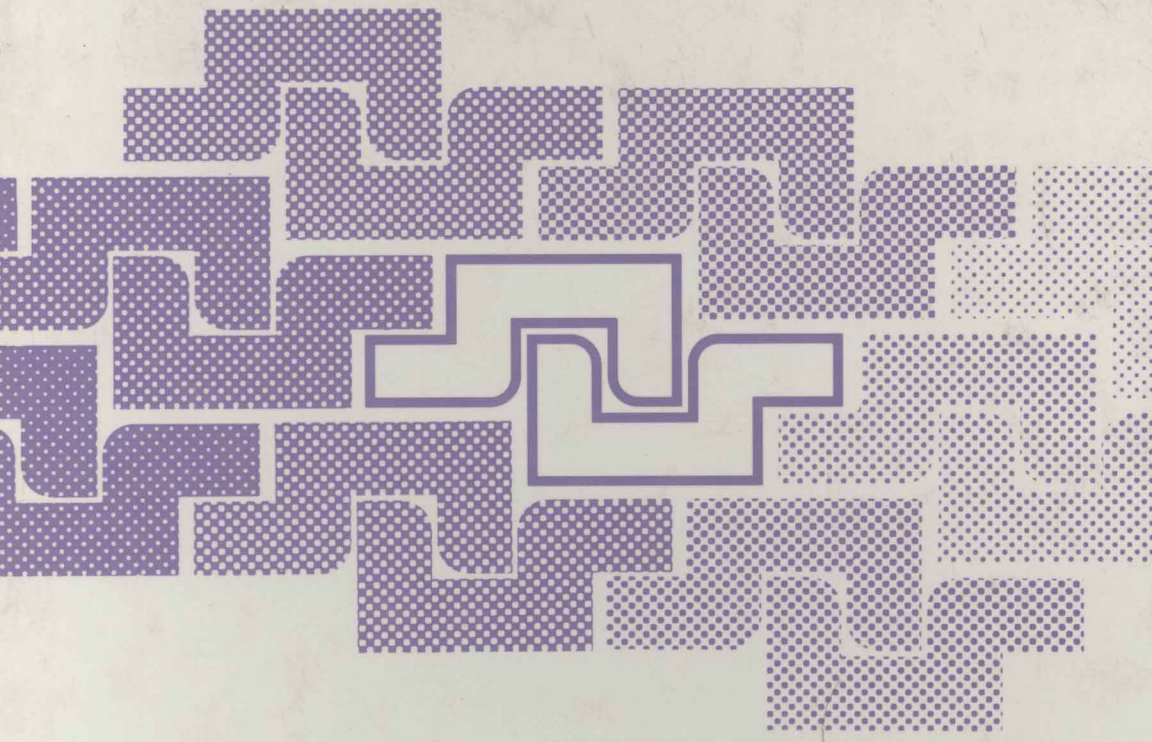

Molecular Modeling and Dynamics of Bioinorganic Systems

Edited by

Lucia Banci and Peter Comba

NATO ASI Series

3. High Technology – Vol. 41

Molecular Modeling and Dynamics of Bioinorganic Systems

edited by

Lucia Banci
University of Florence,
Department of Chemistry,
Florence, Italy

and

Peter Comba
Universität Heidelberg,
Anorganisch-Chemisches Institut,
Heidelberg, Germany

Kluwer Academic Publishers

Dordrecht / Boston / London

Published in cooperation with NATO Scientific Affairs Division

Proceedings of the NATO Advanced Research Workshop on
Molecular Modeling and Dynamics of Biological Molecules Containing Metal Ions
San Miniato (Pisa) Italy
March 15–21, 1997

A C.I.P. Catalogue record for this book is available from the Library of Congress

ISBN 0-7923-4824-9

Published by Kluwer Academic Publishers,
P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

Sold and distributed in the U.S.A. and Canada
by Kluwer Academic Publishers,
101 Philip Drive, Norwell, MA 02061, U.S.A.

In all other countries, sold and distributed
by Kluwer Academic Publishers,
P.O. Box 322, 3300 AH Dordrecht, The Netherlands.

Printed on acid-free paper

Printed in the Netherlands

Molecular Modeling and Dynamics of Bioinorganic Systems

NATO ASI Series

Advanced Science Institutes Series

A Series presenting the results of activities sponsored by the NATO Science Committee, which aims at the dissemination of advanced scientific and technological knowledge, with a view to strengthening links between scientific communities.

The Series is published by an international board of publishers in conjunction with the NATO Scientific Affairs Division

A Life Sciences	Plenum Publishing Corporation
B Physics	London and New York
C Mathematical and Physical Sciences	Kluwer Academic Publishers
D Behavioural and Social Sciences	Dordrecht, Boston and London
E Applied Sciences	
F Computer and Systems Sciences	Springer-Verlag
G Ecological Sciences	Berlin, Heidelberg, New York, London,
H Cell Biology	Paris and Tokyo
I Global Environmental Change	

PARTNERSHIP SUB-SERIES

1. Disarmament Technologies	Kluwer Academic Publishers
2. Environment	Springer-Verlag / Kluwer Academic Publishers
3. High Technology	Kluwer Academic Publishers
4. Science and Technology Policy	Kluwer Academic Publishers
5. Computer Networking	Kluwer Academic Publishers

The Partnership Sub-Series incorporates activities undertaken in collaboration with NATO's Cooperation Partners, the countries of the CIS and Central and Eastern Europe, in Priority Areas of concern to those countries.

NATO-PCO-DATA BASE

The electronic index to the NATO ASI Series provides full bibliographical references (with keywords and/or abstracts) to more than 50000 contributions from international scientists published in all sections of the NATO ASI Series.
Access to the NATO-PCO-DATA BASE is possible in two ways:

– via online FILE 128 (NATO-PCO-DATA BASE) hosted by ESRIN,
Via Galileo Galilei, I-00044 Frascati, Italy.

– via CD-ROM "NATO-PCO-DATA BASE" with user-friendly retrieval software in English, French and German (© WTV GmbH and DATAWARE Technologies Inc. 1989).

The CD-ROM can be ordered through any member of the Board of Publishers or through NATO-PCO, Overijse, Belgium.

3. High Technology – Vol. 41

List of Contributors

Emil Alexov
Department of Physics, City College of New York, 138th Str. and Covent Avenue, New York 0031, USA

Frederic Allain
Laboratoire de Chimie Théorique, Université Pierre et Marie Curie, 4, place Jussieu, 75005 Paris, France

Roger Attias
Laboratoire de Chimie et Biochimie, URA 400 CNRS, 45 Rue des Sants-Peres, 75270 Paris 06, France

B. Asbóth
Institute for Biochemistry and Protein Research, Agricultural Research Center, P.O. Box 170, H-2101 Gödöllõ, Hungary

Pascale Auge-Barrere-Mazouat
Laboratoire de Chimie Théorique, Université Pierre et Marie Curie, 4, place Jussieu, 75005 Paris, France

Lucia Banci
Department of Chemistry, University of Florence, via Gino Capponi, 7, 50121 Florence, Italy; e-mail: lucia@riscl.lrm.fi.cnr.it

Jacqueline Berges
Laboratoire de Chimie Théorique, Université Pierre et Marie Curie, 4, place Jussieu, 75005 Paris, France

P. Beroza
Department of Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037, USA

Ivano Bertini
Department of Chemistry, University of Florence, via Gino Capponi, 7, 50121 Florence, Italy; e-mail: bertini@riscl.lrm.fi.cnr.it

Z. Bõcskei
Department of Theoretical Chemistry, Eotvos Lorand University, P.O. Box 32, 1518 Budapest, Hungary

David Case
Department of Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037, USA; e-mail: case@scripps.edu

Jean-Claude Chottard
Laboratoire de Chimie et Biochimie, URA 400 CNRS, 45 Rue des Sants-Peres, 75270 Paris 06, France

Timothy Clark
Computer Chemie Centrum des Instituts für Organische Chemie, Universität Erlangen-Nürnberg, Nägelsbachstrasse 25, 91052 Erlangen, Germany ;e-mail: clark@organik.uni-erlangen.de

Thomas Cleveland
Department of Chemistry, University of Wisconsin, 1101 W. University Ave., Madison, WI 53706, USA

Peter Comba
Anorganisch-Chemisches Institut, Universität Heidelberg, Im Neuenheimer Feld 270, 69120 Heidelberg, Germany; e-mail: comba@akcomba.oci.uni-heidelberg.de

Csilla Csiki
Chemistry Department, Conneticut College, Box 5624, New London, CT 06320, USA

Robert J. Deeth
Department of Chemistry, Inorganic Computational Chemistry Group, Universtiy of Warwick, Coventry CV4 7AL, UK; e-mail: r.j.deeth@warwick.ac.uk

Miguel-Angel Elizondo-Riojas
Centro Universitario Contra el Cancer, Hospital Universitario "Dr. José Eleuterio Gonzales", Universidad Autónoma de Nuevo León, Monterrey, N. L., Mexico

Timothy K. Firman
Department of Chemistry, University of Wisconsin, 1101 W. University Ave., Madison, WI 53706, USA

M. Fuxreiter
Department of Theoretical Chemistry, Eotvos Lorand University, P.O. Box 32, 1518 Budapest, Hungary

Peter Gedeck
Computer Chemie Centrum des Instituts für Organische Chemie, Universität Erlangen-Nürnberg, Nägelsbachstrasse 25, 91052 Erlangen, Germany

Florence Gonnet
Laboratoire de Chimie Théorique, Université Pierre et Marie Curie, 4, place Jussieu, 75005 Paris, France

Giovanni Gori Savellini
Department of Chemistry, University of Florence, via Gino Capponi, 7, 50121 Florence, Italy

Marilyn R. Gunner
Department of Physics, City College of New York, 138th Str. and Covent Avenue, New York 0031, USA; e-mail: gunner@gunner.sci.ccny.cuny.edu

Michael B. Hall
Department of Chemistry, Texas A&M University, College Station, Texas 77843-3244, USA

Ernst-Walter Knapp
Institut für Kristallographie, Freie Universität Berlin, Takustrasse 6, 14195 Berlin, Germany; e-mail: knapp@chemie.fu-berlin.de

Peter Kollman
Department of Pharmaceutical Chemistry, University of California at San Francisco, 513 Parnassus Ave., San Francisco, Ca 94143, USA; e-mail: pak@cgl.ucsf.edu

Jiri Kozelka
Laboratoire de Chimie et Biochimie, URA 400 CNRS, 45 Rue des Sants-Peres, 75270 Paris 06, France; e-mail: kozelka@citi2.fr

Clark R. Landis
Department of Chemistry, University of Wisconsin, 1101 W. University Ave., Madison, WI 53706, USA; e-mail: landis@chem.wisc.edu

Harald Lanig
Computer Chemie Centrum des Instituts für Organische Chemie, Universität Erlangen-Nürnberg, Nägelsbachstrasse 25, 91052 Erlangen, Germany

J. Li
Department of Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037, USA

Bernd Melchers
Institut für Kristallographie, Freie Universität Berlin, Takustrasse 6, 14195 Berlin, Germany

Ian J. Munslow
Department of Chemistry, Inorganic Computational Chemistry Group, Universtiy of Warwick, Coventry CV4 7AL, UK

Gabor Náray-Szabó
Department of Theoretical Chemistry, Eotvos Lorand University, P.O. Box 32, 1518 Budapest, Hungary; e-mail: naray@para.chem.elte.hu

L. Noodleman
Department of Molecular Biology, The Scripps Research Institute, 10666 N. Torrey Pines Road, La Jolla, CA 92037, USA

Karen M. Norenberg
Chemistry Department, Conneticut College, Box 5624, New London, CT 06320, USA

Wieslaw Nowak
Molecular Biophysics Group - Institute of Physics, N. Copernicus University, ul. Grudziadzka, 5, 87100 Torun, Poland; e-mail: wiesiek@phys.uni.torun.pl

Veronica J. Paget
Department of Chemistry, Inorganic Computational Chemistry Group, Universtiy of Warwick, Coventry CV4 7AL, UK

Fritz Parak
Fakultät für Physik E17, Technische Universität München, James-Frank Straβe, 85747 Garching, Germany

Daniel M. Root
Department of Chemistry, University of Wisconsin, 1101 W. University Ave., Madison, WI 53706, USA

Antonio Rosato
Department of Chemistry, University of Florence, via Gino Capponi, 7, 50121 Florence, Italy

Gudrun Schürer
Computer Chemie Centrum des Instituts für Organische Chemie, Universität Erlangen-Nürnberg, Nägelsbachstrasse 25, 91052 Erlangen, Germany

Christina M. Shoemaker
Chemistry Department, Conneticut College, Box 5624, New London, CT 06320, USA

Per Siegbahn
Department of Physics, University of Stockholm, Box 6730, 11385 Stockholm, Sweden; e-mail: ps@physto.se

H. Süli-Vargha
Research Group of Peptide Chemistry, Hungarian Academy of Sciences, 1518 Budapest 112, P.O. Box 32, Hungary

Andrei L. Tchougréeff
Department of Quantum Chemistry, Karpov Institute of Physical Chemistry, Vorontsovo pole, 10, 103064 Moskow, Russia; e-mail: andrei@cc.nifhi.ac.ru

Katalin Várnagy
Department of Inorganic and Analytical Chemistry, Lajos Kossuth University, 4010 Debreen, P.O. Box 21, Hungary; e-mail: varnagy@tigris.ktle.hu

Claus-Wilhelm von der Lieth
DKFZ, Abteilung Zentrale Spektroskopie, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany; e-mail: w.vonderlieth@dkfz-heidelberg.de

Arieh Warshel
Department of Chemistry, University of Southern California, Los Angeles, CA 90089-1062, USA; e-mail: warshel@invitro.usc.edu

Snezana Zaric
Department of Chemistry, University of Beograd, Studentski trg. 16, 11001 Beograd, Serbia, Yugoslavia; e-mail: zaric@chemvx.tamu.edu

Marc Zimmer
Chemistry Department, Conneticut College, Box 5624, New London, CT 06320, USA; e-mail: mzim@conncoll.edu

Preface

The computation of structures and properties of bioinorganic compounds has experienced enormous progress in recent years. Novel approaches to model metal centers, new techniques to compute charges and solvation, and efficient algorithms to screen energy surfaces of large and complex structures have lead to a situation, in which it has now become possible and useful to discuss structures, electronic properties and reaction mechanisms of metalloproteins on the basis of computational studies. The good agreement between experimentally observed properties and computed parameters has increased the credibility of computational methods to a degree where experimentalists and computational chemists agree that the most demanding problems in bioinorganic chemistry may only be solved in a concerted way. Typical examples are the determination of solution structures of metalloproteins, the insight to the function of metalloenzymes and the design of mutated metalloproteins and transition metal-based drugs.

The chapters in this book are based on lectures presented at an Advanced NATO Research Workshop on Molecular Modeling and Dynamics of Biological Molecules Containing Metal Ions (San Miniato, Pisa, Italy, March 15 - 21 1997), organized by Lucia Banci, Katalin Várnagy, Peter Comba and Gabor Naray-Szabo. The chapters include a review of new force field-based approaches and their application to the computation of structures, electronic properties and dynamics of bioinorganic compounds, the discussion of quantum chemical and integrated QM/MM methods for understanding metalloenzyme functions, the presentation of methods used to compare electrostatic interactions and the evaluation of their importance for enzyme reaction mechanisms and experimental studies. The wide variety of topics and the state-of-the-art approaches presented have stimulated the participants of the NATO workshop, and it is hoped that the same will be true for the readers of this book.

The NATO Advanced Research Workshop was sponsored by the High Technology Division of NATO. We are grateful for this support and additional sponsorship by the University of Florence, the Cassa di Risparmio di San Miniato, IBM Semea and TRIPOS. The help of Dr. Giovanni Gori Savellini and Mrs. Karin Stelzer in the production of this book and the proof-reading by Ms. Anne Ramlow are gratefully acknowledged.

Lucia Banci, Florence — Peter Comba, Heidelberg — June 1997

Table of Contents

SOLUTION STRUCTURES OF PROTEINS CONTAINING PARAMAGNETIC METAL IONS

IVANO BERTINI and ANTONIO ROSATO
Department of Chemistry, University of Florence
via Gino Capponi 7, 50121 Florence, Italy

1. Introduction

Metal ions containing unpaired electrons (i.e. paramagnetic) are rather common in biological systems. In particular, electron transfer metalloproteins must have at least one redox state with unpaired electrons. The quantum-mechanical treatment of these systems is cumbersome and the results are uncertain [1,2], although the density functional approach has provided some interesting data [3].

The solution structure of proteins may normally be obtained by NMR spectroscopy [4,5]. The presence of metal ions containing unpaired electrons causes a broadening of NMR signals [6-9] and therefore makes it difficult to observe NOE's, from which upper distance limits are obtained. In macromolecular systems the line broadening due to paramagnetism depends on the electron relaxation time (τ_S), when the dominating mechanism is the electron-nucleus dipolar coupling [10,11], and on the magnetic field and the rotational correlation time of the molecule (τ_r), when the dominating mechanism is the dipolar coupling between the nucleus and the average magnetic moment induced by the external magnetic field on the metal ion [12,13]. The equation describing

L. Banci and P. Comba (eds.), Molecular Modeling and Dynamics of Bioinorganic Systems, 1–19.

the paramagnetic contribution to nuclear transversal relaxation due to the electron-nucleus dipolar coupling is [10,11]:

$$T_{2M}^{-1} = \frac{1}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r^6}\left[4\tau_s + \frac{13\tau_s}{1+\omega_S^2\tau_S^2} + \frac{3\tau_s}{1+\omega_I^2\tau_S^2}\right] \qquad (1)$$

where S is the spin moment of the nucleus, γ_I is the gyromagnetic ratio of the nucleus, r is the metal to nucleus distance, τ_S is the correlation time for the electron relaxation, ω_I and ω_S are the Larmor frequencies of the nucleus and of the electron, respectively,and all other symbols have their usual meaning. In Table 1 the values of τ_S for various metal ions at room temperature are reported [7,9]. We have set 10^{-11} s as the upper limit of τ_S in order to obtain signal linewidths suitable for high resolution NMR. The other origin of line broadening is Curie relaxation, which is described by eqn. (2) [12,13]:

$$T_{2M}^{-1} = \frac{1}{5}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\omega_I^2 g_e^4 \mu_B^4 S^2(S+1)^2}{(3kT)^2 r^6}\left(4\tau_r + \frac{3\tau_r}{1+\omega_I^2\tau_r^2}\right) \qquad (2)$$

where S is the spin moment of the nucleus, r is the metal to nucleus distance, ω_I is the Larmor frequency of the nucleus, τ_r is the correlation time for the reorientation of the molecule, and all other symbols have their usual meaning. Due to this contribution, when $S > 1/2$ and the magnetic field higher than 500 MHz, the linewidths of the signals of protons close to the metal ion may be broadened beyond detection (depending on the value of τ_r). Indeed, it may happen that the signal of a given proton is detected at 90 MHz more easily than at 600 MHz [14]. Thus, the use of different magnetic fields may be required, depending on the nuclear shell around the metal that we want to focus on.

TABLE 1. Electronic relaxation rates for various paramagnetic metal ions. The line broadening is calculated for a proton at a 5 Å distance from the metal ion, at 500 MHz ^{1}H frequency, assuming the only contribution is that due to the electron-nucleus dipolar coupling (eqn. (1)). Metal ions for which high resolution NMR is feasible are in bold.

Metal ion	τ_S (s^{-1})	Line broadening (Hz)
Ti^{3+}	**10^{-10}-10^{-11}**	**20-200**
VO^{2+}	10^{-8}	10000
V^{3+}	**10^{-11}**	**50**
V^{2+}	10^{-9}	5000
Cr^{3+}	$5\cdot10^{-9}$-$5\cdot10^{-10}$	3000-25000
Cr^{2+}	**10^{-11}-10^{-12}**	**20-150**
Mn^{3+}	**10^{-10}-10^{-11}**	**150-1500**
Mn^{2+}	10^{-8}	100000
Fe^{3+} (H.S.)	**10^{-9}-10^{-11}**	**200-12000**
Fe^{3+} (L.S.)	**10^{-11}-10^{-13}**	**0.5-20**
Fe^{2+} (H.S.)	**10^{-11}-10^{-13}**	**5-150**
Co^{2+} (H.S.)	**10^{-11}-10^{-13}**	**2-100**
Co^{2+} (L.S.)	**10^{-9}-10^{-10}**	**200-1000**
Ni^{2+}	**10^{-10}-10^{-12}**	**5-500**
Cu^{2+}	10^{-9}	1000-5000
Ru^{3+}	**10^{-11}-10^{-12}**	**2-20**
Re^{3+}	**10^{-12}-10^{-13}**	**5-20**
Gd^{3+}	10^{-8}-10^{-9}	20000-200000
Ln^{3+}	**10^{-12}-10^{-13}**	**1-100**

By using the density matrix formalism [15-17], it is possible to simulate most of the NMR experiments and to optimize either the pulse sequences or the various delays in a given pulse sequence, in order to maximize the signal intensities. With this procedure, the detection of distance constraints from NOE's

could be also pursued in unfavorable conditions. At this point the NMR experiment provides the necessary constraints to obtain the solution structure of the protein part [18-20]. We are now going to discuss the use of new additional constraints based on the electron-nucleus hyperfine coupling. These provide proton-metal ion distance information and allow for improving the resolution of the protein part and obtaining direct information on the metal coordinates within the protein frame [20]. A class of these constraints depends on the reciprocal sixth power of the metal to proton distance and another class on the reciprocal third power of the same distance. The different dependences on the distance may provide precious information related to protein mobility, as the two classes of constraints provide different average positions as a function of time.

These approaches allow researchers to obtain accurate solution structures, which may be the starting point for molecular dynamics simulations with the aim of (*i*) further refining the structure and (*ii*) providing models for mobility.

2. The proton-metal ion dipolar coupling

The presence of a paramagnetic center in a protein affects longitudinal nuclear relaxation times essentially through the electron-nucleus dipolar coupling [21], which is described by Solomon's law [10,11]:

$$T_{1M}^{-1} = \frac{2}{15}\left(\frac{\mu_0}{4\pi}\right)^2 \frac{\gamma_I^2 g_e^2 \mu_B^2 S(S+1)}{r^6}\left[\frac{7\tau_s}{1+\omega_S^2\tau_S^2} + \frac{3\tau_s}{1+\omega_I^2\tau_S^2}\right] \tag{3}$$

Eqns. (1) and (2) provide a tool for obtaining metal to proton constraints. It has been discussed [22-26] whether the recovery of magnetization in non-selective inversion-recovery experiments on proteins is exponential, and thus allow us (*i*) to estimate the diamagnetic contribution to nuclear T_1 values, and