Supporting Information

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SI Materials and Methods

Cell Washing Methodology. To determine the validity of the cellular Cd isotope compositions, a series of tests examining the effect of cell washing were carried out. The purpose of cell washing is to remove Cd from cells that is not metabolically assimilated (e.g., passive adsorption to cell exterior; ref. 1). A series of cleaning agents were prepared and tested: 0.05 M trisodium citrate–0.075 M disodium EDTA, 0.85% NaCl–5 mM disodium EDTA, 100 mM disodium oxalate–50 mM disodium EDTA, 0.1 M HCl, 0.1 M EDTA, 0.42 M NaCl, and 18.2 MΩ H_2O. To test the various cleaning agents, a Cd-free starter culture of *Escherichia coli* was grown (*Materials and Methods*) and then transferred to a 1-L container. Immediately (~10 s) following the addition of Cd, 50-mL aliquots of the culture were transferred to centrifuge vials, and the cultures were harvested (*Materials and Methods*). The pellets were resuspended in a cleaning agent, and the suspension was gently agitated for ~1 min. Following this, the cells were centrifuged again, 18.2 MΩ H_2O was added, the cells were centrifuged again, and the final pellet was dried for weighing. The dry mass of recovered pellets for each cleaning agent is shown in Fig. S2. We find that all treatments lead to similar levels of recovery except for the citrate-EDTA treatment (~3.5 times less than the others). However, when the Cd content of each pellet is normalized by recovery mass (Fig. S3 and Table S2), we find that all treatments have essentially identical amounts of Cd present, except for the HCl treatment.

Effect of Cell Washing on Subcellular Cd Isotope Compositions. We also compared the Cd isotopic composition of subcellular isolates that were rinsed in HCl with those rinsed with 18.2 MΩ H_2O (Fig. S4). We found that the total amount of Cd recovered (for a comparable mass of cells) was ~34 times lower, despite all other experimental conditions remaining the same. The partitioning of Cd within the cell was also modified by HCl rinsing, apparently causing a shift in the Cd load from cytosol to membranes (although the total amount of Cd present is lower). Furthermore, the isotopic composition of the membranes was seen to shift to more negative Cd isotopic compositions; but the cytosol remained unchanged. The shift in membrane Cd isotope compositions likely explains why more intense washing techniques led to more fractionated isotopic compositions in whole cells (Fig. 2; Fig. S5). We were unable to isolate Cd from CdCA1 in HCl-rinsed cells, implying that the cleaning techniques were damaging the cells and causing Cd to leak from the cytosol and membranes.

Collectively, the washing data suggest that the rapid uptake of Cd by the cells was not reversible adsorption, but instead reflects some sort of biologically mediated binding of Cd to the cell surface (2), as has been reported for Cu (3). Unlike Cu, however, this does not lead to Cd isotopic fractionation (Fig. 2), in agreement with the (limited) dataset of adsorbed Cd isotopic compositions (4, 5). Because cleaning with 18.2 MΩ H_2O yielded similar Cd contents as the various cleaning agents tested (Fig. S2) but did not demonstrably attack the cells (e.g., HCl or citrate-EDTA mixtures; Fig. S1), we consider the H_2O cleaning to be the most robust in assessing the true Cd isotopic composition of cells studied here.

1. Tang D, Morel FMM (2006) Distinguishing between cellular and Fe-oxide-associated trace elements in phytoplankton. *Mar Chem* 98(1):18–30.
2. Kenney JPL, Fein JB (2011) Importance of extracellular polysaccharides on proton and Cd binding to bacterial biomass: A comparative study. *Chem Geol* 286(3-4):109–117.
3. Navarrete JU, Borrok DM, Viveros M, Ellzey JT (2011) Copper isotope fractionation during surface adsorption and intracellular incorporation by bacteria. *Geochim Cosmochim Acta* 75(3):784–799.
4. Schmitt AD, Galer SJ, Abouchami W (2009) Mass-dependent cadmium isotopic variations in nature with emphasis on the marine environment. *Earth Planet Sci Lett* 277(1-2):262–272.
5. Horner TJ, et al. (2010) Ferromanganese crusts as archives of deep water cd isotope compositions. *Geochem Geophys Geosyst* 11(4):Q04001.

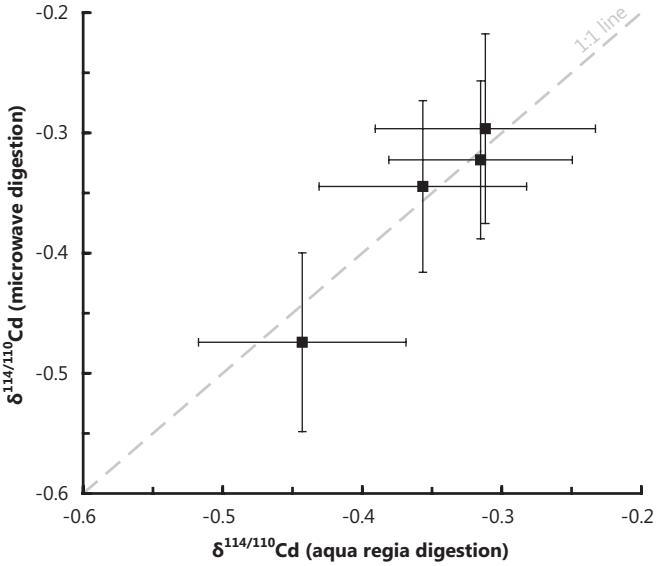

Fig. S1. Cell isotope compositions do not depend on the digestion method. Comparison of Cd isotope compositions for cells digested using the microwave treatment compared with those digested in aqua regia. The similarity of isotopic compositions indicates that the aqua regia digestion was sufficiently aggressive to liberate all Cd from cell organic matter.

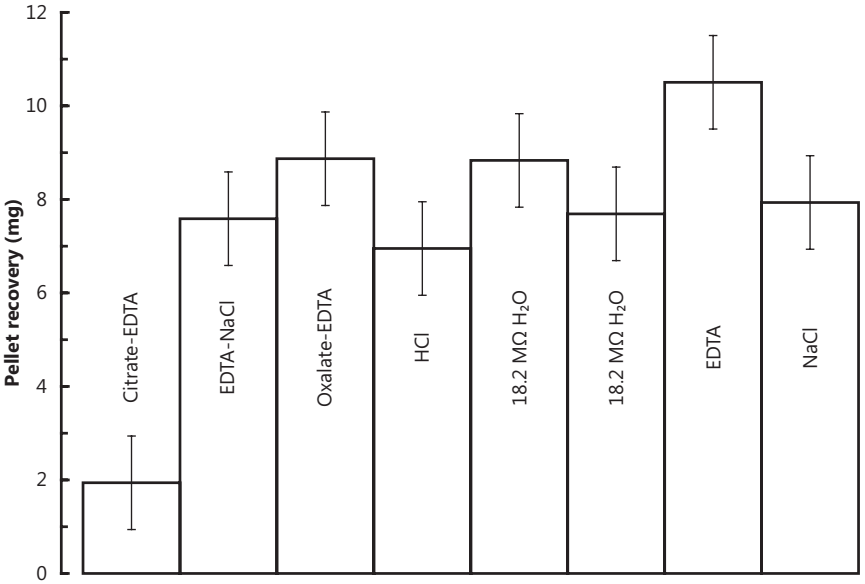

Fig. S2. The mass of bacterial pellets recovered depends on the cleaning strategy used. Dry mass of pellets recovered for a range of different cell washing solutions. All techniques yielded similar recovery masses, except for the citrate-EDTA solution.

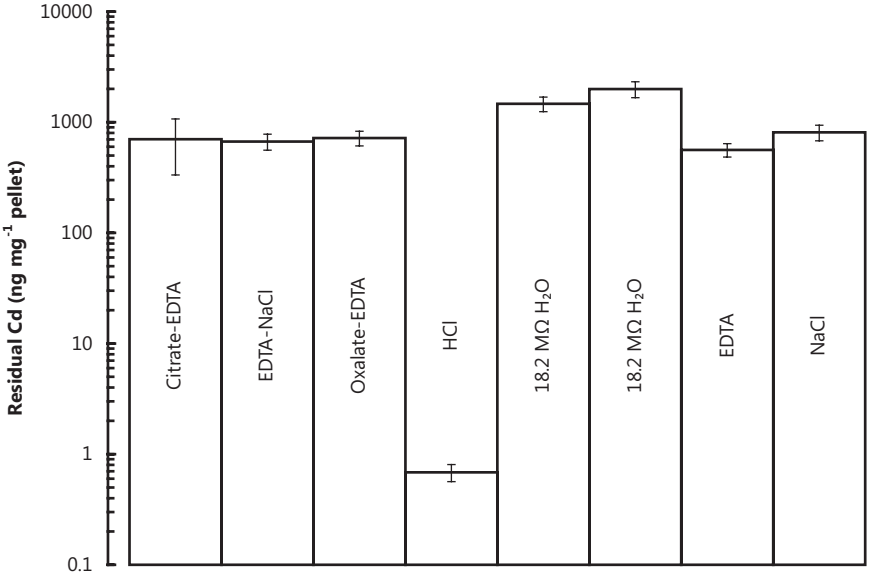

Fig. S3. The Cd content of pellets depends strongly on the cleaning method used. Total Cd (ng) per milligram (dry mass) of bacterial pellets for the same cleaning techniques outlined in Fig. S2. All cleaning techniques yielded similar Cd contents, except for the HCl rinse (~3 orders of magnitude lower Cd content than the other cleaning techniques).

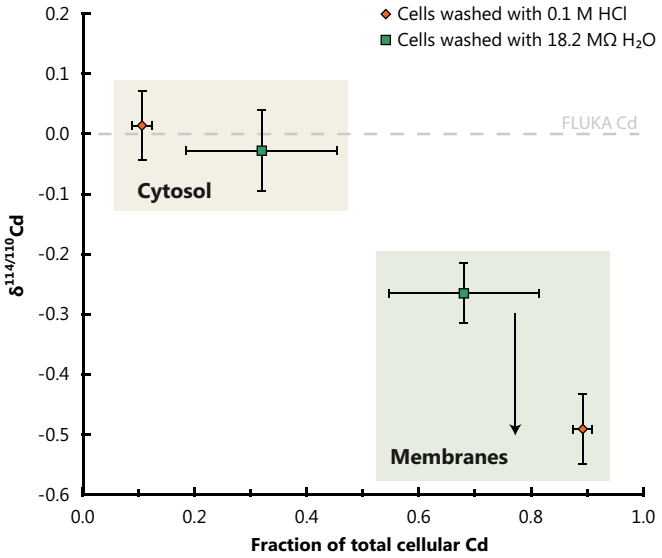

Fig. S4. The isotopic composition of the cell membranes depends on the cleaning method used. Cd isotopic composition vs. fraction of total cellular Cd for cells cleaned in 18.2 MΩ H_2O and 0.1 M HCl. The total Cd recovered from cells cleaned with HCl was ~34 times lower than those cleaned with H_2O, despite all other experimental conditions remaining the same. Furthermore, cleaning with HCl was found to shift the observed partitioning of Cd within cells from the cytosol to the membranes and change the isotopic composition of the membranes (denoted by the arrow), although not the cytosol.

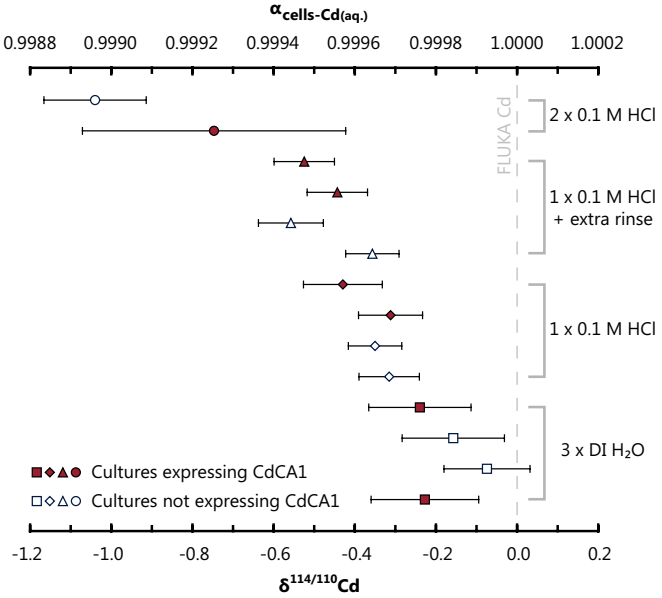

Fig. S5. The isotopic composition of whole cells depends on the cleaning method used. Range of cleaning techniques (relative scale) against whole cell isotopic compositions for induced and uninduced cultures (filled and open symbols, respectively). Increasing the relative ferocity of the cell washing leads to increasingly fractionated Cd isotopic compositions. Similar washing treatments are grouped by symbol. In each case, the induced and uninduced cells cleaned with the same agents exhibit isotopic compositions that are indistinguishable (within uncertainty).

Table S1. BLAST results for the *CdCA1* gene (from *Thalassiosira weissflogii*) in various microorganisms for which the complete genome is available (using Joint Genome Institute and National Center for Biotechnology Information databases)

Lineage	Genus and species	E-value*	Sequence overlap	First hit†	Accession no.
Chlorophyte	*Chlorella* sp. NC64A	—	—	—	—
	Chlamydomonas reinhardtii	—	—	—	—
	Coccomyxa sp. C-169	—	—	—	—
	Volvox carteri f. nagariensis	—	—	—	—
	Micromonas pusilla CCMP1545	$\sim 10^{-79}$	100%	Predicted protein	XP_003063214
	Ostreococcus RCC809	—	—	—	—
	Ostreococcus tauri	—	—	—	—
	Ostreococcus lucimarinus	—	—	—	—
Heterokont	*Fragilariopsis cylindrus*	—	—	—	—
	Phaeodactylum tricornutum CCAP1055	—	—	—	—
	Thalassiosira pseudonana CCMP1335	$\sim 10^{-121}$	99%	Predicted protein	XP_002295227
	Phaeodactylum tricornutum UTEX	Detected via nested PCR		Cadmium-specific carbonic anhydrase	See ref. 1
	Thalassiosira weissflogii	$\equiv 0$	100%	Cadmium-specific carbonic anhydrase	AAX08632
Cyanobacteria	No matches				
Archaea	*Vulcanisaeta distributa* DSM14429	$\sim 10^{0}$	14%	50S ribosomal protein L15	YP_003901354
	No other matches				
Bacteria	*Plesiocystis pacifica* SIR-1	$\sim 10^{-49}$	99%	Hypothetical protein PPSIR1_39640	ZP_01905628
	Nitrosomonas sp. AL212	$\sim 10^{0}$	22%	Carbonic anhydrase, cadmium-binding protein	YP_004295035
	Hoeflea phototrophica	$\sim 10^{0}$	82%	Iron-regulated protein FrpC	ZP_02165618
	No other matches				

*Expected values (number of hits that may occur by chance within a given genome) $>10^{-3}$ are omitted for clarity unless there is >10% sequence overlap.
†Using National Center for Biotechnology Information annotation.

1. Park H, Song B, Morel FMM (2007) Diversity of the cadmium-containing carbonic anhydrase in marine diatoms and natural waters. *Environ Microbiol* 9(2):403–413.

Table S2. Comparison of pellet recoveries and residual Cd for the various cleaning agents tested (data plotted in Figs. S2 and S3)

Cleaning agent	Dry mass of pellet recovered (mg)*	Residual Cd (ng Cd per mg dry pellet)
0.05 M trisodium citrate–0.075 M disodium EDTA	2	702 ± 369
0.85% NaCl–5 mM disodium EDTA	8	669 ± 111
100 mM disodium oxalate–50 mM disodium EDTA	9	720 ± 108
0.1 M HCl	7	1 ± 0†
0.1 M EDTA	11	562 ± 78
0.42 M NaCl	8	809 ± 130
18.2 MΩ H_2O (*i*)	9	1,467 ± 222
18.2 MΩ H_2O (*ii*)	8	1,994 ± 327

*Weighing uncertainty ±1 mg.
†Measurement uncertainty ±0.1 ng/mg pellet.

Table S3. Isotopic data for whole cell washing experiments

Experiment no.	IPTG (+/–)	Washing	[Cd] (ng Cd per mg dry pellet)	$\delta^{114/110}$Cd	±2 SD*	$n^{\dagger}$
CdCA03	+	3× DI H_2O	>2,000 (above calibration)	−0.23	0.13	5
	–		>2,000 (above calibration)	−0.07	0.11	4
CdCA04	–[‡]		1,842	+0.06	0.13	5
	–		2,366	−0.16	0.13	6
	+		2,957	−0.24	0.13	7
CdCA05	+	2× HCl	2	−0.75	0.32	1
	+		<1	—	—	—
	–		<1	—	—	—
	–		18	−1.04	0.08	1
CdCA06	+	1× HCl	44	−0.43	0.10	5
	+		326	−0.31	0.08	5
	–		180	−0.32	0.07	5
	–		129	−0.35	0.08	5
	+	1× HCl + extra rinse	193	−0.44	0.07	5
	+		161	−0.52	0.07	5
	–		74	−0.56	0.07	5
	–		260	−0.36	0.07	5

IPTG, isopropyl-β-D-1-thiogalactopyranoside.
*Uncertainties reported as 2× SD of sample replicates (when $n \geq 5$) or reproducibility of solution standards within the same analysis session, whichever is the larger of the two.
[†]Number of independent isotopic measurements.
[‡]Adsorption-only experiment (cells were harvested after ~10 s).

Table S4. Isotopic data for intracellular separates

Experiment no.	IPTG (+/–)	Washing	Cellular separate	$\delta^{114/110}$Cd	±2 SD	n	Percent cellular Cd*	Percent cytosolic Cd[†]
CdCA05	+	1× DI H_2O	CdCA1	−0.67	0.07	8	—	—
CdCA06	+	1× DI H_2O	Membranes	−0.26	0.05	5	68 ± 13	—
			Cytosol	−0.03	0.07	5	32 ± 13	—
			CdL[‡]	−0.09	0.06	5		98.7 ± 0.2
			CdCA1	−0.59	0.07	5		1.1 ± 0.3
			Denatured CdCA1	+0.17	0.05	2		0.13 ± 0.03
		1× 0.1 M HCl	Membranes	−0.49	0.06	5	89.2 ± 1.7	—
			Cytosol	+0.01	0.06	2	10.6 ± 1.9	—
			CdL[‡]	+0.23	0.17	3		~100[§]

*Percentage of total cellular Cd.
[†]Percentage of total cytosolic Cd.
[‡]CdL refers to nonspecifically bound Cd in the cytosol (i.e., eluted from the His·Bind column before tag cleavage).
[§]Other isolates (e.g., CdCA1) from the cells washed in HCl contained negligible Cd, such that the nonspecifically bound Cd was essentially the only component present in acid-cleaned cells.

Table S5. Isotopic data for cell digestion experiments

Experiment no.	IPTG (+/–)	Cell washing	$\delta^{114/110}$Cd aqua regia digestion	±2 SD	n	$\delta^{114/110}$Cd microwave digestion	±2 SD	n
CdCA06	–	1× 0.1 M HCl	−0.32	0.07	5	−0.32	0.07	5
	+		−0.31	0.08	5	−0.30	0.08	5
	–	1× 0.1 M HCl + extra rinse	−0.36	0.07	5	−0.34	0.07	5
	+		−0.44	0.07	5	−0.47	0.07	5