EUROPEAN ORGANISATION FOR NUCLEAR RESEARCH

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

Cu(I), Ag(I), Cd(II), Hg(II), and Pb(II) binding to biomolecules studied by Perturbed Angular Correlation of γ-rays (PAC) spectroscopy

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L. Hemmingsen[1], Peter W. Thulstrup[1], V. L. Pecoraro[2], A. Jancso[3], Jens Müller[4], K. Johnston[5], Monika Stachura[5], J.G.M. Correia[5]

[1]Department of Chemistry, University of Copenhagen, Denmark
[2]Department of Chemistry, University of Michigan, USA
[3]Department of Inorganic and Analytical Chemistry, University of Szeged, Hungary
[4]Department of Inorganic and Analytical Chemistry, Westfälische Wilhelms-Universität Münster, Germany
[5]ISOLDE, CERN

Spokesperson: L. Hemmingsen (lhe@chem.ku.dk)

Local contact: K. Johnston (karl.johnston@cern.ch)

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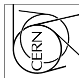

Abstract

Metal ions display diverse functions in biological systems and are essential components in both protein and nucleic acid structure and function, and in control of biochemical reaction paths and signalling. Similarly, metal ions may be used to control structure and function of synthetic biomolecules, and thus be a tool in the design of molecules with a desired function. In this project we address a variety of questions concerning both the function of metal ions in natural systems, in synthetic biomolecules, and the toxic effect of some metal ions. All projects 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.

The isotopes to be employed in the proposal are the following: ^{111m}Cd, ^{111}Ag, ^{199m}Hg, ^{204m}Pb, ^{61}Cu, ^{68m}Cu

Requested shifts: 22 shifts, (split into 4 major runs over 2 years; 2 test runs to be accommodated when possible)

Our group has specialized in the application of PAC spectroscopy in chemistry and biochemistry[1]. In the following we describe the research projects we aim to embark on or continue over the next ~2 years.

I. Binding of Ag(I) to nucleic acids (RNA, DNA) and model systems

Artificial metal-mediated base pairs represent a powerful tool for the site-specific functionalization of nucleic acids with metal ions. The development of applications for the resulting metal-modified nucleic acids depends on the availability of structural information on these double helices. Such nucleic acids are discussed e.g. in the context of nanoscale electronic architectures. Unfortunately, reliable information on the local metal site structure, effects of multiple neighboring metal sites, and intermetallic distances in metal-modified DNA is scarce.[1] The aim of this study is to elucidate molecular and electronic structure at designed Ag(I) binding sites with one and two Ag(I) ions bound, respectively, and thereby to elucidate interactions between the two sites of neighboring Ag(I)-mediated imidazole base pairs in a synthetic oligonucleotide double helix. Using quantum mechanical calculations of spectroscopic properties (electric field gradients) for various possible model structures, we aim to identify molecular structures that agree with the spectroscopic findings, i.e. to characterize the metal ion binding site structures of both mono- and di-nuclear sites, including estimates of the Ag···Ag distance. The findings will be crucial for the future development of applications of this metal-modified nucleic acid.

The local electronic and molecular structure shall be probed by PAC measurements. Towards this end, two DNA duplexes will be prepared comprising one or two neighboring Ag(I)-binding sites, respectively. The duplex with one designated Ag(I)-binding site will be investigated in the presence of one equiv. of Ag(I) or one equiv. of Cd(II). These experiments are performed to ensure that the PAC signal of the DNA with Ag(I)-mediated base pairs is indeed due to Ag(I) rather than to Cd(II), which is the daughter nucleus of ^{111}Ag, and the one which the experiment is effectively carried out for. However, if the structural relaxation from the local structure at the Ag(I) metal site to that of Cd(II) bound to the metal site is slow, the molecular structure will reflect Ag(I) coordination. To further explore the differences between mono- and di-nuclear metal sites, experiments will be conducted at very low concentration of DNA and Ag(I), in order to achieve only single site Ag(I) occupation even in the duplex designed to bind two metal ions, and we will then systematically increase concentrations to finally obtain the fully loaded di-nuclear site, following the transition from mono- to di-nuclear site spectroscopically. The very high sensitivity of PAC spectroscopy allows for such experiments at extremely low concentrations, contrary to other techniques. To aid immobilization of the duplexes, and to trap the local metal site structure in the Ag(I) coordination geometry, all samples will be investigated at –196 °C after flash-freezing in liquid nitrogen.

II. Metal ion binding to *de novo* designed proteins

Over the past decade we have had considerable success exploring the biological chemistry of heavy metal ion binding to designed and naturally occurring proteins[3-8]. The synthetic

proteins are excellent scaffolds to explore and design new metal ion binding sites, but also serve as model systems of naturally occurring regulatory proteins, metal chaperones and the targets of metal toxicity. The strength of this work has been directly dependent on the ability to carry out PAC studies of these systems, and PAC was the only technique to demonstrate that our original design for Cd(II) proteins was complicated by two species. Even metal site dynamics on the biologically important but elusive nanosecond time scale has been revealed[9].

We aim to continue this line of research, and our future studies will focus on 1) Achieving the first unambiguous ^{204m}Pb-PAC results for Pb(II) binding to a biomolecule. Initially we will conduct experiments on the synthetic homotrimeric proteins TRIL16C and TRIL19C, which despite presenting similar metal ion binding sites with three thiols, display very different chemical shifts for the Pb(II) ion in NMR spectroscopy. If successful these studies will be advanced further by experiments including synthetic heterotrimeric systems, from AAA, AAB, ABB, to BBB (where A and B are different monomers), to characterize the structural space of Pb(II) metal site coordination geometries, and thereby acquire basic knowledge about possible structures that Pb(II) may target in biological system, elucidating the toxicity of this metal ion. 2) Continuing to examine Hg(II) coordination geometry and metal site dynamics in HAH1[5], in order to assess how this toxic metal may be transported within human cells. Additionally, both binding of Hg(II) and Cd(II) to synthetic so-called A3D proteins resembling like rubredoxin and possible cupredoxins may shed light on heavy metal ion binding to these proteins families. Finally, the studies with Ag(I) are particularly interesting as we have yet to characterize a monovalent cation in our peptide scaffold using PAC spectroscopy, and because Ag(I) may serve as a probe of Cu(I) coordination to proteins. Cu(I) is spectrocopically silent in most other techniques, and yet one of the most important metal ions in biology. Thus, the combination of different elements should allow us to make significant new advances in studying the basic chemistry of heavy metals in biology and further develop the field of de novo protein design.

III. Metal ion biosensors and model systems

It is believed that the survival and virulence of pathogenic bacteria inside mammalian hosts is largely related to the host metal-ion status and how bacteria respond to it, i.e. how they adapt to, exploit or influence the actual metal ion level[10]. In this bacterial response, the metal ion homeostasis and resistance systems play an important role. The elements of these systems are mediated by metalloregulatory proteins at a transcriptional level. These molecules are able to sense a specific metal ion or a small group of metal ions and this selectivity is, in part, related to the metal ion binding features of the proteins. The copper efflux regulator MerR family member CueR responds to the monovalent group 11 metal ions (Cu^{I},Ag^{I},Au^{I}) but shows no activity in the presence of the divalent ion Hg^{II} or Zn^{II}.[11] Crystal structures of CueR showed that M^{I} ions are restricted to a linear coordination environment by the two Cys-residues of the metal binding loop[11]. In the proposed project the intrinsic metal ion binding characteristics of the protein, i.e. the local structure of metal ions within the effector binding domain are planned to be investigated by means of PAC-spectroscopy. The utilization of various radionuclides (^{111}Ag, ^{199m}Hg, ^{111m}Cd) may allow observing fundamental structural (geometry) changes promoted by the different type of metal ions which necessarily affect the conformation of the protein and thus its activity. Mutations within the metal binding loop of CueR and studying the metal ion binding of the

mutant proteins or relevant model peptides[12] may shed light on the role of individual residues in providing the optimal coordination "pocket" for the cognate metal ions.

Cadmium(II) is often applied as a substitute of zinc(II) ion in biomolecules for better understanding of the local structure of the metal ion and its function. Recently we investigated the metallonuclease colicin E7, the catalytic domain of which is a possible building block of artificial nucleases[13]. To apply it for this purpose we need to know the properties of this nuclease in detail. The metal ion binding, coordination environment and function was already studied and published in the literature, but the results are inconsistent and contradictory. Therefore, we would like to study the role and behaviour of the zinc(II) ion in the active centre in the presence and absence of the substrate. As the most common site-specific artificial nucleases consist of zinc finger DNA binding domains[14], the Zn(II) vs. Cd(II) substitution may also affect the mechanism of action and DNA recognition specificity. The precise description of the structural changes around the metal ion may be achieved by PAC spectroscopy.

IV. Towards a probe for Cu(I) in biomolecules

In order to test the feasibility of using Cu isotopes in PAC studies we propose to run simple tests on two PAC-active copper isotopes: ^{61}Cu and ^{68m}Cu. The motivation is given by presence of Cu in a large number of proteins involved in electron transfer and activation of oxygen and lack of a suitable spectroscopic methods allowing for probing Cu(I). When searching for a suitable PAC probe we have looked at the following aspects: decay type (as isomeric transitions are highly favourable), parent's life time (which defines on-line or off-line experiment), life time of the intermediate state, spin of the intermediate state, γ-γ angular anisotropies and ISOLDE yields. As a result we have selected two isotopes, which have the most favourable properties: ^{61}Cu and ^{68m}Cu, see Table 1. It should be noted that the quadrupole moment for ^{68m}Cu is unknown and for the current proposal it has been roughly estimated to be around 0.1 barn based on the former measurements on different Cu isotopes performed at COLLAPS[15-18].

Table 1. Properties of the chosen Cu isotopes from Nuclear Data Sheets

Isotope	*Parent half-life*	*Decay mode*	*intermediate state*				*γ₁ [keV]*	*γ₂ [keV]*	*anisotropy A_{22}*	*ISOLDE yields*
			spin	*half-life*	*Q [b]*	*$\mu[\mu_N]$*				
^{61}Cu/^{61}Ni	3.4 h	β+→γ	3/2	5.17(6)ns$^{(*)}$	0.20(3)	+0.480(6)	589 (1.2%)	67 (4.2%)	-0.15$^{(*)}$	1x10^8 target: ZrO_2
^{68m}Cu/^{68}Cu	3.75 min	γ→γ	2	7.84 ns	0.1 $^{(**)}$	?	637 (10%)	84 (80%)	-0.15	1x10^8 target: UC_X

$^{(*)}$ *measurement obtained at ISOLDE during first tests in 2012 – J.G. Correia*

$^{(**)}$ *COLLAPS estimation – M. Bissel*

For the test experiment we will use simple inorganic compounds displaying linear Cu(I) coordination, for example with halogens or thiolates as ligands[19], which are expected to provide a large EFG. This will compensate the low value of Q based on $v_Q = \frac{eV_{zz}Q}{h}$. For both isotopes μ values are desirable to be (re) measured, being most likely large enough to probe magnetic interactions, however, the Q values are expected to be rather small, which will lead to poor frequency resolution. Therefore, we only ask for 0.5 shifts for each of the isotopes in order to test feasibility of such experiments.

V. Experimental Methods

Several TDPAC spectrometers are available at ISOLDE, and will be used in the current projects. For the long lived ^{111}Ag isotope we will additionally ship some of the activity to Copenhagen, where a radiochemistry lab as well as a 6-detector PAC instrument are available. The synthesis and purification of the proteins will be done at the laboratory at the University of Michigan, and at the University of Szeged, while synthetic nucleic acids will be produced at the Westfälische Wilhelms-Universität Münster. The samples will be transported to ISOLDE on dry ice (~ -80 °C).

The proposed experiments require the implantation for ~60 minutes into ice at 100 K (in order to guarantee the vacuum in the beamline). For this we use the so-called "biophysics chamber". We have developed our own target holder, cooled by liquid nitrogen, at the collection point. Occasionally, we subsequently add inactive carrier (metal ion salts dissolved in adequate buffer solutions) to the molten ice to increase the metal concentrations from nanomolar to micromolar. After the incubation of the biomolecules with the radioisotope containing-solution with or without carrier for 5 to 30 minutes the biomolecules are immobilized by precipitation/centrifugation, freezing, or adding sucrose. For the radiochemical preparation steps the fume hood of the Solid State Physics Laboratory in building 508 is required. There is also enough space for the cooling centrifuge, a refrigerator/freezer for protein samples, and other basic lab equipment, in the chemistry lab.

For ^{68m}Cu the lifetime is too short to collect and carry out offline chemical sample preparation, therefore we will initially conduct the experiments by directly implanting into the solid Cu(I) salt sample. If this is successful, we will in a later proposal proceed to liquid samples.

VI. Beam Time Request

We ask for a total of 22 shifts within 2 years to be used as follows, all with min. $5 \cdot 10^7$ ions/s:

Isotope	$T_{1/2}$	Target	Yield (ions/µC)	Ion source	Shipping?	# shifts
^{111m}Cd	48 min	Sn	2×10^8	HP (VADIS)	No	6
^{111}Ag	7.45d	UCx	$5x10^7$	RILIS (Ag)	Yes	6
^{199m}Hg	42 min	Pb	2×10^8	HP (VADIS)	No	6
^{204m}Pb	1.13h	UCx	2×10^8	RILIS (Pb)	No	3
^{61}Cu	3.4 h	ZrO_2	1×10^8	RILIS	No	0.5
^{68m}Cu	3.75 min	UC_X	1×10^8	RILIS	No	0.5

If absolutely necessary, we can accommodate cuts in the above plan, although it is – of course – not at all desirable, and will reduce the scientific outcome accordingly. In a breakdown of the shifts applied for, we prioritize PAC experiments on ^{61}Cu and ^{68m}Cu very highly, and next PAC experiments on ^{111}Ag, ^{111m}Cd, ^{199m}Hg, and ^{204m}Pb, where cuts are best made simply proportionally (for example to 4, 4, 4, 2 shifts, respectively, for these isotopes).

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Appendix

DESCRIPTION OF THE PROPOSED EXPERIMENT

The experimental setup comprises: *GLM collection chamber and the biophysics chamber*

Part of the Choose an item.	Availability	Design and manufacturing
GLM	☒ Existing	☒ To be used without any modification
Biophysics chamber	☒ 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			
	GLM	*Biophysics chamber*	*[Part 3 of the*

			experiment/equipment]
Thermodynamic and fluidic			
Pressure	[pressure][Bar], [volume][l]		
Vacuum			
Temperature	[temperature] [K]		
Heat transfer			
Thermal properties of materials			
Cryogenic fluid	[fluid], [pressure][Bar], [volume][l]	LN_2 (cold finger in the chamber and a cryostat outside)	
Electrical and electromagnetic			
Electricity	[voltage] [V], [current][A]		
Static electricity			
Magnetic field	[magnetic field] [T]		
Batteries	☐		
Capacitors	☐		
Ionizing radiation			
Target material	[material]	Ice, frozen liquid	
Beam particle type (e, p, ions, etc)	ions	ions	
Beam intensity			
Beam energy	30-50 keV	30-50 keV	
Cooling liquids	[liquid]	LN_2	
Gases	[gas]		
Calibration sources:	☐		
• Open source	☒		
• Sealed source	☐ [ISO standard]		
• Isotope			
• Activity		< 100LA (Class C) With the exception of 111Ag, considerably less (typically 1-2 LA) NOTE: 204Pb can carry isobaric Fr contamination. To be monitored with the assistance of RP. b	
Use of activated material:			
• Description	☐		
• Dose rate on contact and in 10 cm distance	[dose][mSV]		
• Isotope			
• Activity			
Non-ionizing radiation			
Laser			
UV light			
Microwaves (300MHz-30 GHz)			
Radiofrequency (1-300MHz)			
Chemical			
Toxic	[chemical agent], [quantity]		
Harmful	[chemical agent], [quantity]		
CMR (carcinogens, mutagens and substances toxic to reproduction)	[chemical agent], [quantity]		
Corrosive	[chemical agent], [quantity]		
Irritant	[chemical agent], [quantity]		
Flammable	[chemical agent], [quantity]		

Oxidizing	[chemical agent], [quantity]		
Explosiveness	[chemical agent], [quantity]		
Asphyxiant	[chemical agent], [quantity]		
Dangerous for the environment	[chemical agent], [quantity]		
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			
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

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)*