National Graduate School in Informational and Structural Biology

Seminar in Pyhätunturi, Lappi 9.-12.12.2002

National Graduate School in Informational and Structural Biology

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National Graduate School in Informational and Structural Biology

Receptor Signaling Macromolecular Structure and Biocomputing
Alpha-2, Somastotatin and Integrin Groups

Seminar in Pyhätunturi, Lappi 2002

Place: Hotel Pyhätunturi (130 km from Rovaniemi - 2 h)
Time: December 9 to 12, 2002

Program:

Monday, December 9th

07:55 - 09:15	Leave Helsinki
10:00 - 12:00	From Rovaniemi bus (ISB/ÅA) to the hotel Pyhätunturi (about 2 hours)
13:00 - 14:30	*L u n c h*
14:30 - 15:20	Keynote speaker: Computer Modeling of G-Protein Coupled Receptors (GPCR), Dr. Christoph Schneider, Accelrys, Germany
15:20-16:00	Group leader: Protein Interactions in Trafficking of AMPA receptors, Professor Kari Keinänen, Department of Biosciences, University of Helsinki
16.00 - 16.30	*C o f f e e*
16:30 - 16:50	Prediction of Blood-Brain Barrier using the structural properties of molecule, Kurt Kokko, Juvantia Pharma Ltd.

16:50-17:10 Automated superposition techniques' applications in activity prediction and ligand docking, Samuli-Petrus Korhonen, Department of Chemistry, Kuopio University

17:10-17:30 Bodil modeling environment, Jukka Lehtonen, Department of Biochemistry and Pharmacy, Åbo Akademi University

18:00 - 20:00 *Sauna*

Evening is free - no planned program!

Tuesday December 10th

08:00 - 09:30 Breakfast

09:30 - 10:30 Individual group or project meetings or free time

14:30 - 15:30 *L u n c h*

15:30 - 16:10 Group Leader: Cryo-electron microscopy and image reconstruction - the perfect Finnish occupations.
Docent Sarah Butcher, Institute of Biotechnology, University of Helsinki

16:10 - 16:30 Structural basis of ICF-causing mutations in the methyltransferase domain of DNMT3B, Ilkka Lappalainen, Department of Biosciences, University of Helsinki

16:30 - 16:50 Electron cryo-microscopy structure of a membrane virus PM2, Juha Huiskonen, Institute of Biotechnology and Department of Biosciences, University of Helsinki

16:50 - 17:50 *C o f f e e and P o s t e r S e s s i o n*

17:50 - 18:10 P4 protein of dsRNA bacteriophages is undirectional motor for RNA packaging, Denis Kainov, Institute of Biotechnology and Department of Biosciences, University of Helsinki

18:10 - 18:30 Structure determination of xylose isomerase complexes at 100K, Tarja Parkkinen, Department of Chemistry, University of Joensuu

18:30 - 18:50 Kinetic characterization of Rhodospirillum Rubrum H^+ - pyrophosphatase active site variants, Anssi Malinen, Department of Biochemistry and Food Chemistry, University of Turku

19:00 - 21:00 *Sauna*

21:00 Common dinner

23:00 - 03:00 Karaoke

Wednesday December 11th

08:00-09:30	Breakfast
9:00-10:30	Individual group or project meetings or free time
14:30	*L u n c h*
15:30 - 16:20	Keynote speaker: α2-Adrenergic Receptor Subtype as Drug Targets Professor Mika Scheinin, Department of Pharmacology, University of Turku
16:20 - 17:00	Group Leader: Molecular Interactions Professor Mark Johnson, Department of Biochemistry and Pharmacy, Åbo Akademi University
17:00 - 17:20	Characterisation of poultry egg-white avidins and their potential as tools in pretargeting cancer treatment, Vesa Hytönen, Department of Biological and Environmental Science, University of Jyväskylä
17:20 - 18:20	*C o f f e e and P o s t e r S e s s i o n*
18:20 - 18:40	Ligand recognition by collagen receptor integrins, Mira Tulla, Department of Biological and Environmental Sciences, University of Jyväskylä
18:40 - 19:00	Stress-activated protein kinase P38$^{\alpha/\beta}$ mediates a caspase-independent form of neuronal death induced by glutamate, Jiong Cao, Department of Neurobiology, A.I. Virtanen Institute, University of Kuopio
19:00 - 19:20	Structure of pyruvate formate-lyase with pyruvate, Lari Lehtiö, Institute of Biotechnology, Structural Biology and Biophysics, University of Helsinki
19:20 - 19:40	Stargazin family proteins and their interaction with AMPA-selective glutamate receptors, Lotta von Ossowski, Department of Biosciences, University of Helsinki
20:00 - 22:00	*Sauna*

Thursday December 12th

08:00 - 09:30	*Breakfast*
09:30 - 10:30	Individual group or project meetings or free time
12:00	Bus to Rovaniemi
14:10	Leave for Helsinki
15:25	*Arrive to Helsinki*
17:25	*Arrive to Turku*

KEYNOTE SPEAKERS

α_2-ADRENERGIC RECEPTOR SUBTYPES AS DRUG TARGETS

Professor Mika Scheinin

Department of Pharmacology and Clinical Pharmacology
University of Turku, Finland

The three human α_2-adrenergic receptor (α_2-AR) subtypes, α_{2A}, α_{2B} and α_{2C}, bind their natural ligands, adrenaline and noradrenaline, and many synthetic drug molecules with different affinities. The receptors are therapeutic targets: currently, α_2-AR agonists are used as antihypertensives, to treat various types of dependency and withdrawal syndromes, to treat pain, and as adjuncts to anaesthesia. Antagonists are used as antidepressants. Unfortunately, no current drugs select between the three subtypes of α_2-AR (and thus between the various effects), thus limiting their clinical usefulness. Studies with gene-targeted mice have started to reveal receptor subtype functions. The α_{2A}-AR mediates antihypertensive effects by controlling the activity of the sympathetic nervous system, and activation of α_{2B}-AR counteracts this therapeutic effect by constricting vascular smooth muscle. α_{2A}-ARs are also important for the anaesthetic and analgesic actions of α_2-agonists; the α_{2C}-subtype mediates more subtle effects on CNS integrative functions, and may be a novel neuropsychiatric therapeutic target. Subtype-selective drugs should improve the therapeutic potential of α_2-agonists and –antagonists; it is expected that better understanding of the structure and function of this class of receptors will significantly augment rational target-based drug discovery and development efforts. Mutagenesis experiments combined with molecular modelling have already started to provide detailed atomic-level knowledge of α_2-AR structure and function.

COMPUTER MODELING OF G-PROTEIN COUPLED RECEPTORS (GPCR)

Dr. Christoph Schneider

Accelrys, München, Germany

Today 50% of all recently launched drugs are targeted against GPCRs with annual worldwide sales exceeding $ 30 billion in 2001. The human genome project (HUGO) has revealed several hundred members of the GPCR family, of which only approximately 30 represent targets of currently marketed drugs. Fair enough to have a closer look on current techniques like binding site analysis, homology modeling and structure-based drug design.

GROUP LEADERS

CRYO-ELECTRON MICROSCOPY AND IMAGE RECONSTRUCTION - THE PERFECT FINNISH OCCUPATIONS.

Ph.D. Docent Sarah Butcher

Institute of Biotechnology
University of Helsinki, Finland

"So why did you move to Finland?" my colleagues have often asked. "It's a perfect place for cryo-electron microscopy and image reconstruction" I reply. "After all, there's plenty of water for preserving the biological specimens in their native state, it's almost cold enough in the winter that I can vitrify the specimen without resorting to my normal combination of liquid ethane and nitrogen, and everybody knows that electron microscopists do it better in the dark."

Image processing on the other hand, is all about trying to detect a signal in the presence of noise, or the so called "how to have a conversation in a disco" problem. My talk will thus concentrate on the principles, problems, practice and future expectations of these methods.

MOLECULAR INTERACTIONS

Professor Mark S. Johnson

Department of Biochemistry and Pharmacy
Åbo Akademi University, Turku, Finland

Proteins function through specific recognition of other molecules, including small molecules and macromolecules. Predicting the interactions that take place are not straightforward since both the protein and the ligand generally are flexible. Here I will describe our studies on protein-ligand interactions, including the generation of library of interactions suitable for predicting binding interactions and the structural features of ligands themselves.

PROTEIN INTERACTIONS IN TRAFFICKING OF AMPA RECEPTORS

Professor Kari Keinänen

Department of Biosciences, Division of Biochemistry
University of Helsinki

Activity-dependent modulation of synaptic strength, a key mechanism underlying learning and memory, is largely based on regulation of the number and activity of synaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, a subclass of ligand-gated channels activated by glutamate, the major excitatory neurotransmitter in the brain. In order to better understand this regulation, we analyze the intracellular protein interactions involved in the targeting of AMPA receptors to cell surface and to synapses. Recent work using in vitro protein interaction assays and coimmunoprecipitation experiments to identify and characterize interactions of the GluR-D AMPA receptor subunit with multidomain scaffolding proteins SAP97 and 4.1N will be reviewed.

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Coleman SK, Cai C, Haapalahti JP, Mottershead DG & Keinänen K (2002) Surface expression of GluR-D AMPA receptor is dependent upon an interaction between its C-terminal domain and a 4.1 protein.
J. Neurosci. *(in press*).

PRESENTATIONS

STRESS-ACTIVATED PROTEIN KINASE P38α/β MEDIATES A CASPASE-INDEPENDENT FORM OF NEURONAL DEATH INDUCED BY GLUTAMATE

Cao, J.[1] , Solovyan, V.[1], Coffey, E.T.[2], Courtney, M.J.[1,2]

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2. Turku Centre for Biotechnology, Åbo Akademi University and University of Turku, Turku, Finland

Neuronal death in ischaemia and other brain lesions possesses properties intermediate between classical necrosis and apoptosis. It is suggested that either an array of intermediate pathways exist, or that incomplete caspase-dependent apoptotic cell death may lead to necrosis while still possessing some features of the initial apoptosis. Here, we define a novel mechanism of neuronal death induced in culture by glutamate levels existing in ischaemic brain. We observe delayed pyknosis, lumpy chromatin condensation, somatic shrinkage, neurite degeneration and high molecular weight DNA

fragmentation in the absence of the hallmarks of classical apoptosis, caspase activation and oligonucleosomal DNA fragmentation. Death was insensitive to pan-caspase inhibitor, but Bcl-2 expression prevented loss of viability. The stress-activated p38, implicated in caspase-dependent death, was activated rapidly after and dependent upon increased cytoplasmic free calcium levels induced by glutamate addition, whereas JNK was unaffected. Dominant negative p38 isoforms and MKK3 each prevented loss of viability. A pharmacological inhibitor of p38, like Bcl-2, eliminated death without affecting glutamate-evoked calcium response, suggesting that p38/Bcl-2 act downstream of calcium elevation. These results suggest the existence of a novel mechanism of death, namely p38-mediated but caspase-independent, induced by glutamate. Caspase-independent forms of neuronal cell death may thus be amenable to regulation by signal transduction therapy.

References:

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Kawasaki H, Morooka T, Shimohama S, Kimura J, Hirano T, Gotoh Y, Nishida E. (1997) Activation and involvement of p38 mitogen-activated protein kinase in glutamate-induced apoptosis in rat cerebellar granule cells. J Biol Chem. 1997 Jul 25;272(30):18518-21.

Miller TM, Moulder KL, Knudson CM, Creedon DJ, Deshmukh M, Korsmeyer SJ, Johnson EM Jr (1997) Bax deletion further orders the cell death pathway in cerebellar granule cells and suggests a caspase-independent pathway to cell death. J Cell Biol. 139:205-217.

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Kumar S, McDonnell PC, Gum RJ, Hand AT, Lee JC, Young PR. (1997) Novel homologues of CSBP/p38 MAP kinase: activation, substrate specificity and sensitivity to inhibition by pyridinyl imidazoles. Biochem Biophys Res Commun. 1997 Jun 27;235(3):533-8.

Zhao M, New L, Kravchenko VV, Kato Y, Gram H, di Padova F, Olson EN, Ulevitch RJ, Han J (1999) Regulation of the MEF2 family of transcription factors by p38. Mol Cell Biol 19:21-30.

ELECTRON CRYO-MICROSCOPY STRUCTURE OF A MEMBRANE VIRUS PM2

Huiskonen Juha T., Hanna M. Kivelä, Dennis H. Bamford and Sarah J. Butcher

Institute of Biotechnology and Department of Biosciences, University of Helsinki

Here we present a novel 11.5 Å resolution structure of the membrane virus PM2 based on cryo-electron microscopy and three-dimensional image reconstruction. PM2 is a complex macromolecular assembly with a size of 45 MDa. The icosahedral protein shell consists of the major capsid protein and spikes. The capsid protein trimers surround hexavalent positions organised on a “T=21”-lattice, which has not been observed in any other virus. Underneath the shell is the membrane, which encloses the dsDNA genome. The highly supercoiled genome forms layers with a spacing of 2.8 nm. The first layer is organised and closely juxtaposed to the membrane. Virions with proteolytically truncated spikes were used to build a domain model for the spike protein. The pentameric spikes projecting out from the five-fold vertices are proposed to be composed of three different domains: receptor binding domain,

connector, which forms the interactions between different monomers, and the N-terminal domain, which anchors this complex to the rest of the capsid. The protease cleavage sites on the spike were assigned using mass-spectrometry. Very few icosahedral membrane virus structures have been resolved previously. In comparison to those, PM2 may represent a new paradigm in membrane virus organisation.

San Martin C, Huiskonen JT, Bamford JK, Butcher SJ, Fuller SD, Bamford DH,Burnett RM (2002). Minor proteins, mobile arms and membrane-capsidinteractions in the bacteriophage PRD1 capsid. Nature Structural Biology.Oct;9(10):756-63.

CHARACTERISATION OF POULTRY EGG-WHITE AVIDINS AND THEIR POTENTIAL AS TOOLS IN PRETARGETING CANCER TREATMENT

Hytönen, V.P.[1], Laitinen, O.H.[1], Grapputo, A.[1], Kettunen, A.[1], Savolainen, J.[2], Kalkkinen, N.[3], Marttila, A.T.[1], Nordlund, H.R.[1], Paganelli, G.[4], Kulomaa, M.S.[1]

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[4]*Division of Nuclear Medicine, European Institute of Oncology, Milan, Italy.*

Chicken avidin and bacterial streptavidin are proteins widely used in the various life sciences due to their strong affinity for biotin ($K_d \approx 10^{-15}$ M) [1]. These proteins have been extensively studied and numerous improved forms of (strept)avidin have been designed and produced to further increase their use across a broad field of applications [2]. One extensively studied use for them is in medical pretargeting cancer treatments [3]. However, their pharmacokinetics and immunologic properties are not always optimal limiting their use in these applications [4]. In order to search for potentially beneficial new candidates, we screened egg-white from four different poultry species for avidin. The biotin-binding properties of avidin from the duck, goose, ostrich and turkey were similar to those found in avidin from the chicken. Three of these novel avidins, however, showed different immunological properties when compared to those of the chicken avidin. The patient sera response to duck, goose and ostrich avidin was lower than those observed for chicken and turkey avidins. Ostrich avidin also displayed reduced thermostability in SDS-PAGE-based analysis compared to chicken avidin. Our findings suggest these proteins could offer advantages over the use of chicken avidin and bacterial streptavidin in pretargeting applications. This study gives an insight for the potential in natural proteins as an alternative for biochemically or genetically modified proteins.

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Marttila AT, Hytönen VP, Laitinen OH, Bayer EA, Wilcheck M. and Kulomaa MS. 2002. Mutation of important Tyr-33 residue in chicken avidin: Functional and structural consequences. *Biochem. J.* submitted.
Laitinen OH, Nordlund HR, Hytönen VP, Uotila STH, Marttila AT, Airenne KJ, Livnah O, Bayer EA, Wilchek M and Kulomaa MS. 2002. Rational design of an active avidin monomer. *J. Biol. Chem.* submitted.

P4 PROTEIN OF dsRNA BACTERIOPHAGES IS UNIDIRECTIONAL MOTOR FOR RNA PACKAGING

Kainov, D.E.[1], Mancini, E.J.[2], Grimes, J.M.[2], Stuart, D.I.[2], Bamford, D.H.[1], Makeyev, E.V.[1] and Tuma, R.[1]

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Genomes of many viruses are packaged into preformed empty capsids using molecular motors that convert chemical energy into mechanical work (translocation). A protein P4 is required for for ssRNA packaging into the procapsid of dsRNA bacteriophages from the *Cystoviridae* family. Here we show that the 2 Å resolution X-ray structure of P4 from the double-stranded RNA bacteriophage straight ϕ12 is highly similar to the T7 gene 4 ring helicase and to other hexameric helicases (RecA protein, F1-ATPase), suggesting that packaging enzyme P4 might be a helicase. Biochemical and structural properties of P4 proteins from ϕ12 and related bacteriophages ϕ6, ϕ8 and ϕ13 have been analyzed in order to understand the RNA translocation mechanism. Despite low sequence similarity, all P4 proteins form stable hexamers and catalyze NTP hydrolyses in vitro, which is stimulated by ssRNA. Unwinding of dsRNA and unidirectional RNA translocation was demonstrated for ϕ8 and ϕ13 hexamers *in vitro*. Binding of ssRNA to ϕ8 P4 triggers a conformation change that enables NTP hydrolyzes and assures coupling between NTPase activity and RNA translocation. Similar characteristics have been described for most hexameric helicases suggesting that dsRNA/DNA unwinding and ssRNA packaging share the same mechanism. Our results provide a working model for ssRNA packaging in dsRNA viruses.

PREDICTION OF BLOOD-BRAIN BARRIER USING THE STRUCTURAL PROPERTIES OF MOLECULE

Kokko, K.

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Designing of safer and more effective drugs acting in central nervous system needs optimisation of physicochemical properties of compound. The methodology used for the experimental determination of blood-brain concentration ratio is not a simple task. The experiment is complicated, time-consuming, expensive and it requires a rather large amount of pure compound. For these reasons, it is clear that predictive computational methods are needed to estimate the compounds CNS activity[6]. Several attempts to predict the ratio of blood-brain concentration has been introduced, but the correlation between predicted values and experintal data has not been good enough. This work has compared a method[3], which computes physicochemical properties from a structure, to methods were topological and constitutional descriptors[1][5], solvation free energies[2] and molecular fragments, has been used. This work shows that the physicochemical properties of molecular structure predict rather accurately the ratio of blood-brain concentration and therefore can be used efficiently in various parts of drug development process.

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AUTOMATED SUPERPOSITION TECHNIQUES' APPLICATIONS IN ACTIVITY PREDICTION AND LIGAND DOCKING

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Several widely utilized computational methods used to study pharmaceutically interesting molecules require a precise superposition of molecules. This requires considerable amount of human intervention, which makes these techniques slow and inefficient for large molecule libraries. By using

the The Flexible Ligand Unified Force Field (FLUFF) and it's accompanying QSAR technique called Boundless Adaptive Localized Ligand (BALL) molecules can be semi-automatically superimposed thus reducing the work required for a computational analysis.
The novel FLUFF-BALL technique developed by the author has been tested using a benchmark steroid data set1 and produced results comparable to standard methods widely used in rational drug design and in scanning of large molecular libraries.

The FLUFF-BALL algorithm requires the use of a template structure which can be rigid or flexible allowing it to react to different ligands. The ligands can also be rigid or flexible. The superposition of steroids was performed using a rigid template and ligand, fully flexible template and ligand and with mixed mode in which ligand is flexible and the template is rigid. After the superposition the BALL descriptos were evaluated and PLS fitting performed. The models yelded SDEP/R2 values of 0.687/0.561, 0.502/0.710, and 0.492/0.180 for rigid, flexible and mixed superposition respectively.

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STRUCTURAL BASIS OF ICF-CAUSING MUTATIONS IN THE METHYLTRANSFERASE DOMAIN OF DNMT3B

Lappalainen, I.[1,2] and Vihinen, M.[2,3]

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Mutations in the gene encoding for a de novo methyltransferase, DNMT3B, cause an autosomal recessive Immunodeficiency, Centromeric instability and Facial anomalies (ICF) syndrome. To analyse the structural consequences of ICF-causing mutations, we have modelled the structure of the DNMT3B methyltransferase domain based on *Haemophilus haemolyticus* protein in complex with the cofactor AdoMet and the target DNA sequence [1]. The model shows similar two-subdomain fold as the template where DNA-binding region is situated between the subdomains on a surface cleft having positive electrostatic potentials. The smaller subdomains of the methyltransferases differ in length and sequences and therefore only the target recognition domain -loop was modelled. Based on the model, the DNMT3B recognizes target cytosine from the double stranded DNA and flips it to the catalytic pocket located in the bigger subdomain. The residues in the cofactor and target cytosine binding sites as well as the electrostatic properties of the binding pockets are conserved. Moreover, a registry of all known ICF-causing mutations, DNMT3Bbase, was constructed. The structural principles of the pathogenic mutations based on the modelled structure and the analysis of chi-angle rotation changes of mutated side chains are discussed.

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STRUCTURE OF PYRUVATE FORMATE–LYASE WITH PYRUVATE

Lehtiö, L.[1], Leppänen, V-M.[1], Kozarich, J.[2] and Goldman, A.[1]

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Pyruvate formate-lyase (PFL; E.C., 2.3.1.54) is an anaerobic enzyme that catalyses the conversion of pyruvate and coenzyme A (CoA) to acetylCoA and formate. PFL is a homodimer of 85 kDa subunits displaying half-site reactivity. Activated monomer of PFL contains a relatively stable glycyl radical located at Gly734. Activation of PFL is performed by an activating enzyme (E.C., 1.97.1.4) containing an iron-sulphur cluster, which generates the Gly734 radical using a 5'-deoxyadenosyl radical derived from S-adenosyl methionine. Pyruvate, or its analogue oxamate, is required for activation. The structure of PFL is already known [1,2] and now we have solved a structure at 2.7 Å resolution with pyruvate included in the crystallization mixture [3]. Both active sites of homodimeric enzyme are occupied with pyruvate and additional binding sites were not found. Pyruvate was modeled to a cleft close to the active site cysteines 418 and 419 with the carboxyl group in contact with arginines 176 and 435 and methyl group within van der Waals distance to Phe327. The binding site of pyruvate is not the site when the reaction initiates, because there will be conformational changes during the activation of PFL.

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BODIL MODELING ENVIRONMENT

Lehtonen, J.V.[1], Still, D-J.[1], Rantanen, V-V.[12], Gyllenberg, M.[2] and Johnson, M.S.[1]

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Molecular modeling assists the study and prediction of atomic interactions between molecules. Forces of interaction can be estimated with theoretical models. The results, even erroneous ones, help the researcher to quickly focuse on key aspects of the biological problem. Computer-based visualization is a powerful tool for modeling, since it can reveal properties and relationships that are not directly seen from the numerical results. Therefore, a good molecular modeling software product should provide both computation and visualization tools in a easy to use package.

We have developed a computer program BODIL for molecular modeling. It can visualize the molecular data in several different ways. The focus in our design has been on modularity, extensibility and portability. Our main goal has been to produce a simple, yet powerful tool for protein modeling. We have included basic computational methods (like sequence and structure alignment, electrostatic potential calculations, and homology modeling) as modules within the package and can easily add more when necessary. The program has been developed on Linux and SGI Irix environments, providing good performance on both (inexpensive) PCs and high-end graphics workstations. A MS Windows version is also under development.

The program is free for academic use. A beta version of the program is downloadable from the web page: http://www.abo.fi/fak/mnf/bkf/research/johnson/bodil.html

KINETIC CHARACTERIZATION OF *RHODOSPIRILLUM RUBRUM* H^+-PYROPHOSPHATASE ACTIVE SITE VARIANTS.

Malinen, A.[1], Belogurov, G.A.[1], Salminen, M.[2], Kauko, A.[3], and Lahti, R.[1].

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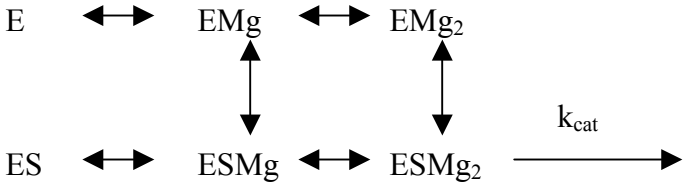
The proton pumping pyrophosphatase (H^+-PPase) is an integral membrane protein that utilizes the energy released upon hydrolysis of inorganic pyrophosphate (PP_i) to transport protons across the membrane against the electrochemical gradient. An effective heterologous expression system in *Escherichia coli* has been developed for H^+-PPase [1] which permits the expression and functional characterization of H^+-PPase mutants. In order to understand the catalytical mechanism of H^+-PPase, we have generated eight single amino acid changes and one double change in the putative catalytic site of *Rhodospirillum rubrum* H^+-PPase. The evolutionary conserved amino acids were identified by sequence alignments and secondary structure prediction programs. We chose to mutate charged residues because they are expected to contribute most to the catalytical activity.

K184R, D187E, D191E, K195R, D642E, K646R and E197D+E649D mutants show less than five percent of pyrophosphate activity compared to wild type and are considered to be inactive. E197D shows about 60 percent and E649D about 40 percent hydrolytic activity which makes them eligble for more detailed kinetic characterization. E197D and E649D are also capable to proton transport.

To find out the role of glutamates 197 and 649 in H^+-PPase we have determined the V_{max} and K_m as a function of free Mg^{2+}, the essential cofactor of H^+-PPases, concentration for wild type enzyme and for E197D and E649D mutants. Mg^{2+} dependence of V_{max} and V_{max}/K_m allowed us to derive the kinetic scheme and parameters of Mg^{2+} binding to enzyme:substrate complex and free enzyme, respectively (*figure 1*). Major conclusion arising from this analysis is that mutation of glutamate 197 to aspartate in *R. rubrum* H^+PPase drastically decreases the binding affinity of first magnesium ion to free enzyme.

Figure 1 *Kinetic scheme of R. rubrum H^+-pyrophosphatase*



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J. Biol. Chem. 277(25):22209-14.

STARGAZIN FAMILY PROTEINS AND THEIR INTERACTION WITH AMPA-SELECTIVE GLUTAMATE RECEPTORS

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Stargazin is a member of calcium channel gamma subunit homologs expressed in the nervous system [1]. It is a membrane protein with 323 amino acid residues, four transmembrane domains and a C-terminal intracellular domain. Stargazin has been reported to be necessary for the synaptic transport of AMPA-type glutamate receptors, but the underlying mechanisms are poorly understood [2]. The purpose of this project is to provide a molecular analysis of stargazin proteins and their interactions with AMPA receptors.

A antiserum against bacterially expressed C-terminal domain of stargazin was prepared to be used as a tool in detection of native stargazin from tissue as well as of recombinant forms expressed in heterologous cells. The interaction of stargazin with AMPA receptor subunits from rat cerebellum was verified by co-immunoprecipitation. Recombinant stargazin and stargazin mutants have successfully been expressed in transfected mammalian cells (HEK293). When co-transfected into HEK 293 cells, stargazin co-immunoprecipitates with GluRD. Use of different deletion mutants of stargazin in co-immunoprecipitations with GluRD has shown that the C-terminal tail, comprising a third of the intact molecule (122 amino acid residues), is dispensable for the interaction. The amino acid sequence of stargazin reveals one putative glycosylation site (asparagine at residue 48). To demonstrate that stargazin is glycosylated a mutant was constructed where asparagine was replaced by glutamine, N48Q. The electrophoretic mobility of wt stargazin was compared to that of the mutant stargazin N48Q. The mutant stargazin N48Q clearly migrated at a lower molecular weight compared to the wt indicating glycosylation of the wt stargazin.

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STRUCTURE DETERMINATION OF XYLOSE ISOMERASE COMPLEXES AT 100 K

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D-Xylose isomerase is an enzyme that catalyses the reversible interconversions of five- and six-carbon aldoses and ketoses The physiological reaction of the enzyme is the interconversion between D-xylose and D-xylulose. The crystal structure of xylose isomerase is well known, it is a tetramer and each subunit folds into a TIM motif. The amino acids in active site are conserved and furthermore the active site contains two metal cations per monomer: structural metal (M1) and catalytic metal (M2). Substrate binds to M1 and M2 has a role in the isomerisation reaction.
All of the D-xylose isomerase structures reported so far have been determined at room temperature. We have found that the crystal diffraction of xylose isomerase weakens rapidly during measurement at room temperature by using rotating anode and it is difficult to collect a complete data set so the resolution limit remains lower than at cryotemperature. In addition, the interpretation of electron densities with ligand is not clear in the active site, so the reliable interpretation might require higher resolution data.
The purpose of cryoprotectants in x-ray diffraction is to prevent freezing of the solvent in the crystals so that they maintain their diffraction ability. Cryoprotectants also reduce the radiation damage of the crystals during the measurement. For xylose isomerase crystals we have tested and measured data with three commonly used cryoprotectants, glycerol, 2-methyl-2,4-pentanediol (MPD) and ethylene glycol. All of those prevents the ice formation and protects crystals for radiation damage, furthermore they improve the resolution compared to the room temperature data. The problem using these cryoprotectants is that they are all binds to the active site and so they remove the cocrystallized substrate. Therefore, these molecules are not suitable for cryoprotection when studying the reaction mechanism of the enzyme with substrate complex structures.

LIGAND RECOGNITION BY COLLAGEN RECEPTOR INTEGRINS

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Integrins are receptors of crucial importance for multicellular organisms. Besides anchoring functions they also mediate signals between cells and the environment. Of the 24 different heterodimeric integrin receptors, four function as primary collagen receptors in cells, namely alpha-1 beta-1, alpha-2 beta-1, alpha-10 beta-1and alpha-11 beta-1. They differ from other extracellular matrix (ECM) receptors in that they all contain an independently folding ligand-binding domain called the I domain.
Collagens are a protein family of more than twenty members. Cellular receptors for many but not all collagen subtypes are known. All integrin type collagen receptors can bind more than one subtype.
Aims of this study are to find out how ligand binding specificity is determined in collagen binding integrins and also to achieve better understanding how these receptors function at the cellular level. Of particular interest are the two most recently found integrins alpha-10 beta-1 and alpha-11 beta-1, whose biology is so far largely unknown.
Mutational studies with recombinant alpha-1, alpha-2 and alpha-10 I domains have given some clues to how selective ligand binding can be achieved. It seems likely that variable charged amino acids in the binding region may make integrin I domains favor different collagen subtypes [1].
The study is widened to include matrix metalloproteinases (MMPs). MMPs are zinc dependent enzymes responsible for the turnover of ECM during development and maintenance processes of the body, and also in pathological conditions. Certain integrins are known to bind MMPs. Possible interactions of MMP and integrin alpha-2 beta-1 are being investigated.

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SEMINAR POSTERS

A LYSINE SUBSTITUTE FOR K^+: A460K MUTATION ELIMINATES K^+-DEPENDENCE IN H^+-PYROPHOSPHATASE OF *CARBOXYDOTHERMUS HYDROGENOFORMANS*

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The H^+-translocating pyrophosphatase (H^+-PPase) family is composed of two phylogenetically distinct types of enzymes: K^+-dependent and K^+-independent. However, to date, the sequence criteria governing this dichotomy have remained unknown. In this study, we describe the heterologous expression and functional characterization of H^+-PPase from the thermophilic bacterium *Carboxydothermus hydrogenoformans*. Both PP_i-hydrolyzing and PP_i-energized H^+ translocation activities of the recombinant enzyme in *Escherichia coli* inner membrane vesicles are strictly K^+-dependent. Here we deduce the K^+ requirement of all available H^+-PPase sequences, based on the K^+ dependence of *C. hydrogenoformans* H^+-PPase in conjunction with phylogenetic analyses. Our data

reveal that K^+-independent H^+-PPases possess conserved Lys and Thr that are absent in K^+-dependent H^+-PPases. We further demonstrate that a A460K substitution in *C. hydrogenoformans* H^+-PPase is sufficient to confer K^+ independence to both PP_i hydrolysis and PP_i-energized H^+ translocation. In contrast, a A463T mutation does not affect the K^+ dependency of H^+-PPase.

Belogurov, G. A. and Lahti, R. (2002) A Lysine substitute for K^+: A460K mutation eliminates K^+-dependence in H^+-Pyrophosphatase of *Carboxydothermus hydrogenoformans*, submitted

Belogurov, G. A., Turkina, M. V., Penttinen, A., Huopalahti, S., Baykov, A. A., and Lahti, R. (2002) H^+-pyrophosphatase of *Rhodospirillum rubrum*. High yield expression in *Escherichia coli* and identification of the Cys residues responsible for inactivation my

SEARCH FOR NOVEL JNK TARGETS IN THE BRAIN

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c-Jun amino-terminal kinases (JNK) typically respond strongly to stress, are implicated in brain development and mediate neuronal apoptosis in response to excitotoxic damage (1). Three genes encode the JNK protein kinases and over ten isoforms are generated through alternative splicing (2). JNKs are strongly activated by extracellular stresses such as cytokines, osmotic shock, redox stress or UV radiation and are widely believed to mediate cellular apoptosis, particularly emphasized in neuronal tissue (3). Given the facts that the JNK mediate apoptosis in response to stress, it would be expected that neurons would have a low basal JNK activity and respond sensitively to apoptotic signals. Surprisingly, recent work from our group has shown that primary cerebellar granule neurons express elevated basal JNK activity that is upregulated during neuronal differentiation and that this pool of highly active JNK activity exists in the absence of stress (4). In these neurons, 95% of the total JNK activity resides outside the nucleus even though the best characterized JNK substrates are nuclear transcription factors. Recent findings from our lab strengthen the idea that specific JNK isoforms are responsible for the regulation of neuronal death and different JNK isoforms take part in other cellular activities like vesicular transport and modulation of cellular architecture. In our ongoing study we are trying to elucidate the nature of these JNK isoforms. We have taken a proteomics based approach to search for and identify novel JNK targets in the brain which may mediate the effects of JNK during stress. In this study we have focused on the brain specific isoform JNK3 because it has been implicated in neurological disorders such as Alzheimer's disease and in excitotoxic damage as occurs during epilepsy and stroke (5, 6). Information on new target molecules for JNK3 could therefore lead to the development of new therapeutic interventions

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SULFUR CONTAINING LIGAND COMPLEXES OF *CHAETOMIUM TERMOPHILUM* XYLANASE

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Xylanases are glycosyl hydrolases that catalyze the cleavage of the glycosidic bond between two adjacent xylopyranose units in hemicellulose chain of xylan. Several structures of xylanase enzymes from different species have been determined[1] but substrate binding remains a question of interest. The purpose of this study was to examine the substrate binding by complexing the enzyme with potential inhibitors, sulfur-containing analogs of native xylan synthesized by Dr. Ibatullin in St. Petersburg.

The enzyme under study, *Chaetomium thermophilum* xylanase (CHX), was crystallized under conditions determined earlier at the University of Joensuu[2]. The potential inhibitor was introduced to the structure by soaking or by co-crystallization. The complex structures of CHX with methoxy-beta-S-xylobiose, methoxy-beta-S-xylotriose, methoxy-beta-S-xylotetrose and methoxy-beta-S-xylopentose were determined by X-ray diffraction. All the potential inhibitors seemed to bind alike to the subsites –1 and -2 of the active site of the enzyme. For the largest three inhibitors the third xylopyranose ring was also visible in the electron density maps. Thioxylotetrose and thioxylopentose probably left a short tail hanging outside the active site, since the intact molecule could not be detected on the maps. No inhibitors bound to the positive subsites, beyond the point of catalytic cleavage. This was considered to be mainly due to a steric hindrance caused by the large methoxy substituent in the reducing end of the inhibitor chain.

The thiooligosaccharides of the type used in this study were found to be potentially useful in examining substrate binding. In order to achieve more information about the binding beyond the site of catalytic cleavage in xylanases, the potential inhibitors used in this study would need to be modified.

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structures of thermophilic β-1,4-xylanases form *Chaetomium thermophilum* and *Actinomadura flexuosa*: comparison of twelve xylanases in relation to their therma stability, submitted

THE STRUCTURAL BASIS OF INTEGRIN ACTION AS A VIRUS RECEPTOR

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Cell surface integrins act as receptors for several microbial pathogens, including picornaviruses such as echovirus 1 (EV1). Structure of the ligand-binding domain of alpha2beta1 integrin, the alpha2I-domain, bound to echovirus 1 was determined by electron cryomicroscopy, 3-dimensional image reconstruction and molecular modeling. The structure shows that alpha2I-domain binds in a surface depression, the so-called canyon, around the fivefold symmetry axis of the icosahedral virus capsid.

The canyon is a common feature of picornaviruses and the binding site of the receptors of eg. rhinoviruses and poliovirus. Comparison of the alpha2I-EV1 complex with the crystal structure of the alpha2I-domain in complex with collagen, the native ligand, shows that alpha2beta1 integrin cannot bind collagen and EV1 simultaneously. The binding affinity of alpha2I-domain to EV1 is approximately tenfold greater than to collagen.

A comparative model was built for the alpha2beta1 integrin heterodimer and superposition of this model with the virus-bound alpha2I-domain shows that EV1 can accommodate full-sized integrin heterodimers on adjacent capsid protomers without steric hindrance. This suggests that EV1 can induce clustering of integrins on the cell surface and thereby trigger intracellular signaling. EV1 capsid is stabilized by alpha2I-domain binding and internalized via caveolae. This is in contrast to most studied picornaviruses, whose capsids are destabilized and uncoating initiated by receptor binding and whose internalization proceeds through the endosomal pathway. Our results suggest that echovirus 1 has evolved to exploit the relatively low affinity of alpha2beta1 integrin for its native ligand as well as the capability of alpha2beta1 signaling to activate virus-receptor complex internalization to caveosomes.

Moreover, the capsid stabilization of EV1 in the receptor complex indicates that its uncoating process

differs from that of picornaviruses that are internalized via endosomes.

LOCATING THE MINOR COMPONENTS OF DSRNA BACTERIOPHAGE Φ6 BY NEUTRON SCATTERING

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The polymerase core of double-stranded (ds) RNA virus provides the molecular machinery for RNA packaging and replication. Procapsid of bacteriophage Φ6 constitutes the best studied model of such dsRNA-processing machine.

A total of 120 copies of protein P1 form the procapsid framework to which other proteins are attached. Although the overall shape of the virus can be determined by cryo-electron microscopy (cryo-EM), minor procapsid constituents, proteins P2 (RNA polymerase) and P7 (packaging factor), have not been localized before this work.

We have applied small angle neutron scattering (SANS) and contrast variation in order to localize the two proteins. Procapsids containing deuterated P2 or P7 were produced in vitro and SANS was measured at several contrast levels. Radial positions of labeled proteins were obtained and modeled within the cryo-EM electron density of the procapsid.
P2 monomers reside at each five-fold vertex just under the RNA packaging complex. P7 was detected at a distance of 160 Å from the procapsid center indicating localization on the inner surface of P1 framework.

Reference:
T. Ikonen, D. Kainov, P. Timmins, R. Serimaa, R. Tuma, Locating the minor components of dsRNA bacteriophage Φ6 by neutron scattering, submitted to Journal of Applied Crystallography

IDENTIFICATION OF IGE-BINDING AMINO ACIDS ON THE CONFORMATIONAL EPITOPES OF HEVEIN (HEV B6.02)

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Hevein (Hev b 6.02), a major small molecular weight (4.7 kDa) allergen in natural rubber latex (NRL) is recognized by IgE of approximately 70 % of latex allergic patients. Hevein is an advantageous molecule for studies of conformational B-cell epitopes, since its 3D structure has been solved, and it can also be considered as a good candidate for studies aimed at allergen specific immunotherapy. We have previously localized conformational IgE-binding areas of hevein to its N- and C- termini by transferring terminal or core regions of hevein to a nonallergenic, structurally homologous adaptor protein (AMP) [1]. In order to confirm the results from our AMP-study and attest the importance of structural epitopes, we produced chimeras containing AMP N- and C-termini within hevein core. Thereafter, we designed and constructed site-specific mutants by QuickChange or megaprimer method to detect and locate IgE-binding epitopes of hevein at amino acid level to those potential antigenic sites. The recombinant proteins were produced in insect cells (Sf9) and weights were determined by mass spectroscopy. A chicken biotin-binding protein, avidin, was fused to the N-terminus of the hevein protein to facilitate effective isolation of the recombinant protein. An ELISA-inhibition method and skin prick test (SPT) provided the linkage from structure modification to immunological responses.

Introduction of the N- and C-terminal parts of AMP to hevein resulted in a remarkable decrease (> 50 %) in the IgE-binding capacity. The terminal regions were scanned with single point mutations that were chosen site-specifically from the naturally occurring hevein-like domains (by "evolutionary approach"). Most of the substitutions caused a decrease in IgE-binding activities at least with some patients. A multifold mutated molecule that shows minimal or no IgE binding but retains the T cell epitopes was constructed by combining the most effective mutations.

In conclusion, we have been able to transfer structurally similar, nonallergenic parts of AMP to the IgE-epitope regions of the originally highly allergenic hevein protein. The B-cell epitopes have been confirmed to localize in the terminal parts of the hevein. They seem to be assisted by amino acid residues that are distant in protein primary structure but proximal in the 3D-fold of the allergen. Homologues to the naturally occurring allergen can be used as a hyposensitizing agent to reduce or diminish the strength of allergic reaction. Selected mutants will be used for tests of immunotherapy of NRL allergy.

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WHAT HAPPENS TO MICROCYSTINS AFTER ACCUMULATION?

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The Past

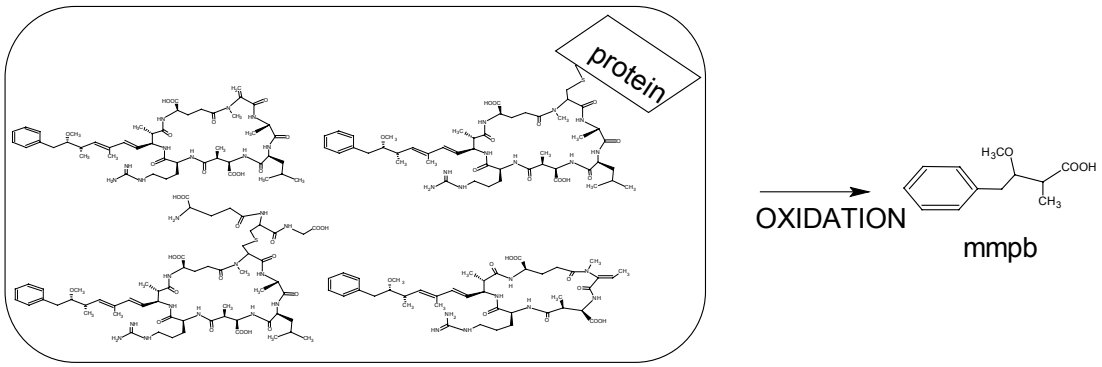
Cyanobacteria produce several kinds of secondary metabolites of which some are toxic to other organisms. Many of these toxins, such as microcystins and nodularins, show several kinds of bioactivity, e.g. hepatotoxicity, protein phosphatase inhibition, tumor initiators and promoters, and affect enzyme activity. Microcystins can bind covalently with thiol-groups in cystein and can not completely be analyzed as intact by extraction of tissue material. It is not known exactly how much of the microcystins is extractable, due to lack of suitable analytical methods. It is also not known what happens to the covalently bound microcystins. Some part of the microcystins form glutathione conjugates and is detoxificated. It is uncertain how big this part is.

The Present

We have developed an LC-MS method for analysis of intact microcystins and nodualrins in tissue. We are currently developing a method to analyze the total microcystin content in tissue, both bound and free toxin. This method involves oxidization of the material and formation of a carboxylic acid from microcystins. This oxidization product is called MMPB and can be detected with LC-MS and quantitatively analyzed as total microcystin or nodularin content. The oxidation is performed with ozone.

The Future

When we have a method to determine the total microcystin amount and the free amount of toxins, we can calculate the amount of bound toxin. Then we will look into the bound toxin more in detail. Can we detect glutathione conjugates conclusively or can we also find protein bound microcystins? Microcystin bound protein can be purified and analyzed on LC-MS both with and without digestion.



Microcystins, bound and free, and nodularins form MMPB when treated with ozone for oxidation. MMPB can be analyzed with LC-MS as total toxin content.

X-RAY CRYSTALLOGRAPHIC STUDIES ON A FERRITIN-LIKE PROTEIN (DPR) FROM *STREPTOCOCCUS SUIS*

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Ferritin-like proteins form a novel group of proteins with diverse functions, such as DNA-binding [1], iron-storage [2] and cell activation [3]. A common structural characteristic of ferritin-like proteins is their ability to form spherical shells that consist of 12 similar subunits instead of 24 in classical ferritin.

Dpr is produced by the gram-positive bacterium Streptococcus suis. Sequence analysis has shown that Dpr may possess an iron-binding site analogous to that found in dodecameric Listeria innocua ferritin. Furthermore, biochemical data suggest that S. suis Dpr may protect the cell from the oxidative stress, possibly by binding the free iron(II) capable of generating highly toxic radicals in the presence of hydrogen peroxide [4]. As S. suis has been associated with meningitis in piglets and humans [5], understanding the survival mechanisms of this bacterium may lead to new targets for antibacterial agents and vaccines.

To better understand the role and mechanistic features of Dpr, we have initiated structural studies using X-ray crystallography. Dpr was expressed in E. coli and purified [6]. Crystals were produced using the hanging drop vapour diffusion method [6]. Complete X-ray diffraction data to 1.95 Å resolution were collected using synchrotron radiation. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit cell parameters a = 104.3, b = 137.6 and c = 142.3 Å. Structure determination is currently in progress.

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STRUCTURAL STUDIES ON RECEPTOR-LIGAND INTERACTIONS

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The aim of my project is to determine the three-dimensional structure of integrin alpha 10 and alpha11 I-domains and hVAP-1 by x-ray crystallography. I will try to crystallize both the native proteins and the protein-inhibitor complexes.

Integrins have a key role in cell-cell interactions and signaling and, thus, they are involved in numerous human disease states, like cancer, inflammation, arthritis, and wound scarring [1]. Inhibitors of integrin function would therefore have therapeutic effect in these diseases but should, however, be integrin subtype specific in order to have the intended wanted effect.

The alpha1beta1, alpha2beta1, alpha10beta1 and alpha11beta1 integrins are collagen-binding integrins, in which a sequence of ~200 residues (I-domain) is responsible for recognition of native collagen. The recently found alpha10 and alpha11 I-domains seem to be closely related to alpha1 and alpha2 I-domains, but there are also some key differences in the ligand-binding site, since they have different ligand preferences [2].

The crystal structures of the alpha10 and alpha11 I-domains would give information about both the ligand preferences and the biological function of integrins. The integrin-inhibitor complexes would reveal the specific binding mode and site of inhibitors to I-domains as well as any structural changes that might occur upon binding. The structures would also be important in the design of integrin subtype specific inhibitors.

Human Vascular Adhesion Protein-1 (hVAP-1) is both a receptor and an enzyme. It is a large homodimer (180 kDa) and functions both as a lymphocyte-endothelial cell adhesion protein and as a copper containing monoamine oxidase [3,4]. These two functions are connected, since small molecular inhibitors of the amine oxidase activity of hVAP-1 are able to prevent the lymphocyte-endothelial cell interaction and, thus, leukocyte emigration in acute inflammatory conditions and chronic diseases like rheumathoid arthritis [5]. The x-ray structure of VAP-1 would be of great importance because there are no mammalian amine oxidase structures published to date. The crystal structure would give us exact information about the active site and the nature of its unknown biological substrate.

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STRCUCTURAL STUDIES OF *YERSINIA* ADHESIN YADA

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Yersinia adhesin, YadA, is a trimeric outer membrane protein encoded by a *Yersinia* virulence plasmid. YadA is one of the main virulence factors as it is largely responsible for the adhesion of *Y. enterocolitica* and *Y. pseudotuberculosis* to the intestinal tissues *in vitro* through binding to the extra cellular matrix proteins on the surface of the host cells [1]. A secondary structure analysis and electron microscope pictures have predicted that YadA has three domains: A collagen binding head domain, a coiled-coil stalk and a membrane anchor [2] We have cloned, produced, purified and crystallised a collagen-binding head domain of YadA.

For the production of the head domain, *yadA* of *Y. enterocolitica O:3* was cloned to expression vector and transformed into *E. coli*. The N-terminally H_6-tagged protein was purified from cytoplasm using a metal chelating affinity column. Soluble protein was separated from the aggregate by a gel filtration and concentrated for the crystallization. Diamond shaped crystals that belong to space group R3 grew within a week. A full data set was collected to 1.55 Å at DESY, Hamburg[3]. For a selenomethionyl-labelled protein, two I→M mutations were designed to collagen-binding SVAIGxxS motifs, which were expected to be on the surface of the protein [4]. Expression of labelled protein yielded to hexagonal crystals, which belonged to space group R32. The full MAD data were collected to 2.0 Å resolution at ESRF, Grenoble.

Two selenium atoms were found per monomer and used for phasing. After a density modification, YadA trimers could be seen in electron density maps, but overall the quality of the maps was poor. However, the selenium atoms could be positioned into the electron density and they turned out to be inside the trimer, and very close to the crystallographic threefold. This resulted to poor map quality, and also explained why the partially built model could not be refined: the phasing power was not enough. To add phasing power, several S→C mutations were designed to surface serines to produce a heavy atom derivative.

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MICROARRAY ANALYSIS OF RAMOS B CELLS

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B cell development or differentiation requires concerted function of a large number of genes and proteins. We have studied the genome wide gene expression in anti-IgM stimulated Ramos B cells. cDNA microarrays were used to follow changes in expression of genes during several days. Genes with significantly altered expression at least in one time point were further studied by clustering them based on expression profiles and by characterizing them based on the functions of the coded proteins. Several groups of genes important for B cells were analyzed. We studied genes involved e.g. in signal transduction and apoptosis, cytokines and their receptors and transcription factors. The proteins were also classified by their yeast homologies in the MIPS functional catalogue. MIPS categories in the clusters were studied and we found out that some of them were statistically enriched to certain clusters e.g. genes involved in protein synthesis were in the clusters which have early expression patterns. The results provide knowledge on the development of humoral immunity. Several new genes were found to be essential for the B cell development. They can be used as targets for research and possibly for drug development.

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STUDIES OF MIXED LIPID BILAYER USING MOLECULAR DYNAMICS SIMULATIONS

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The biophysical properties of biological membranes are known to be modulated by sterols, such as cholesterol [e.g. 1 and 2]. Although the physical properties of lipid bilayers have been extensively studied [3], and phosphatidylcholine (PC) bilayers have been studied using molecular dynamics (MD) simulations [e.g. 4 and 5], there still remains things unexplained. We expect to be able to give plausible explanations for e.g. species-dependent differences in desorptive behaviour of a variety of sterols (e.g. cholesterol and lathosterol) by analysis of the depth distribution and comparisons of the interfacial behaviour of these sterols in simulated PC model bilayers. We hope that MD simulational analyses will provide explanations for a number of other experimentally observable biophysical properties of lipid bilayers, as well.

Once the simulational procedure is under control, the methodology of bilayer MD simulations will be expanded to cover similar aspects in bilayer models consisting of lipids other than PC, such as

sphingomyelin (SM). Little has been published about MD simulations of bilayers consisting of SM, although this lipid is a major constituent in biological membranes and, thus, should be of interest.

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ROLE OF CYTOSKELETON IN NUCLEAR TRANSPORT OF BACULOVIRUSES IN MAMMALIAN CELLS

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Autographa californica multiple nuclear polyhedrsis virus (AcMNPV), a baculovirus, has a 130 kb, double-stranded, circular DNA genome, which is located in a cigar-shaped (25 by 260 nm) enveloped nucleocapsid[1]. Baculoviruses are infectious only to arthropods, mainly insects[2].

Baculovirus enters insect cells via receptor-mediated endosytosis, but the receptor for the viral entry has not yet been identified[1]. In the cytoplasm, baculoviral nucleocapsids seem to induce formation of actin filaments. Moreover, actin and a myosin-like protein may be involved in transport of the nucleocapsid to nucleus[3]. It seems evident that intact microtubules are not required for the intracellular transport of nucleocapsids[1]. However, the detailed mechanism of intracellular movement and nuclear entry of the virus is still unknown. Nucleocapsid of AcMNPV enters host cell nuclei and uncoats within the nucleus[2]. Furthermore, nucleocapsid is likely transported trough the nuclear pore[1].

Baculoviruses can also transduce various mammalian cells[4,5]. Human endothelial aortic hybridoma cell line (EaHy926) is however a cell line which the baculovirus nucleocapsid does not enter the nucleus[6]. AcMNPV transduces both dividing and nondividing mammalian cells[5]. Recent studies have suggested that baculoviruses are potentially useful gene therapy vectors[7,8,9].

The cytoplasm of the cell imposes a substantial barrier to viral transport. Free diffusion is restricted by the presence of organelles and cytoskeleton. Our preliminary results show a clear increase in amount of nuclear import of nucleocapsids in the presence of substances that disrupt microtubules, suggesting that microtubules are able to restrict the cytoplasmic transport of virus towards the nucleus. The data obtained with laser scanning confocal microscopy and electron microscopy (EM) will be quantitated

with EM. The role of actin filaments, ATP-dependent myosin motor protein complex and microtubules will be studied further using assays *in vivo* and *in vitro*.

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BUILDING A PHARMACOPHORE MODEL FOR CB1 CANNABINOID RECEPTOR

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CB1 cannabinoid receptor is mainly located in the central nervous system and it mediates the various physiological effects of cannabinoids [1]. There are at least five structurally different classes of cannabinoid ligands that all exert their effects via CB1 receptor.

The aim of this study was to propose a common pharmacophoric superposition for two different structural classes of cannabinoid ligands, namely, for the classical tricyclic cannabinoids, such as Δ^9-tetrahydrocannabinol (THC), as well as for the highly flexible derivatives of arachidonic acid, such as endocannabinoids. Both classes of ligands are supposed to bind to the same binding site at the CB1 receptor. Several low-energy conformers of the arachidonyl chain have been reported: J-shaped, U-shaped and a helical-shaped conformer as well as an extended conformer [2]. One of those conformers had to be chosen to be superimposed upon the very potent tricyclic cannabinoid, HU-210, which was the rigid template for the whole alignment process. 2-arachidonylglycerol (2-AG) was taken as the template structure for the other endocannabinoid analogues. After comparing all the reported different-shaped conformations of 2-AG with the low-energy conformer of HU-210, the U-shaped conformer was chosen for the pharmacophore model. Criteria for this choice were the good volume overlap of the molecules as well as a reasonably good alignment of the pharmacophoric elements: hydrogen bond

acceptor/donor areas, π-electron rich areas and the aliphatic side chains. The model can then be used to study the QSAR of cannabinoid ligands.

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EFFECTIVE MODELLING FOR MOLECULAR DESIGN

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The performance of the comparative or homology modelling software tools MOE-Homology [1], MODELLER [2] and HOMODGE [3] was evaluated based on a structurally diverse set of test cases with known three-dimensional (3D) X-ray crystal structures. Each of the programs implements a different algorithm for deriving the 3D coordinates of the target from the template structure. Default settings of each program were used. Models were built both with ligand included in and excluded from the template. All models were evaluated by comparison to the actual X-ray crystal structure of the target in four different superimpositionings using root mean square deviation (RMSD) as a measure of error. Emphasis was put on the assessment of correctly reproduced binding site geometry. Results indicate that on the average, using a protein-ligand complex as template does not improve the quality of the resulting model. Difference in the performance of the commercial software is marginal despite the algorithmic divergence.

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EVOLUTION AND STRUCTURE OF THE BIOAMINE RECEPTORS FAMILY.

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The G-Protein Coupled Receptors are integral membrane proteins, typified by a bundle of seven transmembrane helices. They are involved in signal transduction: Following the binding of an agonist ligand, on the extracellular side, the receptor activates molecular effectors, on the intracellular side. Each of the bioamine receptors, observed in many animal species [1], react to a specific messenger (acetylcholine, adrenaline, dopamine, histamine, serotonin or octopamine). Using molecular phylogeny and molecular modeling techniques, the aim of the study was to understand: (A) The evolutionary relationship among the biogenic amine receptors family (B) The residues involved in binding the natural ligands and the co-evolution of the binding sites.

Our results indicate that:
(A) The classification of the receptors using molecular phylogeny is different from the classification derived from pharmacological studies.

For example, on the pharmacological point of view, all the dopaminergic receptors are activated by the natural ligand dopamine and all the adrenergics are activated by the natural ligands adrenaline and noradrenaline. However, evolutionary, the D2 dopaminergic receptors are closer to the alpha-2 adrenergic than to the alpha-1 adrenergic receptors.

(B) In many cases, clear examples of co-evolution between the residues lining the binding site and the chemical properties of the ligands can be found. That observation can be used to propose new residues potentially involved in receptor-ligand interaction.

For example, noradrenaline and dopamine are molecules which differ only by a chemical group, the beta-hydroxyl. The position of the beta-hydroxyl within the adrenergic binding pocket and therefore the orientation of noradrenaline, has been addressed by several experimental studies but remains unclear. We compared subfamilies of dopaminergic and adrenergic receptors, determined as closely related in the previous evolutionary study, to propose new residues interacting with the noradrenaline beta-hydroxyl group within those subfamilies.

Related publication (accepted for publication, Genome Research)
Early Emergence of Multiple alpha2-Adrenergic Receptor Subtypes by Chromosomal Duplications as Revealed by Cloning and Mapping of Five Receptor Subtype Genes in the Zebrafish (*Danio rerio*)
IDENTIFICATION OF A NOVEL, DUPLICATED FOURTH α_2-ADRENERGIC RECEPTOR SUBTYPE

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