EUROPEAN ORGANIZATION FOR NUCLEAR RESEARCH

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

^{111}Ag-Perturbed Angular Correlation of γ-rays (PAC) spectroscopy during the long shutdown 2019-2020: Function of proteins in Cu^+ homeostasis and transport

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Abstract

Metal ions display diverse functions in biological systems and are integral components of a ~1/3 of all proteins. In order to control that metal ions are present in the cells at concentrations that is adequate for binding to the relevant metalloproteins, while not at concentrations so high that the metal ions exert toxic effects, a series of proteins are involved in regulating the concentration of essential metal ions such as zinc, copper and iron. In this project we aim to elucidate metal site structure and dynamics to understand function of proteins involved in regulation and transport of Cu^+. Cu^+ is difficult to observe using standard spectroscopic techniques, and thus we will apply ^{111}Ag PAC spectroscopy: Many of the Cu^+ responsive proteins are similarly sensitive to Ag^+, which exhibits the same charge and a closed d-shell electronic structure. Thus it is expected that Ag^+ is a good spectroscopic probe for the Cu^+ binding sites. To the extent that it is possible, all experiment series will involve other experimental techniques such as NMR, EXAFS, UV-Vis, fluorescence, and CD spectroscopies providing complementary data, as well as interpretation of the experimental data by quantum mechanical calculations of spectroscopic properties.

Requested shifts: No shifts requested – only other infrastructure, vide infra.

Project description

Cu^+ is difficult to observe using standard spectroscopic techniques due to its closed shell electronic structure. It is also one of the most important metal ions in biological systems, involved in both electron transport in photosynthesis and in redox chemistry. Thus, there is a considerable interest in techniques able to elucidate the biochemistry of this metal ion.

Cu^+ sensing by CueR and related proteins

The copper-efflux regulator CueR protein is a member of the MerR-family of transcriptional regulators that provides a sensitive response to monovalent d^{10} metal ions of the copper-group (Cu^+, Ag^+ and Au^+) but remains inactive to the double charged d^{10} ions (e.g. Hg^{2+}, Zn^{2+}) [03Ch]. The crystal structures of CueR with Cu^+, Ag^+ and Au^+ showed that two cysteine residues from the ends of a short metal binding loop (MBL) restricts the bound metal ions to a digonal coordination mode that allosterically affects the interaction of CueR with the regulated DNA, influencing DNA-structure and thus mediating the transcription process [03Ch]. We have postulated that a pH-dependent switch, i.e. the protonation of one of the cysteine residues might play a role in this allosteric mechanism [15Sz]. GolS and CupR are also monovalent metal-responding members of the MerR-family, however, they were recognized to display a metal binding / metal sensing profile with a preference for Au^+ over Cu^+ or Ag^+ [11Ch]. Differences in the sequence of the MBL, relative to CueR was proposed to be the main origin of this fine-tuned metal ion selectivity in GolS [13Ib] whereas the metal-coordinating role of a Cys-Cys-His fragment, located near to the C-terminus, was suggested to alter metal-ion selectivity in favor of Au^+ in CupR [09Ji]. In this project we are aiming at the elucidation of the pH-dependent metal site structure and dynamics of the Ag^+-bound CueR via ^{111}Ag PAC spectroscopy, amongst other techniques. The experiments are planned to be extended to various CueR mutants and potentially to GolS or CupR, enabling us to monitor the effects of modifications at residues that are believed to play a role or modulate metal ion binding and thus metal ion selectivity [15Sz,13Ib,15Ib]. Such modifications may involve e.g. the conserved Ser77, a proposed key residue in the allosteric mechanism of these sensory proteins, amino acids in the sequence of the MBL and modifications or a complete truncation of a short Cys-Cys containing fragment located at the C-terminus of the CueR and CupR proteins. Such experiments may contribute to the understanding of how the selective recognition of monovalent metal ions is achieved by these important molecular elements of bacterial metal homeostasis / resistance. Finally, we wish to conduct experiments both in the presence and absence of the DNA to which the proteins bind, to explore if DNA binding affects the metal site structure

In summary, the proposed experiment series will encompass a variety of Cu^+ sensing proteins and experiments both in the presence and absence of the DNA, thus requiring the possibility of running several PAC instruments in parallel offered at ISOLDE/CERN.

Cu^+ transport by HAH1

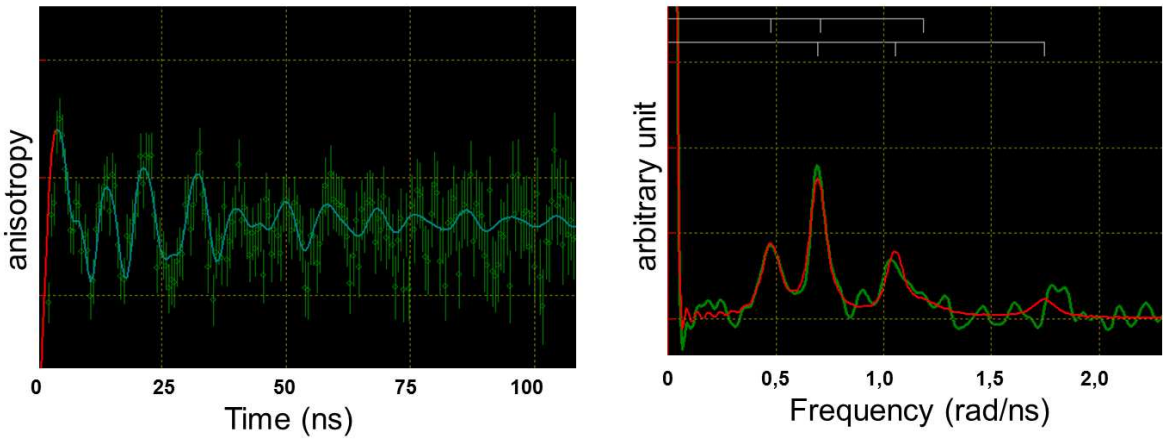
We aim to examine Ag^+ binding to HAH1, a human copper chaperone, in order to elucidate transport and inter-protein transfer of monovalent metal ions. Ag^+ and Cu^+ have similar

coordination preferences, with Ag^+ forming extremely stable linear complexes promoted by hybrid orbitals between 5s and $4dz^2$. Within the copper chaperones a fluxional loop, containing the copper-binding motif positioned between the first beta sheet and the first alpha helix, adapts to at least two limiting conformers for the function of this protein. One is a linear (diagonal) coordination and the other is three coordinated. Ag^+ and Cu^+ can accommodate either coordination preference, keeping in mind the larger ionic radii for Ag^+ (126 vs. 96 pm, Pauling radii). Linear coordination perhaps shelters the monovalent species from adventitious chemistry, whereas the three coordinate species acts at the moment of transfer to the partner protein, for example ATP7B or ATP7A. This is not to rule out a tetrahedral or distorted tetrahedral species, that also might co-exist at the moment of transfer. Another nuance of this copper transfer system is that the coordination environment of the monovalent ions is changing during the copper transiting and transfer events, and interrogation with different ions (like Ag^+) provides insight into this process. We have shown that at high pH a complex between two HAH1 proteins is formed with Hg^{2+} coordinating to both proteins [Lu13], i.e. forming a bridged species, presumably reflecting the process of metal ion transfer between two proteins. With this project we wish to use a spectroscopic probe (^{111}Ag), resembling the native copper ion better than the divalent Hg^{2+}. Thus, we aim to carry out a series of ^{111}Ag PAC experiments at different pH values, and therefore also this project requires the use of several of the PAC instruments present at ISOLDE/CERN.

V. Experimental Methods

Several TDPAC spectrometers are installed permanently at the Solid State Physics Laboratory of ISOLDE at CERN, and maintained by expert personnel. With this proposal we aim to exploit this fact, and carry out series of ^{111}Ag PAC experiments in parallel, making optimal use of the ^{111}Ag produced at ILL, Grenoble, France, and allowing for compilation of data not possible within one experimental campaign anywhere else in the world. We have considerable experience with the use of TDPAC isotopes produced at ISOLDE, like ^{111m}Cd, ^{199m}Hg, and ^{204m}Pb with half-lives of less or about one hour. Additionally, the synthesis and purification of the proteins will be carried out by experts in this facet of the project at the laboratories of University of Szeged, Hungary, (Attila Jancsó) and at the Western Michigan University, USA, (David Huffman).

The radioactive ^{111}Ag will be produced at ILL by irradiation of isotopically enriched ^{110}Pd in a neutron flux of the order of 10^{15} n.cm^{-2}s^{-1}. Neutron capture produces ^{111}Pd and ^{111m}Pd with half-lives of 0.39 h and 5.5 h respectively, these decay then to ^{111}Ag. After the irradiation at ILL the samples are left for decay during 3 days before transport to CERN, so ^{111}Pd and ^{111m}Pd practically disappear by decaying to ^{111}Ag. Subsequently a simple radiochemical separation of the non-carrier-added ^{111}Ag will be performed at ISOLDE. The ^{110}Pd metal target is dissolved in acid and the ^{111}Ag is separated from stable Pd using ion exchange chromatography. In a pilot project in 2016, we have demonstrated that this protocol may be applied successfully, see the ^{111}Ag PAC spectrum below.



^{111}Ag PAC spectrum recorded for the CueR protein in a pilot project, to demonstrate that the experimental protocol was succesful*. Left: The recorded time dependent anisotropy and fit (full line); Right: Fourier transform of data (green) and of fit (red). There are clearly two nuclear quadrupole interactions (indicated in the upper part of the panel (white lines)), reflecting binding of ^{111}Ag in two different coordination geometries.*

We may subsequently add inactive carrier Ag-salt solution prior to adding the biomolecule to increase the metal concentrations from nanomolar to micromolar. After the incubation of the biomolecules with the ^{111}Ag-solution with or without carrier for 5 to 30 minutes the biomolecules are immobilized by precipitation/centrifugation, freezing, or adding sucrose.

This experimental campaign requires CERN services in terms of radioprotection and delivery of liquid nitrogen, and the access to the ISOLDE off-line laboratories (508/R-002 and 508/R-008) infrastructure, mainly the chemistry and PAC laboratories, and integrated equipment. We are thus ready to initiate several production runs aiming optimized experiments, exploiting the parallel use of the four 6D-PAC instruments present at the ISOLDE off-line laboratory 508-r-008.

Summary of requested shifts:

No shifts requested, only other infrastructure at ISOLDE.

References:

[He04] Hemmingsen L., Sas K.N., Danielsen E. Chem. Rev. 2004, 104, 4027-4062.

[03Ch] Changela, A.; Chen, K.; Xue, Y.; Holschen, J.; Outten, C. E.; O'Halloran, T. V.; Mondragón, A. Science 2003, 301, 1383–1387.

[15Sz] Szunyogh, D.; Szokolai, H.; Thulstrup, P. W.; Larsen, F. H.; Gyurcsik, B.; Christensen, N. J.; Stachura, M.; Hemmingsen, L.; Jancsó, A.; Angew. Chem. Int. Ed. 2015, 54, 15756–15761.

[11Ch] Checa, S. K.; Soncini, F. C.; Biometals 2011, 24, 419–427.

[13Ib] Ibáñez, M. M.; Cerminati, S.; Checa, S. K.; Soncini, F. C.; J. Bacteriol. 2013, 195, 3084–3092.

[09Ji] Jian, X.; Wasinger, E. C.; Lockard, J. V.; Chen, L. X.; He, C.; J. Am. Chem. Soc. 2009, 131, 10869–10871.

[15Ib] Ibáñez, M. M.; Checa, S. K.; Soncini, F. C.; J. Bacteriol. 2015, 197, 1606–1613.

[Lu13] Luczkowski M., Zeider B.A., Hinz A.V., Stachura M., Chakraborty S., Hemmingsen L., Huffman D.L., Pecoraro V.L. Chem. Eur. J. 2013, 19, 9042-9049

Appendix

DESCRIPTION OF THE PROPOSED EXPERIMENT

The experimental setup comprises: *(name the fixed-ISOLDE installations, as well as flexible elements of the experiment)*

Part of the Choose an item.	Availability	Design and manufacturing
SSP laboratories (part 1 and 2)	☒ Existing	☒ To be used without any modification
Part 1: Chemical laboratory 508/R-002 (Class C)	☒ Existing	☒ To be used without any modification ☐ To be modified
	☐ New	☐ Standard equipment supplied by a manufacturer ☐ CERN/collaboration responsible for the design and/or manufacturing
Part 2: PAC spectrometers at 508/R-008 (Class C)	☒ Existing	☒ To be used without any modification ☐ To be modified
	☐ New	☐ Standard equipment supplied by a manufacturer ☐ CERN/collaboration responsible for the design and/or manufacturing

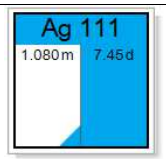
HAZARDS GENERATED BY THE EXPERIMENT

(if using fixed installation) Hazards named in the document relevant for the fixed [COLLAPS, CRIS, ISOLTRAP, MINIBALL + only CD, MINIBALL + T-REX, NICOLE, SSP-GLM chamber, SSP-GHM chamber, or WITCH] installation.

Additional hazards:

Hazards	*Part 1*	*Part 2*	
Thermodynamic and fluidic			
Pressure	atmospheric pressure	atmospheric pressure	
Vacuum			
Temperature	room temperature	room temperature or liquid N temperature	
Heat transfer			
Thermal properties of materials			
Cryogenic fluid	[fluid], [pressure][Bar], [volume][l]	Liquid N (2-4 liters to be refilled every 10 hours)	
Electrical and electromagnetic			
Electricity	Standard 230 V	Standard 230 V	
Static electricity			
Magnetic field	[magnetic field] [T]		
Batteries	☐		

Capacitors	☐		
Ionizing radiation			
Target material	Samples described in the project	Samples described in the project	
Beam particle type (e, p, ions, etc)			
Beam intensity			
Beam energy			
Cooling liquids	[liquid]		
Gases	[gas]		
Calibration sources:	☐		
• Open source	☐		
• Sealed source	☐ [ISO standard]		
• Isotope			
• Activity			
Use of activated material:			
• Description	☒ Chemistry at the chemical laboratory (508/R-002) and transport to the PAC laboratory (508/R-008) in sealed Eppendorf	No manipulation, just measurement using PAC spectrometers	
• Dose rate on contact and in 10 cm distance	**Information for 250 MBq**	**Information for 50 MBq** $H^*(10)$ = 1.426 µSv/h at 40 cm distance $\dot{H}(0.07)$ = 22.91 mSv/h at 10 cm distance	



Dosimetry and Shielding H*(10)

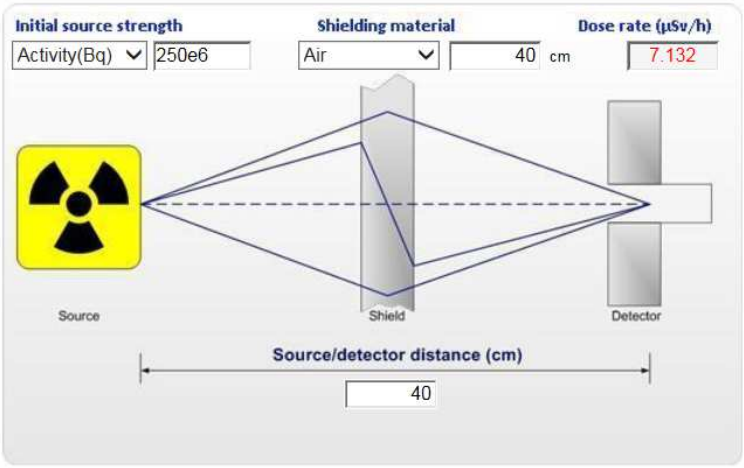
47 Silver

Nuclide / Mixture

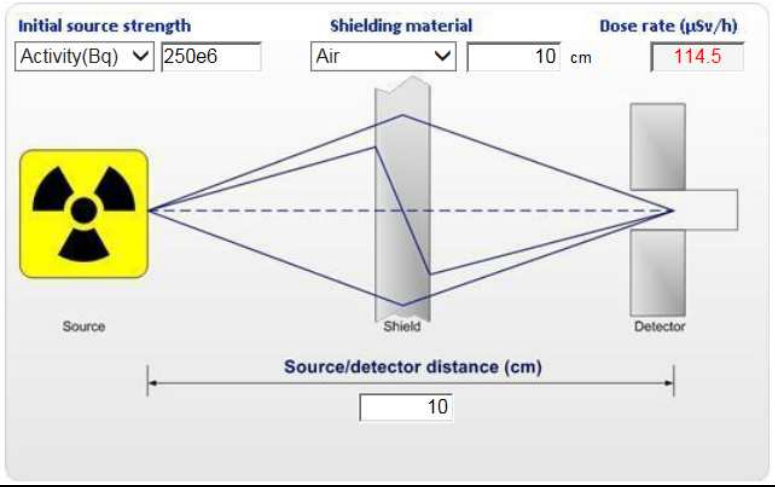
Ag 111

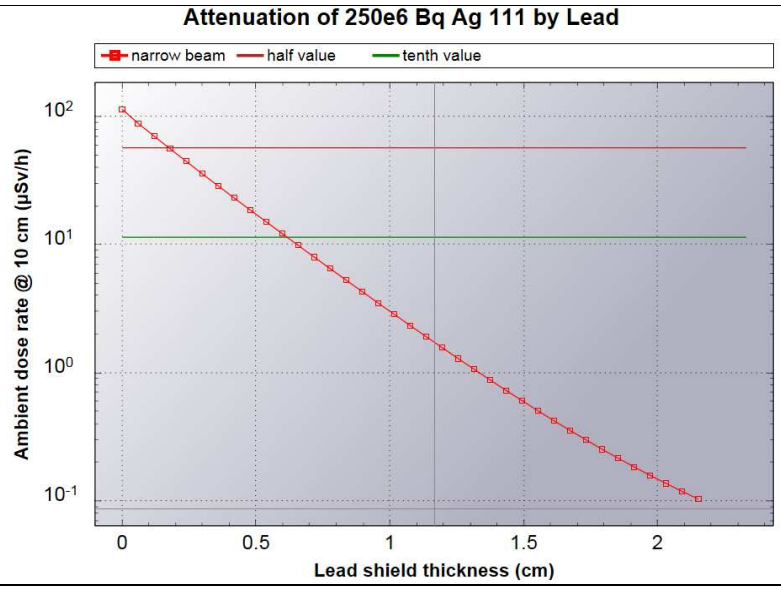
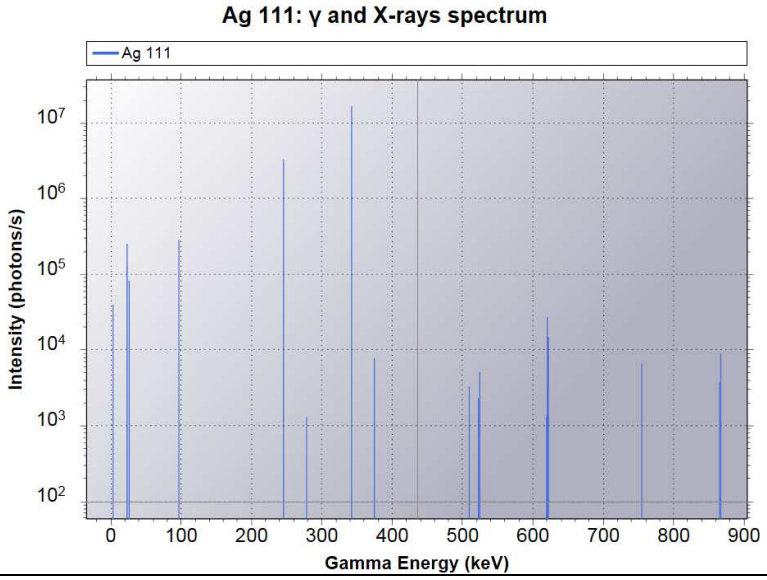
Consider decay

Ambient Dose Rate | Dose rate/Thickness graph | Attenuation & Buildup | Options | Mixture details



Ambient Dose Rate | Dose rate/Thickness graph | Attenuation & Buildup | Options | Mixture details



			
• Isotope	^{111}Ag 	^{111}Ag	
• Activity	**Two times 250 MBq delivered separately** For information: LA = 3 MBq Class C laboratory authorization: 100 LA	maximal 50 MBq per sample LA = 3 MBq Class C laboratory authorization: 100 LA	
Non-ionizing radiation			
Laser			
UV light			
Microwaves (300MHz-30 GHz)			
Radiofrequency (1-300MHz)			
Chemical			
Toxic	[chemical agent], [quantity]		
Harmful	NaClO4 (few g): Harmful if swallowed		
CMR (carcinoge	[chemical agent], [quantity]		

ns, mutagens and substances toxic to reproduction)			
Corrosive	tris-(2-carboxyethyl)phosphine, hydrochloride (TCEP), few g (Skin corrosion/irritation). EU Specific Hazard Statements: R35 causes severe burns Sodium hydroxide **DOT Classification:** Class 8: Corrosive material (the material will be used just in few ml of diluted solutions) HNO3 (the material will be used just in few ml of diluted solutions) AgClO4 and AgNO3 (few g): Causes severe skin burns and eye damage. AgNO3 (few g): corrosive to metals		
Irritant	tris-(2-carboxyethyl)phosphine, hydrochloride (TCEP), few g (Skin corrosion/irritation). EU Specific Hazard Statements: R35 causes severe burns		
Flammable	[chemical agent], [quantity]		
Oxidizing	Perchloric acid **DOT Classification:** CLASS 5.1: Oxidizing material. Class 8: Corrosive material (the material will be used just in few ml of diluted solutions) HNO3 (the material will be used just in few ml of diluted solutions) NaClO4 (few g): Powerful oxidizer AgClO4 and AgNO3: (few g) oxidizer.		
Explosiveness	[chemical agent], [quantity]		
Asphyxiant	[chemical agent], [quantity]		
Dangerous for the environment	AgNO3 (few g): Very toxic to aquatic organisms		
Mechanical			
Physical impact or mechanical energy (moving parts)	[location]		
Mechanical properties (Sharp, rough, slippery)	[location]		
Vibration	[location]		
Vehicles and Means of Transport	[location]		
Noise			
Frequency	[frequency],[Hz]		
Intensity	ISOLDE hall background	Laboratory background	

Physical			
Confined spaces	[location]		
High workplaces	[location]		
Access to high workplaces	[location]		
Obstructions in passageways	[location]		
Manual handling	[location]		
Poor ergonomics	[location]		

0.1 Hazard identification

Chemical waste will be disposed in accordance with CERN control regulations.

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)*