Symposium "Bio-Physics", 23/24 June 2016

THURSDAY JUNE 23, lecture hall Y16-G15

8:00 *Dr. Kathrin Willig, University Medical Center and Max Planck Institute of Experimental Medicine Göttingen*

STED microscopy of the living mouse brain

Far-field light microscopy is a powerful technique for imaging structures inside living cells, tissue or living animals. However, fine details or substructures of the cell cannot be visualized because of the diffraction limited resolution (~ 200 – 350 nm). This barrier had been overcome by a whole family of super-resolution microscopy or nanoscopy concepts such as STED, RESOLFT, PALM, STORM etc. From all super-resolution microscopy techniques available presently, STED microscopy stands out for its imaging capabilities in tissue: It is live-cell compatible, able to record 3D images from inside transparent tissue and the imaging speed is fast.
Here I present an application of STED microscopy to image dendritic spines in the brain of a living mouse. We image the cerebral cortex of a living mouse through a glass window, so that we can observe the dynamics of dendritic spines in the molecular layer of the visual cortex. For example, we reveal filamentous actin in dendrites down to a depth of 40 µm. Time-lapse recordings disclose dynamic changes at a resolution of ~ 60 nm. This talk will give an overview of the current status of *in vivo* STED microscopy dealing with aberrations in scattering brain tissue, labeling of post-synaptic structures and the use of the green, yellow and red-fluorescent protein for live cell and *in vivo* imaging.

8:50 Prof. Dr. Thorben Cordes, University of Groningen

Single-molecule studies of molecular coordination *in vitro* and *in vivo*

Numerous biochemical processes are driven by complex molecular machines that utilize (allosteric) coupling of conformational changes to perform a specific function. Highly regulated cellular pathways, such as protein biogenesis, require a similar “molecular coordination” between DNA, RNA and proteins to always produce the

right mix of proteins during a cell's lifespan. Most classical biophysical and biochemical techniques provide static snapshots of these molecular mechanisms. My group is interested in applying dynamic biophysical tools, with an emphasize on quantitative fluorescence microscopy, to solve problems involving molecular coordination. We recently started to use single-molecule fluorescence microscopy to characterize conformational dynamics of active membrane transporters *in vitro* ("in the test-tube") and spatiotemporal coupling of mRNA and proteins *in vivo* ("in the cell"). In this talk I present selected recent examples from both research areas. Our question-driven approach is complemented by our technological interests and resulting developments in (super-resolution) microscopy, spectroscopy, photophysics, probe- and assay design as well as advanced data analysis, which are presented during the talk in its relevant biological context.

9:40 *Prof. Dr. Sarah Köster, Georg-August-Universität Goettingen*

The Cytoskeleton as a Polymer-Network – from Self-Assembly to Living Cells

Biological cells are pervaded by a dense polymer network of fibrous proteins. The exact composition and network structures are to a great extent important for the mechanical properties of the cells, which in turn support their physiological function. We investigate the relation between molecular structure and mechanics and focus mostly on the so-called intermediate filaments, which are being discussed as strong candidates for the precise definition of the different mechanical properties of different cell types. We employ different microscopy and scattering methods in combination with microfluidic tools to approach the relevant time and length scales and achieve high control of the biological systems

10:30 Coffee Break

10:50 *Dr. Dr. Johann Georg Danzl, Max Planck Institute for Biophysical Chemistry, Göttingen*

Nanoscale fluorescence imaging

Far-field fluorescence nanoscopy techniques are transforming biological imaging. By preparing fluorophores in distinguishable (on and off) states and reading them out sequentially, they "super-resolve" features residing closer than the once fundamental resolution limit given by diffraction of light waves. In coordinate-targeted nanoscopy,

such as stimulated emission depletion (STED) microscopy, state contrast is induced by a pattern of light. Driving all fluorophores to an off-state, except for those residing at intensity minima, sharply confines fluorescence emission and enables high resolution. However, it also exposes fluorohores at the maxima to excess intensities, state cycling, and photobleaching.
I will discuss how transfer to a second off-state that is inert to the excess light enables both protection of fluorophores and superior state contrast. This directly translates into improved repeated imaging and 3D capability as well as superior resolution and image contrast in live cell nanoscopy. I will also explore how such novel concepts will enable us not only to decode the cell's structural features but also its function in terms of signaling and metabolism with diffraction-unlimited resolution.

11:40 *Prof. Dr. Christof Aegerter, University of Zurich*

Biological Physics and Physical Biology

Many Biological questions have answers based in Physics. On the other hand, non-equilibrium Physics can be well studied based on biological problems and tools. After a general introduction on the relation between Biology and non-equilibrium Physics, I will discuss two specific examples from our recent work. First a biological problem that initiates the study of a non-equilibrium process and second a biological problem that is solved physical principles.

FRIDAY JUNE 24, lecture hall Y16-G15

8:00 Dr. Enrico Klotzsch, University of New South Wales, Sydney

Mechanical forces during T-cell activation

When cells make adhesive contacts, they exert mechanical forces to re-arrange proteins laterally, regulate the protrusive activity at the interface, and probe the mechanical properties of the opposing surface. It is reasonable to assume that every interaction of cell surface proteins, including the extracellular matrix and membrane proteins, is put under tension *in vivo*. It appears surprising that molecular forces have been largely overlooked in ligand discrimination processes, especially when one considers that receptor-ligand interactions are largely driven and indeed regulated by cell-applied mechanical stress.

We developed and employ new biological systems to control antigen presentation to T-cells, both biochemically (i.e. ligand density and affinity) and biophysically (i.e. matrix stiffness), and new quantitative imaging approaches to measure mechanical forces that include traction force microscopy, super-resolution microscopy (SRM) and FRET force sensors.

There is growing evidence that the same mechanical forces involved in T-cell activation can even be transmitted to and influence intranuclear events. Since the chromatin structure is already altered when cell actin cytoskeleton is depolymerized, mechanical forces must be transduced from the cell exterior via the cytoskeleton into the nucleus through the dual lipid bilayer and protein rich nuclear lamina. However, little is known about the details of the force transduction processes and how this ultimately regulates gene expression i.e. during T-cell activation. Employing single cell transcriptomics in combination with SRM will help us to understand the molecular function and promote/improve the designs of immune-modulating therapeutics and vaccines.

8:50 *Dr. Sahand Jamal Rahi, The Rockefeller University, New York*

Using dynamics to elucidate biological systems

We used mathematical modeling and fluorescence microscopy to explore three questions: 1) How many oscillators control the cell cycle? During cellular replication, a number of processes have to be coordinated in time, e.g., assembly of a new cell wall, replication of DNA, mitosis, and cytokinesis. Control of these processes involves expression, phosphorylation, dephosphorylation, and degradation of proteins. How many oscillators, which can potentially uncouple, are responsible for this control has been unclear, in particular, whether there is an independent oscillator for cell-cycle periodic transcription. Studying budding yeast cells, we found that the transcription of most cell cycle genes was dictated by the CDK-APC/C cell cycle oscillator, with a few interesting exceptions. Pursuing one of these genes, which oscillates when the CDK-APC/C oscillator is blocked in different states, we found a counter-intuitive interaction between an inhibitor (SIC1) and its targets (mitotic cyclins). 2) Can circuit motifs be deduced from dynamic perturbations? Adaptation, a ubiquitous feature of biological systems, can be displayed by two different circuit topologies, negative feedback loops (NFLs) and incoherent feed forward loops (IFFLs). Based on simple models and a comprehensive computational analysis, we discovered generic, dynamic signatures for NFLs. We observed these NFL signatures in various yeast cell cycle mutants by periodically inducing the Start cyclin CLN2. In the C. elegans olfactory sensory neuron AWA, we also detected the NFL signatures by periodically stimulating worms with odor and found independent evidence for a Calcium-NFL using pharmacological manipulations. 3) Do checkpoints override in predictable patterns? A mathematically optimal checkpoint strategy, which we derived, predicts how cell cycle checkpoints should fail depending on the number of errors a cell encounters. We have begun preliminary experiments to explore checkpoint failure and the model's parameters and predictions in yeast.

9:40 Coffee Break

10:00 *Prof. Dr. Ana-Sunčana Smith, Friedrich-Alexander-Universität Erlangen-Nürnberg*

Challenges and prospects in physics at the interface with biology: The case of cell adhesion

The existence of living matter is based on the defiance of equilibrium while maintaining order at the expense of energy consumption. This makes the cell a prime example of an active system that plays a pivotal role in the development of non-equilibrium physics. Due to their softness, cells exhibits strong fluctuations that are typically comprised from a thermal and an active component. While it is becoming conceptually clear that these fluctuations are coupled to the driving of biological processes, their physiological role remains widely unknown. One reason for this lack of clarity is that both the measurement of fluctuations, and the determination of their relation to a particular biological process, remains a significant challenge. It is precisely this challenge that is tackled in my group across several different systems. Using the example of cell adhesion, I will show how recent experimental advances in measuring cell-surface undulations can be combined with experiments in reconstituted systems and multiscale modelling to demonstrate that active fluctuations of cells can be harnessed for the control of the formation of adhesion clusters and mechanosensing. Furthermore, I will show the effects of mechanosensing on the development of model tissues. In doing so I hope I will demonstrate the competitiveness of my research program and its complementarity to the activities of the Physics department at UZH.

10:50 *Dr. Kurt Michael Schmoller, Stanford University*

The biosynthetic basis of budding yeast cell size control

Cell size is an important physiological trait that sets the scale of all biosynthetic processes. Although physiological studies have revealed that cells actively regulate their size, the molecular mechanisms underlying this regulation have remained unclear. Using quantitative single cell microscopy, we identified the molecular mechanism coupling growth and division in budding yeast. As cells grow, they dilute a cell cycle inhibitor while keeping the upstream activator at a constant concentration, which results in a continuously increasing probability for cell cycle entry. Size control itself is ensured by a differential dependence of activator and inhibitor synthesis rate on cell size. We anticipate that differential size dependence of biosynthesis is a widespread mechanism that allows cells to adjust protein homeostasis according to cell size and encode size dependence into the activity of any regulatory network.