European Cooperation in Science and Technology
- COST -

Secretariat

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COST 4118/12

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action BM1203 : EU-ROS

Delegations will find attached the Memorandum of Understanding for COST Action as approved by the COST Committee of Senior Officials (CSO) at its 185th meeting on 6 June 2012.

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

COST Action BM1203
EU-ROS

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 aim of the Action is to form a multi-disciplinary network of European scientists working synergistically on the fundamental biology of oxygen and reactive oxygen species. Currently separated by different disciplines, various researchers will join forces to achieve world-class breakthroughs in the understanding of the fine balance between health-promoting and disease-triggering effects of reactive oxygen species.

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 64 million in 2012 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 V of the document referred to in Point 1 above.

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A. ABSTRACT AND KEYWORDS

Life requires oxygen. This runs the risk that, when oxygen leaks out from normal metabolism, reactive oxygen species (ROS) are formed, which – when too high – trigger disease. With the idea to overcome this, antioxidants are heavily marketed, yet without proof of their effectiveness. Rather, worrying evidence suggests adverse effects. This paradox is due to the fact that ROS are not only 'bad', but – in tightly regulated amounts – also act as essential signalling molecules. Unravelling the fine balance between ROS acting as a friend or a foe is fundamental to understand aerobic life. To advance this important area of biology and medicine, highly synergistic approaches combining diverse and scattered disciplines are needed. For this, COST provides an ideal framework. EU-ROS will bring together multi-disciplinary experts to enhance the competitiveness of European research. By applying fundamentally new approaches it will generate advanced knowledge and translate this into novel applications ranging from medicine to crop science. With its dynamic structure, EU-ROS will attract further experts and particularly support capacity building of the future European research leaders and talented women. Collectively, EU-ROS will overcome the fragmentation of European R&D on oxygen/ROS research while its translational components will contribute to European societies' economic growth and wellbeing.

Keywords: antioxidants, chronic diseases, crop science, individual therapy and diagnosis, oxidative/reductive stress

B. BACKGROUND

B.1 General background

Definition of the research topic and wider relevance

Oxidative stress is defined as an imbalance between the formation and elimination of so-called reactive oxygen species (ROS). They are formed when oxygen utilisation is uncoupled from its metabolic use. ROS have been suggested as major disease triggers. The concept is very popular and easy to understand, which has lead to a widespread use of antioxidant vitamins by patients with a variety of diseases and by healthy people to improve wellbeing. Indeed, up to half of the population in high-income countries use vitamins supplements, often marketed as antioxidants. However, clinical trials showed that untargeted application of antioxidants, which broadly scavenge ROS, are not only ineffective but may even be harmful. As a result, no antioxidant has ever been approved by a regulatory agency, and their use is confined to alternative medicine and uncontrolled self-prescription. One likely reason why antioxidant supplements do not work is the double-edged role of ROS. They are not only 'bad' disease mediators causing tissue damage, but are also essential signalling molecules. Thus, similar to an excess ('oxidative stress'), a deficiency in ROS results in a redox imbalance (in this case harmful 'reductive stress').

ROS and their (patho-)physiological roles have been intensely studied for many years with, however, little public impact: The consumers' belief in the health benefits of antioxidants is still powerful. There are also devoted scientific societies on ROS (e.g. Society for Free Radical Research, SFRR). Still, the information on antioxidants has not fed through to the general public as, for example, the main objective of these societies is not to change public perception. Therefore, EU-ROS will move away from the antioxidant concept, both scientifically and with respect to public awareness. Rather, EU-ROS aims to identify and then modulate disease-relevant molecular sources of excess ROS, leaving those sources of ROS intact that are essential for adaptation and physiologic signalling. EU-ROS also aims to revert molecular damage caused by oxidative stress. This will be complemented by the development of evidence-based biomarkers and novel imaging technologies. Finally, EU-ROS will elucidate those cases in which antioxidants – in the correct combinations, form, doses and duration – may be useful. Importantly, from day one EU-ROS will actively and continuously inform the public on the facts on antioxidants.

COST offers the appropriate framework for EU-ROS and to provide benefits

To advance the field of redox biology at a world-class level, highly inter-disciplinary teams are needed. With the opportunity provided by COST to develop best practice, enhanced networking and capacity building, this dynamic area of research will benefit from unprecedented interchange of ideas, know-how and technologies. This Action will combine expertise ranging from computational, medicinal and wet chemistry, analytics, imaging, biological chemistry, cell biology, animal models, molecular biology, genetics, proteomics, bioinformatics, drug and biomarker discovery/development, pharmacology as well as the clinics, both from academia and from industry. Leveraging on national research investments, EU-ROS will thus provide a bench-to-bedside approach to achieve outcomes that would not be achievable by a single discipline, individual groups or small-scale co-operations. An important feature of EU-ROS is that it will prevent working in disease silos or silos of individual molecular sources and targets of ROS. Diseases do not necessarily adhere to traditional categories of research or to clinical organ-based classifications. Rather, various diseases ("diseasome") are likely to share common pathways. Establishing that a drug discovered or approved for one disease is also effective for other diseases can be beneficial and cost effective. Thus, linking mechanistic relationships between disease processes holds great potential for future drug development. This has become apparent, as for example tyrosine kinase inhibitors, originally developed for tumour treatment, are also options to treat pulmonary hypertension. Each disease area (e.g. cardiovascular, cancer, neurology) has specific research know-how, methods and technologies. However, there is now a strong need for integration, inter-disciplinary approaches and technology exchange to prevent duplication of efforts. Thus, EU-ROS enables ROS scientists to interact with major benefits on a scientific, commercial and society level. Overall, the structured, but flexible platform of COST for sharing information represents a perfect means for establishing this dynamic network and delivering innovative, meaningful outcomes. It will allow on-going research programs funded by country-specific and international sources to integrate and synergize in order to achieve a much higher level of visibility and impact.

B.2 Current state of knowledge

Summary of the previous research in the field and its current state of the art

Oxidative stress, an excess of radicals and other reactive oxygen species (ROS) relative to antioxidant defence, has been suggested as a major disease mechanism from single cell to multicellular organisms. While minimal changes of the normal redox state evoke adaptive responses, excessive shifts in the redox potential can lead to damage of cell components, including DNA, proteins and lipids. This is considered to promote major diseases representing most causes of death. The famous scientist Linus Pauling – twice Nobel Prize winner (chemistry and peace) – was a pioneer in addressing the connection of disease and oxidative stress. Unfortunately, his idea of using excessive amounts of vitamin C to reduce oxidative stress is not efficient. Clinically, the major clinical trials using antioxidants as therapeutics have been failures, sometimes even with serious side effects. Therefore, antioxidants have failed to impress European Food Safety Authority (EFSA). EFSA's health claims panel has stated: "…no evidence has been provided to establish that having antioxidant activity/content and/or antioxidant properties is a beneficial physiological effect". Still, the concept oxidative stress, probably also thanks to Linus Pauling, is very popular. Up to half the population in high-income countries uses vitamin supplements, often marketed as antioxidants. It is like the Popeye-spinach story, where a health halo around the vegetable was constructed and still believed even when research indicated that the iron in spinach has poor bioavailability.

One reason why antioxidants cannot work is the double-edged role of ROS being both potentially mediators of disease and essential signalling molecules. An excess of ROS can damage cells, but ROS are also essential for host defence. Moreover, a deficiency in ROS can result in similarly harmful reductive stress. Further, there is evidence that ROS not only play a role in the innate immune defence (which would still be toxic, alas directed towards a foreign cell), but are also essential signalling molecules. In a tightly controlled fashion they regulate proliferation, differentiation, metabolic actions, apoptosis and angiogenesis etc. Thus, the roles of ROS in physiology and pathophysiology need to be completely re-defined and much better understood before safe applications are possible.

EU-ROS will apply completely different approaches: First, instead of letting radicals form and then scavenge them EU-ROS will identify their disease-relevant sources and prevent their formation or specifically repair the damage caused by ROS. Second, EU-ROS will differentiate beneficial signalling roles of ROS.

Recently, progress in the redox biology field has been achieved, to which the participants of this Action made significant contributions. In a number of pathological conditions, such as inflammation, type 2 diabetes, cardiovascular diseases, stroke and cancer, producers of ROS – that are part of the normal cellular signal transduction system – are activated. Several of these ROS sources will be addressed by EU-ROS. For example, a growing interest is on the NADPH oxidase (NOX) family. NOX stand out from other ROS sources, e.g. NO synthases, xanthine oxidases, P450 enzymes and mitochondria, as NOX are the only enzymes with the sole function to generate ROS. Seven NOX isoforms exist that demonstrate cell-specificity, differences in sub-cellular localization and regulation. They serve essential physiological roles, e.g. in host defence, cell proliferation, differentiation, adhesion, metabolic action and apoptosis. However, there is convincing evidence of detrimental effects of excessive ROS production by NOX in various diseases. To increase basic knowledge of NOX actions in health and disease highly NOX-specific inhibitors to the various isoforms are urgently needed. These may also serve as therapeutics for a number of major diseases. Recently, members of this Action showed that NOX4 is a detrimental player in stroke in mice, and that this finding can be therapeutically applied by applying a preclinical NOX inhibitor, which was developed by a SME participating in this Action. Using a specific anti-phospho-p47phox antibody, others showed that NOX2 is activated in neutrophils isolated from rheumatoid arthritis patients. An underexplored area, which will be addressed by the synergistic strategy of this Action, is the cross talk between different sources of ROS. NADPH oxidases are the likely initial sources that render other enzymes dysfunctional, which then become ROS sources themselves. Key targets of ROS include uncoupled eNOS and oxidised NO receptor soluble guanylate cyclase (sGC). This is supported by recent pre-clinical data and on-going late-stage clinical developments of sGC activators (phase I) and stimulators (phase III with a likely market entry in 2013), to which EU-ROS members have contributed on a large scale.

Oxidative stress is not only relevant in mammals, but also for example in plants. For example, similar enzymes appear to be involved, e.g. NADPH oxidase in barley's defence against fungi infection. Oxidative stress is also considered as part of plants' response to environmental constraints. As a consequence of plant aerobic metabolic processes, such as respiration and photosynthesis, ROS are produced. However, also in plants, ROS are key components in fundamental plant processes, such as stomatal closure, response to environmental changes or gene expression and modulation of protein activity. Similar to mammals plant have NOXs, the respiratory burst oxidase homologues (RBOHs). The tight regulation of RBOH activity makes these proteins good candidates for the fine-tuning of ROS production in crop science. For example, the role of ROS in the legume Rhizobium symbiosis has been highlighted. Legumes are the only plant family with the ability to establish a symbiotic interaction with soil bacteria, rhizobia, leading to the formation of a new organ, the root nodule, whose primary function is dinitrogen (N_2) fixation. This symbiotic interaction is crucial as (i) legumes are important for food and feed and (ii) biological N_2 fixation is a key component of sustainable agriculture as it strongly limits the use of costly fertilizers and the water pollution they induce.

In plants and mammals, several endogenous cellular antioxidants such as superoxide dismutase (SOD), catalase, glutathione peroxidase, peroxiredoxins and sulfiredoxin exist. EU-ROS members played a prominent role in the discovery of bacillithiol as a unique antioxidant thiol redox buffer that serves as a glutathione surrogate amongst many pathogenic bacteria. This discovery has opened up a whole new research field in microbial thiol redox biology.

Research on the redox biology is hampered by the difficulty to quantify and localise ROS. EU-ROS participants have recently provided progress by developing the first genetically encoded sensor for H_2O_2. Another group has been the first to couple electron paramagnetic resonance (EPR) to micro-computed tomography (CT), which allows 3-D visualisation and overlay of the EPR image to anatomy, e.g. CT.

Despite these recent progresses, many fundamental questions and interrelations of these observations still remain unanswered. One reason is the urgent need for potent and specific research tools, such as animal models and antibodies, as well as specific pharmacological reagents. Mutual access to these would allow scientists to test the oxidative stress hypothesis in an unprecedented manner. Importantly, this Action provides a unique opportunity not only to develop such tools and reagents, but also to establish their efficacy in multiple systems and providing a proof of concept in multiple organisms and disease models. Indeed, a structured public platform for exchanging the respective information will be a major step forward in translating the oxidative stress hypothesis into clinical application.

Innovative aspects

EU-ROS will create a unique network of highly interdisciplinary researchers who have made substantial contributions to the understanding of redox (patho-)physiology. Some are already collaborating with each other on a small scale but never interacted in this constellation. The network includes groups from academia and industry with broad interdisciplinary and synergistic knowledge. The current consortium is comprised of participants from 17 countries. It encourages participation of further non-COST countries. EU-ROS will be innovative in several aspects, for example by:

- Using and develop of innovative and unconventional methodologies;
- Clarifying the notion of antioxidants, the concept of “oxidative stress” and the term “ROS”;
- Providing new therapeutic indications and disease sub-classifications paving the avenue for novel diagnostics and therapeutics;
- Providing the first proof-of-concepts for the feasibility and relevance of the personalised medicine concept in the area of oxidative stress.

B.3 Reasons for the Action

The field of ROS biology and pathology is extremely complex and highly competitive. So far, the (limited) knowledge is fragmented and rather insular, also within Europe. There is an urgent need to 'de-scatter' scientists focused on their specific domains, give them opportunity to work together achieving meaningful outcomes. Often, they do not know whom they should approach if a technology is required. To move the field and its applications forward, we need interactions based on synergies from various angles of expertise. No single discipline can provide meaningful outcomes like a network can that connects leading experts from various disciplines to join forces. Thus, EU-ROS will form a dedicated network of leading European researchers and stakeholders. Indeed, a major advantage of EU-ROS is its flexible and open structure and multidisciplinary participation including a unique starter array of experts from basic to and applied research, from academia and industries. They will combine their expertise in order to not only provide major insights into the exciting field of redox biology and pathology but also into its clinical applications. EU-ROS will provide not only scientific, but also – certainly in the longer term – societal benefits by contributing for example to consumer information and the sustainability of European health care systems. The new collaborations and the insights will clearly enable a large application within European Framework Programmes. In this context, further synergy with the COST Action on gasotransmitters (BM1005) has already been mutually agreed. The participants of EU-ROS have already decided to share and exchange their knowledge, tools and reagents. They are also eager to foster education of early stage researchers. They will establish a website and thus disseminate the novel information to the wider community. Newsletters, regular meetings, Short-Term Scientific Missions (STSMs) and a final international conference will enhance the intensity of scientific exchange.

In summary, EU-ROS will provide several productive outcomes, e.g. for:

- Science and Medicine: EU-ROS will provide one of the first proof-of-concepts for the feasibility and relevance of an integrated concept covering mechanism-based therapeutics, imaging and diagnostics as well as for personalized/precision medicine, the next revolution in medicine and a shared vision of researchers, clinicians and the pharmaceutical and medical devices industries.
- European communities: In the long-term, the research will provide measurable outcomes in improved health, new products, and economic benefits by lowering disease burden. This will contribute to the sustainability of European healthcare systems. EU-ROS will help to meet the challenge of the demographic shift towards an aged population. It will contribute to healthy aging by developing better medical strategies to ensure that citizens enjoy healthy and productive lives.
- Europe's scientific excellence and training: EU-ROS will enhance the competitiveness of European research excellence by collaboration of outstanding European scientists in large networks (including other COST Actions) to achieve high-level objectives that would not be achievable at an individual level. It will inspire young researchers and further attract leading international researchers to Europe. The world-class and collaborative team environment will provide ideal training conditions for Early Stage Researchers. EU-ROS will encourage exchanges in order to provide further opportunities for training.
- European industry and biotech: One of the earliest beneficiaries of personalized medicine is the pharmaceutical industry. EU-ROS expects increased competitiveness by decreased risks and costs in drug development, mid-term direct translation into advanced monitoring of clinical trials followed by therapeutic applications in patient inclusion and exclusion, as well as use of the novel technologies in preventative diagnostics and therapeutic monitoring. Successful completion of this Action will also provide ample opportunity for securing IP. Thus, this Action will facilitate the growth of biotechnology and pharmaceutical industries in Europe.

B.4 Complementarity with other research programmes

EU-ROS will link to other existing programs and networks including the 'RedCat' Initial Training Network. 'RedCat' addresses emerging issues in Medicine and Agriculture, such as disease-preventive antioxidants for an ageing population, antibiotics against bacteria and 'green' pesticides for an ecologically acceptable agriculture. While EU-ROS is unique in its approach and structure, and as such there is no duplication, EU-ROS aims at harnessing the synergy of the other European resources in order to provide maximal benefits.

C. OBJECTIVES AND BENEFITS

C.1 Aim

The main aim of the Action is to form a multi-disciplinary network of European scientists working synergistically on the fundamental biology of oxygen and reactive oxygen species. Currently separated by different disciplines, various researchers will join forces to achieve world-class breakthroughs in the understanding of the fine balance between health-promoting and disease-triggering effects of reactive oxygen species.

Members of this Action will exploit this knowledge in a wide area of applications, ranging from medicine to crop science. EU-ROS will thus enhance the quality, translation and impact of European biomedical research in a highly competitive and relevant area of biology and medicine.

C.2 Objectives

The secondary aims of EU-ROS entail both direct scientific objectives and more general objectives**.**

Direct scientific objectives of EU-ROS mainly achieved through networking activities are:

- Develop and share novel, highly specific tools enabling R&D in the field of ROS;
- Re-define oxidative stress by defining novel roles and functions of ROS and their sources in health and disease of multiple organisms;

- Identify relevant molecular damage caused by ROS;
- Identify and characterise molecules that target the disease-relevant ROS sources and repair molecular damage caused by ROS;
- Establish Markeromes and Diseasomes rather then working in disease-silos with organ-based definition;
- Define predictive biomarker patterns and pattern recognition that accurately predict an individual's risk to develop a disease associated with a disturbed redox balance, to tailor the treatment to the underlying disease mechanism and to monitor success of a therapy;
- Develop novel imaging technologies that allow to localise the risk and disease;
- Validate animal models;
- Advance scientific knowledge and use this for developing novel diagnostics and therapeutics and translating these into the clinics;
- Contribute to improved plant health and hence to a more sustainable agriculture.

Other objectives, which are essential to achieve the direct scientific objectives, are to:

- Increase interactions between research groups with multi-disciplinary and complementary expertise;
- Enhance research efficiency and quality by distributing and sharing knowledge, experience and skills as well as reagents, samples and tools amongst participants and thus overcome hurdles, which currently cause that research on ROS is rather taking place in isolation;
- Enhance Europe's scientific excellence by inspiring young researchers and attracting leading researchers to Europe. EU-ROS will train early career scientists in an interdisciplinary fashion with insight from academia to industry, from basic to clinical research in order to become the future leaders of the European scientific community. EU-ROS will do so by e.g. organising 3 devoted training schools, promoting staff exchange and linking into Summer School networks in SFRR-E;
- Promote equality by supporting female scientists, in particular between post-doc and pre-tenure;

- Advance scientific knowledge and use this for developing novel diagnostics and therapeutics and translating these into the clinics;
- Provide measurable outcomes in improved health, new products, and economic benefits by lowering disease burden, resulting in a net decrease of costs and contribute to the sustainability of European healthcare systems;
- Enhance competitiveness of European industry and biotech;
- Disseminate new knowledge to the international scientific community and to the general public;
- Establish a well-coordinated interdisciplinary team of leading scientists applying for at least one relevant Framework Programme call.

The achievements of EU-ROS will be evaluated using the following indicators:, number of participants, scientific meetings organised, STSM supported, Early Stage Researchers (ESRs) trained, training schools organised, joint publications, hits to website, drug candidates and biomarkers identified, imaging technologies developed, as well as collaborative proposals submitted. An external advisory board will be nominated to provide the critical assessments.

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

The objectives will be achieved by bringing together multi-disciplinary experts that join forces to achieve meaningful outcomes in the field of oxygen and redox biology, pathology and its applications. EU-ROS will enable interactions and cooperation, exchange of information, sharing of tools, model systems and other resources (e.g. patient samples), joint developments and support of technologies, as well as dissemination of knowledge.

The following deliverables/means will enable EU-ROS to achieve these aims:

- Regular (e-)meetings;
- Working group (WG) meetings (1/WG/year);
- Presentation at international conferences ;
- An international EU-ROS conference at the end of year 4;

- Support of ESRs, (3 Training Schools, STSM);
- Attract international top scientists (min. 15, incl. European and non-European);
- An EU-ROS website providing access to the generated knowledge and annual e-newsletters;
- Scientific publications disseminating knowledge and raising awareness of this research field (e.g. high impact, key publications of the field, including at least 3 joint reviews);
- Industry interest and professional translation, economic and patient impact (filed patents, 2 drugs/diagnostics entering clinical development);
- Gender balance (female support; encourage females to present at meetings, inclusion of more women in network);
- Establishing a European research area by implementing a Framework Programme (FP) followed by an application (1 successful FP Program application).

C.4 Potential impact of the Action

Despite the attention that oxidative stress receives, knowledge on ROS sources and targets as well as their time and spatial distribution is insufficient. However, this is needed for insight into ROS (patho-)physiology. Respective research and related drug discovery is hampered by a lack of specific research tools. By developing and exchanging such novel tools, reagents, information, data and expertise, this Action's interdisciplinary team will advance this exciting field. EU-ROS envisages an acceleration of basic and translational research on major disorders, such as inflammation, cardiovascular and neurodegenerative diseases as well as in other areas of biology including crop science and technology. No single research group or field can accomplish this. Only together, such high-level objectives are achievable. EU-ROS will help to enhance competitiveness at the highest scientific and translational level, further contributing to European reputation as place of excellence in research and translation.

In more detail, the benefits will cover the following aspects:

- Cooperation and cross-fertilisation of researchers with synergistic expertise;
- Networks enabling joint applications for European funding programs and other collaborative funding;

- Promotion of the future European research leaders ready to compete within the international scientific community;
- Reduction of disease burden in Europe, also from a economical point of view;
- Enhancement of European biomedical industry's competitiveness.

C.5 Target groups/end users

Several stakeholders will benefit from EU-ROS:

- EU-ROS members;
- Patients suffering from diseases associated directly or indirectly with oxidative stress;
- The general public by dissemination of the facts and foes about antioxidants, evidence-based and personalized medicine;
- Medical experts by gaining insight into and access to state-of-the-art of therapeutic and diagnostic options;
- Early Stage Researchers (ESRs);
- European research community;
- Other synergistic COST Actions, e.g. 'ENOG' (BM1005);
- Industries (several Small Medium Enterprises (SMEs) are already EU-ROS members) as novel biomarkers will allow more focused clinical trials to identify diseases and patients subpopulations that might potentially profit from a redox modulating drug.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

EU-ROS will coordinate 6 major work packages:

- Sources of ROS;
- Molecular mechanisms and targets of ROS;
- Compounds interfering with detrimental ROS generation and the damage caused by ROS;

- Biomarkers of redox status;
- Imaging of redox status;
- Technology transfer.

Each work package will be the major subject of a respective working group (WG; see section D.2). The R&D is highly interdisciplinary, and EU-ROS brings together expertise on physiological, pathophysiological, biochemical, chemical, epidemiological and clinical aspects of ROS in multiple organisms. Further, EU-ROS members provide and further develop state-of-the-art infrastructures, tools, models and technologies. Through participation of clinicians and industries, EU-ROS has access to various clinical cohorts. Together, this will allow achieving the objectives described in section C.

Compared to the failed antioxidant approach, EU-ROS provides alternative, ground breaking different approaches to the biology of ROS and their applications. These include:

- Antioxidants did not work clinically. This approach assumes that all ROS are bad. However, EU-ROS members already showed and will provide further evidence that low levels of ROS are essential. Interfering with these can cause reductive stress and unwanted side effects or worsened outcomes;
- EU-ROS' preferred approach is to inhibit the disease-relevant molecular sources of ROS without eliminating physiological radical formation;
- EU-ROS' members believe that different key sources of physiological signalling and disease-relevant ROS exist, the latter causing oxidative stress. EU-ROS will identify both, physiological and disease-relevant ROS sources in different organisms;
- EU-ROS' next best approach is to specifically revert damage caused by oxidative stress;
- EU-ROS aims to identify patients who potentially benefit from these therapies; markers for oxidative stress will be refined or developed;
- EU-ROS will enable to localise disease processes as imaging techniques will be developed that use new imaging modalities to visualise oxidative stress. This will allow prediction of risk and patient monitoring.

The flexible structure allows for interaction and cross-fertilization between the WGs and enables further interdisciplinary perspectives provided by new EU-ROS members that cannot be foreseen at this stage, but will certainly contribute the success of EU-ROS.

D.2 Scientific work plan methods and means

The major currently pursued and future research activities by EU-ROS members and their allocation to the 6 Working Group (WG) is described below. Further R&D will be covered by current EU-ROS members and by participants expected to join EU-ROS' open and flexible network at a later stage.

WG1: SOURCES

WG1 aims to identify physiological and disease-relevant molecular sources of ROS and antioxidant enzymes by generating and using highly specific tools (e.g. genetic animal models, siRNA, miRNA, antibodies, ROS detection methods) for assessing the contribution (expression regulation, sub-cellular localisation, activity and interaction of ROS and their sources) to ROS production and degradation in different systems (isolated cells, single cell organism, organs, animal models and plants). This is essential to establish strategies that inhibit disease-relevant sources of ROS without eliminating physiological ROS formation. Members of EU-ROS are experts on different ROS producing and antioxidant enzymes and therefore the Action will investigate (intra-) cellular networks in a scope that has never been possible before thus shedding light on these fundamental issues.

Several groups have expertise on NADPH oxidases, major sources of ROS in health and disease, each group focussing on single or very few NOX isoforms or diseases. EU-ROS will enable to integrate the knowledge on all NOX isoforms and a variety of disorders. Thus, tools will be more broadly applied. The results obtained already by some Action member's demonstrated that the NOX isoform NOX4 is the detrimental player in stroke, suggesting NOX4 as a target for stroke therapy while not being relevant for hypertension. Future focus will be on elucidating the effects of NOX in ischemia reperfusion injury (IRI) of different organs and identify the cell types responsible for mediating the effects using e.g. cell-specific NOX KO mice.

Another group will provide complementary expertise on endogenous cardio-protective mechanisms (myocardial ischemic pre-, post-conditioning and remote conditioning). Together, WG1 will work in the field of the double-edged role of ROS in cardio-protection on the one hand and being injurious molecules on the other hand.

An unexplored potential therapeutic target is NOX5, an isoform not present in rats and mice. Thus, NOX5 will be intensely investigated by using humanised NOX5 knock-in mice and NOX5 expressing animals (e.g. rabbits).

Another aspect that will be elucidated is the role of different NADPH oxidases in signalling processes, e.g. in vascular and tumour tissues. The Action members will study the regulatory networks controlling activation and expression of various NADPH oxidases. The researchers will elucidate the role of ROS under conditions of low and very low oxygen availability, which may cause reductive stress.

Phagocytes (polymorphnuclear neutrophils, monocytes, macrophages) and epithelial barriers play a major role in innate immunity and host defence against pathogens. The production of ROS by phagocytes and epithelial cells is essential to mount an immediate defence, but an excessive or inappropriate production of ROS may participate in the physiopathology of inflammatory diseases. EU-ROS members will further elucidate the mechanisms involved in the regulation of ROS production by the phagocyte NADPH oxidase (NOX2) and the epithelial NOX1 and their dys-regulation in inflammatory diseases such as intestinal inflammation and rheumatoid arthritis (RA). The research on the monocyte/macrophage system subjected to heavy intrinsic and extrinsic oxidative conditions specific to this disease is also on-going, including ex vivo and in vitro studies on peripheral and synovial cells from RA patients during treatment with biologic agents as well as interference between ROS-cytokines at the level of the monocyte-Treg network.

This expertise will be supplemented by a group coming from the microbiology side studying host-pathogen interactions. This particular research direction aims to find out how pathogenic bacteria like *Mycobacterium tuberculosis (Mtb)* and *Pseudomonas aerugenosis* are surviving oxidative stress in their interaction with the immune system. Indeed, as a successful intracellular pathogen, *Mtb* has evolved with powerful defence strategies to detoxify ROS and nitric oxide maintaining its viability and achieving long-term persistence in human organs. The mechanisms by which Mtb detoxifies ROS are of particular interest because this knowledge will help to understand the organism's pathogenesis and its ability to persist, which in turn leads to latent infection.

Future work will connect NOX and other sources of ROS to inflammatory and anti-inflammatory cytokines and danger signals that are released in a redox-dependent manner. Synergistic expertise will be available on the characterisation of oxidative post-translational protein modification during chronic inflammation by use of proteomics in order to understand inflammation and anti-inflammatory mechanisms.

NOX are also suggested to be involved in metabolic diseases, such as diabetes mellitus. Therefore, the research will also focus on aspects of ROS-mediated beta cell dysfunction and diabetes-associated kidney and vascular damage and to determine the pathogenic contributions of NOX. However, dissecting the role of other ROS sources is also warranted. Complementary to the respective expertise provided by one research group participating in the Action, another research group will further investigate the role of the brain in sensing and maintenance of glucose homeostasis, and particularly the mechanisms underlying the detection of hypoglycaemia. In addition, EU-ROS will study various interactions between different environmental stressors and hypothalamic glucose sensing. Further understanding and mitigating the effects of ageing-related redox reactions on metabolism and metabolic disorders will be carried with particular focus on characterising oxidative modifications to proteins and lipids. MicroRNAs involved in redox responses will be investigated as well.

The Action members will continue working on the function of metal-containing oxidoreductases, such as peroxidases, including myeloperoxidases, lactoperoxidase, catalases and peroxidasin. Typical research questions that will be addressed are: structure-function analysis of redox-active metalloproteins folding and unfolding of metalloproteins, kinetics and thermodynamics of one- and two-electron transfer reactions, hydrogen peroxide degradation mechanisms, impact of protein matrix on redox chemistry of metalloproteins, role of post-translational polypeptide and heme modifications in catalysis, protein radical formation and migration in heme proteins, enzyme-mediated halogenation reactions and their role in the innate unspecific immune system. Moreover, investigating the formation of oxidants and halogenating species by these heme enzymes and their secondary metabolites will be investigated as well during the course of the Action. In addition, human peroxidases and human peroxidasins might be of special interest due to their role in the innate immune system as well as in oxidative damage of tissue and thus their role in many inflammatory diseases, showing again a synergy of expertise between groups working on other ROS sources, e.g. NADPH oxidases. Indeed, a hypothesis that there is a disease-promoting cross-talk between peroxidases and NADPH oxidases has never been explored in detail before. Further investigating this topic will be made possible due to future interaction with Action members while exchanging respective inhibitors, antibodies and generation of double KO mice.

Moreover, the Action will investigate the iron-sulfur proteins, which successively pass on electrons via complex intra- and intermolecular cascades using thiol-disulfide chemistry. Further focus will be on the recurring thioredoxin motif that catalyses diverse reactions, e.g. exchange of disulfide bonds during oxidative protein folding. The aim will be to identify dys-regulated pathways in several diseases and to define novel therapeutic targets.

Another important aspect will be to gain insight into the mechanisms of redox sensing and signalling in health and disease by identification of novel redox sensor proteins. In this respect, the transcription factor Nrf2, a master regulator of redox homeostasis, is a topic of intense research. Various KO mouse models are used to dissect the mechanisms by which transcription factors Nrf1 and Nrf2 control the basal and stress-inducible expression of antioxidant genes and drug detoxication genes.

Moreover, the Action will investigate the role of mitochondria as sources of ROS. Ongoing research is directed towards oxidative protein folding in the mitochondria intermembrane space, redox regulation of this process and redox signalling in mitochondria biogenesis using non-mammalian model systems such as yeast and bacteria. This sort of expertise is urgently needed as it was recently suggested that NOX4 is located in the mitochondria, but the final proof is warranted.

Finally, the Action members that deal with various plant aspects will further explore the potential of plants in building a sustainable world. The Action will use vast experience of its members in the characterization of regulatory interaction and networks that orchestrate plants responses to environmental stress in general and during oxidative stress. In particular, the ROS-dependent signal transduction mechanisms during abiotic stress will be investigated in this context. A combined top-down and bottom-up genomics approach will be further applied dissecting the gene network governing ROS signal transduction in plants with the aim to pinpoint genes that are potential candidates for innovative molecular breeding strategies with the final aim to develop stress-tolerant crops. These research groups will bring an expertise in areas, which are also highly sought by EU-ROS scientists working in mammalian systems, such as studying the translational control of oxidative stress response, the post-translational modifications during the oxidative stress response, identification and characterization of small molecules provoking oxidative stress resistance, and unravelling ROS-dependent transcriptional gene networks. Moreover, the role of plant NOX homologues (RBOHs) will be further studied in the establishment and functioning of the symbiotic interaction. Based on obtained promising results related to the involvement of a RBOH in the N_2 fixation process and on recent evidence that RBOHs may serve as important molecular “hubs” during ROS-mediated signalling, other RBOHs are currently studied in particular via the generation of transgenic roots. Interestingly, a bacterial infection occurs via the root hairs through the so-called infection threads.

WG2: MOLECULAR MECHANISMS AND TARGETS

WG2 aims to identify molecular mechanisms of ROS actions (redox signalling, direct chemical interactions) and their molecular targets. This will give insight into the role of altered ROS production/signalling in initiation and progression of disease in single-cell up to complex organisms. It will also allow precise identification of molecular defects. EU-ROS will define ROS' physiological roles and post-translational modifications and other changes such as molecular damage caused by oxidative and reductive stress.

Among Action members are world-leading experts on the molecular damage caused by ROS on NO synthase (NOS) and its receptor soluble guanylate cyclase (sGC). As it was demonstrated, both ROS and NOS are oxidised and become dysfunctional by ROS. Oxidative modification of sGC leads to a loss of its heme group and thus to a loss of functionality. Levels of this dysfunctional heme free sGC, apo-sGC, are elevated in patients with Cardiovascular Disease (CVD). Elucidating the precise underlying mechanisms, e.g. which ROS sources are causing this damage, and strategies to repair this damage are focus of intense research, both on a pre-clinical and also by testing novel ways to recouple NOS in a clinical trial. This research will further benefit from the complementary expertise of EU-ROS members. Recently, the first genome wide association study identified sGC as one of the key genes involved in hypertension, and EU-ROS will explore that in more detail.

A transcriptomic study performed by some of the Action members led to the identification of putative H_2O_2 target genes in plants. A special attention will be paid to kinases, which may be involved in the exchange of signals between symbiotic partners and in cell division required for nodule organogenesis as well as to the characterization of H_2O_2 driven post-translational modifications. Obtaining a repertoire of sulfenylated proteins isolated from a symbiotic interaction will help deciphering the signalling role of this ROS in the symbiotic process.

Another scope of the study will be investigating the cross-talk between NO and H_2O_2 signalling. The emphasis will be placed on the peroxiredoxin (Prx)/sulfiredoxin (Srx) system, which controls H_2O_2 fluxes and is modulated by NO. A special attention will be paid to post-translational modifications of Prx.

Moreover, further research will include analysis of oxidative damage to biomolecules and studies of cellular responses to oxidative stress, in particular in inflammation, cardiovascular disease and atherosclerosis through the increased cellular uptake of oxidized low-density lipoprotein (LDL). The research on detection of oxidized phospholipid adducts in LDL is already being carried out.

Moreover, elucidating redox signalling in T-cell function, neurogenesis, synaptic transmission, angiogenesis, and tyrosine kinase cascades are examples of redox signalling functions for future investigation.

WG3: DRUGS

WG3 aims to design, synthesize and pharmacologically test novel inhibitors that selectively block ROS sources (see WG1) as well as serve as enhancers of beneficial ROS signalling pathways, first applied for target validation in pre-clinical models, later for drug development. Proof of concept of the modulators will be provided in models followed by pre-clinical development. EU-ROS will also develop principles for crop science. One major advantage of this Action will be its multifaceted approach allowing pinpointing the best indications/applications for a given therapeutic target, as a drug candidate will be tested in various species and disease models available within EU-ROS. It will also give insight into side effects and indications to be avoided. Together, this allows for a very comprehensive evaluation of a drug candidate and to develop a drug for the application(s) most likely to success in the clinics, or being applied for non-medical purposes e.g. agriculture.

The Action members, which include participants from SMEs, have extensive expertise in HTS assay development, screening of small molecule and natural compound libraries, hit-to-lead optimisation, chemistry, pharmacokinetics, bio-distribution (e.g. by radio-labelling compounds) safety and toxicology/toxicogenomics assessments. Some have already established HTS compatible assays e.g. for individual NOX isoforms, and numerous disulfide reductases implicated in oxidative/reductive stress (bacterial glutathione-, mycothiol-, coenzymeA disulfide reductases, thioredoxin-reductase), which will be used to screen synthetic inhibitors and natural products ("antioxidant" plant metabolites). EU-ROS members have also expertise in the design and synthesis of alternative substrate analogues that are more appropriate for high throughput screening protocols.

Moreover, Action members are further interested in the design of chemical tools that can be used to facilitate the chemical labelling and detection of redox active thiols/metabolites. There is already commitment for sharing tools/methods/ideas to collaboratively optimise and design chemical probes to suit the specific needs of a given drug target. Importantly, several organic, medicinal and *in silico* chemists will participate in the Action as well. This expertise will also be applied to design/synthesis of inhibitors/mechanistic probes of interesting enzymes. Indeed, different approaches have already and will be used to identify specific inhibitors, for example against individual NOX isoforms (particular expertise provided by a SME). This approach includes: computer identified structure-activity relationships to known inhibitors (in case of NOX, this has already let to a set of novel inhibitory compounds); high throughput screening (in case of NOX, this has already resulted in NOX4 inhibitors; 700 hits that were funnelled down to 20 promising compounds). Different compounds are already in hit-to-lead phase, and specificity for NOX2 has been established for a number of development compounds.

The Action members from SMEs and academia have experience in pre-clinical drug development, including also comprehensive investigation of the compounds' impact on the adaptive and innate immune response (immune-toxicological screening, also for identification of secondary targets related to the modulatory effects of the drugs on ROS). This is complemented by other groups and clinicians with expertise in phase I and II clinical trials (e.g. targeting Nrf2 in Parkinson's disease or eNOS recoupling).

WG4: DIAGNOSTICS AND BIOMARKERS

WG4 aims to identify patients at risk for diseases caused by a redox imbalance and who would thus benefit from ROS modulating treatments, and to tailor the therapy to an individual patient (drug monitoring) by gene expression profiling and proteomics as well as oxidative stress and biomarker development (protein arrays, biomarker pattern analysis). Currently used biomarkers and risk factors, in particular for cardiovascular disorders, are hardly causal, but rather surrogate parameters and are not suitable for drug monitoring. Therefore, novel biomarkers are urgently needed to reliably assess the redox status and the individual disease risk. Such novel diagnostics are of major clinical importance to identify patients at risk before symptoms become apparent and to monitor the success of therapies. EU-ROS' bottom-up-approach will also enable identifying novel candidate therapeutic redox targets that will feed into the other WGs. Finally, the research conducted in WG4 will aid the validation of animal models and assessing their suitability for studying human pathologies, i.e. weather they share identical pathomechanisms, a so far neglected area.

EU-ROS will have secured access to samples from various completed clinical trials via cooperation with major pharmaceutical industries and biobanks. These entail samples from patients who had not developed the disease, but subsequently did, to compare with controls that did not develop disease subsequently with later samples at more progressed disease stages also being available. This is essential for the fast development of diagnostics allowing improved differential diagnosis, prognosis and therapeutic monitoring.

Several EU-ROS members will provide proteomic expertise. One strategy will be to develop an antibody-based biochip. Even if technological challenges should prevent EU-ROS from achieving the goal of 4,000 antibodies/chip, the technology will be superior, quicker and more affordable as compared to current ELISA and multiplex-based approaches of diagnostic labs. This particular technology gap is the main reason why so few biomarkers are clinically used and in a rather random fashion. Future EU-ROS' strategy will enable a broad and affordable use of validated biomarkers.

The expertise in protein mass spectrometry, e.g. in the area of protein modifications using various models such as senescent fibroblasts or muscle cells, plant mitochondria, fish muscle proteins, will be made available to various Action members. Moreover, Action members have recently developed a biomarker assay for apo-sGC that has the potential to be applied for therapeutic monitoring of sGC activators. Further expertise will be also available via participating SMEs that developed and launched a diagnostic kit for therapeutic monitoring of antiplatelet drugs, which is considered as the gold standard for this purpose. This kit is also based on the NO-cGMP signalling pathway. On the genetic side, a project has been initiated for the identification of patient subpopulations genetically prone to increased sensitivity to ROS and the development of screening procedures for identification of diabetes patients particularly benefiting from NOX4 inhibition.

WG5: IMAGING

WG 5 aims to localize ROS-triggered physiology and disease processes by developing and applying highly innovative imaging techniques using new modalities (electron paramagnetic resonance, Overhauser-enhanced MRI, near infrared imaging) and new contrast agents. This approach will also help to define the sources of ROS (WG1) and novel biomarkers (WG4).

EU-ROS members will perform ROS imaging by different imaging modalities, all correlated to histological findings. ROS exist in such small amounts and are that short lived that they are very difficult to detect using conventional methods such as bioluminescence and fluorescence. Electron paramagnetic resonance (EPR) is the most direct and sensitive analytical approach for free radical detection, but EPR will not have sufficient penetration depth for human imaging. Therefore, Overhauser-enhanced magnetic resonance imaging (OMRI) will be applied in the final development stage. Here near-infrared imaging (NIRI) is critical in order to link the early and the later phase. OMRI and NIRI represent crucial translational methods for validating animal model-human correlates of disease progression. Pharmacokinetics and tissue distribution of the contrast agent have to be established first. Then, the ROS maps provided by these innovative imaging techniques will be correlated to histological slides that are stained for oxidative stress. An on-going project using *in vivo* EPR imaging is aiming to define biomarkers for the redox status, oxygen, and NO.

Up to date *in vivo* EPR imaging of ROS is very challenging, but feasible with the experience of the EPRI groups within EU-ROS. These Action members have unique expertise in *in vivo* and *in vitro* EPR and EPR imaging from the chemistry of the molecular probes to methodological development and results exploitation in research field such as angiogenesis. In addition, the Action members from SMEs are also expected to bring expertise in EPRI, and recently provided proof-of-concept on brain ROS imaging after strokes in mice. Therefore, it is expected that members of EU-ROS will develop a multiscale approach of ROS measurements based on EPR and spin-trapping. This will be applied *in vitro* on organs using X-band EPR imaging and spin trapping, and on whole body using L-band EPR imaging. Previously conducted research allowed defining the biotransformation pathway of the molecular probes used in EPR.

WG6: TECHNOLOGY TRANSFER

WG6 aims to facilitate collaboration and success of R&D within EU-ROS by exchange of protocols/SOPs, technologies, methods, samples, tools and models.

There will be a very strong synergy between different WGs as the objectives of each WG can only be achieved through continuous exchange of information and materials. WGs1-5 will define the expertise present in their WGs and report this to WG6. Any reported lack of expertise within a particular WG will be dealt with by organizing exchange of staff or protocols. Moreover, EU-ROS will organise multi-centre, pre-clinical studies to test drug candidates and validate animal models in different labs. This approach will increase the likelihood of success of translational research as nowadays too many drug candidates fail in clinical trials due to inherited differences between human and animal model and insufficient quality of preclinical research.WG6 will therefore implement and control quality issues for its scientific activities, such as SOP`s.

E. ORGANISATION

E.1 Coordination and organisation

EU-ROS' participating groups are funded for their research by their institutions as well as national and international sources. EU-ROS provides the coordination to enable intense and cross-fertilizing interactions to the mutual benefit of EU-ROS' members, the scientific community and Europe. To achieve this, EU-ROS will implement the following organisational structures:

Management Committee (MC)

A MC will coordinate the scientific and administrative actions of EU-ROS. At its first (kick-off) meeting, the Chair, Vice-Chair and WG (see E.2) as well as website coordinators will be elected, and the members of the External Advisory Board defined. Also at further MC meetings will be held annually and linked to WG meetings thus reducing travel costs and improve interactions. The National COST Coordinators (CNCs) will nominate the MC (2 representatives/participating COST country). The MC also has the following responsibilities and authorities: promotion of gender balance and ESR involvement; distribution of funds within the Action; draft scientific reports to COST; launch of calls for exchange visits; increase of visibility by publication of EU-ROS activities; recruit new members; organisation, planning and coordination of meetings, conferences, workshops and training schools; monitoring of scientific progress within WG and EU-ROS; establishment of a panel that reviews STSM applications; promoting and establishment of collaborations between EU-ROS members with other COST Actions, European and international programmes; promoting of communication with societies; support of interactions with industrial partners, commercial exploitation and translation of research results.

Steering committee (SC)

EU-ROS will establish a SC composed of the Chair and Vice Chair of the MC, the WG coordinators, the Website coordinator and the STSM Chair. This SC will facilitate efficient communication and decision-making by preparing MC meetings and coordination of EU-ROS' activities. The SC will meet every month through internet-meetings.

Website

EU-ROS' website will be an important communication tool within the Action and beyond. The website coordinator will be responsible for establishment and maintenance (monthly update) of EU-ROS's website. It will be separated in a freely accessible domain and a password protected intranet. The website will entail information about EU-ROS in general and its progress towards reaching its aims. A discussion forum for EU-ROS members will allow them to share their progress, place questions, and seek for tools/technology support. It will list information on and links to the field of redox biology. One major goal is to communicate the novel scientific insights to the broader scientific community and the society in general, thus raising interest and awareness about redox biology, pathology and its applications. The website will also include online education tools for ESR. Within the intranet, activities, decisions and documents generated by the MC and WGs will be uploaded.

External Advisory Board (EAB)

The EAB, composed of experts in the field who are not members of EU-ROS, will monitor the progress, achievements of EU-ROS as well as its instruments and financial aspects. The EAB will prepare evaluations and reports at the end of years 2 and 4, which will be evaluated and approved by the MC.

Monitoring and evaluation

In addition to the EAB, EU-ROS will closely monitor its progress and activities by evaluation forms handed out after each MC and WG meeting as well as TS. The outcomes of this internal evaluation will be available on the website and an agenda item at the next respective meeting. Further, each STSM will conclude with a report generated by the guest and the host for the MC and WG. These reports will allow improving the outcomes of STSM.

WG meetings – see E.2

Scientific Conference

EU-ROS will organise an international scientific conference at the end of the Action. Here, EU-ROS findings will be presented and discussed with international experts from academia, industry and other stakeholders.

Training Schools (TS)

Among the many opportunities that EU-ROS provides, three dedicated TS, each devoted to a specific topic, will give emerging and established scientists a multi-disciplinary and international platform to access the expertise of internationally leading researchers in the field. Each TS, organized by the WGs, will be several days long. The Action will make sure that the leading experts share their knowledge, with particular focus on education of ESR. Hot topic sessions are envisaged. TS provide the opportunity for intensive training and networking, as supported by in depths discussions. These are the foundation for future scientific cooperation. For ESR, special sessions will also be organised that train transferable skills, for example, bioethics, communication, scientific writing and presentation, as today's job market not only demands from ESR that they know how to conduct good research, but also such transferable skills.

Short-Term Scientific Missions (STSM)

STSM are important to facilitate cooperation and initiating collaborations between EU-ROS participants within one WG and between WG (inter-WG STSM). ESRs will visit labs of other members to enable, for example, transfer of technologies. STSMs will provide ESRs with additional opportunities in participation within EU-ROS and skills development, scientific and non-scientific, e.g. cultural.

Major organisational milestones that are crucial to the future direction of EU-ROS

The MC and the EAB will monitor and evaluate the fulfilment of the milestones. (For timing of activities, see section F.)

MS1: Appointment of MC, SC, EAB, WG coordinators, webpage coordinator – Quarter 1
MS2: Website online – Q1
MS3: Newsletters – Q4, Q8, Q12, Q16
MS4: MC meetings held – Q1 (kick-off), Q5, Q9, Q13
MS5: WG meetings held – Q1 (kick-off), Q5, Q9, Q13
MS6: STSM – continuously open
MS7: TS – Q7, Q11, Q15
MS8: Evaluation by EAB – Q8, Q16
MS9: Reports to COST office – Q4, Q8, Q22, Q16
MS8: International Conference – Q 16

E.2 Working Groups

EU-ROS will entail six WGs focusing on the six work packages (see section D1). Current members of EU-ROS have been assigned to WGs according to their expertise and interests. Nevertheless, this assignment will be revisited at the 1st meeting. At this meeting, the WG Coordinators (1/WG) will be nominated. They will be responsible for organizing WG meetings (1/year) and ensuring timely communication with WG members as well as with other WGs and the MC, e.g. by distribution of meeting minutes and progress reports as well as attending MC meetings. Assignment of members to WGs will be flexible. At the WG meetings, both senior and ESRs will be present and discuss their work. Invitation of non EU-ROS experts is encouraged, and many EU-ROS members have strong links to international experts working outside Europe. At the WG meetings, SOPs will be defined, collaborations established (e.g. via exchange of tools, materials, technologies ideas and staff), STSMs planned and contribution to training schools drafted. If possible WG meetings will be scheduled with conferences to increase visibility and reduce travel costs. These conferences also serve as recruitment grounds for additional participants.

E.3 Liaison and interaction with other research programmes

EU-ROS has already links with the COST Action 'ENOG' (BM1005) focussing on gaseous transmitters, including for example NO, as there is a tight interaction between NO and ROS. Indeed, the Chair of ENOG is member of EU-ROS, and several others are members of both Actions. The Action will interact with COST Action CM1001 (on protein modification). There are many European research programs on antioxidants, and links between EU-ROS and not yet identified related European programs will be looked for. EU-ROS will ensure that communication and interaction with other related research initiatives will be continuously supported. This will be of mutual advantage, as both will exchange their state-of-the-art knowledge and promote related research within Europe. Also, new experts and junior members may stem out of external interactions that further strengthen EU-ROS. Nevertheless, the approach of EU-ROS is different compared to other existing programs. Thus, there is no duplication but rather complementarity.

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 ESRs.

In more detail, EU-ROS will ensure that women and ESRs are included in its scientific and organizational activities. With respect to gender balance, the MC will implement a policy to recruit female scientists to the labs of EU-ROS members. Special attention to the gender balance will be given for the Chairs of the MC and WGs. EU-ROS current members entail already a significant proportion of female scientists, who will be role models and mentors for female ESR. EU-ROS will also support high potential female scientist in there critical career phase between senior postdoc and first professorship by providing access to career training programs offered by the members' local institutions' gender programs. For example, EU-ROS seeks contact to and interaction with the newly established centre for gender and diversity research, Boyksen Diversity Research Center at the TU Munich. It promotes research activities related to gender and diversity issues particularly in the technical and life sciences.

This and comparable programs provide e.g. gender consulting, gender issues incentive funds, career building programmes, mentoring and "girl's programmes" (introducing science to young female pupils). Female EU-ROS participants are coordinators of initiatives specifically designed for female life scientists with the aim to enhance their visibility, communication skills and information exchange at a scientific and also at a social and career level. One member of the MC will become a 'gender officer'. The gender officer will be a supportive contact to national/local gender centres and help to create an international network for support of women in the Action with respect to gender aspects related to their career, including promoting access to dual career offices, child care facilities etc. To further actively promote gender related issues in this Action, interaction/cooperation with national/international organisations/associations of female life scientists will be established for the purpose of exchanging ideas and promoting scientific and career-oriented support of female scientists in this Action. Importantly, the topic of EU-ROS is highly linked to gender studies and gender-associated pathologies including cardiovascular diseases. In this regard, a specific emphasis will be laid to promote awareness on the role of gender related ROS effects in the pathogenesis of diseases and to stimulate research in these aspects.

EU-ROS will drive active involvement of women and ESRs in WG meetings, workshops and international meetings. They will be invited and encouraged to present and chair sessions. Each WG will nominate an ESR, who will communicate with other ESRs to seek feedback and ideas. This will then be presented to the MC.

Special attention for the development of ESRs will be given by organization of training schools (see section E1) that will particularly address the needs of ESRs. Beyond these training schools, EU-ROS will support and organize short-term (1-3 months) exchange visits of ESRs between different labs. Particular focus will be placed on including interdisciplinary exchanges across labs working in different work packages. These will facilitate the exchange of materials, methods, and train ESRs in new techniques that they cannot learn at their home labs. Whilst offering excellent international/interdisciplinary research training experiences to ESRs, these exchanges will also underpin the inoculation of new research collaborations/ideas, which will lead to joint research publications as well as prompt new and competitive collaborative grant applications both at the national and international and European level.

F. TIMETABLE

The duration of this Action will be four years. The activities are scheduled as follows:

Activity	**Year 1**				**Year 2**				**Year 3**				**Year 4**			
Kick off meeting: Appointment of Chair, Vice-Chair, WG coordinators, web manager, selection of external advisory board	x															
Dissemination plan set up	x															
Web site establishment (update: continuously)	x															
MC meetings					x				x				x			
WG meetings (1 meeting/WG/year)	x				x				x				x			
Training schools							x				x				x	
STSMs		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Newsletter				x				x				x				x
Progress reports				x				x				x				x
Evaluation								x								x
Final conference																x

Regarding scientific deliverables, EU-ROS expects that by the end of Y4 the targets (see Section D) will be fulfilled or considerably progressed.

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, BE, DE, DK, EL, ES, FR, HU, IE, IT, NL, PL, PT, RO, 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 64 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?

The target audiences of EU-ROS are:

- Patients: One major goal of EU-ROS is to pave the avenue for novel, mechanism-based and individualized treatments of diseases caused by a redox imbalance, such as cardiovascular diseases, neurodegenerative diseases, cancer and immune diseases. Together with novel biomarkers and diagnostics, these therapies will help to reduce the burden of patients;
- Researchers (EU-ROS members and from the international scientific community): EU-ROS is an interdisciplinary network of European scientific experts and ESR providing a critical mass to perform internationally competitive R&D and thus providing scientific insights, tools and technologies in a way that would not be achievable by single disciplines or small scale co-operations. It will provide a proof-of-concepts for the feasibility and relevance of a highly integrated concept covering drugs, diagnostics and imaging technologies. The dissemination methods will trigger the interest of further scientists contributing further expertise, insights, technologies and tools. This provides opportunities that cannot be foreseen at this stage. EU-ROS will pay particular attention to the support of women and ESR, providing world-class and collaborative team environment as ideal training conditions for ESR (see E.4);

- Pharmaceutical and medical devices industries, EU-ROS expects to increase the competitiveness of European industries, as it aims to identify innovative therapeutic targets that can be exploited to develop mechanism-based drugs. Further, EU-ROS aims to develop novel redox biomarkers and imaging technologies. EU-ROS expects increased competitiveness and leadership in preclinical discovery, decreased clinical risk in drug development, mid-term direct translation into advanced monitoring of clinical trials followed by therapeutic applications in patient inclusion and exclusion, as well as use of technologies in preventative diagnostics and therapeutic monitoring. Thus, this Action will facilitate the growth of biotechnology and pharmaceutical industries in Europe. EU-ROS will also stimulate the establishment of new SME building on the scientific deliverables;
- EU member states, policy makers and regulators: EU-ROS will provide excellent training options for the future scientific leaders. EU-ROS is confident to enhance the competitiveness by joined forces of outstanding European scientists in a large network to achieve high-level objectives that would not be achievable at an individual level. Thereby, EU-ROS will contribute to European reputation as place of excellence in biomedical research and translation. EU-ROS addresses diseases that are major burdens of societies, and will help to improve their health. This will result in a net decrease of costs and contribute to the sustainability of European healthcare systems. Finally, EU-ROS will help to develop a more sustainable agriculture;
- Scientific societies, e.g. Society for Free Radical Research (SFRR) in getting access to the latest, ground-breaking R&D results in the field and support of its conferences;
- Medical professionals and the general public: EU-ROS will promote awareness about the limited or even harmful effects of antioxidant supplements. It will further promote knowledge on evidence-based medicine that will help the general public and physicians to distinguish between facts and foes, and to keep up to date with scientific/medical progress.

H.2 What?

EU-ROS will use the following dissemination methods:

- EU-ROS website: The public part website will inform about the latest research results. The password-protected section will be used for exchanging confidential information;
- Presentations at major international conferences and seminars at academic institutions will be given by EU-ROS members. Industry representative will be invited and research results presented to further increase industry involvement;
- Link into societies: Some participants of EU-ROS are society members and support integration;
- Original papers and reviews will be jointly published in peer-reviewed journals;
- Training schools (3) for ESRs will be organized;
- Lay information will be disseminated, e.g. via press releases;
- Exchange of ESRs between EU-ROS laboratories will be organised to train ESRs, promote networking, as well as to exchange/transfer expertise;
- A final international conference will be organised.

H.3 How?

EU-ROS will work out a concrete dissemination plan in the first three months. This plan will be updated and optimized during the course of the Action based on evaluations. EU-ROS will use the following dissemination methods:

- EU-ROS Network logo used on all publications, documents, events and presentations;
- EU-ROS website (set up during the first quarter). The public part, used for spreading the knowledge to all target audiences, will inform about the latest research results, include a discussion forum and a newsletter. It will announce activities and entail a publication database. An intranet section, restricted to EU-ROS members, will be set up for exchanging confidential information amongst EU-ROS members. Documents, such as WG and MC reports and proceeding of EU-ROS meetings will be uploaded;

- Twitter will be used to increase visibility;
- IT websites of the European Commission, e.g. CORDIS, will be used;
- Presentations at major scientific international conferences and seminars at academic institutions by EU-ROS members;
- EU-ROS will involve societies, e.g. to organize special (e.g. hot topic) sessions at conferences and to connect EU-ROS to patient organizations;
- Connection with other networks and service organisations will foster cross-sectional interactions, e.g. with EMMA (mouse models). Further, the Society for Free Radical Research (SFRR) holds summer school, and EU-ROS plans to sponsor speaker and student attendance. Also, at the 2013 (Athens) and 2014 (Paris) annual SFRR conferences, EU-ROS plans to sponsor sessions. To facilitate interaction, some participants of EU-ROS are members, and one is the current honorary secretary of the society. This person will advise and support integration. Hosting a workshop at the Annual Romanian Society of Immunology conference is also envisaged;
- Discuss with educational institutes to include redox biology and its applications into curricula;
- Stalls at scientific conferences will promote the Action among international scientific audiences;
- Original papers and reviews in peer-reviewed journals (8-10/year). A good route for dissemination to a wide international field (ranging from academia to industry to governments) is to publish articles with Research Media www.researchmedia.eu, who prepare professional articles for their research media magazines which is distributed to ~30,000 researchers, policy makers and government bodies, many of those would not be reached by the usual dissemination routes;
- Training schools for ESRs ;
- Exchange of ESRs between EU-ROS laboratories;

- Lay information will be published in newspapers, magazines and leaflets and provided to clinicians for further distribution amongst the general public. Press releases will also be prepared. Dissemination will also via e.g. Scientific American, Science et Vie etc;
- A final international conference will bring together scientists, clinicians, industry partners and other interest groups on redox biology from all over the world. It will thus also promote the outcomes to non-European audiences.

Generally raise interest by policy makers to support R&D on redox biology and its applications by research funds.

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