European Cooperation in Science and Technology
- COST -

Secretariat

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COST 4119/12

MEMORANDUM OF UNDERSTANDING

Subject :	Memorandum of Understanding for the implementation of a European Concerted Research Action designated as COST Action CM1201 : Biomimetic Radical Chemistry

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 CM1201
BIOMIMETIC RADICAL CHEMISTRY

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 implement biomimetic chemical models for understanding free radical biological events. Focus is on: enzymatic activities, DNA damage, membrane stress and bio-inspired synthetic strategies.

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 80 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

Knowledge of chemical reactivity is essential for understanding at a molecular level the mechanistic steps that drive processes in life sciences. This COST Action aims to enhance the role of chemistry as a central discipline for understanding free radical biological events. This goal will be achieved through the implementation of biomimetic chemical models. Four Working Groups will focus on: enzymatic activities via free radical species; the formation and fate of free radicals involving nucleic acids; membrane lipids in stress and ageing; and synthetic methodologies inspired by natural free radical processes. The research groups participating in the Action will create an interdisciplinary framework, in which a younger generation of investigators can broaden their expertise by studying the diversity of free radical interactions in biological systems for metabolic, synthetic and catalytic activities through biomimetic models.

Keywords: Free radical chemical reactivity of biological systems, mechanisms of ageing and biomarkers development, reactive oxygen species and biological damages, antioxidants and repair mechanisms, chemical synthesis and catalysis

B. BACKGROUND

B.1 General background

Learning from Nature has long been a main *leitmotif* of chemists, especially for organic synthesis applied to natural products. Nowadays, the understanding of chemical reactivity is essential for acquiring a deep knowledge of the mechanistic steps that drive living processes in all life sciences. Recent years have witnessed the growth of a strong interest in modelling the chemical reactivity of biological systems that is, improving chemical methodologies and knowledge in order to understand complex reaction pathways related to cellular processes occurring in hydrophobic and hydrophilic environments. In this context, free radical chemistry offers a great challenge to life sciences research, due to the enormous importance of free radical reactivity for a variety of biological events, including ageing and inflammation, as well as for highly selective processes, such as catalysis.

The underpinning chemistry includes expertise in synthetic, kinetic, and mechanistic aspects together with computational methodologies that can successfully address the degree of complexity of biological and catalytic systems. On the other hand, the requisite mixed background includes expertise in verifying molecular interactions, following-up receptor/enzymatic responses, mapping signalling cascades, investigating activation/inhibition pathways, discovering biomarkers and designing biomaterials. This scientific approach needs the integration of different expertise, which has proven quite a difficult task to achieve. In view of strengthening the European Research Area this integration has become a central goal. Indeed, this is a principal task for free radical chemistry, which is one of the scientific fields where European groups have established a highly competitive research environment within the international scenario. For the future of the young scientific generation this is an important context for reaching a holistic vision of chemical sciences and ensures readiness for future challenges in diverse scientific areas. Therefore, this COST project aims at fostering the integration of different expertise and methodological approaches with the participation of outstanding teams that will cooperate in addressing the main subject of Biomimetic Radical Chemistry. Four aspects will be developed, which are currently at the leading edge of life sciences research: radical enzymes, DNA damage and repair, membrane and lipid transformation, bio-inspired synthetic strategies.

B.2 Current state of knowledge

Some of the most interesting aspects of free radical chemistry that have emerged in the last two decades are: (i) radical-based processes in enzymatic and pharmacological activities; (ii) free radical-induced damage of biomolecules and formation of by-products, opening the way for the evaluation of in vivo damage through biomarkers; (iii) synthetic strategies inspired by the biological interactions, such as catalytic processes and reactivity in aqueous systems, either for biological and biotechnological applications, and for green chemistry. Relevant examples of these fields are the formation of the 5'-deoxyadenosyl radical in the functioning of coenzyme B12 [1], the discovery of the function of radicals produced by cyclooxygenase enzymes [2], the identification of hydroxyl radicals as powerful damaging agents for DNA, protein and lipids [3-6], impaired electron transfer processes in macromolecules leading either to damage or signalling events [3,7], the roles of membrane lipids in cellular signalling and homeostasis, providing biomimetic models through liposome technology [8,9], the potential of free radicals in synthetic strategies [10], as well as the simulation of stereocontrol operated by biomolecules applied to organocatalysis and other highly selective synthetic methodologies [11,12]. All together these examples show the flexibility and high applicability of free radical chemistry, and the well-developed state of research teams throughout Europe.

This is a potential to be amplified and expanded in a multidisciplinary approach in order to cope better with new challenges, which are important for the advancement of science and society. Several research groups in Europe deal with myriad aspects of free radical chemistry and reactivity. This scientific community has been able to develop excellence in this subject and compete effectively in the international context. In January 2012, the *Encyclopedia of Radicals in Chemistry, Biology and Materials* (4 volumes, 73 chapters, over 2300 pages) has gathered the significant contributions of several leading European groups in these interdisciplinary fields showing the enormous potential of this chemistry. Moreover, in July 2010, the international meeting 12th EuCheMS (European Association for Chemical and Molecular Sciences) Conference on Organic Free Radicals demonstrated the breadth and immense relevance of free radical research.

In these two occasions, some critical points arose from the community, such as the need of a more concerted effort to address key aspects of free radicals and biomimetic design, to develop a common know-how and shared protocols, which could render chemical knowledge much more applicable and effective in the various fields. Due to the presence of a large number of young researchers among the EuCheMS Conference participants, it was clear that this research area is very attractive for their careers, and that an integrated approach could be effective in expanding its potential and applicability.

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B.3 Reasons for the Action

The reasons for the COST Action on "Biomimetic Radical Chemistry" are:

- the centrality of chemistry to comprehend the molecular level of important free radical processes in life and material sciences;

- the scientific relevance of free radical chemistry and its high potential in life sciences and technology, which is also an important part of the excellence and competitiveness of European research, to be implemented in an interdisciplinary and integrated manner, in order to share and improve methodologies and know-how in different subjects;
- increasing applicability and opportunities of free radical chemical research, especially for the younger generation and their career opportunities;
- the importance of biomimetic models in order to gather substantial knowledge on biological and technological processes relevant to health and material sciences based on free radical chemical research, such as biological damages and repair, signalling and biomarkers, biotechnological applications and novel synthetic approaches;
- the need to address in an interdisciplinary context the development of biomimetic designs, by integrating different disciplines and methodologies for a common large background;
- unifying the efforts of synthetic, physical organic chemists and biochemists and interfacing them with advanced molecular biology approaches and complex organic synthesis challenges;
- increasing the interdisciplinary applications involving chemical science and technologies, such as: enzyme and liposome models, antioxidants in radical chain processes and in repair activity, including the multifaceted role of thiol compounds, design of biomimetic antioxidants and antimicrobial agents, enhanced selectivity with organocatalysts, polymer bioconjugates and organic-inorganic hybrid materials, models for redox processes, lipid reactivity in an oxidative context and biomarkers follow-up, in vitro and in vivo membrane reactivity to free radicals and signalling, oxidative processes in ageing and diseases, etc.

In general, many of the discoveries in biomimetic chemistry have resulted in straightforward utilization in other fields spanning from chemical synthesis to cell biology, medicine and materials. Thus, it is evident that an inter- and multi-disciplinary approach is required. In free radicals to date, this represents the main challenge for rendering chemical observations connected to chemical and biomedical applications. The younger generation of investigators in chemistry will benefit from this Action by widening their chemical competences to adjacent fields of life sciences and materials. Thus, to address this goal, efforts for disseminating and sharing methodologies and protocols have to be pursued, as well as creating a common language facilitating comprehension among disciplines.

B.4 Complementarity with other research programmes

There are several COST Actions addressing inter- and multi-disciplinary subjects having chemistry as the central discipline, such as:

- CM1106 - Chemical Approaches to Targeting Drug Resistance in Cancer Stem Cells
- CM1103 - Structure-based drug design for diagnosis and treatment of neurological diseases: dissecting and modulating complex function in the monoaminergic systems of the brain
- CM1001 - Chemistry of non-enzymatic protein modification - modulation of protein structure and function
- CM1005 - Supramolecular Chemistry in Water
- CM1004 - Synthetic Probes for Chemical Proteomics and Elucidation of Biosynthetic Pathways
- CM0905 - Organocatalysis (ORCA)
- CM1003 - Biological oxidation reactions - mechanisms and design of new catalysts
- TD1003 - Bio-inspired nanotechnologies: from concepts to applications

None of these Actions addresses free radical reactivity, although this subject has a crucial role in biological and technological contexts. European research programmes on free radical reactivity are lacking and the risk of losing competitiveness in this important field is clearly significant. The scientific community working on the various aspects and the younger generation strongly interested in the subject need a stimulating environment to discuss and expand their knowledge.

C. OBJECTIVES AND BENEFITS

C.1 Aim

The aim of the Action is to strengthen the role of chemistry as a central discipline for understanding free radical biological events through the implementation of biomimetic chemical models. The research groups involved in the Action will create an interdisciplinary framework where the younger generation of investigators in chemistry can widen their expertise in order to confront the diversity of free radical problems in biological systems. Four areas will be implemented by the Working Groups focusing on: enzymatic activities via free radical species; the formation and fate of free radicals involving nucleic acids; membrane lipids in stress and ageing, and synthetic methodologies inspired by natural free radical processes.

C.2 Objectives

The four Working Groups will address the topics of the Action with specific objectives as follows:

WG1 – Radical Enzymes

Many biological processes use reactions catalysed by radical enzymes, e.g. biosynthetic processes leading to antibacterial agents and cofactors. Besides their medical importance, radical enzymes are crucial for the environmental degradation of aliphatic (e.g. hexane) and aromatic hydrocarbons (e.g. naphthalene). The primary objectives of this WG will be:

- To elucidate the catalytic pathways and mechanisms of selected radical enzymes.
- To discover new radical enzymes.
- To develop model systems and biomimetic chemistry based on the pathways used by radical enzymes.

These investigations will deploy the full range of chemical (synthesis, mechanistic studies, spectroscopy) and biochemical tools (microbiology, enzymology, structural biology). There are many unanswered questions, which WG1 will seek to resolve through collaborations between the participating groups. In particular, how do radical enzymes:

- tame the high, intrinsic reactivity of radicals?
- guide radical intermediates through specific reaction pathways?
- lower energy barriers within those specific pathways?

WG2 – Models of DNA Damage and Consequences

Studies performed on free radical DNA (deoxyribonucleic acid) damage opened the way to fundamental discoveries of structural and functional consequences on the genetic material, which can be applied to basic processes in ageing and diseases, to development of therapeutic agents, and to biomarker discovery. The main objectives of WG2 will be:

- mechanistic studies elucidating the radical-induced complex lesions, which are the most dangerous in impairing defence systems;
- biomarker discovery and development of the nucleic acid modification, in view of providing a complete screening of DNA damages;
- parallel oxidative and free radical damages with protocols for the analytical identification of the whole lesion pattern in biological samples;
- study of complex DNA lesions' induction mechanisms, repair and biological consequences;
- DNA lesions produced by lipid decomposition products (aldehydes) or through amino acid radicals.

WG3 – Membrane Stress, Signalling and Defences

Membrane stress and remodelling caused by free radicals has been suggested as a crucial signal in the overall homeostatic control of cells. During stress the unsaturated lipids are modified, for example by peroxidation or isomerization. In the latter case, the consequences of the transformation of the natural cis geometry to the trans configuration still needs to be addressed in detail. WG3 will address the following objectives:

- development of biomimetic models of peroxidation and isomerization damages using liposomes; this model will be also used for the tandem lipid-protein and lipid-DNA damage models and for the discovery of new isomerizing agents;
- biomarker discovery and study of the membrane lipid remodelling in cellular models under different pathological (e.g., diabetes, atherosclerosis) and dietary conditions as signalling for the whole metabolic response;
- examination of defence systems based on antioxidant and radical-trapping agents toward the membrane integrity;

WG4 – Bio-Inspired Synthetic Strategies

Bioinspired synthetic strategies based on free radical reactivity demonstrated their importance as models of naturally occurring processes. WG4 will explore:

- applications in catalysis, e.g. C-H activation, using the efficiency of free radical reaction cycles, and in green chemistry, also using the tolerance to aqueous environments of most free radical reagents;
- development of new processes based on oxidative biomimetic catalysis or coupling, either for new synthetic procedures or for new materials (polymers) based on polymer-bioconjugates and organic-hybrid materials;
- chemical modifications of biomolecules, either for simulation of the biological free radical damage or for providing molecular libraries to be used in biomarker discovery;
- applications of free radical bioactive products (such as 4-HNE) as potential bioactivators of nanomaterials (such as carbon nanotubes or bioceramics).

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

From the above described Action objectives it is clear that effective networking will be crucial for accomplishing the Action's aims. Indeed, the multi- and inter-disciplinary character of this Action is the leitmotiv of the collaborations within the Action's participants, in order to achieve a substantial amount of competences and know-how for dissemination and utilization in their research. In this Action the European free radical chemistry community, which has always maintained strong connections among the groups, intends to collaborate with other specialized areas of chemical, biological and biomedical research, in order to address in an integrated manner the specific objectives, listed above. This networking, which is the most important feature to address, will be accomplished by:

- effective distribution of research groups within the WGs in order to provide the right combination of scientific expertise
- interdisciplinary character of the meetings programmed by the Action, and choice of appropriate thematic conferences in chemistry, biology and materials science to link to the Action's initiative;
- organization of events for early-stage researchers in order to disseminate and discuss the experimental methods used in the different areas.

C.4 Potential impact of the Action

The Action addresses several important topics in science but it can also be expected to have a societal impact, since it addresses health and environmental aspects. The main expected effects of the Action can be summarized as follows:

- Affirming the centrality of chemistry for the comprehension at a molecular level of processes in biology and technology and the use of biomimetic models as simplified, yet strictly relevant systems, for studying molecular interactions and effects.
- Promoting contributions from different disciplines, such as analytical, radiation, mechanistic and synthetic chemistry, biochemistry, molecular biology, pharmacology, together with computational tools contributing to the Action's objectives.

- Fostering an innovative multidisciplinary scenario ideal for Early Stage Researchers (ESRs), in order to address research with an array of methods and know-how from different fields, and more importantly to design biomimetic models for solving complex tasks;
- Bringing together scientists with complementary, but distinctive interests in chemical and biological subjects. All groups will gain not only from the reciprocal expertise, but also in providing a whole scenario for improving the biomimetic modelling;
- Reaching a better understanding at a molecular level either in biological and technological areas, such as basic mechanisms underlying a healthy cellular status and efficient defence systems, and new procedures for bio-inspired and bio-compatible materials. Such an integrated vision of chemistry related to other sciences is an important aspect to be reached.
- Addressing important societal needs related to health and technology with favourable impact, and also for clarifying the perception of chemistry's contribution to the overall progress.
- Improving a common language among scientists of different background and experience, creating a common environment for sharing methodologies and solving analytical/mechanistic problems, and enhancing the importance of chemical and molecular aspects within the biological and technological scenarios.
- Last but not least, the Action's participants will publish in the best international scientific journals and this is expected to contribute profoundly to the scientific impact of the Action.

C.5 Target groups/end users

The end users will be a significant cohort of the scientific community, due to the large impact of the science of free radicals and the multidisciplinary approach of the Action. It is expected also that the results and discussions stimulated by the Action will highlight the excellence of European research. Mostly ESRs will participate in the Action with a dedicated STSM (Short-Term Scientific Mission) program, in order to achieve the best inter- and multi-disciplinary training in the various fields. The wider chemical community and scientists from biology and materials science will share in the Action's aims and results, thus providing the opportunity for an integrated vision of several scientific problems. Societal needs for better chemical tools in health and technology will be met by the Action's objectives and so a notable societal impact is also expected.

D. SCIENTIFIC PROGRAMME

D.1 Scientific focus

The scientific subjects in the four WGs have been chosen in order to be the most interdisciplinary ones among their topics in order to foster new ideas and spread research in biomimetic chemistry, hence strengthening ERA development. Key topics in the scientific programme of each WG are summarized below:

WG1 – Radical Enzymes

Radical enzymes are crucial for biosynthetic processes (enzyme cofactors or antibacterial agents) as well as for the environmental degradation of aliphatic (e.g. hexane) and aromatic hydrocarbons (e.g. naphthalene). Still many radical enzymes are to be discovered and investigated. The Action will focus on model studies for the following radical enzyme pathways:

- the reactivity of nucleophilic ketyl radicals derived by one-electron transfer toward a carbonyl group of a thioester or amide. The energy comes either from hydrolysis of ATP, as in 2-hydroxyacyl-CoA dehydratases, or from light.
- The mechanism of H-abstraction by a 5'-deoxyadenosyl radical from SAM, for example from a conserved glycine of a protein, giving a carbon (glycyl) radical within the polypeptide chain of this type of radical enzyme;
- the models for investigation of structure and mechanisms of reductase enzymes [ATP-dependent, FeS benzoyl-CoA reductases, ATP-independent W/Se/FeS/FAD benzoyl-CoA reductases, ATP-independent FAD/FeS naphthoyl-CoA reductases, FeS reductases involved in isoprenoid biosynthesis] and oxidative enzymes (chloroperoxidase). The biotechnological application of these enzymes for the synthesis of building blocks of pharmaceuticals will be investigated;
- the reactivity of organometallic model compounds (derived from B12) to test radical alkylation reactions with structural, mechanistic and theoretical studies in view of the activity of enzymes involved in biological methylation at carbon centres for the biosynthesis of some antibiotics, such as botromycin and fosfomycin;

- reactivity involving the single-electron transferring of flavoenzymes such as NO-synthase and cytochrome P450 reductase in oxidative and partial apoxia conditions;
- reactivity of radical enzymes for the environmental degradation of aliphatic and aromatic hydrocarbons (analogous to the Birch Reduction of aromatic rings);
- the mechanism of conversion of *cis* fatty acid residues to the corresponding *trans* isomers in membranes, a unique biomodification in bacteria by *cis–trans* isomerase (Cti) proteins, will be defined.

WG2 – Models of DNA Damage and Consequences

This WG will focus on the study of the mechanisms of formation of clustered DNA lesions, development of analytical tools to measure such complex lesions at the cellular level, biological consequences of these DNA modifications and related biomarkers, preventive strategies against the harmful effects of complex lesions, application for therapeutic purposes in photo-, radio- or chemo-therapies. The Action will focus on the models of the following reactivity:

- generation and biological consequences of clustered and oxidative damages in specific oligonucleotide model sequences, considering either structural and biological changes due to the damages;
- molecular assays for the detection of bistranded non-double strand break (DSB) oxidative clustered DNA lesions (OCDLs) *in vitro* and, in general, implementation of analytical techniques and protocols for DNA damage quantification also for application in biological samples;
- study of the DNA repair pathways and proteins involved in the processing of lesions after exposure to free radical insult, also deriving from relevant doses of ionizing radiation in radiotherapy;

- models and methodology for the study of clustered DNA lesions at the cellular or tissue level using human or bacterial DNA repair enzymes; the repair will also be studied considering the fact that DNA (and damaged DNA) is present in association with histone proteins in nucleosomes; therefore the repair involves the whole mononucleosome. There are several factors involved in the success of repair: the activity of chromatin re-modellers able to facilitate the enzymes to reach the damage site, the sequence and position of lesions, among others.
- models of DNA-lipid and DNA-protein damages for the development of an integrated vision of damages caused by inflammatory and radiation effects.

WG3 – Membrane Stress, Signalling and Defences

This WG will be dedicated to membrane organization and reactivity, in liposome models as well as extended to biological models, in connection with structural and chemical factors (fatty acid/cholesterol composition, free radical generation, radiations, oxidants, etc…). Both models and living systems will be provided by the WG participants to study the influence of membrane organization and structures including the effects of changes, the main signalling activities departing from membranes with individuation of cascades involved in metabolic situations such as stress, inflammation and aging, the protection given by specific additives with antioxidant/anti-isomerizing properties. Two main lipid transformations, peroxidation and isomerization, will be considered in models and living systems. Moreover, the effect of lipid remodelling will be considered during the follow-up of cellular models, which can occur either favourably or compromising the recovery of the whole membrane homeostasis. The Action will focus on:

- models of liposome vesicles for the study of peroxidation vs. isomerisation reactions under different conditions, simulating the different composition of biological membranes and the presence of additives in the medium, with a particular focus on sulfur- and nitrogen-containing compounds as sources of free radicals;
- cellular models for examination of lipid remodelling during the different nutritional intervention and biological consequences of the membrane transformations, including the events of signalling cascades;

- biomarker identification and influence to be performed in models or organisms related to the membrane transformation and reorganization. This includes molecular libraries and the recognition of modifications in membrane constituents;
- membrane interaction and reactivity with DNA and proteins will also be studied by the design of appropriate biomimetic systems in order to achieve an integrated vision of the free radical reactivity;
- free radical-induced signalling (peroxidation product-mediated) and activation of uncoupling proteins.

WG4 – Bio-Inspired Synthetic Strategies

Reactivity involving free radicals is closely related to processes taking place in Nature. Often, this reactivity occurs in an aqueous environment and this renders free radical chemistry attractive also from an eco-compatibility point of view. The design of biomimetic antioxidants and antimicrobial agents, the modelling of redox processes and the enhanced selectivity obtained with organocatalysts are inspired by the naturally occurring biological activities, and are supported by computational studies. In the field of material science, bio-inspired synthesis is also important to devise polymer bioconjugates and organic-inorganic hybrid materials with a very large range of properties and applications. This WG will focus on the following areas:

- organocatalytic radical reactions (chain reactions, oxidation, single electron transfer processes, activation of radical traps by Brønsted acids, enantioselective radical reactions);
- biomimetic oxidative coupling reactions: mechanistic study and synthetic applications. Conjugate addition reactions concerning alpha,beta-unsaturated acyl azoliums. In biosynthesis such an activation mode is known for C-X bond formation in the beta-position of an aldehyde. Various soft nucleophiles such as indoles, other heteroarenes, nitroalkanes and nitriles will be tested in the reaction with such biomimetic redox-activated *Michael* acceptors for accessing interesting structures under mild conditions;

- C–H activation of complex molecules via radical pathways. Access to modified natural products, fine-tuning of the biological activity of natural products;
- use of antioxidants as reagents in radical chain processes and correlated hydrogen transfer processes;
- development of efficient radical chain processes involving antioxidant mediated repair mechanisms;
- bioinspired synthetic strategies to major autoxidatively formed cyclic natural products, in particular lipid structures, such as IsoP, phytoprostanes and neuroprostanes, as well as heterocyclic and polycyclic compounds;
- Application to biomaterials using polymerisation reactions and biomodulation by bioreactive free radical products, such as 4-hydroxy-2-nonenal (4-HNE).

D.2 Scientific work plan methods and means

The work plan comprises four Working Groups addressing the main topics of the Action, which are:

WG1 – Models for the functioning of enzymes through free radical pathways and intermediates, assaying the outcomes of enzymatic activities by suitably designed systems, which simplify the chemical environment but are strictly related with the naturally occurring transformations. Free radical pathways of enzyme functioning can be assayed by suitably designed models followed by the analysis of the reaction products. This field includes chemical design on the basis of the targeted biological pathways, chemical synthesis of the designed structures and products, analytical protocols for the follow-up of the reaction outcome. The main objectives of this WG are:

- generation and reactivity of the 5'-deoxyadenosyl radical from S-adenosylmethionine (SAM), including the process of one-electron reduction and formation of sulfonium intermediate; the reactivity of this radical by H-abstraction from an amino acid of a polypeptide chain will be also examined;
- reactivity of nucleophilic radicals such as ketyl radicals generated by electron transfer toward a carbonyl group of a thioester or amide, mimicking dehydratase enzymes;

- Models for diol dehydratases and ribonucleotide reductases with reference to the prebiotic formation of deoxyribose;
- reactivity of metal-containing enzymatic sites, such as metalloproteins containing metal-sulfur clusters (reductase or metallothioneins) and organometallic sites (Co-alkyl groups present in cofactors such as B12);
- reactivity of flavo-containing enzymes in the single electron-transfer reactions, relevant for the release of signalling molecules or in reduction processes in presence or absence of oxygen;
- detoxification and environmental degradation by radical enzymes for aliphatic and aromatic hydrocarbons, addressing the potentiality of green methodologies;
- mechanism of chloroperoxidase (CPO)-catalyzed oxidations of alkanes, alkenes or conjugated dienes;
- evaluation of lipid isomerisation of Fe-S clusters under gamma-irradiation mimic the iron-mediated redox isomerisation of *cis–trans* isomerase (Cti).

This WG will be integrated with the activities of WG4 for the synthetic approaches of the model structures needed in enzymatic assays. Some overlaps with WG2 in the DNA damages and WG3 with membrane lipid modification will be also possible.

WG2- Models for a deep understanding of DNA clustered damages due to free radicals, and the functional meaning of these lesions, also in view of the response of repair systems and prevention strategies. This WG is dedicated to the complex lesions generated by free radical stress in DNA. In this approach most of the chemical reactivity is well represented by models, designed in a suitable way in order to simulate strictly the biological environment. This research includes biomarker discovery and damage repair/prevention, and an interdisciplinary approach is required in order to expand the applicability of chemical research. In the Action several groups active in radiation effects are also present therefore the WG addresses also the environmental toxicity due to these phenomena.

The main objectives are:

- elucidation of fundamental DNA damage pathways, such as electron-transfer and oxidative processes, in specific sequences, such as guanine-rich regions (G-quadruplex) present in telomeres;
- expanding knowledge on the cyclopurine lesions, involving also the protocols for recognition of the four diastereoisomers of guanine and adenine 2'-deoxyribo- and ribo-nucleotides;
- expanding knowledge on the structural deformation caused to DNA supramolecular arrangement by the lesions.
- enzymatic repair recognition and activity, with the combined efforts of suitably modified synthetic; oligonucleotide preparation, analytical protocols for digestion/treatment of the biological samples according to available models in the participating group, protocols of molecular biology for the repair enzyme functions. The repair by NER and BER will be assayed using different approaches provided by the participants to this WG. The various lesions will be considered also in a competitive way, and also the enzyme structures in the active site will be considered;
- combined reactivity of DNA radicals with proteins, taking into account the presence of histone proteins in the nucleosomes;
- Mechanisms of formation of covalent DNA-DNA interstrand adduct (cross-links) by one electron oxidation;
- competitive and integrated DNA free radical reactivity, with biomimetic models of liposomes including oligonucleotide or DNA sequences;
- study of radiation effects on the genome integrity, reinforcing this research field with the elucidation of basic mechanistic steps, useful also for preventive or therapeutical applications;
- biomarkers of DNA damage to be expanded in health and diseases.

This WG will be integrated with the activities of WG4 for the chemical synthesis of molecular libraries and WG3 for the liposome technology.

WG3 – Models of membrane lipid transformations due to free radicals, therefore involving mainly the unsaturated moieties, with insights on the lipid remodelling and signalling under stress conditions, both in models and in biological systems. The role of repair and defences will be included. This WG will be dedicated to integrate the reactivity of membrane models, represented by liposome vescicles, with the information related to the membrane structure and functioning in living systems, from cells to organisms. Therefore, this WG gather expertise from groups in chemical research and biomimetic chemistry in combination with biologically oriented groups and medical research. The main objectives of the WG will be:

- study of competitive pathways involving unsaturated lipids, such as peroxidation, and isomerisation, using liposomes with different compositions, to simulate the membrane variability and to envisage the main factors influencing these transformations, to be correlated with the biological environment. Extension of this part will be the effects of radiation damages to simulate the effects of environmental or therapeutical expositions;
- integrated protocols for the evaluation of free radical modifications, developing analytical approaches in models to be extended to biological samples for evaluation of lipid peroxides, isoprostanes and trans lipids. The extension to biological samples will be also considered for biomarker development.
- membrane stress response in different conditions, providing studies in cell cultures or organisms, that can be analysed on the basis of the model studies. This part will include the effects of lipid remodelling consequent to environmental conditions (glucose or hormone stimulation) as well as drugs or dietary factors;
- effects of antioxidant strategies in models and biological systems, including dietary elements and synthetic antioxidants;
- use of liposome technology for combined chemical reactivity such as proposed in the DNA and protein damages and activation.

This WG will be integrated with the activities of WG4 for synthetic approaches and WG2 for the correlation DNA-lipid damages.

WG4 – Synthetic approaches inspired by the naturally occurring processes, where catalysis and stereoselectivity are the most relevant features. Chemical synthesis of molecular libraries and application to bio-materials are also included. This WG will gather the synthetic expertise of the Action, connecting chemical reactivity with the biological processes. Synthetic chemists develop new processes addressing eco-compatibility and sustainability requirements on the basis of biological transformations. Also new bioactive molecules are inspired by the natural biological activity. The main objectives of the WG will be:

- study of organocatalytic reactions such as single electron transfer processes, enantioselective free radical reactions, which are inspired by naturally occurring processes: in this task the modification of natural products and tuning of biological activity will be considered;
- synthetic antioxidants and use in radical chain processes, in view of possible use either in biological and technological processes;
- molecular libraries by synthetic procedures, which mimic what occurs in the biological environment, including biomarker libraries (for example, oxidative transformations involving polyunsaturated fatty acids, cyclonucleosides, trans cholesteryl esters, isoprostanes, phytoprostanes, neuroprostanes, etc…);
- development of new materials (block and graft polymers) and use of polymerization for the synthesis of polymer-bioconjugates and organic-inorganic hybrid materials.

This WG will be integrated with the activities of WG1 for providing synthetic compounds to develop valid models and with WG2 and WG3 regarding the synthesis of active metabolites for the in depth study of their biological profile and as analytical standards.

E. ORGANISATION

E.1 Coordination and organisation

The Action will follow the 'Rules and Procedures for Implementing COST Actions' and will be flexible so that new members can be integrated during the implementation of the Action. The Management Committee (MC) will be responsible for coordinating the Action and in particular for organisation of research topics, preparation of the Action budget and work plan (workshops Training Schools, dissemination of results, STSM applications through the STSM panel) and for reports to the COST Office and the CMST Domain Committee. In the first MC meeting the Chair, Vice-Chair and WG Leaders will be appointed. The Chair will collect the necessary documents for the preparation of reports, such as the list of joint publications and current projects. Each WG will be headed by a Leader and Vice-Leader responsible for specific tasks of the respective WG, while the WG Leaders together with the Action Chair and the Vice-Chair will form the Steering Committee in charge of the preparation and monitoring of the Action activities. All major aspects of the Action, especially its research highlights, will be disseminated by means of a website, which will be established at the earliest stage of the Action's realisation and will be linked to the corresponding websites of the member teams, and other relevant sites reporting on topics related to the Action's themes. The participating groups intend to use the framework offered by COST to implement their new research, either theoretically or methodologically, and expand the topics toward a more interdisciplinary vision. This is for developing a multidisciplinary environment where young investigators can find inspiration for their careers, not only in an academic context but also in entrepreneurial initiatives. The younger participants from the research groups will be stimulated to act (through a small Committee to be set up at the first meeting) so that their ideas and contributions to research are made more visible. They will also be encouraged to participate in specific programs or meetings dedicated to Young Investigators (such as the European Young Investigator Conference (EYIC) that in 2011 reached its 5th edition). They will also be involved in specific parts of the website and their STSM reports will be displayed therein.

The MC will summarize the milestones of the Action every six months, in terms of fulfilment of the objectives that have been cited above for the WG activities and also for the Action as a whole. Exchange and sharing of information, methodologies and protocols, as well as technical tools and common publications are the milestones of the Action that will ensure coordination of the research groups. The mid-term evaluation will allow some activities to be re-enforced or possibly re-oriented, in order to maintain the focus of the overall scientific programme. The following parameters will be measured:

- composition of WGs (increment or variation of the group number), number of exchange visits, access to instrumentation, type of training activities, on-going common projects, sharing of information, etc;
- participation in seminars/conferences/meetings and activation of new initiatives also responding to calls in the European Framework Programme, i.e, the Horizon 2020;
- involvement of other scientific organisations, common initiatives;
- initiatives for early-stage researchers;
- number of publications;
- impact on societal and industrial aspects, spin-off initiatives, patents, etc.

The impact of the Action in the context of European research will be estimated through these indicators, and followed constantly in semester periods, where reports of activities will also be produced and will be disseminated through the website and participation in other meetings and conferences.

E.2 Working Groups

The expertise present in the WGs will be shared throughout the duration of the Action (see Section D). The groups will provide an array of expertise dealing with:

- free radical chemistry and reactivity, including photochemistry and studies on the effects of radiation (radiolysis and photolysis apparatus);
- chemical synthesis and organo-catalysis;

- product studies and tools for identification of simple to complex molecular structures (analytical equipments: NMR, UV-vis, FT-IR, GC, GC/MS, LC/MS, HPLC);
- modelling in biomimetic chemistry including liposome technology and oligonucleotide synthesis;
- biomarker measurement in biological samples;
- antioxidants and free radical scavenging activity from biomolecules;
- biochemistry, molecular biology and related methodologies;
- protein activities and related methodologies;
- omics technologies (mapping by transcriptomics, genomics, lipidomics and metabolomics);
- computational facilities for the study of free radical intermediates;
- pharmacology and related methodologies
- gene responses and activation pathways;
- dietary experiments and whole-body phenotyping of energy metabolism and glucose homeostasis in biological systems;
- nutritional responses, including serum and tissue multiple biomarker analysis;

Each of the four WGs will have a Leader, responsible for documents and reports. The MC will keep the unity of the Action and coordinate the overlapping of the four WGs. The MC will also organize contacts within the disciplines, which are expected to improve during the course of the Action. In fact, supplementary expertise necessary for a better accomplishment of the Action's objectives will be discussed at the joint meetings and among WG Leaders and the Chair of the Action. Besides well-known experts researchers will be involved who are new in the field, thus opening up new ideas and spreading research in these interdisciplinary themes to strengthen ERA development. According to the mutual interests and progress of the Action leading researchers will also be invited from non-COST countries.

E.3 Liaison and interaction with other research programmes

Potential members of the Action have significant experience in National and International programs, including several European projects, such as Marie Curie Training Networks and Collaborative Networks on their themes. Potential participants in the Action fostered its conception because they realized that an interdisciplinary network working on biomimetic radical chemistry could really integrate with the other research programmes in the international scenario. As mentioned above, other COST Actions are addressing biologically active compounds or processes but not the field of free radical chemistry. This Action will interact with the other initiatives on chemical reactivity and biological activity in order to strengthen the interdisciplinarity of research.

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 intends to realize an interdisciplinary platform that will be appropriate for the training of early-stage researchers (ESRs), using the STSM program and Training Schools that will be organized to trigger research collaborations and brainstorming.

F. TIMETABLE

A four-year period is necessary for gaining an effective cross-fertilization between the various WGs and to address the scientific challenges. The timetable divided into one-year intervals is illustrated below. During the 1st MC meeting the MC will plan the calendar of meetings for the following year. The meetings will be organized with the involvement of young researchers, in order to promote scientific exchange and cross-fertilization between the research groups in addition to monitoring the progress of the COST Action.

Timetable of the COST Action on "Biomimetic Radical Chemistry"

Activity	***Year 1***				***Year 2***				***Year 3***				***Year 4***			
	1/4	1/2	3/4	1	1/4	1/2	3/4	1	1/4	1/2	3/4	1	1/4	1/2	3/4	1
Kick-off MC meeting	X															
Establish WGs 1-4	X															
MC meeting		X				X			X					X		
Annual Meeting		X				X			X					X		
WG meeting				X				X				X				
Training School							X								X	
Workshop												X				
STSM		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Final Conference																X

G. ECONOMIC DIMENSION

The following COST countries have actively participated in the preparation of the Action or otherwise indicated their interest: AT, BG, CH, CZ, DE, DK, EL, ES, FR, HR, HU, IL, IT, LT, NL, PL, PT, SE, TR, UK. On the basis of national estimates, the economic dimension of the activities to be carried out under the Action has been estimated at 80 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 targeted audience of the Action are other researchers in the interdisciplinary field of biomimetic processes, involved either in academic or private research bodies, and also companies involved in development of novel chemical processes either for materials or health. The website of the Action will advertise the benefits of the scientific work carried out by the Action participants, which is intended to attract interest from the targeted audience.

H.2 What?

The dissemination plan will be visible on the website of the Action. This is nowadays well accepted as the most efficient way to disperse knowledge and progress in an accessible fashion. One part of the website, specifically concerning on-going research, will only be accessible to the participants. Effective dissemination is very important for the Action, because scientific interaction is the most relevant issue for improving communication among disciplines. The dissemination will be oriented towards several aspects, summarized below:

- METHODOLOGY: the Action will develop specific instruments to improve the understanding and the set-up of the basic methodologies used in different laboratories. This will also respond to a need, generally felt in the field of chemical and biological research, to have protocols that are diffused among the scientific community. An integrative approach among different disciplines is still missing, as previously specified. In this direction, the Action is also ready to increase interest from its targeted audience with the organization of training schools, especially for young investigators.
- RESULTS: scientific results will be disseminated among a large community of researchers, which extend from chemists to biologists in the activities of the participating researchers, to deliver seminars and conferences, as well by publications. Invitation of speakers/observers from outside Europe to the Action annual meetings will be part of the dissemination plan, in order to give an international context to the Action's activities and results. The website will also show the results; mostly underlining those derived from a strong collaborative element, and will highlight the results that can be developed toward industrial applications.
- PROJECTS: from the activity of the Action it is expected that projects will be developed that can attract funding from European calls in the Framework Programme. The dissemination of the ideas and projects delivered by the Action will be put on the website and there will be space for project description and requests for partnerships. The dissemination will concern any other scientific initiative born in each participating group and the website of the Action will provide links to the respective internet resources.

- SOCIETY: an important aspect of the dissemination will concern society and the consequences of the scientific discoveries made through the Action's activities. Due to the disciplines and subjects of the researches, many connections with health and technological applications can be found and some initiatives will be taken in order to spread the scientific content of the Action in the direction of the societal needs. It is also self-evident that communication with non-experts has to be improved, and therefore particular attention will be devoted to science communication tools. The young researchers will be encouraged to contribute to this aspect, also in connection with initiatives at the European level dedicated to the science divulgation.

H.3 How?

Dissemination methods will be used for:

- The Action participants and scientific audience; dissemination of the results will be during the course of the Action through a 6-months report on the achievements of the Actions, in terms of the results of exchange and sharing of information, visits and technical tools. This report will be available on the website of the Action. The STSM fellows will be encouraged to prepare a presentation of their achievements during the STSM period, for dissemination on the website. Also, each meeting organized by the Action will be disseminated through the website, including where possible PowerPoint presentations from the speakers.
- The young generation of scientists; the Action commits itself to organize one training school/year for young researchers during the 4-years period, with the aim of disseminating the trans-disciplinarity of the approach for the study of biomimetic radical chemistry, as well as the methodologies that involves the design of biomimetic models and the set-up of analytical/molecular/biological tools for the evaluation of free radical reactivity.
- Companies and other industrial entities; the dissemination of the results that can have application to industrial processes. Therefore the Action will prepare reports on several important aspects at the molecular level, for example protocols applicable in technological developments or biological applications, such as biomarker development.