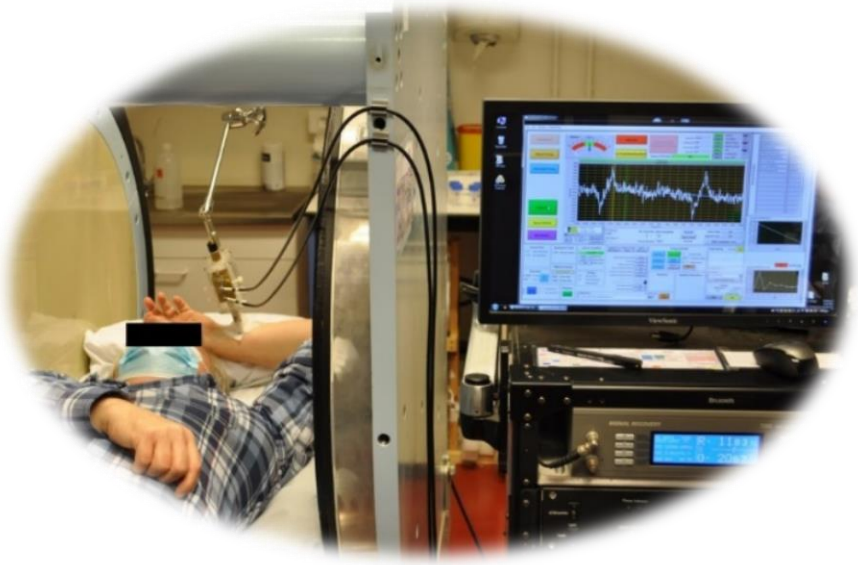
29th Benelux EPR Society Meeting

Brussels, May 25th, 2022



Organized by the Biomedical Magnetic Resonance Research Group

Louvain Drug Research Institute

UCLouvain

29th Meeting of the Benelux EPR Society – Program

May 25th, 2022 – Maisin auditorium – Avenue Mounier 50, B-1200 Brussels

9.45 – 10.30	**Registration – Poster mounting – Coffee/Tea**
10.30 – 10.40	Welcome – General information on the meeting
	Morning scientific session *Chair: Ange Mouithys-Mickalad, ULiège*
10.40 - 11.05	Electrically detected magnetic resonance on 4H-SiC MOSFETs using charge pumping *Jan Lettens, Onsemi*
11.05 – 11.30	Unravelling the magnetic properties of human-liver ferritin *Jacqueline Labra Munoz, Leiden University*
11.30 – 11.55	First-in-man clinical trial to characterize the endogenous free radical melanin in skin melanomas using noninvasive EPR *Bernard Gallez, UCLouvain*
11.55 – 12.00	Group picture in the Maisin Auditorium
12.00 – 14.00	Walking lunch **Poster discussion** Visit of the Nuclear and Electron Spin Technologies (NEST) Platform (12.30-12.50-13.10, by groups of 8 people)
14.00	**Afternoon scientific session** *Chair: Martina Huber, Leiden University*
14.00 – 14.25	Immobilizing neuroglobin in mesoporous materials – monitoring protein structure and dynamics with spin-label electron paramagnetic resonance *Lore Van den Bergh, University of Antwerp*
14.25 – 14.50	Unique biradical intermediate in the mechanism of the heme enzyme chlorite dismutase discovered using microsecond timescale freeze hyperquench *Peter-Leon Hagedoorn, TUDelft*
14.50 – 15.15	Potential colon carcinogenic effects of different micro- and nanoparticles present in food as determined via oxygen radical formation detected by ESR/EPR spectroscopy *Jacco Briede, Maastricht University*
15.15 – 15.40	Light-induced charge transfer in two-dimensional hybrid perovskites *Melissa Van Landeghem*, University of Antwerp (*current, Hasselt University)*
15.40 – 16.00	**Plenary Meeting of the Benelux EPR Society** – News – Next meetings *Henk Vrielinck, Ghent University*
16.00 – 17.00	Farewell drink

Poster communications

R. Cleirbaut, University of Antwerp
Using UV-Vis spectroscopy and EPR as a tool to study the incorporation of heme proteins in titania

B. Mathieu, UCLouvain
Towards magnetic resonance detection of mitochondrial ROS in vivo in solid tumors

I.Serra, University of Antwerp
Reactivity of peroxidases and chlorite dismutases with chlorite and hypochlorite: a spectroscopic investigation of short-lived intermediates trapped by fast freeze-quenching

H. Vrielinck, Ghent University
Paramagnetic centers in LiF:Mg,Cu,P thermoluminescence radiation dosimeters

M. Carbone, UCLouvain
Effects of SDHIs and strobilurins fungicides on the mitochondrial function of human HepG2 cells

P. Shali, Ghent University
Comparative study of short-lived radical species generated in in-plasma and subjacent-plasma treated water

S. Garifo, University of Mons
EPR imaging study of Nanodiamonds

D. d'Hose, UCLouvain
Targeting of the electron transport chain with the mitochondrial targeted biguanide mito-metformin to alleviate tumor hypoxia in prostate cancer

A. Guidetti, Univesity of Antwerp
Development of a combined methodology towards mechanistic investigation of rare metal-free, light activated catalysts

C. Buyse, UCLouvain
Dual measurement of oxygenation and extracellular pH using stable trityl radicals. Application to evaluate the impact of a mitochondrial pyruvate carrier inhibitor on the tumor microenvironment

S. Fardokht Rezayi, University of Antwerp/University of Cardiff
Elucidation of molecular structure of Cu^{II}-TREN complexes with trigonal bipyramidal structure: An exploration by advanced EPR techniques

D. d'Hose, UCLouvain
Repurposing statin use to alleviate tumor hypoxia in prostate cancer: an opportunity for radiosensitization?

P. Nyssen, ULiège
Superoxide anion radical scavenging activity of propofol and its derivatives: a chemiluminescence and EPR spin trapping study

Practical information

Registrations

- from 9.45 to 10.30 AM
- costs: 30 Euros (to be paid by cash on-site)
- certificate of attendance and of payment will be provided on-site

Talks

- 20 minutes presentation + 5 min discussion
- Send your power point presentation to bernard.gallez@uclouvain.be before May 24th, 8.00 PM
- Contact the audiovisual manager, Lionel Mignion, in the auditorium Maisin before your session to check your presentation

Posters

- Size of the boards: height 160 cm x width 120 cm
- Pushpins will be provided
- Posters should be mounted between 9.45 and 10.30
- Please be present in front of your poster between 12.30 and 14.00 (except during the time of the visit of the laboratory, if applicable)
- Please remove your poster after the afternoon session during the farewell drink

Auditorium

- The Auditorium Maisin has been funded by the Foundation Joseph Maisin. Prof. Joseph Maisin was the founder of the Cancer Center in UCLouvain in 1927 grouping activities linked to diagnostic and treatment of cancer, education and scientific research in oncology.
- Foods and drinks are prohibited in the Auditorium

Lunches

- Sandwich lunches, dessert and drinks will be provided in the hall - Vegetarian sandwiches will be available. Please use the trashes provided by the organizers

Visit of the Nuclear and Electron Spin Technologies (NEST) platform

- Visits will be organized by groups of 8 people
- Visits will start at 12.30 – 12.50 – 13.10 from the reception desk
- Book your visit/schedule at the reception desk at your arrival
- During the visit, please follow the instructions of the guide regarding safety in high magnetic field environment
- For people presenting a poster, there is no problem to leave your poster for 20 minutes, the time of the visit. You will have plenty of time to discuss your project and results during the lunch time.

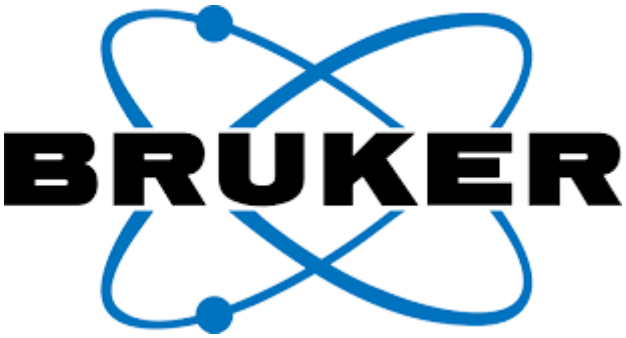
Wifi access

- Please use the following credentials when connecting to the WiFi network:
- User Name: stw001@wifi.uclouvain.be - Password: UHVvc8XzZTtw
- You need to accept the conditions of use of the UCL WiFi network and sign the official form. Return the signed form in the box at the registration desk.

Badges recycling

- Please return your badges in the *ad-hoc* box at the end of the meeting

This event is organized with the support of



Oral communications

Electrically detected magnetic resonance on 4H-SiC MOSFETs using charge pumping

J. Lettens[1), 2), 3)], E. Goovaerts[2)], H. Vrielinck[3)], P. Moens[1)], J. Franchi[4)], S. Cambré[2)]

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After decades of research and development silicon carbide (SiC) technology is ready to produce the next generation of power devices [1]. However, it is still troubled by a poor channel mobility, often linked to defect states at the SiC – gate insulator interface [2]. There is no consensus on the identity of the mobility limiting defects, making it difficult to change process conditions to optimize SiC MOSFET devices.

A very powerful technique to study defects is electrically detected magnetic resonance (EDMR). EDMR is a technique to study spin-dependent recombination processes. In EDMR, an electrical signal is monitored while manipulating the spin states through magnetic resonance transitions. In contrast to electron paramagnetic resonance (EPR) where any unpaired electron can lead to a signal, EDMR is only sensitive to those spins that effect the electrical signal which is monitored. It therefore becomes possible to study a complex structure consisting of multiple materials (such as a MOSFET) yet easily correlate a magnetic resonance to a specific spatial region and electrical characteristic [3].

Here we study n-type lateral MOSFETs with a 40 nm thick dry thermal oxide (SiO_2), 2 um gate length and approximately 80 mm gate width. We monitor the charge pumping (CP) [4] current while measuring EDMR. In charge pumping, the voltage of the MOSFET gate is repeatedly pulsed between a low voltage, to attract holes to the channel, and a high voltage, to attract electrons. When a trap captures an electron during one part and a hole during the other, recombination takes place, which leads to a DC charge pumping current, proportional to frequency. If this recombination is spin- dependent we could expect to see a magnetic resonance [5].

In this work, we report the detection of two different EDMR signals on the same SiC MOSFET using different CP settings (Figure 1), one of which we tentatively assign to the carbon dangling bond center (PbC), and the other to the carbon vacancy (VC) based on the observed hyperfine splitting and their relative intensities, using similar considerations as in [6]. Furthermore, we show that one resonance increases the CP current, while the other surprisingly reduces the CP current.

These findings show that EDMR based on CP can be used to identify the origin of the defects and of the spin dependent recombination process itself.

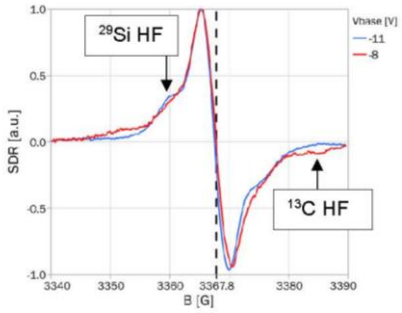

Figure 1: resonances measured at different base voltages where a SNR peak was observed. Signals were 180 +-1 degrees apart in phase.

[1] T. Kimoto and H. Watanabe, Appl. Phys. Express, vol. 13, no. 120101, 2020.
[2] F. Allerstam et al., Semicond. Sci. Technol., vol. 22, pp. 307–311, 2007.
[3] R. L. Vranch, B. Henderson, and M. Pepper, Appl. Phys. Lett., vol. 52, no. 14, pp. 1161–1163, 1988.
[3] E. Ö. Sveinbjörnsson et al., Mater. Sci. Forum, vol. 556–557, pp. 487–492, 2009.
[4] S. J. Brugler and P. G. A. Jespers, IEEE Trans. Electron Devices, vol. 16, no. 3, pp. 297–302, 1969.
[5] F. C. Rong, W. R. Buchwald, E. H. Poindexter, W. L. Warren, and D. J. Keeble, Solid State Electron., vol. 34, no. 8, pp. 835–841, 1991.
[6] T. Wimbauer, B. Meyer, A. Hofstaetter, and A. Scharmann, Phys. Rev. B, vol. 56, no. 12, pp. 7384–7388, 1997.

Unravelling the magnetic properties of human-liver ferritin

Jacqueline A. Labra Muñoz, Vera Calukovic,

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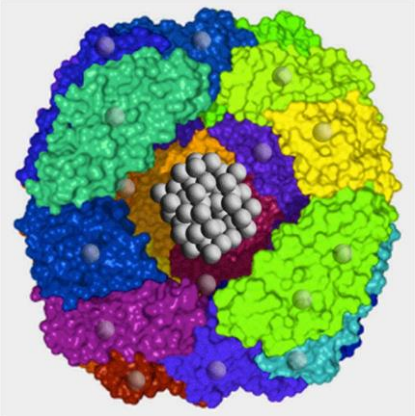
[&] C. J. Gorter Center for High Field MRI, Department of Radiology, Leiden University Medical Center

Ferritin is a protein responsible for iron storage and release within the cell. It encloses iron as an iron-oxide nanoparticle within a ~12 nm shell (see Fig.). The mineral composition of ferritin is directly related to its magnetic properties and indirectly related to its function. Different attempts to magnetically characterize the ferritin core have been pursued, including Electron Paramagnetic Resonance (EPR), magnetometry, and Transmission Electron Microscopy, without, however, being able to capture the complexity of the core's spin system.



In this work, we present the analysis of EPR spectra and magnetometry data acquired on a sample of ferritin purified from human liver. To obtain insight into the spin system in ferritin, we have performed EPR in a range of temperatures from 30 to 190 K and EPR simulations, and compare to magnetometry data.

First-in-man clinical trial to characterize the endogenous free radical melanin in skin melanomas using noninvasive EPR

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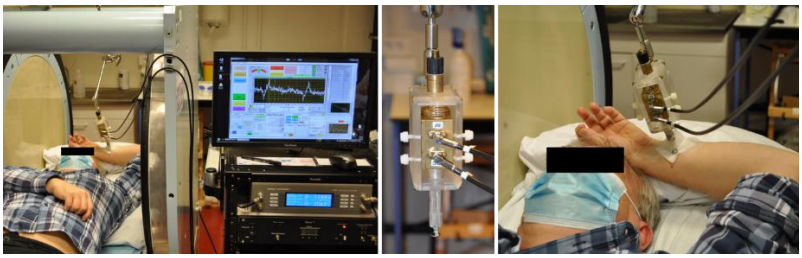
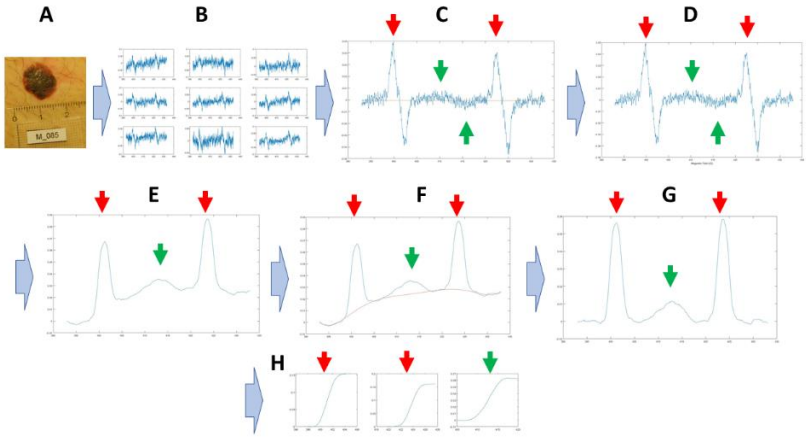
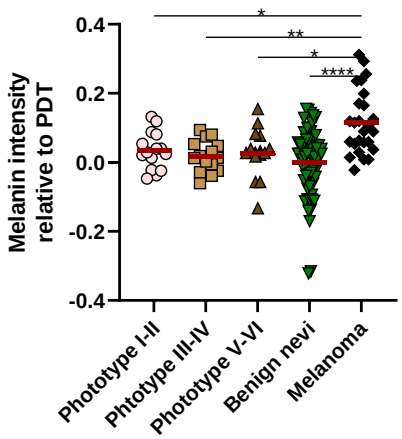
Electron Paramagnetic Resonance (EPR) is a magnetic resonance technology that characterizes species with unpaired electrons. EPR is generally applied at high frequency (≥ 9 GHz) to characterize free radicals *in vitro*. Whole-body low-frequency (1 GHz) EPR spectrometers have recently emerged for measuring oxygen in tissues using exogenous paramagnetic sensors or irradiation-induced free radicals. To enlarge the fields of applications of clinical EPR to endogenous free radicals, we explored the possibility to detect noninvasively melanin (a stable semiquinone free radical) in the skin. EPR spectra were obtained using a spectrometer operating at 1 GHz with a surface coil placed over the area of interest. A first study on healthy volunteers (n=45) presenting different skin phototypes did not show significant difference between groups. Because former *in vitro* studies at 9 GHz on biopsies suggested that the EPR signal from melanin was different when measured in skin melanomas or benign nevi, we conducted a prospective first-in-man clinical EPR study in patients with suspect skin lesions (n=100) requiring surgical resection. EPR data obtained before the surgery were compared with results from the histopathology. 92% of the spectra were analyzable. The EPR signal of melanin was significantly higher ($p<0.0001$) in melanoma lesions (n=26) compared to benign atypical nevi (n=62). A trend to a higher signal intensity (though not significant) was observed in high Breslow depth melanomas (a marker of skin invasion) compared to low Breslow lesions. Our study opens new avenues for evaluating clinical EPR as a potential aid to diagnosis of pigmented skin lesions.







Immobilizing neuroglobin in mesoporous materials – monitoring protein structure and dynamics with spin-label electron paramagnetic resonance

L. Van den Bergh,[1,2] Z. Hafideddine,[1,3] S. Loreto,[2] M. Huysmans,[1] H. Bergmans,[3] J. Wang[2], V. Meynen,[2] H.Y. V. Ching,[1] S. Van Doorslaer[1]

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The developement of biosensing instruments is a booming field of research. Often, these biosensors require the immobilisation of proteins in or upon synthetic matrices, such as metal oxides. Porous materials show a lot of promise due to their high surface area, which may facilicate a high loading of proteins and thus a high amount of active sites as well as sufficient accessibility of the active center.

However, the incorporation of proteins into such porous materials has proven to be non-trivial. The incorporation procedure as well as the characteristics of the support may accelerate protein degradation. In order to warrant a high loading of correctly folded proteins, the characteristics of the porous materials should comply to many demands, requiring controlled pore size and surface properties. Also experimental conditions, like pH and ionic strength, may limit the preperation of protein- loaded materials [1,2]. In this work, we focus on the incorporation of human neuroglobin (NGB) in mesoporous silica and titania. NGB is an iron-containing heme protein with interesting redox properties. Electron paramagnetic resonance (EPR) of the ferric form of the protein allows determining the changes in the electronic structure of the active heme site upon protein incorporation. Furthermore, site-directed spin labeling of the protein with a single nitroxide tag allows determining changes in protein dynamics and polarity of the local environment upon incorporation in the matrix using spin-label EPR. Moreover, pulsed EPR experiments on doubly-labeled proteins reveal support-induced changes in the tertiary structure of the protein. All observed structural and dynamical changes will be correlated to the characteristics of the support materials.

References

[1] S. Loreto, *et al., PCCP*, **2017**, *19*, 13503

[2] S. Loreto, *et al., J. Phys. Chem. C*, **2018**, *122*, 41

Unique biradical intermediate in the mechanism of the heme enzyme chlorite dismutase discovered using microsecond timescale freeze hyperquench

J. Püschmann[a], D. Mahor[a], D.C. de Geus[b‡], M.J.F. Strampraad[a], B. Srour[a†], W.R. Hagen[a], S. Todorovic[c], P.-L. Hagedoorn[a]*

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[‡] The described work is part of activities not related to the current position of DCG at Janssen Vaccines & Prevention, a pharmaceutical company of Johnson & Johnson

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Chlorite dismutase is a unique heme b dependent enzyme that catalyzes the conversion of chlorite (ClO_2^-) to molecular oxygen (O_2) and chloride (Cl^-). This reaction involves O-O bond formation, which is rare in nature. The enzyme catalyzes a single turnover in less than a millisecond, which makes it technically challenging to study the pre-steady state kinetics of this enzyme. The catalytic mechanism of chlorite dismutase was investigated using microsecond timescale mixing techniques and the natural substrate chlorite [1]. Two different in-house developed ultrafast kinetic techniques were used: Nanospec, ultrafast continuous flow UV-vis spectrophotometry; MHQ, microsecond timescale rapid freeze hyperquenching. The dead times of these instruments are 100X shorter than commercially available devices. These techniques allowed us to observe transient intermediates of Cld during a single turnover with its natural substrate chlorite. The UV-visible, EPR and RR spectra of these intermediates were obtained. Distinct intermediates were found that are not observed with the artificial substrate peracetic acid. Most notably a triplet state EPR signal that we attribute to two weakly coupled amino acid based cation radicals, 'compound T', was transiently formed. The formation of compound T is direct evidence of a two electron transfer process which means that the Cl-O bond break is heterolytic, unlike the most recent proposed mechanism for this enzyme. To our knowledge such a triplet state has never been identified in any heme enzyme.

References

1. J. Püschmann, D. Mahor, D.C. de Geus, M.J.F. Strampraad, B. Srour, W.R. Hagen, S. Todorovic and P.-L. Hagedoorn, *ACS Catalysis*, **2021**, *11*, 14533-14544

Potential colon carcinogenic effects of different micro- and nanoparticles present in food as determined via oxygen radical formation detected by ESR/EPR spectroscopy

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These days there is a great concern about the potential health effects related to the presence of micro- (MPs) and nanoparticles (NPs) of different compositions that can been found in food products. Some of these particles are added on purpose to improve the food characteristics, while others are present as a byproduct e.g. because of their presence as environmental pollutants. The complexity and diversity of food matrices, as well as different chemical and physical properties of MPs and NPs makes that it is difficult to assess the potential food safety concerns. We studied the surface reactivity of two different representative examples of these food particles that are widely abundant in many food products, namely E171 (TiO2) and micro/nano-plastics.

E171 is a white colorant used as a food additive and can be found in dairy food products, sauces, sweets, icings, and chewing gums, as well as in personal care products such as toothpaste and pharmaceutical tablets. It consists of at least 10–40% nanoparticles in number–size distribution, with one or more external dimensions in the size range of 1–100 nm. Therefore, many studies have been performed to identify the potential carcinogenicity of E171 after ingestion, as well as other related mechanisms. We studied the potential of E171 as well as TiO2 MPs/NPs to induce oxidative stress via detection of the formation of reactive oxygen radicals by ESR in combination with spin trapping in the absence of cells as well as in the colon cancer cell line, Caco-2. Also we studied the particle reactivity by ESR in a complex food matrix like yoghurt. The results indicate that the detected formation of oxygen radicals might contribute to the creation of a favourable environment for potentiating colon cancer development by E171. Based on the outcome of these studies, as of August 2022 E171 will be banned from food products, particularly due to concerns about its genotoxic potential.

Microplastics are the abundantly present in the ecosystem. Microplastics can be ingested by living organisms, especially aquatic species like sea fish and clamps. The primary pathway of human exposure to microplastics has been identified as gastrointestinal ingestion (mainly seafood for the general population). Microplastics may pollute drinking water, accumulate in the food chain, and release toxic chemicals that may cause disease, including certain cancers. In order to detect the particle reactivity-related oxygen radical formation as an important mechanisms involved in colon carcinogenesis, we detected oxygen radical formation by ESR in combination with the spin trapping technique. We showed that some types of microplastics induce oxygen radical formation and that this can be amplified by incubation in artificial digestive fluids.

Overall this research showed that the detection of oxygen radical formation as detected by ESR in combination with the spin trappings techniques forms a valuable tool to assess the potential (colon) carcinogenic effects of different micro- and nanoparticles present in food.

Light-induced charge transfer in two-dimensional hybrid perovskites

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No photovoltaic material has displayed a more rapid increase in power conversion efficiencies (PCEs) than seen for the hybrid lead-halide perovskites, reaching up to 23% today, only a decade after their first demonstration in solar cells in 2009. Unfortunately, these materials are prone to fast degradation in humid conditions, hindering further technological development. A promising approach to mitigate these stabilization issues is the use of 2D perovskites. The latter consist of alternating layers of lead halide octahedra and organic linker cations, which are hydrophobic and form a natural barrier against water (see Fig. 1). Moreover, in contrast to their 3D counterparts, 2D perovskites have fewer structural constraints regarding the size of the organic cations interleaving consecutive perovskite layers and hence allow for more complex molecules to be embedded in the material. This opens up new possibilities for designing hybrid perovskites with tailored opto-electronic properties.

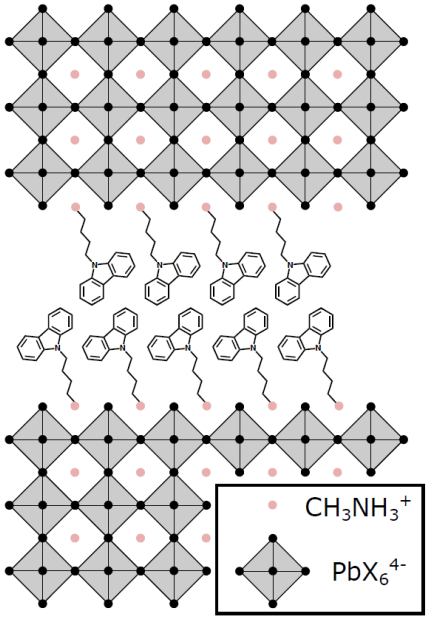

Figure 1. Schematic crystal structure of the carbazole-linked 2D lead-halide perovskites.

In this work [1], we present direct experimental evidence for the light-induced formation of positive polarons in a series of novel 2D perovskites incorporating conjugated linkers based on the carbazole molecule [2]. The combination of dedicated hyperfine spectroscopy and supporting DFT computations allowed to unambiguously identify the charge state of the observed center and to map out the spatial delocalization of the polaron over multiple adjacent carbazole units in the organic layer. Because the applied photo-excitation energies were below the absorption onset of the carbazole linkers, the polarons are proposed to be formed via charge transfer at the organic-inorganic interface from excitons generated in the lead-halide octahedral layer. To the best of our knowledge, this is the first report on charge transfer in layered perovskites, which could potentially be leveraged for the functionalization of novel 2D perovskites with unique synergistic properties of the organic and inorganic layer.

References

1. M. Van Landeghem et al., *J. Phys. Chem. C* (2021) 125, 18317-18327.
2. R. Herckens et al., *J. Mater. Chem. A* (2018) 6, 22899.

Poster Communications

Using UV-Vis spectroscopy and EPR as a tool to study the incorporation of heme proteins in titania

Robine Cleirbaut[1], Ilenia Serra[1], Lore Van den Bergh[1,2], Sabine Van Doorslaer[1]

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Biosensors based on horseradish peroxidase (HRP) have shown to be promising for the detection of phenolic compounds[1]. The key in the development of this kind of devices concerns the immobilization of the proteins on suitable supports, with multiple factors influencing the efficiency of incorporation, such as temperature, pH, type of buffer, support, and the biochemical and biophysical properties of the protein itself. Mesoporous titania (TiO_2) have shown promise to be used as support because of their biocompatibility[2].

In this study, we analyzed the incorporation of different heme proteins, like horse heart myoglobin (hhMb) and HRP, on different kinds of mesoporous and non-porous TiO_2. We carefully checked the effect of various buffer solutions. While UV-Vis spectroscopy of the supernatant provides a facile way to detect the extent of incorporation of the protein, it does not reveal any information on its fate in/on the metal oxide. Using electron paramagnetic resonance (EPR) spectroscopy, the electronic and geometric structure of the active heme site can be investigated. EPR is also a valuable tool to detect paramagnetic centers in titania, such as Ti(III) and O_2^- (surface) centers. This is exploited in this work to understand how incorporation affects the active heme site of the protein as well as its influence on the surface sites. A different pH or different buffers are found to drastically affect the adsorption of the proteins as well as their structure and electrochemical activity. Therefore, it is important to take these factors into consideration when optimizing the adsorption process of heme proteins on mesoporous TiO_2 for biosensor development.

References

1. H.H. Nguyen et al. *Materials* (2019) 12, 121.
2. S. Loreto et al. *The Journal of Physical Chemistry C* (2018) 122, 23393-23404.

Towards magnetic resonance detection of mitochondrial ROS *in vivo* in solid tumors

B. Mathieu [1] , N. Joudiou[2], L. Mignion[1] , P. Sonveaux [3] and B. Gallez [1]

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Reactive oxygen species (ROS) are involved in several diseases such as cancer. A growing body of evidence suggests that mitochondria plays a key role in cancer progression. Mitochondrial ROS production is linked with cellular processes like proliferation, angiogenesis, metastasis and cell death.

In vitro, cyclic hydroxylamines and nitroxides can be used to study ROS production by cells by using electron paramagnetic resonance spectroscopy (EPR). (1)

In vivo, nitroxides can be reduced to the corresponding hydroxylamine form or can be oxidized to oxoammonium cation by reactive oxygen species (ROS). This oxidoreduction can be modified by abnormal tissue redox conditions. (2) By monitoring the decay of the EPR signal of the nitroxide over time, the redox status in vivo can be assessed using low frequency EPR spectroscopy. (3)

Due to their paramagnetism, nitroxides may also serve as contrast agents in magnetic resonance imaging (MRI) by decreasing T1 relaxation time of water protons. Studies have used non targeted nitroxides probes to provide maps of tumors redox status. (4)

A nitroxide targeted to mitochondria (MitoTEMPO°) has been used to provide redox maps in Parkinson's disease and kidneys dysfunction in mice models but never in solid tumors models. (5)

The ambition of this project is to implement an integrative EPR/MRI toolbox for the study of cancer models where mitochondrial ROS are hypothesized to play a central role.

References

1. S. Scheinock et al. *Free radical research* (2018) 52:10, 1182-1196
2. K.I. Matsumoto et al, *Methods Mol. Biol*. (2011), 711, 397-419
3. G. Bacic et al, *Redox Biol.* 2016, 8, 226-242
4. K.I. Matsumoto et al, *Free Rad. Res*. 2018, 52, 248-255
5. Z. Zhelev et al, *ACS Chem Neurosci*. 2013, 4, 1439-45

Reactivity of peroxidases and chlorite dismutases with chlorite and hypochlorite: a spectroscopic investigation of short-lived intermediates trapped by fast freeze-quenching

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Chlorite (ClO_2^-) and hypochlorite (ClO^-) are strong oxidants commonly employed as bleaching agents or disinfectants, however, concerns have been raised around their presence in the environment as pollutants. Chlorite dismutases (Clds) are heme *b*-containing oxidoreductases which possess the unique ability to decompose ClO_2^- into harmless Cl^- and O_2. Investigations into the underlying reaction mechanism thus open ways for potential biotechnological and bioremediation applications.[1] Horseradish peroxidase (HRP), a model enzyme for heme peroxidases, is also known to react with both chlorite and hypochlorite.[2] During the reaction of Clds and HRP with ClO_2^- and ClO^- short-lived intermediates involving transient radicals are formed, making these systems suitable to be studied by electron paramagnetic resonance (EPR) spectroscopy. Given the short time scales of these reactions, a fast freeze-quenching device is needed to trap the transient radical species and collect the frozen sample directly into the EPR tube. In this work we present a comparative EPR investigation of the intermediate states formed during the reaction of HRP and Clds with either chlorite or hypochlorite, to identify common and distinct mechanistic features of these enzymatic systems. This work received funding from the European Union's Horizon 2020 research and innovation program (Marie Skłodowska-Curie Grant Agreement n° 813209) and it was supported by the Austrian Science Funds (FWF-project P30979) and the doctoral program BioToP – Biomolecular Technology of proteins (FWF W1224).

References

1. S. Hofbauer et al., *Biochim Biophys Acta Proteins Proteom.* (2021) Jan;1869(1):140536
2. C. Jakopitsch et. al., *J. Inorg. Biochem*. (2008) 102, 293–302

Paramagnetic centers in LiF:Mg,Cu,P thermoluminescence radiation dosimeters

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LiF doped with magnesium, copper and phosphorus (LiF:Mg,Cu,P) has been introduced as a thermoluminescence dosimeter material for γ-rays at the end of the 1970's [1]. By now it is well-established as high-sensitivity detector material for γ and β-rays, down to the μGy range [2]. The effects of high doses (> 1 kGy) are currently also being explored [3]. All codopants in this material have been found to be essential for obtaining the sensitivity [4]. The exact role of each dopant and how they are incorporated in the LiF matrix is still less clear, though [5].

The room temperature EPR spectrum of LiF:Mg,Cu,P is dominated by a component of an axial paramagnetic center, with $g_{\perp} > g_{//} > g_e$ (= 2.0023), large line width and lacking resolved hyperfine structure. In literature a variety of principal g values have been reported for this center. It has been attributed to Cu^{2+} even though its characteristics are atypical for Cu^{2+} in octahedral F surroundings. In an attempt to characterize this and possible other EPR-detectable defects in this phosphor, we investigated LiF:Mg,Cu,P at room temperature using powder X, Q and W-band EPR, complemented with Q-band ENDOR at 5K. The latter measurements clearly show that a second type of paramagnetic centers is present in this system, which may complicate a consistent analysis of the room temperature spectra.

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References

1. T. Nakajima et al. *Nucl. Instrum. Methods* (1978) 157, 155 – 162.
2. P. Bilski et al. *Proceedings of the IRPA Regional Symposium* (1998) 498–501. https://inis.iaea.org/search/search.aspx?orig_q=RN:30031596
3. P. Bilski et al. Radiat. Meas. (2020) 139, 106486.
4. J.I. Lee et al. *Radiat. Meas.* (2008) 43, 303–308.
5. P.L. Guzzo et al. *J. Lumin.* (2018) 198, 284–288.

Effects of SDHIs and strobilurins fungicides on the mitochondrial function of human HepG2 cells

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SDHIs and strobilurins are fungicides that are widely used in agriculture around the world. They act by inhibiting mitochondrial respiration and, more particularly, the electron transport chain (Li et al., 2021).

The use of these substances raises questions, especially regarding their specificity for pathogenic fungi. Previous studies have shown that these substances have toxic effects on the mitochondrial function of different organisms such as zebrafish embryos, mammalian cells or human cells (Benit et al., 2019; Flampouri et al., 2016; Regueiro et al., 2015; Zhang et al., 2020). The aim of this project is to study the effects of these fungicides on the mitochondrial function of human HepG2 cells.

Electron paramagnetic resonance is the method of choice to study the mitochondrial function of human HepG2 cells providing information on two parameters: oxygen consumption and superoxide production.

We first study the effects of Boscalid and Bixafen (SDHIs) on the mitochondrial function of HepG2 cells. The results show that HepG2 cells exposed during a short time to Bixafen and Boscalid have significantly reduced oxygen consumption and significantly increased superoxide production (d'Hose et al., 2021). This induced mitochondrial dysfunction was associated with an increase in the number of apoptotic cells.

We are now evaluating the effect of exposure of HepG2 cells to strobilurins and SDHI/strobilurins mixtures. The first results obtained for pyraclostrobin (strobilurin) shows a decrease in oxygen consumption in HepG2 cells exposed to this fungicide.

The next step of the project will be to evaluate the effects of pyraclostrobin on superoxide production, on the induction of apoptosis and on metabolic effects such as ATP levels or antioxidant enzyme levels. Another objective will be to evaluate the effects of a SDHI/strobilurin mixture in order to detect additive or synergistic effects between these two fungicides.

References :

Benit, P., Kahn, A., Chretien, D., Bortoli, S., Huc, L., Schiff, M., Gimenez-Roqueplo, A. P., Favier, J., Gressens, P., Rak, M., & Rustin, P. (2019). Evolutionarily conserved susceptibility of the mitochondrial respiratory chain to SDHI pesticides and its consequence on the impact of SDHIs on human cultured cells. *PLoS One*, *14*(11), e0224132. https://doi.org/10.1371/journal.pone.0224132

d'Hose, D., Isenborghs, P., Brusa, D., Jordan, B. F., & Gallez, B. (2021). The Short-Term Exposure to SDHI Fungicides Boscalid and Bixafen Induces a Mitochondrial Dysfunction in Selective Human Cell Lines. *Molecules*, *26*(19). https://doi.org/10.3390/molecules26195842

Flampouri, E., Mavrikou, S., Mouzaki-Paxinou, A. C., & Kintzios, S. (2016). Alterations of cellular redox homeostasis in cultured fibroblast-like renal cells upon exposure to low doses of cytochrome bc1 complex inhibitor kresoxim-methyl. *Biochem Pharmacol*, *113*, 97-109. https://doi.org/10.1016/j.bcp.2016.06.002

Li, X. Y., Qin, Y. J., Wang, Y., Huang, T., Zhao, Y. H., Wang, X. H., Martyniuk, C. J., & Yan, B. (2021). Relative comparison of strobilurin fungicides at environmental levels: Focus on mitochondrial function and larval activity in early staged zebrafish (Danio rerio). *Toxicology*, *452*, 152706. https://doi.org/10.1016/j.tox.2021.152706

Regueiro, J., Olguin, N., Simal-Gandara, J., & Sunol, C. (2015). Toxicity evaluation of new agricultural fungicides in primary cultured cortical neurons. *Environ Res*, *140*, 37-44. https://doi.org/10.1016/j.envres.2015.03.013

Zhang, C., Zhou, T., Xu, Y., Du, Z., Li, B., Wang, J., Wang, J., & Zhu, L. (2020). Ecotoxicology of strobilurin fungicides. *Sci Total Environ*, *742*, 140611. https://doi.org/10.1016/j.scitotenv.2020.140611

Comparative study of short-lived radical species generated in in-plasma and subjacent-plasma treated water

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In recent years, atmospheric pressure plasma technology has been increasingly explored as a possible cancer treatment strategy after being integrated into the medical field as sterilization and wound healing method. In fact, the interaction of plasma species with different living and non-living substrates leads to the formation of a variety of reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as H_2O_2, OH, O and NO_2^- that exhibit a cytotoxic effects to cancer cells and cannot be produced by any other approach[1]. In contrast to the traditional cancer treatments such as radiotherapy and chemotherapy, one of the major advantages of plasma is its selective anti-cancer ability, which has been confirmed over many cancerous versus non-cancerous cell lines.

In a more general perspective, the use of plasma in oncology can be applied following 2 different approaches:

1) The direct treatment in which living cancerous cells, tissue or organs are directly exposed to plasma and are considered as one of the electrodes thus actively participating in the discharge plasma processes.

2) The indirect treatment in which a physiological fluid is first subjected to a plasma treatment then the obtained plasma-activated liquid (PAL) is brought into contact with cancer cells. This approach is currently attracting the interest of researchers since most cells and tissues are surrounded by liquids and PAL in contact with such tissues can lead to several desirable physiological responses.

Up to date, in most studies involving plasma treatment of liquid for cancer treatment, an atmospheric pressure plasma jet (APPJ) generated in the gas phase is placed above the liquid to be treated. This study launches an innovative approach in which plasma is generated in the liquid phase directly. Moreover, the focus in literature is mainly directed towards the identification of stable plasma-induced species and less attention is given on the identification of short-living species such as for example •OH and •H radicals that are also generated in the plasma-exposed solutions.

We present here the first results of a study aiming to compare in-plasma and subjacent-plasma treatment in terms of produced short-lived radical species and their concentrations with EPR, using DMPO as a spin trap. A parametric investigation including a change in the plasma exposure time (2, 5, and 10 min) and the feed gas (He and Ar) carried out in each set-up state. Moreover, The time-dependence of OH and H radical adducts has been followed.

After in-plasma treatment we observe both OH and H species trapped by DMPO. The concentration of the H radical adduct is nearly time-independent and builds up with plasma treatment time. The DMPO-OH radical adduct strongly decays within ~20 min. Rather surprisingly, increasing plasma treatment time leads to lower concentration for this radical. After adjacent-plasma treatment no DMPO-H species could be detected. On the other hand, DMPO-OH is produced in high concentrations and has longer lifetime. The implications of these results for further research will be discussed with you.

References

1. Gorbanev, Yury, Deborah O'Connell, and Victor Chechik, Chemistry–A European Journal 22.10 (2016): 3496-3505.

EPR imaging study of Nanodiamonds

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Recent advances in nanotechnology have attracted considerable interest for nanodiamonds (NDs) in industrial and research areas thanks to their remarkable intrinsic properties including large specific area, poor cytotoxicity, chemical stability, ease of large-scale production and magnetic and optical properties as a result of relative stability of radical-like centers inside the particle. This feature constitutes a main interest for versatile applications, which makes NDs, a relevant platform against non-stable organic radicals (*e.g.*, sensitive to reduction). Two particle origins were of interest: NDs produced by detonation or by grinding of micro-sized crystals obtained by high pressure-high temperature (HPHT) static synthesis. Overall, NDs show a high concentration of radical-like paramagnetic centers (up to 10^{19} spin/g), which are due to structural defects and carbon dangling bonds) inside the diamond core and on the cluster surface. The observed EPR line shapes were characterized with peak-to-peak resonance width (ΔH_{pp}) from 2.3 to 10 Gauss according to some parameters (*e.g.*, NDs origin, treatment). Their experimental EPR spectrum can be assumed as a sum of two components of single lines with the same g-factor (g = 2.0028) but having different linewidths contributions. Since the resolution in EPR imaging is closely proportional to the EPR resonant linewidth, we studied surface modification (*e.g.*, oxidation) and size exclusion to modify the structure and thus reducing the broad component of the signal. Nanodiamond physicochemical properties were evaluated by different characterization techniques including dynamic light scattering (DLS), transmission electron microscopy (TEM), thermalgravimetric analysis (TGA), Fourier transform-infrared (FT-IR) and X-ray photoelectron spectroscopy (XPS) to determine the nature of the particle surface and to monitor the size exclusion and stability of the suspensions.

Here, we describe the development and design of a nanodiamond strategy (*e.g.*, particle origin, surface oxidation, size exclusion) to demonstrate high spectroscopic and imaging feasibilities using these particles for EPR. To achieve this, mathematical and IT procedures were developed and allowed experimental evidence of the conditions required for optimal phantom images resolution (R=0.5 mm, HPHT-sub18).

The ability to perform low frequency EPR (L-band, 1 GHz) resolution imaging in combination with the stable intrinsic properties of nanodiamonds, raises the possibility of performing non-invasive tracking of nanodiamonds.

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References

1. S. Stehlik, *et al.*, *J Phys Chem C*. (2015) 119, 27708-20.
2. E. Rej, *et al.*, *Nat Commun*. (2015) 6, 8459-66.

Targeting the electron transport chain with the mitochondrial targeted biguanide mito-metformin10 to alleviate tumor hypoxia in prostate cancer

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Over the past decades, research on mitochondria witnessed an important gain of interest, highlighting its implication in many physiological and pathological processes like diabetes [1], cardiovascular diseases [2] and neurodegenerative disorders. A growing body of evidence also indicates that mitochondria plays a key role in cancer progression and response to treatment due to its involvement at the crossroad of many cellular pathways. Mitochondrial implication in mediating programmed cell death has led to a great interest in exploiting radio- and chemo-therapeutic agents to trigger cancer cell death [3]. Also, altering the mitochondrial respiration has been associated with alleviation of tumor hypoxia and a consequent increase in radiosensitivity [4]–[6].

With the development of an EPR toolbox at our lab [7], we had the opportunity to test Mito-metformin10, a mitochondria-targeted metformin analogue, which was recently demonstrated to decrease OCR, cell proliferation and tumor growth of PDAC cells *in vitro* and *in vivo* at lower concentrations than Metformin [8]. MitoMetformin10 was also proven to have radiosensitizing effects on the same cell line *in vitro*. So far, no one assessed this new analogue's effect on tumor response to irradiation *in vivo* after treatment.

At our lab, EPR respirometry experiments using MitoMet10 have shown to decrease OCR significantly after 24H at 1µM treatment on PC-3 and DU145 (prostate cancer) cells. We also observed an increasing trend in the production of mitoROS after MitoMet10 exposure on PC-3 but it has yet to be confirmed with a last EPR spectroscopy experiment. Surprisingly, MitoMet10 exposure on DU145 didn't affect mitoROS production despite its significant impact on OCR. However, glutathione tests showed increased GSH levels in DU145 than in PC-3 cells, meaning they can protect themselves from the harm of MitoMet10 induced ROS production.

To evaluate the relevance of these observations *in vivo*, we performed quantitative measurements of tumor oxygenation (using low frequency EPR) in the PC-3 prostate cancer model in mice. This model was found highly hypoxic at the basal level. When mice were treated with MitoMet10 (daily injection of 2 mg/kg), the tumor oxygenation significantly increased 24 hours after initiation of the treatment. Despite the increase in oxygenation, MitoMet10 failed to increase the sensitivity to irradiation in this tumor model.

References

1. P. Newsholme et al. *Adv. Exp. Med. Biol* (2012), 942, 235-247.
2. S. W. Ballinger et al. *Free Radical Biology and Medicine* (2005), 38, 1278-1295.
3. P. Costantini et al. *J. Natl. Cancer Inst* (2000), 92, 1042–1053.
4. B. F. Jordan et al. *Cancer Res* (2002), 62, 3555–3561.
5. C. Diepart et al. *Cancer Res* (2012), 72,482–490.
6. B. Gallez et al. *Biochimica et Biophysica Acta - Bioenergetics* (2017), 1858, 700–711.
7. D.d'Hose et al. *Redox Biol* (2021), 40, 101852.
8. G. Cheng et al. *Cell Biochem Biophys* (2016), 75, 311-317.

Development of a combined methodology towards mechanistic investigation of rare metal-free, light activated catalysts

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Photocatalysis has been an important research field in the last 40 years and has become increasingly more common as an easy way to obtain unusual reactivity and greener synthetic pathways.

While compounds of rare transition metals such as ruthenium and iridium are commonly employed,[1] in recent years the interest has started to shift towards different photocatalysts containing more easily available metals, such as copper, which not only allow for more economically affordable catalysts but also open up reactions and selectivity that were previously inaccessible or unexplored.[2]

In the frame of the European Programme MSCA-Horizon 2020 "Paramagnetic Species in Catalysis Research (PARACAT)" we are working to shed light on synthetic pathways that employ these alternatives. In this talk we will present the insight we were able to obtain for a photocatalytic processes employing a commercially available photosensitizer. This remarkable synthetic protocols can use visible light and mild conditions while resulting in high yield and minimal waste products. EPR and other spectroscopic results will be presented and related to different mechanistic proposals.

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References

1. Prier, C.K et al.; *Chem. Rev.* (2013) 113, 5322-5363.
2. Hossain, A. et al. ; *Science* (2019) 364/6439, eaav9713

Dual measurement of oxygenation and extracellular pH using stable trityl radicals. Application to evaluate the impact of a mitochondrial pyruvate carrier inhibitor on the tumor microenvironment.

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The acidosis of the tumor microenvironment may have profound impact on cancer progression and on the efficacy of treatments. In the present study, we evaluated the impact of a treatment with a mitochondrial pyruvate carrier (MPC) inhibitor on tumor extracellular pH (pHe). MPC disruption was showed to either promote or inhibit cancer progression and impact the extracellular acidification [1, 2]. Corbet et al. [3] showed that MPC inhibition by 7ACC2 decreased the growth of the SiHa cervix cancer cell line. We decided to study the effect of UK-5099, another MPC inhibitor on extracellular pH *in vitro* and *in vivo* using non-invasive Electron Paramagnetic Resonance (EPR). Gluth et al. [4] synthesized a mono-phosphonated tetrathiatriarylmethyl radical, pTAM, whose EPR spectrum is sensitive to multiple parameters: oxygen concentration, pH, and inorganic phosphate concentration.

In the first part of the work, calibration studies were performed on X-Band EPR with solutions at different pH and changing oxygen concentrations. *In vitro* studies were performed on breast cancer 4T1 cells, treated or not with UK-5099 (10 µM) for 24 hours. Extracellular pH values can be obtained from the fraction of the acidic form P_a versus basic form P_b of pTAM whereas peaks linewidths are functions of the oxygen concentration. Oxygen consumption rate (OCR) were measured in sealed capillaries containing control or treated cells and pTAM (200 µM). OCRs obtained with pTAM were compared to experiments with ^{15}N-PDT (4-oxo-2,2,6,6-tetramethylpiperidine-d16-15N-1-oxyl) used as a control sensor for oximetry [5]. For the *in vivo* part of the study, mice bearing the 4T1 tumor model were treated daily during four days with UK-5099 (3 mg/kg). The pHe was evaluated before and after treatment with UK-5099 on L-Band EPR using pTAM.

The *in vitro* results showed that pTAM was able to measure an acidification of pHe values after 4T1 cells were treated for 24 hours with UK-5099. No significant differences in OCR were measured either with pTAM or with ^{15}N-PDT. Preliminary *in vivo* results on 4T1 tumor models showed a significant decrease in tumor pHe in UK-5099-treated mice while there was no change over time for mice treated with the vehicle.

MPC inhibition showed effects on the pHe values *in vitro* and *in vivo* whereas no significant changes were observed in oxygen concentrations. The trityl radical pTAM was able to measure extracellular pH and oxygen concentrations simultaneously *in vitro* and *in vivo*, on X-Band and L-Band EPR, respectively.

References

1. Bender, T. and J.C. Martinou. Biochim Biophys Acta, 2016. **1863**(10): p. 2436-42.
2. Ruiz-Iglesias, A. and S. Manes. Cancers (Basel), 2021. **13**(7).
3. Corbet, C., et al. Nat Commun, 2018. **9**(1): p. 1208.
4. Gluth, T.D., et al. RSC Adv, 2021. **11**(42): p. 25951-25954.
5. d'Hose, D., et al. Molecules, 2021. **26**(19).

Elucidation of molecular structure of Cu^{II}-TREN complexes with trigonal bipyramidal structure: An exploration by advanced EPR techniques

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Copper is a widely used transition metal and the third most abundant trace element in humans after iron and zinc[1], for many years scientists have been interested in investigating copper complexes with multidentate tripodal ligands, such as the ones found in biological systems. This curiosity to study copper-nitrogen containing complexes began when the samples of active copper metalloprotein enzymatic centres, including proteins that transport oxygen, transfer oxygen after O-O bond cleavage or using oxygen as hydrogen atom acceptor such as hemocynaine, tyrosinase and galactose oxidase[2]. Platinum group metals (PGMS) have traditionally been used as catalysts for selective oxidation of alcohols. However, increased concerns about PGMS sustainability are driving researchers to look for cheaper and more environmentally friendly alternatives. In nature, the fungal copper enzyme galactose oxidase (GAO) converts primary alcohol to aldehydes. Inspired by nature, efforts have been put to focus on the use of copper complexes, mimicking Cu enzymes for different catalytic oxidation reactions in organic chemistry[3]. Copper complexes with multidentate tripodal alkylamine, Schiff base, or aza aromatic ligands or their hybrids have been widely used to model the structure and reactivity of active sites in copper proteins[4]. For years, significant attention was paid to study the copper complexes with four coordination number including square planar and square pyramidal geometries. Noticeably fewer studies have been allocated to the five coordinated Cu(II) complexes with trigonal bipyramidal structure. The ligands of the tris(2-aminoethyl) amine (TREN) family are the most important representatives among the tripods which force a metal cation into a trigonal bipyramidal coordination geometry[5]. Several studies have analysed different copper metalloprotein sites, however the coordination chemistry of copper (II) is further complicated because of different coordination spheres and oxidation states possible for this metal ion[6]. Herein this project, Cu^{II}-complexes with TREN as a tripodal ligand coordinated to the metal centre have been studied due to the ability of the multidentate ligands to control the redox activity of the Cu^{II} centres. The geometry and electronic structure of the Cu^{II}-TREN complexes were investigated using a series of advanced EPR techniques like multi-frequency measurements including X, Q and W-band. Afterwards, for obtaining more information about the metal-ligand bonding nature from the hyperfine and nuclear quadrupole couplings we utilized CW Q-band ENDOR and pulsed-ENDOR (Davies) techniques. The studies have been performed in different ligand-to-metal ratios and at different pH values.

References:

1. E. T. Adman, *Adv. Protein Chem.* (1991) 42, 145–197.
2. V. Raab et al. *Inorg. Chem.*(2001) 40, 6964–6971.
3. E. C. Hayes et al. *J. Am. Chem. Soc.* (2016) 138, 4132–4145.
4. S. Schindler, *Eur. J. Inorg. Chem.* (2000), 2311–2326.
5. E. A. Ambundo et al. *Inorg. Chem.* (2003) 42, 5267–5273.
6. E. Carter et al. *Dalton Trans.* (2013) 42, 15088–15096.

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Repurposing statin use to alleviate tumor hypoxia in prostate cancer: an opportunity for radiosensitization?

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Over the past decades, research on mitochondria witnessed an important gain of interest, highlighting its implication in many physiological and pathological processes like diabetes [1], cardiovascular diseases [2] and neurodegenerative disorders. A growing body of evidence also indicates that mitochondria plays a key role in cancer progression and response to treatment due to its involvement at the crossroad of many cellular pathways. Mitochondrial implication in mediating programmed cell death has led to a great interest in exploiting radio- and chemo-therapeutic agents to trigger cancer cell death [3]. Also, altering the mitochondrial respiration has been associated with alleviation of tumor hypoxia and a consequent increase in radiosensitivity [4]–[6].

As a meta-analysis described a beneficial effect of statins on prostate cancer patients treated with radiation therapy (RT), it is crucial to investigate the mechanisms underlying the potential radiosensitizing properties of statins. Our hypothesis was that the alleviation of tumor hypoxia induced by an inhibition of oxygen consumption rate (OCR) could play a role in the improved response to RT in those patients receiving statins. The effect of statins on OCR has been indeed demonstrated in a variety of normal cells, such as cardiomyocytes, skeletal muscle cells, and hepatocytes but has never been described in cancer cells. A screening of five statins (simvastatin, fluvastatin, rosuvastatin, pravastatin, atorvastatin) using Seahorse XF revealed that a 24-hours exposure to simvastatin and fluvastatin significantly decreased the OCR in PC-3 prostate cancer cells. This effect was confirmed for both compounds using EPR respirometry. We also observed an increase in the production of mitoROS after statin exposure as demonstrated by EPR spectroscopy. To evaluate the relevance of these observations in vivo, we performed quantitative measurements of tumor oxygenation (using low frequency EPR) in the PC-3 prostate cancer model in mice. This model was found highly hypoxic at the basal level. When mice were treated by simvastatin or fluvastatin (daily injection of 20 mg/kg), the tumor oxygenation significantly increased 48 and 72 hours after initiation of the treatment. Despite the increase in oxygenation, simvastatin failed to increase the sensitivity to irradiation in this tumor model.

References

1. P. Newsholme et al. *Adv. Exp. Med. Biol* (2012), 942, 235-247.
2. S. W. Ballinger et al. *Free Radical Biology and Medicine* (2005), 38, 1278-1295.
3. P. Costantini et al. *J. Natl. Cancer Inst* (2000), 92, 1042–1053.
4. B. F. Jordan et al. *Cancer Res* (2002), 62, 3555–3561.
5. C. Diepart et al. *Cancer Res* (2012), 72,482–490.
6. B. Gallez et al. *Biochimica et Biophysica Acta - Bioenergetics* (2017), 1858, 700–711.

Superoxide anion radical scavenging activity of propofol and its derivatives: a Chemiluminescence and EPR spin trapping study

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Inflammation is a complex physiological phenomenon involving chemical and enzymatic mechanisms. During this event, PolyMorphonuclear Neutrophil leukocytes (PMNs) play an important role by producing reactive oxygen species (ROS) and releasing oxidant enzymes, which all participate in the host defense against micro-organisms like bacteria and virus.
Inflammatory pathologies such as sepsis, atherosclerosis, Crohn disease, rheumatoid arthritis,… are characterized by the invasion of tissues by PMNs followed by excessive stimulation of the latter ones. The uncontrolled production and release of ROS and enzymes like myeloperoxidase (MPO) in the extracellular medium cause severe damages to the surrounding tissues [1,2].

A possible pathway to control excessive inflammation and treat chronic pathologies is to regulate the PMNs functions [3]. Among the PMNs properties, the respiratory burst plays an essential role by producing superoxide radical anions. These ROS are notably the precursors of hydrogen peroxide, the natural substrate of MPO, another marker of inflammation.

Propofol, an anesthetic agent extensively used in intensive care units, has already shown actions as superoxide anions scavenger [4].
The aim of this study is to complete the investigation of the antioxidant property of propofol. For doing, three propofol derivatives (propofol-β-glucuronide (PPFG), 2,6-diisopropyl-1,4-p-benzoquinone (PPFQ) and 3,5,3',5'-tetraisopropyl-(4,4')-diphenoquinone (PPFDQ)) have been compared on their superoxide radical anions ($O_2^{\cdot-}$) scavenging abilities using EPR spin trapping spectroscopy and Chemiluminescence techniques. Two sources of superoxide anions have been used, a chemical one (KO_2) and an enzymatic one (xanthine/xanthine oxidase).
Overall, our results indicate that the quinone forms of propofol, PPFQ and PPFDQ, act as potent superoxide anions scavenger, whereas its main metabolite, PPFG, is inactive.

References

1. J. A. Smith, *J. Leukoc. Biol.* (1994) 56, 672-686.
2. L. K. Stamp *et al.*, *Rheumatology* (2012) 51, 1796-1803.
3. T. Németh *et al.*, *Nature Reviews – Drug Discovery* (2020) 19, 253-275.
4. I. Gülçin *et al.*, Chem. Pharma. Bull (2005) 53, 281-285.

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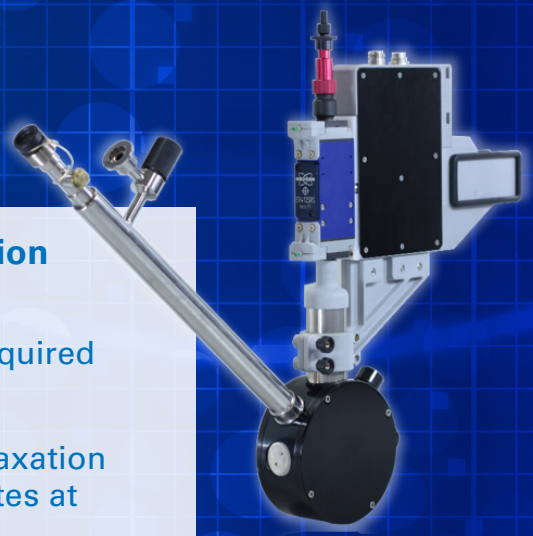
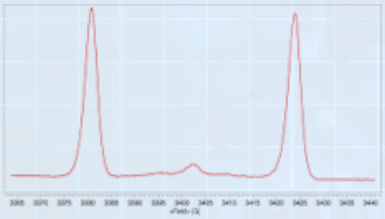
Rapid Scan Measurements at Low Temperatures



Overcoming limitations due to saturation

Rapid Scan spectrum of P doped Si at 5 K



- For P doped Si low temperature is required to see the EPR signal
- However, at low temperature, the relaxation time increases, and the signal saturates at low power in CW-EPR
- Rapid Scan overcomes saturation effects resulting in higher signal amplitude

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