Among chemical elements ingested with food and drinking water, ten are classified as essential trace nutrients in human body. Specific biological activity of any essential element relies on its efficient delivery to the target sites while avoiding undesired sub- or supra- physiological concentrations. Homeostasis of an essential element is tightly regulated by its binding to a series of high and low molecular mass bioligands, which decides on element uptake, metabolization and excretion. Complex chemical equilibria are involved in homeostasis existing in different compartments of the human body with a key role played by different affinity of metal ions to each of the participating ligands. Disrupted homeostasis of essential elements is usually driven by changes in biological pathways of the involved binding ligands and/or by inadequate element intake; the resulting misbalance has been associated with several human pathologies, including different cancer types, diabetes, Parkinson, Alzheimer, etc. In addition to differences in total element concentrations found in patients as compared to healthy subjects, strong evidence has been gathered revealing alterations of stable isotope ratios for some essential elements as Zn, Ca, Fe, Cu. These observations are in line with the nature of isotope effect; since substitution of one isotope by another in a given molecule involves small change of bond's energy, mass-dependent preferences toward lighter or heavier isotopes would exist in metal-binding bioligands, and isotopes fractionation will certainly be affected by the actual status of ligands existing in disrupted homeostasis as compared to health conditions.

As already mentioned before, for some essential elements changes of their isotopes fractionation has been found in human pathologies; however the great majority of studies were carried out by determining isotope ratios in acid-digested sera, tissues or organs of patients with respect to the control group. Despite high relevance, the obtained results do not enable to clarify molecular mechanisms responsible for the observed changes of isotope ratios. To obtain specific information for a given metal-ligand interactions at isotopes level, theoretical calculations have been carried out, but the actual-state-of-the art support small molecules yet not large biomolecules.

Due to the importance of the topic and scarce experimental data available, the goal of this project is to get a new insight into the isotope fractionation of metal/metalloid ions upon binding to individual biomolecules participating in their homeostasis, setting for this purpose suitable analytical procedures. The expected variations of isotope abundances are very small; to meet the required high precision and sensitivity, multiple collector inductively coupled plasma mass spectrometry (MC-ICP-MS) will be applied. This instrument is characterized by very efficient ionization (ICP), high ions transmission (magnetic sector field analyzer) and simultaneous ion detection (MC); these features combined with sample-standard bracketing yield 0.1−0.5 ‰ precision at relatively low concentration levels. On the other hand, MC-ICP-MS is highly demanding in terms of mass discrimination correction and efficient sample purification to avoid spectral interferences.

The scientific methodology to be applied is proposed as follows: (i) setting adequate conditions for metal/metalloid binding to individual biomolecule in a simple in-vitro system followed by the removal of the excess of “free” element form (size exclusion chromatography); (ii) efficient clean-up of the fraction containing metal/metalloid bound to biomolecule based on the previous experience in coupling ion chromatography to MC-ICP-MS; (iii) optimization of instrument operation conditions for isotopes ratio measurements. It should be stressed that the specific focus will be on isotopes fractionation in specific metal/metalloid – biomolecule interaction and on the precise, interference-free MC-ICP-MS isotope ratios measurements. As to the elements selection, in addition to already studied Cu and Fe, other essential elements will be included: Mg, Mo, Se, Cr. Biomolecules participating in metal/metalloid homeostasis whose standards are commercially available, will be studied. To evaluate possible impact of dietary source on isotopes fractionation observed in human body, analysis of selected food products will be undertaken, using the established analytical methodology.

The results obtained in this project will be helpful in better understanding of metal/metalloid isotopes fractionation occurring in normal homeostasis and of changes caused by homeostasis disruption. Foods elaborated from plants or animal tissues are susceptible to contain different ratios of stable isotopes of metals/metalloids as compared to their natural abundances; analysis of foods is necessary to assess the impact of external sources of essential trace elements on the status of stable isotopes ratios in human body.