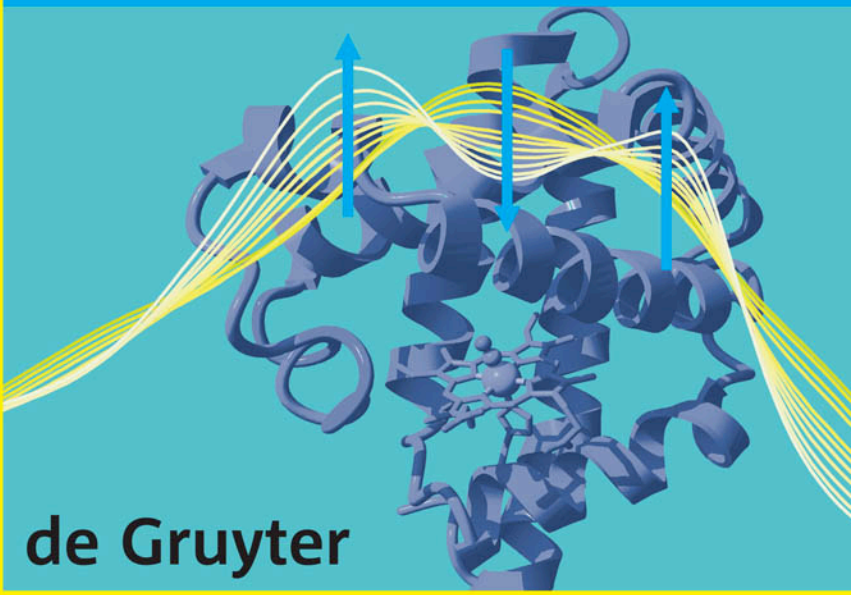
Nils Metzler-Nolte
Ulrich Schatzschneider

Bioinorganic Chemistry: A Practical Course



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Bioinorganic Chemistry: A Practical Course

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Bioinorganic Chemistry

A practical course

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The cover picture shows, in the background, the X-ray crystal structure of sperm whale muscle carbonmonoxy myoglobin at 1.15 Å resolution (PDB code: 1BZR) as determined by Kachalova, Popov, and Bartunik (*Science* **1999**, 284, 473–476). The artwork was generated with Yasara 8.8.12 (E. Krieger et al., *Proteins* **2002**, 47, 39–402) and POV-Ray 3.6.1. Superimposed on this is a trace of UV/Vis spectra from the Q-band region of myoglobin observed upon binding of increasing amounts of carbonmonoxide liberated from a CORM (CO releasing molecule, J. Niesel, U. Schatzschneider et al., *Chem. Commun.* **2008**, 1798–1800). A variation of this experiment is described in Chapter 3.

This work contains 46 figures und 5 tables.

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Preface and Acknowledgements

The topic for this book has emerged, evolved, and grown over the years that we have taught lab courses on *bioinorganic chemistry* (and given related lectures and seminars), first at the University of Heidelberg to students of pharmacy and molecular biotechnology, then at the Ruhr-University Bochum for students of chemistry and biochemistry. After many rounds of writing and revising scripts, printing them, and handing out collections of loose papers to our students, finally the idea for a book emerged. We are grateful to Dr. Stephanie Dawson from the publisher who shared our enthusiasm and helped the project materialize.

As this book is based on our own lab classes, we are grateful to the many co-workers, project students, and of course the students in the course itself, who gave feedback on our manuscripts, helped to improve the experiments, and in the end proof-read the manuscript. There are too many students to name them all here, but we would like to acknowledge our coworkers who helped in the lab course and during the writing of this book: Dr. Srecko Kirin (who was involved in the very early stages of this course in Heidelberg and Chapter 6), Antonio Pinto (Chapters 6, 8, and especially 9), Johanna Niesel (Chapters 3 to 5), Dr. Harmel Peindy N'Dongo (Chapter 6), Heiko Becker (Chapter 3), David Köster (Chapter 8), Katja Steinke and Lotte Holobar (Chapter 2), as well as Anna Sosniak (Chapter 7, thanks also to Dr. Michelle Salmain at ENSC de Paris for helpful discussions). Antonio, Harmel, Johanna, and Lukasz Raszeja have supervised the course in Bochum, and we are grateful for their enthusiasm and support of this project. Finally, the authors wish to thank their research groups for their patience when it was "this time of the year again" and our research needed to be grouped around instrument use by the students in this course (which usually meant late in the evening and on weekends). We are also grateful to colleagues for helpful discussions, in particular Prof. Katrin Sommer (Ruhr-University Bochum). N. Metzler-Nolte gratefully acknowledges an invitation to the ENSC de Paris as a visiting professor, during which time a major part of this book was written and finalized.

Finally, as usual when a major deadline approaches, work at nights and weekends (and during family holidays . . .) becomes inevitable and we are grateful to our families for their patience and support in the last few weeks.

Nils Metzler-Nolte and Ulrich Schatzschneider

Bochum, July 2009

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1 Introduction

Metal ions and their complexes are essential components in all living systems. They serve to enhance the stability of secondary, tertiary, and quarternary structures of biomolecules. They are essential components in the active site of many enzymes. They are vital for signal transduction and they may serve as cofactors for enzymes. Moreover, metal ions and their complexes find use as drugs and in molecular diagnostics. It is this cornucopia of functions, and the diversity of different aspects one might study, that makes the field of *bioinorganic chemistry* so interesting and challenging. Bioinorganic chemistry is also a highly interdisciplinary field of study. Beyond chemists and biochemists, biologists will look at different angles of the field than physicists, who have yet a different perspective from people in medicinal research. This makes the field also appealing to students, who appreciate the broad range of topics, the interdisciplinary contacts, and last but not least the unusually broad range of techniques, which they perceive as a bonus in their education.

Bioinorganic chemistry as a research topic is now firmly established in many places. Beyond the single investigator, there are today joint research efforts and even centers for "Metals in Biology" and the like. There are several regular international meetings devoted to the topic and its various sub-aspects, and at least two journals are entirely devoted to the topic, the *Journal of Biological Inorganic Chemistry* and *Journal of Inorganic Biochemistry*. All indications point to a well-established field of research.

Courses in bioinorganic chemistry have become part of the university curriculum, nowadays, at least for the training of chemists and biochemists. Courses, lectures, and seminars on bioinorganic chemistry are usually held at the advanced Bachelor or Master stage. There are graduate schools where bioinorganic chemistry is an integral part of the curriculum. In terms of practical training, however, there seems to be much less coordinated activity. Students at the Master level are frequently "embedded" in research groups, which means that they become familiar with the techniques and systems of interest in this particular research group. What is lacking, then, is an overview of general topics and common techniques in the field. Concurrently (and maybe not entirely unrelated), there is a tendency towards structured programs (such as PhD schools) and integrated courses. It is the purpose of those activities to counter

the steadily increasing individualization and specialization of research at ever earlier stages in a student's education by providing exposure to a broader background and more diverse topics than a single research group can provide.

With these observations in mind, we have written the present book. Bioinorganic chemistry provides a perfect starting point for an interdisciplinary course in the laboratory, and working on problems of bioinorganic chemistry in the laboratory almost effortlessly gives exposure to a variety of chemical, biochemical, and analytical techniques. For the student, this book complements and extends the range of good textbooks on bioinorganic chemistry which are available but will by their very nature be unspecific about details of experimental techniques or procedures. By having used these techniques in the laboratory themselves, the students will be more comfortable in research seminars or in lectures at conferences. Second, this book is intended to serve as a quick and reliable starting point for establishing a lab course. Naturally, it provides no more than a starting point that will necessarily be modified according to the requirements of the participating scientists and students.

Each chapter in this book is organized in a similar way. A brief summary of the contents is followed by learning targets in a bulleted list. The reader is then introduced to the background of the experiment, as well as guided to some specific information related to the topic of the experiment. We wish to emphasize that this book is *not* a textbook of bioinorganic chemistry, or a substitute for one. The background information is usually brief and condensed as far as general concepts are concerned. The experimentalist should be put in a position to perform the experiment competently and discuss the data that are obtained with confidence. Next, the experiment itself is described, including experimental details and an extensive list of materials (chemicals and laboratory equipment). As this book is aimed at advanced students, we assume knowledge of basic experimental procedures. Also, familiarity with safety-related issues and the use of standard procedures is assumed. Safety data for common procedures or most of the standard chemicals used are *not* provided as part of this book. Safety instructions are only given for special chemicals such as ethidium bromide or cisplatin. Their absence in this book *in no case* implies that a chemical (or procedure) is harmless! In a lab course, collecting the safety data for *all* chemicals prior to beginning the experiment could be made a compulsory part of the course work by the instructor. A number of leading references are provided at the end of each chapter. This list is certainly

not comprehensive and merely a (personal) choice of references which we find useful to get students started on a given topic.

The topics covered in this book can also be used as material for a seminar on bioinorganic chemistry. The introduction for each chapter should provide enough background information and leading references to enable advanced students to work up their own seminar topic. While the choice of topics reflects the research preferences of the authors, an effort was made to present a diversity of topics, chemical synthesis techniques (organic ligands, metal complexes, special techniques), biological systems (peptides, proteins, DNA, small model compounds for enzymes) and experimental techniques (colorimetric, fluorimetric, electrochemical, chromatographic, mass spectrometric analyses, gel electrophoresis, and cell culture techniques).

Chapters 2 and 3 are related to small molecule activation and signaling (O_2, H_2O_2, CO, NO), and to small molecules as models for metallo-enzymes. Chapters 4 and 5 are centered around DNA, using metal complexes to cleave DNA or probe its structure and properties by intercalation. Chapter 6 presents the synthesis of a metal-peptide bioconjugate, and it introduces the reader to the use of organometallic systems in biology, a rapidly growing research area now called *bioorganometallic chemistry*. Chapter 7 deals with the covalent modification of proteins, at which point electron transfer in biological systems is introduced. This chapter is connected to the following Chapter 8, where electrochemical measurements on metal complexes and the conjugates from the two previous chapters are carried out, evaluated, and compared. The final Chapter 9 introduces principles of medicinal inorganic chemistry, in particular anti-tumor drugs and the testing of anti-proliferative activity on tumor cell lines.

The authors of this book strongly believe that there is a genuine need for interdisciplinary training on a practical level in many places in the world. We have therefore compiled this book to encourage the initiative and provide a quick start. On the other hand, there are as many different settings for a lab course on bioinorganic chemistry as there are institutions planning to initiate such a course. Courses will vary in length and number of participants, and also the background of the students (chemists, biochemists, (molecular) biologists, students of biotechnology or molecular medicine, and of related subjects). Most importantly, the instructor will have to define the purpose of the course, for example whether a general introduction is intended or specific topics

and/or experimental techniques should be learned. Accordingly, the experiments can be adjusted in length and depth, and more (or less) theoretical background can be required. A section on variations of the experiment and additional questions has therefore been added to each experiment.

Finally, we have been asked by very senior colleagues as well as junior researchers about experimental details of experiments that we used or reported. While the primary literature is of course extensive, and individual techniques may have been described in volumes like *Methods in Enzymology*, there is no collection of "tested" experimental procedures for common experiments in bioinorganic chemistry. This book is an initial attempt towards this direction, as it collects and describes in detail a number of tested experimental procedures.

The experiments presented herein have emerged from a laboratory course on bioinorganic chemistry that the authors have taught over ten years to students with different backgrounds at different institutions. Currently, we are teaching this course to students in chemistry and biochemistry at the Ruhr-University Bochum during their Master program. Typically, six experiments out of the nine presented herein are performed by groups of two students over a period of two to three weeks. We can accommodate a maximum of six groups, but this number is mainly limited by instrument access and supervision, keeping in mind that the course runs in parallel to the research activities of the group. The presentation of the background and the experiments has evolved over the years, which is to say that they represent a tested and workable base, but they may not be optimal in every aspect. We thus welcome any corrections, additions, and suggestions for improvements to the material presented herein. Also, we will be happy to share our own experience in running a laboratory course on bioinorganic chemistry with interested colleagues and to provide detailed information on the way this course is presently organized and running in our laboratory, ideally in return for sharing your experiences.

So, whatever your intention for reading this book might be, we hope that you find the material useful and interesting, and hope that by reading through the chapters and thinking of your own experiments, you will appreciate the broad and interdisciplinary spirit of our common scientific love, bioinorganic chemistry!