BioNET

Midterm Report

1. July 2004 – 31. March 2007

Foreword

This midterm report comprises the first 2.75 years of activity in the Centre BioNET - Danish Centre for Biophysics. We are extremely grateful to the VILLUM KANN RASMUSSEN Foundation for the generous grant behind the Centre.

This has given us the possibility to initiate numerous projects within biophysics which we could not have started otherwise. In particular, this grant has allowed us to start several common projects between the three node of the Centre thus strengthened the national dimension of the Centre. Furthermore, the BioNET grant has made it possible to apply for additional support at research councils, faculty of sciences at the universities, PhD Schools and other foundations and in particular at other centres of excellence. In that way we have 'boosted' the BioNET grant quite a lot to increase the number of projects. Not all projects within BioNET are included in this report that would have become too comprehensive.

Finally, I should like to thank our international advisory committee professor Joel Stavans, professor Lukas Tamm and professor Luis Serrano for participating in our yearly meetings and for all their advice and encouragement.

Copenhagen, April 2007

Mogens Høgh Jensen
Centre Director

Advisory Committee Report on

Danish Center for Biophysics – BioNET

The Danish Center for Experimental and Theoretical Biophysics held its annual meeting on October 30-31, 2006 at the University of Southern Denmark in Odense. About 50-60 members from the three centers in Alborg, Copenhagen, and Odense participated. Also present were the two undersigned members of the advisory board committee, Joel Stavans from the Weizman Institute in Israel and Lukas Tamm from the University of Virginia in Charlottesville, USA. After a brief introduction by the Center Director Mogens Jensen, Professors, Postdoctoral Research Associates, and Graduate Students gave 21 scientific talks of 15 or 30 min each. The topics ranged from the mathematical modeling of genetic circuits, over single molecule measurements of gene transcription, the physical modeling of the lateral structure of cell membranes, folding of membrane proteins and amyloid fibril formation that can be the cause of neurodegenerative diseases to the mechanism of signal propagation in neurons. The quality of these talks was very high and resulted in stimulating discussions and suggestions for future directions of research. It was obvious that there is a high degree of collaboration between the three Centers of BioNET. In numerous cases students are shared and perform experiments and/or theoretical approaches in other nodes of the network. This interactive mode of research not only benefits the research itself, but also gives the involved students frequent exposures to new techniques and ways of thinking. They are clearly well trained in interdisciplinary approaches to their projects and science in general. We consider this an enormously valuable asset of the program that teaches a future generation of biophysicists in how to perform collaborative interdisciplinary work in an increasingly complex world of science. The program brings together physicists, chemists and biologists. They learn each others languages and way of thinking. Biology needs increasingly more input from the more quantitative sciences as we move into the next decades and the next generation of physicists and chemists must be more proficient in understanding and tackling biological problems than ever before. The advisory committee was also impressed with the presentation skills of the students, including the youngest ones. The committee would encourage further efforts in this direction and a more thorough engagement of students in the scientific discussions would even further improve their participation skills in scientific discourse. It also become clear that the funding mechanism of BioNET provided major support for the more junior research groups involved, most notably those of Lene Oddershede and Thomas Heimburg. These younger researchers have already made significant impacts in their respective fields, and this was clearly facilitated by significant funding through this program. It can only be hoped that these activities can be continued through sustained future support of them and potentially other junior faculty that may be recruited in the future into this emerging field. Last but not least, support of an interdisciplinary graduate student with a civil engineering background and interests in secondary school education should be highly commended. She is investigating new ways of training high school teachers and interest high school students in the interdisciplinary and emerging subject of biophysics. What is true and was said above for graduate students about the need for the quantitative understanding of biology applies even more to the next generation of potential future scientists (and non-scientists!). The seed needs to be planted early and BioNET does an excellent job not only in its research mission, but also in its educational mission at all levels.

In conclusion: We, as the international advisory committee, are very satisfied with the way BioNET is running. We are really impressed by the breath and the quality of the projects which are undertaken within BioNET. We also note the very good collaborations between the three nodes of the center. We therefore strongly suggest to the VKR foundation that BioNET is also granted for the last 2.5 years of the original five year period.

Prof. Luis Serano, EMBL, Heidelberg, Germany, and University of Barcelona, Spain
Prof. Lukas Tamm, University of Virginia, Charlottesville, USA
Prof. Joel Stavans, The Weizmann Institute of Science, Israel

University of Copenhagen, Niels Bohr Institute, NBI

The NBI node of BioNET

Responsible scientist	Mogens Høgh Jensen
Principal scientists in period of report	Thomas Heimburg Lene Oddershede Kim Sneppen
Post-doctoral scientists	Sandeep Krishna (01.11.04-15.11.07) Nader Reihani (01.01.06-30.06.07) P. Sigmundsson (01.1006-01.10.07)
PhD students	Tabita W. Madsen, (01.02.05- 3 år) Anna Anderson (01.12.05 , 50% BioNET/CMOL) Mille Micheelsen (01.01.06, 50% BioNET/CMOL) LiselotteJ.Pedersen (1/3 BioNET, 1/3 KU & 1/3 PhD school) Matthisa Fidorra (50% BioNET/SDU) Mikkel Avlund Matthias Fidorra, (50 % SDU/NBI)

Activities of the Biocomplexity group (M.H. Jensen)

Overview of the activities:

In the biocomplexity group we use modelling techniques in order to describe the behaviour of living systems. We are in particular focussed on systems where we have access to fundamental experimental data. One of out main focus areas is to investigate how the proteins inside a cell might influence each other by means of the so-called genetic networks. Basically these networks tell us which proteins activate or repress each other. A protein might bind to the DNA string and promote the reading of a gene: this is called activation. Alternatively, a protein could bind to the DNA and stop the reading of a gene: this is called repression. We collect all the known data of a given system within the genetic networks of the participating proteins (for example of a NF-κB network see fig. 1). Sometimes the network is studied in terms of the static properties by outlining the specific structure of the network. For instance one can measure how hierarchical the networks acts. Most often, however, we are interested in the dynamical behaviour of the networks as a dynamical response is completely essential for biological functions of living systems. In order to describe such dynamics mathematical equations based on ordinary differential equations are formulated. We directly write up the specific protein-interactions mathematically and in this way model the response of a cell, mostly in terms of feed-back loops. We have been actively involved in the study of proteins related to cell death (p53), segmentation in embryos (Hes1) and inflammation (NF-κB). We have also studied the SOS

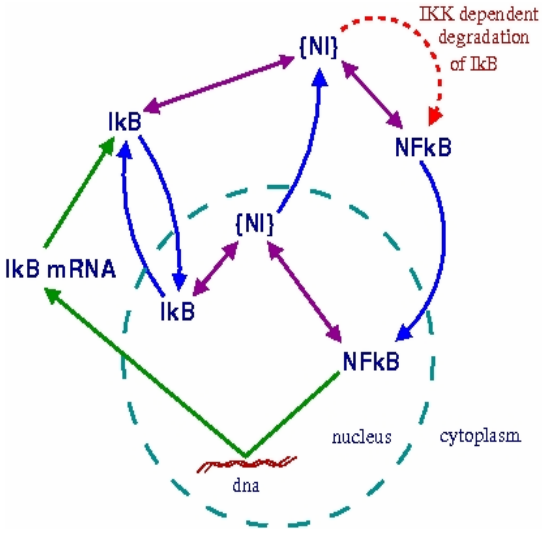


Fig. 1: The feed-back network for the transcription factor NF-κB which regulates many genes related to inflammatory deceases. This reduced network indicates how NF-κB promotes its inhibitor IkB. They subsequently bind to a complex which is a target of the external necrosis factor IKK. This degrades IkB allowing NF-κB to go back into the cell nucleus thus completing the feed-back loop.

response after DNA damage and the iron flow through bacteria cells. All these projects are performed in close collaborations with biologists and we begin to get a clear line of procedures how to attack dynamical biological data from a modelling point of view. Recently, one of our papers on modelling on histone mediated epigenetics has been published in the high profile journal Cell.

Oscillations and temporal signalling in cells

(G. Tiana, S. Krishna, S. Pigolotti, M. H. Jensen and K. Sneppen)
The development of new techniques to quantitatively measure gene expression in cells has shed light on a number of systems that display oscillations in protein concentration. We have studied the different mechanisms which can produce oscillations in gene expression or protein concentration, using a framework of simple mathematical models. We have focussed on three eukaryotic genetic regulatory networks which show "ultradian" oscillations, with time period of the order of hours, and involve, respectively, proteins important for development (Hes1), apoptosis (p53) and immune response (NF-kappaB). We have argued that underlying all three is a common design consisting of a negative feedback loop with time delay which is responsible for the oscillatory behaviour.

Oscillation patterns in negative feedback loops

(S. Pigolotti, S. Krishna, M. H. Jensen)
Organisms are equipped with regulatory systems that display a variety of dynamical behaviours ranging from simple stable steady states, to switching and multistability, to oscillations. Earlier work has shown that oscillations in protein concentrations or gene expression levels are related to the presence of at least one negative feedback loop in the regulatory network. Here we study the dynamics of a very general class of negative feedback loops. Our main result is that in these systems the sequence of maxima and minima of the concentrations is uniquely determined by the topology of the loop and the activating/repressing nature of the interaction between pairs of variables. This allows us to devise an algorithm to reconstruct the topology of oscillating negative feedback loops from their time series; this method applies even when some variables are missing from the data set, or if the time series shows transients, like damped oscillations. We illustrate the relevance and the limits of validity of our method with three examples: p53-Mdm2 oscillations, circadian gene expression in cyanobacteria, and cyclic binding of cofactors at theestrogen-sensitive pS2 promoter.

Spiky oscillations in NF-κB signalling

(S. Krishna, M. H. Jensen, K. Sneppen)
The NF-κB signalling system is involved in a variety of cellular processes including immune response, inflammation, and apoptosis. Recent experiments have found oscillations in the nuclear-cytoplasmic translocation of the NF-κB transcription factor. How the cell uses the oscillations to differentiate input conditions and send specific signals to downstream genes is an open problem. We shed light on this issue by examining the small core network driving the oscillations, which, we show, is designed to produce periodic spikes in nuclear NF-κB concentration. The oscillations can be used to regulate downstream genes in a variety of ways. In particular, we show that genes to whose operator sites NF-κB binds and dissociates fast can respond very sensitively to changes in the input signal, with effective Hill coefficients in excess of 20.

Genetic Regulation of Fluxes: Iron Homeostasis of Escherichia coli

(S. Semsey, A. M. C. Andersson, S. Krishna, M. H. Jensen, E. Massé, K. Sneppen)
Iron is an essential trace-element for most organisms. However, because high concentration of free intracellular iron is cytotoxic, cells have developed complex regulatory networks that keep free intracellular iron concentration at optimal range, allowing the incorporation of the metal into iron-using enzymes and minimizing damage to the cell. We built a mathematical model of the network that controls iron uptake and usage in the bacterium Escherichia coli to explore the dynamics of iron flow. We simulate the effect of sudden decrease or increase in the extra cellular iron level on intracellular iron distribution. Based on the results of simulations we discuss the possible roles of the small RNA RyhB and the Fe-S cluster assembly systems in the optimal redistribution of iron flows. We suggest that Fe-S cluster assembly is crucial to prevent the accumulation of toxic levels of free intracellular iron when the environment suddenly becomes iron rich.

Mutagenesis and the SOS response to DNA damage

(S. Krishna, S. Maslov, K. Sneppen)
Escherichia coli bacteria respond to DNA damage by a highly orchestrated series of events known as the SOS response, regulated by transcription factors, protein–protein binding, and active protein degradation. We

constructed a dynamical model of the UV-induced SOS response, incorporating mutagenesis by the error-prone polymerase, Pol V. In our model, mutagenesis depends on a combination of two key processes: damage counting by the replication forks and a long-term memory associated with the accumulation of UmuD'. Together, these provide a tight regulation of mutagenesis, resulting, we show, in a "digital" turn-on and turn-off of Pol V. Our model provides a compact view of the topology and design of the SOS network, pinpointing the specific functional role of each of the regulatory processes. In particular, we suggest that the recently observed second peak in the activity of promoters in the SOS regulon (Friedman et al., 2005, PLoS Biology 3(7): e238) is the result of positive feedback from Pol V to RecA filaments.

Phage-Bacteria Ecologies

(M. Rosvall, I.B. Dodd, S. Krishna, and K. Sneppen)

Bacteria and their bacteriophages are the most abundant, widespread, and diverse groups of biological entities on the planet. In an attempt to understand how the interactions between bacteria, virulent phages, and temperate phages might affect the diversity of these groups, we developed a stochastic network model for examining the coevolution of these ecologies. In our approach, nodes represent whole species or strains of bacteria or phages, rather than individuals, with "speciation" and extinction modeled by duplication and removal of nodes. Phage-bacteria links represent host-parasite relationships and temperate-virulent phage links denote prophage-encoded resistance. The effect of horizontal transfer of genetic information between strains was also included in the dynamical rules. The observed networks evolved in a highly dynamic fashion but the ecosystems were prone to collapse (one or more entire groups going extinct). Diversity could be stably maintained in the model only if the probability of speciation was independent of the diversity. Such an effect could be achieved in real ecosystems if the speciation rate is primarily set by the availability of ecological niches.

Negative feedback loops involving signalling molecules

(Sandeep Krishna, Anna M. C. Andersson, Szabolcs Semsey, Kim Sneppen)

The molecular network in an organism consists of transcription/translation regulation, protein–protein interactions/modifications and a metabolic network, together forming a system that allows the cell to respond sensibly to the multiple signal molecules that exist in its environment. A key part of this overall system of molecular regulation is therefore the interface between the genetic and the metabolic network. A motif that occurs very often at this interface is a negative feedback loop used to regulate the level of the signal molecules. In this work we use mathematical models to investigate the steady state and dynamical behaviour of different negative feedback loops. We show, in particular, that feedback loops where the signal molecule does not cause the dissociation of the transcription factor from the DNA respond faster than loops where the molecule acts by sequestering transcription factors off the DNA. We use three examples, the bet, mer and lac systems in Escherichia coli, to illustrate the behaviour of such feedback loops.

Nucleosome mediated Epigenetics

(Mille Micheelsen, I. Dodd, G. Thon, K. Sneppen)

Chromosomal regions can adopt stable and heritable alternative states resulting in bi-stable gene expression without changes to the DNA sequence. Such epigenetic control is often associated with alternative covalent modifications of histones. The stability and heritability of the states is thought to involve positive feedback where modified nucleosomes recruit enzymes that similarly modify nearby nucleosomes. We developed a simplified stochastic model for dynamic nucleosome modification based on the silent mating-type region of the yeast *Schizosaccharomyces pombe*. We show that the mechanism of the model indeed can give strong bi-stability that is resistant both to high noise due to random gain or loss of nucleosome modifications, and also to the random partitioning upon DNA replication. However, robust bi-stability requires

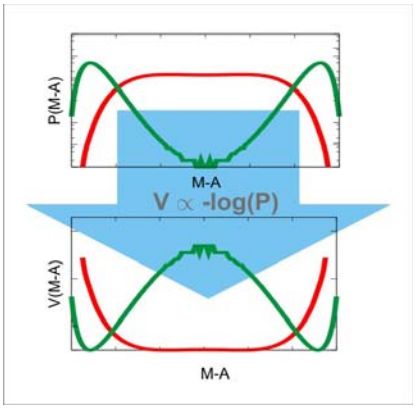


Figure 3: The probability distribution is approx. proportional to the exp of the Potential. The two probability distributions shown in the top of the figure shows a bi-stable (green) or highly cooperative and a random (red) or highly random version of the system. The lower plot illustrates the corresponding potentials where the bi-stable system has two minima with a barrier between them...

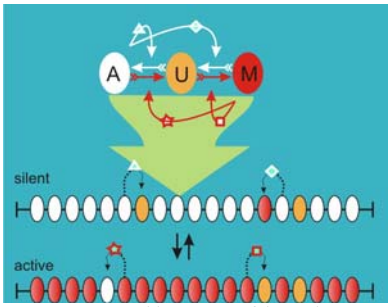


Figure 2: We consider 3 states of the nucleosomes; the modified M the unmodified U and the anti-modified A. Interconversions between the states A and M can only happen through U. Nucleosomes in state A and M will recruit other nucleosomes towards their own state e.g. A recruits M->U and U->A. This in turn results in a bi-stable expression at the gene level as illustrated in the lower part of the figure.

1. cooperativity, the activity of more than one modified nucleosome, in the modification reactions; and

2. that nucleosomes occasionally stimulate modification beyond their neighbor nucleosomes, arguing against a simple continuous spreading of nucleosome modification.

Negative feedback in genetic circuits

(Anna Andersson, J. Paulsson, M.H. Jensen)
So far negative feed back loops have been my main focus, the first project that I worked with was to investigate the role of feed back loops containing small signalling molecules.

In [1] we pointed out the importance of the interactions at the border between the metabolic and protein - DNA transcriptional networks. Previous research has focused on metabolic and protein-interaction networks separately and the link between the two has often been overseen. For example, by studying protein-protein interaction networks alone, one under estimates the number of feedback loops. Does this mean that there is no feedback in the system? No, but it is the small molecules in the metabolic network and not the proteins that are responsible for the feedback. This work inspired more work on feed back loops at the intersection between the two networks. The iron-flow regulation in E-coli is an intricate system consisting of five interlocked negative feedback loops and at least the same number of regulatory proteins. The flow of iron through the cell is approximately 100 times larger than the amount that can be free in the cell without poisoning it. This requires a regulatory system that is very robust and at the same time fast.

In [2] we present a dynamic model that account for most experimental results on the system. An important player in the regulation is the small RNA RhyB that interferes and increases the degradation rate of mRNA for iron storing proteins. This mechanism has recently been discovered experimentally and is believed to make the system faster.

In [3] we generalize the model and looking in particular at the dynamic role of small RNA in feed back loops and how the mechanism can be used for sorting. Presently I am visiting Prof. Johan Paulsson at Harvard Medical School. The lab focuses on understanding stochastic principles of genetic regulation. I am working on the coupled feedback of the toggle switch. Stochastic simulations show that the area of phase space with bi-stability is expanded by noise. The aim is to gain a full understanding for the mechanism responsible for the change in behaviour.

Activities of the Membrane Biophysics Group (T. Heimburg)

Overview of group and the contributions by BioNET

The Membrane Biophysics Group headed by Thomas Heimburg has both an experimental and theoretical orientation. The main interest of the group is the study of artificial and biological membranes and their thermodynamics. The group presently has 11 members, including 1 post doc, 3 PhD students, 3 speciale (masters) students, two project/bachelor students, one technical assistant and the group leader. The equipment of the groups consists of a Fluorescence Correlation (FCS) Spectrometer, a differential calorimeter, a differential densitometer, a Langmuir-Blodgett film balance, a fluorescence microscope, patch clamp amplifiers with Montal-Müller black-lipid membrane cells, and other parts. The group also makes use of theoretical methods including Monte-Carlo simulations.

BioNET presently supports the payment of 50% of a PhD student and 100% of a post doc. Further, BioNET has contributed 50% to acquiring a Fourier Transform spectrometer, a 20% contribution to acquiring a high-sensitivity back-illuminated CCD camera. BioNET has also contributed to material cost (lenses, mirrors and other optical parts for the optical instruments) and to supporting a number of international travels.
The people supported by BioNET are:

- Dr. Kristmundur Sigmundsson, Post doc
- Matthias Fidorra, PhD student (in collaboration with MemPhys, Syddansk Universitet, Odense)

BioNET also supported the guest visit of Dr. Konrad Kaufmann from Göttingen, who is an expert in the thermodynamics of lipid membranes.

Main topics of research

Membranes are thin layers surrounding cells and their organelles consisting of lipids and proteins. They display some very interesting physical features. They are quasi two-dimensional layers embedded into three-dimensional space. This gives rise to the possibility of structural changes of the shapes of lipid vesicles. One

also finds cooperative order transitions of the membrane lipids in which a number of thermodynamic and mechanical properties of the system change. Such transitions are found in biological membranes slightly below body temperature.
Following functions achieve a maximum in the transition regime of biomembranes:

- heat capacity
- isothermal and isentropic volume compressibility
- isothermal and isentropic area compressibility
- bending elasticity
- relaxation times
- permeability

We supported these findings in many experimental studies. In particular, the isothermal compressibilities, the bending elasticity and the relaxation times were shown to display simple functional couplings with the heat capacity. The above relationships have some important implications:

- In the transition regime it is easier to change the shape of membrane vesicles or other membrane structures.
- In the transition regime one finds quantized ion permeation currents through the membranes
- In a membrane above the melting transition one finds the possibility of stable pulse (soliton) propagation.

The latter point belongs to the major developments of our group in the recent three years. We could show theoretically and partially experimentally that one finds stable pulse propagation in lipid cylinders that resemble the properties of nerves. On the basis of this thermodynamic theory of nerves we proposed a new theory for general anesthesia that gives a fundamental physical meaning to this so far un-understood phenomenon. The projects of the last three years are explained in more detail below, with a special focus on the theory of nerves and anesthesia.

Research Details

3.1 Nerve pulse propagation

Biological membranes display melting transitions slightly below body temperature. We have shown that these conditions are necessary but sufficient to allow for the propagation of stable density pulses called solitons. Since the coupling between the heat capacity of lipid membranes and the elastic properties is known, one can write down a hydrodynamic equation that describes the propagation of sound pulses in lipid cylinders. It has the form

$$v^2 \frac{\partial^2}{\partial z^2}\Delta\rho = \frac{\partial}{\partial z}\left[\left(c^2 + p\Delta\rho + q(\Delta\rho)^2\right)\frac{\partial}{\partial z}\Delta\rho\right] - h\frac{\partial^4}{\partial z^4}\Delta\rho$$

$\Delta\rho$ is the change of density in the pulse. The coordinate z=x-vt describes the shape of a density pulse in a cylinder with lateral coordinate x, velocity v of the pulse and time t. z is the spatial coordinate in the a coordinate system moving with the velocity of the pulse. Further, c is the sound velocity in the membrane at physiological conditions, p and q are parameters that describe the dependence of the elastic constants and that can be determined experimentally.

The above equation has an analytical solution. It is shown for typical membrane parameters in Fig. 1.

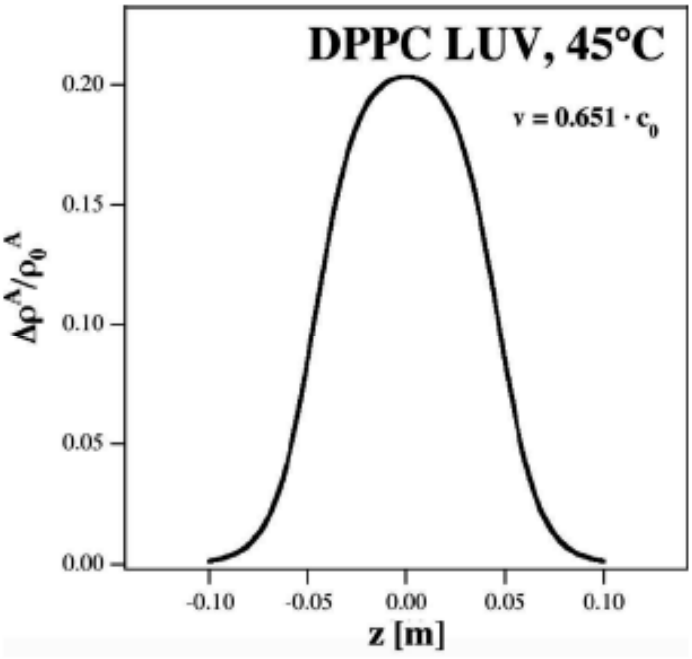


Figure 1. Shape of a propagating density pulse in a membrane cylinder. Propagation velocity is here about 100 m/s and the pulse width is about 10 cm.

The solution consists of a reversible piezoelectric pulse that propagates with about 100m/s. Such a pulse has features that are very similar to those found in many nerves, e.g. they display a reversible release of heat, they display transient thickness changes, and a transient change in voltage. All these features were found for nerves. However, the first two findings were not described by the textbook models. Therefore, these findings have putatively very dramatic consequences for the interpretation of results in neurosciences and the regulation of neurons. On such consequence is described below in a paragraph about anesthesia. These results were published in PNAS (Heimburg & Jackson, 2005) and reviewed in Heimburg & Jackson, 2007a. Other publications about this topic are Lautrup & al. (2006) and Heimburg & Jackson (2007c).

Presently we work in our lab on artificial lipid cylinders, called tethers, and their phase behavior. One can demonstrate the melting behavior in such tether in fluorescence microscopy (Fig. 2). We are presently working on having pulses travel in these artificial models for nerves. If we were able to do so it would strongly support our nerve theory that is exclusively based on the thermodynamic properties of biological membranes, but not on the function of single molecules as in present models.

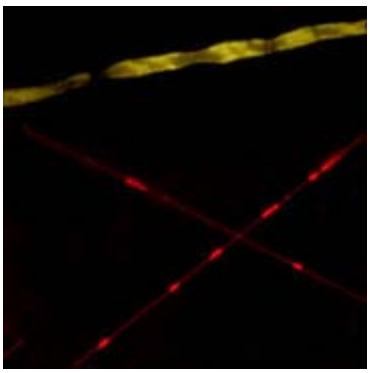


Figure 2. Three lipid tethers as seen in fluorescence microscopy. The diameter of the two red tethers is about 1 □ m, the diameter of the thick yellow tether is about 3-4 □ m. The bright and the dark regions on the tethers are domains of different physical state, such as expected for a propagating pulse. These images, however, are static.

Anesthesia

In a recent paper we have shown that anesthetics lower melting points of membranes in a very coherent simple manner (Heimburg & Jackson, 2007a) that can be described by the well-known physical-chemistry phenomenon called freezing point depression. This is shown in Fig. 3.

We show in this publication that the well-known Meyer-Overton rule that states that the effectiveness of anesthetics is proportional to their solubility in olive oil can be easily translated into the freezing point depression law. In the context of the nerve pulse theory of the previous paragraph this means that the presence of anesthetics increases the amount of free energy needed to trigger a nerve pulse. This is because the melting points are shifted away from physiological temperature and thus it is more difficult to achieve the starting state for a pulse.

It has been observed that anesthetized animals wake up upon application of high hydrostatic pressure (several 10 bars). Our theory is interestingly able to explain the pressure reversal of anesthesia in quantitative terms.

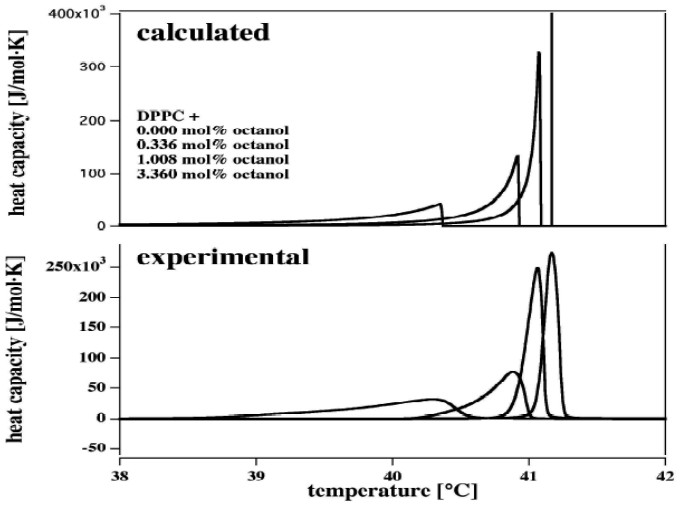


Figure 3. Calculated and experimentally determined heat capacity profiles of an artificial lipid membrane and the influence of increasing amounts of anesthetics in the membrane. The anesthetics lower the melting points of the membrane in a way that is exactly described by a well-known law called freezing-point depression. From Heimburg & Jackson, 2007a.

Monolayers

We have studied in detail the diffusion of lipids and proteins on lipid monolayers using fluorescence correlation spectroscopy on a Langmuir-Blodgett monofilm of lipids (with and without adsorbed proteins). In such experiments one can observe the coexistence of domains/phases of different physical state with fluorescence microscopy (Fig. 4). We could relate the diffusion constants to the later pressure in a simple manner.

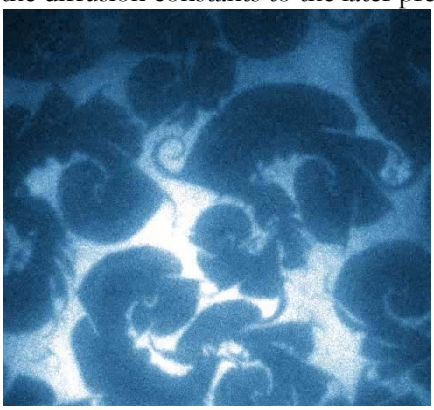


Figure 4. Lipid monolayers in the phase coexistence region. Dark regions correspond to solid domains, bright regions to liquid domains.

We further observed that enzymes called phospholipases hydrolyze the solid domains starting from their edges. This indicates a coupling between protein function and the state of lipid membranes. The results of these investigations have not yet been published but being presented at several conferences.

Relaxation times

In the transition regime of lipid membranes one finds domains formation. A sudden change in temperature or pressure changes the mean domain areas within a typical time that is called the relaxation time. We have made intensive studies with pressure perturbation calorimetry to study such relaxation processes (Seeger et al., 2007). We found that the relaxation time is proportional to the excess heat capacity.

Drugs like antibiotics, neurotransmitters and anesthetics display a pronounced influence on calorimetric profiles, i.e. the typically lower melting points and reduce the cooperativity of the transition. Thus, the straightforward question is whether these drugs influence the relaxation behavior in a coherent manner. In fact, we found that the proportionality between heat capacity and relaxation processes is maintained in the presence of drugs and that the proportional constant is the same. Thus, drugs that lower melting points, and lower the magnitude of the heat capacity maximum lead to a shift in the temperature dependence of the relaxation and faster relaxation processes close to the heat capacity maximum. This indicates that a number of important physical parameters are influenced in a way that can be understood on a thermodynamic basis. Such findings are important for the finding of quantized currents through pure lipid membranes (next section).

Permeability

It is known that lipid membranes become permeable close to the melting transition. Several publications in the literature show this, and we are presently confirming this with FCS (fluorescence correlation spectroscopy) measurements in the framework of a master's project. Interestingly, quantized currents of ions through the membranes are found in the transition regime (Fig. 5). Such events are typical for ion channel proteins but obviously also occur in the transition regime of lipid membranes. Outside of the transition regime no such currents are found.

The typical open time of such channels can be determined by autocorrelation of the current trace. They seem to be closely related to the relaxation times described in the previous paragraph. While at the transition maximum of single lipid membranes, where the heat capacity is high, the typical open times can be up to seconds, they are rather in the range of several 10 ms for lipid mixtures (cf. Fig. 5). These are also the open times expected for biological membranes (judged from their heat capacity). Interestingly, these are also the typical open times of ion channel proteins as reported in the literature.

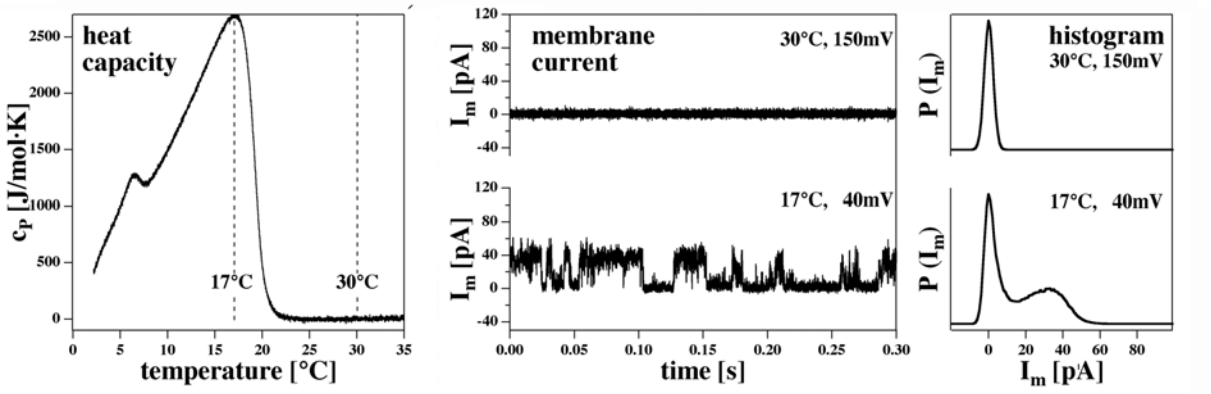


Figure 5. Quantized currents through lipid bilayers (here a DOPC:DPPC mixture). Left: Heat capacity profile of the mixture. The heat capacity maximum is found at 17°C, while at 30°C the membrane is completely fluid. Center: Current traces through the membrane at indicated conditions at 17°C and 30°C. At the heat capacity maximum one finds quantized permeation events, while no such events are seen at 30°C. Right: Current histograms of the traces in the center panel (Fidorra,Winterhalter and Heimburg, 2006).

Perspectives

Our research is very much addressing the thermodynamics of processes like nerve pulse propagation and anesthesia. We are presently building up a cell lab to be able to perform measurements on real nerves, e.g. from crayfish or from other invertebrates. To this purpose with the help of BioNET we have employed a cell biologists as a post doc to perform the cell measurements on nerves with the goal to test predictions on nerves made by our models. We further are on our way to study pulse propagation in artificial nerves (tethers).

Activities of the optical tweezers group (L. Oddershede)

Technical developments

(N. Reihani, A. Richardson, P.M. Hansen, J.K. Dreyer, L. Oddershede)

The optical tweezers facility has been further developed, e.g. we have found a method to improve the axial position detection sensitivity by 2 orders of magnitude simply by adjusting the iris above the condenser to an optimal setting (Appl.Optics, 2004). Also, by another simple method, that is choosing the optimal immersion media for the objective, we have found a new method of making the optical tweezers twice as strong as ever

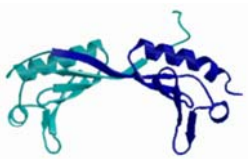
reported in literature (Optics Letters, 2007). This increased performance is allowing for optical trapping of e.g. metallic nanoparticles in a wider range than ever before reported (Nano Letters, 2005). A donation from Carlsberg, supplemented by BioNET has allowed us to buy a Leica TCS SP5 broadband confocal microscope, which was installed in April 2006. We have implemented optical tweezers inside this confocal microscope, so-called 'confocal-tweezers'. With this unique facility confocal images and precise optical force measurements, using photodiodes, can be obtained simultaneously in the x-y plane without moving the objective lens. The unique method by which the confocal images are created facilitates the acquisition of images in areas far from the trapping location. In addition, because the scanning process involves moving galvanic mirrors independently of the objective, the trap is held stable in position and is not subject to any error in position for the x-y scan (SPIE 2006).

Single Molecule Nucleic Acid Research

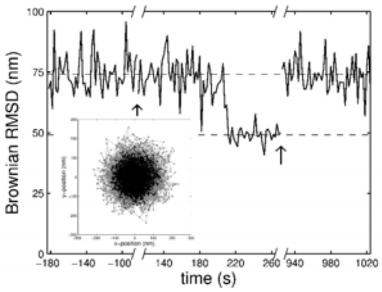
(S.F. Nørrelykke, T.M. Hansen, N. Reihani, M.A. Sørensen, L. Jauffred, L. Oddershede)

We have performed optical investigations on a couple of different systems involving single nucleic acids. The systems are different, but basically studied by the same methods involving optical tweezers, micropipettes and image analysis.

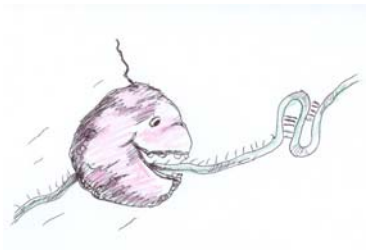
The TATA-binding protein (TBP) is required by all three eukaryotic RNA polymerases for the initiation of transcription from most promoters. TBP recognizes and binds to TATA sequences in the DNA. Upon binding it partially unwinds the DNA and bends DNA by ~80 degrees. This distortion of the DNA is thought to play a crucial role in the recruitment and stabilization of the RNA polymerases.

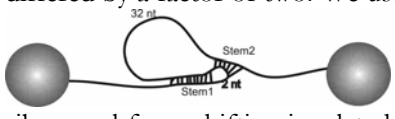


We have studied individual TBPs interacting with single DNA molecules containing the a TATA-box. Using video microscopy we observed the Brownian motion of a bead attached to a short surface-bound DNA tether. When the DNA tether is bound by TBP the effective end-to-end distance is reduced and gives rise to a diminished amplitude of Brownian motion. By monitoring the Brownian motion as a function of time we were able to detect individual binding and unbinding events and derive the kinetics of the process. Unbinding was induced by introducing a large amount of electrolyte or by directly pulling on the tethered bead using optical tweezers. In addition to the well defined bound and unbound states we also observed a third state, intermediate in both time and space between bound and unbound. These observations support a recently published model for the reaction pathway in which an intermediate state is populated a large fraction of the time (Biophysical journal, 2006).



RNA pseudoknots appear in viral mRNA's and cause programmed ribosomal frameshifting, which is often utilized by viral pathogens including HIV. Slippery sequences present in some mRNAs just prior to the pseudoknot cause the ribosome to shift reading frame. The resulting protein is thus encoded by one reading frame upstream from the slippery sequence and by another reading frame downstream from the slippery sequence. We have shown that the efficiency of frameshifting relates to the mechanical strength of the pseudoknot. Two pseudoknots derived from the Infectious Bronchitis Virus were used, differing by one base pair in the first stem. In *Escherichia coli* these two pseudoknots caused frameshifting frequencies that differed by a factor of two. We used optical tweezers to unfold the pseudoknots. The pseudoknot giving rise to the highest degree of frameshifting required a nearly two fold larger unfolding force than the other. The observed energy difference cannot be accounted for by any existing model. We proposed that the degree of ribosomal frameshifting is related to the mechanical strength of RNA pseudoknots. Our observations support the "9Å model" that predicts some physical barrier is needed to force the ribosome into the -1 frame. Also, it supports the recent observation made by cryoelectron microscopy that mechanical interaction between a ribosome and a pseudoknot causes a deformation of the A-site tRNA. The result has implications for the understanding of genetic regulation, reading frame maintenance, tRNA movement, and unwinding of mRNA secondary structures by ribosomes (PNAS 2007).





Colliding RNA polymerases is a fairly new project which was initiated in 2006 in collaboration with the theory group of K. Sneppen. The overall aim of the project is to investigate transcriptional interference of RNA

polymerases through direct single molecule observations in vitro. The binding and firing of one promoter might interfere with the binding and firing of an oppositely directed promoter. This interereence is one ampng many gene regulating mechanisms in both eukaryotic and bacterial cells. Experimentally, we anticipate create a tether, held taut between a micropipette and optical tweezers, with two oppositely directed T7 promoters and then observe the motion of the quantum-dot tagged polymerases along the DNA.

Biological membranes

(T. Winther Madsen, L. Jauffred, S. Brown, T.H. Callisen, L. Oddershede)

The lambda-receptor is a porin transporting nutrients (maltodextrins) across the outer membrane of *E. coli* bacteria. We have studied the energy dependence of the motion of a single λ-receptor. By poisoning the bacteria with arsenate and azide, the bacterial energy metabolism was stopped. The motility of the exact same λ-receptor was measured both before and after poisoning. After poisoning, there was a significant decrease in the width of the distribution of positions visited. We have thus established that there is a difference in membrane protein movements between normal bacteria, and bacteria without a functioning metabolism. This observation is important for the interpretation of diffusion constants especially, if measured in artificial membranes. We have investigated different models of the protein motility and protein attachment to explain the observed differences in the distribution of positions visited. Furthermore, we have repeated this type of investiagtion to study the effect on lambda-receptor motion of the antibiotic ampicillin. The conclusion of this study is that ampicillin also makes the motion of the receptor cease, thus probably stiffening the outer membrane. This work is currently being written up for a scientific submission. The future continuation of this project involves a collaboration between our group and the group of Daniel Otzen to investigate how individual proteins move in spheroblasts made from *E. coli* bacteria.

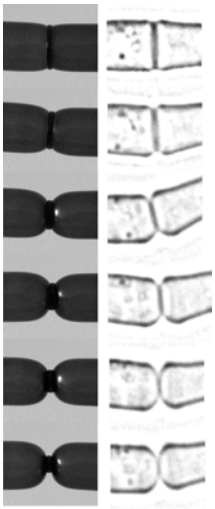
Bacterial tethers were created between a living *E. coli* bacterium and a micron-sized bead by unspecifically attaching the bead to the outer membrane and pulling it away using optical tweezers. Upon release the bead returned to the bacterium thus showing the existence of an elastic tether between the bead and the bacterium. These tethers can be tenths of microns long, several times the bacterial length. Using mutants expressing different parts of the outer membrane structure, we have shown that an intact core lipopolysaccharide is a necessary condition for tether formation, regardless of whether the beads were uncoated polystyrene or beads coated with lectin. A physical characterization of the tethers has been performed yielding visco-elastic tether force-extension relationships: On short timescales the elastic behavior dominates, yielding typical spring constants describing a tether elasticity of 10 +/- 4 pN/micro-m. On longer timescales the tethers exhibit viscous properties as well with typical relaxation timescales of hundreds of seconds. Studies of tether stability in the presence of proteases, lipases, and amylases lead us to propose that the extracted tether is primarily composed of the asymmetric lipopolysaccharide layer of the outer membrane. This unspecific tethered attachment mechanism could be crucial in the initiation of bacterial adhesion. (Biophysical journal 2007).

Cell biophysics:

(P.M. Hansen, A. Richardson, E.L. Munteanu, I. Tolic, K. Berg-Sørensen, G. Thon, L. Oddershede)

Using a library of genetically modified living *S. pombe* yeast cells with different organelles fluorescently marked the group has a long lasting project dedicated to studying the nano-mechanics of cells.

Viscoelastic properties of living yeast cell cytoplasm were investigated by studying the motion of lipid granules naturally occurring in the cytoplasm. A large frequency range of observation was obtained by a combination of video-based and laser-based tracking methods. At time scales from 10^{-4}sec to 10^{2}sec, the granules typically perform subdiffusive motion. This subdiffusive behaviour is thought to be due to the presence of polymer networks and membranous structures in the cytoplasm. Consistent with this hypothesis, we observe that the motion becomes less subdiffusive upon actin disruption. However, also other classes of anomalous diffusion such as super-diffusion and confined motion giving clues about the biological tasks inside the yeast cells were observed (Physical Review Letters 2004).



Nano-mechanics of cell division is an ongoing project for the group. In order to be able to measure the forces involved in cell division in living S. pombe yeast cells, we insert gold beads into the cells and use these as handles for the optical techniques. In order to find the right size of gold beads for this purpose we have optically trapped gold beads in the size range 20-250 nm, which also constitutes world record for optical trapping of gold particles, (Nano Letters 2005). These gold nano particles have been injected into living S.

pombe yeast cells using a micropipette (SPIE 2005). Then, they are attached to various organelles and used as handles for the trapping laser. The future goal of this assay is to perform in vivo measurements of the forces present inside a dividing cell. Also, we have focussed on the topology changes during cell division: During cell division the cell goes from being one to two entities. By studying the outline of the cell we monitor the topology change of the cell during the process with the goal of relating this to other breakup processes as e.g. the breakup process of a water droplet or the pinching of a balloon into two. We reveal the existence of a finite time singularity in the breakup process, the presence of which might facilitate the final splitting. Also, we show a remarkable similarity between the breakup of a cell and the pinching of a balloon, suggesting similar physics in action.

Developing all-atom force-fields for protein structure and dynamic predictions

(J. Ferkinghoff-Borg)

The need for fast and reliable software to elucidate and predict cellular processes at a molecular basis is becoming increasingly important in molecular biology. In collaboration with the structural biology group of prof. L. Serrano (EMBL, Heidelberg and CRG Barcelona) and the SWITCH lab. (VIB, Univ. of Brussel) headed by J. Schymkowitz and F. Rousseau we have extended the scope of the software algorithm *Fold-X* to enable predictions of protein structure, design, docking and dynamics. This work has been conducted as part of the BioNET activities and has been reported in a number of peer-reviewed publications as detailed below. An on-line version of the force field has also been made available:

Protein-ligand binding

Metal-binding proteins play numerous and important roles ranging from recognition to signal transduction. The accurate modelling of metal- and water-binding interactions is critical for the accurate calculation of the energies of proteins and their interactions with other macromolecules like DNA. Molecular dynamics is computationally extremely demanding and approximations are therefore required to allow for higher computational efficiency. In this paper we demonstrate how a semi-empirical extension to Fold-x enables predictions of the positions and binding energies of metal and water molecules in a fast and accurate manner. The method has subsequently been extended to facilitate genome-wide predictions of protein phosphorylation sites as well.

Protein-protein interactions

We have demonstrated the applicability of Fold-x to genome-wide predictions of protein-protein interactions, focusing on identifying true Ras-Binding domains. These domains play a central role in various signal transduction pathways, such as proliferation and differentiation.

Single nucleotide polymorphism

The changes of protein stability upon single point mutations may cause unwarranted phenotypic effects. Fold-x stability calculations have been used as part of a database mapping single nucleotide polymorphism to a variety of phenotypic effects.

Protein dynamics

Recently, we have implemented and effective kinetic sampling method into Fold-x. In this paper we show the applicability of noisy communication channel theory to map the intra-domain signalling pathways based on the simulation data from Fold-x. The method has been demonstrated on the SH2-domain which is an important domain in cellular signalling. We believe, however, that the theory offers a general and systematic description of cellular signalling processes.

Coarse-grained protein models

Despite the speed gained by incorporating semi-empirical "rules" into a protein force-field, all-atom models are still computationally expensive. Consequently, in order to probe large scale conformational changes further careful coarse-graining is most often required. In a number of recent publications we have begun to address this issue more systematically. In particular, we have formulated a simple heteropolymeric c-alpha model which – under particular circumstances - correctly fold to the native state of a protein with a given amino-acid sequence. We have used the model to discuss general properties of design ability and fold ability of different sequences, with particular emphasis on the role played by the unfolded ensemble. This work has been conducted in collaboration with the biophysics group of Prof. R. A. Broglia (Univ. of Milan) and the Biochemistry group of K. Lindorff-Larsen (Univ. of Copenhagen). Studies of unfolded proteins are becoming

increasingly important since this thermodynamic state influences both protein stability and its fold ability. Furthermore, intrinsically disordered proteins have been suggested to play a central role in protein interaction networks and to be implicated in a range of human diseases including Alzheimer, Parkinson and cancer. Despite the immense interests in disordered and unfolded proteins a molecular description is still lacking, mainly due the large ensemble heterogeneities.
The last paper is –to the best of our knowledge- the first to address the question of how to deduce effective interactions in unfolded proteins based on experimental data.

Fragmentation and aggregation kinetics

It has been known for decades that many unrelated human proteins can undergo aberrant aggregation in vivo to form filaments or fibrils, known as amyloids. More than 30 distinct diseases have now been identified which are associated with protein fibrillation, including Alzheimer, Parkinson disease and type 2 diabetes. Furthermore, several recent experiments have demonstrated that any generic proteins under suitable conditions can form other aggregates with morphologies closely resembling amyloid fibrils. In the Biophysics group of prof. M.H. Jensen at NBI we have been studying the mathematical aspects and solutions of various types' fragmentation and aggregation kinetics. Recently, real time data on the fibrillation process of Glucagon has been obtained by the biophysics group of D. Otzen. The data gives a unique opportunity to make accurate and detailed kinetic modelling of this fibrillation process, on the basis of the mean-field equations developed at NBI.

Guests

Andrea,Amatori,Milano,29.03.05 - 30.09.05&01.10.05 - 31.08.06
Luiza,Angheluta,Oslo,07.03.07 - 10.03.07
Rainer,Böckmann,Saarland ,13.09.05 - 15.09.05
Robert ,Cantor,,06.12.06 - 07.12.06
Thomas Callisen, Novozymes, 09.11.05
Roman,Donov,Potsdam,09.08.06 - 27.08.06
Jacob Kisbye Dreyer, Lund University
Matthias,Fidorra,Memphys.SDU,01.10.04 - 01.10.07
Marcela,Gilca,Bucharest,26.06.06 - 30.06.06
Martin,Gudmand,,01.09.04 - 01.09.07
Poul Martin,Hansen,,01.01.07 - 31.01.07
Istvan,Horvathi,Budapest,03.12.06 - 07.12.06- Biomimetic??? Skal med???
Martin J,Howard,London,30.05.06 - 02.06.06
Leo,Kadanoff,,22.11.06 - 25.11.06
Konrad,Kaufmann,Göttingen, several visits in the period
Jan,Kierfeld,Potsdam,24.10.05 - 27.10.05
Paavo,Kinnunen,,14.12.05 - 14.12.05
Lars,Kjær,Aalborg,05.03.07 - 16.03.07
Kristjan Runar,Kristjansson,,17.08.04 - 31.01.05
Ulrich,Kuhl,Marburg,21.08.06 - 25.08.06
Scot,Kuo,Baltimore,31.07.05 - 04.06.05
Zdenek,Lansky,Inst. of Physiology, Acad. Sci. Czech Rep.,01.09.04 - 31.05.05
Christian ,Leirer,Augsburg,11.07.06 - 01.07.07
Boris,Malomed,Tel Aviv,31.05.06 - 05.06.06
Joachim,Mathiesen,Trondheim, several visit in the period
Teresa,Neumaier,Augsburg, 01.07.06 - 31.07.06
Alexandru,Nicolin,Politehnica University of Bucharest,26.08.2002 - 30.04.05
Vitaliy,Oliynyk,Max Planck, 07.01.05 - 28.02.05
Mark,Oxborrow,Middlesex, 08.08.05 - 10.08.05
Sergio,Padilla,Lund,08.08.05 - 15.08.05
Simone,Pigolotti,Rome,30.05.05 - 01.06.05 Biomimetic??? Skal tages med??
Vakhtang,Putkaradze,Colorado,15.10.05 - 16.10.05
Matthias F. ,Schneider,München,21.06.05 - 23.01.05
Joost,Schymkowitz,Brussel,30.05.05 - 02.06.05&16.11.05 - 18.12.05
Heiko,Seeger,Max-Planck Institute for Biophy. Chem,08.09.2003 - 07.09.06
Christine,Selhuber-Unkel,Heidelberg,11.01.07 - 18.01.07
Luis,Serrano,Heidelberg,17.03.05 - 18.03.05
Ingve,Simonsen,Dresden,07.06.06 - 11.06.06
Joel,Stavans,Rohovot,16.03.05 - 19.03.05

Guido,Tiana,Milano,26.09.06 - 01.10.06
Iva Marija,Tolic-Nørrelykke,Dresden,08.05.05 - 10.05.05
Simon Tolic-Nørrelykke, Max Planck Inst., Dresden
Antonio,Trovato,Padova,11.08.05 - 28.08.05
Ala,Trusina,San Francisco,05.02.07 - 19.02.07
J.J.P.,Veerman,Portland,25.08.05 - 01.09.05
Andreas,Wirzba,Jülich,14.05.06 - 21.05.06
Inga,Zins,Mainz,14.03.07 - 18.03.07

Following lectures have taken place at NBI

Date	Person	Affiliation
21/3-07	Alexander Rohrbach	Univ. of Freiburg
7/3-07	Alex Nicolin	Niels Bohr Institute
28/2-07	Jens C. Rekling	Dept. Medical Physiology
24/1-07	Henrik Svensmark	Danish Space Research Institute
13/12-06	Rune W. Berg	MFI Panum Institute
6/12-06	Robert S. Cantor	Dept. Chemistry, Dartmouth College and MEMPHYS
29/11-06	John Hertz	Nordita/NBI
23/11-06	Leo Kadanoff	Univ. of Chicago
22/11-06	Hans Fogedby	Department of Physics and Astronomy, Univ. of Aarhus
15/11-06	Sune Lehmann	IMM DTU
8/11-06	Kåre H. Jensen	NBI
25/10-06	Jacob Bock Axelsen	Weizmann Institute of Science
11/10-06	Anna Andersson	NBI
4/10-06	Liselotte Jauffred	Niels Bohr Institute
27/7-06	Thomas Hamelryck	Bioinformatics, KU
20/9-06	M. Ellegård & Ekelund	Biol. Inst.
13/9-06	Namiko Mitarai	Niels Bohr Institute (visitor)
28/6-06	Marcela Gilca	Politehnica University of Bucharest
16/6-06	Felix Ritort	University of Barcelona
8/6-06	Michael Laessig	Department of Physics,University of Cologne
7/6-06	Søren Brunak	Center for Biological Sequence Analysis, BioCentrum-DTU
31/5-06	Martin Howard	Dept. of Mathematics, Imperial College London
24/5-06	Alexander Mitin	DIKU, Univ. of Copenhagen
3/5-06	Bjarne Andresen	Ørsted Laboratory, Univ. of Copenhagen
26/4-06	Peter Ditlevsen	Niels Bohr Institute
19/4-06	Clive Ellegaard	Niels Bohr Institute
31/3-06	Ian Dodd	Univ. of Adelaide, Australia, NBI guest
24/3-06	Sui Huang	Harvard Medical School, Boston
22/3-06	Ian Dodd	Univ. of Adelaide, Australia, guest visitor
15/3-06	Simone Pigolotti	Niels Bohr Institute
8/3-06	Ian Dodd	Univ. of Adelaide, Australia, NBI guest
22/2-06	Eli Barkai	Bar Ilan University
15/2-06	Mette Høst	Artist in residence at Niels Bohr Institute
10/2-06	Dimitris Stamou	Bio-Nanotech. Lab, Nano-Science Center, Univ. of Copenh
8/2-06	Heiko Seeger	Niels Bohr Institute
1/2-06	Isaac Klapper	Dept. Math. Sciences, Montana State Univ.
27/1-06	Konrad Kaufmann	Max Planck Inst., Biophys Dep.
25/1-06	Thomas Heimburg	Niels Bohr institute
20/1-06	Konrad Kaufmann	Max Planck Inst., Biophys Dep.
18/1-06	Hans Fogedby	Dept. of Physics and Astronomi, Univ. of Aarhus
11/1-06	Prabodh Shukla	North Eastern Hill University, Shillong, India.
14/12-05	Anders Johansen	Niels Bohr Institute
7/12-05	Mads Madsen	Dep. Medic.Biochem.&Genetics,Panum NMR Centre
30/11-05	Andrea Amatori	NBI and Univ. of Milano, Phys. Dep.
23/11-05	Anna Andersson	Niels Bohr Institute
16/11-05	Szabolc Semsey	Niels Bohr Institute, Visitor
9/11-05	Thomas Callisen	Novozymes Research & Development
2/11-05	Jakob Kisbye Dreyer	Phys. Chem, Center for Chem. & Chemical Engi., Lund Univ.
26/10-05	Per Grove Thomsen	DTU, IMM
12/10-05	Sune Danø	Dep. Medic.Biochem.&Genetics,Panum NMR Centre
28/9-05	Ulrich Quaade	Center for individual nanoparticle functionality (CINF)NanoDTU, Department of Physics, DTU
21/9-05	Sandeep Krishna	NBI
14/9-05	Rainer Böckmann	Theo. & Comp. Membrane Biology, Center for Bioinfor., Saar Universität des Saarlandes
7/9-05	J. B. Axelsen &J- Ferkinghoff-Borg	BioComplexity group, NBI
31/8-05	Peter Vermann	Portland State University
6/7-05	Jonas Tegenfeldt	LTH Lund
22/6-05	Matthias Schneider	University of Augsburg
15/6-05	Mia Trolle Borup	Niels Bohr Institute
1/6-05	Scot Kuo	John Hopkins University

25/5-05	Zdenek Lansky	Pragh Univ.
18/5-05	Kresten L.-Larsen	Dept. of Biochemistry, August Krogh, KU
11/5-05	Michael Rudolph	
4/5-05	Fridolin Okkels	DTU, MIC
27/4-05	C. Rygaard-Hjalsted & B. Dam Sørensen	Kommunikationskontoret, NBI
13/4-05	Henrik Bruus	DTU, MIC
30/3-05	Steve Strogatz	Cornell University
9/3-05	Daniel Abrams	Cornell University
2/3-05	T.Bohr, K.Sneppen,P. Ditlevsen	Dep. of Physics, DTU ; NBI & NBI,
23/2-05	Stanley Brown	Mol. Biol. Institute, University of Copenhagen
16/2-05	Rob Delotto	Mol. Biol. Institute, University of Copenhagen
9/2-05	Steve Strogatz	Cornell University
2/2 -05	Kaare Brandt Petersen	DSP/IMM, DTU
26/1-05	Christoffer Johansson	Dept. Theoretical Ecology,Lund University
19/1-05	Eli Barkai	Bar-Ilan University
12/1-05	Nader Reihani	Inst. for Adv. Studies in Basic Sciences, Zanjan, Iran
5/1-05	Anders Andersen	Cornell Univ., Dept. of Theo.and Applied Math.
15/12-04	T. Heimburg & A. Jackson	NBI
8/12-04	Prof. Ian-Max Møller	Risø
1/12-04	Henrik Flyvbjerg	Risø (Plant Research and Danish Polymer Centre)
24/11-04	Sandeep Krishna	NBI
17/11-04		NBI, SDU, AAC

University of Southern Denmark, SDU

The SDU node of BioNET

Responsible scientist	Prof. Ole G. Mouritsen
Principal scientists in period of report	Dr. Matthias Weiss (2004) Prof. Luis Bagatolli (2004-) Dr. Chris Lagerholm (2006-) Prof. Beate Klösgen (2006-)
PhD students	Matthias Fidorra (SDU and NBI, 2004-) Stinne Hørup Hansen (SDU, 2004-) Maria Bloksgaard Mølgaard (SDU, 2005-) Brian Vad (AAU and SDU, 2005-) Eva Arnspang Christensen (SDU, 2006-) Rakhu Sankar (SDU, 2006-)

Major research themes at SDU under BioNET

1. ER exit sites, dynamics of membrane-enzyme interactions, and the mechanics of membrane fluctuations (Dr. Matthias Weiss and Prof. Ole G. Mouritsen)
2. Ceramide containing membranes and Stratum Corneum skin lipid membranes (Prof. Luis Bagatolli, Prof. Beate Klösgen, Prof. Thomas Heimburg (NBI), PhD-students Maria Bloksgaard Mølgaard, Matthias Fidorra, Raghu Sankar) The role of Acyl-CoA Binding Protein in skin − combining biophysics, molecular biology, biochemistry and mouse genetics (PhD-student Maria Bloksgaard Mølgaard, Prof. Luis Bagatolli, Prof. Susanne Mandrup,)
3. An in vivo investigation of the cellular plasma membrane nano-organization (Dr. Christoffer Lagerholm, PhD-student Eva Arnspang Christensen, MSc-student Hanne Matras)
4. Biophysics as a model for inter-disciplinary teaching in Danish high school (PhD-student Stinne Hørup Hansen)
5. Atomic force microscopy studies of membrane proteins (PhD-student Brian Vad (SDU, AAU), Prof. Adam Cohen Simonsen, Prof. Ole G. Mouritsen, Prof. Daniel Otzen (AAU))
6. Instrumentation for advanced microscopy and single molecule imaging (Dr. Chris Lagerholm, Prof. Luis Bagatolli)

PhD-theses supervised under BioNET

1. Matthias Fidorra, University of Southern Denmark (supervised by Luis Bagatolli, Ole G. Mouritsen, and Thomas Heimburg, Niels Bohr Institute): "Confocal microscopy, calorimetry and permeability studies of giant lipid vesicles containing ceramides" (2004-).
2. Stinne Hørup Hansen, University of Southern Denmark (supervised by Claus Michelsen and Ole G. Mouritsen): "Science didactics: Biophysics as a model for cross-disciplinary teaching in high school" (2004-). Maternity leave, August 2005 - August 2006.
3. Brian Vad, University of Aalborg and University of Southern Denmark (supervised by Daniel Otzen and Ole G. Mouritsen): "Atomic force microscopy studies of membrane proteins." (2005-).
4. Maria Bloksgaard Mølgaard, University of Southern Denmark (supervised by Luis Bagatolli and Susanne Mandrup): "The role of Acyl-CoA Binding Protein in skin - a functional investigation by targeted disruption of the gene in mice." (2005-). Maternity leave, September 2006 - August 2007.
5. Eva Arnspang Christensen, University of Southern Denmark (supervised by Chris Lagerholm and Ole G. Mouritsen): "A global investigation of the plasma membrane structure: A large scale *in vivo* investigation of the lateral dynamics of plasma membrane proteins" (2006-).

6. Raghu Sankar, University of Southern Denmark (supervised by Beate Klösgen and Luis Bagatolli): "Structure function interplay of skin: establishing a laboratory model to mimick real skin tissue" (2006).

Initiation and operation of BioNET at the SDU-node

In accordance with the original research plan, the focus of this node is complex membrane phenomena, including sub-cellular dynamics of proteins and vesicles, and the structure of model membranes and models of the skin. In addition, the SDU-node is performing research in science education where biophysics is used as a model topic.

The startup of the activities at the node was complicated by the fact that the first principal scientist hired under BioNET, Dr. Matthias Weiss, at the end of 2004 received and accepted an offer from the Deutsches Krebsforschungszentrum in Heidelberg to build up his own research group there. Four measures were taken in order to remedy this situation.

1. Prof. Luis Bagatolli from the Department of Biochemistry and Molecular Biology of SDU took on the duty to change some of his research directions to fit into the BioNET framework. These directions pertain to structure and dynamics of skin and skin membranes. Prof. Bagatolli's research within BioNET is allocated two partly BioNET-funded PhD-students, Maria Bloksgaard and Mathias Fidorra.
2. The position vacant after Dr. Weiss was announced internationally and filled in January 2006 by Dr. Christoffer Lagerholm from the University of North Carolina. Dr. Lagerholm is an expert in biological imaging using fluorescence techniques and quantum dots applied to study small-scale structure in cell membranes and the dynamics of membrane proteins in living cells. Dr. Lagerholm's research is allocated a partly BioNET-funded PhD-student, Eva Arnspang Christensen.
3. Dr. Weiss continues to be involved in some of the originally planned projects. The ties to Dr. Weiss and his new research group in Heidelberg were partly maintained via a collaboration mediated by a PhD-student, Ask F. Jakobsen, who has been spending time in Heidelberg and who has worked on modelling of dynamic membrane phenomena, enzymatic action, and membrane fusion and fission processes. In the future, the collaboration will be mediated by a German PhD-student to be hired and by Dr. Weiss's visits to SDU.
4. Prof. Beate Klösgen of SDU has joined BioNET and contributes to the study of model skin membranes using scattering techniques and micromechanics. The research of Prof. Klösgen is associated with a partly BioNET-funded PhD-student, Raghu Sankar.

A substantial part of the time since the initiation of BioNET has been allocated to the initiation and setting up novel research programs in complex membrane systems and live cells. This includes the construction of novel instrumentation for two-photon fluorescence microscopy and single molecule imaging techniques, the building of a cell culture laboratory, and the design of molecular biological techniques for specific tagging of membrane proteins.

Hence, the SDU node has had a slow start, recruiting new scientists and students, building up new experimental facilities, and embarking on an ambitious long-term research program that not only builds on already existing facilities. The achievements should be evaluated in this light.

Networking within BioNET and outside

The formal and informal collaboration between SDU and the two other BioNET nodes have been enhanced during the first two years and is now gaining momentum. Similarly, the established BioNET-activities have paved the way for new collaborations nationally and internationally because of the new research profile of the SDU node influenced by BioNET. The following BioNET-induced collaborations should be mentioned

1. Joint NBI-SDU collaboration on membranes containing ceramides and the measurement of water flux through membranes. The PhD-fellowship to Matthias Fidorra is jointly financed by BioNET resources allocated to SDU and NBI.
2. Joint SDU-AAU collaboration on atomic force microscopy studies of membrane proteins in supported membranes. The PhD-fellowship to Brian Vad is jointly financed by BioNET resources allocated to SDU and AAU.
3. Collaboration with Prof. Susanne Mandrup at the Department of Biochemistry and Molecular Biology on the structure of the skin of knock-out mice involving the BioNET-supported PhD-student Maria Bloksgaard Mølgaard.

4. Collaboration between Dr. Christoffer Lagerholm and Prof. Henriette Giese, Dept. of Ecology, Section of Genetics and Microbiology, Faculty of Life Sciences, University of Copenhagen, and Prof. Martin Røssel Larsen, Department of Biochemistry and Molecular Biology, University of Southern Denmark on identifying the function and interaction partners of two unknown gene products in the PKS12 gene cluster responsible for the synthesis of the dye aurofusarin in the filamentous fungi *Fusarium graminearum*. One of these unknown gene products, FG02329, is thought to be a GPI anchored protein.
5. Collaboration with the AAU node on the formation of giant unilamellar vesicles. These vesicles are loaded with different molecular weight fluorophores to study membrane destabilization (leakage events) upon peptide binding to the membrane. The PhD-fellowship to Brian Vad is jointly financed by BioNET resources allocated to SDU and AAU.
6. Collaboration with LEO Pharma on projects regarding liposomal drug delivery.
7. Collaboration with several research groups at the Natural Sciences and Medical Faculties at SDU concerning the establishment of a national instrument center for biomolecular imaging incorporating equipment for light and fluorescence microscopy, atomic force microscopy, electron microscopy, secondary ion mass spectrometry imaging, and positron emission tomography/computed tomography.
8. Collaboration with international partners, specifically Prof. Jonathan Jarvik, Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, USA, Profs. Alan S. Waggoner and Marcel Bruchez, Molecular Biosensor and Imaging Center, Carnegie Mellon University, Pittsburgh, USA, and Prof. Ken Jacobson, Department of Cell and Developmental Biology and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, USA.
9. Collaboration with Prof. Jens Knudsen at the Department of Biochemistry and Molecular Biology on the synthesis of skin ceramides involving a partly BioNET-funded PhD-student, Raghu Sankar.
10. Collaboration with Prof. Jens Knudsen at the Department of Biochemistry and Molecular Biology on the characterization of epidermal lipid composition by use of electrospray ionization mass spectrometry (ESI-MS). This study involves the BioNET-supported PhD-student Maria Bloksgaard Mølgaard.
11. Collaboration of Dr. Christoffer Lagerholm and BioNET-funded PhD-student Eva Arnspang Christensen with Dr. Jakob Lerche Hansen and Christina Lyngsoe at the Laboratory of Molecular and Cellular Cardiology at Copenhagen University Hospital on the migration pattern the G-family receptor Angiotensin II type I receptor (AT1R) in mammalian cells.
12. Collaboration between Dr. Christoffer Lagerholm and Prof. Paul Wiseman at the Department of Physics and Chemistry at McGill University, Montreal, Canada on the analysis of time-lapse image sequences of quantum dots attached to a variety of biological ligands.

Research report

ER exit sites, dynamics of membrane-enzyme interactions, and the mechanics of membrane fluctuations

(Dr. Matthias Weiss, Prof. Ole G. Mouritsen and collaborators)

In 2005 Dr. Weiss in collaboration with the group of Rainer Pepperkok (EMBL Heidelberg, Germany), successfully confirmed their previously preliminary results that the binding kinetics of COPII proteins to single exit sites of the endoplasmic reticulum (ER) is modulated by the presence of cargo and cholesterol. While the typical turn-over time for the involved GTPase Sar-1 increased, the corresponding time for the subsequently recruited coat proteins Sec23/24 decreased. Accompanying the experimental results with kinetic modeling, it was shown that cargo which binds to the coat will retain the Sec23/24 on the ER membrane even after Sar-1 has hydrolyzed its GTP.

Using mesoscopic models for lipid bilayers (so-called dissipative particle dynamics), Dr. Weiss could show in collaboration with A. F. Jakobsen and O. G. Mouritsen, SDU, that the digesting action of phospholipase A2 softens the membrane and enhances the diffusion of lipids as well as the event of flip-flops. In addition it was shown that inclusion of active proteins in lipid membranes leads to a substantial mechanical softening of the bilayers consistent with theoretical predictions.

Ceramide containing membranes and Stratum Corneum skin lipid membranes (Prof. Luis Bagatolli, Prof. Beate Klösgen, Matthias Fidorra, Raghu Sankar, and collaborators)

Ceramide, a sphingosine-based lipid second messenger, is known to be involved in the regulation of several cellular responses to extra cellular stimuli, including differentiation growth suppression, cell senescence, and apoptosis. Ceramides are also related to the formation and function of the permeability barrier of the skin. In particular the barrier properties of the stratum corneum are related to the phase behaviour of the intercellular

lipids, a lipid mixture consisting of ceramides, cholesterol and fatty acids.. This type of lipid, which has a single hydroxyl polar head group, is the most condensed sphingolipid and demonstrate the highest thermal transition temperature. Ceramides may exert their biological activity through changes in membrane structure and organization.

The research plan contains three main goals

1) To complete studies of ceramide-containing artificial lipid mixtures (thermotrophic behavior) using fluorescence spectroscopy and microscopy (confocal/two photon excitation), differential scanning calorimetry, and atomic force microscopy. This will include changes in composition in order to mimic the case of the skin lamellae.
2) To device model systems with full composition (ceramide-containing membranes from skin) to perform correlations with the observed phenomena in artificial mixtures.
3) To explore the lateral structure of skin membranes directly in skin tissue. In this case a new PhD project was started during 2005 (PhD-student Maria Bloksgaard Mølgaard, see below for details).

Artificial lipid mixtures composed of ceramide-based lipids

(Prof. Luis Bagatolli, Prof. Thomas Heimburg (NBI), PhD student Matthias Fidorra)
During 2005, the presence of lateral heterogeneity was demonstrated in mixtures of ceramide and phospholipids (POPC in this case) with and without cholesterol and in mixtures of ceramide/fatty acid cholesterol. As shown in Figure 1, different lipid phases are present in these mixtures. Additionally, mixtures containing cerebrosides (a ceramide-based lipid) instead of ceramide were studied. These results were presented at the 49th Annual Meeting of the Biophysical Society, Long Beach, CA, February 12th -16th 2005: The lateral structures of ceramide containing bilayers as observed by fluorescence microscopy (M. Fidorra, L. Duelund, and L.A. Bagatolli) and at the 50th Annual Meeting of the Biophysical Society, February 18th -22th 2006: Headgroup influence on membrane shapes and lateral membrane structure of POPC/Ceramides and POPC/Cerebrosides mixtures (M. Fidorra, T. Heimburg, and L. A. Bagatolli).

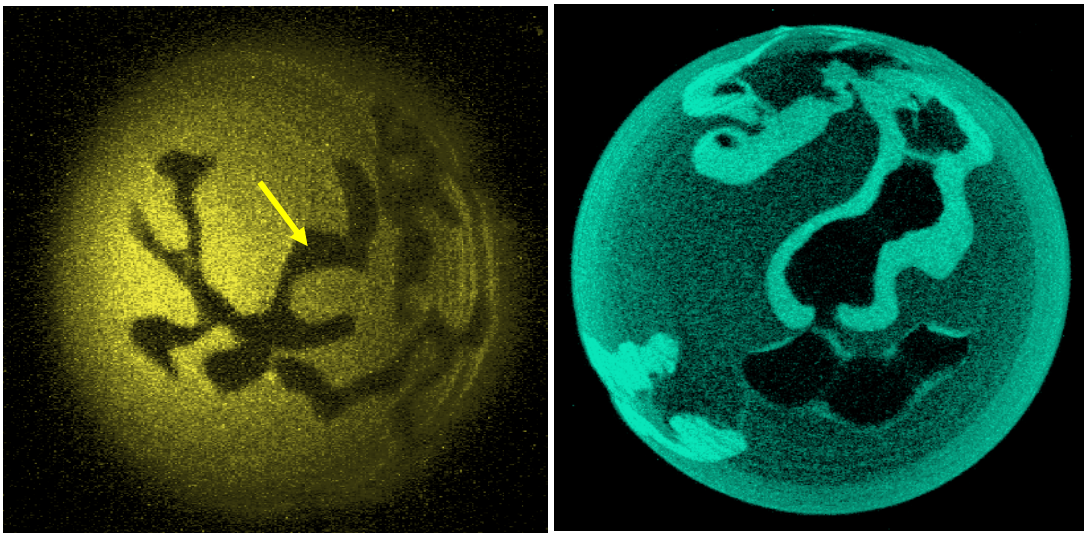


Figure 1: Giant vesicles (GUVs) composed of brain ceramide/POPC mixtures (1:5 mol, right) and of brain ceramide:POPC/cholesterol mixtures (1:5/26% mol, left) displaying phase coexistence. The dark areas in the right image (indicated with the yellow arrow) correspond to ceramide-rich gel phase areas. In the case of the cholesterol-containing mixture, three different areas can be observed in the GUV. The probe DiIC18 was used in this experiment either in two-photon excitation mode (excitation @ 760 nm) or confocal mode (excitation @ 543 nm).

The role of Acyl-CoA Binding Protein in skin - combining biophysics, molecular biology, biochemistry and mouse genetics

(Prof. Luis Bagatolli, Prof. Susanne Mandrup and PhD-student Maria Bloksgaard Mølgaard)
ACBP is a small intracellular lipid-binding protein that binds medium to long chain acyl-CoA esters with a very high affinity. The structure and *in vitro* properties of the protein are very well characterized, but the *in vivo* function of the protein in mammalian species remains poorly characterized. To investigate the function of ACBP *in vivo*, we have generated mice with targeted disruption of the ACBP gene. The mice are viable and fertile, but show a clearly visible phenotype in the skin/fur. Results from metabolic and molecular biological studies support the hypothesis that disruption of ACBP alters the biochemistry and molecular biology of the

skin – probably resulting in altered biophysical properties of the skin. To further characterize the *in vivo* function of ACBP in skin, we have initiated a study of the skin using a combination of techniques from the research field of molecular biophysics, molecular biology and biochemistry. The primary goals of the research plan are:

(i) Characterization of epidermal lipid composition by use of electrospray ionization mass spectrometry (ESI-MS). This work is done in close collaboration with Professor Jens Knudsen, Department of Biochemistry and Molecular Biology, SDU – Odense.

(ii) Evaluation of the variability in skin lipid behavior from the different mouse genotypes by using different biophysical techniques (fluorescent spectroscopy/microscopy and differential scanning calorimetry).

(iii) Direct visualization of the lateral structure of stratum corneum lipid membranes in skin tissue using polarity sensitive fluorescent probes under confocal and 2-photon excitation microscopy.

(iv) Quantification of epidermal mRNA and protein levels in order to correlate the molecular biology of the skin (eg. protein composition) with the lipid composition and physical properties of the skin in order to elucidate the function of ACBP *in vivo*.

The major achievements have been:

(i) Establishment of a standard operating procedure for purification and quantification of epidermal lipids and automated lipid mass search for identification of lipid species in the skin lipid extracts analyzed by ESI-MS.

(ii) A comparison between light and fluorescent microscopy, showing that structures observed in the light microscope easily can be resolved by using the two-photon excitation microscopy techniques (figure 2).

(iii) Confirmation of ultrastructural findings (electron microscopy) by molecular biology techniques, showing that the ACBP knockout mice are having an aberrant expression of at least one protein important for the establishment of the cornified envelope, a lipid-protein complex in the skin responsible for the epidermal water barrier.

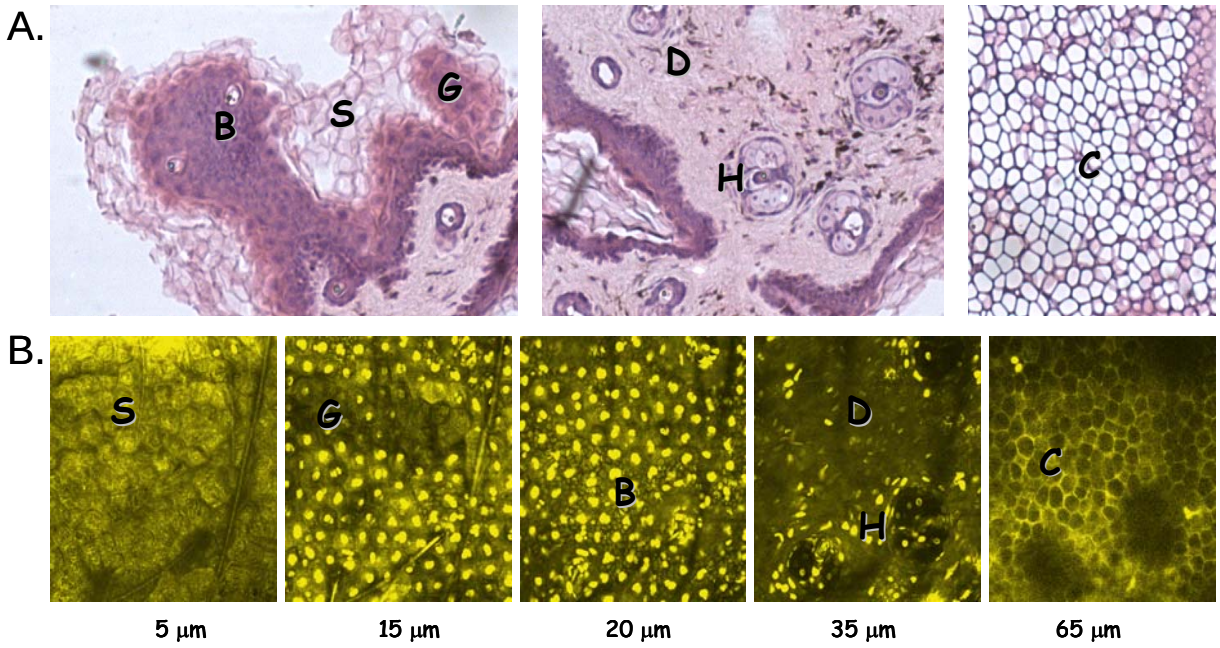


Figure 2: Light microscopy vs. two-photon excitation microscopy on mouse ear tissue: The different layers in the skin visualized by ordinary hematoxylin/eosin light microscopy sections (A) can easily be observed by using TPEM (B). Two-photon excitation was performed at 780 nm. The cell nuclei are labelled with the fluorescent dye sytox 543. Fluorescence emission corresponds to autofluorescence (mainly NAD(P)H) and sytox 543. *Abbreviations:* B: basal layer, C: cartilage, D: dermis, G: granular layer, H: hair follicle, S: surface/stratum corneum.

Biophysics as a model for inter-disciplinary teaching in Upper Secondary School

(PhD student Stinne Hørup Hansen)

The hypothesis of this project is that science education has a dual purpose - to produce future scientists and to initiate the development of scientific literacy in the individual. This study is concerned with both purposes of science education and the aim of the project is to clarify how science education in Upper Secondary School can be improved in order to

- Catch and hold girls' and boys' interest in science and hence improve their scientific literacy
- Enhance the interplay between the sciences in order to improve student interest
- Confront students' prototypical views of people with an interest in science

The project is based on development and implementation of innovative interdisciplinary teaching material in the science subjects in Upper Secondary School with biophysics as the model.

Pilot projects with the purpose of providing research knowledge and experience have been initiated. One pilot project has been designed, implemented and evaluated as an interdisciplinary project about radiation in cooperation with teachers in physics, mathematics, chemistry and biology in a first year class in Upper Secondary School. Part of the results were presented at the 8th Nordic Science Symposia on Science Education (2005) in Ålborg, Denmark (Interdisciplinært undervisningsforløb i matematik og de naturvidenskabelige fag i den danske gymnasieskole, C. H. Hansen)

Stinne H. Hansen has developed teaching material for laboratory experiments on viscosity for first year students at the University of Southern Denmark. The material included background knowledge of viscosity, experimental instructions, data sheets and evaluative questions.

In relation to a science theatre production "Den Magiske Kugle" on the biophysical research involved in the development of liposomes for use in cancer treatment, Stinne H. Hansen has developed teaching material for Upper Secondary School. In addition she has implemented a cross-disciplinary project in physics and biology in a 2nd year class in Upper Secondary School. The project evolved around the science theatre and the students were to write a report within a topic of the science theater, followed by teaching their classmates within their topic and constructing test question. The empirical material derived from the project are questionnaires before and after attending the science theatre, and during the interdisciplinary project, video recordings of group work and class teaching and finally interviews of the student after the project. The qualitative data will be analyzed in order to detect the triggering and maintenance of a situational interest.

Stinne H. Hansen has given a talk with the title: "Samspil mellem matematik og de naturvidenskabelige fag, fire fag – fire lærere (Interplay between mathematic and the sciences, four subject – four teachers)" at the annual meeting for mathematical teachers in the southern region of Denmark. Stinne H. Hansen has also given a talk with the title: "Rollemodeller i naturvidenskab (*Role Models in Science*)" at a course for elementary school physics teachers, organized by University of Southern Denmark.

Currently Stinne H. Hansen is engaged with a project on bridge building between Upper Secondary School and a science study at college. The aim of the project is the improve the transition from high school student to university student by mapping the high school students expectations and competences and organizing the first year of college with regard to these findings.

An in vivo investigation of the cellular plasma membrane organization

(Dr. Christoffer Lagerholm, PhD-student Eva Arnspang Christensen, MSc-student Hanne Matras)
The aim of this project is to investigate the cellular plasma membrane nanostructure, including the possible existence of morphology featureless nano-domains such as lipid rafts, in live mammalian cells by use of native membrane proteins as local reporters and with single molecule fluorescence microscopy. The eventual goal is to perform a large scale analysis of the characteristics of the lateral mobility of 50-100 membrane proteins and to determine the relation of the mobility characteristics to the structure and organization of the cellular plasma membrane. The scope of this project is multi-disciplinary involving recent technology developments in 1) molecular biology and genetics, 2) nanotechnology and 3) single molecule biophysics. In accordance, we have built up new MEMPHYS facilities and created national or international partnerships as needed. New MEMPHYS facilities that have been, or are in the process of being established with support from BioNET include a cell culture facility, basic facilities for molecular biology and protein biochemistry, and a fluorescence microscope optimized for single molecule biophysics.

The key aspect of this project is the use of genetic engineering to generate a novel, maximally versatile model system for *in vivo* studies of native membrane proteins. To accomplish this, we are using a genetic engineering technique termed Central Dogma (CD) tagging. In this approach, custom guest exons containing mammalian splice acceptor and donor signals are inserted at random into genomic DNA with a retroviral delivery system.

During gene splicing and protein translation, only modifications that are inserted in introns in the correct reading frame are detectable at the protein level. For this component, we have established collaboration with the inventor of the CD-tagging technology, Prof. Jonathan Jarvik at the Department of Biological Sciences, Carnegie Mellon University, USA. Our first generation guest exon, Stealth BLAP v1, inserts a total of 47 amino acids including the 9 amino acid *Influenza Hemaglutininin* (HA) epitope tag, a 15 amino acid flexible loop region ((Gly-Gly-Gly-Gly-Ser)$_3$) and the 15 amino acid acceptor peptide for bacterial biotin ligase (BirA) (Figure 3). This guest exon takes advantage of the fact that there is no natural BirA substrate on the cell surface of mammalian cells, and the widely used extremely tight binding interaction of biotin and i.e. streptavidin (sAv).

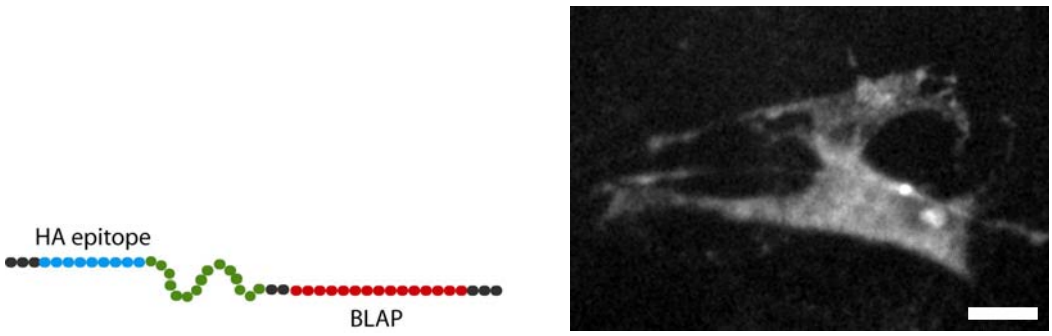


Figure 3: Stealth BLAP v1 guest exon and an example of BLAP annotated membrane protein in live mouse embryo fibroblast detected with Alexa 488 anti-HA (Scale bar = 20 μm).

Initial tests with Stealth BLAP v1 in mouse embryo fibroblasts have resulted in very few observed genetic modification (estimated at < 0.005% of all cells). As a result, we are currently in the process of re-designing this sequence to also include the insertion of the antibiotic selection marker puromycin. This will allow for pre-selection for genetically modified cells with antibiotic resistance followed by a second screen for cells that have accessible modifications at the cell surface. For this modification, we are additionally using flanking 34 base pair lox P sequences such that the 600 base pair pac gene encoding for puromycin resistance can be excised with Cre recombinase hence ensuring that the size of final genetic modification is kept at about 50 amino acids. The current Stealth BLAP 1 will also only successfully modify type 0 introns. Plans are underway to also engineer guest exons to also modify types I and II introns. We have recruited a M. Sc. Student, Hanne Matras, to specifically help us with these modifications. We will also continue our close collaboration with the Jarvik laboratory at Carnegie Mellon University in this work.

In parallel, we are continuing our work with characterizing and optimizing the use of quantum dots for single molecule imaging and with our new microscopy system. Preliminary data of streptavidin conjugated quantum dots immobilized on a glass cover slip indicate signal-to-noise ratios of ≈2, ≈2, ≈4, ≈7, ≈10 and ≈8 of single quantum dots emitting at 525, 565, 585, 605, 655, 705 nm, respectively and which are continuously illuminating over a 1 ms integration period and with a total magnification of 150X (projected camera pixel size of about 110 nm). Under identical illumination conditions but with 100 μsec integration, we find that 655 nm emitting quantum dots can be imaged with a signal-to-noise of ≈ 2. This opens up the eventual possibility of multispecies single molecule experiments with distinct quantum dots at repetition rates. Altenatively, single species experiments can be performed with 655nm emitting quantum dots at frame rates up to 10,000 Hz, rivalling that possible with gold particles of twice the diameter. Such measurements will however require a CCD camera with similar sensitivity but much faster read-out speeds; the current electron multiplied CCD camera (Andor DV887-ECS) has a maximum readout rate of about 250 Hz. We will next continue our characterization by studying the mobility of single biotin lipids in substrate supported membranes of various compositions. We have also initiated collaboration with Dr. Jakob Lerche Hansen and Christina Lyngsoe at the Laboratory of Molecular and Cellular Cardiology at Copenhagen University Hospital on the migration pattern the G-family receptor Angiotensin II type I receptor (AT1R) in mammalian cells. For this, we now have fusion protein consisting of a FLAG epitope tag, the BLAP sequence and the entire AT1R membrane protein sequence. We are currently performing initial tests on this fusion protein to ensure that it is properly transported to the cell membrane in HEK293 cells. This project will allow us to study a defined membrane protein with our BLAP and quantum dot system.

Instrumentation for advanced microscopy and single-particle tracking

(Dr. Chris Lagerholm, Prof. Luis Bagatolli)

We have assembled two new fluorescence microscopes. One of these is a fully dedicated state-of-the-art non-linear microscope. This microscope is equipped for fluorescence intensity, fluorescence lifetime, second harmonic generation imaging, and scanning fluorescence correlation spectroscopy. This equipment, which is unique in Denmark, has a very wide sphere of applicability, and is planned to be used for a wide range of research projects within modern biology and biomedicine. The equipment was built mainly with grants from FNU (equipment grant, applicant: Luis A. Bagatolli) but also with contributions from BIONET. The second microscope is a general purpose automated Olympus IX-81 microscope for light and fluorescence microscope. It is equipped with a high-sensitivity, fast read-out electron multiplied CCD camera capable of single quantum dot imaging at rates up to about 250 Hz with integration times as low as 100 μsec.

Outreach

Many of the Center members have during the year engaged themselves in a variety of outreach activities aimed at stimulating young people, educating teachers, and informing the general public about science in general and the about the research of the Center in particular. The Center aims at addressing individuals at all levels of education and as early as possible. Among the many activities the following deserves mention: public lectures, open-house activities, Sciencelab for high-school students, ScienceOnline, `Forskerspireprogrammet,' `Unge Forskere', writing of popular activities, as well as work on the virtual science exhibition *Science in Your Eyes* (www.scienceinyoureyes.memphys.sdu). As an integral part of `Forskningens Døgn 2006', members of MEMPHYS organized and developed material for a Gastrophysical Treasure Hunt in Odense City. As an example of the recognition of the research communication it could be mentioned that the Center director was nominated as one out of five for the Danish national prize in research communication. Members of the Center continue to be involved in the project *Naturlige Udfordringer* (www.natnet.dk) creating an internet-based database with science challenges for high-school students.

Seven members of the Centre have during late Summer and Fall of 2006 been engaged in a collaboration with the Centre for Art and Science at the University of Southern Denmark and created and developed a science theatre production, *The Magic Bullet,* which describes some of the research of the Centre. The play was put on stage for eleven public performances during January and February 2007. In connection with the play, a Centre member, Stinne Hørup Hansen, developed a comprehensive package of internet-based teaching material for high-school students.

Short-time Visitors and Seminars

- Dr. Pierre Moens, School of Biological, Biomedical & Molecular Sciences, The University of New England, Armidale, Australia, January 8-21, 2006: "Profilin Binding to Sub-micellar Concentrations of Phosphatidylinositol (4,5) bisphosphate and Phosphatidylinositol (3,4,5) trisphosphate".
- Prof. Robert Cantor, Department of Chemistry, Dartmouth College, NH, USA, January 29-February 11, 2006.
- Dr. Zunjing Wang, AMOLF, FOM-Institute, Amsterdam, The Nederlands, February 2-4, 2006: "Channel Formation in Biological Membranes".
- Dr. Josep Pàmies, Computational Physics Group, FOM-Institute for Atomic and Molecular Physics (FOM-AMOLF), Amsterdam, February 7-8, 2006: "Organization of photosynthetic membranes".
- Dr. Norman Packard, ProtoLife, Venice, Italy, February 8-10, 2006: "A geometrical basis for asymmetric diffusion".
- Prof. Mark Bedau, ProtoLife, Venice, February 8-10, 2006.
- Dr. Emily Parke, ProtoLife, Venice, February 8-10, 2006.
- MSc Jesper Bruun, Odense Tekniske Gymnasium, February 20-26, 2006.
- Dr. Julian Shillcock, Max Planck Institute for Colloid and Interface Research, Golm, Germany, February 22-24, 2006: "The computational route from single amphiphiles to vesicle fusion: mesoscopic simulations of membranes, vesicles and fusion".
- Prof. Paavo Kinnunen, Dept. Medical Chemistry, Helsinki University, Finland, March 2-4, 2006.
- Dr. Pramod Pullarkat, Department of Physics, Universität Bayreuth, Germany, March 16-19, 2006: "Osmotically Driven Shape Transformations in Axons".
- CEO PhD Kent Jørgensen, LiPlasome Pharma A/S, Lyngby, March 17 and 22, 2006.
- Dr. Christa Trandum, Dept. of Chemistry and Biology, RUC, Roskilde, April 2006.
- MSc Olli Punkkinen, Physics Institute, Helsinki University of Technology, Finland, May 2-12, 2006.
- Prof. Mark Williams, Department of Physics and Center for Interdisciplinary Research on Complex Systems, Northeastern University, Boston, MA, USA, May 11-12, 2006: "Thermodynamics of force-induced DNA-ligand interactions".

- Dr. Himanshu Khandelia, Department of Chemical Engineering and Materials Science, University of Minnesota, May 21-24, 2006: "Molecular Dynamics Simulations to Guide the Design of Peptide Antibiotics".
- Prof. Ignacio Pagonabarraga, University of Barcelona, Spain, May 21-24, 2006, "Colloidal (hydro) dynamics at fluid interfaces".
- Prof. Enrico Gratton, Laboratory for Fluorescence Dynamics, University of California, Irvine, USA, June 14-17, 2006.
- Prof. Robert Cantor, Department of Chemistry, Dartmouth College, NH, USA, June 18-28, 2006.
- Prof. Salim Abdali, Quantum Protein Center (QuP), DTU, Lyngby, June 21, 2006: "Single molecule Raman and ROA spectroscopy used in membrane protein experiments".
- Prof. Claus Hélix Nielsen, QuP, DTU, Lyngby, June 21, 2006: "Membrane proteins: Studies of structural changes and lipid anchoring".
- Dr. Bjørn G. Nielsen, QuP, DTU, Lyngby, June 21, 2006: Patch-clamp membrane-protein set-up with Raman signals".
- Ruth Montes, University in Bilbao, Spain, August 2-11, November 12-18, 2006.
- Jakob Broder Brodersen, Odense Universitets Hospital, August 2-September 2, 2006.
- Dr. Ilpo Vattulainen, Physics Institute, Helsinki University of Technology, Finland, August 7-18, 2006.
- Dr. Matthias Weiss, Deutches Krebsforschungszentrum, Germany, August 23, 2006: "Getting a handle on active membrane domains in biology".
- Dr. Stephanie Tristram-Nagle, Biological Physics Group, Physics Dept. Carnegie Mellon University, Pittsburgh, PA, September 11, 2006: "Implications of Structure for Water Permeability Through Fully Hydrated Fluid Lipid Bilayers".
- Prof. Martin Zuckermann, Dept of. Physics, Simon Fraser University, Burnaby BC, Canada, September 9-30, 2006: "A polymer-based Brownian motor: how polymers move through a flashing ratchet potential".
- Prof. Hans Fogedby, Department of Physics and Astronomy, University of Aarhus, Friday, September 22, 2006: "DNA bubble dynamics as a quantum Coulomb problem".
- Dr. Tuula Jalonen, Depts. of Physics and Biochemistry, University of Jyväskyla, Finland, Monday, September 25, 2006: "Studies of canine parvovirus-membrane interactions".
- Professor Daniel Otzen, Protein Biophysics Group, Ålborg University, September 28, 2006: "Lectures as part of KE82, Nanobioscience".
- Dr. Byron Ballou, Molecular Biosensor and Imaging Center, Carnegie Mellon University, USA, October 3-6, 2006: "Quantum Dots and Organic Fluorochromes for *in vivo* imaging".
- Prof. Thomas Heimburg, Niels Bohr Institute Copenhagen, October 11, 2006: "Thermodynamics of pulse propagation in nerves and the role of general anesthetics".
- Prof. Kim Sneppen, RUC, Roskilde, October 30-31, 2006: "Models of Life".
- Professor Daniel Otzen, Protein Biophysics Group, Ålborg University, October 30-31, 2006: "Exploring the diversity of protein conformational changes".
- Nader Reihani, October 30-31, 2006: "Stretching of short nucleic acid polymers with optical tweezers".
- Prof. Thomas Heimburg, Niels Bohr Institute Copenhagen, October 30-31, 2006: "Pulse propagation in nerves".
- Mille Michelsen, October 30-31, 2006: "Modeling Epigenetics in Eukaryotes".
- Peter Astrup Christensen, October 30-31, 2006: "Folding of membrane proteins and glucagon fibrillation".
- Anna Andersson, October 30-31, 2006: "Iron homeostatis in *E. coli*".
- Sanne S. Pedersen, October 30-31, 2006: "Associations of OmpA fragments and unfolding of α-lactalbumin in detergents".
- Dr. Sandeep Krishna, October 30-31, 2006: "Patterns in genetic oscillations".
- Dr. Jesper Borg, October 30-31, 2006: "Towards structure prediction of folded and unfolded proteins".
- Dr. Kristmundur Sigmundsson, October 30-31, 2006: "Thermodynamic experiments on nerves".
- Tony Ebdrup, October 30-31, 2006: "Binding of surfactant proteins to membranes".
- Morten Dueholm, October 30-31, 2006: "Bacterial amyloids".
- Dr. Amir Berman, Israel, November 15, 2006: "Biological and Inorganic Biomimetic Interfaces".
- Michael Sonne Hansen, DTU, Lyngby, November 28, 2006: "Tube Representations of Proteins: Using Geometrical Notions to Construct Simplified Models".
- Prof. Søren Nielsen, Aarhus University, December 6, 2006: "Aquaporins and disease".
- Dr. Urban Johanson, University of Lund, Sweden, December 6, 2006: "Structural mechanism of aquaporin gating".
- Peter Holme Jensen, Aquaporin Aps, Copenhagen, December 6, 2006: "Aquaporins for industrial water filtration".
- Dr. Vesa-Matti Loitto, University of Linköping, Sweden, December 6, 2006: "Expression of AQP9 induces a filopodial phenotype and plays a key role in cell motility".
- MSc Kirsi Pakkanen, Nanoscience Center, Department of Biological and Environmental Science, University of Jyväskylä, Finland, December 12-14, 2006: "Virus-membrane interactions: novel ways to cross a semi-liquid barrier".

Aalborg University, AAU

The AAU node of BioNET

Responsible scientist	Prof. Daniel E. Otzen
Post-doctoral scientists funded by BioNET	Dr. Peter A. Christensen (2005-2007) Dr. Uffe B. Westergaard (2005-2007) Dr. Jesper E. Mogensen (2006)
PhD students funded by BioNET *(students in italics subsidized for the last 4 months of their studies)*	Morten S. Dueholm (2006-) Brian Vad (AAU and SDU, 2005-) Magnus Franzmann (2007-) *Tony Ebdrup (2006)* *Lise Nesgaard (2006)* Sanne Pedersen (2004-)
Post-doctoral scientists involved in BioNET activites but funded elsewhere	Dr. Pankaj Sehgal
Ph.D. students involved in BioNET activites but funded elsewhere	Kell K. Andersen (2005-)
M.Sc. students associated with BioNET	Stine K. Knudsen (2005-2006) Mette M. Nielsen (2005-2006) Lars Kjær (2006-2007) Line Aagot Thomsen (2006-2007) Jonas Høgh Hansen (2006-2007) Peter L. Jensen (2006-2007) Anette Yde (2006-2007)

Major research themes at AAU under BioNET

All supervised by Daniel Otzen.

1. Folding and assembly of membrane proteins modulated by an amphiphilic environment (Peter A. Christensen, Uffe B. Westergaard, Brian Vad. Associations: Pankaj Sehgal.)
2. Single-molecule studies of membrane proteins in a lipid environment (Uffe B. Westergaard, Brian Vad in collaboration with Lene Oddershede, BioNET-NBI, Ole G. Mouritsen, BioNET-SDU and Luis Bagatolli, BioNET-SDU)
3. Properties of membrane-anchored proteins in the outer bacterial membrane (Uffe B. Westergaard, M.Sc. student Stine K. Knudsen)
4. Structural and functional studies of outer membrane proteins (Magnus Franzmann, Sanne Pedersen. Associations: Kell K. Andersen)
5. Interactions of water-soluble proteins with membrane-like environments (Jesper E. Mogensen, Sanne Pedersen, M.Sc. students Mette M. Nielsen and Line Aagot Thomsen and Lars Kjær in collaboration with Thomas Heimburg, BioNET-NBI. Associations: Kell K. Andersen)
6. Biophysical properties of surfactant proteins (Tony Ebdrup in collaboration with Beate Klösgen, BioNET-SDU)
7. Functional and structural properties of bacterial amyloid protein (Morten S. Dueholm, M.Sc. students Peter L. Jensen and Annette Yde)
8. Molecular basis of the amyloid disease Familial Danish and British Dementia (Lise W. Nesgaard)
9. Single-particle and spectroscopic analysis of the fibrillation of the glucagon hormone (Peter A. Christensen. Association: Christian B. Andersen. Collaboration with Mogens Høgh Jensen, BioNET-NBI)

PhD-theses supervised under BioNET

All supervised by Daniel Otzen. Tony Ebdrup and Lise Nesgaard subsidized for the last 4 months of their studies.

1. Brian Vad, University of Aalborg and University of Southern Denmark (also supervised by Ole G. Mouritsen): "Atomic force microscopy studies of membrane proteins." (2005-).
2. Morten S. Dueholm: "Functional studies of bacterial amyloid" (2006-)
3. Magnus Franzmann: "NMR studies of bacterial membrane proteins" (2007-)
4. Sanne Pedersen: "Association of fragments of the outer membrane protein OmpA" (2004-)
5. Tony Ebdrup: "Biophysical studies of surfactant proteins" (2003-2007)
6. Lise Nesgaard: "Molecular basis of Familial Danish and British Dementia" (2003-2007)

Networking within BioNET and outside

BioNET-funding has proved invaluable from a project-organizing perspective in several ways:

1. Funding provided by BioNET has provided the seed to initiate and sustain a number of projects. This has occurred by allowing us to hire a number of people that have acted as anchors for related projects carried out by M.Sc. students or post-docs/Ph.D. students hired on other resources, cfr. the overview of people involved in various projects at the beginning of the AAU section of the report.

2. As outlined in the original proposal and subsequently elaborated in several new projects, we have established firm collaborative links to all leading scientists within BioNET, involving both SDU (Ole G. Mouritsen, Luis Bagatolli and Beate Klösgen) and NBI (Mogens Høgh Jensen, Lene Oddershede and Thomas Heimburg).

3. We have also formed and strengthened collaborations with external partners, including academics (Professor Peter Westh, Roskilde University, Dirk Schneider, University of Freiburg and Professor Jesús Pérez-Gil, Universidad Complutense, Madrid) and companies (Dr. Poul Sørensen at Leo-Pharma).

Research report

Folding and assembly of membrane proteins modulated by an amphiphilic environment

(Peter A. Christensen, Uffe B. Westergaard, Lise Nesgaard. Associations: Pankaj Sehgal.)

The inner membrane protein DsbB folds reversibly in mixed micelles, and we have previously shown that this conforms to a simple three-state system involving an unfolding intermediate. We have subsequently carried this folding out over a wide temperature range to perform the first thermodynamic analysis of reversible unfolding of a membrane protein and find that the process to a large extent may reflect the interactions of detergent with protein. Useful predictions with relation to the binding of detergent to the protein surface may be made in this way.

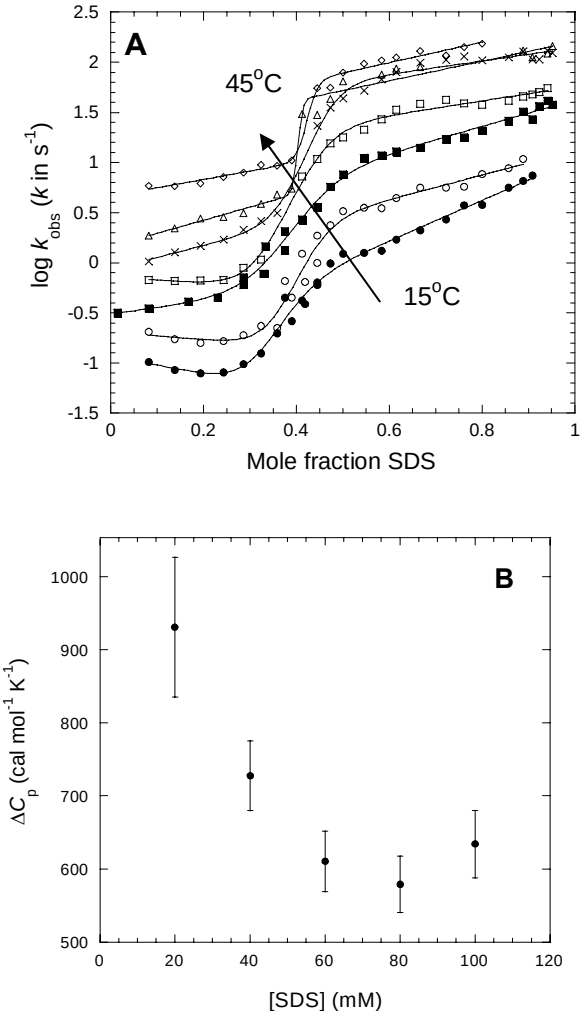


Figure 1. (A) Temperature dependence of the refolding and unfolding kinetics of DsbB between 15 and 45°C, leading to an unexpectedly negative activation heat capacity of unfolding (-1.91 to -2.75 kcal/mol/K between 0 and 0.4 mole fraction SDS). (B) In contrast, the activation heat capacity of unfolding of the water-soluble protein S6 in SDS is positive (0.93 kcal/mol/K in 20 mM SDS).

A subsequent study on the structures of DsbB and the other α-helical membrane protein NhaA in different alcohols has revealed a number of different conformational states, including a very precipitation-prone β-sheet state as well as a soluble "super-α-helix" state. Neither of these states refolds back to the native state. The efficiency of precipitation, and the degree to which DsbB is destabilized at low alcohol concentrations, show the same correlation with alcohol hydrophobicity. Thus, in addition to their effect on the membrane, alcohols perturb membrane proteins directly by solvating the hydrophobic regions of the protein. At intermediate concentrations, this perturbation exposes hydrophobic segments but does not provide sufficient solvation to avoid intermolecular association. Resolubilization requires a reduction in the relative dielectric constant below 65 in conjunction with specific properties of the individual alcohols.

The study was prompted by the desire to find an alternative to mixed micelles to study reversible unfolding of membrane proteins, but the inability to do so indicates that the mixed micelles remain the best approach for these studies.

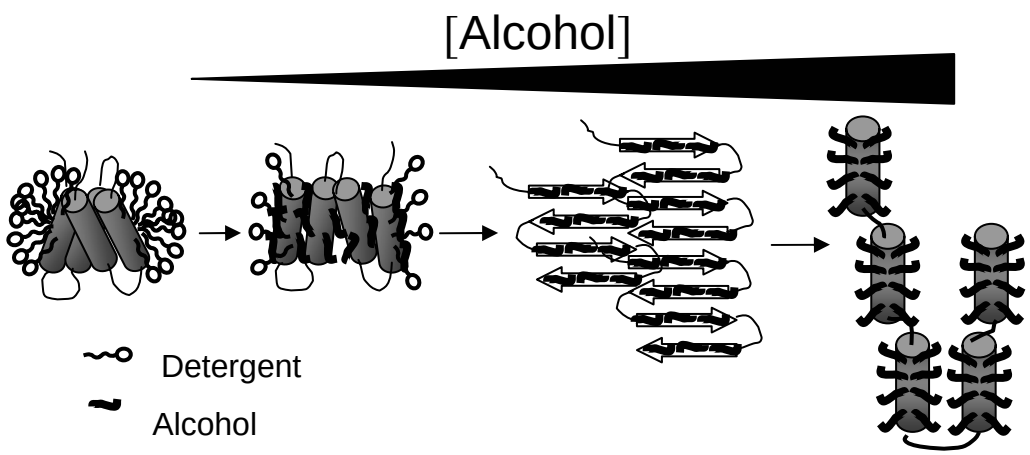


Figure 2. Model for interactions of DsbB with alcohols at different concentrations. At low concentrations, a β-rich precipitated state is induced which is sbubsequently solubilized at higher concentrations.

To strengthen the general applicability of our kinetic studies with the mixed-micelle approach, we have studied to the folding of the membrane protein Mistic, which we cloned, expressed and purified for this purpose. Mistic shows clear differences to DsbB in the folding mechanism which reveals a significant variability in membrane protein folding (P.A.C and D.E.O., manuscript in preparation).
The previous work has focused on monomeric membrane proteins but we would also like to analyze how the stability and association of oligomeric membrane proteins can be modulated by these environments. In collaboration with Dr. Dirk Schneider at the University of Freiburg, we have managed to purify the tetrameric aquaglycerotransporter GlpF from *E. coli* and have developed a simple assay to follow its tetramerisation under different conditions (U.B.W. and D.E.O., in preparation) which we are currently pursuing in more detail.

Single-molecule studies of membrane proteins in a lipid environment

(Uffe B. Westergaard, Brian Vad in collaboration with Lene Oddershede, BioNET-NBI, Ole G. Mouritsen, BioNET-SDU and Luis Bagatolli, BioNET-SDU)
We have managed to clone and express low but workable amounts of biotinylated DsbB in the inner membrane of *E. coli*, which we are currently analyzing using optical tweezers to study the dynamics of their interactions in the membrane. We expect to complete this work within the coming year together with Ph.D. student Tabita Madsen and Dr. Lene Oddershede. Concurrently, we have optimized procedures to label the inner membrane protein NhaA for single-molecule studies in synthetic vesicles, although we have found that it remains a significant limitation that we cannot achieve close to 100% labeling efficiency. This is required when using FRET to investigate interactions between single molecules in large unilamellar vesicles. Consequently, we are in parallel going to investigate how fluorescently labeled variants of the water-soluble but membrane-binding protein α-synuclein aggregate on membrane surfaces.
For progress on Brian Vad's studies on the tethering and folding of DsbB in vesicles, see the SDU part of this report.

Properties of membrane-anchored proteins in the outer bacterial membrane

(Uffe B. Westergaard, M.Sc. student Stine K. Knudsen)
Autotransporters constitute the biggest group of secreted proteins in Gram-negative bacteria and contain a membrane-bound β-domain and an α-domain secreted to the extracellular environment via an unusually long N-terminal sequence. All secretory information is contained within the protein sequence, making them interesting models for cellular folding. Several α-domains are known to be glycosylated by cytosolic glycosyl transferases, promoting bacterial attachment to mammalian cells. We have studied the effect of glycosylation on the extracellular α-domain of the *E. coli* autotransporter Ag43α, which induces frizzy colony morphology and cell settling. We have identified 16 glycosylation sites and suggest two possible glycosylation motifs for Ser and Thr. Glycosylation affects both biophysical and biological properties of Ag43α: it makes Ag43α less prone to C-terminal proteolysis (which occurs around residue 367), stabilizes against thermal and chemical denaturation and increases refolding kinetics. Unexpectedly, glycosylation also reduces the stabilizing effect of Ca^{2+} ions, removes the ability of Ca^{2+} to promote cell adhesion and inhibits the ability of Ag43α-containing cells to form bacterial amyloid. In addition, our data indicate that Ag43α folds without a stable intermediate, unlike pertactin, indicating that autotransporters may arrive at the native state by a variety of different mechanisms despite a

common overall structure. A small but significant fraction of Ag43α can survive intact in the periplasm if expressed without the β-domain, suggesting that it is able to adopt a protease-resistant structure prior to translocation over the membrane. Thus glycosylation plays significant roles in structural and functional properties of bacterial autotransporters at many different levels.

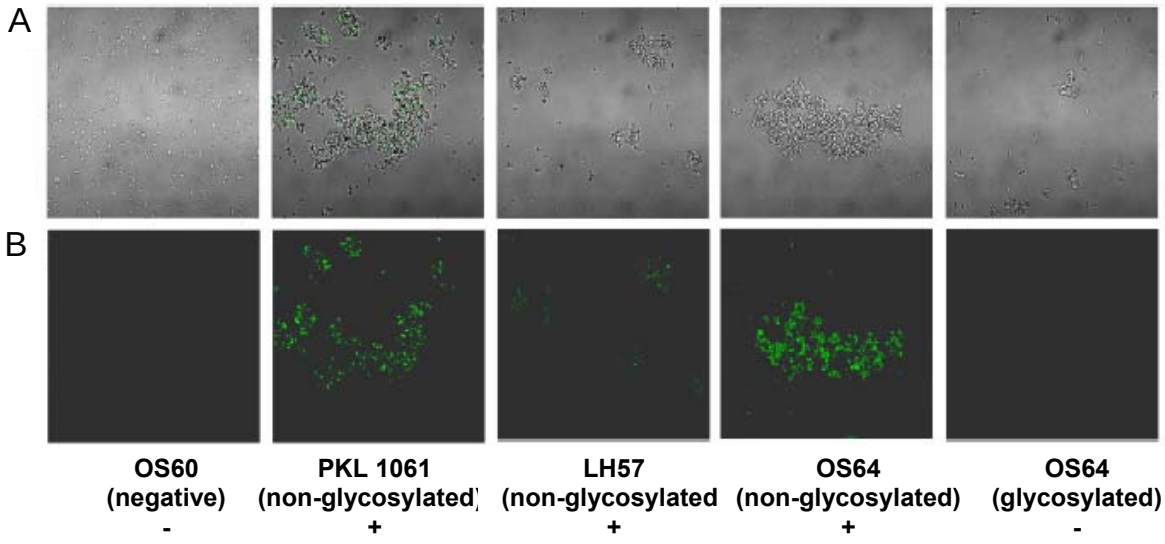


Figure 4. Only non-glycosylated variants of Ag43α are able to form bacterial amyloid structures which stain green with the fibril-binding dye Thioflavin T. This work has been submitted to Journal of Biological Chemistry for publication:

Structural and functional studies of outer membrane proteins

(Magnus Franzmann, Sanne Pedersen. Associations: Kell K. Andersen)

Fragments of α-helical membrane proteins have been shown to associate *in vitro*, supporting the two-stage hypothesis of membrane protein folding. Although the β-barrel membrane protein OmpA can be produced in a biologically viable form in *E. coli* from co-expressed fragments, the fragments have not been demonstrated to associate *in vitro*. We have demonstrated that two separately expressed and purified overlapping fragments of OmpA in both detergents and lipids can form a protease-resistant complex that undergoes the characteristic band-shift upon heating. This demonstrates that although membrane insertion and folding of β-barrel membrane proteins may be a cooperative process, the fragments need not be physically linked although they cannot be folded separately beforehand. This work is submitted to *FEBS Letters* for publication.

We are currently extending this work to obtain fragments of OmpA that have been difficult to express as individual fragments. Instead, we have engineered a genenase-cleavage site between each of the three periplasmic loops to express the three new constructs separately, cleave them with genenase and purify or refold the fragments. We have found that these constructs express to very high levels and can be refolded and cleaved. In this work we are greatly aided by the work by Kell K. Andersen on the mechanism of refolding of OmpA in short-chain detergents which shows a multitude of folding steps (K.K. Andersen and D.E. Otzen, manuscript under preparation).

In addition, we have started a study to elucidate the structure of the membrane-domain of the autotransporter AIDA. This is based on our existing knowledge of the expression, folding and stability of this domain, carried out in a Ph.D. project funded by the Danish Research Councils:

Interactions of water-soluble proteins with membrane-like environments

(Jesper E. Mogensen, Sanne Pedersen, M.Sc. students Mette M. Nielsen, Jonas H. Hansen, Line Aagot Thomsen and Lars Kjær in collaboration with Thomas Heimburg, BioNET-NBI. Associations: Kell K. Andersen).

Within this project, we have used a combination of spectroscopic and proteolytic approaches to characterize the conformational changes that a number of different proteins undergo in contact with either vesicles or detergents.

Bet v 1: The 159 residue Bet v 1 is the major allergen from birch tree pollen. Its natural function is unknown although it is capable of binding several types of physiologically relevant ligands in a centrally placed cavity in the protein structure. We have used circular dichroism and fluorescence spectroscopy to show that Bet v 1 binds to DOPC and DOPG phospholipid vesicles in a pH-dependent manner. Binding is facilitated by low pH, negatively charged phospholipids, and high vesicle curvature, indicating that electrostatic interactions and vesicle surface defects are important parameters for binding. Binding is accompanied by major structural rearrangements, involving an increase in α-helical structure and a decrease in β-structure. A bilayer structure per se is not a prerequisite for these rearrangements, since they also occur in the presence of the micelle-forming lysophospholipids lysoMPC and lysoMPG. Two major bound states (A and B) with distinct secondary structure compositions were identified, which predominate in the pH range ~9.5-6.5 and ~5-2.5, respectively. Despite the high content of secondary structure, the A- and B-states are partially unfolded as they unfold non-cooperatively in CD thermal scans, in contrast to the native state. In addition, the B-state (but not the A-state) shows intermediate proteolysis-resistance and is able to induce complete leakage of calcein from the vesicles indicating that this state is partially inserted into and significantly perturbs the bilayer structure. We conclude that Bet v 1 is a membrane binding protein, highlighting a possible biological function of this protein.

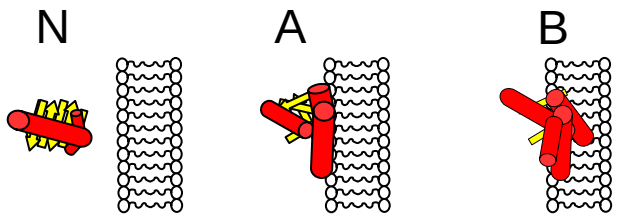


Figure 5.
Different stages of insertion of Bet v 1 into the membrane, depending on the pH and membrane size. State A is predominantly formed with lysolipids and anionic lipids at neutral pH and state B (which is more protected) at lower pH.

Antimicrobial peptide Novispirin: Novispirin G-10 is an 18-residue designed cationic peptide derived from the N-terminal part of an anti-microbial peptide from sheep. This derivative is more specific for bacteria than the parent peptide. We have analyzed Novispirin's interactions with various amphipathic molecules and find that a remarkably wide variety of conditions induce α-helical structure. Optimal structure induction by lipids occurs when the vesicles contain 40-80% anionic lipid, while pure anionic lipid vesicles induce aggregation. SDS also forms aggregates with Novispirin at sub-micellar concentrations but induces α-helical structures above the cmc. Both types of aggregates contain significant amounts of β-sheet structure, highlighting the peptide's structural versatility. The cationic detergent LTAC has a relatively strong affinity for the cationic peptide despite the peptide's net positive charge of +7 at physiological pH and total lack of negatively charged side-chains. Zwitterionic and non-ionic detergents induce α-helical structures at several hundred mM detergent. We have solved the peptide structure in SDS and LTAB by NMR and find subtle differences compared to the structure in TFE, which we ascribe to the interaction with an amphiphilic environment. Novispirin is largely buried in the SDS-micelle, whereas it does not enter the LTAC-micelle but merely forms a dynamic equilibrium between surface-bound and non-bound Novispirin. Thus electrostatic repulsion can be overruled by relatively high detergent concentrations or by deprotonating a single critical side chain, despite the fact that Novispirin's ability to bind to amphiphiles and form α-helical structure is sensitive to the electrostatics of the amphiphilic environment. This emphasizes the versatility of cationic antimicrobial peptides' interactions with amphiphiles.

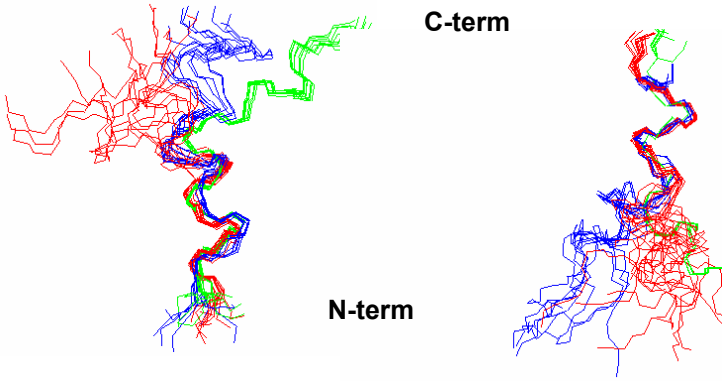


Figure 6. Superposition of the structure of Novispirin in SDS(red), LTAC (blue) and TFE (green).

We have continued this work with the improved variant Novicidin which shows heightened activity towards *E. coli*. By chemically acylating the N-terminal part with acyl chains of 8-16 carbon atoms, we have been able to increase its ability to permeabilize synthetic vesicles. However, the manner of its interaction does not follow a simple linear dependence on the acyl chain length, but may be related to hydrophobic mismatching.
It should be stressed that the collaborations within BioNET has been of major importance for work in our group within this area in particular. Thanks to collaborations with Prof. Luis Bagatolli, we have been able to establish our own facilities for producing Giant Unilamellar Vesicles and analyze their interactions by Confocal Laser Scanning Microscopy, which has become very useful in the analysis of the interactions of antimicrobial peptides with lipid vesicles, as the figure below indicates.

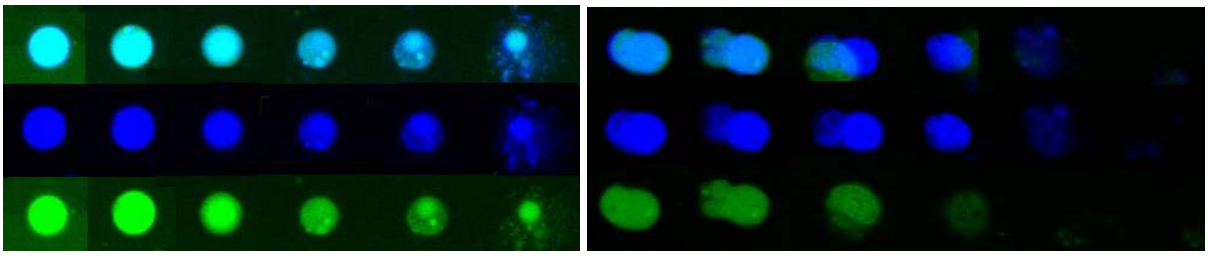


Figure 7. Dissolution of anionic DOPG vesicles by Novispirin (left) and Novicidin (right) over time. The vesicles contain a green lipid (bottom row) as well as a blue fluorescent Alexa dye solubilized in the interior (middle row). The parallel loss of the two dyes (merged in top row) indicates that the vesicles are disrupted by the antimicrobial peptides through general dissolution rather than formation of discrete pores (where the blue dye could diffuse out, leaving the green lipid intact).

α-synuclein: The central protein in Parkinson 's disease, α-synuclein, is known to form α helical structure when in solution with a number of charged lipids. Previous studies have shown that there is a connection between the melting point of the lipid and the binding ability. In collaboration with Dr. Thomas Heimburg, we have shown that there is a complex interplay between membrane structure and α-synuclein conformation. A cooperatively folded structure is only induced above a certain temperature which only partially depends on the vesicle melting temperature, and is subsequently lost at even higher temperatures. However, the structure is not induced at vesicle sizes lower than around 200 nm. We believe these observations may have relevance for both the biological role of α-synuclein in the formation of synaptic vesicles and its modes of interaction with membranes during aggregation.

We are also engaged in very comprehensive studies of the interaction of detergents with soluble proteins. Although these studies employ standard spectroscopic and calorimetric techniques and are not experimentally particularly advanced, the opportunity to compare a large number of different proteins with very thorough investigations have led us to deeper insights into the varied ways in which protein conformations can be modulated by the environment and allowed us to build up specific models for these interactions. It is a great advantage to be able to compare between different model protein systems to test the generality of our observations.

α-lactalbumin: This Ca-binding protein has a remarkable property of specifically targeting tumour cells when bound to specific lipids in the demetellated state. Consequently, we have performed a detailed study of the interaction of the demetellated state of this protein with different detergents. Remarkably, the protein unfolds in the presence of all detergents, although there are clear differences in the mechanism of unfolding, depending on the head group and chain length. However, a combination of monomeric and micellar interactions appear to be necessary to induce unfolding, underlining the versatility of the interaction.

β-sheet proteins: β-sheet proteins are particularly resistant to denaturation by SDS. We have compared unfolding of two β-sandwich proteins TNfn3 and TII27 in SDS. The two proteins show different surface electrostatic potential. Correspondingly, TII27 unfolds below the critical micelle concentration (cmc) via the formation of hemi-micelles on the protein surface, whereas TNfn3 only unfolds around the cmc. Isothermal titration calorimetry (ITC) confirms that unfolding of TII27 sets in at lower SDS concentrations, although the total number of bound SDS molecules is similar at the end of unfolding. In mixed micelles with the non-ionic detergent dodecyl maltoside (DDM), where the concentration of monomeric SDS is insignificant, the behaviour of the two proteins converges. TII27 unfolds more slowly than TNfn3 in SDS and follows a two-

mode behaviour. Additionally TNfn3 shows inhibition of SDS unfolding at intermediate SDS concentrations. Mutagenic analysis suggests that the overall unfolding mechanism is similar to that observed in denaturant for both proteins. Our data confirm the kinetic robustness of β-sheet proteins towards SDS. We suggest this is related to the inability of SDS to induce significant amounts of α-helix structure in these proteins as part of the denaturation process, forcing the protein to denature by global rather than local unfolding.

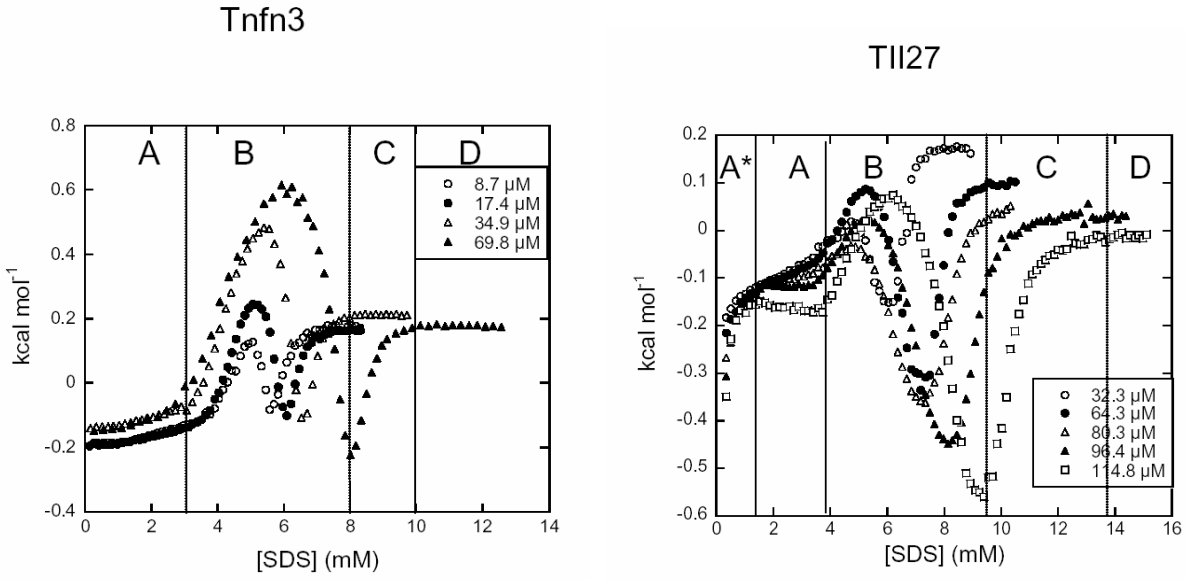


Figure 8. Isothermal titration calorimetry analysis of the binding of SDS to Tnfn3 and TII27. Note the existence of an extra binding phase (AP) for TII27 as well as the difference in relative magnitude of the exothermic and endothermic processes for the two proteins.

Myoglobin: Detergents interact with proteins in multifarious ways which depend on detergent concentration and structure. To obtain a global overview of this process, we have analyzed the interaction of horse myoglobin (Mb) with an anionic (SDS) and cationic (CTAC) detergent, respectively, using a combination of different types of equilibrium titration (isothermal titration, Trp fluorescence, thermal denaturation and pyrene fluorescence) and stopped-flow kinetics. The use of kinetics allows us to extend our observations above the critical micelle concentration as well as providing mechanistic insight into the reaction. Overall, a clear picture emerges, in which interactions proceed through at least 5 distinct stages below the cmc. These phases are broadly similar between the two detergents, despite their difference in head group and chain length. In stage A (below 0.12 mM detergent), fairly weak unspecific binding is only observed through ITC and a linear decrease in thermal stability. This gives way to a more specific binding in stage B, where aggregates (presumably hemi-micelles) form on the protein surface, leading to global denaturation (loss of a thermal transition) and a biphasic unfolding. Stoichiometric (*i.e.* titratable) binding of detergent molecules only occurs in stage C, leading to inhibition of unfolding in CTAC and an altered mode of unfolding in SDS, though still biphasic in both detergents. Stage D (ca. 1-2 mM detergent) is mainly a transition, leading to further uptake of 5 (SDS) or 12 (CTAC) detergent molecules. In Stage E we observe binding of a significantly greater number of detergent molecules (68 SDS and 110 CTAC), presumably as quasi-micellar structures. This stage sees the rise of a very slow unfolding phase in SDS, which as we approach the cmc. Above the cmc, the unfolding rates remain essentially constant in SDS, but increase significantly in CTAC, presumably because binding of bulk micelles removes the inhibition by hemi-micellar aggregates. Our work highlights the fascinating richness of conformational changes that proteins can undergo in detergents.

Biophysical properties of surfactant proteins

(Tony Ebdrup in collaboration with Beate Klösgen, BioNET-SDU)

Lung surfactant is a mixture of approximately 90% phospholipids and 10 % protein which in cooperation prevent alveolar collapse during exhalation. They are presumed to do so via structural rearrangements of the lipid biolayer, which leads to adjustments in surface tension as the alveoli expand and shrink in the respiration. The two very hydrophobic surfactant proteins SP-B and SP-C play a crucial role in this process. Using the quartz crystal microbalance to monitor the collapse of vesicles on a quartz surface, we have been able to demonstrate that physiological concentrations of SP-B completely abolish the normal collapse behaviour, leading to a more elastic coverage. Different fragments of SP-B show different modulations of this behaviour,

indicating that the 79-residue SP-B protein achieves this property by cooperation between different parts of the protein.

We have also carried out extensive spectroscopic analyses using acrylamide quenching and general tryptophan fluorescence to quantitate the degree of burial of the peptides in the membrane environment and find significant variations between the different peptides, which may be correlated to their action on membrane collapse.

We have planned to extend this work with Small Angle Neutron Scattering analyses to further analyze how SP-B interacts with the lipid.

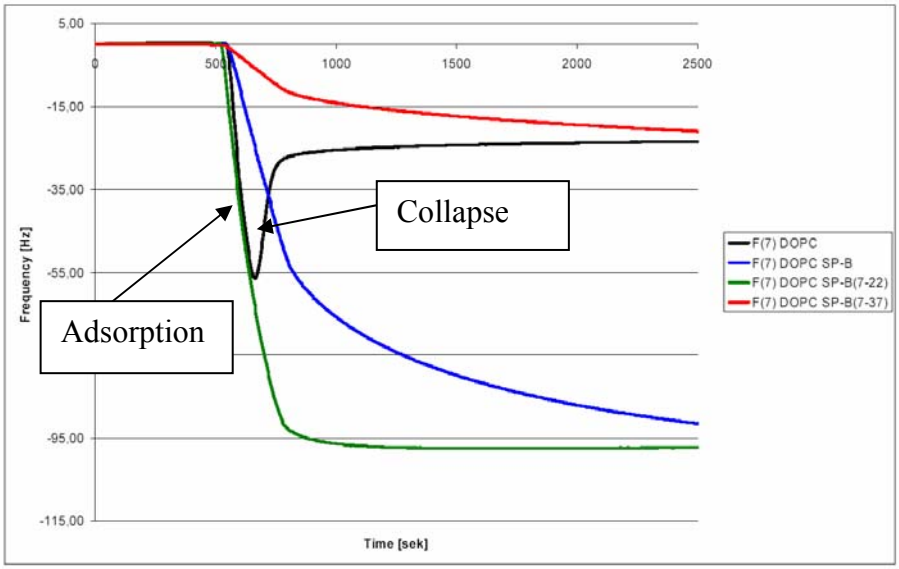


Figure 9. Adsorption and fusion of pure DOPC vesicles (black) on a quartz surface is dramatically hindered by 2% (w/w) full-length SP-B (blue) as well as by different fragments of the protein (red and green). These peptides also show anti-microbial properties according to other studies.

Functional and structural properties of bacterial amyloid protein

(Morten S. Dueholm, M.Sc. students Peter L. Jensen and Annette Yde)

Traditionally, protein aggregation is regarded as a pathological phenomenon, linked to diseases such as Alzheimer's and Parkinson's. However, recent work suggests that bacteria can produce amyloid with beneficial functional properties, relevant for biofilm formation and surface anchoring, although a frequent source of annoyance in the form of plaque, biocorrosion and industrial fouling. We reasoned that these fimbriae will provide an excellent system for *in vivo* studies of amyloid formation, as they are expected to be optimized for fibrillation. Using a combination of fibril-binding dyes, conformationally specific antibodies and fluorescence *in situ* hybridization techniques, we find that bacterial strains producing amyloid-like fimbriae constitutd at least 5-40% of all prokaryotes present in the biofilms, depending on the habitat. Particularly in drinking water biofilms, a high number of amyloid-positive bacteria were identified. The new approach is a very useful tool for further culture-independent studies in mixed microbial communities, where the abundance and diversity of bacteria expressing amyloid adhesins seems much greater than hitherto anticipated. We have studied one of these isolates, a *Pseudomonas* sp., in more detail, using an optimized purification protocol. The amyloid-like structure was verified by Fourier-transform infrared spectroscopy, circular dichroism, and Thioflavin T fluorescence. Fibrillation could be reproduced *in vitro*. Partial sequencing by MS/MS revealed that the fimbrin contained at least one repeated motif. However, this motif was different from those previously found for curli fimbriae monomers described for *E. coli* or the prion proteins. This suggests that sequence repetition may combine with amino acid sequence to regulate the amyloid forming propensity of the fimbrins. Fimbriae from different bacterial species may provide a useful and diverse library of fibrillating sequences that can offer further insight into the fibrillation process.

We have also demonstrated that among Gram-positive species within the *Nocardia* family, functional amyloid appears to be an integrated part of the cell envelope. In addition, fragments of the major component of *E. coli* functional amyloid can modulate the formation of this amyloid *in vitro* and *in vivo*, and we envisage that this may in the longer perspective lead to new strategies to control the growth of these biofilms.

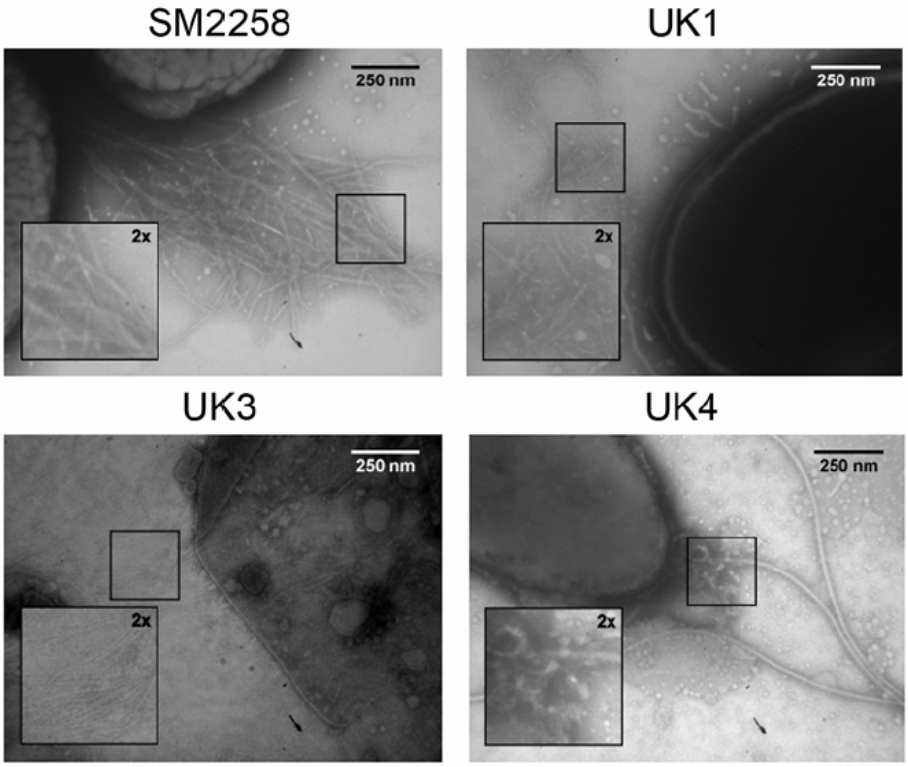


Figure 10. Formation of functional amyloid from a previously characterized E. coli strain (SM2258) as well as 3 previously unknown isolates (UK1-3).

Molecular basis of the amyloid disease Familial Danish and British Dementia

(Lise W. Nesgaard)

The Familial Danish and British Dementias (FDD and FBD) are examples of degenerate neurofibrillar diseases of the same type as the more common and better characterised Alzheimer's disease. Both FDD and FBD involve the formation of 34-amino acid peptides (ADan and ABri), which deposit in the brain as insoluble amyloid fibrils or plaques. The illnesses involve progressive dementia and are fatal with death occurring at the age of 50-60. However, there are differences in the presented symptoms. FDD patients develop cataracts and loss of hearing while FBD is characterised by spastic paralysis. We have carried out a biophysical study of a related peptide termed SerADan in which the two cysteines found in ADan have been changed to serines to emulate the reduced peptide variant isolated from plaques. SerADan aggregates rapidly at pH 5 and 7.5 and subsequently precipitates in a concentration-dependent manner that can be accelerated by addition of preformed aggregates. The structures do not bind amyloid specific dyes but have a high content of β-sheet demonstrated by circular dichroism and infrared spectroscopy. The aggregates are very large and are composed of several morphological types including long, thin fibres similar to amyloid and observable by electron microscopy and atomic force microscopy. The precipitation of SerADan is prevented at neutral pH by the presence of anionic lipids. This effect is partly abolished by increase in ionic strength and does not occur for zwitterionic lipids, indicating an electrostatic mode of interaction. Fresh peptide added to calcein containing vesicles causes vesicle rupture while the peptide appears to interact with the released negatively charged calcein, further supporting lipid interaction and ionic interaction. The lipid bound peptide species isolated with lipid vesicles are also high in β-sheet content, but no fibres can be observed by atomic force microscopy. Thus SerDan appears to rapidly form aggregates with several amyloid-like characteristics but without the typical nucleation-derived lag phase and binding of amyloid specific dyes. Pathologically aggregating peptides of similar sizes may fibrillate by very different mechanisms, and this may be linked to the ensuing clinical symptoms

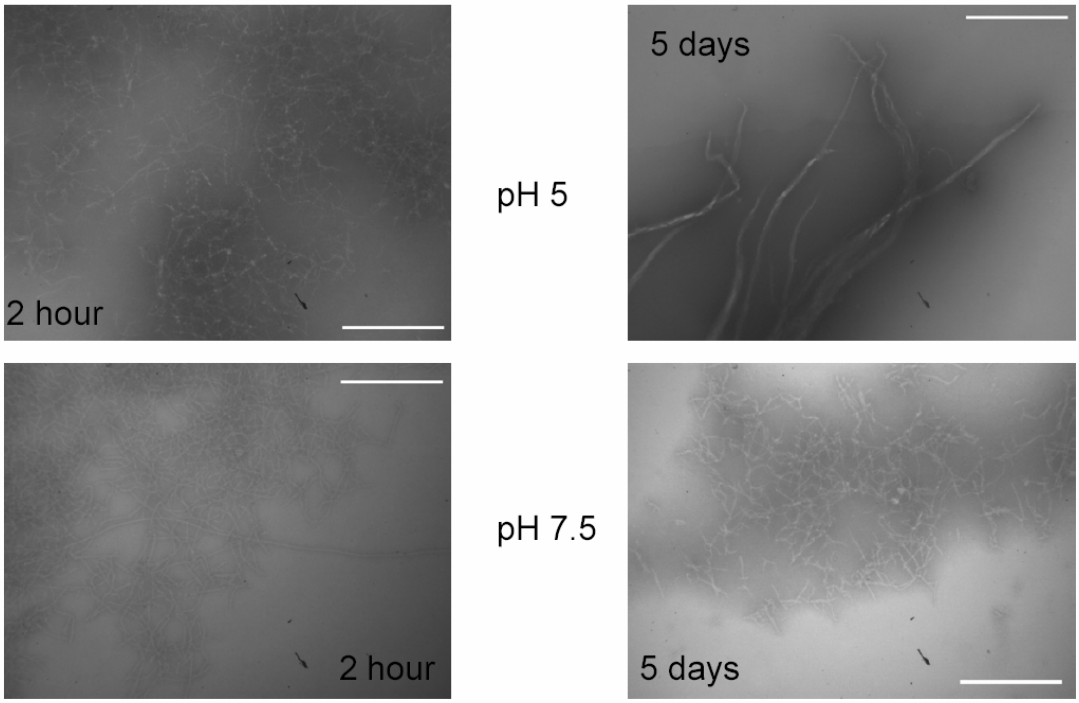


Figure 11. Aggregates of ADan formed after different lengths of incubation at pH 5 and 7.5.

As part of our efforts to describe the structure of the ABri and ADan aggregates, we have turned to vacuum-UV circular dichroism using the synchrotron radiation source at Aarhus University. This facility offers a wider spectroscopic range and can for dry proteins films be applied down to approximately 130nm, which could provide new structural information. In order to build up a data base linking spectra to structure, and thus interpret spectra of new samples, we have recorded spectra down to 130 nm for thirteen model proteins including α-helical, β-sheet and mixed-α/β proteins. Importantly, the proteins do not appear to undergo major structural changes upon drying since spectral features seen in the solution state (down to around 180-175 nm) are retained in the dry state. Synchrotron CD reveals a new low-range peak in the range 130-175nm, which is sensitive to the secondary structure of the protein: while all α-helix containing proteins show the same peak at 140 nm (though with different intensities compared to the major peak at 190 nm, the β-sheet proteins show great variation in this regard. Nevertheless, ABri aggregates and fibrils of lysozyme and α-synuclein all show the same peak top around 140-150 nm, indicating a marked common feature of organization despite subtle variations in architecture. Given a suitable amount of structural information from *e.g.* solid state NMR on a select number of fibrils, we expect that synchrotron CD can provide detailed structural information on a large number of fibrils.

Single-particle and spectroscopic analysis of the fibrillation of the glucagon hormone

(Peter A. Christensen. Association: Christian B. Andersen. Collaboration with Mogens H. Jensen, NBI)
Glucagon is a 29 amino acid peptide used in the treatment of severe hypoglycaemic incidents in diabetics. Glucagon readily fibrillates, and this process is readily monitored the fluorescence from the fibril specific dye Thioflavin T. Glucagon fibrillation is characterized by a very long phase, *the lag phase*, where apparently no fibrillation occurs, followed by a rapid increase in ThT fluorescence. This peculiar behaviour can only be explained by taking so-called *secondary processes* – *e.g.* fibril breakage and fibril branching – into account. However, so far experimental evidence of these proposed secondary processes has been scarce. *Total Internal Reflection Fluorescence Microscopy* (TIRFM) is a technique which enables real time observations of fibrils growing on quartz surfaces. We have recorded real time observations of seeded glucagon solutions during a visit to the lab of Yuji Goto, University of Osaka. The data shows that fibril branching is an important mechanism in glucagon fibrillation.

We plan to investigate these data by studying the size distribution of the fibrils as a function of time. By digitizing snapshots of the recordings we identify each fibril and measure its size and subsequently obtain the size distribution at that particular instant. The distribution will vary in time in a manner that depends on the external conditions (temperature, ph., salt concentration etc.), but we believe that general features of the

dynamics can be elucidated by a proper dimensionless rescaling of the variables. From the experience in related processes of crystal and polymer dynamics, simply dynamical processes such as diffusion, coagulation and fragmentation is expected to govern this time evolution. By identifying these fundamental processes we will learn a lot about fibril dynamics. Of particular interest here will be to extract and model the intermediate oligomeric states in the fibrillation process and to clarify whether these oligomers are on- or off-pathway.

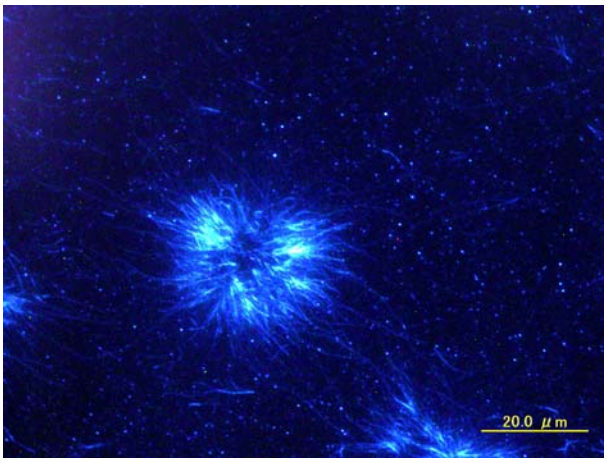


Figure 11. Fibrils of glucagon observed by TIRFM.

Conferences & Workshops

Opening of BioNET
Danish Center for Biophysics

18 March 2005

Preliminary Program

Videnskabernes Selskab,
H.C. Andersens Boulevard 35, Copenhagen

KDVS Meeting room (first floor)

09.30-09.45	Short introduction by Professor Ole Mouritsen, SDU
09.45-12.30:	BioNET's Internationale Committee: Professor Luis Serrano, Heidelberg, Professor Lukas Tamm, Virginia, Professor Joel Stavans, Weizmann, Israel
10.30-11.00	Coffee break
12.30-13.30:	Lunch (third floor)

KDVS Meeting room (first floor)

14.00-15.30	Official Opening Center Director, Professor Mogens Høgh Jensen, University of Copenhagen Contribution by Industrial Manager Professor Daniel Otzen, University of Ålborg

KDVS Meeting room (third Floor)

15.30-16.30:	Reception.

SKIN: Redefining Borders

A one-day multidisciplinary workshop

Thursday December 16th, 2004
University of Southern Denmark
10-16h00, Auditorium 99

Everyone is welcome - no registration necessary!

Programme

9.45h Preworkshop coffee

10.00h Introducing SKIN
10.10 - 11.00h **Lars Norlén** (Stockholm) *Lipid and protein conformations of the human stratum corneum as revealed by cryo-electron microscopy of vitreous sections of native skin*

11.00 - 11.15h Coffee break

11.15 - 11.45h **Karsten Kristiansen** (Odense) *Crosstalk between lipid signaling and inflammation in normal and diseased skin*
11.45 - 12.15h **Mette Ingemann** (LEO Pharmaceuticals) *Choosing excipients for a medicinal product for cutaneous application*
12.15 - 12.30h Discussion Topic led by Jenifer Thewalt

12.30 - 13.15h Lunch

13.15 - 14.05h **Lars Bolund** (Aarhus) *Skin as a model for studies of stem cell biology and degenerative diseases caused by protein conformation problems*
14.05 - 14.25h **Luis Bagatolli** (Odense) *Some practical ideas to address the role of lipid composition in skin tissue: from model systems to the real biological scenario*

14.25 - 14.45h Coffee & fruit break

14.45 - 15.35h **Jenifer Thewalt** (Vancouver) *Drunk and disorderly: stratum corneum model membranes under the influence of ethanol*
15.35 - 16.00h Discussion Topic led by Lars Norlén

16.00h Farewell

Organizers: Amy Rowat and Ole G. Mouritsen, MEMPHYS - Center for Biomembrane Physics
contact: rowat@memphys.sdu.dk http://www.memphys.sdu.dk

beauty is only microns deep

NanoBiC bioTEAM™ south BioNET Danish Center for Biophysics MEMPHYS

Physics of Life

From Single Molecules to Networks

Workshop in biological physics

Krogerup Højskole, Denmark, 21 - 27 August 2005

Scope: This workshop focuses on the dynamics of single molecules and the dynamics of biological networks. Through tutorial lectures and seminars of invited speakers the workshop will cover both experimental techniques and theoretical topics. The workshop includes poster sessions and contributed talks by the participants. The workshop is mainly intended for graduate students and postdoctoral fellows in biological or soft matter physics, and related fields.

Physics of Life - Preliminary PROGRAMME - Krogerup, 21st to 27th August -- Click on speakers' names to view abstracts --							
Time	**Sunday**	**Monday**	**Tuesday**	**Wednesday**	**Thursday**	**Friday**	**Saturday**
08:15 - 08:30		Welcome	Announcements ca. 3 min before lecture			ESF presentation	
08:30 - 10:15		Stavans	Hwa	Schwille	Phillips	Weitz	Breakfast and departure
10:15 - 10:45		Coffee break					
10:45 - 12:30		Dholakia	Palsson	Grubmüller	Seifert	Williams	
12:30 - 15:00	Arrival	Lunch and Leisure					
15:00 - 16:00		Curtis	Maslov	Bagatolli	Wuite	Neumann	
16:00 - 17:00		Losert	Bornholdt	Student talks	Evilevitch	Goksör/Hanstorp	
17:00 - 18:00		Fournier	Brockmann		Dietler	Student talks	
18:00 - 20:00	Mixer with sandwiches	Dinner and Leisure		Conference dinner	Dinner and Leisure	Farewell PARTY	
20:00 -		Student talks and Posters			Posters		

Wed 17 - Sat 20 May 2006, Copenhagen, Denmark

Sponsors: NORDITA, BioNET and Niels Bohr Fond; Nonlinearity

Themes: The workshop focuses on recent advances in physics of complex systems, classical chaos and turbulence, wave and quantum chaos, and much exceptional magic beyond.

Speakers: E Aurell, V Baladi, M V Berry, E Bogomolny, T Bohr, A de Carvalho, A de Wijn, C P Dettmann, B Eckhardt, J-P Eckmann, C Ellegaard, M J Feigenbaum, H Fogedby, T Geisel, P Grassberger, G Gunaratne, F Haake, A G Jackson, J Keating, H Kohler, U Kuhl, P Muratore Ginanneschi, S Nonnenmacher, M Oxborrow, M Paczuski, R Penrose, J Parrondo, M Porter, I Procaccia, A Richter, U Smilansky, N Sondergaard, E A Spiegel, L Tuckerman, F Waleffe, A Wirzba

Scientific direction: B Lautrup (chair), G Tanner (co-chair), P Cvitanović, A Brandenburg, M H Jensen, R Artuso, S Creagh, T Guhr, G Huber, H-H Rugh. **Artistic direction:** P Cvitanović.

Registrations, with a brief description of research interests should be entered into the conference website before **1 April 2006**. Junior researchers applying for partial support should also arrange for letter(s) of reference. For information please contact:

Secretary: Hanne Christensen, Niels Bohr Institute, Blegdamsvej 17, DK - 2100 Copenhagen Ø
www.cats.nbi.dk / Tel: +45:35 32 52 60 / Fax: +45:35 32 52 17 / cats [at] nbi.dk

Nonlinearities '06 - Nordita and Niels Bohr Institute Thematic Workshop

Wednesday, May 17

8:30-8:55	**Registration**	
Chair: **Benny Lautrup**		
9:00-9:25	**Andreas Wirzba**	Casimir effect and trace formula
9:30-9:55	**Clive Ellegaard**	Development in Acoustic Chaos
10:00-10:25	**Niels Søndergaard**	Wave chaos in elastodynamics

Refreshments

Chair: **Thomas Guhr**		
11:00-11:25	**Achim Richter**	Superscars and Nodal Domains in the Barrier Billiard
11:30-11:55	**Eugene Bogomolny**	SLE and nodal lines
12:00-12:25	**Ulrich Kuhl**	Flow structures in open microwave cavities
12:30-12:55	**Stéphane Nonnenmacher**	Resonances and resonant states in chaotic scattering

Lunch

Chair: **Stephen Creagh**		
14:30-14:55	**Michael V. Berry**	Conical diffraction: imaging Hamilton's diabolical point
15:00-15:25	**Fritz Haake**	Semiclassical theory of universal spectral statistics and transport
15:30-15:55	**Mark Oxborrow**	Clocks based on Quantum Chaos (Temporal Order Out Of)?

Refreshments

Chair: **Chris Pethick**		
16:30-16:55	**Mason Porter**	Bose-Einstein Condensates in Optical Lattices and Superlattices
17:00-17:25	**Andy Jackson**	Anaesthetics without pain

20:00 Zappa plays Zappa, Falkoner Salen (not part of the workshop)

Thursday, May 18

Chair: **Mogens H. Jensen**		
9:00-9:25	**Erik Aurell**	Heuristics for satisfiability problems
9:30-9:55	**Astrid de Wijn**	Analytical Lyapunov spectra of high-dimensional billiards
10:00-10:25	**Maya Paczuski**	Turbulence and networks on the earth and elsewhere

Refreshments

Chair: **John Hertz**		
11:00-11:25	**Peter Grassberger**	Mutual information: from phylogenetic trees to spectral analysis
11:30-11:55	**Hans Fogedby**	Growth and Pattern Formation in the KPZ equation
12:00-12:25	**Jean-Pierre Eckmann**	Billiards as heat conductors
12:30-12:55	**Gemunu Gunaratne**	Scaling Distributions and Variable Step Random Walks

Lunch

Chair: **Roberto Artuso**		
14:30-14:55	**Roger Penrose**	An idea for Quantum State Reduction
15:00-15:25	**Juan Parrondo**	Randomness and paradoxes
15:30-15:55	**Uzy Smilansky**	Can one count the shape of a drum?

Refreshments

Chair: **Dwight Barkley**

16:30-16:55	**Theo Geisel**	The Scaling Laws of Human Travel - Anomalous Diffusion in Epidemiology
17:00-17:25	**Itamar Procaccia**	Exact Mapping of Nonlinear Turbulence to Linear Models: The Mechanism of Anomalous Scaling

19:30 Workshop dinner at Spisehuset (Magstræde 12, upstairs, below the cinema)

Friday, May 19

Chair: **Hans Henrik Rugh**

9:00-9:25	**Viviane Baladi**	Ruelle determinants and spectrum for hyperbolic systems: a Paley-Littlewood approach
9:30-9:55	**Andre de Carvalho**	Pruning and infinitely renormalizable Henon maps
10:00-10:25	**Carl P. Dettmann**	Open circular billiards and the Riemann Hypothesis

Refreshments

Chair: **Greg Huber**

11:00-11:25	**Jonathan Keating**	Symmetry's reach
11:30-11:55	**Predrag Cvitanović**	Exceptional magic (a construction of exceptional Lie algebras)
12:00-12:25	**Heiner Kohler**	Many family CMS systems and supersymmetry
12:30-12:55	**Paolo Muratore Ginanneschi**	Inverse (infra-red) renormalisation group for turbulence

Lunch

Chair: **Axel Brandenburg**

14:30-14:55	**Laurette Tuckerman [and Dwight Barkley]**	Can your turbulence theory explain this pattern?
15:00-15:25	**Fabian Waleffe**	The coherence of shear turbulence: self-sustaining process and exact coherent structure
15:30-15:55	**Bruno Eckhardt**	Magic saddles: from quantum chaos to pipe flow

Refreshments

Chair: **Gregor Tanner**

16:30-16:55	**Edward A. Spiegel**	Large-Scale Patterns in Thermal Convection
17:00-17:25	**Tomas Bohr**	The rise and fall of the sap. Complex fluid dynamics in trees
17:30	**<>**	tak og farvel: end of the scientific programme

Saturday, May 20: lectures for general public

NORDITA and Niels Bohr Institute present:

A quartet of science lectures for lay public
Saturday, 20 May 2006
in the Rockefeller Auditorium, Juliane Maries Vej 30, 2100 Copenhagen

Chair: Predrag Cvitanović

09.00-09.05: Welcome address

09.05-09.50: Roger Penrose: Before the Big-Bang; an Outrageous Solution to a Profound Cosmological Puzzle
10.00-10.45: Theo Geisel: Follow the money - New Approaches to the Forecast of Epidemics

11.00-11.30: break

11.30-12.15: Michael V. Berry: Seven Wonders of Physics
12.30-13.15: Mitchell J Feigenbaum: Views from Below the Surface

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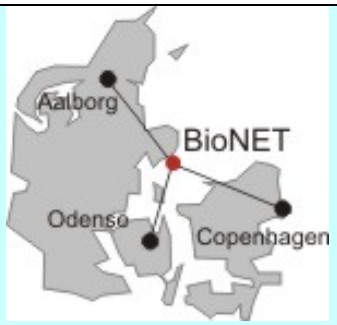
Sponsors: NORDITA, BioNET, Niels Bohr Fond, Nonlinearity

BioNET

Danish Center for Experimental and Theoretical Biophysics

Annual Meeting, October 30-31, 2006
MEMPHYS Center, University of Southern Denmark, Odense



13.00-13.15	Welcome and introduction (Mogens H. Jensen, Center Director)
13.15-13.30	The SDU-node (Ole G. Mouritsen)
13.30-14.00	Models of Life (Kim Sneppen)
14.00-14.30	Exploring the diversity of protein conformational changes (Daniel Otzen)
14.30-15.00	*Coffee break*
15.00-15.30	Stretching of short nucleic acid polymers with optical tweezers (Nader Reihani)
15.30-16.00	Pulse propagation in nerves (Thomas Heimburg)
16.00-16.30	A large-scale investigation of the cellular plasma membrane nanostructure with native plasma membrane protein biosensors (Chris Lagerholm)
16.30-17.00	*Discussion*
17.00-18.00	Business meeting
18.00-18.30	Drinks
18.30 - ?	Sushi Surprise

October 30

October 31

9.15- 9.45	Visit to the MEMPHYS laboratories
9.45-10.00	Modeling Epigenetics in Eucharyotes (Mille Michelsen)
10.00-10.15	Folding of membrane proteins and glucagon fibrillation (Peter Astrup Christensen)
10.15-10.30	Phase separation in phospholipid/cerebrosides/cholesterol model membranes (Matthias Fidorra)
10.30-11.00	*Coffee break*
11.00-11.15	Iron homeostatis in *E. coli* (Anna Andersson)
11.15-11.30	Association of OmpA fragments and unfolding of α-lactalbumin in detergents (Sanne S. Pedersen)
11.30-11.45	Patterns in genetic oscillations (Sandeep Krishna)
11.45-12.00	Current status in the generation of a membrane protein biosensor cell library (Eva Arnspang Christensen)
12.00-12.15	Towards structure prediction of folded and unfolded proteins (Jesper Borg)
12.15-12.30	Force spectroscopy applied to membrane proteins (Brian Vad)
12.30-14.00	*Lunch*
14.00-14.15	*In vitro* investigations of colliding RNA polymerases on DNA (Liselotte Jauffred)
14.15-14.30	Didactical reconstruction of current research on lipid membranes for students in upper secondary school - why is it necessary and how is it done? (Stinne Hørup Hansen)
14.30-14.45	Thermodynamic experiments on nerves (Kristmundur Sigmundsson)
14.45-15.00	Binding of surfactant proteins to membranes (Tony Ebdrup)
15.00-15.30	*Coffee break*
15.30-15.45	Bacterial amyloids (Morten Dueholm)
15.45-16.15	Fluorescence microscopy and membrane structure (Luis Bagatolli)
16.15-16.30	Concluding remarks and good bye

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