EUROPEAN ORGANIZATION FOR NUCLEAR RESEARCH

LETTER OF INTENT
to the ISOLDE and Neutron Time-of-Flight Committee

Beta-NMR as a novel technique for biological applications

L. Hemmingsen[1], M.Stachura[1], M.L. Bissell[2], G. Georgiev[3], A. Gottberg[4] , K. Johnston[5], M. Kowalska[6], K. Kreim[6], M. Luczkowski[7], G. Neyens[2], D.T. Yordanov[5], W.D. Zeitz[8]

[1] University of Copenhagen, Department of Basic Sciences and Environment, Denmark
[2] Katholieke Universiteit Leuven, Nuclear and Radiation Physics Section, Belgium
[3] CSNSM, Orsay, France
[4] Free University of Berlin, Institute of Experimental Physics, Germany
[5] PH-Department, ISOLDE, CERN
[6] Max Planck Institute for Nuclear Physics, Heidelberg, Germany
[7] University of Wroclaw, Faculty of Chemistry, Poland
[8] Helmholtz Institute, Berlin, Germany

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Spokespersons: Lars Hemmingsen, Monika Stachura
Contact Persons: Karl Johnston, Magdalena Kowalska

Introduction and motivation

Many problems of interest to chemists, such as structure and dynamics of molecules in solution can be investigated by NMR spectroscopy on stable isotopes, and it is probably the most versatile of the techniques currently applied to biological systems [Wri96]. One particular drawback however, is that relatively large amounts of sample are required (mg or ~10^{17} molecules), i.e. the sensitivity is usually relatively poor, and this poses constraints on the systems that may be explored. In addition, not all elements are easily accessible by NMR spectroscopy, as the most abundant isotopes display no or poor NMR response.
Beta-NMR, where the NMR resonances are observed as changes in beta-decay anisotropy, offers particular advantages over classical NMR. Most notably, it is extremely sensitive, several orders of magnitude more sensitive than "standard" NMR spectroscopy, and it may be applied for elements which are otherwise difficult to interrogate spectroscopically for certain biologically highly important oxidation states (for example Zn(II) and Cu(I)) [Cri08]. The underlying physics is basically the same as for NMR on stable isotopes. This is a considerable advantage because over the last decades NMR on stable isotopes has proven to work well in the field of biophysics and chemistry [Wri04, Cap04]. Beta-NMR has already been successfully applied in solid state physics [Cho03, Kee08] and the technique holds great promise for successful applications in biology as well, although, there are technical issues to be solved.

Beta-NMR is a technique that has never been applied to biochemistry before, and this is the focus of the current Letter of Intent. The combination of the ISOLDE facility and either optical pumping [Kei00] or tilted-foils (solid state beta-NMR setup) provides a unique opportunity to carry out this project. Furthermore, it would allow for measurements of spectroscopic properties (shielding tensors and electric field gradients) in proteins containing closed shell probe ions that are spectroscopically silent in most other standard techniques, except for X-ray and nuclear techniques, vide infra. Finally, if the project is successful, it is expected to attract additional life-scientists to ISOLDE, and thus, strengthen the society of biophysicists and chemists working in cross-disciplinary projects at the facility.

Summary

The goal of this Letter Of Intent is to explore if beta-NMR can be applied to metal ions bound to biological macromolecules. The requirements for a nucleus to be suitable for NMR studies are: I=1/2 for probing magnetic interaction only or I>1/2 for probing also the quadrupole interactions; well known magnetic moment (and quadrupole moment for electric interactions), good polarization and long relaxation time. In addition, for the beta-NMR method, the half-life should be relatively short (ms – s range) and not much longer than the relaxation time. Therefore, initially we wish to use ^{29}Mg and ^{31}Mg at the beta-NMR setup of the COLLAPS collaboration. Well polarized beams of these nuclides have already been studied at COLLAPS [Kow08] resulting in very precise magnetic moments, and their production is sufficient (> 10^5/s) in order to gain information about both magnetic and quadrupolar interactions in aqueous solutions in a reasonably short time (see Table 1). ^{31}Mg has spin I=1/2, which allows studying only the magnetic interaction whereas the spin of ^{29}Mg is I=3/2, letting us investigate both magnetic and electric interactions. It should be noted that the quadrupole moment of ^{29}Mg has not been yet determined. However, this is not relevant at this stage where we only want to see a general effect of the quadrupole interaction. In a longer time perspective, we are particularly interested in testing the beta-NMR technique on Cu(I) binding proteins, because Cu(I) is essential in many redox processes and in electron transport in biology, and it is invisible to most of the standard spectroscopic techniques applied in biochemistry.

Initially we wish to test how the polarization of the spin can be maintained for as long as possible in an aqueous solution, i.e. we would like to reach the relaxation time in the hundred ms to several s range. This is necessary since the NMR signal is strongest if all nuclei are still polarized when decaying, thus for ^{29,31}Mg the relaxation time should not be much shorter than their half-life (see Table 1). In addition, a prerequisite for experiments on proteins is that the metal ion must bind to the protein (typically in the ms range, depending on the protein concentration) before the spin is depolarized.

We would like to conduct the following tests at the beta-NMR setup of the COLLAPS collaboration using both ^{31}Mg and ^{29}Mg (for the latter, to see the effect of quadrupole interaction):

1) Implant into ice as the first step towards experiments in aqueous solutions, measuring the spin relaxation time.

2) Implant into aqueous solution, and change temperature and viscosity in order to see how this affects the relaxation time (viscosity can be controlled by adding e.g. sucrose, temperature will be controlled directly in the chamber).

3) Check the penetration and scattering of the beam through the rest gas in the chamber also by dosing different "light" gases which should increase the penetration.
4) Check whether degassing the solution (with an inert gas such as N_2 or Ar in order to remove O_2 which is paramagnetic) slows down the relaxation.

Once optimal conditions are found in points 1-4, we wish to verify the relaxation time and the amplitude of the NMR signal after adding:

5) A reference protein (for example lysozyme, which we already use in a different context, and thereby have experience in handling) which is believed not to have specific binding sites for metal ions.

6) Biomolecules, for example proteins or nucleic acids, which are known to interact specifically with Mg(II) (even if the beam consists of Mg(I), the oxidation to Mg(II) is expected to occur very rapidly (faster than μs), and thus, the measurements will effectively be carried out on Mg(II)).

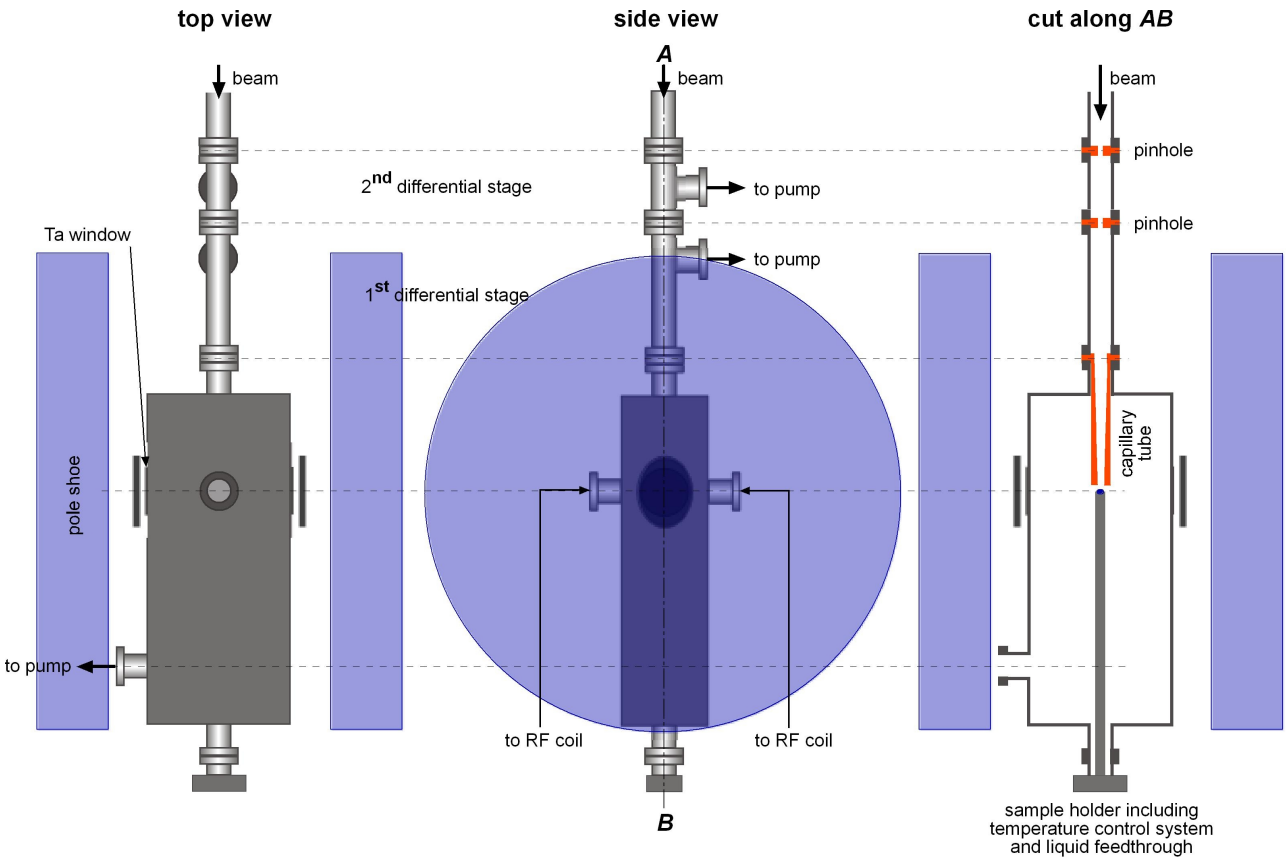
If both parts of the experiment are successful, we plan to submit a proposal to study the binding sites of biologically relevant metal ions (e.g. Cu, Fe, Co, Zn, Mo, or Ni) in biomolecules.

In the future we additionally wish to present an LOI to use the solid state beta-NMR setup which is currently being set up at ISOLDE. On this instrument we will initially implant boron and copper into a solid sample, such as silicon, in order to test if the newly developed chamber is functioning properly.

Experimental setup

The tests will be performed at the beta-NMR setup of the COLLAPS collaboration [Kei00], where short-lived ions are polarized with laser light. However, this setup requires some modifications, since the beam has to be implanted into a liquid. In order to solve the problem of having water (the solution at the target) in vacuum we have developed a “biophysics chamber” which is an upgraded version of the existing collection chamber for the ISOLDE solid state physics beta-NMR setup. This chamber is presently in preparation and it will be assembled and tested at the Institute of Experimental Physics, Free University of Berlin, Germany at the beginning of this year.

This biophysics chamber will replace the chamber used presently for standard beta-NMR studies at the COLLAPS beamline. Its sketch inside the COLLAPS magnet poles is presented below:



A differential pumping system together with pinholes in the setup will allow for a pressure ranging from ~5 mbar at the position of the sample to around 10^{-6} mbar at the beam line. A pressure level of several mbar is required to stay above the water vapor pressure that is needed for a liquid target and will be controlled by a nozzle valve. The first capillary tube will reduce the pressure by approximately one order of magnitude and each differential stage from there on by another two orders of magnitude. A liquid feedthrough included in the sample holder will make it possible to compensate for evaporation of water from the sample.
The biophysics chamber will be designed such that it will not exceed the dimensions required to fit at the COLLAPS beamline and magnet. Temperature will be controlled directly at the sample holder and will be changed via an external temperature control unit. For this purpose we will use one of the temperature controllers available at the solid state setup.
In the case of an approval of this LOI, the prototype of the chamber can be installed and tested before the beginning of the running period 2010.

Beam time request

In total, we ask for 6+2.5 online shifts with ^{29,31}Mg beams using standard UC_x target and laser ionization.

The tests can be performed in two parts:

- Part 1: Tasks 1-4 on both ^{29}Mg and ^{31}Mg. Since the signal-to-noise ratio (S/N) in beta-NMR spectra is directly proportional to the decay asymmetry (up to 7-8% for ^{31}Mg and 2-2.5% for ^{29}Mg) and to the square root of the number of detected betas/channel (^{29}Mg yield is about 10 times larger than for ^{31}Mg), the time spend on tests with ^{29}Mg and ^{31}Mg is comparable. We estimate that we will

require 5h on average per isotope and per task, which gives 2x4x5h = 5 shifts. We will also need 1 shift for stable beam tuning and for optimizing the polarization.

- Part 2: Tasks 5-6 on both ^{29}Mg and ^{31}Mg. Here we will also use both ^{29}Mg and ^{31}Mg, and we will also require 5h per task, giving 2.5 shifts. If this part is separated from part 1, then also here we will need 1 shift for stable beam tuning and for optimizing the polarization.

OUR PREFERENCE is that the tests are performed close in time to the planned ^{21,23}Mg charge-radii run at COLLAPS, which avoids modifying our laser system (However, in this case we need 4-5 days to change and align the beta-NMR chambers, and in addition a target change is required). If such scheduling is not possible, the tests can be combined with any other run on neutron-rich Mg, and it will just require more offline time to set up the COLLAPS laser system without increasing the shift requirements.

Table 1. Properties, yields, target, and ions source for ^{29,31}Mg. Beta asymmetry and yield observed at COLLAPS from [Kow08] and [Bla08], respectively.

Isotope	Half-life	Spin	Beta asymmetry	Target	Ion source	Yield
^{29}Mg	1.3s	3/2	2-2.5 %	UC_x	RILIS	1.2×10^6 μ C^{-1}
^{31}Mg	230ms	1/2	7-8 %	UC_x	RILIS	1.5×10^5 μ C^{-1}

Table 2. Summary of beam request.

Task	shifts
1-4	1+5 shifts
5-6	(1)*+2.5 shifts

* is separate from tasks 1-4

Total: 8.5 (or 9.5)

References:

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