Spectroscopic and Elemental Analysis of *E. coli* 70S Ribosome

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Supporting Information

Experimental Methods

Culture Growth. *E. coli* strain BL21(DE3) was grown in 18 liters of LB broth. An overnight preculture of *E. coli* BL21(DE3) was used to inoculate 1 liter of LB in a 18 X 2 liter flasks. The cultures were allowed to grow with shaking (200 rpm) at 37 ^{o}C until they reached an optical density at 600 nm of 1. Cells were collected by centrifugation.

Preparation of ribosomes. *E. coli* 70S ribosomes were prepared by centrifugation. Bacterial cells were broken by grinding with autoclaved, levigated alumina. The lysis buffer was 20 mM Tris-HCl, pH 7.5, containing 10.5 mM magnesium acetate, 100 mM NH_4Cl, and 3 mM β-mercaptoethanol. The broken cells were removed by centrifugation. The supernatant was then centrifuged at 45 k rpm for 3 hours at 4^{o} C in a Beckman L8-80M ultracentrifuge using a Beckman 80 TI to pellet the ribosomes. The ribosome pellets were then washed 4-6 times with 1 mL of lysis buffer supplemented with 500 mM NH_4Cl. The ribosome pellets were resuspended in 1 mL of lysis buffer supplemented with 500 mM NH_4Cl at 4 ^{o}C with gentle shaking for 8-12 hours. The resuspended ribosomes were centrifuged overnight (16 hours) at 45 k rpm in Bechman 80 TI rotor with an underlay of 9 mL of the lysis buffer supplemented with 500 mM NH_4Cl and 1.1 M sucrose. Pellets were resuspended in lysis buffer without 3 mM β-mercaptoethanol and flash frozen in liquid nitrogen. Ribosomes were quantitated by using absorbance readings at 260 nm and using the conversion factor of $1A_{260}$ unit is equivalent to 23 pmol ribosomes when determined in 20 mM Tris-HCl, pH 7.5, containing10 mM magnesium acetate and 100 mM NH_4Cl.[1] Dialysis of samples was conducted versus 2X1 liter of lysis buffer (20 mM Tris-HCl, pH 7.5, containing 10 mM magnesium acetate, 100 mM NH_4Cl, and 3 mM β-mercaptoethanol) for 4 hours per dialysis.

Preparation of Zn(II)-supplemented and Zn(II)-depleted ribosomes. As isolated ribosomes at a concentration of 10 μM were incubated in lysis buffer supplemented with 1000 μM of Zn(II) for one hour at 4^{o} C. These Zn(II)-supplemented ribosomes were then dialyzed versus 2X1 liter of lysis buffer (20 mM Tris-HCl, pH 7.5, containing 10 mM magnesium acetate, 100 mM NH_4Cl, and 3 mM β-mercaptoethanol) for 4 hours per dialysis at 4^{o} C. As-isolated ribosomes at a concentration of 10

μM were incubated in lysis buffer supplemented with 400 μM EDTA for one hour at 4 ^{o}C. The EDTA-treated ribosomes were then dialyzed versus 2X1 liter of lysis buffer (20 mM Tris-HCl, pH 7.5, containing 10 mM magnesium acetate, 100 mM NH_4Cl, and 3 mM β-mercaptoethanol) for 4 hours per dialysis at 4 ^{o}C.

In vitro translation. *In vitro* translation reactions were performed using the PURESYSTEM Classic II mini alpha kit (without ribosomes). The *in vitro* reaction was carried out as follows: 0.5 μg plasmid DNA containing metallo-β-lactamase L1 gene [2] and *E. coli* 70S ribosomes up to a final concentration of 2.6 μM were added to the reaction and mixed gently. The reaction was centrifuged for 5 sec in an Eppendorf centrifuge to collect the sample in the bottom of the tube and incubated at 37 ^{o}C for 2 hours. The reaction was stopped by placing the mixture on ice. The whole reaction (50 μL) was added to a cuvette with 50 μM nitrocefin and 50 mM cacodylate, pH 7.5, and the formation of the hydrolysis product of nitrocefin was monitored at 485 nm for 20 minutes. The specific activity of L1 (1.3 mmol nitrocefin hydrolyzed per second per μg of L1) was used to calculate the amount of L1 produced in the *in vitro* transcription/translation reactions. Negative controls were performed following the same procedure but without ribosomes or plasmid added to the *in vitro* translation reaction. An additional, non-Zn(II)-containing enzyme, dihydrofolate reductase (DHFR), was also used in the *in vitro* translation experiments. The activity of DHFR was monitored using a dihydrofolate reductase assay kit purchased from Sigma. In brief, 25 μL of the *in vitro* translation reaction was added to a cuvette with 50 μM dihydrofolic acid, 60 μM NADPH, and 50 mM Tris-Cl, pH 7.5, and the decrease in NADPH concentration was observed at 340 nm for 5 minutes. The specific activity of *E. coli* DHFR produced in a bacterial cell free transcription-translation system has been previously reported to be 52.6 units/mg [3] and this was used to calculate the amount of dihydrofolate reductase produced in the *in vitro* transcription/translation reactions. Negative controls were performed following the same procedure but without ribosomes or plasmid added to the *in vitro* translation reaction.

Metal analyses. The metal content of the ribosomes was determined using a PerkinElmer 7300 DV Optical Emission Spectrometer (ICP-OES analyses). Ribosome samples were diluted to 50 nM with Nanopure water immediately before analyses. Metal analyses were conducted in triplicate. A calibration curve was generated with the use of seven standards ranging from 1 to 100 ppb, with correlation coefficients of 0.999 or greater.

X-ray absorption spectroscopy. Samples for extended X-ray absorption spectroscopy (XAS) (24 μM in ribosomes) were prepared with 20% (v/v) glycerol as a cryoprotectant and loaded into Lucite cuvettes with 6-μm polypropylene windows, before rapid freezing in liquid nitrogen. X-ray absorption spectra were measured at the National Synchrotron Light Source (Brookhaven National

Laboratory, Upton, NY), beamline X3B, using a Si(111) double crystal monochromator; harmonic rejection was accomplished using a Ni focusing mirror. Data collection and reduction were accomplished according to published procedures, [4] using E_0 = 9675 eV. The data in Figure 1 (and Figures S1 and S2) represent the average of the 12 scans per sample, from two independently-prepared samples each. Fourier-filtered EXAFS data were fitted utilizing theoretical amplitude and phase functions calculated with FEFF v. 8.00.[5] The Zn-N scale factor and the threshold energy, ΔE_0, were calibrated to the experimental spectrum for *tetrakis*-1-methylimidazole zinc(II) perchlorate, $Zn(MeIm)_4$ and held fixed (at 0.78 and – 16 eV, respectively). First shell fits were then obtained for all reasonable coordination numbers, while allowing the absorber-scatterer distance, R_{as}, and the Debye-Waller factor, σ_{as}^2, for each contribution to vary. The best fits are presented in Table S1 and discussed below.

Table S1. Detailed curve fitting results for Zn K-edge EXAFS of *E. coli* 70S ribosomes.[a]

Fit	Sample	*Zn-N/O*	*Zn-N/O*	*Zn-S*	*Zn-C_{CO_2}*	*Zn-P_{PO_4}*	R_f [b]	R_u [b]
	Zn(II)-depleted (5 Zn/ribosome)							
(-)-1	6 N/O		2.13 (9.2)				363	472
(-)-2	3 N/O + 3 N/O	2.01 (4.7)	2.18 (4.8)				120	321
(-)-3	1 N/O + 2.5 N/O + 1 S	1.93 (2.5)	2.11 (2.4)	2.34 (1.3)			13	233
(-)-3[c]	1 N/O + 2.5 N/O + 1 S						211	233
(-)-4	1 N/O + 2.5 N/O + 1 S + 3 C	1.93 (2.9)	2.11 (3.4)	2.34 (2.7)	3.25 (1.1)		24	171
(-)-5	1 N/O + 2.5 N/O + 1 S + 1 P	1.93 (1.5)	2.11 (3.2)	2.34 (1.2)		3.02 (2.0)	122	203
(-)-6	1 N/O + 2.5 N/O + 1 S + 3 C + 1 P	1.93 (1.6)	2.11 (3.7)	2.34 (2.4)	3.24 (0.7)	3.15 (16)	17	161
	Zn(II)-supplemented (12 Zn/ribosome)							
(+)-1	6 N/O		2.14 (1.2)				419	687
(+)-2	2 N/O + 3 N/O	2.01 (5.9)	2.15 (0.5)				49	449
(+)-3	2 N/O + 3 N/O + 0.5 S	1.96 (2.9)	2.14 (0.3)	2.29 (2.7)			26	436
(+)-3[c]	2 N/O + 3 N/O + 0.5 S						341	436
(+)-4	2 N/O + 3 N/O + 0.5 S + 3 C	1.97 (2.7)	2.16 (0.7)	2.31 (1.3)	3.15 (3.5)		119	371
(+)-5	2 N/O + 3 N/O + 0.5 S + 1.5 P	1.97 (3.7)	2.16 (2.4)	2.32 (1.7)		2.94 (2.5)	80	340
(+)-6	2 N/O + 3 N/O + 0.5 S + 3 C + 1.5 P	1.96 (3.2)	2.15 (1.1)	2.31 (5.0)	3.15 (3.7)	2.97 (1.5)	41	334

[a] Distances (Å) and disorder parameters (in parentheses, σ^2 (10^{-3} Å^2)) shown derive from integer or half-integer coordination number fits to filtered EXAFS data [k = 1.5 – 11.5 Å^{-1}; R = 0.7-2.3 Å (fits 1-3) or 0.7 – 3.3 Å (fits 4-6)].

[b] Goodness of fit (R_f for fits to filtered data; R_u for fits to unfiltered data) defined as $1000 * \frac{\sum_{i=1}^{N}\left\{\left[\mathrm{Re}(\chi_{i_{calc}})\right]^2 + \left[\mathrm{Im}(\chi_{i_{calc}})\right]^2\right\}}{\sum_{i=1}^{N}\left\{\left[\mathrm{Re}(\chi_{i_{obs}})\right]^2 + \left[\mathrm{Im}(\chi_{i_{obs}})\right]^2\right\}}$, where N is the number of data points.

c Fits (+)-3[c] and (-)-3[c] utilized the same parameters as fits (+)-3 and (-)-3, covering the expanded *R*-range of fits 4-6, for direct comparison of fit residuals.

XAS data analyses. From Table S1 and Figure S1, the following conclusions can be drawn. For Zn-depleted ribosomes, the inclusion of two shells of low-Z scatterers improves the filtered fit residual 3-fold (compare (-)-1 and (-)-2), and inclusion of an average of one S scatterer per Zn leads to a 10-fold further improvement (compare (-)-2 and (-)-3). The sulfur's importance to the fit is evidenced by exploration of the coordination space of the two low-Z shells, and finding that one short and 2.5 longer distance scatterers gave the best fit when it is included, (-)-3, but 6 low-Z scatterers are needed in its absence. The single prominent outer shell feature is best fit with a shell of three carbon atoms (fit (-)-4). Modeling this feature with phosphorous, as one would expect for association with phosphate, gave a six-fold higher fit residual (compare (-)-4 and (-)-5); inclusion of both C and P shells did not lead to substantial improvement over the carbon-only fit (compare (-)-4 and (-)-6). In both cases, attempts to include the outer shell scattering expected for coordinated imidazoles did not lead to a significant improvement in the fits.

For Zn-supplemented ribosomes, the primary coordination sphere is also best modeled (fit (+)-3) with a mixed donor set of 2 N/O at short distance, 3 N/O at longer distance and 0.5 sulfurs per Zn. Again, the split first shell of low-Z ligands improves the fit nearly 10-fold (compare (+)-1 and (+)-2); while inclusion of the sulfur contribution is significant (47 % improvement in R; compare (+)-2 and (+)-3), it is not as dramatic as in the Zn-depleted data. The prominent outer shell feature is shifted to low-R

relative to the Zn-depleted sample, and this feature is best fit with a combination of C and P. Inclusion of the carbon shell alone improves the fit 3-fold, while inclusion of the phosphorous shell alone improves the residual more than four-fold. The combined fit, including an average of 3 C and 1.5 P, gives a fit residual that is two-fold better than either single-component fit.

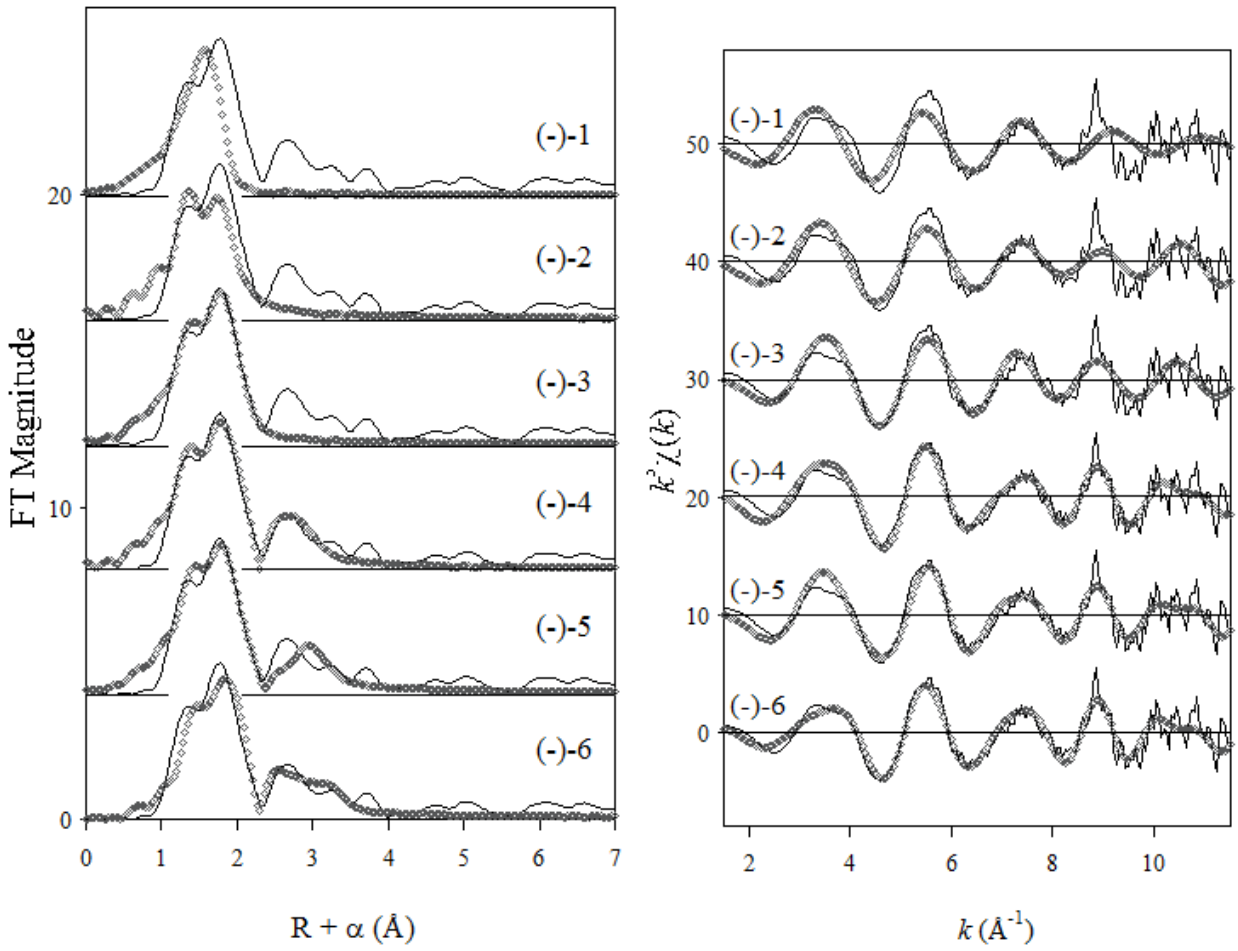

Figure S1. Fourier transforms (left) of k^3-weighted EXAFS for Zn(II)-depleted *E. coli* 70S ribosomes and corresponding fits, according to Table S1. The top three fits focus on the primary coordination sphere, successively showing that two shells of low-Z ligands is better than one (compare (-)-1 and (-)-2), and that inclusion of an average of one S donor per Zn dramatically improves the fit (compare (-)-2 and (-)-3). Fits (-)-4, (-)-5 and (-)-6 aim to identify the source of the prominent outer shell contribution at R + α = 2.7 Å, modeling it first with 3 C atoms (fit (-)-4), then 1 P atom (fit (-)-5) and finally a combination of 3 C and 1 P (fit (-)-6).

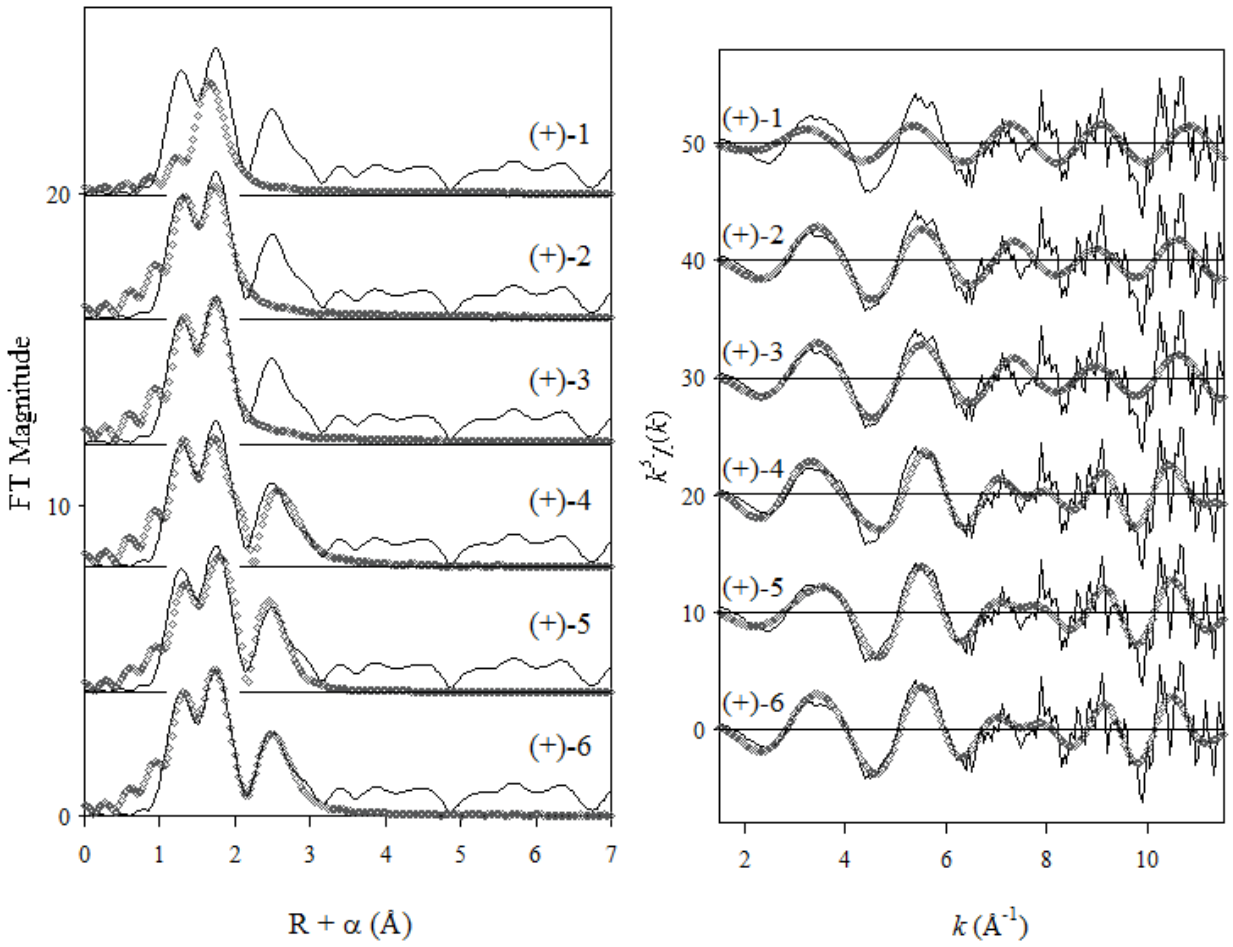

Figure S2. Fourier transforms (left) of k^3-weighted EXAFS for Zn(II)-supplemented *E. coli* 70S ribosomes and corresponding fits, according to Table S1. The top three fits focus on the primary coordination sphere, successively showing that two shells of low-Z ligands is better than one (compare (+)-1 and (+)-2), and that inclusion of an average of 0.5 S donors per Zn further improves the fit (compare (+)-2 and (+)-3). Fits (+)-4, (+)-5 and (+)-6 aim to identify the source of the prominent outer shell contribution at R + α = 2.7 Å, modeling it first with 3 C atoms (fit (+)-4), then 1.5 P atoms (fit (+)-5) and finally a combination of 3 C and 1.5 P (fit (+)-6).

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