European Cooperation in the field of Scientific and Technical Research

- COST -

Brussels, 24 May 2013

COST 008/13

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action CM1303: Systems Biocatalysis

Delegations will find attached the Memorandum of Understanding for COST Action CM1303 as approved by the COST Committee of Senior Officials (CSO) at its 187th meeting on 15-16 May 2013.

MEMORANDUM OF UNDERSTANDING
For the implementation of a European Concerted Research Action designated as

COST Action CM1303
SYSTEMS BIOCATALYSIS

The Parties to this Memorandum of Understanding, declaring their common intention to participate in the concerted Action referred to above and described in the technical Annex to the Memorandum, have reached the following understanding:

1. The Action will be carried out in accordance with the provisions of document COST 4154/11 "Rules and Procedures for Implementing COST Actions", or in any new document amending or replacing it, the contents of which the Parties are fully aware of.

2. The main objective of the Action is to build up a scientific platform to design and construct optimized modular biocatalytic systems for the sustainable synthesis of valuable products.

3. The economic dimension of the activities carried out under the Action has been estimated, on the basis of information available during the planning of the Action, at EUR 68 million in 2013 prices.

4. The Memorandum of Understanding will take effect on being accepted by at least five Parties.

5. The Memorandum of Understanding will remain in force for a period of 4 years, calculated from the date of the first meeting of the Management Committee, unless the duration of the Action is modified according to the provisions of Chapter IV of the document referred to in Point 1 above.

A. ABSTRACT

Systems Biocatalysis is a new approach consisting of organizing enzymes in vitro to generate an artificial metabolism for synthetic purposes. The strategy of this key new platform deals with the analysis of enzymatic systems in vivo, the development and discovery of new biocatalysts, and their assembly in vitro into novel synthetic metabolic pathways. This network aims at the controllable construction of metabolic pathways for the efficient synthesis of valuable chemical products. Tasks involved are the discovery of new biocatalyst, the optimization of their function, mutual compatibility and regulation, and the construction of a continuous flux for high efficiency. These tasks require research in various disciplines, as well as in molecular and engineering aspects of whole synthetic reaction sequences. Therefore this multidisciplinary Action is organized by an innovative grid Working Group structure. The latter will interface vertical thematic pillars with horizontal technology bridges, to foster a profound integration of the European Research Area. The new collaborative research network will develop innovative methods for the discovery, design, optimization, and use of novel biocatalytic modules towards a plug-and-play enzymatic toolbox concept. This platform will be instrumental for the training of the next generation of researchers in cutting-edge technologies and for the rapid translation of results into value creation by European industries.

Keywords: Artificial metabolism. Bioactives. Metabolites and building blocks. Multistepbiocatalytic transformations. Flow reactions.

B. BACKGROUND

B.1 General background

Integration of biocatalysis into chemical processes is one of the three pillars (the “Industrial Biotechnology” pillar) of the European Technology Platform for Sustainable Chemistry. Biocatalysis is also one of the fundamental methods of “Green Chemistry”, a concept to replace conventional chemical processes that are usually characterized by undesired energy consumption from high temperatures or pressures, and by waste generation from using stoichiometric/excess reagents or catalysis with toxic transition metals. However, with the introduction of biocatalysis to reduce the ecological footprint, practical and economical industrial synthetic conversions most often rely on single-step conversions using isolated enzymes or whole cells catalysts (*cell factories*), which transform one set of source compounds into one set of target compounds. In living

organisms, in contrast, chemical conversion of matter proceeds within metabolic flow systems that constitute a highly complex network in which metabolites are interconnected by nested and interdependent reactions. Enzymes have matured by evolution over billions of years into extremely efficient catalysts that can facilitate those reaction cascades with unmatched selectivity at ambient conditions. Despite advances in genetic engineering of production strains to optimize product formation, metabolic streamlining is often hampered by an unpredictable impact on the entire metabolism, because cells have evolved intricate regulatory mechanisms that counteract the genetic mutation by employing alternative pathways. Recent scientific activities in synthetic biology have been directed at simplified organisms, allowing more efficient synthesis of natural products as an endpoint of cellular metabolism. However, more demanding goals, such as expanding the range of accessible products from microorganisms to structures with non-natural constitution or unusual chemical functionalization, have been a major challenge in cellular systems. A variety of systemic interactions both in the chemical as well as biological space, such as e.g. the viability of the organism or the relative expression levels, require new approaches. Recently, problem solving tools applicable to isolated metabolic components, and even to systems-wide parameters, have reached a new qualitative level: (1) design opportunities were promoted by rapid development of knowledge in the molecular sciences, e. g., in proteomics, structural biology, metabolomics and bioinformatic sciences; (2) practical applications were supported by improved technologies, e.g. for enzyme discovery by screening and (meta)genomic mining, protein engineering by directed evolution in vitro, enzyme immobilization and stabilization, non-aqueous enzyme catalysis, flow reactor technology, etc. Attempts to understand and reprogram complex chemical reaction pathways resulted in the concepts of *Systems Chemistry*, which studies the self-assembly of substrates and catalysts for model synthetic systems. However, such systems are difficult to control because of the interference from similar chemical reactivity in substrates, intermediates, and products. Inversely, *Systems Biology* attempts to understand the intricate complexity of biological reaction systems. However, the latter is primarily an analytical top-down approach to metabolic regulation and cell signaling networks.

Systems Biocatalysis - a new approach consisting of organizing enzymes *in vitro* to generate an artificial metabolism for synthetic purposes - steps in as an attempt to bridge the open gap remaining between the concepts of *Systems Chemistry* and *Systems Biology*, merging the synthetic focus of chemistry with the modular design of biological systems. Such novel methodology can only be developed by building on and merging available expertise of research teams and modern technologies on a global, Europe-wide level.

Why COST? The goals of the Systems Biocatalysis (*SysBiocat)* Action will be approached by

merging the complementary expertises such as synthetic, biophysical and biological chemists skills with enzymologists, bioprocess and protein engineers, molecular and structural biologists. The COST mechanism

• rapidly creates a dynamic open network.

• fosters new interconnections between fragmented sub disciplines, e.g. from biosciences and chemistry.

• fosters new cross-disciplinary collaborations that otherwise would never be established.

• merges efforts to tackle big and complex multidisciplinary problems that single groups are unable to pursue.

• maximizes effectiveness by consolidating funding of individual research groups at the national level, thus building up new research capacities within the European Research Area (ERA).

• strongly facilitates cross-disciplinary state-of-the-art education of Early-Stage Researchers (ESR) and Experienced Researchers (ER), via Training Schools and Short-Term Scientific Missions (STSM).

• allows worldwide dissemination of ideas, best practice, materials, and scientific results to a wide audience.

• fosters the development of research careers and creative thinking for young researchers.

• with an open-spirited, bottom-up approach is the most effective scheme for the exchange of knowledge.

• will promote this new initiative by organizing the first ever *Systems Biocatalysis* symposium in 2014.

• will stimulate future joint applications of research projects extending far beyond the Action's funding period.

B.2 Current state of knowledge

The use of enzymes, or whole cells, to transform non-natural compounds has attracted an increasing interest especially during the past two decades: over 10,000 scientific papers concerning new aspects of biocatalysis and biotransformation have been published in peer-review journals, thus creating the basis for innovation in the chemical industry. This approach began to be increasingly applied to the manufacture of high value products including pharmaceuticals, flavors and fragrances, specialty and fine chemicals. Such applications have been primarily carried out in classical stepwise processes consisting of isolation of each intermediate before its use as a starting material for the subsequent reaction. Nevertheless, the potential immanent in the technology has not

yet been fully exploited. One of the major reason is the fact that biocatalytic reactions have often been studied – particularly in academia – without any context to further chemical or enzymatic steps of the particular process so that their full value was not fulfilled. In addition, the operational parameters of many biocatalysts described to date have been rather suboptimal with respect to limits in substrate scope, protein stability, stereoselectivity, substrate loading, and inhibition effects. Also, currently known enzyme classes are limited in the number of possible reaction types, which excludes many valuable operations that are common to industrial organic synthesis. Therefore, there is still an urgent need to promote the viability of biocatalytic processes, thus creating the background for their faster transfer to industrial scale. As a more recent tool, an in vitro version of Darwinian evolution – termed directed evolution – has been brought to the molecular scale, which enables the construction of genetic diversity by randomization techniques followed by selection or screening for the most efficient enzymes. By iterative cycles of improvement, this technology has started to allow targeted modification of single enzymes for improved protein stability, substrate specificity and enantioselectivity. In isolated cases, even in vivo-metabolic pathways could be improved. However, access to such technology is still limited to few specialist laboratories. It is essential for future progress in this field to profoundly disseminate best practice and facilitate access to high-throughput experimental facilities.

Comparison of Europe vs. the rest of the world: Europe has a long-standing tradition in applied biotechnology in the chemical industry, with growing competition from Asia and North America. European institutions are well positioned in the fields of molecular enzymology, structural biology, and rational site-specific protein engineering. Europe also holds a competitive position in prospecting for novel biocatalysts from environmental sources (“metagenomics”) and in advanced engineering of equipment for high-throughput experimentation. However, notwithstanding the seminal conceptual and creative experimental contributions to the recent development of directed evolution within the ERA by several groups and Small and Medium sized Enterprises (SME) working in this field, e.g. for creating enantioselective enzymes, the core technology for directed evolution is a US patented technology, and the largest company applying it for the optimization of enzyme catalysts for industrial applications (Codexis Inc.) is US-based and currently dominating the global market. While research funding of this topical science is prominent in the US, such as the most recent large-scale collaborative project “Enzyme Function Initiative” (EFI, enzymefunction.org), and attains global visibility from political instruments such as the US bioeconomy and the “Presidential Green Chemistry Award”, funding for this field in Europe has been rather limited at national and at the Seventh Framework Programme (FP7) levels. Europe will only be able to remain competitive in industrial applied biotechnology on a global level, if research

efforts in biocatalysis, protein engineering and evolution, reaction biotechnology and organic chemistry are coordinated to achieve maximum impact of its expertise and activity.

B.3 Reasons for the Action

The *SysBiocat* Action is targeted at harnessing the so-called "3rd wave of biocatalysis" (see most recent review article in *Nature* 2012, *485*, 185) to advance the multidisciplinary, fragmented field of applied biocatalysis to a higher level and thereby improving and securing Europe's competitiveness in this field on a longer ranging perspective. The incentive for establishing a concerted COST Action is that most European researchers in the appropriate sub-disciplines are currently working independently with too little interdisciplinary activity. Research in the field is often hindered by proprietary considerations. By serving the need for more effective exchange of information, material, experience and skills within a new collaborative network, this COST Action will profoundly accelerate development of the ERA, by increasing the effectiveness of individual research as well as seeding future collaborations. The aims of the *SysBiocat* Action, by virtue of their ambitious, multi-facetted nature, intrinsically require establishment of a cross-disciplinary forum of experts, ensuring that biocatalysis research in the ERA maintains a leading-edge position. The *SysBiocat* Action will primarily furnish scientific and technological advancement as an immediate outcome, including a deeper understanding of protein structure and catalysis, as well of metabolic flow systems from simplified in vitro systems. On a short to medium timeline, European chemical/pharmaceutical industries are expected to readily adsorb and profit from the practical results of the Action (new biocatalysts, new reaction types, new products, advanced reaction technology) due to their potential economic advantage in comparison to traditional methods of chemical synthesis. Consecutive long-term benefits will arise to serve the need for a sustainable European society: improving the quality of life by more eco-friendly, "green" production processes, and improving the health of citizens by the development of novel products that may accelerate advance in medicinal chemistry and the development of personalized medicines. Indeed, three European SMEs and one large chemical company have been consulted as stakeholders for the definition of the Action's aims and scope, who will also be actively participating in the scientific collaboration. An additional important benefit of this Action will be the cross-disciplinary education of ESRs and ERs in this rapidly developing field of applied biocatalysis, which is essential both for the highly qualified training of the next generation of researchers as well as to counteract against a further fragmentation of European research activities, eventually increasing Europe's competitiveness. Therefore, the activities that will be performed in the *SysBiocat* COST Action are

highly topical to address an innovative approach to an existing problem.

B.4 Complementarity with other research programmes

There are specific activities funded under the Seventh Framework Programme umbrella with complementarity to the aims of the *SysBiocat* application. Among others, these are the training networks *ENEFP* and *BIOTRAINS* on individual aspects of protein engineering and white biotechnology, respectively, as well as the *EngBiocat* (ERA-IB) platform for industrial enzyme engineering; results from these soon ending programs will be directly useful to *SysBiocat* because of dual role overlap of investigators. More recent initiatives are *AMBIOCAS, KYROBIO, BIONEXGEN and HOTZYME,* relevant for the development of various enzymes for industrial applications, the *NewProt* project designated to develop a software portal for in silico protein engineering work, and the *BIOINTENSE* project for bioprocess integration; all the latter activities will be instrumental also for the progress of the *SysBiocat* endeavor due to mutual overlap of principle investigators. Most current programs share in part individual aspects of the *SysBiocat* Action and are directed towards discovery and engineering of specific enzymes, or towards the metabolic engineering of production strains for biotechnology applications. As many consortia only comprise a dozen or less partners, their highly specific focus might be interpreted as evidence for the fragmented state of the ERA, for which the *SysBiocat* network will be providing an overarching platform to integrate the specific individual efforts, for a more effective way of cross-disciplinary, pan-European sharing of methods, materials, technology, and results.

C. OBJECTIVES AND BENEFITS

C.1 Aim

The aim of the Action is to build up a scientific platform to design and construct optimized modular biocatalytic systems for the sustainable synthesis of valuable products.

C.2 Objectives

For the future benefit of European competitiveness the *SysBiocat* network will make use of the most recent scientific and technological developments to integrate biocatalytic synthesis towards a higher qualitative level. The idea is to devise, construct, and optimize new biocatalytic modules along a tool-box concept, that allow processing substrates obtained from renewable resources through

artificial bio-synthetic pathways in cell-free systems in vitro. Thereby, useful products can be obtained in an integrated continuous fashion, avoiding bottlenecks from complex regulation phenomena of cellular systems (*cell factories)* by a bottom-up approach.

Objectives of the *SysBiocat* Action are to develop:

• methods for creating enzyme modules with novel functions to establish a plug-and-play toolbox concept

• the assembly of biocatalytic modules from the toolbox to create novel artificial metabolic pathways in vitro

• enzyme modules with new/modified/improved substrate tolerance from the systems perspective

• a better understanding of regulation of enzyme function, to avoid inhibition in optimized modules

• systems approaches to cofactor-dependent biocatalysis by new/improved/cofactor-independent biocatalysts

• standardized interfaces between important standard enzyme modules

• improved expression systems for biocatalytic modules

• improved mutagenesis strategies for debottlenecking enzyme modules from the systems perspective

• smart assay concepts for high-troughput screening of enzyme function

• improved reaction technology, devices for flow synthesis and in situ reaction monitoring.

Specific steps towards these goals are to:

• identify useful reaction sequences

• optimize enzyme performance by directed evolution

• engineer non-natural enzyme reactivity

• design and optimize multistep artificial bio-synthetic pathways in vitro

• assemble optimized modules in a plug-and-play fashion for specific synthetic targets

• apply process engineering and intensification tools for competitive applications

The task is highly challenging today but will propel a promising "3rd wave technology" into the future. This technology will be instrumental for the production of value-added chemicals that are currently difficult or impossible to produce by whole-cell catalysts or conventional chemical approaches. Such novel methodology can only be developed by merging available complementary expertise of top-level research teams and modern technologies at the interfaces between chemistry–biology–engineering on a Europe-wide level.

C.3 How networking within the Action will yield the objectives?

The Action requires a strong cross-disciplinary approach, and the objectives are not within reach even for the most advanced individual research units. Networking instruments provided by the COST scheme — WG organization, Action Management Committee and Steering Committee meetings, Action Workshops, STSMs for Early-Stage Researchers — provide the ideal communication system to build ideas and accomplish projects.
Various European research groups with complementary expertise in the necessary chemistry, biology, and engineering sub-disciplines have already expressed an interest in participating in the *SysBiocat* Action. Each of them will bring sufficient scientific contribution to reach the objectives. The topics to be addressed are sufficiently broad to allow an extension by incorporating further scientists, within the limits set by the COST rules, once the Action will be launched.

C.4 Potential impact of the Action

A major impact is expected from the high-level education and training of Early Stage Researchers at the forefront of multidisciplinary research in a pan-European scientific network. They will be profiting in their career development from exposure to creative ideas and innovative methods, supported by advanced Training Schools and personal involvement in collaborative STSMs, enabling them to become future leaders in their field. This aspect gains particularly momentum from declining research and development budgets due to the current economic crisis, which could deprive an entire generation of young scientists from developing their perspectives for a scientific career. High-level COST training activities in cutting-edge technologies such as those involved in the *SysBiocat* platform, and opportunities for scientific exchange with top-level research groups, promise to be the ideal instruments to counteract this daunting trend.
The *SysBiocat* Action will make a major contribution to accelerate the development of effective "Green Chemistry" applications in industry, and thus will have a long-term global impact on the quality of life, society and the environment. The toolbox concept of highly optimized biocatalytic modules is expected to profoundly strengthen European competitiveness in biocatalysis research. Combining the dissipated knowledge and financial resources within a strong collaborative network will propel Europe to the forefront of this emerging discipline.
The innovative, interdisciplinary character of the research will enhance research quality and reputation of the contributing teams, creating a stronger European scientific impact by dissemination of results in peer-reviewed journals and presentations at international conferences. Results from process intensification will indicate the boundaries for the *SysBiocat* approach to become industrially relevant and competitive in comparison with existing conventional processes.

Thus, patent applications on the new technology are also to be expected.

C.5 Target groups/end users

The objectives of the *SysBiocat* Action will be of interest to a broad range of target groups in academic research, industrial development, policy makers and the general public. Three European SMEs and one large chemical company have been consulted as stakeholders for the definition of the Action's aims and scope; they will also be actively participating in the scientific collaboration. The Action will actively recruit additional industrial participation. In the preparation of the Action members of three large university-connected European research centres have been involved, who will contribute to disseminate the expected results into the academic and productive world.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

The main objective of the *SysBiocat* Action is to develop an integrated new platform technology for the biocatalytic synthesis of chemicals. Artificial metabolic pathways will be assembled by connecting biocatalytic modules originating from natural metabolisms together with newly designed enzyme modules. The in vitro-approach of *SysBiocat* differs from Synthetic Biology approaches in the architectural principles and enables a wider range of applications at significantly reduced complexity of installation and operation. This method will enable the safe, sustainable processing of renewable resources to produce valuable target products, such as modified bioactives for biomedical purposes and chiral building blocks for industrial purposes. By using continuous flow systems, without a need for isolation of intermediate metabolites, the overall "atom efficiency" and product yield of the chemical transformations becomes strongly improved while avoiding production of waste materials. Major deliverables will be novel enzyme modules, new reaction systems, new molecules and new tools for protein functional analysis. In particular, the most important research goals can be summarized as:

1. New Metabolisms: Newly designed reaction sequences going beyond the single-enzyme catalyzed step for the synthesis of valuable products.
2. Best Biocatalysts: Proteins with required and complementary catalytic functions obtained from directed evolution techniques optimized towards substrates loading, solvent tolerance, broadened pH and T range with as high as possible specific activity.
3. Engineered Bioprocesses: Process conditions allowing successive catalytic steps to occur in the

same reactor or in successive regions of a continuous reactor without product isolation, solvent removal, and purification.

D.2 Scientific work plan - methods and means

Biocatalytic modules from natural metabolisms will be improved for technical applications in vitro and will be joined with newly designed enzyme modules by stepwise assembly into fully modular artificial metabolic pathways. Their behavior should be highly predictable and controllable because of a significantly reduced level of complexity as compared to entire metabolic networks of living organisms. Non-natural metabolites and non-biological target products will become efficiently accessible, by turning knowledge acquired from proteomics and metabolomics into the construction of artificial systems for preparative synthesis.

Scientifically, the *SysBiocat* Action will construct a grid network made up from three vertical pillars of thematic research (Working Groups 1-3), interfaced by two horizontal bridges of technology research (Working Groups 4,5) to best exploit the knowledge from current understanding of protein function and natural metabolic pathways. Each Working Group (WG) will be based on the extensive expertise of the multidisciplinary teams that have actively participated in the conception of the *SysBiocat* Action. Jointly, they draw together an impressive equipment base with state-of-the-art facilities in all diverse subfields of research pertaining to high-throughput experimentation, molecular biology, protein engineering, protein biochemistry, preparative biocatalysis, bioreaction technology that will be available for collaborative research and training within the *SysBiocat* Action.

Beyond the scope of the examples given below, the project framework will remain open to the development of further biocatalytic reaction modules of interest to both European scientific and industrial communities. The Action and its WG grid structure are designed to offer unique opportunities for incorporating new scientific ideas, emerging technologies, and extended collaborations at any stage of the project.

WG1 - New modes of creation and interconversion of chemical functionality in metabolites

The objective of WG1 is to focus on functional group interconversion as an unavoidable step in building an artificial metabolism. Enzyme catalyzed single functional group modifications often suffer from too high substrate specificity, low specific catalytic activity, complex cofactor requirements, and thermodynamic equilibrium constraints. For conversion of an alkene into an amine, e.g., the sequence alkene => alcohol => ketone => amine, which in part mimics a natural pathway, outlines an extremely useful synthetic chemical transformation. This sequence can also be

performed by a combination of three individual enzymes and one shared cofactor but its application is hampered by an incompletely overlapping substrate specificity of the different available enzymes and by an unfavorable overall equilibrium state, which requires extensive reaction engineering. Otherwise, a direct alkene => alkylamine transformation would be even more useful, but except for a very specific precedent in metabolic pathways, generalist enzymes are yet unknown. There are several other chemical transformations in the routine method toolbox of the organic chemists for which there is no standard biocatalytic equivalent in Nature. Therefore, besides the functional group interconversion illustrated above, further aims will be some less explored or emerging reaction types such as C–H bond activation, racemization, (ep)oxidation, hydrogenation, (de)carboxylation, Michael addition, arene alkylation or acylation.

WG2 - Routes to New Key Metabolites

The aim of WG2 is the development of enzyme modules for the interconversion and modification of metabolite components from primary and secondary metabolic pathways that define the efficiency of central carbon flux. The central pathways of metabolism, such as glycolysis and the citric acid cycle, are present in all three domains of life and have been optimized by evolution to sustain life even under extremes of environmental conditions. Cellular metabolic networks are a striking example of a functional architecture able to input a wide range of nutrients and produce a large variety of products such as amino acids, monosaccharides, isoprenoids and nucleotides using relatively few common intermediates. There are multiple levels of self-regulation in metabolic pathways that affect the flux through the system to respond to changes in the levels of substrates or products, often involving indirect allosteric regulation of enzyme activities. The avoidance of regulatory obstacles involved in the metabolic conversion of core building blocks is a central focus of study to accelerate the production of valuable metabolites from simple nutrient substrates along in vitro-assembled metabolic pathways; de-regulation of the sensitivity of enzyme modules to inhibition by substrates, intermediates, and products will be attempted. Also, new pathways will be created by coupling natural parts of metabolic paths to foreign modules to achieve the synthesis of non-natural metabolite analogs. Interesting targets are activated isoprene donor analogs by deviations from the mevalonate pathway, carbon dioxide fixation pathways, metabolic transposition of alpha- to beta-amino acids etc. Stable isotope labelled metabolites will be prepared along artificial pathways for analytical purpose in the investigation of their roles in natural environments, in order to improve our understanding of natural metabolic pathways. Metabolites from pharmaceutical xenobiotics will be prepared as analytical standards to investigate their bioactivity profiles. Enzymes to be developed for new or improved functions as reaction modules for sequential assembly to in vitro-metabolic systems are (de)-carboxylases, aminotransferases,

ammonia-lyases, 2,3-aminomutases, (de)-hydratases, aldolases, isomerases, dehydrogenases, kinases, phosphatases, etc.

WG3 - Constructing pathways towards complex lipid conjugates

The major goal of WG3 is the development of enzyme modules for the synthesis and interconversion of functional components that define the enormous value of lipid conjugates for technical and medical applications. Glycolipids, composed of a hydrophilic polar sugar headgroup and a hydrophobic moiety belonging to different lipid classes (ceramides, phospholipids, fatty acids, terpenes, steroids) are ubiquitous amphiphilic components of cell membranes where they are involved in various important physiological functions, often in modulating cell-cell interactions. The complexity of structural variations in this compound class makes purification of homogenous materials from natural sources extremely difficult. Development of future technical applications for nutrition, functional food, and cosmetics, as well as for biomedical applications for treatment of specific diseases requires a range of enzymes to develop practical syntheses of natural compounds (as well as non-natural analogs having improved technical or therapeutic potential). Exemplary metabolic targets are the modification of constituents making up lipid conjugates and their re-assembly, e.g., fatty acids, ceramides, cyclitols, monosaccharides; immediate studies will concern the regio and stereoselective oxygen functionalization or backbone extension of unsaturated fatty acids towards emerging bioactives (e.g., linolenic acid => eicosapentaenoic acid => docosahexaenoic acid), as well as the formation of medicinally important oligosaccharide epitopes (e.g., GlcNAc => LacNAc => Sia-LacNAc). Enzymes to be developed for new or improved functions as reaction modules for sequential assembly to in vitro-metabolic systems are desaturases, elongases, lipases, phospholipases, aldolases, isomerases, kinases, phosphatases, glycosidases, nucleotidyl transferases, glycosyl transferases etc.

WG4 - Enzyme engineering for novel and optimized functions

The objective of WG4 is the development, improvement, and application of technological tools for the rational and directed engineering of enzyme functions that are required for them to act as efficient catalytic modules within artificial in vitro metabolic systems. Despite continued advances in the understanding of protein structure and function there are many aspects of protein function that cannot be predicted. Nature evolves protein function by iterative cycles of mutation, selection and duplication. Analogous evolutionary strategies can also be profitably exploited in vitro to generate and optimize biocatalysts for diverse biotechnological applications. Based on molecular combinatorial methodologies, directed evolution of enzymes provides a practical means of tailoring biocatalyst properties for new tasks. Optimization strategies require the ability to generate libraries of partially randomized genes and means of screening, or selecting from, proteins produced from

those libraries for improved or modified characteristics. Crucial for rapid progress in combinatorial approaches to alter protein function is the development of innovative library strategies to reduce the high efforts required for screening the large protein sequence space, to build “smaller but smarter” libraries, while avoiding the bias problems arising from the degeneracy of the genetic code. Bioinformatic tools are emerging to provide guidance in the evolutionary pathways (semi-rational design). Discovery and optimization of enzymes for novel and improved functions will be assisted by sophisticated bioinformatics analysis for the mining of gene sequences deposited in public (meta)genome databases, as well as from rich sources of protein structure databases. Another important element is the development of effective assay technology, high-throughput methods that are generic for broader applicability, but highly specific for the desired function. The design of in vivo selection systems would be ideal for the exploitation of large libraries but such strategies are currently underdeveloped due to the complexity of cellular metabolism; thus the design and installation of novel in vitro assays is instrumental for success. A recent example to demonstrate the power of current technologies for the evolution of improved biocatalysts is the most extensive, successful redesign of a microbial transaminase to improve its substrate tolerance and stereoselectivity required to establish an economical, green process for the industrial synthesis of the drug sitagliptin.

WG5 - Process intensification

The aim of WG5 is the development of novel approaches in reaction technology, reaction biotechnology and downstream processing to provide additional advantages for biocatalytic routes by process intensification. The development of novel chemical entities and products in a green and sustainable fashion industry requires new synthetic and screening methods with enhanced speed. Miniaturization and integration of reactors for consecutive steps, and with downstream and process monitoring elements in a single continuous flow system, offer many advantages to the productive industry, which is constantly searching for controllable, information-rich, high-throughput, and environmentally friendly methods for producing products with a high degree of efficiency and selectivity. In all integrated systems from the ‘simplest’ one containing a single or few isolated enzymes used alone or in combination with non-enzyme-catalysed steps, to the most ‘complex’ one in which artificial pathways are created and used as synthetic factories in vitro the advantages of reaction engineering combined with application of miniaturized and compartmentalized systems are manifold. This comprises fewer unit operations, less solvent and smaller reactor volume, shorter cycle times, higher volumetric and space-time yields, and less waste, altogether making up for a much lower environmental footprint (*E* factor). On the other hand, well known physical effects show abnormal effects upon downscaling to the micro domain. Flow, mixing, chemical reactions,

heat transfer all behave differently than in the macroscopic world. Therefore the design and development of micro-devices on the micro-scale requires special care. Such integrated systems are truly emulating Nature where metabolic pathways within living cells perform a series of enzymatic steps in an exquisite multi-catalyst process, without the need for isolation of intermediates. In conventional processes enzyme recovery and recycle is often complicated, application of enzymes is often hampered by a lack of long-term operational stability and downstream processing is difficult. Nature solves these problems by compartmentalization of the various enzymes. Exemplary benefits for artificial integrated and miniaturized systems are expected from improved enzymatic activity and stability by compartmentalization via immobilization, accelerated preparation of compounds with minimum workup, and facilitated scale-up by performing the processes in a highly parallelized mode.

E. ORGANISATION

E.1 Coordination and organisation

The *SysBiocat* Action will provide the means to coordinate the activities required for an effective concerted collaboration of the scientists involved. Research will be carried out in the individual research units, which have their own financial support and only require exchange of knowledge, by way of *a concerted Action*. Organization and management of the Action will strictly follow the "Rules and Procedures for implementing COST Actions" (COST 4154/11) and will make use of its common organizational structures:

The Management Committee **(**MC**)** will be created according to the COST rules based on the nominations by the National COST Coordinators. The members of the MC will elect the Action Chair (AC), Vice-Chair (VC), Working Group (WG) Coordinators, STSM Coordinator, Training School Coordinator and Website Coordinator, who will collectively form the Action Steering Committee (ASC). A person responsible for monitoring the Action (e.g. STSM and budget monitoring) will be elected from the MC members. *Ad hoc* sub-committees to assist those responsible will be established (e.g. organizational board of respective Training School or programme committee of the Action Workshops a special role will be assigned to the ASC which will meet regularly mainly via a *conference call*. The Management Committee will further be responsible for

- budget planning and allocation of funds
- planning of events, such as MC and WG meetings, Action Workshops, and Training Schools
- communicating tasks and milestones

• preparing and approving reports (including final publication)
• conducting evaluation and monitoring of the Action
• communicating with COST Office, public relations matters

MC meetings will be organized annually, preferably together with some other Action activity (WG meeting, Action Workshop, Training School) to reduce travel costs and to improve direct communication across the Action participants. The MC will organize Action Workshops once per year. The MC will make best use of existing structures, e.g. the biannual international Conference on Biocatalysis and Biotransformation *"Biotrans"* organized in odd years (Manchester 2013, Vienna 2015) or on Multi-Enzyme Catalyzed Processes ("*MECP"*) organized in even years (Madrid 2014). Workshops co-organised at these traditional meetings will ensure

• extensive communication with the experts in the field
• higher efficiency in the use of travel funds
• special COST Session as a platform to disseminate COST results at conferences •

The Action Steering Committee will act as a scientific advisor and coordinator of all events of the Action. It will enter into its function at the beginning of the Action, presenting the scientific intentions and scope of each WG and making an analysis of the available and missing competence to achieve the Action ambitious goals. The ASC will meet in a conference call at fixed terms to report about the advancement of the research, the optimization of the utilization of funds for STSMs, and involvement of early-stage researchers.

An Action Website will be established that will act as an Intranet for an exchange of data and ideas among respective WGs inside the Action, and as an Extranet for disseminating the main achievements both to specialists, stakeholders and the general public. A person appointed by the MC will be responsible for coordinating this activity (web-master, editorial coordinator). There will be a regular Webzine (8 editions throughout the duration of the Action), which will promote web updates, provide articles prepared by each WG, point out recent hot topics related to the Action, links (e.g. to conferences and patent reports), information from similar consortia and research centres (biocatalysis, industrial catalysis etc., EU-funded and other projects) and links to other important Action-related web pages (e.g. European Federation of Biotechnology - European Section on Applied Biocatalysis / EFB-ESAB, national associations of biotechnology).

Evaluation and monitoring of all respective Action instruments and activities e.g. STSM, ASC, WG, Action Workshops, and Training Schools etc. will be conducted once they are finished. Written reports from all the activities according to the COST scheme will contain scientific issues and also clear cost breakdown. All reports will be submitted *via* respective coordinators to the MC for evaluation and final approval, followed by publication on the website. Milestones of the Action

will also be carefully monitored and evaluated.

E.2 Working Groups

Five Working Groups will be created and organized into 3 vertical thematic Pillars and 2 horizontal technology Bridges, as detailed in Section D.2, creating an intricate Grid structure that will maximize the scientific interaction and collaboration within the entire *SysBiocat* COST Action, much like a natural metabolic system. The composition and arrangement of the WGs will be decided at the first MC Meeting. Further modifications will be consolidated in the frame of the COST rules during subsequent MC Meetings.
The WGs will be composed in a multidisciplinary manner, typically involving also member(s) from industry. Gender balance will be carefully considered. Close collaboration within each WG will be pursued but also with intense collaboration across the whole Action and will be stimulated by various instruments, e.g. joint WG meetings, STSMs across different WGs, exchange of information *via* restricted membership section of the Action website and all-Action Workshops. In addition to annual WG meetings held to coordinate the research collaboration, also informal meetings of WG members with complementary consortia outside of the COST Action will be encouraged. The WG Leaders appointed by the MC will take on the responsibilities to

- represent the WG actively in ASC and MC meetings
- communicate WG information to the website manager
- coordinate WG meetings together with local organizers
- coordinate WG contributions to annual monitoring progress reports and final Action reports

E.3 Liaison and interaction with other research programmes

The Action Chair, ASC and MC will actively explore opportunities for joint Action Workshops, symposia, and Training Schools together with other COST Actions (and EU funded research programs with complementary focus; see subheading B.4), in which several potential members of the *SysBiocat* Action are already engaged. Thus, the Action will profit from dynamic interactions and synergies with the following COST Actions: CM0804 “Chemical Biology with Natural Products”, CM0903 “Utilisation of Biomass for Sustainable Fuels and Chemicals”, and CM1102 “Multivalent Glycosystems for Nanoscience”, which share an overlapping interest on complementary target substructures, but mostly utilize chemical rather than biocatalytic approaches for synthesis. The *SysBiocat* Action can have possible liaisons and interactions with individual

COST Actions and the Seventh Framework Programme activities mentioned in B.4. On an international level, interactions with the new *Enzyme Function Initiative* funded by the National Institutes of Health (NIH) in the US are possible.

E.4 Gender balance and involvement of early-stage researchers

This COST Action will respect an appropriate gender balance in all its activities and the Management Committee will place this as a standard item on all its MC agendas. The Action will also be committed to considerably involve early-stage researchers. This item will also be placed as a standard item on all MC agendas.
The Action aims to achieve equal gender representation in all its scientific and administrative activities, such as among the ASC, as well as upon allocation of ESR participation in STSMs.

F. TIMETABLE

• The *SysBiocat* Action will last for 4 years.
• Action Chair, Vice-Chair and WG Leaders, together forming the ASC, will be elected during the first MC meeting (year 1, month 1).
• an Action website will be launched (year 1, month 2)
• scientific kick-off Action Workshop to establish a collaboration platform (year 1, month 3-5)
• ASC teleconference will be held at least semi-annually
• annual MC/ASC meetings, jointly held with Action Workshops
• WG meetings held jointly as cross-Action Workshops to interface topical research with technology bridges
• Action Workshops year 1-3 to be held in association to international biocatalysis conferences
• Training Schools for cross-field education of ESRs in best practice, dissemination of results (year 1 and 3)
• STSMs will be open from kick-off until the end of the Action
• final MC meeting for evaluation of the Action's overall performance

Event / Year	**1**	**2**	**3**	**4**
First MC meeting	X			
Kick-off WG meeting	X	X	X	X

MC-ASC meetings	X	X	X	X
ASC meetings	X + X	X + X	X + X	X + X
Website launch	X			
Action Workshops	X	X	X	X
Training School	X		X	
WG meetings	X	X	X	X
STSMs	X X X X X	X X X X X	X X X X X	X X X X X
Final MC meeting				X

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, CH, CZ, DE, DK, ES, FI, FR, HR, HU, IE, IL, IS, IT, NL, SE, UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 68 Million € for the total duration of the Action. This estimate is valid under the assumption that all the countries mentioned above but no other countries will participate in the Action. Any departure from this will change the total cost accordingly.

H. DISSEMINATION PLAN

H.1 Who?

There are several categories of *SysBiocat* target audiences for the dissemination of results:
Cat 1: Scientists and Early-Stage Researchers participating in the *SysBiocat* Action, who represent the immediate beneficiaries of the research activities.
Cat 2: Academic and industrial researchers working in the field of enzyme catalysis, renewable resources, Green Chemistry, fine chemistry, drug design and synthesis, who will be the secondary beneficiaries of the *SysBiocat* research output.
Cat 3: European and national policy makers formulating funding strategies and outlining schemes for new funding opportunities, who will capitalize from the ideas and results created by the *SysBiocat* Action.
Cat 4: The general public, including pupils and young students at all levels with an open mind towards science, will be taking profit of an approach to a "Greener Chemistry", by helping to dismantle false perceptions about the origin and severity of chemical pollution, and by fostering a

better understanding of "Green Opportunities" for solving problems in industrial chemical production.

H.2 What?

• *SysBiocat* WG meetings including joint meetings with other COST Actions (Cat 1 & 2)
• *SysBiocat* Action Workshops and lectures at International specialist meetings and conferences (Cat 1, 2 & 3)
• *SysBiocat* Training Schools and STSMs to introduce Early-Stage Researchers to new concepts, strategies and experimental techniques (Cat 1)
• Undergraduates and postgraduates lectures at host institutions and school visits (Cat 4)
• Public open presentations in scientific events (Cat 4)
• *SysBiocat* Action website with open-access pages for WG presentation, including links for access to original literature (Cat 1-4)
• Scientific publications, periodic reports, final book for disseminating and discussing the results of the *SysBiocat* Action (Cat 1-4)

H.3 How?

A primary task of the MC will be to promote the work of the *SysBiocat* COST Action and encourage all members of the Action to widely participate in dissemination activities. The periodic events like the forecasted participation to *Biotrans* and *MECP* will constitute a particularly strong forum for the dissemination of the results and the illustration of the scientific results and COST Action organization to a wide audience of scientists and various other stakeholders. World leading external experts and members of strategic boards of the European or National funding agencies will be invited to attend the *SysBiocat* Action Workshops in order to be witness of the advancements of the project. The *SysBiocat* website will be regularly updated as an immediate entry to current Action activities and will be the natural link to spread information among the participants and to the general public in an open access version. All publications and oral or poster presentation concerning results arising from the *SysBiocat* Action activity will carry acknowledgments to COST support and, whenever possible, include both the Action acronym and COST logo.