Ultra-Low Field OMRI

Introduction to the ultra-low field imaging techniques

This document aims to present the OMRI (Overhauser enhanced Magnetic Resonance Imaging) at very low field, developed within the European project PrimoGaïa, from H2020 FETOPEN programme. The objective is to target *in-vivo* biochemical activities on small animals (rodents), paving the way of molecular imaging for future personalized diagnosis. This approach is carried out at ultra-low field (ULF), using a static field of ≈200µT, allowing OMRI on large objects.

The settlement of this new imaging modality is based on accumulated experience in OMRI developed at higher magnetic field (0.2T). It was shown that OMRI could be used to detect abnormal proteolysis in mice, but much lower fields are necessary for its use in larger animal or even humans. Whereas a strong advantage of ULF-MRI is cost-efficiency, the counterpart is the lack of sensitivity. Therefore, a preparation step is needed to enhance the NMR signal, either magnetically for standard MRI contrast, or dynamically for OMRI.

OMRI Principle, ULF specificities

The primary goal is to enhance the NMR signal through a phenomenon known as Dynamic Nuclear Polarization (DNP).

Explanation of the principle: Basically, the presence of a free radical, i.e. an unpaired electron with a strong magnetic moment is used to transfer polarization to a much weaker magnetic moment, namely the proton nucleus of water observed in MRI. This polarization transfer occurs when the unpaired electron is submitted to radiofrequency excitation. This step is named Electron Paramagnetic Resonance (EPR). The choice of the appropriate frequency for EPR depends on a variety of physical constraints related to the operating static field and the free radical chemical structure. At ULF the NMR frequency at 200 µT is about 8700 Hz and the EPR frequency will range from 5.6 MHz to 150 MHz according to the free radical used *(see below)*. Such a wide range of EPR frequency is due to the occurrence (or not) of an intramolecular interaction in the radical species called hyperfine coupling. In the absence of this coupling, e.g. in trityl radicals ("one-line nitroxide"), the EPR frequency is about 5.6 MHz and the signal enhancement is only brought by the electron-to-proton ratio of the respective magnetogyric ratios. In nitroxides with one electron-14N coupling ("three-line nitroxide") the EPR irradiation frequency is in the range of 70 MHz and signal enhancements can be much higher. If nitroxides bear two couplings (e.g. with 14N and 31P, "three-line nitroxide") then the irradiation frequency can reach 150 MHz with again more enhancement than with no coupling. See next section for details on contrast agents.

- Contrarily to the *"high field"* situation (0.2T) where the signal enhancement is governed by magnetogyric ratios irrespectively of the presence of hyperfine couplings, the latter plays a prominent role at ULF. In other words, nitroxides give better signal enhancement at very low field, which efficiently counterbalance the intrinsic loss of signal (see references: Parzy_2021, Boudries_2023).

The second goal is to trigger this Overhauser enhancement upon a biological activity, in our case: an enzymatic reaction.

This can be done by casting the nitroxide molecule, processed by the targeted enzyme, so that the EPR irradiation at a given frequency provides the enhancement only when the enzymatic conversion is done. Details on contrast agent properties are given below.

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Chemistry and molecular techniques: Contrast agents

Designing contrasts agents for OMRI

The contrast agents that can be used for OMRI of biomolecular processes *in vivo* should fulfill a number of properties:

- Stability and non-toxicity
- Easy administration to animals
- Water solubility or colloidal stability
- High Overhauser enhancements
- "smart", i.e. enzyme specific Overhauser enhancements
- Fast elimination

High Overhauser enhancements

The Overhauser enhancement (Dynamic Nuclear Polarization Factor) is defined by :

$$DNPF = \varepsilon - 1 = -\rho f s \frac{\left[\langle S_z \rangle - S_0\right]}{I_0} \quad \text{with} \quad \varepsilon = \frac{\langle I_z \rangle}{I_0}$$

where Iz is the enhancement proton magnetization, I0 the unenhanced proton magnetization (not measurable at ULF), Sz the electron magnetization under EPR irradiation, S0 the unperturbed electron magnetization, ρ the coupling factor, f the leakage factor and s the saturation factor.

General rules can be used to design a nitroxide for high DNPFs:

- **ρ**: in liquids the utmost value is 0.5. In highly soluble and small–sized nitroxides, one can reach 0.4 at very low fields. Any slowing of the rotational correlation time is detrimental to ρ.
- **f**: the higher value is 1, when the change in water longitudinal relaxation (T1) in the presence of the nitroxide is only due to this presence. Thus, water-unpaired electron contacts are to be privileged, as well as high nitroxide concentration.
- **s**: the higher value is 1, when the electron resonance is perfectly saturated. This can happen when the EPR field is high but chiefly when the nitroxide EPR resonance linewidth is very low. The best way to achieve good saturation is to use nitroxides with very narrow lines (e.g. rapidly tumbling molecules). Any slowing of the rotational correlation time is detrimental to ρ.
- **Sz**: The Sz magnetization depends on the EPR line at low field. In the presence of hyperfine coupling, Sz can reach much higher value than found for isolated electron. Therefore, the use of nitroxides should be privileged, preferably with a low number of EPR lines. Of note the frequency of EPR irradiation is a trade-off: for a given nitroxide, the higher frequency the better enhancement, the lower the better safe penetration in soft tissues with a corollary usefulness in large animal.

Obtaining "smart" contrasts

Although interesting new contrasts will assuredly be observed with non-targeted nitroxides, this whole project was meant to obtain "smart" contrasts. We emphasized contrast agents that can reveal enzymatic activities in vivo in particular proteolysis. Important pathologies like bacterial-induced inflammation in the context of cystic fibrosis, chronic pancreatitis or cancer can potentially be detected very early by their proteolysis activity. This can be done only if the enzyme activity is able to alter the EPR spectrum of the free radical or create the free radical *de novo.*

We inventoried three ways to reveal enzymatic activities (more are expected)

1. **EPR spectrum alteration through the modification of τc the rotational correlation time of the contrast agent.**

Specifically, this effect is obtained by grafting a nitroxide on a larger molecule or include it in a larger construction (like a micelle). In this situation the tumbling of the nitroxide will be linked to the one of the

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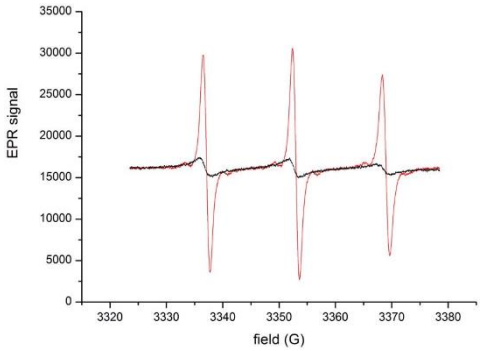
larger structure and be much slower. Consequently, the EPR spectrum will be enlarged, and its amplitude decreased. Such an EPR spectrum prevents the free electron saturation and thus no Overhauser effect occurs. If the larger structure has been chosen to be modified by enzymatic hydrolysis to release free nitroxides or shorter fragments the isotropic EPR spectrum is restored and the Overhauser effect can take place.

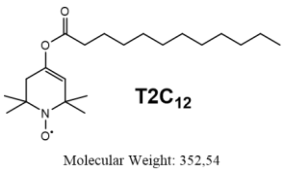
We developed two examples of this:

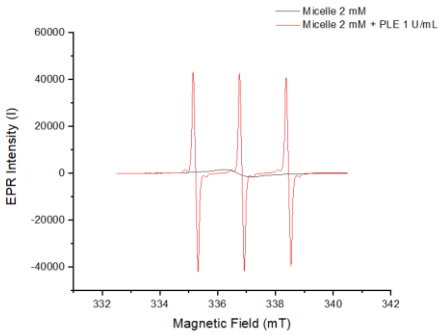
- Elastin fragments over 30 000 Daltons labelled with a nitroxide



EPR spectrum before (black line) and after neutrophile elastase hydrolysis (red line).

- Lipidic nitroxides in micelle form

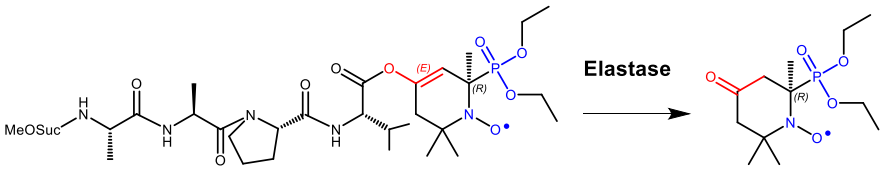




Lipidic nitroxide micelles before and after lipase hydrolysis.

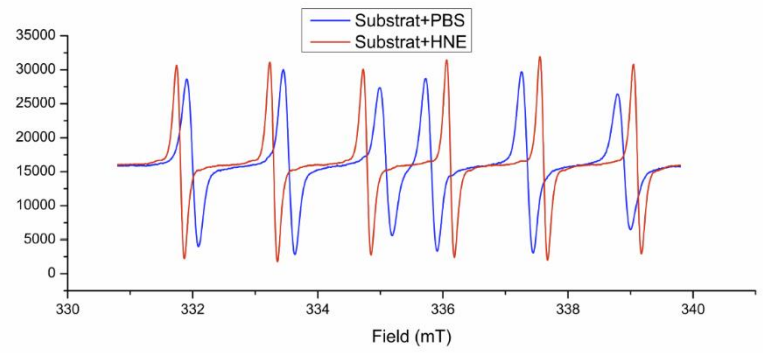
2. EPR spectrum alteration through line shifting upon enzyme activity:

A peptide suitable for the targeted protease in bound covalently to a nitroxide in such a way that the enzyme is able to hydrolyze this bound. The nitroxide is such that the hydrolysis of the peptide triggers an EPR line shift.



Ala Ala Pro Val

Upon elastase activity lines shift sufficiently to allow the record of substrate hdrolysis or product generated.



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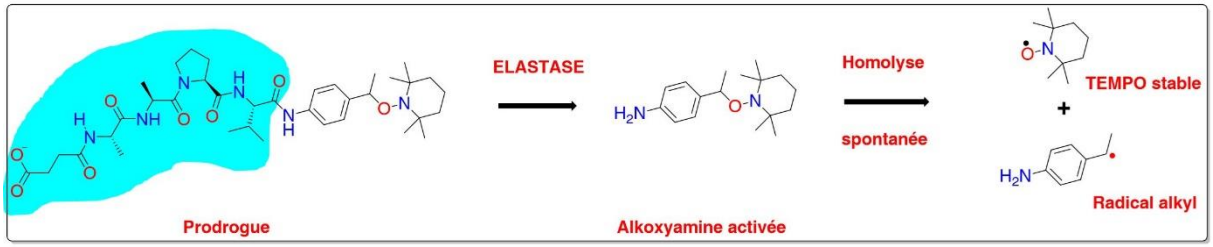
3. Generation of a nitroxide from alkoxyamine homolysis.

The presence of a peptide stabilizes the molecule. Once it is hydrolyzed by the enzyme the activated alkoxyamine undergoes homolysis that generates a free nitroxide. This nitroxide can be further detected by OMRI.



Methodology

The Primogaïa's project proposes a flexible, low-cost molecular imaging strategy, based on the implementation of procedures for non-invasive mapping of enzyme activity in a living animal using ultra-low field OMRI.

The proposed methodology is totally innovative compared to existing standard in clinical MRI. The conventional NMR experiment consists of three steps (polarization, resonance, relaxation). Here, a preparation step is required at ultra-low field, as the signals detected by induction are very weak. This step, also known as pre-polarization, is designed to compensate for low sensitivity, and can be performed using two techniques:

- Magnetic pre-polarization for conventional anatomical imaging.
- Dynamic Nuclear Polarization (DNP), combined with smart nitroxide contrast agents, to reveal specific biological activity. This step can be carried out throughout NMR acquisition, saving considerable preparation time. (figure 1, right).

Typical values of magnetic induction during detection range from the earth's field (45µT) up to 220µT using low-cost Faraday-type detection. Magnetic pre-polarization at 20mT is used for anatomical imaging. EPR irradiation frequencies used with DNP will be in the order of 70MHz for three-line nitroxides and up to 150MHz for 6-line nitroxide. These EPR frequencies, similar to those used in clinical MRI for 1H NMR at 1.5 or 3T, are compatible with the safety and penetration depth requirements.

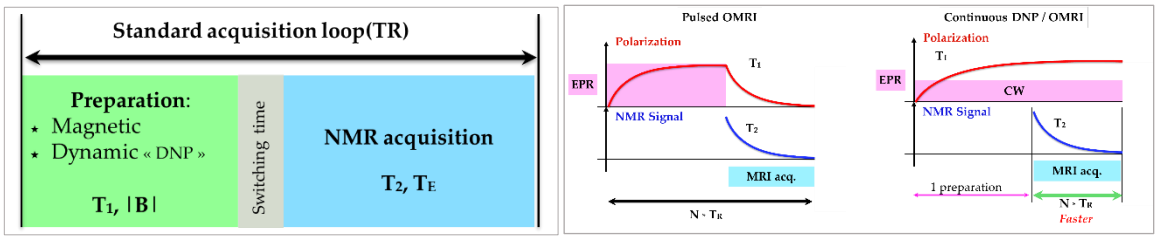


Figure 1 - General scheme for the ULF acquisition time course. Left: conventional sequence with a preparation step prior to NMR acquisition at low field. Right: OMRI acquisition, either with a separated DNP step or throughout the NMR acquisition.

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A typical workflow for OMRI acquisition is described below. The Matlab® interface allows the loading of pulse sequences dedicated to specific tasks:

- Definition of the static Ultralow field value along a given orientation.
- Loading/Overriding of general hardware parameters (proton frequency, time delays, readout)
- EPR hardware calibration: power and frequency
- Probe tuning for NMR and EPR. The EPR frequency depends on the nitroxide used.
- Proton shimming
- Proton RF calibration
- Acquisition (FID, 2D or 3D spin echo, 2D or 3D Gradient echo, 2D or 3D Balanced SSFP, 3D Zero-Echo-Time.
- Reconstruction and display

Instrumentation

The PrimoGaïa project issued two equipment's, at first an OMRI demonstrator with Field Of View (FOV) limited to 60mm in diameter and ≈80mm in length, usable for *in vitro* samples down to 1ml and for *in vivo* imaging of ≈450g rat models (figure 2). In a second step, a "whole-body" OMRI system, limited to 250mm FOV was developed (not shown). These two setups will merge in a single instrumental setup, combining all possibilities.

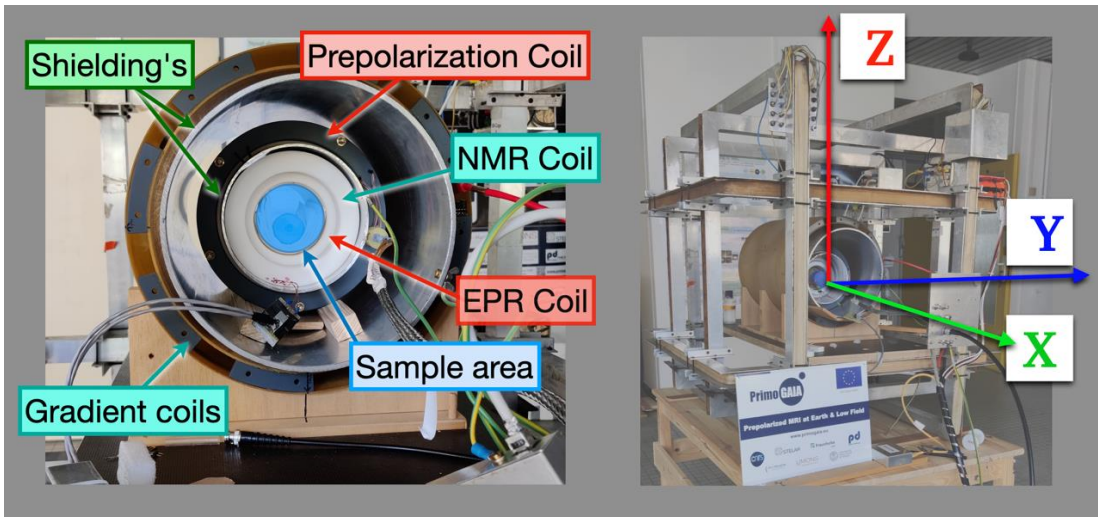

Figure 2: experimental setup (left) and axis directions(right)

Due to the intrinsic weak signal a magnetic prepolarization functionality has been implemented, for standard MRI anatomical image using a 20mT prepolarization field, aligned with the horizontal symmetry axis.

The NMR/MRI acquisition are done at 206µT static B0Z field, vertically oriented (figure 2). Gradient encoding is limited to 500µT/m, it ensures an isotropic resolution down to 3mm, in 2D (no slice selection) or 3D, disregarding the amount of available signal. For small volumes, samples, rat models, the system is equipped with a gradiometer coil configuration, ensuring external noise protection, used as transmit and receive NMR frequency coil.

For OMRI, an additional RF coil for either 70MHz range or 150MHz range EPR saturation is inserted inside the NMR coil, limiting the explorable FOV to 60mm in diameter. A complete controlled RF channel supplies the EPR frequency coil.

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PrimoGaïa results

The methodological, instrumental and biochemical developments carried out during this project enabled *in vitro* and *in vivo* spectroscopy and imaging.

In vitro and *in vivo* experiments were conducted after optimizing the sequences with Cartesian and radial coding for this experimental environment. A preliminary result established the kinetics of an enzymatic activity *in vitro* using Earth's magnetic field in spectroscopy, and recently, kinetics were successfully achieved using imaging at 200uT.

In vivo experiments (figure 3) have enabled:

- **3D anatomical imaging** in live rodents under magnetic pre-polarization.
- **Visualization of the biodistribution of a nitroxide** in 3D OMRI in the kidneys, lungs, and bladder, with a rapid acquisition in less than 10 minutes without significant tissue heating.

- Ongoing work aims to monitor enzymatic activity in a pathological model in the context of inflammatory lung diseases.

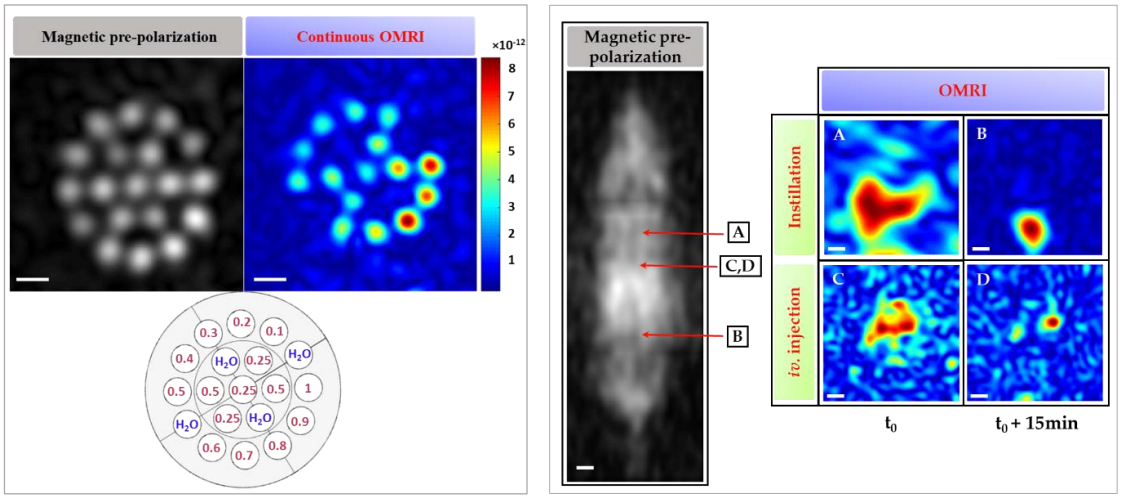

Figure 3 - left, In vitro 3D image of the phantom on the left, 1ml tubes with different concentrations of contrast agent (from 0.1 to 1mM), with 1ml water tubes in between. Right, In vivo 3D images of the rat using magnetic prepolarization and OMRI 3D

Conclusion

The European PrimoGaïa project proposes a very original strategy to map enzymatic activity in small animals (rats). Anatomical and morphological imaging is definitely the strong point of MRI devices equipped with intense magnetic fields (1.5T-3T). On the other hand, although suffering from a problem of lack of sensitivity, the use of systems equipped with weak fields (<1mT), allows obtaining metabolic information in preclinical imaging. The mapping of enzymatic activity is made possible by the use of dynamic polarization (transfer of magnetization between the spin of the electron of a stable and non-toxic radical towards the proton) which is observed in classic MRI.

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