LSHG-CT2003-530203

QUASI

Quantifying signal transduction

Specific targeted research project STREP

Life Sciences, Genomics and Biotechnology for Health

QUASI Final Report

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Project coordinator name: Stefan HOHMANN
Project coordinator organisation name: Goeteborg University UGOT Draft 1

Publishable final activity report

1. Project execution

Summary description of project objectives

The overall objective of QUASI was to assess the dynamic and quantitative operation of signal transduction pathways and to elucidate relevant paradigms. For this it was necessary to measure and quantify different, preferably all, steps in signalling pathways in a time-dependent way taking into account the actual spatial distribution of the components. In order to generate such data QUASI optimised and applied a range of different generally applicable tools and methods that allow to monitor signalling, where possible in single living cells. Those tools will be instrumental in developing quantitative analyses of signal transduction further. QUASI employed the tools to analyse a model MAP kinase signalling network (the yeast HOG and pheromone pathways) with the goal to reach a new level of understanding of its dynamic operation. Crucial to the success of the project was mathematical pathway modelling as well as a realistic illustration of pathway dynamics.

Overall, QUASI has been a most successful project and its results, tools and approaches will have future impact on research on MAPK pathways and quantitative systems biology. Significant achievements are data-based mathematical descriptions of predictive value of yeast osmoregulation encompassing different processes, a model of the yeast pheromone pathway and models describing cross talk between those pathways. Around the generation of those models generally applicable experimental and mathematical tool development was driven ahead significantly and new knowledge was generated.

With its overall project vision, QUASI has been at the forefront of the move from descriptive biology to quantitative and systems biology. In fact, QUASI was started before the EC called for proposals for systems biology the first time. Several countries in Europe as well as individual universities now invest into this “new biology” with major grants (e.g. Germany and the UK) as well as dedicated institutes (e.g. Manchester, Basel/Zurich, Heidelberg, Edinburgh, Barcelona). The aim of quantitative/systems biology is to truly understand and explain the design of biological networks and processes by employing novel measurements with the help of physics and chemistry and to realistically reconstruct in the computer such processes with the help of mathematicians and computer scientists. A major goal of systems biology is to build mathematical models with predictive power, i.e. to correctly simulate experiments that have not previously been done in the laboratory. Predictive models need to be based on appropriate data, which largely cannot be found in the literature or databases. In QUASI biologists, physicists, chemists and mathematicians closely collaborated to generate such data and the mathematical models.

Signal transduction pathways are the cellular information routes with which cells monitor their surrounding as well as their own state and adjust to environmental changes or hormonal stimuli. Signal transduction pathways orchestrate cellular metabolism, establish stress tolerance, control growth, proliferation and development and determine morphogenesis. Consequently, signal transduction pathways are critically involved in disease processes. This is true especially for MAPK (Mitogen-Activated Protein Kinase) pathways. Altered signalling through MAPK pathways by genetic or physiological changes causes diseases or generates fatal disease symptoms. In order to better understand disease development, causes and symptoms, to design drugs that more precisely target MAPK signalling and to correctly apply those drugs and to more reliably predict drug action in qualitative and quantitative terms it is necessary to achieve a a true understanding of the dynamic operation of MAPK signal transduction pathways and to more reliably predict drug action in qualitative and quantitative terms.

MAPK pathways are conserved throughout eukaryotes. They consist of three tiers of protein kinases that sequentially activate each other: a MAPKKK, a MAPKK and a MAPK. This conserved module

is controlled by systems consisting of sensors/receptors, G-proteins and protein kinases, which can be of different type. MAPK mediate responses by controlling further protein kinases and transcriptional regulators, again of different type. Protein phosphatases are negative regulators of MAPK pathways. MAPK pathways are also controlled by both feed-forward and feed-back loops. Each cell type expresses numerous MAPK pathways, which interact and cross-talk in highly complex manners and often share components. In this way, the specific signalling routes are impossible to predict on the basis of protein sequence conservation, which otherwise allows, within limitations, prediction of MAPK pathway components.

Our level of understanding remains restricted largely (and if it all) to the wiring schemes of MAPK pathways. Hence, we may comprehend the direction and sometimes the mechanism of information flow. The parameters that are crucial for the operation of signalling pathways are the amplitude and the period of pathway activity, the spatial organisation and subcellular movements of the signalling components as well as their assembly into larger complexes that insulate signalling and control cross talk between pathways. QUASI has advanced our understanding of several of these quantitative, time-dependent, spatial aspects of signal transduction, which are crucially important to correctly target signalling pathways with existing drugs or to design new drugs. An important issue in monitoring signalling events is that most commonly used techniques are invasive and monitor processes in a whole cell population, tissue of multi-cellular organism. While still suitable, these approaches may not always give a correct impression of the actual time line of events. Therefore it is crucial to monitor, where possible, signal transduction in individual living cells.

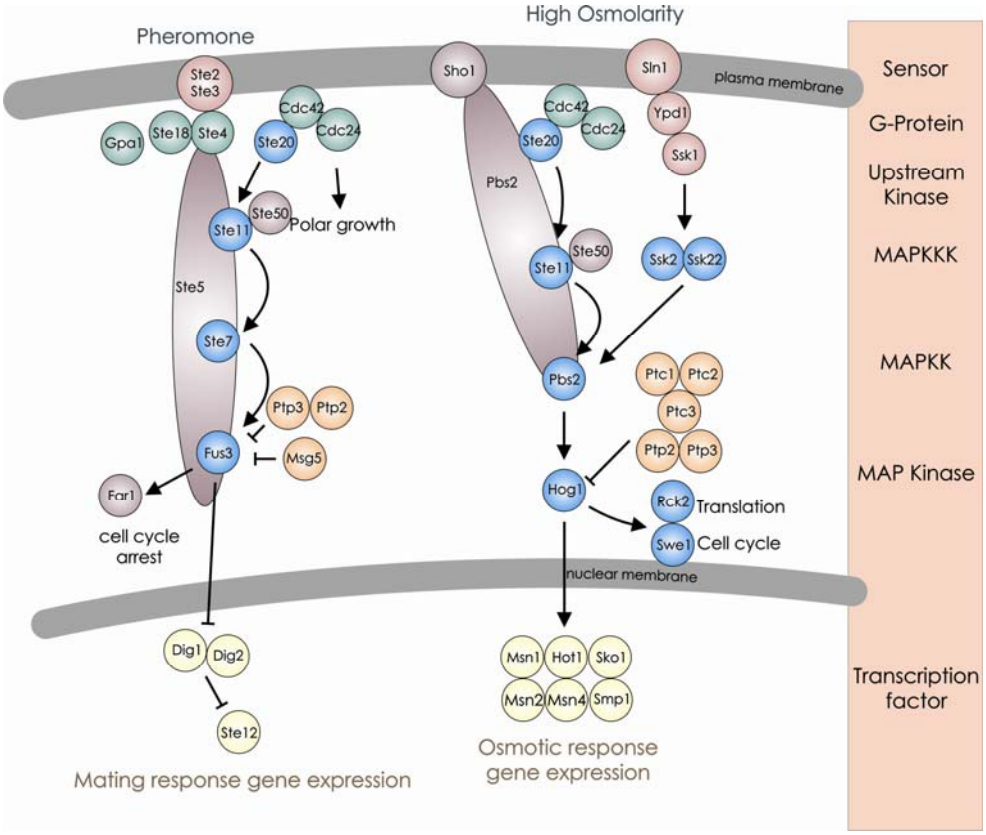


Fig. 1 *The yeast Hog and pheromone response pathways share components and communicate with other but still stimulate stimulus specific responses.*

To address fundamental quantitative questions of MAPK signalling the QUASI consortium chose the yeast HOG (High Osmolarity Glycerol) and mating (pheromone response pathways (Fig. 1). These two pathways, that share components and communicate with each other in not fully understood ways, have been studied extensively by genetic and biochemical approaches. They are regarded as the best-understood MAPK systems. Our knowledge about their quantitative and dynamic features has increased substantially through work performed in QUASI.

QUASI initially phrased eight project objectives when the project was proposed almost five years ago. As described on further pages, in several instances the initially planned approaches were revised to establish new experimental approaches and abandon approaches that were too ambitious or not up-to-date any longer. The initial objectives are briefly discussed on the basis of current state of the art.

1. *To monitor activated protein kinases in the cell. For this, a range of immunoreagents will be used to quantify key phosphorylation events. In addition, a recently established method based on stable isotope labelling and mass spectrometry will be applied.*
   To monitor and quantify protein modifications, in general, remains a major challenge in proteomics these days. Within several large projects the EC invests into development of relevant technology. Indeed, with the development of new mass spectrometry technologies, which were then applied in QUASI, protein phosphorylation can be monitored at proteome-wide scale although it only can partly be quantified. Therefore, a combination of mass spectrometry for detection plus employing immuno-reagents for quantification of specific protein phosphorylation seems to be the most optimal approach for studying the dynamics of signalling pathways at the moment .

2. *To determine dynamic signalling events in single living cells using advanced microscopic and optic tools.*
   Our ability to visualise and quantify biomolecules in individual living cells is still strongly limited. However, at several locations, including QUASI laboratories, developments are driven making use of novel microscopes, optical manipulation tools and microfluid systems. QUASI has made significant contributions in tool and software development.

3. *To follow protein complex formation dynamics in the living cell. For this QUASI will employ protein tags that allow the use of specific cross-linking reagents for protein complexes in solutions as well as on DNA templates in the cell. In addition, we will use activatable GFP variants and advanced microscopy/optics to determine cellular movement and assembly of individual subunits and protein complexes.*
   There are numerous techniques and approaches to study protein complexes. It remains a major challenge to identify functionally relevant and transient protein interactions and to determine timing and extent of such interactions. QUASI planned to approach this problem both by chemical/biochemical as well as microscopic approaches. As described below, the work has been redirected resulting in the development of a novel approach to study and quantify protein interactions in living cells.

4. *To specifically, rapidly and temporarily inhibit signalling components in the living cell. For this QUASI will design functional protein kinase variants that are sensitive to highly specific inhibitory compounds and will also initiate development of inhibitors based on protein kinase target structure.*
   Tools of chemical genetics are superior in many instances to knock-out mutations, which often stimulate compensatory effects in the cell blurring the primary role of the kinase and because knock-outs often disrupt protein complexes. Moreover, such chemical tools often are a first step towards development of lead structures for drug development. The basic concept has been proposed several years ago but so far been applied in research in only a limited number of cases. QUASI has pushed this technique by developing novel chemical inhibitors and using different kinase mutants. At the same time, the approach has been used by several laboratories world-wide together demonstrating is general applicability.

5. *To identify direct protein kinase targets and to quantify kinase-substrate reactions. For this QUASI will develop and verify ATP analogues recognised by specific, modified protein kinases. The combination of kinase and ATP analogue will result in the phosphorylation and labelling of only the immediate protein kinase targets, which will then be determined by gel electrophoresis*

and mass spectrometry.
To identify the targets in the cell of protein-modifying enzymes is another major challenge of proteomics, because relevant recognition motifs in putative target proteins are insufficient as diagnostic tool. The chemical approach, closely related to the one under (4), is extremely promising but so far has been used successfully in only few cases. QUASI has been driving the development by designing new ATP analogues. At the same time, identification of protein kinase targets by mass spectrometry combined with chemical approaches seems to be the most powerful approach at this stage.

6. *To quantify in parallel all possible steps of dynamic signalling from sensing to response. QUASI will employ available tools and those optimised in this project to further elucidate the cellular network of signal transduction through MAP kinase pathways and to monitor and quantify all experimentally accessible events from sensing to response. The project will generate large datasets that describe quantitatively the time-dependent and spatially organised operation of signalling processes.*
   An important part of systems biology and the building of kinetic mathematical models concerns generation of data that allow calculation of realistic model parameters. Rather than taking an undirected global approach, QUASI performed experiments and data generation in close cooperation between experimentalists and model designers, providing an example for multidisciplinary research in systems biology. The project delivered for modelling integrated data sets from a large number of events in the signalling system.

7. *To establish, improve and employ kinetic mathematical models that reproduce the dynamic operation of signalling pathways and generate hypotheses for experimental testing and verification. These models will interpret the datasets generated to describe and reproduce the signalling events and elucidate the rules with which the pathways are activated and deactivated as a system. The models will generate hypotheses for further experimental analysis and will be optimised throughout the project in an iterative manner with experimental studies.*
   Building, using and improving mathematical description and elucidating systems properties is at the essence of quantitative and systems biology. QUASI was well integrated and in contact with numerous other projects and networks in systems biology ensured that up-to-date tools were used in QUASI and that results obtained will be useful for other projects.

8. *To generate visualisation tools based on mathematical models and scientific intuition to communicate the dynamic cellular signalling to the educated and general public.*
   This part of QUASI involved experts in graphical information design. Visualisation and communication is regarded as an essential tool in research development.

Partners of the project continue to work at the forefront in signal transduction as well as quantitative biology, as documented by publications in high impact journals. Moreover, most research groups that took part in QUASI are also involved in the new UNICELLSYS project that will drive the developments initiated in QUASI significantly further in the coming five years.

Contractors involved

Partic. Role*	Partic. No.	Participant name	Participant short name	Country	Date enter project	Date exit project
CO	1	Göteborg University	UGOT	S	Month 1	Month 42
CR	2	Universitat Pompeu Fabra	UPF	E	Month 1	Month 42
CR	3	Institut für Biochemie und Molekulare Zellbiologie der Universität Wien	UNIVIE	A	Month 1	Month 42
CR	4	Eidgenössiche Technische Hochschule Zürich	ETH Zurich	CH	Month 1	Month 42
CR	5	Max Planck Society – Institute for Molecular Genetics	MPIMG	D	Month 1	Month 42
CR	6	Mälardalens Högskola	MDU	S	Month 1	Month 42

*CO = Coordinator

CR = Contractor

Co-ordinator contact details

Prof. Stefan Hohmann
Department of Cell and Molecular Biology
Göteborg University
Box462 (courier: Medicinaregtan 9E)
40530 Göteborg, Sweden
Tel: +46 31 3608488; +46 733547297; Fax: +46 31 7862599; E-mail: stefan.hohmann@gu.se
Web: http://www.gmm.gu.se/groups/hohmann
Project website: http://www.idp.mdh.se/quasi/

Note that phone and fax numbers as well as the coordinator's mail address has changed during the final project period.

Achievements with respect to objectives and the state-of-the-art

The consortium regards QUASI as a major success – significant advances have been made in our understanding of MAPK signalling and the development of quantitative experimental tools and principles of mathematical modelling. The consortium believes that it has shown great flexibility with adjusting the initial project plan to the development of new technologies and abandon approaches that turned out to be too ambitious or out of date. Some work also had to be reduced due to the fact that partner 4 did not receive full funding from the Swiss funding organisation.

1. To monitor activated protein kinases in the cell

QUASI had chosen two approaches to monitor activated protein kinases in the cell,: (a) To generate a range of immunoreagents to quantify key phosphorylation events; (b) To establish a method based on stable isotope labelling and mass spectrometry.

(a) Already during the first reporting period QUASI had produced several useful antisera against the phosphorylated forms of Hog1 target proteins: the transcription factors Msn2 and Sko1 as well as the protein kinase Rck2. With generating the next set of such antisera the consortium decided to wait for the results of a mass spectrometry analysis performed by partner 3 that resulted in a long list of potential Hog1-dependent phosphorylation events. Following this analysis a panel of 20 phospho-peptides was synthesised by a commercial service and used for immunisation of rabbits. Unfortunately those antisera become only available after the end of QUASI but will be extremely useful tools for analysis in future projects. They target the following proteins, in several cases different phosphorylation sites: Pbs2, Sho1, Ptk2, Ste5, Ypk2, Gpd1, Nth1, Rck2, Ede1, Ifh1, Msn2.

(b) It became apparent that the general approaches to quantitative MS would yield at the best only semi-quantitative measurements. Therefore we changed our approach from assaying a collection of known signalling components to a global approach and attempted to identify all proteins targeted by the Hog1 MAPK in yeast. We identified a number of previously unknown Hog1 targets and several interesting regulated phosphorylation events that clearly expand our knowledge about the Hog1 signalling network. Around 90 regulated SP/TP sites were identified, manually verified and assigned as putative Hog1 targets. By monitoring the activation loop of Hog1 we could show that the SILAC data largely conform well to the immunological data. The study performed in QUASI is one of the most comprehensive mass spectrometry studies on signalling performed so far and data compare well with other studies recently published.

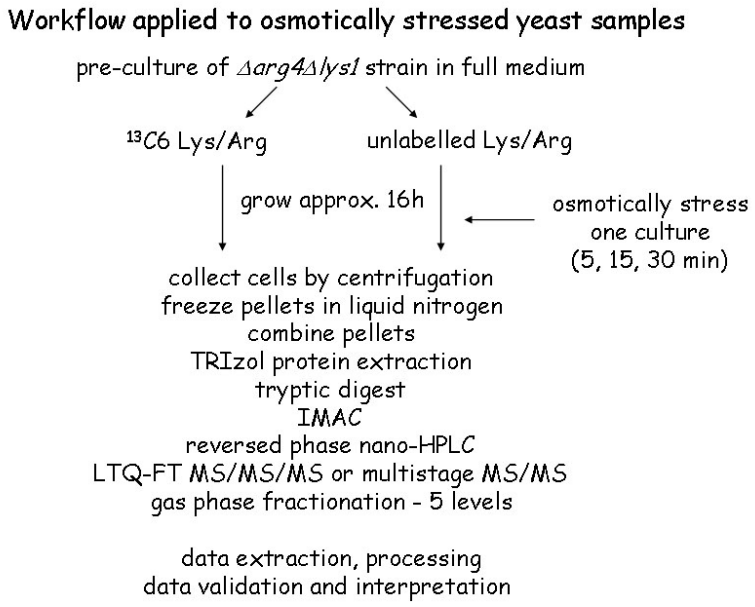


Fig. 2. *Scheme for the determination and quantification of phospo-peptides from yeast by SILAC, IMAC and nano-HPLC ESI-mass spectrometry*

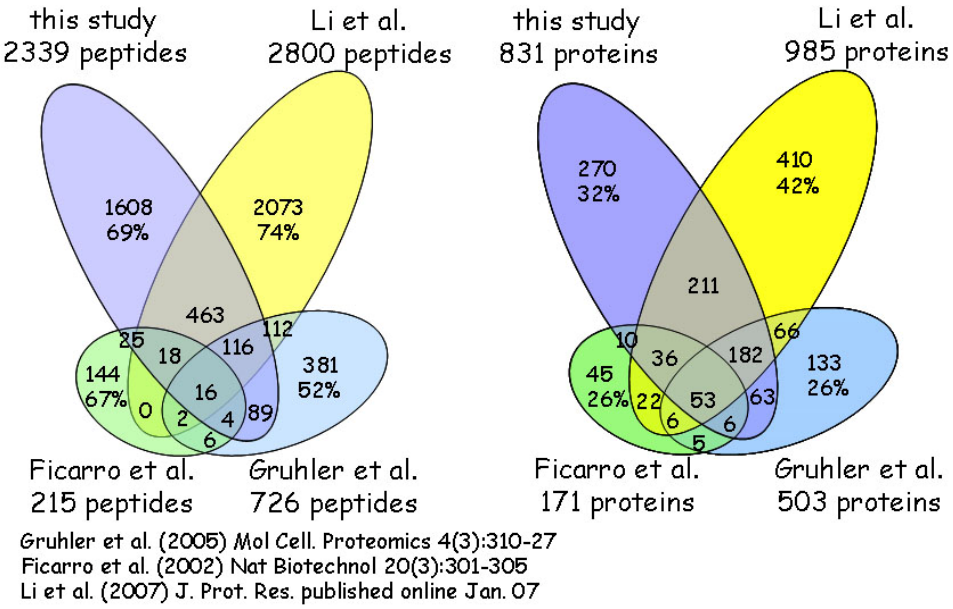


Fig. 3. *Comparison of peptides and proteins from different large-scale MS studies in yeast. Notice the large difference in overlap. It should be mentioned that our collection of phospho-sites was not purged from peptides derived by miss-cleavage or from ambiguous assignments. However, some of the more extensive published studies also do not exclude forms derived by miss-cleavage or ambiguous sequence assignments. Some studies lack estimates for false positive rates. Others did not attempt to measure relative changes in abundance, a fact that makes direct comparisons between the different studies sometimes difficult.*

Summary self assessment: This work initially took a difficult start, has taken far longer than initially thought and has been revised along the way. However, the results eventually obtained are extremely useful for understanding signalling dynamics and the generally applicable tools and reagents developed will be heavily used in future quantitative analyses of signalling.

2. To determine dynamic signalling events in single living cells

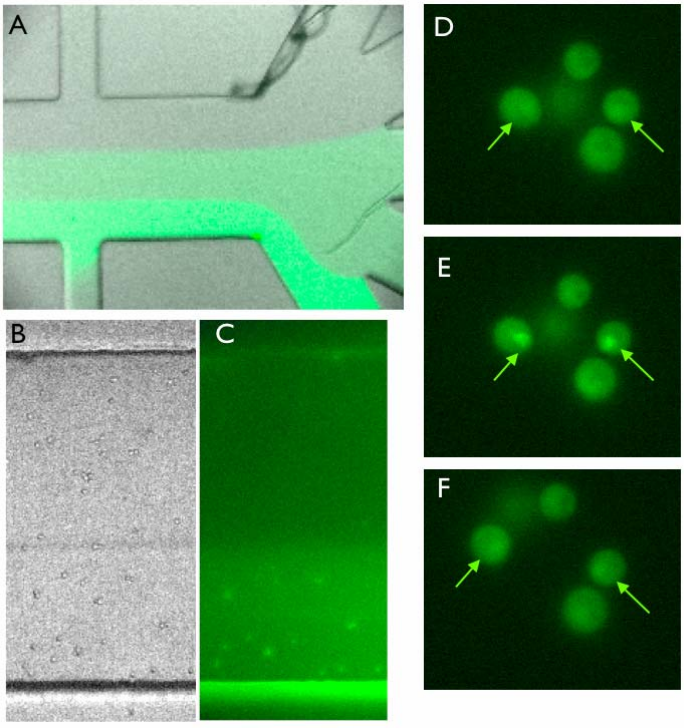


Fig. 4. *Application of microfluidics to biology. A: Image of a microfluidic device where three flow containing different concentrations of a fluorophore merge without mixing. B and C: Phase contrast and fluorescence image of a microfluidic channel were yeast cells have been subjected to two different media, only the cells in the lower part of the channel have started expressed a fluorescent reporter. D, E and F: Hog1-GFP cells in a microfluidic channel before (D), during (E) and after (E) osmotic stress. These three pictures depict the possibility of switching rapidly the media surrounding the cells. Two cell with a clear Hog1 relocation are highlighted with an arrow*

Single cell analyses are becoming centrally important for systems biology. Within a cell population individual cells may respond to environmental changes in a uniform fashion or they may show significant cell-to-cell variation for

instance due to different cell cycle stages. Therefore, response profiles need to be scored in a number of individual cells. In addition, observing signalling in real time requires microscopic analyses. Single cell studies require suitable fluorescent reporters. Development of such reporters and bioimaging tools was a central role of Partner 4.

Partner 4 developed XFP-based reporter systems that allow monitoring of signalling in real time (via nuclear shuttling of Hog1) as well as mapping cell population profiles of cellular responses (via suitable promoter-XFP studies). For the former approach microfluidic devices were developed that allow controlled and rapid changes of the cell environment. In addition, software tools for cell identification and quantification were developed. Both tools will be extremely useful for future research (Fig. 3).

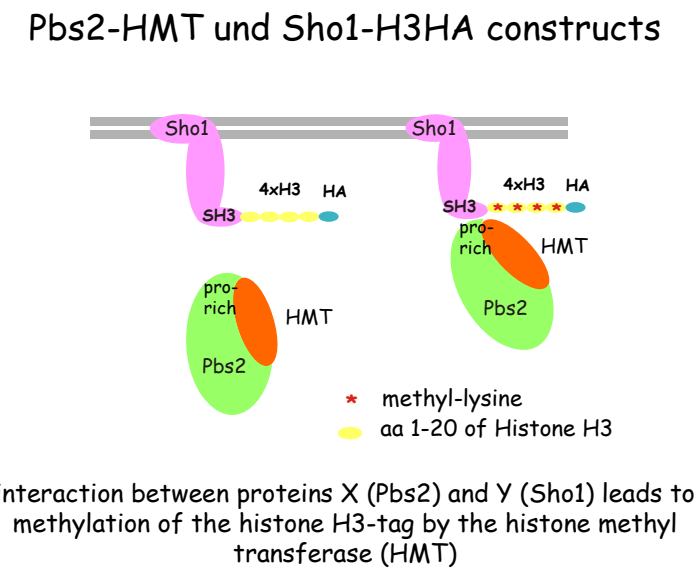
For the latter approach a four-fold venus-YFP was developed in a stable but also an unstable variant. The latter allows for the first time analysis not only of signalling turn-on but also of signalling turn-off. Employing either microfluidics and microscopic investigation or controlled cultivation combined with flow cytometry analysis data were collected that provided interesting new information on the control of signalling and data relevant for improving the structure of mathematical models. For instance, based on data from single cell analysis partner 4 postulated the existence of a novel regulatory mechanism in the nucleus that regulates transcriptional output of activated Hog1.

Summary self assessment: Following a slow start in the first reporting period this part of the project has been extremely successful and is now leading to publications in leading journals. The novel tools developed for single cell analysis will be extremely powerful for future quantitative studies of signalling not only in yeast. An initially planned development of monitoring mRNAs in living cell was abandoned because of technical difficulties and due to under-funding of the Swiss partner.

3. To follow protein complex formation dynamics in the living cell

Signal transduction is based on transient interaction of, for instance, protein kinases and scaffold proteins. QUASI planned to establish or advance the tools for monitoring such transient protein interaction and protein complex formation by (a) chemical cross-linking followed by mass spectrometry as well as (b) by microscopy.

(a) The initially planned approach (employ protein tags that allow specific cross-linking protein complexes in solutions as well as on DNA templates in the cell) was not followed due to technical difficulties.



interaction between proteins X (Pbs2) and Y (Sho1) leads to methylation of the histone H3-tag by the histone methyl transferase (HMT)

Fig. 5. *Principle of the M-tracking protein interaction assay applied to Pbs2 and Sho1.*

Instead, partner 3 established an entirely new method that allows monitoring and quantifying protein interaction in the cell. This variant of the two-hybrid system is not dependent on directing target proteins to the nucleus but rather monitors interaction at the natural location of the relevant proteins. In principle, the technique is also not confined to yeast but requires in the cell absence of histone methyl transferases (HMTs). Partner 3 has now used this new method to monitor, over time, different protein interactions

in the yeast HOG pathway and generated a host of most interesting results that expand our knowledge and provide data for modelling. For instance, it was observed that Hog1 indeed interacts with Sho1 in a Ste11 and Pbs2 dependent way, which suggests a specific mode of feedback regulation.

(b) The technology to monitor protein interactions in cells is well-established at Partner 4 and most of the required GFP (CFP, YFP) fusion proteins are available. Together with the tools developed under (2) it is now possible to monitor in the living cell transient protein interactions. However, this approach has not been used to collect data during the lifetime of the QUASI project.

Summary self assessment: This work had a slow start and initial technical difficulties. However, QUASI has generated a new tool to monitor protein interactions in the living cell and produced most useful data. Hence, although this work took a different direction than initially planned it is regarded as one of the most successful components of the project.

4. To specifically, rapidly and temporarily inhibit signalling components in the living cell

Chemical inhibition of proteins, provided it can be done in a highly specific and rapid manner, is potentially superior over knock-out mutations because cells may adapt to such mutations and the observed phenotype may therefore reflect secondary events. QUASI planned to further advance the so-called Shokat approach (because invented by Kevan Shokat) based on the inhibition by a bulky ATP analogue of a mutant protein kinase (as mutant) with a wider ATP binding pocket. QUASI designed novel ATP analogues and protein kinase mutations and successfully employed those in the project.

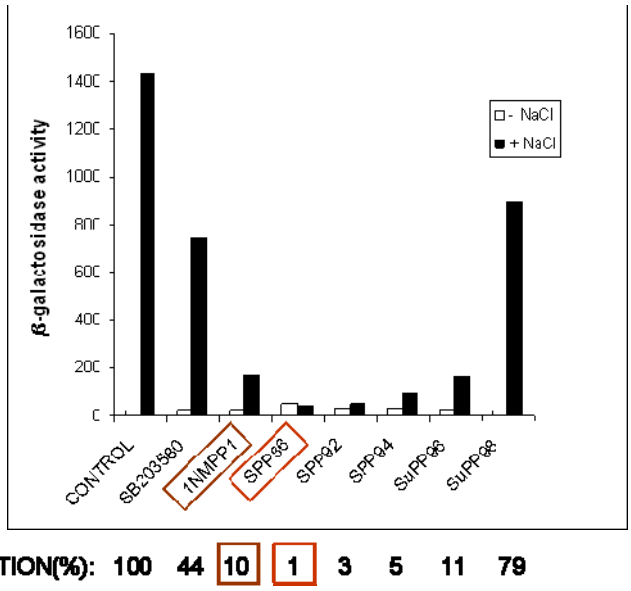


Fig. 6. *Inhibition of gene expression by different ATP-competitive inhibitors in a hog1as modified strain. STL1:LacZ expression was analysed in absence or presence of osmostress and STL1 expression was monitored by B-galactosidase activity.*

Several protein kinases were targeted by mutation (partner 2) and several new ATP analogues were developed (partner 1). Indeed it turned out that one of the new compounds, SPP86, could inhibit the mutant Hog1 kinase (Hog1as) faster and more complete than the previously available compounds (1NMPP1), which is an important achievement. In general it appears that for each kinase a panel of different mutant-inhibitor combinations should be tried. Using as mutants and compounds several new features of HOG pathway signalling could be observed. For instance, it was observed that Hog1 phosphorylation and the degree of gene expression not always correlate. In addition, the novel compounds are presently in use also in other projects, such as in AMPKIN and will be used in the future in UNICELLSYS.

Summary self assessment: QUASI regarded this as an important, though technically challenging part of its work. The goals of this work have been achieved beyond initial expectation and experiments yielded important new information on signal transduction. This work has hence been successfully completed.

5. To identify direct protein kinase targets and to quantify kinase-substrate reactions

The underlying approach, i.e. use of ATP analogues recognised by modified protein kinases is very similar to that under (4), in particular the kinase mutants are identical. Partner 2 has tested in vitro the Hog1 mutant variant with a known ATP analogue that can be used to phosphorylate target proteins. These tests were successful and provided proof-of-principle for the approach. It was then used on a panel of 70 proteins purified by tandem-affinity-purification (TAP) and helped confirming the findings obtained with SILAC and mass spectrometry under (1).

Summary self assessment: The technology has been successfully established and employed with certain modifications. It turned out to be a very useful complement to mass spectrometry. This work has hence been successfully completed.

6. To quantify in parallel all possible steps of dynamic signalling from sensing to response

QUASI has agreed on the use of standard strains as well as a standard experimental setup for data collection. Data collected in this way were then used for mathematical modelling.

On this basis of a range of different types of time-resolved data have been generated that has then been used for modelling. This included:

- Post-translational modifications (chiefly phosphorylations) and ubiquitin-mediated degradation of signalling proteins, using epitope-tagged proteins or antibodies to native proteins: A large amount of time course data have been generated by Western blotting on the MAPK Hog1 and Fus3 under different standard and perturbation conditions. Mass spectrometry has generated semi-quantitative time course data for many other proteins. Degradation has been studied in detail for Ste5-Ste11.
- Protein kinase activity has been monitored mainly for the Hog1 kinase.
- Protein complex formation has been monitored using the newly developed M-tracking method.
- Protein relocalisation has been extensively used to monitor signalling in real time following extensive tool development for microfluidics and image analysis both under standard conditions and following certain perturbations.
- Transcription of target genes using micro arrays as well as Northern analysis and RT-PCR has been monitored under standard conditions and following different perturbations.
- Occupancy of specific responsive promoters using ChIP analyses and real-time PCR techniques has been quantified for the Hog1 kinase.
- Posttranscriptional effects using separation of polysomal mRNA has been extensively monitored under different stress conditions and yielded a wealth of unexpected results.
- Low-molecular weight compounds (glycerol, trehalose, cAMP) have focused on glycerol measurements under standard and different perturbation experiments.
- Systematic examination of the effects on the dynamics and quality of the response of the pathway by changes in the extent, timing, and type of external stimulus provided has been performed extensively by all experimental partners and a large amount data have been provided to modelling.
- Rates of nuclear shuttling of the Hog1 kinase at single cell level.
- Population profiles on Hog1 localisation and Hog1-dependent gene expression were monitored.

Many of the parameters were not only monitored in wild type cells under standard conditions but also following different types of perturbations such as in mutants affected in signalling or adaptation, in the presence of kinase inhibitors or different types or degrees of stress.

QUASI also developed a software tool that allows comparing Western blot data from different experiments.

QUASI has generated a unique dataset that to our knowledge has not been produced before for any other signalling system. In addition, also a range of data were made available on the pheromone pathways, the link of both the HOG and pheromone pathways to cell cycle control as well as for cross-talk between the two pathways.

Summary self assessment: QUASI has not been able to produce data on all steps of the signalling system as initially proposed but still has generated a unique quantitative dataset for mathematical modelling.

7. To establish, improve and employ kinetic mathematical models

A major goal of QUASI was to generate mathematical models that reproduce and predict the dynamic operation of signalling pathways and generate hypotheses for experimental testing and verification. During the first reporting period partner 5 has published a model of the yeast pheromone response pathway, and partners 5 and 1 together have generated an integrative mathematical model of yeast osmoadaptation.

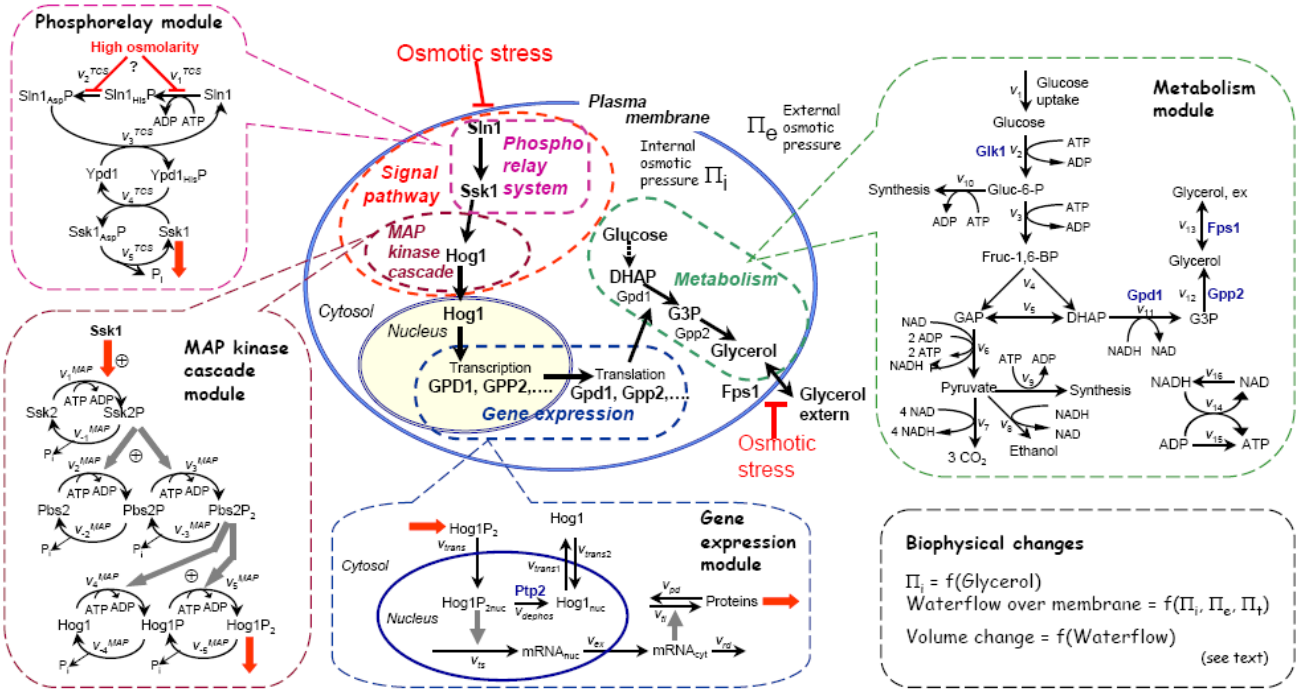


Fig. 7. *Overview of yeast osmoregulation for the purpose of modelling, representing the different modules into which the system was divided.*

In the second project period those models were refined with new data, simplified to generate models better supported by the data. In addition, models were generated that describe and predict cross talk features, describe details of glycerol accumulation and links to cell cycle regulation. A wealth of novel biological information as well as new expertise in modelling has emerged. Moreover, in the second part of the project, all experimental groups had specific collaborations with the modelling partner indicating that QUASI has contributed to wider use of modelling in cell signalling research at the highest level.

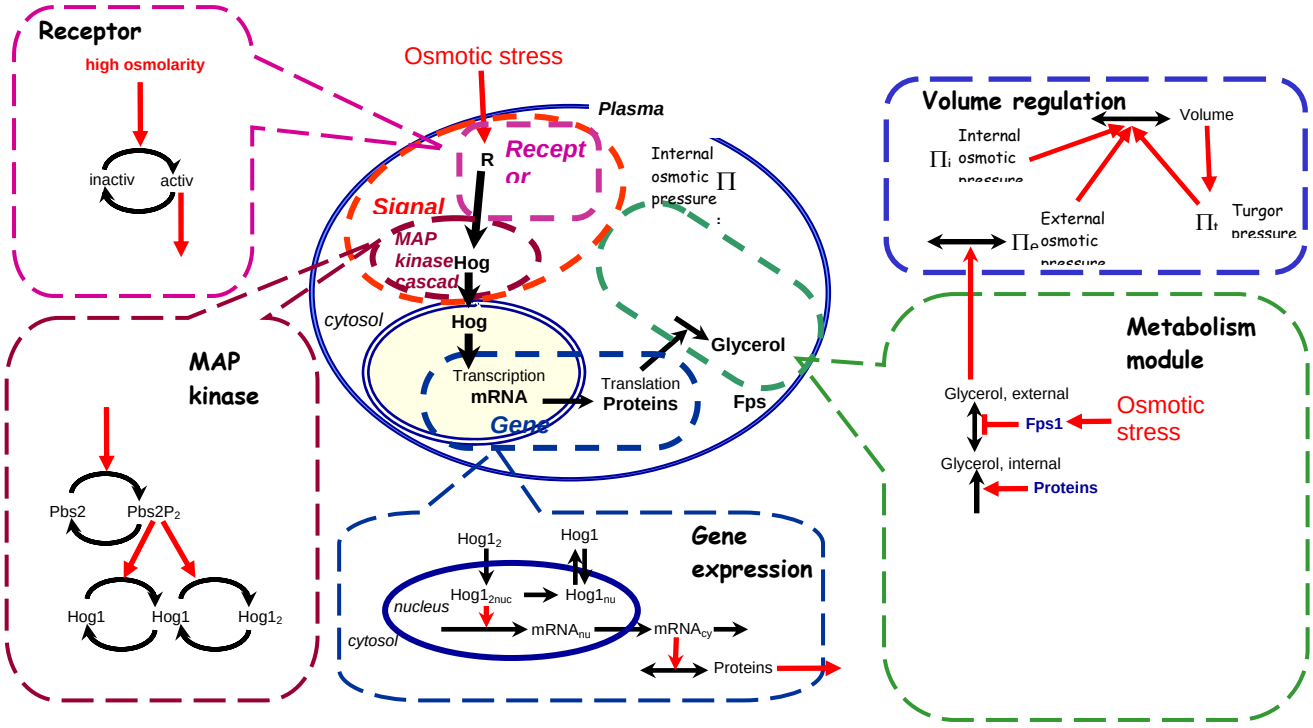


Fig. 8 *Data-centred approach: The core model. The modular structure of the standard model for the HOG pathway served as a starting point but only those components are kept which are indispensable to maintain a modular structure and on which data was available.*

Summary self assessment: This has been a particular successful part of the project. All goals have been achieved and significant work is still to be published.

8. To generate visualisation tools based

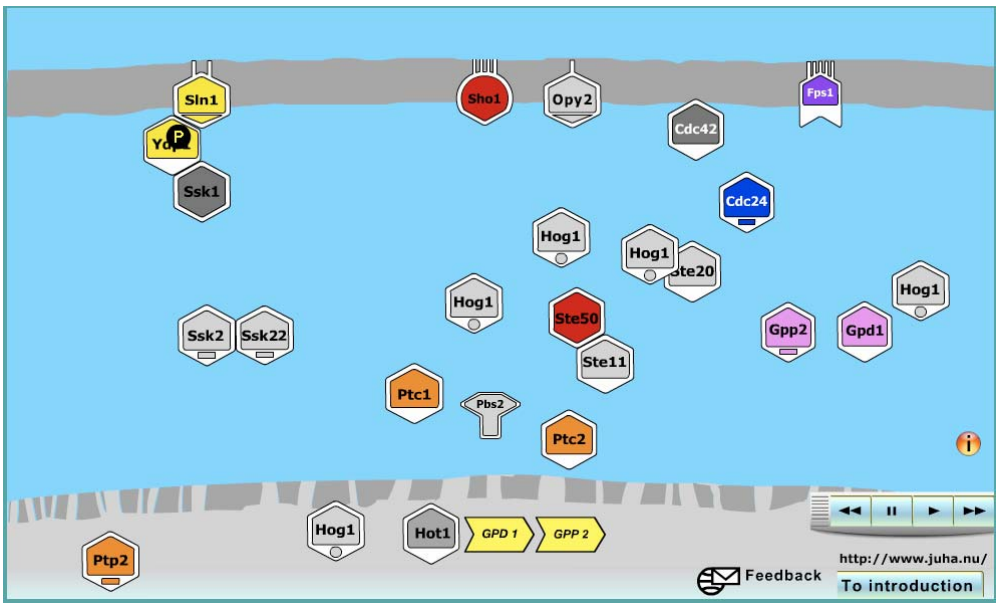


Fig. 9. *The current version of the Hog1-pathway animation (May 2007)*

QUASI has generated animations of yeast signalling pathways (pheromone pathway and HOG pathway for different audiences. The initial idea to could those to real computational models and actual time courses turned out unrealistic, but the animations are anyway most useful for dissemination purposes.

Summary self assessment: This work is been completed to satisfaction of the consortium.

Project impact

The QUASI consortium did not produce results of potential commercial value. Instead, the main goal of QUASI was to increase our understanding of the dynamics of MAPK signalling and to advance quantitative, "systems-level" analyses of signalling pathways. For this, QUASI chose to bring together groups that perform experiments and collect data with a group that generates mathematical models of entire signalling systems are parts thereof.

This integrated approach has been extremely successful. Also groups in the project (partners 2, 3, and 4) that did not previously collaborate with models, did so in different constellations within QUASI to address specific research questions. Also the idea of basing experimental studies on computational simulations was followed to a significant, although perhaps not to the maximally possible extend.

QUASI ensured its impact in the very first place by publication in the scientific literature. Although the project has published a number of individual and joint papers already during its lifetime, numerous of the results of QUASI will only be published within about 12-18 months after the end of the project. It is planned to place publications on the QUASI website as they appear.

QUASI has further ensured its impact via organising two conferences/workshops. The first one was held on Göteborg in 2005 with 140 delegates and a range of invited speakers from Europe and overseas. This conference has generated wide visibility for the project and numerous contacts between QUASI scientists and other researchers interested in quantitative analyses and mathematical modelling. The second workshop was held in Rostock in September 2006 and more confined with about 40 participants and designed as bringing together three related EC-funded projects QUASI, AMPKIN and COSBICS. This workshop has resulted in valuable interaction between those projects.

The QUASI proposal was submitted in spring 2003 following a call for projects "employing multidisciplinary functional genomics approaches to decipher basic mechanisms underlying signal transduction and intracellular communication as well as transcriptional activation". At this time the term "systems biology" was not even used with the EC framework programme; dedicated calls were published only later. Still, QUASI has been regarded as one of the first, or the first, systems biology projects funded by the EC. Therefore it has generated visibility and certainly influenced the design of future programmes as well as the setup of project proposals. QUASI has served as an example of an interdisciplinary systems biology project and its successful completion might have consequences for setting up future projects that aim at increased quantitative understanding.

The tools that QUASI generated for instance for mass-spectrometry, single cell analysis, inhibition of protein kinases and mathematical modelling are generally applicable, i.e. not confined to the use of yeast as experimental organism. Those tools are already being used in other projects both by groups involved in QUASI and other groups. Tools and reagents will be used further in the UNICELLSYS project, due to start in 2008, where kinetic modelling will be pushed significantly ahead within a consortium of leading experimentalists and modellers, including the QUASI partners 1-5.

QUASI has generated new knowledge on MAPK signalling, including knowledge on different feedback mechanisms operating via adaptation or via direct, phosphorylation-mediated feedback on signalling components, the link between signalling and cell cycle control, the integration of different signalling branches as well as cross-talk between different signalling pathways sharing components. Obtained with yeast as a model system this knowledge has general applicability given the conservation of the basic design of MAPK pathway and their importance for human health. QUASI has and will publish its results in high impact journals thereby ensuring dissemination of its results.

QUASI has generated different types of animations of the dynamics of the pathways under study. This has been a useful exercise both for phrasing scientific hypotheses as well as for dissemination.

2. Dissemination and use

Section 1 - Exploitable knowledge and its Use

QUASI did not generate results that will have a potential for industrial or commercial application in research activities or for developing, creating or marketing a product or process or for creating or providing a service. All research tools developed are not of the type that they could be marketed, but instead will be published and made available to the research community.

A possible exception concerns ATP analogues to inhibit protein kinases.

Overview table

Exploitable Knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
ATP analogues for use as protein kinase blockers, and their chemical synthesis	*ATP analogues*	*Biomedical research*	*Could be commercially available in 2008*	*Once suitable compounds are available and a viable synthesis process has been devised: patent application (possibly 2006)*	***Partner 1*** ***(M Grøtli)***

ATP analogues for use as protein kinase blockers, and their chemical synthesis

The exploitable results are the ATP analogues as well as the corresponding mutations in protein kinases, provided those are different from those proposed by K. Shokat. The overall concept has been developed by Shokat, and hence the developments in this project can be regarded as an optimisation.

Exploitable, and protectable, would be the ATP analogue and the process of chemical synthesis. Exploitation would occur via licensing to a company that will produce such compounds.

The market for such compounds would be scientific research in academia and industry, especially research that aims at blocking specific protein kinases in the cell. Commercialisation would involve a company that provides products for research.

Partner 1 (M Grøtli) has designed the compounds and develops their chemical synthesis and hence would be the sole owner of the intellectual property.

Contacts with potential partners for protection/licesning will be taken at a suitable point in the development process.

Section 2 – Dissemination of knowledge

Overview table

Planned/ actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
August 2005	Press release on the occasion of publication in Nat Biotechnol.	General public	Those of partners in the project	Potentially millions	Partner 1
June 2005	Conference	Research	Global	140	Partner 1/All
September 2006	Workshop	Research	Europe	100	Partner 5/All
Constantly	Publications	Research	Global	Hundreds	All
Spring 2004	Project web-site	Research/public	Global	Hundreds	Partner 1 and 6
August 2006	Posters	Research/public	Determined by EC	Thousands	Partner 1
August 2006	Flyers	Research/public	Determined by EC	Thousands	Partner 1
Fall 2005	Animations	Research/public	Global	Hundreds	Partner 6/All

1. Press release

A press release was published in the mid of August 2005 on the occasion of publication a joint article in Nature Biotechnology (see below) and a symposium held in Göteborg on Aug 29 (symposium is not sponsored by QUASI but QUASI will be presented).

2. Conference and workshop

The QUASI conference has taken place in 10-12 June 2005 in Göteborg with 140 delegates. The scientific programme centered around the question of generating the data for systems biology. The speakers were truly top-scientists from all over the world. The conference was partly supported by QUASI as well as by other projects.

In September 2006 the projects COSBICS, AMPKIN and QUASI organised a joint workshop with speakers from the three projects as well as invited speakers. The programme is appended to the report.

Members of the QUASI consortium attend international workshops, conferences and courses on a regular basis and present QUASI results there.

3. Publications

Partner 1:

1. Medrala D, Nordlander B, Geijer C, Hohmann S Gene expression profiles dependent on stress period. In preparation.
2. Nordlander B, Klipp E, Hohmann S and others Control of a eukaryotic osmolyte system. In preparation.
3. Hohmann S, Krantz M, Nordlander B. Yeast osmoregulation. Methods Enzymol. 2007;428:29-45.

4. Eriksson E, Enger J, Nordlander B, Erjavec N, Ramser K, Goksor M, Hohmann S, Nystrom T, Hanstorp D. A microfluidic system in combination with optical tweezers for analyzing rapid and reversible cytological alterations in single cells upon environmental changes. Lab Chip. 2007 Jan;7(1):71-6.

5. Gennemark P, Nordlander B, Hohmann S, Wedelin D. A simple mathematical model of adaptation to high osmolarity in yeast. In Silico Biol. 2006;6(3):193-214.

6. Mustacchi R, Hohmann S, Nielsen J. Yeast systems biology to unravel the network of life. Yeast. 2006 Feb;23(3):227-38. Review.

7. Krantz M, Becit E, Hohmann S. Comparative genomics of the HOG-signalling system in fungi. Curr Genet. 2006 Mar;49(3):137-51.

8. Krantz M, Becit E, Hohmann S. Comparative analysis of HOG pathway proteins to generate hypotheses for functional analysis. Curr Genet. 2006 Mar;49(3):152-65.

9. Klipp E, Nordlander B, Kruger R, Gennemark P, Hohmann S. Integrative model of the response of yeast to osmotic shock. Nat Biotechnol. 2005 Aug;23(8):975-82.

10. Pettersson N, Filipsson C, Becit E, Brive L, Hohmann S. Aquaporins in yeasts and filamentous fungi. Biol Cell. 2005 Jul;97(7):487-500.

11. Hohmann S. The Yeast Systems Biology Network: mating communities. Curr Opin Biotechnol. 2005 Jun;16(3):356-60. Review.

12. Karlgren S, Pettersson N, Nordlander B, Mathai JC, Brodsky JL, Zeidel ML, Bill RM, Hohmann S. Conditional osmotic stress in yeast: a system to study transport through aquaglyceroporins and osmostress signaling. J Biol Chem. 2005 Feb 25;280(8):7186-93.

13. Klein, M, Krainz K, Redwan, IN, Grøtli, M. Synthesis of Chiral 1,4-Disubstituted-1,2,3-Triazole Derivatives from Amino Acids *Submitted*

14. Klein M, Morrilas M, Brive L, Posas F, Grøtli, M. Design, synthesis and evaluation of potent kinase inhibitors by a chemical genetic approach. *In preparation*

15. Klein M, Grøtli M. One pot synthesis of novel 3-triazolyl-1H-pyrazolo[3,4-d]pyrimidines using microwave assisted heating. *In preparation*

16. Hult M, Sunnerhagen P, Posas F and others (2008) Polysomal RNA profiles reveal the complexity of the transcriptional/translational response to stress. In preparation.

Partner 2:

1. de Nadal, E., Zapater, M., Alepuz, P.M., Sumoy, L., Mas, G., & Posas, F. The Hog1 MAP kinase recruits the Rpd3 histone deacetylase to activate osmoresponsive genes. Nature, 427 (6972): 370-374 (2004).

2. Tomas-Cobos L, Casadome L, Mas G, Sanz P & Posas, F. Expression of the HXT1 low-affinity glucose transporter requires the coordinated activities of the HOG and glucose signalling pathways. J. Biol. Chem., 279 (21): 22010-9 (2004).

3. Escoté, X., Zapater, M., Clotet, J. & Posas F. Hog1 mediates cell-cycle arrest in G1 phase by the dual targeting of Sic1. Nat. Cell. Biol., 6 (10): 997-1002 (2004).

4. Zapater, M., Clotet, J., Escoté, X. & Posas F. Control of cell cycle progression by the stress-activated Hog1 MAPK. Cell Cycle, 4 (1):6-7 (2005).

5. Clotet J, Escote X, Adrover MA, Yaakov G, Gari E, Aldea M, de Nadal E, Posas F. Phosphorylation of Hsl1 by Hog1 leads to a G2 arrest essential for cell survival at high osmolarity. EMBO J. 7;25(11):2338-46.

6. Kohler A, Pascual-Garcia P, Llopis A, Zapater M, Posas F, Hurt E, Rodriguez-Navarro S. The mRNA Export Factor Sus1 Is Involved in SAGA-mediated H2B Deubiquitinylation through Its Interaction with Ubp8 and Sgf11. Mol Biol Cell. 17: 4228-36 (2006).

7. Thorsen M, Di Y, Tangemo C, Morillas M, Ahmadpour D, Van der Does C, Wagner A, Johansson E, Boman J, Posas F, Wysocki R, Tamas MJ. The MAPK Hog1 Modulates Fps1p-dependent Arsenite Uptake and Tolerance in Yeast. Mol Biol Cell. 10: 4400-10 (2006).

8. Proft M, Mas G, de Nadal E, Vendrell A, Noriega N, Struhl K, Posas F. The stress-activated Hog1 kinase is a selective transcriptional elongation factor for genes responding to osmotic stress. Mol. Cell. 21;23(2):241-50 (2006).

9. Meritxell Zapater, Marc Sohrmann, Matthias Peter, Francesc Posas & Eulàlia de Nadal. Selective requirement for SAGA in Hog1-mediated gene expression depending on the severity of the external osmostress conditions. Mol. Cell. Biol. 11: 3900-10 (2007).

10. Clotet, J & Posas, F. Control of cell cycle in response to osmostress: lessons from yeast. Methods Enzymol. 428: 63-76 (2007). De Nadal, E., Real, F.X. & Posas, F. Mucins, Osmosensors in Eukaryotic Cells? Trends in Cell Biol. In press (2007).

Partner 3:

1. DeWever, V., W. Reiter, A. Ballarini, G. Ammerer, and C. Brocard. 2005. A dual role for PP1 in shaping the Msn2-dependent transcriptional response to glucose starvation. *EMBO J.* 24: 4115-4123

2. Hosiner, D., H. Lempiäinen, W. Reiter, J. Urban, R. Loewith, G. Ammerer, R. Schweyen, D. Shore, and C. Schüller. Arsenic inhibits TORC1 kinase and differentially modulates the Sfp1 and Msn2/4 transcriptional activators in budding yeast (Current Biology)

3. De Wever, V., A. Petryshyn, A. Rötzer, C. Schüller, C. Brocard and G. Ammerer. Yeast PP2A/Cdc55 protein phosphatase coordinates the transcripional response to osmotic stress via multiple mechanisms.

4. Dohnal, I., C. Friedmann, A. Zuzuarregui, G. Ammerer: Changes in the dynamics of signal protein interactions during osmotic stress in yeast.

5. Dohnal, I., D. Anrather, J. Veis, C. Stingl, K. Mechtler, F. Posas, and G. Ammerer: Changes in the phospho-proteom of yeast during hyper-osmotic stress.

Partner 4:

1. Dard, N. and Peter, M. (2006) Scaffold proteins in MAP kinase signaling: more than simple passive activation platforms. BioEssays, 28, 146-156.

2. Rudolf, F., Pelet, S., and Peter, M., (2007) Regulation of MAPK Signalling in yeast. Topics in Current Genetics, 19, MAPK Signalling, Posas, F., and Nebrada, A. (eds), in press.

3. Kuepfer, L., Peter, M., Sauer, U., and Stelling J., (2007) Ensemble modeling for analysis of cell signaling dynamics. Nature Biotechnology, 25, number 9, in press.

4. Pelet, S, Rudolf, F., and Peter, M. (2008) Kinetic analysis of the MAPK signaling by quantitative single cell microscopy. In preparation.

5. Rudolf, F, Dard, N., Pelet, S., vanDrogen, F., Klipp, E. and Peter, M. (2008) The scaffold Ste5p functions as an E3-ligase to modulate signalling kinetics by degrading the MEKK Ste11p. In preparation.

6. Rudolf, F., Posas, F, Ammerer, G. and Peter, M. (2008) A novel E3-ubiquitin ligase regulates stress signaling by degrading the MEKK Ste11p. In preparation.

Partner 5:

1. Klipp, E. 2007. Modeling dynamic processes in yeast. Review. *Yeast*. **DOI:** 10.1002/yea.1544.
2. Diener, C. (2007) Spatio-temporal simulations for the pheromone signaling pathway in MATa cells of *Saccharomyces cerevisiae*. Bachelor thesis, FU-Berlin.
3. Klipp E, Liebermeister W, Helbig A, Kowald A & Schaber J. 2007. Systems Biology standards – the community speaks. *Nature Biotechnology*, **25**(4), 390-391.
4. Schaber J., Kofahl B., Kowald A., Klipp E. (2006) A modelling approach to quantify dynamic crosstalk between the pheromone and the starvation pathway in baker's yeast. FEBS Journal 273:3520-3533.
5. Klipp E., Schaber J. (2006) Modelling the dynamics of Stress Activated Protein Kinases (SAPK) in cellular stress response. Book chapter. accepted.
6. Klipp E., Schaber J. (2006) Modeling of Signal Transduction in Yeast - Sensitivity and Model Analysis. In 'Understanding and Exploiting Systems Biology in Biomedicine and Bioprocesses'. Fundación CajaMurcia, Mucia, Spain. p.15-30 15-30
7. Uhlendorf, J. (2006) Development of a mathematical model for the occupancy of mRNA with ribosomes in *Saccharomyces cerevisiae*. Bachelor thesis, FU-Berlin.
8. Klipp E & Liebermeister W. 2006. Mathematical modeling of intracellular signaling pathways. BMC Neuroscience. **7** Suppl 1:S10.
9. Klipp, E. & Hohmann, S. 2005. Simulation von Lebensprozessen. *BIOForum*, **10**, 60-61.
10. Klipp E, Nordlander B, Kruger R, Gennemark P, Hohmann S (2005) Integrative model of the response of yeast to osmotic shock. Nat Biotechnol 23:975-82.
11. Kofahl B, Klipp E (2004) Modelling the dynamics of the yeast pheromone pathway. Yeast 30:831-50
12. Klipp E, Nordlander B, Kofahl B, Hohmann S (2004) Shutting the MAP off – and on again? Current Genomics 5: 637-647

Partner 6:

1. Pettersson, R., Meldert, M., & Kroneskog, A. (2005). *Designing a web site for a multinational research project.* Presentation at the International Professional Communication Conference, IPCC 2005 Annual Conference, Limerick, Ireland, July 10-13. Published in: *2005 IEEE International Professional Communication Conference Proceedings.* 0-7803-9028-8/05/
2. Pettersson, R., & Fundberg. B. (2007). *Animation as a tool in information materials.* Presentation at Information design conference. Greenwich, London. March, 29-30.

4. Project website

The project website is running at http://www.idp.mdh.se/quasi/. So far it has mainly served internal purposes but it will be extended with elements for the general public now that the Nat Biotechnol paper has appeared and first animations are available.

5. Posters and flyers

Those were delivered to the EC services.

6. Animations

First versions of the animation of yeast osmoregulation has been produced by Partner 6. This animation will be further optimised. An animation of the mating process will follow. These animations will be used both in scientific lectures, for teaching purposes and for addressing the general public.

Section 3 - Publishable results

QUASI has not produced exploitable results that are ready for publication.

3. Final management report

Form C Financial statement per activity for the contractual reporting period, to be completed by each contractor (see Appendices 5-11)

These items are attached to the report as separate items

Audit certificates
These items are attached to the report as separate items

4. Final report on the distribution of the Community's contribution

To be prepared to the coordinator following the final payment by the EC

5. Final science and society reporting questionnaire

Attached to the report as separate file.

6. Final reporting questionnaire on workforce statistics

Attached to the report as separate file.

7. Final socio-economic reporting questionnaire

Attached to the report as separate file.