EUROPEAN SCIENCE FOUNDATION

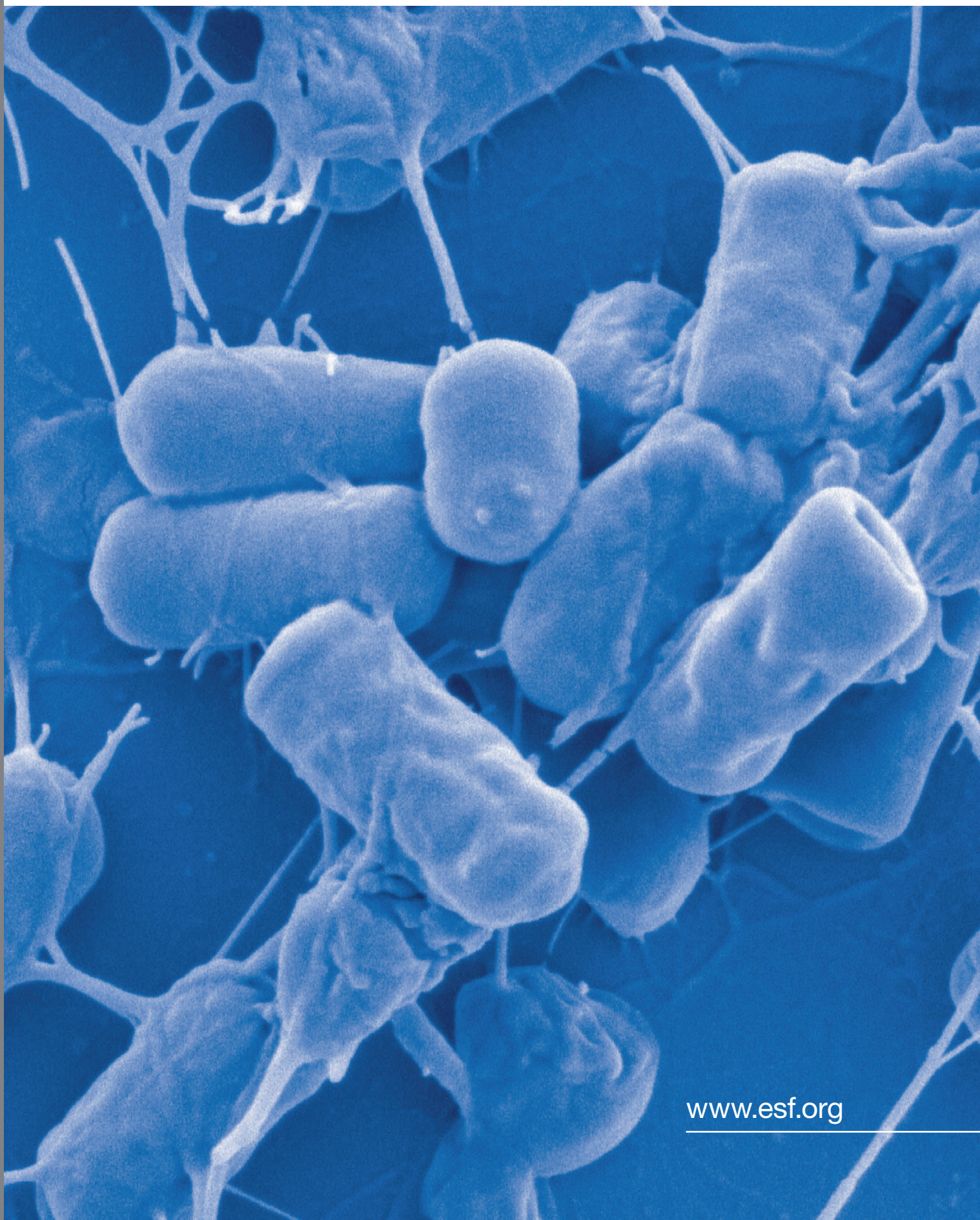
SETTING SCIENCE AGENDAS FOR EUROPE

EUROCORES Programme

EuroSCOPE

Science of Protein Production for Functional and Structural Analysis

Final Report



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Being an interdisciplinary Programme, EuroSCOPE was jointly administered by two of the ESF science units; the Life, Earth and Environmental Sciences Unit and the Medical Sciences Unit.

EUROCORES (European Collaborative Research)

The aim of the European Collaborative Research (EUROCORES) Scheme is to enable researchers in different European countries to develop collaboration and scientific synergy in areas where European scale and scope are required to reach the critical mass necessary for top class science in a global context.

The scheme provides a flexible framework which allows national basic research-funding and -performing organisations to join forces to support excellent European research in and across all scientific areas. The European Science Foundation provides scientific coordination and support for networking activities of funded scientists.

Until the end of 2008, scientific coordination and networking was funded through the EC FP6 Programme, under contract no. ERAS-CT-2003-980409. As of 2009, research funding and the funding for the scientific coordination and networking are provided by participating national organisations.

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Cover Image:
Bacillus subtilis. Courtesy of Professor van Dijl

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1. Description of the EuroSCOPE Programme

1.1 Rationale and objectives

EuroSCOPE was one of the early EUROCORES Programmes that were launched. The theme was proposed as a follow-up of the study report on "Protein Structure and Function in the Post Genomic Era" that resulted in the decision by the ESF Board and Governing Council to focus on the scientific problems linked to protein production and to explore the possibility of proposing a EUROCORES Programme in this field.

Great public, political and academic excitement has been generated over the last couple of years with the release of several complete genome sequences, with new opportunities for dramatically improving human health and biotechnological processes. The tremendous effort required to get such information needs to be realised through the study of the encoded proteins themselves, many of which are potential therapeutic targets as a result of their intimate role in modulating cell responses, and hence their implication in disease.

All major structural genomics activities worldwide include some protein production activity, usually based on fortuitous successes with a limited number of proteins. However, directed activities to address the problems of protein production for functional and structural analysis are much less well supported or coordinated than the genomics and structural activities themselves, despite this being a major limiting factor for many proteins, especially those in complexes and hydrophobic proteins. The EUROCORES Programme EuroSCOPE envisaged bridging this gap for scientific collaboration and innovation, which is generally not foreseen through funding at the national or EU (EC Framework Programme) level.

This Programme's ambition was to tackle the major stumbling blocks in the production of proteins for functional and structural analysis. The focus was put on the basic understanding of the mechanisms underlying protein production, targeting, folding and stability, which may eventually result in the improvement of existing and the design of new expression systems. The work should lead to a firm foundation for the rational engineering of strains for the production of complex proteins such as multi-domain eukaryotic proteins, integral membrane proteins and multi-enzyme complexes. When the Programme was proposed, these complex systems were highly underrepresented in the protein structure databases, because they were difficult to overproduce in a functional form and in amounts suitable for functional and structural work.

The Programme was originally designed to have two phases. The emphasis in Phase I was planned to be on the science of all aspects of protein production to permit structural studies of complex systems to begin. It was not intended to fund projects that aimed at scaling up the processes or setting up protein factories for production on demand, although such initiatives would benefit from basic knowledge of protein production as proposed here. In Phase II of the Programme, the structural analysis of the complex protein systems was intended to become more prominent as this is evidently the second major hurdle in the exploitation of the wealth of genomic information currently available.

Due to the limitation of the available funds the Programme was finally carried out in one stage, where the two aspects were addressed. The Call for Outline Proposals was launched in early 2004.

1.2 Detailed description of sub-fields

1.2.1 Bottlenecks in gene expression

Although many expression systems, ranging from bacterial to eukaryotic and cell free translation systems, are available, the amplification of most proteins is problematic and success is often a matter of trial and error. In particular, eukaryotic membrane proteins and large oligomeric complexes are difficult to produce in functional form and in quantities sufficient for structural analysis. In cases where functional overexpression of membrane proteins fails, the proteins are usually not targeted to the plasma membrane and may be misfolded. Knowledge of bottlenecks in expression will aid the design of new and optimisation of existing systems, for instance, by tailoring particular organisms. A number of recent developments in the utilisation of *Saccharomyces cerevisiae* as host for recombinant protein expression, e.g., construction of tuneable integration vectors and the elucidation of protein quality control mechanisms in the endoplasmic reticulum, suggest rational approaches for the engineering of yeast strains for the high-level expression of functional membrane proteins. Reporter strains have already been constructed that utilise various protein quality control pathways of yeast, like the unfolded protein response, to monitor the levels of unfolded/misfolded proteins in the endoplasmic reticulum. Other strategies may include increasing the secretory capacity or the co-expression of chaperones, foldases or ribosome receptors to overcome limitations in the folding and/or targeting of recombinant soluble and membrane proteins towards the endoplasmic reticulum. Similar approaches may lead to improved biosynthesis of functional proteins in bacterial, insect or animal cells. The goal should be to identify the bottlenecks in gene expression and generate technologies to produce individual proteins, protein complexes and hydrophobic proteins at a high rate.

The structure determination of membrane proteins, large multi-domain eukaryotic proteins and protein complexes is not only lagging behind due to difficulties in the

overproduction, purification and stability of these proteins, but also due to specific problems in crystallisation and labelling of these proteins. Generic methods, based on new approaches towards protein expression, need to be developed to address these problems. For instance, matrix- and interface-assisted 3D crystallisation methods have a strong potential for obtaining high-quality crystals of some proteins for X-ray analysis, but a major bottleneck is the visualisation of non-coloured protein. This could be solved by chromophore labelling of the protein, either biosynthetically (amino acid analogues or tagging at the gene level) or post-translationally.

Major hurdles also exist in finding conditions for expression and in obtaining the media and isotopes required for site specific labelling for use in NMR (13C, 15N, 2H) and other spectroscopic approaches (e.g., 2H for FTIR). Many sources of labels are scarce, still under development and often expensive. Rather less readily available are amino acids for isotopic highly specific labelling of proteins for structural studies, and the price and quantity required for such studies are often wholly prohibitive. In addition, although expression may be successful on defined media, expression on minimal media, which includes isotopically labelled compounds, may not be successful. Each of these hurdles requires breakthroughs in the production of the protein, which may have to come from entirely new expression systems.

1.2.2 Targeting the synthesised protein to a specific cellular location

For high-level expression, the intended sub-cellular localisation of the protein is an important consideration. The simplest strategy is often expression in the cytoplasm, either as a soluble protein or in inclusion bodies, but it can be advantageous to choose other locations in certain situations. Thus, disulphide bonds do not normally form in the cytoplasm, N-linked glycosylation and many other post-translational modifications cannot take place in the cytoplasm, and purification may be easier if the protein is secreted to the medium. For membrane proteins, refolding from inclusion bodies is often a difficult step, and it is better if the protein can be expressed in a functional state in a suitable membrane.

There are considerable difficulties involved in trying to express proteins in non-cytoplasmic compartments, however. Thus, not all proteins can be efficiently translocated across cellular membranes or moved through the secretory pathway, and high-level overexpression may saturate transport machineries and compromise cell viability. In general, the cellular reactions to overexpression of secretory and integral membrane proteins are poorly understood, and more work needs to be done to provide a firm foundation for the rational engineering of strains that can cope with such stress situations.

1.2.3 Folding and stability of expressed proteins

A major problem in the production of recombinant proteins is their low solubility and stability. For instance, many eukaryotic proteins are found in inclusion bodies when overproduced in *Escherichia coli*. In some cases, expression as inclusion bodies and subsequent refolding may be advantageous when large amounts of a labile, toxic or disulphide-bonded protein are needed, especially in labelled form. Here, folding screens similar to those in crystallography have to be established which will allow this methodology to be used on a routine basis. However, the role that the different bacterial chaperones and foldases play in the folding of newly synthesised polypeptide chains has also been well studied, and this knowledge could be used to develop new protocols for the production of soluble proteins in *E. coli*.

The situation is very different for the production of proteins in eukaryotic cells. First, much less is known about the protein folding processes in eukaryotic cells. Some chaperones and foldases have been found but often their precise role in the folding processes is not fully understood. Second, recent work has shown that most proteins are found in one or more multi-protein complexes in the eukaryotic cell. Little is known about the proteins (e.g., chaperones) and processes involved in the *in vivo* formation of these complexes. Studying the functional mechanisms of known chaperones and foldases, and discovering new ones, will increase the understanding of the folding processes in the eukaryotic cell, as well as lead to the development of new protocols for the production of soluble proteins in yeast, and in insect and mammalian cell lines. Further, the proteins involved in the protein complex formation and stabilisation will need to be identified and their roles studied. This knowledge will help to establish protocols for the successful overproduction of multi-protein complexes.

Finally, to obtain protein crystals of a recombinant purified protein, it is important to have mono-disperse samples of fully functional protein at (relatively) high concentration that are stable for long periods of time, and ways of achieving such conditions using genetic strategies need to be standardised. Catalytic activity is an indirect but important indicator of structural integrity of a protein, but for many complex systems this is not easily determined. More work on the development of methods to screen the functional state(s) of proteins and protein complexes in soluble, surface-associated or membrane-embedded state needs to be done.

After an international peer review process that was managed by the ESF, three Collaborative Research Projects (CRPs) were launched in 2006. These three CRPs consisted of 19 Individual Research Projects based in nine different European countries.

Facts and Figures

Deadline for Applications: 15 April 2004
Funded Collaborative Research Projects (CRPs): Three, consisting of 19 Individual Research Projects in nine different countries
Duration of Programme: 2006-2009
Budget for Research: 2 M€
EC Contract Number: ERAS-CT-2003-980409

1.3 EUROCORES quality assurance

1.3.1 Theme selection

New and challenging ideas for EUROCORES Programmes are invited from the scientific community through an annual Call for Theme Proposals. In addition to criteria including scientific quality, novelty and feasibility, the proposals are evaluated on the basis of the requirement for European collaboration: why it is necessary to conduct the Programme at a European level and how the Programme will strengthen and advance Europe's scientific position in a global context. Each proposal is sent for written external assessment to at least three referees. Based on these reviews, the Science Advisory Board (SAB) recommends which themes are to be further developed, a decision which is then ratified by the Governing Council.

1.3.2 Project selection

The peer review of the Collaborative Research Project proposals in a EUROCORES Programme such as EuroSCOPE is a multistage process, including the establishment of an international and independent Review Panel (RP). In response to an open Call for Proposals, Outline Proposals of about three pages are submitted by a team of applicants (minimum of three from three different countries). At that stage, the RP is responsible for the sifting of Outline Proposals prior to the invitation of Full Proposals. At the Full Proposals stage, each proposal is sent for written external assessments to at least three referees, including referees from outside Europe. Applicants are given an opportunity to reply to the anonymous referee reports. Written referees' assessments and replies by applicants are then considered by the RP with scientific quality being the main selection criterion. The RP makes recommendations for funding of Collaborative Research Projects (CRPs), with prioritisation, which ESF communicates to the EUROCORES Funding Organisations (EFOs).

After such an international peer review process three Collaborative Research Projects (CRPs) were selected for EuroSCOPE and launched in 2006.

1.3.3 Management Committee

At the time that the Call for Proposals is published, a Management Committee (MC) is established (see Section 6.1 for the EuroSCOPE MC).

- The MC has overall responsibility for the EUROCORES Programme within the guidelines of the EUROCORES Scheme;
- The MC can request expert advice from the EUROCORES Scientific Committee, Review Panel or any other *ad hoc* advisory group;
- MC members support the EUROCORES review process by nominating the potential Review Panel and external expert referees on behalf of their funding organisation;
- Each MC member is responsible for liaising with their funding organisation, including supervision of the funding process for EUROCORES projects within their organisation;
- Members may attend all meetings of the EUROCORES Programme as observers.

1.3.4 Mid-term and final reviews

Each EUROCORES Programme undergoes two reviews to evaluate its progress at the mid- and final stages. The aim is to assess scientific cooperation and interactions among the investigators and, for the case of a mid-term review, to:

- examine the merits of the EUROCORES Programme and its potential.

In the case of a final review, the aim is to:

- examine the merits of the EUROCORES Programme and the lessons there are to be learned for potential follow-up initiatives.

The Programme is assessed using the following criteria:

- **Novelty/Originality:** Most innovative/original scientific contribution of each CRP to the Programme and to the relevant field of research;
- **Multidisciplinary Research:** How is each CRP working towards (or achieving) multidisciplinary research;
- **Collaborative Research:** Results obtained within the CRP during this reporting period that would not have been achieved (or would have taken longer to achieve) in an individual project;
- **European added value:** a European dimension given to national funding (e.g., building up the European Research Area (ERA); developing a critical mass of expertise; addressing issues of scale and scope). For CRPs involving partners outside Europe: a clear example illustrating their added value to the Programme and their contribution to the relevant field of research in Europe;
- **Relevance to the Call:** Achievement most relevant to the Call.

The EuroSCOPE mid-term review was performed by the Review Panel by written procedure. The final review was based on written reports and a presentation by the Project Leaders during a final Review Panel meeting. The overall comments from the Panel were rather positive. "*Networking, training and dissemination were performed at a satisfactory level. Personnel exchange and training was good within the different CRPs. The make-up of the different CRPs did not lend itself to significant exchanges of personnel between CRPs"*. Regarding the scientific achievements it was mentioned: *"All CRPs have delivered appropriately"* and *"The BACELL project showed that the EUROCORES approach enables standardisation of experimental platforms (e.g., SURE system)".*

1.3.5 EUROCORES acknowledgements

To promote the EUROCORES Programme and the national funding organisations which support it (and prior to 2008, the European Commission), all publications, posters, websites and other dissemination outputs are required to be clearly identified as being Programme-funded or co-funded. This is an important indicator for monitoring the output of the Programmes, particularly peer-reviewed publications.

2. Highlights of the EuroSCOPE Collaborative Research Projects

2.1 Development and exploitation of *Bacillus subtilis* as a host for the production of protein complexes and membrane proteins (BACELL)

Principal Investigators and Associated Partners (APs)

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Funding Organisations

Finland: The Academy of Finland (AKA)
The Netherlands: The Netherlands Organisation for Scientific Research (NWO)
Slovak Republic: Slovak Research and Development Agency (SRDA)

This project was aimed at the development and exploitation of *Bacillus subtilis* as a host for the production of protein complexes and membrane proteins.

The van Dijl team designed and constructed a series of engineered *B. subtilis* strains that allow the high-level production of at least six essential membrane proteins from *B. subtilis* and *Staphylococcus aureus* using the SURE expression system. The research has shown that dispensable membrane-associated stress responsive systems set major limits to membrane protein overproduction. These bottlenecks were successfully removed for the overproduction of several membrane proteins.

The Kontinen team showed that the PrsA protein, a membrane-bound peptidyl-prolyl cis-trans isomerase, determined stability and cellular levels of a set of essential penicillin-binding proteins (PBPs) that are required for lateral cell wall biogenesis in *B. subtilis*. This explained why PrsA is an essential protein in *B. subtilis*. PrsA is not distributed evenly in the membrane, but it is localised in distinct spots that are lined up in spirals along the membrane, a localisation pattern very similar to that of some penicillin-binding proteins. The results can be exploited to improve PrsA technology in protein production. They

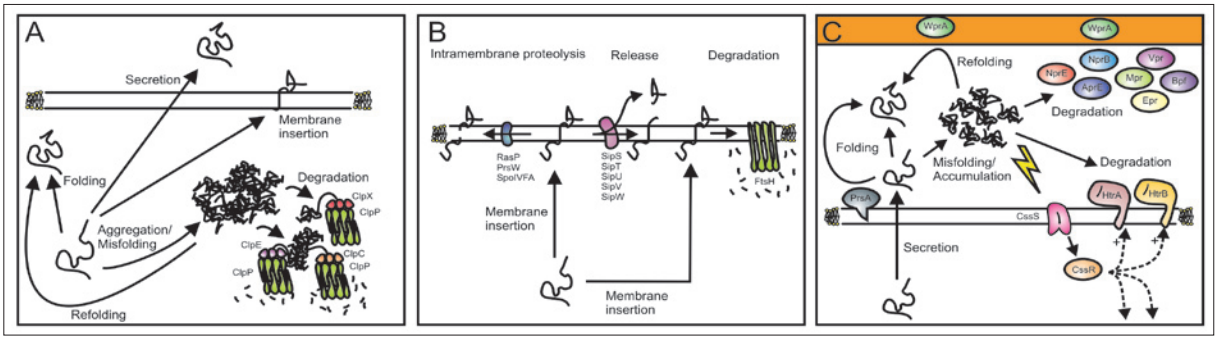

Figure 1. Protein quality control and proteolysis in *Bacillus subtilis.*

A) Model for cytoplasmic protein quality control in *B. subtilis* to which cytoplasmic proteins, membrane proteins and secretory proteins are subject. Depending on the presence or absence of targeting signals, newly synthesised proteins can be targeted for secretion or membrane insertion, or they can remain in the cytoplasm. If control of their folding by chaperones is insufficient these proteins can misfold and/or aggregate. This can lead to degradation by proteases such as ClpCP, ClpEP or ClpXP. Alternatively, misfolded proteins can be refolded with the help of chaperones. **B)** Model for protein quality control and degradation of membrane proteins within the membrane of *B. subtilis*. Proteins targeted to the membrane can be subject to processing by signal peptidases (e.g., SipS-W) or to degradation by membrane-associated proteases such as FtsH, PrsW, RasP or SpoIVFA. **C)** Model for extracytoplasmic protein quality control and degradation in *B. subtilis*. Translocated secretory proteins can fold with the help of folding catalysts such as PrsA. Accumulation of misfolded translocated proteins at the membrane-cell wall interface can trigger a secretion stress response, involving the CssRS two-component regulatory system. If activated, CssRS causes the up regulation of membrane-associated proteases such as HtrA and HtrB. These two proteins can probably catalyse both protein degradation and protein folding. Misfolded proteins are furthermore subject to degradation by cell wall-associated and/or secreted proteases, such as AprE, Bpf, Epr, Mpr, NprB, NprE, Vpr and/or WprA. (Adopted from Zweers et al. 2009, *Microbial Cell Factories* 7, 10, published by BioMed Central)

also open possibilities to search for new cell wall-active antimicrobial agents, PrsA inhibitors.

In the Driessen group multi-spanning membrane proteins (SpoIIIJ and YqjG) were overexpressed to high levels in *B. subtilis* using the SURE system. Furthermore a high molecular mass complex (500 kDa) consisting of SpoIIIJ/YqjG and the entire F1FO- ATP synthase was isolated from membrane fractions. Blue-native gel electrophoresis was successfully used for monitoring the complex. A number of *B. subtilis* knockout strains were created, e.g., *SpoIIIJ* and *YqjG* double knockouts with either xylose-inducible *SpoIIIJ* or *YqjG*. These were analysed in a membrane proteomic approach to identify membrane proteins that depend specifically on these two membrane protein integrases. By combined *in vitro* and *in vivo* assays it was shown that both SpoIIIJ and YqjG play an important role in membrane protein insertion.

In the Barák team efficiency and usefulness of different *B. subtilis* expression systems were compared to study the assembly of *B. subtilis* protein complexes and membrane proteins involved in asymmetric cell division and programmed cell death. A high-level production strain for the cell division protein DivIVA was constructed that allows its isolation and purification in sufficient amounts for further biochemical, electron microscopical and structural analyses. An efficient screening system was developed for identifying mutations that either enhance the production of the sporulation-specific membrane protein SpoIIE, or influence folding, membrane insertion or disulphide bond formation, thereby leading to efficient sporulation.

The Hecker team developed protocols for qualitative and quantitative membrane proteome analyses. These have been effectively implemented in the research of the team to investigate the effects of mutations that enhance membrane protein biogenesis, or to characterise the roles of certain components involved in this process.

Publications directly related to the project

(* Key publications)

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2.2 Folding, production and assembly of viral complexes for high resolution structure analysis (FOLPROCOM)

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Finland: The Academy of Finland (AKA)
The Netherlands: The Netherlands Organisation for Scientific Research (NWO)
Spain: Ministry of Science and Innovation (MICINN)

This project endeavoured to first develop protein expression, folding and assembly systems that were then used to produce viral complexes and to perform high resolution structural analyses of viral intermediary complexes.

The teams of Butcher and Tuma focused on viral protein expression, assembly and structure determination of viruses. They managed to produce viruses from several extremophillic organisms and the structures of these provided novel insights into early evolution of viruses. The expression, functional and structural characterisation of proteins and assemblies from dsRNA viruses yielded potential drug targets. The viruses under study included an avian reovirus, which is a major life stock pathogen.

The Van Raaij and Benavente teams produced viral fibres (sigmaC 117-326) and determined the structure of those and targeted assembly of dsRNA avian reovirus (published in the *Journal of General Virology*, March 2009).

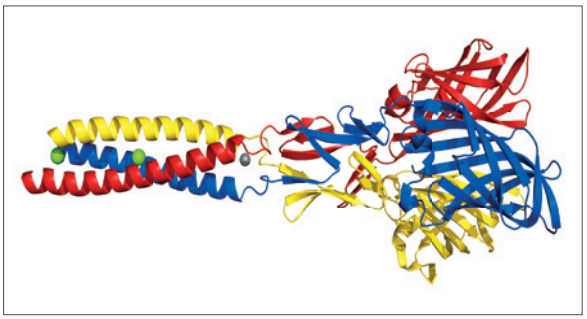
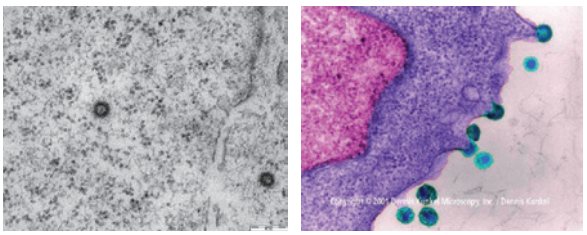


Figure 2. Structure of sigmaC 117-326. Crystals of sigmaC 117-326 were obtained, diffract to 1.9 Å resolution- circular DABC-HEFG sheet, long DE-loop, 2 triple beta-spiral repeats- 2 Cl- (coordinated by 3 Asn each) and Zn2+ ion (coordinated by 3 His).
Courtesy of Dr Mark van Raaij/Professor Javier Benavente

The team of Pichova and Ruml worked on the structural basis of retroviral targeting to the assembly site and mechanism of protease activation. Knowledge about these processes may lead to the development of novel drugs.



M-PMV (B/D type) **HIV (C type)**

Figure 3. Intracellular assembly of M-PMV-capsids. M-PMV assembles immature particles in the cytoplasm and activation of the protease within the immature particles is delayed until viral budding. Courtesy of Dr Iva Pichova

The Braakman team was able to identify, clone and characterise novel ER chaperones – these proteins are essential for antibody production in immune cells.

The Van der Vies team was able to identify and characterise novel parameters that determine the efficiency of GroEL chaperonin-dependent folding reaction – this allowed improved expression and folding of large viral proteins in *E. coli*.

Publications directly related to the project

Brandariz-Nuñez, A., Menaya-Vargas, R., **Benavente,** J. and **Martinez-Costas,** J. Avian reovirus muNS protein forms homo-oligomeric inclusions in a microtubule-independent fashion, which involves specific regions of its C-terminal domain. (Submitted to *J. Mol. Biol.* 4.2009).

Guardado-Calvo, P., Fox, G.C., Llamas-Saiz, A.L. and **van Raaij,** M.J. (2009) Crystallographic structure of the alpha-helical triple coiled-coil domain of avian reovirus S1133 fibre*. J. Gen. Virol.* 90, 672-677.

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Jäälinoja, H.T., Roine, E., Laurinmäki, P., Kivelä, H.M., Bamford, D.H. and **Butcher,** S.J. (2008) Structure and host cell interaction of SH1, a lipid-containing, halophilic euryarchaeal virus. *Proc. Natl. Acad. Sci. (USA)* 105, 8008-8013.

Jaatinen, S.T., Happonen, L.J., Laurinmäki, P., **Butcher,** S.J. and Bamford, D.H. (2008) Biochemical and structural characterisation of membrane-containing icosahedral dsDNA bacteriophages infecting thermophilic *Thermus thermophilus*. *Virology* 379, 10-19.

Ortega Roldan, J.L., Romero Romero, M. L., **Ora**, A., Eiso, A.B., Lopez Mayorga, O., Azuaga, A.I. and van Nuland, N.A.J. (2007) The high resolution NMR structure of the third SH3 domain of CD2AP. *J. Biomol. NMR* 39, 331-336.

Pena, F., Jansens, A. and **Braakman,** I. Calcium as a crucial cofactor for protein folding in the ER. *J. Biol. Chem. (2009, under revision).*

Suchanova, B. and **Tuma,** R. (2008) Folding and assembly of large macromolecular complexes monitored by hydrogen-deuterium exchange and mass spectrometry. *Microb. Cell Fact.* 7, 12.

Van Anken[1], E., Pena[1], F., Hafkemeijer, N., Christis, C., Romijn, E.P., Grauschopf, U., Oorschot, V.M.J., Pertel, V., Engels, S., Ora, A., Lástun, V., Glockshuber, R., Klumperman, J., Heck, A.J.R., Luban, J. and **Braakman**, I. (2009) Efficient IgM assembly and secretion requires the plasma cell induced ER protein pERp1. *Proc. Natl. Acad. Sci.* 106(40), 17019-17024.
[1]equal contribution

Vlach, J., Lipov, L., Rumlová, M., Veverka, V., Lang, J., Srb, P., Knejzlík, Z., **Pichová, I**., Hunter, E., Hrabal, R. and Ruml, T. (2008) D-retrovirus morphogenetic switch driven by the targeting signal accessibility to Tctex-1 of dynein. *Proc. Natl. Acad. Sci.* 105, 10565-10570.

Wildová, M., Hadravová, R., Štokrová, J., Křížová, I., Ruml, T., Hunter, E., **Pichová,** I. and Rumlová, M. (2008) The effect of point mutations within the N-terminal domain of Mason-Pfizer monkey virus capsid protein on virus core assembly and infectivity. *Virology* 380, 157-163.

Zabranská, H., **Tuma,** R., Kluh, I., Svatoš, A., Ruml, T., Hrabal, R. and **Pichová,** I. (2007) The role of the S-S bridge in retroviral protease function and virion maturation. *J. Mol. Biol.* 365, 1493-1504.

2.3 Genome-wide comparison of physiological bottlenecks in multi-subunit protein production in prokaryotic and eukaryotic microbial hosts (GENOPHYS)

Principal Investigators and Associated Partners (APs)

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Professor Merja Elisa Penttilä
VTT, Technical Research Centre of Finland, Biotechnology and Food Research, Espoo, Finland

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Department of Biotechnology and Bioscience, University of Milano-Bicocca, Milan, Italy

Professor Maria-Luisa Tutino (AP)
Molecular and Environmental Biotechology Group, Department of Organic Chemistry and Biochemistry, Faculty of Biotechnology, University of Napoli Federico II, Naples, Italy

Professor Antonio Villaverde
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Funding Organisations

Austria: Austrian Science Fund (FWF)
Finland: Academy of Finland (AKA)
Spain: Ministry of Science and Innovation (MICINN)

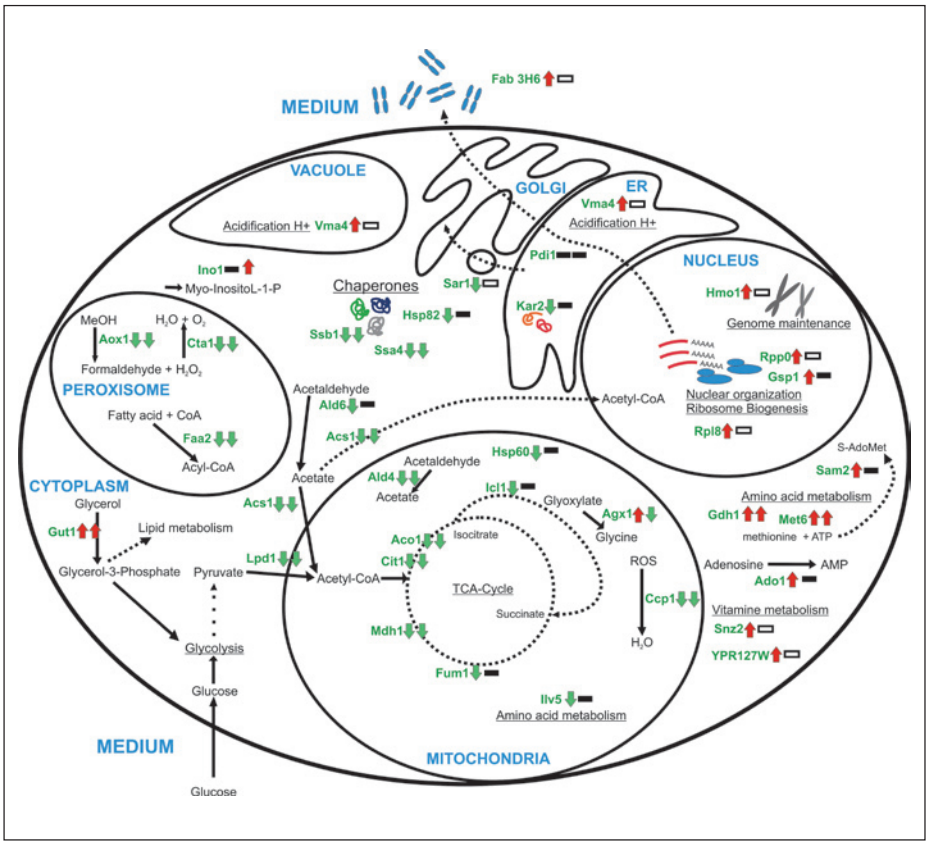

Figure 4. Example of data output of *Pichia pastoris* differential proteomics. Main changes in cellular protein levels upon a temperature shift from 30°C to 20°C in chemostat cultures of *P. pastoris* expressing an antibody Fab fragment. Green downward arrows indicate reduced protein levels at 20°C, red upward arrows indicate higher abundance at 20°C, black blocks indicate no significant change in protein levels at different temperature setpoints and open blocks indicate missing data. Left symbol: expression strain, right symbol: control strain.
Courtesy of Professor Diethardt Mattanovich

The consortium performed the first comparative analysis of microbial protein expression platforms at a genome scale (systems) level as a tool for novel strain engineering strategies for improved complex protein production.

The joint effort represents a critical mass enabling a broad comparison of protein production systems. The multidisciplinary approach of this CRP has allowed systems biotechnology to be established as a tool of great potential to make a breakthrough in the understanding and engineering of cellular processes involved in complex protein synthesis and secretion. This effort would not have been achievable by one single partner, based on national funding.

The European biopharmaceutical industry will benefit from an improved knowledge base for protein production, translated to superior process schemes. European science benefits from new concepts for the production of complex proteins for structure and function research.

This CRP has made an important breakthrough in the application of systems biology tools and methodologies to the systematic quantitative analysis of cellular processes involved in protein synthesis and secretion, and the interaction of such complex processes with key environmental stress factors such as temperature, oxygenation and osmolarity. This allows an important knowledge base to be built for further design of novel cell engineering strategies for improved production of protein complexes. For instance, such a multilevel systems approach is helping the understanding of the cellular mechanisms that lead to increased product formation under a particular environmental condition (e.g., low oxygen levels or low temperature in *Pichia pastoris* cultures), and may reveal hitherto unidentified key factors or major pacemakers of efficient protein production. In this context, strain engineering studies based on the generated knowledge are under way.

Access to joint transcriptomics and proteomics analytical platforms has allowed important qualitative progress to be made towards the application of systems biology concepts and methodologies; for instance, by making a significant contribution to building up a knowledge base that allows new targets to be identified for engineering cellular processes involved in protein expression and secretion in yeast cell factories. This scientific breakthrough had a significant impact on the visibility of the partners' labs, including collaborations with other academic groups and industry (both at international and national levels).

2. Highlights of the EuroSCOPE Collaborative Research Projects

Publications directly related to the project

(* Key publications)

*Baumann, K., Maurer, M., Dragosits, M., Cos, O., **Ferrer,** P. and **Mattanovich,** D. (2008) Hypoxic fed-batch cultivation of *Pichia pastoris* increases specific and volumetric productivity of recombinant proteins. *Biotechnol. Bioeng.* 100, 177-183.

*Carnicer, M., Baumann, K., Töplitz, I., Sánchez-Ferrando, F., **Mattanovich,** D., **Ferrer,** P. and Albiol, J. (2009) Macromolecular and elemental composition analysis and extracellular metabolite balances of *Pichia pastoris* growing at different oxygen levels. *Microb. Cell Fact*. 8, 65.

*Dragosits, M., Frascotti, G., Bernard-Granger, L., Vázquez, F., Giuliani, M., Baumann, K., Rodríguez-Carmona, E., Tokkanen, J., Parrilli, E., Wiebe, M.G., Kunert, R., Maurer, M., Gasser, B., Sauer, M., Branduardi, P., Pakula, T., Saloheimo, M., **Penttilä,** M., **Ferrer,** P., **Tutino,** M.L., **Villaverde,** A., **Porro,** D. and **Mattanovich,** D. Comparative analysis of antibody fragment production in diverse microbial host cells. (2009, under review).

*Dragosits, M., Stadlmann, J., Albiol, J., Baumann, K., Maurer, M., Gasser, B., Sauer, M., Altmann, F., **Ferrer,** P. and **Mattanovich,** D. (2009) The effect of temperature on the proteome of recombinant *Pichia pastoris*. *J. Proteome Res.* 8, 1380-1392.

Ferrer-Miralles, N., Domingo-Espín, J., Corchero, J.L., Vázquez, E. and **Villaverde,** A. (2009) Microbial factories for recombinant pharmaceuticals. *Microb. Cell Fact.* Mar. 24, 8-17.

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*Gasser, B., Maurer, M., Rautio, J., Sauer, M., Bhattacharyya, A., Saloheimo, M., **Penttilä,** M. and **Mattanovich,** D. (2007) Monitoring of transcriptional regulation in *Pichia pastoris* under protein production conditions. *BMC Genomics* 8, 179.

*Gasser, B., Saloheimo, M., Rinas, U., Dragosits, M., Rodríguez-Carmona, E., Baumann, K., Giuliani, M., Parrilli, E., Branduardi, P., **Lang,** C., **Porro,** D., **Ferrer,** P., **Tutino,** M.L., **Mattanovich,** D. and **Villaverde,** A. (2008) Protein folding and conformational stress in microbial cells producing recombinant proteins: a host comparative overview. *Microb. Cell Fact.* 7, 11.

Graf, A., Dragosits, M., Gasser, B. and **Mattanovich,** D. (2009) Yeast systems biotechnology for the production of heterologous proteins. *FEMS Yeast Res.* 9(3), 335-348.

*Graf, A., Gasser, B., Dragosits, M., Sauer, M., Leparc, G.G., Tüchler, T., Kreil, D.P. and **Mattanovich,** D. (2008) Novel insights into the unfolded protein response using *Pichia pastoris* specific DNA microarrays. *BMC Genomics*, 9, 390.

Mattanovich, D., Graf, A., Stadlmann, J., Dragosits, M., Redl, A., Maurer, M., Kleinheinz, M., Sauer, M., Altmann, F., Gasser, B. (2009) Genome, secretome and glucose transport highlight unique features of the protein production host *Pichia pastoris*. *Microb. Cell Fact.* 8, 29.

Resina, D., Maurer, M., Cos, O., Arnau, C., Carnicer, M., Marx, H., Gasser, B., Valero, F., **Mattanovich,** D., and **Ferrer,** P. (2009) Engineering of bottlenecks in *Rhizopus oryzae* lipase production in *Pichia pastoris* using the nitrogen source-regulated FLD1 promoter. *New Biotechnol.* 25(6), 396-403.

3. Networking and Dissemination Activities

Networking and dissemination activities are key characteristics of a EUROCORES Programme. Their aim is to encourage and facilitate scientific collaboration and diffusion across the Collaborative Research Projects (CRPs) within a given domain or, if appropriate, across different domains and programmes. These activities are flexible and can be tailored to the needs of a given Programme.

Networking activities are collaborative activities bringing together scientists from EUROCORES Programmes and colleagues from other relevant programmes in order to discuss, plan and implement future collaboration and interaction.

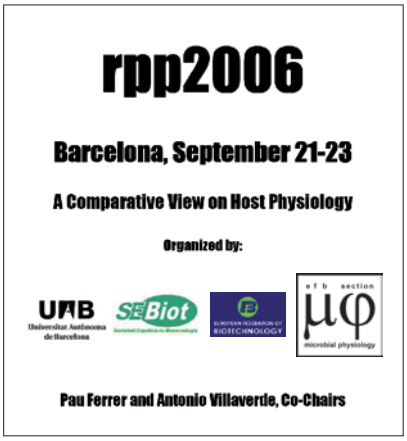
Typical examples are:

- Working group meetings, seminars, workshops, symposia, conferences;
- Summer schools (targeted to the members of academia, the private sector, and governmental or non-governmental organisations);
- Training programmes and specialised courses (graduate-level and continuing education);
- Short visits.

Dissemination activities are all the activities with the aim of raising awareness of and diffusing results of the EUROCORES Programme:

- Leaflets, posters, publications, books, exhibition booths or stands at conferences;
- Invited sessions at larger conferences (when the EUROCORES Programme is directly involved in the conference as a main organiser or co-organiser of the event);
- Dissemination travel grants, to support active participation at conferences (organised outside the EUROCORES Programme), while promoting the EUROCORES Scheme in general and disseminating the achievements of the Programme in particular.

For EuroSCOPE the principal networking and dissemination activities focused on various aspects of protein production during dedicated lectures given at three conferences and also included the production of a programme brochure and banner.



4th EFB (European Federation of Biotechnology) Meeting on Recombinant Protein Production

Barcelona, Spain, 21-23 September 2006

http://www.microbialcellfactories.com/supplements/5/S1

The first dissemination activity was promotion of the EuroSCOPE Programme at the 4th EFB Conference on Recombinant Protein Production, which was attended by more than 250 scientists who presented initial data from the respective EuroSCOPE projects. Additionally, an overview on ESF activities, with a focus on EUROCORES and the EuroSCOPE Programme was given to all conference attendees.

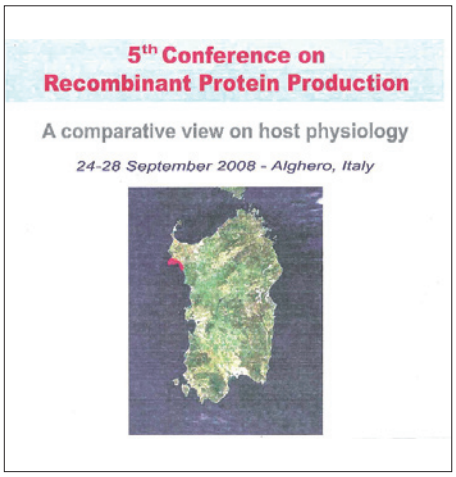
13th European Congress on Biotechnology

Barcelona, Spain, 16-19 September 2007

http://www.ecb13.eu/

The 13th European Congress on Biotechnology, organised by the European Federation of Biotechnology, focused on addressing the great challenges to humanity through the crucial theme of Symbiosis, applying cutting-edge Science and Industry in support of Society.

The EuroSCOPE Satellite Workshop at the 13th European Congress on Biotechnology was the most visible and thus most influential dissemination activity. All three CRPs presented their progress and the ESF gave an overview on EuroSCOPE and EUROCORES in general. Two international experts were invited for keynote lectures on complex protein production and structural biology. The chair of the workshop was Antonio Villaverde, one of the Principal Investigators of GENOPHYS.



5th EFB (European Federation of Biotechnology) Meeting on Recombinant Protein Production

near Alghero (Sardinia, Italy), 24-28 September 2008

The 5th EFB Meeting on Recombinant Protein Production focused on how physiology impacts upon recombinant protein production in all types of cell: bacteria, mammalian cell culture, yeasts and filamentous fungi, plant and insect cell lines as well as from viral vectors.

A EuroSCOPE Satellite Meeting was held at the 5th Recombinant Protein Production Conference. The EUROCORES Programme was briefly introduced, and all CRPs gave presentations on the scientific progress of their projects. External scientists were invited to present their recent work on topics related to EuroSCOPE. Members of GENOPHYS were members of the Scientific Committee and chairs of several scientific sessions.

4. Related ESF and other Activities

The different activities of the EuroSCOPE Programme, like protein production and structure analysis, link to various initiatives in the ESF remit and beyond. In this chapter, only those activities are mentioned that are closely connected and currently running.

4.1 EUROCORES: Membrane Architecture and Dynamics (EuroMEMBRANE)

The aim of the EUROCORES Programme EuroMEMBRANE is to answer long-standing questions in membrane biology using cutting-edge technologies. These will address functional problems in a quantitative manner bringing together experimental tools with theoretical approaches. There will be a special emphasis on lipid-lipid and (glyco)lipid-protein interactions in the membrane region in both health and disease. Using various model organisms would allow cross-species comparison and bring an evolutionary perspective to biomembrane studies. This type of research requires a strong interdisciplinary collaboration that covers biological, chemical, physical and computational aspects of membranology over a broad dynamic range of time and length.

The projects that have been selected for funding started in mid 2009.

More information can be found at:
www.esf.org/euromembrane

4.2 EUROCORES: Synthetic Biology: Engineering Complex Biological Systems (EuroSYNBIO)

Synthetic biology is the rational (re-)design of biological systems with useful properties. It is a highly interdisciplinary endeavour and can be viewed from two angles: first, the engineering perspective, which entertains the hope of transforming biotechnology into a true engineering discipline with the corresponding reliabilities and accuracies in design; second, the synthetic focus provides a unique tool for confirming or challenging our current understanding of molecular events and system function, because only if we can reliably rebuild cellular properties can we claim intellectual mastership ("What I cannot build, I cannot understand", R. Feynman).

Both these aspects of synthetic biology, transforming bioengineering and advancing understanding through synthesis, need to undergo a fundamental transition to be able to tackle systems-level questions. This transformation will happen on two fronts: first, there is the need to transform existing and develop novel computational tools that enable our current computational procedures to move from the analysis of single items to the systems level; second, it is necessary to support the computational change-of-scope with the same change in our workflows towards the "biosystems design laboratory".

The final element in this transition is the societal context, as clearly synthetic biology needs to be aware of and effectively manage its societal impact. Therefore, the societal context will be integrated in its various forms from an early stage of the scientific and engineering endeavour, bearing in mind that it might be a vital element in successfully guiding the future development of synthetic biology.

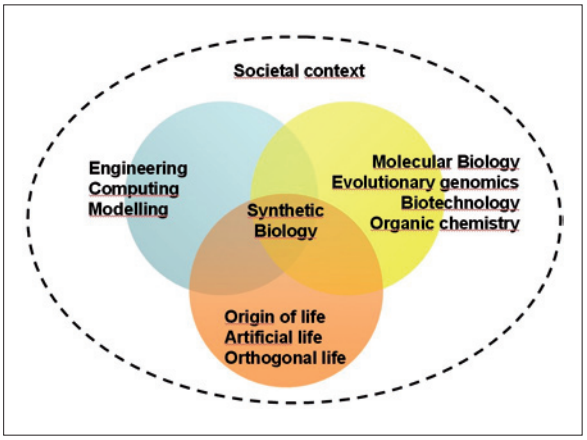


Figure 5. The highly interdisciplinary field of synthetic biology. Courtesy of Professor Sven Panke

The first achievements in synthetic biology include the design and implementation of synthetic genetic circuits, the design of novel biochemical pathways for the production of valuable pharmaceuticals, and the *de novo* synthesis of bacterial genomes. The ultimate ambition of the field is to extend the mastery of biological engineering to systems complex enough to deal with grand challenges such as the design, synthesis and delivery of novel therapeutic treatments, affordable and precise diagnosis of diseases, novel routes to vaccines, production of liquid transportation fuels, bioremediation of pollutants, biocompatible carbon sequestration, and efficient manufacturing of biopharmaceuticals and biochemicals.

The projects that are considered for funding will start in early 2010.

More information can be found at:
www.esf.org/eurosynbio

4.3 Research Networking Programme: The Euroglycoscience Forum (EUROGLYCOFORUM)

Glycoscience is a term used to describe all the areas which relate to complex carbohydrates, their synthesis and biosynthesis, analysis, function and applications. Glycoscience is a rapidly expanding and exciting field that is relevant to many areas of chemistry, biology and medicine. A significant number of European laboratories are considered to be at the forefront of current glycoscience research, and the current programme aims to strengthen that position. Whilst important discoveries have been made in the individual disciplines of glycoscience, it is generally recognised that future successes will rely on large interdisciplinary consortia which have sufficient tools and resources to address the complex questions that lie ahead.

From our current studies it is apparent that a true understanding of the role of glycosylation in complex biological systems will rely on close collaboration between biophysical scientists skilled in glycoscience technologies and scientists working in cell biology and medicine. The nuclei of such consortia have already formed in Europe and the current proposal is to foster such efforts and provide an umbrella organisation, the Euroglycosciences Forum, that will establish a cohesive research landscape for glycoscientists on which to build future successes.

The Euroglycosciences Forum will aim to provide convenient and active links between the leading research groups in the field through a series of meetings and workshops. It will also promote the establishment of key resources for glycoscientists, such as bioinformatics tools, microarray technologies, chemical synthesis of glycans, novel methods in structural analysis of carbohydrates, enzymology of proteins involved in glycan biosynthesis and metabolism, and molecular and cell biology of carbohydrate-binding proteins. Availability of these resources will ultimately lead to wider applications such as carbohydrate-based therapeutics, diagnostics and materials both in academia and industry.

There are quarterly calls for proposals, the 2nd EUROGLYCOFORUM Call for Proposals having the next deadline of 1 March 2010.

For more information, please see
www.esf.org/glycoscience

4.4 Other activities: Bacell consortium

Bacell is the umbrella organisation supporting *Bacillus* research in Europe. The *Bacillus* bacterium has been used for more than 50 years as a model to understand the detailed functions of individual cellular components. As a result, it is one of the best understood of all living organisms. Each component has a specific role and location within the cell. The next challenge is to determine the precise location of the individual components and to model how they interact with each other and their surroundings to form a successful living organism. In addition to its importance to basic science, *Bacillus* contributes to the health and wealth of society and the environment.

Through Bacell, the BACELL EuroSCOPE partners are connected to other European research programmes funded by the European Commission, such as Tat machine, StaphDynamics, BaSysBio, Bacell SysMo and TranSys.

For more information, please see
www.bacell.eu

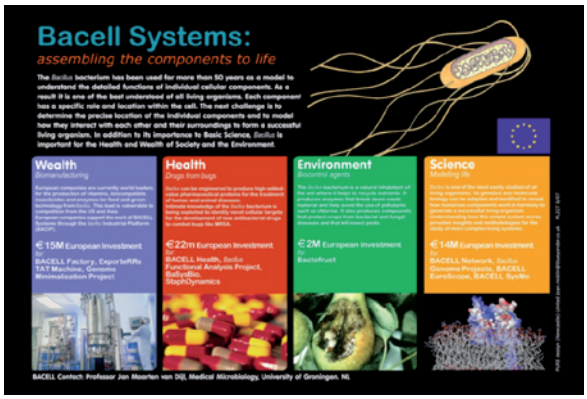


Figure 6. Scope of the Bacell activities.
Source: http://www.bacell.eu/

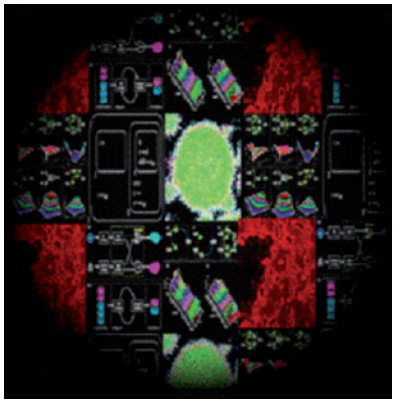


Figure 7. Logo of the Conference

4.5 Research Conference: ESF-UB Conference in Biomedicine

European Conference on Synthetic Biology (ECSB) II
Sant Feliu de Guixols, Spain, 29 March – 3 April 2009

Two communities are emerging within synthetic biology, namely top-down – i.e., knocking out or modifying functions of existing cells, and bottom-up – i.e., construction of artificial systems from first principles, protocells, etc. The aim of this conference was to generate new vigorous interactions between the disciplines that impinge on (and contribute to) Synthetic Biology, and to bring together in the same context top-down and bottom-up researchers. Some of the topics presented at the conference were: DNA sequencing and synthesis, chemical and biological networks, computational techniques (modelling, data mining, optimisation) for synthetic biology, minimal genomes, evolution (natural, directed and simulated), origins of life, biological systems, cell cycles and circuits, and infrastructures for synthetic biology, minimal cells.

For more information, please see
http://www.esf.org/activities/esf-conferences.html

4.6 Upcoming Research Conferences

- **ESF Research Conference**
  Bacterial Networks 2010
  Sant Feliu de Guixols, Spain, 4-9 September 2010

- **ESF-EMBO Symposium**
  Molecular Perspectives on Protein-Protein Interactions
  Sant Feliu de Guixols, Spain, 21-26 November 2010

Information will be made available in due course on
http://www.esf.org/activities/esf-conferences/

5. Conclusions

The Review Panel (RP) conducted a written assessment of the EuroSCOPE Programme in general and specifically the submitted final reports of the CRPs. At the final meeting with the RP, the Project Leaders also presented the outcome of their CRPs and looked forward to future initiatives. The presentations were followed by a discussion with the panel.

With regards to the organisational aspects of the Programme, the RP specifically commented that the subcritical mass of participating European countries that were supporting the programme was a major drawback. The original Programme was designed to cover a broad range of topics having various subfields and multiple calls for proposals. In practice, the Programme had to be reduced in its scope and ambition in order to comply with the limited amount of funding that was committed.

Initially, the Programme suffered from a rather long reviewing procedure (2004-2006) followed by a time delay before the start of funding for the CRPs. This was considered as a start-up problem for the newly developed EUROCORES Programme Scheme, and current improvements of the Scheme should ensure that such delays at the preparatory stage can be avoided in future.

The RP members also indicated that they would have preferred to be more closely involved in the networking and dissemination activities of the Programme.

In terms of scientific achievements the EuroSCOPE Programme delivered a series of valuable outputs. As a main highlight the RP mentioned the optimisation of *B. subtilis* production system for efficient expression of complex membrane proteins. The Programme has allowed a number of European scientists to maintain and extend their leadership in the field of *B. subtilis* research. Moreover, the consortium demonstrated its ability to create synergism by bringing together complementary funding schemes from across Europe.

The proof of principle for the use of the *Picchia pastoris* system for improved production of complex proteins has been delivered. As an important spin-off from the programme, the RP mentioned the possibility of “knowledge transfer” to industrial partners in relation to the hypoxic batchfed system.

A novel chaperone required for specific protein complex assembly was identified, underlining the need for the identification and potential exploitation of specialised cellular components in some cases.

For the whole Programme transnational scientific collaboration was demonstrated by a respectable list of high-quality co-authored research publications.

Networking, training and dissemination was performed at a satisfactory level. Personnel exchange and training was good within the different CRPs. The set-up and objectives of the different CRPs did not lend itself to significant exchanges of personnel between CRPs. It was recommended that in future there should be more inter-CRP exchange to enable participants to access new technologies and approaches that might be beneficial for their work.

6. Governing Bodies

6.1 Management Committee

Dr Olga Dias
Portuguese Science and Technology Foundation (FCT), Portugal

Dr Jan Dijkhof
The Netherlands Organisation for Scientific Research (NWO), The Netherlands

Dr Sonia Ftácnikova
Slovak Research and Development Agency, Slovak Republic

Dr Benno Hinnekint
Research Foundation – Flanders (FWO), Belgium

Ms Elisabeth Kokkelkoren
National Fund for Scientific Research (FNRS), Belgium

Dr Veronika Paleckova
The Czech Science Foundation, Czech Republic (GAČR)

Dr Branislav Peťko
Slovak Academy of Sciences, Slovak Republic

Dr Jukka Reivinen
The Academy of Finland, Finland

Dr Rosa Rodriguez-Bernabé
Ministry of Education and Science (MEC), Spain

Mr Tom Sheedy
Enterprise Ireland, Ireland

Dr Meelis Sirendi
Estonian Science Foundation, Estonia

Dr Graham Tebb
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6.3 International Review Panel

(members in 2006: *, members in 2009: #)

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6. Governing Bodies

6.4 Funding Organisations

Der Wissenschaftsfonds.

Austria: Austrian Science Fund (FWF)

Czech Republic: Czech Science Foundation (GAČR)

ACADEMY OF FINLAND

Finland: Academy of Finland

Nederlandse Organisatie voor Wetenschappelijk Onderzoek

The Netherlands: The Netherlands Organisation for Scientific Research (NWO)

SLOVAK RESEARCH AND DEVELOPMENT AGENCY

Slovak Republic: Slovak Research and Development Agency

MINISTERIO DE CIENCIA E INNOVACIÓN

Spain: Ministry of Science and Innovation (MICINN) [Former Ministry of Education and Science (MEC) / Ministry of Science and Technology (MCYT) / Office for Science and Technology (OCYT)]

6.5 Support team at the ESF

Medical Sciences Unit/
Life, Earth and Environmental Sciences Unit

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