Preface to the Third Edition

I began writing an introduction to the role of metal ions in biology in 2006–07, and here we are, a decade later. The modest 369 pages of that first Introduction, has grown to the 460 pages of the Second Edition published in 2012, and to the more than 650 pages of this present Third Edition (2018), reflecting the growth of this exciting field.

As I pointed out in the Preface to that First Edition, the predominant motivation to write the book came from my lifelong preoccupation with metalloproteins, beginning with cytochrome c in Glasgow, insect haemoglobins in Munich, and proteins of iron storage and transport in Glasgow, Berlin and Louvain-la-Neuve. Iron has always been my major centre of interest; however, my fascination with the role of metals in biology in general has grown out of attending and above all organizing scientific meetings in this exciting field.

In 1983 the first of the series of ICBICs (International Conferences on Bioinorganic Chemistry, today known as International Conferences on Biological Inorganic Chemistry[1]) initiated by Ivano Bertini, Harry Gray, Bo Malmstrom and Helmut Sigel, took place in Florence organized by Ivano Bertini. Coincidentally, also in 1983, the FEBS (Federation of European Biochemical Societies) meeting was held in Brussels, and as a member of the organizing committee, I coorganized two half-day sessions on metalloproteins – the first time that metals had figured as such at a FEBS meeting. At the end of the FEBS meeting, a lively discussion took place over a few beers in the bar of the Sheraton Hotel along with a number of colleagues, including Antonio Xavier, Barry Smith and Cees Veeger among others. The outcome was that, in view of the rapidly growing interest in the role of metals in biology, we should apply to FEBS for support to organize an Advanced Course on Metals in Biology, including both lectures and hands-on practicals on the diverse techniques, which could be used to study metal ions. Cees Veeger and myself were designated as coorganizers and the first course was held in April 1985 in Louvain-la-Neuve with around 30 students. Since then, subsequently with Ricardo Louro as my coorganizer, to date 24 have been organized, training over 1300 students (doctoral and postdoctoral) from all over Europe (and sometimes even further), many of whom are still active in the BIC field. Over the years the opening lecture on metals in biology has been given by Antonio Xavier, Bob Williams, Jan Reedijk, Helmut Sigel, and for the last innumerable courses by myself.

In January 1991 the European Science Foundation (ESF) launched a well-funded Scientific Programme on 'Chemistry of Metals in Biological Systems', which ran from 1991 to 1997. I joined the Steering Committee in 1992, which was made up of Helmut Sigel (Basle, Switzerland) as Chair, Ivano Bertini (Florence, Italy); Bob Crichton (Louvain-la-Neuve, Belgium), Sture Forsen (Lund, Sweden), Dave Garner (Manchester, UK), Carlos Gomez-Moreno (Zaragoza, Spain), Paco Gonzales-Vilchez (Seville, Spain), Imre Sovago (Debrecen, Hungary), Alfred Trautwein, Lübeck, Germany), Jens Ulstrup (Lyngby, Denmark), Cees Veeger (Wageningen, Holland), Raymond Weiss (Strasbourg, France) and Antonio Xavier (Oeiras, Portugal). In the course of the next few years it

[1]This represents a compromise between the chemists 'Bioinorganic Chemistry, and the biochemists 'Inorganic Biochemistry', rather like the name of the research institute to which I finally belonged in the former Chemistry Department at UCL. The fusion of chemists and chemical engineers into a single entity, resulted in a name which satisfied neither – the Institute of Condensed Material and Nanosciences!

supported a number of activities including Conferences[2], Meetings and Workshops, as well as supporting the Advanced Courses, which were still funded by FEBS. However, from the outset there was a growing feeling within the committee, notably represented by Antonio Xavier, that we needed to do something to reinforce the growing importance of BIC within Europe. This attained its climax at the European Research Conference in San Miniato from 22nd to 28th April, 1995 (once again organized by Ivano Bertini). At this meeting, 'the Extended Steering Committee of the ESF, with the approval of a large part of the participants, founded a new Society, the Bioinorganic Chemistry Society, (in reality the Society for Biological Inorganic Chemistry, SBIC), with an associated Journal, the Journal of Biological Inorganic Chemistry (now JBIC)'. This was a monumental event, and over the next year, the statutes of SBIC were established by Dave Garner, Antonio Xavier and Jens Ulstrup, and Ivano became the first editor of JBIC. The launch of SBIC and JBIC was announced at the seventh ICBIC in Lubeck in September 1995 (organized by Alfred Trautwein) – I still have the T-shirt, as I did for the first EuroBIC meeting in Newcastle-on-Tyne, organized by Geoff Sykes the following year (incidentally, both still fit – they were, I should insist, both L, not XL).

The changes in the constantly evolving panoramic view of BIC, which I have highlighted in this Third Edition have been considerable since the Second Edition appeared in 2012, and by way of illustration I have selected a few examples as a sort of literary 'amuse geule[3]'.

Bromine is ubiquitously present in animals (about 200 mg as Br^- in humans), but until quite recently, it had no known essential function. However, it has been demonstrated (McCall et al., 2014) that Br^-, following its conversion to hypobromous acid, forms a bromosulfonium ion intermediate, which is a required cofactor for peroxidasin catalysed formation of sulfilimine crosslinks, a posttranslational modification essential for tissue development and architecture found within the collagen IV scaffold of basement membranes (BMs). This is a critical event for BM assembly and tissue development. Dietary Br deficiency is lethal in *Drosophila*, whereas Br replenishment restores viability. Thus bromine is an essential trace element for animals.

In the previous editions we have discussed the fulgurant developments in genome sequencing, complementing proteomics. Using third-generation DNA sequencing, it is now possible to completely sequence a bacterial genome in a few hours, and as a consequence, the number of published genomes continues to rise at an essentially exponential rate. Since the first bacterial genomes were published in 1995, by early 2015, there were more than 30,000 sequenced bacterial genomes publicly available. A similar fulgurant progress has been evident in the determination of fungal, plant and animal genome sequences. According to the NCBI if we include eukaryotes and prokaryotes, viruses, plasmids and organelles, genomic information is available on some 27,000 organisms.

[2]These included ICBIC 5 and 7 (Oxford, 1991; Lubeck, 1995), EUROBICs 1, 2, 3 and 4 (Newcastle upon Tyne, 1992; Florence, 1994; Noordwijkerhout, 1996 and Seville, 1998), as well as European Research Conferences, including that in San Miniato in 1995.

[3]The French expression for appetizers served before a meal, best translated as 'something to titillate the palate', or more specifically to stimulate our taste buds in anticipation for what is to come afterwards. My apologies, but sometimes French gets the essence of a concept more amusingly than English – another elegant example is the early spring flower, the snow drop, in French 'perce neige', literally meaning that they push through the snow cover, as they indeed do to remind us that Spring is just around the corner.

The number of -omics also continues to increase, including of course metallomics, reflecting the metallome. This was defined by R.J.P (Bob) Williams[4] (Hill and Sadler, 2016), who also formulated the Irving-Williams series (Irving and Williams, 1948) and the concept of the entatic state (Vallee and Williams, 1968), as follows:

> *The selection of the chemical elements by a particular cell from the environment involves a series of steps, the complexity of which depends upon the organism. The variety of paths which individual elements follow in any organism adds to the specific character of the organism. Clearly the paths have evolved to create an element distribution which we shall call* the metallome, *to parallel the nomenclature of protein distribution, the proteome.*
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> **Williams (2001).**

The *metallome* can therefore be loosely defined as the ensemble of all the molecules in a defined biological system, which bind a given metal ion. Metallomics, the study of the metallome, has clearly come of age, illustrated by the Royal Society of Chemistry journal with same name, and a series of international Metallomics Conferences, the first of which was held in 2007, and the latest held in Vienna 2017 at which I gave the opening lecture, as I had done at the third conference in Munster.

The development of targeted genome editing systems and their applications has moved forward enormously in the last decade. However, in the last 5 years it has undergone a quantum leap with the introduction of the clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 (Cas9) system (CRISPR/Cas9), the bacterial immune system, which can be used to edit genomes. It was serendipitously discovered that bacteria contained DNA sequences which were repeated, and interspersed with unique sequences known as Crispr (Clustered Regularly Interspaced Short Palindromic Repeats), and these unique sequences were identified as viral DNA, derived from viruses that had previously infected the bacteria. It was then found that close to the Crispr sequences, genes were located coding for Crisp-associated proteins (Cas), which have nuclease activity. Together with small guide RNAs (crRNAs) that have been transcribed from the Crispr locus, one or more Cas proteins form ribonucleoprotein targeting complexes, which each contain a single guide sequence. The Cas nuclease (usually Cas9) then cleaves the target DNA, marked for degradation by base pairing with the crRNA. Then, in 2012, Emmanuelle Charpentier and Jennifer Doudna proposed that CRISPR/Cas9 could be used for programmable gene editing (Jinek et al., 2012), an idea that has since been further developed by many research groups for potential applications ranging from creating smart model systems for fundamental protein research to enabling bioengineers to modify crops and farm animals, and translational scientists to develop novel treatment approaches for inherited and acquired disorders for which curative treatment options are not yet available (see Mussolino et al., 2017 for a recent review).

[4]Bob Williams, a pioneering figure in the BIC world (for Bob it was definitely BioInorganic Chemistry), probably contributed more ideas and hypotheses to the field than anyone else, including the Irving-Williams series on the stability of metal complexes, carried out while Bob was an undergraduate at Merton College, Oxford, and the concept of the 'entatic' state, derived from the observation that the biological activity of metals within metalloproteins was due to their distorted state.

This progress in molecular biology has been matched by an equally impressive development in structural biology, involving X-ray crystallography, NMR, electron microscopy or hybrid approaches. Among the hybrid approaches we might note the importance of combining X-ray crystallography with spectroscopic methods, when the identity of ligands cannot be determined from the crystallographic data (the electron density of C, N and O cannot easily be differentiated by X-ray crystallography). We already cited the importance of spectroscopy in identifying the three nonprotein diatomic ligands to the Fe in [Ni–Fe] hydrogenase as one CO and two CN^- molecules in the previous edition, and since then the interstitial atom X in the central cavity of the FeMo Cofactor of nitrogenise has been unequivocally identified through combined spectroscopic and structural analysis to be a μ_6-coordinated carbide (C^{4-}) ion (Spatzal et al., 2011; Lancaster et al., 2011).

Whereas in November 2012 there were around 86,000 structures in the Protein Data Bank (PDB), today there are around 134,000, of which 120,000 have been determined by X-ray crystallography. This is in no small measure the result of developments in molecular biology enabling proteins to be produced in sufficient quantities for structural studies, advances in computational techniques, and particularly access to more powerful and tunable X-ray beams such as synchrotrons and, since its inception in 2009 the application of free-electron X-ray lasers (XFELs). Since the development of the XFEL its impact on structure and dynamics in biology has been considerable. XFELs are the most powerful X-ray sources ever built, with peak brightness nine orders of magnitude greater than the beams of third-generation synchrotrons, allowing extremely short (femtoseconds – 10^{-15} s) pulses, which overcome the limitations of radiation damage (Spence, 2017). Using X-ray pulses of 40 fs in duration produces an X-ray diffraction (XRD) pattern before the onset of radiation damage, which subsequently destroys the sample. Since each sample is destroyed by a pulse, a continuously refreshed supply of hydrated microcrystals is therefore needed. XFEL creates new opportunities for crystallography and imaging at atomic resolution on timescales from femtoseconds to seconds, allowing time-resolved diffraction at room temperature, while avoiding most of the effects of radiation damage. In addition, it allows the study of submicrometre crystals that are too small for conventional crystallography (Spence, 2017).

In what follows we will discuss a few of the new metalloprotein structures, which are described in detail in the corresponding chapters. They include a growing number of large membrane proteins. As we illustrate in Chapter 5, An Overview of Intermediary Metabolism and Bioenergetics, the structures of all five membrane bound complexes of the mitochondrial electron transport chain have been determined in atomic detail. Very recently the atomic structure of the entire mammalian Complex I has been solved by cryoelectron microscopy at 3.9 Å resolution. This Complex I has a molecular mass of970 kDa, and in the structure, all 14 conserved core subunits and 31 mitochondria-specific supernumerary subunits are resolved within the L-shaped molecule (Fiedorczuk et al., 2017).

Although the structures of homotetrameric voltage-gated K^- channels have been available for over two decades, structure determination of eukaryotic Na_v and Ca_v channels has proved recalcitrant. The structures of several bacterial Na_v channels, tetramers like their eukaryotic equivalent, but made up of four identical subunits, have now been solved, and the overall topography is similar to the K_v channels. However, in contrast to the dehydrated K^+ ions found in K^+ channels, ion conduction in Na^+ channels appears to occur through a mechanism, which passes hydrated Na^+ ions in preference to hydrated K^- or Ca^{2-} ions. The reasons for this difference remain a mystery.

One of the most exciting challenges in structural biology has been the determination of the structure of photosystem II (PSII), the 700 kDa homodimeric membrane protein complex that catalyses photo-oxidation of water into dioxygen through an S-state cycle of the oxygen evolving complex (OEC). The structure of PSII had been solved by XRD at 1.9 Å resolution, demonstrating that the OEC is a Mn_4CaO_5-cluster coordinated by a well-defined protein environment (Umena et al., 2011). However, extended X-ray absorption fine structure (EXAFS) studies showed that the manganese cations in the OEC are easily reduced by X-ray irradiation, and slight differences were found in the Mn−Mn distances determined by XRD, EXAFS and theoretical studies. Using femtosecond X-ray pulses and hundreds of large highly isomorphous crystals of a thermophile PSII, a 'radiation-damage-free' structure at a resolution of 1.95 Å has been obtained, and its implications for the function of the OEC are described in Chapter 16, Manganese – Oxygen Generation and Detoxification.

A new Ni-cofactor has emerged in the study of the enzyme lactate racemase in lactic acid bacteria, in the form of a (SCS)Ni(II) pincer complex derived from nicotinic acid (Desguin et al., 2016), and while the mechanism of nitrogenase draws ever near, but is not quite there, we can now answer the question 'How many metal does it take to fix N_2?' – 20. Why, over a billion years of evolution has retained this complex system for nitrogen fixation, requiring homocitrate and at least 20 additional proteins, remains unanswered – perhaps as Brian Hartley used to say 'the problem with explaining evolution is that we were not there when it happened'.

The importance of metals in biology, the environment and medicine has become increasingly clear over the last 30 years or so. From electron transfer pathways in photosynthesis and respiration, to water splitting which produces oxygen, to metals like cadmium, manganese and lead which represent a health hazard to metal-based drugs and paramagnetic metal complexes as contrast agents for magnetic resonance imaging, metals pervade our everyday lives. Some 30 or more percent of enzymes require metal ions, and without them, there would be no osmotic regulation, no neurotransmission, cell signalling, fertilization or cell death. This is what I hope I have transmitted to the readers of this book together with the passion, enthusiasm and wonder at the multiple things that metals can do, and I can only hope that they enjoy reading this edition as much as I enjoyed writing it. Among other changes there are short abstracts to each chapter and the list of references at the end of each chapter has been considerably expanded, and I hope that this will be helpful to the reader.

To my many colleagues and friends who, unwittingly or not, have contributed to this text, my sincere thanks, in particular to Ricardo Louro for his critical review of Chapter 2, Basic Coordination Chemistry for Biologists, and to Roberta Ward for her help with the figures. I remain responsible for errors and mistakes that have been perpetrated, and apologize to colleagues whose work has not been cited. As my colleague Ivor Cavill remarked to me a number of years ago 'Bob, 20 years ago we didn't know much, but we sure understood it' – we are now experiencing the opposite situation, confronted by oceans of information, but with less and less understanding of how it all fits together, creating greater difficulties in arriving at a consensus opinion, and thereby making overviews such as this increasingly difficult to achieve.

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