The catalysis, metal specificity and evolution of superoxide dismutases

Although the vast majority of biomass, which is composed of proteins, lipids (fats) and nucleic acids (DNA and RNA), is made up of just six chemical elements (carbon, hydrogen, nitrogen, oxygen, phosphorus and sulfur), all life on Earth is absolutely dependent on a small subset of essential metals. These metals play crucial roles in biological metabolism by performing key functions in enzyme catalysis, in determining protein structure, and in signalling within and between cells. For example, an iron (Fe) atom is key to the function of haemoglobin, which transports oxygen and carbon dioxide in our blood, and this Fe atom gives blood its distinctive red colour. Fe, as well as other metals such as manganese (Mn), copper and zinc, are essential to all living organisms.

It is estimated that approximately one-third of all proteins made by life on Earth requires an essential metal.. Metalloproteins are usually 'metal-specific', i.e. they only function correctly when they are bound to their correct metal. Yet it is unclear how this metal specificity is controlled by proteins. It is assumed that each protein, which folds into a very specific three-dimensional shape, can precisely control the chemistry of its bound metal, thereby optimising its reactivity to maximise its ability to catalyse the desired chemical reaction. Despite decades of studies that have analysed the biochemistry and molecular structure of metalloproteins, there remain few structural clues as to how this metal specificity is achieved by proteins. Understanding this phenomenon is the central goal of my laboratory's research.

In this NCN Maestro project, we will make fundamental discoveries about how proteins achieve this metal specificity by performing detailed biochemical, biophysical and structural studies of a ubiquitous family of metalloproteins, the superoxide dismutases (SODs). These enzymes detoxify superoxide, a reactive oxygen species that is a natural by-product of aerobic metabolism and is thus experienced by all organisms that live in the presence of oxygen. Superoxide is also used as a weapon by the immune system, and therefore pathogenic bacteria are exposed to very high levels of superoxide during infection. This makes SODs a potential target for the development for future antibacterial therapeutics.

In our prior studies, my laboratory has made important discoveries about the structure, function and evolution of SODs. We identified a pair of SODs from one bacterium, *Staphylococcus aureus*, which we have developed into a unique model system to facilitate these studies. These enzymes are very closely related, yet remarkably, they show very different metal specificities; one SOD is absolutely dependent on Mn (called MnSOD), and shows negligible catalytic activity when loaded with Fe, whereas the other staphylococcal enzyme is 'cambialistic', which means it shows equal catalytic activity with either Mn or Fe (called camSOD).

Here, we will exploit this model SOD system, as well as our library of diverse SODs from a range of organisms from bacteria to humans, and our existing robust experimental methods, to further our understanding of how SODs function and how their metal specificity is determined. We will (i) determine the structure of these SODs at unparalleled resolution, including localising essential protons in their active sites, and use these studies to determine the catalytic mechanism; (ii) study how the protein architecture regulates catalysis by precisely controlling the metal's reactivity, resulting in the enzyme's metal specificity; and (iii) study how the properties of SOD enzymes, including their metal specificity, has evolved over the vast period of time in which they have diversified across the evolutionary tree, including performing direct experimental tests of their evolution.

This study will develop our fundamental understanding of how SODs function. Our data will develop models of metalloprotein function that will be applicable to the vast numbers of metalloproteins observed in nature. Our results will also influence the emerging field of synthetic biology, where researchers aim to produce synthetic metalloproteins that perform novel chemical reactions for biotechnological applications such as green chemistry or the biodegradation and decontamination of dangerous long-lived chemicals such as plastics. Furthermore, because SODs play an important role in the immune defence against bacterial pathogens, these studies will also support future drug discovery efforts to develop new antibacterial compounds.