PHYSICS
OF LIVING SYSTEMS

International Meeting
of the Physics of Living Systems
Student Research Network

21–24 July 2014 — Munich
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ORGANIZERS

Prof. Don Lamb
Prof. Dieter Braun (LMU Munich)
&
Prof. Zan Luthey-Schulten (University of Illinois at Urbana-Champaign)

Management Team:
Marilena Pinto, Gabriela Milia (SFB 1032 “Nanoagents”)
Dr. Susanne Hennig, Anna Kager (Center for NanoScience)
Ludwig-Maximilians Universität (LMU) Munich
Geschwister-Scholl-Platz 1
D-80759 Munich, Germany

www.sfb1032.physik.uni-muenchen.de
www.cens.de

VENUE

Deutsches Museum (July 21)
Ehrensaal
Museumsinsel 1
80538 Munich
&
LMU Munich (July 22-24)
Physics Department
Lecture Hall H030
Schellingstr. 4
80799 Munich

FINANCIAL SUPPORT:

Deutsche Forschungsgemeinschaft
National Science Foundation
Monday, July 21
Deutsches Museum, Ehrensaal

9:15 am - 10:55 am
Welcome
Bernd Huber, President Ludwig-Maximilians-Universität (LMU) Munich
Don Lamb, LMU Munich

LMU Munich
Erwin Frey: Pattern Formation and Collective Phenomena in Biological Systems
Dieter Braun: Probing Molecular Evolution, Cellular Kinetics and Biomolecule Binding
with Microthermal Gradients
Ulrike Gaul: Systems Biology of Gene Regulation: Dissecting the Core Promoter

Coffee break

11:20 am -
Technische Universität Munich
12:35 pm
Matthias Rief: Mechanics of Single Protein Molecules
Max Planck Institute of Biochemistry
Elena Conti: Visualizing the Cellular Machines that Degrade RNA Molecules
Wolfgang Baumeister: Electron-Cryomicroscopy: From Molecules to Cells

Lunch break

2:00 pm -
Graduate Students Munich
3:00 pm
Jean-Philippe Sobczak: Investigating DNA Nanostructure Self-Assembly
Johannes Nübler: Including Softness in the Adsorption of Large Molecules on DNA Exhibits New Physics
Frauke Mickler: “Smart” Nanoparticles for Drug and Gene Delivery to Cancer Cells
Fabian Wehnekamp: 3D Real-Time Orbital Tracking in Zebrafish Embryos: High Spatiotemporal Analysis of Mitochondrial Dynamics in Neurons

3:15 pm -
Physics of Living Systems - Establishing International Ties
4:15 pm
(Panel Discussion)

Coffee break

4:45 pm -
CNRS, France
6:30 pm
Vladimir Lorman: Physical Modeling of Viral Assembly
Emmanuel Margeat: Structural Dynamics of Single Metabotropic Glutamate Receptors Dimers
Saurabh Raj: Single-Molecule Study of Ded1 Helicases Using a Hairpin Substrate
Selma Dahmane: Structural Analysis of Tetraspanin Assemblies during HIV-1 Budding Using Correlative AFM-Single Molecule Localization Microscopy
Joachim Rambeau: Modeling Non-Equilibrium Gene Expression Fluctuations during Nutrient Shifts

7:00 pm -
Physik und Leben - Der Magnetsinn des Zugvogels (Public talk by Klaus Schulten)
7:45 pm
(For non German-speaking participants, there will be a short introduction about the Center for New Technologies ZNT at the Deutsches Museum and time to visit the ZNT)

8:00 pm
Welcome reception
**Tuesday, July 22**  
*Lecture Hall H030, Schellingstr. 4, LMU Physics Department*

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<tr>
<th>Time</th>
<th>Location</th>
<th>Speaker(s)</th>
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| 8:30 am  | University of Maryland    | *Dave Thirumalai:* Stepping Kinetics of Myosin Motors: Moving Forward, Backward, and Foot Stomping  
+Wolfgang Losert: Dynamic Contact Guidance of Migrating Cells  
+Arpita Upadhyaya: Forcing it on: the Dynamics of Signaling Activation in Immune Cells  
+Rachel Lee: Quantifying Collective Cell Migration during Cancer Progression  
+David Winogradoff: The Acetylation Landscape of the H4 Histone Tail  
+Huong Vu: All-atom Simulation of a Full Kinesin Docking Process  
+Christina Ketchum: Actin Dynamics and Calcium Signaling in B Cells Respond to Surface Topography |
| 10:15 am |                                                                          |                                                                                                 |
| 10:45 am | Princeton University      | *Thomas Gregor:* Precision and Reproducibility in Development  
+Eric Smith: From Genome to Form: Measuring a Simple Mathematical Input-Output Relation for a 250-bp regulatory DNA Element in the *Drosophila* Embryo  
+Sophie Zhang: Comparing Fungal Foraging Strategies via Simulation  
+Nikolay Ouzounov: The Effect of MreB Polymer Biophysics on *Escherichia coli* Cell Shape  
+Marina Feric: Nuclear Actin Counters Gravity during Cell Growth  
+Farzan Beroz: Physical Limits to Biomechanical Sensing |
| 1:30 pm  |                                                                          |                                                                                                 |
| 2:30 pm  | Georgia Institute of Technology | *Harold Kim:* Probing Elastic Limit of DNA Bending  
+Flavio Fenton: Mapping the Complex Spatiotemporal Dynamics of Electrical Activations in the Heart  
+Curtis Balusek: Simulations of an Outer-Membrane Transporter in a Realistic Environment  
+Patrick Chang: Bottlebrush Swollen Pericellular Matrix Mediates Particle Transport to Cell by Size and Charge  
+Henry Astley: Cybernetic Sidewinders: Modulation of Orthogonal Body Waves Enables Versatile Maneuverability  
+Patricia Yang: Duration of Urination Does not Change with Body Size |
| 4:45 pm  | National University of Singapore | *Chen Chen:* An Introduction to Cryo-Electron Tomography  
+Lu Gan: Applications of Electron Cryotomography  
+Utkur Mirsaidov: Nanoscale Dynamics in Ultrathin Liquids Visualized with TEM  
+Nirmalya Bag: Imaging Fluorescence Correlation Spectroscopy Investigates Biomolecular Dynamics and Organization in 2D and 3D  
+Chee San Tong: Feedback regulation of cortical actin waves  
+Sin Yi Lee: Regulation of Bacterial DNA Packaging in Stationary Phase by Competitive DNA Binding of Dps and IHF |
| 8:00 pm  |                                                                          | Conference dinner (Augustiner Restaurant, Neuhauser Str. 27 - Weißer Saal, first floor)          |
### Wednesday, July 23
Lecture Hall H030, Schellingstr. 4, LMU Physics Department

<table>
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<tr>
<th>Time</th>
<th>Session</th>
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<tr>
<td>8:30 am -</td>
<td><strong>Harvard University</strong></td>
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<tr>
<td>10:15 am</td>
<td><em>Erel Levine</em>: Pathogen Avoidance by Worms as a Collective Behavior</td>
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<td><em>Eugen Frenkel</em>: Competitive Coexistence on Shared Resources Evolves due to Crowded Growth</td>
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<td><em>Matthew Berck</em>: Reconstructing and Visualizing the First Relay of the <em>Drosophila</em> Larva Olfactory System</td>
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<td><em>Lucy Eunju Lee</em>: Gene Regulatory Network Modeling Dynamic Host-Pathogen Interaction of <em>Caenorhabditis elegans</em> and <em>Pseudomonas aeruginosa</em></td>
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<td><em>Alyssa Wilson</em>: Quantifying Synaptic Reorganization in the Developing Cerebellum Using Serial-Section Scanning Electron Microscopy Data</td>
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<td><strong>Coffee break</strong></td>
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<tr>
<td>10:45 am -</td>
<td><strong>Weizmann Institute of Science / Ben-Gurion University of the Negev</strong></td>
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<tr>
<td>12:30 pm</td>
<td><em>Eyal Nir</em>: Fast and Efficient DNA Based Molecular Motors Assisted by Microfluidics and Single-Molecule Fluorescence</td>
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<td><em>Ed Bayer</em>: Cellulosomes – A Structurally Robust Multi-Protein Platform for Broad Nanotechnological Application</td>
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<td><em>Constantin Schöler</em>: Ultrastable Cellulosome-Adhesion Complex Tightens under Load</td>
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<td><em>Anders Barth</em>: Conformational Dynamics in Designer Cellulosomes Studied by Single-Pair FRET</td>
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<td><em>with MFD-PIE</em></td>
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<td><em>Dan Bracha</em>: Direct Observations on Protein-DNA Interactions in Dense and Segregated DNA Phases</td>
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<td><strong>Lunch &amp; cultural program</strong></td>
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<td>2:00 pm -</td>
<td><strong>Guided City Tour</strong></td>
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<td>4:00 pm</td>
<td><em>Meeting Point: Marienplatz, Fischbrunnen</em></td>
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<td>Transportation: underground lines U3 and U6 to Marienplatz</td>
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<tr>
<td>2:00 pm -</td>
<td><strong>Guided Bike City Tour</strong></td>
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<td>≈ 5:00 pm</td>
<td><em>Meeting Point: Marienplatz, Fischbrunnen</em></td>
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<td>Transportation: underground lines U3 and U6 to Marienplatz</td>
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<td><em>Bikes will be provided by the guides at Marienplatz.</em></td>
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<tr>
<td>1:30 pm -</td>
<td><strong>Isar Rafting Tour Lenggries - Bad Tölz</strong></td>
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<tr>
<td>≈ 8:00 pm</td>
<td><em>Meeting Point: Schellingstr. 4, lobby</em></td>
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<td>Transportation: underground lines U3/U6 to Odeonsplatz, change to U4/U5 to Munich main station, Regional train (BOB): departure from Munich 2:05 pm (platform 27-36), arrival in Lenggries 3:11 pm Departure from Bad Tölz 6:48 pm/7:48 pm/8:48 pm, arrival in Munich 7:54 pm/8:54 pm/9:54 pm BOB tickets will be provided.</td>
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<td></td>
<td>Please wear casual clothes and shoes that can cope with some splash water. Spare shoes are recommended. Soft drinks and beer can be purchased on the rafting boats, but you might want to bring a snack with you.</td>
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**Thursday, July 24**  
**Lecture Hall H030, Schellingstr. 4, LMU Physics Department**

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<tr>
<th>Time</th>
<th>Speaker</th>
<th>Institution</th>
<th>Topic</th>
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<tbody>
<tr>
<td>8:30 am</td>
<td>Rice University</td>
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<td><em>Herbert Levine</em>: Introduction</td>
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<tr>
<td>10:15 am</td>
<td><em>José N. Onuchic</em>: The Energy Landscape for Protein Folding and Biomolecular Machines</td>
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<td></td>
<td><em>Ryan Hayes</em>: Reduced Model Captures Mg(^{2+}) Dependence of the RNA Free Energy Landscape</td>
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<td><em>Herbert Levine</em>: Chemotaxis in <em>Dictyostelium</em>– Using Physical Models to Decipher the Mechanisms</td>
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<td><em>Jingchen Feng</em>: Alignment and Nonlinear Elasticity in Biopolymer Gels</td>
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<td><em>Rajeesh Balagam</em>: Role of Mechanical Interactions in Self-Organization of Bacteria in Biofilms</td>
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<td>10:45 am</td>
<td>Yale University</td>
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<td><em>Simon Mochrie</em>: Nucleosome Unwinding and Rewinding: Free Energy Landscapes, First Passages, and Time-Resolved Transition Paths</td>
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<td>12:30 pm</td>
<td><em>Yu Lin</em>: Quantification and Optimization of Image Quality for Single-Molecule Switching Nanoscopy at High Speeds</td>
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<td><em>Junjiajia Long</em>: Bifurcation in the Biased Random Walk of <em>E. coli</em></td>
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<td><em>Peter Koo</em>: A Maximum Likelihood Approach to Extract Underlying Diffusive States from Single Particle Trajectories of Rho GTPase in Live Cells</td>
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<td>1:30 pm</td>
<td>Poster session: Milles - Z</td>
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<td>2:30 pm</td>
<td>Universidade Federal do Rio de Janeiro</td>
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<td>4:15 pm</td>
<td><em>Jerson Silva</em>: The Network Research at the National Institute of Science and Technology for Structural Biology and Bioimaging (INBEB)</td>
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<td><em>Danielly Ferraz da Costa</em>: Prion-like Aggregation of p53 Tumor Suppressor Protein: New Targets for Anticancer Drugs</td>
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<td><em>Guilherme A. P. de Oliveira</em>: “Push and Pull” Hypothesis to Unify the Physical and Chemical Unfolding of Proteins</td>
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<td><em>Fernanda Tovar-Moll</em>: Imaging Brain Connectivity and Plasticity</td>
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<td><em>Mônica Santos de Freitas</em>: Structural Characterization of Transthyretin Protein Misfolding by Solidstate NMR</td>
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<td>4:45 am</td>
<td>University of Illinois at Urbana-Champaign</td>
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<td>6:30 pm</td>
<td><em>Taekjip Ha</em>: Surprising Physics of DNA and Potential Roles in Gene Regulation</td>
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<td><em>Klaus Schulten</em>: The Photosynthetic Membrane of Purple Bacteria - A Clockwork of Proteins and Processes</td>
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<td><em>Jaya Yodh</em>: Next Generation Biophysics Training at University of Illinois at Urbana-Champaign</td>
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<td><em>John Cole</em>: Spatially-Resolved Metabolic Cooperativity within Dense Bacterial Colonies</td>
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<td><em>Neil Kim</em>: Revealing Real-Time, <em>In Vivo</em> Transposable Element Dynamics at both Single Cell and Population Level</td>
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<tr>
<td>6:30 pm</td>
<td>Conclusions</td>
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Pattern Formation and Collective Phenomena in Biological Systems

Erwin Frey
LMU Munich, Physics Department and Center for NanoScience

Reaction-diffusion dynamics endows cells with the capacity for accurate positioning, control of length, and timing of processes. Protein systems in cells employ different types of spatio-temporal patterns to ensure precise cell division and guide intracellular processes. The length of biopolymers is regulated by the interplay between polymerization kinetics and patterns of molecular motors. Under which conditions protein patterns emerge, and how these patterns are regulated by biochemical and geometrical factors are major aspects of current research. We will discuss general design principles of such biological pattern forming systems.

Probing Molecular Evolution, Cellular Kinetics and Biomolecule Binding With Microthermal Gradients

Dieter Braun
LMU Munich, Physics Department and Center for NanoScience

Life signals most of its information by the interaction of molecules. It is important to quantify both the interaction strength and the reaction speed under biological conditions. Interestingly, the movement of proteins in a temperature gradient is a sensitive and versatile way to probe protein interactions. Heating and detection is performed all-optically in a wide range of biological fluids [1]. The technique was commercialized by PhD students of CeNS and shows growing applications in fragment screening of drugs and demonstrates its robust application in biology [2]. The physical basis of thermophoresis could be revealed only recently [3]. Thermal fields also allow to measure the reaction speed inside living cells using fast temperature oscillations and a molecular lock-in method. Maps of the reaction speed reveal both enhanced and slowed reaction speed as compared to in vitro conditions [4]. A spatially moving warm spot allows to also move water all-optically along arbitrary paths, opening the possibility of light driven microfluidics [5]. Combined with natural convection, thermophoresis creates strong natural hydrothermal traps for nucleotides in pores of rock, allowing for example a strong enhancement of oligonucleotide polymerization [6]. Replication and accumulation is combined in thermal traps, offering fascinating potentials for autonomous Darwin evolution [7]. Microthermal fields, besides their new and wide applications in biology could have played a central role in a non-equilibrium origin of life.


Systems Biology of Gene Regulation: Dissecting the Core Promoter

Katja Frühauf, Julia Massier, Mark Heron, Anja Kiesel, Alessio Renna, Alexander Engelhardt, Thomas Walzthöni, Achim Tresch, Johannes Söding, and Ulrike Gaul
Genzentrum LMU, Feodor-Lynen-Strasse 25, 81377 München

Metazoan development and homeostasis requires complex spatio-temporal regulation of gene expression as well as precise tuning of transcription levels. The core promoter plays a crucial role in this process, by positioning the transcription start site and encoding the expression level of the cognate gene, which may range over several orders of magnitude. To unravel this code, we are undertaking a large scale investigation of promoter features, such as sequence motifs, occu-
pancy by nucleosomes and the general transcription machinery, and the resulting transcription rates using a combination of computational, genomic and synthetic biological approaches. Here, we report on the biological insights we have gained to date and discuss the methodological challenges of such a multi-dimensional analysis.

Finally, we will introduce the scientific and academic goals of our new graduate school 'Quantitative Biosciences Munich (QBM)' and report on our first year in operation.

Mechanics of Single Protein Molecules

Matthias Rief
Physik-Department E22, TU Munich, James-Franck-Str. 1, D-85748 Garching, Germany

Proteins are amazing molecular machines that can fold into a complex three-dimensional structure in a self-organization process called protein folding. Even though powerful structural methods have allowed us taking still photographs of protein structures in atomic detail, the knowledge about the folding pathways and dynamics as well as material properties of those structures is rather limited. Over the past 15 years, our group has developed single mechanical methods to study the dynamics and mechanics of protein structures. In my talk I will discuss how these methods can be used to investigate and control the conformational mechanics of individual proteins. Examples include protein folding as well as protein-protein interactions and enzyme mechanics.

Visualizing the Cellular Machines that Degrade RNA Molecules

Elena Conti
Max Planck Institute of Biochemistry, Am Klopferspitz 18, D-82152 Martinsried, Germany

Just like we use shredders for destroying documents that contain potentially damaging information or that are no longer used, cells use molecular machines for degrading defective or unneeded macromolecules. RNAs constitute a large family of macromolecules. They are present in all our cells and have multiple functions, such as allowing the translation of genomic information into proteins. Cells have sophisticated quality control systems to recognize RNAs that are either defective or no longer needed, and to swiftly degrade them.

We study the protein complexes that function as cellular nano-machines for shredding RNAs. These cellular nano-machines are present in low abundance in the cell and are transient in nature, changing in composition and configuration as they perform their functions. We use biochemical and biophysical methods to reconstitute these complexes in defined chemical, functional and structural states. We then use X-ray crystallography to visualize them at nearly atomic resolution, often catching them right as they are carrying out their chemical reactions. The talk will focus on the exosome complex, an essential nano-machine that degrades a wide variety of RNAs into single ribonucleotides, processively and directionally. Our results have shown that the molecular mechanisms used by the exosome complex for degrading RNAs are broadly present across different forms of life, and exhibit conceptual similarities with the mechanisms used by the proteasome, the cellular nano-machine that shreds polypeptides.

Electron-Cryomicroscopy: From Molecules to Cells

Wolfgang Baumeister
Max Planck Institute of Biochemistry, Am Klopferspitz 18, D-82152 Martinsried, Germany

Today’s biomolecular electron microscopy uses essentially three different imaging modalities: (i) electron crystallography, (ii) single particle analysis and (iii) electron tomography. Ideally, these imaging modalities are applied to frozen-hydrated samples to ensure an optimum preservation of the structures under scrutiny. Electron crystallography requires the existence of two-dimensional crystals. In principle, electron crystallography is a high-resolution technique and it has indeed been demonstrated in a number of cases that near-atomic resolution can be attained. Single-particle analysis is particularly suited for structural studies of large macromolecular complexes. The amount of material needed
is minute and some degree of heterogeneity is tolerable since image classification can be used for further ‘purification in silico’. In principle, single particle analysis can attain high-resolution but, in practice, this often remains an elusive goal. However, since medium resolution structures can be obtained relatively easily, it often provides an excellent basis for hybrid approaches in which high-resolution structures of components are integrated into the medium resolution structures of the holocomplexes. Electron tomography can be applied to non-repetitive structures. Most supramolecular structures inside cells fall into this category. In order to obtain three-dimensional structures of objects with unique topologies it is necessary to obtain different views by physical tilting. The challenge is to obtain large numbers of projection images covering as wide a tilt range as possible and, at the same time, to minimize the cumulative electron dose. Cryoelectron tomography provides medium resolution three-dimensional images of a wide range of biological structures from isolated supramolecular assemblies to organelles and cells. It allows the visualization of molecular machines in their functional environment (in situ) and the mapping of entire molecular landscapes.

Investigating DNA Nanostructure Self-Assembly

Jean-Philippe Sobczak, Thomas G. Martin, Thomas Gerling, Hendrik Dietz
Physics Department, Walter Schottky Institute, Technische Universität München, Am Coulombwall 4a, 85748 Garching near Munich, Germany

Molecular self-assembly of DNA nanoscale devices allows us to create objects with molecular weights up to the megadalton regime. This ability to control the position and orientation of molecules on a nanometer-scale in a user-defined way offers a promising route to achieve functionalities to date only seen in natural macromolecular complexes. Improved design rules derived from experimental feedback have shortened week-long assembly protocols to self-assembly on the order of minutes, allowing rapid production at high yields. It has been shown that the choice of sequences, strand lengths, and structure topology influence the self-assembly process. We hope to elucidate this self-assembly process through stochastic computational simulations. Understanding of the intrinsic reaction pathways will help us in the derivation of rational design rules, further improving production rate, yield, and quality in order to advance practical applications of DNA nanotechnology.

Including Softness in the Adsorption of Large Molecules on DNA Exhibits New Physics

Brendan Osberg, Johannes Nuebler, Ulrich Gerland
Physik-Department, Technische Universität München, James-Franck-Str. 1, 85748 Garching, Germany

In physics, real-world systems are often described by simple models that capture the “essential phenomena” and are, ideally, analytically tractable. Within the context of adsorption of extended objects onto a substrate, one such example is the so called “car parking problem”: Particles of unit length perform attachment attempts onto a 1D line that are accepted if no overlap with already adsorbed particles is produced, and otherwise rejected. This simple model captures “jamming” phenomena, where the substrate is not completely covered, but each individual gap is too small to insert another particle. Hard core exclusion is often only a first approximation to biologically relevant situations. For example, a somewhat realistic description of the adsorption of large molecules onto DNA (e.g. histones forming nucleosomes) should take into account softness.
account the possibility of partial overlap due to unwrapping of the outer base pairs. In such a “soft-core” adsorption-desorption model, we analyze to what extent jamming of the line density is avoided by particles that squeeze into progressively smaller gaps. By such “cramming”, in-vivo nucleosome densities can be reached in biologically realistic timescales. Interestingly, the biologically motivated soft-core model also introduces qualitatively new physics: the line density can be non-monotonic, which is never observed in the hard core model. To understand this we come back to the physicist’s approach and identify the simplest possible model: “Soft dimers” on a lattice that can overlap by one site capture the overshoot phenomenon and can be understood in full detail.

“Smart” Nanoparticles for Drug and Gene Delivery to Cancer Cells

Frauke Mickler¹, Veronika Weiss¹, Stefan Niedermayer¹, Alexandra Schmidt¹, Annika Herrmann², Ernst Wagner², Thomas Bein¹ and Christoph Bräuchle¹
1 Department of Chemistry and Center for Nanoscience (CeNS), 2 Pharmaceutical Biotechnology, Center for System-based Drug Research, LMU Munich

Synthetic nanoparticles can be applied as “smart drug delivery ferries” for the controlled delivery of drugs, therapeutic nucleic acids and diagnostic markers to specific sites of the human body and are promising tools for cancer therapy. They improve the solubility of incorporated drugs, protect from degradation in biological fluids and enhance the specific uptake and release of drugs into cancer cells. To improve the selective uptake of nanoparticles into cancer cells, they can be functionalized with custom-designed targeting ligands such as peptides or antibodies, allowing personalized medicine. “Intelligent” linkers or molecules that sense and dynamically adapt to their environment can be attached to overcome intracellular barriers and to mediate the triggered release of a drug inside cancer cells. To improve the efficiency and safety of multifunctional nanoparticles for future clinical application it is important to understand their cellular interactions in detail.

In this talk it will be demonstrated how highly-sensitive fluorescence microscopy can be used to follow the uptake and intracellular trafficking of the described multifunctionalized nanoparticles in living cells. In the acquired movies the cellular attachment, endocytosis and processing of single nanoparticles can be followed in real-time. Due to the high temporal and spatial resolution of this powerful technique mechanistic details of the interaction of nanoparticles with cell components can be revealed. Nanoparticles consisting of DNA complexed by cationic polymers (polyplexes) are investigated as well as mesoporous silica nanoparticles which contain the drug inside the porous network of nanometer-sized channels.

Our data demonstrate that the rational design of “smart” drug delivery ferries can lead to more specific, more efficient and safer drug delivery into cancer cells, in vitro as well as in model animals.

3D Real-Time Orbital Tracking in Zebrafish Embryos: High Spatiotemporal Analysis of Mitochondrial Dynamics in Neurons

Fabian Wehnekamp¹, Gabriela Plucinska², Thomas Misgeld² and Don C. Lamb¹
1 Fablab, Department Chemie, Ludwigs-Maximilians-Universität Munich, Germany; 2 Neuronal Cell Biology, Technical University Munich, Germany

The main function of mitochondria is to provide cells with adenosinetriphosphate (ATP) in regions with high-energy demand. A complex machinery of motor proteins (kinesin, dynein, myosin, etc.) and signaling molecules like calcium ions are responsible for the distribution and recycling of mitochondria in cells. A malfunction in the dynamics of these complexes is one possible reason for neurodegenerative diseases. However, the exact mechanism behind this process has yet to be revealed. To follow the trajectory of individual mitochondria, we use a home-build, three-dimensional, real-time orbital-tracking microscope with a spatial resolution of a few nm in three dimensions and an acquisition speed of up to 500 Hz. Environmental information is recorded simultaneously with a build-in widefield microscope. Photoactiva-
lable GFP (Tracking microscope) and TAGRFP (Widefield microscope) are coexpressed in neuronal mitochondria by coinjecting neuron specific driver constructs with separate UAS responder constructs into fertilized single-cell zebrafish eggs. Zebrafish expressing the markers are measured individually three days past fertilization. By photoactivating mitochondria, we are able to obtain single-mitochondria trajectories with a traveled distance of more than 100µm with nm precision. Due to our high spatial and temporal resolution, we can identify several different dynamic populations involved in mitochondria transport.

Physical Modeling of Viral Assembly

**Vladimir Lorman**
Laboratoire Charles Coulomb, UMR 5221 CNRS – Université Montpellier 2, France

The success of condensed matter physics in the 20th century is largely related to the deep understanding of matter organization, and following elaboration of physical concepts simplifying complex problems. Similar approach applied to the living matter reveals novel unique types of order at all scales, from individual molecules to biological tissues. At the scale typical for biomolecular assemblies, the peculiarities of the organization are often related to their unconventional topology and geometry, and to low dimensionality. Viruses are biological systems with high level of spatial organization well suited to modern physical methods of study. Viral genome is protected by a solid protein shell (capsid) made of many copies of identical subunits. Recent physical and biochemical data rise a whole number of questions concerning unconventional positional order of subunits in the shell, thermodynamics and physical mechanisms of the self-assembly, shape and mechanical stability of the shell. In the present work we develop new principles of modeling which explain and classify the capsid structures for viruses with spherical topology and icosahedral symmetry. We develop an explicit method which predicts the positions of centers of mass for the proteins, including the capsids of unusual viruses discovered quite recently, and discuss the assembly thermodynamics. We show the relation between the protein density distributions obtained and the infectivity properties for several human viruses.

Structural Dynamics of Single Metabotropic Glutamate Receptors Dimers

**Linnea Olofsson¹, Suren Felekyan¹, Etienne Doumazane², Pauline Scholler³,⁴, Ludovic Fabre², Jarriaan Zwier⁴, Philippe Rondard², Claus Seidel¹, Jean-Philippe Pin⁵, and Emmanuel Margeat¹**

¹ Centre de Biochimie Structurale, UMR 5048 CNRS, U1054 INSERM, Universités de Montpellier, France ; 2 Institut de Génomique Fonctionnelle, UMR 5203 CNRS, U 661 INSERM, Universités de Montpellier, France ; 3 Lehrstuhl für Molekulare Physikalische Chemie, Heinrich-Heine-Universität, Düsseldorf, Germany; 4 Cisbio Bioassays, Bagnols-sur-Ceze Cedex, France

Metabotropic glutamate receptors (mGluR) are members of the class C G-protein Coupled Receptors (GPCR) family. They are activated by glutamate, the major excitatory neurotransmitter in the central nervous system. They are homodimeric multidomain proteins stabilized by a disulfide bridge. Each subunit is composed of an extracellular domain (ECD) that binds orthosteric ligands such as glutamate, and a heptahelical transmembrane domain (7TM) common to all GPCRs and responsible for G-protein activation. A major re-orientation of the two ECDs within the dimer appears necessary for receptor activation upon agonist binding. We have established a new method for the purification of soluble mGluR2 ECD dimers, fused at their N-terminus with Snap-tags that can be covalently labeled with organic fluorophores. We then used single molecule Förster Resonance Energy Transfer (smFRET) with multiparametric fluorescence detection (MFD) to measure the conformational changes associated with the binding of agonists, antagonists or partial agonists to these dimers. By monitoring the kinetics of the reorientation of the ECD-dimer at the microsecond timescale, we demonstrate that the receptor exists in a preformed equilibrium of conformational states and that the exclusive role of ligands, including partial agonists, is to modulate the transition rate between states, rather than stabilizing individual conformations at the expense of others. These results were confirmed for receptors belonging to all three groups of receptors, and inspire for a new model describing the activation mechanism as a stochastic process where the probability of activation is intrinsically linked to the frequency of the conformational changes toward the activated state.
We have investigated the mechanical properties of the RNA-helicase-motor proteins in single-molecule assays using Magnetic Tweezers. RNA helicases are fundamental molecular motors that remodel RNA structures, DNA/RNA hybrids and RNA-protein complexes that are essential in gene regulation. So far their mechanism and functioning is very much debated and the classical or bulk assays are not sufficient to answer these questions. This is particularly true for Dead box helicases. We have developed a cyclic assay in parallel on tens of molecules at the same time which allows detecting the unwinding of short RNA/DNA hybrids in real time by Ded1 helicase. The observation of unwinding by the Ded1 helicase in the presence of ATP indicates that the enzyme melt the duplex rather than translocating along ssDNA and strip the complementary strand. Although DEAD-box proteins are most active with substrates containing single-stranded overhangs, they are also able to unwind short blunt-end duplexes. This has led to the proposal that DEAD-box proteins directly interact with the duplex, but they are “activated” by single-stranded RNA bound at another site. Moreover, it has been proposed that the proteins work by localized destabilization of the RNA helical regions. Hence, the proteins lack processivity. We performed the helicase activity measurement at various concentrations of proteins. Our results show that Ded1 activity is maximum in case of RNA oligo with 5’ overhang. The helicase activity is observed only in presence of ATP while the ATP analogues supported annealing activity. We have also confirmed that the enzyme has no visible effect on the unwinding of DNA.
Modeling Non-Equilibrium Gene Expression Fluctuations during Nutrient Shifts

Joachim Rambeau1,2, Ovidiu Radulescu1, Nathalie Declerck3
1 DIMNP, UMR 5235, Université Montpellier 2; 2 Labex Epigenmed, Université Montpellier 1; 3 CBS, CNRS UMR 5048 - UM 1 - INSERM UMR 1054

In bacterial populations, a change of carbon source triggers up and down regulation of appropriate/inappropriate metabolic pathways by modulating the expression of enzymes. Average enzyme levels converge with time to new steady state values. However, even in this steady state, a population of clones shows heterogeneity in protein concentrations from one cell to another. This population heterogeneity emerges from stochastic time-varying protein concentration at the single cell level, due to fluctuations in production events (transcription and translation) and in degradation or dilution (cellular growth).
We address the non-equilibrium dynamics of gene expression fluctuations in a bacterial population when the carbon source is changed. We first use a simple coarse-grained model that accounts for bursting kinetics. By adjusting key parameters to data in steady states (before the switch and long after), we predict the out of equilibrium dynamics of cellular gene products, whose fluctuations obey universal features. For example, fluctuations in the population are enhanced during the switch. These predictions agree with experiments on the glucose/malate switches for Bacillus subtilis made by single molecule microscopy (two-photon fluorescence correlation microscopy). In order to go beyond the coarse-grained level, I will introduce a single-cell-based framework. By relating individual cell properties to population ensemble measurements, I will show how this framework can be used to infer biophysical observables.

Physik und Leben - Der Magnetsinn des Zugvogels

Klaus Schulten
Beckman Institute and Department of Physics, U. Illinois at Urbana-Champaign


Stepping Kinetics of Myosin Motors: Moving Forward, Backward, and Foot Stomping

Dave Thirumalai
University of Maryland, College Park, MD

Myosin V, two-headed motor protein, ferries cellular cargo by walking hand-over-hand on actin filaments. Interplay between ATD-driven conformational changes in the motor head and stress due to load produces a variety of stepping dynamics: the motor can step forward or backward, or “stomp”, where one of the heads detaches and rebinds to the same site. I will present theory that captures all these behaviors, quantitatively matching a wide array of single molecule experiments. The theory lays out the structural and chemical design principles underlying the motor’s robust function, which provides a guide for how bioengineering might alter its dynamics [1]. The theoretical results will be complemented with simulations describing the role the internal dynamics of the motor domain plays in motility [2].


Dynamic Contact Guidance of Migrating Cells

Wolfgang Losert with Xiaoyu Sun, Meghan Driscoll, Can Gaven, Satarupa Das, and John Fourkas
University of Maryland, College Park, MD

How do sense feel and follow the texture of their surrounding environment? In rapidly moving cells, Dictyostelium and neutrophils, we discovered that cells utilize actin waves to sense and follow surface nanotopography. The wave-like character of the sensing allows cells to follow topographical features on the 100s of nm scale, larger than proteins or macromolecular clusters that are involved in other sensing tasks. The use of waves in contact guidance allows us to use physics tools to thoroughly characterize this dynamic contact guidance process. Our nanofabrication platform to measure this actin based dynamic contact guidance allows for rapid prototyping and could be applied to a broad range of cell types.

Forcing it on: the Dynamics of Signaling Activation in Immune Cells

Arpita Upadhyaya
University of Maryland, College Park, MD

The activation of lymphocytes is an essential step in the adaptive immune response. Lymphocyte activation involves the binding of specialized receptors with antigen on the surface of antigen presenting cells. This leads to changes in cell morphology and the movement and assembly of receptors, signaling proteins into nanometer-sized complexes called microclusters. These protein assemblies are essential for immune cell activation. While it has long been recognized that the actin cytoskeleton plays an essential role in this process, much of this understanding has focused on biochemical regulation of the actin cytoskeleton. We will summarize our recent studies from a biophysical perspective to explain the mechanistic role of actin in modulating lymphocyte activation. We highlight the roles of actin filaments in exertion of mechanical stresses that support signaling activation, microcluster assembly and movement in T lymphocytes. We have used traction force microscopy to measure the forces exerted by Jurkat T cells during activated spreading. Jurkat T cells are mechanosensitive with cytoskeletal forces and signaling dynamics both sensitive to the stiffness of the surface. We find that these forces are largely due to actin dynamics with myosin motors playing a limited role. Our studies highlight the potential role of cytoskeletal forces in signaling activation.
Quantifying Collective Cell Migration during Cancer Progression

Rachel Lee¹, Christina Stuelten², Kerstin Nordstrom¹, Douglas Kelly⁴, Nicholas Ouellette⁵, Carole Parent², Wolfgang Losert¹
1 University of Maryland, College Park, MD, 2 National Cancer Institute, Bethesda, MD, 3 University of Rochester, Rochester, NY, 4 Yale University, New Haven, CT

During cancer progression, tumor cells invade the surrounding tissue and migrate throughout the body, forming clinically dangerous secondary tumors. This metastatic process begins when cells leave the primary tumor, either as individual cells or collectively migrating groups. This collective migration is not yet well characterized. Here we present data on the migration dynamics of epithelial sheets composed of many cells. Using quantitative image analysis techniques, we are able to extract motion information from time-lapse images of cell lines with varying malignancy. Adapting metrics originally used to study fluid flows and deformations of soft matter, we are able to distinguish the migration dynamics of these cell lines.

The Acetylation Landscape of the H4 Histone Tail

David Winogradoff², Ignacia Echeverria¹,³ and Garegen Papoian¹,²,³
1 Institute for Physical Science and Technology, 2 Chemical Physics Program, 3 Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742, United States

The DNA of higher organisms must wrap around histone proteins to form nucleosomes, the fundamental structural unit of the DNA-protein complex known as chromatin. Each histone has N- and C-terminal tails that protrude outward from the nucleosomal surface, beyond the surrounding DNA. Histone tails play an important regulatory role for genetic processes, and, because of their high flexibility and intrinsic disorder, they are difficult to analyze experimentally. Furthermore, histone tails exhibit a variety of post-translational modifications that alter their structure and dynamics, as well as their interactions with DNA and other proteins. We investigated the effects of increasing the degree of acetylation on histone tails by performing explicit solvent all-atom simulations to explore the conformational landscapes of WT, mono-, di-, tri-, and tetra-acetylated H4 histone tails. With the exception of the H4 tail solely acetylated at LYS-16, increasing the level of acetylation leads to more compact states and increasing propensities to form helical secondary structure. On the other hand, the sole acetylation of LYS-16 leads to the formation of a prominent, short helix, which favors a more extended conformation and increased structural homogeneity.

Figure A: As we increase the level of acetylation, the H4 histone tail’s propensity to form secondary structure increases. Secondary structure was estimated by the DSSP algorithm, and only structures with 5 or more residues in the beta or helix (3-10 or alpha) conformations were considered.

Figure B: The propensity to form helical secondary structure increases with increasing acetylation. The WT H4 histone tail does not exhibit significant helical propensity. Upon the first level of acetylation at LYS-16, a prominent, short helix forms from residues 7-9. When increasing the level of acetylation further, more and longer helices form, culminating in a continuous 10-residue segment exhibiting significant helical propensity for the tetra-acetylated H4 histone tail. 3-10 and alpha helices are considered.

Figure C: The structures of H4AC (the H4 histone tail solely acetylated at LYS-16) containing a short helix from residues 7-9 favor a more extended conformation and increased structural homogeneity.
All-Atom Simulation of a Full Kinesin Docking Process

Huong T. Vu, Zhechun Zhang, and David Thirumalai
Biophysics Program, University of Maryland, College Park

In a conventional kinesin motor, the docking process of the neck linker in the motor head suggested creation of a power stroke which helps to “pull” the rear head forward, generating the force. This conformational change has been proved that it is not a simple zipping process of a neck linker, but actually a hinge movement of the neck linker and the cover stand together as a bundle (named Cover Neck Bundle). However, the full picture of how does that bundle forms, rotates and docks to generate the sufficient force has still not been clearly understood. We performed a 1 micro second all-atom simulation to investigate this process and did observe the formation of CNB along with a new binding site for the neck linker at the end of the simulation. This alternative binding site is suggested to an opened more possible interactions that favor the neck to dock and generate the force in real dynamic situations.

Actin Dynamics and Calcium Signaling in B Cells Respond to Surface Topography

Christina Ketchum, Xiaoyu Sun, Heather Miller, John Fourkas, Wenxia Song, Arpita Upadhyaya
University of Maryland, College Park, MD

The topography of the cellular environment in recent years has been recognized as an important factor in regulating cell shape and motility, leading to changes in gene expression. B cells become activated by membrane-bound antigen binding to the B cell receptor (BCR), inducing actin dynamics, reorganization of receptors into microclusters, and cell spreading. In vivo B cells gather antigen from a variety of sources which may have different physical characteristics such as mobility, stiffness or topography. However, the effect of these parameters on BCR clustering and signaling activation is not understood. In previous studies we have demonstrated that ligand mobility has a profound effect on B cell activation, as well as BCR and actin organization. Here we have studied the role of topography in cell spreading, actin polymerization and signaling activation. BCR ligand coated substrates presenting ridges of variable spacing were used to probe the interaction of B cells with non-planar surfaces. Using high-resolution TIRF and confocal microscopy of live cells, we followed the movement of BCR clusters and the dynamics of actin as well as the associated signaling using calcium sensors. Ridge spacings ranging from 0.8μm to 5μm resulted in two different modes of actin behavior. Spacings less than 1.5μm induced actin waves that traveled parallel to the ridge, resulting in protrusions and retractions of the cell edge. Spacings of 3μm or greater resulted in large scale contractions of the actin network. Furthermore, cells exhibited a periodic (~30s) calcium flux on flat substrates. The period of the flux was dependent on ridge spacing, with increasing time intervals on smaller spacings. Our results indicate that B cells are sensitive to topographical features, resulting in modulated actin dynamics and signaling activation.

Precision and Reproducibility in Development

Thomas Gregor
Princeton University

The outcome of organismal development is extremely reproducible from one individual to the next. However, the underlying elementary processes that lead to the final organism are prone to environmental and molecular variability, and it is thus unclear how precision is achieved along the developmental path. Recent experimental findings and theoretical descriptions have shed new light on the origin of the reproducibility of molecular and structural patterns. In particular, quantitative measurements have demonstrated that from the earliest to the latest stages of development, molecular precision is maintained at the maximally possible level: molecular fluctuations between adjacent cells and from individual to individual are kept below 10% across successive cellular developmental stages, which translates into a spatial accuracy for cell fate decisions of less than the size of an individual cell, arguably the physical limit that biological systems can achieve. These results argue for an evolutionary design principle in which developmental systems operate near an optimal level of precision.
From Genome to Form: Measuring a Simple Mathematical Input-Output Relation for a 250-bp Regulatory DNA Element in the *Drosophila* Embryo


1 Joseph Henry Laboratories of Physics, Princeton University, Princeton, NJ, USA, 2 Department of Biology, New York University, 100 Washington Square East, New York, NY 10003, USA, and 3 Lewis Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA

The expression of a gene is regulated by the binding of specific proteins, known as transcription factors, on regulatory DNA elements, called enhancers. Despite extensive knowledge of the structure of these enhancers and their binding factors, we cannot quantitatively predict levels of transcriptional output given known inputs in the form of protein–DNA interactions. Here we show progress towards gaining such predictive power by using recently developed methods to measure transcription of a well-characterized 250-bp reporter construct in vivo in the early Drosophila embryo. We measured the effects of single binding sites on mRNA expression by imaging transcription events of this construct, which contains several known transcription factor binding sites that can be individually enabled or disabled. Despite differences in position and sequence, different binding sites show no difference in their contribution to mRNA expression. We find that mRNA expression levels and domain boundaries are both very strongly correlated with the number of functioning binding sites, and we infer from these correlations that the effect of each additional binding site is to recruit 1.8 polymerase/min to the promoter and lower the threshold concentration of the input factor needed for transcriptional activity by roughly 20%. Thus, mRNA expression as a function of transcription factor binding site number is a linear effect: all binding sites contribute equally and additively to the level of mRNA expression and the domain over which mRNA is expressed. Contrary to classical biochemical studies of cooperativity in this enhancer, our data thus imply a lack of cooperativity between binding sites in regulating mRNA expression.

Comparing Fungal Foraging Strategies via Simulation

**Sophie Zhang**, **Ned Wingreen**, **Anne-Florence Bitbol**

1 Department of Physics, Princeton University, 2 Department of Molecular Biology, Princeton University, 3 Lewis-Sigler Institute for Integrative Genomics, Princeton University

Organisms such as fungi are capable of displaying complex foraging and resource allocation behavior, such as proliferation of hyphal strands while searching for food. This is despite being simple organisms without a central nervous system. To explore such strategic behavior, we developed and studied a computational model in which correlated food distributions are generated via Metropolis Monte Carlo using an attractive nearest-neighbor food-food interaction potential. We then explored foraging behavior on such distributions for multiple different correlation strengths and foraging strategies. Assuming the simulated growing organism has only the form of the food-food potential and knowledge of the discovered food to guide it, we nevertheless found foraging strategies that performed substantially better than random search.

The Effect of MreB Polymer Biophysics on *Escherichia coli* Cell Shape

**Nikolay Ouzounov**, **Jeffrey P. Nguyen**, **Benjamin P. Bratton**, **Zemer Gitai** and **Joshua W. Shaevitz**

a - Department of Molecular Biology, b - Lewis-Sigler Institute for Integrative Genomics and c - Department of Physics, Princeton University, Princeton, NJ, 08544, USA

The mechanisms behind how bacteria, such as *E. coli*, maintain a tight distribution of cell diameters remain unknown. The bacterial actin homologue MreB is essential for rod shape and spatially patterns cell wall synthesis. Through the use of fluorescence microscopy and computer vision, we are now able to address questions about how MreB polymer characteristics correlate with cell shape. We have altered cell shape and MreB through mutation and the use of MreB polymerization-inhibiting drugs such as A22. We find an inverse correlation between MreB polymer angle and cell diameter. We also find other correlations such as between MreB polymer length and A22 resistance. These data represent the first
evidence that changes in MreB polymer structure affect cell shape and provide a possible mechanism of regulating cell diameter.

Nuclear Actin Counters Gravity during Cell Growth

Marina Feric and Clifford P. Brangwynne
Department of Chemical & Biological Engineering, Princeton University

Actin forms filaments that primarily serve a structural role in the cytoplasm. The nucleus also contains actin, yet its form and functions are not completely understood. In particular, the large oocytes (immature eggs) of the frog X. laevis actively maintain significantly high concentrations of nuclear actin. We have recently shown that this nuclear actin meshwork stabilizes the liquid-like nuclear bodies from rapid gravitational sedimentation and fusion. However, the mechanical properties of actin remain poorly characterized, despite their apparent importance in kinetically stabilizing the nucleus. Here, we use approaches in active and passive microrheology, in addition to confocal and two-photon excitation microscopy and quantitative image analysis, to probe the local mechanics and microstructure of this nuclear actin network. We find that actin forms a surprisingly soft, viscoelastic scaffold with a mesh size of ~0.5 microns. The distribution of nuclear bodies within the actin network shows signatures of creep during growth. Upon application of forces of ~1 pN, nuclear actin exhibits non-linear, shear-thickening behavior, which could serve a protective role in response to shocks. However, higher forces ultimately lead to mechanical failure and rupture of the actin network. These measurements elucidate mechanical and geometric aspects of cell organization, which suggests that biophysical constraints can play an important role in cell growth and size control.

Physical Limits to Biomechanical Sensing

Farzan Beroz
Princeton University

Experiments have shown that eukaryotic cells such as fibroblasts and mesenchymal stem cells are able to accurately probe and respond to the elastic properties of their microenvironment. These cells navigate across gradients in stiffness, a phenomenon that has been called “durotaxis.” Spatial heterogeneity in the cell’s elastic environment produces sampling noise in local probing of stiffness, which places fundamental limits on the accuracy with which a cell can sense stiffness gradients. To probe the biophysical limits of durotaxis, we develop a quantitative model of a cell as a stiffness-measuring device interacting with a disordered fiber network in two and three dimensions.

Probing the Elastic Limit of DNA Bending

Tung T. Le and Harold D. Kim
School of Physics, Georgia Institute of Technology, Atlanta, GA 30332-0430, USA

Sharp bending of double-stranded DNA (dsDNA) plays an essential role in genome structure and function. However, the elastic limit of dsDNA bending remains controversial. Here, we measured the opening rates of small dsDNA loops with contour lengths ranging between 40 and 200 bp using single-molecule Fluorescence Resonance Energy Transfer (FRET). The relationship of loop lifetime to loop size revealed a critical transition in bending stress. Above the critical loop size, the loop lifetime changed with loop size in a manner consistent with elastic bending stress, but below it, became less sensitive to loop size, indicative of softened dsDNA. We show that our result is in quantitative agreement with the kinkable worm-like chain model. Our findings shed new light on the energetics of sharply bent dsDNA.
Mapping the Complex Spatiotemporal Dynamics of Electrical Activations in the Heart

Flavio Fenton  
School of Physics, Georgia Institute of Technology, Atlanta, GA 30332-0430, USA

The heart contracts in response to electrical waves that propagate through cardiac muscle. Normally, the electrical waves propagate smoothly to effect a coordinated and effective contraction. However, in pathologic states or during very fast pacing a period doubling bifurcation can appear and promote large changes in action potential duration (the time a cardiac is in an excited state) and generate complex spatiotemporal patterns. These patterns affect the normal propagation of the electrical waves and often lead to conduction blocks and initiation of spiral waves that then drive the heart to faster and chaotic rhythms (arrhythmias). In this talk we will describe theoretical some mechanisms that generate the complicated dynamics of these patterns and show quantitative examples of experimental optical mapping recordings from the epicardial and endocardial surfaces of the heart.

Simulations of an Outer-Membrane Transporter in a Realistic Environment

Curtis Balusek and James C. Gumbart  
Georgia Institute of Technology, Atlanta, GA 30332-0430, USA

Gram-negative bacteria possess an asymmetric outer-membrane (OM) bilayer consisting of extracellular (EC) lipopolysaccharide (LPS) and periplasmic phospholipids, analogous to the symmetric phospholipid inner membrane. Simulations of the cobalamin (CBL) transporter BtuB in a symmetric and asymmetric bilayer reveal that the asymmetry of the OM substantially affects EC loops of the BtuB. The oligosaccharide of LPS is found to stabilize the EC loops and key binding residues of BtuB; however, it is further shown that Ca$^{2+}$ ions that bind to the so-called "ASP-cage" induce stability in CBL-binding residues, bringing them closer to their organization found in the CBL-bound structure.

Bottle Brush Swollen Pericellular Matrix Mediates Particle Transport to Cell by Size and Charge

Patrick Chang, Louis McLane, Jennifer Curtis  
Georgia Institute of Technology, Atlanta, GA 30332-0430, USA

Access to the surface of many cell types in vitro as well as some in vivo is impacted by a polymer brush-like structure grafted to the cell surface. Just one polymer layer thick, this often microns thick pericellular matrix (PCM) is comprised primarily of a linear polymer chain, hyaluronan, which is stretched out due to its association with large bottlebrush proteoglycan molecules (eg. aggrecan, versican, neurocan). These highly negatively charged bottlebrush molecules are semi-flexible cylinders with a dimension of ~350x80nm, and they can densely decorate the polymer chain. Our group is interested in how the physical properties of this surface-associated polymer matrix mediates contact with the cell surface in processes like cell adhesion and the transport of molecules and particles to and from the cell.

In this talk, I present the first systematic investigation of transport through the cell coat as a function of particle size versus proteoglycan content in the matrix. Several new biophysical assays are presented, including the quantitative particle exclusion assay, the fluorescent proteoglycan assay, and the exogenous tuning of proteoglycans. Further, I will share compelling data that indicates that positively charged molecules can sequester in the negatively charged pericellular matrix. This preliminary evidence supports the interesting hypothesis that the PCM acts as a local microreservoir that can concentrate and control the timely delivery of critical biomolecules like growth factors and cytokines.

Bradford P Taylor, Michael H Cortez, Joshua S Weitz
1 School of Physics and 2 School of Biology, Georgia Institute of Technology

Virophage are viruses of viruses. In order to propagate, a virophage must coinfect a mutual host with another virus. Virophage achieve coinfection through two different modes: one where a virophage independently enters the host prior to viral infection and one where a virophage attaches to the virus and the pair later enter the host. We construct two population-level models of virophage-virus-host interactions—each model corresponding to a different mode of coinfection. We demonstrate that, in both models, coexistence occurs between host, virus, and virophage is possible and that the presence of the virophage always reduces viral populations and increases host populations. In addition, a model containing both types of virophage, allows for coexistence between virophage strains. This has yet to be observed empirically; however, experimental virophage research has been limited and our results suggest new ecological possibilities.

Cybernetic sidewinders: Modulation of orthogonal body waves enables versatile maneuverability

1 Georgia Institute of Technology; 2 Carnegie Mellon University

Sidewinding is an unusual form of snake locomotion used to move rapidly on yielding substrates such as desert sands. Sidewinding snakes generate horizontal and vertical waves, with a phase offset of ±π/2, resulting in posteriorly-propagating alternating regions of static contact with the substrate and elevated motion, resulting in a “stepping” motion of body segments. Field observations indicate that sidewinding snakes are highly maneuverable, but the mechanisms by which these snakes control their turning are unknown. Motion capture data collected at our facility at Zoo Atlanta from three sidewinder rattlesnakes (Crotalus cerastes) shows two distinct turning methods: “differential turning” and “reversal turning”. In differential turning, the amplitude of the horizontal wave changes along the length, causing one end to move further than the other, resulting in turns of 25.6° ± 12.9° degrees per cycle (maximum: 86.1°). In reversal turning, the vertical wave’s phase suddenly changes by π, causing all lifted segments to drop to the ground while all ground contact segments are lifted and resulting in a sudden, large change in movement direction without any body rotation (77.8° ± 27.4° degrees per cycle, maximum: 160.5°). We then applied these control mechanisms to a 16-link snake robot capable of sidewinding on sand. By modulation of horizontal wave amplitude gradient along the body, we were able to replicate differential turning, and by producing a π phase shift in the vertical wave, we replicated a reversal turn, thereby dramatically improving the maneuverability of the robot. These results show how the combination of two waves in a complex environment can result in the emergence of complex behaviors and, conversely, that highly complex biological systems can be controlled using a limited template.

Duration of Urination Does not Change with Body Size

Patricia Yang, Jonathan Pham, Jerome Choo, David Hu
Georgia Institute of Technology

Many urological studies rely on models of animals, such as rats and pigs, but their relation to the human urinary system is poorly understood. Here, we elucidate the hydrodynamics of urination across five orders of magnitude in body mass. Using high-speed videography and flow-rate measurement obtained at Zoo Atlanta, we discover that all mammals above 3 kg in weight empty their bladders over nearly constant duration of 21 ± 13 s. This feat is possible, because larger animals have longer urethras and thus, higher gravitational force and higher flow speed. Smaller mammals are challenged during urination by high viscous and capillary forces that limit their urine to single drops. Our findings reveal that the urethra is a flow-enhancing device, enabling the urinary system to be scaled up by a factor of 3,600 in volume without compromising its function. This study may help to diagnose urinary problems in animals as well as inspire the design of scalable hydrodynamic systems based on those in nature.
An Introduction to Cryo-Electron Tomography

Chen Chen, Lu Gan
National University of Singapore

Cryo-electron tomography (cryo-ET) has been used to study cellular structures at molecular resolution in 3-D. Cells or proteins are frozen in a near-native state in vitreous ice. The frozen hydrated sample is then imaged by transmission electron microscope under liquid nitrogen temperature. After one projection image is taken, the sample is tilted by 1° or 2° before another image is acquired. This process is repeated from -60° to +60° so that at the end of data collection, a series of 2-D images are acquired at different viewing angles. This tilt series can be computationally reconstructed into a tomogram, which represents the 3-D volume of the sample being studied. An example application of cryo-ET is to study the cell size variation of a single-celled picoplankton, O. tauri.

Applications of Electron Cryotomography

Lu Gan
National University of Singapore, Centre for BioImaging Sciences

Electron cryotomography (also called cryo electron tomography or cryo-ET) enables an unprecedented 3-D view of minimally perturbed cells. We have applied cryo-ET to learn how the tiny algal cells are organized. For example, this technique allowed us to simply count how many organelles and even how many (large) molecular machines exist in each cell. It remains a challenge, however, to document the locations and orientations of the smaller macromolecular complexes, such as nucleosomes residing in the crowded nucleus. Experimental limitations such as the low signal-to-noise ratio, radiation damage, missing data, macromolecular heterogeneity will need to be mitigated. In the future, cryo-ET will provide a census and addresses of key molecular machines, which should enable accurate simulations of subcellular processes.

Nanoscale Dynamics in Ultrathin Liquids Visualized with TEM

Utkur Mirsaidov1,2,3
1 Graphene Research Center and Department of Physics, National University of Singapore, Singapore; 2 Center for BioImaging Sciences, National University of Singapore, Singapore; 3 NUSNNI-Nanocore, National University of Singapore, Singapore

Nanoscale imaging of frozen aqueous specimens and solid materials with transmission electron microscopes (TEM) enabled us to study the structure organic and inorganic materials with atomic resolution. However, the conventional TEM is not suitable for study of dynamic processes in liquids. Since there are many important phenomena in life and physical sciences that occur only in liquid environments there is an advantage of being able to image nanoscale processes directly in liquids [1-3]. I will describe our recent work on the development of a liquid cell platform for imaging soft materials and biological samples in liquids using TEM [4-6]. Liquid cell consists of two ultrathin electron translucent membranes that sandwich thin solution layer and thus effectively protecting it against the vacuum of TEM. We use this platform to study liquid properties at nanoscale. Here we show that the properties of fluids at nanoscale are dominated by its interfacial interaction with the solid substrate surface and drastically differ from the expected bulk behavior. For example, the diffusive movement and rotation of nanocrystals within liquid nanodroplets are severely dampened when compared with macroscopic fluids [7]. We will describe our results on applying this platform towards biological imaging [6]. In addition, we will also describe our attempts at new all-graphene nanofluidic platforms that enable high contrast imaging of nanoscale dynamic processes in liquids [8].

Fluorescence correlation spectroscopy (FCS) is a ubiquitously used single molecule sensitive biophysical tool to quantitatively study molecular dynamics and concentration both in vitro and in vivo. In its conventional realization, FCS is limited to single spot measurements. However, one needs to measure multiple spots simultaneously to study concerted spatial events which are of particular interest for biological specimens. We developed an imaging modality of FCS that provides a spatio-temporal read-out with sufficient time resolution by integrating plane illumination schemes such as total internal reflection (TIR) and single plane illumination microscopy (SPIM) and multiplexed detection by fast and sensitive EMCCD and sCMOS cameras. The detectors capture a series of fluorescence images of the sample with a speed of at least 1000 frames per second, sufficiently fast to capture diffusion dynamics of proteins in the cytosol and in membranes. Imaging FCS offers a number of advantages: i) Real-time spatial multiplexing of dynamics and concentration providing excellent statistics and better quantification, ii) Recording of ‘FCS movies’ to observe temporal evolution of dynamics in space, and iii) Straightforward implementation of FCS diffusion law to extract structural information below resolution limit. We applied Imaging FCS in 2D and 3D to explore structure and dynamics of supported lipid bilayers, giant unilamellar vesicles and live cell membranes. In another application, Imaging FCS was used to investigate the interactions of human islet amyloid polypeptide (hIAPP) with membranes. In addition, we applied Bayesian analysis of Imaging FCS to deconvolve distinct temporal phases of hIAPP-membrane interaction in live cells. These measurements demonstrate that Imaging FCS can provide quantitative images of biological samples. Imaging FCS is therefore a step towards quantitative bioimaging in which image contrast is provided by quantitative molecular properties and not by simple fluorescence intensities.
Pathogen Avoidance by Worms is a Collective Behavior

Erel Levine
Harvard University

Bacterivorous worms faced with noxious food face a dilemma: stay, and risk infection, or move away, and risk starvation. Here we use quantitative experiments and modeling to show that worms can make this decision collectively. We identify molecular and neuronal pathways involved in mediating stigmergic interactions among worms.

Competitive Coexistence on Shared Resources Evolves due to Crowded Growth

Evgeni Frenkel, Michael McDonald, David van Dyken, Katya Kosheleva, Greg Lang, Michael Desai
Harvard University

Identifying the mechanisms that maintain stable coexistence of competing lineages is central to understanding biodiversity. Diversification of microbial populations competing for shared resources typically requires evolution of differences in resource consumption (e.g. cross-feeding). Here we describe a newly-observed mechanism of coexistence caused by differences in growth geometry that evolved spontaneously in laboratory populations of budding yeast. Members of one type are able to grow faster, but limit each other’s access to nutrients in a density dependent manner. Meanwhile, a competing type has evolved to escape this crowding by growing as a dispersed, thin biofilm. We introduce a simple mathematical model of this mechanism of coexistence that quantitatively predicts a variety of experimental data, as well as the future evolution of the system. Crowded growth is known to have the same effect in biofilms and solid tumors, and thus biodiversity in these natural environments may derive from mechanisms similar to the one we report here.

Reconstructing and Visualizing the First Relay of the Drosophila Larva Olfactory System

Matthew Berck,1,2 Avinash Khandelwal,4 Lindsey Claus,3 Guangwei Si,1,2 Christopher Tabone,1,2 Casey Schneider-Mizell,5 Matthieu Louis,4 Albert Cardona,6 Aravinthan Samuel1,2
1 Department of Physics; 2 Center for Brain Science; 3 Department of Mathematics; Harvard University, Cambridge, MA 02138, USA; 4 EMBL/CRG Systems Biology Unit, Center for Genomic Regulation (CRG) and UPF, Barcelona, Spain; 5 Janelia Farm Research Campus, Howard Hughes Medical Institute, Ashburn, Virginia, USA

The antennal lobe is the first processing center for the Drosophila melanogaster larval olfactory system. This brain center contains three broad classes of neurons: the axons of the larva’s 21 odor receptor neurons (ORNs), the dendrites of projections neurons (PNs) that send olfactory information to other brain centers, and local neurons (LNIs) that are responsible for processing the information within the antennal lobe itself. Using a serial section electron microscopy data set, we are reconstructing all of these neurons and elucidating the synapses between them in order to create a map of the first relay of the olfactory system. Analysis of this map reveals that each ORN has distinct downstream connectivity and that there are two separate classes of LNs that regulate two distinct types of PNs. To further analyze the antennal lobe circuitry, we have also designed and utilized a microfluidic device to perform in vivo calcium imaging. With this setup, we can visualize which ORNs respond to known olfactory cues at various concentrations and see the differences in the timescales of adaptation of the ORNs and downstream neurons to long odor stimuli. By performing direct physiological measurements with single-cell resolution throughout the olfactory circuit, we are able to enhance the synaptic connectivity map by adding dynamics that indicate the sign and strength of connections between neurons.
Gene Regulatory Network Modeling Dynamic Host-Pathogen Interaction of *Caenorhabditis elegans* and *Pseudomonas aeruginosa*

*Lucy Eunju Lee*, Javier Irazoqui, Erel Levine

1 Department of Physics, Harvard University, Center for Systems Biology; 2 Laboratory of Comparative Immunology, Center for the Study of Inflammatory Bowel Diseases, Massachusetts General Hospital, Harvard Medical School

The course of infection is a complex control process of gene transcription within a pathogen and its host. The regulatory mechanisms of the host immune reactions and the virulence factors of its pathogen react recursively to each other’s responses, resulting in a spectrum of final consequences from complete remission or chronic infection to death of the host. In this study, we hypothesized that the genes of host and pathogen compose an integrated network of transcriptional control, which continually evolves by interaction among genes over the time course of infection.

We studied the interaction between a nematode *C. elegans* and its pathogen *P. aeruginosa* to study how changes in the gene expression of the host influence changes in that of the pathogen, and vice versa. To test our hypothesis, the time-course gene expression data were obtained by collecting RNA of both *C. elegans* and *P. aeruginosa* from the infected worms using RNA-Seq technique at multiple time points during the infection. Using quantitative data analysis methods including network clustering and inference, we also identified potentially important regulatory gene clusters of the network. To verify the function of these gene groups during the course of infection, we performed the perturbation killing assays using RNAi, and the preliminary data showed that the selected genes in these clusters affected the survival curves of infected worms.

Ultimately, we would like to develop an efficient method to diagnose infectious diseases and to predict their prognoses by analyzing activities of critical gene groups in the pathogen-host regulatory model. Furthermore, we also expect to describe a method to identify the key regulatory pathway, which can change the final outcome of the infectious process by changing or blocking the specific connection in the regulatory network.

Quantifying Synaptic Reorganization in the Developing Cerebellum Using Serial-Section Scanning Electron Microscopy Data

*Alyssa Wilson*, Richard Schalek, Josh Morgan, Ray Jones, Seymour Knowles-Barley, and Jeff Lichtman

1 Department of Physics, Harvard University, Cambridge, MA 02138, USA; 2 Center for Brain Science, Harvard University, Cambridge, MA 02138, USA; 3 Harvard School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138, USA; 4 Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA 02138, USA

Development is physically manifested in parts of the nervous system as a rewiring of synapses between classes of neurons and their targets. For the most part, knowledge of the mechanisms that guide this process is poor. In easily accessible parts of the peripheral nervous system like the neuromuscular junction, techniques like in vivo fluorescence imaging and electrophysiology have revealed some information about the rewiring that occurs there: in general, classes of neurons are initially highly interconnected and over development many connections are pruned, while surviving synapses are strengthened, resulting in a refined neural wiring. This process is called “synapse elimination” and is thought to be driven by synaptic activity and thus experience. Similar techniques have been much less informative in the central nervous system, however. To overcome this barrier to understanding, I am using serial section scanning electron microscopy to produce 3D volumes of high-resolution images of wild-type cerebellum tissue from mice in early postnatal development. Unlike other methods, the resolution of electron microscopy is sufficient to clearly identify all synapses in a tissue sample. Serial section scanning electron microscopy, a recent adaptation of this technique, is capable of imaging blocks of tissue 100s of microns thick with minimal loss, reasonably quickly. The cerebellum is a good system to investigate because it is simple and compact. I am reconstructing Purkinje cells and their climbing fiber inputs, and will measure the numbers, positions, and strengths of their synapses to establish a ground truth for this information. I will use it to provide insights about the mechanisms underlying cerebellar rewiring by determining, first, whether this process is a minor refinement or a major rewiring; and second, whether the mediators of cerebellar rewiring are similar to those of peripheral synapse elimination.
Fast and Efficient DNA Based Molecular Motors Assisted by Microfluidics and Single-Molecule Fluorescence

Toma Tomova, Roman Tsukanov a, Yair Glick b, Miran Liber a, Yaron Berger a, Doron Gerber b and Eyal Nir a

a Department of Chemistry and the Ilse Katz Institute for Nanoscale Science and Technology, Ben-Gurion University of the Negev, Beer Sheva, 84105, Israel, Eynalinr@bgu.ac.il; b Department of Life-Sciences, Bar-Ilan University, Ramat-Gan, Israel

Protein based molecular machines play major roles in important biological processes, often with impressive operational yields and speeds. Inspired by biological bipedal walkers and with the assistance of single-molecule fluorescence (SMF) and microfluidic technology, we design and operate a DNA based bipedal walker that can stride on a DNA origami track over unprecedented distance, processivity and speed. The motor, which can cross between adjacent origami tiles, preformed 36 steps with more than 50% operational yield, which is equivalent to more than 99% yield for chemical reaction, and the stepping rate can be faster than several seconds. In the talk I will demonstrate how the SMF sub-second and nanometer resolutions enables structural dynamics investigation of the motor operation, identification of unwanted side-reactions, which led to rational improvements, and monitoring of the motor progress. I will show how the microfluidics enables computer controlled introduction of fuel and anti-fuel DNA strands providing excellent control of the motor operation. The combination of DNA nanotechnology, SMF and microfluidics makes the realization of fast and efficient DNA based artificial machines much more likely, and can facilitate better understanding of biological machines and the ability to control them.

Cellulosomes – A Structurally Robust Multi-Protein Platform for Broad Nanotechnological Application

Edward A. Bayer
Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot, Israel

Cellulosomes comprise a set of Lego-like multi-modular components – some structural and some enzymatic – that self-assemble into a highly stable, discrete complex. Cellulosomes are composed of one or more structural subunits (the scaffoldins) and numerous functional subunits (both enzymatic and nonenzymatic). The scaffoldins include one or more cohesin modules, which exhibit exquisite high-affinity binding specificity for a dockerin module, borne by each of the various subunits, and the tenacious cohesin-dockerin interaction dictates the configuration of cellulosome assembly and overall architecture. Earlier structural work involved electron microscopic studies of the isolated cellulosomes and cellulosomes on the cell surface of the anaerobic thermophilic cellulolytic bacterium, Clostridium thermocellum. More recently, X-ray crystallography, small-angle X-ray scattering (SAXS), nuclear magnetic resonance (NMR), single-molecule force spectroscopy (SMFS) and molecular dynamics (MD) have been applied to determine the characteristics of the cohesin-dockerin interactions and structural consequences in piecing together the architectural elements of the cellulosomes produced by a variety of cellulolytic bacteria. Using recombinant genetic techniques, artificial complexes (termed designer cellulosomes) can be fabricated by mixing and matching the specificities of cohesin-dockerin pairs and other components, thus creating improved catalytic devices with a virtually unlimited scope of nanoscale application.

Ultrastable Cellulosome-Adhesion Complex Tightens Under Load

Constantin Schoeler a,†, Klara H. Malinowska a, Rafael C. Bernardi a, Lukas F. Milles a, Markus A. Jobst a, Ellis Durner a, Wolfgang Ott b, Daniel B. Fried b, Edward A. Bayer a, Klaus Schulten a,c, Hermann E. Gaub a and Michael A. Nash a

1 Lehrstuhl für Angewandte Physik und Center for Nanoscience, Ludwig-Maximilians-Universität, 80799 Munich, Germany; 2 Theoretical & Computational Biophysics Group, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA; 3 Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel; 4 Department of Physics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA. † Equally contributing.
Challenging environments have guided nature in the development of ultrastable protein complexes. Specialized bacteria produce highly flexible protein networks called cellulosomes to effectively digest lignocellulosic biomass. While network assembly is enabled by protein interactions with commonplace affinities, certain cellulosomal receptor-ligand interactions exhibit extreme resistance to applied force. Through single molecule force spectroscopy and steered molecular dynamics simulations, we characterized the ligand-receptor complex responsible for substrate anchoring in the *Ruminococcus flavefaciens* cellulosome. The complex withstood forces of 600-750 pN, representing the strongest bimolecular interaction reported to date, equivalent to half the mechanical strength of a covalent bond. Our findings demonstrate force activation and stabilization of the complex, and suggest that certain network components serve as mechanical effectors for maintaining network integrity. This detailed understanding of cellulosomal network components could help in the future development of biocatalysts for production of fuels, chemicals, and pharmaceuticals from renewable plant-derived biomass.

Conformational Dynamics in Designer Cellulosomes Studied by Single-Pair FRET with MFD-PIE

*Anders Barth¹, Jelle Hendrix², Edward A. Bayer³ and Don C. Lamb¹⁴*

1 Physical Chemistry, Department of Chemistry, Munich Center for Integrated Protein Science (CiPSM) and Center for Nanoscience (CeNS), Ludwig-Maximilians-Universität München, Butenandtstr. 11, Gerhard-Ertl-Building, 81377 Munich, Germany; 2 Laboratory of Photochemistry and Spectroscopy, Department of Chemistry, University of Leuven, Celestijnenlaan 200F, B-3001 Leuven, Belgium; 3 Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100 Israel; 4 Department of Physics, University of Illinois at Urbana-Champaign, 1110 W. Green St., Urbana, IL 61801, USA

Cellulose is the most abundant biopolymer on earth and holds great potential as a source for bioethanol. The bottleneck for the conversion is the break down of cellulose into oligosugars. In nature, the anaerobic thermophilic bacterium *Clostridium thermocellum* expresses an extracellular multi-enzyme complex that degrades cellulose: the cellulosome. Cellulosomes in general consist of a scaffold that anchors the bacterium to the cellulose and harbors binding sites for cellulose-degrading enzymes. These cellulases bind via their dockerin domain to a complementary cohesin domain on the scaffoldin. An emerging research field aims at engineering ‘designer cellulosomes’: by genetically coupling different types of cohesins, novel minimal cellulosomes with similar, if not better enzymatic activity than the wild-type can be obtained. Little is known about the exact role of the linker peptides between cohesin moieties. To address this, we have site-specifically labeled a designer cellulosome consisting of two cohesin subunits connected by either a natural or shortened peptide linker and performed single pair Förster resonance energy transfer experiments using pulsed interleaved excitation and multi-parameter fluorescence detection. We observe different conformational states of the cohesin dimer, suggesting the presence of conformational dynamics. Relating the conformational dynamics of such designer cellulosomes with their activity will be of great help for understanding and improving their function.

Direct Observations on Protein-DNA Interactions in Dense and Segregated DNA Phases

*Dan Bracha*

Materials & Interfaces Department, Weizmann Institute of Science, Rehovot, Israel

Segregation of basic biochemical reactions into specialized subcellular microenvironments is an important characteristic of the living cell, enabling optimized conditions for specific biochemical activity. Compartimentalization can be achieved even in the absence of a membrane envelope, as exemplified by the bacterial nucleoid that maintains a distinctive composition of soluble proteins, which is different than the surrounding cytosol. By immobilizing DNA onto a surface at controllable DNA density that may reach values similar to those of living cells, we assemble biochemically active units of crowded DNA, and investigate the conditions for and the consequences of boundary-free compartmentalization. Direct measurements of the local concentration of various biomolecules at equilibrium using TIRF microscopy indicate that DNA segregation by itself is sufficient for imposing uneven distributions of biomolecules, where the degree of partitioning depends on the local DNA density. Interestingly, while high affinity DNA associating proteins are confined to the DNA phase, proteins with moderate DNA affinity, such as T7 RNA polymerase are strongly excluded,
apparently due to entropic considerations. Biochemical activity alters these equilibrium partitioning levels, yielding further exclusion or confinement at steady state. Exclusion was also observed for mRNA, suggesting that during active gene expression using cell extract or purified components, nascent mRNA molecules are spontaneously transported out of the DNA compartment and towards the ribosome rich medium of the surrounding reservoir. We speculate that by increasing the macromolecular crowding conditions in the reservoir we may selectively facilitate the penetration of DNA associating proteins into the DNA compartment while keeping DNA-insert biomolecules out, thereby dictating order that promotes efficient gene-expression.

**Abstracts (Talks): Thursday, July 24**

**The Energy Landscape for Protein Folding and Biomolecular Machines**

*José N. Onuchic*
Center for Theoretical Biological Physics, Rice University

It is amazing how cells have created a number of molecular machines specialized for undertaking tasks needed to control and maintain cellular functions with exquisite precision. Due to fact that biomolecules fluctuate via thermal motion and their dynamics is diffusive, biological machines are fundamentally different from those experienced by conventional heat engines or machines in the macroscopic world. One of the key features of biological machines is the conformational changes triggered by the thermal noise under weak environmental perturbation. Therefore we can explain how they behave using ideas borrowed from the energy landscape theory of protein folding and polymer dynamics. This “new view” allows us to envisage the dynamics of molecular motors from the structural perspective and it provides the means to make several quantitative predictions that can be tested by experiments. For the kinesin motor, a prototype of the biological machines in the cell, molecular simulations of an explicit kinesin and microtubule structures show that fluctuations and flexibility inherent to the structure leads to versatile adaptation of the molecular structure, allosteric communication controlled by internal mechanics, and large amplitude stepping motion harnessing the thermal fluctuation.

* Supported by the NSF

**Reduced Model Captures Mg**2+ **Dependence of the RNA Free Energy Landscape**

*Ryan L. Hayes†, Jeffrey K. Noel†, Ana Mandić‡, Paul C. Whitford‡, Udayan Mohanty§, Karissa Y. Sanbonmatsu‖, José N. Onuchic†*
† - Center for Theoretical Biological Physics, Rice University, ‡ - Department of Biomedical Engineering, University of Houston, † - Department of Physics, Northeastern University, § - Department of Chemistry, Boston College, ‖ - Theoretic Biology and Biophysics, Los Alamos National Labs

The RNA energy landscape is sensitive to the ionic environment, and especially to Mg**2+. At physiological ionic concentrations the landscape must be minimally frustrated and funneled to fold on biological time scales; however, variations in ionic concentration can strongly affect the occupancy of competing energy basins. We model the funneled nature and ionic dependence of the energy landscape by adding electrostatic interactions as a perturbation to the unfunneled landscape given by a structure-based model. Mg**2+ is represented explicitly, while KCl is represented implicitly through a Debye screening length and a Manning condensation parameter that effectively rescales RNA charge. The number of excess Mg**2+ ions associated with RNA (Γ2+) is experimentally and computationally measurable. Γ2+ is of thermodynamic relevance because in the presence of excess KCl, Γ2+ can be integrated over log concentration to give the Mg**2+-RNA interaction free energy. Differences in the interaction free energy between conformational basins reveal preferential stabilization by Mg**2+. Simulations of the adenine riboswitch closely reproduce experimental values of Γ2+, suggesting the model is well parameterized thermodynamically. The relevance of electrostatic perturbations to the free energy landscape is demonstrated by simulations of an RNA pseudoknot. While a pure structure-based model yields two-state folding, a structure-based model with electrostatics is able to capture the experimentally observed intermediate.
Alignment and Nonlinear Elasticity in Biopolymer Gels

Jingchen Feng, Herbert Levine, Xiaoming Mao, Leonard Sander
1 Bioengineering Department and Center for Theoretical Biological Physics, Rice University, Houston TX, 77251-1892; 2 Department of Physics, University of Michigan, Ann Arbor MI 48109-1040; 3 Physics & Complex Systems, University of Michigan, Ann Arbor MI 48109-1040

Biopolymer gels such as collagen are complicated materials consisting of a network of cross-linked polymer chains. These materials are important in biology. In particular, they are the medium (the extra-cellular matrix) in which cells move. For example, in the body there is almost always a gel, the extracellular matrix (ECM) which gives tissue its structure. The biopolymer collagen-I is the most common constituent of the ECM. When cells migrate within tissue they crawl by attaching to the ECM. This is often important: wound healing and cancer invasion are well-known examples. When cells move in this fashion they deform the ECM by pulling on it. However deformation also affects cell motility. In particular, if the ECM is partially aligned, cells tend to move along the aligned direction -- this is called contact guidance. An obvious step towards understanding contact guidance is to quantify the alignment of the fibers in the ECM. Almost all of Biopolymer gels show non-linear elasticity, usually strain-stiffening. Here I will present a Landau-type theory for the non-linear elasticity of biopolymer gels with a part of the order parameter describing induced nematic order of fibers in the gel [1]. Our point of view is that all of the non-linear elastic behavior of these materials can be attributed to fiber alignment induced by strain. We suggest an application to contact guidance of cell motility in tissue. We compare our theory to simulation of a disordered lattice model for biopolymers. We treat homogeneous deformations such as simple shear, hydrostatic expansion, and simple extension, and obtain good agreement between theory and simulation. We also treat the case of a localized perturbation which is intended to be a simple model for a contracting cell in a medium.


Role of Mechanical Interactions in Self-Organization of Bacteria in Biofilms

Rajesh Balagam, Douglas B. Litwin, Fabian Czerwinski, Mingzai Sun, Heidi B. Kaplan, Joshua W. Shaevitz, Oleg A. Igoshin
1 Department of Bioengineering, Rice University, TX, USA; 2 Department of Microbiology and Molecular Genetics, University of Texas – Houston Medical School, Houston, TX, USA; 3 Department of Physics and the Lewis-Sigler Institute of Integrative Genomics, Princeton University, Princeton, NJ, USA; *E-mail: rajesh.balagam@rice.edu, igoshin@rice.edu

Bacterial cells self-organize into complex three dimensional multi-cellular structures in biofilms. This process requires coordinated cell movement through variety of chemical and mechanical interactions. Genetic studies have uncovered many of the chemical cues involved; however the role of mechanical interactions is poorly understood. Here, we choose Myxococcus xanthus, a model bacteria for self-organization in biofilms, to study the role of mechanical interactions among cells and with its environment in self-organization. We have developed a computational biophysical model of M. xanthus cell and investigated the mechanism of individual cell motility and cell behavior in groups. Mechanism of gliding motility (smooth movement of cell on solid surface) in M. xanthus is still under investigation. Recent experimental studies proposed two alternative mechanisms of gliding motility that differed in their cell – substrate interactions: elastic coupling vs viscous coupling. We studied the mechanical behavior of model cells in pair-wise cell collisions under two mechanisms of cell motility and observed dramatically different cell behavior. Further comparisons between experimental cell collision behavior and model cells matched only under strong elastic interactions with substrate[1]. Direct measurements on gliding motor-substrate coupling through optical trap experiments further confirmed the elastic nature of this interaction. At high density, M. xanthus cells align themselves into dynamic cell groups (clusters). The exact mechanism underlying the formation of these clusters is unknown. We investigated this process using our biophysical model cells in an agent-based simulation framework. We observe that the formation of these dynamic clusters is a result of the interplay between regular reversal of cell travel direction and cells following the slime paths of other cells. We further investigated the role of elastic substrate interactions in the
Nucleosome Unwinding and Rewinding: Free Energy Landscapes, First Passages, and Time-Resolved Transition Paths

AH Mack,1,2 DJ Schlingman,1,3 M Kamenetska,1,4 M Birger,4 LJ Regan,3,5 SGJ Mochrie2,4
1 Integrated Graduate Program in Physical and Engineering Biology, Yale University, New Haven CT 06511, USA; 2 Department of Applied Physics, Yale University, New Haven CT 06511, USA; 3 Department of Molecular Biophysics and Biochemistry, Yale University, New Haven CT 06511; 4 Department of Physics, Yale University, New Haven CT 06511; 5 Department of Chemistry, Yale University, New Haven CT 06511

In this talk, we present two aspects of recent work on nucleosome forced unwinding: (1) The dynamic packaging of DNA into chromatin is a key determinant of eukaryotic gene regulation. Nucleosomes are the basic unit of chromatin and therefore, the accessible states of the nucleosome must be the starting point for mechanistic models of these key processes. Although the existence of different unwound nucleosome states has been hypothesized, there have been few studies of these states. Here, we show the existence of two distinct states of the unwound nucleosome, which are accessible at physiological forces and ionic strengths. Using optical tweezers, we measure the rates of unwinding and rewinding for these two states and indeed show that the rewinding rates from each state are different. (2) Underpinning our current understanding of biomolecular transitions is the notion of diffusion on a free energy landscape, which models processes ranging from protein and RNA folding, to the stepping of molecular motors, to biomolecular friction. Distilling the complex interactions of biomolecules into a single reaction coordinate on a free energy landscape has led to many accurate predictions. However, the molecular-scale diffusive motions implied by this theoretical picture have never been observed directly until now. To remedy this longstanding deficit, we have analyzed thousands of nucleosome unwinding transitions at microsecond time resolution, at different forces and ionic concentrations, using a fast data acquisition system. Our results permit us to directly observe the time evolution of the molecule along its transition path and determine the mean and variance of the transition time distribution, definitively establishing the existence of a molecular-scale diffusive process.

Quantification and Optimization of Image Quality for Single-molecule Switching Nanoscopy at High Speeds

Yu Lin1, 2, 3, Jane J. Long1, Fang Huang1, Whitney C. Duim1, Stefanie Kirschbaum1, Aleksander Rebane1, 4, Mary Grace M. Velasco1, 3, Alejandro Virrueta1, 3, Daniel W. Mooney1, 3, Junyi Jiao2, 3, Sandy Y. Hernandez2, 3 and Joerg Bewersdorf1, 2, 3,*
1 Department of Cell Biology, Yale University School of Medicine, New Haven, CT 06520, USA, 2 Department of Biomedical Engineering, Yale University, New Haven, CT 06520, USA; 3 Integrated Graduate Program in Physical and Engineering Biology, Yale University, New Haven, CT 06520, USA; 4 Yale College, Yale University, New Haven, CT 06520, USA; 5 Institute for Molecular Biophysics, The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA; 6 Department of Physics, Yale University, New Haven, CT 06520, USA; 7 Department of Mechanical Engineering and Material Science, Yale University, New Haven, CT 06520, USA; 8 Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06520, USA

Single-molecule switching nanoscopy (SMSN) overcomes the diffraction limit of light by stochastically switching single fluorescent molecules on and off, and then localizing their individual positions in recorded camera frames. Recent advances in SMSN have greatly accelerated the speed of data acquisition and improved the temporal resolution of super-resolution images to the sub-second range. However, it has not been quantified whether this speed increase comes at the cost of compromised image quality. The image quality in SMSN depends on many factors, among which camera speed and laser intensity are the two most critical parameters. Here we quantitatively compared the image quality achieved when imaging Alexa 647-immunolabeled microtubules in COS-7 cells over a large range of camera speeds and excitation laser intensities using three criteria – localization precision, density of localized molecules, and resolution of reconstructed images. We found that, with optimized parameters, high-speed SMSN attains the same image quality as conventional
SMSN but in a much shorter time period. Our results demonstrate the importance of optimizing the excitation laser intensity to match the probe switching rate to the used camera frame rate. We provide guidelines for choosing appropriate laser intensities for Alexa 647 at different imaging speeds and a quantification protocol for future evaluations of other probes and imaging parameters.

### Bifurcation in the Biased Random Walk of E. Coli

**Junjiajia Long, Dervis Can Vural, Thierry Emonet**

Yale University

*Escherichia coli* navigates its environment by alternating periods of straight motion called runs with abrupt changes of direction called tumbles. During a run the cell swims forward at a constant speed and its direction of motion is subject to rotational Brownian motion. During a tumble, the speed is zero and the orientation of the cell rapidly changes. To bias its random walk in the desired direction the cell uses the bacterial chemotaxis pathway, which tends to lower the probability to tumble when the cell moves towards higher concentrations of chemoattractant. An adaptation mechanism implemented as an integral feedback ensures that the probability to tumble eventually returns to its original resting state. An interesting consequence of this strategy is that the behavior feeds back onto the input signal. The faster the cell moves up the attractant gradient, the stronger the feedback, causing the cell to respond with an lower average probability to tumble than the resting value. In a recent study we used agent-based stochastic simulations to show that when this feedback is strong (e.g. in a steep gradient) bistability in chemotactic behavior of an individual cell occurs.[2] Here we show that in such cases, the probability to tumble becomes widely distributed. Thus, previous mean field analysis[3] cannot be used in this regime. We derive a master equation for the distribution of the probability to tumble, and find numerical solutions that agree with the stochastic agent-based simulation.


### A Maximum Likelihood Approach to Extract Underlying Diffusive States from Single Particle Trajectories of Rho GTPase in Live Cells

**Peter K. Koo†, Matthew Weitzman‡, Kenneth L. van Golen§, Simon G. J. Mochrie*†**

† Department of Physics, Yale University, New Haven, CT, USA; ‡ Department of Biological Sciences, University of Delaware, Newark, DE, USA; * Department of Applied Physics, Yale University, New Haven, CT, USA

In the heterogeneous environment of a cell, the same protein species may exhibit a number of different diffusive states, depending on location and association state, each with a different underlying diffusion coefficient. Resolving unique diffusive states when analyzing protein trajectories on an individual basis remains challenging because of the limited statistics provided with the relatively short tracks available experimentally. Here, we introduce a new systems-level maximum likelihood approach, based on the Gaussian mixture model, that simultaneously analyzes a population of individual particle tracks to uncover the number of distinct diffusive states, and accurately determines their corresponding diffusivities, localization noises, and population fractions. Our method to locate the global maximum likelihood employs a novel variant of the expectation-maximization algorithm, that we call Perturbation Expectation-Maximization (pEM). We test the performance of pEM on various sets of simulated particle tracks subject to static and dynamic localization noise, that realize different numbers of diffusive states with different diffusion coefficients, static localization noises, and population fractions. We then demonstrate the applicability of pEM to single protein trajectories of Rho GTPase, an integral regulator of cytoskeletal dynamics and cellular homeostasis, in live cells acquired via single particle tracking photo-activated localization microscopy. pEM uncovered 6 normal diffusive states with similar diffusivities and static localization noises conserved across RhoA, RhoC, and various functional mutants. The different population fractions, however, revealed the propensities for each diffusive state, and hence the activation preference for each Rho mutant.
The overall goal of our institute was to create a research network around the Structural Biology, Cell Biology and Tissue Biology, focused on the study of human diseases, such as amyloidogenic diseases, cancer, viral and parasitic diseases, as well degenerative diseases, where it is required the use of new therapies such as cell therapy and rational development of pharmaceuticals and vaccines. One of the achievements in the last five years was to create a unique infrastructure in INBEB where we installed operating advanced equipments assembled in three new buildings. The scientific infrastructure has made possible the study of the structure of biological systems from the macromolecular level to the whole-organism level. Throughout the five years, the Associated Laboratories (ALs) of INBEB focused on the use of multidisciplinary approaches: (1) the study of macromolecules involved in infectious diseases, neurodegenerative illnesses, and cancer; (2) the study of important viruses, such as Dengue, yellow fever, and others; (3) the study of complex cellular structures found in pathogenic protozoan enrolled in neglected diseases such as Leishmaniasis, Chagas’ disease, malaria, and toxoplasmosis; (4) the study of the in vivo behavior of stem cells, which was performed in order to analyze their biodistribution, localization, and functional to treat degenerative diseases. The members of the INBEB ALs have published more than 1000 papers, and several of them resulted from extensive collaborations between researchers among different ALs. We have completed a total of 174 masters’ dissertations and 115 doctoral theses. It is especially gratifying to see that the younger members of the group demonstrate enormous enthusiasm and creativity that holds great promise for a new generation of imaginative leaders in their research areas. The facilities have instruments that are at the forefront of knowledge in the field, some of them the only ones in Latin America. We have also concentrated our efforts in strengthening our ties with IDOR (Instituto D’Or for Research and Teaching, Hospital), thereby closing a gap between basic and clinical research (translational research). The training of undergraduate and graduate students as well as post-docs is crucial if we want to produce high-quality science with publications in high-impact journals. It is worth noting that we have incorporated Professor Kurt Wüthrich, Nobel Prize in Chemistry, as a Special Visiting Researcher of the INBEB. To increase INBEB’s interaction with society, we established a Nucleus for Education and Science Communication (NEDiCi) to disseminate the scientific results from our groups, as well as to promote activities (courses, lectures, workshops, tours, videos and others) designed for teachers and students from primary and secondary schools. The creation of all this infrastructure has increased interaction both among INBEB researchers and researchers from different Brazilian and international institutes, including the INCiT. (Supported by CNPq, FAPERJ, MS-DECIT, FINEP).

Prion-like Aggregation of p53 Tumor Suppressor Protein: New Targets for Anticancer Drugs

Danielly C. Ferraz da Costa1, Luciana P. Rangel1, Guilherme G. A. P. Oliveira1, Jerson L. Silva1
1 Institute of Medical Biochemistry Leopoldo de Meis, National Institute of Science and Technology for Structural Biology and Bioimaging, Universidade Federal do Rio de Janeiro, Rio de Janeiro,RJ, Brazil; * e-mail: dcfcosta@bioqmed.ufrj.br

p53 is a master regulatory protein that participates in cellular processes such as apoptosis, DNA repair and cell cycle control. The function of this tumor suppressor protein is lost in more than 50% of human cancers. Recent studies have suggested that the formation of prion-like aggregates of mutant p53 is associated with loss-of-function, dominant-negative and gain-of-function effects. Studies from our group have shown that p53 aggregation in a mixture of oligomers and fibrils that sequestres the native protein into an inactive conformation, typical of a prionoid behavior. The prionoid properties of p53 aggregates are considered potential targets for drug development. The prion-like properties of nucleation, templating, multiplication and spread have been already considered potential new targets for the development of neurodegenerative disease therapies. We have evaluated whether PRIMA-1, a classical drug described to stabilize mutant p53 structure and function, exerts its effect on aggregated mutant p53. Also, 2-methylene-3-quinuclidinone hydrate (MQ) has been shown to inhibit WT and mutant recombinant p53 central core domain (p53C) aggregation
at 37oC. The WT form has been protected in a lower degree. MQ has also been shown to inhibit the seeding promoted by mutant p53 cellular extracts fractions enriched in oligomers on WTp53C. The same seeding inhibition effect was observed for extracts from cells treated with PRIMA-1. We also found that resveratrol, a natural polyphenol found in grapes and red wine, has the ability to inhibit the wild-type p53 core domain as well as the R248Q p53 mutant to undergo in vitro aggregation. Additionally, resveratrol reduces the formation of nuclear p53 aggregates in MDA-MB231 human breast cancer cells. These findings may pave the way for a better understanding of the mechanisms involved in p53 protein aggregation as a therapeutic strategy for cancer treatment.

“Push and Pull” Hypothesis to Unify the Physical and Chemical Unfolding of Proteins

Guilherme A. P. de Oliveira¹, Jerson L. Silva¹
1 Programa de Biologia Estrutural, Instituto de Bioquímica Médica, Instituto Nacional de Biologia Estrutural e Bioimagem, Centro Nacional de Ressonância Magnética Nuclear Jiri Jonas, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

Proteins undergo changes in conformational states in a multidimensional landscape to search for the global minimum for folding. Groundwork has provided brilliant insight to look for folding intermediates following the downhill trail of the energy landscape. Structural and dynamic information on multiple-stage protein intermediates may increase our knowledge on the misfolding process of proteins associated to the occurrence of several diseases. Different physical and chemical strategies, such as high pressure and urea are commonly used to disturb folding species. Pressure favors reversible unfolding of proteins because of changes in the volumetric properties of the protein-solvent system meanwhile the binding hypothesis of chemical unfolding has clarified our knowledge on the energetic contributions of protein-urea interaction. However, no mechanistic models have been proposed to elucidate urea effects on structure denaturation. Here we provide nuclear magnetic resonance (NMR) spectroscopy and 3D reconstructions from X-ray scattering to introduce the “Push and Pull” hypothesis for the understanding of the mechanism of chemical unfolding of proteins in light of the physical mechanism triggered by high-pressure (HP). Using MpNep2 from Moniliophthora perniciosa we observed that pressure and urea effects are different and it has a major impact on structure denaturation. Using HP-NMR we followed at least two cooperative units during the uphill of the MpNep2 energy landscape, in contrast to the overall structure denaturation up limiting a threshold concentration of urea. These observations explain the differences between the molecular mechanisms for physical and chemical unfolding of proteins, open up new possibilities for the study of protein folding and a new interpretation to explain the nature of the cooperative process of proteins.

Imaging Brain Connectivity and Plasticity

Fernanda Tovar Moll
National Institute for Structural Biology and Bioimaging (INBEB); D’Or Institute for Research and Education (IDOR); Institute of Biomedical Sciences, Federal University of Rio de Janeiro

Although a lot has been learned on the function of specific brain structures, we still know very little about how different structures are interrelated, leading to the emergence of complex mental functions. In addition, several studies using imaging techniques have provided important evidence for the brain capacity to reorganize after environmental influences. These novel techniques have opened new windows for the investigation of changes in brain networks in normal and pathological conditions. We will present and discuss these new concepts and techniques, which allow the study of neural networks. These involve fMRI, especially resting state fMRI, that allows probing functional coupling of networks, and the study of white matter tracts interconnecting nodal structures among networks. Furthermore, changes in these networks can be induced by environmental influences, and were also shown to be present in different developmental, neurological and psychiatric disorders. These will be integrated in the presentation of recent results with the use of modern imaging techniques to study human and non-human brain connectivity and plasticity.
Amyloidosis is a clinical dysfunction caused by extracellular accumulation of proteins that are normally soluble in their original structure, but suffered structural modifications generating insoluble and abnormal fibrils that impair the proper functioning of tissues. Although many challenges have been overcoming in the field of amyloidosis many questions are still waiting for answers. In this work we are interested to evaluate the pathway involved in fibril formation, following structural features that could indicates how soluble proteins undergo conformational changes that result in aggregation.

The diffraction pattern obtained for MTTR fibrils displayed different inter-strand and inter-sheet distances in comparison to others amyloid fibrils described in the literature for other proteins, suggesting a variation on fibrillar architect for transthyretin fibrils. In addition, solid-state NMR has been used to provide more structural information at atomic level. Initial analysis showed up that sample preparation is an important step to get high resolution NMR spectra as observed for u-13C15N-MTTR. As a huge molecular complex composed of several monomeric units, we decided to work separately with the rigid core and the mobile region out of the core of the transthyretin fibrils. In this way, we applied filters for specific parts of the fibrils. Our work has collecting several structural information concerning fibrils formation and organization, as well, structural mobility that could help to solve some question concerning the misfolding pathway.

Surprising Physics of DNA and Potential Roles in Gene Regulation

Thuy Ngo, Jejoong Yoo, Hajin Kim, Qiucen Zhang, Jaya Yodh, Aleksei Aksimentiev and Taekjip Ha
Department of Physics and Center for the Physics of Living Cells, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA

Using advanced single molecule measurements techniques and all atom computational simulations, we discovered two interesting physical properties of DNA. First, DNA sequence and modifications have profound influence on DNA flexibility which in turn determines the asymmetry in mechanical stability of nucleosomes and the accessibility of nucleosomal DNA. Second, DNA sequence and modifications strongly influence association between double stranded DNA molecules without Watson-Crick basepairing, thereby affecting chromosomal organizations. These finding provide novel physical basis for gene expression and genome maintenance.

The Photosynthetic Membrane of Purple Bacteria - A Clockwork of Proteins and Processes

Klaus Schulten, Melih Sener, Johan Strumpfer, Danielle Chandler, Abhishek Singharoy, Angela Barragan-Diaz
Beckman Institute and Department of Physics, U. Illinois at Urbana-Champaign

The chromatophore of purple bacteria is a spherical bioenergetic membrane 70 nm in diameter involving about 130 large protein complexes, generated through invagination of the inner bacterial membrane. Hundreds of chromatophores provide a bacterium with energy in the form of ATP, the synthesis of ATP being driven by sunlight. The transformation of light energy into ATP synthesis comes about through a clockwork of coupled physical processes organized through a multi-million atom macromolecular structure. Recent experimental and computational progress has led to a highly resolved description of the chromatophore down to its atomic and even electronic level, and the physical mechanisms underlying the different participating processes have been largely identified. The coupling of the processes leads to a clockwork with robust and optimal photosynthetic function. This clockwork involves: (1) the quantum biological processes of light absorption, exciton formation, and coherent excitation transfer arising in so-called light harvesting proteins; (2) coupled electron-proton transfer and charging of the quinone-quinole pool in a protein complex called the
reaction center; (3) discharging of the quinone-quinole pool and charging of the chromatophore in terms of an inside-to-outside proton gradient via electron-proton transfer in the bc1 protein complex; and (4) use of the proton gradient by a protein complex called ATP synthase. The lecture exploits the most advanced molecular graphics currently achievable and the most rigorous computational description of the subprocesses possible today (using the authors’ programs VMD, NAMD, and PHI) offering amazing close-up as well as overall views of the clockwork of proteins and processes in the chromatophore. The lecture characterizes living systems through their integration of components and processes; this integration is the main subject of PoLS.

Next Generation Biophysics Training at University of Illinois at Urbana-Champaign

Jaya Yodh, Taekjip Ha, and Klaus Schulten
Department of Physics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

The University of Illinois at Urbana-Champaign (UIUC) plays a leading role in the PoLS community by creating unique opportunities for training the next generation of biological physicists. Of major impact is our annual CPLC Summer School, sponsored by the NSF Center for the Physics of Living Cells, which to date has trained 164 students from 79 institutions worldwide at the UIUC campus. These trainees combined with 74 UIUC teaching assistants have created a global and diverse peer community of early career scientists who are shaping the field of biological physics. The six-day school provides intensive hands-on training to upper level students in cutting-edge tools in experimental single-molecule/cell and theoretical/computational biophysics. Through the Summer School, intramural boot camps, and UIUC Theoretical and Computational Biophysics Group workshops, our graduate students gain valuable experience designing and teaching novel high quality tutorials, software, and experimental training modules at the forefront of the field. Additionally, in education outreach, our CPLC Graduate Teaching Fellows program and school/community partnerships have served 1400 students and 75 teachers, most recently in bilingual classrooms. The PoLS faculty in conjunction with the UIUC Center for Biophysics and Computational Biology directed by T. Ha are leading efforts to modernize Biophysics curriculum and strengthen the interface between biology, chemistry, and physics using frontier, pioneering work from CPLC colleagues. For example, a recently offered graduate Biophysics Laboratory course modeled after Summer School experimental advanced modules is being adapted for undergraduates. UIUC trainees can also explore non-academic careers through industry internships and alumni seminars. Finally, our next generation training promotes gender, minority, and regional representation through these programs as well as SACNAS and Midwest Single-Molecule conferences.

Spatially-Resolved Metabolic Cooperativity Within Dense Bacterial Colonies

John Cole
University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Understanding how environmental conditions can affect cellular function is one of several key challenges for the future of physical systems biology. Many environmental factors, such as oxygen or sugar availability, can vary significantly in both space and time, and in few places is this more evident than within colonies and biofilms. Within a colony, the close proximity of nearby cells competing for the same diffusing resources can create steep gradients in substrate availability that naturally impact every cell’s behavior. This impact is clearly nonlinear; the behavior of a given cell depends on its local microenvironment, but that microenvironment is itself a product of the resources depleted and byproducts
generated by the cell and its neighbors. Here we show that cooperative acetate crossfeeding (wherein one subpopulation produces acetate that another consumes) naturally arises within aerobic glucose-fed *Escherichia coli* colonies under utterly routine laboratory growth conditions. We employ a novel simulation technique marrying the genome-scale detail of flux balance analysis (FBA) with three dimensional spatiotemporal reaction-diffusion modeling in order to study growing colonies *in silico*. These simulations reveal how glucose gradients throughout the colony and extreme hypoxia in the colony interior lead to well-defined regions of fermentative acetate-producing cells and glucose-starved acetate-consuming cells. Our findings are supported by imaging experiments that show reporters associated with key genes involved in acetate crossfeeding upregulated in spatial patterns similar to those predicted. The spatially-resolved dynamic FBA (SR-DFBA) method presented here has applications far beyond simple models of clonal bacterial populations, and we expect rapid adaptation of it to the study of biofilms and other syntrophic microbial consortia, as well as future models of tissues and tumors.

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**Revealing Real-Time, *In Vivo* Transposable Element Dynamics at Both Single Cell and Population Level**

**Hyuneil Kim, Nicholas Sherer, Michael Martini, Nigel Goldenfeld and Thomas E. Kuhlman**

Department of Physics, University of Illinois at Urbana-Champaign, Center for Physics of Living Cells, University of Illinois at Urbana-Champaign

Transposable elements (TEs, also known as jumping genes) have drawn much attention due to their ability to alter gene regulation, drive genomic evolution and provide powerful tools for *in vivo* genomic manipulation. It is also a surprisingly simple system, often requiring only a single protein (transposase) besides TE and target DNA. Despite their importance and wide usage, little quantitative information is available describing their dynamic properties. We present a simple synthetic TE, a derivative of IS608 found in *Helicobacter pylori*, which will permit real-time, *in vivo* observation of TE-related events in *Escherichia coli* through time-lapse fluorescence microscopy. Equipped with microfluidics, the system is capable of tracking large population of cells (>10,000) for ~15 generations, allowing analysis at both single cell level and population level. Fluorescence signals are designed to offer information on real-time transposase expression level, excision events, and rate and location of re-integration of the TE. We will also be able to analyze TE-mediated genetic changes’ effects upon the growth rate of individuals within the population.
Real Time Observation of Lipid-Protein Interactions in Crude Cell Lysates and with Single-Molecule Resolution

Edwin Arauz†, Vasudha Aggarwal†, Ankur Jain‡, Taekjip Ha§*, and Jie Chen†*
† Department of Cell and Developmental Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801; ‡ Center for Biophysics and Computational Biology and Institute for Genomic Biology, Urbana, Illinois 61801; § Department of Physics and Howard Hughes Medical Institute, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

Lipid-protein interactions play key roles in signal transduction. Obtaining new mechanistic insights of these interactions is obligatory for a better understanding of biological processes. Here we use a single-molecule pull-down assay (SiMPull) to probe lipid-protein interactions in crude cell lysates. We demonstrate the applicability of this assay by showing specific interaction between several signaling lipids and their lipid-binding partners. We perform intensive single-molecule data analysis to quantitatively describe the assembly of lipid-binding proteins on their target lipids. Importantly, this assay is applicable to full-length proteins expressed in crude cell lysates, as show for the protein kinase AKT which binds to PI(3,4,5)3 lipid specifically. This new assay lays the foundation to study the interaction of large macromolecular complexes with lipids second messengers in cell lysates, avoiding the need of harsh and lengthy procedures used during protein purification.

Parallel Force Assay for Protein-Protein Interactions

Daniela Aschenbrenner, Diana A. Pippig, Kamila Klamecka and Hermann E. Gaub
Physics Department and Center for Nanoscience (CeNS), LMU Munich, Amalienstr.54, 80799 Munich

One of the main objectives in proteomics is to identify and characterize protein-protein interactions. The extensive number of interactions in any given proteome highlights the need for parallel assays to quantify them. Especially techniques that are able to characterize those interactions in greater detail suffer from low throughput, such as single-molecule force spectroscopy measurements with AFM. To bridge this gap, our lab has recently developed the Molecular Force Assay (MFA). Relying on the principle of comparing the bond in question with a known reference bond, single-molecule measurements can be conducted in parallel. In detail, the two complexes to be compared are attached in series upon which a force is applied. The force directly correlates the mechanical stability of both bonds until, statistically, the weaker bond ruptures. This very sensitive method has already been applied successfully, e.g. to resolve single base-pair mismatches in DNA or characterize the binding of ligands like polyamides or proteins to DNA as well as RNA. Here, we introduce parallelized force measurements of protein-protein interactions utilizing site-specific and covalent integration of a protein pair into the MFA. In a proof-of-principle study, the bonds between different variants of GFP and two GFP-binding nanobodies are quantified by testing them against DNA references. For all GFP constructs, the Minimizer nanobody shows higher binding strength than the Enhancer nanobody. Whereas the binding strength of one type of nanobody to wild type and enhanced GFP is indistinguishable within experimental error, a tendency of weaker binding to the superfolder GFP variant is observed. This difference in binding strength is attributed to alterations in the amino acids that form direct contacts with the nanobodies. In order to enhance the sensitivity, we compare different strategies to adjust the reference DNA strength.

Figure 1: Basic Principle of the Protein Molecular Force Assay
Information flow through allosteric receptors

Ji Hyun Bak and William Bialek
Joseph Henry Laboratory of Physics, and Lewis-Sigler Institute for Integrative Genomics, Princeton University

Signal transduction is a broad and important category of biological communication, where a signaling molecule triggers context-dependent responses which lead to a great variety of vital processes. Although the biochemical details may vary, a signal transduction process typically begins with an elementary event in which the signaling molecule acts as a ligand and binds to a receptor protein. The binding of the signaling molecule induces a change in the protein’s structure, activating its ability to turn on (or off) another pathway. Information can only be lost, from what was conveyed through this initial event, as the signal is further conveyed through the downstream networks. In this work we analyze the information flow through the initial event of signal transduction. Considering the mutual information between the ligand concentration as the regulatory input and the states of the receptors as the output, we derive the structure of this system which would optimize the information transmission. To describe the binding process we use the classical model of allosteric regulation, with two modifications that incorporate (i) the possibility of a protein to be activated by a partial occupation of its binding sites, and (ii) the role of activation barriers as well as the net energy difference between the states. It turns out that there is an optimal, yet energetically costly, solution where each binding event is counted in a sharp and discrete manner. This event-counting solution exploits only the infinitesimally small portion of the dynamic range near zero signal where the response is linear. When the effective output is an integration of different occupation states, there can exist a sub-optimal yet non-extreme solution which uses the full dynamic range.

High-throughput force-fluorescence spectroscopy in zero-mode waveguides

Fabian Baumann, Stephan F. Heucke, Diana A. Pippig, Hermann E. Gaub
Center for Nanoscience & Physics Department, University of Munich, Amalienstrasse. 54, 80799 Munich, Germany

In the last years zero-mode waveguides (ZMW) emerged as an important tool to overcome the concentration limit of optical single-molecule detection. Via their subwavelength geometry in opaque metallic films they prohibit light propagation inside their cavity and hence are capable to confine excitation volumes of conventional optical microscopes drastically. This allows coping with high concentrations of fluorescently labeled ligands as necessary in single-enzyme experiments to monitor their biochemical activity (e.g. DNA real time sequencing). Complementary, mechanical experiments with single-molecule resolution and piconewton sensitivity provide control over a second but important parameter in molecular interactions: force. First combinations of these strong single-molecule techniques, fluorescence and mechanics, provide enormous possibilities in mechanoenzymatic research. Our group addresses the employment of nanoapertures for AFM-based force spectroscopy to establish simultaneous single-molecule fluorescence-force spectroscopy at high fluorescent ligand concentrations. In a proof-of-principle experiment the analysis of a single binding event in a force-activated enzyme (Titin Kinase) has been shown in a ZMW. The methodology is further improved by the implementation of non-invasive tip localization routines to provide automated data acquisition at rates similar to those in standard force spectroscopy. Whereas light incident on the thin cantilever is transmitted with some losses, the fraction of light incident on its high-aspect ratio tip is strongly absorbed. Live superresolution methods applied on this absorption signal are able to securely and centrally navigate the cantilever into a ZMW with an accuracy of few nanometers. The application of high-throughput force-fluorescence spectroscopy in nanoapertures is promising for investigating the mechano-activation of kinases by high-yield recording of fluorescent ATP-binding or of minimum phosphorylation peptides under mechanical stress.

Elucidating the mechanistic details of HIV-1 assembly and membrane budding by quantitative fluorescence and high-resolution imaging

Viola Baumgärtel1, Jens Prescher1, Philipp Messer1, Mikhail Sergeev2, Hans-Georg Kräusslich1, Barbara Mühler1, Don C. Lamb1,2,4
1 Ludwig-Maximilians-University, Department of Chemistry, Center for Integrated Protein Science and NanoScience,
In the past years several structural models for the complex membrane remodeling processes involved in HIV-1 budding from the host cell membrane were derived from in vitro, electron microscopy and siRNA knockdown studies. However, many of these molecular and highly dynamic processes of viral assembly and budding are still not fully understood. During membrane assembly the viral polyprotein Gag hijacks the cellular endosomal sorting complex required for transport (ESCRT) machinery to catalyze virus particle abscission. Thus, we either detected endogenous or expressed fluorescently tagged ESCRT proteins together with fluorescently labelled Gag to analyze the temporal and spatial organization of ESCRT components during all stages of HIV-1 budding. ESCRT membrane assemblies might either drive membrane fission from inside the HIV-1 budding neck, from outside by larger lattices surrounding the neck or by driving membrane neck constriction from within the bud. By applying photoactivation localization microscopy (PALM) to image HIV-1 assembly sites and stochastic optical reconstruction microscopy (STORM) to visualize ESCRT components, we could resolve circular Gag structures (~120 nm) as well as closed, circular structures of Tsg101, ALIX, CHMP4B and CHMP2A, all with average diameters significantly smaller than HIV-1 particles (~50 nm). This clearly supports an internally driven membrane scission process. Using TIRF microscopy to address the underlying dynamic processes during HIV-1 budding, we could detect transient recruitment of the ESCRT associated AAA-ATPase VPS4 to nascent viruses prior to membrane abscission, which points towards an active membrane fusion. Furthermore, we analyzed the number and brightness of each transient fluorescent burst of VPS4 by image correlation spectroscopy (ICS). Interestingly, not just single VPS4 complexes appeared consecutively at each HIV-1 budding site but several complexes associated into a multi-protein structure.

Enhancing FISH through Locating Single Nucleotide Polymorphisms

D. Bo Broadwater, Harold Kim
Georgia Institute of Technology

Single nucleotide polymorphisms (SNPs) are influential in a large number of areas from the study of disease development to utility as genetic markers. Currently, SNPs are detected through many fluorescence-based techniques, including hybridization assays, ligation approaches, and fluorescence resonance energy transfer (FRET). In this study, we test the feasibility of Fluorescence in situ hybridization (FISH) as a tool to detect SNPs in mRNA. We designed a masked DNA probe with a Cy5-quencher pair to increase sequence specificity and signal-to-noise. Using fluorescence dequenching, we measured the displacement rate of the masked probe with a mock mRNA. Our results show a strong dependence of displacement rate on SNP location indicating that SNP location significantly affects the kinetic barrier. Further, these results show that the utility of FISH may be extended to locate SNPs.

Small Angle X-Ray Scattering for Structural Analysis of Biological Macromolecules

Linda Bruetzel and Jan Lipfert
Department of Physics, Nanosystems Initiative Munich, and Center for NanoScience, LMU Munich, Amalienstr.54, 80799 Munich

Small angle X-ray scattering (SAXS) is a powerful technique to unravel the structure and interactions of biological macromolecules such as proteins and nucleic acids. In contrast to X-ray crystallography, the molecules are studied in solution allowing time-resolved investigations and measurements under a broad range of solution conditions, including (near-) physiological environments. Thereby, only small sample volumes (~10 - 30 µl) and moderate protein concentrations (~ 1 mg/ml) are required. SAXS experiments are routinely performed at state-of-the art 3rd generation synchrotron X-ray sources. As a complimentary approach we present an in-house setup, which will be tested for SAXS measurements in collaboration with the lab of P.D. Dr. Bert Nickel. Thereby, standard proteins such as cytochrome c and lysozyme will be used. SAXS yields 1-D scattering profiles. We discuss the different parameters, which can be extracted from a
SAXS profile and their application for the reconstruction of low-resolution (30-10 Å) 3-D models of biomolecules and their assemblies. In this context, we will perform SAXS measurements in order to characterize nucleic acids (RNA, DNA). Here, gold nanocrystals, which can be attached site-specifically to the DNA will be utilized in order to obtain molecular distance distributions.

Feedback regulation of cortical actin waves

Chee San Tong, Maohan Su, Wu Min
Centre for Bioimaging Sciences, National University of Singapore

The formation of well regulated spatial and temporal biological patterns play important roles in many processes, such as cell development and migration. Though the observation of these patterns in many biological systems are widely reported, their underlying mechanisms remain poorly understood. In antigen-stimulated mast cells, we observed that Cdc42 and N-Wasp waves overlap temporally and spatially with FBP17 while actin traveling waves are trailing behind. For recurring pattern initiation and propagation, feedback mechanisms must be present. We investigated the nature of the feedback mechanisms using Total Internal Reflection Fluorescence (TIRF) and Surface Reflective Interference Contrast (SRIC) microscopy in combination with the molecular perturbation, protein knockdown and co-expression of mutant proteins. Our findings provide evidence for the involvement of GTPase, actin cytoskeleton and membrane shaping in the feedback mechanisms which may play important roles in cortical dynamics.

Intrinsic conformational dynamics in substrate-binding proteins regulate transport in ABC importers

Giorgos Gouridis¹, Gea K. Schuurman-Wolters², Evelyn Ploetz¹, Florence Husada¹, Ruslan Vietrov², Bert Poolman², Thorben Cordes¹,*
1 Molecular Microscopy Research Group & Single-molecule Biophysics, Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands; 2 Department of Biochemistry, Groningen Biomolecular Science and Biotechnology Institute (GBB), Proteomics Centre, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

ABC importers play a pivotal role in the physiology of microorganisms. They consist of two transmembrane domains (TMD) forming the translocator and cytoplasmic nucleotide-binding domains (NBD), which power the transport through hydrolysis of ATP. Prokaryotic ABC importers make use substrate-binding domains (SBD) to capture and deliver substrates from the environment to the translocator. To decipher how conformational changes within the subdomains drive the overall membrane transport, we focus on the homodimeric GlnPQ complex, possessing two different SBDs per single translocator. Based on smFRET, ITC and uptake assays, we show that both SBDs have evolved to capture different amino acids by an undocumented type of induced-fit mechanism: they have the capability to intrinsically close in the absence of a ligand. While the presence of high-affinity ligands leads to increased transitions of the SBDs into the closed conformation at constant closed state lifetime, the presence of the low-affinity ligands shortens the closed state lifetime by two orders of magnitude and enhances the overall transport rate. Engineering the ligand’s affinity and hence the close state’s lifetime of each SBD allows us to tune the transport activity. These findings indicate that the intrinsic closing regulates SBD docking to the translocator and following-up transport.

Monitoring actin filament nucleation using zero-mode waveguides

Alvaro H. Crevenna¹², Maria Hoyer¹, Marcelino Arciniega³ and Don C. Lamb⁴
1 Physical Chemistry and Biochemistry, LMU-Munich, 2 Center for NanoScience CeNS, 3 Max Planck Institute of Biochemistry, and 4 University of Illinois at Urbana-Champaign

Actin filament nucleation is fundamental for a vast array of cellular processes, from cell motility to contraction. The very first steps during polymerization, i.e. nucleation, are orders of magnitude slower that filament elongation. The assumed
nucleation pathway, from monomers to dimers and to trimers has remained unclear since its proposal. The main challenge to elucidate the mechanism of nucleation is the high concentration of actin monomers required to form filaments and the low abundance and short lifetime of nuclei (dimers and trimers). Here, using zero-mode waveguides we monitor single actin molecules in real time as they are assembled one at a time to form nuclei and the first six monomers of a filament. We determine the arrival time distributions and kinetic pathway during nucleation generated at either end of the filament. In combination with fluorescence cross-correlation performed also using zero-mode waveguides and computer simulations; we explore the encounter complex during nucleation and reveal the moment where the conformational transition associated with filament formation takes place.

Intramolecular Dynamics Within the N-Cap-SH3-SH2 Regulatory Unit of the c-Abl Tyrosine Kinase Reveal Targeting to the Cellular Membrane

Guilherme A. P. de Oliveira*, Elen G. Pereira, Giulia D. S. Ferretti, Ana Paula Valente, Yraima Cordeiro and Jerson L. Silva
1 Programa de Biologia Estrutural, Instituto de Bioquímica Médica, Instituto Nacional de Biologia Estrutural e Bioimagem, Centro Nacional de Ressonância Magnética Nuclear Jiri Jonas, Universidade Federal do Rio de Janeiro, 21941-902 Rio de Janeiro, RJ; 2 Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, 21941-902 Rio de Janeiro, RJ, Brazil

c-Abl is a key regulator of cell signaling and is under strict control via intramolecular interactions. The c-Abl regulatory mechanisms involve post-translational modifications that act as switches, e.g., intermolecular phosphorylation / dephosphorylation of Y412 and Y245 and G2-myristate association/dissociation with/from the C-terminal cleft of the kinase domain via the N-Cap segment. In this study, we address changes in the intramolecular dynamics coupling within the c-Abl regulatory unit by presenting its N-terminal segment (N-Cap) with an alternative function in the cell as c-Abl becomes activated. Using small angle X-ray scattering, nuclear magnetic resonance and confocal microscopy we demonstrate that the N-Cap and the Src homology (SH) 3 domains acquire μs-ms motions upon N-Cap association with the SH2-L domain, revealing a stabilizing synergy between these segments. The N-Cap-myristoyl tether likely triggers the protein to anchor to the membrane because of this flip-flop dynamic, which occurs in the μs-ms time range. This segment not only presents the myristate during c-Abl inhibition but may also trigger protein localization inside the cell in a functional and stability-dependent mechanism that is lost in Bcr-Abl+ cells, which underlie chronic myeloid leukemia. This loss of intramolecular dynamics and binding to the cellular membrane is a potential therapeutic target.

Thermophoresis for Detection of Autoimmune Antibodies

Evgeniiia V Edeleva and Dieter Braun
LMU Munich, Physics Department, QBM and CeNS, Amalienstraße 54, 80799 Munich, Germany

High blood titers of autoimmune antibodies that are directed against body’s own proteins is a hallmark of many autoimmune diseases. Sensitive detection of such autoantibodies is highly important for reliable diagnostics, disease classification and ultimately for the choice of an optimal treatment strategy. This is true for DEAP-HUS (Deficiency of complement factor H-related plasma proteins and Autoantibody Positive form of Hemolytic Uremic Syndrome), a subtype of hemolytic uremic syndrome characterized by the presence of autoantibodies against the complement immune system inhibitor Factor H. The disease affects children and requires special attention in terms of diagnostics and treatment. A reduction of autoantibody titers via plasmapheresis shows a favorable response in most patients and prevents complications after transplantation surgery [1]. To quantify absolute affinity and concentration values of autoantibodies in DEAP-HUS patients’ plasma, we are developing an assay based on thermophoresis. This directed movement of molecules driven by a temperature gradient allows to analyze antibody-antigen interactions even in complex bioliquids like serum [2]. It has previously been utilized to detect autoantibodies against the β1-adrenoceptor associated with dilated cardiomyopathy [3]. To this end, the exogenously designed, autoantibody-binding peptide COR1 served as a tracer. Though the thermophoretic autocompetition assay has been utilized successfully with spiked antibodies in serum (Figure 1), the affinity of COR1 was too low to resolve the low nM antibody concentrations in patient samples. Based on this work, we are devising a technique to quantify anti-Factor H autoantibodies in patients’ plasma.
using fluorescently labeled Factor H as a tracer. As we use anti-bleaching agents to guarantee high sensitivity [4], we expect to directly detect even pM antibody levels. Such quantitative diagnostics promise to significantly improve the management of DEAP-HUS.

A heated monitored spot was used as a read-out to determine both the concentration and affinity of antibodies.


Figure 1. In an autocompetition approach unlabeled COR1 was titrated against a constant concentration of fluorescently labelled tracer COR1 competing for the binding sites of its ligand – the autoimmune antibody in serum. The change in the thermophoretic depletion of the fluorescent tracer in the heated monitored spot was used as a read-out to determine both the concentration and affinity of antibodies.

**Does Resveratrol Prevent p53 Aggregation?**

*Danielly C. Ferraz da Costa, Nathali P. C. Campos, Luciana P. Rangel, Guilherme G. A. P. Oliveira, and Jerson L. Silva*
Institute of Medical Biochemistry Leopoldo de Meis, National Institute of Science and Technology for Structural Biology and Bioimaging, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ, Brazil

p53 has an essential role in preventing cancer development by inducing cell cycle arrest and/or apoptosis in response to cellular stress. Mutations in the p53 gene are described in over 50% of all human cancers. Besides the mutations, cellular aggregation of p53 can also inactivate the protein, leading to malignancy. Resveratrol, a natural polyphenol found in grapes and red wine, is able to induce p53-dependent cell death in a variety of cell lines. Although several indirect mechanisms of p53 activation by resveratrol have been proposed, there is no evidence that this bioactive compound can interact with p53. Thus, we investigated a possible interaction between resveratrol and the p53 core domain (p53C). In addition, we tested the potential of resveratrol in preventing wild-type and mutated p53 aggregation in vitro and in tumor cells. Experiments were performed by using fluorescence spectroscopy and fluorescence microscopy techniques. Our data suggest that an interaction between resveratrol and the wild-type p53C does occur. We also found that resveratrol has the ability to inhibit the wild-type p53 core domain as well as the R248Q p53 mutant to undergo in vitro aggregation. Additionally, resveratrol (50 and 100µM) reduces the formation of nuclear p53 aggregates in MDA-MB231 human breast cancer cells. Our study provides evidence that resveratrol can directly modulate p53 and may pave the way for a better understanding of the mechanisms involved in p53 protein aggregation as a therapeutic strategy for cancer treatment. Supported by FAPERJ, CNPq, INCT/INBEB and Fundação do Câncer.

**3D Printing Applications In Physics, Engineering, and Biology**

*Catherine McGuinness*<sup>1,7</sup>, *Junjiajia Long*<sup>2,7</sup>, *Nathan Williams*<sup>2,7</sup>, *Tony Wu*<sup>4,7</sup>, *Paul Muir*<sup>3,7</sup>, *Bridget Hegarty*<sup>2,7</sup>, *Jennifer Gaines*<sup>6,7</sup>, and *Jeremiah Johnston*<sup>1,7</sup>

<sup>1</sup> Molecular Biophysics and Biochemistry; <sup>2</sup> Physics; <sup>3</sup> Molecular Cell Biology, Genetics, and Development; <sup>4</sup> Biomedical
The Center for Engineering, Innovation, and Design (CEID) at Yale provides a space for students to explore different engineering techniques and design products as part of course projects or individual research. As part of the Integrated Graduate Program in Physical & Engineering Biology (PEB), we completed several projects including the design and construction of physics teaching tools, a mini-centrifuge, a 3D scanner, and a TPR hydrogel model. Each project involved mastering the basics of SolidWorks and using resources in the CEID. The end products show how cheap, customizable lab equipment and models can be readily created using 3D printing, laser cutting, and other engineering tools.

Single-molecule analysis reveals diverse pathways during early steps of transcription initiation

Andreas Gietl1, Phil Holzmeister1, Fabian Blombach2, Sarah Schulz1, Lena Voith von Voithenberg1,4, Don C. Lamb1,4, Finn Werner3, Philip Tinnefeld1 and Dina Grohmann1

1 Physikalische und Theoretische Chemie - NanoBioSciences, Technische Universität Braunschweig, Hans-Sommer-Strasse 10, 38106 Braunschweig, Germany; 2 RNAP laboratory, University College London, Institute of Structural and Molecular Biology, Division of Biosciences, Gower St, London WC1E 6BT, UK; 3 Center for Nanoscience (CeNS), Schellingstraße 4, 80799 Munich, Germany; 4 Department of Chemistry, Center for Integrated Protein Science Munich (CiPSM), Ludwig Maximilian University, Butenandtstr. 11, 81377 Munich, Germany

During transcription initiation, the promoter DNA is recognized and bent by the basal transcription factor TATA-binding protein (TBP). Subsequent association of transcription factor B (TFB) with the TBP-DNA complex is followed by the recruitment of the RNA polymerase resulting in the formation of the pre-initiation complex. TBP and TFB/TF(II) B are highly conserved in structure and function among the eukaryotic-archaeal domain but intriguingly have to operate under vastly different conditions. Employing single-molecule FRET we monitored TBP-induced DNA bending using eukaryotic and archaeal TBPs in the absence and presence of TFB. We observed that the TBP-DNA interaction is highly adapted to physiological conditions and the lifetime of the complex differs significantly between the archaeal and eukaryotic system. We show that the eukaryotic DNA-TBP interaction is characterized by a stepwise bending mechanism. TF(II)B specifically stabilizes the fully bent TBP-promoter DNA complex and we identify this step as regulatory checkpoint. In contrast, the archaeal TBP-DNA interaction is extremely dynamic and TBP isolated from the archaeal organism *S. acidocaldarius* strictly requires TFB for DNA-bending. We thus demonstrate that transcription initiation follows diverse pathways.\[1\]

Often, single-molecule interrogation requires the immobilization of the molecule, which can lead to a perturbation of its activity causing incongruities between single-molecule and ensemble measurements. Employing the TBP-induced DNA bending assay we developed a method that allows the transfer of a functional biomolecular assay to the single-molecule level without the loss of enzymatic activity. We introduced the recently developed DNA origami as a platform that allows the direct transfer of ensemble assays to the immobilized single-molecule level without changing the nano-environment of the biomolecules.\[2\] nm and preliminary results indicated that CD9 was not randomly distributed at the different budding stages.


The determination of the folding dynamics of polypeptides and proteins is critical in characterizing their functions in biological systems. Numerous computational models and methods have been developed for studying structure formation at the atomic level. Due to its small size and simple structure, deca-alanine is used as a model system in molecular dynamics (MD) simulations, and the free energy of unfolding in vacuum has been studied extensively using the end-to-end distance of the peptide as the reaction coordinate. However, few studies have been conducted in the presence of explicit solvent. Although sufficient in vacuum, we show that end-to-end distance is insufficient in describing the full dynamics of deca-alanine folding in water. Using α-helical content as a second reaction coordinate, we deduce a more descriptive free-energy landscape which reveals a second energy minima in the extended conformations. This work reveals both the necessity and challenge of determining a proper reaction coordinate to fully characterize a system.

Reverse Engineering the Quantitative Shape of Gene Regulation Functions in Yeast

Patrick Hillenbrand, Kerstin Maier, Patrick Cramer, Ulrich Gerland
1 Lehrstuhl für Theorie komplexer Biosysteme, Physik-Department, Technische Universität München, James-Franck-Str. 1, D-85748 Garching, Germany; 2 Gene Center and Department of Biochemistry, Center for Integrated Protein Science CIPSM, Ludwig-Maximilians-Universität München, Munich, Germany

Although much is known about the network structure of transcriptional regulation in yeast, detailed quantitative knowledge of regulatory functions remains scarce. A recently established experimental technique called Dynamic Transcriptome Analysis (DTA) simultaneously measures mRNA levels as well as the rates at which mRNA is transcribed on a genome-wide scale in yeast [1]. The measurements are taken dynamically, at closely spaced time points, and yield a detailed portrait of the nonlinear regulatory dynamics of the genetic network. We leverage these data in conjunction with prior knowledge to quantitatively characterize the regulatory interactions between genes, which are known to be transcriptionally connected. In particular, we estimate the functional form of how multiple input transcription factors act together to generate a combinatorial response in a target gene. We apply this method to specifically investigate transcription factors, which are cell cycle dependently expressed and reconstruct the regulation functions of several target genes within the Clb2 cluster. In previous studies it has been shown that even in the absence of control by the primary cyclin-CDK cell cycle oscillator, many genes are still periodically expressed. This led to the proposition of a secondary, autonomous transcriptional oscillator. We use network-level arguments and quantitative analysis of transcriptional regulation between well-characterized transcription factors to find and rank possible network architectures that could underlie such a cell cycle oscillator. We show that, in principle, the network models we find are capable of generating oscillations even in the absence of a primary cyclin-CDK oscillator.

Single Molecule Force Spectroscopy facilitated by a Microfluidic Lab-Chip for Gene Expression

Markus A. Jobst, Wolfgang Ott, Marcus Otten, Tobias Verdorfer, Lukas F. Milles, Hermann E. Gaub
Physics Department and Center for Nanoscience (CeNS), LMU Munich, Amalienstr.54, 80799 Munich

Mechanical forces acting on proteins play a pivotal role in biological systems. By applying forces to single molecules, conformational changes and energetic barriers along unfolding pathways can be probed by SMFS. Since low experimental throughput has significantly limited the capacity to screen libraries of proteins, we developed a versatile microfluidic system to address these issues. Our platform enables parallelized force spectroscopy utilizing cell-free in vitro gene expression, site-specific protein immobilization and subsequent measurements of mechanical properties at the single molecule level in a streamlined format with one single cantilever. A PDMS microfluidic chip on a glass slide seals engineered DNA spots to provide micro reactors for protein synthesis. Expressed fusion proteins covalently attach to the glass surface at their N-termini and display free dockerin domains at their C-termini. With a single cohesin-functionalized cantilever, unfolding pathways and unbinding characteristics of multiple different proteins can be probed. Our example library contained structural proteins, cytoskeletal constituents, enzymes, and fluorescent proteins, which we were able to detect by their specific unfolding fingerprints. Analysis of contour-length increments and rupture force - loading-rate data characterizes the constructs and are compared with computational methods. As an application of this novel system, mutant variants of individual receptor-ligand proteins can be constructed,
immobilized and measured on a single molecule basis to screen for candidates in protein design. For example, saturation mutagenesis of single residues responsible for binding their counterpart can be performed and characterized on a single device. This method provides a unique means of comparing forced dissociation and unfolding pathway characteristics of engineered proteins.

The elemental composition of virus particles: implications for marine biogeochemical cycles

Luis Jover1, T. Chad Effer2; Alison Buchan1, and Joshua S. Weitz1,3
1 School of Physics, Georgia Institute of Technology, Atlanta, USA; 2 Department of Microbiology, University of Tennessee, Knoxville, USA; 3 School of Biology, Georgia Institute of Technology, Atlanta, USA

Viruses in marine environments have received considerable attention over the last two decades due to their potential role in marine biogeochemical cycles. Viral lysis of host cells releases dissolved and particulate organic materials back into the environment that may then be assimilated by microorganisms. This process - termed the viral shunt -- is at the center of many discussions concerning marine geochemical cycles. For example, viruses are estimated to redirect 150 Gt of Carbon/yr into the dissolved pool in the oceans. Similarly, surveys of viral abundance in the oceans, estimated to range from 109 to 1011 per L, have been used to estimate the total carbon of ocean virus particles as approximately 200 Mt. Viral particles are potential targets for micrograzers and, even when not grazed, may destabilize on the order of days. Hence, viral particles themselves, whether directly or indirectly, may be an important entrance point for carbon (C), nitrogen (N) and phosphorus (P) back into the microbial loop. The basis for estimating virus-associated steady state and flux rates of elements depends, in part, on what it takes to “build” a virus. Viral particles are generally composed of protein and nucleic acids. Proteins are enriched in N, while nucleic acids are enriched in both N and P with respect to the nutrient stoichiometry of whole cells. Here, we propose a biophysical scaling model of the elemental stoichiometry of intact virus particles and, in so doing, hypothesize that virus particles are stronger contributors than previously recognized of marine organic P and N flux and steady-state content.

Unraveling protein-DNA interactions by single molecule force spectroscopy

Kamila Klamecka1,2, Philipp Walker1, Philip Severin1, Stefan Stahl1, Heinrich Leonhardt1, Hermann Gaub2
1 Department of Biology II and Center for Nanoscience, Ludwig Maximilians University, Großhaderner Str. 2, 82152 Martinsried, Germany; 2 Department of Physics and Center for Nanoscience, Ludwig Maximilians University and Center for Nanoscience, Amalienstr. 54, 80799 Munich, Germany

Involved in autism spectrum disorders, Methyl-CpG binding protein 2 (MeCP2) is expressed in all tissues and displays extremely high abundance in neuronal chromatin. Deviations from its optimal expression level have drastic effects on proper brain function. Typically for a member of intrinsically disordered proteins family, MeCP2 recognizes numerous interaction partners. These include DNA, RNA and a number of proteins. Thanks to wide attention MeCP2 has experienced in the past years, its structural composition and biochemistry has been well described, yet the mechanism of its binding to DNA still lacks detailed understanding. One of the few regions of MeCP2 with organized secondary structure is its methyl-CpG binding domain (MBD), responsible for specific binding to methylated CpG sites. We investigate the MeCP2-DNA complex using single molecule techniques: Atomic Force Microscopy and Magnetic Tweezers. In both cases the behavior of specifically designed single DNA molecule during stretching in absence or presence of MeCP2 is being analyzed focusing on intramolecular versus intermolecular DNA crosslinking upon protein binding.

Magnetic Tweezers Experiments to Probe the Mechanics and Interactions of Nucleic Acids

Franziska Kriegel and Jan Lipfert
Department of Physics, Nanosystems Initiative Munich, and Center of NanoScience, LMU Munich, Amalienstr. 54, 80799 Munich
Magnetic tweezers (MT) are a suitable technique to address single-molecules using magnetic forces. In addition to other single-molecule force techniques, torques can be applied to the molecule. External magnetic fields are used to stretch and/or rotate double-stranded DNA. MTs naturally operate in constant force mode. Compared to optical tweezers and atomic force microscopy, small forces (< 1 pN) characteristic of non-covalent macromolecular interactions can readily be applied and measured. Initially the focus on research was on DNA supercoiling but there is no limitation in solving other biological challenges, such as protein-nucleic acid interactions. Combining magnetic tweezers with fluorescence microscopy expands the opportunities of measuring and yields for further interesting projects.

Ultrastable cellulosome-adhesion complex tightens under load

Constantin Schoeler a,†, Klara H. Malinowska a,†, Rafael C. Bernardi b, Lukas F. Milles a, Markus A. Jobst a, Ellis Durner a, Wolfgang Ott a, Daniel B. Fried c, Edward A. Bayer c, Klaus Schulten a,‡, Hermann E. Gaub a and Michael A. Nash a

1 Lehrstuhl für Angewandte Physik and Center for Nanoscience, Ludwig-Maximilians-Universität, 80799 Munich, Germany; 2 Theoretical and Computational Biophysics Group, Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States; 3 Department of Biological Chemistry, The Weizmann Institute of Science, Rehovot 76100, Israel; 4 Department of Physics, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, United States

† Equally contributing.

Challenging environments have guided nature in the development of ultrastable protein complexes. Specialized bacteria produce highly flexible protein networks called cellulosomes to effectively digest lignocellulosic biomass. While network assembly is enabled by protein interactions with commonplace affinities, certain cellulosomal receptor-ligand interactions exhibit extreme resistance to applied force. Through single molecule force spectroscopy and steered molecular dynamics simulations, we characterized the ligand-receptor complex responsible for substrate anchoring in the Ruminococcus flavefaciens cellulosome. The complex withstood forces of 600–750 pN, representing the strongest bimolecular interaction reported to date, equivalent to half the mechanical strength of a covalent bond. Our findings demonstrate force activation and stabilization of the complex, and suggest that certain network components serve as mechanical effectors for maintaining network integrity. This detailed understanding of cellulosomal network components could help in the future development of biocatalysts for production of fuels, chemicals, and pharmaceuticals from renewable plant-derived biomass.

Figure 1: Analysis of binding interface of cohesin (white) - dockein (red) complex from R. flavefaciens cellulosome. The binding surface of cohesin is shown with hydrophobic residues highlighted in gray, polar residues in green, and negative and positive residues in red and blue, respectively. Both Coh and Doc exhibit a hydrophobic patch in the center of the binding surface that is surrounded by polar and charged residues.

Structural Behavior of Cardiac Troponin C Variants Present in Cardiomyopathic Patients


1 Instituto de Bioquímica Médica, IBqM-UFRJ; 2 Florida State University, Department of Biomedical Sciences, College of Medicine

Troponin C (TnC) is a Ca2+ binding protein and plays an important role in regulation of muscle contraction. It is composed by two domains connected by an alpha helix. Mutations in TnC have implicated phenotypic characteristics as hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), and restrictive cardiomyopathy.
Ventricular hypertrophy and fibrillation is common in HCM subjects and is associated with the presence of TnC variants including A8V, D145E, C84Y and A31S. In the other side, DCM is manifested by a ventricular dilation and wall thinning and variants were also reported as Y5H, M103I, I148V, D145E. The main goal of this work is to investigate changes in stability and dynamics of seven specific mutations involved in HCM/DCM using a ensemble of thermodynamic and structural approaches. Calcium titrations followed by bis-ANS fluorescence revealed that D145E mutation reduced the extent of calcium-induced hydrophobic exposure while C84Y substantially enhance it by the N-domain. Circular dichroism data against urea titration showed a greater stability and prominent cooperative effect in N-domain variants as compared to those from the C-domain and wild-type. Shape restorations from small angle scattering were used to evaluate conformational changes induced by mutation. D145E revealed the most affected shape as compared to wild-type and the others and perturbed residues were located at the C-domain as confirmed by chemical shift perturbation analysis for this mutant. D145E secondary structure was not significantly altered by dihedral angles prediction from the NMR assignment data. These observations open up new avenues for the comprehension of the complex behavior of HCM and DCM that has heretofore been not evaluated at structural level.

Quantitative Dye-Comparison for High-speed Nanoscopy

Junjiajia Long, Catherine McGuinness, Lena Schroeder, Yu Lin, Joerg Bewersdorf
Yale University

Single-molecule switching nanoscopy (SMSN) such as PALM and STORM requires dyes which alternate between bright bursts of light and long dark states [1]. These blinking characteristics strongly depend on the laser intensity and other imaging conditions [2]. We performed a quantitative, systematic comparison of two common dyes, Alexa 680 and 700, and compare them to Alexa 647, the most popular SMSN probe, with an emphasis on high-speed imaging. Alexa Fluor 680 and Alexa Fluor 700 perform comparably over the range of laser intensities and camera frame rates tested. Additionally, they are comparable to the current gold standard, Alexa 647. Consequently, these two dyes are promising candidates for dual-color STORM imaging. Our results will be applied to high-speed two-color SMSN.


Force follows Function? - Thermostability and Mechanostability in Carbohydrate Active Enzymes

Lukas F. Milles, Wolfgang Ott, Ellis Durner, Markus A. Jobst, Tobias Verdorfer, Michael A. Nash, Hermann E. Gaub
Lehrstuhl für Angewandte Physik and Center for Nanoscience (CeNS), Ludwig-Maximilians-Universität, 80799 Munich, Germany

Carbohydrate active enzymes break down cellulose fibers into soluble sugars, a process essential to biofuel production. Single amino acid mutations have shown dramatic increases in enzyme thermostability, facilitating the breakdown process. However, it is not known how a protein’s thermostability correlates with its mechanostability, and more fundamentally, if and how carbohydrate active enzyme unfolding pathways correlate with their function. We employ Single Molecule Force Spectroscopy to stretch and break single enzymes while measuring the resulting forces under different conditions by using the high specificity and strong affinity cohesin-dockerin interaction as a pulling handle. In this investigation we study the endoglucanase Cel8a from C. thermocellum as well as its thermostable quadruple mutant, and a xylanase from G. stearothermophilus. Expressed as fusion proteins with the dockerin tag, they are covalently immobilized on a surface. A cantilever fused with a cohesin, that binds to the dockerin, is used to specifically pull and break a single enzyme. The resulting force-extension unfolding traces show various pathways. We sort these with a newly developed classification algorithm and determine possible unfolding pathway clusters through dimensionality reduction techniques.

In the future, parallelizing protein expression and measurement with lab-on-a-chip techniques, as currently pushed forward in our group, should allow a great multitude of mutations to be investigated in one experiment, to test the prevalence of sequence specific unfolding pathways. Ultimately, inferring an enzyme’s structure through comparison with conserved unfolding pathway motifs could be possible.
How sugars control membrane protein dynamics

Leonhard Möckl and Christoph Bräuchle
Department of Physical Chemistry I, LMU Munich

Proteins on the membrane of eukaryotic cells carry large, often very complex sugar structures. Besides their commonly known functions like participation in the quality control during protein folding there is growing evidence that membrane protein glycosylation fulfills also regulation purposes. For example, it was shown that the membrane localization of the EGF receptor depends strongly on its glycosylation which is in turn contingent upon the nutrition state of the cell.

In our work, we use highly sensitive live-cell fluorescence microscopy combined with in-cellulo click-chemistry to enlighten the spatiotemporal dynamics of glycosylated membrane proteins on different cell types. We found out that the turnover rates as well as the mobility of membrane proteins are dependent on the glycosylation type. We could prove that different attachment to the galectin lattice, a network-like structure of carbohydrate binding proteins, is capable to cause these distinct dynamics. We were also able to mimic the lattice formation by streptavidin-mediated crosslinking of biotin-tagged membrane proteins and investigate the resulting lattice at the single molecule level.

Influence of surface modifications on the spatio-temporal distribution of quantum dots in vivo

A. Katharina Nekolla1, Kerstin Pflieger1, Sabine Sellner1, Stefan Zahler1, Dagmar Zeuschner1, Fritz Krombach1, and Markus Rehberg1
1 Walter Brendel Centre of Experimental Medicine, Ludwig-Maximilians-Universität München, Munich, Germany, 2 Department of Pharmacy, Ludwig-Maximilians-Universität München, Munich, Germany, 3 Max Planck Institute for Molecular Biomedicine, Münster, Germany

In nanomedical science, various types of nanoconstructs including quantum dots (QDs) are currently being developed. In this regard, it is crucial to understand the spatio-temporal distribution of these nanomaterials at the microscopic tissue level. In particular, interactions with components of the extracellular matrix might determine their in vivo behavior. As such interactions can be guided by the surface chemistry of the nanoconstructs, we used QDs of equal size (ca. 10 nm), but surface-modified with either carboxyl groups or polyethylene glycol (PEG). The QDs were microinjected (ca. 400 pl, 20 nM) directly into the mouse cremaster muscle and visualized using in vivo fluorescence microscopy. PEG QDs exhibited a dynamic distribution, as they locally diffused inside the tissue for an extended period of time (2 h). In contrast, carboxyl QDs immediately stucked firmly to tissue constituents and remained there during the whole observation period of 2 h. TEM analysis of tissue samples revealed localization of carboxyl QDs at collagen fibers as well as strong attachment to basement membranes located at the basolateral side of blood vessel walls. To specifically test the influence of different extracellular matrix components on the dynamics of QDs, additional in vitro studies were designed. In gels of collagen I the dynamics of QDs closely resembled the in vivo situation: whereas carboxyl QDs tightly adhered to collagen fibers, PEG QDs freely diffused through the matrix. Confocal microscopy of immunostained collagen gels confirmed the colocalization of carboxyl QDs and collagen fibers. Likewise, carboxyl QDs – but not PEG QDs – stucked to the matrigel constituents laminin and collagen IV.

In summary, we found that the spatio-temporal distribution of QDs in skeletal muscle tissue is influenced by their respective surface chemistry. Furthermore, our findings suggest that extracellular matrix components act as a barrier for carboxyl QDs.

MreB localization in E. coli shows enrichment at negative Gaussian curvature

Jeffrey P. Nguyen1, Nikolay Ouzounov2, Benjamin P Bratton2, Zemer Gitai2, Joshua W Shaevitz1,2,3
1 Department of Physics, Princeton University, Princeton, New Jersey; 2 Department of Molecular Biology, Princeton University, Princeton, New Jersey; 3 Lewis Sigler Institute, Princeton University, Princeton, New Jersey

The actin homologue MreB plays a roll in orchestrating the insertion of cell wall material and determining cell shape, but the mechanism of this is largely unknown. The polymeric structure of MreB suggests that it may interact with the cell shape by measuring the local membrane curvature. In order to study this interaction, we developed a novel method
of measuring cell shape in 3D. The method uses the point-spread function of the microscope to find the surface which best describes the 3D image from a fluorescently labeled cell. The accuracy of shape detection is within 30 nm, as measured by atomic force microscopy and correlated electron microscopy. In addition to measuring shape characteristics of the cell, we use a functional fluorescently tagged version of MreB to localize MreB within the cell. This allows us to correlate the localization of MreB with cell shape metrics and estimate the lengths of the polymer structures. We observe that MreB preferentially localizes to areas of negative Gaussian curvature in E. coli. This has led to a model in which rod shape is maintained by MreB directing growth to areas of negative curvature.

### To catch a fly: Viscosity and elasticity-based prey capture by frog tongue projection

**Alexis Noel, Hyun Choe, Jong Ha, David L. Hu**  
Princeton University, Princeton, New Jersey

Many species of amphibian and reptile have developed novel feeding techniques to capture highly elusive prey. Here we elucidate the mechanism through which the animal tongue is projected through inertial elongation, as well as the adhesive methods between soft tissue and lightweight hydrophobic prey. Using high-speed videography, we find that inertial elongation mechanisms can reach accelerations of over fifty times the speed of gravity and the tongue can stretch over 1.5 times its length during prey capture. The tongue has a Young’s modulus of less than 5 kPa; this softness allows for the tongue to deform during impact and maximize adhesive contact area. The salival coating on the tongue is viscoelastic; at slow extension rates, elasticity is prominent, retaining insects in the fluid. At high extension rates, viscous effects are dominant, which help to retain insects during high strain rates. Our findings reveal a novel method for collecting hydrophobic objects with minimal damage due to the soft tissue. This study may help develop high speed actuation mechanisms in the field of soft robotics, as well as new adhesion techniques.

### From genes to protein mechanics on a chip

**Wolfgang Ott, Marcus Otten, Markus A. Jobst, Lukas F. Milles, Tobias Verdorfer, Diana Pippig, Hermann E. Gaub, Michael A. Nash**  
LMU Munich, Center for NanoScience, Chair for Applied Physics, Biophysics, and Molecular Materials

We present a versatile microfluidic platform for on-chip synthesis, covalent immobilization of protein constructs and measurement of protein mechanical properties at the single molecule level. In order to maintain the high throughput idea two requirements are needed. First, a single cantilever measuring all protein constructs is necessary to provide comparable reaction conditions. In order to achieve this goal the cellulosomal derived cohesin and dockerin domains were employed. They are suitable for this approach because of their high binding affinity and specificity. Moreover their relatively small molecular weight (15 and 8 kDa) allows utilizing them in fusion proteins. We found that a single cohesin-modified cantilever remained stable over thousands of pulling cycles, enabling clear identification of each protein in the array by their unfolding fingerprints. To proof the general applicability of the system we expressed a spectrum of proteins with different functions: Structural proteins (e.g., fibronectin tandem repeats), cytoskeletal constituents (e.g.,
alpha spectrin), enzymes (e.g., xylanase), and fluorescent proteins (e.g., GFP). Second, for adaption of the system to the corresponding biological question a highly genetically versatile platform is desired. For proof of principle we used in this work circular plasmid DNA as basis for protein expression. A drawback of the more stable circular DNA is that its manipulation and preparation requires a lot of time and effort. Therefore in future experiments the implementation of double stranded, linear DNA increases the variety of the system. By shuffling, connecting and amplifying certain protein domains the preparation time could be dramatically reduced and the modularity of the system significantly increased.

Characterization of endothelial cell migration in three-dimensional hydrogels focusing on angiogenesis

Kerstin Pflieger, Stefan Zahler
Department of Pharmaceutical Biology, Ludwig-Maximilians-Universität München

Endothelial cell migration is essential to angiogenesis. This motile multistep process is regulated by chemotactic, haptotactic and mechanotactic stimuli as well as degradation of the extracellular matrix (ECM) to enable progression of migrating cells. Most studies of the angiogenic process on a molecular, cellular and multicellular level are based on two-dimensional cell culture models, which allow a good control of experimental conditions, but have turned out to describe the complexity of the in vivo situation only unsatisfactorily. Studies of cell migration in three-dimensional systems have among others revealed differences in morphology, and mechanical and signaling control in comparison to cells investigated in a two-dimensional setup. With a focus on angiogenesis, we investigated cell migration of human umbilical vein endothelial cells (HUVECs) in Matrigel™ and rat tail collagen I gels. Characterizing endothelial tube formation, we analyzed the influence of cell density, hydrogel stiffness and microstructure, growth factors and proteolytic activity. In a three-dimensional setup we prepared comparative studies of single cell migration in Matrigel™ and rat tail collagen I gels. Here, we could show that chemotaxis is unaffected by hydrogel stiffness while velocity is not. We evaluated the hydrogel microarchitecture as the most critical factor for single cell migration and established an immunostaining method to visualize hydrogel structure as well as cells embedded into the gels. By this methodology we figured out the importance of ECM proteins secreted by the cells themselves for directed cell migration.

Advances in Selectively Handling and Probing Individual Proteins in AFM Single-Molecule Force Spectroscopy

Diana A. Pippig, Fabian Baumann, Katherine Erlich, Magnus Bauer and Hermann E. Gaub
Lehrstuhl für Angewandte Physik and Center for Nanoscience (CeNS), Ludwig-Maximilians-Universität, Amalienstrasse 54, 80799 Munich, Germany

Studying proteins or protein networks at the single-molecule level, requires precise targeting, localization and control over the molecular constituents. To address those issues, AFM-based Single-Molecule Cut & Paste (SMC&P), relying on a hierarchical system of unbinding forces for specific biomolecule interactions, has been developed. In initial proof-of-principle experiments utilizing DNA duplex interactions, SMC&P had already been employed e.g. in the controlled deposition of individual fluorophores in well-defined nanometer sized patterns. We have now improved the method to efficiently transport individual proteins (e.g. GFP) from a “storage” area on a functionialized glass surface to a defined position in a “building” area. The system relies on the use of protein-DNA chimeras that are site-selectively and covalently coupled by means of a protein-fused short ybbR-peptide tag and a Coenzyme A modification of the DNA. The linking reaction is catalyzed by Sfp-Synthase. This represents a versatile and robust way of adding a freely programma-
ble and highly selective attachment site to any protein of interest. The achieved Å-precision of protein SMC&P should further enable the deposition of individual enzymes in the centre of Zero-Mode Waveguides (ZMW), which will in the future significantly improve enzymatic studies in the presence of high concentrations of fluorescent substrates that are unmet by other single-molecule fluorescence methods.

Kinetics of Apoptosis Events assessed in High Throughput Microscopy on Cell Lattices

Peter J.F. Röttgermann, Alexandra Fink, Joachim O. Rädler
Fakultät für Physik and Center for Nanoscience (CeNS), Ludwig-Maximilians-Universität, Geschwister-Scholl-Platz 1, 80539 Munich, Germany

Dynamics of molecular processes are heterogeneous at the single-cell level. Hence, time-lapse microscopy becomes increasingly important as it allows measurement of cell fate decisions and cellular responses in general. Micropatterned surfaces help to achieve high-throughput analysis of single cells. For this purpose, a new micro-structured surface has been developed whose surface chemistry and geometry have been optimized to allow self-organization of cells after seeding.[1] First applications has been used for automated image-based analysis of cell death. Time resolved dose response curves of amyloid polystyrene nanoparticles have been measured. Moreover, a temporal dependence between different cell death events has been shown. The knowledge of timing can be used to map various signal pathways of apoptosis (programmed cell death). Microstructured arrays may be furthermore utilized for cell cycle marker independent cell observation over two cell generations, e.g. for studying cell cycle dependence in combined chemotherapy.


Figure 1: Pattern of square lattices are generated with plasma-induced patterning on a 6 channel slide. Cells arrange themselves into an array after seeding. Cells are exposed then to agents (NP or drugs) and monitored with markers for readout with an automated fluorescence microscope up to 72 hours. Automated image processing on the cell lattice enables high-throughput analysis of single cells.

Legless locomotion in lattices

Perrin Schiebel and Daniel I. Goldman
School of Physics, Georgia Institute of Technology, Atlanta, GA 30332

Interaction with the environment is integral to the process of locomotion. However, little is known about the interactions between the animal body and complex terrain during legless, undulatory travel (e.g. snake locomotion). An improved understanding of this interplay of force will improve robotic locomotors and advance understanding of the physics of complex soft materials like sand, debris and leaf litter. The subject of this study, the desert-dwelling Mojave shovel-nosed snake Chionactis occipitalis, demonstrates aptitude navigating a variety of terrains. We tested Chionactis using a simplified model of heterogeneous terrain: square lattices of obstacles. To quantify the effect of heterogeneity on the snake’s performance we measured the effect of lattice spacing on speed for both granular and smooth, hard substrates. Previous studies of C. elegans [Majmudar, T., Keaveny, E.E., Zhang, J., & Shelley, M.J. 2012 Experiments and theory of undulatory locomotion in a simple structured medium. J. R. Soc. Interface 9, doi: 10.1098/rsif.2011.0856] and garter snakes [Kelley, K.C., Arnold, S.J., & Gladstone, J. 1997 The effects of substrate and vertebral number on locomotion in the garter

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snake Thamnophis elegans. Functional Ecology 11, 189-198] observed a lattice density that resulted in maximal speed. We did not observe an optimum but instead found that increased obstacle density in the granular substrate resulted in a decrease in mean forward speed above a critical density. Below this density, speed was comparable to that on open sand. In lattices on hard substrates, lattice density did not affect speed; the speed in the densest lattice was comparable to that on open hard ground. This speed was a factor of three lower than the speed on open sand.

**Describing the RNA polymerase clamp movement throughout the transcription cycle using single-molecule FRET**

Sarah Schulz¹, Andreas Gietl², Richard Ebright², Finn Werner³, Philip Tinnefeld⁴ and Dina Grohmann¹

1 Physikalische und Theoretische Chemie- NanoBioSciences, Technische Universität Braunschweig, Hans-Sommer-Strasse 10, 38106 Braunschweig, Germany; 2 Waksman Institute and Dpt. of Chemistry and Chemical Biology, Rutgers University, Piscataway, NJ08854, USA; 3 RNAP laboratory, University College London, Institute of Structural and Molecular Biology, Division of Biosciences, Gower St, London WC1E 6BT, UK; 4 Center for Nanoscience (CeNS), Schellingstraße 4, 80799 Munich, Germany

In all living organisms transcription is the first step in gene expression. Here, multi-subunit RNA polymerases (RNAPs) utilize genomic DNA as template for RNA synthesis. Transcription can be understood as a cyclic process divided into three steps: initiation, elongation and termination. The transition from one step to the next demands the disruption and constitution of inter- and intramolecular interactions. Therefore, transcription is an inherently dynamic process that cannot be sufficiently described by X-ray crystallography or NMR. We investigated one of the RNAP’s mobile elements - the so called clamp domain. Crystal structures of the RNAP in different contexts indicate that the clamp adopts either an open or closed conformation. Moreover, the clamp is crucially involved in the regulation of RNAP activity as transcription factors like TFE and Spt4/5 bind to the clamp tip. In order to monitor the conformational status of the clamp we site-specifically engineered a donor and acceptor fluorophore into the RNAP using the fully recombinant transcription system derived from the archaeal organism Methanocaldococcus jannaschii. Here we employed single-molecule Förster resonance energy transfer experiments using a multicolour prism-TIRF microscope with alternating laser excitation to investigate the relative position of the clamp throughout the stages of the transcription cycle on immobilized RNAP. We identified hitherto unseen RNAP clamp configurations and show that the clamp can adopt two distinct conformations in both, the initiation and the elongation complex. Spt4/5 and TFE shift the equilibrium towards one of these conformations suggesting that both transcription factors guide a conformational switch thereby regulating the activity of the RNAP.

**Models for Angiogenesis on microstructured surfaces**

Simon Schuster and Stefan Zahler

Centre for Drug Research, Department of Pharmacy, University of Munich LMU

Angiogenesis, the growth and formation of novel blood vessels from preexisting vessels, is an important physiological and pathophysiological process involved in wound healing but also in cancer progression. However, dynamics of angiogenesis in general and the impact of physical factors in angiogenesis are barely understood. We try to model different cellular processes of primary endothelial cells using micro-structured surfaces. With the help of micro contact printing we bring HUVECs (= Human Umbilical Vein Endothelial Cells) into defined cellular shape and stress conditions in order to model 3D migration, tip cell formation and fibronectin-fibrillogenesis. All these processes have been shown to play an important role in angiogenesis.

We use primary endothelial cells, which are not adapted to 2D cell culture, to establish an easy accessible model system for imitating 3D migration on a flat surface. This model has been proposed before for 3T3 fibroblast, but HUVECs show some striking differences. Our results reveal that 1D migrating endothelial cells share a lot of properties compared to 3D migrating cells, regarding their overall morphology as well as their cellular response to selected small molecule inhibitors.
Emergence of Collective Cell Migration on Circular Micropatterns

Felix J. Segerer,1 Florian Thüroff,2 Alicia Piera Alberola,1 Erwin Frey,2 and Joachim O. Rädler1
1 Chair for Soft Condensed Matter, Ludwig Maximilians Universität München, Geschwister-Scholl-Platz 1, D-80539 Munich, Germany; 2 Arnold Sommerfeld Center for Theoretical Physics and Center for NanoScience, Department of Physics, Ludwig-Maximilians-Universität München, Theresienstrasse 37, D-80333 Munich, Germany

The spontaneous emergence of vortices is a hallmark of active cellular matter. Here, we study the emergence of collective circular motion as a function of the number of cells confined in circular micro-patterns. Analyzing the movement of each cell individually, we find distinct states of coherent angular motion (CAMo) and disordered motion (DisMo). The persistence of CAMo increases with increasing cell number, besides a pronounced discontinuity, accompanied by a topological transition towards a conformation featuring a cell in the system center. Supported by computer simulations we suggest that the dipole nature of migrating cells accounts for the occurrence of vortex states and entails the instability of cell configurations with unfavorable dipole arrangement.

Nanoliter-Droplet Thermophoresis for Diagnostics & Drug Discovery

Susanne A.I. Seidel and Dieter Braun
University of Munich, Physics Department, Systems Biophysics, NIM and CeNS, Amalienstraße 54, 80799 Munich, Germany
seidel.susanne@lmu.de, dieter.braun@lmu.de

Biomolecule interactions, which are central to in vitro diagnostics and drug discovery, are quantifiable via thermophoresis, the directed molecule movement along a temperature gradient. It is sensitive for binding induced changes in size, charge, or conformation. Established capillary measurements require at least 0.5μL per sample. We cut down sample consumption 50 fold, using 10nL droplets produced with acoustic droplet robotics (Fig. A).1 In locally heated droplets we analyzed temperature increase, Marangoni flow, and concentration distribution by fluorescence microscopy and numerical simulation (Fig. B), before quantifying AMP-aptamer binding. Miniaturization and the 1536-well plate format make the method high-throughput and automation friendly.

Thus, nL-thermophoresis promotes innovative applications, e.g. when combined with our diagnostic autocompetition assay.2 It allows to directly quantify absolute concentration and affinity of disease related biomarkers in untreated human blood serum. We analyzed autoantibodies against the cardiac β1-adrenoceptor associated with dilated cardiomyopathy (Fig. C). In the future, we are extending thermophoretic serology on lung diseases.3 Diagnostic assays in other bodily fluids seem very feasible as optical thermophoresis even allows for binding studies in crude cell lysate.4 For analyses in bioliquids, fluorescent labels ensure selectivity and sensitivity. However, labels can affect cell number, besides a pronounced discontinuity, accompanied by a topological transition towards a conformation featuring a cell in the system center. Supported by computer simulations we suggest that the dipole nature of migrating cells accounts for the occurrence of vortex states and entails the instability of cell configurations with unfavorable dipole arrangement.

Figure 1: (A) Nanoliter thermophoresis. Droplets of a biomolecule solution (purple) were stabilized in an oil-surfactant mix (brown), locally heated with an IR laser and analyzed by fluorescence microscopy. Inset: 5nL of 1:1 human serum/PBS. (B) Numerical simulation. In a 20nL droplet after 0.2s of heating, the central horizontal plane comprises the boundary of 2 toroidal flow vortices driven by Marangoni convection. (C) Diagnostic autocompetition assay. Antibody concentration and affinity were resolved using the tracer peptide COR1. (D) Label free thermophoresis. The glutamate receptor iGluR2 bound glutamate with KD= 0.84±0.04µM and glu-azo with KD=19±5µM. Preincubation with glutamate prevented glu-azo binding (control).
the binding behavior. We thus established label free ther- 
mo- 
ophoresis utilizing the intrinsic UV-fluorescence of tryp-
tophan residues in proteins. The method is especially suited 
for protein-small molecule analysis (Fig. D). It is thus very 
interesting for pharmacological screening, as the majority of 
today’s drugs are small molecules and most of their targets 
are proteins. Taken together, the presented innovations can 
be expected to considerably widen the application spectrum 
of thermophoresis in drug discovery and laboratory diagnos-
tics.

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DNA nanotubes as intracellular delivery vehicles in vivo

Sabine Sellner¹, Samet Kocabey², A. Katharina Nekolla¹, Fritz Krombach¹, Tim Liedl², Markus Rehberg¹

1 Walter Brendel Centre of Experimental Medicine, Ludwig-Maximilians-Universität München, Marchioninistr. 15, 81377 Munich; 2 Department of Physics & Center for NanoScience, Ludwig-Maximilians-Universität München, Geschwister-Scholl-Platz 1, 80539 Munich, Germany

DNA-based nanoconstructs are promising for biomedical applications. They are biocompatible and can be modified with a plethora of (bio)chemical moieties, e.g. CpG DNA. Unmethylated CpG sequences are a hallmark of microbial DNA and are commonly used as adjuvants for immuno-
stimulation. These CpG oligonucleotides are recognized by 
Toll-like receptor 9 (TLR9), present on lymphocytes and 
antigen-presenting cells, incl. macrophages, and thus initiate 
an immune response. In this study, we investigated the use of DNA-based nanotubes as promising carrier systems (CpG 
delivery) and their effect on immune cells in vivo and in 
real time. DNA nanotubes were designed using the sin-
gle-stranded tile method, mixing 48 unique DNA strands to 
form 8-helix nanotubes with a length of ~40 nm and a diam-
eter of ~8 nm. Half of the strands were labeled with a fluo-
rescence dye for the visualization in vivo. DNA nanotubes, 
CpG-decorated DNA nanotubes, and CpG oligonucleotides 
(500 nM, 300 nl) were microinjected into the cremaster mus-

cle of anesthetized mice. As assessed by in vivo fluorescence 
microscopy, all three types of DNA were rapidly internalized 
by F4/80 positive cells (incl. macrophages and mast cells) and 
colocalized with lysotracker dye in these cells. Only microin-
jection of CpG-decorated DNA nanotubes induced a signifi-
cant increase in leukocyte adhesion and transmigration in 
postcapillary venules of the cremaster muscle as observed 
by in vivo microscopy. Confocal microscopy of immunos-
tained muscle tissue revealed that only after application of 
CpG-decorated DNA nanotubes, nuclei of cells surrounding 
the microinjection site were positive for phosphorylated 
p65, indicating (TLR-9 mediated) activation of the NF-κB 
pathway. Taken together, these in vivo findings suggest that DNA nanotubes are potent delivery vehicles, targeting tissue 
macrophages and mast cells. The immunogenic potential 
apparently depends on the decoration of DNA tubes with 
CpG-oligonucleotides.

Geometry and Dynamics of Diffusion in the Cytoplasm

Wendell Smith¹, Mark Shattuck²,³, Christine Jacobs-Wagner⁴, and Corey O’Hern³,⁵

¹ Department of Physics, Yale University, ² Benjamin Levich Institute and Physics Department, City College of New York, ³ Department of Mechanical Engineering and Materials Science, Yale University, ⁴ Department of Molecular, Cellular and Developmental Biology, Yale University, and ⁵ Department of Applied Physics, Yale University

Recent research indicates that the cytoplasm does not behave 
entirely like a liquid. Thus, simple diffusion alone cannot 
explain the motion of particles through the cell. While small 
particles tend to obey simple diffusion, large particles can 
move through the cell as though in a glass or a solid. While 
these size effects have been documented [1], little is known 
about their origin. In this study, we investigate several dif-
ferent models of the cytoplasm to determine which effects — 
crowding, nucleoid exclusion effects, spatially variable vis-
cosity, or attractive interactions — best explain the dynamics 
of passive transport in the cell.
The Deterministic Information Bottleneck

**DJ Strouse, David Schwab**
Princeton University

A fundamental and ubiquitous task that all organisms face is prediction of the future; accurate predictions of, for example, the locations of food or predators are matters of life and death. The basis of those predictions are, of course, past sensory experience. Since an individual’s memory resources are limited and costly, however, there is a tradeoff between memory cost and predictive payoff. The information bottleneck (IB) method (Tishby, Pereira, & Bialek 2000) formulates this tradeoff as a mathematical optimization problem using an information theoretic cost function. IB encourages storing as few bits of past sensory input as possible while selectively preserving the bits that are most predictive of the future. The optimization is done over the stochastic encoding distribution \( q(m|p) \), where \( p \) and \( m \) are random variables representing past observations and encoded memory, respectively. Algorithmically, the optimization is done via an iterative algorithm, except in a few simple cases where an analytic solution is available. Here we introduce an alternative formulation of the IB method, which we call the deterministic information bottleneck (DIB). First, we argue for an alternative cost function, which better represents the biologically-motivated goal of minimizing required memory resources. Then, we show that this seemingly minor change has the dramatic effect of converting the stochastic encoding distribution \( q(m|p) \) into a deterministic encoding function \( m(p) \). Next, we propose an iterative algorithm for solving the DIB problem. We conclude by discussing the relative advantages and appropriate uses of each method.

Elucidating biological responses to nanoparticles by quantitative live-cell imaging

**Adriano A. Torrano**, Rudolf Herrmann, and Christoph Bräuchle

1 Department of Chemistry and Center for NanoScience (CeNS), University of Munich - LMU, Germany; 2 Institute for Physics, University of Augsburg, Germany

The focus of our research is to perform an interdisciplinary approach to analyze the bioactivity and cellular uptake of distinct nanoparticles in human endothelial cells. Distinct particles of irregular shape and varying size, consisting of carrier material (“washcoat”, e.g. ceria) and noble metal (e.g. platinum) are produced by long-term decomposition of automobile catalyst and released into the environment. However, little is known about the particular biological effects of these decomposition products. We have developed reproducible methods for the synthesis of model compounds consisting of cerium dioxide nanoparticles. Moreover, we succeeded in controlling the agglomeration of small (8-10 nm) ceria nanoparticles and obtaining almost monodisperse agglomerates (40-260 nm) which can be decorated with noble metal nanoparticles (Pt, Pd, Rh, 2-5 nm). For detection by fluorescence microscopy, the models are labeled with a fluorescent dye (ATTO 647N). We use high-resolution live-cell imaging to investigate in great detail the interaction of these nanoparticles with single cells. Our results show that the presence of platinum on the surface of small ceria nanoparticles causes an impressive change in the cellular response: nanoparticles are rapidly internalized by cells and accumulate at mitochondria. Despite the fast and selective targeting to mitochondria, Figure 1: Live-cell fluorescence imaging reveals the interaction between model nanoparticles generated by automobile catalytic converters and human cells. Platinum-decorated ceria nanoparticles (in red) accumulate at mitochondria (in green) and therefore colocalize (in yellow). Lysosomes are shown in blue and are not associated to nanoparticles.
no cytotoxic effect was observed.


Optimization of collective enzyme activity via spatial localization

F. Tostevin, A. Buchner, G. Giunta, U. Gerland
Physik-Department, Technische Universität München, James-Franck-Str. 1, 85748 Garching, Germany

Collaborative enzymes in metabolic pathways are often collected into complex assemblies [1]; the product of one enzymatic reaction becomes the substrate for the next. Both prokaryotic and eukaryotic metabolic pathways include many well-studied examples, such as cellulases [2], pyruvate dehydrogenase complexes [3] and glycolytic enzymes [4]. To fully understand the design and operation of enzymatic pathways, it is therefore crucial to understand how the arrangement of enzymes in space affects pathway function. Here we investigate the effects of different enzyme localization patterns in minimal enzymatic pathway models that explicitly include diffusion and advection of substrate. Our analytic and numerical approaches reveal generic principles of how enzyme distributions can be engineered so as to maximize pathway flux. In particular, the optimal enzyme arrangement transitions from clustering to an extended profile as the relative rates of reaction versus loss of pathway intermediates are varied [5,6]. We identify simple universal

constraints that determine the optimal clustered fraction and critical enzyme density, independent of the specific reaction mechanism and system geometry. The common qualitative behavior of these diverse systems can be related to the underlying stochastic reactions and motion of single substrate molecules. In addition to elucidating the importance of enzyme localization in living cells, our results have implications for the design of synthetic biochemical devices, in which specific enzyme arrangements can be engineered so as to maximize efficiency.


Redox-Initiated Hydrogel System for Detection and Real-Time Imaging of Cellulolytic Enzyme Activity

Tobias Verdorfer, Klara H. Malinowska, Lukas F. Milles, Hermann E. Gaub, Michael A. Nash
Ludwig-Maximilian-University of Munich, Center for Nano Science and Chair of Applied Physics, Biophysics and Molecular Materials

One step on the route to biomass-derived fuels is the enzymatic hydrolysis of cellulosic materials into fermentable sugars. Optimizing pre-treatment conditions and improving enzyme formulations both require assays to quantify saccharification products on solid substrates. Typically such assays are performed using freely diffusing fluorophores or dyes that measure reducing polysaccharide chain ends. These methods have thus far not allowed spatial localization of hydrolysis activity to identifiable morphological substrate features. This work describes a hydrogel reagent signaling (HyReS) system that amplifies saccharification products. It initiates cross-linking of a hydrogel that forms to locations of
cellulose hydrolysis, allowing for imaging of the degradation process in real-time. Optical detection of the gel in a rapid parallel format on synthetic and pre-treated solid substrates was used to quantify activity of T. emersonii and T. reesei enzyme cocktails. When combined with total internal reflection fluorescence (TIRF) microscopy and atomic force microscopy (AFM) imaging, the reagent system provided a means to visualize enzyme activity in real-time with high spatial resolution (<2 µm). This demonstrates the versatility of the HyReS system in detecting and imaging cellulolytic enzyme activity on a wide range of cellulosic substrates. These results taken together establish the HyReS system as a competitive cellulose assay platform and suggest new opportunities in real-time chemical imaging of biomass depolymerization. It provides distinct advantages in studies on susceptibility of cellulose substrates to degradation at specific locations (e.g. branch points, fibril ends or crystalline faces) and cellulase synergy.

Conformational dynamics of proteins involved in RNA maturation and protein folding observed by single-pair FRET

Lena Voith von Voithenberg, Daniela Wengler, Anders Barth, Carolina Sanchez Rico, Lisa Warner, Alina Röhl, Mathias Rosam, Johannes Buchner, Michael Sattler, Don C. Lamb

1 Department of Chemistry, Center for NanoScience (CeNS) and Center for Integrated Protein Science, Munich (CIPSM), Ludwig-Maximilians-Universität, München; 2 Department of Chemistry, Technische Universität, München, Germany

Foerster Resonance Energy Transfer (FRET) is an ideal tool to study properties of objects on the nanoscale, as the distance range of the energy transfer lies in the regime of interest. It is thus well suited for the observation of subpopulations and conformational changes or dynamics of proteins on the single molecule level. Maximum information including FRET efficiency, anisotropy, and lifetime can be collected by multi-parameter fluorescence detection (MFD) on a confocal microscope with polarized, pulsed interleaved excitation. However, observation times of individual molecules are in this case limited, while total internal reflection microscopy (TIRFM) offers extended measurement times up to several hundred seconds with immobilized proteins without the advantage of detecting several parameters simultaneously. Both, solution based confocal microscopy and TIRFM on immobilized molecules were applied to investigate conformational rearrangements of proteins involved in RNA maturation and in protein folding. For one of the first proteins binding to mRNA during the process of splicing, the spliceosomal subunit U2 snRNP auxiliary factor (U2AF65), we could reveal conformational rearrangements of the protein when bound to RNA. Time-resolved measurements showed conformational switching of the protein between distinct states and thus its dynamic motion. Projects on protein folding revealed major conformational changes for the chaperone BiP from the endoplasmic reticulum upon binding to its nucleotide exchange factor (NEF) for different nucleotide- and substrate-bound states. Furthermore, we analyzed the dynamic motion of the chaperone scaffolding protein Sti1 in the cytoplasm to elucidate its mechanistic function in protein maturation. These examples demonstrate that spFRET experiments can be applied to a wide variety of biological systems to gain detailed information on their functional mechanism.

Highly Sensitive mRNA FISH Via a Single Fluorophore

Gable M. Wadsworth, Rasesh Y. Parikh and Harold D. Kim

Georgia Institute of Technology, Atlanta, GA 30332

Phenotypic diversification of genetically identical cells arises from variable mRNA level. Hence, quantification of mRNA transcript number at the single cell level is important for understanding the origin of stochastic gene expression. The mRNA level of individual cells can be measured using Fluorescence In Situ Hybridization (FISH), which has been widely adopted for a variety of model organisms. This method depends on covering a long region of the target mRNA with multiple fluorescently labeled DNA probes with tens of fluorescent dyes. Here, we introduce a FISH protocol for detection of mRNA with a short singly labeled DNA probe in budding yeast. Using highly inclined laser illumination, we achieve single fluorophore sensitivity across a wide range of transcript levels. We also achieve excellent hybridization specificity and improved signal to noise ratio using methanol fixation followed by zymolyase treatment. Our time and cost efficient method has potential applications for detection of short mRNA in higher organisms.
Unwinding dynamics of a helically wrapped polymer: A model for single biomolecules?

J.-C. Walter, M. Baiesi, G. T. Barkema, E. Carlon and H. Schiessel
Laboratory Charles Coulomb, University Montpellier 2, France

This work is inspired by biopolymers displaying winding and more generally entanglement around a fixed object (like e.g. the DNA molecule organised in the chromatin fiber). We characterized the relaxation dynamics of a theoretical model of unwinding dynamics of a polymer as displayed in Fig. 1. Beyond applications to biological processes, it constitutes a fundamental instance of polymer with twist and torque. This model displays an interesting and non-trivial interplay between the dynamics and the conformation of the polymer during the relaxation. We show namely that the relaxation occurs in two time steps, with an initial slow dynamics of the free end followed by an exponential relaxation.

The poster presentation will start by a biological motivation, followed by a presentation of the model. Then I will motivate the use of scaling laws in polymer physics before presenting different results obtained analytically and numerically (Monte Carlo simulations). I will end with a discussion on prediction of the model that could be checked experimentally.

Figure 1: Snapshot of the polymer model in the course of relaxation: initially, the polymer if fully wrapped around a bar in a helical configuration with one end attached (in blue) and the other end free (in red). The relaxation proceeds through the rotation of the free end around the bar with an increasing coil. We give a precise description of the process with an interplay between the dynamics and the shape of the polymer. This model offers predictions for biopolymers that are entangled around a fixed object.

HA Polymer Length Regulation

Wenbin Wei¹, Jennifer Washburn², Paul Weigel², Jennifer Curtis¹
1 School of Physics and Parker H. Petit Institute for Bioengineering and Biosciences, Georgia Institute of Technology, 2 Department of Biochemistry and Molecular Biology, University of Oklahoma Health Sciences Center

Hyaluronan (HA) is an extracellular and cell-surface associated polysaccharide whose tissue distribution is ubiquitous. It is synthesized by HA synthase (HAS), a transmembrane enzyme, which simultaneously elongates and exports HA into extracellular region. Studies in animal models have documented a critical role for HA in embryonic development, healing processes, inflammation and tumor development. This broad spectrum of functions depends strongly on the size of HA polymers, ranging from a few nm to 25 um. At the gene level, HA production is regulated by three isoforms of HA synthase. Each isoform produces a distinct range of size. However, at the single molecule level, HA size regulation is not identified. Detailed biochemical and mutagenesis studies have ruled out most of the common regulation mechanism and people propose the role of polymer tension.

In our work, we use polymer theory to determine whether a length-dependent tension, biases the probability of HA-HAS detachment. In addition, AFM force spectroscopy provides HA-HAS binding properties. The combination of this data will enable us to predict HA size distribution, leading to a concrete biophysical test of the most compelling mechanical force regulation hypothesis. More broadly speaking, if proven true, HA synthase will represent a new class of enzymes whose polymer production is regulated not biochemically but mechanically.
Optimization of Sugar utilization strategies employing regulated phenotypic heterogeneity

Sonja Westermayer¹, Joaquin Gutiérrez¹, Judith Megerle¹, Georg Fritz², Ulrich Gerland³, and Joachim Rädler¹
1 Ludwig-Maximilians-Universität München, Fakultät für Physik, Geschwister-Scholl-Platz 1, 80539 München; 2 Ludwig-Maximilians-Universität München, Biozentrum der LMU, Großhaderner Str. 2-4, 82152 Planegg-Martinsried, 3 Ludwig-Maximilians-Universität München, Institut für Theoretische Physik, Theresienstr. 37, 80333 München

Bacterial gene regulation exhibits stochastic fluctuations at the molecular level that can subsequently lead to heterogeneity in the phenotype. We have previously studied in experiment and theory the phenotypic switching in the arabinose utilization system of single cells in Escherichia coli finding heterogeneity in the timing of the on-switching but a fast, homogeneous off-switching and have identified the small number of transporter proteins as the key source of variability in the on-switching [1], [2]. Given this stochasticity together with diauxic growth by discontinuous switching between different enzymatic states, the response of competitive systems in the presence of multiple sugars is of special interest. Here, we investigate the time-distributed response of sugar utilization systems in the light of evolutionary optimization. We study the situation of two competing sugar utilization systems part of a system with “flux-limited competition”. We are specially interested in the switching mechanism underlying the regulation of such competing sugar utilization systems. As example systems, we concentrate on systems within the Phosphotransferase System (PTS), which catalyzes the uptake and phosphorylation of several different sugars and plays a major role in carbon catabolite repression [3]. When two different sugars are present at the same time, it is assumed that their utilization systems will compete for the phosphor available inside the cell [4]. Using microfluidic set-ups we will expose bacterial cultures to well-defined stable and systematically variable environments and use time-lapse microscopy and single cell tracking to acquire single-cell expression kinetics. In mathematical models using cost-benefit analysis and game theory we compare metabolic networks in their performance as regulatory strategies in such variable environments.


Constructing an energy landscape for the hybridization of short oligonucleotides

Kevin Whitley¹, Matthew J. Comstock²*, and Yann R. Chemla¹,²,³
1 Center for Biophysics and Computational Biology, 2 Center for the Physics of Living Cells, 3 Department of Physics, University of Illinois at Urbana-Champaign, Urbana, IL 61801; * Present address: Department of Physics, Michigan State University, East Lansing, MI 48824

The hybridization of short oligonucleotides plays a critical role in many biological systems, from DNA replication to gene silencing. Despite extensive studies, details on the mechanism of this process on the shortest length scale (~10 bp) remain poorly understood. We use high-resolution optical tweezers with simultaneous fluorescence microscopy to investigate the hybridization of single oligonucleotides under tension. We measure the change in end-to-end extension upon annealing and melting as well as the unbinding kinetics of short (7-12 bp), fluorescently labeled oligonucleotides of DNA and RNA hybridizing to a complementary DNA sequence tethered between trapped beads. Our results allow us to construct an energy landscape of oligonucleotide hybridization along a well-defined reaction coordinate. Interestingly, our measurements of DNA duplex and RNA-DNA melting as a function of oligonucleotide length indicate that the transition state for both has the same end-to-end extension as a ~6 bp duplex. Based on these results and prior studies, we propose that melting occurs through a common transition state in which the two complementary strands partially aligned to each other with a minimal base-paired “core”. Additionally, we find that the change in extension upon hybridization deviates from extensible wormlike-chain model at forces >10 pN. We propose a model combining shear deformation and fraying of the terminal base pairs of the oligonucleotides to explain these results.
Examination of the Impact of Steric Constraints on Protein Core Packing

Bridget E. Hegarty, Paul Muir, Nathan D. Williams, and Tony Wu
Departments of Chemical and Environmental Engineering, Molecular Cell & Developmental Biology, and Cell Biology, Integrated Graduate Program in Physical and Engineering Biology, Yale University, New Haven, CT

A combination of bond and steric contributions largely determines the structures assumed by protein cores. Our project aimed to evaluate the role of steric interactions in constraining the conformational space available to protein cores. We used molecular dynamics (MD) simulations to predict changes in the conformational space explored in WT and mutant structures, and compared these predictions to bond length and angle distributions in crystal structures. Our simulations predicted lower variability of phi and psi angles in variants, which is in agreement with the angles observed in crystallized mutants. These findings illustrate the predictive power of our model, which may aid in future studies on the functional consequences of mutation and in de-novo protein design.

Design of “smart” multifunctional MOF nanocarriers for controlled and targeted drug delivery

A. Zimpel and S. Wuttke
Department of Chemistry and Center for NanoScience (CeNS), University of Munich (LMU), Butenandtstraße 11 (E), 81377 München, Germany

The development and study of novel functionalized nanoparticles as drug delivery systems is the goal of our research. The central idea is to design hybrid nanomaterials based on metal-organic frameworks (MOFs), which could offer a new platform for biomedical applications. These materials are expected to display novel and enhanced properties compared to more established nanomaterials such as polymers, gold nanoparticles, iron oxide nanoparticles, liposomes and mesoporous silica. MOFs are crystalline materials and have regular pores, with a large pore surface area, and a highly designable framework that permit tuning the pore shape, pore size, and internal and external surface functionality. As a result, MOF nanoparticles with well-defined and tuneable structures can be realized. Our research is focus on the design of MOF nanoparticles with inner pore functionalization for controlled interaction with biologically active molecules, as well as outer functionality for target cell uptake, triggered drug release, and with surface shielding against unwanted interactions inside the physiological environment (Figure).

Flow and diffusion in channel-guided cell migration

Anna-Kristina Marel1,4,*, Matthias Zorn1,4,*, Christoph Klingner1, Roland Wedlich-Söldner2, Erwin Frey3,4 and Joachim O. Rädler1,4
1 Fakultät für Physik & Center for NanoScience, Ludwig-Maximilians-Universität, Geschwister-Scholl-Platz 1, 80539 München, Germany; 2 Institute of Cell Dynamics and Imaging, University of Münster, Von-Esmarch-Straße 56, 48149 Münster, Germany; 3 Fakultät für Physik, Arnold Sommerfeld Center & Center for NanoScience, Ludwig-Maximilians-Universität, Theresienstraße 37, 80333 München, Germany; 4 Nanosystems Initiative Munich, Schellingstraße 4, 80799 München, Germany
* These authors contributed equally to this work.

Collective migration of mechanically coupled cell layers plays a notable role during wound healing, embryonic development and cancer progression. Confluent epithelial sheets are well-studied and spontaneous formations of swirls as
well as glass-like dynamic arrest as a function of cell density have been uncovered. In contrast, the flow-like properties of one-sided cell-sheet expansion in confining geometries are not well understood. We studied the short- and long-term flow of Madin-Darby canine kidney (MDCK) cells as they move through microchannels, using single cell tracking and particle image velocimetry (PIV). We found that a defined stationary cell current emerges when data is averaged over characteristic spatial and temporal scales. The averaged flow-field exhibits a velocity gradient in the direction of migration and a plug-flow-like profile across the advancing sheet. The observed flow velocity can be decomposed into two contributions, a constant term stemming from directed cell migration and a diffusion-like contribution that scales with the density gradient. From the density gradient and the speed of front propagation, we extract the collective diffusion coefficient of this diffusive component using the Fisher-Kolmogorov model. In order to connect diffusion mediated transport to underlying cellular motility, single cell trajectories and occurrence of vorticity were studied. We discovered that the directed large-scale cell flow alters fluctuations in cellular motion at short length scales: The formation of swirls is reduced compared to resting tissues. In addition, single-cell trajectories show persistent random-walk behavior superim-

NOTES
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<td>Aschenbrenner</td>
<td>Daniela</td>
<td>LMU</td>
<td><a href="mailto:daniela.aschenbrenner@physik.uni-muenchen.de">daniela.aschenbrenner@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Astley</td>
<td>Henry</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:henry.astley@physics.gatech.edu">henry.astley@physics.gatech.edu</a></td>
</tr>
<tr>
<td>Bag</td>
<td>Nirmalya</td>
<td>National University of Singapore</td>
<td><a href="mailto:a0066359@nus.edu.sg">a0066359@nus.edu.sg</a></td>
</tr>
<tr>
<td>Bak</td>
<td>Ji Hyun</td>
<td>Princeton University</td>
<td><a href="mailto:jhbak@princeton.edu">jhbak@princeton.edu</a></td>
</tr>
<tr>
<td>Balagan</td>
<td>Rajesh</td>
<td>Rice University</td>
<td><a href="mailto:rajesh.balagan@rice.edu">rajesh.balagan@rice.edu</a></td>
</tr>
<tr>
<td>Balusek</td>
<td>Curtis</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:cbalusek3@gatech.edu">cbalusek3@gatech.edu</a></td>
</tr>
<tr>
<td>Barak</td>
<td>Yoav</td>
<td>Weizmann Institute of Science</td>
<td><a href="mailto:yoav.barak@weizmann.ac.il">yoav.barak@weizmann.ac.il</a></td>
</tr>
<tr>
<td>Barth</td>
<td>Anders</td>
<td>LMU</td>
<td><a href="mailto:anders.barth@cup.uni-muenchen.de">anders.barth@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Bässem</td>
<td>Salem</td>
<td>LMU</td>
<td><a href="mailto:baessem.salem@cup.lmu.de">baessem.salem@cup.lmu.de</a></td>
</tr>
<tr>
<td>Bauer</td>
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<td>LMU</td>
<td></td>
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<tr>
<td>Bauer</td>
<td>Marianne</td>
<td>LMU</td>
<td><a href="mailto:Marianne.Bauer@physik.uni-muenchen.de">Marianne.Bauer@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Baumann</td>
<td>Fabian</td>
<td>LMU</td>
<td><a href="mailto:fabian.baumann@physik.uni-muenchen.de">fabian.baumann@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Baumeister</td>
<td>Wolfgang</td>
<td>MPI for Biochemistry</td>
<td><a href="mailto:baumeister@biochem.mpg.de">baumeister@biochem.mpg.de</a></td>
</tr>
<tr>
<td>Baumgärtel</td>
<td>Viola</td>
<td>LMU</td>
<td><a href="mailto:viola.baumgaertel@cup.uni-muenchen.de">viola.baumgaertel@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Bayer</td>
<td>Ed</td>
<td>Weizmann Institute of Science</td>
<td><a href="mailto:ed.bayer@weizmann.ac.il">ed.bayer@weizmann.ac.il</a></td>
</tr>
<tr>
<td>Berck</td>
<td>Matthew</td>
<td>Harvard University</td>
<td><a href="mailto:berck@fas.harvard.edu">berck@fas.harvard.edu</a></td>
</tr>
<tr>
<td>Berezhansky</td>
<td>Taras</td>
<td>TUM</td>
<td><a href="mailto:tarassaverio@yahoo.de">tarassaverio@yahoo.de</a></td>
</tr>
<tr>
<td>Beroz</td>
<td>Farzan</td>
<td>Princeton University</td>
<td><a href="mailto:farzan@princeton.edu">farzan@princeton.edu</a></td>
</tr>
<tr>
<td>Blagoev</td>
<td>Krastan</td>
<td>National Science Foundation</td>
<td><a href="mailto:kblagoev@nsf.gov">kblagoev@nsf.gov</a></td>
</tr>
<tr>
<td>Böhm</td>
<td>Philip</td>
<td>LMU</td>
<td><a href="mailto:philip.boehm@lmu.de">philip.boehm@lmu.de</a></td>
</tr>
<tr>
<td>Bracha</td>
<td>Dan</td>
<td>Weizmann Institute of Science</td>
<td>dan.bracha <a href="mailto:bradadan@gmail.com">bradadan@gmail.com</a></td>
</tr>
<tr>
<td>Bräuchle</td>
<td>Christoph</td>
<td>LMU</td>
<td><a href="mailto:Christoph.Braechle@cup.uni-muenchen.de">Christoph.Braechle@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Braun</td>
<td>Dieter</td>
<td>LMU</td>
<td><a href="mailto:dieter.braun@lmu.de">dieter.braun@lmu.de</a></td>
</tr>
<tr>
<td>Broadwater</td>
<td>Bo</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:bobroadwater@gatech.edu">bobroadwater@gatech.edu</a></td>
</tr>
<tr>
<td>Broda</td>
<td>Ellen</td>
<td>LMU</td>
<td><a href="mailto:ellen.broda@cup.uni-muenchen.de">ellen.broda@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Bruetzel</td>
<td>Linda</td>
<td>LMU</td>
<td><a href="mailto:Linda.K.Bruetzel@campus.lmu.de">Linda.K.Bruetzel@campus.lmu.de</a></td>
</tr>
<tr>
<td>Caldwell</td>
<td>Denise</td>
<td>National Science Foundation</td>
<td><a href="mailto:dcaldwel@nsf.gov">dcaldwel@nsf.gov</a></td>
</tr>
<tr>
<td>Chang</td>
<td>Patrick</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:pchang37@gatech.edu">pchang37@gatech.edu</a></td>
</tr>
<tr>
<td>Chee San</td>
<td>Tong</td>
<td>National University of Singapore</td>
<td><a href="mailto:cheesan.tong@nus.edu.sg">cheesan.tong@nus.edu.sg</a></td>
</tr>
<tr>
<td>Chen</td>
<td>Chen</td>
<td>National University of Singapore</td>
<td><a href="mailto:chen36@nus.edu.sg">chen36@nus.edu.sg</a></td>
</tr>
<tr>
<td>Chitnis</td>
<td>Parag</td>
<td>National Science Foundation</td>
<td><a href="mailto:pchitnis@nsf.gov">pchitnis@nsf.gov</a></td>
</tr>
<tr>
<td>Cole</td>
<td>John</td>
<td>University of Illinois</td>
<td><a href="mailto:cole15@illinois.edu">cole15@illinois.edu</a></td>
</tr>
<tr>
<td>Conti</td>
<td>Elena</td>
<td>MPI for Biochemistry</td>
<td><a href="mailto:conti@biochem.mpg.de">conti@biochem.mpg.de</a></td>
</tr>
<tr>
<td>Cordes</td>
<td>Thorben</td>
<td>Groningen</td>
<td><a href="mailto:t.m.cordes@rug.nl">t.m.cordes@rug.nl</a></td>
</tr>
<tr>
<td>Crevenna</td>
<td>Alvaro</td>
<td>LMU</td>
<td><a href="mailto:a1crpc@cup.uni-muenchen.de">a1crpc@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Dahmane</td>
<td>Selma</td>
<td>CNRS</td>
<td><a href="mailto:dahmane@cnrs.fr">dahmane@cnrs.fr</a></td>
</tr>
<tr>
<td>Dattz</td>
<td>Stefan</td>
<td>LMU</td>
<td><a href="mailto:stefan.datz@cup.uni-muenchen.de">stefan.datz@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Dauloulet</td>
<td>Olivier</td>
<td>CNRS</td>
<td><a href="mailto:olivier.dauloulet@hotmail.com">olivier.dauloulet@hotmail.com</a></td>
</tr>
<tr>
<td>de Oliveira</td>
<td>Guilherme</td>
<td>Federal University</td>
<td><a href="mailto:mrguiba@superig.com.br">mrguiba@superig.com.br</a></td>
</tr>
<tr>
<td>Denk</td>
<td>Jonas</td>
<td>LMU</td>
<td><a href="mailto:jonas.denk@physik.uni-muenchen.de">jonas.denk@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Edeleva</td>
<td>Evgeniia</td>
<td>LMU</td>
<td><a href="mailto:E.Edeleva@campus.lmu.de">E.Edeleva@campus.lmu.de</a></td>
</tr>
<tr>
<td>Engelke</td>
<td>Hanna</td>
<td>LMU</td>
<td><a href="mailto:hanna.engelke@cup.uni-muenchen.de">hanna.engelke@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Erlich</td>
<td>Katherine</td>
<td>LMU</td>
<td><a href="mailto:katherine.erlich@physik.uni-muenchen.de">katherine.erlich@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Feng</td>
<td>Jingchen</td>
<td>Rice University</td>
<td><a href="mailto:fengjc1214@gmail.com">fengjc1214@gmail.com</a></td>
</tr>
<tr>
<td>Fenton</td>
<td>Flavio</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:flavio.fenton@physics.gatech.edu">flavio.fenton@physics.gatech.edu</a></td>
</tr>
<tr>
<td>Feric</td>
<td>Marina</td>
<td>Princeton University</td>
<td><a href="mailto:mferic@princeton.edu">mferic@princeton.edu</a></td>
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<td>Ferraz da Costa</td>
<td>Danielly</td>
<td>Federal University of Rio de Janeiro</td>
<td><a href="mailto:alexandra.fink@physik.uni-muenchen.de">alexandra.fink@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Fink</td>
<td>Alexandra</td>
<td>LMU</td>
<td><a href="mailto:frey@lmu.de">frey@lmu.de</a></td>
</tr>
<tr>
<td>Frenkel</td>
<td>Evgeni</td>
<td>Harvard University</td>
<td><a href="mailto:frenkel@fas.harvard.edu">frenkel@fas.harvard.edu</a></td>
</tr>
<tr>
<td>Frey</td>
<td>Erwin</td>
<td>LMU</td>
<td><a href="mailto:frey@lmu.de">frey@lmu.de</a></td>
</tr>
<tr>
<td>Gaines</td>
<td>Jennifer</td>
<td>Yale University</td>
<td><a href="mailto:jennifer.gaines@yale.edu">jennifer.gaines@yale.edu</a></td>
</tr>
<tr>
<td>Gan</td>
<td>Lu</td>
<td>National University of Singapore</td>
<td><a href="mailto:lu@anaphase.org">lu@anaphase.org</a></td>
</tr>
<tr>
<td>Gaub</td>
<td>Hermann</td>
<td>LMU</td>
<td><a href="mailto:gaub@physik.uni-muenchen.de">gaub@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Gaul</td>
<td>Ulrike</td>
<td>LMU</td>
<td><a href="mailto:gaul@genzentrum.lmu.de">gaul@genzentrum.lmu.de</a></td>
</tr>
<tr>
<td>Gerland</td>
<td>Ulrich</td>
<td>LMU</td>
<td><a href="mailto:gerland@tum.de">gerland@tum.de</a></td>
</tr>
<tr>
<td>Gietl</td>
<td>Andreas</td>
<td>TU Braunschweig</td>
<td><a href="mailto:andreas.gietl@tu-bs.de">andreas.gietl@tu-bs.de</a></td>
</tr>
<tr>
<td>Gregor</td>
<td>Thomas</td>
<td>Princeton University</td>
<td><a href="mailto:tg2@princeton.edu">tg2@princeton.edu</a></td>
</tr>
<tr>
<td>Greiss</td>
<td>Ferdinand</td>
<td>LMU</td>
<td><a href="mailto:Ferdinand.Greiss@physik.lmu.de">Ferdinand.Greiss@physik.lmu.de</a></td>
</tr>
<tr>
<td>Ha</td>
<td>Taekjip</td>
<td>University of Illinois</td>
<td><a href="mailto:tjha@illinois.edu">tjha@illinois.edu</a></td>
</tr>
<tr>
<td>Hayes</td>
<td>Ryan</td>
<td>Rice University</td>
<td><a href="mailto:rh19@rice.edu">rh19@rice.edu</a></td>
</tr>
<tr>
<td>Hazel</td>
<td>Anthony</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:ahaelz3@gatech.edu">ahaelz3@gatech.edu</a></td>
</tr>
<tr>
<td>Hegarty</td>
<td>Bridget</td>
<td>Yale University</td>
<td><a href="mailto:bridge.hegarty@yale.edu">bridge.hegarty@yale.edu</a></td>
</tr>
<tr>
<td>Hennig</td>
<td>Susanne</td>
<td>LMU</td>
<td><a href="mailto:hennig@cens.de">hennig@cens.de</a></td>
</tr>
<tr>
<td>Hillenbrand</td>
<td>Patrick</td>
<td>TUM</td>
<td><a href="mailto:p.hillenbrand@tum.de">p.hillenbrand@tum.de</a></td>
</tr>
<tr>
<td>Hoyer</td>
<td>Maria</td>
<td>LMU</td>
<td><a href="mailto:maria.hoyer@campus.lmu.de">maria.hoyer@campus.lmu.de</a></td>
</tr>
<tr>
<td>Hu</td>
<td>David</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:hu@me.gatech.edu">hu@me.gatech.edu</a></td>
</tr>
<tr>
<td>Jarzembinska</td>
<td>Anita</td>
<td>LMU</td>
<td><a href="mailto:Anita.Jarzembinska@med.uni-muenchen.de">Anita.Jarzembinska@med.uni-muenchen.de</a></td>
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<tr>
<td>Jobst</td>
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<td>LMU</td>
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<tr>
<td>Johnston</td>
<td>Jeremiah</td>
<td>Yale University</td>
<td><a href="mailto:jeremiah.johnston@yale.edu">jeremiah.johnston@yale.edu</a></td>
</tr>
<tr>
<td>Jover</td>
<td>Luis</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:ljover3@gatech.edu">ljover3@gatech.edu</a></td>
</tr>
<tr>
<td>Kager</td>
<td>Anna</td>
<td>LMU</td>
<td><a href="mailto:anna.kager@physik.uni-muenchen.de">anna.kager@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Keil</td>
<td>Lorenz</td>
<td>LMU</td>
<td><a href="mailto:Lorenz.Keil@physik.uni-muenchen.de">Lorenz.Keil@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Ketchum</td>
<td>Christina</td>
<td>University of Maryland</td>
<td><a href="mailto:merrill.blue@gmail.com">merrill.blue@gmail.com</a></td>
</tr>
<tr>
<td>Kim</td>
<td>Harold</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:harold.kim@physics.gatech.edu">harold.kim@physics.gatech.edu</a></td>
</tr>
<tr>
<td>Kim</td>
<td>Hyuneil</td>
<td>University of Illinois</td>
<td><a href="mailto:hkim167@illinois.edu">hkim167@illinois.edu</a></td>
</tr>
<tr>
<td>Klamecka</td>
<td>Kamila</td>
<td>LMU</td>
<td><a href="mailto:klamecka@gmail.com">klamecka@gmail.com</a></td>
</tr>
<tr>
<td>Kneer</td>
<td>Luisa</td>
<td>LMU</td>
<td><a href="mailto:luisa.kneer@physik.lmu.de">luisa.kneer@physik.lmu.de</a></td>
</tr>
<tr>
<td>Koo</td>
<td>Peter</td>
<td>Yale University</td>
<td><a href="mailto:peter.koo@yale.edu">peter.koo@yale.edu</a></td>
</tr>
<tr>
<td>Kopperger</td>
<td>Enzo</td>
<td>TUM</td>
<td><a href="mailto:enzo.kopperger@tum.de">enzo.kopperger@tum.de</a></td>
</tr>
<tr>
<td>Kriegel</td>
<td>Franziska</td>
<td>LMU</td>
<td><a href="mailto:FKriegel@physik.uni-muenchen.de">FKriegel@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Kuhlman</td>
<td>Thomas</td>
<td>University of Illinois</td>
<td><a href="mailto:tkuhlman@illinois.edu">tkuhlman@illinois.edu</a></td>
</tr>
<tr>
<td>Lamb</td>
<td>Don</td>
<td>LMU</td>
<td><a href="mailto:don.lamb@cup.uni-muenchen.de">don.lamb@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Lambrecht</td>
<td>Jana</td>
<td>TUM</td>
<td><a href="mailto:lambrecht@ethris.com">lambrecht@ethris.com</a></td>
</tr>
<tr>
<td>Lanzmich</td>
<td>Simon</td>
<td>LMU</td>
<td><a href="mailto:Simon.Lanzmich@physik.lmu.de">Simon.Lanzmich@physik.lmu.de</a></td>
</tr>
<tr>
<td>Lee</td>
<td>Rachel</td>
<td>University of Maryland</td>
<td><a href="mailto:rmlee@umd.edu">rmlee@umd.edu</a></td>
</tr>
<tr>
<td>Lee</td>
<td>Lucy Eunju</td>
<td>Harvard University</td>
<td><a href="mailto:elee@physics.harvard.edu">elee@physics.harvard.edu</a></td>
</tr>
<tr>
<td>Lee</td>
<td>Sin Yi</td>
<td>National University of Singapore</td>
<td><a href="mailto:mbilsy@nus.edu.sg">mbilsy@nus.edu.sg</a></td>
</tr>
<tr>
<td>Leonhardt</td>
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<td>LMU</td>
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<tr>
<td>Levine</td>
<td>Erel</td>
<td>Harvard University</td>
<td><a href="mailto:elevine@fas.harvard.edu">elevine@fas.harvard.edu</a></td>
</tr>
<tr>
<td>Levine</td>
<td>Herbert</td>
<td>rice University</td>
<td><a href="mailto:herbert.levine@rice.edu">herbert.levine@rice.edu</a></td>
</tr>
<tr>
<td>Liedl</td>
<td>Tim</td>
<td>LMU</td>
<td><a href="mailto:tim.liedl@lmu.de">tim.liedl@lmu.de</a></td>
</tr>
<tr>
<td>Ligon</td>
<td>Thomas</td>
<td>LMU</td>
<td><a href="mailto:Thomas.Ligon@physik.uni-muenchen.de">Thomas.Ligon@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Lin</td>
<td>Yu</td>
<td>Yale University</td>
<td><a href="mailto:yu.lin@yale.edu">yu.lin@yale.edu</a></td>
</tr>
<tr>
<td>Lippok</td>
<td>Svenja</td>
<td>LMU</td>
<td><a href="mailto:Svenja.Lippok@physik.uni-muenchen.de">Svenja.Lippok@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>List</td>
<td>Jonathan</td>
<td>TUM</td>
<td><a href="mailto:jonathan.list@ph.tum.de">jonathan.list@ph.tum.de</a></td>
</tr>
<tr>
<td>Long</td>
<td>Junjiajia</td>
<td>Yale University</td>
<td><a href="mailto:junjiajia.long@yale.edu">junjiajia.long@yale.edu</a></td>
</tr>
<tr>
<td>Lorman</td>
<td>Vladimir</td>
<td>CNRS</td>
<td><a href="mailto:vladimir.lorman@univ-montp2.fr">vladimir.lorman@univ-montp2.fr</a></td>
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<tr>
<td>Losert</td>
<td>Wolfgang</td>
<td>University of Maryland</td>
<td><a href="mailto:wlosert@umd.edu">wlosert@umd.edu</a></td>
</tr>
<tr>
<td>Luthey-Schulten</td>
<td>Zaida</td>
<td>University of Illinois</td>
<td><a href="mailto:zan@illinois.edu">zan@illinois.edu</a></td>
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<tr>
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<td>Malinowska</td>
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<td>LMU</td>
<td><a href="mailto:klara.malinowska@physik.uni-muenchen.de">klara.malinowska@physik.uni-muenchen.de</a></td>
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<tr>
<td>Margeat</td>
<td>Emmanuel</td>
<td>CNRS</td>
<td><a href="mailto:margeat@cbs.cnrs.fr">margeat@cbs.cnrs.fr</a></td>
</tr>
<tr>
<td>Marques</td>
<td>Mayra</td>
<td>Federal University of Rio de Janeiro</td>
<td><a href="mailto:mayra.marques@ymail.com">mayra.marques@ymail.com</a></td>
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<tr>
<td>Mast</td>
<td>Christof</td>
<td>LMU</td>
<td><a href="mailto:mail@christofmast.de">mail@christofmast.de</a></td>
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<tr>
<td>McGuinness</td>
<td>Catherine</td>
<td>Yale University</td>
<td><a href="mailto:catherine.mcguinness@yale.edu">catherine.mcguinness@yale.edu</a></td>
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<td>McInroe</td>
<td>Benjamin</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:bmcinroe3@gatech.edu">bmcinroe3@gatech.edu</a></td>
</tr>
<tr>
<td>Melbinger</td>
<td>Anna</td>
<td>UCSD, LMU</td>
<td><a href="mailto:Anna.melbinger@gmail.com">Anna.melbinger@gmail.com</a></td>
</tr>
<tr>
<td>Messer</td>
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<td>LMU</td>
<td><a href="mailto:philipp.messer@cup.lmu.de">philipp.messer@cup.lmu.de</a></td>
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<td>Mickler</td>
<td>Frauke</td>
<td>LMU</td>
<td><a href="mailto:Frauke.Mickler@cup.uni-muenchen.de">Frauke.Mickler@cup.uni-muenchen.de</a></td>
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<td>Milia</td>
<td>Gabriela</td>
<td>LMU</td>
<td><a href="mailto:gabriela.milia@physik.uni-muenchen.de">gabriela.milia@physik.uni-muenchen.de</a></td>
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<td>Milles</td>
<td>Lukas</td>
<td>LMU</td>
<td><a href="mailto:lukas.milles@physik.uni-muenchen.de">lukas.milles@physik.uni-muenchen.de</a></td>
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<tr>
<td>Mirsaidov</td>
<td>Utkur</td>
<td>National University of Singapore</td>
<td><a href="mailto:simon.mochrie@yale.edu">simon.mochrie@yale.edu</a></td>
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<td>LMU</td>
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<td>Möller</td>
<td>Friederike</td>
<td>LMU</td>
<td><a href="mailto:friedericke.moeller@physik.lmu.de">friedericke.moeller@physik.lmu.de</a></td>
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<td>Yale University</td>
<td><a href="mailto:paul.muir@yale.edu">paul.muir@yale.edu</a></td>
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<td>Larry</td>
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<td>Nekolla</td>
<td>Anna Katharina</td>
<td>LMU</td>
<td><a href="mailto:Katharina.Nekolla@med.uni-muenchen.de">Katharina.Nekolla@med.uni-muenchen.de</a></td>
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<tr>
<td>Nguyen</td>
<td>Jeffrey</td>
<td>Princeton University</td>
<td><a href="mailto:jnguyen@princeton.edu">jnguyen@princeton.edu</a></td>
</tr>
<tr>
<td>Nickel</td>
<td>Bert</td>
<td>LMU</td>
<td><a href="mailto:nickel@lmu.de">nickel@lmu.de</a></td>
</tr>
<tr>
<td>Nir</td>
<td>Eyal</td>
<td>Ben-Gurion University of the Negev</td>
<td><a href="mailto:eyalnir@bgu.ac.il">eyalnir@bgu.ac.il</a></td>
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<tr>
<td>Noel</td>
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<td>Georgia Institute of Technology</td>
<td><a href="mailto:alexis.noel@gatech.edu">alexis.noel@gatech.edu</a></td>
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<tr>
<td>Nübler</td>
<td>Johannes</td>
<td>TUM</td>
<td><a href="mailto:johannes.nuebler@tum.de">johannes.nuebler@tum.de</a></td>
</tr>
<tr>
<td>Onuchic</td>
<td>Jose</td>
<td>Rice University</td>
<td><a href="mailto:jonuchic@rice.edu">jonuchic@rice.edu</a></td>
</tr>
<tr>
<td>Ossato</td>
<td>Giulia</td>
<td>LMU</td>
<td><a href="mailto:giulia.ossato@cup.lmu.de">giulia.ossato@cup.lmu.de</a></td>
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<tr>
<td>Ott</td>
<td>Wolfgang</td>
<td>LMU</td>
<td><a href="mailto:wolfgang.ott@physik.uni-muenchen.de">wolfgang.ott@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Ouzounov</td>
<td>Nikolay</td>
<td>Princeton University</td>
<td><a href="mailto:ouzounov@princeton.edu">ouzounov@princeton.edu</a></td>
</tr>
<tr>
<td>Pflieger</td>
<td>Kerstin</td>
<td>LMU</td>
<td><a href="mailto:kerstin.pflieger@cup.uni-muenchen.de">kerstin.pflieger@cup.uni-muenchen.de</a></td>
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<tr>
<td>Pinto</td>
<td>Marilena</td>
<td>LMU</td>
<td><a href="mailto:marilena.pinto@cern.de">marilena.pinto@cern.de</a></td>
</tr>
<tr>
<td>Pippig</td>
<td>Diana</td>
<td>LMU</td>
<td><a href="mailto:diana.pippig@physik.lmu.de">diana.pippig@physik.lmu.de</a></td>
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<tr>
<td>Prescher</td>
<td>Jens</td>
<td>LMU</td>
<td><a href="mailto:jens.prescher@cup.uni-muenchen.de">jens.prescher@cup.uni-muenchen.de</a></td>
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<tr>
<td>Rädler</td>
<td>Joachim</td>
<td>LMU</td>
<td><a href="mailto:raedler@lmu.de">raedler@lmu.de</a></td>
</tr>
<tr>
<td>Raj</td>
<td>Saurabh</td>
<td>CNRS</td>
<td><a href="mailto:saurabh.raj@eps.ens.fr">saurabh.raj@eps.ens.fr</a></td>
</tr>
<tr>
<td>Ramalho</td>
<td>Tiago</td>
<td>TUM</td>
<td><a href="mailto:tiago.ramalho@tum.de">tiago.ramalho@tum.de</a></td>
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<tr>
<td>Rambeau</td>
<td>Joachim</td>
<td>CNRS</td>
<td><a href="mailto:joachim.rambeau@univ-montp2.fr">joachim.rambeau@univ-montp2.fr</a></td>
</tr>
<tr>
<td>Rank</td>
<td>Matthias</td>
<td>LMU</td>
<td><a href="mailto:matthias.rank@physik.lmu.de">matthias.rank@physik.lmu.de</a></td>
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<td>Rief</td>
<td>Matthias</td>
<td>TUM</td>
<td><a href="mailto:matthias.rief@mytum.de">matthias.rief@mytum.de</a></td>
</tr>
<tr>
<td>Röttgermann</td>
<td>Peter</td>
<td>LMU</td>
<td><a href="mailto:peter.roettgermann@physik.uni-muenchen.de">peter.roettgermann@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Santos de Freitas</td>
<td>Mônica</td>
<td>Federal University of Rio de Janeiro</td>
<td><a href="mailto:msfreitas@bioqmed.ufrj.br">msfreitas@bioqmed.ufrj.br</a></td>
</tr>
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<td>Scheller</td>
<td>Jacques</td>
<td>LMU</td>
<td><a href="mailto:jacques.scheller@physik.uni-muenchen.de">jacques.scheller@physik.uni-muenchen.de</a></td>
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<td>Schiebel</td>
<td>Perrin</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:pschiebel3@gatech.edu">pschiebel3@gatech.edu</a></td>
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<td>Schirnding</td>
<td>Constantin</td>
<td>LMU</td>
<td><a href="mailto:constantin.schirnding@cup.uni-muenchen.de">constantin.schirnding@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Schmidt</td>
<td>Alexandra</td>
<td>LMU</td>
<td><a href="mailto:exspc@cup.uni-muenchen.de">exspc@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Schöler</td>
<td>Constantin</td>
<td>LMU</td>
<td><a href="mailto:constantin.scholer@physik.uni-muenchen.de">constantin.scholer@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Schreiber</td>
<td>Christoph</td>
<td>LMU</td>
<td><a href="mailto:c.schreiber@physik.uni-muenchen.de">c.schreiber@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Schrimpf</td>
<td>Waldemar</td>
<td>LMU</td>
<td><a href="mailto:wscpc@cup.uni-muenchen.de">wscpc@cup.uni-muenchen.de</a></td>
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<td>Schulten</td>
<td>Klaus</td>
<td>University of Illinois</td>
<td><a href="mailto:kschulte@ks.uic.edu">kschulte@ks.uic.edu</a></td>
</tr>
<tr>
<td>Schulz</td>
<td>Sarah</td>
<td>TU Braunschweig</td>
<td><a href="mailto:Sarah.Schulz@tu-bs.de">Sarah.Schulz@tu-bs.de</a></td>
</tr>
<tr>
<td>Schuster</td>
<td>Simon</td>
<td>LMU</td>
<td><a href="mailto:Simon.Schuster@cup.uni-muenchen.de">Simon.Schuster@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Schuster</td>
<td>Cosima</td>
<td>DFG</td>
<td><a href="mailto:cosima.schuster@dfg.de">cosima.schuster@dfg.de</a></td>
</tr>
<tr>
<td>Segerer</td>
<td>Felix</td>
<td>LMU</td>
<td><a href="mailto:felix.segerer@physik.uni-muenchen.de">felix.segerer@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Seidel</td>
<td>Susanne</td>
<td>LMU</td>
<td><a href="mailto:seidel.susanne@physik.uni-muenchen.de">seidel.susanne@physik.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Seifert</td>
<td>Philipp</td>
<td>LMU, TUM</td>
<td><a href="mailto:seifert@tum.de">seifert@tum.de</a></td>
</tr>
<tr>
<td>Sekhavati</td>
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<td>LMU</td>
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<tr>
<td>Sellner</td>
<td>Sabine</td>
<td>LMU</td>
<td><a href="mailto:sabine.sellner@med.uni-muenchen.de">sabine.sellner@med.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Shukla</td>
<td>Kamal</td>
<td>National Science Foundation</td>
<td><a href="mailto:kshukla@nsf.gov">kshukla@nsf.gov</a></td>
</tr>
<tr>
<td>Silva</td>
<td>Jerson</td>
<td>Federal University of Rio de Janeiro</td>
<td><a href="mailto:jerson@bioqmed.ufrj.br">jerson@bioqmed.ufrj.br</a></td>
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<tr>
<td>Smith</td>
<td>Wendell</td>
<td>Yale University</td>
<td><a href="mailto:wendell.smith@yale.edu">wendell.smith@yale.edu</a></td>
</tr>
<tr>
<td>Smith</td>
<td>Eric</td>
<td>Princeton University</td>
<td><a href="mailto:emstthree@princeton.edu">emstthree@princeton.edu</a></td>
</tr>
<tr>
<td>Sobczak</td>
<td>Jean-Philippe</td>
<td>TUM</td>
<td><a href="mailto:j.sobczak@tum.de">j.sobczak@tum.de</a></td>
</tr>
<tr>
<td>Stern</td>
<td>Bodo</td>
<td>Harvard University</td>
<td><a href="mailto:bstern@cgr.harvard.edu">bstern@cgr.harvard.edu</a></td>
</tr>
<tr>
<td>Strouse</td>
<td>DJ</td>
<td>Princeton University</td>
<td><a href="mailto:danieljstrouse@gmail.com">danieljstrouse@gmail.com</a></td>
</tr>
<tr>
<td>Taylor</td>
<td>Bradford</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:bradfordtaylor@gmail.com">bradfordtaylor@gmail.com</a></td>
</tr>
<tr>
<td>Teichmann</td>
<td>Mario</td>
<td>TUM</td>
<td><a href="mailto:mario.teichmann@tum.de">mario.teichmann@tum.de</a></td>
</tr>
<tr>
<td>Thirumalai</td>
<td>Dave</td>
<td>University of Maryland</td>
<td><a href="mailto:thirum@umd.edu">thirum@umd.edu</a></td>
</tr>
<tr>
<td>Torrano</td>
<td>Adriano A.</td>
<td>LMU</td>
<td><a href="mailto:adriano.torrano@cup.uni-muenchen.de">adriano.torrano@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Tostevin</td>
<td>Filipe</td>
<td>TUM</td>
<td><a href="mailto:f.tostevin@tum.de">f.tostevin@tum.de</a></td>
</tr>
<tr>
<td>Tovar-Moll</td>
<td>Fernanda</td>
<td>Federal University of Rio de Janeiro</td>
<td><a href="mailto:tovarmollf@gmail.com">tovarmollf@gmail.com</a></td>
</tr>
<tr>
<td>Upadhyaya</td>
<td>Arpita</td>
<td>University of Maryland</td>
<td><a href="mailto:arpitau@umd.edu">arpitau@umd.edu</a></td>
</tr>
<tr>
<td>Urtel</td>
<td>Georg</td>
<td>LMU</td>
<td><a href="mailto:georg.urtel@physik.uni-muenchen.de">georg.urtel@physik.uni-muenchen.de</a></td>
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<td>Verdorfer</td>
<td>Tobias</td>
<td>LMU</td>
<td><a href="mailto:tobias.verdorfer@physik.uni-muenchen.de">tobias.verdorfer@physik.uni-muenchen.de</a></td>
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<tr>
<td>Voith von Voithenberg</td>
<td>Lena</td>
<td>LMU</td>
<td><a href="mailto:lena.voithenberg@cup.uni-muenchen.de">lena.voithenberg@cup.uni-muenchen.de</a></td>
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<tr>
<td>von Bronk</td>
<td>Benedikt</td>
<td>LMU</td>
<td><a href="mailto:b.bronk@physik.lmu.de">b.bronk@physik.lmu.de</a></td>
</tr>
<tr>
<td>Vu</td>
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<td>University of Maryland</td>
<td><a href="mailto:vuhuong@live.com">vuhuong@live.com</a></td>
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<td><a href="mailto:gwadsworth3@gatech.edu">gwadsworth3@gatech.edu</a></td>
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<td>Walter</td>
<td>Jean-Charles</td>
<td>CNRS</td>
<td><a href="mailto:jean-charles.walter@univ-montp2.fr">jean-charles.walter@univ-montp2.fr</a></td>
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<td>Wehnekamp</td>
<td>Fabian</td>
<td>LMU</td>
<td><a href="mailto:fawepc@cup.lmu.de">fawepc@cup.lmu.de</a></td>
</tr>
<tr>
<td>Wei</td>
<td>Wenbin</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:ww203@gatech.edu">ww203@gatech.edu</a></td>
</tr>
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<td>LMU</td>
<td><a href="mailto:veronika.weiss@cup.uni-muenchen.de">veronika.weiss@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Wengler</td>
<td>Daniela</td>
<td>LMU</td>
<td><a href="mailto:daniela.wengler@cup.uni-muenchen.de">daniela.wengler@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Werkmeister</td>
<td>Franz</td>
<td>LMU</td>
<td><a href="mailto:x.werkmeister@physik.lmu.de">x.werkmeister@physik.lmu.de</a></td>
</tr>
<tr>
<td>Westermayer</td>
<td>Sonja</td>
<td>LMU</td>
<td><a href="mailto:sonja.westermayer@physik.lmu.de">sonja.westermayer@physik.lmu.de</a></td>
</tr>
<tr>
<td>Whitley</td>
<td>Kevin</td>
<td>University of Illinois</td>
<td><a href="mailto:kwhitle2@illinois.edu">kwhitle2@illinois.edu</a></td>
</tr>
<tr>
<td>Williams</td>
<td>Nathan</td>
<td>Yale University</td>
<td><a href="mailto:nathan.d.williams@yale.edu">nathan.d.williams@yale.edu</a></td>
</tr>
<tr>
<td>Wilson</td>
<td>Alyssa</td>
<td>Harvard University</td>
<td><a href="mailto:wilson4@fas.harvard.edu">wilson4@fas.harvard.edu</a></td>
</tr>
<tr>
<td>Winogradoff</td>
<td>David</td>
<td>University of Maryland</td>
<td><a href="mailto:dwino218@gmail.com">dwino218@gmail.com</a></td>
</tr>
<tr>
<td>Wuttke</td>
<td>Stefan</td>
<td>LMU</td>
<td><a href="mailto:stefan.wuttke@cup.uni-muenchen.de">stefan.wuttke@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Yang</td>
<td>Patricia</td>
<td>Georgia Institute of Technology</td>
<td><a href="mailto:peijyang@gatech.edu">peijyang@gatech.edu</a></td>
</tr>
<tr>
<td>Yodh</td>
<td>Jaya</td>
<td>University of Illinois</td>
<td><a href="mailto:jyodh@illinois.edu">jyodh@illinois.edu</a></td>
</tr>
<tr>
<td>Zahler</td>
<td>Stefan</td>
<td>LMU</td>
<td><a href="mailto:stefan.zahler@cup.uni-muenchen.de">stefan.zahler@cup.uni-muenchen.de</a></td>
</tr>
<tr>
<td>Zhang</td>
<td>Sophie</td>
<td>Princeton University</td>
<td><a href="mailto:sz3@princeton.edu">sz3@princeton.edu</a></td>
</tr>
<tr>
<td>Zhao</td>
<td>Yao</td>
<td>Yale University</td>
<td><a href="mailto:yao.zhao@yale.edu">yao.zhao@yale.edu</a></td>
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<td>Matthias</td>
<td>LMU</td>
<td><a href="mailto:matthias.zorn@physik.uni-muenchen.de">matthias.zorn@physik.uni-muenchen.de</a></td>
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Lunch on Monday in the Deutsches Museum is included for all participants. On Tuesday, Wednesday and Thursday, please pick your own lunch spot! Close to the conference location Schellingstr. 4, you will find a multitude of takeaways, snack bars, coffee shops and restaurants in all price ranges. This list is only a selection of recommendable places, all of them also offering vegetarian food. Please feel free to ask the Munich participants for further recommendations.

€ - € € € price level
☐ - ☐☐☐ space

**Atzinger €€, ☐☐☐**
Authentic restaurant with Bavarian & international cuisine
Schellingstraße 9

**Bar Tapas €€, ☐☐☐**
Spanish restaurant
Amalienstraße 97

**Café Schneller €, ☐**
Old-style pastry shop and café, delicious cakes
Amalienstraße 59

**Dean & David €**
Snack bar & takeaway, salads, sandwiches, and curry
Schellingstraße 13

**Der verrückte Eismacher €**
Wide range of delicious and exotic icecream types
Amalienstraße 77

**Essbar €**
Snack bar & takeaway, salads, sandwiches, and quiches
Amalienstraße 93

**Kun Tuk €€, ☐**
Thai restaurant
Amalienstraße 81

**Limoni €€, ☐**
Italian restaurant
Amalienstraße 38

**Max Emanuel Brauerei €€, ☐☐☐**
Restaurant & beer garden, Bavarian and international cuisine
Adalbertstraße 33

**newsbar €€, ☐**
Restaurant, German & international cuisine
Amalienstraße 55

**Pommes Boutique €**
Snack bar & takeaway, home made French fries
Amalienstraße 46

**Turka Kebap €, ☐**
Turkish snack bar & takeaway
Amalienstraße 49

**Hans im Glück €€, ☐**
Hamburger restaurant, even vegetarian hamburgers
Türkenstraße 79

**Zum Koreaner €**
Korean snack bar & takeaway
Amalienstraße 51
Conference Locations

Monday, July 21:
Deutsches Museum, Ehrensaal
Museumsinsel 1
80538 Munich

Transport:
There will be a shuttle bus for international participants to the Deutsches Museum. See page 65 (public transport).

Return:
All S-Bahn train lines from Isartor stop to Marienplatz, at Marienplatz change to U3/U6

Tuesday, July 22 - Thursday, July 24:
LMU Munich, Physics Department, Lecture Hall H030
Schellingstr.4
80799 Munich

Transport:
Underground lines U3 and U6 to Universität
INTERNET ACCESS

At the lecture hall H030, Schellingstr. 4, open WLAN access will be available.
Network name: con, no password required.
At the Deutsches Museum, no internet access can be provided.