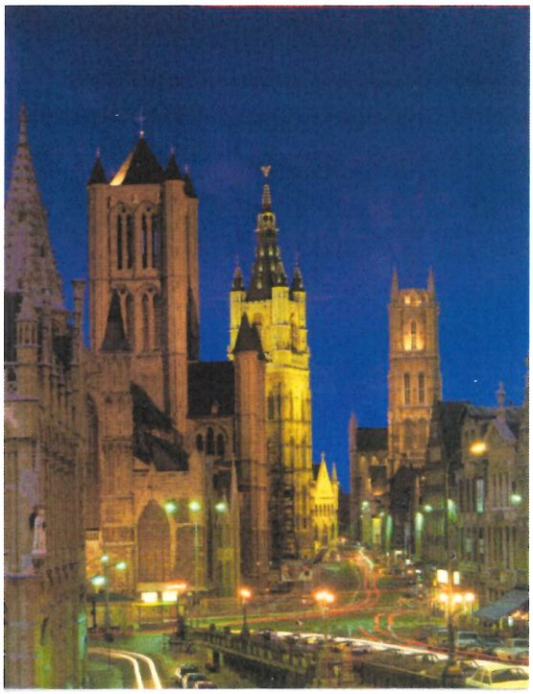
16th Meeting of the Benelux EPR Group 2008

Programme and Abstracts



Ghent University

Department of Solid State Sciences

16 May, 2008

Sponsored by Bruker Belgium

Programme

09:45 Registration and coffee/tea

10.25 Welcome

10:30 Characterisation of cobalt(II)porphyrin/carbon nanotube nanohybrids by electron paramagnetic resonance

Sofie Cambré[1], Wim Wenseleers[1], Jelena Čulin[1,2], Sabine Van Doorslaer[3] and Etienne Goovaerts[1]

[1] *Experimental Condensed Matter Physics Laboratory, University of Antwerp, Universiteitsplein 1, 2610 Antwerp (Belgium)*

[2] *Rudjer Boskovic Institute, 1000 Zagreb (Croatia)*

[3] *Spectroscopy in Biophysics and Catalysis Laboratory, University of Antwerp, Universiteitsplein 1, 2610 Antwerp (Belgium)*

10:55 Combined Electron Magnetic Resonance and Density Functional Theory Study of X-Irradiated β-D-Fructose Single Crystals

Mihaela Tarpan[1], Einar Sagstuen[3], H. Vrielinck[1], E. Pauwels[2], M. Waroquier[2] , F. Callens[1]

[1] *Department of Solid State Sciences, Ghent University, Krijgslaan 281-S1,B-9000 Gent, Belgium*

[2] *Center for Molecular Modeling, Ghent University, Proeftuinstraat 86, B-9000 Gent, Belgium*

[3] *Department of Physics, University of Oslo, P.O. Box 1048 Blindern, N-0316 Oslo, Norway.*

11:20 A multi-frequency cw and pulsed EPR/ENDOR study of cobalt-sulfur coordination

Silvia Sottini[1], J. Mathies[1], D. Maganas[2], S. Milikisyants[1], P. Gast[1], P. Kyritsis[2] and E.J.J. Groenen[1]

[1] *Department of Molecular Physics, Leiden University, The Netherlands*

[2] *Department of Chemistry, University of Athens, Greece*

11:45 EPR analysis of methylbenzylamine stereoselectivity by a chiral copper system

Ignatio Caretti[1], D.M. Murphy[2], I.A. Fallis[2], M. Goebel[2], D.J. Willock[2], J. Landon[2] and S. Van Doorslaer[1].

[1] *University of Antwerp, Department of Physics, SIBAC laboratory, Universiteitsplein 1, B-2610 Wilrijk, Belgium.*

[2] *School of Chemistry, Cardiff University, Main Building, Park Place, Cardiff CF10, 3AT, UK.*

12:10 LUNCH

14:00 The effect of overexpression of UCP3 in muscle on mitochondrial ros production in young versus aged mice

Miranda Nabben[a], Jacob J. Briedé[b], Joris Hoeks[a], Jan F.C. Glatz[c], Matthijs K.C. Hesselink[d], Patrick Schrauwen[a]

[1] *Department of Human Biology, Nutrition and Toxicology Research Institute Maastricht (NUTRIM)*

[2] *Department of Health Risk Analysis and Toxicology, Nutrition and Toxicology Research Institute Maastricht (NUTRIM)*

[3] *Department of Molecular Genetics, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, the Netherlands*

[4] *Department of Human Movement Sciences, Nutrition and Toxicology Research Institute Maastricht (NUTRIM)*

14:25 eNOS enzyme releases significant quantities of NO radicals in septic mice

Annette van de Sandt [1], E. van Faassen [2], A. Gödecke [3], R. Windler [1], S. Becher [1], P. Kleinbongard [1], T. Rassaf [1], M. Kelm [1], J. Schrader [3], M.W. Merx [1]

[1] *Division of Cardiology, Pulmonary Diseases, and Vascular Medicine, Department of Medicine, University Hospital Aachen, Germany;*

[2] *Debye Institute, Surfaces, Interfaces and Devices, Utrecht University, Netherlands;*

[3] *Institute of Physiology, Heinrich-Heine-University Düsseldorf, Germany.*

14:50 Atomic identification of the Si-B5 paramagnetic center by electron spin resonance

Koen Keunen[1] and A. Stesmans[1]

[1] *Department of Physics, University of Leuven, Celestijnenlaan 200D, 3001 Leuven, Belgium*

15.15 Coffee/tea

15.45 New development in structure determination by pulsed EPR

Sergey Milikisyants[1], F. Scarpelli[1], E. J.J. Groenen[1] and M. Huber[1]

[1] *Department of Molecular Physics, Leiden University, The Netherlands*

16.10 General discusson

16.30 Poster session

16.45 Reception

18.30 End

Congress centre "Het Pand"
Onderbergen 1
9000 Gent
Belgium

http://www.ugent.be/en/facilities/pand/how_to_reach/

Poster Presentations

1. **In situ ultrasonic influence on Si nanoparticle formation in SiO_2 through ion implantation**

M. Jivanescu[1], A. Stesmans[1]
[1] *Department of Physics, University of Leuven, Celestijnenlaan 200 D, B-3001 Leuven, Belgium*

2. **Thermal stability of the (111)Si/c-Lu_2O_3 interface probed by electron spin resonance**

P. Somers[1], A. Stesmans[1], and V.V. Afanas'ev[1]
[1] *Department of Physics, University of Leuven, Celestijnenlaan 200D, 3001 Leuven, Belgium*

3. **EPR characterization of organic materials for 3-D heterojunction solar cell applications**

A. Aguirre[1] , E. Goovaerts[1], S. Van Doorslaer[1], G. Janssen[1].
[1] *Department of Physics - CDE, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium.*

4. **W-band transient EPR on the photoexcited states of spin-labelled fullerenes**

H. Moons[1], E. Goovaerts[1], I. Nuretdinov[2], L. Franco[3], C. Corvaja[3]
[1] *Department of Physics-CDE, University of Antwerp, Belgium*
[2] *Arbuzov Institute of Organic and Physical Chemistry, Kazan Scientific Center of the Russian Academy of Sciences, Russia*
[3] *Department of Chemical Sciences, University of Padova, Italy*

5. **Localization of MN^{2+} impurity ions in nanocrystalline ZnS as determined by multifrequency EPR**

S. V. Nistor[1], M. Stefan[1], D. Matescu[1] and E. Goovaerts[2]
[1] *National Institute for Materials Physics, POB MG 7 Magurele, Romania*
[2] *University of Antwerpen-CDE, Physics Department, Antwerpen-Wilrijk, Belgium*

6. **Incorporation of nitrogen in diamond nanograins: Size dependent ?**

A.K. Dhami [1], Igor I. Vlasov [2], and Etienne Goovaerts [1]
[1] *Physics Department – CDE, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium*
[2] *General Physics Institute, Russian Academy of Sciences, 38 Vavilov str., 119991 Moscow, Russia*

7. **High frequency W-Band (94 GHz) EPR study of the hole trapped Bismuth centre in $Bi_{12}MO_{20}$ (M: Si, Ti) single crystals**

I. Ahmad [1], V. Marinova [1,2] and E. Goovaerts [1]
[1] *Experimental Condensed Matter Physics, Department of Physics, University of Antwerp, Universiteitsplein 1,B-2610 Wilrijk, Belgium*
[2] *Central Laboratory of Optical Storage and Processing of Information Sofia, Bulgarian Academy of Sciences, 1784 Sofia, Bulgaria*

8. **Temperature dependence of two Eu-related EPR spectra in CsBr medical image plates**

F. Loncke[1], H. Vrielinck[1], P. Matthys[1], J.-P. Tahon[2], P. Leblans[2], I. Ahmad[3], E. Goovaerts[3] and F. Callens[1]
[1] *Department of Solid State Sciences, Ghent University, Krijgslaan 281 – S1, B-9000 Gent, Belgium*
[2] *Agfa Healthcare NV, Septestraat 27, B-2640 Mortsel, Belgium*
[3] *Physics Department, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen (Wilrijk), Belgium*

9. EPR and TL study of irradiated K_2YF_5 phosphors

D. Zverev[1], H. Vrielinck[1], P.F. Smet[1], D. Poelman[1] and F. Callens[1]

[1] *Department of Solid State Sciences, Ghent University, Krijgslaan 281-S1, B-9000 Gent, Belgium*

10. EPR analysis of vanadium silicate-1 nanoparticles deposited on the mesoporous walls of SBA-15

S. Zamani[1], M. Chiesa[2], V. Meynen[3], X. Yiqun[1], P. Cool[3], E. Vansant[3], S. Van Doorslaer[1]

[1] *University of Antwerp, Laboratory for Spectroscopy in Biophysics and Catalysis, Department of Physics, Universiteitsplein 1, B-2610 Wilrijk, Belgium*

[2] *Dipartimento di Chimica IFM, Università di Torino and NIS Centre of Excellence, via P. Giuria 7, 10125 Torino, Italy*

[3] *University of Antwerp, Laboratory of adsorption and Catalysis, Department of Chemistry, Universiteitsplein 1, B-2610 Wilrijk, Belgium.*

11. Unraveling the activation process of the cobalt Jacobsen's catalyst: A combined EPR-DFT approach

Evi Vinck[1], Sabine Van Doorslaer[1], Damien M. Murphy[2], and Ian A. Fallis[2].

[1]*University of Antwerp, Department of Physics, Universiteitsplein 1, 2610 Wilrijk, Belgium*

[2]*Cardiff University, Department of Chemistry, Cardiff CF10 3TB, Wales, UK*

12. Schonland ambiguity in the determination of hyperfine tensors from angular dependence of ENDOR spectra: application to the T1 radical in sucrose single crystals

Hendrik De Cooman[1] , Henk Vrielinck[1], Sabine Van Doorslaer[2], and Freddy Callens [1]

[1] *Ghent University – Department of Solid State Sciences, Krijgslaan 281-S1, B-9000 Gent, Belgium*

[2] *University of Antwerp – Department of Physics, Campus Drie Eiken, Universiteitsplein 1-N, B-2610 Wilrijk, Belgium*

13. Temperature study of a glycine radical in the solid state: Periodic DFT calculations of vibrational and EPR properties

E. Pauwels[1], T. Verstraelen[1], H. De Cooman[1], V. Van Speybroeck[1] and M. Waroquier[1]

[1] *Center for Molecular Modeling, Ghent University, Proeftuinstraat 86, B-9000, Belgium*

14. Dynamical, structural and functional aspects of neuroglobin

M. A. Ezhevskaya[1], S. Dewilde[2], L. Moens[2], Y. Polyhach[3], G. Jeschke[3], S. Van Doorslaer[1]

[1] *University of Antwerp, Department of Physics, Universiteitsplein 1, B-2610 Wilrijk, Belgium*

[2] *University of Antwerp, Department of Biomedical Sciences, Universiteitsplein 1, B-2610 Wilrijk, Belgium*

[3] *University of Konstanz, Unversitaetsstrasse, 78457 Konstanz, Germany*

15. EPR of the globin domain of the globin-coupled sensor of *Geobacter sulfurreducens*

Filip Desmet[1], Liesbet Thijs[2], Hassane El Mkami[3], Graham Smith[3], Sylvia Dewilde[2], Luc Moens[2], Sabine Van Doorslaer[1]

[1] *Department of Physics, University of Antwerp, Belgium*

[2] *Department of Biomedical Sciences, University of Antwerp, Belgium*

[3] *Milimetre Wave and High Field ESR Group, University of St. Andrews*

16. Impact of a cyclodextrin on liposomes and celles: an ESR evidence

Angeliki A. Grammenos[1], M.A. Bahri[2] , G. Piel[2] and M. Hoebeke[1]

[1] *Laboratory of biomedical spectroscopy, Institute of Physics B5, University of Liège, Belgium*

[2] *Laboratory of Pharmaceutical Technology, Department of Pharmacy, University of Liège , Belgium*

17. Study of neuronal cells preconditioning by ESR

P.-H. Guelluy[1], G. Deby-Dupont[2], M. Hoebeke[1]

[1] *Biomedical Spectroscopy, Institute of Physics, University of Liège*

[2] *Centre for Oxygen Research and Development (CORD), Institute of Chemistry, University of Liège*

18. Effect of statin on liposome and cell membranes fluidity. An ESR spin probe study.

A. Ngendakamuna[1], M. Bahri[2] and M. Hoebeke[1].

[1] *Laboratory of Biomedical Spectroscopy, Department of Physics, Institute of Physics, B5, University of Liège, Sart-Tilman, B-4000 Liège, Belgium, M.Hoebeke@ulg.ac.be*

[2] *Laboratory of Experimental medical Imaging, Department of Physics, Institute of Physics, B5, University of Liège, Sart-Tilman, B-4000 Liège, Belgium*

19. Radicals for life: Nitric Oxide and its reactions

Ernst van Faassen[1]

[1] *Interface Physics, Debye Institute, Utrecht University, the Netherlands.*

20. Detection of nitric oxide in the rat bone marrow by electron paramagnetic resonance

M. Aleksinskaya[1], E. van Faassen[2], T.J. Rabelink[1] and A.J. van Zonneveld[1]

[1] *Department of Nephrology and Einthoven laboratory of experimental vascular medicine, Leiden University Medical Centre, The Netherlands*

[2] *Faculty of Science, Department of Interface Physics, Utrecht University, The Netherlands*

21. Spin density distribution in foodstuff after heat treatment or irradiation studied by EPR imaging

Philippe Levêque[1], Quentin Godechal[1] & Bernard Gallez[1]

[1] *Biomedical Magnetic Resonance Unit, Université catholique de Louvain, Brussels, Belgium*

22. Molecular NMR and EPR in vivo detection of inflammation using specific E-selectin targeted iron oxides

K.A. Radermacher[1], N. Beghein[1], S. Boutry[2], S. Laurent[2], L. Vander Elst[2], R.N. Muller[2], B.F. Jordan[1], and B. Gallez[1].

[1]*Biomedical Magnetic Resonance Unit, Université Catholique de Louvain, Brussels, Belgium.*

[2]*NMR and Molecular Imaging Laboratory, University of Mons-Hainaut, Mons, Belgium*

23. Synthesis and Evaluation of New Persistent Trityl Radicals for Biomedical EPR Applications

Benoît Driesschaert,[1,2] Nicolas Charlier,[2] Bernard Gallez,[2] and Jacqueline Marchand-Brynaert[1]

[1]*Unité de Chimie Organique et Médicinale, Université catholique de Louvain, UCL, place Louis Pasteur 1, B-1348 Louvain-la-Neuve, Belgium*

[2]*Unité de Résonance Magnétique Biomédicale, Université catholique de Louvain, UCL, avenue Mounier 73.40, B-1200 Bruxelles, Belgium*

24. Development and evaluation of a non invasive method to estimate the oxygen consumption by tissues

Caroline Diepart[1], Bénédicte F.Jordan[1], and Bernard Gallez[1,2]

[1] *Laboratory of Biomedical Magnetic Resonance, Université catholique de Louvain, Brussels, Belgium*

[2] *Laboratory of Medicinal Chemistry and Radiopharmacy, Université catholique de Louvain, Brussels, Belgium*

25. Assessment of the liver phagocytosis activity by EPR spectroscopy and imaging

N. Charlier[1], AM. Neyrinck[2], N. Beghein[1], N. Delzenne[2] and, B. Gallez[1]

[1]*Laboratory of Biomedical Magnetic Resonance, Université Catholique de Louvain, Brussels, Belgium.*

[2]*Unit of Pharmacokinetics, Metabolism, Nutrition and Toxicology, Université Catholique de Louvain, Brussels, Belgium.*

26. Arsenic trioxide enhances tumor oxygenation by decreasing oxygen consumption

Caroline Diepart[1], Bénédicte F.Jordan[1], and Bernard Gallez[1,2]

[1] *Laboratory of Biomedical Magnetic Resonance, Université catholique de Louvain, Brussels, Belgium*

[2] *Laboratory of Medicinal Chemistry and Radiopharmacy, Université catholique de Louvain, Brussels, Belgium*

27. A New Method to Distinguish between Blond- and Brown-Haired People: The contribution of Molecular Electron Paramagnetic Resonance Imaging

Nicolas Charlie[1], Mustapha Dinguzli[1], and Bernard Gallez[1]

[1] *Biomedical Margnetic Resonance Unit, Université Catholique de Louvain, Brussels, Belgium*

Oral Contributions

Characterisation of cobalt(II)porphyrin/carbon nanotube nanohybrids by electron paramagnetic resonance

Sofie Cambré[*], Wim Wenseleers[*], Jelena Čulin[*,**], Sabine Van Doorslaer[***] and Etienne Goovaerts[*]

[*]Experimental Condensed Matter Physics Laboratory, University of Antwerp, Universiteitsplein 1, 2610 Antwerp (Belgium)
[**]Rudjer Boskovic Institute, 1000 Zagreb (Croatia)
[***]Spectroscopy in Biophysics and Catalysis Laboratory, University of Antwerp, Universiteitsplein 1, 2610 Antwerp (Belgium)

Tailoring the properties of single-walled carbon nanotubes (SWNTs) with organic molecules by non-covalent functionalisation yields very promising materials for organic electronics. Recent progress in the characterisation of the opening/closing of SWNTs[1] allows for the quantitative preparation of both inclusion and external adsorption complexes. Using paramagnetic molecules, these nanohybrids can be studied by EPR spectroscopy. Here, we present the first EPR investigation of paramagnetic metalloporphyrins adsorbed on the nanotube walls through π-stacking. This functionalisation of SWNTs with porphyrin molecules has attracted much attention due to their use as donor-acceptor building blocks in photovoltaic cells, for their catalytic properties and for their use in biological sensors.[2]

We study multi-component EPR spectra of cobalt(II)porphyrin/SWNT nanohybrids. Their *g*-anisotropy, hyperfine interaction and *g*- and hyperfine strains are monitored under different treatments. Our results affirm the electron acceptor behaviour of the SWNTs in these nanohybrids, in agreement with DFT calculations.[3] In the EPR spectra we can distinguish porphyrins interacting with metallic and semiconducting SWNTS. Solubilising the SWNTs in water using bile salts as surfactants,[4] we can also study the interaction of individual carbon nanotubes with the molecules. The EPR investigation is combined with optical absorption and with steady state and time resolved fluorescence spectroscopy. Large red shifts of the porphyrin absorption bands and a complete quenching of the porphyrin fluorescence indicate strong electronic interactions of the porphyrin system with the SWNTs, as was also evidenced by EPR spectroscopy.

[1] W. Wenseleers, S. Cambré, J. Čulin, A. Bouwen, E. Goovaerts, *Adv.Mater.* **2007**, 19, 2274
[2] D.M. Guldi, *Phys.Chem.Chem.Phys.* **2007**, 9, 1400
[3] E.V. Basiuk, V.A. Basiuk, P. Santiago, I. Puente-Lee, *J.Nanosc.Nanotech.* **2007**, 7, 1
[4] W. Wenseleers, I.I. Vlasov, E. Goovaerts, E.D. Obraztsova, A.S. Lobach, A. Bouwen, *Adv.Funct.Mater.* **2004**, 14, 1107

Combined Electron Magnetic Resonance and Density Functional Theory Study of X-Irradiated β-D-Fructose Single Crystals

M. Tarpan*[a], Einar Sagstuen[§], H. Vrielinck*[b], E. Pauwels[#b], M. Waroquier[#] , F. Callens*

*Department of Solid State Sciences, Ghent University, Krijgslaan 281-S1,B-9000 Gent, Belgium
[#]Center for Molecular Modeling, Ghent University, Proeftuinstraat 86, B-9000 Gent, Belgium
[§] Department of Physics, University of Oslo, P.O. Box 1048 Blindern, N-0316 Oslo, Norway.
[a] Research Assistant and [b] Postdoctoral Fellow of the Flemish Research Foundation (FWO-Vlaanderen)

Radiation-induced radicals in sugars have recently gained considerable interest with respect to both fundamental and applied research. A number of studies are available that focus on the dosimetric characteristics of sugar systems. Other studies, like ours, aim to understand the identity and the structural properties of the involved radicals, and the reactions evolving from the primary radicals.

In the present work, the main objective was to devise a mechanistic link between the presumed primary radicals, detected previously after "in situ" X-irradiation at 10 K, and the stable radicals observed after irradiation at room temperature (RT).

Single crystals of β-D-fructose, X-irradiated at 10 K, 77 K and RT, were examined using Electron Paramagnetic Resonance (EPR), Electron Nuclear Double Resonance (ENDOR) and ENDOR induced EPR (EIE) techniques. For X-irradiations at low temperature (liquid helium or nitrogen) and to measurements at the same or higher temperatures a special X-band EPR/ENDOR setup developed by the EPR group at the University of Oslo was used. By means of Density Functional Theory (DFT) calculations the structures of plausible radical models were examined.

After 10 K X-irradiation three different radicals, labeled T1, T1* and T2 are formed. For the T1 and T1* radicals, the close similarity in hyperfine coupling tensors suggests that they are due to the same type of radical stabilized in two slightly different geometrical conformations. A C3 centered hydroxyalkyl radical model formed by a net H abstraction is found to be appropriate for these radicals. The T2 radical is proposed to be a C5 centered hydroxyalkyl radical, also involving a net hydrogen abstraction. Both identifications are supported by a fairly good agreement between the calculated and the experimental hyperfine tensors.

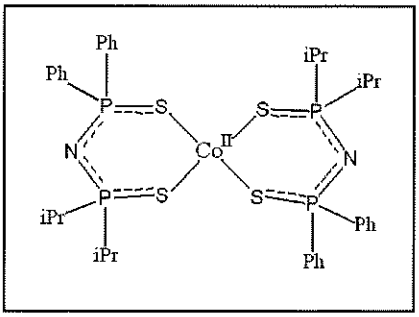
In the case of RT X-irradiation, ENDOR angular variations in the three principal crystallographic planes allowed to determine unambiguously ten proton hyperfine couplings tensors, that could be assigned to six different radicals by means of EI-EPR. Based on DFT calculations a number of trial structures, involving hydrogen/hydroxyl group removal or fructose ring breakage, are proposed and confronted with the measured Electron Magnetic Resonance data.

Results of irradiations and magnetic resonance measurements at temperatures intermediate between 10 K and RT will be discussed in terms of plausible processes/reactions linking the primary and the stable radicals.

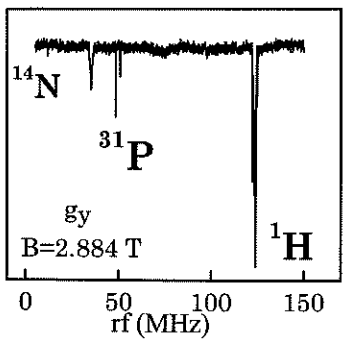
A multi-frequency cw and pulsed EPR/ENDOR study of cobalt-sulfur coordination

S. Sottini*, J. Mathies*, D. Maganas**, S. Milikisyants*, P. Gast*, P. Kyritsis** and E.J.J. Groenen*
*Department of Molecular Physics, Leiden University, The Netherlands
**Department of Chemistry, University of Athens, Greece

The abundance of metal-sulfur coordination in biological systems presents the reason for our EPR and ENDOR studies in this field to try and map the covalent character of this bond for various ligations. Although transition metals like Fe, Ni, Cu, Zn, Mo and W are most prominent, Co has to be considered as well. Recently the active site of ATP sulfurylase has been postulated to contain sulfur-coordinated CoII. In addition, to enable spectroscopic and magnetic investigations, ZnII- and CdII-containing active sites of enzymes have often been reconstituted with CoII. These considerations have prompted us to synthesize and study model complexes of cobalt with sulfur-containing bidentate ligands LH of the type R2P(S)NHP(S)R'2, with R, R' being phenyl (Ph) or isopropyl (iPr).



The large zero-field splitting of divalent cobalt (S=3/2) made a multi-frequency (X, Wand, J-band) EPR approach necessary. We have investigated solutions, powders and crystals of complexes that contain a slightly distorted tetrahedral CoIIS4 coordination of nearly D2d or S4 symmetry. In order to increase the resolution use has been made of diluted (doped) samples in which cobalt is largely replaced by zinc, which is EPR silent.



We have studied the anisotropy of the zero-field splitting, the g-matrix and the cobalt hyperfine interaction. A full quantitative analysis was found possible for a crystal of 1% CoL2 (Ph, iPr) in ZnL2 (Ph, iPr).

Recently a pulsed ENDOR study at 95 GHz was performed on this system and both proton, phosphorus and nitrogen ENDOR signals were observed (see figure). These data allow a detailed investigation into the delocalization of the electronic wavefunction over the ligands, thereby revealing the covalency of the cobalt-sulfur bond. We will describe the phosphorus ENDOR in detail.

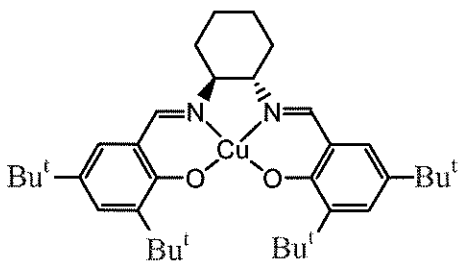
EPR analysis of methylbenzylamine stereoselectivity by a chiral copper system

I.Caretti*, D.M. Murphy**, I.A. Fallis**, M. Goebel**, D.J. Willock**, J. Landon** and S. Van Doorslaer*.

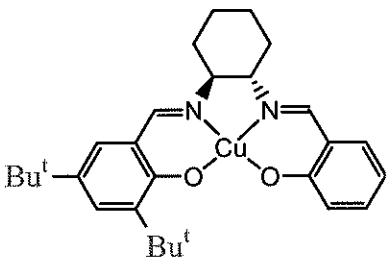
*University of Antwerp, Department of Physics, SIBAC laboratory, Universiteitsplein 1, B-2610 Wilrijk, Belgium.
**School of Chemistry, Cardiff University, Main Building, Park Place, Cardiff CF10, 3AT, UK.

Elucidating the factors that determine chiral selection is a key question to the understanding of the high enantioselective efficiency observed for Jacobsen-type catalysts in many asymmetric reactions [1].Jacobsen *et al* introduced in the nineties the Schiff base N,N'-bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexane-diamine and its corresponding metal complexes (Mn, Co, Cr,...) [2]. The latter ones show astounding enantiomeric excesses in the epoxidation of unfunctionalized alkenes, epoxide ring opening, hydrolytic kinetic resolution of racemic epoxides, and cyclopropanation as well. However, to date, the origins of this striking selectivity are not yet fully understood. In this respect, Electron Paramagnetic Resonance is a very suitable and powerful spectroscopy to analyze the structure, electronic spin distribution and interactions of the paramagnetic transition ion center that are responsible for the stereoselective process.

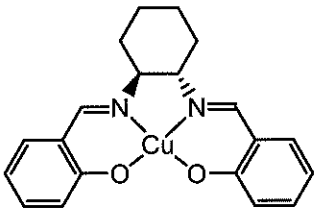
In this work we analyze the enantioselectivity of *R*- and *S*-Methylbenzylamine by a chiral copper (II) Jacobsen complex (Cu**[1]**), as well as by two derivates obtained by removing half (Cu**[2]**) or all (Cu**[3]**) of the *tert*-butyl subtituents of the salen ligand. In the three cases, the continuous wave (CW) EPR and ENDOR experiments enable us to detect differences between the (*SS-S*, *RR-R*) and (*SS-R*, *RR-S*) homochiral Cu**[1,2,3]**/MBA pairs. The degree of symmetry of the central copper atom depends on the number of bulky *tert*-butyl groups, showing a gradual increasing rhombicity from Cu**[1]** to Cu**[3]**. For each of the copper complexes, both the X-Band CW EPR&ENDOR and W-Band CW EPR indicate a preferential formation of the *SS-R* and *RR-S* adducts, whose stability is linked to a more square planar geometry compared to the pair-wise combinations *SS-S* and *RR-R*. DFT calculations of the Cu**[1]**-MBA complex have shown the importance of considering the π-π stacking interaction between the aromatic ring of the amine and that of the ligand.



[1]



[2]



[3]

[1] Laetitia Canali and David C. Sherrington, *Chem. Soc. Rev.*, 1999, **28**, 85.
[2] Tokunaga; M.; Larrow, J.F.; Kakiuchi, F.; Jacobsen, E.N., *Science*, 277, 936 (1997)

THE EFFECT OF OVEREXPRESSION OF UCP3 IN MUSCLE ON MITOCHONDRIAL ROS PRODUCTION IN YOUNG VERSUS AGED MICE

Miranda Nabben[a], Jacob J. Briedé[b], Joris Hoeks[a], Jan F.C. Glatz[c], Matthijs K.C. Hesselink[d], Patrick Schrauwen[a]

Depts of [a]Human Biology, [b]Health Risk Analysis and Toxicology, and [d]Human Movement Sciences, Nutrition and Toxicology Research Institute Maastricht (NUTRIM), and [c]Dept of Molecular Genetics, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, the Netherlands

Introduction: Mitochondrial degradation of substrates leads to the build-up of a proton gradient over the inner mitochondrial membrane, which gradient is used for the formation of ATP. However, the coupling of the proton gradient to the formation of ATP does not show 100% efficiency because leakage of protons occurs causing a release of potential energy that is not coupled to ATP synthesis. This process is called 'mitochondrial uncoupling' and has been suggested to decrease Reactive Oxygen Species (ROS)-production through a reduction of the membrane potential. Uncoupling protein 3 (UCP3) may facilitate this process. Although its exact mechanism is still not fully understood, UCP3 is suggested to protect mitochondria against lipid-induced damage, possibly via modulation of ROS-production. Interestingly, aging has been associated with increased ROS-production, while UCP3 expression decreases with age. We therefore hypothesized that UCP3 overexpression has beneficial effects on aging-related ROS-production. To test this, an ESR-spectroscopic method was set up to specifically measure superoxide anion production in isolated muscle mitochondria.

Methods: Skeletal muscle mitochondria were freshly isolated from ~25- and ~75-week-old male C57Bl6 mice overexpressing UCP3 (UCP3Tg) (n=12, n=5), and their wild type (WT)-littermates (n=10, n=5). Mitochondria were kept in medium and incubated for 5 min at 37°C. After addition of spin trap (5,5-dimethyl-1-pyrolline N-oxide) and glycolytic (glutamate+succinate) substrates with or without 4-hydroxy-2-nonenal (4-HNE), to activate UCP3, ROS-production was measured in glass capillaries, using Bruker EMX 1273 ESR-spectroscopy (CW X-band). Control experiments confirmed that this method directly determines substrate-dependent mitochondrial superoxide anion production.

Results: ROS-production increased with age ($p<0.001$). In the presence of 4-HNE, an activator of UCP3, ROS-production was reduced in UCP3Tg vs WT-mice ($p<0.01$) increased with age ($p=0.001$), and showed a significant time*effect interaction ($p<0.05$), with a blunted increase in ROS-production with age in UCP3Tg mice compared to their WT-controls.

Conclusion: During aging, mitochondrial ROS-production increases. UCP3 overexpression, and when activated with 4-HNE, leads to blunting of the age-induced increase in ROS-production.

This study was supported by the Netherlands Organisation for Health Research & Development (ZonMw).

eNOS enzyme releases significant quantities of NO radicals in septic mice

A. van de Sandt [1], E. van Faassen [2], A. Gödecke [3], R. Windler [1], S. Becher [1], P. Kleinbongard [1], T. Rassaf [1], M. Kelm [1], J. Schrader [3], M.W. Merx [1]

[1] Division of Cardiology, Pulmonary Diseases, and Vascular Medicine, Department of Medicine, University Hospital Aachen, Germany; [2] Debye Institute, Surfaces, Interfaces and Devices, Utrecht University, Netherlands; [3] Institute of Physiology, Heinrich-Heine-University Düsseldorf, Germany.

Purpose: High-level nitric oxide (NO) production plays a central role in the pathogenesis of sepsis. Excessive NO contributes importantly to organ dysfunction, in part through the generation of nitrosating NO metabolites. The high NO levels in sepsis are usually attributed to upregulation of inducible NOS (iNOS). However, we recently demonstrated an absence of hypotension, no signs of septic cardiomyopathy and improved survival in septic $eNOS^{-/-}$ mice. This suggests that endothelial NOS (eNOS) also produces significant quantities of NO in the setting of sepsis. The aim of this study is to elucidate the relative contribution of eNOS as high-capacity NO-producing enzyme leading to organ dysfunction in a clinically relevant polymicrobial cecum ligation and puncture (CLP) model of sepsis.

Methods: B6/c57 wildtype (WT) and $eNOS^{-/-}$ mice were rendered septic by CLP. 12 hours after sepsis induction, endogenous production of NO was detected in liver, heart and kidneys using spin trapping with Fe-DETC complexes. The spin trapping proceeded *in vivo* for 30 min before sacrifice of the mice. The organs were excised, incubated in strong HEPES buffer, snap frozen in liquid nitrogen and stored at 77K for EPR assay. The tissue samples were reduced with dithionite (50 mM, 15min) to remove the overlapping EPR spectra from Cu^{2+}-DETC complexes.

Results: EPR spectroscopy showed the characteristic triplet spectrum of ferrous mononitrosyl iron complexes (MNIC, NO-Fe^{2+}-$(DETC)_2$) in heart, liver and kidneys of $eNOS^{-/-}$ mutants and wildtype mice. Septic wildtype displayed higher MNIC yields in all tissues compared to non-septic wildtype mice. Tissues of septic $eNOS^{-/-}$ mutants contained significantly lower MNIC than those of septic wildtype mice. Controls and sham-operated mice had comparable MNIC yields. In absence of sepsis, WT had similar yields as $eNOS^{-/-}$. However, septic WT had far higher MNIC yields than septic mutants.

Conclusion: Genetic deletion of eNOS led to markedly suppressed MNIC yields when sampled 12 hours after induction of sepsis. The results suggest that eNOS contributes significantly to endogenous NO production and resulting organ dysfunction in sepsis.

Table *MNIC yields WT vs. $eNOS^{-/-}$*

	WT			$eNOS^{-/-}$		
pmol MNIC/ mg wet tissue	Kidney	heart	liver	Kidney	heart	liver
Control	0.20±0.03	0.35±0.01	0.5±0.25	0.22±0.02	0.30±0.03	0.33±0.13
Sham	0.28±0.05	0.34±0.02	1.18±0.63	0.28±0.04	0.34±0.03	0.49±0.12
Clp	0.84±0.00	1.02±0.20	6.00±0.00	0.43±0.04	0.53±0.02	2.63±0.42

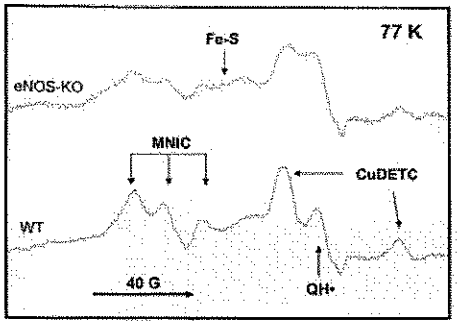


Figure *EPR spectra at 77K of entire hearts from septic mice.* The $eNOS^{-/-}$ mutant (top) contains approx. 0.55 pmol MNIC / mg. The WT (bottom) has twice this amount (1.2 pmol/mg). The tissues also show signals from some residual Cu^{2+} DETC, ubisemiquinone radicals (QH•) and iron-sulfur clusters (Fe-S).

Atomic identification of the Si-B5 paramagnetic center by electron spin resonance

K. Keunen and A. Stesmans
Department of Physics, University of Leuven, Celestijnenlaan 200D, 3001 Leuven, Belgium

During ion implantation, widely used in the fabrication of Si-based devices, intrinsic defects, comprising the vacancy, self-interstitial and their aggregates, are introduced into the c-silicon substrate. Self-interstitials are believed to play an important role in various dynamic phenomena, such as self diffusion and transient enhanced diffusion of dopants, limiting the size of next-generation submicron semiconductor devices. Therefore, accurate identification of such interstitial related defects is essential. While various experimental and theoretical reports have provided an accepted model for the structure of the extended, interstitial related {311} defects, little is known of the first stages of the aggregation process of self interstitials.

Previous electron spin resonance (ESR) studies have revealed several paramagnetic centers in neutron irradiated (100) Si after vacuum annealing in the temperature range T_{an}= 50 °C - 500 °C, such as the Si-P3, Si-P6, Si-H8, Si-B5 and Si-B3. The Si-B5 ESR spectrum has previously been suggested to originate from a tri-interstitial defect, however, the link was not decisive because of the insufficient hyperfine structure information. In the present work, we report on a thorough ESR analysis of the Si-B5 spectrum, using three different frequencies (X, K or Q-band). Five elements of ^{29}Si hyperfine structure, similarly appearing at the three frequencies, are resolved, from where, through comparison with previous theoretical studies, strong evidence is provided for assigning the Si-B5 spectrum to a tri-interstitial defect. Measurements carried out in the range T= 4 K-120K showed the hyperfine splittings and principal g values to be temperature dependent.

New development in structure determination by pulsed EPR

S. Milikisyants, F. Scarpelli, E. J.J. Groenen and M. Huber
Department of Molecular Physics, Leiden University, The Netherlands

In the last decade, structure determination in disordered systems became one of the major directions in the development and application of pulsed EPR spectroscopy. EPR uses unpaired electrons as probes of the geometry of the system, measuring the dipole-dipole interaction strength between the electron spins. The dependence of the dipole-dipole interaction between two radical fragments on distance as well as on their relative orientation opens up the possibility to determine the relative geometry of the coupled radicals.

In recent years, a variety of methods for pulsed EPR has become available, but many problems remained unsolved as yet. Among those are the distance measurements involving electron spins with large g-anisotropy and/or fast relaxing spins, measurements of short distances (<1.5 nm), and the determination of the relative orientation of two radicals.

Here we report recent progress in the development pulsed EPR methods for structure determination. In particular, we will discuss a modification of the widely used double electron-electron resonance (DEER) pulse sequence. A new pulse sequence, observer-selective DEER, is introduced, which makes it possible to use long observer pulses with bandwidth that are smaller than the dipolar interaction.

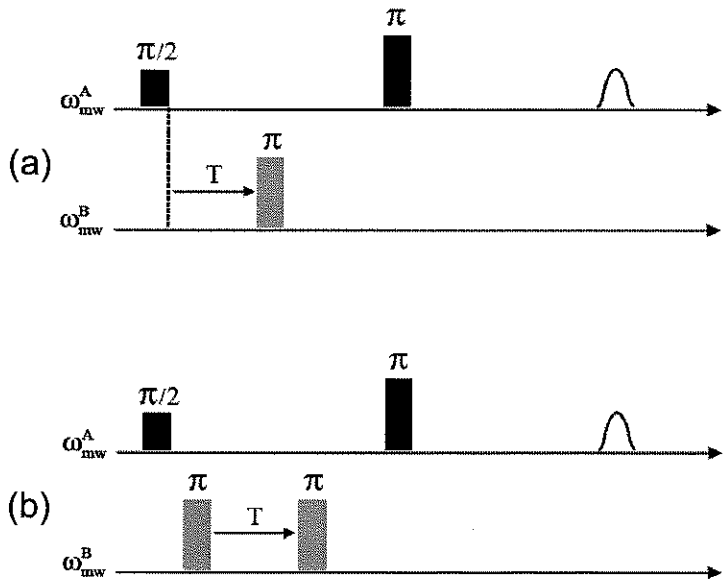


Fig.1. Pulse sequences for DEER experiments: (a) Three-pulse DEER; (b) Four-pulse observer-selective DEER.

Poster Contributions

In situ ultrasonic influence on Si nanoparticle formation in SiO_2 through ion implantation

M. Jivanescu, A. Stesmans

Department of Physics, University of Leuven, Celestijnenlaan 200 D, B-3001 Leuven, Belgium

What keeps Si so attractive for the electronics technology draws on various facts, such as unlimited resources, availability of a superb natural insulator (SiO_2) and good thermal and mechanical properties. The drawback of bulk Si is being an indirect semiconductor, which makes it an inefficient light emitter. Consequently, Si was replaced in optical components with more efficient materials. Si nanoparticles embedded in oxides (mainly SiO_2), combining the strength of bulk Si with the photoluminescence (PL) properties exhibited by porous Si, make Si based optical components possible and fabrication methodologies easier. Electron spin resonance (ESR) has been used to show that the origin of room temperature PL of nanocrystalline Si embedded in SiO_2 can be tuned[1] between quantum confinement and interface point defects[2].

The application of certain ultrasonic frequency treatments during Si^+ implantation of SiO_2 decreases PL, which was not fully understood. Here, we use ESR to study these Si^+ implanted SiO_2 at the atomic level in an attempt to explain the reduced PL in terms of occurring paramagnetic point defects. In the as implanted samples, three types of point defects are observed: E'_γ and EX, which pertain to the oxide, and a previously unreported signal we label IS (Implantation induced Signal). During 1100 °C annealing Si agglomerates, forming Si nanocrystals, information proved by the appearance of P_b-type defects, archetypal Si/SiO_2 interface defects. After annealing the IS signal is no longer visible and the SiO_2 specific defects are present in substantially lower densities than in the as-implanted ones. We observe a correlation between the total number of defects and the optic response, which guides to the idea of point defect originated PL.

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Thermal stability of the (111)Si/c-Lu_2O_3 interface probed by electron spin resonance

P. Somers, A. Stesmans, and V.V. Afanas'ev

Department of Physics, University of Leuven, Celestijnenlaan 200D, 3001 Leuven, Belgium

High-κ materials are expected to replace amorphous a-SiO_2 in the next generation metal-oxide-semiconductor structures. A critical criterion for these high-κ materials, besides having a high dielectric constant, is a high quality interface with Si, since this fundamentally influences device performance. Generally, scientific work has focused on the use of amorphous high-κ materials to replace a-SiO_2, although an intrinsic property of an amorphous/crystalline interface is defect formation due to steric hindrance and bond coordination. Therefore, an interesting approach to control this interface and suppress SiO_x interlayer formation is the use of crystalline high-κ materials epitaxially grown on Si.

In this work, we present for the first time the results of an electron spin resonance analysis of a crystalline high-κ (Lu_2O_3) epitaxially grown on (111)Si by molecular beam epitaxy, with special attention to the crystalline interface in terms of occurring paramagnetic point defects. Three important results are obtained: (1) In the as-grown state, a P_b –type defect (the archetypal defect at the (111)Si/SiO_2 interface) is observed, indicating a non perfect epitaxy. (2) Annealing the c-Lu_2O_3/(111)Si entities at 550°C in vacuum already results in the formation of a SiO_x interlayer, as evidenced by the observation of an increase in P_b defect density and the appearance of the EX-center, a SiO_2 associated defect. (3) After standard H-passivation (405°C; H_2; 1h) a P_b density of $5.76 \pm 0.6 \times 10^{11}$ cm^{-2} was observed i.e. the standard H-passivation technique fails to deactivate the P_b's, fast interface traps, to a sub 10^{11} cm^{-2} eV^{-1} level.

EPR characterization of organic materials for 3-D heterojunction solar cell applications

A. Aguirre , E. Goovaerts, S. Van Doorslaer, G. Janssen.

Department of Physics - CDE, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen, Belgium.

Plastic (organic) photovoltaics is increasingly catching the attention of the scientific community because it offers interesting perspectives for large area / low cost / environmentally friendly / energy production. 3-D heterojunction based organic solar cells comprise donor/acceptor blends, in which a polymer is normally acting as an electron donor while a soluble fullerene derivative acts as acceptor. The light induced charge transfer (from donor to acceptor) gives rise to positive radicals (or polarons) on the polymer chains and negative radicals on the fullerene molecules. EPR is a powerful tool to monitor the charge transfer processes in these blends, and also allowed an in-depth investigation of the radical states in doped P3HT and MDMO-PPV, the standard polymers presently in use in state-of-the-art devices.

Three groups of conjugated polymers have been newly synthesized in order to improve the available organic solar cells technologies, namely: materials with a better solubility (in environmentally more friendly solvents) [1], acceptors (as alternative to PCBM) [2] and materials with a lower band-gap (for better sunlight capture) [3]. CW-EPR measurements at high frequency (95 GHz) on composites of these conjugated polymers with PCBM allow the detection of efficient charge transfer from donor to acceptor and the discrimination of the ***g***-tensor components of the polaron in the different polymers.

Theoretical studies [4] in PPV and thiophene positive radical states (P^+) motivated the study of the polarons formed in MDMO-PPV and P3HT [5]. Knowledge of the relationships between the structure and electronic properties of the polymers can contribute to the development of better photovoltaic devices. Extensive pulsed EPR (ESEEM, HSYCORE and pulsed ENDOR) experiments in a range of frequencies revealed hyperfine couplings with nuclei in the vicinity of the radicals formed and allowed the study of the orientation dependent interactions at the molecular level. These EPR results provide valuable information regarding the orientation of these polymers on the spin coating substrates and the extension of the polaron.

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Corresponding author: aranzazu.aguirre@ua.ac.be

W-band transient EPR on the photoexcited states of spin-labelled fullerenes

H. Moons[*], E. Goovaerts[*], I. Nuretdinov[&], L. Franco[#], C. Corvaja[#]

[*] Department of Physics-CDE, University of Antwerp, Belgium
[#] Department of Chemical Sciences, University of Padova, Italy
[&] Arbuzov Institute of Organic and Physical Chemistry, Kazan Scientific Center of the Russian Academy of Sciences, Russia

Nitroxide radicals can be used as a spin label in different molecular environments to study structural and dynamical effects. Due to the versatile chemistry of fullerenes, it is possible to covalently attach one or more spin labels to the C_{60}-cage. The unpaired electron is localized almost completely on the NO-group and the NO-radicals can be seen as separate from the C_{60}-moiety on the same molecule.

Pulsed laser excitation of C_{60} molecules generates a relatively long lived triplet state, due to efficient intersystem crossing from the first excited singlet state. This property is preserved in the fullerene derivatives. The triplet electron spins ($S = 1$) will interact with the attached nitroxide radicals ($S = ½$), inducing a higher spin system ($S \geq 3/2$). These transient spin systems are investigated by transient EPR[3].

Here we present W-band transient EPR experiments of fullerene derivatives containing one, two or four nitroxide radicals. Spin polarisation effects due to selective population and decay pathways are observed in the EPR spectral components which appear in enhanced absorption and emission. Different linkage from nitroxides to C_{60}-moiety gives rise to different spectra. The use of higher fields allows for a better time and spectral resolution (i.e. g-factor resolution) which are indispensable for the precise assessment of the subsystems that comprise the total spin system and their mutual interactions.

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LOCALIZATION OF Mn^{2+} IMPURITY IONS IN NANOCRYSTALLINE ZnS AS DETERMINED BY MULTIFREQUENCY EPR

S. V. NISTOR[1], M. STEFAN[1], D. MATESCU[1] AND E. GOOVAERTS[2]
[1] *National Institute for Materials Physics, POB MG 7 Magurele, Romania*
[2] *University of Antwerpen-CDE, Physics Department, Antwerpen-Wilrijk, Belgium*

In the explosive development of investigations of nanosized semiconductors doped with activating impurity ions the Electron Paramagnetic Resonance (EPR) spectroscopy plays a central role in determining the localization of the doping transition ions [1]. Since the pioneering work of Bhargava and Gallagher [2] a large number of papers were dedicated to the EPR investigation of the Mn^{2+} ions in nanocrystalline ZnS, a wide band gap II-VI semiconductor, well known for its outstanding optoelectronic properties. An examination of the several publications on this subject [3] shows a large spread in the spin Hamiltonian parameters values and associated local symmetries of the Mn^{2+} centers reported so far. Moreover, what is even more disturbing, in many cases the attempts to reproduce the experimental spectra using lineshape spectra simulation programs with the reported spin Hamiltonian (SH) parameters are failing. This is mainly due to the fact that the experimental investigations and corresponding spectra analysis have been performed only in one microwave frequency band. In such case very little useful information concerning the presence of different paramagnetic centers, as well as their SH parameters, can be obtained from the recorded spectrum, considering that for nanocrystalline powders many of the features specific for single crystal EPR spectra are wiped out by the combined spatial averaging and line broadening mechanisms, which are characteristic for nanomaterials.

We will present here a modality to largerly overcome this difficulty by performing a combined quantitative analysis of the EPR spectra recorded in both low (X-band) and high (W-band) microwave frequency bands with EPR line shape simulation and fitting programs. The nanomaterial under investigation is nanostructured ZnS doped with 0.2% mol Mn^{2+} synthesized at room temperature by a surfactant-assisted liquid-liquid reaction [4]. According to microstructural investigations it has a stable mesoporous structure with pores of 2 nm diameter, its walls being built from 2.5 nm nanocrystals of cubic (blende) ZnS.

The quantitative analysis of the EPR spectra recorded at room temperature has resulted in the unambiguous identification of three types of Mn^{2+} centers, attributed to the different localization of the Mn^{2+} ions in the mesoporous structure and the determination of their spin Hamiltonian parameters. Thus, the Mn^{2+}(I) centers, which are the majority of the observed Mn^{2+} centers, were identified as Mn^{2+} ions localized in the ZnS nanocrystals lattice. The Mn^{2+}(II) centers seem very likely to be localized inside, close to their surface/ at the grain boundaries of the ZnS nanocrystals. Meanwhile, the third type of Mn^{2+}(III) centers, which are more sensitive to thermal and chemical treatments, were attributed to Mn^{2+} ions adsorbed at the pore walls and bound to OH^- molecules. This identification is supported by correlated XRD, TEM and EPR investigations on thermo-chemically treated samples.

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Incorporation of nitrogen in diamond nanograins: Size dependent ?

A.K. Dhami [a], Igor I. Vlasov [b], and Etienne Goovaerts [a]

[a] Physics Department – CDE, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen (Belgium),

E-mail: Etienne.Goovaerts@ua.ac.be;

[b] General Physics Institute, Russian Academy of Sciences, 38 Vavilov str., 119991 Moscow (Russia);

In ultra nano crystalline diamond samples, the effect of nitrogen incorporation has been reported to give interesting results on the various materials properties as well as in controlling the shape of the grains[1]. It has also the potential to be used in quantum computing and coherent coupling [2]. In the present studies, nano grains of diamond powder of different origin and dimensions are compared for the presence of nitrogen in the diamond crystal phase by means of CW EPR and pulsed EPR. The spectra of samples with grain sizes of 5 nm and 55 nm are compared at room temperature and 5 K. Nitrogen gas used during synthesis process has been clearly found to be incorporated into the 55 nm grains of diamond samples, as detected in the CW EPR spectra by a weak hyperfine triplet superimposed on a relatively broad line originated from paramagnetic species at the grain surface or in the intermediate carbon phases. Field-swept electron spin echo (ESE) measurements allow to suppress this signal and obtain selective and sensitive detection of the nitrogen atoms incorporated in diamond. The sample with smaller 5 nm particles does not show any signatures of nitrogen, even in the best detection conditions. Further experiments are underway to determine the limiting particle size above which nitrogen can be successfully incorporated.

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High frequency W-Band (94 GHz) EPR study of the hole trapped Bismuth centre in $Bi_{12}MO_{20}$ (M: Si, Ti) single crystals

I. Ahmad[a], *V. Marinova*[a,b] *and E. Goovaerts*[a]

[a] *Experimental Condensed Matter Physics, Department of Physics, University of Antwerp, Universiteitsplein 1,B-2610 Wilrijk, Belgium*

[b] *Central Laboratory of Optical Storage and Processing of Information Sofia, Bulgarian Academy of Sciences, 1784 Sofia, Bulgaria*

Abstract:

Electron paramagnetic Resonance (EPR) spectroscopy provides valuable information about the microscopic structure of the impurity centers in oxide crystals involved in their opto-electronic properties [1]. The investigated crystals are technological important materials that can be used for holographic data storage purposes and for optical information processing. An EPR investigation of $Bi_{12}SiO_{20}$ (BSO): non-doped BSO, BSO:Ce, BSO:Ru, non-doped $Bi_{12}GeO_{20}$ (BGO) and $Bi_{12}TiO_{20}$ (BTO) doped with Cr has been performed at W-band frequency (94 GHz) at T = 12 K, before and after in-situ illumination. We have observed an isotropic hyperfine spectrum having g-values close to free electron ($g \sim g_e$) and large hyperfine splitting. The observed spectrum is attributed to a hole trapped at an antisite Bi^{3+} defect (Bi^{3+}+h) at the tetrahedral Si or Ti site. To our knowledge no EPR study of Bi defects in BMO crystals has been reported so far however an ODMR study via magnetic circular dichroism (MCD) is reported [2]. We used the defect model proposed by [2] in comparison with our results. In case of non doped BSO and BSO: Ce the EPR spectrum is only observable after Ar^+ ion laser illumination (457 nm) for a few minutes, while in BSO:Ru increase in intensity has been observed after the illumination indicating the raise in charge carrier concentration. An identical line pattern was observed for BTO: Cr with no effect of illumination. The best fit of spin Hamiltonian yields an isotropic g-value (2.0320±0.004) and hyperfine constant (19.315±0.030 GHz) for non-doped BSO. Near identical parameters have been determined for the (Bi^{3+}+h) centre in BSO and in BTO, showing that the delocalization is quite insensitive to the host crystal.
Parallel measurements of photochromism and transient absorption at different temperatures will be presented in correlation with the EPR results.

Financial support from the Fund for Scientific Research, Flanders (FWO, Belgium) is gratefully acknowledged as well as a grant to V. Marinova as an FWO visiting postdoctoral researcher.

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Temperature dependence of two Eu-related EPR spectra in CsBr medical image plates

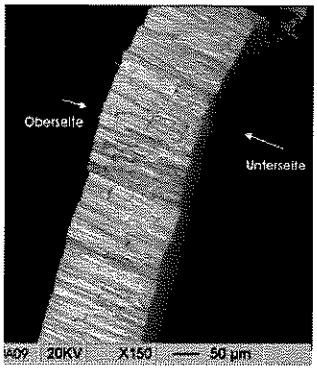
F. Loncke, H. Vrielinck[§], P. Matthys and F. Callens
Department of Solid State Sciences, Ghent University, Krijgslaan 281 – S1, B-9000 Gent, Belgium
[§] Postdoctoral fellow of the Flemish Research Foundation (FWO-Vlaanderen)

J.-P. Tahon and P. Leblans
Agfa Healthcare NV, Septestraat 27, B-2640 Mortsel, Belgium

I. Ahmad and E. Goovaerts
Physics Department, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen (Wilrijk), Belgium

Storage phosphors are nowadays being used in digital image plates for medical X-ray radiography. In many hospitals they have replaced the conventional screen/film technology. $BaFBr:Eu^{2+}$ powder image plates suffer from a loss of resolution due to light scattering. Various other compounds (CsBr, RbBr,...), on the other hand, can be grown in needle image plates (NIPs), which are thermal vapour deposited binderless screens, consisting of needle-shaped microcrystals. Using NIPs strongly reduces the resolution problem and since $CsBr:Eu^{2+}$ has several other favourable X-ray storage properties (high X-ray absorption, high conversion efficiency, low read-out energy, easy erasability, ...), the fastly growing appearance in hospitals of $CsBr:Eu^{2+}$ based NIPs is not surprising. Up till now, however, the physics of the writing and reading processes in these plates are not well understood yet, unlike for the $BaFBr:Eu^{2+}$ based powder plates, where EPR played a major role in identifying the involved radiation-induced as well as dopant-related defects.

Our earlier Q- and W-band room temperature EPR measurements on the CsBr:Eu NIPs revealed the presence of a tetragonal Eu-related defect [1], whose EPR signal intensity is linearly correlated with the sensitivity of the plates [2]. To retrieve more information about the nature of this defect, ENDOR measurements are necessary and were already successfully started. However, lowering the temperature leads to drastic changes in the EPR spectrum, which complicate the analysis of the ENDOR spectra. Detailed analysis demonstrates that below 35 K, the spectrum of a second Eu-related defect, with nearly extreme rhombic symmetry ($E/D \approx 1/3$), emerges and dominates the EPR spectrum at lower temperatures.



SEM picture of a CsBr NIP

In this poster we present the analysis of the temperature dependence of the EPR spectra. For both the tetragonal and the orthorhombic Eu-related defect, zero-field splitting parameters are determined. We conclude with some initial propositions for defect models, inspired by our preliminary ENDOR results.

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[2] "Paramagnetic Eu^{2+} center as a probe for the sensitivity of CsBr X-ray needle image plates", F. Loncke, H. Vrielinck, P. Matthys, F. Callens, J.P. Tahon and P. Leblans, Appl. Phys. Lett., submitted

EPR and TL study of irradiated K_2YF_5 phosphors

D. Zverev, H. Vrielinck[§], P.F. Smet[§], D. Poelman and F. Callens
Department of Solid State Sciences, Ghent University
Krijgslaan 281-S1, B-9000 Gent, Belgium

Recently it was discovered that K_2YF_5 crystals singly doped with rare earth (RE) ions like Ce^{3+}, Dy^{3+}, Tb^{3+} and Tm^{3+} are promising phosphors for thermoluminescence (TL) dosimetry [1,2]. They show relatively high sensitivity to ionising radiation and low fading, while for some K_2YF_5:RE combinations the shape of the TL glow curves depend on the type of radiation field, which offers possibilities for discriminating mixed radiation fields. Here we present the results of our combined TL and EPR study of K_2YF_5:Ce (0.2%) crystals in response to X-ray irradiation at 77 K. Prior to irradiation, the EPR spectra reveal the initial valence state of the RE dopant and unintentional intrinsic and impurity centres.

K_2YF_5 has an orthorhombic crystal structure, its space group being Pnam. YF_7 polyhedra, which have the ab-plane as mirror plane, form chains along the twofold screw c-axis. In principle a large number of distinct intrinsic and dopant-related defects could be formed upon irradiation.

After X-ray irradiation at 77K, signals of at least seven different trapped electron or hole centres are resolved in the EPR spectra. The angular variation of the EPR spectra was studied in the three principal crystallographic planes. Five spectral components have a structure typical of a centre with electron spin S=½ exhibiting a strong hyperfine interaction with two ^{19}F nuclei. Therefore, they may be identified as V- or H-type (F_2^-) intrinsic trapped hole centres. Three V-centres are characterized by a monoclinic g tensor with one principal axis along the crystal's twofold screw axis, and tilted in the ab-plane. Two others have a triclinic g tensor. Via thermal annealing experiments, the role of these centres in the TL processes below room temperature is investigated.

Apart from F_2^- centres, during X-ray irradiation two other centres were formed. For both centres the EPR spectra exhibit a complex multi-line structure. One of them can be described by S=½ and shows an almost isotropic g tensor (g_{iso}=1.969). This centre probably relates to a trapped-electron F-centre. The other centre can also be described by a spin-Hamiltonian with S=½ and anisotropic g-tensor, with the smallest principal value directed close to one of the Y-F orientations. The nature of these centres is, however, not yet entirely clear and is the subject of future ENDOR and pulsed EPR investigations.

[§]Postdoctoral fellow of the Flemish Research Foundation (FWO-Vlaanderen)

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EPR analysis of vanadium silicate-1 nanoparticles deposited on the mesoporous walls of SBA-15

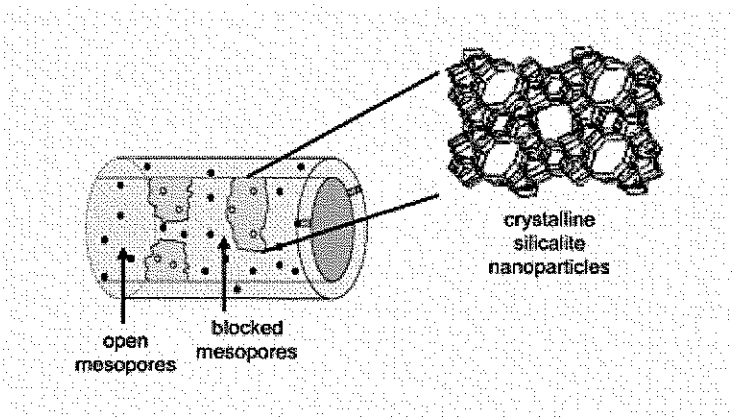
S. Zamani[a], M. Chiesa[b], V. Meynen[c], X. Yiqun[a], P. Cool[c], E. Vansant[c], S. Van Doorslaer[a]

[a] *University of Antwerp, Laboratory for Spectroscopy in Biophysics and Catalysis, Department of Physics, Universiteitsplein 1, B-2610 Wilrijk, Belgium*

[b] *Dipartimento di Chimica IFM, Università di Torino and NIS Centre of Excellence, via P. Giuria 7, 10125 Torino, Italy*

[c] *University of Antwerp, Laboratory of adsorption and Catalysis, Department of Chemistry, Universiteitsplein 1, B-2610 Wilrijk, Belgium.*

Mesoporous supports, such as the hexagonally ordered SBA-15, are generally less stable than microporous materials, but they have the advantage that they allow for the penetration of larger molecules. Recently, a new group of mesoporous materials was synthesized in which vanadium-activated zeolitic nanoparticles (vanadium silicalite-1 (VS-1)) were deposited inside of the mesoporous channels of SBA-VS-15.[1] These materials were reported to have enhanced mechanical stabilities compared to other mesoporous materials and accessibility to large molecules. In our earlier EPR study, we revealed clear differences in the local VO^{2+} environment in the non-calcined VS-1 nanoparticles deposited inside of the mesoporous channels of SBA-15 compared to the non-calcined full-grown VS-1 zeolites.[2] In the present work, we focus on the effects of aging of SBA-VS-15 and VS-1, the changes induced in the vanadium sites after a calcination/reduction cycle, and the adsorption of $^{13}CO_2$ and NH_3 to the non-calcined materials and to those materials that have gone through a calcination/reduction process. Different EPR techniques, such as X-band CW-EPR, ESE-detected EPR, pulsed ENDOR and HYSCORE (Hyperfine Sublevel Correlation) spectroscopy are used to elucidate the local structure and accessibility of the vanadium sites.



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Unraveling the activation process of the cobalt Jacobsen's catalyst: A combined EPR-DFT approach

Evi Vinck[1], Sabine Van Doorslaer[1], Damien M. Murphy[2], and Ian A. Fallis[2].

[1]*University of Antwerp, Department of Physics, Universiteitsplein 1, 2610 Wilrijk, Belgium*
[2]*Cardiff University, Department of Chemistry, Cardiff CF10 3TB, Wales, UK*

The chiral metal-salen complexes [M(**1**)], N,N'-Bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexanediamino-M (M= Co, Cu, Mn, VO, ...), introduced by Jacobsen and coworkers, have been shown to be effective catalysts for a wide range of asymmetric transformations. The [Co(**1**)] catalyst is now widely used to separate the enantiomers in racemic mixtures of terminal epoxides via a hydrolytic kinetic resolution (HKR) reaction. Despite its widespread use, many aspects of the HKR reaction are poorly understood. The structure of the active catalytic species, that is formed upon addition of acetic acid and exposure to air to [Co(**1**)] is unknown. In this work, the structural changes that occur upon activation of the catalyst are probed in detail, using CW and pulsed-EPR techniques. The CW-EPR data show that different molecules are formed upon activation of the catalyst, including a cobalt-bound phenoxyl radical, situated on ligand (**1**). It is the first time that such a species is observed. Detailed information on the electronic structure of the different species that are formed upon activation, including the phenoxyl radical was obtained using pulsed-EPR techniques, such as the four-pulse ESEEM technique HYSCORE (hyperfine sublevel correlation). The EPR data are complemented with density functional theory (DFT) computations, which corroborate that a stable phenoxyl radical can be formed on the salen ligand.

Schonland ambiguity in the determination of hyperfine tensors from angular dependence of ENDOR spectra: application to the T1 radical in sucrose single crystals

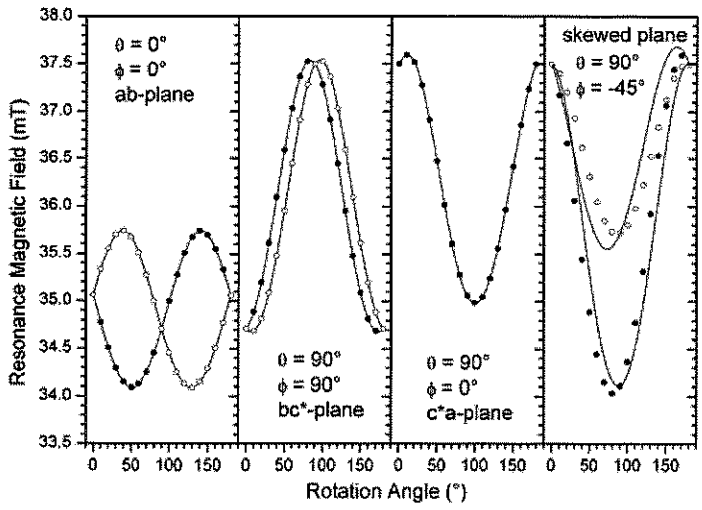
Hendrik De Cooman*, Henk Vrielinck** and Freddy Callens
Ghent University – Department of Solid State Sciences, Krijgslaan 281-S1, B-9000 Gent, Belgium

Sabine Van Doorslaer
University of Antwerp – Department of Physics, Campus Drie Eiken, Universiteitsplein 1-N, B-2610 Wilrijk, Belgium

* Research Assistant and ** Postdoctoral Fellow of the Flemish Research Foundation (FWO-Vlaanderen)

For the analysis of the angular dependence of EPR spectra of low-symmetry centres with S=1/2 in three independent planes, it is well-established that an ambiguity may arise in the best-fit $\vec{\vec{g}}$ tensor result. As illustrated in the figure for a centre with triclinic symmetry in a monoclinic crystal (two sites), two $\vec{\vec{g}}$ tensors (symbols, lines) can be found which fit the angular dependence of the resonance field positions equally well in three planes. Outside of these planes, the EPR angular dependence for these two tensors may differ considerably, demonstrating that only one of the solutions corresponds to the true $\vec{\vec{g}}$ tensor for the paramagnetic centre, while the other merely is a fitting result without physical meaning. Apart from a single crystal angular dependence in a fourth plane the ambiguity can also be lifted by recording powder spectra.

In this poster, we investigate whether this ***Schonland ambiguity*** also arises when determining the hyperfine (HF) $\vec{\vec{A}}$ tensor for nuclei with I=1/2 from angular dependent ENDOR measurements.



Via first order perturbation calculations of resonance frequencies, we show that for each of the M_S ENDOR branches in the angular dependence two best-fit $\vec{\vec{A}}$ tensors can be found, but that in general only one solution fits both branches. The ambiguity thus only arises when experimental data of only one M_S multiplet are used in analysis or in certain limiting cases. We illustrate the problem via the analysis of the HF interaction of a □-proton with an unusual $\vec{\vec{A}}$ tensor shape for the stable T1 radical, produced in sucrose single crystals after X-ray irradiation at room temperature. ENDOR measurements in a well-chosen fourth plane and powder HYSCORE measurements allowed for selection of the correct tensor shape. This specific interaction proved to be the key to the identification of this radical, through comparison between experimental HF tensors and those obtained by density functional theory calculations on certain radical models.

Temperature study of a glycine radical in the solid state: Periodic DFT calculations of vibrational and EPR properties

E. Pauwels, T. Verstraelen, H. De Cooman, V. Van Speybroeck and M. Waroquier
Center for Molecular Modeling, Ghent University, Proeftuinstraat 86, B-9000, Belgium

When biomolecular crystals are treated with ionizing radiation, different kinds of radicals are induced within the lattice. These species are applicable as dosimeters but also serve as model systems to study the effects of irradiation. The induced radicals can be examined with the aid of Electron Paramagnetic Resonance (EPR) spectroscopy. But, because structural assignment based solely on the spectroscopic properties is often complicated and ambiguous, molecular modeling based on density functional theory (DFT) is increasingly being used as a complementary tool to assist in this process.

In this work, we present the results of DFT calculations on a radiation-induced radical in crystalline glycine [1], in which both temperature and solid-state environment are accounted for. Ab-initio molecular dynamics (using the CPMD software) were conducted on the glycine crystal structure under periodic boundary conditions and at simulated temperatures of 100 K and 300 K. The EPR properties were calculated for several snapshots along these trajectories, resulting in a thermal average. In a comparison with experimentally determined spectroscopic properties the calculated EPR parameters were evaluated with respect to both temperature and environment.

In addition, a vibrational analysis was performed based on Fourier transformation of the atomic velocity autocorrelation functions. Using a novel band-pass filtering approach, several vibrational modes were identified and associated with experimental Infrared and Raman assignments. Decomposition of the calculated spectra in terms of radical motion revealed that several vibrational modes are unique to the radical, the most prominent feature at 702 cm-1 corresponding to out-of-plane motion of the paramagnetic center, inversely coupled with similar motion of the CO2 carbon. Since this frequency band is well separated from any other bands due to intact molecules, it might be applicable for discriminatory detection of glycine radicals.

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Dynamical, structural and functional aspects of neuroglobin

M. A. Ezhevskaya*, S. Dewilde**, L. Moens**, Y. Polyhach***, G. Jeschke***, S. Van Doorslaer*

*University of Antwerp, Department of Physics, Universiteitsplein 1, B-2610 Wilrijk, Belgium
** University of Antwerp, Department of Biomedical Sciences, Universiteitsplein 1, B-2610 Wilrijk, Belgium
***University of Konstanz, Unversitaetsstrasse, 78457 Konstanz, Germany

The vertebrate neuroglobins were discovered already 8 years ago, but their functions are still not known. Several interesting hypotheses have been put forward. The observed ability of human neuroglobin (NGB) to form a disulfide bridge and its related change in oxygen affinity led to the assumption that NGB regulates oxygen release in hypoxia conditions. In the disulfide bridge, a Cys located on the flexible CD loop is involved. This flexible loop is found in many globins and has been suggested to modulate the function and accessibility of the heme region. Furthermore, some studies indicate that NGB would give protection against ROS (reactive oxygen species). In this work, we attempt to elucidate some of the above points using EPR. Three NGB variants with an MTSSL spin label attached at one of the three native Cys are constructed using site-directed mutagenesis. In a first step, the mobility of the spin label is studied for these different sites. The experimental correlation times are confronted with theoretical predictions based on the PDB structure of NGB and using the rotamer library approach developed by G. Jeschke and co-workers. In a second set of experiments, the distance between the spin label and the heme iron (Fe(III)) are determined via determination of T_1 and T_2 using saturation recovery, time-dependent CW EPR and two-pulse ESEEM. In a last part, the reaction of neuroglobin with ROS are followed by CW EPR (use of both spin trapping agents and freeze quenching).The results will be related to the protein's possible functions.

EPR of the globin domain of the globin-coupled sensor of *Geobacter sulfurreducens*

Filip Desmet[a], Liesbet Thijs[b], Hassane El Mkami[c], Graham Smith[c], Sylvia Dewilde[b], Luc Moens[b], Sabine Van Doorslaer[a]

[a] *Department of Physics, University of Antwerp*
[b] *Department of Biomedical Sciences, University of Antwerp*
[c] *Milimetre Wave and High Field ESR Group, University of St. Andrews*

We present the results of our EPR investigation of the globin-coupled sensor (GCS) of the bacterium *Geobacter sulfurreducens*. These GCSs are multi-domain heme proteins that combine a heme-containing globin domain with a signal-transduction domain. Gaseous ligands such as O_2, CO and NO can bind to the heme iron atom in the globin domain. This ligand binding triggers a signal-transduction cascade that results in the activation of the second domain, however at present little is known about this mechanism.

We have used X- and Q-band pulsed and CW EPR in combination with different optical techniques to investigate the ferric and NO-ligated ferrous forms of the globin-domain of *Gs*GCS.
The UV/Vis absorption, resonance Raman and CW-EPR spectra of ferric *Gs*GCS showed unambiguously that this protein exhibits a bis-histidine coordination of the heme iron. We used an in-house developed pulsed EPR strategy to determine the orientation of the axial imidazole ligands of the heme iron. This method involves spectral simulations of the experimental HYSCORE and pulsed ENDOR spectra. The resulting structural parameters will be confronted to preliminary XRD results on the protein.
The NO-bound form of *Gs*GCS was studied with CW EPR. Interestingly, this study shows that binding of nitric oxide breaks the bond between the heme iron atom and the evolutionary conserved proximal histidine, which is reflected in the NO-binding kinetics.

IMPACT OF A CYCLODEXTRIN ON LIPOSOMES AND CELLS: AN ESR EVIDENCE

Angeliki A. GRAMMENOS[A], M.A. BAHRI[A] , G.PIEL[B] AND M. HOEBEKE[A]

[A]*Laboratory of biomedical spectroscopy, Institute of Physics B5, University of Liège, Belgium, A.Grammenos@ulg.ac.be*
[B] *Laboratory of Pharmaceutical Technology, Department of Pharmacy, University of Liège , Belgium*

Available knowledge indicates that cellular membranes manage their vast chemical diversity by sorting into specialized micro domains[1]. These domains are now referred to under the general heading of lipid raft. Cholesterol plays a fundamental role in maintaining their structure and function[2]. A way of better understanding these rafts is to use methylated-β-cyclodextrins, known for their capacity of cholesterol extraction. Our study focuses on the action of 2, 6 dimethylated –β-cyclodextrin (Rameb) on liposomial and cellular membrane.

As cellular membrane fluidity is related to the composition in cholesterol and polyunsaturated fatty acids of the membrane, Electron Spin Resonance (ESR) associated with spin labelling seems to be the best way to investigate the microviscosity's changes induced inside membrane at different depths [3].

Microviscosity is defined as the homogenous solution viscosity which results in the same spectrum as that obtained in the microenvironment[3]. Standard curves of microviscosities have been established by calibration of the ESR spectra of three n-doxyl stearic acids (n-DSA: n = 5, 12, 16) probes in glycerol-ethanol mixtures of known viscosities[2]. These curves allow us to quantify the effective microviscosity at different depths inside membrane by measuring the order parameter (S) and the correlation time (τ_c) on n-DSA ESR spectra.

We have shown that addition of Rameb to cholesterol saturated DMPC liposomes solution and HCT116 colon cancer cells leads to the expulsion of n-DSA out of the membrane. That means that Rameb is able to extract phospholipids from biological membrane. Small angle neutron scattering experiences on KWS1 (Jülich) confirm these results.

In consequence, a special protocol has been used in this work to measure microviscosity. All the studied structures have been washed to eliminate Rameb before n-DSA incorporation. In this way, we have shown that liposomes (70% DMPC: 30% CHOL) and HCT116 cells membrane microviscosity decrease in a Rameb dose dependant way. Moreover, addition of Rameb to the same structures dopped with cholestane leads to the expulsion of this probe out of the membrane. As cholestane is a membrane spin label with a chemical structure similar to cholesterol, all these results confirm that Rameb is able to extract cholesterol from biological membrane and permits us to quantify the cholesterol extraction in liposomial membrane. We have also calculated that HCT116 cellular membrane fluidity decrease linearly with the cyclodextrin time of incubation.

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Study of neuronal cells preconditioning by ESR

P.-H. Guelluy[1], G. Deby-Dupont[2], M. Hoebeke[1]

[1] *Biomedical Spectroscopy, Institute of Physics, University of Liège. phguelluy@ulg.ac.be*
[2] *Centre for Oxygen Research and Development (CORD), Institute of Chemistry, University of Liège.*

Preconditioning is a potential protection against stroke, cardiovascular occlusions and many other diseases[1,2]. Second window preconditioning (PC), also known as delayed preconditioning (DPC), is now studied for more than two decades[3] but still remains unclear. The DPC induces many physiological modifications within the cellular organelles, this is why its complete comprehension is so difficult. Some mechanisms seem nevertheless to have a major impact, reactive oxygen species (ROS) production is one of these. Using ESR, we have the possibility to i) quantify the temporal production of ROS following a brief period of sublethal stress (PC) or longer period of lethal stress (anoxic stress in our case), ii) qualify which kinds of ROS are produced (use of different scavengers and different spin trap) iii) measure the resultant PC-mediated modifications of some enzymes like superoxide dismutase, catalase, glutathione peroxidase, and others antioxidant enzymes (use of inhibitors).

In our study, we use a neuroblastoma cell line (neuro-2a), an anoxic stress and POBN spin-trap to assess the generation of ROS by ESR. The cells are always stressed under culture's conditions, this means that the culture medium (DMEM with foetal bovine serum and antibiotics) is not replaced by a classical phosphate buffer saline in order to avoid an uncontrolled additive cellular stress. The anoxic stress is realised by oxygraphic method (a Clark electrode provides the cellular level of O_2 in real time) which is much more realistic and less aggressive than the usual anoxic stress under pure N_2 atmosphere. The POBN spin-trap is combined with ethanol. In those conditions, in presence of $OH\cdot$, a characteristic 6-lines ESR spectrum is observed, reflecting the presence of POBN/$\cdot CH(OH)CH_3$ adduct. After several tests, one hour of anoxic stress has been chosen as preconditioning period, the severe stress consists of a three hours anoxic stress applied 24 hours later .The effect of DPC is significant on the ESR spectrum, indeed the production of ROS by preconditioned cells after the lethal stress is more or less 50% lower than the production of ROS by unpreconditioned cells, those results confirm the intervention of antioxidants into the process of preconditioning.

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Effect of statin on liposome and cell membranes fluidity. An ESR spin probe study.

A. NGENDAKUMANA[1], M. BAHRI[2] AND M. HOEBEKE[1].

[1] Laboratory of Biomedical Spectroscopy, Department of Physics, Institute of Physics, B5, University of Liège, Sart-Tilman, B-4000 Liège, Belgium, M.Hoebeke@ulg.ac.be
[2] Laboratory of Experimental medical Imaging, Department of Physics, Institute of Physics, B5, University of Liège, Sart-Tilman, B-4000 Liège, Belgium

Electron spin resonance (ESR) with nitroxide spin probe was used as a method to probe liposome and cell membranes microenvironments. ESR spectra are very sensitive to the probe anisotropic motion degree and any motional restriction caused by local viscosity is directly seen as a spectrum modification. Thus, absolute values of local viscosity haves been obtained after prior calibration of the ESR spectra of the probes in solvent mixtures of known viscosities (1). In these experiments, the microviscosity is defined as the homogenous solution viscosity, which results in the same spectrum as that in the microenvironment (2).

In the first time, a liposome model has been studied with a mixture of saturated, unsaturated and polyunsaturated phospholipids at a percent equal to those reported for monocyte cell membranes. This liposome model has a double objectives; firstly, to predict the monocyte cell membranes microviscosity and secondly, to see if statin has an effect on phospholipids organisation. The statin, a well-known drug acting as an inhibitor of 3-hydroxy-3methylglutaryl (HMG)-CoA reductase, works by decreasing the level of plasmatic cholesterol. The ESR method described above was used to measure the microviscosity of our liposome model membrane. The incorporation of cholesterol into liposome model increases the viscosity of membrane bilayer. While the statin, at a concentration of 1.9 μM, did not induce any viscosity change not on liposome without cholesterol nor on those doped with cholesterol. Hence, statin doesn't seem to perturb membrane phospholipids organisation. In a second time, we have applied ESR technique to investigate the effect of statin on monocyte cell membrane microviscosity. The microviscosity measured on cell membrane without statin was 270 cP and remained constant during the study period. However, the addition of statin at constant concentration (65.5μM) showed a significant decrease of the cell membrane's microviscosity. The value of microviscosity measured after five weeks of statin treatment was 218 cP.

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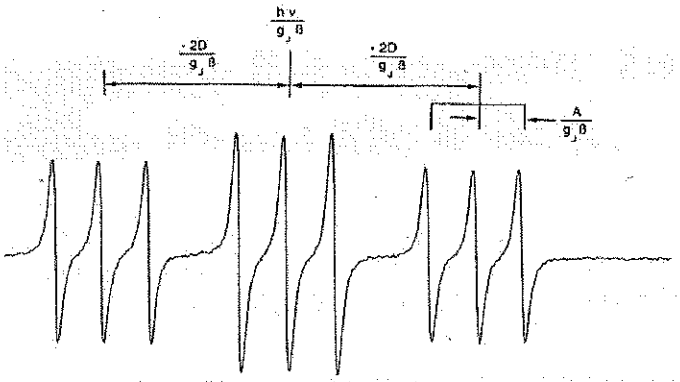
Radicals for life: Nitric Oxide and its reactions

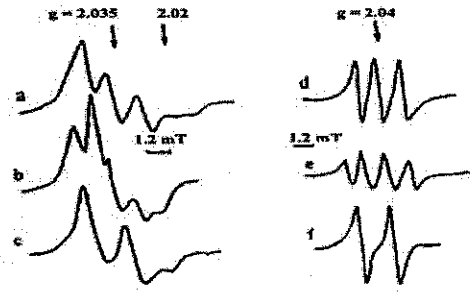
Ernst van Faassen,
Interface Physics,, Debye Institute,
Utrecht University, the Netherlands.

Nitric oxide (NO•) is a peculiar radical : Ground state is not paramagnetic (g = 0 since orbital and spin magnetic moments cancel); low reactivity with other molecules except superoxide (O_2^-•); thermodynamically unstable; dimerizes to N_2O_2; many crucial biological functions discovered after 1986; difficult to detect *in-vivo* and *in-vitro.*

Spin trapping with iron-dithiocarbamate complexes is unique method to detect NO• in tissues [1]: Trapping *in-vivo*, but EPR detection of the paramagnetic mononitrosyl-iron-carbamate adducts (MNIC) is *ex-vivo* in frozen tissue biopts.



X-band EPR spectrum of 1 Torr NO• gas at RT. The spectrum is 250 G wide and centered at 9100 Gauss. The ground state $^2\Pi_{1/2}$ is not paramagnetic (g=0), but the first excited state $^2\Pi_{3/2}$ is paramagnetic with g=4/5.



77 K **RT**

X-band EPR spectra of ferrous MNIC (S=1/2) complexes (g=2.03) depend on isotopes of N and Fe
a,d : ^{14}NO-^{56}Fe-DETC
b,e : ^{14}NO-^{57}Fe-DETC
c,f : ^{15}NO-^{56}Fe-DETC

left column: frozen solutions
right column: liquid solutions

We will discuss the technique for *in-vivo* detection of endogenous NO• radicals, and illustrate the method with three practical examples:

- Functional improvement of kidney transplants (with UMC Utrecht)
- Perinatal therapy of spontaneous hypertension (with UMC Utrecht)
- Nitrite therapy for large area skin burns (with UK Aachen)

[1] *Radicals for life: The various forms of nitric oxide.* Eds. Ernst van Faassen and Anatoly Fyodorovich Vanin, Elsevier, Amsterdam 2007.

Detection of nitric oxide in the rat bone marrow by electron paramagnetic resonance

M. Aleksinskaya[1], E. van Faassen[2], T.J. Rabelink[1] and A.J. van Zonneveld[1]

[1] Department of Nephrology and Einthoven laboratory of experimental vascular medicine, Leiden University Medical Centre, The Netherlands, [2] Faculty of Science, Department of Interface Physics, Utrecht University, The Netherlands.

Introduction. Endothelial nitric oxide (NO) is a key regulator for vasodilation, vascular remodeling and inflammation. Recent studies demonstrated that endothelial nitric oxide synthase (eNOS) expressed by bone marow (BM) stromal cells also facilitates the mobilization of endothelial progenitor cells (EPC). It is well established that patients at risk for cardiovascular disease with e.g. diabetes, hypertension, obesity or renal failure are generally affected by endothelial cell dysfunction in the peripheral vasculature, secondary to a reduced bioavalability of endothelial NO. To begin to explore the hypothesis that eNOS dysfunction is also present in bone marrow stromal cells and may be a causal factor in the impaired mobilization of EPC to the circulation, we have investigated whether electron paramagnetic resonance (EPR) can be used to detect NO levels in rat BM cell suspension.

Materials and methods. Suspensions of total BM cells were prepared by flushing the diaphysis of the bones (anterior and posterior limbs) of adult female Wistar rats. NO trapping in BM cell suspension was initiated by adding 2.5 mM DETC and 20 μM ferrous sulfate (final concentrations). If desired, the BM cell suspension was stimulated with either ionomycin (10 μM), Ca-ionophore (10 μM) or acetylcholine (10 μM). L-NAME (500 μM) was used as a non-selective inhibitor of NOS. After 30 min of NO trapping at 37°C cell pellets with the Fe-DETC complexes were collected in Hepes buffer and snap frozen in liquid nitrogen until the frozen column was assayed with EPR. EPR spectra were recorded at 77 K on a modified X-band ESP 300 radiospectrometer operating near 9.54 GHz with 20 mW power.

Results. About 55×10^6 BM cells were used per sample. Data listed in the table demonstrate that without stimulation no detectable MNIC signal was measured. However, agents known to stimulate NO synthesis by eNOS such as acetylcholine, ionomycin and Ca-ionophore clearly yielded significant MNIC signals. In the presence of the specific eNOS inhibitor L-NAME no signal could be detected.

Conclusions.

Our results demonstrate that EPR can detect NO in cell suspensions of rat BM and suggest that NO is derived from eNOS expressed in BM endothelial cells. In follow-up experiments we will employ EPR to assess the eNOS dependent NO production not only in healthy rats, but also in experimental disease models, like hyperglycemia, hypertension and renal failure.

Stimulus	MNIC (pmol/sample)
no	Not detectable
Acetylcholine (10 μM)	21
Ionomycin (10 μM)	45
Ca-ionophore (10 μM)	49
no + L-NAME (500 μM)	Not detectable
Ca-ionophore (10 μM) + L-NAME (500 μM)	Not detectable

Spin density distribution in foodstuff after heat treatment or irradiation studied by EPR imaging

Philippe Levêque, Quentin Godechal & Bernard Gallez
Biomedical Magnetic Resonance Unit, Université catholique de Louvain, Brussels, Belgium
philippe.leveque@uclouvain.be

Introduction

Free radicals are usually very unstable species which react quickly with other molecules, so that they can hardly be detected in liquid solution. In solid state, on the contrary, their half life is considerably longer and their presence has been widely demonstrated for years by EPR spectroscopy.

In foodstuffs, free radicals are known to be generated by various treatments commonly used in the industry, such as heath treatment or decontamination (sterilization) with ionising radiations. EPR spectroscopy is now recognized internationally as a gold standard method to detect radiation-processed food containing bones, cellulose or crystalline sugar[1].

In this report, we go a step further than standard EPR spectroscopy and investigate the possibilities offered by development of EPR imaging to delineated free radical distribution within biological samples (e.a. foodstuffs).

Method

Several representative samples of commercial foodstuff from various origins (vegetal & animal) were selected because they are known to be treated by heat or ionising radiation during their industrial processing: coffee bean, frog leg, etc.

Frog legs were freeze-dried to remove water before imaging; other samples were directly investigated.

Imaging was performed on a Bruker Elexsys E540 system operating at 9 GHz (100 kHz modulation frequency), equipped with a Super High Sensitivity Probe. Images were acquired either in 2D or 3D mode. Microwave power was selected within the linearity part of the Power-Intensity curve. Amplitude modulation was chosen so that it does not exceed one third of the signal line width.

Results

The spin density of the distal part of a frog hind leg is shown on Fig. 1. The main bones (calcaneum & astragalus) are very well delineated with a rather homogenous signal distribution. Tarsal and part of the metatarsal bones are clearly visible, but not resolved. Surprisingly, muscle tissue and Achilles tendon also give an EPR signal at g=2.0081 & 2.0076, but much lower in intensity, so that they are not visible with the conditions chosen to image the bones.

Roasted coffee bean presents a single band EPR signal at g=2.0100, with a line width of 0.8 mT, whereas this signal is absent from green coffee. Intensity distribution is not homogenous within the bean, the strongest signal arising from the centre (Fig. 2).

Discussion

EPR imaging is a powerful tool to study spatial distribution of spin densities within biological sample. In frog-leg irradiated at 5 kGy, it allows a very good separation of distal bones whose width does not exceed 1-1.5 mm; and smaller structures are also clearly visible even with a rather large signal line width (~1mT).

EPR imaging could be used together with conventional spectroscopy where a regional distribution of radicals is needed, particularly in irradiated samples. The method offers unique capabilities to monitor the fate of these free radicals in biological samples.

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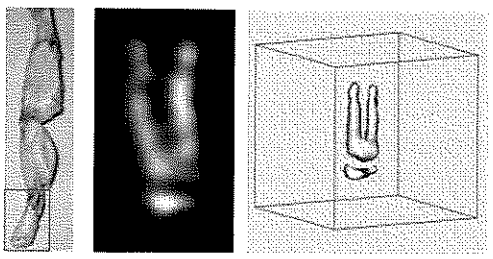

Fig 1: **A** Frog hind leg **B** 2D view of distal part (red square) **C** 3D view

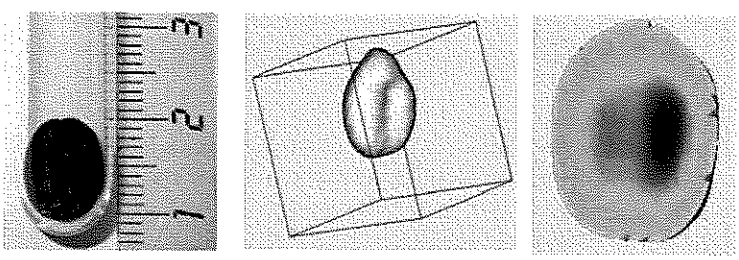

Fig 2: **A** Original roasted coffee bean **B** 3D surface view **C** Coronal slice around the middle of the bean. Color scale: blue is the most intense signal.

Molecular NMR and EPR in vivo detection of inflammation using specific E-selectin targeted iron oxides

K.A. Radermacher[1], N. Beghein[1], S. Boutry[2], S. Laurent[2], L. Vander Elst[2], R.N. Muller[2], B.F. Jordan[1], and B. Gallez[1].

[1]Biomedical Magnetic Resonance Unit, Université Catholique de Louvain, Brussels, Belgium.
[2]NMR and Molecular Imaging Laboratory, University of Mons-Hainaut, Mons, Belgium

Introduction:

The aim of the study was to evaluate a molecular marker for non invasive diagnosis and monitoring in the early phases of inflammation. At the beginning of the inflammatory process, endothelial permeability increases and cell adhesion molecules such as E-selectin are expressed on the endothelial cell surface (1). Leukocytes are slowed down in blood flow by adhesion on E-selectin *in vitro* and *in vivo* (2). A mimetic of the sialyl Lewis X molecule, which is the E-selectin's natural ligand, was coupled to the coating of pegylated ultrasmall iron oxide nanoparticles (USPIO). USPIO possess superparamagnetic properties and are used as negative MRI contrast agent due to strong T_2 and T_2* effects. Besides this method, EPR offers the unique capability of a sensitive and quantitative determination of these particles (3).

Methods:

An inflammation was induced in the right gastrocnemius muscle of male NMRI mice (20-25g) by intramuscular injection of complete Freund's adjuvant. The left muscle serves as internal control. After intravenous injection of either the grafted or ungrafted USPIO (7.7mg Fe/kg BW), we evaluated the USPIO concentration *ex vivo* by a EPR X-band (Bruker, EMX®, 9.4 GHz) and *in vivo* by an EPR L-band (Magnettech®, 1.2 GHz), as well as by a MRI T_2-weighted fast spin echo sequence (Bruker Biospec® 4.7T).

Results:

After establishing a calibration curve by EPR X-band, we determined the mean iron oxide concentration in the inflamed muscles after intravenous injection of grafted or ungrafted UPSIO, which was 0.8% and 0.4% of the initially injected dose respectively (data not shown). By L-band EPR, we observed that the grafted USPIO concentration in inflamed muscles was twice higher than for the ungrafted particles (Fig. 1). Using MRI experiments, a higher signal loss was clearly observed in the inflamed muscle when grafted USPIO were injected in comparison with the ungrafted USPIO (Fig. 2). All these differences were statistically significant.

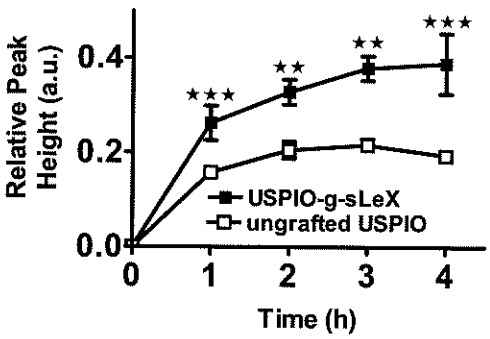

Fig.1: Time course of USPIO concentration in inflamed muscle as measured by in vivo EPR

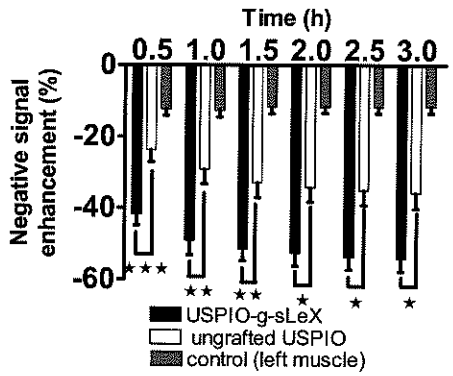

Fig 2. Time course of negative contrast enhancement produced by USPIO.

Discussion:

Using these three methods, we demonstrated that the specific targeting of grafted iron oxides was twice higher than for the ungrafted ones. Interestingly, ungrafted USPIO were also more concentrated in inflamed muscles than in control muscles. This is likely the result of an accumulation of a large number of macrophages in inflamed tissues which can take up USPIO in a non specific manner. Furthermore inflammation causes a vasodilation: the resulting improvement in blood flow carries more iron particles to the inflamed sites. Even taking into account this non specific accumulation of iron oxides, the targeting of USPIO with E-selectin ligands significantly improved the sensitivity of detection of inflamed tissues.

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Synthesis and Evaluation of New Persistent Trityl Radicals for Biomedical EPR Applications

Benoît Driesschaert,[a,b] Nicolas Charlier,[b] Bernard Gallez,[b,*] and Jacqueline Marchand-Brynaert[a,*]

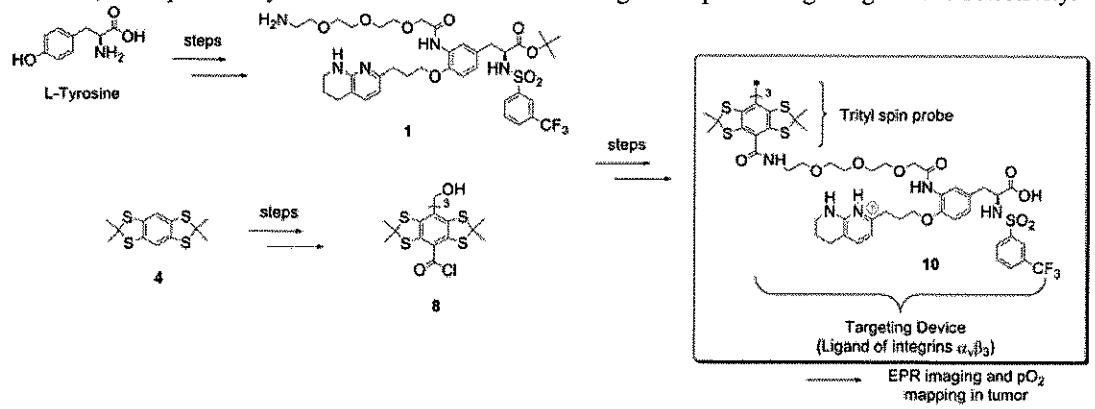
[a]Unité de Chimie Organique et Médicinale, Université catholique de Louvain, UCL, place Louis Pasteur 1, B-1348 Louvain-la-Neuve, Belgium, [b]Unité de Résonance Magnétique Biomédicale, Université catholique de Louvain, UCL, avenue Mounier 73.40, B-1200 Bruxelles, Belgium

1. Assessment of tissue oxygenation by electron paramagnetic resonance (EPR)[1]

Molecular oxygen plays one of the most important roles in the metabolism of living organisms. Abnormal tissue oxygenation is closely linked to number of diseases (e.g., cancer, stroke, ischemic diseases). Therefore, measuring and mapping oxygen in tissues is of great physiological and pathophysiological interest.
EPR oximetry methods are based on the broadening of the EPR signal caused by Heisenberg exchange between molecular oxygen and the spin probe to determine pO_2. A particular material can be calibrated in terms of the effect of oxygen on the EPR linewidth (LW).
When introduced *in vivo*, the measurement of **EPR linewidth can be interpreted in terms of oxygenation** in the vicinity of the spin probe.

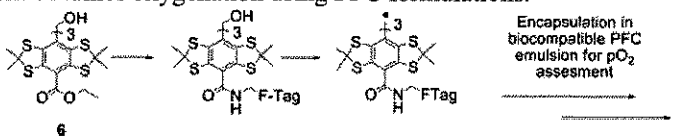
2. Synthesis of a tumor targeted contrast agent for molecular EPR imaging

In this work, we report the synthesis of a new EPR contrast agent **10** possessing a high tissue selectivity.



3. Synthesis of high sensitive oxygen spin probes for monitoring tumor oxygenation[2]

Among solvents, perfluorinated ones are well-known for their excellent capacity to dissolve a high quantity of non polar gases like O_2. We report the synthesis of highly fluorinated trityl radicals specially designed for high sensitive assessment of tumor oxygenation using PFC formulations.



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Development and evaluation of a non invasive method to estimate the oxygen consumption by tissues

Caroline Diepart[1], Bénédicte F.Jordan[1], and Bernard Gallez[1,2]

Laboratory of Biomedical Magnetic Resonance And Laboratory of Medicinal Chemistry and Radiopharmacy, Université catholique de Louvain, Brussels, Belgium

Introduction

Oxygen is a key environmental factor in the development and growth of tumors, and their response to treatment. Tumor oxygenation depends on a balance between oxygen supply and consumption, and both should be considered in developing strategies to reduce hypoxia.

The oxygen consumption rate of tumor cells can be measured by EPR using in vitro or ex vivo EPR measurement (1). The method is based on the variation of the linewidth of a paramagnetic material in the presence of consuming cells.

Purpose

Here, we developed a new EPR method to measure non invasively the tissue oxygen consumption. The protocol used was based on the measurement of pO_2 during a carbogen challenge protocol. The following sequence was used: 1) basal value during air breathing; 2) saturation of tissue with oxygen by carbogen breathing; 3) switch back to air breathing. The assumption was that the kinetics of return to the basal value after oxygen saturation will be mainly governed by the tissue oxygen consumption. This challenge was applied in hyperthyroid mice compared to control mice. This status is known to dramatically affect consumption rate of tumor and muscle cells.

Results

First we checked *ex-vivo*, that the treatment by L-thyroxine was able to change the oxygen consumption by tumors. We observed an oxygen consumption rate significantly enhanced for hyperthyroid mice compared to control mice (Fig.1). Typical evolutions of pO_2 during the breathing challenge are shown in Fig.2. In this figure, it can be seen that the return kinetics to the basal values was faster in hyperthyroid mice than in control mice in tumor and muscle. The quantitative estimation of the return kinetics was carried out using a monoexponential curve. Kinetics constant (k expressed as min^{-1}) are presented in Fig.3. Kinetics constants from hyperthyroid mice were higher than in tumors and muscles control mice.

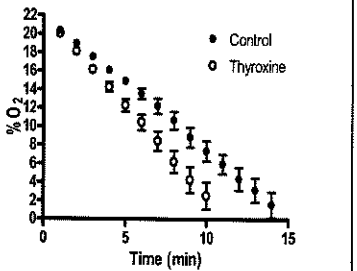

Fig.1: Oxygen consumption rates of tumor from L-thyroxine and control mice measured *ex vivo*.

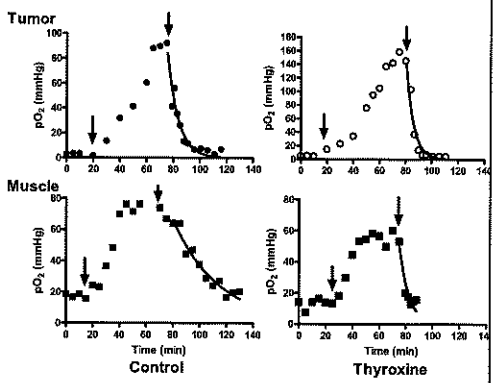

Fig.2: Breathing challenge in tumor and muscle of hyperthyroid and control mice
1st arrow: air switched to carbogen;
2nd arrow: carbogen switched to air.

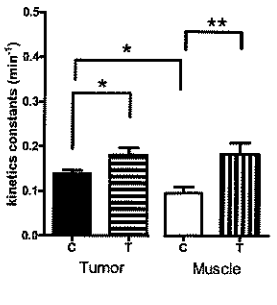

Fig.3: Kinetics constants measured in vivo.
C: control, T: thyroxine

Discussion

Our work was an attempt to provide a new method that may highlight differences in oxygen consumption by different tissues. The use of normal and hyperthyroid mice provided ideal models with tissues presenting differences in oxygen consumption rates. This method has the unique advantage of being non invasive and adapted to *in vivo* studies.

Assessment of the liver phagocytosis activity by EPR spectroscopy and imaging

N. Charlier[1], AM. Neyrinck[2], N. Beghein[1], N. Delzenne[2] and, B. Gallez[1]
[1]Laboratory of Biomedical Magnetic Resonance,
[2]Unit of Pharmacokinetics, Metabolism, Nutrition and Toxicology.
Université Catholique de Louvain, Brussels, Belgium.

Introduction

EPR can be used *in vivo* for the measurement of free radical species. Among the different sensors, India inks are of particular interest since they are characterized by a narrow line width (high SNR). India inks are composed of small carbonaceous particles known as Carbon Blacks (CB) (Lan M, 2004). It ha been reported that India inks can be used to study the phagocytosis activity of liver Kupffer cells. Classically, the CB phagocytosis is assessed by histological analysis (Neyrinck AM, 2000). Blockade of this phagocytosis and/or selective elimination of macrophages are accepted procedures for gaining knowledge about the role of macrophages *in vivo*. The Kupffer cells depletion is usually performed using IV administered $GdCl_3$. Here, we propose the use of EPR spectroscopy and imaging to determine the phagocytosis activity of the Kupffer cells. To do so, we compared the phagocytosis of a control and a $GdCl_3$ treated group using EPR spectrometry/imaging and histological evaluation.

Material and Methods

Rats were administered with saline (control group) or $GdCl_3$ (treated group) 24h before ink IV injection. After 24h, the India ink was injected IV. 20 min after ink injection, the liver was excised, one half was prepared for histological evaluation and the other half was homogenized for further EPR experiments. Measurement of the signal intensity (arbitrary unit) of samples coming from control and treated rats was performed using EPR X-band spectroscopy. Histological assessment of the phagocytosis activity was performed for control and treated group. Precision-Cut Liver Slices (PCLS) were prepared and carefully cut into a square shape to fit the EPR tissue sample cavity. EPR X-band 2D imaging was performed for $GdCl_3$ treated and untreated group.

Results

Using X-band EPR, we were able to detect the differences in signal intensity observed in control group and treated group (Fig. 1). Histological assessment demonstrated obvious differences in phagocyted CB particles between the two groups (Fig. 2). The PCLS X-band EPR 2D imaging allows us to determine the differences in phagocytosis activity between control and treated groups (Fig. 3).

Discussion & Conclusions

We have shown that intravenously administered India ink combined with EPR spectroscopy/imaging were efficient tools to detect differences in groups in which Kupffer cells are depleted or not. The phagocytosis difference was confirmed by histological examination. Moreover, for the first time to our knowledge, 2D EPR images were carried out using India ink as EPR sensor.

References

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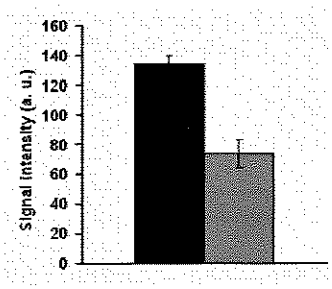

Fig. 1
EPR signal intensities from liver homogenates.
Black column: control group.
Gray columns: $GdCl_3$-treated group

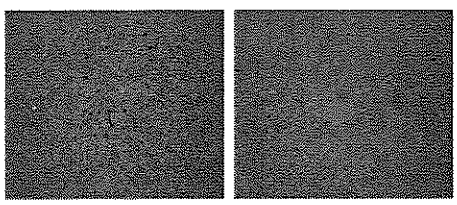

Fig. 2
Histological sections from liver.
Left picture: control group
Right picture: $GdCl_3$-treated group

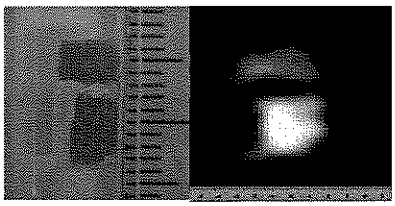

Fig. 3
Photographs (lefts) and 2D EPR images (right) of PCLS.
Top: $GdCl_3$-treated group;
Bottom: control group.

Arsenic trioxide enhances tumor oxygenation by decreasing oxygen consumption

Caroline Diepart[1], Bénédicte F.Jordan[1], and Bernard Gallez[1,2]

Laboratory of Biomedical Magnetic Resonance, Université catholique de Louvain, Brussels, Belgium

Introduction

The partial pressure of oxygen (pO_2) is a crucial factor affecting the response of tumors to irradiation and other cytotoxic treatments. It has been predicted that modification of oxygen consumption is much more efficient at alleviating hypoxia than modification of oxygen delivery.

Arsenic has been reported to have anti-tumor effect in acute promyelocytic leukemia and in solid tumors. As_2O_3 seems also to inhibit mitochondrial respiratory function in human leukemia cells. Thus, we hypothesized that As_2O_3 could be an important modulator of tumor oxygenation by affecting the oxygen consumption of tumors.

Materials and methods

The effects of As_2O_3 were studied in a transplantable tumor model (TLT). Local pO_2 was measured using low frequency EPR (L-Band) and oxygen consumption rate was measured using X-Band EPR. We also measured the perfusion by patent blue staining and the cytotoxicity of As_2O_3 by LDH leakage.

Results

The administration of As_2O_3 increases significantly the pO_2 in TLT tumors, an effect that was not observed for the control group (Fig.1). To explain the increase in pO_2 induced by As_2O_3, the blood perfusion was investigated using Patent blue staining 1h30 after As_2O_3 injection (Fig.2). The colored area observed in tumors 1 min after injection of the dye (Patent blue) showed a decrease in the percentage of coloration in As_2O_3-treated mice compared to control mice. As the increase in pO_2 was not due to an increase in perfusion, the tumor oxygen consumption was investigated. Te administration of As_2O_3 significantly decreased the rate of oxygen consumption (Fig.3). To exclude an possible cytotoxic effect of As_2O_3, we also measured the activity of lactate deshydrogenase (LDH) in TLT cells 1h30 and 4h after the administration of As_2O_3 (Fig.4). Arsenic did not influence the viability of TLT cells.

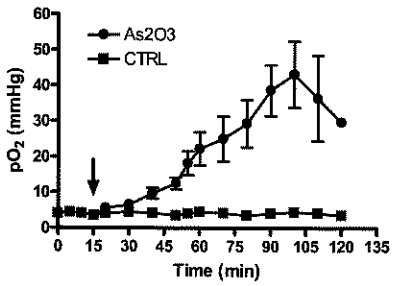

Fig.1: Tumor pO_2 measured by EPR oximetry in TLT tumors as a function of time. Arrows, injection time of the drug.

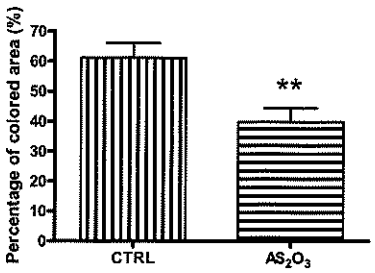

Fig.2: Effect of As_2O_3 on blood perfusion measured by Patent blue staining.

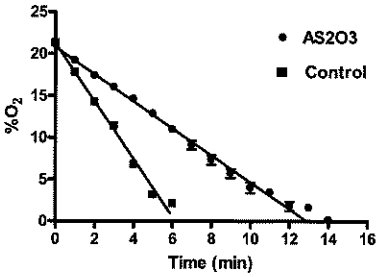

Fig.3: Effect of As_2O_3 administration on tumor oxygen consumption rate in TLT tumor cells.

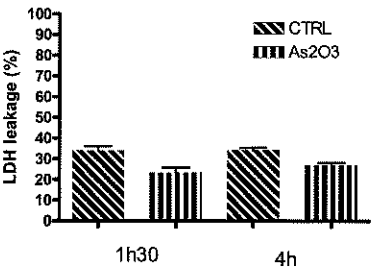

Fig.4: Effect of As_2O_3 on cell survival.

Discussion

Arsenic trioxide is an important modulator of pO_2 by decreasing oxygen consumption. This treatment could enhance tumor radiosensitivity (experiments in progress).

Participants

Aguirre, Aranzazu, Department of Physics, University of Antwerp – UIA, Universiteitsplein 1, B - 2610 Wilrijk, Belgium, aranzazu.aguirre@ua.ac.be

Ahmad, Ijaz, Department of Physics CDE, University of Antwerp – UIA, Universiteitsplein 1, B - 2610 Wilrijk-Antwerpen, Belgium.

Aleksinskaya, Marina, Leiden University Medical Center, Nephrology Department, Einthoven Laboratory of Vascular Medicine, Albinusdreef 2, 2333 ZA Leiden, The Netherlands, M.A.Aleksinskaya@lumc.nl

Briedé, Jacco, Department of Health Risk Analysis & Toxicology, Faculty of Health, Medicine and Life Sciences (FHML), PO Box 616, 6200 MD Maastricht, The Netherlands,Tel. +31 43 3881094, Fax +31 43 3884146, J.Briede@GRAT.unimaas.nl

Callens, Freddy, Universiteit Gent, Dept Solid State Sciences, Krijgslaan 281, S1, B-9000 Gent, Belgium; freddy.callens@ugent.be

Cambre, Sofie, Department of Physics, University of Antwerp – UIA, Universiteitsplein 1, B - 2610 Wilrijk, Belgium, Tel: +32 3820 2482; sofie.cambre@ua.ac.be

Caretti, Ignatio, Department of Physics, University of Antwerp – UIA, Universiteitsplein 1, B - 2610 Wilrijk, Belgium, Tel: +32 3820 2479; ignatio.caretti@ua.ac.be.

Charlier, Nicolas, Catholic University of Louvain, Lab. Chem. Med, Avenue Mounier 73. 40, B-1200 Brussels, Belgium, Nicolas.s.charlier@uclouvain.be.

De Cooman, Hendrik, Universiteit Gent, Dept Solid State Sciences, Krijgslaan 281, S1, B-9000 Gent, Belgium; hendrik.decooman@ugent.be

Desmet, Filip, Department of Physics, University of Antwerp – UIA, Universiteitsplein 1, B - 2610 Wilrijk, Belgium; filip.desmet@ua.ac.be.

Dhami, Amarjot, Physics Department – CDE, University of Antwerp, Universiteitsplein 1, B-2610 Antwerpen (Belgium), amarjot.Dhami@ua.ac.be

Diepart, Caroline, Catholic University of Louvain, Lab. Chem. Med, Avenue Mounier 73. 40, B-1200 Brussels, Belgium, caroline.diepart@uclouvain.be

Ezhevskaya, Maria A. Department of Physics, University of Antwerp – UIA, Universiteitsplein 1, B - 2610 Wilrijk, Belgium; maria.ezhevkaya@ua.ac.be.

Gallez, Bernard, Catholic University of Louvain, Lab. Chem. Med, Avenue Mounier 73. 40, B-1200 Brussels, Belgium; tel: 02 764 2789, bernard.gallez@uclouvain.be

Gast, Peter, Leiden Inst. Physics, University of Leiden, PO Box 9405, 2300 RA Leiden, The Netherlands; tel:+31-715275979; fax:+31-71-5275819; gast@molphys.leidenuniv.nl.

Grammenos, Angeliki, Université de Liège, Institut de Physique - Bâtiment B5, Spectroscopie Biomédicale, Allée du 6-Août , 4000 Liège, A.Grammenos@ulg.ac.be

Groenen, Edgar.J.J., Leiden Inst. Physics, University of Leiden, PO Box 9405, 2300 RA Leiden, The Netherlands; tel:+31-715275914; fax:+31-71-5275819; egroenen@molphys.leidenuniv.nl

Guelluy, Pierre-Henri, Département de Physique, Service de Spectroscopie Biomédicale, Institut de Physique, Université de Liege, B-4000 LIEGE Belgique, Tel: +3243663631, phguelluy@ulg.ac.be

Hoebeke, Maryse, Laboratory of Biomedical Spectroscopy, Department of Physics, Institute of Physics, B5, University of Liège, Sart-Tilman, B-4000 Liège, Belgium, M.Hoebeke@ulg.ac.be

Jivanescu, Mihaela, Department of Physics, University of Leuven, Celestijnenlaan 200D, 3001 Leuven, Belgium; mihalea.jivanescu@fys.kuleuven.ac.be.

Kan, Florentien, Leiden Inst. Physics, University of Leiden, PO Box 9405, 2300 RA Leiden, The Netherlands, tel: +31-715275912; fax: +31-71-5275912; kan@molphys.leidenuniv.nl

Keunen, Koen, Department of Physics, University of Leuven, Celestijnenlaan 200D, 3001 Leuven, Belgium; koen.keunen@fys.kuleuven.ac.be.

Kolbun, Natallia, Catholic University of Louvain, Lab. Chem. Med, Avenue Mounier 73. 40, B-1200 Brussels, Belgium, natasha.kolbun@uclouvain.be

Leveque, Philippe, Catholic University of Louvain, Lab. Chem. Med, Avenue Mounier 73. 40, B-1200 Brussels, Belgium, philippe.leveque@uclouvain.be

Loncke, Frank, Universiteit Gent, Dept Solid State Sciences, Krijgslaan 281, S1, B-9000 Gent, Belgium; frank.loncke@ugent.be

Mathies, Jennifer, Leiden Inst. Physics, University of Leiden, PO Box 9405, 2300 RA Leiden, The Netherlands, tel: +31-715275912; fax: +31-71-5275819; mathies@molphys.leidenuniv.nl

Milkisyant, Sergey, Leiden Inst. Physics, University of Leiden, PO Box 9405, 2300 RA Leiden, The Netherlands, tel:+31-715275912, sergey@molphys.leidenuniv.nl

Moons, Hans, Department of Physics CDE, University of Antwerp – UIA, Universiteitsplein 1, B - 2610 Wilrijk-Antwerpen, Belgium, hans.moons@ua.ac.be

Mouithys-Mickalad, Ange, CORD, University of Liège, Institute of Chemistry, B6a, Allée de la Chimie, 3, Sart Tilman. B-4000 Liège – Belgium, amouithys@ulg.ac.be

Nabben, Miranda, Department of Human Biology, Maastricht University, POBox 616, 6200 MD Maastricht, The Netherlands, Tel. +31 (0)43 3884258, Fax +31 (0)43 3670976, M.Nabben@HB.unimaas.nl

Nevil, Nissy, University of Antwerp, Drie Eiken Campus, Building N, Wilrijk, Antwerpen, 2610 Belgium, Nissy.Nevil@ua.ac.be

Nguyen, Duc, Department of Physics, University of Leuven, Celestijnenlaan 200D, 3001 Leuven, Belgium, Duc.NguyenAhnPhuc@fys.kuleuven.be

Nistor, Sergiu V., National Institute for material Physics, Atomistilor 105bis Street, PO Box MG 7 Magurele RO 077125 Bucharest, Romania, tel: +40-214930195; fax: +40-214930267; snistor@infim.ro.

Niztayev, A., Delft University of Tehnology, Department of Biotechnology, Enzymology Group, A.Niztayev@tnw.tudelft.nl

Pauwels, Ewald, Center for Molecular Modeling, Ghent University, Proeftuinstraat 86, B-9000, Belgium, Ewald.pauwels@ugent.be

Penders, Marc, Bruker Belgium NV, Kolonel Bourgstraat 124 bus 1, 1140 Brussel, marc.penders@bruker.be

Radermacher, Kim, Catholic University of Louvain, Lab. Chem. Med, Avenue Mounier 73. 40, B-1200 Brussels, Belgium, kim.radermacher@uclouvain.be

Scarpelli, Francesco, Leiden Inst. Physics, University of Leiden, PO Box 9405, 2300 RA Leiden, The Netherlands; tel:+31-715275908; fax:+31-71-5275819; scarpelli@molphys.leidenuniv.nl

Somers, Pieter, Department of Physics, University of Leuven, Celestijnenlaan 200D, 3001 Leuven, Belgium, pieter.somers@fys.kuleuven.be.

Sottini, Silvia, Leiden Inst. Physics, University of Leiden, PO Box 9405, 2300 RA Leiden, The Netherlands, tel: +31-715275908; fax: +31-71-5275819; sottini@molphys.leidenuniv.nl.

Stesmans, André, Department of Physics, University of Leuven, Celestijnenlaan 200D, 3001 Leuven, Belgium, andre.stesman@fys.kuleuven.ac.be.

Tarpan, Mihaela, Universiteit Gent, Dept Solid State Sciences, Krijgslaan 281, S1, B-9000 Gent, Belgium; mihaelaadeluta.tarpan@ugent.be

van de Sandt, Annette, Med. Klinik I, Division of Cardiology, Pulmonary Diseases, and Vascular Medicine, University Hospital Aachen, Pauwelsstrasse 30, 52074 Aachen, Germany

Van Doorslaer, Sabine, Department of Physics, University of Antwerp – UIA, Universiteitsplein 1, B - 2610 Wilrijk-Antwerpen, Belgium; sabine.vandoorslaer@ua.ac.be.

Van Faassen, Ernst, Faculty of Science, section Interface Physics, Ornstein laboratory, Utrecht university, 3508 TA Utrecht, The Netherlands; e.e.h.vanfaassen@phys.uu.nl.

van 't Hoff, Ton, BRUKER BioSpin BV PO Box 88, 1530 AB Wormer, The Netherlands; tel: +31-75-6285251; fax:+31-75-6289771; a.n.vanthoff@bruker.nl.

Vinck, Evi, Department of Physics, University of Antwerp – UIA, Universiteitsplein 1, B – 2610 Wilrijk, Belgium, Tel: +32-3820-2482, evi.vinck@uia.ac.be

Vrielinck, Henk, Universiteit Gent, Dept Solid State Sciences, Krijgslaan 281, S1, B-9000 Gent, Belgium; henk.vrielinck@ugent.be

Zamani, Sepideh, Department of Physics, University of Antwerp – UIA, Universiteitsplein 1, B - 2610 Wilrijk, Belgium, sepideh.zamani@ua.ac.be.

Zverev, Dmitry, Universiteit Gent, Dept Solid State Sciences, Krijgslaan 281, S1, B-9000 Gent, Belgium, dmitry.zverev@ugent.be