Functional characterization of native and cobalt-substituted human globins

Globins are a family of globular metalloproteins that contain a heme *b* as prosthetic group and are composed by 5-8 α-helices. Heme *b* can be found in two main coordination forms in globins: in both cases, one axial coordination position, called "proximal", is occupied by the sidechain of a histidine, whereas the other axial coordination position, called "distal", can be unoccupied or occupied by the sidechain of a second histidine residue. According to the coordination state of the iron atom, globins can perform different physiological functions: "penta-coordinated" globins, like myoglobin, are involved in molecules' transport or catalysis; "hexa-coordinated" globins, like neuroglobin, are mainly involved in electron transfer chains. The physiological role and the biochemical mechanisms of some globins, such as myoglobin and hemoglobin, are well-studied, whereas the physiological functions of other globins, as neuroglobin, is still not fully understood.

Furthermore, the interest in exploiting hydrogen as new energy carrier (thanks to its highest energy density, abundance and production of water upon reaction with oxygen) generated a great interest in Co-based organometallic catalysts, which show a relevant catalytic activity for hydrogen reduction. Unfortunately, these compounds are soluble mostly in organic solvent instead of water, which is the solvent of choice. A possible approach to solve this problem is to develop hydrogen evolving water soluble bioinorganic catalysts modifying oligo- or polypeptide matrices by incorporating "artificial" prosthetic groups, mimicking the behaviour of hydrogenases (class of enzymes catalysing the reduction of protons to molecular hydrogen) and overcoming their application limits (as the inactivation by oxygen). In this context, globins are intriguing due to the possibility of easily obtaining Co-substituted derivatives by replacing the non-covalently bound heme *b* with Co-porphyrins.

My PhD project is split into two main fields of research. The first topic deals with the investigation of iron-heme *b* neuroglobin's mutants to study their chemical properties and reactivity for improving knowledge about the physiological role and reaction mechanism of neuroglobin. To this aim, the production of recombinant neuroglobin's mutants by substituting key amino acids will be required, thereafter the spectroscopic and redox properties of the metal centre and the structural properties of mutants will be investigated. Finally, it would be interesting to examine mutants' reactivity towards exogenous molecule involved in biochemical pathways, like H_2O_2 and other ROS, since neuroglobin seems to perform a protective action in neurons.

The second topic is based on the investigation of cobalt adducts of myoglobin and neuroglobin to verify their possible employment for the actual production of Green H_2. Initially, the effect of the axial metal coordination will be explored by comparing Co-adducts of native and mutated human myoglobin and neuroglobin, whose spectroscopic, redox and electrocatalytic properties will be characterized. Afterwards, first, second and third coordination sphere effects will be exploited to fine-tune the catalytic activity and to clarify molecular details of the catalytic mechanism that are still poorly understood. Two approaches will be used, productions of proteins mutated at selected positions and insertion of cobalt-porphyrins with different ring substituents. Finally, solid electrodes surface-modified with controlled layers of recombinant and mutated Co-globins will be produced to verify their possible employment. Different electrode surfaces and immobilization approaches will be analysed, like direct immobilization on surface and covalent immobilization on electrode surface-modified with organic SAMs.