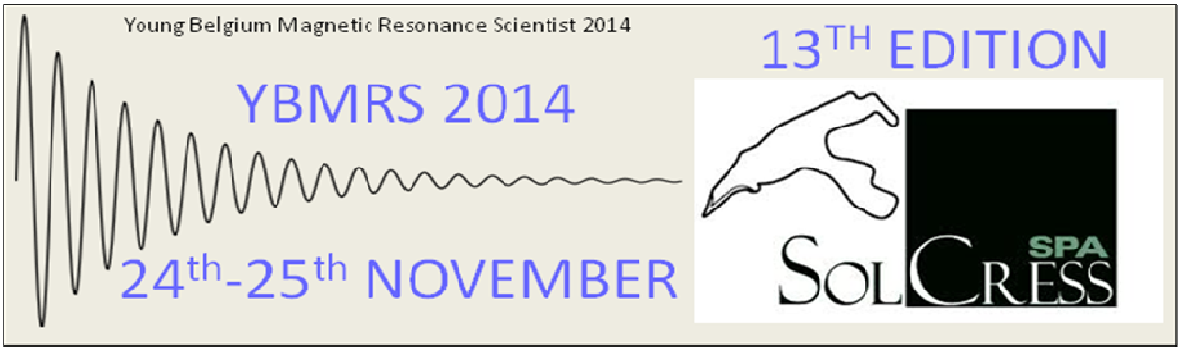


# PROGRAM AND ABSTRACT BOOK

Dear YBMRS Participant,

13TH EDITION

Welcome to the 13th edition of the Young Belgian Magnetic Resonance Scientist symposium series!
We thank you for joining us and hope you will enjoy the conference, as well as your stay here in the heart of the most beautiful Belgian forest. The organizers have strived to present an attractive program, including two educational sessions, two invited lectures and a selection of short oral communications on a wide variety of topics. As always, the goal has been to promote the work of young scientists working in any field related to magnetic resonance, and to create opportunities for interesting discussions and contacts.

## General practical information

All scientific activities are organized around the "Pierre le Grand" conference room, which can be found to the right from the main in the lobby at the level -1. All participating sponsors are located in the meeting area of the Sol Cress, where coffee breaks will be served during the poster sessions and in between the morning sessions. A wardrobe is available, however we urge you not to leave any valuables as we cannot guarantee it will be guarded at all times. Should you have any question during your stay, please address yourself to the conference desk located in the meeting area.

## During sessions

- Please ***switch off your mobile phone*** or put it in silent mode.
- ***Do not take pictures of slides or posters***, but rather approach the author during the meeting and request a reprint or additional information.
- Have the courtesy ***not to use your laptop in the conference room during talks***, but rather use the main lobby, foyer or bar.

## Poster sessions

All posters are to be set up in the "Wellington" and "Sources de la Reine" rooms. The posters panels are numbered. Your poster number is available from the abstract book or on the list provided in the poster room. Participants presenting a poster are kindly requested to set up their poster no later than the lunch Break on Monday, and to remove them after the coffee break on Tuesday afternoon. Transparent adhesive tape is available in the poster room to fix your poster to the panels. Please do not use staples or pushpins in any circumstances.

## Rooms

If you have not done so, please register for your room at the main desk in the lobby of the Sol Cress.
**Check out time:** Please note that all rooms should be vacated no later than 10:00 a.m. on Tuesday morning.

A closed luggage room, next to the poster room, will be available on Tuesday for safekeeping of your luggage. Should you have any question or request concerning your accommodation, please contact the Soll Cress staff.

### Breakfast, lunch and dinner

Breakfast and lunch will be served in the Restaurant. Please follow the indications from the main lobby. Breakfast is served from 07:30 until 09:00 on Monday and Tuesday morning. Lunch starts immediately after the morning session and consists of a buffet with beverages on Monday and of a hot meal on Tuesday.
The main conference dinner will start at 20:30 on Monday evening, with a choice between a vegetarian or a non-vegetarian menu, as indicated during your registration.
If you have any additional dietary requirements, please contact the Sol Cress staff.

### Internet access

Free wireless internet access is available in the entire domain.

### YBMRS party

A party will be organized after dinner on Monday evening, starting around 10 p.m. and featuring the House DJ.
*At the request of the Sol Cress, and as a courtesy to the other guests, please note that the party is set to close at 01:00.*
Please note that the bar remains available to provide more tranquil surrounding. If you have further questions, please contact the conference desk in the foyer.

Yours sincerely,

### The OrganizingCommittee

- Luce Vander Elst, *Université de Mons*
- Christian Damblon, *Université de Liège*
- Céline Henoumont, *Université de Mons*
- Uwe Himmelreich, *Katholieke Universiteit Leuven*
- Yves Gossuin, *Université de Mons*
- Q.L. Vuong, *Université de Mons*
- J. Martins, *Universiteit Gent*

### The Scientific Committee

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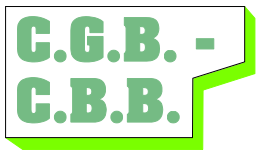
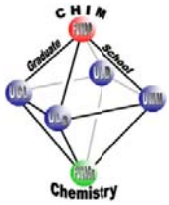
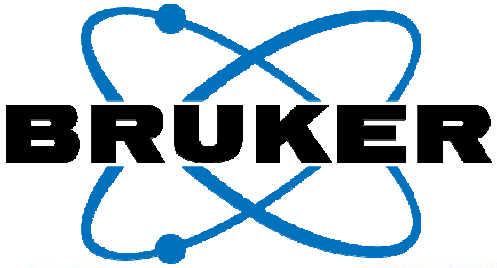
- Nico Van Nuland, *Vrije Universiteit Brussel*

***The organizing committee gratefully acknowledges the following institutions, societies and companies for their kind support***








# Symposium Program

**Monday, 24th November**

9:00 ***Registration, Welcome Coffee and Poster Setup***

10:15 ***Welcome***

10:20 ***Educational Session 1***

**Rudi WILLEM (VUB)**

**High Resolution Magic Angle Spinning (hr-MAS) NMR: investigation of solid-liquid interfaces in materials**

11:20 ***Break***

11:35 ***Educational Session 2***

**Claudio Luchinat (University of Florence)**

**Paramagnetic NMR: a versatile tool in structural biology (Part 1)**

13:00 **Lunch**

14:00 **Symposium Opening**

***Plenary Session 1***

14:10 **Claudio Luchinat (University of Florence)**

**Paramagnetic NMR: a versatile tool in structural biology (Part 2)**

15:20 J. De Roo (Universiteit Gent) "**The surface chemistry of metal oxide nanocrystals; a solution NMR study"**

15:40 D. Henrard (Université de Mons) **"Characterization of iron oxide nanoparticles by NMR relaxometry and magnetometry: effects of size distribution and agglomeration"**

16:00 M. Harris (KU Leuven) **"Dysprosium and terbium magnetofluorescent micellar Complexes as potential bimodal agents for magnetic resonance and optical imaging"**

16:20 ***Poster session 1 – Coffee break***

***Plenary session 2***

17:40 E. Brunetti (Université Libre de Bruxelles) "**NMR Study of the Recognition Properties of a Calix[6]aza-cryptand Incorporated in DPC Micelles"**

18:00 M. Dentamaro (Université de Mons) **"Targeting of Apoptotic Cells by a New Bimodal Probe Based on AGuIX® Nanoparticles"**

18:20 E. Louis( Hasselt University) **"Validation of 1H-NMR-based metabolomics as a tool to detect lung cancer in human blood plasma"**

*19:30* ***Reception***

*20:30* ***Dinner and YBMRS Party***

**Tuesday, 25th November**

07:30 ***Breakfast***

***Plenary session 3***

09:00 D. Buyst (Universiteit Gent) **"Identification of a pKa-regulating motif stabilizing Imidazole modified double stranded DNA"**

09:20 R. Lavendomme (Université Libre de Bruxelles) **"Characterization of calixarene derivatives by liquid-state NMR spectroscopy: challenges and solutions"**

09:40 M. Tassi (Hasselt University) **"Characterization of phosphonic acid grafted titanium dioxide surfaces by 31P NMR and ATR-FTIR"**

10:00 J. Kusakovskij (Universiteit Gent) **"Study of transient radicals created immediately after room temperature X-ray irradiation in single crystal sucrose"**

10:20 **Gérald REMAUD (Université de Nantes)**
**Isotopic NMR and analysis of food : $^{2}H$ and $^{13}C$ NMR**

11:20 ***Poster session 2- Coffee Break***

*13:00* ***Lunch***

***Plenary session 4***

14:00 **Klaas Nicolaij (Eindhoven University of Technology)**
**MR techniques for guiding cancer therapy**

15:00 B. Cuypers (University of Antwerp) **"An investigation of Antarctic fish cytoglobins using EPR and optical spectroscopy"**

15:20 R. Garcia Ribeiro (KU Leuven) **"Synthesis and characterization of functionalized magnetoliposomes for Magnetic Resonance Imaging and theranostics applications"**

15:40 N. Sauwen (KU Leuven) **"Hierarchical non-negative matrix factorization for brain tumor characterization using multi-parametric MRI"**

*16:00* ***Coffee break***

16:20 A. De Rache (Université de Bordeaux) **"G-quadruplex in the HIV promoter region: UV-spectroscopy and NMR insight"**

16:40 A. Kretschmer (Dow Corning) **"Application of NMR Spectroscopy in the Development of Silicone Materials"**

*17:00* ***Awards/ Closure***

**LIST OF POSTERS**

P1 F. Chain, R. Ribić, D. Sinnaeve, J. C. Martins, S. Tomić, K. Fehér
**Targeted delivery of peptidoglycan immunomodulators using liposomal carriers: NMR study of the lipid encapsulation**

P2 M. De Vleeschouwer, N. Matthijs, D. Sinnaeve, T. Coenye, J.C. Martins, A. Madder
**First insights in the structure-function relationship of a natural cyclic lipodepsipeptide by synthetic modification and NMR investigation**

P3 Matthias Ceulemans, Wim De Borggraeve, Luce Vander Elst, Sophie Laurent, Robert N. Muller and Tatjana N. Parac-Vogt **Synthesis of bimodal MRI contrast agents based on metallostar complexes**

P4 G. Circelli , L. Vander Elst , R. N. Muller , S. Laurent
**Design of contrast agent for neurodegenerative diseases diagnosis**

P5 C. Desmet, A. Lafosse, S. Vériter, Ph. Levêque, D. Dufrane and B. Gallez
**Development of EPR oximetry in diabetic wound healing models**

P6 S. Delangre, Q.L. Vuong, C. Po, B. Gallez and Y. Gossuin
**Theoretical and Experimental study of the Off-Resonant Saturation, an MRI Sequence for Positive Contrast With Superparamagnetic Particles**

P7 N. Geudens, K. Fehér, M. De Vleeschouwer,J-M Crowet, M.N. Nasir, A. Madder, L. Lins, J.C. Martins and D. Sinnaeve
**Membrane interactions of natural cyclic lipodepsipeptides**

P8 L. Fusaro, M. Luhmer, G. Casella and A. Bagno
**$^{17}$O NMR Study of Diamagnetic and Paramagnetic Lanthanide(III)− DOTA Complexes in Aqueous Solution**

P9 L. Capette, S. Laurent, L. Granato, R. N. Muller and L. Vander Elst
**Design and characterization of a dendrimeric contrast agent dedicated to the imaging of the nervous central system**

P10 S. Gillet, M. Aguedo, C. Blecker, N. Jacquet, A. Richel
**Use of $^{13}$C-NMR in structural elucidation of polysaccharides: case of locust bean gum.**

P11 A. Hannecart, L. Vander Elst, R. N. Muller, S. Laurent
**Conception of superparamagnetic polymersomes for potential drug delivery and magnetic resonance imaging applications**

P12 J. Kay, D. C. Thorn, C. Pain, N. Scarafone, C. Huynen, S. Preumont, A. Corazza, C. Damblon, and M. Dumoulin
**Investigation of the effects due to the insertion of a polyQ tract increasingly long on the structure and dynamic of BlaP using NMR spectroscopy**

P13 V. Marchand, J. Magat, G. De Preter,P. Sonveaux, B. Jordan, B. Gallez.
**Evaluation of extracellular pH andenergetic status response tohyperglycemia and MIBG treatment on oxidative versus glycolytic tumor phenotype: An *in vivo* 31P MRS study**

P14 G. Maniet, N. Jacquet, S. Gillet, A. Richel
**Impact of steam explosion treatment on chemical configuration of Festuca L. lignin : structural elucidation using NMR spectroscopy**

P15 C. Henoumont, S. Laurent, R.N. Muller, L. Vander Elst
**DOSY in HR-MAS : a tool to be used with caution.**

P16 U. B. le Paige, P. S. Mercuri, A. I. Karsisiotis, J-D. Docquier, M. Galleni, C. Damblon
**Metalloβ-lactamase Cau-1 : Interaction with Adenosine Triphosphate and Metal-binding studies**

P17 R. Michez, L. Fusaro, M. Luhmer, Th.Doneux and C. Buess-Herman
**Electrochemical degradation of imidazolium based ionic liquids studied by NMR spectroscopy**

P18 Maité Callewaert, Cyril Cadiou, Valérie G. Roullin, Elodie Millart, M.C. Andry, Christophe Portefaix, Michael Molinari, Françoise Chuburu, Robert. N. Muller, Sophie Laurent, Céline Henoumont, Luce Vander Elst
**A nanohydrogel approach to boost the relaxivity of conventional MRI Gd contrast agents**

P19 M.-A. Neveu, V. Bol, A. Bol, V. Grégoire and B. Gallez
**Impact of oxygenation status on $^{18}$F-FDG uptake inside solid tumors**

P20 D. Sinnaeve
**Simultaneous solvent and J-modulation suppression in PFGSTE diffusion measurements**

P21 I. Nevjestić, H. Depauw, K. Leus, V. Kalendra, I. Caretti, G. Jeschke, S. Van Doorslaer, F. Callens, P. Van Der Voort, H. Vrielinck
**Confirming vanadium dopant incorporation in an Al-Metal-Organic Framework**

**MIL-53 by EPR and ENDOR spectroscopy**

P22 S. Montante, L. Vander Elst, R. N. Muller, S. Laurent
**Nanodiamond Particles for Medical Imaging: Chemical Oxidative Treatment and Analysis of the Surface**

P23 D. Sinnaeve, K. Van Hecke, I. Van Driessche and P. Lommens
**Analysis of the multimerization of $Cu^{2+}$ complexes in aqueous solution by $^{1}H$ NMR and Evans' method**

P24 L. Van Lokeren, C. Stassen, G. Desmet, K. Broeckhoven, S. Eeltink
**Investigation of Total Pore-Blocking Conditions in Polymer Monolithic Columns**

P25 C. Wauters, A. Tatton, T. Defize, P. Lecompte, C. Damblon
**Solid-state NMR characterisation of a shape memory material**

P26 D. Stanicki , S. Boutry, L. Vander Elst, R. N. Muller , S. Laurent
**Polysiloxane coated nanoparticles, an innovative platform for bimodal molecular imaging**

P27 N. Álvarez, G. Alejandro, J. Gómez, E. Goovaerts and A. Butera
**Relaxation dynamics in ferromagnetic resonance for chemically disordered FePt thin films**

P28 A.Weerasekera, T. Dresslaers, D M Sima, U.Himmelreich
**Non-invasive assessment of the onset and progression of amyotrophic lateral sclerosis in transgenic animal models using magnetic resonance spectroscopy.**

P29 M. Retout, Th. Doneux, G. Bruylants
**Development of a colorimetric biosensor specific to the oncoprotein Mdm2**

P30 A. S. Tatton, R. Wechselberger, H. Nova De Armas and C. Damblon
**Using solid-state NMR to characterise pharmaceutical polymorphs**

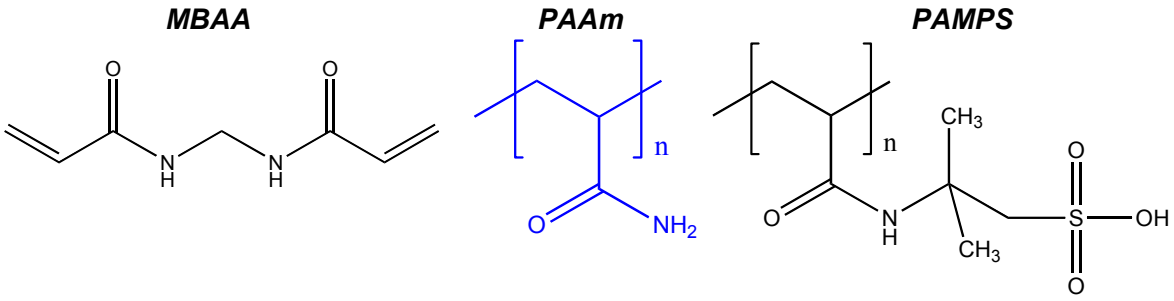
# INVITED LECTURES

# High Resolution Magic Angle Spinning (hr-MAS) NMR: investigation of solid-liquid interfaces in materials

Rudolph WILLEM

*High Resolution NMR Centre, Department of Materials and Chemistry, Vrije Universiteit Brussel, Pleinlaan 2, 1050 Brussel (Belgium)*

High resolution Magic Angle Spinning (hr-MAS) NMR, applied to proton, carbon-13 and other nuclei, enables *in situ* analysis at solid-liquid interfaces of chemical functionalities grafted onto insoluble solid supports and dipped in a suitable solvent.[1] Swelling of the solid material by the solvent ensures fast isotropic rotational and/or conformational mobility of the graft, resulting in natural cancellation of dipolar and chemical shift anisotropy interactions for the graft but not for the solid support. Hence, upon application of usual liquid NMR techniques, 1D or 2D spectra of only the mobile graft are obtained within standard chemical shift ranges of liquids, resonances from the solid support being smeared out into the noise of the spectral baseline. Magic Angle Spinning at moderate frequencies (2 – 4 kHz) cancels residual line broadening due exclusively to magnetic susceptibility heterogeneity at the interface, while application of diffusion filters edits resonances from grafts, those of translationally mobile species being suppressed. These techniques are illustrated on synthesis and activity monitoring of grafted organotin catalysts used, in particular, in ring opening polymerization of ε-caprolactone.[2] The power of the technique is also emphasized by the ability to observe unusual $^{1}J(^{1}H\text{-}^{14}N)$ and $^{1}J(^{2}H\text{-}^{14}N)$ scalar couplings in $^{1}H$ and $^{2}H$ hr-MAS NMR spectra of Double Network (DN) hydrogels composed of *poly*(2-acrylamido-2-methyl-1-propanesulfonic acid) (***PAMPS***) and *poly*(acrylamide) (***PAAm***) cross-linked by N,N'-methylene *bis*(acrylamide) (***MBAA***).[3] This enabled not only a complete identification of the chemical structure and morphology of such materials, but also insight into the origin of the exceptional mechanical strength and stress resistance of such materials in comparison with single network gels.[3]

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# Paramagnetic NMR: a versatile tool in structural biology

**Claudio Luchinat**

CERM and Department of Chemistry, University of Florence,
via Sacconi 6, 50019 Sesto Fiorentino, Italy

Paramagnetic centers such as metal ions have long been known to strongly affect NMR parameters and sometimes even prevent signal detection. For this reason, in structural biology studies in solution, metal ions are often avoided. Indeed, the presence of a paramagnetic center in *e.g.* a protein increases the linewidths of the protein nuclei and therefore makes it more difficult to detect signals and collect the NMR-based restraints necessary to solve the three-dimensional structure. At the same time, however, paramagnetism-induced relaxation, contact and pseudocontact shifts, residual dipolar couplings and cross-correlation effects provide novel restraints that can compensate for the losses in diamagnetic restraints. In addition, being of a different nature, paramagnetism-based restraints can provide information that cannot be possibly obtained otherwise. The purpose of my lectures is to provide the basic information necessary to plan the experiments in such a way as to minimize the adverse effects and maximize the extra structural information. To do so, the various paramagnetic effects and their physical principles will be reviewed, and their exploitation described with examples from the author's own experience. Then, examples from recent work carried out at CERM, both in solution and in the solid state, will be presented.

It has to be kept in mind that about 1/3-1/4 of all proteins encoded in the genomes are metalloproteins, a non-negligible fraction of which contain paramagnetic metals. So, sometimes dealing with metals cannot be avoided. It should be also appreciated that, even in the absence of paramagnetic line broadening, *i.e.* when the metal is diamagnetic, the very presence of the metal prevents the obtainment of NOE restraints: a metal ion surrounded by liganded protein side chains constitutes a "black hole" across which NOEs are hardly measured. Furthermore, with a few exceptions, metal-protein restraints are not available, so that the metal coordination cage is often ill-determined. In this case, substitution of the diamagnetic metal with a paramagnetic one may provide a dramatic improvement in i) defining the protein ligands and ii) defining the metal coordinates in the structure. The first information can be provided by contact shifts, as they depend on the presence of metal-donor atom coordination bonds, while the second is provided by pseudocontact shifts. Finally, contact shifts can provide information on dihedral angles, in much the same way as $^{3}J$ coupling measurements do in diamagnetic systems.

Another instance where paramagnetic relaxation enhancement may be beneficial is in the study of relatively weak protein-protein and protein-small molecules interaction, where the strong relaxation enhancement permits the detection and the partial characterization of the interaction even in the presence of high molar ratios between unbound and bound forms.

One of the most promising exploitations of paramagnetism in metalloproteins is based on the combination of the various pieces of information derived from the anisotropic magnetic susceptibility tensor (pseudocontact shifts and residual dipolar couplings) to learn about the relative degrees of freedom of one protein domain with respect to another. Broadly speaking, NMR is in principle able to provide information on unstructured or partially structured protein systems, thereby complementing other structural techniques. More and more efforts are

dedicated to understand the behavior of unfolded proteins that may be natively lacking tertiary structure. In parallel, there is a continuing interest in understanding the dynamics of proteins that perform their function by changing their structure. The presence of a paramagnetic metal ion helps acquiring information on, *e.g.*, global order parameters of one domain with respect to another, or on the relative population of different conformers.

Finally, paramagnetic effects have been recently shown to be a very promising tool for the determination of protein structures by solid state NMR. Indeed, Curie relaxation, often the major source of paramagnetic line broadening, is absent in the solid state. Pseudocontact shifts are measured as easily as in solution, while distance restraints of NOE type are much less readily obtained. As a consequence, the relative importance of pseudocontact shifts as structural restraints is higher in the solid state than it is in solution. Furthermore, when the magnetic susceptibility tensor is strongly anisotropic, the pseudocontact shifts in microcrystalline materials may reach out neighboring molecules in the crystal, permitting the obtainment of information on the reciprocal disposition of the molecules, in a sort of “NMR crystallography”.

# Isotopic NMR and analysis of food : $^{2}H$ and $^{13}C$ NMR

Gérald S. Remaud

EBSI Team, CEISAM UMR-CNRS 6230, University of Nantes, France.

The traceability of a given product may be defined as the "ability to trace the history, application or location of manufactured or distributed products" [1]. Industry today faces major problems such as product substitution or copy and adulterations. Techniques employing stable isotope analyses [2], using isotope ratio mass spectrometry (IRMS), have found increasing popularity in forensic science [3]. However, it can only determine the global isotope content of a given element, leading to the loss of much valuable data. The development of isotopic NMR spectrometry at natural abundance enables the quantification each isotopomer constituting a given molecule for a given element.

Measurement of $^{2}H/^{1}H$ ratios by NMR is a well-established technique for food authentication and is used for the official control of wine, spirits, fruit juices and flavors [4]. The possibility of measuring site-specific $^{13}C/^{12}C$ ratios directly using $^{13}C$ NMR has been established more recently [5]. The main difficulty of isotopic $^{13}C$ NMR is meeting the requirement for a high level of precision: better than 1‰! Advanced protocols have been developed that overcome several obstacles and allow its successful application to a number of fields [6, 7, 8].

Recent technological developments have made further improvements, specifically by the exploitation of polarization transfer techniques, in which the abundance of the $^{1}H$ atom is exploited to enhance sensitivity [9]. The relative $^{13}C$ distribution within the molecule is characteristic and sufficient for establishing an isotope fingerprint. The representation of the data can then be considered analogous to that carried out in 'metabolomic' (or any 'omic' approaches): comparison of data built up from a multiplicity of variables and collected from several samples using the same protocol for which the precision has been established. Thus the isotope profile could be considered as an 'isotopomic' study.

**References**

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[3] Gentile, N., Besson, L., Pazos, D., Delémont, O., Esseiva, P., *Forensic Sci. Int.* 2011, **212** 260-271.
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## MR techniques for guiding cancer therapy

Prof. Klaas Nicolay

*Biomedical NMR Group*
*Department of Biomedical Engineering*
*Eindhoven University of Technology, Eindhoven, The Netherlands*

Traditionally, MR imaging has a lot to offer to the diagnostics of a wide variety of diseases. In recent years, MRI is also intensely explored for its utility to steer therapeutic interventions, particularly in the setting of cancer therapy. Real-time MRI guidance is being exploited for thermal interventions (like High-Intensity Focused Ultrasound), photodynamic therapy and radiotherapy. This presentation will describe how MRI is being used to steer and monitor tumor treatment and also highlight the use of MRI for the early prediction of the efficacy of the therapy.

# ORAL COMMUNICATIONS

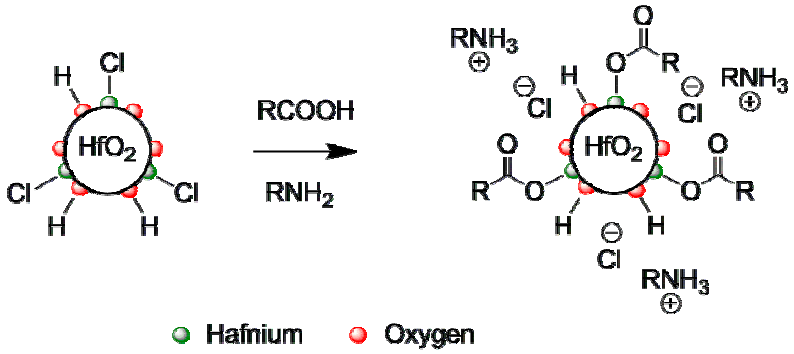
# The surface chemistry of metal oxide nanocrystals; a solution NMR study

J. De Roo[1,2,3], F. Van den Broeck[1], K. De Keukeleere[2], I. Van Driessche[2], Z. Hens[3] and J. C. Martins[1]

[1] *NMR and Structure Analysis Unit, Vakgroep Organische Chemie, Universiteit Gent*
[2] *SCRIPTS, Vakgroep Anorganische en Fysische Chemie, Universiteit Gent*
[3] *PCN, Vakgroep Anorganische en Fysische Chemie, Universiteit Gent*

Ceramic nanocrystals (NCs) are of general interest because of their potential applications in catalysis, gas sensing, LED's, and other areas. However, the formed particles need to be processed in order to be used in a specific application. Gaining knowledge about the surface chemistry of the NCs is therefore of special importance and NMR provides us with the tools to address this due to the extensive $T_2$ relaxation of bound ligands.

$HfO_2$ NCs are solvothermally synthesized in benzyl alcohol with microwave heating. The surface of the obtained charge stabilized NCs can subsequently be modified with fatty acids and oleylamine to allow solubility in nonpolar solvents.[1] During this process, clusters are broken up and the constituent particles are obtained. We present here a detailed study of the fundamental acid/base processes during the surface modification, using 1D proton, NOESY and DOSY NMR. We demonstrate that there is a crucial difference in surface chemistry between metal oxide NCs and the more widely studied chalcogenide NCs, such as PbS and CdSe.[2] The electronegative property of oxygen allows for protons to be accommodated at the surface of $HfO_2$ NCs which was shown with the help of exchange experiments with deuterated acid. The binding event of a carboxylic acid on a metal oxide NC is thus a dissociative process, see scheme 1.




**Scheme 1: Surface reaction from charge stabilized to sterically stabilized particles**

Subsequently, we show the practical implications of these findings. First, we show that ligand exchange reactions, which were considered impossible, do take place at the metal oxide NC surface. We examined the exchange of X-type ligands for L-type ligands by performing titration experiments which were followed by 1D proton and DOSY NMR. Secondly, we demonstrate that acid catalyzed organic reactions could proceed in the presence of the $HfO_2$ NCs due to the surface attached protons. As an example of such catalytic process, we monitored in situ the esterification of ethanol and oleic acid with 1D proton NMR and characterized the final compound with HMBC, HSQC and COSY.

In conclusion, metal oxide NCs hold protons on the surface, in contrast to metal selenides or sulfides. This property allows unexpected catalytic activity and ligand exchange to take place.

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## Characterization of iron oxide nanoparticles by NMR relaxometry and magnetometry: effects of size distribution and agglomeration.

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Iron oxide nanoparticles (NP) are of great interest in nanomedicine. They are used in Magnetic Resonance Imaging (MRI) as negative contrast agent, in tumor targeting, in drug delivery and in magnetic hyperthermia. With suited sequences they could even generate positive contrast. In this work, we explore the effects of size distribution, magnetization and agglomeration of different-sized magnetite NP ($Fe_3O_4$) on NMR relaxation and magnetometry experiments. Indeed, these parameters play a key role in their efficiency as MRI contrast agents.

First, using a Vibrating Sample Magnetometer (VSM), Zero-Field-Cooling (ZFC) measurements are carried out. The particle size distribution can be obtained by fitting the obtained curves with the standard ZFC theory[1]: each NP is considered to be either blocked or superparamagnetic, depending on its size and on the temperature.

Then, nuclear magnetic relaxation dispersion (NMRD) profiles were recorded at 37°C using a Fast Field Cycling (FFC) relaxometer. Fitting the data with the RMG model[2] allows to get the average NP radius and the saturation magnetization. NMRD profiles are also more sensitive to the agglomeration of the NP. Finally, direct measurements of the size distribution and of the NP agglomeration were carried out by transmission electron microscopy (TEM).

The comparison of these three sets of results allows to finely characterize iron oxide NP in aqueous solutions since the different techniques are sensitive to different factors. For example, we obtained larger NP radii and lower saturation magnetizations from the NMRD profiles than those obtained from magnetometric experiments. This is consistent since magnetometry is sensitive to the iron oxide monocrystals (even if clustered in a larger structure), while relaxometry will consider a cluster as a global, less magnetized particle. NP radii measured by TEM are very similar to those obtained from magnetometric experiments which is consistent too. On the other side, distribution widths are larger in magnetometric experiments than in TEM.

In a later work, biological samples containing iron oxide NP will be studied in order to optimize protocols of iron quantification by magnetometry.

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## Dysprosium and terbium magnetofluorescent micellar complexes as potential bimodal agents for magnetic resonance and optical imaging

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Most of the contrast agents currently used in magnetic resonance imaging (MRI), are based on complexes of gadolinium(III) with diethylenetriaminepentaacetic acid (DTPA) or 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), which cause shortening of the longitudinal relaxation time ($T_1$) of water protons resulting in a positive contrast. Alternatively, iron oxide particles can be used to accelerate the transverse relaxation time ($T_2$) resulting in a negative contrast. The high spatial resolution of the MRI technique unfortunately suffers from low sensitivity and a decrease in relaxation efficiency of current contrast agents with increasing magnetic field strengths. Decreasing the molecular tumbling rate has been identified as a method to increase their performance.[1-4]

Recently, contrast agents combining paramagnetic and luminescent properties have been investigated for the purpose of enhancing imaging performance because optical imaging has a high sensitivity due to the low detection limit together with the good resolution of MR imaging.[1,2,5] The use of lanthanide systems circumvents restrictions of bio-conjugates such as short luminescence lifetimes, small Stokes shifts, and photobleaching. Our research group has recently published a review of the topic in Chemical Society Reviews.[5]

In our most recent work, two diethylene triamine pentaacetic acid (DTPA) and four 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) bisamide derivatives functionalized with amphiphilic *p*-dodecylaniline and *p*-tetradecylaniline. For the DOTA derivatives this was in a differing cis- and trans-orientation. The DOTA derivatives were coordinated to dysprosium (III) and both DTPA and DOTA were coordinated to terbium (III). The complexes were assembled into mono-disperse micelles of approx. 10 nm. For the first time terbium (III) has been evaluated as a single lanthanide negative bimodal contrast agent for MRI, dysprosium (III) DOTA complexes are compared with similar DTPA complexes[1] and the magnetic and optical properties of the complexes were examined in detail. The complexes show characteristic $Dy^{III}$ and $Tb^{III}$ emission. The transverse relaxivity $r_2$ per $Dy^{III}$ and $Tb^{III}$ ion at 500 MHz and 310 K reaches maximum values around 20 $s^{-1}$ $mM^{-1}$ and 15 $s^{-1}$ $mM^{-1}$ respectively. The efficient $T_2$ relaxation especially at high magnetic field strengths is sustained by the high magnetic moment of the lanthanide ions.

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# NMR Study of the Recognition Properties of a Calix[6]aza-cryptand Incorporated in DPC Micelles

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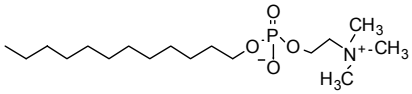
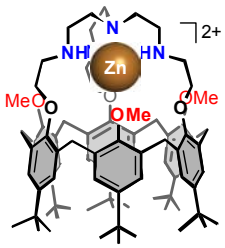
Water is a unique solvent and the design of selective artificial hosts that can efficiently work in an aqueous medium is a challenging task.[1] The solubilisation of organo-soluble receptors in micelles is an elegant and very simple strategy to obtain water compatible nanosized supramolecular recognition devices which can be prepared via a straightforward self-assembly process.[2] It was notably shown that calix[6]tren complex **1.Zn$^{2+}$** (Figure) can efficiently and selectively bind neutral guests in $CDCl_3$.[3,4] In order to study this complex in water, **1.Zn$^{2+}$** was incorporated into dodecylphoscholine micelles (DPC) and intensive NMR binding studies were undertaken.



**DPC**



**1.Zn$^{2+}$**

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# Targeting of Apoptotic Cells by a New Bimodal Probe Based on AGuIX® Nanoparticles

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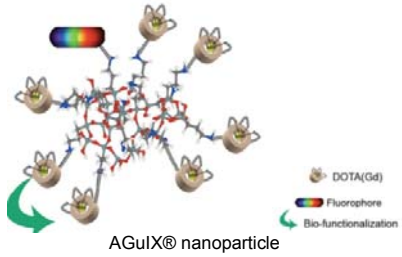
AGuIX® nanoparticles are small and rigid platforms of polysiloxane recently developed and used in various applications of medical imaging. These nanoparticles are formed after the dissolution in aqueous solution, of gadolinium oxide nanoparticles with a core/shell structure of polysiloxane. The coating contains several amine functions used for the fixation of DOTA ligands on nanoparticle surface. The dissolution of $Gd_2O_3$ is followed by the complexation of the $Gd^{3+}$ ions by approximately 70 percent of DOTA ligands [1]. These paramagnetic platforms have a diameter less than 5 nm and a low transmetalation [2]. Different studies have moreover been achieved in the biomedical domain, showing that they allow to combine multimodal and theragnostic properties. A passive tumoral targeting has already been observed by EPR effect (Enhanced Permeation Effect). Otherwise, their small size allows a quick elimination by the kidney [3].




AGuIX® nanoparticle

Previously phage display studies showed that TLVSSL peptide has a high affinity for phosphatidylserine, a phospholipid overexpressed on membrane of apoptotic cells. Apoptosis is a natural process of cell death [4]. The targeting of apoptotic cells is interesting in following the efficiency of an antitumoral therapy and for diagnosis of diseases related to this process. This peptide has been fixed on nanoparticles AGuIX® by activation with EDC of carboxylic functions available on nanoparticle surface. Furthermore, previous addition of an optical dye allows their applications in optical imaging. Different techniques such as PCS, fluorescence spectroscopy, TGA, HPLC and relaxometry were used to characterize this platform. Relaxometric studies by NMRD profiles were mostly used to confirm the increase of the rotational correlation time after linking of the peptide and to study the time stability of the platform. The biological efficiency of this novel bimodal agent to target apoptotic cells was evaluated by fluorescence microscopy on lymphablastic human T cell line. *In-vitro* cell apoptosis was chemically induced by incubation with campthothecin.

These characterizations and biological tests confirm the substituent linking and the efficient targeting of apoptotic cells. Further applications will be achieved in *in-vivo* systems.

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# Validation of $^{1}$H-NMR-based metabolomics as a tool to detect lung cancer in human blood plasma

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**Background.** Until today no effective method permits the early detection of lung cancer. Evidence has shown that disturbances in biochemical pathways which occur during the development of cancer provoke changes in the metabolic phenotype. Recently, our research group has constructed a statistical classifier by means of multivariate orthogonal partial least squares-discriminant analysis (OPLS-DA). This classifier (constructed with 110 spectral integration regions as variables) allows to discriminate between 209 lung cancer patients (70% male, 30% female, age: 68 ± 10, BMI: 25.8 ± 4.6) and 199 controls (52% male, 48% female, age: 67 + 11, BMI: 28.2 ± 5.1) with a sensitivity of 81%, a specificity of 92%, and an area under the curve (AUC) of 0.86. When only the 28 most discriminating variables (VIP value > 0.8) were selected to construct a classifier (i.e. regions representing glucose, lactate, myo-inositol, β-hydroxybutyrate, threonine, citrate and lipids) a sensitivity of 72%, a specificity of 88% and an AUC of 0.80 is achieved. The present study aims to examine the predictive accuracy of these classifiers in an independent cohort of 50 lung cancer patients (58% male, 42% female, age: 67 ± 9, BMI: 25.7 ± 4.2) and 64 controls (44% male, 56% female, age: 70 ± 10, BMI: 28.3 ± 6.1). **Methods.** The classification of this independent cohort is accomplished by means of the classifiers described above. The predictive accuracy of these classifiers is further evaluated by means of a receiver operating characteristic (ROC) curve, using the independent cohort as a hold-out dataset. **Results.** By using the classifier constructed with all variables, 86% of the lung cancer patients and 72% of the controls are correctly classified, with an AUC of 0.93. When the classifier constructed with the 28 most discriminating variables is used, a sensitivity of 90%, a specificity of 83% and an AUC of 0.86 is achieved. **Conclusions.** Both statistical classifiers show a good predictive accuracy. Further experiments are ongoing to investigate whether the constructed classifiers have potential as valid screening tool.

# Identification of a pKa-regulating motif stabilizing imidazole modified double stranded DNA

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Inspired by nature, the de novo design of artificial enzymes using physico-chemical principles intuition and computational methods is rapidly coming of age (1). A major design problem is the requirement to precisely engineer the position of the various functionalities required for catalysis in a productive arrangement capable of providing the basic reactivity. An alternative approach has been to simplify the design by grafting onto a simpler 'template' structure, which is selected to ensure the formation of a sufficiently rigid unit bearing the catalytic core (2,3). Using a 14mer DNA duplex as a rigid scaffold for the precise and predictable positioning of catalytic functionalities, our systems of interest can be classified as first generation hydrolase-like DNAzymes equipped with one histidine mimicking functionality based on a modified thymine nucleotide building block ($T^{Im}$).

Depending on the position of this peptide-like functionality, a significant increase in stability with respect to the non-modified wild type duplex due to the contribution of a single modification has been observed using UV melting experiments. In addition, an increase in $pKa_H$ of the imidazole functionality depending on its position inside the DNA framework has been demonstrated. Most notably this is the case in the $T_8^{ImH+}$ system, where both a significant increase in stability and $pKa_H$-value is perceived. Following complete $^1H$ NMR assignments of all modified systems, an initial view on the exact position and interactions of the imidazole moiety with the duplex is achieved using chemical shift difference and nOe-contact mapping. In a second stage, GPU-accelerated unrestrained molecular dynamics trajectories in the AMBER FF12SB force field with explicit water have been used to obtain an atomic view on the systems at hand. The overall quality and validity of the simulations was assessed by extracting relevant parameters (e.g. sampling of the α/γ conformational space (4)) for each system. Subsequently, distances between the imidazole and duplex hydrogen atoms were monitored during the trajectory and confronted with the available nOe data. Using this integrated methodology, a new $pKa_H$-regulating DNA motif has been identified in $T_8^{ImH+}$ and subsequently validated in other duplexes.

When integrated into a DNA sequence, this generic motif enables a specific interaction and $pKa_H$-regulation of the imidazole functionality within the major groove. Simultaneous introduction with non-interacting tethered imidazoles should allow specific tuning of relative $pKa_H$ in multiple modified systems.

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# Characterization of calixarene derivatives by liquid-state NMR spectroscopy: challenges and solutions

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Calixarenes are polyphenolic macrocycles used in host-guest chemistry for the design of molecular receptors.[1] We are currently developing a new strategy for the tailored, and highly selective, functionalization of such compounds.[2]

Calixarenes exhibit high conformational flexibility originating from the rotation of the phenolic units through the macrocycle annulus (Figure 1). Due to the coexistence of several atropisomers or conformers, and/or as a consequence of inherent chirality, the characterization of calixarene derivatives by solution-state NMR spectroscopy may look like a nightmare at first sight. Indeed, depending on the nature and number of substituent groups, as well as on the experimental conditions, calixarene derivatives may yield numerous and/or broadened $^{1}$H NMR signals, leading to poorly resolved overall spectra.

These issues will be overviewed, hints for obtaining suitable NMR spectra will be given, the strategy used for signal assignment will be presented and additional topics related to the characterization of some calixarene derivatives that were synthesized in our laboratory will be illustrated.

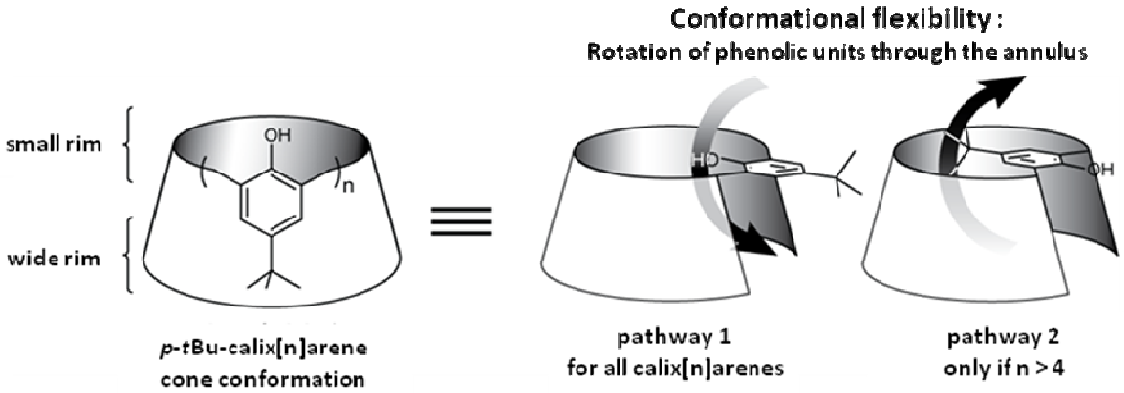


**Figure 1:** General structure of *p-t*Bu-calix[n]arenes and origin of their conformational flexibility.

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# Characterization of phosphonic acid grafted titanium dioxide surfaces by $^{31}$P NMR and ATR-FTIR

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The reaction between titanium dioxide ($TiO_2$) and aromatic or alkyl phosphonic acids results in the formation of a hydrophobic layer on the transition metal oxide surface[1]. The phosphonic acids can be physically or chemically adsorbed to the surface. The chemical adsorption is characterized by the presence of covalent bonds between the titanium and the phosphonic group[2]. The mode of these bonds is strongly dependent on the reaction conditions and has been investigated using ATR-FTIR and $^{31}$P CP-MAS SS-NMR[3]. The binding mode is important since it is correlated to the stability of the surface layer[4]. Generally it is possible to distinguish three different binding modes: monodentate, bidentate and tridentate[5]. The detailed results coming out of an in depth investigation of several reaction conditions provide information on the correlation between the reaction temperature and reaction mechanism. We demonstrated that the formation of bidentate structures is possible at room temperature via heterocondensation reactions, while the formation of the tridentate structure at the $TiO_2$ surface starts at 45°C and increases as a function of the temperature. At 150°C, both the aromatic and alkyl phosphonic acids are bonded prevalently via the tridentate binding mode. In order to explain the formation of tridentate structures, a reaction mechanism is hypothesized in which a nucleophilic attack of water at the phosphorus atom of a bidentate structure takes place leading to the formation of the third P-O-Ti bond. A qualitative monitoring of the tridentate structure formation is possible by ATR-FTIR due to the presence of two dedicated absorptions (1023 $cm^{-1}$, 1080 $cm^{-1}$) and a typical resonance signal in the solid-state (SS) $^{31}$P CP-MAS NMR spectrum (-8 ppm for aromatic phosphonic acids and 4 ppm for alkyl phosphonic acids). In addition to this qualitative investigation, we also developed a measuring protocol to absolutely quantify the reaction between $TiO_2$ and phenylphosphonic acid by SS-$^{31}$P-MAS NMR.

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## Study of transient radicals created immediately after room temperature X-ray irradiation in single crystal sucrose

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Radicals induced by high-energy radiation in sucrose are interesting for a number of reasons. From a practical standpoint, the electron paramagnetic resonance (EPR) spectrum of stable radicals has properties that make it relevant for dosimetric applications, i.e. low detection limit and considerable linear dose response range [1]. On the other hand, there exists a fundamental interest in identifying radical structures to gain insight into the radiation chemistry of biologically relevant sugar-containing systems, e.g. DNA or RNA. Understanding processes leading to damage of these molecules is foremost important from a fundamental point of view, but in the long run this knowledge can lead to practical advances, e.g. improvements in radiation therapy.
In this work, radicals with limited stability at room temperature [2] have been studied using EPR and electron-nuclear double resonance (ENDOR) spectroscopy. Measurements were performed in Q-band (34 GHz) at 50 K, while the rotation planes were determined at 110 K by fitting the angular variations of ENDOR transitions associated with the largest hyperfine couplings (HFC) of the three known stable radicals [3]. Contributions of individual radicals to the EPR spectrum at main crystallographic orientations have been resolved by comparing simulated EPR spectra to measured ENDOR-induced EPR spectra. Experimental HFC tensors of proton couplings were compared to density functional theory calculation results, which led to the identification of the minority species as a radical formed by an H abstraction from C4 (Figure 1). The dominant species has not been definitively identified yet, but we present compelling evidence that it should be a radical formed by an H abstraction from C6.

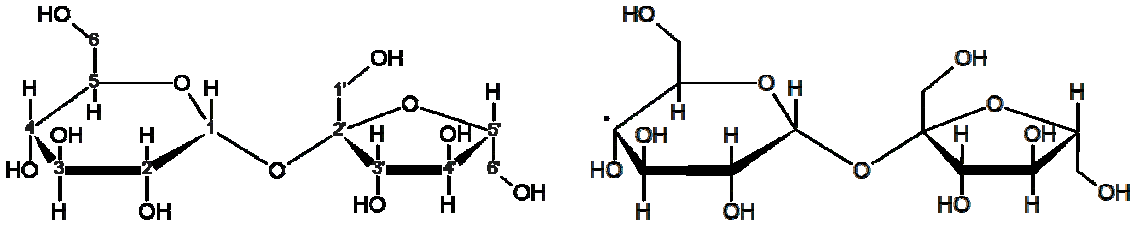

Figure 1: (left) Chemical structure of a sucrose molecule with the numbering scheme used in this work and (right) chemical structure of the U2 radical.

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## An investigation of Antarctic fish cytoglobins using EPR and optical spectroscopy

B. Cuypers[1], S. Vermeylen[2], A. De Schutter[1], V. Rahemi[3], C. Verde[4], K. De Wael[3], S. Dewilde[2] and S. Van Doorslaer[1]

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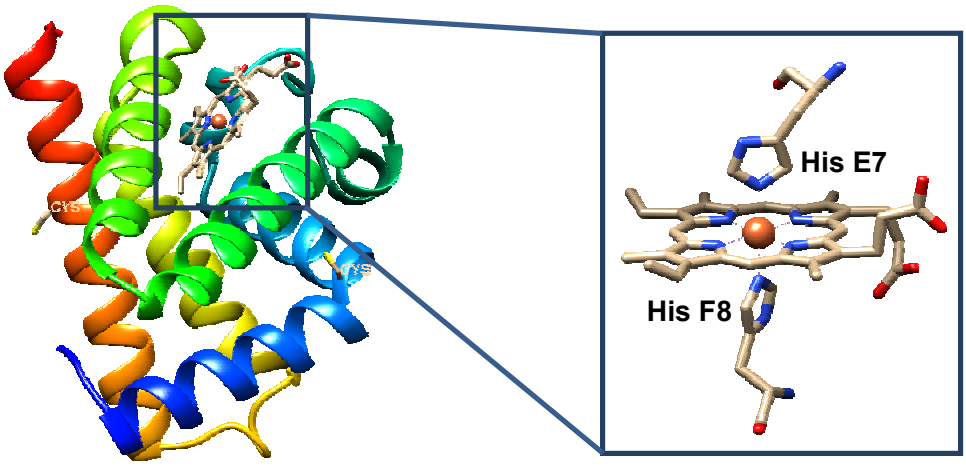
Up to now, five different globins have been identified in humans: hemoglobin, myoglobin, neuroglobin, cytoglobin and androglobin. Both neuroglobin and cytoglobin are hexacoordinated globins (HisE7-Fe-HisF8), which means that the heme iron has no free binding site in comparison with hemoglobin and myoglobin. Cytoglobin occurs in different tissues in a rather low concentration. In this work, two Antarctic fish cytoglobins (*Dissostichus mawsoni* and *Chaenocephalus aceratus*) are investigated using different spectroscopic techniques: optical absorption spectroscopy, electron paramagnetic resonance (EPR) and resonance Raman spectroscopy (RRS). Both Antarctic fish lack myoglobin and *C.ace* also lacks hemoglobin, which makes their corresponding cytoglobins interesting candidates to investigate and compare with their human variant.[1] Unlike in human neuroglobin, where the presence of an intramolecular disulfide bond is found to modulate the gas binding affinity through a change in the heme-pocket structure, the formation of a disulfide bridge in human cytoglobin has only a negligible effect.[2] The Antarctic fish and human cytoglobins have cysteines at different positions. By comparing the principal *g*-values of the ferric cytoglobins with those of their Cys→Ser mutants, the effect of possible intra- and/or intermolecular disulfide bridges on the heme-pocket structure can be determined. Combination of all the data from the above mentioned spectroscopic techniques reveals slight differences in the heme-pocket structure of human and Antarctic fish cytoglobins. The most pronounced difference is found in the stabilization of the CO-ligand.

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# Synthesis and characterization of functionalized magnetoliposomes for Magnetic Resonance Imaging and theranostics applications

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Magnetoliposomes (MLs), which were earlier developed in our lab, consist of nanosized, magnetisable iron oxide cores (magnetite, $Fe_3O_4$) which are individually enveloped by a bilayer of phospholipid molecules. In the past, it was already shown that these structures are biocompatible imaging agents, resulting in a highly efficient labeling of cells without evoking toxic effects and with a powerful imaging signal that remains stable over a long time period (1). A unique feature of MLs is that the coating can be easily modified, for instance by inserting fluorescent or radioactive labels and/or targeting molecules into the lipid bilayer. Thus, these highly sophisticated MLs offer exciting possibilities for multimodal molecular imaging and drug delivery. Till now, in-house synthetized MLs were functionalized and characterized by Transmission Electron Microscopy (TEM) and Dynamic Light Scattering (DLS). The results were satisfactory in both techniques, with TEM defining the size (30-35 nm) and DLS the hydrodynamic diameter (45 nm) of the MLs, and with the morphology of the phospholipidic bilayers very discernible in the TEM images. These MLs were further employed for cell labeling and longitudinal in vivo imaging of prelabeled INS-1E cells and Pancreatic Islets (PIs). To test the MR detectability of cells labeled under different conditions, agarose phantoms were prepared and MR imaging was performed. Phantoms were scanned using a 3D T2*-weighted gradient echo sequence with a Fast Low Angle Shot sequence (FLASH, TR = 200 ms, TE = 15 ms) resulting in an isotropic resolution of 234 $\mu m^3$. First proof-of-principle experiments were also successfully conducted to transplant the prelabeled cells and PIs in the kidney capsule and portal vein of C57Bl6 adult mice. Animals were scanned on the day of the islet engraftment and until 14 days post islet transplantation. A respiration-gated FLASH sequence (TE= 2.3ms, TR= 202.56ms, six slices with a thickness of 1mm and an in-plane resolution of 136µm2) was used to determine the decrease in the signal intensity due to labeled islets at the site of transplantation. All MR measurements were performed using a 9.4 T Bruker Biospec small animal MR scanner (Bruker Biospin, Ettlingen, Germany). The results obtained here show that MLs have highly sensitive T2/T2* MR contrast and can be successfully used for prelabeling or cells/ islets and subsequent in vivo imaging. MLs express lower level of toxic effects compared to other iron oxide particles (2) and functionalized MLs using cell recognizing ligands such as peptides (e.g. cRGD peptide), small molecules (e.g. lactose moieties) and vitamins (e.g vitamin A) were already validate by our group for the in vitro and in vivo visualization of hepatocytes and hepatic setellate cells, respectively (3). The later were used for the early detection of liver fibrosis. The focus of our future work is on *in vivo* targeting of beta cells for diabetes therapy using molecules that target receptors of beta cells, including the GPR-40 receptor using a thio-derivative of TAK-875, a potent GPR-40 agonist.

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# Hierarchical non-negative matrix factorization for brain tumor characterization using multi-parametric MRI

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Advanced MR modalities such as MRSI, PWI (perfusion-weighted imaging) and DWI (diffusion-weighted imaging) have shown their added value to characterize brain tumors in a non-invasive way, detect full tumor extent and assess early success of therapy. The combination of MR modalities in a multi-parametric MRI (MP-MRI) approach provides us with complementary information and allows us to answer clinical questions more specifically and unambiguously [1]. Tissue characterization within gliomas is challenging due to the co-existence of several intra-tumoral tissue types within the same region and the high spatial heterogeneity in high-grade gliomas. Previous advanced MR studies have often neglected this aspect of tissue complexity. An accurate and reproducible method for brain tumor characterization and the detection of the relevant tumor substructures could be of great added value for the diagnosis, treatment planning and follow-up of individual patients. This study presents a hierarchical non-negative matrix factorization (hNMF) technique, providing a voxelwise tissue characterization and incorporating the concept of tissue mixtures. The hNMF algorithm was originally developed to process MRSI data only [2], but has been modified to cope with an extended set of MP-MRI data. Tissue-specific patterns are obtained and the spatial distribution of each tissue type is visualized by means of abundance maps. The hNMF algorithm is applied to the MP-MRI data of 13 non-necrotic glioma patients and 11 patients with glioblastoma multiforme. Dice-scores were calculated by converting the abundance maps into a tissue segmentation and comparing it to the manual segmentation by a radiologist. Correlation coefficients were calculated between the pathologic tissue sources and the average feature vector within the corresponding tissue region. For the non-necrotic patients, an average dice-score of 88% and an average correlation coefficient of 0.97 were found for the tumor region. For the GBM patients, average dice-scores of 76%, 69% and 84% were obtained for active tumor, necrosis and the whole tumor region. The average correlation coefficients were 0.90 for active tumor and 0.96 for necrosis. hNMF can be applied on a patient-by-patient basis, it does not require large training datasets nor data normalization and it provides a more refined tissue characterization compared to black-and-white segmentation techniques.

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## G-quadruplex in the HIV promoter region: UV-spectroscopy and NMR insight

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G-quadruplexes are nucleic acid structures formed by stacking of a minimum of two G-quartets. Each G-quartet is composed of four guanines interacting through Hoogsteen H-bonds. Several G-quadruplex forming aptamers (nucleic acid sequences selected in vitro for their affinity towards a given target) are able to interact with key proteins in the HIV replication cycle like the integrase, the reverse transcriptase, the gp120, etc. They inhibit the HIV infectivity at various steps of the viral cycle.

We have demonstrated that the sequence HIV_PRO1, located in the promoter of the virus, is prone to adopt a G-quadruplex folding in vitro and particularly conserved among the HIV variants.[1]

In the present work we investigate the biophysical characteristics of a second sequence, HIV_PRO2, located in the viral promoter region. The formation of a G-quadruplex structure is attested by UV spectroscopy and by the presence of twelve imino protons in the 1H NMR spectrum. Indications related to the topology (i.e. the organization of the G-quartet and base pairs) of the G-quadruplex were obtained by circular dichroism and NMR (including H2O to D2O exchange, HMBC and NOESY experiments). Single base 13C and 15N labelling are used to assess key assignments.

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# Application of NMR Spectroscopy in the Development of Silicone Materials

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Silicones bringing inorganic (Si-O-Si bonds) with organic chemistry (Si-C bonds) together, show very special properties like thermal and chemical stability, resistance to light, low surface tension and others. Beside well known poly (dimethyl siloxanes), the silicone backbone can be linked to other organic groups like phenyl, polyether and others; the silicone can be branched by incorporation of tri- or tetra-functional silanes.
The silicone materials, their formation, their reactions like hydrosilylation or condensation, can be monitored by mainly $^{29}$Si NMR spectroscopy, but also $^{1}$H and $^{13}$C NMR methods are of interest. The NMR characterization of silicones, their theoretical and practical aspects, were developed in detail in the 1970ies and 1980ies by Marsmann, Radeglia, Engelhardt, Jancke, Harris, Schraml and others [1-5].
The chemical shift range in $^{29}$Si NMR allows to distinguish between different functionalities (M,D,T and Q units), neighborhoods; quantification allows to determine molecular weights, branching levels, average structures [6]. In addition to classic solution state NMR, a few examples of characterizing emulsions and solids will be presented. Solid applications do not only reflect structures, but also dynamic aspects. The relative low glass transition temperature of poly (dimethyl siloxane) with its high mobility at room temperature makes investigations of copolymers with rather rigid components like poly amides very interesting for $^{1}$H solid state applications [7].

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# POSTERS

**Targeted delivery of peptidoglycan immunomodulators using liposomal carriers: NMR study of the lipid encapsulation**

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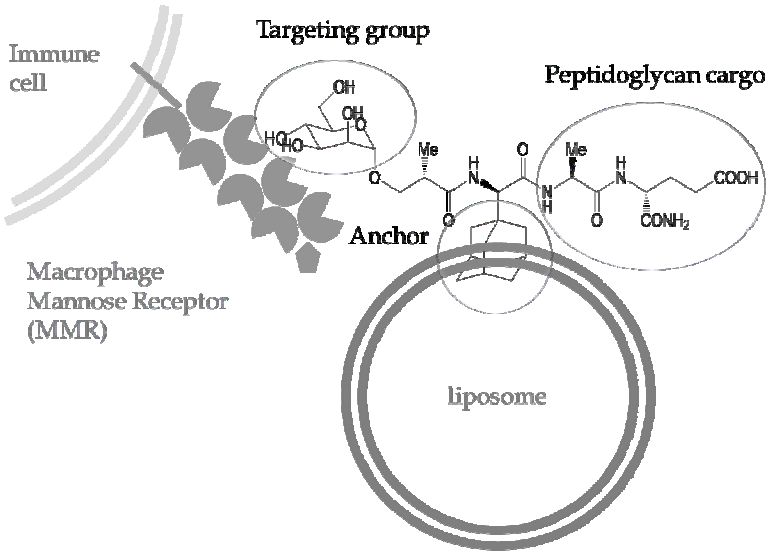
Peptidoglycan (PGN) as well as fragments and substances derived from it, have well-documented immunomodulating properties[1] exerted by interaction with innate immune receptors. Such immunomodulators are urgently needed in many medical interventions[2], such as adjuvants in vaccines or in aiding cancer therapies.
In order to selectively modulate a certain subset of immune cells using PGN fragments, we have developed actively targeted delivery of PGN-based immunomodulants using liposomes as carriers.




We have investigated the encapsulation of targeting compounds into lipid bilayers and the interaction between them on a molecular level using NMR spectroscopy. We have shown that the PGN derivatives are incorporated in the studied lipid bilayers by the change of the sign and intensity of the nOe as well as by the observation of slower translational diffusion in PFG-NMR spectra. We have determined the encapsulation efficiency and have found that the chirality of the adamantly group attachment had a large influence on the entrapment efficiency. We have utilised STD experiments for the characterisation of the orientation of the studied derivatives in the bilayer. We have found that the adamantyl group does penetrate the lipid core of the bilayer and acts as an anchor of the PGN derivative cargo, while the hydrophilic peptidoglycan fragment is exposed on the surface.

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# First insights in the structure-function relationship of a natural cyclic lipodepsipeptide by synthetic modification and NMR investigation

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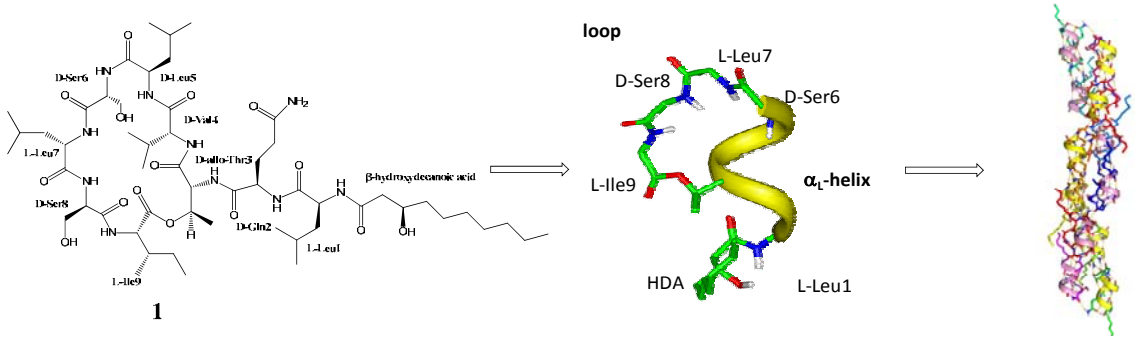
Pseudodesmin A (**1**) is a secondary metabolite produced by *Pseudomonas* bacteria, belonging to the viscosin group of cyclic lipodepsipeptides. It displays moderate antibiotic activity, including against MRSA and vancomycin-resistant *Enterococcus*. Extensive NMR studies revealed that individual molecules self-assemble into well-defined supramolecular structures in non-polar solvents.[1,2]




Our goal is to investigate in detail the molecular structure of the self-assembly and its role in biological activity, which involves membrane interaction. For this, a rapid, efficient solid-phase synthesis strategy for pseudodesmin A was developed.[3] The newly developed route allows the straightforward production of analogues for structure-activity relationship studies, including an Ala-scan and modifications to the fatty acid moiety. Using NMR diffusion measurements, the modulation of the self-assembly could be monitored, revealing fundamental intermolecular contacts. Additionally, the enantiomer of pseudodesmin A was produced, revealing identical biological activity, for the first time demonstrating that no chiral interactions mediate these compounds' mode of action.

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## Synthesis of bimodal MRI contrast agents based on metallostar complexes

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During the last decades, *Magnetic Resonance Imaging* became a well established medical imaging technique. Most contrast agents used today are complexes of $Gd^{3+}$ with diethylenetriaminepentaacetic acid ($[Gd(DTPA)]^{2-}$), 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid $(Gd(DOTA)]^{-}$) or structural analogues. The paramagnetic gadolinium(III) with its seven unpaired electrons and sphere symmetric ground state ($^8S_{7/2}$) is an ideal metal for this application. The relaxivity of the molecule is determined by several parameters such as the number of inner-sphere water molecules, the residence time of the coordinated water molecules and the rotational correlation time of the contrast agent.

Multimodal contrast agents are gaining in demand as combination of different imaging techniques allows analysis in more exquisite details. Compounds being both luminescent and paramagnetic hold potential as bimodal contrast agents for MRI and optical imaging. Luminescence is known to have a high sensitivity, while MRI shows a much better spacial resolution and deeper penetration depth. In this work we present a ligand consisting of a parasubstituted pyridine-2,6-dicarboxylate derivative of DTPA. In the envisioned complex, water molecules are able to approach the paramagnetic entity in order to achieve efficient relaxation enhancement, while water is excluded from the first coordination sphere of central lanthanide(III) resulting in a bright emissive compound. Furthermore, the sensitivity difference between optical and MRI is encountered by assembling three paramagnetic components around one luminescent metal ion.

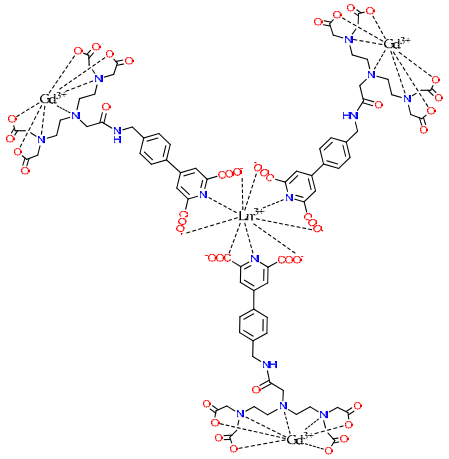

**Figure 1. Structure of the metallostar complex**

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## DESIGN OF CONTRAST AGENT FOR NEURODEGENERATIVE DISEASES DIAGNOSIS

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In the last decades, molecular imaging technologies began to attract increased interest for the targeting of diseases. In particular, Alzheimer disease diagnosis is nowadays investigated through the design of tailor-made contrast agents able to cross over the blood-brain barrier (BBB). Conventional contrast agents are composed of three parts: contrast agent, spacer and vector. Over the last three decades, gadolinium complex of DOTA macrocycle has exhibited enhanced stability properties as compared to other first generation contrast agents. In addition, the versatility of the complexes confers to DOTA-based contrast agents the ability to be used in a large range of imaging modalities, including magnetic resonance (MR), positron emission tomography (PET), single photon emission computed tomography (SPECT) and fluorescence imaging.[1] Variations on the arms of the DOTA macrocycle appears as the way towards the generation of highly stable responsive and selective probes.[2] In this context, the specific targeting of diseases has been drastically enhanced by the incorporation of peptides as vectors. In the present study, the design of L-DOPA functionalized Gd-DOTA complex as a bluilding block for further modifications is under consideration. The synthetic strategy involves the following steps : (i) the selective three-arms protection of cyclene by *tert*-butyl acetate moeities; (ii) the incorporation of a fourth arm exhibiting a primary amine moeity; (iii) amidation reaction on the fourth arm with Boc-L-DOPA; (iv) BOC deprotection; (v) $Gd^{3+}$ complexation by the modified DOTA macrocycle. For instance, the two first steps were successfully achieved. Both NMR and ESI-MS characterization techniques were used to follow the progress of the reactions. The next steps are under progress.

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# Development of EPR oximetry in diabetic wound healing models

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[2] *Endocrine Cell Therapy Unit, Center of Tissue/Cell Therapy, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels.*
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**Introduction:** One major complication of diabetes is the development of skin ulcers with impaired healing. Indeed, 15 % of diabetic patients will suffer from lower extremity ulcers during their life and 7 to 20 % of these patients with foot ulcers will require amputation [1]. A main cause of the nonhealing of foot ulcers is tissue ischemia [2] as it is known that oxygen is a key parameter in wound healing. Nevertheless, the variation of the $pO_2$ in the wound remains uncertain as there still lacks non invasive methods for absolute and repeated $pO_2$ measurements in situ. EPR oximetry is a technique that allows repeated measurements of the absolute tissue $pO_2$. The technique is based on the measurement of the linewidth of the EPR signal recorded with a biocompatible oxygen sensor like LiPc implanted in the tissue.
**Aim:** To investigate whether EPR oximetry with LiPc is a possible tool to follow the $pO_2$ in the wound in diabetic wound healing.
**Materials and methods:** Two diabetes models were used: chemically-induced type I diabetes (with streptozotocin) and genetically-induced type II diabetes (db/db mice).
Two types of wounds were tested: a 30 x 8 mm pedicled flap and a 6 mm diameter excisional wound.
LiPc crystals were inserted in different positions inside the wound and the EPR signal recorded repeatedly during the wound healing process.
**Results:** In the flap model, the $pO_2$ decreased the first day after wounding due to the flap surgery. Then, the $pO_2$ increased during the healing process. Interestingly, this increase was more rapid for non diabetic mice than for diabetic mice (fig. 1). For the excisional wound model, a variation of the $pO_2$ was observed in the wound periphery during the healing process but it did not differ from what was observed in the control non wounded tissue. In the center of the excisional wound, the $pO_2$ was elevated the first days after the wounding without any difference between the diabetic and non diabetic state (fig. 2).

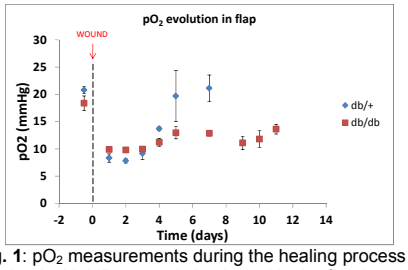


**Fig. 1**: $pO_2$ measurements during the healing process obtained with LiPc crystals implanted in the flap in control db/+ and db/db mice.

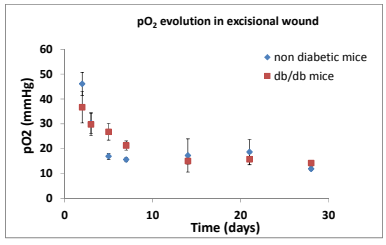


**Fig. 2**: $pO_2$ measured in the wound during the healing process in non diabetic mice and db/db mice.

**Conclusion:** EPR oximetry using LiPc is a suitable methodology for monitoring the variation of the $pO_2$ over time in wounds. The flap model containing LiPc in db/db mice is an adequate wound model to study the tissue $pO_2$ during the wound healing process in diabetic animals.

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# Theoretical and Experimental study of the Off-Resonant Saturation, an MRI Sequence for Positive Contrast With Superparamagnetic Particles

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Magnetic Resonance Imaging (MRI) is a powerful non invasive medical imaging technique that provides images with excellent resolution and intrinsic contrast. However, MRI sometimes necessitates contrast agents to highlight regions of interest such as tumors. The Superparamagnetic Iron Oxide Nanoparticles (SPM particles)[1] are one kind of contrast agent: these biocompatible particles appear as hypointense spots in MRI images obtained with conventional imaging sequences due to their high $r_2/r_1$ ratio – and thus, belong to the negative contrast agent category.

Unfortunately, negative contrast can be difficult to interpret because it can also be produced by many sources which are independent of the SPM nanoparticles – such as air bubbles or tissue interfaces. To overcome this problem, new imaging sequences producing positive contrast with SPM nanoparticles were developed during the last few years. One of them is the Off-Resonance Saturation (ORS) technique[2]. The idea behind this sequence is to saturate the signal near the SPM particles and to subtract the resulting image from a classical image obtained without saturation. This computation yields an image with a positive contrast near the SPM nanoparticles.

Different studies have experimentally shown that ORS is able to generate positive contrast both *in vitro* and *in vivo*[2,3]. However, a complete theoretical study of the ORS sequence is still missing, which makes impossible the optimization of the ORS sequence. For this reason, this work proposes a theoretical study of the ORS sequence, validated by numerical simulations and verified by experiments on agarose gel phantom on a 11.7 T MRI scanner system. An analytical expression describing the contrast dependence on the sequence parameters and the SPM particles properties was developed. This expression provides a fundamental comprehension of the mechanisms leading to the ORS contrast and allows an optimization of the ORS sequence. Therefore, the theoretical model can be useful for future in-vivo applications.

The influences of the SPM particles relaxivities and concentration, the echo time and the saturation pulse parameters on the contrast were investigated. The best contrast was achieved for SPM particles possessing the lowest transverse relaxivity, for specific values of particles concentration predicted by the theory and for the lowest echo times.

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## Membrane interactions of natural cyclic lipodepsipeptides

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Cyclic lipodepsipeptides (CLPs) are non-ribosomal peptides produced by bacteria, mainly *Pseudomonas* and *Bacillus* spp. They consist of a short sequence of both D- and L-amino acids that forms a cyclic structure by means of an ester bond between the C-terminus and a side-chain alcohol. They possess antimicrobial activity against clinically relevant Gram-positive bacteria and certain agricultural relevant plant pathogens.

In the past, efforts have been made in analysing the conformations and self-assembling properties of a collection of CLPs known as the viscosin group. [1-4] It is hypothesized that the antimicrobial activity of CLPs can be related to their ability to interact with cell membranes. We apply a multidisciplinary approach to investigate the CLP's working mechanism, focussing either on the peptides themselves or the impact on the lipid bilayer.

NMR spectroscopy has emerged as a powerful tool for the structure determination of peptides in the presence of model membranes. In liquid-state NMR, it is important that the model membrane is sufficiently small to obtain good spectral resolution. This requirement imposes a restriction on the types of model membranes that are suitable. In this respect, isotropic bicelles have been developed as model membranes for NMR, combining the advantageous NMR properties of micelles with the characteristics of lipid bilayers. [5]

Complementary biophysical experiments are performed, including fluorescence spectroscopy and circular dichroism. Additionally, preliminary Molecular Dynamics simulations in the presence of bilayers have been performed to better understand the molecular mechanism of CLPs at an atomic level.

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# 17O NMR Study of Diamagnetic and Paramagnetic Lanthanide(III)− DOTA Complexes in Aqueous Solution

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*3 Dipartimento di Scienze della Terra e del Mare (DiSTeM), Università di Palermo, Italy.*
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The complexes between the 17O-enriched polyaminocarboxylate DOTA ligand and the whole series of stable lanthanide-(III) metal ions were studied in aqueous solution by 17O NMR:

I) The complex between DOTA and praseodymium(III) (Pr3+) was studied in aqueous solution by variable-temperature 17O NMR. pH effects as well as the influence of metal ions free in solution were investigated. The 17O NMR signals of both the nonchelating (O1) and chelating (O2) oxygen atoms could be detected. At low temperature, the signals of both the square antiprismatic (SAP) and twisted square antiprismatic (TSAP) conformational isomers were also observed. At high temperature, the spectra exhibit signal broadening that reveals the interchange of the O1 and O2 oxygen atoms of the carboxylate groups. The linewidths measured for O1 were deconvolved into contributions from quadrupole relaxation and chemical exchange, allowing the corresponding activation barriers to be determined.1

II) For all of the paramagnetic systems, except Gd3+, the 17O NMR signals of both O1 and O2 could be detected, and for some of them, the signals of both the SAP and TSAP (TSAP′) conformational isomers were also observed. Line width data analysis reveals that signal broadening is not dominated by paramagnetic relaxation enhancement, as it was believed to be. The data indicate that quadrupole relaxation and, for some complexes, chemical exchange between the SAP and TSAP isomers are the major contributions to the 17O NMR line width at 25 °C. Besides, the Fermi contact and pseudocontact contributions to the observed lanthanide-induced shifts could be extracted.2

III) The 17O NMR spectrum of the non-coordinated carboxyl oxygen in the Gd(III) DOTA complex has been observed experimentally. Its line width is essentially unaffected by paramagnetic relaxation due to Gd, and due only to the quadrupole pathway. The results are supported by the relevant parameters (hyperfine and quadrupole coupling constant) calculated by relativistic DFT methods. This finding opens new avenues for investigating the structure and reactivity of paramagnetic Gd(III) complexes used as contrast agents in MRI.3

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# Design and characterization of a dendrimeric contrast agent dedicated to the imaging of the nervous central system

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Dendrimers are a class of particular polymer with a large number of functions and a rigid quasi-spherical structure. Their properties make them ideal for a wide range of biomedical applications.
The aim of this work is therefore the development of a macromolecular contrast agent with several paramagnetic centers with a reduced mobility, able to be transported by neurons. Indeed, previous studies showed that the reduction of the mobility of a Gd-complex increases its efficiency in the magnetic fields of medical imaging. Our objective is thus to synthesize a second generation dendrimer and to graft it with a paramagnetic chelates and a vector for the neuronal uptake and transport.
The zero, the first and the second generations of dendrimer were obtained by optimizing different synthesis parameters such as the solvents, the temperature, the reaction time and the reagents. Finally, all generations were characterized by mass spectrometry and by nuclear magnetic resonance (NMR) spectroscopy (**Fig. 1**).
The macrocycle which is a DOTA derivative (a well-known contrast agent) was also obtained and fully characterized.
In the near future, we plan to graft on the second generation dendrimer the Gd paramagnetic complex and also the biocytin or the wheat germ agglutinin (WGA) in order to obtain an MRI contrast agent able to undergo uptake and transport by neurons.

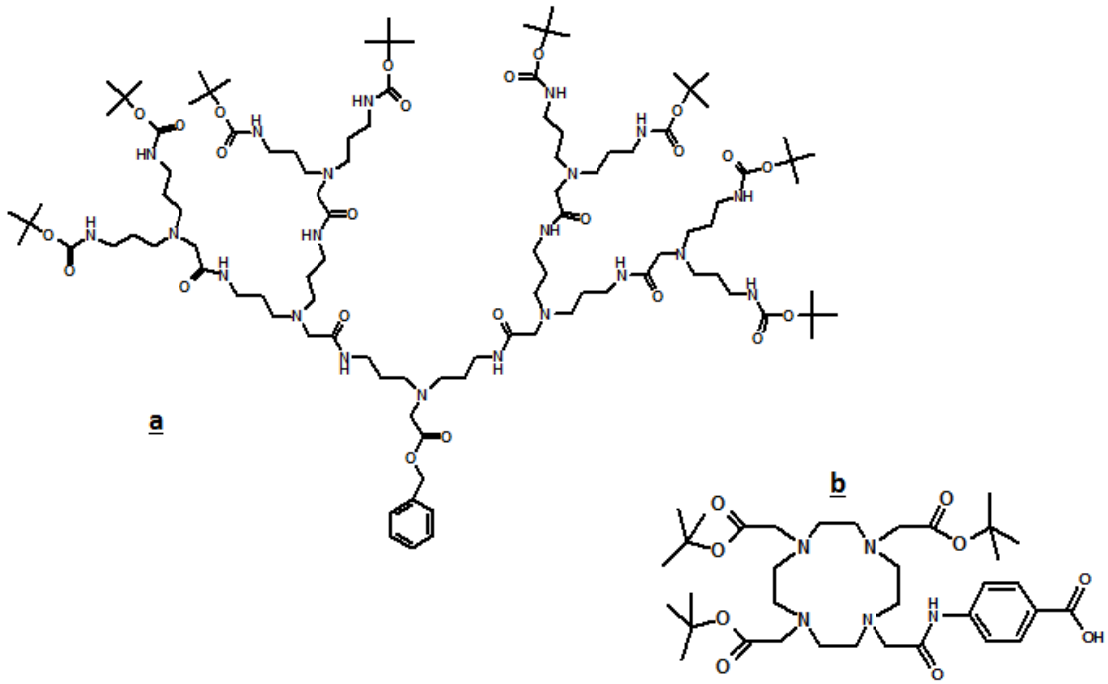


**Fig.1** : Design of **(a)** second generation dendrimer and **(b)** DOTA derivative

# Use of $^{13}$C-NMR in structural elucidation of polysaccharides: case of locust bean gum.

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Locust bean gum (LBG) galactomannans are polysaccharides consisting of a β-(1→4) D-mannopyranosyl backbone substituted to varying degrees in α-(1→6) with single D-galactopyranosyl residues. This basic structure is the same for all galactomannans (Fig. 1). However, when locust bean gum is extracted at different temperatures, the generated fractions exhibit different properties in aqueous solution (viscosity, viscoelasticity, gel formation, thermohydrolysis resistance, etc.). This means that there are differences within the fine structure of the polymers (although the basic structure is the same).

Analysis of [$^{13}$C]-NMR spectra of galactomannans, in combination with other techniques, can provide capital information about fine structural elucidation of the polymers. The method specifies the distribution of lateral galactosyls along the main chain of mannans.

Two fractions extracted from locust bean gum at 25 and 80 °C (respectively GM25 and GM80) were comparatively studied by [$^{13}$C]-NMR. Mannosyls/Galactosyls (M/G) ratios can be determined by considering the intensities of C-1 mannose and galactose signals in [$^{13}$C]-NMR spectra. This method provides results relatively close to those obtained by GC-MS analysis. Spectra also showed that resonance from $C_4$ of D-mannose residues were split, in evident dependence upon the nearest-neighbor probabilities ("diad frequencies") of D-galactosyl groups along the mannan chains (Fig. 1). Diad frequencies were obtained by integrating $C_4$(Man) peak areas. $F_{11}$, $F_{21}/F_{12}$ and $F_{22}$ gave respectively the di-, mono- or non-substituted mannose pairs proportions. High percentages of $F_{11}$ and $F_{22}$ therefore indicate a more non-homogeneous distribution of lateral galactosyls along the polysaccharide backbone as observed for GM80. The percentages of total lateral substituents obtained by $C_4$(Man) peak analysis [$F_{11}$ + ($F_{21}$ or $F_{12}$)/2] were fairly well correlated with M/G ratios. Splitting of the C-6 substituted D-mannose resonance provides, therefore the basis for determining the next-nearest-neighbor probabilities (triad frequencies) (Fig. 1). However, the spectrum is often not sufficiently resolved to accurately quantify and interpret the results.

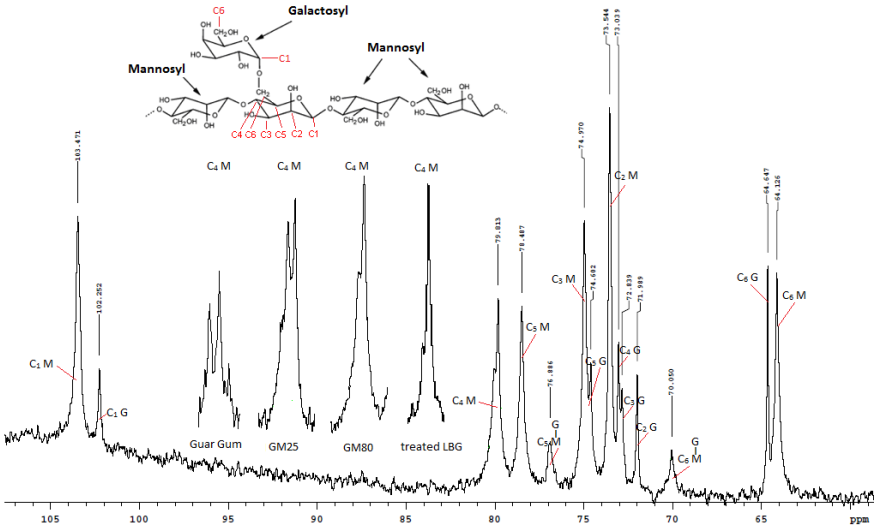


**Fig. 1 : [$^{13}$C]-NMR spectrum of GM80 and superimposed mannosyl C4 signals of different galactomannans.**

# Conception of superparamagnetic polymersomes for potential drug delivery and magnetic resonance imaging applications

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Ultra-small superparamagnetic iron oxide nanoparticles (USPIO) have been extensively investigated for biomedical applications due to their numerous advantages like their high magnetization, their biodegradability and their biocompatibility. Thanks to their high magnetic moment, USPIO lead to a Magnetic Resonance Imaging (MRI) contrast enhancement by accelerating the relaxation of water protons. Besides their use as MR imaging contrast agents, USPIO can also create an increase in temperature by applying an oscillating magnetic field (magnetic hyperthermia).

The objective of this study is to develop nanoscale drug delivery systems encapsulating iron oxide nanoparticles. Among the different delivery systems, polymer vesicles called polymersomes have emerged as promising nanocarriers thanks to their robustness and their ability to load hydrophilic as well as hydrophobic compounds [1]. Firstly, commercial copolymers of poly(ethylene oxide) (PEO) and poly(caprolactone) (PCL) were used to prepare polymersomes. Then iron oxide nanoparticles were synthesized by a coprecipitation method and stabilized by an organosilane (3-triethoxysilylpropyl succinic anhydride (TEPSA)). Transverse ($r_2$) and longitudinal ($r_1$) relaxivities of the USPIO-TEPSA (describing their efficiency as MRI contrast agent) were measured at 20 and at 60 MHz. A nuclear magnetic relaxation dispersion (NMRD) profile reporting the longitudinal relaxivity over a magnetic field range extending from 0.15 MHz to 60 MHz was recorded on these USPIO-TEPSA. The saturation magnetization ($M_s$) and the size of superparamagnetic crystals ($R_{NMR}$) were determined by fitting this NMRD curve by a theoretical model [2].

The next step in further studies will be the encapsulation of iron oxide nanoparticles into the polymersomes which could be used to release a drug in a controlled manner due to an increase of the fluidity of the semicrystalline membrane by heat produced by magnetic nanoparticles. The attachment of an active targeting group such as an RGD-containing peptide to the polymersome surface is also considered.

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## Investigation of the effects due to the insertion of a polyQ tract increasingly long on the structure and dynamic of BlaP using NMR spectroscopy

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Polyglutamine (polyQ) diseases, which include the well-known Huntington's disease, arise from an anomalous expansion of a polyQ tract within proteins specific to each disease[1]. When the length of this polyQ tract is higher than a characteristic threshold (~35-45Q), it becomes pathogenic by triggering the aggregation of disease-associated proteins into amyloid fibrils which are deposited in neurons as nuclear inclusions and lead to the death[2]. Although the polyQ repeat was thought to be the only determinant factor that mediates the aggregation, recent studies have demonstrated that non-polyQ regions of these proteins play also a significant role, either preventive or facilitative, into the aggregation process[3-4]. In order to better understand the complex interplay between the ability of the polyQ tract to mediate the aggregation and the modulating effect of non-polyQ regions, we engineered in our lab chimeric proteins by inserting at two positions (i.e. 197 and 216) a polyQ tract of various lengths (i.e. 23Q, 30Q, 55Q and 79Q) into the β-lactamase BlaP from Bacillus licheniformis 749/C[5]. Both sets of BlaP chimeras recapitulate the aggregation behaviour of disease-associated proteins: indeed there is a repeat threshold length above which the chimeras readily assemble into amyloid fibrils and the rate of assembly increases with polyQ length. While having little impact on the overall structure, the location of the polyQ tract affects the tertiary structure, enzymatic activity, unfolding cooperativity and stability of the chimeric proteins, and more importantly, it significantly affects their aggregation propensity. Indeed, the 216 chimeras exhibit a significantly higher fibril-forming propensity than their 197 counterparts[6].

The aim of my work was to use NMR to investigate, at the molecular level, the effects of polyQ insertions on the structure and the dynamics of BlaP, in order to better understand the differences in the aggregating properties of the two sets of chimeras. First, I assign 98.5% of the backbone 1H-15N correlations in the 2D-HSQC spectrum of BlaP216Q0 using a series of triple resonance NMR experiments. Then, the regions of BlaP affected by the insertion of the dipeptide PG at position 197 and 216 were identified by comparing their respective [1H-15N] 2D-HSQC spectrum with that of the wild-type been introduced within the gene of BlaP, at position 197 or 216, to allow poly(CAG) sequence insertion. Changes in the chemical shift of each residue due to the PG insertion were determined. In both cases, the perturbations are essentially located around the insertion site (i.e. helix H8 for BlaP197Q0 and helix H9 for BlaP216Q0); they are, however, more significant when the dipeptide is inserted at position 216 than at position 197. Perturbations also occur in the three left-located helixes (i.e. H3, H4 and H9) that seem to laterally propagate the perturbation because of side chains interactions. In order to investigate eventual changes in the dynamic of BlaP upon the insertion of the dipeptide PG, real time hydrogen/deuterium exchange experiments were carried out. The preliminary analysis indicates that the dynamics of BlaP is more perturbed by the insertion at position 216 than at position 197; this result is in agreement with the lower thermodynamic stability of this chimera. Finally, the perturbations due to the presence of a polyQ tract increasingly long were also investigated. Globally, polyQ insertion at position 197 induces perturbations essentially located in the α-domain of BlaP while the perturbations due to insertion at position 216 are located in both domains. For both sets of chimera, the longer the polyQ, the farther are the perturbations on the surface of BlaP. These differences may explain the different aggregation propensity of the two sets of chimeras.

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# Evaluation of extracellular pH and energetic status response to hyperglycemia and MIBG treatment on oxidative versus glycolytic tumor phenotype: An *in vivo* 31P MRS study

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**Context:** While normal cells generate ATP and biosynthetic precursors through a combination of glycolytic and oxidative metabolism, tumor cells reprogram their metabolism depending on their microenvironment to support a rapid, invasive and metastatic growth. Indeed, it has been shown that solid tumors show an increased glycolytic metabolism involved in tumor aggressiveness.

There are two types of glycolytic phenotype: some tumor cells switch from oxidative phosphorylation to glycolysis under hypoxic conditions (Pasteur effect, anaerobic glycolysis), and some cells remain glycolytic even when oxygen availability is restored (Warburg effect, aerobic glycolysis) .This Warburg phenotype is a hallmark of aggressive cancers. So the evaluation of metabolic pathways in solid tumors and their interaction is very important for the understanding of mechanisms of transformation, malignant tumor resistance to cancer chemotherapy and developing new treatments

**Purpose:** Our project aims to establish an *in vivo* non-invasive dynamic analysis of tumor metabolism by $^{31}P$ magnetic resonance spectroscopy. We study the effect of mitochondrial inhibition by hyperglycemia and MIBG on the pHe and energetic status. We assume that the response of these metabolic biomarkers would discriminate an oxidative metabolism (Pasteur Effect) of a glycolytic metabolism (Warburg Effect).

**Methods and results:** The NMR spectroscopy is performed with 11.7 T Bruker Biospec system and a 1H/$^{31}P$ Transceiver Surface Coil with a single pulse sequence. The pHe measurement is based on chemical shift of $^{31}P$ exogenous probe (3-APP) and energetic status is measured via the ratio of NTP/total phosphate. The choice of tumor models is based on *in vitro* phenotypic characterization performed beforehand in the laboratory. (De Preter et al.) The oxidative phenotype is represented by the TLT and SiHa models and glycolytic phenotype by MDA-MB-231 model. The treatment with hyperglycemia caused a significant acidification in both oxidative models but not in MDA-MB-23. No significant differences were observed in all three models for the energetic status response. The combination of hyperglycemia with MIBG induced an acidification in each model and decreased energetic status for SiHa and MDA-MB-231. Moreover, there are no significant differences in pH variations induced between the three lines.

**Conclusion:** We observe a different pHe response to hyperglycemia alone (Crabtree Effect) between oxidative and glycolytic metabolism

# Impact of steam explosion treatment on chemical configuration of Festuca L. lignin : structural elucidation using NMR spectroscopy

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In the economic and energy context of our society, it is universally recognized that alternatives to petrochemicals products must be found. To overcome this problem, renewable lignocellulosic biomass could be used to produce high value products. To achieve this objective, pretreatment processes are required to allow the breakdown of lignocellulosic structure and increase accessibility of the material. In this way, steam explosion is a thermo-mechano-chemical pretreatment which allows the opening of lignocellulosic material structural components and includes modifications of the physical properties of the material, hydrolysis of hemicellulosic components and modification of the chemical structure of lignin [1].
This study is focused on the impact of various steam explosion treatments on the chemical configuration of Festuca L. lignin. NMR-2D analyses perform on the Festuca L. pretreated samples show a slight increase of the β-β link with the treatment intensities, which traduce a partial re-polymerization of the lignin structure. In parallel, HPSEC analyses show modifications in the molecular weight of the lignin obtained after the steam explosion treatment.

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## DOSY in HR-MAS : a tool to be used with caution.

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Our laboratory is developping since several years iron oxide nanoparticles for molecular imaging by MRI. Molecular imaging aims to visualize at an early stage molecular entities which are expressed or overexpressed in pathological conditions. To achieve this, iron oxide nanoparticles are vectorized in order to recognize specifically these molecular targets. The vectorization is performed by grafting at the nanoparticle surface small peptides selected in our laboratory by the phage display technique.

Before testing these nanoparticles *in vitro* and *in vivo*, it is important to fully characterize them, and particularly it is important to verify the covalent grafting of the small vectors on the nanoparticle surface. A method of choice to achieve it could be NMR, and particularly DOSY. A significant decrease of the diffusion coefficient of the small organic molecules should indeed be observed when they are grafted at the nanoparticle surface.

Iron oxide nanoparticles being superparamagnetic, HR-MAS spectroscopy is needed to obtain 1D and 2D spectra of grafted organic molecules, as it has been shown in an article of Polito et al.[1] Nevertheless, we will show here that the recording of DOSY spectra in HR-MAS is not so easy and requires to take some precautions, as it was already demonstrated in the literature[2]. We will also show that it becomes impossible to obtain accurate diffusion coefficients on iron oxide nanoparticles grafted with a test molecule, polyethylene glycol (PEG).

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# Metallo β-lactamase Cau-1 : Interaction with Adenosine Triphosphate and Metal-binding studies

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Cau-1 is a class 3 metallo β-lactamase constitutively expressed by *Caulobacter crescentus.* The enzyme uses two Zinc cations as cofactors in the active site, but valuable information can be obtained through replacement of these ions with chemically similar metals such as Cadmium.
In order to investigate the Zinc-binding process and the reversibility of such cofactor removal, Zinc titration of the apoenzyme form has been realized and followed by NMR. The results of this experiment allowed the Zinc replacement by Cadmium to be performed, which will eventually lead to obtaining a $^{113}$Cd NMR probe in the active site of the enzyme.

*Caulobacter crescentus* living in an antibiotic-free environment, the expression profile of Cau-1 suggests a yet unknown additional function for the protein. Several known enzymes are exhibit strong structural similarity to metallo β-lactamases, especially class-3, but have an activity upon different compounds such as nucleic acids, glyoxals, and even the insecticide carbofuran. The possibility of an interaction of Cau-1 with nucleic acids derivatives has been investigated.
Kinetic studies show that Adenosine Triphosphate (ATP) interacts with Cau-1 and even increases its β-lactamase activity. NMR titration of Cau-1 with ATP has been used in order to further study this interaction.

# Electrochemical degradation of imidazolium based ionic liquids studied by NMR spectroscopy

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During the last years, ionic liquids have attracted increasing attention within the scientific community and in particular amongst the electrochemists. Imidazolium based ionic liquids, which are known for their high ionic conductivity and their relatively low viscosity at room temperature, appear to be good electrolytes for various electrochemical processes such as metal deposition or electrocatalysis.

We are currently investigating the conversion of $CO_2$ at a gold electrode in [BMIm][$NTf_2$] (BMIm = 1-butyl-3-methylimidazolium $NTf_2$ = bis(trifluoromethanesulfonyl)imide), a room temperature ionic liquid selected for its capacity to fairly solubilize carbon dioxide. Voltammetric experiments have revealed that carbon dioxide reduction occurs at potentials for which reduction of the ionic liquid cannot be excluded. Little is known on the cathodic decomposition of imidazolium based ionic liquids at gold electrodes and, therefore, the electrochemical stability of [BMIm][$NTf_2$] was first investigated. Long-time electrolysis experiments were performed at controlled potentials in the absence of carbon dioxide and the medium was then analyzed by $^{1}H$, $^{13}C$ and $^{19}F$ NMR spectroscopy. Several NMR signals ascribable to the reduction of the ionic liquid were detected. The structure of the major reduction product of the BMIm cation was elucidated thanks to various one- and two-dimensional NMR experiments and its output could be quantified as a function of the electrolysis time and the consumed charge. Additional experiments were conducted with two other imidazolium based ionic liquids: [BDMIm][$NTf_2$] (BDMIm = 1-butyl-2,3-dimethyl-imidazolium) selected in order to assess the role of carbene formation and [BMIm][DCA] (DCA = dicyanamide) in order to examine the possible influence of the anion on the cathodic stability of the ionic liquid.

**A nanohydrogel approach to boost the relaxivity of conventional MRI Gd contrast agents**

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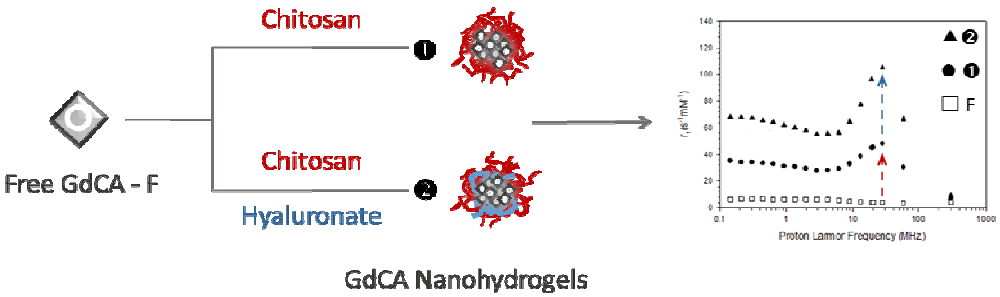
Magnetic resonance imaging (MRI) is one of the most powerful non-invasive techniques for clinical diagnostic, mainly due to its high anatomical resolution and its speed. MRI relies upon the perturbation by a radio frequency of the relaxation times of water protons, along the longitudinal ($T_1$) and transverse ($T_2$) components of the main applied magnetic field.[1] Contrast in MR image results then from the natural variations of the relaxation times ($T_1$ and/or $T_2$) between healthy and diseased tissues. Because these variations are weak, the use of paramagnetic contrast agents (CAs) is often required. The role of these CAs is to shorten the $T_1$ and/or $T_2$ relaxation times of water protons in the tissues.[1] The most commonly used contrast agents are gadolinium chelates (GdCAs) and their efficiency to accelerate the relaxation rate is given by their relaxivity $r_1$. Even if these agents have enabled significant progresses they still suffer from some limitations because their relaxivities are far from the ones predicted by the theory.[1]
In order to circumvent these limitations, we have developed a series of GdCA-loaded polysaccharide-based nanohydrogels (GdCA⊂NPs).[2] These biocompatible nanohydrogels, elaborated by an easy and robust ionotropic gelation process[3] involving chitosan (CH) and hyaluronan (HA), encapsulate GdCAs such as GdDOTA, GdDOTP and MS325 in a highly hydrated nanostructure. In the poster presentation, we will demonstrate that according to the nature of the polymer matrix and to the cross-linking ability of the GdCA, $r_1$ relaxivities per Gd centre as high as 100 $s^{-1}$ $mM^{-1}$ at 30 MHz can be reached. The NMRD profiles will confirm that molecular motions of the Gd chelate are effectively restricted and that water access to the inner core of these nanogels is not limited. On $T_1$- and $T_2$-weighted images recorded at 3Teslas, we will show that this relaxation enhancement is clearly translated into a magnified contrast, demonstrating the powerful dual mode imaging capability of such nanosytems.

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# Impact of oxygenation status on $^{18}$F-FDG uptake inside solid tumors

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Hypoxia is for long known as a major factor in tumor progression, metastasis and response to treatments in solid tumor. It has also a notable impact on tumor metabolism since tumor cells can adapt their metabolism according to the oxygen content. The issue of the relationship between hypoxia within tumors and glucose uptake has been debated in the literature. In this context we tried to assess the influence of acute changes in tumor oxygenation on the uptake of $^{18}$F-FDG. For this purpose, we evaluated the uptake of $^{18}$F-FDG using micro-PET imaging under different oxic conditions in parallel with true $pO_2$ measurements with EPR oximetry in two different human tumor models, MDA-MB-231 and SiHa models (n=16 and n=12 respectively). Mice were scanned twice for the breathing challenge, air versus carbogen breathing, with one day between each condition. For each breathing condition, EPR measurements were performed before PET imaging.

We found acute changes in $^{18}$F-FDG uptake linked to the carbogen challenge. We observed a lower uptake of $^{18}$F-FDG under hyperoxic conditions compared to normoxic conditions in both tumor models. In Fig. 1, we present the relationship between global $^{18}$F-FDG uptake and pO2 measurements obtained from individual tumors (mice breathing air or carbogen). The $^{18}$F-FDG uptake was higher in hypoxic tumors compared to tumors with $pO_2$ larger than 10 mmHg.

We assessed the correlation between global PET uptake and $pO_2$ values for each tumor model during the breathing challenge. We found that the uptake of $^{18}$F-FDG was higher in tumors with a $pO_2$ value inferior to 10 mm Hg. We also found that the uptake of $^{18}$F-FDG was lower after a short period of carbogen breathing. This observation emphasizes that the uptake is not only depending on the GLUT-1 expression but also depends on the rapid adaptation of the metabolism of the tumor cells when oxygen became available.

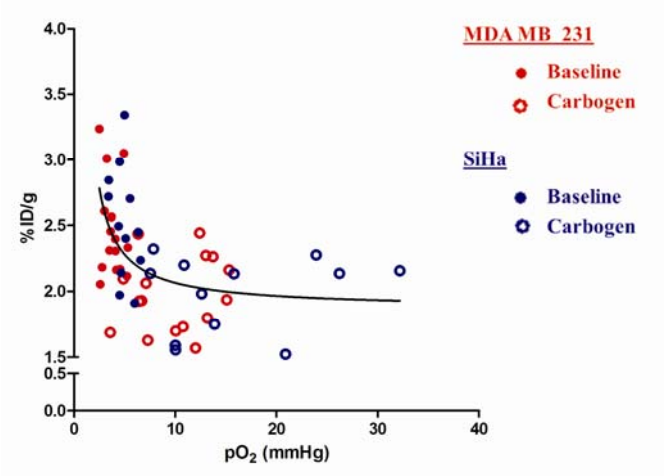


**Fig. 1 : Relationship between $^{18}$F-FDG uptake and $pO_2$ values during air (filled symbol) and carbogen breathing (open symbol).**

# Simultaneous solvent and J-modulation suppression in PFGSTE diffusion measurements

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The measurement of molecular translational diffusion in solution by NMR pulsed field gradient experiments has become an established methodology with a broad range of applications, such as the study of intermolecular interactions or complex mixture analysis. One of the greatest sources of error during the extraction of diffusion coefficients is spectral overlap between peaks of different compounds. Homonuclear scalar coupling evolution during the pulse sequence (J-modulation) is particularly notorious in this respect, as the introduced dispersive line-shape contributions decrease the spectral resolution that is of prime importance for 2D DOSY analysis. [1]

WATERGATE and excitation sculpting have been the most successful solvent suppression schemes applied in diffusion experiments. [2,3] However, the long spin echoes to accommodate the soft rf-pulses lead to significant J-modulation. These line-shape artefacts can be suppressed by means of the perfect-echo element, [4,5] which has already been applied in solvent suppression ("Perfect-Echo WATERGATE"). [6] Here, a PFGSTE-based diffusion NMR experiment based on the Perfect-Echo WATERGATE is presented that suppresses both the solvent peak and homonuclear J-modulation artefacts. [7] An excellent quality solvent suppression is achieved next to near-perfect in-phase line-shapes. The sequence allows for long soft pulses to be applied without introducing severe J-modulation artefacts, making DOSY experiments with narrow bandwidth solvent suppression possible.

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# Confirming vanadium dopant incorporation in an Al-Metal-Organic Framework MIL-53 by EPR and ENDOR spectroscopy

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Metal organic frameworks (MOFs) are often called “superzeolites” or “the next generation of porous materials”. MOFs are constructed of metal ions or clusters linked by organic ligands to form an infinite three-dimensional network. The possibility of designing MOF to desired functionality, by combining different metal ions and organic linkers, makes these materials very interesting for a wide range of applications, from catalysis and gas storage to purification. Two characteristic types of MOFs, exhibiting one-dimensional pores, are MIL-47[1] [VO(BDC); BDC = terephthalate or 1,4-benzenedicarboxylate, MIL = Materials of the Institute Lavoisier] and MIL-53[2] [Al(OH)(BDC)], originally synthesized by Ferey’s group.

Recently we reported that V-MIL-47 can be a highly selective catalyst in the epoxidation of cyclohexene[3]. However, water exposure should be avoided, as it causes framework instability. A solution for this problem may be doping the highly stable MIL-53 with catalytically active $V^{IV}$ ions. In order to understand the catalytic activity of such doped framework, it is necessary to verify where dopant ions actually are incorporated in the framework. As $V^{IV}$ ($3d^1$) is a paramagnetic ion, and EPR and ENDOR spectroscopy give information about the local coordination environment and the site symmetry of paramagnetic centers, these characterization methods can provide the answer.

The EPR spectra of V-doped MIL-53 samples after synthesis are dominated by just one EPR component. It exhibits a nicely resolved 8-lines hyperfine (HF) structure due to interactions between unpaired electron and $^{51}V$ nucleus (Fig. 1.). From multifrequency (S, X, Q and W-band) EPR spectra it is extracted that $V^{IV}$ center has a rhombic g and the principal axes of the g tensor and $^{51}V$ HF tensor do not coincide. The ENDOR spectra (Q-band) of $V^{IV}$ in MIL-53 reveal HF interactions with $^{1}H$ and $^{27}Al$ nuclei, whose anisotropy allows reconstructing the nearest environment of the dopant ions, thus yielding direct information on whether and where the dopant is actually incorporated in the MIL-53 lattice.

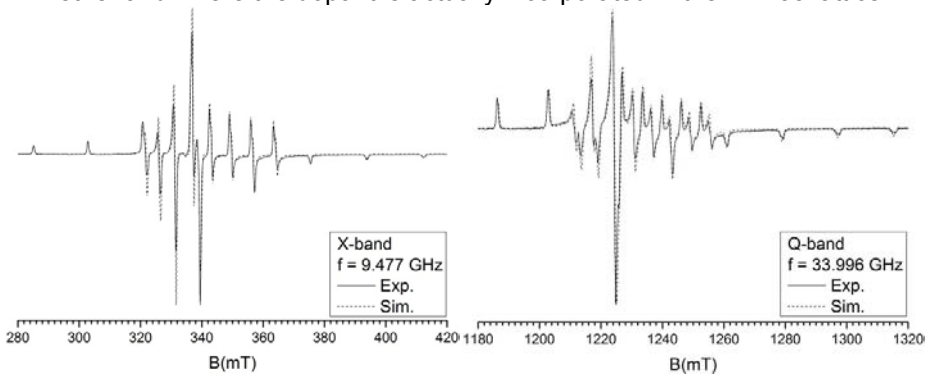


**Figure 2 - X and Q-band spectra of V-doped MIL-53 at RT**

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# Nanodiamond Particles for Medical Imaging: Chemical Oxidative Treatment and Analysis of the Surface

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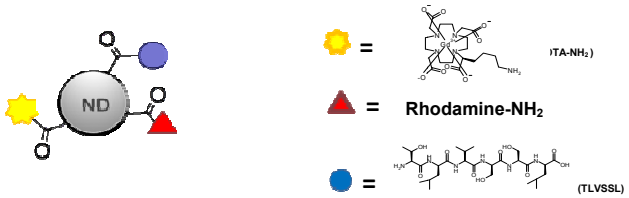
Nanotechnology is a multidisciplinary research field involving the use of devices reduced to the nanoscale. These nanomaterials are new objects whose reactivity and properties are different from those observed at the micro- or macroscopic scale. These "nano-objects" found many applications in various domains including biomedical research[1].
This research program aims to develop and validate a tool for the diagnosis based on a nanodiamond platform which is specific (biological vector) and active (contrast agent and fluorochrome) for medical imaging techniques (magnetic resonance imaging (MRI) and optical imaging (OI)). The benefit of this multimodal nanoprobe results from the combination of MRI and OI properties in terms of high spatial resolution and high sensitivity.




Surface functionalization of nanoparticles is a strategy allowing targeted applications. The scientific method adopted in this work starts with a chemical modification of the surface of diamond nanoparticles (two treatments tested[2,3]). Then, the platform is made specific by grafting molecules of interest (contrastophore, fluorophore). The specificity of multimodal probe to apoptosis will be made possible through the grafting of a peptidic vector. The oxidative treatments have been tested to ensure uniform presence of carboxylic acids at the surface of the nanoparticles. These functions will allow the loading of specific molecules of interest (contrast agent, fluorochrome and peptide).
The surface composition of the studied materials (treated and untreated NDs) was then analyzed by thermogravimetric analysis (TGA) and X-ray photoelectron spectroscopy (XPS) to define the efficiency of the oxidation method. The quantitative determination of the carboxylic groups added by the surface oxidizing treatment was conducted following the method of adsorption / desorption of a selected dye, toluidine blue[4] and performed using the UV-visible spectroscopy.
The grafting of a gadolinium complex on the nanodiamond was performed to obtain a MRI contrast agent. The choosen gadolinium complex, Gd-DOTA-$NH_2$, has been synthesized. The different intermediates have been characterized by mass spectrometric and NMR techniques. The surface functionalization of ND-COOH with the MRI contrast agent was characterized by relaxometry and NMRD profiles. The designed multifunctional probe showed promising relaxation rate for MRI applications. Results demonstrated the effectiveness of the MRI nanodiamond-based probe for medical imaging.

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## Analysis of the multimerization of $Cu^{2+}$ complexes in aqueous solution by $^{1}H$ NMR and Evans' method

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Ceramic high temperature superconductors are near-perfect conductors for electricity and promising materials for applications in magnets, generators and transformers in the context of green energy generation and energy saving industrial devices. To allow their integration in conducting wires, these brittle materials are deposited on flexible substrates using precursor-containing chemical solutions. The development of such precursor solutions is a time-consuming and complicated task that would greatly benefit from being able to predict the solution's stability as a function of factors such as pH and concentrations of metal ions and additives[1].

Triethanolamine (TEA) is often used to stabilize metal ions in aqueous $YBa_2Cu_3O_{7-x}$ (YBCO) precursor solutions. However, the nature of the complexation of $Cu^{2+}$ ions with TEA is still a matter of debate. To increase the accuracy in predicting the solution's stability, clarification on this issue is of course desirable. Although crystal structures of dimeric and tetrameric complexes have already been described, there exists no data concerning the existence of such complexes in solution.

Here, we present NMR evidence of the pH-dependent formation of TEA complexes containing at least two $Cu^{2+}$ ions in solution. By measuring the magnetic moment per $Cu^{2+}$ ion using $^{1}H$- NMR spectroscopy and Evans' method[2,3], we demonstrate that multi-nuclear copper-triethanolamine complexes form in aqueous solution at pH 4 or higher. This information can be obtained accurately in complex mixtures with large counterion and background species concentrations by making use of diamagnetic corrections from reference measurements and tabulated data. Our approach will be most useful to further improve and develop speciation models that simulate the stability of precursor solutions for the deposition of Cu-based ceramic films.

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# Investigation of Total Pore-Blocking Conditions in Polymer Monolithic Columns

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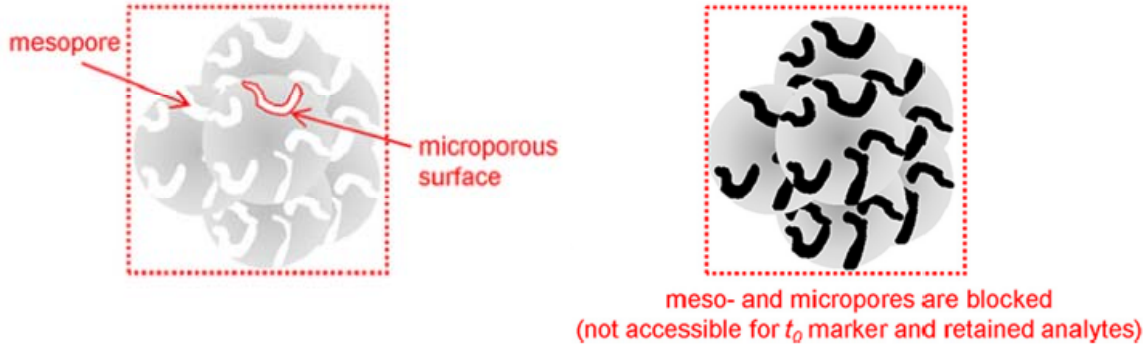
Cross-linked polymer monolithic materials for chromatography were introduced in the early 1990s, and the resulting polymer monolithic columns have been successfully applied for the HPLC separation of synthetic polymers and large biomolecules. In contrast, the performance of such columns for the separation of small molecules is generally poor. This unfavourable performance (for small molecules) is attributed to the absence of stagnant pores (and consequently the lower surface area) and to mass-transfer effects. The surface area can be increased using a polymer monolithic column with a broad bimodal pore-size distribution (*i.e.* having macro- and mesopores) rather than a typical narrow monomodal macropore size. Mass-transfer effects causing band broadening are for instance restricted surface diffusion, related to the gel porosity and swelling of the polymer.

An answer to these problems was formulated as the total pore-blocking method,[1] introduced in 2007 as an alternative method to inverse size-exclusion chromatography. This method is based on the filling of the stagnant pores of the stationary phase with a hydrophobic solvent, which is immiscible with the mobile (water) phase and remains in the pores due to strong interactions, hence blocking the pores.

To demonstrate in situ total pore-blocking in polymer monolithic columns, HR-MAS NMR is an excellent technique to investigate the swollen cross-linked polymer monolithic columns, since the broad bands from the polymer matrix are eliminated. Consequently, $T_2$ relaxometry and PGSE NMR proved the wetting of mesopores by common organic solvents like acetonitril and dodecane, as evidenced by different $T_2$ relaxation times and diffusion coefficients, corresponding to the macro- and mesopores. In contrast, when water was used only one $T_2$ relaxation time and one diffusion coefficient were found, corresponding to the macropores.[2]

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# Solid-state NMR characterisation of a shape memory material

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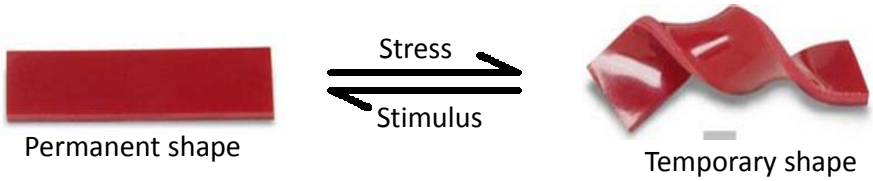
Solid-state NMR is used to study a PCL network. This network is obtained by covalently bonding; through thermoreversible Dields-Alder adducts, 4 arm star-shaped PCL end-functionalised by furan and maleimide moieties. Since PCL is a semi-crystalline polymer, PCL networks exhibits shape memory properties. Shape memory is the ability of a material to change shape from a temporary shape to a permanent shape upon application of an external stimulus, such as heat or light.




A range of solid-state NMR experiments are implemented to characterise the precursors and the shape memory material. A considerable level of motion in these samples was showed by high spectral resolution in the $^{1}H$ spectra and a reduced effective dipolar coupling in the $^{13}C$ cross-polarisation spectra for some components. Prove of the network formation was given by $^{1}H$ direct polarisation experiments performed at 65°C. In fact, the dramatically improved spectral resolution was not observed for the network, which indicates that the sample did not melt.

Solid-state NMR can be used to extract information specific to the solid state, such as identifying crystalline versus amorphous phases. The different phases present in the samples were determined by different $^{13}C$ experiments and $T_{1C}$ measurements. In fact, direct polarisation and cross polarisation do not show the same components, the first one shows fast relaxing components with high mobility whereas the cross polarisation experiment shows components with reduced mobility where the dipolar coupling is not average. The determination and quantification of more mobile regions and less mobile regions was achieved and the mass fraction of the crystalline components is in good agreement with the degree of crystallinity determined by DSC measurements.

## Polysiloxane coated nanoparticles, an innovative platform for bimodal molecular imaging

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Multimodality molecular imaging is now playing a pivotal role in biomedical research for noninvasive, quantitative visualization of fundamental molecular and cellular *in vivo* processes. Such emerging technology uses the strength of different imaging techniques and yields a hybrid imaging platform with benefits superior to those of any of its individual components. As a prototype, we decided to combine the benefits of a fluorescent probe to the synthetized MRI platform. Superparamagnetic iron oxide nanoparticles were prepared by co-precipitation of iron salts in diethyleneglycol as described elsewhere. Stabilization has been achieved by coating the particles with TEPSA. The as-obtained stabilized nanoparticles have then been decorated with chemically modified rhodamin in order to obtain the bimodal probe. At each step of the synthesis, the particles have been characterized by conventional techniques including DLS, infrared spectroscopy, TGA, TEM and relaxometry. The resulting ferrofluid has been administrated to mice suffering from a tumor. As hoped, the bimodal probe could be localized by MRI and optical imaging. Grafting a specific vector in order to obtain a probe for molecular imaging studies appear as an exciting research axe to develop. Previous studies performed in our laboratory have already highlighted specific vetcors able to target, among others, inflammation process or apoptosis

# Relaxation dynamics in ferromagnetic resonance for chemically disordered FePt thin films

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FePt intermetallic alloys have been thoroughly studied in the past years, mainly because of possible applications in the field of magnetic recording. This interest arises from the presence of a very large coercitivity and anisotropy in the ordered crystalline phase. However, films deposited by sputtering techniques at room temperature tend to grow in an FCC crystalline structure with the atoms randomly distributed and with relatively soft magnetic properties. Although the coercive field and the magnetocrystalline anisotropy are not as high as in the ordered phase, this system presents a magnetic behavior with many unusual properties from the fundamental point of view.
It has been reported that as-made FePt thin films presents a component of the anisotropy perpendicular to the plane of the film that can give a particular kind of magnetic structure called “stripe-domains”. This configuration presents a small out-of-plane component of the magnetization which periodically changes from the “up” to the “down” direction inducing the formation of a stripe structure. The origin of the out-of-plane anisotropy is due to the combined effects of magnetocrystalline anisotropy and magnetoelastic energy.
The dynamic behavior of a set films has been studied using ferromagnetic resonance (FMR) at room temperature in order to analyze the damping of the magnetization as a function of the excitation frequency. As expected, an increment in the microwave frequency resulted in higher resonance fields and broader linewidths. Also, all the magnitudes involved show an anisotropic response to the angle between the perpendicular direction of the film and the external field, although for a homogeneous ferromagnetic alloy the linewidth should remain constant. This apparently odd behavior was explained by adding to the Gilbert broading an inhomogeneous contribution, independent of the frequency .

Related work:

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# Non-invasive assessment of the onset and progression of amyotrophic lateral sclerosis in transgenic animal models using magnetic resonance spectroscopy.

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**Introduction:** Amyotrophic lateral sclerosis (ALS) is a neurodegenerative neuromuscular disorder characterized by rapidly progressive loss of motor neurons. There is increasing interest in using advanced MR neuroimaging techniques such as proton magnetic resonance spectroscopy ($^1$H-MRS), diffusion tensor imaging (DTI) and functional MR imaging (fMRI) in diagnosing ALS[1]. Visualizing corresponding metabolic changes in the brain of patients with ALS with $^1$H-MRS could provide bio markers for an early disease detection, for monitoring the progression and for evaluating a treatment response. In this ongoing longitudinal study we employ MRS to reveal biochemical changes in the novel transgenic ALS mouse model hTDP43$^{A315T}$. TAR DNA-binding protein (TDP-43) is found to be a major pathological protein in ALS[2]. Transgenic mice, overexpressing mutant (A315T) human TDP-43 have developed motor neuron disease with involvement of both cortical and spinal motor neurons reminiscent of human ALS. Here we report the first results of *in vivo* MRS at 9.4T in the initial phase of this

**Methods**: Localized proton spectra of motor cortex were collected using a voxel size of 1.5x 2.5x 2.5 mm via a PRESS sequence with TR / TE of 1.8s / 20ms. Shimming was performed using FASTMAP. Data were processed and analyzed using jMRUI software package with an in house recorded metabolite profile using QUEST combined with the 'Subtract' approach for background modeling[3]. Quantitative data are reported as a ratio to unsuppressed water signal. Brain metabolite concentrations in motor cortex of female TDP-43$^{A315T}$ mice were recorded 150,180 and 210 days postpartum (126 days median survival).

**Results and discussion**: Within the studied age range no significant differences in metabolite levels could be observed (see fig1). Previous studies[4] in another ALS model (G93A-SOD1 mice) have reported significantly decreased levels of N-acetyl aspartate 34 days postpartum and significant decrease of glutamine and γ-aminobutyric acid at Day 75 (50% survive at 128 days). Since the average metabolite levels varied considerably less we should be able to observe similar metabolic changes. The lack of significant differences in metabolite concentrations (Figure 1) and large variances might reflect a subject dependent disease progression. Alternatively, the processing method used, in particular the background modelling, could be suboptimal. We intend to employ an optimized macromolecule-lipid based quantification method in parallel with the current method. We will continue to characterize the novel hTDP43-A315T mouse model to non-invasively identify and visualize the neuronal degeneration and correlate other *in vivo* MRI readouts with $^1$H-MRS.

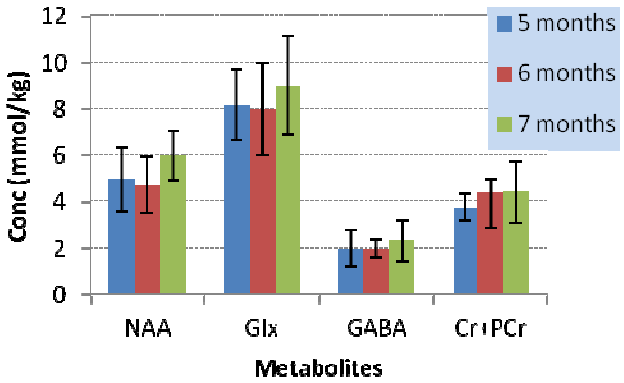


**Figure** 1 TDP-43 +/- mice motor-cortex metabolite levels. n=5, av±SD

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# Development of a colorimetric biosensor specific to the oncoprotein Mdm2

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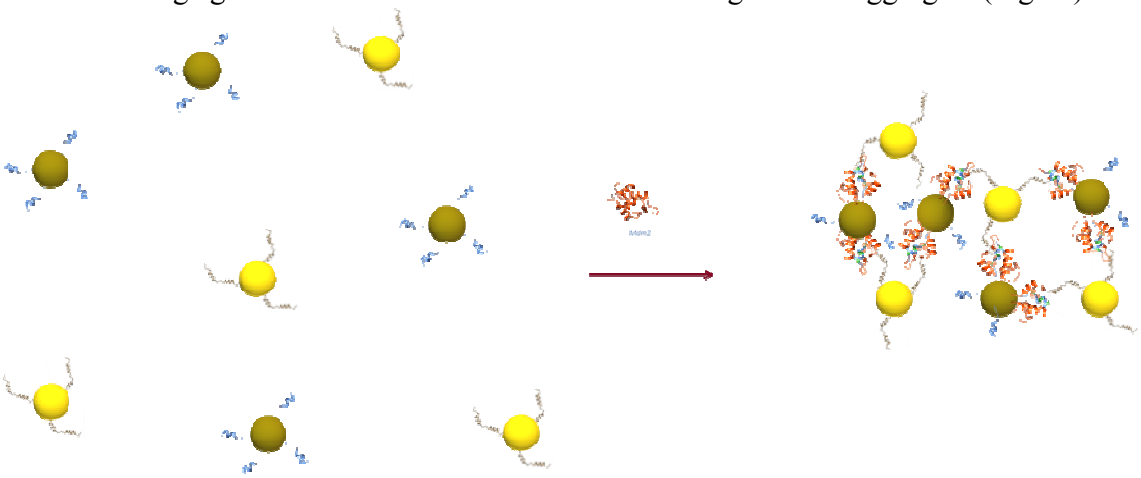
The protein Mdm2 plays a critical role in human cells as it is the main negative regulator of the tumor suppressor protein p53. This latter protein is deeply involved in the cellular response to the stress that can lead to the apparition of cancerous cells.[1,2] In a number of human cancers, overexpression of the Mdm2 protein has been observed [3] and its detection could be used in cancer diagnosis. Its regulation could furthermore lead to new therapies. Our aim is to set up a biosensor specific to Mdm2 which takes advantage of the optical properties of gold nanoparticles (GNPs), i.e. their Localized Surface Plasmon Resonance (LSPR). Indeed, GNPs present a LSPR band which is strongly dependent on their dielectric environment.

GNPs were synthesized and characterized (by TEM, DLS, UV-Vis) and then functionalized with peptide aptamers containing sequences identified in proteins known to interact naturally with Mdm2. A protocol was developed for the grafting of the aptamers on the GNPs surface and for the quantification of the grafting level. We observed that it was not possible to detect Mdm2 in solution using a single set of GNPs functionalized with a peptide aptamer as the change in the LSPR band was not significant. An alternative approach based on the use of two sets of GNPs, each functionalized with a different peptide aptamer but able to bind Mdm2 simultaneously was therefore envisaged. In the presence of Mdm2, but not of a control protein (BSA) a significant change in the LSPR band was observed. This can be explained by the fact that Mdm2 plays the role of a linking agent between the two sets of GNPs forcing them to aggregate (Figure).



**Aggregation of the two sets of GNPs after addition of Mdm2.**

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## Using solid-state NMR to characterise pharmaceutical polymorphs

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Polymorphism is most commonly described as the possibility for the existence of different crystal structures for the same chemical structure.[1] Understanding polymorphism is of particular interest in the pharmaceutical industry, as different polymorphs may have significantly different physical properties, such as changes in solubility or bio-availability. Therefore correct identification of all possible forms is an important step for both patient safety and patent concerns.
Herein various solid-state NMR experiments are presented to identify the differences between two pharmaceutical polymorphs, in particular probing intermolecular interactions, such as aromatic $\pi$-$\pi$ effects and hydrogen bonding. The proton chemical shift is well known to be sensitive to changes in intermolecular configurations that govern self-assembly in the solid state. However, the resolution of a typical proton spectrum in the solid state obtained at a moderate Magic-Angle Spinning (MAS) frequency is typically poor, due to the presence of strong, non-commuting homonuclear dipolar couplings. Herein, fast MAS ($\nu_r$ > 30 kHz) or homonuclear decoupling schemes are applied during 2-D correlation experiments to improve resolution and therefore identify differences in intermolecular interactions that govern polymorphism. Clear differences in the aromatic proton chemical shifts were noted between forms **I** and **II** via various 1D and 2D experiments, indicating differences in the $\pi$-$\pi$ stacking between the two forms, whereas no significant differences in intermolecular hydrogen bonding interactions were noted.

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