

BP 18: Regulation and Signaling

Time: Thursday 9:30–13:15

Location: HÜL 186

Invited Talk BP 18.1 Thu 9:30 HÜL 186
Systems biology of yeast cell signaling and response to stress — ●EDDA KLIPP — Humboldt-Universität zu Berlin, Dept. of Biology, Theoretical Biophysics

Life is change. In order to study and understand life, it is necessary, but not sufficient to study genes, proteins or metabolites, and networks thereof in static conditions. Instead, we must handle the dynamic action. Stress and external perturbations are means to study the wiring of biochemical networks or signal transduction pathways and to understand the underlying regulatory principles.

Over the last years, we have studied various signal transduction and regulatory pathways in a model organism, the yeast *Saccharomyces cerevisiae*, and investigated the response of cells to external perturbations on various levels. To this end, we have established mathematical models, reflecting physical properties such as reaction kinetics, thermodynamic constraints as well as fluxes and forces. They are mainly in form of ordinary differential equation systems. Their structure and parameters are based on publicly available information and new dynamic data measured by our experimental collaborators. Here, I will focus on results with respect to interaction of different signaling and regulatory pathways. Specifically, new aspects in cell cycle regulation and the interaction of stress-activated signaling pathways with cell cycle progression will be discussed. The results indicate that yeast cells have developed different mechanisms for coping with external stress during different periods of their life time.

Invited Talk BP 18.2 Thu 10:00 HÜL 186
Towards an understanding of membrane and protein traffic in living cells — ●MATTHIAS WEISS — Cellular Biophysics Group, German Cancer Research Center, Heidelberg, Germany

Sorting of transmembrane proteins is a central task of the secretory pathway in eucaryotic cells. Here, the multitude of transmembrane proteins have to utilize self-organization processes on the molecular scale to decide whether they participate in transport along the secretory pathway or rather reside in their current compartment (e.g. the endoplasmic reticulum, ER, or the Golgi apparatus, GA). Using advanced light microscopy techniques and coarse-grained membrane simulations, we have addressed two of the key issues in membrane and protein trafficking. First, we elucidated the interaction between unfolded proteins and chaperones in the ER. As a result, we found that unfolded proteins show a strongly obstructed diffusion that can be altered to the diffusion behavior of folded proteins, e.g. by blocking the interaction with chaperones. Accompanying simulations indicate that this behavior reflects the obstructed diffusion of a cluster of chaperones and unfolded proteins due to (almost immobile) translocon pores in the ER. Second, we asked for generic mechanisms that support the sorting of folded transmembrane proteins into emerging vesicles at the ER and/or the GA. We showed by means of coarse-grained membrane simulations that hydrophobic mismatching can drive cluster formation of transmembrane proteins and even the demixing of a heterogeneous population of proteins. Based on this result, we propose a generic way to make and fill vesicular carriers with a well-defined cargo of proteins.

BP 18.3 Thu 10:30 HÜL 186
Perfect robust network design of the KaiABC circadian clock — ●CHRISTIAN BRETTSCHEIDER and MARKUS KOLLMANN — Institut für theoretische Biologie, Humboldt Universität zu Berlin

The simple circadian oscillator found in cyanobacteria can be reconstituted *in vitro* using three proteins - KaiA, KaiB, KaiC. It has been shown that four forms of KaiC differing in their phosphorylation state appear in an ordered pattern. Importantly, it has been revealed that phase, frequency and amplitude of the oscillations do not change under concerted severalfold over- and underexpression of its components. Consequently, the circadian clock is invariant with respect to concerted fluctuations in total protein concentrations of all Kai proteins. This observation is a strong constraint for modeling the circadian clock.

We will present the first quantitative model that includes the ordered pattern of the four KaiC states as well as the measured invariance of concerted variations. The model is systematically developed from intrinsic KaiC autokinase and autophosphatase rates that are modulated by KaiA and KaiB. A formal description shows that the invariance of concerted variations can be achieved by inactivation. In

this case, inactivation of KaiA leads to a negative feedback oscillator, that in turn causes synchronization between different KaiC as well as high amplitudes.

BP 18.4 Thu 10:45 HÜL 186
Physical constraints on cooperative transcription factor-DNA interaction — ●NICO GEISEL¹ and ULRICH GERLAND² — ¹Departament de Física Fonamental, Universitat de Barcelona — ²Arnold Sommerfeld Center for Theoretical Physics, LMU München

DNA-binding proteins often interact not only with the genomic DNA, but also with each other. In particular for the case of transcription factors (TFs), cooperative binding is fundamental to the nonlinear and combinatorial control of gene expression. Here, we focus on the simplest case of two TFs binding specifically to two neighboring functional sites, in the background of the quasi-random genomic DNA sequence. Within a coarse-grained theoretical model, we characterize both the equilibrium occupancy of the target sites and the non-equilibrium cooperative search kinetics of the TFs. Based on our model and analysis, we identify physical constraints on the optimal choice of protein-protein and protein-DNA interaction parameters in the context of bacterial gene regulation.

BP 18.5 Thu 11:00 HÜL 186
Influence of chemical modifications on siRNA strand separation and RISC target interaction studied by fluorescence cross-correlation spectroscopy *in vivo* — ●WOLFGANG STAROSKE, THOMAS OHRT, and PETRA SCHWILLE — Biophysics Group, BIOTEC, TU Dresden, Germany

Short double stranded RNA molecules have emerged as key regulators of gene expression in various organisms, both in the context of controlling developmental programs and as a defence mechanism to protect the genome against viruses and transposons. Short interfering RNAs use Argonaute-containing complexes called RNA-Induced Silencing Complex (RISC) to identify cognate RNA transcripts, whose expression is to be silenced. By combining laser scanning microscopy, fluorescence correlation and cross-correlation spectroscopy (FCS/FCCS) and biochemical methods, we have exploited the interaction of short interfering RNAs with RISC and a target RNA *in vivo*. We used a stable EGFP-Ago2 expressing 293 cell line, with endogenous expression levels suitable for FCS/FCCS measurements and designed a fluorescently labelled RNA, mimicking a target mRNA. By investigating the EGFP-Ago2 cell line and delivered fluorescently labelled siRNAs or targetRNA *in vivo*, we were able to gain new insights into siRNA strand separation, RISC loading and RISC target interactions. Our analysis of various chemical modified and fluorescently labelled siRNAs showed a correlation between chemical modification, passenger strand separation and gene silencing.

15 min. break

BP 18.6 Thu 11:30 HÜL 186
Transfection on the Single Cell Level: Interplay of Stochastic Delivery and Deterministic Expression — ●JAN-TIMM KUHR^{1,2,3}, GERLINDE SCHWAKE³, SIMON YOUSSEF^{1,3}, JOACHIM O. RÄDLER^{1,3}, and ERWIN FREY^{1,2,3} — ¹Center for NanoScience (CeNS) — ²Arnold Sommerfeld Center for Theoretical Physics — ³Fakultät für Physik, Ludwig-Maximilians-Universität, Munich, Germany

Non-viral delivery of exogenous genes to cells, known as transfection, is a key technology in gene therapy. To analyze transfection on the single cell level we used complexes of cationic lipids/polymers and fluorophore-encoding plasmids. Statistical analysis of abundant expression curves permits conclusions on key properties of complex delivery.

Expression onset time distributions depict strong cell phase dependence of successful transfection.

Distributions in maximal expression are analyzed within a theoretical model, which describes plasmid delivery as a multi-step stochastic process followed by deterministic gene expression. The model suggests that noise in transfection is primarily caused by small number fluctuations intrinsic to gene delivery. We infer the steady state ratio of proteins per plasmid, the number of activated plasmids per complex, and the average number of delivered complexes from single cell data.

Simultaneous transfection with plasmids coding for distinct proteins yields consistent percentages of non-fluorescent, mono- and, dichromatic cells, substantiating our semi-stochastic model of transfection and the resulting distribution of active plasmids per cell.

BP 18.7 Thu 11:45 HÜL 186

Dynamics of receptor-mediated signal transduction in living cells analyzed by correlation spectroscopy — ●STEFFEN STEINERT¹, FELIX NEUGART¹, ANDREA ZAPPE¹, DEBORAH BUK², LUTZ GRAEVE², PETER SCHEURICH³, and JÖRG WRACHTRUP¹ — ¹3. Physikalisches Institut, Universität Stuttgart — ²Biologische Chemie und Ernährungswissenschaften, Universität Hohenheim — ³Institut für Zellbiologie und Immunologie

Malfunctions of signaling cascades can cause serious diseases such as Alzheimer or Multiple Sclerosis. Thus, it is of high relevance to have an elementary understanding of functionality, stoichiometry and dynamics of signaling processes. However, membrane dynamics of many important hormone and cytokine receptors are still poorly understood. A drawback of biochemical in-vitro techniques is their potential introduction of artifacts due to the analysis of dead cells and the usage of several reagents. Instead, we employ highly sensitive optical methods which are capable of detecting proteins even at the single molecule level in living cells. Among the applied optical techniques are pulsed Total-Internal-Reflection-Fluorescence-microscopy (TIRF), Fluorescence-Correlation-Spectroscopy (FCS) and Fluorescence-Cross-Correlation-Spectroscopy (FCCS). By these optical techniques we can determine spatial and temporal parameters of signaling components in-vivo at physiological concentrations and temperatures. As model systems we are looking particularly into the receptors of Ciliary-Neurotrophic-Factor(CNTF) and Tumor-Necrosis-Factor(TNF).

BP 18.8 Thu 12:00 HÜL 186

Cell stimulation with optically manipulated microspheres — ●HOLGER KRESS¹, JIN-GYU PARK¹, CECILE MEJEAN¹, JASON FORSTER¹, JASON PARK¹, SPENCER WALSE^{1,2}, DIANQING WU¹, ORION WEINER³, TAREK FAHMY¹, and ERIC DUFRESNE¹ — ¹Yale University, New Haven, USA — ²US Department of Agriculture, Parlier, USA — ³UC San Francisco, USA

Many cells can sense spatial and temporal heterogeneities in concentrations of soluble molecules. The cellular signal transduction which forms the basis of this ability consists of signaling cascades and loops whose length and time scales are largely unknown. The systematic investigation of these networks requires control over the chemical microenvironment of cells. We present a novel technique to create molecular concentration patterns that are chemically, spatially and temporally flexible. Our approach uses optically manipulated colloidal particles which act as microspheres of soluble molecules. This technique for flexible cell stimulation is combined with quantitative live cell microscopy measurements of cellular responses. We demonstrate the method by inducing chemotaxis in neutrophils. We quantify the intracellular calcium release, actin distribution, shape and motility of single cells. The possibility for quantitative stimulus-response measurements on single cells makes this method applicable to a wide range of cell biological studies.

BP 18.9 Thu 12:15 HÜL 186

Non-optimal microbial response to antibiotics underlies drug interactions — ●TOBIAS BOLLENBACH and ROY KISHONY — Harvard Medical School, Boston, MA, USA

Bacterial cells respond to antibiotic stress by regulating gene expression. Of key importance for survival and growth is the regulation of ribosomal genes, which control the overall cellular translation rate. While ribosome production is known to be tuned to different nutrient conditions to maximize growth, much less is known about the optimality of ribosome production under antibiotic stress. Inhibition of translation by drugs can relieve the inhibitory effect of antibiotics that target DNA synthesis, suggesting a greater-than-optimal expression of ribosomal genes when under DNA stress. Here we test this hypothesis by measuring and manipulating gene expression in *Escherichia coli* under various antibiotic stresses. We find that cells down-regulate ribosomal gene expression in response to antibiotics that inhibit DNA replication. The hallmark of non-optimality is the possibility for im-

provement: using strains with genetically manipulated ribosomal gene expression, we show that decreased ribosomal expression can increase survival and growth under DNA stress. Further, we find that genetically optimizing ribosomal expression removes the suppression between DNA and protein synthesis inhibitors, demonstrating that these drug interactions result from non-optimal gene regulation. We present a mathematical model which shows how optimal growth rate-dependent regulation of ribosome synthesis can lead to (1) non-optimal regulation in response to antibiotics and (2) suppressive drug interactions.

BP 18.10 Thu 12:30 HÜL 186

Quorum signal integration in the *B. subtilis* sporulation phosphorelay — ●ILKA BISCHOF¹, JOSH HUG², AIWEN LIU², DENISE WOLF¹, and ADAM ARKIN^{1,2} — ¹Lawrence Berkeley Lab, Berkeley, USA — ²UC Berkeley, Berkeley, USA

The phosphorelay is a central signal transduction structure in *B. subtilis* that integrates numerous cues including starvation and cell density signals in order to determine whether to commit to spore formation. Based on a theoretical model we demonstrate that the phosphorelay can act as a computational machine performing a sensitive division operation of inductive kinase encoded signals by instructive quorum modulated phosphatase signals, indicative of cells computing a "food per cell" estimate. In addition, we show experimentally that at least one quorum operon is heterogeneously induced in sporulating microcolonies. Cells delaying sporulation sustain quorum signal expression during periods of active growth, while cells committing to sporulation do not. Together with the model these findings suggest that the phosphorelay normalizes environmental signals by the size of the subpopulation actively competing for nutrients.

BP 18.11 Thu 12:45 HÜL 186

Networks for cell division and their neutral mutants — ●GUNNAR BOLDHAUS and KONSTANTIN KLEMM — Bioinformatics Group, Department of Computer Science, University of Leipzig, Härtelstraße 16-18, D-04107 Leipzig, Germany

The functioning of a living cell is largely determined by the structure of its regulatory network, comprising complex, typically not pairwise interactions between regulatory genes. An important factor for the stability and evolvability of such regulatory systems is neutrality – typically a large number of alternative structures exhibit the same dynamical behaviour. Here we study the regulatory networks for the cell cycles of the yeast species *S. cerevisiae* and *S. pombe*. A coarse-grained Boolean approach allows us to abstract from biochemical details such as precise binding constants and degradation rates.

We exhaustively enumerate all networks performing the same function as the yeast wildtype. For both species, the wildtype is close to optimal with respect to sparse wiring; almost all neutral mutants have more regulatory connections than the wildtype. Furthermore we analyse the reachability of the alternative networks from the wild type. This neutral space of regulatory networks is fragmented under point mutations which establish or delete single interactions.

BP 18.12 Thu 13:00 HÜL 186

Drug absorption in a three-compartment model — ●NIKO KOMIN and RAÚL TORAL — IFISC (UIB-CSIC), Palma de Mallorca, Spain

For the understanding of pharmacological phenomena, a variety of compartment models were and are developed. The concept of interconnected pools, into which the drug is administered, searches solutions for the concentration evolution over time in the different compartments. The connections can be linear or non-linear, the system can be open or closed, concerning its interchange with the environment.

An analytic solution for any of the models would be of great value for understanding experimental data and refining the underlying assumptions. Here we want to present a mathematical way of transforming the system into a different picture and propose an adequate approximation to it. As this is a general approach we will explicitly do that on a closed three-compartment model with a Michaelis-Menten non-linearity, as a representation of a P-gp limited antibiotic absorption [1], and show how it can be extended to other models.

References:

1. Gonzalez-Alvarez et al (2005) Xenobiotica.