

Talks

Cell movements and cytoskeletal self-organization

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Many movements of eukaryotic cells are driven by the cytoskeleton. This cellular structure plays essential roles in various vital processes like cell division, cell locomotion, or the internal organization of subcellular components.

It consists of filamentous proteins, notably microtubules and actin filaments, which interact with a host of proteins affecting filament lengths, acting as cross-linkers, or functioning as molecular motors. While a lot is now known about the biochemistry of individual cytoskeletal proteins, we still lack a thorough understanding of how these components are organized on a cellular scale. In this talk, theoretical descriptions of cytoskeletal dynamics will be discussed. The central finding is that collective effects emerging from interactions between a few key cytoskeletal components can lead to spatio-temporal structures similar to those observed in cells. Specifically, I will first discuss the cytoskeleton-dependent organization of pigments in cells that allow fish to change color. Secondly, I will discuss the role played by self-organized polymerization waves in the organization of the actin cytoskeleton in crawling neutrophils and amoeba.

Modelling the role of shape in orientational ordering and collective motion of myxobacteria

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Motivated by observations regarding collective motion of gliding myxobacteria on a substrate, we have derived various mathematical models to describe these phenomena. We present results a model describing emergence of orientational order in rod-shaped self-propelled particles and compare the results to experiments. The model takes into account explicitly the shape of the rods and assumes interaction due to volume exclusion. Simulations with up to 500 rods exhibit spontaneous formation of clusters indicated by a bimodal cluster size distribution already at low densities. Clustering is shown to require both the rod-shape and the active motion of the particles and is hence a genuine non-equilibrium phenomenon. Equations for the cluster size distribution are derived and reproduce the simulation findings well. In addition, they yield a simple criterion for the onset of clustering depending only on the aspect ratio of the particles and their packing fraction. Effects of boundaries and diffusive contribution to particle motion are also considered. Preliminary experimental results indicating a transition to clustering with increasing packing fraction are also presented and compared to the modelling predictions.

Functional Dynamics in *Physarum*: Towards an understanding of the cellular behavioral intelligence

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The plasmodium of the true slime mold *Physarum polycephalum* is a single multi-nucleate giant amoeboid cell. This naked aggregate of protoplasm senses various environmental stimuli, judges the information, and acts behaviorally by changing its shape as well as moving toward or away from the stimuli. By observing the dynamics of behavior, we notice that this primitive organism exhibits a rather high level of “intelligence”. In my talk, the ability of finding (forming) the shortest path, signaling pathways or networks for judging the multiple stimulation, spatiotemporal patterns of rhythmic contraction for controlling the cell behavior, etc will be discussed. Discussions will also be made on the further possibility of this organism as a model system for systems biology.

Dynamical quorum sensing

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Synchronous metabolic oscillations in yeast constitute one of the best characterized examples of emerging dynamics in biological populations. Well-stirred suspensions of *S. cerevisiae* only display collective oscillations if their density is sufficiently high. In this work, we study the mechanism at the basis of the macroscopic oscillations loss. We perform a quantitative comparison of a theoretical model with experimental measures on cellular suspensions at different dilution. We show that cell-to-cell interaction through an external medium can entrain a qualitative change of the intracellular dynamical state. Analogously to the mechanism of quorum sensing, the collective oscillations can thus encode an information on the population density.

Cells as stochastic media

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Text books on Mathematical Physiology describe cells as deterministic continuous reaction diffusion systems obeying mean field dynamics. However, there is growing evidence that cell physiology does not take place at the thermodynamic limit. While in some cases small numbers of molecule copies might be the reason for large fluctuations, even sub-systems with large molecule numbers may behave stochastically. They do so because of the existence of concentration gradients which are functionally relevant. Thus rather than deterministic dynamics we find concepts of coherence resonance applied in cell physiology. I will present our experimental studies with the example Calcium dynamics, will point out other cellular system exhibiting strong gradients and will present first ideas for a new theory of reaction diffusion processes inside cells which can account for large gradients and molecular fluctuations.

Circadian rhythms and molecular noise

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Most living organisms have developed the capability of generating autonomously sustained oscillations with a period close to 24h. These circadian rhythms are generated at the molecular level. The core mechanism responsible for these circadian rhythms relies on the negative regulation exerted by a protein on the expression of its own gene. In most organisms, however, the detailed mechanism of the clock involves interlocked positive and negative feedback loops. Minimal as well as detailed deterministic models based on these experimentally established genetic regulations account for circadian oscillations in constant environmental conditions (i.e. in continuous darkness), entrainment of these rhythms by light-dark cycles, and phase shifts induced by light pulses. When the numbers of mRNA and protein molecules are reduced, it is necessary to resort to stochastic simulations to determine the influence of molecular noise on circadian oscillations. It is indeed possible that the autoregulatory mechanism of genetic expression can not produce stable rhythms, because of fluctuations when the number of molecules involved in the clock mechanism is low. We have performed a comparison between deterministic and stochastic approaches for a core model based on negative autoregulation of a clock gene. We show that robust circadian oscillations can already be produced with maximum numbers of molecules in the order of tens and hundreds for mRNA and protein, respectively. Furthermore, the results indicate that the cooperativity characterizing the repression of transcription increases the robustness of the rhythm while entrainment by light-dark cycles stabilizes the phase of circadian oscillations. Stochastic simulation of more detailed models show that interlocked positive and negative feedback loops do not contribute much to overcome the effect of noise. On the contrary, the coupling between oscillators leads to a higher robustness of the oscillations.

Phase tracking and restoration of circadian rhythms by model-based optimal control

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Periodic cellular processes and especially circadian rhythms governed by the oscillating expression of a set of genes based on feedback regulation by their products have become an important issue in biology and medicine. The central circadian clock is an autonomous biochemical oscillator with a period close to 24 h. Research in chronobiology demonstrated that light stimuli can be used to delay or advance the phase of the oscillator, allowing it to influence the underlying physiological processes. Phase shifting and restoration of altered rhythms can generally be viewed as open-loop control problems that may be used for therapeutic purposes in diseases. A circadian oscillator model of the central clock mechanism is studied for the fruit fly *Drosophila* and show how model-based mixed-integer optimal control allows for the design of chronomodulated pulse-stimuli schemes achieving circadian rhythm restoration in mutants and optimal phase synchronisation between the clock and its environment.

Stability of reaction networks with applications to biochemical reactions

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The concept of stoichiometric network analysis (SNA) is based on examining a possibly complex (bio)chemical reaction by decomposing the corresponding network into a set of basic subnetworks representing elementary pathways. On assuming mass action kinetics, stability of a steady state corresponding to an open system where the studied reaction takes place can be assessed by examining stability of each basic subnetwork separately, pairwise, etc. This procedure provides a set of unstable subnetworks, i. e., subnetworks that possess unstable steady states for proper values of rate coefficients. By combining the unstable subnetworks with the remaining stable subnetworks the whole network can be adjusted to display various bifurcations that are available for the examined system. In this way, nonlinear dynamical phenomena such as multiple steady states and oscillatory dynamics can be predicted. This method can be to certain extent used for estimating stability of non-mass action kinetics frequently used in bioreactions. In addition, any oscillatory reaction can be classified according to the topological features of its network and a specific role of each reacting species in generating the oscillations is implied. These features are related to positive and negative feedbacks available in the network. Using this approach several categories are identified, corresponding to distinct topological features displayed by any network capable of oscillations. Simple examples of biochemical networks are discussed and their instabilities elucidated in terms of mechanistic features.

Analysis of cell cycle models using the shapes of local sensitivity functions

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Two cell cycle models, the Chen et al. budding yeast cell cycle model [1] and the generic cell cycle model of Csikász-Nagy et al. [2] were investigated. Time dependence of the local sensitivity coefficients was calculated for all variables and parameters of these models. The sensitivity functions of the Chen model are periodic, while the sensitivity functions of the generic cell cycle model could be transformed using the method of Zak et al. [3] to become periodic functions. For both models, most of the local sensitivity coefficient-time functions could also be obtained from another one by multiplying it with a constant, which means that these functions exhibit global similarity [4]. Local similarity of the sensitivity functions was also detected. The distance of the shapes of two scaled sensitivity functions was defined by the integrated squared difference of these functions. The distance matrices of function shapes were interpreted by a clustering method and the shapes could be sorted to few groups for each model variable.

Presence of the global similarity of sensitivity functions means that the change of some enzyme activities can be fully compensated by changing the activity of other enzymes. This feature can be related to the robustness of living organisms.

[1] K.C. Chen, A. Csikász-Nagy, B. Györfy, J. Val, B. Novák, J.J. Tyson, Kinetic analysis of a molecular model of the budding yeast cell cycle, *Molecular Biology of the Cell*, 11, 369-391 (2000)

[2] A. Csikász-Nagy, D. Battogtokh, K.C. Chen, B. Novák, J.J. Tyson, Analysis of a generic model of eukaryotic cell cycle regulation. *Biophysical Journal* 90, 4361-437(2006)

[3] D.E. Zak, J. Stelling, F.J. Doyle, III., Sensitivity analysis of oscillatory (bio)chemical systems. *Comp. Chem. Engng.* 29, 663-673(2005)

[4] A. Lovrics, I. Gy. Zsély, A. Csikász-Nagy, J. Zádor, T. Turányi, B. Novák, Analysis of a budding yeast cell cycle model using the shapes of local sensitivity functions, *Int.J.Chem.Kinetics*, 2008, in press

The autocatalytic reaction cycle of hydrogenase: evidences, models and possible physiological importance

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Hydrogenases are metalloenzymes that catalyze the reaction $H_2 = 2p^+ + 2e^-$. Despite the many features described in the hydrogenase reaction, the activity of this class of enzymes has not yet been thoroughly explained. The characteristics and the members of the enzyme cycle are still a matter of dispute. There is a consensus, however, that hydrogenase needs activation to attain full activity. We have strong experimental evidence regarding the autocatalytic behavior of hydrogenase catalysis. A moving front has been observed as a special pattern during the reaction of hydrogenase uptake in a thin-layer reaction chamber, which is an indisputable sign of the autocatalytic reaction step in the enzyme reaction. The front speed depends on the enzyme concentration as a square root function. There is also a threshold enzyme concentration value for the front speed below which the reaction does not start. We conclude that there is at least one autocatalytic reaction step in the hydrogenase reaction cycle. This is the first experimentally proven case when autocatalytic behavior has been observed in the pure form of an enzyme.

Several mathematical models of the hydrogenase reaction cycle were investigated by means of theoretical calculations and model simulations and compared with the experimental findings.

To find a physiological explanation for an autocatalytic reaction in the bacterial membrane, where hydrogenase resides, is a challenge. Hydrogenase has a very complicated structure which is accomplished with a sophisticated maturation process. Considering these properties, one can conclude that a hydrogenase molecule is a very valuable enzyme for the cell and the autocatalytic behavior can help in the survival of the enzyme molecule. Experimental indications and the possibility of such a mechanism are also discussed.

Modeling of globally coupled arrays of oscillators

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Arrays of coupled oscillators are widely spread in complex biological or chemical systems. We considered an array of oscillators, and studied the function that can be played by a time dependent global coupling. Investigations were done using an array of globally coupled FitzHugh-Nagumo oscillators [1]. The coupling can be tuned by changing the frequency and the amplitude of the coupling term. Changing these parameters, a sequence of complex dynamical patterns were obtained, when the dynamics of the array is qualitatively different with respect to the dynamics of an uncoupled oscillator. Thus, the time dependent global coupling provides a valuable tool in controlling the dynamics of the array of oscillators.

The dynamics of the array was explored using the following methods [2,3]:

- Analytical signal approach (Hilbert transform);
- animated phase portraits;

- stroboscopic maps;
- phase distributions.

One of the features of the system studied by us was the multiplicity of states, i.e., for given values of the control parameters, several different dynamics are possible, depending on the initial conditions.

1. S. Strogatz, *Nonlinear Dynamics and Chaos*, Addison Wesley, Reading, 1994.
2. H. Kantz, T. Schreiber, *Nonlinear Time Series Analysis*, Cambridge University Press, 1997.
3. A. Pikovsky, M. Rosenblum, J. Kurths, *Synchronization – a Universal Concept in Nonlinear Sciences*, Cambridge University Press, 2001.

Model-based design of structured reactive particles for controlled release

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The pharmacodynamic and pharmacokinetic effects of an active pharmaceutical ingredient (API) are closely related to the dynamics of the API in dissolution and release from its dosage form into the body. The most commonly used dosage forms – solid tablets – are composite materials with a complex hierarchical microstructure: a tablet is compressed from a blend of granules, lubricants, disintegrants and other ingredients. Granules, in turn, are typically composed of at least three ingredients: the active drug substance (API), an excipient, and a binder. It has been shown that the internal microstructure of a granule, which is determined during its manufacture, has a measurable effect on the release kinetics of the API from a tablet.

In recent years we have developed an integrated methodology for computer-aided design of granule and tablet microstructure [1-3], which allows *in silico* optimisation of alternative formulations, microstructures and related manufacturing processes that result into those microstructures. We have also developed novel granule formation processes, based on fluidised bed in-situ melt granulation, that allow precise direct control over the evolved structures and their dissolution characteristics [4-6].

In the present contribution we will highlight further developments in the area of controlled release of unstable or highly potent actives that cannot be present in the granule structure in its final chemical form, but have to be formed in situ from precursors. The above-mentioned approach will be used to produce granules that contain compartmentalised reactive particles. These granules will then be subjected to a dissolution test to study the release behaviour of the API that would be produced in-situ as a result of the interaction between the reactive precursor particles contained within the granule. Since granule properties profoundly influence the tablet dissolution characteristics, the idea of compartmentalised granules would offer a range of applications in the area where in-situ API are essential formulation or physiological requirements.

1. Štěpánek F. “Computer-aided product design: granule dissolution”, Chem. Eng. Res. Des. 82, 1458-1466 (2004)
2. Štěpánek F., Ansari M.A. “Computer simulation of granule microstructure formation”, Chem. Eng. Sci. 60, 4019-4029 (2005)
3. Štěpánek F., Loo A., Lim, T.S. “Multiscale modelling methodology for virtual prototyping of effervescent tablets”, J. Pharm. Sci. 95, 1614-1625 (2006)
4. Ansari M.A., Štěpánek F. “Design of granule structure: Computational methods and experimental realisation”, AIChE J. 52, 3762-3774 (2006)
5. Ansari M.A., Štěpánek F. “Formation of hollow core granules by fluid bed in-situ melt granulation: Modelling and experiments”, Int. J. Pharm. 321, 108-116 (2006)
6. Ansari M.A., Štěpánek F. “The evolution of microstructure in three-component granulation and its effect on dissolution”, Part. Sci. Technol. 26, 55-66 (2008)

Modeling Protein Dilution Experiments: stochastic effects

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Recent experiments have studied protein expression/regulation at the single cell level. Simply stated, cells are "filled" with a protein's repressor molecule that dilutes out due to cell growth and division. As a consequence, protein expression can be tracked as a function of time (i.e. as a function of the repressor concentration). Protein and repressor levels are characterized by fluorescence microscopy (CFP and YFP resp.) thus allowing the quantification of the relation between repressor levels and the protein production rate: the gene regulatory function. The latter is crucial for understanding and modelling gene regulatory networks. Herein we present a modeling approach towards this system. Thus, we propose a system of equations that describes, at the single cell level, the production rate of the protein as a function of the concentration of its repressor and the interplay of the protein operator sites. By means of a Kramers Moyal expansion we are able to formally derive an effective Langevin description that accounts for the protein dynamics inside a given cell. In addition, cell cycle effects are taken into account. Our results show protein regulation and production as experimentally measured. We also provide evidence that cooperativity between protein operator sites (repressor binding sites) is required for obtaining the observed gene regulatory function. Importantly, we show that the autocorrelation of the protein production rate presents a resonant effect as a function of the "amount of stochasticity" in the cell cycle: there is an optimal value of the stochastic component of the cell life time that synchronizes (coordinates) protein production within the cell population.

Calibrating two reaction-diffusion models describing the formation of the gradient of the protein Bicoid in the *Drosophila* embryo

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We present two reaction-diffusion models describing the establishment of the gradient of the protein Bicoid along the antero-posterior axis the *Drosophila* embryo. Our aim is to elucidate the following three questions: Does the Bicoid protein stay localised near ribosomes, as high definition images show? Why does the measured diffusion coefficient of protein Bicoid differ by one order of magnitude from the diffusion coefficient obtained with reaction-diffusion models? At which cleavage stage does the Bicoid protein reach a stable gradient?

In order to answer these questions, we calibrate the parameters of the two reaction-diffusion models with experimental data, using a Monte-Carlo optimization algorithm.

Predicting Recombinant Protein Expression in the Baculovirus/Insect Cells System: A Stochastic/Structured Modeling Approach

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Rotavirus-like particles (RLPs) are a vaccine candidate against rotavirus disease which currently has a higher mortality than AIDS/HIV in under-five children. These triple-layered particles are produced using the baculovirus/insect cells system. This extremely complex process has assembly efficiencies of the three constitutive viral proteins of RLPs (VP2, VP6 and VP7) normally around 11-15%. In order to optimize batch performance, it is essential to evaluate the effect of critical process parameters as the multiplicity of infection ($\text{MOI} = \#\text{virus} \cdot \text{cell}^{-1}$) on process kinetics. Therefore, a stochastic/structured mathematical model was implemented in MATLABTM to find optimal asynchronous infection regimes. Results indicate that maximum productivity for VP2 (used as a model for the remaining viral proteins) is achieved at $\text{MOI}=0.01 \text{ virus} \cdot \text{cell}^{-1}$ while the minimum is obtained at $\text{MOI}=10 \text{ virus} \cdot \text{cell}^{-1}$. Moreover, model simulations show that high MOIs have no advantage over low ones in protein expression due to maximum translation capacity of mRNA blocks coming from the polyhedrin promoter. One can conclude that optimal protein production is a compromise between high intracellular protein templates, obtained at high MOIs, and high infected cell densities, obtained at low MOIs. These findings demonstrate the potential of mathematical models in process design and will show its usefulness in RLPs process optimization. In a second study, several baculovirus titration methods were evaluated to find the one estimating viral titers with best accuracy as it strongly affects MOI variability and consequently batch consistency. The techniques that better combined titer accuracy, cost *per* titration, titration time and labor intensity were the TCID_{50} , MTT and flow cytometric assays. Nevertheless, it is compulsory to confirm titers accuracy by at least two different titrations methods.

Modelling cardiac dynamics: What can we learn from simple models?

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In cardiac muscle, a change in transmembrane cellular potential produces a response known as action potential, that propagates along tissue. Many cardiac malfunctions are associated to problems in propagation, sometimes inducing the formation of rotors. When unstable, they can give rise to ventricular fibrillation, in which synchronous excitation is lost among different parts of the ventricle, impeding contraction, and causing death in a few minutes. To understand this transition, it is necessary to construct models that help us bridge the gap from cellular electrophysiology to propagating properties in tissue, and through whole heart models, to clinical manifestations. This often involves creating very detailed electrophysiological models to study the effect of, for instance, a given mutation. However, the complexity of such models makes sometimes difficult to get insight into the exact arrhythmogenic mechanisms. For this, simplified descriptions can help, as has been the case, for instance, in the study of alternans rhythms.

In this talk, we will consider the effects of electro-mechanical coupling in cardiac tissue. We show that mechano-electric models which describe both the electric propagation and the mechanic contraction of the heart naturally lead to close systems of equations with global coupling among the variables. We exemplify this using the Nash-Panfilov model, which reduces to a Fitzgugh-Nagumo type equation with global coupling in the linearly elastic regime. The simplicity of the resulting model allows us to get a better understanding of the different mechano-electric behavior of cardiac tissue both from an analytical and numerical perspective.

Controlled wave-emission from endogenous sites terminates malignant cardiac wave dynamics

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Spatially extended excitable media like cardiac tissue exhibit defect (or phase singularity) mediated turbulence in terms of irregular wave fronts or turbulent spiral dynamics. In heart dynamics this spatio-temporal chaotic state corresponds to an electro-mechanical malfunction of the heart and may result in sudden cardiac death. Low frequency arrhythmias (up to 4 Hz) can be terminated gently and effectively by pacing from a single site only. It has been demonstrated, that the termination of high frequency - and often lethal - arrhythmias is possible, but requires a very large number of pacing sites. Pacing from many sites could not be achieved yet, because installing and connecting many leads may damage the contracting heart.

Here we show, that multisite pacing can be achieved non-invasively by applying a pulsed low-energy electric field to the cardiac tissue. We found in canine heart preparations that such a pulsed far-field may result in the emission of waves from tissue heterogeneities, which are anatomical objects of various sizes present within the cardiac muscle. How many of these objects may act as a wave source depends on the electric field strength. We demonstrate that this approach can be used to terminate turbulent spiral dynamics in cardiac tissue.

Generation and suppression of calcium-induced arrhythmias in a model of heart ventricular myocyte

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Several types of arrhythmias are induced by defective calcium handling by the cardiac cell. It has been suggested that increased calcium sensitivity and/or enhanced basal activity of the ryanodine receptor calcium release channel upon adrenergic stimulation may constitute the primary defect; the resulting increase in diastolic calcium levels in the cytosol then leads to activation of the sodium/calcium exchanger, thus initiating cell depolarization and generation of delayed afterdepolarizations and/or spontaneous action potentials. Here we have investigated the effect of changed basal activity and changed luminal regulation of the ryanodine receptor (RyR) on the propensity of the cardiac myocyte to arrhythmias. A model of rabbit ventricular myocyte action potential (AP), based on the Shannon-Bers AP model [1, 2], was developed and the influence of changed RyR channel properties on initiation of heart arrhythmias was investigated.

The simulation was run for 300 s at a predefined stimulation frequency to obtain steady-state values of variables characterizing ion currents, membrane potential, and calcium concentration in individual cell compartments. External stimulation was then excluded and spontaneous behavior of the cell was observed. Under basal conditions, no spontaneous APs were observed at a stimulation frequency of 1 – 2 Hz. Upon adrenergic stimulation, i.e., upon increase in the calcium content of the intracellular stores and increased calcium sensitivity and basal activity of the ryanodine receptor, spontaneous APs could be observed. The number and frequency of spontaneous APs increased with the preceding stimulation frequency. Increasing the basal activity of the RyR led to a further increase in the frequency of spontaneous APs. Spontaneous activity was highly dependent on the calcium dynamics of the diffusion-restricted junctional space near the RyRs and both the number and frequency of spontaneous APs decreased with increasing rate of Ca^{2+} diffusion from the junctional space.

In conclusion, we have demonstrated that changes in RyR gating (without changes in other properties of the myocyte) may lead to generation of spontaneous action potentials. Two strategies – lowering the

heart rate and speeding up calcium diffusion – may prove useful to prevent RyR-generated arrhythmias

[1] T. R. Shannon, et al, *Biophys. J.* 87 (2004) 3351-3371.

[2] T. R. Shannon, F. Wang, D. M. Bers, *Biophys. J.* 89 (2005) 4096-4110.