Supplemental Table 1. Summary of the best-fit parameters from the ArsD109-As EXAFS fitting analyses[a]. Fits in bold are the best fit for each sample.

	Ligand Environment[b]				Ligand Environment[b]				
Sample	**Atom[c]**	**R(Å)[d]**	**C.N.[e]**	**Å^2 [f]**	**Atom[c]**	**R(Å)[d]**	**C.N.[e]**	**Å^2 [f]**	***F' [g]***
ArsD109	O/N	2.08	3.0	0.9					3.86
	S	2.24	3.0	2.4					0.89
	O/N	2.01	2.0	4.1	O/N	2.13	1.0	7.8	6.01
	O/N	2.06	0.5	4.4	S	2.24	3.0	2.6	0.84
	O/N	**2.06**	**0.5**	**2.9**	**S**	**2.24**	**3.0**	**2.9**	**0.78**
	C	**2.71**	**1.0**	**4.7**	**C**	**3.14**	**1.0**	**1.3**	
	C	**3.61**	**1.0**	**1.4**					

[a] Data were fit over a *k* range of 1 to 13 Å^{-1}.

[b] Independent metal-ligand scattering environment

[c] Scattering atoms: O (oxygen), N (nitrogen), S (sulfur) and C (carbon), Cu (Copper)

[d] Average metal-ligand bond length from three independent samples

[e] Average metal-ligand coordination number from three independent samples

[f] Average Debye-Waller factor in Å^2 x 10^3 from three independent samples

[g] Number of degrees of freedom weighted mean square deviation between data and fit.

SUPPLEMENTAL FIGURES

FIGURE 1S. Circular dichroism spectra of ArsD109 and tryptophan derivatives. CD spectra were collected with (●) 10 μM ArsD109, (Δ) tryptophan-free W35Y/W97Y, (■) T15W and (◊) V17W.

FIGURE 2S. Activation of ArsA ATPase activity by ArsD109 and single tryptophan derivatives. Activated ArsA ATPase activity was determined at the indicated concentrations of sodium arsenite in the presence or absence of ArsD109 derivatives, with the basal rate of hydrolysis subtracted from each set of data, as described under Materials and Methods. The concentration of ArsA was 0.3 μM, and ArsD was 6 μM. The concentration of As(III) required for half maximal activation is given in parentheses. (●), no ArsD109 ($K_{1/2}$ = 260 μM); (○), parental ArsD109 ($K_{1/2}$ = 25 μM); (▼), tryptophan-free W35Y/W97Y ($K_{1/2}$ = 15 μM); (Δ), T15W ($K_{1/2}$ = 16 μM). The data were fitted using SigmaPlot 9.0, with error bars representing the standard deviation from three assays.

FIGURE 3S. Effect of monothiols on binding of As(III) to ArsD109. Fluorescence of V17W was measured with excitation and emission wavelengths of 295 and 345 nm, respectively. At the arrow the following additions were made: (●), 50 μM As(III); (▽), 5 mM L-cysteine followed by 50 μM As(III); (■), 5 mM β-mercaptoethanol followed by 50 μM As(III); (◊), 2.5 μM Sb(III).

FIGURE 4S. $As(GS)_3$ does not activate ArsA. Activated ATPase activity of 0.3 μM ArsA was determined at the indicated concentrations of sodium arsenite, with the basal rate of hydrolysis subtracted from each set of data, as described under Materials and Methods. (●), no addition; (○), 5 mM GSH; (■), 6 μM ArsD109. The data were fitted using SigmaPlot 9.0, with error bars representing the standard deviation from three assays.

Figure 1S

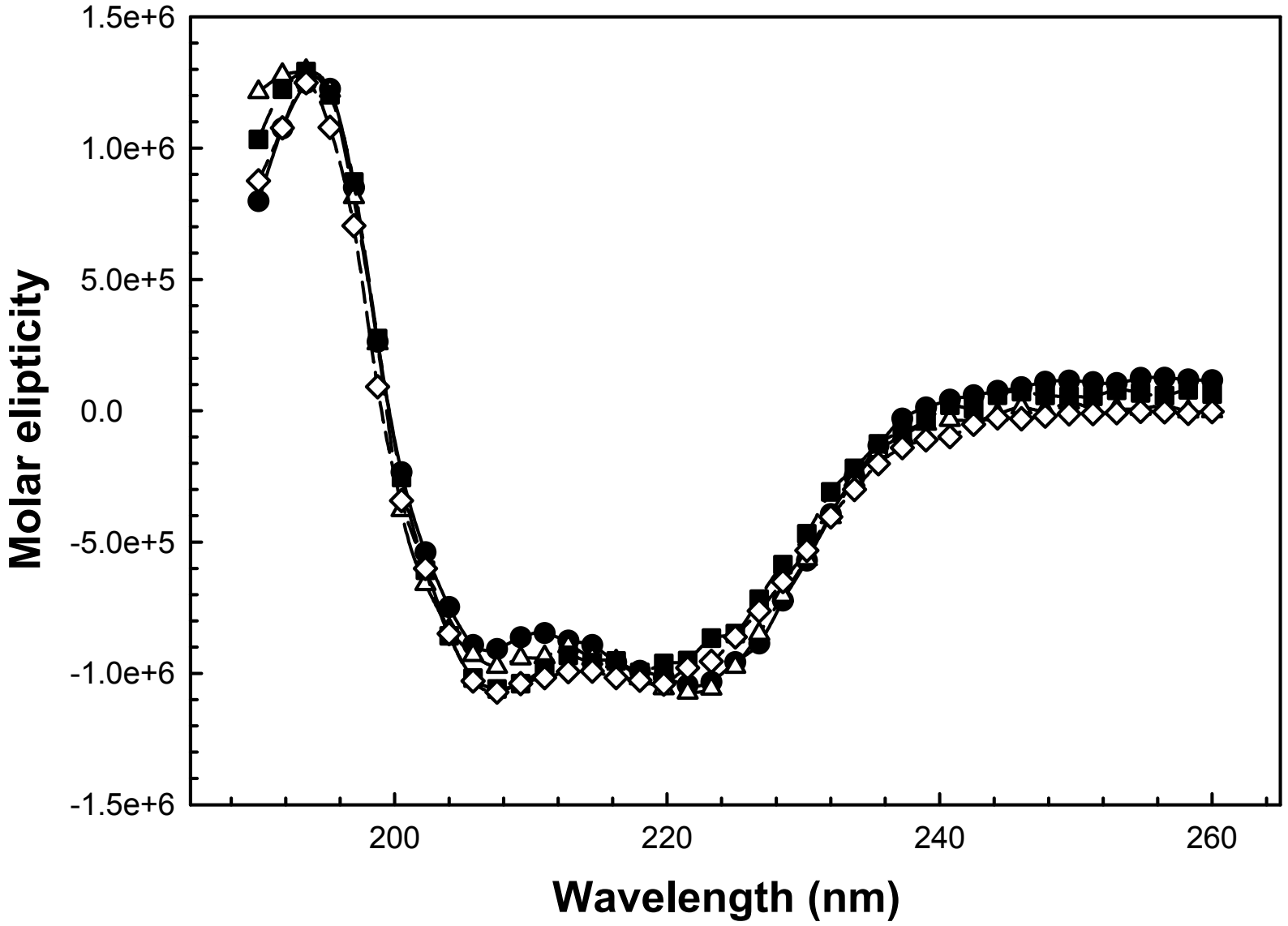

1.5e+6
1.0e+6
5.0e+5
0.0
-5.0e+5
-1.0e+6
-1.5e+6
Molar elipticity
200
220
240
260
Wavelength (nm)

Figure 2S

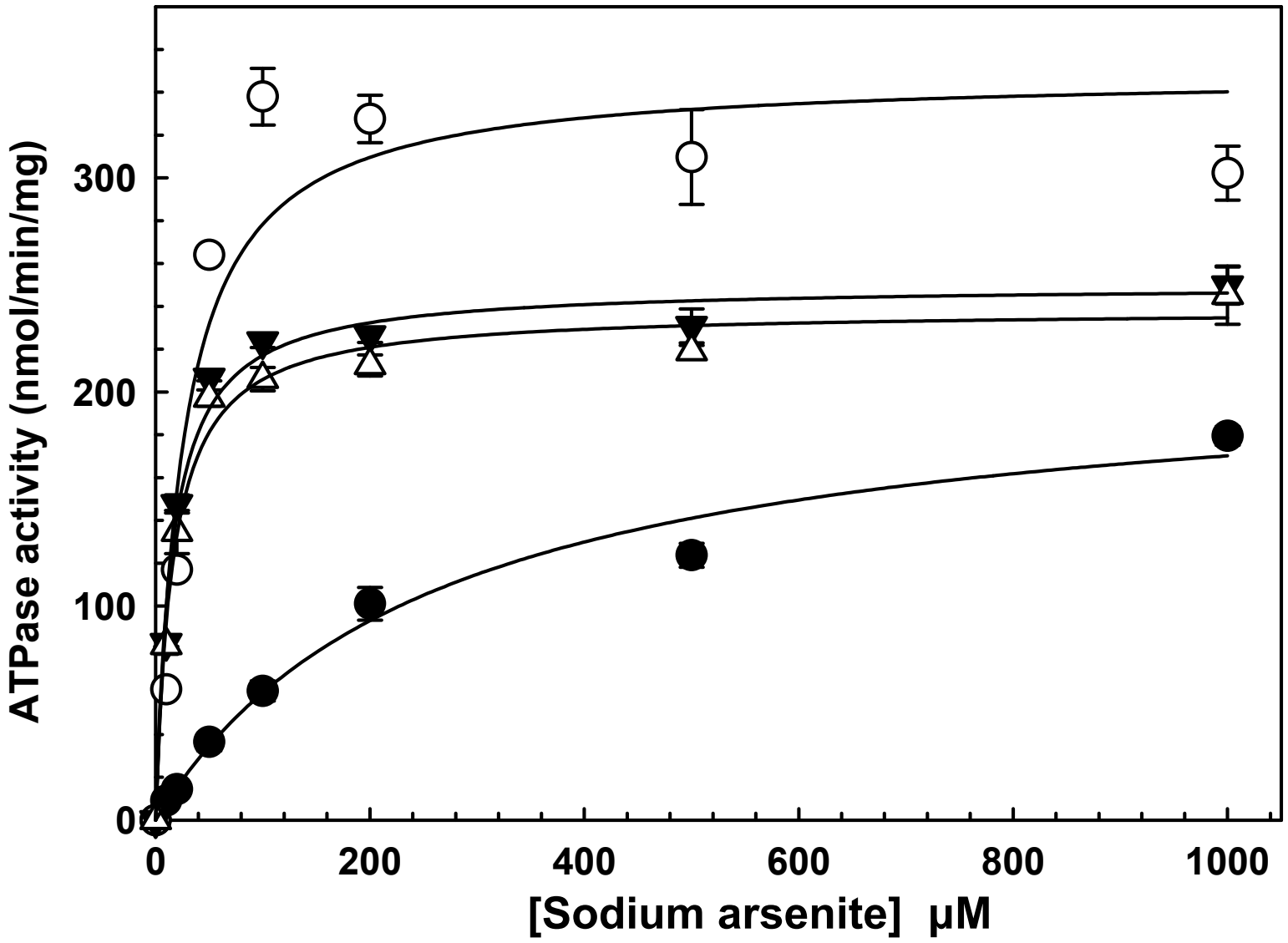

ATPase activity (nmol/min/mg)
0
100
200
300
0
200
400
600
800
1000
[Sodium arsenite] µM

Figure 3S

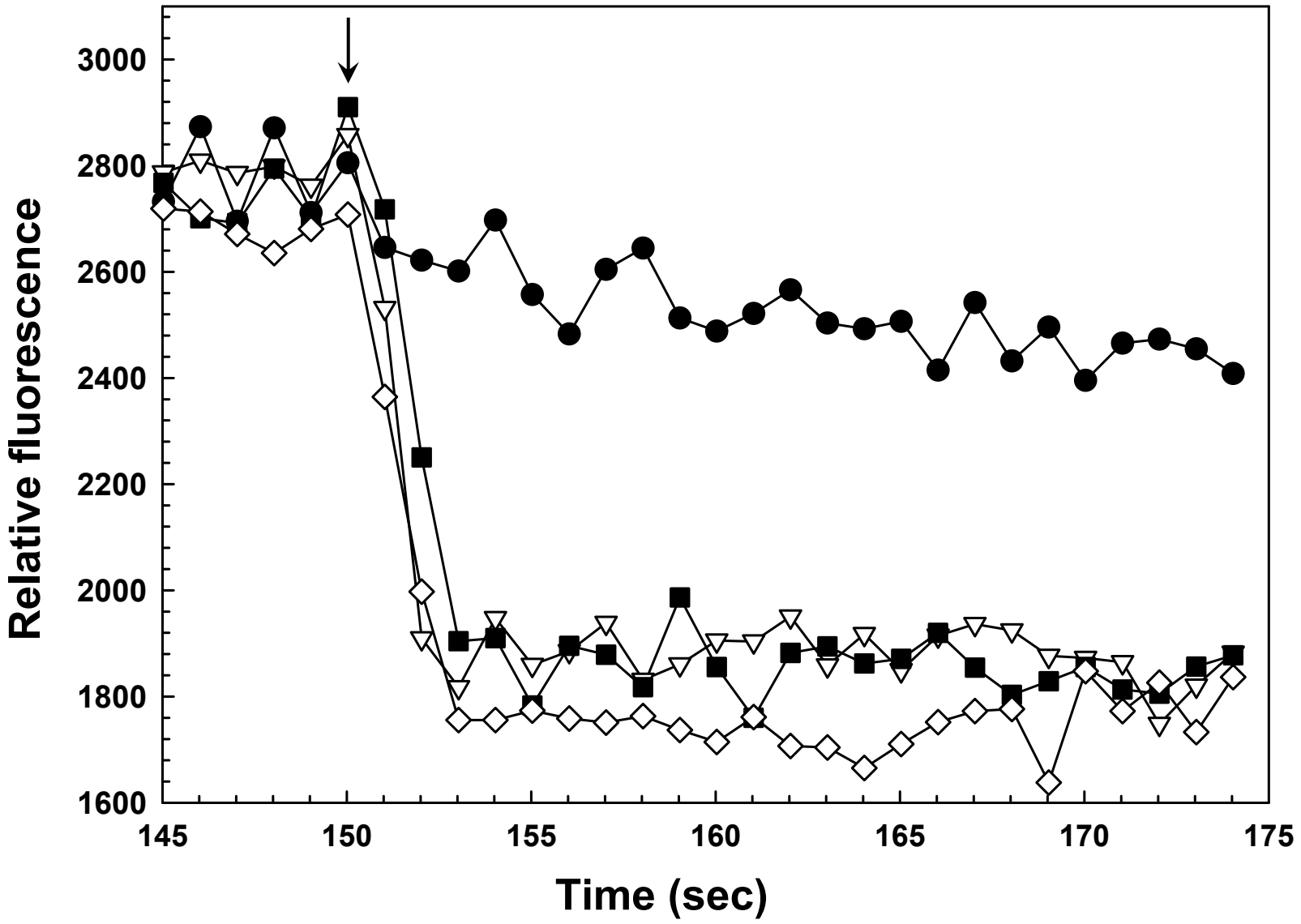
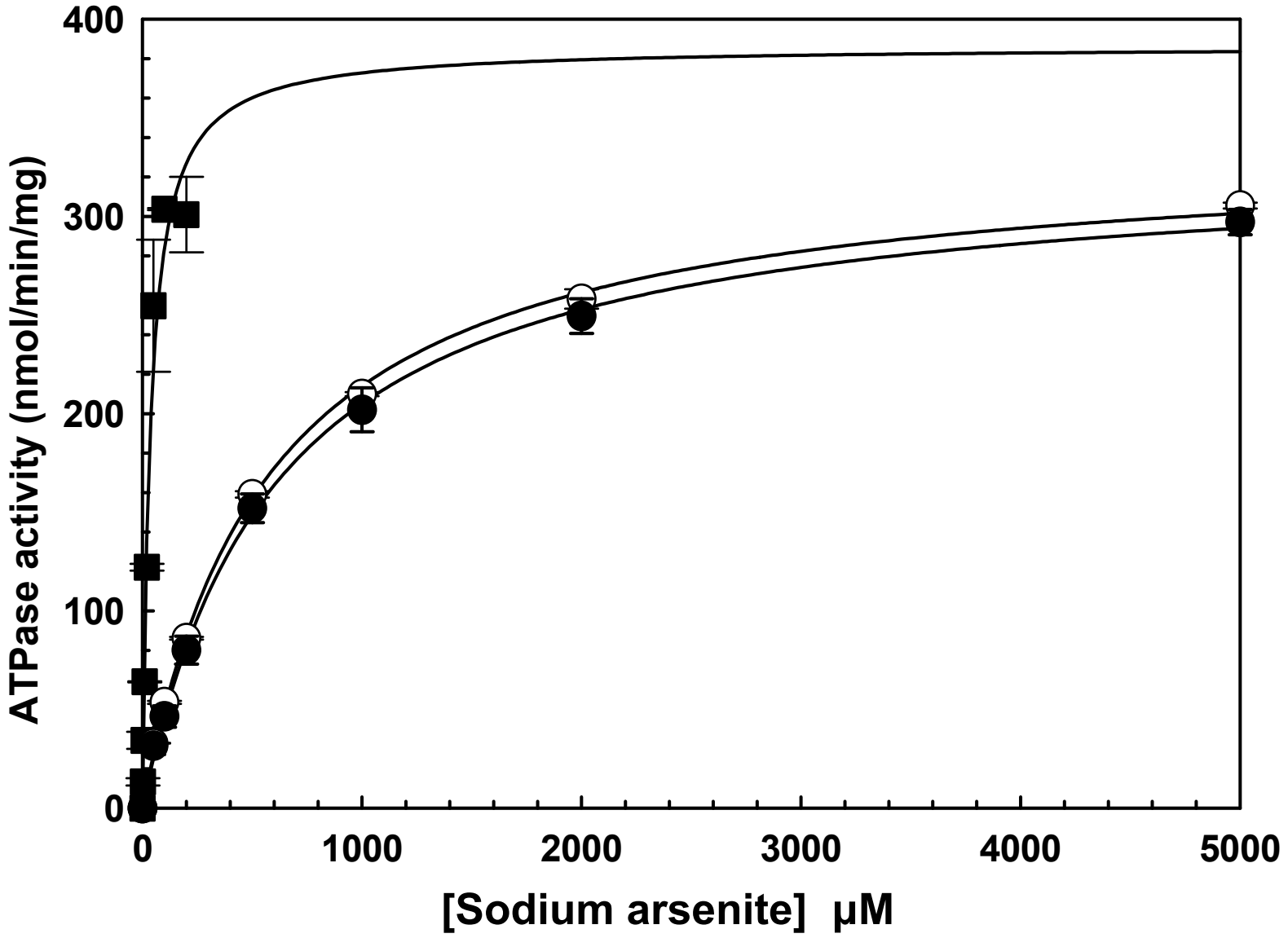

Relative fluorescence
3000
2800
2600
2400
2200
2000
1800
1600
145
150
155
160
165
170
175
Time (sec)

Figure 4S


400
300
200
100
0
ATPase activity (nmol/min/mg)
0
1000
2000
3000
4000
5000
[Sodium arsenite] μM