PROJECT FINAL REPORT

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4.1 Final publishable summary report

4.1.1 Executive Summary

Metabolic engineering is an applied science focusing on developing new cell factories or improving existing ones. Metabolic engineering distinguishes itself from applied genetic engineering by the use of advanced analytical tools for identification of appropriate targets for genetic modifications and the use of mathematical models to perform *in silico* (computer based) design of optimized cell factories. In recent years, there has been increasing focus on using mathematical models for design and SYSINBIO has been coordinating European activities in the field of model driven metabolic engineering and activities on other technologies required for state of the art metabolic engineering, e.g. metabolomics (measurements of internal metabolites) and fluxomics (measurements of metabolic fluxes). The coordination of activities has resulted in the establishment of a database containing metabolic models for different industrially important microorganisms (the BioMet ToolBox available at www.sysbio.se). The database also contains different simulation tools required for use of these models to identify metabolic engineering targets and use of these models for analysis of omics data (high throughput measurements of biologically relevant entities such as proteins or messenger RNAs). SYSINBIO has also worked on coordinating further development of techniques required for metabolic engineering, such as metabolomics, fluxomics and identification of mutations in evolved strains through whole genome sequencing using second generation sequencing approaches. Several publications, in particular reviews, summarizing the state of the art in the field have been published in connection with SYSINBIO.

A key part of SYSINBIO was to coordinate activities on developing new and better mathematical models of metabolism that can be used for metabolic engineering. Activities on both stoichiometric models and kinetic models have been coordinated. The use of stoichiometric models has been further advanced and we have defined methods that allows for rapid setting up this kind of models. A fundamental problem with stoichiometric models is that it is necessary to perform a manual annotation and curation of the many different reactions, which is time consuming and laborious. We have therefore formulated methods and standards that will allow for faster development of this kind of models, and we have also developed a database of existing models that can be used as scaffolds for building new models. In the field of kinetic models we have performed a detailed review of the state-of-the art on the use of these models, and based on this we have drafted recommendations, in particular for use in focussed industrial metabolic engineering projects.

4.1.2 Concept and Objectives

Metabolic engineering is an applied science focusing on developing new cell factories or improving existing ones. There are several definitions, but most of these are consistent with: *the use of genetic engineering to perform directed genetic modifications of cell factories with the objective to improve their properties for industrial application.* In this definition the word improve is to be interpreted in its broadest sense, i.e. it also encompasses the insertion of completely new pathways with the objective to produce a heterologous product in a given host cell factory. Metabolic engineering is an enabling science, and distinguishes itself from applied genetic engineering by the use of advanced analytical tools for identification of appropriate targets for genetic modifications and the use of mathematical models to perform *in silico* design of optimized cell factories. Metabolic engineering is therefore often seen as a cyclic process, where the cell factory is analyzed and based on this an appropriate target is identified (the design phase). This target is then experimentally implemented and the resulting strain is analyzed again. Thus, metabolic engineering involves a continuous iteration between design and experimental work. In recent years, there has been increasing focus on using mathematical models for design. Hereby, it is expected that metabolic engineering will become faster and more efficient through the development of robust and reliable mathematical models describing the function of cell factories.

In recent years metabolic engineering has adapted tools from functional genomics and systems biology. There are many definitions of systems biology, but most of these contain elements such as mathematical modelling, global analysis (or “ome” analysis), mapping of interactions between cellular components, and quantification of dynamic responses in living cells. In most cases the objective of systems biology is to obtain a quantitative description of the biological system under study, and this quantitative description may be in the form of a mathematical model[1,2]. In some cases, the model may be the final result of the study, i.e. the model captures key features of the biological system and can hence be used to predict the behaviour of the system at

conditions different from those used to derive the model. In other cases, mathematical modelling rather serves as a tool to extract information of the biological system, i.e. to enrich the information content in the data. There is not necessarily a conflict between the two, and generally, mathematical modelling goes hand in hand with experimental work. This partnership exemplifies the essence of systems biology, *obtaining new insight into the molecular mechanisms occurring in living cells or sub-systems of living cells, through the combination of mathematical modelling and experimental biology*. This does not say anything about the use of high-throughput data, e.g. transcriptome or proteome data, and clearly there are many systems biology studies that do not rely on such data. Mathematical models have, however, shown to be particularly useful for analysis of high-throughput data, as the complexity and integrative nature of biological systems makes it difficult to extract information on molecular processes from such data without the use of models as either scaffolds for the analysis or for hypothesis driven analysis of the data.

In the field of industrial biotechnology there is much focus on how systems biology can impact the development of efficient cell factories, and in particularly speed up the development process, and hereby ensure that new products can be brought to the market faster or there can be a faster improvement of existing bioprocesses. This interaction between systems biology, metabolic engineering and bioprocess development, and in particular how model driven metabolic engineering interacts with modern process development is illustrated in Figure 1.

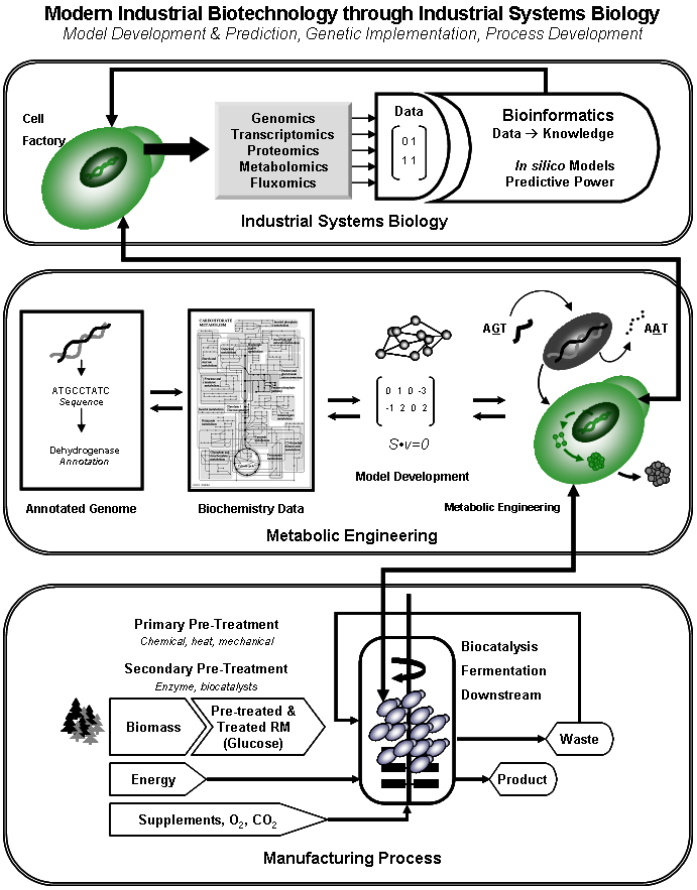

Figure 1. Metabolic engineering is the use of molecular biology tools to implement targeted and specific genetic perturbations to re-direct fluxes towards the desired product. Metabolic engineering has been used to improve cell factories for about 10 years, and recently systems biology tools have been incorporated in the design and development of novel and efficient cell factories. Referred to as industrial systems biology, this approach is iterative and aims at enhancing current metabolic engineering approaches to produce a greater variety of products through biotechnology with significantly reduced time and resources required for commercialization. In particular there is interest in the use of systems biological tools to improve metabolic models that can be used to identify complex metabolic engineering targets[2].

The objectives of SYSINBIO was to coordinate research activities in the field of model driven metabolic engineering in Europe and according to the concept described above coordinate how systems biology can be used to improve the performance of metabolic models and further how mathematical models can be used for improved design of cell factories used in industrial biotechnology. The overall goal was divided into 11 specific objectives that were dealt with in 11 different workpackages that formed the basis of the project:

- Research efforts in the field of model guided metabolic engineering within Europe were coordinated through organizing yearly meetings of leading European research groups working in this field.
- Advanced education in the field of metabolic engineering in Europe was coordinated. This has involved setting up and running dedicated courses and workshops on mathematical modelling and metabolic engineering, but it also involved advanced research training through exchange of researchers between the involved laboratories.
- SYSINBIO organized an international conference with focus on the use of model guided metabolic engineering for development of efficient cell factories. The conference ensured dissemination of new results generated by the participating research groups and further pushed forward the research field by bridging between academia and industry.
- SYSINBIO coordinated the development of metabolic models and has provided a database for existing models. Concepts and methods for setting up genome-scale metabolic models were exchanged and developed resulting in implementation of best practices and efficient work flows in the partner laboratories.
- SYSINBIO also coordinated the development of dynamic models for design of metabolic engineering strategies. Concepts and methods for setting up and analyzing dynamic models was exchanged and developed. Efficient workflows for setting up dynamic models of metabolic reaction networks including parameter estimation, model validation and discrimination, parameter estimation, and simulation were defined for different types of dynamic models.
- SYSINBIO coordinated the development of novel simulation tools that allow the use of metabolic models for design of metabolic engineering strategies. Different algorithms for identification of metabolic engineering targets through the use of genome-scale metabolic models were compared and evaluated, and based on the evaluation, general guidelines for the use of metabolic models for metabolic engineering was specified.
- SYSINBIO coordinated the integration of omics data with metabolic models in order to improve their predictive strength. Different concepts and methods for integration of "omics" data into metabolic models was compared and evaluated leading to guidelines on how such data can be used for the reconstruction and evaluation of genome-scale metabolic models.
- SYSINBIO coordinating the development of novel tools for ^{13}C-based fluxomics (both experimental and computational tools). Different tools for fluxomics were compared and evaluated, and guidelines for quantification of metabolic fluxes in microorganisms, during industrial fermentations, have been proposed.
- SYSINBIO coordinated the development of novel tools for metabolomics. Different methods for sampling and analysis of key intracellular metabolites was compared and evaluated, and guidelines for use of different analytical tools for the analysis of intracellular metabolites, in different industrially important microorganisms, was set up.
- SYSINBIO coordinated the use of statistical methods and metabolic models for upgrading the information content in "omics" data. Different methods and concepts were evaluated for their ability to upgrade the information content in "omics" data, and in particularly it was evaluated how this kind of data can be used to identify novel metabolic engineering targets.
- Finally, SYSINBIO coordinated research activities on the use of DNA arrays, tiling DNA arrays and DNA sequencing for rapid identification of mutations that lead to improved phenotypic properties, e.g. arising in connection with adaptive evolution. The use of these different methods for mapping of mutations in strain lineages was evaluated.

4.1.3 Main Results from S/T

The consortium behind SYSINBIO involved all the leading groups on metabolic engineering in Europe, and it had particularly strong competence on metabolic engineering of the yeast *Saccharomyces cerevisiaae* and the bacterium *Escherichia coli*. For these two microorganisms a number of different technologies had already been developed and implemented before the start of the project, and during the project there was further advancement in technologies. However, the strength of SYSINBIO was that there was also much experience on working with other industrially important microorganisms. The key microorganisms studied within SYSINBIO were:

- *Saccharomyces cerevisiae*, which is used for the production of **bioethanol** and a range of **pharmaceutical proteins**. *S. cerevisiae* is also considered as a general production platform for a range of different chemicals, e.g. **isoprenoids** (flavours, fragrances, pharmaceuticals), bulk chemicals (**succinate**), **nutraceuticals** (resveratrol and poli-unsaturated fatty acids known as PUFAs), in some of the partner laboratories. *S. cerevisiae* also serves as an important model organism for studying eukaryotic cells.[1,3]
- *Escherichia coli*, which is used for production of many different **recombinant proteins**, e.g. human growth hormone and various interleukins, and a wide range of different chemicals like amino acids, e.g. **phenylalanine** (used in the production of Aspartame®) and **methionine** (used as a feed). *E. coli* is also considered as a general production platform for chemical production, and it has traditionally served as the most important model organism for studying bacterial physiology and genetics.
- *Aspergillus niger*, which is used for the production of **organic acids** (citric acid and gluconic acid) and a wide range of **industrial enzymes**.
- *Aspergillus oryzae*, which is used for the production of a wide range of **industrial enzymes**.
- *Trichoderma reesei*, which is used for the production of **celluloses** used for processing of fibers and for biomass egradation in connection with production of fuels and chemicals from biomass.
- *Corynebacterium glutamicum*, which is used for the production of **lysine** and other amino acids used in the feed industry.
- *Lactococcus lactis*, which is used in **starter cultures** and also serves as an important model organism for lactic acid bacteria.
- *Bacillus subtilis*, which is used for the production of **industrial enzymes**, different chemicals, e.g. **riboflavin**, and different **antibiotics**, e.g. **bacitracin**.
- *Streptomyces* species, which are used for the production of **antibiotics** like nystatin, **industrial enzymes**, and **recombinant proteins**.

Besides broad competence on different industrially important microorganisms the partners of SYSINBIO covered competence on all key technologies that are required for state of the art metabolic engineering, namely:

- **Well controlled fermentation experiments**: The ability to perform well controlled fermentation experiments, e.g. using chemostat cultures or computer controlled fed-batch experiments, is essential for metabolic engineering, as it allows for detailed quantitative analysis of the cell factory. The consortium covered extensive experience in this field for a wide range of microorganisms.
- **Transcriptome analysis**: The partners have performed genome-wide transcription analysis of many different industrially important microorganisms, and in particularly demonstrated how this omics technique can be used for gaining insight into the operation of metabolic networks.
- **Metabolome analysis**: Several of the partners are among the World leaders in metabolomics of industrially important microorganisms, and has been involved in defining standards and protocols in this field.
- **Fluxome analysis**: The consortium includes most of the World leading research groups in the field of fluxome analysis. Partners of the consortium have pioneered the use of GC-MS analysis for measurement of isotopomers and they have recently brought the field further forward by using isotopomer measurements of key intermediates in the central carbon metabolism.
- **Genome-scale metabolic models**: The consortium has played an active role in setting up genome-scale metabolic models for a range of industrially important microorganisms.

- **Thermodynamic analysis**: The consortium has demonstrated the use of thermodynamics for analysis of large metabolic networks and illustrated how thermodynamics can be used to identify flux control coefficients in key biosynthetic pathways.
- **Dynamic models**: Based on its strong competences the consortium has evaluated the use of dynamic models for analysing the operation of metabolic networks and has developed several different modelling concepts for simulation of dynamic operation of metabolism.
- **Model simulation and bioinformatics**: The partners in the consortium have developed several new algorithms for analysis of omics data in the context of metabolic models as well as they have developed different simulation platforms that allow simulation of both stoichiometric and dynamic mathematical models. SYSINBIO ensured further advancement in this field.

Based on combination of these strong competences SYSINBIO was able to significantly advance the field and bridge stronger between academia and industry through the coordination activities described further in the following.

Training and Education

SYSINBIO was involved in organizing several advanced courses and student workshops:

- The International Yeast Course on Systems Biology that was held June 1-18, 2009 in Gothenburg. The course was sponsored by FEBS. 4 days of this course were focusing on metabolic engineering technologies, such as metabolic flux analysis, metabolic modelling, and metabolic control analysis. The course had 24 full time students, but parts of the course were offered to local students, of which there were more than 15. The 24 full time students came from 19 different countries.
- The International Course on Systems Biology of Metabolism that ran in the period from May 24 to June 11, 2010 at Chalmers University of Technology. The course had 22 participants from all over Europe. The course consisted of five blocks of teaching: 1) An introduction to systems biology; 2) Setting up and applying genome scale metabolic models; 3) Analysis of omics data and their integration with genome-scale models; 4) Dynamic mathematical models of metabolism and signalling pathays; 5) Examples in biotechnology and medicine. More information about the course can be found at: http://www.sysbio.se/ICSBM2010/.
- A student workshop that was held on December 8-9 in Istanbul . The workshop was chaired by Prof. Vassily Hatzimanikatis, EPFL. At the workshop the 20 participants (PhD students and post docs) each gave a 30 min presentation about their research work.
- A student workshop that was held on January 18-19 at the DECHEMA-Haus in Frankfurt. The workshop was chaired by Prof. Ralf Takors, University of Stuttgart, and Dr. Sergio Velasco, Chalmers. At the workshop the 20 participants (PhD students and post docs) each gave a 30 min presentation about their research work. The presentations were followed by extensive discussions involving both the participants and the organizers. In connection with the workshop there were two special lectures:
  - Probabilistic Boolean framework to decrypt gene regulatory networks – case studies with primary human hepatocytes and *E. coli*, by Prem Kumar
  - Metabolome-based ^{13}C-metabolic flux analysis; A tool for measuring *in vivo* metabolic activities, by Katharina Nöh

  Besides the scientific presentations the workshop also allowed the participant to network through a social event organized over dinner.
- A PhD course entitled “Industrial Biotechnology for lignocellulose based processes” that was held at Chalmers, Gothenburg, in the period 16th to 21st October 2011 and organized by Professor Lisbeth Olsson. The course was relevant for PhD students and researchers working in the field of lignocellulose based processes, i.e. the production of fuels and other chemicals using plant cell wall as raw material. The idea of the course was to encompass all parts of the subject from raw material composition and sources, to pretreatment and hydrolysis, enzymes that act on plant cell wall material, microorganisms and their improvement for production of targeted biofuels and chemicals, the fermentation process and last but not least analytics. The course program included an evening poster session, where the participants had the chance to present their research and visit Professor Olsson laboratories. The course program was closed with a day of seminars on “Hot topics in industrial biotechnology”. The course was attended by 25 students of 17 different nationalities, coming from

institutions in the following countries: Sweden, Denmark, Norway, Finland, United Kingdom, Belgium, Germany, Italy, Spain and Turkey. SYSINBIO sponsored 7 students from the SYSINBIO consortium. 15 lecturers from Sweden, Denmark, Finland, Norway and Italy was involved in the course. Several of them were partners of the SYSINBIO.

International Conference

SYSINBIO organized a 2 day conference "Industrial Systems Biology: Sustainable production of fuels and chemicals". The conference was held at Chalmers University of Technology, Gothenburg, Sweden August 18-19, 2010 (the conference was organized as one of the conferences in the Gothenburg Life Science Conference series). In connection with the conference there was also organized a dedicated student workshop on August 17 (3 hours) where one speaker from academia (Professor Gregory Stephanopoulos, MIT, USA) and one speaker from industry (Dr. Alan Berry, Novozymes, USA/Denmark) discussed job prospects and industrial demand for future students.

At the conference there were 20 oral presentations and 40 poster presentations. There were more than 170 participants at the conference. Among the speakers were: **Jens Nielsen**, Chalmers, Gothenburg Sweden; **Vassily Hatzimanikatis**, EPFL, Lausanne, Switzerland; **Kiran Patil**, DTU, Lyngby, Denmark; **Matthias Heinemann**, ETH Zürich, Switzerland*;* **Elmar Heinzle**, Saarland University, Germany*;* **Sang Yup Lee**, KAIST, Daejon, Korea; **Christoph Wittmann**, TU Braunschweig, Braunschweig, Germany*;* **Andrew C. Eliot**, DuPont, Wilmington, USA*;* **Jack Pronk**, TU Delft, The Netherlands; **Alan Berry**, Novozymes, Davis, USA*;* **Verena Siewers**, Chalmers, Gothenburg, Sweden; **Jochen Förster**, Fluxome, Copenhagen, Denmark*;* **Eric Appelman**, Perstorp, Sweden. A detailed report from the conference has been published in Biotechnology Journal[4]. Furthermore, based on oral presentations several papers were published in a special issue of Biotechnology journal (issue 3, 2011) entitled "Systems biology for industrial applications", and the editorial provides perspectives based on conclusions of the conference[5].

Mathematical Models and Their Use in Industrial Biotechnology

A key task of SYSINBIO was to coordinate activities on developing new and better mathematical models of metabolism that can be used for metabolic engineering. Activities on both **stoichiometric models** and on **kinetic models** have been coordinated. Stoichiometric models, often also referred to as genome-scale metabolic models (GEMs), have the advantage that they are directly linked to genomic information[6,7], and methods for set up and use of GEMs has been further advanced. In particular SYSINBIO was active in defining methods that allows for faster setting up this kind of models[8]. A fundamental problem with stoichiometric models is that it is necessary to perform a manual annotation and curation of the many different reactions, and this is time consuming and laborious. We have therefore formulated methods and standards that will allow for faster development of this kind of models, and we have also developed a database of existing models that can be used as scaffolds for building new models[9]. We have also worked on different user-friendly graphical interfaces that allows for visualization of these complex models. For kinetic models we have performed an extensive survey of existing models in the partner laboratories (and of published models), and we have used this to discuss how these models can be used in metabolic engineering[8,10]. A fundamental problem with kinetic models is the requirement for parameter estimation, but partners within SYSINBIO are using different modelling concepts that partly circumvent this problem, e.g. Monte Carlo simulations and use of linearized kinetics in the logarithmic domain[11,12]. Furthermore, workflows for setting up kinetic models are in the process of being defined and these will be used to define the requirements for experimental data for validation and parameter estimations (unpublished).

The coordination of the development of more reliable and standardized microbial models has allowed for the improvement of model predictions and therefore made it possible to apply **simulation and optimization algorithms** that can identify gene modifications that can increase the production yields of desired compounds. We have coordinated the development of reliable and effective computational and mathematical methods for the design of rational metabolic engineering strategies[13,14,15,16]. Through activities we have ensured that the simulation and optimization tools developed obey the following requisites: 1) robustness regarding the different models used (stoichiometric versus dynamic models) and metabolic engineering objectives; 2) compatibility with existing model storage standards (SBML and other formats); and 3) straightforward incorporation in user-friendly software tools to facilitate its use by non-expert users. Another important part of the activities has been the coordination of the development of user friendly software tools, something that is

allowing for a wider use of mathematical modelling in the field of metabolic engineering[16]. Finally SYSINBIO has been working on improving the predictive strength of GEMs through incorporation of regulatory information[17].

Integration of Omics Data

A key challenge in metabolic engineering is to be able to integrate omics data, e.g. transcriptomics, proteomics and metabolomics, with mathematical models with the objective to identify novel metabolic engineering targets. In SYSINBIO there were several ways to advance this. Flux data obtained from ^{13}C-labelled experiments have been shown to allow constraining the fluxes in GEMs and hereby obtain better constraints on the operation of these large metabolic networks[18]. We have further used a combination of thermodynamics and experimental fluxes to identify flux constraining reactions[19]. This has substantially improved the predictive strength of these models for finding metabolic engineering targets. SYSINBIO also demonstrated how a combination of random sampling of GEMs combined with transcriptome data can be used to identify whether flux control is at the transcriptional or metabolic level[20].

Another key challenge in this field is visualization of the rather complex data, but through the use of GEMs as scaffold it has been possible to easily visualize how omics data link to specific enzymatic reactions[21], and this has been provide in a user-friendly software algorithm.

Due to the high connectivity of the different metabolic reactions within the metabolic network, there has been much interest in exploiting tools from functional genomics for mapping of global regulatory structures or even using high-throughput experimental techniques provided by the various omics for dissecting how the fluxes through the different branches of the metabolic network are controlled. In particular it is interesting to map the level of control of fluxes, i.e. is it at the metabolism level or is there control of flux at the transcriptional, translational or post-translational level. This kind of information can only be obtained through a combination of mathematical models and high-quality experimental data on metabolites and fluxes, and possible other omes. The partners of SYSINBIO have in several studies demonstrated how integrative analysis of omics data through the use of GEMs and other network structures can lead to new biological insight, in particular on how metabolic fluxes are controlled. This has involved both the identification of key transcription factors controlling parts of metabolism in yeast[22,23] and how the key protein kinase Snf1 control yeast metabolism[24]. These concepts were also demonstrated to be useful to gain insight into how different yeast strains control their energy metabolism[25]. However, these concepts have not only been used for studies of yeast, as the concepts have also been used to gain new insight into enzyme production by *Aspergillus oryzae*[26]. As part of these studies there was also performed a detailed characterization of different transcriptome platforms for yeast, and even though these results were specific for yeast they are likely to have translational character and hence some general guidelines for transcriptome analysis in industrial microorganisms has been proposed[25].

Fluxomics

Metabolic fluxes can either be estimated through the use of flux balance analysis (FBA) using GEMs or through the use of ^{13}C-labelled substrate feeding followed by analysis of the labelling patterns in intracellular metabolites. Due to their abundance and stability, ^{13}C-based methods have conventionally used proteinogenic amino acids to detect labelling patterns. Recently, however, methods for direct analysis in the free pool of metabolites have been developed. Both methods require a tight integration of experiments and model simulations for estimation of the fluxes, and in particular the mathematical complexity involved in flux estimation has been a barrier for a wider use of fluxomics. Besides providing general information on how the metabolic network is operating at different growth conditions, metabolic flux analysis is very well suited for analysis of the effects of growth on different media, specific mutations and screening of different mutants. Thus, flux analysis today represents a standard technique for rapid phenotypic characterization of metabolically engineered strains, and this tool is likely to gain even wider use in the future. Within SYSINBIO there has been coordination of developments in this field, and this has resulted in several key reviews and papers specifying guidelines for how to perform metabolic flux analysis[27,28,29,30].

Metabolomics

Another important analytical tool in metabolic engineering is metabolome analysis, as this allows for direct quantification of the levels of intracellular metabolites. As these levels determine the kinetics of the different reactions, the metabolite levels together with the fluxes represents key information on how metabolism can be

engineered. SYSINBIO has coordinated activities on metabolome analysis in the different partner laboratories. Many of the partner labs were involved in a thorough inter-laboratory comparison of metabolome analysis of the yeast *Saccharomyces cerevisiae* and this allowed to provide an overview of the advantage and disadvantage of different methods for metabolome analysis[25]. Based on this we have provided an overview of best practice in the field and also provide guidelines for performing metabolome analysis in other industrially important microoganisms. This inter-laboratory study has been supplemented with more specific studies on extraction of metabolites from yeast by SYSINBIO partners[31,32]. Whereas there has been good progress on metabolomics of yeast it has been found to be far more difficult to obtain truly quantitative data on intracellular metabolite levels in bacteria, as illustrated in a study on *E. coli*[33].

Adaptive Evolution

Random mutagenesis has traditionally been used to optimize the performance of microbial strains used in industry, and it is still being used as a very powerful tool. Also in metabolic engineering it is often necessary to evolve strains further in order to adapt them to certain environmental conditions. Often directed genetic modifications that results in a desirable phenotype with respect to production of a certain metabolite lead to a reduction of the specific growth rate of the cells, and this may often result in low productivities of the strain. However, there have been several examples illustrating that following such directed genetic modifications resulting in engineering of the metabolic network, it is possible to evolve the recombinant strains towards faster growth using the concept of adaptive evolution. Hereby the productivity is increased and a strain with both high yield and productivity is obtained. In cases where random mutagenesis or evolution is used to identify microbial strains with desirable phenotypes there is much interest to identify the underlying mutations, as this information can be used to further improve our models of microbial metabolism and hereby this information can be used actively to further metabolic engineer the production strain. With the development of next generation sequencing technologies it has become possible to sequence microbial strains and hereby identify mutations acquired during adaptive evolution. In connection with the SYSINBIO different strategies for use of sequencing have been discussed and this has resulted in the several studies evaluated the use of sequencing for SNP identification has been evaluated. This has led to the development of a specific genome-browser for a widely applied *S. cerevisiae* strain (CEN.PK), and this genome-browser can be used as a database for queering specific questions[34]. The use of genome-sequencing has furthermore been demonstrated for identification of governing mutations acquired during adaptive evolution of yeast growth on galactose[35].

4.1.4 Potential Impact

The SYSINBIO project has ensured coordination of research efforts in the field of model guided metabolic engineering within Europe, and in particular ensured improved dialogue between academia and industry. This has led to a substantially improved transfer of ideas and technology from academia to industry and hence contributed to improve the competiveness of the European biotech industry. The project has also been instrumental in coordinating advanced education in the field of metabolic engineering in Europe by stimulating the exchange of students and by sponsoring workshops that have the graduate student as their main focus. It is further expected that this will have increased the awareness among all the partners of existing courses in the different partner countries, and this will have sustained impact following the project end.

In the last period of the project an international conference was organized that ensured the dissemination of new results generated by the participating research groups and further pushed forward the research field by bridging between academia and industry. The project has also resulted in coordination of the development of metabolic models and has led to the establishment of a database for existing models. This has led to exchange of concepts and methods for setting up genome-scale metabolic models. In the last part of the project this database has been further populated and it will be maintained by the partner groups following the project, which represents a major outcome of the project with a wide dissemination potential.

We have coordinated the development of dynamic models for design of metabolic engineering strategies. The concepts and methods for setting up and analyzing dynamic models have been summarized in a review paper. This involved defining efficient workflows for setting up dynamic models of metabolic reaction networks including parameter estimation, model validation and discrimination etc. We have in the project discussed the application of different simulation tools that allow the use of metabolic models for design of metabolic engineering strategies. This has helped to define some general guidelines for the use of metabolic models for

metabolic engineering and move towards a consensus between the different research groups working on this field in Europe. We have evaluated different concepts for integration of "omics" data into metabolic models and we will in the last part of the project provide guidelines on how such data can be used for the reconstruction and evaluation of genome-scale metabolic models. These guidelines will allow for improved and faster model reconstructions in the future.

Very solid reports on novel tools for 13C-based fluxomics (both experimental and computational tools) have been drafted. These reports have already been disseminated to several companies. We have coordinated research activities on the use of DNA arrays, tiling DNA arrays and DNA sequencing for rapid identification of mutations that lead to improved phenotypic properties. In the last part of the project we will further evaluate the use of genome-sequencing for analysis of evolved mutants. This will lead to development of guidelines for which methods are preferential to use for analysis of evolved mutants in connection with strain design and optimization.

SYSINBIO has also attempted to raise the public awareness of industrial biotechnology. Several news and cover stories have been featured in Swedish newspapers, including Dagens Nyheter and Ny Teknik. In each case this was handled by providing press releases through assistance from Chalmers central administration.

As a global conclusion we can say that SYSINBIO has been instrumental to advance the industrial applications of systems biology in Europe both by coordinating research among different groups in academia and between industry and academia and by facilitating transfer of ideas and technology from academy to industry. SYSINBIO has also played a very relevant role in education of graduate students by facilitating exchanges and by giving graduate students in the partner academic groups a broad overview of the activities that are being carried out by different actors in the field.

4.1.5 Contacts

Further information about SYSINBIO can be obtained by contacting the coordinator:

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12. Kotte O, Zaugg JB, Heinemann M (2010) Bacterial adaptation through distributed sensing of metabolic fluxes. *Mol. Systems Biol.* **6**:355
13. Rocha M, Maia P, Mendes R, Pinto JP, Ferreira EC, Nielsen J, Patil KR, Rocha I (2008) Natural computation meta-heuristics for the *in silico* optimization of microbial strains. *BMC Bioinformatics* **9**:499
14. Vilaça P, Maia P, Rocha I, Rocha M (2010) Metaheuristics for strain optimization using transcriptional information enriched metabolic models. *Lecture Notes in Computer Science* **6023**:205-216
15. Evangelista P, Rocha I, Ferreira EC, Rocha M (2009) Evolutionary approaches for strain optimization using dynamic models under a metabolic engineering perspective. *Lecture Notes in Computer Science* **5483**:140-151
16. Rocha I, Maia P, Evangelista P, Vilaça P, Soares S, Pinto JP, Nielsen J, Patil KR, Ferreira EC, Rocha M (2010) OptFlux: an open-source software platform for *in silico* metabolic engineering. *BMC Systems Biology* **4**:45
17. Vilaça P, Rocha I, Rocha MA (2011) Computational tool for the simulation and optimization of microbial strains accounting integrated metabolic/regulatory information. *BioSystems* **103**:435-441
18. Bordel S, Nielsen J (2010) Identification of flux control in metabolic networks using non-equilibrium thermodynamics. *Metab. Eng.* **13**:369-377
19. Hadlich F, Nöh K, Wiechert W (2010) Determination of flux directions by thermodynamic network analysis: computing informative metabolite pools. Math. Comp. Sim. **10**:1016
20. Bordel S, Agren R, Nielsen J (2010) Sampling the Solution Space in Genome-Scale Metabolic Networks Reveals Transcriptional Regulation in Key Enzymes. 2010. *PLoS Comput. Biol*. **6**:e1000859.
21. Droste P, Miebach S, Niedenführ S, Wiechert W, Nöh K (2011) Visualizing multi-omics data in metabolic networks with the software Omix - A case study. *BioSystems* **105**:154-161
22. Fendt SM, Oliveira AP, Christen S, Picotti P, Dechant RC, Sauer U (2010) Unraveling condition-dependent networks of transcription factors that control metabolic pathway activity in yeast. *Mol. Systems Biol.* **6**:432
23. Fazio A, Jewett M, Daran-Lapujade P, Mustacchi R, Usaite R, Pronk JT, Workman C, Nielsen J (2008) Transcription factor control of growth rate dependent genes in *Saccharomyces cerevisiae*: A three factor design. *BMC Genomics* **9**:341
24. Usaite R, Jewett MC, Oliveira AP, Yates JR, Olsson L, Nielsen J (2009). Reconstruction of the yeast Snf1 kinase regulatory network reveals its role as a global energy regulator. *Mol. Systems Biol.* **5**:319
25. Vongsangnak W, Hansen K, Nielsen J (2011) Integrated analysis of the global transcriptional response to α–amylase over-production in *Aspergillus oryzae*. *Biotechnol. Bioeng.* **108**:1130-1139
26. Canelas A.B., Burton N., Fazio A., Zhang J., Pitkänen J., van den Brink J., Bakker B., Bouwman J., Castrillo J., Cankorur A., Chumnanpuen P., Daran-Lapujade P., Dikicioglu D., van Eunen K., Ewald J.C., Heijnen J.J., Kirdar B., Niebel A., Penttilä M., Pronk J., Reuss M, Rintala E., Sauer U., Sherman D., Siemann-Hertzberg M., Westerhoff H., Petranovic D., Oliver S., Workman C., Zamboni N., Nielsen J. (2010) Integrated multilaboratory systems biology reveals differences in protein metabolism between two reference yeast strains. *Nature Comm.* **1**:145
27. Rantanen A, Rousu J, Jouhten P, Zamboni N, Maaheimo H, Ukkonen E (2008) An analytic and systematic framework for estimating metabolic flux ratios from 13C tracer experiments. *BMC Bioinformatics* **9**:266
28. Zamboni N, Fendt SM, Rühl M, Sauer U (2009) (13)C-based metabolic flux analysis. *Nat. Protoc.* **4**:878–92
29. Wahl SA, Nöh K, Wiechert W (2008) 13C labeling experiments at metabolic nonstationary conditions: an exploratory study. *BMC Bioinformatics* **9**:152
30. Christen S, Sauer U (2011) Intracellular characterization of aerobic glucose metabolism in seven yeast species by 13C flux analysis and metabolomics. *FEMS Yeast Res* **11**:263-272
31. Canelas AB, ten Pierick A, et al. (2009) Quantitative evaluation of intracellular metabolite extraction techniques for yeast metabolomics. *Anal. Chem.* **81**:7379-7389
32. Ewald JC, Heux S, Zamboni N (2009) High-throughput quantitative metabolomics: workflow for cultivation, quenching, and analysis of yeast in a multiwell format. *Anal. Chem.* **81**:3623-3629.
33. Vielhauer O, Zakhartsev M, Horn T, Takors R, Reuss M (2012) Simplified absolute metabolite quantification by gas-chromatography dilution mass spectrometry on the basis of commercial available source material. *J. Chromatography B,* in press
34. Otero JM, Vongsangak W, Asadollahi MA, Olivares-Hernandes R, Maury J, Farinelli L, Barlocher L, Østerås M, Schalk M, Clark A, Nielsen J (2010) Whole genome sequencing of *Saccharomyces cerevisiae*: from genotype to phenotype for improved metabolic engineering applications. *BMC Genomics* **11**:723
35. Hong K-K, Vongsangnak W, Vemuri GN, Nielsen J (2011) Unravelling evolutionary strategies of yeast for improving galactose utilization through integrated systems level analysis. *Proc. Nat. Acad. Sci. USA* **108**:12179-12184

4.2 Use and dissemination of foreground

Section A (public)

TEMPLATE A1: LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES										
NO.	Title	Main author	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Year of publication	Relevant pages	Permanent identifiers[2] (if available)	Is/Will open access[3] provided to this publication?
1	A system-level approach for metabolic engineering of yeast cell factories	*Nielsen J*	*FEMS Yeast Res.*	*In press*			*2012*			No
2	Meeting report: Gothenburg Life Science Conference XI: Industrial Systems Biology	Siewers V	*Biotechnol. J*	6			2011	259-261		No
3	Editorial: Industrial Systems Biology	Nielsen J	*Biotechnol. J*	6			2011	255		No
4	Use of genome-scale metabolic models for understanding microbial physiology	Nielsen J	*FEBS Lett*.	584			2010	2556-2564		No
5	Fifteen years of large scale metabolic modeling of yeast: Developments and impacts	Nielsen J	*Biotechnol. Adv.*	*In press*			2012			No
6	Mathematical models of cell factories: Moving towards the cores of industrial biotechnology	Nielsen J	*Microbiol. Biotechnol.*	4			2011	572-584		No

[2] A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).

[3] Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

7	BioMet Toolbox: Genome-wide analysis of metabolism	Nielsen J	*Nuc. Acid Res.*	38			2010	W144-W149		No
8	Mechanistic pathway modeling for industrial biotechnology: challenging but worthwhile	Noack S	*Current Opinion Biotechnol.*	22			2011	604-610		No
9	A divide–and–conquer approach to analyze underdetermined biochemical models	Heinemann M	*Bioinformatics*	25			2009	519-525		No
10	Bacterial adaptation through distributed sensing of metabolic fluxes	Heinemann M	*Mol. Systems Biol.*	6			2010	355		No
11	Natural computation meta-heuristics for the *in silico* optimization of microbial strains	Rocha I	*BMC Bioinformatics*	9			2008	499		Yes
12	Metaheuristics for strain optimization using transcriptional information enriched metabolic models	Rocha M	*Lecture Notes in Computer Science*	**6023**			2010	205-216		No
13	Evolutionary approaches for strain optimization using dynamic models under a metabolic engineering perspective	Rocha M	*Lecture Notes in Computer Science*	**5483**			2009	140-151		No
14	OptFlux: an open-source software platform for *in silico* metabolic engineering	Rocha M	*BMC Systems Biology*	4			2010	45		Yes
15	Computational tool for the simulation and optimization of microbial strains accounting integrated metabolic/regulatory information	Rocha MA	*BioSystems*	**103**			2011	435-441		No
16	Identification of flux control in metabolic networks using non-equilibrium thermodynamics	Nielsen J	*Metab. Eng*	**13**			2010	369-377		No
17	Determination of flux directions by	Wiechert W	Math. Comp. Sim	**10**			2010	1016		No

	thermodynamic network analysis: computing informative metabolite pools									
18	Sampling the Solution Space in Genome-Scale Metabolic Networks Reveals Transcriptional Regulation in Key Enzymes	Nielsen J	*PLoS Comput. Biol*.	**6**			2010	e1000859		Yes
19	Visualizing multi-omics data in metabolic networks with the software Omix - A case study	Nöh K	*BioSystems*:	**105**			2011	154-161		No
20	Unraveling condition-dependent networks of transcription factors that control metabolic pathway activity in yeast	Sauer U	*Mol. Systems Biol.*	**6**			2010	432		No
21	Transcription factor control of growth rate dependent genes in *Saccharomyces cerevisiae*: A three factor design	Nielsen J	*BMC Genomics*	**9**			2008	341		Yes
22	Reconstruction of the yeast Snf1 kinase regulatory network reveals its role as a global energy regulator	Nielsen J	*Mol. Systems Biol.*	**5**			2009	319		No
23	Integrated analysis of the global transcriptional response to α–amylase over-production in *Aspergillus oryzae*	Nielsen J	*Biotechnol. Bioeng*.	**108**			2011	1130-1139		No
24	Integrated multilaboratory systems biology reveals differences in protein metabolism between two reference yeast strains	Nielsen J	*Nature Comm.*	1			2010	145		No
25	An analytic and systematic framework for estimating metabolic flux ratios from 13C tracer experiments	Ukkonen E	*BMC Bioinformatics*	**9**			2008	266		Yes
26	(13)C-based metabolic flux	Sauer U	*Nat. Protoc.*	**4**			2009	878–92		No

	analysis									
27	13C labeling experiments at metabolic nonstationary conditions: an exploratory study	Wiechert W	*BMC Bioinformatics*	**9**			2008	152		Yes
28	Intracellular characterization of aerobic glucose metabolism in seven yeast species by 13C flux analysis and metabolomics	Sauer U	*FEMS Yeast Res*	**11**			2011	263-272		No
29	Quantitative evaluation of intracellular metabolite extraction techniques for yeast metabolomics	Heijnen JJ..	*Anal. Chem.*	**81**			2009	7379-7389		No
30	High-throughput quantitative metabolomics: workflow for cultivation, quenching, and analysis of yeast in a multiwell format	Zamboni N	*Anal. Chem.*	**81**			2009	3623-3629		No
31	Simplified absolute metabolite quantification by gas-chromatography dilution mass spectrometry on the basis of commercial available source material	Reuss M	*J. Chromatography B*,	in press			2012			No
32	Whole genome sequencing of *Saccharomyces cerevisiae*: from genotype to phenotype for improved metabolic engineering applications	Nielsen J	*BMC Genomics*	**11**			2010	723		Yes
33	Unravelling evolutionary strategies of yeast for improving galactose utilization through integrated systems level analysis	Nielsen J	*Proc. Nat. Acad. Sci. USA*	**108**			2011	12179-12184		No

Most of the scientific journals are not freely accessible and need to be consulted from a university or a public library (they are therefore publicly available). Publicly available does not mean that they open access but that they can be accessed from a public institution.

TEMPLATE A2: LIST OF DISSEMINATION ACTIVITIES								
NO.	Type of activities[4]	Main leader	Title	Date	Place	Type of audience[5]	Size of audience	Countries addressed
1	*Conference*	*Nielsen J*	*Industrial Systems Biology: Sustainable production of fuels and chemicals*	*26 February 2010*	*Chalmers University of Technology*	*Scientific Community, Medias*	*>170*	All
2	*Dry course on met eng (Part of the 4th ICYSB)*	*Nielsen J*	*International Course on Yeast Systems Biology*	*1-18 June 2009*	*Gothenburg, Sweden*	*Scientific Community*	*30*	All
3	*Workshop*	*Nielsen J*	*First SYSINBIO workshop*	*8-9 December 2009*	*Istanbul, Turkey*	*Scientific Community*	*25*	EU countries
4	*Course*	*Nielsen J*	*International Course on SB of Metabolism*	*24 May – 11 June 2010*	*Gothenburg, Sweden*	*Scientific Community*	*25*	All
5	*Advanced Course*		*Applied genomics of industrial fermentation*	*11 - 15 October 2010*	*Delft, the Netherlands*	*Scientific Community*	*25*	All
6	*Workshop*	*R.Takors*	*Second SYSINBIO workshop*	*17-18 January 2011*	*Frankfurt am Main, Germany*	*Scientific Community*	*30*	EU countries

[4] A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

[5] A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias ('multiple choices' is possible.

Section B (Confidential[6] or public: confidential information to be marked clearly)
Part B1

DUE TO THE NATURE OF THE PROJECT (COORDINATION ACTION) THERE HAS NOT BEEN GENERATED IP DIRECTLY AS A RESULT OF THE PROJECT.

TEMPLATE B1: LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.					
Type of IP Rights[7]:	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Application reference(s) (e.g. EP123456)	Subject or title of application	Applicant (s) (as on the application)

[6] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.

[7] A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

Part B2

Please complete the table hereafter:

Type of Exploitable Foreground[8]	Description of exploitable foreground	Confidential Click on YES/NO	Foreseen embargo date dd/mm/yyyy	Exploitable product(s) or measure(s)	Sector(s) of application[9]	Timetable, commercial or any other use	Patents or other IPR exploitation (licences)	Owner & Other Beneficiary(s) involved
	Ex: New superconductive Nb-Ti alloy			*MRI equipment*	*1. Medical* *2. Industrial inspection*	*2008* *2010*	*A materials patent is planned for 2006*	*Beneficiary X (owner) Beneficiary Y, Beneficiary Z, Poss. licensing to equipment manuf. ABC*

DUE TO THE NATURE OF THE PROJECT (COORDINATION ACTION) THERE HAS NOT BEEN GENERATED IP DIRECTLY AS A RESULT OF THE PROJECT.

[19] A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

[9] A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

4.3 Report on societal implications

A General Information *(completed automatically when **Grant Agreement number** is entered.*	
Grant Agreement Number:	
Title of Project:	
Name and Title of Coordinator:	

B Ethics	
1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	***0Yes X No***
2. Please indicate whether your project involved any of the following issues (tick box) :	***YES***
RESEARCH ON HUMANS	
• Did the project involve children?	
• Did the project involve patients?	
• Did the project involve persons not able to give consent?	
• Did the project involve adult healthy volunteers?	
• Did the project involve Human genetic material?	
• Did the project involve Human biological samples?	
• Did the project involve Human data collection?	
RESEARCH ON HUMAN EMBRYO/FOETUS	
• Did the project involve Human Embryos?	
• Did the project involve Human Foetal Tissue / Cells?	
• Did the project involve Human Embryonic Stem Cells (hESCs)?	
• Did the project on human Embryonic Stem Cells involve cells in culture?	
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	
PRIVACY	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	
• Did the project involve tracking the location or observation of people?	
RESEARCH ON ANIMALS	
• Did the project involve research on animals?	
• Were those animals transgenic small laboratory animals?	
• Were those animals transgenic farm animals?	
• Were those animals cloned farm animals?	
• Were those animals non-human primates?	
RESEARCH INVOLVING DEVELOPING COUNTRIES	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	
• Was the project of benefit to local community (capacity building, access to healthcare, education etc)?	
DUAL USE	
• Research having direct military use	0 Yes 0 No
• Research having the potential for terrorist abuse	

C Workforce Statistics

3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	2	1
Work package leaders	2	4
Experienced researchers (i.e. PhD holders)	10	15
PhD Students	30	45
Other		

4. How many additional researchers (in companies and universities) were recruited specifically for this project?	**2**
Of which, indicate the number of men:	1

D Gender Aspects

5.	**Did you carry out specific Gender Equality Actions under the project**?	○ X	Yes No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	○	○	○
❑	Set targets to achieve a gender balance in the workforce	○	○	○	○	○
❑	Organise conferences and workshops on gender	○	○	○	○	○
❑	Actions to improve work-life balance	○	○	○	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

X No

E Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

○ Yes- please specify

X No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

○ Yes- please specify

X No

F Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

○ Main discipline[10]: 2.3

○ Associated discipline[10]: 1.5 ○ Associated discipline[10]:

G Engaging with Civil society and policy makers

11a	**Did your project engage with societal actors beyond the research community?** *(if 'No', go to Question 14)*	○ X	Yes No

11b If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?

○ No

○ Yes- in determining what research should be performed

○ Yes - in implementing the research

○ Yes, in communicating /disseminating / using the results of the project

[10] Insert number from list below (Frascati Manual).

11c In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?	○ X	Yes No

12. Did you engage with government / public bodies or policy makers (including international organisations)

X	No
○	Yes- in framing the research agenda
○	Yes - in implementing the research agenda
○	Yes, in communicating /disseminating / using the results of the project

13a Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?

○	Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
○	Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
X	No

13b If Yes, in which fields?

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
		Foreign and Security Policy		Space	
Education, Training, Youth		Fraud		Taxation	
Employment and Social Affairs		Humanitarian aid		Transport	

13c If Yes, at which level?

- ○ Local / regional levels
- ○ National level
- ○ European level
- ○ International level

H Use and dissemination

14. How many Articles were published/accepted for publication in peer-reviewed journals?	**33**
To how many of these is open access[11] provided?	**7**
How many of these are published in open access journals?	**7**
How many of these are published in open repositories?	
To how many of these is open access not provided?	**26**
Please check all applicable reasons for not providing open access:	
❑ publisher's licensing agreement would not permit publishing in a repository X no suitable repository available ❑ no suitable open access journal available X no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[12]: ……	

15. How many new patent applications ('priority filings') have been made? *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*		**0**
16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).	Trademark	**0**
	Registered design	**0**
	Other	**0**
17. How many spin-off companies were created / are planned as a direct result of the project?		**0**
Indicate the approximate number of additional jobs in these companies:		

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑	Increase in employment, or	❑	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	❑	None of the above / not relevant to the project
X	Difficult to estimate / not possible to quantify		

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:*

[11] Open Access is defined as free of charge access for anyone via Internet.
[12] For instance: classification for security project.

Difficult to estimate / not possible to quantify	X

I Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes X No

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes X No

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

❑	Press Release	X	Coverage in specialist press
❑	Media briefing	❑	Coverage in general (non-specialist) press
❑	TV coverage / report	X	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
❑	Brochures /posters / flyers	❑	Website for the general public / internet
❑	DVD /Film /Multimedia	❑	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

X	Language of the coordinator	❑	English
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]

1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)

1.3 Chemical sciences (chemistry, other allied subjects)

1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)

1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)

2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as

geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]