D structures [1,2]. The force sensitivity is around 50 fN.


Some examples of nanotools used as AFM probes. Two-photon polymerisation has been used to generate a range of structures, the designs of which were informed by detailed simulations of the EM field and hydrodynamics.
We investigated the translocation mechanisms and dynamics of single dsDNA and dsDNA-protein complexes upon threading through a solid-state nanopore (NP) by quantitative 3D-optical tweezers (OT) (Fig. 1). The combination of OT force mechanics with electrophysiology allows experiments at sub-pN force sensitivity, ms time resolution and pA ionic current sensitivity. In our single molecule translocation experiments, we find distinct asymmetric and retarded force signals that can be associated to individual proteins and depend on the protein charge, the DNA elasticity and its counter-ionic screening in the buffer [1]. A theoretical model where an isolated charge on an elastic, polyelectrolyte strand is experiencing an anharmonic nanopore potential was developed. Its results compare very well with the measured force curves and explain the experimental findings that the force depends linearly on the applied electric field and exhibits a small hysteresis during back and forth translocation cycles. Moreover, the translocation dynamics reflects the stochastic nature of the thermally activated hopping between two adjacent states in the NP that can be adequately described by Kramers rate theory [2].

In our presentation, we will also report on a recent tedious analysis of single DNA translocation experiments where we quantified the effective dsDNA threading force through He-ion-microscope drilled Si$_3$N$_4$-solid-state NPs with diameters ranging from 6 nm to 70 nm [3, 4] as well as the working progress of our experiments with biopore toxins.

Fig.1. a) Single molecule NP force spectroscopy: a microbead with an attached DNA molecule is optically trapped in the vicinity of a solid-state NP. A membrane voltage drives the negatively charged DNA into the NP. The
electrokinetic forces on the DNA are balanced by the force acting on the bead in the optical trap. The distance between trap and membrane can continuously be varied with nm precision. b) In the force-distance curve, two isolated DNA-bound proteins can be detected as distinct signals by unthreading the DNA strand out of the NP. c) Dependence of threading force on NP diameter (red dots: NPs fabricated at UNCG, black dots: fabricated at UBi, blue line as guide to the eye).

References

Upon focusing intense lasers of a few hundreds mW into a diffraction limit, small objects such as molecular clusters, polymers, and nanoparticles can be trapped in solution at room temperature, leading to new applications. We have been exploring new laser trapping phenomena in view of chemistry and material science, and here summarize our recent results and discuss future perspective.

1. Crystallization and liquid-liquid phase separation of amino acids by laser trapping
When intense CW 1064 nm laser is focused at an interface between a thin heavy water solution film of glycine and a glass substrate, the assembled molecules nucleate and evolve to a liquid-liquid phase separation, while they will crystallize if the trapping laser is focused on the solution surface (1). We can control the polymorph of the formed glycine crystal selectively by tuning trapping laser polarization and power.

2. Optical trapping and polarization-controlled scattering by femtosecond laser pulses
Optical trapping behavior of 50-nm-sized polystyrene beads in water was investigated by irradiating femtosecond 800 nm laser pulses. Their laser trapping is more efficient than that by CW laser, and further the nanoparticles are scattered out of the focal spot to the surrounding areas, in an alternating manner, perpendicular to the laser polarization (2). To understand these phenomena, we analyzed radiation (gradient and scattering) force of femtosecond laser pulses and their temporal force exerted on the nanoparticles by taking into account the impulsive peak power and the axial component of electric light field produced by high numerical aperture of objective lens.

3. Future perspective
We have also reported polymerization and solidification confined into small volume (3), J-aggregate formation (4), and so on, and are studying resonance effects in laser trapping. On the basis of these works we will discuss our future perspective.

Poster Session
P1 – Single influenza virus tracking reveals cell specific modes of movement – a key to understand cellular tropism

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Following single viruses on their journey through a host cell provides valuable insights that help to understand the infection process. Fluorescent labeling techniques together with high-resolution microscopic imaging allows tracking of single viruses on living cells with high temporal and spatial accuracy.

Influenza A viruses were fluorescently labeled without interfering with their biological activity. Single-virus trajectories were recorded on the surface and inside living cells of different permissive cell lines. By using conventional computational methods we extracted the position of viruses within each image frame. In addition, we have applied a recently developed mathematical tool to further analyze a special type of recurrent trajectory obtained at the surface of living cells. Using this approach we can identify and characterize microdomains on the cell surface. We show that the viruses are surprisingly mobile and display a variety of different types of movement, which depend on the studied cell line. The cell surface was characterized by scanning electron microscopy (SEM) and revealed a unique topography that also strongly depends on the cell type and the cells growth state.

By comparing trajectories obtained from two permissive cell lines we were able to correlate the distinctive behavior of single viruses with the cell surface ultrastructure. We used cells that are infected to a different degree and combine this with virus uptake and intracellular fusion kinetics. This allows us to draw a comprehensive picture of the first steps of virus infection. Further we can draw conclusions about the infectivity and could explain why some cells are permissive while others are not.
P2 – Epitope mapping of monoclonal antibody HPT-101 using Optical Tweezers

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For diagnostic procedures that rely on monoclonal antibodies (mAb) it is imperative to know whether the antibody (e.g. HPT-101) recognizes the epitope of its target peptide/protein (e.g. tau-protein) specific, or whether possible cross-reactivity may occur with other forms of the ligand. In Wagner et al. [1] non-specific interactions between mAbs and tau-peptides with different phosphorylation pattern were detected. Based on this result, it can be assumed that the specificity of these antibodies refer not only to a specific isolated phosphorylation site, but also to the surrounding amino acid sequence in the tau-peptide. Here we present Optical tweezers-assisted dynamic force spectroscopy (DFS) measurements to investigate the specific binding between the biphosphorylated peptide tau[Thr231/Ser235] and its monoclonal antibody HPT-101 on a molecular level. By replacing single amino acids in the ligand sequence by alanine and analyzing the interactions with the DHS-model [2], conclusions about the existence of essential and secondary amino acids can be drawn.

References:
P3 – Nanomechanics of protein-DNA hybrids

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In current nanobiotechnology optical tweezers are used to hold and manipulate various biological objects such as cells, organelles, biomolecular machines or DNA tethered to microspheres. DNA handles are often used as spacers to avoid unspecific interactions between the biological objects of interest. We here present optical trapping of novel protein-labelled DNA hybrids in a microfluidic environment. Double stranded DNA handles of 4056 bp length with digoxigenin (DIG) and biotin end groups were labelled with streptavidin or neutravidin. The DIG end of the hybrid molecules was linked to surface-modified polystyrene beads with the antibody anti-digoxigenin. In optical force measurements with various physiological buffers the novel DNA constructs exhibited very reliable mechanical characteristics with long dissociation times of the DNA constructs. We applied these hybrid handles to interconnect them with biotinylated DNA molecules. Thus, we could show that the novel protein-labelled DNA handles are excellent candidates to grasp single molecules, which expose tags suitable for molecular recognition, in time critical molecular motor studies.

Protein-labelled DNA hybrids are used as handles to facilitate the study of two molecular machineries with angstrom spatial resolution, namely the mechanics of the ribosome translation process and folding of the nascent polypeptide chain during translation, as well as the mitochondrial protein import, a process involving several molecular machines working in tandem. In order to be able to study these molecular motors an optical tweezers instrument had to be built with the necessary spatial and temporal resolution. A device based on Bustamante’s dual trap optical tweezers design has been built and tested. Following successful force spectroscopy measurements with the ribosomes and mitochondria, the setup will be used to investigate inhibition of the ribosomal translation by drugs and to study mitochondria from tissue related to mitochondrial dysfunction and neuro-degeneration.
P4 – Forces of interaction between blank, grafted and blank-grafted colloids by using optical tweezers

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Optical tweezers are experimental tools with extraordinary resolution in positioning a micron-sized colloid and in the measurement of forces acting on it – without any mechanical contact. Here, we report the direct measurements of the interactions between blank (partially measured with the JPK NanoTracker), grafted and blank-grafted colloids. The forces are repulsive at all measured distances in all three cases, decaying with distance. For the symmetric blank colloids (SiO$_2$), the experimental data are well described by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory.

In the case of symmetric grafted colloids (SiO$_2$ grafted with poly(acrylic acid)), the data are quantitatively described by Jusufi et. al. model for spherical polyelectrolytes brushes which takes into account the entropic effect of the counterions. In the asymmetric case (blank against grafted) the experimental data are described by Alexander de Gennes model which only takes into account the steric force.
P5 – Probing adhesion properties of malaria parasites on tunable substrates using Holographic Optical Tweezers

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We investigate the adhesion properties of the \textit{Plasmodium} sporozoites, the infective stage of the malaria parasite. Sporozoites have the ability to penetrate host tissues and invade host cells using a unique type of locomotion called gliding motility. It employs a submembranous actin myosin-based gliding machinery. The force generated by this motor machinery is transferred to the substrate at distinct adhesion sites along the parasite. Within these adhesion sites transmembrane proteins of the apicomplexan-specific thrombospondin-related anonymous protein (TRAP) family regulate the adhesion characteristics of the parasite (Hegge et al., 2010).

We employed Optical Tweezers to probe these adhesion characteristics of individual parasites on bare glass substrates. Furthermore, Holographic Optical Tweezers allow to manipulate the parasite simultaneously by multiple optical traps.

In order to mimic the soft tissue, the parasites encounter during infection, we use soft PEG hydrogels of different elasticity, which have been shown to affect sporozoite gliding motility (Perschmann et al., 2011; Hellmann et al., 2013). Those tunable substrates are more comparable to physiological conditions and might allow for probing the adhesion strength of established adhesion sites by rupturing, which was not possible on glass substrates. By comparing the phenotype of different sporozoite mutants lacking specific surface proteins, we shed light on the complex adhesion and motility of apicomplexan parasites.

Reference List


P6 – Surface force measurement between polyelectrolyte brushes in aqueous solution

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Polymers chemically grafted to the surface of substrates are known as polymer brushes. A polyelectrolyte brush surface, a class of polymer brush consisting of polyelectrolyte, is particularly attractive because of its potential applications for adhesion, antifouling, water lubrication systems, and so on. In this study, we aimed to investigate the surface property of the polyelectrolyte brush through the surface force measurements by the optical tweezers. Cationic poly(2-(methacryloyloxy)ethyltrimethylammonium chloride) (PMTAC) brush was prepared on the surface of silica particles (radius \( R = 1.5 \mu \text{m} \)). The silica particles with brushes were dispersed in water or in NaCl aqueous solutions, and manipulated with the optical tweezers (NanoTracker\textsuperscript{TM}, JPK Instruments). The IR laser (3W, wavelength \( \lambda = 1064\text{nm} \)) was split to two laser passes to independently manipulate two particles at the same time. The force between two particles was detected as they approached with approaching rate of 0.5 \( \mu \text{m/sec} \). (Figure 1). The stiffness (spring constant) of optical tweezers was determined by the power spectrum method.\textsuperscript{2}

Obtained force (normalized by radius \( R \) of silica particle) was plotted against distance between the two silica particles in Figure 2. In the case of bare silica particles, rapid increase in the force was observed at the distance where the two particles contacted. This was obviously attributed to the steric repulsion between the hard spheres. In contrast, the increase in force was rather mild and occurred from longer distance in the case of PMTAC coated particles. Fitting the force-distance curves by a theoretical equation suggested that the change was due to the electrostatic interaction between the charged surfaces. Debye length, \( 1/\kappa \), surface potential, \( \psi_0 \), and surface charge density \( \sigma \) were obtained from the fitting analysis. In the case of polymer grafted particles, \( 1/\kappa \) and \( \psi_0 \) decreased with increasing the salt concentration in the medium. The \( \sigma \) value was 0.2 Cm\(^2\) for densely grafted polymer brush (~0.1 chains/\text{nm}^2), which was only c.a. 10% of the predicted value of full dissociation of ionic groups. Furthermore, the increase in the degree of ionic dissociation was observed with decreasing the grafting density of polymer at the surface. These results indicated that the large portion of counter ions (chloride ions) was immobilized in the dense polyelectrolyte brush layer to neutralize its considerably high charge density.
References


Protofilament switching of Kinesin-8 investigated with Optical Tweezers

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The budding yeast Kinesin-8 Kip3 is a highly processive motor protein that walks to the end of microtubules and shortens them in a collective manner. Microtubules usually consist of 12 to 15 circularly-arranged tubulin polymer chains, called protofilaments. Left-handed rotations of microtubules in Kip3 gliding assays indicate sideward motion of Kip3 perpendicular to the microtubule axis, i.e. a switching between single protofilaments. Here, we used a high-resolution optical tweezers setup in a force feedback mode to apply sideward loads on single motor proteins. Our studies show that Kip3 stepped sideward in both directions under alternating sideward loads. In control experiments with immobilized Kip3 and not protofilament switching kinesin-1, we measured no effective sideward motion. Statistical analysis and comparison with simulations propose a diffusive motion of Kip3 on the microtubule lattice with a preference to the left with respect to the direction of forward motion. This is consistent with the gliding assays. Protofilament switching has implications for the suggested mechanical signaling role of Kinesin-8 in budding yeast with respect to its ability to bypass obstacles.


2 Quantum-dot-assisted characterization of microtubule rotations during cargo transport, B. Nitzsche, F. Ruhnow and S. Diez, nature nanotechnology, 3 (2008)
P8 – Reflected differential interference contrast microscopy to visualize sub-resolution phase objects

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The visualization of objects smaller than the diffraction limit has become a broad field, especially in life science. However, current technologies often require a fluorescent label and/or complicated optical setups. Here, we report the observation of single, non-labeled microtubules with interference reflection microscopy (IRM) using a light emitting diode for illumination.

IRM can visualize sub-diffraction-limited objects, provides three-dimensional (3D) information with nanometer resolution in the axial direction, and a superior signal-to-noise ratio compared to differential interference contrast (DIC) microscopy. In comparison to other techniques such as DIC microscopy, IRM does not require any (expensive) polarizing optics, is independent of the object orientation, and can easily be implemented into conventional inverted microscopes. Also, IRM provides free access to the upper side of the sample and can be combined with other optical techniques such as fluorescence microscopy and optical tweezers.
P9 – Video-based and interference-free force analysis

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We have developed a video-based axial force detection and analysis system for optical tweezers for measuring the minute forces exerted on single molecules during controlled translocation through nanopores with sub-piconewton precision [1]. Because of the limited sample rate of the video signal of 123 Hz, we integrated Allan variance analysis [2] for trap stiffness calibration.

When manipulating a microbead in the vicinity of a weakly reflecting surface with simultaneous axial force detection, interference effects have to be considered [3]. Therefore, we measured and analyzed the backscattering light properties of polystyrene and silica microbeads with different diameters and propose distinct and optimized experimental configurations (microbead material and diameter) for minimal light backscattering and virtually interference-free microbead position detection.

As a proof of principle, we investigated the nanopore threading forces of a single dsDNA strand attached to a trapped microbead with an overall force resolution of 0.5 pN.

Fig. 1: a) Still frame of a polystyrene bead with circular regions of interest, detected edges and fitted circle. b) Dependency of the (apparent) bead size on the amount of backscattered light. c) Controlled dsDNA threading with 3.05 µm bead (55 nm pore diameter, 50 mV applied voltage)

References
P10 – Force spectroscopy of DNA-bound histones

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We investigated the dynamic structure of individual nucleosomes by optical tweezers with single molecule force spectroscopy. 16.4µm long biotinylated dsDNA is attached to two streptavidin coated microspheres, one held by a micropipette, as well as the other by the optical trap, respectively.

The histones in our measurement contain all of the natural proteins (H2a, H2b, H3, H4) including the linker histone-like protein (H1) to form higher order structures. We observed distinct sawtooth patterns, that can be interpreted as the release of individual nucleosome complexes and will discuss the observed phenomena in the framework of histone complex formation.

Fig.1: a) setup b) Stretching process of 16.4µm dsDNA with histones and native.
P11 — Elongation and melting of double stranded DNA can be identified by transient kinetics measured with 2-30 pN force steps and temperature

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SUMMARY
Double stranded (ds) DNA pulled to ~65 pN undergoes an overstretching transition from the basic conformation (B-form) to a 1.7 times longer conformation. Elongated ds-DNA and single stranded DNA are fundamental stages in the structural transitions involved in DNA recombination, replication and repair; thus understanding the mechanisms that control the relative stability of these different states is of basic importance. By using a dual laser optical tweezers with a fast force feedback, we recorded the length transient following 2-30 pN force steps imposed on the λ-phage DNA with different degrees of melting and at different temperatures (10-25°C). The load-dependence of the extent and rate of the length transient shows that the whole 70% lengthening is a two state reaction (B-S transition) with a cooperativity of 22 bp. The shortening during the transient following a 20-30 pN stepwise force drop is accounted for by the backward two state reaction, while the re-annealing of the melted fraction is a time consuming step that does not imply shortening. The temperature dependence of the transient elongation following 2 pN steps shows that (i) there is an entropic contribution to the free energy change of the reaction that at room temperature is ~1/2 of the enthalpy change (~8.2 k_BT per bp) and only 1/3 of the entropy change expected from thermal melting; (ii) there is no enthalpic contribution to the transition energy barrier; (iii) the cooperativity is independent of temperature, suggesting that it arises from structural factors, such as the nucleic acid sequence.
P12 – Specific single-molecular interaction between apoA-I and ABCA1 on living cells measured by optical tweezers

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Apolipoprotein A-I (ApoA-I) plays a crucial role in the human atheroprotective system. It facilitates cellular lipids efflux in the process of reverse cholesterol transport (RCT). ApoA-I has been demonstrated to function by either directly forming a complex with the membrane lipid transporter—ATP binding cassette transporter A1 (ABCA1) or indirectly binding with a plasma membrane (PM) lipid domain created by ABCA1 activity. However, the molecular mechanism of specific apoA-I/ABCA1 interaction is still largely unknown. Here optical tweezers is utilized to quantitatively investigate the interaction between ABCA1 on THP-1 cells and dysfunctional apoA-I from diabetes patients. The specific interaction between apolipoprotein A-I (apoA-I) and ABCA1-expressing THP-1 cells has been measured on the single-molecular level by optical tweezers in situ. The inhabitation effect for the specific interaction by the monoclonal antibody (ab18180) was studied also. Through single-molecular force spectroscopy analysis we demonstrate that the specific interaction of the dysfunctional apoA-I of diabetes patients with ABCA1 took place at two different binding sites on the cells. One is apoA-I - ABCA1 with a binding strength of 56.7±4.1 pN, which accounts for a small part. Another is the high capacity binding site (HCBS) with binding strength of 26.5±4.9 pN. The results will be helpful to understand the mechanisms underlying apoA-I interactions with ABCA1. This method we presented also would be applied in other specific single molecule interaction on single living cells. This work was supported by National Science and Technology Infrastructure Program of China (2012BAF14B14), and National Key Technologies R&D program of China (2012ZX09303005003).

Keywords: single-molecular force spectroscopy; optical tweezers; receptor-ligand interaction; apolipoprotein A-I
P13 — Studying HIV-1 Protease folding pathways at the single molecule level using optical tweezers

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Abstract

Optical tweezers (OT) is an established tool in the field of life sciences and enable us to revisit protein folding with a completely new approach. Protein folding is one of the major unsolved challenges for modern biophysics. Retroviral protease from human immunodeficiency virus type 1 (HIV-1-PR) is essential for the maturation of the virus and it has been identified as potential target for structure-based drug design. In present study, we used OT to investigate the folding pathways of HIV-1-PR at the single molecule level. The results of our experiments reveal that this protein reaches its native state following multiple folding routes characterized by different intermediate structures. The insight obtained from our measurements, which is consistent with the results of earlier molecular dynamic (MD) simulation studies, might help us design effective folding inhibitor drugs for HIV-1-PR.

Keywords: Protein folding, single molecule, optical tweezers (OT), human immunodeficiency virus type 1 (HIV-1-PR).
P14 – Resonant optical tweezers with anti-refection coated Titania microspheres

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Brownian motion is exhibited by an optically tapped particle due to the thermally driven molecules of the surrounding medium. This motion is often considered to be a frequency-independent phenomenon which is known as a white noise process. However, fluid entrainment influences the particle in the trap and results in a frequency-dependent motion. Therefore, the power spectral density (PSD) of the noise that drives the motion is “colored”.

The “colored noise” of the Brownian motion can change the behavior of an optical trap from an overdamped oscillator to a resonant one. Here, our goal was to amplify this resonance. Theoretical calculations predict that particles with a large diameter and a high trap stiffness enhance the resonance effect. Therefore, we synthesized large anti-reflection coated titania microspheres. These microspheres have a high trap stiffness in the optical trap.

In comparison to our previous work [Jannasch et al., PRL 2011], the results showed a roughly 1.5 and 3 times enhancement of the resonance in water and acetone, respectively. The resonant behavior could be used as a sensor in analogy to other resonant probes such as an atomic force microscope cantilever.
P15 – Changing the state of nano-particles in ferrofluid with optical tweezers

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Ferrofluids consist of nanoparticles (diameter ~10nm) which are dispersed in a liquid often using the surfactants [1-4]. Thus ferrofluids are colloidal suspensions and have both liquid and magnetic properties, which are useful for various applications. The ferrofluids are stable, which means that there is no particles aggregation or phase separation even in strong external magnetic fields. However, under conditions when surfactants break down the nanoparticles will aggregate.

In this study we report on the behavior of the ferrofluid in the strong optical field. The experiments were performed using homemade holographic optical tweezers. We have shown that the focused laser beam has strong influence on the magnetic nanoparticles, provided that the light energy density is high enough. In particular, at some threshold of the light density the magnetic nanoparticles are removed from the center of the focused beam and form a ring magnet around the light spot. A few seconds after the laser was switched small clusters of nanoparticles traveling through the medium was observed. These clusters are large enough to be observed by our microscope.

References
P16 — Studies of DNA-protein interactions at the single-molecule level using optical tweezers

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Optical trapping is a powerful tool to study the mechanical properties of biological molecules. A highly focused laser beam allows for precise manipulation of microscopic dielectric objects such as polystyrene or glass microbeads that for example can be attached to single DNA molecules.

A method to study the mechanical properties of single DNA molecule has been developed at the Institute for Nanobiotechnology (Saint-Petersburg Polytechnical State University). Specially designed multiflow microfluidic chamber in combination with double-trap optical tweezers apparatus is used for preparing a single “DNA-dumbbell” complex in flow. A λ-DNA biotinylated on both ends is attached to two streptavidin coated polystyrene beads. The beads position is controlled by means of two optical traps. The trap stiffness is calibrated using a drag force calibration and the measurement of force applied to the beads is performed using a high-speed CCD camera.

Rapid change of the reaction conditions by moving the “DNA-dumbbell” between different laminar flows allows for the real time measurement of the dynamics of DNA-protein interactions. Force-extension curves of naked DNA and DNA upon protein binding can be measured in the wide range of applied forces (up to 90 pN) including the high-force regime when the DNA undergoes structural changes during the overstretching at 65 pN. The method was successfully tested on well-studied DNA-binding protein RecA and YOYO-1 intercalating dye. Present work is focused on studies of eukaryotic proteins Pontin and Reptin and their interaction with DNA.
P17 – Diffusion inside living cells and wormlike micellar solutions

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Abstract:
We report results of single tracer particle tracking by optical tweezers and video microscopy in micellar solutions [1] and inside living human cells [2]. Wormlike micellar solutions are a model system for living polymers and we find tracer beads undergoing free anomalous diffusion which is ergodic and consistent with a description in terms of the generalized Langevin equation with a power-law memory kernel. With optical tweezers tracking, we unveil a power-law relaxation over several decades in time to the thermal plateau value under the confinement of the harmonic tweezer potential. After the subdiffusive motion in the millisecond range, the motion becomes faster and turns either back to normal Brownian diffusion or to even faster superdiffusion, depending on the size of the tracer beads. Additionally, we track lipid granules which diffuse in the cytoplasm of living cells in order to map out the viscoelastic landscape. Using optical trapping and single particle tracking we find that lipid granules exhibit anomalous diffusion inside human umbilical vein endothelial cells. For these cells the exact diffusional pattern of a particular granule depends on the physiological state of the cell and on the localization of the granule within the cytoplasm.
