31st Benelux EPR Society Meeting
Ghent, June 6th, 2024

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Campus Sterre, Building S1, Lecture room 1.1 (First floor, Entrance facing building S2)

Program

9:45 – 10:30 Opening registration + coffee

10:30 – 10:40 Opening meeting

10:40 – 11:05 Barbara Mathieu (UCLouvain): Non-invasive in vivo discrimination of mitochondrial ROS from global ROS production in solid tumors using EPR spectroscopy

11:05- 11:30 Lore Van den Bergh (UAntwerpen): The tricky story of black titania - A spectroscopic study on the reduction and reoxidation of titania

11:30 – 11:55 Peter-Leon Hagedoorn (TUDelft): EPR spectroscopy for Biocatalysis

11:55 –12:30 Your question to the audience – session 1

12:30 –13:30 **Lunch break + photo + poster session**

13:30 – 14:00 Your question to the audience – session 2

14:00 – 14:25 Thilo Hetzke (Bruker): Horizons in EPR Studies Enabled by Novel Technology

14:25 – 14:50 Ilias Vandevenne (UAntwerpen): Electrical detection of magnetic resonance on a Chip (EDMRoC): A low-cost and sensitive characterization tool for defects in SiC MOSFETs

14:50 – 15:30 **Coffee + poster session**

15:30 – 15:55 Leonardo Passerini (ULeiden): DEER spectroscopy on multi-spin copper complexes

15:55 – 16:20 Sofie Cambré (UAntwerpen): Monitoring triplet state engineering in (6,5) single-walled carbon nanotubes by optically detected magnetic resonance

16:20 – 16:30 General meeting

16:30 –17:30 Reception and farewell

Posters session (12:30 – 13:30 and 14:50 – 15:30)

Mohammad Wehbi (UCLouvain): Highly Sensitive Detection of Melanin in Melanomas Using Multi-harmonic Low Frequency EPR

Mélina Carbone (UCLouvain): Cocktails of fungicides targeting cellular respiration : benefits/risks balance ?

Cloe Buysse (UCLouvain): Monitoring the effect of anti-cancer drugs on the extracellular pH measurements using magnetic resonance

Abstracts -Oral presentations

Noninvasive discrimination of mitochondrial ROS from global ROS production in solid tumors using EPR spectroscopy

Barbara Mathieu[1], Justin Rondeau[2], Lionel Mignion[3], Pierre Sonveaux[2,4], Bernard Gallez[1]
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Mitochondria are major producers of ROS in cells. In healthy cells, mitochondrial ROS (mtROS) are maintained at stable concentration by antioxidant systems. When the balance is disrupted and lead to oxidative stress, mtROS are implied in cell death pathways. However, in cancer cells, mtROS are maintained at an intermediate level and promote cancer progression by triggering proliferation, angiogenesis and metastasis, key hallmarks of cancer(1).Electron paramagnetic resonance (EPR) spectroscopy allows detection of ROS in vivo but has never been used for specific mtROS detection in solid tumors.
ROS detection by EPR spectroscopy is based on the reduction of nitroxides (EPR detectable) to hydroxylamine (EPR indetectable) by ROS. Nitroxides are injected and the EPR signal decay is recorded over time(2). To be specific of mitochondria, we used mitoTEMPO, a nitroxide accumulating in mitochondria, and worked in comparison with 3CP, a nitroxide non-targeting mitochondria.
To implement this new toolbox, we worked by steps of increasing complexity. First, we worked in vitro on whole cells exposed to redox modulators. In a second time, in vivo, we were able to follow the decay of the signal of the two nitroxides for 30 minutesafter local injection in tumor.
Then, we modulated mitochondrial redox status with Antimycin A (ETC inhibitior) and we depleted cytosolic antioxidant GSH with L-BSO. We measured the signal decay rates of nitroxides over 3 days. MitoTEMPO signal decay rates in 4T1 tumors increased after 24 hours of treatment with Antimycin A but not with L-BSO. On the contrary, 3CP decay rates didn't change with Antimycin A but increased with L-BSO treatment. The ex-vivo analysis showed that nitroxides are well converted into hydroxylamines but that the total concentration of product remaining in the tumors stays stable over time, indicating no impact from blood circulation on the signal decay.
Finally, to assess the specificity for superoxide we genetically modified cells to over-express SOD2. The signal decay was lower in tumor cells over-expressing SOD2. These results were confirmed in vivo where mitoTEMPO signal decay rate was lower. It shows that the method is partially selective for superoxide and confirmed that the method can detect changes in mitochondrial redox status.
Regarding these data, we can say that the use of EPR spectroscopy and mitoTEMPO allows dynamic measurements over time of mitochondrial redox status in response to therapeutic agents.

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The tricky story of black titania – A spectroscopic study on the reduction of titania

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Titanium-dioxide materials are known semiconductors with many prospects in chemical catalysis, the food industry and energy conversion. Most of these applications use the photocatalytic property of titania, which is mostly active in the UV part of the electromagnetic spectrum. By chemically reducing the normally white titania, it can acquire colour, which makes it active in also the visible part of the electromagnetic spectrum [1]. However, in literature there is a lot of contradiction on the most appropriate reduction process and its influence on the properties and photocatalytic activity of the coloured titania. In this project, titania is reduced using a thermal process with NaBH4 as reducing agent [2]. It appears the reduction is sensitive to many different parameters of the process which are hard to monitor, leading to problems with reproducibility. Another key parameter of reduction is the crystal structure of the titania material. By characterizing the reduced titania with different spectroscopic techniques, such as EPR, XRD, XPS, EELS, in-situ drift-FT-IR and UV-Vis DR, it is attempted to create a clear overview on the influence of the crystal structure of titania on the reduction. EPR, as one of the key techniques reveals insight in the nature of the Ti(III) centres formed upon reduction of the titania. EELS measurements deliver complimenting data on the nature of the Ti centres in the reduced materials.

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EPR for biocatalysis

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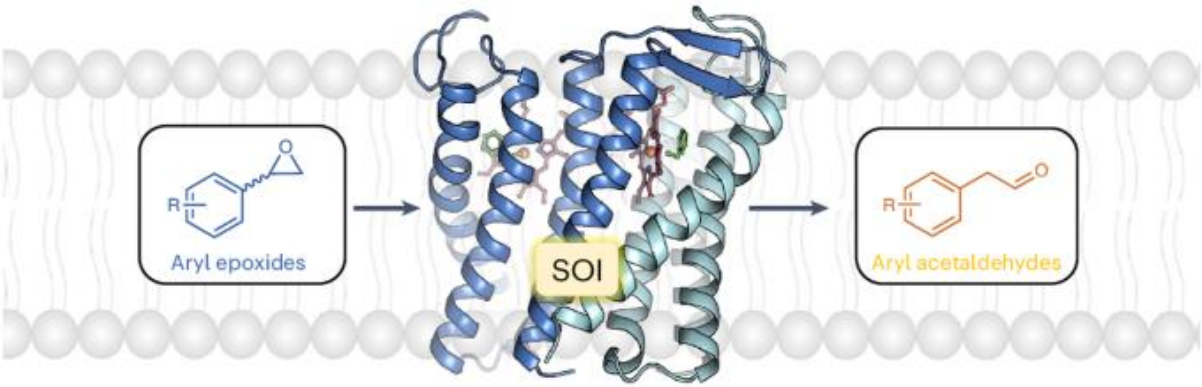
Roughly one third of all enzymes contain one or more metal cofactor, and the identity, oxidation state and coordination environment of the metal cofactor provides essential functional information. EPR spectroscopy is very useful to measure metal cofactors in enzymes even in complex matrices such as whole cells, can be used to determine the redox state and obtain information on the direct environment of the metals sites.

We have explored the mechanism of metalloenzymes using ultrafast mixing and freezing techniques and used EPR spectroscopy to examine enzyme catalytic intermediate states of a heme enzyme chlorite dismutase chlorite dismutase and a tungsten-cofactor enzyme Benzoyl-coA reductase [1,2]. Furthermore we have provided direct evidence on the role of a heme cofactor in an unexpected heme enzyme Styrene oxide isomerase (figure) [3].



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Horizons in EPR Studies Enabled by Novel Technology

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Multi-harmonic detection is a novel method for improving CW-EPR experiments, which at times can require lengthy collection times to achieve good signal-to-noise (S/N). Multiple-harmonics of an overmodulated EPR spectrum can be reconstructed into the 1st derivative spectrum with increased sensitivity. Using this technology, we show significant S/N gains in samples ranging from organic radicals to transition metals. The multi-harmonic method also allowed for detection of melanin radicals in a single zebrafish embryo, an otherwise difficult feat with traditional CW-EPR detection [1]. CW-EPR can also be improved in terms of time resolution. The detection is often not fast enough to track reaction kinetics for processes such as chemical, physical or photo-reactions. We will show several examples of Rapid Scan EPR being used in each of these applications, displaying the advantage of this technique for fast reactions [2,3]. In each respective section, we will also cover some basic theory on each of these methods and how they are integrated into CW-EPR spectrometers.

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Electrical detection of magnetic resonance on a Chip (EDMRoC):
A low-cost and sensitive characterization tool for defects in SiC MOSFETs

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Recently, the integration of a microwave chip into an application-specific integrated circuit (ASIC) led to the development of so-called electron paramagnetic resonance on a chip (EPRoC), allowing for extremely compact and low-cost EPR instrumentation [1-2]. This method has recently demonstrated its potential for electrical detection of magnetic resonance (EDMR) in a thin a-Si:H solar cell, by detection of the EDMR signal through the change in conductivity in the photoactive layer [3]. Here, we extend EDMRoC spectroscopy to measurements of EDMR in lateral SiC MOSFETs, combining it with the powerful charge pumping (CP) characterization technique [4-5]. In CP, the gate voltage is periodically changed between inversion and accumulation so that a current can be extracted from the transistor channel region originating from recombination of charges at defect trapping sites. CP-EDMR has demonstrated the capability of identifying and quantifying charge traps within the transistor channel of SiC MOSFETs [6-7], but it requires advanced instrumentation and is therefore not generally applicable in fundamental and applied research as well as in industrial environments.
In this work, we present CP-EDMRoC as a versatile, fast, and sensitive technique to detect EDMR in SiC MOSFET devices. Figure 1 shows the experimental configuration of the device positioned in front of the microwave chip on the ASIC (permanent magnet not shown for clarity). The compact ASIC (total dimensions 6×12 cm) operates in a region around 13 GHz.
In contrast to conventional EDMR, in EDMRoC the microwave frequency is not fixed, allowing for scanning of either magnetic field or microwave frequency. Moreover, for sensitive detection, conventional EDMR uses magnetic field modulation, which induces currents in the device circuitry that yield a background signal, while in EDMRoC this can be avoided using microwave frequency modulation. A direct comparison of EDMRoC and cavity-based EDMR spectra, as well as between magnetic-field and microwave frequency scans, will be presented, showing very similar signal-to-noise ratio's for EDMRoC and cavity-based EDMR.
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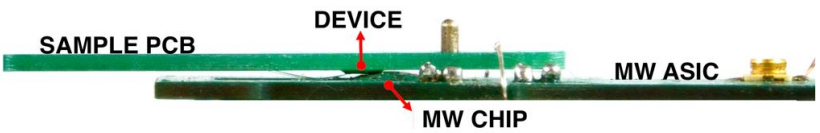

Fig. 1: Sample printed circuit board (PCB) with bonded MOSFET device positioned in close proximity to the microwave (MW) chip at one end of the ASIC.

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Multi-copper-DEER on synthetic, self-assembled copper cages

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In recent years, double-electron-electron resonance spectroscopy (DEER) has proven to be an excellent tool to obtain nanometer-distance constraints for molecular systems containing paramagnetic centers. The technique was especially used in biomolecular nitroxide-based systems. This study focuses on investigating multi-spin interactions in inorganic, self assembled copper(II) cages, containing six, respectivbely eight, copper ions[1]. In this context, studying multi-spin interactions presents challenges, particularly in accurately determining the distances between more than two spins.

We show that DEER gives reliable structural information even on the copper cages, which are systems that are not conventionally studied with DEER. We obtain information that relates with molecular modeling, and determine the role of multispin effects in the cages using a theory that was developed on multispin nitroxide based systems[2].

Overall, this study demonstrates the applicability of DEER spectroscopy in resolving structural details and multi-spin interactions in copper(II) supramolecular systems, extending the use of DEER beyond traditional nitroxide-based biological studies. These findings provide insights into the future applicability of DEER spectroscopy to a broader variety of compounds.

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Monitoring triplet state engineering in (6,5) single-walled carbon nanotubes by optically detected magnetic resonance

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Single-walled carbon nanotubes (SWCNTs) are promising one-dimensional platforms for functional materials since the fine-tuning of their optical properties can be done by introducing different molecules in their interior or on their external wall [1]. The diversity of possible functional groups that can be introduced provides a playing room to create new functionalities. Controlled *sp*3-functionalization on the outer wall was shown to increase emission efficiency by more than an order of magnitude, accompanied by a red shift of 100-300 nm [2,3]. While this research mostly focused on the bright singlet excitons, little information is available on how the dark triplet excitons are affected by the creation of these sp^3-defects along the nanotube wall.

Here, we take advantage of the sensitivity of optically detected magnetic resonance (ODMR) to monitor the effects of functionalization on triplet excitons in a series of samples with different functionalization density and functional groups. Experimentally, we find significant differences in ODMR intensities and in zero-field splitting (ZFS) of the triplet spectra. While pristine (6,5) SWCNTs hold triplet excitons with a purely axial symmetry and a ZFS inversely proportional to the nanotube diameter [4], the spin-density distribution of triplet excitons trapped in the sp3-defects changes significantly, losing the axial symmetry of the nanotubes. These results are corroborated by theoretical DFT calculations of the trap-induced triplet states and their spin density distribution.
Finally, we demonstrate first steps towards tuning of intersystem crossing by changing the attached functional group. Decoration of (6,5) SWCNTs with the paramagnetic, redox active, fluorescent, and chiral perchlorotriphenylmethyl (PTM) radical was recently found to partially quench the emission from the *sp*3 defects [5]. Our ODMR experiments reveal changes in symmetry and in ZFS demonstrating the creation of triplet excitons localized at these defects, as is also supported by DFT calculations. We furthermore observe an increase of ODMR intensity from the excitons trapped at the radical sites, corresponding well to the observed quenching of the emission of the defect stqte upon functionalization of the SWCNTs.

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Abstracts -Posters

Monitoring the effect of anti-cancer drugs on the extracellular pH and pO_2 measurements using magnetic resonance

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The acidosis of the tumor microenvironment may have profound impact on cancer progression and on the efficacy of treatments. Noninvasive technologies may help in assessing the efficacy of treatments targeting tumor metabolism. Here, we evaluated the effects of two different pharmacological treatments on tumor extracellular pH (pHe): UK-5099, a mitochondrial pyruvate carrier (MPC) inhibitor and syrosingopine, an monocarboxylate transporter (MCT) inhibitor.
In the first studies [1,2], we first analyzed *in vitro* the consequences of an exposure of cervix cancer SiHa cells and breast cancer 4T1 cells to UK-5099 (10 µM). Glucose consumption, lactate release and ECAR were increased in both cell lines after UK-5099 exposure. Mice bearing the 4T1 tumor model were treated daily during four days with UK-5099 (3 mg/kg). The pHe was evaluated in vivo before and after treatment using Chemical Exchange Saturation Transfer (CEST)-MRI. CEST-MRI provided high resolution parametric images (0.44 µl/voxel) of pHe highlighting the heterogeneity of response within the tumor when exposed to UK-5099. CEST-MRI showed a significant decrease in tumor pHe of 0.22 units in UK-5099-treated mice while there was no change over time for mice treated with the vehicle. As the MPC inhibition may lead to an alteration of oxygen consumption (and consequently alleviate tumor hypoxia), we developed an innovative method to measure simultaneously pHe and pO2 simultaneously using electron paramagnetic resonance (EPR). In vitro, we found an extracellular acidification and a significant reduction in OCR after 4T1 cells were treated for 24 hours with UK-5099. In vivo, in 4T1 tumor models, we observed a significant decrease in tumor pHe in UK-5099-treated mice but no significant changes were observed in pO_2 values. In the second part of our work [3], the impact of syrosingopine was studied on several metabolic processes. Glucose consumption, lactate secretion, ECAR, pHi, cell proliferation, viability and apoptosis were measured in vitro after treatment of human breast cancer MDA-MB-231 cells and squamous cell carcinoma FaDu cells with syrosingopine. Syrosingopine led to a decrease of glucose consumption, lactate secretion and ECAR after exposure to 10 µM in MDA-MB-231 cells. Only ECAR was reduced in FaDu cells. Intracellular pH, cell proliferation and viability were measured after exposure to increasing doses of syrosingopine. Decreases in all parameters were observed starting at 10 µM in MDA-MB-231 cells and 25 µM in FaDu cells. Apoptosis was increased after exposure to 25 µM in both cell types. Despite promising results obtained in vitro, no significant effect of treatment with syrosingopine (5 mg/kg) on pHe was measured in vivo using CEST-MRI, nor on tumor growth or survival.

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Boscalid, pyraclostrobin and their mixture induce a mitochondrial dysfunction in human hepatocytes

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Fungicides are extensively used in agriculture for crop protection. The most commonly used classes of fungicides are inhibitors of the electron transport chain, including succinate dehydrogenase inhibitors (SDHIs) and strobilurins which act by blocking complex II and complex III, respectively. In addition, to deal with the emergence of resistance to conventional pesticides, the agrochemical industry resorts to the use of cocktails of phytosanitary products.
In a first study, we analyzed the effect of boscalid and bixafen (two SDHIs) on the mitochondrial function of human hepatocytes. We observed that both SDHIs (1 µM concentration) induced a decrease in oxygen consumption rate (OCR) and an increase in mitochondrial superoxide. Flow cytometry revealed an increase in the number of early apoptotic cells in human hepatocytes exposed to both SDHIs [1].
In a second study, we analyzed then the impact of the exposure of human hepatocytes to pyraclostrobin, a fungicide belonging to the class of strobilurins. Using electron paramagnetic resonance (EPR), we observed a decrease in OCR and an increase in mitochondrial superoxide levels after 24 h exposure to 0.5 µM concentration. As a consequence, the content in ATP amount in the cells was reduced, the ratio reduced/oxidized glutathione was decreased, and a decrease in cell viability was observed using three different assays [2].
As SDHIs and strobilurins are commonly associated in commercial preparations, we evaluated a potential “cocktail” toxic effect. For this purpose, we selected low concentrations of boscalid (0.5 µM) and pyraclostrobin (0.25 µM) that did not induce a mitochondrial dysfunction in liver cells when used separately. In sharp contrast, when both compounds were used in combination at the same concentration, we observed a decrease in OCR, an increase in mitochondrial superoxide production, a decrease in the ratio reduced/oxidized glutathione, and a decrease in cell viability in three different assays [2].

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Highly Sensitive Detection of Melanin in Melanomas Using Multi-Harmonic Low Frequency EPR

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Over the last decades, the incidence of melanoma has been continuously increasing. Today, melanoma remains the most aggressive skin cancer, significantly reducing survival rates for patients in its advanced stages. Therefore, early diagnosis remains the key to change the prognosis of patients with melanoma. The incidence of amelanotic and hypomelanotic melanomas being very low (with less than 2% of newly diagnosed melanomas each year [1]) justifies to mainly focus on pigmented melanoma. Eumelanin the main pigment present in melanomas, is paramagnetic and detectable by EPR. We previously described that images obtained using 9 GHz EPR imaging could be overlaid on histological images [2]. In parallel, ex-vivo measurements of human biopsies showed that the EPR signal in benign nevi was significantly lower than that in malignant melanomas and found a correlation between the EPR signal and Breslow depth (tumor thickness in the skin) [3]. This led us to succeed in detecting noninvasively the melanin signal from skin melanoma models in mice at low frequency EPR (1GHz) [4,5]. We performed a clinical study using a **whole-body EPR system** (*ClinEPR*), in patients on skin lesions suspicious of melanoma. EPR data obtained before surgery were compared with histopathology results. **The EPR signal of melanin was significantly higher ($p<0.0001$) in melanoma lesions (n=26) than that in benign atypical nevi (n=62)**. A trend toward a higher signal intensity (though not significant) was observed in high Breslow depth melanomas (a marker of skin invasion) than in low Breslow lesions [6]. Because the melanin signal recorded was at the limit of the noise, there was a clear room for boosting the sensitivity of the method through improvement in instrumentation.

Our clinical EPR system has been very recently upgraded with the capability to apply larger modulation amplitude and to record/analyze the EPR signal in **multi-harmonics** mode (*Novilet*) [7]. We have compared the melanin signal obtained on phantoms using classical CW-EPR (1st harmonic) and multi-harmonics mode. We observed a boost in sensitivity by a factor about 10. The same result was obtained when these phantoms were placed at the surface of human skin. In nude hairless mice (n=8) with implanted skin B16 melanomas, we observed a **boost in sensitivity *in vivo* similar to that *in vitro* with the capability to detect melanoma cells in the skin at an earlier stage of development**. **Multi-harmonic EPR was also able to detect non-invasively a signal coming from a lymph node tumor** (nude mice n=8) **as well as metastatic tumor in the lungs** (nude mice n=3)**. The boost in the sensitivity compared to CW EPR was clearly significant.** We confirmed the improvement of multi-harmonic technology in signal acquisition for melanin *in vivo* and *in vitro* to be implemented in clinical studies for early melanoma diagnosis [8].

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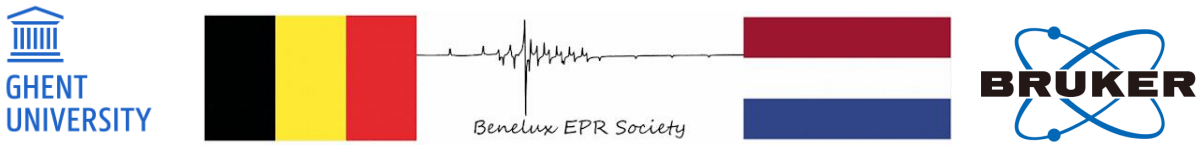

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