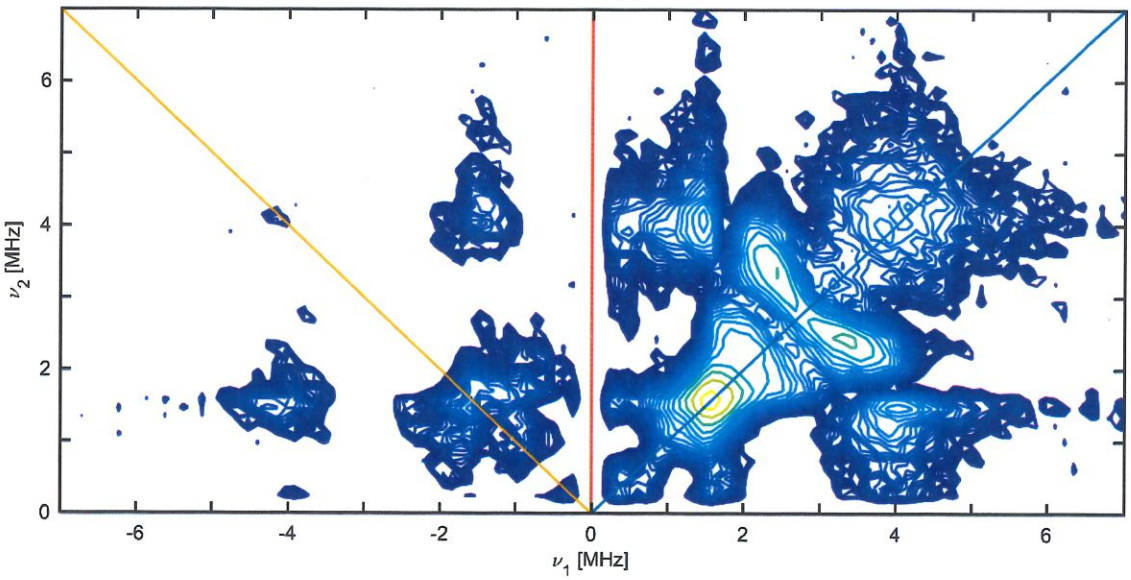
Universiteit Antwerpen




# 26th Meeting of the Benelux EPR Society

## Antwerp, 6th June 2018

BRUKER

## 26th Meeting of the Benelux EPR Society Program

**9:45** **Registration Tea/Coffee**

**10:30** **Opening: Henk Vrielinck**

**Chair: Bernard Gallez**

**10:40** When stronger magnets don't help: Methods for disentangling overlapping high-field EPR spectra illustrated in record organic solar cell blend PBDB-T:ITIC

*Melissa Van Landeghem (Antwerp, BE)*

**11:10** Effects of gas exposure on the EPR spectrum of V(IV) doped into the Metal Organic Framework MIL-53(Al).

*Kwinten Maes (Gent, BE)*

**11:40** EPR: a tool to investigate for soil and air pollution

*Manuela Liberi (Bruker, IT)*

**12:10** **Lunch & Posters**

**Chair: Edgar Groenen**

**13:30** Power Saturation in EPR Made Easy

*Fred Hagen (Delft, NL)*

**14:00** Effect of a water soluble curcumin-hydroxypropyl-β-cyclodextrin complex on peroxidase-catalyzed reaction: luminescence and EPR studies

*Ange Mouithys-Mickalad (Liege, BE)*

**14:30** New insights into the B-class dye-decolorizing peroxidases: An EPR study of the heme and the radical sites in a DyP from K. pneumoniae

*Kevin Nys (Antwerp, BE)*

**15:00** **Tea/Coffee & Posters**

**Chair: Freddy Callens**

**15:30** EPR signals of Ferritin, an Iron Storing Protein – More Questions than Answers

*Martina Huber (Leiden, BE)*

**16:00** Towards improved detection of mitochondrial superoxide using EPR

*Samantha Scheinok (Louvain, BE)*

**16:30** Thermal stability and temperature dependent ESR characteristics of the As acceptor in geological 2H-$MoS_2$

*B. Schoenaers (Leuven, BE)*

**17:00** **Closing Remarks and Drinks**

## ORAL ABSTRACTS

Call Leiden!

# When stronger magnets don't help: Methods for disentangling overlapping high-field EPR spectra illustrated in record organic solar cell blend PBDB-T:ITIC

M. Van Landeghem[1], W. Maes[2], E. Goovaerts[1], S. Van Doorslaer[1]

[1] *Department of Physics, University of Antwerp, Universiteitsplein 1, 2610 Antwerpen, Belgium*

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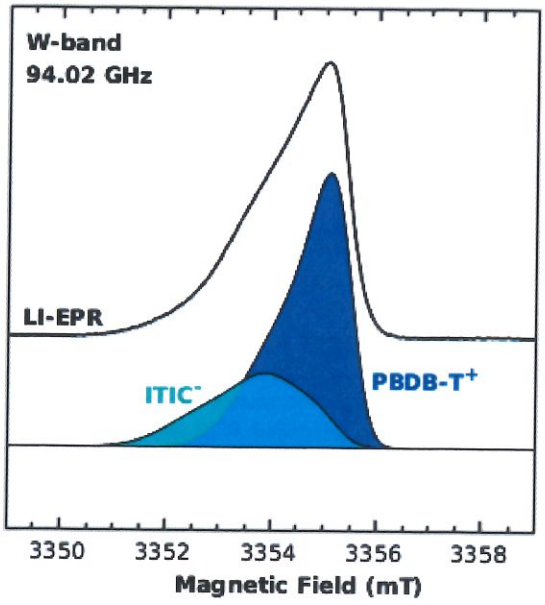
Spectral overlap, even at high field, is a problem generally encountered in many EPR studies. In the specific case of bulk-heterojunction (BHJ) organic solar cells (OSCs), the paramagnetic species of interest are light-induced radicals which are created as a pair after charge transfer at the interface between the donor polymer and molecular acceptor regions making up the BHJ blend. Hence, the similar *g*-values expected for the positive and negative organic radicals often lead to strong spectral overlap complicating the unambiguous assignment of the light-induced (LI) EPR spectrum.

The donor-acceptor combination studied here, PBDB-T:ITIC, was the first fullerene-free OSC to recently achieve >11% efficiency, challenging the state-of-the-art polymer-$PC_{71}BM$ devices [1]. For this blend, the two-component structure of the LI-EPR spectrum could not even be resolved at W-band frequency (94 GHz). Therefore we separated the two contributions to the total EPR spectrum by exploiting two different properties of the charge-transfer radicals, namely the (small) difference in their longitudinal ($T_1$) relaxation times and the presence of a unique magnetic nucleus, $^{14}N$, in the ITIC molecule. For the $T_1$-based method, we applied an inversion-recovery filter to selectively suppress one component in the spin echo analogously to the relaxation-filtered hyperfine spectroscopy (REFINE) technique first proposed by Maly et al. [2]. Sensitive detection of the $^{14}N$ hyperfine couplings at W-band frequency was achieved by means of electron-electron double resonance (ELDOR)-detected NMR (EDNMR). Here we demonstrate the application of EDNMR-induced EPR to obtain a field-swept EPR spectrum containing only contributions from the ITIC radical [3]. Both approaches are validated by LI-EPR spectra on related blends which yield better resolved spectra of the individual PBDB-T and ITIC radicals.




1. W. Zhao et al., *Adv. Mater.*, **2016**, 28, 4734-4739.
2. T. Maly, T. F. Prisner, *J. Magn. Reson.*, **2004**, 170, 88-96.
3. M. Van Landeghem et al., *J. Magn. Reson.*, **2018**, 288, 1-10.

powder!
this is still missing in your speach.

Hz

**Effects of gas exposure on the EPR spectrum of V(IV) doped into the Metal Organic Framework MIL-53(Al).**

K. Maes, I. Nevjestić, H. Damien, H. Vrielinck, F. Callens

*Ghent University, Dept. of Solid State Sciences, Krijgslaan 281/S1, 9000 Gent, Belgium.*

Metal-Organic Frameworks (MOFs) are ordered porous crystalline materials constructed of metal ions connected by organic linkers. Because of their many interesting properties, a diverse scale of applications are being explored (e.g., catalysis, gas adsorption, separation and storage). For the research presented here we use MIL-53(Al), which consists of Al(OH) chains linked together by BDC (1,4-benzenedicarboxylate) creating a lattice with large onedimensional pores. After activation (emptying the pores) the structure exhibits the breathing phenomenon: the framework can reversibly change from an open (large pore) to a closed (narrow pore) structure depending on conditions like temperature and pressure. EPR spectroscopy using V(IV) as a paramagnetic probe is able to distinguish between the large pore and the narrow pore state of V-doped MIL-53(Al). Recently the g and $^{51}V$ A tensors for these dopant ions were determined in both states and the transition between both was investigated using EPR and XRD measurements.[1-3]

In the present study we investigate the changes to the structure when introducing different gases at various temperatures. First, the effect of air on the large pore structure was studied. Increasing air pressure leads to a clear broadening of the spectrum. Measuring the temperature dependence of this broadening showed increase of the linewidth with decreasing temperature, following an Arrhenius behaviour with an activation energy of 12 kJ/mole. Secondly, we found that exposure of the large pore structure to $CO_2$ causes most of the sample to convert to the narrow pore state, with a nonnegligible part still in the large pore state. Exposing the narrow pore state to $CO_2$ and $O_2$ has no effect on the EPR spectrum, which suggests that these gases cannot access the pores for this state.

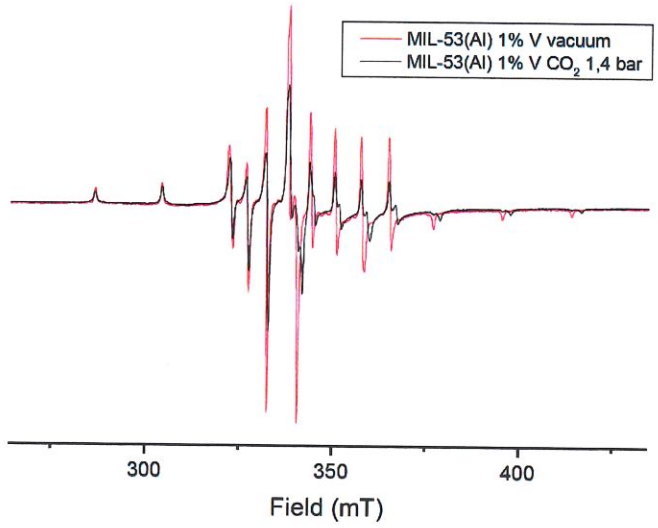



*Figure 1: X-band EPR spectrum of V-doped MIL-53(Al) before and after exposure to $CO_2$. The structure started in the large pore state and changed to mostly the narrow pore state.*

1. Nevjestic, H. Depauw, K. Leus, V. Kalendra, I. Caretti, G. Jeschke, S. Van Doorslaer, F. Callens, P. Van Der Voort and H. Vrielinck, Chemphyschem **16** (14), 2968-2973 (2015).
2. Nevjestić, H. Depauw, K. Leus, G. Rampelberg, C. A. Murray, C. Detavernier, P. Van Der Voort, F. Callens and H. Vrielinck, The Journal of Physical Chemistry C **120** (31), 17400-17407 (2016).
3. Nevjestic, H. Depauw, P. Gast, P. Tack, D. Deduytsche, K. Leus, M. Van Landeghem, E. Goovaerts, L. Vincze, C. Detavernier, P. Van Der Voort, F. Callens and H. Vrielinck, Phys Chem Chem Phys **19** (36), 24545-24554 (2017).

Taalverzorging!

→ P. Gast



→ B. Galler

Toch vroeger beginnen met oefenen volgende keer

Magical site

EPR dissmination

**EPR: a tool to investigate for soil and air pollution**

Manuela Liberi

*Bruker Biospin, Italy*

Identifying and monitoring generation of free radicals from ambient particulate matter and determining their oxidative potential is already of great concern due to adverse effects on human health. In the meantime, the existence of potentially toxic EPFRs now questions the long held belief that sorption of an organic pollutant into a soil matrix is a method of mitigating its environmental impact.

With EPR one gains insight into the mechanisms generating toxic radicals from inhalable ambient particulate matter: it detects, identifies, and quantifies ROS, PAHs, EPFRs, and transition metals involved in the particulate matter chemistry

On the other hand, EPR provides as well details to study the impact of pollution from industrial and agricultural sources on the soil environment, understanding the mechanisms and roles of the inorganic, organic, and biological components of soil.

EPR, with the EMXNano, is the solution for investigating, evaluating, and better understanding this important branch of chemistry.

• Take up in

air pollution, contains radicals!

soil / clay

advanced oxidation processes AOP eff.

inspiration ?!

automibile

What is spin count accuracy?

better than 10%

CW EPR

(Fe-S) proteins

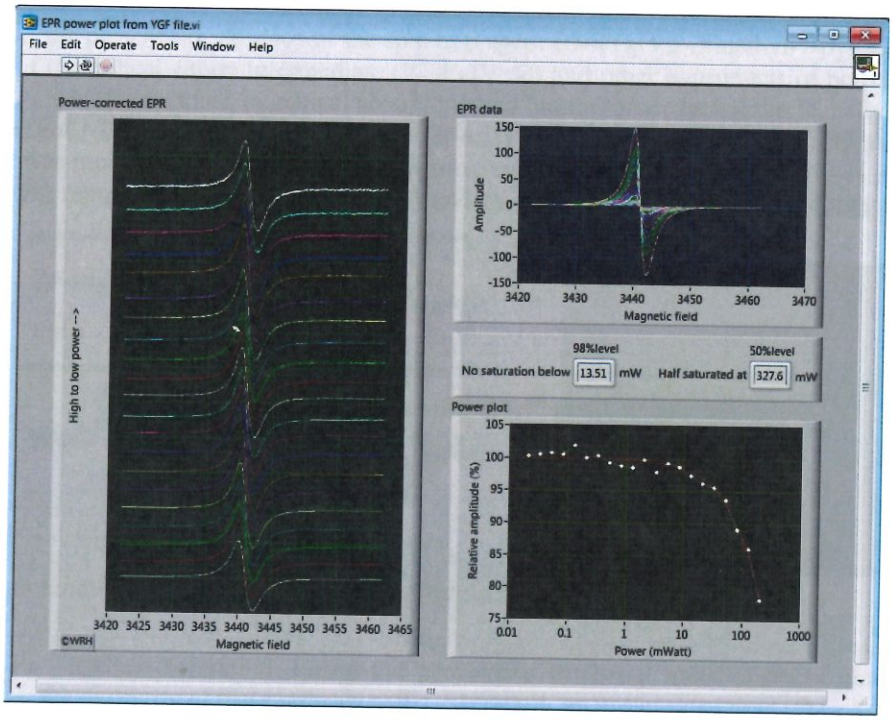
# Power Saturation in EPR Made Easy

Fred Hagen

*Delft University of Technology, Department of Biotechnology,*
*Van der Maasweg 9, 2629HZ Delft, The Netherlands*

EPR spectroscopy has always played a central role in the study of iron-sulfur proteins from their initial discovery in 1960 till today. An inspection of the 6-decade spanning literature indicates considerable variations in quality of the research; overall the value level does not necessarily increase over time [see: (2018) EPR of complex biological iron-sulfur systems; open access: http://link.springer.com/article/10.1007/s00775-018-1543-y]. The most pressing problem appears to be a frequent misunderstanding, or at least improper handling, of the phenomenon of power saturation. One of the underlying causes, and possibly the central culpit, is the manner in which power saturation is, and has been, dealt with (or ignored) in the software of commercial computerized spectrometers over the last some 25 years.

I will briefly explain the physical background of power saturation, discuss its relevance for quantitative EPR spectroscopy of iron-sulfur proteins, indicate how research literature affected by improper power handling can readily be recognized, and present a simple and straightforward approach, in the form of freely available software, to practically deal with the matter with minimal effort.



## Effect of a water soluble curcumin-hydroxypropyl-β-cyclodextrin complex on peroxidase-catalyzed reaction: luminescence and EPR studies

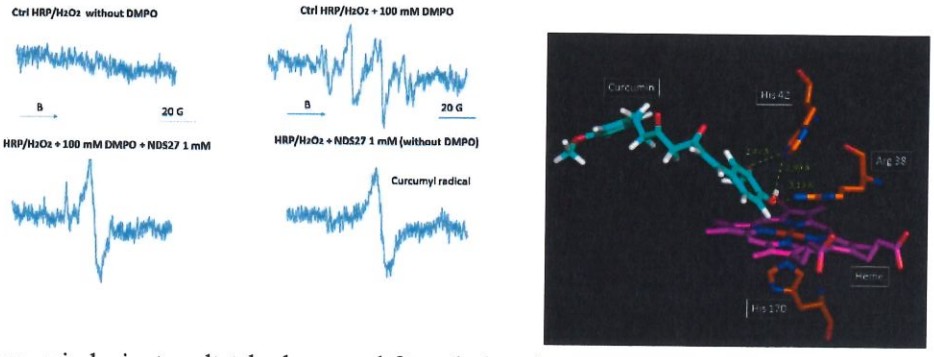
T. Franck,[1] P. Nyssen,[2] P. Neven,[1] M. Hoebeke,[2] D. Serteyn,[1] A. Mouithys-Mickalad[1]

[1]*Centre for Oxygen, R&D (CORD)-CIRM, University of Liège, Institute of Chemistry, B6a, Quartier Agora. Domaine du Sart-Tilman. 4000 Liège – Belgium.*

[2]*Laboratory of Biomedical Spectroscopy, Institute of Physics, B5a, Domaine du Sart-Tilman. 4000 Liège-Belgium.*

It is well known that peroxidase catalyzed reactions of some drugs, xenobiotics result in the formation of radical species (e.g. phenoxyl radicals) which may in turn cause sometime toxicity within cellular compartments(1). Amongst the popular flavonoid widely used, curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene- 3,5-dione), isolated from the spice turmeric *curcuma longa*, is described to have numerous beneficial properties, including antioxidant, anti-inflammatory and anti-cancer ones (2). However, curcumin in its initial form suffers from low solubility and very weak bioavailability thus reducing its therapeutic relevance. To improve both solubility and bioavailability of curcumin, various new compounds are in the pipeline and based on nanoparticles, liposomes, metal complexed and encapsulated cyclodextrin. Recently, our group has prepared and patented a new water soluble curcumin complex named NDS27** (3).

Herein we investigated for the first time, using EPR spectroscopy, the ability of encapsulated curcumin (NDS27) to produce the curcumyl (phenoxyl) radical when exposed to the peroxidase (HRP)-$H_2O_2$ system in the presence or absence of the spin trap agent DMPO (5,5-dimethyl-1-pyrroline-N-oxide). The reaction of the radical cation intermediate ($P^{\bullet+}$-FeIV=O) and other byproducts of peroxidase activity with NDS27 were also monitored by optical absorption and luminescence technique. Finally, the potential inhibitory effect of NDS27 against reactive oxygen species (ROS) produced by PMA-activated monocytes (HL-60 cells) was monitored by chemiluminescence technique in the presence of L-012 as probe. On the figures below are represented on left (EPR spectra) and on right (docking of HRP and Curcumin).




**NDS27 : Curcumin lysinate salt + hydropropyl-β-cyclodextrin

1. O'Brien, PJ. Radical formation during the peroxidase catalyzed metabolism of carcinogens and xenobiotics; the reactivity of these radicals with GSH, DNA, an unsaturated lipid. *Free Radic Biol Med*, 4, 169-183 (1988).
2. Aggarwal BB, Sung B. Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. *Trends Pharmacol Sci*, 30, 85–94 (2009).

Neven P, Serteyn D, Delarge J, Kiss R, Mathieu V, Cataldo D, Rocks N. Water soluble curcumin compositions for use in anti-cancer and anti-inflammatory therapy. Patent N° WO2009144220A1. December 03, 2009.

→ porphyrin radical only in wt

2.0098
2.0046
2.

Porphyrin

# New insights into the B-class dye-decolorizing peroxidases: An EPR study of the heme and the radical sites in a DyP from *K. pneumoniae*

K. Nys[1], V. Pfanzagl[2], G. Mlynek[3], H. Vrielinck[4], P. Gast[5], K. Djinović-Carugo[3], C. Obinger[2] & S. Van Doorslaer[1]

[1] *Department of Physics, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium*

[2] *Division of Biochemistry, BOKU – University of Natural Resources and Life Sciences, Muthgasse 18, 1190 Vienna, Austria*

[3] *Max F. Perutz Laboratories, University of Vienna, Dr. Bohr-Gasse 9, 1030 Vienna, Austria*

[4] *Department of Solid State Sciences, Ghent University, Krijgslaan 281/S1, 9000 Ghent, Belgium*

[5] *Department of Physics, Leiden University, Niels Bohrweg 2, 2333 Leiden, the Netherlands*

Dye-decolorizing peroxidases (DyPs) are heme *b*-containing proteins that occur in the genomes of several fungi and bacteria. DyPs can be divided into three distinct classes (A, B, C/D) and are hailed for their biotechnological potential, including the degradation of textile dyes and lignin. However, reported peroxidase activity with conventional substrates is often low and the reaction mechanism remains confuse [1].

Here we have studied *Kp*DyP, a class B enzyme from the bacterium *Klebsiella pneumoniae*, which has been expressed recombinantly in *E. coli*. Kinetic studies showed that, upon activation of the protein with equimolar $H_2O_2$, an extremely stable Compound I (an oxoiron(IV) porphyrin π-cation radical) is formed. Subsequent addition of specific substrates led to the first observation of an oxoiron(IV)-type Compound II in a B-class DyP [2]. This challenged the current hypothesis on the reduction steps [3].

Electron paramagnetic resonance (EPR) is highly suited to investigate the different intermediates during enzyme turnover. The resting state protein features distinct high-spin ($S$ = 5/2) Fe(III) signals with varying rhombicity upon site-directed mutation of the catalytically important residues Arg232 and Asp143. Surprisingly, an organic radical is also observed in the resting state of the wild-type enzyme (Fig. 1). Multi-frequency CW and pulsed EPR reveals the presence of at least one tyrosyl radical. An amino acid radical has, to our knowledge, never been reported in the resting state of DyPs.

Addition of a stoichiometric excess of $H_2O_2$ provides a Compound I spectrum similar to what was observed for HRP, demonstrating the presence of a porphyrin π-cation radical [4].

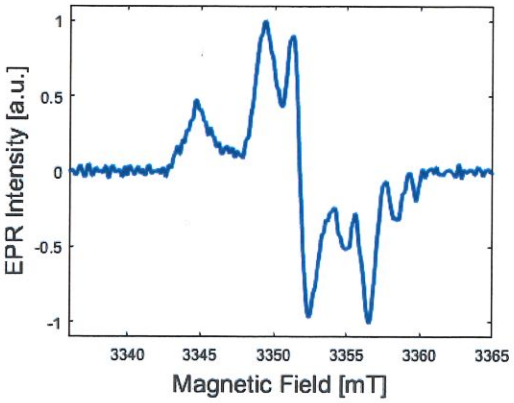



*Fig. 1: W-band 1st derivative ESE-detected EPR of ferric wt KpDyP at 80 K.*

1. S. Hofbauer, I. Schaffner, P.G. Furtmüller and C. Obinger, *Biotechnol. J.*, **2014**, 9, 461-473
2. V. Pfanzagl, K. Nys, M. Bellei, H. Michlits, G. Mlynek, G. Battistuzzi, K. Djinovic-Carugo, S. Van Doorslaer, P.G. Furtmüller, S. Hofbauer and C. Obinger, *manuscript submitted*
3. R. Schrestha, G. Huang, D. Meekins, B.V. Geisbrecht and P. Li, *ACS Catalysis*, **2017**, 7, 6352-6364
4. C.E. Schulz, P.W. Devaney, H. Winkler, P.G. Debrunner, N. Doan, R. Chiang, R. Rutter and L.P. Hager, *FEBS Letters*, **1979**, 103, 102-105

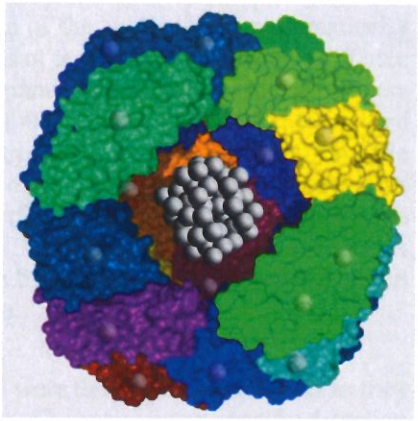
## EPR signals of Ferritin, an Iron Storing Protein – More Questions than Answers

Dipro Mondal[1], Lucia Bossoni[1,2] Martina Huber[1]

[1] *Department of Physics, Huygens-Kamerlingh-Onnes Laboratory, Leiden University, PO Box 9504, 2300 RA Leiden, The Netherlands*

[2] *Leiden University Medical Center, Leiden, The Netherlands*

Ferritin, an iron storing protein in humans and animals, consists of the core, a mineral form of iron (grey in the centre of the figure), and a shell made up of two or three different proteins, dependent on the species, which assemble into a multimeric shell that encloses the core (coloured in the figure). Biochemistry and physiology have shown that the shell not just the encasing, but also is active in oxidizing and transporting iron ions into the core and to assemble the iron to the proper mineral form, for a recent review, see [1]. Here we take a closer look at the EPR properties of the core. Interest in the subject arose recently, because of the suspected iron imbalance in the brains of Alzheimer's disease patients.



The EPR properties of the core of ferritin derive from its superparamagnetic behaviour. Signals centred at $g = 2$ and spanning several hundreds of Gauss are observed above the blocking temperature of the iron core, which in some ways resembles an iron-nanoparticle, as it has a diameter of around 8 nm[2]. Such signals were described by theory and experiment[3][4]. We will discuss the challenges involved with working with these kinds of signals.

[1] W.R. Hagen, P.-L. Hagedoorn, K. Honarmand Ebrahimi, The workings of ferritin: a crossroad of opinions, Metallomics. 9 (2017) 595–605. doi:10.1039/C7MT00124J.

[2] T.J. Smith, S.D. Erickson, C.M. Orozco, A. Fluckiger, L.M. Moses, J.S. Colton, et al., Tuning the band gap of ferritin nanoparticles by co-depositing iron with halides or oxo-anions, J. Mater. Chem. A. 2 (2014) 20782–20788. doi:10.1039/C4TA04588B.

[3] M. Fittipaldi, R. Mercatelli, S. Sottini, P. Ceci, E. Falvo, D. Gatteschi, Sensing the quantum behaviour of magnetic nanoparticles by electron magnetic resonance, Phys. Chem. Chem. Phys. 18 (2016) 3591–3597. doi:10.1039/C5CP07018J.

[4] M.P. Weir, T.J. Peters, J.F. Gibson, Electron spin resonance studies of splenic ferritin and haemosiderin, Biochim. Biophys. Acta (BBA)/Protein Struct. Mol. 828 (1985) 298–305. doi:10.1016/0167-4838(85)90311-5.

## Towards improved detection of mitochondrial superoxide using EPR

Samantha Scheinok[1], Philippe Leveque[1], Pierre Sonveaux[2], Benoit Driesschaert[1], Raphaël Robiette[3] and Bernard Gallez[1]

[1]*Biomedical Magnetic Resonance Research Group, Louvain Drug Research Institute, UCL Brussels*

[2]*Pole of Pharmacology (FATH), Institut de Recherche Expérimentale et Clinique (IREC), UCL, Brussels*

Superoxide is a reactive oxygenated species that is mainly produced by the electron leak from the mitochondrial transport chain (complex I and III) and NADPH oxidases family (NOXs). Recently, mitochondrial superoxide has gained an increasing interest because several studies suggested its involvement in several pathologies such as Parkinson's disease, hypertension and cancer metastasis. Mitochondrial superoxide is difficult to detect due to its intracellular localization and its short lifetime. In order to assess the modulation of mitochondrial superoxide level, there is a crucial need for qualifying new approaches allowing its sensitive and specific detection. The first aim of the study was to systematically compare the existing EPR approaches for detecting superoxide in terms of sensitivity and specificity. Three main classes of EPR probes were used in the comparison, including paramagnetic superoxide scavengers (such as nitroxides TEMPOL and mitoTEMPO as well as trityl CT-03), a spin trap (DIPPMPO), and diamagnetic superoxide scavengers (such as cyclic hydroxylamines CMH and mitoTEMPO-H). We analyzed the reactivity of the different probes in the presence of a constant production of superoxide or hydroxyl radical in buffers and in cell lysates. We also assessed the performances of the different probes to detect superoxide produced by macrophages stimulated by phorbol 12-myristate 13-acetate (PMA). A second aim was to synthesize new probes targeting the mitochondria by linking a triphenylphosphonium moiety to CPH and to assess their performance in detecting superoxide.

### Results

Our comparison study demonstrated that DIPPMPO and CMH were the two best candidates as they both showed a high stability in complex media (lysates) and both allowed a sensitive and specific detection of superoxide in the PMA-stimulated macrophages. We then synthetized new mitochondria-targeted hydroxylamines: mitoCPH ether and mitoCPH ester **(Fig.1)**. We compared the new probes to the existing hydroxylamines CMH and MitoTEMPO-H. mitoCPH ester provided a higher sensitivity than mitoTEMPO-H in PBS and lysates to detect superoxide. However, mitoCPH ether link was much less sensitive compared to mitoCPH ester **(Fig.2)**. Appropriate controls should be used to non-unambiguously demonstrate the role of superoxide in the induced EPR signal.

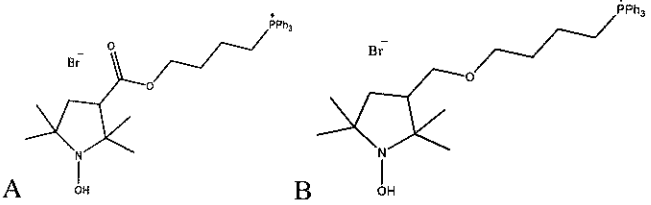



**Fig1:** A. mitoCPH ester B. mitoCPH ether

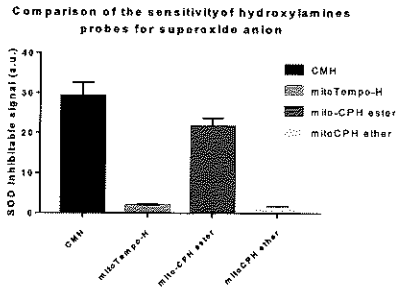



**Fig2**

### Perspectives

We are now testing mitoCPH-ester on wild-type (WT) SiHa cells and metastatic SiHa cells (supposed to produce more mitochondrial superoxide than the WT) to confirm the implication of mitochondrial superoxide in cancer metastasis.

1.6K – RT

**Thermal stability and temperature dependent ESR characteristics of the As acceptor in geological 2H-$MoS_2$**

B. Schoenaers, A. Stesmans, and V. V. Afanas'ev
*Department of Physics and Astronomy, University of Leuven*
*3001 Leuven, Belgium*

Electron spin resonance (ESR) results are presented on the thermal stability and temperature ($T$) dependence of the spectral characteristics of the As acceptor dopant (As substituting for S site) in geological 2H-$MoS_2$. Under sequential isochronal heating in $H_2$ (1.1 atm), the As dopant density is found to remain unaffected for anneal temperatures ($T_{an}$s) up to 525 °C, above which the density moderately decreases (~3 times) for $T_{an} \rightarrow$ 840 °C. In turn, vacuum annealing is seen to result in a gradual increase (2 – 3 times) of the As acceptor density for $T_{an}$ increasing from 400 °C → 840 °C, pointing to a positive 'regain' of misconfigured As impurities in pristine geo-$MoS_2$.Finally, meticulous monitoring of the ESR signal intensity vs.$T$ at X-band confirms the previously inferred As acceptor activation energy of 0.7 ± 0.2 meV at K-band. In light of these findings, As is exposed as a promising candidate for stable p-type doping of $MoS_2$.

## POSTER ABSTRACTS

## P1: Towards melanin detection in melanomas by EPR in patients: a feasibility approach

C. Desmet, P. Danhier, and B. Gallez

*Biomedical Magnetic Resonance Research group, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium*

Melanoma is a skin cancer characterized by an uncontrolled proliferation of melanocytes. Until now, melanoma diagnosis is based on visual examination, dermatoscopy and biopsy. A potentially interesting technique in melanoma diagnosis is EPR [1]. Indeed, melanin produced by melanocytes is a pigment with paramagnetic properties which is measurable by EPR. A previous study showed that EPR spectrometry is applicable to detect melanin in melanomas ex vivo [2]. Also, a correlation between the signal amplitude and the tumor stage was observed [2]. In addition, EPR imaging was successfully used to detect and map melanin in melanomas ex vivo [3, 4]. Moreover, a proof-of-concept study showed the feasibility to record an EPR image from melanoma in vivo [5].

The aim of the present work was to assess the feasibility to detect melanin in vivo using an L-band EPR spectrometer dedicated to clinical measurements.

First, the limit of detection of melanin on the clinical EPR spectrometer was determined. Then, in vivo measurements of the EPR signal of melanin were performed repeatedly in mice inoculated with B16 tumors (pigmented melanomas) or WM2664 tumors (non-pigmented melanomas). To assess the applicability of the technique for measurements in humans, we used a phantom containing melanin placed at several positions of the human body and the reproducibility of the measurements was assessed.

The limit of detection observed on the clinical spectrometer Clin-EPR was 0.08 mg of melanin. An EPR signal was observed in vivo in B16 melanomas. The amplitude of this signal was increasing with the tumor size. On the contrary, no signal was observed in WM2664 melanomas that do not contain melanin. Finally, reproducible measurements of the EPR signal were recorded from the phantom containing melanin placed at several positions of the body of a volunteer.

In conclusion, we could evidence EPR signal of melanin in vivo with our clinical spectrometer. The amplitude of the EPR signal was correlated with the melanoma size, and the technique was applicable in a volunteer with a good reproducibility. These results are promising for the application of the technique for the detection and classification of melanomas in humans.

1. Godechal, Q. et al. *J Skin Cancer* 2011, 273280.
2. Godechal, Q. et al. *Contrast Media Mol Imaging* 2011, 6, 282-288.
3. Godechal, Q. et al. *Exp Dermatol* 2012, 21, 341-346.
4. Godechal, Q. et al. *Mol Imaging* 2013, 12, 218-23.
5. Vanea, E. et al. *NMR Biomed* 2008, 21, 296-300.

## P2: Spectroscopic analysis of myoglobin and neuroglobin immobilized on mesoporous materials

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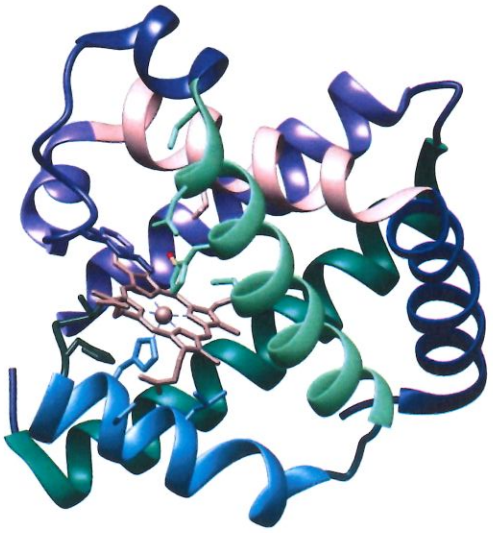
The incorporation of globins in mesoporeus materials is an important and promising direction for biosensing and biocatalysis. The immobilization cannot only stabilize the heme proteins but also improve the activity in the appropriate environment. In particular, the properties of these immobilization matrices should avoid loss of the protein activity and leaching or degeneration of the proteins. The electrochemical activity is enclosed in the heme group of the globin, where the change of the oxidation state of the heme iron can be detected in biosensing applications. These type of biosensors can be used for the detection of small molecules like H2O2, H2S and NO2-. Two different globins will be incorporated, namely horse heart myoglobin (hhMb) and human neuroglobin (NGB). Mb, one of the best-known proteins, is an oxygen-storage protein found in muscle cells with dimensions about 45 x 35 x 25 Å. In contrast, the function of NGB is still uncertain. NGB is predominantly expressed in the nervous system and the structure of NGB displays the typical globin fold as Mb. NGB is extremely stable under pH and temperature conditions known to denature other globins and, under in vitro conditions, it is redox active. For the encapsulation of Mb and NGB, SBA-15-type silica and titanium dioxide are used. These mesoporous materials show high biocompatibility and good retention of the protein activity. Besides the large surface area and pore volume, the size and the morphology of the mesopores are easily fine-tuned. Electron paramagnetic resonance (EPR) allows to study the ferric heme center and thus check the stability and state of the globin and its heme group before and after immobilization. Surprising differences in the behavior of the two proteins upon incorporation become apparent via this technique.



## P3: Investigation of Plasma-Induced Chemistry in Organic Solutions by EPR

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Plasma technology has been shown that it can induce physical and chemical modifications in polymeric electrospinning solutions [1–5]. In our recent work [3], polylactic acid (PLA) organic solutions were modified by using an atmospheric-pressure plasma jet and plasma-induced chemistry in organic solutions of PLA, and their effects on the resultant PLA nanofibers were analyzed in detail by using a broad range of analyzing techniques like EPR, NMR, OES, EEM, UV-vis, SEM, XPS, etc.

To prepare PLA electrospinning solutions, PLA was dissolved in a binary mixture of chloroform (CHL) and *N*,*N*-dimethylformamide (DMF) with a volume ratio of 8:2. It was therefore important to investigate the plasma-induced effects on CHL, DMF, CHL/DMF, and PLA solutions, separately. The detection of the radical species in the plasma-treated solutions was performed by EPR. Two different spin traps, 5-Dimethyl-1-pyrroline *N*-oxide (DMPO) and *N*-tertbutyl-α-phenylnitrone (PBN), were used to produce more stable and thus detectable radical adducts via reactions with the otherwise too short-living free radical species. It is noteworthy to mention that the untreated solutions containing the spin traps only showed EPR peaks with very low intensities which do not exceed the noise level of the EPR spectrometer. The assignment of the most prominent radical adducts (both nature and percentage) was based on the best simulation fitting. Most important radical adducts, which have been found in the solutions, are $CHCl_2^{\bullet}$, $CH_3^{\bullet}$, $CHO^{\bullet}$, $CCl_3^{\bullet}$, $CON(CH_3)_2^{\bullet}$.

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## P4: Elucidation of the mechanism of the intramolecular cyclisation of allyl 2-bromobenzyl ether by *in-situ* EPR spectroelectrochemistry and DFT calculations

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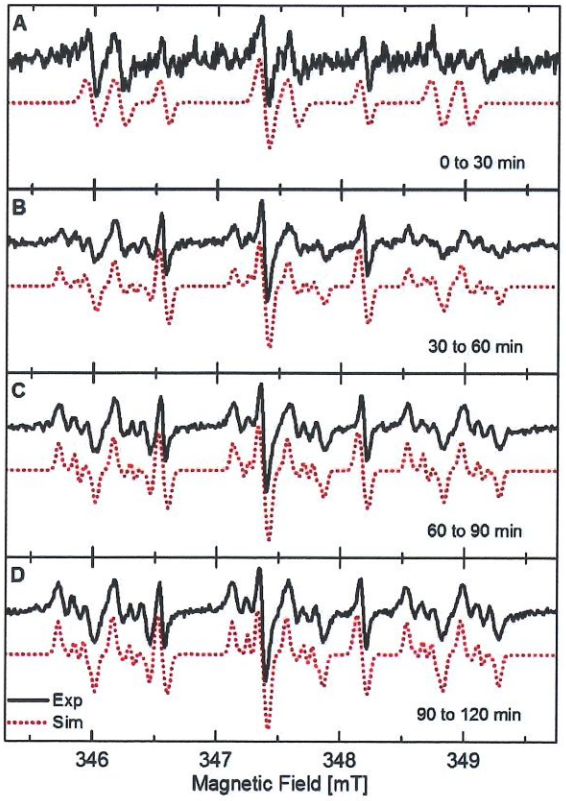


*Figure 2. Accumulated experimental (black) and simulated (red) CW EPR spectra of the radicals spin-trapped during allyl 2-bromobenzyl ether cyclization reaction in different time steps*

Heterocyclic compounds are vital in the pharmaceuticals, pesticides, dyes and synthetic applications. Organic electrosynthesis offers a clean, catalytic, inexpensive and environmentally friendly method for the synthesis of these compounds [1]. A profound understanding of the reaction mechanism and the influence of the electrocatalyst allows optimal tuning of the electrocatalytic process. This can be achieved by combining electrochemical techniques with electron paramagnetic resonance (EPR) spectroscopy.

In this work, EPR was used to study the electrochemical cyclisation of allyl 2-bromobenzyl ether in a home-built inexpensive electrochemical cell adapted to *in-situ* EPR. The organic radicals that were produced *in-situ* during the electrochemical process were spin-trapped by phenyl-tert-butyl-nitrone (PBN), and EPR spectra were recorded at different time steps (Figure 1). The EPR parameters of the different spin-trapped radical adducts were determined via spectral simulations using EasySpin. Possible adduct structures, possible conformations and corresponding EPR parameters were calculated using DFT computations. A comparison of these results and their implications on the cyclisation mechanism will be discussed.

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## P5: Probing local surface interactions in organophosphonic modified mesoporous titania

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Inorganic materials ($TiO_2$, $SiO_2$) possess advantages like mechanical, thermal and chemical stability but generally lack variation in their surface functionality. An elegant way to combine all those properties in a single material is to graft organic functional groups onto the surface. They have many potential applications e.g. in catalysis, separation, as adsorbents or sensors. Although organosillilation is the best known method to graft $SiO_2$, it is less suitable for $TiO_2$ because Ti-O-Si-R bonds are prone to hydrolysis. Moreover, organosilanes easily polymerize, leading to pore blocking or multilayer formation. Alternatively, grafting of $TiO_2$ with organopphosphonic acids generates more stable Ti-O-P-R bonds. Moreover, depending on the synthesis conditions applied, the surface properties can be tuned to a large extent, even though the same type of organic functional group is applied. The research presented, aims at gaining insights into the interaction and mobility of molecules with the surface. The main focus is to correlate synthesis conditions to the resulting surface properties and its impact on the specific interactions of the surface with probe molecules. This detailed knowledge allows to design improved tuned surface properties having enhanced performance of modified products in many potential applications. To reach this goal, local information on the interactions occurring at the surface are required. Therefore, in-situ EPR analysis with spin-probes is combined with in-situ spectroscopy and information of synthetic control, to correlate the surface modification properties (type and amount of surface groups, packing density and homogeneity of these groups and the bonding modes of phosphonic acid) to the resulting specific interaction with probe molecules. Similarly, the surface grafting mechanisms itself can also be explained by spin-probe mobility and interaction knowledge during surface modification.

## P6: Photodegradation processes in fullerene-free organic solar cells investigated by Electron Paramagnetic Resonance

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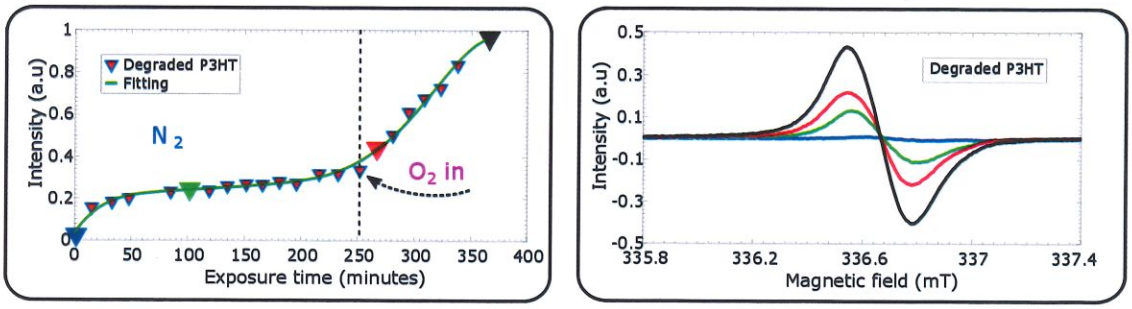
The constantly growing energy demand and disastrous damage to the environment forces humanity to find a renewable energy source which can be used safely and responsibly. Organic Solar Cells (OSC) can be a suitable technology provided that high efficiencies combined with low fabrication cost and *long lifetimes are in place*. Today, single junction OSCs based on non-fullerene acceptors reach PCE over 13% [1], while efficient roll-to-roll processes for OSCs offering high throughput and low labor costs are already up and running [2].

However, the technology is not yet dominant on the market as the long-term stability under real-life operating conditions remains the main obstacle. The photoactive layer of OSC consist of a nanoscale interpenetrating network of acceptor and donor materials. This layer deteriorates due to several (un)known external factors: oxygen, moisture, light irradiation, mechanical stress and heating [3].

In this work we use multi-frequency (X- and W-band) electron paramagnetic resonance (EPR) as a sensitive and **non-destructive** method to monitor photochemical degradation of a P3HT donor polymer in combination with FBR acceptor molecules under different gas contents. First, the light-induced EPR signal of the positive and negative polarons were characterized in freshly made blends. Next, signals were compared to the EPR spectra observed upon degradation of the pristine materials. While no radical signal could be observed for degraded FBR, clear contributions were found for pristine degraded P3HT.




We found that degradation of the pristine P3HT involves a radical pathway and becomes significant only by combination of light and oxygen. This EPR signal was resolved in two components as previously assigned to positive polaron on $P3HT^{+}$ and negative polaron on $[P3HT{:}O_2]^{-}$ charge transfer (CT) complex. These features were also observed in degraded P3HT:FBR blends. Light illumination at 20K still revealed CT and associated creation of polarons, necessary for the solar cell application.

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## P7: Nitrite reductase activity of GLB-33, a unique chimeric globin in *Caenorhabditis elegans examined with EPR and UV-vis spectroscopy*

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Globins are versatile proteins widespread throughout life which have many biologically relevant functions aside from oxygen binding and transport, such as the involvement in redox signaling, NO metabolism and reproduction. Out of the 34 glb genes which are all expressed in the nematode *C. elegans* [1], our interest goes to the glb-33 gene which is the largest of its kind. It is unique within the globin family because it consists of a globin domain connected to a G-protein coupled receptor domain (GPCR). Here we show the globin domain (GLB-33GD) of the full-length glb-33 gene that was expressed in the bacterium *E. coli,* purified and examined using optical (UV-vis absorption spectroscopy), CW and pulsed EPR methods. Spectrophotometric analysis of GLB-33GD shows a tenfold higher nitrite reductase activity compared with other globins [2]. This may be due to the highly hydrophobic environment of the heme pocket, the high reducing potential of the heme or the easy accessibility of the heme iron by the $NO_2^-$ anion. Especially the Arg E10 residue is thought to play a stabilizing role in OH ligation, but its role in $NO_2^-$ stabilization has yet to be resolved. An integrative toolbox of optical and EPR methods is necessary to fully characterize the heme pocket of this interesting globin [3]. In a first approach, the nitrite and NO ligation will be discussed and site-directed mutagensis of heme-surrounding amino acids allows to dissect the molecular mechanism behind the fast nitrite reductase activity.

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**P8: Rigid Spin Labels for Improved Distance and Dynamics in Intrinsically Disordered Proteins and Peptides**

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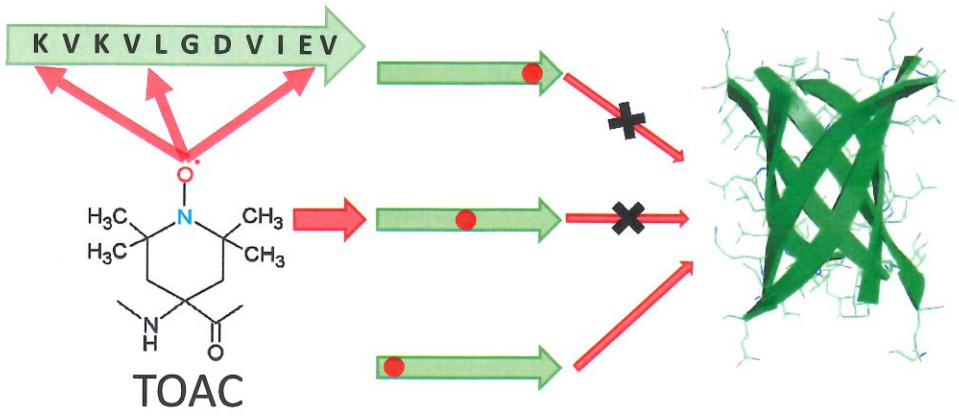
Amyloid biomacromolecules are intrinsically disordered proteins associated with many neurodegenerative diseases such Alzheimer's and Parkinson's diseases. The process by which they influence the brain is still largely unknown and requires deeper studies.

Spin-labeling and Electron Paramagnetic Resonance spectroscopy (EPR) have become powerful tools for structure determination in proteins and biomacromolecules. We investigate a series of peptides, derived from the KVKVLGDVIEV peptide[1], with a spin label rigidly linked to the backbone: the 4-amino-1-oxyl-2,2,6,6-tetramethylpiperidine-4-carboxylic acid (TOAC). These peptides mimic amyloid-aggregation proteins. They were found to form stable oligomers that are cell-toxic, and enabled X-ray crystallography to determine their structures[1].

We synthesized and characterized three TOAC-peptides and one reference peptide (EZ). We show that the aggregation reaction can be studied in detail by EPR combined with Circular Dichroism (CD). We propose that for rigid spin labels such as TOAC, the spin label position and the peptide host are crucial and that the TOAC label may interfere with peptide properties. The results are essential to design spin-labeled constructs for the investigation of intrinsically disordered proteins.

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