BP 9: Regulation and Signaling

Time: Tuesday 9:30–13:00 Location: H43

Invited Talk BP 9.1 Tue 9:30 H43

Single molecule recognition in regulatory systems — •Robert Ros — Experimental Biophysics, Physics Department, Bielefeld University, 33615 Bielefeld, Germany

Molecular recognition between proteins and nucleic acids is fundamental for many aspects of cellular regulation. For instance, a central issue for the regulation of gene expression is the specific recognition of DNA target sequences by transcription regulators. Furthermore, the protein expression of a cell can be regulated on the post-transcriptional level by the interaction of proteins with RNA. We are using atomic force microscopy (AFM) based force spectroscopy to investigate the interaction of proteins with DNA [1-4] and RNA target sequences at the single molecule level. For analyzing our force spectroscopy data, we apply and improve the concept of chemical bond heterogeneity [5], giving insights into the molecular binding mechanism and resulting in quantitative characterization of the interactions in terms of rate constants and dissociation lengths.

[1] F.W. Bartels et al.; J. Struct. Biol. 143: 145 (2003). [2] B. Baumgarth et al.; Microbiology 151: 259 (2005). [3] R. Eckel et al.; Ang. Chem. Int. Ed.44: 3921 (2005). [4] F.W. Bartels et al.; Biophys. J. (accepted). [5] Raible et al. ; Biophys.J. 90: 3851 (2006).

Invited Talk BP 9.2 Tue 10:00 H43

Transcription by RNA Polymerase II — •Stephan Grill[1,2,3], Eric Galburt[3,1], Martin Depken[1], and Carlos Bustamante[3] — [1]Max-Planck-Institut für Physik komplexer Systeme, Dresden — [2]Max-Planck-Institut für molekulare Zellbiologie und Genetik, Dresden — [3]University of California, Berkeley

RNA polymerase II (RNAP II) is responsible for transcribing all mRNAs in eukaryotic cells in a highly regulated process that serves as a central control point for cellular function. We have investigated the transcription dynamics of single RNAP II molecules against force in the presence and absence of TFIIS, a transcription elongation factor that enables the enzyme to remove copy errors. Using a single-molecule dual-trap optical-tweezers assay, we found that the response of RNAP II to force is entirely determined by enzyme backtracking. We show that backtrack pause durations follow a $t^{-3/2}$ power law, implying that during backtracking RNAP II performs a random walk in discrete base-pair steps and suggesting that backtracks may account for most of RNAP II pauses. We discuss the implications of our results in light of an optimal balance between speed and accuracy in decoding the genetic information during transcription.

15 min. break.

BP 9.3 Tue 10:45 H43

Basal promoter activity of comK sets a switching-window into the K-state of Bacillus subtilis — •Madeleine Leisner[1,2], Joachim Rädler[1], and Berenike Maier[2] — [1]LMU, Department für Physik, LS Rädler, Geschwister-Scholl-Platz 1, Munic, Germany, — [2]WWU, Institut für allgemeine Zoologie und Genetik, Am Schlossplatz 5, Münster, Germany

Bacillus subtilis cell population divides into a competent fraction and a non-competent fraction in the stationary phase. The transition from the non-competent state (with basal ComK concentration) to the K-state (with high ComK concentration) behaves like a bistable switch. To determine factors that set the fraction of cells that switch into the K-state (K-fraction), we characterized the basal comK expression in individual non-competent cells and found a broad Gaussian distribution whose center shifted towards higher values before entry into stationary phase. Basal promoter activity increased exponentially, reached a maximum and decreased towards zero in the stationary phase. The intrinsic switching rate increased and decreased with a time lag. When switching was induced prematurely by down-regulation of ComK proteolysis, the K-fraction increased strongly. Our data supports a model in which the basal ComK concentration increases during the exponential phase and the fraction at the high end triggers the autocatalytic feedback for ComK transcription. Shut-down of basal promoter activity sets a 'time-window' for switching and is thus involved in determining the K-fraction in the bimodal population.

BP 9.4 Tue 11:00 H43

Yeast cell cycle: Stable network dynamics despite molecular fluctuations — •Stefan Braunewell and Stefan Bornholdt — Institute for Theoretical Physics, University of Bremen, Otto-Hahn-Allee, 28359 Bremen

Regulatory systems in living cells consist of many components, which interact through intricate cascades of molecular processes. In spite of the stochastic nature of these processes, a robust functioning of the regulatory machinery is required for the survival of the cell. On the basis of the model organism S. cerevisiae, we investigate the stability of the network dynamics under such noisy conditions [1]. We extend a recently proposed synchronous Boolean model of the yeast cell-cycle control network [2] to continuous time and allow for stochastic noise on the signal transmission times. Further we incorporate a low-pass filter to account for typical characteristics of transcriptional regulation. As a result, one finds that the cell-cycle network shows a remarkable stability against timing fluctuations and exhibits specific features that aid this stability.

[1] S. Braunewell and S. Bornholdt, Superstablity of the yeast cell cycle dynamics: Ensuring causality in the presence of biochemical stochasticity, J. Theor. Biol. (2006), doi:10.1016/j.jtbi.2006.11.012

[2] F. Li et al., The yeast cell-cycle network is robustly designed. Proc. Natl. Acad. Sci. USA (2004), 101(14):4781-4786

BP 9.5 Tue 11:15 H43

How general is the Boolean network approach for predictive models? A case study of the yeast cell-cycle. — •Maria Davidich and Stefan Bornholdt — Institute for Theoretical Physics, Bremen University, Bremen, Germany

Boolean networks once were a mere toy model analogy for how regulatory processes in living cells could in principle work [1]. However, today there are examples of Boolean networks predicting regulatory processes in living organisms as the cell-cycle control in yeast [2] or some developmental modules in Drosophila [3]. We here pose the question whether Boolean networks have the potential to provide a new general method for predicting biological regulatory networks and discuss this question by comparing alternative Boolean models for the yeast cell-cycle.

[1] Kauffman, S.A The Origins of Order: Self-Organization and Selection in Evolution (Oxford, UK: Oxford University Press), (2003).

[2] Li F, Long T, Lu Y, Quyang Q, Tang C. The yeast Cell-Cycle Network Is Robustly Designed. PNAS, April 6, 2004, vol. 101, no. 14, 4781-4786.

[3] Albert R, Othmer H.G. The topology of the regulatory interactions predicts the expression pattern of the Drosophila segment polarity genes. Journal of Theoretical Biology 223, 1-18 (2003).

BP 9.6 Tue 11:30 H43

Ca^{2+} signaling: order from disorder — •Alexander Skupin and Martin Falcke — Hahn-Meitner-Institut, Glienicker Str. 100, 14109 Berlin

In the last years, the understanding of the influence and importance of noise in biological systems has substantially increased. Here we show how microscopic fluctuations, i.e. the stochastic manner of ion channels, effect the global behavior of cells. Therfore we present biological experiments which have been done and analyzed in a physical way to characterize the underlying stochastic process and to show the importance of noise. We have measured oscillations of the cytosolic Ca^{2+} concentration in different cell types under different conditions. These oscillations are caused by the stochastic opening of ion channels releasing Ca^{2+} from internal stores into the cytosol. This liberated Ca^{2+} can activate adjoining channels resulting in a global Ca^{2+} wave within the cell. We analyzed the periods of these oscillations and the influence of Ca^{2+} buffer to specify the stochastic mechanism. It turns out that cells use array enhanced coherence resonance to create the wide spectrum of observed Ca^{2+} oscillations. Thus we demonstrate a first exampale of the constructive role of noise in cell signalling.

BP 9.7 Tue 11:45 H43

Hybrid models and simulations of intracellular calcium dynamics — •Sten Rüdiger — Hahn-Meitner-Institut, Glienicker Str. 100, 14109 Berlin

Intracellular calcium release is a prime example for the role of stochastic effects in cellular systems. Recent models consist of deterministic reaction-diffusion equations coupled to stochastic transitions of calcium channels. The resulting dynamics is of multiple time and spatial scales, which complicates far-reaching computer simulations. In this contribution we introduce a novel hybrid scheme that is especially tailored to accurately trace events with essential stochastic variations, while deterministic concentration variables are efficiently and accurately traced at the same time. We use finite elements to efficiently resolve the extreme spatial gradients of concentration variables close to a channel. We describe the algorithmic approach and we demonstrate its efficiency compared to conventional methods. Our single channel model matches experimental data by Mak et al. (PNAS 95, 15821, 1998) and results in intriguing dynamics if calcium is used as charge carrier. Random openings of the channel accumulate in bursts of calcium blips that may be central for the understanding of cellular calcium dynamics. We further discuss calcium release from clusters of channels and and the effects of calcium-binding proteins on the dynamics.

BP 9.8 Tue 12:00 H43

The Mechanism of Gene Repression Revealed by spFRET Investigations of TBP-NC2 Complexes — •Peter Schluesche[1], Gertraud Stelzer[2], Elisa Piaia[2], Christoph Braeuchle[1], Michael Meisterernst[2], and Don C. Lamb[1] — [1]Department for Physical Chemistry, Ludwig-Maximilians-University, Butenandtstr. 11, 81377 München — [2]GSF-National Research Center for Environment and Health, Department of Gene Expression, Marchioninistr. 25, 81377 München

The initiation of DNA transcription starts with the binding of the TATA-Box Binding Protein (TBP) to the gene promoter site on the DNA. The transcription can be inhibited by the regulatory protein Negative Cofactor 2 (NC2) which forms a complex with the DNA bound TBP and represses gene expression. Recent results of biochemical experiments suggest that the TBP becomes mobile along the DNA upon the binding of NC2. Thus, transcription inhibition by NC2 could be explained by the dislocation of TBP from the promoter site rather than by steric hindrances as currently thought. To test this hypothesis, we have performed fluorescence resonance energy transfer (FRET) experiments on single TBP-NC2 complexes bound to DNA using Total Internal Reflection Fluorescence Microscopy with dual color detection. TBP was labeled specifically with a FRET-donor molecule and a TATA sequence containing dsDNA was labeled with a FRET-acceptor. By observing fluctuations in the FRET efficiency of individual complexes upon NC2 binding, we confirmed the mobility of the TBP-NC2 complexes along the DNA.

BP 9.9 Tue 12:15 H43

Analysis of bacterial gene regulatory networks by time-lapse fluorescence microscopy — •Judith Leierseder[1], Georg Fritz[1], Kirsten Jung[2], and Joachim Rädler[1] — [1]Department für Physik, Ludwig-Maximilians-Universität München — [2]Department Biologie I, Ludwig-Maximilians-Universität München

The vision of artificial genetic circuits with well defined response functions to external signals requires a quantitative understanding and description of the function of gene regulatory networks. Gene expression is, however, not only determined by the mere network structure, but also influenced by stochastic effects that lead to significant cell to cell variations. The single cell studies that are thus required to resolve these variations are greatly facilitated by the use of the green fluorescent protein (GFP) as network output marker. Using semi-automated time-lapse fluorescence microscopy combined with quantitative image processing we measured expression kinetics for many single bacterial cells. For our model system, the pBAD/AraC module in Escherichia coli, we analyzed the response following different induction concentrations and compared the kinetics to a simple theoretical gene expression model of the network.

BP 9.10 Tue 12:30 H43

Detection of functional RNA in genomic sequences — •Bernd Burghardt and Alexander Hartmann — Institut für Theoretische Physik, Universität Göttingen

Some ten years ago it was believed that RNA mainly acts by transferring genetic information, i.e. essentially its primary structure is relevant. However, many RNAs provide functionality by its secondary and tertiary structure. Such non-coding RNA (ncRNA) is coded in genomic DNA sequences and includes well known types, e.g. tRNA and rRNA as well as recently discovered ones, e.g. miRNA. An important issue is, how to detect regions in the DNA which code for such functional RNAs, i.e. to distinguish it from gene-coding and other parts.

We present here a efficient numerical method to detect such functional RNAs in long RNA (or DNA) sequences. The method is based on the ground-state calculations of RNA secondary structures for all subsequences up to a certain length. By analysing the average ground-state energy one gets strong evidence where such such functional RNA fragments are embedded.

BP 9.11 Tue 12:45 H43

Coarse-Grained Lattice Model for Molecular Recognition — •Hans Behringer, Andreas Degenhard, and Friederike Schmid — Fakultät für Physik, Universität Bielefeld, D-33615 Bielefeld

Equilibrium aspects of molecular recognition are investigated using coarse-grained models for the recognition process of two rigid biomolecules. To this end, a two-stage approach is adopted. First, the structure of the target molecule is fixed and learned by a probe molecule resulting in an ensemble of probe sequences. In a second step the recognition ability of the designed probe ensemble with respect to the chosen target sequence is tested by comparing the free energy of association with the previously fixed target structure and a different competing structure. Particular attention is paid to the influence of cooperative effects accompanying the association of the target biomolecule and the probe molecules. Cooperativity is found to enhance selectivity. In addition it is discussed how correlated hydrophobicity distributions affect the recognition ability.

Behringer, H., A. Degenhard, F. Schmid 2006, Coarse-Grained Lattice Model for Molecular Recognition, *Phys. Rev. Lett.* **97**, 128101.