

EUROPEAN ORGANIZATION FOR NUCLEAR RESEARCH

Proposal to the ISOLDE and Neutron Time-of-Flight Committee

β -NMR of copper isotopes in ionic liquids

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Abstract

We propose to test the feasibility of spin-polarization and β -NMR studies on several short-lived copper isotopes, ⁵⁸Cu, ⁷⁴Cu and ⁷⁵Cu in crystals and liquids. The motivation is given by biological studies of Cu with β -NMR in liquid samples, since Cu is present in a large number of enzymes involved in electron transfer and activation of oxygen. The technique is based on spin-polarization via optical pumping in the new VITO beamline. We will use the existing lasers, NMR magnet and NMR chambers and we will prepare a new optical pumping system. The studies will be devoted to tests of achieved β -asymmetry in solid hosts, the behaviour of asymmetry when increasing vacuum, and finally NMR scans in ionic liquids. The achieved spin polarization will be also relevant for the plans to measure with high precision the magnetic moments of neutron-rich Cu isotopes.

Requested shifts: 27 shifts (split into 2-3 runs over 2-3 years)



Introduction and motivation:

Most biological macromolecules are folded into a characteristic 3D structure in their native state, of which the proper fold and function often requires the presence of a metal ion [Poz01]. Therefore, to understand protein function, it is essential to elucidate the structure and dynamics in the proximity of the metal binding site.

Solution-phase NMR is one of the most widely used techniques in biochemistry and life sciences [Wüt01]. It allows for studying macromolecules, such as nucleic acids and proteins, in their native environment and hence makes it possible to gain information on both the structure and the dynamics of biological macromolecules at the same time [Wüt90, Dys96]. Conventional NMR has, however, several limitations which often make the experiments extremely difficult or nearly impossible to perform. The major constraint is the amount of sample required, which is crucial when using biological samples. Typically a sample in the range of mg (approx. 10^{17} - 10^{18} probe nuclei) is needed. Furthermore, nuclei with nuclear spin $\frac{1}{2}$ are favourable as nuclei with higher spins yield quadrupolar interactions making the spectra rather difficult to interpret. Finally, standard NMR is limited to stable nuclei which are highly abundant in nature. Most of the experiments are therefore carried out on ^1H or ^{13}C . Thus, studying the binding environment of transition metal ions in biological samples via conventional NMR is hence quite challenging [Phi86].

β -detected NMR provides particular advantages as compared to conventional NMR when studying biological macromolecules. In β -NMR experiments much fewer probe nuclei are required (approx. 10^5 - 10^8 molecules as compared to 10^{17} - 10^{18} for standard NMR) and almost any short-lived nuclei decaying through β -decay in favourable time range (\sim half-life $< 1\text{s}$) may be investigated in principle. The nuclei studied do not have to be highly abundant in the sample, as β -NMR is about nine orders of magnitude more sensitive than standard solution-phase NMR. In 2012 we demonstrated the feasibility of using β -NMR in the first studies on liquid samples [Sta12, Bul12, Got13]. **Based on the successful experiments with ^{31}Mg , we are therefore proposing β -NMR experiments on Cu ions implanted into liquid solvents.**

Copper as one of the most abundant trace elements in living organisms is essential for many metabolic pathways including cellular respiration, photosynthesis, removal of reactive oxygen species, etc. [Ral03]. The two naturally occurring oxidation states Cu(I) and Cu(II) allow copper ions to serve as a vital electron transfer species when bound to proteins [Tap03]. Both deficiency and excess of Cu is pathological and is linked to various severe diseases including Alzheimer's, Wilson's, Menkes and prion diseases [Bie07, Gag06, Squ12]. Therefore, strict control mechanisms exist in living organisms to ensure the exact concentration and oxidation state ratio is maintained [Tap03]. A wide variety of transport and storage proteins are involved in the metal ion homeostasis and understanding the native binding properties is crucial for further applied research and potential drug design. However, Cu(I) as a closed shell ion is irresponsive in most spectroscopic methods. Out of the applicable spectroscopic techniques X-ray and solid-state NMR do not give desired information on the macromolecule in solution. Solution-phase NMR of the Cu ions has several major limitations. Firstly, only two stable copper isotopes ^{63}Cu and ^{65}Cu are active in NMR [Ma199]. Secondly, both of these nuclei are quadrupole nuclei (spin $> \frac{1}{2}$) with low gyromagnetic ratio, causing low sensitivity and broad NMR lines. Only a very few works on Cu solution-phase NMR are published due to the technical challenges of the experiments and the limited information obtained [Phi86]. Most of the works published were performed

with small inorganic compounds [Ma199, Yam70], which gives valuable information on the metal binding, but does not exactly describe the coordination and binding environment in native macromolecules. β -NMR spectroscopy provides the same information as conventional NMR: chemical shifts, line broadening or relaxation times providing information about energies and dynamics of chemical bonding. But in addition, it can overcome the above stated problems in studies of Cu(I) bound to proteins in solution.

Experimental details:

When searching for a suitable β -NMR Cu isotope, we have looked above all at the following aspects: decay type, life-time, spin and the ISOLDE yields. For comparison, ^{31}Mg on which the proof-of-principle experiment was performed has $I = 1/2$, $t_{1/2} = 230$ ms, and yield of about $1.5 \cdot 10^5$ ions/ μC . As a result, we have selected three candidate isotopes with favourable properties: ^{58}Cu , ^{74}Cu and ^{75}Cu , see Table 1. All of these isotopes decay through β -decay in favourable time ranges (1-3 s) and have sufficient yields, see Table 2. All of the nuclei have a spin larger than $1/2$ allowing for studying both magnetic and quadrupolar interactions (there is no known Cu isotope with spin $1/2$). The quadrupolar interactions may, however, also be a challenge, as they may lead to rapid depolarization of the nuclear spin, and thus loss of β -NMR signal – an aspect to be explored in this proposal.

Table 1: Properties of the chosen Cu isotopes, from [TOI, Vin10, Fla10]

| Isotope | Half-life [s] | Spin | Decay mode | Magnetic moment [μ_N] | Quadrupole moment [$e \cdot \text{fm}^2$] | Q_β [MeV] |
|------------------|---------------|------|---------------------|-----------------------------|---|-----------------|
| ^{58}Cu | 3.2 | 1 | β^+/EC | +0.570(2) | -15(3) | 8.6 |
| ^{74}Cu | 1.6 | 2 | β^- | -1.068(3) | +26(3) | 9.8 |
| ^{75}Cu | 1.2 | 5/2 | β^- | +1.0062(13) | -26.9(16) | 8.1 |

Concerning the expected β -decay asymmetries and the background, the angular distribution of β -radiation from a polarized sample can be represented in the 1st approximation as:

$$W(\theta) = 1 + a_\beta \frac{v}{c} P_I \cos(\theta)$$

where a_β is an asymmetry factor, v is the β -particle speed, P_I a nuclear polarization and θ – the angle of the observation with respect to the polarization symmetry axis. The asymmetry factor a_β depends on the decay details. For ^{58}Cu the β^+ decay is rather well known and the a_β is very high: -0.88. For ^{74}Cu and ^{75}Cu the decay is more fragmented and not many spins are assigned in the daughter nuclei. Thus, we have not been able to estimate a_β . The test will show how high asymmetries can be reached (even if the asymmetry parameter is lower than in ^{58}Cu , these isotopes have the advantage of a shorter $t_{1/2}$, which is important if relaxation times are also short).

The laser excitation schemes have already been used online at COLLAPS [Vin10], see Figure 1, and these are suitable transitions for “online” polarization tests on the VITO beam line [VITO-INTC].

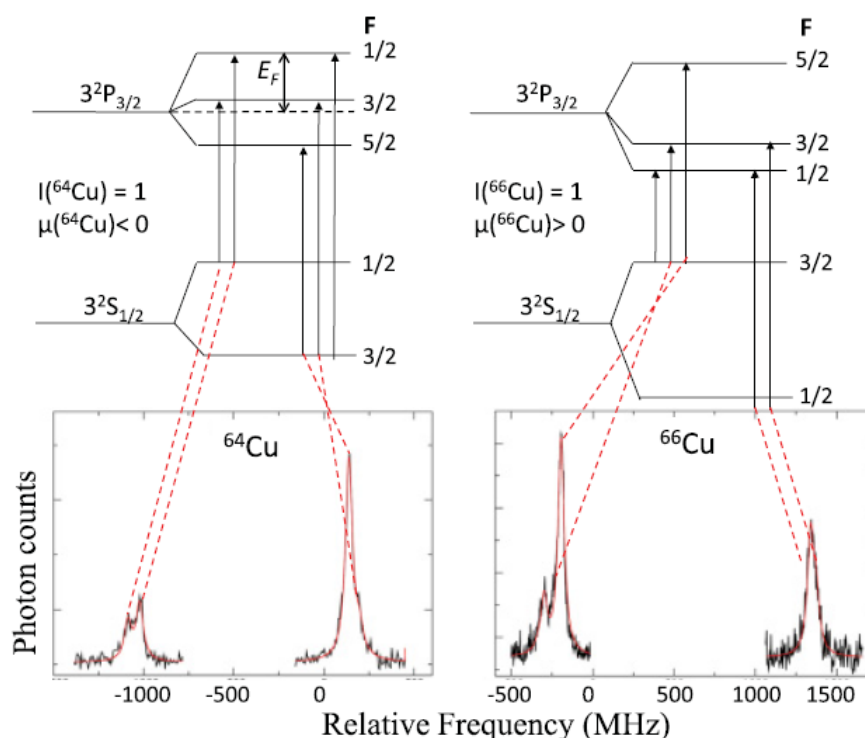


Fig. 1 Top: Hyperfine splitting of the $^2S_{1/2}$ and $^2P_{3/2}$ levels for a Cu isotope with nuclear spin $I=1$. Bottom: Hyperfine spectra for ^{64}Cu and ^{66}Cu , observed in fluorescence (not β -asymmetry) [Vin10].

The first choice solvent for the liquid-phase experiment is of course water, as almost all biochemistry occurs in water. The differential-pumping section and β -NMR chamber are now being optimized (development of the “biophysics chamber”) and several technical challenges have to be overcome in order to reach at least several % beam transmission with the pressure in the range of several mbar at the sample site (just above the water vapour pressure). The existing setup shows acceptable transmission up to 0.1 mbar, therefore other solvents are also taken into account in this proposal. Ionic liquids (ILs) are an excellent first step towards applications in liquid solution, most notably due to their extremely low vapour pressure, allowing for experiments at vacuum beam lines. ILs are a new class of solvents, low melting organic salts possessing several highly favourable properties [Wei13]. The advantage of ILs is the versatility of their properties which can be achieved by varying their composition. Nowadays hundreds of different ionic liquids are commercially available (approx. 1000 ILs described in literature, approx. 300 commercially available) allowing for choosing the exact properties of interest for particular applications [ChemFiles Sigma-Aldrich]. The use of ionic liquids for β -NMR experiments was already successfully tested by us with ^{31}Mg in 2012 and it proved the feasibility of this experimental setup [Sta12, Got13].

The planned spin polarization and observation of β -NMR resonances on Cu isotopes is also relevant for nuclear structure studies. Namely, following the presently proposed experiments, a proposal to the INTC is envisaged to measure with very high precision the magnetic moments of neutron-rich Cu isotopes using the β -NMR technique in solid hosts.

Technique and the experimental setup:

The proposed experiments will be performed at the β -NMR spectrometer placed at the end station of the VITO beam line [VITO-INTC, VITO-safety]. The elements of the line relevant for this proposal are presented in Figure 2.

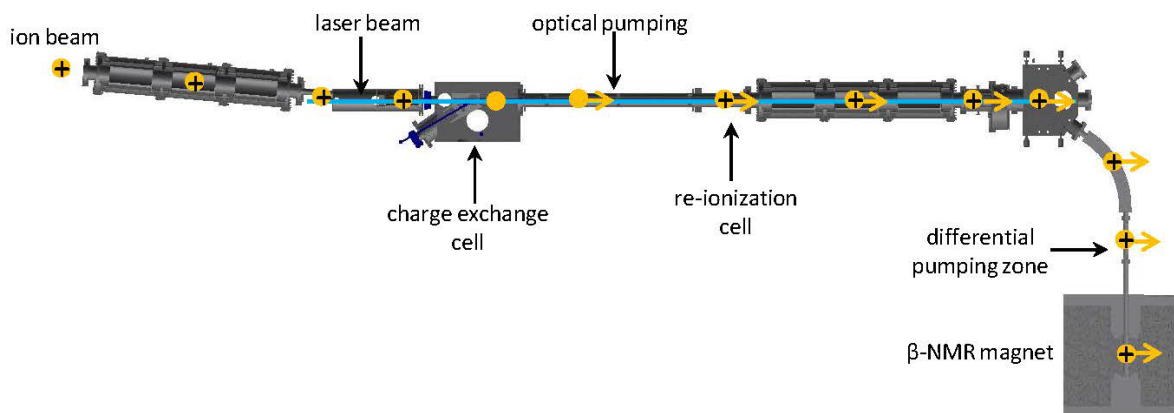


Fig. 2. Elements of the VITO beam line relevant for β -NMR studies on liquid samples.

The β -NMR experiments will be based on nuclear spin polarization via optical pumping. This way of spin polarization induced by circular polarized laser light [Neu06] does not require stopping or extreme cooling of the ion, and it has provided very good and reproducible results for over a dozen of isotopes. The planned setup is a slight modification of the COLLAPS β -NMR system, as shown in Figure 2. First, the ions pass via the Doppler tuning region where one scans across the optical resonances by changing the ion velocity and keeping the laser frequency fixed. For spin-polarizing of the Cu atoms we will use the COLLAPS lasers in the VITO optical pumping line. The transitions to be used are the same as in previous experiments devoted to Cu fluorescence spectroscopy – the Cu^+ are neutralized in the charge exchange cell and get excited in the $3d^{10}4s^2S_{1/2} \rightarrow 3d^{10}4p^2P_{3/2}$ transition at 324.754 nm (for details, see [Vin10]). Next, the resulting atoms are being polarized in the optical pumping section, and further downstream they are re-ionized to Cu^+ before entering the switchyard. After the bent by 90°, the polarized Cu^+ ions pass through the differential pumping section, consisting of 3 small apertures with powerful vacuum pumps between. The pressure in this section is around 10^{-5} - 10^{-6} mbar when working with solid samples inside the NMR magnet and it gradually increases in each section when working with mbar pressures in the NMR chamber. At the end the ion beam is implanted into the NMR sample placed inside a magnetic field of some kG. The NMR chamber was used in the proof-of-principle experiment in 2012 and it can host both solid and liquid samples at the same time. The liquid can be introduced into the chamber via a glass tube which end is placed in the middle of the magnet. After establishing the liquid flow, a drop is formed right before the last aperture and the beam is being implanted into it. The pressure inside the chamber can be changed by use of a leakage valve. For the NMR resonances, a small coil is placed around the sample and the rf circuit is tuned with a set of variable capacitors. In addition, for the reference scans on stable Cu isotopes, two photomultipliers will detect the fluorescence light, while the isotopes of interest will be guided to the NMR magnet and will be implanted into a host crystal (or later a liquid) placed inside the magnet.

Due to both, the high degree of nuclear polarization and the sensitive detection of asymmetry via the beta decay as few as 10^7 nuclei in total are required for observing a resonance, compared to 10^{17} for conventional NMR spectroscopy.

Summary of requested shifts:

The initial stage of the project will study ^{58}Cu (9 shifts) using Zirconia fibers and the laser ionization ion source (RILIS). Then the measurements with ^{74}Cu and ^{75}Cu (9 + 9 shifts) requiring the standard UC_x target in conjunction with the RILIS will be undertaken. The measured yields are shown in Table 2.

Table 2. Properties, yields, target, and ions source for ^{58}Cu , ^{74}Cu and ^{75}Cu .

| Isotope | Target | Ion source | Yields [ions/ μC] | Ref |
|------------------|--------------------|------------|-------------------------------|---------|
| ^{58}Cu | Zirconia fibers/YO | RILIS | $3 \cdot 10^5$ | [Kös03] |
| ^{74}Cu | UC _x | RILIS | $5 \cdot 10^5$ | [Äys02] |
| ^{75}Cu | UC _x | RILIS | $1 \cdot 10^5$ | [Äys02] |

Most of the beam time will be devoted to optimizing and carrying out the β -NMR measurements of the three different Cu isotopes in a chosen solid host material. After selecting the best study-case Cu isotope, the measurement on Cu ions implanted into an ionic liquid will be performed.

Each of the three isotopes offers certain advantages: for ^{58}Cu the asymmetry parameter is known to be very high; $^{74,75}\text{Cu}$ have shorter $t_{1/2}$ and are thus less affected by relaxation effects; $^{74,75}\text{Cu}$ asymmetry parameters are not yet known, but might be also high. Therefore, for each of the three isotopes the following steps will be undertaken (shift estimates based on the previous experiments with ^{31}Mg , see ^{31}Mg proposal [31Mg-INTC].):

- 1) HFS optical detection of a stable Cu isotope (reference) and the isotope of interest (1 shift)
- 2) Measurements of β -asymmetry and β -NMR in cubic-lattice crystals - Si, MgO or Al (4 shifts):
 - a) Recording HFS in β -asymmetry for both laser polarizations
 - b) β -asymmetry maximization: selection of the transition and laser polarization, and crystal. Measurement of relaxation time is different hosts.
 - c) β -asymmetry measurement as a function of the rest gas pressure in the chamber.
 - d) β -NMR resonance in good and bad vacuum in the optimal host crystal.
- 3) Measurements of β -asymmetry and β -NMR in an ionic liquid (4 shifts):
 - a) β -asymmetry measurements in an ionic liquid in good vacuum.
 - b) β -asymmetry measurements as a function of the rest gas pressure in the chamber.
 - c) β -NMR measurement in good and bad vacuum.

In total, we ask for 27 online shifts with ^{58}Cu , ^{74}Cu and ^{75}Cu beams to be spread over two runs (with ZrO and UC_x target) in a period of two – three years years.

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Appendix

DESCRIPTION OF THE PROPOSED EXPERIMENT

The experimental setup comprises: *VITO beamline and the β -NMR spectrometer*

| Part of the Choose an item. | Availability | Design and manufacturing |
|--|---|--|
| VITO +COLLAPS lasers + β -NMR spectrometer | <input checked="" type="checkbox"/> Existing (in preparation) | <input type="checkbox"/> To be used without any modification <input checked="" type="checkbox"/> To be modified |
| VITO beamline | <input checked="" type="checkbox"/> Existing | <input type="checkbox"/> To be used without any modification <input checked="" type="checkbox"/> To be modified |
| | <input type="checkbox"/> New | <input type="checkbox"/> Standard equipment supplied by a manufacturer <input checked="" type="checkbox"/> CERN/collaboration responsible for the design and/or manufacturing |
| COLLAPS lasers | <input checked="" type="checkbox"/> Existing | <input checked="" type="checkbox"/> To be used without any modification <input type="checkbox"/> To be modified |
| | <input type="checkbox"/> New | <input type="checkbox"/> Standard equipment supplied by a manufacturer <input type="checkbox"/> CERN/collaboration responsible for the design and/or manufacturing |
| β -NMR spectrometer | <input checked="" type="checkbox"/> Existing | <input checked="" type="checkbox"/> To be used without any modification <input type="checkbox"/> To be modified |
| | <input type="checkbox"/> New | <input type="checkbox"/> Standard equipment supplied by a manufacturer <input type="checkbox"/> CERN/collaboration responsible for the design and/or manufacturing |

HAZARDS GENERATED BY THE EXPERIMENT

Hazards named in the document relevant for the fixed VITO installation (in preparation).

Additional hazards:

| Hazards | | | |
|---------------------------------------|---------------------------------|----------------|---------------------------------|
| | VITO | COLLAPS lasers | B-NMR spectrometer |
| Thermodynamic and fluidic | | | |
| Pressure | | | |
| Vacuum | $10^{-9} - 10^{-6}$ mbar | | $10^{-6} - 1$ mbar |
| Temperature | RT | | RT |
| Heat transfer | | | |
| Thermal properties of materials | | | |
| Cryogenic fluid | LN ₂ , 1.2 Bar, 20 l | | LN ₂ , 1.2 Bar, 20 l |
| Electrical and electromagnetic | | | |
| Electricity | 220V, 10kW | | |
| Static electricity | Less than 10kV | | |
| Magnetic field | Less than 50 G | | 0.03 [T] |
| Batteries | <input type="checkbox"/> | | |
| Capacitors | <input type="checkbox"/> | | |
| Ionizing radiation | | | |
| Target material | | | Crystals, ionic liquid |
| Beam particle type (e, p, ions, etc) | ions | | ions |
| Beam intensity | $\leq 10^7$ ions/s | | $\leq 10^7$ ions/s |

| | | | |
|--|---|--------------------------------|------------------|
| Beam energy | 10-50 kV | | 10-50 kV |
| Cooling liquids | water | | water |
| Gases | Ar | | He |
| Calibration sources: | <input type="checkbox"/> | | |
| • Open source | <input type="checkbox"/> | | |
| • Sealed source | <input type="checkbox"/> [ISO standard] | | |
| • Isotope | | | |
| • Activity | | | |
| Use of activated material: | | | |
| • Description | <input type="checkbox"/> | | |
| • Dose rate on contact and in 10 cm distance | | | |
| • Isotope | 58Cu, 74Cu, 75Cu | | 58Cu, 74Cu, 75Cu |
| • Activity | 100 – 1000 kBq | | 100 – 1000 kBq |
| Non-ionizing radiation | | | |
| Laser | | Nd:YAG (10W) dye laser (1W) | |
| UV light | | 325 nm (less than 100mW) | |
| Microwaves (300MHz-30 GHz) | | | |
| Radiofrequency (1-300MHz) | | | NMR strength |
| Chemical | | | |
| Toxic | | methanol for lasers | |
| Harmful | | Laser dyes | |
| CMR (carcinogens, mutagens and substances toxic to reproduction) | | | |
| Corrosive | | | Ionic liquids |
| Irritant | | | |
| Flammable | Ethanol, acetone | | Ethanol, acetone |
| Oxidizing | | | |
| Explosiveness | | | |
| Asphyxiant | | | |
| Dangerous for the environment | | | |
| Mechanical | | | |
| Physical impact or mechanical energy (moving parts) | | | |
| Mechanical properties (Sharp, rough, slippery) | | | |
| Vibration | | | |
| Vehicles and Means of Transport | | | |
| Noise | | | |
| Frequency | | | |
| Intensity | | | |
| Physical | | | |
| Confined spaces | | | |
| High workplaces | | | |
| Access to high workplaces | | | |
| Obstructions in passageways | | | |
| Manual handling | | | |
| Poor ergonomics | | | |

3.1 Hazard identification

3.2 Average electrical power requirements (excluding fixed ISOLDE-installation mentioned above):
(make a rough estimate of the total power consumption of the additional equipment used in the experiment)