

## **BioNET, Annual Report**

**1 January – 31 December 2007**



## 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 (01.11.04-31.12.07) Nader Reihani (01.01.06-30.06.07) P. Sigmundsson (01.1006-01.)
PhD students	Tabita W. Madsen, (01.02.05- 3 år) Anna Andersson (01.12.05 , 50% BioNET/CMOL, pt orlov) 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) Andreas Blicher Peter. Ahlgren

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

### Overview of the activities:

The main focus in the biocomplexity group is to model the behaviour of living systems, in particular systems where we have access to experimental data. How the proteins inside a cell influence each other is characterized by the underlying genetic network. We collect all the known data of a given system within the genetic networks of the participating proteins. We are mainly interested in the dynamical behaviour of the networks. 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 feedback loops. We have been actively involved in the study of proteins related to cell death (p53), segmentation in embryos (Hes1) and inflammation (NF-kB). We have also studied the SOS response after DNA damage and the iron flow through bacteria cells. Recently, we have initiated a project in embryo segmentation modelled by a mathematical system of two oscillators, Wnt and Notch, which are essential in somite formation in embryos. All these projects are performed in close collaborations with biologists and in 2007 we have expanded our interactions with experimental colleagues, in particular Profs. Alexander Hoffmann, San Diego, Arnie Levine, Princeton, Galit Lahav, Harvard, Ian Dodd, Adelaide, and Szabolcs Semsey, Budapest. In particular we have in collaboration with Prof. Levine organized a two day workshop at Institute of Advanced Studies in Princeton, in Nov. 2007, where our group presented the modelling of NF-kB, p53 and Wnt-Notch systems and other participants presented the experimental data. This workshop has initiated several collaborations on these interesting issues.

## Projects:

### **Oscillatory gene expressions in embryos: The Wnt and Notch Systems**

(Peter B. Jensen, Mogens H. Jensen & Sandeep Krishna)

Oscillating gene expressions are essential for somite segmentations in embryos of mice. It is well known that two oscillators are important, the Wnt and Notch, and that these are out of phase. We formulate a mathematical model of the Wnt oscillator based on a feed-back loop where the protein Axin2 is the main player. This protein also binds to the Wnt ligand and is effectively degraded this way. A complex formation with beta-catenin and GSK completes the feed-back loop. The model produced nice damped and sustained oscillations with the correct period. We further introduce a coupling to the Notch oscillator over the GSK protein. Then the Wnt system drives oscillations in the Notch system which is indeed out of phase and observed in experiments.

### **Dynamics of uptake and metabolism of small molecules in cellular response systems preprint**

(Maria Werner, Szabolcs Semsey, Kim Sneppen & Sandeep Krishna)

Proper cellular function requires uptake of small molecules from the environment. In response to changes in extra cellular conditions cells alter the import and utilization of small molecules. For a wide variety of small molecules the cellular response is regulated by a network motif that combines two feedback loops, one which regulates the transport and the other which regulates the subsequent metabolism. We analyze the dynamic behaviour of two widespread but logically distinct two-loop motifs. These motifs differ in the logic of the feedback loop regulating the uptake of the small molecule. We find that the negative feedback to transport is accompanied by overshoot in the intracellular amount of small molecules, whereas a positive feedback to transport removes overshoot by boosting the final steady state level. On the other hand, the negative feedback allows for a rapid initial response, whereas the positive feedback is slower.

### **Combinatorics of feedback in cellular uptake and metabolism of small molecules**

(Szabolcs Semsey, Sandeep Krishna & Kim Sneppen)

We analyze the connection between structure and function for regulatory motifs associated with cellular uptake and usage of small molecules. Based on the boolean logic of the feedback we suggest four classes: the socialist, consumer, fashion, and collector motifs. We find that the socialist motif is good for homeostasis of a useful but potentially poisonous molecule, whereas the consumer motif is optimal for nutrition molecules. Accordingly, examples of these motifs are found in, respectively, the iron homeostasis system in various organisms and in the uptake of sugar molecules in bacteria. The remaining two motifs have no obvious analogs in small molecule regulation, but we illustrate their behavior using analogies to fashion and obesity. These extreme motifs could inspire construction of synthetic systems that exhibit bistable, history-dependent states, and homeostasis of flux (rather than concentration).

## Oscillations and temporal signalling in cells

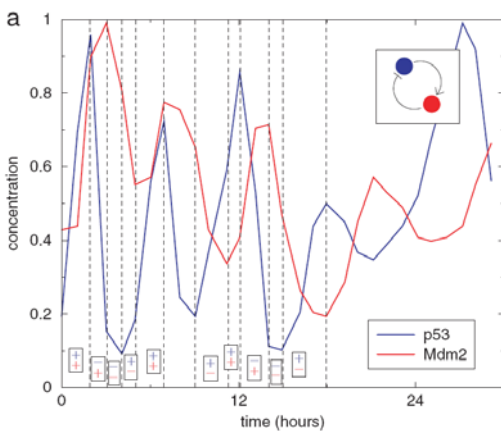
(G Tiana, S Krishna, S Pigolotti, M H Jensen & K Sneppen)

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. Here we review the different mechanisms which can produce oscillations in gene expression or protein concentration using a framework of simple mathematical models. We focus on three eukaryotic genetic regulatory networks which show 'ultradian' oscillations, with a time period of the order of hours, and involve, respectively, proteins important for development (Hes1), apoptosis (p53) and immune response (NF- $\kappa$ B). We argue that underlying all three is a common design consisting of a negative feedback loop with time delay which is responsible for the oscillatory behaviour.

## Signal Integration in the Galactose Network of Escherichia coli

(Szabolcs Semsey, Sandeep Krishna, Kim Sneppen & Sankar Adhya)

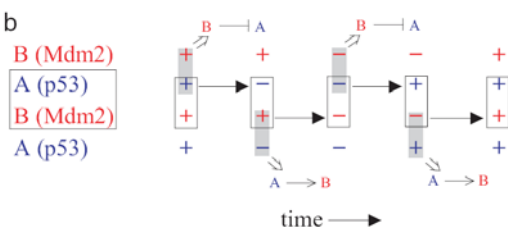
The gal regulon of Escherichia coli contains genes involved in galactose transport and metabolism. Transcription of the gal regulon genes is regulated in different ways by two iso-regulatory proteins, Gal repressor (GalR) and Gal isorepressor (GalS), which recognize the same binding sites in the absence of galactose. DNA binding by both GalR and GalS is inhibited in the presence of galactose. Many of the gal regulon genes are activated in the presence of the adenosine cyclic-3',5'-monophosphate (cAMP)-cAMP receptor protein (CRP) complex. We studied transcriptional regulation of the gal regulon promoters simultaneously in a purified system and attempted to integrate the two small molecule signals, d-galactose and cAMP, that modulate the isoregulators and CRP respectively, at each promoter, using Boolean logic. Results show that similarly organized promoters can have different input functions. We also found that in some cases the activity of the promoter and the cognate gene can be described by different logic gates. We combined the transcriptional network of the galactose regulon, obtained from our experiments, with literature data to construct an integrated map of the galactose network. Structural analysis of the network shows that at the interface of the genetic and metabolic network, feedback loops are by far the most common motif.



## Oscillation patterns in negative feedback loops

(Simone Pigolotti, Sandeep Krishna & Mogens H. Jensen)

Organisms are equipped with regulatory systems that display a variety of dynamical behavior 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, when



a single negative feedback loop dominates the dynamical behavior, the sequence of maxima and minima of the concentrations exhibit a pattern that uniquely identifies the interactions of the loop. This allows us to devise an algorithm to (i) test whether observed oscillating time series are consistent with a single underlying negative feedback loop, and if so, (ii) reconstruct the precise structure of the loop, i.e., the activating/repressing nature of each interaction. 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 the estrogen-sensitive pS2 promoter.

## **UV-induced mutagenesis in the Escherichia coli SOS response: A quantitative**

(Sandeep Krishna, Sergei Maslov & Kim 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 present 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<sup>2</sup>. 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.

## **Efficient degradation and expression prioritization with small RNAs**

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

We build a simple model for feedback systems involving small RNA (sRNA) molecules based on the iron metabolism system in the bacterium E. coli, and compare it with the corresponding system in H. pylori which uses purely transcriptional regulation. This reveals several unique features of sRNA-based regulation that could be exploited by cells. Firstly, we show that sRNA regulation can maintain a smaller turnover of target mRNAs than transcriptional regulation, without sacrificing the speed of response to external shocks. Secondly, we propose that a single sRNA can prioritize the usage of different target mRNAs. This suggests that sRNA regulation would be more common in more complex systems which need to co-regulate many mRNAs efficiently.

## **Dynamics of Opinions and Social Structures**

(M. Rosvall & K. Sneppen)

Social groups with widely different music tastes, political convictions, and religious beliefs emerge and disappear on scales from extreme subcultures to mainstream mass-cultures. Both the underlying social structure and the formation of opinions are dynamic and changes in one affect the other. Several positive feedback mechanisms have been proposed to drive the diversity in social and

economic systems, but little effort has been devoted to pinpoint the interplay between a dynamically changing social network and the spread and gathering of information on the network. Here we analyze this phenomenon in terms of a social network-model that explicitly simulates the feedback between information assembly and emergence of social structures: changing beliefs are coupled to changing relationships because agents self-organize a dynamic network to facilitate their hunter-gatherer behaviour in information space. Our analysis demonstrates that tribal organizations and modular social networks can emerge as a result of contact-seeking agents that reinforce their beliefs among like-minded. We also find that prestigious persons can streamline the social network into hierarchical structures around themselves.

## **Analyzing a stochastic model for evolving regulatory networks by unbiased gene duplication**

(Jakob Enemark & Kim Sneppen)

**Background:** Duplication of genes is important for evolution of molecular networks. Many authors have therefore considered gene duplication as a driving force in shaping the topology of molecular networks. In particular it has been noted that growth via duplication would act as an implicit way of preferential attachment, and thereby provide the observed broad degree distributions of molecular networks. **Results:** We extend current models of gene duplication and rewiring by including directions and the fact that molecular networks are not a result of unidirectional growth. We introduce upstream sites and downstream shapes to quantify potential links during duplication and rewiring. We find that this in itself generates the observed scaling of transcription factors for genome sites in prokaryotes. The dynamical model can generate a scale-free degree distribution,  $p(k) \propto 1/k^{\gamma}$ , with exponent  $\gamma=1$  in the non-growing case, and with  $\gamma>1$  when the network is growing. **Conclusions:** We find that duplication of genes followed by substantial recombination of upstream regions could generate main features of genetic regulatory networks. Our steady state degree distribution is however too broad to be consistent with data, thereby suggesting that selective pruning acts as a main additional constraint on duplicated genes. Our analysis shows that gene duplication can only be a main cause for the observed broad degree distributions, if there also are substantial recombinations between upstream regions of genes.

## **Modelling transcriptional interference and DNA looping in gene regulation**

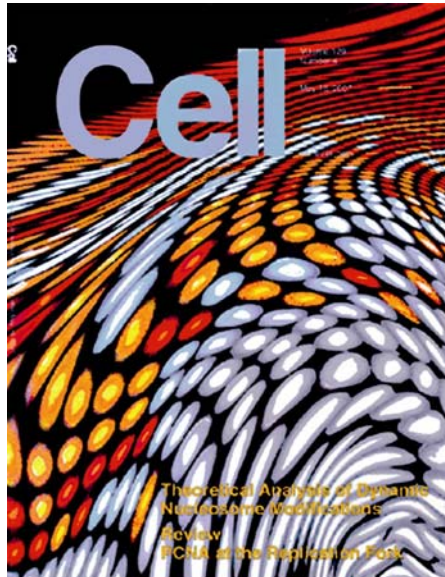
(I.B. Dodd, K.E. Shearwin & K. Sneppen)

We describe a hybrid statistical mechanical and dynamical approach for modelling the formation of closed, open and elongating complexes of RNA polymerase, the interactions of these polymerases to produce transcriptional interference, and the regulation of these processes by a DNA-binding and DNA-looping regulatory protein. As a model system, we have used bacteriophage 186, for which genetic, biochemical and structural studies have suggested that the CI repressor binds as a 14-mer to form alternative DNA-looped complexes, and activates lysogenic transcription indirectly by relieving transcriptional interference caused by the convergent lytic promoter. The modelling showed that the original mechanisms proposed to explain this relief of transcriptional interference are not consistent with the available in vivo reporter data. However, a good fit to the reporter data was given by a revised model that incorporates a novel predicted regulatory mechanism: that RNA polymerase bound at the lysogenic promoter protects itself from transcriptional interference by recruiting CI to the lytic promoter. This mechanism and various estimates of in vivo biochemical

parameters for the 186 CI systems should be testable. Our results demonstrate the power of mathematical modelling for the extraction of detailed biochemical information from in vivo data.

## Theoretical Analysis of Epigenetic Cell Memory by Nucleosome Modification

(Ian B. Dodd, Mille A. Micheelsen, Kim Sneppen & Geneviève Thon)



Chromosomal regions can adopt stable and heritable alternative states resulting in bistable 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 are 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 can give strong bistability that is resistant both to high noise due to random gain or loss of nucleosome modifications and to random partitioning upon DNA replication. However, robust bistability required: (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. (Picture: Frontcover chosen by Cell, in col. with artist Mette Høst, NBI)

## Spreading out of perturbations in reversible reaction networks

(Sergei Maslov, Kim Sneppen & Iaroslav Ispolatov)

Using an example of physical interactions between proteins, we study how a perturbation propagates in the equilibrium of a network of reversible reactions governed by the law of mass action. We introduce a matrix formalism to describe the linear response of all equilibrium concentrations to shifts in total abundances of individual reactants, and reveal its heuristic analogy to the flow of electric current in a network of resistors. Our main conclusion is that, on average, the induced changes in equilibrium concentrations decay exponentially as a function of network distance from the source of perturbation. We analyze how this decay is influenced by such factors as the topology of a network, binding strength, and correlations between concentrations of neighboring nodes. We find that the minimal branching of the network, small values of dissociation constants, and low equilibrium free (unbound) concentrations of reacting substances all decrease the decay constant and thus increase the range of propagation. Exact analytic expressions for the decay constant are obtained for the case of equally strong interactions and uniform as well as oscillating concentrations on the Bethe lattice. Our general findings are illustrated using a real network of protein-protein interactions in baker's yeast with experimentally determined protein concentrations.

## Activities of the the Optical Tweezers Group (Lene Oddershede)

### Biological membranes

Through the BioNET collaboration the optical tweezers group at NBI has mainly focused on studying properties of the living bacterial membrane. Using optical tweezers we grabbed polystyrene beads unspecifically attached to the bacterial outer membrane. By pulling on these beads tethers consisting of membrane material were extracted out from the bacterial body. Through force-extension relationships we performed a physical characterization of the viscoelastic properties of the membrane tethers. Through enzymatic studies we examined the biochemical constituents of the tethers, also, we used different mutants deficient in various parts of the outer membrane structure. The conclusion was that the tethers mainly consisted of lipopolysaccharides, and that they behaved elastic on short time scales but viscous on timescales approaching hundreds of seconds, our results were published in Jauffred et al. *Biophysical Journal*, 2007. In parallel, we have continued our investigation of protein motility in bacterial membranes. The new angle on this comes about through collaboration with the BioNET partner Daniel Otzen, iNANO, Århus. The goal is to monitor the mobility of the DsbB protein in the inner phospholipid membrane of *E. coli* bacteria. To this end the protein has been biotinylated to attach a streptavidin coated bead to it to be used as a handle for the optical methods. The status of the project is that we are able to revive spheroblasts expressing the protein.

### Technical improvements

A major fraction of our activities focused on improving the technique of optical trapping. The major de-stabilizing factors of an optical trap are the spherical aberrations, which in particular weaken the trap in the axial direction. We found that by a clever choice of immersion media, the spherical aberrations intrinsically present in the system can be compensated with an exceptionally strong trap as a result; our findings were published in *Optics Letters*, 2007. This improved optical trap also has made it possible for us to perform optical trapping of silver nanoparticles (results accepted for publication in *Nano Letters* 2008) as well as trapping of quantum dots. The project concerning optical manipulation of quantum dots is in collaboration with BioNET partner Chris Lagerholm, SDU.

### In vitro single molecule studies

Finally, our efforts related to BioNET in 2007 also encompassed single molecule investigations of the mechanical stability of mRNA pseudo knots and the correlation between pseudo knot strength and degree of ribosomal frame shifting. These results were published in *PNAS*, 2007.

**People financially supported by BioNET in 2007:** Tabita Winther, Nader Reihani, Liselotte Jauffred.

## Activities of the Membrane Biophysics Group, (Thomas Heimburg)

The main research interests of the “Membrane Biophysics Group” are cooperative events in biological membranes and their thermodynamics. This includes domain formation processes, permeability studies, pulse propagation in nerves, but also topics of medical interest as general and local anesthesia. Most of these phenomena are in a logical manner connected to the phase transition temperatures of lipid membranes.

In 2005, we proposed a theory of nerve pulse propagation that is based on such cooperative events (in collaboration with A. D. Jackson, NBI). This was the starting point of a series of papers published in 2007. We propose that the nerve pulse is a piezoelectric soliton that can exist close to the lipid phase transitions. Lipid phase transitions have been found in native biomembranes slightly below physiological temperatures. We base this theory on a number of thermodynamic data on nerves reported in the literature. The most striking of these data is that one finds a reversible heat release during the nerve pulse. This implies that the nerve pulse is based on reversible physics. However, the text book models rely on the flux of ions along gradients, which is an irreversible process. In our group many studies circle around this and related issues. Presently, one post doc is investigating nerve pulse propagation in real nerves of invertebrates, and one master student is investigating pulse propagation on monolayer systems. Interestingly, this theory also implies an explanation of anesthesia. The effect of anesthetics is explained to be rooted in the effect of melting point depression caused by dissolving anesthetics molecules within the membranes. This melting point plays an essential role in the soliton theory.

The present textbook understanding of many processes in biomembranes – including the nerve pulse - is based on the opening and closing of ion channel proteins. Both, our theories on nerves and on anesthesia, challenge the conventional views of such processes and work without objects such as ion channels. A theory for nerves based on the thermodynamics of biological membrane systems is new and necessarily controversial. Therefore, these developments from our group found a lot of coverage in the international media (listed below). Ion channel protein conductance can be investigated in electrophysiology recordings (either patch clamp or ‘black lipid membranes’, BLM). The currents measured across biological membranes show up as discrete and quantized steps. This has been taken as evidence for the existence of macromolecules (ion channel proteins) that are responsible for such events. In a masters project (and hopefully continuing as a PhD project within BioNET) we have constructed a ‘black lipid membrane (BLM)’ setup to record such conductance events. We found that artificial membranes not containing any proteins can display very similar phenomena as reported for ion channel proteins if one is close to the phase transition temperature. One finds quantized currents through such membranes that resemble those reported for ion channel proteins both in conductance and lifetime. This fact is surprising, but there are also some reports in the literature about this. We found that the lipid ion channels respond in a very coherent manner to changes in the thermodynamic variables. For instance, they can be switched on and off with anesthetics.



Other activities of our group include studies on charged lipid membrane systems that form extended lipid bilayer networks, and the formation of lipid tethers and their phase behavior.

**Press reports about the Science of the Membrane Biophysics Group:**

16.01.2008	Illustreret videnskab 2/2008: 44-47.	Nervesignaler er lydbølger
25.06.2007	Ingeniøren	Nerver kommunikerer med lydbølger
11.06.2007	Wired Magazine	Nerves might run on sound, not electricity
11.06.2007	Corriere della Sera	La musica del sistema nervoso
04.03.2007	Business Week	Of neural sound waves
12.03.2007	Stereophile Magazine	The Nerve
09.03.2007	Canad. Broadcast.Corp, CBC	Scientists say nerves use sound, not electricity
07.03.2007	Politiken	Smerte er lyd

**Co-financing of collaborators by BioNET:**

Matthias Fidorra, 0.5 Ph.D. stipend, Ph.D. defense in November 2007. He studied domain formations. Thesis title “Confocal microscopy, calorimetry and permeability studies on giant lipid vesicles containing ceramides” at SDU.

Kristmundur Sigmundsson, Postdoc since October 2006. His duty is to study nerve pulse propagation in invertebrates

**First paper using the FTIR spectrometer co-financed with 250 kDKK by BioNET in 2004:**

M. Fidorra, T. Heimburg and H. M. Seeger. 2007. *Melting of Single Lipid Components in Binary Lipid Mixtures: A Comparison between FTIR Spectroscopy, DSC and Monte Carlo Simulations.* submitted ([arXiv:0712.0064](http://arxiv.org/abs/0712.0064))

## The SDU node of BioNET

Responsible scientist	Prof. Ole G. Mouritsen
Principal scientists in period of report	Prof. Luis Bagatolli (2004-) Dr. Chris Lagerholm (2006-) Prof. Beate Klösgen (2006-) Prof. John Hjort Ipsen (2007-)
Postdoc	Dr. Kristian Boye (2007)
PhD students	Matthias Fidorra (SDU/NBI, 2004-2007) Stinne Hørup Hansen (SDU, 2004-) Maria B. Mølgaard (SDU, 2005-maternity leave) Brian Vad (AAU & SDU, 2005-) Eva Arnsfang Christensen (SDU, 2006-)
MSc student	Rakhu Sankar (SDU, 2006-) Hanne Matras (2007-)

## Activities of the SDU node

### Major research themes at SDU under BioNET

#### **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, Rakhu 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,)

#### **An *in vivo* investigation of the cellular plasma membrane nano-organization**

(Dr. Christoffer Lagerholm, PhD-student Eva Arnsfang Christensen, MSc-student Hanne Matras)

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

(PhD-student Stinne Hørup Hansen)

#### **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))

#### **Instrumentation for advanced microscopy and single molecule imaging**

(Dr. Chris Lagerholm, Prof. Luis Bagatolli)

#### **Bio-probe force spectroscopy and ligand-receptor interactions**

(Dr. Kristian Boye, Prof. John H. Ipsen)

## PhD-theses supervised under BioNET

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" (2004-2007).

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-).

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-)

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.

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-).

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-).

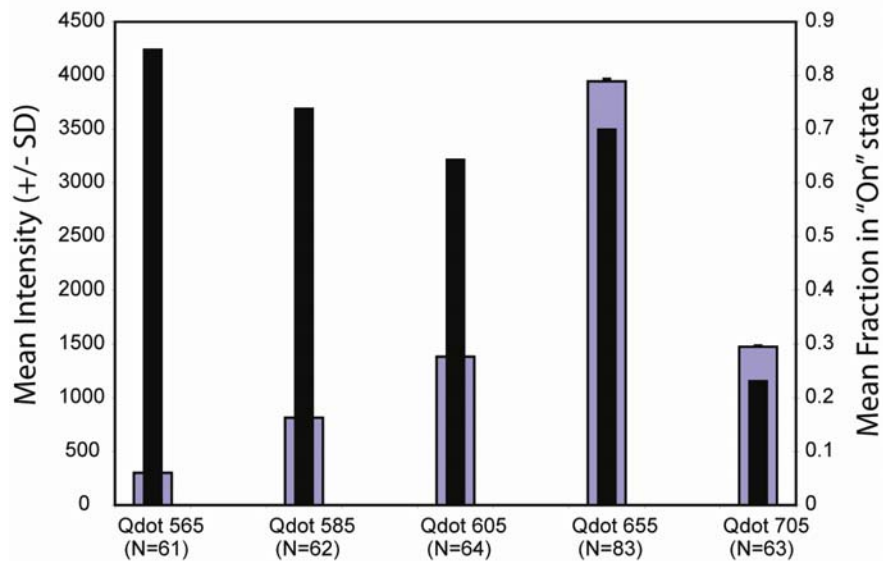
## Research progress report

### Membrane proteins and imaging using quantum dots

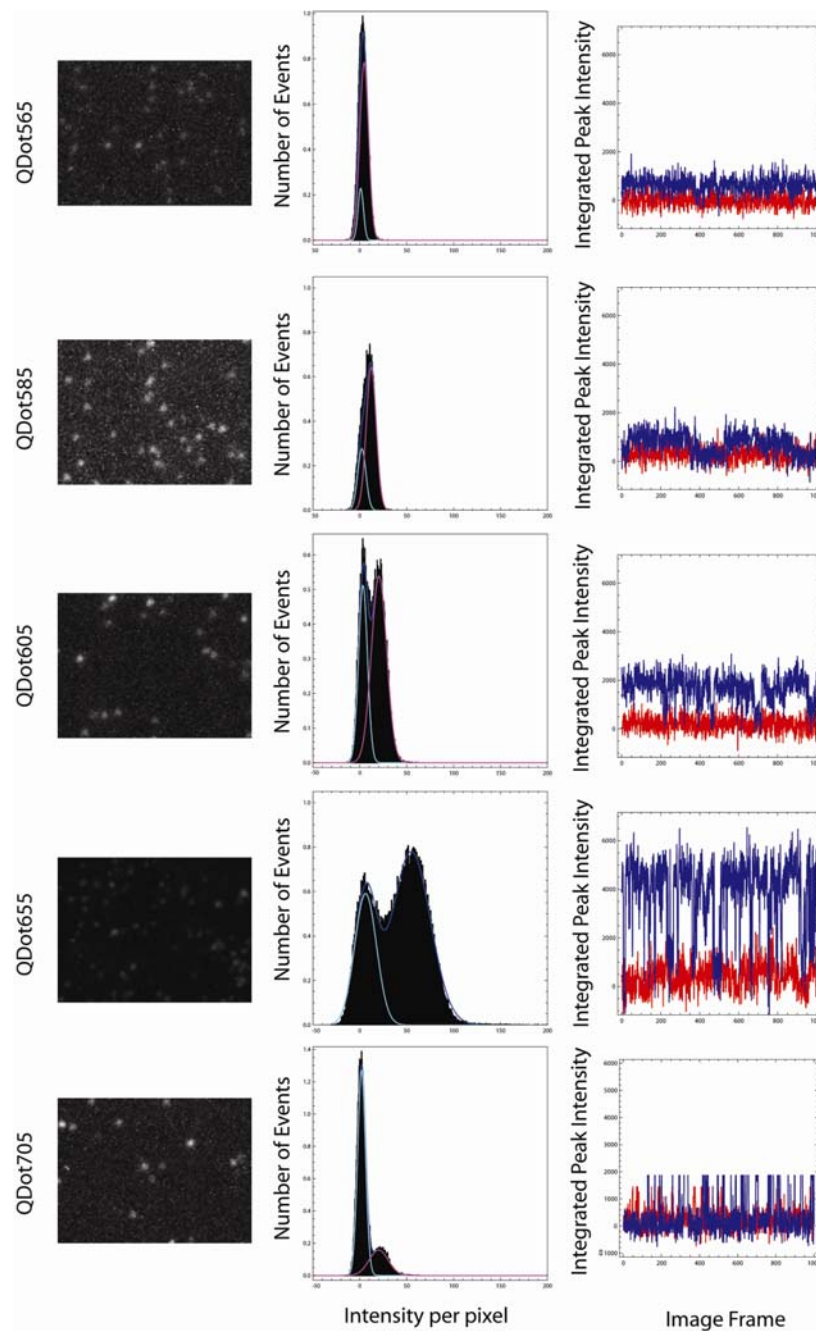
(Dr. Christoffer Lagerholm, PhD-student Eva Arnspang Christensen, MSc-student Hanne Matras)

#### Characterization of optical properties of single quantum dots

Quantum dots (Qdots) have several advantages compared to other probes for single molecule imaging. These include enhanced brightness and photostability as well as in some cases smaller size. The major advantage of Qdots for single molecule imaging is the possibility of simultaneous imaging of multiple species at fast repetition rates over long periods of time. With this in mind, we have assembled a microscopy system capable of imaging multiple colors of single quantum dots at high repetition rates over long periods of time in cells and substrate-supported planar membranes. This system, which consists of an Olympus IX81 microscope equipped with an electron-multiplied CCD (Andor DV887-ECS) for detection, we can image single Qdots with 100  $\mu$ s signal integration or at rates up to 250 Hz. However, we have found that these results are very dependent on the particular emission color characteristics of the Qdots, as we find that certain quantum dot colors are dimmer and/or primarily in a non-fluorescent state (Figures 1.1 and 1.2). These results were presented by Ph. D. student Eva Christensen Arnspang at the 2007 6th European Biophysics Congress in London, UK; a presentation that was also awarded by a Poster Prize. A manuscript of these results is currently in preparation.



**Figure 1.1.** Mean on intensity (grey bars) and mean fraction that Qdots were in on intensity state (black bars). : Intensity comparison of streptavidin Qdots adsorbed on glass. Images were acquired with 1 ms integration and an acquisition frame rate of 94 Hz. Under these conditions, the order of Qdot brightness is 655 nm > 605 nm, 705 nm > 585 nm > 565 nm. The Qdot “on-intensity” frequency is approximately equal at ~70-80% for the 565, 585, 605, and 655 nm Qdots while the 705 nm Qdots are only in the “on-intensity” state 25% of the time.

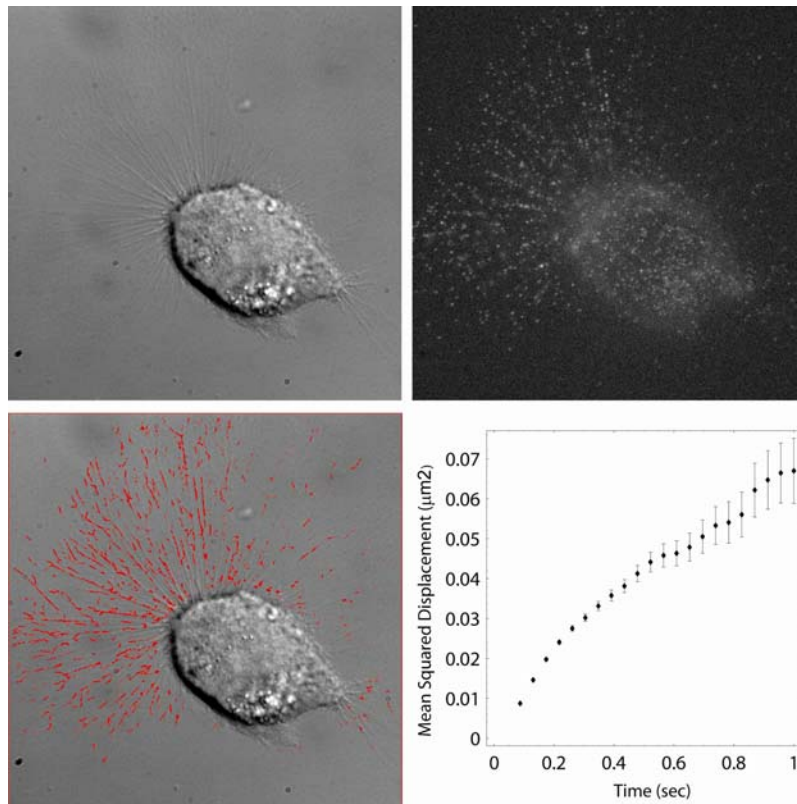


**Figure 1.2:** Raw data for Figure 1. (left) Maximum intensity images of from top to bottom, 565 nm, 585 nm, 605 nm, 655 nm and 705 nm Qdots. (center) Histograms of integrated peak intensities per pixel of all single Qdots in an image series. (right) Representative plots of the integrated peak intensity of single Qdots as a function of frame number showing characteristic Qdot blinking behavior.

## Random genetic targeting of native membrane proteins

In a majority of diseases, the plasma membrane constitutes a key component in the search for new therapeutics. While the composition of the plasma membrane has been well characterized less is

known of the structure. The nano-organization of the plasma membrane including the hypothesized existence of lipid raft cell signaling nano-domains in the plasma membrane has generated much interest during the last decade. Our goal is to develop a new *in vivo* system in order to investigate the possible the nano-organization of the plasma membrane including the possible existence of lipid rafts. In this work we are using a genetically engineered retrovirus to insert a specific peptide sequence in native membrane protein which allows for universal targeting with a combination of bacterial biotin ligase and any streptavidin based probe (Figure 1.3).



**Figure 1.3.** Genetically modified mouse embryo fibroblast (MEF) specifically labeled with biotin ligase and streptavidin (sAv) conjugated Qdots. (top left) DIC image of MEF. (top right) Single image (10 ms integration, 23 Hz acquisition) of sAv-Qdots specifically bound to unknown genetically modified native membrane protein. (bottom left) Fit trajectories of all single Qdots from first 120 image frames (~5.2 sec). (bottom right) Mean squared displacement of all trajectories of all single Qdots in the first 120 image frames.

During the course of the year, we have used molecular biology to make improvements to the inserted peptide sequence in order to increase our chances of generating cell lines containing genetically modified native membrane proteins. This includes generating a reading frame independent biotin ligase acceptor peptide insert and a combined reading frame independent insert that also confers puromycin resistance to modified cells. We are currently evaluating both of these constructs.

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

(PhD-student Stinne Hørup Hansen)

In January and February 2007 67 students from 2 high schools attended performances of *The Magic Bullet* at the theater and participated in this study. Several qualitative methods were used to collect and analyze data. Currently the empirical data is being analyzed and the theater play itself is

analyzed with regard to narrative and didactical reconstruction theory. Two follow-up teaching sequences were conducted post-performance, a three week project and a three hour trail. The citations below are student comments on the sequences:

Project: *I think that there has been a great collaboration – much more than I had ever imagined biology and physics could collaborate. (Interview 16, S2N).*

Trail: *It was really good. Some of the questions made me think that, argh, I should have taken notes. But then in the group we all remembered different things and then we talked about it and it was like a repetition and that was really great. (Interview 35, C2B).*

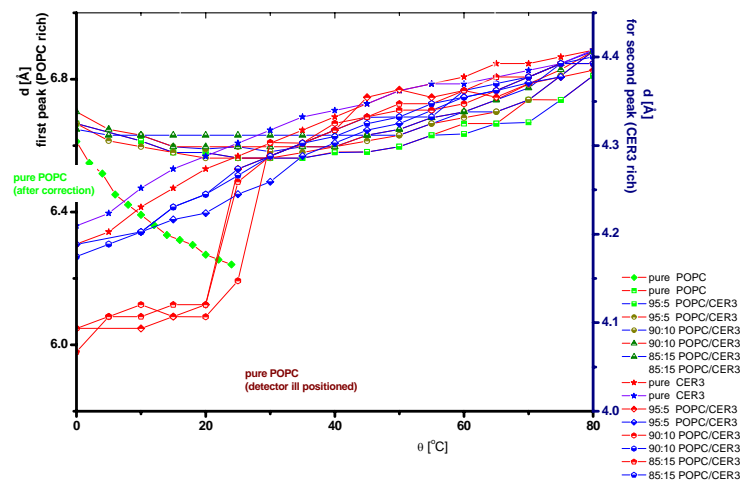
## Structure function interplay of skin: establishing a laboratory model to mimic real skin tissue

(Prof. Beate Klösgen and PhD-student Rakhu Sankar)

### The effect of the ceramide CER3 into a fluid host membrane of POPC

The ceramide CER3 was chosen as a model ceramide and embedded at different molar fraction (5%, 10%, 15% into samples of constant lipid contents of lipid/water: 5mg/ml) into the well-known model membrane of POPC. The system was then studied using X-ray diffraction, differential scanning calorimetry, and confocal microscopy.

### X-ray diffraction:



**Figure 3.1:** Temperature dependency of the lamellar repeat distance  $d$  from a stack of membranes consisting either of pure POPC or CER3, and mixtures of CER3 into POPC. Two coexisting structures are obtained in all mixtures that can be distinguished by their repeat distances  $d_{I,II}$ . The respective values are entered on the left axis.  $d_{II}$  signifies a narrow packing with almost no bulk water contained; the related values are found on the right axis. Red curves represent results from the first heating of the samples; blue curves correspond to the cooling scan following the heating. Details see text.

Two coexisting structures are obtained in all mixtures that can be distinguished by their repeat distances  $d_{I,II}$ .  $d_I$  signifies a high distance that is comparable to the one of pure POPC (bright green: pure POPC, heating scan; red and blue stars: pure CER§, heating and cooling scan).

The course of pure POPC exhibits pronounced characteristics due to the interplay of membrane layer shrinkage and water expansion. For higher temperatures, the  $d_I$ -values increase again as a consequence of the increasing undulation repulsion. The course of the related pure ceramide is almost linearly increasing: the system is all the time below its main transition temperature: therefore, undulations are almost absent, and the increased can be attributed only mostly to the normal temperature dependent expansion of the system.

In the mixed systems the behaviour is totally different: the small distance values  $d_{II}$  exhibit a jump around 25°C at first heating where the distance goes from ~4.1Å to ~4.25Å that is absent later on. This jump can most probably be attributed to a sudden rupture of the system accompanied by some water uptake. Thereafter the peak of this ceramide rich domain almost follows the course of the pure ceramides and does so as well upon subsequent cooling as also upon further heating. No swelling is observed.

The high distance values  $d_I$  (left axis) of the mixed system start at about the value for the pure POPC at low temperature but do not exhibit its pronounced temperature course, for all mixtures. Instead there is only a slight swelling from 6.73Å to 6.81Å that exhibit traces of the interplay among chain melting / water expansion and undulation repulsion. No sudden jump of water uptake at any temperature occurs.

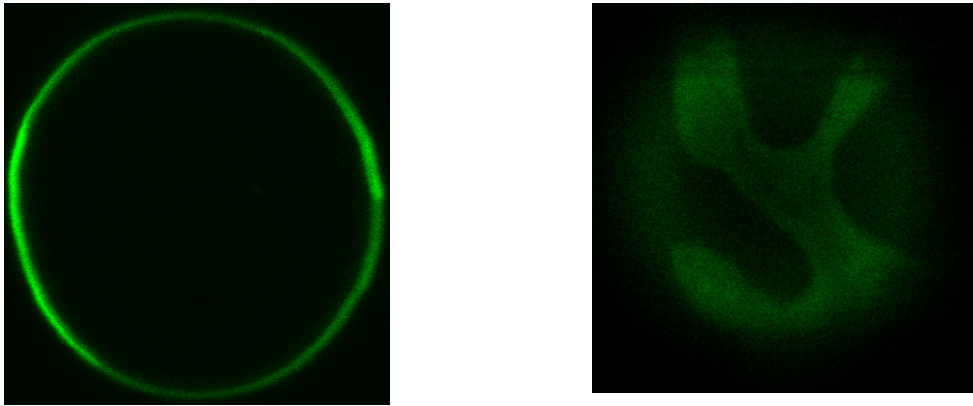
### **DSC-experiments:**

Identically composed systems were scanned many times by DSC. Pure POPC is characterized by a phase transition around -5°C, whereas pure CER3 melts above 100°C where the method reaches its limit of precision. The high temperature region is therefore not easy to analyse but in all cases a broad peak around 108°C was seen.

The peak that signifies the transition of the initially pure POPC is slightly shifted towards higher transition temperatures with increasing CER3 contents of the whole sample: the presence of small amounts of CER3 seems to support the stability of the gel phase of POPC. Moreover, a very broad new peak emanates in the course of time. The details must still be clarified, and especially the chemical stability of the sample if being checked just now.

### **Confocal microscopy:**

Giant vesicles obtained from electro-swelling were labelled by DiI and observed for the morphologies and domain structure in the confocal microscope. For all compositions, domains were seen with flower-like shapes and smooth domain borders. The domains shown below represent the POPC rich regions of the membrane.



**Figure 3.2:** Single vesicles prepared from a sample containing ~15% (mol/mol) CER3 in a host system of POPC. Rigid, partially faceted domains can be distinguished in elsewhere strongly fluctuating vesicle membranes. Left: equatorial view; right: top view

Incorporation of CER3 into lipid host membranes of POPC already at small molar fractions induces the formation of domains in the host membrane. The effect of the ceramide is solidifying: this is seen by all methods. Especially the diffraction data hint towards the presence of a mechanically stabilized membrane conformation still exhibiting some features of fluidity. This is a major feature of skin: being soft and stable at a time, and as well providing the option of lateral and transversal transport.

The future plans include: a) More detailed analysis of the calorimetry data. b) Further diffraction experiments and their analysis towards the acquisition of the density profile. From these experiments the relative extension of the hydrophobic core and the more hydrophilic membrane interface as well as the water distribution will be extracted; micromechanical studies of the mixed membranes will be attempted. c) further analysis of the confocal images; possibly transport in the distinguishable domain will be tried (FRAP)

## Bio-probe force spectroscopy and ligand-receptor interactions

(Dr. Kristian Boye, Prof. John H. Ipsen)

In an international collaboration with Institute for Bio- and Nanosystems at Research Centre Jülich and a polish colleague from the Jagiellonian University in Cracow, a biological relevant receptor-ligand system of integrin  $\alpha7\beta1$  and invasin has been studied with the Biomembrane Force Probe technique – a technique for measuring minuscule forces at biological interfaces. Recently we were able to complete an extensive series of measurements investigating the role of divalent cations on this particular interaction, and a full paper draft on this study has been prepared from my side and is currently being perused by my collaborators in Jülich. The paper is to be submitted to the Journal of Biological Chemistry in the near future. Some results from the study have already been published on my poster contribution for the 52<sup>nd</sup> annual meeting of the Biophysical Society in Long Beach (which I attended in February 2008). My involvement in the project embraces modeling, experimental planning and - to a high degree - data analysis and paper writing. However, I have also spent a couple of weeks in 2007 conducting measurements at the micromanipulation setup in Jülich. A force dynamical study of the same protein system aimed at investigating the binding energy landscape of

the interaction has been initiated recently, and a second joined publication covering this new project is planned for publication later this year.

Work is also in progress that investigates theoretical aspects of dynamic force measurements obtained with the Biomembrane Force Probe technique. The modeling has reached a final stage, and paper writing has been initiated.

Bio-probe force instrumentation has been furnished with technical improvements of the local micromanipulation setup. The aim has been a refinement of already implemented procedures as well as the development of new techniques. Some of the achievements are listed below:

1. Installation and integration of a new and highly accurate pressure system.
2. Implementation of a video recording routine for the permanent micromanipulation setups.
3. Development and application of a mobile micromanipulation setup for our local confocal microscope (together with PhD student Olav Garvik).
4. Development of new software for Vesicle Fluctuation Analysis (together with John Ipsen and PhD student H el ene Bouvrais).
5. Development of analysis software for the Biomembrane Force Probe technique.

## Imaging of giant unilamellar vesicles (GUV)

(PhD-student Matthias Fidorra , Prof. Luis Bagatolli)

We developed a new image analysis for confocal microscopy image stacks of Giant Unilamellar Vesicles composed of diverse lipid mixtures. Deconvolution and segmentation routines followed by 3D surface models were used to reconstruct the surfaces and from 3D sphere projections the area fractions of the coexisting lipid phases were calculated. These results were utilized to fit the lever rule along particular tie lines obtained from the lipid mixture phase diagram of binary lipid mixtures. This study not only adds a quantitative component to the so far qualitative inspection of GUVs but demonstrates that the coexisting phases are in real thermodynamic equilibrium in these model membrane systems. At present the same analysis is being applied with some modifications in order to compute tie lines from the area fraction measured from the GUVs composed of canonical raft mixtures.



**Figure 5.1** GUVs composed of canonical raft mixtures.

## Skin biophotonic

(PhD-student MariaBloksgard Mølgard, Prof. Luis Bagatolli)

An implementation of a lipidomics approach was realized during 2007 in order to determine the lipid composition of skin stratum corneum membranes from mouse skin. The information obtained is combined with the diffusion coefficient measured at different depths in skin using scanning FCS techniques and pH and polarity profiles of skin obtained using FLIM (pH sensitive probes) and LAURDAN two photon excitation GP images. This general approach is currently applied to Acyl CoA knock-out mouse skin in order to determine if the compositional and physico-chemical information differs from wild type mice.

## Outreach

During the period of report, members of BioNET-SDU have been actively engaged in a wide range of outreach activities where the research has been propagated to students and teachers in elementary school and high-school, to researchers and students in other fields, as well as to the general public. In particular, BioNET-PhD-student Eva Arnspang Christensen and Ole G. Mouritsen were engaged in the science theatre performance *The Magic Bullet* during the months of January and February of 2007. The performance, which was organized and produced by the Center for Science and Art at the University of Southern Denmark was seen by about 1200 people, including school kids, students, faculty and members of the public.

Ole G. Mouritsen was awarded the National Prize for Science Communication 2007 and the University of Southern Denmark Prize for Research Communication for 2007. BioNET-PhD-student Stinne Hørup Hansen was the Winner of the 2007 Researcher Grand Prix (Competition on research communication among PhD-students). PhD-student Eva Arnspang Christensen was awarded an award for best poster at the 6th European Biophysics Congress in London in July.

## Short-time visitors and seminars related to BioNET

1. Dr. Daniel Wüstner, Dept. of Biochemistry and Molecular Biology, SDU, March 8, 2007: "Molecular assembly, membrane insertion and intracellular transport of a fluorescent cholesterol analog".
2. Ruth Montes, Ph.D. fellow, University of Bilbao, Spain, March 18-23, 2007.
3. Prof. Paavo Kinnunen, Dept. Medical Chemistry, Helsinki University, Finland, May 24-26, 2007: "Apoptosis, amyloid formation and antimicrobial peptides".
4. Prof. Evan Evans, Departments of Physics and pathology, University of British Columbia, Canada and Department of Biomolecular Engineering, Boston University, USA, June 20-23, 2007: "New aspects of mechanical tests with lipid: cholesterol membranes and folding/unfolding of protein domains".
5. Prof. Lene Oddershede, CELCOM, Niels Bohr Institute, Copenhagen, June 21, 2007.
6. Prof. Alan Waggoner, Molecular Biosensor and Imaging Center, Carnegie Mellon University, June 21-25, 2007: "Fluorescent probes for imaging".
7. Dr. Matthias Weiss, Deutsches Krebsforschungszentrum, Germany, August 15, 2007.
8. Dr. Kerstin Wagner, Max Planck Institute of Colloids and Interfaces, Potsdam, Germany, August 20-21, 2007: "The regulation of phospholipase activity by lipid membrane structure".

9. Dr. Marcel Bruchez, Associate Research Professor/Program Manager, Technology Center for Networks and Pathways, Carnegie Mellon University, August 30-September 1, 2007: "Design and use of quantum dots for measurements in complex biological systems".
10. Stinne Hørup Hansen, Ph.D. fellow, IMADA, DREAM and MEMPHYS, SDU, September 4, 2007: "Science theatre as a way of communicating biophysics to students in upper secondary school".
11. Laura Rodríguez Arriaga, Ph.D. fellow, Dept. of Physics and Chemistry, Universidad Complutense de Madrid, Spain, September 26 - December 19, 2007: "Membrane Mechanics: Role of Cholesterol on the Elastic Properties".
12. Dr. Byron Ballou, Molecular Biosensor and Imaging Center, Carnegie Mellon University, USA, October 1-3, 2007.
13. Dr. Manuel Prieto, Portugal, November 4-6, 2007.
14. Prof. Felix Goñi, Unidad de Biofísica, Universidad del País Vasco, Spain, November 4-6, 2007.
15. Dr. Emma Sparr, Fysikalisk Kemi 1, Lunds Universitet, Sweden, December 6, 2007: "Diffusion in Responding Lipid Membranes".
16. Dr. Matthias Fidorra, Hamburg, Germany, December 7-8, 2007: "Christmas Seminar".
17. Brian Vad, Ph.D. fellow, Aarhus University, December 7, 2007: "Christmas Seminar".
18. Dr. Byron Ballou, Molecular Biosensor and Imaging Center, Carnegie Mellon University, USA, December 13, 2007.
19. Prof. Dr. Lars Norlén, Department of Cellular and Molecular Biology (CMB), Karolinska Institute, Stockholm, Sweden, December 20-21, 2007.

### **Workshops and conferences organized in relation to BioNET**

1. 2nd Annual Biophysics PhD-meeting, Bridging Theoretical and Experimental Science, Holbæk, Denmark, May 23-25, 2007.
2. Wenner-Gren Foundation's International Symposium on The Human Skin Barrier as a Biomembrane Model, Stockholm, Sweden, June 27-30, 2007.
3. MEMPHYS BioNano Workshop, University of Southern Denmark, Odense, Denmark, October 3, 2007.
4. MEMPHYS Biophysics Mini-workshop, University of Southern Denmark, Odense, Denmark, November 1, 2007.

### **Talks**

Biological Applications of Quantum Dots, Christoffer Lagerholm, Joint meeting of the Danish Society for Cyto- and Histochemistry and Danish Society for Flow Cytometry, March 2007

Genetic Engineering of Native Mammalian Proteins, Christoffer Lagerholm, Membrane proteins: Structure, stability and folding, Aalborg University PhD Course, May 2007

## Conference and symposia participation

Stinne Hørup Hansen: Videnskabsteater som formidlingsværktøj i gymnasiet. Hvordan påvirker det elevernes holdning til faget?., Symposia for high school teachers at The Elmuseet i Bjerringbro, September 6, 2007

Science Theater as a Way of Communicating Biophysics to Students in Upper Secondary School, Stinne Hørup Hansen, NNORSC 4, Fourth Nordic Network of Researchers in Science Communication Symposium, June 15-17, 2007

Challenges for Interdisciplinary Teaching of Mathematics and the Sciences in Upper Secondary School, Stinne Hørup Hansen, MACAS 2, Mathematics and its Connection to the Arts and Sciences, May 29-30, 2007

## Poster Presentations

A large-scale investigation of the cellular plasma membrane nanostructure with native, genetically modified membrane proteins as molecular reporters, Eva Arnsfang Christensen, Hanne Matras and Christoffer B. Lagerholm, Membrane proteins: Structure, stability and folding, Aalborg University PhD Course, May 2007

Applications of quantum dots for single molecule imaging in cells and substrate-supported planar membranes, Eva Arnsfang Christensen, Hanne Matras and B. Christoffer Lagerholm, European Biophysics Conference, July 2007

A large-scale investigation of the cellular plasma membrane nanostructure with native genetically modified proteins as molecular reporters, Eva Arnsfang Christensen, Hanne Matras and Christoffer B. Lagerholm, EMBO workshop New Methods in Membrane Protein Research, Stockholm, August 2007

A large-scale investigation of the cellular plasma membrane nanostructure with native, genetically modified, membrane proteins as molecular reporters., Eva Arnsfang Christensen, Hanne Matras, Sally Adler, Jonathan Jarvik, and B. Christoffer Lagerholm., Centre for Structural Biology: TRAMP-6 2007 Symposium, Aarhus University, 2007



## 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 <i>(students in italics subsidized for the last 4 months of their studies)</i>	Morten S. Dueholm (2006-) Brian Vad (AAU and SDU, 2005-) Magnus Franzmann (2007-) <i>Tony Ebdrup (2006)</i> <i>Lise Nesgaard (2006)</i> Sanne Pedersen (2004-)
Post-doctoral scientists involved in BioNET activities but funded elsewhere	Dr. Pankaj Sehgal
Ph.D. students involved in BioNET activities 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)

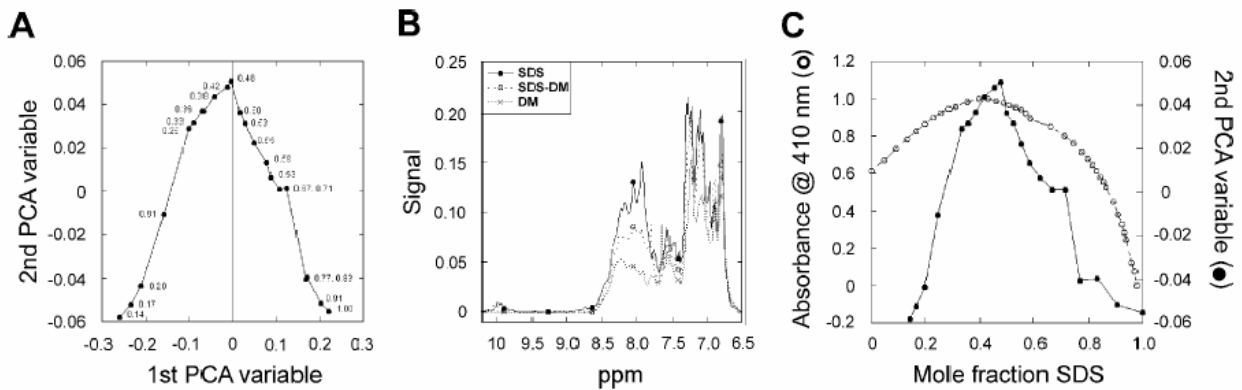
## Activities of the Daniel Otzen group

### Folding and assembly of membrane proteins modulated by an amphiphilic environment

(Peter A. Christensen, Uffe B. Westergaard, Brian Vad. Associations: Pankaj Sehgal.)

Together with our collaborators at Aarhus University, we have developed an NMR-based method to monitor conformational transitions for proteins in response to changes in the environment. In this procedure, known as Principal Component Analysis, we record simple one-dimensional NMR spectra of a given protein as we systematically vary the conditions (*e.g.* temperature, addition of a denaturant or surfactant, change in pH, *etc.*). Subsequently the spectra are analyzed to elucidate how many different conformational transitions are involved and the structural characteristics of these transitions (*e.g.* whether they primarily involve changes in hydrogen-bonding or aromatic side chain interactions). This analysis can in principle be performed on any protein that yields a reasonably disperse NMR signal, which opens the door to include membrane proteins in mixed micelles of

varying compositions. As proof of concept, we have demonstrated for the membrane-bound heme-protein PsbF that the relatively complex changes around the heme group in response to changes in the mixed micelle (SDS and dodecyl maltoside) composition are matched by changes in the protein conformation. This is a very useful technique as it with one stroke abolishes the artifacts associated with fluorescence (where simple binding of surfactants near Trp side chains can markedly affect Trp fluorescence without necessarily involving conformational changes) and the generally small and insignificant changes in circular dichroism associated with unfolding. We are about to apply this approach to a number of other membrane proteins that we study in our group.



1D NMR spectra of the PsbF peptide at different SDS-DM concentrations subjected to Principal Component Analysis.

(A) Plot of the two major components describing the variation in 1D NMR spectra of apo-PsbF in SDS upon stepwise addition of DM. PC1 and PC2 are obtained by Principal Component Analysis.

(B) 1D NMR spectra corresponding to the three corners in panel A, namely pure SDS, 48 SDS:52 DM and 14 SDS : 86 DM.

(C) Plot of PC2 and absorbance at 410 nm versus mole fraction SDS.

## Properties of membrane-anchored proteins in the outer bacterial membrane

(Uffe B. Westergaard & M.Sc. student Stine K. Knudsen)

The article on Ag43 glycosylation (Knudsen, S. K., Westergaard, U. B., Stensballe, A. & Otzen, D. E. Effect of glycosylation on biophysical and flocculative properties of the extracellular domain of Ag43.) has been accepted for publication in *Biochemical Journal*. Thanks to this publication, we have already established links with a leading group within the autotransporter field, paving the way for possible future collaborations.

## Structural and functional studies of outer membrane proteins

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

We have combined previous work by S. Pedersen on association of separately purified fragments of OmpA fragments with the work by Dilip Debnath on the production of complementary fragments of OmpA. These complementary fragments are produced by cleavage of a protease-recognition sequence introduced in different periplasmic loops of OmpA. We have in this way produced 3 complementary fragment pairs of OmpA which can associate to form a folded complex according to the SDS band-shift assay, although the process only goes to around 30% completion. We are complementing these studies with cell-free transcription-translation systems which allow us to study

the folding of a membrane protein from the biologically relevant denatured state (the nascent chain on the ribosome). Our preliminary data indicate that the type of lipid can influence the yield of both native and denatured protein.

### **Interactions of water-soluble proteins with membrane-like environments** (Jesper

(E. Mogensen, Sanne Pedersen, M.Sc. students Mette M. Nielsen and Line Aagot Thomsen & Lars Kjær in col. with Thomas Heimborg, NBI. Associations: Kell K. Andersen)

Our work on  $\alpha$ -synuclein interactions with lipids has been greatly boosted by a close collaboration with our BioNET-colleague Thomas Heimborg, who has developed a thermodynamic theory that is able to predict many of the observations we have made on  $\alpha$ -synuclein structural transitions in the presence of lipids. In view of the central role of lipid interactions in  $\alpha$ -synuclein's proposed biological and pathological activity, we believe this work will have a significant impact on the field.

### **Biophysical properties of surfactant proteins**

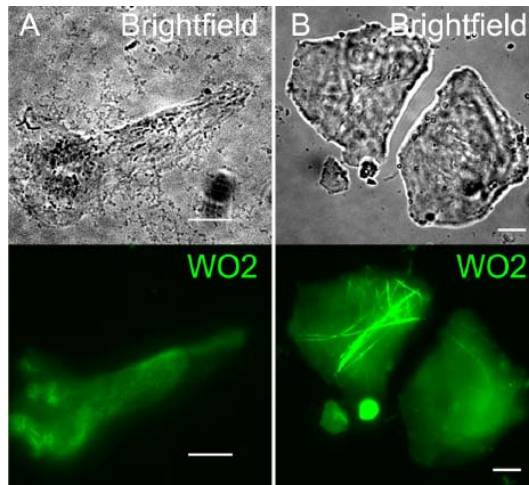
(Tony Ebdrup in collaboration with Beate Klösgen, SDU)

We have arranged for a Ph.D. student from Professor Jesús Perez-Gil's lab to visit Daniel Otzen's lab in May-June 2008 in order to carry out some complementary experiments that will finish the work largely completed by Tony Ebdrup during his Ph.D. project.

### **Functional and structural properties of bacterial amyloid protein**

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

Amyloid fibrils are usually associated with protein misfolding and neurodegenerative diseases. Recently, however, functional bacterial amyloid (FuBA) has been detected and characterized in a few bacterial species. We have substantially added to this by reporting the wide-spread abundance of amyloid adhesions in numerous microbial systems. Spores from *Streptomyces*, *Nocardia* and *Bacillus* were all coated with amyloids. We have developed a purification method to analyze several of these amyloid species. FuBA purified from *Gordonia amarae* (from the cell envelope) and *Geodermatophilus obscurus* showed the morphology, tinctorial properties and  $\beta$ -rich structure typical of amyloid and, for the latter species, the dominance of simple amino acids seen in the *E. coli* curli protein. We conclude that amyloid is widespread among Gram positive bacteria and may in many species constitute a hitherto overlooked integral part of the spores and the cellular envelope. For FuBA isolated from a *Pseudomonas* species, partial sequencing by MS/MS revealed that the fimbriae 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 fimbriae. Fimbriae from different bacterial species may provide a useful and diverse library of fibrillating sequences that can offer further insight into the fibrillation process.



Velvet-like appearance and large fibrils of FuBA. Brightfield and WO2 (amyloid-specific antibody) labeling of A) *M. avium*, where velvet-like substances strongly bind WO2, and B) *T. spumae*, where long (>50 $\mu$ m) WO2 binding fibers can be seen.

In addition to these more discovery-based studies, we have managed to purify sufficient amounts of isotope-labelled CsgA fibrils from *E. coli* for ongoing solid state NMR studies, as well as establishing a recombinant expression system in which aggregation of CsgA monomers is inhibited while it is in fusion with the SUMO protein. Proteolytic release of CsgA by the SUMO protease subsequently triggers aggregation and provides a useful switch.

We have established a robust expression system for the production of recombinant ADan, a peptide produced as a result of proteolytic processing of an aberrant form of the Bri protein. ADan leads to Familial Danish Dementia, a hereditary neurodegenerative illness with clinical symptoms similar to Alzheimer's disease. Unusually, the peptide does not form classic amyloid fibrils despite a fibrillar morphology. We have shown by X-ray fiber diffraction that the ADan aggregates possess regular  $\beta$ -strand spacings (leading to conventional circular dichroism spectra) but lack well-defined  $\beta$ -sheet distances. The cytotoxic effects of ADan are associated with membrane interactions. We have completed a biophysical study indicating that anionic lipids can inhibit the large-scale aggregation of ADan, suggesting that they stall the proteins at an early stage in the aggregation process.

ADan peptides have also been studied as part of an independent project to exploit the world-class facilities for synchrotron radiation circular dichroism (SRCD) at Aarhus University. SRCD can extend the spectral range down to approximately 130nm for dry proteins, potentially providing new structural information. Using a selection of dried model proteins, including  $\alpha$ -helical,  $\beta$ -sheet and mixed-structure proteins, we observe a low-wavelength band in the range 130-160nm, whose intensity and peak position is sensitive to the secondary structure of the protein and may also reflect changes in super-secondary structure. This band has previously been observed for peptides but not for globular proteins, and is compatible with previously published theoretical calculations related to  $\pi$ -orbital transitions. We also show that drying does not lead to large changes in the secondary structure and does not induce orientational artifacts. In combination with principal component analysis, our SRCD data allow us to distinguish between two different types of protein fibrils, highlighting that *bona fide* fibrils formed by lysozyme are structurally more similar to the non-classical fibrillar aggregates formed by the SerADan peptide than with the amyloid formed by  $\alpha$ -synuclein. Thus, despite the lack of direct structural conclusions, a comprehensive SRCD-based database of dried protein spectra may provide a useful method to differentiate between various types of supersecondary structure and aggregated protein species.

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

(Peter A. Christensen. Association: Christian B. Andersen. Col. with Mogens Høgh Jensen, NBI)

The model peptide glucagon continues to be a highly inspiring system to delve into the details of protein and peptide fibrillation. Based on a comprehensive series of studies published in 2006, we have proposed a Darwinian view of the *in situ* evolution of different types of fibril morphologies which forms the basis of fibrillar polymorphism. We continue to characterize the molecular basis for this polymorphism using techniques such as X-ray fiber diffraction, calorimetry, synchrotron radiation CD and solid state NMR. This will be aided by a system for recombinant glucagon expression currently under construction.

We are in the process of completing a calorimetric study on the energetic of fibril elongation, based on the model peptide glucagon, which highlights the enthalpy of proton ionization as central to the process. Thus the deprotonation of acidic side chains appears to be the main barrier to fibril elongation at pH values (2-3) well below the side chain  $pK_a$  (3-4). We have identified an early stage oligomer using a specific fluorophor incorporated into glucagon, which is corroborated by an ongoing Small Angle X-ray Scattering study.

### PhD Dissertations:

1. Rajiv Vaid Basaiawmoit (DO main supervisor): *Molecular Mechanisms of Corneal Dystrophies*. Aalborg University, April 2007.
2. Ditte Maria Simondson Lundvig (DO co-supervisor): *Investigation of the normal function of the brain protein p25 $\alpha$  and its relation to demyelinating disorders*. Aarhus University, July 2007.

### M.Sc. dissertations:

1. Peter Lüttge Jensen. Detection and characterization of amyloid in mycolata species
2. Anette Yde: Degradation of bacterial amyloid.
3. Line Aa. H. Thomsen. Acylation of the antimicrobial peptide Novicidin
4. Lars Kjær: A biophysical study of the interaction of  $\alpha$ -synuclein with phospholipid membranes.
5. Jonas H. Hansen: Limited proteolysis as a tool to probe protein conformation in surfactants.
6. Marie Thomsen: "A biophysical investigation of a conformational disease: "Antithrombin III Aalborg"
7. Anders Dahl Knudsen: Expression proteomic analysis of cell-lines expressing proteins implicated in Parkinson's Disease.

### External funding:

October 2007: 4 Ph.d. stipends co-funded by the Research Training Committee (2 mio DKK) and matched with external funding from Novo Nordisk A/S, Novozymes A/S, ALK-Abelló A/S and Lundbeck A/S.

### Invited talks:

1. Towards a cure for Parkinson's Disease: The case for aggregation inhibition. Winter



Conference in Brain Research, Snowmass, Colorado, January 2007.

2. Aggregation of  $\alpha$ -synuclein and its inhibition. Wyeth A/S, Princeton, New Jersey. March 2007.

3. Mod en kur for Parkinson's Sygdom. Dansk Parkinson Forening årsmøde, Fredericia. March 2007.

4. Functional and pathogenic amyloid. Dortmund University, April 2007.

5. The intricacies of protein aggregation and structural polymorphism. Niels Bohr Institute, April 2007.

6. *In vitro* and *in vivo* folding of membrane proteins, TRAMP Symposium, Aarhus University, October 2007.

Ph.D. courses:

1. Biophysics of membrane proteins (main organizer). Aalborg University, May 2007.

2. Frontiers in molecular biology (organizing committee). EMBO, Heidelberg, September 2007.

Refereed articles 2007:

22 original articles, 1 book proposal (Oxford University Press) and 1 grant proposal.

## Publications:

Namiko Mitarai, Anna M. C. Andersson, Sandeep Krishna, Szabolcs Semsey, & Kim Sneppen "Efficient degradation and expression prioritization with small" l RNAs Phys. biol. 4:164 (2007)

Sergei Maslov, Kim Sneppen & Iaroslav Ispolatov, Spreading out of perturbations in reversible reaction networks New J. Phys. 9:273 (2007)

Karin Stibius & Kim Sneppen, "Modeling the Two-Hybrid Detector: Experimental Bias on Protein Interaction Networks" Biophysics J. 93:1 (2007)

Ian B. Dodd, Mille A. Micheelsen, Kim Sneppen & Geneviève Thon Theoretical Analysis of Epigenetic Cell Memory by Nucleosome Modification Cell 129:813-822 (2007)

Jakob Enemark, Kim Sneppen  
Analyzing a stochastic model for evolving regulatory networks by unbiased gene duplication JSTAT 0:P11007 (2007)

I.B. Dodd, K.E. Shearwin & K. Sneppen  
Modelling transcriptional interference and DNA looping in gene regulation J. Mol. Biol. 369:1200 (2007)

Namiko Mitarai, Anna M. C. Andersson, Sandeep Krishna, Szabolcs Semsey, & Kim Sneppen Efficient degradation and expression prioritization with small RNAs Phys. biol. 4:164 (2007)

Szabolcs Semsey, Sandeep Krishna, Kim Sneppen  
Combinatorics of feedback in cellular uptake and metabolism of small molecules Proc. Natl. Acad. Sci. (USA) 104:20815 (2007)

Sandeep Krishna, Sergei Maslov, Kim Sneppen  
UV-induced mutagenesis in the Escherichia coli SOS response: A quantitative model PLoS Comput. Biol. 3:e41 (2007)

Szabolcs Semsey, Sandeep Krishna, Kim Sneppen, Sankar Adhya Signal Integration in the Galactose Network of Escherichia coli Molecular Microbiology 65:465 (2007)

S. Pigolotti, S. Krishna & M.H. Jensen,  
"Oscillation patterns in negative feedback loops", Proc.Nat.Acad.Sci., **104**, 6533-6537 (2007).

G. Tiana, S. Krishna, S. Pigolotti, M.H. Jensen & K. Sneppen, "Oscillations and temporal signalling in cells", Physical Biology **4**, R1-R17 (2007).

I. Simonsen, PTH Ahlgren, M.H. Jensen, R. Donangelo R & K Sneppen, "Fear and its implications for stock market" Eur. Phys. Jour. **57** 153-158 (2007).

J. Fonslet, K. Rud-Petersen, S. Krishna & M.H. Jensen, "Pulses and Chaos: Dynamical Response in a Simple Genetic Oscillator, Int. Journ. Mod. Phys. B **21**, 4083 - 4090 (2007).

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