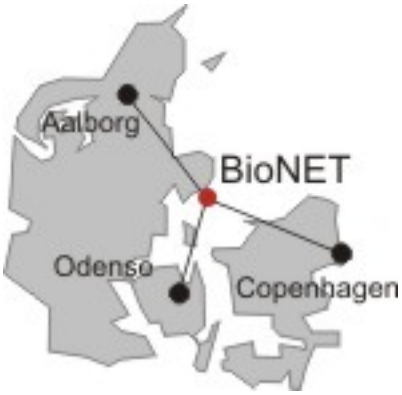
BioNET Activity Report

1. January – 31. December 2005



University of Copenhagen:

Theoretical Biophysics, Kim Sneppen, Mogens Høgh Jensen

Oscillating gene expression in feed-back networks

M.H. Jensen, K. Sneppen, S. Krishna

Oscillating gene expressions have in recent years been observed in several systems involving eukaryotic cells. Examples are the response to DNA damage given by the cell regulating protein p53 leading to apoptosis; the oscillations of the protein Hes1 which regulates embryo segmentations and the oscillations of the transcription factor NF-kappaB which is of importance for the control of inflammations. In these three cases the core of the genetic feed-back loop has been outlined and specific models have been formulated in terms of the involved components (proteins, mRNAs, enzymes, etc) leading to a set of coupled ordinary differential equations. The oscillatory state can be triggered by two very different biological mechanism, namely by means of a time delay due to transcription and translations or by means of a non-linear saturated degradation of the inhibitors.

We build upon this knowledge by in particular investigating how abrupt responses and spiky oscillations are necessary for giving rise to a clear biological signal. Three specific cases will be investigated. In the case of the transcription factor NF-kappaB previous studies have indicated that the spiky oscillations in the density of NF-kappaB in the cell nucleus may cause a very abrupt response of downstream genes. In particular it is possible to obtain effective Hill coefficients of the order of 20. Such high Hill coefficients may be of fundamental importance to be able to control inflammation and this will be investigated by means of theoretical modelling.

It is well known that hormones in living animals are produced in oscillating spikes, in humans for instance often with a period of hours. It is still not known why the body 'prefers' to be subjected to spikes instead of a steady rate. One explanation could be that a constant rate puts the body under too much stress whereas the oscillations leave time for the body to recover. This kind of behaviour will also be investigated by means of mathematical modelling.

The last part of the project deals with the response to iron (Fe) in the cells of bacteria. Fe is necessary for the cell to function. However, it is toxic in too high concentrations. It turns out that non-coding RNA (socalled small RNA - sRNA) are very important for the regulations of Fe in the cell. In particular if the external source of Fe is diminished sRNA will act and lead a quick non-linear response to increase the Fe level in the cell. By deleting a node in the feed-back Fe-network (named Iscs) it has been observed that the Fe level will oscillate, with a period of around one hour. We investigate first of all why such oscillations appear and next what are the biological implications. Also, studies of how such oscillating signals will change when moving across the network will be conducted.

Feed-back systems regulated by small RNA

Anna Anderson, Mogens Høgh Jensen, Kim Sneppen:

Negative feed back is a mechanism that counteracts the initial signal and is useful to control flows and concentrations of metabolic molecules inside the cell and fluctuations and chocks in these concentrations have to be regulated at time scales which are shorter than one cell generation for the cell to survive. We point out the importance of the interactions at the border between the metabolic and protein networks as well as investigate two apparently different similar mechanisms showing different dynamical behaviour. Earlier much effort has been put in to the separate study of metabolic and protein protein interaction networks. The link between the two is often overseen or argued to be inferior mechanisms on the time scales considered. In the protein

protein interaction network this viewpoint leads to the conclusion that feed back loops in protein regulatory networks are very rare. A cumbersome statement can be understood by looking at the time scales at which the protein production takes place which is of the order of a few cell generation. If the negative feed back would be a pure protein interaction mechanism the response would be too slow.

This work inspired us to work with the network intersection. The Iron flow regulation in E-coli is an intricate system consisting of five interlocked 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 in the cell with out poisoning it. This requires a regulatory system that is very robust and at the same time fast. 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 found and is believed to make the system faster. We believe we have solidified this by simulations. The RhyB regulation is especially interesting in this case since similar bacteria living in richer media have another mechanism than we have in our simulations and they are observed slower. The future for this project is first to find a full model that describes the whole system and then to try to simplify the system and maintain the main features of it.

Modelling the effect of histone modifcation in epigenetics of eucaroytic systems.

Mille Micheelsen, Kim Sneppen

Information of the regulatory state of a parent cell is kept down several generations of cell division. The information is usually stored in concentrations of cytoplasmic regulators that keep a gene silent or active. The transition of a gene from a silent to an active state is regulated by genetic switches. A different way of storing information has been found in eukaryotic organisms, where the storage is directly bound to the DNA. In this case domains on the DNA string will wrap around protein complexes called nucleosomes. These nucleosomes can be modified or unmodified which affect the expression of genes in this region to be either silent or active. Upon DNA replication the nucleosomes are distributed to the daughter DNA molecules and unmodified nucleosomes are added to the empty sites. The daughter DNA quickly regains the state of the parent of either modified or unmodified nucleosomes, thus on average staying in one state (either modifed or unmodified) for 100 or more generations. The time between cell divisions depend on the conditions of the environment the cells live in and this should give us some constraint on the robustness against variations of external parameters. In yeast the modification is methylation and demethylation of the nuclesome. We are proposing a model for this system where our first prediction is that methylation plays an important role in the stability of the system and active demethylation is important for the robustness.

Personal:

Post Doc, Sandeep Krishna, Bangalore, India, 01.11.04 – 01.11.06, shared 50% with CMOL
PhD Anna Anderson, Sweden, from 01.12.05 , shared 50 % with CMOL
PhD Mille Micheelsen, Denmark, from 01.01.06, shared 50% with CMOL

Guests:

Jacob Sparre Andersen, 31.10-09.11 & 03.08.-27.08. & 26.02.-09.03
Sebastian Bernhardsson 17.10. – 09.11
Raul Donangelo 24.10. – 05.11.
Joachim Mathiesen, 12 – 17. 09 & 23.03. – 04.04. & 14.05. – 23.05. & 26.01. – 13.02
Adam Palmer (student)
Vakhtang Putkaradze 15.10 -16.10
Joost Schymkowitz, 16.11. – 18.11. & 30.05. – 02.06.
Luis Serrano, 17.03. – 18.03.
Joel Stavans, 16.03. – 19.03.
Lukas Tamm, Virginia,
Antonio Trovato, 11.08. – 28.08.

J.J.P. Veerman, 25.08. – 01.09.
Mark Oxborrow, 08.08.-10.08.

Publications:

- Borg, J., Jensen, M.H., Olesen, P., Mathiesen, J.K.: Diffusion, fragmentation and merging processes in ice crystals, alpha helices and other systems, Proceedings of the Geilo ASI School on " Dynamics of Complex Interconnected Sys. -, i: Networks and Bioprocesses s. -. A.T. Skjeltrop and A.V. Belushkin. Kluwer Academic, Dordrecht 2005. Holland 2005.
- Fogedby H., Jensen, M.H.: Weak noise approach to the logistic map, J. Stat. Phys. 121, s. 759-778. 2005.
- Jensen, M.H., Sneppen, K., Tiana, G.: "Oscillating Gene Expressions in Regulatory Network", in Proceedings of the Geilo ASI School on "Forces, Groth and Form in Soft Condensed Matter: At the Interface between Physics and Biology", Proceedings of the Geilo ASI School -, i: At the Inetrface between Physics and Biology s. 195-202. A.T. Skjeltrop and A.V. Belushkin. Geilo ASI School. Kluwer Academic, Holland 2005.
- Olesen, P., Borg, J., Jensen, M.H., Mathiesen, J.K.: Diffusion, fragmentation, and coagulation processes: Analytical and numerical results, Physical Review E 72, s. 031103-13. 2005.
- Donangelo R., Hansen A., Sneppen, K., Souza S.R.: Need, greed and noise: competing strategies and in a trading model, Physica A : statistical and Theoretical Physics 348, s. 496-504. 2005.
- Jensen, M.H., Sneppen, K., Tiana, G.: "Oscillating Gene Expressions in Regulatory Network", in Proceedings of the Geilo ASI School on "Forces, Groth and Form in Soft Condensed Matter: At the Interface between Physics and Biology", Proceedings of the Geilo ASI School -, i: At the Inetrface between Physics and Biology s. 195-202. A.T. Skjeltrop and A.V. Belushkin. Geilo ASI School. Kluwer Academic, Holland 2005.
- Kim B.J., Trusina A., Minnhagen P., Sneppen, K.: Self organized scale-free networks from merging and regeneration, The European Physical Journal B - Condensed Matter 43(3), s. 369-372. 2005.
- Krishna S., Jensen, M.H., Sneppen, K., : Spiky oscillations in NF-KB signalling, Quantitative Biology, abstract q-bio.MN/0509017 -, s. 1-11. 2005.
- Maslov S., Sneppen, K.: Computational architecture of the yeast regulatory network, Physical Biology 2, s. S94-S100. 2005.
- Rosvall M., Grönlund A., Minnhagen P., Sneppen, K.: Searchability of networks, Physical Review E 72, s. 046117. 2005.
- Rosvall M., Minnhagen P., Sneppen, K.: Navigating netwoks with limited information, Physical Review E 71(6), s. 066111. 2005.
- Rosvall M., Trusina A., Minnhagen P., Sneppen, K.: Networks and Cities: An Information Perspective, Physical Review Letters 94(2), s. 028701. 2005.
- Sneppen, K., Dodd J., Shearwin K., Palmer A., Schubert R., Callen B., Egan B.: A Mathematical Model for Transcriptional Interence by RNA Polymerase Traffic in Escherichia coli, Journal of Molecular Biology 346(2), s. 399-409. 2005.
- Sneppen, K., Trusina A., Rosvall M.: Hide-and-seek on complex network. Europhysics Letters 69(5), s. 853-859. 2005.
- Sneppen, K., Zocchi, G.: Physics in Molecular Biology, Physics in Molecular Biology, Kim Sneppen, Giovanni Zocchi. Cambridge University Press. Cambridge University Press, UK 2005.
- Trusina A., Rosvall M., Sneppen, K.: Communication Boundaries in Networks, Physical Review Letters 94(23), s. 238701. 2005.
- S. Krishna, A. M. C. Andersson S. Semsey, K. Sneppen, Structure and function of negative feedback loops at the interface of genetic and metabolic networks. Nucleic Acid Research (in press).

Membrane Biophysics Group, Thomas Heimburg

The work of our group focuses cooperative behavior of lipid membranes, lipid-protein complexes, and biological membranes. In particular we perform calorimetric measurements, fluorescence correlation spectroscopy experiments, infrared spectroscopy, confocal microscopy and atomic force microscopy. In particular in 2005 we started investigating the propagation of density pulses (solitons) in biomembranes. Related to this activity, we also study the action of anesthetics on these pulses. A further related topic is the permeability of membranes induced by phase transitions. M. Fidorra started to measure series of infrared spectra of lipids from skin to understand the phase behavior in mixtures with phosphatidylcholines. Infrared spectroscopy allows investigating the lipids in the mixtures independently if one species is deuterated. Recently, he added permeability measurements using the “black lipid membrane” technique.

In detail:

Solitons: Biomembranes are mainly composed of lipids and proteins. Both molecule classes display order-disorder transition close to physiological temperature. While proteins unfold slightly above body temperature, the membranes display a melting transition about 10-15 degrees below body temperature. We found such transitions for various bacterial membranes, the spinal cord of chicken and for lung surfactant. We further know that in the lipid melting transition the elastic constants of the membrane change. We could show that a membrane located slightly above the melting transition implies the possibility of the propagation of density pulses (solitons) along cylindrical membranes that strongly resemble nerve pulses. Our comparison with nerve pulses is based on the following observation made by various authors: During the action potential one finds a heat release immediately followed by a heat re-absorption. These heat changes are exactly in phase with the voltage changes during the nerve pulse. This indicates that the nerve pulse is isentropic, i.e. based on reversible processes. However, the Hodgkin-Huxley theory that is the accepted picture for the mechanism of nerves is exclusively based on dissipative processes. Therefore, it is not in agreement with the reversible heat. Furthermore, one finds that during nerve pulses the thickness of the nerve changes, as do fluorescence anisotropy of the lipids and other properties. All these changes are in agreement with a propagating density pulse during which the nerve membrane is reversibly pushed through the melting transition. Such a process would automatically display a reversible heat. Also, the propagation velocity of a solitons is exactly that of the action potential in a myelinated nerve. This work was published in PNAS, 2005. It created various studies resulting from this.

Anesthetics: We have now established an alternative model for the nerve pulse based on reversible compression and the presence of a melting transition close to physiological conditions (see above). This implies an immediate explanation of the action of anesthetics that has been unexplained for 150 years. It is known that the potency of anesthetics is proportional to their solubility in lipid membranes. What we have established in the last year is that this implies that the anesthetics lower the melting temperature of membranes following an easy physical-chemistry law known as freezing-point-depression. Presently, we study the action of anesthetics on lipid membranes theoretically, and experimentally by using calorimetry and infrared spectroscopy (using the spectrometer partially financed by BioNET).

Domain formation in skin lipids: Matthias Fidorra is Ph.D. student who is financed to 50% by BioNET in collaboration with Syddansk Universitetet in Odense. He studies to domain formation in giant lipid vesicles using confocal microscopy. Furthermore, he investigates the mixing of the lipids by Fourier transform infrared spectroscopy using deuterated lipids. In infrared spectroscopy one can investigate the melting of lipids by studying the position of vibrational bands, e.g. the C-H stretching vibrations. This allows us to understand the phase behavior of the lipids independently of each other. We will use this information in combination with Monte-Carlo simulations of the melting behavior of lipid mixtures that we have been performing in the recent

decade. The simulations are exclusively based on thermodynamic information from calorimetry. They permit to investigated domain formation but also the melting of the individual lipid components. We intend to compare this information with the information from infrared spectroscopy to gain a better understanding of the domain formation as such.

Permeability measurements using black lipid membranes: Since the elastic constants largely change in the melting regime of lipid membranes, the likelihood to find spontaneous pore formation is also drastically increased. We study the permeability by fluorescence correlation spectroscopy (FCS) and recently also with black lipid membranes. In the first technique we study the diffusion of fluorescent markers entrapped in vesicles and the diffusion of free markers. Due to membrane permeability the ratio of the two diffusing species changes during time. We find that in the melting regime the permeability is largely increased. This permeability can be affected by proteins in a direct correlation to their influence on the melting profiles of the lipid membranes. Recently, Matthias Fidorra also started investigating ion permeation across membranes using current measurements. In the transition one finds quantized currents that resemble the current traces from ion channel proteins both in magnitude and timescale. However, no proteins are present in such membranes. It seems difficult so far to understand why these processes are quantitatively so similar and one may wonder whether they are intimately related to each other. Since we have successfully modeled the nerve pulse without proteins we investigate the possibility whether the currents measured through biomembranes are related to the fluctuations in the lipid membrane.

Personal:

Matthias Fidorra continued his Ph.D. project about the formation of domains in lipid mixtures from skin. This project is a combined effort by the group of Luis Bagatolli from the Memphys group in Odense (project leader: O.G. Mouritsen). In our group (Copenhagen) he is performing infrared measurements on the skin lipid mixtures, calorimetric measurements and recently also membrane permeability measurements in collaboration with Prof. Dr. M. Winterhalter from the University of Bremen, Germany. We soon will have a guest from Germany investigating the propagation of solitons in membrane thethers.

Guests:

- Rainer Böckmann 13.09. – 15.09.
- Matthias Schneider, 21.06. – 23.06.
- Konrad Kaufmann, 12.12. – 23.12.
- Paavo Kinnunen , 14.12.
- Ivan Makarov, Max-Planck Inst. for Bio. Chemistry Göttingen, Germany, April-June 2005.
- Vitaliy Oliynyk, 07.01. – 28.02.

Publications:

- A. Hac, H. Seeger, M. Fidorra, and T. Heimburg. 2005.
  Diffusion in two-component membranes - A fluorescece correlation spectroscopy and Monte Carlo simulation study, Biophysical Journal 88: 317-333.
- T. Heimburg, and M Gudmand. 2005
  Selvorganisering i Biomembraner, Kvant 3(05): 22-24.
- H. Seeger, M. Fidorra, and T. Heimburg. 2005. Domain size and fluctuations at domain interfaces in lipid mixtures. Macromolecular Symposia (Wiley) 219: 85-96
- T. Heimburg, and A. D. Jackson: 2005.
  On soliton propagation in biomembranes and nerves, Proceedings of the National Academy of Sciences of the USA 102: 9790-9795.
- B. Lautrup, A. D. Jackson, and T.Heimburg. 2005.
  The stability of solitons in biomembranes and nerves. Submitted.

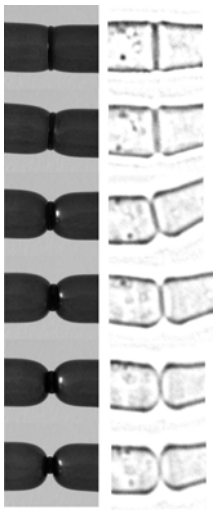
Optical Tweezers Group, Lene Oddershede

Single molecule studies:

RNA pseudoknots are assumed to induce ribosomal frame shifting. We have genetically constructed two different pseudoknots and shown that they induce different degrees of frame shifting. By optical tweezers we performed a mechanical unfolding of the pseudoknot such mimicking the action of the ribozyme. Our hypothesis, that the degree of frameshifting is related to the mechanical strength of the pseudoknot, has been confirmed by these experiments. Also, the non-equilibrium thermodynamics of the process has been investigated.

As a continuation of earlier studies on the motility of the lambda receptor in the outer membrane of living *E. coli* bacteria, experiments have been performed where the mobility of the exact same protein in a living and a dead bacteria has been monitored. The bacteria was killed by various means such as azide, arsenate and ampicillin. The results consistently show that the motion of the receptor stops upon killing the organism; that the motion is not purely thermal, there is an active component too.

Cell biophysics:



In order to be able to measure the forces involved in cell division in living S. pombe yeast cells, we wish to 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). These gold nano particles have been injected into living S. pombe yeast cells using a micropipette. Then, they are attached to various organelles and used as handles for the trapping laser. The goal of this setup 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.

Publications relevant for BioNET published in 2005:

- Hansen, P., Dreyer J., Borg, J., Oddershede, L.B.: Novel optical and statistical methods reveal colloid-wall interactions incosistent with DLVO and Lifshitz theories. Journal of Colloid and Interface Science 287(2), s. 561-571. 2005.
- Hansen, P.H., Bhatia, V.K., Harrit N., Oddershede, L.B.: Expanding the Optical Trapping Range of Gold Nanoparticles, Nano Letters 5, s. 1937-1942. 2005.
- Hansen, P.H., Oddershede, L.B.: Optical Trapping Inside Living Organisms, SPIE Proceedings 5930, s. 1-9. 2005.
- Hansen, T.M., Reihani, R., Oddershede, L.B.: Combining optiacl tweezers and micropipettes for DNA stretching: Elasticity of micropipette crucial, NATO Springer proceedings (2005).
- Tolic-Nørrelykke, S.F., Rasmussen, M.B., Pavone, F.S., Berg-Sørensen, K.B., Oddershede, L.B.: Stepwise bending of DNA by a single TATA-box binding protein, accepted for publication in Biophysical Journal (2005).
- Flyvbjerg, H., Oddershede, L.B., Berg-Sørensen, K.: Brownske bevægelser, fra Einstein til optiske picetter, KVANT 2, s.6-9 (2005).
- Hansen, P.M., Madsen, T.W., Oddershede, L.B.: Vild med lys, KVANT 4, s.14-17 (2005).

- Oddershede, L.B.: Optisk manipulation af nanopartikler, GAMMA 140, s.11-17 (2005)

BioNET financed Personel

Tabita W. Madsen, PhD-student, started February 1, 2005 is involved in the projects on mobility of proteins in bacterial membranes.
Nader Reihani, visited 3 months in 2005, has started as a post doc 1.1.2006.

Guests

- Visiting phd-student Zdenek Lansky, Prag, partly financed by BioNET, September 2004 to June 2005. Involved in the topology study of dividing cells.
- Jan Kierfeld, Pottsdam, 24.10 – 27.10
- Scot Kuo, Johns Hopkins, Baltimore, 31.05. – 04.06.
- Iva Mariija Tolic-Nørrelykke, Max Planck Inst., Dresden, 08.05. – 10.05.
- Thomas Callisen, Novozymes, 09.11.
- Sergi Padilla-Parra, Barcelona, 08.08-15.08.
- Jakob Kisbye Dreyer, Lund University
- Simon Tolic-Nørrelykke, Max Planck Inst., Dresden

The following lectures have taken place at NBI

in 2005:

Date	Person	Affiliation
14.12	Anders Johansen	Niels Bohr Institute
7.12	Mads Madsen	Dep. Medical Biochemistry and Genetics, Panum NMR Centre
30.11	Andrea Amatori	Niels Bohr Institute and University of Milano, Phys Dep.
23.11	Anna Andersson	Niels Bohr Institute
16.11	Szabolc Semsey	Niels Bohr Institute
09.11	Thomas Callisen	Novozymes Research & Development
02.11	Jakob Kisbye Dreyer	Center for Chemistry and Chemical Engi. Lund Univ.
26.10	Per Grove Thomsen	DTU, IMM
12.10	Sune Danø	Dep. Medical Biochemistry and Genetics, Panum NMR Centre
28.09	Ulrich Quaade	Center for individual nanoparticle functionality (CINF), NanoDTU, Department of Physics, DTU
21.09	Sandeep Krishna	Niels Bohr Institute
14.09	Rainer Böckmann	Theoretical & Computational Membrane Biology, Center for Bioinformatics, Saar Universität des Saarlandes
07.09	Jacob Bock Axelsen Jesper Ferkinghoff-Borg	BioComplexity group, NBI
31.08	Peter Vermann	Portland State University
06.07	Jonas Tegenfeldt	LTH Lund
22.06	Matthias Schneider	University of Augsburg
15.06	Mia Trolle Borup	Niels Bohr Institute

01.06	Scot Kuo	John Hopkins University
25.05	Zdenek Lansky	Visiting phd-stud from Pragh Univ.
18.05	Kresten L.-Larsen	Dept. of Biochemistry, August Krogh, KU
11.05	Michael Rudolph	
04.05	Fridolin Okkels	DTU, MIC
27.04	Camilla Rygaard-Hjalsted, Betina Dam Sørensen	Kommunikationskontoret, NBI
13.04	Henrik Bruus	DTU, MIC
30.03	Steve Strogatz	Cornell University
09.03	Daniel Abrams	Cornell University
02.03	Thomas Bohr, Kim Sneppen, Peter Ditlevsen	Dep. of Physics, DTU ; Niels Bohr Inst.; Niels Bohr Inst, Dep. of Geophysics
23.02	Stanley Brown	Mol. Biol. Institute, University of Copenhagen
16.02	Rob Delotto	Mol. Biol. Institute, University of Copenhagen
09.02	Steve Strogatz	Cornell University
02.02	Kaare Brandt Petersen	DSP/IMM, DTU
26.01	Christoffer Johansson	Dept. Theoretical Ecology, Lund University
19.01	Eli Barkai	Bar-Ilan University
12.01	Nader Reihani	Institute for Advanced Studies in Basic Sciences, anjan, Iran
05.01	Anders Andersen	Cornell University, Dept. of Theoretical and Applied Mathematics

University of Aalborg

Department of Life Sciences, Daniel Otzen:

(A) Interactions of pro-aggregator p25α with α-synuclein and other molecules

Our collaborator Poul Henning Jensen, Aarhus University, has identified a number of molecules that bind to the major component in Parkinson's Disease, α-synuclein, which is natively unfolded. We have characterized the nature of its interactions with α-synuclein (*1*) and find that it enhances the protein's aggregation as well as co-localizing with α-synuclein in the cell. p25α may represent an important new class of proteins that modulate the aggregative properties of fibrillogenic proteins such as α-synuclein. Based on p25α's primary sequence and preliminary sequence analysis, the protein has been predicted to be natively unfolded, but we have shown that despite the protein's inherent dynamic structure and pronounced sensitivity to proteases, it behaves in many ways like a conventional globular protein and also binds to tubulin in a ca. 4 p25α: 1 tubulin molar ratio (*2*). We are now engaged in analyzing the interactions of p25α with other natively unfolded proteins.

(B) Folding and misfolding of a bacterial autotransporter

We have used the outer membrane protein AIDA as a model for folding of outer membrane proteins. The protein cannot be folded spontaneously in solution, but requires a solid support, without which it forms an only partially folded state that is sensitive to proteases, although it folds and unfolds in a cooperative fashion (*3, 4*). However, the folded state – once formed – is extremely stable and only unfolds at high temperatures in the presence of the anionic detergent SDS. We have devised a simple linear extrapolation method to assess the stability of AIDA in mixed micelles based on mole fractions, and find that it is necessary to operate with the micellar (as opposed to bulk) mole fraction to obtain linear correlations (and thus reliable extrapolations) between melting temperatures and micelle composition (*5*). We have also written a review on the subject of outer membrane proteins folding in the periplasm (*6*).

(C) Thermodynamic stability of a membrane protein

The inner membrane protein DsbB folds reversibly in mixed micelles, and we have shown that this conforms to a simple three-state system involving an unfolding intermediate. We have now 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 (*7*).

(D) Structure of an antimicrobial peptide in different detergents

The cationic antimicrobial peptide Novispirin forms an α-helical structure in the presence of anionic lipids and detergents. Surprisingly, positively charged detergents are also able to induce this structure. We have determined binding affinities for different amphiphiles and have used NMR to obtain the solution structure of Novispirin in both anionic and cationic detergents, finding that the peptide is (nor surprisingly) more deeply embedded in the negative than the positively charged detergent (*8*); however, it is remarkable that the peptide nonetheless can override electrostatic repulsion to interact with an interface of the same charge.

(E) Fibrillation of glucagon and bacterial amyloidin: fibrillar polymorphism and structural hyperstability

We have ascertained that the small peptide hormone glucagons, which fibrillates readily at both low and high pH, forms fibrils whose ulstrastructure as well as secondary and tertiary structure is very sensitive to the conditions under which it is formed. This fibrillar polymorphs also differ significantly in stability towards denaturation by heat or chemical denaturants, highlighting the structural variance of evolutionarily non-adapted fibrils and the ability to "select" for fibrils of different strength depending on the conditions under which they are formed (*9, 10*). In contrast, we have started to identify a large number of naturally occurring bacterial fibrils which may prove to be extremely stable, testifying to the ability of evolution to evolve sequences optimally tuned for stable fibril structures. We have written two reviews on fibrillation (*11, 12*)

(F) Folding of model proteins: S6

We have shown that the model protein S6 has some unusual properties in that it possesses structural antagonism which can be relieved by the introduction of suitable mutants (*13*). We have determined the X-ray structure of these variants, and a deeper ongoing analysis of these structures may reveal the basis for this difference. In addition, the folding behaviour of S6 in stabilizing additives such as sodium sulfate (*14*) and trehalose (*15, 16*) serve to highlight how the folding landscape may be modulated significantly by solvent conditions.

(G) Miscellaneous

We have also reported on the unusual activation properties of monomeric (as opposed to micellar) detergents on the hydrolytic properties of the usually interfacially activated lipase from *T. lanuginosus* (*17*) and have published some analytical studies on the interactions of detergents with each other and cyclodextrins (*18, 19*).

(H) Ongoing studies

We hope to be able to carry out optical tweezer studies with the membrane protein DsbB in 2006 based on encouraging *in vitro* biotinylation results. We have unfortunately not been able to express fragments of DsbB in fusion with Green Fluorescent Protein variants, but are currently focusing on single transmembrane helix fragments of DsbB which have been produced by solid state synthesis. Fragments of the outer membrane protein OmpA will hopefully be produced by specific proteolytic cleavage via novel inserted protease sites, after attempts to purify several of these fragments by direct expression in *E. coli* have proved unsuccessful.

Publications:

(1) Lindersson, E., Lundvig, D., Petersen, C., Madsen, P., Højrup, P., Moos, T., Otzen, D. E., Gai, W.-P., and Jensen, P. H. (2005) P25a is co-expressed with a-synuclein in a-synucleinopathies and stimulates its aggregation. *J. Biol. Chem. 280*, 5703-5715.

(2) Otzen, D. E., Lundvig, D., Wimmer, R., Hatting, L., Pedersen, J. R., and Jensen, P. H. (2005) p25alpha is flexible but natively folded and binds tubulin in an oligomeric complex. *Prot. Sci. 14*, 1396-409.

(3) Mogensen, J. E., Tapadar, D., Schmidt, M. A., and Otzen, D. E. (2005) Barriers to folding of the Transmembrane Domain of the *Escherichia coli* Autotransporter Adhesin involved in diffuse adherence. *Biochemistry 44*, 4533-45.

(4) Mogensen, J. E., Kleinschmidt, J. H., Schmidt, M. A., and Otzen, D. E. (2005) Misfolding of a Bacterial Autotransporter. *Prot. Sci. 14*, 2814-27.

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University of Southern Denmark:

Memphys and Physics Department, Ole G. Mouritsen

Principal scientists in period of report: Dr. Matthias Weiss, Prof. Luis Bagatolli

PhD students: Matthias Fidorra (jointly with SDU and NBI)
Stinne Hørup Hansen
Maria Bloksgaard Mølgaard (from February 1)

1. Status

After having joined the Deutsches Krebsforschungszentrum in Heidelberg to build up his own research group there, Dr. Weiss has continued his involvement with BioNET mainly via PhD-students studying ER exit-site dynamics, dynamics of membrane-enzyme interactions, and the mechanics of membrane fluctuations. The succession of Dr. Weiss has been complicated by the fact that the appointment of Dr. Nicolette Kaya from May 1 was cancelled due to personal reasons. After an intensive search for a replacement, we have luckily been able to attract Dr. Christoffer Lagerholm from the University of North Carolina. Dr. Lagerholm is an expert in biological imaging using fluorescence techniques and quantum dots. Dr. Lagerholm will assume his position on January 15, 2006. His project with BioNET was planned during the end of 2005.

The main contributions to BioNET during the period of report have been by Prof. Luis Bagatolli from the Department of Biochemistry and Molecular Biology of SDU. Dr. Bagatolli has together with PhD-student Matthias Fidorra and others embarked on a comprehensive study of dynamics of skin and skin membranes. The research on skin is allocated a BioNET PhD-fellowship filled by cand.scient. Maria Bloksgaard Mølgaard.

The engagement in the national activities concerning science education has been continued by a PhD-student Stinne Hørup Hansen who has been on maternity leave part of the year (from August 2005).

2. Research report

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

[Dr. Matthias Weiss 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

Publications:

Forster, R., Weiss, M., Zimmermann, T., Reynaud, E. G., Verissimo, F., Stephens, D. J., and Pepperkok, R. Curr Biol **16**, 173-179 (2006)].

Also, 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 at MEMPHYS-Center

for Biomembrane Physics, 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 has been shown that inclusion of active proteins in lipid membranes leads to a substantial mechanical softening of the bilayers consistent with theoretical predictions.

Publications:

1. Close-up view on the the modifications of fluid membranes due to phospholipase PLA2 (A. F. Jakobsen, O. G. Mouritsen, and M. Weiss) J. Phys.: Condens. Matter **17**, S4015-S4024 (2005).
2. Activation of interfacial enzymes at membrane surfaces (O. G. Mouritsen, T. L. Andresen, A. Halperin, Per Lyngs Hansen, A. F. Jakobsen, U. B. Jensen, M. Ø. Jensen, Kent Jørgensen, T. Kaasgaard, C. Leidy, A. C. Simonsen, G. H. Peters, M. Weiss) J. Phys.: Condens. Matter (in press)
3. Non-equilibrium fluctuations of biomembranes with active inclusions (M. Weiss, A. F. Jakobsen, and O. G. Mouritsen) Phys. Rev. Lett. (submitted).

2.2 Ceramide containing membranes and Stratum Corneum skin lipid membranes

[Assoc. Prof. Luis Bagatolli, PhD-students Maria Bloksgaard Mølgaard and Matthias Fidorra 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 may exert their biological activity through changes in membrane structure and organization. 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 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 behavior of the intercellular lipids, a lipid mixture consisting of ceramides, cholesterol and fatty acids.

The research plan contains three main goals

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

1) Ceramide containing artificial lipid mixtures

[Assoc. Prof. Luis Bagatolli, Dr. Thomas Heimburg (NBI, Copenhagen), PhD student Matthias Fidorra]

During the 2005 period 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 instead of ceramide were studied. These results were presented at the 49th and 50th Biophysical Society Meeting:

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.

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, L. A. Bagatolli

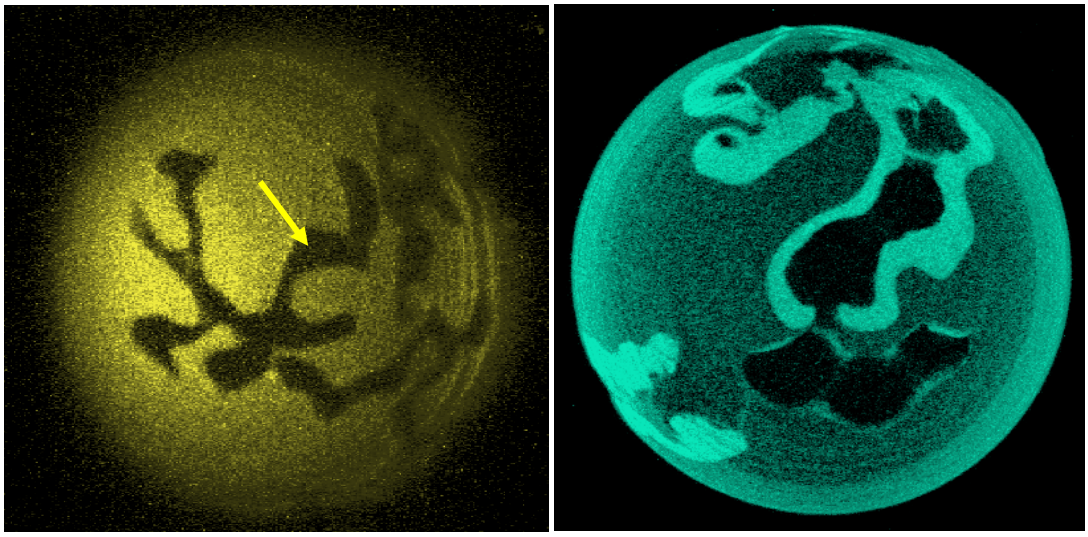

Figure 1: Giant vesicles composed of brain ceramide/POPC mixtures (1:5 mol, right) and of brain ceramide:POPC/cholesterol mixtures (1:5/26% mol, left) displaying phase coexistence. The dark areas in the right image (indicated with the yellow arrow) correspond to ceramide rich gel phase areas. In the case of the cholesterol containing mixture tree 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).

Additionally the results obtained for the ceramide containing mixtures was accepted to be published in Biophysical Journal (see below):

Publications:

M. Fidorra, L. Duelund, C. Leidy, A. C. Simonsen and L.A. Bagatolli, Absence of fluid-ordered/fluid-disordered phase coexistence in ceramide/POPC mixtures containing cholesterol, 2006. *Biophys. J. (In press, schedule for the June 2006 issue)*.

2) and 3) The role of Acyl-CoA Binding Protein in skin - combining biophysics, molecular biology, biochemistry and mouse genetics

[Assoc. Prof. Luis Bagatolli, Assoc. 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 biophysical techniques, such as fluorescent spectroscopy and differential scanning calorimetry.

(iii) Direct visualization of the lateral structure of giant vesicles composed of either artificial mixtures mimicking stratum corneum lipid composition or natural lipid extracts from the mice by 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*.

During the period April 1st 2005 - April 1st 2006, 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 technique (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.

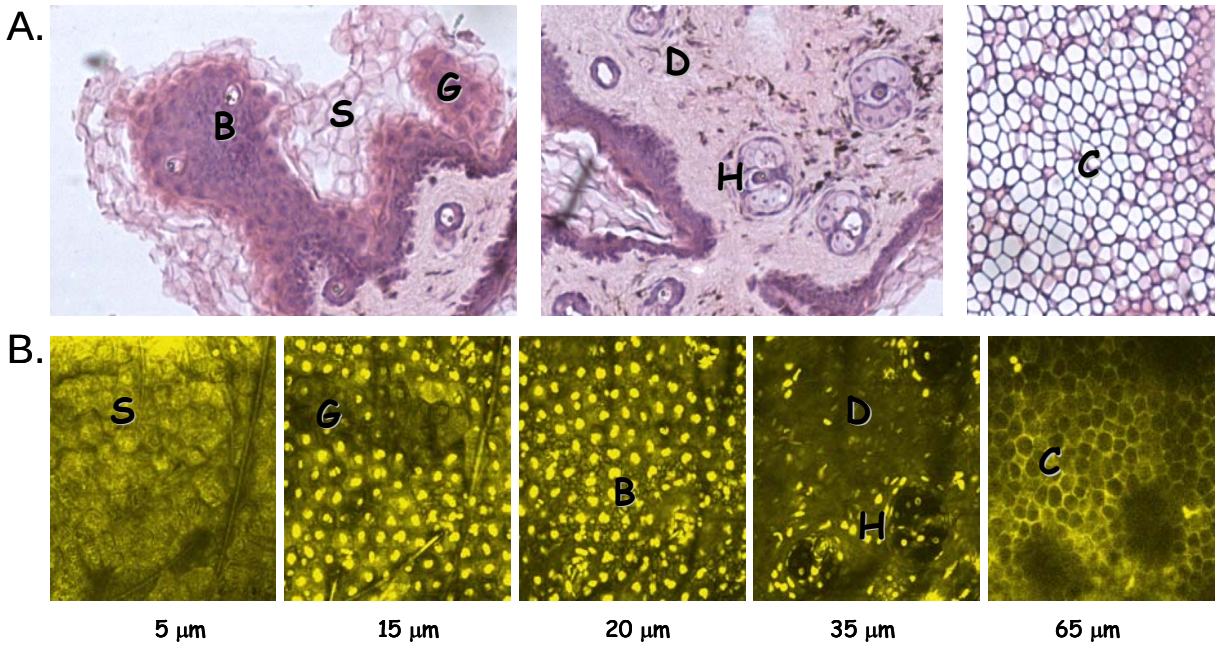

Figure 2: LIGHT MICROSCOPY vs. TWO-PHOTON EXCITATION MICROSCOPY, mouse ear tissue: The different layers in the skin visualized by ordinary hematoxylin/eosin light microscopy sections (A) can easily be observed by using TPEM (B). Two-photon excitation was performed at 780 nm. The cell nuclei are labelled 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.

2.3 Biophysics as a model for inter-disciplinary teaching in Danish high school

[PhD student Stinne Hørup Hansen]

PhD-project was initiated on September 1, 2004, by Stinne (Christina) Hørup Hansen. During the time of report, Stinne Hørup Hansen has continually been studying the litterature, and planning the project with the following title and aim: *Design-based research of interdisciplinary science teaching in Upper Secondary School*.

According to Stinne Hørup Hansen 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)

Additionally Stinne and her collaborators are writing a research paper with the title: How interesting can it get? Interest based teaching in Science in Upper Secondary School. C.H.Hansen, M. Kofoed, N.B.Dohn, and C.Michelsen.

Stinne participated in a seminar about a project with the title: I have fun (IFUN) concerning science teaching. The project is developed in collaboration with a German research group from IPN-Leibniz Institute for Science Education.

In addition Stinne 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.

2.4 A global in vivo investigation of the cellular plasma membrane organization

[BioNET postdoc Christoffer Lagerholm]

This project was planned during the late 2005 and will be initiated in January 2006. The goals of this project work are: 1) to characterize the role of protein-protein and protein-lipid interactions in the observed organization and dynamics of individual plasma membrane proteins, and 2) to perform a large scale analysis of the characteristics of 50-100 membrane proteins to determine their relation to the structure and organization of the cellular plasma membrane. To accomplish this I have initiated a series of collaborations with researchers at Carnegie Mellon University (Prof. Jonathan Jarvik), Massachusetts Institute of Technology (Prof. Alice Ting) and at Syddansk Universitet (Prof. Ole Nørregard Jensen). In this project, I in collaboration with Jonathan Jarvik, will create a library of cell lines each of which has had a guest exon inserted randomly into a single membrane protein. This guest exon will be specifically biotinylated by a bacterial enzyme, biotin ligase. High-throughput FACS will enable specific selection of cell lines that have insertions that are accessible to the enzyme and to the fluorescent probes. I will exploit these specific biotin sites to label tagged membrane protein with a wide range of probes of various sizes and

valencies to 1) investigate effects on the organization and dynamics of tagged plasma membrane proteins with single molecule imaging microscopy; 2) identify protein-protein and protein-lipid interactions of tagged membrane proteins by mass spectrometry; and thus 3) obtain a global spatial and temporal map of the organization of the cellular plasma membrane. I anticipate that this experimental approach will dramatically increase existing knowledge of the organization and dynamics of known membrane proteins as well as help identify and characterize novel membrane proteins. I further believe that this project will be an important step towards resolving ambiguities concerning the existence of plasma membrane microdomains such as lipid rafts. Work is currently underway at Carnegie Mellon University (with Jonathan Jarvik) to modify existing mouse retroviruses to randomely insert the required guest exon into mouse fibroblasts. Plans are then to transfer plasmid DNA and/or modified cell lines to Syddansk Universitet for characterization by single molecule microscopy and mass spectometry (with Ole Nørregard Jensen). For our single molecule experiments, we have a assembled a fluorescence microscope (Olympus IX70) and a CCD camera (Andor DV-887) suitable for low level imaging and at fast dynamic rates.

Conferences, meetings & 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.

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.

Program

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		

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

Københavns Universitet, Niels Bohr Institutet
Syddansk Universitet
Aalborg Universitet

BioNET Regnskab 2005

	VKR Bevilling	Forbrug	Overført til 2006
NBI	2.565.000	2.108.138	456.862
SDU	2.036.767	1.412.711	624.056
AAU	1.408.000	1.963.000	-555.000
	6.009.767	**5.483.849**	**525.918**

Budgetskema for 2006

	Revideret budget 2006	Overført fra 2005	Til udbetaling 2006
NBI	2.950.000	456.862	2.493.138
SDU	2.149.056	624.056	1.525.000
AAU	1.691.000	-555.000	1.136.000
			5.154.138

Samlet budget for hele perioden

	2004	2005	2006	2007	2008	2009	I alt
NBI	735.062	2.108.138	2.950.000	2.880.000	2.045.000	1.030.000	11.748.200
SDU	318.233	1.412.711	2.149.056	1.780.000	1.130.000	710.000	7.500.000
AAU	371.542	1.963.000	1.691.000	1.009.000	558.000	157.000	5.749.542
I alt	1.424.837	5.483.849	6.790.056	5.669.000	3.733.000	1.897.000	**24.997.742**

BioNET - Center for Biofysik: NBI

Faktisk forbrug for 2005 er angivet i skarp parentes [].
Medfinansiering er angivet i rund parentes ().

Alle beløb er i DKK.

	2004	2005	2006	2007	2008	2009	Financieret af
VIP	(400)	(800)	(800)	(800)	(800)	(800)	NBI
Sekretær	100 [117.945]	200 [242.624]	250	250	250	125	NBI bet.10 t
Tekniker	75 (130) [0]	205(300)[205]	150 (300)	150 (300)	150 (300)	75 (150)	BioNET/NBI
Ansættelser							
PhD T.Winther	140	420 [311.259]	420	420	35		BioNET
Postdoc Reihani			500				BioNET
Postdoc No Name				500			BioNET
Samfinansiering							
Postdoc Krishna	280 [175.814]	500 [351.602]	220	220	220	110	CMOL/BioNET
Phd Fidorra	140 [45.082]	420 [45.082]	430	140			SDU/BioNET
Phd Micheelsen		130	210	210	210		CMOL/BioNET
Phd Andersson		100	210	210	210		CMOL/BioNET
PhD No name			50	140	140	90	FS/KU/BioNET
PhD No name			50	140	140	90	FS/KU/BioNET
PhD No name							
PhD stip. Total	**140 [45.082]**	**1070 [356.341]**	**1370**	**1260**	**735**	**190**	
PostDoc Total	**280 [175.814]**	**500 [351.602]**	**720**	**720**	**220**	**110**	
Eks.udstyr/drift	320 (50) [338.605]	250 (1100) [435.267,53]	150 (100)	150 (100)	280 (100)	250 (50)	
Rejser	0 [9.308]	110 [168.110]	150	150	150	70	
Gæster	40 [48.309]	150 [239.825]	100	100	130	80	
Workshop	40	50 [109.368]	60	60	100	100	
Reserve	40	30		40	30	40	
Lab./kontorer	(95)	(150)	(190)	(190)	(190)	(190)	
Computer. Kap.	(75)	(150)	(150)	(150)	(150)	(75)	
Ialt	**1175 (770) [735.062]**	**2565 (1540) [2.108.138]**	**2950 (1540)**	**2880 (1540)**	**2045 (1540)**	**1030 (865)**	

VKR bev. 2005	DKK	2.565.000
Forbrug 2005	-	2.108.138
Overskud overført	DKK	456.862

FS=Forskerskole NBI=Niels Bohr Instituttet, KU=Københavns Universitet, CMOL= Center for Models of Life

- PhD stud. Mille Micheelsen startede som PhD 01.01.06
- PhD stud. Anna Andersson bliver i december 05 betalt af CMOL, herefter finansieres 50% af BioNET/CMOL
- M. Fidorras løn for 16 mdr., 01.09.04-31.12.05, DKK 183.595, er budgetteret i 2006 sammen med hans løn i 2006 da vi ikke har modtaget regninger fra SDU

BioNET - Center for Biofysik: SDU Årsregnskab 2005

Faktisk forbrug er angivet i skarp parantes [ ].
Medfinanicering fra institiutionen er angivet i rund parantes ( ).

	2004	**2005**	**2006**	**2007**	**2008**	**2009**
VIP	(200.000)	(200.000)	(200.000)	(200.000)	(200.000)	(100.000)
TAP	(50.000)	(50.00)	(50.000)	(50.000)	(50.000)	(25.000)
Postdoc Matthias Weiss Nicoletta Kahaya	225.000 [181.792]	 [0] 300.000	 450.000	 450.000	 450.000	 225.000
1. PhD (2/3) Maria Bloksgaard	225.000 [0]	250.000 [228.052]	300.000	300.000	50.000	
2. PhD (1/3)		150.000	150.000	150.000		
3. PhD (2/3) Stine Hørup Hansen	150.000 [128.641]	300.000 [385.106]	300.000	200.000		
4. PhD (1/3)			75.000	150.000	150.000	75.000
5. PhD (2/3)				300.000	300.000	300.000
Medfinanicering af PhD studerende	(150.000)	(200.000)	(200.000)	(200.000)	(200.000)	(100.000)
Computerkapacitet	(100.000)	(100.000)	(100.000)	(100.000)	(100.000)	(50.000)
Rejser	40.000 [3.293]	70.000 [24.060]	70.000	70.000	70.000	30.000
App./Drift	110 (150) [4.507]	926.767 (150.000) [775.493]	782.056 (150.000)	120.000 (150.000)	110.000 (150.000)	40.000 (75.000)
Workshop		40.000 (100.000)	40.000 (100.000)	40.000 (100.000)		40.000 (100.000)
Ialt	**750.000** **(650.000)** **[318.233]**	**2.036.767** **(800.000)** [1.412.711]	**2.149.056** **(800.000)**	**1.780.000** **(800.000)**	**1.130.000** **(700.000)**	**710.000** **(600.000)**

Budget 2005	2.036.767
Faktisk forbrug	1.412.711
Overføres til app./drift	624.056
Budget 2006	1.525.000
I alt 2006	2.149.056

BioNET - Center for Biofysik: AAU

Budget for perioden 2005-2009, opdateret marts 2006.

Faktisk forbrug for 2004 og 2005 er angivet i skarp parentes [].
Medfinansiering fra AAU og eksterne midler (forskningsstyrelsemidler til ph.d. stipendier) er angivet i rund parentes ().

Alle beløb er i kkr.

	2004	**2005**	**2006**	**2007**	**2008**	**2009**
Professorat	(300)	(600)	(600)	(600)	(600)	(300)
Postdoc 1	225 [245]	450 [391]	450	78	0	0
Postdoc 2	113 [0]	480 [303]	415	120	0	0
Ph.d. 1[a]	225 [0] (150)	150 [427] (0)	23 (427)	0 (323)	0	0
Ph.d. 2[b]	0 [0]	0 [113] (150)	337 (113)	0 (450)	0 (337)	0
Ph.d. 3[c]	0 [0]	0	150	480	330 (150)	(300)
Apparatur	44 [100] (100)	100 [605] (100)	88 (300)	103 (100)	0 (100)	0 (100)
Drift	82 [17]	173 [95]	163	163	163	81
Rejser	20 [10]	30 [29]	40	40	40	20
Workshop	13 [0]	25 [0]	25	25	25	12
Samlet	722 [372]	1408 [1963]	1691	1009	558	157

Kommentarer

- **Ph.d. stipendium 2** deles med Ole Mouritsen (SDU), som bidrager med 1 års løn via et delt ph.d. budget
- **Ph.D. stipendium 3** er stadig budgetteret med 2/3 medfinansiering fra BioNET og 1/3 fra AAU. Vi søger dog Forskningsstyrelsen om medfinansiering på 2 ph.d. stipendier; lykkes dette, vil det være muligt at ekspandere det oprindelige 3. stipendium til hele to ph.d. stipendier.
- **Apparatur:** Vi har anskaffet en større mængde apparater i år, deriblandt et FTIR apparat (330.000 kr), et fluorimeter (100.000 kr), inkubatorer (110.000 kr), en bordcentrifuge m.v. Vi har i 2006 planer om yderligere anskaffelser til ca. 300.000 kr, så den samlede apparatur regning kommer op på ca. 900.000 kr. Disse tages dog ikke primært fra BioNET budgettet. AAU medfinansierer 1:1, således at vi vil have 450.000 til gode fra AAU, svarende meget fint til de planlagte 440.000 kr til apparatur i AAU budgettet for den samlede BioNET periode 2004-2009. Det nuværende forbrug på 604,740 kr indeholder altså ikke bidrag fra AAU medfinansiering; i praksis er der derfor kun et reelt forbrug på det halve. Evt. yderligere apparat anskaffelser vil dækkes via driftskontoen (dette skaber ikke problemer, jvf. underforbrug af drift i år) og vil også kunne medfinansieres via AAU.
- **Post-doc løn:** Vi har haft 3 post-docs ansat i 2005 (for overskuelighedens skyld holdt sammen som 2 post-docs i overensstemmelse med det oprindelige budget). Een er fratrædt i slutningen af 2005 efter en meget produktiv indsats, mens de to resterende overgår til anden projekt-ansættelse i løbet af vinter/forår 2007. Derved bliver den samlede postdoc udgift holdt indenfor AAU budgettet.