Junior Nanotech Network

A Self-Organized Graduate Student Exchange Program

2010

Center for the Physics of Living Cells

CeNS
Center for NanoScience | LMU

Welcome

Research in the fields of nanoscience and nanobiosciences is extraordinarily diverse and interdisciplinary. PhD students in these areas must have a distinct open-mindedness and engagement and must constantly acquire new expertise and technical know-how from a variety of disciplines.

At the Center for NanoScience (CeNS) in Munich, we offer a multidisciplinary platform to these students, allowing for access to a large range of research topics. In an effort to exchange ideas and scientific knowledge, as well as to broaden one's horizon, one approach is the participation in an international exchange program such as the Junior Nanotech Network (JNN).

The JNN was established in 2006 as a PhD exchange between CeNS and partners at McGill University in Montreal. This unusual and unique program is strongly focused on an intensive exchange on both the scientific and cultural level since students from both parties are hosts in their labs as well as at their homes. This guarantees strong interactions between all participants and mitigates requirements on third-party funding. I am convinced that the experience from this exchange, and the network that was formed from it, will aid the students in their further scientific and personal development. We were especially delighted to find the CPLC in Urbana, Illinois, as a partner in this exchange program. The strength of both institutions in the field of single-molecule biophysics promises strong synergetic effects.

I had the chance to visit the CPLC in Urbana-Champaign during the exchange program and was greatly impressed by the facilities, the students' knowledge, and the hospitality I experienced. I can state without doubt that the exchange met my highest expectations and that the students took this chance with great enthusiasm. I am very grateful to Taekjip Ha and Klaus Schulten and all our partners in Illinois whose hospitality and scientific expertise have been outstanding. I am convinced that the exchange program is just the beginning of long-lasting interactions and networking between our two institutions.

Prof. Philip Tinnefeld
Center for NanoScience, Germany

The Center for the Physics of Living Cells (CPLC) in Urbana strives to understand how molecules come to life using physical concepts and experimental tools. Many of the key molecules in biology are tiny, just a few nanometers across, challenging our abilities to measure them. On the other hand, these molecules are huge, made of thousands of atoms, defying straightforward theoretical analysis. In short, the mission of our Center requires advances in nanosciences, both experimental and theoretical, to make major breakthroughs. It was fortunate that we were able to participate in the JNN program this year so that our students could get hands-on experiences on the latest technologies in Munich and at the same time teach their counterparts from Munich. We particularly thank Philip Tinnefeld and Hermann Gaub who strongly supported the program from the Munich side. And more thanks for choosing wonderful students to participate! All nine students from Munich were uniformly excellent and they are a fun-loving bunch too!

All participating students had to take six weeks off from their regular PhD training, but it is clear to me that they actually worked even harder during the JNN program and I believe that they will look back and consider this an invaluable experience. One learns so much by teaching, and from looking at problems using different approaches. The network of students formed during the program will endure too. The organization of the program also allowed the student to engage in several memorable cultural activities. I wish I were one of the students who went on the bike ride to Hermann Gaub's house in the Alps! The program gave me another opportunity to appreciate how great our students are and how intensely they live their lives. We will certainly build on this most amazing experience and will organize similar exchange programs with Munich and other places in the future.

Prof. Takjip Ha
Center for the Physics of Living Cells, U.S.A.

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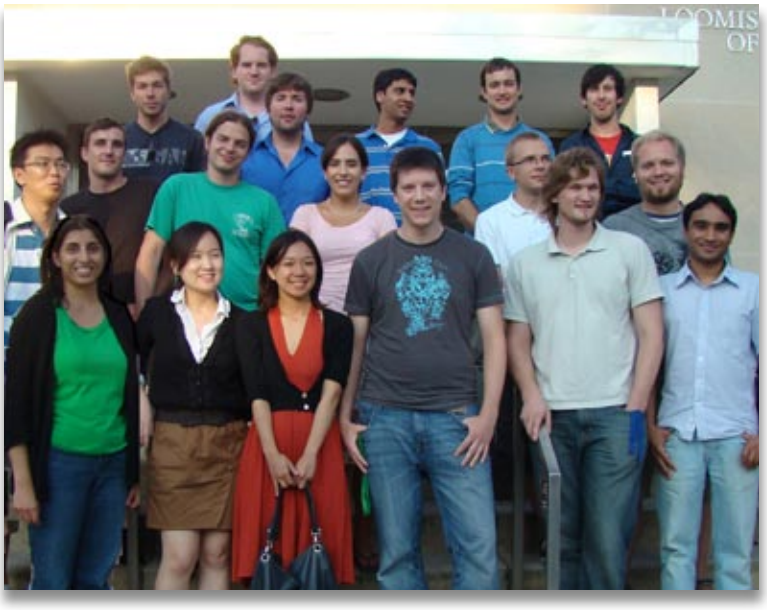


Participants of the JNN 2010: Top Row (l-r): Johannes Stigler (CeNS), Stephan Heuke (CeNS), Rogan Carr (UIUC), Chaitanya Sathe (UIUC), Ingo Stein (CeNS), Brendan Osberg (CeNS), Middle Row (l-r): Kyung Suk Lee (UIUC), Wolfgang Kügel (CeNS), Christian Osseforth (CeNS), Markita Landry (UIUC), Stefan Stahl (CeNS), Björn Hellenkamp (CeNS), Bottom Row (l-r) Jaya Yodh (CPLC Outreach Director), Jeehae Park (UIUC), Helen Hwang (UIUC), Nikolaus Naredi-Rainer (CeNS), Johan Strumpfer (UIUC), Ankur Jain (UIUC).

About the Junior Nanotech Network

Advanced research in the fields of nanosciences and nanobiosciences requires joint efforts of enthusiastic scientists trained in different disciplines as well as a strong international network enabling the researchers to work closely together and exchange knowledge and innovative ideas. The aim of the Junior Nanotech Network (JNN) is to exchange knowledge of world class nanoscience and nanobioscience between graduate students from different institutions worldwide.

In 2010, PhD students from the Center for NanoScience (CeNS, LMU Munich) teamed up with their peers at the Center for the Physics of Living Cells (CPLC, University of Illinois at Urbana-Champaign, UIUC) for a third round of the Junior Nanotech Network. This program was established in 2006 together with partners from McGill University (Canada) and repeated with different students from the same institutions in 2008. The JNN project takes advantage of local expertise and facilities and aims at initiating scientific and personal exchange between junior nano-researchers in Germany and partner institutions abroad, linking the research efforts in the field of nanoscience of the two universities and fostering the interaction between the partnering countries. The participants of the JNN are preselected based on excellence and compatibility of the proposed projects.

For the program between CPLC and CeNS in 2010, eight University of Illinois graduate students served as scientific and housing hosts for nine CeNS PhD students from July 11-31, 2010, while the German student group reciprocally hosted the UIUC students for a three week European visit from September 17 to October 9, 2010.

Lectures and Lab work

The scientific programs in both locations involved two main elements:

- Hands-on experimental and computational training by their host peers
- Conferences and poster sessions

At the conferences, the participants were exposed to presentations from internationally renowned speakers and were given the chance to engage in discussions with researchers from all over the world. During the extended period of laboratory and computational work, the students were acquainted with new techniques and were introduced to many different subjects in the field of nanosciences and biophysics.

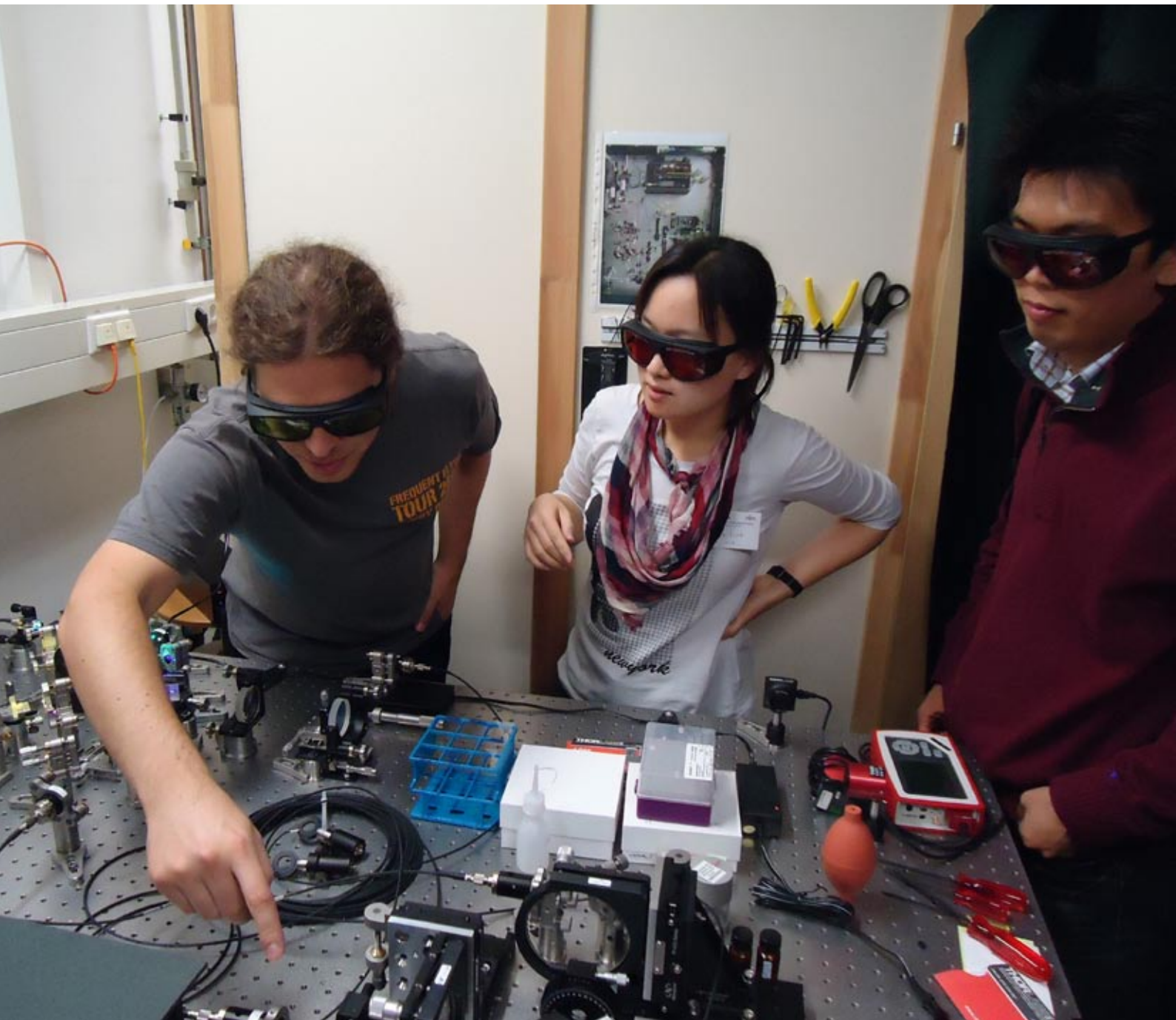
Learning From Peers

One interesting feature of the JNN is the self-organized character of the project, getting the PhD students involved in the organization and realization of the exchange program. In particular, the students were asked to conceive, teach, and supervise the scientific experiments in an independent manner. The laboratory work was carried out by the guest students under the supervision of the host students. In this way, students coming from the different areas of nanosciences and biophysics were exposed to the cutting-edge of a variety of domains via the guidance of the host students. The scientific projects allowed the transfer of broad knowledge and new technical skills and gave the hosts the opportunity to demonstrate their advanced research to a small and enthusiastic audience.

Intercultural Exchange

The scientific program was complemented and enriched by the social, cultural and outdoor activities which were organized by the host students. To guarantee social integration of the guests, the visiting researchers were housed at the homes of their local exchange partners. This close interaction fostered the intercultural exchange and stimulated informal scientific discussions. Combining joint lab work with common leisure activities enabled the creation of strong bonds between the young people from the U.S.A. and Germany.

Research Projects



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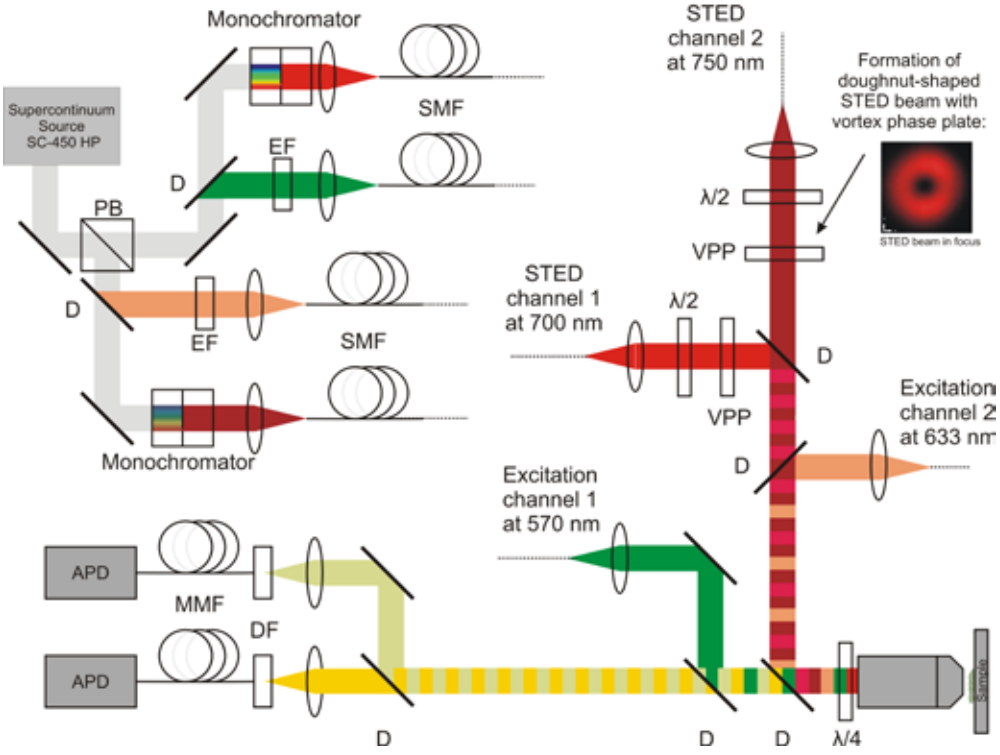
CHRISTIAN OSSEFORTH

Super-Resolution Stimulated Emission Depletion (STED) Microscopy

Optical analysis of cellular structures has recently gained a lot of attention due to the ability to break the diffraction limit of light. Different approaches allow researchers to image objects in the far-field with conventional optics with a resolution of only tens of nanometers. In the past, this ability was only attained by using electron microscopy which does not allow the direct observation of dynamic processes in cells.



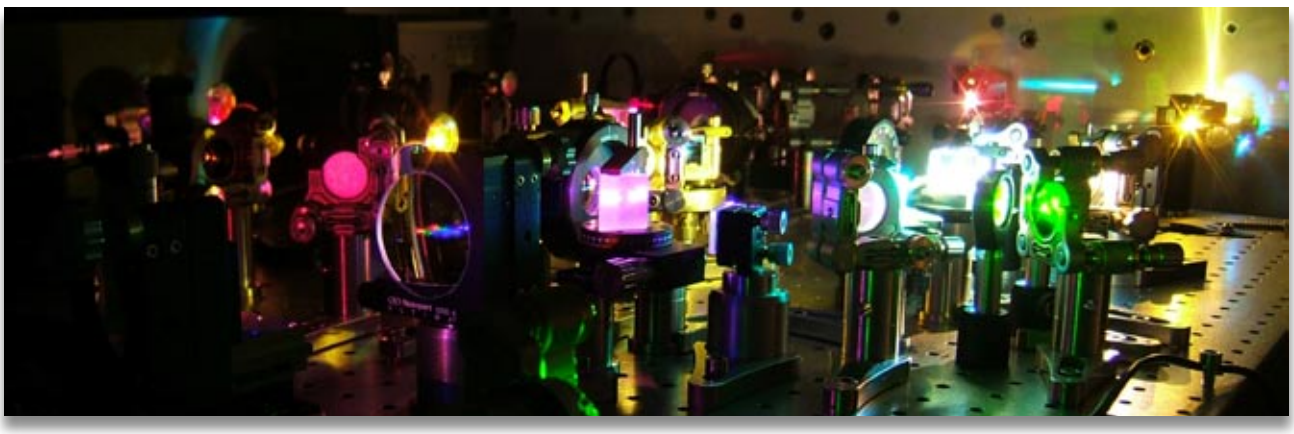
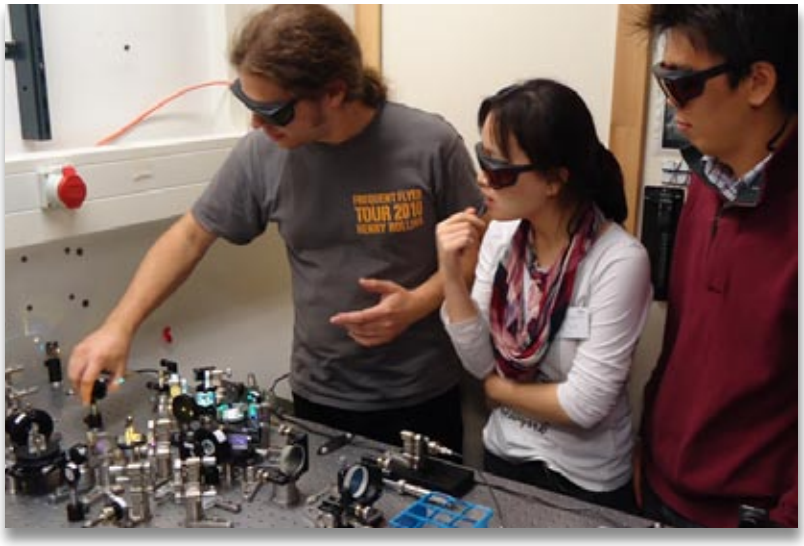


Fig. 1: Schematic representation and real set-up of current dual color STED microscope which was used in the JNN module.

One technique to overcome the diffraction limit of light is stimulated emission depletion microscopy (= STED Microscopy). In essence fluorophores are excited by a diffraction limited spot and immediately depleted by a second laser pulse with the spatial distribution of a donut featuring a central zero of intensity. Turning up the intensity of the STED-beam results in a smaller fluorescent spot in two dimensions in the focus. The two beams are overlaid and raster-scanned across the sample of choice.



This module took the students through each step of aligning a self-built STED microscope. First it was shown how to select a certain wavelength range for the depletion beam using a prism-based monochromator and its importance for the success of the experiment was discussed. Furthermore the adjustment of the donut beam and its crucial contribution to the setup was explained and performed by using specialized components to analyze its circular polarization.

Last but not least, after alignment of the instrument small fluorescent beads (20 nm diameter) were imaged as test samples to demonstrate the increase in resolution and the concomitant gain in information one can extract from the super-resolved images. The attained resolution was measured from the recorded images and was shown to depend on the wavelength used for the depletion beam. Furthermore recent advances of our lab in dual-color and 3D-STED microscopy and its implications were discussed in the module.

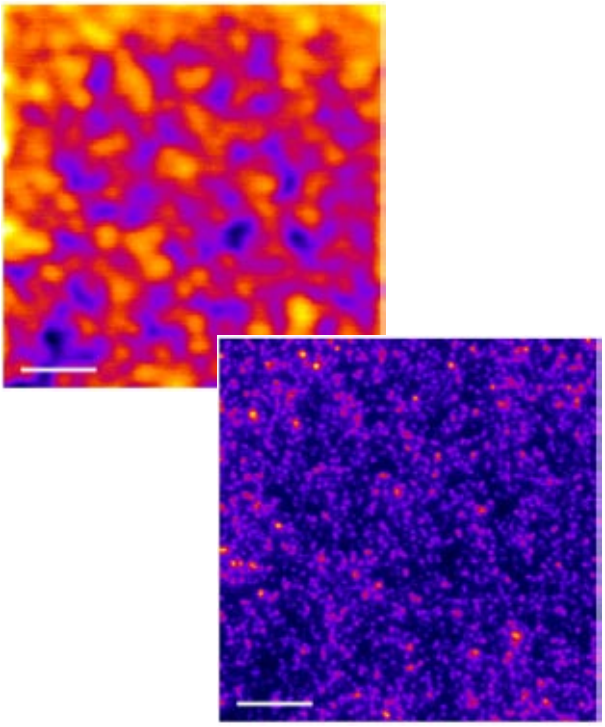


Fig. 2: Conventional confocal image of 20 nm fluorescent beads limited by the diffraction limit of light (upper left side) and corresponding super-resolved STED image of the same area (lower right side). Scale bars 1 µm.

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MARKITA LANDRY

Optical Trapping Study of ssDNA Binding Protein – DNA Interactions

Protein-DNA interactions lie at the heart of many essential cellular processes such as replication, recombination, and repair. Recent advances in optical "tweezers" have made it possible to resolve motions on the scale of a single base pair of DNA, 3.4 Å. These high-resolution optical traps have the potential to reveal these interactions at their fundamental length scales and should reveal how certain proteins bind to DNA or recognize target sequences.

Telomerases are enzymes that have been actively studied in various organisms because of their fundamental involvement with both cancer and aging (figure 1) [1]. There is a variety of different telomerase enzymes whose broad function is to protect the integrity of chromosomal ends by various different means. In prokaryotic organisms, the study of DNA hairpins has proven to be particularly important. DNA hairpin structures regulate gene expression, and therefore play an active role in protecting DNA and controlling mutagenesis [2]. Unlike many proteins that perform similar catalytic functions, TelK is not an ATP dependent enzyme, which is surprising given the large energy barrier intrinsic in DNA hairpin formation.

Students in this module learned to take measurements using high-resolution optical tweezers. These measurements allow one to measure the forces associated with TelK interaction with DNA, and provide fundamental insights into the nature and importance of the electrostatic interactions between TelK and its DNA substrate.

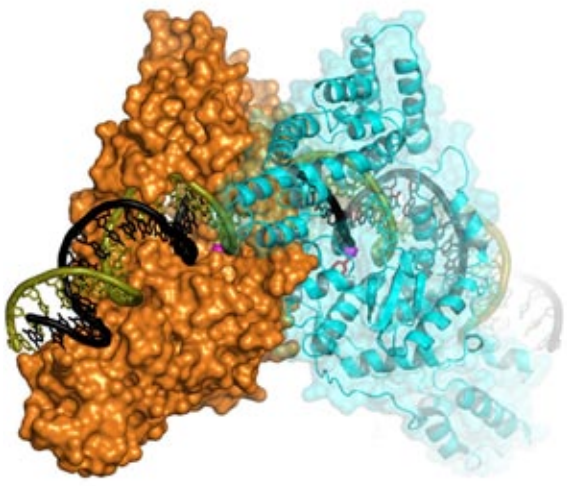

Figure 1. TelK complexed with DNA at the target site.

First, the students learned to synthesize a DNA substrate using polymerase chain reaction, through the amplification of a 3.4 kb segment of a pBR322 DNA plasmid. This DNA product had a biotinylated end and a digoxigenin modification on the opposite end for attachment to modified microspheres. The students analyzed the yield of the PCR reaction through agarose gel electrophoresis with ethidium bromide staining, and purified the DNA using a commercially available DNA purification kit.

Next, the students attached the DNA to the microspheres, built microfluidic chambers, and learned to properly install these chambers into a high-resolution dual optical trap setup by collimating the laser to form two optical traps. The students first took force-extension curves of tethered DNA, and fit these curves to the worm-like chain model of polymer elasticity. The students then introduced TelK protein into one stream of a laminar flow chamber, allowing for careful control of exposure of the DNA tether to TelK. Students took time traces of DNA tethers held in blank buffer, and subsequently took time traces of DNA tethers held in buffer containing TelK.

Results show that, contrary to the currently accepted model for SSDBP-DNA interactions, TelK distorts nonspecific DNA (figure 2). These DNA distortions are likely to correspond to the DNA bending observed in the TelK-DNA crystal structure (Figure 1). Students hypothesized as to why, unlike other SSDBPs, TelK distorts nonspecific DNA sequences. TelK may use an indiscriminate tight binding mechanism as a form of potential energy storage for the eventual energy-expensive formation of DNA hairpins, particularly since it does so in the absence of an external energy cofactor. These attributes may also be general characteristics of single-turnover proteins, which have yet to be well studied at the single-molecule level.

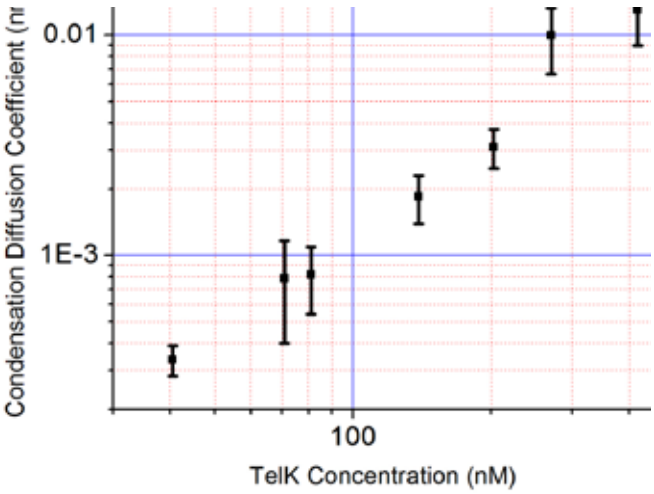

Figure 2. Nonspecific DNA condensation, in nm^2/s, as a function of TelK concentration.

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References:

[1] Shay, J. et al. Rad. Res. 155, 188 (2001).
[2] Zazopoulous, E. et al. Nature 390, 311 (1997).

INGO STEIN

Confocal Microscopy: Analysis of Fluorophore Properties and Single-Molecule FRET

Fluorescence spectroscopy is widely used to address a variety of questions in biophysics and related fields. Due to technical advances, in many of the applications the quality of fluorescent dyes has become the limiting factor. In this project an approach for the reduction of blinking and bleaching of dyes was studied and an application for single molecule FRET on DNA nanostructures was shown.

In the first part of the project the properties of immobilized Cy5 molecules were investigated. Specifically, the working principle of a reducing and oxidizing system (ROXS) for increased dye stability and minimized blinking was demonstrated. Figure 1 illustrates the effect on the fluorophores:

- fast bleaching without oxygen removal
- strong blinking with oxygen removal and ascorbic acid added (1mM)
- constant fluorescence with oxygen removal and ascorbic acid plus methyl viologen added (1mM each)

Especially for fluorescence resonance energy transfer (FRET) studies it is very important to have stable fluorescence to get high quality data and to avoid falsely identifying photophysical intensity fluctuations as biomolecular distance changes.
In the second part of the project rectangular DNA nanostructures were assembled by employing a novel technique for bottom-up fabrication of nanostructures that is based on folding a long DNA strand into a designed structure by around 200 short staple strands. The unique feature of this fabrication method, termed DNA origami and introduced by Paul Rothemund in 2006, is the individual addressability of each position within the structure by modifying the corresponding staple strand. In figure 2 a schematic drawing of the rectangular DNA origami structure is shown along with the positions of two incorporated dyes that act as a FRET pair. For assembly the long scaffold DNA strand is mixed with the short staple strands, heated

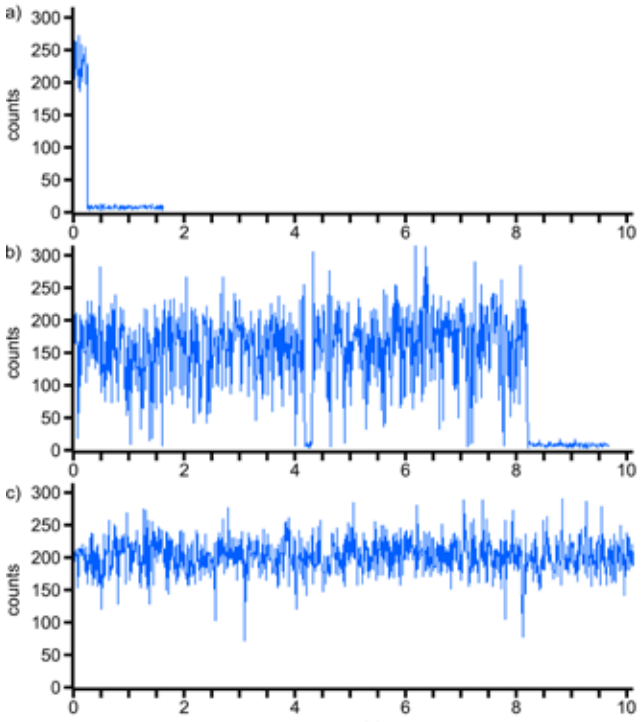

Figure 1: Fluorescence time transients of surface immobilized Cy5 molecules (10 ms binning). Traces a), b) and c) show different buffer conditions as described in the text.

and slowly cooled down to allow annealing of the complementary sequences.

To check the correct folding of the constructs the FRET signal can be used as a read out: Only if the construct folded, the donor and acceptor dye are in close proximity. By using alternating laser excitation (ALEX) of origami molecules diffusing through the confocal volume of a fluorescence microscope, the energy transfer between the dyes was measured. The histogram of the obtained energy transfer values is depicted in figure 2 and clearly shows a significant energy transfer that indicates correctly folded structures.

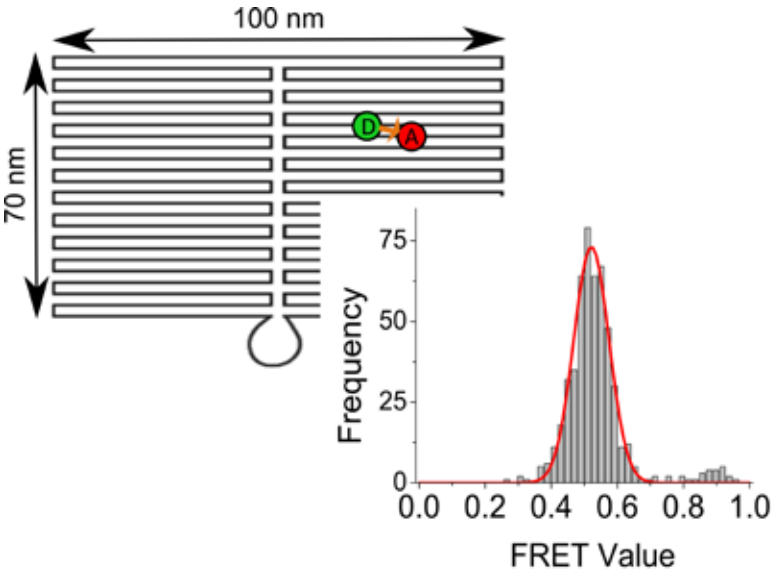

Figure 2: Scheme of a rectangular DNA origami with two incorporated fluorescent dyes.
The energy transfer histogram was obtained from origami molecules diffusing through the laser focus.

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JEEHAE PARK

Single-Molecule FRET Study of a DNA Motor Protein Using TIR Microscopy

Inside a cell, there are families of motor proteins that move along the DNA or RNA and regulate important cellular functions such as replication, transcription, recombination and repair. Motor proteins hydrolyze ATP and convert its energy to directional movement however their detailed mechanism of motion is poorly understood. Here we used Single Molecule FRET to directly monitor a DNA translocase, PcrA pulling ssDNA as it moves in a directional manner.

Observing dynamics of individual molecules provides information on state distribution of population as well as heterogeneous, non-synchronized dynamics of individual molecules. Of many single molecule methods, single-molecule FRET has unique advantage in that highly sensitive distance change (Å) of individual molecules can be detected in parallel for massive populations (200-600 molecules). Here we used Total Internal Reflection (TIR) microscope to illuminate the evanescent field to reject background fluorescence and used a dual color detection scheme to detect fluorescence signals from a FRET pair of fluorophores.

We passivated the surface with polyethylene glycol (PEG) to reject non-specific binding. Biotin-neutravidin linker method is used for specific immobilization of fluorescently labeled DNA on the surface. As PcrA protein and ATP are added to the DNA, the FRET signal increase was observed indicating that the ssDNA is pulled by the action of PcrA. ATP concentrations were varied to identify that these dynamics are driven by ATP hydrolysis.

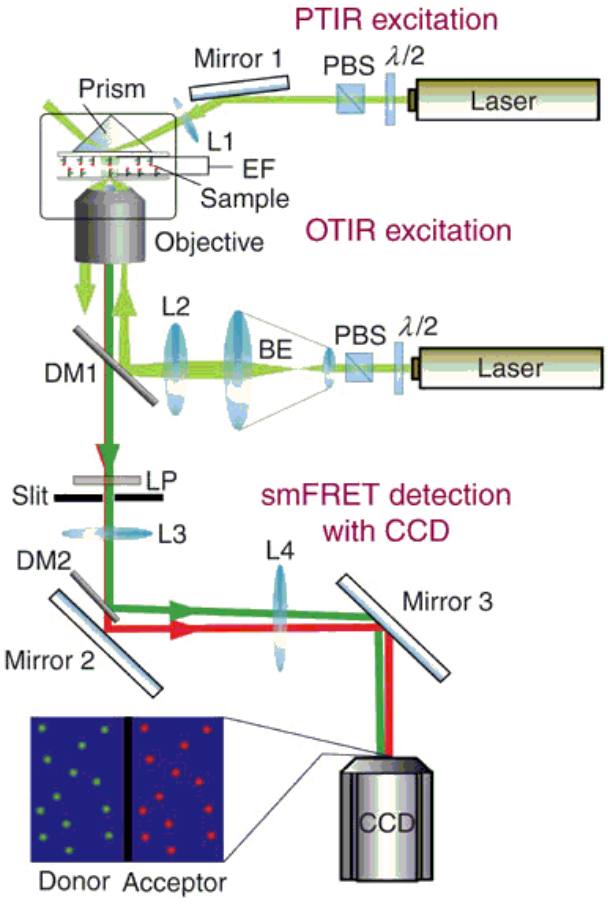
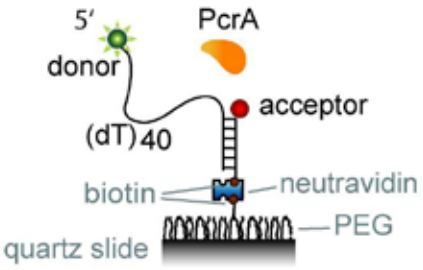


Figure 1: Total Internal Reflection Microscopy for Single-Molecule FRET: In order to observe single molecule, background fluorescence is rejected by illuminating only the evanescence field using total internal reflection (TIR) microscopy. Fluorescence signals that come from the surface immobilized molecules are collected through the objective. After filtering the scattered excitation light using long-pass filter, the donor and the acceptor fluorescence is split by the dichroic mirror and are sent to CCD camera side by side. TIR can be achieved by a prism or simply by a high numerical aperture objective lens.



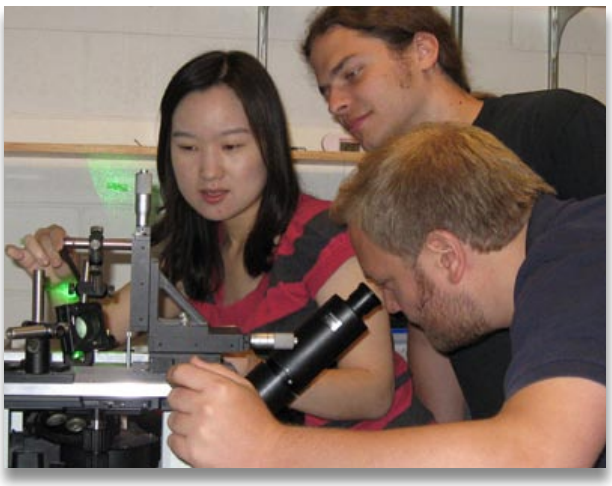
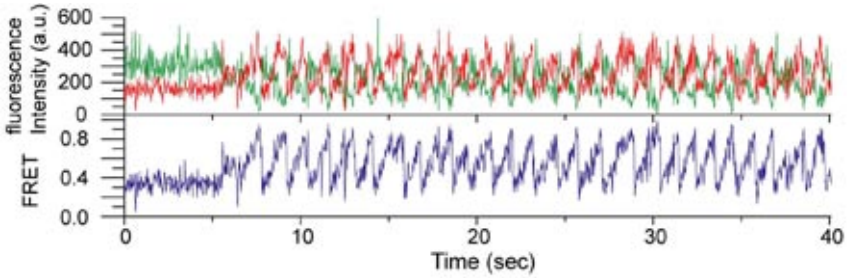
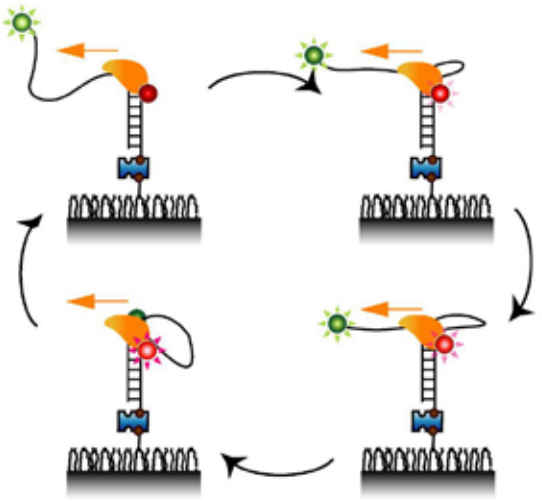


Figure 2: A partial duplex DNA is immobilized through biotin-avidin linkage on a PEG passivated surface to achieve specific binding. When PcrA protein is added with ATP on the immobilized substrate, single strand DNA was pulled through repeatedly resulting repeated sawtooth pattern in FRET signal. FRET is a molecular ruler that reports distance change between the two labeled positions.





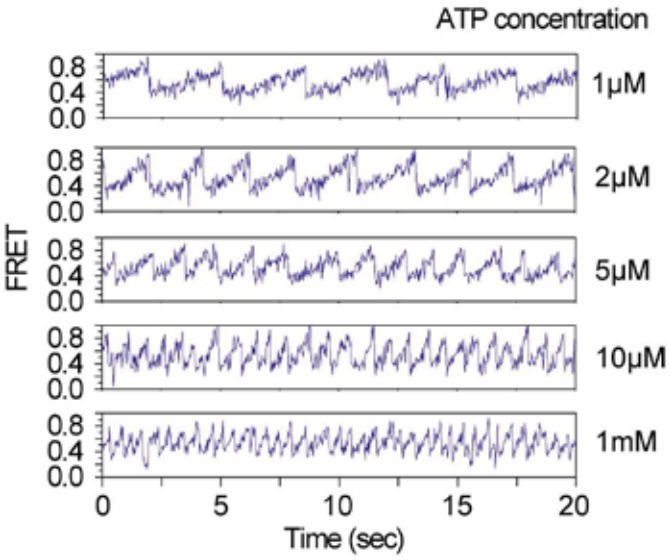


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Figure 3: A model describing PcrA protein reeling-in single strand DNA (left). Reeling-in activity is a result of directional translocation activity which is dependent on ATP hydrolysis (right).

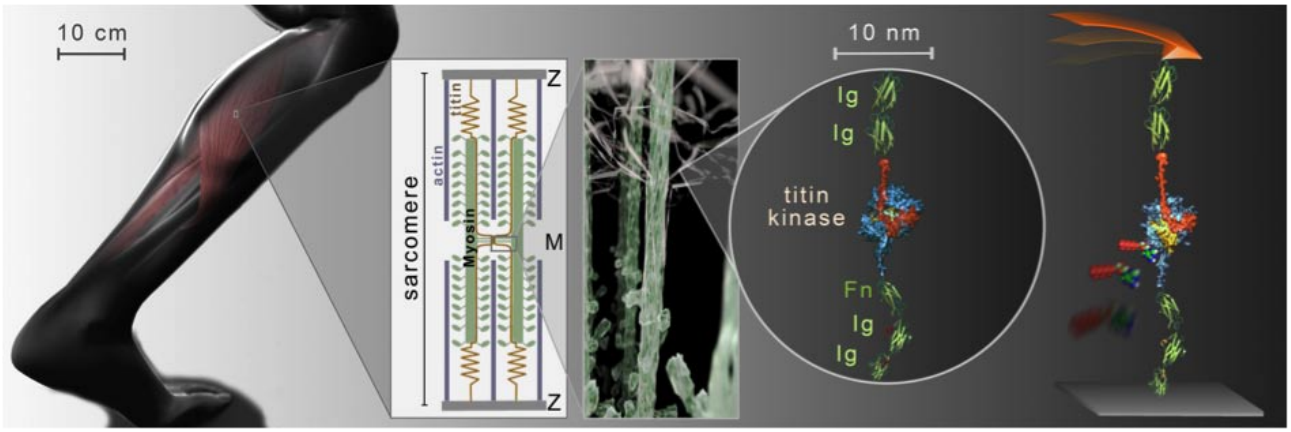
STEFAN STAHL

Single-Molecule Force Spectroscopy (SMFS) on Protein Kinases

Atomic-Force-Microscopy (AFM) based force spectroscopy provides a powerful tool to manipulate single molecules and study their behavior under load. As more and more recent investigations point out, force plays an important role in cellular regulation mechanisms. Biological responses to mechanical stress are often based on force induced conformational changes of single molecules, which serve as force sensors. The aim of this project is to demonstrate the application of SMFS for the study of such molecular force sensors based on the knowledge that has been developed in our group on the model system titin kinase.

The autoinhibited enzyme titin kinase is embedded at an ideal position in the sarcomeric M-band structure to sense force. When pulling on titin kinase by means of single-molecule force spectroscopy one observes a very regular unfolding pattern with substep openings at physiological forces. The occurence of one of the barriers in the pattern is influenced both by the presence of ATP and pulling velocity. The underlying model which is supported by molecular dynamic simulations is that the ATP binding pocket, which is blocked in the not stretched conformation gets mechanically opened. Furthermore it is possible to measure the barrier positions along the sequential activation pathway up to a few amino acids. Such AFM based force spectroscopy allows a structural understanding of this force-sensor that regulates gene expression and protein turnover.

In the JNN project, single-molecule pulling experiments were performed on myosin-light-chain-kinase, a protein with a sequential proximity to titin kinase. The question arises whether similar working principles are present for this construct for which no structural data is available. Overlays of unfolding traces show that there is a sequential unfolding as well and preliminary data suggest that one of the elements causing substeps in protein unfolding is stabilized in the presence of ATP.



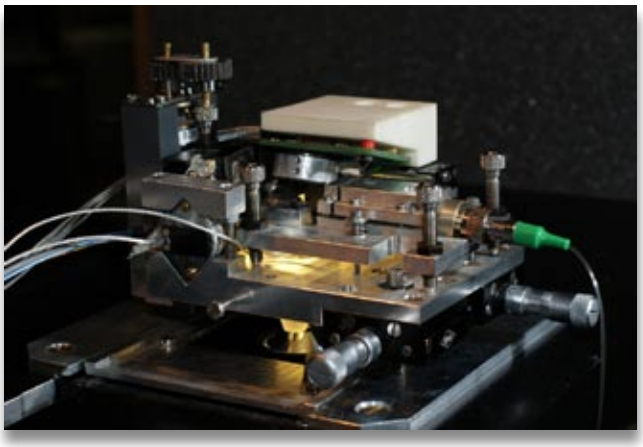


Figure 1: Experimental setup. The force-extension traces were recorded with a custom built AFM head specialized for long term SMFS data aquisition.

Figure 2: Superposition of titin kinase unfolding traces at 720 nm/s without ATP in solution. 5 characteristic barriers are observed around 50 pN until the whole kinase is streched. Unfolding of the surrounding marker domains (Ig-/Fn like) occurs at higher forces.

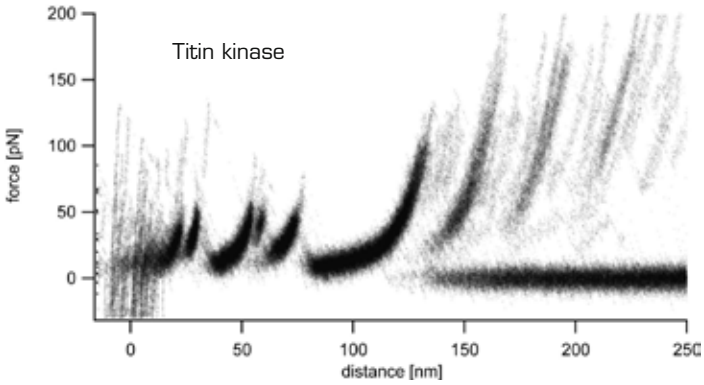
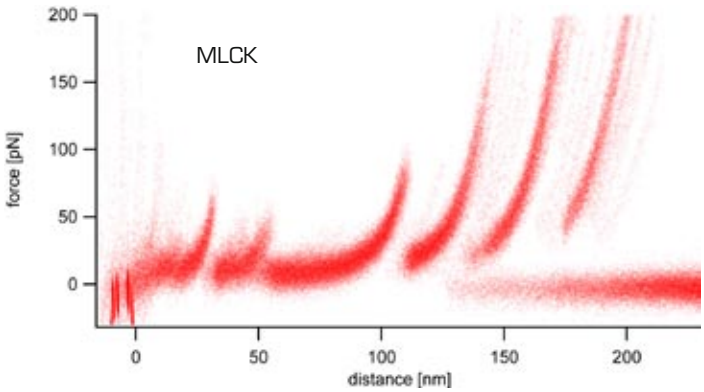


Figure 3: Superposition of MLCK unfolding traces at 1 µm/s pulling speed with 200 µM ATP in solution shows that MLCK unfolding is sequential with disinct characteristic engery barriers as well.



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ANKUR JAIN

Single-Molecule Immunoprecipitation

Immunoprecipitation (IP) is the technique of precipitating a protein out of solution using an antibody that specifically binds to the protein of interest. It is one of the most widely immunochemical techniques and forms the basis for various methods for detecting protein-protein (co-immunoprecipitation) and protein-nucleic acid (ChIP, RIP) interaction. We will perform immunoprecipitation at a single molecule level. Individual immunoprecipitated molecules are imaged using TIRF microscopy, enabling direct visualization of protein-protein and protein-DNA interaction.

We start with PEG (poly ethylene glycol) passivated quartz surfaces. PEG forms a dense polymer brush that prevents non-specific adsorption of cellular proteins and antibodies to the surface (1). A small amount of biotinylated PEG is incorporated while surface preparation to immobilize NeutrAvidin, which can be used for immobilizing biotinylated proteins and DNA. Flow chambers are prepared as described in Joo et al. (2).

Biotinylated antibodies against the protein of interest are immobilized and crude cell lysate (unpurified, protein of interest) is flowed in. The protein of interest (bait) binds to the antibody and co-precipitates its interacting partners. The identity of proteins bound to the bait is verified either by using fluorescent protein fusion constructs or through antibodies against anticipated targets. For a multimeric protein complex, fluorophore labeled antibodies against its subunits colocalize in the same diffraction limited spot. Using multicolor fluorescence colocalization we can ascertain the molecular composition of the complex and comment on interaction heterogeneity

We use protein kinase A(PKA) as the model system for this experiment. PKA is a serine threonine kinase and regulates signal transduction downstream of GPCRs. PKA is a heterotetramer with two catalytic and two regulatory subunits bound with a sub-nanomolar dissociation constant, in the absence of cyclic AMP. PKAC-HA-YFP and PKARIIb-FLAG-mCherry are overexpressed in HEK293T cells and cell lysates are prepared.

We immobilize antibodies against FLAG tag on the surface. PKARIIb-FLAG-mCherry is the bait and is visualized by mCherry fluorescence. Interacting partner, PKAC-HA-YFP is visualized via YFP fluorescence. YFP and mCherry colocalize in the same diffraction limited spot. Upon adding cAMP, PKAC-HA-YFP dissociates leaving behind mCherry spots only.

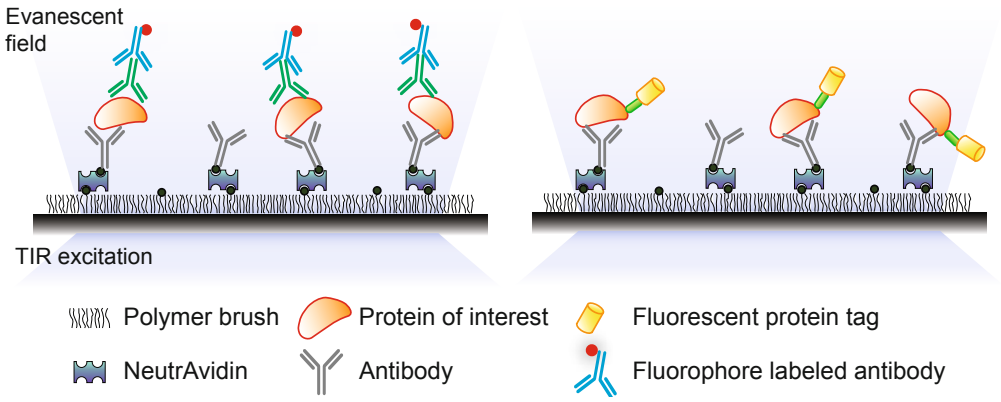
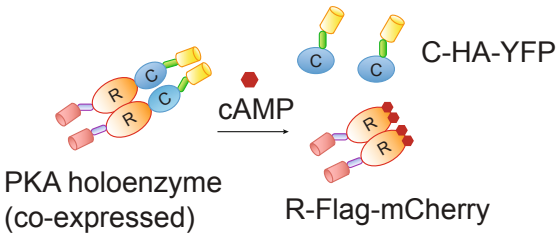
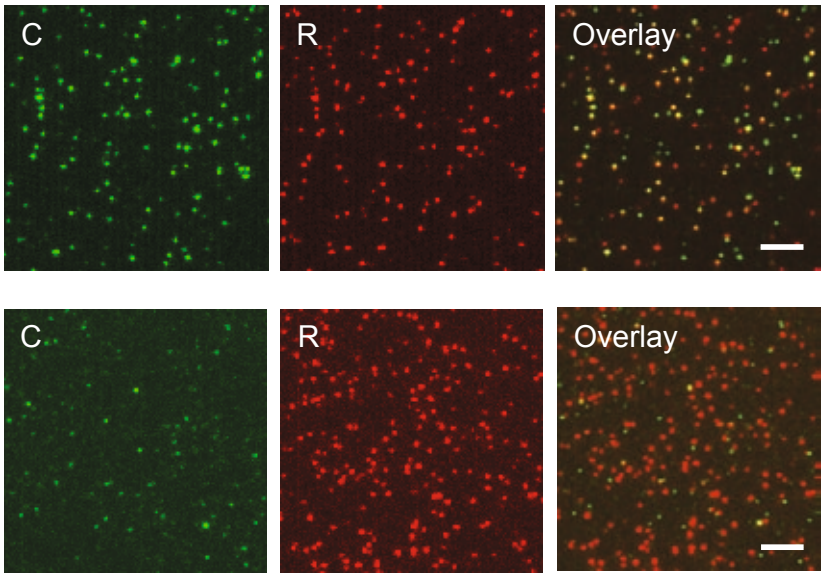


Figure 1: Schematic for single molecule immunoprecipitation.





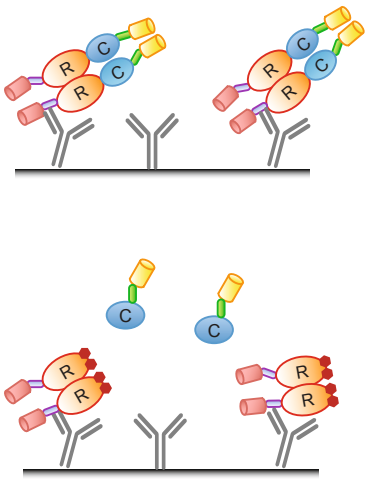


Figure 2: PKA constructs and TIRF images of C-YFP, R-mCherry and overlay.

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References:

1) Ha, T., I. Rasnik, W. Cheng, H. P. Babcock, G. Gauss, T. M. Lohman, and S. Chu, "Initiation and Re-initiation of DNA Unwinding by the Echerichia Coli Rep Helicase", Nature, 419, 638-641 (2002).

2) Joo C., and Ha T., "Single-Molecule FRET with Total Internal Reflection Microscopy", in Single-Molecule Techniques: A Laboratory Manual, pp 3-36, ed. Ha T. and Selvin P., Cold Spring Harbor Laboratory Press, NY (2008).

STEPHAN HEUCKE

Single-Molecule Cut-and-Paste

Spatial isolation of the investigated molecule is a prerequisite in optical single-molecule experiments. Commonly this is done with simple dilution. However, this way it is impossible to position the single-molecules relative to objects or secondary molecules whose influence might be of scientific interest. A novel bottom-up assembly method is Single-Molecule Cut-and-Paste (SMCP). Here single molecules are picked up from a depot and are positioned in a previously empty target area. This is accomplished with the tip of an atomic force microscope (AFM) and the help of differentially coupled force anchors made from DNA. Thus SMCP provides the ability to isolate single-molecules at designated positions with nanometer precision. As an exemplary application SMCP could be used to set up networks of different interacting enzymes in designed geometries.

In the JNN project the visiting students first needed to functionalize the specimen slides, AFM tips and dyes required for SMCP: The silanized glass slides were passivated with polyethylene glycol. Following, a depot and a target region of different DNA-force anchors were written using a micro-spotter. The depot-DNA had a fluorescent dye attached as depicted in Figure 1. AFM cantilevers were functionalized with a similar recipe.
With these samples the students went to the optics laboratory and used a homebuilt AFM to pick up single dyes from the depot region and deposit them in the target area. Force curves of the pick up and deposition process were recorded by the AFM and monitored simultaneously by the students (Fig. 2). The written pattern, a smiley face (Fig. 3), was then observed by using a total internal reflection fluorescence microscope built into the setup.

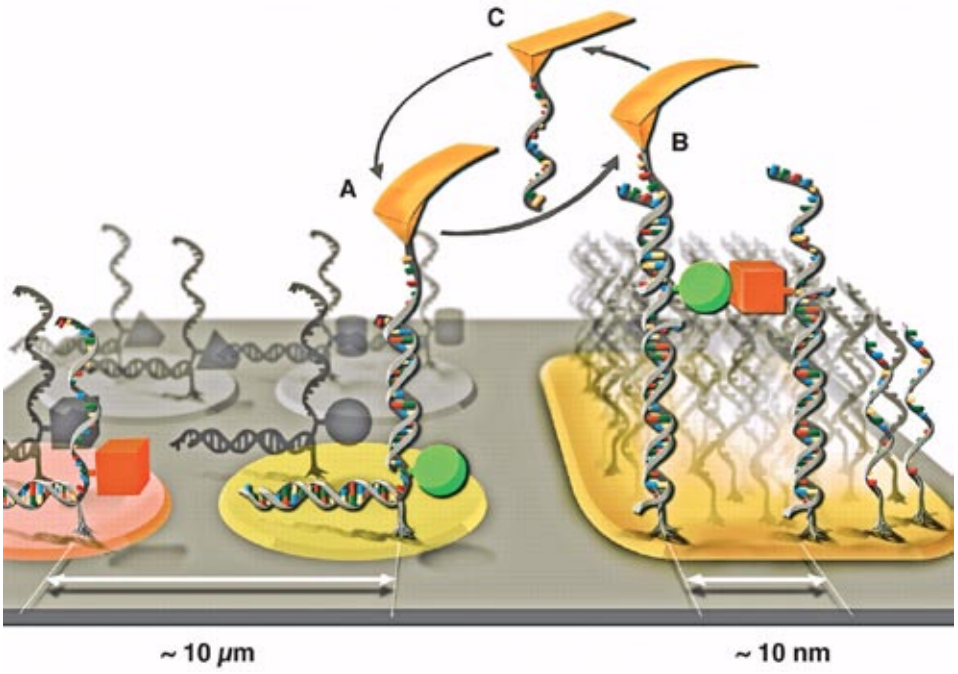
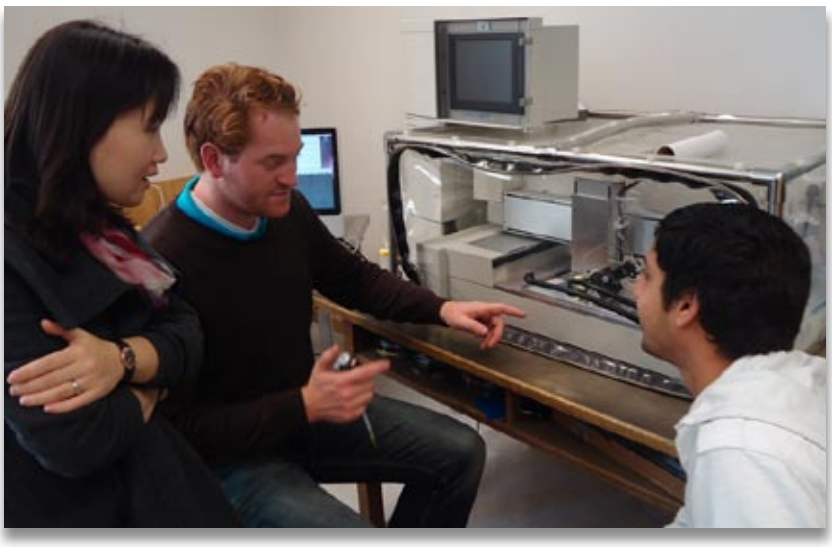


Figure 1: Cartoon of the Single-Molecule Cut-and-Paste principle: A functionalized tip of an AFM cantilever binds to a DNA-anchored single-molecule in a depot region (A). Pick-up and deposition are accomplished via hierarchical forces (B). The cantilever can then be used for transportation of the next molecule. (C).



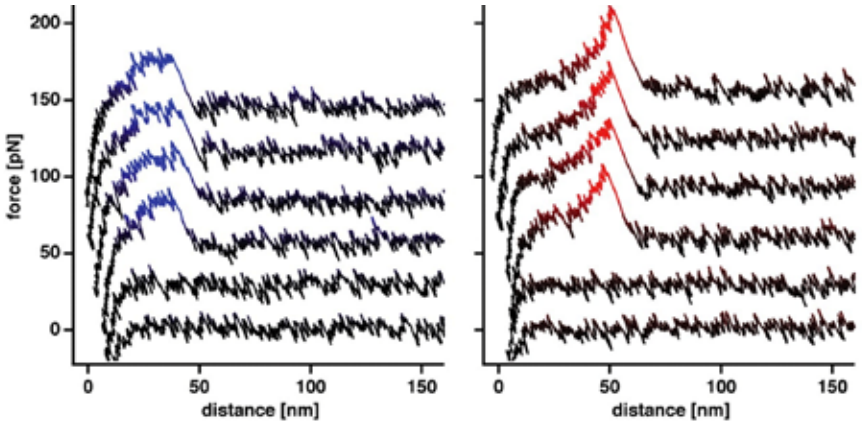


Figure 2: Force curves of the unzipping DNA in the depot (left) and target area respectively.

Reference:
Kufer, SK, Puchner, EM, Gumpp, H, Liedl, T, Gaub, HE.
Single-molecule cut-and-paste surface assembly.
Science 2008;319 (5863):594-6.

Figure 3: Smiley written with single molecules.

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HELEN HWANG

Characterization of Single-Molecule Protein Induced Fluorescence

Protein Induced Fluorescence Enhancement (PIFE) was recently developed as an alternative single molecule assay that circumvents protein labeling where a single fluorophore reports on the proximity of the interacting protein. This photophysical effect is mediated by increases in local viscosity that reduces the cis "non-fluorescent" isomerization of the dye, thereby increasing its quantum yield and lifetime. However, the distance sensitivity of PIFE assay is still unknown.

In this experiment, we employ a restriction enzyme BamHI, which binds site-specifically to dsDNA (GGATCC). By designing DNA constructs to bear Cy3 fluorophore on one end and the BamHI recognition sites at several different locations away from Cy3, we can measure the intensity change of the dye before and after the addition of BamHI.

Raw intensities from a single molecule are normalized to the non-protein bound DNA intensity and a cumulative intensity fold change is calculated for each DNA construct. Seemingly, PIFE displays a sharp distance-sensitive range under the 4nm range where FRET is insensitive. Combined with FRET, PIFE can reveal hidden dynamics in short distances such as several base pairs and nucleotides.

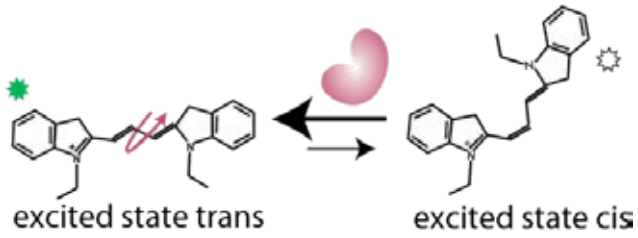


Figure 1: In the presence of protein, the trans state is favored in the cis-trans isomerization of Cy3 fluorophore.

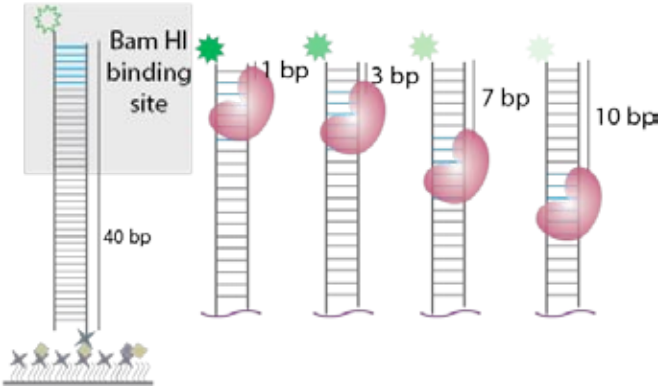
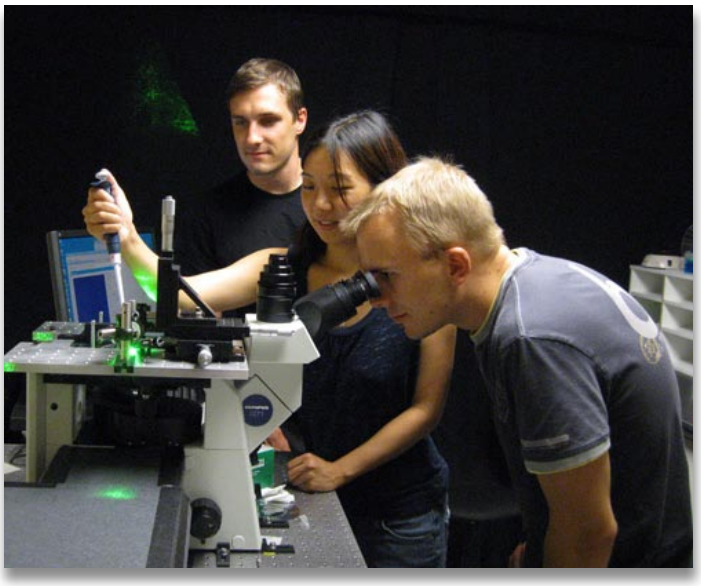


Figure 2: BamHI recognition sequence (blue) at a certain distance away from the Cy3 (green) on a 40-bp double stranded DNA.



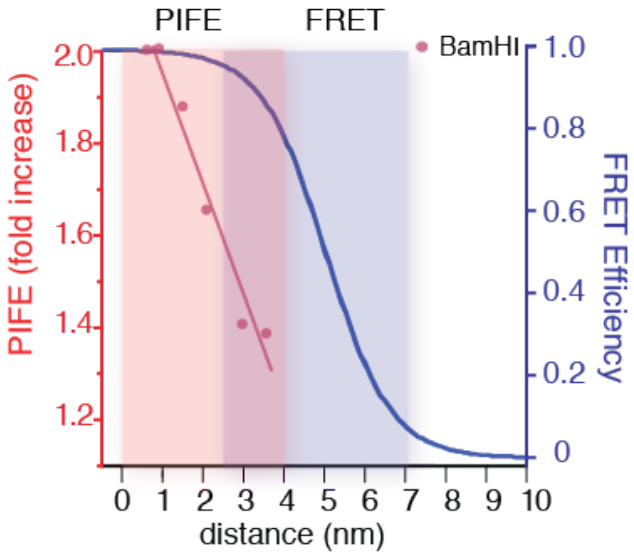


Figure 3: Distance sensitivity of PIFE vs. FRET. PIFE is sensitive in regions where FRET is insensitive.

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NIKO NAREDI-RAINER

Exploring the Folding Efficiency of GFP with Dual-Color Fluorescence-Correlation-Spectroscopy

As our understanding of cellular processes increases, there is a need for methodologies to ascertain molecular concentration, dynamics, and organization at high temporal and spatial resolution. A common approach to study cellular dynamics uses fluorescent molecules that can be observed by confocal microscopy. Single-point fluorescence correlation spectroscopy (FCS) originally developed by Magde et al. for solutions provides high temporal resolution information about protein concentrations and dynamics within a small volume (~1fL) of the cell. In recent years, not only have new artificially dyes proven to be useful, but also fluorescent proteins (e.g. GFP – figure 1) have become more and more important - as seen by the Nobel prize in chemistry in 2008 for the discovery of GFP.

In this project, the folding efficiency of GFP is determined with the help of fluorescence cross-correlation spectroscopy by using a GFP-linker-mCherry construct that is expressed in CHO cells. With the help of a global fit applied to the measurement, shown in figure 2, it is possible to calculate the amount of protein that carries only one fluorescent protein and the amount that have two fluorescent proteins. This ratio allows a lower estimate of the percentage of correctly folded proteins.

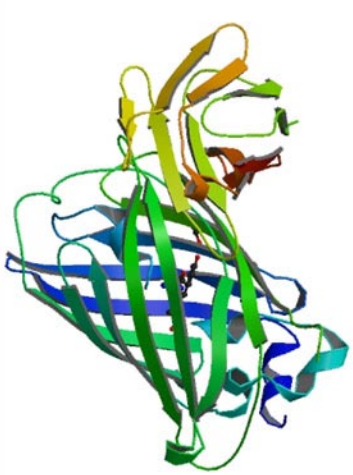

Figure 1: Crystal structure of GFP.

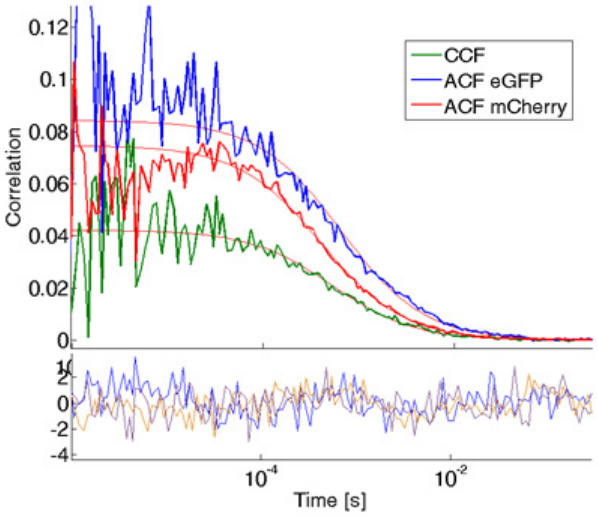

Figure 2: Auto- and Cross correlation curves.

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JOHAN STRÜMPFER, CHAITANYA SATHE, ROGAN CARR

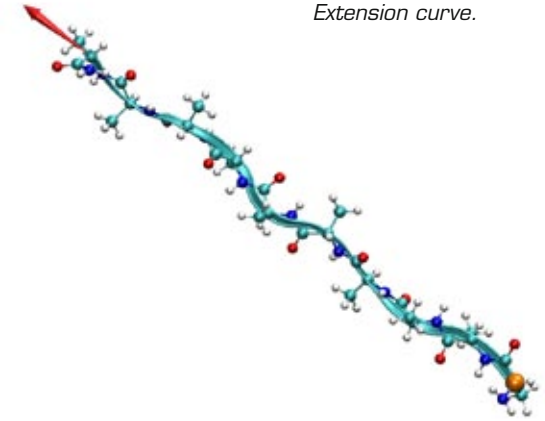
Molecular Dynamics as a Research Tool

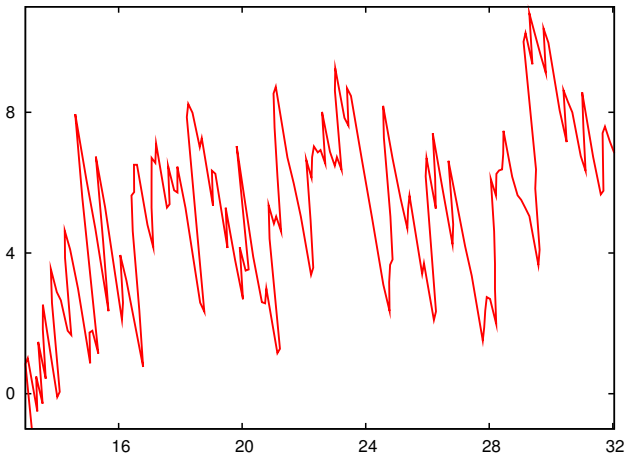
While the spatial resolution available with current experimental techniques is ever improving, it has not yet reached that of identifying individual atoms for many techniques. Molecular dynamics (MD) offers a complete description of all dynamically interacting atoms in a system. By modeling experimental systems and being able to analyze and visualize the dynamics of each individual atom, researchers can gain an unparalleled perspective and understanding in their research.
In the "Theory" workstations the students were taught the basic concepts of MD and how to set up and run MD simulations using NAMD [1]. Being able to set up and run a simulation, however, does not teach one how to extract useful information that is useful in research. They were also shown some of the typical analysis techniques that are available in VMD [2].
In addition to being introduced to the basics of MD simulations, students were also taught 3 more advanced techniques: (1) How to simulate pulling forces, in emulation of optical tweezer or atomic force-microscopy experiments; (2) How to build and analyze water permeation through carbon-nanotubes; (3) How to build and analyze a hybrid biological-inorganic systems using coarse grained molecular dynamics.

Pulling Deca-Alanine

Testing the response of biological systems to mechanical forces is one of the basic methods used in biophysics today. Experimental techniques such as AFM and optical tweezers provide great insight into the force response of individual macromolecules. Being able to visualize how the macromolecules behave when forces are applied, with a resolution unobtainable in experiments, offer us an even greater understanding. In this advanced technique, students were taught how to pull on deca-alanine during a molecular dynamics simulation and how to obtain the resulting force-extension curve, such as that shown in Figure 1.

Figure 1: Deca-alanine and a resulting Force-vs-Extension curve.





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Water Permeation Through Carbon Nanotubes

Carbon nanotubes are nano structures, which can be used as templates for understanding water permeation through transmembrane channels. Molecular dynamics can be used as a microscope to look at the orientation of water dipoles inside the tubes and also study the effect of modification in nanotubes on the ordering of water molecules. Simulating such a system can enhance our understanding of many biological and artificial water channels. In this module students build a system comprising of nanotube filled with water molecules and then analyze the diffusion of water through the nanotube. The nanotube is decorated with charges and the effect of charges on the flow of water through the tube is studied. This simple example also allows the researchers to play with the Van der Waals parameters, which govern the interaction of the water molecules with the nanotubes. The template is also used to introduce the researchers to the AutoIMD feature of NAMD, where one can automatically setup a NAMD simulation that can be visualized in real-time in VMD.

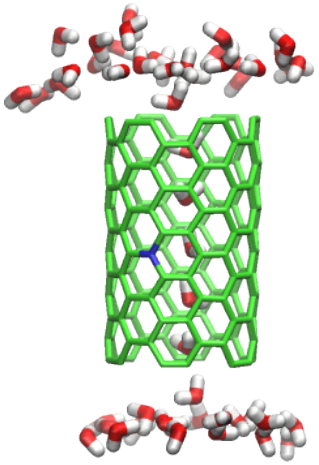
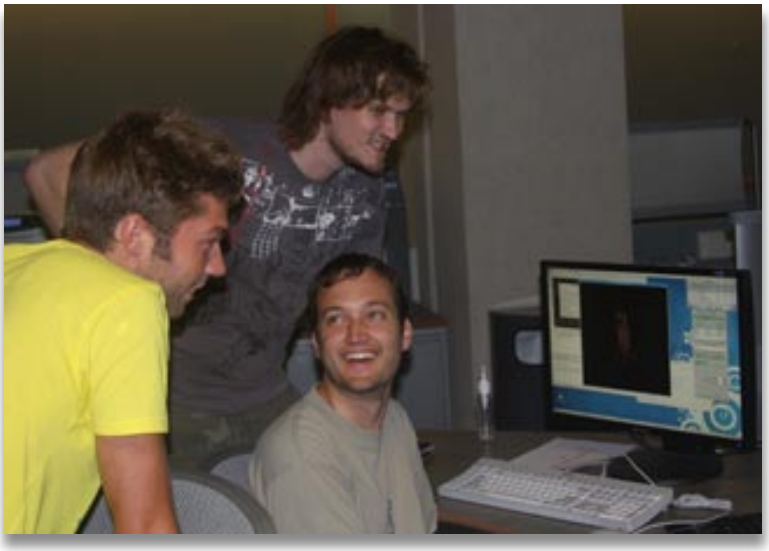


Figure 2: Decorated nanotube water channel.



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Modeling Hybrid Biological–Inorganic Systems

Today it is of great interest to combine biological and inorganic components to make devices that take advantage of the strengths of both biological machinery and solid-state technology. Molecular dynamics offers a platform by which these devices can be modeled and simulated, allowing researchers to refine the design of their hybrid devices. In this advanced technique, each researcher recreated the study, "Synthetic Ion Channels via Self-Assembly: a Route for Embedding Porous Polyoxometalate Nanocapsules in Lipid Bilayer Membranes" [3], detailed in Fig. 3, and were taught how to design and implement a research project involving the interface between biological and inorganic systems using coarse-grain molecular dynamics (MD) as a tool.

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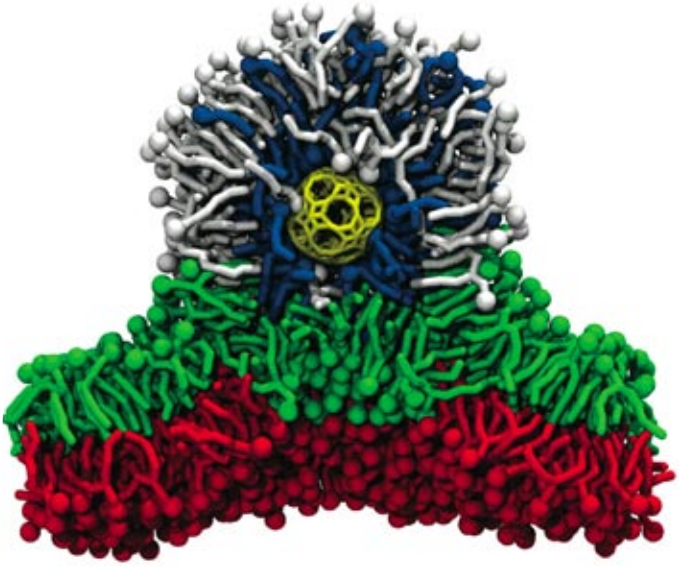


Figure 3 Simulating lipid membrane fusion. A liposomal structure containing a polyoxomolybdate nanocapsule (yellow), dimethyldioctadecylammonium surfactant (blue) and Palmitoyloleoyl-phosphatidylcholine (POPC) lipids (white) fuses with a POPC lipid bilayer membrane, with the upper and lower leaflets shown in green and red.

References:
[1] J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kale, and K. Schulten. J. Comp. Chem., 2005 26, pp 1781-1802.
[2] W. Humphrey, A. Dalke, and K. Schulten. J. Mol. Graph., 1996, 14, pp 33-38.
[3] R. Carr, I.A. Weinstock, A. Sivaprasadarao, A. Müller and A. Aksimentiev. Nano Lett., 2008, 8 (11), pp 3916–3921.

WOLFGANG KÜGEL

Single Molecule Multiparameter Fluorescence

The aim of the project was to give an introduction to confocal multi-parameter fluorescence detection (MFD). Photons emitted by molecules diffusing through the confocal volume are detected according to their color as well as their polarization relative to the excitation as shown in Figure 1. Combined with a burst search this allows for the simultaneous access to a number of parameters such as FRET efficiency, fluorescence lifetime and anisotropy on a single molecule level.

DNA hairpins can exist in a closed and an open conformation and buffer conditions as well as the choice of the dye molecules change the equilibrium. Here it is demonstrated how MFD can reveal the distribution of conformations (Figure 2).

While normal single molecule counting experiments are limited to the analysis of Stoichiometry and FRET, MFD gives access to additional parameters such as lifetime and anisotropy. An example from RNA Polymerase II shows that these are important to distinguish different states.

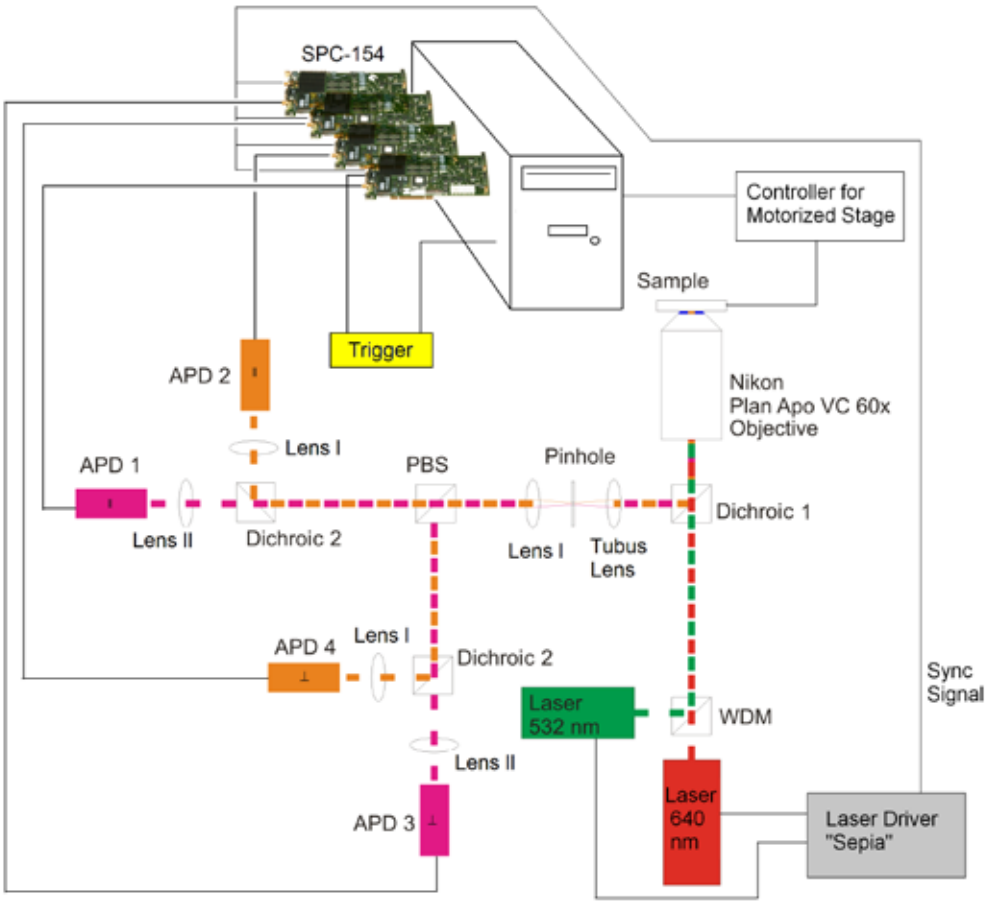


Figure 1: Experimental scheme.

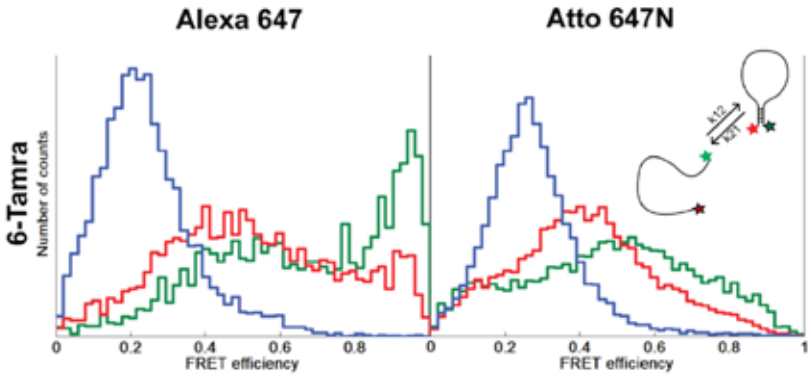


Figure 2: Exemplary comparison of DNA hairpin FRET efficiencies of selected dye combinations at NaCl concentrations of 10 mM (blue) 160 mM (red) and 320 mM (green).

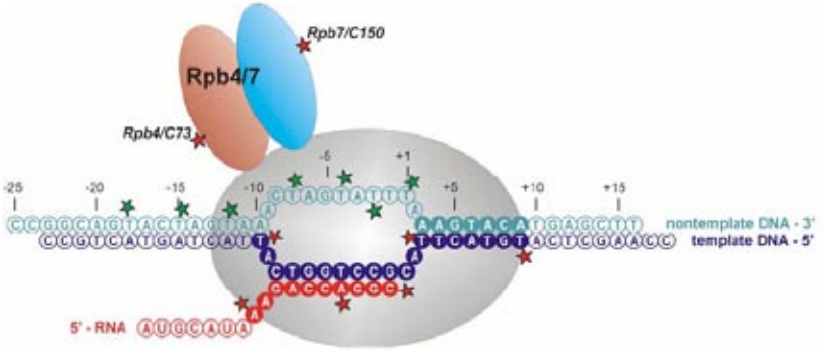
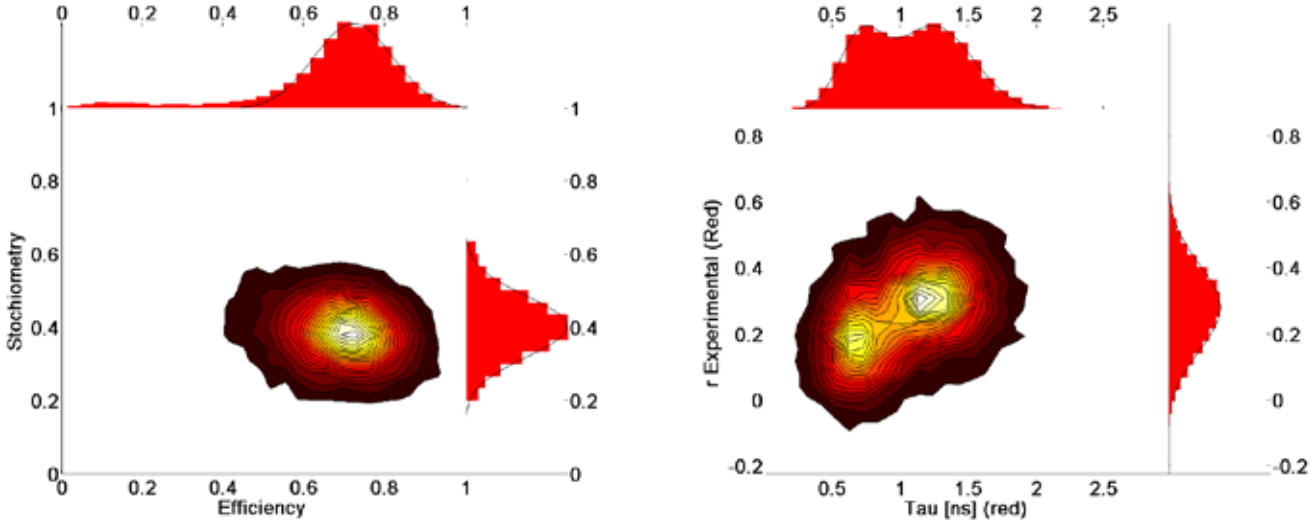


Figure 3: Artificially mismatched DNA constructs with (high lifetime and anisotropy) and without (low lifetime and anisotropy) RNA polymerase II cannot be resolved in the FRET-Stoichiometry parameter space but are clearly visible in anisotropy and lifetime.



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KYUNG SUK LEE

Single Molecule Fluorescence Combined With Optical Tweezers

This project shows that the combination of two single molecule techniques can provide complementary information and be useful: while the binding and dissociation of fluorophore-labeled single stranded DNA binding proteins (SSB) to a DNA is monitored by fluorescence microscopy, optical tweezers manipulate and record the tension applied on DNA. The dependence of binding and dissociation probability of the SSB to the applied tension can be studied in a single experiment.

Single stranded DNA binding proteins (SSB) are the proteins that preferentially bind to ssDNA. Their role is to protect the single stranded DNA (ssDNA) from degradation and to control the accessibility of ssDNA to other proteins. *E. coli* SSB has been selected to study the interaction between SSB and DNA because it forms a stable homo-tetramer. E. coli SSB binds to ssDNA in two major binding modes: 65-binding mode and 35-binding modes as shown in Figure 1.

In this project, the binding and dissociation of E. coli SSB to the 70 nt long ssDNA (dT70) in the 65-binding mode is observed. Alexa555-labeled SSB binds to and dissociates from the 70 nt ssDNA region in the DNA tether, while various tensions are applied, as shown in Figure 2.

By analyzing the tension-fluorescence trajectories (Figure 3), the SSB binding events and the corresponding tension at the moment of binding can be recorded, and the same goes with the SSB dissociation events. The statistics of these events reveals the tension-dependence of the binding and dissociation probability of the SSB to the ssDNA.

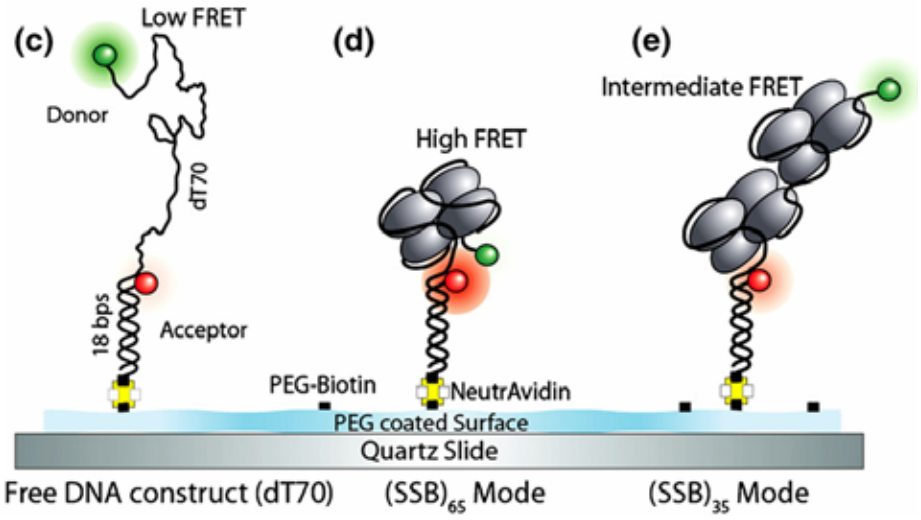
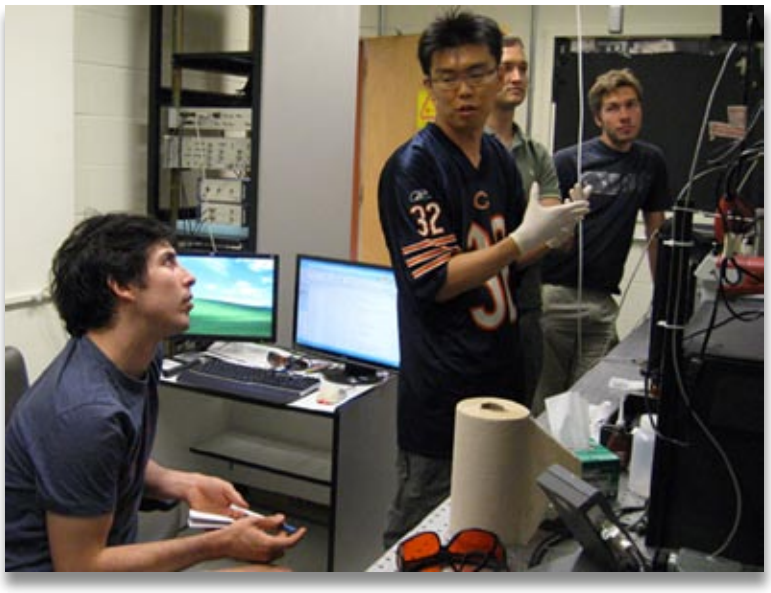


Figure 1: Two binding modes of SSB.



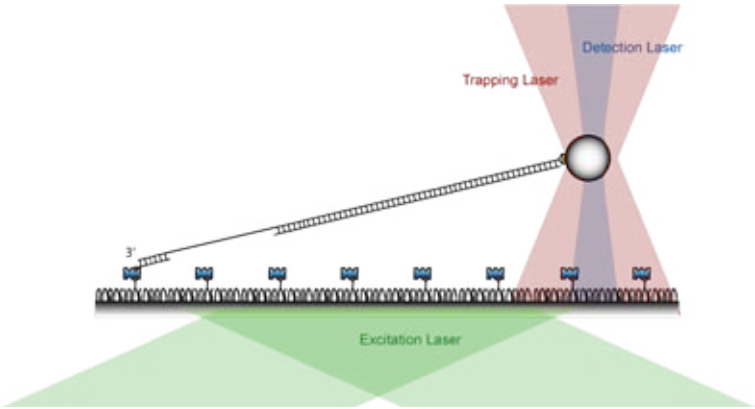


Figure 2: Experimental scheme.

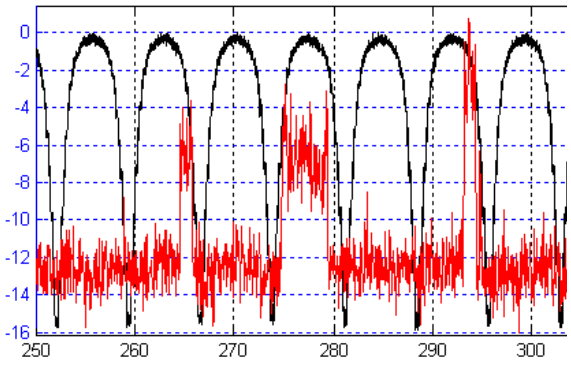


Figure 3: Tension – Fluorescence Trajectory.

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JOHANNES STIGLER

Force Spectroscopy on Calmodulin with Optical Tweezers

Optical Tweezers are a sensitive tool to measure forces in the piconewton regime and lengths on the nanometer scale. Low drift along with specific attachment allow for the measurement of a single molecule on the timescale of several minutes. We employed this technique to investigate the folding properties of Calmodulin (CaM), a ubiquitous calcium signal transducing protein.

The protein was attached N- and C-terminally to dsDNA handles of 370 nm length. The ends of the DNA were then bound to micron-sized glass beads by biotin-streptavidin and digoxigenin-antibody linkage. Measurements were performed on a dual-beam infrared trap with oil immersion objectives and back focal plane detection (Figure 1). In stretch-and-relax probe experiments we first ensured the formation of a proper dumbbell configuration with only one protein-DNA hybrid bound to the beads. The fast refolding kinetics of CaM allowed us to measure folding and refolding close to equilibrium conditions. We observed unfolding of several subdomains of the protein with various pathways. In constant-distance-mode we then are able to separate at least five different states (Figure 3). Subsequent analysis of the recorded traces allowed us to extract kinetic parameters such as transition rates, position of transition barriers and equilibrium energies.

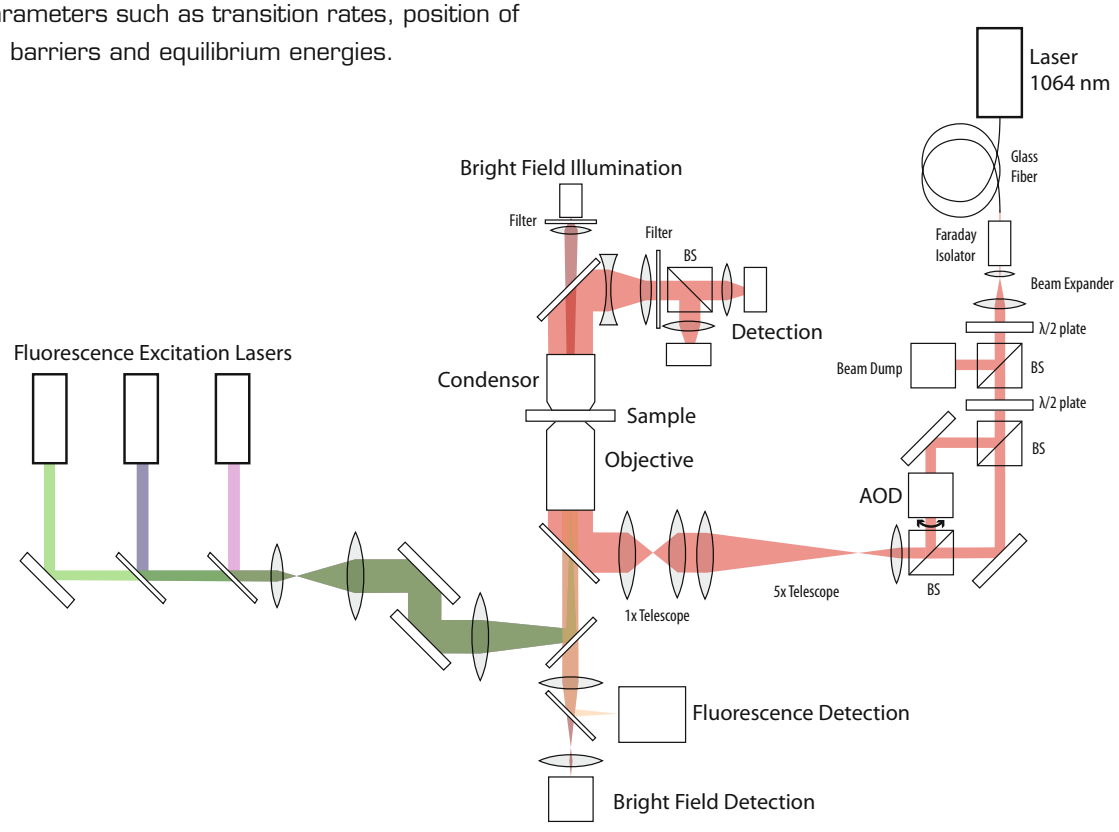

Figure 1: Experimental setup.

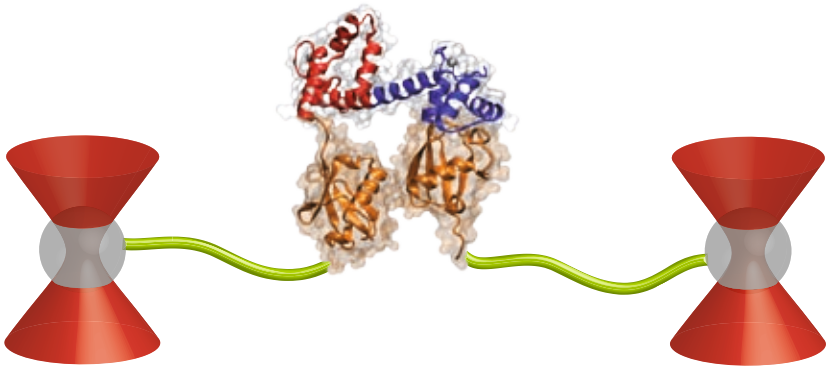

Figure 2: Zoom-in on the dumbbell configuration during the measurement. The protein is linked with ubiquitin-DNA hybrid handles (orange and green) to functionalized glass beads. Shown in red is the CaM N-domain, in blue the C-domain.

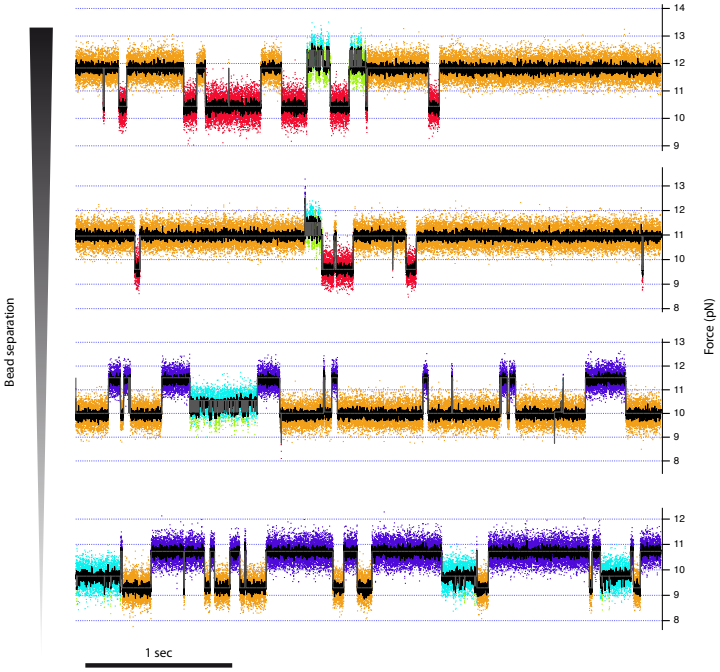

Figure 3: Rapid transitions between several states can be observed. By changing the trap center positions different pretensions can be applied to the protein, thus shifting the equilibrium between the states.

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BJÖRN HELLENKAMP

Structure and Dynamics of Hsp90 Investigated by Single Molecule FRET

Hsp90 is a molecular chaperone required for survival of the cell under physiological conditions, during heat shock and for the activation of a large set of client proteins. It consists of two monomeric chains with dimerization interfaces at the C and N-terminal end [1]. We investigated the N- and C-terminal dimerization kinetics of Hsp90 with smFRET [2,3]. Surprisingly, we found nucleotide dependent C-terminal dissociation/association kinetics on the timescale of seconds although the nucleotide binding pocket is far away in the N-terminal domain [3]. The crystal structure of the completely closed state has been solved [1], but both the C- and N-terminal open states have not been crystallized yet. We use PIE-FRET (Pulsed Interleaved Excitation FRET) to triangulate the position of these domains in the open state.

PIE-FRET

The Hsp90 dimer is labelled with donor- and acceptor dye one per monomer and then FRET is measured with a self-made confocal setup that is well suited to avoid surface interactions and to have a high throughput. The proteins are diffusing freely through the confocal volumes of two appropriate lasers (Fig 1) that are pulsed interleaved with 80 MHz to measure also fluorescence lifetimes. For each photon burst that increases certain threshold FRET efficiency and stoichiometry S are calculated and added to a 2D histogram. By distinguishing events from single labelled or denatured proteins we calculate the corrected FRET efficiency (Fig 2). Furthermore selecting bursts for diffusion times could be helpful for aggregation studies.

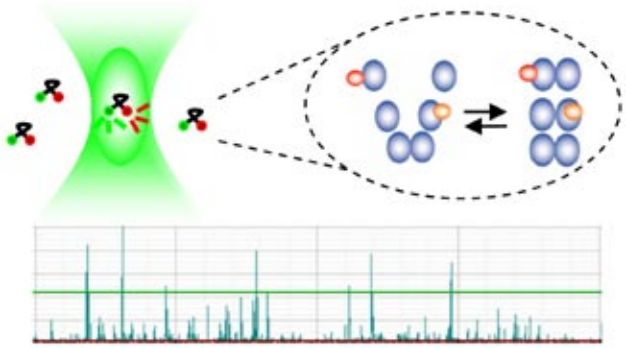


Fig 1: Hsp90 dimers diffusing through the confocal volumes of two lasers; burst selection by intensity.

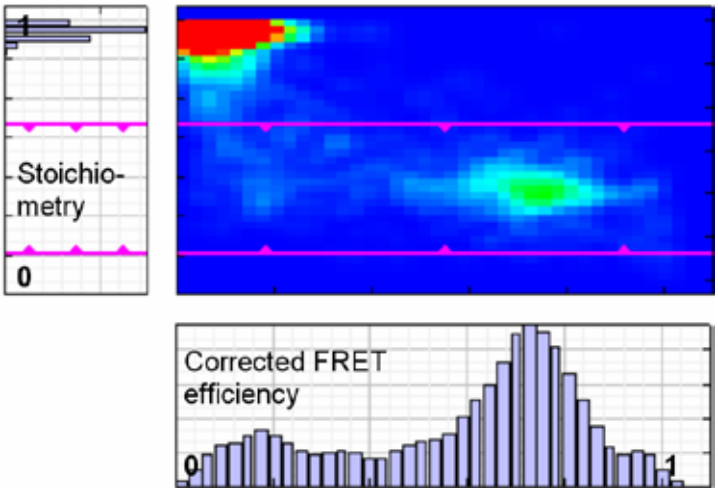
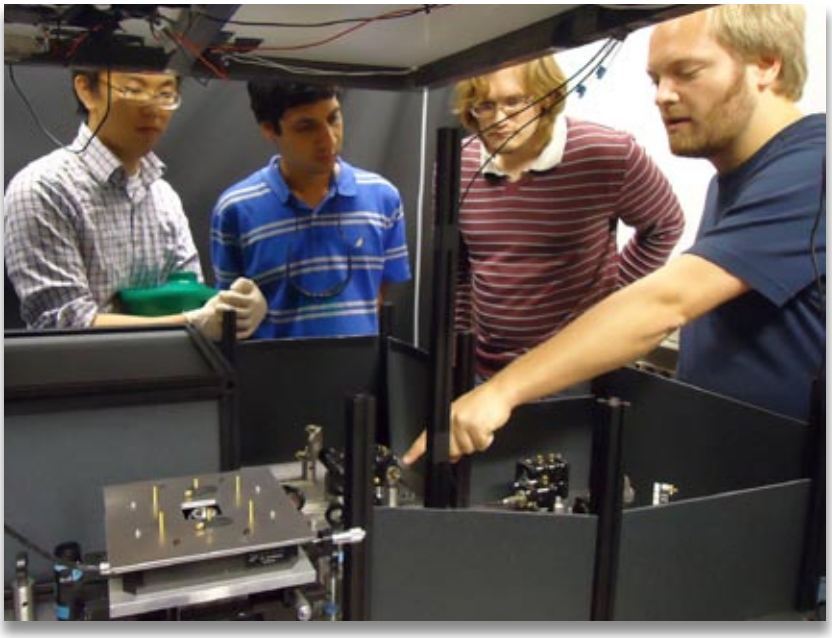


Fig 2: Stoichiometrie-FRET-correlogram of Hsp90.



Triangulation

Measuring distances of six donor-acceptor-pairs or more and using a nano-positioning system [4] we get position and torsion of the N-domain in the open state. The distances of the closed state can be used for validation (Fig 3).

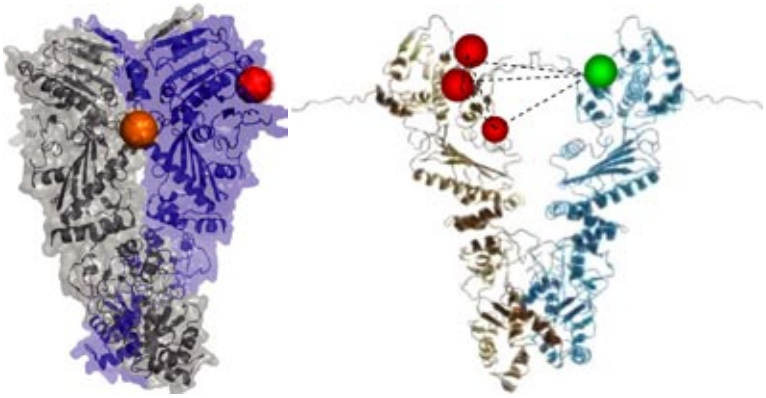


Fig 3: Left: crystal structure of Hsp90 in the closed state; right: triangulation to solve the open state.

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References:
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[4] A Muschielok, J. Andreacka, A. Jawhari, F. Brückner, P. Cramer, J. Michaelis, Nature Methods (2008)

BRENDAN OSBERG

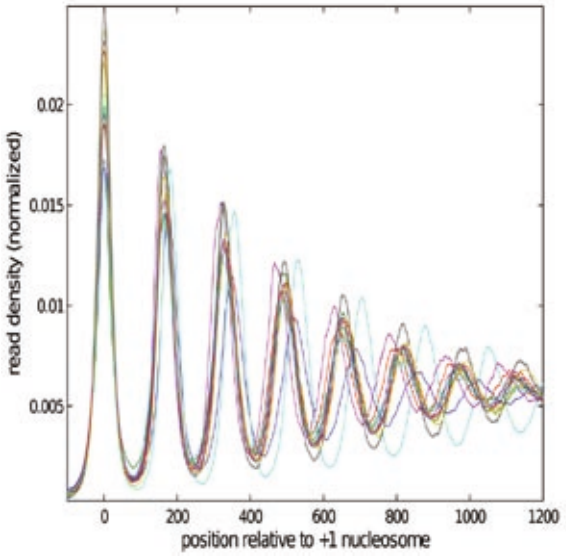
Computational Methods and Elementary Chromatin Structure

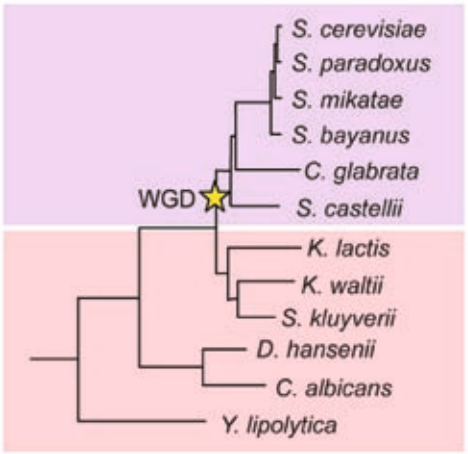
Nucleosomes are the functional unit of chromatin. They serve a vital role in both compacting the DNA into the nucleus of the cell, and in regulating the expression (transcription) of genes via acetylation/deactylation. Their density and positioning determine the site exposure of eukaryotic genes, and their distribution along the DNA also has implications for higher-order chromatin structure and geometry. Experimentally observed pattern-formation of nucleosome distribution (as, for example, in the phylogeny of yeast species in figure 1) may yield insight into these structures.

To that end, we seek a unified model of nucleosome distribution; a task which we undertake by applying an Ising model to the convenient 1-dimensional lattice of DNA nucleotides. The project is introduced to the JNN group at different levels ranging from introduction to statistical mechanics to full derivation of the partition function, and autocorrelation function, depending on the experience of the participants.

To show the methodology of the work, and to provide a more generally useful tool, participants were taken through a tutorial of the maple mathematical package, with emphasis on the numerical analysis employed on this project. Through the introduction of the software, and derivation of the interaction, the goals of this module were to a) provide familiarity with a general computational resource that could be used in other research, b) to showcase the utility of statistical-mechanics methods as a resource in solving stochastic problems, c) To provide insight into the primary structure of chromatin. As shown by the agreement between theory and experiment, this problem lends itself quite well to tractable theoretical analysis.

Figure 1: (left) Probability distribution of nucleosomes along the 1-dimensional contour of the DNA for 12 species of yeast shown at right. The pattern shown here has implications both on gene regulation and higher-order chromatin structure.





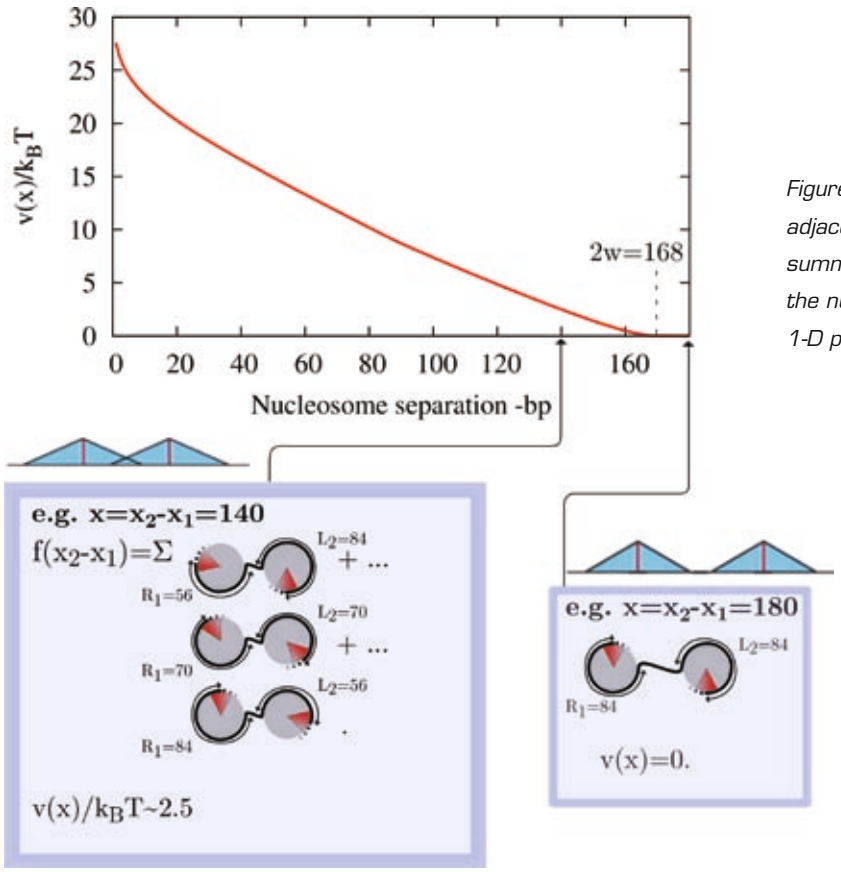


Figure 2: Interaction potential of two adjacent nucleosomes derived from thermal summations of possible bond formations of the nucleotides with the histone proteins -a 1-D particle model.

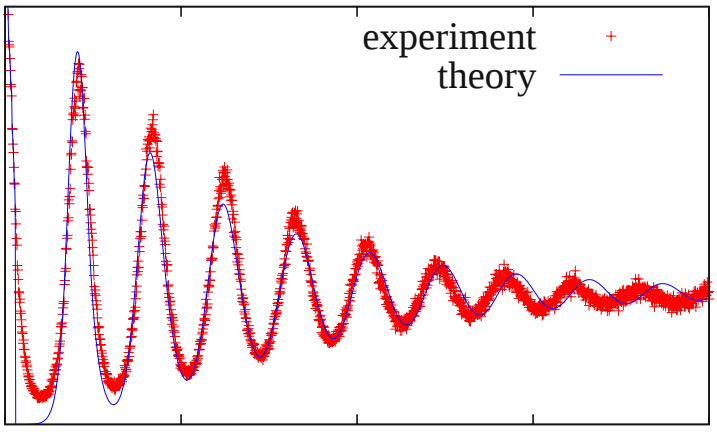


Figure 3: Typical plot of experimental and theoretical nucleosome probability distribution profiles for the species S. kluyverii. Derivation of the blue curve provides not only the values shown, but also insight into the formation of the pattern as a whole.

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CPLC Co-Director, Prof. Taekjip Ha, introducing the CPLC summer school and lectures on single-molecule fluorescence and STORM

PhD students discussing scientific results with PostDocs and Professors at a poster session at the CeNS workshop in Venice.

JNN student Ingo Stein (CeNS), explaining his research poster to Prof. Taekjip Ha at the CPLC summer school.

Chaitanya Sathe (CPLC) and other PhD students from CeNS in discussions with Prof. Mildred Dresselhaus (MIT) at the CeNS workshop in Venice.

Lectures & Discussions

In addition to the 2-weeks period of hands-on experimental and computational research work, the participants of the JNN in 2010 followed an intense program of top-notch conferences. On these occasions, the students could listen to a large number of scientific talks of internationally renowned speakers and had the opportunity to discuss their own research at serveral poster sessions organized at the conferences.

As such, the organizers from the University of Illinois invited the students to participate in the 2010 summer school of the CPLC at Urbana and to travel to the Midwest Single-Molecule Workshop in St. Louis. Offering their guests from ths U.S. an Italian flair, the Munich-based Center for NanoScience invited all JNN participants to its annual workshop taking place at the Venice International University on the island of San Servolo.

Center for the Physics of Living Cells

CPLC Summer School 2010

As part of the JNN exchange program the students from Munich participated in the 2010 summer school of the Center for the Physics of Living Cells (CPLC). This workshop is mainly aimed at graduate students and postdoctoral fellows in biophysics and the chemical and life sciences and totaled roughly 40 participants, coming from all around the US as well as from International institutions. A number of the JNN hosts also actively participated as TAs and tutors in the workshops. The weeklong program included "basic training" elements such as introductory mini-courses to Visual Molecular Dynamics (VMD), optics and the widely used software Matlab and Labview. Additionally, CPLC faculty presented their current research projects giving the students a broad overview of the range of topics covered in the CPLC. During the poster session all JNN students presented their own research and had the opportunity to discuss with faculty as well as with other summer school students.

Besides lectures and mini courses, so-called "advanced modules" made up a major part of the CPLC summer school, where the participants were assigned to small groups of around three to four people in every project. These modules covered a whole range of topics such as sophisticated theoretical concepts in molecular dynamics and dynamical network analysis, a variety of single-molecule fluorescence techniques (FRET, FIONA, STORM, FReI, etc.) and force related measurements such as optical trapping of single-molecules or even whole cells. For the JNN students the module was chosen such that it did not directly match their research topics in order to give them a better understanding of a technique they were so far not familiar with.

After an introduction into the topic by the teaching assistants of the corresponding labs, the modules allowed the students to perform their own experiments within three days. During that time the TAs assisted the students with lots of good advice and additional information. The participants got to know the labs and techniques of the faculty instructors organizing the summer school in a very direct and hands-on manner. On the last day the teams summarized their results in short presentations that they introduced to the whole group. This gave everyone the opportunity to get an overview over what was done in the other modules.

Apart from the scientific program there were lively discussions amongst the participants of the summer school, also after the end of the official program each day. Mostly on initiative of JNN hosts the word was spread as to where to meet each evening for a casual end of the day. Many of the other summer school students gladly joined in to make the evening discussions and activities truly diversified, and surely everyone got to know new interesting people - scientifically as well as personally. Last but not least, the summer school ended with a nice and lively party in Taekjip Ha's house and garden.

Midwest Single-Molecule Workshop

The Washington University in Saint Louis hosted this first workshop of single molecule physicists from Midwestern universities. It was held on July 26 – 27 and gave the German JNN students the opportunity to gain insight into the newest research done in this area.

The Keynote by Julio Fernandez from Columbia University was followed by 16 other oral presentations. The topics ranged from single-molecule force spectroscopy to fluorescence microscopy of protein-DNA interactions. The discussions were lively and two poster sessions gave the opportunity to meet and exchange with the post docs, PhD-students and professors participating.

Besides this scientific benefit, the trip to Saint Louis was also a chance for the JNN students to further explore the unique culture and history of the American Midwest: The three hour bus journey from Urbana to St. Louis exhibited the significance of the agricultural and rural dimensions of the USA. The City of Saint Louis itself offered a free outdoor musical ("The Sound of Music"), a walk along the famous blues clubs of Delmar Boulevard or a visit of the town´s landmark, the Gateway Arch. The latter is a parabolic 192 m tall concrete gate one can climb up. The underground museum of westward expansion underneath covered the exciting history of the exploration and settling of the Midwest.

The Urbana physicists ended the trip with a tour at the headquarters of the Budweiser brewery at St Louis and had a pleasant and safe bus ride home to Illinois.

CeNS Workshop 2010: "Nanosciences - Merging Disciplines"

During their stay in Munich, the UIUC students were invited to participate in the CeNS Workshop "Nanosciences - Merging Disciplines" taking place at the Venice International University (VIU) on San Servolo in Venice, Italy from September 20 – 24, 2010.

CeNS had invited 24 internationally renowned scientists to the sunny and beautiful island of San Servolo for one week of intensive exchange on nanosciences over a broad array of topics. In their stimulating talks, cutting-edge researchers from all over the world presented an overview of current research topics on nanometer-scale science and presented their latest research highlights to the graduate students, postdoctoral researchers and professors of CeNS. The theme being 'merging disciplines', a wide assortment of research areas were covered, from bionanotechnology to quantum optics, to condensed matter among many others. These talks provided an excellent overview and complemented the narrow perspective of one's own research field.

Being a member of the CeNS advisory board, Prof. Gerd Binnig, Nobel Laureate in Physics in 1986, joined the conference and took special interest in encouraging the students to take part in the discussions after the presentations.

In addition to the talks, PhD students and junior researchers presented their scientific results to their colleagues and to the guest speakers at two lively poster sessions which took place in the evenings. Many young researchers used the session to start conversations with professional scientists and to get some new ideas and inspirations for their research theme. Furthermore, several JNN participants had the honor to be selected by the conference committee for short oral presentations: Helen Hwang and Johan Strumpfer (CPLC) as well as Stefan Stahl, Ingo Stein and Christian Osseforth from CeNS.

During this workshop, the JNN participants had the opportunity to join in many lively discussions between young researchers and experienced scientists from the fields of physics, chemistry and biology taking place inside of the lecture hall, but also during the breaks on the green yards of San Servolo, as they paused to savour apricot drinks overlooking the dazzling bay. Of course, lively informal discussions proceeded throughout the canals of Venice, as the JNN participants took in the magnificent city. People who had not enough time to get in contact during the poster session now had the chance while having excellent Italian food or while meeting at Campo Santa Margherita. Whether it was Johan's take on prime numbers, or Chaitanya's musings on numeric computations, science was never far from their minds, as the participants happily got lost amongst the winding canals of the city. The relaxed atmosphere facilitated starting interesting and extensive conversations, for example how to use nanoscience to prevent the flooding of Venice.

Returning to the conference, the participants found out the good news that one of the speakers, Prof. Kostya Novoselov, had just received the Nobel Prize 2010 in the field of physics. Clearly, this event was an opportunity for students from CPLC and CeNS to network and to create new contacts over the range of fields that were, with internationally renowned researchers as well as with their fellow students.

Intercultural Exchange

Getting to know People and Culture

One of the key intentions of the JNN was establishing a social network between students from Munich and Urbana/Champaign. Outside the lab, this was accomplished through a wide variety of activities. UIUC provides excellent sports facilities which were frequently used by all JNN students for team sport activities, such as soccer, basketball and volleyball. Also, some of the exchange students took the chance to gain first experience on the local golf range and to adventure through Bavarian mountain valley by bike. The overall program, but especially the leisure activities led to numerous new friendships and a continuing scientific network.

USA: Urbana/Champaign, Chicago and more....

As a college town Urbana/Champaign offers a number of great restaurants, bars and clubs. During the JNN exchange a local county fair took place and the German students experienced and greatly enjoyed a typical Mid-west happening, a demolition derby. Prof. Ha surprised the German students with a traditional American BBQ, where karaoke was a great part of the entertainment.
CPLC organized a weekend trip to the beautiful city of Chicago, where the inner city was occupied by the movie stage of Transformers 3. The fascinating skyline was explored on a boat trip and as a rule of thumb: one well-known Chicago pan pizza is enough to feed four Germans!

Germany: Munich, the Bavarian Alps and more....

Coincidently, the American students were visiting Munich during the time of Oktoberfest, which was greatly appreciated by all of the JNN students. Not only the visit of a soccer game in the Allianz Arena and a classical concert, but also historical sites such as Dachau concentration camp, Münchner Residenz and Deutsches Museum were part of the Munich leisure activities.
Another highlight was the invitation of Prof. Gaub (CeNS Spokesman) to a mountain bike tour from Schliersee to Tegernsee and back again to his home, where an amazing dinner waited for all participants.

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