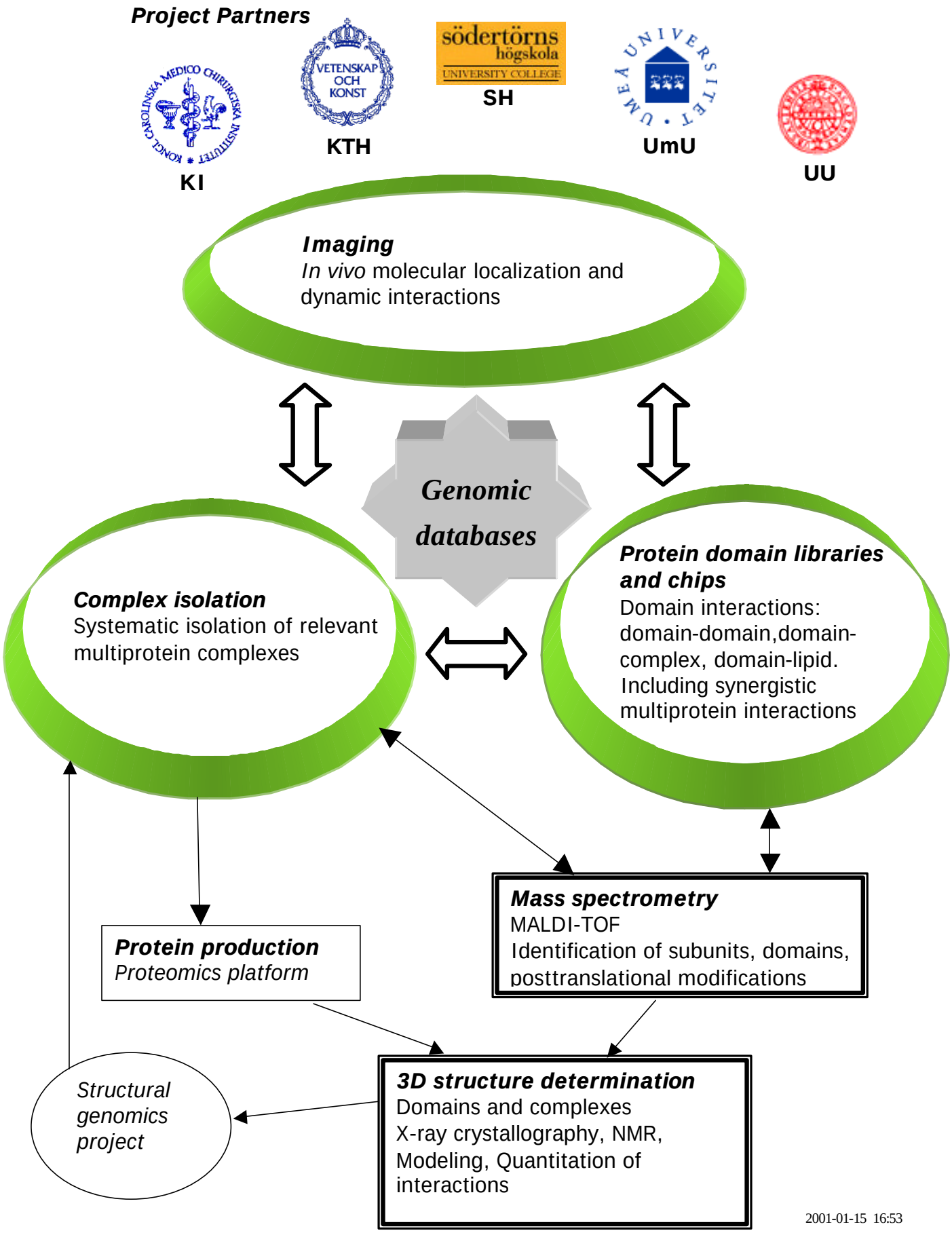
# INTEGRATED PROTEOMICS: MULTIPROTEIN COMPLEXES IN SIGNAL TRANSDUCTION

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# INTEGRATED PROTEOMICS: MULTIPROTEIN COMPLEXES IN SIGNAL TRANSDUCTION

Karolinska Institutet (KI), Kungliga Tekniska Högskolan (KTH), Södertörns University College (SH), Umeå Universitet (UmU), and Uppsala Universitet (UU)

## SUMMARY

In addition to the genome itself, protein-protein interactions form the basis for life as we know it. This novel program integrates proteomics in order to determine the location and composition of dynamic multi-protein complexes. A parallel approach will be applied to interpret signals in a context-dependent manner. The principle is to identify complexes in a temporal and spatial fashion as they are generated in activated cells using fluorescent fusion proteins. Complexes will be isolated, using tandem affinity purification, and the complexes are subsequently analyzed by mass spectrometry. The identified proteins are transferred to protein/lipid chips in order to identify novel partners or the corresponding genes are made as fusions for a new round of selection of multi-protein complexes. Genomic databases are fundamental in the project both for mass spectrometric identification of proteins and in searching for signaling proteins using consensus motifs found in signaling protein modules as detailed below.

The collaborating groups currently are actively studying complexes related to hedgehog signaling proteins, histone deacetylase, integrins, Mediator, the nuclear pore complex, nuclear receptors, SWI-SNF chromatin remodeling and tyrosine kinases. Aided by the joint resources within the project the groups will be able to pursue their studies at a new level and using approaches, which could not be otherwise achieved.

Taking off from the currently studied systems we expect to be able to identify several novel domains, novel complexes and novel interactions in signaling proteins during the course of the project.

## INTRODUCTION

Cellular behavior is regulated by external stimuli and the majority of polypeptides corresponding to the vast number of new genes, which the ongoing genome sequencing projects have identified, are involved in processes that require protein-protein interactions. Signaling pathways pass information via protein-protein interactions from the plasma membrane to structures in the cells, including the nucleus. These signals regulate cell proliferation, apoptosis, differentiation, motility, and more. The proteasome regulates the degradation of proteins, the apoptosome regulates cell death, the Mediator governs transcriptional regulation, and several chromatin remodeling complexes regulate the chromatin structure of individual genes. Some of these interactions are high-affinity, whereas in many cases they are transient. In particular this is true for complexes generated during cellular signaling processes. The very nature of signal transduction is to rapidly form and subsequently disassemble complexes in a dynamic and adaptable fashion.

Signaling proteins contain structural modules that are evolutionary conserved. They form the basis for different protein scaffolds that are required for localized, ordered and dynamic enzymatic reactions within the cell. Since only a minor part of the signaling proteins has yet been identified, our understanding of signaling pathways is fragmentary.

The unraveling of the human genome will allow analysis of signaling proteins in human cells and of their importance for diseases in man. We propose that the wealth of information now available for understanding cell regulation should be explored in a systematic and efficient way. By searching human gene databases with consensus motifs of signaling protein modules, hundreds to thousands of novel signaling proteins are likely to be identified.

This proposal aims to identify these proteins and to assemble complementing technologies in resource centers within a framework of collaborating groups in order to provide a functional genomics approach applicable to the study of a large number of signaling processes. The emphasis is on techniques allowing the study of dynamic, multiprotein complexes. Due to the complexity of these approaches and the multitude of new techniques it is difficult for any single scientific group to keep up with the rapid development.

## RESOURCE CENTERS

### In vivo *molecular localization and dynamic interactions*


To further the understanding of the function of the human genome, methods for rapid screening of the expression of candidate genes in a cellular environment would provide novel clues as to their potential function. The applicants presently have access to seven confocal microscopes and are currently working on complexes listed in the Summary. This new program will allow the development of an integrated analysis of complexes based upon imaging ↔ complex isolation ↔ protein/lipid chips.

### *Development of a highly standardized system for high throughput screening of expressed GFP-tagged genes*

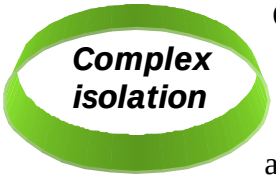
We suggest taking advantage of expression vectors carrying mutants of GFP (green fluorescent protein) for rapid cloning and expression of candidate genes in the 3´or 5´end of the reporter, in all reading frames. An optimized cell transduction protocol for each particular combination of vector and cell type is established. An automated epifluorescence microscopic screening platform equipped with a high precision motorized X, Y and Z control stage will be used. An image analysis system based on the concepts of FISH analysis workstations will be developed in collaboration with Andreas Plesch of Metasystems GmbH, Germany. Today, techniques exist for the automated analysis of cells showing or lacking fluorescence.

We wish to develop a novel screening system allowing high throughput enumeration and characterization of cells expressing proteins according to cellular location such as membrane, cytoplasmic and nuclear fluorescence. The software will be developed for database storage of expression profiles for new genes and cluster analysis-based result reporting. The aim is to perform high throughput analysis of proteins following ligand-induced redistribution. This provides key information concerning spatial and temporal organization of functional molecular complexes, which may form a basis for the isolation of relevant multi-protein complexes using *Complex isolation* or *Protein domain libraries and chips* (see these sections**)**. Following the identification of new proteins in such complexes by mass spectrometry (see flow chart) the corresponding genes are expressed as fluorescent fusion proteins and subjected to high throughput imaging analysis to allow for a new round of search for proteins forming the complexes. The technology also enables the analysis of any set of genes for subcellular distribution of the corresponding proteins and could be used as a general tool for functional genome analysis.

### *Detailed analysis of in vivo molecular interaction using GFP- based reporter constructs*

In addition to rapid screening of gene expression patterns, analysis of the dynamics and interactions of components of multimeric complexes in intact cells are of importance to complement molecular and structural studies. Recently, useful color variants of fluorescent proteins have become available for simultaneous analysis of the expression of multiple genes. The use of selected combinations of these color variants also allows analysis of molecular interactions, based on Fluorescence Resonance Energy Transfer (FRET), particularly in combination with Fluorescence Life Time Imaging (FLIM).

A unique technology for this kind of laser based fluorescence lifetime imaging for multiple fluorophores is the intensity-modulated multiple wavelength scanning system, developed at the Dept of Biomedical Physics, KTH, by Kjell Carlsson and co-workers. The advantages provided by these optical principles for fluorescent microscopy are particularly important for the analysis of in vivo molecular interactions in their almost complete suppression of signal cross talk. Moreover, FRAP (Fluorescence Recovery After Photobleaching) can be used to measure rates of diffusional mobility of fluorescent molecules in biological systems. A chosen area of a living cell – e.g., a portion of a membrane containing a membrane protein tagged with GFP – is bleached by a very intensive laser pulse and the time for unbleached fluorescing molecules from adjacent areas to diffuse into the bleached area is measured. FRAP can both give valuable information of a specific protein's tendency to associate with complexes or other cellular structures and its range of distribution during different experimental conditions. Collectively, this non-invasive approach should generate a large set of biologically relevant observations complementing the other two subprograms of this application.




Our high throughput analysis of ligand-based redistribution of proteins will identify putative complexes and conditions under which these complexes are formed (see previous section). On the basis of this information, proteins of interest will be tagged to simplify purification, and subsequently isolated together with any directly or indirectly interacting components. Complex isolation allows for the identification of all directly or indirectly interacting components in a single experiment. In addition, the purification provides information about the approximate stoichiometry of the proteins present in the given complex, allows for direct biochemical analysis, and identification of any co-purifying ligands.

Traditionally signaling complexes are identified by immuno-precipitation techniques. However, due to the gain in analytical sensitivity, many more proteins are found in the immuno-precipitates than anticipated. This is to a large part due to nonspecific protein-proteins interactions. For a high-throughput approach to complex purification it is therefore necessary to find an alternative to immuno-precipitation. The method most suitable for standardization is affinity purification based on the fusion of a tag, usually a peptide or a small protein, to the target protein. However, protein over-expression is not possible for heteromeric complexes of unknown composition and may also lead to the assembly of over-expressed proteins in non-physiological complexes. Protein complex purification therefore requires expression of the target protein at, or close to, its natural expression levels. Thus, a combination of high-affinity tags will be required for purification.

We intend to employ the newly developed and highly efficient tandem affinity purification (TAP) tag (Rigaut *et al*, Nat. Biotechnol. 1999, 17:1030-2). This technique has previously been used successfully to isolate complexes such as U1 snRNP, Transcription Factor II H, and the Srb/Mediator. The TAP tag consists of a protein A moiety which is linked via a

specific tobacco etch virus (TEV) protease recognition sequence to the second affinity tag, a calmodulin binding peptide. During a first purification, the labeled protein of interest together with specifically and nonspecifically associated proteins is captured on IgG beads. The TEV protease is then used to cleave off the protein A moiety and the complex can be eluted onto the second affinity column, consisting of $Ca^{2+}$/calmodulin beads. After washing the protein complex is eluted under native conditions with EDTA in a highly pure form. Combined with mass spectrometry methods currently available, the TAP methods will allow us to rapidly characterize multiprotein complexes in intracellular signaling. In particular the activity of mutant complexes can easily be analyzed.

Genomic tagging of proteins is easily accomplished in yeast with the use of genetic recombination. For work with human complexes, we intend to generate cell lines stably expressing tagged versions of proteins from a separate promoter. Cell lines will be selected, which express the tagged versions of the proteins at close to physiological levels. For some complexes, however, the introduction of specifically tagged proteins can prove difficult, due to negative effects on complex formation and activity. In these cases we intend to employ an alternative approach, using a combination of either affinity purified polyclonal antibodies or monoclonal antibodies for immunoaffinity purification.

To isolate protein complexes specifically interacting with known protein sequences, e.g. proteins containing *SH2* domains, *SH3* domains, *WW* domains or *PDZ* domains, we will use synthetic peptides immobilized to a matrix. The adsorbed proteins and protein complexes will be resolved on SDS-PAGE and the identity of individual protein bands elucidated. For this purpose and for the purpose of identifying possible posttranslational modifications, we will employ MALDI-TOF mass spectroscopy as well as ESI Ion Trap mass spectroscopy that are available at the Ludwig Institute in Uppsala.


### *Expression of tagged signaling domains*

The approach of searching DNA databases for conserved modules of signaling proteins offers two important possibilities: a) Identification of <u>all</u> signaling proteins, some of which may be difficult to identify even by the most potent alternative methods, e.g. mass spectroscopy analysis of isolated protein complexes. b) Elucidation of functions of the protein, by identification of interaction partners to these domains. We therefore propose to *i*) identify complete sets of selected signaling protein domains in the human genome, and *ii*) express tagged domains for further interaction studies.

In order to achieve these aims, human genome databases will be searched for amino acid sequence motifs with the help of expertise at the Bioinformatics center at Uppsala University. In many cases these domains are easily recognized, but the identification of certain types of modules and atypical variants will require sophisticated sequence analysis methods. A large number of domains of known and unknown proteins are already listed at various databases and available for study. The identified DNA sequences encoding modules of signaling proteins will be amplified by PCR and cloned by highly efficient and rapid methods into vectors for expression as fusion proteins in *E. coli*. We will use the Echo cloning system (Invitrogen) which takes advantage of the possibilities to ligate PCR products directly (without restriction cutting) into a topoisomerase-linked vector, and to directionally transfer the insert from this donor vector to any of a variety of acceptor vectors via in vitro Cre-Lox recombination (bypassing traditional sub-cloning steps). This simple system will allow us to rapidly make plasmid constructs for both bacterial, yeast and mammalian gene expression. Genes of interest can be expressed fused to GFP, GST, 6xHis, or the TAP-tag and used for the various experimental procedures described in this proposal.

The folding of these intracellular protein domains is not dependent on disulfide bridges and can often be produced efficiently in bacteria. Thus, the expression and isolation of 6xHis and GST tagged fusion proteins will follow established protocols and will be relatively cost-efficient. The isolated GST-tagged protein modules will be powerful tools for characterization of interactions with numerous applications. For example, in similar manners as antibodies are used, they can be employed in pull-down assays, western blotting, Biacore applications etc. The proteins that are recognized in these assays will be identified by mass spectroscopy techniques at the Proteomics platforms at Uppsala University and Karolinska Institutet. Selected interactions will be further characterized by structural and biophysical analysis.

### *Protein and lipid chips*

One important application for collections of large numbers of expressed signaling domains is in development of protein chips. Immobilization of many tagged signaling domains on such chips will allow high-throughput screening of, for example, *i*) interactions of selected proteins towards many (all) domains of a particular family, *ii*) binding specificity of potential drugs to members of a domain family, *iii*) the domains as substrates for kinases or other enzymes, *iv*) which signaling pathways are active in a particular cell type or in a disease situation. These assays will require different types of detection and may therefore require different types of chips. In several cases, e.g. in all examples above except *iii*), plasmon resonance appears to be the superior detection method, having the potential of providing information on affinity and kinetics. Biacore, which presently offers chips with up to four channels, are in the process of developing chips with higher capacity. They have expressed a great interest in using the protein domain libraries generated in our project for that purpose. Thus, in a joint effort the Biacore technology will be developed for our proteomics program (see Letter of support in the Appendix section).

A related approach that we intend to explore is to immobilize different membrane lipids on hydrophobic chips available from Biacore. These will be incubated with cell lysates, and any interacting protein(s) will be analyzed by mass spectroscopy. Protein-lipid interactions is an obviously important area that is yet poorly investigated, and our strategy may result in the identification of novel lipid-binding protein domains. These two approaches will complement each other.

### *Proteins to put on chips*

The number of different types of structural modules that so far has been identified in signaling proteins is quite large and growing. As a start, we therefore have to focus our efforts to the following groups of modules of high priority, based on present knowledge of their functions and on the research direction of the applicants behind this proposal. The *SH2* (src homology 2) module is an extensively studied protein domain that specifically recognizes phosphorylated tyrosines together with a few C-terminally adjacent amino acids. The *PTB* (phosphotyrosine binding) domain has the NPXY motif as an essential part of the recognition structure, where the tyrosine in some, but not all, cases has to be phosphorylated. Together, the *SH2* and *PTB* domains are responsible for most interactions that are regulated by tyrosine kinases, including those triggered by growth factor receptors and adhesion receptors. *SH3* (src homology 3) domains are present in numerous adapter proteins and interact with different types of proline-rich motifs. *PH* (pleckstrin homology) modules often mediate membrane localization of proteins by binding to phosphorylated forms of phosphatidylinositol lipids, but interactions with proteins are also common for this domain family. *PDZ* domains bind to several different types of structural motifs, some of which are yet poorly defined. Like *PH* proteins, *PDZ*-containing proteins are often involved in organization of protein complexes at the plasma membrane, one example being synapses in nerve cells. *Death domains* and *Death effector domains* are present in receptors and signaling

proteins that activate cell death. *BIR* is a novel module found in anti-apoptotic proteins mediating cell survival via inhibition of caspases. The LXXLL motif is found in many coactivator complexes and is structurally crucial in the interaction of other proteins with hormone-bound receptors.

## PROTEIN PRODUCTION

It is of course a vital interest that proteins, once identified, can also be purified and produced in sufficient quantities for further study, which for structure determinations means 10-100 mg. The Proteomics platform is planning a high capacity protein production facility that would be very valuable for this purpose.

## MASS SPECTROMETRY

Mass spectrometry today is an established high throughput technique for the identification of proteins based on protease digestion fragment signatures coupled to genomic sequence databases. The ability of mass spectrometry to detect posttranslational modifications, in particular phosporylations, is highly relevant for studies of signaling proteins where such events are key aspects of the process. The availability of both MALDI-TOF and ESI Ion Trap mass spectrometry instrumentation, and expertise, at the Ludwig Institute for Cancer Research in Uppsala, as well as at the Wallenberg funded technology platforms, will be invaluable in the study of protein complexes.

## STRUCTURAL AND BIOPHYSICAL ANALYSES

A central theme of modern research in molecular biology aims at describing the structure, organization and function of well defined macroscopic processes in physical and chemical terms at the molecular or even atomic level. Insight into the functions of proteins, including their interactions in large complexes, requires an understanding of how they acquire and maintain their conformation. It has become increasingly clear that knowledge about mobility, dynamics and energetics must be added to atomic resolution structures in order to obtain a complete picture. This means that no single experimental technique will be sufficient to provide answers to these difficult questions.

In signal transduction pathways with their many interacting protein components it is of importance to characterize individual proteins/domains as well as the interactions between two, or more, components. At the low resolution level one needs to screen for correctly folded proteins and to identify and *quantify* non-covalent interactions, tasks where CD-spectroscopy, fluorescence polarization, microcalorimetry, surface plasmon resonance and analytical ultra centrifugation are powerful methods. Analysis of the raw experimental data from the above techniques requires that the molecularity of the interaction be known. Experimental determination of the molecular mass is often a critical component in this analysis. While mass spectrometry promises unparalleled precision, it is still unreliable in determining the mass of non-covalent complexes. For this, sedimentation equilibrium is the method of choice. The dedicated analytical ultracentrifuge (Beckman XL-I) is capable of analyzing up to 21 samples per day. Using this technique, the masses of the components, the complex and equilibrium binding constant are obtained. Relatively pure components at known concentrations are required.

3-dimensional structure determinations by X-ray crystallography or NMR spectroscopy will be undertaken for domains and interaction complexes, with priority given to domains with novel folds (i.e. low sequence identity to proteins with a known fold), or unexpected properties. The *Structural Genomics Project* would provide a natural collaboration in this context, with a broad approach allowing rapid structure determination of many proteins in a

standardized fashion, allowing expertise within this project to focus on especially interesting or more complex cases.

As it will not always be possible to determine experimentally the 3D structures of all proteins involved in a given biological context, homology based structural modeling will be undertaken to complement experimental work. In contrast to the experimental procedures, comparative modeling can be performed in high throughput-mode, extending structural knowledge from one protein to its homologues. Complexes present even more of a challenge, and here protein-protein and protein-ligand docking algorithms will be applied to screen for potential interactions. More advanced, and more time consuming, modeling can in certain cases be undertaken to carefully define an interaction site, thus going beyond fold characterization.

## PROJECT ORGANIZATION

The multifaceted approach outlined in this proposal is not possible without a close collaboration between different groups with expertise in the main areas. Collaboration between several groups is also required to handle the large amounts of laboratory work necessary for the project.

As indicated in the Budget section funds will mainly be allocated to three major resource centers (imaging, complex isolation, and protein domain libraries/chips) and to the analysis units (MS, structure determination, quantitative interaction analysis) available to the collaborating groups.

For each resource center there will be one or two coordinators, who together with the project principal investigator form the steering group for the project:

| | |
|---|---|
| PI: | Lennart Nilsson (KI) |
| Imaging: | Edvard Smith (KI) and Kjell Carlsson (KTH) |
| Complex isolation: | Claes Gustafsson (KI) and Lars Rönnstrand (UU) |
| Protein/lipid chips: | Staffan Johansson (UU) |

The steering group will be supported by an international advisory board, for which 4-5 renowned experts covering the main aspects of the project will be recruited. Funds are allocated in the budget for regular meetings with the advisory board, 1-2 times/year.

To aid the collaborating groups in their recruitment of research personnel, short-term recruitment fellowships will be made available by the steering group upon application.

It will be the responsibility of the PI to establish contacts with relevant Platforms and other Projects within the Consortium, and also with the South-West Consortium.

Information about the project, its progress and available tools, will be presented on web pages updated monthly. Meetings will be arranged for all participants annually and for smaller constellations as the need arises.

We believe that this project is unique in Sweden, and with its biological focus combined with the expertise of the partners we believe that it is also internationally competitive.

### *Interactions with the Wallenberg Consortium North*

Several facilities within the ***proteomics platform*** would be very useful for our project, including protein production and mass spectrometry.

The ***structural genomics project***, which also intends to focus on signaling pathways, would be a natural partner in that signaling proteins of unknown fold identified within the present project could be added to the target list of the structural genomics project.

# APPENDICES

## COLLABORATING GROUPS

| *Area* | *Name and affiliation* |
|---|---|
| **S** | Ass. Prof. Kurt Berndt, Södertörns University College, Natural Sciences Section |
| **P** | Ass. Prof. Stefan Björklund, Umeå University, Dept. of Medical Biosciences, Medical Biochemistry |
| **I** | Prof. Kjell Carlsson, KTH, Department of Biomedical and X-ray Physics |
| **I** | Prof. Birger Christensson, Karolinska Institutet, IMPI |
| **P** | Prof. Ted Ebendal, Uppsala University, Developmental Neuroscience |
| **I,C** | Ass. Prof. Karl Ekwall, Södertörns University College, Natural Sciences Section |
| **I,C** | Ass. Prof. Donald Gullberg, Uppsala University, Cell and Molecular Biology |
| **C** | Ass. Prof. Claes Gustafsson, Karolinska Institutet, Department of Medical Nutrition |
| **I,C** | Prof. Jan-Åke Gustafsson, Karolinska Institutet, Department of Medical Nutrition |
| **I** | Ass. Prof. Einar Hallberg, Södertörns University College, Natural Sciences Section |
| **C,P,M** | Prof. Carl-Henrik Heldin, The Ludwig Institute for Cancer Research, Uppsala Branch |
| **C,P** | Prof. Staffan Johansson, Uppsala University, Medical Biochemistry and Microbiology |
| **S** | Prof. Rudolf Ladenstein, Karolinska Institutet, Department of Bioscience |
| **I,P** | Prof. Dan Lindholm, Uppsala University, Developmental Neuroscience |
| **S** | Prof. Lennart Nilsson, Karolinska Institutet, Department of Bioscience |
| **C,P** | Prof. Lars Rask, Uppsala University, Medical Biochemistry and Microbiology |
| **I,C,P** | Prof. Kristofer Rubin, Uppsala University, Medical Biochemistry and Microbiology |
| **C,P,M** | Prof. Lars Rönnstrand, Ludwig Institute for Cancer Research, Uppsala Branch |
| **I** | Prof. Edvard Smith, Karolinska Institutet, Department of Bioscience |
| **I,C** | Prof. Rune Toftgård, Karolinska Institutet, Department of Bioscience |
| **C,S** | Prof. Anthony Wright, Södertörns University College, Natural Sciences Section |

**I**=Imaging, **C**=Complex isolation, **P**=Protein/lipid chips, **M**=Mass spectrometry, **S**=Structural and biophysical studies

### Partners

Prof. Anders Blomberg, Gothenburg University, Department of Microbiology (Mass spectrometry)

## BUDGET JUSTIFICATION

The amount of work which can be done is more or less proportional to the available resources – about two thirds of the budget is personnel and consumables.

*Imaging year 1*

Inverted Zeiss epifluorescence microscope, will be equipped with a high precision motorized X, Y and Z control stage and a back illuminated or intensified very high sensitivity CCD camera controlled by a computer workstation. 1.1 MSEK.

One engineer, software development 0.6MSEK; one engineer, microscope development 0.6MSEK; one engineer with expertise in biophysics 0.6MSEK; 0.5 research technician, generation and supply of cloning vectors for high throughput analysis 0.2 MSEK.

Total cost year 1: 3.1MSEK

*Imaging year 2*

413 nm Kr-ion laser (Enterprise II k Discmaster VI from COHERENT) for FRET studies 0.8 MSEK

One engineer, software development 0.6MSEK; one engineer, microscope development 0.6MSEK; one engineer with expertise in biophysics 0.6MSEK; 0.5 research technician, generation and supply of cloning vectors for high throughput analysis 0.2 MSEK.

Total cost year 2: 2.8MSEK

*Complex isolation year 1*

BioFlo 5000 fermentor, 40 l (New Brunswick Scientific Co) (0.97 MSEK)

0.5 research technician for fermentor/bioreactor work (0.2 MSEK)

2 postdoctoral fellows (0.5 MSEK) + 1 graduate student (0.4MSEK) + 2 research technicians (0.4 MSEK) for tagging, purification, and analysis of protein complexes.

Consumables 0.4 MSEK

Total cost year 1: 3.4 MSEK

*Complex isolation year 2*

Bioreactor CelliGen Plus, 5 l (New Brunswick Scientific Co) (0.44MSEK)

0.5 research technician for fermentor/bioreactor work (0.2 MSEK)

2 postdoctoral fellows (0.5 MSEK) + 1 graduate student (0.4MSEK) + 2 research technicians (0.4 MSEK) for tagging, purification, and analysis of protein complexes.

Consumables 0.4 MSEK

Total cost year 2: 3.2 MSEK

*Protein chips year 1*

4 computers (100 kSEK), incubator, shakers for bacteria culture (75 kSEK), freezer (75 kSEK), temperature cycler for PCR (100 kSEK), electrophoresis units for DNA and protein (100 kSEK), centrifuges (100 kSEK). Subtotal 0.55 MSEK.

Consumables: Reagents for PCR, echo-cloning, culture media for bacteria, affinity-Sepharose, electrophoresis chemicals, etc, etc (0.9 MSEK), charge for mass spectroscopy analysis (0.1 MSEK). Subtotal 1MSEK.

Personnel: 2 postdoctoral fellows (1 MSEK), 2 research technicians/graduate students (0.8MSEK).

Total cost year 1: 3.35MSEK

*Protein chips year 2*

Personnel: As above (1.8 MSEK)

Consumables: As above (1MSEK), Chips (0.2MSEK)

Total cost year 2: 3.0MSEK

*Physical characterization of interactions year 1*

High speed CCD camera for X-ray diffraction data collection ca 0.8 MSEK. This would substantially reduce the time for structure determinations once crystals are obtained; it is not absolutely essential to the project and can be assigned a lower priority.

2 postdoctoral fellows (1 MSEK)

Running costs 0.3 MSEK

Total cost year 1: 2.1MSEK

*Physical characterization of interactions year 2*

Beckman XL-I Raleigh optics and rotor (1.8 MSEK)

2 postdoctoral fellows (1 MSEK)

Running costs (0.3 MSEK)

Total cost year 2: 3.1MSEK

*General*

Recruitment support, 8 short-term fellowships( 0.8 MSEK/year)

Advisory board meetings (0.2 MSEK/year)

Travel and meetings within the project (0.2 MSEK/year)

**BUDGET SUMMARY**

| Amounts in kSEK | **2001** | **2002** | **Priority** |
|---|---|---|---|
| **Equipment (> 150 kSEK)** | **2 870** | **3 040** | |
| *Imaging* | | | |
| Inverted Zeiss epifluorescence microscope | 1 100 | | 1 |
| 413 nm Kr-ion laser for FRET | | 800 | 1 |
| *Complex isolation* | | | |
| Bioflo 5000 fermentor, 40 litres | 970 | | 1 |
| Bioreactor CelliGen Plus, 5 litres | | 440 | 1 |
| *Structural and biophysical studies* | | | |
| Beckman XL-I Raleigh optics and rotor | | 1 800 | 1 |
| High speed CCD camera for X-ray diffraction data | 800 | | 2 |
| | | | |
| **Equipment (< 150 kSEK)** | **550** | | |
| Computers, incubator, shakers | 175 | | |
| Freezer, centrifuges | 175 | | |
| PCR temperature cycler | 100 | | |
| Electrophoresis units | 100 | | |
| | | | |
| **Personnel** | **7 200** | **7 200** | |
| Postdocs, graduate students | 3 400 | 3 400 | |
| Research engineers | 1 800 | 1 800 | |
| Technicians | 2 000 | 2 000 | |
| | | | |
| **Running costs** | **2 900** | **3 100** | |
| Advisory board meetings | 200 | 200 | |
| Travel and meetings within the project | 200 | 200 | |
| Recruitment fellowships (8x100kSEK) | 800 | 800 | |
| Consumables | 1 700 | 1 900 | |
| | | | |
| **GRAND TOTAL:** | **13 520** | **13 340** | |

## LETTER OF SUPPORT

To

Evaluation committee

Wallenberg Consortium North

After a contact with Prof. Staffan Johansson about the application called: ”Integrated Proteomics: Multiprotein Complexes in Signaling Transduction” we have decided to add a letter of support. The presented research area is of great interest for future technical application and product improvement. However, it must be noted that some applications and studies are ordinary applications that can be performed by established BIACORE techniques whereas others considerably can improve future product development. It is applications of the latter type that is of interest for the R&D function in the company.

Our future development is directed towards array chip analysis, faster mass spectrometry combinations and drug – protein interactions that join function with structure. We have announced that we will develop real-time surface plasmon resonance detection technology for array analysis of protein interactions. Important aspects besides the technical solutions are purified molecular targets and a biological knowledge of consequences of changed concentrations and molecular properties. Therefore, a close interaction with this project would certainly increase the speed in product development.

It should also be mentioned that we have an active participation in a project called:” Broad scale implementation of proteo-chemometrics aiming for mapping the chemical space in the proteins”, organized by Prof. Jarl Wikberg, Uppsala University. There can also be other projects that can generate interesting results for future product development based on surface plasmon resonance technology. We would certainly be interested to follow results generated in this large effort to support functional genomics research in Sweden to further strengthen Swedish biotech industry.

Yours sincerely

Magnus Malmqvist

Senior Scientific Advisor

BIACORE AB

Technical Operations