# A report on the study of biological systems at ISOLDE IS448 and IS488: “Ag(I), Pb(II) and Hg(II) binding to biomolecules studied by Perturbed Angular Correlation of γ-rays (PAC) spectroscopy: Function and toxicity of metal ions in biological system.”

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This report summarizes the achievements of the IS448 and IS488 collaboration work in 2009-2011 and new plans and ideas, to be pursued out over the following years, are briefly described.
*The main purpose of both projects is and was to understand the fundamental chemistry of heavy metal-protein interactions and thus the mechanisms underlying heavy metal toxicity at the molecular level.* The IS488 project (21 shifts approved in 2009, still ongoing) is basically a continuation of the IS448 (24 shifts approved in 2007), however, it includes several new systems, such as RNA, DNA and plants, and in addition to the $^{199m}$Hg and $^{204m}$Pb it also includes application of the $^{111}$Ag ion beam. So far all experiments with the $^{199m}$Hg ion beams were very successful (all together ~ 75 experiments carried out within 9 shifts in 2009 and 2010). The $^{204m}$Pb runs have proven more complicated, partly due to technical problems in the isotope production, partly because the spectra are difficult to interpret reliably, as there are many (up to 36) lines for an intermediate spin of 4, and line-broadening is inherent to many of the systems investigated. In order to use the allotted beam time as efficiently as possible two PAC-Cameras at the Solid State Physics Laboratory at ISOLDE were used in parallel for all of the $^{199m}$Hg- and $^{204m}$Pb-PAC experiments while the $^{111}$Ag samples were shipped to Copenhagen.

The first $^{111}$Ag collections in 2009 were successful, nevertheless, radioactivity shipment to Denmark was delayed by Logistics, and the received samples had low activity. The results were therefore inconclusive. It was however, possible to test of how the implantations of 111Ag and subsequent extraction of the radioactivity for biochemical sample preparation was optimised. Therefore, most of the scientific highlights briefly described below are based on the experiments carried out with the use of $^{199m}$Hg ion beams:

1. Small model complexes
   The purpose of carrying out experiments on a group of about 10 different Hg(II) containing coordination compounds with biologically relevant ligands, such as: S, N, O, was two-fold. The results obtained from these experiments 1) allow us to test the quality of density functional theory based (DFT) calculations of electric field gradients, and 2) the structural

interpretation of the more complex systems, e.g. natural proteins, relies on similarity of the spectra with those recorded for the small model complexes. One Pb(II) containing coordination compound was also successfully characterized (manuscript in preparation).

2. *De novo* designed proteins and peptides
   These proteins, designed from the scratch by the group of Prof. Vincent Pecoraro, are much simpler than the naturally occurring proteins they mimic and at the same time they are more complex than the small model compounds mentioned above. Moreover, their composition can be controlled and thus they are perfect candidates for systematic studies of the heavy metal binding to proteins, which most likely prevents the normal physiological functions of these biomolecules. In this part of the project we aimed to study different coordination geometries of Hg(II), depending on the loading state and a degree of flexibility of potential metal binding site, hence elucidated different toxicities of a single metal ion [1-3] (two more manuscripts in preparation). In addition, we hope to design a peptide which selectively and strongly binds Hg(II) ions potentially leading to biotechnological purification of water. From a different perspective these data imply that metal ions can be used to control the structure and dynamics of structurally promiscuous proteins (manuscript in preparation), and this is probably the single most prominent discovery from our work in 2009-2011 from a biological viewpoint.
3. Natural proteins
   By characterizing the Hg(II) binding to zinc fingers, metallothioneins, other naturally occurring proteins, as well as in vivo in plants, we aim to elucidate the origin of Hg(II) toxicity at the molecular level. This is believed to be related to the substitution of naturally occurring metal ion from functional and structural sites in proteins leading to mis-folding and mis-function. And indeed the particular ligand preferences and structural features of Hg(II) bound to the investigated proteins indicate that this is the case, although the picture may be surprisingly more complex, and involve the effect of Hg(II) on intermolecular interactions [4,5].
4. Nucleic acids
   Nucleic acids find increasing applications in nanotechnology: DNA and RNA can serve as scaffolds to align metal ions in a specific way and hence to create electronic tools, e.g. molecular wires or magnets. Here we investigate the alignment of Ag(I) and Hg(II) ions within natural and modified RNA and DNA duplexes, e.g. stacked uracil-Hg-uracil base pairs. Initially these experiments presented considerable problems in terms of sample preparation, but we believe, that this has been overcome, and interpretation of the data is ongoing.

Plans for future projects and experiment series include a continuation of the characterization of binding of heavy metal ions to designed and naturally occurring biomolecules as well as in vivo binding to bacteria, plants and similar systems. A novel aspect of the investigations will be the role of metal ions in control of intermolecular interactions and transfer of metal ions between biomolecules, fields that are largely unexplored, and attracting increasing attention within biological inorganic chemistry.

A publication list:

1. Hemmingsen L., Stachura M., Thulstrup P.W., Christensen N.J., Johnston K. *Selected applications of perturbed angular correlation of gamma-rays (PAC) spectroscopy in biochemistry* Hyperfine Interactions **2010**, 197, 255-267
2. Iranzo O., Chakraborty S., Hemmingsen L., Pecoraro V.L. *Controlling and Fine Tuning the Physical Properties of Two Identical Metal Coordination Sites in De Novo Designed Three Stranded Coiled Coil Peptides* J. Am. Chem. Soc. **2011**, 133, 239-251

3. Chakraborty S., Kravitz J.Y., Thulstrup P.W., Hemmingsen L., DeGrado W.F., and Pecoraro V.L. *Realization of a Designed Three-Helix Bundle Capable of Binding Heavy Metals in a Tris(Cysteine) Environment* Angew. Chem. Int. Ed. **2011**, 50, 2049-2053
4. Heinz U., Hemmingsen L., Kiefer M., Adolph H.W. *Structural Adaptability of Zinc Binding Sites: Different Structures in Partially, Fully, and Heavy Metal Loaded States* Chem. Eur. J. **2009**, 15, 7350-7358
5. Selevsek N., Rival S., Tholey A., Heinzle E., Heinz U., Hemmingsen L., Adolph H.W. *Zinc ion-induced domain organization in metallo-beta-lactamases: A flexible "zinc arm" for rapid metal ion transfer?* J. Biol. Chem. **2009**, 284, 16419-16431