

20th meeting of the
Benelux EPR Society
Leiden May 24th, 2012.

Programme
Book of Abstracts
List of participants



Universiteit Leiden



Program

May 24th, 2012, Leiden University

Einsteinweg 55, 2333 CC Leiden Lecture hall C1 (main entrance of the Gorlaeus Labs)

10.00		<i>coffee/tea and registration</i>	
10.30		welcome (room C1)	
10.35	Ernst van Faassen	On the role of nitric oxide radicals in septic myocardial dysfunction	LUMC, ULeiden
11.00	Martin van Son	Doubly spin-labeled peptides: Models for short distances	ULeiden
11.25	Sevdalina Lyubenova	Realities and Perspectives in modern EPR	Bruker BioSpin Germany
12.00		<i>depart for lunch, 10 min walk</i>	
14.00	Anna Dimitrova	Reversible activation of Mechanosensitive Channels of Large Conductance studied by EPR spectroscopy	RU Groningen
14.25	Philippe Levêque	Extended Spin Trapping strategy: can the use of a cocktail of spin traps speed up the current step by step approach?	UCLouvain
14.50		poster session and coffee	
15.45	Biniam Tedlla	Spin dependent conductivity in MDMO-PPV based diodes studied by electrically detected magnetic resonance	UAntwerpen
16.10	Gauthier Vanhaelewyn	EMR study of X-irradiated L-threonine single crystals – tentative radical models	Ghent University
invited lecture:			
16.35	Daniella Goldfarb	Gd ³⁺ - nitroxide and Mn ²⁺ - nitroxide distance measurements for structural studies of biomacromolecules	Weizmann Institut of Chemistr, Rehovot, Israel
<i>farewell drinks and discussions</i>			

List of Posters

(*alphabetic by presenting author, abstract p.9 ff*)

1	Pierre	Danhier	UCLouvain	"Oxygen Consumption Assessment of Adherent Cells Using Electron Paramagnetic Resonance"
2	Pierre	Danhier	UCLouvain	"Ex Vivo Monitoring of Spontaneous Metastasis of Iron Oxide-labeled Cancer Cells using EPR"
3	Geraldine	De Preter	UCLouvain	"Screening of tumor cell metabolism: comparison between O ₂ consumption (EPR oximetry) and glycolysis efficiency (enzymatic assays)"
4	Quentin	Godechal	UCLouvain	"Differentiation between pigmented and non-pigmented human skin melanoma using electron paramagnetic resonance (EPR) spectrometry and imaging"
5	Maryam	Hashemi Shabestari	ULeiden	"The effect of a membrane mimicking detergent on Alzheimer's Amyloid peptide aggregation studied by EPR"
6	Feng	Lin	UAntwerp	"EPR investigation of pore formation and metal incorporation in periodic mesoporous organosilicas"
7	Yun	Ling	UAntwerpen	"Efficient charge transfer in blends of donor para-phenylenevinylene polymer and acceptor thiazolothiazole oligomers for organic photovoltaics"
8	Irina	Lobysheva	University of Louvain Medical School	"EPR study of cross-talk between superoxide anion and nitric oxide in vasculature based on cyclic hydroxylamine oxidation"
9	D.	Nguyen	KULeuven	"3d-metal near-interface Si substrate contamination during ALD processing detected by electron spin resonance"
10	Dorith	Wunnicke	RUGroningen	"Ligand induced conformational capture of a synthetic tetracycline riboswitch revealed by pulse EPR"

DO this!

On the role of nitric oxide radicals in septic myocardial dysfunction.

Ernst van Faassen, Leiden University Medical Centre, NL.

Severe sepsis presents with an uncontrolled release of nitric oxide radicals. It initiates a lethal cascade of collapse of blood pressure, multi-organ failure and severe heart dysfunction. In this work we question the usual assumption that the nitric oxide be due to excessive expression of inducible Nitric Oxide Synthase (iNOS) isoform. We investigated the role of various NOS isoforms in a mouse model of acute septic shock where intestinal sepsis was induced by caecum ligation and puncture. The levels of nitric oxide in various organs were measured by EPR using in-vivo spin trapping with Fe-DETC complexes. Comparing the physiological response of wild-type and mutant mice, we find that the endothelial NOS isoform (eNOS) contributes very significantly to the pool of nitric oxide. Contrary to conventional expectation, we find that eNOS aggravates the septic insult to cardiac function. Our findings have important implications for therapeutic treatment of septic shock with NOS inhibitors.

Spin trap!

Bloedvergiftiging 25%

↳ organ failure / dysfunction!

Nitric oxide synthases (NOS)

Nitride NO (new field?!)

inhibition NOS enzymes →

↳ extra → blood press ↓

by C. → 100 mM. → EPR
200

Spin trapping

→ trapping in vivo
→ after death
→ get signal
out of very
large dist Cu²⁺

Padova

goede presentatie, vragen!

DOUBLY SPIN-LABELED PEPTIDES: MODELS FOR SHORT DISTANCES

Martin van Son^a, Maryam Hashemi Shabestari^a, Enrica Venturelli^b,
Alessandro Moretto^b, Claudio Toniolo^b, Martina Huber^{a*}

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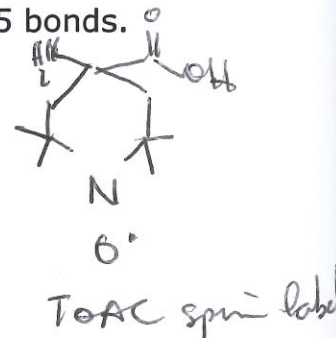
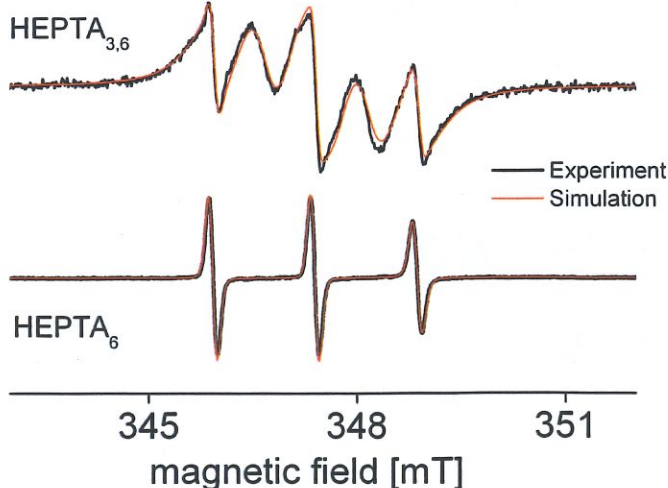
^bDepartment of Chemistry, University of Padova, Italy

$w_{dip} \propto \frac{1}{r^3}$
↓
dip.

$J \sim e^{-\alpha r^2} \rightarrow$ kan niet echt uit deze studie, eerder # bindingen!?

Measuring distances is important to determine structural features in biological systems. We synthesized a series of rigid peptides that serve as models for distance determination. These were measured in liquid solution and at room temperature with electron paramagnetic resonance. The peptides contain two identical nitroxide spin-labels (TOAC) that are separated by two, three, four, or five amino acids. We elaborate the spin-spin interaction, i.e. exchange interaction, on the spectra and relate it to the relative position of the spin labels. The results suggest that the exchange interaction is facilitated by the helical peptide backbone and is carried through no less than 15 bonds.

exchange!
[]



81% - 19% spec.

Peptides



Realities and perspectives in modern EPR (latest developments)

Sevdalina Lyubenova

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Xenon: software significant
34 GHz → power probe
263 GHz ← J-band more power!

The progress in the performance of digital components in the last years had a considerable influence in the development of EPR instrumentation. In the mid 90's a high speed signal averager which could accumulate 1 million transients per second with 250 MHz sampling rate was the limit of the technology. Today the maximum sampling rate for a high speed averager is at 1 GHz. In addition to high speed averaging, the speed of new signal processors allows even real time applications of mathematical functions for signal processing, e.g. FFT, windowing, phase cycling. All these developments have resulted in a performance jump in modern pulse-EPR instruments.

For the low frequency analog-to-digital converters (ADC) the main increase in performance relates to the amplitude resolution. An 8 bit ADC with 100 kHz sampling rate, as used in field modulated experiments, was quite common 20 years ago. Currently this is at 32 bit and gives a tremendous dynamic range in amplitude resolution. Furthermore, experiment handling has become much more forgiving as, for example, receiver gain settings are now almost irrelevant.

Another development related to digital-to-analog converters (DAC) has emerged in the last couple of years giving completely new perspectives to pulse-EPR. Specifically, arbitrary waveform generators (AWG) with clock speeds in the order of several Gs/sec and 12 bit vertical resolution became the results of this progress. An AWG is not only limited to rectangular pulses as typically used in pulse-EPR up to now. It can generate pulses with almost any feature which can be described mathematically, e.g. pulse shapes, amplitude ramps or frequency chirps. By combining the AWG analog output signal with a microwave source of about 9 GHz all the AWG features can be transferred to a typical X-Band EPR frequency. This opens up completely new experimental possibilities in pulse-EPR and the first results of an X-Band implementation will be reported.

A. Valin-

EPR
F
b

Application examples on ELEXSYS E780, the first commercial 263 GHz EPR spectrometer which enables routine operations at high fields will be also presented. Furthermore, different microwave power options in the light of performance in pulse ELDOR experiment at Q-Band will be discussed.

→ high resolution impressive, but what
SPU → abs disp. multi.

Anja Cunnels
JEM 2009

→ Simulative quantitative EPR → 6k! interesting
münchen tech cen high level det 200 kosten !!

Add Waveform Generator → in progress → pulse EPR. ± 200 MHz

Q-Pulse:

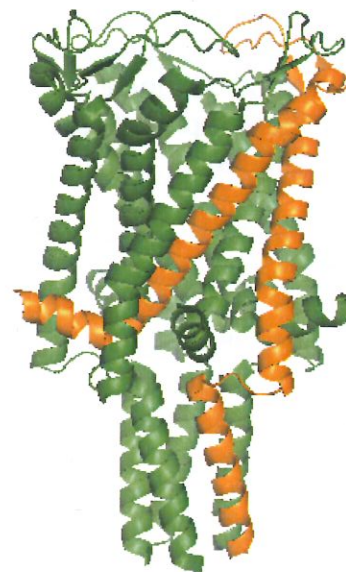
PELDOR / DEER ↓ in single shot.

Following the mobility changes of G22R1 MscL upon opening and closing of the channel

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Bacterial cells are protected from the life threatening risks of hypo-osmotic shock by a family of membrane spanning Mechanosensitive (MS) channels. One of the best characterized MS channels is MechanoSensitive Channel of Large conductance (MscL) from *Esheria coli*. Its sequence, structure from a homologue (Figure) and electrophysiological characteristics are well known. If cell faces sudden hypo-osmotic stress, water enters into the cells and the turgor pressure increases. MscL senses the resulting changes in the physical properties of the lipid bilayer and acts as a safety valve. It undergoes significant structural changes and opens one of the largest pores in nature – allowing passage of water, ions and even small protein molecules. Thus, MscL (and MS channels in general) is able to convert the mechanical force directly into helical movements.



*Crystal structure of
Mycobacterium tuberculosis MscL*

Even though a model of MscL opening exists, the real mechanism is still unknown.

Here, we present a method to follow helical movements of the channel towards gaining insight into the gating mechanism of MscL. We follow the structural rearrangements of reconstituted MscL by activating (and deactivating) the channel by external addition of different L- α -lysophosphatidylcholine (LPC) concentrations and, trapping the channel in semi-closed, expanded and open states. Deactivation is achieved by the addition of bovine serum albumin (BSA). Our strategy combines Site-Directed Spin Labelling mutagenesis together with EPR spectroscopy and fluorescence dequenching assay. By labeling with (1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl)-methanethiosulfonate (MTSSL) Cys-MscL mutant in the narrowest part of the channel (G22R1), we use the changes in mobility and accessibility of R1 as a detector for the structural rearrangements of the pore-segment.

SDSL

EXTENDED SPIN TRAPPING STRATEGY: CAN THE USE OF A COCKTAIL OF SPIN TRAPS
SPEED UP THE CURRENT STEP BY STEP APPROACH?

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Reactive free radicals in biological media are currently mainly detected using Fluorescence based assay, or by EPR detection. Fluorescence assays are easy and widely available, but also widely criticized so that results should be used with caution. Detection of reactive free radicals by EPR requires the use of the so called Spin Traps agents, which will react with free radicals to form a more stable adduct which will be subsequently more easily detectable with EPR. Many different STs are available, with different properties and reactivity, so that the design of a correct experimental set-up can be quite intricate and time consuming because based on a trial-error approach. Consequently false negatives are not uncommon when studying biological media. It would thus be desirable to have a straightforward method that would allow the detection of radicals in many different situations, whatever the chemical studied or the exact mechanism.

We propose a new approach which gives more easily an answer to the question asked by non EPR specialist investigators: is a reactive free radical produced in the process studied?

In this proof-of-concept study, we compared the signals obtained using spin traps individually, one at a time, or on the contrary as a cocktail of all the ST used in combination altogether. To validate this approach, we used a simple in vitro system composed of K562 leukemic cells and several toxics known to produced radicals representative of the main families, namely C•, O• or N• centred radical. The complexity of the recorded signal was then compared.

Four Spin Traps were selected to cover a large range of lipophilicity and reactivity against the main family of radicals. Toxics were chosen to produce radicals representative of the main types commonly observed (C, O or N centred radicals): menadione bisulfite, hydrogen peroxide, tert-butylhydroperoxide, phenylhydrazine and acetaldehyde. Toxic was added directly to the medium and incubated to generate radicals in situ. In control experiments, when applicable, specific enzymes were used to degrade the toxic or the radical formed by the toxic and consequently suppress the EPR signal.

We show here that indeed the cocktail gave a signal in all the conditions tested, whereas individual STs did not systematically respond. The complex signal observed was the sum of individual responses, with no particular distortion nor alteration of the resultant spectrum. Because of its composition, the cocktail of STs should give an EPR signature in most of the situations, whatever the nature of the chemical to be tested

Spin dependent conductivity in MDMO-PPV based diodes studied by electrically detected magnetic resonance.

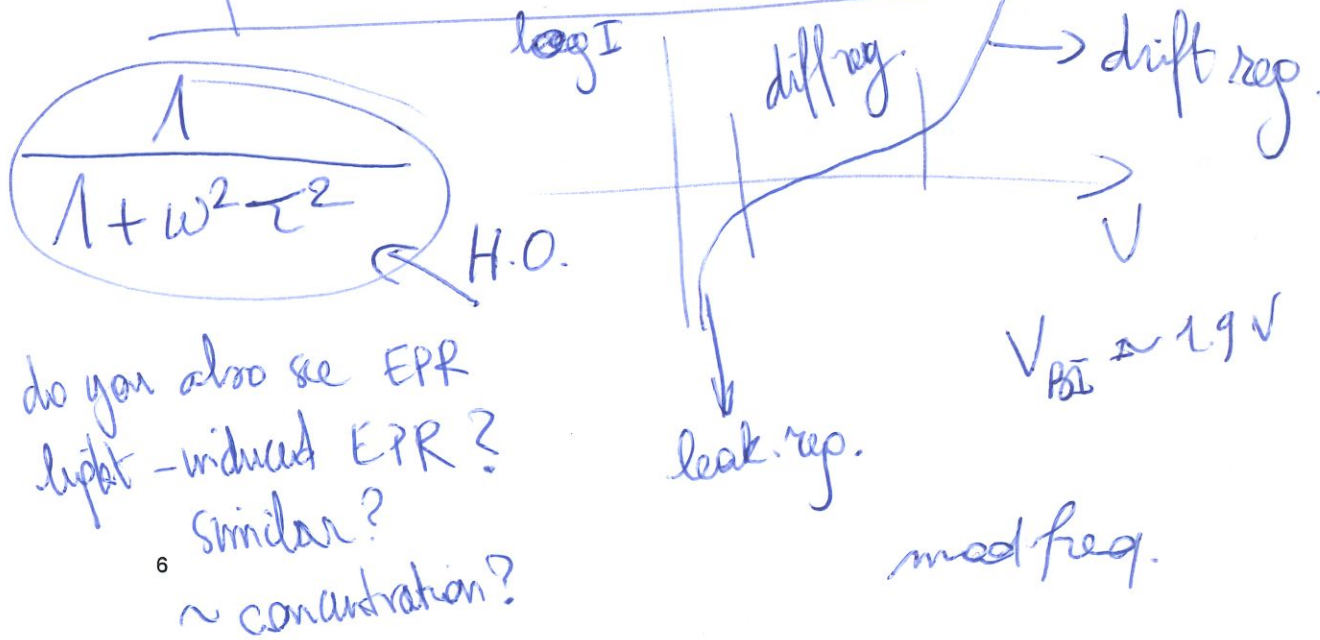
Biniam Zerai Tedlla, and Etienne Goovaerts

Department of physics, University of Antwerpen, Antwerpen

Change in conductance of organic devices with non-magnetic electrodes in an applied magnetic field (OMAR: organic magneto-resistance) has triggered renewed interest in the role of the spin degree of freedom in charge transport and recombination processes. The mechanism that governs the OMAR effect is being actively debated, and different spin-dependent models have been proposed to account for experimental results. The observation of the phenomenon in organic diodes and solar cells has led to the proposals of magnetic field induced singlet-to-triplet interconversion (MIST), and of triplet-exciton polaron quenching models. However, the presence of the effect in unipolar devices cast doubt over the models, and the alternative bipolaron model has been proposed. This model doesn't rely on the presence of two types of charge carriers and is based on "spin blocking" during hopping charge transport as a result of the Pauli's exclusion principle. By electrically detected magnetic resonance (EDMR) one detects changes in the conductivity of the active layer of an electrical device due to rearrangement of the spins of charge carriers by resonant microwave excitation. Here we apply the EDMR technique to MDMO-PPV devices to identify charge carriers that contribute to the observed EDMR signals. We also investigate the correlation between the onset of minority charge carrier injection and a sign reversal of the EDMR signal.

Organic Magneto-resistance effect (OMAR) Bloom *APL* 93 (263302) (2008) ^{art no'}

→ FM mod. } What is most convenient?
 → MW mod. }



EMR study of X-irradiated L-threonine single crystals – tentative radical models

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Electron Magnetic Resonance (EMR) is a prominent research tool for detecting, quantifying and especially identifying radiation induced paramagnetic defects in organic (poly) crystalline materials (amino acids, sugars, sugar phosphates, etc.). This type of research is motivated, on the one hand by the suitable dosimetric properties of some organic materials, and on the other hand by need to understand the radiation effects in some important biological relevant molecules such as deoxyribonucleic acid (DNA) and proteins.

In the past decennia radiation-induced radicals were successfully identified in sugars (sucrose, fructose, sorbose, glucose phosphate, trehalose dehydrate) and amino acids (alanine, glycine, lysine, arginine, serine phosphate). Thereby, the number of accurately studied systems gradually increases to a satisfactory level allowing underlying principles to become clear with respect to the formation of certain type of radicals.

A recently started EMR study on L-threonine reveals promising results for dosimetric applications and fundamental research. In this presentation we will focus on some of the preliminary Q-band ENDOR and EIE results obtained on X-irradiated threonine single crystals measured at 20 K. Four different radicals could be detected. Most of the hyperfine coupling (HFC) tensors could be deduced from the ENDOR experiments for three of the four radicals. The dominant radical seems to exhibit HF interactions with the CH₃ group of the molecule. Interestingly in irradiated alanine, the so called Stable Alanine Radical also exhibits CH₃ group HF interactions. A radical model for the dominant radical in threonine is proposed. DFT calculated HFC tensors using Gaussian (for single molecule calculations) and CP2K (for periodic calculations) are compared with the experimental HFC tensors in order to support the proposed radical model.

↳ R1 dominant in list der EPR.

Why not ORCA

Ⓡ1 → is het model wel zo evident?



Gd³⁺ - nitroxide and Mn²⁺ - nitroxide distance measurements for structural studies of biomacromolecules.

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High field DEER (double electron-electron resonance) distance measurements between half integer high spin ions with an isotropic g, such as Mn²⁺ (S=5/2) and Gd³⁺ (S=7/2) and a nitroxide (S=1/2) may offer a number of advantages the corresponding nitroxide-nitroxide or Gd³⁺ -Gd³⁺ (Gd³⁺ -Gd³⁺). In this work we explore this possibility on two different systems. A protein dimer with mixed nitroxide-Gd³⁺ labeling and and spin-labeled RNA and Mn²⁺ that binds to a specific binding site in the RNA oligonucleotide. We discuss the pros and cons and the instrumental requirements needed for optimizing such measurements.

distance measurements NO[•] — $\begin{matrix} \text{Gd}^{3+} \\ \text{Mn}^{2+} \end{matrix}$ → g_n anisotropic.

Pannier JMR 142, 331 (2000).

$\begin{matrix} \uparrow \\ \text{Fe}^{3+} \end{matrix}$

RNA - helicase 1100 # / 10-10 nmol.

Chelate [ki ~~plate~~ ite]

Kaminker 2012 PCCP

Bensari dual freq. cavity.

Oxygen Consumption Assessment of Adherent Cells Using Electron Paramagnetic Resonance

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Oxygen consumption is a major biochemical process studied in various fields such as tumor biology where oxygen consumption rate (OCR) of tumor cells is correlated with aggressiveness. Most OCR determination assays require cell harvesting, which is a potential stressful step. The aims of the present study are (i) to use (collagen-coated) dextran microbeads (cytodex) in combination with highly sensitive X-band Electron Paramagnetic Resonance¹ (EPR) to measure OCR in intact adherent cells and (ii) to see the influence of various harvesting procedures (trypsin and collagenase) on OCR of two cell lines (melanoma B16F10-luc and HUVEC cells).

Briefly, microbeads covered by cells are mixed with an oxygen sensing probe nitroxide (¹⁵N-PDT) in a dextran rich (10% w/v) medium and gently drawn into a glass capillary. This capillary is sealed and inserted in the EPR cavity and linewidth of the nitroxide (proportional to %O₂) is recorded every minute. The same experiment was also carried out using trypsin for detaching cells from microbeads or collagenase for dissolving the coating of cytodex (Fig. 3). Each detachment procedure resulted in a net impairment of OCR in both B16F10-luc and HUVEC cells (Fig. 2).

In summary, using microbeads with EPR allows reliable OCR measurements in adherent cells. Indeed cells remain intact during EPR records (Fig. 1). Impact of detaching agents on OCR highlights the crucial importance of sample preparation for biochemical assays.

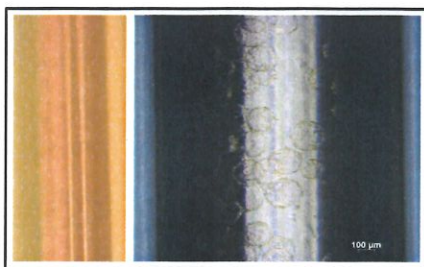


Figure 1 : Cells remain adherent in the EPR tube

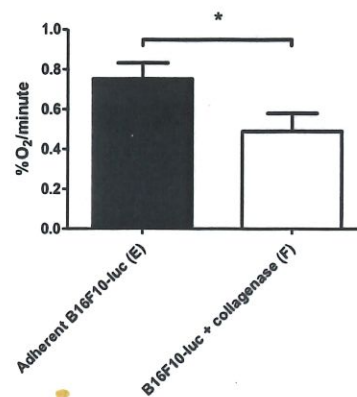


Figure 2 : Collagenase impairs cell oxygen consumption

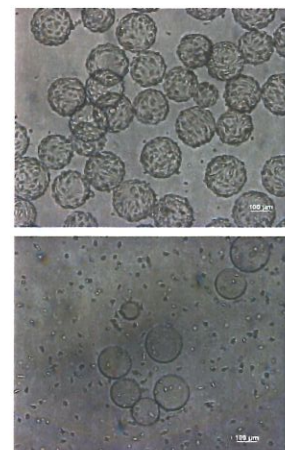


Figure 3 : collagenase dissolves coating of cytodex and harvests cells

Reference : (1) Diepart J, Verrax J, Calderon P et al, Comparison of methods for measuring oxygen consumption in tumor cells in vitro (2010), Analytical Biochemistry, 396, 250-256

Ex Vivo Monitoring of Spontaneous Metastasis of Iron Oxide-labeled Cancer Cells using EPR

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Cancer metastasis is the most important cause of cell death linked to cancer. There is currently a lack of quantitative assays to evaluate accurately cell spreading from the primary tumor and cell homing to distant sites. We propose to use cell labeling (using superparamagnetic iron oxides) in combination with X-band EPR to evaluate metastatic properties of a murine renal adenocarcinoma cell line expressing the luciferase (RENCA-luc). Briefly, RENCA-luc were incubated overnight with iron oxide particles (Molday Ion Rhodamine B, MIRB). The next day, cells containing MIRB were collected, washed several times and injected in the left kidney or spleen of Balb/cJRJ mice. Organs were subsequently harvested and freeze-dried at day 1, 3 and 8 post injection and then analyzed using X-band EPR operating at 9 GHz. In the same time, similar experiments were carried out using bioluminescence imaging (BLI) in order to compare EPR and BLI. Results show that metastatic cells were already detectable in lungs and liver using *ex vivo* EPR one day post injection whereas BLI images display hot spots only after 8 days (Figure 1). However, intracellular particles are rapidly diluted owing cell proliferation. This feature restricts use of *ex vivo* EPR for short term studies.

In conclusion, EPR is a promising quantitative and specific tool (there is no iron oxide EPR signal in control organs) for measuring metastasis.

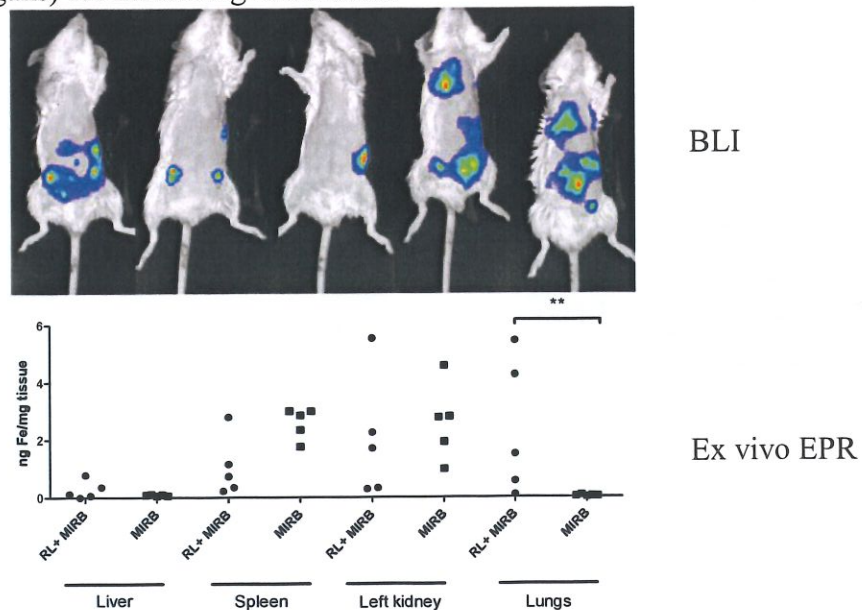


Figure 1 : positive lungs for metastasis are visible several days post injection (BLI, upper panel) while MIRB-labeled cells are present in lungs one day post injection (EPR results, lower panel)

Screening of tumor cell metabolism: comparison between O₂ consumption (EPR oximetry) and glycolysis efficiency (enzymatic assays)

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Cancers show extensive heterogeneity in almost all phenotypic features, due to mutations that appear frequently in response to microenvironmental stresses. From a metabolic point of view, some solid tumors rely almost exclusively on glycolysis even when oxygen is present (i.e., Warburg phenotype) instead of using oxidative phosphorylation to produce energy [1]. As a consequence, to keep sufficient ATP level, it involves higher intake of glucose, higher release of lactate with limited oxygen consumption because of abnormal mitochondrial activity. This “glycolytic switch” has been associated with poor prognosis, since it has been demonstrated that it confers advantages for proliferation, invasion and drug resistance [2]. The present study aims to investigate the metabolic activity of six different tumor cell lines. Oxygen consumption rate was assessed using Electron Paramagnetic Resonance (EPR) oximetry as already described [3] (Fig. 1) and the glycolysis efficiency, i.e., lactate produced per glucose consumed, was measured using enzymatic assays (CMA600 Microdialysis Analyser) *in vitro*. We show that the correlation between these two parameters allows the distinction of two groups of tumor cell lines characterized by different metabolic profiles (Fig. 2). This study indicates that EPR, which is more sensitive than other methods [3] could be considered as a valuable tool to screen tumor cell metabolism. Moreover, these results are beneficial for future research focusing on the link between energy production and tumor cells abilities, as well as for developing new therapeutic strategies, especially those that rely on modulating the metabolism of cancer.

Figure 1

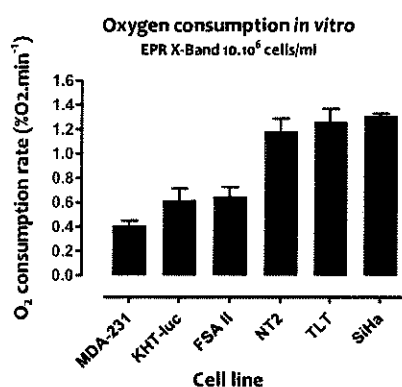
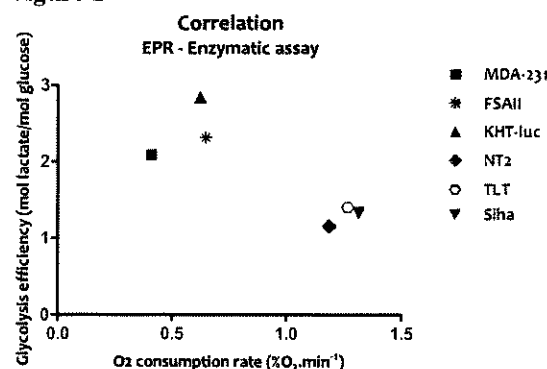


Figure 2



[1] O. Warburg, F. Wind, E. Negelein. The metabolism of tumors in the body (1927). *J Gen Physiol*, 8:519-530.

[2] R.J. DeBerardinis, J.J. Lum, G. Hatzivassiliou et al. The biology of cancer: metabolic reprogramming fuels cell growth and proliferation (2008). *Cell Metab*, 7:11-20.

[3] C. Diepart, J. Verrax, PB. Calderon et al. Comparison of methods for measuring oxygen consumption in tumor cells in vitro (2010). *Anal Biochem*, 396:250-256.

Distinction between pigmented and non-pigmented human skin melanoma using EPR spectrometry and imaging

Quentin Godechal¹, Ghanem E. Ghanem², Martin G. Cook³ and Bernard Gallez¹

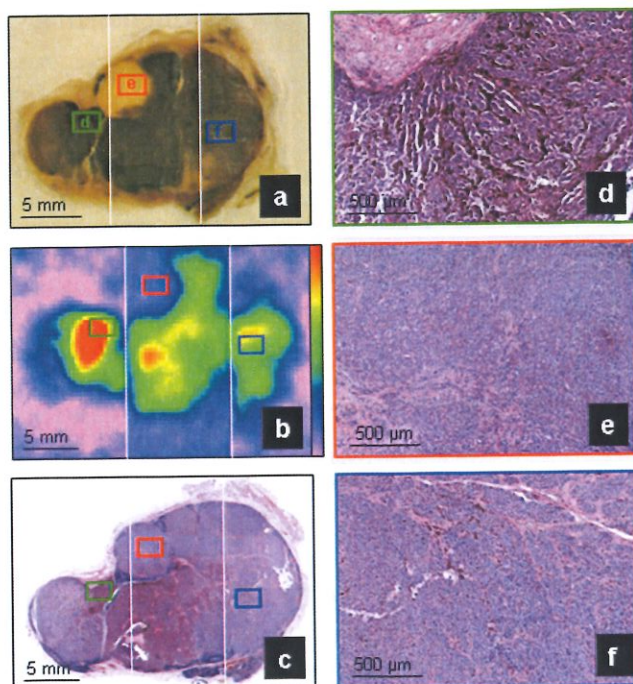
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Abstract

It is known for a long time that the melanin pigments present in normal skin, hair and most of malignant melanomas can be detected by electron paramagnetic resonance (EPR) spectrometry. In this study, we proposed to use EPR imaging as a tool to map the concentration of these pigments inside *ex-vivo* human pigmented and non-pigmented melanomas and to compare the EPR cartography with histology. As results, we obtained accurate mappings of the melanin inside pigmented melanoma samples. The signal intensity observed the EPR images could be correlated with the concentration of melanin within the tumors, visible on the histological sections. Such a quality for EPR images was never achieved before for the distribution of melanin, and, to our knowledge, for the distribution of an intrinsic organic compound. In opposition, no EPR signal coming from melanin could be observed from non-pigmented melanomas, demonstrating therefore the absence of any kind of pigments inside these particular cases of skin cancer and the importance of pigmentation for ulterior EPR studies on melanoma.



Pigmented human melanoma: EPR and Histology

A 500 μm -thick slice of a paraffin-embedded pigmented human melanoma was cut into three parts following the white lines drawn on the figure (A). The three parts were measured by EPR imaging and the images were jointed to reconstruct the total image of the sample (B). A 5 μm -thick histological section adjoining to the sample was cut (C) and used to correlate the pigmentation to the EPR intensity, especially on three selected areas: A highly pigmented area (D), a non-pigmented area (E), and a moderately pigmented area (F).

The effect of a membrane mimicking detergent on Alzheimer's Amyloid peptide aggregation studied by EPR

M. Hashemi Shabestari^a, N.J. Meeuwenoord^b, D.V. Filippov^b, M. Huber^{a*}

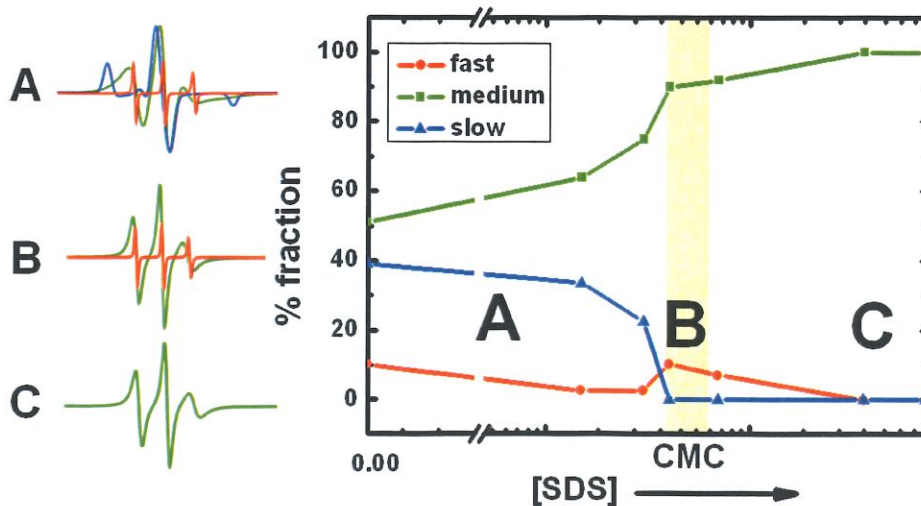
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The aggregation of the β -Amyloid (A β) peptide into fibrils and plaques is the chief indicator of Alzheimer's disease. Specific interest in oligomers stems from the suggestion that small, oligomeric aggregates and protofibrils, rather than fully formed fibrils could be responsible for the toxicity of the A β -peptide. Membrane interaction could also have an effect, so here the membrane-mimicking detergent SDS is studied.

Spin-label-mobility EPR enables us to measure the changes of A β aggregation over the whole range of SDS concentrations. It monitors all peptide in the sample and is not limited to particular aggregate sizes.

The high-SDS form found (C) is consistent with the one-peptide/micelle model [1-2]. In the absence of SDS (A) the high concentration of A β (0.55 mM) most certainly enforces fibrils. At intermediate SDS (B), EPR reveals an increase in particle size suggestive of oligomer formation, eventually involving detergents. Thus a first glimpse into the behavior of SDS at sub-CMC concentrations, where high resolution techniques, such as NMR, fail because of particle size limitation, [1-2] is obtained.



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EPR investigation of pore formation and metal incorporation in periodic mesoporous organosilicas

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Since the discovery of M41S materials in 1992, there has been an explosive growth in the development of micelle-templated mesoporous silicas (pore diameters between 2 and 50 nm). However, for sorption and catalytic applications it is necessary to activate the mesoporous silica structures. One of the most effective ways is to incorporate organic functionalities directly into the silica structure. Among the most recent innovations, a novel hybrid organic-inorganic mesoporous materials with a homogeneous distribution of organic fragments and well-ordered mesostructure have been synthesized by condensation of bridged silsesquioxane in presence of structure-directing agents, namely periodic mesoporous organosilicate (PMO)^[1]. Numerous potential applications exist for PMO materials, e.g. in the chemical industry (catalysis), in environmental applications (metal scavenging) and in medical applications (controlled drug release). Moreover, besides introducing organic functionalities, the incorporation of additional metal atoms in the framework is also viable for producing solid acid/base or redox catalysts. Therefore, combining organic groups and active sites of metal atoms in the hybrid framework with ordered mesoporosity, a desirable catalyst could be put into practice.

Copper complexes have long been found to be effective catalysts and are used in a wide range of reactions. Cu-containing mesoporous materials such as Cu-MCM-41^[2] and Cu-SBA-15^[3] have been successfully synthesized and show efficient catalytic performance in liquid phase oxidation of aromatic compounds. Although, Cu-containing mesoporous materials have received considerable attentions, only a few Cu-containing PMO materials have been reported. Furthermore, the coordination, dispersion and aggregation degree of copper species in mesoporous silica materials are still not well understood.

Here, we show that electron paramagnetic resonance (EPR) is an excellent tool to obtain mechanistic insight into the periodic mesoporous organosilica (PMO) materials. On the one hand, the EPR method allows to monitor directly copper species in the PMO materials. On the other hand, one can add spin probes (nitroxide molecules) to the reaction mechanism. These probes function as EPR spy molecules that can monitor the changing structure of the material during the synthesis process.

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Efficient charge transfer in blends of donor para-phenylenevinylene polymer and acceptor thiazolothiazole oligomers for organic photovoltaics

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Organic conjugated compounds are promising for the realization of low-cost, large-area electronic products such as organic solar cells. The 3D bulk heterojunction concept for solar cells starts from a blend of donor ('p-type') and acceptor ('n-type') compounds. While a large number of p-type organic semiconductors is now available, reports about n-type organic oligomers or polymers are still scarce.

Here, we present a light-induced electron paramagnetic resonance(EPR) investigation of a series of hexyl-substituted bithiophene compounds containing a thiazolothiazole(5, 4-d) unit[1]. The molecules were blended in different ratios with the standard p-type polymer MDMO-PPV (poly[2-methoxy-5-(3,7-dimethyloctyloxy)]-1,4-phenylenevinylene) to investigate electron transfer under illumination, an essential step in the photovoltaic process. X-band(9.5 GHz) and W-band(95 GHz) EPR of these polymer/oligomer blends were measured before and after illumination (argon ion laser, 488nm). This is compared with the results in pure oligomers and in MDMO-PPV. In order to unravel the identity of the observed signals, the pure oligomers and the MDMO-PPV are chemically doped with iodine and investigated with CW and pulsed EPR and corroborated with DFT computations. The acceptor capacity of the oligomers as observed in the light-induced experiments in the blends is anti-correlated with the efficiency of iodine doping of the pure oligomers.

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EPR study of cross-talk between superoxide anion and nitric oxide in vasculature based on cyclic hydroxylamine oxidation.

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Development of endothelial dysfunction (ED) under metabolic diseases such as atherosclerosis, diabetes and aging as a disbalance between vessel vasodilation and vasoconstriction, is tightly associated with decrease of nitric oxide bioavailability. Potential reaction between endothelial nitric oxide and superoxide anions, generated by uncoupled eNOS, NOX enzymes and mitochondria, with formation of peroxynitrite is often discussed as an important source of ED. Cyclic hydroxylamine, 1-Hydroxy-3-methoxycarbonyl-2,2,5,5-tertramethylpyrrolidine (CM-H), was reported to penetrate into tissue, and can be oxidized by superoxide or peroxynitrite anions to form paramagnetic nitroxide ($A_N = 16.1$ G) reflecting redox status of environment. CM-H as redox active spin probe was used in numerous biological studies, but selectivity was not deeply analyzed in tissues. We studied effects of addition of SOD and NO in situ, using aortic rings, isolated from C57Bl/6 mice (12-14 weeks old). We observed: 1) that in oxygenated solution spontaneous CM-H oxidation was increased and partly SOD-sensitive; 2) added NO-donor (0.5 $\mu\text{mol/L/min}$) did not change auto-oxidation; 3) additional radical formation in aortic rings in situ at 37°C was significantly decreased by preincubation with free superoxide dismutase (SOD, 100U/mL) or L-NAME, 2 mM, (to 16 \pm 16%, N=10, and 72 \pm 12%, N=6, respectively, $P < 0.05$), indicating discrete formation of superoxide anion and peroxynitrite. This method can add useful information about site and mechanism of radical formation, and a good basis for evaluation of ED model in situ.

3d-metal near-interface Si substrate contamination during ALD processing detected by electron spin resonance

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The excellent electrical, optical, and chemical properties of Si dioxide, and the superb stable interface it forms with Si, have led to the dominance of the Si/SiO₂ structure as basic entity in modern integrated circuit technology. Still, with the furthering downscaling to the nanoscale, new low temperature methods for well controlled deposition of device grade quality thin SiO₂ layers are still explored, e.g., to comply with maximally allowed thermal budgets.

In this perspective, a K- and Q-band electron spin resonance (ESR) study has been carried out on (100)Si/SiO₂ entities manufactured by low temperature (150 °C) atomic layer deposition (ALD) of a high-quality SiO₂ layer on Si using 3-aminopropyltriethoxysilane, H₂O, and ozone in a three-step process. More details about the deposition process can be found elsewhere [1].

Whereas previous work has demonstrated the high quality of the deposited SiO₂ layer, the current ESR analysis reports on the tracing of growth-related contamination of near interface Si substrate layers by two transition metals. This includes, first, detection of the signal of interstitial Cr⁺ (S=5/2) impurities in c-Si, characterized by an isotropic central g value of 1.9980 ± 0.0002 , an isotropic ⁵³Cr (I=3/2) hyperfine interaction of splitting $A_{\text{iso}} = 11.8$ G, and cubic crystal field splitting parameter $a = +32.2$ G, well in agreement with the known bulk c-Si case [2]. The total Cr⁺ defect density is inferred as $\sim 5 \times 10^{11}$ cm⁻². Second, a single signal is observed at isotropic $g = 2.070 \pm 0.001$, corresponding to interstitial Fe impurities (Fe_i)⁰ (S=1) positioned in a c-Si matrix. Defect density depth profiling reveals the impurities to be confined to a few μm thick Si substrate top layer, the density decaying exponential-like from the Si/SiO₂ interface inward the Si substrate.

The total of the results points to a contamination of reactor-environment origin, connected with the layer deposition process. It concerns a weak contamination, in which detection the ESR technique emerges as a powerful technique to identify very low levels of contamination of near-surface Si substrate layers.

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Ligand induced conformational capture of a synthetic tetracycline riboswitch revealed by pulse EPR

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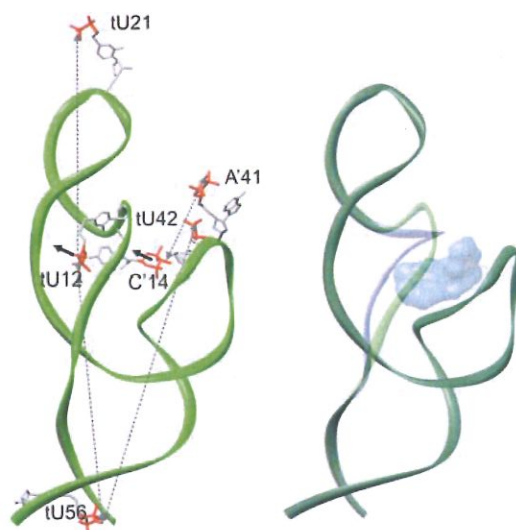
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RNA aptamers are *in vitro* selected binding domains which recognize their respective ligand with high affinity and specificity.^[1] They are characterized by complex three-dimensional conformations providing preformed binding pockets which undergo conformational changes upon ligand binding. Small molecule binding aptamers have been exploited as synthetic riboswitches for conditional gene expression in various organisms. In the present study, double electron electron resonance (DEER) spectroscopy combined with site directed spin labeling^[2,3] was used to elucidate the conformational transition of a tetracycline binding aptamer. Different sites were selected for post-synthetic introduction of either the (1-oxyl-2,2,5,5-tetramethylpyrroline-3-methyl) methanethiosulfonate by reaction with a 4-thiouridine modified RNA or of 4-isocyanato-2,6-tetramethylpiperidyl-N-oxid spin label by reaction with 2'-aminouridine modified RNA. The results of the DEER experiments reveal the presence of a thermodynamic equilibrium between two aptamer conformations in the free state and capture of one conformation upon tetracycline binding.

In the outlook we will report on DEER experiments on DNA containing deoxyadenine residues which were functionalized with the spin label 4-azido-2,2,6,6-tetramethyl-piperidine-1-oxyl *via* azide-alkyne 'click' chemistry.



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