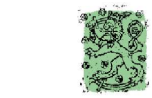
isb 05

WINTER SCHOOL PROGRAM & ABSTRACTS

SAARISELKÄ 12 - 15 DECEMBER

NATIONAL GRADUATE SCHOOL IN INFORMATIONAL AND STRUCTURAL BIOLOGY

ISB thanks the following sponsors for their grants and funding support for 2005 Winter School.

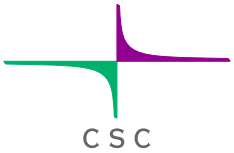


Ministry of Education

Academy of Finland

Åbo Akademi University

Biotie Therapies Corp.



CSC - Finnish IT center for science

FBD Ltd.

NordForsk

ISB 2005 Winter School Organizing Committee

Markus Linder, VTT Biotechnology
Jussi Meriluoto, Åbo Akademi University
Outi Salo, University of Kuopio

Coordinators: Fredrik Karlsson and Kaija Söderlund
Web site: Susanna Repo
Web programming & book: Mikko Huhtala

Contents

Program **1**

Participants **4**

Speaker abstracts **9**

Poster abstracts **29**

Index of persons **69**

Program

Monday, December 12th

11.20- 13.00 Departure from Helsinki-Vantaa airport (AY 463)

13.15 Bus (ISB) Ivalo - Saariselkä, Tunturihotelli (45 min.)

14.30 *Lunch*

15:30 **Session I, chair Jussi Meriluoto**

15.30 ISB Director Mark Johnson

15.32 Stefan Höger (University of Konstanz, Konstanz, Germany) *(p. 10)*

16.30 Matts Nylund *(p. 12)*

17.00 *Coffee*

17.30 **Session II, chair Peter Mattjus**

17.30 Johanna Rinne *(p. 13)*

18.00 Bohdana Térová *(p. 14)*

18.30 Pia Vesterkvist *(p. 16)*

19.00 Lenita Viitanen *(p. 17)*

21.00 - 24.00 *Sauna in the Kontio building*

Tuesday, December 13th

07:00 - 09:30 *Breakfast*

09:00 - 11:00 Individual group or project meetings or free time

14:00 *Lunch*

15:00 **Session III, chair Markus Linder**

15.00 Janne Lehtiö (Karolinska Institute, Stockholm, Sweden) *(p. 18)*

16.00 Lotta Amundsen *(p. 19)*

16.30 Susanna Repo *(p. 20)*

17.00 *Coffee*

17.30 **Session IV, chair Markku Kulomaa**

17.30 Heidi Tuominen *(p. 21)*

18.00 Kai Fredriksson *(p. 22)*

18.30 Mikko Purmonen *(p. 23)*

19.00 Virve Rauhamäki *(p. 24)*

21:00 *Dinner*

Wednesday, December 14th

07:00 - 09:30 *Breakfast*

09:00 - 11:00 Individual group or project meetings or free time

14:00 *Lunch*

15:00 Session V, chair Outi Salo

15.00 Björn Windshügel (Martin Luther University, Halle-Wittenberg, Germany) *(p. 25)*

16.00 Johanna Hakanpää *(p. 26)*

16:30 *Coffee and poster session*

18:00 **Session VI, chair Antti Poso**

18.00 Merja Niemi *(p. 27)*

18.30 Esko Oksanen *(p. 28)*

19.00 ISB Director Mark Johnson

21.00 - 24.00 *Sauna in the Kontio building*

Thursday, December 15th

07:00 - 09:30 *Breakfast*

09:30 - 11:00 Individual group or project meetings or free time

12:00 Bus to Ivalo (45 min)

13.30 Departure from Ivalo airport for Helsinki (AY464)

15.05 Arrival at Helsinki-Vantaa airport

Participants

Airenne, Tomi	Department of Biochemistry and Pharmacy, Åbo Akademi University
Airenne, Kari	A.I. Virtanen Institute, University of Kuopio
Alanko, Sonja	Department of Biochemistry and Pharmacy, Åbo Akademi University
Amundsen, Lotta	VTT Processes
Annila, Arto	Institute of Biotechnology, University of Helsinki
Bicskei, Zoltán	Department of Medical Physics, University of Turku
Björkblom, Benny	Turku Centre for Biotechnology, University of Turku and Åbo Akademi University
Björkqvist, Jenny	Department of Biochemistry and Pharmacy, Åbo Akademi University
Brandt, Anna-Maria	Department of Biochemistry and Pharmacy, Åbo Akademi University
Denessiouk, Konstantin	Department of Biochemistry and Pharmacy, Åbo Akademi University
Denesyuk, Alexander	Department of Biochemistry and Pharmacy, Åbo Akademi University
Filén, Jan-Jonas	Turku Centre for Biotechnology, University of Turku and Åbo Akademi University
Fredriksson, Kai	Institute of Biotechnology, University of Helsinki
Gorbikova, Elena	Institute of Biotechnology, University of Helsinki
Hakanpää, Johanna	Department of Chemistry, University of Joensuu
Halling, Katrin	Department of Biochemistry and Pharmacy, Åbo Akademi University
Halttunen, Vera	Institute of Biotechnology, University of Helsinki
Hatahet, Feras	Department of Biochemistry, University of Oulu
Heikinheimo, Pirkko	Institute of Biotechnology, University of Helsinki
Heikkinen, Outi	Department of Chemistry, University of Helsinki
Heino, Jyrki	Department of Biochemistry and Food Chemistry, University of Turku
Hoffrén, Anna-Marja	FBD Ltd.
Huhtala, Mikko	Department of Biochemistry and Pharmacy, Åbo Akademi University
Huiskonen, Juha	Institute of Biotechnology, University of Helsinki

continued on next page...

Participants, continued

Hänninen, Pekka	Medical Physics and Chemistry, University of Turku
Höger, Stefan	Environmental Toxicology, University of Konstanz, Germany
Johnson, Mark	Department of Biochemistry and Pharmacy, Åbo Akademi University
Jokinen, Johanna	MediCity Research Laboratory, University of Turku
Jäälinoja, Harri	Institute of Biotechnology, University of Helsinki
Kallio, Pauli	Department of Biochemistry and Food Chemistry, University of Turku
Kankaanpää, Pasi	Department of Biochemistry and Food Chemistry, University of Turku
Katajamaa, Mikko	Turku Centre for Biotechnology, University of Turku and Åbo Akademi University
Kauko, Anni	Turku Centre for Biotechnology, University of Turku and Åbo Akademi University
Keinänen, Kari	Department of Biological and Environmental Sciences, University of Helsinki
Kidron, Heidi	Department of Biochemistry and Pharmacy, Åbo Akademi University
Korhonen, Samuli-Petrus	Department of Chemistry, University of Kuopio
Kulomaa, Markku	Institute of Medical Technology (IMT), University of Tampere
Kuokkanen, Elina	Department of Biochemistry and Food Chemistry, University of Turku
Käpylä, Jarmo	Department of Biochemistry and Food Chemistry, University of Turku
Lahesmaa, Riitta	Turku Centre for Biotechnology, University of Turku and Åbo Akademi University
Lahti, Reijo	Department of Biochemistry and Food Chemistry, University of Turku
Laurikainen, Katri	VTT Biotechnology
Lehtiö, Janne	Karolinska Institutet/Karolinska University Hospital, Solna, Sweden
Lehtiö, Lari	Institute of Biotechnology, University of Helsinki
Lehtovuori, Pekka	CSC - Finnish IT center for science
Linder, Markus	VTT Biotechnology
Lisal, Jiri	Institute of Biotechnology, University of Helsinki
Lähde, Milla-Riina	Department of Biochemistry and Pharmacy, Åbo Akademi University

continued on next page...

Participants, continued

Lönnberg, Tapio	Turku Centre for Biotechnology, University of Turku and Åbo Akademi University
Malinen, Anssi	Department of Biochemistry and Food Chemistry, University of Turku
Marjamäki, Anne	Biotie Therapies Corp
Mattjus, Peter	Department of Biochemistry and Pharmacy, Åbo Akademi University
Meriluoto, Jussi	Department of Biochemistry and Pharmacy, Åbo Akademi University
Mäntsälä, Pekka	Department of Biochemistry and Food Chemistry, University of Turku
Määttä, Juha	Institute of Medical Technology, University of Tampere
Niemi, Jarmo	Department of Biochemistry and Food Chemistry, University of Turku
Niemi, Merja	Department of Chemistry, University of Joensuu
Nikula, Tuomas	Turku Centre for Biotechnology, University of Turku and Åbo Akademi University
Niskanen, Einari	Department of Biological and Environmental Science, University of Jyväskylä
Nylund, Matts	Department of Biochemistry and Pharmacy, Åbo Akademi University
Nyrönen, Tommi	CSC - the Finnish IT Center for Science
Oksanen, Esko	Institute of Biotechnology, University of Helsinki
Palonen, Elina	Department of Biochemistry and Pharmacy, Åbo Akademi University
Patana, Anne-Sisko	Institute of Biotechnology, University of Helsinki
Poso, Antti	Department of Pharmaceutical Chemistry, University of Kuopio
Puranen, Santeri	Department of Biochemistry and Pharmacy, Åbo Akademi University
Purmonen, Mikko	Department of Chemistry, University of Joensuu
Rauhamäki, Virve	Helsinki Bioenergetics Group, Institute of Biotechnology, University of Helsinki
Repo, Susanna	Department of Biochemistry and Pharmacy, Åbo Akademi University
Rinne, Johanna	NanoScience Center, Molecular Biology, University of Jyväskylä

continued on next page...

Participants, continued

Rouvinen, Juha	Department of Chemistry, University of Joensuu
Ruddock, Lloyd	Department of Biochemistry, University of Oulu
Salminen, Tiina	Department of Biochemistry and Pharmacy, Åbo Akademi University
Salo, Outi	Department of Pharmaceutical Chemistry, University of Kuopio
Scheinin, Mika	Department of Pharmacology and Clinical Pharmacology, University of Turku
Seitsonen, Jani	Institute of Biosciences, University of Helsinki
Soini, Juhani	Turku Centre for Biotechnology, University of Turku and Åbo Akademi University
Szilvay, Géza	VTT Biotechnology
Söderholm, Annu	CSC - the Finnish IT Center for Science
Tiikkainen, Pekka	VTT
Tulla, Mira	Department of Biological and Environmental Science, University of Jyväskylä
Tuominen, Heidi	Department of Biochemistry and Food Chemistry, University of Turku
Térová, Bohdana	Department of Biochemistry and Pharmacy, Åbo Akademi University
Vainio, Mikko	Department of Biochemistry and Pharmacy, Åbo Akademi University
Vesterkvist, Pia	Department of Biochemistry and Pharmacy, Åbo Akademi University
Vihinen-Ranta, Maija	NanoScience Center, University of Jyväskylä
Viitanen, Lenita	Department of Biochemistry and Pharmacy, Åbo Akademi University
Windshügel, Björn	Institute for Pharmaceutical Chemistry, Martin-Luther-University Halle-Wittenberg, Germany
Xhaard, Henri	Department of Biochemistry and Pharmacy, Åbo Akademi University

Speaker abstracts

in order of appearance

PEPCY-Toxic and Bioactive Peptides in Cyanobacteria

Stefan Höger

Environmental Toxicology, University of Konstanz, Germany

PEPCY is an 11-partner EU project and dedicated to investigate the toxic properties and occurrence of cyanobacteria. Cyanobacteria grow either as single cells or as trichomes, dispersed in marine and freshwater environments. Some of them are buoyant and occur sometimes on the surface, forming scums, one of the many features of the so-called 'water blooms'. Cyanobacteria occur worldwide in nearly every environment under nearly every condition, even in very cold and very hot deserts. Some of these cyanobacterial species produce complex compounds, however their benefits from synthesising these energetically costly compounds are still unknown. Many of these compounds are very small alkaloids or peptides, with a molecular weight of below 2kD, mostly with a complex structure and, in case of the peptides, composed of uncommon amino acids. The peptides are of specific interest, as the mammalian digestive tract is incapable to digest these peptides, due to the incompatibility of the amino acid structures with the mammalian peptidases. Some of the amino acids have D-configuration and many of the peptides are cyclic and therefore quite stable even at low pH. Moreover due to the cytotoxicity or peptidase inhibition capacity of some of these peptides, gastro-intestinal problems e.g. acute diarrhoea are the logical consequences, albeit the acute, subchronic and chronic consequences of the systemic availability of these peptides i.e. gastrointestinal absorption and transport to the liver and the rest of the body via the portal vein, uptake into the liver cells, the kidneys and possibly the brain can be even worse. Apart from the influence on the livers detoxification system, resulting in a depleted detoxification potential of the hepatocytes for other potentially negative influences such as oxidative stress, some of these toxic peptides are known to bind and specifically inhibit important enzymes, the protein phosphatases 1 and 2a. Via this inhibition the cell loses its capability to maintain its structural integrity and thus the contact to the neighbouring cells as well as the proper regulation of the cell cycle which can either ensue in acute hepatotoxicity or under chronic conditions in the generation of preneoplastic lesions and liver tumors.

In order to assess the health hazard to humans and livestock, PEPCY aims to achieve a better understanding of the occurrence and toxic properties of cyanobacteria in general. Specifically, PEPCY aims to characterise the health hazard caused by peptides in cyanobacteria, to understand the

occurrence of these peptides and the key environmental factors influencing occurrence, to develop methods for monitoring and surveillance, to provide a risk assessment framework developed in communication with end users in the public and in environmental health sectors and finally to provide information to the general public and to professional groups.

The role of disulfide bonds in the activity of the glycolipid transfer protein

Matts Nylund, Elina Palonen, Peter Mattjus

Department of Biochemistry and Pharmacy, Åbo Akademi University

Glycosphingolipids (GSLs) are important molecules that together with phospholipids and cholesterol make up the basic lipid core structure of mammalian cell membranes, and also of the membranes of some intracellular organelles as a minor component. GSLs act as cell surface receptors for hormones, bacterial toxins and viruses. They are also important in stimulating cell growth and differentiation. The glycolipid transfer protein (GLTP) has been shown to possess specific transfer activity on the in vitro transfer of glycosphingolipids with sugars beta-linked to the ceramide backbone. Mammalian GLTPs have a length of 209 amino acids, of which there are three conserved cysteine residues. Two of the cysteines are buried inside the protein and can make an intra-subunit disulfide bond. The third one is located on the surface of the protein and can build a inter disulfide bond with another protein. Here, we have treated the protein with different reagents to form cysteine-cysteine bonds. We treated the protein with copper sulfate to oxidize the surface cysteine and therefore promote formation of a GLTP dimer, also the inner cysteines are oxidized so that we get two forms of monomer GLTP, with and without an internal disulfide bridge. By treating GLTP with NEM, which binds to the surface cysteine and prevents the promotion of dimers and therafter treat the protein with copper sulfate, we‘ll get more GLTPs with an internal disulfide bond. Nonreducing SDS-PAGE and glycolipid transfer activity measurements was done on the differently treated GLTPs.

Internalization of TAT-Streptavidin Fusion Protein in Mammalian Cells

Johanna Rinne[1], Brian Albarran[2], Juulia Jylhävä[1], Teemu Ihalainen[1], Pasi Kankaanpää[1], Vesa P. Hytönen[1], Patrick S. Stayton[2], Markku S. Kulomaa[3], Maija Vihinen-Ranta[1]

[1] NanoScience Center, Molecular Biology, University of Jyväskylä, [2] Department of Bioengineering, University of Washington, Seattle, WA 98195, USA, [3] Institute of Medical Technology, University of Tampere, Tampere

The cell penetrating peptide derived from the Human immunodeficiency virus-1 Tat protein possesses the capacity to promote the effective uptake of various cargo molecules across the plasma membrane into cells and tissues. The objective of this study was to characterize the uptake mechanism and intracellular localization of a novel TAT-streptavidin (TAT-SA) construct. Confocal and immunoelectron microscopy showed that the majority of internalized TAT-SA accumulated in perinuclear vesicles, and only a minority in the nucleus, at four hours post transduction. Studies in living cells with various fluorescent endocytic markers demonstrated that within 15 minutes after uptake a small proportion of TAT-SA colocalized with internalized transferrin, a clathrin-mediated endocytic marker, whereas the majority localized with the macropinocytic marker dextran. When endosomal release of TAT-SA was enhanced through the incorporation of a biotinylated, pH-responsive polymer poly(propylacrylic acid) (PPAA), nuclear localization of TAT-SA was markedly improved. In conclusion these results suggest that TAT-SA enters cells using both clathrin-mediated endocytosis and macropinocytosis. Furthermore, TAT-SA combined with PPAA may be utilized in protein therapeutics to deliver a wide range of biotinylated molecules into mammalian cells.

Formation of the sterol-rich domains in complex lipid bilayers containing PCPE or NAPEs

Bohdana Térová

Department of Biochemistry and Pharmacy, Åbo Akademi University

In our studies we are interested in formation of heterogeneities (domains) in model lipid membranes. We used cholestatrienol as a fluorescent reporter molecule to study the presence of sterol rich ordered domains in complex lipid bilayers. As a quencher we used 1-palmitoyl-2-stearoyl-(12-doxyl)-phosphatidylcholine (12SLPC) which is known to be located mainly outside the ordered phase (Ahmed et al., 1997). The melting of cholesterol rich domains was detected by quenching of cholestatrienol fluorescence when the probe is exposed to the quencher. With this essay we were able to confirm formation of sterol rich domains in complex lipid mixtures containing sphingomyelins or saturated phosphatidylcholines (Björkqvist et al., 2005). Similarly, we followed formation of sphingolipids-rich domains using N-*trans*-parinoyl-sphingomyelin as a fluorescent probe or ceramide-rich domains using N-*trans*-parinoyl-sphingosine (Alanko et al., 2005).

Formation of ordered domains in mixed lipid bilayers is favored by presence of saturated sphingolipids and by presence of cholesterol. Molecular basis of preferential interaction between sphingomyelin and cholesterol is not yet fully understood although presence of saturated acyl chains and hydrogen bond donating and accepting groups in molecular structure of sphingomyelin seems to be important. I have studied how the headgroup size and properties affect the membrane properties of sphingomyelin and interactions with cholesterol. N-palmitoyl ceramide phosphoethanolamine (PCPE) is a naturally occurring lipid which lacks three methyl groups from sphingomyelin phosphocholine head group. By cholestatrienol quenching essay we showed that PCPE failed to form sterol-riched domains, suggesting that the size and/or properties of the headgroup are important for stabilizing sphingolipid/sterol interaction (Térová et al, 2005a). The weak interaction between PCPE and cholesterol was further confirmed by high rate of cholesterol desorption from mixed monolayers to βCyD in subphase as compared to sphingomyelin.

I have also studied the effect of the N-linked acyl chain of N-acyl phosphatidylethanolamines (NAPE) on the lateral distribution of cholesterol in membranes. The abundance of NAPEs in membranes of mammals is very low but their content markedly increases with stress and cell injury. In our

cholestatrienol quenching essay NAPEs failed to form ordered domains in presence of cholesterol, despite the presence of two or three saturated acyl chains. In contrast, they showed good miscibility with sphingomyelin and partially displaced sterol from ordered sphingomyelin and cholesterol-rich domains (Térová et al, 2005b). This finding may have implications particularly for neural cell membrane function during stress and injury.

Ahmed, S.N., D.A. Brown, and E. London. 1997. On the origin of sphingolipid/cholesterol-rich detergent soluble cell membranes: physiological concentrations of cholesterol and sphingolipid induce formation of a detergent insoluble, liquid ordered lipid phase in model membranes. *Biochemistry* 36:10944-53.

Alanko, S., K. Halling, S. Maunula, J.P. Slotte, and B. Ramstedt. 2005. Displacement of sterols from sterol/sphingomyelin domains in fluid bilayer membranes by competing molecules. *Biochim. Biophys. Acta* 1715:111-121.

Björkqvist, Y.J., T.K.M. Nyholm, J.P. Slotte, and B. Ramstedt. 2005. Domain formation and stability in complex lipid bilayers as reported by cholestatrienol. *Biophys. J.* 88:4054-4063.

Térová, B., R. Heczko and J.P. Slotte. 2005. On the importance of the phosphocholine methyl groups for sphingomyelin/cholesterol interactions in membranes: A study with ceramide phosphoethanolamine. *Biophys. J.* 88:2661-9.

Térová, B., G. Petersen, H.S. Hansen and J.P. Slotte. 2005. N-acyl phosphatidylethanolamines affect the lateral distribution of cholesterol in membranes. *Biochim. Biophys. Acta* 1715:49-56.

Interaction between cyanotoxins and lipid monolayers

Pia Vesterkvist, Jussi Meriluoto

Department of Biochemistry and Pharmacy, Åbo Akademi University

Toxins produced by certain cyanobacterial species are able to create serious water quality concerns. Cyanotoxins can according to their chemical structure be divided into three main groups: cyclic peptides, alkaloids and lipopolysaccarides. Microcystins and nodularins, the most frequently found toxins, are amphipathic molecules believed to require an active transport mechanism to penetrate lipid membranes of cells. Toxicity and behaviour of microcystins have been predicted according to experiments conducted with one rather hydrophilic variant, MC-LR. Here we try to elucidate if minor changes in the microcystin structure alters their biophysical properties. Anatoxin-a and cylindrospermopsin are alkaloids included in this study and whose membrane interactions still are unrevealed. Surface activity studies using a surface barostat showed that the more hydrophobic microcystins have membrane penetrating capacities which are dependent on surface pressure. This was confirmed for one toxin variant with fluorescence emission maximum measurements. Experiments conducted using TMA-DPH, laurdan and cholestatrienol as fluorescent probes did not offer supporting evidence regarding membrane interaction. Finally, RP-HPLC with a dynamic lipid coating was performed in order to include both polar and hydrophobic interactions in the retention behavior of the toxins.

Structural modeling of sterol carrier protein-2 from plants.

Lenita Viitanen, Tiina A. Salminen

Department of Biochemistry and Pharmacy, Åbo Akademi University

Sterol carrier protein-2 (SCP-2) is a small cytoplasmic protein, originally described as a cholesterol transfer protein. Later it has been shown to bind a variety of lipids but its biological function is unclear. SCP-2-like proteins have been found in various organisms from bacteria to vertebrates, and recently also in plants. Our collaborators have studied the ligand binding preferences of *Arabidopsis thaliana* SCP-2 (AtSCP-2) and *Euphorbia lagascae* SCP-2 (ElSCP-2) by a competition transfer assay [1]. Ergosterol was one of the preferred substrates for AtSCP-2, whereas ElSCP-2 did not transfer any of the tested sterols. ElSCP-2, on the other hand, showed a high palmitic acid transfer activity, while AtSCP-2 could not transfer it at all. Considering their high sequence identity (67.5%), it is surprising that these two proteins preferably bind different lipids. We have built structural models of AtSCP-2 and ElSCP-2 in order to find out properties, which would explain the differences in ligand binding preference.

[1] Edqvist J., Rönnberg E., Rosenquist S., Blomqvist K., Viitanen L., Salminen T.A., Nylund M., Tuuf J. and Mattjus P. (2004) *J. Biol. Chem.*, 279, 53544-53553

Detection of novel proteins as diagnostic and therapy predictive markers in lung cancer

Janne Lehtiö

Karolinska Institutet/Karolinska University Hospital, Solna, Sweden

Among cancer diseases, lung cancer (LC) is the number one cause of mortality and the occurrence continues to increase word wide. Main treatment in early phase of lung cancer is surgical removal combined with adjuvant chemo (CT) and radio therapy (RT). Non-surgical cases are treated with CT and kinase inhibitors still having very low impact on survival. Since both radio- and chemotherapy are extensively used in more advanced cases of LC it is important to know who will benefit from the therapy. Today, unfortunately, only 20 – 40 % of the cases respond to various therapies including modern kinase inhibitors. At Karolinska Biomics Center (KBC) lung cancer project we have lung cancer tumour tissue and serum bank material and are prospectively collecting additional material, relating it to patient survival, metastatic behaviour and sensitivity to given therapy. Additionally we are collecting data using in vitro tested (drug resistance test) lung cancer material, which can be used to assist in biomarker discovery as related to drug sensitivity.

These lung cancer related samples are utilized for protein profiling and identification of plasma and cellular proteins relevant to LC distinguishing them from other lung diseases. In cases with surgically respectable disease and/or cases suitable for precision radiotherapy proteomic analyses of tumour and plasma may offer novel guidance for therapy. Our research programmes therefore focus on understanding which proteins or protein modifications are important in the efficacy of treatments in LC. We are currently using our in house developed plasma pre-fractionation and MS-peak detection algorithm combined to mass spectrometry based proteomics for biomarker discovery. For proteomic profiling using cell line model systems and tumour tissue we are using both 2DE based proteomics and MS-profiling.

Study presenting biomarker discovery from cell line model system to clinical validation will be presented. General considerations working with clinical proteomics, including study design, method development and data-analysis, are discussed.

Analysis of Testosterone using Immunoaffinity Capillary Electrophoresis and Microchips

Lotta Amundsen[1], Tarja Nevanen[2], Kristiina Takkinen[2], Stella Rovio[1], Ari Hokkanen[3], Santeri Tuomikoski[4], Sami Franssila[4], Heli Sirén[1]

[1] VTT Processes, [2] VTT Biotechnology, [3] VTT Microelectronics, [4] Helsinki University of Technology

A new testosterone analysis method, based on immunoaffinity capillary electrophoresis (IACE), is introduced. Testosterone is an androgen that affects male sexual differentiation and fertility, in addition to muscle, bone and brain development. IACE, a technique developed in early 1990s, is based on the combination of interactions between an antibody and an antigen, and capillary electrophoresis. In general, IACE offers many advantages over traditional analysis methods (liquid chromatography, gas chromatography). The principal benefit is the reduction of sample pre-treatment. In this study, a testosterone specific antibody Fab fragment was developed and covalently immobilized to cobalt-iminodiacetic acid-coupled sepharose via 6-histidine tags. A 1-ul aliquot of Fab-coupled sepharose was placed in a Tygon-tube between two fused silica electrophoresis capillaries. During sample injection, testosterone was bound to Fab fragments, while other sample components ran through the immunomatrix. After injection, the immunomatrix was rinsed with capillary electrophoresis running buffer. Subsequently, testosterone was eluted from the immunomatrix with a micellar solution and transferred to a diode array detector by applying an electric field.

The IACE method was also applied in polymer and silicon microchips. For this purpose, testosterone was labeled with fluorescein-5-isothiocyanate, eluted with methanol, and detected with a laser induced fluorescence detector.

This study has been very interdisciplinary, and the results look promising. Next step will be testosterone determination from biological fluids.

The design of novel ligands for estrogen receptor-related receptors

Susanna Repo[1], Juha Pulkkinen[2], Reino Laatikainen[2], Paavo Honkakoski[3], Mark S. Johnson[1]

[1] Department of Biochemistry and Pharmacy, Åbo Akademi University, [2] Department of Chemistry, University of Kuopio, [3] Department of Pharmaceutics, University of Kuopio

Estrogen receptor-related receptors (ERRs), initially found due to their homology with estrogen receptor α (ERα), have been suggested to share common biological pathways with the ERs. ERRs, together with the estrogen receptors, belong to the superfamily of nuclear receptors (NRs), a family of transcription factors, which mediate gene expression in various biological processes. The sequence similarity between the three ERR subtypes, α, β and γ, is considerably high, however, each isoform is encoded by a different gene. The endogenous ligands of ERRs remain to be identified and only a handful of synthetic ligands are known. We have synthesized a set of novel ligands, which have been computationally evaluated for their possible affinity for the ERRs. The selected ligands are tested experimentally and further computational and experimental studies are based on modifying the ligand properties to design truly subtype selective ligands. In this study, our ultimate aim is to characterize novel, subtype selective ligands, both agonists and antagonists for the NR subfamily of ERRs.

This research has been supported by TEKES, ISB, Emil Aaltonen Foundation and Magnus Ehrnrooth Foundation.

CBS-pyrophosphatases: a new subfamily of family II pyrophosphatases

Heidi Tuominen, Joonas Jämsen, Georgiy Belogurov, Anu Salminen, Reijo Lahti

Department of Biochemistry and Food Chemistry, University of Turku

Inorganic pyrophosphatase (PPase) is essential for life and catalyzes the interconversion of inorganic pyrophosphate and orthophosphate. Two types of PPase have been identified: soluble and integral membrane-bound. Soluble PPases are further divided into two families with completely different primary and tertiary structures. Family II PPases were discovered quite recently (in 1998) and have been only partially characterized so far. Most family II PPases are two-domain proteins, but 16 of the 63 family II PPase sequences currently listed in the GenBank have a large ($\sim$ 250 amino acid) insertion within the 200-residue-long N-terminal domain. The insertion includes two CBS (Cystathionine Beta Synthase) domains ($\sim$ 60 residues each) and one DRTGG domain ($\sim$ 120 residues). This subfamily of family II PPases is called CBS-PPases. CBS-PPase from *Moorella thermoacetica* is unique in possessing CBS domains but lacking a DRTGG domain. The CBS domains are abundant in proteins of all three kingdoms of life and, in some cases, are the targets for regulation by adenosine derivatives, whereas DRTGG domains are rare and have unknown functions.

Our work on CBS-PPases will focus on finding out the role of the long insertion. This work will involve structural, mutational and functional studies. We have cloned, expressed and purified CBS-PPases from *Clostridium perfringens* (CBS-*cp*PPase) and *M. thermoacetica* (CBS-*mt*PPase). Crystallization of these two enzymes has turned out to be quite difficult, probably because of the high flexibility of the long insertion. We have shown that both CBS-*cp*PPase and CBS-*mt*PPase are significantly inhibited by AMP and that CBS-*mt*PPase is activated by ATP. We are currently studying the mechanism of AMP inhibition and ATP activation by kinetic and binding approaches to elucidate whether these enzymes are subject to allosteric regulation. Parallel detailed solution studies with CBS-*cp*PPase and CBS-*mt*PPase will be important because they will allow determination of the individual roles of these two additional domains.

Order amid disorder - Nascent structural motifs examined by residual dipolar couplings

Kai Fredriksson, Martti Louhivuori, Arto Annila

Institute of Biotechnology, University of Helsinki

Emergence of primitive structural elements in a nascent polypeptide is thought to reduce the number of conformational states considerably and to prompt protein folding. Provided that these evanescent states can be stabilized, residual dipolar couplings offer means to characterize these motifs amid non-structured segments. We have studied how residual dipolar couplings (RDCs) reflect concurrent appearance of local structure and arousal of coherence in fragmental alignments.

We show how residual dipolar couplings report from a helix-coil transition and from a loop closure in a 21 amino acid polypeptide. In our simple model system the helix-coil transition is driven by pH change and the loop closure by the formation of a disulfide bridge. We are able to identify the local conformational states and to extract fragmental alignments using residual dipolar couplings that correspond to inter nuclear vector directions in the Cα sites. We realized that an RDC value measured from a dispersed ensemble is essentially a weighted average that is biased towards elongated conformations. Perhaps the best way to interpret the convoluted data is to use restrained simulations to create a concise family of conformations that reproduce largely the measured couplings. In this way residual dipolar couplings can be used to monitor early events in folding processes and to describe the intermediate states to provide information about nascent structural motifs that emerge from random segments.

Molecular dynamics studies on thermostability of family 11 xylanases

Mikko Purmonen[1], Jarkko Valjakka[2], Juha Rouvinen[1]

[1] Department of Chemistry, University of Joensuu, [2] Institute of Medical Technology, University of Tampere

Xylan is one of the main building components of the plant cell wall of deciduous trees. Xylans are polysaccharides, which are formed from 1,4-linked xylopyranosidic units. The most important enzymes taking part to the degradation of xylan polymers are endo-1,4-xylanases (E.C. 3.2.1.8). Endo-1,4-xylanases degrade xylan to short xylo-oligosaccharides units of variable lengths. The most important protein families of xylanases are families 10 and 11 because of their commercial purposes in the paper-, pulp-, feed-, baking- and brewing industry. We have focused on the protein family 11 in this study.

Molecular dynamics (MD) is one tool of computational chemistry to study biological problems e.g. protein folding and unfolding. By following the conformational changes as a function of time, it is possible to investigate denaturation pathway. In this study the 12 xylanases of family 11 have been simulated by using the MD program Amber 7. Simulations were performed in water environment in the octahedral box in NVT environment (constant number of atoms, volume and temperature). Typical length of the simulation was 4.5 ns long and required one month of CPU time. During this project we have found several factors that affect on the results of MD-simulations

(1) Influence of the initial structure for unfolding was studied, for example, by changing places of the atoms arbitrarily. Initial structure was found to affect slightly on the pathway of denaturation, if only all the trails end up with similar final result. This study is in agreement with the previous papers that have shown the initial structure dependence to the unfolding process.

(2) Another question is the effect of used temperature to the results. Because of the short time-scale in MD simulations very high temperatures must be used to fasten unfolding. We supposed that the use of higher temperature does not change unfolding pathway significantly. In this study the xylanases have been simulated in four different temperatures: 300, 400, 500 and 600 K and we found clear similarities in the unfolding pathways. On the basis of simulations we have been able to suggest a general pathway for denaturation of family 11 xylanases. In addition, the most mesophilic xylanases unfolded usually more rapidly than thermostable xylanases. These results can be used in order to design new mutants to increase thermostability of Family 11 xylanases.

Protein chemical analysis of the histidine-tyrosine cross-link in the active site of the cbb$_3$-type cytochrome c oxidase from Rhodobacter sphaeroides

Virve Rauhamäki[1], Marc Baumann[2], Mårten Wikström[1]
[1] Institute of Biotechnology, University of Helsinki, [2] Institute of Biomedicine, Biomedicum, University of Helsinki

Haem-copper oxidases are a superfamily of terminal enzymes located in the inner mitochondrial membrane or in bacterial cell membrane. These enzymes catalyse the final step of energy conservation in the respiratory chain. The catalytic subunit, which contains two haems and a copper as redox centres forming the active site, is homologous between all oxidases in this group of enzymes. The *cbb*$_3$-type cytochrome *c* oxidase is a distant member of the haem-copper oxidase family. Even though several key structural features are missing from the *cbb*$_3$-type oxidases, the mechanism with which they perform the four-electron reduction of oxygen to water is thought to be the same as in the canonical haem-copper oxidases.

In the canonical haem-copper oxidases, there is a fully conserved tyrosine residue in the active site of the enzymes which cannot be found in the *cbb*$_3$-type oxidases. The residue forms a covalent bond to a close by histidine residue which acts, at the same time, as a ligand of one of the redox centres of the active site. This modified tyrosine residue has been proposed to have a critical role in the catalytic mechanism of the enzyme. The presence of the bond in the canonical haem-copper oxidases was first shown by protein chemical analysis (Buse et al., 1999). Recent results from homology modelling suggest that a tyrosine residue, which is fully conserved among the *cbb*$_3$-type oxidases and which is situated in another near by transmembrane helix, could replace the missing tyrosine in the active site and thereby fulfil the requirement for the tyrosine to be mechanistically important (Hemp et al., 2005). The methods of protein chemistry and mass spectrometry have been applied to obtain direct evidence of the cross-link in the catalytic centre of the *cbb*$_3$-type oxidases.

1. Buse, G., Soulimane, T., Dewor, M., Meyer, H. E., Bluggel, M. (1999) Evidence for a copper-coordinated histidine-tyrosine cross-link in the active site of cytochrome oxidase, *Protein science 8*, 985-990.

2. Hemp, J., Christian, C., Barquera, B., Gennis, R. B., Martinez, T. J. (2005) Helix switching of a key active-site residue in the cytochrome *cbb*$_3$ oxidases, *Biochemistry 44*, 10766-10775.

Deciphering the basal acticity of constitutive androstane receptor (CAR) - how far can modelling go?

Björn Windshügel[1], Johanna Jyrkkärinne[2], Antti Poso[3], Paavo Honkakoski[2], Wolfgang Sippl[4]

[1] Institute for Pharmaceutical Chemistry, Martin-Luther-University Halle-Wittenberg, Germany, [2] Department of Pharmaceutics, University of Kuopio, FINLAND, [3] Department of Pharmaceutical Chemistry, University of Kuopio, FINLAND, [4] Institute for Pharmaceutical Chemistry, Martin-Luther-University Halle-Wittenberg, GERMANY

The constitutive androstane receptor (CAR) belongs to the superfamily of nuclear hormone receptors that function as ligand-activated transcription factors. CAR plays an essential role in the metabolism of xenobiotics which makes it an interesting pharmaceutical target. In contrast to most other known nuclear receptors CAR shows constitutive activity for which the structural basis was unknown.

When starting our project no 3D information for CAR was available. Therefore, a homology model of the CAR ligand binding domain (LBD) was established based on the related pregnane X (PXR) and vitamin D receptor (VDR). Molecular dynamics (MD) simulations were carried out to validate the model and to examine the molecular basis for constitutive activity[1].

Our studies revealed a tyrosine residue to be essential for basal activity of CAR. Docking of known agonists and site-directed mutagenesis (in vitro and in silico) supported the hypothesis of a 'molecular mimicry' in which a tyrosine side chain mimics a bound agonist. Additionally a hydrogen bond was predicted to be essential for constitutive activity which could be verified by experimental studies[2].

Very recently the X-ray structures of human and mouse CAR complexed with various ligands have been solved and published. This gave us the opportunity to verify the quality of the generated homology model. The CAR X-ray structures and the homology model show a nearly identical structural organisation. This is reflected by a low root mean square deviation (1.9 Å) for all atoms forming the ligand binding pocket (LBP). Other relevant stereochemical parameters are also almost identical.

[1]Windshügel et al. (2005) J Mol Mod **11** 69-79

[2]Jyrkkärinne et al. (2005) J Biol Chem **280** 5960-5971

Hydrophobin HFBII in detail - ultra-high resolution structure of 0.75 Å

Johanna Hakanpää[1], Markus Linder[2], Alexander Popov[3], Andrea Schmidt[3], Juha Rouvinen[4]

[1] Department of Chemistry, University of Joensuu, [2] VTT Biotehcnology, [3] EMBL-Hamburg, Germany, [4] Department of chemistry, University of Joensuu

The structure of *Trichoderma reesei* hydrophobin HFBII has been determined to ultra-high resolution of 0.75 Å. The structure was refined conventionally and analyzed for any special features that might distinguish the ultra-high resolution structures from the more modest resolution ones. A previously collected atomic resolution data (1.0 Å) was used as a reference to highlight the special features that are unique for ultra-high resolution data only.

Both the ultra-high and atomic resolution structures of HFBII were refined with program Shelx. Hydrogen atoms were included in the refinement as were the anisotropic temperature factors. The ultra-high resolution structure was then analyzed for quality of electron density maps, disorder, identification of atoms by atom type, observed hydrogen atoms, hydrogen bonds (regular and weak), inter- and intramolecular interactions, solvent content and water contacts, B-factors and anisotropy and bond lengths and angles. In addition, comparison was made with the ultra-high resolution structure of HFBII with respect to other ultra-high resolution protein structures.

During the analysis, clear differences could be found between the ultra-high and the atomic resolution structure. The ultra-high resolution structure had residual density around the peptide bond and carbonyl oxygens of the backbone, considerably lower B-factors, more observed hydrogen atoms and a bit higher R-factors at the end of the Shelx-refinement when compared with the atomic resolution structure. Some of these facts suggested, that the refinement of the ultra-high resolution structure was not yet complete but needed to be refined in a way that takes into account the deformation of the valence electrons due to bonding. The program Mopro was used for this purpose and the refinement is in progress.

Structural studies of the anti-morphine Fab fragment M1

Merja Niemi[1], Timo Pulli[2], Kristiina Takkinen[2], Juha Rouvinen[1]

[1] Department of Chemistry, University of Joensuu, [2] VTT Biotechnology

The immune systems primary function is to protect vertebrates against microorganisms such as viruses and bacteria. The recognition elements of the humoral immune response are soluble proteins, antibodies, which bind to antigens and inactivate them or mediate their destruction. The overall structure of antibodies is conserved with the exception of six hypervariable loops known as complementarity determining regions (CDR), which contain most of the antigen binding amino acid residues. The conformations and also the length and amino acid compositions of these regions determine mainly the specificity and binding affinity of the antibody. These specific interactions of an antibody with an antigen form the basis of immunological technologies widely used in diagnostic and medical applications.

We have been studied the anti-morphine Fab fragment M1 from the IgG antibody. M1 Fab has been produced by VTT. The main goal has been to develop a quick and inexpensive method for testing of drug abuse. The anti-morphine Fab fragment complexed with morphine has been crystallized using PEG3350 as a precipitant at pH 6.5. Data from frozen crystal has been collected to 2.65 Å resolution using synchrotron radiation on beamline BW7B at the EMBL, Hamburg. The structure of the M1 Fab fragment was solved by molecular replacement. Clear electron density corresponding to the morphine was found in the binding site of the M1 Fab fragment. Morphine is a compact cationic ligand; a hydrophobic central part of the molecule separates two polar areas, cationic nitrogen atom and three polar oxygen atoms. Hence, binding pocket is substantially hydrophobic and consists of aromatic side chains, but also serine and glutamic acid seems to take part in binding of morphine.

Recently we have crystallized M1 Fab fragment complexed with heroin and collected the data to 2.8 Å resolution at the ESRF, Grenoble. Furthermore, we have also managed to collect the higher resolution diffraction data (2.2 Å) for the M1 Fab-morphine complex. We are expecting to get better view on Fab-antigen interactions using these freshly collected data sets.

Yeast pyrophosphatase: structures of the B and F states complete the catalytic cycle of the enzyme

Esko Oksanen[1], Anna-Karoliina Ahonen[2], Heidi Tuominen[3], Alex Baykov[4], Reijo Lahti[3], Adrian Goldman[1], Pirkko Heikinheimo[1]

[1] Institute of Biotechnology, University of Helsinki, [2] Institutt for Kjemi, Universitetet in Tromsø, Norge, [3] Dept. of Biochemistry and Food Chemistry University of Turku [4] A.N. Belozersky Institute of Physico-Chemical Biology and School of Chemistry, Moskow State University

Inorganic pyrophosphatases (PPases) catalyze the hydrolysis of pyrophosphate (PP_i) to two phosphates (P_i). Family I PPases are found in all kinds of organisms, including *Saccharomyces cerevisiae*. The enzyme is an obligate dimer and requires four divalent metal cations per monomer for catalysis; magnesium provides highest activity, but also manganese, cobalt and zinc activate the enzyme with altered catalytic properties. The structure of the product complex is known at 1.15 Å resolution [1] and structures corresponding to other catalytic stages are known.

We solved the structures of eight active site mutants (K56R, Y93F, D117E, D120E, D120N, E48D and D115E) and the wild type protein crystallised with magnesium and phosphate. The resolution of the structures is between 1.5 Å and 1.9 Å. The structures contain two molecules per asymmetric unit, which correspond to distinct catalytic states. The open and closed conformations of the active site contain different numbers of metal and phosphate ions.The catalytic activity of these mutants is reduced and their pH optimum is switched [2]. As there are no significant differences in the main chain conformation, the mutations seem to affect the binding of the metals and the hydrogen bonding network in the active site.

These new structures allow the completion of the reaction scheme for yeast PPase. The structure of the wild type shows an active site where one of the two product phosphates has already dissociated (state F). The structure of D115E shows the active site with two waters instead of one between the two catalytic metal ions (state B). This arrangement has previously been observed only in the absence of substrate or product. In addition to this, these structures provide data on the conformations available to the active site residues. This information allows a more detailed analysis of the active site dynamics.

References
[1] P. Heikinheimo, V. Tuominen, A-K. Ahonen, A. Teplyakov, B.S. Cooperman, A.A. Baykov, R. Lahti, A. Goldman, Proc. Nat. Acad. Sci. 98, 3121, (2001)
[2] P. Pohjanjoki, R. Lahti, A. Goldman, B.S. Cooperman, Biochemistry. 37, 1754, (1998)

Poster abstracts

in alphabetical order

Removal of cyanobacterial toxins by lactic acid bacteria

Sonja Alanko, Jussi Meriluoto

Department of Biochemistry and Pharmacy, Åbo Akademi University

Cyanobacterial toxins such as microcystins are widely recognized as a public health problem due to their acute and chronic toxicity. Removal of these toxins during drinking water treatment processes is therefore of increasing concern.

We currently assess the ability of food grade lactic acid bacteria to remove cyanobacterial toxins from aqueous solutions by selecting a number of different bacterial strains and assessing their capacity to bind toxins. The bacterial strains which have been screened for their toxin-binding capacity so far are *Lactobacillus casei* Shirota, *Lactobacillus fermentum* ME3, *Lactobacillus rhamnosus* strains GG and LC-705, *Lactobacillus plantarum* Lp-115, *Lactobacillus salivarius* Ls-33, *Lactobacillus acidophilus* NCFM, *Bifidobacterium lactis* Bb12 and *Bifidobacterium longum* 46. The effects of incubation temperature and pH on the binding properties have also been investigated. The *Bifidobacterium lactis* strain Bb12 was shown to be the most efficient of the tested strains with a maximum removal of about 35% of the cyanobacterial peptide toxin microcystin-LR (toxin concentration 100 μg/l, bacterial concentration 2 g dw/l, 37 °C, 24 h). In addition to microcystin-LR, the cyanobacterial toxins microcystin-RR and microcystin-LF will also be tested (10 to 100 μg/l). Furthermore the molecules or molecular mechanisms responsible for toxin binding will be studied and identified.

Toxin removal by lactic acid bacteria may provide significant human health protection against microcystins either as a method to decontaminate drinking water or as a natural barrier against toxin exposure in the gastrointestinal tract. Demonstration of the basic properties will enable us to screen and select specific bacterial strains for both food and technological uses.

On-line simulation and optimization of immunoreagents production

Zoltán Bicskei, Pilvi Ylander, Juhani Soini, Pekka Hänninen

Department of Medical Physics, University of Turku

Computers and automated instruments have been introduced in the entire process of immunotesting from sample collection to final report generation and validation. However, great part of the variance is emerging from reagent production and preparation. We monitor the immunoassay reagent production with a software package based on mathematical and statistical methods. These methods describe biomolecular binding reactions by mechanistic-models in different stages of the production process path. The model takes into account the effects of basic reagents and the conditions of reagent and assay preparation. New approaches[1], such as, mathematical models based on underlying reaction mechanism of bioaffinity assays have been studied. With known parameters and with help of the new mathematical models, it is possible to further describe and simulate various conditions without performing the actual experiments[2]. Components for modelling of immunoreactions, variations, and degradation effects are linked together into a flow chart to simulate the theoretical signal of an immunoassay.

By using this aspect of variance control, we hope that it will be possible to determine the parameters and the extent they affect the precision of routine immunoassay production procedures. Potential applications are: what-if scenarios for reagents production planning, finding out critical conditions and improved possibilities for mastering these processes in an optimal way.

The application is defined in the Orange[3] graphical framework for data analysis and is based on the information flow between user definable widgets. These Orange components are linked to the R[4] statistical environment for execution of statistical methods.

References:
1. Pekka Hänninen, Matti Waris, Mika Kettunen and Erkki Soini, Reaction kinetics of a two-photon excitation microparticle based immunoassay - from modelling to practice, Biophysical Chemistry 105 (2003) 23-28.
2. Zoltán Bicskei, Pilvi Ylander, Pekka Hänninen, Calibration of bioaffinity assays using kinetic data, Journal of Biochemical and Biophysical Methods (2005), Submitted, Manuscript number: JBBM-D-05-00141
3. Demsar J, Zupan B, Leban G (2004) Orange: From Experimental Machine Learning to Interactive Data Mining, White Paper (www.ailab.si/orange), Faculty of Computer and Information Science, University of Ljubljana.
4. R Development Core Team (2003). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-00-3, URL http://www.R-project.org.

Cytoplasmic JNK1 is a Dominant Regulator of Dendritic Architecture; Role of MAP2 as an effector.

Benny Björkblom[1], Nina Östman[1], Vesa Hongisto[1], Vladislav Komarovski[2], Jan-Jonas Filén[1], Tuula A Nyman[1], Tuula Kallunki[3], Michael J Courtney[2], Eleanor T Coffey[1]

[1] Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, [2] AIV institute, University of Kuopio, [3] The Danish Cancer Society, Copenhagen, Denmark.

cJun N-terminal kinases (JNKs) contribute to stress-induced neuronal cell death. Of the 3 JNK genes expressed in the nervous system, JNK1 displays elevated activity that is not stress-related (1). This activity predominates in the cytoplasm where it regulates neuritic architecture (2). To reveal potential mediators of JNK action on neuronal morphology, we have taken a proteomics approach and identified the dendrite-specific high molecular weight microtubule-associated protein 2 (HMW-MAP2) as a JNK substrate in brain. We subsequently show that HMW-MAP2 is phosphorylated efficiently by JNK in intact cells. We developed compartment-targeted JNK inhibtitors to define whether a functional relationship exists between the physiologically active, cytosolic pool of JNK and dendritic architecture. Using these, we demonstrate that cytosolic but not nuclear JNK determines dendritic length and arbor complexity in cultured neurons. Moreover, we confirm that MAP2-dependent process elongation is enhanced upon activation of JNK. These results suggest that JNK phosphorylation of MAP2 plays an important role in defining dendritic architecture in the brain.

Ref.
1) Coffey ET, et al. (2000) J Neurosci. 20:7602-7613.
2) Coffey ET, et al. (2002) J Neurosci. 22:4335-4345.

Exploring domain formation and stability in model membranes using fluorescent and fluorescence quenching lipids

Jenny Björkqvist, Stina Maunula, J. Peter Slotte, Bodil Ramstedt

Department of Biochemistry and Pharmacy, Åbo Akademi University

We have developed a method for exploring lateral heterogeneity in model membrane systems with the use of specific fluorescent molecules. Cholesterol, the major sterol in mammalian cells, interacts preferentially with saturated sphingolipids in membranes under formation of ordered cholesterol-rich domains, which separate from unsaturated phospholipids existing in more disordered membrane regions. This kind of membrane phase separation seem to be physically important for a number of cell functions, e.g. protein sorting and signal transduction (1), where involved proteins associate specifically with either ordered or disordered membrane domains.

We studied formation of cholesterol-rich membrane domains using a fluorescent cholesterol analog, cholestatrienol (CTL), which has been shown to mimic the behaviour of cholesterol in membranes well (2). Our model membrane systems also included a fluorescence quenching phospholipid, 7SLPC, which exists in the disordered membrane phase since the bulky structure of the lipid prevents it from packing tightly in membranes (3). Therefore, fluorescent lipids residing in ordered lipid domains are less quenched than fluorophores in the disordered phase. By measuring the fluorescence intensity of CTL in different membrane systems, it is possible to examine the extent of cholesterol-rich domains formed by various membrane lipids (4). Similarly, we have used a fluorescent ceramide, *trans*-parinoyl sphingosine (tParCer), and sphingomyelin, *trans*-parinoyl sphingomyelin (tParSM), to explore formation of ordered domains enriched in ceramides and sphingomyelins, respectively (5).

Our results show that cholesterol-rich domains formed together with saturated sphingomyelins are more stable than phosphatidylcholine/cholesterol domains. We were also able to show that saturated galactosyl and lactosyl ceramides do not form cholesterol-rich domains alone but stabilize sphingomyelin/cholesterol domains. By using tParCer we confirmed that ceramides can displace cholesterol from ordered domains formed together with sphingomyelins.

(1) Brown, D.A. and London, E. 2000. *J. Biol. Chem.* 275: 17221-17224. (2) Scheidt, H.A., Muller, P., Herrman, A. and Huster, D. 2003. *J. Biol. Chem.* 278: 45563-45569. (3) Ahmed, S.N., Brown, D.A. and London, E. 1997. *Biochemistry* 36: 10944-10953. (4) Björkqvist, Y.J.E., Nyholm, T.K.M., Slotte, J.P. and Ramstedt, B. 2005. *Biophys. J.* 88: 4054-4063. (5) Alanko, S.M.K., Halling, K.K., Maunula, S., Slotte, J.P. and Ramstedt, B. 2005. *Biochim. Biophys. Acta* 1715: 111-121.

Structural studies on collagen binding integrin αI domains

Anna-Maria Brandt, J. Santeri Puranen, Yvonne Nymalm, Tiina Salminen, Mark S. Johnson

Department of Biochemistry and Pharmacy, Åbo Akademi University

Integrins are cell adhesion receptors that mediate cell-cell or cell-extracellular matrix interactions by bidirectional signaling. The $\alpha\beta$ heterodimeric glycoproteins are composed of 19 different α subunits and 8 different β subunits. The collagen binding integrin family consists of four collagen receptors that have a common β1 subunit non-covalently bound to either α1, α2, α10 or α11 subunit.

The α subunits contain an inserted domain of 200 amino acids, also called the I domain, which is the key ligand-binding domain. The αI domain folds into a Rossman fold, which forms a metal ion-dependent adhesion site, referred to as MIDAS. The I domain alternates between two conformations, which is important for regulating the affinity of the ligand. In the open conformation collagen is bound mediated via MIDAS, while in the closed conformation the binding is hindered.

The crystal structure of α1I and α2I, and also the complex structure of α2I bound to a collagen-like peptide, have been solved [1,2,3]. Comparison of the α2I in complex with a collagen-like peptide (open conformation) and α2I without ligand (closed conformation) showed that conformational changes occur, when the ligand is bound.

We are studying the conformational changes and the binding of collagen in the open conformation. We have used mutagenesis to lock the structure in the open conformation and expect this to facilitate crystallization. We have also modeled the open conformation of α1I in complex with a collagen-like peptide and characterized the binding of the ligand [4].

[1] Salminen TA., Nymalm Y., Kankare J., Käpylä J., Heino J., Johnson MS. (1999) *Acta Crystallogr D Biol Crystallogr.* 55:1365-7.

[2] Emsley J., King SL., Bergelson JM., Liddington RC. (1997) *J Biol Chem.* 272:28512-7.

[3] Emsley J., Knight CG., Farndale RW., Barnes MJ., Liddington RC. (2000) *Cell* 101:47-56.

[4] Nymalm Y., Puranen JS., Nyholm TK., Käpylä J., Kidron H., Pentikäinen OT., Airenne TT., Heino J., Slotte JP., Johnson MS., Salminen TA. (2004) *J Biol Chem.* 279:7962-70.

Determination of Interleukin-4 Regulated Proteins in the Microsomal Fraction of CD4+ cells using Isotope-Coded Affinity Tags with Tandem Mass Spectrometry

Jan-Jonas Filén

Turku Centre for Biotechnology, University of Turku and Åbo Akademi University

T helper cells (Th) are divided into Th1 and Th2 subsets based upon their cytokine profiles and function. Naïve Th cells differentiate into Th1 and Th2 subsets depending on the antigens, co-stimulatory molecules, and cytokines they encounter. Cytokine interleukin (IL)-4 enhances the generation of Th2 lymphocytes and inhibits the production of Th1 subset. Th2 cells participate in humoral immunity and their selective activation plays an important role in the pathogenesis of allergy and asthma.

In this study, isotope-coded affinity tag labeling combined with chromatographic techniques and tandem mass spectrometry was used to find IL-4 regulated proteins in microsomal fraction of CD4+ Th cells isolated from umbilical cord blood. A total of 557 proteins were confidently identified by two or more peptides, with quantitative data for 293 of these. Changes in expression were indicated for 33 of these proteins, out of which we have focused on three biologically interesting candidates. Of the latter, further validation has been achieved using Western blotting. Currently we are designing functional studies to determine the significance of these observations.

Redox reactions of cytochrome c oxidase. Combined FTIR-electrochemical approach

Elena Gorbikova, Kai Vuorilehto, Michael I. Verkhovsky

Institute of Biotechnology, University of Helsinki

With the final aim to construct the whole thermodynamic model of functioning of cytochrome c oxidase (CcO), electrochemical titration of the enzyme from *Paraccocus denitrificans* was performed by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy for the first time.

The redox titration (changes of enzyme properties depending on applied potential) of CcO by FTIR allowed direct estimation of thermodynamic constants such as midpoint potentials (E_ms) and potential interaction which are necessary for the construction of the thermodynamic model.

As a result of redox titration of the enzyme by FTIR, surfaces 'optical density-potential-wavenumber' were generated for three pHs: 6.5, 8.0, and 9.0. Applied potentials were in range 0:+480 mV vs NHE with step of 40 mV. Analyzed infrared region was 1800-1000 cm-1. Redox titration curves (dependence of optical density on potential) were extracted from the surfaces at each peak (and trough).

Majority of the absorption peaks was devided into four groups depending on their behavior. These four groups were shown to correlate with the redox transitions of four redox centers of the enzyme (CuA, heme a, heme a3, and CuB). Infrared spectroscopy gave us an advantage to measure redox centers independently, which could not be well separated or even observed ('invisible' CuB) by any other spectroscopic techniques.

Combined FTIR-electrochemical approach provided new way of assignment of infrared bands of redox active enzymes. Practically all infrared bands in redox-induced difference FTIR spectra of CcO were found to be complex but with one major component in each. Most of these bands were assigned to specified vibrations of redox centers themselves (heme groups) and their ligands (all four centers).

As a result of assignment of bands to each of four redox centers, we have found 12 bands which were titrating with the highest midpoint redox potential (E_m) +412 mV at pH 6.5. They had pH dependence (52 mV per pH unit) very close to the theoretical one (60 mV) and may be assigned to 'invisible' Cu_B center. To the Cu_A center we assigned bends, titration of which showed pH independent Em =+250 mV (10 bends resolved). Two other groups reflecting redox transition of the hemes had more complex behavior.

Each of them included two parts corresponding to the high and low potential transitions. For the bands representing heme a (6 bands), the ratio of high to low potential components was ca. 3:2, and for a_3 (8 bends) this ratio was ca. 2:3. Taking into account the redox interactions between hemes, such ratio gave the difference in the redox potentials of hemes of 9 mV. The value of interaction was estimated to be negative and pH-dependent. The pH dependence of Ems for two hemes were the same (30 mV) and practically twice smaller than theoretical one. This indicates that the hemes have common group which binds proton upon reduction either of the hemes.

Displacement of sterols from sterol/sphingomyelin domains in fluid bilayer membranes by competing molecules

Katrin Halling, Sonja M.K. Alanko, Stina Maunula, J. Peter Slotte, Bodil Ramstedt

Department of Biochemistry and Pharmacy, Åbo Akademi University

Lipids and proteins in biological membranes are organized into lateral regions with different physical and chemical properties. Domains with a relatively high degree of order, often rich in cholesterol and sphingolipids, are of importance in e.g. signal transduction, membrane trafficking and protein sorting [1]. In model membranes, the favorable interactions between sphingomyelin and cholesterol cause phase separation and the formation of ordered sphingomyelin- and cholesterol-rich domains. In recent studies, it has however been shown that ceramide is able to compete with cholesterol for association with ordered domains in bilayer membranes [2].

The aim of this model membrane study [3] was to explore the ability of several membrane intercalators to affect the distribution of sterol between ordered domains and the surrounding lipid matrix. The experiments were based on fluorescence quenching, cholesterol efflux from monolayers, and differential scanning calorimetry. We observed that sterols were displaced from ordered domains by a variety of saturated, single- and double-chain membrane intercalators with a small polar group as a common denominator. Palmitoyl ceramide and palmitoyl dihydroceramide, the two double-chain intercalators included in the study, were both able to displace sterols from ordered domains. Of the single-chain intercalators, hexadecanol and hexadecyl amide displaced the sterol from sterol/sphingomyelin domains, whereas palmitic acid, sphingosine and sphinganine failed to do so. All molecules examined stabilized the sphingomyelin-rich, ordered domains in our model membrane system.

References:

1. Rietveld, A. and Simons, K., Biochim. Biophys. Acta 1376 (1998) 467-479.

2. Megha and London, E., J. Biol. Chem. 279 (2004) 9997-10004.

3. Alanko, S.M.K., Halling, K.K., Maunula, S., Slotte, J.P. and Ramstedt, B., Biochim. Biophys. Acta 1715 (2005) 111-121.

NMR structure of the 17^{th} domain of human filamin A

Outi Heikkinen[1], Pudas, R.[2], Permi, P.[3], Tossavainen, H.[3], Ylänne, J.[2], Kilpeläinen, I.[1]

[1] Department of Chemistry, University of Helsinki, [2] Department of Biochemistry, University of Oulu, [3] Institute of Biotechnology, University of Helsinki

Human filamin A (FLNA) is a 24 subdomain protein which binds to actin filaments [1]. It has a role in cytoskeleton and cell signaling. We have solved the solution structure of the 17th domain of human filamin A (FLNA17) using high-resolution NMR spectroscopy. The domain 17 was chosen under investigation since earlier studies have pointed out its role in FLNAs interactions with glycoprotein-Ibα (GP-Ibα) [2]. GP-Ibα is a part of the von Willebrand factor receptor complex which initiates platelet adhesion to injured blood vessel wall [3].

A set of 3D triple-resonance NMR experiments was recorded for sequential backbone and side chain resonance assignment. 3D ^{13}C- and ^{15}N-edited NOESY spectra were recorded to obtain the structural constraints. The assignment of ^{1}H, ^{15}N and ^{13}C resonances was done manually using the program Sparky [4]. The NOE constraint based structure calculations were performed with automatic NOE assignment and structure calculation mode of the program CYANA 2.0 [5].

The rod-like structure of FLNA17 consists of antiparallel β-strands and a short helical segment. The structure closely resembles the immunoglobulin-like fold of F-actin cross-linking gelation factor [6]. The solution structure of free FLNA17 was compared with the structure of FLNA17 complexed with GP-Ibα peptide (the structure of the complex solved by X-ray diffraction). GP-Ibα peptide binds next to the one of the β-strands. However, FLNA17 does not undergo any major structural changes upon interaction with the peptide.

[1] A. van der Flier and A. Sonnenberg, Biochim. Biophys. Acta, 1538, 99 (2001).
[2] S. C. Meyer, S. Zuerbig, C. C. Cunningham, J. H. Hartwig, T. K. Gardner and J. E. B. Fox, J. Biol. Chem., 272, 2914 (1997).
[3] I. Canobbio, C. Balduin and M. Torti, Cellular Signalling, 16, 1329 (2004).
[4] T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco.
[5] P. Güntert, C. Mumenthaler and K. Wüthrich, J. Mol. Biol., 273, 283 (1997).
[6] P. Fucini, C. Renner, C. Herberhold, A. A. Noegel and T. A. Holak, Nat. Struct. Biol., 4, 223 (1997).

Studies on the structure of bacteriophage PRD1 spike complex

Juha Huiskonen[1], John Briggs[2], Michael Merckel[1], Adrian Goldman[1], Dennis Bamford[1], Roman Tuma[1], Stephen Fuller[2], Sarah Butcher[1]

[1] Institute of Biotechnology, University of Helsinki, [2] Division of Structural Biology, University of Oxford

PRD1 is a bacterial membrane virus targeting antibiotic resistant bacteria. An atomic model of the PRD1 capsid has been built based on X-ray crystallographic electron density map of the whole virion at 4-Å resolution (Abrescia et al., 2004). In this map, the receptor binding spike complexes composed of proteins P2 and P5 are, however, unresolved. This is due to symmetry mismatches between the spike components and the viral capsid, in addition to spike flexibility.

We solved an X-ray structure of the purified spike protein P5 C-terminal fragment (Merckel et al., 2005). A P5 mutant with reduced flexibility constructed earlier was crucial to the structure determination of the whole fragment (Huiskonen et al., 2003). The trimeric structure consists of a globular knob domain and a shaft. A DALI search revealed a striking similarity between the knob domain and the TNF-α fragment.

While atomic models for the component structures P2 (Xu et al., 2003) and P5 are now available, their spatial relationship in the complex remains unknown. We have developed a novel method to study symmetry-mismatched objects from cryo-electron microscopy data (Briggs et al., 2005). This method should yield a low resolution three-dimensional shape of the spike complex, where the atomic models of the component structure could be docked. This is turn should provide insight into the function of this receptor binding complex.

Abrescia, N. G., Cockburn, J. J., Grimes, J. M., Sutton, G. C., Diprose, J. M., Butcher, S. J., Fuller, S. D., San Martin, C., Burnett, R. M., Stuart, D. I., et al. (2004). Insights into assembly from structural analysis of bacteriophage PRD1. Nature 432, 68-74.

Briggs, J. A., Huiskonen, J. T., Fernando, K. V., Gilbert, R. J., Scotti, P., Butcher, S. J., and Fuller, S. D. (2005). Classification and three-dimensional reconstruction of unevenly distributed or symmetry mismatched features of icosahedral particles. J Struct Biol 150, 332-339.

Huiskonen, J. T., Laakkonen, L., Toropainen, M., Sarvas, M., Bamford, D. H., and Bamford, J. K. (2003). Probing the ability of the coat and vertex protein of the membrane-containing bacteriophage PRD1 to display a meningococcal epitope. Virology 310, 267-279.

Merckel, M. C., Huiskonen, J. T., Bamford, D. H., Goldman, A., and Tuma, R. (2005). The structure of the bacteriophage PRD1 spike sheds light on the evolution of viral capsid architecture. Mol Cell 18, 161-170.

Xu, L., Benson, S. D., Butcher, S. J., Bamford, D. H., and Burnett, R. M. (2003). The receptor binding protein P2 of PRD1, a virus targeting antibiotic-resistant bacteria, has a novel fold suggesting multiple functions. Structure (Camb) 11, 309-322.

Structural requirements of integrin outside-in signaling in multivalent ligand binding

Johanna Jokinen[1], Liisa Nissinen[1], Daniel J. White[2], Santeri Puranen[3], Jyrki Heino[4]

[1] MediCity Research Laboratory, University of Turku, [2] Department of Biological and Environmental Science, University of Jyväskylä, [3] Department of Biochemistry and Pharmacy, Åbo Akademi University, [4] Department of Biochemistry and Food Chemistry, University of Turku

Integrins are a structurally elaborate family of adhesion molecules that transmit bidirectional signals across the plasma membrane by undergoing large-scale structural rearrangements. Integrin mediated cell-cell and cell-extracellular matrix contacts lead to the activation of intracellular signaling pathways resulting in a wide range of biological functions from development to tissue repair and homeostasis. Integrin $\alpha2\beta1$ is an abundant collagen receptor expressed on many mesenchymal and epithelial cell types. We have studied its signaling mechanisms and several observations have indicated a crucial role for p38 mitogen activated protein kinase pathway. Here, we provide further insights into the requirements of $\alpha2\beta1$ mediated outside-in signaling elicited by a multivalent ligand.

p38 activation was studied in human osteosarcoma and Chinese hamster ovary cells transfected to express wild type $\alpha2\beta1$ integrin or $\alpha2\beta1$ integrin with either a mutant $\alpha2$ cytoplasmic domain or an $\alpha2$I domain substitution mutation Glu-336-Ala. The p38 pathway was triggered with collagen or with secondary antibody induced integrin clustering. The phosphorylation level of p38 was determined by Western blot analysis.

The C-terminal $\alpha7$-helix of the $\alpha2$I domain and the linker connecting it to the $\beta1$ I-like domain are proposed to be crucial for integrin activation. Glu-336, located in the linker region, is a conserved amino acid in vertebrate integrins containing αI domain. Mutation of this residue has been shown to prevent the conformational change needed for αL$\beta2$ integrin ligand binding (Yang *et al.* 2004, *Proc. Natl. Acad. Sci.* 101, 2906-2911). Here, we show that both collagen and antibody-mediated clustering of the $\alpha2\beta1$ integrin induced a rapid phosphorylation of p38 that was dependent on the $\alpha2$ integrin cytoplasmic tail. However, when the Glu-336-Ala mutation was introduced into the $\alpha2$I domain, the phosphorylation promoted by $\alpha2\beta1$ integrin was substantially diminished. Thus, the activation of p38 seems to require the conformational change in $\alpha2\beta1$ integrin induced by a multivalent ligand, and integrin clustering alone is not sufficient for generating this signaling.

Three-dimensional reconstructions of the virion and core of bacteriophage Phi8

Harri Jäälinoja[1], Juha Huiskonen[1], John Briggs[2], Vibhor Kumar[1], Leonard Mindich[3], Stephen Fuller[1], Dennis Bamford[1], Sarah Butcher[1]

[1] Institute of Biotechnology, University of Helsinki, [2] Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Headington, Oxford, OX3 7BN, UK, [3] Department of Microbiology, PHRI, 225 Warren Street, Newark, NJ 07103, USA

Phi8 belongs to the Cystoviridae family of dsRNA bacteriophages. The members of the family have in common a virion core particle that packages, encapsulates and transcribes the genome. The complete genomes of Phi8 and its relatives Phi6 and Phi13 have been sequenced, and comparison of the genomes shows that Phi6 and Phi13 are more closely related to each other than to Phi8.

In Phi6, the core and a surrounding T=13 layer of protein P8 constitute a nucleocapsid that is in turn enveloped by a lipid membrane1. In viral entry, the virus membrane fuses with the host cell outer membrane, and the nucleocapsid is released into the periplasm. P8 mediates the entry of the core through the inner membrane into the cytoplasm2. In contrast, the Phi8 P8 is a membrane-associated protein. The core alone can infect sphaeroplasts 3, so it is unclear whether or not there is a T=13 layer in Phi8.

Here we report the cryo-EM reconstruction of the Phi8 virion to 28 Å resolution, revealing the multilayered nature of the virion. An 8.5Å resolution reconstruction of the core shows that it consists of an icosahedral T=1 shell formed of asymmetric dimers with hexamers of the packaging protein P4 at the vertices. We developed a method4 to reconstruct the hexamers on the core, to see the relationship of the P4 to the T=1 shell despite the symmetry mismatch.

1. Butcher, S.J., Dokland, T., Ojala, P.M., Bamford, D.H. & Fuller, S.D. Intermediates in the assembly pathway of the double-stranded RNA virus ϕ6. EMBO J. 16, 4477-4487 (1997).
2. Poranen, M.M., Daugelavicius, R., Ojala, P.M., Hess, M.W. & Bamford, D.H. A novel virus-host cell membrane interaction. Membrane voltage-dependent endocytic-like entry of bacteriophage phi6 nucleocapsid. J Cell Biol 147, 671-82 (1999).
3. Sun, Y., Qiao, X., Qiao, J., Onodera, S. & Mindich, L. Unique properties of the inner core of bacteriophage phi8, a virus with a segmented dsRNA genome. Virology 308, 354-61 (2003).
4. Briggs, J.A.G. et al. Classification and three-dimensional reconstruction of unevenly distributed or symmetry mismatched features of icosahedral particles. Submitted to J Struct Biol.

Homologous polyketide cyclases SnoaL and AknH: implications for catalytic mechanism and product stereoselectivity

Pauli Kallio[1], Azmiri Sultana[2], Jarmo Niemi[1], Pekka Mäntsälä[1], Günter Schneider[2]

[1] Department of Biochemistry and Food Chemistry, University of Turku,

[2] Department of Medical Biochemistry and Biophysics, Karolinska Institutet

Aromatic polyketides form a diverse group of naturally occurring bioactive compounds, majority of which are secondary metabolites of gram-positive soil bacteria of the genus *Streptomyces*. Certain aromatic polyketides, namely antracyclines, have commercial and medical significance due to their antitumoral properties and applications in cancer therapy. Polyketide antibiotics have been intensively studied since the late 60's until today, and over time focus has shifted from the search for novel natural compounds by simple screening of soil samples towards detailed understanding of the genetics and the enzymology of the biosynthetic pathways.

AknH and SnoaL are small homologous polyketide cyclases which catalyse the closure of the fourth carbon ring in the biosynthesis of aclacinomycin (*S. galilaeus*) and nogalamycin (*S. nogalater*), respectively. Detailed comparison of these two enzymes reveals a high level of similarity at structural as well as mechanistic level. This is also apparent at functional level as AknH can convert the natural substrate of SnoaL, nogalonic acid methyl ester, *in vitro* and in constructs *in vivo*. However, despite the highly conserved features between the enzymes, the reaction products are stereochemically clearly distinct. AknH product has a C9-R stereochemistry common to most aromatic polyketides, whereas the SnoaL product has the opposite C9-S stereochemistry. High-resolution crystal structure comparison, combined with mutagenesis and stereospecific functional studies, reveal two active site amino acids which are involved in determining the product stereoselectivity. Substitution of AknH residues Tyr15 and Asn51 by the respective SnoaL residues (Phe15 and Leu51) results in a complete loss of stereoselectivity, yielding a racemic mixture of the two stereoisomers as reaction products.

The crystal structure and detailed mechanistic features of SnoaL have been published earlier (Sultana et al 2004), followed by a comparative study of the enzymes, revealing factors involved in the stereochemical differences between the products of the two enzymes (JMB submitted November 2005).

Sultana, A., Kallio, P., Jansson, A., Wang, J-S., Niemi, J., Mäntsälä, P. and Schneider, G. (2004) Structure of the polyketide cyclase SnoaL reveals a novel mechanism for enzymatic aldol condensation EMBO J. 5;23(9):1911-21.

BioImageXD – A new tool for 4-dimensional bioimaging

Pasi Kankaanpää, Kalle Pahajoki, Jyrki Heino, Varpu Marjomäki, Daniel White

Department of Biochemistry and Food Chemistry, University of Turku

Advanced four dimensional (3 spatial dimensions + time) imaging is one of the most powerful techniques in the modern era of informational biology. It is becoming increasingly important to visualize biomolecules and other elements in living cells in research ranging from the elucidation of the structure and function of proteins to the development of new drugs and gene therapeutics. There is a myriad of advanced multidimensional imaging techniques already available and in frequent use – examples include confocal, electron and atomic force microscopies. However, irrespective of the target of the imaging and the technique used, special computer software is required for the proper visualization, interpretation and analysis of the results. Existing (commercial) programs are often very expensive, rigid, function inadequately and use undisclosed algorithms. We have developed new software, named BioImageXD, as a solution to these problems.

A key principle with BioImageXD is that it offers nearly everything necessary for the processing, analysis and visualization of multidimensional image data in one software package, and that this package has open source code. As the name implies, it is primarily intended for biological data, but is fully suitable for working with any kind of images. The software is developed by a multidisciplinary group of scientists from the Universities of Turku and Jyväskylä, with collaborators world wide. Collaboration is also being started with several Finnish companies and some major international companies working in the field of bioimaging. BioImageXD is written in Python and C++, with wxPython being used for the graphical user interface (GUI) and the Visualization Toolkit (VTK) for image processing and rendering.

Current plans for the development of BioImageXD extend to the year 2009, but the software already has a wealth of features and functions. It has a simple, intuitive user interface based on a single large window, and its operations have been designed to be consistent and functional. The software offers advanced file input/output functions and multiple visualization modes (including several 3D rendering algorithms), that can be freely combined with any of the numerous task modes (including channel merging, colocalization analysis, intensity correction and noise filtering). Results can be finalized and stunning movies created with the built-in animator that allows full control of

camera paths, viewing angles, time point changes etc. BioImageXD utilizes numerous previously published algorithms as well as new algorithms that we have created. In all of them, all relevant parameters are adjustable, and unlike with commercial programs, the user can always know precisely what is done to the data, what kind of algorithms are used, and change them if he so wishes.

BioImageXD can rival and even out-perform commercial competitors, and we believe it has the potential to become a globally used tool in advanced bioimaging. It is free and operating system independent and comes with a comprehensive manual, so basically any scientist in need of this type of software should be able to utilize it. Indeed, it has garnered considerable attention already, prior to publication. The first beta version is slated for release soon.

LC/MS data normalization using a learning method involving multiple internal standard compounds

Mikko Katajamaa[1], Tuulikki Seppänen-Laakso[2], Matej Oresic[2]

[1] Turku Centre for Biotechnology, University of Turku and Åbo Akademi University ,
[2] VTT Biotechnology

One of the biggest problems in differential profiling using any of the modern high-throughput technologies is to differentiate between effects with biological relevance and those that are result of technical imperfection in the measurement process. In the case of liquid chromatography coupled to mass spectrometry (LC/MS) technology, the unwanted variation is usually introduced during the manual sample preparation stages or by changes in the instrument performance. Normalization methods aim at removing any technical variation within a measurement and between multiple measurements while leaving all biological variability untouched.

We present a new normalization method for LC/MS data, which is based on multiplicative error model and covariance structure of the internal standard profiles. This approach assumes that the contribution of each internal standard to the individual peak normalization can be determined from a prior repeatability study where a typical sample (e.g. serum) with internal standards is run multiple times to calculate the covariance between the internal standards and with the other peaks.

We have conducted an experiment to demonstrate two steps of the normalization method: 1) estimation of model parameters from a set of repeated measurements on a single sample, and 2) utilization of the model to normalize peak intensities in a profiling experiment involving similar types of samples. We also use data from the first step to point out the need for LC/MS data normalization in a differential profiling scenario.

Implementation of the new normalization method will be made available in a future release of the *MZmine* toolbox for processing of LC/MS profile data.

Structural studies on Dps-like peroxide resistance protein Dpr from Streptococcus suis

Anni Kauko[1], Arto Pulliainen[2], Sauli Haataja[2], Jukka Finne[2], Anastassios C. Papageorgiou[1]

[1] Turku Centre for Biotechnology, University of Turku and Åbo Akademi University,
[2] Department of Medical Biochemistry and Molecular Biology, University of Turku

Pathogens have developed a variety of defence mechanisms to survive in the presence of high concentration of reactive oxygen species, such as hydrogen peroxide secreted by the immune system of the host. Dps-like peroxide resistance protein (Dpr) from the meningitis-associated bacterium *Streptococcus suis* belongs to the Dps-family, which is widely spread among prokaryotes. Most Dps-family members are involved in peroxide resistance and iron binding. Peroxide resistance is achieved by the removal of Fe^{2+} from the cytoplasm; if present, Fe^{2+} degrades H_2O_2 to highly toxic hydroxyl radicals by Fenton reaction. Members of the Dps-family are characterised by a common overall structure of a hollow sphere formed by 12 identical four α-helix bundles similar to those of ferritin monomers. Similarly to ferritins, Dps-proteins form an iron core inside the protein shell. Twelve putative ferroxidase centres have been identified in the inner surface of the sphere and in clefts formed at the interface of neighbouring monomer. To elucidate the mechanistic details of the Dpr's function, multidisciplinary studies are indispensable.

We have previously characterised the iron-free and iron-bound structure of Dpr at 1.95 Å and 1.8 Å resolution, respectively. In the iron-free structure, another metal, probably Ca^{2+} was found $\sim$ 6 Å from the active site. Upon iron binding, Ca^{2+} leaves and three active site residues, Asp74, Glu78 and Asp63 change their conformation. These changes demonstrate some flexibility of the active site and may suggest different stages of iron binding.

We have recently initiated characterisation of the iron core using X-ray absorption spectroscopy (XAS). Preliminary results have shown the presence of a ferrihydrate structure similar to that found in ferritins. The conditions however, under which the core is formed appear to affect its stoichiometrical composition. Detailed analysis is currently underway.

Phylogenetic analysis of three structurally related amino acid decarboxylases

Heidi Kidron, Susanna Repo, Mark S. Johnson, Tiina A. Salminen

Department of Biochemistry and Pharmacy, Åbo Akademi University

Aliphatic polyamines are involved in growth and developmental processes of many organisms. The biosynthesis of putrescine, the precursor of other polyamines, is different in animals, plants and bacteria. In animals there seems to be only one pathway for putrescine biosynthesis: the direct synthesis of putrescine from L-ornithine by ornithine decarboxylase (ODC). In plants and bacteria there is an indirect additional pathway to putrescine, where L-arginine is first decarboxylated to agmatine by arginine decarboxylase (ADC). In plants agmatine is then modified in a two-step process to produce putrescine, but in bacteria agmatine is directly converted to putrescine. Both enzymes, ODC and ADC, belong to the alanine racemase structural family, which in addition to alanine racemase consists of eukaryotic ODC, prokaryotic biosynthetic ADC and diaminopimelate decarboxylase (DapDC). The sequence identity between the amino acid decarboxylases is relatively low (20-30%), but they are all pyridoxal-5'-phosphate (PLP) dependent enzymes that function as dimers. We have collected protein sequences from the alanine racemase structural family of amino acid decarboxylases and constructed a multiple sequence alignment. Based on this alignment we have inferred phylogenetic trees, which provide understanding of the evolutionary relationships within the enzyme group.

Improving the performance of SOMFA by use of standard multivariate methods

Samuli-Petrus Korhonen, Kari Tuppurainen, Reino Laatikainen, Mikael Peräkylä

Department of Chemistry, University of Kuopio

Self-Organizing Molecular Field Analysis (SoMFA) comes with a built-in regression methodology, the Self-Organizing Regression (SOR), instead of relying on external methods such as PLS. However, it can be proven that SOR is equivalent to SIMPLS and NIPALS with one principal component. Therefore the modest performance of SOMFA on complex datasets can be primarily attributed to the low performance of the SoMFA regression methodology. In order to improve the performance of SoMFA, a multi-component extension of the original SOR methodology (MCSOR) is introduced, and the performances of SOR, MCSOR and SIMPLS are compared using several datasets including a large and diverse xenoestrogen dataset. The effect of polarizibility descriptor and two superposition techniques on the predictive ability of SoMFA is also evaluated. The results indicate that in general the performance of SoMFA models is greatly improved if SOR is replaced with a more sophisticated regression method. On the other hand no clear difference was observed between the two superposition techniques. The polarizability descriptor generated predictive models as a stand-alone descriptor but clearest improvement in the accuracy of the prediction is achieved when the polarizability descriptor is combined with the electrostatic field descriptor. Also, the behaviour of the Cramer (CBG) dataset further underline the fact that it is a very poor benchmark dataset and should not be used to evaluate the performance of QSAR techniques.

Characterisation of Recombinant Human Neutral α-Mannosidase

Elina Kuokkanen[1], Heidi Tuominen[1], Marika Mäkinen[2], Wesley Smith[2], Sandrine Duvet[3], Eija Jokitalo[2], Pirkko Heikinheimo[2]

[1] Department of Biochemistry and Food Chemistry, University of Turku, [2] Institute of Biotechnology, University of Helsinki, [3] Unite de Glycobiologie Structurale et Fonctionnelle, Universite des Sciences et Technologies de Lille

Human neutral α-mannosidase (NAM) hydrolyses soluble sugars containing nine mannose residues and one N-acetylglucosamine ($Man_{6-9}GlcNAc$). These oligosaccharides either originate from misfolded proteins or are side products of N-glycosylation. NAM is mostly regarded as a cytosolic enzyme but the activity has also been reported from ER and blood circulation. Its sequence belongs to a glycoside hydrolase family 38 (GH38) and like other family members, NAM hydrolyses the artificial PNP-α-D-mannoside, but distinctively using cobalt and with the pH optimum close to 7. The well-known lysosomal and Golgi GH38 α-mannosidases are key cellular enzymes with medical importance. NAM is less known, but possibly has a role in symptoms of lysosomal α-mannosidosis and certain types of cancer. GH38 enzymes in general have been difficult to heterologously over express and NAM has also been unstable and tricky to purify. It has been isolated from several different organisms, but a true characterisation has suffered from contaminating activities from other cellular GH38 enzymes. In order to structurally and functionally characterize this enzyme, we have expressed and purified the recombinant human NAM.

We have cloned a cDNA for human NAM from its native source and expressed the N-terminally His-tagged NAM intracellularly in *Pichia pastoris*. From the yeast culture, NAM can be purified by Co^{2+} affinity chromatography and proteolytic cleavage of the metal affinity tag. Initial characterisation by gel filtration shows a molecular weight of 440 kDa and a tetrameric structure. Since the characterised GH38 members are metalloproteins we studied the effect of different cations for hydrolysis of $Man_9GlcNAc$. It appeared that Co^{2+} and Fe^{2+} are strong and activators, Mn^{2+} is weaker and Cu^{2+} totally blocks the reaction. With Co^{2+} and Fe^{2+} NAM cuts off five mannose residues from the substrate but even without any activators it is able to release one mannose from the substrate. We also report that the cloned NAM hydrolyses $Man_9GlcNAc$ substrates much faster than $Man_9GlcNAc_2$, providing a natural selection mechanism towards the catabolic break down pathways and

protecting the native protein glycosylation. Further characterisation and metal ion usage with the artificial PNP-substrate will also be shown.Another part of the NAM characterisation concerns studies of the subcellular localisation. By immuno-electron microscopy and confocal microscopy we attempt to find an unambiguous answer for the question whether NAM is solely a cytosolic protein or also located in ER.

Crystal structure of a glycyl radical enzyme from Archaeoglobus fulgidus

Lari Lehtiö[1], Günter J. Grossmann[2], Bashkim Kokona[3], Robert Fairman[3], Adrian Goldman[1]

[1] Institute of Biotechnology, University of Helsinki, [2] CLRC Daresbury Laboratory, United Kingdom, [3] Department of Biology, Haverford College, USA

We have solved the crystal structure of a PFL2 from *Archaeglobus fulgidus* at 2.9 Å resolution. Of the three previously solved enzyme structures of glycyl radical enzymes, pyruvate formate lyase (PFL), anaerobic ribonucleotide reductase and glycerol dehydratase (GD), the last one is clearly most similar to our model. We observed an electron density in the active site of PFL2, which we modelled as glycerol.

Although the orientation of the glycerol is different than that in GD, overall active site composition suggests that PFL2 is a dehydratase. The actual substrate of PFL2 is perhaps a slightly bigger molecule than glycerol. Crystal packing, small angle scattering and ultracentrifugation experiments show that PFL2 is tetrameric protein unlike other glycyl radical enzymes. *A. fulgidus* is a hyperthermophile and PFL2 appears to be stabilized by several factors including increased number of ion pairs, differences in buried charges, a truncated N-terminus, anchoring of loops and N-terminus via salt bridges, changes in the oligomeric interface and perhaps also the higher oligomerization state of the protein.

Enzymatic mechanism of bacteriophage phi12 RNA packaging motor: Cooperativity of ATP hydrolysis and RNA translocation

Jiri Lisal, Roman Tuma

Institute of Biotechnology, University of Helsinki

P4 is a hexameric ssRNA packaging motor of dsRNA bacteriophages from Cystoviridae family. P4 shares sequence and structural similarities with hexameric helicases. Structure based mechanism of single translocation step has been recently proposed but coordination of ATP hydrolysis and RNA translocation within the hexamer remains elusive. Here we present detailed enzymatic study of nucleotide binding, hydrolysis and product release by phi12 P4 in the presence of different RNA and DNA substrates. Binding affinities for ATP and ADP are independent of RNA binding. The force generating step appears to be the ATP hydrolysis. ATP hydrolysis is cooperative involving at least three neighboring P4 subunits. Similarly, three neighboring subunits have to bind to RNA substrate in order to achieve translocation. Binding of nucleotide by neighboring subunits does not change the subunit affinity for ATP but it increases the rate of ATP hydrolysis. The mechanism of ATP hydrolysis coordination during RNA translocation is delineated. "Stochastic cooperativity" model consistent with all the experimental data is introduced. In the absence of RNA, ATP hydrolysis requires the coincidence of two random events - binding of three ATP molecules next to each other, and the right conformation of subunits. Binding of RNA presets the subunits in the conformation suitable for hydrolysis, when ATP hydrolysis by one subunit stimulates ATP hydrolysis by the following subunit.

Quantitative LC-MS of Cyanotoxins and their Metabolites in Animal and Human Samples

Milla-Riina Lähde, Jussi Meriluoto

Department of Biochemistry and Pharmacy, Åbo Akademi University

Cyanobacteria (blue-green algae) produce hundreds of bioactive compounds, many of which are peptides with enzyme inhibition activities. Problems caused by toxic cyanobacteria, e.g. related to the production of safe drinking water, are encountered worldwide. Many cyanotoxins are also capable of bioaccumulation. The main toxins of freshwater cyanobacteria, cyclic peptides called microcystins, are hepatotoxins and tumour promoters. The presence of microcystins in drinking water has been recognized as a human health hazard by the World Health Organization. Chronic exposure is believed to promote liver tumour formation, and microcystins are risk factors in the development of liver cancer.

The project will develop validated quantitative liquid chromatography - mass spectrometry (LC-MS) detection of cyanobacterial peptide hepatotoxins and their metabolites present in animal and human tissues and biofluids. In addition, immunostaining of toxins in tissue biopsies is planned.

Elucidating cell signalling pathways leading to the development of T helper cell subset 2 phenotype

Tapio Lönnberg[1], Riitta Lahesmaa[1], Matej Oresic[2]

[1] Turku Centre for Biotechnology, University of Turku and Åbo Akademi University ,
[2] VTT Biotechnology

During the first years of the 21st century, we have witnessed an unprecedented flood of biological data, resulting mainly from various genome sequencing efforts and high throughput gene expression profiling studies. However, at the same time it has become increasingly evident that cellular functions, being ultimately determined by interactions between gene products, cannot be extrapolated from this data alone. Investigating protein interactions, and signalling pathways formed by them, has proved to be an efficient way of exploring complex cellular processes.

One of the key areas of current signal transduction research is the process by which T helper lymphocytes differentiate into functionally distinct Th1 and Th2 subpopulations. In addition to representing a general model of cellular decision-making, the process holds exceptional medical significance; the involvement of defective T helper cells in the pathogenesis of atopic allergies and various autoimmune diseases has been widely documented. Better understanding of the development of the Th1 and Th2 subsets will hopefully lead to new diagnostic methods and therapies for these disorders.

Our goal is to uncover and characterise new cell signalling pathways that are involved in the lineage commitment of T helper cells. The basis of the research consists of coaffinity-purification of protein complexes and protein identification with mass spectrometry. To maximize accuracy and coverage of these studies, we are applying several complementary methods, including well established ones, such as GST-pulldowns, together with more novel methods, such as strep-tag purification, that has only recently been applied in mammalian cells. Interactions are studied in both primary human T cells and T cell lines, using both endogenous protein populations and tagged fusion proteins. New findings are further analysed with functional methods, focus being on the role of post-translational modifications, and ultimately integrated into a framework of existing knowledge to make a pathway map of Th2-specific signal transduction.

Transient kinetics of membrane-bound pyrophosphatase from Thermotoga maritima

Anssi Malinen, Georgiy Belogurov, Reijo Lahti

Department of Biochemistry and Food Chemistry, University of Turku

Membrane-bound pyrophosphatase of the hyperthermophilic bacterium *Thermotoga maritima* (Tm-PPase), a homologue of H^+-translocating pyrophosphatase, has an absolute requirement for Na^+ but displays the highest activity in the presence of millimolar levels of both Na^+ and K^+. In this study, we employ pre-steady-state kinetics to elucidate the details of Tm-PPase activation by these cations. Quenched-flow analysis is used to resolve substrate binding, pyrophosphate (PP_i) cleavage and product release steps in millisecond time scale. The rate constants for individual reaction steps will be determined at different concentrations of Na^+ and K^+ for both wild-type and variant enzymes containing a reduced number of Na^+-binding sites, allowing identification of the reaction steps controlled by these cations and their role in catalysis.

Our preliminary data define the chemical PP_i cleavage step as solely rate-determining in the catalytic mechanism. This is in contrast to the well-known soluble PPases in which the overall rate is a combination of both PP_i hydrolysis and product release steps. In the absence of an obligatory activator Na^+, Tm-PPase is able to bind substrate but not to catalyse its cleavage. Also the optional activator K^+ operates by directly increasing the rate of PP_i cleavage step. The catalytic mechanism has a strong forward commitment in the presence of K^+ but weaker in its absence as substrate dissociation rate from the active site is increased at least 5-fold in absence of K^+. K^+ has only a minor effect on substrate binding rate and Michaelis constant K_m. Altogether the transient kinetic data indicate that the main reaction step controlled by the activating cations Na^+ and K^+ is substrate cleavage step. This is further supported by our studies of the temperature dependence of maximal reaction rate that may indicate K^+ binding to Tm-PPase to tune the catalysis to follow a different, energetically more favourable, reaction coordinate.

Towards dual ligand avidin

Juha Määttä[1], Vesa Hytönen[2], Tiina Paldanius[1], Henri Nordlund[1], Markku Kulomaa[1]

[1] Institute of Medical Technology, University of Tampere, [2] Department of Materials, ETH Zurich, Hönggerberg, CH-8093 Zürich, Switzerland

The homotetrameric chicken avidin from the egg-white is well characterised protein showing extremely high affinity (K_d=10^{-15}) to a water-soluble vitamin, biotin. Avidin is widely used tool in applications ranging from life sciences to nanotechnology, and a subject of numerous research projects. Up to date, different kinds of variations of avidin have also proved to have potential in applications. Since the crystal structure of avidin (and some of its mutants) is solved, the rational modification of ligand-binding site has become feasible (Livnah et al. 1993).

Besides biotin, avidin binds several different molecules, such as azo dye 4-hydroxyazobenzene-2-carboxylic acid (HABA). Avidin binds HABA with moderate affinity (K_d= 6 x 10^{-6} M). Cleavage of loop connecting β-strands 3 and 4 in avidin by proteinase K has been suggested to enhance the binding of HABA to avidin. The reason for improved affinity is described by increased solvent accessible surface and space for HABA molecule in binding site (Ellison et al. 1995).

We created circularly permuted avidin, where loop between β-strand 3 and 4 (cp43Avd) was cleaved and partially deleted, and tested its capability to bind HABA and biotin. Furthermore, we joined this novel avidin circularly permuted form in the context of previously published dual chain avidin as a fusion with other circularly permuted avidin cp54Avd (Nordlund, et al. 2004) and studied its properties. Advantage of dual chain avidin is that fusion of circularly permuted forms, which are joined covalently in a polypeptide chain, makes it possible to independently modify binding sites within quaternary unit. Therefore, our aim is to employ this novel avidin scaffold to generate dual ligand avidin.

Affinities to both ligands, biotin and HABA, were measured and other physico-chemical properties were also analysed. Novel circularly permuted avidin (cp43Avd), indeed, showed increased affinity to HABA. Furthermore, it showed reduced affinity to biotin as compared to wild type avidin. Combination of cp43Avd with wt-like cp54Avd in dual chain avidin had also altered binding properties to the biotin and HABA. This strategy has previously showed to be successful when avidins with varying affinities for biotin between binding sites were generated (Hytonen et al. 2005).

These kinds of dual ligand avidins could have potential use in nanoscale methods as self-organising structures and they also provide possibilities to develop more efficient methods in traditional biotechnology.

Ellison, D., Hinton, J., Hubbard, S. J. and Beynon, R. J. (1995) Limited proteolysis of native proteins: the interaction between avidin and proteinase K. Protein Sci. 4, 1337-145.
Hytonen, V. P., Nordlund, H. R., Horha, J., Nyholm, T. K., Hyre, D. E., Kulomaa, T., Porkka, E. J., Marttila, A. T., Stayton, P. S., Laitinen, O. H. and Kulomaa, M. S. (2005) Dual-affinity avidin molecules. Proteins in press.
Livnah, O., Bayer, E. A., Wilchek, M. and Sussman, J. L. (1993) Three-dimensional structures of avidin and the avidin-biotin complex. Proc. Natl. Acad. Sci. U. S. A. 90297, 5076-5080.
Nordlund, H. R., Laitinen, O. H., Hytönen, V. P., Uotila, S. T., Porkka, E. and Kulomaa, M. S. (2004) Construction of a dual chain pseudotetrameric chicken avidin by combining two circularly permuted avidins. J. Biol. Chem. 279, 36715-36719.

Mononuclear cells from patients with type 1 diabetes have altered response to IL-12 and IL-4, the key cytokines in the regulation of Th cell differentiation.

Tuomas Nikula

Turku Centre for Biotechnology, University of Turku and Åbo Akademi University

Type 1 diabetes mellitus (T1D) is the most common metabolic-endocrine disorder in children in western countries and is caused by T-cell-mediated autoimmune attack against insulin producing pancreatic β-cells in the islets of Langerhans. The molecular mechanisms underlying the disease are not known. Our hypothesis is that individuals who develop T1D diabetes have an altered response to IL-12 and/or IL-4, the key cytokines in the regulation of Th cell differentiation.

To test the hypothesis experimentally cytokine response of peripheral blood mononuclear cells isolated from T1D patients and controls was studied. Lithium-heparin blood was collected from 12 patients who have developed T1D before the age of five years and matched control samples (date of birth, sex, location of birth, HLA risk group). Peripheral blood mononuclear cells were isolated with gradient centrifugation (Ficoll-Paque PLUS) and macrophages were attached on a plate. Non-adherent cells were cultured for 48 h in the presence of PHA and cytokines (IL-12 or IL-4). Total RNA from cytokine stimulated T cells and untreated samples were hybridized on Affymetrix U133A oligonucleotide arrays for gene expression analysis. Conventional gene expression analysis, analysis on a pathway level and learning algorithms were used to compare the cytokine induced gene expression in T1D patients and controls.

The results indicate that cells isolated from patients with T1D have altered response to both IL12 and IL4 as compared to cells isolated from controls. The study was repeated with a new collection of cells from 5 T1D patients and their controls and the results of the first experiment were confirmed. The results are consistent with our hypothesis. The studies in progress aim at elucidating the molecular mechanisms and candidate genes responsible for the altered cytokine response characteristic for T1D.

Functional hetero-oligomerization among the human UDP-glucuronosyltransferases may attenuate the consequences of the pathological Y486D mutation

Anne-Sisko Patana[1], Mika Kurkela[2], Adrian Goldman[1], Moshe Finel[2]

[1] Institute of Biotechnology, University of Helsinki, [2] Viikki Drug Discovery Technology Center, University of Helsinki

The pathological Y486D mutation in the human UDP-glucuronosyltransferases (UGTs) affects all the 9 UGTs of subfamily 1A in individuals who are homozygous carriers for it. In recombinant enzymes that were produced in baculovirus-infected insect cells, this mutation lowered the scopoletin glucuronidation activities of UGTs 1A1 and 1A6 (where it is Y485D) by about 89% and 96%, respectively. UGT1A4 is virtually inactive in scopoletin glucuronidation and coinfecting insect cells with the mutated UGT1A1 (1YD) together with UGT1A4 had no significant effect on the scopoletin glucuronidation activity. In contrast, coinfecting the mutated UGT1A6 (6YD) with 1A4 greatly increased the normalised glucuronidation rate of scopoletin in comparison to infection with 6YD alone. Among the human UGTs, serotonin glucuronidation is almost exclusively catalysed by UGT1A6 and the Y to D mutation decreased this activity by about 99%. Subsequently, we have examined the capacity of 14 different recombinant human UGTs, namely 1A1, 1A3-1A10, 2B4, 2B7, 2B10, 2B15, 2B17 and 2B28, to affect the serotonin glucuronidation activity upon coinfection with the 6YD. The results of this hetero-oligomerization screen revealed that all the tested UGTs, apart from UGT2B28, significantly increased the serotonin glucuronidation activity with respect to the expression level of 6YD (normalised activity), albeit at variable efficiency. Similar functional hetero-oligomerization may also occur in carriers of the Y486D mutation, protecting them against more severe consequences than the moderate elevation of unconjugated bilirubin level in the serum.

Structural comparison of integrin receptors may reveal the basis of α/β partner selection

Santeri Puranen[1], Daniel J. White[2], Jyrki Heino[3], Mark S. Johnson[1]

[1] Department of Biochemistry and Pharmacy, Åbo Akademi University, [2] Cell Biology, Department of Biological and Environmental Science, University of Jyväskylä, [3] Department of Biochemistry and Food Chemistry, University of Turku

Integrins are heterodimeric cell surface receptors involved in adhesion to components of the extracellular matrix, as well as to receptors on other cells. This function is accompanied by elaborate signaling (both inside-out and outside-in signals, with respect to the cellular membrane).

In man, eighteen α subunits and eight β subunits have been observed to form 24 different receptors. While some subunits are promiscuous in terms of dimer-formation (such as αV, β1 and β2) others form only very specific pairs. The structural basis of partner selection is not clear. There are clear indications that there may be a hierarchy in the formation of the heterodimers: not all α/β combinations are equally favored, or even allowed, since distinct partners may bind to the promiscuous subunits with different affinities.

The phylogeny of the integrins does not correlate with their pair-forming preferences. Our statistically founded method of predicting potential interactions of proteins, in this case the structural models of all integrin α subunits, has yielded similar clustering as the phylogenetic methods. Our hypothesis is, however, that structural features will provide an explanation for the reported pair-forming preferences of integrins, although the involvement of other mechanisms for regulating dimer formation cannot yet be ruled out.

The case may be that only a few amino acid residues located in a large interface are responsible for either allowing or preventing the formation of a dimer. Support for this idea can be found e.g. in diseases such as Glanzmann thrombasthenia, where single residue mutations adversely affect αIIbβ3 dimer formation. Patterns of this type would not be detected by phylogenetic methods or the predicted interactions-based clustering and could easily be interpreted merely as noise during subjective analysis. Our future strategies therefore include fine-tuning of property mapping-based clustering for detecting more subtle structural patterns and the use of equilibrium ensembles of molecules for more realistic modeling of interactions.

This research was supported by Academy of Finland, Tekes, Sigrid Juselius Foundation and ISB.

Structure of coxsackievirus A9 by cryo-EM and single particle reconstruction and structural differences with coxsackievirus B3

Jani Seitsonen

Institute of Biosciences, University of Helsinki

Coxsackievirus A9 is a member of the picornaviridae family and a common human pathogen. Aminoacid sequence alignments have shown that coxsackievirus A9 is over 90% similar to coxsackievirus B3. The high correlation of sequences indicates that the structure of these two viruses should also be quite similar. I solved the structure of coxsackievirus A9 to a resolution of 1.2nm using cryo-EM and single particle reconstruction. Comparison of x-ray model of coxsackievirus B3 with the cryo-EM model of coxsackievirus A9 showed that structures of these two viruses are actually very similar. Differences between the two viruses were determined by calculating difference maps of the two viruses and comparing the differences with the two models. By using the difference maps it was possible to isolate the locations in the coxsackievirus B3 aminoacid sequence that correspond to the differences between the structures of these two viruses.

Multimerisation of hydrophobins in solution determined with FRET

Géza Szilvay, Tiina Nakari-Setälä, Markus B. Linder

VTT Biotechnology

Filamentous fungi produce amphiphilic proteins called hydrophobins to manage with their surroundings interfacial forces [1]. Hydrophobins are surface active proteins that lower the surface tension of water to help fungal hyphae to grow from the water phase into the air. At air-water interfaces hydrophobins self-assemble to form ordered protein monolayers. The amphiphilicity of hydrophobins derives from hydrophobic and aliphatic amino acids on the surface of the protein that form a planar hydrophobic patch [2]. The hydrophobins HFBI and HFBII from *Trichoderma reesei* have been shown to form multimers in solution [3]. This multimerisation may enhance the solubility of hydrophobins by clustering the hydrophobic patches and reducing their exposure to water.

To enable site-specific chemical conjugation to hydrophobins we genetically engineered HFBI to have one additional Cys residue in the amino terminus yielding an NCys-HFBI variant. The introduced sulfhydryl can be specifically conjugated with a maleimide group containing molecule resulting in a stable thioether bond.

Förster resonance energy transfer (FRET) was used to study the homomultimer formation of fluorescently labelled NCys-HFBI monomers. FRET between monomers was concentration dependent and nonlabelled HFBI was able to decrease FRET between the labelled NCys-HFBI. The results show that the intermolecular association of labelled NCys-HFBI increases up to about 20 μM total hydrophobin concentration after which no appreciable increase in association can be seen. The method enables interaction studies between hydrophobins and other proteins or other molecules.

1. Linder, M. B., Szilvay, G. R., Nakari-Setälä, T., and Penttilä, M. (2005) *FEMS Microbiol Rev* In press.

2. Hakanpää, J., Paananen, A., Askolin, S., Nakari-Setälä, T., Parkkinen, T., Penttilä, M., Linder, M. B., and Rouvinen, J. (2004) *J Biol Chem* 279(1), 534-9

3. Linder, M., Szilvay, G. R., Nakari-Setälä, T., Söderlund, H., and Penttilä, M. (2002) *Protein Sci* 11(9), 2257-66

Identification of novel androgen receptor binding compounds using virtual screening

Annu Söderholm, Pekka Lehtovuori, Tommi Nyrönen

CSC - the Finnish IT Center for Science

Virtual screening (VS) can be defined as computational tools applied for selection of potential drug candidates from large databases of chemical compounds [1]. The objective with VS is to identify novel active compounds (leads) for a target protein and to weed out compounds that are unlikely to interact with the target. VS focuses research on the most promising compounds, thus reducing the costs and time needed for drug discovery projects.

We have performed VS of a chemical database to identify novel androgen receptor (AR) binding compounds. Initially, compounds that do not possess drug-like characteristics according to structural and physicochemical properties were filtered out from the database. Subsequently, 2D substructure search was performed on the reduced data set to identify compounds with specified structural moieties. Next the compounds containing e.g. reactive chemical groups were filtered out and the remaining compound set was submitted to a docking analysis. The selection of compounds for purchase and biological testing was primarily based on activity prediction with our previously generated 3D QSAR model of AR ligands [2].

Using the above described approach we chose 50 nonsteroidal compounds from a database of ca 220 000 compounds for *in vitro* testing. IC_{50} values below 3 μM were measured for 10 compounds, with the best compound having IC_{50} of 440 nM. Additionally, novel nonsteroidal core structures were identified. The results clearly demonstrate the value of 3D QSAR models as part of the VS procedure in finding new lead compounds for drug discovery projects.

[1] Böhm H-J and Schneider G Eds. *Virtual Screening for Bioactive Molecules.* Vol. 10 of *Methods and Principles in Medicinal Chemistry*, Wiley-VCH Verlag GmbH, Weinheim, 2000.

[2] Söderholm AA, Lehtovuori PT and Nyrönen TH. *J. Med. Chem.*, **48** (2005) 917-925.

Pharmacogenomic and chemoinformatic analysis of cancer chemotherapy response in vitro

Pekka Tiikkainen[1], Henrik Edgren[1], Rolf Skotheim[2], Antti Poso[3], Olli Kallioniemi[1]

[1] VTT, [2] Department of Genetics, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway, [3] Department of Pharmaceutical Chemistry, University of Kuopio

A key approach to improve future cancer therapies involves the identification of drugs that are specifically effective against genetically altered cancer cells. Such drugs could be prescribed to patients based on the information acquired from the analysis of tumour samples. We have launched an in vitro pharmacogenomic and chemoinformatic study to integrate cancer therapy response data in vitro with the molecular properties of cancer cells.

As a basis for this study, we used data on the response of a panel of 60 different cancer cell lines to 44,145 compounds. First, the compound structure data from SDF-files were used to calculate a set of 3D conformations. Based on the structures, a set of Almond descriptors were derived that describe compound's pharmacodynamic properties. Second, we acquired data on the expression levels (by gene expression microarrays) and genetic changes (by sequencing of key cancer genes and by copy number analysis using array-based comparative genomic hybridization). This forms an excellent base to study the relationship of genetics and transcriptomics with cancer cells' response to a large number of compounds. To our knowledge, this is the first time that such an integrated profiling of all kinds of molecular data are integrated with 3D compound structures and chemical parameters derived. Using this approach, we have completed the first version of an integrated database, and applied SOM analysis to find informative clusters linking specific genetic changes with responses to either existing or experimental drugs or to novel compounds.

These studies will focus on the identification of hypothetical gene-drug relationships that could be then be tested with wet lab experimentation in vitro. For example, if a particular gene deletion or amplification is suggested to correlate with a response to a chemical agent, we will seek to knock down or overexpress the target in that cell line, testing the impact that this may have on drug response. At the same time, we will also explore the chemical space around the major hits to identify chemical determinants that are associated with anti-cancer efficacy in the context of defined genetic features of cancer cells.

Evolution of collagen recognition: An ascidian αI domain integrin cannot recognize the GFOGER site in collagens, but may act as a Mg $^{2+}$/MIDAS independent collagen receptor

Mira Tulla[1], Mikko Huhtala[2], Juha Jäälinoja[3], Jarmo Käpylä[4], Richard Farndale[5], Leena Ala-Kokko[3], Mark Johnson[2], Jyrki Heino[4]

[1] Department of Biological and Environmental Science, University of Jyväskylä, [2] Department of Biochemistry and Pharmacy, Åbo Akademi University, [3] Collagen research unit, University of Oulu, [4] Department of Biochemistry and Food Chemistry, University of Turku, [5] Department of Biochemistry, University of Cambridge

Sequencing the genome of the urochordate *Ciona intestinalis* has revealed the presence of αI domain containing integrins, previously known to exist in vertebrates only. In vertebrates, integrins with αI domains function either as collagen receptors or as leukocyte-specific mediators of inflammatory processes. Based on phylogenetic analysis the ascidian αI domain integrins segregate to neither of these two subgroups and their function has remained unknown. Here, one *Ciona intestinalis* αI domain (α1) was produced as a recombinant protein. It could not recognize fibril-forming collagens purified from vertebrate tissues. Molecular modeling indicated that its structure does not allow binding to the GFOGER motif or other well-known adhesion sequences in collagens. This was also confirmed in binding assays using triple helical peptides. Furthermore, no GFOGER motifs were found in *Ciona* collagens. Thus, the results indicate that the high-affinity binding of collagen receptors to GFOGER and related motifs has required concomitant evolution of both the collagens and the integrins. Suprisingly, in solid phase assays α1 was found to bind to collagen IX, a prototype fibril associated collagen with interruptions in triple helix (FACIT). The approximated Kd of this selective interaction was within the same range we have measured for some human collagen receptor αI domains, but the interaction was not dependent on divalent cations. Furthermore, a mutation targeted to the metal ion dependent coordination site (MIDAS) in α1 did not impair the binding. Thus it is possible that before the development of GFOGER dependent collagen receptors in vertebrates, αI domain integrins might have been able to bind to selective collagen sequences with alternative mechanisms.

Protein-Ligand Interactions: Pharmacophores and Receptor-Ligand Docking

Mikko Vainio[1], Jukka V. Lehtonen[2], Santeri Puranen[1], Mark S. Johnson[1]

[1] Department of Biochemistry and Pharmacy, Åbo Akademi University, [2] FBD Ltd.

Proteins involved in cellular signalling, gene regulation, metabolism, immunity, and many other vital processes perform their functions by binding to some substrate molecule. If we wish to understand and perhaps intervene in these processes on the molecular level, we need a fast, efficient way to find the structural motifs and interactions necessary for the binding. Laboratory techniques are expensive and usually low-throughput. It is necessary to perform simulated interaction screening for e.g. finding new ligands from a database of small molecules or the proteome, or for understanding the physicochemical process of molecular recognition in a certain complex.

The Structural Bioinformatics Lab develops software to support our in-house 'wet' projects and as general tools to be used in structural biology and drug development. The most recent effort aims to combine two well established simulated screening methods; pharmacophore screening and receptor-ligand docking.

Structural modeling of adrenoceptors

Henri Xhaard[1], Rantanen V-V[2], Laurila J[3], Scheinin M[3], Johnson MS[1]

[1] Department of Biochemistry and Pharmacy, Åbo Akademi University, [2] Department of Biochemistry and Pharmacy, Åbo Akademi, [3] Department of Pharmacology and Clinical Pharmacology, University of Turku

Alpha-2 adrenoceptors (alpha-2 ARs) belong to the rhodopsin-like class of G-protein coupled receptors (GPCRs) characterized by seven transmembrane (TM) helices. In human, alpha-2 ARs are divided into three subtypes called alpha-2A, alpha-2B and alpha-2C. Through their interaction with naturally occurring ligands, alpha-2 ARs mediate a variety of physiological effects like anaesthesia, analgesia, vasodilation, vasoconstriction, bradycardia and mood effects and are thus key targets for pharmaceutical development. Alpha-2 ARs have therapeutic applications in a variety of diseases in the treatment of, for example, hypertension, pain, depression, anxiety and obesity.

No experimentally determined x-ray structure for an alpha-2 AR has so far been reported. In this work, the functional, structural and experimental data that exists in the literature, together with the previously unavailable crystal structure of bovine rhodopsin (Palczweski et al. 2000), were combined to construct atomic resolution models of diverse adrenoceptors. Atomic resolution models are used to provide insights on the structure-function relationship of a receptor, and for example help to understand the binding mode of natural or non-natural (pharmaceutical drugs) ligands, or to elucidate the structural changes that occur upon activation of the receptor.

Index

Ahonen, Anna-Karoliina, 28
Ala-Kokko, Leena, 66
Alanko, Sonja, 30, 38
Albarran, Brian, 13
Amundsen, Lotta, 19
Annila, Arto, 22

Bamford, Dennis, 40, 42
Baumann, Marc, 24
Baykov, Alex, 28
Belogurov, Georgiy, 21, 56
Bicskei, Zoltán, 31
Björkblom, Benny, 32
Björkqvist, Jenny, 33
Brandt, Anna-Maria, 34
Briggs, John, 40, 42
Butcher, Sarah, 40, 42

Coffey, Eleanor T., 32
Courtney, Michael J., 32

Duvet, Sandrine, 50

Edgren, Henrik, 65

Fairman, Robert, 52
Farndale, Richard, 66
Filén, Jan-Jonas, 32, 35
Finel, Moshe, 60
Finne, Jukka, 47
Franssila, Sami, 19
Fredriksson, Kai, 22
Fuller, Stephen, 40, 42

Goldman, Adrian, 28, 40, 52, 60
Gorbikova, Elena, 36
Grossmann, Günter J., 52

Haataja, Sauli, 47
Hakanpää, Johanna, 26
Halling, Katrin, 38
Heikinheimo, Pirkko, 28, 50
Heikkinen, Outi, 39
Heino, Jyrki, 41, 44, 61, 66
Hokkanen, Ari, 19
Hongisto, Vesa, 32
Honkakoski, Paavo, 20, 25
Huhtala, Mikko, 66
Huiskonen, Juha, 40, 42
Hytönen, Vesa, 13, 57
Hänninen, Pekka, 31
Höger, Stefan, 10

Ihalainen, Teemu, 13

Johnson, Mark S., 20, 34, 48, 61, 66–68
Jokinen, Johanna, 41
Jokitalo, Eija, 50
Jylhävä, Juulia, 13
Jyrkkärinne, Johanna, 25
Jäälinoja, Harri, 42
Jäälinoja, Juha, 66
Jämsen, Joonas, 21

Kallio, Pauli, 43
Kallioniemi, Olli, 65
Kallunki, Tuula, 32
Kankaanpää, Pasi, 13, 44
Katajamaa, Mikko, 46
Kauko, Anni, 47
Kidron, Heidi, 48
Kilpeläinen, Ilkka, 39
Kokona, Bashkim, 52
Komarovski, Vladislav, 32
Korhonen, Samuli-Petrus, 49
Kulomaa, Markku S., 13, 57
Kumar, Vibhor, 42
Kuokkanen, Elina, 50
Kurkela, Mika, 60
Käpylä, Jarmo, 66

Laatikainen, Reino, 20, 49

Lahesmaa, Riitta, 55
Lahti, Reijo, 21, 28, 56
Laurila, Jonne, 68
Lehtiö, Janne, 18
Lehtiö, Lari, 52
Lehtonen, Jukka V., 67
Lehtovuori, Pekka, 64
Linder, Markus B., 26, 63
Lisal, Jiri, 53
Louhivuori, Martti, 22
Lähde, Milla-Riina, 54
Lönnberg, Tapio, 55

Malinen, Anssi, 56
Marjomäki, Varpu, 44
Mattjus, Peter, 12
Maunula, Stina, 33, 38
Merckel, Michael, 40
Meriluoto, Jussi, 16, 30, 54
Mindich, Leonard, 42
Määttä, Juha, 57
Mäkinen, Marika, 50
Mäntsälä, Pekka, 43

Nakari-Setälä, Tiina, 63
Nevanen, Tarja, 19
Niemi, Jarmo, 43
Niemi, Merja, 27
Nikula, Tuomas, 59
Nissinen, Liisa, 41
Nordlund, Henri, 57
Nylund, Matts, 12
Nymalm, Yvonne, 34
Nyman, Tuula A., 32
Nyrönen, Tommi, 64

Oksanen, Esko, 28
Oresic, Matej, 46, 55

Pahajoki, Kalle, 44
Paldanius, Tiina, 57
Palonen, Elina, 12
Papageorgiou, Anastassios C., 47
Patana, Anne-Sisko, 60
Peräkylä, Mikael, 49
Permi, P., 39
Popov, Alexander, 26
Poso, Antti, 25, 65
Pudas, R., 39
Pulkkinen, Juha, 20
Pulli, Timo, 27
Pulliainen, Arto, 47
Puranen, J. Santeri, 34
Puranen, Santeri, 41, 61, 67
Purmonen, Mikko, 23

Ramstedt, Bodil, 33, 38
Rantanen, Ville-Veikko, 68
Rauhamäki, Virve, 24
Repo, Susanna, 20, 48
Rinne, Johanna, 13
Rouvinen, Juha, 23, 26, 27
Rovio, Stella, 19

Salminen, Anu, 21
Salminen, Tiina A., 17, 34, 48
Scheinin, Mika, 68
Schmidt, Andrea, 26
Scneider, Günter, 43
Seitsonen, Jani, 62
Seppänen-Laakso, Tuulikki, 46
Sippl, Wolfgang, 25
Sirén, Heli, 19
Skotheim, Rolf, 65
Slotte, J. Peter, 33, 38
Smith, Wesley, 50
Soini, Juhani, 31
Stayton, Patrick S., 13
Sultana, Azmiri, 43
Szilvay, Géza, 63
Söderholm, Annu, 64

Takkinen, Kristiina, 19, 27
Térová, Bohdana, 14
Tiikkainen, Pekka, 65
Tossavainen, H., 39

Tulla, Mira, 66
Tuma, Roman, 40, 53
Tuomikoski, Santeri, 19
Tuominen, Heidi, 21, 28, 50
Tuppurainen, Kari, 49

Vainio, Mikko, 67
Valjakka, Jarkko, 23
Verkhovsky, Michael I., 36
Vesterkvist, Pia, 16
Vihinen-Ranta, Maija, 13
Viitanen, Lenita, 17
Vuorilehto, Kai, 36

White, Daniel J., 41, 44, 61
Wikström, Mårten, 24
Windshügel, Björn, 25

Xhaard, Henri, 68

Ylander, Pilvi, 31
Ylänne, J., 39

Östman, Nina, 32