

Motion of the Zinc Ions in Catalysis by a di-Zinc Metallo- β - Lactamase

**Robert M. Breece,¹ Zhenxin Hu,² Brian Bennett,^{3*} Michael W. Crowder,^{2*}
and David L. Tierney^{1*}**

¹*Department of Chemistry and Chemical Biology, B80 Clark Hall, MSC03 2060,
University of New Mexico, Albuquerque, New Mexico 87131-0001*

²*Department of Chemistry and Biochemistry, 160 Hughes Hall, Miami University,
Oxford, Ohio 45056,*

³*National Biomedical EPR Center, Department of Biophysics, Medical College of
Wisconsin, 8701 Watertown Plank Road, Milwaukee, Wisconsin 53226-0509*

Supporting Information

Experimental Procedures

Sample Preparation. Samples for EXAFS studies were generated using a modified Update Instruments (Madison, WI) rapid-freeze-quench (RFQ) system. Equal volumes of 1 mM L1 and 5 mM nitrocefin, each containing 20 % glycerol in 50 mM HEPES, pH 7.0, were rapidly mixed (< 1 ms), incubated at 2° C, and freeze-quenched in 2-methylbutane at -130° C.¹ The total effective reaction time was 10 ms; this was calibrated by comparing the development of a low-spin Fe(III) EPR signal and the disappearance of a high-spin Fe(III) EPR signal with the associated optical changes at 636 nm using stopped-flow spectrophotometry, upon mixing myoglobin with an excess of sodium azide. The syringes, mixer, and tubing were all contained in a watertight bath that was maintained at 2° C using ice water. Immediately prior to sample collection (< 10 s), the nozzle and attached mixer were removed from the bath and held 5 mm above the surface of 2-methylbutane (Fisher) contained in a collecting funnel and maintained at -130° C by a surrounding bath (Update Instruments) of liquid nitrogen-cooled 2-methylbutane. Samples were packed into EXAFS cuvettes at -130° C and stored in liquid nitrogen. Samples were shipped in liquid nitrogen-soaked dry ice and otherwise stored in liquid nitrogen.

EXAFS Data Collection and Analysis. X-ray absorption spectra were measured at the National Synchrotron Light Source (NSLS), beamline X3B, with a Si(111) double crystal monochromator; harmonic rejection was accomplished using a Ni focusing mirror. Fluorescence excitation spectra for all samples were measured with a 13-element solid-state Ge detector array. Samples were held at ~ 15 K in a Displex cryostat during XAS measurements. X-ray energies were calibrated by reference to the absorption spectrum of the appropriate metal foil, measured concurrently with the protein spectra. The data shown represent the average of 16 total scans, from two independently prepared samples. Data collection and reduction were performed according to published procedures² with E_0 set to 9675 eV for Zn. The unfiltered and Fourier-filtered EXAFS were fit to Equation 1 using the nonlinear least-squares engine of IFEFFIT that is distributed with SixPack.^{3,4}

$$\chi(k) = \sum \frac{N_{as} A_s(k) S_c}{k R_{as}^2} \exp(-2k^2 \sigma_{as}^2) \exp(-2R_{as} / \lambda) \sin[2k R_{as} + \phi_{as}(k)] \quad (1)$$

In Eq. 1, N_{as} is the number of scatterers within a given radius ($R_{as}, \pm \sigma_{as}$), $A_s(k)$ is the backscattering amplitude of the absorber-scatterer (as) pair, S_c is a scale factor, $\phi_{as}(k)$ is the phase shift experienced by the photoelectron, λ is the photoelectron mean free-path, and the sum is taken over all shells of scattering atoms included in the fit. Theoretical amplitude and phase functions, $A_s(k) \exp(-2R_{as}/\lambda)$ and $\phi_{as}(k)$, were calculated using FEFF v. 8.00.⁵ The scale factor (S_c) and ΔE_0 for Zn-N ($S_c = 0.78$, $\Delta E_0 = -16$ eV) and Zn-S (0.85, -21 eV) scattering were determined previously and held fixed throughout this analysis.² Fits to the current data were obtained for all reasonable integer or half-integer coordination numbers, refining only R_{as} and σ_{as}^2 for a given shell. Multiple scattering contributions from histidine ligands were approximated according to published procedures, fixing the number of imidazole ligands per metal ion at half-integral values while varying R_{as} and σ_{as}^2 for each of the four combined ms pathways (see Table S1).² Zn-Zn scattering was modeled by fitting calculated amplitude and phase functions to the experimental EXAFS of $\text{Zn}_2(\text{sapln})_2$.

The fits shown in **Table S1** and plotted in **Figure S1** show, in order, (i) the most representative coordination number with only N/O scatterers (Fits S1-S5), (ii) that an inhomogeneous first shell of resolved N and O scattering is not statistically justified (compare Fit S6 to Fit S3, 23 % improvement), (iii) that any mixture that includes Zn-S scattering is not statistically significant (compare Fit S7 to Fit S3 (9 %), and Fit S8 to Fit S6 (9 %)), (iv) that inclusion of a Zn-C scattering interaction from a coordinated carboxylate is required for an acceptable fit (compare Fits S9 and S10, 34 %), (v) that inclusion of a Zn-Zn vector at 3.72 Å leads to a further 68 % reduction in the fit residual (compare Fits S10 and S11). As described in the main text, a second, shallower minimum was observed with a refined Zn-Zn distance of 3.37 Å, but the fit residual improved by only 20 %. In a fit including both a 3.37 Å and a 3.72 Å Zn-Zn vector (Fit S12), allowing the coordination numbers to refine freely gave values of 0.12 at 3.37 Å and 0.93 at 3.72 Å, indicating that any mixed population is at most 11 % of the species at 3.37 Å.

References:

1. Garrity, J. D.; Bennett, B.; Crowder, M. W., *Biochemistry* **2005**, *44*, 1078-1087.
2. Thomas, P. W.; Stone, E. M.; Costello, A.; Tierney, D. L.; Fast, W., *Biochemistry* **2005**, *44*, 7559-7569.
3. Sixpack is available free of charge from its author, Sam Webb, at <http://www-ssrl.slac.stanford.edu/~swebb/sixpack.htm>.
4. IFEFFIT is open source software available from <http://cars9.uchicago.edu/ifeffit>.
5. Ankudinov, A. L.; Ravel, B.; Rehr, J. J.; Conradson, S. D., *Phys. Rev. B* **1998**, *58*, 7565-7576.

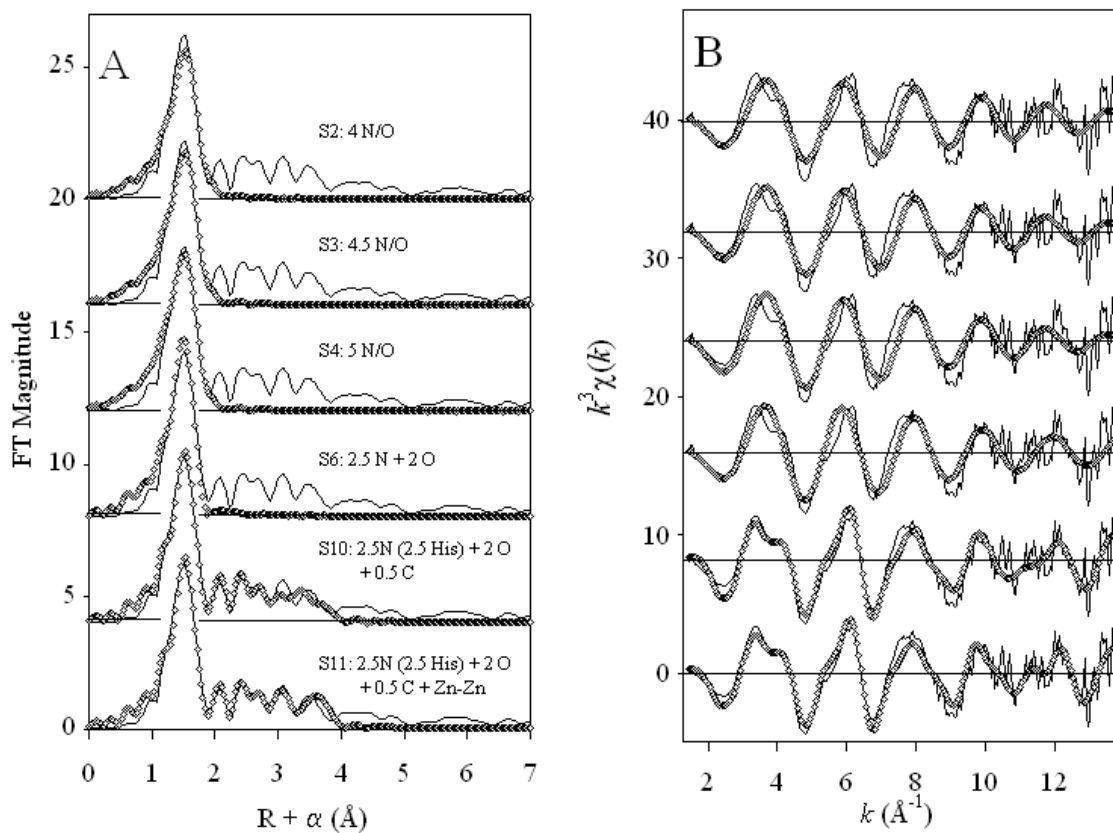


Figure S1. Fourier transforms (A) of k^3 -weighted EXAFS (B) for ZnZn-L1, freeze-quenched after 10 ms reaction with nitrocefin (solid lines), and corresponding curve fits (open symbols) from Table S1.

Table S1. Detailed EXAFS curve fitting results for freeze-quenched ZnZn-L1.^a

Fit	Model	Zn-N/O	Zn-S	Zn-His ^b	Zn-Zn	R _f ^c	R _u ^c
S1	3 N/O	2.02 (2.8)				152	355
S2	4 N/O	2.03 (4.8)				116	331
S3	4.5 N/O	2.03 (5.6)				112	330
S4	5 N/O	2.03 (6.3)				115	335
S5	6 N/O	2.03 (7.8)				135	355
S6	2.5 N + 2 O	[N] 2.08 (3.8) [O] 1.94 (1.3)				86	300
S7	4 N/O + 0.5 S	2.00 (4.2)	2.27 (4.8)			101	322
S8	2 N + 2 O + 0.5 S	[N] 2.18 (24) [O] 1.97 (1.3)	2.24 (3.1)			78	289
Fit	Model	Zn-N/O	Zn-C _{CO₂}	Zn-His ^b	Zn-Zn	R _f ^c	R _u ^c
S9	4.5 N/O (2.5 His)	2.03 (10)		2.90 (4.3) 3.15 (10) 4.13 (20) 4.36 (15)		124	216
S10	4.5 N/O (2.5 His) + Zn-C	2.03 (10)	2.45 (0.3)	2.89 (4.4) 3.12 (13) 4.11 (16) 4.36 (17)		82	192
S11	4.5 N/O (2.5 His) + Zn-C + Zn-Zn ^d	2.03 (10)	2.45 (0.2)	2.89 (4.2) 3.10 (10) 4.12 (8.7) 4.35 (14)	3.72 (6.6)	26	159
S12	4.5 N/O (2.5 His) + Zn-C + 2 Zn-Zn ^e	2.03 (5.1)	2.45 (0.2)	2.86 (6.1) 3.05 (10) 4.10 (8.6) 4.28 (13)	3.38 (6.1) 3.71 (7.2)	22	156

^a Distances (Å) and disorder parameters (in parentheses, σ^2 (10^{-3} Å²)) shown derive from integer or half-integer coordination number fits to filtered EXAFS data [$k = 1.5$ - 13.7 Å⁻¹; $R = 0.7$ - 2.2 Å (Fits 1-8) or 0.2 - 4.0 Å (Fits 9-11)].

^b Multiple scattering paths represent combined paths, as described previously (see Materials and Methods).

^c Goodness of fit (R_f for fits to filtered data; R_u for fits to unfiltered data) defined as

$$1000 * \frac{\sum_{i=1}^N \{ [\text{Re}(\chi_{i_{calc}})]^2 + [\text{Im}(\chi_{i_{calc}})]^2 \}}{\sum_{i=1}^N \{ [\text{Re}(\chi_{i_{obs}})]^2 + [\text{Im}(\chi_{i_{obs}})]^2 \}}, \text{ where } N \text{ is the number of data points.}$$

^d A second, shallower minimum (R_f = 66, compared to R_f = 26 in Fit S11) is observed at a refined Zn-Zn distance of 3.36 Å ($\sigma^2 = 1.3 \times 10^{-3}$ Å²).

^e Fit S12 included two independent Zn-Zn vectors at ~ 3.4 and 3.7 Å, and their coordination numbers were allowed to refine, along with R_{as} and σ^2 .