Supplementary Materials:

Supplemental Fig. 1. Stoichiometry of Cd(II) and Zn(II) binding to wild type and mutant CadCs. (A) Zn(II) (circles) and Cd(II) (triangles) titration of wild type CadC. The open and closed figures represent the results from four independent assays. The x-axis indicates the added concentration of either Cd(II) or Zn(II). The data that the dimer can either bind a 4 Zn(II) or 2 Cd(II). (B) Comparison of Cd(II) (open bars) and Zn(II) (closed bars) binding stoichiometry between wild type, Site 1 and site 2 mutants. Each protein binds 2 Cd(II) per dimer. The Site 1 and Site 2 mutants bind 2 Zn(II) per dimer, compared with 4 for the wild type.

Supplemental Fig. 2. CadC Zn EXAFS, Fourier transforms of the EXAFS and fitting analysis. Unfiltered EXAFS with respective Fourier transforms are: (A, F) wild type CadC with 1 Zn bound, (B, G) wild type CadC with 2 Zn bound, (C, H) wild type CadC with 1 Zn and 1 Cd bound, (D, I) Site 1 mutant with 1 Zn bound and (E, J) Site 2 mutant with 1 Zn bound. Empirical data are shown as solid lines while simulations are given in dashed lines.

Supplemental Fig. 3. CadC Cd EXAFS, Fourier transforms of the EXAFS and fitting analysis. Unfiltered EXAFS data figures include: (A) wild type CadC with 1 Cd bound, (B) wild type CadC with 2 Cd bound, (C) wild type CadC with 1 Zn and 1 Cd bound, (D) Site 1 mutant with 1 Cd bound and (E) Site 2 mutant with 1 Cd bound. Fourier transforms of the respective EXAFS spectra are adjacent to the EXAFS figure. Empirical data are shown as solid lines while simulations are given in dashed lines.

Supplemental Fig. 4. Multiple alignment of CadC, SmtB and ArsR. The sequence of plasmid pI258 CadC (P20047), *Synechocystis sp*. PCC 7942 SmtB (P30340) and plasmid R773 ArsR (P15905) were aligned with CLUSTAL W. The cysteine residues of Site 1 and the four residues of Site 2 are indicated, as is Gly^{84} of CadC, Gly^{68} of ArsR and Arg^{87} of SmtB. Accession numbers are given in parentheses.

Supplemental Table 1. X-ray data and structure refinement statistics. X-ray data and structure refinement statistics of the CadC Site 2 mutant. For comparison, statistics for wild type CadC are also given.

Parameter	**Site 2 mutant**	**Wild type**
Data		
Space group	$P4_3$	$P4_1$
Cell dimensions	a=b=89.5, c=148.5	a=b=116.5, c=41.8
Beamline	21-ID-D (APS)	14-BM-C (APS)
Wave length (Å)	0.9787	0.9
Resolution (Å)*	2.3 (2.38-2.30)[1]	1.9
No. of unique reflections*	50010(4033)	40691
Completeness (%)*	97.3 (79)	95.6
Redundancy*	7.3 (5.1)	3.5
I/σ *	12.3 (6.7)	28.0
R_{sym} (%)*	9.8 (43)	4.5
Refinement		
No. of molecules in the asymmetric unit	3	2
No. of residues	622	432
No. of ions	5	3
No. of water molecules	142	245
R factor / R_{Free} (%)	23.7 / 28.8	23.0 / 29.0
RMS deviations		
Bond (Å)	0.032	0.029
Angles (°)	2.53	2.24

[1]Numbers in parenthesis are values in the highest resolution shell

Supplemental Table 2. Summary of Zn EXAFS fitting analysis for the CadC dimer. Data fit over a k range of 1 to 13.0 $Å^{-1}$. Best-fit parameters for each sample are indicated in bold.

		Ligand Environment[a]				Ligand Environment[a]				
Sample	**Fit #**	**Atom[b]**	**R(Å)[c]**	**C.N.[d]**	**σ^{2} [e]**	**Atom[b]**	**R(Å)[c]**	**C.N.[d]**	**σ^{2} [e]**	**F' [f]**
Wild Type	1.1	S	2.30	1.0	3.06					2.25
2 Zn/dimer	1.2	S	2.31	1.0	1.64	O/N	1.98	2.0	3.93	0.60
	1.3	S	2.30	1.5	3.80	O/N	1.97	1.5	2.28	0.59
		C	3.49	1.0	4.58					
	1.4	**S**	**2.30**	**1.5**	**3.73**	**O/N**	**1.97**	**2.0**	**3.85**	**0.41**
		C	**3.18**	**1.0**	**4.66**	**C**	**4.00**	**3.0**	**3.18**	
Wild Type	2.1	S	2.30	1.0	3.81					1.99
4 Zn/dimer	2.2	S	2.30	1.5	4.34	O/N	1.97	2.0	4.45	0.44
	2.3	S	2.30	1.5	4.33	O/N	1.97	2.0	4.4	0.39
		C	3.98	2.0	4.07					
	2.4	**S**	**2.30**	**1.5**	**4.41**	**O/N**	**1.97**	**2.0**	**4.43**	**0.38**
		C	**3.00**	**1.0**	**5.69**	**C**	**3.98**	**1.0**	**1.35**	
Wild Type	3.1	O/N	1.97	3.0	4.65					0.77
2 Zn + 2 Cd/dimer	3.2	O/N	1.95	3.0	2.97	O/N	2.09	1.5	3.35	0.49
	3.3	**O/N**	**1.95**	**3.0**	**2.98**	**O/N**	**2.09**	**1.5**	**3.41**	**0.38**
		C	**3.95**	**2.0**	**4.2**					
Site 1 mutant	4.1	O/N	1.96	4.0	4.06					0.69
2 Zn/dimer	**4.2**	**O/N**	**1.96**	**4.0**	**4.02**	**C**	**3.93**	**2.5**	**4.7**	**0.50**
Site 2 mutant	5.2	S	2.30	3.0	4.96					0.70
2 Zn/dimer	5.2	S	2.31	2.5	3.70	O/N	2.04	1.0	3.24	0.49
	5.3	S	2.31	2.0	2.56	O/N	1.94	1.0	3.91	0.42
		O/N	2.08	1.5	1.10					
	5.4	**S**	**2.31**	**2.0**	**2.55**	**O/N**	**1.94**	**1.0**	**3.75**	**0.38**
		O/N	**2.08**	**1.5**	**1.00**	**C**	**4.04**	**2.0**	**4.30**	

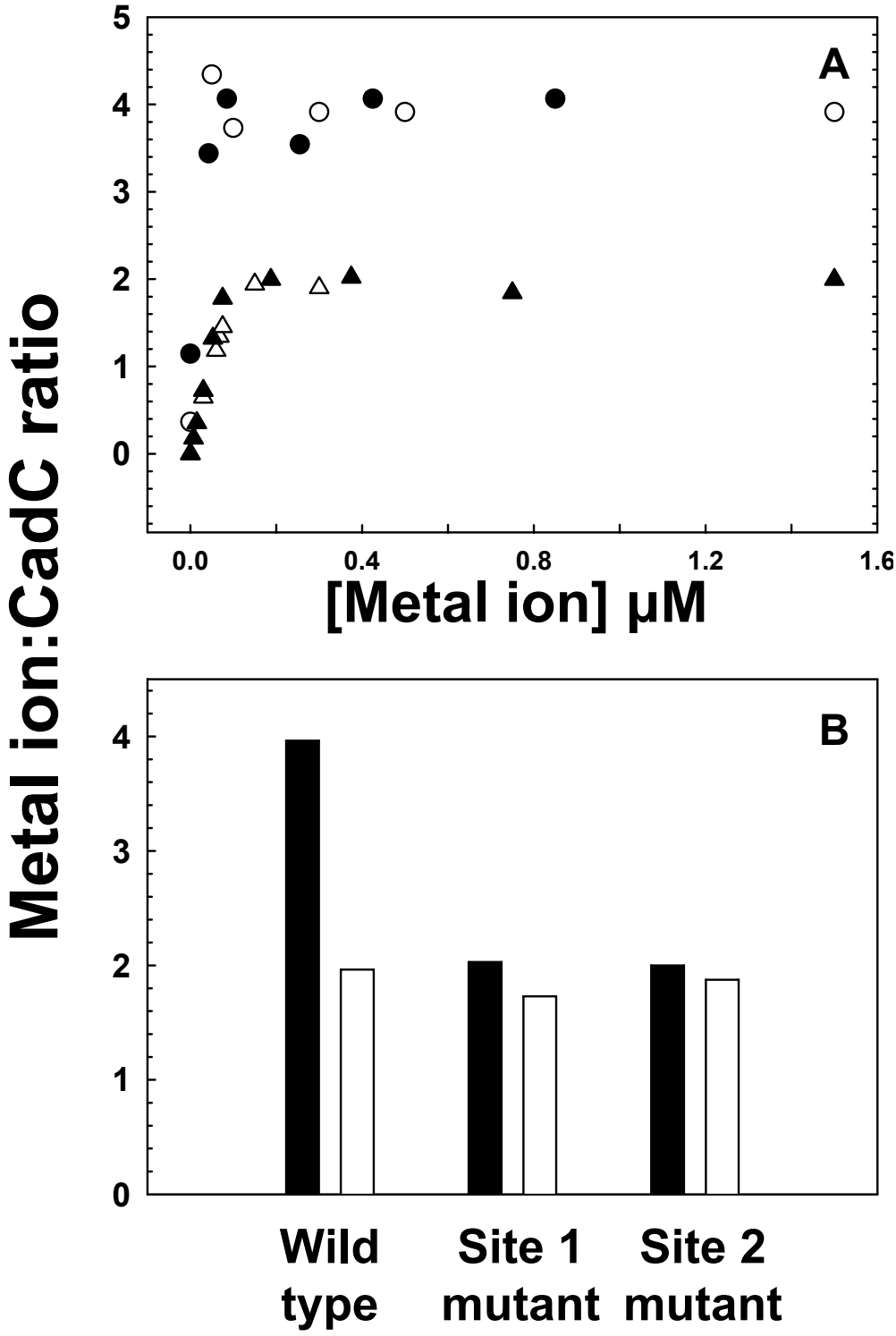
[a] Independent metal-ligand scattering environment
[b] Scattering atoms: O (oxygen), N (nitrogen), C (carbon), S (sulfur)
[c] Average metal-ligand bond length for 2 independent samples
[d] Average metal-ligand coordination number for 2 independent samples
[e] Average Debye-Waller factor in $Å^2$ x 10^3 for 2 independent samples
[f] Number of degrees of freedom weighted mean square deviation between data and fit

Supplemental Table 3. Summary of Cd EXAFS fitting analysis for the CadC dimer. Data fit over a *k* range of 1 to 13.0 $Å^{-1}$. Best-fit parameters for each sample are indicated in bold.

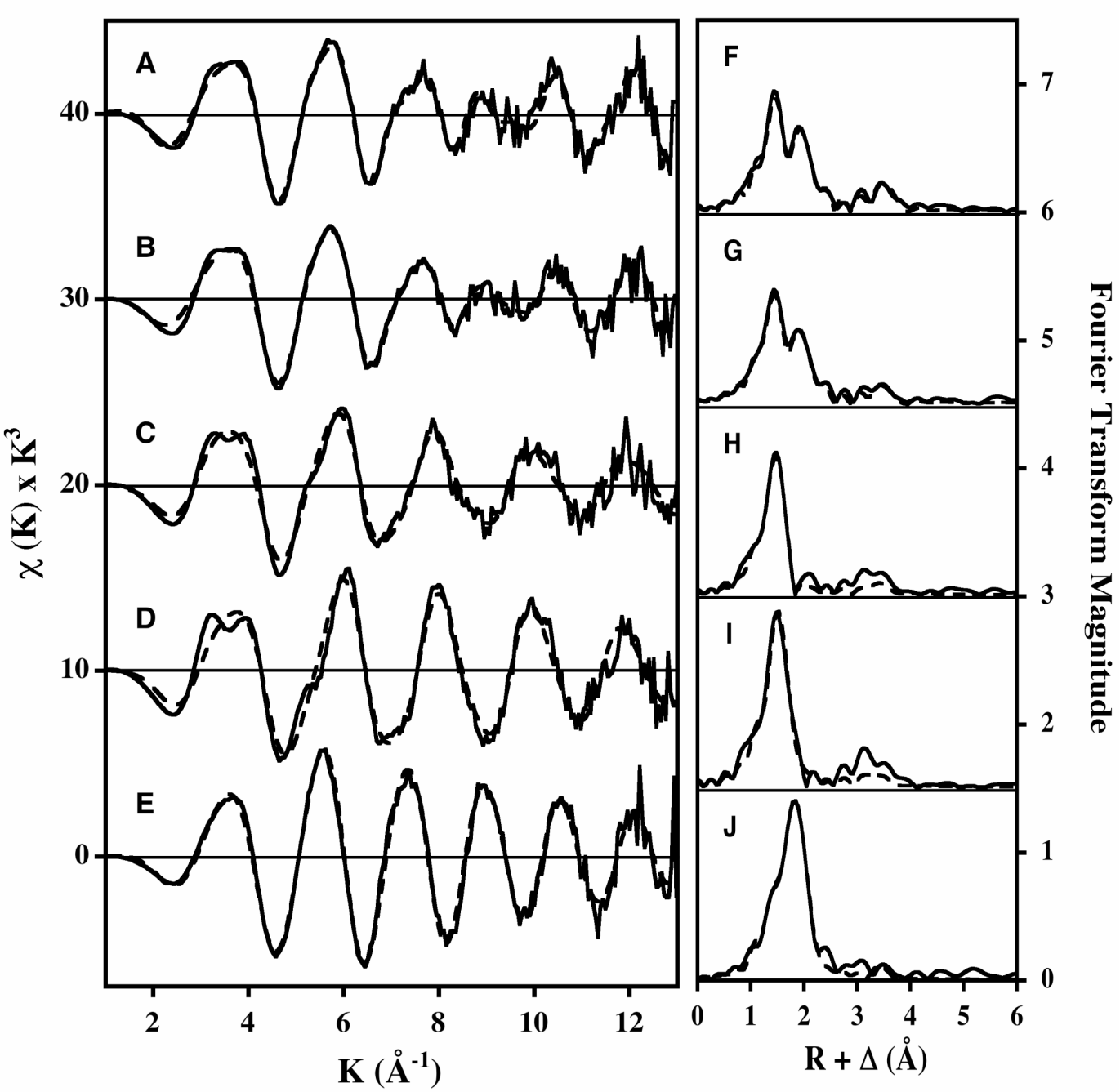
Sample	Fit #	Ligand Environment[a] Atom[b]	R(Å)[c]	C.N.[d]	σ^2 [e]	Ligand Environment[a] Atom[b]	R(Å)[c]	C.N.[d]	σ^2 [e]	F' [f]
Wild Type	6.1	S	2.48	3.5	4.43					1.05
2 Cd/dimer	6.2	S	2.50	2.5	5.03	O/N	2.30	2.0	1.01	0.27
	6.3	**S**	**2.50**	**2.5**	**5.34**	**O/N**	**2.30**	**2.0**	**1.31**	**0.26**
		C	**3.25**	**1.0**	**4.49**					
Wild Type	7.1	S	2.51	3.0	4.87					0.60
4 Cd/dimer	7.2	S	2.52	2.5	4.79	O/N	2.28	4.5	3.51	0.18
	7.3	**S**	**2.52**	**2.5**	**4.79**	**O/N**	**2.31**	**1.5**	**4.65**	**0.16**
		C	**3.70**	**1.0**	**2.18**					
Wild Type	8.1	S	2.51	4.0	4.54					0.98
2 Zn + 2 Cd/dimer	8.2	S	2.51	3.0	4.59	O/N	2.31	1.5	4.25	0.70
	8.3	**S**	**2.51**	**3.0**	**4.58**	**O/N**	**2.31**	**2.0**	**2.37**	**0.68**
		C	**3.83**	**2.0**	**2.17**					
Site 1	9.1	O/N	2.28	3.0	4.56					0.71
2 Cd/dimer	9.2	O/N	2.25	3.0	2.60	O/N	2.39	2.0	3.44	0.55
	9.3	**O/N**	**2.25**	**3.0**	**2.25**	**O/N**	**2.39**	**2.0**	**3.28**	**0.51**
		C	**3.93**	**2.5**	**4.7**					
Site 2	10.1	S	2.51	4.0	4.45					0.39
2 Zn/dimer	10.2	S	2.52	2.0	1.34	O/N	2.29	2.0	2.81	0.18
	10.3	**S**	**2.52**	**2.0**	**1.35**	**O/N**	**2.29**	**2.0**	**2.79**	**0.17**
		C	**3.79**	**1.0**	**3.53**					

[a] Independent metal-ligand scattering environment
[b] Scattering atoms: O (oxygen), N (nitrogen), C (carbon), S (sulfur)
[c] Average metal-ligand bond length for 2 independent samples
[d] Average metal-ligand coordination number for 2 independent samples
[e] Average Debye-Waller factor in $Å^2$ x 10^3 for 2 independent samples
[f] Number of degrees of freedom weighted mean square deviation between data and fit

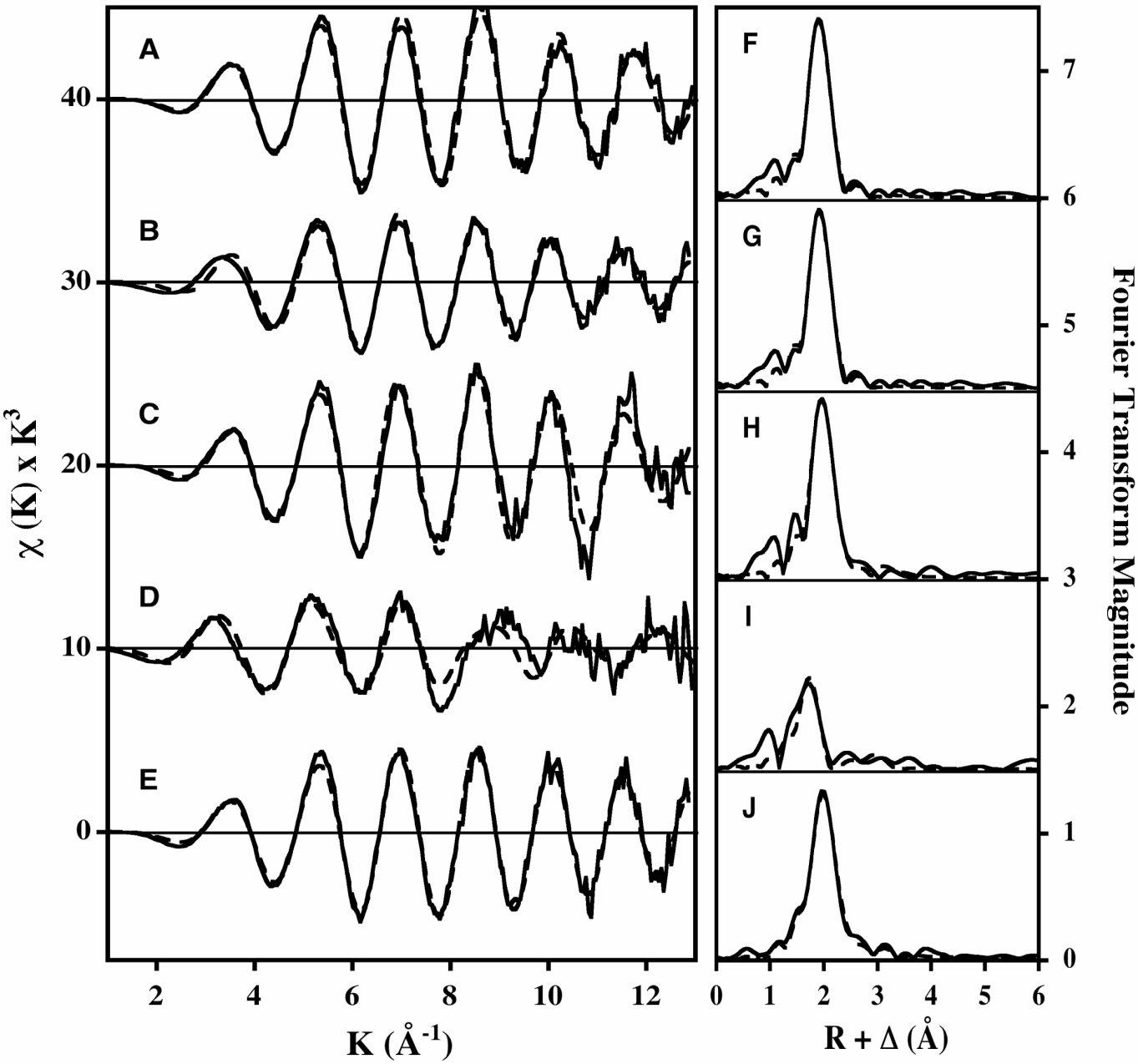
Supplemental Fig. 1



Supplemental Fig. 2



Supplemental Fig. 3



Supplemental Fig. 4