Preface

Metals are involved in most segments of the chemistry of life, including respiration, numerous steps of metabolism, photosynthesis, nitrogen fixation, nerve transmission, signal transduction, muscle contraction, oxygen transport and protection from xenobiotic compounds. In addition, metals are used in medicine as therapeutic agents. This volume presents chapters that describe several of these functions of metals in biology.

Although the protein structures of enzymes can provide a wide range of catalytic chemistry, many biological reactions would be inefficient or blocked if the residues of the 20 or so amino acids that are used were the only catalytic tools. For example, reactions involving oxygen are almost non-existent with most amino acids, and electron-transfer reactions would be very inefficient. Nature has thus augmented its catalytic repertoire by incorporating a considerable variety of additional tools. The vitamin cofactors, such as flavins (B_2), niacin (B_1) and cobalamin (B_{12}), carbohydrates and metals all contribute to providing a rich diversity of chemistry required for efficient biological function. Metals allow proteins to react with oxygen, carry out radical reactions and facilitate rapid electron transfer, and they are often excellent in promoting catalysis. In addition, metals such as zinc, calcium and magnesium are crucial to these roles for the maintenance of structures of proteins and nucleic acids. A side benefit of metals is that they serve as spectroscopic handles for helping the researcher monitor various chemical processes. This has led to a multitude of techniques being applicable to the study of metalloproteins in addition to those commonly used for other proteins. These techniques include UV-visible, EPR, electron-nuclear double resonance (ENDOR), NMR, Mössbauer, extended X-ray absorption fine structure (EXAFS), infrared and Raman spectroscopies, magnetic susceptibility and redox measurements, and X-ray crystallography. Most of these techniques will be mentioned in the chapters of this volume.

This volume attempts to show many of the above roles of metals in a variety of circumstances that are used widely in biology. An objective was to present each of the topics from the viewpoint of chemistry. The first two chapters describe processes that bacteria use to rid themselves of toxic metals by either actively pumping them out of the cell or converting them, e.g. mercury, into unharmful forms that can be excreted. Barry Rosen and Susan Miller lucidly present some strategies of how protein side chains can be used to bring about gentle and efficient chemistry that effects detoxification. Chapters 3–6, 8, 10 and 11 are largely devoted to metalloproteins that react with or transport oxy-

gen. Eric Coulter and David Ballou describe a range of non-haem iron-containing oxygenases that are prevalent in the microbial world and which participate in the biodegradation of aromatic compounds. A theme that emerges is that the iron-based active sites in these types of enzymes employ co-ordination environments that exchange ligands during catalysis to permit the various kinds of chemistry to occur. Chapter 4, by Issa Isaac and John Dawson, describes haem iron-containing peroxidases. This chapter covers some of the history of how the mechanisms of peroxidase chemistry were elucidated. A concerted attempt to describe the various oxidation states of the haem prosthetic group provides a solid foundation for understanding the various modes of how iron participates in oxidase and oxygenative chemistry. This chapter serves as an introduction to the 'language' of iron–oxo species, which has been valuable as a framework for thinking about these complex systems. Chapter 5 on cytochromes P450 by Stephen Sligar briefly covers the history of this rich field and captures the spirit of the multiple facets of research on these systems as it builds on the information in Chapter 4. Donald Kurtz describes three very different chemical approaches that have been applied to the transport of oxygen in biological systems. He presents a firm basis of the chemistry and how the structural motifs of proteins are used to bind, but not react irreversibly with, such a strong oxidant as O_2. Grant Mauk (Chapter 6) shows how metalloproteins are so important in carrying out biological electron-transfer reactions. He summarizes theoretical principles in terms that chemists not specializing in physical chemistry can understand, and describes experimental approaches to studying this exciting field. Russ Hille's presentation on molybdenum-containing enzymes (Chapter 8) focuses on oxidative chemistry. He shows how the multiple oxidation states of this oft-neglected metal are well suited for many processes in biology. Neil Marsh writes about how the unique properties of cobalamins promote the formation of metal–carbon bonds whose scission often leads to radical chemistry. This permits a variety of curious carbon-skeleton rearrangements that are vital to metabolism. James Whittaker's chapter on oxygen reactions of the copper oxidases first deals with the unique chemistry of oxygen. His chapter serves well as an introduction to the chemical properties of oxygen. In addition to the mechanisms of catalysis, in several cases, copper enzymes transform themselves by modifying certain residues. This process gives the enzymes a wider range of properties than is provided by the original amino acids. Joan Broderick shows how catechol dioxygenases, which are central to the aerobic biodegradation of most aromatic compounds, catalyse with specificity the cleavage of aromatic rings. The nature of the iron ligation determines what type of cleavage is carried out. Finally, Elizabeth Trimmer and John Essigmann tell us about the chemistry of cisplatin, a metal-containing drug that has been used successfully for the treatment of certain types of cancer. The chemistry of how this drug interacts with

DNA to disrupt the machinery of rapidly dividing cells and the processes whereby cells become resistant to these drugs gives us pause to think about the pharmacological difficulties of dealing with this terrible disease.

This volume is intended mainly for chemistry or biochemistry students in their third or fourth years of undergraduate degrees, or for those beginning postgraduate studies. In addition, many of the articles may be of value to chemists or biochemists who specialize in other fields, and who want to learn what is happening in contemporary bioinorganic chemistry research. The 12 chapters of this volume are written by several of the best-known experts in their specialties. They have tried to present their subjects with a view to teaching principles of chemistry and showing methods of how to develop an understanding of the functions of metals, outlining the roles of metals in a variety of biological functions and presenting perspectives on the future of research in bioinorganic chemistry. No attempt was made to be exhaustive in the coverage of these topics. Only references that appeared crucial to understanding the articles are included, and references to more extensive reviews are given so that readers can go into more depth as desired. The field of bioinorganic chemistry has grown exponentially in the past 20 years, so that only a fraction of this field has been presented here. I would refer the reader to *Principles of Bioinorganic Chemistry* by S.J. Lippard and J.M. Berg (1994), University Science Books, Mill Valley, CA, or to *Chemical Review* (1996), volume **96** (a thematic issue on bioinorganic enzymology), for anyone who wishes to explore these areas further. I hope that the readers of these chapters will begin to feel the excitement of these areas of research and thereby be encouraged to explore them further, and even participate in future research efforts. I am sure that all of the authors will be happy to reply to any questions about their research areas or to receive inquiries about possible research positions.

David P. Ballou
Michigan, 1999

Authors

Barry P. Rosen received his B.Sc. degree from Trinity College, Hartford, CT, U.S.A., in 1965, and his M.Sc. and Ph.D. in Biochemistry from the University of Connecticut in 1968 and 1969 respectively. He was a Public Health Service Postdoctoral Fellow at Cornell University from 1969 to 1971. He became a faculty member in the Department of Biological Chemistry at the University of Maryland School of Medicine in 1972. In 1987 he assumed his current position of Professor and Chairman of the Department of Biochemistry and Molecular Biology at Wayne State University School of Medicine.

Susan M. Miller is currently Assistant Professor of Pharmaceutical Chemistry at the University of California, San Francisco. She received her B.Sc. in Chemistry from the University of Missouri, Columbia, her Ph.D. in Organic Chemistry/Enzymology from the University of California, Berkeley, and postdoctoral training in enzymology at the University of Michigan, Ann Arbor. Her research interests are focused on the study of mechanisms of enzyme catalysis with an interest in both the chemical mechanisms of transformations and how protein-structural features contribute to catalysis. Mechanistic studies of mercuric ion reductase, as well as its interaction with other proteins in the mercury-detoxification pathway, continue to be a significant focus of the laboratory. New areas of interest in the laboratory include the physiological function of a redox-active oestrogen-binding protein from *Candida albicans*, and the mechanism of action of orotate monophosphate decarboxylase, a key enzyme in the uridine biosynthesis pathway.

Eric Coulter is a native of Northern Ireland, where he obtained a B.Sc. in Biological Chemistry from the University of Coleraine in 1991. In 1996, he received a Ph.D. in Chemistry under the direction of Professor John Dawson at the University of South Carolina. Following postdoctoral studies with Professor David Ballou at the University of Michigan, he is currently a postdoctoral associate with Professor Donald Kurtz at the University of Georgia. His current research interests include the mechanism of iron regulation by bacterioferritins and structure-and-function relationships in several mono- and dinuclear non-haem iron proteins.

David P. Ballou received his B.Sc. in Chemistry with a minor in Music from Antioch College in 1965. He did his M.Sc. (1967) and Ph.D. (1971) in Biological Chemistry at the University of Michigan under Graham Palmer. After combined postdoctoral study with M.J. Coon and Vincent Massey, he became an instructor, and is now Professor of Biological Chemistry at Michigan. His research interests are in biological redox systems, especially

oxygenations involving flavins and/or metals. His specialities are in physical biochemistry, with an emphasis on spectroscopy and kinetic characterization of intermediates. His other interests include road and mountain biking, downhill and cross-country skiing, music, dancing, sailing and tennis (and most sports).

Issa Isaac was born in Kuwait and his family resides in Palestine. He did his undergraduate studies at Hesston Junior College in Hesston, Kansas, and at Goshen College in Goshen, Indiana, where he obtained a B.A. degree. He then attended Southern Illinois University at Edwardsville and received an M.S. degree in Chemistry. He is a candidate for the Ph.D. degree in Chemistry at the University of South Carolina in Columbia, where he has done his research in the laboratory of Professor John H. Dawson. A significant part of his Ph.D. research has involved a collaborative project that he carried out with Professor David Ballou at the Department of Biological Chemistry at the University of Michigan Medical School in Ann Arbor.

John H. Dawson received an A.B. degree with a major in Chemistry from the University of Columbia and a Ph.D. degree in Chemistry with a minor in Biochemistry from the University of Stanford. He was a National Institutes of Health (NIH) Postdoctoral Fellow in Chemistry at the California Institute of Technology. In 1978, he joined the Department of Chemistry and Biochemistry at the University of South Carolina where he is now Carolina Distinguished Professor with a joint appointment in the School of Medicine. Professor Dawson has been a Camille and Henry Dreyfus Teacher/Scholar, an NIH Research Career Development Awardee and an Alfred P. Sloan Research Fellow. He has been named Outstanding South Carolina Chemist by the SC Section of the American Chemical Society, has received the Russell Award for Research Excellence in Science from the University of South Carolina, and has been elected a Fellow of the American Association for the Advancement of Science. He has recently received the Basic Science Faculty Research Award from the University School of Medicine and has received the Governor's Award for Excellence in Science Discovery from the SC Academy of Science. He was Chair of the Organizing Committee for the Tenth International Conference on Cytochrome P450: Biochemistry, Biophysics and Molecular Biology and is Editor-in-Chief of the *Journal of Inorganic Biochemistry*. Professor Dawson's research interests focus on the structure and function of haem iron oxygenase and peroxidase enzymes and on the application of magnetic circular dichroism to the study of haem proteins. His research is supported by grants from the NIH and NSF.

Stephen G. Sligar received his Ph.D. in Physics from the University of Illinois in 1975. He was an Assistant Professor at Yale University and is now a Professor in the Departments of Chemistry and Biochemistry at the University of Illinois at Urbana-Champaign and a part-time Beckman Institute faculty member in the Advanced Chemical Systems Group. His research is in

the area of molecular recognition and the fundamental principles of protein–protein, protein–nucleic-acid and protein–small-molecule interactions using a combination of site-directed mutagenesis, computer modelling and structure determination. His work extends to biomolecular electronics centres, and the synthesis and physical characterization of highly ordered protein superlattices and how they might be useful as building blocks for optically coupled sensors, storage elements and processors. He uses a combination of site-directed mutagenesis, thin-film generation, optical and vibrational polarized spectroscopy, bio-organic chemistry and semi-conductor technology in these endeavours.

Donald M. Kurtz, Jr. received his B.Sc. in Chemistry at the University of Akron in 1972 and his Ph.D. in Physical Biochemistry from Northwestern University under Professor Irving M. Klotz in 1977. After postdoctoral research in the laboratory of Professor Richard H. Holm at Stanford University, he joined the faculty in the Department of Chemistry at Iowa State University in 1979. In 1986 he moved to the University of Georgia where he is currently Professor of Chemistry, Biochemistry and Molecular Biology. His research interests involve the chemistry, enzymology and molecular biology of non-haem iron proteins and enzymes, especially those that interact with oxygen.

Grant Mauk is a Professor of Biochemistry at the University of British Columbia. He obtained his undergraduate degree in Chemistry at Lawrence University in Wisconsin and then completed the M.D.–Ph.D. (Biochemistry) programme at the Medical College of Wisconsin. Following postdoctoral research at the California Institute of Technology, he joined the faculty at the University of British Columbia. His research group studies the kinetic, thermodynamic and spectroscopic properties of genetically modified electron-transfer proteins and the mechanisms by which such proteins form electrostatically stabilized binary complexes.

Russ Hille was born in Tyler, Texas. He did his undergraduate work at Texas Tech University (in Chemistry) and Ph.D. work at Rice University (in Biochemistry, with Dr. John S. Olson). After postdoctoral work at the University of Michigan (in the Department of Biological Chemistry with Dr. Vincent Massey), he joined the faculty of the Department of Medical Biochemistry at the Ohio State University, where he currently holds the position of Professor. His major research interests have been in the application of physical biochemical approaches to studying complex biological redox systems, including molybdenum-containing enzymes and iron–sulphur flavoenzymes.

Neil Marsh received his undergraduate education at Cambridge University and subsequently obtained a Ph.D. in Biochemistry, also from Cambridge. He spent 2 years as a Postdoctoral Fellow in the Chemistry Department at the Johns Hopkins University, Baltimore, before returning as a senior research scientist to the Department of Biochemistry at Cambridge,

where he held a Royal Society University Research Fellowship. Since 1995 he has been a member of the faculty in the Department of Chemistry at the University of Michigan.

James Whittaker is an Associate Professor of Biochemistry and Molecular Biology at the Oregon Graduate Institute of Science and Technology in Portland, Oregon. He graduated from the University of Minnesota (Ph.D. in Biochemistry) and was an NIH Postdoctoral Fellow at Stanford University (in the Department of Chemistry). He joined the Chemistry faculty of Carnegie Mellon University in 1986 and moved to Oregon in 1996. His research interests include the electronic structures and dynamics of metalloenzyme active sites and the application of spectroscopic and computational approaches to biomolecular structures.

Joan B. Broderick received a B.Sc. in Chemistry from Washington State University while doing research in both Inorganic Chemistry (with Roger Willett) and Biochemistry (with Tom Okita). She received an N.S.F. predoctoral fellowship to pursue graduate studies in Inorganic Chemistry at Northwestern University (she gained her Ph.D. in 1992). At Northwestern she worked under the direction of Thomas O'Halloran studying spectroscopic and mechanistic aspects of chlorocatechol dioxygenase. She then moved to the Massachusetts Institute of Technology (MIT) as an American Cancer Society Postdoctoral Fellow in the laboratory of Joanne Stubbe, where she investigated the mechanism of the adenosylcobalamin-dependent ribonucleotide reductase. Dr. Broderick spent 5 years on the faculty at Amherst College before moving to Michigan State University in the summer of 1998. Her current research focuses on understanding the role of metal centres, particularly iron–sulphur clusters, in the initiation of radical chemistry.

Elizabeth Trimmer graduated with a B.Sc. degree in Chemistry from Carleton College (Northfield, MN, U.S.A.) in 1988 and obtained her Ph.D. in Biological Chemistry from MIT in 1997. Her doctoral research focused on the interactions of cellular proteins with DNA adducts of the anti-cancer drug cisplatin. She is currently a Postdoctoral Research Fellow in the laboratory of Dr. Rowena Matthews in the Biophysics Research Division and Department of Biological Chemistry at the University of Michigan, Ann Arbor, where she is investigating the mechanism of the flavoenzyme, methylenetetrahydrofolate reductase.

John Essigmann is Professor of Chemistry and Toxicology at MIT. He received his B.Sc. in Biology in 1970 from Northeastern University (Boston, MA, U.S.A.) and S.M. and Ph.D. degrees in Toxicology from MIT in 1972 and 1976, respectively. Following postdoctoral work at MIT, he took a faculty position at the same institution in 1981. His research interests centre on the responses of cells to DNA-damaging agents.

Abbreviations

AdoCbl	adenosylcobalamin
BphC	2,3-dihydroxybiphenyl 1,2-dioxygenase
CCD	chlorocatechol dioxygenase
CCP	cytochrome *c* peroxidase
CCP-ES	original name for compound I of CCP
CPO	chloroperoxidase
CPO-I	CPO compound I
CTD	catechol 1,2-dioxygenase
DDP	diamminedichloroplatinum(II)
ENDOR	electron-nuclear double resonance spectroscopy
ES	enzyme–substrate
EXAFS	extended X-ray absorption fine structure spectroscopy
Hb	haemoglobin
Hcy	haemocyanin
HMG domain	high-mobility group domain
HOMO	highest-occupied molecular orbital
Hr	haemerythrin
HRP	horseradish peroxidase
HRP-I, -II, -III	HRP compounds I, II and III, respectively
LUMO	lowest-unoccupied molecular orbital
Mb	myoglobin
MeCbl	methylcobalamin
Me-H_4-folate	methyltetrahydrofolate
Me-H_4-MPT	methyltetrahydromethanopterin
MFP	membrane fusion protein
MMO	methane mono-oxygenase
myoHr	myohaemrythrin
NDO	naphthalene 1,2-dioxygenase
OMF	outer-membrane factor
PCD	protocatechuate 3,4-dioxygenase
PDO	phthalate dioxygenase
PDR	phthalate dioxygenase reductase
ppt	pyranopterin
RNAP	RNA polymerase
SV40	simian virus 40
TPQ	topaquinone
XP	xeroderma pigmentosum