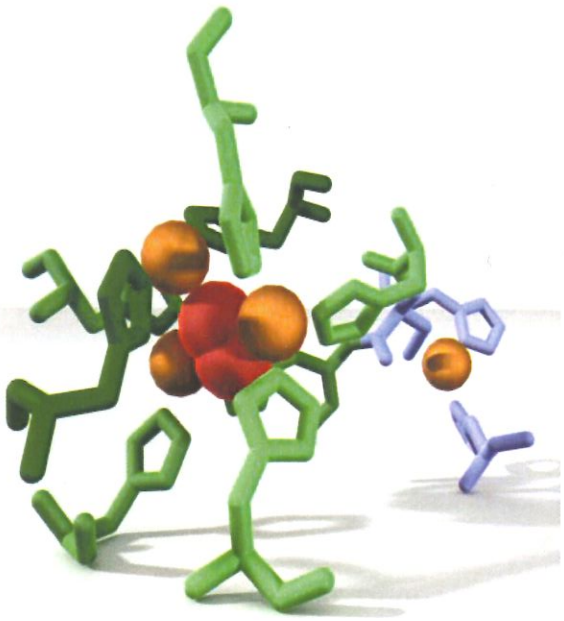
Henk Vrielink

Seventeenth Meeting of the Benelux EPR Group



Programme
Book of Abstracts
List of participants

May 15, 2009

BRUKER

Huygens Laboratory,
Niels Bohrweg 2, Leiden.

Universiteit Leiden

17th Meeting of the Benelux EPR Group 2009

Leiden – May 15

10:00 **Registration and coffee/tea**

10:30 **Welcome by Edgar Groenen**

10:35 **EPR detection of basal NO production in bone marrow: Hypertensive rats have lower production of nitric oxide.**
M. Aleksinskaya, E. van Faassen, T.J. Rabelink and A.J. van Zonneveld (Leiden)

11:00 **Structural characterization of a highly active superoxide-dismutase mimic**
M. Ezhevskaya, V. Balasubramanian, H. Moons, M. Neuburger, C. Cristescu, C. Palivan and S. Van Doorslaer (Antwerp)

11:25 **Identification of a radical intermediate in the enzymatic reduction of oxygen by a small laccase.**
A. W.J.W. Tepper, S. Milikisyants , S. Sottini, E. Vijgenboom, E. J.J. Groenen, and G. W. Canters (Leiden)

11:50 **W-band (94 GHz) time resolved EPR spectroscopy of optically excited fullerene/spin probe complexes**
H. Moons, E. Goovaerts, I. Nuretdinov, L. Franco, C. Corvaja (Antwerp)

12:15 **LUNCH**

14:00 **Alkene oxidation with H_2O_2 efficient Dinuclear Manganese Catalysts - what lessons can we take from EPR spectroscopy in understanding mechanisms.**
J. W. de Boer, P. L. Alsters, R. Hage, B. L. Feringa and W. R. Browne (Groningen)

14:25 **Quantitative EPR spectroscopy for dose measurements in radiotherapy.**
B. Schaeken, S. Lelie, W. Schroeyers, S. Schreurs (Diepenbeek)

14:50 **The sunny side of life: EPR detection of NO radicals in human skin.**
E. van Faassen (Utrecht)

15:15 **ESR: a powerful non-destructive method to study the interaction of Cyclodextrins on cell membrane.**
A.Grammenos, M.Lismont, P-H Guelluy and M. Hoebeke (Liège)

15:40 **EPR and thermoluminescence investigation of $K_2YF_5:Tb^{3+}$ after X-ray exposure**
D.G. Zverev, S. Cromphout, H. Vrielinck and F. Callens (Ghent)

16.05 **General discussion**

16.10 **Poster session & Tour EPR lab & drinks**

18.00 **End**

ABSTRACTS

Oral presentations

EPR detection of basal NO production in bone marrow: Hypertensive rats have lower production of nitric oxide.

M. Aleksinskaya[1], E. van Faassen[2], T.J. Rabelink[1] and A.J. van Zonneveld[1]
[1] Department of Nephrology and Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Centre [2] Faculty of Science, Department of Interface Physics, Utrecht University.

Introduction.
Nitric oxide (NO$^{\bullet}$) radicals activate various proteinases that assist in the release of stem cells (repair cells) from bone marrow (BM). Cardiovascular disease (e.g. hypertension, obesity, diabetes or renal failure) indicates endothelial dysfunction associated with insufficient production of NO radicals by endothelial nitric oxide synthase (NOS).

Hypothesis:
NO production in bone marrow stromal cells is depressed in hypertensive rats. This impairs mobilization of endothelial progenitor cells from the marrow into the circulation.

Materials and methods.
We used electron paramagnetic resonance (EPR) and iron-dithiocarbamate complexes (Fe-DETC) to trap and quantify NO radicals in suspensions of BM cells of male Wistar rats (fig 1). Hypertension was artificially induced by implantation of a silicone pellet containing 100 mg deoxycorticosterone acetate (DOCA) and adding 0.9% saline and 0.2% KCl to the drinking water. Hypertension developed after 6 weeks.

BM cells were collected by flushing the bone with a syringe. NO trapping was initiated in suspensions of ca 100×10^6 BM cells by adding 2.5 mM DETC and 20 μM ferrous sulfate. If desired the BM cell suspension was stimulated with Ca-ionophore (10 μM). L-Nitro-Arginine Methyl Ester (L-NAME, 500 μM) was used as a non-selective inhibitor of NOS. After 30 min of NO trapping at 37°C, the cell fraction was harvested by centrifugation, resuspended in 300 μl HEPES buffer (150 mM, pH7.4) and snap frozen in liquid nitrogen for EPR assay. CW-EPR spectra were recorded at 77 K on a X-band ESP-300E spectrometer operating at 20 mW power (fig 2).

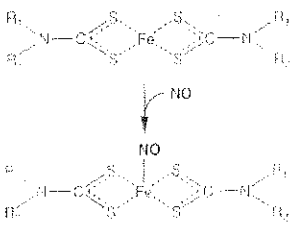


Fig 1: trapping of short-lived NO radical by Fe-DETC complexes. The MNIC adduct (S=1/2, I_N=1) is paramagnetic.

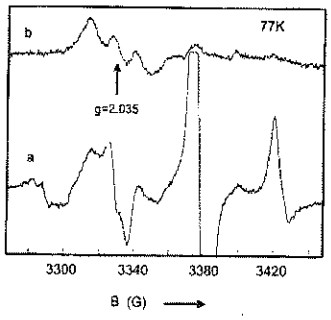


Fig 2: EPR spectrum of 180 mg rat brain is dominated by Cu^{2+}-DETC (a). After reduction with dithionite, the triplet of MNIC (NO-Fe^{2+}-DETC) is visible (b).

Results.
Table 1 contains yields of paramagnetic MNIC (NO-Fe(II)-DETC). Experimental sensitivity is just sufficient to quantify the basal (nonstimulated) NO production in rat BM cells suspension. The yields confirm our hypothesis that NO levels in BM from DOCA-treated hypertensive rats are significantly lower than in healthy rats. The MNIC is cancelled by adding L-NAME and shows that the NO is produced by NOS enzymes.

Conclusions.
- The EPR assay is sufficiently sensitive to detect basal NO production in 10^8 BM cells
- NO levels in DOCA-treated hypertensive rats are significantly lower than in healthy rats.
- NO production is dominated by NOS enzymes (L-NAME inhibition).
- We demonstrated that NO production may be upregulated via the Ca-dependent pathways.

Table 1. Relative MNIC yields in bone marrow from adult Wistar rats.
(b.d: below detection limit of ca 15 pmol MNIC/sample)

	healthy	DOCA	units
Basal MNIC	**35 ± 5**	**14 ± 8**	**pmol/100×10^6 cells**
Stimulated with Ca-ionophore	**47 ± 7**	**30 ± 12**	**pmol/100×10^6 cells**
Inhibited with L-NAME	**b.d.**	**b.d.**	

Structural characterization of a highly active superoxide-dismutase mimic

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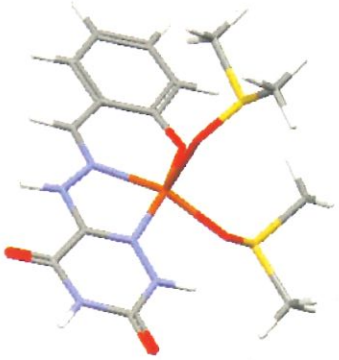
Cu,Zn superoxide dismutase (SOD) is an enzyme that efficiently catalyzes the dismutation of superoxide anions into oxygen and hydrogen peroxide. A lot of effort has been put in the synthesis of copper complexes having SOD activity, because of their potential pharmaceutical applications. In this work, one of so-called SOD mimics (SODm), namely a copper complex of 6-(2-hydroxy-benzaldehyde) hydrazono-as-triazine-3,5-dione, has been studied, which shows an extremely high SOD-like activity in solution.

X-ray diffraction reveals that the complex adopts a di-copper structure in the solid state. However, in solution, the complex is broken, forming a mono-copper center as follows from UV/Vis absorption and, more clearly, from electron paramagnetic resonance (EPR) experiments. Using pulsed EPR techniques in combination with DFT (density functional theory) computations, the electronic structure of the complex in solution is analyzed in detail.

X-band CW EPR reveals an essential axial g tensor. The principal g and copper hyperfine values are typical of a type-2 mono Cu^{II} complex, *i.e.* a square planar ligation of the Cu^{II} ion with possible additional weak axial ligation. This structure differs from the tetrahedral surrounding of the copper ion in bovine Cu,Zn-SOD. In order to obtain more detailed information on the Cu^{II}SODm, a number of pulsed EPR experiments were set up (ESEEM, ENDOR, ELDOR-detected NMR). In order to interpret the EPR data, five different models of Cu^{II}SODm are assumed. A likely model of Cu^{II}SODm in solution is given in Scheme 1. All observed EPR and DFT data point towards axial and equatorial ligation of solvent molecules to the complex and five coordination of the copper ion is more likely to happen than six-coordination. Probably this mono-nuclear conformation of the complex provides an easy accessibility to the O_2^- anion and contributes to its high catalytic activity.



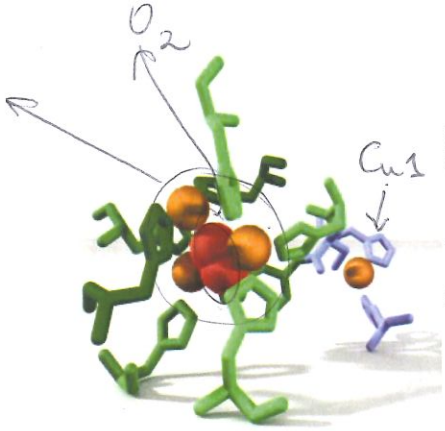
Scheme 1 Schematic representation of Cu^{II}SODm in solution with double DMSO ligation.

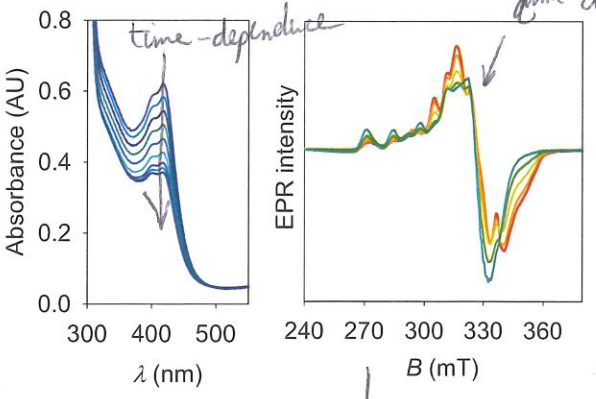
Identification of a radical intermediate in the enzymatic reduction of oxygen by a small laccase.

*A*rmand W.J.W. Tepper[1], Sergey Milikisyants[2], Silvia Sottini[2], Erik Vijgenboom[1], Edgar J.J. Groenen[2], and Gerard W. Canters[1]

[1]*Leiden Institute of Chemistry and* [2]*Leiden Institute of Physics, Leiden University, Leiden, the Netherlands*

The enzyme mechanism of the Cu containing small laccase (SLAC) from *Streptomyces coelicolor* has been investigated by optical and EPR spectroscopy. A new intermediate could be identified after the reaction of molecular oxygen with the reduced trinuclear site of the type 1 depleted (T1D) form of the enzyme.





It has the fingerprint of a biradical with a triplet ground state. One of the spins resides on a Cu in the trinuclear site, tentatively identified as the type 2 site, while the other spin derives from a protein based radical. The latter is tentatively identified as a tyrosyl radical on the basis of the similarity of the optical characteristics with those observed for a Cu tyrosyl radical pair. The spin-spin distance is found to be about 5.0 Å.

W-band (94 GHz) time resolved EPR spectroscopy of optically excited fullerene/spin probe complexes

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The combination of a chromophore and a nitroxide radical can lead to organic photo-responsive spin systems. Here we present a W-band (~94 GHz) time resolved EPR (TR-EPR) investigation of molecules comprising a C_{60}-cage connected with one or two nitroxide radicals through different covalent linkers. All measurements are performed in oxygen-free toluene solution at room temperature. This work complements previous X-band (~9.4 GHz) TR-EPR experiments[1]. The use of higher microwave frequency/magnetic field 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.

Pulsed laser excitation of C_{60} molecules generates a relatively long lived (in the μs range) triplet state, due to efficient intersystem crossing from the first excited singlet state. This property is preserved in the fullerene derivatives under study. The triplet electron spins ($S = 1$) will interact with the attached nitroxide radicals ($S = ½$), inducing higher-spin levels ($S \geq 3/2$) in this system. Spin polarization effects due to selective population and decay pathways are observed in the EPR spectral components which appear in enhanced absorption and emission. From field dependent data obtained by slicing the field/time dependent data, spin states with different spin multiplicities could be clearly resolved and their spectra simulated. Besides the accurate determination of the g-values and hyperfine parameters, a well-defined range could be set for the magnitude of the exchange interaction between the two moieties. The stronger exchange interaction in one of the molecules is assigned to the presence of a phosphor atom in the connection between the nitroxide radical and the C_{60}-cage. The molecule with a phosphor-free connection displays an intermediate exchange coupling. Analysis of the time dependences yields unexpected results in the rise of the spin polarization of the coupled state. The decay of the spin polarization is attributed to spin-lattice relaxation and intrinsic decay of the electronically excited state.

From these measurements it is clear that the use of different frequencies is essential for the complete characterization of exchange coupled systems, not only because of higher time and field resolution, but also for proper analysis of exchange interactions and polarization mechanisms.

[1] Franco L., Mazzoni M., Corvaja C., Gubskaya V.P., Berezhnaya L.Sh., Nuretdinov I.A., Mol. Phys., 104, 1543-1550, 2006

Alkene oxidation with H_2O_2 efficient Dinuclear Manganese Catalysts - what lessons can we take from EPR spectroscopy in understanding mechanisms.

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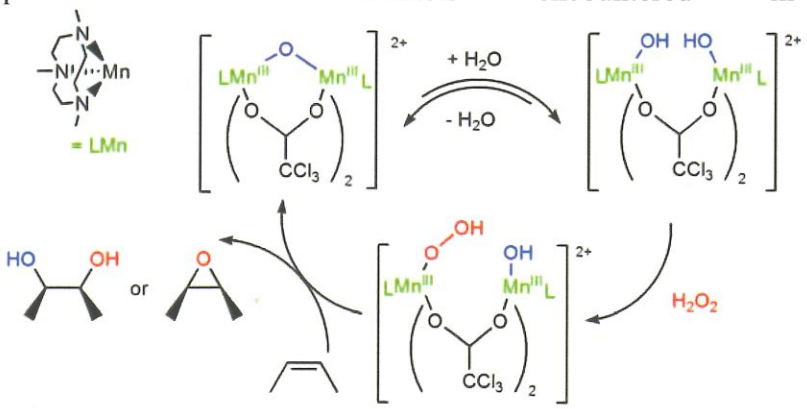
The dinuclear manganese(IV) complex $[Mn^{IV}_2(\mu\text{-}O)_3(tmtacn)_2]^{2+}$ (**1**, where tmtacn = N,N',N''-trimethyl-1,4,7-triazacyclononane) was recently shown to be highly effective in catalyzing the *cis*-dihydroxylation and epoxidation of alkenes with H_2O_2 as terminal oxidant.[1] Key to its activity and selectivity is the role played by carboxylic acids as ligands to the active form of the complex formed in situ which has the general formula $[Mn_2(\mu\text{-}O)(\mu\text{-}R\text{-}CO_2)_2(tmtacn)_2]^{2+}$. High turnover numbers (t.o.n. >2000) can be achieved especially w.r.t. *cis*-dihydroxylation for which the use of 2,6-dichlorobenzoic acid allows for the highest t.o.n. reported thus far for *cis*-dihydroxylation of alkenes catalyzed by a 1st row transition metal and high efficiency w.r.t. the terminal oxidant (H_2O_2). Tuning of the activity of the catalyst by variation in the carboxylato bridging ligands is dependent on both the electron withdrawing nature of the ligand and on steric effects. By contrast, the *cis*-diol/epoxide selectivity is dominated by steric factors. Speciation analysis and isotope labeling studies will confirm that the complexes of the type $[Mn_2(\mu\text{-}O)(\mu\text{-}R\text{-}CO_2)_2(tmtacn)_2]^{2+}$ are the resting state of the catalytic system and that they retain a dinuclear structure throughout the catalytic cycle. The mechanistic understanding obtained from these studies holds considerable implications for both homogenous manganese oxidation catalysis and in understanding related biological systems such as dinuclear catalase and arginase enzymes. In this presentation aspects of EPR spectroscopic studies employed in studying the mechanism of this system will be explored with attention paid to the artifacts encountered in these studies.



[1] J. W. de Boeretal. *J. Am. Chem. Soc.* **2005** *127*, 7990-7991; J. W. de Boer, et al. *Inorg. Chem.* **2007**, 6353-6372; J. W. de Boer et al. Dalton, 2008, 44, 6283-6295; J. W. de Boer et al. Chem. Commun. 2008, 3747-3749.

Quantitative EPR spectroscopy for dose measurements in radiotherapy.

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Abstract

Background: Cancer treatment with radiotherapy is quickly evolving to complicated treatments. In this context, modern radiation therapy techniques such as the so-called Intensity Modulated Radiation Therapy, radiosurgery or tomotherapy present a challenge (non reference dosimetry). Alanine-EPR dosimetry bears a large potential to solve persistent difficulties in the dosimetry for modern radiation therapy. In January 2009, the Nuclear Technological Center (NuTeC) of the XIOS Hogeschool Limburg has started up a laboratory for alanine-EMR dosimetry.

Materials&methods: Radiation detectors are available as L-α-alanine pellets (d = 4.9 mm, l = 3.0 mm; Harwell) and dose is evaluated using four detectors for each dose measurement. Detectors are read out using an EMX^{micro} spectrometer (Bruker), equipped with a high sensitivity resonant cavity. The spectrometer parameter settings are: P= 0.25 mW; B_{mod} = 0.5 mT; Time constant = 40.96 ms, t_{sweep}= 84 s; N_{sweep}= 5. The dosimeters are read out at a fixed, well controlled, position into the cavity corresponding to maximum amplitude. Five spectra are acquired after subsequent rotations of 72°. The recorded spectrum is a superposition of the pure alanine spectrum with a singlet line of a reference substance. The ratio of the amplitude of the central line in the alanine spectrum to the amplitude of the reference signal is used as a measure of absorbed dose.
The spectrometer is calibrated in terms of "Dose to water" (D_w) using a set of dosimeters irradiated against the primary standard of water calorimetry at PTB. In addition, the alanine-EPR system was benchmarked to classic ionisation chambers (IC) measurements.
As an example, the possibilities of alanine-EPR dosimetry is illustrated for the case of a total body irradiation (TBI), using an anthropomorphic phantom.

Results: EPR-dosimetry proves to be a reliable method for dose measurements: correspondence between EMR and IC dose measurement is within 0.1% in reference (calibration) conditions. Our method was validated in 4 different clinical photon beams at 2 institutions and the overall uncertainty on dose measurement is comparable to an IC measurement: $U_r(D_w$= 4Gy) = 1.3%. The TBI treatment was evaluated at a prescription dose of 10 Gy, reducing lung dose to 7.5 Gy. Measurements show that the average measured medial dose corresponds well to the prescribed dose (Dw= 10.10 Gy; sd=0.48 Gy), but the average medial lung dose is 8.05 Gy; sd=1.55 Gy. We observed an important dose gradient of 3 Gy from the upper lung lobe (6.90 Gy) to the lower lung lobe (10.00 Gy), suggesting that lung shielding should be improved at the lower lobe by adding shielding material at that location.

The sunny side of life: EPR detection of nitric oxide in human skin.

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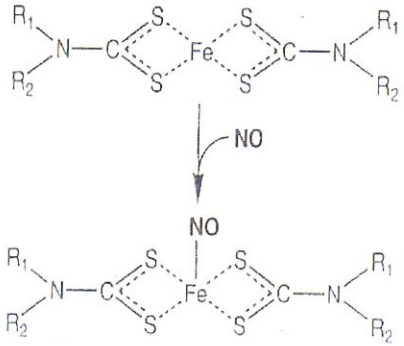
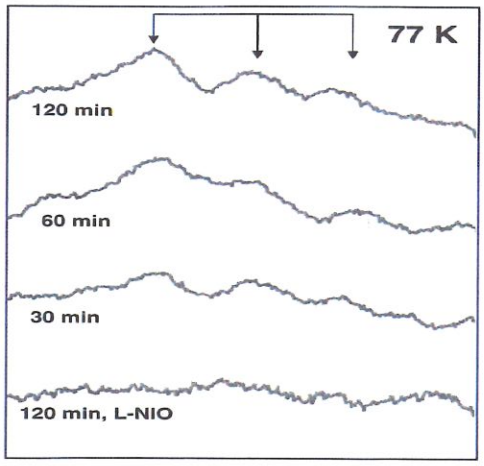
Physiology: Nitric oxide radicals ($NO^{\bullet}$) fulfil many physiological roles in human tissues like brain, vasculature, and skin. It acts locally as disinfectant and promoter of wound healing and blood circulation. Normally, $NO^{\bullet}$ is produced from arginine by the NOS-enzyme, but under hypoxia (eg infarct) or acid conditions (eg stomach, bladder) $NO^{\bullet}$ may be released from nitrite anions (NO_2^-). Endogenous $NO^{\bullet}$ has very low concentration and is too short lived (<ms) for direct detection with EPR. It is known that UVA light (320 – 400 nm) promotes repair and blood circulation in skin, and enhances risk of melanoma.

Aim: to study the effect of UVA in sunlight on levels of $NO^{\bullet}$ in human skin.

Method: $NO^{\bullet}$ levels in fresh human skin biopts are quantified by spin-trapping with Fe-DETC complexes. After incubation (30 min at 37 °C, dark or illuminated), the biopts are snap frozen in liquid nitrogen for CW-EPR assay.



Trapping of $NO^{\bullet}$ by Fe-DETC complex results. in stable MNIC adduct ($NO\text{-}Fe^{2+}$-DETC with $S=1/2$, $I_N=1$.



Formation of ca 70 pmol MNIC in 160 mg female breast skin in the dark.
Formation of MNIC is suppressed by adding NOS-inhibitor L-NIO.

Findings: Basal (dark) $NO^{\bullet}$ production is dominated by NOS-enzyme. Under UVA illumination (dose 20 J/cm^2 ~ 1 hrs sun exposure*), the yields increase 4-5 fold due to photolysis of nitrite anions in the epidermis. The yields may be artificially enhanced further tenfold by application of nitrite-skin cream.
In addition to $NO^{\bullet}$ radicals, EPR reveals radiative induction of carbon-based and pyrimidine radicals in collagen matrix, as well as radical oxygen ($OH^{\bullet}$) species in skin tissue.

* threshold UVA dose for erythema (sunburn) is ca 60 J/cm^2.

ESR: a powerful non destructive method to study the interaction of Cyclodextrins on cell membrane

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Cyclodextrins (CD) are molecule formed by six, seven, or eight D-glucopyranose units denominated respectively α-,β-,γ-cyclodextrin. These amphiphilic molecules present an internal hydrophobic cavity that permits to encapsulate a large number of organic molecules. It has been reported that Methylated beta-CD like randomly methylated β-cyclodextrin (RAMEB) are able to extract cholesterol from cell membrane[1].

Electron spin resonance (ESR) is not commonly used in the study of cyclodextrin. However, ESR combined with spin labelling is a powerful non-invasive way to probe cell membranes and to quantify microviscosity modifications induced by addition of cyclodextrin.

Microviscosity is defined as the homogenous solution viscosity, which results in the same spectrum as that obtained in the microenvironment. The quantification of these microviscosity variations is obtained thanks to a prior calibration of the ESR spectra of n-doxyl stearic acids (n-DSA: n=5, 12, 16) in solvent mixture of known viscosities. This calibration allows 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 [2, 3].

In the present work, the Rameb ability to extract membrane cholesterol and phospholipids was demonstrated. For the first time, the percentage of cholesterol extracted by Rameb thorough a wide range of concentration has been monitored in a non invasive way. A dosage of cellular cholesterol using Amplex Red Cholesterol Assay Kit has also been done and confirms the ESR results.
This study shows that ESR is a method able to quantify the cholesterol depletion in cells membrane and is a good tool for the study of CD.

[1] G. Piel, M. Piette, V. Barillaro, D. Castagne, B. Edvard, L. Delattre, Int. J. Pharm. 338 (2007) 35-42

[2] M. Bahri, M.D. Hoebeke, A.Grammenos, L. Delanaye, N.Vandewalle, A.Seretin *Colloïds and Surf. A:*
Physicochemical and Engineering aspects (2006).

[3] M.A. Bahri, J Heyne, P. Hans, A. Seret, A. A. Mouithy-Mickalad and M.D. Hoebeke in *Biophys. Chem.* 114(2005): 53-61

EPR and thermoluminescence investigation of $K_2YF_5:Tb^{3+}$ after X-ray exposure

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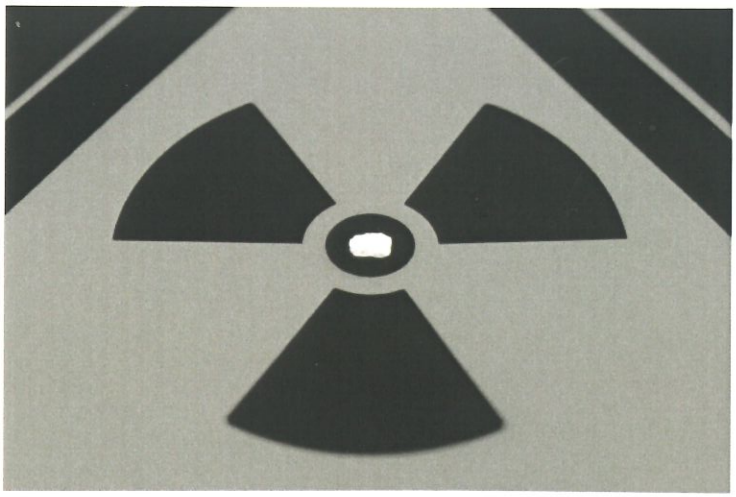
Wide interest in K_2YF_5 based thermoluminescence (TL) phosphors doped with rare earth (RE) impurities has been generated by the possibility of forming stable trapped electron (e^-) and hole (h^+) defects by ionizing irradiation (α, β, γ, X-rays) at room temperature (RT). After recombination of the defects and transfer of the energy to the RE impurities when the sample is heated, one could observe a strong RE^{3+} TL signal.[1] Among the RE^{3+} dopants, Tb^{3+} was found to produce the highest sensitivity and it was argued that e^- and/or h^+ trapping at the RE^{3+} activator ions might play a role in this. However, it still remains unclear which defects are responsible for the storage and what their role in the TL process is.

Therefore, the main purpose of the research reported here is to unravel the TL mechanisms in K_2YF_5 crystals through the study of the structure of radiation-induced paramagnetic defects using EPR and ENDOR, and to establish correlations with TL by simultaneously monitoring the EPR spectrum and the light emitted by the crystals in thermal bleaching experiments. Our focus lies on TL events below RT, where the thermal bleaching experiments can be performed in situ with the crystal remaining in the EPR cavity.

Prior to irradiation, the EPR spectra only reveal contributions of unintentional impurities: Gd^{3+}, Er^{3+} and Yb^{3+}, while the Tb^{3+} dopant ions ($4f^8$ ground configuration) are EPR-silent. By X-ray irradiation at 77K, several new spectral components are produced, five of which are identified as F_2^- type trapped h^+ centres. In addition, at low temperatures (T=10K) EPR signals of a centre with high spin (S = 7/2) are detected, very probably to be attributed to Tb^{4+}. Thermal bleaching experiments indicate that the two most stable F_2^- centres are involved in two TL peaks in the T=100-150K range. At temperatures above 140K the TL free decay of the high spin centre is observed, accompanied by the appearance and growth of a second centre with S = 7/2. For both high spin centres, the zero-field splitting parameters are determined which provide information on the microscopic structure of these defects. The second centre is shown to be thermally stable up to at least 250°C and decays at temperatures above 300°C.

[1] H. W. Kui, D. Lo, Y. C. Tsang, N. M. Khaidukov and V. N. Makhov, *Journal of Luminescence*, 2006, **117**, 29-38.



ABSTRACTS

Posters

Endohedral copper(II)acetylacetonate/single-walled carbon nanotube hybrids characterized by Electron Paramagnetic Resonance

Sofie Cambré, Wim Wenseleers and Etienne Goovaerts

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The encapsulation of organometallic paramagnetic molecules, copper(II) acetylacetonate ($Cu(acac)_2$), inside single walled carbon nanotubes (SW CNTs) is studied using continuous wave electron paramagnetic resonance (EPR). By preparing samples from either fully opened or closed SW CNTs[1], the EPR spectra of encapsulated and non-encapsulated molecules can be clearly identified. The EPR spectrum originating from the encapsulated molecules is unchanged by dispersion of the endohedral nanohybrids in a solvent or by solubilization of the nanohybrids in water using bile salt surfactants[2], demonstrating that the CNTs protect the encapsulated molecules from changes in the external environment. The EPR parameters obtained for the encapsulated molecules show that these molecules experience an extremely apolar environment. From the EPR spectra of the encapsulated molecules the distances between the encapsulated molecules inside the CNTs can be estimated by adapting Van Vleck's method of moments[3] for the case of a one-dimensional array of molecules. Furthermore, orientation dependent EPR experiments in combination with polarized Raman scattering experiments of preferentially aligned endohedral CNT hybrids inside stretched polymer film samples, yield information on the orientational distribution of the encapsulated molecules inside the SW CNTs.

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"EPR investigation of the globin-coupled sensor of Geobacter sulfurreducens"

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We present the results of our EPR investigation of the globin-coupled sensor of the bacterium *Geobacter sulfurreducens*. These globin-coupled sensors (GCS) 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-band pulsed and CW EPR to investigate the ferric and NO-ligated ferrous forms of the globin-domain of *Gs*GCS.
Our optical and CW-EPR study of ferric *Gs*GCS showed that this protein has a bis-histidine coordination of the heme iron. We used a method developed in this group that uses spectral simulations of the experimental HYSCORE and pulsed ENDOR spectra to determine the orientation of the axial imidazole ligands of the heme iron. Our results will be compared with the x-ray structure of the protein.
The NO bound form of *Gs*GCS was studied with CW EPR, this study shows that binding of nitric oxide breaks the bond between the heme iron atom and the evolutionary conserved proximal histidine.

Electron paramagnetic resonance study of free radicals in X-irradiated trehalose dihydrate single crystals

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Carbohydrates are important constituents of several biological systems including DNA and membrane systems. Elucidating their radiation chemistry is therefore of general importance.

Recently, the chemical structures of three dominant radicals obtained after room temperature (RT) irradiation in sucrose single crystals, were identified by De Cooman et al. [1,2,3] All three radicals are characterized by a glycosidic bond cleavage. The T1 radical is an allylic-type radical with approximately half of the spin density localized at the C2' carbon atom of the fructose unit. On the other hand the T2/T3 radicals are glucose-centered with the major spin density localized at the C1 carbon atom.

In order to check if the cleavage of the glycosidic bond is a common process produced by irradiation in disaccharides, free radicals produced by X-irradiation in trehalose single crystals are currently under study with electron paramagnetic resonance (EPR) techniques in our group. Trehalose is a disaccharide composed of two α-D-glucosyl units linked by a glycosidic oxygen bridge between their two anomeric carbon atoms, C1 and C1'.

After RT irradiation of trehalose single crystals three dominant radicals are present. One radical species is characterized by a rather isotropic triplet that indicates the interaction between the unpaired electron and two almost equivalent protons in β positions. The formation of a radical that exhibits two equivalent large beta hyperfine couplings can be simply explained by a net hydrogen abstraction from one of the carbon atoms in the ring, most probably C2 or C2'. The other two radical species exhibit only proton hyperfine couplings smaller than 20 MHz and therefore are characterized by a broad EPR singlet. In addition to these free radicals, two other less dominant species characterized by a doublet are present.

After keeping the irradiated trehalose crystal at RT for three month of after warming it to 40° for three days, the EPR spectrum changes completely. The dominant species in this case is characterized by a doublet of doublets. In a previous study it was suggested that this radical is obtained after a beta elimination starting from the dominant radical that exhibits a triplet immediately after irradiation. [4]

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

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In this work, the activation process of the metal salen catalyst *N,N'*-Bis(3,5-di-tert-butylsalicylidene)-1,2-cyclohexanediamino cobalt^{II} ([Co(**1**)]) has been studied using a combined electron paramagnetic resonance (EPR) – density functional theory (DFT) approach. The [Co(**1**)] catalyst is activated by the addition of acetic acid and subsequent exposure to air. The resulting complex is widely used to separate the enantiomers in racemic mixtures of terminal epoxides via a hydrolytic kinetic resolution (HKR) reaction. Despite its widespread use, the nature of the activated species is still poorly understood.
The CW-EPR data, performed in this work, 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.

EPR investigation of cationic radicals for organic electronics applications

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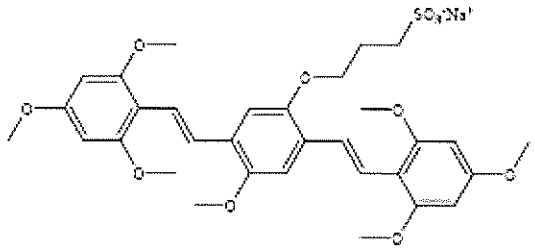
Poly(*p*-phenylene vinylene)-like (PPV-like) oligomers are found to be promising materials for the use as gas sensors, such as electronic noses. "Sensing" is done via the detection of changes in resistance of the active oligomer layer upon contact with the molecules of interest. However, in order to perform these conductimetric measurements, the PPV-like oligomers need to be oxidized, creating positive cations (polarons).

In this work, we present a combination of an X- and W-band continuous-wave and pulsed electron paramagnetic resonance (EPR) study and a DFT analysis of the material obtained after electrochemical oxidation of the sodium salt of *E,E*-2-(3-sulfopropoxy)-5-methoxy-1,4-bis[2-(2,4,6-trimethoxyphenyl)ethenyl]benzene (scheme).



The W-band CW-EPR analysis allowed for the determination of the rhombic ***g*** tensor of the obtained paramagnetic species. As expected, the principal *g* values are similar to those obtained earlier for polarons formed by I_2-doping of PPV-type polymers.[1] Surprisingly, the X-band ENDOR and HYSCORE analysis revealed proton interactions with maximum couplings well below the ones detected previously in the polymers[1]. Since the polaron is extended over far more subunits in a polymer than in the oligomer under study (containing only three subunits), the inverse was expected, as corroborated by the DFT computations. Furthermore, the lack of a half-field signal in the CW-EPR spectra seems to exclude the formation of coupled polaron systems. Finally, a comparative study of the EPR line-widths and the relaxation properties of the electrochemically treated and I_2-doped oligomers indicate that we are dealing with magnetically diluted polarons in the former case. Furthermore, the X-band HYSCORE spectra of the I_2-doped oligomers revealed a much larger proton coupling than for the electrochemically induced polarons.

Our results thus clearly show that the simple picture of the formation of isolated oligomer polarons does not hold in the case of the electrochemically induced polarons. Several possible explanations will be given.

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ESEEM and ENDOR study of stereoselective binding of methylbenzylamine by a chiral cobalt salen complexes

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The reliable preparation of enantiomerically pure epoxides, as extremely valuable chemical compounds, is very important in areas such as pharmaceutical and food industries. In 1990's, E. Jacobsen *et al.* discovered a new chiral *N,N'*-bis(3.5-di-*tert*-butylsalicylidene)-1,2-cyclohexane-diamino-cobalt(II) catalyst (known as cobalt Jacobsen's catalyst) with very high enantioselectivity in the hydrolytic kinetic resolution of racemic epoxides. Although widely used, the mechanism of this asymmetric reaction is still not clear. In this work, we use different EPR techniques to study the subtle differences in the geometric and electronic structure when *R*- or *S*-Methylbenzylamine (MBA) bind to the chiral cobalt (II) Jacobsen complex ((*R,R*)-Co[**1**] or (*S,S*)-Co[**1**]). The X-band CW EPR spectra of the homo-chiral pairs, *R*-MBA–(*R,R*)-Co[**1**] (hereafter denoted as (*RR-R*)) and *S*-MBA–(*S,S*)-Co[**1**] (SS-S), are identical. However, there are clear differences between these homo-chiral pairs and the hetero-chiral pairs (*SS-R, RR-S*). The EPR spectra of the racemic-racemic mixture of the Co[**1**] with MBA indicate the preferential binding of *S*-MBA by (*R,R*)-Co[**1**] with very high selectivity (90%). Furthermore, ESEEM and pulsed ENDOR experiments were applied on the *RR-S* and *RR-R* pairs in order to unravel the specific changes in the hyperfine and nuclear quadrupole couplings with the surrounding protons and nitrogens.

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