

Determination of metal-metal distances: significance and accuracy

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Nature utilises a variety of metal clusters as catalytic centers. Some of them make use of two or more metals in the catalytic site. The variation of metal-metal distances plays an important role in several processes like charge transfer and weakening of bonds. X-ray absorption spectroscopy can determine these metal-metal distances in several states (crystal, solution or amorphous). However sometimes backscattering from light elements hides the metal-metal contribution to the fine structure. Here we point out significance and accuracy of metal-metal distances in a model system. Therefore a number of different refinement protocols are applied to the data. These protocols will be discussed focusing on the significance and accuracy of the metal-metal distances extracted from the data.

Keywords: Debye-Waller factors, Exafs, model systems.

1. Introduction

Frequently, the accurate determination of metal-metal distances in multi-nuclear catalytic centers of metalloenzymes is the key to the correct understanding of the catalytic mechanism. Studies on model systems have been helpful in determining optimum conditions for the experiments. Furthermore these measurements point out possible problems and restrictions, for example: Zhang *et al.* (1996). Here we focus on the superimposition of metal-metal contributions with the backscattering of carbon atoms, like in metalloproteins, where carbon atoms of the amino acids are at similar distances to those of the metal atoms.

Our approach includes not only multiple K-edge refinements [Zhang *et al.* (1996), Meyer-Klaucke *et al.* (1999)] but also the temperature dependence of the disorder, which we describe by the Debye model. Thus more information is available to extract the metal core structure. Instead of only two measurements at different metal K-edges, we use measurements at three different temperatures for each K-edge in the refinement procedure.

2. Experimental

Model samples (Fig. 1) of thiophenolate-bridged complexes of general formula [LFeNiFeL]ⁿ⁺ with n=2,3,4 where L³⁻ is the trianionic form of the ligand 1,4,7-tris (4-tertbutyl-2-mercaptobenzyl)-1,4,7 triazocyclononane [Glaser *et al.* (1999)] were measured by XAFS.

Fe- and Ni-K edge X-ray absorption spectra at 20, 85 and 178K were recorded at the beamline D2 of the EMBL Outstation Hamburg at DESY. The Synchrotron was operating between 85 and 140 mA. A Si (111) double crystal monochromator and a focusing mirror were used. The data were collected in absorption mode by ionization chambers.

Data reduction was achieved with the program package EXPROG [Nolting & Hermes (1992)]. The data were refined using Feff6.01 [Zabinsky *et al.* (1995)] and Feffit2.32 [Newville *et al.* (1995)]. Multiple scattering (MS) paths up to 4.5 Å were included and the corresponding Debye-Waller factors of each MS contribution were estimated by the square root of the sum of the squares of the single scattering ones.

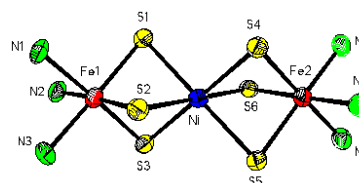


Figure 1
Overall structure of the [LFeNiFeL]ⁿ⁺ core.

As a statistical test for the significance of the results the F-test introduced by Joyner *et al.* (1987) is applied. We make use of the implementation of Michalowicz *et al.* (1999).

3. Refinement method

Exafs measurements on similar compounds with Cr instead of Ni have been reported by Meyer-Klaucke *et al.* (1999). Essentially the same structural model is put in practice here. For the Fe-K edge the model comprises a first shell of 3 N, a second shell of 3 S, a shell for the Fe-Ni scattering and four shells with 3 ligands (C) each. The contribution of the carbon groups have been refined using a rigid movement of their relative distances. For the Ni-K edge fit only a first shell including 3 S and the Ni-Fe contribution are included.

The flexibility of the FEFFIT program was used to compare three different approaches:

- I) a single edge refinement for each metal site,
- II) simultaneous Ni- and Fe-K edge refinements for each temperature, and
- III) a fit using in addition the Debye model for the thermal vibrations in a simultaneous refinement of measurements taken at three temperatures and two absorption edges.

Approach I

First, a multiple scattering single edge fit was performed on each sample for each temperature for the Fe-K edge. This represents the refinement procedure applied frequently to heteronuclear metalloenzymes. In Table 1 the results for the three different samples at three different temperatures are presented. All of the nine refinements significantly improve including the metal-metal contribution. This can be concluded from the statistical F-test results given in Table 3. With the oxidation state of the metal core the metal-metal distances vary. Thus these series allow analysing the significance of the metal-metal contribution for different distances. The variation of the metal-metal distance is the important step in the systematic analysis. The superimposition of the metal and carbon backscattering contribution reduces the accuracy for refinements of [LFeNiFeL]³⁺ and [LFeNiFeL]⁴⁺. Already at 178K the significance for the metal-metal signal is rather poor. Hence single temperature measurements should at least be carried out at liquid nitrogen temperature. Interestingly the Fe-Ni distance is refined to the same value for 20 K and 85 K with a slightly increased error for the higher temperature. The Debye-Waller factor again has similar errors for both temperatures, but its absolute value increases by about 30%.

The Fe-C contributions for three C atoms at about 3Å superimpose with the metal-metal contribution. Thus the results for the carbon groups deserve some attention. Only the result for the nearest C is shown since the other Fe-C distances were varied rigidly, taking the crystallographic data as a starting point [Glaser *et al.* (1999)]. The results for each separate temperature arising from a single edge fit (Table 2) give reasonable inaccuracies in the Fe-C distances but rather arbitrary values for the Debye-Waller factors,

Table 1

Fe edge fit of the metal-metal distances and the σ^2 , both as free parameters.*

	2+		3+		4+	
	Fe-C	$\sigma^2 * 10^{-2}$	Fe-C	$\sigma^2 * 10^{-2}$	Fe-C	$\sigma^2 * 10^{-2}$
20K	3.047 (7)	0.35(7)	2.972(9)	0.37(10)	2.971(9)	0.37(9)
85K	3.047(9)	0.48(10)	2.971(9)	0.46(9)	2.976(10)	0.46(11)
178K	3.056(7)	0.42(7)	2.983(16)	0.48(16)	3.008(19)	0.65(20)

Table 2

Fe edge fit of the Fe-C distances and the σ^2 , both as free parameters (only iron edge shown). Results for the nearest C are shown.

	2+		3+		4+	
	Fe-C	$\sigma^2 * 10^{-2}$	Fe-C	$\sigma^2 * 10^{-2}$	Fe-C	$\sigma^2 * 10^{-2}$
20K	2.823 (10)	0.44(14)	2.811(15)	0.55(22)	2.825(16)	0.64(22)
85K	2.822(12)	0.46(18)	2.832(15)	0.74(21)	2.827(15)	0.59(21)
178K	2.819(7)	0.27(10)	2.792(16)	0.43(25)	2.804(11)	0.09(13)

Table 3

Approach I: F- test probability values for the Fe edge fit with metal-metal distances and the σ^2 , both as free parameters. Approach II: F- test probability values for the Fe edge fit with metal-metal distances and the σ^2 , both as free parameters (multiple edge fit). Approach III: F- test probability values for the multiple edge fit a Debye model for the σ^2 .

Approach I			
F/p(F,12,10)	2+	3+	4+
20K	3.5/0.996	2.03/0.939	2.37/0.97
85K	2.32/0.966	2.67/0.98	2.02/0.938
178K	3.32/0.995	1.29/0.71	1.32/0.73
Approach II			
F/p(F,12,10)	2+	3+	4+
20K	3.08/0.999	2.39/0.997	2.03/0.988
85K	2.36/0.997	2.42/0.997	1.85/0.976
178K	2.12/0.992	1.58/0.932	1.37/0.846
Approach III			
F/p(F,12,10)	2+	3+	4+
	1.98/0.999	1.93/0.999	1.68/0.999

e.g. its decrease with increasing temperature can not be justified. This is due to mathematical correct but physically unreasonable global minima for the refinement. These minima are independent of the starting parameters. Thus a strategy is needed to overcome such misleading results.

Approach II

Applying a multiple K-edge refinement is appropriate for heteronuclear metal cores. For $[\text{LFeNiFeL}]^{2+}$ and $[\text{LFeNiFeL}]^{3+}$ the significance for the metal-metal contributions increases considerably, but the F-test results (Table 3) for $[\text{LFeNiFeL}]^{4+}$ show that in critical cases significance has to be improved. For all the metal-metal distances given in Table 4 the error margins for the distances were lowered by this approach by at least 20 to 30 %. Interestingly this is not the case for the Debye-Waller factors, which have similar errors for approach I and II. This can be explained by the low correlation they show with other refined parameters, e.g. the highest correlation is obtained for the carbon atoms Debye-Waller factor with the metal-metal distance (<0.4 in each refinement). The errors in the Debye-Waller factors reflect the goodness of the fit. Therefore a further improvement of the significance must be

achieved if several light atoms hide the metal backscattering contribution or if the two metals have no bridging ligands.

For the carbon atoms the results are similar to the approach I because no additional information is available for the refinement of their positions and Debye-Waller factors compared to the single edge fits (Table 5).

Approach III

Which additional information is available to refine the metal core coordination?

We suggest applying an appropriate model for the temperature dependence of the individual backscattering contributions.

In Fig. 2 the metal backscattering signal at two different temperatures is plotted. Whereas the amplitude changes only slightly for the carbon backscattering, it is lowered by a factor of more than 2 for the metal backscattering. This different temperature dependence is the additional information we need to improve the significance of the refinement. The Debye as well as the Einstein models are reasonable estimates for the change of the Debye-Waller factors with temperature. Use of one of these models reduces the number of free parameters and thereby increases the significance of the refined distances and Debye-Waller factors.

Assuming the metal-metal distance to be constant over the temperature range under study allows even a further restriction of the number of free parameters. Especially if the error margins are quite high, like for the multiple K-edge refinement of $[\text{LFeNiFeL}]^{4+}$, this is an adequate method to obtain a well defined metal-metal distance.

Table 4

Multiple edge fit of the metal-metal distances and the σ^2 , both as free parameters (only Fe K-edge shown).

	2+		3+		4+	
	Fe-Ni	$\sigma^2 * 10^{-2}$	Fe-Ni	$\sigma^2 * 10^{-2}$	Fe-Ni	$\sigma^2 * 10^{-2}$
20K	3.046 (5)	0.33(5)	2.971(7)	0.37(7)	2.982(8)	0.41(8)
85K	3.046 (7)	0.34(6)	2.973(7)	0.47(7)	2.981(9)	0.53(9)
178K	3.052 (7)	0.45(7)	2.979(12)	0.68(12)	2.999(15)	0.89(17)

Table 5

Multiple edge fit of the Fe-C distances and the σ^2 , both as free parameters (only iron edge shown). Results for the nearest C are shown.

	2+		3+		4+	
	Fe-C	$\sigma^2 * 10^{-2}$	Fe-C	$\sigma^2 * 10^{-2}$	Fe-C	$\sigma^2 * 10^{-2}$
20K	2.823 (9)	0.46(13)	2.812(20)	0.55(26)	2.824(24)	0.60(33)
85K	2.819(14)	0.48(20)	2.832(17)	0.73(24)	2.826(24)	0.55(32)
178K	2.820(10)	0.30(13)	2.800(20)	0.46(30)	2.807(16)	0.10(20)

The results of the simultaneous refinement of six different spectra for each sample are summarised in tables 6 and 7. The significance for the metal-metal contribution exceeds 0.99 for all cases (Table 3). The accuracy for the metal-metal distance is in the order of 0.005 \AA^{-1} . Especially for the comparison of a series of samples such a relative accuracy is extremely helpful. Nevertheless it should be pointed out that this distance represents an average over a temperature range. The authors do not expect the distance to be constant over the temperature range under study. But this approximation allows probing the significance of the presence of a metal-metal contribution with high accuracy.

Furthermore the relative errors for the Debye-Waller factors are reduced by 30 to 60%. Interestingly, a tendency in the disorder of the metal-metal distance could already be suggested on the basis of the multiple K-edge refinement. Using our temperature dependent approach it is even possible to extract a Debye temperature from the data and quantify the differences. The three

* 2+ refers to the compound $[\text{LFeNiFeL}]^{2+}$, 3+ to $[\text{LFeNiFeL}]^{3+}$, and 4+ to $[\text{LFeNiFeL}]^{4+}$.

Debye temperatures for the metal-metal path are $\Theta_{\text{Debye}}(2+) = 424\text{K}$, $\Theta_{\text{Debye}}(3+) = 330\text{K}$, and $\Theta_{\text{Debye}}(4+) = 294\text{K}$, respectively.

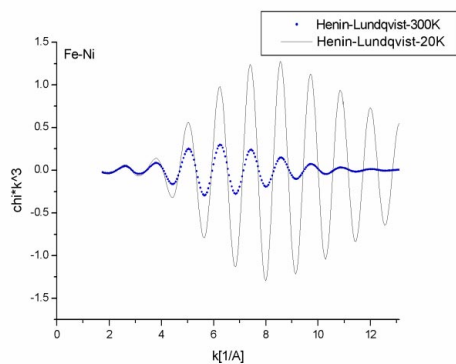


Figure 2
Simulation of the EXAFS signal for the Fe-Ni.

Table 6

Multiple edge fit of the metal-metal distances as free parameter and the σ^2 following a Debye model (only iron edge shown).

**The metal-metal distance was assumed to be constant over the temperature range.

	2+		3+		4+	
	Fe-Ni	$\sigma^2 * 10^{-2}$	Fe-Ni	$\sigma^2 * 10^{-2}$	Fe-Ni	$\sigma^2 * 10^{-2}$
20K	3.047(4)	0.29(2)	2.972(4)	0.38(2)	2.982(5)	0.43(3)
85K	*	0.33(3)	*	0.45(4)	*	0.54(5)
178K	*	0.46(6)	*	0.71(8)	*	0.88(11)

Table 7

Multiple edge fit of the Fe-C distances as free parameter and the σ^2 following a Debye model (only iron edge shown). Results for the nearest C are shown.

** The Fe-C distance was assumed to be constant over the temperature range.

	2+		3+		4+	
	Fe-C	$\sigma^2 * 10^{-2}$	Fe-C	$\sigma^2 * 10^{-2}$	Fe-C	$\sigma^2 * 10^{-2}$
20K	2.824(7)	0.41(8)	2.824(12)	0.59(12)	2.825(13)	0.44(14)
85K	*	0.42(9)	*	0.62(15)	*	0.45(16)
178K	*	0.47(13)	*	0.78(25)	*	0.51(23)

Can similar conclusions be drawn for the carbon backscattering? Indeed, the accuracy of the Debye-Waller factors is rather poor for the refinements at a single temperature. Again the application of the Debye model guides the fitting routine to a reasonable minimum. Thus the error margins are reduced by up to 40%.

4. Conclusions

The different approaches discussed in this paper differ considerably in the amount of measurements required. If the objective of the measurements focuses on the metal-metal contributions there is no need for a temperature dependent measurement in many cases. Nevertheless sometimes differences between results extracted from protein crystallography and X-ray absorption spectroscopy are reported or the significance of a metal-metal backscattering signal is rather poor. Here the temperature-dependent study increases the significance of the EXAFS results. Moreover the flexibility of the metal positions described by the Debye temperature for the metal-metal contribution is quantified.

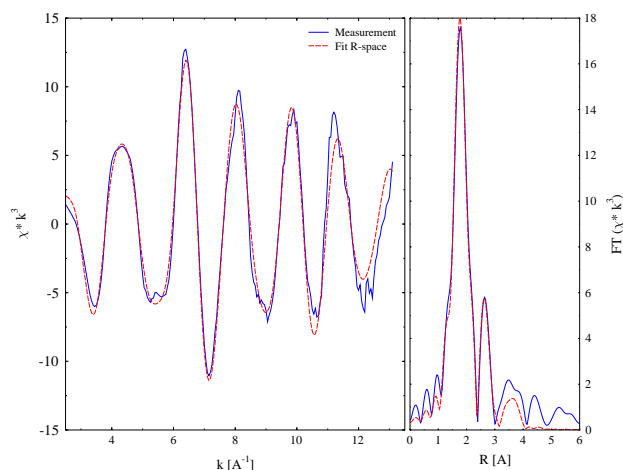


Figure 3
EXAFS fit for the $[\text{LFeNiFeL}]^{3+}$ sample at 178K.

Thus, this approach supplies information about metal-ligand bond properties. Thereby it is possible to add more information to the discussion of the metal core in enzymes. This information on the bond strength is a first step towards a more detailed understanding of dynamic properties of the metal-ligand interaction.

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