3rd Annual Scientific Meeting

&

4th *Management Committee Meeting*

May 11-13, 2015
Royal Olympic Hotel, Athens, Greece

Chair of the Action: Chryssostomos Chatgilialoglu

Organization Committe: *Chryssostomos Chatgilialoglu*
Vasso Zatta

GENERAL INFORMATION

The conference takes place on the beautiful city of Greece, in Athens. The accommodations and the lecture facilities are situated in the Royal Olympic Hotel, closed to Plaka area. Information about the hotel you can find at the following website www.royalolympichotel.com.

Athens is a walking city. So, get your comfortable shoes and go for it. The most significant part of the walk, is the one from "Akropoli" subway station on the Red metro line, ending up at "Monastiraki" station. It's an arched walk on three pedestrian roads: "Dionyssiou Areopagitou", "Apostolou Pavlou" and "Adrianou". It ends up at Thission area which has many cafes and awesome views of the Acropolis and at Monastiraki Square with its vibrant shopping areas and many shops. Plaka with its neoclassical buildings is aside.

Athens has also several important museums. We suggest:

The New Acropolis Museum, is an archaeological museum focused on the findings of the archaeological site of the Acropolis of Athens. The museum was built to house every artifact found on the rock and on its feet, from the Greek Bronze Age to Roman and Byzantine Greece. It also lies on the archaeological site of Makrygianni and the ruins of a part of Roman and early Byzantine Athens. **The National Archaeological Museum**, the largest archaeological museum in the country, and one of the most important internationally, as it contains a vast collection of antiquities; its artifacts cover a period of more than 5,000 years, from late Neolithic Age to Roman Greece, **The Benaki Museum** with its several branches for each of its collections including ancient, Byzantine, Ottoman-era, Chinese art and beyond, **The Byzantine and Christian Museum**, one of the most important museums of Byzantine art, **The Numismatic Museum**, housing a major collection of ancient and modern coins.

SOCIAL ACTIVITIES PROGRAM

The registration desk will be open:

- on Monday, May 11th, 2015 from 09:00 to 18:30
- on Tuesday from 09:00 to 16:00
- on Wednesday May 13th from 09:00 to 13:00

Monday May 11th, 2015

➢ **Festive Dinner**

It takes place at a traditional tavern in the Plaka area. We'll enjoy local foods, desserts and Greek music. *Departure from the hotel at 20:00*

Wednesday, May 13th, 2015

Athens is a walking city. So we can wear comfortable shoes and begin to explore the surroundings.

➢ **Walk to Acropolis** or **Visit at the Acropolis Museum**
Departure from the hotel at 16:30

➢ **Official Conference Dinner**
We will cross the distance which covers the entire area from where our hotel is located, up to Thission.
Departure from the hotel at 19:30

SCIENTIFIC PROGRAM

Monday, May 11th 2015: Royal Olympic Hotel, Athens

09:00-10:00	**Registration** **Welcome, Action Chair Cryssostomos Chatgilialoglu**
Session 1: Chair Chryssostomos Chatgilialoglu	
10:00-11:00	Wolfgang Buckel *Radicals involved in flavin-based electron bifurcation*
11:00-11:30	**COFFEE BREAK**
11:30-12:00	Bernard T. Golding *Prebiotic radical chemistry connecting a Ribonucleotide to a Deoxyribonucleotide*
12:00-12:30	Bernie Creaven *Dual-functioning mimetics, possible new therapeutics?*
12:30-13:00	Jozsef Kaizer *Oxoiron(IV)-mediated Baeyer-Villiger oxidation of cyclic ketones generated by dioxygen with cooxidation of aldehydes*
13:00-15:00	**LUNCH**
Session 2: Chair Philippe Renaud	
15:00-16:00	Ioulia Smonou *Exploring the potential of bioreductions for the synthesis of high-added value compounds*
16:00-16:30	Anna Croft *Time and Motion - Using the Virtual Lab to unpick the subtleties of the mechanism of QueE*
16:30-17:00	**COFFEE BREAK**
17:00-17:30	Krzysztof Bobrowski *Stabilization of monomeric sulfur radical cations in methionine-containing peptides with oligoprolines backbones*
17:30-18:00	Gabriela Ionita *Processes involving globular proteins investigated by EPR spectroscopy*
18:00-18:30	Ioannis N. Lykakis *Green organic transformations catalyzed by supported gold nanoparticles*
18:30-19:00	Massimo Bietti *Reactivity and Selectivity Patterns in Hydrogen Atom Transfer from Amino Acid C-H Bonds to the Cumyloxyl Radical*
20:00 -	**FESTIVE DINNER AT THE PLAKA AREA**

Tuesday, May 12th 2015: Royal Olympic Hotel, Athens

Session 3: Chair Urlich Jahn	
09:00-10:00	Michael Davies *Investigation of mechanisms of action of nitroxide radicals as protective agents in human disease*

10:00-10:30	Aldo Tomasi *Proteomics in the clinic: the search for biomarkers*
10:30-11:00	Shlomo Sasson *The search of endogenous activators of PPARδ: from cell-based studies of oxidized metabolites of fatty acids to computer modeling and in silico analysis*
11:00-11:30	**COFFEE BREAK**
11:30-12:00	Elena E. Pohl *UCP4: compete or not compete?*
12:00-12:30	Tomris Ozben *Epoxomicin sensitizes resistant osteosarcoma cells to TRAIL induced apoptosis*
12:30-13:00	Itziar Tueros *Can lipidomics assist food product innovation? Contribution to personalised nutrition*
13:00-15:00	**LUNCH**
Session 4: Chair Bischoff Rainer	
15:00-15:30	Nataliya Rohr-Udilova *The role of radical stress in liver disease*
15:30-16:00	Kyriakos E. Kypreos *Novel functions of apolipoprotein A-I beyond coronary heart disease and atherosclerosis*
16:00-16:30	Carla Ferreri *Membrane Lipidomics for Personalized health*
16:30-17:00	**COFFEE BREAK**
17:00-19:30	4nd Management Committee Meeting
	FREE EVENING

Wednesday, May 13th 2015: Royal Olympic Hotel, Athens

Session 5: Chair Andrew Kellett	
9:00-9:30	Jean-Luc Ravanat *DNA and repair, an overview*
9:30-10:00	Alexandros Georgakilas *Applying bioinformatics for the understanding of the radiation response and repair mechanisms*
10:00-10:30	Andrea Peluso *Hole transfer in DNA: thermodynamic and kinetic aspects*
10:30-11:00	Michael Terzidis *Radiation-induced purine lesion formation in DNA*
11:00-11:30	**COFFEE BREAK**
11:30-12:30	Yuan Liu *Oxidative DNA damage repair and repeat sequence instability*
12:30-13:00	Tsvetan G. Gantchev *Interaction of hydrated electrons, e_{aq}^- with cisplatinum intra- and inter-strand cross-linked DNA studied by molecular modeling and molecular dynamics*
13:00-15:00	**LUNCH**
Session 6	
15:00-16:30	Working Groups Meetings
16:30	**Walk to the Acropolis or Visit at the Acropolis Museum**
20:30	**OFFICIAL CONFERENCE DINNER**


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Abstracts

Formation of Radicals by Flavin-Based Electron Bifurcation

Wolfgang Buckel

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Flavin-based electron bifurcation has been recognized as a novel strategy of energy coupling in strict anaerobic bacteria and methanogenic archaea [1]. In butyric acid forming Clostridia an electron transferring flavoprotein (Etf) and butyryl-CoA dehydrogenase (Bcd) catalyze the bifurcation of the two electrons of NADH (E' = −320 mV); one goes exergonically to the high potential crotonyl-CoA (E' = −10 mV) and the other endergonically to the low potential ferredoxin (E' = −500 mV). Ferredoxin, the 'energy rich' electron carrier of anaerobes, can also be reduced by bifurcation of NADH to the higher potential caffeyl-CoA or pyruvate, NADPH to NAD^+, H_2 to NAD^+ or heterodisulfide (in methanogens), and formate to NAD^+. Some of these systems are reversible, e.g. in *Thermotoga* NADH and reduced ferredoxin confurcate to 2 H_2. The crystal structures of Etf and Bcd from *Acidaminococcus fermentans* (Negativicutes of Clostridia) gave clues to the mechanism of electron bifurcation [2]. The heterodimeric EtfAB contains α-FAD in subunit α and β-FAD in subunit β. β-FAD is the bifurcating cofactor, which accepts the hydride of NADH. α-FAD is able to approach β-$FADH^-$ and takes up one electron yielding a stable anionic semiquinone, α-$FAD^{\bullet -}$, which donates this electron further to δ-FAD of Bcd. The remaining non-stabilized neutral semiquinone, β-$FADH^\bullet$ immediately reduces ferredoxin. Repetition of this process affords a second reduced ferredoxin and δ-$FADH^-$ of Bcd that converts crotonyl-CoA to butyryl-CoA. Under aerobic conditions, Etf and Bcd act as NADH oxidase, but only the presence of catalytic amounts of crotonyl-CoA or butyryl-CoA. Thereby oxygen replaces ferredoxin as acceptor and is reduced to superoxide, whereas a second molecule of oxygen oxidizes butyryl-CoA to crotonyl-CoA and H_2O_2 is formed. Hence, under air electron bifurcation can be regarded as an Achilles' heel of anaerobes.

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Prebiotic Radical Chemistry Connecting a Ribonucleotide to a Deoxyribonucleotide

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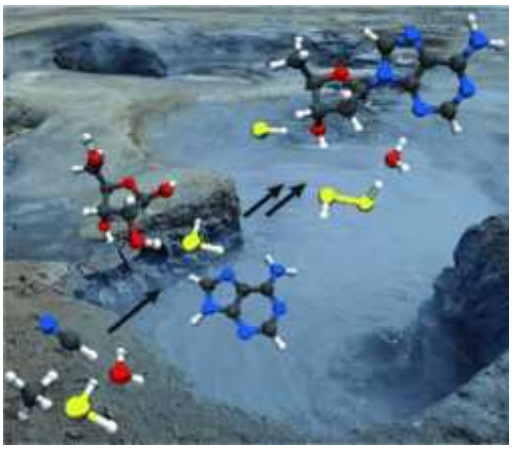
Most discussions of life's origin focus on heterolytic chemistry even though today many radical processes in biology have been recognised [1]. This study takes today's extant biology (ribonucleotide reductase mechanism) and extrapolates back, attempting to identify prebiotic radical chemistry, the possibility of which has been explored using computational chemistry.

Model studies of prebiotic chemistry have revealed compelling routes for the formation of the building blocks of proteins and RNA, but not DNA. Today, deoxynucleotides required for the construction of DNA are produced by reduction of nucleotides catalysed by ribonucleotide reductases, which are radical enzymes. This study considers potential non-enzymatic routes via intermediate radicals for the ancient formation of deoxynucleotides. In this context, several mechanisms for ribonucleotide reduction, in a putative H_2S/HSC environment, are characterized using computational chemistry. A bio-inspired mechanistic cycle involving a keto intermediate and HSSH production is found to be potentially viable. An alternative pathway, proceeding through an enol intermediate is found to exhibit similar energetic requirements. Non-cyclical pathways, in which HSSC is generated in the final step instead of HSC, show a markedly increased thermodynamic driving force (~ 70 kJ mol^{-1}) and thus warrant serious consideration in the context of the prebiotic ribonucleotide reduction.

It is hoped that this study [cf. ref. 2] will inspire the development of experimental model systems to validate the proposed role for H_2S in the prebiotic formation of deoxyribonucleotides.



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Copper(II) and Manganese(II) Biomimetics: A New Therapeutic Approach?

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Human metabolism generates free radicals and other reactive species such as superoxide radical $O_2^{\bullet-}$, hydroxyl radical $OH^{\bullet}$ and hydrogen peroxide H_2O_2. SOD and CAT enzymes regulate oxidative stress through the dismutation of these damaging species to less harmful compounds. When oxidative stress is excessive and the enzyme defence is overwhelmed, ROS induce cell damage through attack of lipid and protein structures which can lead to disease. Copper(II) complexes with have shown anti-candida[1,2], antibacterial activity[3] and cytoxicity[4,5] on selected cancer cell lines. Previous chemical assays have shown these copper(II) & similar manganese(II) complexes have exhibited superoxide, SOD, and catalase, CAT, mimetic activity[5,6,7,8,9]. Inflammatory conditions such as Alzheimer's, arthritis and tumours are characterised by high levels of ROS, therefore these copper & manganese complexes could provide a novel therapeutic approach as low molecular weight biomimetics. A series of copper(II) and manganese complexes containing N_2O_2 donor ligands have been synthesised. *Saccharomyces cerevisiae* has been used previously as a eukaryotic model to determine the SOD & CAT activity of metal complexes with good results[7,10,11]. In this research, the model has been optimised for less soluble complexes. Using the BY4741 wild type strain under oxidative stress, this assay has been developed to evaluate the SOD and CAT mimetic activity of these complexes and their protective effect, if any, determined.

References

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Oxoiron(IV)-mediated Baeyer-Villiger oxidation of cyclic ketones generated by dioxygen with cooxidation of aldehydes

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The Baeyer-Villiger oxidation of ketones to lactones or esters is one of the main reaction in organic chemistry owing to very wide range of possible applications, for example in the production of polymers, pharmaceuticals and herbicids[1]. The most important industrial process for the production of -caprolactone is the oxidation of cyclohexanone with *m*-chloroperbenzoic acid [2].
Over the past four decades transition metal complexes of a variety of ligand systems have been reported as active catalysts for the catalytic oxygen transfer reaction [3,4], and their catalytic cycles often involve oxoiron(IV) intermediates as oxidants [5,6].
A novel catalytic method for the Baeyer-Villiger oxidation of cyclohexanone derivatives (cyclohexanone, 2-methyl-cyclohexanone, 3-methyl-cyclohexanone, 4-methyl-cyclohexanone and 4-*tert*-butyl-cyclohexanone) has been investigated, with non-heme iron(II) complex ($[Fe^{II}(CH_3CN)(N4Py)](ClO_4)_2$ N4Py = *N,N*-bis(2-pyridylmethyl)-N-bis(2-pyridyl)methyl-amine) as catalyst, aldehydes (isobutyraldehyde, benzaldehyde, 4-methylbenzaldehyde and 4-chlorobenzaldehyde) as oxygen acceptors and dioxygen as oxidant. The experimental results clearly indicated the formation of a high-valent metal-oxo intermediate (Fe^{IV}=O), and its role in the oxidation process. Reactions were monitored and products were determined using a gas chromatograph.

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Exploring the potential of bioreductions for the synthesis of high-added value compounds

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Our continuing interest on stereoselective ketoreductase-catalyzed reductions of various carbonyl compounds has resulted in the synthesis of optically active keto alcohols, diols and hydroxy esters, useful chiral synthons for the synthesis of high added value compounds. Some ketoreductases have shown interesting reactivity toward the carbon-carbon double bond reduction. Our latest results on the enzymatic reductions of -unsaturated carbonyl compounds will be presented. This ene-reductase activity combined with the ketoreductase activity, which can be used for the synthesis of optically pure allylic alcohols, -unsaturated hydroxy esters or other valuable chiral synthons will be discussed.

We will also present our work relating to the chemoenzymatic synthesis of chiral precursors of a large variety of β-hydroxy-δ-lactones.

Time and Motion - Using the Virtual Lab to unpick the subtleties of the mechanism of QueE

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The mechanisms of radical enzymes that contain S-adenosyl methionine as the radical-generating cofactor are only just being revealed in detail. A thorough knowledge of such mechanisms can lay the foundation for rational protein engineering, with the potential for opening up a wealth of novel biologically-accessible chemistry. More importantly, consideration of the overall dynamics of such systems, with different individual residues playing key roles at various stages of the mechanism, can uncover details of both the reaction kinetics and the overall thermodynamics of the enzyme's mechanism not apparent from 'snapshot' calculations.

We have focussed on the enzyme 7-carboxy-7-deazaguanine (CDG) synthase (QueE), which catalyses the rearrangement of 6-carboxy-5,6,7,8-tetrahydropterin (CPH4) into CDG as a key step in queosine biosynthesis. This intermediate is a precursor to a number of interesting *Streptomyces* antibiotics, and molecules with anti-viral and anti-cancer properties.[1]

We have examined a number of key dynamic processes for the mechanism of QueE, including Mg^{2+} binding and coordination, which is known to be important for activity,[2] and changes that occur on cofactor binding. Results will be presented in the context of both DFT and molecular dynamics computations, with an outlook to the future and scope for engineering the QueE reaction.

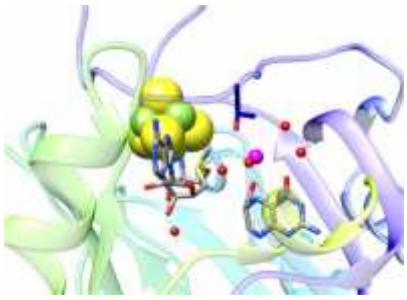

Figure 1. *The active site of the rSAM enzyme QueE, as determined by X-ray crystallography,[3] depicting both the Mg^{2+} (in pink) and substrate binding.*

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Stabilization of monomeric sulfur radical cations in methionine-containing peptides with oligoprolines backbones

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Factors goverining the ultimate course of methionine (Met) oxidation are fairly well understood. The functional groups adjacent to the sulfur center, the primary site of initial oxidation, are clearly a key to how the reaction is initiated and how it proceeds. It has turned out that stabilization of monomeric sulfur radical cations (Met>$S^{+\bullet}$) within particular and protein domains depends on geometrical and conformational properties of peptide molecules. The L-Met-$(Pro)_n$-L-Met peptides, investigated in the current work are oligopeptides that contain two Met residues located on the N and C-termini, and that are separated by a defined number (n = 0 – 4) of proline (Pro) residues. The use of such petides allows distance control between sulfur atoms located in the side chains of Met residues. The motivation to study the oxidation mechanism of these peptides is related to the previous studies of cyclic Met-Met dipeptides where the distance between sulfur atoms was controlled by optical isomerism (L/D) of the Met residues and turned out to be a key factor in the formation of intramolecular S∴S-bonded radical cations. In particular, where various competing pathways leading to stabilization of Met>$S^{+\bullet}$ are considered, it is important to know how the tendency towards formation of the transients with -type 2c-3e S∴S-bonds changes with the separation distance. This tendency can be additionally probed by the observation of other transients with -type 2c-3e S∴N and/or S∴O-bonds as well as of -(alkylthio)alkyl radicals. All of these species are formed competitively, and at some point their formation supposedly can be kinteically preferred over the formation of intramolecular S∴S-bonded radical cations. In addition, there is a key difference in the primary products when oxidation occurs by strong one-electron oxidant such as triplet of 4-carboxybenzophenone (^{3}CB*) as compared to oxidation by hydroxyl radicals ($^{\bullet}$OH). In this work, we investigated the oxidation processes starting from the transients formed either by radiationally induced $^{\bullet}$OH radicals or by photochemically induced ^{3}CB* at two pH values 1 and 5.7, respectively, in order to unravel the mechanism of oxidation of Met residues in peptides with the changing distance between them. The statistical distributions of distances separating the reactive thioether-sulfur atoms in L-Met-$(Pro)_n$-L-Met peptides were calculated by means of umbrella sampling calculations. These oligopeptides can also serve as primary models for protein molecules containing multiple Met residues where, for example, higher-order structures, may locate thioether functional groups at various distances.

Processes involving globular proteins investigated by EPR spectroscopy

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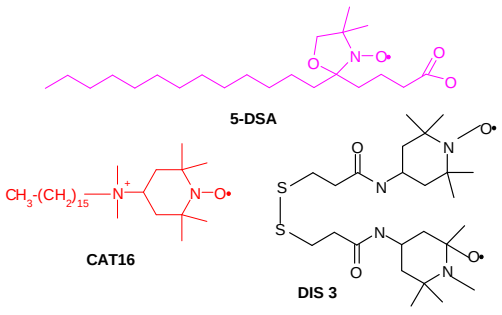
Serum albumins constitute a significant class of proteins in the circulatory system acting as carriers for a broad spectrum of compounds or assemblies such as nanoparticles. Herein we present the information revealed by electronic paramagnetic resonance (EPR) spectroscopy regarding: 1) the formation of Au nanoparticles (AuNps) in solutions of albumins and 2) the transfer of low molecular weight compounds between pluronic micelles and albumin. The EPR studies have been complemented by other physico-chemical methods.

1) Given the ability of albumin to transport various exogenous substrates and the application of AuNPs in therapeutics and diagnosis, the formation of AuNPs in the presence of albumins and probing the structural changes of the protein became important. The formation and the growth of AuNPs were monitored in buffer solution (pH 7) of bovine serum albumin (BSA) at room temperature using UV-Vis, CD, IR, Raman and EPR spectroscopy. Formation of AuNPs in buffer solution at pH 7 was not observed in the absence of protein and this proves that BSA is involved in the first step of Au(III) reduction. The size of AuNPs formed in a solution of BSA increases slowly with time. Thus, formation of nanoparticles with different shapes was observed and after one month, the size of nanoparticles was about 50 nm. Monitoring of AuNPs growth and the protective role of albumin can be proved by changes in spin probes EPR spectra (CAT16 and DIS3 – fig. 1). The EPR spectrum of CAT16 shows a two component feature in BSA solution. The ratio between two components changes during the AuNPs growth. Once the gold particles form, a third component with slow dynamic emerges in the EPR spectra of CAT16. The diradical DIS3 in an aqueous solution of BSA gives rise to a spectrum with five lines, due to spin-spin interactions. As AuNPs grow, the intensity of the lines, attributed to spin-spin interactions, decreases.



The spin probes used in the EPR studies

2) EPR spectroscopy represents a good tool to investigate the interactions in proteins /surfactant systems. Our previous studies proved the suitability of the spin probe method in investigation of the interactions between ionic surfactants and albumins. The effect of nonionic surfactants like pluronic block copolymers is less studied compared with the one induced by ionic surfactants on proteins structures. Here we present the results of an EPR study on the pluronic F127/human

serum albumin (HSA) systems using as spin probes 5-DSA and CAT16. The analyses of the EPR spectra of the spin probes used in this study led to the conclusion that spin probe locations in the protein/F127 system depends on the polymer phase. Thus, at temperatures below critical micellar temperature (*cmt*), the spin probes are located on the protein surface, while at temperatures above *cmt,* the spin probes are transferred to the polymer micelles. CD spectra, rheological measurements and micro DSC traces complemented the EPR study.

Reactivity and Selectivity Patterns in Hydrogen Atom Transfer from Amino Acid CJH Bonds to the Cumyloxyl Radical

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Hydrogen atom transfer (HAT) from peptides and proteins to free radicals are of fundamental importance and play an essential role in a variety of biochemical processes. HAT can take place from the peptide backbone and/or the side-chains of amino acid residues and, depending on the abstraction site and on the reactions that follow the initial HAT step, a variety of structural and functional alterations can occur. Along these lines, a detailed understanding of the factors that govern the selectivity of HAT reactions from peptides and proteins appears to be of great importance and accordingly, considerable efforts have been devoted to the study of this aspect. However, due to the complexity of protein substrates and to the multitude of reactive sites, simpler model substrates such as oligopeptides and amino acids have been often employed for this purpose, and, most importantly, the available results have been mostly obtained through product and computational studies, whereas limited direct kinetic information on these reactions is presently available.

Along these lines, in order to obtain direct kinetic information on the reactivity and selectivity patterns observed in these reactions, we have carried out a detailed time-resolved kinetic study on the reactions of the cumyloxyl radical (CumO$^{\bullet}$) with a series of *N*-*tert*-butoxycarbonyl (*N*-Boc) protected proteinogenic and non-proteinogenic amino acids bearing aliphatic side-chains. With all the amino acids investigated HAT to CumO$^{\bullet}$ exclusively occurs from the C–H bonds.

The measured rate constants are discussed showing the important role of structural effects and polar effects on these reactions, and have been compared with the relative rates obtained previously for the corresponding reactions of different hydrogen abstracting species (Cl$^{\bullet}$, Br$^{\bullet}$, $^{\bullet}$OH and dioxiranes) providing a general framework for the description of the factors that govern reactivity and selectivity patterns in HAT reactions from amino acid C–H bonds.

Green organic transformations catalyzed by supported gold nanoparticles

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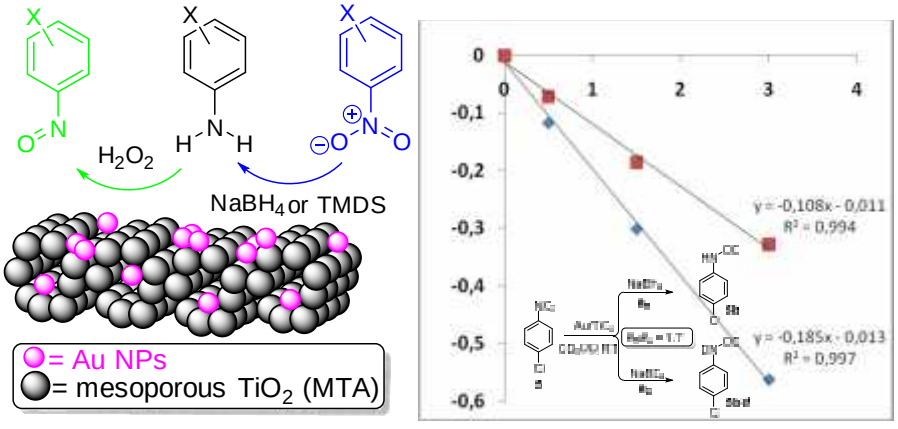
For catalytic processes, an attractive approach is the use of a solid, recyclable catalyst and environmentally friendly reagents. The use of supported catalysts on a well ordered metal oxide surface for heterogeneous catalysis offers several advantages on the catalyst reusability and the regio- and chemo-selectivity of the reaction process. For this reason, herein we outline a research study aimed to gain insight into the catalytic applications of new AuNPs heterogeneous systems,[1] in order to afford new chemical transformations, based on the combination of AuNPs and the surface of the support properties.[1,2] Such materials are expected to exhibit superior catalytic activities compare to the commercial ones. For this reason: a) catalytic reduction of nitro-aromatic compounds using $NaBH_4$ and 1,1,3,3-tetramethyldisiloxane (TMDS) as hydrogen donor molecules, b) chemoselective reduction of nitroalkenes into the corresponding oximes or nitroalkanes using TMDS and AuNPs or AgNPs-supported catalysts, and c) selective oxidation of aryl amines into nitrosoarenes with H_2O_2 catalyzed by Au/TiO_2, were studied.[3] In addition, a detail mechanistic study based on kinetic isotope effects, on Hammett type-kinetics and product analysis was proposed.[4] The findings of this work are expected to provide a tool of knowledge in order to understand the role of AuNPs activities in fundamental chemistry reactions.



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Radical inhibition of a radical enzyme

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Myeloperoxidase (MPO) is a member of the peroxidase superfamily of heme enzymes. It is released by activated neutrophils, monocytes and some macrophages at sites of inflammation, and is postulated to contribute to oxidation damage to proteins and other biological targets. The enzyme has multiple catalytic cycles and can generate a wide range of oxidants, including both radical and non-radical (2-electron) species. In its halogenation cycle, MPO catalyses the oxidation of halide and thiocyanate ions (SCN^-) to the powerful oxidants hypochlorous acid (HOCl) and hypothiocyanous acid (HOSCN) using H_2O_2 as a co-factor, via the intermediacy of a Fe^{4+}=O heme$^{+\cdot}$ (Compound I) species generated from the resting Fe^{3+} species. The Compound I intermediate can also oxidise a wide range of phenols and indole compounds and other electron-rich materials (e.g. ascorbic acid, urate, NO_2^-) to radicals, via sequential single electron reactions involving the intermediacy of a Fe^{4+}=O, Compound II species. These enzymatic cycles are of major significance in the human immune response to invading pathogens, such as bacteria. Whilst this intentional oxidant formation is of major importance in preventing infections, excessive, mistimed or misplaced generation of these oxidants can cause host tissue damage and for this reason elevated levels of myeloperoxidase (MPO) are associated with multiple inflammatory human pathologies, including cardiovascular disease, rheumatoid arthritis, asthma, cystic fibrosis, some cancers and neurodegenerative conditions including Parkinson's and Alzheimers diseases. As a consequence there is considerable academic and pharmacological interest in the inhibition of the oxidant-generating capacity of MPO.

Recent studies have demonstrated that multiple agents can modulate both the halogenating and peroxidase activity of MPO, including the use of substrates that enhance enzyme turnover through the peroxidase cycle. These include both (poly)phenols that are converted to phenoxyl radicals, and nitroxide radicals that are oxidized to oxo-ammonium cations. Inhibition by nitroxides has been investigated in detail, with the extent of inhibition being critically dependent on the structure of the nitroxide radical. Subsequent testing of one of these nitroxides in an animal model of cardiovascular disease, shows significant protective effects, though not all of these may arise from MPO inhibition.

Proteomics in the clinic: the search for biomarkers

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In this first contribution of our research group to COST action CM1201 we like to introduce our laboratory work in the development of diagnostic biomarkers, the main problems we meet, and the techniques applied. Biomarkers are molecules that exist naturally in the body that can help to predict or reflect the presence of a disease, the relapse risk of the disease, and/or response to treatment. The search for new biomarkers is intense, however the success is scarce. An ideal target molecule should be measurable in a non-invasive sample source such as blood or urine, present high sensitivity and specificity, should be amenable to reliable, robust, and reproducible measurement techniques, and should be validated across a broad range of populations. Systematic study of the set of proteins produced (expressed) by an organism, tissue or cell, and the changes in protein expression patterns in different environments and conditions constitutes the object of proteomics studies Recent developments in mass spectrometry made the study of complex molecules possible; particularly useful were advances in protein separation techniques, triple quadrupole, time of flight and orbitrap mass analyser. Examples of clinical proteomics studies on-going in our lab will be given, in particular referring to renal disease patients.

The search for endogenous activators of PPARδ: From cell-based studies of fatty acids and their metabolites to computer modelling and in silico analysis

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Peroxisome proliferator-activated receptor-δ (PPARδ) is a ligand activated receptor that dimerizes with another nuclear receptor of the retinoic acid receptor (RXR) family. The formed dimers interact with other co-activator proteins and form active complexes that bind with PPAR-response elements and promote transcription of genes involved in lipid metabolism. It appears that various natural fatty acids and their metabolites serve as endogenous activators of PPARδ; however, there is no consensus in the literature on the nature of the prime activators of the receptor. In vitro and cell based assays of PPARδ activation by fatty acids and their derivatives produce often conflicting results. The search for synthetic and selective PPARδ agonists, which may be pharmacologically useful, is intense. Current rational modelling used to obtain such compounds relies mostly on crystal structures of synthetic PPARδ ligands with the recombinant ligand binding domain of (LBD) the receptor. We have developed an original computational prediction model for ligand binding with PPARδ LBD to calculate binding probabilities of 82 different natural and synthetic compounds from the literature that were independently tested in cell-free and cell-based assays for their capacity to bind or activate PPARδ*. The model was built based on EC_{50} data of 16 ligands with available crystal structures and validated by calculating binding probabilities of 82 different natural and synthetic compounds from the literature that were independently tested in cell-free and cell-based assays, leading to prediction accuracy of between 70% and 93% (depending on ligand type). This new computational tool could therefore be used in the search for natural and synthetic agonists of the receptor.

UCP4: competitor or not?

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Stochastic optical reconstruction microscopy (STORM) is a single-molecule superresolution imaging technique, which has allowed us to visualize mitochondrial uncoupling protein 4 (UCP4) in primary mice neurons, and to study its relative position to other mitochondrial proteins porin, cytochrome c oxidase and F_0F_1-ATP synthase. Uncoupling proteins (UCP2-UCP4) are members of the mitochondrial anion carrier protein superfamily. They have been shown to transport protons in artificial systems that support their putative function in ROS regulation and cell metabolism[1, 2]. In this study[3], we have proved the hypothesis that UCP4 and ATP-synthase are localized in different mitochondrial compartments. It rules out any competition between them for the proton gradient that may result in serious damage to ATP-dependent cell functions. Our results reveal that different levels of UCP4 expression exist in different mitochondria and that both UCP4 and ATP synthase are spatially separated within the mitochondrial inner membrane. Being able to differentiate between the localization of functionally connected proteins in this remarkably narrow space allows us to gain new insights in regulative mechanisms and signaling pathways *in vivo*. The data suggest that UCP4 may selectively govern reactive oxygen production instead of restricting ATP production.

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Epoxomicin sensitizes osteosarcoma cells to TRAIL resistance on apoptosis

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Background: Osteosarcoma (OS) is the second most common primary malign neoplasm of bone after multiple myeloma. OS is a high-grade neoplasm and develops mainly in the second decade with 60% of the patients under the age of 25. The estimated incidence of OS is 4-5 cases per million in the population, with a peak incidence at 18 years. Despite systemic chemotherapy, OS may give rise to local recurrences and metastases. Resistance to chemotherapy is not rare and likely to occur in many of the patients. Novel therapeutic approaches are required for osteosarcoma treatment. Tumour necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL) and proteasome inhibitors (epoxomicin, MG132, bortezomib) represent a new promising approach in cancer treatment. The aim of our study is to elucidate the effects of epoxomicin alone or in combination with TRAIL in two TRAIL-resistant OS cell lines, Saos-2 and MG-63 namely.

Methods: We determined the cytotoxic effects of epoxomicin and/or TRAIL on two types of OS cells using dimethylthiazolyl 2,5 diphenyltetrazolium bromide (MTT) test and measured apoptosis markers such as pro-apoptotic Bax levels and caspase-3, -8, -9 activities. We used TUNEL assay to demonstrate apoptosis. We investigated dose and time dependent survival rates of OS cells and determined LD_{50} doses of epoxomicin and TRAIL on OS cell viability after 24, 48, and 72 hour incubations.

Results: Concurrent incubation with TRAIL and epoxomicin for 24 hour significantly increased caspase-3, caspase-8, caspase-9 activities and Bax protein levels compared to groups incubated with only TRAIL or epoxomicin treated group ($p<0.01$).

Conclusions: Our study revealed that combining TRAIL with epoxomicin enhances apoptosis and overcomes TRAIL resistance and gives promising results for OS threapy in the future.

Can lipidomics assist food product innovation? Contribution to personalised nutrition

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European Food Industry is trying to adapt consumers' needs into innovative products, bringing to market more convenient and safe products, with new formats. At the same time, the public is advised to consume a healthy diet in order to prevent or delay the onset of chronic disease or cancer AZTI's Food Division has a multidisciplinary team working closely with the food industry, transferring knowledge and offering integrated solutions for the development and launch into market of new food products.

Future research in nutrition will focus on the use of new "omics" tools (genomics, trancriptomics, proteomics and metabolomics). These tools are useful to select bioactive compounds and to provide new biomarker concepts and strategies to characterize and quantify physiological functions related to food and health. In this sense, we present an ongoing project that evaluates the usefulness of membrane lipidome (lipidomics) as biomarker with potential correlation with dietary and chemosensory alterations (taste and smell) in cancer patients under chemotherapy. Subjects were recruited from the Oncology Outpatient Unit of Onkologikoa hospital (n=150). Membrane lipidome is expressed as the fatty acid composition of patients' erythrocyte phospholipids (n=50). Combination of qualitative study of chemosensory impairments with quantitative determination of molecular profiles connected to diet and metabolism can provide a multidisciplinary and effective strategy to address oncological patients' needs. This valuable information will help to design specific food products, capable of alleviating symptoms, and above all, preventing malnutrition.

On the other hand, we also present some preliminary results regarding the translation of membrane lipid profile analysis for the development of personalised food products. During the last years, AZTI has been applying a valuable *in vivo* model for the assessment of bioactive compounds´ effectiveness in metabolic diseases: the zebrafish. We will study the applicability of lipid profiling in this model, which allows the prescreening of molecules in a fast and cost-effective way.

The use of lipidomics in food research paves the way for the development of a new generation of food products in a personalised nutrition approach.

The role of radical stress in liver disease

Nataliya Rohr-Udilova, Dagmar Stoiber, Eva Bauer, Wen Li, Martha Seif, Hubert Hayden, Regina Brigelius-Flohe, Klaus Stolze, Gerald Timmelthaler, Robert Eferl, Markus Peck-Radosavljevic

Background: Glutathione peroxidase 4 (GPx4) is a selenium containing antioxidative enzyme able to reduce lipid hydroperoxides. Whereas some evidence links GPx4 expression levels to colon cancer risk, the role of GPx4 in liver cancer remains to be investigated.

Methods: Expression plasmids with the porcine GPx4 gene under control of the CMV promoter were transfected into human Huh7 and HCC-3 hepatocarcinoma cells. The GPx4 transfection efficiency was evaluated by real-time PCR, western blotting, and activity measurements. Free radical formation was measured by electron spin resonance spin labelling. Cell migration was assessed both in a two-chamber assay as well as in a scratch assay. Intrinsic and induced oxidative stress, cell cycle progression, and expression of IL-8 and VEGF genes were investigated. The effect of GPx4 on tumour growth *in vivo* was assessed by xenotransplantation into NSG recipient mice. After immunohistochemical staining, the expression of GPx4, cell proliferation and vessel formation were determined by histomorphometric analysis of paraffin embedded tumour tissues.

Results: *In vitro*, GPx4 overexpression increased the resistance of cells to oxidative stress induced either by hydrogen peroxide or by linoleic acid peroxide (LOOH). Internal radical formation both at base line and at prooxidative challenge by LOOH was reduced in GPx4 overexpressing cells. GPx4 prevented LOOH-induced IL-8 but not VEGF formation. GPx4 reduced migration of tumour cells in a two-chamber assay by 35±5% in HCC-3 and by 64±11% in Huh7. Moreover, LOOH treatment increased the percentage of HCC cells in G2/M phase of the cell cycle which was prevented by GPx4 overexpression.

In vivo, smaller tumours were formed by GPx4 overexpressing HCC cells in NSG mice compared to cells expressing control plasmid. Median tumour weight after 6 weeks of growth was reduced by GPx4 overexpression from 0.82±0.52 g to 0.32±0.24 g for HCC-3 cells (n=16, p=0.002) and from 0.85±0.66 g to 0.40±0.37 g for Huh7 cells (n=18, p=0.01).

Higher expression of GPx4 in tumours formed from overexpressing cells was confirmed both by PCR and by immunohistochemistry. GPx4 influenced the vascularization parameters of tumours.

Among molecules regulating vascular architecture, no difference in human VEGF expression was observed between groups. In contrast, human thrombospondin 1 was increased in GPx4 overexpressing tumours.

Mouse VEGF and the IL-8 analogue CXCL1 were expressed at lower levels in tumours derived from GPx4 overexpressing cells.

Conclusion: GPx4 overexpression and decreased radical formation interferes with the malignant potential and with vascularisation of HCC *in vitro* and *in vivo*.

This work was supported by a grant from Herzfelder Familienstiftung to N.R.U., project No. AP**0**0585OFF.

	Vessel Density (1/mm²)	Vessel with Lumen Density (1/mm²)	Average Vessel Size (µm²)	Average Vessel with Lumen Size (µm²)	Average Vessel without Lumen Size (µm²)	Average Vessel Wall Thickness (µm)	% Vessel Small	% Vessel Medium	% Vessel Large
MW Control	**183**	**2,95**	**280**	**1411**	**231**	**2,20**	**7,0**	**58,1**	**35,0**
Stdev	**23**	**0,36**	**18**	**405**	**17**	**0,10**	**1,0**	**2,6**	**3,1**
Mw GPx4	**296**	**2,52**	**197**	**1115**	**166**	**1,83**	**11,8**	**66,1**	**22,1**
Stdev	**43**	**0,63**	**68**	**494**	**46**	**0,23**	**4,3**	**3,7**	**6,4**
p	**0,0001**	**0,0910**	**0,0083**	**0,1419**	**0,0044**	**0,0025**	**0,0110**	**0,0007**	**0,0006**

HDL quality and functionality as a central contributor to the development of metabolic syndrome

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Epidemiological and clinical studies have established over the years that dyslipidemia constitutes the main risk factor for atherosclerosis. The inverse correlation between HDL cholesterol (HDL-C) levels and coronary heart disease morbidity and mortality identified HDL-C as an alternative to LDL cholesterol pharmacological target and a potential anti-atherosclerosis marker. However, more recent data reinforced the principle of "HDL quality" in atherosclerosis that refers to the functionality of HDL particle, as defined by its protein and lipid content, rather than HDL-C levels in plasma. Since HDL functionality depends the genes and proteins of the HDL metabolic pathway, its apoprotein composition may serve as a surrogate marker of atheroprotection. In an effort to identify measurable qualitative structural parameters of HDL that reflect its atheroprotective functionality, we turned our attention to the study of relevant clinical examples where we found that a dramatic reduction in the ratio of apoA-I/apoC-III and apoA-I/apoE in HDL resulted in HDL particles with modified biological functions. In addition, our functional data studies in experimental mice indicate that qualitative alterations in HDL may influence other pathological conditions associated with metabolic syndrome such as hypertriglyceridemia, nonalcoholic fatty liver disease, diabetes and bone metabolic diseases. Taken together our results indicate that in subjects with metabolic syndrome, the coexistence of reduced HDL levels with other metabolic dysfunctions in more than just a mere coincidence and underlays a strong causative relationship.

Membrane lipidomics for personalized health

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Each organism and biological compartment has its own lipid composition, defined as the lipidome, which can be monitored by lipidomics. In particular, lipidomics revealed the precious information embedded in the correct assembly of phospholipids, to provide the best organization and functionality of the complex and homeostatic system of cell membranes.

Membrane lipidomics is influenced by various metabolic and environmental conditions and the extreme flexibility and adaptation of membrane composition for its optimal functioning is also the novel aspect that attracted a lot of research and medical interest. This has been translated into a practical tool for personalised medicine by using the profiles determined from the mature red blood cell membranes, isolated from blood samples by an innovative robotics, as reporter of the health status. Inadequate diets and life styles can perturb the membrane lipidomics, and this is also connected with further disease onset.

The power of membrane lipidomics as molecular diagnostic tool to highlight fatty acids changes and unbalances is evident in diseases, such as obesity, neurological and cardiovascular diseases, as well as in health status such as pregnancy, fertility and many other conditions. It also evidenced that membranes can be “repaired” from incorrect profiles, due to their natural turnover and membrane remodelling processes, therefore they are an ideal site of personalised intervention by nutritional and nutraceutical strategies.

A new book will be soon published by Wiley giving an overview of the state of art in the field and the perspectives for this innovative molecular profiling useful for personalised health strategies.

A new book is coming soon:

Carla Ferreri and Chryssostomos Chatgilialoglu. Membrane Lipidomics for Personalized Health, First Edition, Publisher: John Wiley, July 2015.

DNA damage and repair, an overview

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Due to its key role in the maintenance of the genetic information, any modification that induces damage the DNA macromolecule could have severe biological consequences, inducing mutations or even cell death. Several endogenous and exogenous agents could damage DNA producing different types of lesions, involving base damages, base losses or abasic sites, single or double strand breaks, intra or inter-strand crosslinks, DNA protein cross-links and DNA adducts. Many cellular defenses mechanisms have been developed through evolution to first reduce the vulnerability of DNA and also to repair the integrity of the macromolecule in order to minimize the effect of the produced lesions.

During the first part of the presentation dedicated to genotoxicology, the different types of damages will presented and examples will be given to illustrate at least partly the mechanisms involved in the formation of the lesions. Particular attention will be focused on the effects of reactive oxygen species (ROS), which are produced endogenously by biological processes and could be also produced by exogenous stress such as ionizing radiations. The particularity of ionizing radiations to produce so-called cluster lesions or multiple damage site will be also presented.

In the second part of the presentation, emphasis will be placed on the DNA repair processes that are involved in cell in the removal of the lesions and restoration of the integrity of the macromolecule. A general overview of the DNA repair mechanisms will be presented and again examples will be given to indicate how DNA repair could be determined or followed in cells and recent methodological development will be also described.

Targeting ionizing radiation response using bioinformatical approaches

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Exposure to ionizing radiation induces a variety of responses in the cell ranging from DNA repair, apoptosis and inflammatory response. This multiparameter system is highly complex and in many cases the different subpathways interact with each other. This situation raises a great difficulty in understanding and studying radiation response (RR). In this presentation, I will present our latest work on the use of bioinformatical approaches towards the delineation of ionizing radiation response in the cell. We have identified gene products involved in immune and inflammatory responses upon exposure to ionizing radiation by using specific bioinformatic tools. Genes implicated both in radiation and immune/inflammatory responses were collected manually from the scientific literature with a combination of relevant keywords. The experimentally validated and literature-based results were inspected and genes involved in radiation, immune and inflammatory response were pooled. This kind of analysis was performed for the first time for both healthy and tumor tissues. In this way, a set of 24 genes common in all three different phenomena was identified. These genes were found to form a highly connected network. In addition, common gene products in complex DNA damage repair were identified in an attempt to target possible DNA repair pathways for inducing 'synthetic lethality' will be discussed.

Hole transfer in DNA: thermodynamic and kinetic aspects

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The rates of hole transfer between guanines in DNA have been analyzed using the Fermi golden rule and Kubo's generating function approach for evaluating the Franck-Condon weighted density of states.[1] Hole site energies and intra-strand electronic coupling elements have been estimated by differential pulse voltammetries of isolated nucleobases in solutions and of A rich and G rich oligonucleotides, both in single strands and duplex configurations.[2] Voltammetric measurements point toward the establishment of delocalized adenine hole domains, which efficiency promote coherent hole transfer between guanines.

Radiation-induced purine lesion formation in DNA

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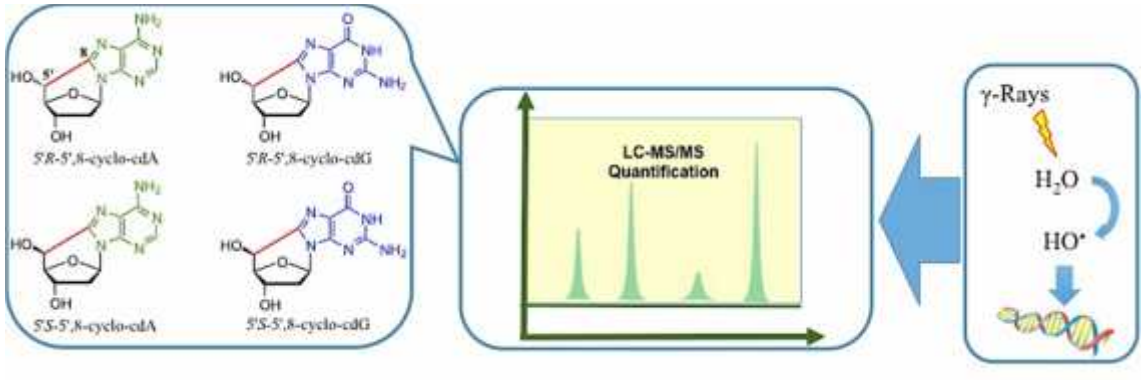
In all living organisms DNA, the unmodified, natural, purine 2'-deoxynucleosides consist of a base linked to a sugar via one single carbon nitrogen bond. The presence of a second bond between the C5' carbon of the sugar and the C8 carbon of the base moieties, changes significantly the chemical signature of the molecules in respect to the parent ones, with dramatic consequences for both the translation and the integrity of genetic information itself.[1] The extent of the, so called purine 5',8-cyclo-2'-deoxynucleosides, formation caused by the selective C5' radical generation after the DNA sugar backbone insult by the hydroxyl radical, is still under debate due to complexity of the quantification methodologies reported in the literature to date. Herein, will be described the application of a newly developed protocol for the quantification of the 5'*R* and 5'*S* purine 5',8-cyclo-2'-deoxynucleosides based on the efficient enzymatic digestion of DNA treated with γ-rays.[2] The isotope dilution LC-MS/MS technique, enabled to monitor also the 7,8-dihydro-8-oxo-2'-deoxyguanosine and 7,8-dihydro-8-oxo-2'-deoxyadenosine apart from the four cyclopurines, provides significant insights on the radiation induced formation of these bulky lesions in DNA, which are known to be among of the smallest activating the Nucleotide Excision Repair (NER) mechanism for their removal in cells.[3]



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Oxidative DNA damage repair and repeat sequence instability

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Oxidative DNA damage including 8-oxoguanine and 5',8-cyclodeoxypurines are DNA lesions that occur frequently in the genome of mammalian cells. Repeat sequences such as trinucleotide repeats that are rich of purines, are hot spots of oxidative DNA damage. Instability of trinucleotide repeats (TNRs) is associated with neurodegenerative diseases and cancer and is modulated by DNA damage and repair. We have previously discovered that oxidative DNA damage located in the context of CAG repeats can lead to repeat expansion via DNA base excision repair (BER). Recently, we have further demonstrated that oxidative DNA base lesions can also result in TNR deletion during BER. Moreover, we have found that the repeat instability is governed by the locations of DNA base lesions in a TNR tract. Further characterization of the activities of BER enzymes during repair of a base lesion located at the 5'-end of a TNR repeat tract has shown that DNA polymerase β (pol β) can synthesize more repeats than those removed by flap endonuclease 1 (FEN1), thereby leading to repeat expansion, whereas pol β can synthesize fewer repeats than FEN1 removes when a lesion located in the middle of a TNR tract, thus leading to repeat deletion. Interestingly, we have found that BER of an oxidized base lesion located at the loop region of a TNR hairpin results in the removal of the hairpin preventing or attenuating TNR expansion. Most recently, we have discovered that a 5',8 cyclo-deoxyadenosine lesion can specifically induce TNR deletion by inducing the formation of a TNR loop on the template strand that allows pol β to skip over the hairpin and FEN1 to remove more repeats than pol β synthesizes. Our results indicate that TNR instability can be modulated by oxidative DNA base lesions via BER, and this can be developed as a novel target of treatment of neurodegenerative diseases caused by TNR expansion.

Interaction of hydrated electrons, e_{aq}^- with cisplatinum intra- and inter-strand cross-linked DNA studied by molecular modeling and molecular dynamics

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Cis-diamminedichloroplatinum (II) (cisplatin, CPT) is a classical chemotherapeutic drug used in the treatment of various cancers. CPT binds to N7 purine sites in DNA, preferentially on guanine creating cytotoxic intra- and inter-strand crosslinks (ICL) in between other damages. In clinic, concomitant CPT-chemo-radiotherapy enhances tumor control and patient survival. The synergetic effects have been manifested by enhanced cell kill and correlate with increased DNA base damage and DNA fragmentation (formation of SS and DS breaks). It has been shown that among different radiation-induced species hydrated electrons, e_{aq}^- play important role in the radiosensitization of CPT-modified DNA, *i.e.* Pt-atom acting as an electron-affinity center; Pt(II)→Pt(I) reduction, eventually followed by cisplatin detachment with the generation of -based free radicals via electron-transfer processes.

To elucidate the basics of e_{aq}^- interaction with CPT-modified DNA (intra and inter-strand cross-linked CL-DNA) we applied molecular modeling and molecular dynamics MD (including meta-MD). The e_{aq}^- was modeled as a distance-constrained $^-(H_2O)_6$ water cluster, *i.e.* Kevan's octahedral cavity, single water shell structure of -1 total charge, distributed as Mulliken partial charges along the six water molecules. The dynamic conformational properties of both intra- and inter-G*G* cross-linked DNAs show common structural deviations from native DNA, including pronounced DNA-axis bend, unwinding and minor groove widening at the CPT site, with an important difference: the intra-CL-DNA is bent towards the major groove, while the inter-CL-DNA axis is bent toward the minor groove with the G*- pairing cytosines flipped out from the DNA helix. In both cases, bending direction follows the individual groove location of the CPT unit. Consecutively, meta-MD results are consistent with e_{aq}^- frequent entrance (close VDW atomic interaction) within the major groove of the former and within the minor groove of the later, while no close interactions between native DNA and e_{aq}^- are found. Structural DNA deviations which control long- and short-range interactions between CPT-DNAs and e_{aq}^- are examined.


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Index

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