Each generation has its unique needs and aspirations. When Charles Wiley first opened his small printing shop in lower Manhattan in 1807, it was a generation of boundless potential searching for an identity. And we were there, helping to define a new American literary tradition. Over half a century later, in the midst of the Second Industrial Revolution, it was a generation focused on building the future. Once again, we were there, supplying the critical scientific, technical, and engineering knowledge that helped frame the world. Throughout the 20th Century, and into the new millennium, nations began to reach out beyond their own borders and a new international community was born. Wiley was there, expanding its operations around the world to enable a global exchange of ideas, opinions, and know-how.

For 200 years, Wiley has been an integral part of each generation’s journey, enabling the flow of information and understanding necessary to meet their needs and fulfill their aspirations. Today, bold new technologies are changing the way we live and learn. Wiley will be there, providing you the must-have knowledge you need to imagine new worlds, new possibilities, and new opportunities.

Generations come and go, but you can always count on Wiley to provide you the knowledge you need, when and where you need it!

William J. Pesce
President and Chief Executive Officer

Peter Booth Wiley
Chairman of the Board
To my young friends
Allie, Andy, Anna, and Sebastian
CONTENTS

Preface xiii
Acknowledgments xix

1 Inorganic Chemistry Essentials 1
   1.1 Introduction, 1
   1.2 Essential Chemical Elements, 1
   1.3 Metals in Biological Systems: A Survey, 3
   1.4 Inorganic Chemistry Basics, 6
   1.5 Biological Metal Ion Complexation, 8
      1.5.1 Thermodynamics, 8
      1.5.2 Kinetics, 9
   1.6 Electronic and Geometric Structures of Metals in Biological Systems, 13
   1.7 Bioorganometallic Chemistry, 19
   1.8 Electron Transfer, 22
   1.9 Conclusions, 26
       References, 27

2 Biochemistry Fundamentals 29
   2.1 Introduction, 29
   2.2 Proteins, 30
      2.2.1 Amino Acid Building Blocks, 30
      2.2.2 Protein Structure, 33
2.2.3 Protein Sequencing and Proteomics, 39
2.2.4 Protein Function, Enzymes, and Enzyme Kinetics, 43

2.3 Nucleic Acids, 47
2.3.1 DNA and RNA Building Blocks, 47
2.3.2 DNA and RNA Molecular Structures, 47
2.3.3 Transmission of Genetic Information, 53
2.3.4 Genetic Mutations and Site-Directed Mutagenesis, 56
2.3.5 Genes and Cloning, 58
2.3.6 Genomics and the Human Genome, 61

2.4 Zinc-Finger Proteins, 63
2.4.1 Descriptive Examples, 67

2.5 Summary and Conclusions, 73
References, 74

3 Instrumental Methods 76

3.1 Introduction, 76
3.1.1 Analytical Instrument-Based Methods, 76
3.1.2 Spectroscopy, 77

3.2 X-Ray Absorption Spectroscopy (XAS) and Extended X-Ray Absorption Fine Structure (EXAFS), 78
3.2.1 Theoretical Aspects and Hardware, 78
3.2.2 Descriptive Examples, 81

3.3 X-Ray Crystallography, 83
3.3.1 Introduction, 83
3.3.2 Crystallization and Crystal Habits, 84
3.3.3 Theory and Hardware, 88
3.3.4 Descriptive Examples, 95

3.4 Nuclear Magnetic Resonance, 98
3.4.1 Theoretical Aspects, 98
3.4.2 Nuclear Screening and the Chemical Shift, 101
3.4.3 Spin–Spin Coupling, 104
3.4.4 Techniques of Spectral Integration and Spin–Spin Decoupling, 106
3.4.5 Nuclear Magnetic Relaxation, 107
3.4.6 The Nuclear Overhauser Effect (NOE), 108
3.4.7 Obtaining the NMR Spectrum, 110
3.4.8 Two-Dimensional (2D) NMR Spectroscopy, 111
3.4.9 Two-Dimensional Correlation Spectroscopy (COSY) and Total Correlation Spectroscopy (TOCSY), 112
3.4.10 Nuclear Overhauser Effect Spectroscopy (NOESY), 115
3.4.11 Multidimensional NMR, 116
3.4.12 Descriptive Examples, 117

3.5 Electron Paramagnetic Resonance, 122
3.5.1 Theory and Determination of g-Values, 122
3.5.2 Hyperfine and Superhyperfine Interactions, 127
3.5.3 Electron Nuclear Double Resonance (ENDOR) and Electron Spin-Echo Envelope Modulation (ESEEM), 129
3.5.4 Descriptive Examples, 129
3.6 Mössbauer Spectroscopy, 132
3.6.1 Theoretical Aspects, 132
3.6.2 Quadrupole Splitting and the Isomer Shift, 134
3.6.3 Magnetic Hyperfine Interactions, 136
3.6.4 Descriptive Examples, 137
3.7 Other Instrumental Methods, 139
3.7.1 Atomic Force Microscopy, 139
3.7.2 Fast and Time-Resolved Methods, 143
3.7.2.1 Stopped-Flow Kinetic Methods, 143
3.7.2.2 Flash Photolysis, 144
3.7.2.3 Time-Resolved Crystallography, 146
3.7.3 Mass Spectrometry, 148
3.8 Summary and Conclusions, 153
References, 154

4 Computer Hardware, Software, and Computational Chemistry Methods

4.1 Introduction to Computer-Based Methods, 157
4.2 Computer Hardware, 157
4.3 Molecular Modeling and Molecular Mechanics, 160
4.3.1 Introduction to MM, 160
4.3.2 Molecular Modeling, Molecular Mechanics, and Molecular Dynamics, 161
4.3.3 Biomolecule Modeling, 166
4.3.4 A Molecular Modeling Descriptive Example, 167
4.4 Quantum Mechanics-Based Computational Methods, 170
4.4.1 Introduction, 170
4.4.2 Ab Initio Methods, 170
4.4.3 Density Function Theory, 171
4.4.4 Semiempirical Methods, 173
4.5 Computer Software for Chemistry, 174
4.5.1 Mathematical Software, 180
4.6 World Wide Web Online Resources, 181
4.6.1 Nomenclature and Visualization Resources, 181
4.6.2 Online Societies, Online Literature Searching, and Materials and Equipment Websites, 183
4.7 Summary and Conclusions, 185
References, 185
5 Group I and II Metals in Biological Systems: Homeostasis and Group I Biomolecules

5.1 Introduction, 189
5.2 Homeostasis of Metals (and Some Nonmetals), 192
  5.2.1 Phosphorus as Phosphate, 192
  5.2.2 Potassium, Sodium, and Chloride Ions, 193
  5.2.3 Calcium Homeostasis, 194
5.3 Movement of Molecules and Ions Across Membranes, 195
  5.3.1 Passive Diffusion, 195
  5.3.2 Facilitated Diffusion, 197
    5.3.2.1 Gated Channels, 197
  5.3.3 Active Transport—Ion Pumps, 197
5.4 Potassium-Dependent Molecules, 199
  5.4.1 Na+/K⁺ ATPase: The Sodium Pump, 199
  5.4.2 Potassium (K⁺) Ion Channels, 203
    5.4.2.1 Introduction, 203
    5.4.2.2 X-Ray Crystallographic Studies, 205
5.5 Conclusions, 235
References, 235

6 Group I and II Metals in Biological Systems: Group II

6.1 Introduction, 238
6.2 Magnesium and Catalytic RNA, 238
  6.2.1 Introduction, 238
  6.2.2 Analyzing the Role of the Metal Ion, 241
  6.2.3 The Group I Intron Ribozyme, 244
  6.2.4 The Hammerhead Ribozyme, 261
6.3 Calcium-Dependent Molecules, 301
  6.3.1 Introduction, 301
  6.3.2 Calmodulin, 302
    6.3.2.1 Introduction, 302
    6.3.2.2 Calmodulin Structure by X-Ray and NMR, 303
    6.3.2.3 Calmodulin Interactions with Drug Molecules, 308
    6.3.2.4 Calmodulin–Peptide Binding, 313
    6.3.2.5 Conclusions, 326
6.4 Phosphoryl Transfer: P-Type ATPases, 327
  6.4.1 Introduction, 327
  6.4.2 Calcium P-Type ATPases, 327
    6.4.2.1 Ca²⁺-ATPase Protein SERCA1a and the Ca²⁺-ATPase Cycle, 329
6.5 Conclusions, 337
References, 338
7 Iron-Containing Proteins and Enzymes

7.1 Introduction: Iron-Containing Proteins with Porphyrin Ligand Systems, 343
7.2 Myoglobin and Hemoglobin, 343
  7.2.1 Myoglobin and Hemoglobin Basics, 345
  7.2.2 Structure of the Heme Prosthetic Group, 347
  7.2.3 Behavior of Dioxygen Bound to Metals, 348
  7.2.4 Structure of the Active Site in Myoglobin and Hemoglobin: Comparison to Model Compounds, 349
  7.2.5 Some Notes on Model Compounds, 352
  7.2.6 Iron-Containing Model Compounds, 353
  7.2.7 Binding of CO to Myoglobin, Hemoglobin, and Model Compounds, 356
  7.2.8 Conclusions, 359
7.3 Introduction to Cytochromes, 359
7.4 Cytochrome P450: A Monooxygenase, 361
  7.4.1 Introduction, 361
  7.4.2 Cytochrome P450: Structure and Function, 363
  7.4.3 Cytochrome P450: Mechanism of Activity, 365
  7.4.4 Analytical Methods: X-Ray Crystallography, 369
  7.4.5 Cytochrome P450 Model Compounds, 372
    7.4.5.1 Introduction, 372
    7.4.5.2 A Cytochrome P450 Model Compound: Structural, 372
    7.4.5.3 Cytochrome P450 Model Compounds: Functional, 374
  7.4.6 Cytochrome P450 Conclusions, 382
7.5 Cytochrome b(6)f: A Green Plant Cytochrome, 382
  7.5.1 Introduction, 382
  7.5.2 Cytochrome b(6)f Metal Cofactor Specifics, 386
7.6 Cytochrome bc₁: A Bacterial Cytochrome, 388
  7.6.1 Introduction, 388
  7.6.2 Cytochrome bc₁ Structure, 389
  7.6.3 Cytochrome bc₁ Metal Cofactor Specifics, 391
  7.6.4 The Cytochrome bc₁ Q Cycle, 395
  7.6.5 Cytochrome bc₁ Inhibitors, 397
  7.6.6 Cytochrome bc₁ Conclusions, 408
7.7 Cytochromes c, 408
  7.7.1 Introduction, 408
  7.7.2 Mitochondrial Cytochrome c (Yeast), 411
  7.7.3 Mitochondrial Cytochrome c (Horse), 416
  7.7.4 Cytochrome c Folding, Electron Transfer, and Cell Apoptosis, 422
    7.7.4.1 Cytochrome c Folding, 422
7.7.4.2 Electron Transfer in Cytochrome c and Its Redox Partners, 424
7.7.4.3 Apoptosis, 427
7.7.5 Cytochrome c Conclusions, 429

7.8 Cytochrome c Oxidase, 429
  7.8.1 Introduction, 429
  7.8.2 Metal-Binding Sites in Cytochrome c Oxidase, 432
  7.8.3 Dioxygen Binding, Proton Translocation, and Electron Transport, 434
  7.8.4 Cytochrome c Oxidase Model Compounds and Associated Analytical Techniques, 440
  7.8.5 Cytochrome c Oxidase Conclusions, 453

7.9 Non-Heme Iron-Containing Proteins, 454
  7.9.1 Introduction, 454
  7.9.2 Proteins with Iron–Sulfur Clusters, 454
    7.9.2.1 The Enzyme Aconitase 455
  7.9.3 Iron–Oxo Proteins, 458
    7.9.3.1 Methane Monooxygenases 459

7.10 Conclusions, 465
References, 466

Index 477
PREFACE

This second edition of *Bioinorganic Chemistry: A Short Course* adopts the same philosophy as the first—that is, chapters of introductory material followed by chapters featuring detailed discussions of specific bioinorganic chemistry topics. This approach foregoes any attempt to exhaustively survey the enormous range of bioinorganic topics that occupy the attention and research of theoreticians and experimentalists currently engaged in the field. In this second edition, introductory Chapters 1 and 2 cover inorganic chemistry essentials and biochemistry fundamentals for bioinorganic chemistry students whose background in these topics may be less than ideal. Chapter 3 (Instrumental Methods) concentrates on the physical and analytical methods used to describe the bioinorganic systems discussed in Chapters 5 through 7. Chapter 4 (Computer Hardware, Software, and Computational Chemistry Methods) describes some of the vast array of computer hardware, software, and drawing, visualization, computational, and modeling programs used by every researcher studying bioinorganic systems. Computational chemistry, for instance, allows researchers to predict molecular structures of known and theoretical compounds and to design and test new compounds on computers rather than at the laboratory bench. Chapter 5 (Group I and II Metals in Biological Systems: Homeostasis and Group I Biomolecules) discusses the vital roles of sodium and potassium ions in maintaining cellular integrity, and features the Nobel Prize-winning work of Roderick MacKinnon’s research group on potassium ion channels. More structural work by the MacKinnon group confirming the selectivity of potassium ion channels for K⁺ over Na⁺ can be found in a recent *Science* magazine article (*Science* 2006, *314*, 1004–1007). Chapter 6 (Group I and II Metals in Biological Systems: Group II) describes the importance of
magnesium ions in catalytic RNA (ribozymes). Readers interested in the “RNA World hypothesis”, a theory connecting the origin of life with self-replicating ribozymes, will want to read the recent article by Michael Robertson and William Scott (Science 2007, 315, 1549–1553). A background perspective on this article has been written by Gerald Joyce (Science 2007, 315, 1507–1508). In addition, Chapter 6 discusses two calcium-containing biomolecules—calmodulin, a primary receptor for intracellular calcium ions and a switch in Ca²⁺-dependent signaling pathways, and Ca²⁺-ATPase, a major player in muscle contraction-relaxation cycles. Chapter 7 (Iron Containing Proteins and Enzymes) devotes much of its descriptive material to proteins and enzymes that contain their iron ions within a heme ligand system. This chapter extends the first edition’s discussion of myoglobin and hemoglobin, then reports on some members of the ubiquitous cytochrome family—cytochrome P450, a monoxygenase, cytochrome b(6)f, a green plant constituent, bacteria-based cytochrome bc₁, members of the cytochrome c superfamily, and cytochrome c oxidase (CcO), the terminal electron transferring enzyme in the mitochondrial respiratory chain. An update reported recently by the Collman group (Science 2007, 315, 1565–1568) connects the redox-active centers of cytochrome c oxidase—Fe₃₃, Cu₄, and tyr244—to the rapid accumulation of four electrons. The four accumulated electrons are needed to reduce dioxygen, O₂, to two oxide, O²⁻, ions while avoiding the production of partially reduced, tissue-damaging superoxide, O₂⁻, or peroxide, O₂²⁻, ions. A shorter section in Chapter 7 discusses non-heme iron-containing proteins and enzymes, many of which, like aconitase, feature iron-sulfur clusters. Lastly, Chapter 7 reports on the enzyme methane monoxygenase (MMO), utilized by methanotrophic bacteria to oxidize methane to methanol with incorporation of one O₂ oxygen atom.

Many exciting bioinorganic topics are not covered in either the first or the present editions of Bioinorganic Chemistry: A Short Course. The new field of nanobioinorganic chemistry has become a prominent research area, especially in the medical field. Readers who wish to research this area might start with the review article: “Metal Nanoshells” in the Annals of Biomedical Engineering 2006, 34(1), 15–22. In this article, Jennifer L. West and coworkers describe a new class of nanoparticles that have tunable optical properties. Chad Mirkin and coworkers describe oligonucleotide-modified gold nanoparticles that are being developed as intracellular gene regulation agents (Science, 2006, 312, 1027–1030; J. Am. Chem. Soc. 2006, 128(29), 9286–9287; J. Am. Chem. Soc. 2006, 128(27), 8899–8903). These agents may eventually find applications in controlling the expression of specific proteins in cells for medical diagnostic and therapeutic purposes. The International Council on Nanotechnology (ICON) maintains a website at http://icon.rice.edu/research.cfm that includes links to other databases of interest, such as NIOSH (National Institute for Occupational Safety and Health) and the nanomedicine portal. ICON is particularly interested in informing researchers and nanotechnology users on environmental and safety issues related to this new, rapidly expanding field.
Readers interested in the connection between bioinorganic chemistry and catalysis might begin by reading an article entitled: “Better than Platinum? Fuels Cells energized by enzymes.” written by Marcetta Darensbourg, Michael Hall, and Jesse Tye (Proc. Natl. Acad. Sci. U.S.A. 2005, 102(47), 16911–16912.) This article briefly describes the interest of bioinorganic chemists in the hydrogenase enzymes that biologically and reversibly accomplish proton reduction and dihydrogen oxidation. Since their discovery, hydrogenase enzymes, containing sulfur-bridged di-iron or nickel-iron active sites, have been presented as possible substitutes for expensive noble-metal based catalysts in the $2\text{H}^+ + 2\text{e}^- \leftrightarrow \text{H}_2$ reaction. More recently, these researchers have published studies of synthetic di-iron(I) complexes as structural models of reduced Fe-Fe hydrogenase (Inorg. Chem. 2006, 45(4), 1552–1559) and computational studies comparing computed gas-phase and experimental solution phase infrared spectra of Fe-Fe hydrogenase active site models (J. Comput. Chem. 2006, 27(12), 1454–1462).

Readers with a more structural biology bent might be interested in the 2006 achievement of Jennifer A. Doudna’s group at the University of California, Berkeley in obtaining the first crystal structure of Dicer, an enzyme that initiates RNA interference (RNAi). This work, published in Science (2006, 311, 195–198), helps confirm that two metal ions—in the X-ray crystallographic structure, Er$^{3+}$ substitutes for the naturally occurring Mn$^{2+}$ ions—participate in Dicer’s catalytic mechanism.

Researchers continue to extend their ability to study and analyze complex bioinorganic systems as new experimental and instrumental methods are developed and current ones are improved. For instance, protein structure determination in solution by nuclear magnetic resonance, NMR, received a boost in 2006 through a technique developed at Tokyo Metropolitan University. This technique, stereo-array isotope labeling, SAIL, will make it possible to routinely determine protein structures at least twice as large as those being determined using current NMR methods (Kainosho, M., Torizawa, T., Iwashita, Y., Terauchi, T., Ono, A. M., Guntert, P. *Nature* 2006, 440, 52–57, PDB: 1X02). The solution structure of the Ca$^{2+}$-containing protein calmodulin described in the *Nature* article, as determined by the SAIL method, is compared to X-ray crystallographic structures in Section 6.3.2.2—see especially Figure 6.23.

In some cases, the increasing complexity of bioinorganic systems studied, and the increasing sophistication of the analytical methods used, has led to controversy over the interpretation of biomolecular structures and behaviors. In this text, variations in experimental results and their interpretations among different research groups are found in the discussions of potassium ion channels (Section 5.4.2), group I intron ribozymes (Section 6.2.3), and the hammerhead ribozyme (Section 6.2.4). This author has attempted to present material on all existent interpretations by different research groups working in good faith to solve thorny experimental problems. All researchers, including newcomers to these complicated subjects, should maintain an open mind, a continuing interest in and exploration of the problems, and a civil manner of discourse within the scientific literature.

Admission of errors can be part of this discourse, although, to my knowledge, these have not been called for in the research areas mentioned in the previous paragraph. Recently, however, retractions appeared in *Science* magazine concerning incorrect interpretations of X-ray crystallographic data gathered on the MsbA protein, an important member of a class of molecules that use energy from adenosine triphosphate, ATP, to transport molecules across cell membranes—the so-called ABC transporters. The erroneous structures arose not because of any fault in the data collection scheme or the protein crystals themselves, but because of a faulty data-analysis program used to massage the data into visualized molecular structures. The incorrectly visualized MsbA protein structures were featured in at least five journal articles now being retracted (Miller, G., News of the Week, *Science* 2006, 314, 1856–1857; Chang, G., Roth, C. B., Reyes, C. L., Pornillos, O., Chen, Y-J., Chen, A. P. Letters, *Science* 2006, 314, 1875; Miller C. Letters *Science* 2007, 315, 459. No MsbA protein structures, faulty or otherwise, are discussed in this text. However, as will be said numerous times herein, the techniques of X-ray crystallography provide snapshots of biomolecules frozen into a solid crystalline lattice, not a
normal biomolecular physical state of being, and certainly not representative of every possible molecular conformation in the biological milieu. If errors in data interpretation are also introduced, one sees how incorrect biomolecule structure visualizations find their way into the literature. Confirmation of X-ray crystallographic structural results through experimental biochemistry and by the use of multiple analytical techniques—nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR), and Mössbauer spectroscopies to name a few—should always be sought by bioinorganic researchers.

Lastly, and importantly, researchers, academicians, and their students want to maintain ethical behaviors in their scientific endeavors. Although science practitioners have historically been self-policing in this regard, and continue to be so, science writers and thinkers now call for more consideration of ethical topics, especially for students in graduate and post-graduate years as well as for early-career scientists. Readers who wish more information on ethical issues may consult a recent article entitled: “A Code of Ethics for the Life Sciences” by Nancy Jones, an American Association for the Advancement of Science/National Institutes of Health (NIH) Science Policy Fellow and a faculty member at Wake Forest University School of Medicine. The article has been published in Science and Engineering Ethics, by Springer Netherlands, January 30, 2007, online at http://www.springerlink.com.

This text is appropriate for use in one-semester bioinorganic chemistry courses offered to fourth year undergraduate chemistry, biochemistry and biology majors or first year graduate students concentrating in inorganic and biochemical subject areas. After presentation of some introductory material in inorganic, biochemistry, and a review of selected instrumental and computer-based topics, I suggest choosing one to three bioinorganic chemistry topics from Chapters 5 through 7 for thorough discussion. Following that, students should be encouraged to choose their own bioinorganic topics for research and study. Their endeavors could lead to classroom presentations, laboratory experimentation, and submission of written term papers. Certainly, the subject area provides great opportunities for introducing the use of primary literature sources and the application of computer- and internet-based searching, visualization, and modeling techniques.

A website to accompany the second edition of Bioinorganic Chemistry: A Short Course can be found at http://chemistry.washcoll.edu/roat/. The website contains the book’s table of contents, a listing of online resources organized by chapter and subject area, additional figures organized by chapter section (best viewed while studying the section’s material), updated bibliographic references, study questions for each chapter, and communication links for questions, comments, and corrections submitted by instructors and students.

Rosette M. Roat-Malone
Washington College
Many groups of people contributed to the creation and realization of this book. Thanks to former bioinorganic chemistry students, whose enthusiasm for the subject material inspired the book’s manner of presentation. Professional colleagues at Washington College and other universities worldwide helped in many ways—as critical readers, and as advisers on important subject areas to be included. The book would not exist without the expert assistance of Wiley editors—Anita Lekhwani, Rebekah Amos, Danielle Lacourciere, Nancy Heimbaugh, and Dean Gonzalez. Lastly, I express heartfelt gratitude to family and friends for their patience during the many months of gestation.
INORGANIC CHEMISTRY ESSENTIALS

1.1 INTRODUCTION

Bioinorganic chemistry involves the study of metal species in biological systems. As an introduction to the basic inorganic chemistry needed for understanding bioinorganic topics, this chapter will discuss the essential chemical elements, the occurrences and purposes of metal centers in biological species, the geometries of ligand fields surrounding these metal centers, and ionic states preferred by the metals. Important considerations include equilibria between metal centers and their ligands and a basic understanding of the kinetics of biological metal–ligand systems. The occurrence of organometallic complexes and clusters in metalloproteins will be discussed briefly, and an introduction to electron transfer in coordination complexes will be presented. Since the metal centers under consideration are found in a biochemical milieu, basic biochemical concepts, including a discussion of proteins and nucleic acids, are presented in Chapter 2.

1.2 ESSENTIAL CHEMICAL ELEMENTS

Chemical elements essential to life forms can be broken down into four major categories: (1) bulk elements (H/H⁺, C, N, O²⁻/O₂⁻/O₂⁻, P, S/S²⁻); (2) macro-minerals and ions (Na/Na⁺, K/K⁺, Mg/Mg²⁺, Ca/Ca²⁺, Cl⁻, PO₄³⁻, SO₄²⁻); (3) trace
elements (Fe/Fe^{II}/Fe^{III}/Fe^{IV}, Zn/Zn^{II}, Cu/Cu^{I}/Cu^{II}/Cu^{III}); and (4) ultratrace elements, comprised of nonmetals (F/F^{−}, I/I^{−}, Se/Se^{2−}, Si/Si^{IV}, As, B) and metals (Mn/Mn^{II}/Mn^{III}/Mn^{IV}, Mo/Mo^{IV}/Mo^{VI}/Mo^{V}, Co/Co^{II}/Co^{III}, Cr/Cr^{III}/Cr^{VI}, V/V^{II}/V^{IV}/V^{V}, Ni/Ni^{II}/Ni^{III}, Cd/Cd^{2+}, Sn/Sn^{II}/Sn^{IV}, Pb/Pb^{2+}, Li/Li^{+}). In the preceding classification, only the common biologically active ion oxidation states are indicated. (See references 3 and 21d for more information.) If no charge is shown, the element predominately bonds covalently with its partners in biological compounds, although elements such as carbon (C), sulfur (S), phosphorus (P), arsenic (As), boron (B), selenium (Se) have positive formal oxidation states in ions containing oxygen atoms; that is, S = +6 in the SO_{4}^{2−} ion or P = +5 in the PO_{4}^{3−} ion. The identities of essential elements are based on historical work and that done by Klaus Schwarz in the 1970s. Other essential elements may be present in various biological species. Essentiality has been defined by certain criteria: (1) A physiological deficiency appears when the element is removed from the diet; (2) the deficiency is relieved by the addition of that element to the diet; and (3) a specific biological function is associated with the element. Table 1.1 indicates the approximate percentages by weight of selected essential elements for an adult human.

Every essential element follows a dose–response curve, shown in Figure 1.1, as adapted from reference 2. At lowest dosages the organism does not survive, whereas in deficiency regions the organism exists with less than optimal function. After the concentration plateau of the optimal dosage region, higher dosages cause toxic effects in the organism, eventually leading to lethality. Specific daily requirements of essential elements may range from microgram to gram quantities as shown for two representative elements in Figure 1.1. Considering the content of earth’s contemporary waters and atmospheres, many questions arise as to the choice of essential elements at the time of life’s origins 3.5 billion or more years ago. Certainly, sufficient quantities of the bulk elements were available in primordial oceans and at shorelines. However, the concentrations of essential trace metals in modern oceans may differ considerably from those found in prebiotic times. Iron’s current approximate 10^{−4} mM

### Table 1.1 Percentage Composition of Selected Elements in the Human Body

<table>
<thead>
<tr>
<th>Element</th>
<th>Percentage (by weight)</th>
<th>Element</th>
<th>Percentage (by weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>53.6</td>
<td>Silicon, magnesium</td>
<td>0.04</td>
</tr>
<tr>
<td>Carbon</td>
<td>16.0</td>
<td>Iron, fluorine</td>
<td>0.005</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>13.4</td>
<td>Zinc</td>
<td>0.003</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>2.4</td>
<td>Copper, bromine</td>
<td>2. × 10^{−4}</td>
</tr>
<tr>
<td>Sodium, potassium, sulfur</td>
<td>0.10</td>
<td>Selenium, manganese, arsenic, nickel</td>
<td>2. × 10^{−5}</td>
</tr>
<tr>
<td>Chlorine</td>
<td>0.09</td>
<td>Lead, cobalt</td>
<td>9. × 10^{−6}</td>
</tr>
</tbody>
</table>

*Source: Adapted from reference 2.*
concentration in seawater, for instance, may not reflect accurately its pre-life-
forms availability. If one assumes a mostly reducing atmosphere contemporary
with the beginnings of biological life, the availability of the more soluble
iron(II) ion in primordial oceans must have been much higher. Thus, the essen-
tiality of iron(II) at a concentration of 0.02 mM in the blood plasma heme
(hemoglobin) and muscle tissue heme (myoglobin) may be explained. Besides
the availability factor, many chemical and physical properties of elements and
their ions are responsible for their inclusion in biological systems. These
include: ionic charge, ionic radius, ligand preferences, preferred coordination
geometries, spin pairings, systemic kinetic control, and the chemical reactivity
of the ions in solution. These factors are discussed in detail by Frausto da Silva
and Williams.\textsuperscript{3}

\section*{1.3 METALS IN BIOLOGICAL SYSTEMS: A SURVEY}

Metals in biological systems function in a number of different ways. Group 1
and 2 metals operate as structural elements or in the maintenance of charge
and osmotic balance (Table 1.2). Transition metal ions that exist in single oxi-
dation states, such as zinc(II), function as structural elements in superoxide
dismutase and zinc fingers, or, as an example from main group +2 ions, as trig-
gers for protein activity—that is, calcium ions in calmodulin or troponin C

![Dose–response curve for an essential element. (Used with permission from reference 2. Copyright 1985, Division of Chemical Education, Inc.)](image-url)
Transition metals that exist in multiple oxidation states serve as electron carriers—that is, iron ions in cytochromes or in the iron–sulfur clusters of the enzyme nitrogenase or copper ions in cytochrome c oxidase (Cuₐ site), azurin and plastocyanin (Table 1.4); as facilitators of oxygen transport—that is, iron ions in hemoglobin or copper ions in hemocyanin (Table 1.5); and as sites at which enzyme catalysis occurs—that is, copper ions in superoxide dismutase or cytochrome c oxidase (Cuₐ site), iron ions in heme a₃ of cytochrome c oxidase, or iron and molybdenum ions in nitrogenase (Table 1.6). Metal ions may serve multiple functions, depending on their oxidation state or location within the biological system so that the classifications in Tables 1.2–1.6 are necessarily incomplete, arbitrary, and/or overlapping.⁴,⁵

<table>
<thead>
<tr>
<th>TABLE 1.2 Metals in Biological Systems: Charge Carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal</td>
</tr>
<tr>
<td>Sodium, Na⁺</td>
</tr>
<tr>
<td>Potassium, K⁺</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 1.3 Metals in Biological Systems: Structural, Triggers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metal</td>
</tr>
<tr>
<td>Magnesium, Mg²⁺</td>
</tr>
<tr>
<td>Calcium, Ca²⁺</td>
</tr>
<tr>
<td>Zinc, Zn²⁺ (d¹⁰)</td>
</tr>
<tr>
<td>Zinc, Zn²⁺ (d¹⁰)</td>
</tr>
<tr>
<td>Manganese, Mn²⁺ (d⁵)</td>
</tr>
<tr>
<td>Manganese, Mn³⁺ (d⁶)</td>
</tr>
</tbody>
</table>
### TABLE 1.4 Metals in Biological Systems: Electron Transfer

<table>
<thead>
<tr>
<th>Metal</th>
<th>Coordination Number, Geometry</th>
<th>Preferred Ligands</th>
<th>Functions and Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron, Fe(^{2+}) ((d^6))</td>
<td>4, tetrahedral</td>
<td>S-Thiolate</td>
<td>Electron transfer, nitrogen fixation in nitrogenases</td>
</tr>
<tr>
<td>Iron, Fe(^{2+}) ((d^6))</td>
<td>6, octahedral</td>
<td>O-Carboxylate, alkoxide, oxide, phenolate</td>
<td>Electron transfer in oxidases</td>
</tr>
<tr>
<td>Iron, Fe(^{3+}) ((d^5))</td>
<td>4, tetrahedral</td>
<td>S-Thiolate</td>
<td>Electron transfer, nitrogen fixation in nitrogenases</td>
</tr>
<tr>
<td>Iron, Fe(^{3+}) ((d^5))</td>
<td>6, octahedral</td>
<td>O-Carboxylate, alkoxide, oxide, phenolate</td>
<td>Electron transfer in oxidases</td>
</tr>
<tr>
<td>Copper, Cu(^{+}) ((d^{10})), Cu(^{2+}) ((d^9))</td>
<td>3, trigonal planar 6, tetragonal</td>
<td>N-Imidazole</td>
<td>Electron transfer in Type III heme–copper oxidases (Cu(_B) in cytochrome c oxidase, for example)</td>
</tr>
<tr>
<td>Copper, Cu(^{+}) ((d^{10})), Cu(^{2+}) ((d^9))</td>
<td>4, tetrahedral</td>
<td>S-Thiolate, thioether, N-imidazole</td>
<td>Electron transfer in Type I blue copper proteins and Type III heme–copper oxidases (Cu(_A) in cytochrome c oxidase, for example)</td>
</tr>
</tbody>
</table>

### TABLE 1.5 Metals in Biological Systems: Dioxygen Transport

<table>
<thead>
<tr>
<th>Metal</th>
<th>Coordination Number, Geometry</th>
<th>Preferred Ligands</th>
<th>Functions and Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper, Cu(^{2+}) ((d^9))</td>
<td>5, square pyramid 6, tetragonal</td>
<td>O-Carboxylate, N-imidazole</td>
<td>Type II copper oxidases, hydroxylases Type III copper hydroxylases, dioxygen transport in hemocyanin Dioxygen transport in hemoglobin and myoglobin</td>
</tr>
<tr>
<td>Iron, Fe(^{2+}) ((d^6))</td>
<td>6, octahedral</td>
<td>N-Imidazole, porphyrin</td>
<td>Type III copper hydroxylases, dioxygen transport in hemocyanin Dioxygen transport in hemoglobin and myoglobin</td>
</tr>
</tbody>
</table>
Ligand preference and possible coordination geometries of the metal center are important bioinorganic principles. Metal ligand preference is closely related to the hard–soft acid–base nature of metals and their preferred ligands. These are listed in Table 1.7.\(^6\)

In general, hard metal cations form their most stable compounds with hard ligands, whereas soft metal cations form their most stable compounds with soft ligands. Hard cations can be thought of as small dense cores of positive charge, whereas hard ligands are usually the small highly electronegative elements or ligand atoms within a hard polyatomic ion—that is, oxygen ligands in \((\text{RO})_2\text{PO}_2^-\), or \(\text{CH}_3\text{CO}_2^-\). Crown ethers are hard ligands that have cavities suitable for encapsulating hard metal ions. The [18]-crown-6 ether shown in Figure 1.2 with its 2.6 to 3.2-Å hole provides a good fit for the potassium ion, which has a radius of 2.88 Å.\(^6\)

It is possible to modify a hard nitrogen ligand toward an intermediate softness by increasing the polarizability of its substituents or the \(\pi\) electron cloud.
### TABLE 1.7 Hard–Soft Acid–Base Classification of Metal Ions and Ligands

<table>
<thead>
<tr>
<th>Metals, Ions, Molecules</th>
<th>Ligands</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HARD</strong></td>
<td></td>
</tr>
<tr>
<td>H⁺, Mg²⁺, Al³⁺, SO₃⁻</td>
<td>Oxygen ligands in H₂O, CO₃²⁻, NO₃⁻, PO₄³⁻, R₂O, and crown ethers</td>
</tr>
<tr>
<td>Na⁺, Ca²⁺, Co³⁺, CO₂</td>
<td></td>
</tr>
<tr>
<td>K⁺, Mn²⁺, Ga³⁺, VO³⁺</td>
<td>Nitrogen ligands in NH₃, N₂H₄, RNH₂, or Cl⁻</td>
</tr>
<tr>
<td>Fe²⁺, Ni²⁺, Zn²⁺, Cu²⁺, Pb²⁺, Sn²⁺, Ru²⁺, Au³⁺, SO₂, NO⁺</td>
<td></td>
</tr>
<tr>
<td><strong>INTERMEDIATE</strong></td>
<td></td>
</tr>
<tr>
<td>Fe³⁺, Ni²⁺, Zn²⁺, Co²⁺, Cu²⁺, Pb²⁺, I⁻, Br⁻, SO₃²⁻, Nitrogen ligands in NO₂, N₃⁻, N₂⁻</td>
<td></td>
</tr>
<tr>
<td><strong>SOFT</strong></td>
<td></td>
</tr>
<tr>
<td>Cu⁺, Pt²⁺, Pt⁴⁺</td>
<td>Sulfur ligands in RSH, RS⁻, R₂S, R₃P, RNC, CN⁻, CO, R⁻, H⁻, I⁻, S₂O₅²⁻, (RS)₂PO₂⁻, (RO)₂P(O)S⁻</td>
</tr>
<tr>
<td>Au⁺, Pb³⁺</td>
<td></td>
</tr>
<tr>
<td>Tl⁺, Hg²⁺</td>
<td></td>
</tr>
<tr>
<td>Ag⁺, Cd²⁺</td>
<td></td>
</tr>
<tr>
<td>Hg₂²⁺, Pd²⁺</td>
<td></td>
</tr>
</tbody>
</table>

**Source:** Adapted from references 4 and 6.

![Figure 1.2](image-url) [18]-Crown-6 ether.

about it, an example being the imidazole nitrogen of the amino acid histidine. Increasing the softness of phosphate ion substituents can transform the hard oxygen ligand of (RO)₂PO₂⁻ to a soft state in (RS)₂PO₂⁻. Soft cations and anions are those with highly polarizable, large electron clouds—that is, Hg²⁺, sulfur ligands as sulfides or thiolates, and iodide ions. Also, note that metal ions can overlap into different categories. Lead as Pb²⁺, for instance, appears in both the intermediate and soft categories. The Fe³⁺ ion, classified as a hard
cation, coordinates to histidine (imidazole) ligands in biological systems, whereas Fe$^{2+}$, classified as intermediate, can coordinate to sulfur ligands and the carbon atom of CO (see Section 7.2, for example, in which hemoglobin and myoglobin are discussed).

### 1.5 BIOLOGICAL METAL ION COMPLEXATION

#### 1.5.1 Thermodynamics

The thermodynamic stability of metal ions is denoted by stepwise formation constants as shown in equations 1.1–1.3 (charges omitted for simplicity):

\[
M + L \leftrightarrow ML \quad K_1 = \frac{[ML]}{[M][L]} \tag{1.1}
\]

\[
ML + L \leftrightarrow ML_2 \quad K_2 = \frac{[ML_2]}{[M][L]} \tag{1.2}
\]

\[
ML_2 + L \leftrightarrow ML_3 \quad K_3 = \frac{[ML_3]}{[ML_2][L]} \tag{1.3}
\]

Alternately, they are indicated by overall stability constants as shown in equations 1.4–1.6:

\[
M + L \leftrightarrow ML \quad \beta_1 = \frac{[ML]}{[M][L]} \tag{1.4}
\]

\[
M + 2L \leftrightarrow ML_2 \quad \beta_2 = \frac{[ML]}{[M][L]^2} \tag{1.5}
\]

\[
M + 3L \leftrightarrow ML_3 \quad \beta_3 = \frac{[ML]}{[M][L]^3} \tag{1.6}
\]

The equation relating the stepwise and overall stability constants is indicated by equation 1.7:

\[
\beta_n = K_1 K_2 \ldots K_n \tag{1.7}
\]

In biological systems, many factors affect metal–ligand complex formation. Hard–soft acid–base considerations have already been mentioned. Concentrations of the metal and ligand at the site of complexation are determined locally through concentration gradients, membrane permeability to metals and ligands, and other factors. Various competing equilibria—solubility products, complexation, and/or acid–base equilibrium constants—sometimes referred to as “metal ion speciation,” all affect complex formation. Ion size and charge, preferred metal coordination geometry, and ligand chelation effects all affect metal uptake. To better measure biological metal–ligand interactions, an