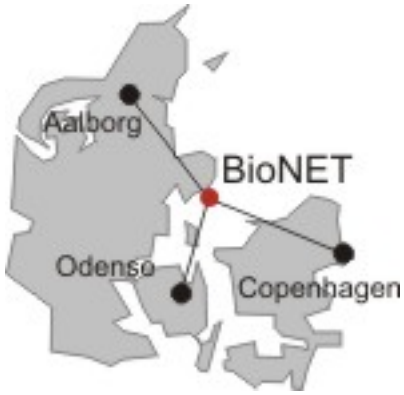
BioNET Activity Report

1. July – 31. December 2004



University of Copenhagen:

Theoretical Biophysics, Kim Sneppen and Mogens Høgh Jensen

Genetic networks:
Biological systems need to continually respond to information from the environment in order to function and survive. The information which enters a cell through its signal transduction pathways has to be decoded and processed by various genetic networks in a specific and robust way. Genetic circuits which have oscillatory behaviour have particularly interesting ways of encoding information in the frequency, amplitude and phase of the oscillations. Such oscillations are known to be involved in a number of cellular processes such as the segmentation clock, cell cycle, circadian rhythms, stress response and apoptosis. Our approach to understand these systems has been to construct models consisting of effective dynamical equations of fairly few degrees of freedom. We use these models to understand, analytically and computationally, the role of different types of feedback and time delays in producing oscillations. We have successfully applied such models to oscillations of p53-Mdm2 and Hes1. We are currently applying this approach to oscillations of the NFkB family of transcription factors in mammalian cells, which appear to be involved in many cellular processes ranging from cell growth, cell survival, embryo development, stress response and cell death.

Protein networks:
We investigate the mechanism of translation regulation in the Unfolded Protein Response in yeast. This response system circumvents the inherent bottleneck in ordinary transcription by having a constitutive production of passive mRNA. We found that a limiting parameter of a system of this kind is the conversion rate of passive to active mRNA compared to the lifetimes of both active and passive mRNA. We have developed the mathematical framework for capturing the overshooting capabilities of systems where translation regulation is present. We speculate that translation regulation is still to be discovered in many biological systems.

Personal:
Post Doc, Sandeep Krishna, Bangalore, India, 01.11.04 – 01.11.06

Guests:
Joakim Mathiesen, NTNU, Trondheim, Norway, 26.01. – 13.02
Ralf Akermann, University of Potsdam, Germany, 16.11. – 23.12. 2005

Travels:
Poul Martin Hansen, partly paid trip to Denver

Publications:

- (T.C. Halsey) and M.H. Jensen, Hurricanes and Butterflies, Nature Vol. 428 (2004) 127
- (D.K. Fygenson), (D.J. Needleman) \& K. Sneppen, Variability alignment identifies functional regions in tubulin paralogs, Protein Science 13 (2004) 25-31
- (S. Maslov), K. Sneppen & (A. Zaliznyak), Pattern Detection in Complex Networks: Correlation Profile of the Internet Physica A Vol 333 (2004) 529-540
- Simonsen), (K.A. Eriksen), (S. Maslov) and K. Sneppen, Diffusion on Complex Networks: a Way to Probe their Large-scale Topological Structures, Physica A vol 336, 1-2 (2004) 163-173

- J.B. Axelsen and K. Sneppen, Quantifying the Benefits of Translation regulation the unfolded protein Response, Physical Biology 1 (2004) 159-165
- P. Minnhagen, M. Rosvall, K. Sneppen \& A. Trusina, Self-organization of Structures and Networks, From Merging and Small-scale Fluctuations, Physica A vol 340, 4 (2004) 725-732
- Trusina, (S. Maslov), P. Minnhagen \& K. Sneppen, Hierarchy and Anti-Hierarchy in Real and Scale Free networks, Pysical Review Lett. vol 92 (2004) 178702
- (S. Maslov), K. Sneppen and K. A. Eriksen, Upstream Plasticity and Downstream Robustness in Evolution of Molecular Networks, BMC Evolutionary Biology (2004) 4(1):9
- (Kasper Astrup Eriksen), (Michael Hörnquist) and Kim Sneppen, Visualization of Large-scale Correlations in Gene Expressions, Functional \& Integrative Genomics 4, 4 (2004) 241 - 245
- K. Sneppen, M. Rosvall, A. Trusina and P. Minnhagen, A simple Model for Self Organization of Bipartite Networks, Europhys. Lett. 67 (2004) 349 ~

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.

Personal:

Matthias Fidorra was hired for a Ph.D. project on 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. Furthermore, he shall perform patch clamp experiments on the skin systems to understand the permeability of those membranes for water and other molecules.

Instrumentation:

The VKR-Foundation partially sponsored the acquisition of a Fourier transform infrared (FTIR) spectrometer by Bruker Optics. The total value of the instrument is 500.000 DKK. VKR paid 50% and the SNF paid the other 50%. This instrument is designed to record vibrational spectra of biomolecules. Since the spectral features of the main components of biomembranes (lipid and proteins) are different, FTIR is able to simultaneously monitor changes in lipid and in protein structure. We plan to measure the permeability of membranes, the insertion of peptides, and relaxation processes in membranes.

Travels sponsored by BioNet:

T.Heimburg:

- Conference on lipid-peptide interaction in Hamburg, invited talk, 9.-12.2 2005
- American Biophysical Society Meeting, Long Beach, California, 12.-16.2.2005
- Visit and talk: National Institutes of Health, Bethesda, Maryland, 18.2.2005

M.Fidorra:

- American Biophysical Society Meeting, Long Beach, California, 12.-16.2.2005

Guests:

Ivan Makarov, Max-Planck Institute for Biophysical Chemistry Göttingen, Germany, April-June 2005.

Publications:

- A. Hac, H. Seeger, M. Fidorra, and T. Heimburg. 2005. Diffusion in two-component lipid membranes - A Fluorescence Correlation Spectroscopy and Monte-Carlo simulation study. Biophys. J. 88: 317-333
- 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. Submitted.

Poster Contribution:

- M. Fidorra and L. Bagatolli, Biophysical Society Meeting Long Beach, February 2005. Confocal microscopy, calorimetry and permeability studies on giant lipid vesicles containing ceramides

Other:
Creation of a Web site for Bionet: **www.bionet.dk**

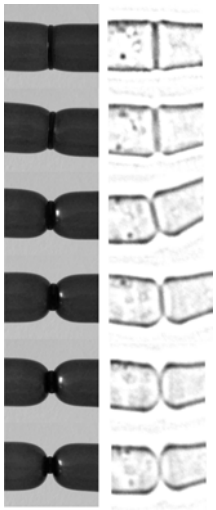
Optical Tweezers Group, Lene Oddershede

At the single molecule level we have had great improvements of our two major projects:

RNA pseudoknots are assumed to induced ribosomal frame shifting. We have genetically constructed two different pseudoknots and shown that they induce different degrees of frame shifting. Our hypothesis is that the degree of frameshifting is related to the mechanical strength of the pseudoknot. Therefore, by using optical tweezers and micropipettes we are presently pulling RNA pseudoknots to measure their mechanical strength. In 2004 (December 27th) we observed the first succesful rip of the pseudoknot and later, this was reproduced.

As a continuation of our investigations on how the lambda receptor moves in the outer membrane of 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. This has shown the intriguing result, that the motion is not purely thermal, there is an active component too.

At the whole cell level we also had significant improvements:



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 made and optically trapped gold beads in the size range 20-250 nm which constitutes world record for optical trapping of gold particles. These nano particles have been injected into living S. pombe yeast cells using a micropipette. These nano-probes will be 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. The results

show that the curvature scales with time going towards a singular point at the exact breakup. To relate the microscopic world to the macroscopic and to determine the role of surface tension, we have performed similar experiments using baloons. To a large extent, cells behave as baloons. From this we get the intriguing result that surface tension is largely responsible for the outline of the cell during division.

Publications relevant for BioNET published in 2004:

- Iva Marija Tolic-Nørrelykke, Emilia-Laura Munteanu, Genevieve Thon, Lene Oddershede and Kirstine Berg-Sørensen: "Anomalous diffusion in living yeast cells", Phys. Rev. Lett. 93 (2004). 078102-1 - 4
- Jakob Kisbye Dreyer, Kirstine Berg-Sørensen, and Lene Oddershede: "Improved axial position detection in optical tweezers measurements", Applied Optics 43 issue (2004). 10 pp.1991-1996

Personel

Tabita W. Madsen, forskningsassistent Dec.04 + Jan. 05, started as a phd-student February 1, 05. Involved in the mobility of proteins in bacterial membranes.

Guests

- Phd-student, Nader Reihani, financed partly by BioNET, has been here October 04-January 05. Involved in the RNA pseudoknot project.
- Visiting phd-student Zdenek Lansky, partly financed by BioNET, has been here from September 2004 and will leave June 2005. Involved in the topology study of dividing cells.

University of Aalborg

Department of Life Sciences, Daniel Otzen:

Currently following people are employed at BioNET:

Post-doc Peter Astrup Christensen (since June 2004)
PAC works on two projects:

(1) *Biophysics of human aquaporin (AQP2) folding*. The long-term aim is to compare folding properties of wildtype AQP2 and mutants giving rise to nephrogenic diabetes. PAC's preliminary results indicate that expression in *E. coli* has not yielded significant yields, so we are instead concentrating initially on a biophysical characterization of AQP2 purified from bovine kidneys. This is done in collaboration with Ass. Prof. Jan Enghild, Aarhus University, who routinely purifies this protein. We are also in contact with Ass. Prof. Torsten Kristensen, Aarhus University, who is setting up aquaporin expression in insect cells.

(2) *Association of fragments of the membrane protein DsbB*. PAC has supervised a M.Sc. project involving *stud. Polyt.* Søren Mølgaard, in which two fragments of DsbB were each fused to a variant of Green Fluorescent Protein. Association of the fragments would be expected to lead to Fluoresence Resonance Energy Transfer. Unfortunately, this project has been hampered by extremely low expression levels of the fusion proteins. However, in collaboration with Jan Enghild, PAC has chemically synthesized peptides corresponding to the individual transmembrane segments of DsbB, and has started an analysis of their structural properties in different micellar and lipidic environments.

Post-doc Uffe Bendfeldt Westergaard (since February 2005)
UBW has started up the following projects:

(1) *Biotinylation of DsbB to follow molecular motion.* This is a collaboration with BioNET member Lene Oddershede, NBI. UBW will insert an amino acid sequence into DsbB that leads to *in vivo* biotinylation . The *E. coli* cells expressing DsbB in the inner membrane will then be stripped of their outer membrane, leaving DsbB exposed for labeling with streptavidin-labeled polystyrene/gold cells. This will be analyzed by Lene Oddershede's equipment to obtain more insight into protein motion in the bacterial inner membrane.

(2) *In vivo association of DsbB fragments.* DsbB fragments will be fused to fragments of the murine enzyme dihydrofolate reductase (DHFR); DHFR activity, which leads to resistance against trimethoprim, presupposes DsbB fragment association. We have obtained the DHFR clones from Dr. Dirk Schneider, University of Freiburg, and cloning has started.

Post-doc Jesper Emil Mogensen (December 2004-August 2005)
JEM is employed on a short-term contract after completion of his Ph.D. thesis in order to carry out *in vitro* studies of the interactions between the outer membrane protein AIDA and different periplasmic chaperones, which we have already purified in our laboratory. During this period he has written a review on the interactions between chaperones and outer membrane proteins which has been preliminarily approved for publication in *Molecular Microbiology.*

Ph.D. student Sanne Schaldemose Pedersen (co-financed by BioNET, AAU and the Danish Research Councils)
SSP has now expressed five of the 6 fragments of the outer membrane protein OmpA in *E. coli*; the last fragment will be produced by chemical synthesis. She has managed to refold several fragment combinations *in vitro* not only in detergents, but also in lipid vesicles.

University of Southern Denmark

Memphys and Physics Department, Ole G. Mouritsen

Responsible scientist	Prof. 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 (from September 1)

1. Initiating BioNET at the SDU-node
In accordance with the research plan, the main focus of this node has been biogenesis, sub-cellular dynamics, and the membrane aspects of these phenomena. Dr. Matthias Weiss was hired on July 1 as a research assistant professor to be in charge of work on the dynamics and structure formation of biomembranes, in particular in association with ER (endoplasmic reticulum) exit sites. The other line of the SDU activities within BioNET concerning science didactics was initiated by the announcement of a PhD-fellowship which was won by cand.polyt. Stinne Hørup Hansen who started on September 1 to work on a project entitled "Biophysics as a model for inter-disciplinary teaching in Danish high school: interest arousal and gender perspectives". The fellowship received one third co-financing support from the

Danish Graduate School of Upper-secondary Education. During the period of report, Dr. Weiss received an offer from the Deutsches Krebsforschungszentrum in Heidelberg to build up his own research group there, and he left BioNET at the end of the year. Three measures have been taken in order to remedy this situation. Firstly, Prof. Luis Bagatolli from the Department of Biochemistry and Molecular Biology of SDU took on the duty to change some of his research directions to fit into the BioNET framework. These directions pertain to structure and dynamics of skin and skin membranes. The research on skin is allocated a BioNET PhD-fellowship which was recently announced and won by cand.scient. Maria Bloksgaard. This fellowship is co-financed by one third from SDU. Secondly, the postion vacant after Dr. Weiss was announced and we managed to attract a highly competent biophysicist, Dr. Nicoletta Kahya, who will join BioNET on May 1, 2005. Thirdly, the involvement of Dr. Weiss in BioNET continues on the planned projects. The ties to Dr. Weiss and his new research group will be maintained by the association of a joint PhD-student who will be co-financed between BioNET and Dr. Weiss, and by collaboration by another PhD-student, Ask F. Jakobsen, who will be spending part of the Spring 2005 with Dr. Weiss in Heidelberg doing model calculation on membrane curvature in relation to the COPI/COPII vesicle machinery at the ER exit sites. Concerning establishment of new instrumentation in relation to the experimental parts of the projects at SDU, it has been decided to pool the resources from 2004 and 2005 to be used in 2005 for building a new experimental two-photon laser scanning facility.

2. Research report

2.1 ER exit sites [Dr. Matthias Weiss and collaborators]

In 2004 Dr. Weiss and his collaborators succeeded in elucidating the binding kinetics of COPII proteins to single exit sites of the endoplasmic reticulum (in collaboration with the group of R. Pepperkok, EMBL Heidelberg, Germany). It turned out, that the presence of cargo molecules that have to enter the emerging bud/vesicle strongly alters the binding kinetics in a nontrivial way: while the typical turn-over time for the involved GTPase increased, the corresponding time for the subsequently recruited coat proteins decreased. This effect could be described by two alternative models, one of which could be ruled out by subsequent experiments. From the model and the accompanying experimental data, we are now able to tell that coat and GTPase are dissected on the membrane, i.e. cargo molecules retain the coat on the membrane while the GTPase can already dissociate (Forster, Weiss, Zimmerman, Reynaud, Stephens, Pepperkok, submitted).

2.2 Ceramide containing membranes: skin and mitochondrial membranes [Prof. Luis Bagatolli, PhD-student 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 and mitochondria) to perform correlations with the observed phenomena in artificial mixtures.
3) Explore the lateral structure of skin membranes directly in skin tissue. Preliminary results from our laboratory are available at present (see below).

During the 2004 period the presence of lateral heterogeneity was demonstrated in mixtures of ceramide and phospholipids (POPC in this case) and in mixtures of ceramide/fatty acid cholesterol. As shown in Figure 1, different lipid phases are present in these mixtures. Part of these results were presented at the 49th *Biophysical Society Meeting* (2005) in Long Beach, CA (The lateral structure of ceramide containing bilayers as observed by fluorescence microscopy, M. Fidorra, L. Duelund and L.A. Bagatolli).

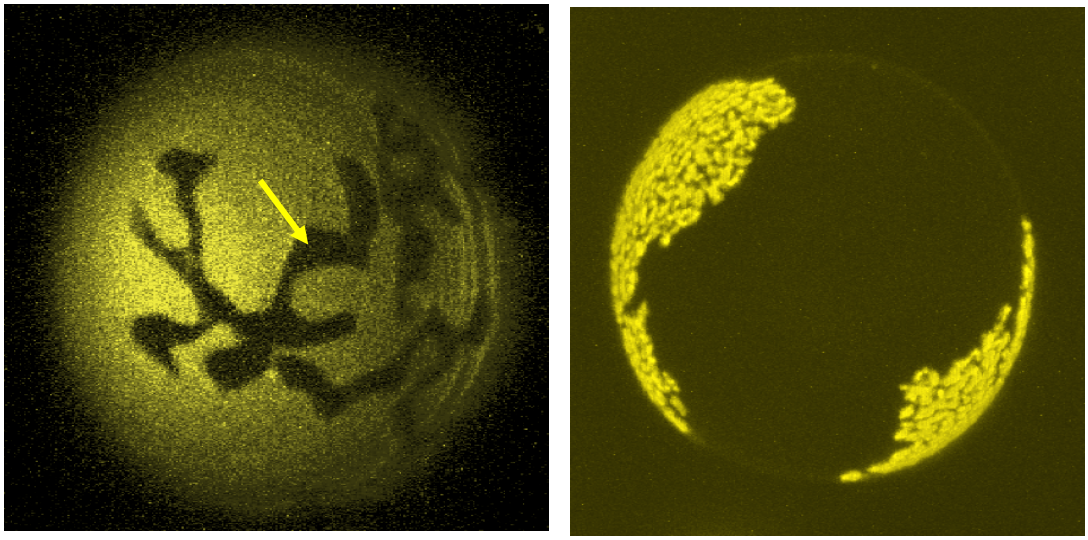


Figure 1: Giant vesicles composed of brain ceramide/POPC mixtures (1:5 mol, right) and choleterol/ceramide/palmitic acid (1:1:1 mol, left) displaying phase coexistence. The dark areas in the right image (indicated with the yellow arrow) correspond to ceramide rich gel phase areas. The probe DiIC18 was used in this experiment either in two-photon excitation mode (excitation @ 760 nm) or confocal mode (excitation @ 543 nm).

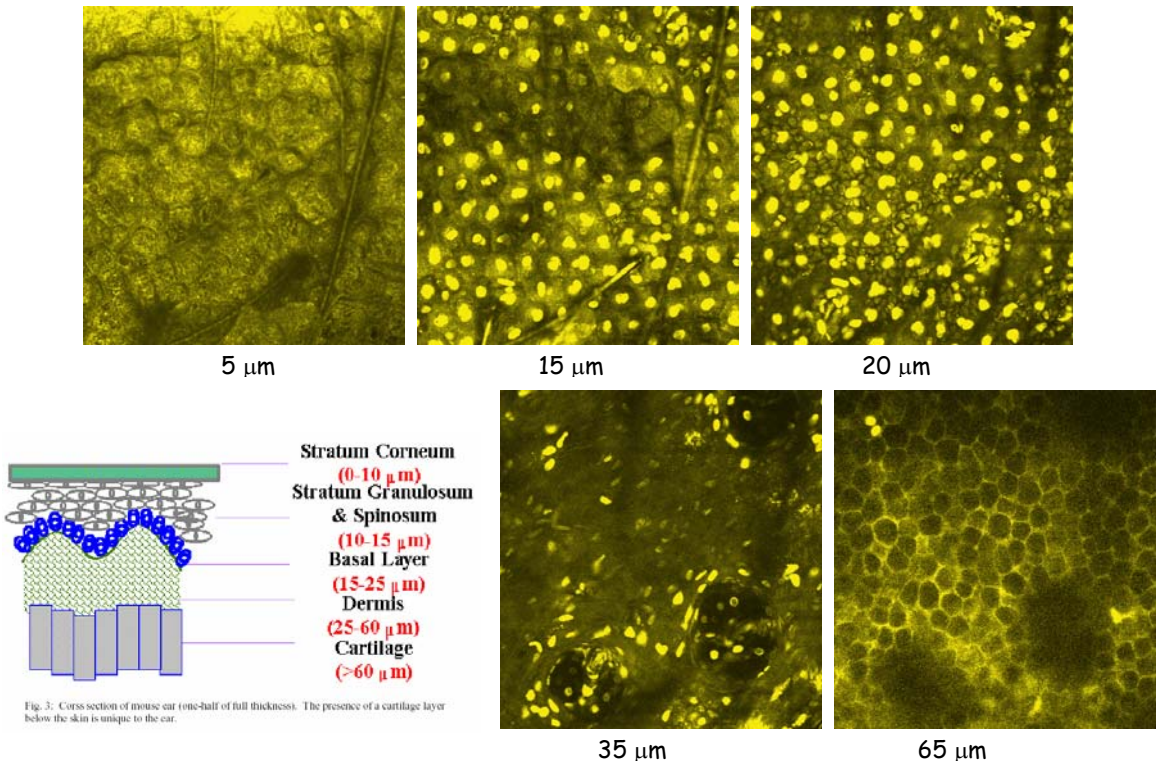


Figure 2: Two-photon excitation fluorescence images of skin tissue from rat ear. The pictures were taken at different z positions (from the outer surface of the tissue deep inside - top left to down right). The excitation wavelength was 780 nm. The fluorescence is coming mainly from natural components of the skin (NADH). Cell nuclei were labeled with the fluorescent probe sytox that intercalates in the cell DNA (molecular probes).
Additionally, preliminary results were also obtained in skin tissue using multi-photon excitation fluorescence microscopy. In these experiments we were able to solve the different layers of the dermis/epidermis of skin using natural molecules present in the tissue as fluorescence probes (such is the case of NADH). The main goal of this part of the project is to characterize membrane phase state in situ (i.e., in the tissue) using UV excited polarity sensitive probes (where two-photon excitation microscopy is also required). Figure 2 summarizes some of the preliminary observations.

2.2 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 Hørup Hansen. During the time of report, Stinne Hørup Hansen has been studying the literature and planning the project with the following title and aim: *Design-based research of interdisciplinary science teaching in Upper Secondary School*.

Science has apparently lost its attraction for many young people in Denmark. Only half of the students feel that they learn something of relevance in science class. In addition boys' and girls' areas of interest in science show remarkable differences and in the science curriculum these interests are not being met.

The aim of the project is to clarify how science teaching in Upper Secondary School can be improved in order to

- Catch and hold girls' and boys' interest in science
- Improve the interplay between the sciences
- Confront students prototypical views of scientists

The project is based on design and implementation of innovative interdisciplinary teaching material in the science subjects in Upper Secondary School. It is proposed to use biophysics as a model for the interdisciplinary teaching material. In the process, knowledge of interest, gender and prototypical views will be utilized.

A pilot project has been designed and implemented as an interdisciplinary project about radiation in cooperation with teachers in physics, mathematics, chemistry and biology in a class in Upper Secondary School. In the material for the project Stinne Hørup Hansen has elucidated the subject, radiation, from several angels in order to emphasize the relevance of learning about radiation. Topics like cancer and radiation therapy, radiation from cell phones and microwaves, colors and the electromagnetic waves, as well as radioactivity were included in the material.

The students worked in groups on a radiation topic of their own choice. There were several criteria that had to be met for each subject and included in a final report. In mathematics they had to make calculations on radioactive decay by using an exponential function. In physics they had to make an experiment with a Geiger-Müller Counter and a radioactive source. In biology the students cultured seeds that had been exposed to different levels of radiation, and finally in chemistry they either made an experiment or calculated the energy needed to brake a molecular bond and found the corresponding wavelength.

During the project, Stinne Hørup Hansen participated in all lessons and observed how the students worked with their projects. The main emphasis was on their abilities to transfer and exploit knowledge from one subject to the other and on their general engagement in the project. The project was evaluated with a report and oral presentation and a questionnaire.