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19th edition of YBMRS

13th and 14th of November 2023

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BOOK OF ABSTRACTS

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SCIENTIFIC PROGRAM

Monday, 13th of November

9:00 *Registration, welcome coffee*

10:00 *Opening*

10:10 Tribute to Jean Jeener (**M. Luhmer**)

10:20 ***Educational Talk 1:*** Quantitative NMR spectroscopy of complex mixtures - Prof. **Patrick Giraudeau** (Université de Nantes)

11:10 ***Educational Talk 2:*** Fast Field Cycling MR imaging - Prof. **Lionel Broche** (Aberdeen University)

12:00 Lunch break

Oral Session 1: NMR spectroscopy

13:00 **Lennert Cools** (UHasselt) - Exploring the interactions and microstructure of amorphous solid dispersions containing structural analogues of diflunisal using 1D and 2D solid-state NMR

13:20 **Sofie Schellinck** (UGent) - Unlocking the Secrets of Aptamer-Small Molecule Complexes through NMR Spectroscopy

13:40 **Manon Campas** (ULiège) - Establishment of a NMR-based metabolomics protocol for salivary samples

14:00 **Romain Carpentier** (ULB) - Development and characterization of calixarene-based macrocyclic systems for the recognition of primary ammoniums in an aqueous environment

14:20 ***Poster Session 1***, odd numbers - coffee break

16:00 ***Plenary Lecture 1:*** Hyperpolarized NMR metabolomics - Prof **Patrick Giraudeau** (Université de Nantes)

Oral Session 2: EPR

16:50 **Lore Van den Bergh** (UAntwerpen) - The tricky story of black titania – A spectroscopic study on the reduction and reoxidation of titania

17:10 **Chloé Buyse** (UCLouvain) - Characterization of the impact of an inhibitor of the mitochondrial pyruvate carrier (MPC) using in vivo EPR and CEST-MRI

17:30 **Andrea Guidetti** (UAntwerpen) - Development of a combined methodology towards the investigation of sustainable light-activated catalysts

17:50 *End of the scientific presentations*

18:00 *Discussion about the future of YBMRS + meeting of the "Groupe de contact FNRS"*

19:30 *Reception*

20:30 *Dinner and party*

SCIENTIFIC PROGRAM

Tuesday, 14th of November

9:00 ***Plenary Lecture 2:*** The mechanisms of lipid-targeting antibiotics - Prof **Marcus Weingarth** (Utrecht University)

Oral Session 3: Imaging and low-field NMR

9:50 **Thomas Gevart** (UMons)- In vitro and in vivo study of iron oxide nanoparticles designed for theranostic targeting EGFR-overexpressing tumors

10:10 **R. de Oliveira-Silva** (KULeuven) - A low-field benchtop MRI system for general applications

10:30 **Marie Bernardi** (UMons) - Benchtop NMR Relaxometry for monitoring Cu^{2+} removal using ion exchange resins and commercial activated charcoal

10:50 **Ziyou Yu** (KULeuven) - The application of low field NMR relaxometry in iron-rich materials

11:10 ***Poster Session 2***, even numbers, coffee break

12:50 *Lunch break*

13:50 ***Plenary Lecture 3:*** Medical imaging with fast field-cycling: how and why? - Prof **Lionel Broche** (University of Aberdeen)

Oral Session 4: Spectroscopy

14:40 **Maxime Kolkman** (ULiège) - Warburg-associated acidification represses lactic fermentation independently of lactate, contribution from Real-Time NMR on cell-free systems

15:00 **Maik Derks** (Utrecht University) - Plectasin kills bacteria by a Ca^{2+}-sensitive supramolecular mechanism

15:20 **Rosan de Winter** (Utrecht University) - Revisiting the mode of action of teixobactin

15:40 *Coffee break, poster removal*

16:20 *Closure, awards of the best presentations and posters*

ABSTRACTS - TUTORIALS AND PLENARY LECTURES

Quantitative NMR spectroscopy of complex mixtures

Patrick Giraudeau

Nantes Université, CNRS, CEISAM UMR 6230, 44000 Nantes, France

Complex mixtures are ubiquitous and include pharmaceutical samples, biological fluids or extracts, and also samples undergoing chemical reactions. NMR is ideally suited to address the challenge of quantifying mixture components with a broad diversity of chemical structures and concentrations. 1D ^{1}H spectroscopy, the most widespread approach, is a simple and rapid approach for quantitative analysis. It can yield very accurate results provided that quantitative NMR (qNMR) experimental conditions are implemented (sufficient signal-to-noise, long relaxation delay, internal or external reference signal, etc.). However, the accuracy of quantitative 1D NMR strongly limited by the numerous peak overlaps that prevent the accurate quantification of mixture components. This limitation can be overcome by 2D NMR, albeit at the cost of a longer experiment time and of several specificities associated with the use of multi-pulse sequences.

In the last few years, an ensemble of fast and quantitative 2D NMR approaches have been developed for the accurate analysis of complex mixtures. Most of these methods combine accelerated 2D pulse sequences such as ultrafast NMR, spectral aliasing or non-uniform sampling, with analytical chemistry methods such as external calibrations or standard additions. They provide efficient solutions for the accurate quantification of targeted compounds in mixtures, but also for the untargeted analysis of large sample collections. Alternative methods rely on “intrinsically quantitative” 2D NMR heteronuclear methods that provide quantitative results relying on a single internal standard, similarly to 1D NMR and without the need for a calibration procedure.

In this lecture, we will describe the basic principles of quantitative NMR in one and two dimensions, then we will illustrate the principle of these different quantitative 2D NMR approaches and discuss their practical advantages and limitations for a variety of practical situations where complex mixtures are involved.

1. Giraudeau, P., Chem. Commun., 2023, 59, 6627-6642.

Fast Field Cycling MR imaging

L. Broche

Aberdeen Biomedical Imaging Centre, School of Medicine Medical Sciences and Nutrition, University of Aberdeen, United Kingdom

The acquisition of MRI images requires numerous adjustments to ensure image quality. This tutorial explains basic methods for calibrating fundamental device parameters, such as field homogeneity, gradient linearity, temporal precision of RF and gradient pulses, or RF signal distortion, and demonstrates typical artifacts associated with setpoint drifts and how to address them.

Hyperpolarized NMR metabolomics

Patrick Giraudeau

Nantes Université, CNRS, CEISAM UMR 6230, 44000 Nantes, France

NMR spectroscopy is a central method for metabolomics. However, it mostly relies on 1D ^{1}H spectroscopy, which provides an acceptable sensitivity but suffers from ubiquitous overlap between complex metabolite patterns. NMR offers a wide range of multi-nuclear and multi-dimensional techniques for analyzing complex samples, which have the potential to profoundly change the way metabolomics studies are conducted.[1] In the past few years, we showed how fast 2D NMR methods could be systematically incorporated into metabolomics workflows, providing improved sample classification and/or biomarker identification.[2] These methods –mainly homonuclear 2D experiments for sensitivity reasons– provide a first stage of dispersion improvement. ^{13}C NMR spectroscopy would be even more advantageous as it provides narrow singlets spread over a broad spectral range. In fact, ^{13}C NMR would be ideal for metabolomics, were it not for the fact that its low sensitivity is not compatible with the detection of low-concentrated analytes at natural abundance.

In this context, we recently showed that Dissolution Dynamic Nuclear Polarization (d-DNP) could provide a unique way to detect ^{13}C NMR metabolomics spectral signatures with a sensitivity enhanced by several orders of magnitude.[3] After reaching excellent repeatability with a prototype d-DNP equipment, we showed that ^{13}C NMR at natural abundance could be applied to plant extracts and incorporated in a full metabolomics workflow.[4] We then systematically optimized the parameters involved in our d-DNP setting, leading to major sensitivity and resolution improvements.[5] Thanks to this optimization, we recently reported the first d-DNP-enhanced ^{13}C NMR analysis of a biofluid -urine- at natural abundance, offering unprecedented resolution and sensitivity for this challenging type of sample.[6] We also showed that accurate quantitative information on multiple targeted metabolites could be retrieved through a standard addition procedure.

These results open many perspectives for ^{13}C NMR-based metabolomics at natural abundance, but also raise a number of analytical challenges in terms of metabolite identification, cost and throughput. We will discuss the potential of this new approach, as well as undergoing methodological developments based on multiple receivers[7] or ultrafast 2D NMR[8] that could further improve its performance.

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6. Ribay, V., Dey, A., Charrier, B., Praud, C., Mandral, J., Dumez, J.-N., Letertre, M. P. M., Giraudeau, P., Angew. Chem. Int. Ed., 2023, 62, e202302110.
7. Dey, A., Charrier, B., Ribay, V., Dumez, J.-N., Giraudeau, P., *under revision*
8. Praud, C., Ribay, V., Charrier, B., Mandral, J., Farjon, J., Dumez, J.-N., Giraudeau, P., *under revision*

The Mechanisms of Lipid-targeting Antibiotics

Markus Weingarth, Utrecht/NL

Utrecht University, Department of Chemistry, Utrecht, the Netherlands

Antimicrobial resistance is a global health threat, calling for new antibiotics. Good candidates could be compounds that target special lipids that only exist in bacterial, but not in human cell membranes. These drugs kill pathogens without detectable resistance, which has generated considerable interest.

Using ssNMR and microscopy, our group has introduced approaches to study lipid-targeting antibiotics across different length-scales in membranes[1]. Recently, we determined the killing mechanism of teixobactin[2,3], considered the first new antibiotic in 30 years. We showed that teixobactin kills bacteria by forming supramolecular fibrils that compromises the bacterial membrane. In addition, we show the molecular mechanism of Clovibactin, a new antibiotic from 'unculturable' bacteria[4].

References:

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Medical imaging with fast field-cycling: how and why?

L. Broche

Aberdeen Biomedical Imaging Centre, School of Medicine Medical Sciences and Nutrition, University of Aberdeen, United Kingdom

It is well known that the magnetic relaxation rate T_1 observed in MRI systems varies with the strength of the magnetic field. The causes for this effect are varied and the study of T_1 variations, also termed T_1 relaxometry, offers a rich and unique source of information that has been exploited almost as soon as NMR spectroscopy was established.

T_1 relaxometry is closely related to the dynamics of molecules and can probe timescales from milli- to nanoseconds, non-invasively. This proves useful in a variety of applications, from the design of contrast agents to the formulation of concrete or the detection of fraud in foodstuff. It also has much to offer in the context of medicine.

Our research unit is exploring the development of an MRI system that can perform T_1 relaxometry mapping in-vivo, to probe molecular dynamics non-invasively. The latest iteration of this system, the Field-Cycling Imaging scanner, is currently being built in the Aberdeen hospital.

This presentation will explain what T_1 relaxometry has to offer to medicine, how relaxometer and FCI scanners operate, and what applications we have found for in-vivo T1 relaxometry to date, particularly in the detection of cancer and stroke. It will provide some insights about the type of molecular dynamics that this technique can detect and how this is relevant in clinics.

ABSTRACTS - ORAL CONTRIBUTIONS

ORAL 1

Exploring the interactions and microstructure of amorphous solid dispersions containing structural analogues of diflunisal using 1D and 2D solid-state NMR

Lennert Cools[a,b], Felien Reniers[c], Elien Derveaux[b], Wim Dehaen[c], Guy Van den Mooter[a], Peter Adriaensens[b]

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[b] *Applied and Analytical Chemistry Department, Institute for Materials Research, UHasselt, Diepenbeek*
[c] *Sustainable Chemistry for Metals and Molecules, Department of Chemistry, KU Leuven*

Drug-polymer intermolecular interactions, and H-bonds specifically, play an important role in the stabilization process of a compound in an amorphous solid dispersion (ASD). However, it is still difficult to predict whether or not interactions will form and what the strength of those interactions would be, based on the structure of drug and polymer. Therefore, in this study, structural analogues of diflunisal (DIF) were synthesized and incorporated in ASDs with poly(vinylpyrrolidone-co-vinyl acetate) (PVPVA) as a stabilizing polymer. The respective DIF derivatives (see Figure 1) contained different types and numbers of H-bond donor groups, which allowed to assess the influence of these structural differences on the phase behavior and the actual interactions formed in the ASDs. ASDs containing a set drug loading of a derivative were manufactured with spray drying. These spray dried ASDs were subjected to an in-depth solid-state nuclear magnetic resonance (ssNMR) study, including 1D spectroscopy, relaxometry, and 2D HETCOR ssNMR techniques. A preliminary drug loading study revealed that it was possible to reach the highest loading, of 50 wt%, in PVPVA with native DIF. The methoxy DIF derivative reached the second highest drug loading of 35 wt%. Methylating the carboxylic acid group of DIF led to a sharp decrease in the maximum loading, to around 10 wt% only. Unexpectedly, the maximum loading increased again when both donor groups were methylated in the dimethyl DIF derivative, up to around 30 wt%. The study of the $T_{1\rho H}$ relaxation times and 2D HETCOR experiments of the spray dried ASD samples indicated that the systems containing native DIF and methoxy DIF formed a homogenous system with PVPVA. The derivatives where the carboxylic acid group was not available, i.e., dimethyl DIF and DIF methyl ester, formed heterogenous ASD systems with PVPVA. Analysis of the 1D ^{13}C-spectra and the 2D ^{1}H-^{13}C HETCOR spectra indicated that the driving force for mixing between the components was most likely the formation of H-bonds.

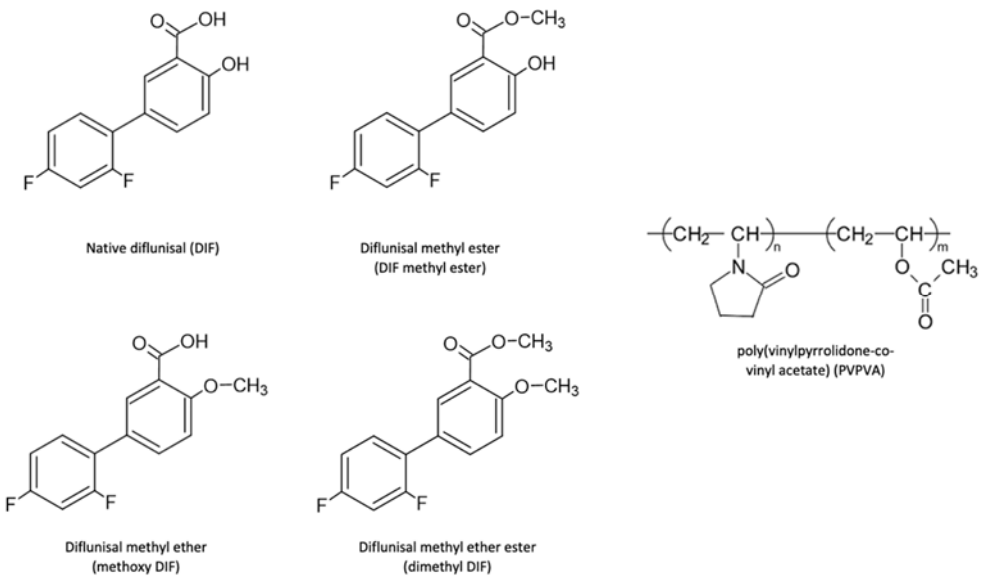

Figure 1: Structural formulas of PVPVA, diflunisal and the synthesized DIF derivatives

Unlocking the Secrets of Aptamer-Small Molecule Complexes through NMR Spectroscopy

S. Schellinck[1], N. Medved[2], D. Buyst[1,3], K. De Wael[4], A. Madder[5], J. Plavec[2], J. C. Martins[1,3]

[1] *NMR and Structure Analysis Research Group, Ghent University, Ghent.*
[2] *Slovenian NMR Centre, National Institute of Chemistry, Ljubljana, Slovenia.*
[3] *NMR Expertise Centre, Ghent University, Ghent.*
[4] *A-Sense Lab, University of Antwerp, Antwerp.*
[5] *Organic and Biomimetic Chemistry Research Group, Ghent University, Ghent.*

Aptamers are more present now than ever in scientific research.
Since their discovery in 1990 [1,2], aptamers have piqued the interest of scientists looking for bioreceptors for small molecules. Numerous aptamers, capable of binding their small molecule target with high affinity and selectivity are described in literature. They can be implemented into biosensors by using a fairly simple and logical design that links a target induced conformational change of the aptamer to a signal generation that can be 'read' electrochemically or by fluorescence. Although a number of these biosensors appear successful, many challenges remain to be addressed for these to break-through in real world applications [3]. One challenge concerns addressing the lack of insight at the molecular level of the factors determining affinity and selectivity, as well as going beyond the simplified, cartoon-like approach in describing the underlying sensing mechanism. We make an effort to contribute towards this goal by developing NMR based strategies, using the structure-switching testosterone binding TESS.1 DNA aptamer [4] as model system.

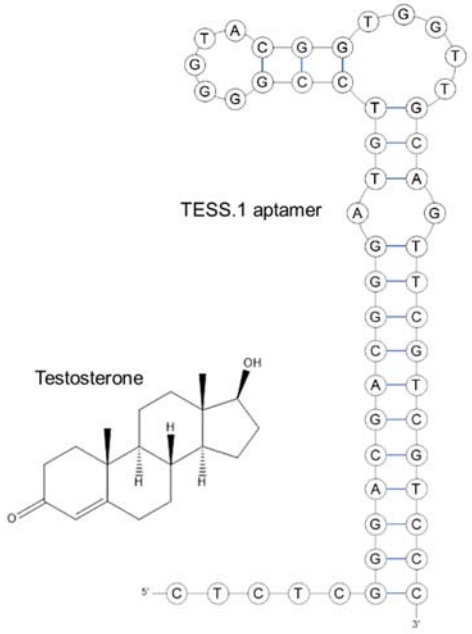

Figure 1: The proposed 2D representation, based on [4], of the TESS.1 aptamer and its target testosterone.

To verify interaction between the TESS.1 aptamer and its said target testosterone, we analysed and identified its corresponding complexation patterns in different regions of the proton NMR spectrum. To allow improved analysis and interpretation of the aptamer-target interaction, a two-step optimisation was performed, generating a more 'NMR optimal' construct, which we labelled TESS.1_s_mod. It interacts with the target in a similar fashion as the originally sized TESS.1 aptamer. A more complete assignment of the system was secured by introducing single nucleotide ^{13}C and ^{15}N uniform labelling of G and T nucleotides in TESS.1_s_mod. Besides a more in-depth analysis of binding events, these assignments will also allow 3D structure determination.
We will present and discuss these developed NMR based strategies and recent results that provide the first detailed molecular view on the TESS.1 aptamer and its interaction with the target testosterone.

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ORAL 3

Establishment of a NMR-based metabolomics protocol for salivary samples

M. Campas [1], C. Germay [1], A. Cirillo[1], M. Schoumacher[1], P. de Tullio[1]

[1]Centre for Interdisciplinary Research on Medicines (CIRM), Clinical Metabolomics Group, University of Liège, Belgium

In contrast to the most used biofluids such as blood and urine, saliva is rarely studied in metabolomics. However, this biofluid seems to be of great interest because of its non-invasive, simple and fast collection. Moreover, it is perfectly suited for self-sampling, well-adapted to a personalized approach of medicine and is expected to be complementary with other biofluids information. This is why we aim to develop and optimize a protocol to analyse salivary samples by NMR.

Therefore, this work focused on the identification of the best suitable protocol allowing to obtain the most pertinent and huge metabolomics information. For this purpose, four methods were selected according to the literature and to in-house processes and were compared. On the basis of several analytical criteria such as the number and the concentration of identified metabolites, the repeatability and the robustness, the best method was selected. Freeze-drying step followed by ultrafiltration led to the most informative and repeatable protocol. Finally, this optimized workflow was applied to a real-case study (fasting *vs* non-fasting volunteers) in order to prove the pertinence of the method and of the saliva's analysis in metabolomics. This study showed significant differences between the groups and discriminant metabolites were identified.

In conclusion, results obtained in this work are encouraging and highlight the real interest of using saliva samples in metabolomics. In the future, it will be interesting to apply the developed protocol to metabolomics studies and to combine it with the usual blood and urine's metabolomics analyses.

Development and characterization of calixarene-based macrocyclic systems for the recognition of primary ammoniums in an aqueous environment

R. Carpentier[1,2], C. Testa[3], A. Pappalardo[3], I. Jabin[2], and K. Bartik[1]

[1] *Engineering of Molecular NanoSystems, Université libre de Bruxelles.*
[2] *Laboratoire de Chimie Organique, Université libre de Bruxelles.*
[3] *Laboratory of Supramolecular Organic Synthesis, University of Catania.*

The recognition and complexation of charged species in aqueous media is particularly challenging due to the competitive nature of water.[1] An appealing strategy for the design of ammonium ion receptors consists in using calixarene-based systems, which provide an π-electron-rich pocket that can participate in the stabilization of the ammonium−receptor complex through cation−π and CH−π interactions while ensuring size and shape cavity-based selectivity. They can furthermore easily be functionalized on their upper and lower rims.[2]

We present our recent work on the selective complexation of primary ammonium ions, over secondary, tertiary and quaternary ammoniums, in an aqueous environment by homooxacalix[3]arene **1**[3] and calix[5]arene **2** (Figure 1). Both receptors can be directly solubilized in water, following the deprotonation of their carboxylic acid groups but exhibit different complexation properties as only receptor **2** binds primary ammoniums. However, when incorporated in DPC micelles, recognition of primary ammoniums is observed with both systems (Figure 1).

Through extensive ^{1}H NMR (DOSY, PRE) and molecular modeling studies, we have been able to unravel the role of the microenvironment on the recognition properties, which are counterion-dependent due to the energy penalty for the dissociation of certain ammonium salts in the apolar micellar core.

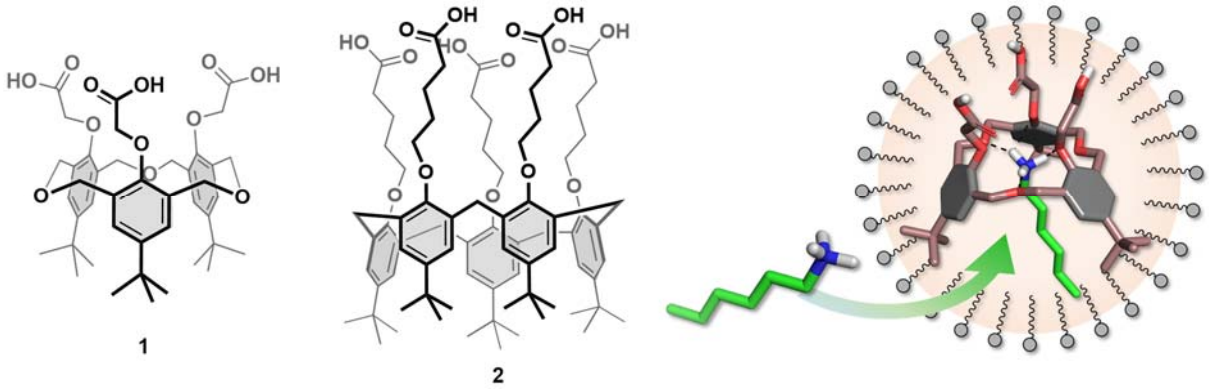

Figure 1. Homooxacalix[3]arene **1**, calix[5]arene **2** and schematic representation of the recognition of primary ammonium in micelles.

References

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

L. Van den Bergh[1,2], N. Gauquelin[3], J. Verbeeck[3], S. Van Doorslaer[2], V. Meynen[1]

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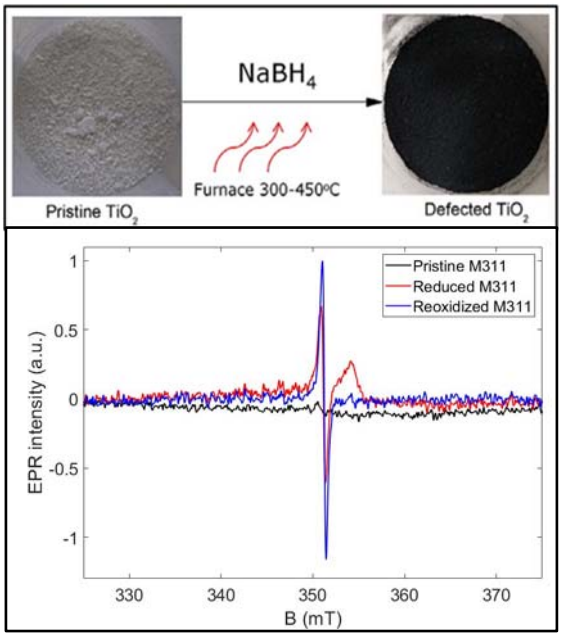

***Figure 1**: (a) Reduction of titania [2]. (b) cw EPR spectra of pristine, reduced and reoxidized titania at RT.*

Titanium-dioxide materials are known semiconductors with many prospects in chemical catalysis, the food industry and energy conversion. Most of these applications use the photocatalytic property of titania, which is mostly active in the UV part of the electromagnetic spectrum. By chemically reducing the normally white titania, it can acquire colour, which makes it active in also the visible part of the electromagnetic spectrum [1]. However, in literature there is a lot of contradiction on the most appropriate reduction process and its influence on the properties and photocatalytic activity of the coloured titania. In this project, titania is reduced using a thermal process with $NaBH_4$ as reducing agent [2]. It appears the reduction is sensitive to many different parameters of the process and the properties of the used titania. To discover which parameters and properties actually influence the reduction, they are evaluated by systematically changing them and characterizing the reduced titania with different spectroscopic techniques, such as EPR, XRD, XPS, EELS, in-situ drift-FT-IR and UV-Vis DR. By combining the results of these techniques, it is attempted to figure out the reduction process and the importance of the reduction parameters. EPR, as one of the key techniques reveals insight in the nature of the Ti(III) centres formed upon reduction of the titania.

References

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ORAL 6

Characterization of the impact of an inhibitor of the mitochondrial pyruvate carrier (MPC) using in vivo EPR and CEST-MRI

[1]Chloé Buyse, [2]Nicolas Joudiou, [2]Lionel Mignion, [3]Cyril Corbet, [3]Olivier Feron, [4]Julien Flament, [1]Samia Melloul, [5]Benoit Driesschaert, and [1]Bernard Gallez

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[4]Commissariat à l'Energie Atomique et aux Energies Alternatives (CEA), Centre National de la Recherche Scientifique (CNRS), Molecular Imaging Research Center (MIRCen), Laboratoire des Maladies Neurodégénératives, Université Paris-Saclay, Fontenay-aux-Roses, France
[5]West Virginia University, Morgantown, WV, USA

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The acidosis of the tumor microenvironment may have profound impact on cancer progression and on the efficacy of treatments. We evaluated the impact of a treatment with a mitochondrial pyruvate carrier (MPC) inhibitor on tumor extracellular pH (pHe) and pO_2. MPC disruption was showed to either promote or inhibit cancer progression and impact the extracellular acidification [1, 2]. Corbet et al. [3] showed that MPC inhibition by 7ACC2 decreased the growth of the SiHa cervix cancer cell line. We decided to study the effect of UK-5099, another MPC inhibitor on extracellular pH *in vitro* and *in vivo,* first using non-invasive Chemical Exchange Saturation Transfer (CEST)-MRI.

Glucose consumption, lactate secretion and Extracellular acidification rate (ECAR) were measured *in vitro* after exposure of cervix cancer SiHa cells and breast cancer 4T1 cells to UK-5099 (10 µM). Mice bearing the 4T1 tumor model were treated daily during four days with UK-5099 (3 mg/kg). The pHe was evaluated *in vivo* using chemical exchange saturation transfer (CEST)-MRI with iopamidol as pHe reporter probe. MR protocols were applied before and after 4 days of treatment. Glucose consumption, lactate release and ECAR were increased in both cell lines after UK-5099 exposure. CEST-MRI showed a significant decrease in tumor pHe of 0.22 units in UK-5099-treated mice while there was no change over time for mice treated with the vehicle. Parametric images showed a large heterogeneity in response with 16 % of voxels shifting to pHe values under 7.0.

We then evaluated the impact of UK-5099 on pHe and pO_2 using Electron Paramagnetic Resonance (EPR). Gluth et al. [4] synthesized a mono-phosphonated tetrathiatriarylmethyl radical, pTAM, whose EPR spectrum is sensitive to multiple parameters: oxygen concentration, pH, and inorganic phosphate concentration. The *in vitro* results showed that pTAM was able to measure an acidification of pHe values after 4T1 cells were treated for 24 hours with UK-5099. A significant reduction in OCR was measured with pTAM and with ^{15}N-PDT. For the *in vivo* studies, we decided to combine pTAM with Lithium Phthalocyanine (LiPc) to measure pO2. The results on 4T1 tumor models showed a significant decrease in tumor pHe in UK-5099-treated mice while there was no change over time for mice treated with the vehicle. No significant changes were observed in pO_2 values.

The effects of an MPC inhibition were measured using CEST-MRI and EPR, are two noninvasive methods able to measure changes in pHe.

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Development of a combined methodology towards the investigation of sustainable light-activated catalysts

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

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

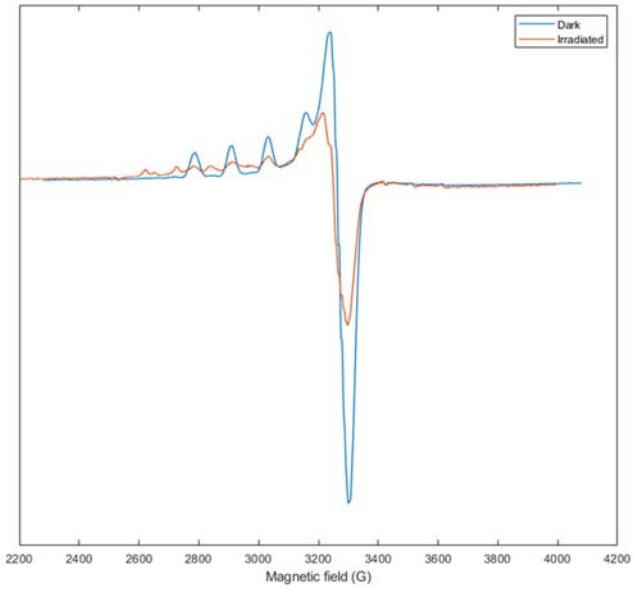

Figure 1: Effect of blue light irradiation on the CW-EPR spectrum of the copper-based photocatalyst, showing structural changes and a decrease in intensity

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

We gratefully acknowledge funding from Marie Skłodowska-Curie Joint Doctorate grant agreement No 813209.

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In vitro and *in vivo* study of iron oxide nanoparticles designed for theranostic targeting EGFR-overexpressing tumors

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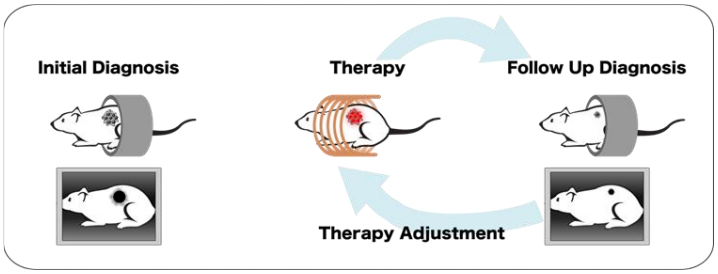
Aim of the study is to develop targeted multifunctional nanoplatforms designed for theranostic (diagnosis, therapy and follow-up diagnosis) in cancer context. MRI and magnetic hyperthermia are used to diagnosis and treat solid tumors respectively. This work is focused on the diagnostic part. Iron oxide nanoparticles (IONPs) are synthesized by thermal decomposition method and coated with dendrons (DNPs)[1]. A targeting ligand, peptide 22 (P22), is conjugated to DNPs. P22 binds specifically to EGFR overexpressing cancer cell such as triple negative breast tumors[2].



First the cytotoxic effect of DNPs was measured on tumoral cell lines. The amount of internalized nanoparticles with and without P22 have been measured by iron quantification in biological matrix methods[3]. In a second time, DNP's biodistribution have been observed on standard mouse strain with a 9.4T MRI machine; mice have been monitored for one month after nanoparticle injection. At last, EGFR-overexpressing cells have been inoculated into to mice. When the tumor size was sufficient, nanoparticles were intravenously injected to mice. The contrast effect of DNPs was evaluated using 9.4T T_2^*-weighted-imaging MRI. Tumors, liver and spleen were collected after sacrifice. Those tissue and organs were digested in hydrochloric acid, and the amount of iron was measured by ICP analysis.
Cytotoxicity tests prove that DNPs are weakly toxic below 150 μg_{iron}/mL after 24h exposure. The presence of P22 on DNPs surface increases the amount of nanoparticles internalized by cells. *In vivo,* no sign of toxicity has been seen during a one-month monitoring. Nanoparticles are mostly found in sinusoid organs (liver and femoral bone marrow) in which cells of the mononuclear phagocytic system should participate in the nanoparticle uptake process. After one month, the signal decrease is still observable in these organs. In the tumoral model, nanoparticle accumulation in tumors is noticeable 1 hour after iv. injection. After 24h, the darkening in the tumor region is stronger for DNPs conjugated with P22 than without. *Ex vivo* ICP analysis of organs also points in this direction; the iron amount in tumors is greater for DNPs+P22 than for DNPs alone.
In conclusion, DNPs are safe to use for *in vitro* and *in vivo* experiments at a controlled iron concentration (<150µg/mL). *In vivo* observations suggest a nanoparticle accumulation in liver but without toxicity signs or side effects on mice. Peptide 22 is of interest as its binding increases the cellular internalisation of DNPs and allows tumor contrast enhancement on T_2^*-weighted images.

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ORAL 9

A low-field benchtop MRI system for general applications

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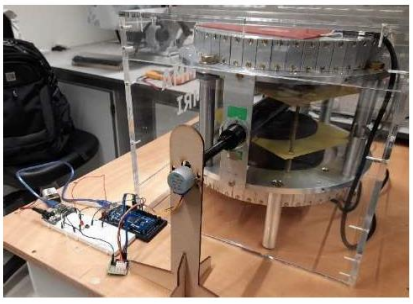
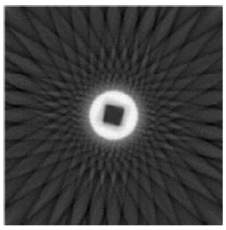
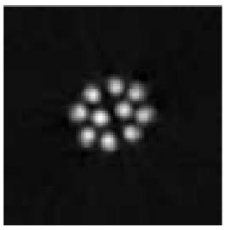
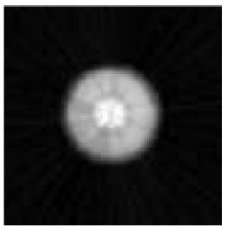
Low-field NMR instruments are made of an assembly of magnetic pieces that are placed together to generate the desired magnetic field in a specific region known as the “sweet spot”. The design of these instruments can be object-oriented, thus avoiding the concept of one instrument that fits all. In this project, an H-shape 2 MHz magnet with a bore of 16 cm and containing a 3 cm diameter hole at the centre of the pole pieces was designed to be applied in hyphenated MRI experiments with other techniques (e.g. CT, PET, etc.). Furthermore, these construction constraints imposed further design constrains for the gradients and shimming coils designs which are not found in commercially available instrumental solutions.

Here, it is presented the resulting MRI system composed of the magnet, probe, and gradients. The sweet spot is intended to perform images inside a sphere of 2 cm diameter, having less than 200 ppm homogeneity (without the shimming coils). Moreover, back-projection reconstruction images were recorded with this system, showing its capabilities to perform MRI experiments. Preliminary results can be observed in the figures below, from the left showing the magnet system, the image of a rectangular phantom with a square 1 cm cross-section surrounded by water, the image of an ensemble of 5 mm NMR tubes, filled with liquid and 2 cm in length, and a 5 mm carrot slice.

ORAL 10

Benchtop NMR Relaxometry for monitoring Cu^{2+} removal using ion exchange resins and commercial activated charcoal.

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Water pollution by heavy metals is a major environmental problem [1]. To address this issue, the removal of heavy metals from wastewater often requires the use of ion exchange resins or adsorbents. However, current methods for assessing ion exchange/adsorption efficiency are often indirect and destructive. Some heavy metal ions, such as Cu^{2+}, exhibit paramagnetic properties that influence the NMR relaxation times T_1 and T_2 of water protons. Benchtop NMR relaxometry can thus provide a means to monitor the removal of paramagnetic heavy metals by sorbents thanks to the measurement of T_1 and T_2 [2-3].

This research focuses on studying the removal of Cu^{2+} using Amberlite IR120; Amberlite IRC748; Dowex Marathon MSC resin and commercial activated charcoal (AC). Batch experiments were conducted to investigate ion exchange and adsorption isotherms. Samples containing 5.5 mg of IR120/MSC resin; 5 mg of IRC748 resin or 45 mg of AC were exposed to 350 µL of aqueous solutions with varying Cu^{2+} concentrations and then shaken until equilibrium was reached. Upon reaching equilibrium, T_1 measurements of the solution were carried out to determine the amount of adsorbed metal. Additionally, the study of the loaded resin was also carried out using a larger amount of resin/AC, which was first dried and then rehydrated before being analyzed.

The equilibrium isotherms for Cu^{2+} were satisfactorily described by the Langmuir model for resins and by the Freundlich model for AC. The longitudinal and transverse relaxation of the wet resin exhibited biexponential behavior. The relaxation rates of the fast-relaxing water fraction of Cu^{2+} loaded sulfonic resins showed an excellent correlation with their Cu^{2+} content determined independently via Atomic Emission Spectroscopy. The impact of Cu^{2+} loading on relaxation rates in the case of IRC748 was less pronounced due to Cu^{2+} complexation. Loaded activated charcoal, due to its heteroporosity displayed a more complex relaxation behavior. The relaxation curves of loaded AC exhibit more than two fractions. To eliminate the contribution of intergranular water, centrifugation was employed. The residual contribution from intragranular water was biexponential. The relaxation rates of the slowest relaxing fraction increased with the Cu^{2+} content in AC.

In the future, it will be interesting to carry out a so-called NMR column experiment in order to follow the loading of adsorbent in real-time through the measurement of the NMR signal inside the column.

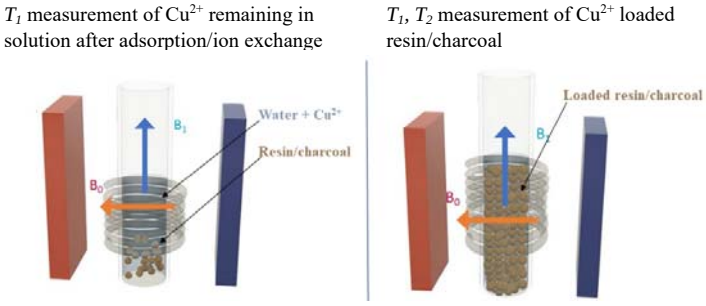

Figure 1. Experimental set-up

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The application of low field NMR relaxometry in iron-rich materials

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Iron is the most abundant transition element on Earth, occurring as Fe^{2+} and Fe^{3+} in a variety of rock and soil minerals. The magnetic properties of iron make iron-rich materials unsuitable for nuclear magnetic resonance (NMR) measurements due to the paramagnetic influence of unpaired electrons in iron ions. The paramagnetic influence increases with the magnetic field. Although it is difficult to apply high-field NMR in the characterization of iron-rich materials, it is still possible to exploit the low paramagnetic influence of low-field NMR to characterize certain properties of iron-rich materials.

One of the application of low-field NMR relaxometry we proposed is to determine the iron redox ratio in materials. Accurate determination of the iron redox ratio is essential for understanding the formation of a mineral and for further utilization of iron-containing materials. The measurements depend on the different paramagnetic influences of Fe^{2+} and Fe^{3+} ions on the relaxation of bulk water. Experiments have been carried out to calibrate the relationship between the concentration of Fe^{3+} and Fe^{2+} ions and the measured relaxation times on prepared standard samples. Solid samples (*eg*. Rocks, soils, and slags) were first digested in an acid to produce solution. Hydrogen peroxide (H_2O_2) was then added to the digested samples to oxidize any Fe^{2+} ions to Fe^{3+} ions. The concentration of Fe^{2+} and Fe^{3+} ions in solution and thus the redox ratio of iron can be calculated by measuring the relaxation times before and after oxidation.

Another application we proposed is to estimate the durability of construction materials by monitoring the dissolving behaviour of Fe^{3+} ions in an acid solution. The non-destructive and non-invasive nature of NMR techniques allows us to observe the dissolution behaviour of materials in acid solution *in situ*. By calibrating the relation between relaxation rates and Fe^{3+} ion concentrations, we can quantitatively measure the real-time release of Fe^{3+} ions into the acid solution. The dissolution process was observed as a high temporal resolution, which is an advantage for the construction of a mathematical model.

Warburg-associated acidification represses lactic fermentation independently of lactate, contribution from Real-Time NMR on cell-free systems

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Lactate accumulation and acidification in tumours are a cancer hallmark associated with the Warburg effect. Lactic acidosis correlates with cancer malignancy, and the benefit it offers to tumours has been the subject of numerous hypotheses. Strikingly, lactic acidosis enhances cancer cell survival to environmental glucose depletion by repressing high-rate glycolysis and lactic fermentation, and promoting an oxidative metabolism involving reactivated respiration. We used Real-Time NMR to evaluate how cytosolic lactate accumulation up to 40 mM and acidification up to pH 6.5 individually impact glucose consumption, lactate production and pyruvate evolution in isolated cytosols. We used a reductive cell-free system (CFS) to specifically study cytosolic metabolism independently of other Warburg-regulatory mechanisms found in the cell. We assessed the impact of lactate and acidification on the Warburg metabolism of cancer cytosols, and whether this effect extended to different cytosolic phenotypes of lactic fermentation and cancer. We observed that moderate acidification, independently of lactate concentration, drastically reduces the glucose consumption rate and halts lactate production in different lactic fermentation phenotypes. In parallel, for Warburg-type CFS lactate supplementation induces pyruvate accumulation at control pH, and can maintain a higher cytosolic pyruvate pool at low pH. Altogether, we demonstrate that intracellular acidification accounts for the direct repression of lactic fermentation by the Warburg-associated lactic acidosis.

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Plectasin kills bacteria by a Ca^{2+}-sensitive supramolecular mechanism

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The rise of multidrug-resistant bacteria is a severe threat to human health and calls for the development of antibiotics that use new mechanisms. Plectasin is a host defense peptide from the fungus *Pseudoplectania nigrella* and was discovered in 2005[1]. Plectasin and its variants[2-4] show high activity against clinically relevant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae*, or M*ycobacterium tuberculosis*, including in animal infection models[1] . The peptide was previously shown to enact its bactericidal activity by targeting the peptidoglycan precursor Lipid II[5], blocking the cell wall biosynthesis. Despite the prominence of plectasin, its mechanism is insufficiently understood, primarily because structural investigations of such systems is technically challenging. We have investigated the plectasin-Lipid II complex using solid-state NMR in order to elucidate its mode of action in a near-native membrane environment. Using modern ssNMR methodologies, including ^{1}H-detection, DNP hyperpolarization, ^{43}Ca-NMR, and experiments at ultra-high magnetic field (28.2 T, 1200 MHz ^{1}H-frequency), we show that plectasin uses a new type of supramolecular antimicrobial action that is modulated by calcium ions and we provide insights into the unexplained improved activity of a plectasin variant called NZ2114 that displays improved activity against MRSA.

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ORAL 14

Revisiting the mode of action of teixobactin

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The antibiotic teixobactin is produced by the previously uncultured bacterium *Eleftheria terrae* and acts against Gram-positive bacteria such as *Staphylococcus aureus* [1]. Resistance is difficult for bacteria to achieve since teixobactin acts on an essential lipid involved in peptidoglycan synthesis, Lipid II. Recently, we showed that teixobactin targets LII and then assembles into supramolecular fibrils that compromise bacterial membranes [2,3]. Here, using a combination of solid-state NMR spectroscopy and calorimetry techniques, we discover a major influence of membrane composition on teixobactin's mode of action.

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ABSTRACTS - POSTER CONTRIBUTIONS

POSTER 1

EPR characterization of the heme pocket structure of nonsymbiotic hemoglobins from the model legume *Lotus japonicus*

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Legumes hold significant agricultural importance due to two primary reasons. Firstly, they serve as a vital protein source for both human and animal nutrition. Secondly, they can form nitrogen-fixing symbiotic relationships with soil rhizobia, thereby reducing the need for expensive and polluting nitrogen fertilizers.

Plant hemoglobins (Hbs) are found in legume nodules and actinorhizal plants and are also present in nonsymbiotic organs of monocots and dicots. Nonsymbiotic Hbs (nsHbs) have been categorized into two phylogenetic groups: class 1 and class 2. Class-1 nsHbs have a very high affinity for O_2 and are induced by hypoxia and nitric oxide (NO) [1,2], while class-2 nsHbs have moderate O_2 affinity and are induced by cold and cytokinins [3]. Various functions of nsHbs depend on the ability of hemes to bind diatomic ligands and catalyze the NO dioxygenase reaction, which converts oxyferrous Hb and NO to ferric Hb and nitrate.

In this work, we focus on the analysis of the ferric form of three nsHbs from *Lotus japonicus*, more specifically two class-1 nsHbs (*Lj*Glb1-1 and *Lj*Glb1-2) and one class-2 nsHb (*Lj*Glb2-1) [4]. *L. japonicus* is used as a model legume to facilitate the transfer of genetic and biochemical information into crops. The class-1 variants have Cys residues at specific positions in the protein chain. Using the variants *Lj*Glb1-1 C78S, *Lj*Glb1-2 C79S and *Lj*Glb1-1 C8S we earlier showed that mutation of the Cys at position 78 (79) in LjGlb1-1 (1-2) induced significant changes in both protein dynamics and stability, while the impact of the mutation *Lj*Glb1-1 C8S was relatively minor [4]. Here, we focus on the difference in the heme environments of the ferric wild type nsHbs and their Cys variants using continuous-wave electron paramagnetic resonance (CW EPR), hyperfine sublevel correlation spectroscopy (HYSCORE), resonance Raman spectroscopy and 3D modelling of the protein structure. All ferric forms show the heme iron predominantly in a hexacoordinated low-spin state, with proximal and distal His ligation. The difference in the EPR parameters between the three nsHbs reflects the difference in the relative orientation of the imidazole planes of the His residues. The data are compared to those of other plant Hbs and related to our earlier resonance Raman data of the deoxy ferrous forms of these proteins.

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POSTER 2

Unravelling the folding of a large monomeric model protein at high resolution through HDX-MS and HDX-NMR

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The detailed characterization of the folding of a large protein is conducted on a model enzyme, namely the BS3 class A β-lactamase. The principal goal is to determine the order of formation of its secondary structure elements using quenched-flow HDX pulse-labelling experiments, combined with both NMR and mass spectrometry (MS) measurements. Class A β-lactamases (Mr ca. 29000) are among the largest proteins studied in terms of their folding properties. Thus, the folding of β-lactamases has been characterized in some detail, and, as observed with most large proteins, folding is not kinetically two-state and intermediate partially folded species are observed. The highly conserved cis peptide bond between residues 166 and 167, in a long Ω-loop at the active site of these enzymes, controls important steps in the refolding reaction. The sequence of the two structural domains implies a substantial movement of the polypeptide chain during the folding process. Indeed, one of the two domains (composed of both α-helices and β-sheet) is made up of the N and C-terminal parts of the protein, whereas the other domain (all α) is formed by the central part of the polypeptide chain. HDX-MS experiments revealed the initial formation of molecules with native-like protection against exchange (EX1) in the secondary structural elements closest to the N- and C-terminal parts of the sequence, then propagating to the core of the protein. These results suggest the presence of an intermediate species with a folding nucleus that allows a productive process toward the formation of fully active enzyme molecules.

Monte Carlo simulations of transverse relaxation induced by superparamagnetic iron oxide nanoparticles with a semipermeable coating

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In Nuclear Magnetic Resonance (NMR), diffusion of protons in magnetic field inhomogeneities can be a cause of transverse relaxation. Indeed, as protons move, the magnetic field they experience changes, as their Larmor frequency, leading to a progressive dephasing of their magnetic moments. This causes a decrease of the global transverse magnetization of the sample.

Therefore, when contrast needs to be enhanced on Magnetic Resonance Images, one option is to add magnetic material, acting as a contrast agent, in the region of interest in the body. Indeed, under the static magnetic field of the NMR imaging system, that material is magnetized, and therefore increases the magnetic field inhomogeneity in the region of interest, which in turns modifies the transverse relaxation time, and therefore the signal magnitude.

For biomedical applications, superparamagnetic iron oxide nanoparticles are excellent candidates for use as contrast agents, due to their low toxicity, high saturation magnetization, and null remnant magnetization [1].

To ensure the stability of these particles, and to allow their functionalization, those nanoparticles are coated with sugars, silica or polymers. Those coatings are usually semipermeable to water: water can penetrate the coating, but its diffusion is slower than in the solvent. As diffusion in the magnetic field inhomogeneities is at the origin of the magnetic moment relaxation induced by these nanoparticles, limited diffusion in the coating is expected to impact relaxation times of water. Few experimental studies have evaluated the impact of coating nature and thickness on relaxation, but those who did found an effect of these parameters on relaxation times indeed [2,3].

In this work, the influence of the diffusion coefficient in the coating and of the coating thickness on water transverse relaxation caused by coated iron oxide nanoparticles is probed, thanks to Monte Carlo simulations. Nanoparticles are modelled as impermeable spheres producing a dipolar magnetic field and surrounded by a semipermeable region, in which the entry and exit of a proton is conditioned by coating permeability. Diffusion of the protons is modelled by a random walk, whose step is defined by the simulation time step and the diffusion coefficient in its environment.

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POSTER 4

Sustainable Photocatalysis with Copper Complexes: mechanistic studies through ^{1}H NMR and EPR spectroscopies

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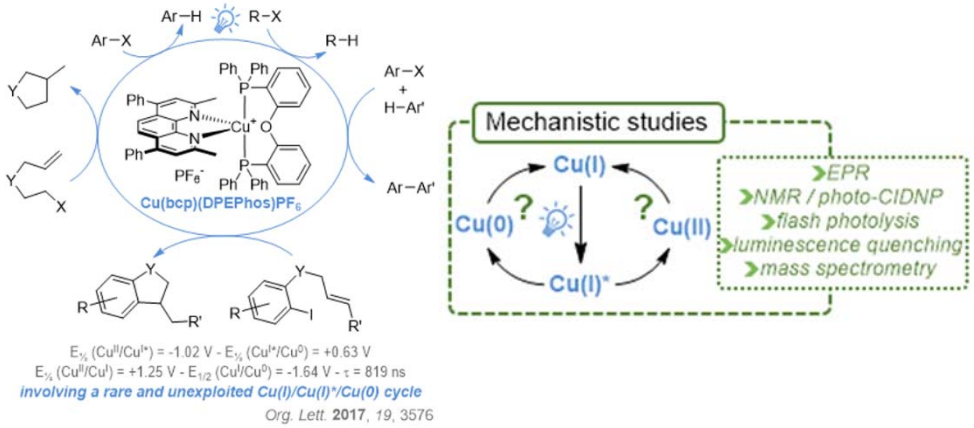
Organic synthesis is clearly today a central science with deep implications in various domains such as biology, medicine, agrochemistry, energy or material science. There is thus a high and growing demand for efficient procedures to assemble complex molecules or pharmaceuticals from simple building blocks. The need for environmentally benign and more sustainable chemical practices also poses new challenges and requires new ways of carrying out synthesis. In order to design efficient, innovative and sustainable processes for the synthesis of organic molecules, a global and systematic approach, involving inorganic chemistry, photochemistry, physical chemistry as well as homogeneous and heterogeneous catalysis, was used for the development of copper-based complexes and their use as photocatalysts.[1]

After extensive screenings of the best conditions and the most versatile copper complexes for photocatalytic reactions[2,3,4,5,6] the heteroleptic complex Cu(bcp)(DPEPhos)PF_6 was selected as the most interesting candidate to perform mechanistic studies. ^{1}H NMR and EPR studies we carried out for a model debromination reaction in order to clearly identified the oxidative or reductive pathway of the corresponding catalytic cycle and to understand the key parameters for further ligand design. NMR measurements were conducted after brief illumination to allow an *in-situ* monitoring of the photocatalyzed reaction. The occurrence of the Cu(I)/Cu(II) or Cu(I)/Cu(0) catalytic cycles was assessed by EPR and spectro-electrochemistry.



Acknowledgements

This work was supported by the Université libre de Bruxelles (ULB), the Région de Bruxelles Capitale-Innoviris (2019-BRIDGE-5 PhotoCop) and the Fonds de la Recherche Scientifique – FRS-FNRS (EQP2019-35305690). We gratefully thank Pr G. Evano, Pr C. Moucheron, Dr J. Beaudelot, F. Urbain (ULB) and Pr S. Van Doorslaer (UAntwerpen) for their help and/or fruitful discussions.

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POSTER 5

HIGH-RESOLUTION NMR ALLOWS VIRTUAL SEPARATION OF COMPLEX MIXTURES

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Organic molecules and (bio-)polymers produced by biological and catalytic processes often occur as complex mixtures of structurally similar organic components. Identification and quantification of the individual components in complex mixtures is essential to chemical research and quality control in an industrial, biomedical or food safety setting. NMR spectroscopy achieves component identification by measuring the precession frequency of every NMR-active nucleus when the sample is placed in a magnetic field. Mixtures of biopolymer components complicate this method, as overlap of crowded, near identical NMR spectra makes analysis difficult. Therefore, a physical separation can be beneficial to simplify the spectra. However, physical separation techniques may alter the chemical structure of the compounds, which influences the subsequent measurements and results.

NMR spectroscopy can counter this problem as it enables a virtual separation of the components in the complex mixtures in multiple ways. Increasing the resolution of standard two-dimensional NMR experiments allows identification of highly similar components in a mixture if only one or a few nuclei differ in chemical environment. To achieve this, a high field spectrometer and experiment optimization are critical. Another way of virtual separation is obtained by using Diffusion Ordered SpectroscopY (DOSY) experiments. DOSY-NMR monitors the apparent self-diffusivity of each compound in solution. As such, differences in self-diffusion act as a separation tool to discriminate compounds virtually while retaining the mixture in its initial state. Finally, relaxation rates of the magnetization of the nuclei can be included as another dimension along which virtual separation occurs. Combining these experimental techniques allows for a high-dimensional spectrum, able to virtually separate the complex mixtures.[1]

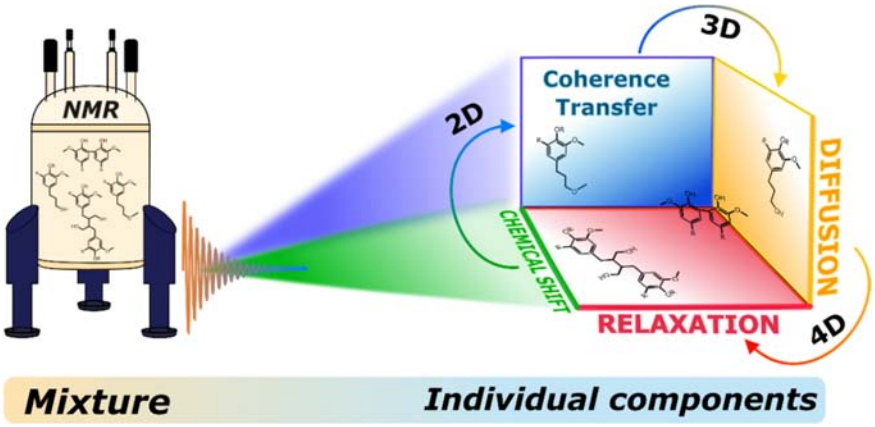

Figure 1. Virtual separation of components in a mixture by high-resolution NMR spectroscopy.

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POSTER 6

Towards the understanding of the formation mechanism of hollow silica nanotubes and nanospheres: an NMR approach

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In the last 20 years, many low-dimensional porous silica-based nanomaterials have been synthesized via the sol-gel process and the micelles-templated syntheses of mesoporous solids. Among them, hollow nanospheres and nanotubes have withdrawn a great interest because of their elevated specific surface area and large pores diameter, which are favourable features for catalytic applications.[1] Moreover, the isomorphic substitution of the Si with a metal cation allows obtaining an acid heterogeneous catalyst.[2]

It is interesting to highlight that the synthesis protocol suggested by Kruk *et al.* for both silica nanotubes and hollow nanospheres, is very similar. These materials are obtained by using triblock copolymer (Pluronic F127) micellar systems as soft templates, in combination with a swelling agent (toluene, in this particular case). The only significantly different parameters in the two syntheses are the stirring speed and the time between the addition of a swelling agent and the silica precursor (tetraethyl orthosilicate, TEOS). In particular, nanotubes are synthesised at a lower stirring speed, without any delay in the addition of toluene and TEOS, while for the synthesis of hollow nanospheres, it is necessary to increase the stirring speed and/or delay the addition of TEOS.[1]

Certainly, gaining a thorough understanding of the mechanism behind the formation of hollow silica nanospheres and nanotubes is essential for the improvement, optimization, tuning and eventually scale-up of their synthesis. Earlier research had proposed that hollow nanospheres are formed through the fragmentation of nanotubes. More recently, our research group definitively established that the crucial parameter determining whether tubular or spherical nanostructures form is the amount of surfactant-stabilized toluene present in the reaction mixture.[3]

Nonetheless, a deeper understanding of the aforementioned mechanism is required. Herein, the synthesis of Sn-doped silica nanospheres is presented. The use of several NMR techniques is employed to understand more in-depth how the process of Pluronic F127 micelles formation is influenced by the toluene amount and the temperature, and how these parameters affect the formation of hollow nanospheres. Specifically, 1D and 2D NMR experiments have been acquired at different temperatures and toluene concentrations to obtain structural and dynamic information. Finally, the synthesized nanomaterials have been characterized through static and MAS solid-state NMR experiments of ^{29}Si and ^{119}Sn.

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POSTER 7

Insights into the extraordinary self-assembly of fampridine by multinuclear NMR

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Small organic molecules generally form simple supramolecular arrangements, producing crystal structures with relatively small unit cells. However, we have recently isolated four new crystalline phases (**1**-**4**) of fampridine hydrochloride (4-APH$^+$Cl$^-$), a simple organic compound whose crystalline phases can adopt an incredibly complex self-assembly.[1,2]
Interestingly, **1** and **2** represent the first observation of Frank-Kasper (FK) phases in small organic systems. FK is a remarkable class of crystalline phases previously observed only in metal alloys[3] and different types of supramolecular soft matter. The two FK structures crystallised from a dense liquid phase (DLP) obtained after liquid-liquid phase separation.

To understand how such a simple molecule may crystallise as an FK phase, we monitored the DLP precursor of complex phase **1** and the aqueous precursor of simple phase **3** by liquid-state NMR. ^{1}H, ^{13}C, ^{14}N and ^{35}Cl NMR experiments were carried out as a function of the concentration of 4-APH$^+$Cl$^-$ until the crystallisation occurred. The results are compared to Molecular Dynamics simulations of DLP, investigating the relevant solute/solvents interactions. In addition, these phases were studied also by ^{13}C solid-state NMR, showing the potential of NMR to explore such complex crystalline phases.[2]

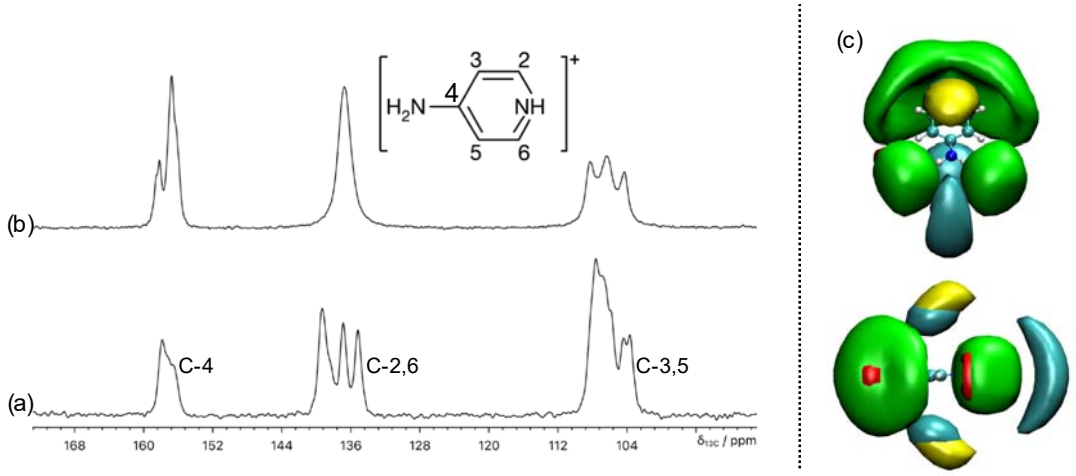

*Figure 1. ^{13}C Solid state NMR of phase **3** (a) and **1** (b) recorded at 14.1 T and room temperature. Spatial Density Functions (SDF) around 4-APH$^+$ cation (c). SDF are shown for the Cl$^-$ (green), water (oxygen atom, red), acetone (carbon atom of carbonyl, yellow), and 4-APH$^+$ (carbon C4, cyan).*

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POSTER 8

Impact of exposure to pyraclostrobin and to a mixture pyraclostrobin / boscalid on the mitochondrial function of human hepatocytes

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Fungicides are widely used in agriculture for crop protection. Succinate dehydrogenase inhibitors (SDHIs) and strobilurins inhibit mitochondria electron transport chain (ETC) in fungi, by blocking the complex II and complex III, respectively. Questions regarding their selectivity of action for fungi have been raised in the literature, and we previously showed that boscalid and bixafen (SDHIs) alter the mitochondrial function of human hepatocytes. Here, we analyzed the impact of the exposure of human hepatocytes to pyraclostrobin, a fungicide belonging to the class of strobilurins. Using Electron Paramagnetic Resonance (EPR), we observed a decrease in oxygen consumption rate (OCR) and an increase in mitochondrial superoxide levels after 24 hours exposure to 0.5 µM concentration. As a consequence, the content in ATP amount in the cells was reduced, the ratio reduced/oxidized glutathione was decreased, and a decrease in cell viability was observed using three different assays (Presto Blue, crystal violet and annexin V assays). In addition, as SDHIs and strobilurins are commonly associated in commercial preparations, we evaluated a potential “cocktail” toxic effect. We selected low concentrations of boscalid (0.5 µM) and pyraclostrobin (0.25 µM) that did not induce a mitochondrial dysfunction in liver cells when used separately. In sharp contrast, when both compounds were used in combination at the same concentration, we observed a decrease in OCR, an increase in mitochondrial superoxide production, a decrease in the ratio reduced/oxidized glutathione, and a decrease in cell viability in three different assays.

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POSTER 9

Understanding the acidity properties of different silica-based materials via ^{31}P ssNMR using TMP as probe molecule.

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Nowadays, various industrial processes are performed with the aid of acid catalysts. In the past decades Brønsted acid such as H_2SO_4, H_3PO_4 and HF have been broadly used as homogeneous catalysts due to their acidic strengths. Despite their excellent catalytic performances, these homogeneous catalysts display serious drawbacks manly related to equipment corrosion, difficult waste treatment and high toxicity. To solve the above-mentioned problems, solid heterogeneous acid such as zeolites, functionalized silica and supported heteropolyacids (HPAs) have been developed and largely employed in various catalytic chemical processes.[1] In general, the heterogeneous solid acids display two different kinds of acidity: Lewis (L) and Brønsted (B). Silica-based materials are among the most used heterogeneous catalyst due to high specific surface area and pore size distribution. Moreover, the L/B acid balance can be tuned via the insertion of a metal cation in the structure or modifying the ratio between silicon and the selected metal cation. In this context, different analytical methods based on well known spectroscopic techniques have been developed with the aim to characterize these acidic features. Indeed, the overall acidity can be studied via the use of probe molecules (e.g. pyridine and ammonia) in combination with temperature programme desorption approach (TPD) or coupled to infrared spectroscopy (FT-IR).[2] Recently, it has emerged that detailed acid features such as nature, concentration, and strength of acid sites can be investigated by solid state nuclear magnetic resonance (ss NMR) selecting a suitable probe molecule containing NMR-sensitive nucleus such as ^{13}C, ^{15}N, or ^{31}P. Among all the possible probe molecules, trimethylposphine (TMP) and trimethylposphine oxide (TMPO) are largely employed in this field. The use of these two molecules is quite advantageous due to the wide chemical shift range of ^{31}P NMR allowing the clear identification of the different acid sites, and because ^{31}P is a dipolar nucleus with an abundance of 100%.[3] Herein, we show the characterization of the L and B acidity of different silica-based materials using TMP as probe molecule. Initially, a comparison between Hf, Ga and Sn silica hallow nanotubes is done. Afterwards, Ga and Sn nanotubes are compared with the respective silica hollow nanospheres, to evidence a possible modification on the acid properties due to the different morphology of the solids. Finally, an oxidation treatment in air allows converting TMP in TMPO which can employed to better understand the strength of the different acid sites.

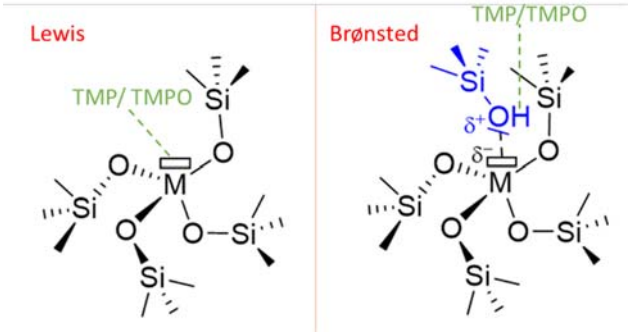

Figure 1. General schematic representation of Lewis (left) and Brønsted (right) acid active site on silica-based materials.

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POSTER 10

Structural and Dynamic Insights into the Loss of Activity of Tolaasin I

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Cyclic lipopeptides (CLiPs) are a class of secondary metabolites that consist of a peptide moiety with an N-terminal lipid tail and a macrocycle formed via an ester bond (depsi) between the C-terminal carboxyl group and a side chain hydroxyl group. They are produced by multiple genera of bacteria including *Pseudomonas* and *Bacillus*. CLiPs exhibit a multitude of biological functions in nature such as improving bacterial motility, antibacterial activity, and antifungal activities among others.[1] Tolaasin, a CLiP produced by *Pseudomonas tolaasii*, is the causative agent for brown blotch disease in mushrooms. The sequence of tolaasin I is comprises of 18 mostly non-proteinogenic amino acids, with the 5 residues at C-terminal involved in the macrocycle. Its 3D structure has been determined by Jourdan et al, tolaasin unstructured in water, it adopts a stable amphipathic left-handed a-helix ending in a flattened macrocycle loop when exposed to micelle forming detergents such as SDS.[2] Recent studies have shown that a natural defence mechanism exists where the antagonistic properties of tolaasin are lost by hydrolysis of the depsi bond closing the macrocycle[3].

In this project we aim is to gain a better understanding of why the loss of the macrocycle has such a significant impact on tolaasin activity by comparing the conformation adopted by hydrolysed tolaasin with that of the native molecule, both studied in SDS micelles using NMR spectroscopy. To enable advanced multidimensional structural analysis, we first produced ^{13}C and ^{15}N isotope-enriched tolaasin by growing the producing bacterium on a minimal medium and suitable labeled isotopically enriched precursors. Subsequently, we synthesized the isotopically enriched hydrolyzed form through controlled alkaline hydrolysis. Following full resonance assignment, Long-range HNCO experiments are recorded to investigate the presence of long-lived hydrogen bonds through $^{3h}J_{NC'}$ scalar couplings while HNHA measurements are used to obtain the φ torsion angle dependent $^{3}J_{HNHA}$ coupling. In addition, amide proton longitudinal relaxation rates are determined in absence and presence of paramagnetic relaxation agent to map the PRE wave of the tolaasin. This allows for the extraction of the tilt angle and azimuth angle, enabling the mapping of helix orientations. Furthermore, ^{15}N T_1, T_2, and het-nOe experiment are performed to investigate the backbone dynamics of the peptide. Ordered parameters(S^2), overall(τ_c) and effective(τ_e) corelation times are derived from model-free analysis (MFA) of the relaxation data. Reduced spectral density mapping (RSDM) was used to calculate the spectral density at $J\omega_0$, $J\omega_N$, and $J\omega_{0.87H}$. By studying the dynamics of the peptide backbone, we have gained insight into molecular motions occurring on a nanosecond to picosecond timescale. This has enabled us to identify residues with distinct rigidity and flexibility profiles in both forms. This comprehensive set of NMR experiments provides crucial information about the conformational changes and backbone mobility that occur after the linearization process and the impact of the presence of the macrocycle on the structural defense of native Tolaasin.

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POSTER 11

^{207}Pb MAS NMR as a probe of the structure and dimensionality of lead halide perovskites

Thomas J.N. Hooper, Dimitrios Sakellariou

Lead halide perovskites (LHPs) have been the subject of fervent research over the last 10 years due their potential in optoelectronic applications. The high structural flexibility provided by the labile Pb-X bonds and substitutable charge-balancing cations allows multiple revenues for material design including 0D, 1D and 2D monomeric configurations of the $[PbX_6]^{-4}$ octahedral units. However, in the rush for novel material synthesis, structural characterisation is often neglected, especially as LHPs are routinely synthesized as nanocrystals or thin-films, which are difficult to examine with traditional XRD. Here, we demonstrate the potential of ^{207}Pb MAS NMR for structural characterisation of a variety of LHPs, from revealing dynamic behaviour in the archetypal 3D LHPs to tracking the lower dimensionality of novel bulky cation hybrid LHPs.

POSTER 12

Dried blood spot sampling: a new tool for NMR-based metabolomics approach

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Introduction

The metabolomics study of whole blood has been since long time subject of several research because of the extremely diverse and complex nature of biological specimens. This field of "omics" sciences find a wide range of applications in personalized medicine approach going from clinical diagnostics for early disease detection, and from therapy prediction to the patient's follow-up. In the optic of the incoming personalized medicine era, the need for robust self-made bio-specimens' collection methods are mandatory.

Materials and methods

In this work the use of dried blood sample (DBS) device was tested for NMR-based metabolomics approach. The idea of combining DBS sampling with NMR-based metabolomics will enlarge the analytical available options for the personalized method approach. In particular, by focusing on a fast and simple biofluids analysis through a NMR platform, we propose the use of DBS as device for a non-invasive and poorly time-consuming blood samples collection and analysis.

In order to verify the applicability of DBS to NMR-based metabolomics, in our study we set up an extraction protocol from DBS in way to maximize the number and the quantity of extracted metabolites. Once the protocol was set, its reproducibility and the repeatability were evaluated. The extraction from DBS paper was performed at three different time points in way to evaluate the reproducibility of extraction on our device. In way to test the repeatability several DBS spots were extracted and compared by measuring the levels of extracted metabolites. Moreover, the performance of the methods was assessed through a spiking experience by comparing it with the gold-standard method (plasma analysis). Finally, a proof of concept study was also realized in way to determine the usefulness and applicability of our methodology.

Results and conclusion

Taking together, our results assess DBS sampling as a simple, robust and reliable method for NMR-based metabolomics purpose. Our proof of concept study clearly shows that relevant information can be extracted by using this method. Finally, the DBS device could represent a valid auto-sampling method and a proper alternative to common practices easily applicable to metabolomics studies with higher complexity's experimental design.

POSTER 13

Monte Carlo Simulations of the T2 relaxivity induced by Cubic-Shaped Superparamagnetic Nanoparticles

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Nanoscale materials have garnered immense scientific interest for the past few decades due to their wide range of applications [1] and their unique properties, such as enhanced surface reactivity and quantum effects. At this scale, composite materials of magnetite and maghemite exhibit superparamagnetic behavior at room temperature in addition to a high surface area-to-volume ratio. Those superparamagnetic iron oxide nanoparticles (SPION) are predominantly used as T2 or T2* contrast agents to detect tumors in magnetic resonance imaging (MRI) [1]. In MRI, the image quality is closely tied to the enhancement of contrast between two distinct tissue types. To artificially increase the contrast, SPION can be introduced inside the tumors via targeting methods to reduce the transversal relaxation time (T2), making the tumor appear darker on images.

In this work, the effect of the SPION shape [2] on T2 is theoretically studied in a high magnetic field at room temperature. Monte Carlo simulations of CPMG sequences using cubic nanoparticles ranging from 20 to 500nm have been simulated via a well-known methodology from reference [3]. The analytical magnetic stray field of cubic particles is implemented [4], and results are compared volume-wise to spherical particles to keep the magnetic moment constant. The diffusion of protons is modeled by a random walk and their spins as vectors rotating around the stray field of the cubic particles.

Our results indicate that the transverse relaxation time does not significantly change between a cubic particle and a spherical particle for sizes over 20 nm, corresponding to the static diffusion regime and the partial refocusing regime. For particles below the 20 nm threshold, corresponding to the motional average regime (MAR), a 10% increase in T2 is observed for the cubic nanoparticles.

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POSTER 14

Controlling the synthesis and understanding the structure of porous metal phosphonates via crystalline reference structures

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Porous metal phosphonates (PMPs) are a promising class of hybrid inorganic-organic materials with appealing properties such as a high specific surface area, an extended organic-inorganic network and versatility in tuning the bulk and interfacial chemical reactivity and properties. PMPs incorporating tetravalent metal ions with phosphonate coupling molecules introduce strong Lewis and Brønsted acids with chemically robust M-O-P bonds, providing high potential in catalysis and sorption.

The amorphous nature of PMPs, the variety of possible binding modes and the challenging reactivity control hinder their structural elucidation. In order to understand the correlation between the synthesis conditions and the resulting structure of disordered PMPs, a methodology based on crystalline metal phosphonates (*i.e.*, metal-organic frameworks (MOFs)) is studied. This way, analytical signatures in liquid- and solid-state NMR spectroscopy (^{31}P, ^{13}C, ^{1}H) can be correlated to their respective binding modes and chemical environments, and used as reference fingerprints to unravel amorphous PMPs. Hereto, the approach involves phosphonate grafting on a well-studied carboxylate-based MOF (*e.g.,* NU-1000) to obtain controlled metal-phosphonate environments. The Zr-nodes that compose the carboxylate MOF can be tuned via post-synthetic grafting with organophosphonic acids to synthesize a MOF with desirable properties for targeted applications.[2] (Figure 1) To benchmark the importance of the reference structures, a comparison with organophosphonic acid grafted TiO_2 (P25) has been realised and studied by solid-state ^{31}P MAS NMR. Hereby, grafted NU-1000 revealed sharper and less broadened ^{31}P signals than grafted P25, indicates the well-defined structural and chemical environments in the MOF reference structure (Figure 1).

The obtained analytical signatures will be further discussed in the framework of an improved PMP structural elucidation. It is expected that the structural features and binding modes can steer the development of both MOFs and amorphous PMP towards towards a broader applicability in acid-catalysed biorefinery reaction pathways.

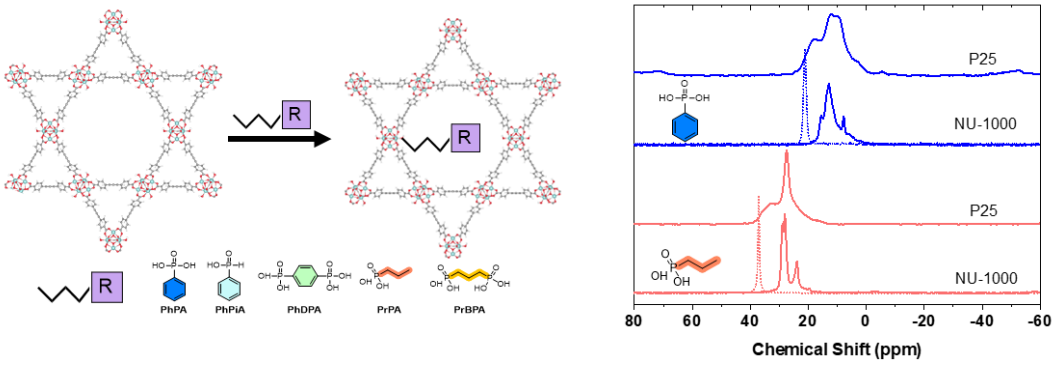

Figure 1 : Graphical representation of the phosphonate grafting approach the NU-1000 and solid state 31P MAS NMR of surface modified P25 TiO2 and postfunctionalised NU-1000 with organophosphonic acids; ppm calibration with KH_2PO_4 at 3.9ppm; Spinning at 14kHz

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POSTER 15

Analyzing mechanisms of action of vancomycin like glycopeptide antibiotics by solid-state nuclear magnetic resonance

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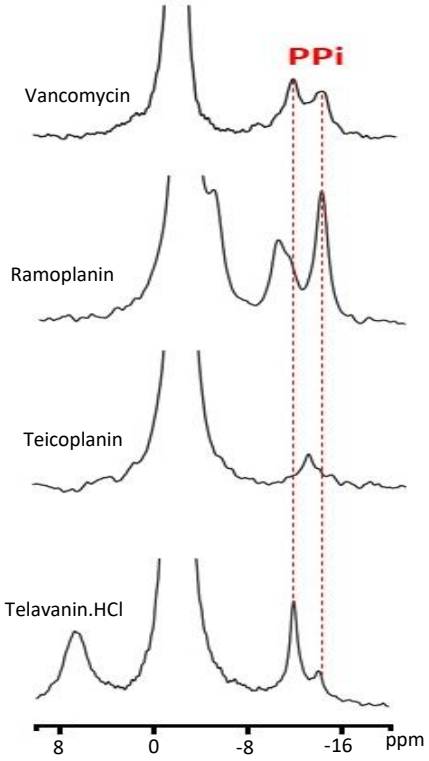
Glycopeptide antibiotics are used for the treatment of serious infections caused by Gram-positive pathogens including Methicillin-resistant Staphylococcus aureus (MRSA). These antibiotics inhibit the peptidoglycan synthesis. However, the emergence of resistance to glycopeptide antibiotics has led to various efforts and research to develop new antimicrobial therapeutics.

Previous studies have shown that vancomycin targets lipid II in bacteria membrane. Lipid II is an essential precursor of the bacteria cell wall synthesis. However, these studies have been conducted in the absence of membranes. Therefore, there is no precise picture of how vancomycin and related glycopeptides bind to lipid II in native conditions. Having knowledge about mechanism of action of these glycopeptide antibiotics at the molecular level and biological relevant membrane will enable us to design the next-generation glycopeptide antibiotics in order to overcome resistance.

In this study, we measured the interact of Vancomycin, Ramoplanin, Teicoplanin and Tevalanin.HCl, as glycopeptide antibiotics with lipid II by ^{31}P ssNMR. This measurement enables us to study this interact in native like condition. This preliminary 31PssNMR data suggests that these glycopeptide antibiotics target pyrophosphate in lipid II.



^{31}P solid-state NMR spectra , acquired in liposomes of lipid II bound to vancomycin, ramoplanin, teicoplanin and telavanin.HCl

POSTER 16

Towards magnetic resonance detection of mitochondrial reactive oxygen species in vivo in solid tumors.

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

In vitro, cyclic hydroxylamines and nitroxides can be used to study ROS production by cells by using electron paramagnetic resonance spectroscopy (EPR). [1] *In vivo*, nitroxides can be reduced to the corresponding hydroxylamine form or can be oxidized to oxoammonium cation by reactive oxygen species (ROS). This oxidoreduction can be modified by abnormal tissue redox conditions. [2] By monitoring the decay of the EPR signal of the nitroxide over time, the redox status *in vivo* can be assessed using low frequency EPR spectroscopy. [3] A nitroxide targeted to mitochondria (MitoTEMPO°) has been used to provide redox maps in Parkinson's disease and kidneys dysfunction in mice models but never in solid tumors models. [5]

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

We have worked by steps of increasing complexity and by comparison with another nitroxide, 3 Carbamoyl-Proxyl (3CP) which distributes both in extra- and intra-cellular compartments. First, we followed the decay of the signal of Mitotempo when superoxide is produced by an enzymatic system. After that, we worked on whole cells exposed to either L-Buthionine sulfoximine (a glutathione synthesis inhibitor) or Antimycin A (a mitochondria complex III inhibitor). We observed that the Mitotempo decay rate of the signal was increased when cells were exposed to antimycin A.
In vivo, we were able to follow the decay of the signal of the two nitroxides for 30 minutes after local injection in tumor.
We observed a change in the decay rate of 3CP but not Mitotempo when mice were treated for 2 days with L-BSO. Interestingly, we also observed a change in Mitotempo decay rate but not 3CP when mice were treated with Antimycin A for 2 days. *Ex-vivo* analysis allowed us to determine which part of the decay of the signal is due to blood-wash out or transformation of the nitroxide in its corresponding hydroxylamine form.

In further investigations, we will also assess the specificity of the method by using cell-models over-expressing SOD2 (mitochondrial isoform of superoxide dismutase) or a catalase variant targeting mitochondria.

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Radiosensitizing Magnetic Nanoparticles as a Targeted Theranostic Agent for Cancer Therapy

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[2]*Center for Microscopy and Molecular Imaging (CMMI)*
[3]*Medical Physics Department, Institute Jules-Bordet, Free University of Brussels (ULB)*
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High-Z nanoparticles (NPs) have attracted increasing attention as radiosensitizing substances since 2004. According to reports up to this point, these nanoparticles can accelerate tumor cell death in response to radiation, increasing the effectiveness of radiation therapy. The mechanism(s) underlying the radiosensitization effect of gold nanoparticles (GNPs) remain poorly known, with the majority of research focusing on physical impacts, despite some considerable studies conducted in the field (particularly focused on GNPs). Recent research points to specific metabolic processes as being crucial to the observed radiosensitizing impact [1]. The quality of radiosensitizing impact and the inhibition of one enzyme that act as a metabolic hub (*i.e.* the thioredoxin reductase (TrxR)) in cells treated with GNP have been found to be significantly correlated [2].

With respect to these considerations, our lab previously demonstrated that a similar enzymatic behavior could be observed in cells exposed to iron oxide nanoparticles (IONPs) which appear as a promising theragnostic nanoplatform due to their biocompatibility and magnetic properties [3]. As an improvement of our previously described nanoplatform, we evaluated *in vitro* the biological impact of various vectorization strategies for the IONPs.

To achieve this objective, we compared the internalization levels of IONPs between two cellular models namely a lung cancer derived cell line (A549) and a non-cancerous lung derived cell line (NL20) with a colorimetric assay involving the Prussian Blue reaction.

The inhibition of TrxR induced by IONPs was measured by measuring its activity using a commercial kit as previously described [3]. For this, the cell lines were incubated for 24 hours at a concentration of 50 µg Fe/mL with various formulations. 225kV X-rays were used to irradiate cells at a rate of 2 Gy/min (X-Rad 225 XL, PXi Precision x-ray, USA).

The Nuclear Magnetic Dispersion (NMRD) profiles created by a field cycling relaxometer (STELAR, Italy) were used to derive the longitudinal relaxation rates (R_1) of the previously synthesized PEGylated IONPs. A Biospec 9,4T (Bruker, Germany) was used to capture MR images of mice and phantoms. In CD1 mice, IONPs were intravenously given at a rate of 70 µmoles Fe/Kg.

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POSTER 18

X-Pulse Broadband NMR Spectrometer in a Benchtop Solution

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[2] Oxford Instruments Magnetic Resonance, U.K.
[3] Oxford Instruments, Concord, U.S.

The **X-Pulse** is a benchtop NMR spectrometer built around a 60 MHz permanent magnet. Combining many of the features and capabilities usually found on high-field systems, with the convenience and ease of use of a benchtop system. These include a fully tuneable broadband (X-)channel, an external ^{2}H lock, three-axis gradients, a user removable probe; and the option to operate in a variable temperature configuration, with a flow cell, or with a 25 position autosampler. This flexibility provides great potential for increasing the usefulness of benchtop NMR spectroscopy in both industrial and academic environments.

X-Pulse is a true broadband system which gives the user the opportunity to manually adjust the tuning and matching to individual samples. This includes such nuclei as: ^{13}C, ^{31}P, ^{11}B, ^{7}Li, ^{23}Na, ^{27}Al and ^{29}Si all observable on a single instrument. The standard **X-Pulse** can provide a wide range of one-dimensional experiments such as DEPT; PGSE (Pulsed field Gradient Spin Echo) for measurement of diffusion coefficients; Inversion Recovery and CPMG, for measurements of T1 & T2 respectively (run with or without heteronuclear decoupling, or with solvent suppression) as well as two-dimensional homo- and hetero-nuclear correlation experiments including COSY, TOCSY, NOESY, HSQC-ME, HMBC, and HMQC. In addition, the system is capable of true variable temperature operation in a range of at least 0°C to 65°C, with the sample temperature varied using a temperature-controlled external gas flow while maintaining the magnet temperature constant.

POSTER 19

YBMRS 2023

A realistic outer membrane model of the bacterium *Escherichia coli*

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The rapid emergence of Gram-negative 'superbugs' adds significantly to the antimicrobial resistance (AMR) crisis and has an overwhelming impact on human health worldwide. The development of novel drugs that can overcome AMR requires the understanding of the atomic-scale structural processes that drive the killing mechanisms. Gram-negative bacteria possess unique characteristics related to the cell envelope which make it a daunting task to design new drugs against them.

The cell envelope of Gram-negative bacteria consists of an inner membrane (IM), a thin peptidoglycan (PG) cell wall and an outer membrane (OM), with the latter being the target of lipopeptide antibiotics of last resort for treating multi-drug resistant infections (e.g., polymyxins). The OM is a unique asymmetric bilayer and the complex lipopolysaccharides (LPS) render its structure and dynamics still poorly understood.

In this study, we present a computational approach to build a realistic and asymmetric OM model of the Gram-negative bacterium *Escherichia coli* (*E. coli*) using all-atom molecular dynamics simulations and high-performance computing. Resistant *E. coli* strains are frequently associated with extra-intestinal human infections, e.g., bloodstream or urinary tract infections. This model will serve for the understanding of the killing mechanism of antibiotics that target the OM, through the integration of solid-state NMR and computational chemistry approaches. Highlighting the molecular mechanisms that lead to AMR in pathogenic Gram-negative bacteria can provide the rational for designing new therapeutic strategies.

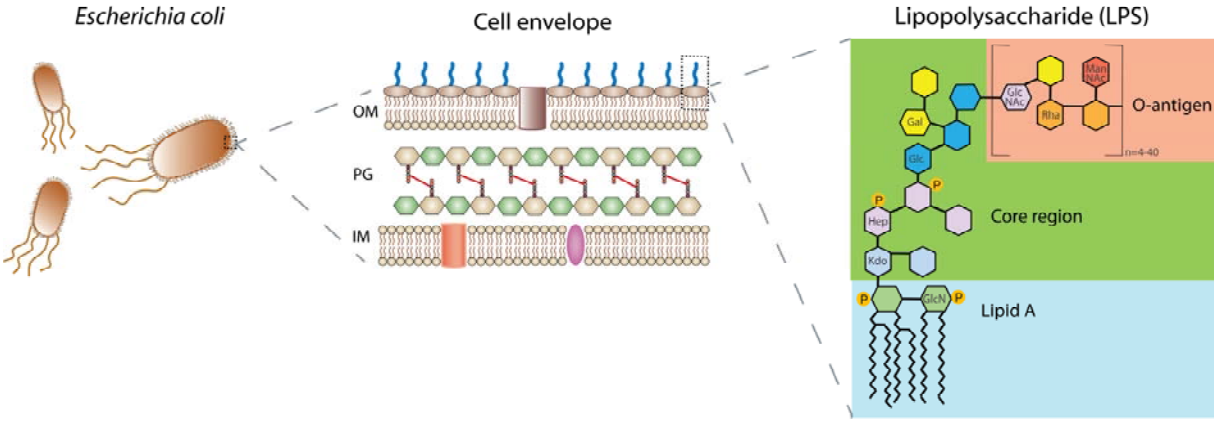

Figure: The cell envelope of *E. coli* along with the LPS structure with the R1 type core region and the O1 antigen type that was used in the current study.

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COMBINING THEORY AND EXPERIMENTS: PRELIMINARY RESULTS OF SPECTRA VALIDATION THROUGH DFT STUDIES OF METAL-PHOSPHONATE PROPERTIES IN NU-1000 STRUCTURE

Roberth Mateo Narvaez[1,2], Jennifer Theissen[3,4], Nick Gys[1,3], Elien Derveaux[4], Peter Adriaensens[4], Wouter Marchal[4], Rob Ameloot[3], Tom Hauffman[1], and Frederik Tielens[2]

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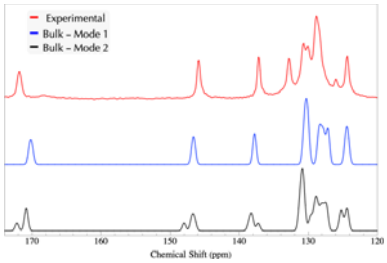
Metal-organic frameworks (MOFs) have emerged as a revolutionary class of materials with remarkable versatility and wide-ranging applications across multiple scientific disciplines. MOFs, consisting of metal nodes interconnected by organic linkers, possess exceptional tunability, high surface areas, and intriguing porosity. These distinctive characteristics bestow upon MOFs extraordinary properties, making them highly desirable for diverse applications encompassing gas storage and separation, catalysis, sensing, and drug delivery systems.

Amid the extensive assortment of MOFs, the NU-1000 structure has garnered substantial attention due to its exceptional properties and potential in various fields. NU-1000 is an exceptionally sturdy and highly stable MOF characterized by a well-defined structure comprising 558 atoms (264 C, 180 H, 96 O, 18 Zr) within its unit cell. The deliberate design and synthesis of NU-1000 provide a platform for investigating the fundamental principles governing MOF behavior and offer a pathway for the development of advanced functional materials.

In this study, density functional theory (**DFT**) calculations are employed to investigate the electronic structure, adsorption energies, and structural parameters of the NU-1000 metal-organic framework (**MOF**) in the presence of phenyl phosphonic acid (PhPA) molecules adsorbed on its metallic nodes. Molecular dynamics simulations, accounting for temperature effects, are utilized to compute infrared (**IR**) spectra, while nuclear magnetic resonance (**NMR**) and X-ray photoelectron spectroscopy (**XPS**) spectra are also calculated. A comparison between the simulated and experimentally measured spectra establishes the reliability of the DFT calculations. This work, conducted as part of the PHOSPORE project, advances the understanding of MOFs and facilitates the tailored design of MOFs for a wide range of applications in catalysis and functional materials.

(a) 

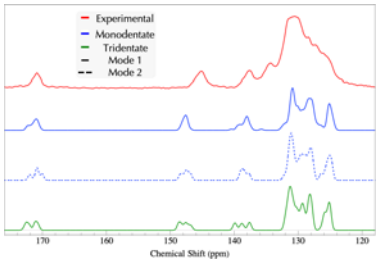
(b) 

Figure1. Comparison of theoretical-experimental ^{13}C NMR spectra of the bulk structure (a) of NU-1000, and most probable structures in presence of PhPA adsorbed on its metallic nodes (b).

POSTER 21

Rapid Structural Elucidation Of *Pseudomonas* Cyclic Lipopeptides: Methodology, Results, and Relevance

Niels Geudens[1], Durga Prassad[1], Penthip Muangkaew[2], Vic De Roo[1], Yentl Verleysen[1,2], Benjámin Kovács[1], Lu Zhou[3], Léa Girard[4], Catherine Cesa-Luna[4], René De Mot[4], Monica Höfte[3], Annemieke Madder[2] and José C. Martins[1]

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Cyclic lipopeptides (CLiPs) are secondary metabolites that are produced and secreted by a range of bacterial genera including *Pseudomonas* and *Bacillus*. Well over 100 CLiPs originating from *Pseudomonas* spp. have been described at varying levels of structural and biological activity details and their numbers keep rising. Structural variations are very diverse, including total amino acid sequence length, size of the macrocycle, amino acid identity and stereochemistry (e.g. D- vs. L-amino acids). [1,2]

CLiPs and their producing bacteria are ubiquitous in Nature and reports detailing the discovery of novel or already characterized CLiPs from new sources appear regularly in literature. However, the lack of characterisation detail threatens to cause considerable confusion. Using NMR fingerprint matching, we have introduced a rapid and facile way of characterizing existing CLiPs coming from novel bacterial sources. [3] Using this approach, the identity of CLiPs can be established by simple comparison of their NMR spectral fingerprint recorded under standardized conditions. All reference NMR data is made freely available on https://www.rhizoclip.be, such that researchers can quickly match data of their newly isolated compounds with that of the reference compounds, leading to facile dereplication.

This NMR fingerprint approach led to stereochemical characterization of many different Pseudomonas [3,4], including MDN-0066 (8:6), orfamides (10:8), xantholysin (14:8), putisolvin (12:4), newly isolated tanniamide (12:10), and entolysins (14:5). For the latter, it was established that two configurational isoforms, that only differ by D/L configuration of the same serine residue, are biosynthesized by a single NRPS enzyme, indicating the presence of an intermittently epi-active E/C domain. The validation of stereochemical make-up of the CLiPs is a first crucial step in determining their 3D structure and structure-activity relations.

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POSTER 22

Enhanced non-invasive detection of melanin radicals in melanomas *in vivo* and *in vitro* by Multi-Harmonic Electron Paramagnetic Resonance (EPR)

Mohammad Wehbi[1], Evelyne Harkemanne[2], Lionel Mignion[3], Nicolas Joudiou[3], Isabelle Tromme[2], Jean-François Baurain[4], and Bernard Gallez[1,3*]

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

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

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Probing Water Confined in Nanopores: Integrating Dielectric Measurements and NMR Spectroscopy

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Water is a green solvent for polar compounds in numerous chemical and biological processes. However, its high polarity limits its ability to solubilize non-polar compounds, thus very often demanding the use of environmentally harmful organic solvents when the use of water is not possible for a targeted chemical/industrial process. Enhancing water's capacity to dissolve non-polar compounds is a promising avenue for minimizing the chemical industry's dependence on hazardous solvents [1].

The solvation power of water is governed by its hydrogen-bonding network. Under nanoconfinement, the hydrogen bonding network of water is drastically modified, opening up the possibility to tune its solvation properties [1]. The dielectric permittivity is a quantity directly impacted by modifications in the hydrogen-bonding network of solutions and is closely linked to the solvation properties of liquids. For instance, while bulk water features a high room temperature dielectric permittivity of 80, aliphatic alcohols such as 1-propanol, 1-pentanol, and 1-octanol, in which the presence of the organic chain hinder the formation of extensive hydrogen of a comprehensive hydrogen bonding network, feature dielectric permittivity's as low as 21, 14 and 10, respectively[2].

For the present investigation, we developed an *in situ* multi-diagnostic method to probe both the NMR spectrum and the dielectric properties of water under confinement using standard Magic Angle Spinning (MAS) NMR probes. I will discuss the fundamentals of this technique and proof of concept experiments demonstrating its validity. Further, I will show that confinement of water in the micropores of MFI-type zeolites of different Si/Al ratios results in a drastically decreased dielectric permittivity of the nano-confined water phase. Confined in these micropores water features values as low as those usually found for organic solvents like methanol, ethanol, and 1-propanol. The NMR investigation of these confined systems backs the interpretation of the results. In the micropores of these zeolites, water adsorbs to Brønsted acid sites and defect sites, which decreases the polarizability of the water molecules involved in the interaction. Aside from confined water systems, we expect that the new *in situ* multi-diagnostic platform developed by us will also benefit the fields of battery research, food quality control, and sensing, where both NMR and EIS are common characterization techniques.

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POSTER 24

Evaluation of hyperosmolar Blood-Brain Barrier opening in Glioblastoma using histology with Evans Blue and DCE-MRI

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Glioblastoma (GB) is the most common and aggressive malignant tumor of the central nervous system. This tumor shows a high proliferation rate, variability in tumor histopathology, and diffuses in the adjacent brain tissue (parenchyma), making GB a very challenging cancer to treat. While the Blood-Brain Barrier (BBB) is often compromised in GB, the perfusion and consequent delivery of drugs are highly heterogeneous. Moreover, the accessibility of drugs is largely impaired in the margins of the tumor and for infiltrating cells at the origin of the tumor recurrence[1]. In this work, we have evaluated if a hyperosmolar shock[2] may change hemodynamics parameters in the core and the margins of a tumor in a GB model. The osmotic shock was induced with an intracarotid infusion of a hypertonic solution of mannitol in mice grafted with U87MG cells. To evaluate the effect of the hyperosmolar shock, the distribution of a fluorescent vascular leakage marker (Evans Blue) within the brain was assessed by histology[3]. Dynamic contrast-enhanced (DCE)-MRI[4] with an injection of Gd-DOTA as contrast agent was also used to evaluate effect on hemodynamic parameters and diffusion of the contrast agent outside the tumor area.

The histological study revealed that the fluorescent dye diffused much more largely outside the tumor area after osmotic shock than in control tumors. However, the study of tumor hemodynamic parameters by DCE-MRI did not reveal any change in the permeability of the BBB, whatever the MRI parameter studied.

The use of hypertonic mannitol infusion seems to be a promising method to increase the delivery of compounds in the margins of GB. Nevertheless, the DCE-MRI analysis method using gadolinium-DOTA as contrast agent seems of limited value for determining the opening of the BBB in GB after an osmotic shock.

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POSTER 25

Title: Mechanism of enzyme inhibition by nanobody: from molecular structure to molecular dynamic

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The most common and important mechanism of bacterial resistance to penicillin-based antibiotics is the production of β-lactamases that inactivate β-lactam ring of penicillin. TEM-1 is one of the most well-studied and diversified β-lactamases [1]. At the present time, more than 200 mutants of TEM-1 were detected [1]. The emergence of these mutants led to search for β-lactamase inhibitors to develop diagnostic tests. In this purpose, we have initiated a study in order to use nanobody cAb_{TEM-1}, as β-lactamase inhibitor [2]. First step of this study was the understanding of TEM-1 inhibition mechanism by the nanobody.

To understand this inhibition mechanism, we have used a multidisciplinary approach such as enzyme kinetics, crystallography, *in-silico* molecular dynamics and NMR. X-ray diffraction of the bounded enzyme TEM-1/cAb_{TEM-1} has revealed that, the nanobody takes place near one loop that borders the entrance of active site. This loop known as the “hinge region” (residues 213-218), has been shown to be dynamic in the free enzyme [3-4]. Interestingly, x-ray structure of the bounded enzyme has revealed rigidification of the hinge region. This rigidification was seen as well by *in-silico* molecular dynamics.
In order to confirm this rigidification and to understand the inhibition mechanism, NMR study of the proteins molecular dynamic has been initiated. Nuclear spin relaxation study of the free and bounded enzyme at us-ms timescale, has partially validated molecular dynamic results. In addition, NMR relaxation results has highlighted dynamic perturbation distant to the “hinge region”.

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Study of Protein Dynamics by NMR Relaxation Experiments

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Nuclear Magnetic Resonance (NMR) relaxation experiments can provide major insights into the study of protein dynamics. Over the years, various experiments have been developed to measure the dynamic phenomena that animate proteins. Relaxation experiments such as R_1, R_2 and *hn*NOE provide information on the dynamics occurring at the picosecond to the nanosecond time-scale [1]. Other experiments like relaxation dispersion (R_2 CPMG) and CEST allow to measure dynamics taking place at the microsecond to millisecond time-scale [2]. The complementarity of these experiments allows to gain a general view of the dynamic phenomena exhibited by proteins.

To explore the importance of these experiments, the dynamics of three different proteins, varying in size, have been studied through the forementioned relaxation experiments. The smallest proteins studied are Cold Shock Proteins or CSP (14kDa), a class of proteins produced by organisms when they are submitted to sudden temperature drops [3]. The second protein investigated is TEM-1 (29kDa), a beta-lactamase responsible for the resistance to antibiotics [4]. Finally, a complex of TEM-1 bound to a nanobody, which drastically increases the size of the protein to 45kDa, will be studied [5]. Performing various NMR relaxation experiments on these samples will reveal the potential of these experiments for the measure and characterization of the dynamics that animate various proteins.

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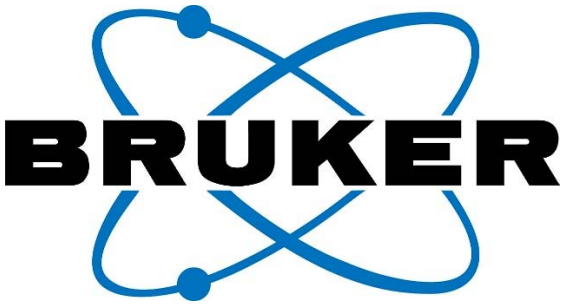
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