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X-RAY ABSORPTION SPECTROSCOPIC STUDIES OF THE ACTIVE SITES OF NICKEL-AND COPPER-CONTAINING METALLOPROTEINS

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June 1993

Prepared for the Department of Energy under contract number DE-AC03-76SF00515

Printed in the United States of America. Available from the National Technical Information Service, U.S.
Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161

* Ph.D. thesis

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Abstract

X-ray absorption spectroscopy (XAS) is a useful tool for obtaining structural and chemical information about the active sites of metalloproteins and metalloenzymes.

Information may be obtained from both the edge region and the extended X-ray absorption fine structure (EXAFS) or post-edge region of the K-edge X-ray absorption spectrum of a metal center in a compound. The edge contains information about the valence electronic structure of the atom that absorbs the X-rays. It is possible in some systems to infer the redox state of the metal atom in question, as well as the geometry and nature of ligands connected to it, from the features in the edge in a straightforward manner. The EXAFS modulations, being produced by the backscattering of the ejected photoelectron from the atoms surrounding the metal atom, provide, when analyzed, information about the number and type of neighbouring atoms, and the distances at which they occur.

In this thesis, analysis of both the edge and EXAFS regions has been used to gain information about the active sites of various metalloproteins. The metalloproteins studied were plastocyanin (Pc), laccase and nickel carbon monoxide dehydrogenase (Ni CODH). Studies of Cu(I)-imidazole compounds, related to the protein hemocyanin, are also reported here.

Hemocyanin (Hc) is an oxygen transport protein containing two copper atoms in its active site. In oxy Hc (when dioxygen is bound), the EXAFS interaction between the two Cu atoms is readily seen, but in deoxy Hc (no O₂ is bound and both Cu ions are in the Cu(I) state) the Cu–Cu interaction is weak and difficult to distinguish from the strong EXAFS signal produced by imidazole rings (from histidine) ligated to both Cu. Some studies of Cu(I)-imidazole compounds were thus made, with a view towards accurately interpreting the EXAFS spectrum of hemocyanin in the reduced or deoxy state. The crystal structure of Cu(I)(*N*-methylimidazole)₂BF₄ was solved and is reported here. We also made an attempt to better characterize the EXAFS of Cu-imidazole by relating the EXAFS Debye-Waller factor from spectra of Cu(I)(*N*-methylimidazole)₄ClO₄ measured over 10–200 K to the vibrational frequencies of the Cu-imidazole interactions. We found that the EXAFS Debye-Waller factor is not determined with sufficient accuracy for us to solve an EXAFS temperature series for a unique value of a bond-stretch frequency or force constant.

Plastocyanin is a small (99-amino acid residue) protein, containing one copper atom, that functions as a electron carrier in the process of photosynthesis. This protein, along with other "blue copper" proteins, is of special interest to inorganic chemists because of its unique spectroscopic features, including an unusually intense blue colour, which arise from

an unusual tetrahedral Cu site, in which Cu is ligated with two histidines (N-ligands), a cysteine and a methionine (S-ligands). Crystal structures of this protein in both the oxidized and reduced states at several pH values have been obtained. These show that the Cu site in the reduced protein undergoes significant structural changes as the pH is varied. Most notably, the Cu–SMet bond, which is 2.90 Å at pH 7.8, shortens to 2.52 Å at pH 3.8. Our work attempted to obtain more accurately the Cu–ligand distances in the reduced protein at high and low pH's by measuring and analyzing EXAFS spectra on frozen solutions of reduced plastocyanin at pH 7.2 and pH 4.8. For both high and low pH, we found two fit minima, and while for both minima 1 S was found at 2.13–2.21 Å, the other S occurred at either 2.3–2.4 Å or greater than 2.7 Å from Cu, and for *both* high and low pH the short Cu–S' minimum fitted as well as or slightly better than the long Cu–S' minimum. This is unlikely to be a true representation of the actual Cu–SMet bond length, and most likely EXAFS is unable to resolve the weak long Cu–S contribution with sufficient accuracy given the quality and range of the data we were able to obtain.

The multicopper oxidases perform the four-electron reduction of dioxygen to water while concomitantly carrying out various other biochemical functions. Laccase is the simplest of the multicopper oxidases, containing four copper atoms, one of Type 1 (T1) copper ("blue copper", similar to the Cu in plastocyanin), one of Type 2 (T2) copper ("normal" copper) and two of Type 3 (T3) copper (in a coupled binuclear site). The T2 and T3 Cu ions occur close to each other, in a triangular array, and are jointly referred to as the trinuclear site. To aid in the elucidation of the complex chemistry of this protein, two derivatives of laccase can be prepared, one by removing the T2 Cu (Type 2-depleted or T2D laccase) and the other by replacing the T1 Cu with Hg (Type 1 Hg or T1Hg laccase). In this work, samples of T2D and T1Hg laccase were fully reduced under anaerobic conditions and then reoxidized by exposure to air. At various time points, aliquots were withdrawn and frozen, and XAS spectra were collected on the frozen samples. The edges of these XAS spectra were analyzed in order to quantitate the total redox state of the Cu in the samples. We were able to conclude that T1Hg laccase is totally oxidized when exposed to air, but in T2D laccase only the T1 Cu is oxidized while the T3 Cu remain reduced. Taken in the context of the known chemistry of laccase, this suggested that the trinuclear site is the minimum site required to perform reduction of dioxygen to water. Some studies of a similar nature on the oxygen intermediate in T1Hg laccase are also reported.

The nickel carbon monoxide dehydrogenase (Ni CODH) in *Rhodospirillum rubrum*, containing 1 Ni, ~8 Fe and ~8 inorganic S, carries out the oxidation of carbon monoxide to carbon dioxide in the presence of a one-electron reductant. It is known that CO binds at the

nickel atom, and EPR studies showed that the Fe atoms occurred as Fe_4S_4 -type cubane clusters and that Ni was furthermore probably coupled with the cubane clusters in some way. Some structural possibilities were that Ni occurred in a NiFe_3S_4 cubane cluster, or that Ni was ligated to one or two Fe_4S_4 clusters. Ni K and Fe K edges of the oxidized and reduced protein both showed no significant shift in energy with the change in redox state, and thus we concluded that Ni does indeed reside in an electronically delocalized system such as a multinuclear cluster. Using EXAFS analysis, we were able to conclude beyond doubt that Ni was not in a NiFe_3S_4 cluster. We were able to fit the data with a weak Fe-wave at ~ 2.7 Å from Ni, but since S- and N-waves fitted the data equally well, we were not able to confirm the presence of Fe at ~ 2.7 Å. We also determined that the coordination environment of the nickel atom contained two N/O scatterers at 1.85–1.89 Å and two S-type scatterers at 2.23–2.25 Å. Comparisons of the Ni K edge of Ni CODH with edges of known model compounds showed that the geometry about Ni was intermediate between square-planar and tetrahedral.

Acknowledgements

I can hardly believe that the time has come for me to leave Stanford. I have been here almost seven years, and they have been extremely good years. This is not least because I had the good fortune to join the Hodgson Group.

An integral part of being in the Hodgson Group is the "beam time experience". Oddly enough, I have come to enjoy measuring spectra at the Stanford Synchrotron Radiation Laboratory (SSRL) at all hours of the day or night, for weeks on end. I think it must be because of the camaraderie fostered by the shared enterprise of running a beam line (or two), trying as that can sometimes be. I've also enjoyed the atmosphere of SSRL itself. SSRL is full of wonderful huge machines all humming together, and also lots of wonderful, friendly people who keep the machines going, assist hapless users and somehow carry out their own experiments as well. Science doesn't get more exciting than this! SSRL radiates the feeling of a community of people who really do believe in what they are doing and love doing it, and so it's been a congenial place to work, and I think I'll actually miss it when I'm gone.

I am deeply grateful to my advisor, Prof. Keith Hodgson, for his trust in me and his patient guidance and leadership. Under him, I learned not only the technique of X-ray absorption spectroscopy, but also how to motivate myself and to be responsible for the results of my research. I really value the opportunity he gave me to grow in this way.

I also owe a debt of gratitude to the two "secret weapons" of the Hodgson Group, Drs. Britt Hedman and Pat Frank, who incidentally are also about the nicest people you could get to know. Their inexhaustible stores of knowledge, and willingness to teach, help and encourage me have meant a great deal to me. I especially thank Pat for repeatedly shoring up my confidence in myself as a scientist. He's been a real friend to me, as well as a valued colleague.

I also want to thank the other people of the Hodgson Group : Chrisie Stanfel, David Eliezer, Susan Shadle, Kent Nakagawa, Lingling Chen, Isaac Liu, Tami Westre, Heather Bufford, Kendra Rose and Holly Zhang. It's been a pleasure to know all of them. I wish them all success in completing their degrees and in their careers thereafter! I would also like to remember some members of the group who have already graduated. Jane DeWitt graduated a few months before me, and her example and encouragement were very helpful to me in the final stretch of writing up and preparing to leave. I also greatly enjoyed knowing Trevor Tyson, Soichi Wakatsuki and Uwe Spann while they were here.

I would like to thank my collaborators in the various projects that are described in this thesis. Prof. Thomas Sorrell and Dr. Martha Garrity supplied the Cu(I)-imidazole compounds studied in Chapters 3 and 4. Prof. Hans C. Freeman came all the way from Australia with samples of plastocyanin (Chapter 5), and also brought with him Paul Ellis, whose flair for computer programming has made life easier for all of us. Prof. Edward I. Solomon and his hard-working team (Dr. Jim Cole, Ed Yang, Dr. Pat Clark and Brooke Hemming) provided the many laccase samples studied in Chapter 6. And Prof. Paul Ludden, Dr. Scott Ensign and Jeff Fox supplied the samples of Ni CODH for the study in Chapter 7. Several crucial Ni model compounds for this study were supplied by Prof. Richard H. Holm.

My time at Stanford has been important to me not only for the things I have learned about Chemistry and science. I have had such fun learning ballroom dancing from Robin Rebello, together with thoroughly nice people, especially Joe Kahn. I have also had the rich joy of singing great choral works with the Stanford Symphonic Choir under the batons of Profs. William Ramsey and Gregory Wait. There's nothing quite like the thrill of launching into the "Ode to Joy" at the end of Beethoven's 9th Symphony, together with 200 other voices and 100 orchestral instruments.

But best of all has been the joy of getting to know my God and Creator more intimately. In the Chemistry Department, I have had a true sister in Christ in Cynthia Jesudason, and also Christian brothers in Will Watkins, Jon Stocker and Tom Schoch. Many friends at Peninsula Bible Church have encouraged me and taught me how to live joyfully and richly as I walk with Christ and grow in Him. Doug Goins, Chip and Frederica Stearns, Greg Donoho, Steve and Erica Lawry, and Dick and Carol Haines are particularly dear to me for their faithfulness not only in encouraging and advising me, but also in generously giving of their time and resources when I really needed them. I also have many dear friends in the Chinese Christian Fellowship at Stanford, who taught me what it means to be in the family of God. Truly, they have been to me a family away from my family, and a home away from home. With them I have also learned the joy of giving back what I have received from them, in encouragement and practical help. I would particularly like to acknowledge June Loh, who for four years was the best house-mate I could ever have asked for, and who has been a wonderful sister and friend to me. Also Charmaine Wang, my house-mate this year, another dear sister in Christ.

As I think about my time at Stanford, I also want to remember my family in Singapore, who little knew when they sent me off how long I would be gone! I really want to thank them, especially my parents, Dr. N. C. Tan and Dr. Dixie Tan, and also my sister

Jacinta Lim (like me newly a doctor, albeit a medical one) for loving me from beginning to end, and supporting me in such a long absence from home.

Above and beyond all the wonderful people I have come to know at Stanford, and the rich and unforgettable experiences that I have shared with them, is the LORD God, Who has given me all of them. He brought me here, and stayed with me and guarded me as I got to know a strange and beautiful new country and a culture less familiar than it seemed. I knew no-one when I came, but He gave me better friends here than I have ever had before. Most of all, He gave me Himself. In all ways I have been loved and given so much more than I can ever repay. Now it is time for me to leave, and while I must leave all the wonderful people and places that I have come to know here, I know that the Lord will look after me just as wonderfully well wherever He may take me next.

All the way my Saviour leads me,
What have I to ask beside?
Can I doubt His tender mercy,
Who through life has been my guide?
Heavenly peace, divinest comfort,
Here by faith in Him to dwell!
For I know, whatever befall me,
Jesus doeth all things well.

Table of Contents

Abstract	iv
Acknowledgements	vii
Table of Contents.....	x
List of Tables	xv
List of Figures	xvii
Chapter 1. Introduction	1
(A) The Scope and Organization of this Thesis	2
(B) The Usefulness of X-ray Absorption Spectroscopy in the Study of Metalloproteins	3
References	5
Chapter 2. Methodology of X-ray Absorption Spectroscopy — Data Collection, Reduction and Analysis	6
(A) What is X-ray Absorption Spectroscopy?.....	7
(1) Information From Studying XAS Edges.....	7
(2) Information From Analyzing the EXAFS Region.....	7
(B) Interpreting EXAFS Spectra.....	8
(1) The EXAFS Equation	8
(2) <i>k</i> -Weighting of EXAFS Data	9
(3) Phase Shifts and Fourier Transforms.....	10
(4) Limitations of EXAFS Analysis.....	10
(C) Details of Methodology — Experiment and Analysis.....	11
(1) Data Collection	11
(a) Sample Preparation	11
(b) Synchrotron X-rays	11
(c) Transmission Mode and Fluorescence Mode.....	13
(d) Temperature Used for Data Collection	13
(e) EXAFS Data Range	14
(2) Data Reduction	14

(a) Background Subtraction and Splining.....	15
(b) Normalized Data	16
(3) EXAFS Analysis.....	16
(a) Fourier Filtering of EXAFS Data.....	17
(b) Extraction of Parameters.....	17
(c) Parameterized Curve-Fitting of EXAFS Data	18
(d) The Parameter c_2	19
(e) The Fit Index F.....	20
References	21

Chapter 3. Crystal Structure of Bis(*N*-methylimidazole)copper(I)

Tetrafluoroborate.....	25
(A) Relevance of Simple Cu-Imidazole Compounds to Biological Studies.....	26
(B) Experimental Details	27
(C) Results and Discussion.....	28
References	29

Chapter 4. Studies of the EXAFS of a Cu(I)-Imidazole Compound

Over a Range of Temperatures.....	34
(A) Brief Overview of Hemocyanin Structure.....	35
(C) The Temperature Behaviour of the Debye-Waller Factor as a Tool For Obtaining Physically Meaningful Results From EXAFS Curve-Fitting	37
(D) Experimental Details	38
(1) Preparation of Samples.....	38
(2) Data Collection	38
(3) Data Analysis	38
(E) Results and Discussion	38
(F) Conclusion	42
References	43

Chapter 5. EXAFS Studies of Reduced Poplar Plastocyanin at High

and Low pH	53
(A) Structure and Function of Plastocyanin	54
(1) Overall Structure of Plastocyanin	54
(2) Structure of the Copper Site and Its pH Dependence	54
(3) Some Questions About the Copper Site	55

(B) Experimental Details	56
(1) Preparation of Samples	56
(2) Data Collection	56
(3) Data Analysis	56
(C) EXAFS Fits to the Data — Results and Discussion	57
(1) Extraction of Parameters and Testing on a Cu(I)-N ₂ S ₂ Model.....	57
(2) Fits to Reduced Plastocyanin at pH 7.2 and 4.8	59
(a) Fourier Transform Windows and Types of Fits Done	59
(b) Results of Two-Wave Fits.....	59
(c) Results of Three-Wave Fits.....	60
(D) Conclusion.....	63
References	65

Chapter 6. Cu Difference Edge Analysis of *Rhus vernicifera* Laccase

— A Continuing Study	81
(A) A Brief Overview of the Structure and Function of Laccase.....	82
(1) Structure and General Reaction Mechanism of Laccase	82
(2) The Usefulness of X-ray Edge Analysis	83
(3) EXAFS Studies on Laccase.....	84
(B) Experimental Details	85
(1) Preparation of Samples.....	85
(2) Data Collection	86
(3) Data Analysis	86
(a) Data Reduction	86
(b) Corrections Applied to the Laccase Edges.....	87
(c) Construction of Difference Edges.....	88
(C) Results and Discussion.....	89
(1) Reaction of T2D and T1Hg Laccase with Dioxygen.....	89
(2) The T1Hg Laccase Oxygen Intermediate.....	90
(3) Probable Geometry of T2 and T3 Cu in Laccase Samples	92
(D) Conclusion.....	93
References	94

Chapter 7. Edge and EXAFS Studies of <i>Rhodospirillum rubrum</i>	
Nickel Carbon Monoxide Dehydrogenase and Nickel	
Model Compounds	113
(A) Brief Overview of <i>Rhodospirillum rubrum</i> Ni Carbon Monoxide	
Dehydrogenase	114
(1) Information Known From Other Studies.....	114
(2) Some Questions About the Ni CODH Active Site.....	115
(B) Some Results of XAS Studies on Ni Proteins and Model Compounds	
Carried Out by Other Workers.....	115
(1) Edge Studies on Ni Models.....	115
(2) XAS Studies on Ni Proteins.....	116
(C) Experimental Details	117
(1) Preparation of Samples.....	117
(2) Data Collection	118
(3) Data Analysis	119
(D) Edge Studies.....	119
(1) Further Observations About Model Compound Edges	119
(2) Ni K and Fe K Edges of Ni CODH.....	121
(E) EXAFS Analysis of Ni CODH Samples and Related Model Compounds	122
(1) Extraction and Testing of Parameters on Model Compounds.....	122
(a) Extraction of Parameters and Tests on "Single-Shell" Compounds	123
(b) Further Testing of Parameters on Ni–N,S Compounds	124
(c) Testing for the Presence of Fe Using (Et ₄ N) ₃ [NiFe ₃ S ₄ (SEt) ₄].....	126
(2) EXAFS Analysis of <i>Rhodospirillum rubrum</i> Ni CODH Data	128
(a) Results of One-Wave Fits.....	129
(b) Results of Two-Wave Fits to Window 2	130
(c) Results of Two-Wave Fits to Window 3.....	130
(d) Results of Two- and Three-Wave Fits to Window 6.....	133
(e) Fits to the Fourier Peaks at R' = 3–5 Å From Ni in CODHRX.....	135
(F) Conclusion	137
References	139

Appendices.....181

Appendix I. Complete Tables of Fits to EXAFS Spectra of Reduced Poplar Plastocyanin at pH 7.2 and 4.8 and a Cu(I)-N,S Model Compound.....	182
Appendix II. Error Analysis for Laccase Difference Edge Analysis	197
Appendix III. Complete Tables of Fits to EXAFS Spectra of Oxidized and Reduced <i>Rhodospirillum rubrum</i> Ni CODH and Associated Model Compounds ..	204

List of Tables

Chapter 3

Table 3.1. Crystal data for Cu(I)(<i>N</i> -methylimidazole) ₂ BF ₄	30
Table 3.2. Atomic positions and equivalent isotropic displacement factors for non-hydrogen atoms.	31
Table 3.3. Bond lengths (Å) and angles (°).	32

Chapter 4

Table 4.1. Some of the more probable scattering paths for a Cu-imidazole system.	45
Table 4.2. Values of <i>c</i> ₂ obtained by using parameters extracted from data collected at 10 K and varying <i>c</i> ₂ only.	46
Table 4.3. Amplitude parameters obtained by varying six amplitude and phase parameters (<i>c</i> ₀ , <i>c</i> ₂ , <i>c</i> ₃ , <i>a</i> ₀ , <i>a</i> ₁ and <i>a</i> ₂) for the closest fit to data at each temperature.	47

Chapter 5

Table 5.1. Least-squares fits to CuI ₂ ((CH ₃ NCH ₂ CH ₂ SSCH ₂ CH ₂ -N(CH ₃) ₂) ₂ (BF ₄) ₂	66
Table 5.2. Results of Type 2 fits (varying distances and coordination numbers while fixing Debye-Waller factors) made to CuI ₂ ((CH ₃ NCH ₂ CH ₂ S-SCH ₂ CH ₂ N(CH ₃) ₂) ₂ (BF ₄) ₂	67
Table 5.3. Fourier transform windows filtering data used in curvefitting of reduced Pc data	68
Table 5.4. Fits made to reduced Pc data at pH 7.2 (PCHI) and pH 4.8 (PCLO)	68
Table 5.5. Two-wave Type 2 fits (varying distances and coordination numbers while keeping Debye-Waller factors fixed) to reduced Pc at pH 7.2 (PCHI) and pH 4.8 (PCLO).	69
Table 5.6. Three-wave Type 2 fits (varying distances and coordination numbers while keeping Debye-Waller factors fixed) to PCHI and PCLO.	70
Table 5.7. Results of some Type 3 fits (varying distances and Debye-Waller factors while keeping coordination numbers fixed) to reduced Pc at high pH (PCHI) and low pH (PCLO).	71
Table 5.8. Type 2 (varying R's and CN's) and Type 3 (varying R's and <i>c</i> ₂ 's) fits to Window 3 of reduced Pc at pH 7.2 (PCHI), made to compare with the distances found in the crystal structure at pH 7.0.	72

Chapter 6

Table 6.1. Samples of laccase for which edge spectra were collected	96
Table 6.2. Edge spectra of laccase used in difference edge analysis, with corrections for native content and oxidation of samples as indicated	99
Table 6.3. Difference edges constructed from corrected laccase edges — "100% reduced Δ standards"	101
Table 6.4. Difference edges constructed from corrected laccase edges — amount of reduced Cu is determined from standards in Table 6.3.....	102
Table 6.5. Normalized difference edge energies and amplitudes at peak maxima	103

Chapter 7

Table 7.1. Samples of Ni CODH on which data were collected (does not include one sample collected in April 1990, which was not analyzed).	142
Table 7.2. Ni model compounds used in this study.....	143
Table 7.3. Results from tests of Ni–N, Ni–S and Ni–Fe parameters on "single- shell" compounds (data range $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$).....	145
Table 7.4. Crystallographic bond distances for some Ni compounds of various coordination geometries	146
Table 7.5. Fits made to Ni–N,S compounds	148
Table 7.6. Results of Type 2 fits (varying CN's and fixing c_2 's) made to Ni–N,S models.....	149
Table 7.7. Results of some Type 3 fits (fixing CN's and varying c_2 's) made to Ni– N,S model compounds.....	150
Table 7.8. Results of Type 2 (varying CN's and fixing c_2 's) and Type 3 (fixing CN's and varying c_2 's) fits made to $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$	151
Table 7.9. Fourier transform windows for $R' < 3 \text{ \AA}$ used for curvefitting of Ni CODH data.	152
Table 7.10. Fits made to Ni CODH data for $R' < 3 \text{ \AA}$	153
Table 7.11. Results of some of the Type 2 fits (varying CN and fixing c_2) for $R' <$ $3 \text{ \AA}$ made to five Ni CODH data sets.	154
Table 7.12. Results of some of the Type 3 fits (fixing CN and varying c_2) for $R' <$ $3 \text{ \AA}$ made to five Ni CODH data sets.	156
Table 7.13. Some Type 2 fits (varying CN's and fixing c_2 's) made to signals at $R' = 3\text{--}5 \text{ \AA}$ in CODHRX.	158

List of Figures

Chapter 2

Figure 2.1. A typical X-ray absorption spectrum.	22
Figure 2.2. EXAFS of a compound where the absorbing atom is surrounded by four equidistant scatterers.....	23
Figure 2.3. Schematic representation of the experimental setup used to collect XAS data in (a) transmission mode, (b) fluorescence mode.	24

Chapter 3

Figure 3.1. Molecular geometry and atomic numbering.....	33
Figure 3.2. Stereographic projection of the unit-cell contents.	33

Chapter 4

Figure 4.1. Schematic of a Cu–imidazole ring.....	45
Figure 4.2. Averaged EXAFS spectra of Cu(I)(<i>N</i> -methylimidazole) ₄ ClO ₄	48
Figure 4.3. Fourier transforms over $k = 3.5\text{--}16.5$ (0.1) Å ⁻¹ of the averaged EXAFS spectra of Cu(I)(<i>N</i> -methylimidazole) ₄ ClO ₄	49
Figure 4.4. Contour plot of $F(\mu, \nu)$ for c_2 values (listed in Table 4.2) from fits to the first shell.....	50
Figure 4.5. Contour plot of $F(\mu, \nu)$ for c_2 values (listed in Table 4.2) from fits to the second shell.	51
Figure 4.6. Contour plot of $F(\mu, \nu)$ for c_2 values (listed in Table 4.2) from fits to the third shell.	52

Chapter 5

Figure 5.1. (a) A stereoview of the C α framework of Poplar Cu ^{II} -plastocyanin. The Cu-binding residues are drawn in black. (b) Stereoview of the Cu site in Cu ^{II} -plastocyanin, showing all non-hydrogen atoms of each ligand residue. (c) Dimensions (in Å) of the copper site in reduced plastocyanin at six pH values.....	73
Figure 5.2. EXAFS data and Fourier transform for Cu ^I ₂ ((CH ₃ NCH ₂ CH ₂ S- SCH ₂ CH ₂ N(CH ₃) ₂)(BF ₄) ₂	74
Figure 5.3. EXAFS data and Fourier transform for reduced Pc at pH 7.2 and 4.8.....	75
Figure 5.4. Reduced Pc EXAFS data Fourier-transformed over $k = 3.5\text{--}12.5$ (0.1) Å ⁻¹	76
Figure 5.5. N+S+S' fit (short-S' minimum) to Pc data at pH 7.2.	77

Figure 5.6. N+S+S' fit (long-S' minimum) to Pc data at pH 7.2.....	78
Figure 5.7. N+S+S' fit (short-S' minimum) to Pc data at pH 4.8.....	79
Figure 5.8. N+S+S' fit (long-S' minimum) to Pc data at pH 4.8.....	80

Chapter 6

Figure 6.1. X-ray absorption edges of Cu(I) model compounds.....	104
Figure 6.2. Difference edges of Cu(I) compounds when the edge of [Cu ₂ (L ₈ - Et)(OAc)](ClO ₄) ₂ is subtracted.....	105
Figure 6.3. Edges of resting and totally oxidized (H ₂ O ₂ -treated) laccase.....	106
Figure 6.4. T2D laccase sample series showing reactivity with dioxygen.	107
Figure 6.5. T1Hg laccase sample series showing reactivity with dioxygen.....	108
Figure 6.6. X-ray edges of some T1Hg O ₂ intermediate samples, with fully reduced and fully oxidized T1Hg laccase.	109
Figure 6.7. Corrected difference edges of "100% reduced ΔT1Hg" (see Table 6.3) and of some T1Hg O ₂ intermediates with fully oxidized T1Hg subtracted.	110
Figure 6.8. Difference edges of "100% reduced ΔT2", constructed by various methods (see Table 6.3), and a corrected difference edge of one sample of T1Hg O ₂ intermediate.....	111
Figure 6.9. Corrected difference edges of various forms of laccase with CUL2 subtracted.	112

Chapter 7

Figure 7.1. Possibilities for the Ni environment in <i>Rhodospirillum rubrum</i> Ni CODH.....	159
Figure 7.2. Edges of square-planar and tetrahedral Ni compounds.....	160
Figure 7.3. Edges of trigonal bipyramidal and octahedral Ni compounds.	161
Figure 7.4. Ni compounds with edges of unusual appearance.	162
Figure 7.5. Edges of a series of trigonal bipyramid compounds with Ni-NS ₃ L ligation.	163
Figure 7.6. Edge of CODH (ox) (1992) compared with model compound edges.....	164
Figure 7.7. Ni K edges of oxidized and reduced Ni CODH samples measured in 1991 and 1992.	165
Figure 7.8. Ni K edges of oxidized CODH samples measured in 1989, 1991 and 1992.....	166
Figure 7.9. Fe K edges of oxidized and reduced Ni CODH (1991 data), and (Et ₄ N) ₃ [NiFe ₃ S ₄ (SEt) ₄].	167

Figure 7.10. EXAFS spectra of Ni CODH protein.	168
Figure 7.11. Fourier transformed Ni CODH protein data.	169
Figure 7.12. Fourier transforms over $k = 3.0\text{--}12.0$ (0.1) $\text{\AA}^{-1}$ of CDHOX (oxidized, July 1992) and CODHRX (reduced, July 1991), showing the Fourier windows used to generate filtered EXAFS data for curve- fitting.	170
Figure 7.13. Examples of reverse Fourier transformed data ("filtered" data), to which various fits were made	171
Figure 7.14. Type 2 fits (varying R and CN) made to Window 3 of CODHX (oxidized, June 1989).	172
Figure 7.15. Type 2 fits (varying R and CN) made to Window 3 of CODHRX (reduced, July 1991).	173
Figure 7.16. EXAFS spectra of the cubanes $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$ and $(\text{Et}_4\text{N})_2[\text{NiFe}_3\text{S}_4(\text{PPh}_3)(\text{SEt})_3]$, and CODHX (oxidized CODH, June 1989)	174
Figure 7.17. Fourier-transformed spectra of the cubanes $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$ and $(\text{Et}_4\text{N})_2[\text{NiFe}_3\text{S}_4(\text{PPh}_3)(\text{SEt})_3]$, and CODHX (oxidized CODH, June 1989).	175
Figure 7.18. Filtered EXAFS data from CDHOX (Window 6), with two- and three-wave Type 3 fits (varying R and c_2).	176
Figure 7.19. Filtered EXAFS data from CODHRX (Window 6), with two- and three-wave Type 3 fits (varying R and c_2).	177
Figure 7.20. Fourier transforms of filtered (Window 6) and unfiltered protein data and some Type 3 N+S+S' fits to the filtered data.	178
Figure 7.21. Fourier transforms of filtered (Window 6) and unfiltered protein data and some Type 3 N+S+Fe fits to the filtered data.	179
Figure 7.22. The core of $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$, with Ni attached to one of the S(Ph) atoms.	180

Chapter 1

Introduction

(A) The Scope and Organization of this Thesis

This thesis presents results of a portion of the experiments in which I have been involved for the past six years. Except for the crystal structure reported in Chapter 3, all of these experiments utilized X-ray absorption spectroscopy (XAS) to extract structural and, to a lesser extent, chemical information about metalloproteins. The information was extracted by empirical comparison with small-molecule complexes of known structure (either direct visual comparison (for edges, *vide infra*) or curvefitting using empirical parameters extracted from known compounds (for EXAFS, *vide infra*), rather than through simulation of the XAS spectra by *ab initio* calculations. As such, there is also considerable discussion of the XAS spectra of these "model" compounds.

Chapter 2 gives an introduction to the technique of X-ray absorption spectroscopy. It explains in brief how it occurs, how the XAS data are measured, and how the data are analyzed. It is not possible to give a complete account of the technique in the space of one chapter, but hopefully sufficient background has been provided to render the rest of the thesis intelligible without recourse to outside references.

All the experiments described were undertaken in the context of continuing research into the chemistry of the metalloproteins studied by various methods. Since four different metalloprotein systems were studied, background information on the respective proteins (their chemistry and, where relevant, results from XAS studies undertaken by others) is briefly described at the beginning of the chapters dealing with results of XAS studies on them, rather than in this introduction. Once again, these reviews are not exhaustive, aiming only to provide a context for evaluation of the results discussed in that chapter.

Information from XAS studies may be subdivided into two major categories : information from the edge region, and information from the extended X-ray absorption fine structure (EXAFS) or post-edge region of the spectrum of a metal center in a compound. The edge region contains information about the valence electronic structure of the atom that absorbs the X-rays and so yields information of a chemical nature about the metal atom. On the other hand, the EXAFS region can yield metrical information about the environment of the metal atom, giving the number, type and distances of atoms surrounding the metal atom in question. Analyses in both categories are reported in this thesis.

Some portions of this thesis, as indicated by the references following, have already been published. Other portions, in particular most of Chapter 7, will be published soon. Chapters 3 and 4 describe crystallographic¹ and EXAFS studies undertaken on Cu(I)-imidazole compounds. These compounds were studied as models for the binuclear copper

protein hemocyanin. Chapter 5 describes an EXAFS study of poplar (*Populus nigra*, var. *italica*) plastocyanin, a small protein containing one copper atom. Chapter 6 describes some edge studies² of Japanese lacquer tree (*Rhus vernicifera*) laccase, which contains four copper atoms. Chapter 7 reports an extensive edge and EXAFS study³ of the nickel site of *Rhodospirillum rubrum* (a photosynthetic bacterium) nickel carbon monoxide dehydrogenase (Ni CODH).

(B) The Usefulness of X-ray Absorption Spectroscopy in the Study of Metalloproteins

X-ray absorption spectroscopy provides information of a nature complementary to that from more widely used spectroscopic techniques such as UV-visible or EPR spectroscopy. Since it is an absorption spectroscopy, rather than a diffraction or scattering technique, it can also be performed on compounds in any physical state, whether crystalline, amorphous, in solution, in membranes, bound to a surface or even gaseous. For proteins, X-ray absorption spectra are usually measured on solutions.

Features in the XAS edge can be interpreted as bound-state electronic transitions, in a manner analogous to those in optical spectroscopy. However, since the transitions are from the core electron orbitals to the valence orbitals, rather than within the valence shell, different selection rules apply. For example, Cu(I), which is difficult to probe spectroscopically because of its filled 3d shell (3d¹⁰), displays 1s→4p transitions which are easily interpreted for geometrical and electronic structural information (see Chapter 6).

EXAFS spectroscopy is one of the few techniques that directly yields metrical details (distances and numbers of atoms) about compounds being studied. As such, its value is obvious in the study of systems which cannot be, or have not yet been, crystallized for study by X-ray crystallography. Even for proteins for which the crystal structure has been solved, EXAFS analysis still has a contribution to make.

Protein crystallography gives a three-dimensional structure of the whole protein molecule. However, since the protein molecule is often extremely large, the accuracy with which it can do this is often limited. Interatomic distances are known with accuracies of 0.05 Å at best⁴ for small proteins (such as plastocyanin). For large proteins (such as hemocyanin), the accuracy is considerably worse, being typically about ±0.2–0.4 Å.^{5,6} Also, it can be hard to detect the presence of small moieties bound to the active site, such as water molecules or hydroxide ions, by crystallography, especially with lower resolution data.

On the other hand, EXAFS spectra give information only about the local environment for atoms of a specific type within a protein. If metal atoms of different types occur in a

protein (e.g. Ni and Fe in Ni CODH), XAS can be performed at different energies to give information on the environment around first one type of atom (say, Ni), then the other (say, Fe). Interatomic distances of up to about 4 Å from the metal atom (in protein systems) can be determined to an accuracy of ± 0.03 Å or better.⁷⁻⁹ This improvement in accuracy makes a great difference in the ability to understand and make models for the reactions at the active site of a protein. The numbers of atoms around the metal atom absorbing the X-rays can generally be determined with an accuracy of about 25%.⁷⁻⁹ This also is often sufficient to resolve details that protein crystallography cannot. Chapter 5 reports work in which EXAFS spectroscopy was used on a protein for which the structure has already been determined by X-ray crystallography.

EXAFS spectroscopy is, however, limited in that where two or more atoms of the same type are found, it will give information about the averaged environment for all of them, regardless of their individual redox states or chemical environments. Also, whereas crystallography produces a three-dimensional structure, EXAFS spectroscopy on proteins in solution yields only one-dimensional information, simply giving the radial distance from the origin atom at which other atoms occur without giving any indication of the angles between them. Thus, if several different types of atoms occur at nearly the same distance from the origin atom, EXAFS analysis may not be able to resolve their positions. It also gives no direct information about the geometry around the origin atom, though it may be combined with observations made about the edge region to infer geometrical information. (This is done in Chapter 7.) Geometrical information can, however, be directly obtained if polarized EXAFS spectroscopy is performed on protein crystals.

X-ray absorption spectroscopy therefore occupies a unique place in the set of tools that we may bring to bear on a problem of chemistry. It is furthermore a relatively young technique, having been used to provide structural information for only about the past twenty years, and its full potential as a tool for structural analysis is still being explored. For example, analysis of multiple-scattering EXAFS interactions, besides obtaining more accurate distance information, can provide information about the angular distribution of the other atoms around the atom absorbing the X-rays. This technique is not yet fully reliable when applied to systems as complicated as proteins, but it is still evolving. This thesis does not include multiple-scattering analyses, but otherwise provides ample demonstration of both the strengths and limitations of X-ray absorption spectroscopy in its current state when applied to extract information about metalloproteins.

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Chapter 2

Methodology of X-ray Absorption Spectroscopy — Data Collection, Reduction and Analysis

(A) What is X-ray Absorption Spectroscopy?

X-ray absorption spectroscopy (XAS) involves the measurement of the absorption coefficient μ of a compound of interest as a function of energy. However, instead of probing the transitions among partially filled orbitals, X-rays have high enough energy (measured in eV) to excite electrons out of completely filled inner shell orbitals. An X-ray absorption spectrum has a general decreasing trend, interrupted by sharp upward steps (Figure 2.1(a)). These sharp rises in absorption, referred to as "edges", are observed when the monochromatized X-ray beam has an energy equal to or greater than the ionization energy of an electron being excited. Edges are labelled according to the orbital from which the electron has been excited, e.g. a Cu K edge refers to the excitation of an electron from the innermost (K) shell of Cu. Immediately after an edge, the absorption manifests a series of oscillations, or fine structure, of decreasing amplitude (Figure 2.1(b)). This region of the spectrum is called the extended X-ray absorption fine structure, or EXAFS, region. The region of the spectrum just before an edge is referred to as the pre-edge region.

(1) Information From Studying XAS Edges

If the "edge" is examined with good energy resolution, features within the "edge jump" can be discerned (Figure 2.1(c)), and these can be interpreted in terms of bound-state transitions. In particular, in K edge XAS on first-row transition metal systems, these "edge features" can be interpreted in terms of the promotion of a 1s electron to the partially filled 3d orbitals and vacant 4p orbitals, and so the appearance of the edge contains information about the valence electronic structure of the compound. The edge is thus a valuable tool for probing the geometry, oxidation state or nature of ligands for a sample in which these are not known. In some cases, ligand field arguments can be used to correlate features in the edge to the geometry of the compound in a straightforward manner. For example, the spike at ~8337 eV in Figure 2.1(c) indicates that the compound is a square-planar nickel compound. Work done along these lines in this thesis can be found in Chapter 6, Section (A.2) and Chapter 7, Sections (B.1) and (D.1) – (D.2).

(2) Information From Analyzing the EXAFS Region

The EXAFS region occurs at energies just above the edge. At these energies, the photoelectron escapes from the absorbing atom into the continuum. The photoelectron wave then scatters off neighbouring atoms and some of it returns to the absorbing atom, interfering with the outgoing wave and modulating the absorption μ of the compound. Thus, the EXAFS region contains information about the atoms surrounding the absorbing atom — the number of neighbours, the types of atoms and the distances at which they

occur.^{1,2} The manner in which this information is encoded is explained in greater detail in the next section, and the manner in which the information is extracted is explained in the section after that.

(B) Interpreting EXAFS Spectra

(1) The EXAFS Equation

The photoelectron ejected from the atom absorbing the X-rays may be pictured as a spherical wave propagating outwards from the origin (Figure 2.2). This spherical wave impinges upon the atoms surrounding the absorbing atom and some of the wave is scattered back to the absorbing atom. (Hence, the neighbouring atoms are sometimes referred to as "scattering atoms" or "scatterers".) The energy of the ejected electron increases with the energy of the incident X-rays, and the wavelength of the photoelectron becomes shorter as we scan to higher energies. Thus the backscattered waves oscillate between constructive and destructive interference with the outgoing electron wave at the absorbing atom (Figures 2.2 (a) and (b) respectively), and we observe the sinusoidal modulations of the absorption spectrum that are characteristic of EXAFS.^{1,2}

The amplitude of the EXAFS signal depends on the number and size (in terms of electron density) of scatterers, and the phase of the signal depends both on the types of the scatterers and the distance from the absorbing atom at which they occur. These relations are expressed in the EXAFS equation.³ For the case where the outgoing wave is scattered only once before returning to the absorbing atom (called "single scattering"), the EXAFS equation takes the following form :

$$\chi(k) = \sum_s N_s \frac{|f_s(\pi, k)|}{k R_{as}^2} \exp(-2\sigma_{as}^2 k^2) \exp\left(\frac{-2R_{as}}{\lambda}\right) \sin[2kR_{as} + \alpha_{as}(k)] \dots\dots\dots(2.1)$$

where N_S is the number of scattering atoms of type S at an average distance R_{as} from the absorbing atom. $|f_S(\pi, k)|$ is the backscattering amplitude and $\alpha_{as}(k)$ is the phase shift of the scattering atom; both are dependent on backscattering atom type. λ is the mean free path which accounts for the finite lifetime of the photoelectron, and k is the photoelectron vector, explained below. The Debye-Waller factor, $\exp(-2\sigma_{as}^2 k^2)$, accounts for thermal and structural disorder in a given coordination sphere, with σ_{as}^2 defined as the root-means-square variation of atomic distances from the average R_{as} .

The EXAFS for any absorbing atom/scattering atom pair is represented as a damped sine wave with amplitude $\{N_S |f_S(\pi, k)| \exp(-2\sigma_{as}^2 k^2) \exp(-2R_{as}/\lambda) / [k(R_{as}^2)]\}$, frequency $(2R_{as})$ and phase shift $(\alpha_{as}(k))$ characteristic of the atoms involved. The total EXAFS is

given by the summation of the individual sine waves which describe each absorbing atom/scattering atom interaction.

As shown above, the EXAFS signal is expressed as a function of k , the photoelectron vector, in units of $\text{\AA}^{-1}$, but the absorption data are measured as a function of energy (in eV). The relationship between energy and k is given by :

$$k = \sqrt{\frac{2m_e(E - E_0)}{\hbar^2}} \dots\dots\dots(2.2)$$

where m_e is the electron mass, E is the photon energy (of the incident X-rays), $\hbar$ is Planck's constant divided by 2π , and E_0 is the threshold energy (at which the photoelectron has just sufficient energy to escape the absorbing atom), where k is defined to be zero.

The observed modulation of the absorption coefficient μ is therefore the superposition of the various damped sine waves resulting from the interference of the outgoing photoelectron wave with the wave backscattered from the neighbouring atoms, as measured at the location of the absorbing atom. The types of scattering atoms surrounding the absorbing atom can be recognized by analyzing the EXAFS spectrum for the characteristic amplitude $|f_S(\pi, k)|$ and phase shift ($\alpha_{as}(k)$), the number of scatterers by analyzing for N_S , and the distances at which the scatterers occur by analyzing for R_{as} .

(2) k -Weighting of EXAFS Data

The amplitude of the EXAFS weakens at higher values of k due to the $1/k$ dependence of the EXAFS expression, and also because of the damping by the exponential Debye-Waller and lifetime limit (mean free path) terms. This damping effect can be compensated for in the data analysis by k -weighting the EXAFS signal $\chi(k)$. Also, the characteristic amplitude $|f_S(\pi, k)|$ for each type of scattering atom has a maximum at some value of k , and this maximum occurs at higher k for heavy scatterers such as metal atoms. Therefore, k -weighting enhances the contribution the the EXAFS from heavier scatterers, and this is particularly desirable for analysis of metalloprotein EXAFS. Another reason to k -weight the EXAFS is that at higher values of k the single-scattering approximation holds more accurately, and so the simple form of the EXAFS equation shown above can be used to analyze the data. Therefore, all the EXAFS analysis reported in this thesis was carried out on EXAFS spectra weighted by k^3 . This weighting scheme has proven to be the best compromise, enhancing the data at higher values of k without overemphasizing the increased noise level at higher k .

(3) Phase Shifts and Fourier Transforms

The contribution of each scatterer to the EXAFS can be conveniently visualized by applying a Fourier transform to the k -weighted data. However, the distances at which the Fourier transform peaks appear (denoted R') do not represent accurately the distances between absorber and scatterers. This is because the EXAFS signal for each scatterer includes a k -dependent phase shift ($\alpha_{as}(k)$ in Equation 2.1), which results in a sine-wave frequency of $[2R_{as} + \alpha_{as}(k)/k]$ or $[2R_{as} + a_1]$, shifting the Fourier peak by $a_1/2$ Å. The magnitude of the shift depends on the type of scatterer, but, generally, R' is about 0.4 Å shorter than the true distance R_{as} (which is simply designated R in later chapters).

The Fourier transform is also a valuable tool for separating contributions to the EXAFS at different R_{as} values, so that components of the EXAFS may be isolated and fitted separately, simplifying the analysis.

(4) Limitations of EXAFS Analysis

Since the EXAFS amplitude is proportional to $(R_{as})^2$, the same atom at longer distances would contribute less to the EXAFS than one nearby. Thus EXAFS analysis can only yield structural information within a limited radius of the absorbing atom. Also, in complex environments like those found in metalloproteins, disorder in the scatterer distances results in further reduction of the EXAFS signal, especially at higher k values. The result is that EXAFS on proteins is usually limited to providing information within 4 Å of the absorbing atom. Also, if metal atoms are found in two or more different environments in a metalloprotein, EXAFS will provide structural information averaged over all sites.

The analysis done in this thesis is also limited in that it takes no account of multiple scattering, wherein the outgoing photoelectron wave scatters from more than one neighbouring atom before returning to overlap with the outgoing wave at the origin. This effect is more significant at lower values of k , where the photoelectron may be pictured as travelling more slowly and more apt to be deflected by neighbouring atoms. Thus our EXAFS analysis does not include data below $k = 3$ Å⁻¹. However, even above $k = 3$ Å⁻¹ significant multiple scattering occurs in rigid systems, in particular the imidazole ring of histidine, which is known to be a ligand in almost all the proteins studied in this thesis. Work is in progress to characterize the EXAFS of imidazole more accurately, but it is only now reaching a stage where it can be reliably applied to proteins. Bearing all this in mind, however, careful use of the single-scattering paradigm can still produce reliable results from EXAFS analysis.

(C) Details of Methodology — Experiment and Analysis

(1) Data Collection

(a) Sample Preparation

XAS data were collected on two types of samples for this thesis: protein solutions, and solid samples of small-molecule compounds which served as models for the metal environment in the protein.

Model compounds samples were prepared by grinding the solid sample together with boron nitride powder, which was used as a diluent. The uniformly mixed powder was packed into an aluminium spacer, made out of a piece of aluminium 1 mm thick with a slot $4 \times 18 \text{ mm}^2$ in size cut through the thickness. The slot was covered with mylar tape both front and back, so that the sample was held between the tape windows, and the path length of the sample was 1 mm. The amount of sample considered optimum was calculated by estimating the absorption coefficient of the sample above and below the edge, and finding the amount of sample that would produce a significant absorption without decreasing the beam intensity too much.

All protein samples were run as dilute frozen solutions, in lucite or lexan cells with a front face of thin mylar or Kapton tape that allowed X-ray fluorescence to escape. A more concentrated sample gave a larger and less noisy fluorescence signal, but the concentrations achievable were limited by the viscosity of the protein and the tendency to denature above certain concentrations. The quality of data collected was also affected by the homogeneity of the sample. A clear frozen glass was most desirable, but not always achievable. Oftentimes the glass produced had cracks or bubbles in it, both of which increase noise in the fluorescence signal collected. Less often, a sample froze as ice instead of glass. Ice increases the noise in the data collected, and can sometimes produce diffraction peaks and larger background features which completely ruin an EXAFS scan. In order to avoid ice formation, glycerol or glycol was usually added to a sample. Unfortunately, glycerol also produces free radicals in the X-ray beam, which cause photoreduction in some susceptible proteins (such as T1Hg laccase, see Chapter 6).

(b) Synchrotron X-rays

All data were collected using synchrotron radiation, either at the Stanford Synchrotron Radiation Laboratory (SSRL) or at the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratories. The high intensity of synchrotron radiation, especially at wiggler beam lines (which have an order of magnitude more flux than bending

magnet beamlines),⁴ makes for better signal to noise in the data, especially when measuring the weak fluorescence signals produced by protein samples.

As produced by the synchrotron ring, synchrotron radiation is broad-spectrum, "white" light. For the purpose of collecting EXAFS spectra, it is normally monochromatized by Bragg diffraction at an angle θ from a crystal :

$$n\lambda = 2d\sin\theta \quad \dots\dots\dots(2.3)$$

For the data in this thesis, a double crystal monochromator was used. It consists of two silicon crystals mounted parallel so that the beam is diffracted twice, first from one, then from the other, so that the exit beam propagates in the same direction as the incident beam. For a flat crystal monochromator, the energy resolution δE of the monochromatized X-ray beam is given by⁵ :

$$\delta E = E \cot\theta \delta\theta \quad \dots\dots\dots(2.4)$$

where θ is the angle for Bragg diffraction from the crystal, and $\delta\theta$ is the angular divergence of the X-ray beam. Sometimes, the [111] planes of the crystal are presented to the beam, and sometimes, the [220] planes are presented. In the latter case, the d -spacing of the lattice is smaller (therefore, for a given energy E or wavelength λ , the Bragg angle θ is larger), and so the monochromatized beam that emerges has a narrower bandpass, and spectra collected using this beam have a higher energy resolution than spectra collected using a Si[111] monochromator. Another factor in the monochromaticity of the beam is the degree to which it has been collimated prior to diffraction from the monochromator crystals (represented by $\delta\theta$ in Equation 2.4). Thus, a beam passing through a 1-mm monochromator entrance slit has a higher energy resolution than one passing through a 2-mm slit. δE is also influenced by many other factors, such as the mosaicity of the crystal, which affect the rocking curve of the crystal.

Higher harmonics (at double or triple the energy) in the X-ray beam can be excluded either by using mirrors, which are designed to absorb rather than reflect all radiation above a cutoff energy, or by "detuning" the monochromator, that is, very slightly misaligning one of the two parallel monochromator crystals. The higher harmonics are excluded to a much greater extent than the fundamental because of their much narrower rocking curves. All the samples reported in this thesis were measured with 50% detuning, that is, the incident intensity was reduced 50% by detuning in order to exclude harmonics.

(c) Transmission Mode and Fluorescence Mode

As alluded to above, data were collected in either transmission mode or fluorescence mode (see Figure 2.3). The former was used for model compounds, where the absorption by the metal atom is large relative to the total flux, and the latter for the dilute protein samples, where the edge jump due to the metal atom is small, not only because the sample is dilute, but also in comparison with the total absorption by the large protein molecule. The X-ray fluorescence measured is emitted when electrons in higher orbitals of the absorbing atom descend to fill the vacancy in the 1s orbital.

In both cases, incident and transmitted flux were measured by using ionization chambers filled with flowing N₂ gas (I_0 , I_1 and I_2 in Figure 2.3).⁶ Internal calibration standards were collected simultaneously with each scan on every sample by placing a thin foil of the appropriate element (copper or nickel) behind the sample, between I_1 and I_2 .⁷ In transmission mode, the absorption spectrum of the compound is given by $[\ln(I_0/I_1)]$. In fluorescence mode, the spectrum is given by (I_F/I_0) , and I_1 and I_2 are required only to measure the calibration standard.

The quality of data collected by fluorescence can be improved by using a more sensitive detector. Two types of detectors were used in collecting protein data. The first was an ionization chamber⁸ filled with a flow of argon gas, and equipped with a filter (to exclude fluorescence background at energies below the signal of interest) and a Soller slit, which is a screen of silver vanes in a fanlike pattern (to minimize detection of randomly scattered radiation from sources other than the sample, in particular, fluorescence produced in the filter). This detector was suitable for more concentrated proteins, since it was less apt to reach saturation. For more dilute samples, a 13-element germanium solid-state detector array⁹ was used. This detector was more sensitive, and could also be windowed to include only the energies of interest, i.e. the K α fluorescence from copper or nickel.

(d) Temperature Used for Data Collection

Because the EXAFS signal is damped by thermal disorder in the scatterers (see Equation 2.1), data were collected at as low a sample temperature as possible in order to maximize the EXAFS signal. We used an Oxford Instruments CF1208 continuous-flow liquid helium cryostat with a temperature range of 4–200 K to collect all our data. Most of our EXAFS data were collected at 10 K, for which we reckoned that all atoms would be at or close to zero-point motion.

(e) EXAFS Data Range

When collecting data, every effort was made to collect EXAFS to as high a k value as possible. The greater the k -range of EXAFS data, the more information it contains. A longer k -range allows more accurate determination of scatterer distances, and the possibility of distinguishing scatterers that are at similar distances. For example, data to $k = 15 \text{ \AA}^{-1}$ can be used to distinguish two scatterers of the same type with R_{as} differing by $0.1 \text{ \AA}$. It also allows a more accurate determination of the number of scatterers N_S , because the effect on the amplitude of the linear term N_S and the exponential terms $\exp(-2\sigma_{as}^2 k^2)$ (Debye-Waller factor) and $\exp(-2R_{as}/\lambda)$ (lifetime) can be better distinguished.

However, the range of protein EXAFS data collected is limited by two factors. Firstly, the metalloprotein solution is dilute, and so at a certain value of k the noise overwhelms the EXAFS signal. However, the signal-to-noise ratio in the data can be improved to a certain extent simply by collecting as many scans as time allows (up to 30 or 40) on the sample. In extremely favourable circumstances (a concentrated protein solution and/or a clear glass), data may be collected as far as $k \sim 15 \text{ \AA}^{-1}$. More usually, the scan-averaged EXAFS signal can only be traced out as far as $k \sim 12$ or $13 \text{ \AA}^{-1}$. Secondly, many proteins contain more than one type of metal. In particular, most copper proteins contain some zinc. Thus, when scanning a copper protein, the EXAFS can be seen until the zinc edge is reached, and then the small copper EXAFS signal is swamped by the rising zinc edge, which occurs just above $k = 13 \text{ \AA}^{-1}$.

(2) Data Reduction

Data reduction and analysis were accomplished using the software package XFPACK.¹⁰

First, each scan is subjected to a one-point energy calibration. The monochromator motorstep value at which a well-defined feature of the calibration standard (in this case the first inflection point of copper or nickel foil) occurs is set to be equal to a known energy value (8980.3 eV and 8331.6 eV for copper and nickel respectively), and all the other data points are assigned energy values relative to this one point.

The individual scans in a data set are each inspected and then averaged together. Monochromator glitches are then removed if necessary from the averaged data set. This data set is then subjected to background reduction and normalization, allowing the EXAFS to be extracted.

(a) Background Subtraction and Splining

EXAFS ($\chi(k)$) is defined as the modulation of the absorption coefficient, μ , and can be expressed as

$$\chi(k) = (\mu - \mu_0)/\mu_0 \quad \dots\dots\dots(2.5)$$

where μ_0 is the free-atom absorption coefficient which would be observed if the sample contained only the absorbing atom without any neighbouring atoms. In Equation 2.5, the EXAFS modulations are isolated from, and normalized to, the free-atom absorption, effectively yielding information on a per-atom basis. In an XAS experiment, the absorption (or fluorescence) measured consists of the absorption coefficient, μ , plus a background contribution, μ_{back} , arising from absorption from lower Z atoms in the sample and scatter from the sample, as well as absorption by windows, tape or air path. The absorption background μ_{back} decreases monotonically with increasing energy. In principle, the absorption of a blank sample containing everything except the absorbing species of interest should be used to subtract μ_{back} . In practice, it is difficult to create an accurate blank. So μ_{back} is approximated by a polynomial, which is fitted to the data in either the pre-edge or the post-edge region, extrapolated through the rest of the data, and then subtracted. By this method, there is still some residual background, μ_{res} , left in the data, and this is removed in the next step.

The free-atom absorption μ_0 cannot be measured and so must be approximated in some way. Since μ_0 is a smooth curve on which the EXAFS oscillations are superimposed, it can be modelled by a polynomial spline fitted to the data in the post-edge or EXAFS region. This method also removes the residual background μ_{res} . A different value of μ_0 must be used in the denominator of Equation 2.5 because the spline approximation to μ_0 includes μ_{res} . The value of μ_0 used for normalization is usually calculated by using the Victoreen formula.¹¹

The determination of μ_0 by the spline curve-fitting method is one of the most subjective parts of EXAFS data analysis. At the border of each spline region, polynomials are constrained to meet with equal value and equal slope. Beyond this, evaluation of a spline is guided by experience and common sense. For data ranges up to $k = 15 \text{ \AA}^{-1}$, a three-region spline was found to work best, for ranges longer than that a four-region spline was used.

Optimizing the spline involves changing the lengths of the regions and the orders of the polynomials in each region and observing the effect of such changes on the appearance of the spline, of the EXAFS that results after subtraction of the spline and of the Fourier

transform produced from the EXAFS. Care must be taken to insure that the curve used to mimic the background does not distort the resulting EXAFS when it is subtracted. A sound spline should flex with the background but not be susceptible to curving with the EXAFS oscillations. Thus, no spline regions should be made too short or too long (spline regions are usually of roughly equal length in k -space), nor should the order of the polynomial in each region be too high (orders 2 and 3 work well). The optimum spline representing $\mu_0 + \mu_{res}$ should maximize the intensity of the peaks while minimizing the low- R noise in the Fourier transform, and the amplitude of the EXAFS above and below the zero line should show no ultra-low-frequency structure.

Improper background subtraction can result in errors in the structural information obtained by EXAFS analysis. Distortion of the EXAFS can be manifested as changes in both the frequency and the amplitude of the data, leading to erroneous determinations of distances and coordination numbers.

(b) Normalized Data

All the data discussed in this thesis have been normalized, that is, the edge jump has been scaled to one. The data can be then interpreted on a per atom basis and different samples can be compared with each other. The edge region is sensitive to the chemical environment, and so the point at which the data is scaled must lie above the edge region so that the normalization procedure is independent of the chemical nature of the sample. In practice the data are normalized at E_0 , where the continuum begins, and at which $k = 0 \text{ \AA}^{-1}$. In reality, E_0 varies with the effective nuclear charge of each compound, but by convention it is set at 8350 eV for Ni and 9000 eV for Cu in all the work done in this thesis. The scale factor which is used to normalize the data corresponds to the difference between the spline fitted to the postedge region (mimicking μ_0) and the polynomial used to mimic and remove the absorption background μ_{back} , as measured at E_0 .

(3) EXAFS Analysis

For each "shell" of scatterers of one type at the same distance, the form of the EXAFS Equation (Equation 2.1) utilized by XFPACK is :

$$\chi_s(k) = \frac{N_s Scf_s Trf_s}{kR_{as}^2} Amp_s(k) \exp(-2\sigma_{as}^2 k^2) \sin[2kR_{as} + Phs_s(k)] \quad \dots\dots\dots(2.6)$$

where the amplitude factors characteristic of the scatterer S are now subsumed into $Amp_s(k)$, and there are two new empirical scaling factors Scf_s and Trf_s . A non-linear least-squares curvefitting algorithm is used to curve-fit the EXAFS data by varying certain parameters in Equation 2.6 in the manner described below.

$Amp_S(k)$ and $Phs_S(k)$ may be provided in a number of ways. They could be obtained by looking up tables of theoretical EXAFS amplitudes and phases. In this case, especially if plane-wave parameters¹² are used, Scf_S and Trf_S have to be varied to scale the theoretical signal to the experimental data. A second option is to extract the amplitude and phase shift as data files from a model compound with a known structure. A third option, and the one used throughout this thesis, is to parameterize $Amp_S(k)$ and $Phs_S(k)$ so :

$$Amp_S(k) = c_0[\exp(c_1k + c_2k^2)]k^{c_3} \dots\dots\dots(2.7)$$

$$Phs_S(k) = \frac{a_{-1}}{k} + a_0 + a_1k + a_2k^2 \dots\dots\dots(2.8)$$

(a) Fourier Filtering of EXAFS Data

In order to simplify the curve-fitting process, EXAFS "shells" of interest (that is, groups of scatterers occurring at the same distance from the absorbing atom) are isolated by Fourier-transforming the data to produce a Fourier transform of the spectrum with peaks in R-space (in Å), and then applying a Fourier transform again in the reverse direction, using a "Fourier transform window" to include only the peak(s) of interest, and producing a "filtered" EXAFS spectrum in k -space (in Å⁻¹) once more, which is then used for EXAFS curve-fitting analysis.

Fourier filtering distorts the data somewhat because of artifacts introduced by truncating the data. These truncation effects can be minimized by using a window smoothing function to taper the data to zero at the minimum and maximum limits of the Fourier transform window. Thus, throughout this thesis, a "Gaussian window" of width 0.1 Å⁻¹ (for forward transform from k -space to R-space) or 0.1 Å (for reverse transform) was used. That is, half-Gaussians of the appropriate width were used at both ends of the window to taper the data to zero for Fourier transform.

(b) Extraction of Parameters

The choice of the model compound from which to extract the parameters is critical. The assumption implicit in this method is that the parameters describe a generic absorber/scatterer interaction that is transferable to other compounds. However, this is not always true. Thus, models from which parameters are extracted should bear a reasonable chemical similarity to the unknown system that they will be used to curve-fit. The model compound used for this purpose should have all the scatterers of interest occurring at the same distance from the absorbing atom, and no other atoms occurring at similar distances (within 0.5 Å) from the origin.

The values of c_1 and a_1 were always set to zero in the work described in this thesis. Values for the other six parameters were obtained in the following way. The EXAFS signal of interest (e.g. Cu–N) in the EXAFS spectrum of a compound of known structure was isolated by Fourier transform. The EXAFS equation in (2.6) was then used to fit it, with Scf_S and Trf_S being fixed at 1, and N_S and R_{as} fixed at the correct values. Initial values of c_0 , c_2 , c_3 , a_0 , a_1 and a_2 were obtained from parameters optimized for a similar absorber-scatterer pair (e.g. parameters optimized for Cu–N interactions were used as initial guesses for Ni–N interactions). The six parameters were then optimized in an iterative manner, with all the phase terms (a 's) being varied for the best fit, then all the amplitude terms (c 's), then the phase terms again. Two cycles between varying phase and amplitude terms was usually sufficient to produce a fit that was not improved upon further iteration. All six terms were then varied at once, and the resulting values of c_0 , c_2 , c_3 , a_0 , a_1 and a_2 — the "extracted parameters" — were saved for further use.

This process was repeated for all the absorber/scatterer interactions we wish to investigate in the EXAFS analysis of data from structurally unknown systems.

Parameters should be extracted from data covering the same k -range and similar Fourier transform ranges as the data for the unknown system, both to minimize truncation artifacts due to the Fourier-filtering technique, and because of the k -dependence of the parameters. Parameters extracted from the "first shell" (i.e. the atoms directly coordinated to the absorbing atom, at ~ 2 Å) are less reliable if used to fit the "outer shells" (atoms 3 Å or more from the absorbing atom).

(c) Parameterized Curve-Fitting of EXAFS Data

When parameters are used for fitting a compound of unknown structure, the parameters are held fixed, and variables are R_{as} , E_0 , N_S and σ_{as}^2 . Non-linear least-squares curve-fitting techniques are subject to correlation between variables, especially between R_{as} and E_0 (which affect the phase of the EXAFS) and N_S and σ_{as}^2 (which affect the amplitude). For the empirical method used here, the phase parameters already take the effective nuclear charge into account, and so E_0 is never varied. R_{as} and N_S (called the coordination number CN in later chapters) are varied at the same time, since there is little danger that they would correlate with each other.

Despite the danger of correlation with N_S , it is necessary to vary σ_{as}^2 , since different compounds containing the same absorber/scatterer pairs have bonds of differing length and stiffness, or with a different radial distribution, and so σ_{as}^2 is not transferable. Usually, when N_S is varied together with R_{as} , σ_{as}^2 is held at a fixed value, and vice versa.

Using this technique of curve-fitting on structurally characterized model compounds has yielded distances with an estimated accuracy of ± 0.03 Å and coordination numbers with an estimated accuracy of about 25%.^{1,2,13} Care must be taken to step through a sufficient range of distances and coordination numbers to identify a global, rather than a local, minimum corresponding to the best fit.

The more shells of scatterers are summed together to fit a given data file, the better the fit that can be achieved. This is always true, since there are always more parameters that can then be varied for a good fit. Therefore, a better fit with more "EXAFS waves" is not necessarily a more meaningful fit to the data. Also, when more waves are used to fit the same data, the danger of correlation of R_{as} with R_{as}' , and N_S with N_S' , etc. is increased. So the results of multiwave fits have to be examined with particular care to ensure that they make sense physically.

If the EXAFS signal of a compound is extremely complex, and the Fourier transform reveals two or more peaks corresponding to shells of scatterers at different distances, then each peak is windowed and Fourier backtransformed to be curvefitted in isolation with one or two waves. When a good fit has been achieved for each shell, then a wide Fourier window is used to include all the peaks, and all the waves are then combined to fit the data. In a well-behaved fit, the results for the filtered data from combined window should not differ much from the results obtained from fits to each shell in isolation. This procedure works best when the peaks in the Fourier transform are well-separated from each other. If there is overlap of Fourier transform peaks, then fits to isolated peaks will not give trustworthy results. They are still done to give a first approximation of the possible contributions to the EXAFS, but results from fits done to the data from the combined Fourier peaks are often different from the results of fits to isolated peaks.

(d) The Parameter c_2

As can be seen from Equations 2.6 and 2.7, the parameter c_2 occurs in the same functional form as the Debye-Waller factor, i.e., $\exp(xk^2)$, where $x = c_2$ or $x = -2\sigma_{as}^2$. Thus, a value of σ_{as}^2 is already included in the value of c_2 in the parameters extracted from the model compound. Because of this, in practice, σ_{as}^2 is set to zero and never varied, and c_2 is varied in its place. The absolute value of c_2 (always negative) cannot be directly correlated to σ_{as}^2 , but, in comparing results from different sets of data, we may say that $\Delta c_2 = -2\Delta\sigma_{as}^2$. A more negative value of c_2 denotes a larger value of σ_{as}^2 and so a weaker bond or a greater distribution of distances for scatterers, and a less negative value of c_2 denotes a stronger or stiffer bond, or else a narrow distribution of scatterer distances. As

will be seen in Chapters 5 and 7, scrutiny of c_2 values is important in the interpretation of fits to EXAFS data.

(e) The Fit Index F

The goodness-of-fit is evaluated by the minimization function value F , which is referred to as the fit index in later chapters.

$$F = \sqrt{\frac{\sum_i^n [k_i^3 (\text{EXAFS}_{\text{obs}} - \text{EXAFS}_{\text{calc}})_i]^2}{n}}, \text{ where } n = \text{no. of data points } i \quad \dots\dots\dots(2.9)$$

F is thus evaluated over the entire range of data fitted, and it is weighted by k^3 to give due consideration to data at higher values of k . F is not, however, adjusted for the overall amplitude of the signal, so care must be taken when comparing F values for fits made to different data files. For fits made to the same data, a smaller value of F corresponds to a closer match of the calculated fit to the data. However, the closest match is not necessarily the best or most reasonable fit of the data. The distances, coordination numbers and Debye-Waller factors obtained must all also be taken into account in judging the best fit. Examples of the application of such fit criteria are presented in Chapters 5 and 7.

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Figure 2.1. A typical X-ray absorption spectrum. (a) Generalized X-ray absorption spectrum, showing the K edge ($1s \rightarrow \text{continuum}$) and the L_I - L_{III} edges ($2s, 2p \rightarrow \text{continuum}$). (b) Inset from (a), showing the pre-edge, edge and EXAFS regions for a nickel compound. (c) Inset from (b), showing features in the edge which are diagnostic of a square-planar geometry.

(a) Constructive interference

(b) Destructive interference

Figure 2.2. EXAFS of a compound where the absorbing atom is surrounded by four equidistant scatterers. (a) At this energy, the spherical photoelectron wave has a wavelength such that the amplitudes of the outgoing wave and of waves scattered back overlap constructively at the origin, producing a maximum in the absorption. (b) At this lower energy, the wavelength of the photoelectron wave is longer, and destructive interference between outgoing and backscattered waves occurs, producing a minimum in the absorption. (Wavefront schematic diagrams reproduced from *Prog. Inorg. Chem.* **1979**, 25, 1–39, by courtesy of K. O. Hodgson.)

(a) Transmission Mode

(b) Fluorescence Mode

Figure 2.3. Schematic representation of the experimental setup used to collect XAS data in (a) transmission mode, (b) fluorescence mode. I_0 , I_1 and I_2 are ionization chambers containing N_2 gas. A potential of 300 V is applied across each chamber. I_F is the fluorescence detector, which may be either an ionization chamber-type detector or a 13-element Ge solid-state array detector. The liquid helium cryostat for the samples has a temperature range of 4–200 K.

Chapter 3

Crystal Structure of Bis(*N*-methylimidazole)copper(I) Tetrafluoroborate

(A) Relevance of Simple Cu-Imidazole Compounds to Biological Studies

Many vital biological functions (e.g., oxygen transport, electron transport and catalysis of biochemical reactions) are carried out by metalloproteins, that is, proteins or enzymes with one or more metal atoms located within the coils and sheets formed by the peptide sequence which comprise the bulk of the protein. Although these metal atoms occupy a small volume in the protein, their properties readily make them the focus of the function of the metalloprotein, with catalysis, binding or redox action occurring at or through the metal atoms. Thus, the metal atoms and their immediate environment merit particular study. For this purpose, metalloproteins may be thought of as particularly large coordination complexes of metals, which may be approximated by smaller complexes synthesized to model the immediate coordination sphere of the metal atoms. These smaller compounds are useful because they can be characterized with much greater accuracy, particularly by X-ray crystallography, than the large protein molecules.

Histidine is one of the amino acids that is commonly found coordinated to the metal atom in a metalloprotein. In the binuclear copper protein hemocyanin (see Chapter 4) and the trinuclear copper site of laccase (see Chapter 6), histidine is the primary ligand coordinated to the copper atoms. In the mononuclear "blue copper" site, found in plastocyanin (Chapter 5) and also the Type 1 site of laccase (Chapter 6), histidine again accounts for two out of four ligands to the copper atoms.

Histidine coordinates to a metal atom through the imidazole ring, and thus many model compounds have utilized imidazole as a ligand. Some model compounds attempt to mimic the metalloprotein active site in its entirety (e.g., binuclear copper compounds with bridging oxygen ligands to mimic the active site in hemocyanin), while others focus on one aspect of the site, in order to study that aspect in isolation.

Simple Cu-imidazole compounds play an essential role in the EXAFS analysis of copper metalloproteins like hemocyanin and plastocyanin. This is because the rigid imidazole ring produces a strong EXAFS signal that is perceived in Fourier transforms at $\sim 2\text{--}4\text{ \AA}$ from the metal atom, and there is a need to characterize and understand this complex signal on its own in order to reliably distinguish it from any signals due to other types of ligands, or neighbouring metal atoms within $4\text{ \AA}$, in the protein active site.

In the course of our study of deoxyhemocyanin (see Chapter 4), it became necessary for us to solve the crystal structure of $\text{Cu(I)(N-methylimidazole)}_2\text{BF}_4$. The structure we obtained was eventually published in *Acta Crystallographica* as a short paper.¹

(B) Experimental Details

$\text{Cu(I)}(N\text{-methylimidazole})_2\text{BF}_4$ crystals were provided to us by Professor Thomas N. Sorrell of the University of North Carolina at Chapel Hill. The compound was prepared under an inert atmosphere by treating a solution of *N*-methylimidazole in dry methanol with a half equivalent of solid $\text{Cu}(\text{CH}_3\text{CN})_4\text{BF}_4$.² Colourless parallelepipeds were obtained by slow cooling of a hot ethanol solution of the complex. The density of the crystals was not measured because of their extreme sensitivity to dioxygen.

A crystal measuring approximately $0.50 \times 0.25 \times 0.15 \text{ mm}^3$ was mounted in a glass capillary and employed to collect X-ray intensity data on a Siemens $P2_1$ diffractometer, using graphite-monochromated $\text{Mo } K\alpha$ radiation. Some crystal structure data are given in Table 3.1. Unit-cell dimensions were obtained from 50 reflections, with $2.9 < \theta < 18.6^\circ$. There were no systematic absences, and the space group was determined to be $P\bar{1}$ by intensity statistics and satisfactory structure solution and refinement. 2796 unique reflections were collected, with $1.0 < \theta < 27.5^\circ$; $[(\sin\theta)/\lambda]_{\text{max}} = 0.6497 \text{ \AA}^{-1}$, and $-10 \leq h \leq 10$, $-9 \leq k \leq 9$, $-11 \leq l \leq 11$. There were 1163 reflections with $I > 3\sigma(I)$. Three intensity standards checked every 50 reflections showed a small decline in intensity (maximum decline was 6%) during data collection; the data were scaled accordingly. Lorentz and polarization corrections were applied, but no absorption corrections were considered necessary for a crystal of this size and composition.

The copper atom was located from a Patterson map. All other non-hydrogen atoms were located from a subsequent difference Fourier map. Hydrogen atoms were included in calculated positions ($\text{C-H} = 0.96 \text{ \AA}$). The program used for F_{obs} refinement was *SHELX76*.³ Final refinement values were $w = 1.0/[\sigma^2(F) + 2.05 \times 10^{-4}F^2]$ (for 165 parameters), $R = 0.060$, $wR = 0.056$, $S = 2.437$ and $\Delta_{\text{max}}/\sigma = 0.03$ for non-hydrogen atoms. In the final difference map, the maximum $\Delta\rho$ excursion was $0.62 \text{ e \AA}^{-3}$ (near $[\text{BF}_4]^-$) and the minimum $\Delta\rho$ was $-0.41 \text{ e \AA}^{-3}$.

Calculations were performed on a MicroVAX 3600 computer. Diagrams were produced using *ORTEPII*.⁴ Atomic scattering factors used were those of Cromer and Mann⁵ for F, O, N, C and B, the *International Tables for X-ray Crystallography*⁶ for Cu and H, and Cromer and Liberman⁷ (for all $\Delta f'$ and $\Delta f''$).

(C) Results and Discussion

Atomic positions are given in Table 3.2, and selected bond lengths and angles are listed in Table 3.3. The molecular geometry and atomic numbering are depicted in Figure 3.1.

Within each cation, the copper atom is nearly linearly coordinated to the two nitrogen donors on the two imidazole groups, with $\text{Cu-N}(1) = 1.863(7) \text{ \AA}$, $\text{Cu-N}(3) = 1.855(7) \text{ \AA}$ and $\text{N}(1)\text{--Cu--N}(3) = 173.6(3)^\circ$. The imidazole rings are disposed such that the two methyl substituents project to the same side of the molecule, rather than adopting the alternative centrosymmetric arrangement (see Figure 3.1).

This linear geometry contrasts with the geometry of tetrahedral $\text{Cu(I)(N-methylimidazole)}_4\text{ClO}_4$,⁸ where $\text{Cu-N} = 2.054(2) \text{ \AA}$, but is quite similar to the geometries for other two-coordinated Cu(I) -heteroaromatic compounds. For example, $\text{Cu(I)(1-methylpyrazole)}_2^+$ and $\text{Cu(I)(1,3,5-trimethylpyrazole)}_2^+$, have Cu-N and N-Cu-N values of $1.873(3) \text{ \AA}$, $1.879(3) \text{ \AA}$, $178.2(2)^\circ$ and $1.878(3) \text{ \AA}$, $1.863(4) \text{ \AA}$, $173.8(2)^\circ$ respectively,² and $\text{Cu(I)(2,4-dimethylpyridine)}_2^+$ has $\text{Cu-N} = 1.86(1) \text{ \AA}$ and $\text{N-Cu-N} = 170(1)^\circ$.⁹

Each of these four linear complexes shows a different degree of aggregation in the solid state. In $\text{Cu(I)(2,4-dimethylpyridine)}_2^+$, there is a weak interaction with the perchlorate counterion ($\text{Cu}\cdots\text{O} = 2.72(4) \text{ \AA}$) to form a pseudo three-coordinate structure. In $\text{Cu(I)(1,3,5-trimethylpyrazole)}_2^+$, there are no long-range interactions involving the copper atoms. In $\text{Cu(I)(1-methylpyrazole)}_2^+$, the flat molecules are partially overlapped in a weak dimeric structure, with copper in each molecule being almost directly opposite the middle of a C–C bond in the molecule that it is paired with ($\text{Cu}\cdots\text{C}(2a') = 3.150(4) \text{ \AA}$, $\text{Cu}\cdots\text{C}(3a') = 3.221(4) \text{ \AA}$). This is suggestive of a weak metal- π (alkene) type of interaction, giving a pseudo three-coordinated copper.

In the present complex, there is a more pronounced dimeric unit, with $\text{Cu}\cdots\text{Cu} = 3.198(2) \text{ \AA}$, and completely overlapping stacked rings (see Figure 3.2). As shown in Figure 3.2, the deviation of the $\text{N}(1)\text{--Cu--N}(3)$ angle from 180° is probably due at least in part to mutual repulsion between the stacked imidazole rings, indicating that there is indeed some weak Cu–Cu interaction and so a pseudo three-coordinate environment for copper.

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Table 3.1. Crystal data for Cu(I)(*N*-methylimidazole)₂BF₄

Formula	C ₈ H ₁₂ N ₄ BF ₄ Cu
Space group	$P\bar{1}$
a (Å)	7.811 (2)
b (Å)	8.993 (3)
c (Å)	10.136 (3)
α (°)	77.95 (2)
β (°)	77.91 (2)
γ (°)	72.11 (2)
V (Å ³)	654.5 (3)
Z	2
D_x (g cm ⁻³)	1.60
$\lambda(\text{Mo } K\alpha)$ (Å)	0.71073
$\mu(\text{Mo } K\alpha)$ (cm ⁻¹)	17.71
T (K)	293
Crystal size (mm ³)	0.50 × 0.25 × 0.15
Collection range	$\pm h, \pm k, \pm l$ $1.0^\circ < \theta < 27.5^\circ$ $[(\sin\theta)/\lambda]_{\text{max}} 0.6497 \text{ \AA}^{-1}$
No. of unique reflections	2796
No. of reflections $I > 3\sigma(I)$	1163
$F(000)$	316
$R(F)$	0.060
$wR(F)$	0.056

Table 3.2. Atomic positions and equivalent isotropic displacement factors for non-hydrogen atoms.

$$U_{\text{eq}} = \frac{1}{3} \sum_{ij} U_{ij} a_i^* a_j^* \mathbf{a}_i \cdot \mathbf{a}_j$$

	<i>x</i>	<i>y</i>	<i>z</i>	<i>U</i> _{eq} (Å ²)
Cu	0.1703(1)	0.0643(1)	0.4235(1)	0.102
N(1)	0.0437(9)	0.2167(9)	0.2968(7)	0.082
N(2)	-0.1326(8)	0.4415(9)	0.2112(8)	0.080
N(3)	0.3097(8)	-0.0711(9)	0.5503(8)	0.087
N(4)	0.4342(11)	-0.1530(13)	0.7377(8)	0.103
C(1)	-0.0493(12)	0.3618(13)	0.3174(8)	0.079
C(2)	0.0156(12)	0.2049(12)	0.1705(10)	0.086
C(3)	-0.0930(11)	0.3421(12)	0.1183(8)	0.079
C(4)	-0.2550(12)	0.6043(11)	0.2016(10)	0.135
C(5)	0.3180(13)	-0.0372(12)	0.6705(12)	0.105
C(6)	0.4255(13)	-0.2189(13)	0.5434(10)	0.100
C(7)	0.5023(12)	-0.2683(13)	0.6585(13)	0.113
C(8)	0.4742(15)	-0.1580(15)	0.8736(10)	0.160
B	-0.1821(21)	0.2783(17)	0.7621(14)	0.119
F(1)	-0.2734(8)	0.3991(7)	0.8329(6)	0.149
F(2)	-0.0535(8)	0.1837(8)	0.8450(8)	0.194
F(3)	-0.2916(8)	0.1832(7)	0.7634(6)	0.167
F(4)	-0.1087(16)	0.3192(12)	0.6448(7)	0.274

Table 3.3. Bond lengths (Å) and angles (°).

Cu–N(1)	1.863(7)	N(4)–C(5)	1.325(11)
Cu–N(3)	1.855(7)	N(4)–C(7)	1.354(11)
N(1)–C(1)	1.320(9)	N(4)–C(8)	1.463(11)
N(1)–C(2)	1.374(10)	C(2)–C(3)	1.340(10)
N(2)–C(1)	1.334(9)	C(6)–C(7)	1.353(11)
N(2)–C(3)	1.357(10)	B–F(1)	1.356(14)
N(2)–C(4)	1.478(10)	B–F(2)	1.404(12)
N(3)–C(5)	1.334(11)	B–F(3)	1.379(14)
N(3)–C(6)	1.365(10)	B–F(4)	1.240(14)
N(1)–Cu–N(3)	173.6(3)	N(1)–C(1)–N(2)	111.2(8)
Cu–N(1)–C(1)	124.6(7)	N(1)–C(2)–C(3)	109.3(8)
Cu–N(1)–C(2)	129.9(8)	N(2)–C(3)–C(2)	106.9(7)
C(1)–N(1)–C(2)	105.3(7)	N(3)–C(5)–N(4)	111.8(9)
C(1)–N(2)–C(3)	107.2(7)	N(3)–C(6)–C(7)	108.2(9)
C(1)–N(2)–C(4)	126.0(9)	N(4)–C(7)–C(6)	108.1(9)
C(3)–N(2)–C(4)	126.7(8)	F(1)–B–F(2)	103.0(9)
Cu–N(3)–C(5)	124.7(8)	F(1)–B–F(3)	110.8(12)
Cu–N(3)–C(6)	129.8(8)	F(1)–B–F(4)	114.7(12)
C(5)–N(3)–C(6)	105.5(8)	F(2)–B–F(3)	103.8(10)
C(5)–N(4)–C(7)	106.4(8)	F(2)–B–F(4)	111.8(14)
C(5)–N(4)–C(8)	126.7(12)	F(3)–B–F(4)	112.0(11)
C(7)–N(4)–C(8)	126.9(11)

Figure 3.1. Molecular geometry and atomic numbering. Anisotropic ellipsoids represent 33% probability boundaries.

Figure 3.2. Stereographic projection of the unit-cell contents.

Chapter 4

Studies of the EXAFS of a Cu(I)-Imidazole Compound

Over a Range of Temperatures

(A) Brief Overview of Hemocyanin Structure

To understand our interest in the EXAFS of Cu-imidazole compounds, we will look briefly at the copper metalloprotein hemocyanin, and at efforts to elucidate structural details of its active site.

Hemocyanin (Hc) is the oxygen transport protein in the blood of molluscs and arthropods.¹⁻³ Although it corresponds to hemoglobin in its biological function, the structure of its active site is completely different, containing two copper atoms, magnetically coupled to each other,⁴ and ligated primarily with histidine.

When dioxygen is bound (this form is called oxyhemocyanin, or oxy Hc), both copper atoms are oxidized, and so the O₂ moiety is bound as a peroxide. However, this blue species is EPR-silent, indicating an antiferromagnetic coupling between the two Cu (3d⁹) ions. When dioxygen is not bound to the protein (this form is called deoxy Hc), the protein is colourless, and the Cu ions are reduced (3d¹⁰). This species is of course EPR-silent, and can only be indirectly probed by UV-visible spectroscopy through charge-transfer transitions in binding studies with exogenous ligands such as CO. Optical and EPR methods, while giving insight into the electronic structure of the active site, could not give metrical information. In particular, the distance between the two Cu ions, of vital importance for understanding the chemistry of hemocyanin, could not be ascertained.

Crystal structures have been solved on hexameric deoxy hemocyanin from *Panulirus interruptus* (spiny lobster) at a resolution of 3.2 Å,^{5,6} and on oxy hemocyanin from *Limulus polyphemus* (horseshoe crab) at a resolution of 2.4 Å.⁷ The crystal structure of deoxy Hc shows the two Cu atoms 2.9–3.8 Å apart, coordinated by six histidine ligands (three on each Cu) arranged in an antiprismatic fashion. Four histidine nitrogens lie in a plane with the two Cu atoms, and for these ligands Cu–N = 1.94–2.26 Å. The remaining two Cu–N(His) bonds, one on each Cu, project in opposite directions from this plane and are extremely weak, being 2.5–2.9 Å long. No ligand bridging the two Cu atoms could be found. Not all details of the oxy Hc structure have been published yet, but it is known that the distance between the two Cu atoms is 3.4 ± 0.2 Å, and that they are bridged by O₂ in an unusual side-on $\mu\text{-}\eta^2\text{:}\eta^2$ configuration to form a planar, symmetrical, Cu–O₂–Cu core, where O–O provides two ligands to *both* the Cu atoms in the site.

c2.(B) Using EXAFS to Examine the Structure of Hemocyanin — Multiple Scattering by Imidazole

Even though crystal structures have now been solved for both oxy and deoxy Hc, EXAFS spectroscopy is still a valuable means of obtaining more accurate structural

information about the active site. The exact distance between the two copper atoms also remains of particular interest.

In the early 1980's, a number of EXAFS studies on both oxy and deoxy hemocyanin, from various species of molluscs and arthropods, were published.⁸⁻¹¹ In the case of oxy Hc, all studies concurred in finding about four N/O scatterers coordinated to the copper ions (2 N at 2.0 Å and 2 O at 1.9 Å), and an unambiguous Cu–Cu interaction at 3.55–3.68 Å in Hc from all species measured.^{8,10,11} However, the EXAFS spectra of deoxy Hc proved to be more difficult to interpret in an unambiguous way. All studies found about two N/O scatterers coordinated to the Cu ions at 1.90–2.00 Å. However, some studies (by Brown, *et al.* and Woolery, *et al.*) found a Cu–Cu interaction (which could also be modelled as a Cu–N interaction) at 3.39–3.48 Å,^{8,11} while another study (Co, *et al.*) found no evidence of a Cu–Cu interaction within 4 Å.⁹

Conflicting results were obtained because of the difficulty in accurately accounting for the "outer shell" signal from the histidine imidazoles ligated to Cu. Most ligands display little significant EXAFS signal beyond the first shell. However, imidazole has a characteristic EXAFS signal which is manifested in Fourier transforms as a three-peak group over $R = 2\text{--}4$ Å (Figure 4.3). The significant imidazole EXAFS signal in the region of $R = 3\text{--}4$ Å must be accurately modelled in order to distinguish a weak metal-metal interaction occurring at this distance. Woolery, *et al.* and Co, *et al.* did employ techniques which fitted the EXAFS spectrum of imidazole as a unit. However, it was recognized that these models oversimplified the imidazole signal, taking no account of the multiple-scattering which occurs in imidazole.

The reason the "outer shell" imidazole EXAFS signal is so strong is that multiple-scattering significantly enhances the backscattering from imidazole atoms at 3–4 Å from Cu (see Figure 4.1 and Table 4.1). Thus, an outer shell atom such as C₁ is perceived not only through the single-scattering interaction Cu–C₁–Cu, but through the multiple-scattering interactions Cu–N₁–C₁–Cu (double-scattering) and Cu–N₁–C₁–N₁–Cu (triple-scattering). Multiple-scattering is only significant in rigid moieties such as imidazoles or porphyrin rings because each leg of the scattering path is subject to its own Debye-Waller factor, and so multiple-scattering signals are damped out in less rigid systems. Also, multiple-scattering occurs most strongly if the scattering path is linear, e.g. in Cu–C≡O. As the scattering path deviates from 180°, the amplitude of the multiple-scattering signal diminishes.¹² In Cu-imidazole, there are no linear scattering paths, but the two atoms at ~4 Å (N₂ and C₃ in Figure 4.1) do provide almost-linear scattering paths (e.g. Cu–N₁–N₂–Cu

or Cu-N₁-N₂-N₁-Cu), so that the EXAFS signal from these atoms is considerably enhanced.

Several studies have attempted to take into account multiple-scattering in modelling metal-imidazole EXAFS spectra.¹³⁻¹⁶ However, these theoretical simulation methods cannot yet be relied upon to consistently model even compounds of known structure accurately, and they also suffer from having too many parameters that need to be varied for a fit. In particular, no satisfactory way has yet been found to determine Debye-Waller factors for the imidazole ring, and so these have thus far been varied in an entirely empirical fashion to fit the data, even in so-called theoretical simulations.

(C) The Temperature Behaviour of the Debye-Waller Factor as a Tool For Obtaining Physically Meaningful Results From EXAFS Curve-Fitting

In order to constrain at least some EXAFS modelling parameters in a physically meaningful way, there is a need to correlate the EXAFS Debye-Waller factor ($\exp(-2\sigma_{as}^2 k^2)$, see Chapter 2) with the actual physical behaviour of the molecule, that is, with factors such as bond stiffness (σ_{vib}^2 , vibrational or thermal disorder) and root-mean-square distribution of atom distances (σ_{stat}^2 , static disorder).

$$\sigma_{as}^2 = \sigma_{vib}^2 + \sigma_{stat}^2 \quad \dots\dots\dots(4.1)$$

An idea of the true thermal contribution to the Debye-Waller factor can be obtained from EXAFS data collected on the same compound over a wide range of temperatures. The temperature behaviour of thermal disorder for some types of material (mainly solid-state) has been described by various mathematical models. For solid samples of molecular compounds, it seems best to use the simple harmonic oscillator as an approximate model. The temperature dependence of σ_{vib}^2 in this model is then

$$\sigma_{vib}^2 = \left(\frac{h}{8\pi^2 \mu \nu} \right) \coth \left(\frac{h\nu}{2kT} \right) \quad \dots\dots\dots(4.2)$$

where h is Planck's constant, ν is the stretching frequency of the bond in question, and reduced mass $\mu = \frac{m_1 m_2}{m_1 + m_2}$ in the case of a simplistic diatomic stretch model. For the Cu-imidazole or Cu-methylimidazole stretch, a value for $m_1 = 63.5$ a.m.u. (for Cu) and $m_2 = \sim 70-80$ a.m.u. (for imidazole) may be used to give $\mu \cong 35$ a.m.u. as a reasonable estimate.

For the temperatures at which we are making our study (10–200 K), the static disorder for a given compound should remain much the same, but the vibrational disorder will increase with temperature. Therefore, any change in σ_{as}^2 should be due to changes in

σ^2_{vib} , not σ^2_{stat} . In principle, therefore, fits of the EXAFS of the same compound at different temperatures should be able to yield $\sigma^2_{vib}(\Delta T)$, which can then be solved for ν and μ in a known compound, and ν and μ can then be compared with literature values from vibrational (IR and Raman) studies. Once this relation is established, $\sigma^2_{vib}(\Delta T)$ can then be constrained to give a consistent (ν, μ) value in EXAFS curve-fitting of data at different temperatures simultaneously, and ν and μ can be checked to make sure they are reasonable for a given system.

(D) Experimental Details

(1) Preparation of Samples

Cu(I) model compounds were ground with boron nitride and packed into aluminum spacers in the manner described in Chapter 2.

(2) Data Collection

Data for Cu(I) models were collected at the Stanford Synchrotron Radiation Laboratory at the unfocussed 8-pole wiggler beam line 7-3 in transmission mode at 10, 45, 80, 130 and 200 K, using an Oxford Instruments CF1208 continuous-flow liquid helium cryostat, to $k = 17 \text{ \AA}^{-1}$. For a temperature series on one compound, the cryostat was stepped from one temperature to the other without disturbing the sample.

(3) Data Analysis

Data were reduced and analyzed using XFPACK, in the manner described in Chapter 2. Scans were calibrated using copper foil as an internal standard, taking the first inflection point of the foil as 8980.3 eV. Subtraction of the background was done by fitting a polynomial to the EXAFS region and then applying this at the bottom of the "edge step" and extending this into the pre-edge region. The same four-region spline to $k = 17 \text{ \AA}^{-1}$ was used for data at all temperatures for one compound. For all data analyzed in this chapter, a data range of $k = 3.5\text{--}16.5 \text{ \AA}^{-1}$ was used for the forward Fourier transform (Figure 4.3), and the data were then backtransformed to $k = 4\text{--}16 \text{ \AA}^{-1}$ for curvefitting. Parameters were also extracted over the same data ranges.

(E) Results and Discussion

The EXAFS temperature series for Cu(I)(*N*-methylimidazole)₄ClO₄ was examined. As shown in Figures 4.2 and 4.3, this series displays an extremely regular trend, with EXAFS and FT magnitudes for all features decreasing in a monotonic fashion with increasing temperature. For each of the first (FT window $R' = 1.00\text{--}2.00 (0.1) \text{ \AA}$), second

($R' = 2.10\text{--}3.20$ (0.1) Å) and third ($R' = 3.00\text{--}4.10$ (0.1) Å) shells, amplitude and phase parameters (c_0 , c_2 , c_3 , a_0 , a_1 and a_2) were extracted from the data collected at 10 K in the manner described in Chapter 2. On the principle that only σ_{as}^2 should vary as the temperature changed, the parameters were then applied to the data collected at 45, 80, 130 and 200 K and only c_2 was varied (c_2 varies as σ_{as}^2 , see Chapter 2). The trend in c_2 with temperature is shown in Table 4.2. At each temperature, after the c_2 result was obtained, R only was then varied to check that the fit was good. For all three shells there was indeed little shift in R with temperature. It is extremely interesting to note that the c_2 values are less negative (i.e., σ_{as}^2 is smaller) for the third shell than they are for the second shell. This is consistent with our expectation that multiple-scattering magnitudes are greater for the almost linear paths to the third shell.

An error was made in extracting the parameters at 10 K for the first shell. The phase parameter a_1 was not set to zero when the other three phase parameters (a_0 , a_1 and a_2 , see Chapter 2) were varied. However, since the phase parameters and the amplitude parameters (including c_2) do not interact much, and the values of all phase parameters were the same for fits to data at all temperatures, this should not have a significant effect on the results for c_2 .

An additional step was also performed in the fits to the first-, second- and third-shell data. All the characteristic amplitude and phase parameters (c_0 , c_2 , c_3 , a_0 , a_1 and a_2) extracted from the data collected at 10 K were varied for the best fit to data collected at each temperature. The results for the amplitude parameters (c_0 , c_2 and c_3) are listed in Table 4.3. It can be seen that, while in principle only c_2 should change, in fact the best fit is achieved with significant changes in all three amplitude parameters (the c_0 – c_3 values). Since c_2 is affected by correlation with c_0 and c_3 , c_2 by this method is less reliable than c_2 obtained by the simpler method above. Indeed, it can be seen that the trend in c_2 is not so regular as that in Table 4.2, especially for the second shell.

These results show that the variation of the EXAFS with temperature is accounted for by changes in c_2 or σ_{as}^2 with good, but not complete accuracy. This is not surprising for the second and third shells, since they in fact contain several contributions from both single- and multiple-scattering paths. The fact that correlation occurs even in the first shell most probably simply shows the limit of accuracy achievable by the Fourier filtering/least-squares curve-fitting approach to EXAFS analysis within the large degree of freedom chosen.

In order to correlate the temperature trends observed for c_2 with possible ν and μ values for $\text{Cu(I)(N-methylimidazole)}_4\text{ClO}_4$, a comparison was made between the value of

$\Delta\sigma_{vib}^2(T)$, as calculated from Equation 4.2, and the observed $\Delta c_2(T)$, as calculated from the values in Table 4.2. The best fit with the experimental c_2 values were determined by evaluating

$$F(\mu, \nu) = \sum_{T_1, T_2} \left\{ \left[\frac{h}{8\pi^2\mu\nu} \left(\coth\left(\frac{h\nu}{2kT_2}\right) - \coth\left(\frac{h\nu}{2kT_1}\right) \right) \right] - \left(-\frac{1}{2}\right)[c_2(T_2) - c_2(T_1)] \right\}^2 \quad \dots\dots\dots(4.3)$$

over a grid of (μ, ν) values. The $(-1/2)$ factor is included since $\Delta c_2 = -2\Delta\sigma_{as}^2$ (see Chapter 2). Since there were c_2 values for five temperatures, $F(\mu, \nu)$ was summed over four sets of $\Delta(T_1, T_2)$ values at each (μ, ν) point.

Given the precision to which c_2 values are trusted ($\sim 1 \times 10^{-3} \text{ \AA}^2$, or $\sim 5 \times 10^{-4} \text{ \AA}^2$ for $c_2/2$), all values of (μ, ν) for which $[F(\mu, \nu) - F(\mu, \nu)_{\min}] < \sim 5 \times 10^{-7} \text{ \AA}^4$ fall in the solution space. As shown in Figures 4.4, 4.5 and 4.6, the solution space turned out to cover a rather large range of (μ, ν) values in a narrow valley along a "1/x line".

The summation was made by evaluating $[f(45) - f(10)]$, $[f(80) - f(45)]$, $[f(130) - f(80)]$ and $[f(200) - f(130)]$ and adding these terms. This gives the most weight to the c_2 values of the three temperatures in the middle (45 K, 80 K and 130 K). A narrower minimum could be obtained by maximizing the $[f(T_1) - f(T_2)]$ terms in the expression for $F(\mu, \nu)$. This was done by taking all (T_1, T_2) differences with respect to 10 K, i.e. $[f(45) - f(10)]$, $[f(80) - f(10)]$, $[f(130) - f(10)]$ and $[f(200) - f(10)]$. This gives much more weight to the c_2 value at 10 K than at the other temperatures, which is not entirely inappropriate, since the EXAFS amplitude at low temperatures is larger, and c_2 values obtained from low-temperature data should then in fact be more accurate. Changing the weighting of the c_2 values from different temperatures, though it made the minimum narrower, did not change the location of the minimum, and the minimum still included a large range of (μ, ν) values along the "1/x line".

The results then show that, for the first shell (Figure 4.4), ν may be anywhere between 50–200 cm^{-1} , with most of the (μ, ν) solutions occurring along $\nu \sim 50\text{--}100 \text{ cm}^{-1}$, and μ may be anywhere above 20 a.m.u. They are related to one another along the "1/x line", but the "valley" for which $F(\mu, \nu) < \sim 5 \times 10^{-7} \text{ \AA}^4$ is $\sim 50 \text{ cm}^{-1}$ wide, and so there is considerable latitude in the possible solutions. The value of μ ($\cong 35$ a.m.u.) estimated above for a Cu–N(imidazole) stretch lies within the solution space, but not in the center of it. Better fits are obtained with $\mu > 60$ a.m.u. The values of ν from far-infrared studies of Cu(II)-imidazole compounds¹⁷⁻¹⁹ (not Cu(I)) are 271–307 cm^{-1} , with one tentative assignment at 237 cm^{-1} , while those for Zn(II)-imidazole compounds^{17,19,20} (since Zn(II)

is isoelectronic with Cu(I)) are 232–261 cm^{-1} . For the proteins hemocyanin and tyrosinase,^{21,22} $\nu(\text{Cu(II)}-\text{N(His)})$ has been assigned at 220–320 cm^{-1} . Even though, from chemical considerations, Cu(I)–imidazole bonds are expected to be weaker than either Cu(II)– or Zn(II)–imidazole bonds, they would be expected to have a frequency nearer to 200 cm^{-1} (about 170–220 cm^{-1}) than to 100 cm^{-1} .²³ It may be that, for this particular compound, Cu(I)(*N*-methylimidazole)₄ClO₄, where Cu(I)–N is longer than usual (2.054(2) Å),²⁴ ν is indeed $\sim 100 \text{ cm}^{-1}$. On the other hand, if we use $\mu \equiv 35 \text{ a.m.u.}$ as a constraint, then $\nu \sim 170 \text{ cm}^{-1}$, which is more reasonable, though it occurs at one end of the minimum "valley" and not in the center. If infrared spectroscopy is undertaken on this compound, a comparison of the ν value obtained will help to show whether it is useful to constrain μ .

It can be seen from Figures 4.5 and 4.6 that the $F(\mu, \nu)$ minima obtained for the second and third shells are narrower than that for the first shell. This is rather surprising, since with the many scattering interactions contributing to the EXAFS from these shells, we would have expected the temperature trend to deviate more from the harmonic oscillator model and produce broader minima. Also, despite the difference in the c_2 values for the second and third shells, the $F(\mu, \nu)$ minima for both shells occur over the same values of (μ, ν) , with ν being between 30–200 cm^{-1} , but mostly occurring between 30–100 cm^{-1} , and μ being once again anywhere above 20 a.m.u. Closer inspection of Table 4.2 shows that similarity in the $F(\mu, \nu)$ minima is because Δc_2 actually turns out to be rather similar for both shells. Only the components of c_2 due to the intrinsic amplitude envelope (see Equation 2.7 in Chapter 2) and σ^2_{stat} (Equation 4.1) differ. The similarity in Δc_2 for the second and third shells is unexpected; we would have expected a shell at longer distance, and more affected by multiple-scattering, to be more susceptible to thermal damping than a nearer shell. At present we have no explanation for this result. Constraining μ to be $\sim 35 \text{ a.m.u.}$ once again gives a higher ν value ($\sim 150 \text{ cm}^{-1}$) at one extreme of the solution space. As before, the value of ν should be compared with IR studies on this compound, followed by a normal coordinate analysis.

Relative to the value of ν for the first shell, the value of ν obtained for the second and third shells is similar and only slightly lower. This probably shows that, while Cu–N–C bending modes are now convoluted with the Cu–N stretch in the observed EXAFS Debye-Waller factor, the imidazole ring can still be treated as a rigid unit in EXAFS analysis. Also, even in a Cu(I)-imidazole compound with a relatively long Cu–N bond, wagging or rotating motions about the Cu–N bond by the ring as a whole do not seem to have a major impact on the EXAFS Debye-Waller factor.

(F) Conclusion

For all three shells, it can be seen that c_2 is not very useful for obtaining a good value of μ . However, a physically correct value of μ , if a good estimate can be made, may possibly be useful in constraining c_2 values in fits to EXAFS data. At present we cannot explain why a reasonable value for μ should occur at the extreme end rather than the center of the solution space. The stretching frequency ν could be more useful in constraining c_2 values, but it still remains to be proven that the ranges of values for ν we obtained correctly bracket the true values of ν as it would be measured in an IR experiment. In the absence of such results, some indication of the reliability of these results could be obtained by fitting the EXAFS temperature series of a similar compound such as $\text{Cu(I)(N-methylimidazole)}_2\text{BF}_4$ (for which the appropriate data have already been collected). Since the Cu(I)–N bond is shorter (avg. 1.859(7) Å, see previous chapter) and presumably stiffer, the ν value obtained should be closer to 200 cm^{-1} . If this does indeed turn out to be the result, then it may be worth trying a value of ν as a constraint on the variation of c_2 in a temperature series of data.

This analysis has also shown that imidazole rings can to a good approximation be treated as rigid bodies for EXAFS analysis, ignoring their ring vibrational modes. Similarly, that wagging or rotation of the imidazole ring as a whole about the Cu–N bond does not occur to a sufficient degree to have much impact on the EXAFS Debye-Waller factor.

All this is still a long way from our goal of fitting the imidazole EXAFS accurately in a system, such as hemocyanin, for which scatterer distances and vibrational data are not accurately known, and for which imidazoles as well as other scatterers occur with greater spreads of distances and angles. Furthermore, it is not often that there will be data at multiple temperatures available for trying this fitting procedure. All the same, in the process, much insight into the EXAFS behaviour of imidazole will be gained, and this cannot but lead us closer to our final goal.

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Table 4.1. Some of the more probable scattering paths for a Cu-imidazole system. Similar paths involving C₂ and C₃ also occur.

Single-scattering	Double-scattering	Triple-scattering
Cu-N ₁ -Cu	Cu-N ₁ -C ₁ -Cu	Cu-N ₁ -C ₁ -N ₁ -Cu
Cu-C ₁ -Cu	Cu-C ₁ -N ₂ -Cu	Cu-C ₁ -N ₂ -C ₁ -Cu
Cu-N ₂ -Cu	Cu-N ₁ -N ₂ -Cu	Cu-N ₁ -N ₂ -N ₁ -Cu
		Cu-N ₁ -N ₂ -C ₁ -Cu

Figure 4.1. Schematic of a Cu-imidazole ring. N₁ is in the first shell, C₁ and C₂ are in the second shell and N₂ and C₃ are in the third shell. The second and third shell together are also referred to as the outer shells.

Table 4.2. Values of c_2 obtained by using parameters extracted from data collected at 10 K and varying c_2 only.

Temperature	Value of c_2 ($\text{\AA}^2$)		
	First Shell	Second Shell	Third Shell
10 K	-0.01231	-0.03589	-0.02374
45 K	-0.01235	-0.03636	-0.02427
80 K	-0.01293	-0.03768	-0.02526
130 K	-0.01495	-0.04006	-0.02768
200 K	-0.01713	-0.04323	-0.03076

Table 4.3. Amplitude parameters obtained by varying six amplitude and phase parameters (c_0 , c_2 , c_3 , a_0 , a_1 and a_2) for the closest fit to data at each temperature.

Temperature		c_0	c_2	c_3
First Shell [†]	10 K	0.98428	-0.01231	-1.07515
	45 K	0.83541	-0.01285	-0.97802
	80 K	0.90618	-0.01312	-1.02831
	130 K	0.73034	-0.01570	-0.90375
	200 K	0.97287	-0.01503	-1.14167
Second Shell	10 K	0.00192	-0.03589	+2.40278
	45 K	0.00111	-0.04150	+2.84896
	80 K	0.00043	-0.04887	+3.50962
	130 K	0.01191	-0.03065	+1.21972
	200 K	0.01096	-0.03424	+1.28778
Third Shell	10 K	0.40400	-0.02374	-0.51130
	45 K	0.39316	-0.02430	-0.49638
	80 K	0.19256	-0.02997	+0.00494
	130 K	0.09958	-0.03666	+0.46615
	200 K	0.06515	-0.04180	+0.74227

[†] — These values may be slightly erroneous because there was an error in setting the value of the phase parameter a_1 ($a_1 = 16.33$ instead of 0) while extracting these parameter sets.

Figure 4.2. Averaged EXAFS spectra of $\text{Cu(I)(N-methylimidazole)}_4\text{ClO}_4$, measured at 10, 45, 80, 130 and 200 K to $k = 17 \text{ \AA}^{-1}$. There is a monotonic decrease in the overall amplitude of the EXAFS signal as the temperature is raised.

Figure 4.3. Fourier transforms over $k = 3.5\text{--}16.5$ (0.1) $\text{\AA}^{-1}$ of the averaged EXAFS spectra of $\text{Cu(I)(N-methylimidazole)}_4\text{ClO}_4$, measured at 10, 45, 80, 130 and 200 K, shown in Figure 4.2. There is a monotonic decrease in the Fourier transform magnitude as the temperature is raised.

Figure 4.4. Contour plot of $F(\mu, \nu)$ for c_2 values (listed in Table 4.2) from fits to the first shell. $F(\mu, \nu)$ was evaluated by taking differences between adjacent temperatures (i.e., $[f(45)-f(10)]$, $[f(80)-f(45)]$, $[f(130)-f(80)]$ and $[f(200)-f(130)]$). The thin lines show where $F(\mu, \nu) < 5 \times 10^{-7} \text{ \AA}^4$, and the heavy lines show where $F(\mu, \nu) < 1 \times 10^{-6} \text{ \AA}^4$.

Figure 4.5. Contour plot of $F(\mu, \nu)$ for c_2 values (listed in Table 4.2) from fits to the second shell. $F(\mu, \nu)$ was evaluated by taking differences between adjacent temperatures (i.e., $[f(45)-f(10)]$, $[f(80)-f(45)]$, $[f(130)-f(80)]$ and $[f(200)-f(130)]$). The thin lines show where $F(\mu, \nu) < 5 \times 10^{-7} \text{ \AA}^4$, and the heavy lines show where $F(\mu, \nu) < 1 \times 10^{-6} \text{ \AA}^4$.

Figure 4.6. Contour plot of $F(\mu, \nu)$ for c_2 values (listed in Table 4.2) from fits to the third shell. $F(\mu, \nu)$ was evaluated by taking differences between adjacent temperatures (i.e., $[f(45)-f(10)]$, $[f(80)-f(45)]$, $[f(130)-f(80)]$ and $[f(200)-f(130)]$). The thin lines show where $F(\mu, \nu) < 5 \times 10^{-7} \text{ \AA}^4$, and the heavy lines show where $F(\mu, \nu) < 1 \times 10^{-6} \text{ \AA}^4$.

Chapter 5

EXAFS Studies of Reduced Poplar Plastocyanin at High and Low pH

(A) Structure and Function of Plastocyanin

(1) Overall Structure of Plastocyanin

Plastocyanin is a small protein, containing one copper atom, that functions as an electron carrier in the process of photosynthesis.¹ It is the last carrier in a chain of electron transport from Photosystem II to Photosystem I, transferring one electron between the membrane-bound proteins cytochrome *f* and P700 (Photosystem I). This protein, along with other "blue copper" proteins, is also of special interest to inorganic chemists because of its unique spectroscopic features, including an unusually intense blue colour and a very small EPR hyperfine splitting in the $g_{\parallel}$ region, which are not easily matched in any small-molecule copper compound.²⁻⁵

The crystal structure of Poplar plastocyanin (*Populus nigra*, var. *italica*, henceforth Pc) has been determined (Figure 5.1(a)), in both oxidized and reduced forms, at various pH's, at resolutions ranging from 1.33 Å to 2.15 Å.⁶⁻⁸ This protein has 99 amino acid residues, and takes the form of a slightly flattened β -barrel, with almost no α -helix structure. The copper atom is found near one end of the barrel, ligated by two histidines (residues 37 and 87), a cysteine (residue 84) and a methionine (residue 92). The intense blue colour of the protein is ascribed to ligand-to-metal charge transfer from S(cysteine) to Cu. Recently, X-ray absorption spectroscopy at the sulfur K edge has shown that the small hyperfine splitting in the $g_{\parallel}$ region is due to the highly covalent nature of the Cu–Cys84 bond.⁹ Histidine 87 protrudes from the surface of the protein at one end, and may provide a redox pathway to or from the copper.

(2) Structure of the Copper Site and Its pH Dependence

The geometry of the copper atom is unusual, being a distorted tetrahedron rather than tetragonal (Figure 5.1(b)). Another distinctive feature is the Cu–Met92 bond, which is 2.82 Å long in the oxidized protein.⁸ As confirmed by the high reduction potential of 370 mV,¹⁰ this unusual geometry optimizes the Cu for electron transfer, facilitating oxidation and reduction with little change in geometry.

The redox potential of Pc is strongly pH dependent below pH 5.4.¹⁰ The presence of a protonated redox-inactive form of Pc at low pH has been suggested.¹¹ Crystal structures determined at two pH's on oxidized Pc⁶ show that the structure of oxidized Pc is essentially unchanged between pH 4.2 and pH 6.0. However, crystal structure determinations of reduced Pc⁷ at six pH's between 3.8 and 7.8 show that the copper site changes significantly while the rest of the protein remains much the same. Between pH 7.8 and pH 3.8, the Cu–Met92 bond in the crystal structures changes from 2.90 Å to 2.52 Å,

while the Cu–His87 bond breaks, and the coordination of the Cu becomes trigonal planar (Figure 5.1(c)). At pH 3.8, the distance between Cu and the nearest atom of His87 is 3.15 Å, and the imidazole ring of His87 has actually rotated 180° so that carbon rather than nitrogen is the nearest atom to Cu. This suggests that His87 has been protonated and that Pc at pH 3.8 may indeed be inactive. The lengths of the Cu–His37 and Cu–Cys84 bonds do not change significantly with pH.

(3) Some Questions About the Copper Site

Although the crystal structure of Pc has been solved, questions about the structure and function of the copper site remain, some of which may be addressed by X-ray absorption spectroscopy. One such question is whether the gradual changes observed in the Cu site geometry are really due to a gradual shifting with pH, or to a shifting equilibrium between two extreme positions. The latter is more chemically reasonable. However, the crystallographic data alone were not sufficient to rule out either possibility, and while evidence from the pH-dependent reduction potential and reactivity of Pc was suggestive of an equilibrium, it was not conclusive.

While X-ray crystallography reveals the position of the Met92 sulfur equally well whether it is 2.90 Å or 2.52 Å from Cu, in EXAFS the Cu–S contribution would be stronger for a shorter and stiffer Cu–S bond. Thus, S at 2.5 Å from Cu will be much more easily detected than S at 2.9 Å. So, if the variation in Cu–S bond length is truly due to a gradual change in Cu–S bond length, we would find S at intermediate bond lengths for intermediate pH's. On the other hand, if it is due to a shifting equilibrium, EXAFS would perceive a partial atom at the shorter bond length.

Another major reason to perform an EXAFS study of reduced Pc is that EXAFS is capable of giving bond lengths to a precision of 0.03 Å or better, while the crystal structures of Pc have Cu–ligand bond lengths with standard deviations varying from 0.04 Å to more than 0.07 Å.⁸ In particular, the resolutions of the crystal structures at pH 3.8 and pH 7.8, which would give the best view of the two extreme geometries, are such that the standard deviations are greater than 0.07 Å. Thus, the extra precision afforded by EXAFS would be valuable in helping to understand the chemistry of plastocyanin. Also, we wished to see if the structure of reduced Pc in solution, especially at low pH, was the same as in the crystal structure.

(B) Experimental Details

(1) Preparation of Samples

Two samples of Poplar plastocyanin (at pH 7.2 and 4.8) were provided by Professor Hans C. Freeman of the University of Sydney, Australia. Two bands of plastocyanin were separated by ion-exchange chromatography of the reduced Pc on DEAE resin or Q-Sephadex using a NaCl gradient column.¹² The pH 7.2 sample was taken from Band 1, and dialyzed with a 0.1 M sodium phosphate buffer with 0.05 M sodium ascorbate to keep the sample reduced. The solution was made 40% in glycerol by dialysis, and the final concentration was 4–4.2 mM. The pH 4.8 sample was from Band 2, in a 0.1 M sodium acetate buffer with 0.05 M sodium ascorbate, which was added as ascorbic acid. The pH was adjusted to 4.8 by addition of sodium hydroxide solution. This solution was also made 40% in glycerol. The concentration of the pH 4.8 sample is not precisely known since some precipitation of the protein occurred as the pH was lowered, but it is estimated to be ~3 mM.

Each solution was loaded into an EXAFS cell made of lucite (outer dimensions $2 \times 4 \times 28$ mm³, volume ~180 μ l) with an X-ray-transparent front face of 60 μ m Mylar tape for X-ray fluorescence measurements. The samples were stored in liquid nitrogen and also mounted for data collection under liquid nitrogen.

Cu(I) model compounds were ground with boron nitride and packed into aluminum spacers in the manner described in Chapter 2.

(2) Data Collection

Protein data were collected at the Stanford Synchrotron Radiation Laboratory at the unfocussed 8-pole wiggler beam line 4-2 in fluorescence mode, using a Lytle detector¹³ with a nickel filter, and argon gas in the chamber. Ring current was between 25 and 49 mA. The temperature of the samples during data collection was 10–12 K. No photoreduction or oxidation of either sample was observed during data collection, but in both cases, when the samples were removed from the X-ray beam, the originally clear and colourless frozen glass had turned brown where the beam had passed through it. Data for Cu(I) models were collected in transmission mode at 10 K.

(3) Data Analysis

Data were reduced and analyzed using XFPKAG, using the approach described in Chapter 2. Scans were calibrated using copper foil as an internal standard, taking the first inflection point of the foil as 8980.3 eV. Twenty-one scans were averaged together for the

high pH sample, and 29 scans for the low pH sample. A major glitch was removed from both the averaged data sets at $k = 10.9 \text{ \AA}^{-1}$. Subtraction of the background was done by fitting a polynomial to the EXAFS region and then applying this at the bottom of the "edge step" and extending this into the pre-edge region. The same three-region spline to $k = 13 \text{ \AA}^{-1}$ was used for both data sets (Figure 5.3). For all data analyzed in this chapter, a data range of $k = 3.5\text{--}12.5 \text{ \AA}^{-1}$ was used for the forward Fourier transform (Figures 5.2 and 5.4), and the data were then backtransformed to $k = 4\text{--}12 \text{ \AA}^{-1}$ for curvefitting. Phase and amplitude parameters were also extracted over the same data ranges.

(C) EXAFS Fits to the Data — Results and Discussion

(1) Extraction of Parameters and Testing on a Cu(I)–N₂S₂ Model

Using the method for parameter extraction described in Chapter 2, Cu(I)–N parameters were extracted from data collected at 10 K on Cu^I(*N*-methylimidazole)₄ClO₄,¹⁴ which has four crystallographically equivalent methylimidazoles ligated about Cu(I) in a tetrahedral geometry. The Cu(I)–N distance in this compound is 2.054 Å. Similarly, Cu(I)–S parameters were extracted from [Cu^I(diethylthiourea)₃]₂SO₄,¹⁵ which has three crystallographically equivalent diethylthioureas ligated at a Cu(I)–S distance of 2.241 Å. Data collected on Cu₂I₂((CH₃NCH₂CH₂SSCH₂CH₂N(CH₃)₂)₂(BF₄)₂)¹⁶ (Figure 5.2), a compound with Cu(I)–N₂S₂ ligation, were used to test these parameters. The two Cu atoms in this dimer are not crystallographically equivalent, but they have very similar bond lengths. Cu(I)–N bond lengths range between 2.089–2.184 Å, and Cu(I)–S bonds between 2.283–2.326 Å.

A Fourier window over 1.35–2.35 Å was used to filter out the main peak of the data on the Cu(I)–N₂S₂ compound (henceforth referred to as CUNS2) for data analysis (Figure 5.2). It was not possible to widen the window in a manner analogous to the treatment of the protein data (*vide infra*) without including contributions from second-shell carbon atoms, so this was not done. One-, two- and three-wave fits were performed on the filtered data, as shown in Table 5.1. (See also Appendix I for complete fit results.)

The relative Debye-Waller factor is represented by the parameter c_2 , which is always negative (see Chapter 2). A larger negative value of c_2 indicates more disorder in the distances of scatterers, or more vibrational disorder and a weaker Cu–X bond. Coordination numbers and c_2 values were evaluated together to determine the most reasonable fit to a data set.

Fits were done either by floating coordination numbers and fixing c_2 (Type 2 fits), or varying c_2 and fixing coordination numbers (Type 3 fits), or occasionally by varying both

coordination numbers and Debye-Waller factors (Type 1 fits). In all fits, the Cu–X distances of all waves were varied. In two- or three-wave Type 1 fits, there is considerable correlation between the coordination numbers and c_2 values, and so these fits do not have much physical significance as such. However, they can be useful in finding out what minimum the data tend towards.

As can be seen from Table 5.1, fits were done not only for scatterer combinations that we expected to find (such as Cu to N and S), but also for site geometries that we did not expect to find (such as Cu–N only or Cu–S only). How closely such "incorrect" or "negative" fits can model the data gives an indication of how significant the "correct" fits are. Table 5.2 summarizes the results for Type 2 fits. From it we can see that the two-wave fit which has the smallest fit function (fit index $F = 0.134$) is indeed the physically correct one, namely N+S with 1.6 N at 2.11 Å and 1.4 S at 2.28 Å. Two other minima were found for the N+S fit. However, these not only fit much more poorly (fit index $F = 0.665$ and 0.301 respectively), but they are physically quite implausible, having an unreasonably high coordination number for N and a very long Cu–S bond in one case, and an unreasonably long Cu–N bond in the other. Also, fits with N+N' and S+S' waves either fitted the data very poorly or gave manifestly unphysical results (such as negative coordination numbers).

Fits of Type 3 behaved in a similar fashion, that is, N+S fits fitted the data much better than either N+N' or S+S' fits. The Type 3 fit with the lowest fit index was achieved using 1N at 2.14 Å with 2S at 2.27 Å rather than 2N at 2.17 Å with 2S at 2.26 Å, which proved to have the next closest fit. However, the Debye-Waller factor for the N-wave in the 2N+2S fit ($\Delta\sigma^2 = +0.001$) makes better physical sense than that in the 1N+2S fit ($\Delta\sigma^2 = -0.003$), because the Cu(I)–N length has increased relative to that in $\text{Cu}^{\text{I}}(\text{N-methylimidazole})_4\text{ClO}_4$, and this should be accompanied by an increase in the Debye-Waller factor. Therefore, the 2N+2S fit may still be considered the most plausible fit.

A couple of three-wave fits (N+N'+S) were tried, but they yielded either totally unphysical results or the very same minimum that was achieved with the N+S fit.

Thus, we may conclude that the most reasonable fit to CUN2 (2N+2S) is the one that we know to be physically correct, and that the parameters we extracted are working as they should and giving us the right answers. We also gain a good idea of the precision of coordination numbers obtained using these parameters.

(2) Fits to Reduced Plastocyanin at pH 7.2 and 4.8

(a) Fourier Transform Windows and Types of Fits Done

The averaged data sets for the two protein samples were curvefitted in parallel. By and large, a fit that was done on the high pH data (hereafter PCHI) was also done on the low pH data (PCLO) and vice versa. Our working assumption was that the solution structure of reduced Pc at pH 7.2 would most probably be similar to the crystal structure, with one long Cu–Met92 bond, while there was less certainty about the structure of reduced Pc at lower pH. Thus PCHI served as an internal reference against which to gauge the reliability of fits to PCLO. Wishing to search for a sulfur scatterer between 2.5 Å and 2.9 Å, but uncertain if scattering from the carbon atoms of the imidazole rings of the ligated histidines would instead be detected, we decided to try fits on Fourier transform windows of various widths, starting from a bare main peak and widening the shell by stages to include the first small peak to higher R, then the second as well (see Figure 5.4, Table 5.3). Since a low-R shoulder of significant height seen in the Fourier transform for the PCLO data could not be removed by splining, fits were also done on a window for both PCHI and PCLO including it.

Various one-, two- and three-wave fits were performed using Cu(I)–N and Cu(I)–S parameters (see Table 5.4, also Appendix I for complete fit results). These fits were done either by floating coordination numbers and fixing Debye-Waller factors (Type 2 fits in Tables 5.5 and 5.6), or by varying Debye-Waller factors and fixing coordination numbers (Type 3 fits in Table 5.7), or occasionally by varying both coordination numbers and Debye-Waller factors (Type 1 fits). Type 1 fits were mainly done in the initial stages of data analysis to obtain a feel for the minima that the data tend towards. For two-wave and three-wave fits, Type 1 fits cannot be relied on to produce physically trustworthy results because of high parameter correlation, and so only Type 2 and Type 3 fits are discussed. As it turned out, results from Type 2 and Type 3 fits led to the same conclusions, so most of the following discussion is in terms of Type 2 fits.

Once again, besides using combinations of waves that we expected to find in the data (such as N+S+S'), we used combinations that we did not expect to find (such as N+N') to test the significance of the fits we obtained.

(b) Results of Two-Wave Fits

For PCHI, Type 2 N+S fits on all windows gave 1.9 N at 2.03 Å and 0.5 S at 2.20 Å. For PCLO, the same fits gave two minima : -0.4 N at 1.85 Å with 0.9 S at 2.16 Å and

1.9–2.4 N at 2.17 Å with 1.4–1.5 S at 2.14 Å. The latter is by far the better fit to the data. Type 3 N+S fits were also done, and in PCHI they tended to come up with longer Cu–N distances (2.08 Å) than the Type 2 fits did. Of the fits tried, the most physically believable fit (2N+1S) did not produce the closest fit, but it did produce the most sensible Debye-Waller factors. In PCLO, there were again two minima, one with essentially no N-wave (a huge Debye-Waller factor) and 1S at 2.13–2.15 Å and another, much better-fitting minimum suggesting 2N (rather than the expected 1N) at 2.13 Å and 1S at 2.15 Å. It is obvious that the poorly-fitting minimum with no or negative N signal is not physically believable. It is only mentioned here because these two minima for PCLO occur again in the three-wave fits (*vide infra*).

Comparing all the Type 2 two-wave fits done (see Table 5.5, also Appendix I), it is clear that, for PCHI, N+N' and S+S' do not fit the data as well as N+S, but since their fit indices are less than twice that for N+S, the fits may not be considered completely unsuccessful. For fits to PCLO, N+N' produced extremely poor fits, and so N+N' is easily ruled out as a viable fit. However, S+S' actually produced fits as good as N+S, or even slightly better. For both PCHI and PCLO, two minima could be obtained by varying the initial distance for S'. In both these minima, for both PCHI and PCLO, R_S remained constant at 2.16–2.19 Å, but $R_{S'}$ changed considerably, being found at either 2.33–2.37 Å or 2.74–2.96 Å. These two minima are also found in the N+S+S' fits (*vide infra*). However, unlike in the three-wave fits, the short-S' minimum is a much better fit than the long-S' fit, so the S+long-S' fit can be dismissed as unlikely to be a true solution for either PCHI or PCLO. In PCHI, since S+short-S' is a worse fit than N+S, we may conclude that short-S' is doing its best to fill in for the absent N. In PCLO, S+short-S' fits the data as closely as N+S, so the fit index F cannot be used to choose the more likely fit of the two. However, only 0.4 S' are found at 2.37 Å in the S+short-S' fit, whilst 1.9–2.1 N are found in the N+S fit (~1 S occurs at 2.15 Å for both fits). The N+S fit thus seems more plausible, if we are using only two waves to account for all the ligands coordinated to Cu. If we consider that perhaps two waves are not enough to model all the different contributions to the EXAFS of PCLO, then both the N+S and the S+short-S' fits may be accurately reflecting the contributions of different scatterers.

(c) Results of Three-Wave Fits

The N+S+S' fits (see Tables 5.6 and 5.7) are of particular interest, since they most closely model the Pc site as shown by the crystal structures. We discovered that, simply by varying the initial distance for the S'-wave, different minima could be reached.

If $R_{S'}$ were initially set at 2.5 Å, then the fit would refine to a minimum of $R_{S'} = 2.3\text{--}2.4$ Å. But if $R_{S'}$ was initially set at a higher value (2.8 Å for PCHI, 2.6 Å for PCLO), the fit invariably refined to a value of $R_{S'}$ greater than 2.7 Å. This was true of both PCHI and PCLO, for fits of both Type 2 and Type 3, and it was true for every Fourier window that the N+S+S' fit was performed on (Windows 1–3), even though Windows 1 and 3 do not include data above $R' \sim 2.4$ Å.

If the short $R_{S'}$ fit were either as good or better than the long $R_{S'}$ fit for PCLO, and the reverse were true for PCHI, then we might be able to conclude that S' in PCLO is indeed at a shorter distance than it appears in the crystal structure at low pH. However, in most of the N+S+S' fits performed, on PCHI as well as on PCLO, the short $R_{S'}$ fit is as good as or better than the long $R_{S'}$ fit. Since we are not expecting to find both sulfurs at short distances in Pc at high pH, we would tend to regard the fit minimum with the short $R_{S'}$ for PCHI as a false minimum not reflecting the true physical arrangement of scatterers about the copper atom. If we so regard this minimum in PCHI, we need good reasons to regard the similar minimum in PCLO differently. In the absence of such reasons, we cannot conclude for sure that the Cu–S' distance is truly now less than 2.4 Å in PCLO.

One possible reason for the good fit with a short $R_{S'}$ is that, in Windows 1 and 3, data from $R' > 2.4$ Å were not included. However, if we examine Window 2 alone, we still find that, for Type 2 fits, the short- $R_{S'}$ fit is as good or better than the long- $R_{S'}$ fit at both pH's. In the Type 3 fits, however, we do find a possible hint of a meaningful trend (see Table 5.7). In Windows 1 and 3, the short- $R_{S'}$ fits achieve a closer fit to the data (with fit indices being 15–60% lower) than the long- $R_{S'}$ fits. In Window 2, the short- $R_{S'}$ fit has a fit index that is 16% higher than the long- $R_{S'}$ fit for PCHI, but 10% lower for PCLO. This is certainly suggestive of a truly long Cu–S' bond in PCHI and a truly shorter Cu–S' bond in PCLO. However, the differences in the fit indices are not enough to be considered firmly significant, especially since this trend is confounded rather than confirmed by the Type 2 fits.

While the behaviour of the Cu–S' wave in N+S+S' fits is remarkably similar in both PCHI and PCLO, this is not so for the Cu–N wave in these same fits. Considering only Type 2 fits (Table 5.6), in PCHI, the contribution from N remains fairly stable (1.5–1.9 N at 2.01–2.03 Å in all fits except one on Window 3, where 1.0 N occurs at 1.99 Å) for both the long-S' and short-S' minima. Also, no fit component exceeds the magnitude of the total fit (Figures 5.5 and 5.6).

However, in PCLO, in behaviour similar to that in the N+S fits (*vide supra*), for the short-S' minimum we find 0.3 N at 2.02 Å (except in Window 3), while for the long-S'

minimum we find 1.5–2.3 N at 2.10–2.19 Å. In the corresponding Type 3 fits, the N-wave makes even less of a contribution in the short-S' minimum, disappearing to almost nothing at an improbably short distance of 1.75–1.80 Å in Windows 1 and 2, whereas in the long-S' minimum it acquires a Debye-Waller factor that is far too low for a Cu(I)–N bond of over 2 Å. In Window 3, the presence of the contribution from the large low-R shoulder results in a larger component of N in the fit, about 0.4 N at 1.85 Å. Nor does the N-wave disappear in the Type 3 fits. Overlaying the backtransformed data with the N-, S- and S'-waves in a typical fit (Figures 5.7 and 5.8), it can be seen that massive cancellation occurs in order to produce both minima in the fits to PCLO. In both cases, the S-wave (modelling the short Cu–Cys84 bond) is actually larger than the total EXAFS. For the short-S' minimum, the S-wave is partly cancelled by the S'-wave, while in the long-S' minimum the S-wave is partly cancelled by the N-wave. Thus there is considerable correlation between the N-wave and the S'-wave, and we hesitate to conclude that the numbers for either the N- or S'-wave produced by these fits are a true indication of their location around Cu in PCLO. However, the S-wave is remarkably little affected by the dance that the N- and S'-waves are performing around it; we consistently find it at 2.13–2.17 Å, and in general it shows the same coordination number for both minima, for a given Fourier transform window.

Some N+S+N' fits were also made (see Table 5.6). For both PCHI and PCLO, these fits are slightly better than the N+S+S' fits for Window 1, and slightly worse for Window 2. There is always less than 0.5 N' present, and $R_{N'}$ occurs at up to three different distances for the same window, and so it can be seen that the minima it found are very shallow. On the other hand, a substantial N-wave (more than 1 N, usually closer to 2 N) at about 2.1 Å is always present, along with the stable S-wave at about 2.15 Å. These two waves look rather like the N- and S-waves in the long-S' minima discussed above. The N+S+N' fits may thus be interpreted as basically the same N+S fit found for the long-S' solution above, plus an N'-wave trying to fill up the little spaces left. The uncertainty in $R_{N'}$ shows that no firm conclusions should be based on it. The fact that the N+S+N' fit works about as well as the N+S+S' fit for both PCHI and PCLO also indicates that caution is warranted in believing the S' results in the long-S' N+S+S' fit, even in PCHI.

The results we have obtained so far on PCHI (~2 N at 2.02 Å and ~1 S at 2.20 Å) do not resemble the reduced pH 7.0 crystal structure distances that closely (see Figure 5.1(c)). Some 1N+1N'+1S fits on Window 3 of PCHI were carried out (see Table 5.8), using initial distances ($R_N = 2.15$ Å, $R_{N'} = 2.40$ Å and $R_S = 2.15$ Å) close to the crystal structure distances ($R_N = 2.16$ Å, $R_{N'} = 2.37$ Å and $R_S = 2.15$ Å). When only R's were varied, we

obtained $R_N = 2.11 \text{ \AA}$, $R_{N'} = 2.63 \text{ \AA}$ and $R_S = 2.17 \text{ \AA}$. A Type 2 fit (varying R's and CN's) led to the implausible result of 8.4 N at $2.07 \text{ \AA}$, 2.3 N' at $2.34 \text{ \AA}$ and 1.5 S at $2.03 \text{ \AA}$. A Type 3 fit gave $R_N = 2.11 \text{ \AA}$, $R_{N'} = 2.04 \text{ \AA}$ and $R_S = 2.17 \text{ \AA}$ ($c_{2N} = -0.0267$, $c_{2N'} = -0.0326$ and $c_{2S} = -0.0255$). The distances are reasonable in themselves, but we would have expected that if $R_{N'} < R_N$, then $|c_{2N'}| < |c_{2N}|$, and we find that the reverse is true. So this result, also, should be regarded with caution. So, for the N+N'+S fits N and S are well-behaved (though Cu–N is somewhat shorter than the crystal structure value of $2.16 \text{ \AA}$), but N' does not fall anywhere near the crystal structure value of $2.37 \text{ \AA}$ in a reasonable fit.

We also tried adding S' at $2.9 \text{ \AA}$ for a few N+N'+S+S' fits (see Table 5.8). When only R's were varied, results were similar to the N+N'+S fit ($2.11 \text{ \AA}$, $2.59 \text{ \AA}$, $2.17 \text{ \AA}$ and $2.91 \text{ \AA}$ respectively). When all R's and two CN's (N' and S') were varied, the result for N' was again implausible (-0.9 N at $2.37 \text{ \AA}$). A Type 3 fit gave $R_N = 2.11 \text{ \AA}$, $R_{N'} = 2.59 \text{ \AA}$, $R_S = 2.17 \text{ \AA}$ and $R_{S'} = 2.95 \text{ \AA}$. So, even with a four-wave fit, N' could not be found anywhere between $2.3 \text{ \AA}$ and $2.4 \text{ \AA}$ except with a negative CN.

The crystal structure at pH 7.0 was determined with a resolution of $1.8 \text{ \AA}$, which gives a standard deviation σ of $0.07 \text{ \AA}$ in Cu–X bond lengths, with 3σ being $\sim 0.2 \text{ \AA}$. So Cu–S and Cu–S' from EXAFS ($2.17/2.95 \text{ \AA}$) are not significantly different from Cu–S and Cu–S' in the crystal structure ($2.15/2.88 \text{ \AA}$). One of the N's is also found by EXAFS at a distance not significantly different from the crystal structure distance (i.e., $2.11 \text{ \AA}$ and $2.16 \text{ \AA}$ respectively), while the other N, being at either $2.59 \text{ \AA}$ or $2.04 \text{ \AA}$, may truly occur at a different distance from that in the crystal structure ($2.37 \text{ \AA}$).

This suggests either that, at pH ~ 7 , the distances of at least one Cu–ligand bond are indeed different in solution from what was found in the crystal structure, or that it is not possible to resolve the true distances of these ligands over this data-fitting range.

D) Conclusion

A thorough and cautious analysis of data from solution samples of reduced Pc at pH 7.2 and pH 4.8 shows that it is difficult to resolve the contribution of the sulfur scatterer from Met92. At pH 7.2, there are 1.5–1.9 N at $2.01\text{--}2.03 \text{ \AA}$ and 0.5–1.2 S at $2.20\text{--}2.21 \text{ \AA}$. At pH 4.8, all that can be stated with certainty is that there is 0.9–1.6 S at $2.13\text{--}2.17 \text{ \AA}$.

The distances obtained from the pH 7.2 data differ from those found in crystal structure at pH 7.0, where the 2 N's are found at $2.15 \text{ \AA}$ and $2.37 \text{ \AA}$. Attempts to find a fit with 2 N-waves at these distances failed; reasonable fits found the N's at $2.11/2.59 \text{ \AA}$, or possibly $2.11/2.04 \text{ \AA}$. So the Cu–ligand distances in our solution sample of reduced Pc at

high pH may truly differ from those found in the crystal structure, or perhaps the data range used for the fits is not sufficient to resolve the true distances of the ligands.

The other question we had was whether we could confirm that there is a shifting equilibrium between two extreme Cu–SMet bond lengths, rather than a gradual shift in the bond length itself, with pH. There are tantalizing indications that S from Met92 might indeed be found at two extreme positions, since two fit minima were found which placed it at either 2.3–2.4 Å or greater than 2.7 Å from Cu. However, since both these minima appeared in the data at high pH as well as at low pH, and also there was correlation with the N-wave in the low-pH data, these minima cannot be confirmed to be physically significant.

We have thus not been able to answer with certainty the questions that we posed at the beginning of this chapter. Indeed, it is doubtful that EXAFS analysis of Pc solutions, at least given the range and quality of the available data, can reliably discern a weak Cu–SMet interaction among so many other stronger contributions occurring at differing distances. It is possible that polarized EXAFS spectroscopy performed upon Pc crystals may be able to discern the methionine S at an orientation where the EXAFS contributions from the other coordinating atoms are reduced. Extreme care must be taken, however, to avoid being deceived by the sort of physically plausible, but not physically significant, solutions that we encountered in this study.

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Table 5.1. Least-squares fits to $\text{CuI}_2((\text{CH}_3\text{NCH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{N}(\text{CH}_3)_2)_2(\text{BF}_4)_2$ (CUNS2)

Waves used	Fit Type
N	1,2
S	1,2
N + S	(1,2)x3,(3)x2
N + N'	1,(2)x2
S + S'	(1,2)x2
N + N + S'	(2)x2,(3)

- 1 — Distances, coordination numbers and Debye-Waller factors are varied
- 2 — Distances, coordination numbers are varied while Debye-Waller factors (c_2 in the EXAFS equation) are fixed at initial (model) values
- 3 — Distances and Debye-Waller factors are varied while coordination numbers are fixed at various sets of values
- ()x2 — 2 fit minima are explored

Table 5.2. Results of Type 2 fits (varying distances and coordination numbers while fixing Debye-Waller factors) made to $\text{Cu}^{\text{I}}_2((\text{CH}_3\text{NCH}_2\text{CH}_2\text{SSCH}_2\text{CH}_2\text{N}(\text{CH}_3)_2)_2(\text{BF}_4)_2$ (CUNS2). Fixed values of $c_{2\text{N}} = -0.0253$ and $c_{2\text{S}} = -0.0216$.

				Fit index [†]
N only :	5.2 N	@	2.13 Å	0.848
S only :	1.9 S	@	2.28 Å	0.354
N + S :	1.6 N	@	2.11 Å	0.134
	1.4 S	@	2.28 Å	
	5.2 N	@	2.13 Å	0.665
	0.6 S	@	2.65 Å	0.301
	0.7 N	@	2.64 Å	
	1.9 S	@	2.27 Å	
N + N' :	2.4 N	@	2.13 Å	0.848
	2.8 N'	@	2.13 Å	
	4.8 N	@	2.13 Å	0.432
	2.5 N'	@	2.51 Å	
S + S' :	-0.3 S	@	2.05 Å	0.172
	1.7 S'	@	2.28 Å	
	1.9 S	@	2.28 Å	0.322
	0.2 S'	@	2.76 Å	
N + N' + S	2.4 N	@	2.13 Å	0.035
	-1.8 N'	@	2.30 Å	
	0.7 S	@	2.29 Å	
	1.2 N	@	2.11 Å	0.134
	0.4 N'	@	2.11 Å	
	1.4 S	@	2.28 Å	

[†] — The fit index (F) is defined in Chapter 2.

Table 5.3. Fourier transform windows filtering data used in curvefitting of reduced Pc data

Data set	Window 0	Window 1	Window 2	Window 3
PCHI	1.20–2.12 Å	1.20–2.45 Å	1.20–2.85 Å	0.85–2.45 Å
PCLO	1.25–2.12 Å	1.25–2.40 Å	1.25–3.05 Å	0.85–2.40 Å

Table 5.4. Fits made to reduced Pc data at pH 7.2 (PCHI) and pH 4.8 (PCLO)

		Window 0	Window 1	Window 2	Window 3
N	PCHI	1,2	1,2	1,2	
	PCLO	1,2	1,2	1,2	
S	PCHI	1,2	1,2	1,2	
	PCLO	1,2	1,2	1,2	
N+S	PCHI	1,2	1,2,3	1,2,3	1,2,3
	PCLO	1,2	(1,2,3)x2	1,3,(2)x2	1,2,3
N+N'	PCHI	1,2	(1,2)x2	(1,2)x2	
	PCLO	1,2	(1,2)x2	(1,2)x2	
S+S'	PCHI	1,2	(1,2)x2	(1,2)x2	
	PCLO	1,2	(1,2)x2	(1,2)x2	
N+S+S'	PCHI		(2,3)x2	(2,3)x2	(2,3)x2
	PCLO		(2,3)x2	(2,3)x2	(2,3)x2
N+S+N'	PCHI		2,3	2,3	
	PCLO		(2,3)x2	(2)x3,(3)x2	
N+N'+S	PCHI				2,3
	PCLO				
N+N'+S+S'	PCHI				2,3
	PCLO				

- 1 — Distances, coordination numbers and Debye-Waller factors are varied
2 — Distances, coordination numbers are varied while Debye-Waller factors (c_2 in the EXAFS equation) are fixed at initial (model) values
3 — Distances and Debye-Waller factors are varied while coordination numbers are fixed at various sets of values
()x2 — 2 fit minima are explored

Table 5.5. Two-wave Type 2 fits (varying distances and coordination numbers while keeping Debye-Waller factors fixed) to reduced Pc at pH 7.2 (PCHI) and pH 4.8 (PCLO). Fixed values of $c_{2N} = -0.0253$ and $c_{2S} = -0.0216$.

Reduced Pc, pH 7.2				Reduced Pc, pH 4.8			
	Window 0	Fit Index		Window 0	Fit Index		
N + S	1.9 N @ 2.03 Å 0.5 S @ 2.20 Å	0.098		-0.3 N @ 1.85 Å 0.9 S @ 2.16 Å	0.173		
N + N'	0.7 N @ 1.87 Å 3.7 N' @ 2.05 Å	0.156		0.6 N @ 2.02 Å 1.9 N' @ 2.02 Å	0.400		
S + S'	1.3 S @ 2.18 Å 0.6 S' @ 2.33 Å	0.144		1.1 S @ 2.16 Å 0.3 S' @ 2.35 Å	0.096		
	Window 1	Fit Index		Window 1	Fit Index		
N + S	1.9 N @ 2.03 Å 0.5 S @ 2.20 Å	0.167		1.9 N @ 2.16 Å 1.4 S @ 2.14 Å	0.094		
				-0.4 N @ 1.86 Å 0.9 S @ 2.16 Å	0.233		
N + N'	0.8 N @ 1.87 Å 3.7 N' @ 2.05 Å	0.219		2.6 N @ 2.03 Å 0.9 N' @ 2.35 Å	0.324		
	3.1 N @ 2.04 Å 0.5 N' @ 2.73 Å	0.336		2.6 N @ 2.02 Å 0.4 N' @ 2.68 Å	0.427		
S + S'	1.4 S @ 2.18 Å 0.5 S' @ 2.35 Å	0.221		1.2 S @ 2.17 Å 0.4 S' @ 2.37 Å	0.076		
	1.1 S @ 2.19 Å 0.2 S' @ 2.89 Å	0.428		0.9 S @ 2.16 Å 0.1 S' @ 2.78 Å	0.272		
	Window 2	Fit Index		Window 2	Fit Index		
N + S	1.9 N @ 2.03 Å 0.5 S @ 2.20 Å	0.201		2.1 N @ 2.17 Å 1.4 S @ 2.15 Å	0.163		
				-0.4 N @ 1.86 Å 0.9 S @ 2.16 Å	0.289		
N + N'	0.8 N @ 1.87 Å 3.7 N' @ 2.05 Å	0.236		2.6 N @ 2.03 Å 0.9 N' @ 2.35 Å	0.356		
	3.1 N @ 2.04 Å 0.4 N' @ 2.75 Å	0.357		2.6 N @ 2.02 Å 0.3 N' @ 2.68 Å	0.463		
S + S'	1.4 S @ 2.18 Å 0.5 S' @ 2.35 Å	0.239		1.2 S @ 2.17 Å 0.4 S' @ 2.37 Å	0.154		
	1.1 S @ 2.19 Å 0.1 S' @ 2.96 Å	0.454		0.9 S @ 2.16 Å 0.1 S' @ 2.74 Å	0.326		
	Window 3	Fit Index		Window 3	Fit Index		
N + S	1.9 N @ 2.03 Å 0.5 S @ 2.20 Å	0.375		2.4 N @ 2.16 Å 1.5 S @ 2.14 Å	0.336		

Table 5.6. Three-wave Type 2 fits (varying distances and coordination numbers while keeping Debye-Waller factors fixed) to PCHI and PCLO. Fixed values of $c_{2N} = -0.0253$ and $c_{2S} = -0.0216$.

Reduced Pc, pH 7.2				Reduced Pc, pH 4.8			
	Window 1		Fit Index	Window 1		Fit Index	
N + S + S'	1.6 N @	2.01 Å	0.108	0.4 N @	2.02 Å	0.060	
	0.8 S @	2.21 Å		1.0 S @	2.17 Å		
	0.3 S' @	2.42 Å		0.3 S' @	2.38 Å		
	1.8 N @	2.03 Å	0.135	1.5 N @	2.10 Å	0.059	
	0.5 S @	2.20 Å		0.9 S @	2.13 Å		
	0.1 S' @	2.87 Å		0.1 S' @	2.80 Å		
N + S + N'	1.8 N @	2.03 Å	0.101	1.3 N @	2.09 Å	0.026	
	0.5 S @	2.20 Å		0.8 S @	2.14 Å		
	0.5 N' @	2.71 Å		0.4 N' @	2.66 Å		
				1.5 N @	2.12 Å	0.079	
				1.2 S @	2.14 Å		
				0.4 N' @	2.23 Å		
	Window 2		Fit Index	Window 2		Fit Index	
N + S + S'	1.5 N @	2.01 Å	0.147	0.3 N @	2.02 Å	0.149	
	0.8 S @	2.21 Å		1.0 S @	2.17 Å		
	0.3 S' @	2.41 Å		0.3 S' @	2.38 Å		
	1.9 N @	2.03 Å	0.181	2.3 N @	2.19 Å	0.149	
	0.5 S @	2.20 Å		1.5 S @	2.16 Å		
	0.1 S' @	3.02 Å		0.1 S' @	2.70 Å		
N + S + N'	1.9 N @	2.03 Å	0.179	2.2 N @	2.17 Å	0.127	
	0.5 S @	2.20 Å		1.5 S @	2.15 Å		
	0.4 N' @	2.72 Å		0.5 N' @	2.92 Å		
				2.3 N @	2.21 Å	0.156	
				1.6 S @	2.16 Å		
				0.3 N' @	2.55 Å		
				1.7 N @	2.13 Å	0.155	
				1.2 S @	2.14 Å		
				0.4 N' @	2.24 Å		
	Window 3		Fit Index	Window 3		Fit Index	
N + S + S'	1.0 N @	1.99 Å	0.317	0.4 N @	1.85 Å	0.301	
	1.2 S @	2.20 Å		1.4 S @	2.17 Å		
	0.5 S' @	2.39 Å		0.6 S' @	2.37 Å		
	1.8 N @	2.03 Å	0.360	2.3 N @	2.15 Å	0.334	
	0.5 S @	2.20 Å		1.4 S @	2.14 Å		
	0.1 S' @	2.88 Å		0.1 S' @	2.81 Å		

Table 5.7. Results of some Type 3 fits (varying distances and Debye-Waller factors while keeping coordination numbers fixed) to reduced Pc at high pH (PCHI) and low pH (PCLO).

Reduced Pc, pH 7.2				Reduced Pc, pH 4.8			
Window 1				Window 1			
	R	c ₂	F		R	c ₂	F
2 N	2.08	-0.0322	0.140	1 N	2.14	-0.0238	0.139
1 S	2.17	-0.0254		1 S	2.15	-0.0204	
2 N	2.06	-0.0374	0.066	1 N	1.75	-0.0980	0.039
1 S	2.18	-0.0243		1 S	2.15	-0.0206	
1 S'	2.37	-0.0623		1 S'	2.32	-0.0420	
2 N	2.08	-0.0329	0.082	1 N	2.15	-0.0244	0.096
1 S	2.17	-0.0251		1 S	2.15	-0.0201	
1 S'	2.92	-0.0661		1 S'	2.84	-0.0697	
Window 2				Window 2			
	R	c ₂	F		R	c ₂	F
2 N	2.08	-0.0322	0.175	1 N	2.14	-0.0224	0.204
1 S	2.17	-0.0251		1 S	2.14	-0.0199	
2 N	2.06	-0.0373	0.102	1 N	1.80	-0.1086	0.146
1 S	2.18	-0.0242		1 S	2.15	-0.0203	
1 S'	2.37	-0.0614		1 S'	2.33	-0.0407	
2 N	2.08	-0.0319	0.088	1 N	2.20	-0.0061	0.162
1 S	2.17	-0.0254		1 S	2.16	-0.0119	
1 S'	2.98	-0.0609		1 S'	2.98	-0.0819	
Window 3				Window 3			
	R	c ₂	F		R	c ₂	F
2 N	2.07	-0.0333	0.331	1 N	2.12	-0.0172	0.377
1 S	2.17	-0.0253		1 S	2.13	-0.0204	
2 N	2.03	-0.0438	0.258	1 N	1.90	-0.0359	0.291
1 S	2.18	-0.0225		1 S	2.16	-0.0185	
1 S'	2.35	-0.0476		1 S'	2.34	-0.0328	
2 N	2.07	-0.0333	0.302	1 N	2.11	-0.0165	0.355
1 S	2.17	-0.0250		1 S	2.13	-0.0207	
1 S'	2.92	-0.0624		1 S'	2.86	-0.0651	

Table 5.8. Type 2 (varying R's and CN's) and Type 3 (varying R's and c_2 's) fits to Window 3 of reduced Pc at pH 7.2 (PCHI), made to compare with the distances found in the crystal structure at pH 7.0. Fixed values of $c_{2N} = -0.0253$ and $c_{2S} = -0.0216$.

N+N'+S				N+N'+S+S'			
Only R's varied			Fit Index	Only R's varied			Fit Index
1 N @	2.11 Å		0.477	1 N @	2.11 Å		0.419
1 N' @	2.63 Å			0.6 N' @	2.59 Å		
1 S @	2.17 Å			1 S @	2.17 Å		
				0.13 S' @	2.91 Å		
Type 2 fits (R's and CN's varied)				Type 2 fits (R's and CN's varied) [†]			
			Fit Index				Fit Index
8.4 N @	2.07 Å		0.215	(1 N) @	2.09 Å		0.347
2.3 N' @	2.34 Å			-0.9 N' @	2.37 Å		
1.5 S @	2.03 Å			(1 S) @	2.17 Å		
				0.2 S @	2.90 Å		
Type 3 fits (R's and c_2 's varied)				Type 3 fits (R's and c_2 's varied) [‡]			
	R	c_2	Fit Index		R	c_2	Fit Index
1 N	@ 2.11 Å	-0.0267	0.331	1 N	@ 2.11 Å	(-0.0253)	0.398
1 N'	@ 2.04 Å	-0.0326		1 N'	@ 2.59 Å	-0.0340	
1 S	@ 2.17 Å	-0.0255		1 S	@ 2.17 Å	(-0.0216)	
				1 S'	@ 2.95 Å	-0.0497	

[†] — CN's enclosed in brackets (e.g., (1 N)) were not varied

[‡] — c_2 's enclosed in brackets were not varied

Figure 5.2. EXAFS data and Fourier transform for CuI₂((CH₃NCH₂CH₂SSCH₂CH₂N(CH₃)₂)₂(BF₄)₂ (CUNS2). Top : Averaged EXAFS data for CuI₂((CH₃NCH₂CH₂SSCH₂CH₂N(CH₃)₂)₂(BF₄)₂ (CUNS2), with spline applied up to $k = 13 \text{ \AA}^{-1}$. Bottom : Fourier transform over $k = 3.5\text{--}12.5 \text{ \AA}^{-1}$, with vertical lines showing the Fourier backtransform window ($R = 1.35\text{--}2.35 (0.1) \text{ \AA}$) used to filter data for curvefitting.

Figure 5.3. EXAFS data and Fourier transform for reduced Pc at pH 7.2 and 4.8. Top : Averaged EXAFS data for reduced Pc at pH 7.2 (PCHI). Bottom : EXAFS data for reduced Pc at pH 4.8 (PCLO). A glitch at $k = 10.9 \text{ \AA}^{-1}$ was removed from both spectra.

Figure 5.4. Reduced Pc EXAFS data Fourier-transformed over $k = 3.5\text{--}12.5$ (0.1) $\text{\AA}^{-1}$. Vertical lines show the Fourier backtransform windows used to filter the data for curvefitting (see also Table 5.3). Top : Reduced Pc at pH 7.2 (PCHI). Bottom : Reduced Pc at pH 4.8 (PCLO).

Figure 5.5. N+S+S' fit (short-S' minimum) to Pc data at pH 7.2 (PCHI). Top : Backtransformed PCHI data (Window 3), with the components of the short-S' minimum of the N+S+S' fit (Type 3) and the total fit. Bottom : Fourier transforms over $k = 4.25\text{--}11.75$ (0.1) $\text{\AA}^{-1}$ of the data and fit components shown above.

Figure 5.6. N+S+S' fit (long-S' minimum) to Pc data at pH 7.2 (PCHI). Top : Backtransformed PCHI data (Window 3), with the components of the long-S' minimum of the N+S+S' fit (Type 3) and the total fit. Bottom : Fourier transforms over $k = 4.25\text{--}11.75$ (0.1) $\text{\AA}^{-1}$ of the data and fit components shown above.

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Figure 3.7. N+S+S' fit (short-S' minimum) to Pc data at pH 4.8 (PCLO). Top : Backtransformed PCLO data (Window 3), with the components of the short-S' minimum of the N+S+S' fit (Type 3) and the total fit. Bottom : Fourier transforms over $k = 4.25\text{--}11.75$ (0.1) $\text{\AA}^{-1}$ of the data and fit components shown above.

Figure 5.8. N+S+S' fit (long-S' minimum) to Pc data at pH 4.8 (PCLO). Top : Backtransformed PCLO data (Window 3), with the components of the long-S' minimum of the N+S+S' fit (Type 3) and the total fit. Bottom : Fourier transforms over $k = 4.25\text{--}11.75$ ($0.1 \text{ \AA}^{-1}$) of the data and fit components shown above.

Chapter 6

Cu Difference Edge Analysis of *Rhus vernicifera* Laccase — A Continuing Study

(A) A Brief Overview of the Structure and Function of Laccase

(1) Structure and General Reaction Mechanism of Laccase

The multicopper oxidases (laccase, ascorbate oxidase and ceruloplasmin) catalyze the four-electron reduction of dioxygen to water. Laccase is the simplest of these, and it may be found in trees, or in fungi. *Rhus vernicifera* laccase comes from the Japanese lacquer tree, where the reduction of dioxygen to water occurs concomitantly with the oxidation of quinones and catechols in tree sap to radicals which then polymerize, hardening the sap into lacquer. In fungi, laccase, in concert with other fungal enzymes, serves the opposite function of degrading tree lignin.

Each unit of laccase contains four copper atoms, named according to their spectral characteristics : one Type 1 (T1) "blue" copper ($\lambda_{\text{max}} \equiv 610 \text{ nm}$, $A_{\parallel} \leq 95 \times 10^{-4} \text{ cm}^{-1}$), one Type 2 (T2) "normal" copper (no known UV-visible absorption band, $A_{\parallel} > 140 \times 10^{-4} \text{ cm}^{-1}$) and two Type 3 (T3), in a coupled binuclear site ($\lambda_{\text{max}} \equiv 330 \text{ nm}$, EPR undetectable). Spectroscopic studies of laccase and ascorbate oxidase showed that the T2 and T3 Cu were so close to each other as to form a single trinuclear site.¹⁻⁶ This is supported by a recent crystal structure of ascorbate oxidase,^{7,8} in which the T1 Cu is 12.2–14.9 Å away from the other three Cu ions, while the T2 and T3 Cu ions form a triangle, with all three Cu ions being within 3.9 Å of each other. The T1 site is linked to the trinuclear site through the amino acid sequence His506–Cys507–His508; the His are each ligated to one of the T3 Cu, while the Cys is ligated to the T1 Cu.

The reaction cycle of laccase may be divided into a reduction leg and an oxidation leg.⁹ Each leg can be studied separately, the reduction leg in the presence of anaerobic substrate (catechol, etc.) and the oxidation leg in the presence of dioxygen. In the reduction leg, the T1 Cu receives electrons from the substrate and passes them to the other three Cu. The exact sequence in which the reduction of all the Cu's occurs is pH-dependent.⁹ Our studies are concerned with elucidating details of the oxidation leg. In this part of the cycle, dioxygen binds to fully reduced laccase at the trinuclear site. Three electrons are very rapidly transferred to it, so that it forms an "oxygen intermediate" (indicated by an optical absorption band near 360 nm), and at the same time the T1 Cu is reoxidized.¹⁰⁻¹² The oxygen intermediate decays slowly ($t_{1/2} \sim 1$ to 15 s), and there is a corresponding growing in of the EPR signal due to oxidized T2 Cu.⁵ A mass spectroscopic study of the reaction of laccase with $^{18}\text{O}_2$ shows that one water molecule is released quickly, but the second oxygen remains bound and is slowly exchanged with the bulk solution with $t_{1/2} \sim 30$ minutes.⁴

It thus seems clear that, upon binding of O₂ to the trinuclear site, three electrons are quickly transferred to it from the T1 and T3 Cu's. However, the apparent oxidation of the T2 Cu is too slow to be catalytically relevant, and so it was suggested that the T1 Cu was re-reduced and that the fourth electron required to completely reduce O₂ was donated from T1. Nevertheless, there is evidence that T2 Cu has a role to play; both it and the T3 Cu ions are affected when azide is bound to the trinuclear site,^{1,2} and the second water molecule formed in the reduction remains bound to the T2 Cu.⁶ (A recent study suggests that in the oxygen intermediate the oxygen species has been reduced by four electrons rather than three, and that the fourth electron may come from the T2 Cu.¹³)

To shed light on this matter, two enzymatically inactive derivatives of native (four-Cu) laccase have been studied; in one derivative (Type 2-depleted or T2D laccase) the T2 Cu has been removed, and in the other (T1Hg laccase) the T1 Cu has been replaced with Hg. The T1Hg laccase derivative is particularly interesting because it, like native laccase, exhibits an absorption band (at 340 nm rather than 360 nm) due to an oxygen intermediate ($t_{1/2} = 6.4 \pm 2$ min.) when the fully reduced form is exposed to air.¹⁴ Enzymatically active native laccase can easily be reconstituted from T2D laccase, or, indeed, from apo laccase (all four Cu removed) by adding Cu ions. However, it is not possible to reconstitute native laccase once Hg has bound to the T1 site.

(2) The Usefulness of X-ray Edge Analysis; Previous Work on Laccase

When probed with optical, MCD or EPR spectroscopy, Cu(I) gives no signal (except for metal-to-ligand charge transfers), since it has a completely filled 3d shell (i.e., 3d¹⁰). Furthermore, the T3 Cu ions in laccase give no EPR signal, even when they are oxidized, because they are then antiferromagnetically coupled, and the T2 Cu has no known UV-visible band in any oxidation state. However, the X-ray absorption edges of Cu(I) compounds can be easily differentiated from those of Cu(II) compounds, and furthermore show features, especially at ~8984 eV, that allow determination of the Cu coordination geometry (see Figure 6.1).

Kau, *et al.*¹⁵ showed that the feature at 8984 eV is a sharp spike for linear two-coordinated Cu(I) compounds, and appears as a shoulder of significant amplitude for three- and four-coordinated compounds (Figure 6.1). The differences in the edges of the three- and four-coordinated compounds can be more accurately discerned by subtracting the edge of a standard Cu(II) compound from the Cu(I) edge and examining the resulting derivative-shaped "difference edge" (Figure 6.2); the difference edges of three-coordinate compounds

show a peak at 8983-8984 eV, while those of four-coordinate compounds show no peak below 8985 eV.

Cu(II) edges show a weak $1s \rightarrow 3d$ transition at ~ 8979 eV. Also, the rising edge occurs at higher energy, and so the edge amplitude at 8984 eV is much lower. The covalency of the Cu(II)-ligand bond can be judged from the position of the rising edge; the lower the energy at which it occurs, the greater the covalency of the bond, because the electrons then donated by the ligand lower the effective nuclear charge of Cu. There is some overlap between the possible edge shapes of four-coordinate Cu(I) compounds and those of covalent Cu(II) compounds. In such cases, the $1s \rightarrow 3d$ transition, together with other methods of spectroscopy, should also be relied on to determine the oxidation state and ligation of Cu.

Besides determining the geometry of reduced Cu (i.e., Cu(I)), difference edges can be used to determine the proportion of reduced Cu in a sample. For example, in previous work done by this group,¹⁵ it was determined that the T3 Cu are $98 \pm 22\%$ reduced in resting (i.e., as-prepared) T2D, with three- or (2+4)-coordination, and that oxidation of T3 (as opposed to the formation of a peroxy complex) increases proportionately as T2D is treated with increasing excess (up to 60 $\times$) of H_2O_2 . This showed that resting T2D laccase does not react with O_2 . The same study found that, in resting native laccase, the T3 sites are $22 \pm 3\%$ reduced, being completely oxidized upon addition of 30 $\times$ H_2O_2 .

(3) EXAFS Studies on Laccase

Some EXAFS studies have been carried out on laccase. In 1983, Spira *et al.*¹⁶ studied the EXAFS spectrum of met T2D (i.e., oxidized T2D laccase with no O_2 bound) and azide-treated met T2D laccase. In both samples, they found 3.0–3.5 N at 1.99–2.00 Å and 0.33 S at 2.07 or 2.09 Å. When the contribution of the T1 Cu, approximated by a plastocyanin EXAFS spectrum, was subtracted, they found 3.2–3.5 N at 2.01–2.02 Å, and the contribution from S was 0.13 or less. They concluded that 1 S was bound to the T1 site (which is consistent with the subsequent crystal structures of plastocyanin and ascorbate oxidase, since the second Cu–S bond is extremely long). Comparison with the EXAFS results from the Cu binuclear protein, hemocyanin, showed that the N/O-ligation found for the T3 Cu's was similar, but unlike for hemocyanin the T3 site was not much perturbed by the binding of azide, and they could not find any Cu closer than 3.8 Å.

In 1984, Woolery, *et al.*¹⁷ studied the EXAFS of oxidized and reduced native laccase and also the resting (as-prepared) and H_2O_2 -oxidized forms of T2D laccase. They made fits to the first shell (i.e. the shell of scatterers directly coordinated to Cu), and obtained results

agreeing with those of Spira, *et al.*, though 0.3 S was found at 2.19 Å rather than 2.09 Å (this difference is within the experimental error, because the contribution of S is small). For both forms of laccase, oxidized and reduced forms gave the same total coordination. The outer-shell EXAFS signals ($R = 3\text{--}4$ Å), were fitted with an imidazole outer-shell wave and a Cu wave. They were able to account for the outer-shell EXAFS of both forms of T2D laccase using imidazole alone, but they needed both imidazole and Cu (0.5 Cu at 3.4 Å) to obtain a good fit of the native laccase outer-shell EXAFS. They thus concluded that the removal of the T2 Cu causes a change in the T3 site, with the two Cu ions perhaps moving further apart from each other.

More recently (1989), Klemens, *et al.*¹⁸ studied the Hg-substituted blue copper site in plastocyanin, azurin, laccase and stellacyanin using EXAFS at the Hg L₃ edge. They provided the first direct evidence that, when incorporated, the Hg occupies only the T1 site, and that T1 coordination in all four proteins was two imidazole groups and one thiolate sulfur. Furthermore, when considered together with the results for the crystal structure of Hg-substituted plastocyanin, the data showed that the coordination geometry in the T1 site was the same, whether Cu or Hg was bound. This confirmed the usefulness of the T1Hg derivative for the study of laccase.

(B) Experimental Details

(1) Preparation of Samples

Rhus vernicifera laccase was isolated from acetone powder (Saito and Co., Osaka, Japan) to a purity ratio of $A_{280}/A_{614} = 14.5\text{--}15.5$.¹⁹ Laccase activity was assayed spectrophotometrically with *N,N*-dimethyl-*p*-phenylenediamine as the substrate. T2D laccase was prepared according to the procedure of Graziani, *et al.*,²⁰ as modified by Spira-Solomon and Solomon.¹⁹ T1Hg laccase was prepared according to published procedures,^{21,22} as modified by Cole, *et al.*¹⁴ Reduction of T2D laccase was carried out in a N₂-purged cuvette by adding 2 equivalents of ascorbate. T1Hg laccase was reduced by anaerobic dialysis against 5 mM dithionite, and excess dithionite was removed by anaerobic dialysis against 0.1 M potassium phosphate buffer (the buffer used for all samples) of pH 7.4 (unless otherwise stated), before the protein was transferred into a cuvette for optical absorption measurements. An aliquot each of fully reduced T2D and T1Hg was transferred under Ar into XAS protein cells (outer dimensions $2 \times 4 \times 28$ mm³, volume ~180 µl, with an X-ray-transparent front face of 60 µm Mylar tape) and frozen in liquid N₂. Both T2D and T1Hg laccase were then reoxidized by exposing the cuvette to air, and at the time points indicated in Table 6.1, UV-visible spectra were measured, and aliquots of protein

were withdrawn and frozen in lucite XAS cells. EPR spectra were measured on the frozen samples both before and after measuring the XAS spectra, and UV-visible spectra were also re-recorded after thawing the samples to 298 K. For the study on the TlHg laccase oxygen intermediate, reoxidation of the fully reduced TlHg laccase was initiated by addition of dioxygen-saturated buffer, and aliquots were withdrawn and frozen in the same manner. For the study on the native laccase oxygen intermediate, dioxygen-saturated buffer and reduced laccase were simultaneously injected into the XAS protein cell, which was then frozen within a few seconds. Several repeat samples of both types of oxygen intermediates were prepared.

(2) Data Collection

Laccase edge data were collected at the Stanford Synchrotron Radiation Laboratory in five sessions from 1987 to 1991 (see Table 6.1), at the unfocussed 8-pole wiggler beam line 7-3 in fluorescence mode, using a Lytle detector²³ with a nickel filter, and argon gas in the chamber, except in March 1989, when N₂ gas was mistakenly used. (N₂ gas is not as readily ionized as Ar gas by Cu K α fluorescence, and so gives a smaller and noisier signal.) Ring current on these occasions varied between 8 and 69 mA. To maintain consistency with data previously collected, all spectra were run using Si[220] monochromator crystals and a vertical monochromator slit width of 2 mm. The temperature of the samples during data collection was either 10–12 K or 85 K. (The temperature made no difference to the appearance of the edges.) Scans to $k = 9 \text{ \AA}^{-1}$ were collected, except in December 1987, when scans were collected to $k = 8 \text{ \AA}^{-1}$. The number of scans collected depended on the concentration of the sample, the ring current at the time, and whether any photoreduction was observed. Photoreduction (manifested by an increase in the edge amplitude at 8984 eV) was not observed in any of the samples of native and T2D laccase. However, it was significant (~1% per scan) in many of the samples of TlHg laccase (those containing glycerol; see Table 6.1), and only the first scans of these samples were used in subsequent analyses.

(3) Data Analysis

(a) Data Reduction

Data were reduced and analyzed using XFPKKG, in the manner described in Chapter 2. Scans were calibrated using copper foil as an internal standard, taking the first inflection point of the foil as 8980.3 eV. In the laccase samples where no photoreduction was observed, all good scans were averaged. In those TlHg laccase samples where photoreduction occurred, only the first scan was used in the difference edge analysis.

All laccase spectra were normalized in exactly the same manner as spectra used in previous work : A polynomial of order -2 was fitted to 8770–8970 eV, the pre-edge region of the spectrum, then a one-region spline of order 1 was applied over 9045–9309 eV, or 9045–9243 eV (for spectra ending at $k = 8 \text{ \AA}^{-1}$).

(b) Corrections Applied to the Laccase Edges

In the process of preparing and fully reducing the Cu in the two derivatives, a certain amount of native laccase was reconstituted, and had to be taken into account during data analysis. The native laccase was assumed to be fully reduced before the samples were exposed to air, and then either 89% oxidized (in T2D samples) or fully oxidized (in T1Hg samples) once the samples had been exposed to air (see Table 6.2). The proportion of native laccase (x) remaining in "fully reduced" T2D and T1Hg laccase was determined by several methods : assaying laccase activity (in both T2D and T1Hg), monitoring the absorption band at 600 nm and the EPR signal due to oxidized T1 Cu (which is absent in T1Hg), and atomic absorption measurements (for T2D, where the ratio of copper found to the moles of protein could be solved for x , since native laccase has four copper atoms, while T2D has only three).

The proportion of T1Hg oxidized (y) in the "fully reduced" sample MLRD (*vide infra*) was deduced from quantitating the EPR signal due to oxidized T2 (z) and subtracting x . (So, $y = z - x$.) The EPR signal due to oxidized T2 was also used for determination of z and y for all T1Hg oxygen intermediate samples. See Table 6.2 for values of x and z that were determined for each sample measured.

All T2D and T1Hg laccase edges were "corrected for native" and renormalized in the following manner :

$$\text{Proportion of Cu edge signal due to native laccase} = \frac{\left(\frac{4}{3}\right)x}{\left[\left(\frac{4}{3}\right)x + (1 - x)\right]} = x' \quad \dots\dots\dots(6.1)$$

(The factor 4/3 allows for the native laccase having 4 Cu as opposed to the 3 Cu in the T2D and T1Hg derivatives.)

$$\text{Native-corrected edge} = \frac{[\text{Edge} - x'(\text{Native edge})]}{1 - x'} \quad \dots\dots\dots(6.2)$$

(The $(1-x')^{-1}$ factor scales the corrected edge back to 1. Table 6.2 shows which native edge was appropriate for which sample. Generally, the fully reduced

native edge NTFRD was used on fully reduced samples, and the fully oxidized native edge NTFOX was used on the rest.)

Where necessary (see Table 6.2 and next section), the reduced T2D and T1Hg samples were further corrected to "100% reduction" in the following way :

$$\begin{aligned}
 & \text{100\% reduced corrected edge} \\
 &= \left[(\text{Native - corrected edge}) - \frac{y}{(1-x)} (\text{Native - corrected fully oxidized edge}) \right] \frac{1-x}{1-z} \\
 &= \frac{[(1-x)(\text{Native - corrected edge}) - y(\text{Native - corrected fully oxidized edge})]}{1-z} \dots\dots\dots(6.3)
 \end{aligned}$$

(Table 6.2 shows which fully oxidized edge was appropriate for which sample. T2D and T1Hg samples treated with sufficient excess of H₂O₂ were considered fully oxidized. These edges were themselves corrected for native before being used here. The (1-x)/(1-z) factor renormalizes the corrected edge to 1.)

After carrying out these corrections, the edges were ready to be used in difference edge analysis.

(c) Construction of Difference Edges

In the discussion below, difference edges are denoted by "Δ" to distinguish them from regular edges.

Difference edges were made for two purposes : (i) for quantitation of the amount of reduced copper in a sample; (ii) for estimation of the possible geometry of Cu.

In Case (i), "100% reduced Δ standards" were first made by subtracting a 100% oxidized edge from a 100% reduced edge :

$$\begin{aligned}
 & \text{"100\% reduced } \Delta \text{ standard"} = \text{"Corrected totally reduced edge"} \\
 & \quad \quad \quad - \text{"Corrected totally oxidized edge"} \dots\dots\dots(6.4)
 \end{aligned}$$

In this way, "100% reduced Δ standards" were made for native laccase, T2D laccase, T1Hg laccase and "T3 Cu" (see Table 6.3). Then, the amount of reduced Cu in a sample could be determined by subtracting the appropriate 100% oxidized edge from it and comparing the height of the resulting difference edge at 8984 eV to the height of the "100% reduced Δ standard".

In Case (ii), we used the criteria of height and edge slope at 8984 eV set forth in Table III of the paper by Kau, *et al.*¹⁵ (reproduced in part here in Table 6.5) to estimate the geometry of Cu(I). Difference edges were made for this purpose by subtracting the same Cu(II) compound edge that they had used in their analysis, namely that of [Cu₂(L8-Et)(OAc)](ClO₄)₂,²⁴ hereafter referred to as CUL2 :

$$\text{"Difference edge } \Delta \text{ wrt CUL2"} = \text{"Corrected laccase edge"} - \text{CUL2} \quad \text{.....(6.5)}$$

In some cases, say for T3 or T2 Cu, the difference edges had to be scaled up to "one full Cu". Here CUL2 could not be directly subtracted from the edge. Instead, the "difference edge Δ wrt CUL2" (wrt = "with respect to") had to be constructed by adding together two other difference edges, so :

$$\begin{aligned} \text{"Difference edge } \Delta \text{ wrt CUL2"} &= q[\text{"Difference edge } \Delta \text{ wrt fully oxidized laccase"}] \\ &\quad + \text{"Difference edge } \Delta \text{ of fully oxidized wrt CUL2"} \\ &\quad \text{.....(6.6)} \end{aligned}$$

where q is the factor needed to scale the difference edge back up to 1.

$$\begin{aligned} \text{e.g., "}\Delta\text{T3 Cu wrt CUL2"} &= 1.5(\text{"resting T2D"}_{\text{corr}} - \text{"T2D + 60}\times\text{H}_2\text{O}_2\text{"}_{\text{corr}}) \\ &\quad + (\text{"T2D + 60}\times\text{H}_2\text{O}_2\text{"}_{\text{corr}} - \text{CUL2}) \end{aligned}$$

(The factor 1.5 was required because the T1 Cu is oxidized in both resting T2D and fully oxidized T2D, and so we produced a difference edge of only 2 out of 3 Cu when one was subtracted from the other.)

Results for Cases (i) and (ii) are given in Tables 6.3, 6.4 and 6.5.

An error analysis was carried out for the work reported in Section (C.1) below when it was published in 1990.¹⁴ This analysis is repeated with some corrections in Appendix II. No error analysis was carried out for the work reported in Sections (C.2) and (C.3), but the errors are almost certainly greater, since the difference edge analysis proceeded through more steps of adjustment, subtraction and addition.

(C) Results and Discussion

(1) Reaction of T2D and T1Hg Laccase with Dioxygen

In 1987, the T1Hg derivative had not yet been much characterized, and we measured two samples, of resting T1Hg and H₂O₂-treated T1Hg, to determine the redox states of the various Cu ions. After due correction for native content as described above, and assuming that the H₂O₂-treated sample was 100% oxidized, we found that only $1 \pm 8\%$ of all Cu's in

the resting T1Hg were reduced (as compared with resting T2D laccase, in which T1 was 100% oxidized and T3 was 100% reduced, see Figure 6.3). This was an indication that the resting T1Hg, unlike resting T2D, might have completely reacted with dioxygen.

It remained to be seen how the fully reduced derivatives would react with dioxygen. So, in 1989, we collected two series of data on laccase, one each on T2D and T1Hg laccase at various times after exposing the fully reduced protein to air.

The T2D series consisted of fully reduced T2D (T2D1), T2D exposed to air for 15 minutes (T2D2) and T2D exposed for 63 hours (T2D3). A small amount of oxidation was observed between T2D1 and T2D2. This can be entirely accounted for by the rapid reoxidation of native laccase. After correction for native, it can be seen that T2D1 and T2D2 were both equally reduced (Figure 6.4). T2D3 was oxidized to a greater extent, but by no means totally oxidized. EPR and optical spectra showed that the T1 Cu in T2D3 was completely oxidized. Taking the difference edge of T2D3 with respect to H₂O₂-treated T2D (T26A), we calculated that $88 \pm 14\%$ of the T3 Cu were still reduced, even after 63 hours (Figure 6.4).

The T1Hg series consisted of a 90% reduced sample (MLRD) and a sample exposed to air for 30 minutes (MLOX). MLRD was corrected for native content and for 10% oxidation. Using corrected MLRD as the standard for 100% reduction, we found that the reoxidized sample (MLOX) contained $2 \pm 11\%$ reduced Cu (see Figure 6.5, Table 6.4).

It is clear that the oxidation of the T3 site in T2D laccase proceeds too slowly to be biologically relevant. On the other hand, all the Cu(I) ions in T1Hg laccase were completely oxidized within 30 minutes. This shows that the trinuclear T2+T3 site, rather than the T1+T3 assembly suggested by kinetic studies, is the minimal active site required for the multielectron reduction of dioxygen.¹⁴

(2) The T1Hg Laccase Oxygen Intermediate

It is known that, when O₂ binds to fully reduced native laccase, the T2 Cu is oxidized more slowly than the T1 and T3 Cu ions, and that when the reaction with O₂ is completed all the Cu are oxidized. We hoped that if we could freeze the reaction at the point when the oxygen intermediate was formed, we might catch the system in a state wherein the T2 Cu had not yet oxidized. We have not yet been able to achieve reproducible results for native laccase, but the kinetics of T1Hg laccase are somewhat slower than those of native laccase, and we have consistent results, obtained in 1991, for this oxygen intermediate.

Two corrections were applied to the O₂ intermediate spectra. The first was the correction for totally oxidized native laccase, as explained above. This correction was quite

small, being between 3.5% and 7%. However, there was 12–22% of oxidized T2 (see Table 6.2), with the proportion of oxidized T2 increasing with the time the sample had been exposed to air. This was considered to be due to protein that had passed through the O₂ intermediate stage and was now fully oxidized. The contribution of this state of T1Hg laccase was thus subtracted using fully oxidized T1Hg laccase (MLFOX, itself corrected for native laccase), in the manner shown in Equation 6.3.

All five O₂ intermediate samples appeared very similar, even before any corrections were applied, and it was clear that the majority of the Cu ions in the samples were oxidized (Figure 6.6). Indeed, when their heights at 8983.4 eV were measured against a "100% reduced T1Hg Δ standard", we found that only 13–17% of copper was in the Cu(I) state (Figure 6.7). However, this is only an overall number which assumes that all the Cu in T1Hg laccase are equivalent. For the true percentage of reduced Cu, we had to break down the contributions of the different Cu ions to the edge.

Based on our understanding of laccase chemistry, we assumed that 100% of the T3 Cu ions were oxidized, so that any difference between the edges of the O₂ intermediates and fully oxidized laccase would be due to reduced T2 Cu. We attempted to determine what percentage of T2 was reduced in the oxygen intermediates, by comparing difference spectra of the T1Hg O₂ intermediates (" Δ T1Hg O₂ int." = "T1Hg O₂ int._{corr}" – "T1Hg(ox)_{corr}") with a "100% reduced Δ standard" for reduced T2 (" Δ T2 Cu"). Unfortunately, " Δ T2 Cu" could not be directly obtained from the data we had collected. The best we could do was to construct the " Δ T2 Cu" standard out of other difference edges (which were in turn constructed according to Equation 6.4). Two ways were found for doing this :

$$\begin{aligned} \text{"}\Delta\text{T2 Cu"}_1 &= 4\text{"}\Delta\text{T1}_{\text{red}}\text{T2}_{\text{red}}2(\text{T3}_{\text{red}})\text{"} - 3\text{"}\Delta\text{T1}_{\text{red}}2(\text{T3}_{\text{red}})\text{"} \\ &= 4(\text{"100% reduced native } \Delta \text{ standard"}) - 3(\text{"100% reduced T2D } \Delta \text{ standard"}) \\ &\dots\dots\dots\text{Method (i)} \end{aligned}$$

$$\begin{aligned} \text{"}\Delta\text{T2 Cu"}_2 &= 3[\text{"}\Delta\text{T2}_{\text{red}}2(\text{T3})_{\text{red}}\text{"} - \text{"}\Delta 2(\text{T3})_{\text{red}}\text{"}] \\ &= 3[\text{"100% reduced T1Hg } \Delta \text{ standard"} \\ &\quad - (\text{"Corrected resting T2D"} - \text{"Corrected fully oxidized T2D"})] \\ &= 3[\text{"100% reduced } \Delta\text{T1Hg"} - 2/3(\text{"100% reduced } \Delta\text{T3 Cu"})] \\ &\dots\dots\dots\text{Method (ii)} \end{aligned}$$

The " Δ T2 Cu" difference edges constructed by these two methods have some similar features below 8986 eV, but also look considerably different at 8990 eV (Figure 6.8). A third " Δ standard" (" Δ T2 Cu"_{avg}) was also constructed by averaging these two. The peak

heights at 8983.4 eV of the O₂ intermediate difference edges were evaluated against all three standards, and by these criteria between 59% and 106% of T2 was reduced (see Tables 6.3, 6.4). However, the shapes of the "ΔT2 Cu" standards we constructed do not closely resemble the shapes of the O₂ intermediate difference edges, and so we should regard these percentages as a first guess at the redox state of T2 Cu in the T1Hg oxygen intermediate, rather than the final word.

Although the "ΔT2 Cu" standard is not very firm, it is nevertheless clear from visual inspection that all the T1Hg O₂ intermediate samples contain a small but reproducible amount of Cu(I), which possibly represent a reduced T2 Cu.

(3) Probable Geometry of T2 and T3 Cu in Laccase Samples

For estimation of Cu (I) geometry, difference edges with respect to CUL2 were constructed according to Equations 6.5 and 6.6. Since the "ΔT2 Cu" standards were constructed out of native, T1Hg and T2D edges, there was no clear candidate for the appropriate "difference edge Δ of fully oxidized wrt CUL2", and NTFOX was used. The difference edges thus constructed (see Figure 6.9) were then compared with Table III in the paper by Kau, *et al.* (reproduced in part here in Table 6.5). The results are given in Table 6.5. They show that the fully reduced native, T2D and T1Hg edges are remarkably similar, and they would fit into two of the possible geometries listed by Kau, *et al.* : (i) 3-coordinate, or (ii) (3 and 2+4)-coordinate (i.e., (3+3+2+4)-coordinate). The "ΔT3 Cu" difference edge fits well with a 2+3 Cu geometry, but the "ΔT2 Cu" we constructed does not fit into any of the categories of Cu(I) geometry surveyed by Kau, *et al.* This is not that surprising when we consider how many difference edges were pasted together to construct "ΔT2 Cu wrt CUL2", with small residuals furthermore being scaled up, magnifying the errors in the resultant edge.

We can thus conclude that the reduced T3 Cu ions in T2D laccase are probably 2+3-coordinated. Since the native, T2D and T1Hg laccase forms are (3 and 2+4)-coordinate (or its 3-Cu equivalent), and the T1 Cu (a blue copper site) is almost certainly 4-coordinate, it is possible that T2 is either 3- or 4-coordinate. However, we could not confirm this by examining our "ΔT2 Cu" standards.

(D) Conclusion

We have been able to show that reduced T1Hg is completely oxidized by dioxygen, while under the same conditions the T3 Cu in T2D laccase remain reduced. This shows that the trinuclear T2+T3 site, rather than the T1+T3 combination, is the minimal active site required for reduction of dioxygen.

We have also found that a small amount of Cu is reduced in the oxygen intermediate of T1Hg laccase. This reduced Cu is from chemical considerations most probably T2, but we were not able to confirm this because we did not have a reliable standard against which to measure the reduced Cu in the T1Hg O₂ intermediate.

The difficulty in obtaining a reliable " $\Delta T2$ Cu" reduced standard shows that, in trying to determine one copper out of three, we are operating near the limit to which we can trust the method of difference edges. The amplitude of " $\Delta T2$ Cu" is small enough so that errors in spline, noise and sample variation can and do distort it significantly, as attested by the fact that " $\Delta T2$ Cu wrt CUL2" does not at all resemble any known class difference edges.

On the other hand, conclusions reached about the overall geometries of native, T2D, and T1Hg laccase (3-and-2+4-coordinate), as well as T3 Cu ions in T2D laccase (2+4-coordinate), can be held with some confidence, because the difference edges obtained are large in comparison to the errors they contain. This is also true of the conclusions reached in the study comparing the reactivities of T2D and T1Hg laccase with dioxygen.

Thus, the method of difference edge analysis of X-ray absorption spectra has proven extremely useful in elucidating details of the chemistry of laccase that would otherwise have been inaccessible to study, since it provides direct information on the averaged oxidation states and geometries of the Cu ions present in laccase. In our use of the difference edge method to study laccase, XAS edge studies complement information from more conventional forms of spectroscopy to an even greater extent than is usual in studies of metalloproteins. However, care should be taken not to push this method beyond what its inherent errors allow.

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Table 6.1. Samples of laccase for which edge spectra were collected (continued on the next two pages).

Date collected	Name of data set	Description	Concentration, buffer, pH	Comments
Dec 1987	1ML01	Resting T1Hg laccase	3.2 mM Cu, pH 6.0, 100 mM potassium phosphate buffer	Photoreduction occurred; only the 1st scan was used
Dec 1987	2ML01	T1Hg laccase + H ₂ O ₂	3.2 mM Cu, pH 6.0, 100 mM potassium phosphate buffer	Photoreduction occurred; only the 1st scan was used
Dec 1987	1T2D*	Fully reduced T2D		Analysis not reported here
Dec 1987	2T2D*	T2D exposed to air for 15 minutes		Analysis not reported here
Dec 1987	2T2D*	T2D exposed to air for 3.5 days		Analysis not reported here
Mar 1989	MLOX	Fully reduced and reoxidized T1Hg	4.5 mM Cu, pH 6.0, 100 mM potassium phosphate buffer	N ₂ in the Lytle detector
Mar 1989	MLRD	Fully reduced T1Hg	4.5 mM Cu, pH 6.0, 100 mM potassium phosphate buffer	N ₂ in the Lytle detector
Mar 1989	NLRD	Fully reduced native	3.6 mM Cu, pH 6.0, 100 mM potassium phosphate buffer	N ₂ in the Lytle detector
Mar 1989	T2D1	Fully reduced T2D	3.3 mM Cu, pH 6.0, 100 mM potassium phosphate buffer	N ₂ in the Lytle detector
Mar 1989	T2D2	T2D exposed to air for 15 minutes	3.3 mM Cu, pH 6.0, 100 mM potassium phosphate buffer	N ₂ in the Lytle detector
Mar 1989	T2D3	T2D exposed to air for 63 hours	3.3 mM Cu, pH 6.0, 100 mM potassium phosphate buffer	N ₂ in the Lytle detector

* — Too much reductant (i.e., 5 eq. ascorbic acid) was added to these samples, putting their reliability in doubt.

Table 6.1. Samples of laccase on which edge spectra were collected (continued from the previous page, continued next page)

Date collected	Name of data set	Description	Concentration, buffer, pH	Comments
Apr 1990	MLEX	T1Hg reduced and reoxidized	10.2 mM Cu, pH 6.0, 50% glycerol, 50% 200 mM potassium phosphate buffer	EXAFS sample, 13 good scans measured on BL 4-2, ~20% photoreduction
Apr 1990	MLOI‡	T1Hg O ₂ intermediate	2.2 mM Cu, pH 6.0, 100 mM potassium phosphate buffer	Analysis not reported here
Apr 1990	NLO1†	Native O ₂ intermediate #1, frozen 5.6 s after mixing	~4 mM Cu, pH 7.4, 100 mM potassium phosphate buffer	Detailed analysis not done
Apr 1990	NLO2†	Native O ₂ intermediate #2, frozen 3.9 s after mixing	~4 mM Cu, pH 7.4, 100 mM potassium phosphate buffer	Detailed analysis not done
Jun 1991	MLFRD	Fully reduced T1Hg	3.5 mM Cu, pH 7.4, 100 mM potassium phosphate buffer	
Jun 1991	MLFOX	Fully oxidized T1Hg	1.8 mM Cu, pH 7.4, 100 mM potassium phosphate buffer	
Jun 1991	O21ML	T1Hg O ₂ intermediate #1 — exposed to O ₂ for 8 minutes	1.8 mM Cu, pH 7.4, 100 mM potassium phosphate buffer	
Jun 1991	O22ML	T1Hg O ₂ intermediate #2 — exposed to O ₂ for 27 minutes	1.8 mM Cu, pH 7.4, 100 mM potassium phosphate buffer	
Jun 1991	NTFRD	Fully reduced native	7–8 mM Cu, pH 7.4, 50% ethylene glycol, 50% potassium phosphate buffer	
Jul 1991	NTFOX	Fully oxidized native (with 30× H ₂ O ₂)	7–8 mM Cu, pH 7.4, 50% ethylene glycol, 50% potassium phosphate buffer	

‡ — This edge was different from edges of T1Hg O₂ intermediates run the following year.

† — Reproducible edges of native laccase O₂ intermediates could not be obtained.

Table 6.1. Samples of laccase on which edge spectra were collected (continued from the previous pages).

Date collected	Name of data set	Description	Concentration, buffer, pH	Comments
Jul 1991	O21NT [†]	Native O ₂ intermediate #1, rapid mix, 6–8 s	2.4 mM Cu, pH 7.4, 50% ethylene glycol, 50% 100 mM potassium phosphate buffer	Detailed analysis not done
Jul 1991	O22NT [†]	Native O ₂ intermediate #2, rapid mix, 8–10 s	2.4 mM Cu, pH 7.4, 50% ethylene glycol, 50% 100 mM potassium phosphate buffer	Detailed analysis not done
Jul 1991	O23NT [†]	Native O ₂ intermediate #3, partially oxidized past the intermediate stage	2.4 mM Cu, pH 7.4, 50% ethylene glycol, 50% 100 mM potassium phosphate buffer	Detailed analysis not done
Oct 1991	ML11	T1Hg O ₂ intermediate #1 — exposed to O ₂ for 9 minutes	2.1 mM Cu, pH 7.4, 20% glycerol, 80% 100 mM potassium phosphate buffer	Photoreduction occurred; only the 1st scan was used
Oct 1991	ML21	T1Hg O ₂ intermediate #1 — exposed to O ₂ for 27 minutes	2.1 mM Cu, pH 7.4, 20% glycerol, 80% 100 mM potassium phosphate buffer	Photoreduction occurred; only the 1st scan was used
Oct 1991	ML31	T1Hg O ₂ intermediate #1 — exposed to O ₂ for 90 minutes	2.1 mM Cu, pH 7.4, 20% glycerol, 80% 100 mM potassium phosphate buffer	Photoreduction occurred; only the 1st scan was used

[†] — Reproducible edges of native laccase O₂ intermediates could not be obtained.

Table 6.2. Edge spectra of laccase used in difference edge analysis, with corrections (see text) for native content and oxidation of samples as indicated (continued next page).

Name of data set	Description	Percentage of native (100x)	Data file used for native correction	Percentage of oxidized Cu [†] (100z)	Data file used for oxidation correction [£]
NTVL [‡]	Resting native laccase	-----	-----	100 (T1, T2) 78 (T3) ¹⁵	-----
NT30 [‡]	Native + 30× H ₂ O ₂ (fully oxidized)	-----	-----	100	-----
T2DS [‡]	Resting T2D laccase	7-8	NTVL	100 (T1) 98 (T3) ¹⁵	-----
T26A [‡]	T2D + 60× H ₂ O ₂ (fully oxidized)	7-8	NT30 [§] , NTFOX [⌘]	100 (T1) 0 (T3) ¹⁵	-----
1ML01	Resting T1Hg laccase	15	NTVL	See Table 6.4	-----
2ML01	T1Hg + 30× H ₂ O ₂	15	NT30	100	-----
NLRD	Fully reduced native	-----	-----	~4 (T2)	-----
MLOX	Fully reduced and reoxidized T1Hg	4-7 (T1)	NTVL	See Table 6.4	-----
MLRD	Fully reduced T1Hg	4-7 (T1)	NLRD	10 (T2) T3 assumed to be 10	2ML01
T2D1	Fully reduced T2D	10 (AA)	NTFRD	~0 (T1)	-----
T2D2	T2D exposed to air for 15 minutes	10 (AA)	NTVL	15-20 (T1)	-----
T2D3	T2D exposed to air for 63 hours	10 (AA)	NTVL	100 (T1)	-----

† — the type of Cu on which these figures are based is shown in brackets. Oxidized T1 was measured either by optical absorption spectra or by EPR, usually the former, oxidized T2 was measured by EPR alone, and oxidized T3 by optical spectra alone.
AA : atomic absorption

£ — These data sets were themselves first corrected for native content

‡ — These data sets were collected by previous workers

§ — This data set was used where corrected T26A (denoted T26A_{corr1}) was subsequently subtracted from T2DS for "T3 Cu" difference edge (see text, Table 6.3), so that all the data going into the difference edge was collected at the same time (Nov 1983).

⌘ — This data set was used where corrected T26A (denoted T26A_{corr2}) was subsequently subtracted from the 1989 T2D edges.

Table 6.2. Edge spectra of laccase used in difference edge analysis, with corrections (see text) for native content and oxidation of samples as indicated (continued from previous page).

Name of data set	Description	Percentage of native (100x)	Data set used for native correction	Percentage of oxidized Cu [†] (100z)	Data set used for oxidation correction [£]
NTFRD	Fully reduced native	-----	-----	0	-----
NTFOX	Native +30× H ₂ O ₂	-----	-----	100	-----
MLFRD	Fully reduced T1Hg	7 (T1)	NTFRD	0 (T2)	-----
MLFOX	Fully oxidized T1Hg	7 (T1)	NTFOX	100 (T2)	-----
O21ML	T1Hg O ₂ intermediate #1 — exposed to O ₂ for 8 minutes	7 (T1)	NTFOX	22.4 (T2)	MLFOX
O22ML	T1Hg O ₂ intermediate #2 — exposed to O ₂ for 27 minutes	7 (T1)	NTFOX	21.4 (T2)	MLFOX
ML11	T1Hg O ₂ intermediate #1 — exposed to O ₂ for 9 minutes	3.5 (T1)	NTFOX	12 (T2)	MLFOX
ML21	T1Hg O ₂ intermediate #1 — exposed to O ₂ for 27 minutes	3.5 (T1)	NTFOX	15 (T2)	MLFOX
ML31	T1Hg O ₂ intermediate #1 — exposed to O ₂ for 90 minutes	3.5 (T1)	NTFOX	18 (T2)	MLFOX

£ — These data sets were themselves first corrected for native content

† — the type of Cu on which these figures are based is shown in brackets. Oxidized T1 was measured either by optical absorption spectra or by EPR, usually the former, oxidized T2 was measured by EPR alone, and oxidized T3 by optical spectra alone.
AA : atomic absorption

Table 6.3. Difference edges constructed from corrected laccase edges — "100% reduced Δ standards".

No.	Description	Edge data sets used	Height at 8984 eV	% of Cu reduced
I	100% reduced T1Hg, 1989	MLRD _{corr} – 2ML01 _{corr}	0.4496	100
II	100% reduced T1Hg, 1991	MLFRD _{corr} – MLFOX _{corr}	0.4787 0.5000 [†]	100
III	100% reduced native, 1989	NLRD – NT30	0.4524	100
IV	100% reduced native, 1991	NTFRD – NTFOX	0.4234	100
V	100% reduced T2D, 1989	T2D1 _{corr} – T26A _{corr2}	0.4751	100
VI	100% reduced "T3 Cu"	1.5(T2DS _{corr} – T26A _{corr1})	0.6188	100
VII	100% reduced "T2 Cu" ₁	4×[III] – 3×[IV]	0.2582 0.3184 [†]	100
VIII	100% reduced "T2 Cu" ₂	3×[II] – 2×[V]	0.2037 0.2415 [†]	100
IX	100% reduced "T2 Cu" _{avg}	0.5"T2 Cu" ₁ + 0.5"T2 Cu" ₂	0.2319 0.2831 [†]	100

† — This difference edge height was measured at 8983.4 eV (where the 100% reduced T1Hg standard is maximum) for greater accuracy.

Table 6.4. Difference edges constructed from corrected laccase edges — amount of reduced Cu is determined from standards in Table 6.3.

Description	Edge data sets used	Height at 8984 eV	% of Cu reduced [‡]
T2D exp. 15 min.	T2D2 _{corr} – T26A _{corr}	0.4776	100 wrt [V]
T2D exp. 63 hrs.	T2D3 _{corr} – T26A _{corr}	0.3628	76 wrt [V], 88 wrt 2/3[VI]
Resting T1Hg	1ML01 _{corr} – 2ML01 _{corr}	0.0060	1 wrt [I]
Reduced and reoxidized T1Hg	MLOX _{corr} – 2ML01 _{corr}	0.0082	2 wrt [I]
T1Hg O ₂ int. #1	O21ML _{corr} – MLFOX _{corr}	0.06303 [†]	13 wrt [II] 59 wrt 1/3[VII] 78 wrt 1/3[VIII] 67 wrt 1/3[IX]
T1Hg O ₂ int. #2	O22ML _{corr} – MLFOX _{corr}	0.07564 [†]	15 wrt [II] 71 wrt 1/3[VII] 94 wrt 1/3[VIII] 80 wrt 1/3[IX]
T1Hg O ₂ int. #3	ML11 _{corr} – MLFOX _{corr}	0.07730 [†]	15 wrt [II] 73 wrt 1/3[VII] 96 wrt 1/3[VIII] 82 wrt 1/3[IX]
T1Hg O ₂ int. #4	ML21 _{corr} – MLFOX _{corr}	0.08498 [†]	17 wrt [II] 80 wrt 1/3[VII] 106 wrt 1/3[VIII] 90 wrt 1/3[IX]
T1Hg O ₂ int. #5	ML31 _{corr} – MLFOX _{corr}	0.08498 [†]	17 wrt [II] 80 wrt 1/3[VII] 106 wrt 1/3[VIII] 90 wrt 1/3[IX]

[†] — These difference edge heights were measured at 8983.4 eV (where the 100% reduced T1Hg standard is maximum) for greater accuracy.

[‡] — "wrt" = "with respect to"

Table 6.5. Normalized difference edge energies and amplitudes at peak maxima (the first part is from Table III in the Cu edge analysis paper by Kau, *et al.*¹⁵).

Coordination Number	Energy [†] (eV)	Amplitude [†]	Slope [§] (units/eV)
2	8983.6–84.2	0.99 ± 0.13	-0.20 to -0.45
3	8983.1–83.8	0.54 ± 0.08	0.0 to -0.06
4	8984.7–86.3	0.49 ± 0.11	0.10 to 0.08
covalent Cu(II)	8985.8–88.0	0.42 ± 0.05	0.12 to 0.09
2 + 3	8983.6–84.2	0.75 ± 0.10	-0.07 to -0.11
2 + 4	8983.6–84.4	0.65 ± 0.07	-0.05 to -0.08
3 + 4	8983.7–85.8	0.48 ± 0.10	0.03 to 0.04
3 and 2 + 4	8983.1–84.4	0.59 ± 0.13	0.0 to -0.08
2 + 3 and 2 + 4	8983.6–84.4	0.71 ± 0.13	-0.05 to -0.11
100% reduced native laccase [£]	8983.6	0.58	-0.047 [◇]
100% reduced T2D laccase [£]	8983.7	0.57	-0.045 [◇]
100% reduced T1Hg laccase [£]	8983.6	0.58	-0.044 [◇]
100% reduced "T3 Cu" [‡]	8983.7	0.72	-0.08
100% reduced "T2 Cu" ₁ [‡]	8982.5	0.46	-0.08
100% reduced "T2 Cu" ₂ [‡]	8982.8	0.39	-0.02
100% reduced "T2 Cu" _{avg} [‡]	8982.8	0.43	-0.05

† — at peak maxima

§ — The slope is defined as the best fit line extending between 8984 and 8986 eV of the spectrum and the unit is (normalized difference edge amplitude)/(eV)

£ — This difference edge is constructed by direct subtraction of the edge of the Cu(II) model compound [Cu₂(L₈-Et)(OAc)](ClO₄)₂²⁴ (CUL2), as described in Equation 6.5.

‡ — This difference edge is scaled up to "one full Cu" and is with respect to the Cu(II) model compound [Cu₂(L₈-Et)(OAc)](ClO₄)₂²⁴ (CUL2), as described in Equation 6.6.

◇ — The extra decimal place is included to reveal the similarity of the slopes obtained for the three different forms of laccase.

Figure 6.1. X-ray absorption edges of Cu(I) model compounds : Cu(I)(*N*-methylimidazole)₂BF₄,²⁵ linear 2-coordinate (· · ·); [Cu(I)₂(mxyN₆)](BF₄)₂,²⁶ 3-coordinate (- - -); Cu(I)(*N*-methylimidazole)₄ClO₄,²⁷ tetrahedral 4-coordinate (— · — · —); and [Cu₂(L₈-Et)(OAc)](ClO₄)₂,²⁴ Cu(II) N/O-ligated (CUL2) (——).

Figure 6.2. Difference edges of Cu(I) compounds when the edge of $[\text{Cu}_2(\text{L8-Et})(\text{OAc})](\text{ClO}_4)_2$ ²⁴ (CUL2) is subtracted : $\text{Cu(I)}(\text{N-methylimidazole})_2\text{BF}_4$,²⁵ linear 2-coordinate (---); $[\text{Cu(I)}_2(\text{mxyN}_6)](\text{BF}_4)_2$,²⁶ 3-coordinate (—); and $\text{Cu(I)}(\text{N-methylimidazole})_4\text{ClO}_4$,²⁷ tetrahedral 4-coordinate (— · — · —).

Figure 6.3. Edges of resting (—) and totally oxidized (H₂O₂-treated) (- - -) laccase. Top : T2D laccase. Resting sample is corrected T2DS, totally oxidized sample is corrected T26A. Bottom : T1Hg laccase. Resting sample is corrected 1ML01, totally oxidized sample is corrected 2ML01.

Figure 6.4. T2D laccase sample series showing reactivity with dioxygen. Top : Corrected X-ray absorption edges. Bottom : Difference edges made by subtracting totally oxidized T2D laccase (T26A). Totally reduced T2D (T2D1) (—), T2D exposed to air for 15 minutes (T2D2) (- - - -), T2D exposed for 63 hours (T2D3) (- - -) and totally oxidized T2D (T26A) (- . - .).

Figure 6.5. T1Hg laccase sample series showing reactivity with dioxygen. Top : Corrected X-ray absorption edges. Bottom : Difference edges made by subtracting totally oxidized T1Hg laccase (2ML01). Totally reduced T1Hg (MLRD) (—), T1Hg exposed to air for 30 minutes (MLOX) (- - - -), and totally oxidized T1Hg (2ML01) (— - — - —).

Figure 6.6. X-ray edges of some T1Hg O₂ intermediate samples, with fully reduced and fully oxidized T1Hg laccase. Edges are normalized, but no corrections for either native content or oxidation of T2 have been applied.

Figure 6.7. Corrected difference edges of "100% reduced Δ TlHg" (see Table 6.3) and of some TlHg O₂ intermediates with fully oxidized TlHg (MLFOX, corrected) subtracted.

Figure 6.8. Difference edges of "100% reduced $\Delta T2$ ", constructed by various methods (see Table 6.3), and a corrected difference edge of one sample of $T1Hg O_2$ intermediate. " $\Delta T2 Cu$ "₁ (---), " $\Delta T2 Cu$ "₂ (---), " $\Delta T2 Cu$ "_{avg} (—) and $T1Hg O_2$ intermediate #5 (ML31) (—).

Figure 6.9. Corrected difference edges of various forms of laccase with CUL2 subtracted. Fully reduced native (—), T2D (- - -) and T1Hg (- - -) laccase, " Δ T3 Cu" (- - - —) and " Δ T2 Cu"_{avg} (- - - -) with the edge of $[\text{Cu}_2(\text{Lg-Et})(\text{OAc})](\text{ClO}_4)_2^{24}$ (CUL2) subtracted (see Table 6.5) for the purpose of determining the geometry of Cu(I) in laccase.

Chapter 7

Edge and EXAFS Studies of *Rhodospirillum rubrum*

Nickel Carbon Monoxide Dehydrogenase

and Nickel Model Compounds

(A) Brief Overview of *Rhodospirillum rubrum* Ni Carbon Monoxide Dehydrogenase

(1) Information Known From Other Studies

It has been known for some time that trace amounts of nickel are essential for healthy growth in plants and animals,¹ but the first nickel-containing enzyme, urease, was not identified until 1975. Since then, four classes of nickel-containing enzymes have been identified: urease (in plants), hydrogenase (in sulfate-reducing bacteria), methyl-S-coenzyme-M reductase (in methanogenic bacteria) and carbon monoxide dehydrogenase (in photosynthetic and acetogenic bacteria). As yet, no nickel enzymes have been discovered in animals.

The two classes of nickel-containing carbon monoxide dehydrogenase (henceforth CODH) so far characterized both run the following reaction :

In acetogenic bacteria (the most intensively studied of which is *Clostridium thermoaceticum*), CODH is extremely flexible, and this reaction may proceed both ways, with concomitant synthesis (or at times degradation) of acetyl-coenzyme A from either CO or H₂-CO₂ at another site on the CODH enzyme.^{2,3} However, in photosynthetic bacteria (including *R. rubrum*), this reaction proceeds only in the forward direction, generating CO₂ and H⁺ from CO and H₂O, and the CODH does not itself carry out synthesis of any larger molecules.⁴

R. rubrum Ni CODH is a monomeric protein of 62 kDa containing one Ni, 8 Fe and approximately 8 inorganic S. It is associated *in vivo* with a 22-kDa subunit which contains four Fe in an Fe-S center.⁵ *In vitro*, without the 22-kDa subunit, the 62-kDa unit is capable of converting CO to CO₂ in the presence of a low-potential one-electron reductant such as methyl viologen.⁶ *In vivo*, the 22-kDa subunit serves to mediate electron transfer from CO to the hydrogenase in *R. rubrum*, thus enabling the latter to generate H₂. Beyond this, the physiological role of Ni CODH in *R. rubrum* is not yet clear.

An apo form of the 62-kDa unit can be obtained by growing *R. rubrum* in the absence of nickel.⁷ Studies on this form of the protein show conclusively that Ni is required for the protein to be active, and strongly suggest that Ni is the site where CO binds.^{8,9} EPR studies at liquid helium temperatures have been done on both the holo and apo forms, in oxidized and reduced states.^{4,7,10} In the reduced state, an Fe₄S₄-type signal (seen at *g* = 1.90–2.05) due to about two such units was observed in both holo- and apo-Ni CODH. EPR on the oxidized state of the holo-Ni CODH showed two clusters of signals, one with *g* = 2.04, 1.90 and 1.71 and the other with *g* = 4.24, 4.32 and 4.40, while oxidized apo-

Ni CODH showed no signals at all. (Fe_4S_4 is EPR-silent when oxidized.) The signals observed in the oxidized holo-Ni CODH are therefore due to the Ni itself. Furthermore, the $g = 2.04$ feature was observed to broaden upon labelling with either ^{61}Ni or ^{57}Fe . Thus Ni is associated with the Fe-S clusters in some way.

(2) Some Questions About the Ni CODH Active Site

Although the studies mentioned above established that the active site of *R. rubrum* CODH must contain Ni associated with Fe-S clusters in some manner, before we undertook this XAS study nothing was known about the immediate environment of Ni — its geometry, ligation or relation to the Fe-S clusters. Is Ni actually part of a cubane cluster (i.e., NiFe_3S_4 , shown in Figure 7.1(a))? Or is it instead attached to an Fe_4S_4 cluster through a bridge (Figure 7.1(b))? Also, we wanted to determine the coordination environment about the Ni, as a step towards understanding the structure and function of this enzyme.

(B) Some Results of XAS Studies on Ni Proteins and Model Compounds Carried Out by Other Workers

(1) Edge Studies on Ni Models

Major work in surveying the edges of Ni compounds of various coordination geometries and ligand types has been carried out by Robert A. Scott^{11,12} and Michael J. Maroney.¹³ Maroney's survey is more extensive, but, unfortunately, the energy resolution of the edge data collected by him is considerably lower than that collected by Scott. All Maroney's data were collected using Si[111] monochromator crystals, whereas Scott used Si[220] crystals and a 1-mm monochromator entrance slit. This means that Scott's edge data reveal more features and details than Maroney's do, and so afford more accurate conclusions.

The first feature of a nickel edge is the selection-rule-forbidden $1s \rightarrow 3d$ transition, which occurs at 8332-8333 eV, at the onset of the rising edge (Figure 7.2, bottom). This feature is extremely weak for compounds with centrosymmetric geometries (octahedral and square-planar), but grows much larger as the geometry deviates from centrosymmetry, because some mixing of the 3p and 3d orbitals then occurs, and so the transition becomes allowed in some degree. It reaches maximum height (still less than 10% of the total edge jump) with tetrahedral geometry.

For nitrogen-type ligands, octahedral compounds show a steep, featureless rising edge which often peaks sharply at a normalized amplitude of above 1.5 units (the overall

edge jump is scaled to 1 unit, see Chapter 2) and then dips well below 1.0 units (Figure 7.3, bottom). In square-planar compounds, on the other hand, the rising edge is interrupted by a spike at about 8337 eV, variously assigned as $1s \rightarrow 4s$, $1s \rightarrow 4p_z$, or $1s \rightarrow 4p$ plus shakedown, and the edge does not finally rise much above 1.2 units, or peak sharply at the top (Figure 7.2, top). The rising edge for tetrahedral compounds does not have the characteristic signature of a spike half-way up, nor does it rise to as tall a maximum, but it may show a shoulder half-way up (Figure 7.2, bottom). Five-coordinate compounds, as might be expected, show edges that are intermediate between all these, with medium-sized $1s \rightarrow 3d$ transitions, a relatively smooth rising edge that is not very steep and, usually, a slightly taller profile than edges of square-planar compounds (Figure 7.3, top). Where Ni(II) and Ni(III) compounds with the same ligands and ligating geometry can be compared, the Ni(III) edge shows similar features to the corresponding Ni(II) edge, only shifted to higher energy by 1–2 eV. However, this shift is not useful for identifying the Ni oxidation state in an unknown compound because the edge position is also affected by the polarizability of the ligands attached to Ni (*vide infra*).

Maroney further observed that rising edges of square-pyramidal compounds have a shoulder about halfway up, while those of trigonal-bipyramidal compounds rise featurelessly to a peak. He also noted that features for the Ni(III) compound edges are generally broader and less distinct, and the areas of their $1s \rightarrow 3d$ transitions are about 50% larger, than those for the corresponding Ni(II) compounds.

When compared with edges of nitrogen-ligated compounds, edges of compounds with sulfur-type ligation also show the same characteristic transitions mentioned for the various geometries, but have lower maximum edge amplitudes (Figures 7.2 and 7.3). Thus, octahedral compounds lack a tall sharp peak. Edges of square planar compounds are still distinguished by spikes at 8337 eV, but are generally flatter on top. Another effect of sulfur ligation is that the edge is shifted to lower energy by 1–2 eV, which may be explained as due to the reduction of the positive charge density on the Ni atom by the more polarizable sulfur ligands. For Ni compounds with mixed nitrogen-type and sulfur-type ligands, Maroney found a smooth change in the characteristics the edges displayed as the proportion of sulfur ligation was increased.

(2) XAS Studies on Ni Proteins

EXAFS studies on all four classes of Ni-containing enzymes have been carried out.

In jack bean urease, Ni is coordinated with 5–6 N/O-type ligands, and a Ni-Ni interaction was detected in the 2-mercaptoethanol-inhibited enzyme.¹⁴ In the S-methyl

coenzyme-M reductase of *Methanobacterium thermoautotrophicum*, the nickel "corphin" factor F₄₃₀ has a Ni-tetrapyrrole structure. In free F₄₃₀, the pyrrole ring is ruffled and the Ni geometry is square-planar, but when the F₄₃₀ is incorporated into the protein the coordination number rises to 5–6 N/O, in an octahedral geometry, and the pyrrole ring lies flat.^{11,15}

In the hydrogenases, the most recent results show that Ni is coordinated with a mixture of nitrogen-type and sulfur-type ligands. In *Desulfovibrio baculatus* hydrogenase there are 3–4 N/O, 1–2 S/Cl, and also one selenium, coordinated to Ni in a pseudo-octahedral or pentacoordinate geometry.¹⁶ In *Thiocapsa roseopersicina* hydrogenase, Ni is ligated by ~3 N/O and ~2 S in a trigonal bipyramidal geometry, and the EXAFS Fourier transform furthermore shows peaks at 4.3 Å and 6.2 Å that can be fitted with Fe and S, and so are suggestive of a Ni-Fe-S cluster.¹⁷

Two independent studies of the *Clostridium thermoaceticum* Ni CODH have also been published.^{12,18} Data ranges were rather limited (to $k \sim 10 \text{ Å}^{-1}$) in both studies, which may explain why one study (Bastian, *et al.*) suggested Ni–S₄ ligation (S at 2.16 Å) and one Fe at ~3.25 Å, while the other study (Cramer, *et al.*) suggested Ni–N₂S₂ ligation (N/O at 1.97 Å, S at 2.21 Å). Based on the edge of the protein, the latter study also suggested a distorted square-planar or square-pyramidal geometry for Ni. (A complicating factor, however, is that there are six Ni in each $\alpha_3\beta_3$ protein unit, and at least two different Ni sites.³)

(C) Experimental Details

(1) Preparation of Samples

In all, we collected data on six samples of *R. rubrum* Ni CODH, four oxidized and two reduced, over three years. (Only five of these were subsequently analyzed; see next section.) All samples were provided by Professor Paul W. Ludden of the University of Wisconsin at Madison. *R. rubrum* CODH was purified and assayed as described in the literature.^{4,9} The final step of purification, native gel electrophoresis, yielded two bands of CODH with differing activity and Fe content. The majority of CODH purified as peak 1, the more active band, and this band was used in all experiments. Activities were measured in terms of μmol of CO oxidized per minute per mg protein. Samples were prepared for spectroscopy in an anaerobic glove box [Vacuum Atmospheres (Hawthorne, CA) Dri-Lab glove box model HE-493] containing an N₂ atmosphere with <1 ppm O₂. The samples were oxidized with methyl viologen or indigo carmine on a DE52 column, eluted with sodium chloride solution, then concentrated if necessary in a collodion ultrafiltration

apparatus (Schleicher & Schuell). To produce reduced samples, sodium dithionite was added, for a final concentration of 5 or 10 mM dithionite. See Table 7.1 for the final concentrations of the samples and buffers, and for the specific activities of CODH. The redox states of the samples were not characterized by EPR or other spectroscopic methods before XAS data were measured on them.

The oxidized and reduced samples were each loaded into EXAFS cells made of lucite (outer dimensions $2 \times 4 \times 28$ mm³, volume ~ 180 μ l) with an X-ray-transparent front face of 40 μ m Kapton tape for X-ray fluorescence measurements. The samples were stored in liquid nitrogen and also mounted for data collection under liquid nitrogen.

Some of the Ni(II) model compounds we used were obtained commercially, some were provided by collaborators, most notably Professor Richard H. Holm of Harvard University, and some were synthesized by us (see Table 7.2). The solid compounds were ground with boron nitride and packed into aluminum spacers in the manner described in Chapter 2.

(2) Data Collection

Protein data were collected at the unfocussed bending magnet beam line X19A at the National Synchrotron Light Source (NSLS), and at the unfocussed 8-pole wiggler beam line 7-3 at the Stanford Synchrotron Radiation Laboratory (SSRL) (see Table 7.1). (One data set, collected in April 1990 on beam line 4-2 at SSRL, proved much noisier and more glitch-ridden than all the other data sets, and it was not curvefitted, nor is it included in Table 7.1.) Si[220] monochromator crystals were used on all beam lines. For all the edge spectra shown, including those of model compounds and Fe edges measured on two of the protein samples in 1991, the monochromator entrance slit at SSRL BL 7-3 was set at a 1-mm vertical gap. (NSLS BL X19A lacked a monochromator entrance slit, but the hutch slit was set at a 1-mm gap.) EXAFS spectra were collected separately, employing a 2-mm or 1.5-mm gap in the slit (at both beam lines) for increased flux, except in 1992 at SSRL BL 7-3, when a 1-mm gap was maintained for combined edge-and-EXAFS scans (see Table 7.1). Besides the energy resolution of the data, edge scans also differed from EXAFS scans in that 6 motorsteps/eV rather than 3 motorsteps/eV were taken in the edge region of the scan. Edge spectra were measured to $k = 9$ $\text{\AA}^{-1}$ (Fe K edges were measured to $k = 11$ $\text{\AA}^{-1}$) and EXAFS spectra were measured to $k = 15$ $\text{\AA}^{-1}$.

All the model compound data were measured at SSRL on unfocussed beam lines, most of them on BL 7-3. All edges and most of the EXAFS were measured with a 1-mm monochromator slit; a few compounds had EXAFS scans measured with a 2-mm slit.

All data that we collected, both protein and model compound, were measured at 10 K. Some model compound data provided to us by Professor Robert A. Scott were measured at 4 K, under the same conditions as our data, at SSRL. Protein data were measured in fluorescence mode, using a 13-element Ge solid-state detector array.¹⁹ Model compound data were measured in transmission mode.

No photoreduction or photooxidation (as seen by a change in the Ni or Fe edge) of any of the samples was observed during data collection.

(3) Data Analysis

Data were reduced and analyzed using XFPACK, in the manner described in Chapter 2. Scans were calibrated using nickel foil as an internal standard, taking the first inflection point of the foil as 8331.6 eV. We made repeated attempts to obtain protein EXAFS data usable to $k = 15 \text{ \AA}^{-1}$, but were unable to do so because of a monochromator glitch at $k = 12.8 \text{ \AA}^{-1}$, which manifested itself as a large step in the two data sets with the lowest noise (CODHX and CODHRX, see Table 7.1). It was less severe in the other data sets, but we still considered it inadvisable to trust data beyond it. Subtraction of the background was done by fitting a polynomial to the EXAFS region and then applying this at the bottom of the "edge step" and extending this into the pre-edge region. A different three-region spline to $k = 12.7 \text{ \AA}^{-1}$ was applied to each protein data set. Model compound data were splined (also with three regions) to the limit of the data (usually $k = 14.5 \text{ \AA}^{-1}$ or $15 \text{ \AA}^{-1}$, unless cut short by the monochromator glitch at $k = 12.8 \text{ \AA}^{-1}$) and then Fourier-transformed over the same range as the protein data for analysis. For all data analyzed in relation to the proteins, a data range of $k = 3.0\text{--}12.0 \text{ \AA}^{-1}$ was used for the forward Fourier transform, and the data were then backtransformed to $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$ for curvefitting. Parameters were also extracted over the same data ranges. (An earlier analysis over a shorter data range, using only CODHX, has been published.²⁰)

For comparisons of the edges of the proteins and model compounds, the same one-region spline to $k = 9 \text{ \AA}^{-1}$ was applied to most of the files, since normalization of peak heights can be greatly affected by spline curvature.

(D) Edge Studies

(1) Further Observations About Model Compound Edges

Apart from the observations of trends in Ni edges already made by Scott and Maroney (*vide supra*), it is difficult to pick out systematic trends based on finer details of geometry. We tried correlating features such as the height of the spike at 8337 eV or the

size and location of the $1s \rightarrow 3d$ transition with details of geometry such as the extent of chelation, bite size of chelating ligands, bond length and degree of saturation in the ligands. We could not find any trends that held for our entire library of edges. However, there were a number intriguing trends, or rather, exceptions to trends observed by Scott and Maroney, in subsets of related compounds.

One such trend may be observed in the edges of $\text{Ni}(\text{N}_2\text{S}_2\text{C}_2)$ (**5**), $\text{Ni}(\text{N}_2\text{S}_2\text{C}_3)$ (**6**) and $\text{Ni}(\text{N}_2\text{S}_2\text{C}_4)$ (**7**) (see Table 7.2 for all compounds discussed), provided to us by Professor Robert D. Bereman of North Carolina State University (Figure 7.4, bottom). These compounds differ only in that the stepwise lengthening of an alkane chain (from C_2 to C_4) in the tetradentate ligand forces increasing deviation from square-planar towards tetrahedral geometry. As we might expect, the progressive deviation from centrosymmetry is accompanied by a corresponding increase in the size of the $1s \rightarrow 3d$ transition in the edge as the $3d$ orbitals acquire more p character. We might also expect, based on the appearance of tetrahedral compound edges (*vide ante*), that the ~ 8337 eV spike should decrease in size or sharpness as the geometry deviated from planarity. However, this clearly does not happen, raising the possibility that the size of the spike does not depend primarily on the geometry of the compound.

On the other hand, we have measured two square-planar compounds where the ~ 8337 eV spike seems to have been broadened and reduced to a shoulder. $\text{Ni}(\text{tsalen})$ (**8**) differs from $\text{Ni}(\text{ebmba})$ (**9**) (Figure 7.4, middle) only in that two bonds in the ligand are unsaturated, allowing for conjugation around the bite of the chelate. Both compounds are truly square-planar; in both cases no weakly bonding interactions between molecules perturb the coordination geometry. In the other case, $[\text{Ni}(\text{SS}_2)]_2$ (**15**) (Figure 7.4, top), the Ni coordination is nominally square-planar, but the two Ni atoms are quite close to each other ($2.74 \text{ \AA}$), and so there is probably some interaction between them.

From this small sampling, we may hypothesize that delocalization of valence electrons is responsible for broadening of features in the Ni edge spectrum, in particular of the ~ 8337 eV transition. This is consistent with the appearance of the edges of NiFe_3S_4 cubanes (highly delocalized systems) (Figure 7.4, top) which, apart from the $1s \rightarrow 3d$ transition, do not show sharp features anywhere along the edge.

The edges of a series of tripodal $\text{Ni}-\text{NS}_3\text{L}$ compounds provide another fascinating exception to the trends (Figure 7.5). The edge for $[\text{Ni}(\text{NS}_3^{\text{tBu}})\text{Cl}](\text{BPh}_4)$ (**22**) is that of a typical trigonal bipyramidal compound, as described by Maroney. However, edges for all the other compounds in this series (where Cl is replaced by H, Me, CO and COMe respectively (**18–21**)) do not fit this description at all. They all rise at lower energy, have a

much lower profile, and show six transitions from the bottom of the edge (the $1s \rightarrow 3d$ transition) to the top of the edge (at ~ 8360 eV). The transition at ~ 8360 eV changes slightly in energy, but the other transitions remain at about the same energy for all four compounds, varying only in height. Of the five compounds in this series, only $[\text{Ni}(\text{I})(\text{NS}_3^{\text{tBu}})\text{CO}](\text{BPh}_4)$ is a Ni(I) compound; all the rest are Ni(II) compounds. Thus, the difference between $\text{L} = \text{Cl}$ and all the rest cannot be explained by the Ni oxidation state, nor in terms of the spectrochemical series, which would predict a splitting in the $1s \rightarrow 3d$ feature for $\text{L} = \text{Cl}$ (Ni should be high-spin in this case) rather than H, as we observed. Clearly, there are complex electronic effects at play, which cannot easily be accounted for by a simple ligand field theory.

Therefore, besides geometry and hardness of binding atoms, the appearance of Ni edges is affected by other electronic effects. The presence of a spike at ~ 8337 eV does not always mean that a compound is square-planar, nor does its absence always mean that the compound is not square-planar. However, in all the compounds we examined, the $1s \rightarrow 3d$ feature still proved a reliable indicator of centrosymmetry, so that it is still quite easy to distinguish square-planar compounds from tetrahedral compounds. Edges of trigonal bipyramid compounds can vary considerably in appearance, and we have no explanation that accounts for all of our data.

(2) Ni K and Fe K Edges of Ni CODH

The Ni edge of oxidized Ni CODH has the same sort of "smeared-out, low resolution" appearance as the edges of the NiFe_3S_4 cubanes and $[\text{Ni}(\text{SS}_2)]_2$ (Figure 7.6, top), though not the same precise shape. It has a $1s \rightarrow 3d$ feature of the same size as the cubane edges, which suggests that it may, like the cubanes, have a distorted tetrahedral coordination about Ni. Also, the edge rises at the same energy as the cubane edges. However, further up the edge rise, the Ni CODH edge does not resemble the cubane edge so closely. The $[\text{Ni}(\text{SS}_2)]_2$ edge does not match the CODH edge as closely even as the cubanes do, since the $1s \rightarrow 3d$ transition is a lot smaller and the edge rises at lower energy. We were not able to find any compounds that resembled the Ni CODH edge more closely than the cubanes or $[\text{Ni}(\text{SS}_2)]_2$. All the mononuclear compound edges that we compared the Ni CODH edge with had steeper edge rises and more sharply resolved transitions (Figure 7.6, bottom). All this suggests that, consistent with findings from EXAFS analysis,²⁰ Ni is not part of a NiFe_3S_4 cubane (*vide infra*), and also that the Ni atom is part of a highly delocalized electronic environment. Also, the coordination geometry of Ni is clearly not centrosymmetric, but is distorted four-coordinate (possibly tetrahedral) or perhaps five-coordinate.

A highly delocalized electronic environment would also explain why no shift in the edge could be observed when the Ni CODH was reduced (Figure 7.7). Indeed, the edges of oxidized and reduced Ni CODH, especially for the samples measured in 1992 (CDHOX and CDHRD), are practically identical. There is a small difference in the spectra for the oxidized and reduced samples measured in 1991 (CODHOX and CODHRX). However, there is just as much variation in the edges for the three oxidized samples measured in different years (Figure 7.8). Thus, all the Ni edges show that the Ni environment undergoes no major changes when the protein is reduced (despite indications to the contrary from EXAFS analysis, *vide infra*). The Ni atom may in fact be in the same oxidation state for both oxidized and reduced forms of the protein, rapidly transferring any electrons it receives to the Fe-S units it is associated with.

We also measured Fe K edges (Figure 7.9) on oxidized and reduced CODH in 1991 (samples CODHOX and CODHRX). Consistent with results from EPR spectra, the Fe's in Ni CODH are definitely shown to be in an Fe₄S₄-cubane-like environment. Again, we could not observe any shift with redox state in these edges. The edge of the reduced protein is a little more "smeared out" in appearance, possibly reflecting the presence of two extra electrons (for two reduced Fe₄S₄ units). It is unlikely that a difference of two electrons spread out over 9 metal and 8 S atoms would be detectable by a shift in the X-ray edges, though it can be detected by EPR.

Thus the Ni K and Fe K edges of Ni CODH show that Ni in CODH has a distorted four-coordinate or five-coordinate environment, and that it is unlikely to be occupying one corner of a cubane. They also show that there is no significant change in the effective oxidation state of Ni, or the Ni ligand environment, upon changing the redox state of the protein.

(E) EXAFS Analysis of Ni CODH Samples and Related Model Compounds

(1) Extraction and Testing of Parameters on Model Compounds

Parameters extracted over a range of $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$ (*vide infra*) were thoroughly tested on model compounds in order to gauge their reliability. One-, two- and three-wave fits were carried out on filtered data from model compounds as shown in Tables 7.3 and 7.5–7.8. (See also Appendix III for complete fit results.)

The relative Debye-Waller factor is represented by the parameter c_2 , which is always negative (see Chapter 2). A larger negative value of c_2 indicates more disorder in the distances of scatterers, or more vibrational disorder and a weaker Ni–X bond.

Coordination numbers and c_2 values were evaluated together to determine the most reasonable fit to a data set.

Fits were done either by floating coordination numbers and fixing c_2 (Type 2 fits), or varying c_2 and fixing coordination numbers (Type 3 fits), or occasionally by varying both coordination numbers and Debye-Waller factors (Type 1 fits). In all fits, the Ni–X distances of all waves were varied. In two- or three-wave Type 1 fits, there is considerable correlation between the coordination numbers and c_2 values, and so these fits do not have much physical significance as such. However, they can be useful in finding out what minimum the data tend towards.

(a) Extraction of Parameters and Tests on "Single-Shell" Compounds

Parameters over $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$ were extracted from Ni(dmegly)₂ (**2**), (Ni–N = 1.85 Å), Ni(dedtc)₂ (**14**) (average Ni–S = 2.201 Å) and (Et₄N)₂[NiFe₃S₄(PPh₃)(SEt)₃] (**10**) (average Ni–Fe = 2.689 Å).

In order to gauge a reasonable range of values for the Debye-Waller factors, the Ni–N and Ni–S parameters were fitted to several "single-shell" compounds, i.e. compounds with only one type of ligand, all occurring within a narrow range of distances. Testing was carried out on 12 compounds with Ni–N ligation and 5 with Ni–S ligation. The results are summarized in Table 7.3 and listed in full in Appendix III.

Distances were obtained with good accuracy, within 0.014 Å at worst and often within 0.006 Å. Also, the shorter the Ni–X bond, the lower the magnitude of the Debye-Waller factor (as shown by c_2). (The square-pyramidal Ni–N appears to be an exception to this trend. However, this compound has a cyclam-like ring ligated to the Ni, with a pyridine molecule in the apical position, probably at a longer distance. It is quite likely that we are seeing mainly the contribution from the cyclam ring nitrogens, and that the apical nitrogen actually interferes destructively with this signal, especially at higher values of k .) These fits also showed that the Ni–S parameters give much more reliable coordination numbers than the Ni–N parameters, with coordination numbers within 0.4 units (as opposed to 1.5 units) of the correct value. The range of reasonable c_2 values for four-coordinate Ni–N compounds is -0.0221 to -0.0265. For octahedral Ni–N compounds this range is -0.0253 to -0.0280. For Ni–S compounds, the corresponding ranges in reasonable c_2 values are -0.0201 to -0.0214 and -0.0217 (for one octahedral compound) respectively.

It can be seen that there is considerable latitude in the coordination numbers and relative Debye-Waller factors obtained, and that it would be hard to make a reliable judgement of the true coordination number from these alone. It is therefore helpful to also

take the length of the Ni–L bond into account when estimating a coordination number. To give us criteria for making such judgements, we surveyed 126 crystallographically determined model compounds with various geometries and ligand combinations, and the results are summarized in Table 7.4. As can be seen, square-planar compounds have the shortest bond lengths (e.g., Ni–N = 1.85–2.066 Å in Ni–N₄ compounds) and octahedral compounds the longest (e.g., Ni–N = 2.035–2.151 Å in Ni–N₆ compounds), and tetrahedral and five-coordinate compounds are somewhere in between. Also, the length of Ni–N does not seem to depend on whether there are S ligands also coordinated to Ni (e.g., Ni–N = 1.857–2.003 Å in square-planar Ni–N₂S₂ compounds), or how many S there are. The same is true of Ni–S with respect to the presence of N ligands. Rather, bond lengths are more affected by the steric and electronic character of the ligand for each compound. For square-planar Ni–S₄ compounds, the bond length shortens from 2.165–2.240 Å to 2.101–2.122 Å as the formal oxidation state of Ni increases from +2 to +4. This trend is also present, but not so well-substantiated, in Ni–N₂S₄ compounds.

The only compound available for testing the Ni–Fe parameters was (Et₄N)₃[NiFe₃S₄–(SEt)₄] (**11**). When the data for it were Fourier transformed, we obtained two peaks that were not very well separated and thus not optimal for testing the Ni–Fe parameters. Nevertheless, the second peak was windowed out and fitted with the Ni–Fe parameters. A good fit was obtained, with 2.6 Fe at 2.75 Å. A Type 3 fit (*vide supra*) with 3 Fe's gave a c_2 value of -0.0169 (result in Table 7.3).

(b) Further Testing of Parameters on Ni–N,S Compounds

Next, the Ni–N and Ni–S parameters were tested on compounds with mixed N,S ligation, namely Ni(tsalen) (**8**), [Ni(NS₃^{tBu})H](BPh₄) (**18**) and [Ni(N₃S₂)] (**16**). Ni(tsalen) is a fairly regular compound, with Ni–N = 1.85 Å, 1.86 Å and Ni–S = 2.174 Å, 2.139 Å. [Ni(NS₃^{tBu})H](BPh₄) has a different ratio of N and S ligands, but is still fairly regular (Ni–N = 2.02 Å, Ni–S = 2.234, 2.227, 2.218 Å). [Ni(N₃S₂)] is ligated by a strained terpyridine, and it also has two crystallographically inequivalent molecules, both of which make for more disorder in the ligand distances (Ni–N = 1.968–2.125 Å, Ni–S = 2.274–2.332 Å).

For all three compounds, the main Fourier transform peak was filtered out, and fits of Type 1, 2 and 3 (*vide supra*), using various combinations of N and S waves, were done on the backtransformed data (see Tables 7.5–7.7, and Appendix III for a complete list of fit results). For N+S and S+S' combinations, several Type 3 fits were made, exploring several possible combinations of N-type and S-type ligation. Results agreed with those of

the Type 2 fits, and also showed how shallow or deep the minimum was for numbers of N and S coordinated. In the case of $[\text{Ni}(\text{NS}_3^{\text{tBu}})\text{H}](\text{BPh}_4)$, the main Fourier transform peak was narrower than that for the other two compounds, so fits were tried on data from two Fourier transform windows, one of the same width as for the other two compounds, and including a small low-R' peak, and the other narrower, including only the main peak. Results from both these windows were quite similar. The wider window showed somewhat less of undesirable correlation between coordination numbers (CN's) and relative Debye-Waller factors, so only the results from this window will be discussed. A summary of the results of the Type 2 fits is in Table 7.6, selected Type 3 fits are listed in Table 7.7, and results for all fits done are in Appendix III.

As in Chapter 5, fits using incorrect combinations of N and S waves as well as correct combinations were done. As expected, fits using one or two N waves were extremely poor, with either a high fit index, or unreasonable R, CN or c_2 values. Fits using one or two S waves do better. In $\text{Ni}(\text{tsalen})$ and $[\text{Ni}(\text{N}_3\text{S}_2)]$, which have two S ligated to Ni, one S wave alone could not produce a reasonable fit, that is, no fit produces a combination of R, CN and c_2 that is physically reasonable (as judged from the results in Tables 7.3 and 7.4). Only 2 S are found, and they by themselves cannot constitute a coordination shell. However, in $[\text{Ni}(\text{NS}_3^{\text{tBu}})\text{H}](\text{BPh}_4)$, which has three S ligated to Ni, 4 S at 2.22 Å gives a very reasonable fit, with a good fit index ($F = 0.492$). Again, for S+S' fits, a reasonable fit could not be obtained in $\text{Ni}(\text{tsalen})$ and $[\text{Ni}(\text{N}_3\text{S}_2)]$, but $[\text{Ni}(\text{NS}_3^{\text{tBu}})\text{H}](\text{BPh}_4)$ gave reasonable fits with a two-wave total of 3 or 4 S.

For all three compounds, reasonable N+S fits had a fit index which was 25% lower than reasonable S+S' fits. Also, distances obtained for Ni–N and Ni–S are all within 0.03 Å of the averaged crystallographic bond distances.

For $\text{Ni}(\text{tsalen})$ and $[\text{Ni}(\text{NS}_3^{\text{tBu}})\text{H}](\text{BPh}_4)$, Type 2 fits produced the correct coordination numbers (2N/2S and 1N/3S respectively), and, of the various Type 3 fits tried, the most reasonable c_2 values were also obtained when the correct combination of CN's was used. However, the most reasonable Type 3 fit did not produce the lowest fit index. A slightly (less than 5%) lower fit index was achieved by fixing a larger CN for the N wave (3N/2S and 2N/3S respectively), which also resulted in a more negative $c_{2\text{N}}$ value. Nevertheless, applying the criteria in Table 7.3 in a straightforward manner gives us the correct distances and coordination numbers.

In the case of $[\text{Ni}(\text{N}_3\text{S}_2)]$, however, the Type 2 fit gives 1.9 N and 1.9 S. There is no Type 3 fit in which the c_2 values fit within the criteria listed in Table 7.3. A fit with 2N/2S comes the closest to being "reasonable", with c_2 values just barely outside the range

given in Table 7.3 ($c_{2N} = -0.0270$, $c_{2S} = -0.0220$). The fit with the correct CN's (3N/2S) gives $c_{2N} = -0.0340$, $c_{2S} = -0.0233$, while the fit with the lowest fit index (4N/2S) gives $c_{2N} = -0.0406$, $c_{2S} = -0.0244$. All three fits have fit indices within 10% of each other. From CN and c_2 values alone, then, we can tell that there are two S in the coordination shell, but the number of N is anywhere between two and four. If we take the Ni–S bond length (2.30 Å) into account, we can eliminate an octahedral 4N/2S geometry as highly unlikely, and then the shape of the edge spectrum (which is in this case typically trigonal bipyramidal) tips the balance towards the correct answer of three N and two S (although we cannot totally eliminate the possibility of tetrahedral coordination).

In summary, Ni–N and Ni–S distances can be obtained with a high degree of accuracy (within 0.03 Å for Ni–N and 0.01 Å for Ni–S). The number of S in a coordination shell can also be ascertained with some accuracy (within 0.1 units in these compounds), but the number of N was less accurate (being within 0.1 units for Ni(tsalen) and $[\text{Ni}(\text{NS}_3^{\text{tBu}})\text{H}](\text{BPh}_4)$, but off by 1 unit for $[\text{Ni}(\text{N}_3\text{S}_2)]$). It can be seen from the fits to $[\text{Ni}(\text{N}_3\text{S}_2)]$ that static disorder in the bond distances raises the Debye-Waller factor considerably, so that we have to exercise care in using Table 7.3 to eliminate possible geometries, especially with regard to N coordination. The c_2 values in Table 7.3 can be used to set a lower bound for the possible number of N or S coordinated to Ni. The bond distances in Table 7.4 can also be used to confirm or eliminate octahedral coordination. There is less difference in Ni–N and Ni–S bond distances between four and five-coordinated compounds of various geometries, but the variation in distances (together with the appearance of the edge) can still be useful in judging the more likely geometry.

(c) Testing for the Presence of Fe Using $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$

Since a major question about the Ni site in CODH is whether it is part of a cubane, we carried out EXAFS analysis on such a cubane, namely $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$ (**11**). For a more complete comparison with the fits done on the protein data (*vide infra*), reverse Fourier transforms were calculated including only the two major peaks ($R' = 1.37\text{--}2.85$ (0.1) Å — Window 2) and also including the two major peaks with a substantial shoulder to lower R ($R' = 1.00\text{--}2.85$ (0.1) Å — Window 3). S+Fe, S+S'+Fe and N+S+Fe fits were carried out on data from both backtransforms. A summary of selected fits is given in Table 7.8, with the complete results being listed in Appendix III. Results of fits to Window 2 are discussed first, then compared with results from Window 3.

S+Fe fits show that $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$ (**11**) has more disorder in Ni–scatterer distances than $(\text{Et}_4\text{N})_2[\text{NiFe}_3\text{S}_4(\text{PPh}_3)(\text{SEt})_3]$ (**10**) (from which Ni–Fe parameters were

extracted), showing only 2.3 Fe at a longer distance (2.75 Å vs. 2.689 Å). Unfortunately, no crystal structure of (11) is available with which to compare this result, which seems to indicate that (11) is a slightly less rigid or regular cubane than (10). A Type 3 fit of (11) with 4S/3Fe gives $c_{2\text{Fe}} = -0.0184$. The first shell (i.e., the scatterers directly coordinated to Ni) is also somewhat disordered, since a Type 2 fit gives 2.9 S, rather than 4 S, at 2.25 Å. A certain amount of disorder is expected, since the Ni–S(Et) distance is certainly different from the Ni–S(cubane) distance.

We hoped to distinguish the Ni–S(Et) and the Ni–S(cubane) bond distances with three-wave S+S'+Fe fits. We obtained two minima. One had S and S' at exactly the same distance (2.25 Å) with a total CN of 2.9, being in effect the same as the two-wave S+Fe fit. The other minimum had 3.5 S at 2.26 Å and 1.0 S' at 2.47 Å. It is possible that Ni–S(Et) is 2.47 Å long, and that we really are seeing the two Ni–S distances present in this compound. However, as Table 7.4 shows, the typical Ni–S bond in a tetrahedral compound is 2.25–2.30 Å long, so Ni–S(Et) would then be unusually long.

The N+S+Fe fits were carried out to make a comparison with the N+S+Fe fits done on the protein data (*vide infra*). We wished to see whether the N detected in the protein EXAFS spectra is real, or whether it is just filling in spaces, as it would be in fits to $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$. The N+S+Fe fits have higher fit indices than the S+S'+Fe fits. In these fits, the N-wave correlates with the S-wave, so that S is shifted to shorter distance (2.7 S at 2.22 Å), and N makes a rather large contribution (3.7 N at 2.16 Å). The Fe-wave, being at much longer distance, is not affected by this correlation. In the corresponding Type 3 fit with 2N/3S/1Fe, the N-wave shifts to 2.26 Å, but it is clear from the too-low c_2 values ($c_{2\text{N}} = -0.0140$, $c_{2\text{S}} = -0.0153$) that the same type of correlation is going on.

Results from fits on Window 3 were very similar to those from Window 2. In general CN's for S and N were a little higher, but not to any significant degree, and CN's for Fe were very slightly lower. In the S+S'+Fe fits, starting from the same initial values as in Window 2, we did not obtain the minimum wherein the two S waves go to exactly the same R. For both initial values, the other minimum, with 3.7 S at 2.26 Å and 1.2 S' at 2.47 Å, was obtained. (CN_S = 3.7 is rather high, since we expect to find only 3 S(cubane) atoms. This may indicate that some correlation occurs between the two S-waves.) The N+S+Fe fits once again did not match the data as closely as the S+S'+Fe fits. They also behaved in the same way as for Window 2, with the Type 2 fit giving 4.4 N at 2.19 Å and 3.4 S at 2.22 Å. The shift in R_{N} in the Type 3 fit with 2N/3S/1Fe is less dramatic this time, with $R_{\text{N}} = 2.23$ Å. The $|c_2|$ values are still too low ($c_{2\text{N}} = -0.0184$, $c_{2\text{S}} = -0.0171$), but they

are closer to the reasonable values set forth in Table 7.3. Thus, while the Type 2 fit would suggest that Window 3 contains more N than Window 2, the Type 3 fit suggests the opposite. We conclude that there really is the same amount of "N-wave contribution" in both windows.

In summary, fits to $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$ all show a strong Fe contribution, as expected for fits to a cubane, even though the contribution has been lessened by disorder in the Ni-Fe distances. Results from fitting Fourier Windows 2 and 3 turned out to be very similar. It is rather disconcerting that such a large N-wave should be obtained when no N is in fact present, but its effect on the S-wave, and the long Ni-N distance, are signs that it is not modelling any real presence of N ligands. Neither the number of S present, nor the number of N found, was much affected by the inclusion or exclusion of the "left shoulder of the main peak". It is also important to note that the N+S+Fe fits do not fit the data as closely as the S+S'+Fe fits in both windows. This is significant when compared with the different results obtained by including the "left shoulder" of the main peak in CODH data.

(2) EXAFS Analysis of *Rhodospirillum rubrum* Ni CODH Data

The EXAFS spectra for the five protein data sets are shown in Figure 7.10. It can be seen that the data for all the oxidized samples, and for CDHRD, have a beat node at $k \sim 7-8 \text{ \AA}^{-1}$. However, the EXAFS of CODHRX is qualitatively different, being much larger and not showing the beat node, although the presence of more than one shell of scatterers is shown by shoulders in the EXAFS. The Fourier transforms for the five data sets (Figure 7.11) show that CODHRX does indeed have a much taller peak than the other data sets, and that it alone also has three peaks above the noise at $R' = 3-5 \text{ \AA}$. However, all five Fourier transforms can be considered in terms of one main peak with "shoulders" on either side. These shoulders can be quite large compared with the main peak, and they are not in most cases easily separable from the main peak. Since we were not sure what contribution these shoulders might make to the EXAFS of the coordinating shell of Ni, various Fourier windows were used, both including and excluding the shoulders on either side, to obtain backtransformed data for curvefitting (see Table 7.9 and Figures 7.12 and 7.13). In particular, CODHX, the first data set to be collected and analyzed, shows a large, well-separated peak to the left of the main peak, which could not be reduced by splining. This peak was analyzed separately, as well as together with the main peak, and parallel fits were also done on the other data sets. The types of fits done for each window are shown in Table 7.10.

Results of some Type 2 and Type 3 fits are shown in Tables 7.11 and 7.12 respectively, and complete fit results are listed in Appendix III. Once again, for N+S and S+S' combinations, several Type 3 fits were made, exploring several possible combinations of N-type and S-type ligation. Again, Type 3 fit results generally agreed with those of the Type 2 fits, and also showed how shallow or deep the minimum was for numbers of N and S coordinated. Some variation in the Ni-X distance (usually Ni-N) with differing contributions of N and S was observed. Those fits for which the Ni-X distance differs by more than 0.02 Å from the corresponding Type 2 fit are considered to be less sound. These fits always also have c_2 values that are well outside the range set forth in Table 7.3. Generally, discussion of results will first be in terms of Type 2 fits, followed by additional insights from Type 3 fits, if any.

When using the fit index F (defined in Chapter 2) to evaluate the goodness of a fit to the data, we have to bear in mind that the fit index is not adjusted for the size of the signal. Thus, for equally good fits, we would expect the fit indices for fits to CODHRX to be larger than those for fits to the smaller signals in other data sets. The fit index is also affected by the noise level in a data set. So the fit index should only be used to compare different fits made on the same data. However, it can be seen from comparing Figures 7.14 and 7.15 (and also Figures 7.18 and 7.19) that the fits to CODHRX do in general achieve a worse match in phase and amplitude than corresponding fits in the other data sets.

(a) Results of One-Wave Fits

One-wave fits were performed on data from Windows 1 and 2. No attempt was made to fit data from Windows 3 or 6 with one wave because these had a beat node in the EXAFS (see Figure 7.13), and one wave clearly would not give a good fit.

Even though the Fourier transform peak of CODHX Window 1 looked quite different from that for the other data sets, when curvefitted with N or S all five data sets gave much the same result : either 1 N at about 1.9 Å, or less than 0.5 S at about 1.6 Å. In all cases it was obvious that the parameters did not match the signal in the backtransform at all, either in phase or in amplitude, and so this peak really does not contain either an N-type or an S-type wave. And so, even for CODHX, it is clear that the "first peak" or "left shoulder" cannot stand on its own.

For one-wave fits to data from Window 2 (see Table 7.11), N fits fared poorly, but S fits were able to give a fairly close fit of the data. Between 1.3 S and 1.8 S at 2.23 Å or 2.24 Å were obtained, except for CODHRX, which had 2.3 S at 2.25 Å. This suggests that the "main peak" does contain S.

(b) Results of Two-Wave Fits to Window 2

We turn to two-wave fits for a better indication of whether the "main peak" in Window 2 can account for a complete coordination of Ni by itself. N+N', S+S' and N+S fits were done on this window.

N+N' could not achieve a close fit of the data, and the fits achieved often had a total coordination number of more than six, which is chemically unlikely.

Of the two-wave fits tried, S+S' fits achieved the closest match to the Window 2 data. One of the S-waves was found at about the same distance (2.24–2.25 Å) and slightly higher CN (1.5–2.1 S, 3.1 S for CODHRX) as in the one-wave S fit. However, in all five data sets, the other S-wave was found at $R < 2.1$ Å. This is true for all Type 3 fits with reasonable c_2 values as well as for Type 2 fits. Since no Ni–S bond length of less than 2.1 Å has been found even for Ni^{IV}–S compounds (see Table 7.4), we reject S+S' as being a physically reasonable fit of the data.

In the N+S fits, two initial distances for N were tried: 2.1 Å and 1.9 Å. In the former case, the coordination number for N always refined to a negative value, so these fits will not be considered further. In the latter case, the S wave reached more or less the same R and CN as it did in the S only fit, and the contribution of the N wave varied between 0.4 N to 0.7 N (1.1 N for CODHRX). Except for CODHX, the N wave was at 1.89–1.91 Å. In CODHX, N was at 1.82 Å, which is shorter than Ni–N distances found for any model compound (see Table 7.4). In this case, especially when compared with fits done on other windows of CODHX (*vide infra*), it is doubtful that this represents a true N-wave. For CODHOX, the Type 3 1N/2S fit has a Ni–N distance of 1.84 Å (see Table 7.12), which is substantially different from the result for the Type 2 fit (0.4 N at 1.91 Å), and so it is also doubtful that this window contains a true N-wave. For the other three samples, some component of a true N-wave may be present. For CDHOX and CDHRD, the total coordination adds up to 1N and 2S, which is not quite sufficient for a complete coordination shell. For CODHRX, we observe 1N and 3S, which may indeed comprise a complete coordination shell.

(c) Results of Two-Wave Fits to Window 3

Window 3 includes both the major peaks in CODHX or, in the other data sets, the main peak plus its left shoulder. Again, N+N, S+S' and N+S fits were done on the data.

N+N' fits were actually able to achieve the closest fits to the data in the cases of CODHX and CODHOX (see Figure 7.14). However, these fits had total CN's of 11 (CODHX) or 8 (CODHOX), so they are not physically reasonable. For the other three data

sets, N+N' had the worst match to the data (see Figure 7.15), and total CN's ranged from 9 to 12. Once again, N+N' may be dismissed as physically implausible for the coordination shell of Ni.

In contrast to Window 2, S+S' fits were generally a poorer match to the data than N+S fits (see Figure 7.14). The one exception to this is CODHRX, where S+S' provides a slightly (15–20%) better match (see Figure 7.15). However, for all five data sets we again find that while one of the S-waves is at 2.25–2.27 Å, the other is always found at < 2.1 Å, both in Type 2 fits and in Type 3 fits with reasonable c_2 values. So S+S' is again not a reasonable coordination shell for this Fourier window.

N+S fits are the only ones wherein the R, CN and c_2 values of both waves used are all physically reasonable at the same time. With the exception of CODHRX, these fits also provide the closest match to the data. Again, CODHRX is different from the other data sets in that it shows a contribution of 3 S (2.8 S at 2.25 Å), while the rest show 2 S (2.0–2.1 S at 2.23–2.24 Å). (CODHOX shows 1.6 S, but a 1N/2S Type 3 fit gives $c_{2S} = -0.0252$, which is more reasonable than $c_{2S} = -0.0162$, which was obtained using 1N/1S.)

The contribution from the N-wave is rather weak by comparison, and so it is harder to firmly establish the number of N coordinated to Ni. It is possible that the presence of N is merely an artifact of the Fourier transform, an effect of including the left shoulder of the main Fourier peak in the backtransform. However, the tests done on $(Et_4N)_3[NiFe_3S_4(SET)_4]$ (*vide ante*), with Fourier windows both excluding and including the left shoulder, showed that where no N's were ligated to Ni, no extra N's were found when the left shoulder was included in the transform. On the other hand, we found in all the CODH data sets that the amount of N-wave contribution was doubled or tripled when the left shoulder was included. We believe that this indicates the real presence of Ni–N coordination at ≤ 1.9 Å.

Our analysis also suggests a reason for the large, well-separated first peak in the CODHX Fourier transform. The Ni–N distance in CODHX was found to be 1.85 Å, while for the other data sets it was 1.89 Å (1.87 Å in CDHRD). Perhaps a greater spacing between the N and S distances in CODHX is the cause of the more pronounced beat pattern in the EXAFS of CODHX, which manifested itself as the split between the two peaks when the Fourier transform was applied.

In CODHX, we found 2.1 N at 1.85 Å. In the other two oxidized samples, we found 1.3 N at 1.89 Å. In Type 3 fits (Table 7.12), 2N/2S gave $c_{2N} = -0.0216$ for CODHX and $c_{2N} = -0.0314$ for both CODHOX and CDHOX. A Type 3 fit with 1N/2S gave $c_{2N} =$

-0.0219 and $c_{2N} = -0.0218$ for CODHOX and CDHOX respectively. Yet the edges of all three samples look very similar, so we do not expect a major change in the Ni geometry. It could be argued that, since the Ni–N bond length for CODHX (1.85 Å) is 0.04 Å shorter than those for CODHOX and CDHOX (1.89 Å), we would expect the bond to be stiffer and the c_2 value to be less negative for CODHX. This would allow us to interpret the low c_{2N} magnitude of -0.0216 as reasonable for CODHX. We may also further argue, based on Table 7.4, that a Ni–N distance of 1.85 Å is extremely unlikely with five-coordinate ligation, which we would have to accept if we concluded that 3 N's were present in CODHX. To accept a value of $c_{2N} = -0.0314$ as reasonable for the other two oxidized samples, however, we would also have to postulate a certain amount of static disorder in the Ni–N distances. If all this is true, then we can reconcile the differences in the EXAFS of these three samples with the similarity in their edges, and conclude that the most likely ligation for oxidized *R. rubrum* Ni CODH is two nitrogens and two sulfurs.

Reconciliation of edge and EXAFS data is even more difficult when the reduced CODH samples are considered. Besides the difference in the number of S coordinated, we find in CODHRX 1.9 N at 1.89 Å, while in CDHRD we find only 1.2 N at 1.87 Å. A Type 3 fit to CODHRX with 2N/3S gives $c_{2N} = -0.0245$, while a fit to CDHRD with 1N/2S gives $c_{2N} = -0.0242$. Yet the edges of both samples are almost identical, not only with each other, but with the edges of all the oxidized samples. In this case, it is not possible to reconcile this discrepancy, and so we have no definite conclusion about the numbers of N or S coordinated to Ni in reduced CODH from EXAFS analysis.

Instead, we have to conclude that either CODHRX or CDHRD is not a good reduced Ni CODH sample. Since CDHRD has exactly the same edge (see Figure 7.7), and also almost exactly the same EXAFS results as CDHOX, it may be that CDHRD is in fact an oxidized sample of Ni CODH. In this case, then, there is a real change in the Ni environment upon reduction, with Ni now being coordinated to 2 N at 1.89 Å and 3 S at 2.25 Å. It is amazing, though, that the addition of one sulfur ligand (or two, *vide infra*) should have made so little difference to the Ni edge of CODHRX. Alternatively, it may be CODHRX that is unreliable — perhaps denatured — and if so then the Ni site in Ni CODH shows no change upon reduction that is perceivable by EXAFS spectroscopy. A third reduced CODH sample, redox-characterized by optical or EPR spectroscopy, should be measured in the future to resolve this problem.

(d) Results of Two- and Three-Wave Fits to Window 6

The Fourier backtransform Window 6 includes the main peak, with both left and right shoulders. Since fits to Window 3 sufficiently proved N+S as the only plausible ligation for all data sets, N+N' and S+S' fits were not repeated on Window 6. However, N+S fits were carried out to see if including the right shoulder would have any effect on the coordination numbers or distances for N or S. As Table 7.11 shows, there is no significant difference in R's or CN's for either N or S. The fit indices are a little higher, but that is normal when more data are included in a Fourier window.

Three-wave fits to Window 6 were made in order to search for any Fe present within 3 Å of Ni, specifically in the "right shoulder" of the main peak, which is quite large for CODHOX, CDHOX and CDHRD (see Figure 7.11). It is pretty obvious, simply from visual inspection of the EXAFS and Fourier transforms of $(\text{Et}_4\text{N})_2[\text{NiFe}_3\text{S}_4(\text{PPh}_3)(\text{SEt})_3]$ and $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$ compared with those of any CODH data set (Figures 7.16 and 7.17), that Ni in *R. rubrum* CODH does not reside in an NiFe_3S_4 cubane. This is confirmed by the results of fits done on $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$, where at least 2 Fe were always found (*vide ante*), and the fits following. Note that, except for CODHRX, Window 6 does not include data above $R' = 2.6$ Å, meaning that we should not expect scatterers found at distances above 2.7 Å to have much physical meaning. The next peak at higher R in the Fourier transforms was not included because in most of the data sets this peak was barely above noise (see Figure 7.11).

N+S+S', N+S+Fe and N+S+N' fits were performed (see Figures 7.18 and 7.19). The N+S+S' and N+S+N' fits are control fits, to estimate the significance of "finding" any Fe with the N+S+Fe fits. In evaluating all these fits, we do not have any firm criteria for the maximum likely c_2 value for N' and S', since we have no model data for "second-shell" N or S. Since CODHRX has a right shoulder that is much less distinct than those for the other data sets, the results for it differ from the results for the rest.

With the N+S+S' fits, it was possible to obtain a minimum which was basically the same as the N+S fit, with the two S waves at the same distance having a total S coordination of the same magnitude as in the N+S fit. This fit is not shown in Table 7.11 or 7.12 (except for CODHX), since it adds no new information. The other minima that were obtained had N+S at about the same R's and CN's as in the two-wave N+S fits, with S' occurring at various distances. In all five data sets, 0.3–0.5 S' could be found at ~2.9 Å. In other fits, it was also possible to find about 0.4–0.6 S' at 2.43–2.48 Å (CODHOX and CDHOX) or 0.8 S' at 2.62 Å (CODHRX), or 0.7 S' at 2.16 Å (CDHRD).

The minimum with S' at ~ 2.9 Å was the closest fit to the data in the cases of CODHX, CDHOX and CDHRD. For CODHOX and CODHRX S' at ~ 2.4 – 2.6 Å produced a closer fit. However, in all the data sets except CODHRX S' at ~ 2.9 Å was better able to fit the right shoulder of the main peak (see Figure 7.20, top). For CODHRX, the best fit was obtained with S' at 2.6 Å (Figure 7.20, bottom). Considering both Type 2 and Type 3 fits, another minimum can be obtained in the reduced CODH data sets, in which the 2 S-waves correlate and one of them refines to $R \leq 2.18$ Å, while the other is found at $R \sim 2.3$ Å. CODHRX and CDHRD also have one more minima in the Type 3 fits: in CODHRX, S' finds a minimum at 3.23 Å (but the fit to the data is poor), while in CDHRD, S' finds a minimum at 2.31 Å, with $c_2 = -0.0612$, which means that it makes essentially no contribution to the EXAFS signal.

In the N+S+Fe fits, two minima were again found. In both cases, N+S had the same R's and CN's as they did in the two-wave N+S fits, and were essentially unaffected by the location of the Fe-wave. The closer fit was achieved with 0.3 – 0.4 Fe at 2.70 – 2.76 Å (except for CODHRX, with 0.6 Fe at 2.80 Å). This minimum also produced the better fit of the right shoulder of the main peak (see Figure 7.21, top), except in CODHRX, where both minima fail to achieve a good fit to the right shoulder (Figure 7.21, bottom). Type 3 fits with 1 Fe gave $c_{2\text{Fe}}$ values between -0.0256 and -0.0333 (except $c_{2\text{Fe}} = -0.0184$ for CODHRX). These values are well above those obtained with the NiFe_3S_4 cubanes (where $c_{2\text{Fe}} = -0.0153$ to -0.0194 , from both (10) and (11)). However, since a single Fe connected by a single bridge to Ni would not be in as rigid a structure as Fe's in a cubane, these c_2 values are not improbable. Another minimum, with 0.1 – 0.2 Fe (0.6 Fe for CODHRX) at 3.06 – 3.09 Å, could also be obtained. Not surprisingly (since this is outside the range included in Window 6, except for CODHRX), the contribution of Fe is so slight as to be below the noise level (except in CODHRX), and also this fit does not manage to reproduce the right shoulder of the main peak in the Fourier transform (except, again, in CODHRX, where it actually produces a slightly better match in this region).

N+S+N' fits were carried out because N-waves are known to have phase shifts very similar to Fe-waves, and so it is easy to mistake one for the other when curvefitting. In this case, the N-wave parameters were so similar to the Fe-wave parameters that N+S+N' fits were almost indistinguishable from N+S+Fe fits. The N and S waves in these fits gave almost exactly the same R's, CN's and c_2 's in corresponding fits. The CN's for the N'-wave were of course higher than those for the Fe wave, since N' is a lower Z scatterer; about 3 N's correspond to 1 Fe. We thus found a minimum with 1.1 – 1.3 N' at 2.71 – 2.76 Å, and another with 0.2 – 0.5 N' at 3.09 – 3.11 Å. (CODHRX is again different from the

rest, with 1.2 N' at 2.81 Å and 1.3 N' at 3.09 Å respectively, so that only 2 N's correspond to 1 Fe.)

Comparing the three classes of fits overall, we find that N+S+S', N+S+Fe and N+S+N' achieved comparably close fits to the data (as shown by the fit indices), except for CODHRX, where N+S+S' fitted a little more closely (see Tables 7.11 and 7.12).

Thus, for the four data sets giving similar results, while there may very well be 1 Fe at ~2.7 Å, there may equally well be 1 S at ~2.5 Å or more likely ~2.9 Å (since this fits the "right shoulder"), or several N at ~2.7 Å. Second-shell N-type scatterers (as, for instance, in imidazole rings) usually occur at 2.9–3.1 Å. Ni is most probably four-coordinate and thus would not have any ligating S further away than 2.3 Å (though we cannot rule out an unusual structure such as that found in plastocyanin, see Chapter 5). At ~2.7 Å, therefore, Fe is chemically the most plausible scatterer. Since N or Fe at ~2.7 Å rather than ~3.1 Å accounts better for the "right shoulder" in the Fourier transforms, and it is more likely that we see Fe than N at 2.7 Å, it is possible that 1 Fe is present in Ni CODH at ~2.7 Å from Ni. However, this conclusion is tentative.

For CODHRX, the best three-shell fit is N+S+S', with S' at 2.61–2.62 Å. Even so, this three-shell fit does not match the data as closely as the three-shell fits for the other data sets. However, if we accept this as the most plausible fit, then we have six-coordinate (Ni-N₂S₄) ligation, in which five of the bonds (Ni–N = 1.88–1.89 Å, Ni–S = 2.25 Å) are much shorter than Ni–N or Ni–S bonds in any octahedral model compound surveyed (see Table 7.4). It does not seem likely that so many ligands could crowd within such a short distance from Ni, and furthermore it is unlikely that a change from four-coordinate to six-coordinate geometry should have so little impact on the appearance of the edge. This suggests that, of the two reduced samples, it is CODHRX that is unreliable.

(e) Fits to the Fourier Peaks at R' = 3–5 Å From Ni in CODHRX

Since one possible model for the Ni site in *R. rubrum* CODH involves Ni bridged to a Fe₄S₄ cubane, we investigated whether the three peaks seen at R' = 3–5 Å in CODHRX might be due to backscattering from atoms in a cubane-like array.

Each peak was individually windowed and backtransformed (R' = 2.85–3.60 (0.1) Å — Window 7, R' = 3.52–4.20 (0.1) Å — Window 8, R' = 4.10–5.07 (0.1) Å — Window 9) and all three peaks were also included in a wide backtransform (R' = 2.85–5.07 (0.1) Å — Window 10). Fits using S-waves and Fe-waves were carried out on all four windows. (Fits using N-waves were not performed because N-waves and Fe-waves have such similar phase shifts.) Since the S and Fe parameters are being used at distances far larger

than the distances in the compounds from which they were extracted, the results from these fits are not definitive. At such distances, the CN values obtained would be only a rough indication of the presence or absence of S or Fe. The intrinsic EXAFS backscattering from atoms at longer distances is less than from shorter distances, so that CN values found would be lower than the actual number of scatterers present (e.g. 1 S found at 4 Å would suggest the presence of more than 1 S at that distance, but, without calibration on models of known structure, we cannot tell how many there in fact are). Results for some of these fits are shown in Table 7.13, and listed in full in Appendix III.

On each of Windows 7–9, fits were done using an S-wave only, an Fe-wave only, and S+Fe. In all cases, whether one- or two-wave, the parameters were able to achieve a fair-to-good match to the phase of the data above $k \sim 8 \text{ Å}^{-1}$, where the amplitude was greatest, but at lower values of k the phase match was worse, being quite poor in some cases. In the one-wave fits, R_S was usually $\sim 0.2 \text{ Å}$ longer than R_{Fe} . In the S+Fe fits, it was possible to obtain two minima, one with R_S about the same as R_{Fe} and the other with R_S being $\sim 0.2 \text{ Å}$ longer than R_{Fe} .

On Window 7, S and Fe parameters both performed about as well as each other on the data, and combining them did not solve the problem of poorly matching phases at lower k . This suggests that Window 7 does not in itself completely contain either S or Fe, but does have elements of both, at $\sim 3.5 \text{ Å}$ (both S and Fe possible) or $\sim 3.7 \text{ Å}$ (for S). On Window 8, there were still problems with phase matching at lower values of k , but the S-wave did match the data better than the Fe-wave, and so we may conclude that Window 8 probably does contain the equivalent of 1.5 S at 4.19 Å, and not much Fe (particularly since CN_{Fe} becomes negative in one of the S+Fe fits). On Window 9, the Fe-wave matched the data much better than the S-wave, so in this case it is obvious that the dominant signal is an Fe-type signal, with the equivalent of 2.3 Fe at 4.87 Å.

On Window 10, only Type 2 fits were carried out. Between three and six waves were used at one time. When so many waves are used to fit EXAFS data, the risk of correlation between different waves is high, and, once again, the results should not be regarded as conclusive. Not all the possible combinations were explored, but, of the fits tried, we can make the following observations. We found five waves to be the minimum necessary to reproduce all the oscillations in the data. These had either S or Fe in the region of the first peak and both S and Fe for each of the other two peaks. Two solutions obtained were : (0.5 Fe at 3.54 Å, 1.8 S at 4.18 Å, 0.6 Fe at 3.83 Å, 1.1 S at 4.65 Å, 1.8 Fe at 4.88 Å) and 0.8 S at 3.73 Å, 2.0 S at 4.17 Å, 1.1 Fe at 3.82 Å, 1.0 S at 4.66 Å, 1.9 Fe at 4.87 Å). The latter is a slightly closer fit to the data.

To see whether S's and Fe's at the above distances could be due to a cubane, we used the crystal structure coordinates²¹ for $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$ as a model Fe_4S_4 cubane. Ni was attached by a bond 2.21 Å long to one of the S from SPh, such that the Ni–S–Fe angle was 103° and Ni–Fe was 3.52 Å. Ni was then rotated around the cubane by rotating the S–Fe bond (Figure 7.22). With Ni in various positions, the distances from Ni to the other Fe's and S's in the cubane were then measured. Apart from the Fe fixed at 3.52 Å, the other Fe's in the cubane were found at distances ranging from 4.7 Å to 6.3 Å, and the cubane S's at distances between 3.4 Å and 5.7 Å (not including the apical S furthest from Ni, which is at 6.7–7.2 Å from Ni), depending on the orientation of the cubane towards Ni.

This exercise makes it obvious that the Fe_4S_4 cubane is far too large a moiety to fit within the three high-R Fourier peaks in CODHRX. It is possible that we are seeing EXAFS signals from only one face of such a cubane. At certain orientations, our model shows as many as 2 Fe and 2 S within $R = 3.5\text{--}4.9$ Å. However, no orientation of the model shows 2 S at 4.2 Å or 2 Fe at 4.9 Å (these being the most stable waves in the fits to Window 10); at most only one atom occurs at each of these distances.

We may thus conclude that the Fourier peaks observed at $R' = 3\text{--}5$ Å are probably not due to an Fe_4S_4 cubane bridged to Ni through a terminal S, such as is shown in Figure 7.1(b), and probably not even due to two such cubane units. The origin of the Fourier peaks is in any case doubtful, since this sample shows contradictory results for its edge and its Ni coordination shell.

(F) Conclusion

Edge and EXAFS investigations of five samples of *Rhodospirillum rubrum* Ni CODH were carried out. Results from four of these samples (three oxidized and one of two reduced samples) show consistently that the nickel in the active site is most probably coordinated with two nitrogens (or oxygens) at 1.85–1.89 Å and two sulfurs at 2.23–2.25 Å, in a geometry that is considerably distorted from planarity, perhaps pseudo-tetrahedral. If this reduced sample is truly reduced, these four samples suggest that there is no significant change in ligation upon reduction of Ni CODH. Data from the other reduced sample were best fitted with two nitrogens (or oxygens) at 1.89 Å, but three sulfurs at 2.25 Å and a fourth sulfur at 2.61 Å. Most of these Ni–ligand distances are extremely short for a six-coordinate compound and suggest that this sample is unreliable. Otherwise, there is a major change in Ni ligation upon reduction of Ni CODH.

This, however, would not be consistent with the Ni and Fe K edges, which show no significant change with redox state, suggesting not only that the Ni ligation remains the

same, but also that there is extensive delocalization of electrons throughout the Ni/Fe/S cluster. The latter is also consistent with the "smeared-out, low-resolution" appearance of the Ni edge. The Fe edge of CODH shows a strong resemblance to the Fe edge of a NiFe_3S_4 cubane, confirming the presence of Fe_4S_4 -type moieties in the protein.

Leaving aside the reduced sample that is most likely unreliable, edge and EXAFS results together suggest that the oxidation state and coordination shell of Ni do not change upon reduction or oxidation.

It is established beyond doubt that Ni is not part of an NiFe_3S_4 cubane. There is a possibility that 1 Fe was detected at $\sim 2.7 \text{ \AA}$ in four of the samples, but this is by no means certain. It was found that a group of peaks at $R' = 3\text{--}5 \text{ \AA}$ in the Fourier transform of one of the samples (whose reliability is doubtful) could not be fitted by modelling an Fe_4S_4 cubane ligated to Ni through a thiolate S. Thus, EXAFS analysis gives no firm evidence for an Fe_4S_4 cubane or two near Ni (as suggested by EPR and other studies), but does not preclude their existence.

Studies on more samples of Ni CODH, especially in the reduced state, would be desirable, to resolve the discrepancies that we have found. It would also be extremely interesting to interpret the edge and EXAFS spectra of Ni CODH with CO or CN^- bound to the site. Ni edges have many intriguing features, and these would also be worth investigating in the future.

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Table 7.1. Samples of Ni CODH on which data were collected (does not include one sample collected in April 1990, which was not analyzed).

Data Set Name	CODHX	CODHOX*	CDHOX	CODHRX*	CDHRD
Redox State	oxidized	oxidized	oxidized	reduced	reduced
[Ni ²⁺]	0.85 mM	1.4 mM	1.5 mM	1.4 mM	1.5 mM
Buffer	50 mM Mops/D ₂ O with 1 mM indigo carmine, pH 7.5	100 mM Mops, pH 7.5, in 10% glycerol	25 mM Mops, pH 7.5, 15% glycerol, 400 mM NaCl	100 mM Mops, pH 7.5, in 10% glycerol, 5 mM Na ₂ S ₂ O ₄	25 mM Mops, pH 7.5, 15% glycerol, 400 mM NaCl, 10 mM Na ₂ S ₂ O ₄
Activity [†]	~4000 units/mg	5200 units/mg	3600 units/mg	5200 units/mg	3600 units/mg
Date measured	June 1989	July 1991	July 1992	July 1991	July 1992
Beam Line	NLS X19A	SSRL 7-3	SSRL 7-3	SSRL 7-3	SSRL 7-3
Ring Current	92-170 mA	24-39 mA	50-92 mA	19-52 mA	38-77 mA
Good scans	6 edge 16 EXAFS	10 edge 29 EXAFS	11 edge/EXAFS 20 EXAFS	13 edge 34 EXAFS	30 edge/EXAFS
(9 good detectors)					

* — Fe K edges were also measured, to $k = 11 \text{ \AA}^{-1}$.

† — Activity was measured in $\mu\text{mol CO oxidized per minute per mg protein}$

Table 7.2. Ni model compounds used in this study (continued on next page).

No.	Short name	Full name	Ni ligation	Supplier	Ref.
1	K ₂ Ni(CN) ₄	Potassium tetracyanonickelate(II)	C ₄	commercial	23
2	Ni(dmegly) ₂	Bis(dimethylglyoximate)nickel(II)	N ₄	commercial	24,25
3	Ni(corphin)	Nickel(II) <i>cccc</i> -octaethylpyrrocorphinato	N ₄	Scott	26,27
4	[Ni(TC-6,6)]	[Ni(tropocoronand-6,6)]	N ₄	Scott	28
5	Ni(N ₂ S ₂ C ₂)	[<i>N,N'</i> -ethylenebis(methyl-2-amino-1-cyclopentene-dithiocarboxylato)]nickel(II)	N ₂ S ₂	Bereman	29
6	Ni(N ₂ S ₂ C ₃)	[<i>N,N'</i> -trimethylenebis(methyl-2-amino-1-cyclopentene-dithiocarboxylato)]nickel(II)	N ₂ S ₂	Bereman	29
7	Ni(N ₂ S ₂ C ₄)	[<i>N,N'</i> -tetramethylenebis-(methyl-2-amino-1-cyclopentene-dithiocarboxylato)]nickel(II)	N ₂ S ₂	Bereman	29
8	Ni(tsalen)	[<i>N,N'</i> -ethylenebis-(thiosalicylidenaminato)]nickel(II)	N ₂ S ₂	Holm	30
9	Ni(ebmiba)	[<i>N,N'</i> -ethylenebis-(<i>o</i> -mercaptobenzylaminato)]nickel(II)	N ₂ S ₂	Holm	31
10	(Et ₄ N) ₂ [NiFe ₃ S ₄ (PPh ₃)(SEt) ₃]	(Et ₄ N) ₂ [NiFe ₃ S ₄ (PPh ₃)(SEt) ₃]	PS ₃	Holm	32,33
11	(Et ₄ N) ₃ [NiFe ₃ S ₄ (SEt) ₄]	(Et ₄ N) ₃ [NiFe ₃ S ₄ (SEt) ₄]	S ₄	Holm	32
12	[Ni(SPh) ₄] ²⁻	[(C ₆ H ₅) ₄ P] ₂ Ni(SC ₆ H ₅) ₄	S ₄	Scott	34
13	(Bu ₄ N) ₂ [Ni(mnt) ₂]	((C ₄ H ₉) ₄ N) ₂ [Ni(maleonitriledithiolate) ₂]	S ₄	Scott	35
14	Ni(dedtc) ₂	Bis(<i>N,N'</i> -diethyldithiocarbamate)-nickel(II)	S ₄	Hodgson	36
15	[Ni(SS ₂) ₂]	Di-μ-(bis-2-mercaptoethyl sulfide)-dinickel(II)	S ₄	Hodgson	37,38
16	[Ni(N ₃ S ₂)]	[Ni(terpyridine)(S-2,4,6-(<i>i</i> -Pr) ₃ -C ₆ H ₂) ₂]-1.5CH ₃ CN	N ₃ S ₂	Hodgson	39
17	[Ni(N ₂ S ₃)]	Ni[S ₂ P(OMe) ₂] ₂ (2,9-dimethyl-1,10-phenanthroline)	N ₂ S ₃	Hodgson	40

Bereman — Prof. Robert D. Bereman of North Carolina State University supplied these compounds

Holm — Prof. Richard H. Holm of Harvard University supplied these compounds

Scott — Prof. Robert A. Scott of the University of Georgia at Athens supplied data for these compounds

Hodgson — Synthesized by Grace Tan in the Hodgson Group

Table 7.2. Ni model compounds used in this study (continued)

No.	Short name	Full name	Ni ligation	Supplier	Ref.
18	[Ni(NS ₃ ^t Bu)H] (BPh ₄)	{Ni ^{II} [N(CH ₂ CH ₂ S- <i>t</i> -Bu) ₃]H} (BPh ₄)	NS ₃ H	Holm	22
19	[Ni(NS ₃ ^t Bu)Me] (BPh ₄)	{Ni ^{II} [N(CH ₂ CH ₂ S- <i>t</i> -Bu) ₃]Me} (BPh ₄)	NS ₃ C	Holm	22
20	[Ni(NS ₃ ⁱ Pr)COMe] (BPh ₄)	{Ni ^{II} [N(CH ₂ CH ₂ S- <i>i</i> -Pr) ₃]COMe} (BPh ₄)	NS ₃ C	Holm	22
21	[Ni(NS ₃ ^t Bu)CO] (BPh ₄)	{Ni ^{II} [N(CH ₂ CH ₂ S- <i>t</i> -Bu) ₃]CO} (BPh ₄)	NS ₃ C	Holm	22
22	[Ni(NS ₃ ^t Bu)Cl] (BPh ₄)	{Ni ^{II} [N(CH ₂ CH ₂ S- <i>t</i> -Bu) ₃]Cl} (BPh ₄)	NS ₃ Cl	Holm	22
23	[Ni([9]aneN ₃)] ²⁺	Bis(1,4,7-triazacyclononane) nickel(II)	N ₆	Scott	41
24	[Ni([9]aneS ₃)] ²⁺	Bis(1,4,7-trithiacyclononane) nickel(II)	S ₆	Scott	42
25	NiCl ₂	Nickel(II) chloride, anhydrous	Cl ₆	commercial	43

Holm — Prof. Richard H. Holm of Harvard University supplied these compounds

Scott — Prof. Robert A. Scott of the University of Georgia at Athens supplied data for these compounds

Table 7.3. Results from tests of Ni-N, Ni-S and Ni-Fe parameters on "single-shell" compounds (data range $k = 3.25-11.75 \text{ \AA}^{-1}$).

Coordination geometry	$R(\text{cryst})_{\text{av}}^*$	$R_{\text{EXAFS}}^\#$	$\Delta R_{\text{cryst,EXAFS}}^\dagger$	CNEXAFS §	c_2 value ‡	No. of cpds. curve-fitted
Ni-N compounds :						
Approx. sq. planar	1.861-1.921 $\diamond$	1.859-1.961	0.009-0.014 $\diamond$	3.3-5.4	-0.0221 to -0.0265	6
Tetrahedral	1.947	1.931	0.016	3.8-4.6	-0.0242	1
Sq. pyramidal	-----	1.856-1.857	-----	3.2-3.7	-0.0269	1
Octahedral	2.090-2.124 $\diamond$	2.070-2.118	-0.002 to 0.011 $\diamond$	4.3-7.5	-0.0253 to -0.0280	4
Ni-S compounds :						
Approx. sq. planar	2.175-2.18	2.170-2.175	0.000-0.010	3.7-4.1	-0.0201 to -0.0214	2
Sq. planar, NiIII	2.149	2.143	0.006	4.2-4.3	-0.0193	1
Tetrahedral	2.288	2.287	0.001	4.0-4.2	-0.0207	1
Octahedral	2.386	2.380	0.006	5.6-6.4	-0.0217	1
Ni-Fe compound :						
Dist. tetrahedral	-----	2.755	-----	2.5-2.6	-0.0169	1

* — The $R(\text{cryst})_{\text{av}}$ values are the averaged crystallographic bond distances for each compound. The range shown is the range of $R(\text{cryst})_{\text{av}}$ seen over different compounds.

— Similarly, R_{EXAFS} is shown as a range where more than 1 compound was fitted. The R_{EXAFS} values shown are from Type 1, 2 and 3 fits. The 3 types of fits produce practically identical R_{EXAFS} values.

† — $\Delta R_{\text{cryst,EXAFS}}$ is calculated for each compound, and is also given as a range of values to indicate the possible goodness of fit for different compounds.

§ — The CN values shown were obtained from fits of both Type 1 and Type 2. (Although Type 1 fits are generally less reliable, in fits to compounds with regular, simple coordination spheres, better CN values can sometimes be obtained.)

‡ — The c_2 values shown were obtained from Type 3 fits.

$\diamond$ — Only two of the six square-planar and three of the four octahedral Ni-N compounds fitted have had their crystal structures determined.

Table 7.4. Crystallographic bond distances for some Ni compounds of various coordination geometries (continued on next page). Unless otherwise noted, compounds have a formal Ni oxidation state of +2.

Coordination geometry		$R(\text{Ni-N})_{\text{av}}^{\dagger}$	$R(\text{Ni-S})_{\text{av}}^{\dagger}$	No. of cpds
Ni-N ₄	sq. pl.	1.85–2.066	-----	24
Ni-N ₄	T _d	1.9470	-----	1
Ni-N ₅	sq. py.	1.871(eq), 2.663(ax)	-----	1
Ni-N ₆	O _h	2.035–2.151	-----	8
Ni-N ₂ O ₂	sq. pl.	1.826–1.92	1.817–1.85 (O)	4
Ni-N ₂ O ₂	T _d	1.960	1.896 (O)	1
Ni-N ₃ O ₃	O _h	2.059	2.131 (O)	1
Ni-O ₆	O _h	2.028–2.053	-----	3
NiI-N ₃ S	sq. pl.	1.980	2.143	1
Ni-N ₂ S ₂	app. sq. pl.	1.857–2.003	2.151–2.204	11
Ni-N ₂ S ₂	app. D _{2d}	1.913–1.985	2.152–2.167	4
Ni-NOS ₂	sq. pl.	1.848–1.917 1.865–1.904 (O)	2.178–2.184 (S)	2
Ni-NS ₃	sq. pl.	1.901–1.953	2.170–2.184	3
Ni-OS ₃	sq. pl.	1.852 (O)	2.189	1
Ni-N ₄ Cl	tbpy.	2.123	2.295 (Cl)	1
Ni-N ₃ S ₂	tbpy.	2.057	2.302	1
Ni-N ₂ S ₃	tbpy.	2.00	2.30, 2.42, 2.58*	1
Ni-NS ₃ L [‡]	tbpy.	2.02–2.208	2.226–2.368	5
Ni-N ₃ SCl	sq. py.	2.047	2.296(S), 2.275(Cl)	1
Ni-NS ₄	sq. py.	2.06	2.42	1

[†] — Averages of the crystallographic bond distances were calculated for each compound, and the range shown encompasses the possible values that these averaged bond distances can take for the different compounds surveyed.

* — no averages made in this case

[‡] — These compounds are the [Ni(NS₃^R)L](BPh₄) compounds (**18–22**) listed in Table 7.2. Since the character of L varies so widely (L = H⁺, CH₃⁺, CO, CH₃CO⁺, Cl⁺), distances for the Ni–L bond are not given.

Table 7.4. Crystallographic bond distances for some Ni compounds of various coordination geometries (continued from previous page). Unless otherwise noted, compounds have a formal Ni oxidation state of +2.

Coordination geometry		$R(\text{Ni-N})_{\text{av}}^{\dagger}$	$R(\text{Ni-S})_{\text{av}}^{\dagger}$	No. of cpds
Ni-N ₃ OS ₂	O _h	2.110(N), 2.249(O)	2.378	1
Ni-N ₄ S ₂	O _h	2.041–2.050	2.413–2.516	5
Ni-N ₃ S ₃	O _h	2.065–2.118	2.452–2.476	2
Ni-N ₂ S ₄	O _h	2.047–2.11	2.412–2.50	6
Ni ^{III} -N ₂ S ₄	O _h	2.037	2.279	1
<hr/>				
Ni ^{II} -S ₄	sq. pl.	-----	2.165–2.240	19
Ni ^{III} -S ₄	sq. pl.	-----	2.135–2.149	4
Ni ^{IV} -S ₄	sq. pl.	-----	2.101–2.122	2
Ni-S ₄	T _d	-----	2.281–2.292	4
Ni-PS ₃	T _d	2.174 (P)	2.258	1
Ni-PS ₄	sq. py.	2.114 (P)	2.281	1
Ni-S ₅	sq. py.	-----	2.200(eq), 2.741(ax)	1
Ni-S ₆	O _h	-----	2.386–2.438	4

† — Averages of the crystallographic bond distances were calculated for each compound, and the range shown encompasses the possible values that these averaged bond distances can take for the different compounds surveyed.

Table 7.5. Fits made to Ni–N,S compounds : Ni(tsalen) (**8**), [Ni(NS₃^tBu)H](BPh₄) (**18**) and [Ni(N₃S₂)] (**16**). The same fits were performed on all three compounds.

Waves used	Fit Type [†]
N	1,2,3
S	1,2,3
N + S	1,2,3*
N + N'	1,2,3
S + S'	1,2,3*

- 1 — Distances, coordination numbers and Debye-Waller factors are varied
- 2 — Distances, coordination numbers are varied while Debye-Waller factors (c_2 in the EXAFS equation) are fixed at initial (model) values
- 3 — Distances and Debye-Waller factors are varied while coordination numbers are fixed at various sets of values
- 3* — Several possible combinations of N and/or S coordination numbers were explored

Table 7.6. Results of Type 2 fits (varying CN's and fixing c_2 's) made to Ni-N,S models. Fixed $c_{2N} = -0.0230$, $c_{2S} = -0.0204$.

Crystallographic distances	EXAFS results
Ni(tsalen) (8) :	Window : 0.95–2.35 (0.1) Å
	Fit Index
Ni–N = 1.85 Å 1.86 Å	3.8 N @ 1.97 Å 1.628
	1.9 S @ 2.14 Å 1.032
Ni–S = 2.174 Å 2.139 Å	1.9 N @ 1.87 Å 0.324
	2.0 S @ 2.16 Å
avg. 2 N @ 1.86 Å 2 S @ 2.157 Å	3.4 N @ 1.96 Å 1.226
	2.8 N' @ 2.39 Å
	1.2 S @ 2.04 Å 0.438
	2.3 S' @ 2.17 Å
[Ni(NS ₃ ^t Bu)H](BPh ₄) (18) :	Window : 0.95–2.35 (0.1) Å
	Fit Index
Ni–N = 2.02 Å	6.7 N @ 2.06 Å 1.719
Ni–S = 2.234 Å 2.227 Å 2.218 Å	3.2 S @ 2.22 Å 0.575
	1.0 N @ 1.99 Å 0.454
Ni–H = 2.0 Å	3.0 S @ 2.23 Å
avg. 1 N @ 2.02 Å 3 S @ 2.226 Å (1 H @ 2.0 Å)	3.4 N @ 1.88 Å 0.929
	9.8 N' @ 2.06 Å
	-0.3 S @ 1.94 Å 0.441
	3.2 S' @ 2.22 Å
[Ni(N ₃ S ₂)] (16) :	Window : 1.00–2.30 (0.1) Å
	Fit Index
Ni–N = Mol. 1 2.113 Å 1.974 Å 2.086 Å	4.1 N @ 2.11 Å 1.258
	1.9 S @ 2.28 Å 0.879
Ni–S = 2.274 Å 2.332 Å	1.9 N @ 2.03 Å 0.461
	1.9 S @ 2.30 Å
avg. 3 N @ 2.057 Å 2 S @ 2.302 Å	3.1 N @ 1.97 Å 0.382
	6.5 N' @ 2.13 Å
	1.4 S @ 2.21 Å 0.571
	1.8 S' @ 2.33 Å

Table 7.7. Results of some Type 3 fits (fixing CN's and varying c_2 's) made to Ni-N,S model compounds.

Crystallographic distances	EXAFS results			
Ni(tsalen) (8) :	Window : 0.95–2.35 (0.1) Å			
			c ₂	Fit Index
Ni–N = 1.85 Å	2 N @ 1.87 Å	-0.0242	0.319	
1.86 Å	2 S @ 2.16 Å	-0.0206		
Ni–S = 2.174 Å	3 N @ 1.88 Å	-0.0309	0.303	
2.139 Å	2 S @ 2.16 Å	-0.0217		
avg. 2 N @ 1.86 Å				
2 S @ 2.157 Å				
[Ni(NS ₃ ^t Bu)H](BPh ₄) (18) :	Window : 0.95–2.35 (0.1) Å			
			c ₂	Fit Index
Ni–N = 2.02 Å	1 N @ 1.99 Å	-0.0232	0.456	
	3 S @ 2.23 Å	-0.0203		
Ni–S = 2.234 Å	2 N @ 2.00 Å	-0.0359	0.449	
2.227 Å	3 S @ 2.23 Å	-0.0209		
2.218 Å				
Ni–H = 2.0 Å				
avg. 1 N @ 2.02 Å				
3 S @ 2.226 Å				
(1 H @ 2.0 Å)				
[Ni(N ₃ S ₂)] (16) :	Window : 1.00–2.30 (0.1) Å			
			c ₂	Fit Index
Mol. 1	Mol. 2			
Ni–N = 2.113 Å	2.075 Å	1 N @ 2.02 Å	-0.0182	0.520
1.974 Å	1.968 Å	2 S @ 2.29 Å	-0.0207	
2.086 Å	2.125 Å	2 N @ 2.03 Å	-0.0270	0.437
Ni–S = 2.274 Å	2.298 Å	2 S @ 2.30 Å	-0.0220	
2.332 Å	2.302 Å	3 N @ 2.04 Å	-0.0340	0.404
		2 S @ 2.30 Å	-0.0233	
avg. 3 N @ 2.057 Å				
2 S @ 2.302 Å				

Table 7.8. Results of Type 2 (varying CN's and fixing c_2 's) and Type 3 (fixing CN's and varying c_2 's) fits made to $(Et_4N)_3[NiFe_3S_4(SEt)_4]$ (**11**). For the Type 2 fits, values of fixed $c_{2N} = -0.0230$, $c_{2S} = -0.0204$, and $c_{2Fe} = -0.0153$. No crystal structure was available for comparison.

Window 2 (1.37–2.85 Å)				Window 3 (1.00–2.85 Å)			
Type 2 fits			Fit Index				Fit Index
	2.9 S @	2.25 Å	0.634	3.0 S @	2.25 Å	0.948	
	2.3 Fe @	2.75 Å		2.3 Fe @	2.75 Å		
	0.7 S @	2.25 Å	0.634				
	2.3 S' @	2.25 Å					
	2.3 Fe @	2.75 Å					
	3.5 S @	2.26 Å	0.298	3.7 S @	2.26 Å	0.687	
	1.0 S' @	2.47 Å		1.2 S' @	2.47 Å		
	2.2 Fe @	2.76 Å		2.1 Fe @	2.76 Å		
	3.7 N @	2.16 Å	0.399	4.4 N @	2.19 Å	0.780	
	2.7 S @	2.22 Å		3.4 S @	2.22 Å		
	2.4 Fe @	2.76 Å		2.4 Fe @	2.76 Å		
Type 3 fits	R	c_2	Fit Index	R	c_2	Fit Index	
4 S	2.26 Å	-0.0244	0.750	2.26 Å	-0.0243	0.962	
3 Fe	2.75 Å	-0.0184		2.75 Å	-0.0185		
3 S	2.25 Å	-0.0187	0.222	2.25 Å	-0.0185	0.694	
1 S'	2.44 Å	-0.0283		2.43 Å	-0.0283		
3 Fe	2.76 Å	-0.0194		2.76 Å	-0.0194		
2 N	2.26 Å	-0.0140	0.272	2.23 Å	-0.0184	0.744	
3 S	2.25 Å	-0.0153		2.24 Å	-0.0171		
3 Fe	2.76 Å	-0.0190		2.76 Å	-0.0193		

Table 7.9. Fourier transform windows for $R' < 3 \text{ \AA}$ used for curvefitting of Ni CODH data.

Data set	Window 1	Window 2	Window 3	Window 6
CODHX*	1.00–1.58 $\text{\AA}$	1.50–2.25 $\text{\AA}$	1.00–2.25 $\text{\AA}$	1.00–2.55 $\text{\AA}$
CODHOX	0.80–1.60 $\text{\AA}$	1.45–2.20 $\text{\AA}$	0.80–2.20 $\text{\AA}$	0.80–2.60 $\text{\AA}$
CDHOX	1.05–1.60 $\text{\AA}$	1.40–2.25 $\text{\AA}$	1.05–2.25 $\text{\AA}$	1.05–2.60 $\text{\AA}$
CODHRX	1.00–1.60 $\text{\AA}$	1.45–2.60 $\text{\AA}$	1.00–2.60 $\text{\AA}$	1.00–2.95 $\text{\AA}$
CDHRD	1.00–1.57 $\text{\AA}$	1.40–2.25 $\text{\AA}$	1.00–2.25 $\text{\AA}$	1.00–2.60 $\text{\AA}$

Window 1 : left (or low-R) shoulder of main peak only

Window 2 : main peak only

Window 3 : main peak + left shoulder

Window 6 : main peak + left shoulder + right shoulder

* — The "left shoulder" in CODHX is in fact a well-separated peak of substantial height.

Table 7.10. Fits made to Ni CODH data for $R' < 3 \text{ \AA}$. Except where noted, the same fits were performed on all five data sets

	Window 1	Window 2	Window 3	Window 6
N	1,2,3*	1,2,3*		
S	1,2,3*	1,2,3		
N+S		(1,2,3)x2 [†]	1,2,3	1,2,3
N+N'		1,2,3	1,2,3	
S+S'		1,2,3 [#]	1,2,3	
N+S+S'				(2,3)x3 [§]
N+S+Fe				(2,3)x2
N+S+N'				(2,3)x2

1 — Distances, coordination numbers and Debye-Waller factors are varied

2 — Distances, coordination numbers are varied while Debye-Waller factors (c_2 in the EXAFS equation) are fixed at initial (model) values

3 — Distances and Debye-Waller factors are varied while coordination numbers are fixed at various sets of values

()x2 — Two fit minima are explored

* — Type 3 fit not done for CODHX and CODHOX

† — Only one minimum explored for Window 2 of CDHRD

— Two minima explored for (1,2,3) for CODHRX only

§ — Four minima found for CODHRX and CDHRD, three minima found for the rest

Table 7.11. Results of some of the Type 2 fits (varying CN and fixing c_2) for $R' < 3 \text{ \AA}$ made to five Ni CODH data sets (continued on the next page). Values of fixed $c_{2N} = -0.0230$, $c_{2S} = -0.0204$, and $c_{2Fe} = -0.0153$.

CODHX				CODHOX				CDHOX				CODHRX				CDHRD			
		CN	R(Å)	F			CN	R(Å)	F			CN	R(Å)	F			CN	R(Å)	F
Window 2	N only	3.1 N	2.08	1.139	2.5 N	2.08	0.917	3.0 N	2.07	1.206	4.2 N	2.10	2.177	3.5 N	2.08	1.240			
	S only	1.6 S	2.23	0.481	1.3 S	2.24	0.360	1.6 S	2.23	0.449	2.3 S	2.25	1.186	1.8 S	2.24	0.379			
	N+S	0.6 N	1.82	0.261	0.4 N	1.91	0.257	0.7 N	1.91	0.236	1.1 N	1.89	0.989	0.5 N	1.89	0.253			
		1.7 S	2.23		1.4 S	2.24		1.8 S	2.23		2.6 S	2.25		1.9 S	2.24				
	N+N'	1.4 N	2.08	1.139	1.8 N	1.89	0.529	2.3 N	1.89	0.701	3.3 N	1.90	1.667	2.4 N	1.89	0.738			
Window 3	S+S'	1.7 N'	2.07		4.1 N'	2.08		5.2 N'	2.07		7.2 N'	2.09		5.7 N'	2.08				
		0.4 S	1.99	0.143	0.3 S	2.08	0.203	0.4 S	2.07	0.136	0.8 S	2.05	0.827	0.3 S	2.06	0.184			
		1.8 S'	2.26		1.5 S'	2.25		2.0 S'	2.24		3.1 S'	2.25		2.1 S'	2.24				
	N+S	2.1 N	1.85	0.438	1.3 N	1.89	0.558	1.3 N	1.89	0.299	1.9 N	1.89	0.859	1.2 N	1.87	0.430			
		2.1 S	2.23		1.6 S	2.24		2.0 S	2.23		2.8 S	2.25		2.1 S	2.24				
Window 6	N+N'	4.3 N	1.86	0.380	3.1 N	1.89	0.473	3.3 N	1.88	0.508	4.4 N	1.89	1.523	3.4 N	1.88	0.600			
		6.5 N'	2.07		5.1 N'	2.08		6.0 N'	2.07		8.0 N'	2.09		6.5 N'	2.07				
	S+S'	1.0 S	2.01	0.781	0.7 S	2.07	0.641	0.7 S	2.06	0.388	1.2 S	2.05	0.704	0.6 S	2.05	0.513			
		2.5 S'	2.22		1.9 S'	2.25		2.2 S'	2.23		3.4 S'	2.25		2.4 S'	2.24				
	N+S	2.2 N	1.85	0.527	1.4 N	1.88	0.616	1.4 N	1.88	0.474	1.9 N	1.89	0.897	1.3 N	1.87	0.520			
	2.1 S	2.23		1.7 S	2.24		2.0 S	2.23		2.8 S	2.25		2.2 S	2.24					

Table 7.11. Results of some of the Type 2 fits (varying CN and fixing c_2) for $R' < 3 \text{ \AA}$ made to five Ni CODH data sets (continued from previous page). Values of fixed $c_{2N} = -0.0230$, $c_{2S} = -0.0204$, and $c_{2Fe} = -0.0153$.

CODHX				CODHOX				CODHRX				CDHRD			
CN R(Å) F				CN R(Å) F				CN R(Å) F				CN R(Å) F			
Window 6 N+S+S'	2.2 N	1.85	0.523	1.8 N	1.88	0.488	1.6 N	1.89	0.416	1.7 N	1.89	0.544	1.2 N	1.85	0.482
	8.8 S	2.24		2.2 S	2.24		2.2 S	2.24		2.6 S	2.25		2.1 S	2.26	
	-6.8S'	2.25		0.6 S'	2.43		0.4 S'	2.48		0.8 S'	2.62		0.7 S'	2.16	
N+S+Fe	2.2 N	1.85	0.455	1.4 N	1.88	0.522	1.4 N	1.88	0.277	1.9 N	1.89	0.872	1.3 N	1.87	0.375
	2.1 S	2.22		1.7 S	2.24		2.0 S	2.23		2.8 S	2.25		2.2 S	2.24	
	0.4 S'	2.88		0.4 S'	2.89		0.5 S'	2.90		0.3 S'	2.94		0.5 S'	2.92	
N+S+Fe	2.1 N	1.85	0.441	1.4 N	1.89	0.549	1.3 N	1.88	0.278	1.9 N	1.89	0.755	1.3 N	1.87	0.399
	2.1 S	2.23		1.7 S	2.24		2.0 S	2.23		2.8 S	2.25		2.2 S	2.24	
	0.3 Fe	2.70		0.3 Fe	2.71		0.4 Fe	2.74		0.6 Fe	2.80		0.4 Fe	2.76	
N+S+N'	2.2 N	1.85	0.522	1.4 N	1.88	0.606	1.4 N	1.88	0.440	2.0 N	1.89	0.789	1.3 N	1.87	0.494
	2.1 S	2.23		1.7 S	2.24		2.0 S	2.23		2.8 S	2.25		2.2 S	2.24	
	0.1 Fe	3.06		0.1 Fe	3.08		0.2 Fe	3.07		0.6 Fe	3.09		0.2 Fe	3.09	
N+S+N'	2.2 N	1.85	0.403	1.4 N	1.89	0.499	1.4 N	1.89	0.245	1.9 N	1.89	0.830	1.3 N	1.87	0.383
	2.1 S	2.23		1.7 S	2.24		2.0 S	2.23		2.8 S	2.25		2.2 S	2.24	
	1.1 N'	2.71		1.2 N'	2.71		1.3 N'	2.74		1.2 N'	2.81		1.2 N'	2.76	
N+S+N'	2.2 N	1.85	0.525	1.4 N	1.88	0.611	1.4 N	1.88	0.458	1.9 N	1.89	0.841	1.3 N	1.87	0.509
	2.1 S	2.23		1.7 S	2.24		2.0 S	2.23		2.8 S	2.25		2.2 S	2.24	
	0.2 N'	3.09		0.3 N'	3.10		0.5 N'	3.09		1.3 N'	3.09		0.4 N	3.11	

Table 7.12. Results of some of the Type 3 fits (fixing CN and varying c_2) for $R' < 3 \text{ \AA}$ made to five Ni CODH data sets (continued on the next page)

the next page)

CODHX			CODHOX			CODHRX			CDHRD			
Window	R	c ₂	F	R	c ₂	F	R	c ₂	F	R	c ₂	F
2	2.23	-0.0228	0.612	2.24	-0.0276	0.331	2.23	-0.0240	0.403	2.26	-0.0179	1.162
3 (6) [†] N												
2 S												
4 S												
1 N	1.82	-0.0277	0.334	1.84	-0.0393	0.170	1.89	-0.0320	0.224	1.93	-0.0199	0.947
2 S	2.23	-0.0223		2.24	-0.0265		2.23	-0.0227		2.26	-0.0163	
2 N	1.81	-0.0417	0.341	1.84	-0.0580	0.161	1.88	-0.0490	0.230	1.92	-0.0318	0.937
2 S	2.23	-0.0219		2.24	-0.0266		2.24	-0.0231		2.26	-0.0166	
2 N										1.88	-0.0314	1.030
3 S										2.25	-0.0219	
Window 3												
1 N	1.84	-0.0128	0.390	1.87	-0.0219	0.470	1.88	-0.0218	0.299	1.91	-0.0179	0.998
2 S	2.22	-0.0216		2.24	-0.0252		2.23	-0.0217		2.25	-0.0165	
2 N	1.85	-0.0216	0.428	1.88	-0.0314	0.402	1.89	-0.0314	0.202	1.91	-0.0266	0.889
2 S	2.22	-0.0199		2.24	-0.0245		2.24	-0.0215		2.26	-0.0162	
3 N	1.86	-0.0278	0.522	1.89	-0.0381	0.379	1.90	-0.0385	0.182	1.91	-0.0327	0.822
2 S	2.23	-0.0193		2.25	-0.0245		2.24	-0.0217		2.26	-0.0162	
2 N										1.89	-0.0412	0.309
3 S										2.24	-0.0204	
										1.85	-0.0304	0.239
										2.24	-0.0259	

[†] — The number in brackets refers to the fixed-CN value for CODHRX only.

Table 7.12. Results of some of the Type 3 fits (fixing CN and varying c_2) for $R' < 3 \text{ \AA}$ made to five Ni CODH data sets (continued from previous page)

	CODHX			CODHOX			CODHOX			CODHRX			CDHRD		
	R	c_2	F	R	c_2	F	R	c_2	F	R	c_2	F	R	c_2	F
Window 6															
2 N	1.85	-0.0208	0.492	1.88	-0.0294	0.560	1.89	-0.0288	0.460	1.88	-0.0242	0.900	1.89	-0.0319	0.490
2 (3) [†] S	2.22	-0.0192		2.24	-0.0233		2.24	-0.0203		2.25	-0.0213		2.24	-0.0194	
2 N	1.86	-0.0196	0.316	1.88	-0.0267	0.427	1.89	-0.0269	0.395	1.82	-0.0275	0.578	1.87	-0.0303	0.423
2 (3) [†] S	2.22	-0.0181		2.23	-0.0215		2.23	-0.0195		2.18	-0.0236		2.24	-0.0197	
1 S'	2.38	-0.0318		2.40	-0.0378		2.40	-0.0506		2.31	-0.0080		2.31	-0.0612	
2 N	1.85	-0.0208	0.420	1.88	-0.0295	0.451	1.89	-0.0290	0.291	1.88	-0.0241	0.869	1.88	-0.0319	0.350
2 (3) [†] S	2.22	-0.0192		2.24	-0.0233		2.23	-0.0203		2.25	-0.0212		2.24	-0.0195	
1 S'	2.90	-0.0390		2.90	-0.0328		2.90	-0.0300		3.23	-0.0279		2.92	-0.0325	
2 N										1.88	-0.0280	0.444			
3 S										2.25	-0.0236				
1 S'										2.61	-0.0186				
2 N	1.85	-0.0210	0.386	1.88	-0.0301	0.391	1.89	-0.0300	0.196	1.88	-0.0246	0.826	1.89	-0.0326	0.333
2 (3) [†] S	2.22	-0.0194		2.24	-0.0236		2.24	-0.0208		2.25	-0.0216		2.24	-0.0197	
1 Fe	2.72	-0.0333		2.72	-0.0273		2.74	-0.0256		2.80	-0.0184		2.75	-0.0289	
2 N	1.85	-0.0208	0.492	1.88	-0.0294	0.560	1.89	-0.0288	0.458	1.88	-0.0240	0.830	1.89	-0.0319	0.488
2 (3) [†] S	2.22	-0.0192		2.24	-0.0233		2.24	-0.0203		2.25	-0.0212		2.24	-0.0194	
1 Fe	3.50	-0.1115		3.47	-0.1072		3.56	-0.1084		3.09	-0.0193		3.52	-0.0982	

† — The number in brackets refers to the fixed-CN value for CODHRX only.

Table 7.13. Some Type 2 fits (varying CN's and fixing c_2 's) made to signals at $R' = 3-5 \text{ \AA}$ in CODHRX. Values of fixed $c_{2N} = -0.0230$, $c_{2S} = -0.0204$, and $c_{2Fe} = -0.0153$.

Window 7 (2.85–3.60 Å)				Window 10 (2.85–5.07 Å)			
			Fit Index				Fit Index
0.8 S	@	3.68 Å	0.183	0.5 Fe	@	3.54 Å	0.072
0.7 Fe	@	3.53 Å	0.203	1.8 S'	@	4.18 Å	
0.5 S	@	3.64 Å	0.164	0.6 Fe'	@	3.83 Å	
0.4 Fe	@	3.56 Å		1.1 S''	@	4.65 Å	
				1.8 Fe''	@	4.88 Å	
0.6 S	@	3.53 Å	0.155				
1.1 Fe	@	3.54 Å					
Window 8 (3.52–4.20 Å)							
			Fit Index				Fit Index
1.44 S	@	4.19 Å	0.234	0.8 S	@	3.73 Å	0.080
1.0 Fe	@	4.04 Å	0.364	2.0 S'	@	4.17 Å	
2.1 S	@	4.18 Å	0.203	1.1 Fe'	@	3.82 Å	
-0.7 Fe	@	4.00 Å		1.0 S''	@	4.66 Å	
				1.9 Fe''	@	4.87 Å	
1.7 S	@	4.08 Å	0.216				
1.9 Fe	@	4.08 Å					
Window 9 (4.10–5.07 Å)							
			Fit Index				
2.3 S	@	5.03 Å	0.418				
2.3 Fe	@	4.87 Å	0.178				
-1.3 S	@	4.88 Å	0.073				
1.6 Fe	@	4.85 Å					
0.6 S	@	4.66 Å	0.107				
2.0 Fe	@	4.88 Å

Figure 7.1. Possibilities for the Ni environment in *Rhodospirillum rubrum* Ni CODH: (a) NiFe₃S₄ cubane and (b) Ni ligated to Fe₄S₄ cubane.

Figure 7.2. Edges of square-planar and tetrahedral Ni compounds. Square planar (top) : $(\text{Bu}_4\text{N})_2[\text{Ni}(\text{mnt})_2]$ (**13**) (—), and $\text{Ni}(\text{corphin})$ (**3**) (----). Tetrahedral (bottom) : $[\text{Ni}(\text{SPh})_4]^{2-}$ (**12**) (—), and $[\text{Ni}(\text{TC-6,6})]$ (**4**) (----).

Figure 7.3. Edges of trigonal bipyramidal and octahedral Ni compounds. Trigonal bipyramidal (top) : [Ni(N₃S₂)] (**16**) (—), and [Ni(N₂S₃)] (**17**) (----). Octahedral (bottom) : [Ni([9]aneS₃)]²⁺ (**24**) (—), and [Ni([9]aneN₃)]²⁺ (**23**) (----).

Figure 7.4. Ni compounds with edges of unusual appearance. Top : Ni in a multinuclear cluster. $[\text{Ni}(\text{SS}_2)]_2$ (**15**) (—), and $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$ (**11**) (---). Middle : Ni- N_2S_2 square planar. Ni(ebmbs) (**9**) (ligand not conjugated) (—), and Ni(tsalen) (**8**) (conjugated ligand) (---). Bottom : Ni- N_2S_2 stepwise distortion from square planar towards tetrahedral geometry. Ni($\text{N}_2\text{S}_2\text{C}_2$) (**5**) (distorted 3.4° from planarity) (---), Ni($\text{N}_2\text{S}_2\text{C}_3$) (**6**) (distorted 18.6°) (-.-.-), and Ni($\text{N}_2\text{S}_2\text{C}_4$) (**7**) (distorted 38.6°) (—). Inset shows $1s \rightarrow 3d$ feature in compounds (**5**), (**6**) and (**7**).

Figure 7.5. Edges of a series of trigonal bipyramid compounds with Ni-NS₃L ligation. Top to bottom : [Ni(NS₃^tBu)Cl](BPh₄) (**22**), [Ni(NS₃^tBu)CO](BPh₄) (**21**), [Ni(NS₃ⁱPr)COMe](BPh₄) (**20**), [Ni(NS₃^tBu)Me](BPh₄) (**19**) and [Ni(NS₃^tBu)H](BPh₄) (**18**). The vertical grid lines aid in comparing the six transitions discernible in these edges.

Figure 7.6. Edge of CODH (ox) (1992) (— in both top and bottom) compared with model compound edges. Top (Ni in clusters) : $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$ (**11**) (----) and $[\text{Ni}(\text{SS}_2)]_2$ (**15**) (---). Bottom : $(\text{Bu}_4\text{N})_2[\text{Ni}(\text{mnt})_2]$ (**13**) (square planar) (----), $[\text{Ni}(\text{SPh})_4]^{2-}$ (**12**) (tetrahedral) (---), and $[\text{Ni}(\text{N}_3\text{S}_2)]$ (**16**) (trigonal bipyramidal) (— —).

Figure 7.7. Ni K edges of oxidized and reduced Ni CODH samples measured in 1991 and 1992.

Figure 7.8. Ni K edges of oxidized CODH samples measured in 1989, 1991 and 1992.

Figure 7.9. Fe K edges of oxidized and reduced Ni CODH (1991 data), and $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$ (**11**).

Figure 7.10. EXAFS spectra of Ni CODH protein. (a) CODHX (oxidized, June 1989), (b) CODHOX (oxidized, July 1991), (c) CDHOX (oxidized, July 1992), (d) CODHRX (reduced, July 1991), (e) CDHRD (reduced, July 1992).

Figure 7.11. Fourier transformed Ni CODH protein data, using a Fourier window of $k = 3.0\text{--}12.0$ (0.1) $\text{\AA}^{-1}$. Top to bottom : CODHX (oxidized, June 1989), CODHOX (oxidized, July 1991), CDHOX (oxidized, July 1992), CODHRX (reduced, July 1991), CDHRD (reduced, July 1992).

Figure 7.12. Fourier transforms over $k = 3.0\text{--}12.0$ (0.1) $\text{\AA}^{-1}$ of CDHOX (oxidized, July 1992) and CODHRX (reduced, July 1991), showing the Fourier windows used to generate filtered EXAFS data for curve-fitting. Windows 7–10 were only used for CODHRX. Note : While the peak at $R' \sim 2.8$ $\text{\AA}$ looks significant in CDHOX, in most of the data sets it is barely above the noise (see Figure 7.11).

Figure 7.13. Examples of reverse Fourier transformed data ("filtered" data), to which various fits were made : Windows 1, 2, 3 and 6 from CDHOX (oxidized, July 1992).

Figure 7.14. Type 2 fits (varying R and CN) (----) made to Window 3 of CODHX (oxidized, June 1989) (—). Top to bottom : N+N', S+S' and N+S.

Figure 7.15. Type 2 fits (varying R and CN) (----) made to Window 3 of CGDHRX (reduced, July 1991) (—). Top to bottom: N+N', S+S' and N+S.

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Figure 7.15. Type 2 fits (varying R and CN) (----) made to Window 3 of CODHRX (reduced, July 1991) (—). Top to bottom: N+N', S+S' and N+S.

Figure 7.16. EXAFS spectra of the cubanes $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$ (**11**) (top) and $(\text{Et}_4\text{N})_2[\text{NiFe}_3\text{S}_4(\text{PPh}_3)(\text{SEt})_3]$ (**10**) (middle), and CODHX (oxidized CODH, June 1989) (bottom)

Figure 7.17. Fourier-transformed spectra of the cubanes $(\text{Et}_4\text{N})_3[\text{NiFe}_3\text{S}_4(\text{SEt})_4]$ (**11**) (top) and $(\text{Et}_4\text{N})_2[\text{NiFe}_3\text{S}_4(\text{PPh}_3)(\text{SEt})_3]$ (**10**) (middle), and CODHX (oxidized CODH, June 1989) (bottom). Fourier transform range : $k = 3.0\text{--}12.0$ (0.1) $\text{\AA}^{-1}$

Figure 7.18. Filtered EXAFS data from CDHOX (Window 6) (—), with two- and three-wave Type 3 fits (varying R and c_2) (----). Top to bottom : $2N+2S$, $2N+2S+1S'$ (S' at $2.90 \text{ \AA}$) and $2N+2S+1Fe$ (Fe at $2.74 \text{ \AA}$).

Figure 7.19. Filtered EXAFS data from CODHRX (Window 6) (—), with two- and three-wave Type 3 fits (varying R and c_2) (----). Top to bottom : $2\text{N}+3\text{S}$, $2\text{N}+3\text{S}+1\text{S}'$ (S' at $2.61 \text{ \AA}$) and $2\text{N}+3\text{S}+1\text{Fe}$ (Fe at $2.80 \text{ \AA}$).

Figure 7.20. Fourier transforms of filtered (Window 6) and unfiltered protein data and some Type 3 N+S+S' fits to the filtered data. Fourier transform range : $k = 3.5\text{--}11.5$ (0.1) $\text{\AA}^{-1}$. Top : CDHOX data and two $2N+2S+1S'$ fits to it. S' is found at $2.40 \text{ \AA}$ or $2.90 \text{ \AA}$. Bottom : CODHRX data and two $2N+3S+1S'$ fits to it. S' is found at $2.61 \text{ \AA}$ or $3.23 \text{ \AA}$.

Figure 7.21. Fourier transforms of filtered (Window 6) and unfiltered protein data and some Type 3 N+S+Fe fits to the filtered data. Fourier transform range : $k = 3.5\text{--}11.5$ (0.1) $\text{\AA}^{-1}$. Top : CDHOX data and two $2N+2S+1\text{Fe}$ fits to it. Fe is found at 2.74 $\text{\AA}$ or 3.56 $\text{\AA}$. Bottom : CODHRX data and two $2N+3S+1\text{Fe}$ fits to it. Fe is found at 2.80 $\text{\AA}$ or 3.09 $\text{\AA}$.

Figure 7.22. The core of $[\text{Fe}_4\text{S}_4(\text{SPh})_4]^{3-}$, with Ni attached to one of the S(Ph) atoms.

Appendices

Appendix I. Complete Tables of Fits to EXAFS Spectra of Reduced Poplar Plastocyanin at pH 7.2 and 4.8 and a Cu(I)-N,S Model Compound

The following tables list the distances (R), coordination numbers (CN), c_2 values and fit indices (F) for the fits made for the EXAFS curve-fitting results discussed in Chapter 5. In the parameterized EXAFS equation employed by our analysis package (see Chapter 2), c_2 values vary in the same way as the squared term σ_{as}^2 in the Debye-Waller factor, such that $\Delta c_2 = -2\Delta\sigma_{as}^2$. Thus, though the sign is reversed, the magnitude of c_2 is proportional to σ_{as}^2 .

When using the fit index F to evaluate the goodness of a fit to the data, we have to bear in mind that F is not adjusted for the size of the signal. Thus, for equally good fits (as judged by viewing the match of phase and amplitude), we would expect the fit indices for fits to a larger EXAFS signal to be larger than those for fits to smaller EXAFS signals. So the fit index F should only be used to compare different fits made on the same data.

The windows used on the data sets are denoted by their data file names. Thus, fits to PCHI.FIL2 correspond to fits on Window 2 of PCHI, PCHI.FIL3 contains data filtered using Window 3, etc.

The tables also show what initial R, CN and c_2 values were used for beginning each fit. Unless otherwise stated, a fit result shown had as its initial values the results of the fit shown in the line immediately above. Thus, in a given table (e.g., N+S fits on PCHI.FIL2), the values listed as initial values were first used in a fit varying R's only. Then the results of this fit were in turn used in a fit varying R and CN (referred to as Type 2 fits in the text), whose results are listed below it. The results of this Type 2 fit were in turn used as initial values for the Type 1 fit (varying R's, CN's and c_2 's) whose results are listed immediately below. An exception to this is that Type 3 fits (varying R's and c_2 's), listed below Type 1 fits, generally used the results of fits varying R only as initial values. Bracketed CN or c_2 values were held fixed at the values shown.

List of Tables :

Results of fitting a $\text{Cu}_1\text{N}_2\text{S}_2$ model with N & S	183
Results of fitting Reduced Pc with N & S	186

Results of fitting a Cu^I-N₂S₂ model with N & S

Grace Tan

14/12/90

(Numbers checked and corrected 17/4/93)

These fits are discussed in Chapter 5, Section (C.1).

Data to $k = 17 \text{ Å}^{-1}$, but has some interesting problems :

- (1) 9 scans collected, but Scans 3 & 4 were ruined by small earthquakes!
- (2) A node in the EXAFS occurs at $k = 13$. EXAFS in this region underwent a change between Scans 5 & 6, when detuning changed from counterclockwise (the usual) to clockwise (required for this particular run on BL 4-2).
- (3) Chose to use scans collected before the change occurred in the EXAFS.
- (4) Spline to $k = 17 \text{ Å}^{-1}$ looks different from spline to $k = 13 \text{ Å}^{-1}$ because of the node at $k = 13 \text{ Å}^{-1}$.
- (5) Decided to use spline to $k = 13 \text{ Å}^{-1}$ because the glitch at $k = 13.25 \text{ Å}^{-1}$ may be due to Zn.
 Pre-edge : 9043–9640 (-2)
 Spline : 9025 (2) 9138 (3) 9343 (3) 9640

Forward FT : $k = 3.5\text{--}12.5 (0.1) \text{ Å}^{-1}$

Reverse FT (to $k = 4.0\text{--}12.0 \text{ Å}^{-1}$) :

CUNS2.FIL0 : $R' = 1.35\text{--}2.35 (0.1) \text{ Å}$ — main peak only

CUNS2.FIL2 : $R' = 1.35\text{--}3.00 (0.1) \text{ Å}$ — main peak + 1 higher-R peak

Parameters used in fits :

Cu ^I -N : Cu ^I (N-meim) ₄ ClO ₄ ,	10 K,	$k = 4.0\text{--}12.0 \text{ Å}^{-1}$ (data to $k = 17 \text{ Å}^{-1}$)
Cu ^I -S : [Cu ^I (detu) ₃] ₂ SO ₄ ,	10 K,	$k = 4.0\text{--}12.0 \text{ Å}^{-1}$ (data to $k = 17 \text{ Å}^{-1}$)

Fits of CUN2.FIL0 :

N only :

	R	CN	c ₂	F
Initial	2.0	4	-0.02528	----
	2.1269	5.1923	"	0.847597
	2.1285	4.5318	-0.02291	0.833506

S only :

	R	CN	c ₂	F
Initial	2.2	3	-0.02161	----
	2.2755	1.9126	"	0.354235
	2.2760	2.3186	-0.02454	0.284316

N + S :

	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
Initial	2.0	2	-0.02528	2.2	2	-0.02161	-----
##	2.0643	"	"	2.2922	"	"	0.676022
	2.1145	1.5789	"	2.2795	1.4440	"	0.133608
	2.1070	0.5572	-0.01348	2.2830	1.8822	-0.02399	0.0716634
	2.0882	(1)	-0.01288	2.2955	(1)	-0.01586	0.245786
	2.1025	(2)	-0.02257	2.2890	(1)	-0.01803	0.180088
	2.1127	(3)	-0.03032	2.2803	(1)	-0.01851	0.229882
	2.1377	(1)	-0.01950	2.2741	(2)	-0.02625	0.105672
	2.1731	(2)	-0.02782	2.2615	(2)	-0.02545	0.173140
	2.1819	(3)	-0.05073	2.2693	(2)	-0.02336	0.233801
Initial	2.2	4	-0.02528	2.6	1	-0.02161	-----
	2.1283	"	"	2.6543	"	"	0.922859
	2.1284	5.2084	"	2.6466	0.5793	"	0.665233
	2.1308	5.0578	-0.02454	2.6921	2.1575	-0.04688	0.652876
Initial	2.6	1	-0.02528	2.2	3	-0.02161	-----
	2.6788	"	"	2.2775	"	"	1.35061
	2.6350	0.6904	"	2.2741	1.9086	"	0.300635
	2.6343	0.0303	+0.01088	2.2766	2.1750	-0.02306	0.215093

-- these R values used as starting values in each of the fixed-CN fits.

N + N' :

	R _N	CN _N	c _{2N}	R _{N'}	CN _{N'}	c _{2N'}	F
Initial	2.0	2	-0.02528	2.2	2	-0.02528	-----
	2.1262	"	"	2.1260	"	"	0.988750
	2.1269	2.4006	"	2.1270	2.7915	"	0.847597
(Made a few attempts to vary c ₂ 's, but program crashed each time.)							
Initial	2.2	4	-0.02528	2.6	1	-0.02528	-----
	2.1274	"	"	2.5123	"	"	0.756658
	2.1306	4.8439	"	2.5082	2.4561	"	0.432334
	2.1285	4.7205	-0.02505	2.5068	1.7462	-0.01980	0.426004

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
Initial	2.0	1	-0.02161	2.2	2	-0.02161	-----
	1.9950	"	"	2.2506	"	"	1.48276
	2.0528	-0.3031	"	2.2806	1.6904	"	0.171698
	2.0680	-0.1227	-0.01126	2.2805	2.1372	-0.02494	0.0559465
Initial	2.2	2	-0.02161	2.6	1	-0.02161	-----
	2.2746	"	"	2.7711	"	"	0.768813
	2.2752	1.9060	"	2.7604	0.1780	"	0.321687
	2.2767	2.2599	-0.02395	2.7425	0.0029	+0.01876	0.240476

N + N' + S : (these fits are exploratory)

Fixed c_2's							
	$c_{2N} = -0.02528$		$c_{2N'} = -0.02528$		$c_{2S} = -0.02161$		F
	R_N	CN_N	$R_{N'}$	$CN_{N'}$	R_S	CN_S	
Initial	2.1	1	2.2	1	2.3	2	-----
	2.0893	"	2.3154	"	2.2794	"	0.234863
	2.1314	2.3885	2.3014	-1.8216	2.2877	0.7262	0.0350366
Initial	2.0970*	1	2.1635*	1	2.2998*	2	-----
	2.0893	"	2.3155	"	2.2794	"	0.234863
	2.1144	1.1570	2.1145	0.4215	2.2795	1.4443	0.133608
Fixed CN's							
	$CN_N = 1$		$CN_{N'} = 1$		$CN_S = 2$		F
	R_N	c_{2N}	$R_{N'}$	$c_{2N'}$	R_S	c_{2S}	
Initial	2.0970*	-0.02528	2.1635*	-0.02528	2.2998*	-0.02161	-----
	4.3544	-0.33503	-0.0505	-0.49496	2.2754	-0.02266	0.318068

* --- averages of the crystallographic values

Results of fitting Reduced Pc with N & S

Grace Tan

includes fits done 2/2/91 & 14/4/93

(numbers checked and corrected 17/4/93)

The following fits are discussed in Chapter 5, Sections (C.2.a) – (C.2.c). All fits made to the data set PCHI are first listed, followed by fits to PCLO.

Reduced Pc at pH 7.2 : PCHI — 21 acceptable scans
Reduced Pc at pH 4.8 : PCLO — 29 acceptable scans

Data to $k = 13 \text{ \AA}^{-1}$

Final pre-edge and spline for both (to $k = 13 \text{ \AA}^{-1}$) :

Pre-edge : 9045–9640 (2)
Spline : 9030 (2) 9146 (3) 9350 (3) 9640

Forward FT : $k = 3.5\text{--}12.5 (0.1) \text{ \AA}^{-1}$ (but note large glitches removed at $k = 10.9 \text{ \AA}^{-1}$)

Reverse FT (to $k = 4.0\text{--}12.0 \text{ \AA}^{-1}$) :

PCHI.FIL0 : $R = 1.20\text{--}2.12 (0.1) \text{ \AA}$ PCLO.FIL0 : $R = 1.25\text{--}2.12 (0.1) \text{ \AA}$
— main peak only

PCHI.FIL1 : $R = 1.20\text{--}2.45 (0.1) \text{ \AA}$ PCLO.FIL1 : $R = 1.25\text{--}2.40 (0.1) \text{ \AA}$
— main peak + 1 higher-R bump

PCHI.FIL2 : $R = 1.20\text{--}2.85 (0.1) \text{ \AA}$ PCLO.FIL2 : $R = 1.25\text{--}3.05 (0.1) \text{ \AA}$
— main peak + 2 higher-R bumps

[PCHI.FIL3 : $R = 0.85\text{--}2.45 (0.1) \text{ \AA}$ PCLO.FIL3 : $R = 0.85\text{--}2.40 (0.1) \text{ \AA}$
— main peak + low-R shoulder + 1 higher-R bump] *

* Of questionable reliability, because of spline dependence of low-R shoulder. However, fits show that the low-R shoulder probably really does contain some Cu-scatterer signal.

Parameters used in fits :

Cu^I-N : Cu^I(N-meim)₄ClO₄, 10 K, $k = 4.0\text{--}12.0 \text{ \AA}^{-1}$ (data to $k = 17 \text{ \AA}^{-1}$)
Cu^I-S : [Cu^I(detu)₃]₂SO₄, 10 K, $k = 4.0\text{--}12.0 \text{ \AA}^{-1}$ (data to $k = 17 \text{ \AA}^{-1}$)

Fits of PCHI :

<u>N only :</u>	R	CN	c ₂	F
Initial	1.9, 2.0	3	-0.02528	----
PCHI.FIL0	2.0396	3.0794	(-0.02528)	0.319982
	2.0387	3.4195	-0.02715	0.311351
PCHI.FIL1	2.0401	3.0926	(-0.02528)	0.358759
	2.0393	3.3990	-0.02697	0.352564
PCHI.FIL2	2.0402	3.1012	(-0.02528)	0.369850
	2.0395	3.4078	-0.02697	0.363911

<u>S only :</u>	R	CN	c ₂	F
Initial	2.0, 2.2	3, 3, 2	-0.02161	----
PCHI.FIL0 :	2.1883	1.0524	(-0.02161)	0.449532
	2.1917	1.6187	-0.02842	0.358746
PCHI.FIL1 :	2.1880	1.0637	(-0.02161)	0.448154
	2.1905	1.4886	-0.02688	0.394862
PCHI.FIL2 :	2.1879	1.0642	(-0.02161)	0.466378
	2.1904	1.4754	-0.02673	0.419230

<u>N + S :</u>	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2s}	F
PCHI.FIL0 :							
Initial	2.0	2	-0.02528	2.2	2	-0.02161	-----
	1.9245	"	"	2.2137	"	"	1.47935
	2.0283	1.9372	"	2.2042	0.4824	"	0.0981685
	2.0704	2.4140	-0.03140	2.1643	0.9035	-0.02695	0.0390049
PCHI.FIL1 :							
Initial	2.0	2	-0.02528	2.2	1	-0.02161	-----
	1.9930	"	"	2.2213	"	"	0.575079
	2.0316	1.8568	"	2.1987	0.5050	"	0.166545
	2.1536	3.6166	-0.04308	2.1695	1.6997	-0.02709	0.0722466
	2.0815	(2)	-0.03219	2.1685	(1)	-0.02537	0.140060
	2.0907	(3)	-0.04227	2.1701	(1)	-0.02379	0.0882840
	2.2784	(1)	-0.01301	2.2061	(2)	-0.02505	0.151783
	2.2324	(2)	-0.01342	2.1883	(2)	-0.01956	0.0759371
PCHI.FIL2 :							
Initial	2.0	2	-0.02528	2.2	1	-0.02161	-----
	1.9934	"	"	2.2211	"	"	0.592753
	2.0331	1.8853	"	2.1972	0.4931	"	0.201165
	2.1667	3.9492	-0.04234	2.1698	1.8823	-0.02754	0.101359
	2.0401	(1)	-0.02071	2.1930	(1)	-0.02821	0.244280
	2.0840	(2)	-0.03219	2.1679	(1)	-0.02509	0.175027
	2.0926	(3)	-0.04197	2.1695	(1)	-0.02365	0.124069
	2.2793	(1)	-0.01288	2.2073	(2)	-0.02516	0.184585
	2.2336	(2)	-0.01362	2.1889	(2)	-0.01967	0.121799

	R_N	CN_N	c_{2N}	R_S	CN_S	c_{2S}	F
PCHI.FIL3 :							
Initial	2.0	2	-0.02528	2.2	1	-0.02161	-----
	1.9915	"	"	2.2199	"	"	0.632023
	2.0291	1.8725	"	2.1992	0.5297	"	0.374580
	2.0840	8.9573	-0.07794	2.1774	1.0164	-0.02270	0.218851
	2.0742	(2)	-0.03328	2.1712	(1)	-0.02531	0.331454

N + N' :

	R_N	CN_N	c_{2N}	$R_{N'}$	$CN_{N'}$	$c_{2N'}$	F
PCHI.FILO :							
Initial	2.0	2	-0.02528	2.2	2	-0.02528	-----
	1.9969	"	"	2.0831	"	"	0.335411
	1.8670	0.7403	"	2.0454	3.6946	"	0.156056
	1.9758	2.0898	-0.02579	2.0846	1.3695	-0.01512	0.0745650
PCHI.FIL1 :							
Initial	2.0	2	-0.02528	2.2	2	-0.02528	-----
	1.9978	"	"	2.0831	"	"	0.375270
	1.8678	0.7541	"	2.0460	3.7184	"	0.218781
	1.9879	2.2975	-0.02461	2.0942	0.9503	-0.01176	0.124775
Initial	2.2	2	-0.02528	2.8	2	-0.02528	-----
	2.0371	"	"	2.7321	"	"	0.727990
	2.0398	3.0930	"	2.7322	0.4783	"	0.336372
	2.0378	3.3018	-0.02642	2.6971	0.0293	+0.01086	0.280630
PCHI.FIL2 :							
Initial	2.0	2	-0.02528	2.2	2	-0.02528	-----
	1.9982	"	"	2.0830	"	"	0.385517
	1.8680	0.7564	"	2.0462	3.7288	"	0.235564
	1.9972	2.5680	-0.02601	2.0961	0.7106	-0.01020	0.148635
Initial	2.2	2	-0.02528	2.8	2	-0.02528	-----
	2.0375	"	"	2.7491	"	"	0.756576
	2.0401	3.1037	"	2.7471	0.3720	"	0.357205
	2.0377	3.3230	-0.02650	2.6824	0.0061	+0.02307	0.293565

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
PCHI.FILO :							
Initial	2.0	1	-0.02161	2.2	1	-0.02161	-----
	2.1390	"	"	2.2628	"	"	0.387970
	2.1755	1.3453	"	2.3315	0.5753	"	0.144278
	2.1630	0.9474	-0.02073	2.2805	1.1740	-0.03412	0.129104
PCHI.FIL1 :							
Initial	2.0	1	-0.02161	2.2	1	-0.02161	-----
	2.1388	"	"	2.2606	"	"	0.456676
	2.1808	1.3698	"	2.3471	0.5210	"	0.220856
	2.1794	1.5809	-0.02616	2.3475	2.0418	-0.05590	0.0996645
Initial	2.2	1	-0.02161	2.8	1	-0.02161	-----
	2.1873	"	"	2.9121	"	"	0.767793
	2.1878	1.0660	"	2.8937	0.1720	"	0.428490
	2.1889	1.3842	-0.02580	2.9302	2.9945	-0.08332	0.331988

	R_S	CN_S	c_{2s}	$R_{S'}$	$CN_{S'}$	$c_{2s'}$	F
PCHI.FIL2 :							
Initial	2.0	1	-0.02161	2.2	1	-0.02161	-----
	2.1389	"	"	2.2606	"	"	0.481035
	2.1816	1.3862	"	2.3507	0.5349	"	0.239043
	2.1792	1.5288	-0.02599	2.3440	2.5588	-0.06276	0.124938
Initial	2.2	1	-0.02161	2.8	1	-0.02161	-----
	2.1887	"	"	2.9884	"	"	0.770744
	2.1880	1.0670	"	2.9620	0.1455	"	0.454291
	2.1886	1.3874	-0.02578	2.9773	7.2110	-0.10937	0.322347

N + S + S' :

PCHI.FIL1 :

Fixed c_2's							
	$c_{2N} = -0.02528$		$c_{2s} = -0.02161$		$c_{2s'} = -0.02161$		
	R_N	CN_N	R_S	CN_S	$R_{S'}$	$CN_{S'}$	F
Initial	2.0	2	2.2	1	2.5	1	-----
	2.0064	"	2.2390	"	2.4507	"	0.532334
	2.0145	1.5767	2.2106	0.7994	2.4181	0.2607	0.107835
Initial	2.0	1	2.2	1	2.8	1	-----
	1.9999	"	2.2009	"	2.9296	"	0.736591
	2.0320	1.8321	2.1977	0.5106	2.8660	0.1253	0.135402
	2.0309	1.6898	2.1966	0.5504	2.9080	1.2912*	0.0939606
					[* $c_2 = -0.06838$]		

Fixed CN's							
	$CN_N = 2$		$CN_S = 1$		$CN_{S'} = 1$		
	R_N	c_{2N}	R_S	c_{2s}	$R_{S'}$	$c_{2s'}$	F
Initial	2.0	-0.02528	2.2	-0.02161	2.5	-0.02161	-----
	2.0592	-0.03735	2.1760	-0.02430	2.3658	-0.06231	0.0659733
Initial	2.0	-0.02528	2.2	-0.02161	2.8	-0.02161	-----
	2.0820	-0.03286	2.1689	-0.02510	2.9186	-0.06612	0.0821691

PCHI.FIL2 :

Fixed c_2's							
	$c_{2N} = -0.02528$		$c_{2s} = -0.02161$		$c_{2s'} = -0.02161$		
	R_N	CN_N	R_S	CN_S	$R_{S'}$	$CN_{S'}$	F
Initial	2.0	2	2.2	1	2.5	1	-----
	2.0069	"	2.2376	"	2.4481	"	0.536572
	2.0133	1.4933	2.2088	0.8491	2.4118	0.2935	0.147050
Initial	2.0	2	2.2	1	2.8	1	-----
	1.9920	"	2.2227	"	3.0214	"	0.818127
	2.0322	1.8873	2.1984	0.4951	3.0181	0.1267	0.180653
	2.0320	1.6423	2.1946	0.5609	2.9669	5.0354*	0.0879317
					[* $c_2 = -0.10999$]		

Fixed CN's							
	$CN_N = 2$		$CN_S = 1$		$CN_{S'} = 1$		
	R_N	c_{2N}	R_S	c_{2s}	$R_{S'}$	$c_{2s'}$	F
Initial	2.0	-0.02528	2.2	-0.02161	2.5	-0.02161	-----
	2.0605	-0.03729	2.1756	-0.02417	2.3677	-0.06143	0.101716
Initial	2.0	-0.02528	2.2	-0.02161	2.8	-0.02161	-----
	2.0807	-0.03187	2.1680	-0.02544	2.9822	-0.06090	0.0884258

PCHI.FIL3 :

Fixed c_2's							
	$c_{2N} = -0.02528$		$c_{2S} = -0.02161$		$c_{2S'} = -0.02161$		F
	R_N	CN_N	R_S	CN_S	$R_{S'}$	$CN_{S'}$	
Initial	2.0	2	2.2	1	2.5	1	-----
	2.0056	"	2.2356	"	2.4457	"	0.623402
	1.9858	0.9573	2.2007	1.2182	2.3874	0.4824	0.317458
Initial	2.0	2	2.2	1	2.8	1	-----
	1.9906	"	2.2196	"	2.9593	"	0.896392
	2.0297	1.8404	2.1979	0.5375	2.8764	0.1342	0.360093
Fixed CN's							
	$CN_N = 2$		$CN_S = 1$		$CN_{S'} = 1$		F
	R_N	c_{2N}	R_S	c_{2S}	$R_{S'}$	$c_{2S'}$	
Initial	2.0	-0.02528	2.2	-0.02161	2.5	-0.02161	-----
	2.0271	-0.04380	2.1797	-0.02246	2.3470	-0.04755	0.257595
Initial	2.0	-0.02528	2.2	-0.02161	2.8	-0.02161	-----
	2.0732	-0.03432	2.1722	-0.02502	2.9225	-0.06240	0.302079

N + S + N' :

PCHI.FIL1 :

Fixed c_2 's							
	$c_{2N} = -0.02528$		$c_{2S} = -0.02161$		$c_{2N'} = -0.02528$		F
	R_N	CN_N	R_S	CN_S	$R_{N'}$	$CN_{N'}$	
Initial	2.0	2	2.2	1	2.8	1	-----
	1.9921	"	2.2228	"	2.6798	"	0.538301
	2.0288	1.8401	2.2016	0.5189	2.7060	0.5028	0.101347
	2.0297	"	2.1997	"	2.7165	1.5021*	0.0838297
	[* $c_2 = -0.04865$]						
Fixed CN 's							
	$CN_N = 2$		$CN_S = 1$		$CN_{N'} = 1$		F
	R_N	c_{2N}	R_S	c_{2S}	$R_{N'}$	$c_{2N'}$	
Initial	2.0	-0.02528	2.2	-0.02161	2.8	-0.02528	-----
	2.0759	-0.03141	2.1690	-0.02610	2.7271	-0.04075	0.0592036

PCHI.FIL2 :

Fixed c_2 's							
	$c_{2N} = -0.02528$		$c_{2S} = -0.02161$		$c_{2N'} = -0.02528$		
	R_N	CN_N	R_S	CN_S	$R_{N'}$	$CN_{N'}$	F
Initial	2.0	2	2.2	1	2.8	1	-----
	1.9934	"	2.2211	"	2.8764	"	0.605381
	2.0316	1.8863	2.1990	0.4964	2.7198	0.3538	0.178645
	2.0318	"	2.1969	"	2.7639	7.6189*	0.103527
					[* $c_2 = -0.10007$]		
Fixed CN 's							
	$CN_N = 2$		$CN_S = 1$		$CN_{N'} = 1$		
	R_N	c_{2N}	R_S	c_{2S}	$R_{N'}$	$c_{2N'}$	F
Initial	2.0	-0.02528	2.2	-0.02161	2.8	-0.02528	-----
	2.0792	-0.03132	2.1678	-0.02577	2.7755	-0.04667	0.114648

N + N' + S :

PCHI.FIL3 :

Vary R's only							
	$c_{2N} = -0.02528$		$c_{2N'} = -0.02528$		$c_{2S} = -0.02161$		F
	R_N	CN_N	$R_{N'}$	$CN_{N'}$	R_S	CN_S	
Initial	2.15	1	2.40	1	2.15	1	-----
	2.1081	(1)	2.6267	(1)	2.1709	(1)	0.477251
Fixed c_2 's							
	$c_{2N} = -0.02528$		$c_{2N'} = -0.02528$		$c_{2S} = -0.02161$		F
	R_N	CN_N	$R_{N'}$	$CN_{N'}$	R_S	CN_S	
Initial	2.15	1	2.40	1	2.15	1	-----
	2.0695	8.4489	2.3378	2.3380	2.0275	1.4595	0.214600
Fixed CN 's							
	$CN_N = 1$		$CN_{N'} = 1$		$CN_S = 1$		F
	R_N	c_{2N}	$R_{N'}$	$c_{2N'}$	R_S	c_{2S}	
Initial	2.15	-0.02528	2.4	-0.02528	2.15	-0.02161	-----
	2.1062	-0.02673	2.0390	-0.03260	2.1674	-0.02552	0.330759

N + N' + S + S' :

PCHI.FIL3 :

Vary R's only									
	$c_{2N} = -0.02528$		$c_{2N'} = -0.02528$		$c_{2S} = -0.02161$		$c_{2S'} = -0.02161$		F
	R_N	CN_N	$R_{N'}$	$CN_{N'}$	R_S	CN_S	$R_{S'}$	$CN_{S'}$	
Initial	2.15	1	2.40	0.6	2.15	1	2.9	0.13	-----
	2.1094	(1)	2.5926	(0.6)	2.1699	(1)	2.9116	(0.13)	0.419486
Fixed c_2 's									
	$c_{2N} = -0.02528$		$c_{2N'} = -0.02528$		$c_{2S} = -0.02161$		$c_{2S'} = -0.02161$		F
	R_N	CN_N	$R_{N'}$	$CN_{N'}$	R_S	CN_S	$R_{S'}$	$CN_{S'}$	
Initial	2.15	1	2.40	0.6	2.15	1	2.9	0.13	-----
	2.0916	(1)	2.3732	-0.9257	2.1661	(1)	2.9020	0.1727	0.346906
Fixed CN 's									
	$CN_N = 1$		$CN_{N'} = 0.6$		$CN_S = 1$		$CN_{S'} = 0.13$		F
	R_N	c_{2N}	$R_{N'}$	$c_{2N'}$	R_S	c_{2S}	$R_{S'}$	$c_{2S'}$	
Initial	2.15	-0.02528	2.4	-0.02528	2.15	-0.02161	2.9	-0.02161	-----
	2.1084 (-0.02528)		2.5925	-0.02420	2.1702 (-0.02161)		2.9034	-0.01323	0.411057
Fixed CN 's									
	$CN_N = 1$		$CN_{N'} = 1$		$CN_S = 1$		$CN_{S'} = 1$		F
	R_N	c_{2N}	$R_{N'}$	$c_{2N'}$	R_S	c_{2S}	$R_{S'}$	$c_{2S'}$	
Initial	2.15	-0.02528	2.4	-0.02528	2.15	-0.02161	2.9	-0.02161	-----
	2.1109 (-0.02528)		2.5862	-0.03395	2.1685 (-0.02161)		2.9514	-0.04971	0.397720

Fits of PCLO :

<u>N only :</u>	R	CN	c ₂	F
Initial	1.9, 2.0	3	-0.02528	----
PCLO.FIL0 :	2.0185	2.5357	(-0.02528)	0.399961
	2.0210	1.9444	-0.02074	0.366448
PCLO.FIL1 :	2.0187	2.5608	(-0.02528)	0.442943
	2.0216	1.9001	-0.02021	0.405129
PCLO.FIL2 :	2.0189	2.5732	(-0.02528)	0.467742
	2.0218	1.9000	-0.02013	0.431117

<u>S only :</u>	R	CN	c ₂	F
Initial	2.0, 2.2	3	-0.02161	----
PCLO.FIL0 :	2.1625	0.9153	(-0.02161)	0.250405
	2.1626	0.9214	-0.02171	0.250375
PCLO.FIL1 :	2.1616	0.9256	(-0.02161)	0.297636
	2.1611	0.8330	-0.02004	0.291756
PCLO.FIL2 :	2.1614	0.9271	(-0.02161)	0.345433
	2.1608	0.8154	-0.01970	0.338042

N + S :

	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
PCLO.FIL0 :							
Initial	2.0	2	-0.02528	2.2	2	-0.02161	-----
	1.8214	"	"	2.1673	"	"	1.58810
	1.8471	-0.3484	"	2.1601	0.8616	"	0.173218
	1.8735	-0.2489	-0.01424	2.1590	1.1207	-0.02623	0.145435
PCLO.FIL1 :							
Initial	2.0	2	-0.02528	2.2	1	-0.02161	-----
	1.9644	"	"	2.1972	"	"	0.884473
	2.1615	1.9275	"	2.1447	1.3502	"	0.0936219
	2.1471	1.7627	-0.02449	2.1403	1.1668	-0.02040	0.0756268
	2.1372	(1)	-0.02379	2.1453	(1)	-0.02042	0.138895
	2.1290	(2)	-0.03591	2.1453	(1)	-0.02112	0.0946570
	2.1285	(3)	-0.05201	2.1509	(1)	-0.02171	0.133749
	2.3059	(1)	-0.01230	2.2005	(2)	-0.03125	0.0819654
	2.2652	(2)	-0.01816	2.1893	(2)	-0.02495	0.139340
Initial	2.0	1	-0.02528	2.2	2	-0.02161	-----
	1.8085	"	"	2.1647	"	"	1.41993
	1.8559	-0.3635	"	2.1584	0.8765	"	0.232731
	1.8620	-0.3410	-0.02317	2.1581	0.9109	-0.02223	0.232553
	1.5304	(1)	-0.09279	2.1612	(1)	-0.02237	0.295742
	2.1290	(2)	-0.03591	2.1453	(1)	-0.02112	0.0946571
	2.1285	(3)	-0.05200	2.1509	(1)	-0.02170	0.133749
	1.6686	(1)	-0.04401	2.1622	(2)	-0.03308	0.482981
	1.6465	(2)	-0.06356	2.1622	(2)	-0.03284	0.479582

	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
PCLO.FIL2 :							
Initial	2.0	1	-0.02528	2.2	1	-0.02161	-----
	1.9702	"	"	2.1765	"	"	0.583442
	2.1724	2.1481	"	2.1474	1.4496	"	0.162931
	2.1553	1.8842	-0.02204	2.1400	1.2364	-0.01957	0.150964
	2.1425	(1)	-0.02241	2.1449	(1)	-0.01986	0.204154
	2.1301	(2)	-0.03395	2.1437	(1)	-0.02080	0.164210
	2.1297	(3)	-0.05019	2.1500	(1)	-0.02152	0.185921
	2.3063	(1)	-0.01194	2.2015	(2)	-0.03122	0.152135
	2.2662	(2)	-0.01772	2.1903	(2)	-0.02481	0.191943
Initial	2.0	1	-0.02528	2.2	2	-0.02161	-----
	1.8078	"	"	2.1645	"	"	1.42996
	1.8573	-0.3732	"	2.1581	0.8780	"	0.288522
PCLO.FIL3 :							
Initial	2.0	2	-0.02528	2.2	1	-0.02161	-----
	1.9587	"	"	2.1948	"	"	0.908152
	2.1582	2.3640	"	2.1407	1.4811	"	0.336404
	2.1053	2.5088	-0.01352	2.0905	1.2693	-0.01700	0.289273
Initial	2.2	1	-0.02528	2.2	1	-0.02161	-----
	2.1144	"	"	2.1432	"	"	0.387362
	2.1150	(1)	-0.01718	2.1311	(1)	-0.02042	0.377158

N + N' :

	R _N	CN _N	c _{2N}	R _{N'}	CN _{N'}	c _{2N'}	F
PCLO.FIL0 :							
Initial	2.0	2	-0.02528	2.2	2	-0.02528	-----
	1.9691	"	"	2.0696	"	"	0.575192
	2.0186	0.6003	"	2.0185	1.9354	"	0.399961
	2.0621	0.8075	-0.00931	1.9512	1.3789	-0.02159	0.119313
PCLO.FIL1 :							
Initial	2.0	2	-0.02528	2.2	2	-0.02528	-----
	2.0305	"	"	2.3685	"	"	0.544147
	2.0255	2.5835	"	2.3534	0.9337	"	0.324132
	2.0168	2.4271	-0.02614	2.3560	0.1359	+0.00142	0.203679
Initial	2.2	2	-0.02528	2.6	2	-0.02528	-----
	2.0165	"	"	2.6841	"	"	0.653670
	2.0185	2.5554	"	2.6821	0.4364	"	0.426829
	2.0187	1.8495	-0.01985	2.6609	0.0287	+0.01245	0.308331
PCLO.FIL2 :							
Initial	2.0	2	-0.02528	2.2	2	-0.02528	-----
	2.0309	"	"	2.3669	"	"	0.569470
	2.0260	2.6100	"	2.3506	0.9423	"	0.356093
	2.0166	2.5137	-0.02680	2.3530	0.1158	+0.00335	0.235593
Initial	2.2	2	-0.02528	2.6	2	-0.02528	-----
	2.0166	"	"	2.6790	"	"	0.711757
	2.0187	2.5692	"	2.6760	0.2552	"	0.462544
	2.0183	1.8886	-0.02018	2.6516	0.0160	+0.01775	0.326867

S + S' :

	R _S	CN _S	c _{2S}	R _{S'}	CN _{S'}	c _{2S'}	F
PCLO.FILO :							
Initial	2.0	1	-0.02161	2.2	1	-0.02161	-----
	2.1126	"	"	2.2413	"	"	0.584524
	2.1624	1.1098	"	2.3479	0.3051	"	0.0961859
	2.1432	0.6282	-0.01680	2.2528	0.6050	-0.02925	0.0686127
PCLO.FIL1 :							
Initial	2.0	1	-0.02161	2.2	1	-0.02161	-----
	2.1121	"	"	2.2391	"	"	0.651926
	2.1661	1.1643	"	2.3695	0.3890	"	0.0764028
	2.1548	0.9637	-0.02016	2.3231	0.8132	-0.03902	0.0533031
Initial	2.2	1	-0.02161	2.6	1	-0.02161	-----
	2.1598	"	"	2.8123	"	"	0.757800
	2.1614	0.9221	"	2.7756	0.1463	"	0.271934
	2.1601	0.7649	-0.01900	2.8546	2.0684	-0.07725	0.230939
PCLO.FIL2 :							
Initial	2.0	1	-0.02161	2.2	1	-0.02161	-----
	2.1123	"	"	2.2391	"	"	0.680619
	2.1664	1.1829	"	2.3705	0.4185	"	0.153913
	2.1544	0.9564	-0.01989	2.3229	0.8485	-0.03867	0.147856
Initial	2.2	1	-0.02161	2.6	1	-0.02161	-----
	2.1619	"	"	2.7510	"	"	0.807994
	2.1615	0.9245	"	2.7405	0.1365	"	0.325527
	2.1622	0.8630	-0.02056	2.7111	0.0438	-0.00591	0.311699

N + S + S' :

PCLO.FIL1 :

Fixed c_2 's							
	$c_{2N} = -0.02528$		$c_{2S} = -0.02161$		$c_{2S'} = -0.02161$		
	R_N	CN_N	R_S	CN_S	$R_{S'}$	$CN_{S'}$	F
Initial	2.2	1	2.2	1	2.5	1	-----
	1.9838	"	2.1972	"	2.4170	"	0.543114
	2.0247	0.4289	2.1662	0.9663	2.3806	0.3007	0.0604921
Initial	2.2	1	2.2	1	2.6	1	-----
	2.2998	"	2.1756	"	2.7382	"	0.750976
Initial	2.0	1	2.2	1	2.6	1	-----
	2.1158	"	2.1417	"	2.8242	"	0.724957
	2.1044	1.4764	2.1340	0.9445	2.8011	0.1017	0.0592774
	2.1044	"	2.1339	"	2.7991	0.0893*	0.0591259
					[* $c_{2S'} = -0.01971$]		
Fixed CN 's							
	$CN_N = 1$		$CN_S = 1$		$CN_{S'} = 1$		
	R_N	c_{2N}	R_S	c_{2S}	$R_{S'}$	$c_{2S'}$	F
Initial	2.2	-0.02528	2.2	-0.02161	2.5	-0.02161	-----
	1.7500	-0.09796	2.1544	-0.02057	2.3222	-0.04199	0.0394407
Initial	2.0	-0.02528	2.2	-0.02161	2.6	-0.02161	-----
	2.1470	-0.02443	2.1473	-0.02011	2.8353	-0.06972	0.0961449

	CN _N = 2		CN _S = 1		CN _{S'} = 1		F
	R _N	c _{2N}	R _S	c _{2S}	R _{S'}	c _{2S'}	
Initial	2.0	-0.02528	2.2	-0.02161	2.5	-0.02161	-----
	1.9820	"	2.2162	"	2.4265	"	0.535456
	1.7545	-0.12775	2.1547	-0.02057	2.3239	-0.04190	0.0395483
Initial	2.0	-0.02528	2.2	-0.02161	2.6	-0.02161	-----
	1.9629	"	2.1976	"	2.7850	"	1.15020
	2.1429	-0.03901	2.1491	-0.02092	2.7847	-0.07436	0.0438933

PCLO.FIL2 :

Fixed c ₂ 's							
	c _{2N} = -0.02528		c _{2S} = -0.02161		c _{2S'} = -0.02161		F
	R _N	CN _N	R _S	CN _S	R _{S'}	CN _{S'}	
Initial	2.1	1	2.1	1	2.5	1	-----
	1.9850	"	2.1961	"	2.4151	"	0.549125
	2.0237	0.3432	2.1666	1.0247	2.3783	0.3483	0.149209
	2.0433	"	2.1614	"	2.3685	0.4314*	0.148365
					[*c _{2S'} = -0.02635]		
Initial	2.2	1	2.2	1	2.6	1	-----
	2.2999	"	2.1754	"	2.7220	"	0.747763
	2.1945	2.2600	2.1556	1.5355	2.6955	0.0965	0.149291
	2.1936	"	2.1554	"	2.6974	0.0709#	0.148648
					[#c _{2S'} = -0.01729]		

Fixed CN's							
	CN _N = 1		CN _S = 1		CN _{S'} = 1		F
	R _N	c _{2N}	R _S	c _{2S}	R _{S'}	c _{2S'}	
Initial	2.1	-0.02528	2.1	-0.02161	2.5	-0.02161	-----
	1.8028	-0.10862	2.1549	-0.02034	2.3277	-0.04069	0.146289
Initial	2.2	-0.02528	2.2	-0.02161	2.6	-0.02161	-----
	2.1973	-0.00614	2.1574	-0.01187	2.9765	-0.08189	0.162084
	CN _N = 2		CN _S = 1		CN _{S'} = 1		F
	R _N	c _{2N}	R _S	c _{2S}	R _{S'}	c _{2S'}	
Initial	2.1	-0.02528	2.1	-0.02161	2.5	-0.02161	-----
	2.0851	-0.01872	2.0993	-0.02245	2.4104	-0.08165	0.174820
Initial	2.1	-0.02528	2.1	-0.02161	2.6	-0.02161	-----
	1.8288	-0.14572	2.1551	-0.02039	2.3296	-0.04104	0.146606

PCLO.FIL3 :

Fixed c ₂ 's							
	c _{2N} = -0.02528		c _{2S} = -0.02161		c _{2S'} = -0.02161		F
	R _N	CN _N	R _S	CN _S	R _{S'}	CN _{S'}	
Initial	2.0	1	2.2	1	2.5	1	-----
	1.9799	"	2.1951	"	2.4148	"	0.660304
	1.8533	0.4149	2.1683	1.4006	2.3670	0.6005	0.301035
Initial	2.0	1	2.2	1	2.6	1	-----
	1.9563	"	2.1750	"	2.8029	"	0.951849
	2.1548	2.2829	2.1397	1.4438	2.8117	0.0537	0.333885
Fixed CN's							
	CN _N = 1		CN _S = 1		CN _{S'} = 1		F
	R _N	c _{2N}	R _S	c _{2S}	R _{S'}	c _{2S'}	
Initial	2.2	-0.02528	2.2	-0.02161	2.5	-0.02161	-----
	1.8953	-0.03594	2.1611	-0.01854	2.3373	-0.03278	0.291028
Initial	2.2	-0.02528	2.2	-0.02161	2.6	-0.02161	-----
	2.3027	"	2.1744	"	2.7349	"	0.849961
	2.1109	-0.01647	2.1283	-0.02065	2.8622	-0.06505	0.354557

N + S + N' :

PCLO.FIL1

Fixed c_2 's							
	$c_{2N} = -0.02528$		$c_{2S} = -0.02161$		$c_{2N'} = -0.02528$		
	R_N	CN_N	R_S	CN_S	$R_{N'}$	$CN_{N'}$	F
Initial	2.0	1	2.2	1	2.5	1	-----
	1.9633	"	2.1787	"	2.6341	"	0.510012
	2.0900	1.2523	2.1375	0.8462	2.6560	0.3982	0.0256428
Initial	2.0	1	2.2	1	2.35	1	-----
	2.0657	"	2.1479	"	2.2063	"	0.107831
	2.1247	1.4735	2.1413	1.1693	2.2256	0.4279	0.0790783
	2.1342	"	2.1460	"	2.2611	0.2155*	0.0768155
					[* $c_{2N'} = -0.01411$]		
Fixed CN 's							
	$CN_N = 1$		$CN_S = 1$		$CN_{N'} = 1$		
	R_N	c_{2N}	R_S	c_{2S}	$R_{N'}$	$c_{2N'}$	F
Initial	2.0	-0.02528	2.2	-0.02161	2.5	-0.02528	-----
	2.1391	-0.02630	2.1477	-0.02064	2.6388	-0.04286	0.0701327
Initial	2.0	-0.02528	2.2	-0.02161	2.35	-0.02528	-----
	2.1108	-0.04604	2.1350	-0.01980	2.1305	-0.01953	0.0823582

PCLO.FIL2

Fixed c_2 's							
	$c_{2N} = -0.02528$		$c_{2S} = -0.02161$		$c_{2N'} = -0.02528$		
	R_N	CN_N	R_S	CN_S	$R_{N'}$	$CN_{N'}$	F
Initial	2.0	1	2.2	1	2.8	1	-----
	1.9690	"	2.1771	"	2.9136	"	0.586307
	2.1735	2.2397	2.1475	1.4776	2.9230	0.4552	0.126604
	2.1737	"	2.1476	"	2.9270	0.3261*	0.124790
					[* $c_{2N'} = -0.01952$]		
Initial	2.0	2	2.2	1	2.5	1	-----
	1.9650	"	2.1782	"	2.6265	"	0.554268
	2.2061	2.2805	2.1596	1.5588	2.5452	0.3492	0.155786
	2.2040	"	2.1594	"	2.5531	0.1824†	0.153544
					[† $c_{2N'} = -0.01589$]		
Initial	2.0	2	2.2	1	2.35	1	-----
	2.0659	"	2.1481	"	2.2077	"	0.172550
	2.1337	1.7369	2.1409	1.2420	2.2434	0.4025	0.154740
	2.1484	"	2.1455	"	2.2875	0.1040‡	0.152302
					[‡ $c_{2N'} = -0.00844$]		
Fixed CN 's							
	$CN_N = 1$		$CN_S = 1$		$CN_{N'} = 1$		
	R_N	c_{2N}	R_S	c_{2S}	$R_{N'}$	$c_{2N'}$	F
Initial	2.0	-0.02528	2.2	-0.02161	2.8	-0.02528	-----
	2.1393	-0.02279	2.1446	-0.02006	2.7283	-0.06890	0.183819
Initial	2.0	-0.02528	2.2	-0.02161	2.5	-0.02528	-----
	2.1393	-0.02276	2.1445	-0.02006	2.7276	-0.06892	0.183819
Initial	2.0	-0.02528	2.2	-0.02161	2.35	-0.02528	-----
	2.1027	-0.04155	2.1334	-0.01824	2.1414	-0.01636	0.153919

Appendix II. Error Analysis for Laccase Difference Edge Analysis

The following error analysis was originally carried out for the paper published in 1990. (Cole, J. L.; Tan, G. O.; Yang, E. K.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1990**, *112*, 2243-2249.) As such, errors were only calculated for samples that were measured in 1987 and 1989, i.e. T2D1, T2D2 and T2D3 (and also T2DS and T26A, old data sets) for T2D laccase and 1ML01, 2ML01, MLRD and MLOX for T1Hg laccase (see Tables 6.1 and 6.2 in Chapter 6). Some inconsistencies in the original error analysis have been remedied, so that errors are now a little higher than originally reported.

Errors may be related either by addition or by multiplication. The propagation of errors was treated according to the following equations (from *Data Reduction and Error Analysis for the Physical Sciences* by Philip R. Bevington, McGraw-Hill, New York, pp. 56–65) :

Where x is the weighted sum of u and v , i.e., $x = au \pm bv$, then

$$\sigma_x^2 = a^2 \sigma_u^2 + b^2 \sigma_v^2, \text{ where } u \text{ and } v \text{ are independent} \quad \dots\dots\dots(\text{II.1})$$

Where x is the weighted product of u and v , i.e. $x = \pm auv$, then

$$\sigma_x^2 = a^2 v^2 \sigma_u^2 + a^2 u^2 \sigma_v^2, \text{ or } \frac{\sigma_x^2}{x^2} = \frac{\sigma_u^2}{u^2} + \frac{\sigma_v^2}{v^2},$$

where u and v are independent $\dots\dots\dots(\text{II.2})$

Where x is obtained by division, i.e. $x = \pm \frac{au}{v}$,

$$\frac{\sigma_x^2}{x^2} = \frac{\sigma_u^2}{u^2} + \frac{\sigma_v^2}{v^2}, \text{ where } u \text{ and } v \text{ are independent} \quad \dots\dots\dots(\text{II.3})$$

Sources of error :

- (1) Error from the noise of the data, based on measuring the half-height of typical noise.
At most (in 1989), it is $\pm 1\%$ of the normalized amplitude. More usually, it is $\pm 0.5\%$.
- (2) Spline or normalization error. A conservative estimate is $\pm 5\%$ of the normalized amplitude (from the notes of James E. Penner-Hahn).

(3) Error in estimating content of native laccase in T2D and T1Hg samples, as measured by optical or EPR spectroscopy. Error is proportional to the amount of native present, and it is usually ~5% of all laccase present. However, this error can be neglected in most cases because the native edge used to correct the T2D or T1Hg edge is rather similar to the latter (i.e., a reduced edge is used to correct reduced samples, and an oxidized edge for oxidized samples), and so applying the correction makes very little difference to the shape of the edge, or its amplitude, as measured at 8984 eV. The only cases where this is not true is in the corrections applied to T2D2 and T2D3 (and T2DS) (see Table 6.2), where a largely oxidized edge is used to correct largely reduced edges. In this case, native is $10 \pm 2\%$ (6-7% for T2DS) of all laccase.

(4) Error in determining the redox state of "standard" samples. This is measured by optical or EPR spectroscopy, and is proportional to the signal measured. This error is:

Considered 0% error for fully oxidized (H₂O₂-treated) samples.

For reduced T2D (T2D1) : 0% (because no laccase was oxidized).

For reduced T1Hg (MLRD) : $10 \pm 2\%$ of T2 was oxidized, ~10% of T3 was oxidized. So, in terms of total Cu : $\sigma(T2) = \left(\frac{1}{3}\right) \times 0.02$, $\sigma(T3) = \left(\frac{2}{3}\right) \times 0.1$.

Errors not accounted for in this analysis :

(5) Systematic error in estimation of fully oxidized edges = 8% of all Cu present (from Kau, L.-S.; Spira-Solomon, D. J.; Penner-Hahn, J. E.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1987**, *109*, 6433-6442). This is the error involved in judging a laccase sample (e.g. T1Hg + 60× H₂O₂) to be fully oxidized when the edge is the only spectroscopic criterion used.

(6) Systematic error in estimation of fully reduced edges due to the range in amplitude of Cu(I) models at ~8984 eV (*ibid.*). This is the error involved in judging a laccase sample (e.g., fully reduced native laccase) to be fully reduced when the edge is the only spectroscopic criterion used.

(7) Systematic error due to range in heights (at 8984 eV) of various resting T2D samples (including T2DS), and various fully oxidized native laccase samples, run in the past (unpublished data).

- (8) Error in measuring the edge amplitudes at 8984 eV. This was done by hand, using a pencil and a ruler marked in mm. The error in an individual measurement was about 0.5 mm. The impact this had on the amplitude calculated depended on the scale over which the graph was plotted, heights measured were usually about 50 mm when plotted.

Propagation of errors :

Errors in 100% oxidized edges (T26A and 2ML01) :

Only the spline and the noise contribute errors. So :

Normalized signal $E_N = SE_R$, where S = spline scaling factor, E_R = raw signal
(II.4)

Amplitude of oxidized edges at 8984 eV is ~0.1 normalized units, so the spline error is scaled down by this amount, while the noise error is not reduced.

So, using Equation II.2,

$$\begin{aligned} \text{Error in totally oxidized laccase edge } \frac{\sigma_{E_N}}{E_N} &= \sqrt{\left(\frac{\sigma_{E_R}}{E_R}\right)^2 + \left(\frac{\sigma_S}{S}\right)^2} \\ &= \sqrt{\left(\frac{0.005}{0.1}\right)^2 + (0.05)^2} = 0.071 \equiv 7\% \text{ (of normalized amplitude)} \end{aligned}$$

Errors in 100% reduced edges :

Amplitude of reduced edges (both T2D and T1Hg) at 8984 eV is ~0.6 units, so the spline error is scaled down by this amount, while the noise error is not reduced.

Amplitude of T2DS at 8984 eV is ~0.5 units.

(a) For fully reduced T2D (T2D1) :

Only the spline and the noise contribute errors. So, using Equations II.2 and II.4,

$$\begin{aligned} \text{Error in T2D1 } \frac{\sigma_{E_N}}{E_N} &= \sqrt{\left(\frac{\sigma_{E_R}}{E_R}\right)^2 + \left(\frac{\sigma_S}{S}\right)^2} \\ &= \sqrt{\left(\frac{0.005}{0.6}\right)^2 + 0.05^2} = 0.051 \equiv 5\% \text{ (of amplitude at 8984 eV)} \end{aligned}$$

(b) For fully reduced T1Hg (MLRD) :

Errors are from the spline, the noise and the redox state. So

Normalized signal $E_N = SRE_R$, where S = spline scaling factor, E_R = raw signal
and R = proportion of reduced Cu.
.....(II.5)

Using Equation II.1,

$$\begin{aligned} \text{Error in redox state of MLRD} &= \sigma_R = \sqrt{a^2 \sigma_{R_1}^2 + b^2 \sigma_{R_2}^2} \\ &= \sqrt{\left(\frac{1}{3}\right)^2 0.02^2 + \left(\frac{2}{3}\right)^2 0.1^2} = 0.067 \equiv 7\% \text{ (of total Cu)} \end{aligned}$$

Using Equation II.2,

$$\begin{aligned} \text{Error in MLRD} &= \frac{\sigma_{E_N}}{E_N} = \sqrt{\left(\frac{\sigma_S}{S}\right)^2 + \left(\frac{\sigma_R}{R}\right)^2 + \left(\frac{\sigma_{E_R}}{E_R}\right)^2} \\ &= \sqrt{0.05^2 + 0.067^2 + \left(\frac{0.01}{0.6}\right)^2} = 0.085 \equiv 9\% \text{ (of amplitude at 8984 eV)} \end{aligned}$$

(c) For resting T2D laccase ($\equiv$ "fully reduced T3 Cu") :

The native correction cannot be neglected in this case. So

$$\text{Corrected T2DS} = 1.111[\text{T2DS}] - 0.111[\text{NTVL}] \quad (\text{Equation 6.2 in Chapter 6})$$

$$\begin{aligned} \text{The combined spline and noise error in NTVL} &= \frac{\sigma_{E_N}}{E_N} = \sqrt{\left(\frac{\sigma_{E_R}}{E_R}\right)^2 + \left(\frac{\sigma_S}{S}\right)^2} \\ &= \sqrt{\left(\frac{0.005}{0.2}\right)^2 + 0.05^2} = 0.056 \equiv 6\% \end{aligned}$$

The combined spline and noise error in T2DS is approximated by the error found for T2D1 above ($= 0.051$).

Error in corrected T2DS

$$= \sqrt{1.111^2(0.051)^2 + 0.111^2(0.056)^2} = 0.057 \equiv 6\% \text{ (of normalized amp.)}$$

Errors in 100% reduced difference edge standards :

100% reduced difference edge $\Delta E_{100} = E_{\text{red}} - E_{\text{ox}}$, where E_{red} is the reduced edge
and E_{ox} is the oxidized edge.

$$\text{So } \sigma_{\Delta E_{100}} = \sqrt{(\sigma_{E_{\text{red}}})^2 + (\sigma_{E_{\text{ox}}})^2} \quad (\text{from Equation II.1})$$

(a) 100% reduced $\Delta T2D$ laccase :

$$\text{Error in (T2D1 - T26A)} = \sqrt{(0.051)^2 + (0.071)^2} = 0.087 \equiv 9\% \text{ (of amp. at 8984 eV)}$$

(b) 100% reduced $\Delta T1Hg$ laccase :

$$\text{Error in (MLRD - 2ML01)} = \sqrt{(0.085)^2 + (0.071)^2} = 0.111 \equiv 11\% \text{ (amp. at 8984 eV)}$$

(c) 100% reduced $\Delta T3$ Cu :

$$\text{Error in } 1.5(\text{T2DS} - \text{T26A}) = \sqrt{1.5^2[(0.057)^2 + (0.071)^2]} = 0.136 \equiv 14\% \\ \text{(of amplitude at 8984 eV)}$$

Errors in difference edges of reoxidized samples (T2D2-T2D3 series or MLOX) :

Difference edge $\Delta E_D = E_N - E_S$, where E_N = edge with redox state to be determined
 E_S = standard (100% oxidized) edge

(a) Samples close to totally reduced (T2D2 and T2D3):

Correction for native must be first taken into account (*vide supra*). $10 \pm 2\%$ of native were subtracted and the edge renormalized in the following equation :

$$\text{Corrected T2D2 or T2D3} = 1.1481[\text{T2D}(2,3)] - 0.1481[\text{NTVL}] \\ \text{(Equation 6.2 in Chapter 6)}$$

The combined spline and noise error in T2D2, T2D3 is approximated by the error found above for T2D1 (0.051 units).

So, using Equation II.1, error due to native correction in E_N (T2D2 and T2D3)

$$= \sqrt{1.1481^2(0.051)^2 + 0.1481^2(0.056)^2} = 0.059 \equiv 6\% \text{ (of normalized amp.)}$$

Then, error in $\Delta E_D = \sigma_{E_D} = \sqrt{\sigma_{E_N}^2 + \sigma_{E_S}^2}$
 $= \sqrt{0.059^2 + 0.071^2} = 0.092 \equiv 9\%$ (of normalized amplitude at 8984 eV)

(b) Samples close to totally oxidized (MLOX):

Amplitudes of both E_N and E_S are ~ 0.1 at 8984 eV. Spline error σ_S is scaled down to 0.5%, while the noise error σ_R remains at 0.5%.

For spline and noise error (Equation II.3),

$$\begin{aligned} \frac{\sigma_{E_S}}{E_S} &= \sqrt{\left(\frac{\sigma_S}{S}\right)^2 + \left(\frac{\sigma_{E_R}}{E_R}\right)^2} \\ &= \sqrt{(0.05)^2 + \left(\frac{0.005}{0.1}\right)^2} = 0.071 \equiv 7\% \text{ (of normalized amplitude at 8984 eV)} \end{aligned}$$

$$\begin{aligned} \frac{\sigma_{E_N}}{E_N} &= \sqrt{\left(\frac{\sigma_S}{S}\right)^2 + \left(\frac{\sigma_{E_R}}{E_R}\right)^2} \\ &= \sqrt{(0.05)^2 + \left(\frac{0.01}{0.1}\right)^2} = 0.112 \equiv 11\% \text{ (of normalized amplitude at 8984 eV)} \end{aligned}$$

So, error in (MLOX - 2ML01) difference edge

$$\begin{aligned} \sigma_{\Delta E_D} &= \sqrt{\sigma_{E_N}^2 + \sigma_{E_S}^2} \\ &= \sqrt{(0.071)^2 + (0.112)^2} \\ &= 0.132 \equiv 13.2\% \text{ (of normalized amplitude at 8984 eV)} \end{aligned}$$

Errors in quantitating proportion of reduced Cu :

Proportion of Cu(I) determined, "Cu" = $\frac{\Delta E_D}{\Delta E_{100}}$

So, using Equation II.3, $\frac{\sigma_{\text{"Cu"}}^2}{\text{"Cu"}^2} = \frac{\sigma_{\Delta E_D}^2}{\Delta E_D^2} + \frac{\sigma_{\Delta E_{100}}^2}{\Delta E_{100}^2}$

Since the errors for E_D and E_{100} already take into account the amplitudes of the component edges at 8984 eV, the errors are divided by $\Delta E_D / E_N$ and $\Delta E_{100} / E_{\text{red}}$ rather than just ΔE_D or ΔE_{100} .

(a) For the T2D series (T2D2 and T2D3) :

Error in proportion of Cu determined in T2D3 (T2D2 is taken to be similar)

$$= \frac{\sigma_{\text{"Cu"}}}{\text{"Cu"}} = \sqrt{\left(\frac{0.092}{0.4/0.5}\right)^2 + \left(\frac{0.087}{0.5/0.6}\right)^2} = 0.155 \equiv 16\% \text{ (of Cu(I) determined)}$$

This works out to a Cu(I) content of $76 \pm 12\%$ in T2D laccase exposed for 63 hours (T2D3).

Error in proportion of T3 Cu determined in T2D3

$$= \frac{\sigma_{\text{"T3 Cu"}}}{\frac{2}{3}\text{"T3 Cu"}} = \sqrt{\left(\frac{0.092}{0.4/0.5}\right)^2 + \left(\frac{\frac{2}{3} \times 0.136}{0.4/0.5}\right)^2} = 0.161 \equiv 16\% \text{ (of Cu(I) determined)}$$

This works out to a Cu(I) content of $88 \pm 14\%$ of T3 Cu in T2D laccase exposed for 63 hours (T2D3).

(b) For T1Hg reoxidized (MLOX) :

Error in proportion of Cu determined

$$= \frac{\sigma_{\text{"Cu"}}}{\text{"Cu"}} = \sqrt{\left(\frac{0.132}{0.01/0.1}\right)^2 + \left(\frac{0.111}{0.5/0.6}\right)^2} = 1.329 \equiv 133\% \text{ (of Cu(I) determined)}$$

This works out to a Cu(I) content of $2 \pm 3\%$ in reoxidized T1Hg laccase (MLOX).

Appendix III. Complete Tables of Fits to EXAFS Spectra of Oxidized and Reduced *Rhodospirillum rubrum* Ni CODH and Associated Model Compounds

The following tables list the distances (R), coordination numbers (CN), c_2 values and fit indices (F) for the fits made for the EXAFS curve-fitting results discussed in Chapter 7. In the parameterized EXAFS equation employed by our analysis package (see Chapter 2), c_2 values vary in the same way as the squared term σ_{as}^2 in the Debye-Waller factor, such that $\Delta c_2 = -2\Delta\sigma_{as}^2$. Thus, though the sign is reversed, the magnitude of c_2 is proportional to σ_{as}^2 .

When using the fit index F to evaluate the goodness of a fit to the data, we have to bear in mind that F is not adjusted for the size of the signal. Thus, for equally good fits (as judged by viewing the match of phase and amplitude), we would expect the fit indices for fits to a larger EXAFS signal to be larger than those for fits to smaller EXAFS signals. So the fit index F should only be used to compare different fits made on the same data.

The windows used on the data sets are denoted by their data file names. Thus, fits to CODHX.FIL2 correspond to fits on Window 2 of CODHX, CODHX.FIL3 contains data filtered using Window 3, etc.

The tables also show what initial R, CN and c_2 values were used for beginning each fit. Unless otherwise stated, a fit result shown had as its initial values the results of the fit shown in the line immediately above. Thus, in a given table (e.g., N+S fits on CODHX.FIL2), the values listed as initial values were first used in a fit varying R's only. Then the results of this fit were in turn used in a fit varying R and CN (referred to as Type 2 fits in the text), whose results are listed below it. The results of this Type 2 fit were in turn used as initial values for the Type 1 fit (varying R's, CN's and c_2 's) whose results are listed immediately below. An exception to this is that Type 3 fits (varying R's and c_2 's), listed below Type 1 fits, generally used the results of fits varying R only as initial values. Bracketed CN or c_2 values were held fixed at the values shown.

List of Tables :

One-shell N or S fits of Ni models	205
Results of fitting Ni-N,S models with N & S.....	208
Fits to (Et ₄ N) ₃ [NiFe ₃ S ₄ (SEt) ₄].....	216
Results of fitting Ni CODH with N & S	219
Three-Wave Fits of CODH data, using N, S and Fe	241

One-shell N or S fits of Ni models

Grace Tan

2nd Feb 1993

(fits checked and correct on 3rd Feb 1993)

These fits are discussed in Chapter 7, Section (E.1.a).

Parameters used in fits :

Ni^{II}-N : [Ni^{II}(dimethylglyoxime)₂], 10 K, $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$ (data to $k = 15 \text{ \AA}^{-1}$)
 Ni^{II}-S : Ni^{II}(S₂CN(CH₂CH₃)₂)₂, 10 K, $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$ (data to $k = 15 \text{ \AA}^{-1}$)

Ni-N compounds :

		R(cryst)	Coord.
NBC004	[Ni(isobacteriochlorin)]	1.915–1.934, av. 1.921	sq pl
NPC004	[Ni(phthalocyanine)]	-----	sq pl
NTP004	[Ni(tetraphenylporphyrin)]	-----	sq pl
NTM004	[Ni(tetramethylcyclam)](ClO ₄) ₂	-----	sq pl
NC4004	[Ni(cyclops)](ClO ₄)	-----	sq pl
NC5004	[Ni(cyclops)(py)](ClO ₄)	1.859–1.897, av. 1.874*	sq py
N33004	[Ni(tropocoronand-3,3)]	1.854–1.868, av. 1.861	dist. sq pl
N66004	[Ni(tropocoronand-6,6)]	1.944–1.950, av. 1.947	dist. T _d
N6C004	[Ni(corphin)NCS] ₂	-----	O _h
NEN004	[Ni(ethylenediamine) ₃] ²⁺	2.124	O _h
NPH004	[Ni(phen) ₃]Cl ₂ ·7H ₂ O	2.078–2.106, av. 2.090	O _h
NTA004	[Ni([9]aneN ₃) ₂](ClO ₄) ₂	2.093–2.116, av. 2.1052	O _h
N4C004#	[Ni(octaethylpyrrocorphin)]	1.899–1.914, av. 1.909	sq pl

* — for equatorial N, from crystal structure of [Ni(cyclops)I](ClO₄)

— data extremely noisy, no fits done

Ni-S compounds :

		R(cryst)	Coord.
N2M004	[(n-Bu) ₄ N] ₂ [Ni(mnt) ₂]	2.173–2.176, av. 2.175	sq pl
N3M004	[(n-Bu) ₄ N][Ni(mnt) ₂]	2.147–2.151, av. 2.149	sq pl
NI2S	[Ni(SC ₂ H ₄ SC ₂ H ₄ S)] ₂	2.14–2.22, av. 2.18	sq pl*
NS4004	[Ni(S-Ph) ₄] ²⁻	2.272–2.303, av. 2.288	T _d
NTT004	[Ni([9]aneS ₃) ₂] ²⁺	2.377–2.400, av. 2.386	O _h

* — 2 square planar coordinated Ni hinged together with folding angle of 111.3°

N only :

	R	CN	c ₂	F
NBC004	1.9 1.9107 1.9119 1.9111	4 3.3206 5.0781 (4)	-0.02297 " -0.02987 -0.02652	----- 0.467720 0.206093 0.302562
NPC004	1.9 1.8764 1.8768 1.8764	4 4.0022 4.6482 (4)	-0.02297 " -0.02534 -0.02338	----- 0.250106 0.171814 0.238654
NTM004	1.9 1.9607 1.9608 1.9609	4 3.3551 3.8249 (4)	-0.02297 " -0.02506 -0.02566	----- 0.409008 0.389323 0.391376
NTP004	1.9 1.9111 1.9114 1.9108	4 4.4215 5.4100 (4)	-0.02297 " -0.02617 -0.02232	----- 0.344177 0.220816 0.415830
NC4004	1.9 1.8595 1.8594 1.8594	4 4.1748 4.0824 (4)	-0.02297 " -0.02262 -0.02236	----- 0.331356 0.330108 0.330946
NC5004	1.9 1.8560 1.8557 1.8567	5 3.6654 3.1738 (5)	-0.02297 " -0.02074 -0.02690	----- 0.298606 0.248012 0.554885
N33004	1.9 1.8469 1.8469 1.8467	4 4.2980 4.3164 (4)	-0.02297 " -0.02304 -0.02206	----- 0.180921 0.180831 0.202419
N66004	2.0 1.9314 1.9315 1.9313	4 3.7921 4.6106 (4)	-0.02297 " -0.02608 -0.02421	----- 0.282243 0.181014 0.231207
N6C004	1.9 2.0705 2.0706 2.0703	6 5.2572 7.5424 (6)	-0.02297 " -0.02886 -0.02565	----- 0.529425 0.257256 0.375924
NEN004	2.1 2.1163 2.1182 2.1176	6 4.7950 6.5761 (6)	-0.02297 " -0.02812 -0.02680	----- 0.786185 0.706368 0.712755

	R	CN	c ₂	F
NPH004	2.1	6	-0.02297	-----
	2.0791	5.1554	"	0.632345
	2.0793	5.8675	-0.02502	0.609498
	2.0793	(6)	-0.02532	0.610100

NTA004	2.1	6	-0.02297	-----
	2.1054	4.3436	"	0.671186
	2.1067	5.4507	-0.02663	0.631083
	2.1073	(6)	-0.02803	0.637859

S only :

	R	CN	c ₂	F
N2M004	2.2	4	-0.02044	-----
	2.1750	4.1131	"	0.464713
	2.1750	4.0588	-0.02023	0.463380
	2.1750	(4)	-0.02005	0.464650

N3M004	2.2	4	-0.02044	-----
	2.1425	4.3384	"	0.571261
	2.1425	4.1975	-0.01993	0.563203
	2.1425	(4)	-0.01934	0.576458

NI2S*	2.2	4	-0.02044	-----
	2.1698	3.7044	"	0.704884
	2.1698	3.7293	-0.02054	0.704696
	2.1697	(4)	-0.02143	0.721648

NS4004	2.3	4	-0.02044	-----
	2.2869	3.9708	"	0.390313
	2.2869	4.2157	-0.02137	0.365219
	2.2868	(4)	-0.02071	0.381030

NTT004	2.4	6	-0.02044	-----
	2.3801	5.6034	"	0.519830
	2.3803	6.3948	-0.02254	0.344368
	2.3802	(6)	-0.02171	0.384669

* — bad fits may be due to some contribution from the Ni-Ni signal, which was not completely separable from the Ni-S signal over this data range.

Results of fitting Ni-N,S models with N & S

Grace Tan

9th February, 1993

(checked and corrected 9th February, 1993)

These fits are discussed in Chapter 7,
Section (E.1.b).

Models used :

Ni(tsalen) or Ni (S-salen) (**SSALEN**)

Ni-N = 1.85, 1.86, av. 1.86 Å
Ni-S = 2.174, 2.139, av. 2.157 Å

[Ni(NS₃^{t-Bu})H][BPh₄] (**NS3BH**)

Ni-N = 2.02 Å
Ni-S = 2.234, 2.227, 2.218, av. 2.226 Å
Ni-H = 2.0 Å

[Ni(terpy)(S-2,4,6-(iPr)₃-
C₆H₂)₂].1.5CH₃CN (**N3S2**)

Molecule 1:

Ni-N = 2.113 Å, 1.974 Å, 2.086 Å
Ni-S = 2.274 Å, 2.332 Å

Molecule 2 :

Ni-N = 2.075 Å, 1.968 Å, 2.125 Å
Ni-S = 2.298 Å, 2.302 Å

Average bond distances :

Ni-N = 2.057 Å
Ni-S = 2.302 Å

Ni(tsalen) or Ni (S-salen) (SSALEN)

Crystal structure : T. Yamamura, M. Tadokoro & R. Kuroda, *Chem. Lett.*, 1245-6 (1989).

The compound was supplied by R. H. Holm. Data was collected at BL 7-3 in July 1992 to $k = 15 \text{ \AA}^{-1}$.

Pre-edge : 8360–9211 (2)

Spline : 8356 (2) 8515 (3) 8793 (3) 9211

[Ni(NS₃t-Bu)H][BPh₄] (NS3BH)

Crystal structure : P. Stavropoulos, M. C. Muetterties, M. Carrie, R. H. Holm, *J. Am. Chem. Soc.* **1991**, **113**, 8485-92.

The compound was supplied by R. H. Holm. Data was collected at BL 7-3 in July 1991 to $k = 15 \text{ \AA}^{-1}$.

Pre-edge : 8380–9211 (-2)

Spline : 8383 (2) 8515 (3) 8765 (3) 9211

[Ni(terpy)(S-2,4,6-(iPr)₃-C₆H₂)₂].1.5CH₃CN (N3S2)

Crystal structure : Narayan Baidya, Marilyn Olmstead, Pradip K. Mascharak, *Inorg. Chem.* **1991**, **30**, 929-37.

The compound was synthesized by Grace Tan. There is some doubt about the purity about the compound, since microanalysis gave 64.69 % C instead of the expected 69.93 %. Data was collected at BL 7-3 in June 1991 to $k = 15 \text{ \AA}^{-1}$.

Pre-edge : 8399–9219 (-2)

Spline : 8382 (2) 8557 (3) 8825 (3) 9219

Forward FT on all compound data : $k = 3.0\text{--}12.0 (0.1) \text{ \AA}^{-1}$

Reverse FT (to $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$) :

SSALEN.FIL: $R' = 0.95\text{--}2.35 (0.1) \text{ \AA}$ — main peak, including some smudged-in shoulders

NS3BH.FIL1: $R' = 0.95\text{--}2.35 (0.1) \text{ \AA}$ — main peak, includes low-R shoulder

NS3BH.FIL2: $R' = 1.25\text{--}2.35 (0.1) \text{ \AA}$ — main peak, excludes low-R shoulder

N3S2.FIL: $R' = 1.00\text{--}2.30 (0.1) \text{ \AA}$ — main peak, including a smudged-in low-R shoulder

Parameters used in fits :

Ni^{II}-N : [Ni^{II}(dimethylglyoxime)₂], 10 K, $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$ (data to $k = 15 \text{ \AA}^{-1}$)

Ni^{II}-S : Ni^{II}(S₂CN(CH₂CH₃)₂)₂, 10 K, $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$ (data to $k = 15 \text{ \AA}^{-1}$)

Fits of SSALEN.FIL :

<u>N only :</u>	R	CN	c ₂	F
Initial	2.0	4	-0.02297	-----
	1.9671	3.8085	"	1.62750
	1.9486	10.4802	-0.04133	1.20930
	1.9564	(6)	-0.03190	1.35256

<u>S only :</u>	R	CN	c ₂	F
Initial	2.2	3	-0.02044	-----
	2.1384	1.9095	"	1.03187
	2.1358	3.7131	-0.03169	0.572879
	2.1362	(3)	-0.02838	0.634010

<u>N + S :</u>	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.8729	"	"	2.1599	"	"	0.328662
	1.8702	1.9292	"	2.1589	2.0396	"	0.323872
	1.8880	3.6795	-0.03209	2.1643	1.6257	-0.01956	0.293489
	1.8848	(1)	-0.01009	2.1644	(1)	-0.01030	0.824633
	1.8935	(2)	-0.01840	2.1707	(1)	-0.01161	0.614017
	1.8983	(3)	-0.02435	2.1728	(1)	-0.01287	0.469283
	1.8626	(1)	-0.01540	2.1533	(2)	-0.01960	0.426399
**	1.8719	(2)	-0.02418	2.1587	(2)	-0.02058	0.319308
	1.8784	(3)	-0.03092	2.1603	(2)	-0.02169	0.302882
	1.8438	(1)	-0.02095	2.1475	(3)	-0.02607	0.364792
	1.8555	(2)	-0.03123	2.1514	(3)	-0.02701	0.413729
	1.8670	(3)	-0.04078	2.1509	(3)	-0.02827	0.479455

— these R values used as starting values in each of the fixed-CN fits.

** — Of the fixed-CN fits, this has the best initial fit (F=0.328662). Next best initial fit has F=0.703896 (for Ni-N₁S₂).

<u>N + N' :</u>	R _N	CN _N	c _{2N}	R _{N'}	CN _{N'}	c _{2N'}	F
Initial	1.9	2	-0.02297	2.2	4	-0.02297	-----
	1.9546	"	"	2.3823	"	"	1.50744
	1.9601	3.4254	"	2.3882	2.7790	"	1.22597
	1.9506	13.9697	-0.05127	2.3562	2.6270	-0.02074	0.453035
	1.9558	(3)	-0.02432	2.3827	(3)	-0.02144	1.23441

S + S' :

	R _S	CN _S	c _{2s}	R _{S'}	CN _{S'}	c _{2s'}	F
Initial	1.9	1	-0.02044	2.2	2	-0.02044	-----
##	2.0479	"	"	2.1721	"	"	0.505807
	2.0401	1.1897	"	2.1745	2.2855	"	0.437660
	1.9804	0.2472	-0.00796	2.1437	3.0153	-0.02380	0.410389
	2.0616	(1)	-0.00993	2.1985	(1)	-0.00726	0.649207
	2.0844	(2)	-0.02124	2.2099	(1)	-0.01279	0.438570
**	2.0359	(1)	-0.01485	2.1766	(2)	-0.01650	0.427572
	2.0661	(2)	-0.02707	2.1862	(2)	-0.02180	0.494142

— These R values used as starting values in each of the fixed-CN fits.

** — Of the fixed-CN fits, this has the best initial fit (F=0.505807). Next best initial fit has F=1.47535 (for Ni-S₂S₂).

Fits of NS3BH.FIL1 :

<u>N only :</u>	R	CN	c ₂	F
Initial	1.9	6	-0.02297	-----
	2.0582	6.6969	"	1.71884
	2.0549	8.4918	-0.02683	1.67317
	2.0586	(6)	-0.02224	1.75080

<u>S only :</u>	R	CN	c ₂	F
Initial	2.2	4	-0.02044	-----
	2.2209	3.2004	"	0.575304
	2.2208	3.7716	-0.02307	0.479932
	2.2209	(4)	-0.02387	0.491639
	2.2207	(3)	-0.02013	0.620913

N + S :

	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2s}	F
Initial	1.9	1	-0.02297	2.2	3	-0.02044	-----
##	1.9909	"	"	2.2275	"	"	0.456864
	1.9863	1.0212	"	2.2277	3.0490	"	0.454281
	2.0002	1.8359	-0.03209	2.2265	2.9106	-0.02047	0.446785
	1.9862	(1)	-0.01179	2.2368	(2)	-0.01437	0.661055
	1.9966	(2)	-0.02088	2.2367	(2)	-0.01567	0.549644
	2.0040	(3)	-0.02784	2.2341	(2)	-0.01652	0.499962
	2.0093	(4)	-0.03377	2.2311	(2)	-0.01694	0.488908
**	1.9872	(1)	-0.02319	2.2274	(3)	-0.02028	0.455875
	2.0046	(2)	-0.03594	2.2251	(3)	-0.02091	0.449014
	2.0165	(3)	-0.04625	2.2229	(3)	-0.02101	0.466119
	2.3964	(1)	-0.03444	2.2227	(4)	-0.02414	0.435986
	2.3775	(2)	-0.04760	2.2231	(4)	-0.02389	0.416295

— these R values used as starting values in each of the fixed-CN fits.

** — Of the fixed-CN fits, this has the best initial fit (F=0.456864). Next best initial fit has F=0.713959 (for Ni-N₂S₃).

N + N' :

	R_N	CN_N	c_{2N}	$R_{N'}$	$CN_{N'}$	$c_{2N'}$	F
Initial	1.9	1	-0.02297	2.2	5	-0.02297	-----
**	2.0576	"	"	2.0577	"	"	1.75944
	1.8836	3.4180	"	2.0636	9.7620	"	0.929487
	1.9652	6.8178	0.02974	2.0950	4.2223	-0.01463	0.811436
Initial	1.9	2	-0.02297	2.1	4	-0.02297	-----
	1.9393	(2)	-0.00920	2.0860	(4)	-0.00955	1.12285

** — The program crashed when this was used as an initial value for a fixed-CN fit.

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
Initial	1.9	1	-0.02044	2.2	3	-0.02044	-----
##	1.9644	"	"	2.2074	"	"	1.82054
	1.9391	-0.2634	"	2.2244	3.1562	"	0.440953
	1.9518	-0.7737	-0.04276	2.2225	3.2172	-0.02132	0.405034
	2.2180	(1)	-0.01554	2.2230	(1)	-0.01548	1.11624
	2.2191	(2)	-0.02016	2.2239	(1)	-0.02005	0.620909
	2.2171	(1)	-0.02018	2.2225	(2)	-0.02009	0.620910
**	2.2242	(1)	-0.02398	2.2197	(3)	-0.02383	0.491639
	2.3565	(2)	-0.12748	2.2203	(3)	-0.02015	0.605174

— These R values used as starting values in each of the fixed-CN fits.

** — Of the fixed-CN fits, this has the best initial fit ($F=1.82054$). Next best initial fit has $F=2.67538$ (for Ni-S₁S₂).

Fits of NS3BH.FIL2 :**N only :**

	R	CN	c_2	F
Initial	1.9	6	-0.02297	-----
	2.0583	6.6288	"	1.65568
	2.0560	7.9550	-0.02591	1.62596
	2.0587	(5)	-0.02227	1.68174

S only :

	R	CN	c_2	F
Initial	2.2	4	-0.02044	-----
	2.2210	3.1635	"	0.461855
	2.2209	3.6537	-0.02274	0.367634
	2.2210	(4)	-0.02397	0.402886
	2.2208	(3)	-0.02023	0.501004

N + S :

	R_N	CN_N	c_{2N}	R_S	CN_S	c_{2S}	F
Initial	1.9	1	-0.02297	2.2	3	-0.02044	-----
##	1.9931	"	"	2.2277	"	"	0.339631
	1.9964	1.0067	"	2.2276	2.9620	"	0.338022
	1.9683	0.1267	-0.00088	2.2255	3.2370	-0.02034	0.301583
	1.9865	(1)	-0.01042	2.2388	(2)	-0.01416	0.466062
	1.9986	(2)	-0.01965	2.2388	(2)	-0.01575	0.412076
	2.0082	(3)	-0.02704	2.2350	(2)	-0.01686	0.430708
	2.0154	(4)	-0.03349	2.2307	(2)	-0.01734	0.476941
**	1.9947	(1)	-0.02124	2.2286	(3)	-0.02054	0.335679
	2.0240	(2)	-0.03644	2.2236	(3)	-0.02130	0.394777
	2.0347	(3)	-0.04999	2.2212	(3)	-0.02106	0.448007
	2.4049	(1)	-0.03156	2.2229	(4)	-0.02437	0.309284
	2.3910	(2)	-0.04287	2.2237	(4)	-0.02415	0.270586

— these R values used as starting values in each of the fixed-CN fits.

** — Of the fixed-CN fits, this has the best initial fit (F=0.339631). Next best initial fit has F=0.677349 (for Ni-N₂S₃).

N + N' :

	R_N	CN_N	c_{2N}	$R_{N'}$	$CN_{N'}$	$c_{2N'}$	F
Initial	1.9	2	-0.02297	2.2	4	-0.02297	-----
**	2.0579	"	"	2.0579	"	"	1.69004
	1.8851	2.9742	"	2.0637	9.2827	"	1.07902
	1.9845	5.9620	-0.02472	2.1062	2.9362	-0.01104	0.924210
##	2.1194	(2)	-0.00529	1.9936	(4)	-0.01534	1.02939

** — The program crashed when this was used as an initial value for a fixed-CN fit.

— This fit started from scratch, with the initial values in the first line.

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
Initial	1.9	1	-0.02044	2.2	3	-0.02044	-----
##	1.9508	"	"	2.2065	"	"	1.72846
	1.9600	-0.2441	"	2.2244	3.0676	"	0.350825
	1.9505	-0.0282	+0.00357	2.2238	3.3162	-0.02088	0.305316
	1.7514	(1)	-0.03599	2.2217	(1)	-0.00870	1.70386
	1.6766	(2)	-0.07169	2.2207	(1)	-0.00890	1.76198
	2.2162	(1)	-0.02028	2.2231	(2)	-0.02018	0.500995
	2.2194	(2)	-0.02405	2.2226	(2)	-0.02388	0.402885
**	2.6621	(1)	-0.03425	2.2206	(3)	-0.02039	0.395206
	1.7137	(2)	-0.15933	2.2206	(3)	-0.02024	0.490190

— These R values used as starting values in each of the fixed-CN fits.

** — Of the fixed-CN fits, this has the best initial fit (F=1.72846). Next best initial fit has F=2.50533 (for Ni-S₁S₂).

Fits to N3S2.FIL :

<u>N only :</u>	R	CN	c ₂	F
Initial	1.9	6	-0.02297	-----
	2.1096	4.0888	"	1.25793
	2.0958	9.9822	-0.03902	0.921433
	2.1021	(6)	-0.03051	1.04192

<u>S only :</u>	R	CN	c ₂	F
Initial	2.2	3	-0.02044	-----
	2.2785	1.9258	"	0.879282
	2.2785	3.5311	-0.03055	0.567713
	2.2783	(3)	-0.02804	0.594806

<u>N + S :</u>	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
Initial	2.0	3	-0.02297	2.3	2	-0.02044	-----
##	2.0335	"	"	2.3126	"	"	0.663878
	2.0255	1.8546	"	2.3001	1.9201	"	0.460677
	2.0639	7.0138	-0.04208	2.3008	0.9038	-0.01656	0.330640
	2.0333	(1)	-0.01158	2.3036	(1)	-0.01134	0.793849
	2.0431	(2)	-0.01982	2.3088	(1)	-0.01286	0.620257
	2.0490	(3)	-0.02577	2.3097	(1)	-0.01426	0.499180
	2.0534	(4)	-0.03074	2.3085	(1)	-0.01545	0.415118
	2.0160	(1)	-0.01815	2.2935	(2)	-0.02066	0.520464
**	2.0285	(2)	-0.02698	2.2973	(2)	-0.02196	0.437079
	2.0386	(3)	-0.03397	2.2969	(2)	-0.02327	0.403855
	2.0487	(4)	-0.04055	2.2937	(2)	-0.02437	0.397933
	2.0065	(1)	-0.02614	2.2882	(3)	-0.02729	0.488580
	2.0345	(2)	-0.03837	2.2879	(3)	-0.02893	0.490095
	2.0976	(3)	-0.05682	2.2772	(3)	-0.02958	0.490976

— these R values used as starting values in each of the fixed-CN fits.

** — Of the fixed-CN fits, this has the best initial fit (F=0.605521). Next best initial fit has F=0.663878 (for Ni-N₃S₂).

<u>N + N' :</u>	R _N	CN _N	c _{2N}	R _{N'}	CN _{N'}	c _{2N'}	F
Initial	2.0	3	-0.02297	2.3	3	-0.02297	-----
	2.0489	"	"	2.1607	"	"	1.03334
	1.9653	3.1425	"	2.1303	6.4630	"	0.382125
	2.0215	6.2990	-0.03290	2.1586	3.4295	-0.01875	0.361463
	2.0108	(3)	-0.01856	2.1560	(3)	-0.01346	0.587170

S + S' :

	R _S	CN _S	c _{2S}	R _{S'}	CN _{S'}	c _{2S'}	F
Initial	2.0	2	-0.02044	2.3	2	-0.02044	-----
##	2.2198	"	"	2.3503	"	"	0.687830
	2.2129	1.3882	"	2.3297	1.8072	"	0.570914
	2.0925	0.0202	-0.00031	2.2780	3.4237	-0.02928	0.559072
	2.2129	(1)	-0.01096	2.3428	(1)	-0.00950	0.716779
	2.2400	(2)	-0.02232	2.3535	(1)	-0.01761	0.579599
	2.2758	(3)	-0.02807	2.4063	(1)	-0.07312	0.549273
	2.1988	(1)	-0.01805	2.3191	(2)	-0.02035	0.575700
**	2.2721	(2)	-0.02633	2.2972	(2)	-0.04346	0.571999
	2.2755	(3)	-0.02801	2.4293	(2)	-0.08963	0.523333
	2.2737	(1)	-0.02312	2.2830	(3)	-0.03812	0.576022
	2.2725	(2)	-0.02470	2.3098	(3)	-0.05969	0.592135
	2.2753	(3)	-0.02799	2.4392	(3)	-0.10131	0.505789

— These R values used as starting values in each of the fixed-CN fits.

** — Of the fixed-CN fits, this has the best initial fit (F=0.687830). Next best initial fit has F=1.18764 (for Ni-S₂S₁).

Fits to (Et₄N)₃[NiFe₃S₄(SEt)₄]

Grace Tan

30th Mar 1993

(checked and corrected 30th Mar 1993)

These fits are discussed in Chapter 7, Sections (E.1.a) and (E.1.c).

There is no crystal structure for (Et₄N)₃[NiFe₃S₄(SEt)₄]; however, there is a structure for the related compound (Et₄N)₂[NiFe₃S₄(PPh₃)(SEt)₃], for which the average Ni-S distance (in the cubane) is 2.258 Å and the average Ni-Fe distance is 2.689 Å. Data were collected in July 1991 on SSRL BL 7-3 to $k = 15 \text{ Å}^{-1}$. The Fourier transform shows two large peaks which are not well-separated. Although the second peak was fitted on its own as a test for Ni-Fe parameters, reliable results can really only be obtained by fitting both peaks together.

Pre-edge : 8380–9218 (2)

Spline : 8370 (2) 8512 (3) 8790 (3) 9218

Forward FT range :

2NFS.FT-K12 : $k = 3.0\text{--}12.0 (0.1) \text{ Å}^{-1}$

Reverse FT ranges (to $k = 3.25\text{--}11.75 (0.1) \text{ Å}^{-1}$):

2NFS.FIL-K12 : $R' = 2.03\text{--}2.80 (0.1) \text{ Å}$ (2nd peak only)

2NFS.FIL2 : $R' = 1.37\text{--}2.85 (0.1) \text{ Å}$ (1st and 2nd peaks)

2NFS.FIL3 : $R' = 1.00\text{--}2.85 (0.1) \text{ Å}$ (1st + 2nd peaks + left shoulder of 1st peak)

Parameters used in fits :

Parameters were extracted over the same ranges as the compound data they were used to fit.

Ni ^{II} -N :	Ni ^{II} (dimethylglyoxime) ₂ ,	10 K,	data to $k = 15 \text{ Å}^{-1}$
Ni ^{II} -S :	Ni ^{II} (S ₂ CN(CH ₂ CH ₃) ₂) ₂ ,	10 K,	data to $k = 15 \text{ Å}^{-1}$
Ni ^{II} -Fe :	[Et ₄ N] ₂ [NiFe ₃ S ₄ (PPh ₃)(SEt) ₃],	10 K,	data to $k = 15 \text{ Å}^{-1}$

Fits to 2NFS.FIL-K12 :

Fe only :

	R _{Fe}	CN _{Fe}	c _{2Fe}	F
Initial	2.689	3	-0.01530	-----
	2.7551	"	"	0.753763
	2.7546	2.5759	"	0.649238
	2.7545	2.5133	-0.01500	0.648880
	2.7553	(3)	-0.01694	0.665921

Results of fitting Ni CODH with N & S

Grace Tan

30th Mar 1993

(Numbers checked and corrected : 30th Mar 1993)

These fits are discussed in Chapter 7, Sections (E.2.a) – (E.2.e). The fits are listed by data set in the following order : CODHX, CODHOX, CDHOX, CODHRX and CDHRD. However, the three-wave fits to Window 6 (discussed in Section (E.2.d)) of all five data sets are listed separately in landscape mode, after all the other fits.

Several data sets being curve-fitted over :

$$k = 3.25\text{--}11.75 \text{ \AA}^{-1} \text{ (Forward FT : } k = 3.0\text{--}12.0 (0.1) \text{ \AA}^{-1}\text{)}$$

Parameters used in fits :

Parameters were extracted over the same ranges as the protein data they were used to fit.

Ni ^{II} -N :	Ni ^{II} (dimethylglyoxime) ₂ ,	10 K,	data to $k = 15 \text{ \AA}^{-1}$
Ni ^{II} -S :	Ni ^{II} (S ₂ CN(CH ₂ CH ₃) ₂) ₂ ,	10 K,	data to $k = 15 \text{ \AA}^{-1}$
Ni ^{II} -Fe :	[Et ₄ N] ₂ [NiFe ₃ S ₄ (PPh ₃)(SEt) ₃],	10 K,	data to $k = 15 \text{ \AA}^{-1}$

(1) CODHX (oxidized CODH) :

Data were collected at NSLS Beam Line X19A in June 1989 using 13-element germanium detector. 16 EXAFS scans collected, with 11 good detectors. Problem with energy calibration due to bug in software, so calibration may be slightly off, but calibration accuracy is quite acceptable for EXAFS. Data to $k = 15 \text{ \AA}^{-1}$, but, owing to huge monochromator glitch at $k = 12.8 \text{ \AA}^{-1}$, only useable up to $k = 12.0 \text{ \AA}^{-1}$. This data set was initially fit over $k = 4\text{--}11 \text{ \AA}^{-1}$, but now it is being fit over the maximum range that the glitch will allow, $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$. (The data were resplined for this purpose.) This data set is the only one that shows a large peak at very low R in the Fourier transform.

Final pre-edge : 8390.2–8970.8 (2)

Final spline : 8384.5 (2) 8518.9 (3) 8716.3 (3) 8970.8

Forward FT over $k = 3.0\text{--}12.0 (0.1) \text{ \AA}^{-1}$

Reverse FT (to $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$) :

CODHX.FIL1 : R' = 1.00–1.58 (0.1) $\text{\AA}$ — 1st large peak only

CODHX.FIL2 : R' = 1.50–2.25 (0.1) $\text{\AA}$ — 2nd large peak only

CODHX.FIL3 : R' = 1.00–2.25 (0.1) $\text{\AA}$ — 2 main peaks included

CODHX.FIL6 : R' = 1.00–2.55 (0.1) $\text{\AA}$ — 2 main peaks + right shoulder of 2nd peak

(2) CODHOX (oxidized CODH) :

Data were collected in July 1991 at SSRL on BL 7-3, using the 13-element Ge detector. 29 good scans and 9 detectors were averaged together. This data set is noisier than CODHX, but on the other hand there is no glitch to cut the data short and it is good up

to $k = 14.5 \text{ \AA}^{-1}$ or so. However, it was not considered worthwhile to attempt fits to the limit of the data, because of the noise.

Final pre-edge : 8365-9213 (2)

Final spline : 8381 (2) 8554 (3) 8774 (3) 9213

Forward FT over $k = 3.0\text{--}12.0 (0.1) \text{ \AA}^{-1}$

Reverse FT (to $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$) :

CODHOX.FIL1 : $R' = 0.80\text{--}1.60 (0.1) \text{ \AA}$ — left shoulder of main peak only

CODHOX.FIL2 : $R' = 1.45\text{--}2.20 (0.1) \text{ \AA}$ — main peak only

CODHOX.FIL3 : $R' = 0.80\text{--}2.20 (0.1) \text{ \AA}$ — main peak + left shoulder

CODHOX.FIL4 : $R' = 2.07\text{--}2.60 (0.1) \text{ \AA}$ — peak or right shoulder on 2nd peak

CODHOX.FIL5 : $R' = 1.45\text{--}2.60 (0.1) \text{ \AA}$ — main peak + right shoulder

CODHOX.FIL6 : $R' = 0.80\text{--}2.60 (0.1) \text{ \AA}$ — main peak + both shoulders

(Note: CODHOX.FIL4 and CODHOX.FIL5 were not used in curvefitting.)

(3) CDHOX (oxidized CODH) :

This sample was prepared at very short notice by Jeff Fox of Paul Ludden's group, who does not usually work on Ni CODH. Data were collected in July 1992 at SSRL on BL 7-3, using the 13-element Ge detector. 31 good scans and all 13 detectors were averaged together. This data set is less noisy than CODHOX, and it does not have a huge step glitch like CODHX. Unfortunately, it still does have enough of a glitch at $k = 12.9 \text{ \AA}^{-1}$ to make it unwise to curvefit the data beyond $k = 12 \text{ \AA}^{-1}$.

Final pre-edge : 8392.7-8962.4 (2)

Final spline : 8386.9 (2) 8506.1 (3) 8695.0 (3) 8962.4

Forward FT over $k = 3.0\text{--}12.0 (0.1) \text{ \AA}^{-1}$

Reverse FT (to $k = 3.25\text{--}11.75 \text{ \AA}^{-1}$) :

CDHOX.FIL1 : $R' = 1.05\text{--}1.60 (0.1) \text{ \AA}$ — left shoulder of main peak only

CDHOX.FIL2 : $R' = 1.40\text{--}2.25 (0.1) \text{ \AA}$ — main peak only

CDHOX.FIL3 : $R' = 1.05\text{--}2.25 (0.1) \text{ \AA}$ — main peak + left shoulder

CDHOX.FIL4 : $R' = 2.07\text{--}2.60 (0.1) \text{ \AA}$ — right shoulder of main peak

CDHOX.FIL5 : $R' = 1.40\text{--}2.60 (0.1) \text{ \AA}$ — main peak + right shoulder

CDHOX.FIL6 : $R' = 1.05\text{--}2.60 (0.1) \text{ \AA}$ — main peak + both shoulders

(Note: CDHOX.FIL4 and CDHOX.FIL5 were not used in curvefitting.)

(4) CODHRX (reduced CODH):

Data were collected in July 1991 at SSRL on BL 7-3, using the 13-element Ge detector, at the same time as CODHOX. 34 good scans and all 13 detectors were averaged together. This data set is low in noise, but the same glitch that cut short CODHX also affects this data set, so it can only be curve-fit to around $k = 12 \text{ \AA}^{-1}$. Of all the data sets analyzed, only CODHRX shows 3 Fourier peaks at higher R. These peaks are analyzed separately.

Final pre-edge : 8365-8970 (2)

Final spline : 8387.1 (2) 8520.8 (3) 8738.9 (3) 8970

Forward FT over $k = 3.0\text{--}12.0$ (0.1) $\text{\AA}^{-1}$

Reverse FT (to $k = 3.25\text{--}11.75$ $\text{\AA}^{-1}$) :

CODHRX.FIL1 : $R' = 1.00\text{--}1.60$ (0.1) $\text{\AA}$ — left shoulder of main peak only

CODHRX.FIL2 : $R' = 1.45\text{--}2.60$ (0.1) $\text{\AA}$ — main peak only

CODHRX.FIL3 : $R' = 1.00\text{--}2.60$ (0.1) $\text{\AA}$ — main peak + left shoulder

CODHRX.FIL4 : $R' = 2.45\text{--}2.95$ (0.1) $\text{\AA}$ — right shoulder of main peak

CODHRX.FIL5 : $R' = 1.45\text{--}2.95$ (0.1) $\text{\AA}$ — main peak + right shoulder

CODHRX.FIL6 : $R' = 1.00\text{--}2.95$ (0.1) $\text{\AA}$ — main peak + both shoulders

CODHRX.FIL7 : $R' = 2.85\text{--}3.60$ (0.1) $\text{\AA}$ — first high-R peak

CODHRX.FIL8 : $R' = 3.52\text{--}4.20$ (0.1) $\text{\AA}$ — second high-R peak

CODHRX.FIL9 : $R' = 4.10\text{--}5.07$ (0.1) $\text{\AA}$ — third high-R peak

CODHRX.FIL10 : $R' = 2.85\text{--}5.07$ (0.1) $\text{\AA}$ — all three high-R peaks

(Note: CODHRX.FIL4 and CODHRX.FIL5 were not used in curvefitting.)

(4) CDHRD (reduced CODH) :

This sample was prepared at very short notice by Jeff Fox of Paul Ludden's group, who does not usually work on Ni CODH. Data were collected in July 1992 at SSRL on BL 7-3, using the 13-element Ge detector, at the same time as CDHOX. 30 good scans and 13 detectors were averaged together. This data set is noisier than CODHRX, but it does not have a huge step glitch. Unfortunately, it still does have enough of a glitch at $k = 12.9$ $\text{\AA}^{-1}$ to make it unwise to curvefit the data beyond $k = 12$ $\text{\AA}^{-1}$. Another problem is that it looks more like the EXAFS of the oxidized spectra than it does like CODHRX. In particular, it looks extremely similar to CDHOX.

Final pre-edge : 8417.5-8960.6 (2)

Final spline : 8362.0 (2) 8519.7 (3) 8721.2 (3) 8960.6

Forward FT over $k = 3.0\text{--}12.0$ (0.1) $\text{\AA}^{-1}$

Reverse FT (to $k = 3.25\text{--}11.75$ $\text{\AA}^{-1}$) :

CDHRD.FIL1 : $R' = 1.00\text{--}1.57$ (0.1) $\text{\AA}$ — left shoulder of main peak only

CDHRD.FIL2 : $R' = 1.40\text{--}2.25$ (0.1) $\text{\AA}$ — main peak only

CDHRD.FIL3 : $R' = 1.00\text{--}2.25$ (0.1) $\text{\AA}$ — main peak + left shoulder

CDHRD.FIL4 : $R' = 2.07\text{--}2.60$ (0.1) $\text{\AA}$ — right shoulder of main peak

CDHRD.FIL5 : $R' = 1.40\text{--}2.60$ (0.1) $\text{\AA}$ — main peak + right shoulder

CDHRD.FIL6 : $R' = 1.00\text{--}2.60$ (0.1) $\text{\AA}$ — main peak + both shoulders

(Note: CDHRD.FIL4 and CDHRD.FIL5 were not used in curvefitting.)

Fits of CODHX.FIL1 :

<u>N only :</u>	R	CN	c ₂	F
Initial	1.8	2	-0.02297	----
	1.8701	1.2232	"	0.836605
	1.8954	2.6490	-0.03643	0.815026

<u>S only :</u>	R	CN	c ₂	F
Initial	1.8	1	-0.02044	----
	1.6437	0.4447	"	0.556344
	1.6445	0.4068	-0.01905	0.554704

Fits of CODHX.FIL2 :

<u>N only :</u>	R	CN	c ₂	F
Initial	2.2	3	-0.02297	----
	2.0791	3.1020	"	1.13913
	2.0880	1.6474	-0.01356	1.04482

<u>S only :</u>	R	CN	c ₂	F
Initial	2.2	2	-0.02044	----
	2.2342	1.5821	"	0.481026
	2.2350	1.1153	-0.01515	0.363842
	2.2341	(2)	-0.02275	0.611829

N + S :

	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
Initial	2.1	2	-0.02297	2.2	2	-0.02044	----
##	2.1915	"	"	2.2130	"	"	0.697786
	2.2439	-2.1515	"	2.2503	0.9185	"	0.344041
	2.1136	1.8605	-0.01288	2.1503	2.0125	-0.03314	0.279135
	2.1136	(2)	-0.01333	2.1466	(2)	-0.03264	0.279658
Initial	1.9	2	-0.02297	2.2	2	-0.02044	----
##	1.8390	"	"	2.2253	"	"	0.866683
	1.8235	0.6429	"	2.2306	1.6824	"	0.261428
	1.8504	4.3139	-0.08322	2.2368	1.1533	-0.01532	0.141425
	1.8774	(1)	-0.04505	2.2360	(1)	-0.01358	0.250479
	1.8770	(2)	-0.06024	2.2367	(1)	-0.01365	0.205969
**	1.8180	(1)	-0.02767	2.2307	(2)	-0.02226	0.334292
	1.8122	(2)	-0.04170	2.2330	(2)	-0.02190	0.341081
	1.8041	(1)	-0.02247	2.2274	(3)	-0.02910	0.633917
	1.7967	(2)	-0.03447	2.2297	(3)	-0.02830	0.643040

— these R values used as starting values in the fixed-CN fits following them.

** — Of the fixed-CN fits with short Ni-N, this one has the best initial fit (F = 0.481030).
Next best initial fit is F = 0.866683 (for Ni-N₂S₂).

N + N' :

	R_N	CN_N	c_{2N}	$R_{N'}$	$CN_{N'}$	$c_{2N'}$	F
Initial	2.0	2	-0.02297	2.2	2	-0.02297	-----
##	2.0485	"	"	2.1087	"	"	1.20713
	2.0791	1.4209	"	2.0709	1.6805	"	1.13913
	1.9403	(2)	-0.02383	2.0980	(2)	-0.01129	0.614664
	1.9415	4.2253	-0.04126	2.0985	1.9373	-0.01288	0.579619

— these R values used as starting values in the fixed-CN fit.

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
Initial	2.0	2	-0.02044	2.2	2	-0.02044	-----
##	1.9725	"	"	2.1958	"	"	2.08342
	1.9869	0.4128	"	2.2253	1.8304	"	0.143003
++	2.0316	0.8998	-0.04393	2.2361	1.4597	-0.01730	0.100094
††	2.0708	1.5587	-0.06854	2.2380	1.1833	-0.01551	0.100935
	2.1025	(1)	-0.05626	2.2385	(1)	-0.01370	0.158999
	2.1031	(2)	-0.07897	2.2379	(1)	-0.01385	0.114662
**	2.0001	(1)	-0.03615	2.2314	(2)	-0.02082	0.185063
	2.0059	(2)	-0.05747	2.2360	(2)	-0.02123	0.292066
	1.9701	(1)	-0.02765	2.2215	(3)	-0.02699	0.405871
	1.9804	(2)	-0.04456	2.2302	(3)	-0.02632	0.496987

— these R values used as starting values in all the fixed-CN fits.

++ — Initial values from fixed- c_2 fit.

†† — Initial values from fixed-CN fit ($CN_S=2,2$).

** — Of the fixed-CN fits, this one has the best initial fit ($F = 0.978747$). Next best initial fit is $F = 1.59468$ (for Ni-S₁S₃).

Fits of CODHX.FIL3 :**N + S :**

	R_N	CN_N	c_{2N}	R_S	CN_S	c_{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.8527	"	"	2.2257	"	"	0.447884
	1.8534	2.1108	"	2.2257	2.0834	"	0.437524
	1.8328	0.8571	-0.01029	2.2140	2.7689	-0.02723	0.297795
	1.8613	(1)	-0.01594	2.2235	(1)	-0.01238	0.765617
	1.8781	(2)	-0.02555	2.2309	(1)	-0.01151	0.693149
	1.8889	(3)	-0.03224	2.2356	(1)	-0.01142	0.661207
	1.8421	(1)	-0.01284	2.2172	(2)	-0.02158	0.390134
**	1.8532	(2)	-0.02158	2.2244	(2)	-0.01993	0.427638
	1.8614	(3)	-0.02783	2.2301	(2)	-0.01934	0.521801
	1.8323	(1)	-0.01178	2.2145	(3)	-0.02817	0.316082
	1.8403	(2)	-0.02011	2.2211	(3)	-0.02612	0.478031
	1.8468	(3)	-0.02605	2.2268	(3)	-0.02521	0.637938

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit ($F = 0.447884$). Next best initial fit is $F = 0.777492$ (for Ni-N₃S₂).

N + N' :

	R_N	CN_N	c_{2N}	$R_{N'}$	$CN_{N'}$	$c_{2N'}$	F
Initial	1.9	2	-0.02297	2.2	2	-0.02297	-----
##	1.8463	"	"	2.0615	"	"	1.65487
	1.8647	4.2687	"	2.0659	6.5082	"	0.380288
	1.8975	(2)	-0.01642	2.0817	(2)	-0.00906	0.833410
	1.8967	5.6339	-0.02913	2.0880	3.9911	-0.01636	0.295387

— these R values used as starting values in the fixed-CN fit.

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
Initial	1.9	2	-0.02044	2.2	2	-0.02044	-----
##	1.9948	"	"	2.2056	"	"	1.99164
	2.0126	1.0278	"	2.2215	2.4526	"	0.781242
	1.9526	0.1889	-0.00015	2.1971	3.9120	-0.03676	0.495614
	2.0790	(1)	-0.02753	2.2414	(1)	-0.01081	0.962526
	2.1062	(2)	-0.04126	2.2469	(1)	-0.01220	0.921942
	2.1201	(3)	-0.05188	2.2471	(1)	-0.01306	0.930681
**	2.0227	(1)	-0.02019	2.2243	(2)	-0.01750	0.807401
	2.0552	(2)	-0.03326	2.2407	(2)	-0.01787	0.909897
	2.0721	(3)	-0.04387	2.2472	(2)	-0.01919	1.02340
	1.9966	(1)	-0.01786	2.2131	(3)	-0.02364	0.753216
	2.0273	(2)	-0.02948	2.2319	(3)	-0.02235	0.945813
	2.0451	(3)	-0.03921	2.2423	(3)	-0.02321	1.10665

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 1.03979). Next best initial fit is F = 1.40465 (for Ni-S₂S₃).

Fits of CODHX.FIL6 :**N + S :**

	R_N	CN_N	c_{2N}	R_S	CN_S	c_{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.8519	"	"	2.2253	"	"	0.552835
	1.8531	2.1949	"	2.2252	2.1440	"	0.527269
	1.8390	0.9872	-0.01149	2.2149	2.4268	-0.02389	0.434658
	1.8638	(1)	-0.01508	2.2230	(1)	-0.01158	0.748252
	1.8785	(2)	-0.02446	2.2300	(1)	-0.01076	0.669697
	1.8880	(3)	-0.03101	2.2346	(1)	-0.01066	0.642017
	1.8441	(1)	-0.01215	2.2164	(2)	-0.02082	0.460528
**	1.8542	(2)	-0.02077	2.2234	(2)	-0.01924	0.492443
	1.8616	(3)	-0.02691	2.2290	(2)	-0.01866	0.583967
	1.8340	(1)	-0.01119	2.2137	(3)	-0.02748	0.472033
	1.8413	(2)	-0.01943	2.2202	(3)	-0.02548	0.598408
	1.8472	(3)	-0.02529	2.2257	(3)	-0.02458	0.740544

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.553835). Next best initial fit is F = 0.825130 (for Ni-N₃S₂).

Fits of CODHOX.FIL1 :

<u>N only :</u>	R	CN	c ₂	F
Initial	1.8	2	-0.02297	----
	1.9234	0.8445	"	0.734775
	1.9704	7.6276	-0.06843	0.514273
<u>S only :</u>	R	CN	c ₂	F
Initial	1.8	1	-0.02044	----
	1.6259	0.2584	"	0.669872
	1.6086	1.2005	-0.04969	0.436320

Fits of CODHOX.FIL2 :

<u>N only :</u>	R	CN	c ₂	F
Initial	2.2	3	-0.02297	----
	2.0813	2.4597	"	0.917343
	2.0768	3.0740	-0.02666	0.909784
<u>S only :</u>	R	CN	c ₂	F
Initial	2.2	2	-0.02044	----
	2.2431	1.2726	"	0.359707
	2.2416	(2)	-0.02762	0.330690
	2.2421	1.6663	-0.02483	0.305961

N + S :

	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
Initial	2.1	2	-0.02297	2.2	2	-0.02044	-----
##	2.2391	"	"	2.2304	"	"	0.544938
	2.3147	-1.6805	"	2.2198	0.8705	"	0.220784
	2.1352	(2)	-0.01809	2.1714	(2)	-0.03193	0.197806
	2.1270	1.6866	-0.01771	2.1775	2.4321	-0.03886	0.171607
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.9181	"	"	2.2509	"	"	0.962368
	1.9093	0.4489	"	2.2444	1.3751	"	0.256763
	1.8752	5.8284	-0.10235	2.2461	1.5305	-0.02343	0.120416
**	1.9612	(1)	-0.03552	2.2500	(1)	-0.01763	0.306386
	1.9584	(2)	-0.05022	2.2498	(1)	-0.01803	0.262811
	1.8417	(1)	-0.03934	2.2424	(2)	-0.02654	0.169631
	1.8363	(2)	-0.05798	2.2443	(2)	-0.02663	0.160718
	1.8038	(1)	-0.03314	2.2383	(3)	-0.03369	0.345112
	1.7912	(2)	-0.04963	2.2404	(3)	-0.03338	0.343644

— these R values used as starting values in the fixed-CN fits following them.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.722955). Next best initial fit is F = 0.739005 (for Ni-N₁S₂).

N + N' :

	R_N	CN_N	c_{2N}	$R_{N'}$	$CN_{N'}$	$c_{2N'}$	F
Initial	2.0	2	-0.02297	2.2	2	-0.02297	-----
##	2.0201	"	"	2.1303	"	"	0.935261
	1.8938	1.7802	"	2.0823	4.0884	"	0.528860
	1.9536	(2)	-0.02427	2.1079	(2)	-0.01442	0.531512
	1.9445	5.4822	-0.04956	2.1039	2.4353	-0.01979	0.456391

— these R values used as starting values in the fixed-CN fit.

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
Initial	2.0	2	-0.02044	2.2	2	-0.02044	-----
##	2.1780	"	"	2.3257	"	"	1.17627
	2.0822	0.3138	"	2.2488	1.5191	"	0.202850
	2.1331	2.2509	-0.08405	2.2482	1.3623	-0.02214	0.0677134
**	2.1731	(1)	-0.04251	2.2550	(1)	-0.01898	0.168878
	2.2471	(2)	-0.02594	2.0492	(1)	-0.05515	0.102657
	2.0492	(1)	-0.05154	2.2471	(2)	-0.02594	0.102657
	2.0536	(2)	-0.08130	2.2473	(2)	-0.02680	0.145034
	1.9890	(1)	-0.04044	2.2383	(3)	-0.03166	0.225618
	1.9946	(2)	-0.06463	2.2439	(3)	-0.03222	0.288993

— these R values used as starting values in the fixed-CN fit.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.795829). Next best initial fit is F = 1.17627 (for Ni-S₂S₂).

Fits of CODHOX.FIL3 :

N + S :

	R_N	CN_N	c_{2N}	R_S	CN_S	c_{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.8898	"	"	2.2405	"	"	0.724252
	1.8881	1.3284	"	2.2400	1.6277	"	0.557644
	1.8520	1.3382	-0.02417	2.2352	2.9839	-0.03133	0.355326
	1.9110	(1)	-0.02509	2.2444	(1)	-0.01622	0.743935
	1.9252	(2)	-0.03393	2.2504	(1)	-0.01625	0.627362
	1.9329	(3)	-0.04009	2.2537	(1)	-0.01655	0.541367
	1.8675	(1)	-0.02194	2.2363	(2)	-0.02520	0.469848
**	1.8826	(2)	-0.03136	2.2439	(2)	-0.02449	0.401736
	1.8925	(3)	-0.03810	2.2491	(2)	-0.02451	0.379323
	1.8473	(1)	-0.02049	2.2325	(3)	-0.03201	0.361065
	1.8592	(2)	-0.02989	2.2398	(3)	-0.03079	0.370358
	1.8684	(3)	-0.03677	2.2455	(3)	-0.03052	0.418617

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.724253). Next best initial fit is F = 0.891128 (for Ni-N₁S₁).

N + N' :

	R_N	CN_N	c_{2N}	$R_{N'}$	$CN_{N'}$	$c_{2N'}$	F
Initial	1.9	2	-0.02297	2.2	2	-0.02297	-----
##	1.8839	"	"	2.0741	"	"	1.30700
	1.8852	3.1127	"	2.0767	5.1437	"	0.473235
	1.9249	(2)	-0.02126	2.0969	(2)	-0.01323	0.828709
	1.9296	8.5826	-0.04645	2.1088	3.4690	-0.02164	0.238650

— these R values used as starting values in the fixed-CN fit.

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
Initial	1.9	2	-0.02044	2.2	2	-0.02044	-----
##	2.1674	"	"	2.3161	"	"	1.28882
	2.0666	0.7031	"	2.2462	1.9111	"	0.640577
	1.9694	0.1772	-0.00681	2.2177	4.1988	-0.04112	0.447074
**	2.1405	(1)	-0.02991	2.2657	(1)	-0.01660	0.715005
	2.1583	(2)	-0.04383	2.2654	(1)	-0.01864	0.611635
	2.2414	(3)	-0.02837	2.0386	(1)	-0.02778	0.557399
	2.0790	(1)	-0.02921	2.2527	(2)	-0.02287	0.594757
	2.1099	(2)	-0.04308	2.2631	(2)	-0.02500	0.599888
	2.1275	(3)	-0.05615	2.2631	(2)	-0.02719	0.645506
	2.0386	(1)	-0.02778	2.2414	(3)	-0.02837	0.557399
	2.0743	(2)	-0.04133	2.2574	(3)	-0.02942	0.637388
	2.0949	(3)	-0.05399	2.2628	(3)	-0.03187	0.725129

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 1.10817). Next best initial fit is F = 1.28882 (for Ni-S₂S₂).

Fits of CODHOX.FIL6 :**N + S :**

	R_N	CN_N	c_{2N}	R_S	CN_S	c_{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.8854	"	"	2.2387	"	"	0.726064
	1.8838	1.4193	"	2.2385	1.7025	"	0.615818
	1.9804	15.8992	-0.07700	2.2534	0.3605	-0.00682	0.380030
	1.9066	(1)	-0.02254	2.2422	(1)	-0.01482	0.780674
	1.9193	(2)	-0.03180	2.2483	(1)	-0.01482	0.679282
	1.9268	(3)	-0.03829	2.2517	(1)	-0.01510	0.611608
	1.8677	(1)	-0.01998	2.2344	(2)	-0.02406	0.599412
**	1.8799	(2)	-0.02938	2.2417	(2)	-0.02331	0.560426
	1.8885	(3)	-0.03618	2.2468	(2)	-0.02329	0.559547
	1.8489	(1)	-0.01896	2.2310	(3)	-0.03106	0.574874
	1.8583	(2)	-0.02828	2.2380	(3)	-0.02982	0.593449
	1.8661	(3)	-0.03512	2.2435	(3)	-0.02951	0.637468

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.726064). Next best initial fit is F = 0.820817 (for Ni-N₁S₂).

Fits of CDHOX.FIL1 :

<u>N only :</u>	R	CN	c ₂	F
Initial	1.9	2	-0.02297	----
	1.9445	0.8477	"	0.600747
	1.9760	6.5532	-0.06415	0.342871
	1.9556	(2)	-0.03875	0.459719

<u>S only :</u>	R	CN	c ₂	F
Initial	1.9	1	-0.02044	----
	2.1375	0.2860	"	0.688747
	2.2066	3.0869	-0.07163	0.536515
	2.1702	(1)	-0.04305	0.583757
Initial	1.8	1	-0.02044	----
	1.6538	0.2179	"	0.618596
	1.6233	0.9682	-0.05034	0.499593
	1.6226	(1)	-0.05120	0.499676

Fits of CDHOX.FIL2 :

<u>N only :</u>	R	CN	c ₂	F
Initial	2.2	3	-0.02297	----
	2.0692	3.0407	"	1.20585
	2.0622	4.1465	-0.02816	1.18758
	2.0683	(3)	-0.02351	1.20466

<u>S only :</u>	R	CN	c ₂	F
Initial	2.2	2	-0.02044	----
	2.2318	1.6076	"	0.448623
	2.2305	1.9988	-0.02397	0.403157
	2.2305	(2)	-0.02398	0.403157

N + S :

	R_N	CN_N	c_{2N}	R_S	CN_S	c_{2S}	F
Initial	2.1	2	-0.02297	2.2	2	-0.02044	-----
##	2.1925	"	"	2.2096	"	"	0.619535
	2.1055	-2.0064	"	2.2429	2.3162	"	0.145000
	2.0788	-2.2232	-0.02710	2.2347	2.7240	-0.02330	0.116052
	2.1539	(2)	-0.01269	2.1714	(2)	-0.02076	0.275736
	2.1433	0.8533	-0.01067	2.1934	2.3879	-0.02844	0.199087
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.9299	"	"	2.2442	"	"	0.762130
	1.9050	0.6785	"	2.2341	1.7583	"	0.236180
	1.9022	1.6042	-0.04108	2.2363	1.7982	-0.02147	0.219064
	1.9650	(1)	-0.02226	2.2441	(1)	-0.01380	0.394268
	1.9685	(2)	-0.03425	2.2443	(1)	-0.01472	0.335090
	1.9679	(3)	-0.04347	2.2431	(1)	-0.01520	0.314259
**	1.8857	(1)	-0.03198	2.2337	(2)	-0.02265	0.224250
	1.8815	(2)	-0.04898	2.2352	(2)	-0.02306	0.230234
	1.8264	(1)	-0.03180	2.2286	(3)	-0.02915	0.389999
	1.8111	(2)	-0.04913	2.2304	(3)	-0.02918	0.397881

— these R values used as starting values in the fixed-CN fits following them.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.458946). Next best initial fit is F = 0.762130 (for Ni-N₂S₂).

N + N' :

	R_N	CN_N	c_{2N}	$R_{N'}$	$CN_{N'}$	$c_{2N'}$	F
Initial	2.0	2	-0.02297	2.2	4	-0.02297	-----
	1.8946	"	"	2.0745	"	"	0.845906
	1.8882	2.3220	"	2.0732	5.1551	"	0.701418
	1.9645	4.8807	-0.03550	2.1066	1.9580	-0.01374	0.607712
	1.9071	(2)	-0.01902	2.0806	(4)	-0.01842	0.674207

— these R values used as starting values in the fixed-CN fit.

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
Initial	2.0	2	-0.02044	2.2	2	-0.02044	-----
##	2.1670	"	"	2.3036	"	"	1.08142
	2.0668	0.4378	"	2.2368	1.9652	"	0.135596
	2.1279	1.1006	-0.03733	2.2462	1.5212	-0.01962	0.118222
**	2.1580	(1)	-0.02437	2.2620	(1)	-0.01501	0.181291
	2.1715	(2)	-0.04650	2.2460	(1)	-0.01655	0.143265
	2.0850	(1)	-0.03975	2.2406	(2)	-0.02231	0.141492
	2.0878	(2)	-0.06913	2.2375	(2)	-0.02349	0.214243
	2.0123	(1)	-0.03817	2.2311	(3)	-0.02751	0.285323
	2.0113	(2)	-0.06371	2.2338	(3)	-0.02855	0.356797

— these R values used as starting values in all the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.977790). Next best initial fit is F = 1.08142 (for Ni-S₂S₂).

Fits of CDHOX.FIL3 :

N + S :

	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.8906	"	"	2.2325	"	"	0.521377
	1.8867	1.2987	"	2.2313	1.9554	"	0.298998
	1.8794	1.9001	-0.03028	2.2332	2.3246	-0.02348	0.178573
	1.9313	(1)	-0.02252	2.2379	(1)	-0.01330	0.669272
	1.9403	(2)	-0.03135	2.2420	(1)	-0.01365	0.523433
	1.9448	(3)	-0.03762	2.2439	(1)	-0.01404	0.413173
**	1.8788	(1)	-0.02180	2.2294	(2)	-0.02166	0.298819
	1.8916	(2)	-0.03143	2.2353	(2)	-0.02147	0.201606
	1.8992	(3)	-0.03847	2.2388	(2)	-0.02171	0.181675
	1.8513	(1)	-0.02052	2.2249	(3)	-0.02793	0.210301
	1.8615	(2)	-0.03045	2.2308	(3)	-0.02728	0.274092
	1.8685	(3)	-0.03793	2.2349	(3)	-0.02731	0.359848

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.376892). Next best initial fit is F = 0.521377 (for Ni-N₂S₂).

N + N' :

	R _N	CN _N	c _{2N}	R _{N'}	CN _{N'}	c _{2N'}	F
Initial	1.9	2	-0.02297	2.2	4	-0.02297	-----
##	1.8806	"	"	2.0683	"	"	0.859557
	1.8815	3.3056	"	2.0699	5.9759	"	0.508232
	1.9421	8.2590	-0.04383	2.1027	2.6796	-0.01676	0.362955
	1.8924	(2)	-0.01691	2.0722	(4)	-0.01788	0.669458

— these R values used as starting values in the fixed-CN fit.

S + S' :

	R _S	CN _S	c _{2S}	R _{S'}	CN _{S'}	c _{2S'}	F
Initial	1.9	2	-0.02044	2.2	2	-0.02044	-----
##	2.1627	"	"	2.2997	"	"	1.13989
	2.0565	0.7045	"	2.2349	2.2471	"	0.388308
	1.9787	0.2140	-0.00813	2.2124	3.4706	-0.03137	0.287360
	2.1428	(1)	-0.02431	2.2590	(1)	-0.01377	0.554025
	2.1577	(2)	-0.03930	2.2538	(1)	-0.01591	0.400694
	2.2327	(3)	-0.02520	2.0388	(1)	-0.02697	0.379396
	2.0839	(1)	-0.02740	2.2438	(2)	-0.02012	0.387140
**	2.1085	(2)	-0.04238	2.2489	(2)	-0.02222	0.413168
	2.1222	(3)	-0.05771	2.2455	(2)	-0.02377	0.493842
	2.0388	(1)	-0.02697	2.2327	(3)	-0.02520	0.379396
	2.0676	(2)	-0.04152	2.2435	(3)	-0.02646	0.515051
	2.0812	(3)	-0.05712	2.2442	(3)	-0.02841	0.639731

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 1.13989). Next best initial fit is F = 1.15599 (for Ni-S₁S₁).

Fits of CDHOX.FIL6 :

N + S :

	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.8870	"	"	2.2320	"	"	0.623468
	1.8843	1.4143	"	2.2315	2.0452	"	0.474256
	1.9614	9.0689	-0.05927	2.2460	0.7367	-0.01104	0.379624
	1.9274	(1)	-0.01973	2.2381	(1)	-0.01191	0.715149
	1.9358	(2)	-0.02892	2.2425	(1)	-0.01227	0.589091
	1.9400	(3)	-0.03541	2.2445	(1)	-0.01267	0.504941
**	1.8806	(1)	-0.01926	2.2292	(2)	-0.02049	0.497701
	1.8906	(2)	-0.02879	2.2352	(2)	-0.02026	0.459532
	1.8968	(3)	-0.03578	2.2388	(2)	-0.02046	0.467319
	1.8556	(1)	-0.01855	2.2247	(3)	-0.02688	0.537332
	1.8631	(2)	-0.02821	2.2306	(3)	-0.02622	0.574973
	1.8684	(3)	-0.03545	2.2346	(3)	-0.02619	0.631741

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.536094). Next best initial fit is F = 0.623468 (for Ni-N₂S₂).

Fits of CODHRX.FIL1 :

N only :

	R	CN	c ₂	F
Initial	1.8	2	-0.02297	----
	1.9367	0.6988	"	0.608393
	1.9775	7.4559	-0.07223	0.357312
	1.9520	(2)	-0.04196	0.468848

S only :

	R	CN	c ₂	F
Initial	1.8	1	-0.02044	----
	1.6309	0.2018	"	0.576076
	1.6068	1.0291	-0.05302	0.418188
	1.6074	(1)	-0.05224	0.418275

Fits of CODHRX.FIL2 :

N only :

	R	CN	c ₂	F
Initial	2.2	6	-0.02297	----
	2.0962	4.1545	"	2.17745
	2.1056	2.7231	-0.01652	2.14610
	2.0891	(6)	-0.02775	2.24697

<u>S only :</u>	R	CN	c ₂	F
Initial	2.2	4	-0.02044	----
	2.2546	2.3448	"	1.18616
	2.2563	1.9716	-0.01777	1.16202
	2.2562	(2)	-0.01794	1.16215
	2.2502	(4)	-0.02795	1.49473

<u>N + S :</u>	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
Initial	2.1	2	-0.02297	2.2	2	-0.02044	----
	2.1643	"	"	2.2335	"	"	1.44535
	2.2916	-5.6858	"	2.4138	-0.6560	"	0.412677
	2.2765	-5.3165	-0.01918	2.4992	-0.0198	+0.01546	0.233357
Initial	2.0	2	-0.02297	2.2	2	-0.02044	----
##	1.8871	"	"	2.2504	"	"	1.47229
	1.8932	1.1491	"	2.2532	2.6127	"	0.988918
	1.9144	3.4693	-0.04486	2.2606	1.9513	-0.01669	0.933126
	1.9964	(1)	-0.01133	2.2734	(1)	-0.00737	1.03944
	1.9956	(2)	-0.02284	2.2727	(1)	-0.00870	1.02904
	1.9913	(3)	-0.03132	2.2709	(1)	-0.00938	1.03122
	1.9295	(1)	-0.01985	2.2576	(2)	-0.01627	0.946596
	1.9232	(2)	-0.03181	2.2596	(2)	-0.01661	0.937056
	1.9151	(3)	-0.04116	2.2602	(2)	-0.01688	0.933812
**	1.8859	(1)	-0.01986	2.2511	(3)	-0.02195	1.03819
	1.8777	(2)	-0.03144	2.2538	(3)	-0.02189	1.03010
	1.8683	(3)	-0.04065	2.2550	(3)	-0.02199	1.02630

— these R values used as starting values in the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 1.13032). Next best initial fit is F = 1.13388 (for Ni-N₂S₃).

<u>N + N' :</u>	R _N	CN _N	c _{2N}	R _{N'}	CN _{N'}	c _{2N'}	F
Initial	2.0	2	-0.02297	2.2	4	-0.02297	----
	1.8911	"	"	2.0902	"	"	2.03341
	1.8986	3.3374	"	2.0929	7.1910	"	1.66695
	1.9965	4.8515	-0.02714	2.1366	1.8881	-0.00796	1.45968
	1.9408	(2)	-0.01281	2.1084	(4)	-0.01285	1.53757

— these R values used as starting values in the fixed-CN fit.

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
Initial	2.0	2	-0.02044	2.2	2	-0.02044	-----
	1.9972	"	"	2.2241	"	"	2.73166
	2.0505	0.8463	"	2.2509	3.0671	"	0.826914
	2.2090	1.4835	-0.01257	2.3263	0.3689	+0.00163	0.679031
Initial	2.2	2	-0.02044	2.3	2	-0.02044	-----
##	2.2013	"	"	2.3063	"	"	1.56607
	2.0505	0.8463	"	2.2509	3.0671	"	0.826914
	2.1686	(1)	-0.00922	2.3001	(1)	-0.00447	0.707893
	2.1785	(2)	-0.02681	2.2877	(1)	-0.00943	0.727397
	2.2542	(3)	-0.01954	2.0611	(1)	-0.02215	0.810885
	2.1151	(1)	-0.01950	2.2691	(2)	-0.01402	0.745128
**	2.1180	(2)	-0.03713	2.2685	(2)	-0.01601	0.742070
	2.2599	(3)	-0.02033	2.0687	(2)	-0.03714	0.815432
	2.0611	(1)	-0.02216	2.2542	(3)	-0.01954	0.810884
	2.0687	(2)	-0.03716	2.2599	(3)	-0.02033	0.815433
	2.0660	(3)	-0.04956	2.2608	(3)	-0.02103	0.846089

— these R values used as starting values in the fixed-CN fit.

** — of the fixed-CN fits, this one has the best initial fit (F = 1.56607). Next best initial fit is F = 1.64489 (for Ni-S₁S₂).

Fits of CODHRX.FIL3 :**N + S :**

	R_N	CN_N	c_{2N}	R_S	CN_S	c_{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.8805	"	"	2.2486	"	"	1.31657
	1.8879	1.9134	"	2.2507	2.8097	"	0.859414
	1.9135	5.7409	-0.04453	2.2622	2.0813	-0.01687	0.756178
	1.9733	(1)	-0.01634	2.2658	(1)	-0.00840	1.28502
	1.9687	(2)	-0.02645	2.2666	(1)	-0.00892	1.17295
	1.9660	(3)	-0.03279	2.2671	(1)	-0.00918	1.09040
	1.9083	(1)	-0.01791	2.2532	(2)	-0.01645	0.998284
	1.9120	(2)	-0.02663	2.2568	(2)	-0.01622	0.888951
	1.9141	(3)	-0.03269	2.2592	(2)	-0.01621	0.822320
	1.8802	(1)	-0.01598	2.2468	(3)	-0.02202	0.943755
**	1.8834	(2)	-0.02453	2.2508	(3)	-0.02140	0.858024
	1.8856	(3)	-0.03056	2.2536	(3)	-0.02116	0.823796

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.900116). Next best initial fit is F = 1.08034 (for Ni-N₃S₃).

N + N' :

	R_N	CN_N	c_{2N}	$R_{N'}$	$CN_{N'}$	$c_{2N'}$	F
Initial	1.9	2	-0.02297	2.2	4	-0.02297	-----
##	1.8845	"	"	2.0879	"	"	2.05387
	1.8930	4.4335	"	2.0891	7.9991	"	1.52312
	1.9545	10.8089	-0.04436	2.1235	3.1923	-0.01418	1.31894
	1.9236	(2)	-0.01329	2.1003	(4)	-0.01360	1.63201

— these R values used as starting values in the fixed-CN fit.

S + S' :

	R _S	CN _S	c _{2S}	R _{S'}	CN _{S'}	c _{2S'}	F
Initial	1.9	2	-0.02044	2.2	2	-0.02044	-----
##	2.0101	"	"	2.2283	"	"	2.67054
	2.0517	1.1972	"	2.2495	3.3967	"	0.703665
	2.1158	2.5260	-0.03589	2.2707	2.1041	-0.01588	0.641231
	2.1622	(1)	-0.01187	2.2928	(1)	-0.00556	0.958742
	2.1688	(2)	-0.02740	2.2850	(1)	-0.00893	0.778885
	2.1684	(3)	-0.03724	2.2799	(1)	-0.00980	0.717124
	2.1036	(1)	-0.01846	2.2656	(2)	-0.01361	0.789916
	2.1173	(2)	-0.03088	2.2709	(2)	-0.01492	0.660279
	2.1216	(3)	-0.04004	2.2716	(2)	-0.01578	0.648337
**	2.0597	(1)	-0.01866	2.2514	(3)	-0.01879	0.715752
	2.0792	(2)	-0.02964	2.2615	(3)	-0.01892	0.667176
	2.0874	(3)	-0.03844	2.2655	(3)	-0.01962	0.716611

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 1.23011). Next best initial fit is F = 1.69110 (for Ni-S₁S₂).

Fits of CODHRX.FIL6 :**N + S :**

	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
	1.8813	"	"	2.2491	"	"	1.35061
	1.8885	1.9373	"	2.2511	2.8263	"	0.896653
	1.9170	5.8737	-0.04476	2.2631	1.9980	-0.01629	0.790356
	1.9743	(1)	-0.01558	2.2665	(1)	-0.00813	1.28807
	1.9695	(2)	-0.02583	2.2672	(1)	-0.00871	1.17837
	1.9665	(3)	-0.03226	2.2677	(1)	-0.00899	1.09739
	1.9101	(1)	-0.01747	2.2536	(2)	-0.01627	1.02046
	1.9134	(2)	-0.02620	2.2572	(2)	-0.01605	0.914357
	1.9152	(3)	-0.03227	2.2596	(2)	-0.01605	0.850497
	1.8820	(1)	-0.01570	2.2472	(3)	-0.02185	0.981302
**	1.8848	(2)	-0.02424	2.2511	(3)	-0.02125	0.899519
	1.8868	(3)	-0.03026	2.2540	(3)	-0.02102	0.867523
	1.8674	(2)	-0.02286	2.2468	(4)	-0.02562	1.02764

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.931386). Next best initial fit is F = 1.10296 (for Ni-N₃S₃).

Fits of CODHRX.FIL7 :**S only :**

	R	CN	c ₂	F
Initial	3.5	1	-0.02044	-----
	3.6809	"	"	0.198078
	3.6804	0.8295	"	0.182519
	3.6813	0.4925	-0.01265	0.152753
	3.6806	(1)	-0.02127	0.196553

<u>Fe only :</u>	R	CN	c ₂	F
Initial	3.5	1	-0.01530	----
	3.5316	"	"	0.277812
	3.5301	0.6571	"	0.202757
	3.5308	0.5963	-0.01411	0.202273
	3.5275	(1)	-0.01991	0.215713

<u>S + Fe :</u>	R _S	CN _S	c _{2S}	R _{Fe}	CN _{Fe}	c _{2Fe}	F
Initial	3.7	1	-0.02044	3.5	1	-0.01530	----
	3.7589	"	"	3.4859	"	"	0.458880
	3.6446	0.5076	"	3.5597	0.4429	"	0.164241
	3.5427	1.0796	-0.04451	3.5378	0.8948	-0.01674	0.147789
	3.5358	(1)	-0.04091	3.5372	(1)	-0.01754	0.148316
Initial	3.5	1	-0.02044	3.5	1	-0.01530	----
	3.5280	0.5703	"	3.5384	1.0963	"	0.155372

Fits of CODHRX.FIL8 :

<u>S only :</u>	R	CN	c ₂	F
Initial	4.2	1	-0.02044	----
	4.1890	"	"	0.278934
	4.1896	1.4360	"	0.233608
	4.1895	1.4640	-0.02074	0.233562
	4.1918	(1)	-0.01624	0.246277
	4.1871	(2)	-0.02504	0.244550

<u>Fe only :</u>	R	CN	c ₂	F
Initial	4.2	1	-0.01530	----
	4.0377	"	"	0.363886
	4.0377	0.9811	"	0.363797
	4.0289	1.8351	-0.02338	0.349329
	4.0363	(1)	-0.01644	0.361547

<u>S + Fe :</u>	R _S	CN _S	c _{2S}	R _{Fe}	CN _{Fe}	c _{2Fe}	F
Initial	4.2	1	-0.02044	4.0	1	-0.01530	----
	4.2361	"	"	3.9957	"	"	0.371268
	4.1766	2.0881	"	3.9983	-0.6701	"	0.202677
	4.1985	(1)	-0.01509	3.8881	(1)	-0.03640	0.207320
Initial	4.0	1	-0.02044	4.0	1	-0.01530	----
	4.1255	"	"	4.0817	"	"	0.250436
	4.0825	1.7013	"	4.0769	1.9476	"	0.216222
	4.0873	2.7728	-0.04071	4.0615	1.6667	-0.01906	0.197129
	4.1149	(1)	-0.00696	4.1114	(1)	-0.00488	0.244849
	4.0775	(2)	-0.03081	4.0639	(2)	-0.01909	0.205390

Fits of CODHRX.FIL9 :

<u>S only :</u>	R	CN	c ₂	F
Initial	5.0	1	-0.02044	----
	5.0293	"	"	0.524656
	5.0300	2.3089	"	0.417930
	5.0208	1.0199	-0.00866	0.351502

<u>Fe only :</u>	R	CN	c ₂	F
Initial	5.0	1	-0.01530	----
	4.8714	"	"	0.423678
	4.8725	2.3228	"	0.177896
	4.8724	2.2378	-0.01485	0.177619
	4.8731	(3)	-0.01805	0.193257

<u>S + Fe :</u>	R _S	CN _S	c _{2S}	R _{Fe}	CN _{Fe}	c _{2Fe}	F
Initial	4.8	1	-0.02044	4.9	2	-0.01530	-----
	4.6519	"	"	4.8790	"	"	0.149722
	4.6552	0.5958	"	4.8756	2.0401	"	0.106902
	4.6340	0.4021	-0.01181	4.8820	1.4982	-0.01117	0.0996890
	4.6526	(1)	-0.03082	4.8729	(2)	-0.01460	0.107332
Initial	5.0	1	-0.02044	4.9	2	-0.01530	-----
	4.9787	"	"	4.8890	"	"	0.280214
	4.8847	-1.3039	"	4.8521	1.5836	"	0.0730962
	5.2231	(1)	-0.04212	4.8714	(2)	-0.01339	0.143901

Fits of CODHRX.FIL10 :

S + Fe, 3 to 6 Waves :

	R _S	CN _S	c _{2S}	R _{Fe}	CN _{Fe}	c _{2Fe}	F
Peak 1	3.7638	(1)	(-0.02044)	3.4841	(1)	(-0.01530)	0.581478
Peak 2	4.1926	(1)	(-0.02044)	----	----	----	
Peak 3	----	----	----	4.8752	(2)	(-0.01530)	
Peak 1	3.8484	-0.4090	(-0.02044)	3.5268	0.4649	(-0.01530)	0.348254
Peak 2	4.1875	(1)	(-0.02044)	----	----	----	
Peak 3	----	----	----	4.8732	(2)	(-0.01530)	
Peak 1	3.8134	-0.6876	(-0.02044)	3.5566	0.5286	(-0.01530)	0.263842
Peak 2	4.1864	1.6581	(-0.02044)	----	----	----	
Peak 3	----	----	----	4.8711	2.3584	(-0.01530)	
Peak 1	----	----	----	3.5250	(1)	(-0.01530)	0.428777
Peak 2	4.1795	(1)	(-0.02044)	----	----	----	
Peak 3	----	----	----	4.8719	(2)	(-0.01530)	
Peak 1	----	----	----	3.5264	0.6217	(-0.01530)	0.336138
Peak 2	4.1838	1.4222	(-0.02044)	----	----	----	
Peak 3	----	----	----	4.8722	2.2728	(-0.01530)	

	R_S	CN_S	c_{2S}	R_{Fe}	CN_{Fe}	c_{2Fe}	F
Peak 1	3.6739	(1)	(-0.02044)	----	----	----	0.423233
Peak 2	4.1878	(2)	(-0.02044)	----	----	----	
Peak 3	----	----	----	4.8706	(2)	(-0.01530)	
Peak 1	3.6741	0.7426	(-0.02044)	----	----	----	0.343948
Peak 2	4.1879	1.4389	(-0.02044)	----	----	----	
Peak 3	----	----	----	4.8727	2.3210	(-0.01530)	
Peak 1	3.9185	(1)	(-0.02044)	3.5235	(1)	(-0.01530)	0.214485
Peak 2	4.1877	(2)	(-0.02044)	3.9187	(1)	(-0.01530)	
Peak 3	4.6511	(1)	(-0.02044)	4.8777	(2)	(-0.01530)	
Peak 1	3.7559	0.5039	(-0.02044)	3.5223	0.2110	(-0.01530)	0.0841238
Peak 2	4.1761	(2)	(-0.02044)	3.8289	(1)	(-0.01530)	
Peak 3	4.6558	(1)	(-0.02044)	4.8752	(2)	(-0.01530)	
Peak 1	----	----	----	3.5704	0.4897	(-0.01530)	0.133686
Peak 2	4.1743	2.0977	(-0.02044)	3.8271	(1)	(-0.01530)	
Peak 3	4.6614	(1)	(-0.02044)	4.8723	(2)	(-0.01530)	
Peak 1	----	----	----	3.5374	(0.4897)	(-0.01530)	0.0829290
Peak 2	4.1765	1.7783	(-0.02044)	3.8376	0.5638	(-0.01530)	
Peak 3	4.6489	(1)	(-0.02044)	4.8773	(2)	(-0.01530)	
Peak 1	----	----	----	3.5379	(0.4897)	(-0.01530)	0.0721235
Peak 2	4.1753	(1.7783)	(-0.02044)	3.8349	(0.5638)	(-0.01530)	
Peak 3	4.6522	1.0658	(-0.02044)	4.8769	1.8254	(-0.01530)	
Peak 1	3.7337	0.7867	(-0.02044)	----	----	----	0.0878662
Peak 2	4.1742	1.9878	(-0.02044)	3.8184	1.0766	(-0.01530)	
Peak 3	4.6563	(1)	(-0.02044)	4.8748	(2)	(-0.01530)	
Peak 1	3.7332	(0.7867)	(-0.02044)	----	----	----	0.0796796
Peak 2	4.1736	(1.9878)	(-0.02044)	3.8176	(1.0766)	(-0.01530)	
Peak 3	4.6587	1.0291	(-0.02044)	4.8742	1.8529	(-0.01530)	

Fits of CDHRD.FIL1 :

<u>N only :</u>	R	CN	c_2	F
Initial	1.8	2	-0.02297	----
	1.9201	0.6334	"	0.637959
	1.9740	7.4909	-0.07422	0.415094
	1.9435	(2)	-0.04329	0.506762
<u>S only :</u>	R	CN	c_2	F
Initial	1.8	1	-0.02044	----
	1.6224	0.2243	"	0.533847
	1.6059	0.9354	-0.04840	0.385836
	1.6048	(1)	-0.05016	0.386334

Fits of CDHRD.FIL2 :

<u>N only :</u>	R	CN	c ₂	F
Initial	2.2	3	-0.02297	----
	2.0798	3.5449	"	1.24005
	2.0791	3.6788	-0.02357	1.23968
	2.0821	(3)	-0.02088	1.24820

<u>S only :</u>	R	CN	c ₂	F
Initial	2.2	2	-0.02044	----
	2.2403	1.8164	"	0.379339
	2.2399	2.0361	-0.02226	0.360653
	2.2400	(2)	-0.02202	0.361055
	2.2389	(3)	-0.02807	0.531841

<u>N + S :</u>	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.9750	"	"	2.2599	"	"	0.858254
	1.8890	0.4937	"	2.2401	1.9343	"	0.253392
	1.8711	3.7475	-0.07541	2.2430	1.9986	-0.02167	0.153089
	1.9931	(1)	-0.02260	2.2523	(1)	-0.01314	0.482859
	1.9931	(2)	-0.03557	2.2503	(1)	-0.01385	0.429123
	1.9895	(3)	-0.04505	2.2487	(1)	-0.01407	0.404089
**	1.8777	(1)	-0.03842	2.2415	(2)	-0.02133	0.199267
	1.8763	(2)	-0.05466	2.2427	(2)	-0.02147	0.166762
	1.8732	(3)	-0.06728	2.2430	(2)	-0.02161	0.154976
	1.8229	(1)	-0.03090	2.2374	(3)	-0.02726	0.316474
	1.8147	(2)	-0.04621	2.2393	(3)	-0.02706	0.317938
	1.8074	(3)	-0.05859	2.2403	(3)	-0.02709	0.330015

— these R values used as starting values in the fixed-CN fit.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.612090). Next best initial fit is F = 0.858254 (for Ni-N₂S₂).

<u>N + N' :</u>	R _N	CN _N	c _{2N}	R _{N'}	CN _{N'}	c _{2N'}	F
Initial	2.0	2	-0.02297	2.2	4	-0.02297	-----
##	1.8870	"	"	2.0777	"	"	1.04605
	1.8879	2.3569	"	2.0791	5.7030	"	0.738293
	1.9528	5.8440	-0.04212	2.1044	2.8989	-0.01699	0.642506
	1.9168	(2)	-0.01953	2.0898	(4)	-0.01743	0.720253

— these R values used as starting values in the fixed-CN fit.

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
Initial	2.0	2	-0.02044	2.2	2	-0.02044	-----
##	2.1808	"	"	2.3094	"	"	1.03127
	2.0573	0.3326	"	2.2411	2.1054	"	0.184136
	2.0888	1.2864	-0.05663	2.2454	2.0398	-0.02169	0.0816660
	2.1853	(1)	-0.02467	2.2654	(1)	-0.01543	0.254993
	2.1942	(2)	-0.04635	2.2502	(1)	-0.01530	0.196338
	2.0916	(1)	-0.04862	2.2457	(2)	-0.02128	0.0928909
**	2.0949	(2)	-0.07426	2.2446	(2)	-0.02175	0.100208
	2.0098	(1)	-0.03894	2.2388	(3)	-0.02593	0.184192
	2.0152	(2)	-0.06191	2.2423	(3)	-0.02649	0.266388

— these R values used as starting values in the fixed-CN fit.

** — of the fixed-CN fits, this one has the best initial fit ($F = 1.03127$). Next best initial fit is $F = 1.07154$ (for Ni-S₁S₁).

Fits of CDHRD.FIL3 :**N + S :**

	R_N	CN_N	c_{2N}	R_S	CN_S	c_{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
	1.8700	"	"	2.2341	"	"	0.743142
	1.8713	1.1897	"	2.2363	2.1245	"	0.429883
	1.8462	1.4783	-0.02572	2.2347	3.0249	-0.02634	0.226235
	1.9439	(1)	-0.02831	2.2447	(1)	-0.01283	0.834657
	1.9502	(2)	-0.03653	2.2470	(1)	-0.01301	0.714210
	1.9532	(3)	-0.04233	2.2482	(1)	-0.01320	0.621341
**	1.8690	(1)	-0.02420	2.2369	(2)	-0.02069	0.450366
	1.8847	(2)	-0.03413	2.2416	(2)	-0.02034	0.357920
	1.8941	(3)	-0.04118	2.2445	(2)	-0.02042	0.309399
	1.8413	(1)	-0.02056	2.2324	(3)	-0.02676	0.247253
	1.8520	(2)	-0.03040	2.2371	(3)	-0.02586	0.239318
	1.8601	(3)	-0.03765	2.2408	(3)	-0.02563	0.301789

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit ($F = 0.463157$). Next best initial fit is $F = 0.743142$ (for Ni-N₂S₂).

N + N' :

	R_N	CN_N	c_{2N}	$R_{N'}$	$CN_{N'}$	$c_{2N'}$	F
Initial	1.9	2	-0.02297	2.2	4	-0.02297	-----
##	1.8714	"	"	2.0719	"	"	1.06640
	1.8786	3.3869	"	2.0742	6.5165	"	0.599615
	1.9309	9.6969	-0.04838	2.1030	3.7629	-0.01922	0.382274
	1.8963	(2)	-0.01826	2.0805	(4)	-0.01744	0.831311

— these R values used as starting values in the fixed-CN fit.

S + S' :

	R_S	CN_S	c_{2S}	$R_{S'}$	$CN_{S'}$	$c_{2S'}$	F
Initial	1.9	2	-0.02044	2.2	2	-0.02044	-----
	1.9976	"	"	2.2108	"	"	2.42063
	2.0459	0.6244	"	2.2379	2.3938	"	0.512846
	1.9721	0.3346	-0.01151	2.2205	3.9545	-0.03214	0.348967
	2.1691	(1)	-0.02776	2.2596	(1)	-0.01425	0.688567
	2.1762	(2)	-0.04251	2.2542	(1)	-0.01487	0.534805
	2.1800	(3)	-0.05303	2.2513	(1)	-0.01494	0.463262
**	2.0919	(1)	-0.03247	2.2490	(2)	-0.01980	0.512119
	2.1150	(2)	-0.04741	2.2520	(2)	-0.02110	0.481359
	2.1278	(3)	-0.06086	2.2505	(2)	-0.02192	0.510134
	2.0346	(1)	-0.02847	2.2386	(3)	-0.02422	0.436179
	2.0649	(2)	-0.04307	2.2484	(2)	-0.02493	0.525038
	2.0793	(3)	-0.05639	2.2507	(3)	-0.02614	0.628742

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 1.20716). Next best initial fit is F = 1.24502 (for Ni-S₁S₃).

Fits of CDHRD.FIL6 :**N + S :**

	R_N	CN_N	c_{2N}	R_S	CN_S	c_{2S}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	-----
##	1.8697	"	"	2.2346	"	"	0.799125
	1.8720	1.3047	"	2.2368	2.2096	"	0.519996
	1.9583	11.9063	-0.07304	2.2489	1.0066	-0.01325	0.400760
	1.9391	(1)	-0.02534	2.2454	(1)	-0.01173	0.856339
	1.9451	(2)	-0.03419	2.2478	(1)	-0.01194	0.738258
	1.9482	(3)	-0.04040	2.2490	(1)	-0.01216	0.650253
**	1.8734	(1)	-0.02222	2.2370	(2)	-0.01979	0.564461
	1.8851	(2)	-0.03190	2.2416	(2)	-0.01943	0.489761
	1.8926	(3)	-0.03883	2.2445	(2)	-0.01947	0.460015
	1.8470	(1)	-0.01936	2.2326	(3)	-0.02591	0.507370
	1.8550	(2)	-0.02889	2.2371	(3)	-0.02505	0.495076
	1.8613	(3)	-0.03588	2.2406	(3)	-0.02480	0.529538

— these R values used as starting values in each of the fixed-CN fits.

** — of the fixed-CN fits, this one has the best initial fit (F = 0.581252). Next best initial fit is F = 0.799125 (for Ni-N₂S₂).

Three-Wave Fits of CODH data, using N, S and Fe

(checked and corrected 1st Mar 1993)

Grace Tan

25th Feb 1993

These fits are discussed in Chapter 7, Section (E.2.d).

Fits of CODHX.FIL6 :

N + S + S' :

Initial ##	R _N	CN _N	C _{2N}	R _S	CN _S	C _{2S}	R _{S'}	CN _{S'}	C _{2S'}	F
	1.9	2	-0.02297	2.2	1	-0.02044	2.3	1	-0.02044	-----
	1.8519	"	"	2.2252	"	"	2.2253	"	"	0.552837
**	1.8531	2.1949	"	2.2252	1.0693	"	2.2252	1.0747	"	0.527269
**	1.8544	2.1777	"	2.2415	8.8200	"	2.2467	-6.7720	"	0.523152
**	1.8544	2.1778	"	2.2415	9.0004	"	2.2466	-6.9521	"	0.523151
**	1.8652	(2)	-0.01966	2.2043	(1)	-0.01158	2.2885	(1)	-0.02212	0.404753
**	1.8571	(2)	-0.01955	2.2182	(2)	-0.01806	2.3784	(1)	-0.03812	0.315513
Initial	1.9	2	-0.02297	2.2	2	-0.02044	2.7	1	-0.02044	-----
	1.8505	"	"	2.2242	"	"	2.8832	"	"	0.663708
	1.8525	2.1711	"	2.2248	2.1280	"	2.8799	0.3614	"	0.455441
	1.8540	(2)	-0.02075	2.2232	(2)	-0.01924	2.9048	(1)	-0.03899	0.419662

** — Use ## as initial value, but reset R_{S'} = 2.3 Å

N + S + Fe :

Initial	R _N	CN _N	C _{2N}	R _S	CN _S	C _{2S}	R _{Fe}	CN _{Fe}	C _{2Fe}	F
	1.9	2	-0.02297	2.2	2	-0.02044	2.7	1	-0.01530	-----
	1.8522	"	"	2.2253	"	"	2.7153	"	"	0.781714
	1.8533	2.1387	"	2.2256	2.1073	"	2.7044	0.3085	"	0.440529
	1.8542	(2)	-0.02099	2.2239	(2)	-0.01935	2.7153	(1)	-0.03331	0.385578
Initial	1.9	2	-0.02297	2.2	2	-0.02044	2.9	1	-0.01530	-----
	1.8496	"	"	2.2236	"	"	3.0557	"	"	0.857158
	1.8529	2.1971	"	2.2250	2.1448	"	3.0620	0.1017	"	0.522017
	1.8542	(2)	-0.02077	2.2234	(2)	-0.01924	3.4961	(1)	-0.11151	0.491808

<u>N + S + Fe :</u>										
Initial	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	R _{Fe}	CN _{Fe}	c _{2Fe}	F
	1.9	2	-0.02297	2.2	2	-0.02044	2.7	1	-0.01530	-----
	1.9032	"	"	2.2494	"	"	2.6783	"	"	0.950351
	1.8867	1.3724	"	2.2397	1.6676	"	2.7144	0.3022	"	0.548799
Initial	1.8817	(2)	-0.03006	2.2431	(2)	-0.02350	2.7192	(1)	-0.02733	0.391462
	1.9	2	-0.02297	2.2	2	-0.02044	3.0	1	-0.01530	-----
	1.8817	"	"	2.2372	"	"	3.1012	"	"	0.932091
	1.8830	1.4218	"	2.2382	1.7050	"	3.0784	0.1483	"	0.606296
1.8800	(2)	-0.02940	2.2417	(2)	-0.02331	3.4663	(1)	-0.10724	0.559856	
<u>N + S + N' :</u>										
Initial	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	R _{N'}	CN _{N'}	c _{2N'}	F
	1.9	2	-0.02297	2.2	2	-0.02044	2.7	2	-0.02297	-----
	1.8912	"	"	2.2422	"	"	2.6974	"	"	0.675035
	1.8870	1.4140	"	2.2402	1.7052	"	2.7092	1.1674	"	0.498797
Initial	1.8814	(2)	-0.02970	2.2427	(2)	-0.02336	2.7197	(2)	-0.02948	0.370963
	1.9	2	-0.02297	2.2	2	-0.02044	3.0	2	-0.02297	-----
	1.8843	"	"	2.2383	"	"	3.1184	"	"	0.811230
	1.8835	1.4237	"	2.2384	1.7055	"	3.1026	0.3345	"	0.610661
1.8800	(2)	-0.02940	2.2417	(2)	-0.02331	3.4085	(2)	-0.09839	0.559161	

Fits of CDHOX.FIL6 :

<u>N + S + S' :</u>										
Initial	R _N	CNN	C _{2N}	R _S	CNS	C _{2S}	R _{S'}	CNS'	C _{2S'}	F
	1.9	2	-0.02297	2.2	1	-0.02044	2.3	1	-0.02044	----
	1.8870	"	"	2.2320	"	"	2.2320	"	"	0.623468
	1.8818	1.4035	"	2.2190	1.3286	"	2.2525	0.7842	"	0.473538
	1.9055	(2)	-0.03056	2.2309	(1)	-0.03690	2.2390	(1)	-0.01406	0.445932
Initial	1.9	2	-0.02297	2.2	2	-0.02044	2.3	1	-0.02044	----
	1.8663	"	"	2.2022	"	"	2.3037	"	"	0.634603
	1.8913	1.5767	"	2.2372	2.2308	"	2.4802	0.3624	"	0.416485
	1.8748	(1)	-0.01880	2.2267	(2)	-0.02080	2.2904	(1)	-0.07375	0.451111
	1.8903	(2)	-0.02690	2.2320	(2)	-0.01950	2.4028	(1)	-0.05055	0.394554
Initial	1.9	2	-0.02297	2.2	2	-0.02044	2.7	1	-0.02044	----
	1.8845	"	"	2.2305	"	"	2.9019	"	"	0.613206
	1.8831	1.3766	"	2.2308	2.0265	"	2.8983	0.5303	"	0.276810
	1.8899	(2)	-0.02902	2.2348	(2)	-0.02034	2.9040	(1)	-0.03004	0.290559
<u>N + S + Fe :</u>										
Initial	R _N	CNN	C _{2N}	R _S	CNS	C _{2S}	R _{Fe}	CN _{Fe}	C _{2Fe}	F
	1.9	2	-0.02297	2.2	2	-0.02044	2.7	1	-0.01530	----
	1.8861	"	"	2.2299	"	"	2.7472	"	"	0.747356
	1.8842	1.3305	"	2.2307	1.9893	"	2.7422	0.4263	"	0.278355
	1.8916	(2)	-0.03001	2.2358	(2)	-0.02079	2.7360	(1)	-0.02557	0.195659
Initial	1.9	2	-0.02297	2.2	2	-0.02044	3.0	1	-0.01530	----
	1.8815	"	"	2.2298	"	"	3.0791	"	"	0.817655
	1.8831	1.4150	"	2.2311	2.0492	"	3.0732	0.2424	"	0.440239
	1.8906	(2)	-0.02880	2.2352	(2)	-0.02026	3.5570	(1)	-0.10840	0.458161

<u>N + S + N' :</u>										
	R _N	CNN	C _{2N}	R _S	CN _S	C _{2S}	R _{N'}	CNN'	C _{2N'}	F
Initial	1.9	2	-0.02297	2.2	2	-0.02044	2.7	2	-0.02297	-----
	1.8905	"	"	2.2331	"	"	2.7382	"	"	0.522501
	1.8858	1.3835	"	2.2320	2.0265	"	2.7388	1.3338	"	0.245315
	1.8919	(2)	-0.02960	2.2359	(2)	-0.02059	2.7369	(2)	-0.02802	0.187309
Initial	1.9	2	-0.02297	2.2	2	-0.02044	3.0	2	-0.02297	-----
	1.8852	"	"	2.2314	"	"	3.1016	"	"	0.705976
	1.8838	1.4194	"	2.2314	2.0494	"	3.0946	0.5123	"	0.458226
	1.8907	(2)	-0.02880	2.2352	(2)	-0.02026	3.4796	(2)	-0.10393	0.457038

Fits of CODHRX.FIL6 :

<u>N + S + S' :</u>										
Initial	R _N	CN _N	c _{2N}	R _S	CN _S	c _{2S}	R _{S'}	CN _{S'}	c _{2S'}	F
	1.9	2	-0.02297	2.2	2	-0.02044	2.3	1	-0.02044	-----
	1.8863	"	"	2.2392	"	"	2.2749	"	"	0.906768
	1.8886	1.9373	"	2.2511	1.8835	"	2.2512	0.9428	"	0.896653
	1.8373	(2)	-0.02378	2.1825	(2)	-0.01279	2.3168	(1)	-0.00384	0.367692
Initial	1.9	2	-0.02297	2.2	3	-0.02044	2.3	1	-0.02044	-----
	1.8765	"	"	2.2309	"	"	2.3360	"	"	1.15023
	1.8886	1.9373	"	2.2512	2.2484	"	2.2509	0.5778	"	0.896653
	1.8152	(2)	-0.02753	2.1845	(3)	-0.02364	2.3070	(1)	-0.00802	0.578308
Initial	1.9	2	-0.02297	2.2	3	-0.02044	2.7	1	-0.02044	-----
	1.8906	"	"	2.2519	"	"	2.6230	"	"	0.725640
	1.8877	1.6913	"	2.2514	2.6116	"	2.6224	0.8430	"	0.544102
	1.8765	(2)	-0.02800	2.2531	(3)	-0.02359	2.6104	(1)	-0.01857	0.444381
Initial	1.9	2	-0.02297	2.2	3	-0.02044	2.9	1	-0.02044	-----
	1.8873	"	"	2.2504	"	"	2.9466	"	"	1.02479
	1.8878	1.9341	"	2.2508	2.8243	"	2.9423	0.2933	"	0.872221
	1.8846	(2)	-0.02409	2.2509	(3)	-0.02120	3.2334	(1)	-0.02794	0.868847

<u>N + S + Fe :</u>										
	R_N	CNN	$c_{2N}^{c_{2N}}$	R_S	CNS	$c_{2S}^{c_{2S}}$	R_{Fe}	CNFe	$c_{2Fe}^{c_{2Fe}}$	F
Initial	1.9	2	-0.02297	2.2	3	-0.02044	2.7	1	-0.01530	-----
	1.8840	"	"	2.2485	"	"	2.8020	"	"	0.880066
	1.8852	1.9038	"	2.2495	2.7983	"	2.7982	0.5573	"	0.755406
	1.8799	(2)	-0.02455	2.2494	(3)	-0.02157	2.7970	(1)	-0.01838	0.825711
Initial	1.9	2	-0.02297	2.2	3	-0.02044	3.0	1	-0.01530	-----
	1.8863	"	"	2.2501	"	"	3.0907	"	"	0.860810
	1.8866	1.9567	"	2.2503	2.8376	"	3.0882	0.5898	"	0.788659
	1.8834	(2)	-0.02397	2.2503	(3)	-0.02119	3.0882	(1)	-0.01926	0.829862

<u>N + S + N' :</u>										
	R_N	CNN	$c_{2N}^{c_{2N}}$	R_S	CNS	$c_{2S}^{c_{2S}}$	$R_{N'}$	CNN'	$c_{2N'}^{c_{2N'}}$	F
Initial	1.9	2	-0.02297	2.2	3	-0.02044	2.7	2	-0.02297	-----
	1.8878	"	"	2.2506	"	"	2.8147	"	"	0.886183
	1.8875	1.9325	"	2.2507	2.8219	"	2.8120	1.1764	"	0.829866
	1.8816	(2)	-0.02443	2.2501	(3)	-0.02147	2.8078	(2)	-0.02122	0.858402
Initial	1.9	2	-0.02297	2.2	3	-0.02044	3.0	2	-0.02297	-----
	1.8884	"	"	2.2509	"	"	3.0958	"	"	0.877716
	1.8878	1.9487	"	2.2508	2.8328	"	3.0936	1.2968	"	0.841414
	1.8840	(2)	-0.02408	2.2506	(3)	-0.02121	3.0928	(2)	-0.02284	0.860931

Fits of CDHRD.FIL6 :

<u>N + S + S' :</u>		R_N	CNN	C_{2N}^{2N}	R_S	CNS	C_{2S}^{2S}	$R_{S'}$	CN S'	$C_{2S'}^{2S'}$	F
Initial ##	1.9	1.8698	2	-0.02297	2.2	1	-0.02044	2.3	1	-0.02044	-----
		1.8492	1.2103	"	2.2345	"	"	2.2346	"	"	0.799128
		1.8763	(2)	-0.02942	2.1561	0.6860	"	2.2554	2.0532	"	0.481828
Initial	1.9	1.8576	2	-0.02297	2.1852	(1)	-0.01066	2.3006	(1)	-0.01019	0.477713
		1.8492	"	"	2.2	2	-0.02044	2.3	1	-0.02044	-----
		1.8747	1.2110	"	2.2084	"	"	2.3016	"	"	0.599538
Initial	1.9	1.8693	(2)	-0.03031	2.1563	0.6880	"	2.2555	2.0513	"	0.481828
		1.8493	1	-0.02297	2.2380	(2)	-0.01968	2.3052	(1)	-0.06122	0.422606
		1.8517	"	"	2.2	1	-0.02044	2.3	1	-0.02044	-----
Initial	1.9	1.9645	1.2117	"	2.2368	"	"	2.2368	"	"	0.573818
		1.8492	(1)	-0.01866	2.2555	2.0495	"	2.1565	0.6890	"	0.481829
		1.8746	2	-0.02297	2.1682	(1)	-0.00872	2.2967	(1)	-0.00741	0.516684
Initial	1.9	1.8661	"	"	2.2	2	-0.02044	2.7	1	-0.02044	-----
		1.8703	1.2097	"	2.2671	"	"	2.5365	"	"	1.10544
		1.8843	(2)	-0.03194	2.1559	0.6848	"	2.2554	2.0548	"	0.481828
Initial	1.9	1.8661	(2)	-0.03031	2.2379	(2)	-0.01968	2.3051	(1)	-0.06118	0.422606
		1.8703	2	-0.02297	2.2	2	-0.02044	2.9	1	-0.02044	-----
		1.8843	"	"	2.2331	"	"	2.9192	"	"	0.798418
Initial	1.9	1.8661	1.2870	"	2.2361	2.1980	"	2.9158	0.5007	"	0.375228
		1.8703	(2)	-0.03194	2.2413	(2)	-0.01945	2.9200	(1)	-0.03250	0.350360
		1.8843	"	"	2.2413	(2)	-0.01945				

** — Initial values in ##, except that $R_S = 2.2 \text{ \AA}$ and $R_{S'} = 2.3 \text{ \AA}$.

<u>N + S + Fe :</u>									
Initial	R _N	CNN	C _{2N}	R _S	CNS	C _{2S}	R _{Fe}	CN _{Fe}	C _{2Fe}
	1.8614	2	-0.02297	2.2	2	-0.02044	2.7	1	-0.01530
	1.8692	"	"	2.2305	"	"	2.7706	"	"
	1.8853	1.2500	"	2.2356	2.1686	"	2.7634	0.3787	"
Initial	1.9	(2)	-0.03263	2.2419	(2)	-0.01970	2.7504	(1)	-0.02892
	1.8660	2	-0.02297	2.2	2	-0.02044	3.0	1	-0.01530
	1.8712	"	"	2.2334	"	"	3.0939	"	"
	1.8851	1.3182	"	2.2365	2.2176	"	3.0912	0.2249	"
		(2)	-0.03190	2.2416	(2)	-0.01943	3.5213	(1)	-0.09818
									F

									0.905008
									0.398986
									0.332579

									0.960752
									0.493907
									0.487815

<u>N + S + N' :</u>									
Initial	R _N	CNN	C _{2N}	R _S	CNS	C _{2S}	R _{N'}	CN _{N'}	C _{2N'}
	1.8689	2	-0.02297	2.2	2	-0.02044	2.7	2	-0.02297
	1.8718	"	"	2.2342	"	"	2.7620	"	"
	1.8858	1.2732	"	2.2368	2.1917	"	2.7563	1.1703	"
Initial	1.9	(2)	-0.03236	2.2420	(2)	-0.01957	2.7474	(2)	-0.03195
	1.8689	2	-0.02297	2.2	2	-0.02044	3.0	2	-0.02297
	1.8718	"	"	2.2344	"	"	3.1171	"	"
	1.8852	1.3128	"	2.2367	2.2145	"	3.1125	0.4487	"
		(2)	-0.03191	2.2416	(2)	-0.01943	3.4447	(2)	-0.09193
									F

									0.763094
									0.383414
									0.326899

									0.871425
									0.509074
									0.485990

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