

# 22nd Benelux EPR Society Meeting

Maastricht, May 22<sup>nd</sup> 2014



**Sponsored by Bruker (NL)** 

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# 22<sup>nd</sup> Benelux EPR Society Meeting – Program

#### 10:00. Registration

#### 10:25. Opening

#### Session 1 :

- 10:30. Bert Cuypers (University of Antwerp)"Differences and similarities between human and Antarctic fish cytoglobin"
- 10:50. Agnieszka Adamska (MPI CEC Mülheim) "Pulsed EPR studies of an [FeFe] hydrogenase with a non-native cofactor"
- 11:10. Maurice van Gastel (MPI CEC Mülheim)
  "Calculation of g values by density functional theory. Application to small model systems, blue copper proteins and the Ni-A state [NiFe] Hydrogenases"

#### 11:30. Coffee break

#### Session 2 :

- 11:45. Philippe Levêque (Université Catholique de Louvain)
  "Comparison of Monte-Carlo simulation and EPR/CT imaging for retrospective dosimetry in heterogeneous bone tissue: first results"
- 12:05. Jevgenij Kusakovskij (Ghent University)
  "Towards understanding of the dosimetric EPR spectrum of irradiated sucrose: ENDOR characterization of a fourth stable radical species"
- 12:25. Serena Iacovo (KU Leuven) "Inherent Si dangling bond defects at the thermal (211)Si/SiO<sub>2</sub> interface"

#### 12:45. Lunch break + Poster session

#### Session 3 :

- 14:30. Sevdalina Lyubenova (Bruker Germany) "EPR from 1 to 263 GHz"
- 14:50. Fred Hagen (Technical University Delft) "Development of a broadband transmission EPR spectrometer"
- 15:10. Faezeh Nami (Leiden University) "Rapid freeze-quench EPR study of intermediates in the enzymatic reduction of oxygen by small laccase"

#### 15:30. Coffee break

#### Session 4 :

- 15:45. Jan Gielis (University of Antwerp)"Discovery of two temporal distinct free radical bursts during pulmonary ischemiareperfusion injury"
- 16:05. Ernst van Faassen (Leiden University Medical Center) "NO radical signaling in bone marrow mobilizes progenitor cells for endothelial repair"
- 16:25. Shashi Singam (University of Antwerp, ECM)"Towards implementation of selective NV-nanodiamond imaging in a neurobiology experimental set-up"

#### 16:45. General meeting of the Benelux EPR Society

#### 17:00. Reception + poster session

#### **Poster session**

P1. Flavia Dei Zotti and Irina Lobesheva (Catholic University of Louvain-la-Neuve)

"Quantification of erythrocyte nitric oxide by electron paramagnetic resonance: Biochemical determinants"

P2. Céline Desmedt (Catholic University of Louvain-la-Neuve)

"Development of EPR oximetry in diabetic wound healing models"

P3. Pravin Kumar (Leiden University)

"Long-range distance constraints for the fibril fold of alpha-synuclein"

P4. Mykhailo Azarkh (Leiden University)

"A mononuclear Mn(II) pseudo-clathrochelate complex studied by multi-frequency electronparamagnetic-resonance spectroscopy"

P5. Irena Nevjestić (Ghent University)

"Resolving the relative tilting of hyperfine and g tensors for vanadyl complexes in the Almetal-organic framework MIL-53 by a multifrequency (S, X, Q, and W-band) EPR approach"

P6. Ignacio Caretti (University of Antwerp)

"Photoinduced high-field electron paramagnetic resonance of TiO<sub>2</sub>-based nanocatalysts"

P7. Feng Lin (University of Antwerp)

"Spin-probe EPR analysis probing framework-guest interactions in different periodic mesoporous organosilica"

P8. Elena Morra (University of Antwerp – University of Torino)

"EPR analysis of model compounds of Ziegler-Natta catalysts"

P9. Biniam Tedlla (University of Antwerp)

"Triplet formation pathways in polymer:fullerene based organic solar cells"

P10. Jacek Kepa (KU Leuven)

"Structural damage in thin SLIM-cut c-Si foils fabricated for solar cell purposes: atomic assessment by electron spin resonance"

P11. Ed Reijerse (MPI – CEC Mülheim)

"EPR probeheads for very small samples: developments and prospects"

- Posters from the Sponsor Bruker
- P12. Pulse Q-band
- P13. Cryogen-free VT systems

P14. SpinJet-AWG

**Abstracts Oral Presentations** 

# Differences and similarities between human and Antarctic fish cytoglobin

**B.** Cuypers<sup>1</sup>, S. Vermeylen<sup>2</sup>, A. De Schutter<sup>1</sup>, V. Rahemi<sup>3</sup>, C. Verde<sup>4</sup>, K. De Wael<sup>3</sup>, S. Dewilde<sup>2</sup>, and S. Van Doorslaer<sup>1</sup>

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Cytoglobin is a recently discovered globin in both mammalian and non-mammalian vertebrates. In this work, Antarctic fish cytoglobins are investigated using different spectroscopic techniques (optical spectroscopy, electron paramagnetic resonance (EPR) and resonance Raman spectroscopy (RRS)) and compared to earlier findings about their human variants.<sup>1</sup> The cytoglobins of red-blooded (Dissostichus mawsoni) and white-blooded (Chaenocephalus aceratus) Antarctic fish are analysed. These fish are special since, except for the lack of hemoglobin in C.ace, they both lack myoglobin. In human cytoglobin, a negligible effect of an intramolecular disulfide bridge on the gas binding kinetics is observed.<sup>2</sup> This is in contrast with human neuroglobin, where the presence of an intramolecular disulfide bond is found to clearly modulate the gas binding affinity through a change in the heme-pocket structure.<sup>2</sup> The most important difference between the Antarctic fish cytoglobins and the human form is found in the positions of the cysteines; and thus their ability to form intra- and/or intermolecular disulfide bridges. Comparison of the principal g-values of the ferric fish cytoglobins with those of their Cys-Ser mutants allows determining the effect of such disulfide bridges on the heme-pocket structure. Furthermore, the combined CW/Pulsed EPR and RRS analysis reveals slight differences in the heme-pocket structure of human and Antarctic fish cytoglobins. The most pronounced difference is found in the stabilization of the CO ligand for the different cytoglobins. Finally, although the cytoglobins have no direct catalase activity (reaction with  $H_2O_2$  to form the ferryl state of the protein), they are able to reduce  $H_2O_2$  to water when combined with cytochrome c peroxidase.

<sup>&</sup>lt;sup>1</sup>E. Vinck, S. Van Doorslaer, S. Dewilde and L. Moens, "Structural change of the heme pocket due to disulfide bridge formation is significantly larger for neuroglobin than for cytoglobin," *J. Am. Chem. Soc.*, 126, 4516-4517, 2004.

<sup>&</sup>lt;sup>2</sup>D. Hamdane, L. Kiger, S. Dewilde. B. Green, *et al.*, "The redox state of the cell regulates the ligand binding affinity of human neuroglobin and cytoglobin," *J. Biol. Chem.*, 278, 51713-51721, 2003.

#### Pulsed EPR studies of an [FeFe] hydrogenase with a non-native cofactor.

Edward Reijerse<sup>1</sup>, Agnieszka Adamska-Venkatesh<sup>1</sup>, Trevor Simmons<sup>2</sup>, Judith Siebel<sup>1</sup>, Vincent Artero<sup>2</sup>, Wolfgang Lubitz<sup>1</sup>

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Hydrogenases are enzymes which catalyze the oxidation of H<sub>2</sub> as well as the reduction of protons to produce H<sub>2</sub>. The active site of [FeFe] hydrogenase is referred to as the "H-cluster" and consists of a "classical" [4Fe4S] cluster connected via a protein cysteine side group to a unique [2Fe]<sub>H</sub> sub-cluster containing CN<sup>-</sup> and CO ligands as well as a dithiol bridging ligand. It was recently shown that various biomimetic complexes of the diiron sub-cluster can be inserted into "apo" [FeFe] hydrogenase which contains only the [4Fe-4S] part of the Hcluster [1,2]. The mimic complex with a aza-dithiol bridging ligand activated the enzyme to full activity proving that the amine function is essential as proton accepting base. In a more recent study we discovered that oxidized [FeFe] hydrogenase from C.reinhardtii maturated with non-natural mimic complex  $[Fe_2(CO)_4(CN)_2(pdt)]^2$  in which the bridging amine is replaced by  $CH_2$  strongly resembles active oxidized ( $H_{0x}$ ) state of the native protein[3]. The  $H_{ox}$  state is EPR active and the signal originates from the mixed valence Fe<sup>I</sup>Fe<sup>II</sup> state of the diiron subcluster [4]. Taking advantage of the readily available isotope labeled mimic complex as well as possibility to obtain a pure redox state we performed HYSCORE and ENDOR studies of the <sup>13</sup>C and <sup>15</sup>N labeled non-natural H-cluster. The <sup>13</sup>C hyperfine couplings of both CN<sup>-</sup> ligands were observed for the first time. The <sup>15</sup>N/<sup>14</sup>N coupling of the distal CN was detected and compared to those of the native enzyme [4].

- G. Berggren, A. Adamska, C. Lambertz, T. Simmons, J. Esselborn, M. Atta, S. Gambarelli, J. Mouesca, E. Reijerse, W. Lubitz, T. Happe, V. Artero, M. Fontecave, *Biomimetic assembly and activation of [FeFe]-hydrogenases*, Nature **2013**, 499 (7456), 66-69.
- [2] J. Esselborn, C. Lambertz, A. Adamska-Venkatesh, T. Simmons, G. Berggren, J. Nothl, J. Siebel, A. Hemschemeier, V. Artero, E. Reijerse, M. Fontecave, W. Lubitz, T. Happe, *Spontaneous activation of [FeFe]-hydrogenases by an inorganic [2Fe] active site mimic*, Nature Chemical Biology 2013, 9 (10), 607-609.
- [3] A. Adamska-Venkatesh, D. Krawietz, J. Siebel, K. Weber, T. Happe, E. Reijerse, W. Lubitz, *Artificially maturated [FeFe] hydrogenase reveals new redox states*, (submitted)
- [4] A. Silakov, B. Wenk, E. Reijerse, W. Lubitz, (14)N HYSCORE investigation of the Hcluster of [FeFe] hydrogenase: evidence for a nitrogen in the dithiol bridge, Physical Chemistry Chemical Physics 2009, 11 (31), 6592-6599.

# Calculation of g values by density functional theory. Application to small model systems, blue copper proteins and the Ni-A state [NiFe] Hydrogenases

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The prediction of g values by Density Functional Theory in the last 10 years has been very successful. Yet, systematic differences in the calculated and experimentally observed g shifts are still present, and are typically on the order of 30%.<sup>1</sup> A particular example is neutral TiF<sub>3</sub> (d<sup>1</sup>) for which the experimentally observed g shift is recovered by DFT calculations to only 50%. In this contribution we investigate the cause of these differences, with the possibilities being either spin polarization or errors in orbital energies. For neutral TiF<sub>3</sub>, high level CCSD(T) calculations of the excitation energies are found to differ by a factor of 2, thus pointing to inaccurate excitation energies as the main source of error. For blue copper proteins, a d<sup>9</sup> system related to the d<sup>1</sup> system, CCSD(T) calculations are underway. Pencil and paper estimates of the g values based on the VIS absorption spectra give good agreement with experiment (as has also been performed previously).<sup>2</sup> Spin polarization effects cannot occur for d<sup>1</sup> and d<sup>9</sup> systems, but may contribute up to ~15% if excited doublet states with 3 unpaired electrons are possible. Finally, the acquired knowledge is applied to the oxidized Ni-A and Ni-B states of [NiFe] hydrogenases. These states are structurally very similar and are even characterized by almost identical ENDOR spectra, but differ significantly in their middle g value (g<sub>y</sub> = 2.24 for Ni-A and g<sub>y</sub> = 2.16 for Ni-B).<sup>3</sup>

[1] Neese, F. J. Chem. Phys. 115 (2001), 11080-11096

- [2] Sinnecker, S.; Neese, F. J. Comput. Chem. 27 (2006) 1463-1475
- [3] Lubitz, W.; Reijerse, E.J.; van Gastel, M. Chem. Rev. 107 (2007), 4331-4365

# Comparison of Monte-Carlo simulation and EPR/CT imaging for retrospective dosimetry in heterogeneous bone tissue: first results.

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lonizing radiations are known to generate free radicals in the mineral part of tooth enamel and in bones. Those  $CO_2^-$  radicals are quite stable and can be detected and quantified by EPR spectroscopy. As the EPR signal is proportional to the dose, EPR spectroscopy is widely used for after-the-fact or retrospective nuclear dosimetry in human. Unfortunately the spectroscopy mode does not give any information about the spatial dose distribution in the tissue. In some circumstances, when the irradiation is heterogeneous or involving a strong gradient, multiple sampling (biopsies) is required to get the information. A technique offering a spatial distribution of the dose would therefore be desirable.

We have previously demonstrated that EPR Imaging (EPRI) could potentially be used, as EPRI was shown to reflect the dose distribution in phantoms of hydroxyapatite, in compact bone samples homogeneously irradiated, as well as in bone samples submitted to a steep gradient of dose around brachytherapy sources [1]. Nevertheless, when human phalanges, homogeneously irradiated, were analyzed with EPRI, the image revealed an heterogeneous distribution of the free radicals.

As the bone tissue in a phalanx is quite heterogeneous, we hypothesized that this heterogeneous distribution of the bone density could be a confounding factor that led to the heterogeneous EPR image.

To test this hypothesis, we measured the bone density distribution in the phalanx using CT imaging, performed CT/EPRI coregistration, and normalized the EPR image using the density map obtained from the CT (Fig. 1). Results were compared with the energy and the dose distribution obtained with Monte Carlo simulation.

The raw EPR image mainly reflected the energy deposition and was in close agreement with the energy deposition simulation computed with Monte Carlo simulation. After normalization with the bone density matrix obtained with the calibrated CT, the normalized dose image showed a much more homogeneous dose distribution, as predicted by the Monte Carlo dose map. Some heterogeneities remained in limited areas, that might be due to variations in the hydroxyapatite content. Their origin should be further investigated to validate this approach.



Fig. 1. Illustration of the developed methodology for visualization of the dose absorbed by an human phalanx retrospectively using EPR/CT coregistration and Monte Carlo simulation.

[1]. Levêque Ph, Godechal Q, Bol A, Trompier F, Gallez B., X-band EPR imaging as a tool for gradient dose reconstruction in irradiated bones. Med Phys. 2009 Sep;36(9):4223-9.

## Towards understanding of the dosimetric EPR spectrum of irradiated sucrose: ENDOR characterization of a fourth stable radical species

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EPR dosimetry is based on the fact that the intensity of the radiation-induced EPR spectrum grows with the absorbed dose. For a couple of decades sucrose has been considered interesting from this standpoint: its radiation-induced spectrum is stable and detectable at room temperature, while its dose detection limit (of ca. 200 mGy) and linear dose response (of up to 10 kGy) [1] are relevant for a range of practical applications, e.g. emergency dosimetry or characterization of radiation-sterilized foodstuffs. The multicomponent character of the spectrum and complex hyperfine (HF) structure of individual components complicate the establishment of unambiguous dose assessment protocols. A thorough understanding of the radiation-induced stable EPR spectrum of sucrose would be helpful in this respect, but has not been achieved yet, more than 50 years after it was first reported [2].

In a previous major contribution [3] we fully characterized and identified the three stable radicals that dominate the central part of the X-ray induced EPR spectrum of sucrose – see Figure below. Here, we present our findings on a fourth stable radical species in sucrose single crystals. From the analysis of angular variations of ENDOR and ENDOR-induced EPR spectra in Q-band (34 GHz) at 110 K, we determined the g tensor and four  $\beta^{-1}$ H HF coupling tensors. Comparison of the measured and simulated powder patterns including this contribution suggests that features in the wings of the spectrum can now also be explained.



Figure: Stable EPR spectrum of irradiated sucrose measured at 9.4 GHz and 110K (full line), simulation including the contributions of radicals T1-T3 and T1-T4.

- 1. Karakirova, Y., Yordanov, N. D., De Cooman, H., Vrielinck, H., Callens, F., *Radiat. Phys. Chem.* 2010, **79**, 654-659.
- 2. Ueda, H., Kuri, Z., Shida, S., J. Chem. Phys. 1961, 35, 2145.
- 3. De Cooman, H., Keysabyl, J., Kusakovskij, J., Van Yperen-De Deyne, A., Waroquier, M., Callens, F., Vrielinck, H., J. Phys. Chem. B 2013, **117**, 7169-7178.

#### Inherent Si dangling bond defects at the thermal (211)Si/SiO<sub>2</sub> interface

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Aggressive scaling not only has led to the introduction of new materials in the Si-based device technology but also to the introduction of three dimensional channel structures such as finFETs [1] and nanowires [2]. Recent work has revealed that the (211)Si surface can be used to grow lattice-ordered and defect-free II-VI or III-Sb crystals [3]. In general, a better understanding of the semiconductor/insulator interface nature, e.g., occurring point defects, may substantially support tracing methods to improve the quality of interfaces in devices, and hence device performance. In light of the above results, an in-depth study of the (211)Si/SiO<sub>2</sub> interface quality in terms of inherently occurring defects would be much useful, for which the electron spin resonance technique (ESR) is used. This method has previously allowed the identification Si dangling bond (DB) interface defects  $-P_b$ -type centers (generic entity Si<sub>3</sub>=Si<sup>•</sup>) as detected by ESR– which have shown to represent the dominant system of intrinsic (mismatch induced) interface defects [4, 5] operating as electrically detrimental traps [4].

Conventional cw K-band and Q-band measurements have been performed at low temperatures (~4.3 K) to atomically identify the types of intrinsic point defects occurring at the (211)Si/SiO<sub>2</sub>. The data reveal the presence of two species of P<sub>b</sub>-type interface defects, exhibiting a significant difference in defect density. On the basis of the pertinent ESR parameters and interface symmetry, the basic defect is typified as  $P_{b0}^{(211)}$ , close to the P<sub>b0</sub> center observed in standard (100)Si/SiO<sub>2</sub>.

Interface properties such as the strain induced lattice-mismatch, have been extensively explored through monitoring the defect density and lineshape temperature dependence in (211)Si/SiO<sub>2</sub> entities thermally grown in the temperature range  $T_{ox}$ = 320-1066°C. It is found that  $T_{ox} \ge 750$  °C is required to minimize the  $P_b^{(211)}$  defect density through relaxation of the oxide (interface).

Reliable identification of a point defect from the ESR point of view requires the observation of hyperfine structure resulting from the interaction of the unpaired electron with nearby magnetic nuclei. Q-band saturation spectroscopy measurements have enabled to reveal a <sup>29</sup>Si hyperfine doublet centered at the central  $P_{b0}^{(211)}$  Zeeman line, identifying the defect as an unpaired electron with strongest interaction with a single Si nucleus.

Multifrequency spectroscopy, combining X, K and Q band ESR, has been applied to infer, through the analysis of the ESR signal shape and width, precise information on the strain-induced distribution in g factor. This reveals a difference between the low- $T_{ox}$  ( $\leq 400$  °C) and high- $T_{ox}$  ( $\geq 750$  °C) grown thermal (211) Si/SiO<sub>2</sub> interfaces.

<sup>[1]</sup> M. Jurczak, N. Collaert, A. Veloso, T. Hoffmann, S. Biesemans, in Proc. IEEE Int. SOI Conf., Foster City, California (2009)

<sup>[2]</sup> E. Meng, W. Li, K. Nakane, Y. Shirahashi, Y. Hayakawa, AIP Adv. 3, 092107 (2013)

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## EPR from 1 to 263 GHz

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For more than one decade Bruker has been continuously extending the range of commercial CW/FT-EPR towards higher and lower frequencies with respect to X-Band. The increase in operation frequency is typically driven by the expected higher concentration and effect sensitivity (PELDOR/DEER, HYSCORE) and by the increase in resolution for g-factor and ENDOR. The technical challenges are diverse but resonator design and available power are always major issues when increasing the frequency. The Bruker intermediate frequency concept has served well for all frequency extensions including the most recent step to 263 GHz. The 263 GHz, mm-wave system includes a 12 T magnet which is based on cryogen-free technology.

Recent progress at Q-band includes new resonator design and higher power (150 W).

A summary of the latest developments and current status in Q-band and mm-wave instruments will be presented.

### **Development of a Broadband Transmission EPR Spectrometer**

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For historical and technical reasons X-band has become the standard frequency and starting point of EPR spectroscopic research. However, molecular EPR spectra are determined both by interactions linear in the microwave source frequency (Zeeman, g-strain) and by interactions independent of the frequency (hyperfine, zero-field). It is therefore generally useful if not mandatory for meaningful spectral interpretation to collect data at more than one frequency. This typically implies the use of several different spectrometers when based on single-mode resonators. We are interested in the application of EPR to the study of metal ions in biomolecular systems, in particular in the analysis of magnetic interactions in multi-center systems and the effects of protein conformational distributions. In our experience these matters are most favorably studied in an approximate frequency range of 1-15 GHz. Our interest has led us to consider the design of a continuously frequency tunable EPR spectrometer in the quoted range. Previous attempts described in the literature have not resulted in practical spectrometers for the systems of our interest.

Our basic concept is to substitute the high-Q reflection resonator with a piece of transmission line in which the dielectric is (partially) replaced with a paramagnetic sample. The concomitant loss of sensitivity is compensated in part by an increased cell length and increased microwave powers. In an initial study we showed proof of principle using an all-digital 0-2.7 GHz vector network analyzer (VNA) in combination with coaxial transmission cells of considerable length (up to 12 meter) [1]. In an ongoing follow-up study we have replaced the coax cells with strip-line cells of much smaller dimensions. Also, the VNA has been replaced with a 0-6 GHz vector signal transceiver (VST) which is extendable for high-power operation up to 16 GHz using active multipliers and dividers. The present state of the project will be shown and expected near-future developments will be discussed.



*Figure: (L) Front end of the 0-6 GHz Vector Signal Transducer and auxiliary equipment; (R) 2D plot of 251 absorption EPR spectra of DPPH at 20 MHz frequency steps from 1-6 GHz.* 

[1] WR Hagen (2013) Broadband Transmission EPR Spectroscopy. PLOS ONE 8 (3) 15p, e59874

#### Rapid freeze-quench EPR Study of Intermediates in the

#### **Enzymatic Reduction of Oxygen by Small Laccase**

<u>Faezeh Nami</u>, Mykhailo Azarkh, Ankur Gupta, Gerard W. Canters and Edgar J. J. Groenen Leiden Institute of Physics, Leiden University, Leiden, The Netherlands

#### Abstract

SLAC, small laccase, belongs to the family of multicopper oxidase (MCO) enzymes, which catalyze the reduction of molecular oxygen to water at a trinuclear copper cluster. Recently, our group has identified a transient biradical intermediate during reaction of type 1 Cu depleted (T1D) SLAC with oxygen on the time scale of minutes (1). Here, electron paramagnetic resonance (EPR) spectroscopy in conjunction with the rapid freeze-quench (RFQ) technique has been used to trap and monitor the intermediates that are formed during the reaction of reduced T1D SLAC with molecular oxygen on the time scale of milliseconds. Besides a copper signal, a narrow EPR line (1.2 mT) centered at g = 2.0045 with the features of a tyrosyl radical was detected at the early stage of reaction. As the reaction progresses, the narrow radical signal disappears and is replaced by a broad structured signal at g = 2 and a half-field signal in the g = 4 region. We are currently performing multi-frequency EPR (9.5, 95 and 275 GHz) on SLAC samples prepared by the rapid freeze-quench technique to obtain new information about the enzymatic mechanism of the oxygen-to-water conversion.



#### Reference

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#### JF Gielis<sup>1,2</sup>, JJ Briedé<sup>3</sup>, G Boulet<sup>2</sup>, T Horemans<sup>2</sup>, P Cos<sup>2</sup>, P Van Schil<sup>1</sup>

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# Discovery of two temporal distinct free radical bursts during pulmonary ischemia-reperfusion injury

#### Objectives

Pulmonary ischemia-reperfusion injury (IRI) can severely compromise lung transplantation. Reactive molecules such as superoxide, nitric oxide (NO) and peroxynitrite are believed to contribute to the IRI pathogenesis, but this assumption is based on indirect measurements. We use electron spin resonance (ESR) to directly quantify free radical formation during pulmonary IRI.

#### Methods

Four groups of Swiss mice were subjected to left pulmonary hilum clamping for one hour of ischemia or ischemia followed by 1, 4 and 24 hours of reperfusion. A control group only underwent sham thoracotomy. For ESR quantification, pulmonary NO was trapped with iron-DETC, while the spin probe CMH was used to capture free radicals in peripheral blood. Pulmonary peroxynitrite was indirectly monitored with nitrotyrosine Western blot.

Experimental groups		
Group	Ischemia	Reperfusion
Sham	No	No
1I	1h	No
1I1R	1h	1h
1I4R	1h	4h
1I24R	1h	24h

#### Results

Peripheral blood showed a significant free radical burst after one hour of ischemia  $(11,77\pm0,72x10^3 \text{ A.U. vs.} 6,66\pm0,83 x10^3 \text{ A.U. in sham}; p<0,001)$ . After one hour of reperfusion, a pulmonary NO peak $(14,69\pm0,91x10^4 \text{ A.U. vs.} 1,84\pm0,75 x10^4 \text{ A.U. in sham}; p<0,001)$  coincided with a significant increase in nitrosylated proteins  $(105,01\pm15,16 x10^{-3} \text{ A.U. vs.} 46,57\pm6,48 x10^{-3} \text{ A.U. in sham}; p<0,005)$ .



#### Conclusion

Longitudinal quantification of free radicals during IRI indicated the occurrence of two major radical bursts. The peripheral radical peak after ischemia may be related to systemic hypoxia. After one hour of reperfusion, the lung tissue showed a significant increase of NO and its reaction products, which may play an important role in the IRI pathogenesis.

# NO-radical signalling in bone marrow mobilizes progenitor cells for endothelial repair.

Ernst van Faassen Nephrology Department, Leiden University Medical Centre The Netherlands.

The functionality of the endothelial layer of blood vessels depends on the balance between degenerative and regenerative processes. Many diseases (atherosclerosis, diabetes, kidney malfunction) present with low counts of endothelial progenitor cells in the circulation and impaired regeneration of the endothelium. Such progenitors are offspring of specialized stem cells in the bone marrow, and are released into the bloodstream via controlled activity of matrix metallopeptidases (eg. MMP9).

We use NO-spin trapping for the first ever detection of NO radicals in bone marrow cells from healthy as well as hypertensive rats. The NO levels correlate closely with MMP9 activity in the bone marrow niche. Our observations support the hypothesis that the manifest progenitor deficiency in hypertension results from impaired signalling in the NOS/NO/MMP9 cascade. We also give proof of principle that the progenitor deficiency may be rescued *in-vivo* by pharmacological intervention [1].

[1] PLOSone 8(3), 2013, e57761

#### Towards implementation of selective NV-nanodiamond imaging in a neurobiology experimental set-up

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The charged nitrogen-vacancy (NV<sup>-</sup>) centre is a remarkable defect in diamond which allows discrimination of its magnetic sublevels through its fluorescence intensity that can be altered by external fields coupling to the electronic spins. Among the many proposed applications, background-free fluorescence bio-imaging based on NV fluorescent nanodiamond (FND) particles was recently demonstrated [1-3]. The highly efficient non-blinking FNDs can be discriminated from background fluorescence by switching on resonant microwaves and/or an external magnetic field [1-3], as demonstrated in cells [1] and potentially in small animals [2].

Here we present an overview of how we are going to implement NV-nano diamond imaging in a microscope for neuron research incorporating patch clamp measurements of electrical activity. A circular antenna (i.d. 1mm,  $50\Omega$  terminated) fed by waveguides on a print plate is inserted and its position adjusted underneath the cell for the neuron culture which can thus be placed in the near field radiation. A synthesizer and amplifier combination provides 2.3-3.2GHz microwaves (up to ~30dBm). For FND detection, a pointer-type 532nm laser delivers an adjustable spot on the field of view of the microscope and an NV<sup>-</sup> selective filter is inserted detection. In test samples contrast ratios of FND emission of up to 10 % have been found in this configuration upon on-off switching of resonant microwaves.

This opens the way for in vivo imaging of extra- or intracellular FNDs parallel with of neuron cells observation and monitoring of their activity.

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# **Abstracts Posters**

#### Quantification of erythrocytic nitric oxide by Electron Paramagnetic Resonance: Biochemical determinants.

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The bioavailability of nitric oxide in circulating blood can reflect the severity of endothelial dysfunction in metabolic and cardiovascular diseases. Therefore a quantitative assay of circulating bioavailable NO is highly desirable. Nitric oxide can form iron-nitrosyl complexes with hemoglobin in red blood cells (RBCs). We hypothesized that 5-coordinate- $\alpha$ -HbNO that predominantly formed in venous blood, reflects bioavailability of vascular NO as well as vascular endothelial function *in vivo*. We developed a modified subtraction method using EPR spectroscopy to quantify this complex in erythrocytes from venous blood. We propose to characterize the physico-chemical determinants of formation and accumulation of 5-coordinate- $\alpha$ -HbNO (HbNO) in intact human RBCs.

**Methods and Results**: We tested the capacity of erythrocytes to take up extracellular NO, generated from exogenous NO-donor, and to accumulate HbNO under different oxygen levels. The RBCs were collected from venous blood of healthy volunteers, washed with isotonic solution and after centrifugation reconstituted at 50% of hematocrit. They were preincubated at 1% and at 21% of O<sub>2</sub>, at 37°C during 1 hour, and with NO-donor (Spermine-NONOate) at different concentrations during next 1 hour. Then the samples were frozen in liquid nitrogen for measurements of accumulated HbNO by low-temperature EPR. The formation of HbNO was significantly higher in anaerobic condition than in aerobic (0.018 +/-0.002  $\mu$ mol HbNO/ $\mu$ mol NO-donor under 1% of O<sub>2</sub>; 0.0036+/-0.0004  $\mu$ mol HbNO/ $\mu$ mol NO-donor in open air) and inversely correlated with the ratio of oxy- to deoxy- forms of Hb measured by gasometry in the same volunteers. We also observed that NO diffusion into RBCs was limited. Only about 1.8 % of NO liberated from NO-donor was capable to form HbNO in anaerobic condition in our experiments.

Subsequently, we analysed the physico-chemical determinants (pO<sub>2</sub>, pH and temperature) that can influence the HbNO stability in intact RBCs. Venous blood was collected from healthy volunteers into vacuum tubes and Spermine-NONOate solution (100 µmol/L) was added. The tubes were then incubated at proper venous O<sub>2</sub> level during 45 minutes, centrifuged, in order to discard the plasma, opened at 1% or 21% of O<sub>2</sub> and the RBCs samples were frozen every 10 min for low-temperature EPR measurements. We observed that the stability of HbNO was significantly higher in hypoxic condition: only 17 +/-0.4 % of HbNO was degraded under 1% of O<sub>2</sub> in comparison to 49 +/-0.2 % under 21% during 30 minutes after vacuum tainer tube opening. On the other hand, the presence of a column of plasma, obtained after blood centrifugation, can slow down the O<sub>2</sub> diffusion into the erythrocytes allowing a better stability of deoxy-Hb with NO. We determined also the effect of the temperature and pH on the HbNO formation and stability incubating RBCs at different temperatures (9°C, 20°C, 37°C) or at different pH (7.6, 7.4, 6.8, 6.5 and 6.2), after addition of Spermine-NONOate in blood and RBCs collection. We observed that HbNO complex is more stable at 20°C, and under incubation with acidic pH < 6.5.

**Conclusion**: The study demonstrates that physico-chemical determinants such as  $pO_2$ , pH and temperature influence the stability of 5-coordinate- $\alpha$ -HbNO complex in human erythrocytes opening the way to further understanding of in vivo determinants of NO bioavailability in human circulation.

# Development of EPR oximetry in diabetic wound healing models <u>C. Desmet</u><sup>1</sup>, A. Lafosse<sup>2, 3</sup>, S. Vériter<sup>2</sup>, Ph. Levêque<sup>1</sup>, D. Dufrane<sup>2</sup>, B. Gallez<sup>1</sup>

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<u>Introduction</u>: One major complication of diabetes is the development of skin ulcers with impaired healing. Indeed, fifteen percent of diabetic patients will suffer from lower extremity ulcers during their life and 7 to 20 % of these patients with foot ulcers will require amputation [1]. A main cause of the nonhealing of foot ulcers is tissue ischemia [2] as it is known that oxygen is a key parameter in wound healing. Nevertheless, the variation of the pO<sub>2</sub> in the wound remains uncertain as there still lacks non invasive methods for absolute and repeated pO<sub>2</sub> measurements in situ. EPR oximetry is a technique that allows repeated measurements of the absolute tissue pO<sub>2</sub>. The technique is based on the measurement of the linewidth of the EPR signal recorded with a biocompatible oxygen sensor like LiPC implanted in the tissue.

<u>Aim</u>: To investigate whether EPR oximetry with LiPC is a possible tool to follow the  $pO_2$  in the wound in diabetic wound healing.

<u>Materials and methods</u>: Two diabetes models were used: chemically-induced type I diabetes (with streptozotocin) and genetically-induced type II diabetes (db/db mice).

Two types of wounds were tested: a  $30 \times 8$  mm pedicled flap and a 6 mm diameter excisional wound. LiPC crystals were inserted in different positions inside the wound and the EPR signal recorded repeatedly during the wound healing process.

<u>Results:</u> In the flap model, the  $pO_2$  decreased the first day after wounding due to the flap surgery. Then, the  $pO_2$  increased during the healing process. Interestingly, this increase was more rapid for non diabetic mice than for diabetic mice (fig. 1). For the excisional wound model, a variation of the  $pO_2$  was observed in the wound periphery during the healing process but it did not differ from what was observed in the control non wounded tissue. In the center of the excisional wound, the  $pO_2$  was elevated the first days after the wounding without any difference between the diabetic and non diabetic state (fig. 2).

![](_page_18_Figure_8.jpeg)

![](_page_18_Figure_9.jpeg)

Fig. 1:  $pO_2$  measurements during the healing process obtained with LiPC crystals implanted in the flap in control db/+ (blue) and db/db mice (red).

![](_page_18_Figure_11.jpeg)

<u>Conclusion</u>: EPR oximetry with LiPC seems to be suitable to follow the variation of the  $pO_2$  in a wound. The flap model containing LiPC in db/db mice seems to be an adequate wound model to study the tissue  $pO_2$  in diabetic wound healing.

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# LONG-RANGE DISTANCE CONSTRAINTS FOR THE FIBRIL FOLD OF ALPHA- SYNUCLEIN

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#### Abstract:

The fibril-fold of  $\alpha$ -synuclein ( $\alpha$ S), relevant for the understanding of fibrilization and Parkinson's disease, is still not known. Long-range distance constraints from Double Electron Electron Resonance (DEER) are beginning to emerge. Fibrillizing doubly spin labelled proteins in a background of wild-type, intramolecular distances are emphasized, but intermolecular distances cannot be suppressed completely, leading to superpositions difficult to disentangle. We compare distances between 56-69, 56-90 and 69-90<sup>1</sup> to recent results of Pornsuwan et al.<sup>2</sup>.

Some of these constraints are inconsistent with the simplest model that the protein is in a plane perpendicular to the fibril axis, with all  $\beta$ -sheets parallel. Reasons could be different fibril structures, e.g. discrepancies in morphologies. Also,  $\beta$ -sheets at an angle or extension of the protein over different planes could be the cause. In that case, combining the present constraints with ss-NMR derived ones would be needed.

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### A mononuclear Mn(II) pseudo-clathrochelate complex studied by multifrequency electron-paramagnetic-resonance spectroscopy

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The correlation between structural and spectroscopic properties in transition-metal complexes is of great importance to design catalysts and single molecular magnets. The recently synthesized mononuclear Mn(II) complex,  $[Mn(PzOx)_3BC_6H_5]Cl$  belongs to the class of pseudo-chlathrochelates, which attract particular interest as molecular scaffolds for medicinal diagnostics and radiotherapy as well as MRI shift reagents. Most of the clathrochelates are formed by polyazomethine caging ligands, which determine the geometry of the metal coordination by means of their high chemical stability and mechanical rigidity. This coordination geometry corresponds to a particular electronic structure and splitting of the energy levels of the metal ion. The electronic structure of the  $[Mn(PzOx)_3BC_6H_5]Cl$  complex has been characterized by means of high-frequency electron-paramagnetic-resonance spectroscopy (275 GHz). For the first time, these studies allowed to determine the magnitude and sign as well as the distribution of the zero-field splitting parameters for Mn(II) in the trigonally prismatic coordination. Careful consideration of the distribution in the zero-field splitting parameters is found to be a key point in understanding and interpreting the experimental EPR spectra.

#### Resolving the relative tilting of hyperfine and g tensors for vanadyl complexes in the Al-metal-

#### organic framework MIL-53 by a multifrequency (S, X, Q, and W-band) EPR approach

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Metal organic frameworks (MOFs) are ordered porous materials built of metal ions or clusters connected by organic linkers. These materials are interesting for many applications because they provide possibilities of designing structure, pore size or shape to desired functionality. MOFs have potential use in catalysis, gas storage and gas separation. Two characteristic types of MOFs are MIL-47<sup>[1]</sup> [VO(BDC); BDC = terephthalate or 1,4-benzenedicarboxylate, MIL = Materials of the Institute Lavoisier] and MIL- $53^{[2]}$  [Al(OH)(BDC)], originally synthesized by Ferey's group.

Recently we reported that V-MIL-47 can be a highly selective catalyst in the liquid phase oxidation of cyclohexene <sup>[3]</sup>. This framework, however, exhibits limited stability in aqueous environments. This problem may be solved by doping the highly stable MIL-53 with catalytically active  $V^{IV}$  ions. In order to understand the catalytic activity of such doped framework, it is necessary to verify whether dopant ions are actually incorporated in the framework. As  $V^{IV}$  (3d<sup>1</sup>) is a paramagnetic ion, EPR and ENDOR spectroscopy are excellent tools for this purpose. In Fig. 1 the evolution of the powder EPR spectrum with microwave frequency for V-doped MIL-53 is shown. The spectra are dominated by just one  $V^{IV}$  center with rhombic g and <sup>51</sup>V hyperfine (HF) tensors whose principal axes do not coincide. The ENDOR spectra of  $V^{IV}$  in MIL-53 reveal HF interactions with <sup>1</sup>H and <sup>27</sup>Al nuclei, strongly suggesting that the  $V^{IV}$  ions are indeed incorporated in the framework.

![](_page_21_Figure_11.jpeg)

Figure 1: S-band, X-band, Q-band and W-band EPR spectrum of V-doped (1%) MIL-53 at RT

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#### Photoinduced high-field electron paramagnetic resonance of TiO<sub>2</sub>-based nanocatalysts

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 $TiO_2$  is a naturally abundant, inert, cheap, non-toxic, highly photoactive and photostable semiconductor, whose large band-gap (~3eV) requires illumination with light in the near ultraviolet (UV) region for catalytic activation. The main strategies to improve the photocatalytic performance of  $TiO_2$  comprise the synthesis and morphologic control of nanoscopic 1D to 3D architectures (nanotubes, nanosheets, nanoparticles, etc)<sup>1</sup> to increase the specific surface area and reduce the e-/h+ diffusion range, and its visible-light activation via irradiation-induced defects, metal and non-metal doping, dye sensitization, metal surface plasmon resonance and inorganic-organic hybridization<sup>2</sup>. This yields a wide variety of TiO<sub>2</sub>-based materials that are among the most promising candidates for solar energy conversion and remediation of environmental pollution through photocatalytic processes.

In this work we will use continuous wave (CW) and pulsed Electron Paramagnetic Resonance (EPR) to study two sate of the art photocatalysts: i) Pure anatase TiO<sub>2</sub> nanosheets with well-defined (001) facets and ii) P90 commercial TiO<sub>2</sub> nanoparticles loaded with different ratios of Au/Ag nanoparticles. The highest surface energy of (001) facets is expected to result in an increased catalytic performance, but only limited research has supported this hypothesis and controversy still stands on the origin of enhanced catalysis<sup>3</sup>. Regarding Ag/Au loaded P90, the role of the metal-semiconductor junction and metal surface plasmon excitation remain subject of active research<sup>4</sup>. X- and W-band UV and visible light-induced EPR will be used to discern the origin of the radicals species created and their time evolution during photoexcitation of these materials. In this way, a detailed view on the charge separation, transfer and trapping mechanisms related to the photocatalytic activity will be obtained.

![](_page_22_Figure_7.jpeg)

X- and W-band CW EPR of P90 commercial TiO<sub>2</sub> powder under UV illumination

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# Spin-probe EPR analysis probing framework-guest interactions in different periodic mesoporous organosilica

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Periodic mesoporous organosilicas (PMOs) are a new class of porous inorganic-organic hybrid materials with highly ordered mesoporous structures, and most importantly, with the organic groups as an integral part of the inorganic-oxide framework<sup>1-2</sup>. Different from the organically functionalized mesoporous silicas, the inorganic and organic moieties are covalently linked to each other and homogeneously distributed in the framewall of PMOs, which indeed have extended the research of mesoporous materials from "chemistry of the void space" to the "chemistry of the walls". Due to the presence of the organic groups within the framewall, PMOs have a tunable surface hydrophobicity/hydrophilicity, unique mechanical and hydrothermal stability, and adsorption capacity, leading to materials with promising potentials in catalysis, metal scavenging and controlled drug release. Indeed, all potential applications of mesoporous material. In order to generate novel applications, it is of crucial importance to have a better insight into the framework-guest interactions in the PMO materials.

Among the different techniques available for material characterization, spin-probe electron paramagnetic resonance (EPR) has revealed itself to be an excellent tool to analyze the surface properties of porous materials. By analyzing the EPR spectra of paramagnetic spin probes, mostly nitroxide radicals, that can interact with the surface sites of the porous materials, information about the structure, accessibility, surface polarity and surface charge can be obtained. Recently we investigated the formation mechanism and surface properties of ethane-bridged PMOs by means of spin probe EPR.<sup>3</sup> Here we show the spin-probe EPR study of the framework-guest interactions in different PMO materials, including ethane-bridged PMOs and benzene-bridged PMOs with different molecular-scale ordering and different pore structures.

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### EPR Analysis of Model Compounds of Ziegler-Natta Catalysts

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Multifrequency CW and Pulse EPR techniques at the X (9.4 GHz), Q (33 GHz) and W band (94 GHz) have been applied for studying a  $TiCl_3(py)_3$  complex in dry powder form, in pyridine solution and supported on MgCl<sub>2</sub>.

The evolution of the EPR spectra is related to the different nature and nuclearity of  $Ti^{3+}$  ions. The  $TiCl_3Py_3$  complex in dry powder form is characterized by a well-defined anisotropic CW-EPR spectrum (as already reported in literature <sup>[1]</sup>) and a strong temperature dependence of the signal has been observed in the range 10K - RT. The Q and W band CW-EPR spectra of the  $TiCl_3Py_3$  dry complex, although more resolved, are consistent with the corresponding X band spectrum. In the case of the  $TiCl_3Py_3$  complex dissolved in pyridine the presence of dimers has been observed, as also reported for  $TiCl_3$  in pyridine solution<sup>[2]</sup>. By means of Q-band HYSCORE spectroscopy the interaction of  $Ti^{3+}$  ions with <sup>14</sup>N nuclei belonging to the pyridine ligands was revealed for both  $TiCl_3Py_3$  dry complex and  $TiCl_3Py_3$  dissolved in pyridine; the interaction of  $Ti^{3+}$  ions with <sup>35,37</sup>Cl nuclei is not evident, probably due to suppression effects.

The analysis of these compounds is part of a bottom-up approach of investigation of model systems for MgCl<sub>2</sub>-supported Ti-based Ziegler-Natta catalysts with the aim of obtaining reference data helpful in the study of real-world industrial high-yield Ziegler-Natta catalysts.

![](_page_24_Figure_10.jpeg)

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# Triplet Formation Pathways in Polymer:Fullerene Based Organic Solar Cells

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Triplet formation has been considered as one of terminal charge-loss pathways in polymer: fullerene based bulk heterojunction solar cells. Triplets in these devices could be produced by direct intersystem crossing from excited singlet states in the constituent materials, or as a result of decay of charge transfer (CT) pairs to energetically low lying triplet states. The latter has been controversially assigned as a major interfacial charge-loss pathway with detrimental impact on photocurrent whenever the CT states lie energetically above the triplet states of the polymer or fullerene molecule [1]. A systematic study has been made in three prototype SY-PPV:PC(70)BM and SY-PPV:ICBA films and devices using complementary optical and magnetic resonance spectroscopies. Both CT and PCBM fluorescence bands are observed in SY-PPV:PCBM films/devices, while only ICBA fluorescence is detected in SY-PPV:ICBA films/devices. Using electrical detected magnetic resonance with excitation laser of different wavelengths, we detect fullerene triplets in all devices and conclude that the TEs are populated by intersystem crossing from the singlet excitations (SEs) in the fullerene domains. The singlet states in the fullerene domains are populated by three different pathways: direct photoexcitation from the ground state, energy transfer from SE in SY-PPV, and CT pair transfer. Although the CT states lie energetically above the fullerene triplet states, triplet production from direct photoexcitation of the CT states has not been observed. Therefore, we conclude that CT to triplet pathway is kinetically suppressed. This has the implication that direct CT to triplet transitions may have minimal effect on interfacial charge loss and photocurrent [2]. Rather, the severe photocurrent reduction observed in polymer:fullerene devices when the open circuit voltage is pushed to or above 1 V can be ascribed to increasing energy alignment of CT states to the fullerene singlet states [3-5]. This accidental alignment unfortunately favors both recombination of SEs to the ground state and formation of triplets by ISC in the fullerene domains.

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## Structural damage in thin SLIM-cut c-Si foils fabricated for solar cell purposes: Atomic assessment by electron spin resonance.

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Thin (< 90  $\mu$ m) silicon foils intended for photovoltaic applications have been fabricated from standard (100)Si wafers using a low-temperature (< 150°C) stress-induced lift-off process aiming to reduce production costs [1]. An electron spin resonance (ESR) study was performed in order to evaluate the quality of the material in terms of occurring defects showing paramagnetic activity.

Initial tests revealed a complex spectrum, which upon further inspection appeared to be a superposition of 3 separate signals. The most prominent one (~91 % of total signal intensity) concerns the D-line, corresponding to  $Si_3\equiv Si \cdot dangling$  bonds (DBs) in disordered Si environment; A second (~3 %), highly anisotropic, signal is ascribed to Si dislocations [2], generated as a result of the plastic deformation of strained Si; The third signal (~6 %), composed of a triplet, was identified as originating from a N-related center –similar to the Si-SL5 defect [3]– generated as a result of trapping of N atoms into the Si foil, possibly at tiny fissures, during the manufacturing ("cooling-off" in liquid N<sub>2</sub>) process.

Step by step Si etch back experiments, carried out to trace the location of these defects within the Si foils, indicated all signals to disappear after etching off a ~33-µm thick Si layer. This study revealed the signals to be equally distributed along the top third of the Si foil viewed from the lift-off side. Additionally, by separately analyzing different parts of the wafer, the distribution of the dislocations was revealed to be non-uniform, supporting the notion that the dislocation formation spreads out from the "crack initiation" point.

It is thus found that the SLIM-cut method for fabrication of thin Si foils as studied in this work results in the introduction of potentially detrimental defects that may impair the functionality of photovoltaic cells built on these substrates. Fortunately, the defects may be removed by etching off a top Si layer.

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### EPR probeheads for very small samples: developments and prospects

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In the study of paramagnetic centers in radical- and metallo-proteins one of the main targets is the determination of all magnetic interaction tensors of the center including their relative orientations. This task can be best performed when single crystals are available. Since protein crystals are usually very small (i.e. typically 0.1-0.4 mm), the development of EPR probeheads adapted to very small samples is of key importance for such investigations. Also other sample limited EPR applications (such as titration series with isotope labeled materials) would benefit from targeted designed EPR micro-resonators. Our project in the German SPP1601 priority program aims to investigate the practical limits in sample size and sensitivity of EPR micro-resonators. Several designs will be analyzed, e.g. 1) Planar microresonators (PMR) operating at X-band <sup>1,2</sup>, 2) loopgap resonators at 35 GHz<sup>3</sup>, 3) Solenoid microcoils and planar microstrip probe-heads<sup>4</sup>. Each of these designs can be optimized for particular sample shapes and particular EPR experiments. Several of these options will be discussed and preliminary experiments on single crystals will be presented<sup>5</sup>. We have constructed an X-band (cryogenic) PMR probehead (sample diameter 0.4 or 0.8 mm) and performed a systematic comparative study with standard EPR resonators using a nitroxide probe in a range of sample sizes. As expected, the PMR was superior in absolute sensitivity but some commercial resonators also performed remarkably well. The implications of these measurements with respect to further optimizations of the design will be discussed.

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